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CHEMICAL CONSTITUENTS OF *ERYTHRINA FUSCA* AND *ERYTHRINA SUBEROSA* STEM BARK AND THEIR BIOLOGICAL ACTIVITIES

Miss Pranorm Khaomek

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ปรานอม ขาวเมฆ: องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของเปลือกต้นทองโหลงและทองบกและฤทธิ์ทางชีวภาพ (CHEMICAL CONSTITUENTS OF *ERYTHRINA FUSCA* AND *ERYTHRINA SUBEROSA* STEM BARK AND THEIR BIOLOGICAL ACTIVITIES) อาจารย์ที่ปรึกษา: รศ. ดร. นิจศิริ เรืองรังษี, อาจารย์ที่ปรึกษาร่วม: รศ. ดร. เอกรินทร์ สายฟ้า, 239 หน้า. ISBN: 974-17-5109-5

การศึกษาทางพฤกษเคมีของเปลือกต้นทองโหลง สามารถแยกสารใหม่ได้ 1 ชนิด คือ 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan และสารที่มีรายงานมาแล้ว 11 ชนิด ได้แก่ sandwicensin, lupinifolin, citflavanone, lonchocarpol A, erythrisenegalone, liquiritigenin, daidzein, 8-prenyldaidzein, cerinic acid, 1-octacosanol และ erythrinassinate B ส่วนการศึกษาทางพฤกษเคมีของเปลือกต้นทองบกพบสารที่มีรายงานมาแล้ว 6 ชนิดคือ erythrabyssin II, sandwicensin, erythrinassinate B, 5,7,4'-trihydroxy-8,3',5'-triprenylflavanone, erythratidinone, และสารผสมของ β-sitosterol กับ stigmasterol การพิสูจน์โครงสร้างทางเคมีของสารที่แยกได้นี้อาศัยการวิเคราะห์สเปกตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว จากการศึกษาฤทธิ์ต้านเชื้อมาลาเรีย, ฤทธิ์ต้านจุลชีพ, ฤทธิ์จับอนุมูลอิสระ, ฤทธิ์ต้านเชื้อวัณโรค และ ความเป็นพิษต่อเซลล์มะเร็งของสารทดสอบทั้งหมดพบว่ามีฤทธิ์ต่ำในการต้านเชื้อจุลชีพ ยกเว้น lonchocarpol A และ lupinifolin มีฤทธิ์แรงในการต้านจุลชีพ *Bacillus subtilis* มีฤทธิ์ปานกลางต่อ *Enterococcus faecalis* และ *Staphylococcus aureus* และพบว่า lonchocarpol A มีฤทธิ์ต้านเชื้อมาลาเรียสายพันธุ์ K_1ที่แรงที่สุด (EC_{50} 1.6 μg/ml) เมื่อเปรียบเทียบกับ erythrabyssin II, 8-prenyldaidzein และ citflavanone (EC_{50} 3.9, 5.0 และ 5.0 μg/ml ตามลำดับ) แต่ไม่มีฤทธิ์ในสัตว์ทดลอง (ที่ 20 mg/kg) นอกจากนี้สารทดสอบเกือบทั้งหมดมีฤทธิ์ต่ำในการจับอนุมูลอิสระและการต้านเชื้อวัณโรค (H37Ra) ในขณะเดียวกันพบว่า erythrisenegalone และ lupinifolin มีความเป็นพิษที่แรงต่อเซลล์มะเร็งเต้านม (BC)

สาขาวิชา เภสัชเคมีและผลิตภัณฑ์ธรรมชาติ ลายมือชื่อนิสิต..
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PRANORM KHAOMEK: CHEMICAL CONSTITUENTS OF *ERYTHRINA FUSCA* AND *ERYTHRINA SUBEROSA* STEM BARKS AND THEIR BIOLOGICAL ACTIVITIES. THESIS ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. EKARIN SAIFAH, Ph.D., 239 pp. ISBN: 974-17-5109-5

Phytochemical study of the stem bark of *Erythrina fusca* Lour. led to the isolation of a new pterocarpan, 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan, together with 11 known compounds: sandwicensin, lupinifolin, citflavanone, lonchocarpol A, erythrisene galone, liquiritigenin, daidzein, 8-prenyldaidzein, cerinic acid, 1-octacosanol and erythrinassinate B. Phytochemical study of the stem bark of *E. suberosa* Roxb. yielded 6 known compounds, erythrabyssin II, sandwicensin, erythrinassinate B, 5,7,4'-trihydroxy-8,3',5'-triprenylflavanone, erythratidinone, and a mixtures of β-sitosterol and stigmasterol. The structures of all these isolates were determined by extensive spectroscopic studies, including comparison of their UV, IR, MS and NMR properties with previously reported data. Some of these compounds were evaluated for its antimalarial activity, antimicrobial activity, free radical scavenging activity, antituberculosis activity and cytotoxic activity against cancer cell. All of the tested compounds showed weak antimicrobial activity except lonchocarpol A and lupinifolin which were strongly active against *Bacillus subtilis* and moderate active against *Enterococcus faecalis* and *Staphylococcus aureus*. Lonchocarpol A showed the highest *in vitro* antimalarial activity against K1 strain (EC$_{50}$ 1.6 μg/ml), when compared with 8-prenyldaizein, erythrabyssin II and citflavanone (EC$_{50}$ 3.9, 5.0 and 5.0 μg/ml, respectively). However, lonchocarpol A exhibited no *in vivo* antimalarial activity (at 20 mg/kg). In addition, all of tested compounds showed marginal free radical scavenging activity. Almost all of the tested compounds showed weak antituberculosis activity against H37Ra strain, whilst erythrisenegalone and lupinifolin displayed strong cytotoxic activity against breast cancer (BC) cell line.

Field of study Pharmaceutical Chemistry and Natural Products

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LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]^{25}_{D}$ = Specific rotation at 25° and sodium D line (589 nm)

Acetone-d_6 = Deuterated acetone

ax = Axial

br = Broad (for NMR spectea)

°C = Degree Celsius

calcd = Calculated

$CDCl_3$ = Deuterated chloroform

$CHCl_3$ = Chloroform

CH_2Cl_2 = Dichloromethane

CD_3OD = Deuterated methanol

CH_3CN = Acetonitile

Cm = Centimeter

cm^{-1} = Reciprocal centimeter (unit of wave number)

^{13}C NMR = Carbon-13 Nuclear Magnetic Resonance

d = Doublet (for NMR spectra)

dd = Doublet of doublets (for NMR specta)

DEPT = Distortionless Enhancement by Polarization Transfer

DPPH = 1,1-Diphenyl-2-picrylhydrazyl

DMSO-d_6 = Deuterated dimethylsulfoxide

δ = Chemical shift

ε = Molar absorptivity

ED_{50} = Median Effective Dose

EIMS = Electron Impact Mass Spectrometry

eq = Equatorial

ESIMS = Electrospray Ionization Mass Spectrometry

EtOAc = Ethyl acetate

EtOH = Ethanol

FABMS = Fast Atom Bombardment Mass Spectrometry

g = Gram

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

GC/MS = Gas Chromatrography/Mass Spectrometry

hr = Hour

^{1}H NMR = Proton Nuclear Magnetic Resonance

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

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

H_2O = Water

HPLC = High Performance Liquid Chromatography

HRFABMS = High Resolution Fast Atom Bombardment Mass Spectrometry

HREIMS = High Resolution Electron Impact Mass Spectrometry

Hz = Hertz

IC_{50} = Median Inhibitory Concentration

IR = Infrared Spectrum

J = Coupling constant

KBr = Potassium bromide

Kg = Kilogram

μg = Microgram

μL = Microliter

μM = Micromolar

λ_{max} = Wavelength at maximal absorption

M^+ = Molecular ion

m = Multiplet (for NMR spectra)

MHA = Mueller Hinton agar

MeOH = Methanol

mg = Milligram

MHz = Megahertz

MIC = Minimum Inhibitory Concentration

MBC = Minimum Bactericidal Concentration

min = Minute

mL = Milliliter

mM = Millimolar

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

MW = Molecular weight

m/z = Mass to charge ratio

MS = Mass Spectrometry

nm = Nanometer

NMR = Nuclear Magnetic Resonance

NOE = Nuclear Overhauser Effect

NOESY = Nuclear Overhauser Effect Spectroscopy

NSS = Normal saline solution

ODS = Octadecylsilane

ppm = Part per million

PTLC = Preparative Thin Layer Chromatography

q = Quartet (for NMR spectra)

SDA = Sabouraud dextrose agar

spp. = Species

ν_{max} = Wave number at maximal absorption

s = Singlet (for NMR spectra)

TFA = Trifluoro acetic acid

t = Triplet (for NMR spectra)

TLC = Thin Layer Chromatography

UV = Ultraviolet

UV-VIS = Ultraviolet and Visible Spectrometry

CHAPTER I

INTRODUCTION

The genus *Erythrina* (coral tree) belongs to the subfamily Papilionoideae of the family Leguminosae. The 108 species of deciduous and semi-evergreen trees and shrubs in this genus occur wild in tropical and subtropical regions around the world, though with most species in the America and Africa. Belonging to the bean tribe of the legumes, they are grown as ornamentals for their vividly hued flowers. Their trunks and branches are protected by short, sharp prickles; many species have weak branches that tend to fall in storms. Leaves are pinnately 3-foliolate often diamond-shaped leaflets, stipules small. Flowers in axillary and terminal racemes, handsome, usually bright red, twin or fascicled along the rhachis, bracts and bracteoles small or zero. Bean-like flowers in scarlet, crimson or orange are borne in racemes towards the ends of the branches at varying times of the year (some species in mid-winter), followed by narrow seed pods that dry and brown as they ripen (Warren, 1998). Calyx with an oblique mouth, splitting down to the base, or campanulately bilabiate. Petals unequal; standard much exserted, considerably exceeding the keel and wings. Vexillary stamen free nearly to the base or connate with the others half-way up the filaments; anthers uniform. Ovary stalked; ovules many; style incurved, subulate at the apex, beardless; stigma, terminal. Pod stalked, falcate at the apex, beardless; stigma small, terminal (Kirtikar *et al*., 1981).

According to Smitinand (1980), there are six species of the genus *Erythrina* found in Thailand as follows.

E. crista-galli Linn.	ทองหลางฮ่องกง (Thong laang hong kong)
E. fusca Lour.	ทองโหลง (Thong long)
E. stricta Roxb.	ทองเดือนห้า (Thong duean haa)
E. suberosa Roxb.	ทองบก (Thong bok)
E. subumbrans Merr.	ทองหลางป่า (Thong laang paa)
E. variegata Linn.	ทองหลางลาย (Thong laang laai)

***Erythrina suberosa* Roxb.** (Figure 1) is a medium-sized deciduous tree with deeply cracked corky bark. Branches armed are white or pale yellow prickles. Young parts are undersurface of leaflets and inflorescence softly tomentose. Leaflets have 7.5-20 cm broad, often broader than deep, green and glabrous above, glaucous and matted with grey cottony pubescence beneath, rhomboid, entire or lobed, acute, base broadly deltoid. Racemes dense are terminating the branches. Calyx campanulate is deeply 2-labiate, standard 3.8-5 cm long, oblong, narrowed into a short claw and keel-petals connate is less than half the length of the standard. Upper stamen is free from low down. Pod has 12.5-15 cm long, terete, tapering at the ends, torulose; seeds 4-5 black. This plant is distributed in Central and South India, Pakistan and Northern to the Central Thailand (Kirtikar *et al*., 1981; Tanaka *et al*., 2001; Nanakorn *et al*., 2003). *E. suberosa* Roxb. is cultivated extensively in India as an ornamental and medicinal plant. The juice of leaf and bark is reported to have anti-tumor activity (Miana, Sultana and Khan, 1972).

Previous phytochemical studies of the roots, leaves, wood and seeds of *E. suberosa* have been reported (Singh and Chawla, 1970; Miana, Sultana and Khan, 1972; Tanaka *et al*., 1998; 2001). However, chemical constituents from the stem bark of this plant have not been reported. The major components of *E. suberosa* seeds have been known to be erythrinan alkaloids. Phytochemical studies of non-alkaloidal secondary metabolites from the roots, leaves and wood of *E. suberosa* yielded pterocarpans (erysubin C, erysubin D and cristacarpin) and isoflavones such as, wighteone, alpinumisoflavone, erythrinin C, erysubin A, erysubin B, erysubin E, erysubin F, euchrenone b_{10} and senegalensin.

***Erythrina fusca* Lour.** (Figure 2) found in many tropical areas. This deciduous tree grows to 80 ft (24 m) tall with a crooked trunk. Its pinnate leaves have 8 inches (20 cm) long. The flowers are rich scarlet with creamy green wings and keel. They are followed by slim pods, which are up to 12 (30 cm) long (Warren, 1998). Previous phytochemical studies of the stem bark, seeds and leaves of *E. fusca* during the past two decades have indicated the major components of its seeds and leaves as erythrinan alkaloids (Barton *et al*., 1973; Hargreaves *et al*., 1974). Three pterocarpans, erythrabyssin-I, 3-*O*-methylcalopocarpin and sandwicensin, were also isolated from the stem bark of this plant (Fomum *et al*., 1986; McKee *et al*., 1997).

Several *Erythrina* species have been used medicinally in many countries. For example, the East African has been used *E. abyssinaica* in various folk remedies, such as malaria and syphilis, and 60% methanol extract of the roots was found to possess strong activity against gram positive bacteria (Kamat, *et al*., 1981). *E. addisoniae* is widely used in traditional medicine in Cameroon to treat various diseases, including dysentery, asthma, veneral diseases, boils and leprosy (Talla, *et al*., 2003). *E. indica*, a plant used extensively in African folk medicine for the treatment of several diseases, including microbial infections (Nkengfack, *et al*., 2000). *E. sigmoidea* has been used in Cameroonian traditional medicine for the treatment of various diseases, dysentery, asthma, stomach pain, female infertility and microbial infections (Nkengfack, *et al*., 1994).

There are many reports concerning biological activities of flavonoids from *Erythrina* spp. including antimicrobial (Kamat, *et al*., 1981; Mitscher, *et al*., 1988; Nkengfack, *et al*., 1995; Waffo, *et al*., 2000), anti-plasmodial (Yenesew, *et al*., 2003), cytotoxicity against human KB cells (Nkengfack, *et al*., 2001), anti-tuberculosis (Tanaka, *et al*., 2003) and antioxidant (Talla, *et al*., 2003) activities. Pharmacological screening of the extracts from *E. suberosa* and *E. fusca* showed them as possessing significant activities as well. It is therefore of interest to investigate these two plants for the above mentioned activities.

The main objectives of this investigation were as follows.

1. To isolate and purify compounds from the stem bark of *E. suberosa* Roxb. and *E. fusca* Lour.
2. To determine the chemical structure of each isolated compound
3. To evaluate anti-microbial, anti-malarial, free radical scavenging, antituberculosis and cytotoxic activities of each isolated compound

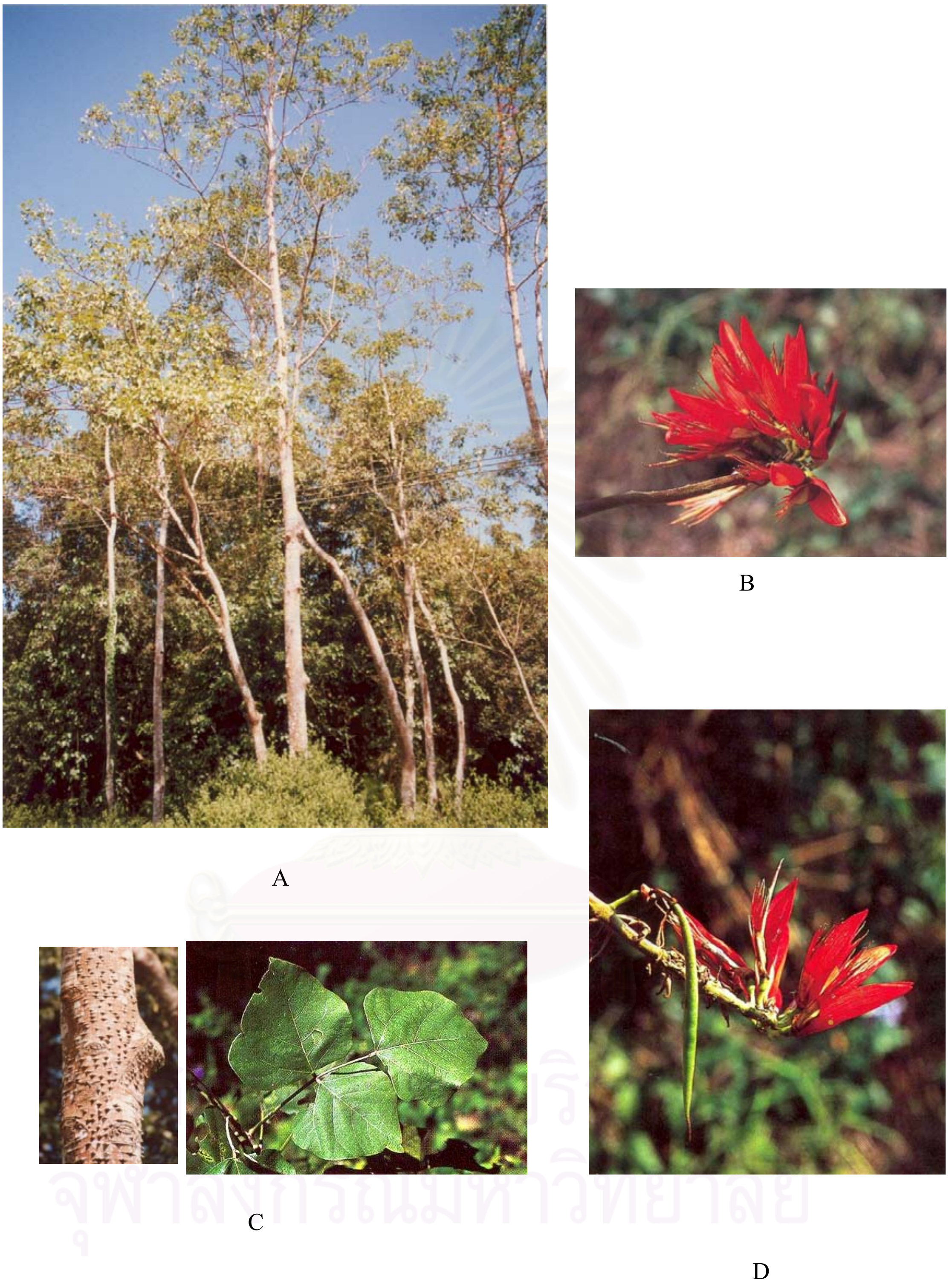


Figure 1 *Erythrina suberosa* Roxb.

A) Whole plant B) Inflorescence, C) Stem bark and Leaves, D) Flowers andPods

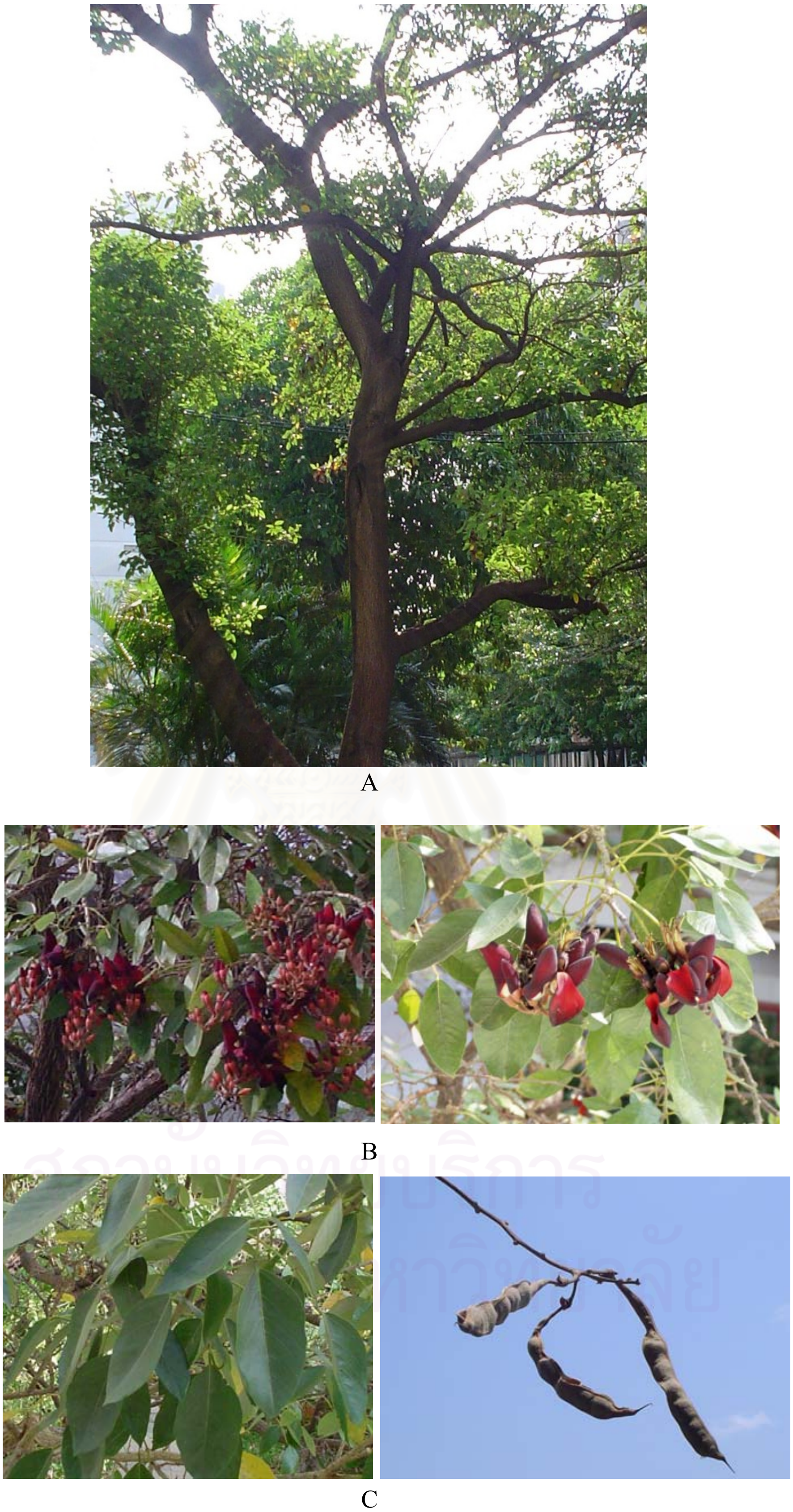


Figure 2 *Erythrina fusca* Lour.

A) Whole plant, B) Inflorescence, C) Leaves and Pods

CHAPTER II

HISTORICAL

1. Chemical Constituents of *Erythrina* spp.

A number of compounds have been isolated from plants in the genus *Erythrina*. They can be classified as flavonoids, alkaloids, terpenoids, steroids and miscellaneous substances (Tables 1-3).

Table 1 Distribution of flavonoids in *Erythrina*

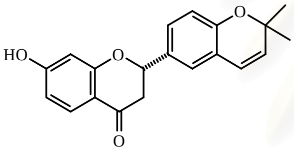
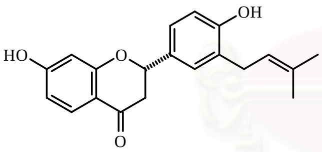
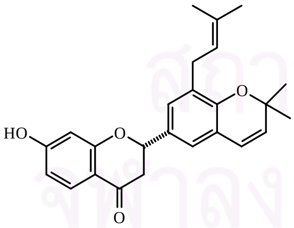
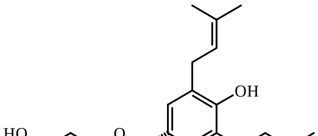
Plant and chemical compound	Category	Plant part	Reference
***Erythrina abyssinica* DC.**			
Abyssinone-I **[1]** 	Flavanone	Root	Kamat *et al.*, 1981
Abyssinone-II **[2]** 	Flavanone	Root	Kamat *et al.*, 1981
Abyssinone-III **[3]** 	Flavanone	Root	Kamat *et al.*, 1981
Abyssinone-IV **[4]** 	Flavanone	Root	Kamat *et al.*, 1981

Table 1 (continued)

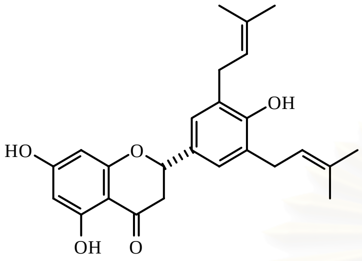
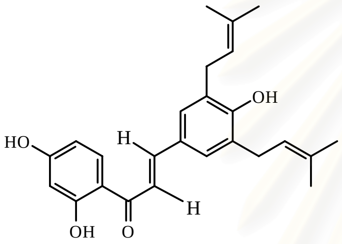
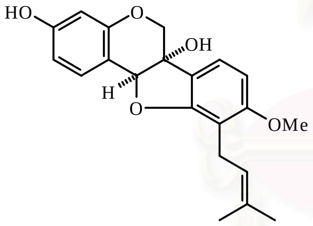
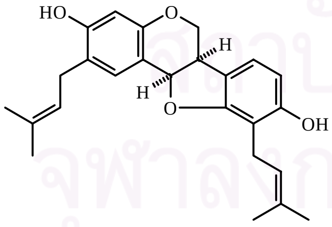
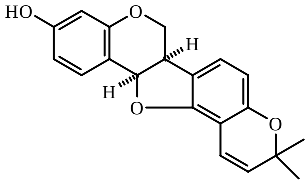
Plant and chemical compound	Category	Plant part	Reference
***E. abyssinica* DC.**			
Abyssinone-V **[5]** 	Flavanone	Root	Kamat *et al.*, 1981
Abyssinone-VI **[6]** 	Chalcone	Root	Kamat *et al.*, 1981
Erythrabyssin-I **[7]** 	Pterocarpan	Root	Kamat *et al.*, 1981
Erythrabyssin-II **[8]** 	Pterocarpan	Root	Kamat *et al.*, 1981
Phaseollin **[9]** 	Pterocarpan	Root	Kamat *et al.*, 1981

Table 1 (continued)

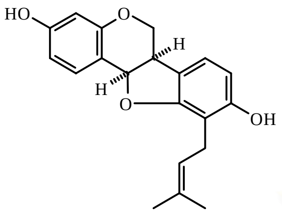
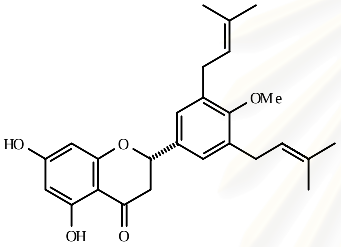
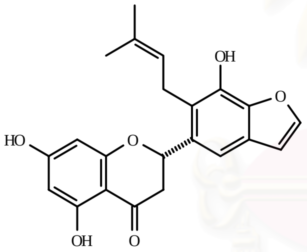
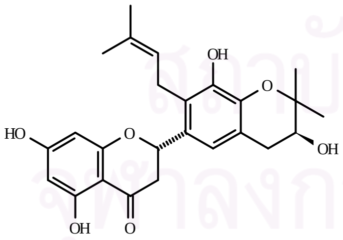
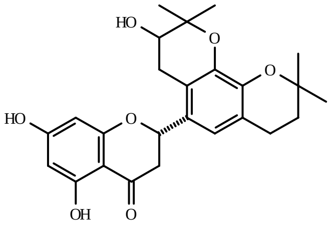
Plant and chemical compound	Category	Plant part	Reference
***E. abyssinica* DC.**			
Phaseollidin **[10]** 	Pterocarpan	Root	Kamat *et al.*, 1981
Abyssinone-V 4'-methyl ether **[11]** 	Flavanone	Stem Bark	Moriyasu *et al.,* 1998
Abyssinoflavanone IV **[12]** 	Flavanone	Stem Bark	Moriyasu *et al.,* 1998
Abyssinoflavanone V **[13]** 	Flavanone	Stem Bark	Moriyasu *et al.,* 1998
Abyssinoflavanone VI **[14]** 	Flavanone	Stem Bark	Moriyasu *et al.,* 1998

Table 1 (continued)

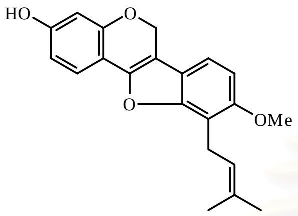
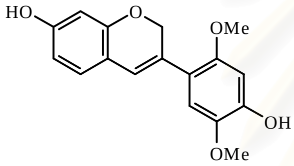
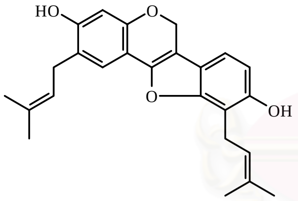
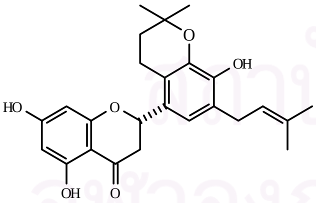
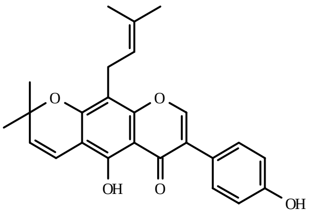
Plant and chemical compound	Category	Plant part	Reference
***E. abyssinica* DC.**			
3-Hydroxy-9-methoxy-10-(3,3-dimethylallyl)pterocarpene **[15]** 	Pterocarpan	Root bark	Yenesew *et al.,* 2003
7,4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene **[16]** 	Isoflav-3-ene	Root bark	Yenesew *et al.,* 2003
Erycristagallin **[17]** 	Pterocarpan	Root bark	Yenesew *et al.,* 2003
Sigmoidin D **[18]** 	Flavanone	Bark	Moriyasu *et al.,* 1998
***E. addisoniae* Hutchinson & Dalziel**			
Warangalone **[19]** 	Isoflavone	Stem bark	Talla *et al*., 2003

Table 1 (continued)

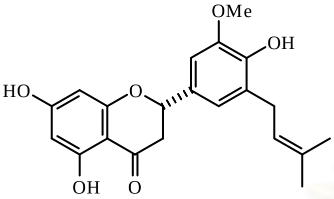
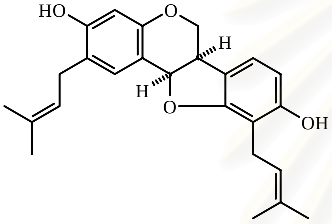
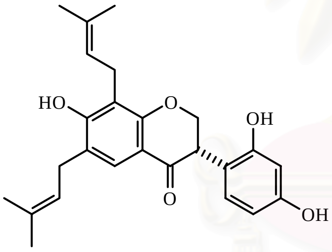
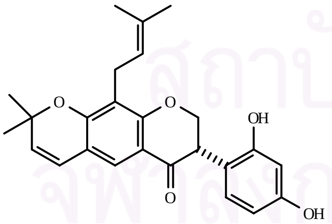
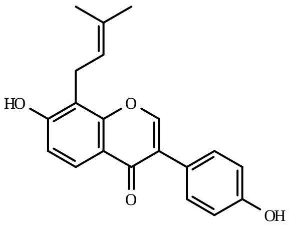
Plant and chemical compound	Category	Plant part	Reference
***E. berteroana* Urb.** Sigmoidin B **[20]** 	Flavanone	Root bark	Maillard, Gupta and Hostettmann, 1987
E. x bidwillii Erythrabyssin-II **[8]** 	Pterocarpan	Root bark	Iinuma *et al*, 1992
Bidwillon A **[21]** 	Isoflavanone	Root bark	Iinuma *et al*, 1992
Bidwillon B **[22]** 	Isoflavanone	Root bark	Iinuma *et al*, 1992
8-γ,γ-Dimethylallyldaizein (8-prenyldaidzein **[23]** 	Isoflavone	Root bark	Iinuma *et al*, 1992

Table 1 (continued)

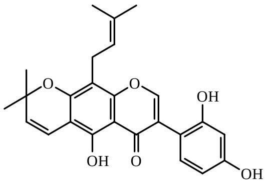
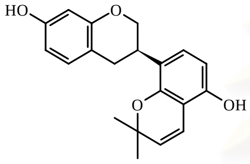
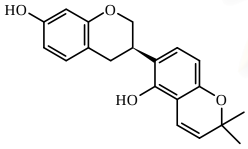
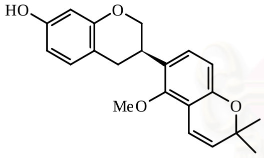
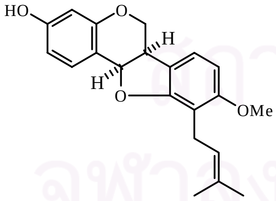
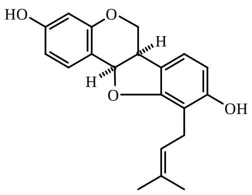
Plant and chemical compound	Category	Plant part	Reference
E. x bidwillii			
Auriculatin **[24]** 	Isoflavone	Root bark	Iinuma *et al*, 1992
Erythbidin A **[25]** 	Isoflavan	Wood	Tanaka *et al*., 1998
Phaseollinisoflavan **[26]** 	Isoflavan	Wood	Tanaka *et al*., 1998
2'-Methoxyphaseollinisoflavan **[27]** 	Isoflavan	Wood	Tanaka *et al*., 1998
Sandwicensin **[28]** 	Pterocarpan	Wood	Tanaka *et al*., 1998
***E. burana* Chiov.**			
Phaseollidin **[10]** 	Pterocarpan	Bark	Dagne, Gunatilaka and Kingston, 1993

Table 1 (continued)

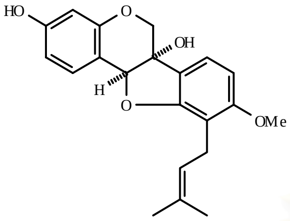
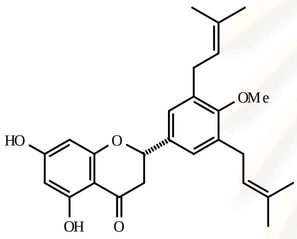
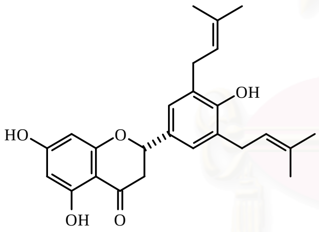
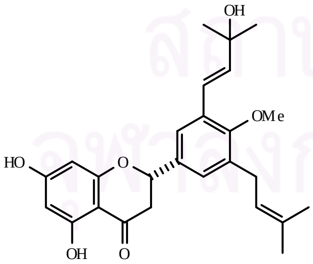
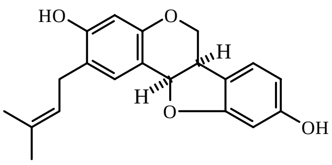
Plant and chemical compound	Category	Plant part	Reference
E. burana **Chiov.** Cristacarpin **[29]** 	Pterocarpan	Bark	Dagne, Gunatilaka and Kingston, 1993
E. burttii **Ball.** Abyssinone V-4'-methyl ether **[11]** 	Flavanone	Stem bark	Yenesew *et al*., 1998
Abyssinone-V **[5]** 	Flavanone	Stem bark	Yenesew *et al*., 1998
Burttinone **[30]** 	Flavanone	Stem bark	Yenesew *et al*., 1998
Calopocarpin **[31]** 	Flavanone	Stem bark	Yenesew *et al*., 1998

Table 1 (continued)

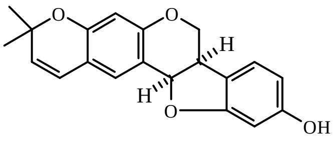
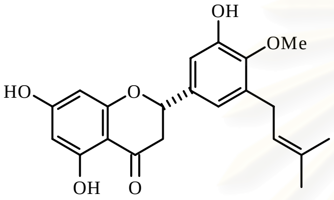
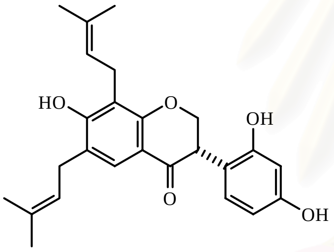
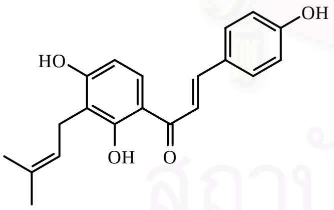
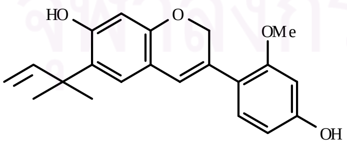
Plant and chemical compound	Category	Plant part	Reference
***E. burttii* Ball.**			
Neorautenol **[32]** 	Pterocarpan	Stem bark	Yenesew *et al*., 1998
4'-*O*-Methylsigmoidin B **[33]** 	Flavanone	Stem bark	Yenesew *et al*., 1998
Bidwillon A **[21]** 	Isoflavone	Stem bark	Yenesew *et al*., 1998
Isobavachalcone **[34]** 	Chalcone	Stem bark	Yenesew *et al*., 1998
Burttinol A **[35]** 	Isoflav-3-ene	Root bark	Yenesew *et al*., 2002

Table 1 (continued)

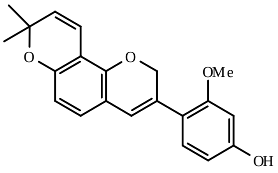
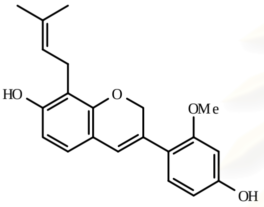
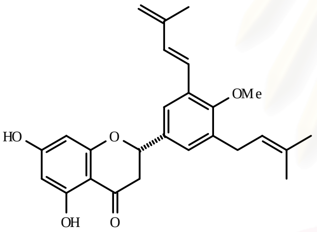
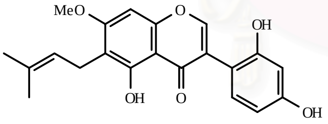
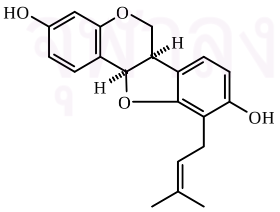
Plant and chemical compound	Category	Plant part	Reference
***E. burttii* Ball.**			
Burttinol B **[36]** 	Isoflav-3-ene	Root bark	Yenesew *et al.*, 2002
Burttinol C **[37]** 	Isoflav-3-ene	Root bark	Yenesew *et al.*, 2002
Burttinonedehydrate **[38]** 	Flavanone	Stem bark	Yenesew *et al.*, 2003
7-*O*-Methylluteone **[39]** 	Isoflavanone	Stem bark	Yenesew *et al.*, 2003
***E. crista-galli* L.**			
Phaseollidin **[10]** 	Pterocarpan	Seed Wood	Ingham and Markham, 1980 Tanaka and Etoh, 1997

Table 1 (continued)

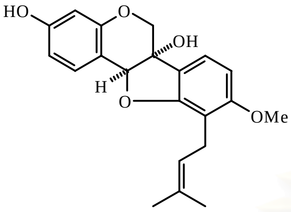
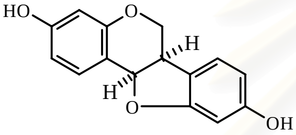
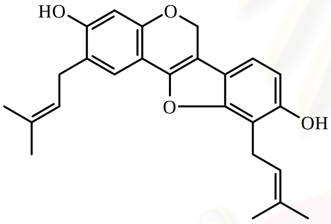
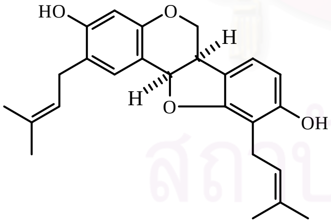
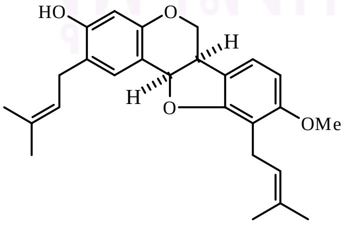
Plant and chemical compound	Category	Plant part	Reference
***E. crista-galli* L.**			
Cristacarpin **[29]** 	Pterocarpan	Seed	Ingham and Markham, 1980
Demethylmedicarpin **[40]** 	Pterocarpan	Seed Wood	Ingham and Markham, 1980; Tanaka and Etoh, 1997
Erycristagallin **[17]** 	Pterocarpan	Bark	Mitscher *et al.*, 1988
Erythrabyssin-II **[8]** 	Pterocarpan	Bark Wood	Mitscher *et al.*, 1988; Tanaka and Etoh, 1997
Erycristin **[41]** 	Pterocarpan	Stem bark	Mitscher *et al.*, 1988

Table 1 (continued)

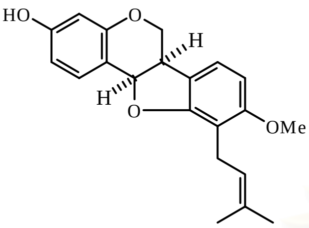
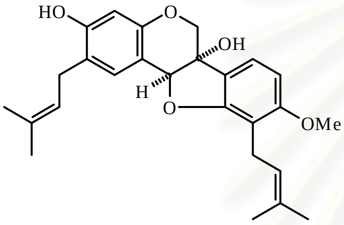
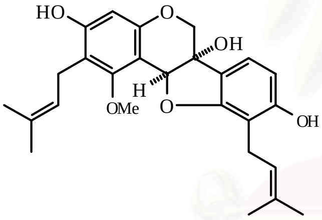
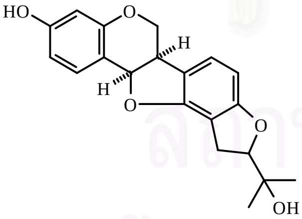
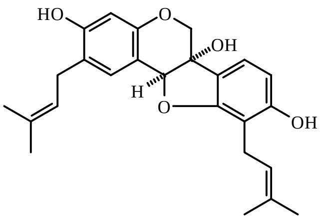
Plant and chemical compound	Category	Plant part	Reference
***E. crista-galli* L.**			
Sandwicensin **[28]** 	Pterocarpan	Stem bark	Mitscher *et al*., 1988
Erystagallin A **[42]** 	Pterocarpan	Wood	Tanaka and Etoh, 1997
Erystagallin B **[43]** 	Pterocarpan	Wood	Tanaka and Etoh, 1997
Erystagallin C **[44]** 	Pterocarpan	Wood	Tanaka and Etoh, 1997
2-(γ,γ-Dimethylallyl)-6a-hydroxy phaseollidin **[45]** 	Pterocarpan	Wood	Tanaka and Etoh, 1997

Table 1 (continued)

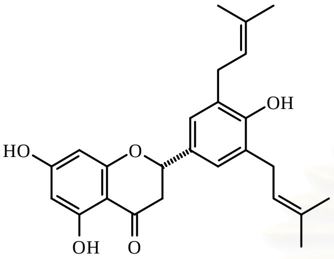
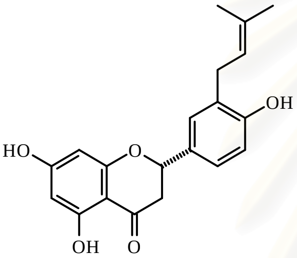
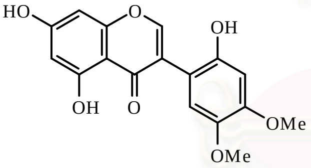
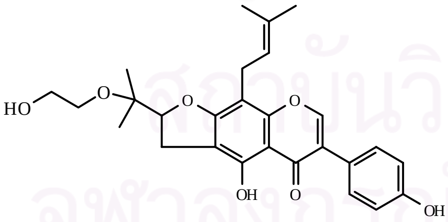
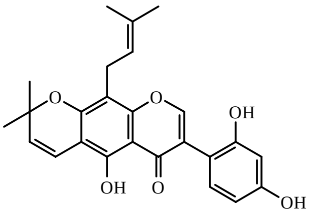
Plant and chemical compound	Category	Plant part	Reference
***E. eriotriocha* Harms.**			
Abyssinone V **[5]** 	Flavanone	Stem bark	Nkengfack *et al*., 1989
3'-Prenylnaringenin **[46]** 	Flavanone	Stem bark	Nkengfack *et al*., 1989
2'-Hydroxy-5'-methoxybiochanin A **[47]** 	Isoflavone	Stem bark	Nkengfack *et al*., 1989
Eriotriochin **[48]** 	Pterocarpan	Stem bark	Nkengfack and Fomum, 1990
Auriculatin **[24]** 	Pterocarpan	Stem bark	Nkengfack and Fomum, 1990

Table 1 (continued)

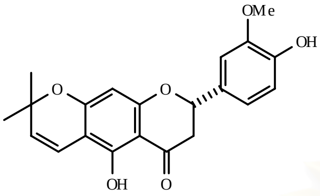
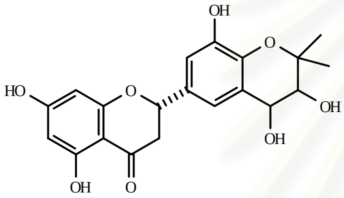
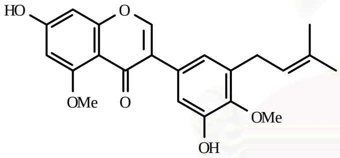
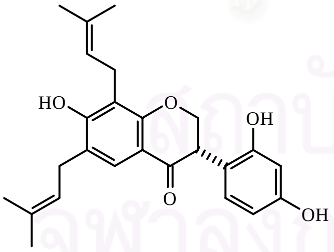
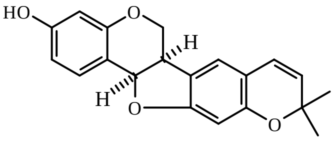
Plant and chemical compound	Category	Plant part	Reference
***E. eriotriocha* Harms.**			
Eriotrinol **[49]** 	Flavanone	Stem bark	Nkengfack *et* al., 1993
Sigmoidin G **[50]** 	Flavanone	Stem bark	Nkengfack *et* al., 1993
5,4'-Dimethoxy-3'-prenylbiochanin A **[51]** 	Isoflavone	Stem bark	Nkengfack and Fomum, 1990 Nkengfack *et* al., 1993
Eriotrichin B **[52]** 	Isoflavanone	Root bark	Nkengfack *et al.*, 1995
Isoneorautenol **[53]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1995

Table 1 (continued)

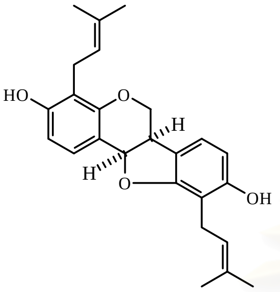
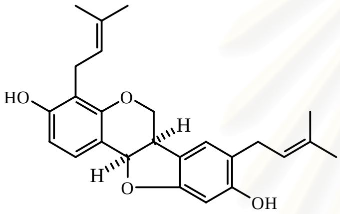
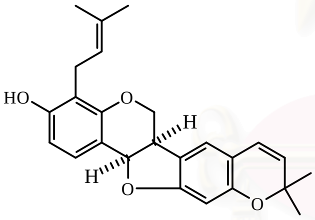
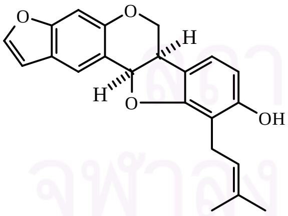
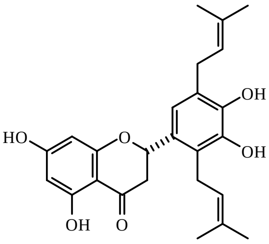
Plant and chemical compound	Category	Plant part	Reference
***E. eriotriocha* Harms.**			
Erybraedin A **[54]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1995
Erybraedin C **[55]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1995
Erybraedin D **[56]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1995
Erybraedin E **[57]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1995
Sigmoidin A **[58]** 	Flavanone	Stem bark	Nkengfack *et al.*, 1997

Table 1 (continued)

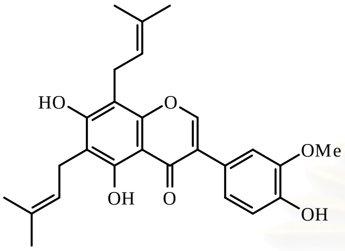
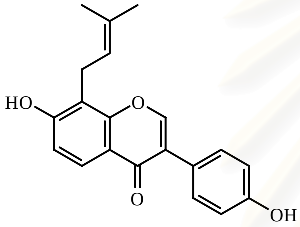
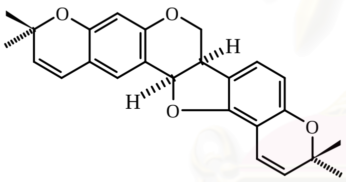
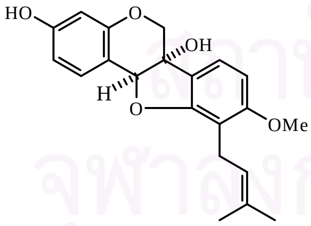
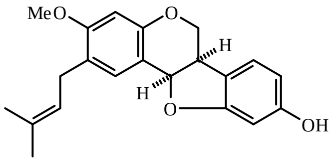
Plant and chemical compound	Category	Plant part	Reference
***E. eriotriocha* Harms.**			
Fleminphilippinin B **[59]**  	Isoflavone	Stem bark	Nkengfack *et al.*, 1997
8-Prenyldaidzein **[23]**  	Isoflavone	Stem bark	Nkengfack *et al.*, 1997
Gangetinin **[60]**  	Pterocarpan	Stem bark	Nkengfack *et al.*, 1997
***E. gluca* Willd.**			
Erythrabyssin-I **[7]**  	Pterocarpan	Stem bark	Fomum *et al.*, 1986
3-*O*-Methylcalopocarpin **[61]**  	Pterocarpan	Stem bark	McKee *et al.*, 1997

Table 1 (continued)

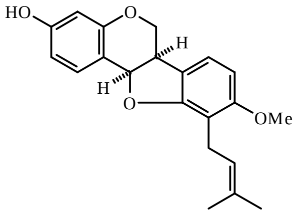
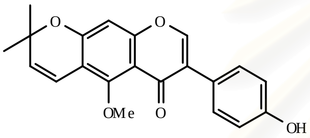
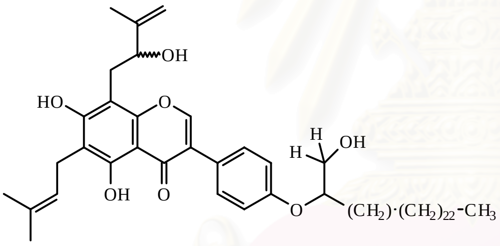
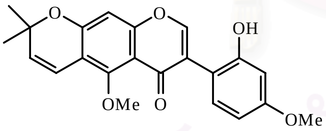
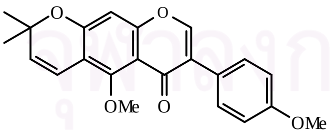
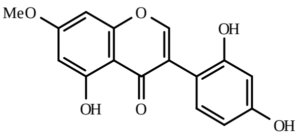
Plant and chemical compound	Category	Plant part	Reference
E. gluca **Willd.**			
Sandwicensin **[28]**  	Pterocarpan	Stem bark	McKee *et al.*, 1997
E. indica **Lam.**			
Indicanine C **[62]**  	Isoflavone	Root bark	Waffo *et al.*, 2000
Indicanine D **[63]**  	Isoflavone	Stem bark	Nkengfack *et al.*, 2001
Indicanine E **[64]**  	Isoflavone	Stem bark	Nkengfack *et al.*, 2001
5,4'-Di-*O*-methylalpinumisoflavone **[65]**  	Isoflavone	Root bark	Waffo *et al.*, 2000
Cajanin **[66]**  	Isoflavone	Root bark	Waffo *et al.*, 2000

Table 1 (continued)

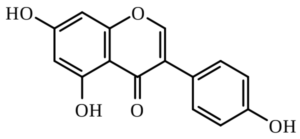
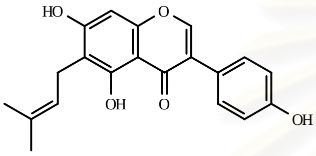
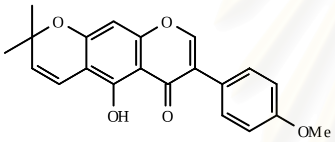
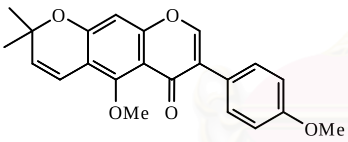
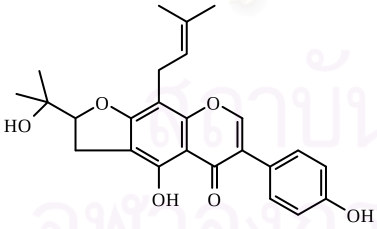
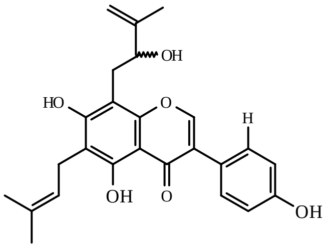
Plant and chemical compound	Category	Plant part	Reference
***E. indica* Lam.**			
Genistein **[67]**  	Isoflavone	Stem bark	Nkengfack *et al*., 2001
Wighteone **[68]**  	Isoflavone	Stem bark	Nkengfack *et al*., 2001
Alpinumisoflavone **[69]**  	Isoflavone	Stem bark	Nkengfack *et al*., 2001
Dimethylalpinumisoflavone **[70]**  	Isoflavone	Stem bark	Nkengfack *et al*., 2001
8-Prenylerythrinin C **[71]**  	Isoflavone	Stem bark	Nkengfack *et al*., 2001
Erysenegalensein E **[72]**  	Isoflavone	Stem bark	Nkengfack *et al*., 2001

Table 1 (continued)

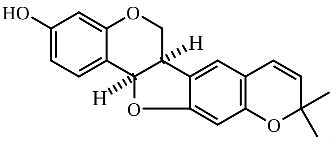
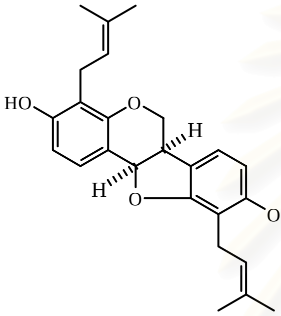
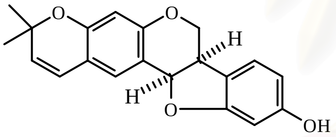
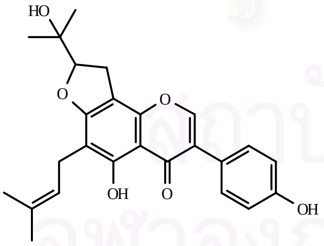
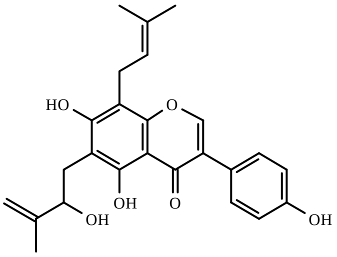
Plant and chemical compound	Category	Plant part	Reference
***E. latissima* E. Meyer**			
Isoneorautenol **[53]** 	Pterocarpan	Root bark	Wanjala *et al.*, 2001
Erybraedin A **[54]** 	Pterocarpan	Root bark	Wanjala *et al.*, 2001
Neorautenol **[32]** 	Pterocarpan	Root bark	Wanjala *et al.*, 2001
***E. lysistemon* Hutch.**			
Isosenegalensein **[73]** 	Isoflavone	Stem bark	Masry *et al.*, 2002
Isoerysenegalensein E **[74]** 	Isoflavone	Stem bark	Masry *et al.*, 2002

Table 1 (continued)

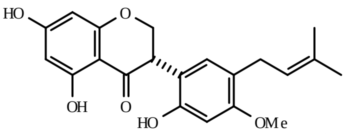
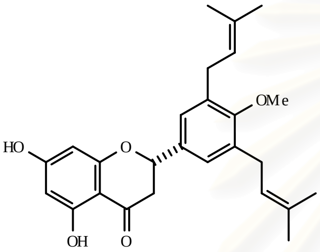
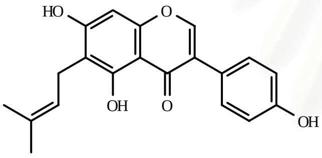
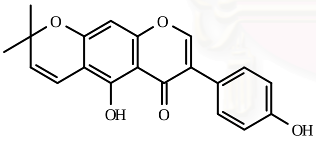
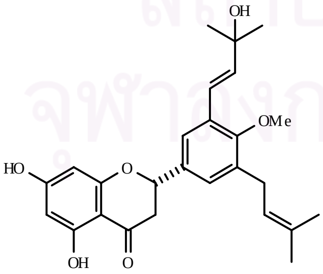
Plant and chemical compound	Category	Plant part	Reference
***E. lysistemon* Hutch.**			
Lysisteisoflavanone **[75]**  	Isoflavanone	Stem bark	Masry *et al.*, 2002
Abyssinone V-4'-methylether **[11]**  	Flavanone	Stem bark	Masry *et al.*, 2002
Wighteone **[68]**  	Isoflavone	Stem bark	Masry *et al.*, 2002
Alpinumisoflavone **[69]**  	Isoflavone	Stem bark	Masry *et al.*, 2002
Burttinone **[30]**  	Flavanone	Stem bark	Masry *et al.*, 2002

Table 1 (continued)

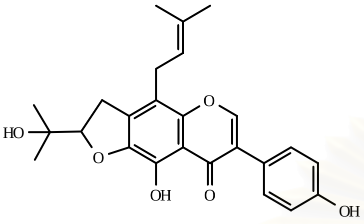
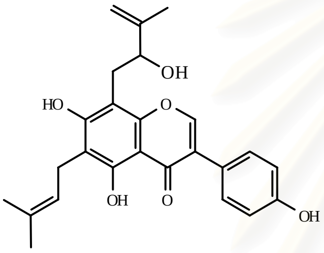
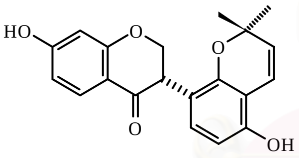
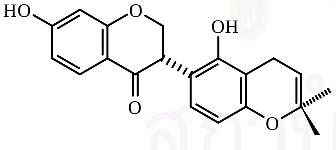
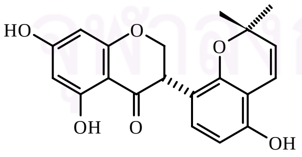
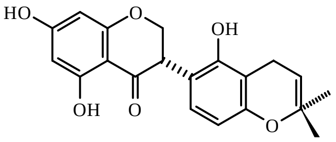
Plant and chemical compound	Category	Plant part	Reference
***E. lysistemon* Hutch.**			
Senegalensin **[76]** 	Isoflavone	Stem bark	Masry *et al*., 2002
Erysenegalensein E **[72]** 	Isoflavone	Stem bark	Masry *et al*., 2002
5-Deoxyglyasperin F **[77]** 	Isoflavanone	Root	McKee *et al*., 1997
5-Deoxylicoisoflavanone **[78]** 	Isoflavanone	Root	McKee *et al*., 1997
Glyasperin F **[79]** 	Isoflavanone	Root	McKee *et al*., 1997
Licoisoflavanone **[80]** 	Isoflavanone	Root	McKee *et al*., 1997

Table 1 (continued)

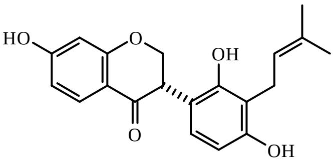
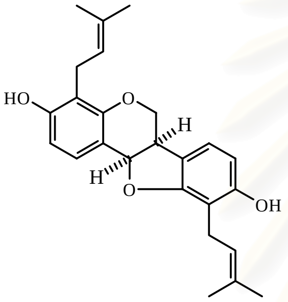
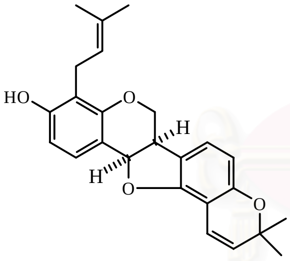
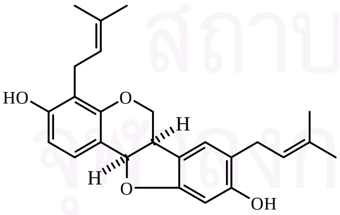
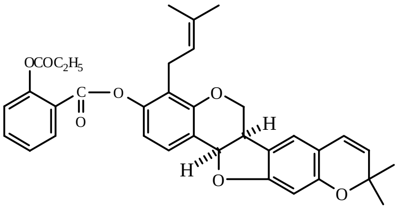
Plant and chemical compound	Category	Plant part	Reference
E. lysistemon **Hutch.**			
5-Hydroxyneobavaisoflavanone **[81]** 	Isoflavanone	Root	McKee *et al.*, 1997
E. mildbraedii **Harms.**			
Erybraedin A **[54]** 	Pterocarpan	Leaves	Mitscher *et al.*, 1988
Erybraedin B **[82]** 	Pterocarpan	Leaves	Mitscher *et al.*, 1988
Erybraedin C **[55]** 	Pterocarpan	Leaves	Mitscher *et al.*, 1988
Erybraedin D **[56]** 	Pterocarpan	Leaves	Mitscher *et al.*, 1988

Table 1 (continued)

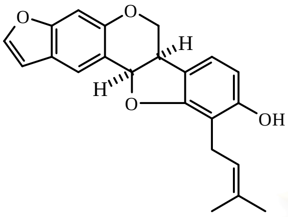
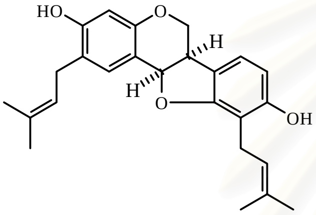
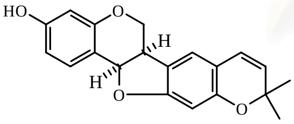
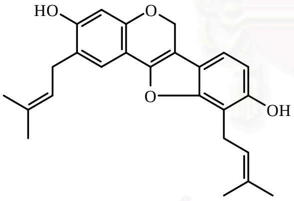
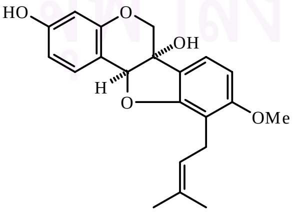
Plant and chemical compound	Category	Plant part	Reference
***E. mildbraedii* Harms.**			
Erybraedin E **[57]** 	Pterocarpan	Leaves	Mitscher *et al*., 1988
Erythrabyssin-II **[8]** 	Pterocarpan	Leaves	Mitscher *et al*., 1988
Isoneorautenol **[53]** 	Pterocarpan	Leaves	Mitscher *et al*., 1988
Erycristagallin **[17]** 	Pterocarpan	Root bark	Njamen *et al*., 2003
***E. orientalis* L.**			
Cristacarpin **[29]** 	Pterocarpan	Wood	Tanaka and Etoh, 1996 and 1997

Table 1 (continued)

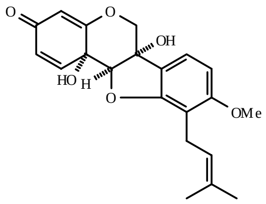
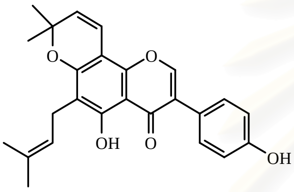
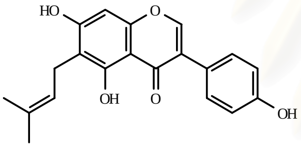
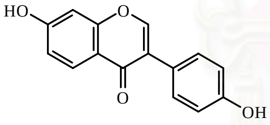
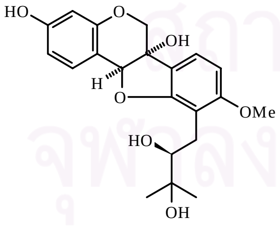
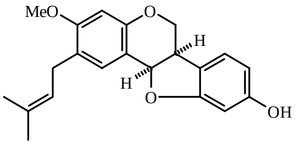
Plant and chemical compound	Category	Plant part	Reference
***E. orientalis* L.**			
Hydroxycristacarpone **[83]** 	Pterocarpan	Wood	Tanaka and Etoh, 1996
Osajin **[84]** 	Isoflavone	Wood	Tanaka and Etoh, 1996
Wighteone **[68]** 	Isoflavone	Wood	Tanaka and Etoh, 1996, and 1997
Daidzein **[85]** 	Isoflavone	Wood	Tanaka and Etoh, 1997
Orientanol A **[86]** 	Pterocarpan	Wood	Tanaka and Etoh, 1997
Orientanol B **[87]** 	Pterocarpan	Root	Tanaka and Etoh, 1998

Table 1 (continued)

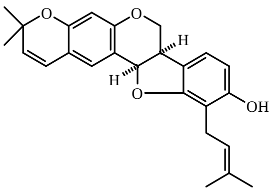
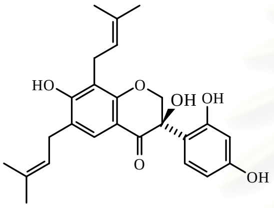
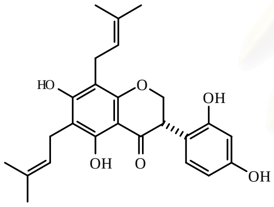
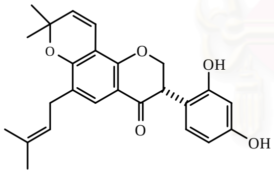
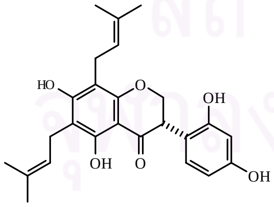
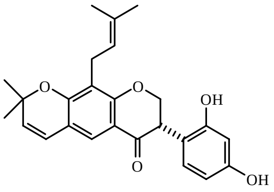
Plant and chemical compound	Category	Plant part	Reference
E. orientalis **L.**			
Orientanol C **[88]** 	Pterocarpan	Root	Tanaka and Etoh, 1998
Orientanol D **[89]** 	Isoflavanone	Root	Tanaka, *et al.*, 1998
Orientanol E **[90]** 	Isoflavanone	Root	Tanaka, *et al.*, 1998
Orientanol F **[91]** 	Isoflavanone	Root	Tanaka, *et al.*, 1998
Bidwillon A **[21]** 	Isoflavanone	Root	Tanaka, *et al.*, 1998
Bidwillon B **[22]** 	Isoflavanone	Root	Tanaka, *et al.*, 1998

Table 1 (continued)

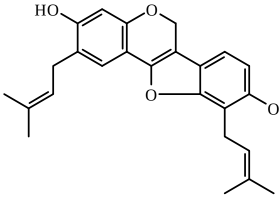
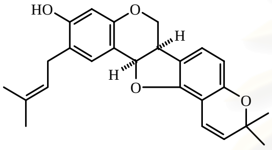
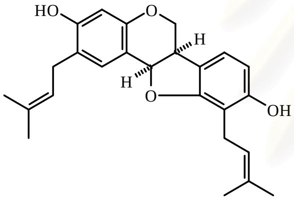
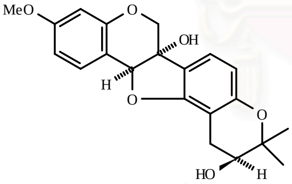
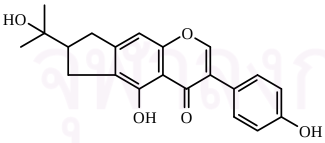
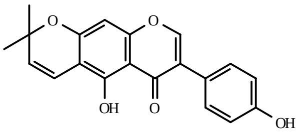
Plant and chemical compound	Category	Plant part	Reference
***E. orientalis* L.**			
Erycristagallin **[17]** 	Pterocarpan	Root	Tanaka and Etoh, 1998
Folitenol **[92]** 	Pterocarpan	Root	Tanaka and Etoh, 1998
Erythrabyssin II **[8]** 	Pterocarpan	Root	Tanaka and Etoh, 1998
***E. poeppigiana* Walp.**			
Eryvarin A **[93]** 	Pterocarpan	Wood	Tanaka *et al.*, 2001
Erythrinin C **[94]** 	Isoflavone	Wood	Tanaka *et al.*, 2001
Alpinumisoflavone **[69]** 	Isoflavone	Wood	Tanaka *et al.*, 2001

Table 1 (continued)

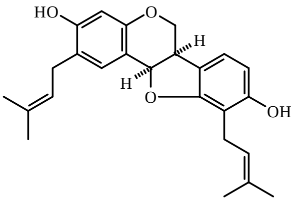
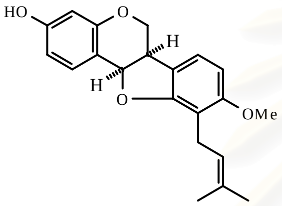
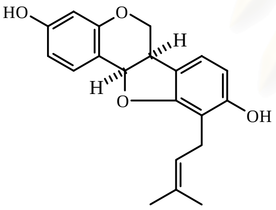
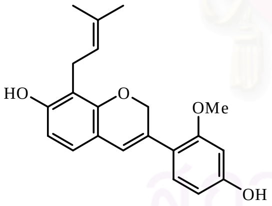
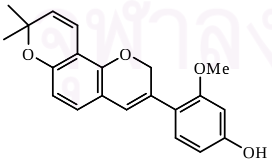
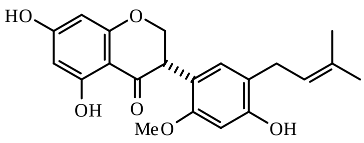
Plant and chemical compound	Category	Plant part	Reference
E. poeppigiana **Walp.**			
Erythrabyssin II **[8]** 	Pterocarpan	Root	Tanaka *et al.*, 2002
Sandwicensin **[28]** 	Pterocarpan	Root	Tanaka *et al.*, 2002
Phaseollidin **[10]** 	Pterocarpan	Root	Tanaka *et al.*, 2002
Erypoegin A **[95]** 	Isoflav-3-ene	Root	Tanaka *et al.*, 2002
Erypoegin B **[96]** 	Isoflav-3-ene	Root	Tanaka *et al.*, 2002
Erypoegin C **[97]** 	Isoflavanone	Root	Tanaka *et al.*, 2002

Table 1 (continued)

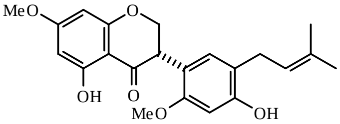
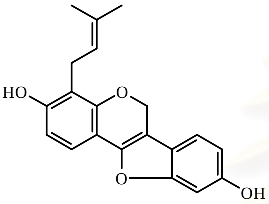
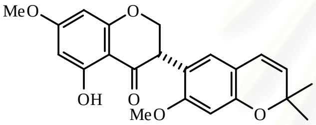
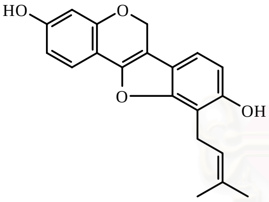
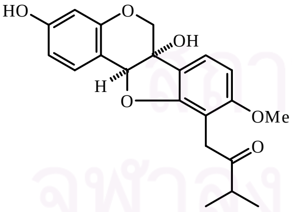
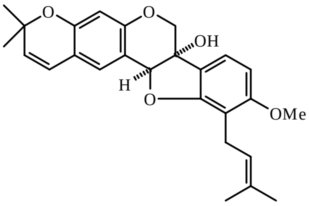
Plant and chemical compound	Category	Plant part	Reference
E. poeppigiana **Walp.**			
Erypoegin D **[98]** 	Isoflavanone	Root	Tanaka *et al*., 2002
Erypoegin E **[99]** 	Pterocarpan	Root	Tanaka *et al*., 2002
Erypoegin G **[100]** 	Isoflavanone	Root	Tanaka *et al*., 2002
Erypoegin H **[101]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Erypoegin I **[102]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Erypoegin J **[103]** 	Pterocarpan	Root	Tanaka *et al*., 2003

Table 1 (continued)

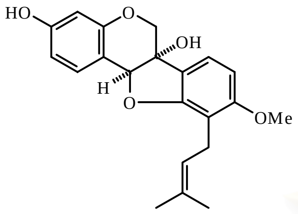
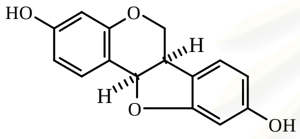
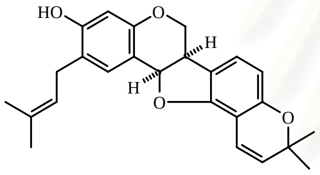
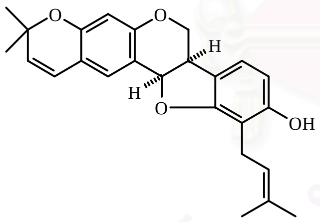
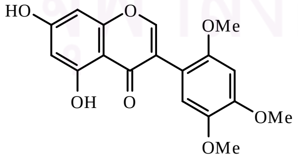
Plant and chemical compound	Category	Plant part	Reference
***E. poeppigiana* Walp.**			
Cristacarpin **[29]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Demethylmedicarpin **[40]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Folitenol **[92]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Orientanol C **[89]** 	Pterocarpan	Root	Tanaka *et al*., 2003
***E. sacleuxii* Hua.**			
7-Methylrobustigenin **[104]** 	Isoflavone	Stem bark	Yenesew *et al*., 1998

Table 1 (continued)

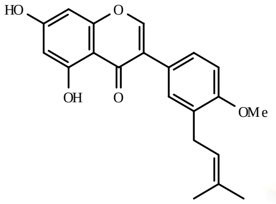
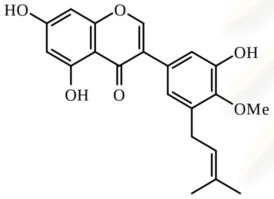
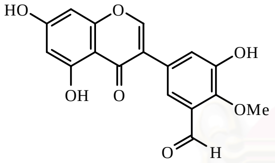
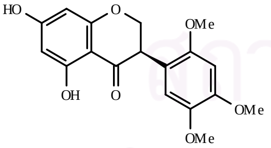
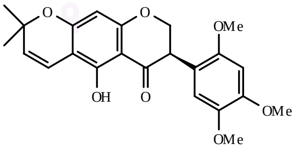
Plant and chemical compound	Category	Plant part	Reference
***E. sacleuxii* Hua.**			
3'-(3-Methylbut-2-nyl)biochanin A **[105]** 	Isoflavone	Stem bark	Yenesew *et al*., 1998
5'-(3- Methylbut-2-enyl) pratensein **[106]** 	Isoflavone	Stem bark	Yenesew *et al*., 1998
5'-Formylpratensein **[107]** 	Isoflavone	Stem bark	Yenesew *et al*., 1998
(R)-2,3-Dihydro-7-demethylrobustigenin **[108]** 	Isoflavavone	Stem bark	Yenesew *et al*., 2000
(R)-Saclenone **[109]** 	Isoflavavone	Stem bark	Yenesew *et al*., 2000

Table 1 (continued)

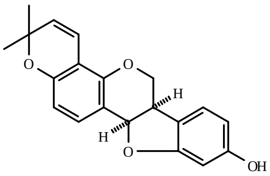
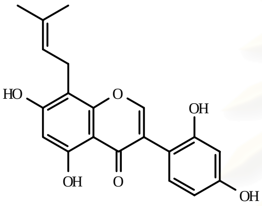
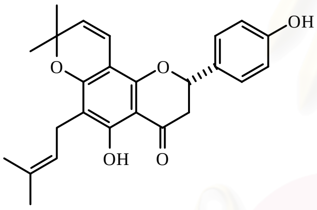
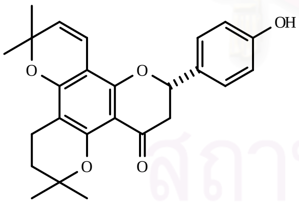
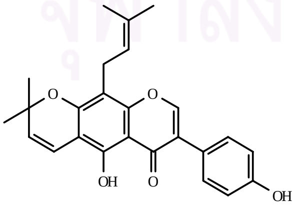
Plant and chemical compound	Category	Plant part	Reference
***E. sacleuxii* Hua.**			
Shinpterocarpin **[110]** 	Pterocarpan	Stem bark	Yenesew *et al.*, 2000
2,3-Dehydrokievitone **[111]** 	Isoflavone	Stem bark	Yenesew *et al.*, 2000
***E. senegalensis* DC.**			
Erythrisenegalone **[112]** 	Flavanone	Stem bark	Fomum, *et al.*, 1985
Cycloerythrisenegalone **[113]** 	Flavanone	Stem bark	Fomum, *et al.*, 1985
Warangalone **[19]** 	Isoflavone	Stem bark	Fomum, *et al.*, 1985 Taylor *et al.*, 1986

Table 1 (continued)

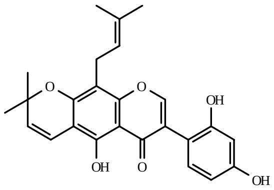
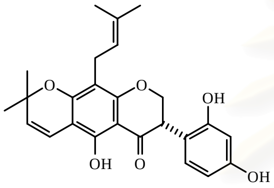
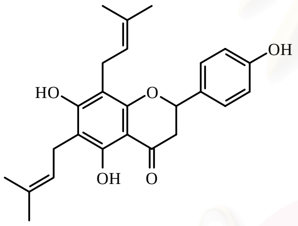
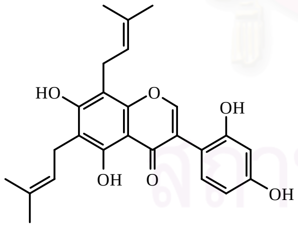
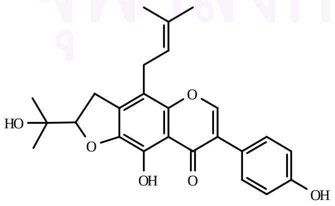
Plant and chemical compound	Category	Plant part	Reference
***E. senegalensis* DC.**			
Auriculatin **[24]** 	Isoflavone	Stem bark	Wandji *et al*., 1994
2,3-Dihydroauriculatin **[114]** 	Isoflavanone	Stem bark	Taylor *et al*., 1986 ; Wandji *et al*., 1994
Senegalensein (Lonchocarpol A) **[115]** 	Flavanone	Stem bark	Taylor *et al*., 1986; Fomum, Ayafor and Wandji, 1987; Wandji *et al*., 1994
8-Prenylluteone **[116]** 	Isoflavone	Stem bark	Wandji *et al*., 1994
Senegalensin **[76]** 	Isoflavone	Stem bark	Wandji *et al*., 1994

Table 1 (continued)

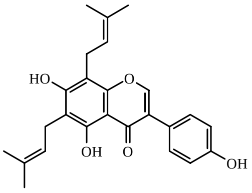
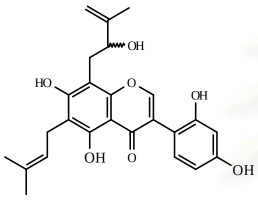
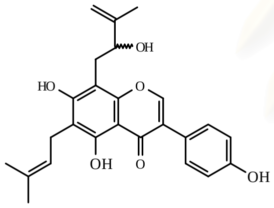
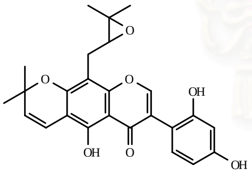
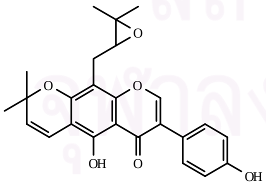
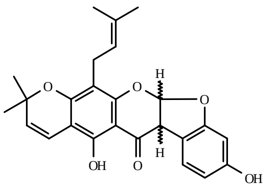
Plant and chemical compound	Category	Plant part	Reference
E. senegalensis **DC.**			
6,8-Diprenylgenistein **[117]** 	Isoflavone	Stem bark	Wandji *et al*., 1994
Erysenegalensein D **[118]** 	Isoflavone	Stem bark	Wandji *et al*., 1994
Erysenegalensein E **[72]** 	Isoflavone	Stem bark	Wandji *et al*., 1994
Erysenegalensein F **[119]** 	Isoflavone	Stem bark	Wandji *et al*., 1994
Erysenegalensein G **[120]** 	Isoflavone	Stem bark	Wandji *et al*., 1994
Erysenegalensein J **[121]** 	Isoflavavone	Stem bark	Wandji *et al*., 1995

Table 1 (continued)

