# การศึกษาทางพฤกษเคมีของรากพญารากเดียว

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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# PHYTOCHEMICAL STUDY OF *MALLOTUS SPODOCARPUS* ROOTS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmaceutical Botany
Department of Pharmaceutical Botany
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เพิ่มสุข สุขเกษม : การศึกษาทางพฤกษเคมีของรากพญารากเดียว (PHYTOCHEMICAL STUDY OF *MALLOTUS SPODOCARPUS* ROOTS) อ. ที่ปรึกษา : รศ. ดร. เอกรินทร์ สายฟ้า, อ. ที่ปรึกษาร่วม : รศ. ดร. ภาคภูมิ เด็งอำนวย, 105 หน้า.

จากการศึกษาองค์ประกอบทางเคมีของรากพญารากเดียว (วงศ์ Euphorbiaceae) สามารถแยกสารในกลุ่มไตรกลีเซอไรด์ชนิดใหม่ 1 ชนิด คือ 1,3-dilauroyl-2-linolenoylglycerol นอกจากนี้ยังพบสาร $\beta$-sitosterol, $\beta$-sitosterol glucoside และ bergenin การพิสูจน์โครงสร้างทางเคมีของสารทั้งหมดที่สกัดแยกได้โดยอาศัยการวิเคราะห์เชิงสเปคตรัมของUV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่มีการรายงานมาแล้ว



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| ภาควิชา | เภสัชพฤกษศาสตร์ | ลายมือชื่อนิสิต..... |
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KEY WORD: *MALLOTUS SPODOCARPUS*/ GALLIC ACID DERIVATIVE/ STEROID/TRIGLYCERIDE

PERMSUK SUKKHASEM : PHYTOCHEMICAL STUDY OF *MALLOTUS SPODOCARPUS* ROOTS. THESIS ADVISOR: ASSOC. PROF. EKARIN SAIFAH, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. PARKPOOM TENGAMNUAY, Ph.D., 105 pp.

Chemical investigation of the roots of *Mallotus spodocarpus* (family Euphorbiaceae) led to the isolation of one new triglyceride, namely 1,3-dilauroyl-2-linoleoylglycerol along with $\beta$-sitosterol, $\beta$-sitosterol glucoside and bergenin. The structure determination of these compounds was accomplished by spectroscopic methods, including UV, IR, MS and comparison with previously reported data.



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# LIST OF ABBREVIATION AND SYMBOLS

| | | |
|---|---|---|
| $[\alpha]^{25}_{D}$ | = | Specific rotation at 25° and sodium D line (589 nm) |
| α | = | Alpha |
| β | = | Beta |
| *br t* | = | Broad triplet (for NMR spectra) |
| °C | = | Degree Celsius |
| calcd | = | Calculated |
| CC | = | Column chromatography |
| $CDCl_3$ | = | Deuterated chloroform |
| $CH_2Cl_2$ | = | Dichloromethane |
| cm | = | Centimeter |
| $cm^{-1}$ | = | Reciprocal centimeter (unit of wave number) |
| $^{13}$C-NMR | = | Carbon-13 Nuclear Magnetic Resonance |
| COSY | = | Correlated Spectroscopy |
| 2D NMR | = | Two dimensional Nuclear Magnetic Resonance |
| *d* | = | Doublet (for NMR spectra) |
| *dd* | = | Doublet of doublets (for NMR spectra) |
| DPPH | = | 1,1-diphenyl-2-picrylhydrazyl |
| DEPT | = | Distortionless Enhancement by Polarization Transfer |
| DMSO-$d_6$ | = | Deuterated dimethylsulfoxide |
| δ | = | Chemical shift |
| ε | = | Molar absorptivity |
| EIMS | = | Electron Impact Mass Spectroscopy |
| EtOAc | = | Ethyl acetate |
| eV | = | electron volt |
| g | = | Gram |
| $^{1}$H-NMR | = | Proton Nuclear Magnetic Resonance |
| $^{1}$H-$^{1}$H COSY | = | Homonuclear (Proton-Proton) Correlation Spectroscopy |
| HMBC | = | $^{1}$H-detected Heteronuclear Multiple Bond Coherence |
| HMQC | = | $^{1}$H-detected Heteronuclear Multiple Quantum Coherence |
| Hz | = | Hertz |

| | | |
|---|---|---|
| IR | = | Infrared Spectrum |
| *J* | = | Coupling constant |
| KBr | = | Potassium bromide |
| Kg | = | Kilogram |
| L | = | Liter |
| $\lambda_{max}$ | = | Wavelength at maximum absorption (nm) |
| $[M^+]$ | = | Molecular ion |
| m | = | Meter |
| *m* | = | Multiplet (for NMR spectra) |
| MeOH | = | Methanol |
| mg | = | Milligram |
| MHz | = | Megahertz |
| ml | = | Milliliter |
| mm | = | Millimeter |
| m.p. | = | Melting point |
| MS | = | Mass Spectrometry |
| MW | = | Molecular weight |
| *m/z* | = | Mass to charge ratio |
| nm | = | Nanometer |
| NOESY | = | Nuclear Overhauser Effect Spectroscopy |
| n.s. | = | Not specified |
| ppm | = | Part-per-million |
| *s* | = | Singlet (for NMR spectra) |
| $\nu_{max}$ | = | Wavenumber at maximum absorption |
| sp. | = | Species |
| *t* | = | Triplet (for NMR spectra) |
| TLC | = | Thin layer Chromatography |
| UV | = | Ultraviolet |