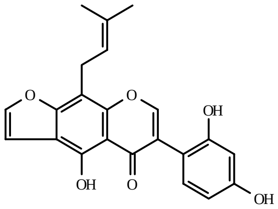
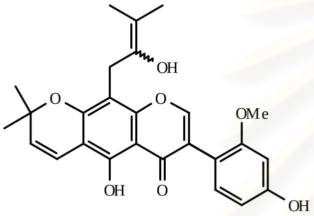
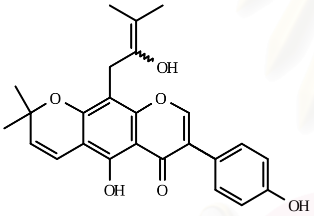
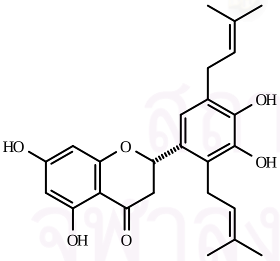
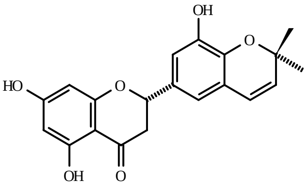
Plant and chemical compound	Category	Plant part	Reference
***E. senegalensis* DC.**			
Erysenegalensein K **[122]** 	Isoflavone	Stem bark	Wandji *et al*., 1995
Erysenegalensein L **[123]** 	Isoflavone	Stem bark	Wandji *et al*., 1995
Erysenegalensein M **[124]** 	Isoflavone	Stem bark	Wandji *et al*., 1995
***E. sigmoidea* Hua.**			
Sigmoidin A **[58]** 	Flavanone	Stem bark	Promsattha *et al*., 1989
Sigmoidin C **[125]** 	Flavanone	Stem bark	Promsattha and Tempesta, 1986

Table 1 (continued)

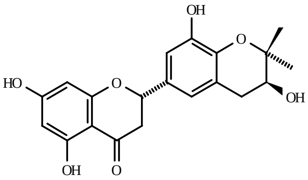
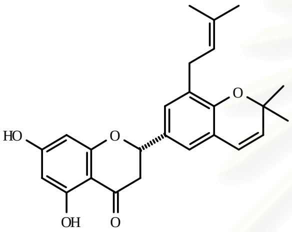
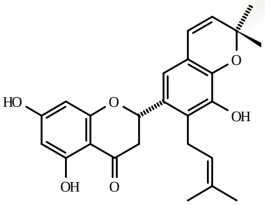
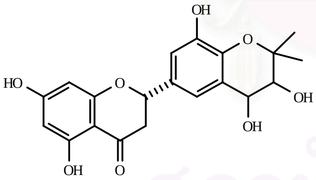
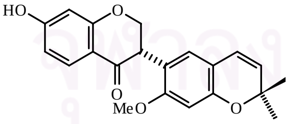
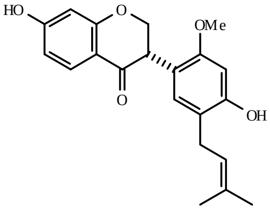
Plant and chemical compound	Category	Plant part	Reference
***E. sigmoidea* Hua.**			
Sigmoidin D **[18]** 	Flavanone	Stem bark	Promsattha and Tempesta, 1986
(-)-Sigmoidin E **[126]** 	Flavanone	Root bark	Promsattha and Tempesta, 1988
Sigmoidin F **[127]** 	Flavanone	Stem bark	Promsattha *et al.*, 1989
Sigmoidin G **[50]** 	Flavanone	Stem bark	Nkengfack *et al.*, 1993
Sigmoidin H **[128]** 	Isoflavanone	Stem wood	Nkengfack *et al.*, 1994
Sigmoidin I **[129]** 	Isoflavanone	Root bark	Promsattha and Tempesta, 1988

Table 1 (continued)

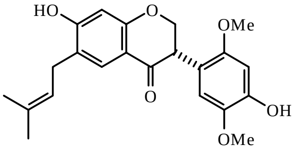
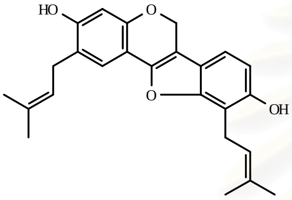
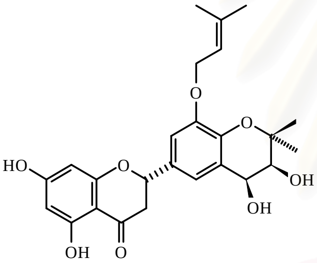
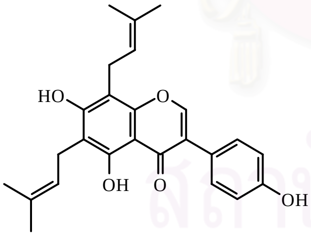
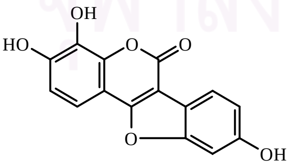
Plant and chemical compound	Category	Plant part	Reference
***E. sigmoidea* Hua.**			
Sigmoidin J **[130]** 	Isoflavanone	Root bark	Nkengfack *et al.*, 1994
Sigmoidin K **[131]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1994
Sigmoidin L **[132]** 	Flavanone	Stem bark	Nkengfack *et al.*, 1994
6,8-Diprenylgenistein **[117]** 	Isoflavone	Stem wood	Nkengfack *et al.*, 1994
4-Hydroxycoumestrol **[133]** 	Coumestan	Stem bark	Nkengfack *et al.*, 1997

Table 1 (continued)

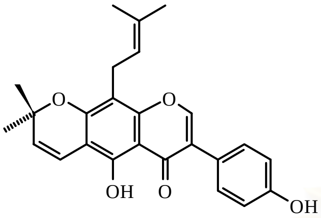
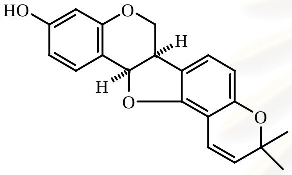
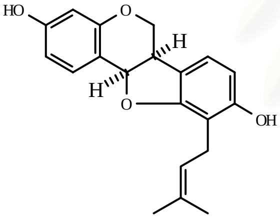
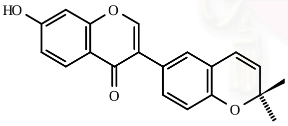
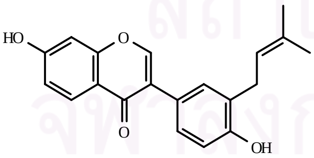
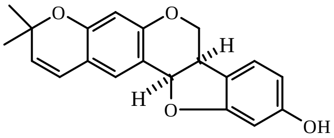
Plant and chemical compound	Category	Plant part	Reference
E. sigmoidea **Hua.**			
Scandenone (warangalone) **[19]** 	Isoflavone	Stem wood	Nkengfack *et al.*, 1994
Phaseollin **[9]** 	Pterocarpan	Stem wood	Nkengfack *et al.*, 1994
Phaseollidin **[10]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1994
Corylin **[134]** 	Isoflavone	Root bark	Nkengfack *et al.*, 1994
Neobavaisoflavone **[135]** 	Isoflavone	Root bark Stem wood	Nkengfack *et al.*, 1994; Nkengfack *et al.*, 1994
Neorautenol **[32]** 	Pterocarpan	Root bark	Nkengfack *et al.*, 1994

Table 1 (continued)

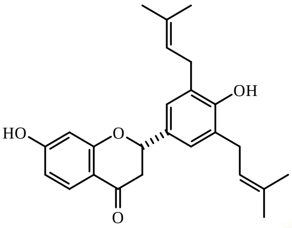
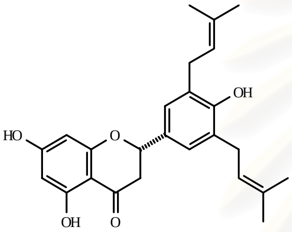
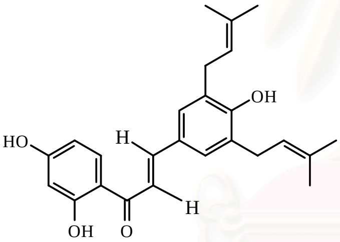
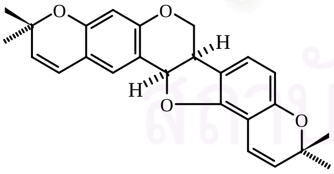
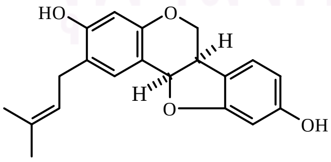
Plant and chemical compound	Category	Plant part	Reference
***E. sigmoidea* Hua.**			
Abyssinone IV **[4]** 	Flavanone	Stem wood	Nkengfack *et al.*, 1994
Abyssinone V **[5]** 	Flavanone	Root	Promsattha and Tempesta, 1988
Abyssinone VI **[6]** 	Chalcone	Root bark Stem wood	Nkengfack *et al.*, 1994; Nkengfack *et al.*, 1994
Gangetinin **[61]** 	Pterocarpan	Stem bark	Nkengfack *et al.*, 1997
Calopocarpin **[31]** 	Pterocarpan	Stem bark	Nkengfack *et al.*, 1997

Table 1 (continued)

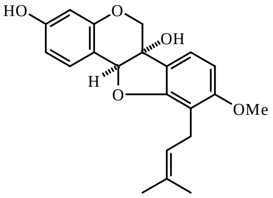
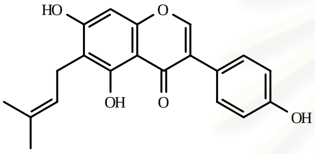
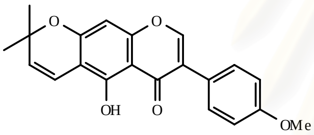
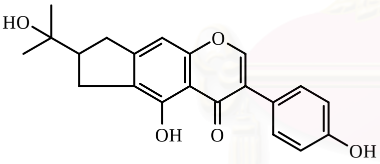
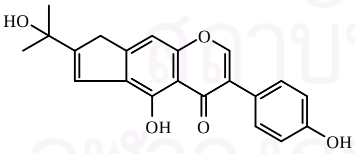
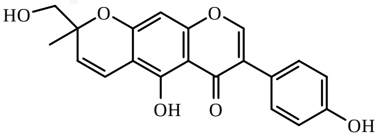
Plant and chemical compound	Category	Plant part	Reference
E. suberosa* var. *glabrescences			
Cristacarpin **[29]** 	Pteroccarpan	Wood Root	Tanaka *et al*., 1998; Tanaka *et al*., 2001
Wighteone **[68]** 	Isoflavone	Wood Root	Tanaka *et al*., 1998; Tanaka *et al*., 2001
Alpinumisoflavone **[69]** 	Isoflavone	Wood Root	Tanaka *et al*., 1998; Tanaka *et al*., 2001
Erythrinin C **[94]** 	Isoflavone	Wood	Tanaka *et al*., 1998
Erysubin A **[136]** 	Isoflavone	Wood	Tanaka *et al*., 1998
Erysubin B **[137]** 	Isoflavone	Wood	Tanaka *et al*., 1998

Table 1 (continued)

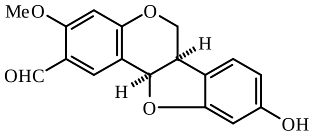
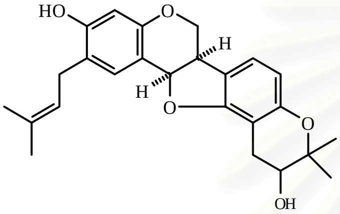
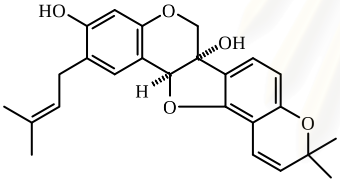
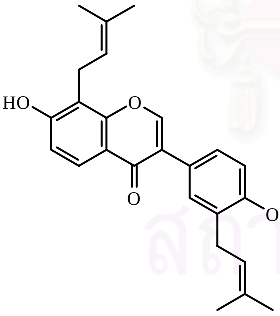
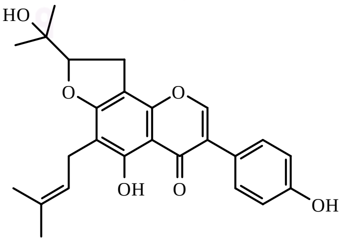
Plant and chemical compound	Category	Plant part	Reference
E. suberosa* var. *glabrescences			
Erysubin C **[138]** 	Pterocarpan	Root	Tanaka *et al*., 2001
Erysubin D **[139]** 	Pterocarpan	Root	Tanaka *et al*., 2001
Erysubin E **[140]** 	Pterocarpan	Root	Tanaka *et al*., 2001
Erysubin F **[141]** 	Isoflavone	Root	Tanaka *et al*., 2001
Euchrenone b_{10} **[142]** 	Isoflavone	Root	Tanaka *et al*., 2001

Table 1 (continued)

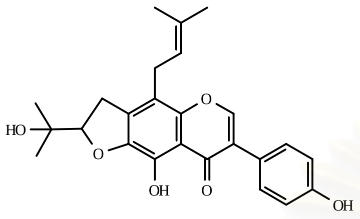
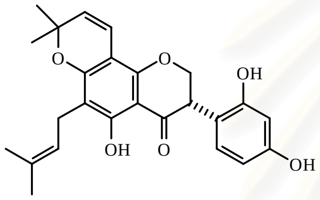
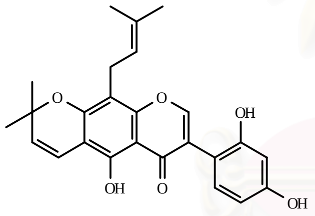
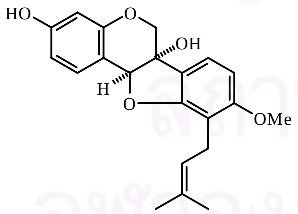
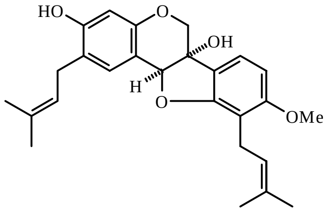
Plant and chemical compound	Category	Plant part	Reference
E. suberosa* var. *glabrescences			
Senegalensin **[76]** 	Isoflavone	Root	Tanaka *et al*., 2001
***E. variegata* L.**			
Bidwillon A **[21]** 	Isoflavanone	Bark Root	Kobayashi *et al*., 1997; Tanaka *et al*., 2003
Auriculatin **[24]** 	Isoflavone	Root	Tanaka *et al*., 2003
Cristacarpin **[29]** 	Pterocarpan	Bark Root	Kobayashi *et al*., 1997; Tanaka *et al*., 2003
Erystagallin A **[42]** 	Pterocarpan	Wood Root	Tanaka *et al*., 2000; Tanaka *et al*., 2002

Table 1 (continued)

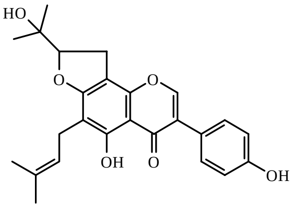
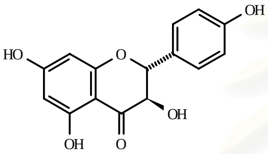
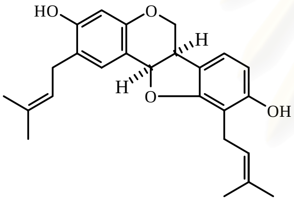
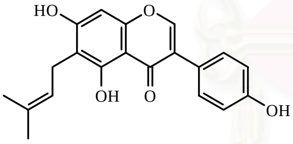
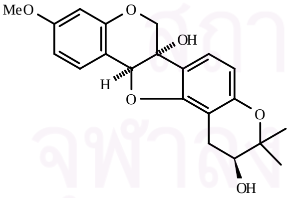
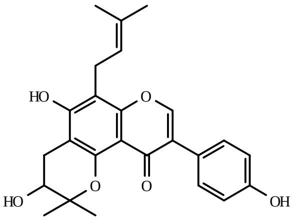
Plant and chemical compound	Category	Plant part	Reference
E. variegata **L.**			
Euchrenone b_{10} **[142]** 	Isoflavone	Bark	Kobayashi *et al*., 1997
(+)-Aromadendrin **[143]** 	Flavanonol	Bark	Kobayashi *et al*., 1997
Erystagallin (Erythrabyssin II) **[8]** 	Pterocarpan	Root	Tanaka *et al*., 2002
Erythrinin B **[144]** 	Isoflavone	Bark Root	Kobayashi *et al*., 1997; Tanaka *et al*., 2002
Eryvarin A **[93]** 	Pterocarpan	Bark Root	Tanaka *et al*., 2000; Kobayashi *et al*., 1997
Eryvarin B **[145]** 	Isoflavone	Wood	Tanaka *et al*., 2002

Table 1 (continued)

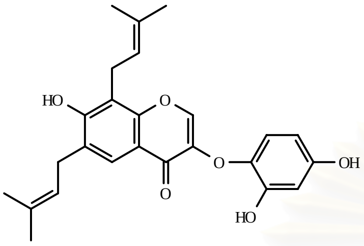
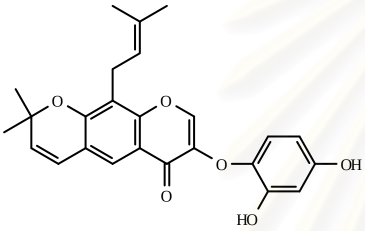
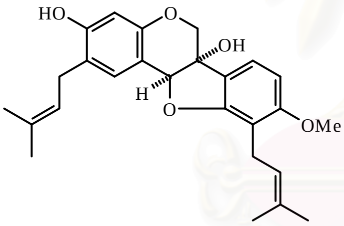
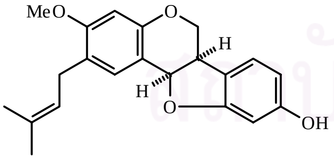
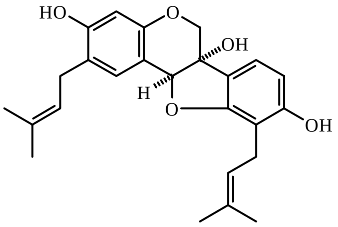
Plant and chemical compound	Category	Plant part	Reference
E. variegata **L.**			
Eryvarin F **[146]** 	3-Phenoxychromones	Root	Tanaka *et al*., 2003
Eryvarin G **[147]** 	3-Phenoxychromones	Root	Tanaka *et al*., 2003
Erystagallin A **[42]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Orientanol B **[87]** 	Pterocarpan	Root	Tanaka *et al*., 2002
2-(γ,γ-Dimethylallyl)-6a-hydroxyphaseollidin **[45]** 	Pterocarpan	Root	Tanaka *et al*., 2002

Table 1 (continued)

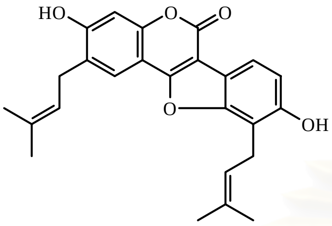
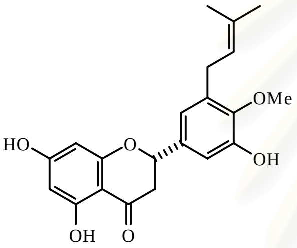
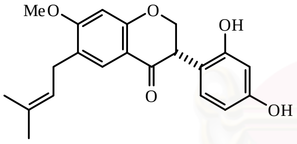
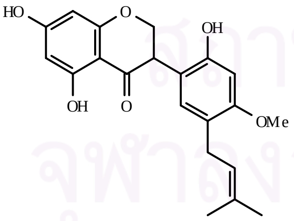
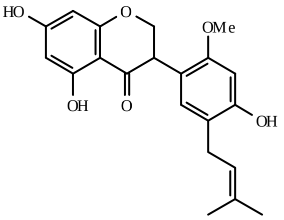
Plant and chemical compound	Category	Plant part	Reference
E. variegata **L.**			
Sigmoidin K **[131]** 	Coumestan	Root	Tanaka *et al.*, 2002
E. vellutina **Willd.**			
4'-*O*-Methylsigmoidin **[148]** 	Flavanone	Stem bark	Da-Cuhna *et al.*, 1996
Eryvellutinone **[149]** 	Isoflavavone	Stem bark	Da-Cuhna *et al.*, 1996
E. vogelii **Hook. f.**			
Vogelin A **[150]** 	Isoflavanone	Root	Atindehou *et al.*, 2002
Vogelin B **[151]** 	Isoflavanone	Root	Atindehou *et al.*, 2002

Table 1 (continued)

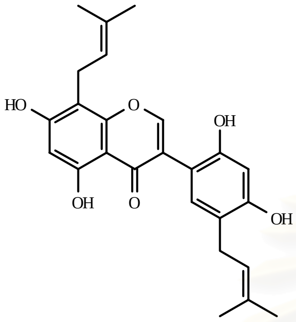
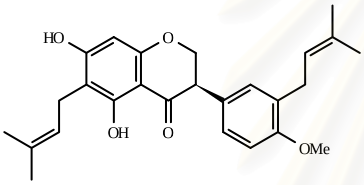
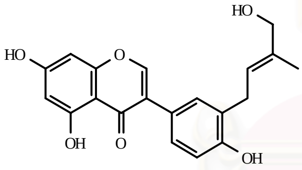
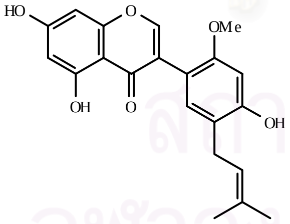
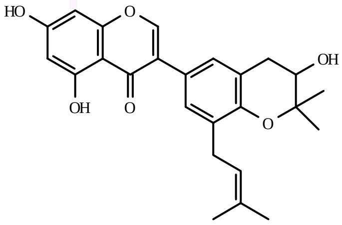
Plant and chemical compound	Category	Plant part	Reference
E. vogelii **Hook. f.**			
Vogelin C **[152]** 	Isoflavone	Root	Atindehou *et al*., 2002
Vogelin D **[153]** 	Isoflavanone	Root	Queiroz *et al*., 2002
Vogelin E **[154]** 	Isoflavone	Root	Queiroz *et al*., 2002
Vogelin F **[155]** 	Isoflavone	Root	Queiroz *et al*., 2002
Vogelin G **[156]** 	Isoflavone	Root	Queiroz *et al*., 2002

Table 1 (continued)

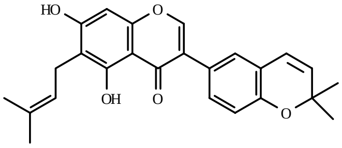
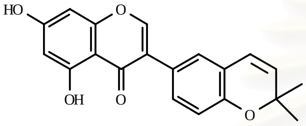
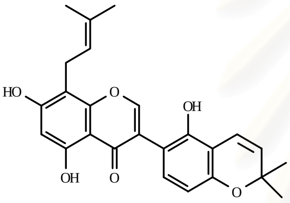
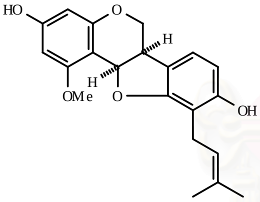
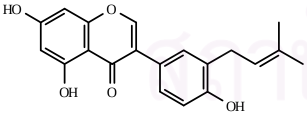
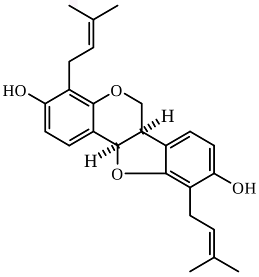
Plant and chemical compound	Category	Plant part	Reference
***E. vogelii* Hook. f.**			
Isochandalon **[157]** 	Isoflavone	Root	Queiroz *et al.*, 2002
Isoderrone **[158]** 	Isoflavone	Root	Queiroz *et al.*, 2002
Ulexone A **[159]** 	Isoflavone	Root	Queiroz *et al.*, 2002
1-Methoxyphaseollidin **[160]** 	Pterocarpan	Root bark	Atindehou *et al.*, 2002
Isowighteone **[161]** 	Isoflavone	Root bark	Atindehou *et al.*, 2002
E. zeyheri			
Erybraedin A **[54]** 	Pterocarpan	Root	Tanaka *et al.*, 2003

Table 1 (continued)

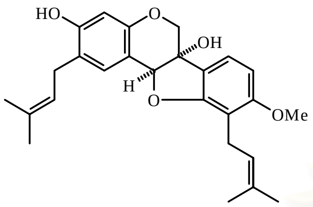
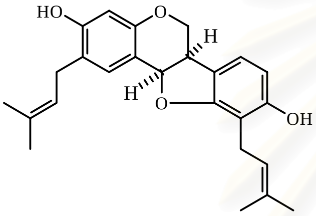
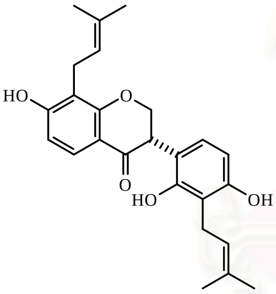
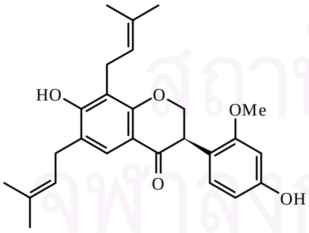
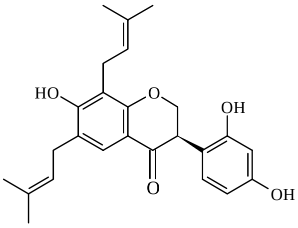
Plant and chemical compound	Category	Plant part	Reference
E. zeyheri			
Erystagallin A **[42]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Erythrabyssin II **[8]** 	Pterocarpan	Root	Tanaka *et al*., 2003
Eryzerin A **[162]** 	Isoflavanone	Root	Tanaka *et al*., 2003
Eryzerin B **[163]** 	Isoflavanone	Root	Tanaka *et al*., 2003
Eryzerin C **[164]** 	Isoflavanone	Root	Tanaka *et al*., 2003

Table 1 (continued)

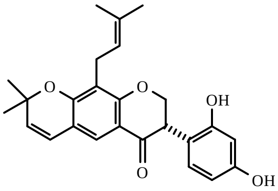
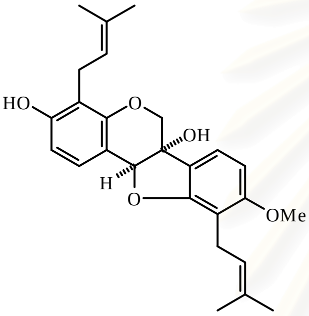
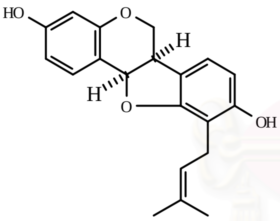
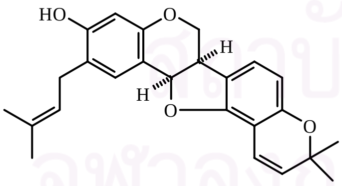
Plant and chemical compound	Category	Plant part	Reference
E. zeyheri			
Eryzerin D **[165]** 	Isoflavanone	Root	Tanaka *et al.*, 2003
Eryzerin E **[166]** 	Pterocarpan	Root	Tanaka *et al.*, 2003
Phaseollidin **[10]** 	Pterocarpan	Root	Tanaka *et al.*, 2003
Folitenol **[92]** 	Pterocarpan	Root	Tanaka *et al.*, 2003

Table 2 Distribution of erythrina alkaloids

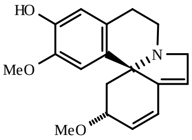
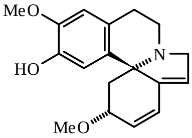
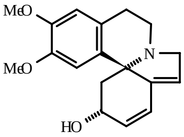
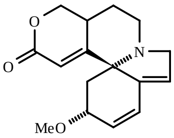
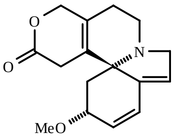
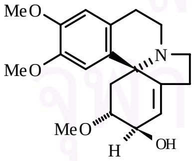
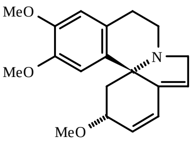
Plant and chemical compound	Category	Plant part	Reference
E. americana			
Erysodine **[167]** 	Dienoid	Seed	Sotelo *et al*., 1993 Mateos, *et al*., 1996
Erysovine **[168]** 	Dienoid	Seed	Sotelo *et al*., 1993 Mateos *et al*., 1996
Erythravine **[169]** 	Dienoid	Seed	Sotelo *et al*., 1993 Mateos *et al*., 1996
α-Erythroidine **[170]** 	Lactonic dienoid	Seed	Sotelo *et al*., 1993 Mateos *et al*., 1996
β-Erythroidine **[171]** 	Lactonic dienoid	Seed	Sotelo *et al*., 1993 Mateos *et al*., 1996
Erythratidine **[172]** 	Alkenoid	Seed	Sotelo *et al*., 1993 Mateos *et al*., 1996
Erysotrine **[173]** 	Dienoid	Seed	Sotelo *et al*., 1993 Mateos *et al*., 1996

Table 2 (continued)

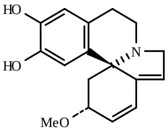
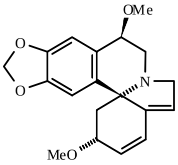
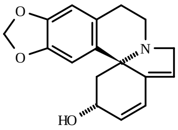
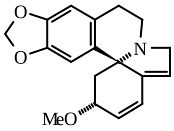
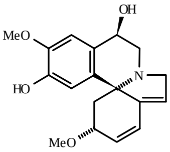
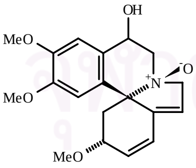
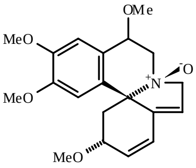
Plant and chemical compound	Category	Plant part	Reference
E. americana			
Erysopine **[174]** 	Dienoid	Seed	Altamirano *et al.*, 1877 Sotelo *et al.*, 1993
11β-Methoxyerythraline **[175]** 	Dienoid	Seed	Altamirano *et al.*, 1877
Erythrocarine **[176]** 	Dienoid	Seed	Sotelo *et al.*, 1993
Erythraline **[177]** 	Dienoid	Seed	Sotelo *et al.*, 1993
11β-Hydroxyerysovine **[178]** 	Dienoid	Seed	Mateos *et al.*, 1996
Erythratine-N-oxide **[179]** 	Dienoid	Seed	Mateos *et al.*, 1996
Erythristemine-N-oxide **[180]** 	Dienoid	Seed	Mateos *et al.*, 1996

Table 2 (continued)

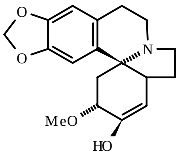
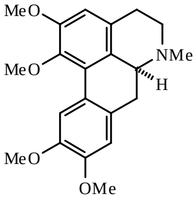
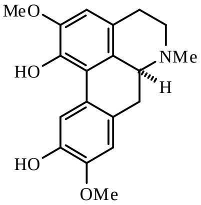
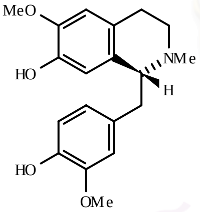
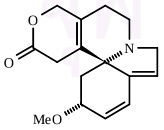
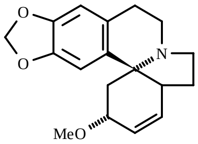
Plant and chemical compound	Category	Plant part	Reference
***E. abyssinica* DC.**			
Erythratine **[181]** 	Alkenoid	Seed	Barton *et al*., 1973
(+)-Glaucine **[182]** 	Aporphine	Seed	Barton *et al*., 1973
Isoboldine **[183]** 	Aporphine	Leaves	Barton *et al*., 1973
Orientaline **[184]** 	Tetrahydrobenzyl isoquinoline	Leaves	Barton *et al*., 1973
***E. arborescens* Roxb.**			
β-erythroidine **[171]** 	Lactonic dienoid	Seed	Ghosal *et al*., 1972
Erythramine **[185]** 	Alkenoid	Seed	Ghosal *et al*., 1972

Table 2 (continued)

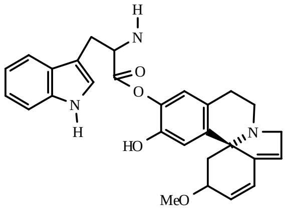
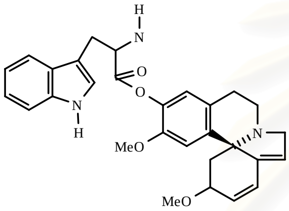
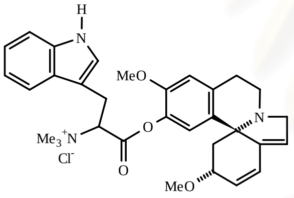
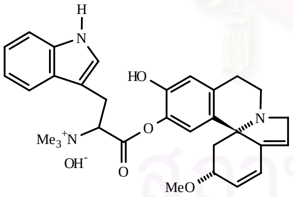
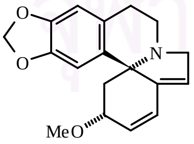
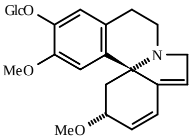
Plant and chemical compound	Category	Plant part	Reference
***E. arborescens* Roxb.**			
Erysopinophorine hydroxide **[186]** 	Dimeric dienoid	Seed	Ghosal *et al.*, 1972
Erysodinophorine hydroxide **[187]** 	Dimeric dienoid	Seed	Ghosal *et al.*, 1972
Erysophorine chloride **[188]** 	Dimeric dienoid	Seed	Tiwari and Masood, 1979
Isoerysopinophorine hydroxide **[189]** 	Dimeric dienoid	Seed	Tiwari and Masood, 1979
Erythraline **[177]** 	Dienoid	Seed	Barton *et al.*, 1973
Glucoerysodine **[190]** 	Dienoid	Seed	Barton *et al.*, 1973

Table 2 (continued)

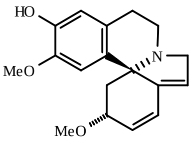
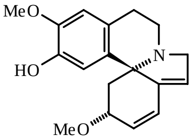
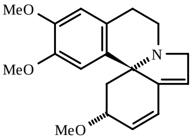
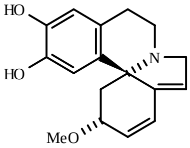
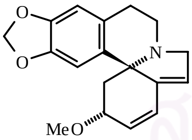
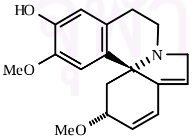
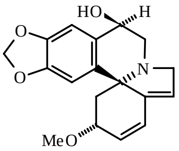
Plant and chemical compound	Category	Plant part	Reference
***E. berteroana* Urb.**			
Erysodine **[167]** 	Dienoid	Seed	Hernandez and Jackson, 1994
Erysovine **[168]** 	Dienoid	Seed	Hernandez and Jackson, 1994
Erysotrine **[173]** 	Dienoid	Seed	Hernandez and Jackson, 1994
Erysopine **[174]** 	Dienoid	Seed	Hernandez and Jackson, 1994
E. bidwillii			
Erythraline **[177]** 	Dienoid	Flower	Tanaka *et al.*, 1998
Erysodine **[167]** 	Dienoid	Flower	Tanaka *et al.*, 1998
Erythrinine **[191]** 	Dienoid	Flower	Tanaka *et al.*, 1998

Table 2 (continued)

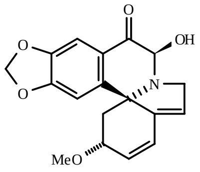
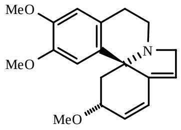
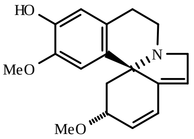
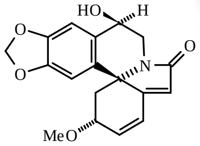
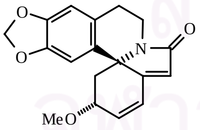
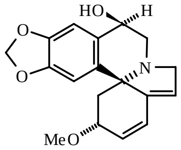
Plant and chemical compound	Category	Plant part	Reference
E. x bidwillii			
Erythbidin B **[192]** 	Dienoid	Flower	Tanaka *et al.*, 1998
E. blakei			
Erysotrine **[173]** 	Dienoid	Bark	Singh *et al.*, 1981
Erysodine **[167]** 	Dienoid	Bark	Singh *et al.*, 1981
***E. brucei* Schweinf.**			
8-Oxoerythrinine **[193]** 	Dienoid	Flower	Dagne and Steglich, 1984
8-Oxoerythraline **[194]** 	Dienoid	Flower	Dagne and Steglich, 1984
Erythrinine **[191]** 	Dienoid	Flower	Dagne and Steglich, 1984

Table 2 (continued)

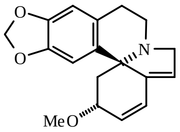
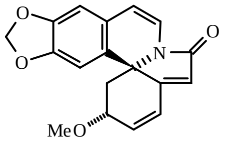
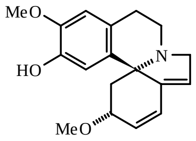
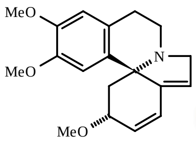
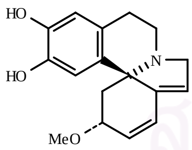
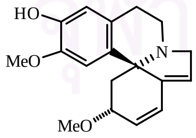
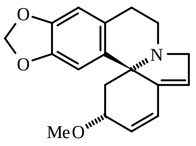
Plant and chemical compound	Category	Plant part	Reference
***E. brucei* Schweinf.**			
Erythraline **[177]** 	Dienoid	Flower	Dagne and Steglich, 1984
Crystamidine **[195]** 	Dienoid	Flower	Dagne and Steglich, 1984
***E. fusca* Lour.**			
Erysovine **[168]** 	Dienoid	Leaves Seed	Barton *et al*., 1973; Hargreaves *et al*., 1974
Erysotrine **[173]** 	Dienoid	Leaves	Barton *et al*., 1973
Erysopine **[174]** 	Dienoid	Seed	Hargreaves *et al*., 1974
Erysodine **[167]** 	Dienoid	Seed	Hargreaves *et al*., 1974
Erythraline **[177]** 	Dienoid	Seed	Hargreaves *et al*., 1974

Table 2 (continued)

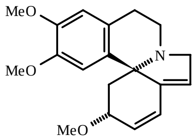
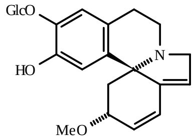
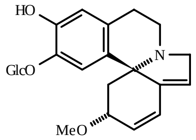
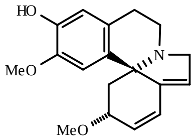
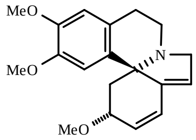
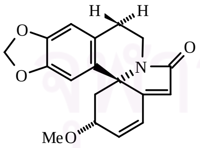
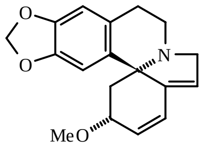
Plant and chemical compound	Category	Plant part	Reference
***E. latissima* E. Meyer**			
(+)-10,11-Dioxoerysotrine **[196]** 	Dienoid	Seed pod	Wanjala *et al.*, 2001
(+)-16β-D-Glucoerysopine **[197]** 	Dienoid	Seed pod	Wanjala *et al.*, 2001
(+)-15β-D-Glucoerysopine **[198]** 	Dienoid	Seed pod	Wanjala *et al.*, 2001
Erysodine **[167]** 	Dienoid	Seed pod	Wanjala *et al.*, 2001
Erysotrine **[173]** 	Dienoid	Seed pod	Wanjala *et al.*, 2001
8-Oxoerythraline **[195]** 	Dienoid	Seed pod	Wanjala *et al.*, 2001
Erythraline **[177]** 	Dienoid	Seed pod	Wanjala *et al.*, 2001

Table 2 (continued)

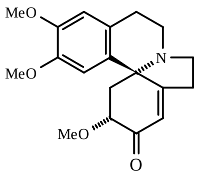
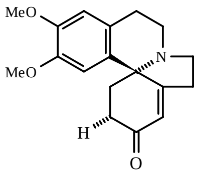
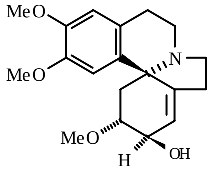
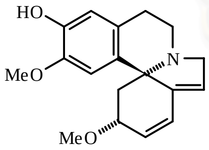
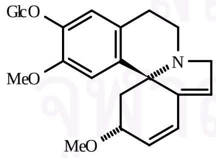
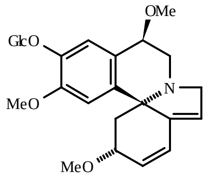
Plant and chemical compound	Category	Plant part	Reference
***E. lithosperma* Blume.**			
Erythratidinone **[199]** 	Alkenoid	Leaves	Barton *et al*., 1973
3-Demethoxyerythratidinone **[200]** 	Alkenoid	Leaves	Barton *et al*., 1973
Erythratidine **[172]** 	Alkenoid	Leaves	Barton *et al*., 1973
***E. lysistemon* Hutch.**			
Erythristemine **[201]** 	Dienoid	Fruit	Amer and El-Masry, 1991
(+)-Glucoerysodine **[190]** 	Dienoid	Leaves	Barton *et al*., 1973
(+)-11β-Methoxyglucoerysodine **[202]** 	Dienoid	Fruit	Amer and El-Masry, 1991

Table 2 (continued)

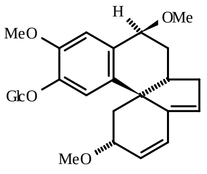
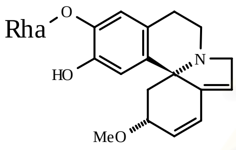
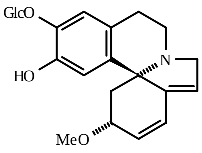
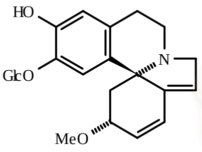
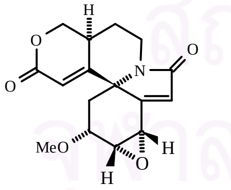
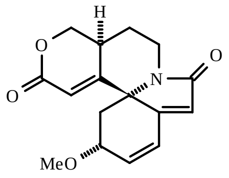
Plant and chemical compound	Category	Plant part	Reference
***E. lysistemon* Hutch.**			
(+)-11β-Methoxyglucoerysovine **[203]** 	Dienoid	Fruit	Amer and El-Masry, 1991
(+)-Rhamnoerysodine **[204]** 	Dienoid	Fruit	Amer and El-Masry, 1991
(+)-16β-D-Glucoerysopine **[197]** 	Dienoid	Seed	Wanjala and Majinda, 2000
(+)-15 β-D-Glucoerysopine **[198]** 	Dienoid	Seed	Wanjala and Majinda, 2000
***E. poeppigiana* Walp.**			
8-Oxo-α-erythroidine epoxide **[205]** 	Lactonic dienoid	Wood	Tanaka *et al.*, 2001
8-Oxo- α-erythroidine **[206]** 	Lactonic dienoid	Wood	Tanaka *et al.*, 2001

Table 2 (continued)

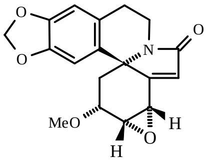
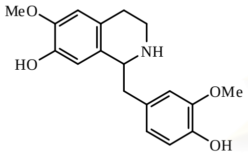
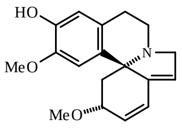
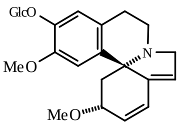
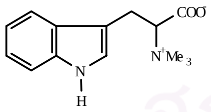
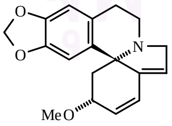
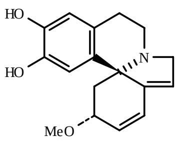
Plant and chemical compound	Category	Plant part	Reference
***E. poeppigiana* Walp.**			
8-Oxoerythraline epoxide **[207]** 	Lactonic dienoid	Wood	Barton *et al.*, 1973
N-Nororientaline **[208]** 	Tetrahydrobenzyl Isoquinoline	Leaves	Tanaka *et al.*, 2001
E. senegalensis			
Erysodine **[167]** 	Dienoid	Seed	Wandji *et al.*, 1995
Glucoerysodine **[190]** 	Dienoid	Seed	Wandji *et al.*, 1995
Hypaphorine **[209]** 	Indole	Seed	Wandji *et al.*, 1995
***E. stricta* Roxb.**			
Erythraline **[177]** 	Dienoid	Seed	Singh *et al.*, 1981
Erysopine **[174]** 	Dienoid	Seed	Singh *et al.*, 1981

Table 2 (continued)

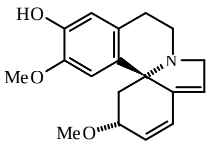
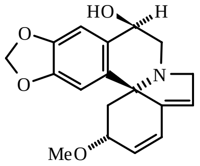
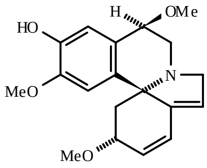
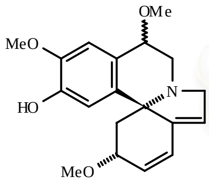
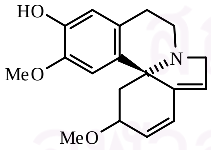
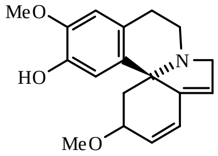
Plant and chemical compound	Category	Plant part	Reference
E. stricta **Roxb.**			
Erysodine **[167]** 	Dienoid	Seed	Singh *et al*., 1981
Erythrinine **[191]** 	Dienoid	Seed	Singh *et al*., 1981
11β-Methoxyerysodine **[202]** 	Dienoid	Seed	Singh *et al*., 1981
11-Methoxyerysovine **[210]** 	Dienoid	Seed	Singh *et al*., 1981
Erysodine **[167]** 	Dienoid	Bark	Singh *et al*., 1981
Erysovine **[168]** 	Dienoid	Bark	Singh *et al*., 1981

Table 2 (continued)

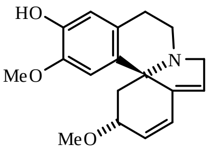
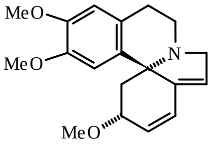
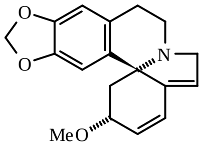
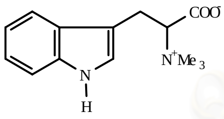
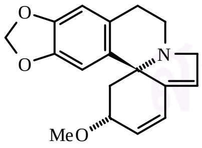
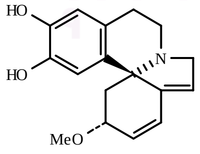
Plant and chemical compound	Category	Plant part	Reference
***E. suberosa* Roxb.**			
Erysodine **[167]** 	Dienoid	Seed	Singh and Chawla, 1970
Erysotrine **[173]** 	Dienoid	Seed	Singh and Chawla, 1970;
Erythraline **[167]** 	Dienoid	Seed	Singh and Chawla, 1970
Hypaphorine **[209]** 	Indole	Seed Leaves	Singh and Chawla, 1970; Singh and Chawla, 1971
***E. variegata* L.**			
Erythraline **[177]** 	Dienoid	Bark	Chawla *et al.*, 1988
Erysopine **[174]** 	Dienoid	Bark	Ghosal, Dutta and Bhattacharya, 1972; Hernandez and Jackson, 1994

Table 2 (continued)

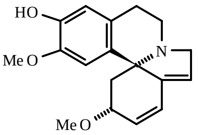
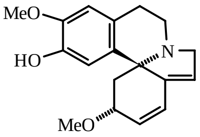
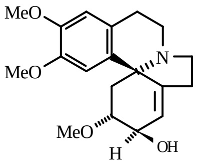
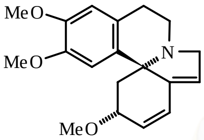
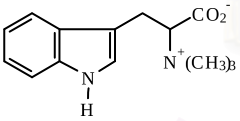
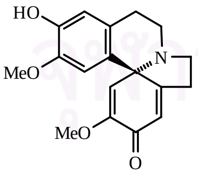
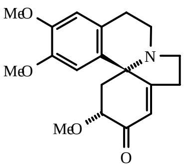
Plant and chemical compound	Category	Plant part	Reference
***E. variegata* L.**			
Erysodine **[167]** 	Dienoid	Bark	Ghosal, Dutta and Bhattacharya, 1972; Chawla *et al*., 1988
Erysovine **[168]** 	Dienoid	Bark	Chawla *et al*., 1988 Hernandez and Jackson, 1994
Erythratidine **[172]** 	Alkenoid	Bark	Ghosal, Dutta and Bhattacharya, 1972; Chawla *et al*., 1988
Erysotrine **[173]** 	Dienoid	Bark	Ghosal, Dutta and Bhattacharya, 1972; Chawla *et al*., 1988
Hypaphorine **[209]** 	Indole	Bark	Ghosal, Dutta and Bhattacharya, 1972
Erysodienone **[211]** 	Alkenoid	Bark	Ghosal, Dutta and Bhattacharya, 1972
Erythratidinone **[212]** 	Alkenoid	Bark	Ghosal, Dutta and Bhattacharya, 1972

Table 2 (continued)

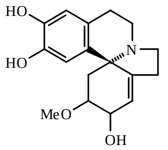
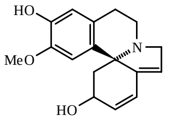
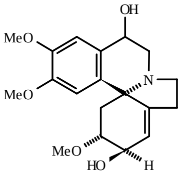
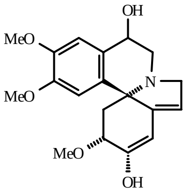
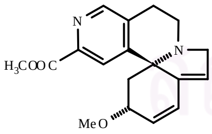
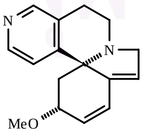
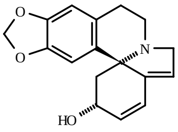
Plant and chemical compound	Category	Plant part	Reference
***E. variegata* L.**			
Erysopitine **[213]** 	Alkenoid	Bark	Ghosal, Dutta and Bhattacharya, 1972
Erysonine **[214]** 	Dienoid	Bark	Ghosal, Dutta and Bhattacharya, 1972
11-Hydroxy-epi-erythratidine **[215]** 	Alkenoid	Bark	Chawla *et al*., 1988
11-Hydroxyerythratidine **[216]** 	Alkenoid	Seed	Hernandez and Jackson, 1994
Erymelanthine **[217]** 	16-Azo erythrinane	Seed	Hernandez and Jackson, 1994
Demethoxycarbonylerymelanthine **[218]** 	16-Azo erythrinane	Seed	Hernandez and Jackson, 1994
Erythrocarine **[219]** 	Dienoid	Seed	Hernandez and Jackson, 1994

Table 3 Distribution of miscellaneous compounds in *Erythrina* plants

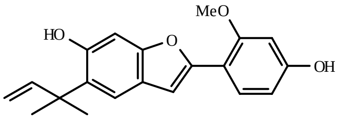
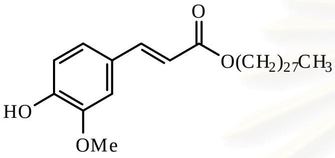
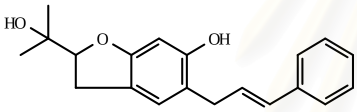
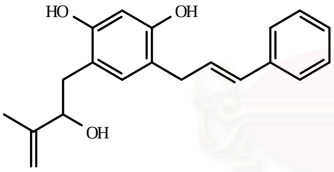
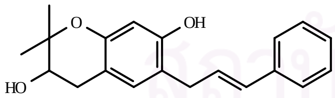
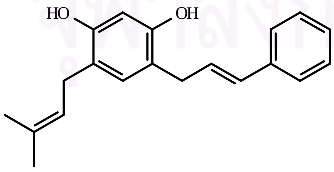
Plant and chemical compound	Category	Plant part	Reference
***E. burttii* Ball.**			
Burttinol D **[220]** 	Arylbenzofuran	Root bark	Yenesew *et al*., 2002
Erythrinassinate B **[221]** 	Long chain ester	Stem bark	Yenesew *et al*., 1998
E. crista-galli			
Erycristanol A **[222]** 	Cinnamylphenol	Heartwood	Iinuma *et al*., 1994
Erycristanol B **[223]** 	Cinnamylphenol	Heartwood	Iinuma *et al*., 1994
Erycristanol C **[224]** 	Cinnamylphenol	Heartwood	Iinuma *et al*., 1994
Eryvariestyrene **[225]** 	Cinnamylphenol	Heartwood	Iinuma *et al*., 1994

Table 3 (continued)

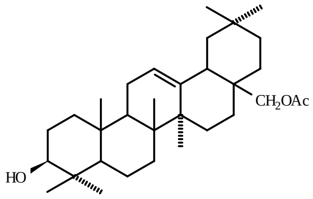
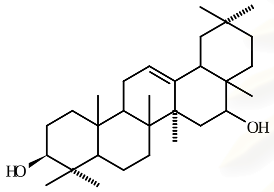
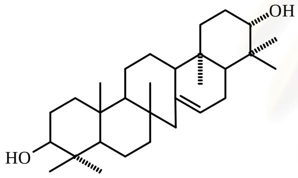
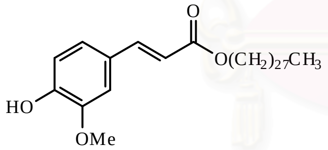
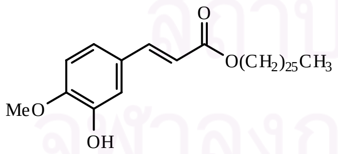
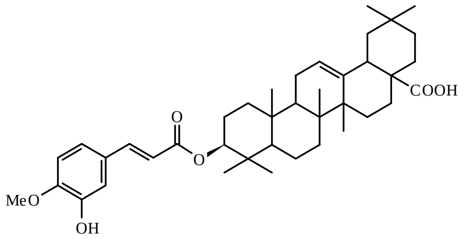
Plant and chemical compound	Category	Plant part	Reference
***E. eriotriocha* Harms.**			
28-Acetoxyerythrodiol **[226]**  	Triterpenoid	Stem bark	Nkengfack and Fomum, 1990
Maniladiol **[227]**  	Triterpenoid	Stem bark	Nkengfack and Fomum, 1990
Serrat-14-ene-3β,21α-diol **[228]**  	Triterpenoid	Stem bark	Nkengfack and Fomum, 1990
Erythinassinate B **[221]**  	Long chain ester	Stem bark	Nkengfack *et al.*, 1989
Erythinassinate D **[229]**  	Long chain ester	Stem bark	Nkengfack *et al.*, 1997
3β-*O*-(*E*)-Isoferuloyl oleanolic acid **[230]**  	Triterpenoid	Stem bark	Nkengfack *et al.*, 1997

Table 3 (continued)

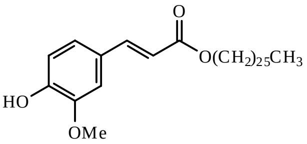
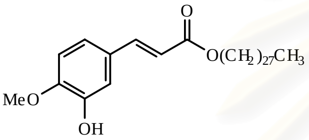
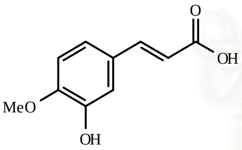
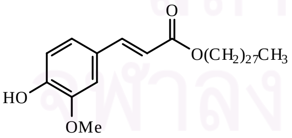
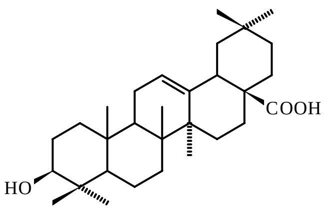
Plant and chemical compound	Category	Plant part	Reference
E. excelsa			
n-Hexacosanyl isoferulate **[231]** 	Long chain ester	Stem bark	Wandji *et al*., 1990
***E. glauca* Willd.**			
Erythrinasinate **[232]** 	Long chain ester	Stem bark	Fomum *et al*., 1986
1-Octacosanol **[233]** $HO(CH_2)_{27}Me$	Long chain alcohol	Stem bark	Fomum *et al*., 1986
3-Hydroxy-4-methoxy cinnamic acid **[234]** 	Acid	Stem bark	Fomum *et al*., 1986
***E. indica* Lam.**			
Erythrinassinate B **[221]** 	Long chain ester	Stem bark	Nkengfack *et al*., 2001
Oleanolic acid **[235]** 	Triterpenoid	Stem bark	Nkengfack *et al*., 2001

Table 3 (continued)

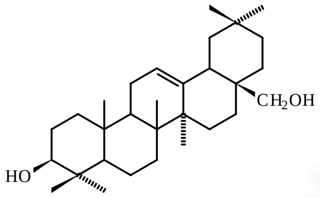
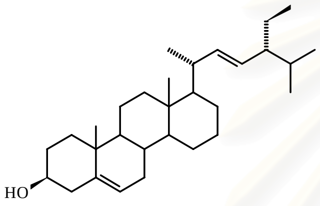
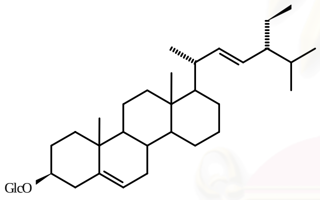
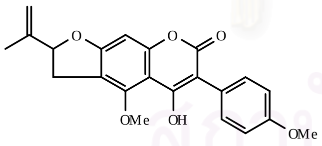
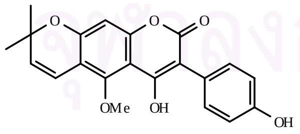
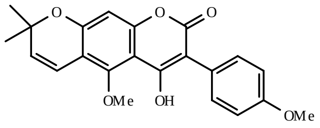
Plant and chemical compound	Category	Plant part	Reference
***E. indica* Lam.**			
Erythrodiol **[236]** 	Triterpenoid	Stem bark	Nkengfack *et al*., 2001
Stigmasterol **[237]** 	Steroid	Stem bark	Nkengfack *et al*., 2001
Stigmasteriol 3-*O*-β-D-glucopyranoside **[238]** 	Steroid	Stem bark	Nkengfack *et al*., 2001
Indicanine A **[239]** 	3-Phenyl coumarin	Root bark	Nkengfack *et al*., 2001
Indicanine B **[240]** 	3-Phenyl coumarin	Root bark	Waffo *et al*., 2000
Robustic acid **[241]** 	3-Phenyl coumarin	Root bark Stem bark	Waffo *et al*., 2000; Nkengfack *et al*., 2000

Table 3 (continued)

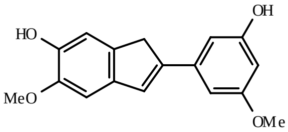
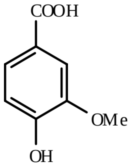
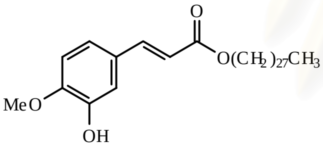
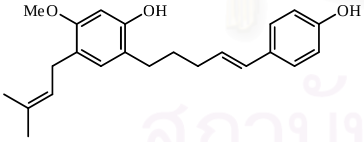
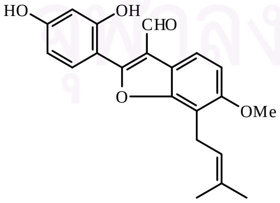
Plant and chemical compound	Category	Plant part	Reference
***E. latissima* E. Meyer** 2-(5'-Hydroxy-3'-methoxyphenyl)-6-hydroxy-5-methoxybenzofuran **[242]**  Vanillic acid **[243]** 	Arylbenzofuran Acid	Seed pod Seed pod	Wanjala *et al.*, 2001 Wanjala *et al.*, 2001
***E. mildbraedii* Harms.** Erythrinasinate **[232]** 	Long chain ester	Stem bark	Fomum *et al.*, 1986
***E. poeppigiana* Walp.** Erypostyrene **[244]**  Erypoegin F **[245]** 	Cinnamylphenol Arylbenzofuran	Root Root	Sato *et al.*, 2003 Tanaka *et al.*, 2003

Table 3 (continued)

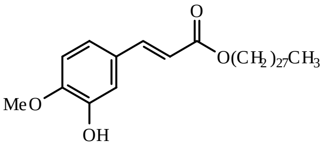
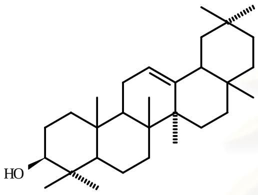
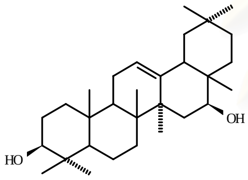
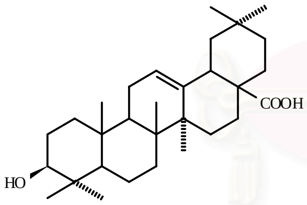
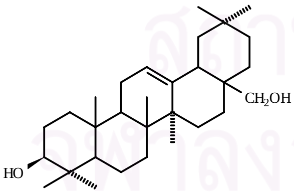
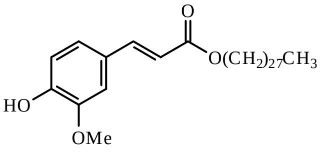
Plant and chemical compound	Category	Plant part	Reference
***E. sensegalensis* DC.**			
Erythrinasinate **[232]** 	Long chain ester	Stem bark	Fomum *et al*., 1986; Wandji *et al*., 1990
β-Amyrin **[246]** 	Triterpenoid	Stem bark	Wandji *et al*., 1995
Maniladiol **[227]** 	Triterpenoid	Stem bark	Wandji *et al*., 1995
Oleanolic acid **[235]** 	Triterpenoid	Stem bark	Wandji *et al*., 1995
Erythrodiol **[236]** 	Triterpenoid	Stem bark	Wandji *et al*., 1995
Erythrinasinate B **[221]** 	Long chain ester	Stem bark	Wandji *et al*., 1990

Table 3 (continued)

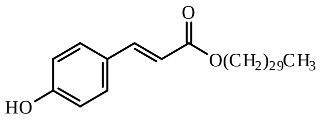
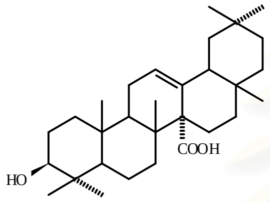
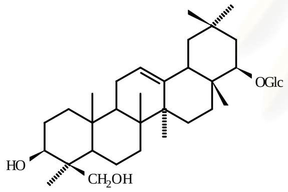
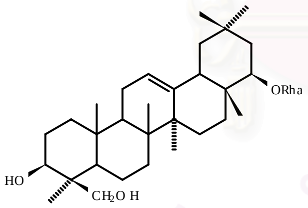
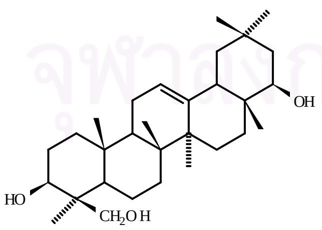
Plant and chemical compound	Category	Plant part	Reference
***E. sensegalensis* DC.**			
p-Coumaric acid **[247]** 	Long chain ester	Stem bark	Wandji *et al.*, 1990
Cornulacic acid **[248]** 	Triterpenoid	Stem bark	Wandji *et al.*, 1995
***E. sigmoidea* Hua.**			
Sigmoside C **[249]** 	Triterpenoid	Stem bark	Mbafor *et al.*, 1997
Sigmoside D **[250]** 	Triterpenoid	Stem bark	Mbafor *et al.*, 1997
Soyasapogenol B **[251]** 	Triterpenoid	Stem bark	Mbafor *et al.*, 1997

Table 3 (continued)

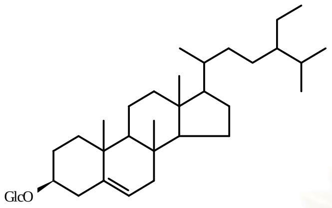
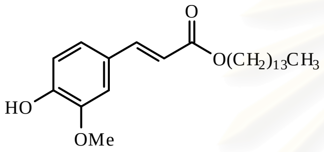
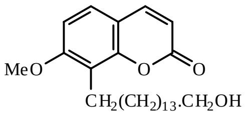
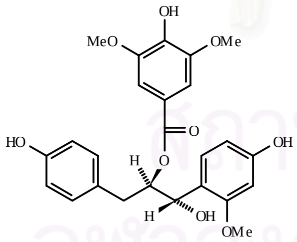
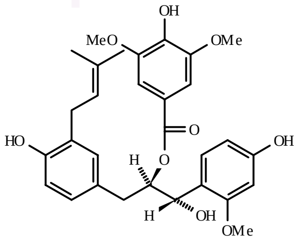
Plant and chemical compound	Category	Plant part	Reference
***E. sigmoidea* Hua.**			
3-*O*-[β-D-glycopyranosyl]-sitosterol **[252]**  	Steroid	Stem bark	Mbafor *et al*., 1997
Erythinassinate C **[253]**  	Long chain ester	Root bark	Nkengfack *et al*., 1997
***E. stricta* Roxb.**			
7-Methoxy-8-(15-hydroxypentadecyl)-coumarin **[254]**  	Coumarin	Bark	Singh *et al*., 1981
***E. variegata* L.**			
Eryvarinol A **[255]**  	Diphenyl-propandiol	Root	Tanaka *et al*., 2002
Eryvarinol B **[256]**  	Diphenyl-propandiol	Root	Tanaka *et al*., 2002

Table 3 (continued)

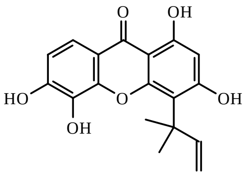
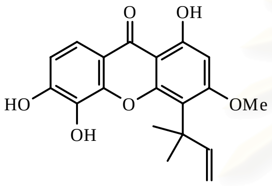
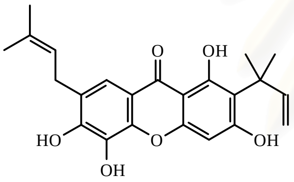
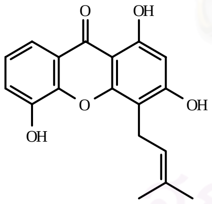
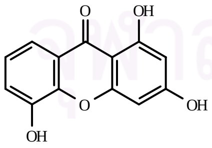
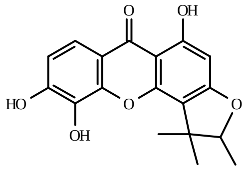
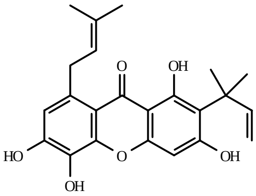
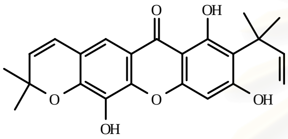
Plant and chemical compound	Category	Plant part	Reference
***E. variegata* L.**			
Isocudraniaxanthone A **[257]** 	Xanthone	Bark	Kobayashi *et al.*, 1997
Isocudraniaxanthone B **[258]** 	Xanthone	Bark	Kobayashi *et al.*, 1997
Isoalvaxanthone **[259]** 	Xanthone	Bark	Kobayashi *et al.*, 1997
1,3,5-Trihydroxy-4-(3-methylbut-2-enyl)xanthen-9-one **[260]** 	Xanthone	Bark	Kobayashi *et al.*, 1997
1,3,5-Trihydroxyxanthone **[261]** 	Xanthone	Bark	Kobayashi *et al.*, 1997
Deprenylated rheediaxanthone **[262]** 	Xanthone	Bark	Kobayashi *et al.*, 1997

Table 3 (continued)

Plant and chemical compound	Category	Plant part	Reference
E. variegata **L.**			
Alvaxanthone **[263]** 	Xanthone	Bark	Kobayashi *et al.*, 1997
Gerontoxanthone B **[264]** 	Xanthone	Bark	Kobayashi *et al.*, 1997

2. Biosynthetic Relationship among Flavonoids

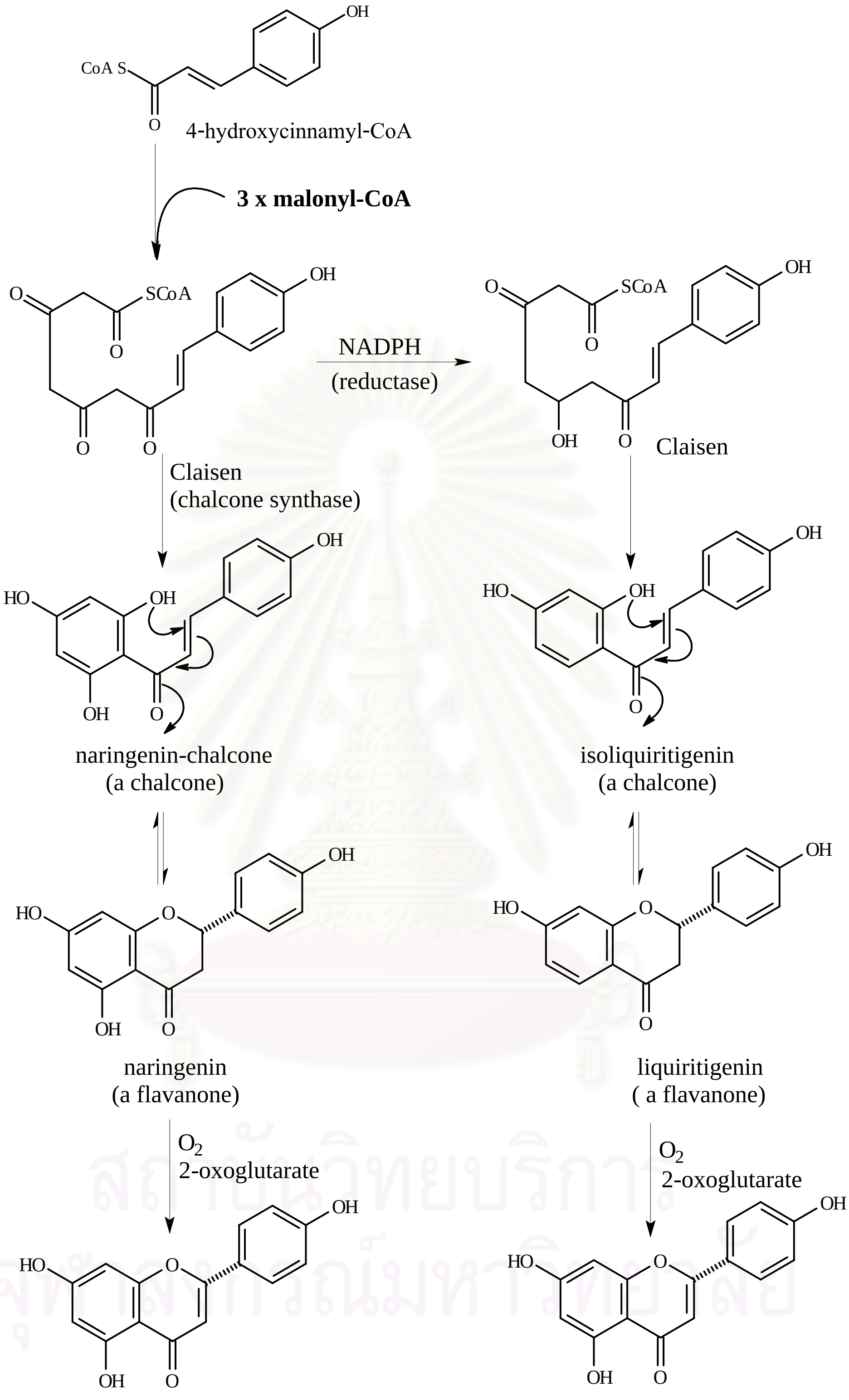
The flavonoids: chalcones, flavones, isoflavones, rotenoids and pterocarpan share the same biosynthetic pathway as shown in **Schemes 1-4** (Dewick, 2002; Harborne, 1994).

Flavonoids are products from a cinnamoyl-CoA starter unit, with chain extension using three molecules of malonyl-CoA. This initially gives a polyketide, according to the nature of the enzyme responsible. This allows aldol or Claisen-like reactions to occur, generating aromatic rings. Enzyme chalcone synthase couples a cinnamoyl-CoA unit with three malonyl-CoA units giving naringenin, chalcone or isoliquiritigenin. Both structures nicely illustrate the different characteristic oxygenation patterns in aromatic rings derived from the acetate or shikimate pathways. Chalcones act as precursors for a vast range of flavonoid derivatives. Most contain a six-membered heterocyclic ring, formed by Michael-type nucleophilic attack of a phenol group on the unsaturated ketone giving the flavanone, naringenin or liquiritigenin. Flavanones can then give rise to flavones **(Scheme 1)**.

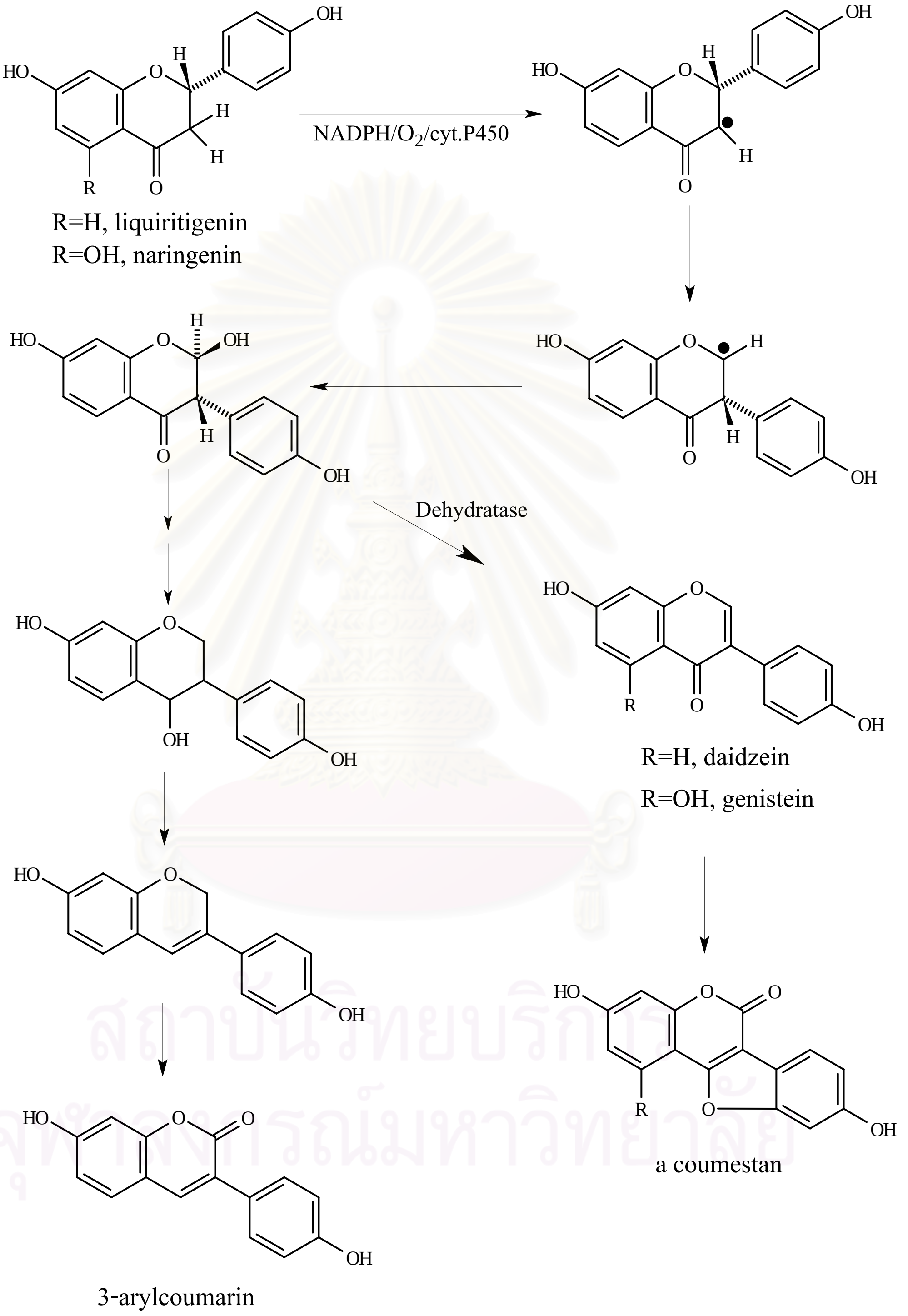
The isoflavonoids form a quite distinct subclass of flavonoid compounds, being structural variants in which the shikimate-derived aromatic ring has migrated to the adjacent carbon of the heterocycle. This arrangement process is brought about by a cytochrome P-450-dependent enzyme requiring NADPH and O_2 cofactors, which transforms the flavanones liquiritigenin or naringenin into the isoflavone daidzein or genistein, respectively *via* an intermediate hydroxyisoflavanones. A radical mechanism has been proposed. Hundreds of different isoflavonoids have been identified and their structural complexity is brought about by hydroxylation and alkylation reactions, varying the oxidation level of the heterocyclic ring to occurring of 3-arylcoumarin **(Scheme 2)**.

The rotenoids take their name from the first known example, rotenone, and are formed by ring cyclization of a methoxyisoflavone. Rotenone itself contains a C_5 isoprene unit introduced *via* dimethylallylation of demethylmunduserone. The isopropenylfurano system of rotenone is formed *via* rotenoic acid **(Scheme 3)**.

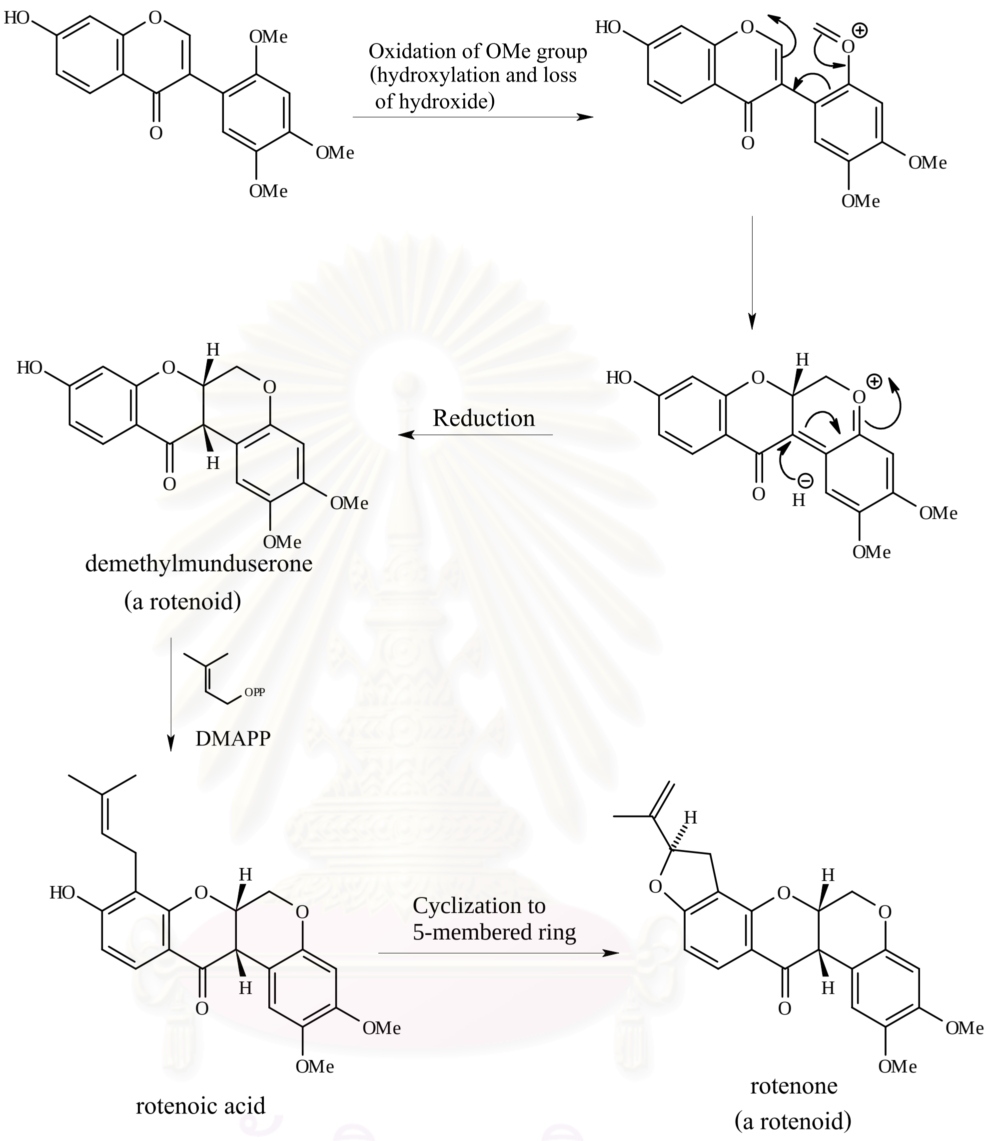
Pterocarpans contain a tetracyclic ring system derived from the basic isoflavonoid skeleton by an ether linkage between the 4- and 2'-positions. The systematic numbering of rather than that for simple isoflavonoids is used, however. Convenient subdivisions into pterocarpans, 6a-hydroxypterocarpans is made for this group **(Scheme 4)**.



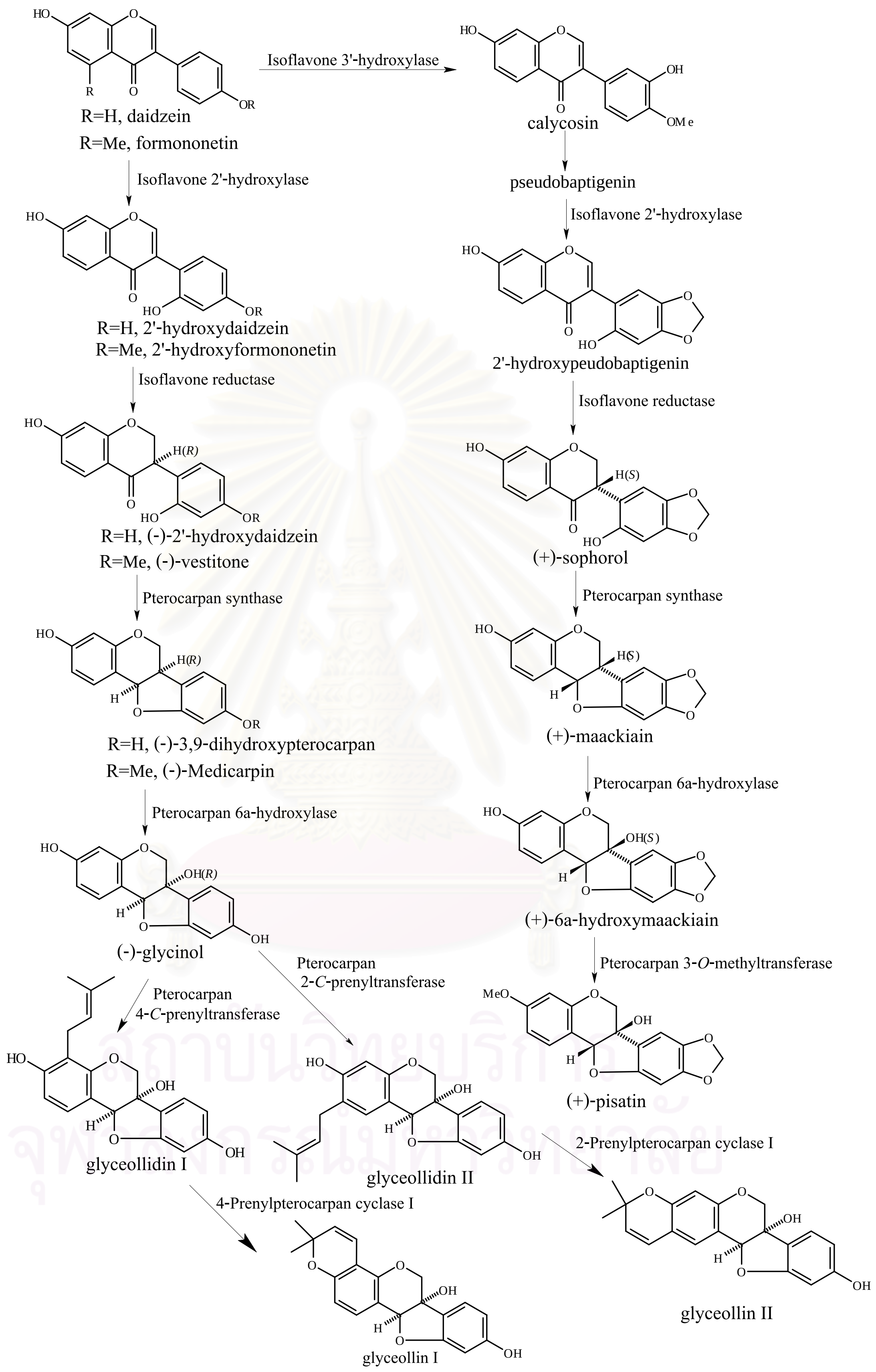
Scheme 1 Biosynthetic relationship among chalcones and flavones



Scheme 2 Biosynthetic relationship among flavanones and isoflavones



Scheme 3 Biosynthetic relationship among isoflavones and rotenoids



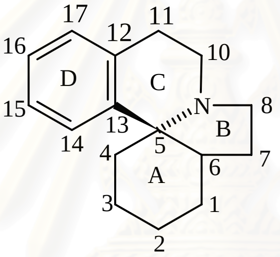
Scheme 4 Biosynthetic relationship among isoflavones and pterocarpan

3. *Erythrina* alkaloids

Erythrina plant species are the main source for the tetracyclic *Erythrina*-type alkaloids. The distribution of these alkaloids is unusual because except for some isolations from the genus *Cocculus*, family Menispermaceae, of closely related alkaloids which differ from the true *Erythrina* alkaloids in their oxygenation pattern in ring A, no other isolations have been reported outside the genus *Erythrina* (Cordell, 1981; Amer, 1991).

3.1 Nomenclature

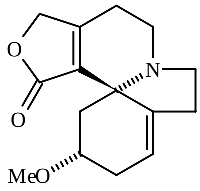
An interesting insight into the possible catabolism of the erythrina-type alkaloids is provided by such lactonic compounds as cocculolidine **[265]**, α-erythroidine **[170]**, β-erythroidine **[171]**, 8-oxo-α-erythroidine **[206]** and erymelanthine **[217]**, which are most probably products of *in vivo* oxidation of the aromatic ring D of the classical skeleton **I** below.



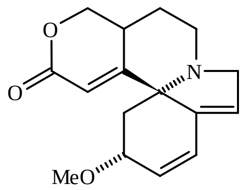
erythrinane skeleton **I**

The nomenclature of the erythrina-type alkaloids is interesting. The prefix eryso- usually denotes the presence of phenolic function. The prefix erythroi- indicates that ring D is lactonic, while the prefix erythra- points to the classical skeleton **I** as above. So-called dienoid alkaloids (the second subdivision) possess one carbon-carbon double bond in ring A and another in ring B, but alkenoids (the first subdivision) incorporate only one double bond, usually in ring A.

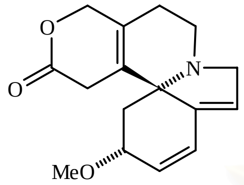
The lactonic alkaloids mentioned above represent the third subdivision of *erythrina*-type alkaloids. The *in vivo* oxidation of classical type alkaloids possessing skeleton **I** may also explain the biogenesis of the 16-azoerythrinanes such as erymelanthine **[217]**, because in such instances oxidation could be followed by ammonia uptake and recyclization to form an aminated ring D. Finally, a few so-called dimeric alkaloids are known such as isoerysopinophorine hydroxide **[189]** that incorporates a tryptophan moiety.



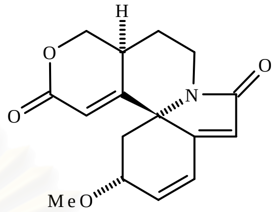
cocculolidine **[265]**



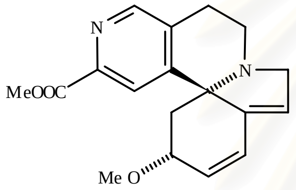
α-Erythroidine **[170]**



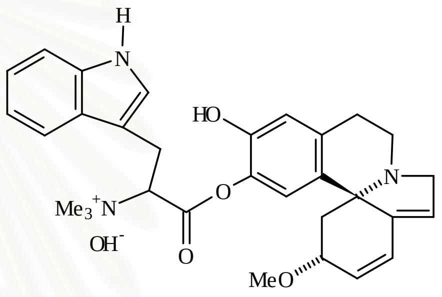
β-Erythroidine **[171]**



8-Oxo- α-erythroidine **[206]**



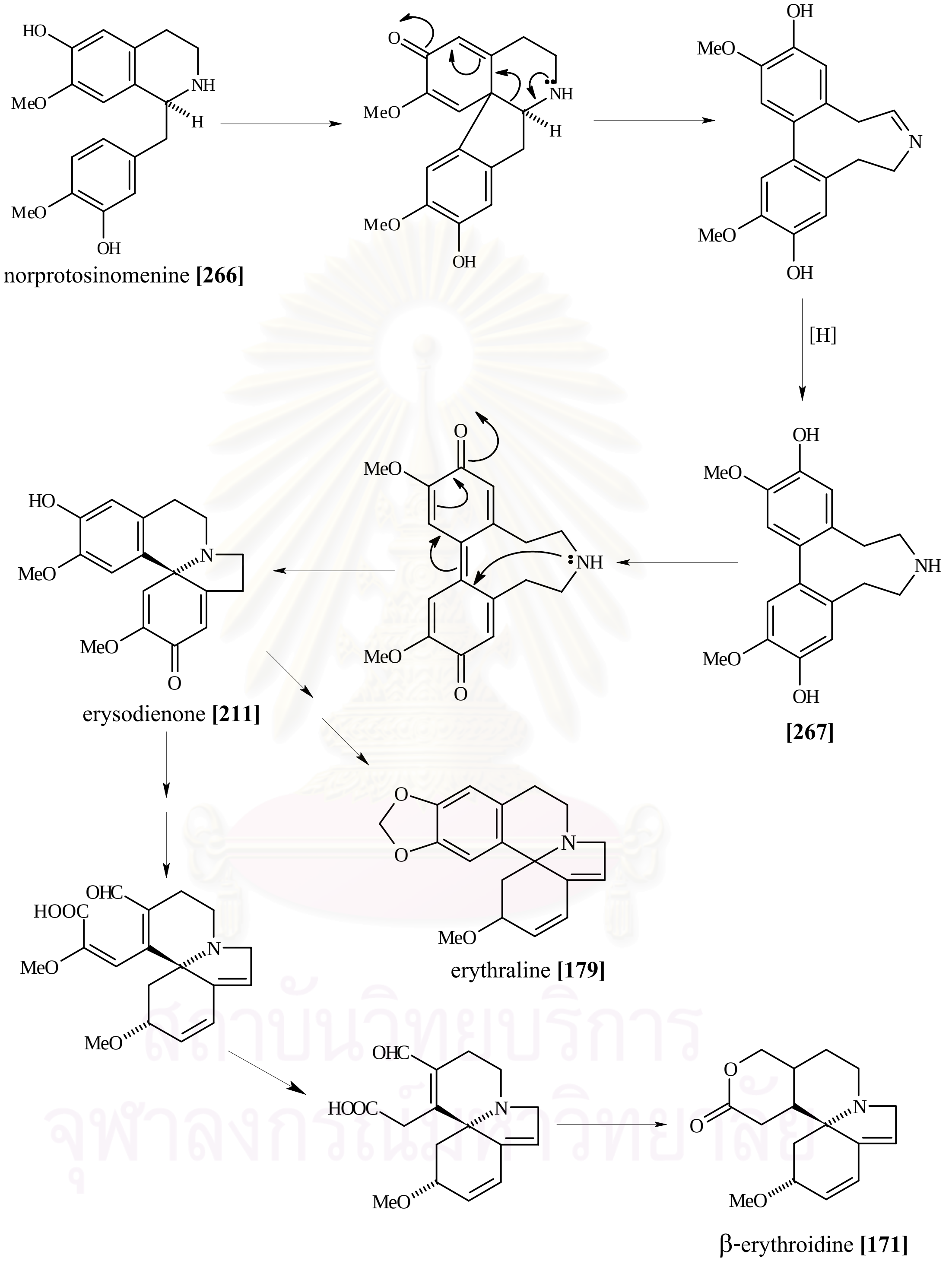
erymelanthine **[217]**



isoerysopinophorine hydroxide **[189]**

3.2 Biosynthesis

Norprotosinomenine **[266]** is the precursor of erythraline **[179]**. A symmetrical intermediate **[267]** is involved in the biosynthetic pathway. The pathway involves phenolic oxidative coupling and ring opening to form a dibenzazonine, which subsequently ring closure to erysodienone **[211]**. It is thought that formation of lactonic alkaloids occurs at relatively late stage in the pathway as tracer studies show that C_{16} was lost in the oxidative cleavage process. The overall scheme for the formation of the *Erythrina* skeleton was shown in **Scheme 5**. (Barton, 1973; Cordell, 1981; Phillipson, 1985).



Scheme 5 Biosynthesis of the *Erythrina* alkaloids

4. Traditional Uses and Biological Activities of *Erythrina* Constituents

Erythrina plants have been used in traditional medicine in many countries for several purposes. Extracts of the leaves, stem bark and roots of *E. addisoniae* are used in indigenous medicinal practice for the treatment of pathological inflammations. The stem bark of this plant has been used for the treatment of dysentery, asthma, venereal diseases, boils, and leprosy in Cameroon (Talla, 2003). The crushed branches of *E. berteroana* have been used as a fish poison and crude extracts of the stem bark showed antifungal activity against *Cladosporium cucumerinum* (Maillard, 1987). The root bark of *E. burttii* showed significant antimicrobial activity against oral bacteria (Iinuma, 1994). The stem bark and root of *E. latissima* are burnt to powder and used as a dressing for open wounds (Wanjala, 2000). The root, seed and stem bark of *E. popeppigiana* and *E. senegalensis* have been used in indigenous folk medicine for treatment of microbial infections and neuromuscular transition blocking activity (curare-like action) (Mitscher, 1987; Tsuda, 1996; Tanaka, 2001a; Dalziel, 1937; Dyke, 1981). *E. sigmoidea* has been used in Cameroonian traditional medicine for the treatment of several conditions such as female infertility, stomach pain and gonorrhea (Ayensu ,1978). *E. suberosa* has been used in Pakistan as an ornamental plant and folk medicine, and in India as the treatment of various ailments. The ethanol extract of the leaves from this plant has been reported to have antitumor activity (Miana, 1972; Tanaka, *et al*., 2001). *E. zeyheri* is utilized to cure ailments such as asthma, tuberculosis and rheumatism in South Africa (Tanaka, *et al*., 2003).

Traditiondl uses of other Erythrina species have been recorded. In Kenya and East Africa, seed and root extract of *E. abyssinica* are most widely used to treat microbial infection and malarial. Abyssinone IV **[4]**, Erythrabyssin II **[8]**, 3-hydroxy-9-methoxy-10-(3,3-dimethylallyl)pterocarpene **[15]**, 7,4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene **[16]** and erycristagallin **[17]** showed antimalarial against *Plasmodium* falciparum (W2 and D6 strains) at IC_{50} value ranged from 6.5 ± 0.6 μM to 27.7 ± 1.8 μM (whesras that of chloroquine was 0.93 ± 0.005 μM) (Yenesew, 2003), while abyssinone-I **[1]**, abyssinone-II **[2]**, abyssinone-III **[3]** abyssinone-IV **[4]**, abyssinone-V **[5]**, erythrabyssin I **[7]**, erythrabyssin II **[8]**, phaseollin **[9]** and phaseollidin **[10]** showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtillis* and *Micrococcus lysodeikticus* at MIC values of 3.13 to 50 μg/ml (Kamat, 1981).

Isoflavones from the stem bark of *E. indica*, indicanine D **[63]**, wighteone **[68]**, alpinumisoflavone **[69]**, 8-prenylerythrinin C **[71]** and erysenegalensein E **[72]** were cytotoxic against KB cells at ED_{50} values of 12.5, 0.78, 4.13, 13 and 6.25 μg/ml, respectively (Nkengfack, 2001). The pterocarpan from the bark of *E. burana* (found only in Ethiopia), phaseollidin **[10]** and cristacarpin **[29]** were cytotoxic activity against P-338 (murine leukemia cells), CHOC (Chinese hamster ovary cells) and CHOC-PGO (glycoprotein overproducing Chinese hamster ovary cell) (Dagne, 1993).

The anti-inflammatory activites of *Erythrina* flavonoid have been studied by testing these compounds for their inhibitory actions on several models, including both phospholiphase A_2 (PLA_2) and carrageenan-induced mouse paw edema, 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema, and a model of chronic dermatitis caused by repeated administration of TPA. Warangalone **[19]** from the stem bark of *E. addisoniae* (Talla, 2003) was as effective as the standard drug ciproheptadine on the PLA_2- induced mouse paw edema at 60 min. *In vivo*, erycristagallin **[17]** (from the root bark of *E. midbraedii*) inhibited the PLA_2 -induced mouse paw edema as well as the mouse ear oedema induced by TPA (ID_{50}< 10 μg/ear). *In vitro*, it inhibited arachidonic acid metabolism *via* the 5-lipoxygenase pathway in rat polymorphonuclear leukocytes at IC_{50} value of 23.4 μM (Njamen, 2003).

Euchrenone b_{10} **[142]**, erythrinin B **[145]** and 1,3,5-trihydroxy-4-(3-methylbut-2-enyl)xanthen-9-one **[259]** from the bark of *E. variegata* inhibited the Na^+/H^+ exchanger system of arterial smooth muscel cells, with minimum inhibitory concentrations of 1.25, 1.25 and 10 μg/ml, respectively. However, erythrinin B **[144]** also showed moderate cytotoxic activity against normal cells at the concentration of 2.5 μg/ml.

Anti-HIV activity in the NCI's XTT-based primary screening of 5-deoxylicoiso flavanone **[78]** and 5-hydroxyneobavaisoflavanone **[81]** from the root extract of *E. lysistemon* showed the two compounds as having EC_{50} of 11.5 and 7.6 μg/ml, respectively (McKee, 1997).

Some flavonoids from *E. crista-galli*, such as erycristin **[41]** and erythrabyssin-II **[8]** showed antibacterial activity against *S. aureus* at MIC values of 6.25 and 3.12 μg/ml, and *Mycobacterium smegmatis* at MIC values of 12.5 and 0.78 μg/ml, respectively (Mitscher, 1988). Eriotrichin B **[52]**, isoneorautenol **[53]**, erybraedin A **[54]**, erybraedin C **[55]**, erybraedin D **[56]** and erybraedin E **[57]** isolated from the root bark of *E. eriotricha* demonstrated antibacterial activity against the gram-positive pathogenic bacteria *S. aureus* at MIC of 8.3, 28.4, 13.6, 12.8, 78.3 and 22.1 μg/ml, respectively (Nkengfack, 1995). Indicanine B **[240]**, indicanineC **[62]**, cajanin **[66]** and dimethylalpinumisoflavone **[70]** found in the root bark of *E. indica* displayed activity against several bacteria at MIC as low as 9.7 μg/ml (Waffo, 2000). In the cases of erythrabyssin-II **[8]**, erybraedin A **[81]**, erybraedin B **[82]**, erybraedin C **[55]**, erybraedin D **[56]**, erybraedin E **[57]** and isoneorautenol **[53]** from the root bark of *E. mildbreaedii*, these compounds showed activity against *S. aureus* at MIC 3.12, 12.5, 12.5, 12.5, 12.5, 100, and 25.0 μg/ml, respectively, and *M. smegmatis* at MIC 0.78, 6.25, 12.5, 12.5, 25.0, >100 and 25.0 μg/ml, respectively (Mitscher, 1988). Sandwicensin **[28]**, demethylmedicarpin **[40],** erypoegin A **[95]** and erypostyrene **[244]** from the root of *E. sigmoidea* had activity against *S. aureus* at MIC 6.25, 6.25, 25 and 50 μg/ml, respectively (Nkenfack, 1994).

Flavonoids from the stem bark of *E. glauca*, sandwicensin **[28]** and 3-*O*-methylcalopocarpin **[61],** inhibited the cytopathic effects of *in vitro* HIV-1 infection in a human T-lymphoblastoid cell line (CEM-SS). 3-*O*-Methylcalopocarpin was cytoprotective over a modest concentration range (EC_{50} 0.2 μg/ml; IC_{50} 3.0 μg/ml) with a maximum of 80-95 % protection. Sandwicensin was less effective (EC_{50} 2 μg/ml, IC_{50} 7 μg/ml), with a maximum protection of only 50-60 % (McKee, 1997).

CHAPTER III

EXPERIMENTAL

1. Sources of Plant Materials

The stem bark of *Erythrina suberosa* Roxb. was collected from Nakhon Ratchasima province, Thailand, in April 2001. Authentication was achieved by comparison with the herbarium specimen (BKF No. 115610) at the Royal Forest Department, Ministry of Agriculture and Cooperative, Thailand. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

The stem bark of *Erythrina fusca* Lour. was collected from Muang District, Pathumthani Province, Thailand, in February 2002. Authentication was achieved by comparison with the herbarium specimen (BKF No. 112379) at the Royal Forest Department, Ministry of Agriculture and Cooperative, 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 : Silica gel F_{254} (E. Merck) precoated plate
Layer thickness : 0.2 mm
Distance : 5.0 cm
Temperature : Laboratory temperature (28-35 °C)
Detection : 1. Ultraviolet light (254 and 365 nm)
2. Anisaldehyde-acetic acid reagent and heating at 105 °C for 10 min

2.2 Preparative Thin-Layer Chromatography (PTLC)

Technique : One dimension, ascending
Adsorbent : Silica gel F_{254} (E. Merck) precoated plate
Layer thickness : 1 mm
Distance : 15 cm

Temperature : Laboratory temperature (28-35 °C)

Detection : Ultraviolet light (254 and 365 nm)

2.3 Column Chromatography

2.3.1 Vacuum Liquid Column Chromatography

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

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 top of the column.

Detection : Fraction were examined by TLC observing under UV light (254 and 365nm)

2.3.2 Flash Column Chromatography

Adsorbent : Silica gel 60 (No.9385) particle size 0.040-0.063 nm (70-230 mesh ASTM) (E. Merck)

Packing method : Wet packing

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

Detection : Fraction were examined in the same manner as described in section 2.3.1.

2.3.3 Gel Filtration Chromatography

Adsorbent : 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 poured into the column and allowed to set tightly.

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

Detection : Fraction were examined in the same manner as described in section 2.3.1.

2.3.4 High Pressure Liquid Chromatography (HPLC)

Column (Semi-preparative): SenShu Pak. PEGASIL ODS No.980172T SSC

(Analytical) : SenShu Pak. PEGASIL ODS No.9306215T SSC

Flow rate : 1. 8 mL/min for semi-preparative column
2. 1 mL/min for analytical column

Mobile phase : 1. Isocratic: 55 % and 85 % acetonitrile-H_2O + 0.05% trifluoro acetic acid (TFA) for semi-preparative column
2. Gradient: acetonitrile-H_2O + 0.05% TFA for analytical column

Sample preparation : The sample was dissolved in a small amount of eluent and filtered through Millipore filter paper before injection.

Injection volume : 1. 200-300 μL for semi-preparative column
2. 1 μL for analytical column

Pump : 1. LC-6A (Shimadzu)
2. LC-10A (Shimadzu)

Detector : 1. SPD-6A UV Detector (Shimadzu)
2. SPD-10A UV Detector (Shimadzu)

Recorder : C-R6A Chromatopac (Shimadzu)

Temperature : Room temperature (25 °C)

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Absorption Spectra

UV (in methanol) spectra were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.2 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and film) were recorded on a Perkin Elmer FT-IR 1760X and 2000 series spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University and Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, respectively).

2.4.3 Mass Spectra

Electron impact and fast atom bombardment mass spectra (EIMS and FABMS) were obtained with a JEOL JMS-AX505HA spectrometer (Kitasato University, Japan), high-resolution electron impact and high-resolution fast atom bombardment mass spectra (HR-EIMS, HR-FABMS) on a JEOL JMS-700 Mstation spectrometer (Kitasato University, Japan) and electrospray ionization time of flight mass spectra (ESITOFMS) on a Micromass LCT mass spectrometer (National Center for Genetic Engineering and Biotechnology, BIOTEC.

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

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

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained with a Varian Unity 400 NMR spectrometer (Kitasato University, Japan).

^{1}H NMR (500 MHz) and ^{13}C NMR (125MHz) spectra were obtained with a JEOL JMN-A 500 NMR spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

Solvents for NMR spectra were deuterated chloroform (choroform-*d*), deuterated methanol (methanol-d_3) and deuterated acetone (acetone-d_6). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.5 Physical Properties

2.5.1 Melting Points

Melting points were obtained on a Yanaco MP Melting Point Apparantus (Kitasato University, Japan) and a Fisher-Johns Melting Point Apparantus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 Optical Rotations

Optical rotations were measured on a Perkin Elmer Polarimeter 341 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.3 Elemental Analysis

Elemental analysis was performed on a Perkin Elmer Series II CHNS/O Analyzer 2400 (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.6 Solvents

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

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from the Stem Bark of *Erythrina suberosa*

3.1.1 Extraction

The dried stem bark of *E. suberosa* (5.7 kg) were chopped, ground and macerated 4 times, each for 3 day period, at room temperature with hexane (14 L), ethyl acetate (14 L) and 95% EtOH (14 L) and filtered. The filtrates were pooled and evaporated *in vacuo* until dryness to give a hexane extract (59.21 g, 1.04 % based on dried weight of bark), an ethyl acetate extract (394.79 g, 6.93 %) and a 95% EtOH extract (180.23 g, 3.16 %).

3.1.2 Isolation

3.1.2.1 Isolation of Compounds from Hexane Extract

The hexane extract (54 g) was fractionated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No. 7734, 540 g). Elution was performed in a polarity gradient manner with mixtures of hexane/CH_2Cl_2 (1:0 to 0:1), and CH_2Cl_2/MeOH (1:0 to 0:1). The eluates were collected as 500 mL as fractions and examined by TLC (silica gel, hexane/CH_2Cl_2 = 1:4). Thirty-six fractions were collected and similar chromatographic patterns were combined to yield eleven fractions: A-1 (2.6 g), A-2 (0.5 g), A-3 (3.4 g), A-4 (1.1 g), A-5 (3.9 g), A-6 (8.1 g), A-7 (2.7 g), A-8 (11.2 g), A-9 (10.4 g), A-10 (0.8 g) and A-11 (0.4 g).

3.1.2.1.1 Isolation of Compound ES1 (Mixture of Stigmasterol and β-Sitosterol)

Fraction A-8 (11.2 g) was separated by column chromatography (silica gel, mixtures of hexane/CH_2Cl_2 4:1 to 0:1). Eight combined fractions were collected. Fraction A-8-5 (1.37 g) was further purified on a silica gel column (40 % hexane in CH_2Cl_2) and the fourth fraction 4 from this column, after drying, was re-crystallized from MeOH to give 97.4 mg of compound ES1 (colorless needles, R_f 0.38, silica gel, CH_2Cl_2/MeOH = 99:1). It was subsequently identified as a mixture of stigmasterol **[268]** and β-sitosterol **[269]**.

Fraction A-9 (5.3 g) was subjected to flash column chromatography (silica gel 530 g, CH_2Cl_2/MeOH with increasing polarity). Eighteen fractions (50 mL per fraction) were combined according to their TLC behavior (silica gel, CH_2Cl_2/MeOH 5:95): to give 11 fractions A-9-1 (47.4 mg), A-9-2 (71.5 mg), A-9-3 (133.8 mg), A-9-4 (155.6 mg), A-9-5 (153.3 mg), A-9-6 (713.8 mg), A-9-7 (406.3 mg), A-9-8 (223.0 mg), A-9-9 (323.0 mg), A-9-10 (530.1 mg), A-9-11

(222.5 mg), A-9-12 (850.5 mg), A-9-13 (111.3 mg), A-9-14 (142.0 mg), A-9-15 (329.0 mg), A-9-16 (159.3 mg), A-9-17 (156.0 mg) and A-9-18 (84.7 mg).

Fraction A-9-7 (404.3 mg) was further purified on a silica gel column with gradient elution (40 % CH_2Cl_2 in hexane to 1% MeOH in CH_2Cl_2) to afford 84.6 mg of compound ES 1 (colourless needles, R_f 0.38, silica gel, CH_2Cl_2/MeOH = 99:1). The compound was identified as mixtures of stigmasterol **[268]** and β-sitosterol **[269]**.

3.1.2.1.2 Isolation of Compound ES2 (Erythrabyssin II)

Fraction A-9-9 (323 mg) was subjected to column chromatography using silica gel 60 (No. 9385, 35 g) as adsorbent. Mixtures of CH_2Cl_2 and MeOH (1:0 to 19:1) were used as mobile phase. Fractions with similar chromatographic patterns were combined to yield twelve fractions. Fraction 2 (111.1 mg) was purified by gel filtration chromatography, using a Sephadex LH-20 column with mixture of MeOH and $CHCl_3$ (1:2) as the eluent to afford 20 mg of compound ES2 (colorless needles, R_f 0.36, silica gel, CH_2Cl_2-MeOH = 19:1). ES2 was identified as erythrabyssin II **[8]**.

3.1.2.2 Isolation of Compounds from Ethyl Acetate Extract

The ethyl acetate extract (62 g) was separated by vacuum liquid column chromatography (silica gel No. 7734, 620 g). Elution was performed in a polarity gradient manner with mixtures of hexane/CH_2Cl_2 (1:0 to 0:1) and CH_2Cl_2/MeOH (1:0 to 0:1). The eluates were collected (500 mL per fraction) and examined by TLC (silica gel, hexane-CH_2Cl_2 = 1:4). Fractions with similar chromatographic patterns were combined to yield eleven fractions: B-1 (33.6 mg), B-2 (28.5 mg), B-3 (32.8 mg), B-4 (98.1 mg), B-5 (145.3 mg), B-6 (147.1 mg), B-7 (467.9 mg), B-8 (4558.0 mg), B-9 (1100 mg), B-10 (1700 mg) and B-11 (223.1 mg).

3.1.2.2.1 Isolation of Compound ES3 (Erythrinassinate B)

Fraction B-7 (467.9 mg) was separated on a silica gel 60 (No. 9385, 50 g) column chromatography. Gradient elution (hexane/CH_2Cl_2 2:3 to 0:1) was performed (50 mL per fraction) to give six fractions: B-7-1 (149.8 mg), B-7-2 (89.7 mg), B-7-3 (26.3 mg), B-7-4 (11.7 mg), B-7-5 (37.9 mg) and B-7-6 (24.3 mg)

Fraction B-7-1 (149.8 mg) was purified on a silica gel column (50 g) with 60 % CH_2Cl_2 in hexane as eluent to afford 46 mg of compound ES 3 (white amorphous powder, R_f 0.62, silica gel, hexane/CH_2Cl_2 = 1:4). This compound was subsequently identified as erythrinassinate B **[221]**.

3.1.2.2.2 Isolation of Compounds ES4 (Sandwicensin) and ES5 (5,7,4'-Trihydroxy-8-3'-5'-triprenylflavanone)

Fraction B-8 (11.7 mg) was subjected to flash column chromatography (silica gel, 550g) and eluted with mixtures of hexane/$CHCl_3$ (2:3 to 0:1) and $CHCl_3$/MeOH (1:0 to 0:1). The eluates were collected (300 mL each) and then combined according to their TLC patterns (silica gel, 5% MeOH in $CHCl_3$) to give 10 fractions: fractions B-8-1 (53.7 mg), B-8-2 (39.1 mg), B-8-3 (97.9 mg), B-8-4 (54.8 mg), B-8-5 (485.4 mg), B-8-6 (413.8 mg), B-8-7 (4.4 g), B-8-8 (1.5 g), B-8-9 (2.0 g) and B-8-10 (1.9 g).

Further fractionation of fraction B-8-5 (485.4 mg) was performed by column chromatography (silica gel No. 9385, CH_2Cl_2) to give 8 combined fractions. Fraction C-8-5-8 from this column was purified by a Sephadex LH-20 column with MeOH as eluent and PTLC silica gel F_{254} with 5% MeOH in $CHCl_3$ to give 6.2 mg of compound ES4 as brown gum (R_f 0.50, silica gel, 1% MeOH in $CHCl_3$) and 50.3 mg of compound EF5 as yellow powder (R_f 0.43, silica gel, 1% MeOH in $CHCl_3$).

3.1.2.3 Isolation of Compounds from 95% EtOH Extract

The ethanol extract (70 g) was fractionated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (700 g). Elution was performed in a polarity gradient manner with mixtures of hexane/CH_2Cl_2 (1:0 to 0:1), and CH_2Cl_2/methanol (1:0 to 0:1). The eluates were collected (500 mL per fraction) and examined by TLC (silica gel, CH_2Cl_2-MeOH = 19:1). Fractions with similar chromatographic patterns were combined to yield nine fractions: C-1 (0.01 g), C-2 (0.4 g), C-3 (0.1 g), C-4 (4.1 g), C–5 (0.03 g), C-6 (26.4 g), C-7 (3 g), C-8 (14.2 g) and C-9 (20.3 g).

3.1.2.3.1 Isolation of Compound ES1 (Mixture of Stigmasterol and β-Sitosterol)

Fraction C-4 (4.1 g) was separated by column chromatography (silica gel, mixtures of hexane/CH_2Cl_2 4:1 to 0:1) to give twelve fractions: C-4-1 (14.8 mg), C-4-2 (52.3 mg), C-4-3 (10.8 mg), C-4-4 (28.3 mg), C-4-5 (39.1 mg), C-4-6 (45.5 mg), C-4-7 (87.3 mg), C-4-8 (869.9 mg), C-4-9 (1400.0 mg), C-4-10 (133.6 mg), C-4-11 (95.2 mg) and C-4-12 (222.9 mg).

Fraction C-4-7 (87.3 mg) was further fractionated on a silica gel column (eluted with 60 % hexane in CH_2Cl_2). Fraction C-4-7-3, after drying, was re-crystallized in MeOH to give

14.2 mg of compound ES1 (colourless needles, R_f 0.38, silica gel, MeOH- CH_2Cl_2 = 1:99). ES1 was identified as mixtures of stigmasterol **[268]** and β-sitosterol **[269]**.

3.1.2.3.2 Isolation of Compound ES6 (Erythratidinone)

Fraction C-7 (3 g) was subjected to column chromatography using silica gel column (150 g). Elution was performed with CH_2Cl_2/MeOH gradient (1:0 to 0:1, 50 mL per fraction) to give thirteen fractions: C-7-1 (20.8 mg), C-7-2 (239.4 mg), C-7-3 (44.6 mg), C-7-4 (26.7 mg), C-7-5 (80.2 mg), C-7-6 (167.0 mg), C-7-7 (47.3 mg), C-7-8 (234.1 mg), C-7-9 (98.4 mg), C-7-10 (37.7 mg), C-7- 11 (61.0 mg), C-7-12 (57.8 mg) and C-7-13 (72.9 mg).

Fraction C-7-9 (98.4 mg) was further separated using silica gel column (30 g , 1% MeOH in $CHCl_3$) to give nine fractions. Fraction F-9-5 was purified by PTLC silica gel F_{254} with 5% MeOH in $CHCl_3$ to give 19.9 mg of compound ES5 as yellowish oil (R_f 0.48, silica gel, 10 % MeOH in $CHCl_3$) and later identified as erythratidinone **[212]**.

3.2 Extraction and Isolation of Compounds from the Stem Bark of *Erythrina fusca*

3.2.1 Extraction

The pulverized, dried stem bark of *E. fusca* (5.1 kg) was macerated 4 times (3 days each) at room temperature with n-hexane (14 L), EtOAc (14 L) and 95% EtOH (14 L), respectively. The obtained extracts were evaporated to dryness to give a hexane extract (45 g, 0.88% based on dried weight of the stem bark), ethyl acetate extract (300 g, 5.88%) and 95% EtOH extract (172 g, 3.37%).

3.2.2 Isolation

3.2.2.1 Isolation of Compounds from Hexane Extract

The hexane extract (14.6 g) was subjected to flash column chromatography (silica gel No. 7734, 550 g). Elution was performed in a polarity gradient manner with mixtures of hexane/$CHCl_3$ (1:0 to 0:1) and $CHCl_3$/MeOH (1:0 to 0:1). The eluates were collected (500 mL per fraction) and examined by TLC (silica gel, dichloromethane). Fractions with similar chromatographic pattern were combined to yield 15 fractions: D-1 (0.1 g), D-2 (2.1 g), D-3 (0.4 g), D-4 (0.3 g), D-5 (0.02 g), D-6 (0.01 mg), D-7 (1.4 g), D-8 (0.4 g), D-9 (0.02 g), D-10 (5.0 g), D-11 (0.4 g), D-12 (0.07 g), D-13 (0.1 g), D-14 (0.5 g) and D-15 (0.1 g).

3.2.2.1.1 Isolation of Compound EF1 (1-Octacosanol)

Fraction D-7 (1.4 g) was separated by silica gel column chromatography (100g, $CHCl_3$). The fractions (20 mL each) were collected and combined according to their TLC pattern (silica gel, 5% MeOH in $CHCl_3$) to give 271.3 mg of white amorphous powder of compound EF1, identified as 1-octacosanol **[233]**

3.2.2.1.2 Isolation of Compound EF2 (Sandwicensin)

Fraction D-10 (5 g) was subjected to column chromatography using silica gel (500g) as adsorbent. Elution was performed with mixture of MeOH/$CHCl_3$ (1:19) to give five fractions: fractions D-10-1 (0.1 g), D-10-2 (0.07 g), D-10-3 (0.2 g), D-10-4 (2.9 g) and D-10-5 (1.6 g).

Fraction D-10-4 (2.9 g) was re-chromatographed on a silica gel (500g) column. Gradient elution (hexane/$CHCl_3$ 2:3 to 0:1 and $CHCl_3$/MeOH 1:0 to 19:1) was performed to give 3 fractions: D-10-4 -1 (990.9 mg), D-10-4 -2 (313.1 mg) and D-10-4 -3 (408.9 mg).

Purification of fraction D-10-4-1 (990.9 mg) was performed by HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm), eluted with 85% acetonitrile in H_2O + 0.05% TFA at the flow rate of 8 mL/min). Two fractions were obtained: D-10-4-1-1 (40.3 mg) and D-10-4-1-2 (527.9 mg). Fraction D-10-4-1-2 (527.9 mg) was chromatographed on a silica gel column (5% MeOH in $CHCl_3$) to give fractions D-10-4-1-2-1 (8.2 mg), D-10-4-1-2-2 (17.2 mg), D-10-4-1-2-3 (63.5 mg), D-10-4-1-2-4 (320.2 mg) and D-10-4-1-2-5 (18.3 mg). Fraction D-10-4-1-2-4 (320.2 mg) was re-separated by HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm), eluted with 85% acetonitrile in H_2O + 0.05% TFA at the flow rate of 8 mL/min to give fractions G-10-4-1-2-4-1 (208.3 mg) and D-10-4-1-2-4-2 (4.6 mg). Fraction D-10-4-1-2-4-1 was purified by PTLC silica gel F_{254} with 5% MeOH in $CHCl_3$ to afford 159 mg of compound EF 2 as a brown gum (R_f 0.48, silica gel, 5% MeOH in $CHCl_3$, R_t 9.5 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min). This compound was subsequently identified as sandwicensin **[28].**

3.2.2.1.3 Isolation of Compound EF3 (3-Hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan)

Fraction D-10-4-1-2-5 (18.3 mg) was separated by HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm), eluted with 85% acetonitrile in H_2O + 0.05% TFA with flow rate 8 mL/min) to give one major fractions (5.09 mg). This fraction was purified using PTLC Silica gel F_{254} using 5% MeOH in $CHCl_3$ as developing solvent to give 2.7 mg of compound EF 3 as brown gum (R_f 0.25, silica gel, 5% MeOH in $CHCl_3$, R_t 3.3 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA at the flow rate of 1 mL/min). EF3 was identified as a new pterocarpan, 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan **[271]**.

3.2.2.1.4 Isolation of Compound EF4 (Lupinifolin)

Purification of fraction D-10-4-2 (313.1 mg) was performed using HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm) and eluted with 85% acetonitrile in H_2O + 0.05% TFA at the flow rate of 8 mL/min). Seven major fractions were obtained: D-10-4-2-1 (17.5 mg), D-10-4-2-2 (10.8 mg), D-10-4-2-3 (70.6 mg), D-10-4-2-4 (3.11 mg), D-10-4-2-5 (11.1 mg), D-10-4-2-6 (21.4 mg) and D-10-4-2-7 (13.6 mg). Fraction D-10-4-2-6 (21.4 mg) was purified using PTLC silica gel with 5% MeOH in $CHCl_3$ as developing solvent to give 8.5 mg of compound EF 4 yellow needles (R_t 35.6 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min). EF4 was identified as a lupinifolin **[272]**.

Further isolation of fraction D-10-4-3 (408.9 mg) was performed by HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm), eluted with 85% acetonitrile in H_2O + 0.05% TFA at the flow rate of 8 mL/min). Ten major fractions were obtained: fractions D-10-4-3-1 (43.6 mg), D-10-4-3-2 (18.1 mg), D-10-4-3-3 (10.3 mg), D-10-4-3-4 (28.0 mg), D-10-4-3-5 (44.8 mg), D-10-4-3-6 (46.6 mg), D-10-4-3-7 (30.0 mg), D-10-4-3-8 (6.0 mg), D-10-4-3-9 (66.9 mg) and D-10-4-3-10 (3.5 mg).

Fraction D-10-4-3-9 (66.9 mg) was re-chromatographed on silica gel column (25 g, 5% MeOH in $CHCl_3$) to give 3 fractions: D-10-4-3-9-1 (1.93 mg), D-10-4-3-9-2 (34.2 mg) and D-10-4-3-9 -3 (6.7 mg). Fraction D-10-4-3-9-2 (34.2 mg) was re-purified by PTLC silica gel with 5% MeOH in $CHCl_3$ as developing solvent to give 12.5 mg of EF 4 yellow needles, lupinifolin **[272]** (R_t 35.5 min SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min).

3.2.2.2 Isolation of Compounds from Ethyl Acetate Extract

The ethyl acetate extract (150 g) was separated by vacuum liquid column chromatography (silica gel, 1.5 kg). Elution was performed in a polarity gradient manner with mixtures of hexane/CH_2Cl_2 (1:0 to 0:1) and CH_2Cl_2/MeOH (1:0 to 0:1). Five hundred milliliter fraction of eluates were collected. Fractions of similar TLC patterns (silica gel, CH_2Cl_2-MeOH, 99:1) were combined to yield 17 fractions: E-1 (0.05 g), E-2 (0.07 g), E-3 (4.7 g), E-4 (4.5 g), E-5 (2.5 g), E-6 (16.6 g), E-7 (17.5 g), E-8 (39.5 g), E-9 (10.2 g), E-10 (4.6 g), E-11 (2.1 g), E-12 (7.5 g), E-13 (7.4 g), E-14 (2.6 g), E-15 (2.1 g), E-16 (2.3 g) and E-17 (1.3 g).

3.2.2.2.1 Isolation of Compound EF5 (Erythrinassinate B)

Fraction E-4 (4.5 g) was subjected to column chromatography (silica gel, 500 g, mixture of hexane/CH_2Cl_2 1:4 to 0:1) to afford 277.6 mg of compound EF 5 (R_f 0.23, silica gel, hexane-CH_2Cl_2 1:4) as colorless amorphous powder. It was identified as erythrinassinate B **[221]**.

3.2.2.2.2 Isolation of Compound EF4 (lupinifolin), EF6 (cerylic acid or cerinic acid), EF7 (citflavanone), EF8 (senegalensein or lonchocarpol A) and EF9 (erythrisenegalone or nariginin)

Fraction E-9 (10.2 mg) was purified by flash column chromatography (silica gel 500 g). Gradient elution with mixture of hexane/$CHCl_3$ (1:4 to 0:1) and $CHCl_3$/MeOH (1:0 to 0:1) was performed. Nine fractions were obtained: E-9-1 (0.03 g), E-9-2 (0.05 g), E-9-3 (0.07 g), E-9-4 (4.5 g), E-9-5 (2.3 g), E-9-6 (0.06 g), E-9-7 (0.02 g), E-9-8 (0.3 g) and E-9-9 (0.07 mg).

Fraction E-9-4 (4.5 g) was subjected to column chromatography using silica gel column (500 g, 5% MeOH in $CHCl_3$) to give 200 fractions (20 mL each) and combined according to their TLC pattern (silica gel, 5% MeOH in $CHCl_3$) as leading to 6 major fractions: E-9-4-1 (145.9 mg), E-9-4-2 (251.1 mg), E-9-4-3 (159.4 mg), E-9-4 -4 (247.8 mg), E-9-4 -5 (2.2 g) and E-9-4 -6 (154.3 mg).

Fractions E-9-4-5 (2.2 g) was dissolved in MeOH to give the powder and solution. The powder was cleaned on methanol to give compound FE6 as a white amorphous powder. It was subsequently identified as cerylic acid (cerinic acid) **[273]**.

The MeOH soluble of fraction E-9-4-5 (1.5 g) was further purified by HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm) and eluted with 85% acetonitrile in H_2O + 0.05% TFA at flow rate of 8 mL/min. Six major fractions were obtained: fractions E-9-4-5-1 (38.7 mg), E-9-4-5-2 (473.0 mg), E-9-4-5-3 (20.2 mg), E-9-4-5-4 (10.2 mg), E-9-4-5-5 (289.6 mg) and E-9-4-5-6 (17.9 mg).

Fractions E-9-4-5-1, E-9-4-5-2 and E-9-4-5-5 were re-purified on a silica gel (5% MeOH in $CHCl_3$) column and PTLC silica gel F_{254} with 5% MeOH in $CHCl_3$.

Compound EF7 (citflavanone **[274]**, 1 mg) was obtained as yellow powder from fraction E-9-4-5-1 (R_f 0.37, silica gel, 5% MeOH in $CHCl_3$; R_t 10.7 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min).

Compound EF8 (senegalensein (lonchocarpol A)) **[115]** (319.6 mg) was obtained as yellow powder from fraction E-9-4-5-2 (R_f 0.39, silica gel, 5% MeOH in $CHCl_3$; R_t 21.2 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min).

Compound EF4 (lupinifolin **[272]**, 200.1 mg) was obtained as yellow needles from fraction E-9-4-5-5 (R_f 0.45, silica gel, 5% MeOH in $CHCl_3$; R_t 35.7 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min).

Compound EF9 (erythrisenegalone (nariginin) **[112]**, 17.9 mg) was obtained as yellow powder from fraction E-9-4-5-6 (R_f 0.38, silica gel, 5% MeOH in $CHCl_3$; R_t 40.9 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min).

3.2.2.2.3 Isolation of Compound EF10 (liquiritigenin) , EF11 (diadzein) and EF12 (8-prenyldaidzein)

Fraction E-12 (7.5 g) was isolated by flash column chromatography (silica gel 60 No. 7734, 500 g, gradient mixtures of $CHCl_3$/MeOH 1:0 to 0:1). Fraction with similar chromatographic patterns were combined (silica gel, TLC: 10 % MeOH in $CHCl_3$) to give nine fractions: E-12-1 (0.3 g), E-12-2 (0.07 g), E-12-3 (0.04 g), E-12-4 (0.05 g), E-12-5 (1.2 g), E-12-6 (1.7 g), E-12-7 (2.1 g), E-12-8 (0.3 g) and E-12-9 (1.5 g).

Fraction E-12-5 (1.2 g) was subsequently separated by column chromatography (silica gel, 120 g, 5% MeOH in $CHCl_3$) to give nine fractions: E-12-5-1 (30.65 mg), E-12-5-2 (98.35 mg), E-12-5-3 (528.4 mg), E-12-5-4 (37.7 mg), E-12-5-5 (97.1 mg), E-12-5-6 (43.4 mg), E-12-5-7 (38.0 mg) and E-12-5-8 (56.8 mg).

Isolation of fraction E-12-5-5 (97.1 mg) was performed by HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm) and eluted with 55% acetonitrile in H_2O + 0.05% TFA at flow rate of 8 mL/min. Five major fractions were obtained: E-12-5-5-1 (2.1 mg), E-12-5-5-2 (1.5 mg), E-12-5-5-3 (3.5 mg), E-12-5-5-4 (2.5 mg) and E-12-5-5-5 (20.7 mg). Fraction E-12-5-5-1 (2.1 mg) was purified by PTLC silica gel F_{254} (E. Merck, precoated plate, 5% MeOH in $CHCl_3$) to give 1 mg of compound EF10 as yellow powder (R_f 0.28, 10 % MeOH in $CHCl_3$; R_t 3.1 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 40% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min). This compound was identified as liquiritigenin **[275]**.

Fraction E-12-5-5-5 (20.7 mg) was purified on a silica gel (5% MeOH in $CHCl_3$) column and PTLC silica gel F_{254} (5% MeOH in $CHCl_3$) to give 1.4 mg of compound EF11 and 1 mg of compound EF12.

Compound EF11 (daidzein **[85]**, 1.4 mg) was obtained as pale yellow powder (R_f 0.17, 10 % MeOH in $CHCl_3$; R_t 6.2 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 40% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min).

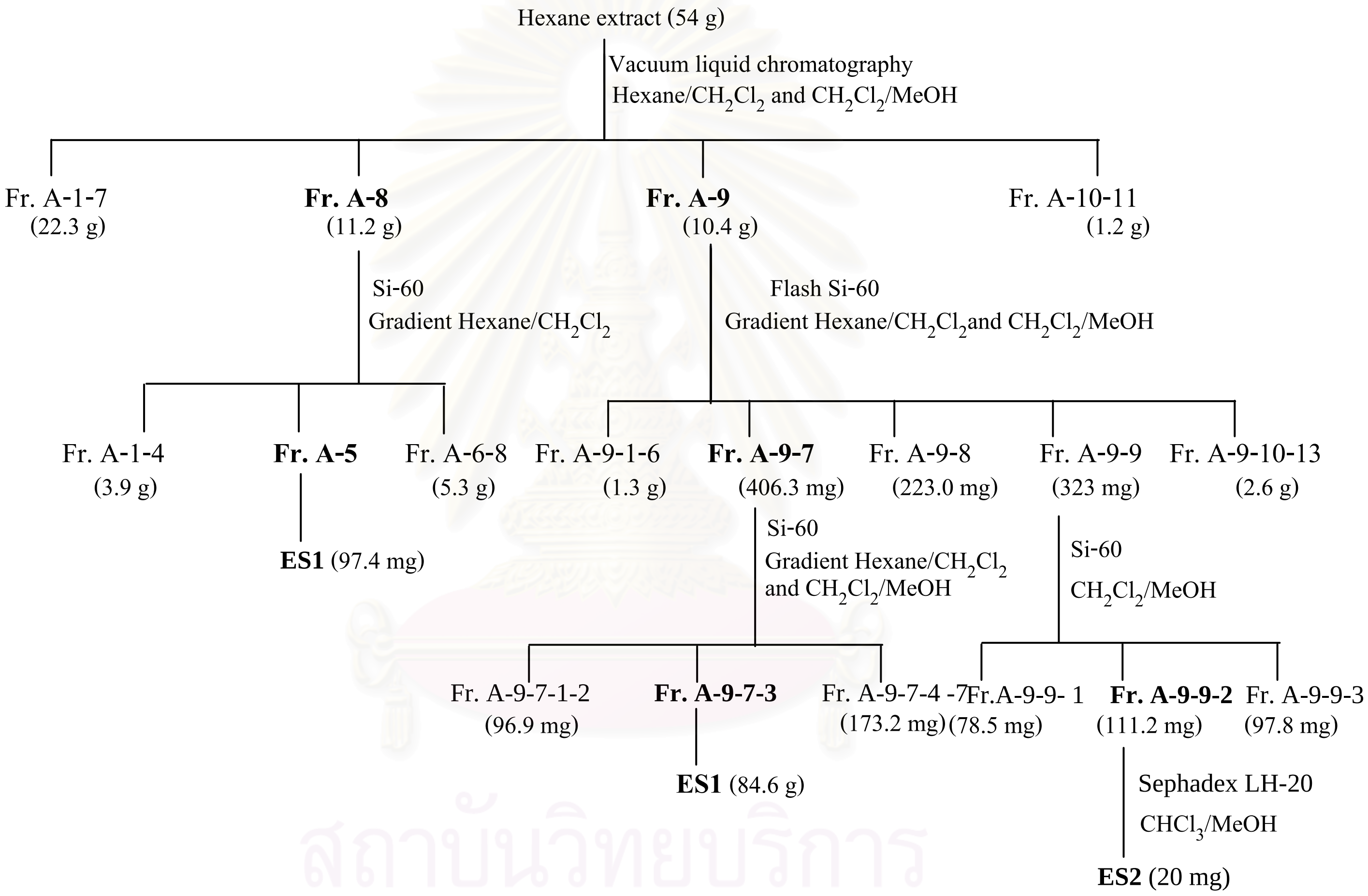
Compound EF12 (8-γ,γ-dimethylallyldaidzein or 8-prenyldaidzein **[23]**, 1 mg) was obtained as yellow powder (R_f 0.28, 10 % MeOH in $CHCl_3$; R_t 10.7 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 40% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min).

3.2.2.3 Isolation of Compounds from 95 % EtOH Extract

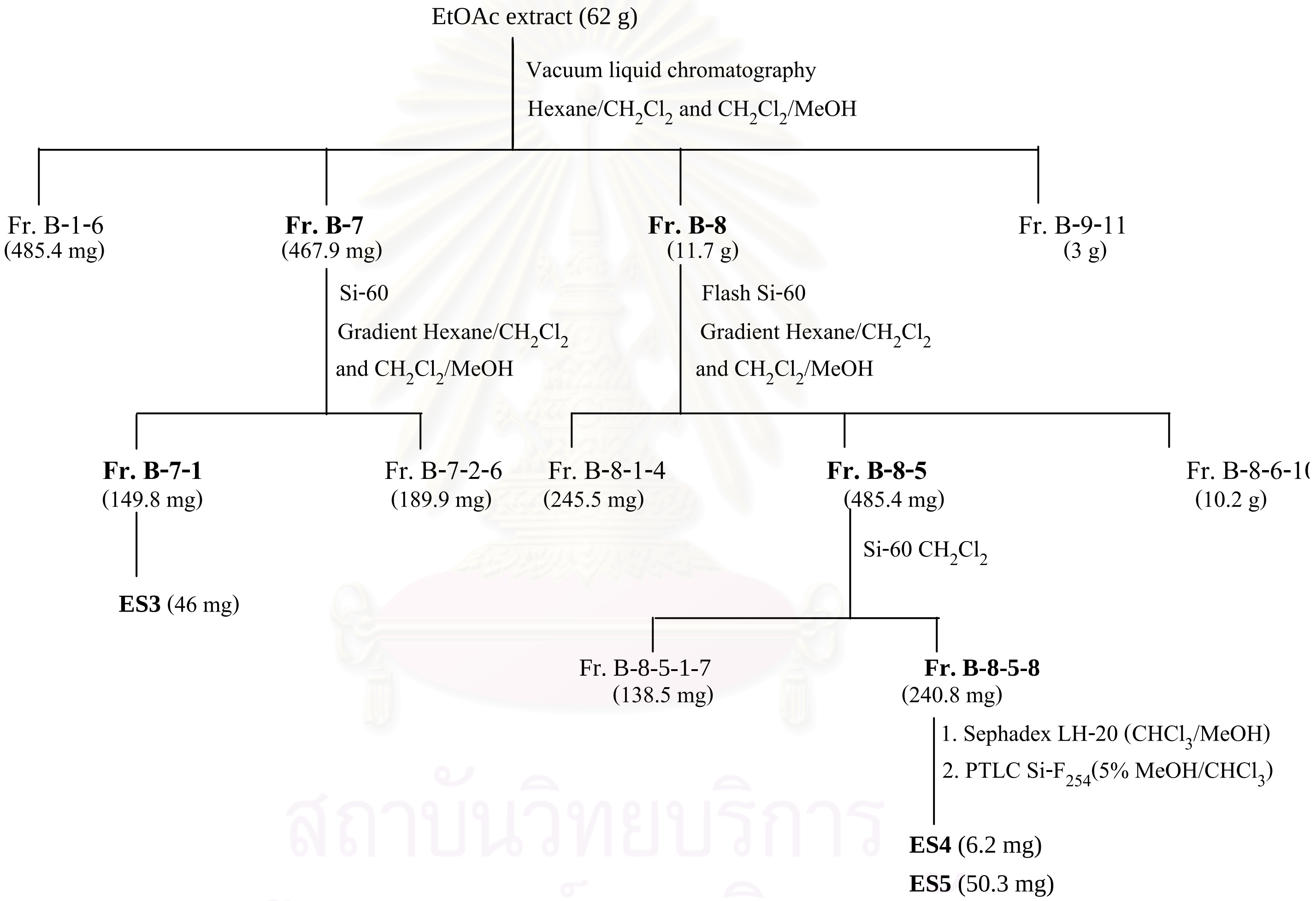
The ethanol extract (15 g) was separated by flash column chromatography (silica gel, 550 g), with polarity gradient elution using mixture of hexane/$CHCl_3$ (1:0 to 0:1) and then $CHCl_3$/MeOH (1:0 to 0:1). Thirteen fractions were collected according to their TLC behavior (silica gel, CH_2Cl_2-MeOH, 99:1): F-1 (0.2 g), F-2 (0.07 g), F-3 (0.03 g), F-4 (0.09 g), F-5 (0.5 g), F-6 (0.3 g), F-7 (0.2 g), F-8 (0.9 g), F-9 (0.7 g), F-10 (1.6 g), F-11 (2.0 g), F-12 (3.9 g) and F-13 (1.6 g).

3.2.2.3.1 Isolation of Compound EF8

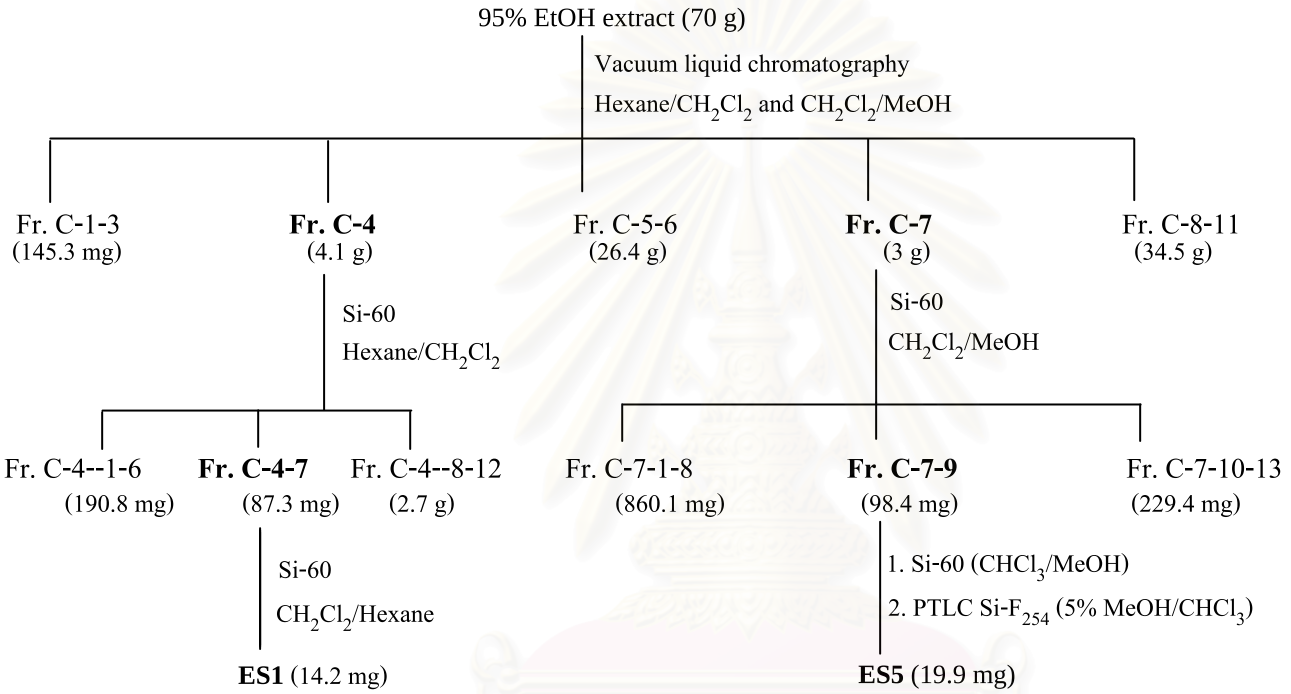
Fraction F-5 (0.5 g) was subjected on silica gel column chromatography (5% MeOH in $CHCl_3$). Fractions showing similar chromatographic patterns were combined (silica gel, TLC, 5% MeOH in $CHCl_3$) to yield five fractions: F-5-1 (71.9 mg), F-5-2 (16.8 mg), F-5-3 (220.1 mg), F-5-4 (9.54 mg) and F-5-5 (25.1 mg). Fraction F-5-3 (220.1 mg) was separated by HPLC (SenShu Pak., PEGASIL ODS, 20 x 250 mm) eluted with 85% acetonitrile in H_2O + 0.05% TFA with flow rate 8 mL/min) to give five fractions. F-5-3-1 was re-purified by PTLC silica gel F_{254} (5% MeOH in $CHCl_3$) to give 2.4 mg of compound EF8 as yellow powder (R_f 0.39, silica gel, 5% MeOH in $CHCl_3$; R_t 21.0 min, SenShu Pak. PEGASIL ODS, 4.6 x 150 mm, 55% acetonitrile in H_2O + 0.05% TFA with flow rate 1 mL/min). This compound was identified as senegalensein (lonchocarpol A) **[115]**.



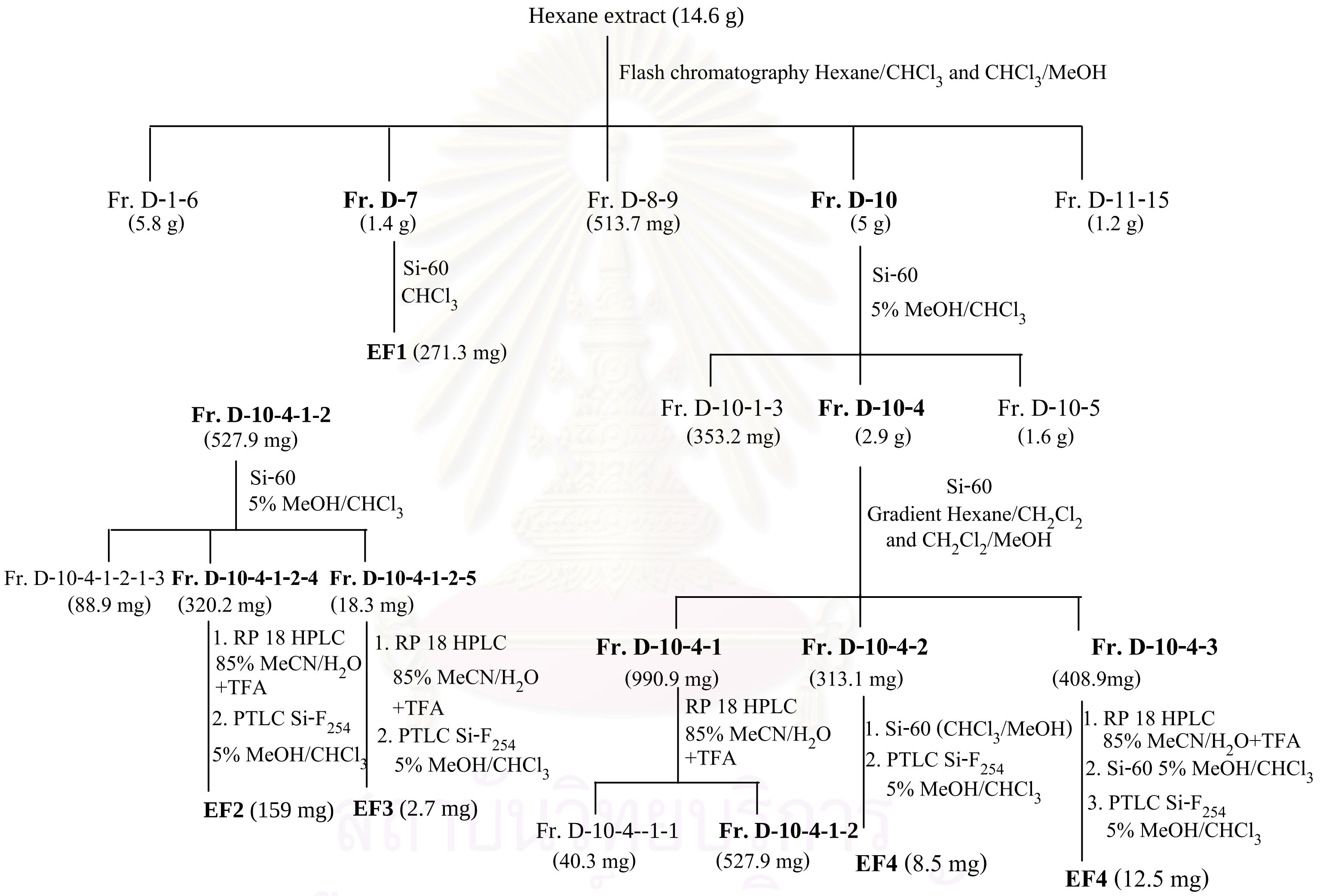
Scheme 6 Separation of Hexane extract from the stem bark of *Erythrina suberosa*



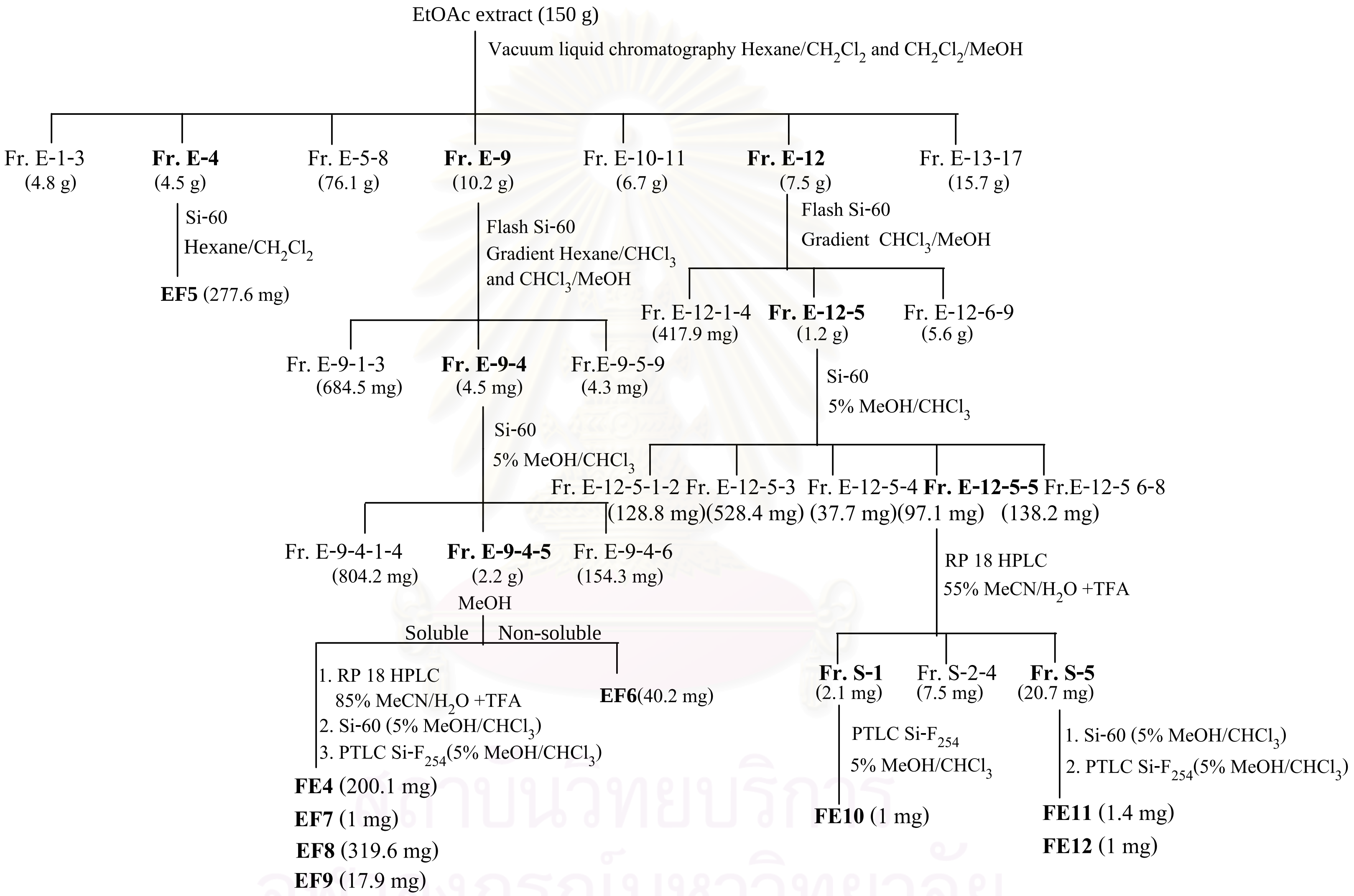
Scheme 7 Separation of the EtOAc extract from the stem bark of *Erythrina suberosa*



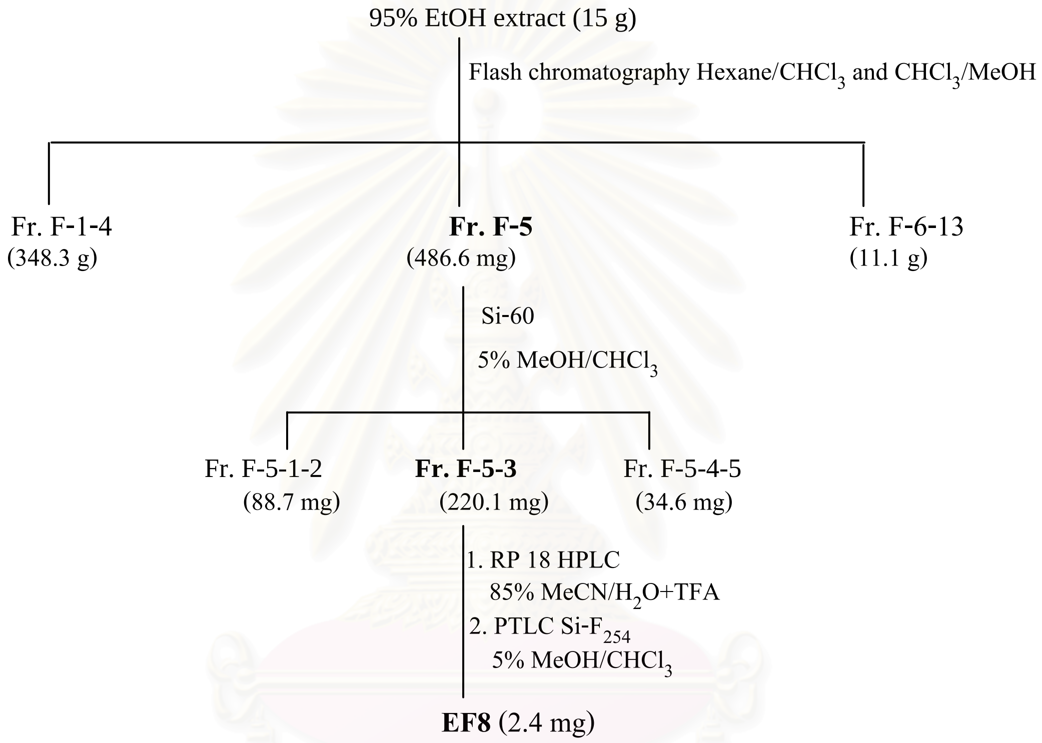
Scheme 8 Separation of the 95 % EtOH extract from the stem bark of *Erythrina suberosa*



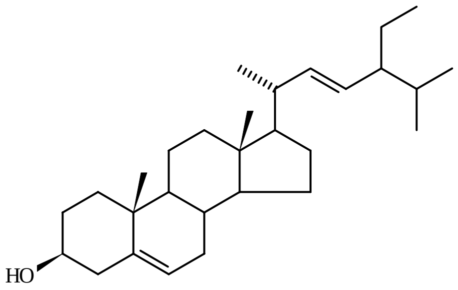
Scheme 9 Separation of the hexane extract from the stem bark of *Erythrina fusca*



Scheme 10 Separation of the EtOAc extract from the stem bark from *Erythrina fusca*

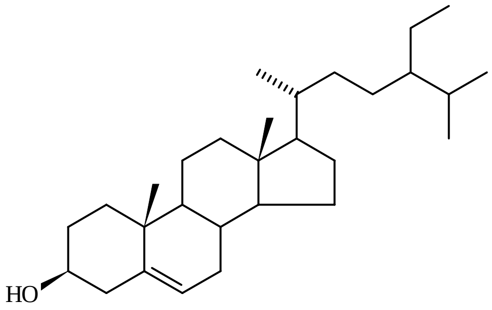


Scheme 11 Separation of the EtOH extract from the stem bark of *Eryhrina fusca*



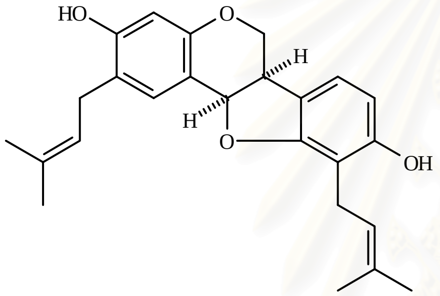
ES1

Stigmasterol **[268]**



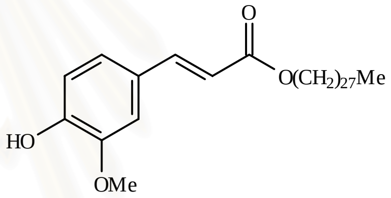
ES1

β-Sitosterol **[269]**



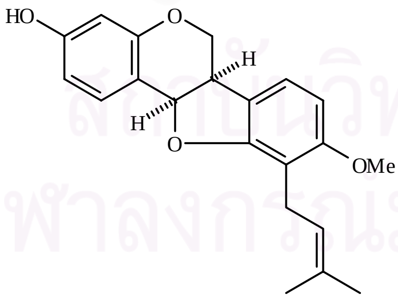
ES2

Erythrabyssin II **[8]**



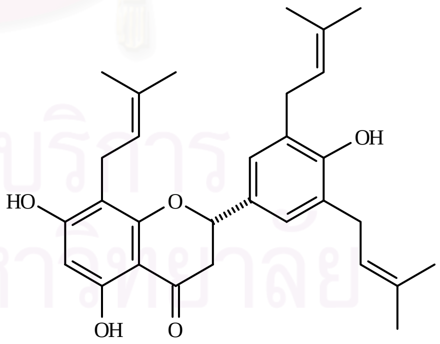
ES3

Erythrinassinate B **[221]**



ES4

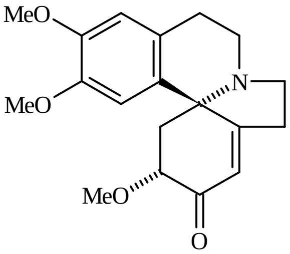
Sandwicensin **[28]**



ES5

5,7,4'-Trihydroxy-8-3'-5'-triprenylflavanone **[270]**

Figure 3 Structures of compounds isolated from the stem bark of *Erythrina suberosa*



ES6

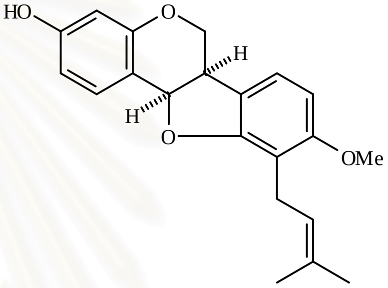
Erythratidinone **[212]**

Figure 3 (Continued)

$CH_3(CH_2)_{27}OH$

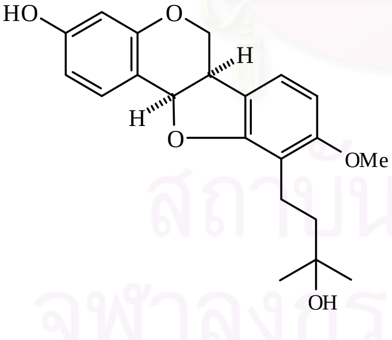
EF1

1-Octacosanol **[233]**



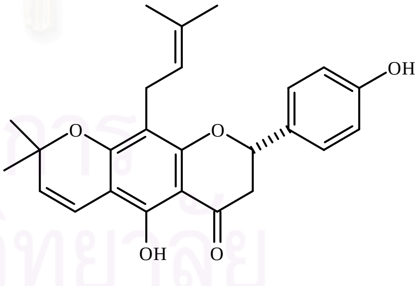
EF2

Sandwicensin **[28]**



EF3

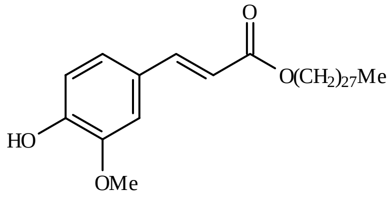
3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan **[271]**



EF4

Lupinifolin **[272]**

Figure 4 Structures of compounds isolated from the stem bark of *Erythrina fusca*



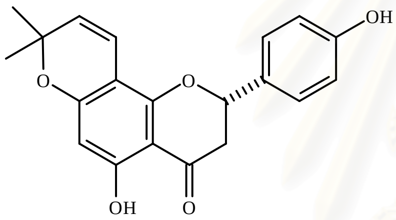
$CH_3(CH_2)_{24}COOH$

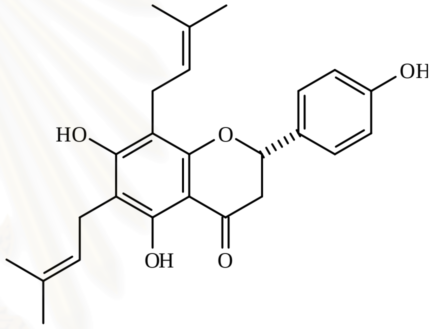
EF5

EF6

Erythrinassinate B **[221]**

Cerinic acid **[273]**



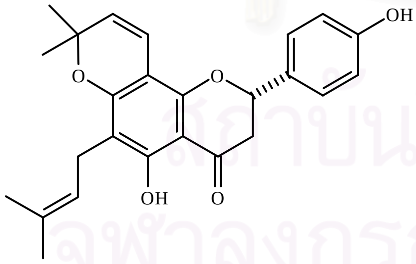


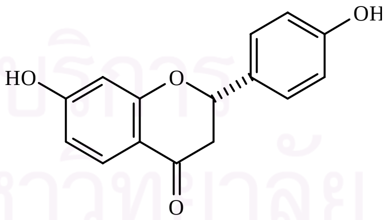
EF7

EF8

Citflavanone **[274]**

Lonchocarpol A (Senegalensein) **[115]**





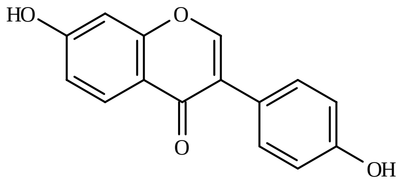
EF9

EF10

Erythrisenegalone (nariginin) **[112]**

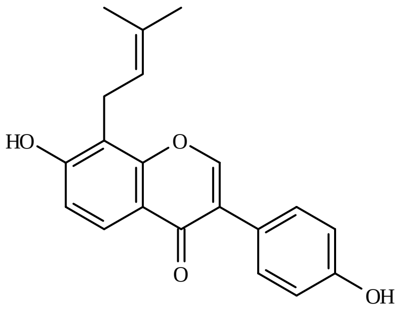
Liquitigenin **[275]**

Figure 4 (continued)



EF11

Daidzein **[85]**



EF12

8-prenyldaidzein **[23]**

Figure 4 (continued)

4. Physical and spectra data of isolated compounds

4.1 Compound ES1 (mixtures of β-sitosterol and stigmasterol)

Compound ES1 was obtained as colorless needles, soluble in chloroform (196.2 mg, 3.44 x 10^{-3}% based on dried weight of stem bark).

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 6, Table 4

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 7, Table 4

4.2 Compound ES2 (Erythrabyssin II)

Compound ES2 was obtained as colorless needles, soluble in chloroform (20 mg, 3.51 x 10^{-4} % based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 392.1978 (calcd for $C_{25}H_{28}O_4$ 392.1980)

EIMS : *m/z* (% relative intensity); Figure 8

392 (M^+, 100), 375 (36), 336 (46), 281 (36), 161 (10), 32 (23), 28 (78)

$[\alpha]^{28}_{D}$: -218.1 (*c* 0.21, MeOH)

Melting point : 149-151 °C

UV : λ_{max} nm (log ε), in methanol; Figure 9

218 (4.36), 287 (3.76)

IR : ν_{max} cm^{-1}, KBr disc; Figure 10

3404, 1621, 1526, 1449, 1374, 1253, 1191, 1162, 1093, 1031, 912, 839

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 11, Table 5

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 12, Table 5

4.3 Compound ES3 (Erythrinassinate B)

Compound ES3 and EF5 were obtained as white amorphous powder, soluble in chloroform (323.6 mg, 6.25 x 10^{-3}% based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 586.4961 (calcd for $C_{38}H_{66}O_4$ 586.4961)

EIMS : *m/z* (% relative intensity); Figure 15

586 (M^+, 74), 558 (100), 196 (34), 194 (81), 177 (58), 137 (44), 28 (90)

Melting point : 64-65 °C

UV : λ_{max} nm (log ε), in methanol; Figure 16

328 (3.03)

IR : ν_{max} cm^{-1}, KBr disc; Figure 17

3501, 2918, 2849, 1724, 1639, 1603, 1519, 1471, 1281,1175, 1027,718

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 18, Table 6

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 19, Table 6

4.4 Compound ES4 and EF2 (Sandwicensin)

Compound ES4 and EF2 were obtained as brown gum, soluble in chloroform (165.2 mg, 3.23 x 10^{-3} % based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 338.1509 (calcd for $C_{21}H_{22}O_4$ 338.1512)

EIMS : *m/z* (% relative intensity); Figure 22

338 (M^+, 100), 323 (18), 295(25), 283(45), 282 (92), 147(20), 73(11), 55(18)

$[\alpha]^{28}_{D}$: -173.4 (*c* 0.20, MeOH)

UV : λ_{max} nm (log ε), in methanol; Figure 23

218 (4.23), 286 (3.11)

IR : ν_{max} cm^{-1}, film; Figure 24

3423, 2967, 2932, 1621, 1509, 1486, 1351, 1265, 1164, 1082, 794

^{1}H-NMR : δ ppm, 500 MHz, in $CDCl_3$; Figure 25, Table 7

^{13}C-NMR : δ ppm, 125 MHz, in $CDCl_3$; Figure 26, Table 7

4.5 Compound ES5 (5,7,4'-Trihydroxy-8-3'-5'-triprenylflavanone)

Compound ES5 was obtained as yellow powder, soluble in chloroform (50.2 mg, 9.84 x 10^{-4} % based on dried weight of stem bark).