# CHAPTER I

# INTRODUCTION

*Mallotus spodocarpus* Airy Shaw (Figure 1) is a plant belonging to the family Euphorbiaceae. In Thailand, this plant is called Phaya rak diao (Eastern), Ta khe khum wang (Southwestern) and Tao tua mia (Central). (ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้, 2544). The description of *Mallotus spodocarpus*, according to Thai Forest Bulletin (Welzen et al., 2000), is as follows:

> "Shrublets up to 50 cm high. Indumentum of sparse, long simple and stellate hairs, glandular scales whitish. Stipules triangular, 2.3-7 by 0.6-1 mm. Leaves opposite; petiole 0.3-5.5 cm long; blade elliptic, 2.2-16 by 1.2-8 cm, length/width ratio 1.8-2,drying brownish,base broadly shallowly emarginate to truncate, margin subentire to denticulate with glandular teeth, apex rounded to bluntly acuminate, upper surface glabrous to some hairs on especially midrib, no extrafloral nectaries, lower surface subglabrous to somewhat hairy on venation, usually hair tuft domatia, glandular scales sparse, venation triplinerved. Inflorescences mainly axillary, very short, single, not branching. Staminate inflorescences up to 2 cm long, usually dichasial and forming large clumps of flowers, flowers c. 5 per node; bracts elliptic, 1.6-2.3 by 0.4–0.7 mm. Staminate flowers: pedicel 1–2 mm long; sepals 3–5, elliptic to obovate, c. 4 mm long; stamens c. 25, filaments still young. Pistillate inflorescences usually reduced to (1)2(-4)-flowered spikes, up to 2 cm long; bracts c. 3 by 0.7 mm. Pistillate flowers c. 3.3 mm in diameter; pedicel 1(-4.5 in fruit) mm long; sepals 3 or 4, ovate, c. 3.3 by 1.1 mm; ovary 3-locular, c. 2 by 2.7 mm, not armed, densely villose; style 0.9–1 mm long; stigmas 1.7–2 mm long. Fruits lobed capsules,10-12 by 7-8 mm, unarmed, densely villose; column not seen. Seeds subglobose, 5-6 mm in diameter."

According to seventeen species of *Mallotus* found in Thailand are as follows:

| | |
|---|---|
| *M. barbatus* Müll. Arg. | ตองเต๊า (Tong tao) |
| *M. brevipetiolatus* Gage. | ปอหุ้นก้านสั้น (Po hun kan san) |
| *M. cuneatus* Ridl. | โผ (Pho) |
| *M. floribundus* Müll. Arg. | ปริก (Prik) |
| *M. kongkandae* Welzen & Phattarahirankanok | ประกายแสด (Pra kai saet) |
| *M. macrostachyus* Müll. Arg. | เปล้าใหญ่ (Plao yai) |
| *M. oblongifolius* Müll. Arg. | หลอดเถื่อน (Lot thuean) |
| *M. paniculatus* Müll. Arg. | สอยดาว (Soi dao) |
| *M. peltatus* Müll. Arg. | สลัด (Salat) |
| *M. philippinensis* Müll. Arg. | คำแสด (Kham saet) |
| *M. repandus* Müll. Arg. | มะกายเครือ (Makai khruea) |
| *M. spinihispidus* Welzen & Chayamarit | มะต่อง (Ma tong) |
| *M. spodocarpus* Airy Shaw | ตะเข้คุ้มวัง (Ta khe khum wang), พญารากเดียว (Phaya rak diao), เต่าตัวเมีย (Tao tua mia) |
| *M. stipularis* Airy Shaw | นูด (Nut) |
| *M. subpeltatus* (Blume) Müll. Arg. | ช้าเงาะผี (Cha ngo phi) |
| *M. thorelii* Gagnep | ฝายน้ำ (Fai nam) |
| *M. viridis* Welzen & Chayamarit | มะสอย (Ma soi) |

*Mallotus spodocarpus* is distributed in Thailand and Vietnam. In Thailand, it can be found in the northern (Nakhon Sawan), eastern (Nakhon Ratchasima), south-western (Kanchanaburi, Phetchaburi) and central parts(Chai Nat, Saraburi) at altitude of 20-250 m, along sunny waysides, mixed forest, deciduous forest, bamboo forest and limestone soil.

The people in the northeastern part of Thailand use the powdered roots of this plant for skin whitening. The chloroform extract from the roots of *Mallotus spodocarpus* was demonstrated as exhibiting analgesic effect and anti-inflammatory activity on both the acute and chronic phases of inflammation.

Currently, phytochemical study on the roots of *Mallotus spodocarpus* has never been reported. However, preliminary screening of the crude extract of the roots has shown significant scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH). Therefore, the purpose of this investigation was to isolate, identify and to evaluate the antioxidant activity of the compounds from the roots of *Mallotus spodocarpus*. The results may serve as an additional information on the chemical nature of this plant family, its chemotaxonomy and biological activities.

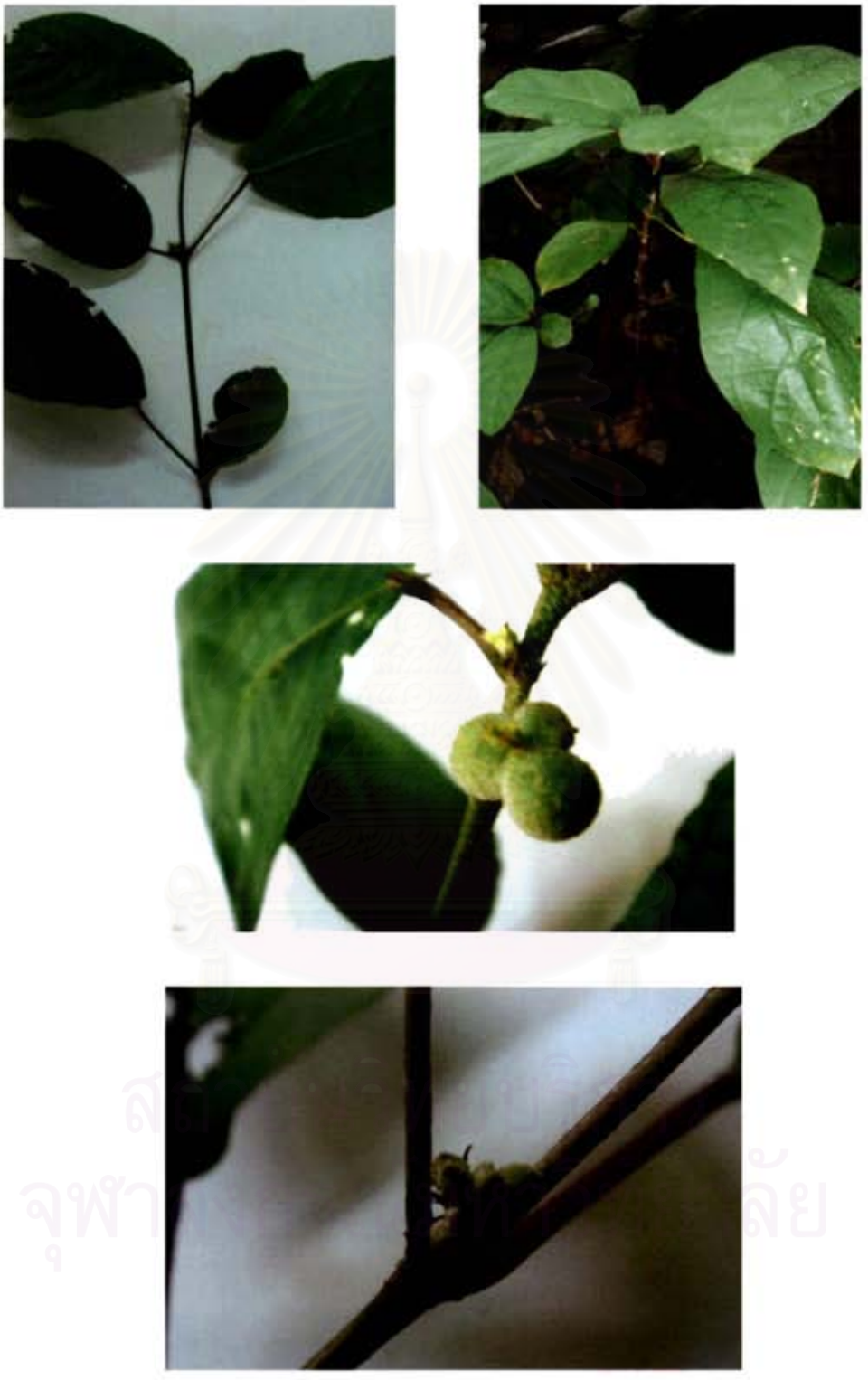


**Figure 1.** *Mallotus spodocarpus* Airy Shaw

# CHAPTER II

# HISTORICAL

## 1. Botanical description of euphorbiaceous plants

The family Euphorbiaceae is large and diverse, with about 300 genera and 8,100 species. They grow extensively in the tropical and warmer regions of the world.

The plants in this family can be described as highly variable with plants growing as herbs, shrubs or tree with fleshy stems and milky or colored latex that can be irritating or toxic.

The leaves of these plants are usually alternate, but can also be opposite or even whorled. The stipules are present, they can be large or small and gland-like. The leaves are usually simple but some are palmately compound.

The flowers are regular and usually monoecious, but they can also be dioecious although this form is rare. The inflorescences are various in type, often compacted to form a special flower cluster called a cyathium. The perianth is usually 5-merous, distinct or connate. The androecium consists of one to many stamens that are free or united. The filaments are distinct or connate. Sometimes the nectary disk is present. The opening of anther is by longitudinal slits. The gynoecium of a flower consists of a compound pistil of 3 united carpels (but they can have 2 or 4), with as many locules. The ovary is superior and commonly 3-lobed. There are 1 or 2 ovules in each locule attached to apical-axile placentas. The styles can be distinct or connate into a single style.

The fruits is usually a dehiscent capsule, but can occasionally be an indehiscent utricle in 1-celled species. This schizocarp separates elastically into usually 3 segments that split ventrally.

The seeds are abundant, having fleshy endosperm with the embryo straight or curved (Dennis, 2000).

## 2. Ethnomedicinal Uses of *Mallotus* species

Plants of the genus *Mallotus* have been known for their uses in traditional medicine of several countries, as follows.

The roots of *M. apelta* are used in traditional Chinese medicine to treat chronic hepatitis (Cheng, Meng and Chen, 1998).

The leaves of Chinese plant, *M. furetianus* ("Shan Ku Cha" in Chinese), are used as a folk medicine for the treatment of cholecystitis (Wei *et al.*, 2004).

The bark *of M. japonicas*, a dioecious and deciduous tree distributed throughout tropical and temperate Asia, is used as a cure for ulcers and its leaves are used as a treatment of pimples (Ishii *et al.*, 2001).