EIMS : *m/z* (% relative intensity); Figure 30

476 (M^+, 55), 459 (100), 421 (31), 282 (9), 243 (23), 221 (9), 220 (11), 219 (20), 205 (16), 203 (18), 191 (15), 177 (14), 165 (8), 149 (22), 91 (36), 77 (43)

$[\alpha]^{28}_{D}$: -8.7 (*c* 0.10, MeOH)

Melting point : 115-117 °C

UV : λ_{max} nm (log ε), in methanol; Figure 31

219 (4.54), 292 (4.25)

IR : ν_{max} cm^{-1}, film; Figure 32

3435, 2974, 1639, 1441, 1383, 1269, 1172, 1079

^{1}H-NMR : δ ppm, 500 MHz, in $CDCl_3$; Figure 33, Table 8

^{13}C-NMR : δ ppm, 125 MHz, in $CDCl_3$; Figure 34, Table 8

4.6 Compound ES6 (Erythratidinone)

Compound ES6 was obtained as yellowish oil, soluble in chloroform (19.9 mg, 3.49 x 10^{-4} % based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 329.1604 (calcd for $C_{19}H_{23}O_4N$ 329.1627)

EIMS : *m/z* (% relative intensity); Figure 38

329 (M^+, 4), 301 (10), 271 (100), 256 (30), 228 (38), 197 (39), 97 (12), 55 (15)

$[\alpha]_{D}^{28}$: +132.8 (*c* 0.12, MeOH)

UV : λ_{max} nm (log ε), in methanol; Figure 39

229 (4.09), 281 (3.43), 423 (2.71)

IR : ν_{max} cm^{-1}, film; Figure 40

3435, 2931, 1678, 1509, 1463, 1253, 1205, 1102, 1021, 891

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 41, Table 9

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 42, Table 9

4.7 **Compound EF1** (1-Octacosanol)

Compound EF1 was obtained as white amorphous powder, soluble in chloroform (271.3 mg, 5.31 x 10^{-3} % based on dried weight of stem bark).

GC/MS : *m/z* (% relative intensity); Figure 46

410(M^+, 8), 364(2), 281(3), 207(5), 139(12), 125(28), 111(54), 97(91), 83(96), 69 (81), 57 (100), 55(86), 43(87)

Melting point : 89-91 °C

IR : ν_{max} cm^{-1}, KBr disc; Figure 47

3307, 2917, 2848, 1473, 1463, 1123, 1062, 730, 720

^{1}H-NMR : δ ppm, 500 MHz, in $CDCl_3$; Figure 48, Table 10

^{13}C-NMR : δ ppm, 125 MHz, in $CDCl_3$; Figure 49, Table 10

4.8 **Compound EF2** (Sandwicensin)

Compound EF2 was obtained as same as ES4 (see **4.4 compound ES4**).

4.9 **Compound EF3** (3-Hydroxy-10-(3-hydroxy-3-methylbutyl)- 9-methoxypterocarpan)

Compound EF3 was obtained as brown gum, soluble in chloroform (2.7 mg, 5.29 x 10^{-5} % based on dried weight of stem bark).

ESITOFMS : $[M+Na]^+$ *m/z* 379.1523 (calcd for $C_{21}H_{24}O_5$+Na 379.1514)

EIMS : *m/z* (% relative intensity); Figure 51

356(M^+, 43), 338 (100), 282 (77), 267 (22), 253 (20)

$[\alpha]_D^{28}$: -317.8 (*c* 0.10, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 52

219 (4.41), 285 (3.91)

IR : ν_{max} cm^{-1}, film; Figure 53

3400, 2969, 2933, 1621, 1487, 1351, 1265, 1161, 1120, 1080, 757

^{1}H-NMR : δ ppm, 500 MHz, in $CDCl_3$; Figure 54, Table 11

^{13}C-NMR : δ ppm, 125 MHz, in $CDCl_3$; Figure 55, Table 11

4.10 Compound EF4 (Lupinifolin)

Compound EF4 was obtained as yellow needles, soluble in chloroform (221.1 mg, 4.34 x 10^{-3}% based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 406.1773 (calcd for $C_{25}H_{26}O_5$ 406.1780)

EIMS : *m/z* (% relative intensity); Figure 59

406 (M^+, 42), 391 (75), 313(3), 286 (4), 271 (27), 258 (5), 243 (15), 216 (6), 147(3), 120 (3), 119 (3), 29 (100)

Melting point : 105-106 °C

$[\alpha]^{28}_{D}$: -4.3 (*c* 0.22, MeOH)

UV : λ_{max} nm (log ε), in methanol; Figure 60

274 (4.29), 311 (3.75), 367 (3.21)

IR : ν_{max} cm^{-1}, film; Figure 61

3251, 2973, 2912, 1644,1618, 1519, 1449,1380, 1240, 1196, 1124, 835, 733

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 62, Table 12

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 63, Table 12

4.11 Compound EF5 (Erythrinassinate B)

Compound EF5 was obtained as same as ES3 (see **4.3 compound ES3**).

4.12 Compound EF6 (Cerinic acid)

Compound EF6 was obtained as white amorphous powder, soluble in chloroform (40.2 mg, 7.88 x 10^{-4}% based on dried weight of stem bark).

EIMS : *m/z* (% relative intensity); Figure 68

424 (M^+, 17), 396 (100), 353 (34), 297 (28), 185 (38), 129 (94), 97 (62), 85 (68)

Melting point : 96-97 °C

IR : ν_{max} cm^{-1}, KBr disc; Figure 69

2918, 2849, 1707, 1473, 1463, 1298, 934, 729, 720

^{1}H-NMR : δ ppm, 500 MHz, in $CDCl_3$; Figure 70, Table 13

^{13}C-NMR : δ ppm, 125 MHz, in $CDCl_3$; Figure 71, Table 13

4.13 Compound EF7 (Citflavanone)

Compound EF7 was obtained as yellow powder, soluble in chloroform (1 mg, 1.96 x 10^{-5} % based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 338.1154 (calcd for $C_{20}H_{18}O_5$ 338.1379)

EIMS : *m/z* (% relative intensity); Figure 73

338 (M^+, 37), 323 (85), 218 (4), 203 (100), 161 (6), 120 (3) 83 (16), 73 (10)

$[\alpha]_D^{28}$: -5.3 (*c* 0.05, MeOH)

Melting point : 141-142 °C

UV : λ_{max} nm (log ε), in methanol; Figure 74

271 (4.19), 362 (2.98)

IR : ν_{max} cm^{-1}, KBr disc; Figure 75

3443, 1614, 1261, 1154, 1094, 802

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 76, Table 14

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 77, Table 14

4.14 Compound EF8 (Senegalensein)

Compound EF8 was obtained as yellow powder, soluble in chloroform (322 mg, 6.31 x 10^{-3}% based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 408.1928 (calcd for $C_{25}H_{28}O_5$ 408.1937)

EIMS : *m/z* (% relative intensity); Figure 81

408 (M^+, 100), 393 (20), 353 (40), 337 (50), 297 (29), 217 (50), 189 (69)

$[\alpha]^{28}_{D}$: -11.3 (*c* 0.22, MeOH)

Melting point : 79-80 °C

UV : λ_{max} nm (log ε), in methanol; Figure 82

296 (4.21), 349 (3.53)

IR : ν_{max} cm^{-1}, KBr disc; Figure 83

3332, 2912, 1603, 1519, 1448, 1378, 1223, 1180, 1075, 832, 735

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 84, Table 15

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 85, Table 15

4.15 Compound EF9 (Erythrisenegalone)

Compound EF9 was obtained as yellow powder, soluble in chloroform (17.9 mg, 3.51 x 10^{-4}% based on dried weight of stem bark).

HRFABMS : $[M]^+$ *m/z* 406.1776 (calcd for $C_{25}H_{26}O_5$ 406.1780)

EIMS : *m/z* (% relative intensity); Figure 91

406 (M^+, 42), 391 (70), 363 (6), 271 (24), 243 (11), 215 (43), 57 (26), 28 (100)

$[\alpha]^{28}_{D}$: -9.3 (*c* 0.12, MeOH)

Melting point : 118-119 °C

UV : λ_{max} nm (log ε), in methanol; Figure 92

218 (4.26), 272 (4.45), 363 (3.46)

IR : ν_{max} cm^{-1}, KBr disc; Figure 93

3412, 2973, 2918, 1626, 1596, 1519, 1448, 1378, 1195, 1167, 1117, 836, 573

^{1}H-NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 94, Table 16

^{13}C-NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 95, Table 16

4.16 Compound EF10 (Liquitigenin)

Compound EF10 was obtained as yellow powder, soluble in chloroform (1 mg, 1.96 x 10^{-5} % based on dried weight of stem bark).

HREIMS : $[M]^+$ *m/z* 256.0736 (calcd for $C_{15}H_{12}O_4$ 256.0732)

EIMS : *m/z* (% relative intensity); Figure 98

256 (M^+, 100), 239 (75), 335 (6), 271 (24), 215 (43), 83 (8), 57 (10)

$[\alpha]^{28}_{D}$: -6.7 (c 0.03, MeOH)

Melting point : 134-135 °C

UV : λ_{max} nm (log ε), in methanol; Figure 99

274 (3.61), 311 (3.31)

IR : ν_{max} cm^{-1}, KBr disc; Figure 100

3393, 2929, 1660, 1601, 1516, 1462, 1330, 1240, 1158, 1120, 713

^{1}H-NMR : δ ppm, 400 MHz, in acetone-d_6; Figure 101, Table 17

^{13}C-NMR : δ ppm, 100 MHz, in acetone-d_6; Figure 102, Table 17

4.17 Compound EF11 (Daidzein)

Compound EF11 was obtained as pale yellow powder, soluble in mixture of chloroform : methanol = 1:1 (1.4 mg, 2.74 x 10^{-5} % based on dried weight of stem bark).

EIMS : *m/z* (% relative intensity); Figure 105

254 (M$^+$, 29), 238 (100), 210 (70), 181 (8), 152 (10), 136 (45), 108 (35), 76 (10)

$[\alpha]^{28}_{D}$: -14.9 (c 0.02, MeOH)

Melting point : 165-167 °C

UV : λ_{max} nm (log ε), in methanol; Figure 106

247 (4.11)

IR : ν_{max} cm^{-1}, KBr disc; Figure 107

3224, 1631, 1596, 1518, 1460, 1388, 1307, 1279, 1240, 1192, 1097, 843, 547

^{1}H-NMR : δ ppm, 500 MHz, in CD$_3$OD:MeOH-d_4; Figure 108, Table 18

^{13}C-NMR : δ ppm, 125 MHz, in CD_3OD:MeOH-d_4; Figure 109, Table 18

4.18 Compound EF12 (8-prenyldaidzein)

Compound EF12 was obtained as yellow powder, soluble in acetone (1 mg, 1.96×10^{-5} % based on dried weight of stem bark).

HREIMS : $[M]^+$ *m/z* 322.1205 (calcd for $C_{20}H_{18}O_4$ 322.1200)

EIMS : *m/z* (% relative intensity); Figure 112

322(M^+, 83), 307 (24), 267(91), 149 (64), 97 (38), 71 (48), 55 (52), 29 (100)

$[\alpha]^{28}_{D}$: -25 (*c* 0.03, MeOH)

Melting point : 97-98 °C

UV : λ_{max} nm (log ε), in methanol; Figure 113

251 (3.46)

IR : ν_{max} cm^{-1}, KBr disc; Figure 114

3384, 2927, 1685, 1623, 1515, 1437, 1385, 1272, 1209, 1140, 1030, 840

^{1}H-NMR : δ ppm, 400 MHz, in acetone-d_6; Figure 115, Table 19

^{13}C-NMR : δ ppm, 100 MHz, in acetone-d_6; Figure 116, Table 19

5. Determination of Antimicrobial Activity

Antimicrobial activity of the crude extracts and pure compounds were tested by microdilution method (Murray *et al*., 1999) against *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. The microorganisms were streaked on agar petri dish for isolation. The plates were incubated in a humidified incubator at 37 °C for 24 hours, during which colonies were formed. With a sterile loop, the colonies were picked, dispersed in broth and incubated in humidified incubator at 37 ^{O}C for 24 hours. After incubation, the concentration of the microorganisms was adjusted by standardization to match a turbidity of 0.5 McFarland standard (80% transmittance at 625 nm), which provided approximately 1 x 10^{8} CFU/ml (colony forming unit/ml). The cell concentration was adjusted to 10^{6} cells (bacteria) and 10^{3} (yeast) per 1 ml for use as the inoculum. Solution of the tested compound was added to the wells of 96-well microplate and serially diluted two-fold. The final concentrations of tested compound ranged from 0.39 to 200 μg/ml. After inoculation (50 μl/well for bacteria, 100 μl/well for yeast, and none for control wells), the 96-well microplate was incubated at 37 ^{O}C for 18 hours. Then, 20 μl of 0.5 mg/ml *p*-iodonitrotetrazolium violet (INT) solution was added into each well. The microdilution plates were further incubated for 1 hour for bacteria and overnight for *C. albicans*. The microbial growth was confirmed if the violet color was developed. The lowest concentration of isolated compounds that inhibited visible growth of tested microorganisms was regarded as the minimal inhibitory concentration (MIC, μg/ml). All inhibitory concentrations were re-checked by addition of each solution showing activity into agar plate, and incubated at 37 ^{O}C for 24 hours. The lowest concentration of the test compounds which kill these microorganisms were defined as minimum bactericidal concentration (MBC, μg/ml). Tetracycline and nystatin were used as positive control for antibacterial and antifungal activities, respectively.

6. Determination of Antimalarial Activity (Otoguro, *et al*., 2001)

6.1 Assay for *In vitro* Antimalarial Activity

The antimalarial activity of the crude extracts and pure compounds were evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), which was cultured continuously according to the method of Trager and Jansen (1976). *P. falciparum* stain were cultured in the human erythrocytes in RPMI medium (RPMI-1640 with 25 mM HEPES buffer, 24 mM $NaHCO_3$, 0.2 % glucose, 0.05 % L-glutamine, 50 μg/ml hypoxanthine, and 25 μg/ml gentamicin) supplemented with 10% human plasma at 37 ^{O}C, under 93% N_2, 4% CO_2, and 3% O_2. Antimalarial activity of the tested compound have been achieved by dose response curve using the parasite lactate dehydrogenase (pLDH) assay according to the method of Makler *et al*., (1993). One hundred ninety microliters of asynchronous parasites (2.0% hematocrit and 0.5 or 1% parasitaemia) was seeded in a 96-well microplate and 100 μl the solution of the tested compounds (dissolved in 25% ethanol or 5% DMSO) was added. After incubation at 37 ^{O}C for 72 hours under 93% N_2, 4% CO_2 and 3% O_2, the plate was immediately frozen at –20 ^{O}C for 18 hours. The plate was then thawed at 37 ^{O}C, and 20 μl of haemolyzed parasite suspension was transferred to another plate containing 100 μl of Malstat reagent. The plate was futher incubated for 15 minutes at room temperature, and 20 μl of a 1:1 mixture of nitroblue tetrazolium and phenazine ethosulfate (2 mg and 0.1 mg/ml respectively) was added to each well. After incubation for 2 hours in the dark at room temperature, the blue formazan product was measured at 655 nm by an iEMS microplate reader. The 50% inhibitory concentration (IC_{50}) was estimated from a dose response curve.

6.2 Assay for *In vivo* Antimalarial Activity

In vivo antimalarial activity was determined against rodent malaria-derived *P. berghei* strain N according to the 4-day suppressive test of Peter *et al.* (1975). Male CD-1 (ICR) mice (Charles River Japan Inc., Japan) weighting 18-20g were inoculated with 10^6 parasitized red blood cells intravenously. Test compounds were dissolved in 10% DMSO-water and subcutaneously injected to the mice two hours after the infection (day zero). Tested compounds were successively injected to the mice once a day for 3 consecutive days (Day 1 to 3). Five mice were tested at each dosage, and another 5 infected mice were injected with 10% DMSO-water as a control. The day after the last treatment (Day 4), thin blood films were made from the tail blood of the infected mice, and the parasitaemia was determined. The 50% effective dose (ED_{50}) was estimated from a dose response curve. The values were tested for statistical significance by Dunnett protocol.

6.3 Cytotoxicity Tests

Cytotoxicity of the tested compound was measured by the colorimetric MTT assay (Mossman, 1983; Otoguro, 1991) in 96-well microplates. In brief, 100 μl of MRC-5 cell suspension was added to 96-well microplates at 1 x 10^3 cells/well, and cultivated for 24 hours. Then, 90 μl of standard culture medium (MEM+10% FCS), with or without 10 μl of tested compound solutions, which were dissolved in 25% ethanol or 5% DMSO were added to each well. The plate was then incubated at 37^{O}C for 4 hours under 5% CO_2-95% air. Then, the incubation medium was aspirated, and 100 μl of DMSO was added to solubilise the MTT formazan product. After mixing, absorbance at 540 nm was measured with an iEMS microplate reader. The 50% inhibitory concentration (IC_{50}) was estimated from a dose response curve.

7. Determination of Free Radical Scavenging Activity

The reduction of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) as described by Talla *et al*. (2003); Yen and Hsieh (1997) was performed. Quercetin was used as reference compound.

7.1 TLC autographic assay

Thin layer chromatograms of the tested compounds were developed. After drying, the plates were sprayed with 0.2% DPPH solution in MeOH, then examined. Active compounds appear as yellow spots against purple background.

7.2 Spectrophotometric assay

The tested compounds were mixed with 0.022% DPPH solution in MeOH and left at room temperature for 30 minutes. Absorbance at 510 nm was then determined and percentage of activity was calculated.

8. Determination of Antimycobacterial Activity

In vitro antimycobacterial activity was performed by a microplate alamar blue assay (Collins and Franzblau, 1997). *Mycobacterium tuberculosis* H37Ra was used as the tested microorganism. The minimum inhibitory concentrations (MICs) of the tested compounds were measured in μg/ml.

9. Determination of Cytotoxicity Activity Against Tumor Cell Lines

In vitro cytotoxicity test (Skehan *et al*., 1990) was assessed using the sulforhodamine B (SRB)-assay using human tumor cell lines of KB (oral human epidermoid carcinoma) and BC (breast cancer). The cell lines were incubated at 37 °C for 72 h, at which time the SRB was added. The results were expressed as IC_{50} of the tested compounds.

CHAPTER IV

RESULT AND DISCUSSION

The pulverized stem bark of *Erythrina suberosa* Roxb. (5.7 kg) was extracted with hexane, ethyl acetate and 95% ethanol, successively, to give hexane extract (59.21 g), ethyl acetate extract (394.79 g) and ethanol (180.23 g) extract. Then extracts were further separated by several chromatographic techniques to afford six compounds (ES1-ES6).

The dried stem bark of *Erythrina fusca* Lour. (5.1 kg) was extracted in the same manner as above, to give hexane extract (45.36 g), ethyl acetate extract (300.38 g) and the ethanol (172.84 g) extract. Then extracts were further purified using several chromatographic techniques to yield thirteen compounds (EF1-EF13).

The structure of all isolates were determined by interpretation of their UV, IR, NMR and MS data, and further confirmed by comparison with literature values.

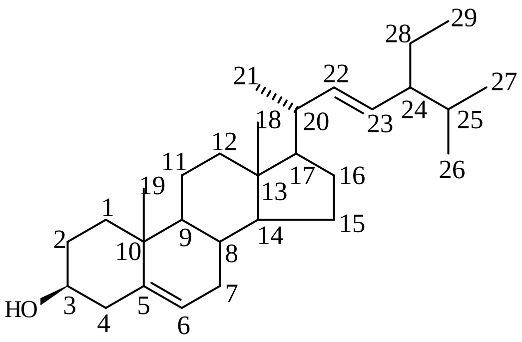
1. Structure Determination of Isolated Compounds

1.1 Structure Determination of Compound ES1

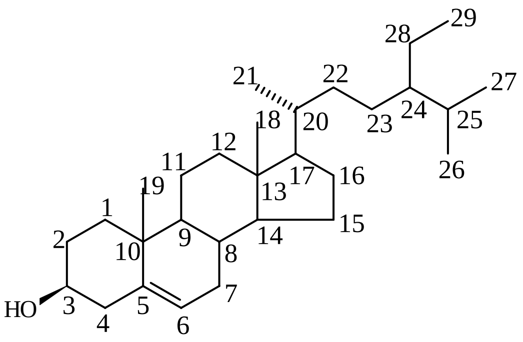
Compound ES1 was obtained as colorless needles. This compound gave purple coloration upon spraying with anisaldehyde reagent. The NMR data of ES1 were in full agreement with the published valued mixture of β-sitosterol and stigmasterol.

In the ^{1}H-NMR spectrum (Table 4 and Figure 6), the signal at δ 5.01 (1H, *dd*, *J* = 8.4, 15.2 Hz), 5.15 (1H, *dd*, *J* = 8.4, 15.2 Hz) and 5.35 (2H, *d*, *J* = 5.2 Hz) could be assigned to H-22 and H-23 of stigmasterol and H-6 of β-sitosterol and stigmasterol, respectively. The integration value for H-6 was twice those of H-22 or H-23. Therefore, it could be deduced that ES1 was a 1:1 mixture of β-sitosterol and stigmasterol.

The ^{13}C-NMR spectrum (Figure 7) of ES1 displayed 43 signals. Comparison of its carbon NMR data with reported values of β-sitosterol and stigmasterol mixture (Wright *et al*., 1978) is shown in Table 4.



stigmasterol **[268]**



β-sitosterol **[269]**

Table 4 ^{13}C NMR Spectral data of compound ES1 as compared with mixture of β-Sitosterol and Stigmasterol (in CDCl$_3$)

Position	Chemical shift (ppm)		Compound ES1
	β-Sitosterol	Stigmasterol	
1	37.3	37.3	37.3
2	31.6	31.7	31.7
3	71.7	71.8	71.8
4	42.5	42.4	42.2
5	140.8	140.8	140.8
6	121.6	121.7	121.7
7	31.9	31.9	31.9
8	31.9	31.9	31.9
9	50.2	50.2	50.2
10	36.5	36. 6	36.5
11	21.1	21.1	21.1
12	39.8	39.7	39.7, 39.8
13	42.3	42.4	42.3
14	56.7	56.9	56.8, 56.9
15	24.3	24.4	24.3, 24.4
16	28.3	28.9	28.2, 28.9
17	56.1	56.1	55.9, 56.1
18	11.9	12.1	11.8, 12.0

Table 4 (Continued)

Position	Chemical shift (ppm)		Compound ES1
	β-Sitosterol	Stigmasterol	
19	19.4	19.4	19.4
20	36.2	40.5	36.1, 40.5
21	18.8	21.1	18.9, 21.1
22	33.9	138.3	33.9, 138.3
23	26.1	129.3	26.1, 129.3
24	45.9	51.3	45.8, 51.2
25	29.2	31.9	29.2, 31.9
26	19.8	21.3	19.8, 21.2
27	19.0	19.0	19.0
28	23.1	25.4	23.1, 25.4
29	12.3	12.3	12.2

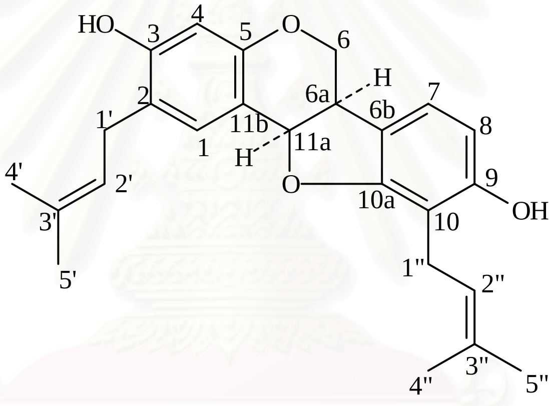
1.2 Structure Determination of Compound ES2

Compound ES2 was obtained as colorless needles. A molecular formula of $C_{25}H_{28}O_4$ was deduced from its M^+ ion at *m/z* 392.1978 (calcd for $C_{25}H_{28}O_4$ 392.1980) in the HRFABMS. The UV absorptions (Figure 9) at 218 and 287 nm were characteristics of a pterocarpan (Kamat *et al*., 1981). The IR spectrum (Figure 10) showed the presence of hydroxyl (3404 cm^{-1}), olefinic (1621 cm^{-1}), aromatic ring (1526, 1449 cm^{-1}) and ether (1253, 1162 cm^{-1}) groups.

The ^{1}H NMR spectrum (Table 5 and Figure 11) of ES2 displayed a set of four protons at δ 3.49 (1H, *m*, H-6a), 3.59 (1H, *t*, *J* = 10.8 Hz, H-6eq), 4.19 (1H, *dd*, *J* =10.8, 5.0 Hz, H-6ax) and 5.43 (1H, *d*, *J* = 6.8 Hz, H-11a) characteristic of a pterocarpan derivative (Tanaka *et al*., 1998). The singlet at δ 7.25 was assigned to H-1. It should be *para* to singlet at the δ 6.41 (H-4) because of absence of coupling. Proton spectrum showed *ortho*-coupled aromatic protons at δ 6.37 (1H, *d*, *J* = 7.6 Hz, H-8) and 6.95 (1H, *d*, *J* = 8.0 Hz, H-7). Two γ,γ-dimethylallyl groups appeared as signals at δ 1.81, 1.81, 1.79 and 1.75 (3H each, s, Me-4', Me-5', Me-4'' and Me-5''), 3.34 (2H, *d*, *J* = 7.2 Hz, H-1'), 3.34 (1H, *dd*, *J* = 13.1, 7.2 Hz, H-1''), 3.37 (1H, *dd*, *J* = 13.1, 7.2 Hz, H-1''), 5.27 (1H, *t*, *J* = 7.2 Hz, H-2') and 5.30 (1H, *t*, *J* = 7.2 Hz, H-2'') which correlated to the ^{13}C NMR (Table 5 and Figure 12) and HMQC (Figure 13) signals at δ 17.8, 25.8, 17.9, 25.8, 29.2, 23.2, 121.4 and

121.9 respectively. The HMBC (Table 5 and Figure 14) correlations between H-1' (δ 3.34) and C-1 (δ 132.0), C-2 (δ 120.9) and C-3 (δ 155.1), and between H-1'' (δ 3.34 and 3.37) and C-9 (δ 155.9), C-10 (δ 110.2) and C-10a (δ 158.4) indicated that the two γ,γ-dimethylallyl groups should be placed at C-2 and C-10, respectively. The absolute stereochemistry at C-6a and C-11a was *R* base on the negative optical rotation value (Tanaka *et al*., 1997). Compound ES2 was identified as erythrabyssin II **[8]** (Kamat *et al*., 1981).

This compound has previously been found in the roots, woods, leaves and stem bark of several *Erythrina* plants such as *E. abyssinica* (Kamat *et al*., 1981), *E. x bidwillii* (Iinuma *et al*, 1992), *E. crista-galli* (Mitscher *et al*., 1988; Tanaka and Etoh, 1997), *E. mildbraedii* (Mitscher *et al*., 1988), *E. orientalis* (Tanaka and Etoh, 1998), *E. poeppigiana* (Tanaka *et al*., 2002) and *E. zeyheri* (Tanaka *et al*., 2003). This compound was the first isolated from the stem bark of *E. suberosa*.



[8]

Table 5 NMR Spectral data of compound ES2 as compared with erythrabyssin II (in $CDCl_3$)

Position	Compound ES2		Erythrabyssin II		HMBC of ES2 (correlation with ^{13}C)
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	
1	7.25 (*s*)	132.0	7.25 (*s*)	132.0	C-3, C-5, C-1'
2	-	120.9	-	121.0	-
3	-	155.1	-	155.0	-
4	6.41 (*s*)	103.9	6.41 (*s*)	103.9	C-2, C-11b
5	-	155.7	-	155.7	-
6eq	3.59 (*t*-like, 10.8)	66.6	3.59 (*t*-like, 11.0)	66.6	C-6a* and C-11a
6ax	4.19 (*dd*, 10.8, 5.0)	-	4.20 (*dd*, 11.0,5.1)	-	C-6a* and C-11a
6a	3.49 (*m*)	40.1	3.49 (*m*)	40.1	C-6b* and C-10a
6b	-	118.8	-	118.8	-
7	6.95 (*d*, 8.0)	122.4	6.95 (*d*, 8.1)	122.4	C-9, C10a
8	6.37 (*d*, 7.6)	108.1	6.37 (*d*, 8.1)	108.4	C-6b, C-9* and C-10
9	-	155.9	-	155.9	-
10	-	110.2	-	110.2	-
10a	-	158.4	-	158.4	-
11a	5.43 (*d*, 6.8)	78.2	5.44 (*d*, 7.3)	78.2	C-1, C-5, C-11b*
11b	-	112.4	-	112.4	-
1'	3.34 (*d*, 7.2)	29.2	3.34 (*d*, 7.3)	29.2	C-1, C-2*, C-2'*and C-11b*
2'	5.27 (*t*, 7.2)	121.4	5.29 (*t*, 7.3)	121.4	C-2, C-1' and C-4'
3'	-	134.9	-	134.8	-
4'	1.81 (*s*)	17.8	1.81 (*s*)	17.9	C-3*, C-2', C-3' and C-5'
5'	1.81 (s)	25.8	1.80 (*s*)	25.8	C-2', C-3' and C-5'
1"	3.34 (*dd*, 13.1, 7.2)	23.2	3.35 (*dd*, 13.2,7.3)	23.2	C-10*, C-10a, C-3" and C-4"
	3.37 (*dd*, 13.1, 7.2)	-	3.40 (*dd*, 13.2, 7.3)	-	C-10*, C-10a, C-3" and C-4"
2"	5.30 (*t*, 7.2)	121.9	5.34 (*t*, 7.3)	121.9	C-10 and C-1"*
3"	-	135.2	-	135.2	-
4"	1.79 (*s*)	17.9	1.79 (*s*)	17.9	C-2" C-3" and C--5"
5"	1.75 (*s*)	25.8	1.75 (*s*)	25.8	C-2", C-3" and C-4"

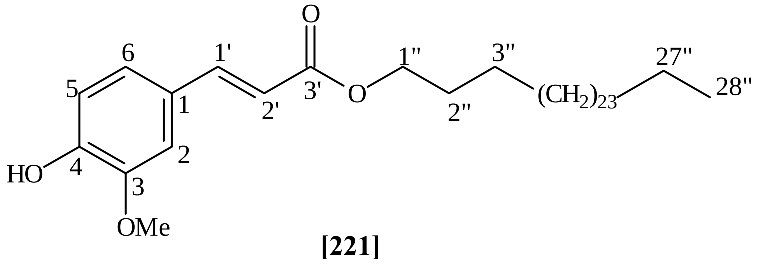
* Two-bond coupling

1.3 Structure Determination of Compound ES3 and EF5

Compound ES3 and EF5 were obtained as white amorphous powder. A molecular formula of $C_{38}H_{66}O_4$ was deduced from its M^+ ion at *m/z* 586.4961 (calcd for $C_{38}H_{66}O_4$ 586.4961) in the HRFABMS. Its UV spectrum (Figure 16) showed λ_{max} at 328 nm similar to that of long chain aromatic ester with one unsaturation on its side chain (Wandji *et al*., 1990). The IR spectrum (Figure 17) showed absorption bands at free hydroxyl (3510 cm^{-1}), carbonyl (1724 cm^{-1}), olefinic (1639 cm^{-1}), aromatic ring (1603, 1519, 1471 cm^{-1}), ether (1281, 1175 cm^{-1}) and methylene (718 cm^{-1}) groups.

The ^{1}H-NMR spectrum (Table 6 and Figure 18) of this compound in $CDCl_3$ revealed the presence of an ABX splitting system at δ 6.91 (1H, *d*, *J* = 8.0 Hz, H-5), 7.03 (1H, *d*, *J* = 2.0 Hz, H-2) and 7.07 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-6). The methyl signal at δ 0.88 (3H, *t*, *J* = 8.0 Hz, H-28'') and methylene protons at δ 4.19 (2H, *t*, *J* = 6.8 Hz, H-1''), 1.69 (2H, *m*, H-2''), 1.39 (2H, *m*, H-3''), 1.25 (46H, br, H-4'' to H-26'') and 1.30 (2H, *m*, H-27'') clearly showed the presence of hydrocarbon side-chain. The methoxy group was located at C-3 (δ 3.93), as shown by its NOE (Figure 20) interaction with H-2 (δ 7.03). The trans-olefinic proton signals of a cinnamoyl moiety showed at δ 7.61 (1H, *d*, *J* = 16.0 Hz, H-1') and 6.29 (1H, *d*, *J* = 16.0 Hz, H-2'). The ^{13}C spectrum appeared (Figure 19) showed peak at δ 167.4 (C-3') due to the carbonyl group of an ester function and the olefinic C=C of the side chain at δ 144.6 (C-1') and 115.7 (C-2'). This was confirmed by the HMBC (Figure 21) correlations of H-1' (δ 7.61) and C-2' (δ 115.7), H-2' (δ 6.29) and C-1 (δ 127.1), H-1' (δ 7.61) and H-2' (δ 6.29) and C-3' (δ 167.4). Further confirmation of this skeleton came from the EIMS (Figure 15) spectrum of this compound, which showed significant fragment peaks at *m/z* 194 and 177, both characteristic of a hydroxy- and methoxy-substituted of cinnamic moiety. The successful was supported by application of 2D-NMR as HMQC and HMBC.

From all of the above spectroscopic data in comparison with reported values (Nkengfack *et al*., 1989; Wandji *et al*., 1990), compounds ES3 and EF5 were identified as erythrinassinate B **[221]**. This compound has been found in the stem bark of several *Erythrina* species such as *E. burttii* (Yenesew *et al*., 1998), *E. eriotriocha* (Nkengfack *et al*., 1989), *E. indica* (Nkengfack *et al*., 2001) and *E. sensegalensis* (Wandji *et al*., 1990).



[221]

Table 6 NMR Spectral data of compounds ES3 and EF5 as compared with erythrinassinate B (in $CDCl_3$)

Position	Compounds ES3 and EF5		erythrinassinate B		HMBC of ES3 and EF5
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	(correlation with ^{13}C)
1	-	127.1	-	127.0	-
2	7.03 (*d*, 2.0)	**109.3**	7.04 (*d*, 1.3)	**115.7**	C-1*, C-3* and C-1'
3	-	147.9	-	147.9	-
4	-	146.7	-	146.7	-
5	6.91 (*d*, 8.0)	114.7	6.94 (*d*, 8.1)	114.6	C-1 and C-4*
6	7.07 (*dd*, 2.0,8.0)	123.0	7.07 (*dd*, 1.3,8.1)	123.1	C-2
1'	7.61 (*d*, 16.0)	144.6	7.61 (*d*, 16)	144.6	C-2, C-6*, C-2' and C-3'
2'	6.29 (*d*, 16.0)	**115.7**	6.28 (*d*, 16)	**109.3**	C-1 and C-3'*
3'	-	167.4	-	167.4	-
1"	4.19 (*t*, 6.8)	64.6	4.19 (*t*, 7.5)	64.6	C-3', C-3" and C-4" to 26"
2"	1.69 (*m*)	28.8	-	28.8	C-1" and C-3"*
3"	1.39 (*m*)	26.0	-	26.0	C-2"*
4" to 26"	1.25 (*br*)	29.7	1.26 (*br*)	29.7	C-27" and C-27"
27"	1.30 (*m*)	22.7	1.30 (*m*)	22.7	-
28"	0.88 (*t*, 6.8)	14.1	0.88 (*t*, 6.7)	14.1	C-27"
3-MeO	3.93 (*s*)	55.9	3.89 (*s*)	55.9	C-4
4-OH	5.88 (*s*)	-	6.98 (*s*)	-	C-3, C-4 and C-5

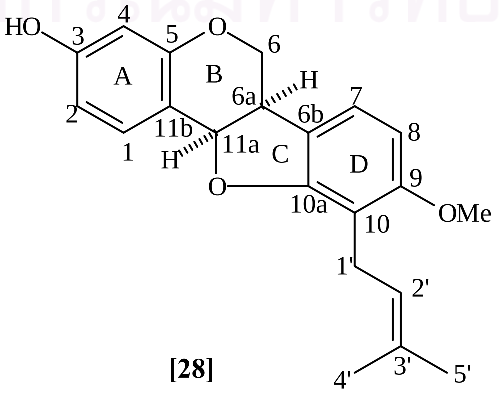
* Two-bond coupling

The bold values are revised assignments.

1.4 Structure Determination of Compounds ES4 and EF2

Compound ES4 and EF2 were obtained as brown gum. A molecular formula of $C_{21}H_{22}O_4$ was deduced from its M^+ ion at *m/z* 338.1509 (calcd for $C_{21}H_{22}O_4$ 338.1512) in the HRFABMS. The UV absorptions (Figure 23) at 218 and 286 nm were characteristics of a pterocarpan (Kamat *et al.*, 1981). The IR spectrum (Figure 24) showed the presence of hydroxyl (3423 cm^{-1}), olefinic (1621 cm^{-1}), aromatic ring (1509, 1449 cm^{-1}) and ether (1265, 1239 cm^{-1}) groups.

The ^{1}H-NMR signals (Table 7 and Figure 25) at δ 3.65 (1H, *t*, *J* = 11.0 Hz), 4.22 (1H, *dd*, *J* = 5.0, 11.0 Hz), 3.51 (1H, *m*) and 5.44 (1H, *d*, *J* = 6.5 Hz) were assignable to the H-6eq, H-6ax, H-6a and H-11a protons of the pterocarpan ring, suggesting a *cis* arrangement of the 6a and 11a protons (Pachler and Underwood, 1967). The ^{13}C-NMR (Figure 26) signals at δ 66.4, 39.9 and 77.8 were in agreement with the signals assigned to the C-6, C-6a and C-11a, respectively, of the pterocarpan ring in the reference (McKee *et al.*, 1997). A pair of *ortho* coupled protons at δ 7.01 (1H, *d*, *J* = 8.0 Hz) and 6.41 (1H, *d*, *J* = 8.0 Hz, H-8) for ring D, and a group of ABX aromatic proton signals at δ 7.38 (1H, *d*, *J* = 8.0 Hz, H-1), 6.52 (1H, *dd*, *J* = 8.0, 2.5 Hz, H-2) and 6.39 (1H, *d*, *J* = 2.5 Hz, H-4) could clearly be seen in the proton spectrum. Additionally, a signal at δ 3.80 (3H, *s*) was assigned to methoxyl groups at C-9, as shown by its NOESY (Figure 27) correlation with H-8 (δ 6.41). The remaining signals at δ 3.30 (2H, *t*, *J* = 7.3 Hz, H-1'), 5.24 (1H, *t*, *J* = 7.3 Hz, H-2'), 1.76 (3H, *s*, H-4') and 1.66 (3H, *s*, H-5') together with certain peaks in the mass spectrum ($[M-15]^+$, $[M-43]^+$ and $[M-55]^+$) indicate the presence of one isoprenyl group located at C-10, which was confirmed by the HMBC (Figure 28) correlations of H-8 (δ 6.41), H-1' (δ 3.30) and C-10 (δ 113.3); H-7 (δ 7.01) and C-9 (δ 158.5); H-1' (δ 3.30) and 9-MeO (δ 3.80). Compounds ES4 and EF2 were therefore identified as sandwicensin **[28]**. This compound has been found in the stem bark, wood and root of *Erythrina* species such as *E. x bidwillii* (Tanaka *et al.*, 1998), *E. crista-galli* (Mitscher *et al.*, 1988), *E. gluca* (McKee *et al.*, 1997) and *E. poeppigiana* (Tanaka *et al.*, 2002).



[28]

Table 7 NMR Spectral data of compound ES4 and EF2 as compared with sandwicensin (in $CDCl_3$)

Position	Compound ES4 and EF2		Sandwicensin		HMBC of ES4 and EF2
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	(correlation with ^{13}C)
1	7.38 (*d*, 8.0)	132.3	7.38 (*d*, 8.4)	132.4	C-3, C-5 and C-11a
2	6.52 (*dd*, 2.5,8.0)	109.7	6.55 (*d*, 8.4)	109.6	C-4
3	-	**157.0**	-	**158.5**	-
4	6.39 (*d*, 2.5)	103.5	6.39 (*s*)	103.0	C-2
5	-	156.4	-	156.6	-
6eq	3.65 (*t*, 11.0)	66.4	3.63 (*dd*, 11.0)	66.5	C-6b and C-11a
6ax	4.22 (*dd*, 11.0, 5.0)	66.4	4.20 (*dd*, 11.0, 4.8)	66.5	C-5, C-6b and C-11a
6a	3.51 (*m*)	39.9	3.48 (*m*)	39.9	C-7 and C-10a
6b	-	119.3	-	119.3	-
7	7.01 (*d*, 8.0)	121.6	6.98 (*d*, 7.8)	121.6	C-9 and C-10a
8	6.41 (*d*, 8.0)	103.1	6.40 (*d*, 7.8)	103.5	C-6b and C-10
9	-	**158.5**	-	**156.6**	-
10	-	113.3	-	113.3	-
10a	-	158.4	-	158.6	-
11a	5.44 (*d*, 6.5)	77.8	5.44 (*d*, 6.9)	77.8	C-1, C-5 and C-6
11b	-	112.9	-	113.3	-
1'	3.30 (*t*, 7.3)	22.8	3.27 (*br*)	22.9	C-9, C-10*, C-10a and C-3'
2'	5.24 (*t*, 7.3)	122.2	5.21 (*t*, 7.2)	122.2	C-1', C-3', C-4' and C-5'
3'	-	131.6	-	131.6	-
4'	1.76 (*s*)	17.7	1.73 (*s*)	17.8	C-2', C-3'and C-5'
5'	1.66 (*s*)	25.7	1.64 (*s*)	25.8	C-2', C-3'and C-4'
MeO-9	3.80 (*s*)	55.9	3.78 (*s*)	55.9	C-9

* Two-bond coupling

The bold values are revised assignments.

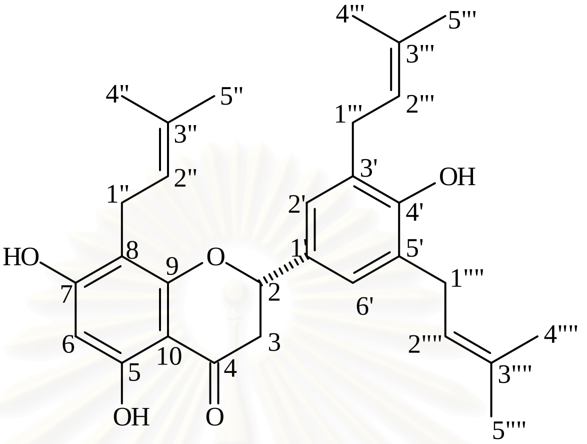
1.5 Structure Determination of Compounds ES5

Compound ES5 was obtained as yellow powder. A molecular formula of $C_{30}H_{36}O_5$ was deduced from its M^+ ion at *m/z* 476 in the EIMS. The UV adsorptions (Figure 31) showed at 219 and 292 nm. The IR spectrum (Figure 32) exhibited absorption bands for hydroxyl (3435 cm^{-1}), carbonyl (1639 cm^{-1}), aromatic ring (1441 and 1383 cm^{-1}) and ether (1269 and 1172 cm^{-1}) functionalities.

The ^{1}H-NMR signals (Table 8 and Figure 33) at δ 2.76 (1H, *dd*, *J* = 17.5, 3.0 Hz, H-3eq), 3.04 (1H, *dd*, *J* = 17.0, 13.0 Hz, H-3ax) and 5.28 (1H, *dd*, *J* = 13.0, 3.0 Hz, H-2), and ^{13}C-NMR signals at δ 79.0 for C-2, δ 43.2 for C-3 and δ 196.7 for C-4, further confirmed a flavanone structure. A signal for the chelated OH (exchangeable with D_2O) appeared at δ 12.00. Double signals in the aromatic region at δ 7.31 (2H, *s*, H-2' and H-6') suggested the presence of *para*- and *meta*-substituted aromatic ring. The ^{1}H NMR spectrum showed two more hydroxy signals at δ 5.49 (sharp, *s*) and 6.39 (br, *s*), which located at C-4' and C-7. The assignment of C-7 was based on its HMBC (Figure 35-36) correlation between 7-OH (δ 6.39) with C-6 (δ 96.7), C-7 (δ 163.6) and C-8 (δ 106.3), while HMBC correlation of H-2', H-6' (δ 7.03), H-1''', H-1'''' (δ 3.34) 4'-OH (δ 5.49) and C-4' at δ 153.0 (oxygenated carbon) with confirmed the attachment of the hydroxyl group at C-4'.

Furthermore, the ^{1}H NMR spectra showed signals of three γ,γ-dimethylallyl group (two were equivalent) at 1.70 and 1.76 (3H each, s, Me-4'' and Me-5'') and two sharp singlet signals at 1.75 and 1.76 (6H each, *s*, Me-4''', Me-4'''', Me-5''' and Me-5''''), 3.27 (2H, *d*, *J* = 7.5 Hz, H-1''), 3.34 (4H, *d*, *J* = 7.0 Hz, H-1''' and H-1''''), 5.19 (2H, *t*, *J* = 7.0 Hz, H-2''), and 5.30 (2H, *t*, *J* = 7.0 Hz, H-2'''and H-2'''') correlated with the carbon (Table 5 and Figure 33) signals at δ 25.8 (C-4'') , 17.8 (C-5'') , 25.8 (C-4''' and C-4'''') , 17.9 (C-5''' and C-5''''), 21.8 (C-1'') , 29.7 (C-1''' and C-1'''',) , 121.7 (C-2'') and 121.6 (C-2''' and C-2'''',) respectively. The HMBC (Table 8 and Figure 37) correlation between H-1'' (δ 3.27) with C-9 (δ 159.9), and H-1''' (δ 3.34) with C-3' (δ 127.4) and C-4' (δ 153.0) indicated that three γ,γ-dimethylallyl group should be placed at C-8, C-3' and C-5', respectively.

On the basis of the above spectroscopic data, compound ES5 was identified as 5,7,4'-trihydroxy-8,3',5'-triprenylflavanone **[270]**. Its ^{1}H NMR data are in good agreement with earlier published data (Baruah *et al*., 1984). This compound was the first isolated from the genus *Erythrina*.



ES5

[270]

Table 8 NMR Spectral data of compound ES5 as compared with 5,7,4'-trihydroxy-8-3'-5'-triprenylflavanone (in $CDCl_3$)

Position	Compound ES5		5,7,4'-trihydroxy-8-3'-5'-triprenylflavanone	HMBC of ES5 (correlation with ^{13}C)
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	
2	5.28 (*dd*, 17.0,3.0)	79.0	5.28 (*dd*, 17.0,3.0)	-
3ax	3.04 (*dd*, 17.0,13.0)	43.2	3.04 (*dd*, 17.0,13.0)	C-2* and C-4*
3eq	2.76 (*dd*, 17.5,3.0)	-	2.77 (*dd*, 17.0, 3.0)	-
4	-	196.7	-	-
5	-	162.1	-	-
6	5.99 (*s*)	96.7	6.01 (*s*)	C-5, C-7, C-8 and C-10
7	-	163.6	-	-
8	-	106.3	-	-
9	-	159.9	-	-
10	-	103.2	-	-
1'	-	130.2	-	-
2'	7.03 (*s*)	125.7	7.05 (*s*)	C-2, C-3', C-6', C-1''' and C-1''''
3'	-	127.4	-	-
4'	-	153.0	-	-
5'	-	127.4	-	-
6'	7.03 (*s*)	125.7	7.05 (*s*)	C-2, C-2', C-3', C-1''' and C-1''''
1"	3.27 (*d*, 7.5)	21.8	3.28 (*brd*, 6.0)	C-8*, C-9, C-2"* and C-3"
2"	5.19 (*t*, 7.0)	121.7	5.28 (*brt*, 6.0)	C-4" and C-5"
3"	-	134.5	-	-
4"	1.70 (*s*)	25.8	1.76 (*s*)	C-2" and C-3"
5"	1.76 (*s*)	17.8	1.76 (*s*)	C-2"
1''', 1''''	3.34 (*d*, 7.0)	29.7	3.36 (*brd*, 6.0)	C-3', C-4', C-5', C-3''' and C-3''''
2''', 2''''	5.303 (*t*, 7.0)	121.6	5.32 (*brt*, 6.0)	C-4''', C-4'''', C-5''' and C-5''''
3''', 3''''	-	134.8	-	-
4''', 4''''	1.75 (*s*)	25.8	1.76 (*s*)	C-3'''*, C-3''''*, C-5'''* and C-5''''*
5''', 5''''	1.76 (*s*)	17.9	1.76 (*s*)	C-3'''*, C-3''''*, C-4'''* and C-4''''*
5-OH	12.00 (*s*)	-	12.02 (*s*)	C-5, C-6 and C-10
7-OH	6.39 (*s*)	-	-	C-6 and C-8
4'-OH	5.49 (*s*)	-	-	C-3 and C-5'

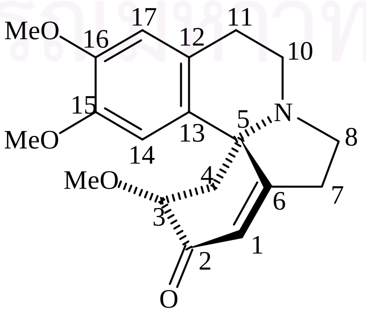
* Two-bond coupling

1.6 Structure Determination of Compound ES6

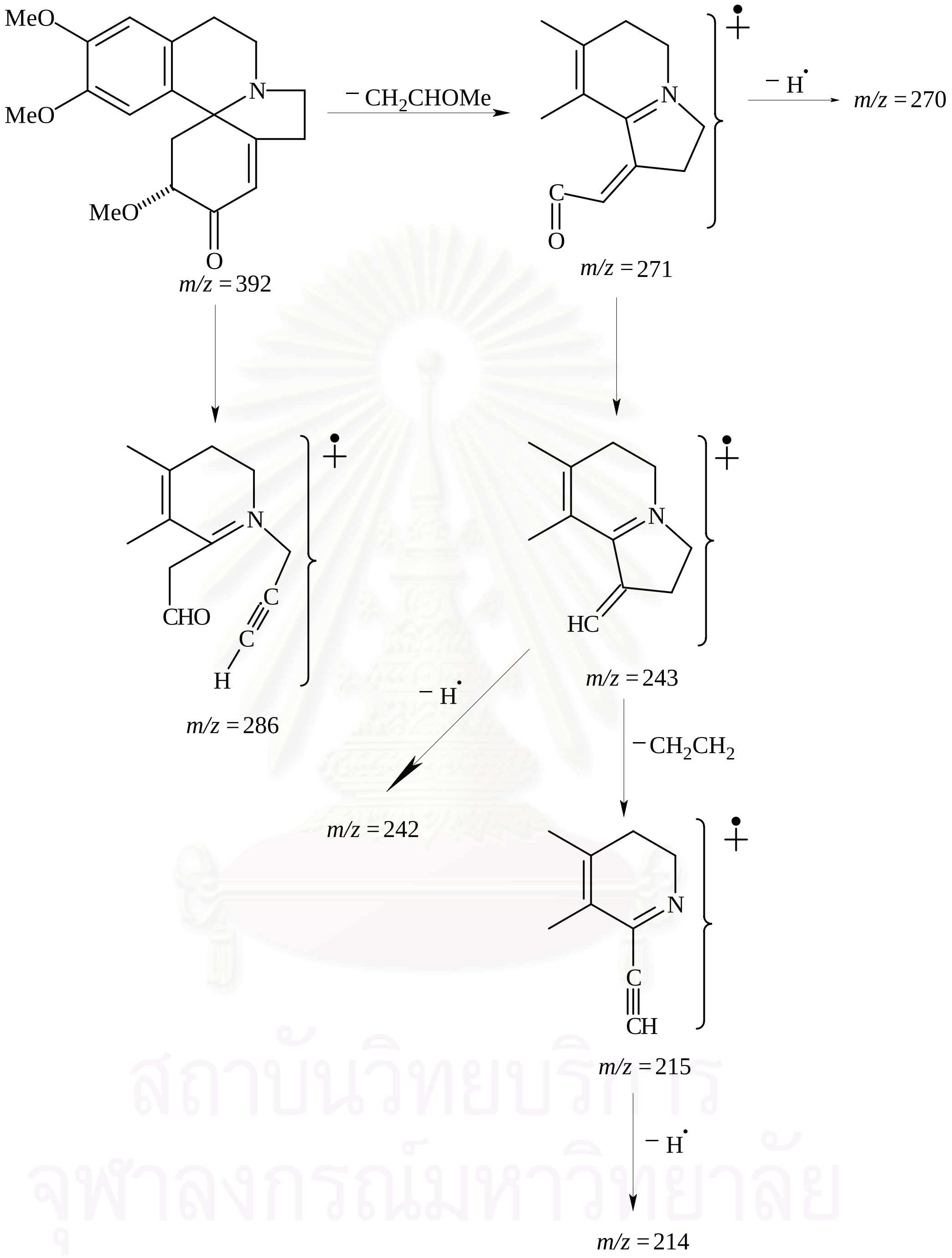
Compound ES6 was obtained as yellowish oil. A molecular formula of $C_{19}H_{23}O_4N$ was deduced from its M^+ ion at *m/z* 329.1604 (calcd for $C_{19}H_{23}O_4N$ 329.1627) in the HRFABMS. The UV absorptions (Figure 39) at 229 and 281 nm were characteristics of enone and dioxyaryl groups, respectively (Barton *et al*., 1973). The IR spectrum (Figure 40) showed the presence of carbonyl (1678 cm^{-1}), aromatic ring (1509, 1463 cm^{-1}) and ether (1253, 1205 and 1102 cm^{-1}) groups.

The ^1H-NMR spectrum (Table 9 and Figure 41) indicated the presence of three methoxy groups at δ 3.48, 3.86 and 3.76 (3-OMe, 15-OMe and 16-OMe), two aromatic protons at δ 6.52 and 6.66 (H-14 and H-17), the α-proton of an α,β-unsaturated ketone at δ 6.10 (H-1) and a proton α to oxygen at δ 4.03 (H-3), in addition to the methylene envelope. The placements of methoxy signal were done using HMBC (Figure 44) correlations between the methoxy protons and the corresponding carbon atoms. ^1H decoupling (Figure 45) experiments enabled the NMR absorptions to be assigned further. Irradiation of the olefinic proton (H-1) at δ 6.10 sharpened proton signals at δ 2.50 and 2.69 (H-7) by removing the coupling between H-1 and H-7. The signals at δ 4.03 (1H, *dd*, *J* = 13.2, 5.2 Hz), 2.15 (1H, *dd*, *J* = 13.2, 11.6 Hz) and 2.61 (1H, *dd*, *J* = 11.6, 5.2 Hz) were assigned to the H-1, H-4ax and H-4eq protons of ring A. The mass spectrum of this compound showed the characteristic fragmentation pattern of the 1(6)-ene-2-one alkaloids (**Scheme 12**) and it almost identical to that of erythratidinone when allowance is made for the mass difference of 28.

From all of the above spectroscopic data in comparison with reported values (Barton *et al*., 1973), compound ES6 was identified as erythratidinone **[199]**. This compound has previously been found only in the leaves of *E. lithosperma* (Barton *et al*., 1973).



[199]



Scheme 12 Mass spectra fragmentations of erythratidinone

Table 9 NMR Spectral data of compound ES6 as compared with erythratidinone (in $CDCl_3$)

Position	ES6		Erythratidinone	HMBC of ES6
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	(correlation with ^{13}C)
1	6.10 (*m*)	122.9	6.13 (*m*)	C-5 and C-7
2	-	197.7	-	-
3	4.03 (*dd*, 13.2,5.2)	77.1	4.05 (*m*)	C-2
4ax	2.15 (*dd*, 13.2,11.6)	42.6	2.28-3.32	C-2, C-3, C-5, C-6 and C-13
4eq	2.61 (*dd*, 5.2, 11.6)	-	2.28-3.32	-
5	-	64.8	-	-
6	-	168.2	-	-
7a	2.50 (*m*)	28.6	2.28-3.32	C-1, C-5 and C-6*
7b	2.69 (*m*)	-	2.28-3.32	-
8a	3.21 (*m*)	39.9	2.28-3.32	C-5 and C-10
8b	3.45 (*m*)	-	2.28-3.32	-
9	-	-	-	-
10a	2.80 (*m*)	45.9	2.28-3.32	C-5, C-8 and C-12
10b	3.01 (*m*)	-	2.28-3.32	-
11a	2.62 (*m*)	21.3	2.28-3.32	C-12 and C-13
11b	3.05 (*m*)	-	2.28-3.32	-
12	-	124.7	-	-
13	-	126.0	-	-
14	6.52 (*s*)	109.8	6.57(*s*)	C-5 and C-13*
15	-	149.0	-	-
16	-	147.0	-	-
17	6.66 (*s*)	113.0	6.68(*s*)	C-11
3-OMe	3.48 (*s*)	58.5	3.50 (*s*)	C-3
15-Ome	3.86 (*s*)	55.9	3.88 (*s*)	C-15
16-OMe	3.76 (*s*)	56.1	3.78 (*s*)	C-16

* Two-bond coupling

1.7 Structure Determination of Compound EF1

Compound EF1 was obtained as white amorphous powder. It molecular formula, $C_{28}H_{58}O$, was assigned from elemental analysis and mass fragment (Figure 46), which showed the $[M^{+}]$ ion at *m/z* 410. The IR spectrum exhibited absorption bands at OH stretching (3307 cm^{-1}), aliphatic C-H (2917, 2848 cm^{-1}) and CH bending (1473 and 1463 cm^{-1}) (Figure 47).

The ^{1}H-NMR spectrum (Table 10 and Figure 48) displayed signals for a methyl group at δ 0.86 (*t*, *J* = 7.0 Hz), methylene protons at δ 1.23 (52H, *brs*), 1.55 (2H, *m*) and 3.62 (2H, *t*, *J* = 7.0 Hz). The ^{13}C-NMR (Figure 49) and DEPT spectra (Figure 50) showed carbon signals, corresponding to a methyl group, methylene carbons, are one methylene alcohol. These ^{1}H and ^{13}C NMR data of EF1 was shown in Table 6. From all of the above spectroscopic data, compound ES1 was identified as octacosanol **[233]**. This compound has been reported as a constituent of the stem bark of *E. glauca* Willd. (Fomum *et al.*, 1986).

$CH_3(CH_2)_{27}OH$

[233]

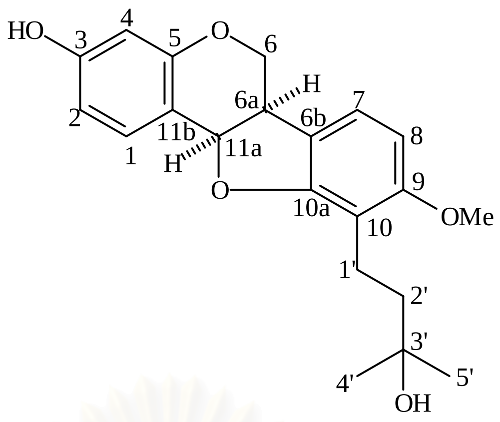
Table 10 NMR Spectral data of compound EF1 (in $CDCl_3$)

Position	Compound EF1	
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)
1	3.62 (*t*, 7.0)	63.1
2	1.55 (*m*)	22.7
3 to 27	1.23 (*brs*)	29.7
28	0.86 (*t*, 7.0)	14.1

1.8 Structure Determination of Compound EF3

Compound EF3 was obtained as brown gum. A molecular formula of $C_{21}H_{24}O_5$ was deduced from its $[M+Na]^+$ ion at *m/z* 379.1523 (calcd for $C_{21}H_{24}O_5$+Na 379.1514) in the HR-TOF-FSI-MS. The UV absorptions (Figure 52) at 218 and 286 nm were characteristics of a pterocarpan (Kamat *et al*., 1981). The IR spectrum (Figure 53) showed the presence of hydroxyl (3423 cm^{-1}), olefinic (1621 cm^{-1}), aromatic ring (1509, 1449 cm^{-1}) and ether (1265, 1239 cm^{-1}) groups.

The ^{1}H-NMR signals (Table 11 and Figure 54) at δ 3.50 (1H, *m*, H-6a), 3.60 (1H, *t*, *J* = 11.0 Hz, H-6eq), 4.20 (1H, *dd*, *J* = 11.0, 5.0 Hz, H-6ax) and 5.44 (1H, *d*, 7.0, H-11a) indicated it to be a pterocarpan derivative. Five aromatic protons at δ 6.36 (1H, *d*, *J* = 2.5 Hz, H-4), 6.40 (1H, *d*, *J* = 8.0 Hz, H-8), 6.50 (1H, *dd*, *J* = 8.0, 2.5 Hz, H-2), 7.00 (1H, *d*, *J* = 8.0 Hz, H-7) and 7.37 (1H, *d*, *J* = 8.0 Hz, H-1) on rings A and D were assigned by comparison of the ^{1}H NMR and ^{13}C NMR spectra with those of sandwicensin (EF2). The remaining signals were assignable to two methylene groups at δ 1.70 (H-2$'$) and 2.67 (H-1$'$), two methyl groups at δ 1.21 (H-4$'$) and 1.22 (H-5$'$) on a carbinol carbon. These partial structures were fully compatible with a 3-hydroxy-3-methylbutyl side chain as also shown by the ^{13}C NMR spectral (Figure 55) assignments at δ 18.4 (C-1$'$), 29.0 (C-5$'$), 29.1 (C-4$'$), 42.4 (C-2$'$), 55.9 (9-OMe) and 71.3 (C-3$'$)of this compound. The assignment of this side chain at the C-10 (δ 114.0) position was confirmed from the HMBC experiment, revealing that the H-1$'$ aliphatic protons (δ 2.67) correlated with C-9 (δ 158.5), C-10 (δ 114.0) and C-10a (δ 158.4). A methoxyl group was located at the C-9 position, as deduced from the HMBC correlation between C-9 (δ 158.5) and the methoxyl proton (δ 3.79). The unambiguous assignment of all the ^{1}H NMR and ^{13}C NMR signals of EF3 was accomplished by analyses of its HMQC (Figure 56), HMBC (Figure 57) and NOESY (Figure 58) spectra. The absolute stereochemistry at both C-6a and C-11a was deduced as *R* from the negative optical rotation. Therefore, the structure of EF3 can be represented by the structure **[271]** and was thus elucidated as a new compound, 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan **[271]**.



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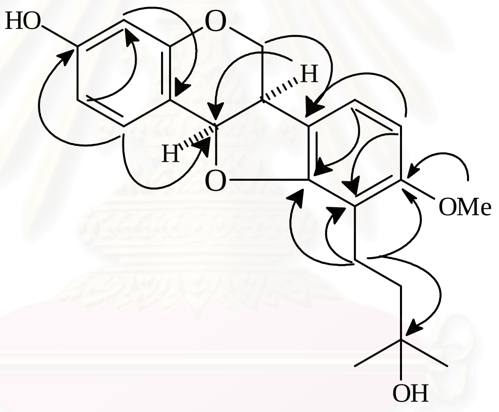


Figure 5 Selected HMBC correlations of **EF3**

Table 11 NMR Spectral data of compound EF3 as compared with sandwicensin (in CDCl$_3$)

Position	Compound EF3		Sandwicensin		HMBC of EF3
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	(correlation with ^{13}C)
1	7.37 (*d*, 8.0)	132.3	7.38 (*d*, 8.4)	132.4	C-3, C-5 and C-11b
2	6.50 (*dd*, 2.5,8.0)	109.7	6.55 (*d*, 8.4)	109.6	C-4 and C-11b
3	-	157.8	-	158.5	-
4	6.36 (*d*, 2.5)	103.6	6.39 (*s*)	103.0	C-2 and C-11b
5	-	156.6	-	156.6	-
6eq	3.60 (*t*, 11.0)	66.5	3.63 (*dd*, 11.0)	66.5	C-5 and C-11a
6ax	4.20 (*dd*, 11.0, 5.0)	-	4.20 (*dd*, 11.0, 4.8)	-	C-11a
6a	3.50 (*m*)	39.9	3.48 (*m*)	39.9	C-10a
6b	-	119.2	-	119.3	-
7	7.00 (*d*, 8.0)	121.7	6.98 (*d*, 7.8)	121.6	C-6a, C-9 and C-10a
8	6.40 (*d*, 8.0)	103.0	6.40 (*d*, 7.8)	103.5	-
9	-	158.5	-	156.6	-
10	-	114.0	-	113.3	-
10a	-	158.4	-	158.6	-
11a	5.44 (*d*, 7.0)	77.1	5.44 (*d*, 6.9)	77.8	C-1 and C-11b*
11b	-	112.7	-	113.3	-
1'	2.67 (*t*, 8.0)	18.4	3.27 (br)	22.9	C-9, C-10, C-10a and C-3'*
2'	1.70 (*m*)	42.4	5.21 (*t*, 7.2)	122.2	C-10, C-1'*, C-4' and H-5'
3'	-	71.3	-	131.6	-
4'	1.21 (*s*)	29.1	1.73 (*s*)	17.8	C-2' and C-3'
5'	1.22 (*s*)	29.0	1.64 (*s*)	25.8	C-2' and C-3'
MeO-9	3.79 (*s*)	55.9	3.78 (*s*)	55.9	-

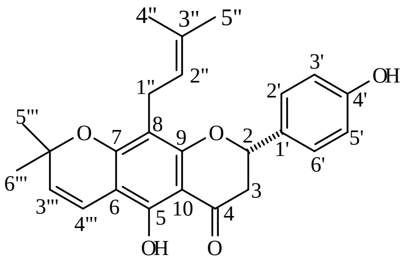
* Two-bond coupling

1.9 Structure Determination of Compound EF4

Compound EF4 was obtained as yellow needles. A formula of $C_{25}H_{26}O_5$ was deduced from its M^+ ion at *m/z* 406.1773 (calcd for $C_{25}H_{26}O_5$ 406.1780) in the HRFABMS. The IR spectrum (Figure 61) exhibited absorption bands for hydroxyl (3251 cm^{-1}), conjugated carbonyl (1644 and 1618 cm^{-1}), aromatic ring (1519, 1449 and 1380 cm^{-1}) and ether (1240 and 1196 cm^{-1}) functionalities. The UV absorptions (Figure 60) at 274, 311 and 367 nm were indicative of a flavanone skeleton (Roussis, V., Ampofo, S.A. and Wiemer, D.F., 1987).

The ^{1}H-NMR signals (Table 12 and Figure 62) at δ 2.80 (1H, *dd*, *J* = 17.0, 3.2 Hz, H-3eq), 3.04 (1H, *dd*, *J* = 17.0, 12.8 Hz, H-3ax) and 5.33 (1H, *dd*, *J* = 12.8, 3.2 Hz, H-2), and ^{13}C-NMR signal (δ 78.5 for C-2, δ 43.2 for C-3 and δ 196.5 for C-4) further confirmed a flavanone structure. A signal for the chelated OH (exchangeable with D_2O) appeared at δ 12.24. Two sharp singlet signals at δ1.43 and 1.45 (3H each, *s*, Me-5''' and Me-6''') and the AB pattern of two doublets at δ 5.50 (1H, *d*, *J* = 10 Hz, H-3''') and 6.63 (1H, *d*, *J* = 10 Hz, H-4''') provided evidence for a 2,2-dimethylchromene ring. The ^{1}H-NMR data also revealed the presence of a γ,γ-dimethylallyl group with signals at δ 1.68, 1.65 (3H each, *s*, Me-4" and 5"), δ 3.20 (2H, *d*, *J* = 7.2 Hz, H-1") and δ 5.14 (1H, *t*, *J* = 7.6 Hz, H-2") which correlated to the ^{13}C-NMR (Table 12 and Figure 63) signals at δ 25.8 (C-4"), 17.8 (C-5"), 21.4 (C-1") and 122.5 (C-2"), respectively. Four protons on a *para*-substituted aromatic ring (AA'BB' pattern) appeared at δ 7.32 (2H, *d*, *J* = 8.4 Hz, H-2' and H-6') and 6.87 (2H, *d*, *J* = 8.4 Hz, H-3' and H-5'). The HMBC (Figure 65) correlations of C-4' at δ 155.9 (oxygenated carbon) with H-2' (δ 7.32) and H-6' (δ 7.32), confirmed the attachment of the hydroxyl at C-4'.

The γ,γ-dimethylallyl unit was located at C-8 position, as suggested by the fragment ion at *m/z* 351 (loss of C_4H_7) in the mass spectrum, together with the HMBC (Figure 65-66) correlations of H-1" with C-7 (δ 159.9), and H-4''' (on the 2,2-dimethylchromene ring) with C-5 (δ 156.5) and C-7 (δ 159.9). Moreover, the HMBC (Figure 67) correlation of 5-OH (δ 12.24) with C-5 (δ 156.5) supported the assignment of C-9 at 159.3. From all of the above spectral data, compound EF4 was identified as lupinifolin **[272]** (Lin *et al*., 1991). This compound was the first isolated from the genus *Erythrina*.



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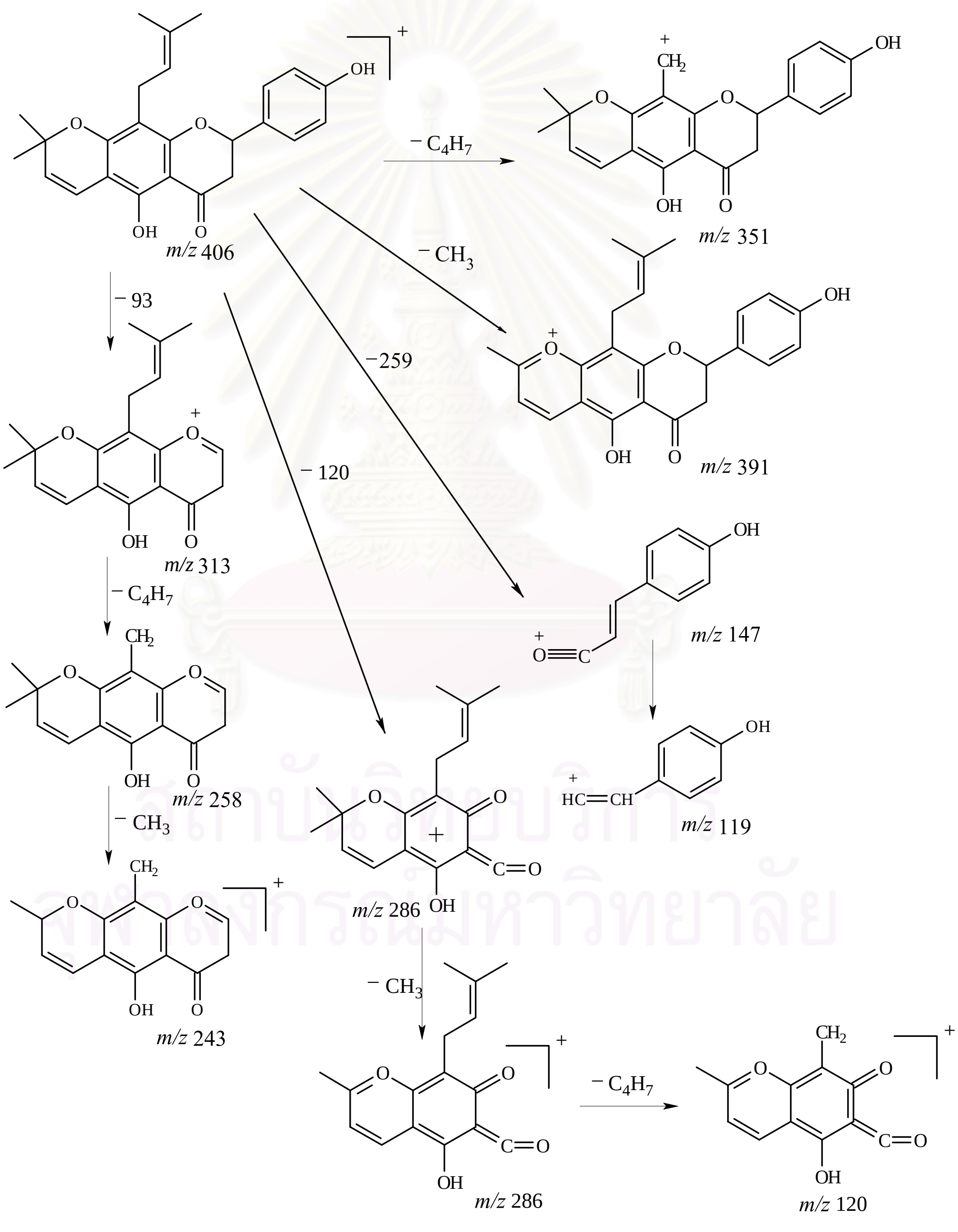


Scheme 13 Mass spectra fragmentations of lupinifolin

Table 12 NMR Spectral data of EF4 as compared with lupinifolin (in $CDCl_3$)

Position	Compound EF4		Lupinifolin		HMBC of EF4
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	(correlation with ^{13}C)
2	5.33 (*dd*, 3.2,12.8)	78.5	5.31 (*dd*, 3.0,12.9)	78.5	C-3, C-4, C-1'*, C-2' and C-6'
3ax.	3.04 (*dd*, 12.8,17.0)	43.2	3.00 (*dd*, 12.9,17.0)	43.1	C-4* and C-10
3eq.	2.80 (*dd*, 3.2,17.0)	43.2	2.77 (*dd*, 3.0,17.0)	43.1	-
4	-	196.5	-	196.1	-
5	-	**156.5**	-	**159.4**	-
6	-	102.8	-	102.8	-
7	-	159.9	-	160.0	-
8	-	108.6	-	108.7	-
9	-	**159.3**	-	**156.5**	-
10	-	102.6	-	102.6	-
1'	-	**131.1**	-	**126.0**	-
2'	7.32 (*d*, 8.4)	127.7	7.30 (*d*, 9.0)	127.7	C-2, C-3'* and C-4'
3'	6.87 (*d*, 8.4)	115.5	6.61 (*d*, 9.0)	115.5	C-1' and C-4'*
4'	-	155.9	-	156.0	-
5'	6.87 (*d*, 8.4)	115.5	6.61 (*d*, 9.0)	115.5	C-1'*
6'	7.32 (*d*, 8.4)	127.7	7.30 (*d*, 9.0)	127.7	C-4' and C-5'*
1"	3.20 (*d*, 7.2)	21.4	3.10 (*d*, 7.0)	21.4	C-7, C-9, C-2"* and C-3"
2"	5.14 (t, 7.6)	122.5	5.12 (*t*, 7.0)	122.4	C-8, C-1" and C-4"
3"	-	131.0	-	130.7	-
4"	1.68 (*s*)	25.8	1.63 (*s*)	25.7	C-3"* and C-5"
5"	1.65(*s*)	17.8	1.63(*s*)	17.6	C-2", C-3"* and C-4"
2"'	-	78.1	-	78.1	-
3"'	5.50 (*d*, 10.0)	**126.0**	5.48 (*d*, 9.9)	**131.1**	C-6, C-2"'*, C-5"' and C-6"'
4"'	6.63 (*d*, 10.0)	115.6	6.61 (*d*, 9.9)	115.6	C-5, C-6*, C-7 and C-2"'
5"'	1.45 (*s*)	28.4	1.43 (*s*)	28.4	C-2"'*, C-3"' and C-6"'
6"'	1.43 (*s*)	28.3	1.42 (*s*)	28.3	C-2"'*, C-3"' and C-6"'
HO-5	12.24 (*s*)	-	-	-	C-4 and C-5

* Two-bond coupling

The bold values are revised assignments.

1.10 Structure Determination of Compound EF6

Compound EF6 was obtained as colorless amorphous powder. It molecular formula, $C_{26}H_{52}O_2$ was assigned from elemental analysis and EIMS (Figure 68), which showed the [M^+] ion at *m/z* 396. The IR spectrum (Figure 69) exhibited absorption bands of aliphatic C-H (2918, 2849 cm^{-1}), carboxylic (1707 cm^{-1}) and C-H bending (1473 and 1463 cm^{-1}).

The ^{1}H-NMR spectrum (Table 13 and Figure 70) displayed signals for a methyl group at δ 0.86 (3H, *t*, *J* = 7.0 Hz, H-26), methylene protons at δ 1.24 (2H, *m*, H-25), 1.62 (44H, *brs*, H-3 to H-24) and 2.33 (2H, *t*, *J* = 7.5 Hz, H-2). The ^{13}C-NMR (Figure 71) and DEPT spectra (Figure 72) showed carbon signals, corresponding to a methyl group and methylene carbons, which linked to carboxylic acid. These ^{1}H and ^{13}C NMR data were showed in Table 10. From all of the above spectroscopic data, compound EF6 was identified as cerinic acid **[273]** (SciFinder database).

$CH_3(CH_2)_{24}COOH$

[273]

Table 13 NMR Spectral data of compound EF6 (in $CDCl_3$)

Position	Compound EF6	
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)
1	-	178.8
2	2.33 (*t*, 7.5)	33.8
3 to 24	1.62 (*m*)	29.7
25	1.24 (*brs*)	22.7
26	0.86 (*t*, 7.0)	14.1

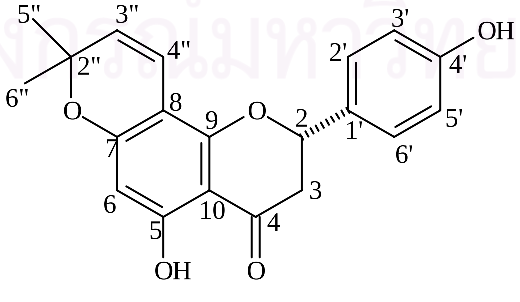
1.11 Structure Determination of Compound EF7

Compound EF7 was obtained as yellow powder. A formula of $C_{20}H_{18}O_5$ was deduced from its M$^+$ ion at *m/z* 338.1154 (calcd for $C_{25}H_{26}O_5$ 338.1379) in the HRFABMS. The IR spectrum exhibited absorption bands for hyhroxyl (3443 cm^{-1}), conjugated carbonyl (1614 cm^{-1}) and ether (1261 and 1154 cm^{-1}) functionalities (Figure 75). The UV absorptions (Figure 74) at 271 and 362 nm were indicative of a flavanone skeleton (Roussis *et al*., 1987).

The ^{1}H NMR spectrum exhibited the signals of 1,4-disubstituted aromatic ring at δ 7.33 (2H, *d*, *J* = 8.4 Hz, H-2' and H-6') and 6.88 (2H, *d*, *J* = 8.4 Hz, H-3' and H-5'), and two hydroxy groups at δ 12.29 (1H, *s*, hydrogen-bonded, 5-OH) and δ 6.43 (1H, *s*, 4'-OH). The ABX type signals at δ 2.77 (1H, dd, *J* = 17.2, 3.2 Hz, H-3eq), 3.07 (1H, dd, *J* = 17.2, 12.8 Hz, H-3ax) and 5.33 (1H, dd, *J* = 13.0, 3.2 Hz, H-2) are characteristic of protons attached to C-3 and C-2 of the flavanone ring (Ito *et al*., 1988).

The ^{1}H and ^{13}C NMR spectra (Table 14, Figures 76 and 77) also revealed the presence of a dimethylpyran ring at δ 1.43 (3H, s, H-5")/δ 28.4 (C-5"), 1.44 (3H, s, H-6")/δ 28.3 (C-6"), 5.50 (1H, d, *J* = 10.0 Hz, H-3")/δ 126.3 (C-3") and 6.62 (d, *J* = 10.0 Hz, H-4")/δ 115.6 (C-4"). The appearance of diagnostic EI-MS fragment peaks at *m/z* 217 and 120, produced by retro-Diels-Alder process at the B-ring in the flavanone nucleus, suggested the location of a dimethylpyran ring on the A-ring. The dimethylpyran ring should be fused in an angular position at C-7 and C-8, as supported by the presence of the singlet signal at δ 5.95 (H-6) and the HMBC (Figure 78-80) correlations of H-4" (δ 6.62) and C-7 (δ 162.1) and between H-3", H-6 and C-8 (δ 102.6).

On the basis of the above spectroscopic data, compound EF7 was identified as citflavanone **[274]**. These were revised from previously report that has been found only in the root bark of *Citrus sinensis* and *C. nobilis* (Ito, 1988; Wu, 1989).



[274]

Table 14 NMR Spectral data of compound EF7 as compared with citflavanone (in $CDCl_3$)

Position	Compound EF7		Citflavanone		HMBC of EF7 (correlation with ^{13}C)
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	
2	5.33 (*dd*, 13.0,3.2)	78.8	5.36 (*dd*, 12.9,3.0)	78.8	-
3ax.	3.07 (*dd*, 17.2,12.8)	43.2	3.07 (*dd*, 17.1,12.9)	43.0	C-2, C-4* and C-1'
3eq.	2.77 (*dd*, 17.2,3.2)	-	2.79 (*dd*, 17.1,3.0)	-	-
4	-	196.2	-	196.1	-
5	-	**158.4**	-	**156.2**	-
6	5.95 (*s*)	96.3	6.00 (*s*)	97.6	C-7* and C-8
7	-	162.3	-	163.7	-
8	-	102.6	-	102.0	-
9	-	**162.0**	-	**156.9**	-
10	-	102.8	-	102.9	-
1'	-	130.5	-	130.4	-
2'	7.33 (*d*, 8.4)	127.9	7.32 (*d*, 8.4)	127.8	C-2, C-4' and C-6'
3'	6.88 (*d*, 8.4)	115.6	6.89 (*d*, 8.4)	115.5	C-1', C-4' and C-5'
4'	-	156.5	-	156.2	-
5'	6.88 (*d*, 8.4)	115.6	6.89 (*d*, 8.4)	115.5	C-1', C-3' and C-4'*
6'	7.33 (*d*, 8.4)	127.9	7.32 (*d*, 8.4)	127.8	C-2, C-2' and C-4'
2"	-	78.3	-	78.3	-
3"	5.50 (*d*, 10.0)	126.3	5.46 (*d*, 10.0)	127.8	C-8 and C-2"
4"	6.62 (*d*, 10.0)	115.3	6.52 (*d*, 10.0)	115.6	C-7, C-9 and C-2"
5"	1.43 (*s*)	28.4	1.42 (*s*)	28.5	C-2", C-3" and C-6"*
6"	1.44 (*s*)	28.3	1.44 (*s*)	28.2	C-2", C-3" and C-5"*
5-OH	12.29 (*s*)	-	12.10 (*s*)	-	C-5 and C-10
4'-OH	6.43 (*s*)	-	6.00 (*s*)	-	-

* Two-bond coupling

The bold values are revised assignments.

1.12 Structure Determination of Compound EF8

Compound EF8 was obtained as yellow powder. A formula of $C_{20}H_{18}O_5$ was deduced from its M^+ ion at *m/z* 408.1928 (calcd for $C_{25}H_{28}O_5$ 408.1937) in the HRFABMS. UV absorptions (Figure 82) at 296 and 349 nm were indicative of a flavanone moiety (Roussis *et al*., 1987). The IR spectrum exhibited absorption bands for hydroxyl (3332 cm^{-1}), conjugated carbonyl (1603 cm^{-1}), aromatic ring (1519, 1448 and 1378 cm^{-1}) and ether (1223 and 1180 cm^{-1}) functionalities (Figure 83).

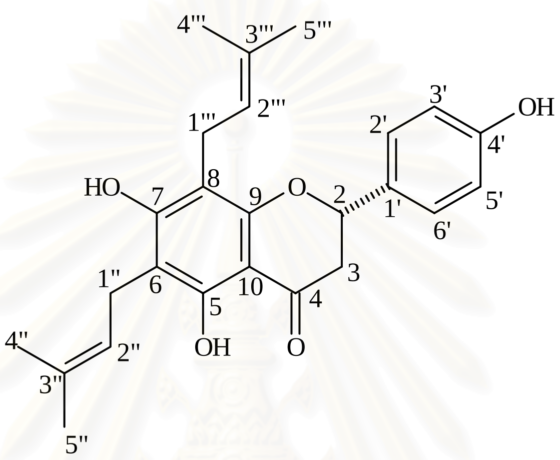
The ^1H NMR spectrum (Table 15 and Figure 84) showed characteristic signals for a flavanone nucleus at δ 2.80 (1H, *dd*, *J* = 17.2, 3.2 Hz), 3.04 (1H, *dd*, *J* = 17.2, 12.8 Hz) and 5.32 (1H, *dd*, *J* = 12.8, 3.2 Hz), assignable to H-3eq, H-3ax and H-2, respectively. This was confirmed by the HMQC spectrum (Figure 86) in which the first of two signals correlated with a carbon signal at 43.2 (C-3) and the H-2 proton correlated with a signal at δ 78.5 (C-2). The ^1H NMR signal at δ 12.34 was assigned to a chelated hydroxyl proton at H-5. Two doublets signals (AA'BB' spin system) in the aromatic region at δ 7.31 (2H, *d*, *J* = 8.8 Hz, H-2' and H-6') and δ 6.87 (2H, *d*, *J* = 8.8, H-3' and H-5') suggested the presence of a *para*-substituted aromatic ring. The ^1H NMR also displayed signals of two other hydroxy groups at δ 5.59 and 6.40 located at C-4' and C-7, respectively. The assignment of C-7 was based on its HMBC (Figure 87-88) correlations between 7-OH (δ 6.40) with C-6 (δ 107.3), C-7 (δ 162.4) and C-8 (δ 106.5), while HMBC correlation of H-2' and H-6' and C-4' at δ 156.0 (oxygenated carbon), which confirmed the attachment of the hydroxyl at C-4'.

Furthermore, the ^1H spectrum showed the presences of two γ,γ-dimethylallyl groups at δ 1.70, 1.71, 1.81 and 1.75 (3H each, s, Me-4'', Me-5'', Me-4'''and Me-5'''), 3.34 (2H, *brd*, *J* = 6.4 Hz, H-1''), 3.29 (2H, *brd*, *J* = 6.8 Hz, H-1'''), 5.23 (1H, *t*, *J* = 7.2 Hz, H-2''), and 5.18 (1H, *d*, *J* = 7.2 Hz, H-2''') which correlated to the ^{13}C NMR (Table 12 and Figure 85) signals at δ 17.8 (C-4'') , 25.8 (C-5'') , 17.8 (C-4'''), 25.8(C-5''') , 21.2 (C-1''), 21.9 (C-1'''), 121.7 (C-2'') and 121.9 (C-2''') respectively. The HMBC (Table 15 and Figure 89-90) correlation between H-1''(δ 3.34) and C-5 (δ 159.3), and between H-1''' (δ 3.29) and C-7 (δ 162.4), C-8 (δ 106.5) and C-9 (δ 157.8) indicated that the γ,γ-dimethylallyl groups should be placed at C-6 and C-8, respectively.

On the basis of the above data, compound EF8 was identified as lonchocarpol A or senegalensein **[115]**. Its ^1H NMR data are in good agreement with earlier published data

(Roussis *et al*., 1987). The complete assignment was managed by performing HMQC and HMBC experiment in the first time for this compound.

This compound has been found in the stem bark of *E. senegalensis* (Taylor *et al.*, 1986; Fomum *et al*, 1987; Wandji *et al*., 1994). This is the first report of its occurrence in the stem bark of *E. fusca*.



[115]

Table 15 NMR Spectral data of compound EF8 as compared with lonchocarpol A (in CDCl$_3$)

Position	Compound EF8		Lonchocarpol A		HMBC of EF8
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	(correlation with ^{13}C)
2	5.32 (*dd*, 3.2,12.8)	78.5	5.28 (*dd*, 3.0,12.8)	78.6	C-4, C-1'*, C-2' and C-6'
3ax.	3.04 (*dd*, 12.8,17.2)	43.2	3.03 (*dd*, 12.8,17.1)	43.1	C-2*, C-4, C-10 and C-1'
3eq.	2.80 (*dd*, 3.2,17.2)	-	2.78 (*dd*, 3.0,17.1)	-	-
4	-	196.7	-	196.8	-
5	-	**159.3**	-	**156.2**	-
6	-	107.3	-	107.4	-
7	-	**162.4**	-	**157.5**	-
8	-	106.5	-	106.7	-
9	-	**157.8**	-	**159.3**	-
10	-	102.8	-	102.9	-
1'	-	131.0	-	130.8	-
2'	7.31 (*d*, 8.8)	127.7	7.27 (*d*, 8.5)	127.7	C-2, C-3'*, C-4' and C-6'
3'	6.87 (*d*, 8.8)	115.5	6.86 (*d*, 8.5)	115.6	C-4'* and C-5'
4'	-	**156.0**	-	**162.5**	-
5'	6.87 (*d*, 8.8)	115.5	6.86 (*d*, 8.5)	115.6	C-2', C-3' and C-4'
6'	7.31 (*d*, 8.8)	127.7	7.27 (*d*, 8.5)	127.7	C-2, C-2', C-4' and C-5'
1"	3.34 (*d*, 6.4)	**21.2**	3.33 (*d*, 7.1)	**25.8**	C-5, C-6, C-2"* and C-3"
2"	5.23 (*t*, 7.2)	121.7	5.22 (*t*, 7.1)	121.8	C-6, C-1"*, C-4" and C-5"
3"	-	**134.7**	-	**133.8**	-
4"	1.70 (*s*)	17.8	1.73 (*s*)	17.8	C-3"*
5"	1.71 (*s*)	**25.8**	1.68 (*s*)	**21.3**	C-2" and C-3"*
1'''	3.29 (*d*, 6.8)	**21.9**	3.29 (*d*, 6.8)	**25.8**	C-7, C-8, C-9, C-2'''* and C-3'''
2'''	5.18 (*d*, 7.2)	121.9	5.18 (*d*, 7.2)	122.0	C-8, C-1'''*, C-4''' and C-5'''
3'''	-	**134.0**	-	**134.6**	-
4'''	1.81 (*s*)	17.8	1.80 (*s*)	17.9	C-5'''
5'''	1.75 (*s*)	**25.8**	1.69 (*s*)	**21.9**	C-4'''
HO-5	12.32 (*s*)	-	12.28 (*s*)	-	C-4, C-5, C-6 and C-10
HO-7	6.40 (*s*)	-	-	-	C-6, C-7 and C-8
HO-4'	5.59 (*s*)	-	-	-	C-4'

* Two-bond coupling

The bold values are revised assignments.

1.13 Structure Determination of Compound EF9

Compound EF9 was obtained as yellow powder. A formula of $C_{25}H_{26}O_5$ was deduced from its M$^+$ ion at *m/z* 406.1776 (calcd for $C_{25}H_{26}O_5$ 406.1780) in the HRFABMS. The IR spectrum (Figure 93) exhibited absorption bands for hydroxyl (3412 cm^{-1}), conjugated carbonyl (1626 and 1596 cm^{-1}), aromatic ring (1519, 1448 and 1378 cm^{-1}) and ether (1195 and 1167 cm^{-1}) functionalities. The UV absorptions (Figure 92) at 272 and 363 nm were indicative of a flavanone skeleton (Fomum *et al*., 1985).

The ^{1}H-NMR signals (Table 16 and Figure 94) at δ 2.78 (1H, *dd*, *J* = 16.8, 3.2 Hz, H-3eq), 3.04 (1H, *dd*, *J* = 17.0, 12.8, Hz, H-3ax) and 5.32 (1H, *dd*, *J* = 12.8, 3.0 Hz, H-2), and ^{13}C-NMR signals (δ 78.7 for C-2, δ 43.2 for C-3 and δ 196.1 for C-4) further confirmed a flavanone structure. A signal for the chelated OH (exchangeable with D_2O) appeared at δ 12.34. Two sharp singlet signals at 1.45 and 1.42 (3H each, *s*, Me-5''' and Me-6''') and the AB pattern of two doublets at δ 5.46 (1H, *d*, *J* = 10 Hz, H-3''') and δ 6.54 (1H, *d*, *J* = 10 Hz, H-4''') provided evidence for a 2,2-dimethylchromene ring. The ^{1}H-NMR data also revealed the presence of a γ,γ-dimethylallyl group with signals at δ 1.68, δ 1.79 (3H each, *s*, Me-4'' and 5''), δ 3.25 (2H, *d*, *J* = 7.2 Hz, H-1'') and δ 5.21 (1H, *t*, *J* = 7.4 Hz, H-2'') which correlated to the ^{13}C-NMR (Table 13 and Figure 95) signals at 25.8, 17.8, 20.9 and 122.3, respectively. Four protons on a *para*-substituted aromatic ring (AA'BB' pattern) of the spectrum showed at δ 7.32 (2H, d, *J* = 8.4 Hz, H-2' and H-6') and 6.88 (2H, *d*, *J* = 8.8 Hz, H-3' and H-5'). The HMBC (Figure 96) correlation of C-4' at δ 156.0 (oxygenated carbon) with H-2' and H-6', confirmed the attachment of the hydroxyl at C-4'.

The γ,γ-dimethylallyl (prenyl group) unit was located at C-6 position, as suggested by the HMBC correlations (Figure 96-97) of H-1'' (δ 3.25) and C-5, C-6, C-7, and between H-2'' (δ 5.21) and C-6. The location of the 2,2-dimethylchromene ring (dimethylpyran ring) could be fused in an angular position at C-7 and C-8, as supported by the presence of the HMBC correlations of H-3''' (δ 5.46) and C-8 (δ 101.7), and between H-4''' (δ 6.54) and C-7 (δ 159.8), C-8 (δ 101.7), C-9 (δ 155.0).

From all of the above spectroscopic data in comparison with reported values (Fomum *et al*., 1985), compound EF9 was identified as erythrisenegalone **[112]**. This compound has been found only in the stem bark of *E. sensegalensis* (Fomum *et al*., 1987).

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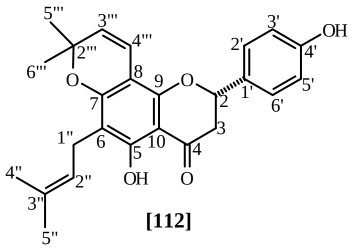


[112]

Table 16 NMR Spectral data of compound EF9 as compared with erythrisenegalone (in CDCl$_3$)

Position	Compound EF9		Erythrisenegalone	HMBC of EF9
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	(correlation with ^{13}C)
2	5.32 (*dd*, 12.8, 3.0)	78.7	5.0-5.54 (*m*)	C-4, C-1', C-4' and C-6'
3ax.	3.04 (*dd*, 17.0, 12.8)	43.2	3.10 (*dd*, 16.5, 4.0)	C-2, C-4, C-10, C-1' and C-4'
3eq.	2.78 (*dd*, 16.8, 3.2)	43.2	2.80 (*dd*, 16.5, 4.0)	-
4	-	196.1	-	-
5	-	161.1	-	-
6	-	109.7	-	-
7	-	159.8	-	-
8	-	101.7	-	-
9	-	155.0	-	-
10	-	102.5	-	-
1'	-	130.8	-	-
2'	7.32 (*d*, 8.4)	127.7	7.34 (*d*, 8.0)	C-2, C-1'*, C-3' and C-4'
3'	6.88 (*d*, 8.8)	115.6	6.86 (*d*, 8.0)	H-4'*
4'	-	156.0	-	-
5'	6.88 (*d*, 8.8)	115.6	6.86 (*d*, 8.0)	C-4'* and C-3'*
6'	7.32 (*d*, 8.4)	127.7	7.34 (d, 8.0)	C-1'* and C-4'
1''	3.25 (*d*, 7.2)	20.9	3.20 (*d*, 7.0)	C-5, C-6, C-7 and C-3''
2''	5.21 (*t*, 7.4)	122.3	5.0-5.54 (*m*)	C-6, C-1''*, C-4'' and C-5''
3''	-	131.3	-	-
4''	1.68 (*s*)	25.8	1.70 (*s*)	C-2'', C-3'' and C-5''*
5''	1.79 (*s*)	17.8	1.70 (*s*)	C-2'', C-3'' and C-4''*
2'''	-	77.9	-	-
3'''	5.46 (*d*, 10.0)	126.1	5.50 (*d*, 10.0)	C-8, C-2'''* and C-4'''*
4'''	6.54 (d, 10.0)	116.0	6.65 (*d*, 10.0)	C-7, C-8, C-9 and C-2'''
5'''	1.45 (*s*)	28.5	1.45 (*s*)	C-2''', C-3''' and C-6'''*
6'''	1.42 (*s*)	28.2	1.45 (*s*)	C-2''', C-3''' and C-5'''*
5-OH	12.34 (*s*)	-	12.35 (*s*)	C-5 and C-10

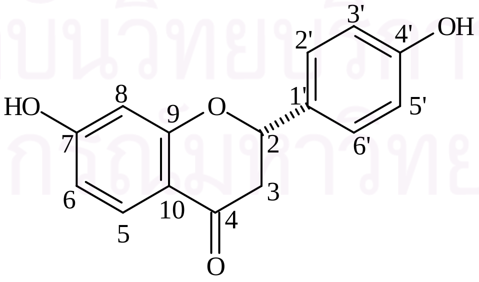
* Two-bond coupling

1.15 Structure Determination of Compound EF10

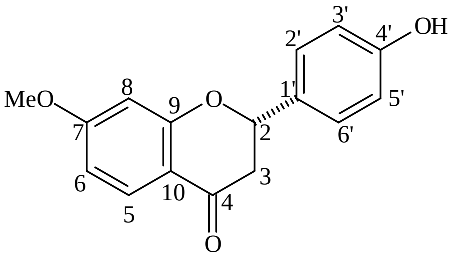
Compound EF10 was obtained as yellow powder. A formula of $C_{15}H_{12}O_4$ was deduced from its M$^+$ ion at *m/z* 256.0736 (calcd for $C_{15}H_{12}O_4$ 256.0732) in the HRFABMS. The UV absorptions (Figure 99) showed absorption at 212, 275 and 312 nm. The IR spectrum (Figure 100) exhibited absorption bands for hydroxyl (3393 cm^{-1}) and carbonyl (1601 cm^{-1}) functionalities.

The ^{1}H-NMR signals (Table 17 and Figure 101) at δ 2.67 (1H, *dd*, *J* = 17.0, 2.8 Hz, H-3eq), 3.05 (1H, *dd*, *J* = 17.0, 13.2 Hz, H-3ax) and 5.45 (1H, *dd*, *J* = 13.2, 2.8 Hz, H-2), and ^{13}C-NMR signal (δ 80.5 for C-2, δ 44.7 for C-3 and δ 190.4 for C-4) indicated a flavanone structure. The ABX splitting system consisting of two doublets at δ 6.42 (1H, *d*, *J* = 2.4 Hz, H-8) and δ 7.73 (1H, *d*, *J* = 8.6 Hz, H-5) and a double doublet at δ 6.58 (1H, *dd*, *J* = 8.6, 2.4 Hz, H-6), together with the HMBC correlation (Figure 103) of H-5 (δ 7.73) with C-4 (δ 190.4), suggested the location of the oxygenated carbon at C-7 (δ 165.5). The presence of an AA'BB' spin system at δ 7.41 (2H, *d*, *J* = 8.8 Hz, H-2' and H-6') and 6.91 (2H, *d*, *J* = 8.8 Hz, H-3' and H-5') indicated a simple *para*-substituted B ring. The positions of H-2' and H-6' were assigned on the basis of its HMBC correlation (Figure 103-104) with C-2, and correlation of C-4' at δ 158.5 (oxygenated carbon) with both H-2' and H-6', confirmed the attachment of the hydroxyl group at C-4'.

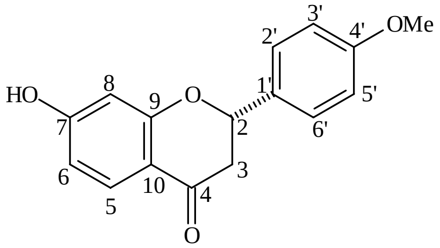
From all of the above spectroscopic data and comparison with reported values (Achenbach *et al*., 1988), compound EF10 was identified as liquiritigenin **[275]**. This is the first time report of the compound as a constituent of an *Erythrina* plant.



[275]



liquiritigenin 7-methyl ether



liquiritigenin 4'-methyl ether

Table 17 NMR Spectral data of compound EF110 as compared with liquiritigenin 7 -methyl ether and liquiritigenin 4'-methyl ether (Acetone-d_6)

Position	Compound EF10		Liquiritigenin 7-methyl ether	Liquiritigenin 4'-methyl ether	HMBC of EF11 (correlation with ^{13}C)
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	^{1}H (mult., *J* in Hz)	
2	5.45 (*dd*, 2.8,13.2)	80.5	5.48 (*dd*, 3.5, 12.5)	5.50 (*dd*, 3.5,12.5)	C-4, C-1' and C-2'
3ax.	3.05 (*dd*, 13.2,17.0)	44.7	3.05 (*dd*, 12.5,16.5)	3.06 (*dd*, 12.5,16.5)	C-2, C-4, C-10 and C-1'
3eq.	2.67 (*dd*, 17.0,2.8)	44.7	2.70 (*dd*, 16.5,3.5)	2.75 (*dd*, 16.5,3.5)	-
4	-	190.4	-	-	-
5	7.73 (*d*, 8.6)	129.4	7.77 (*d*, 8.5)	7.73 (*d*, 8.5)	C-5*, C-7 and C-9
6	6.58 (*dd*, 8.6,2.4)	111.2	6.64 (*dd*, 8.5,2.5)	6.58 (*dd*, 8.5,2)	C-7*, C-8 and C-10
7	-	165.5	-	-	-
8	6.42 (*d*, 2.4)	103.6	6.53 (*d*, 2.5)	6.43 (*d*,2)	C-6, C-7*, C-9* and C-10
9	-	164.5	-	-	-
10	-	115.1	-	-	-
1'	-	131.3	-	-	-
2'	7.41 (*d*, 8.8)	128.9	7.41 (*d*, 8.5)	7.49 (d, 8.5)	C-2, C-3'* and H-4'
3'	6.91 (*d*, 8.8)	116.1	6.90 (*d*, 8.5)	6.99 (*d*, 8.5)	C-1', C-2' and C-4'*
4'	-	158.5	-	-	-
5'	6.91 (*d*, 8.8)	116.1	6.90 (*d*, 8.5)	6.99 (*d*, 8.5)	C-1', C-4'* and C-6'*
6'	7.41 (*d*, 8.8)	128.9	7.41 (*d*, 8.5)	7.49 (*d*, 8.5)	C-2, C-4' and C-5'*
7-OH	-	-	-	9.42 (*s*)	-
4'-OH	-	-	8.49 (*s*)	-	-
7-OMe	-	-	3.87 (*s*)	-	-
4'-OMe	-	-	-	3.83 (*s*)	-

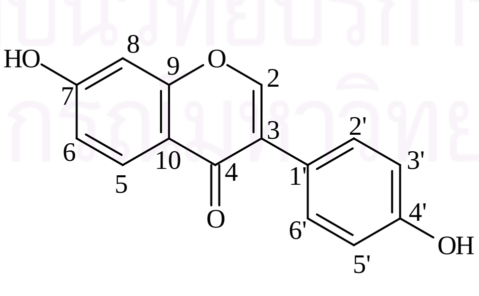
* Two-bond coupling

1.16 Structure Determination of Compound EF11

Compound EF11 was obtained as pale yellow powder. It molecular formula, $C_{15}H_{10}O_4$ was determined from the [M^+] ion at *m/z* 254. The UV absorptions (Figure 106) at 247 nm that was characteristic of an isoflavone. The IR spectrum (Figure 107) exhibited absorption bands for hydroxyl (3224 cm^{-1}), conjugated carbonyl (1631 and 1596), aromatic ring (1518, 1460 and 1388) and ether (1279 and 1240 cm^{-1}) functionalities.

A sharp proton singlet signal at δ 8.04 (H-2 of isoflavone) and the carbon signal at δ 153.7 (C-2) (Table 18 and Figures 108-109) confirmed the existence of the isoflavone nucleus. The ABX splitting system consisting of two doublets at δ 6.82 (1H, *d*, *J* = 2.0 Hz, H-8) and δ 7.99 (1H, *d*, *J* = 8.9 Hz, H-5) and a double doublet at δ 6.91 (1H, *dd*, *J* = 8.9, 2.0 Hz, H-6), together with the HMBC correlation (Figure 110) of H-5 and C-4 (δ 177.8), C-7 (δ 163.7) and C-9 (δ 159.2), suggested the location of the oxygenated carbon at C-7 (δ 163.7). The presence of an AA'BB' spin system at δ 7.33 (2H, *d*, *J* = 8.8 Hz, H-2' and H-6') and 6.84 (2H, *d*, *J* = 8.8 Hz, H-3' and H-5') indicated a simple *para*-substituted B ring. The positions of both H-2' and H-6' were assigned on the basis of its HMBC correlation (Figure 111) with C-3, and correlation of C-4' at δ 157.8 (oxygenated carbon) with H-2' and H-6', confirmed the attachment of the hydroxyl at C-4'.

Based on the above spectral data, this compound was identified as daidzein **[85]**. Its ^{13}C NMR properties are in good agreement with previously published values (Pelter and Ward, 1978). This compound has been isolated from the stem bark of *E. eriotriocha* (Tanaka and Etoh, 1997).



[85]

Table 18 NMR Spectral data of compound EF11 ($CDCl_3$:CD_3OD) as compared with daidzein ($CDCl_3$–DMSO-d_6 1:1)

Position	Compound EF11		Daidzein	HMBC of EF11
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{13}C (mult.)	(correlation with ^{13}C)
2	8.04 (*s*)	153.7	152.2	C-3*, C-4 and C-1'
3	-	125.5	123.9	-
4	-	177.8	175.0	-
5	7.99 (*d*, 8.9)	128.1	127.2	C-4*, C-7 and C-9
6	6.91(*dd*, 2, 8.9)	116.0	115.1	C-8 and C-10
7	-	163.7	162.5	-
8	6.82 (*d*, 2)	102.9	102.2	C-6 and C-9*
9	-	159.2	157.6	-
10	-	117.8	116.8	-
1'	-	123.7	122.7	-
2'	7.33 (*d*, 8.8)	130.9	130.0	C-3, C-4' and C-6'
3'	6.84 (*d*, 8.8)	115.9	115.1	C-1', C-4'*and C-5'
4'	-	157.8	157.3	-
5'	6.84 (*d*, 8.8)	115.8	115.1	C-3' and C-4'*
6'	7.33 (*d*, 8.8)	130.9	130.0	H-2' and C-4'

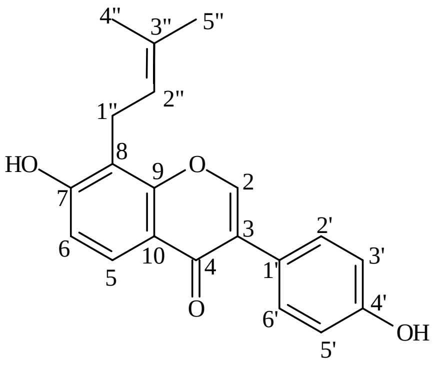
* Two-bond coupling

1.17 Structure Determination of Compound EF12

Compound EF12 was obtained as yellow powder. A formula of $C_{20}H_{18}O_4$ was deduced from its M^+ ion at *m/z* 322.1205 (calcd for $C_{20}H_{18}O_4$ 322.1200) in the HRFABMS. The UV absorptions (Figure 113) showed absorption at 251 nm that was characteristic of an isoflavone (Hakamatsuka *et al*., 1991). The IR spectrum (Figure 114) exhibited absorption bands for hydroxyl (3385 cm^{-1}) and conjugated carbonyl (1685 and 1623 cm^{-1}) functionalities.

A sharp singlet proton signal at δ 8.24 (H-2 of isoflavone) and the carbon signal at δ 153.2 (C-2) in the ^{1}H and ^{13}C NMR spectra (Table 19 and Figures 115-116) confirmed the existence of the isoflavone nucleus. The presence of an AA'BB' spin system at δ 7.50 (2H, *d*, *J* = 8.8 Hz, H-2' and H-6') and δ 6.90 (2H, *d*, *J* = 8.8 Hz, H-3' and H-5') indicated a simple *para*-substituted of ring B. The positions of H-2' and H-6' (δ 7.50) were assigned on the basis of its HMBC correlation (Figure 118) with C-3 (δ 124.7). The ^{1}H NMR spectrum also exhibited two doublets at δ 7.93 (1H, *d*, *J* = 8.8 Hz) and δ 7.10 (1H, *d*, *J* = 8.8 Hz), assignable to the two *ortho*-coupled aromatic protons at H-5 (δ 7.93) and H-6 (δ 7.10) of ring A. The assignment was based on the HMBC correlation (Figure 117) of H-5 (δ 7.93) and C-4 (δ176.1), C-7 (δ 160.4) and C-9 (δ 156.6). The ^{1}H NMR signals at δ 1.67 (3H, *s*, H-4''), 1.84 (3H, *s*, H-5''), 3.58 (2H, *d*, *J* = 7.2 Hz, H-1'') and 5.30 (1H, *t*, *J* = 7.2 Hz, H-2'') correlation to ^{13}C NMR signals at δ 25.8 (C-4''), 17.9 (C-5''), 22.6 (C-1'') and 122.6 (C-2''), indicated the presence of a γ,γ-dimethylallyl group. In the mass spectrum, a fragment ion peak appeared at *m/z* 267 [M-55] supporting the prenylated structure. The placement of γ,γ-dimethylallyl group at C-8 position was confirmed by HMBC correlation (Figure 119-120) of H-1''and C-7 (δ 160.4) and C-9 (δ 156.6).

Compound EF12 was identified as 8-prenyldaidzein **[23]** based on the above spectral data. Its ^{1}H NMR properties are in good agreement with previously published values (Hakamatsuka *et al*., 1991). This compound has been isolated from the root bark and stem bark of several plants such as *E. x bidwillii* (Iinuma *et al*, 1992) and *E. eriotriocha* (Nkengfack *et al*., 1997). It is the first time that 8-prenyldaidzein was separated from the stem bark of *E. fusca*.



[23]

Table 19 NMR Spectral data of compound F12 as compared with 8-prenyldaidzein (in acetone-d_6)

Position	Compound EF12		8-Prenyldaidzein	HMBC of EF12
	^{1}H (mult., *J* in Hz)	^{13}C (mult.)	^{1}H (mult., *J* in Hz)	(correlation with ^{13}C)
2	8.24 (*s*)	153.2	8.29 (*s*)	C-9 and C-1'
3	-	124.7	-	-
4	-	176.1	-	-
5	7.93 (*d*, 8.8)	125.2	7.97 (*d*, 8.6)	C-4*, C-7 and C-9
6	7.10 (*d*, 8.8)	114.9	7.08 (*d*, 8.6)	C-8 and C-10
7	-	160.4	-	-
8	-	116.0	-	-
9	-	156.6	-	-
10	-	118.7	-	-
1'	-	124.4	-	-
2'	7.50 (*d*, 8.8)	131.0	7.54 (*d*, 8.5)	C-1'*
3'	6.90 (*d*, 8.8)	115.8	6.94 (*d*, 8.5)	C-3, C-1' and C-4'
4'	-	158.5	-	-
5'	6.90 (*d*, 8.8)	115.8	6.94 (*d*, 8.5)	C-6'*
6'	7.50 (*d*, 8.8)	131.0	7.54 (*d*, 8.5)	C-3, C-1', C-3' and C-4'*
1"	3.58 (*d*, 7.2)	22.6	3.63 (*d*, 7.3)	C-7, C-8*, C-9, C-4', C-2"* and C-3"
2"	5.30 (*t*, 7.2)	122.6	5.34 (*t*, 7.3)	C-4" and C-5"
3"	-	132.4	-	-
4"	1.67 (*s*)	25.8	1.71 (*s*)	C-2", C-3"and C-5"
5"	1.84 (*s*)	17.9	1.88 (*s*)	C-2", C-3"and C-4"

* Two-bond coupling

2. Determination of biological activities

The biological activities including antimicrobial, antimalarial, free radical scavenging activity, antituberculosis and cytotoxic activities of crude extracts and the isolated compounds were determined. A few of the isolated compounds were not investigated for these activities due to their limited quantities and solubility.

2.1 Antimicrobial Activity

The compounds investigated for this activity against *Enterococcus faecalis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were erythrabyssin II **[8]**, sandwicensin **[28]**, erythrisenegalone **[112]**, lonchocarpol A **[115]**, erythratidinone **[199]**, erythrinassinate B **[221]**, 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan **[271]**, lupinifolin **[272]** and citflavanone **[274]**. Compounds **[8]**, **[272]** and **[112]** showed weak activity against *E. faecalis*, compared to the positive control, tetracycline (MIC 16 μg/ml), while lonchocarpol A **[115]** exhibited stronger activity (MIC and MBC of 6.25 μg/ml) than tetracycline. Erythrabyssin II **[8]** and erythrisenegalone **[112]** displayed weakly activity against *B. subtilis*, whereas compounds **[8]**, **[272]**, **[274]** and **[115]** showed moderate activity (MIC and MBC 12.5, 6.25, 12.5 and 3.125 μg/ml, respectively), when compare to tetracycline (MIC 0.06 μg/ml). Tetracycline inhibited *S. aureus* at MIC 0.125 μg/ml, while compound **[8]**, **[272]**, **[274]** and **[115]** inhibited and kill at MIC 50, 12.5, 12.5 and 6.25 μg/ml, respectively.

All of the test compounds possessed no activity against *E. coli* and *C. albicans* at the maximum concentration of 100 μg/ml. This results are summarized in Table 20.

Compounds **[115]** and **[272]** showed better activity better than other tested compounds. Both compounds were prenylated flavanone substituted at C-6 (only for **[115]**) and C-8, suggesting that the prenylated substituents might be important for the activity.

Table 20 *In vitro* antimicrobial activity of compounds isolated from *E. fusca* and *E. suberosa*

Compound	Microorganism*									
	1		2		3		4		5	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
[115]	6.25	6.25	3.125	3.125	6.25	>100	>100	>100	>100	>100
[199]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
[221]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
[271]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
[272]	50	50	6.25	6.25	12.5	>100	>100	>100	>100	>100
[274]	>100	>100	12.5	12.5	12.5	>100	>100	>100	>100	>100
Tetracycline	0.125	0.125	16	16	< 0.25	<0.25	1.0	1.0	ND	ND
Nystatin	ND	ND	ND	ND	ND	ND	ND	ND	2	ND

ND: Not determined

MIC: Minimum Inhibitory Concentration in µg/ml

MBC: Minimum Bactericidal Concentration in µg/ml

*The microorganisms employed are; 1 *Enterococcus faecalis* (ATCC 29212), 2 *Bacillus subtilis* (ATCC 6633), 3 *Staphylococcus aureus* (ATCC25923), 4 *Escherichia coli* (ATCC25922), 5 *Candida albicans* (ATCC 10231)

2.2 Antimalarial Activity

Ten compounds, comprising erythrabyssin II **[8]**, 8-prenyldaidzein **[23]**, sandwicensin **[28]**, erythrisenegalone **[112]**, lonchocarpol A **[115]**, erythratidinone **[199]**, erythrinassinate B **[221]**, lupinifolin **[272]**, citflavanone **[274]** and liquiritigenin **[275]** were subjected to *in vitro* antimalarial activity test against *Plasmodium falciparum* (K1 strain). Compounds **[28]**, **[112]**, **[199]**, **[221]**, **[272]** and **[275]** were inactive at the maximum concentration (EC$_{50}$12.5 µg/ml), whereas compounds **[8]**, **[274]**, **[115]** and **[23]** showed activity with EC$_{50}$ values of 5.0, 5.0, 1.6 and 3.9 µg/ml, respectively. These results are summarized in Table 21. Compounds exhibiting EC$_{50}$ of not more than 1.6 µg/ml were further analyzed for *in vivo* antimalarial activity and cytotoxicity.

Compound **[115]** was selected for *in vivo* antimalarial activity assay, but showed no activity either by oral or subcultaneous administration in mice at 20 mg/kg. This compound exhibited cytotoxicity at 12.0 µg/ml.

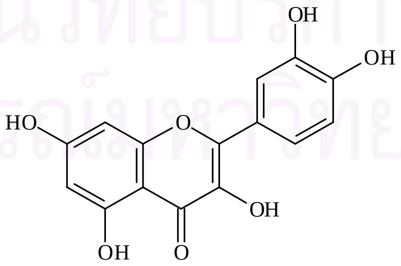
Table 21 *In vitro* antimalarial activity of compounds isolated from *E. fusca* and *E. suberosa*

Compound	EC_{50} (μg/ml)	Cytotoxicity (μg/ml)
erythrabyssin II **[8]**	5.0	ND
8-prenyldaidzein **[23]**	3.9	ND
sandwicensin **[28]**	>12.5	ND
erythrisenegalone **[112]**	>12.5	ND
lonchocarpol A **[115]**	1.6	12.0
erythratidinone **[199]**	>12.5	ND
erythrinassinate B **[221]**	>12.5	ND
lupinifolin **[272]**	>12.5	ND
citflavanone **[274]**	5.0	ND
liquiritigenin **[275]**	>12.5	ND

ND: Not determined

2.3 Free Radical Scavenging Activity

Thirteen compounds (erythrabyssin II **[8]**, 8-prenyldaidzein **[23]**, sandwicensin **[28]**, daidzein **[85]** erythrisenegalone **[112]**, lonchocarpol A **[115]**, erythratidinone **[199]**, 5,7,4'-trihydroxy-8,3',5'-triprenylflavanone **[270]**, 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxy pterocarpan **[271]**, lupinifolin **[272]**, citflavanone **[274]** and liquiritigenin **[275]**) were investigated for this activity. Quercetin was used as positive control. The results are summarized in Table 22.



Quercetin

Table 22 Free radical scavenging activity of compounds isolated from *E. fusca* and *E. suberosa*

Compound	% Scavenging activity at 31.25 μg/mL[a]	IC_{50} (μM)
erythrabyssin II **[8]**	27.02	ND
8-prenyldaidzein **[23]**	7.09	ND
sandwicensin **[28]**	10.20	ND
daidzein **[85]**	6.68	ND
erythrisenegalone **[112]**	8.91	ND
lonchocarpol A **[115]**	12.02	ND
erythratidinone **[199]**	9.13	ND
5,7,4'-trihydroxy-8-3'-5'-triprenylflavanone **[270]**	7.37	ND
3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan **[271]**	9.53	ND
lupinifolin **[272]**	6.51	ND
citflavanone **[274]**	6.93	ND
liquiritigenin **[275]**	6.96	ND
quercetin **[276]**[b]	81.4	1.98

[a] Compound with >50% inhibition were further analyzed for IC_{50} values.

[b] Concentration 2.0 x 10^{-4} μg/mL

ND: Not determined because % scavenging activity at 31.25 μg/mL less than 50 % inhibition

All of pure compounds showed very weak free radical scavenging activity. The structures of these compounds were composed of only one to three free hydroxyl substitute with high molecular formula when compared with quercetin. This functional group should be important for the activity.

2.4 Antituberculosis Activity

The compounds investigated for this activity against *Mycobacterium tuberculosis* H37Ra were erythrabyssin II **[8]**, sandwicensin **[28]**, erythrisenegalone **[112]**, lonchocarpol A **[115]**, erythratidinone **[199]**, erythrinassinate B **[221]** and lupinifolin **[272]**. The results are summarized in Table 23.

Table 23 Antituberculosis activity of compounds isolated from *E. fusca* and *E. suberosa*

Compound	Activity at 200 μg/mL	MIC (μg/mL)
erythrabyssin II **[8]**	Active	100
sandwicensin **[28]**	Active	100
erythrisenegalone **[112]**	Active	50
lonchocarpol A **[115]**	Active	50
erythratidinone **[199]**	Inactive	-
erythrinassinate B **[221]**	Inactive	-
lupinifolin **[272]**	Active	25

2.5 Cytotoxic Activity

Seven compounds: erythrabyssin II **[8]**, sandwicensin **[28]**, erythrisenegalone **[112]**, lonchocarpol A **[115]**, erythratidinone **[199]**, erythrinassinate B **[221]** and lupinifolin **[272]**, were subjected to cytotoxicity test against oral human epidermoid carcinoma (KB) and breast cancer (BC) cell lines. The results are summarized in Table 24.

Compounds **[112]** and **[272]** exhibited strong activity against BC cell line. Both compounds were flavanones with dimethylpyran ring substituents, suggesting that the dimethylpyran ring might be important for the activity.

Table 24 Cytotoxicity Activity of compounds isolated from *E. fusca* and *E. suberosa*

Compounds	KB		BC	
	Cytotoxicity[*]	ED_{50} (μg/mL)	Cytotoxicity[*]	ED_{50} (μg/mL)
erythrabyssin II **[8]**	Inactive	-	Weakly active	18.00
sandwicensin **[28]**	Inactive	-	Weakly active	10.69
erythrisenegalone **[112]**	Weakly active	10.98	Strongly active	3.93
lonchocarpol A **[115]**	Weakly active	13.56	Moderate active	6.03
erythratidinone **[199]**	Inactive	-	Inactive	-
erythrinassinate B **[221]**	Inactive	-	Inactive	-
lupinifolin **[272]**	Weakly active	12.02	Strongly active	3.06

[*] Concentration (μg/mL) >20 = Inactive, >10-20 = Weakly active, 5-10 = Moderate active and <5 = Strongly active

CHAPTER V

CONCLUSION

In this investigation, six known compounds were isolated from the stem bark of *Erythrina suberosa* Roxb. Three flavonoids, erythrabyssin II **[8]**, sandwicensin **[28]** and 5,7,4'-trihydroxy-8-3'-5'-triprenylflavanone **[270]** were found, together with an erythrina alkaloid, erythratidinone **[212]**, one long chain aromatic ester, erythrissinate B **[221]** and a mixture of β-sitosterol **[268]** and stigmasterol **[269]**. From the stem bark of *E. fusca* Lour., a new compound, 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan **[271]** was isolated, along with 8 other known flavonoids, i.e. sandwicensin **[28]**, lupinifolin **[272]**, citflavanone **[274]**, lonchocarpol A **[115]**, erythrisenegalone **[112]**, liquiritigenin **[275]**, daidzein **[85]** and 8-prenyldaidzein **[23]**, and three long chain compounds (acid, alcohol and aromatic ester): cerinic acid **[273]**, 1-octacosanol **[233]** and erythrissinate B **[221]**. All of the tested compounds showed weak and no antimicrobial activity except lonchocarpol A **[115]** and lupinifolin **[272]** showed strongly active against *B. subtillis* and lonchocarpol A was moderately active against *E. faecalis* and *S. aureus*. Lonchocarpol A **[115]** also showed the highest *in vitro* antimalarial activity (EC$_{50}$ 1.6 μg/ml) against K1 strain, when compared with the other active compounds: 8-prenyldaidzein **[23]**, erythrabyssin II **[8]** and citflavanone **[274]** (EC$_{50}$ 3.9,5.0 and 5.0 μg/ml, respectively). However, lonchocarpol A exhibited no *in vivo* antimalarial activity in both oral and subcutaneous administration (at 20 mg/kg) to mice. In addition, all of tested compounds showed only marginal free radical scavenging activity. Almost all of the tested compounds showed weak Antituberculosis activity against H37Ra. Erythrisenegalone **[112]** and lupinifolin **[272]** showed strong cytotoxicity against breast cancer (BC) cell line.

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APPENDICES

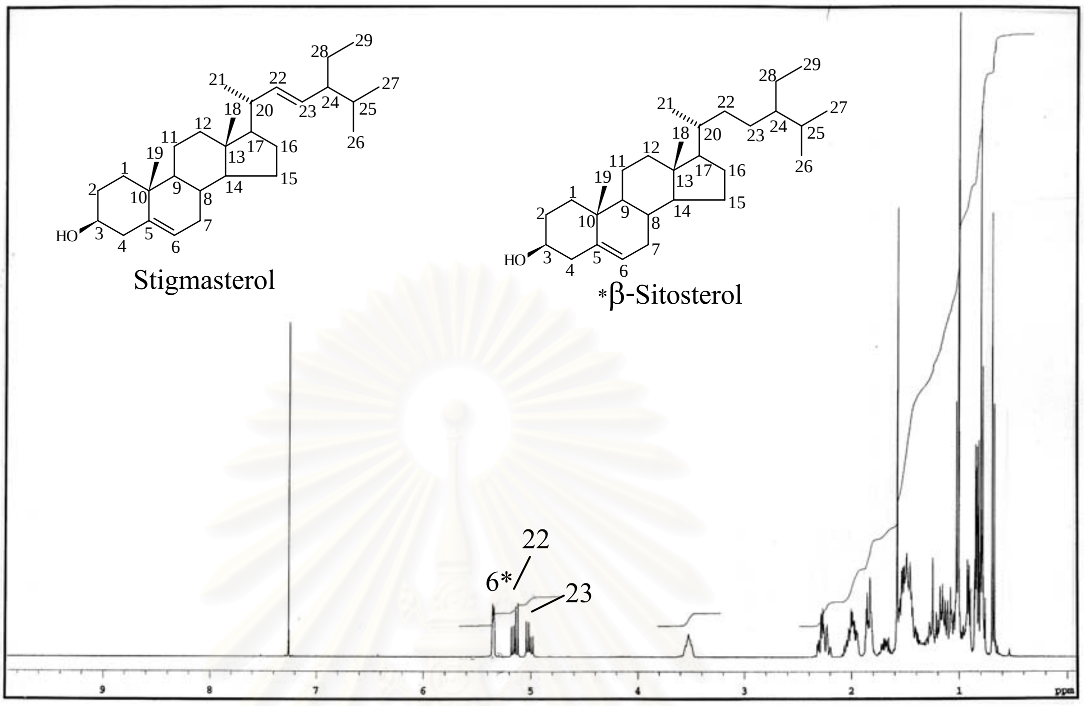


Figure 6 ^{1}H NMR (400 MHz) Spectrum of compound ES1 ($CDCl_3$)

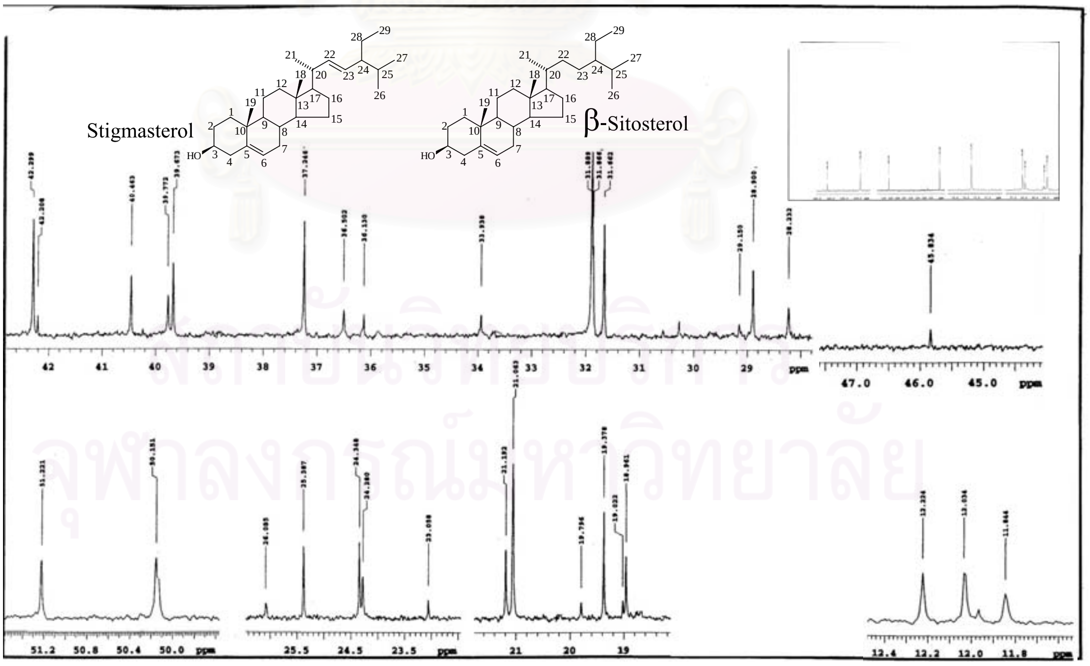


Figure 7 ^{13}C NMR (100 MHz) Spectrum of compound ES1 ($CDCl_3$)

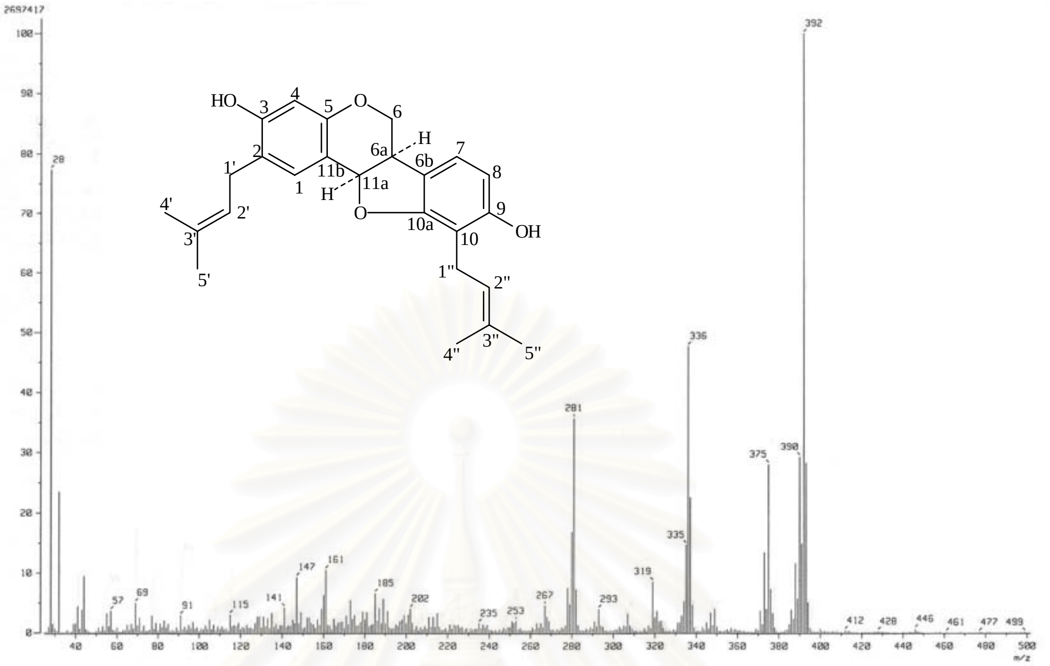


Figure 8 EI Mass spectrum of compound ES2

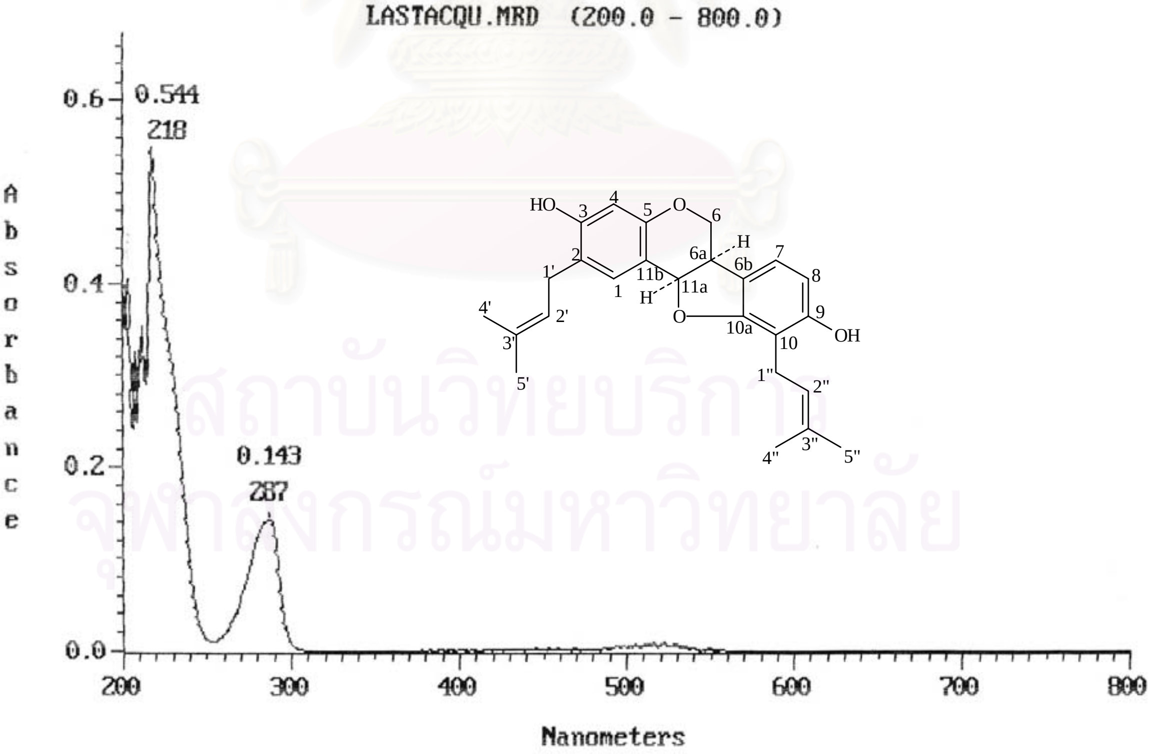


Figure 9 UV Spectrum of compound ES2 (MeOH)

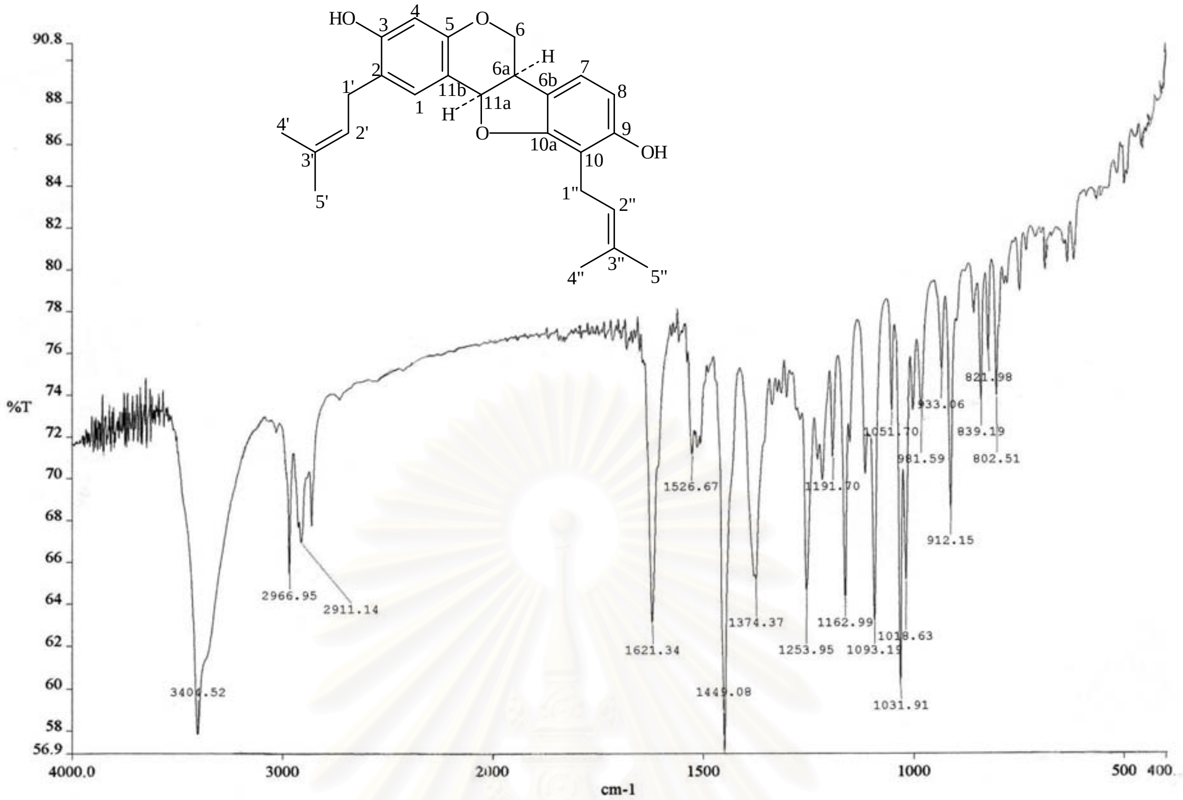


Figure 10 IR Spectrum of compound ES2 (KBr disc)

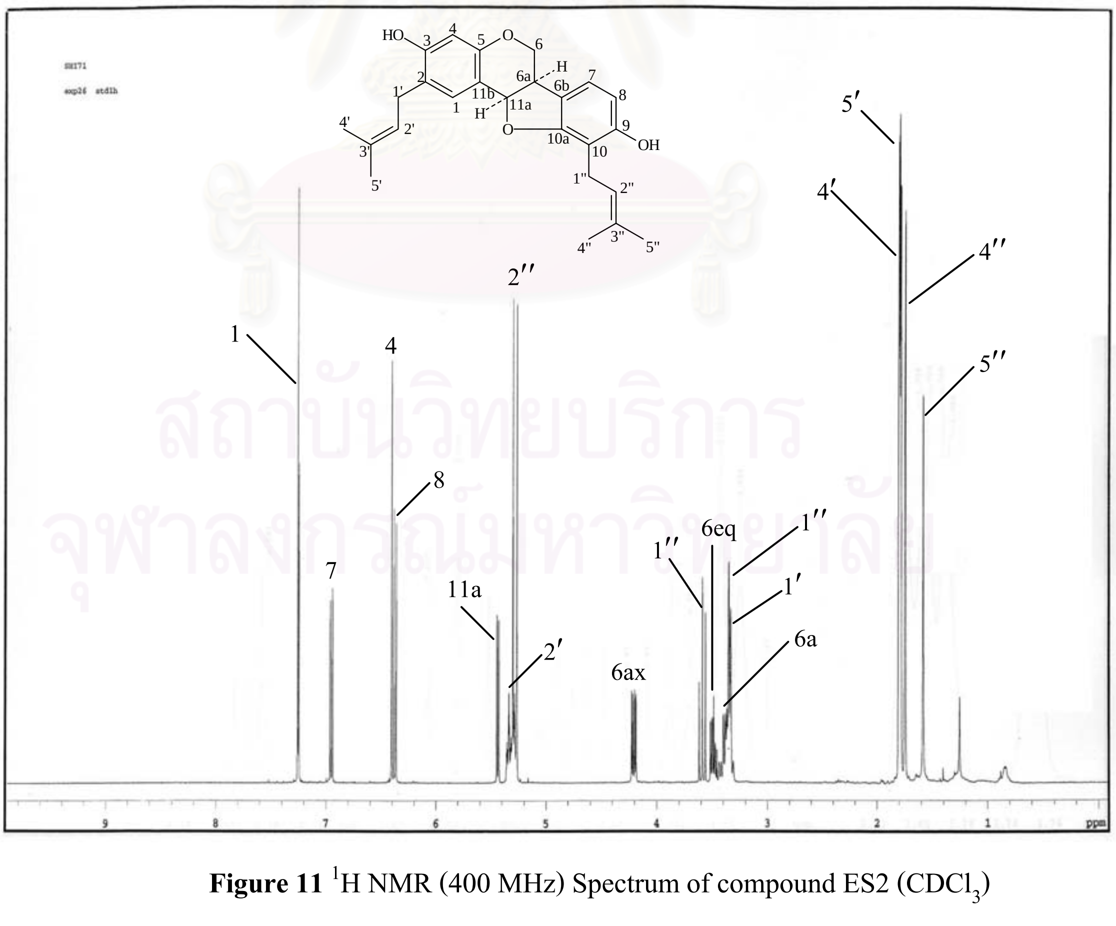


Figure 11 ^{1}H NMR (400 MHz) Spectrum of compound ES2 ($CDCl_3$)

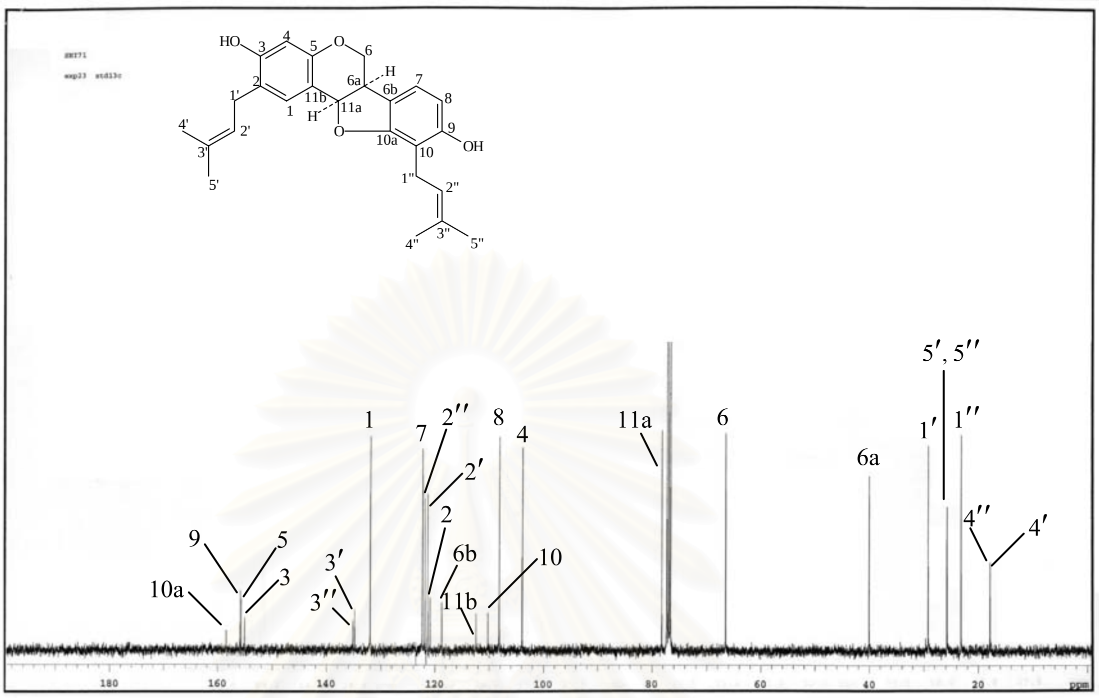


Figure 12 ^{13}C NMR (100 MHz) Spectrum of compound ES2 ($CDCl_3$)

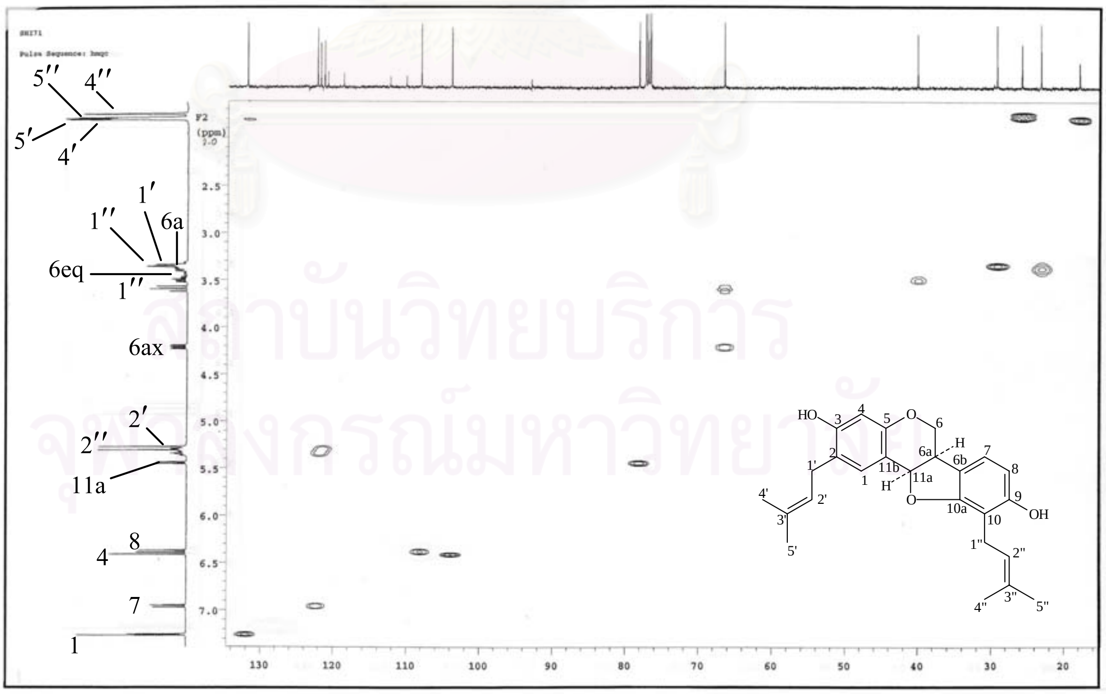


Figure 13 HMQC Spectrum of compound ES2 ($CDCl_3$)

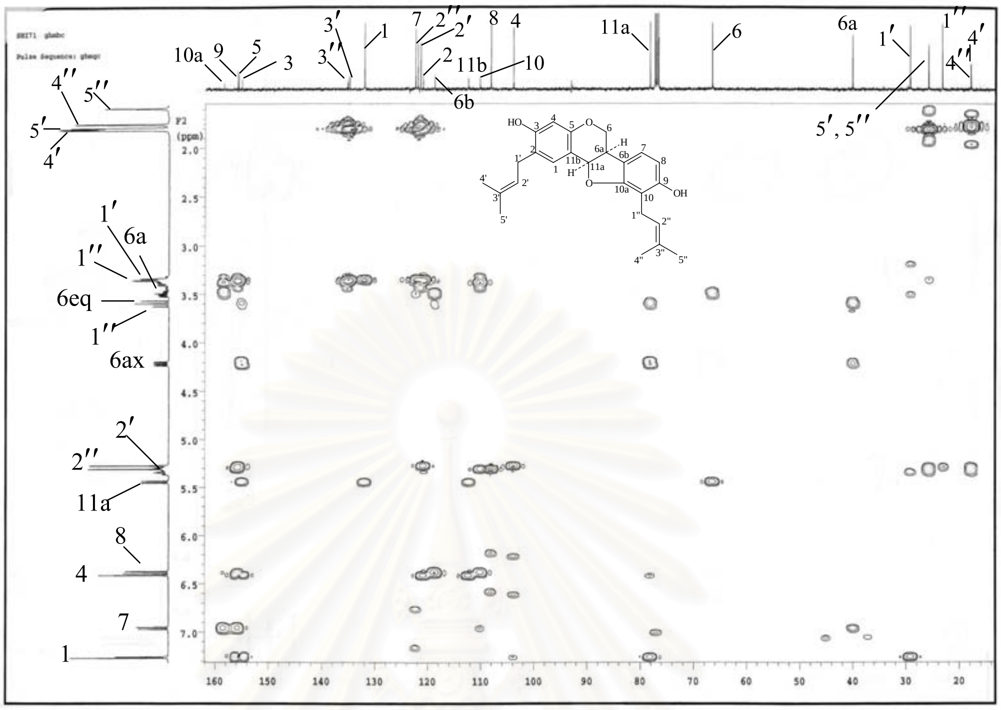


Figure 14 HMBC Spectrum of compound ES2 ($CDCl_3$)

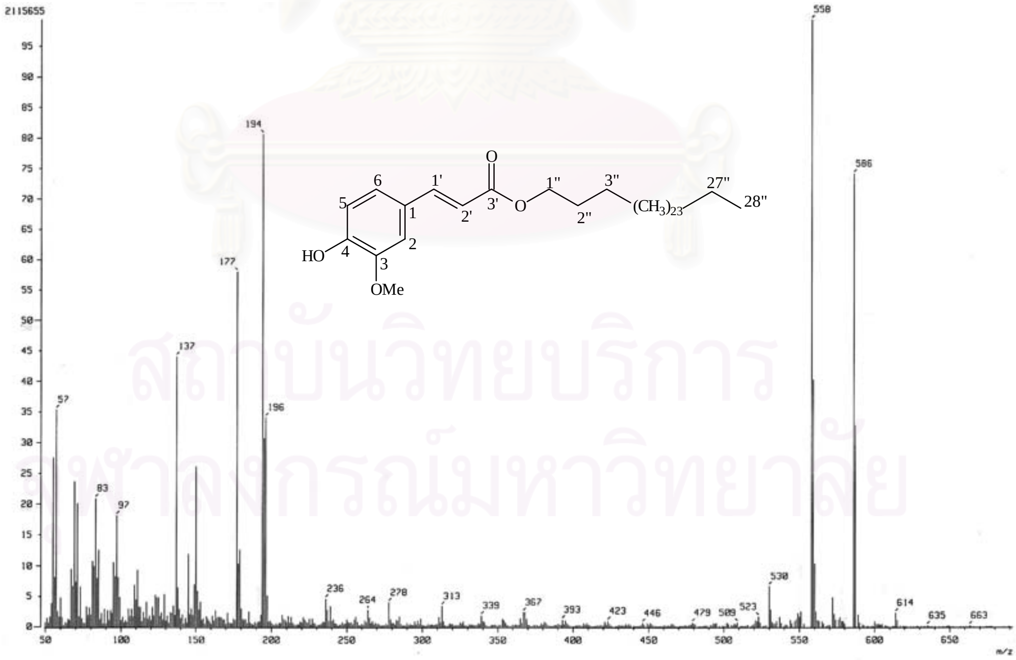


Figure 15 EI Mass spectrum of compound ES3 and EF5

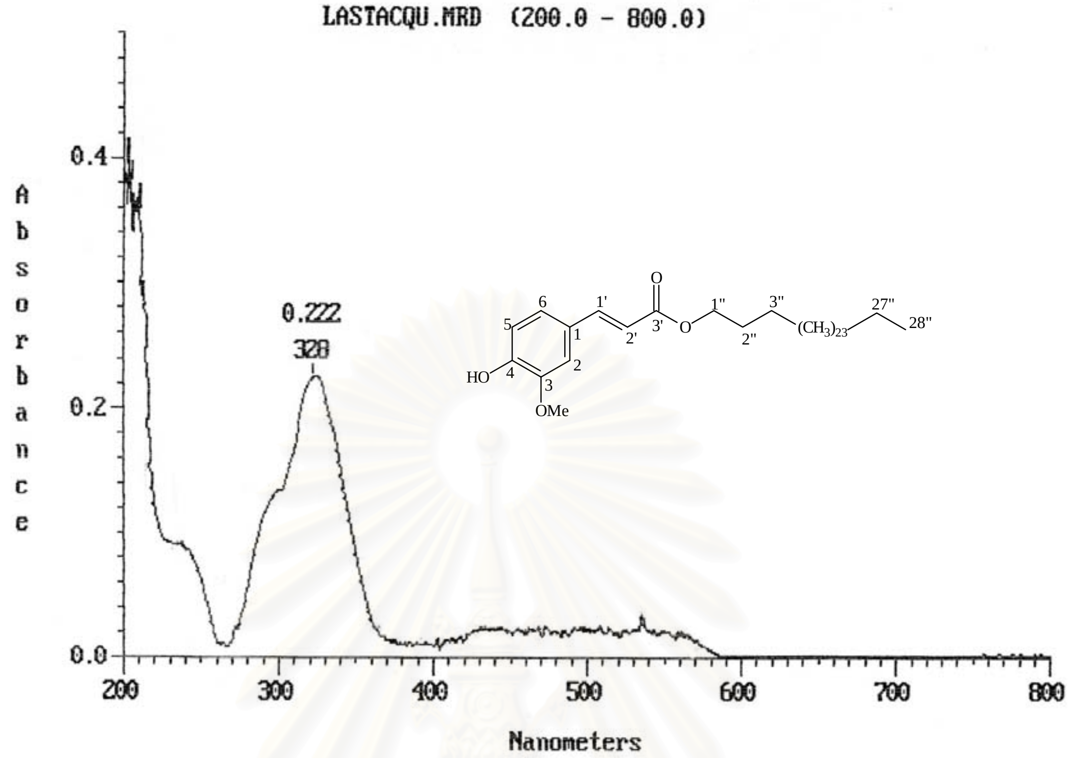


Figure 16 UV Spectrum of compound ES3 and EF5 (MeOH)

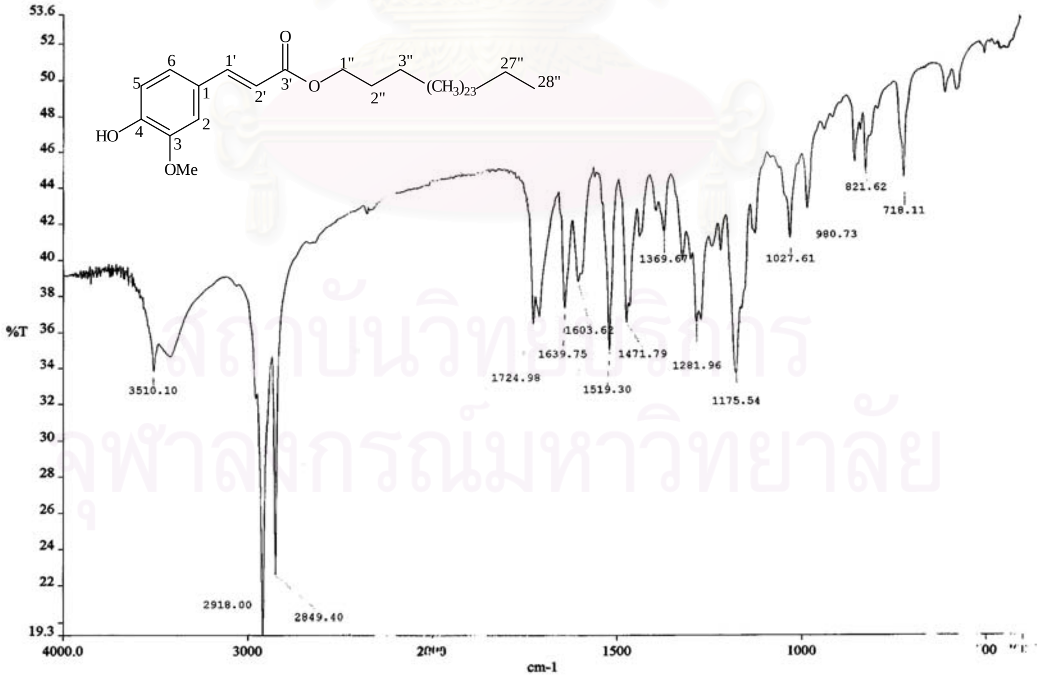


Figure 17 IR Spectrum of compound ES3 and EF5 (KBr disc)

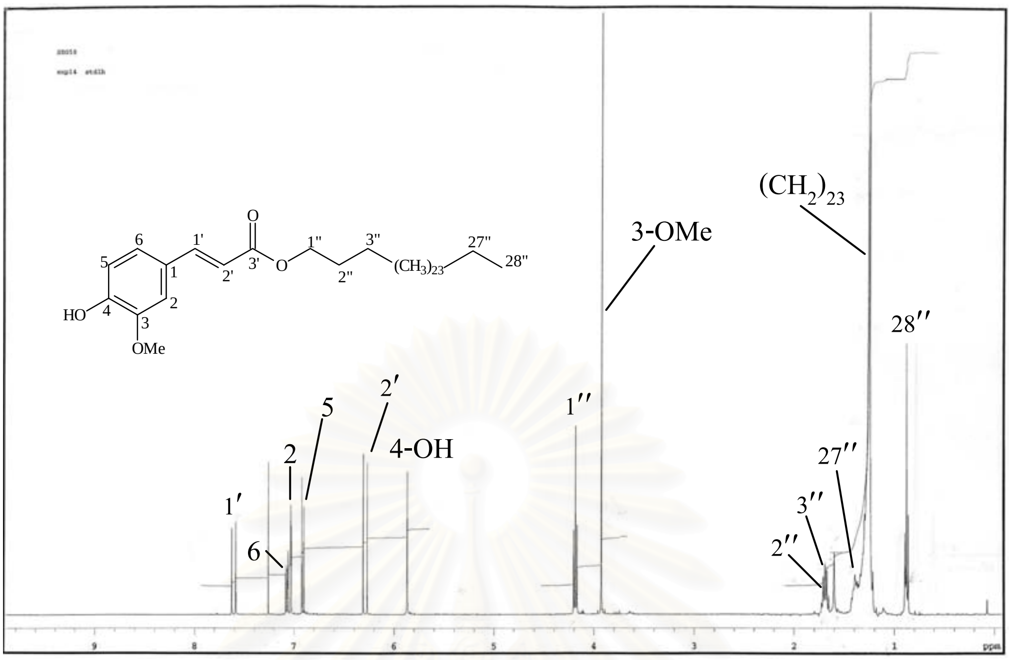


Figure 18 ^{1}H NMR (400 MHz) Spectrum of compound ES3 and EF5 ($CDCl_3$)

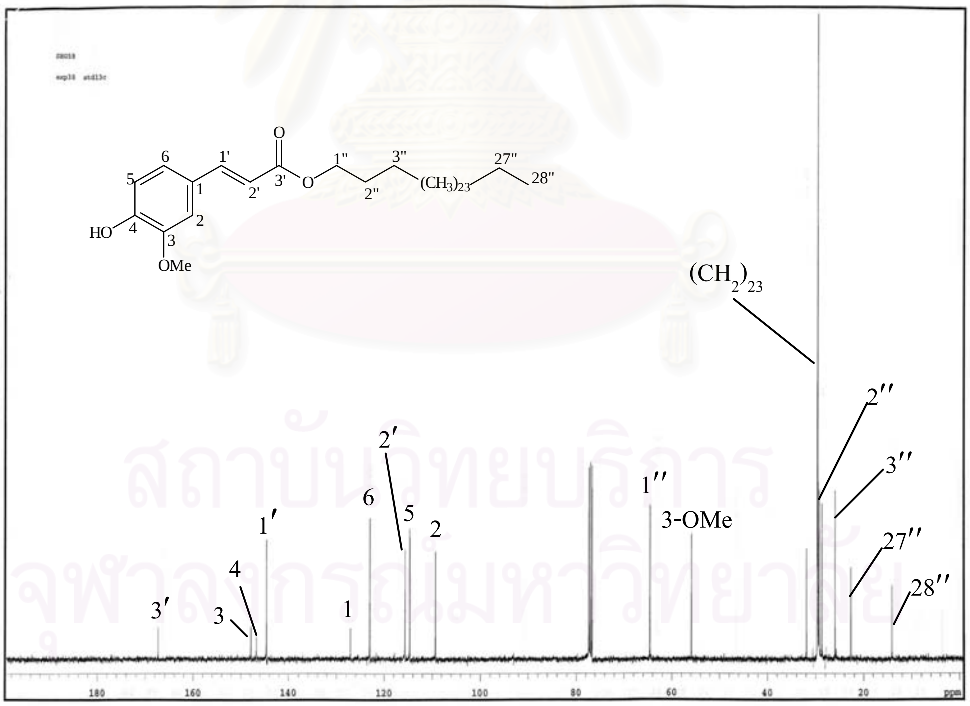


Figure 19 ^{13}C NMR (100 MHz) Spectrum of compound ES3 and EF5 ($CDCl_3$)

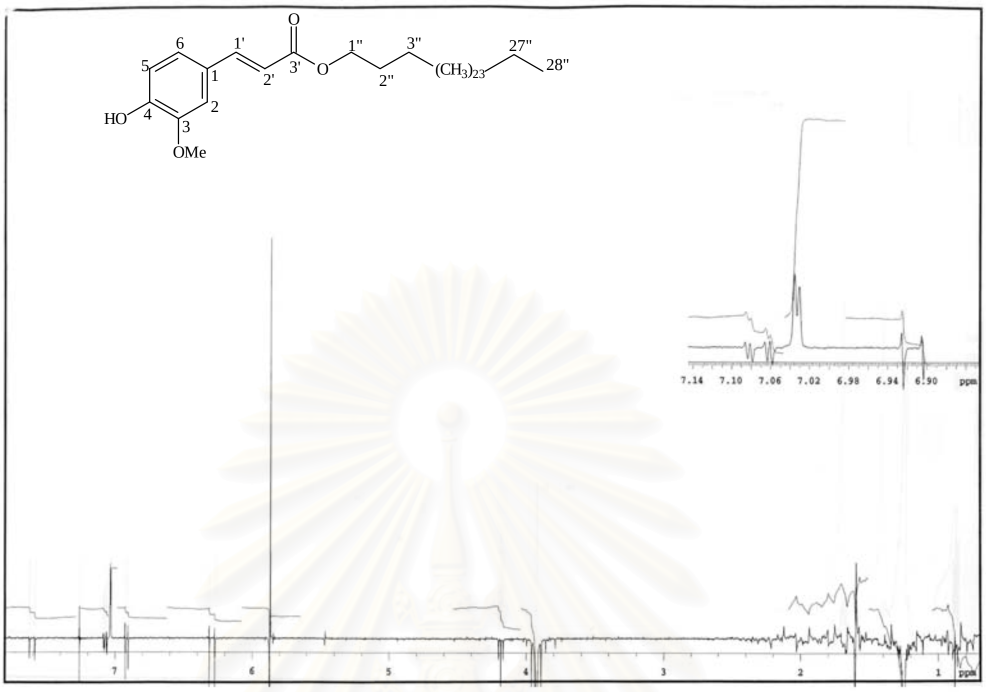


Figure 20 NOE Spectrum of compound ES3 and EF5 ($CDCl_3$)