*M. peltatus* is widely used among the tribal population of Bay Islands in India. The decoction of its leaves is used for the treatment of stomach ache, intestinal ailments and skin infections (Chattopadhyay *et al.*, 2002).

A red powder consisting of glandular hairs from the capsules of *M. philippinensis* , locally known as "Kamala", has long been used as an anthelmintic and anti-cestodal in tropical Asia (Akhtar and Ahmad, 1992; Jost *et al.*, 1996; Daikonya *et al.*, 2004).

In Thailand the aerial part of *M. repandus* has been used as an anti-inflammatory, antigastric ulcer, hepatotoxic drug. Its dried roots have been used as insecticide and as a treatment for rheumatic arthritis, hepatitis, liver cirrhosis and snakebite (Sutthivaiyakit *et al.*, 2001).

In the northeastern part of Thailand, the powdered roots of *M. spodocarpus* are used for skin whitening (Intahphuak *et al.*, 2004).

### 3. Chemical constituents of the *Mallotus* species

Chemical investigation of *Mallotus* species revealed the presence of phloroglucinol derivatives as major compounds. Other compounds found in plants of this genus are benzopyran derivatives, triterpenoids, flavonoids, coumarins, coumarinolignoids, alkaloids, tannins, lignan glycosides, galloylglucosides and some miscellaneous phytochemicals. The distribution of these compounds in *Mallotus spp.* is summarized in Table 1.

**Table 1.** Chemical constituents of the genus *Mallotus*

| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| **1. Alkaloids** | | | |
| Malloapeltine **[1]** | *M. apelta* | root | Cheng, Meng and Chen, 1998 |
| **2. Benzopyran derivatives** | | | |
| 4-Hydroxy-2,6-dimethyl-6-(3,7-dimethyl-2,6-octadienyl)-8-(3-methyl-2-butenyl)-2H-1-benzopyran-5,7(3H,6H)-dione **[2]** | *M. apelta* | leaves | An *et al.*, 2001 |
| 4-Hydroxy-2,6,8-trimethyl-6-(3,7-dimethyl-2,6-octadienyl)- 2H-1-benzopyran-5,7(3H,6H)-dione **[3]** | *M. apelta* | leaves | An *et al.*, 2001 |
| 5-Hydroxy-2,8-dimethyl-6-(3-methyl-2-butenyl)- 8-(3,7-dimethyl-2,6-octadienyl)- 2H-1-benzopyran-4,7(3H,8H)-dione **[4]** | *M. apelta* | leaves | An *et al.*, 2001 |
| 5-Hydroxy-2,6,8-trimethyl-8- (3,7-dimethyl-2,6-octadienyl)- 2H-1-benzopyran-4,7(3H,8H)-dione **[5]** | *M. apelta* | leaves | An *et al.*, 2001 |
| 2,3-Dihydro-5,7-dihydroxy-2,6-dimethyl-8-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one **[6]** | *M. apelta* | leaves | An *et al.*, 2001 |

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| 2,3-Dihydro-5,7-dihydroxy-2,8-dimethyl-6-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one **[7]** | *M. apelta* | leaves | An *et al.*, 2001 |
| 2,3-Dihydro-5,7-dihydroxy-2,6,8-trimethyl-4H-1-benzopyran-4-one **[8]** | *M. apelta* | leaves | An *et al.*, 2001 |
| 6-[1'-Oxo-3'(R)-hydroxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran **[9]** | *M. apelta* | leaves | Kiem *et al.*, 2005 |
| 6-[1'-oxo-3'(R)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran **[10]** | *M. apelta* | leaves | Kiem *et al.*, 2005 |
| **3. Coumarinolignoids** | | | |
| Aquillochin **[11]** | *M. apelta* | root | Cheng and Chen, 2000 |
| Cleomiscosin A **[12]** | *M. apelta* | root | Cheng and Chen, 2000 |
| 5′-Demethyl aquillochin **[13]** | *M. apelta* | root | Cheng and Chen, 2000 |
| **4. Coumarins** | | | |
| Esculetin **[14]** | *M. resinosus* | root | Ma, Jones and Hecht, 2004 |
| 6,7-Dimethoxycoumarin **[15]** | *M. resinosus* | root | Ma, Jones and Hecht, 2004 |
| Isoscopoletin **[16]** | *M. resinosus* | root | Ma, Jones and Hecht, 2004 |
| Scopoletin **[17]** | *M. resinosus* | root | Ma, Jones and Hecht, 2004 |