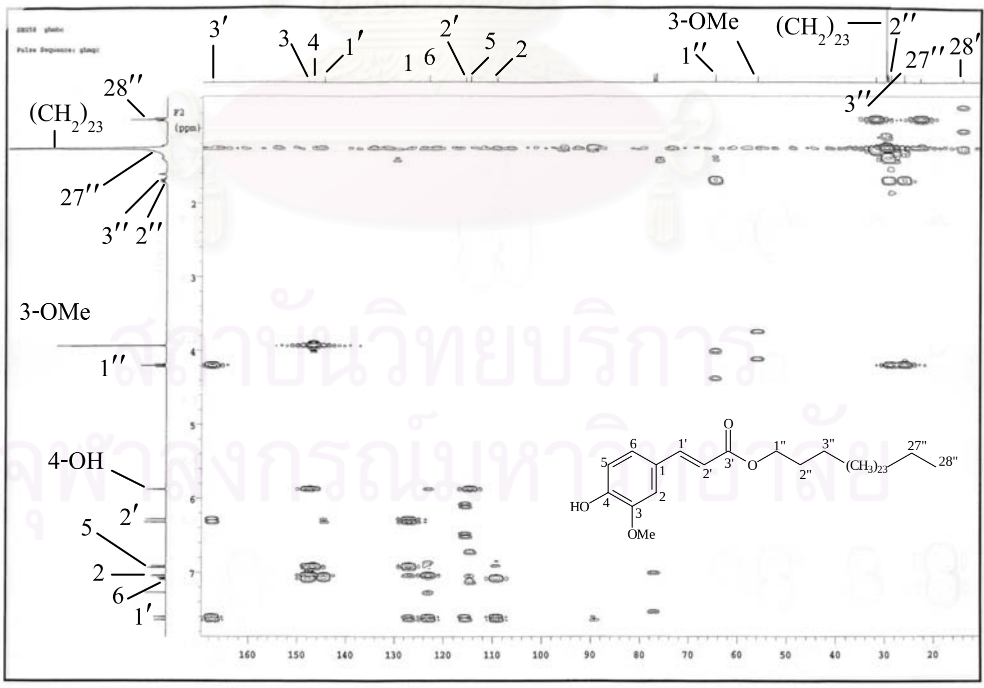


Figure 21 HMBC Spectrum of compound ES3 and EF5 ($CDCl_3$)

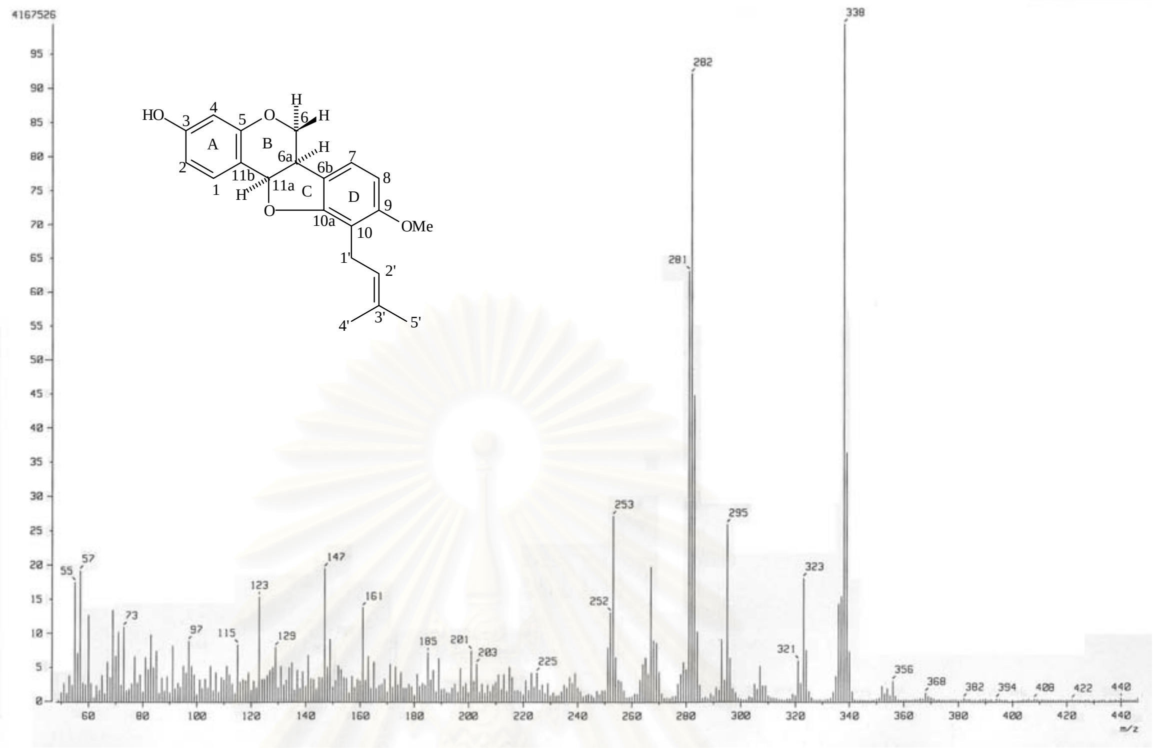


Figure 22 EI Mass spectrum of compound ES4 and EF2

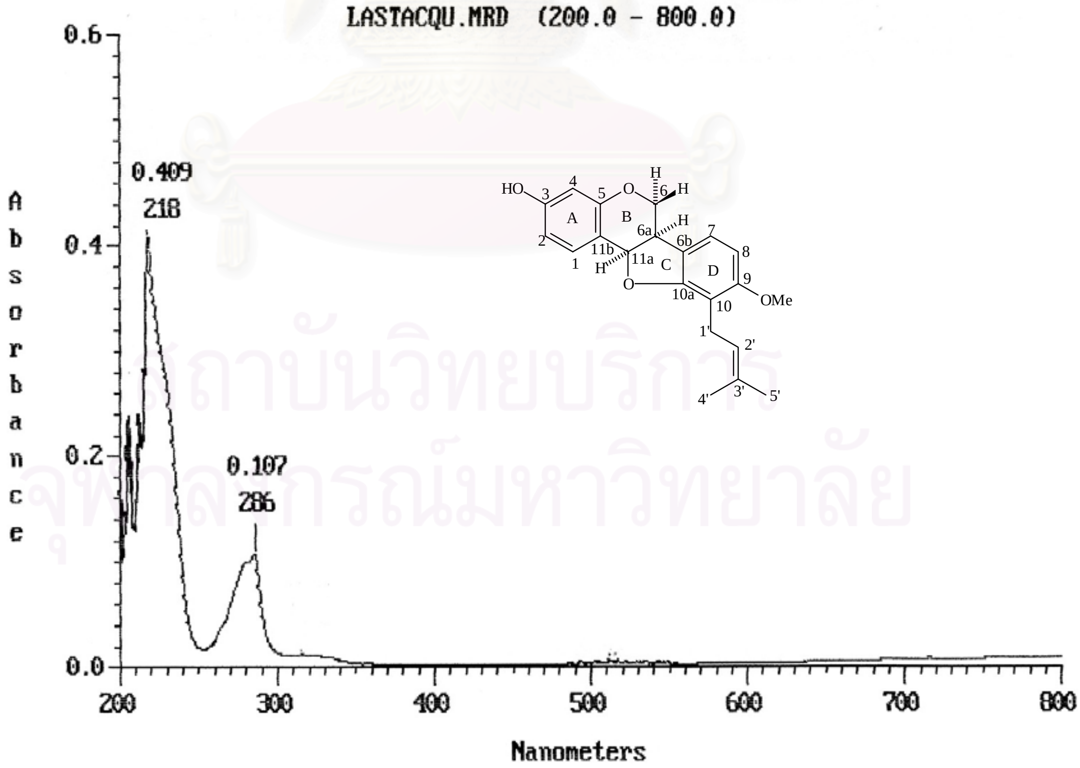


Figure 23 UV Spectrum of compound ES4 and EF2 (MeOH)

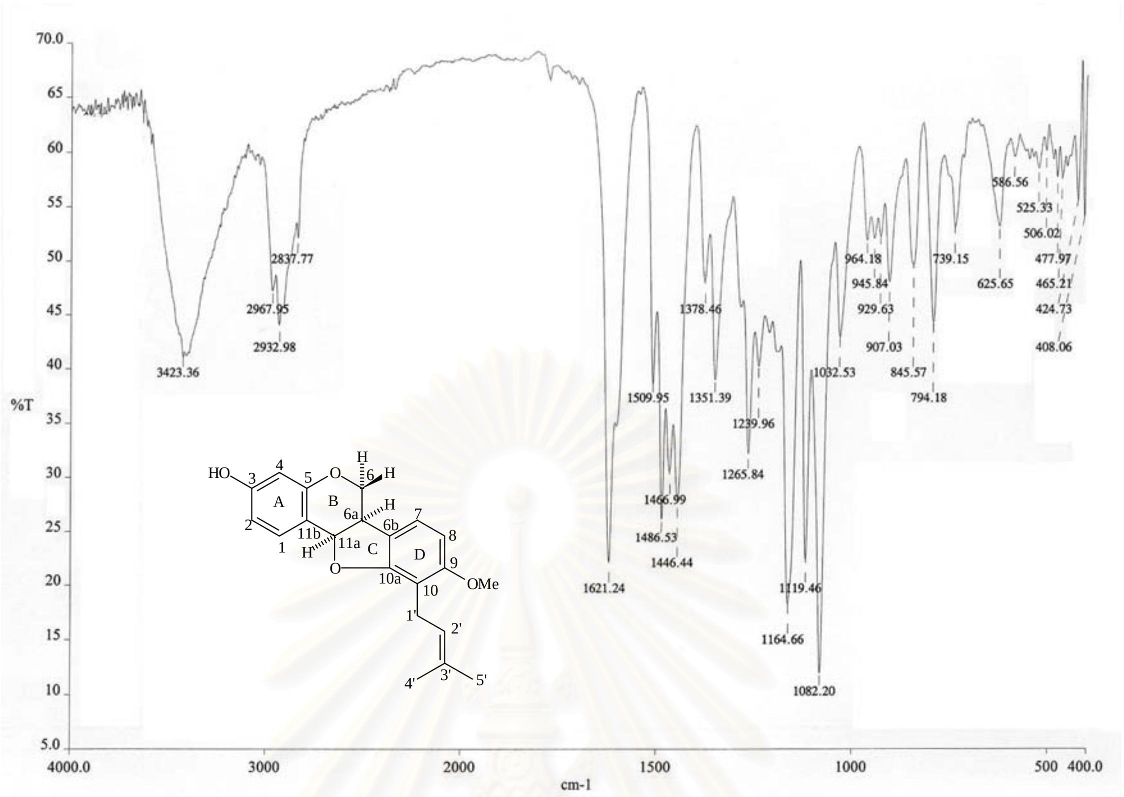


Figure 24 IR Spectrum of compound ES4 and EF2 (Film)

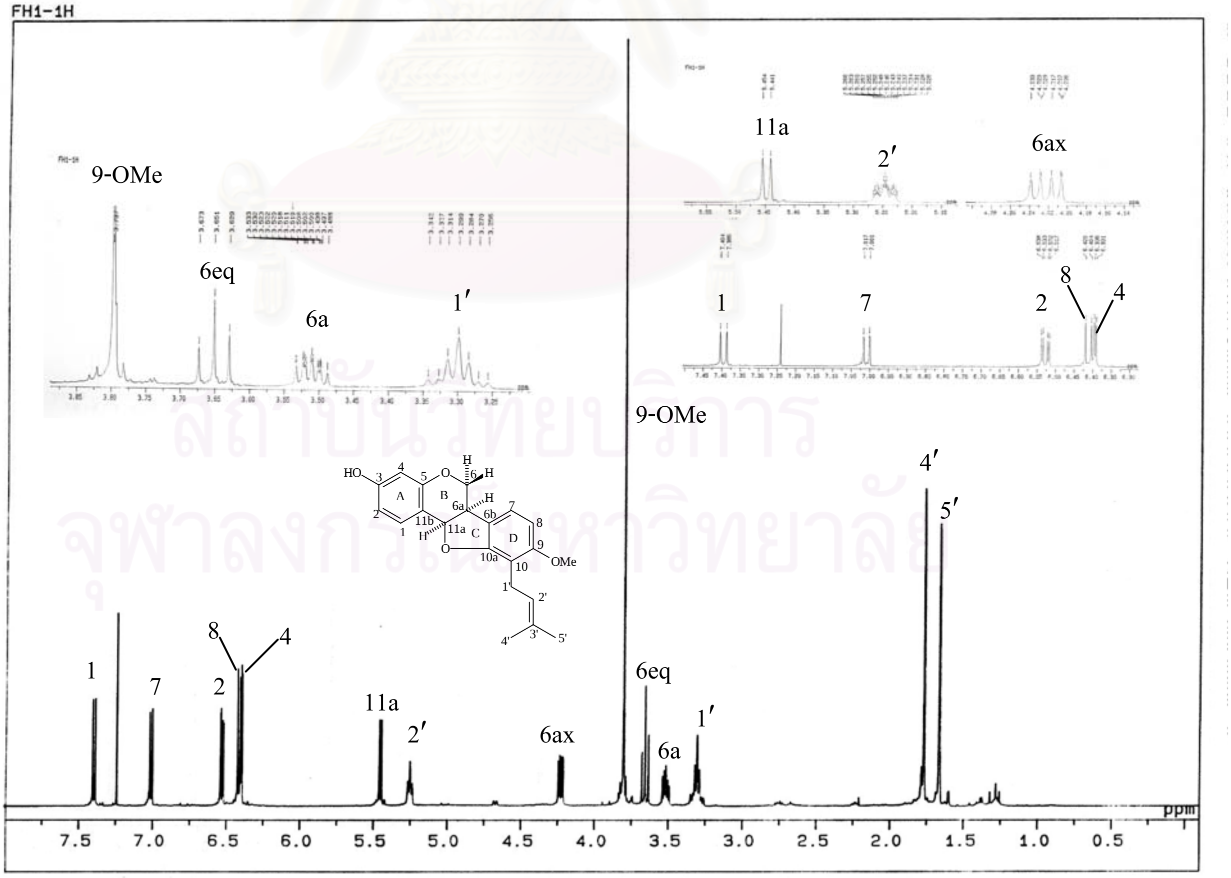


Figure 25 ^{1}H NMR (500 MHz) Spectrum of compound ES4 and EF2 ($CDCl_3$)

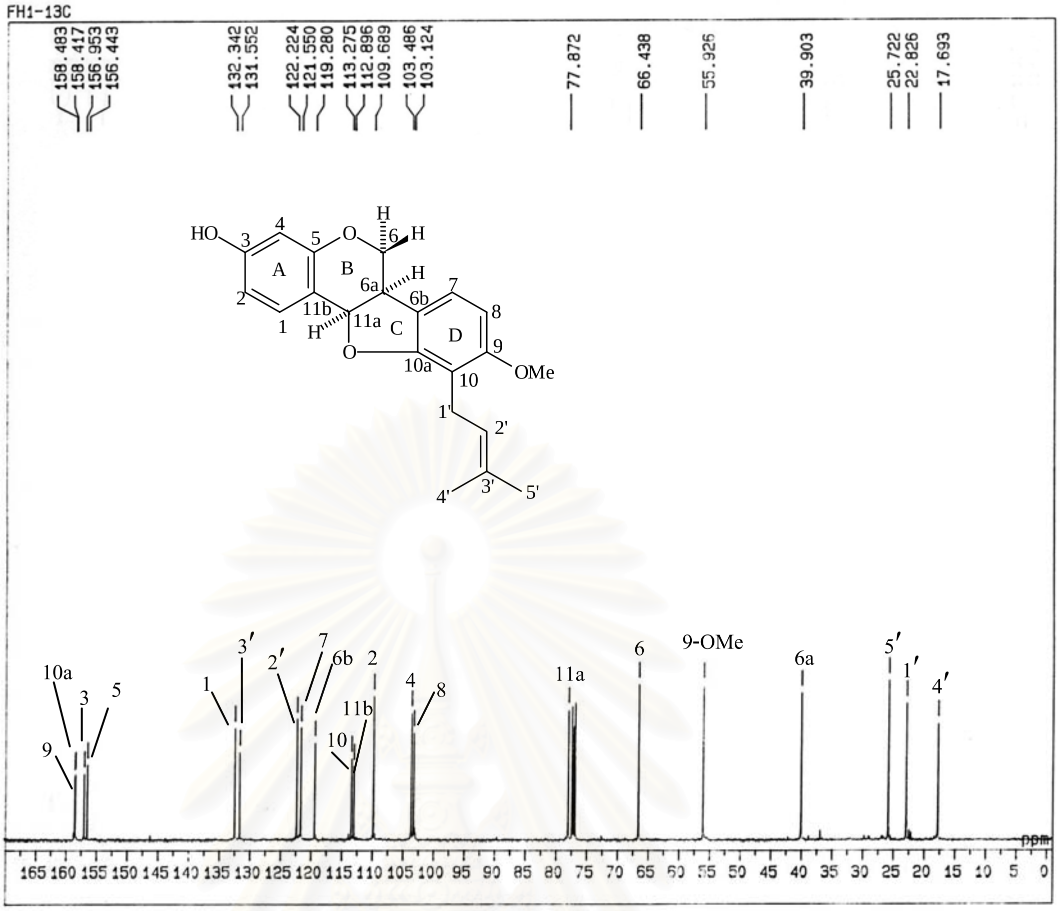


Figure 26 ^{13}C NMR (125 MHz) Spectrum of compound ES4 and EF2 (CDCl$_3$)

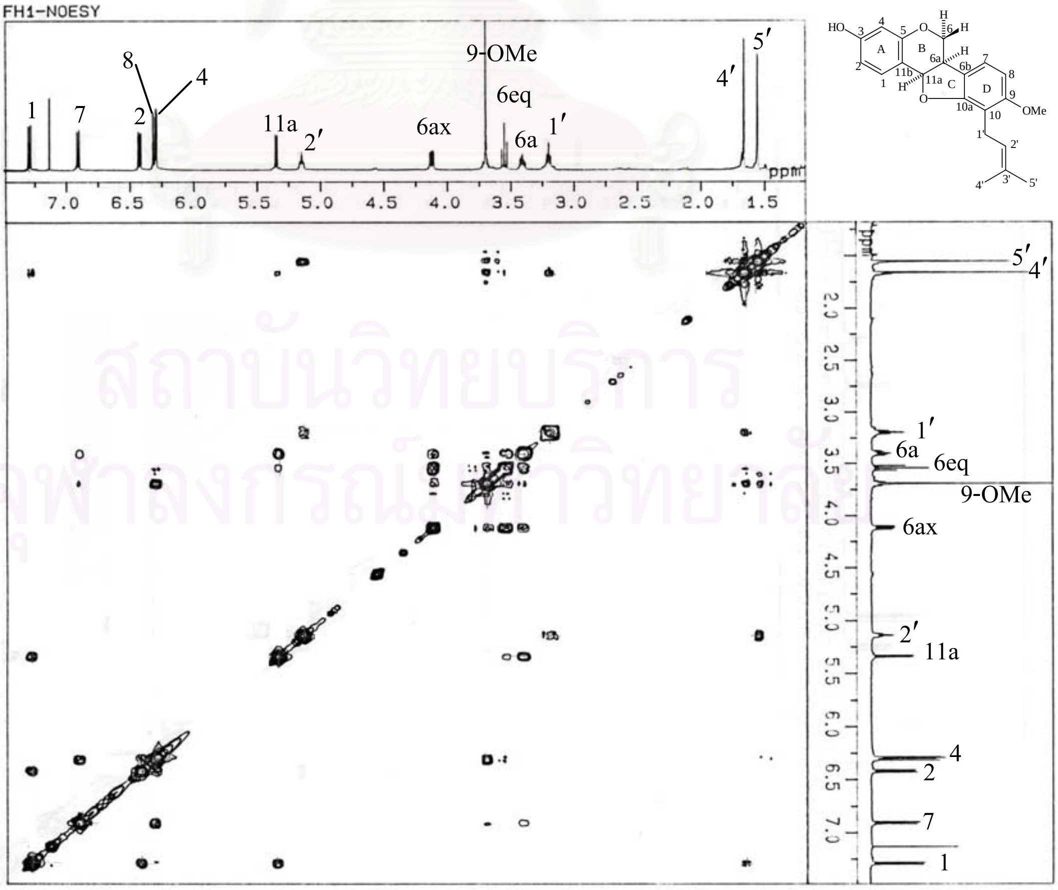


Figure 27 NOESY Spectrum of compound ES4 and EF2 (CDCl$_3$)

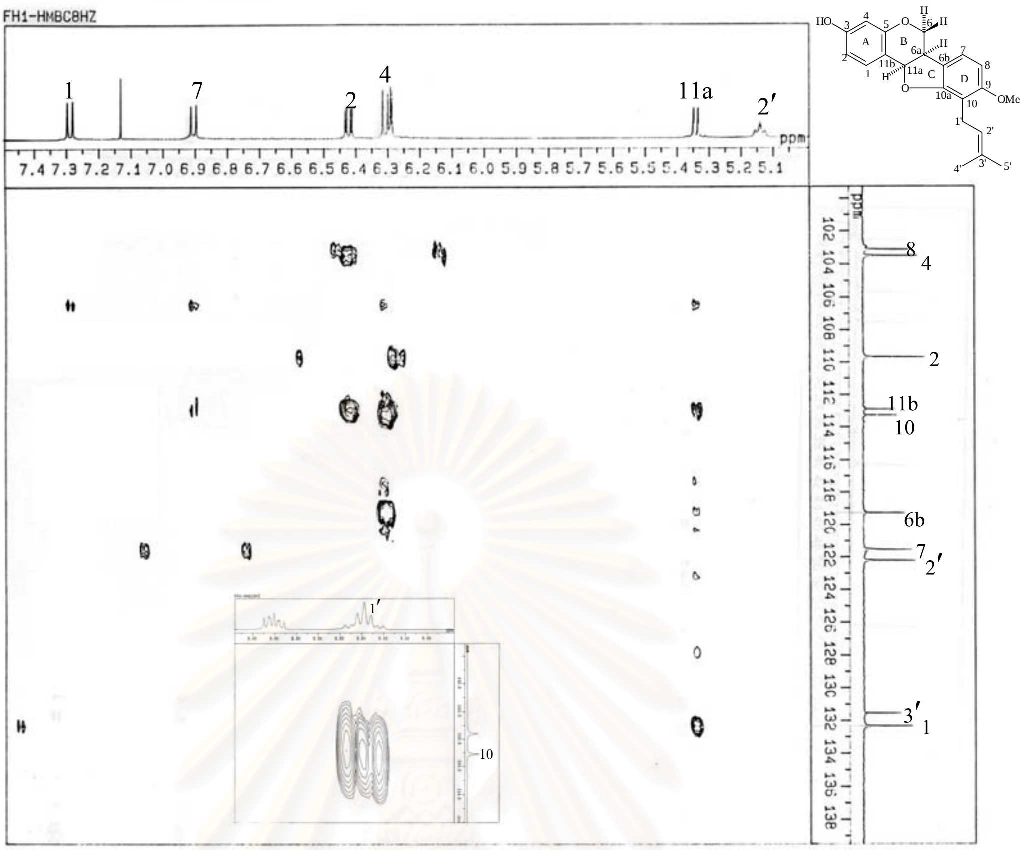


Figure 28 HMBC Spectrum of compound ES4 and EF2 ($CDCl_3$)

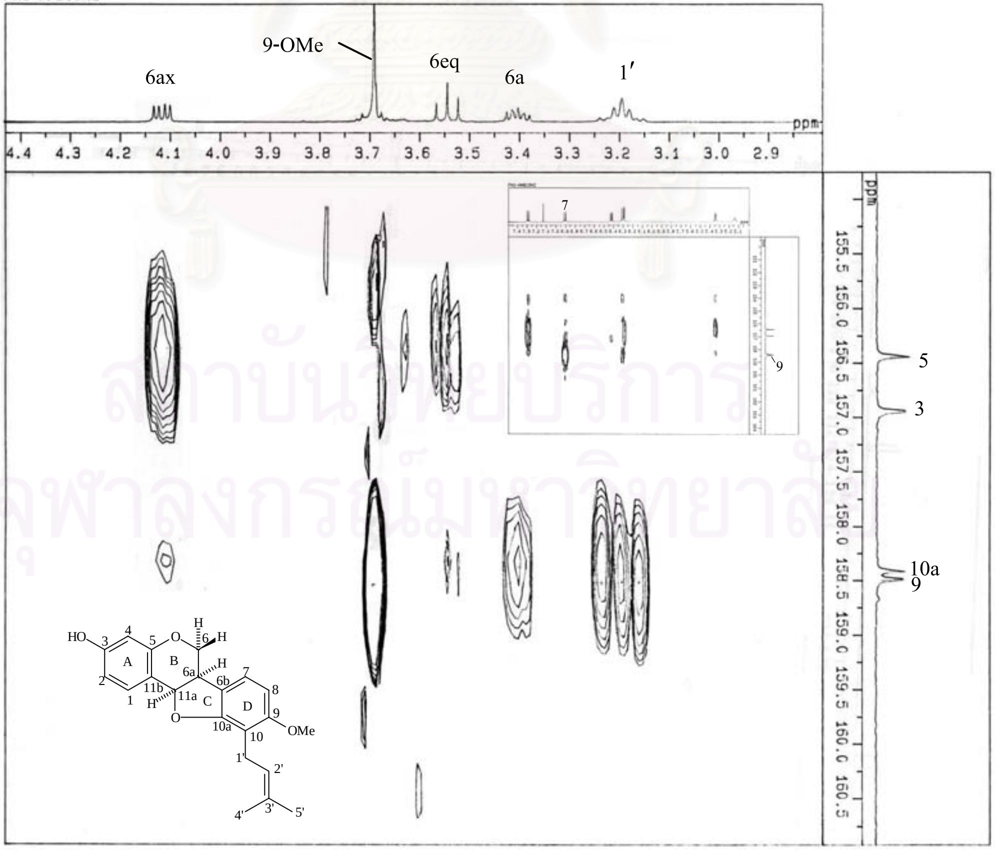


Figure 29 HMBC Spectrum of compound ES4 and EF2 ($CDCl_3$)

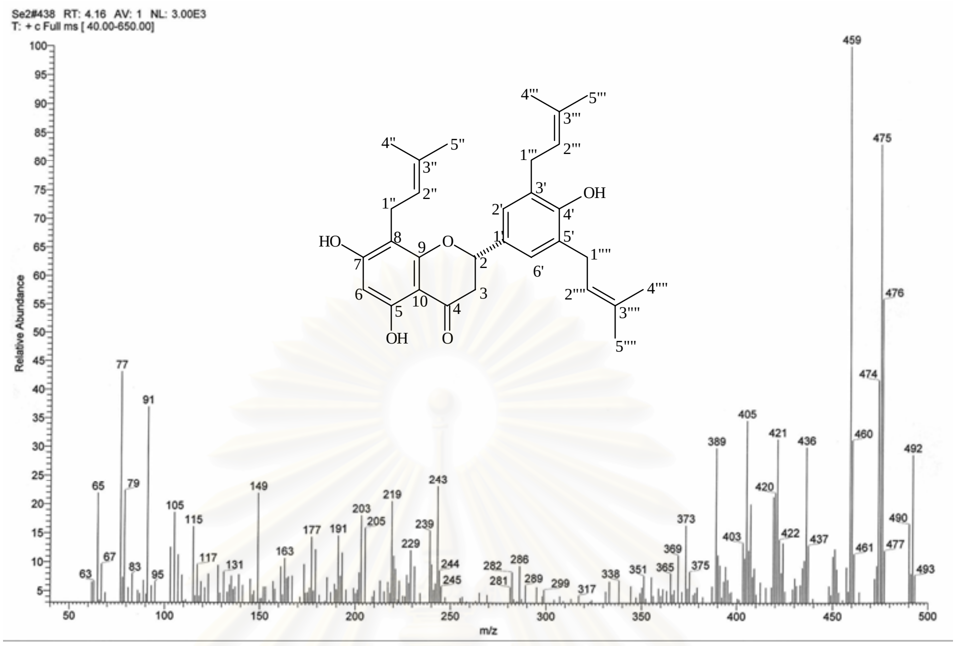


Figure 30 EI Mass spectrum of compound ES5

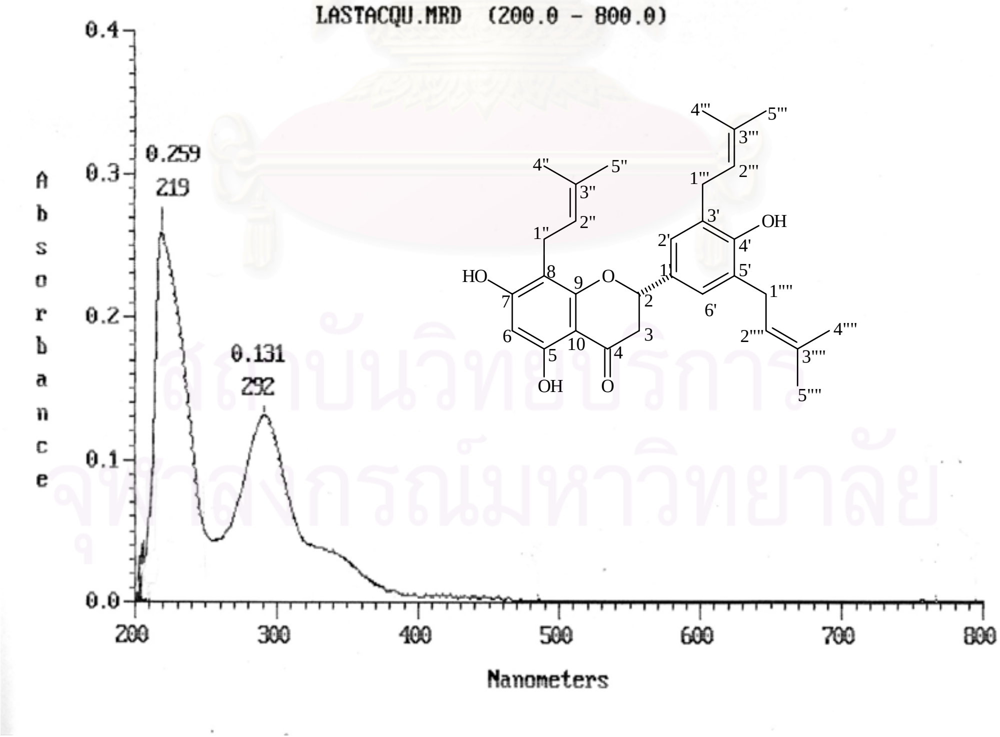


Figure 31 UV Spectrum of compound ES5 (MeOH)

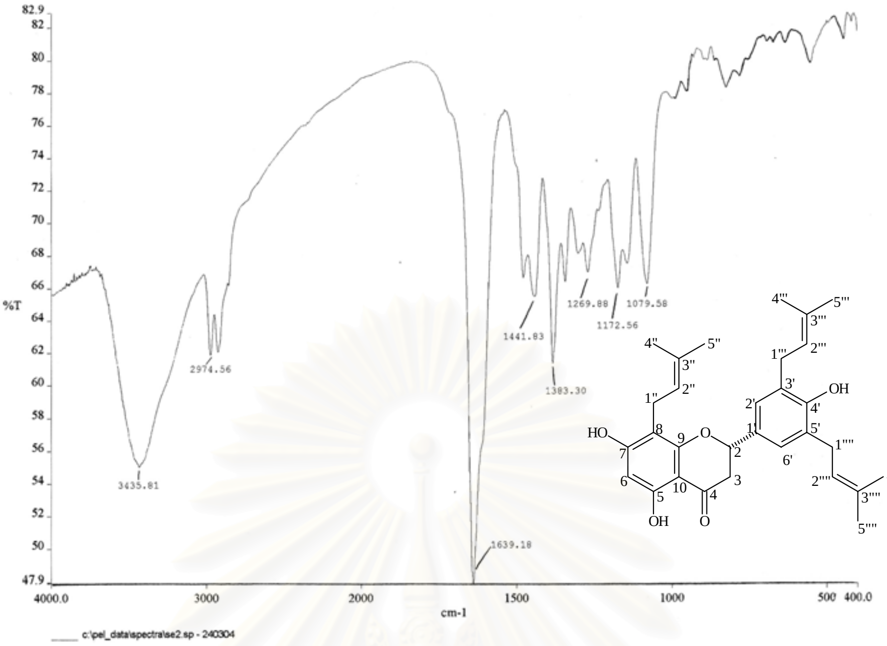


Figure 32 IR Spectrum of compound ES5 (Film)

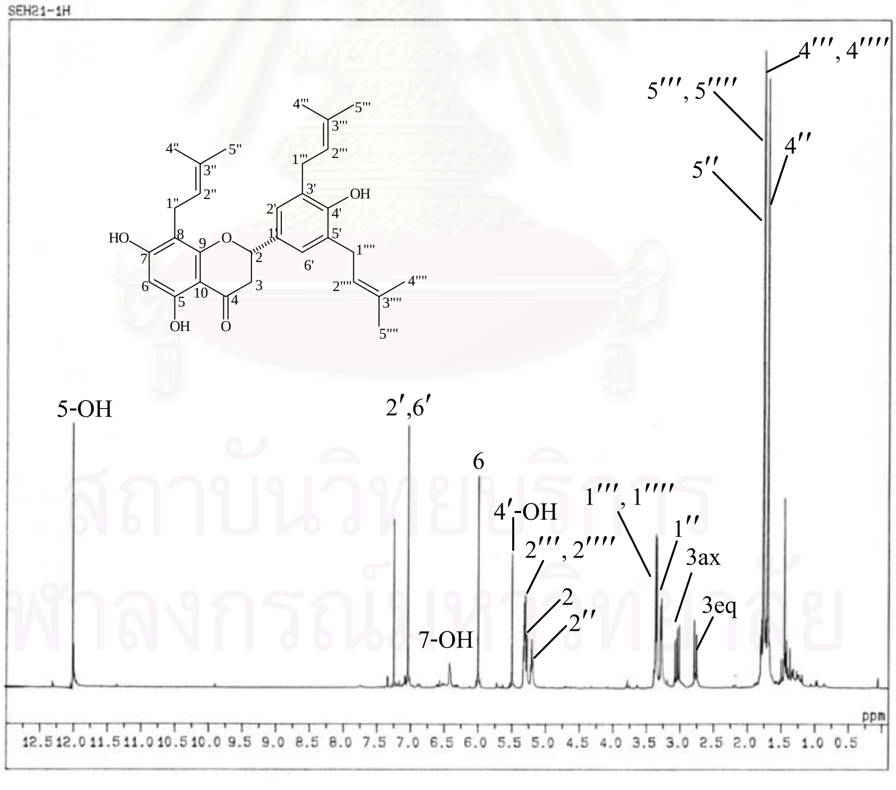


Figure 33 ^{1}H NMR (500 MHz) Spectrum of compound ES5 ($CDCl_3$)

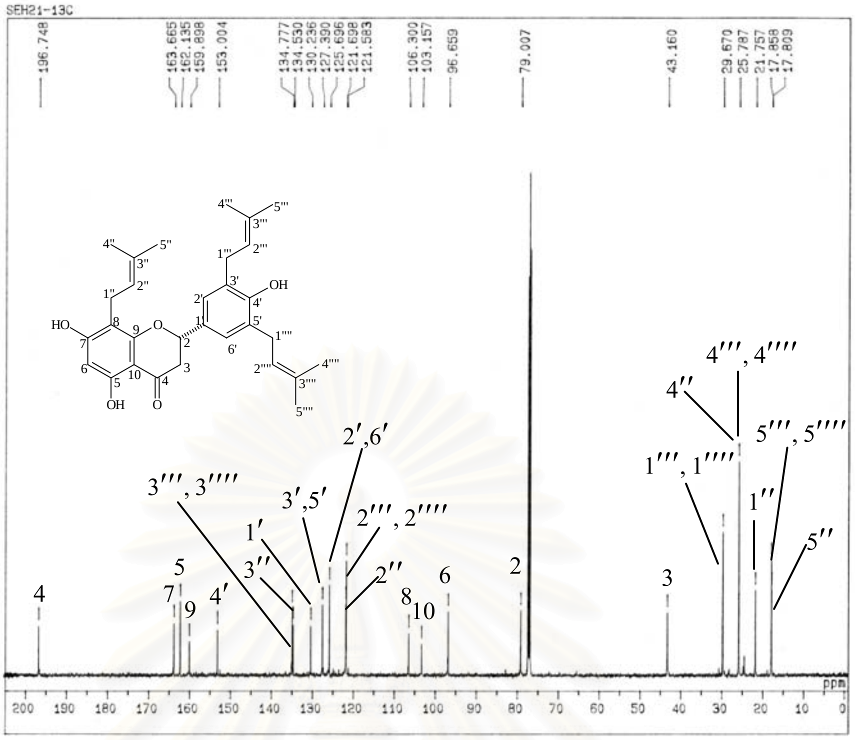


Figure 34 ^{13}C NMR (125 MHz) Spectrum of compound ES5 ($CDCl_3$)

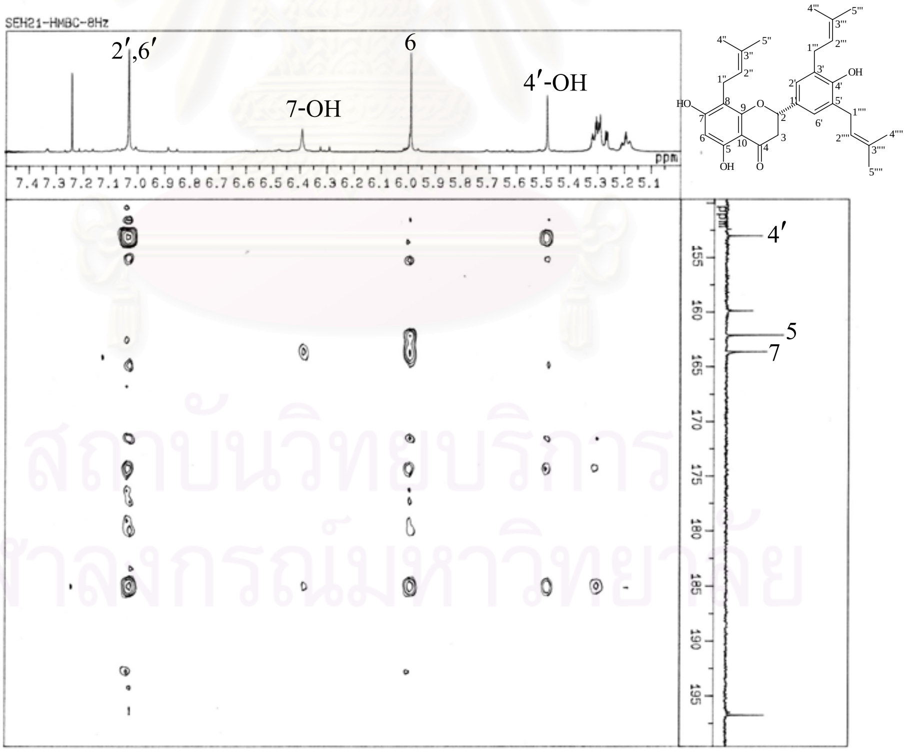


Figure 35 HMBC Spectrum of compound ES5 ($CDCl_3$)

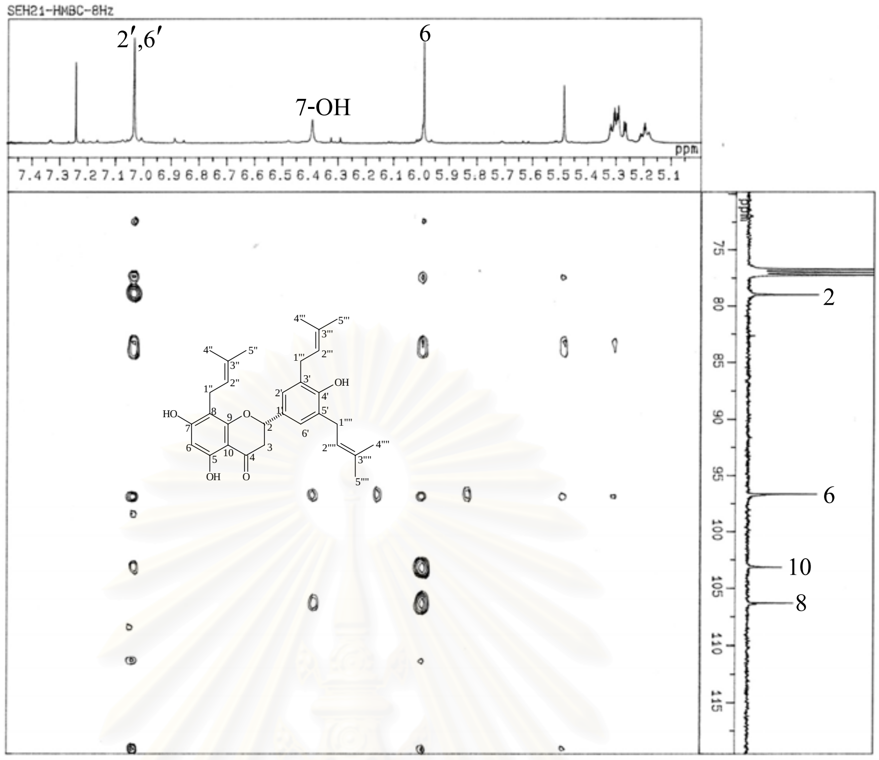


Figure 36 HMBC Spectrum of compound ES5 ($CDCl_3$)

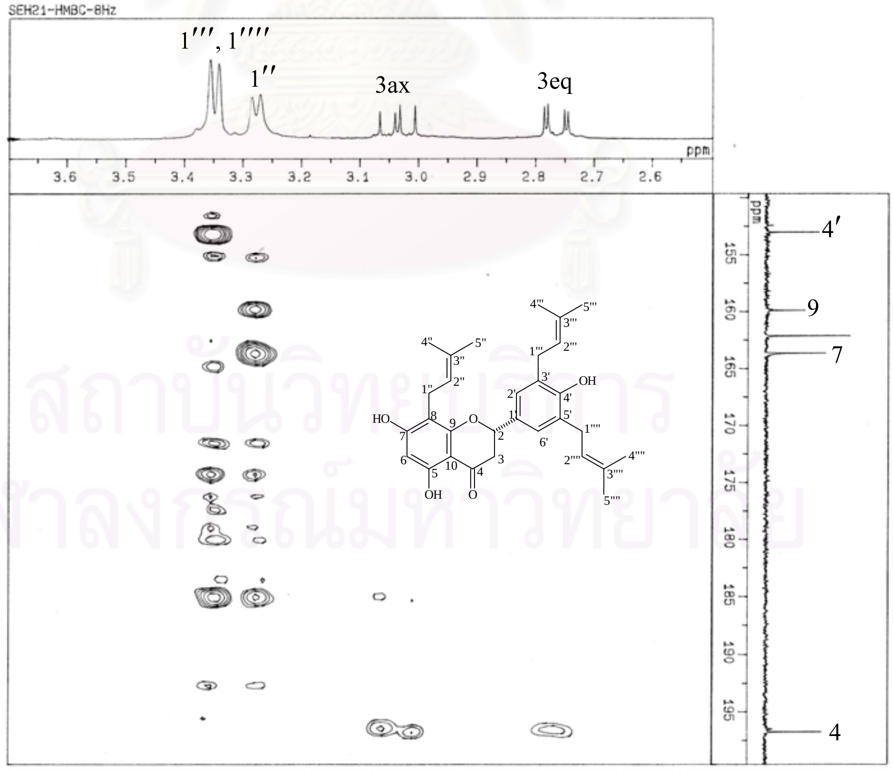


Figure 37 HMBC Spectrum of compound ES5 ($CDCl_3$)

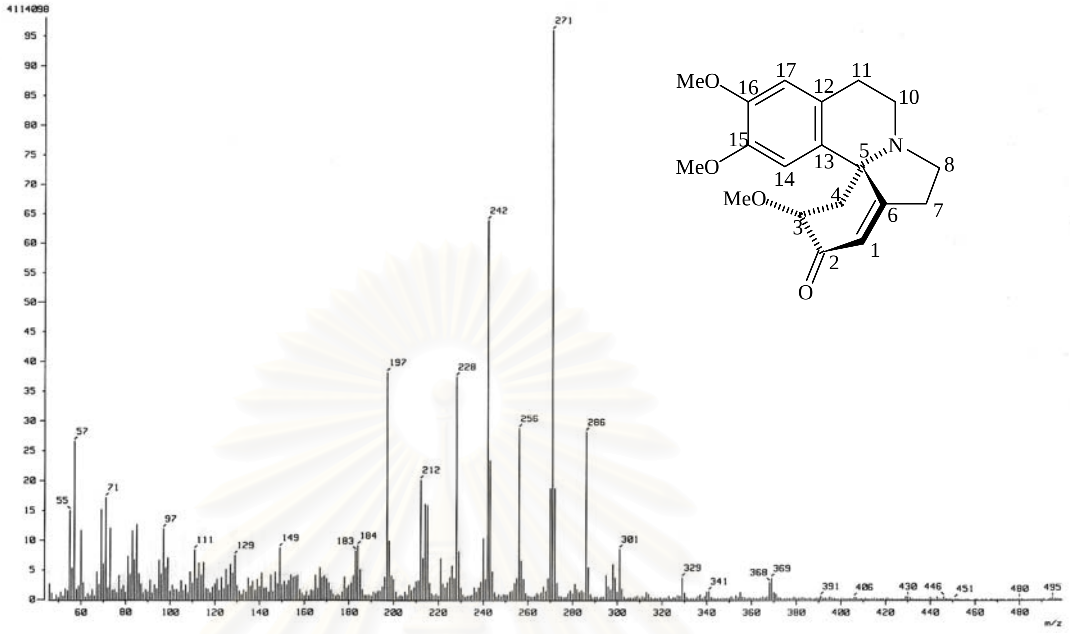


Figure 38 EI Mass spectrum of compound ES6

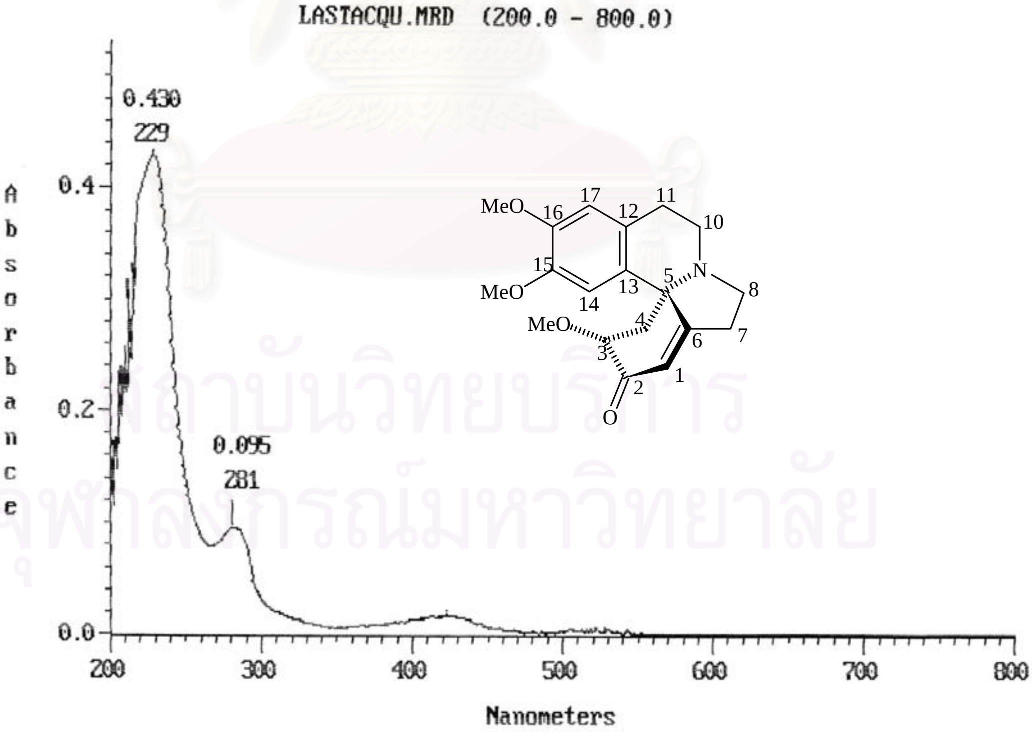


Figure 39 UV Spectrum of compound ES6 (MeOH)

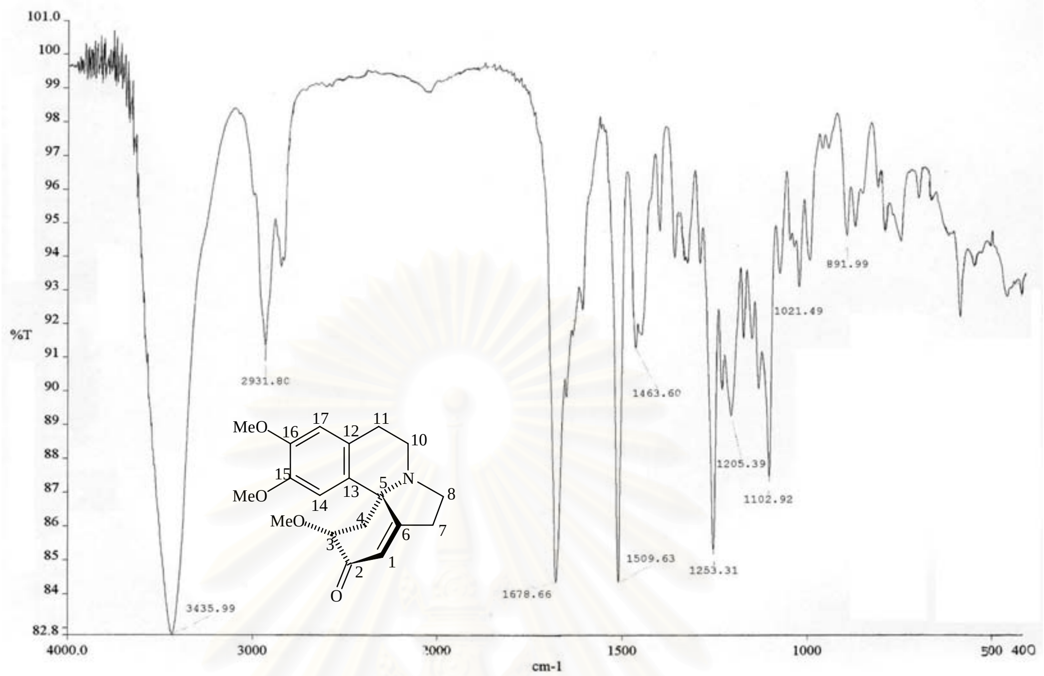


Figure 40 IR Spectrum of compound ES6 (Film)

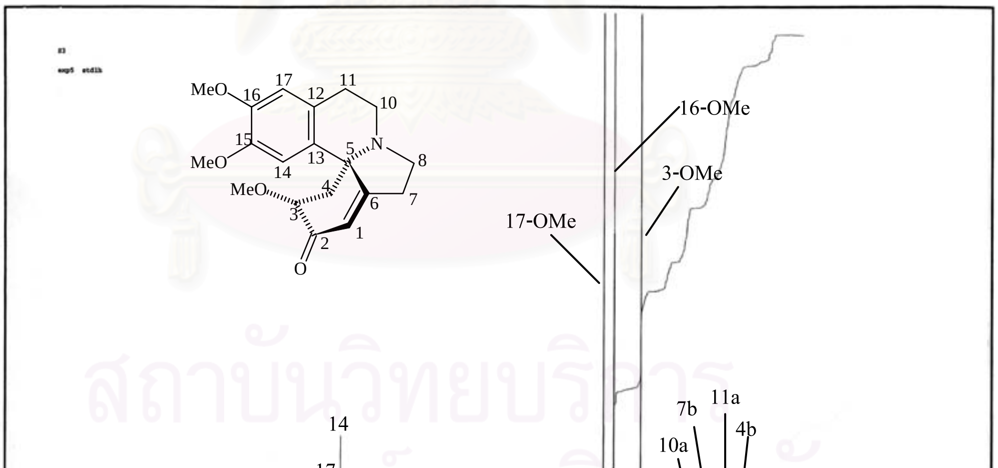


Figure 41 ^{1}H NMR (400 MHz) Spectrum of compound ES6 ($CDCl_3$)

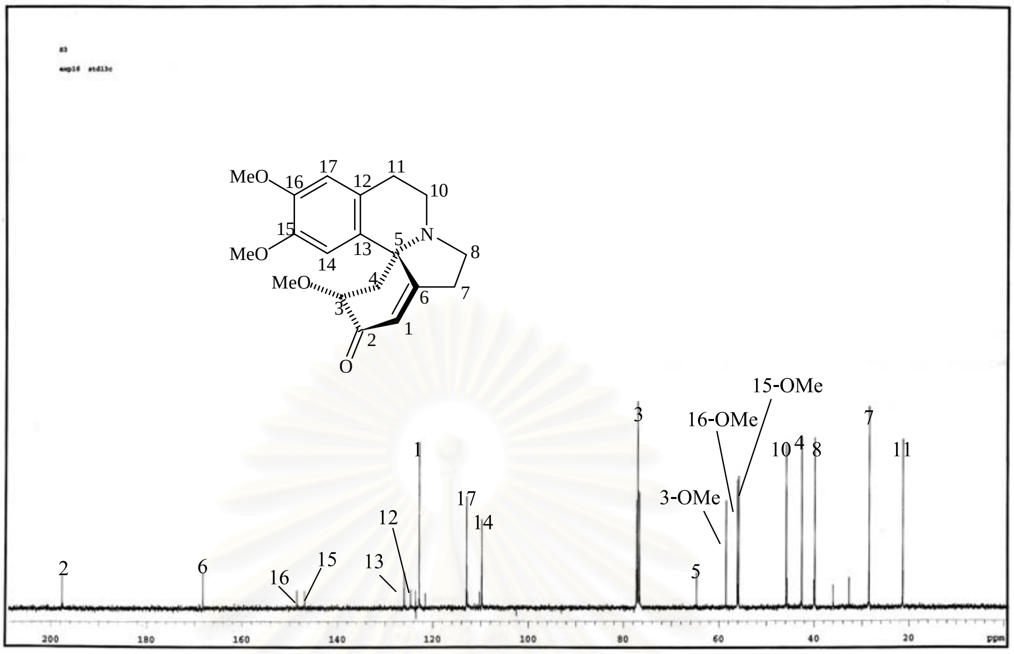


Figure 42 ^{13}C NMR (100 MHz) Spectrum of compound ES6(CDCl$_3$)

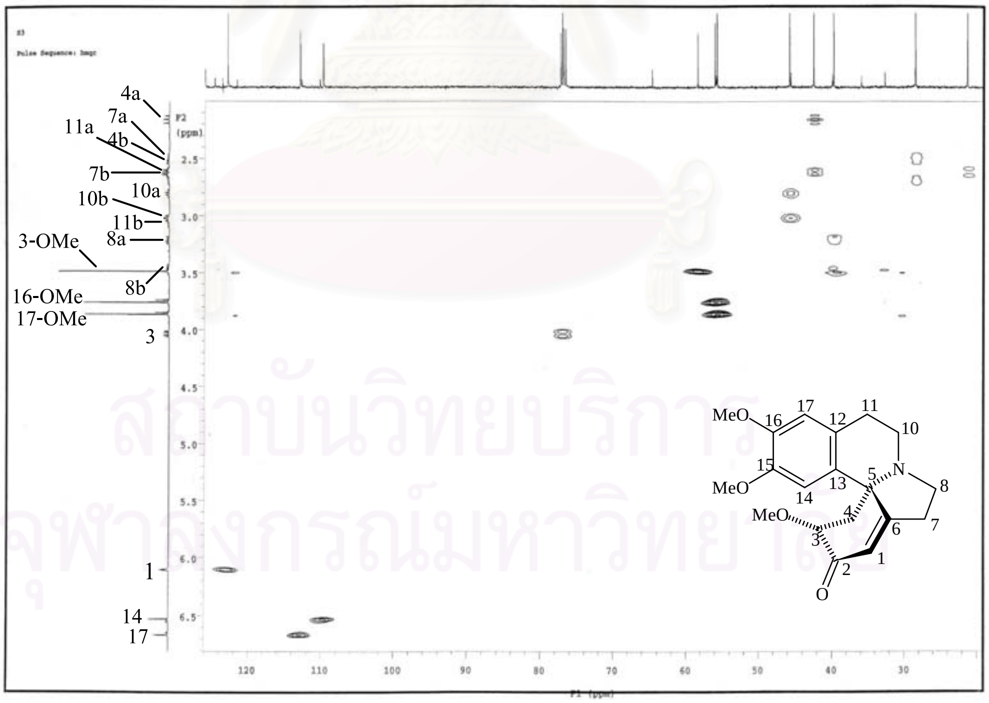


Figure 43 HMQC Spectrum of compound ES6 (CDCl$_3$)

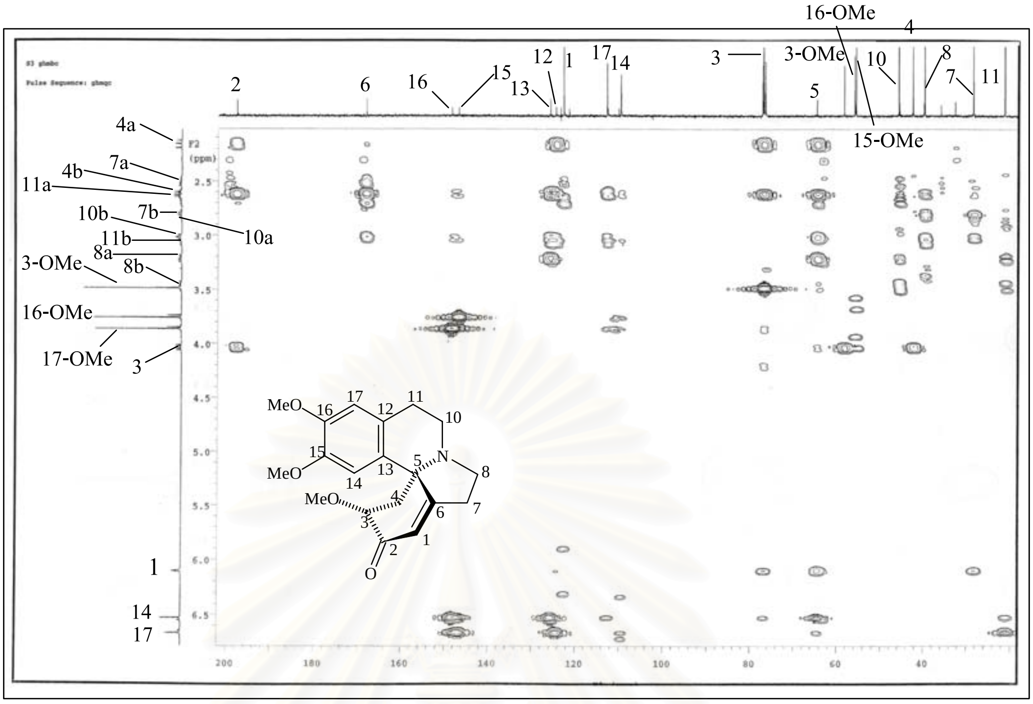


Figure 44 HMBC Spectrum of compound ES6 ($CDCl_3$)

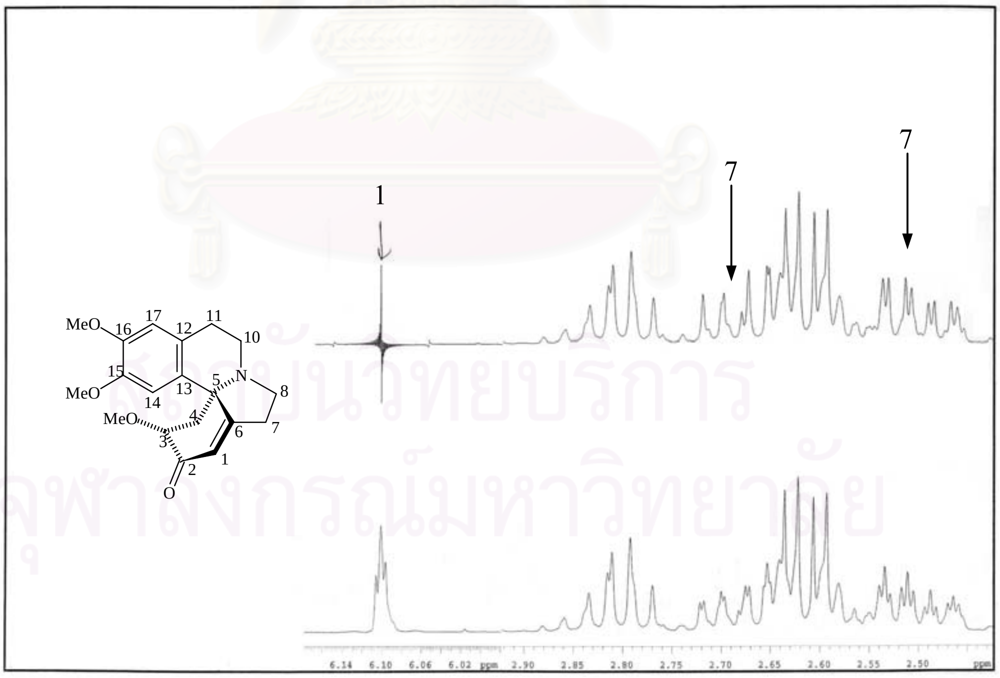


Figure 45 ^1H Decoupling Spectrum of compound ES6 ($CDCl_3$)

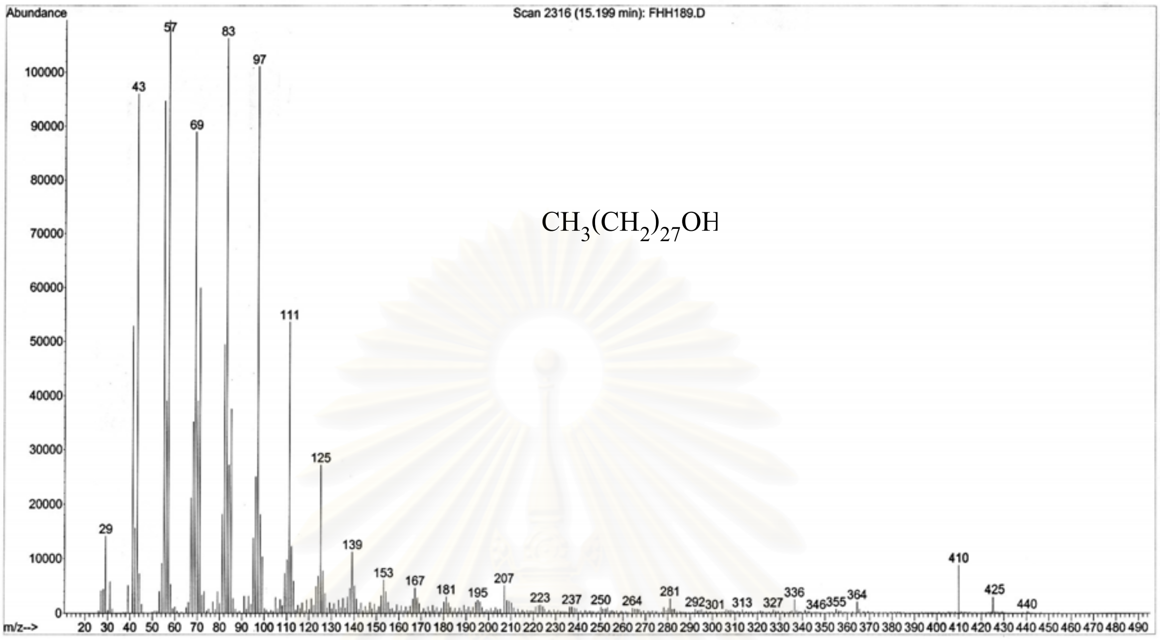


Figure 46 GC Mass spectrum of compound EF1

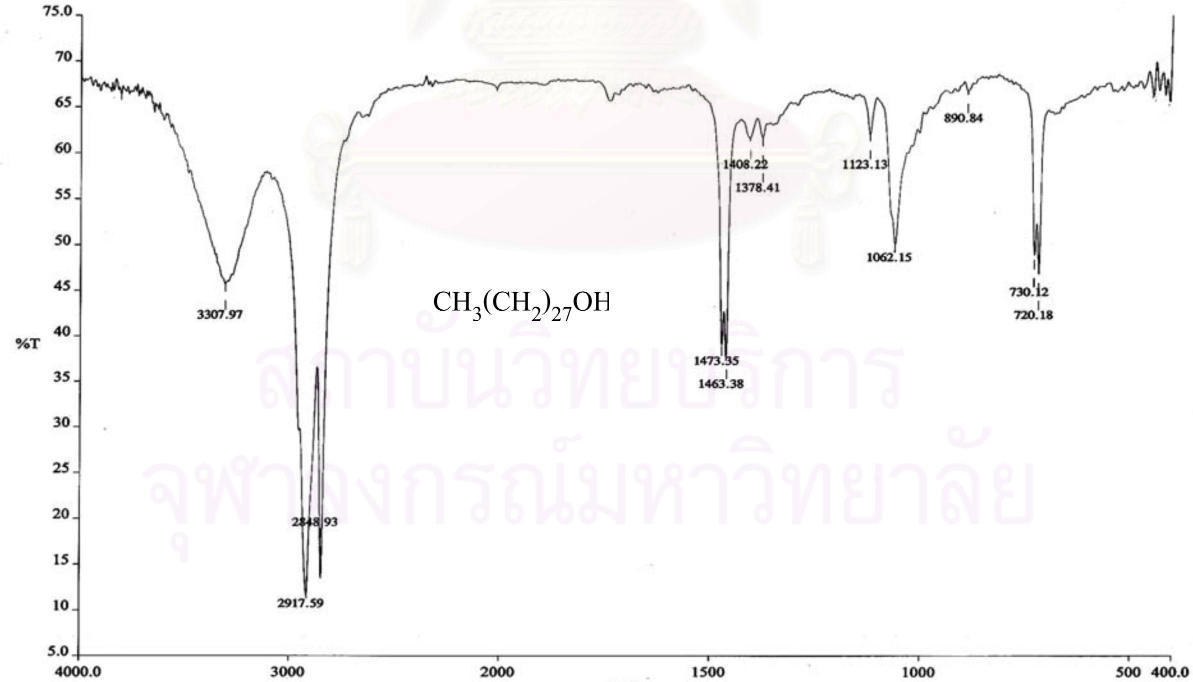


Figure 47 IR Spectrum of compound EF1 (KBr disc)

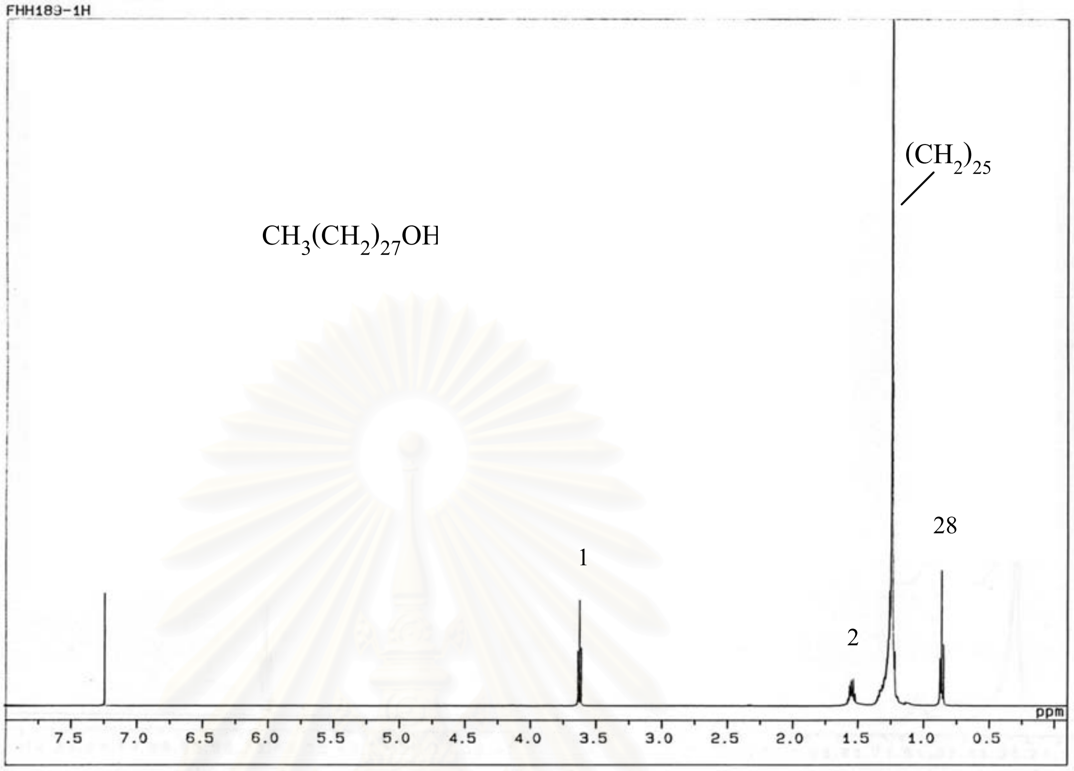


Figure 48 ^{1}H NMR (500 MHz) Spectrum of compound EF1 ($CDCl_3$)

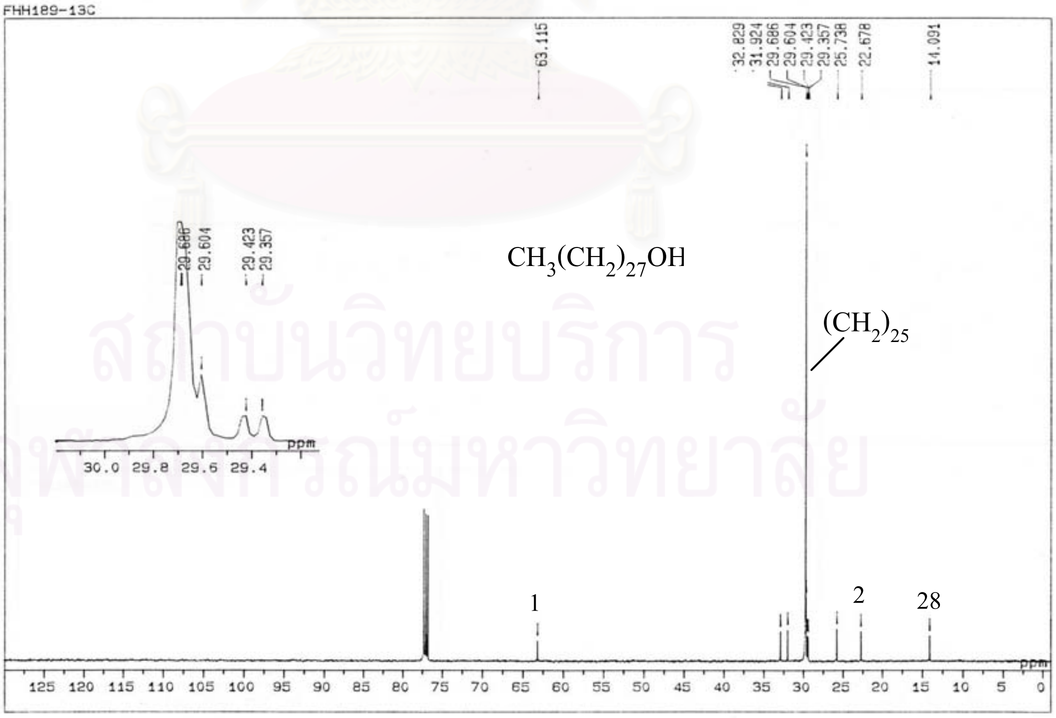


Figure 49 ^{13}C NMR (500 MHz) Spectrum of compound EF1 ($CDCl_3$)

$CH_3(CH_2)_{27}OH$

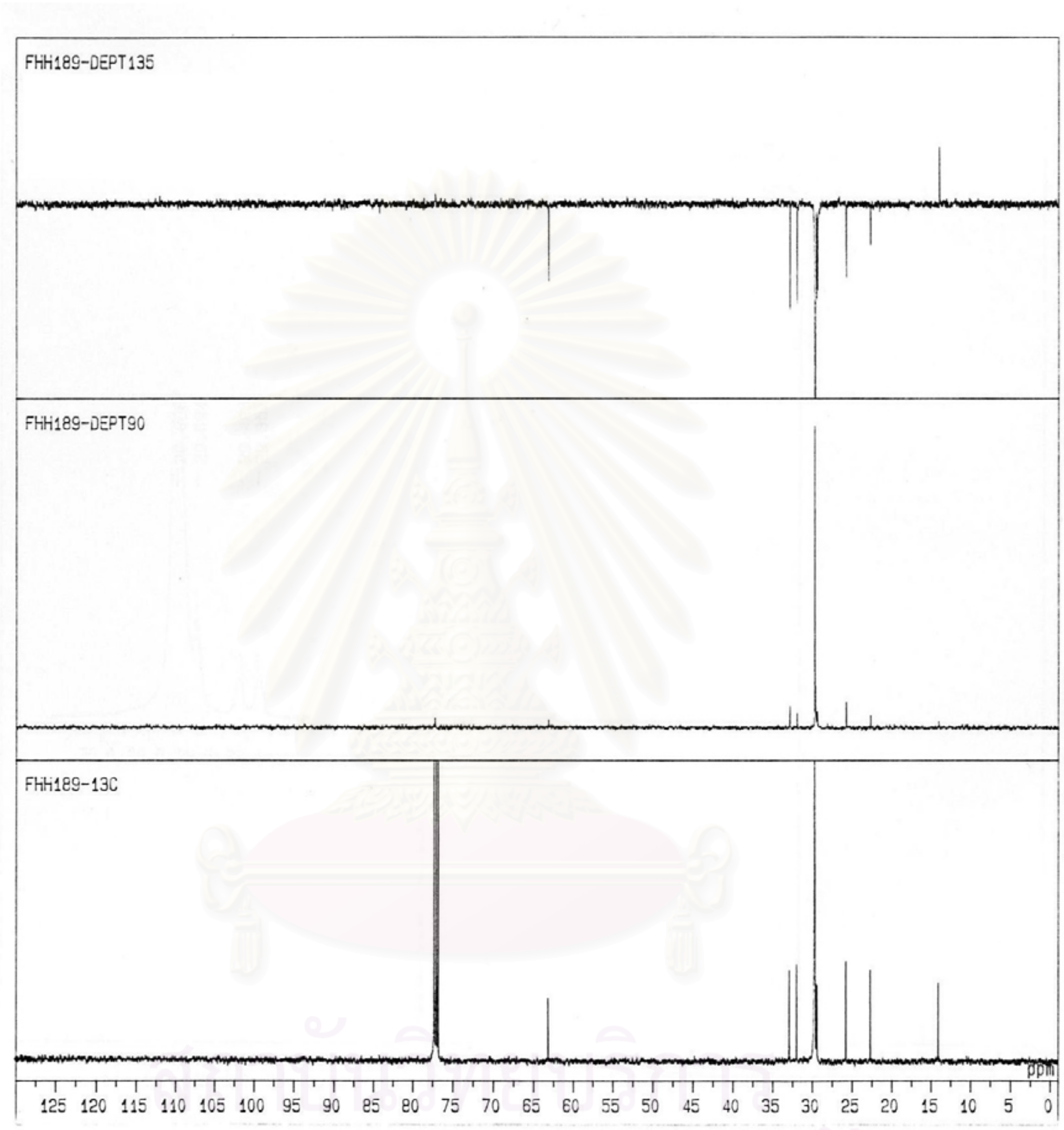


Figure 50 DEPT 90 and DEPT 135 Spectra of compound EF1 ($CDCl_3$)

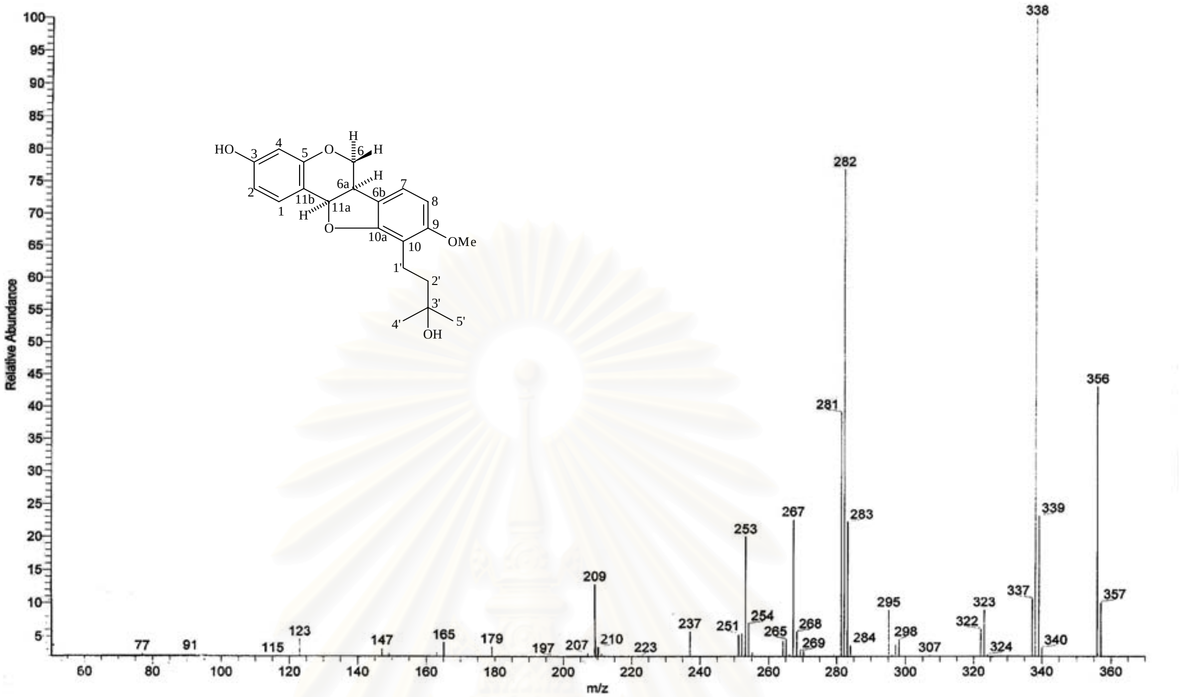


Figure 51 EI Mass Spectrum of compound EF3

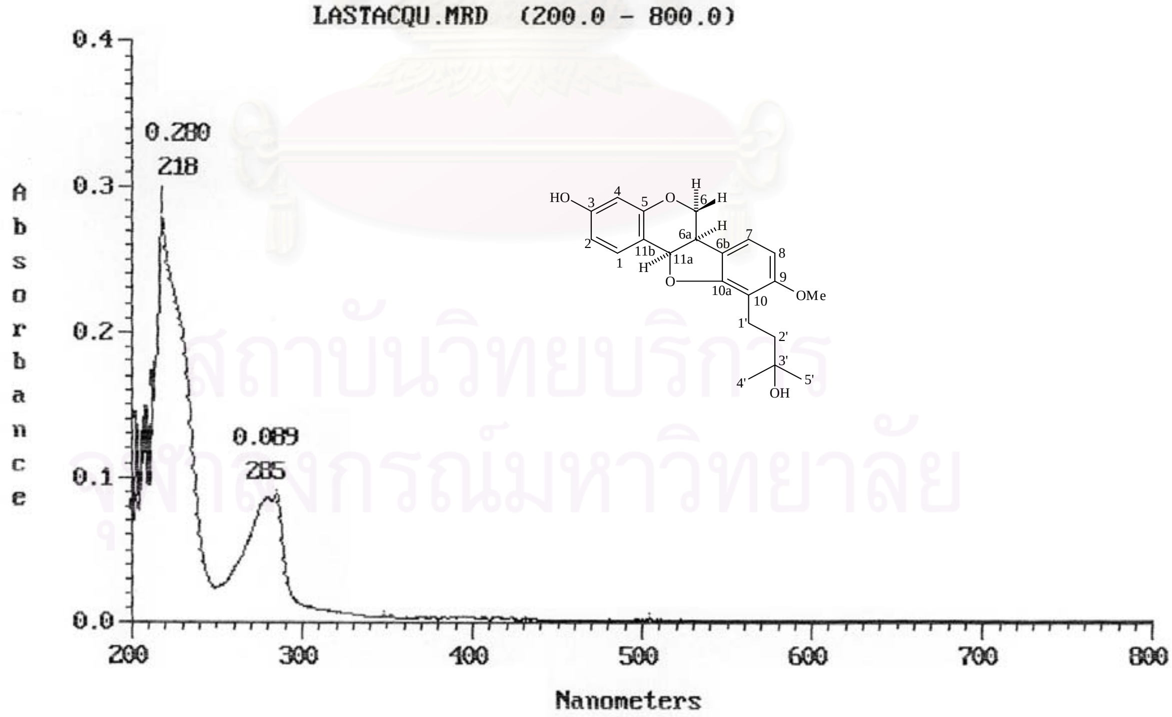


Figure 52 UV Spectrum of compound EF3 (MeOH)