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| **5. Diterpenoids** | | | |
| Hookerianolide A **[18]** | *M. hookerianus* | n.s. | Bai, Yang and Ye, 2006 |
| Hookerianolide B **[19]** | *M. hookerianus* | n.s. | Bai, Yang and Ye, 2006 |
| Hookerianolide C **[20]** | *M. hookerianus* | n.s. | Bai, Yang and Ye, 2006 |
| **6. Flavonoids** | | | |
| Apigenin **[21]** | *M. apelta* | n.s. | Xu *et al.*, 2006 |
| 5,7-Dihydroxy-8-methyl-6-prenylflavone **[22]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| 6,6-Dimethylpyrano(2″,3″: 7,6 )-5-hydroxy-8-methylflavone **[23]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| 4′-Hydroxyisorottlerin **[24]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| Isoallorottlerin **[25]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| Isorottlerin **[26]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| Kamalachalcone A **[27]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| Kamalachalcone B **[28]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| Kamalachalcone C **[29]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| Kamalachalcone D **[30]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| | *M. japonicus* | pericarps | Arisawa *et al.*, 2003 |
| Rottlerin **[31]** | *M. philippinensis* | fruits | Furasawa *et al.*, 2005 |
| Vicenin **[32]** | *M. apelta* | n.s. | Zhu *et al.*, 2007 |
| **7. Galloylglucosides** | | | |
| Mallophenol A **[33]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| Mallophenol B **[34]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| (6*S*,9*R*)-Roseoside **[35]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| **8. Lignan glycosides** | | | |
| Aviculin **[36]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| (+)-Lyoniresinol-3-$\alpha$-*O*-L-rhamnopyranoside **[37]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| **9. Phloroglucinol derivatives** | | | |
| Butyrylmallotolerin **[38]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| Dehydropallidusol **[39]** | *M. pallidus* | leaves | Supudompol, Likhitwitayawuid and Houghton, 2004 |
| 2,6-Dihydroxy-3-methyl-4-methoxy acetophenone **[40]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Homopallidol **[41]** | *M. pallidus* | leaves | Supudompol, Likhitwitayawuid and Houghton, 2004 |
| Isobutyrylmallotochromanol **[42]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Isomallotochromanol **[43]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Isomallotochromene **[44]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Mallopallidol **[45]** | *M. pallidus* | leaves | Supudompol, Likhitwitayawuid and Houghton, 2004 |
| Mallotochroman **[46]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Mallotochromanol **[47]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Mallotochromene **[48]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001; Satomi *et al.*, 1994 |

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

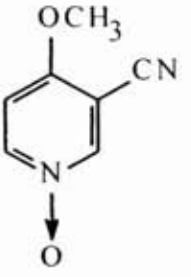
| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| Mallotojaponin **[49]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Mallotophenone **[50]** | *M. japonicus* | pericarps | Ishii *et al.*, 2001 |
| Mallotophilippen A **[51]** | *M. philippinensis* | fruits | Daikonya *et al.*, 2002 |
| Mallotophilippen B **[52]** | *M. philippinensis* | fruits | Daikonya *et al.*, 2002 |
| Mallotophilippen C **[53]** | *M. philippinensis* | fruits | Daikonya, Katsuki and Kitanaka, 2004 |
| Mallotophilippen D **[54]** | *M. philippinensis* | fruits | Daikonya, Katsuki and Kitanaka, 2004 |
| Mallotophilippen E **[55]** | *M. philippinensis* | fruits | Daikonya, Katsuki and Kitanaka, 2004 |
| Pallidol **[56]** | *M. pallidus* | leaves | Supudompol, Likhitwitayawuid and Houghton, 2004 |
| Pallidusol **[57]** | *M. pallidus* | leaves | Supudompol, Likhitwitayawuid and Houghton, 2004 |

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

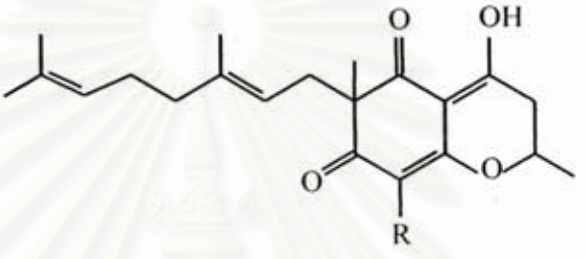
| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| **10. Steroids** | | | |
| $\beta$-Sitosterol **[58]** | *M. roxburghianus* | leaves | Rana *et al.*, 2005 |
| $\beta$-Sitosterol-$\beta$-D-glucoside **[59]** | *M. roxburghianus* | leaves | Rana *et al.*, 2005 |
| Stigmasterol **[60]** | *M. roxburghianus* | leaves | Rana *et al.*, 2005 |
| **11. Tannins** | | | |
| Bergenin **[61]** | *M. roxburghianus* | leaves | Rana *et al.*, 2005 |
| | *M. japonicas* | cortex | Lim *et al.*, 2000; 2001 |
| | *M. milliettii* | cane | Li *et al.*, 2007 |
| | *M. repandus* | stems | Huang, Wang and Lim, 1999 |
| Gallic acid **[62]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| 2,3,8,9,10-Pentahydroxydibenzo[b,d]pyran-6-one **[63]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| 4,5,4′-Trimethoxyl-ellagic acid **[64]** | *M. apelta* | root | Cheng, Meng and Chen, 1998 |

**Table 1.** Chemical constituents of the genus *Mallotus* (continued)

| Groups/Compounds | Source | Plant part | Reference |
|---|---|---|---|
| **12. Triterpenoids** | | | |
| 3$\alpha$-Benzoyloxy-D:A-friedo-oleanan-27,16$\alpha$-lactone **[65]** | *M. repandus* | stems | Sutthivaiyakit *et al.*, 2001 |
| Betulinic acid **[66]** | *M. roxburghianus* | leaves | Rana *et al.*, 2005 |
| 3$\alpha$- Benzoyloxy-28$\beta$-methoxy-13$\alpha$-ursan-28,12$\beta$-epoxide 3-benzoate **[67]** | *M. repandus* | stems | Huang, Wang and Lim, 1999 |
| 3$\alpha$- Hydroxy-13$\alpha$-ursan-28-oic acid **[68]** | *M. repandus* | stems | Huang, Wang and Lim, 1999 |
| 3$\alpha$- Benzoyloxy-13$\alpha$-ursan-28,12$\beta$-olide 3-benzoate **[69]** | *M. repandus* | stems | Huang, Wang and Lim, 1999 |
| 3-Oxo-D:A-friedo-oleanan-27,16$\alpha$-lactone **[70]** | *M. repandus* | stems | Sutthivaiyakit *et al.*, 2001 |
| Ursolic acid **[71]** | *M. repandus* | stems | Huang, Wang and Lim, 1999 |
| **13. Miscellaneous** | | | |
| (*Z*)-3-Hexenyl-$\beta$-D-glucopyranoside **[72]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |
| 3-Hydroxy-4,5(*R*)-dimethyl-2(5H)-furanone **[73]** | *M. furetianus* | leaves | Wei *et al.*, 2004 |

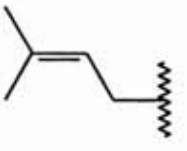



Malloapeltine **[1]**



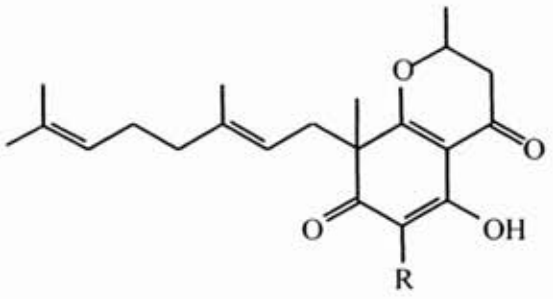

**R**

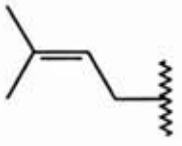
4-Hydroxy-2,6-dimethyl-6-(3,7-dimethyl-2,6-octadienyl)-8-(3-methyl-2-butenyl)-2H-1-benzopyran-5,7(3H,6H)-dione **[2]**

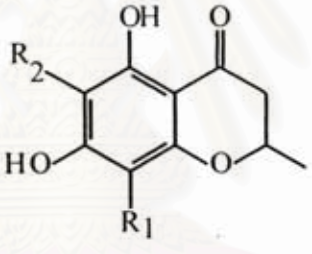


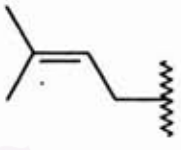
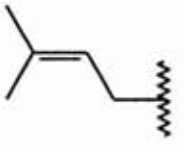
4-Hydroxy-2,6,8-trimethyl-6-(3,7-dimethyl-2,6-octadienyl)-2H-1-benzopyran-5,7(3H,6H)-dione **[3]**

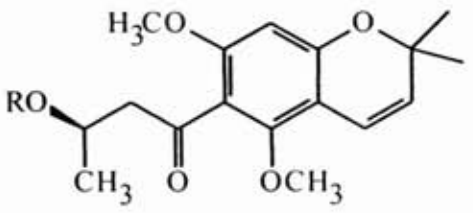
$CH_3$



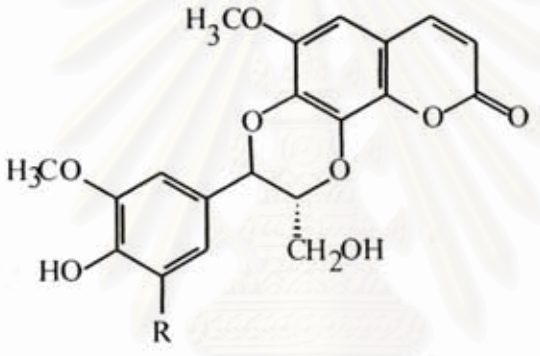

| | R |
|---|---|
| 5-Hydroxy-2,8-dimethyl-6-(3-methyl-2-butenyl)- 8-(3,7-dimethyl-2,6-octadienyl)- 2H-1-benzopyran-4,7(3H,8H)-dione **[4]** |  |
| 5-Hydroxy-2,6,8-trimethyl-8- (3,7-dimethyl-2,6-octadienyl)- 2H-1-benzopyran-4,7(3H,8H)-dione **[5]** | $CH_3$ |



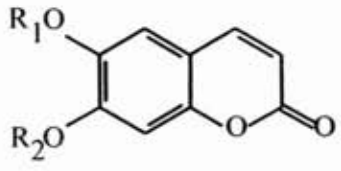

| | $R_1$ | $R_2$ |
|---|---|---|
| 2,3-Dihydro-5,7-dihydroxy-2,6-dimethyl-8-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one **[6]** |  | $CH_3$ |
| 2,3-Dihydro-5,7-dihydroxy-2,8-dimethyl-6-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one **[7]** | $CH_3$ |  |
| 2,3-Dihydro-5,7-dihydroxy-2,6,8-trimethyl-4H-1-benzopyran-4-one **[8]** | $CH_3$ | $CH_3$ |



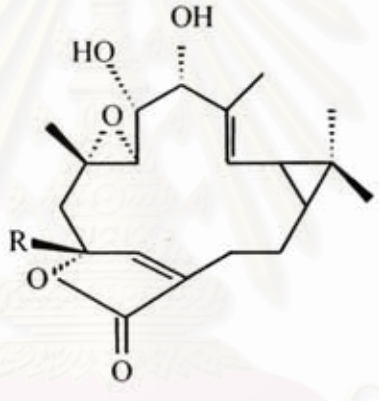

| | R |
|---|---|
| 6-[1'-Oxo-3'(R)-hydroxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran **[9]** | H |
| 6-[1'-Oxo-3'(R)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran **[10]** | $CH_3$ |