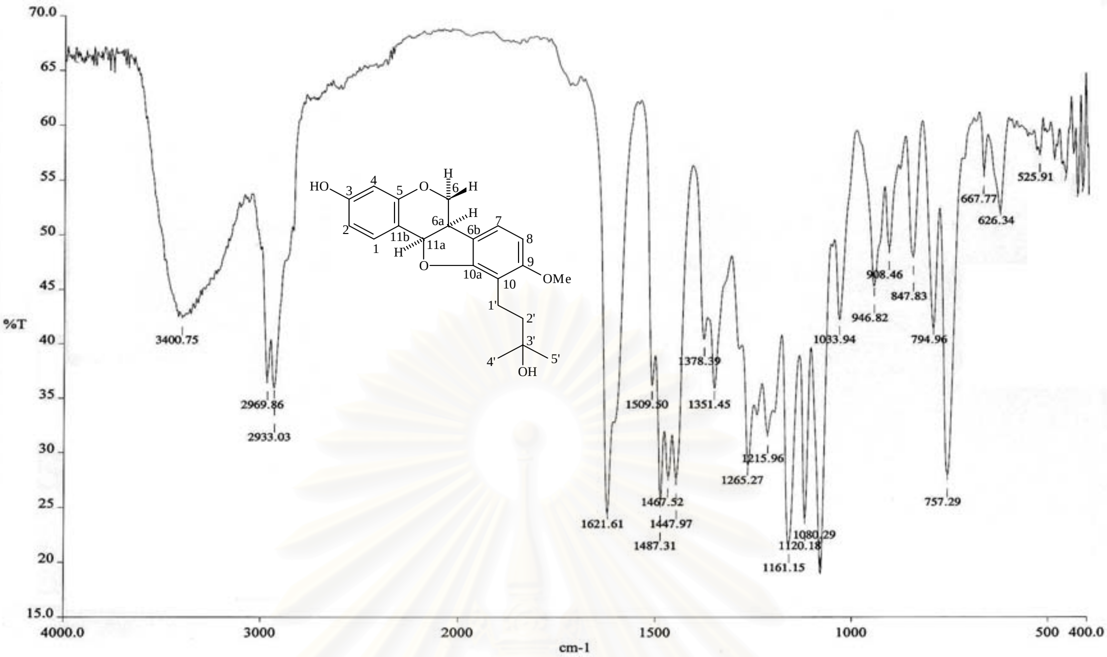


Figure 53 IR Spectrum of compound EF3 (Film)

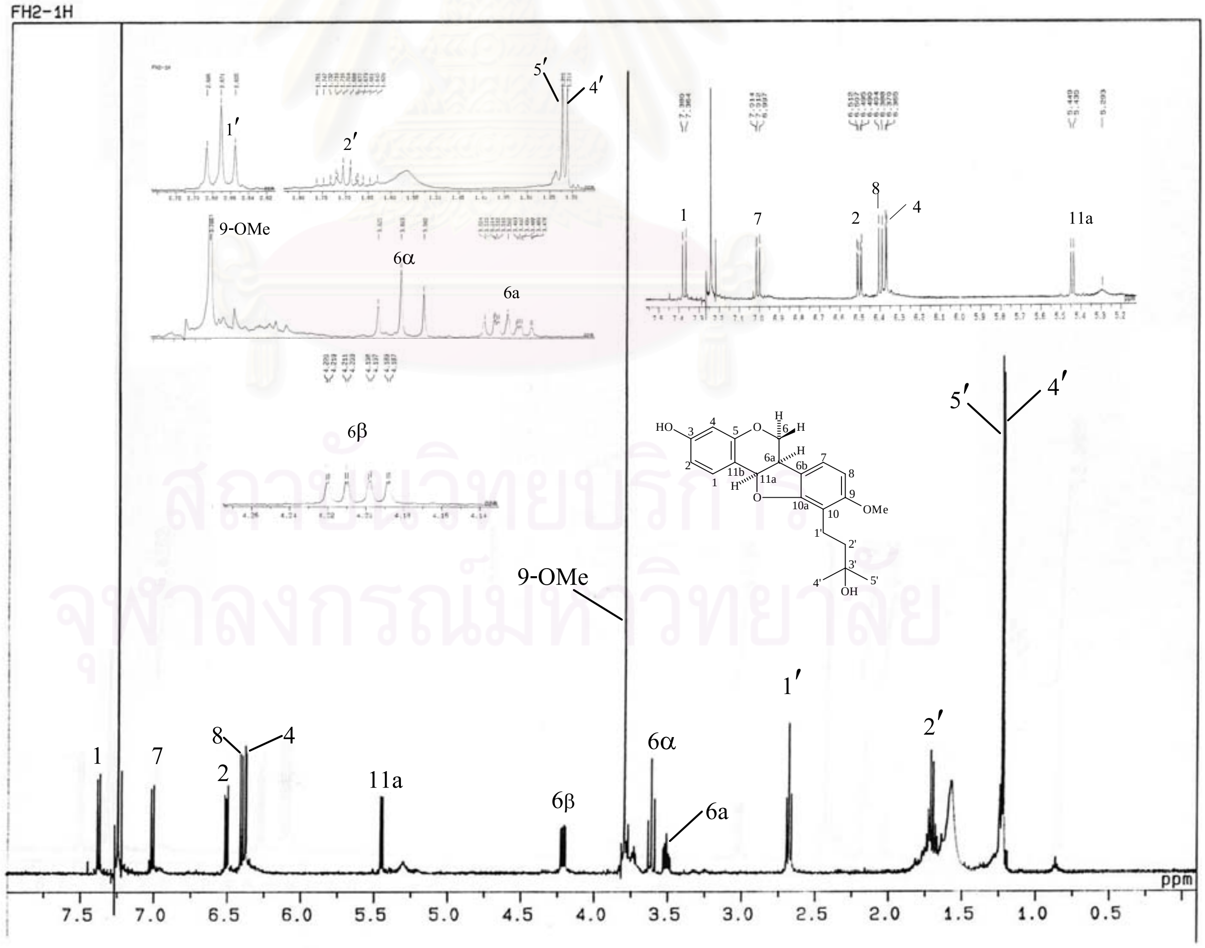


Figure 54 ^{1}H NMR (500 MHz) Spectrum of compound EF3 ($CDCl_3$)

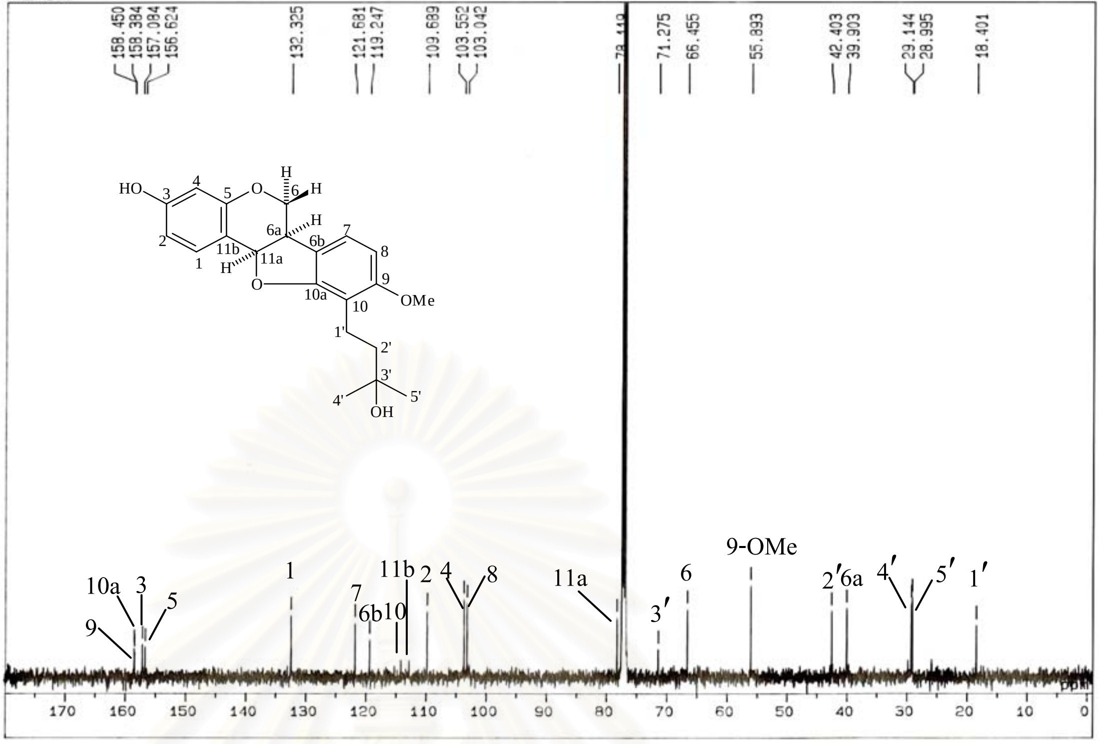


Figure 55 ^{13}C NMR (125 MHz) Spectrum of compound EF3 (CDCl$_3$)

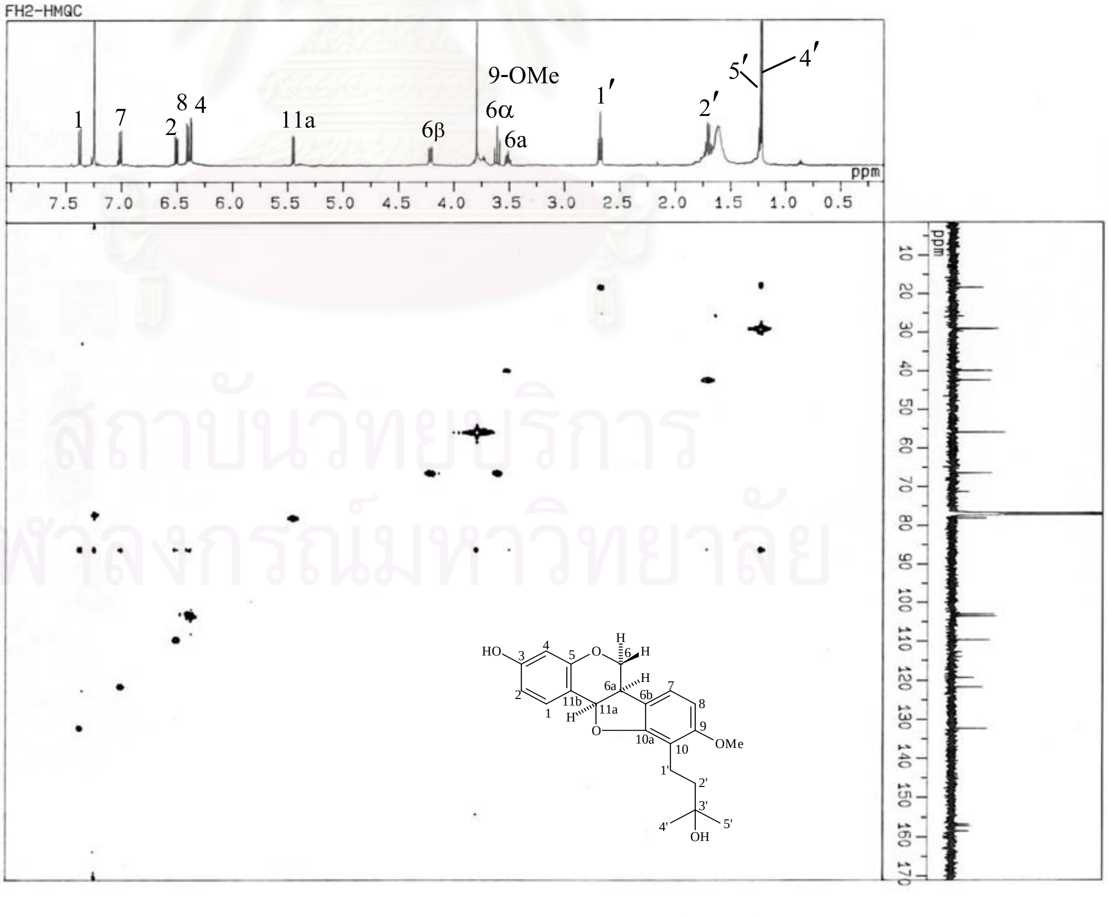


Figure 56 HMQC Spectrum of compound EF3 (CDCl$_3$)

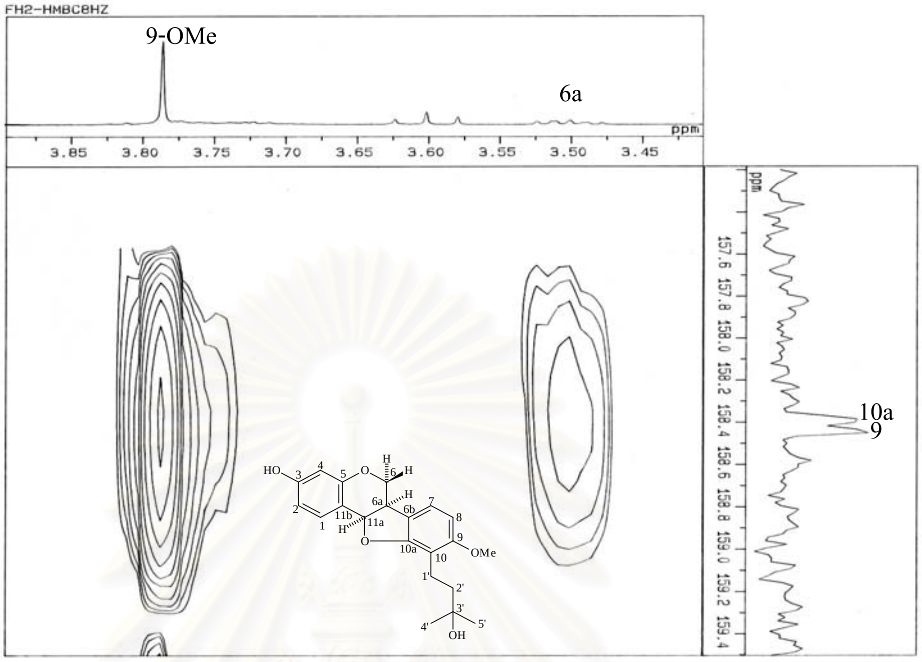


Figure 57 HMBC Spectrum of compound EF3 ($CDCl_3$)

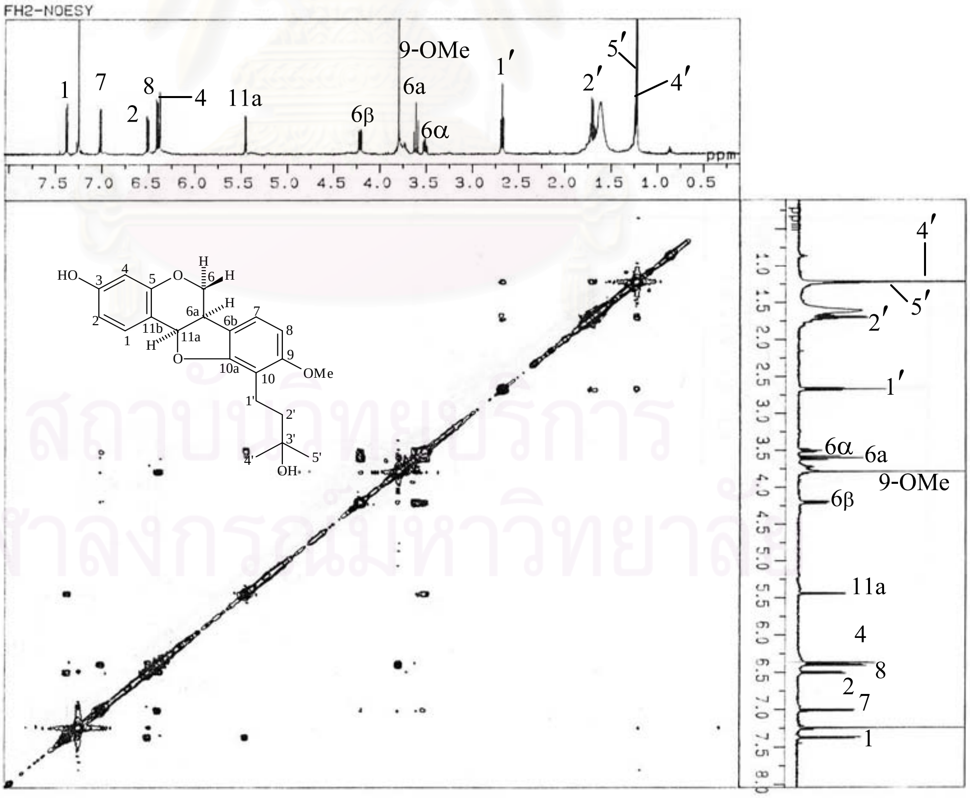


Figure 58 NOESY Spectrum of compound EF3 ($CDCl_3$)

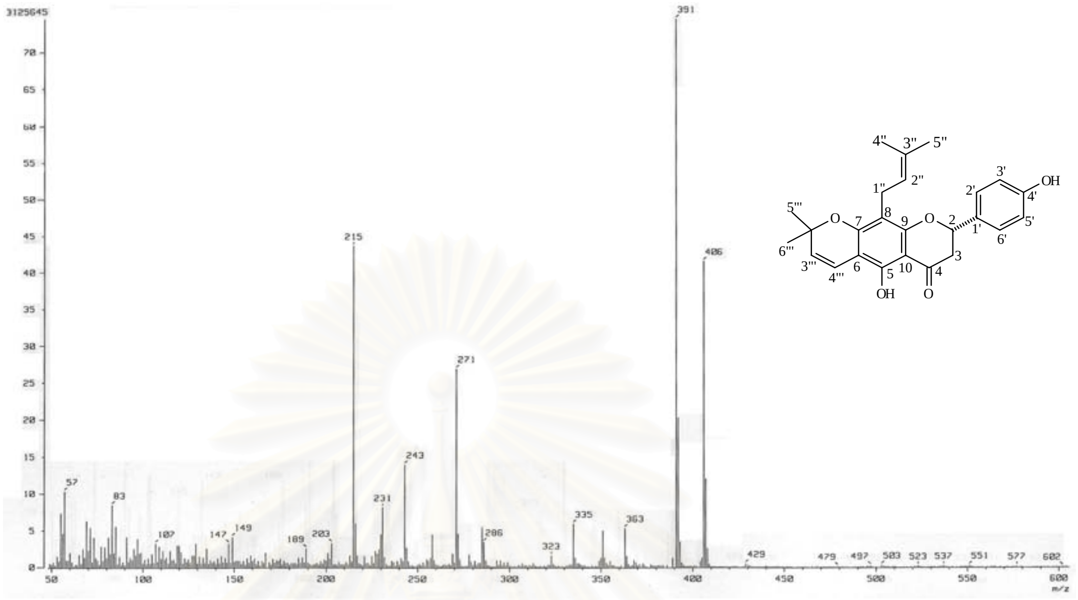


Figure 59 EI Mass spectrum of compound EF4

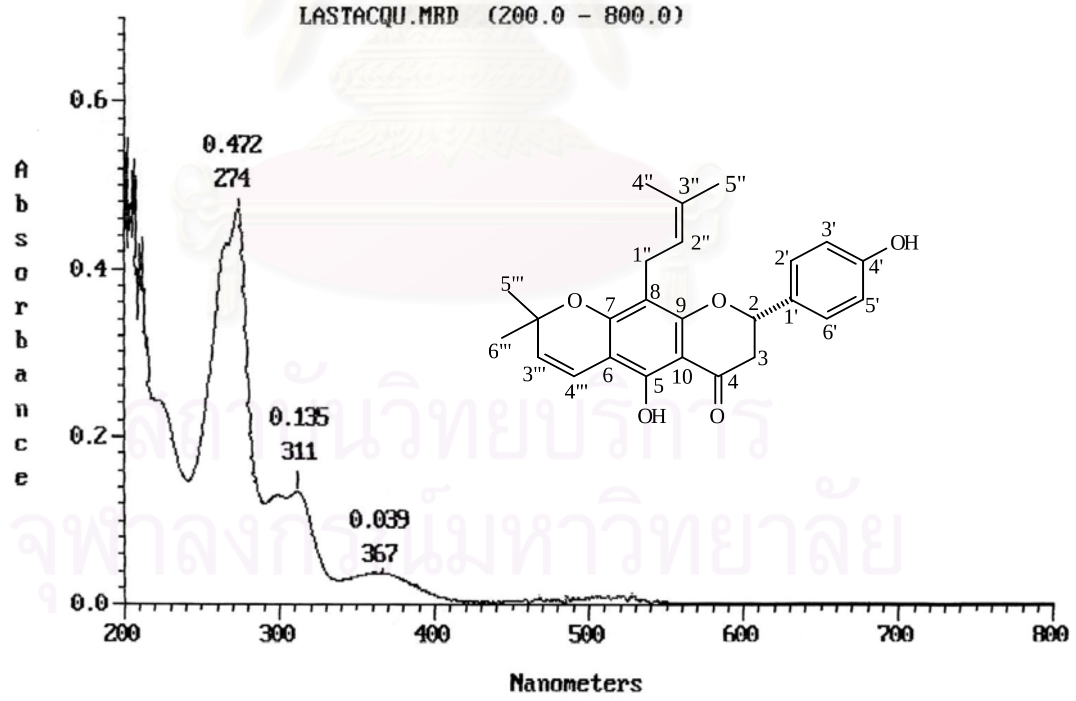


Figure 60 UV Spectrum of compound EF4 (MeOH)

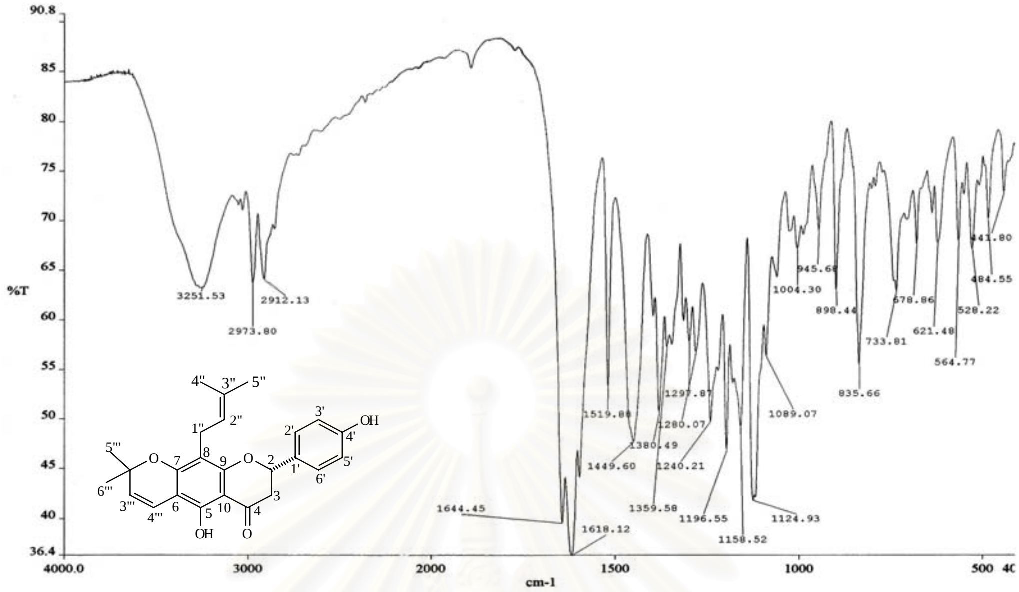


Figure 61 IR spectrum of compound EF4 (Film)

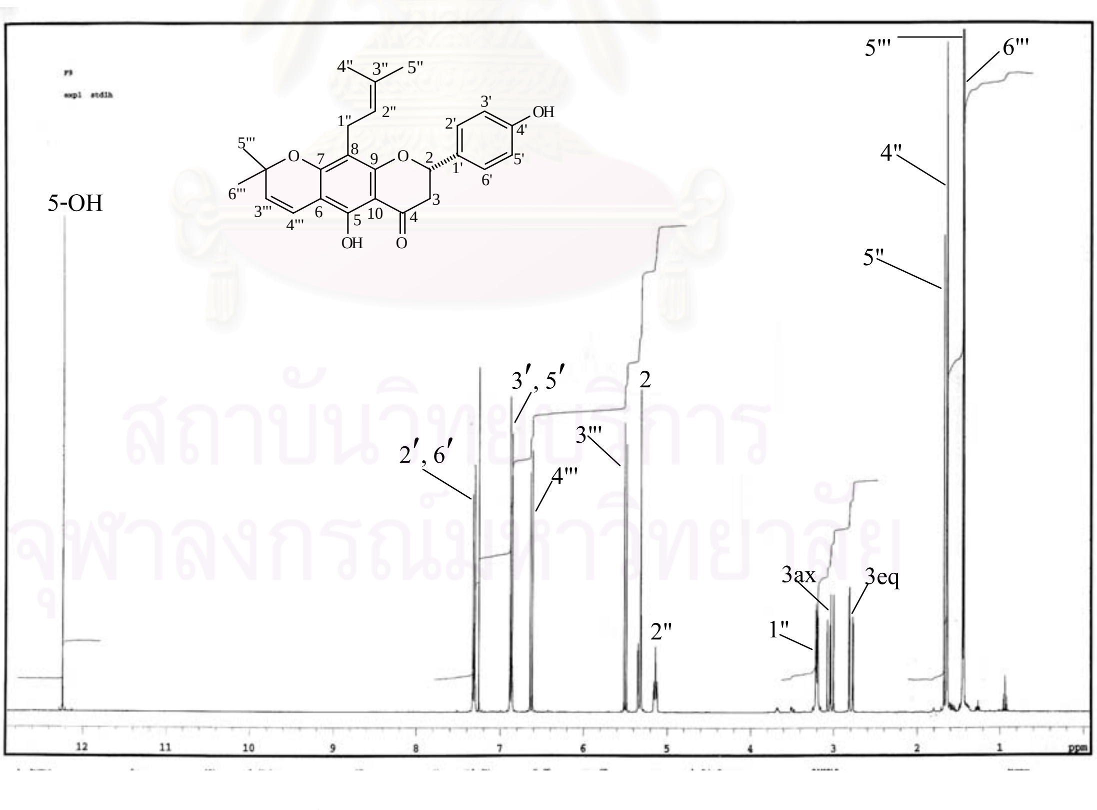


Figure 62 ^{1}H NMR (400 MHz) Spectrum of compound EF4 ($CDCl_3$)

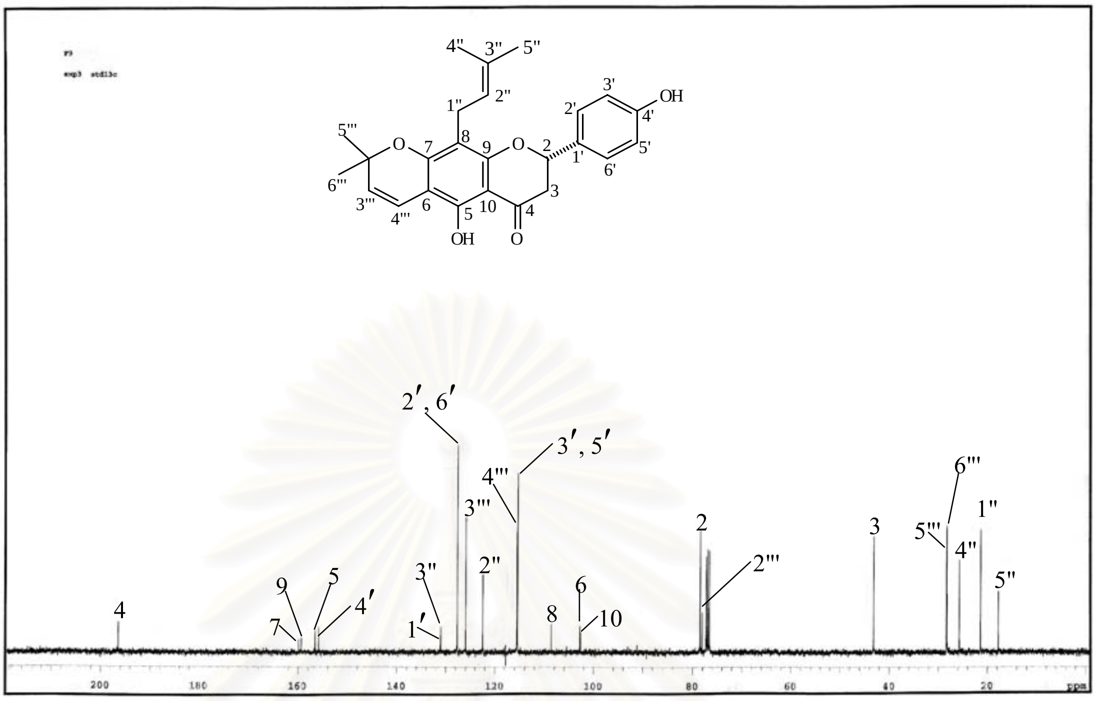


Figure 63 ^{13}C NMR (100 MHz) Spectrum of compound EF4 (CDCl$_3$)

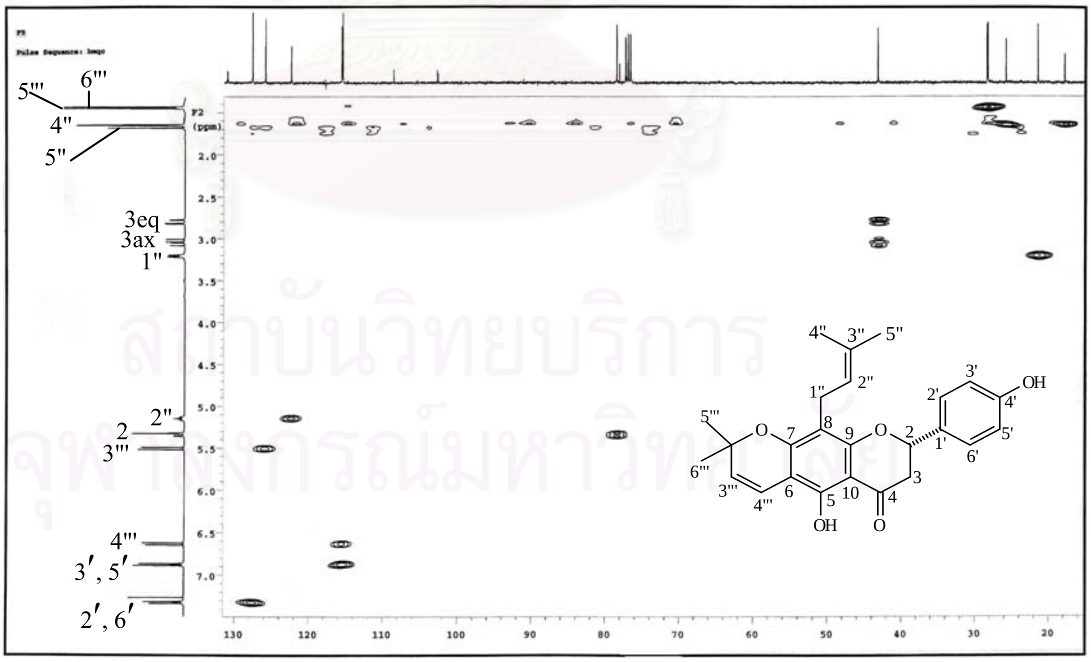


Figure 64 HMQC Spectrum of compound EF4 (CDCl$_3$)

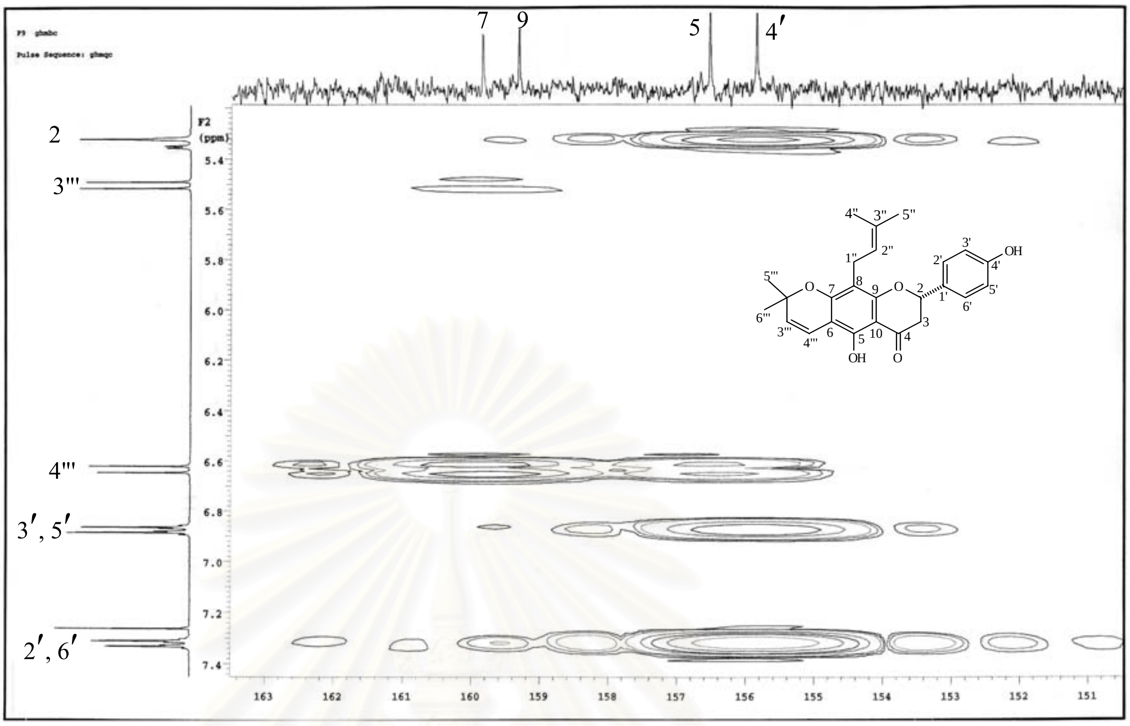


Figure 65 HMBC Spectrum of compound EF4 ($CDCl_3$)

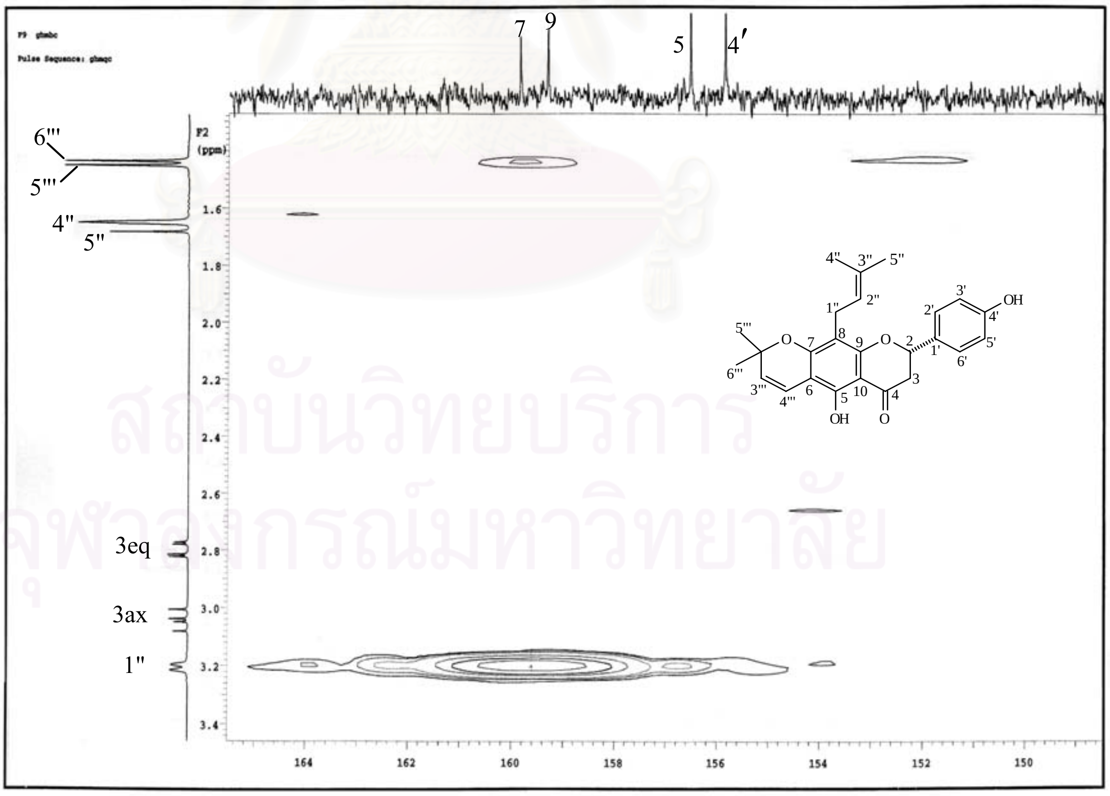


Figure 66 HMBC Spectrum of compound EF4 ($CDCl_3$)

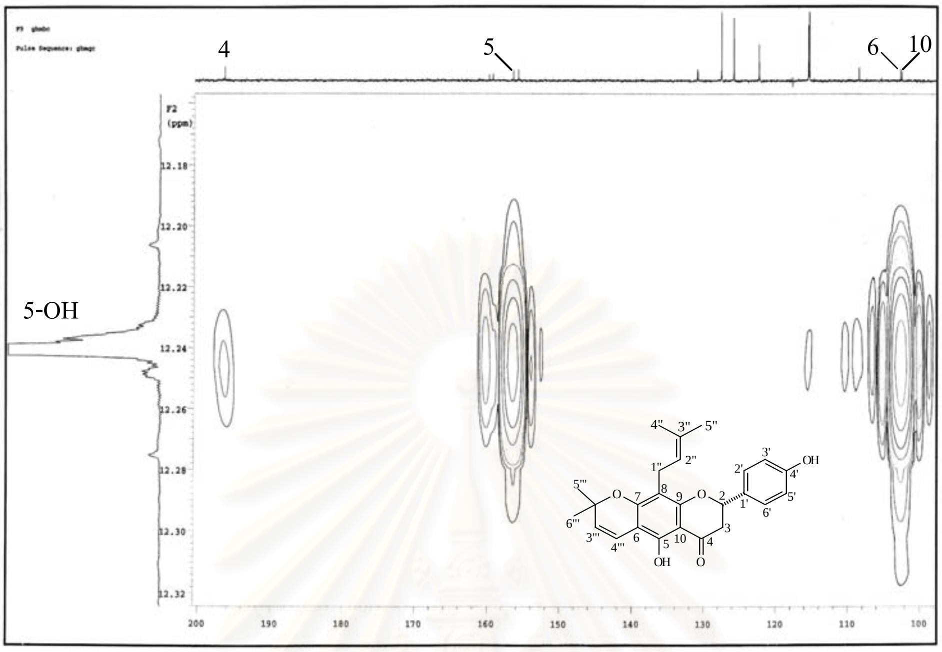
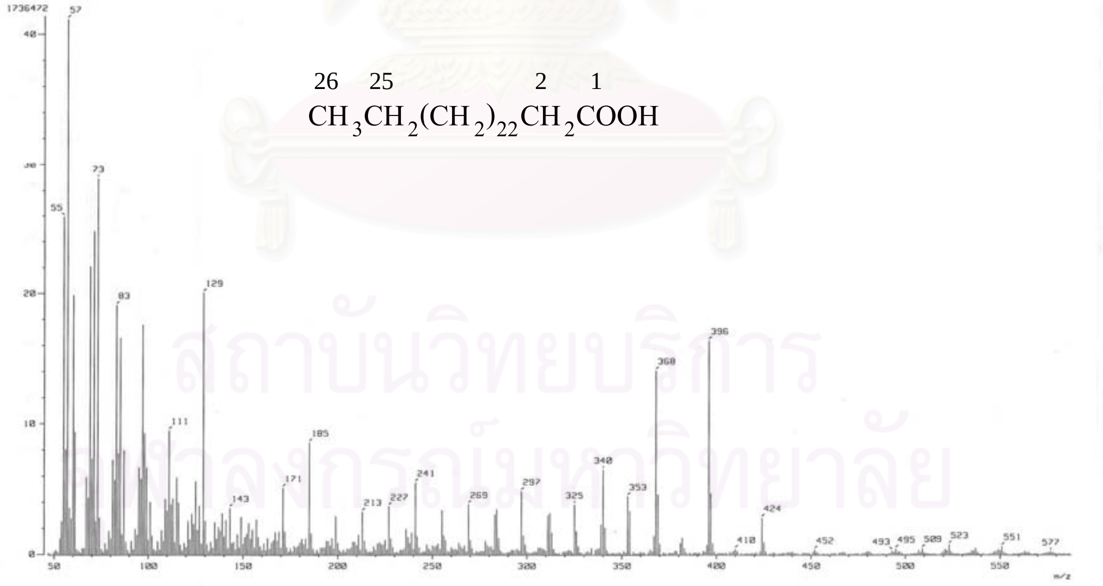


Figure 67 HMBC Spectrum of compound EF4 ($CDCl_3$)



$$\overset{26}{CH_3}\overset{25}{CH_2}(CH_2)_{22}\overset{2}{CH_2}\overset{1}{COOH}$$

Figure 68 EI Mass spectrum of compound EF6

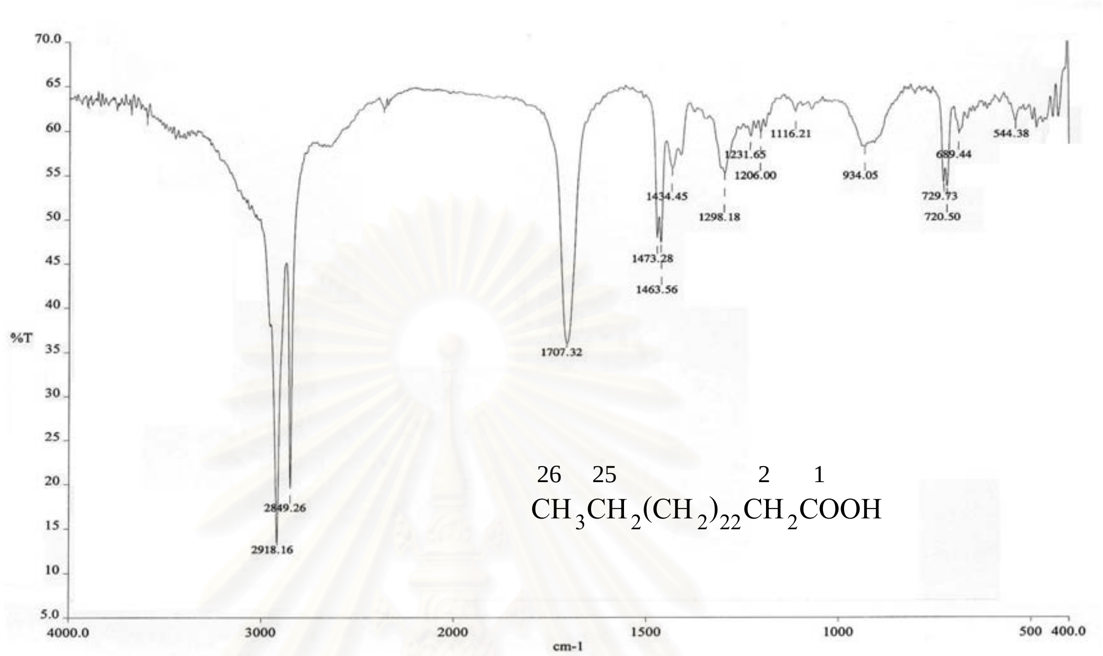


Figure 69 IR Spectrum of compound EF6 (KBr disc)

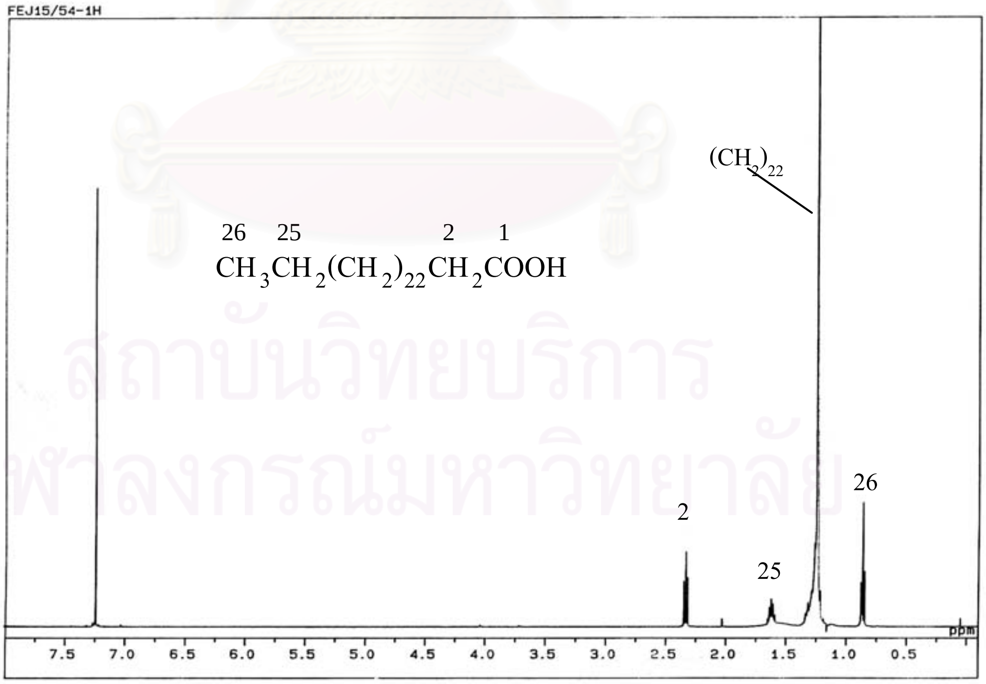


Figure 70 ^{1}H NMR (500 MHz) Spectrum of compound EF6 ($CDCl_3$)

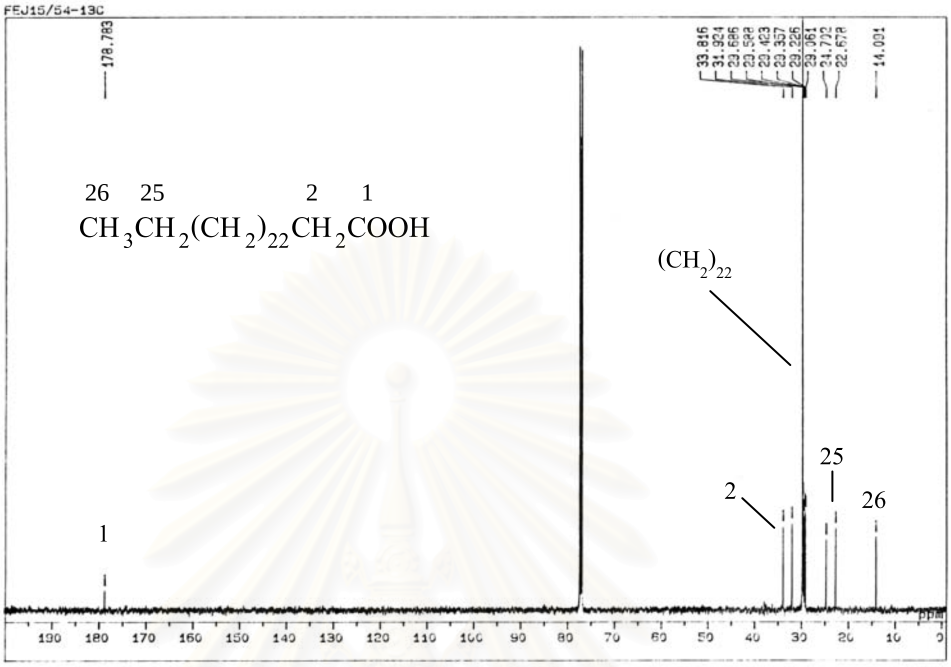


Figure 71 ^{13}C NMR (500 MHz) Spectrum of compound EF6 ($CDCl_3$)

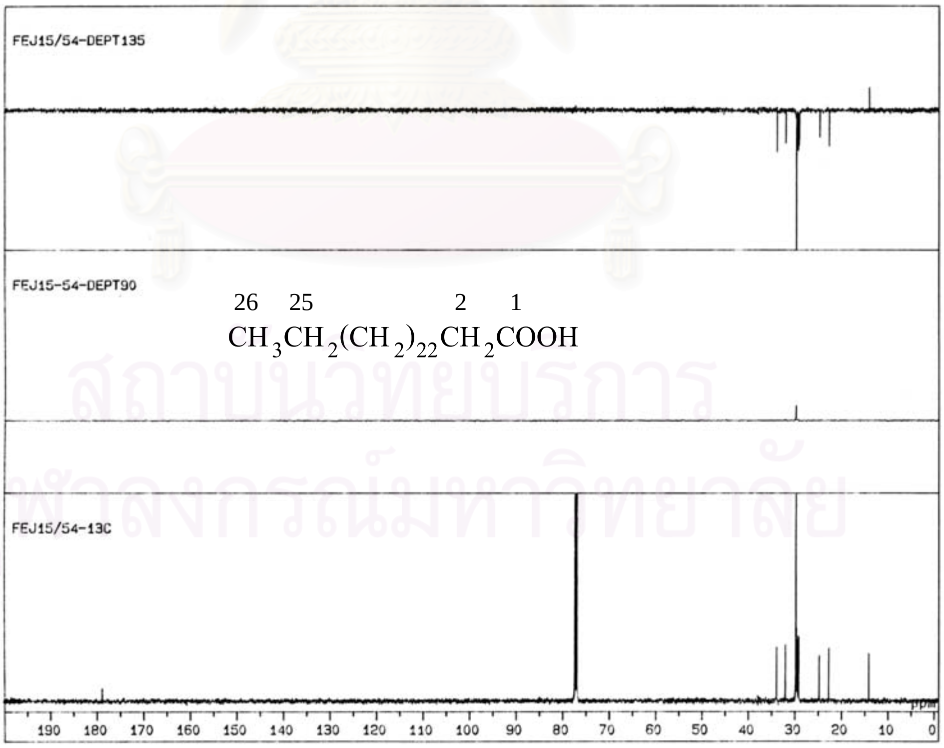


Figure 72 DEPT 90 and DEPT 135 Spectra of compound EF6 ($CDCl_3$)

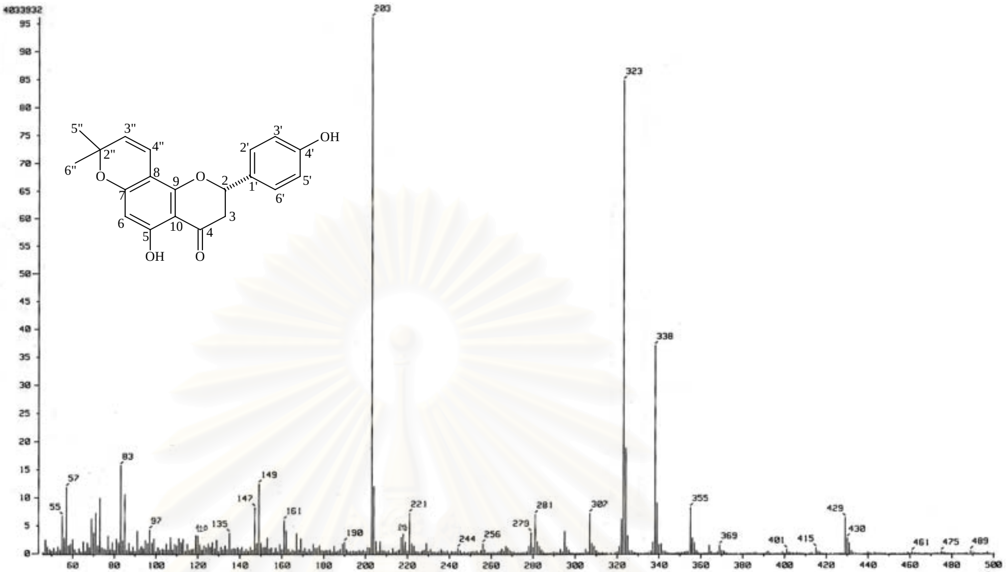


Figure 73 EI Mass spectrum of compound EF7

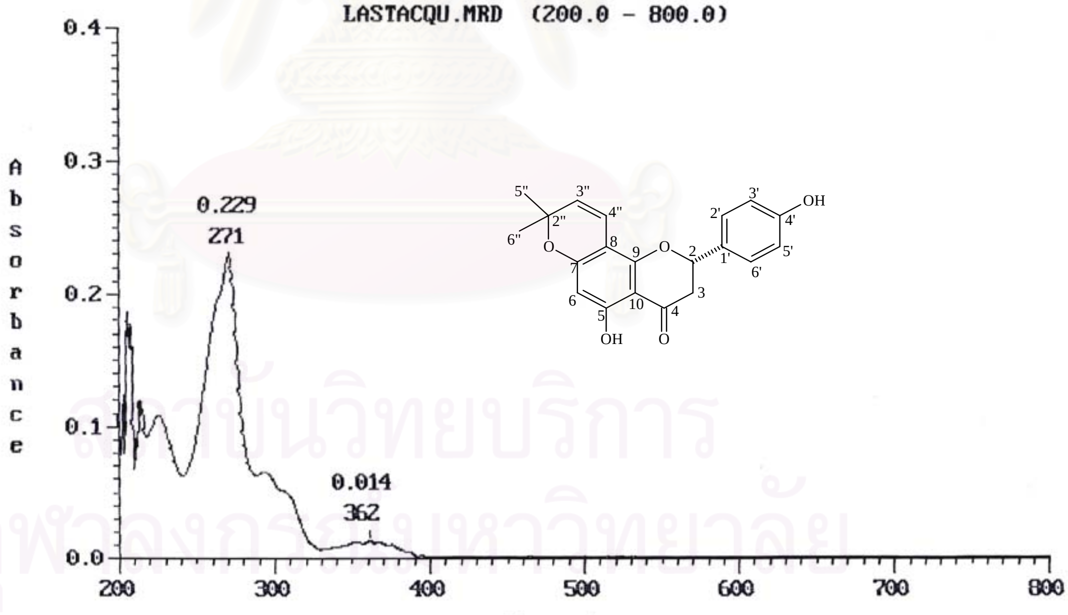


Figure 74 UV Spectrum of compound EF7 (MeOH)

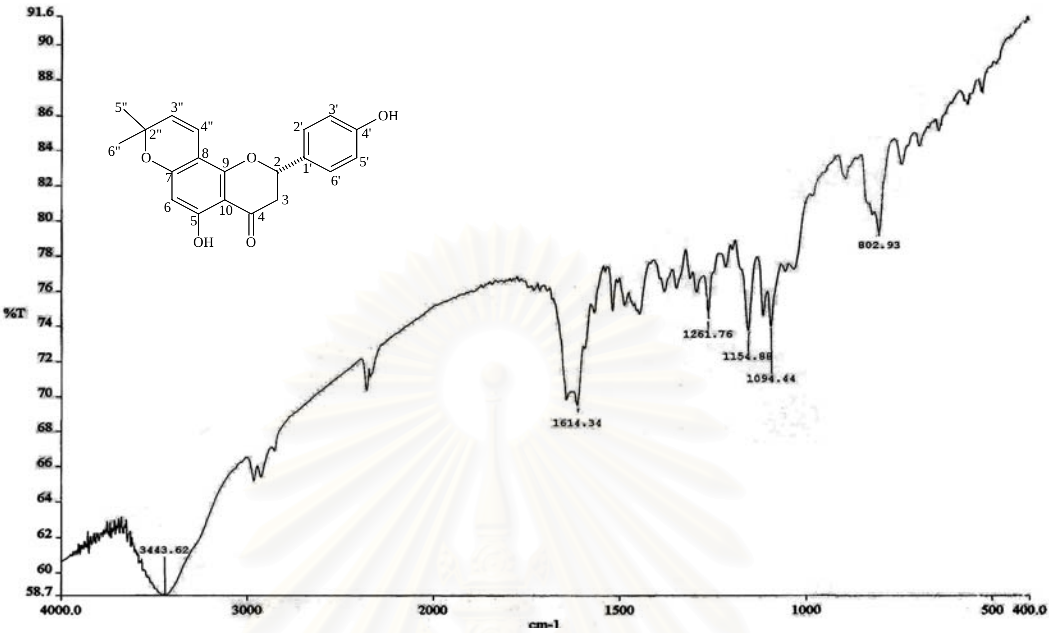


Figure 75 IR Spectrum of compound EF7 (KBr disc)

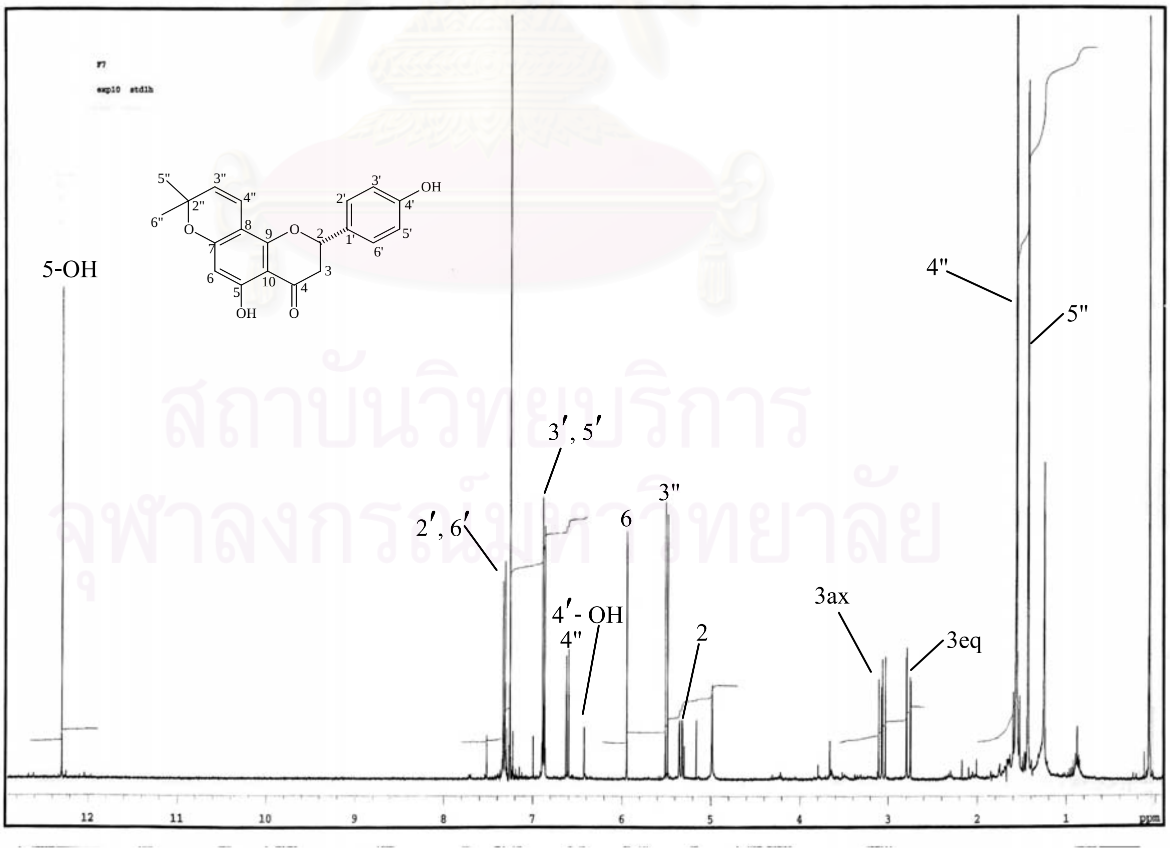


Figure 76 ^{1}H NMR (400 MHz) Spectrum of compound EF7 (CDCl$_3$)

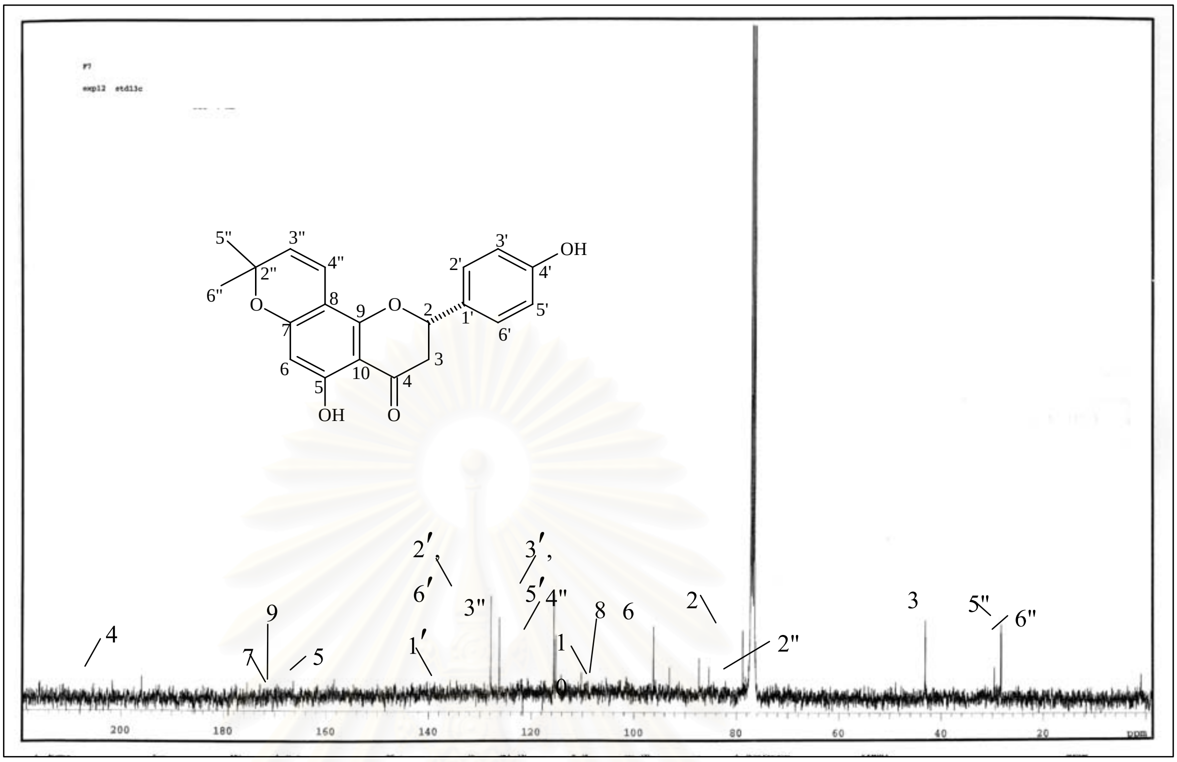


Figure 77 ^{13}C NMR (100 MHz) Spectrum of compound EF7 ($CDCl_3$)

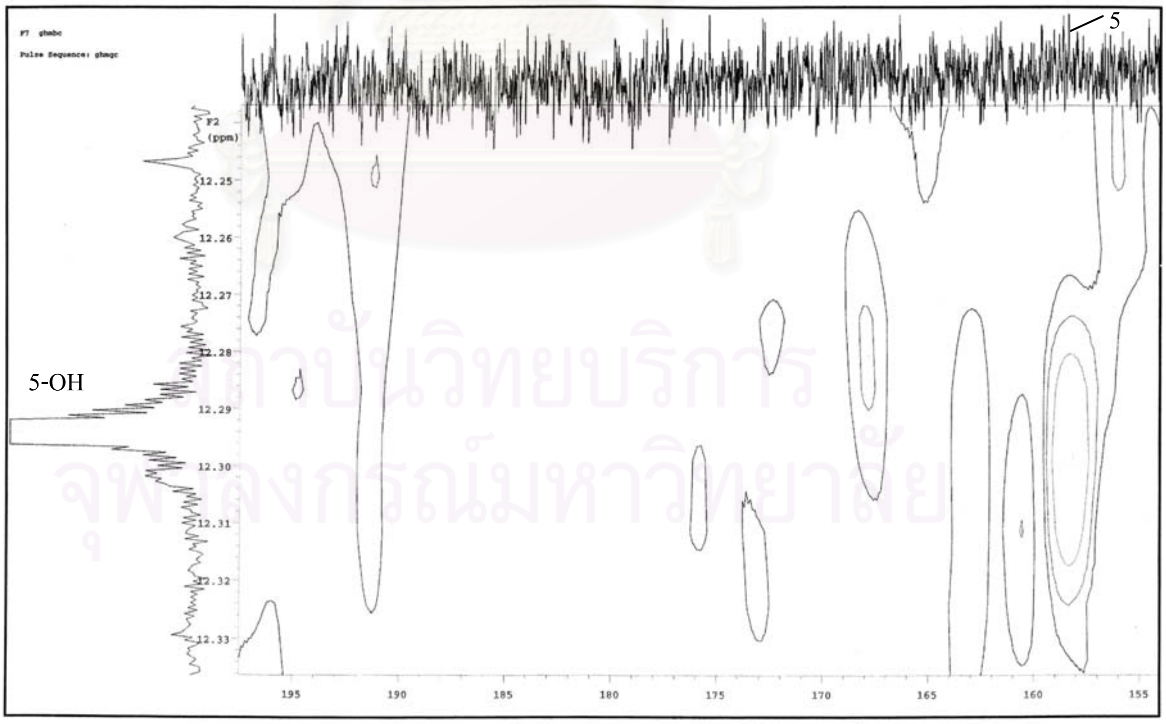


Figure 78 HMBC Spectrum of compound EF7 ($CDCl_3$)

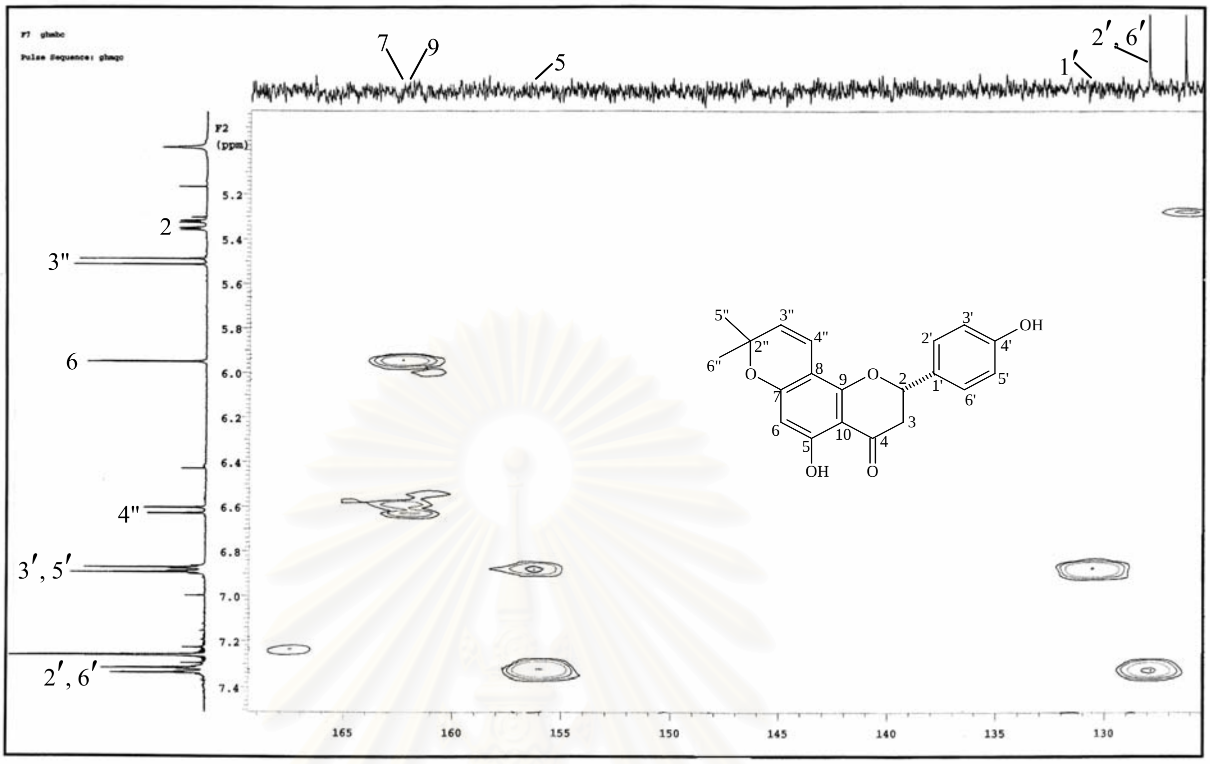


Figure 79 HMBC Spectrum of compound EF7 ($CDCl_3$)

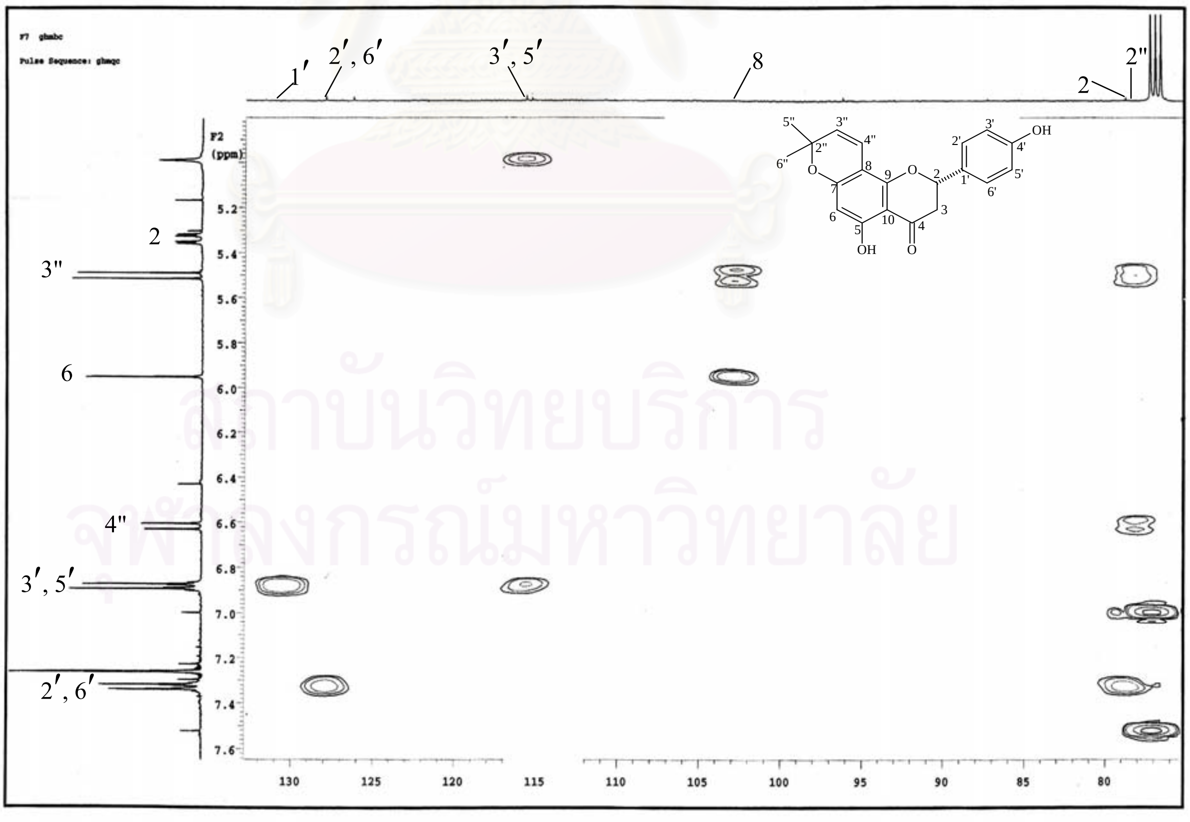


Figure 80 HMBC Spectrum of compound EF7 ($CDCl_3$)

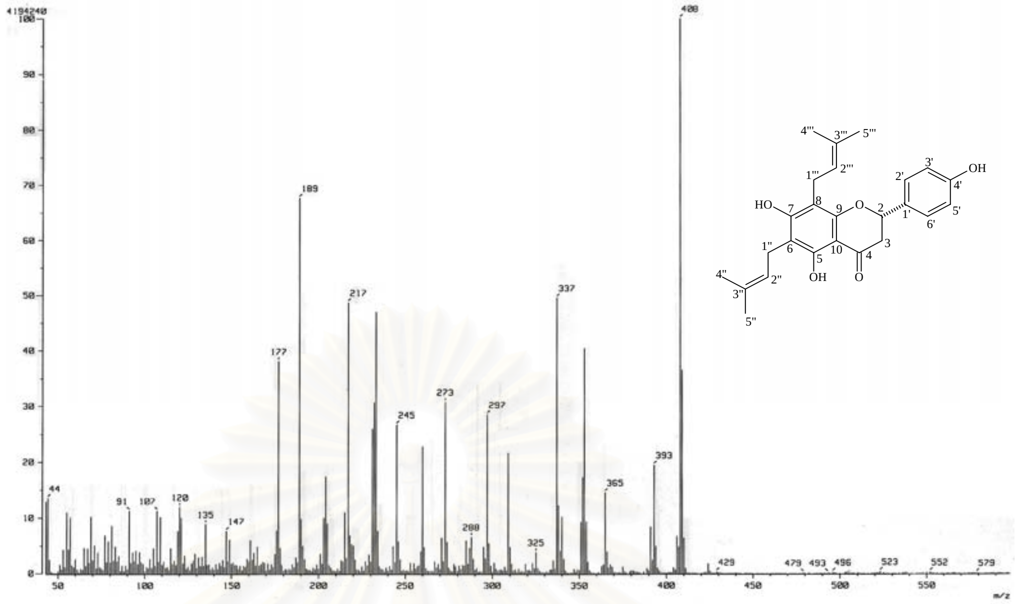


Figure 81 EI Mass spectrum of compound EF8

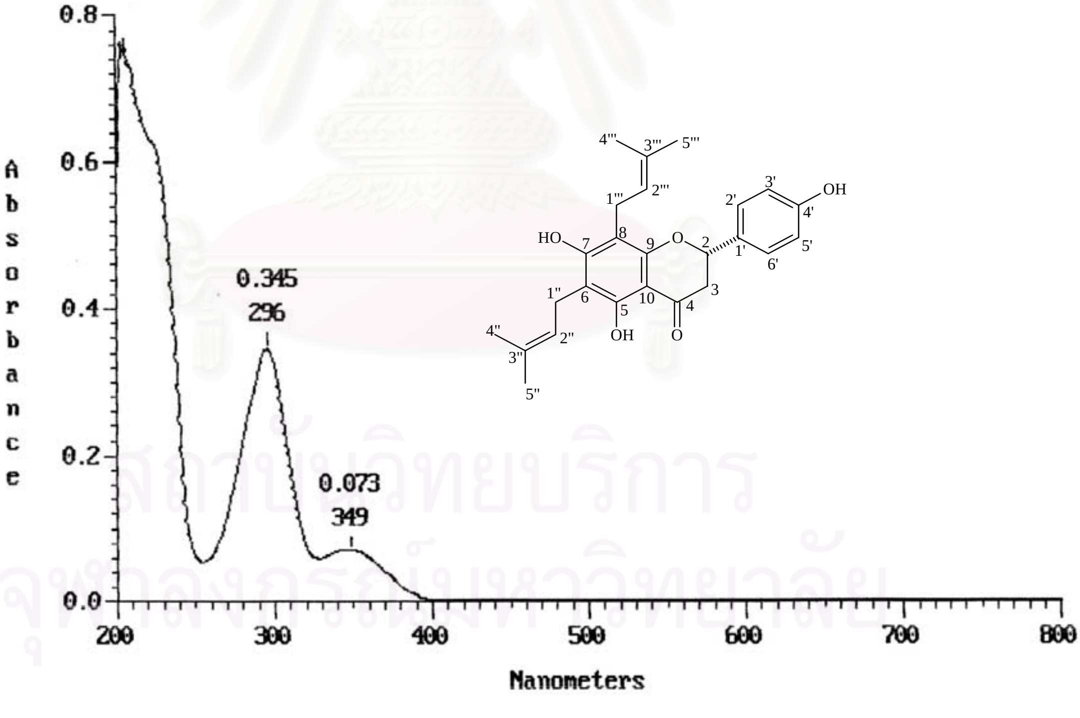


Figure 82 UV Spectrum of compound EF8 (MeOH)

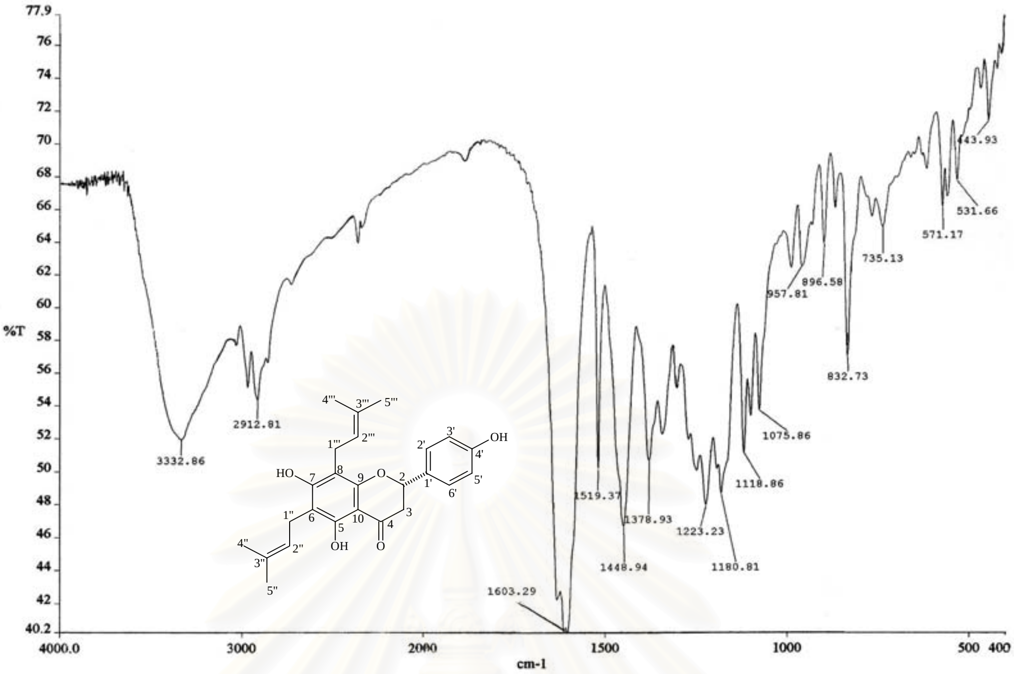


Figure 83 IR Spectrum of compound EF8 (KBr disc)

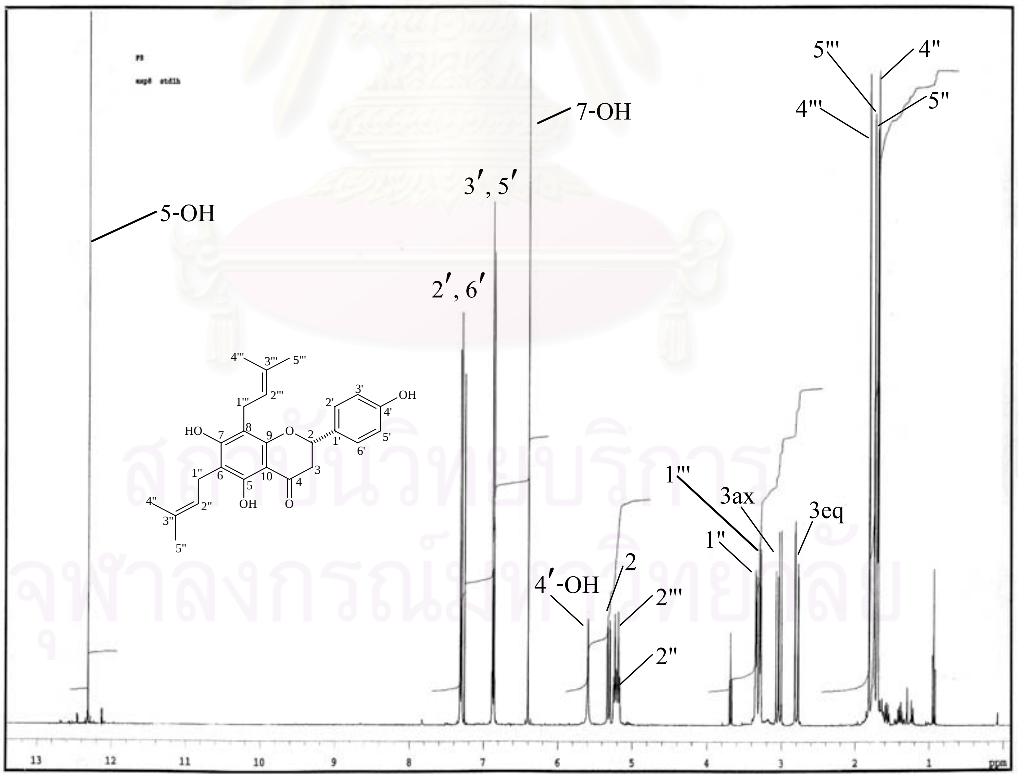


Figure 84 ^{1}H NMR (400 MHz) Spectrum of compound EF8 ($CDCl_3$)