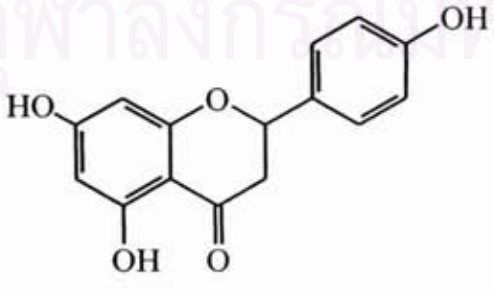

| | R |
|---|---|
| Aquillochin **[11]** | $OCH_3$ |
| Cleomiscosin A **[12]** | H |
| 5'-Demethyl aquillochin **[13]** | OH |



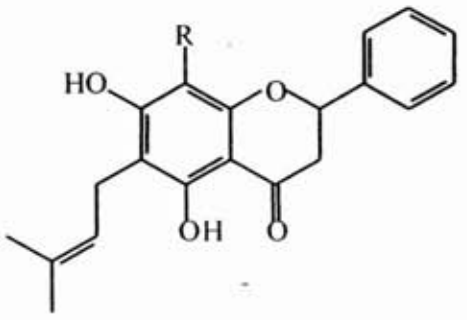
| | $R_1$ | $R_2$ |
|---|---|---|
| Esculetin **[14]** | H | H |
| 6,7-Dimethoxycoumarin **[15]** | $CH_3$ | $CH_3$ |
| Isoscopoletin **[16]** | H | $CH_3$ |
| Scopoletin **[17]** | $CH_3$ | H |

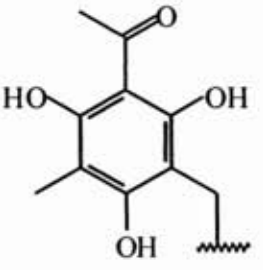


| | R |
|---|---|
| Hookerrianolide A **[18]** | OH |
| Hookerrianolide B **[19]** | H |
| Hookerrianolide C **[20]** | $OC_2H_5$ |

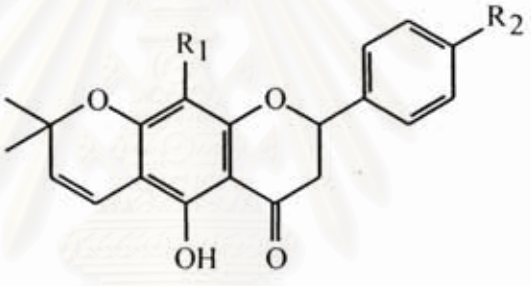


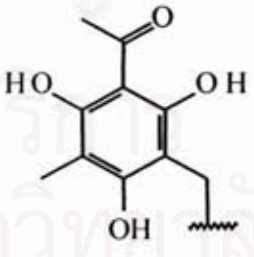
Apigenin **[21]**



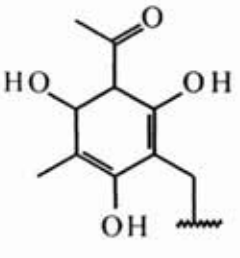

| | **R** |
|---|---|
| 5,7-Dihydroxy-8-methyl-6-prenylflavanone **[22]** | $CH_3$ |
| Isoallorottlerin **[25]** |  |

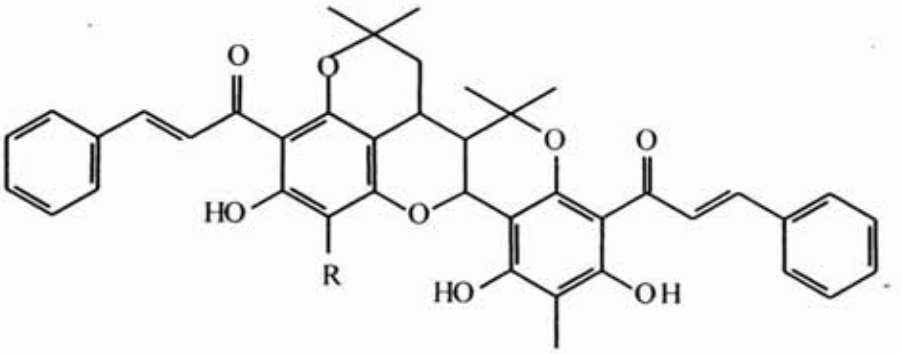





| | **$R_1$** | **$R_2$** |
|---|---|---|
| 6,6-Dimethylpyrano(2″,3″: 7,6 )-5-hydroxy-8-methylflavanone **[23]** | $CH_3$ | H |
| 4′-Hydroxyisorottlerin **[24]** |  | OH |