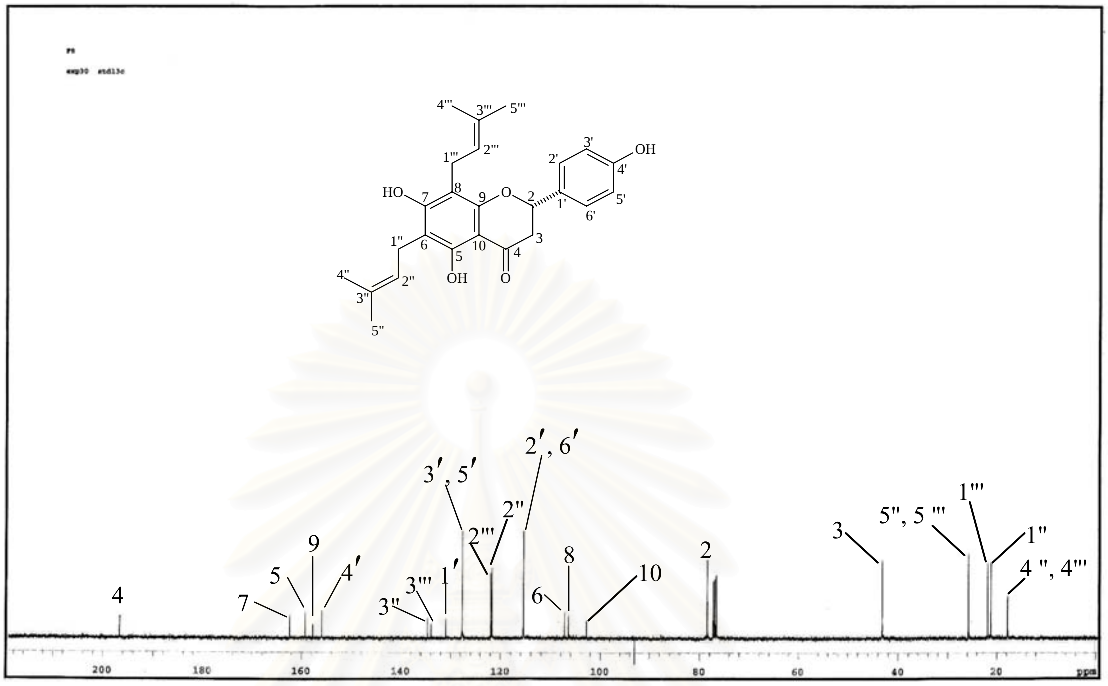


Figure 85 ^{13}C NMR (100 MHz) Spectrum of compound EF8 (CDCl$_3$)

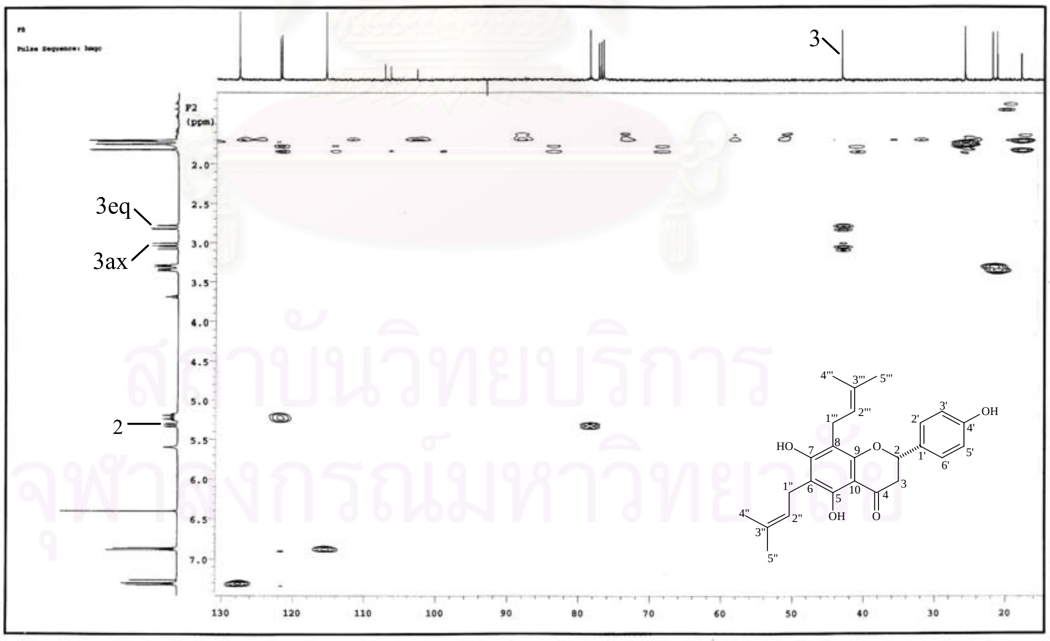


Figure 86 HMQC Spectrum of compound EF8 (CDCl$_3$)

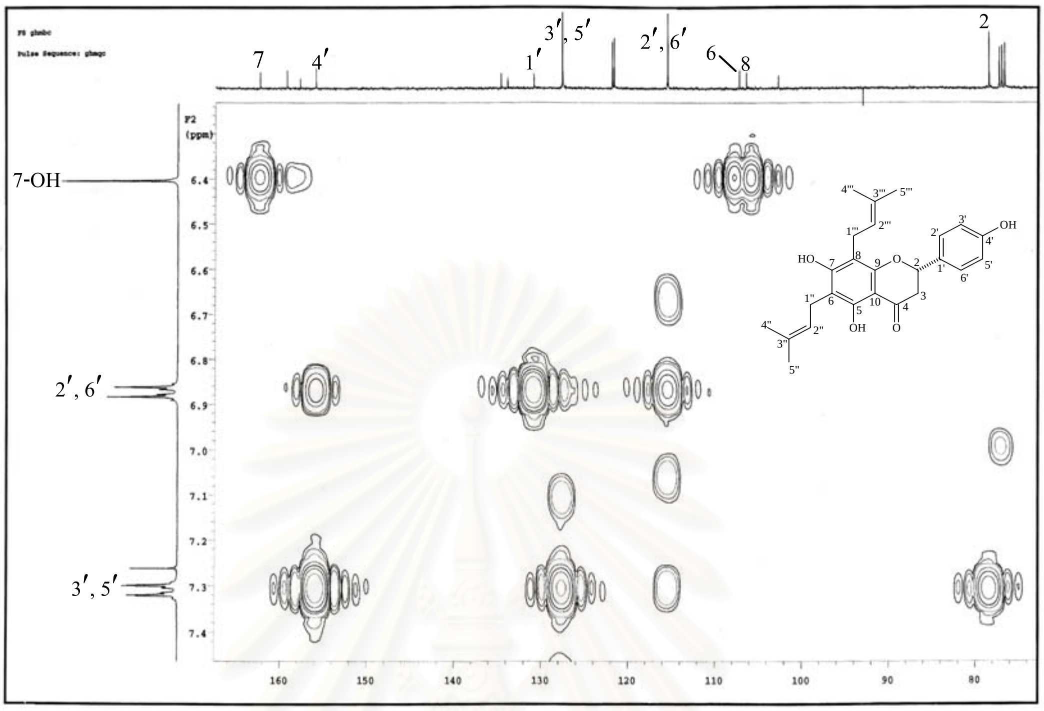


Figure 87 HMBC Spectrum of compound EF8 ($CDCl_3$)

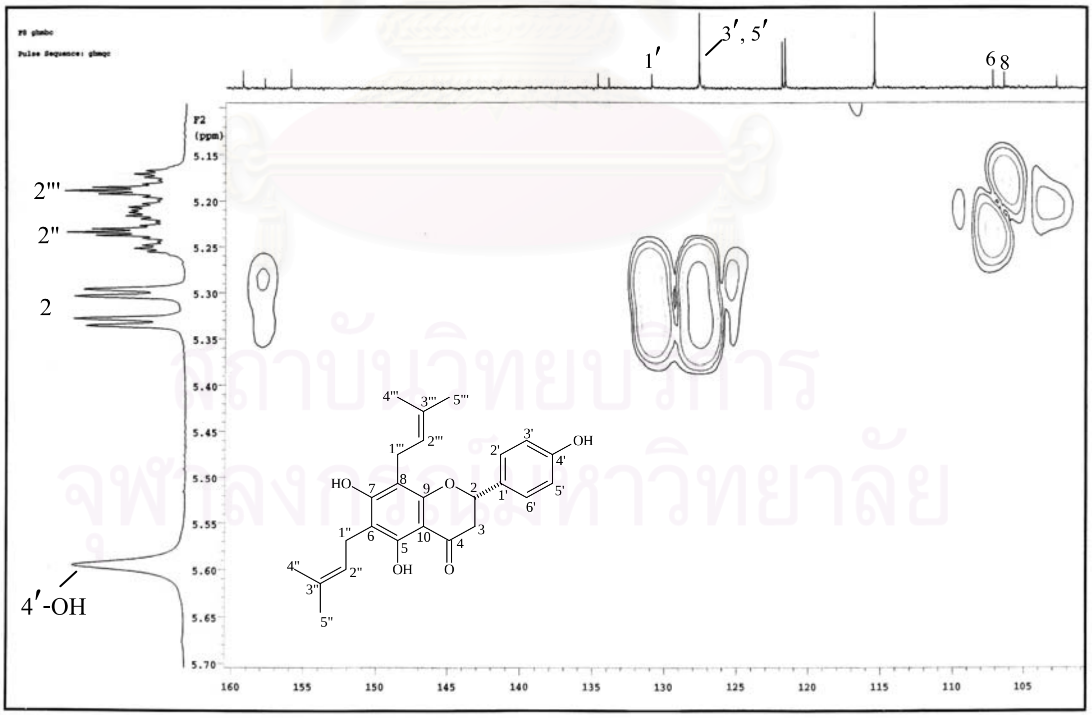


Figure 88 HMBC Spectrum of compound EF8 ($CDCl_3$)

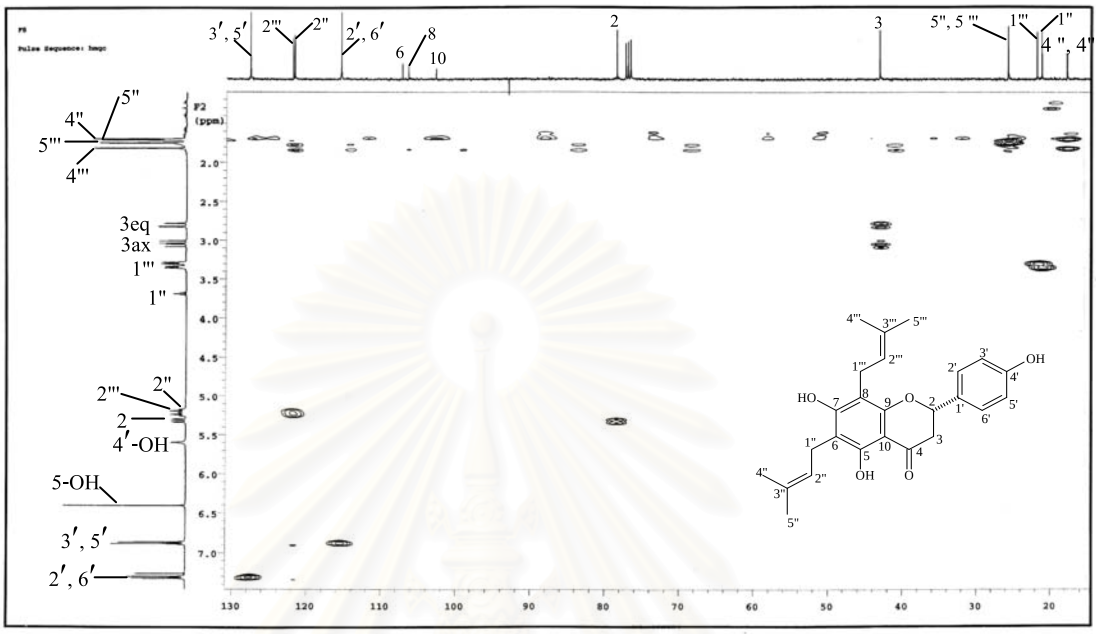


Figure 89 HMBC Spectrum of compound EF8 ($CDCl_3$)

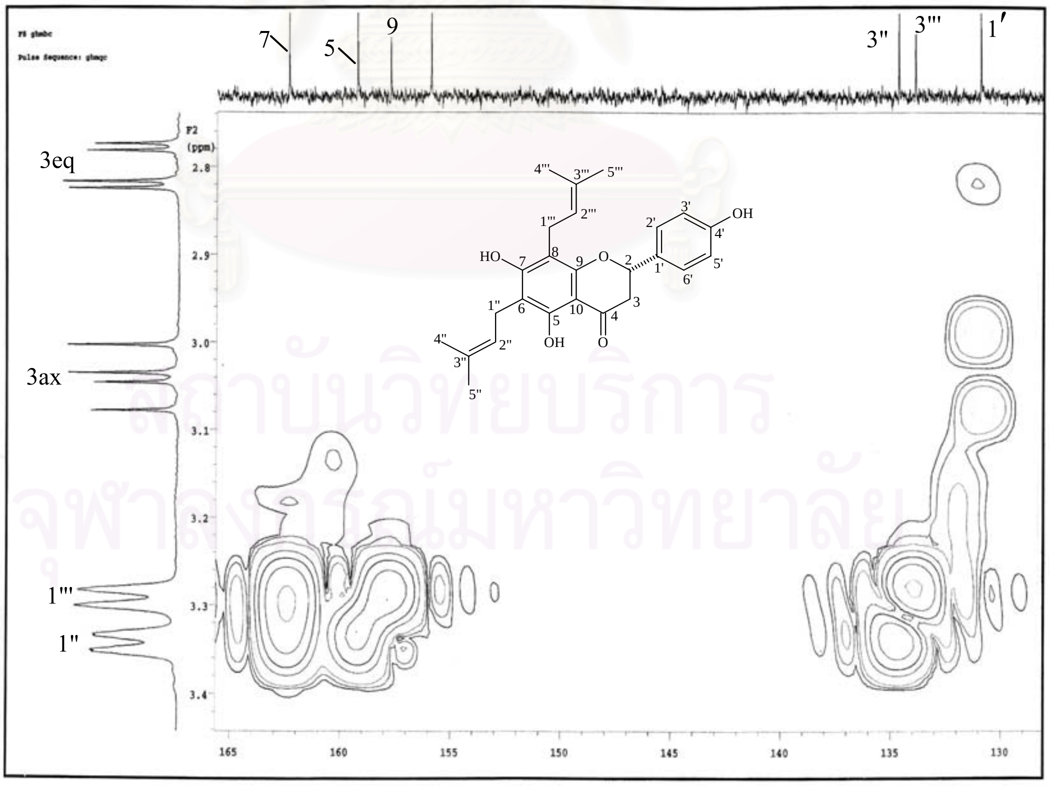


Figure 90 HMBC Spectrum of compound EF8 ($CDCl_3$)

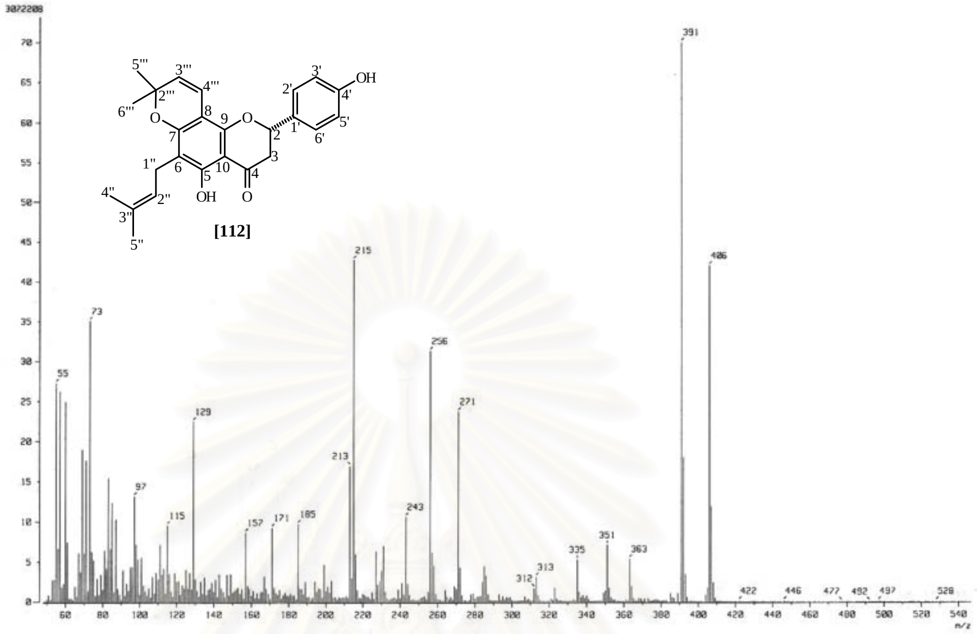


Figure 91 EI Mass spectrum of compound EF9

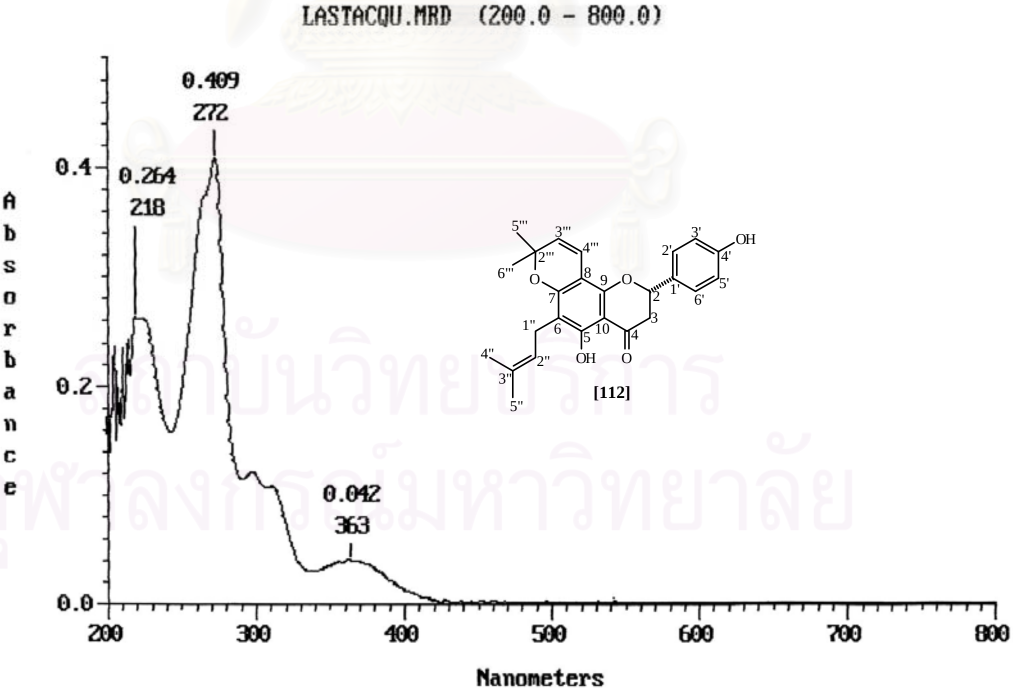


Figure 92 UV Spectrum of compound EF9 (MeOH)

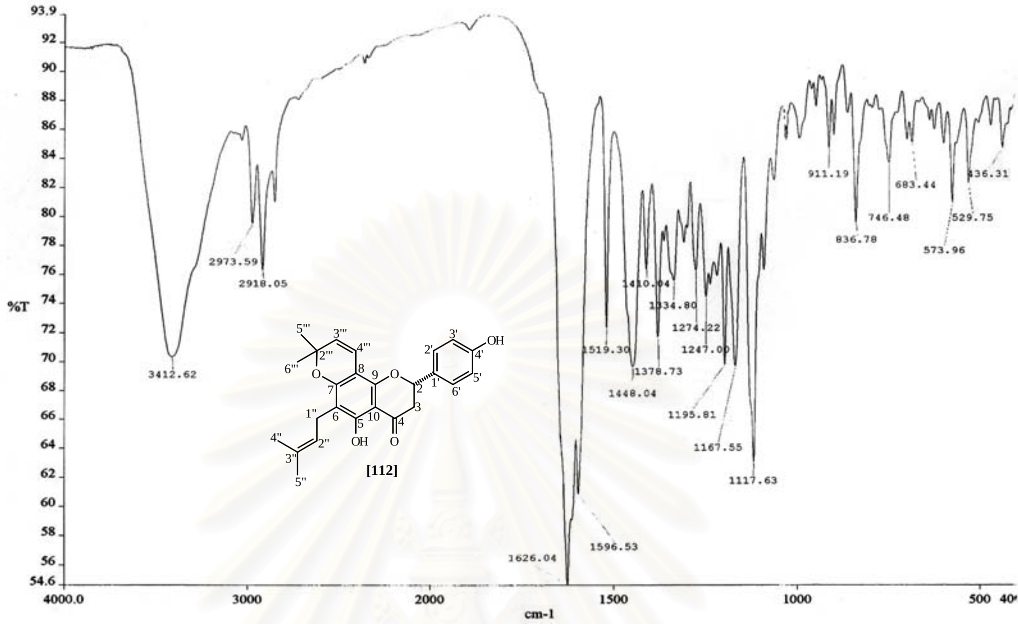


Figure 93 IR Spectrum of compound EF9 (KBr disc)

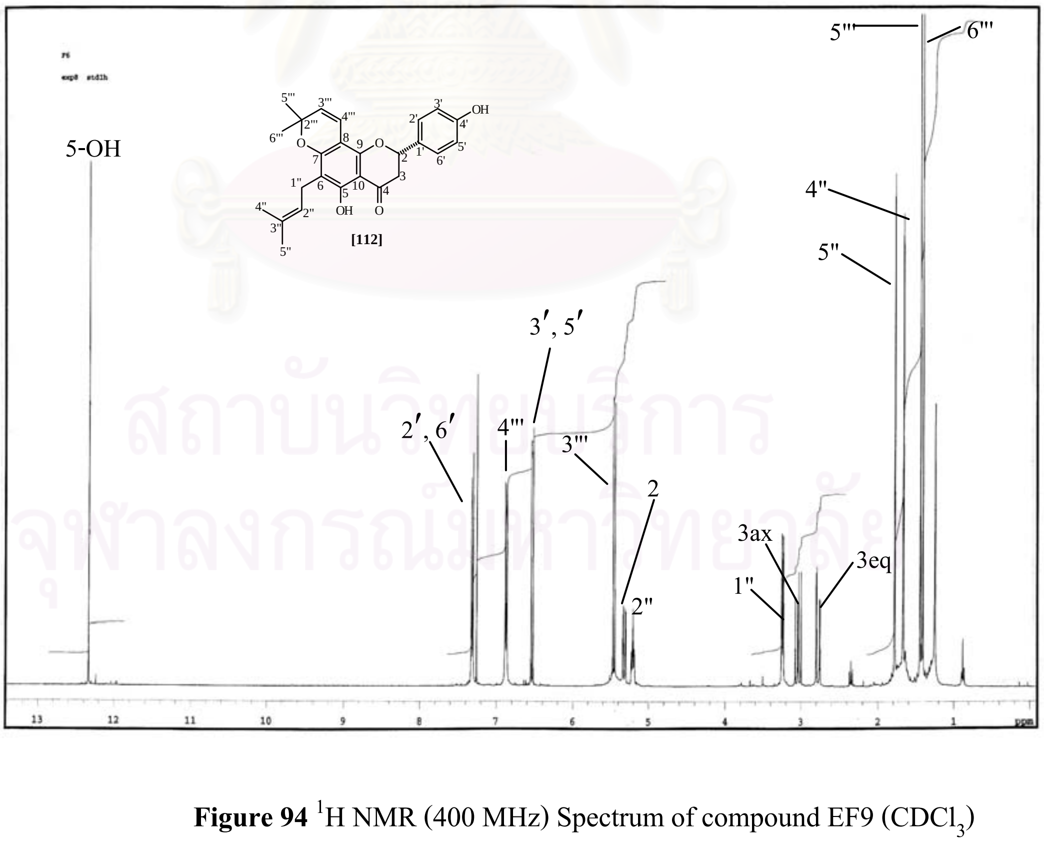


Figure 94 ^{1}H NMR (400 MHz) Spectrum of compound EF9 (CDCl$_3$)

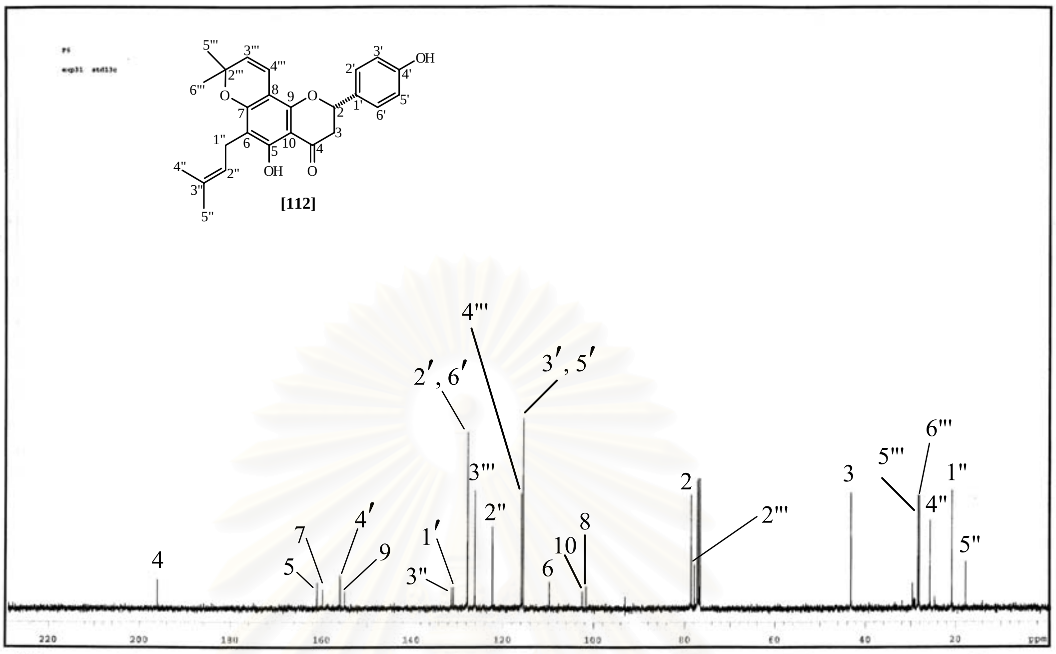


Figure 95 ^{13}C NMR (100 MHz) Spectrum of compound EF9 ($CDCl_3$)

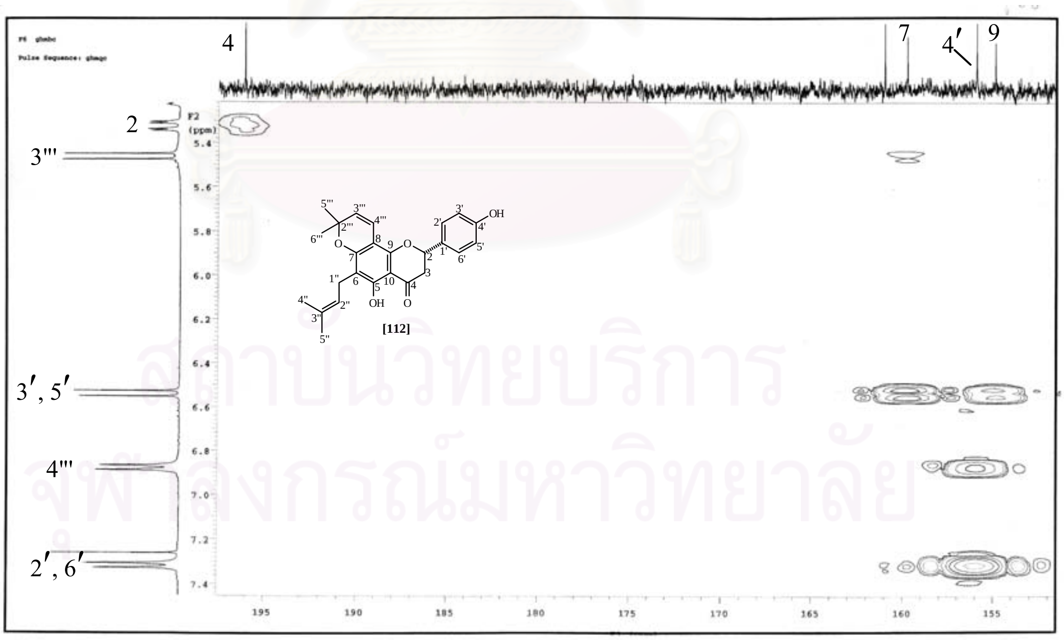


Figure 96 HMBC Spectrum of compound EF9 ($CDCl_3$)

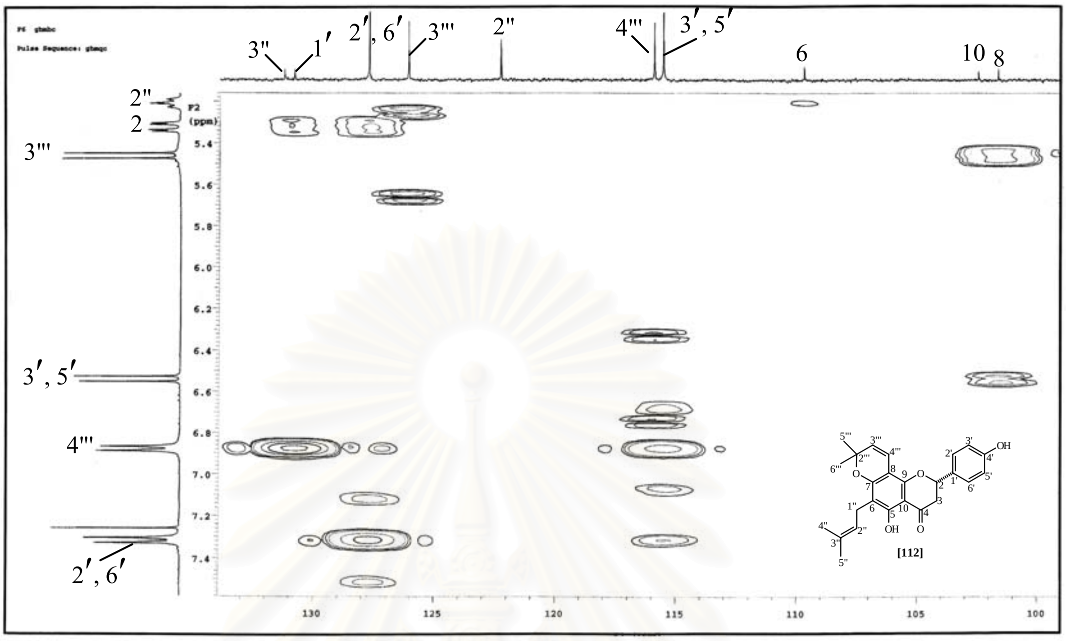


Figure 97 HMBC Spectrum of compound EF9 ($CDCl_3$)

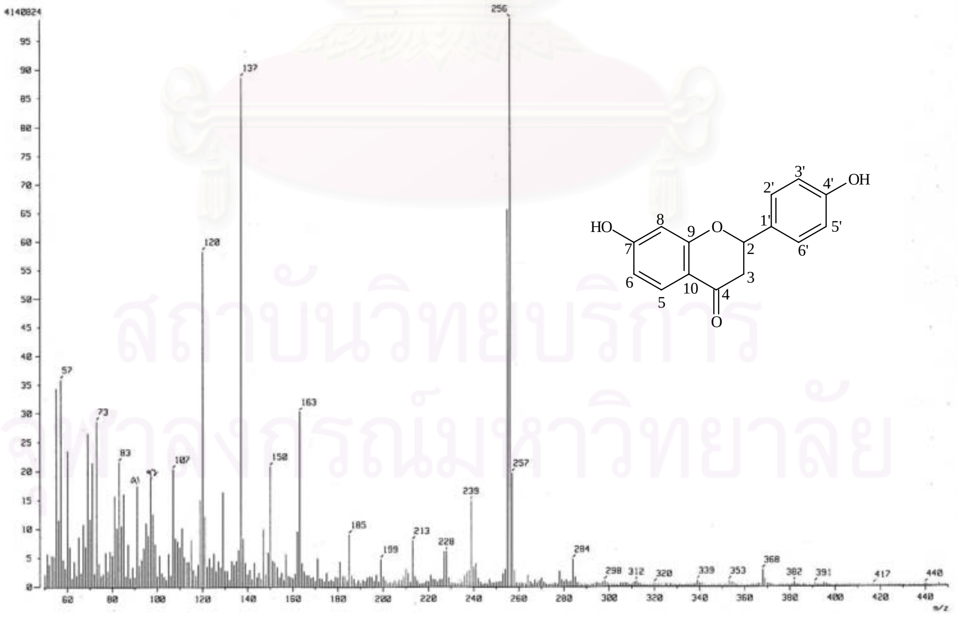


Figure 98 EI Mass spectrum of compound EF10

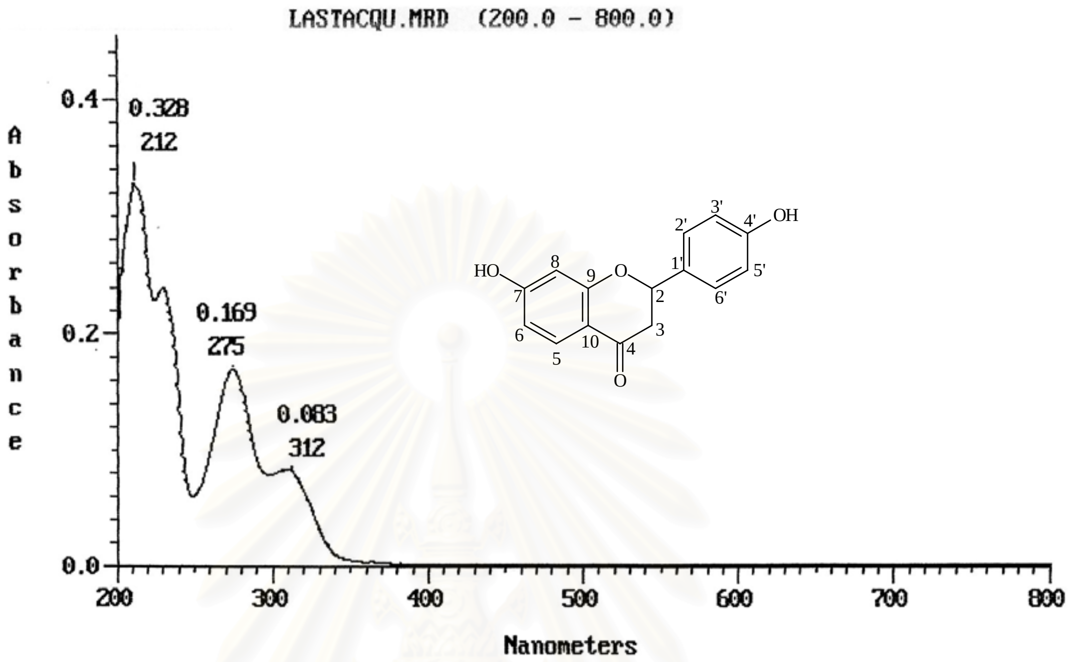


Figure 99 UV Spectrum of compound EF10 (MeOH)

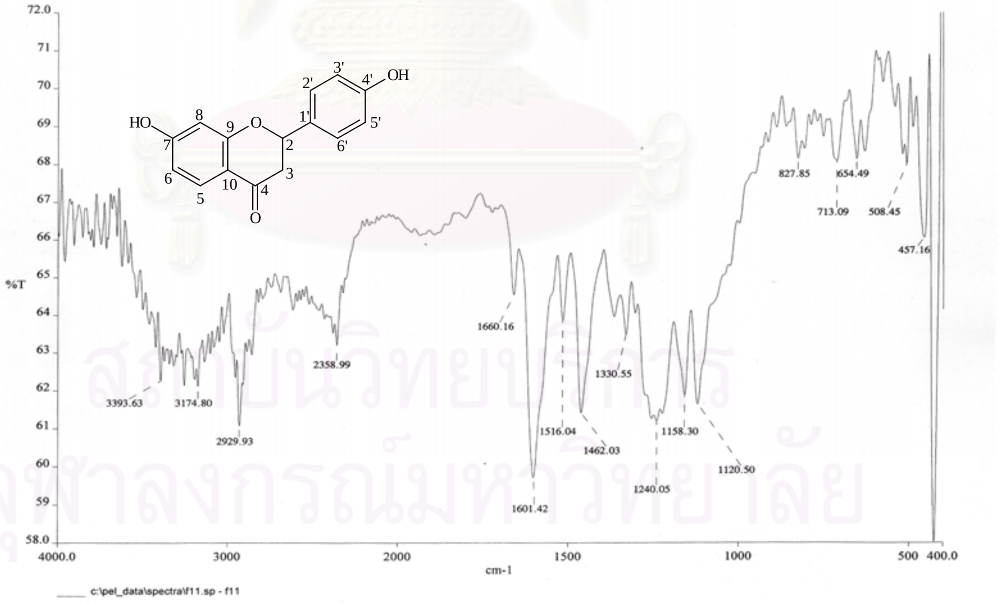


Figure 100 IR Spectrum of compound EF10 (Film)

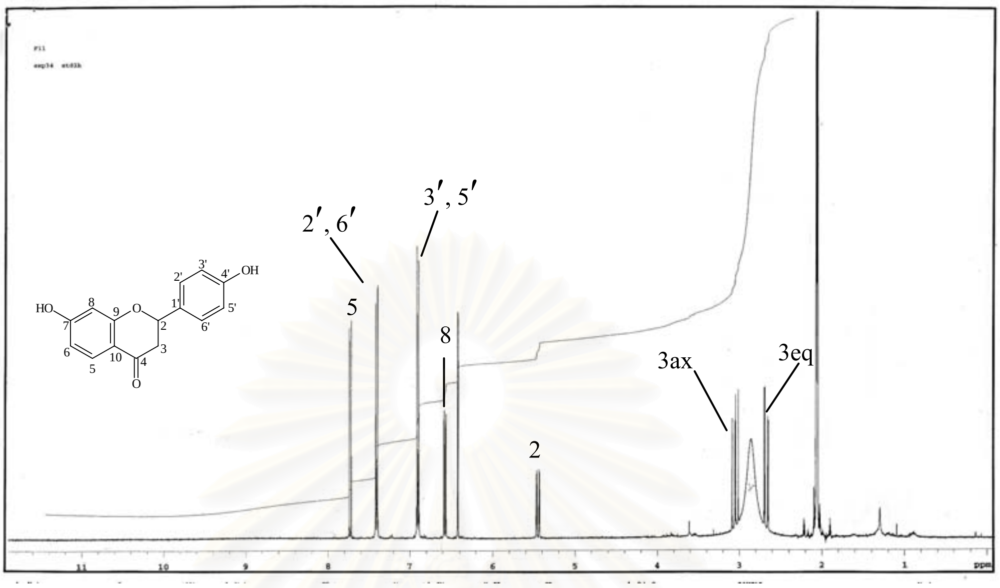


Figure 101 ^{1}H NMR (400 MHz) Spectrum of compound EF10 (Acetone-d_6)

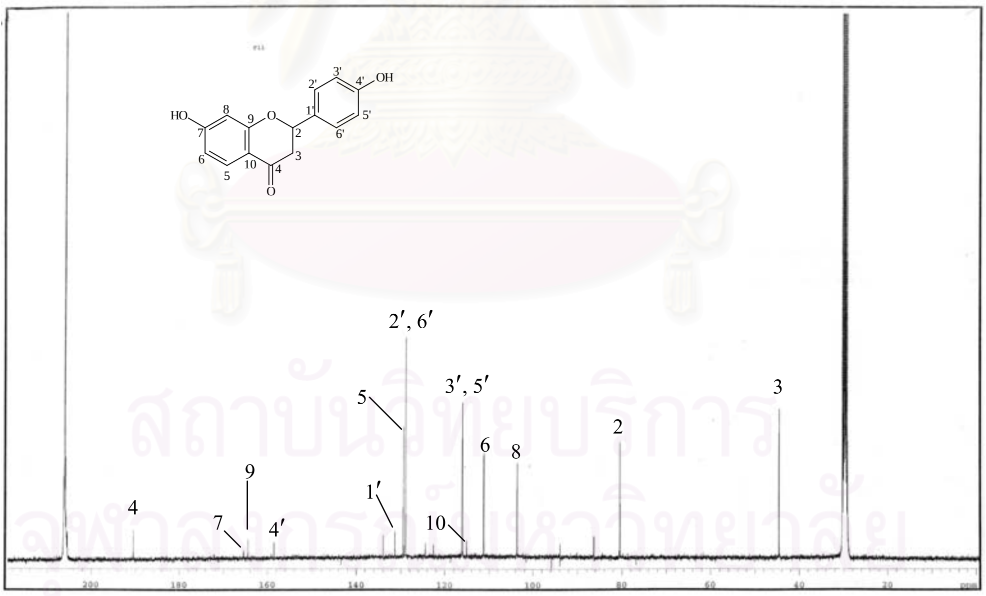


Figure 102 ^{13}C NMR (100 MHz) Spectrum of compound EF10 (Acetone-d_6)

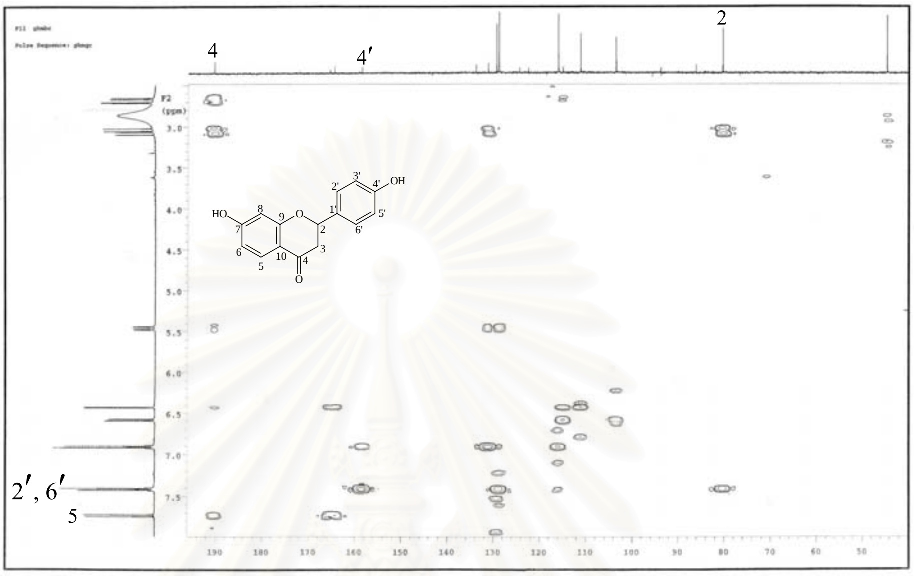


Figure 103 HMBC Spectrum of compound EF10 (Acetone-d_6)

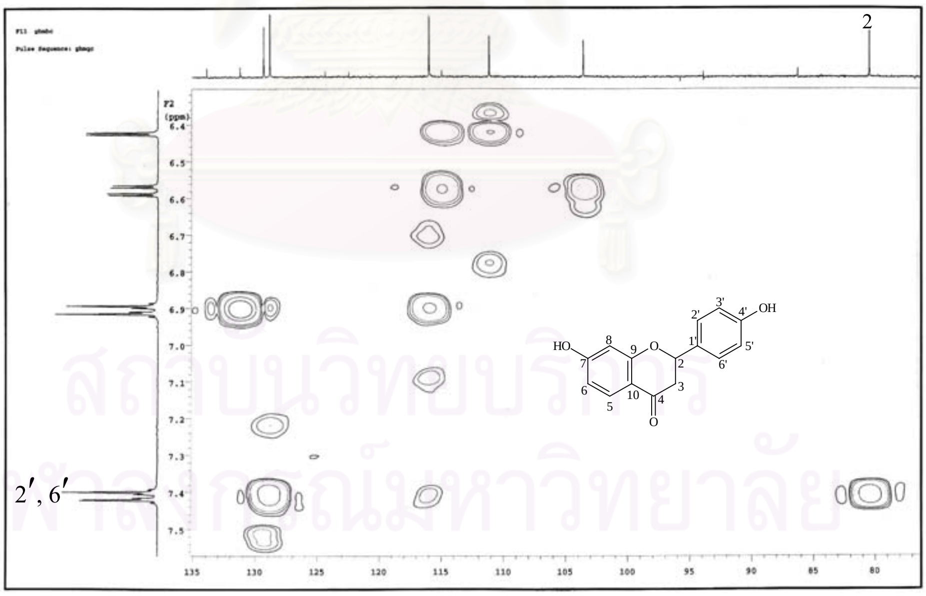


Figure 104 HMBC Spectrum of compound EF10 (Acetone-d_6)

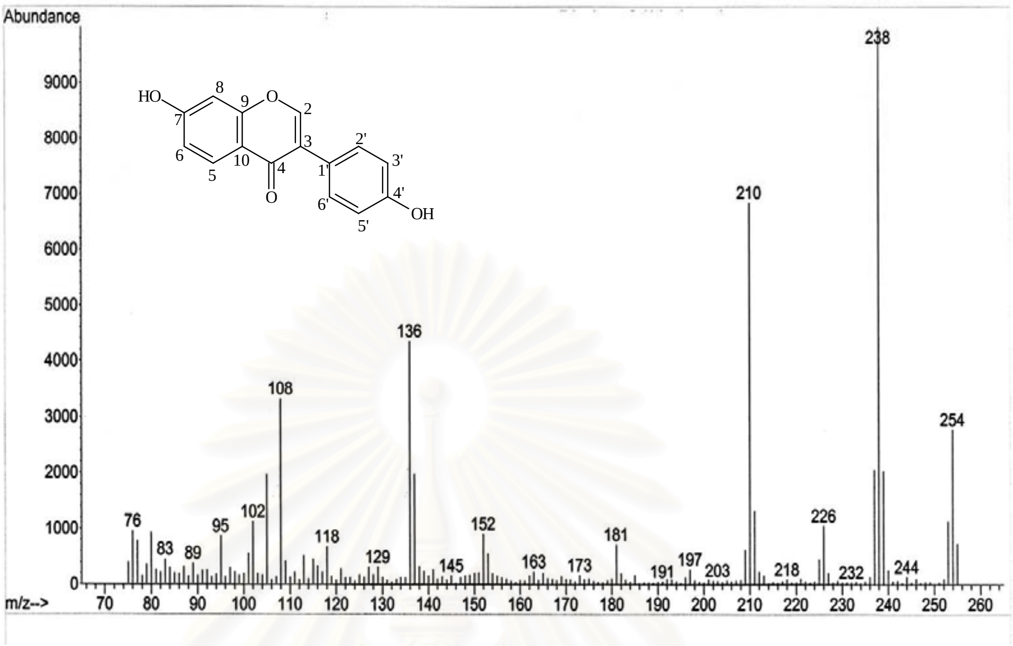


Figure 105 EI Mass spectrum of compound EF11

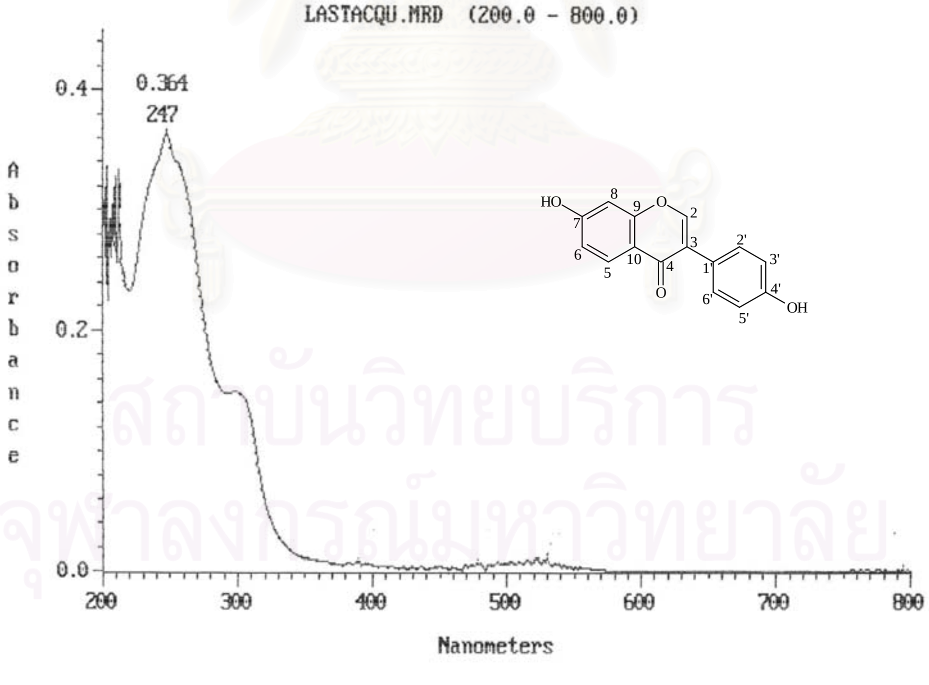


Figure 106 UV Spectrum of compound EF11 (MeOH)

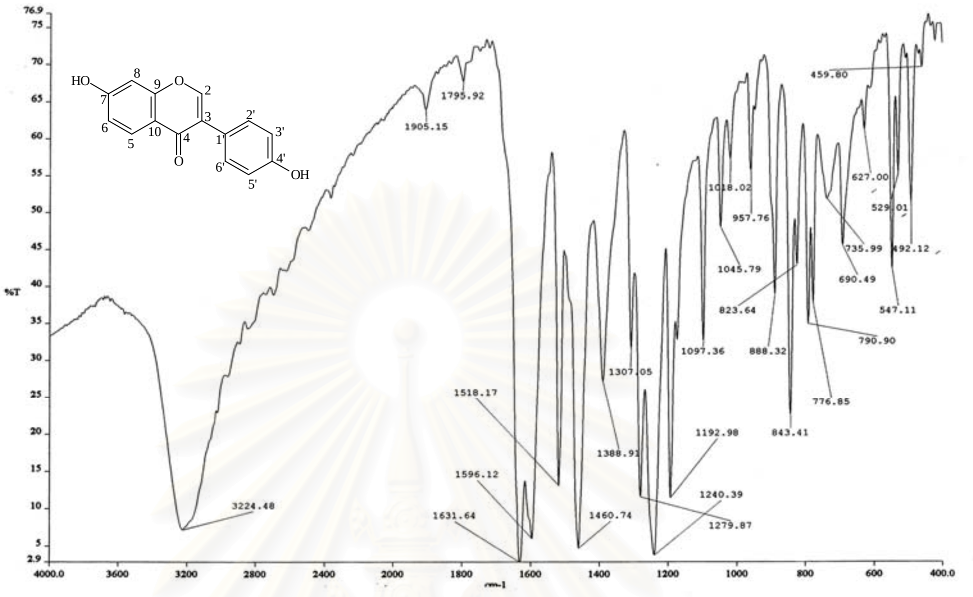


Figure107 IR Spectrum of compound EF11 (KBr disc)

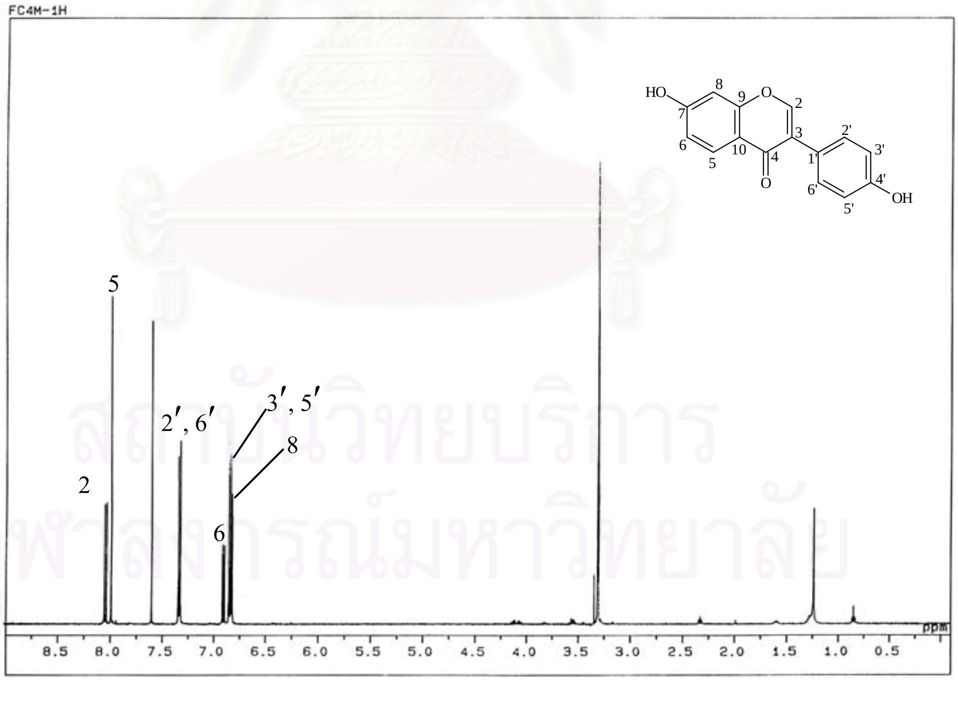


Figure 108 ^{1}H NMR (500 MHz) Spectrum of compound EF11 ($CDCl_3$:CD_3OD)

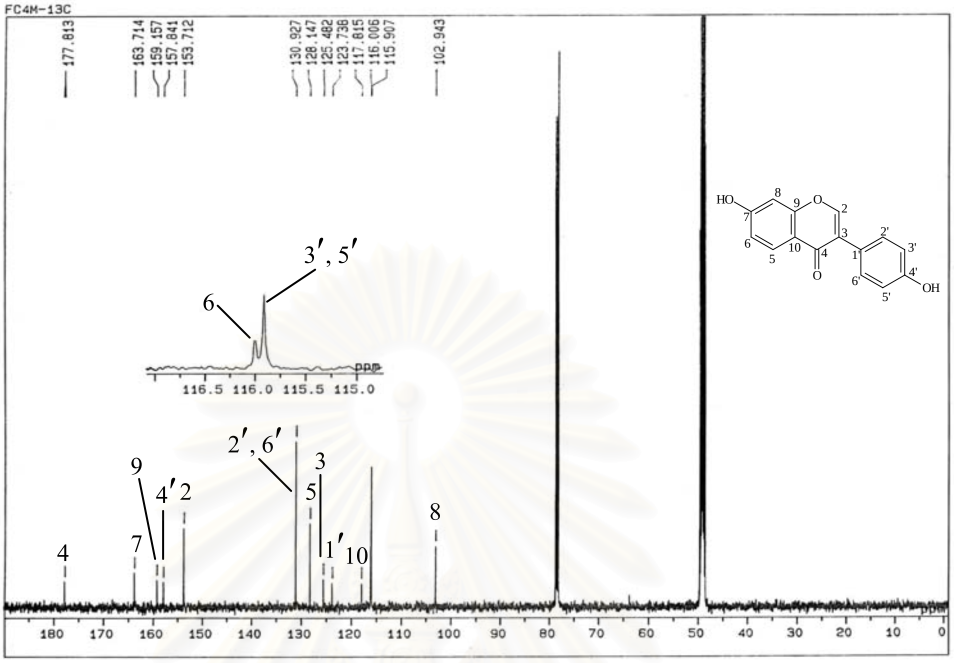


Figure109 ^{13}C NMR (125 MHz) Spectrum of compound EF11 (CDCl$_3$:CD$_3$OD)

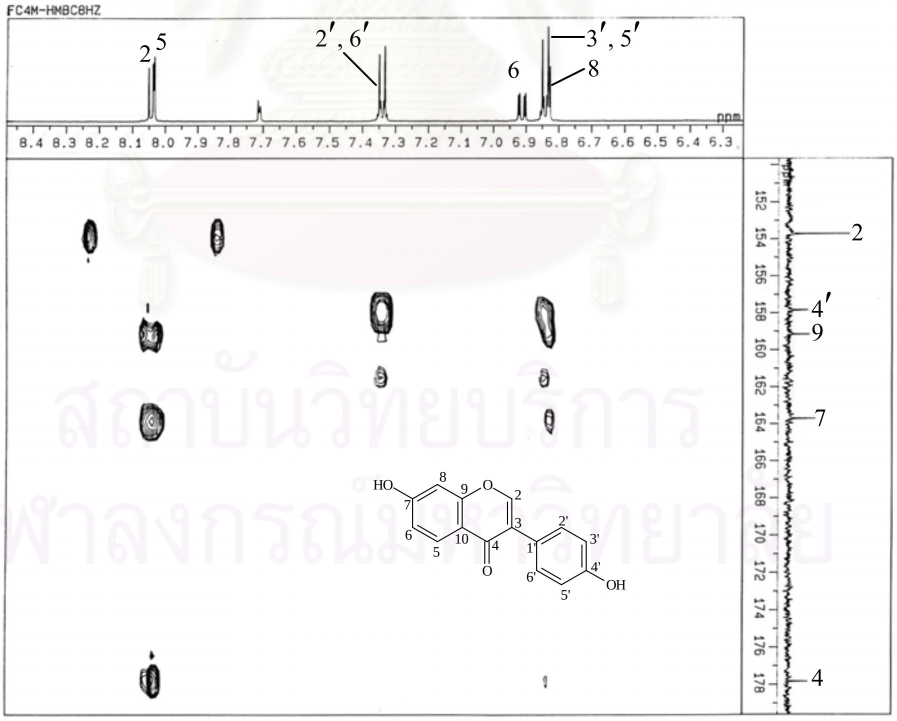


Figure 110 HMBC Spectrum of compound EF11 (CDCl$_3$:CD$_3$OD)

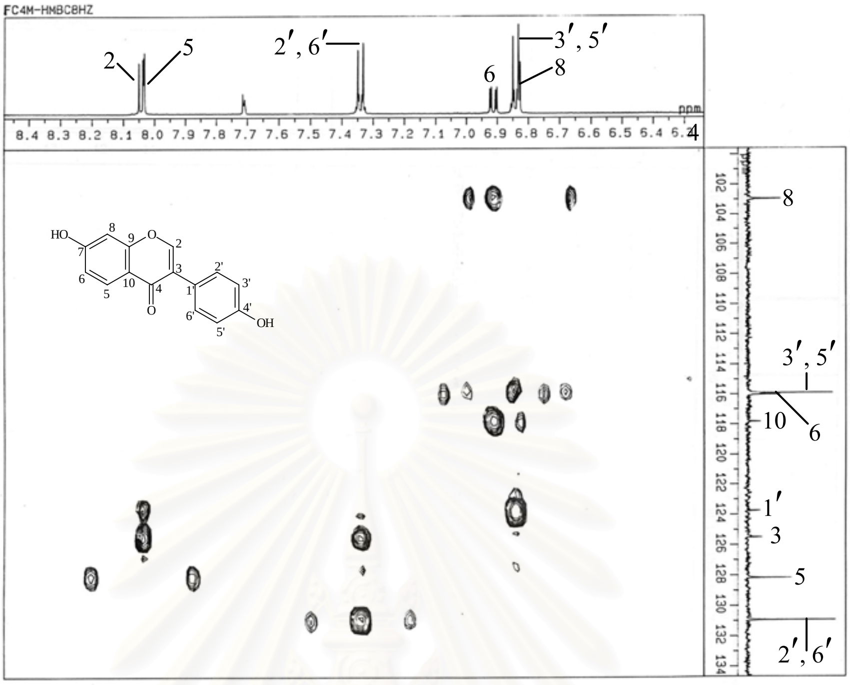


Figure 111 HMBC Spectrum of compound EF11 ($CDCl_3$:CD_3OD)

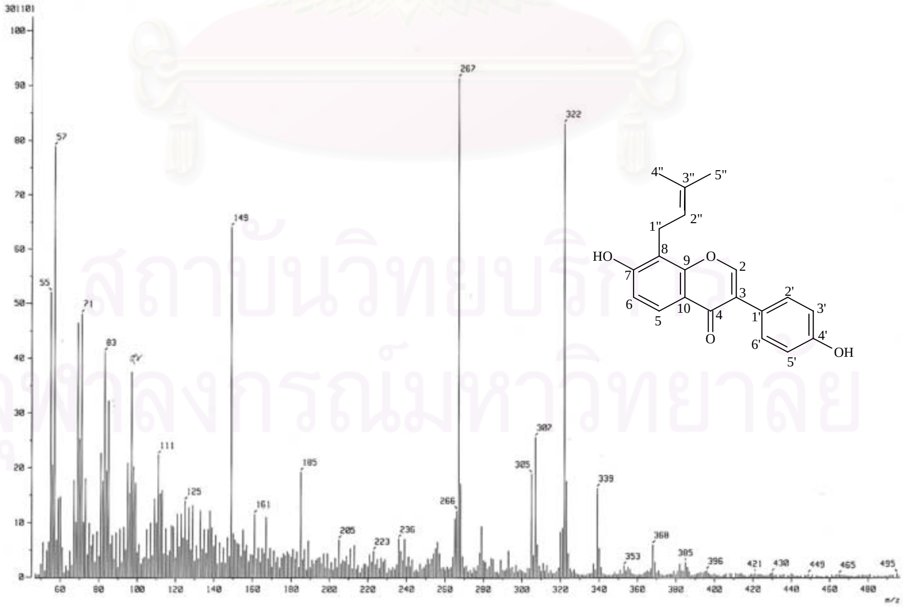


Figure 112 EI Mass spectrum of compound EF12

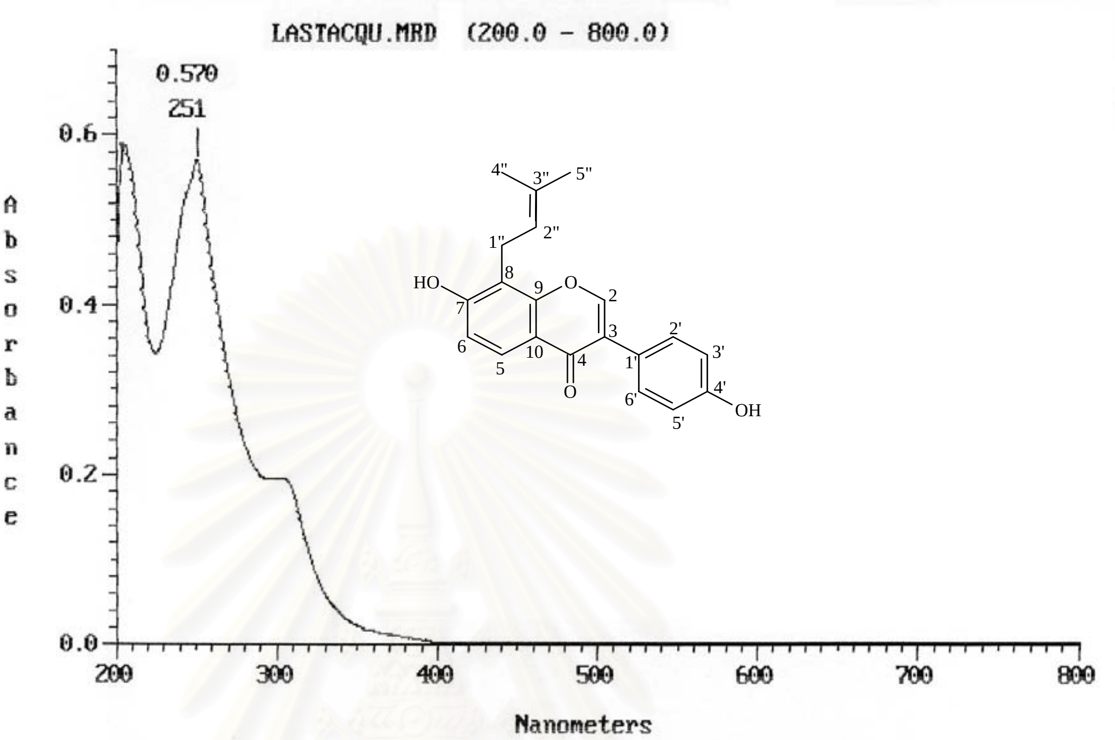


Figure 113 UV Spectrum of compound EF12 (methanol)

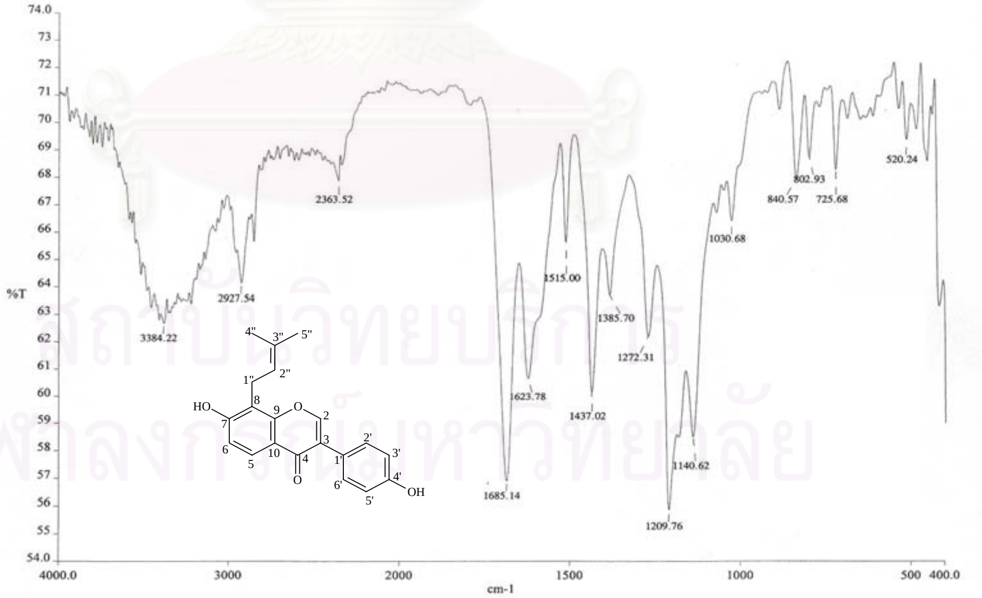


Figure 114 IR Spectrum of compound EF12 (Film)

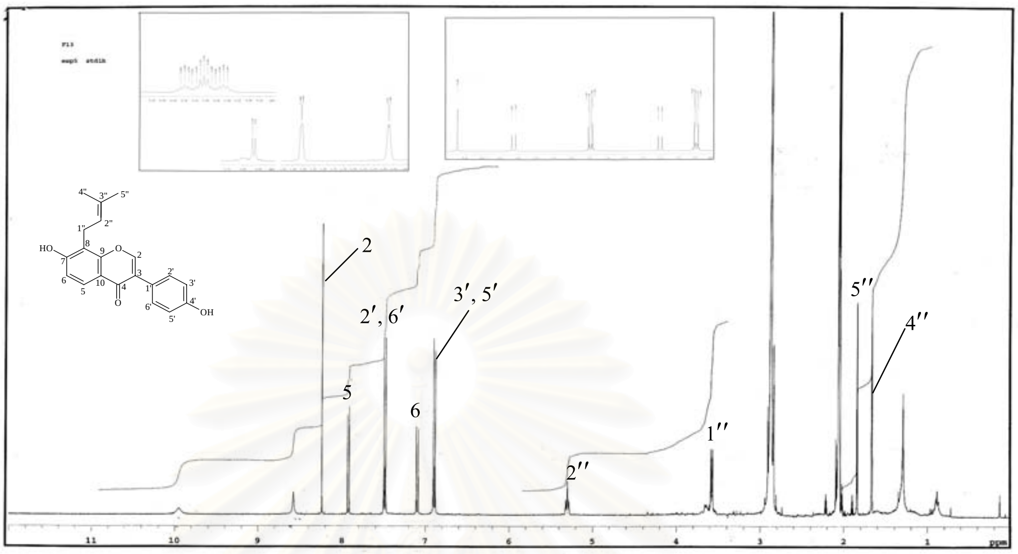


Figure 115 ^{1}H NMR (400 MHz) Spectrum of compound EF12 (Acetone-d_6)

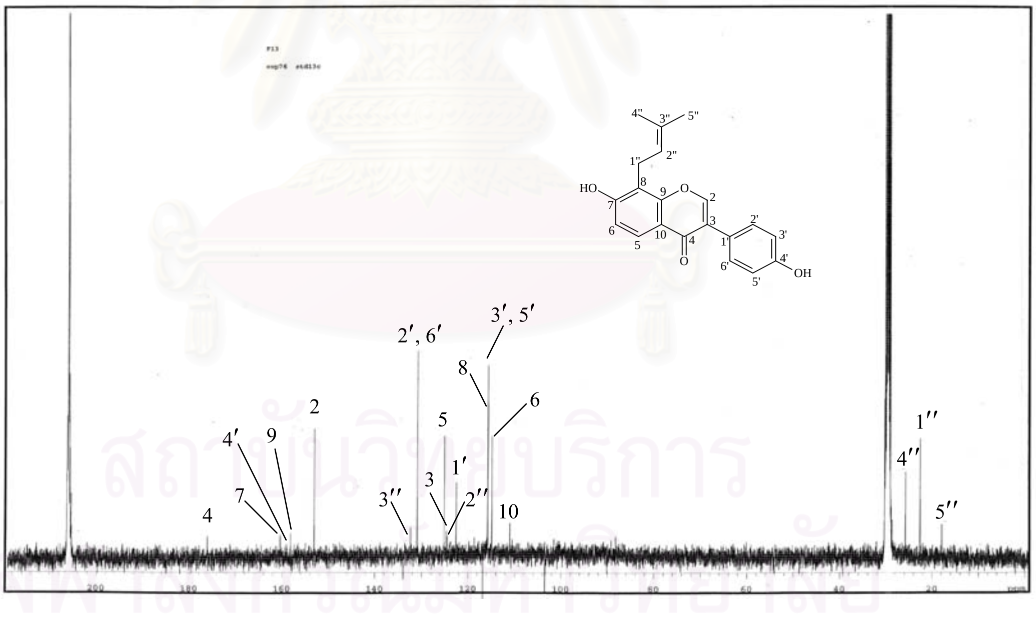


Figure 116 ^{13}C NMR (100 MHz) Spectrum of compound EF12 (Acetone-d_6)

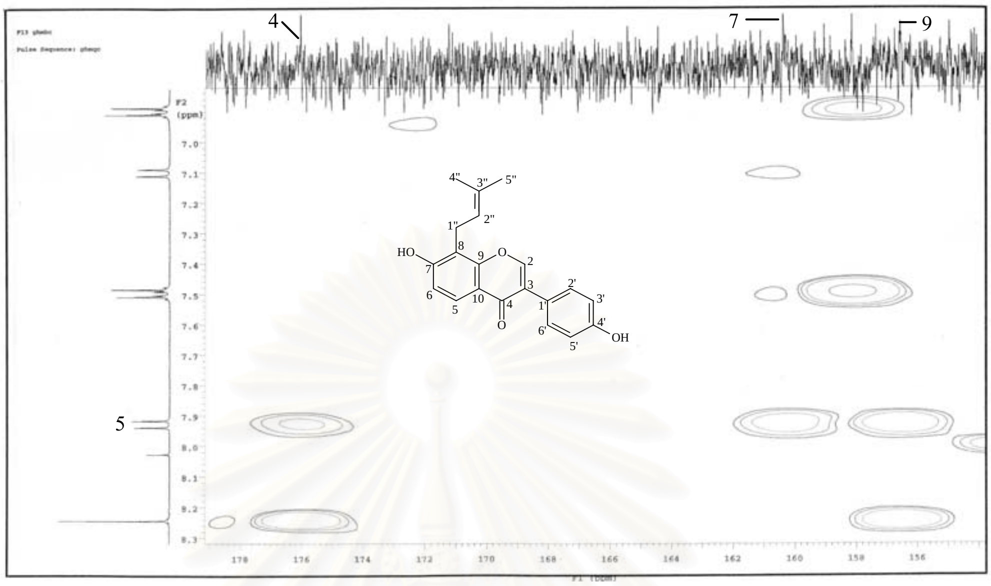


Figure 117 HMBC Spectrum of compound EF12 (Acetone-d_6)

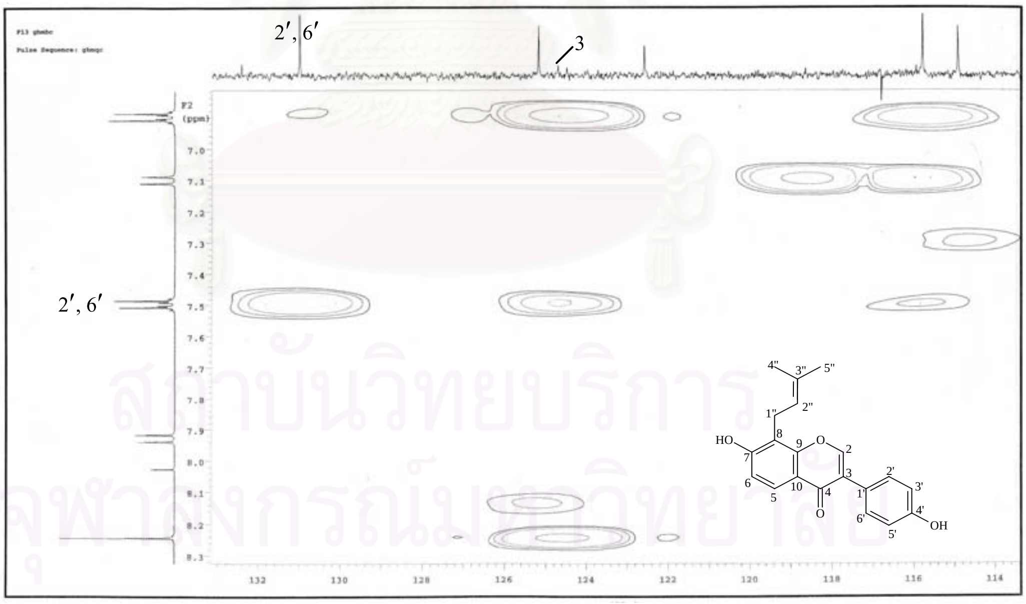


Figure 118 HMBC Spectrum of compound EF12 (Acetone-d_6)

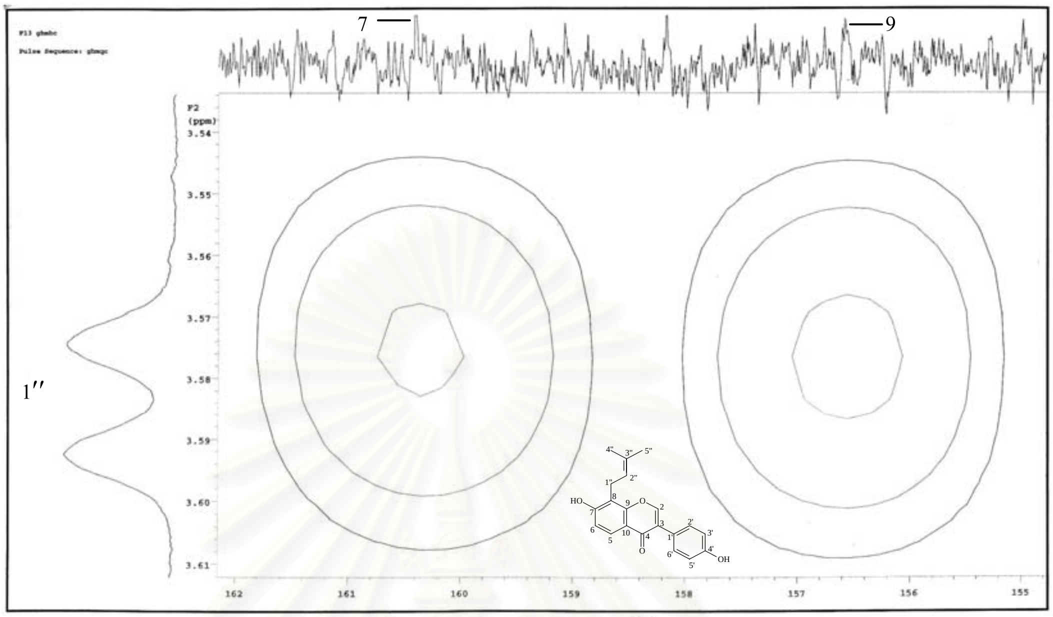


Figure 119 HMBC Spectrum of compound EF12 (Acetone-d_6)

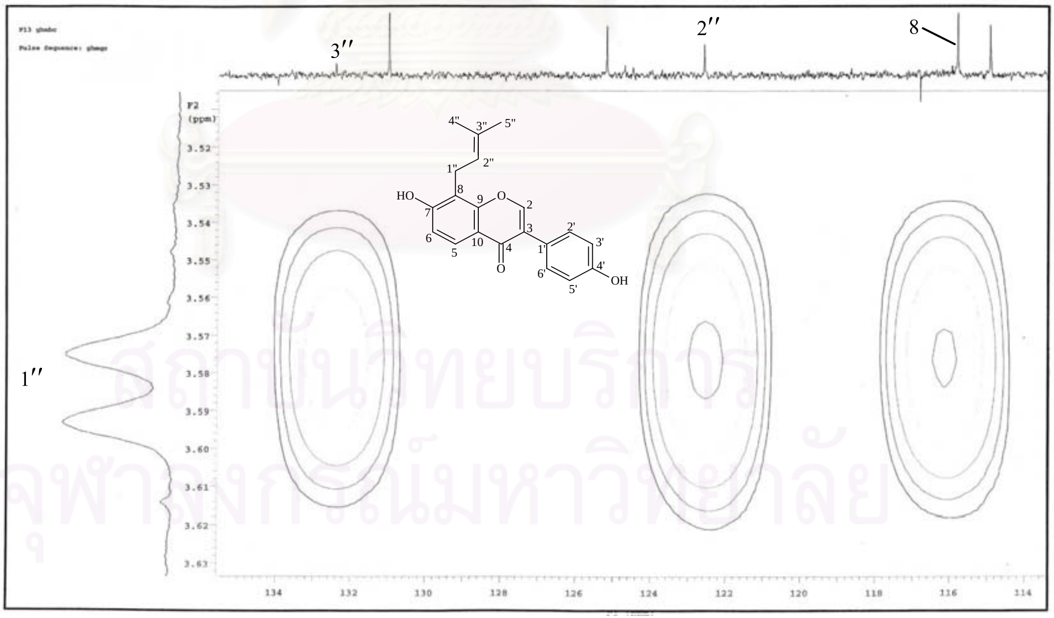


Figure 120 HMBC Spectrum of compound EF12 (Acetone-d_6)

VITA

Miss Pranorm Khaomek was born on July 28, 1969 in Pathumthani, Thailand. She received her Bachelor's degree of Science in Chemistry in 1991 from the Faculty of Science, Sri-Nakarinwirot University and Master's degree of Science in Analytical Chemistry in 1995 from Chulalongkorn University, Thailand. She was awarded a 1999 Staff Development from Rangsit University, Thailand and a 2003 research grant from the UNDP/World Bank/ WHO Special Program for Research and Training in Tropical Diseases (grant ID A10124) and the NCRT-JSPS at Kitasato University, Tokyo, Japan. She is currently a member of Faculty of Science, Rangsit University.

Publications

1. Khaomek, P., Ruangrungsi, N., Saifah, E., Sriubolmas, N., Ichino, C., Kiyohara, H. and Yamada, H. 2004. A new pterocarpan from *Erythrina fusca*. Heterocycles 63: 879-884.
2. Khaomek, P., Ruangrungsi, N., Saifah, E., Ichino, C., Kobayashi, M., Suzuki, M., Kiyohara, H., Otoguro, K., Yamada, H. and Omura, S. 2004. Chemical consitutents of *Erythrina suberosa*. Natural Medicines, In Press.
3. Khaomek, P., Ruangrungsi, N., Saifah, E., Ichino, C., Kobayashi, M., Suzuki, M., Kiyohara, H., Otoguro, K., Yamada, H. and Omura, S. 2004. *In vitro* antimalarial activity of flavonoids from *Erythrina fusca*. Planta Med., In Press.

Poster Presentrations

1. Khaomek, P., Ruangrungsi, N., Saifah, Kiyohara, H. and Yamada, H. Chemical consitutents of *Erythrina suberosa.* p.140. NRCT-JSPS Core University System on Pharmaceutical Sciences The Sixth Joint Seminar Recent Advances in Natural Medicine Research, December 2-4, 2004, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.
2. Khaomek, P., Ruangrungsi, N., Saifah, E., Sriubolmas, N., Kiyohara, H. and Yamada, H. Flavonoids from the stem bark of *Erythrina fusca*. p.141. NRCT-JSPS Core University System on Pharmaceutical Sciences The Sixth Joint Seminar Recent Advances in Natural Medicine Research, December 2-4, 2004, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.
3. Khaomek, P., Ruangrungsi, N., Saifah, Kiyohara, H. and Yamada, H. Chemical consitutents of *Erythrina suberosa.* p.P77. The 20th Annual Research Meeting in Pharmaceutical Sciences, December 1, 2004, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.
4. Khaomek, P., Ruangrungsi, N., Saifah, E., Sriubolmas, N., Kiyohara, H. and Yamada, H. Flavonoids from the stem bark of *Erythrina fusca*. p.P78. The 20th Annual Research Meeting in Pharmaceutical Sciences, December 1, 2004, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.