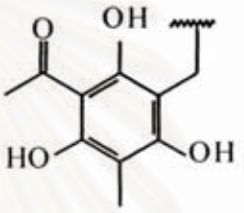


| | |
|---|---|
| Isorottlerin **[26]** |  H |

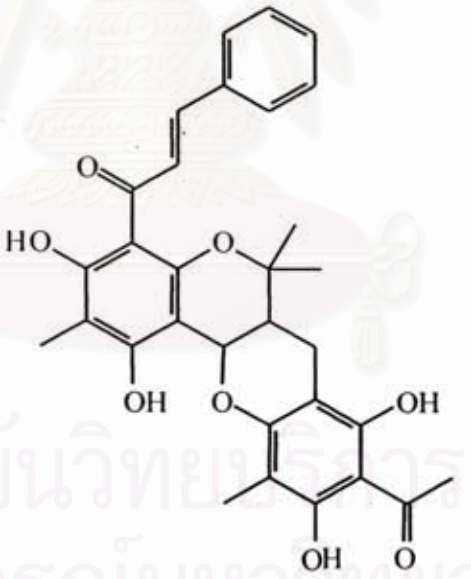


**R**

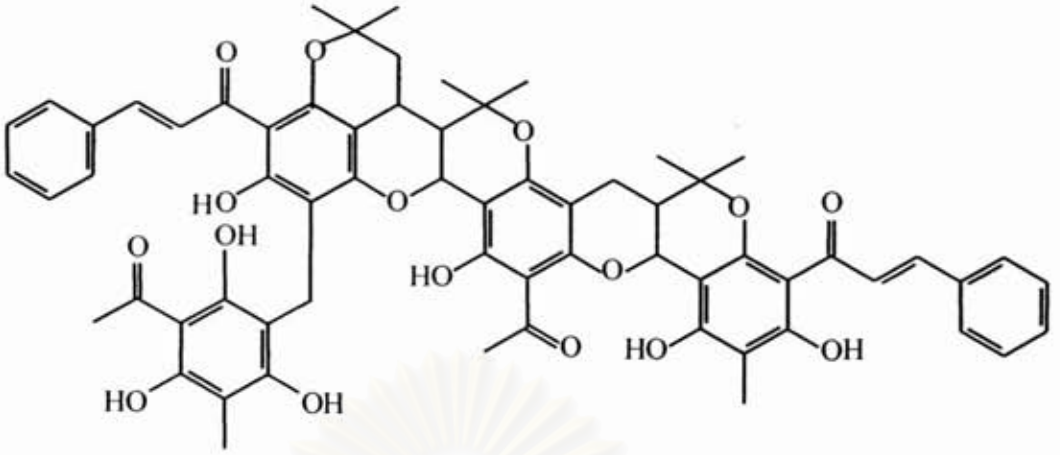
Kamalachalcone A **[27]** $CH_3$



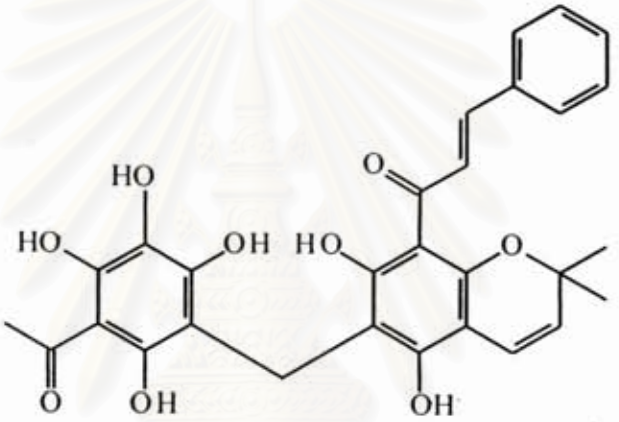
Kamalachalcone B **[28]**



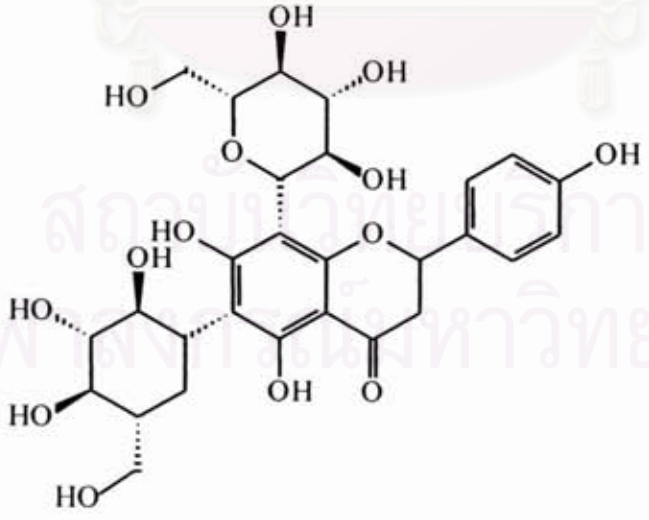
Kamalachalcone C **[29]**



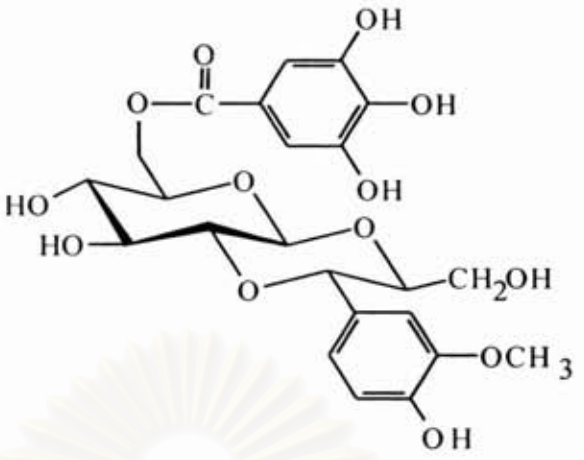
Kamalachalcone D **[30]**



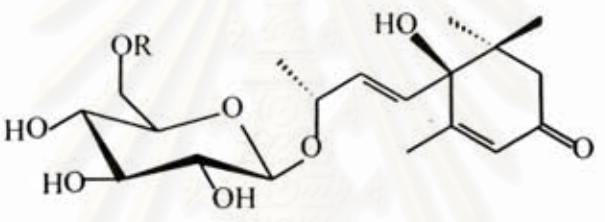
Rottlerin **[31]**



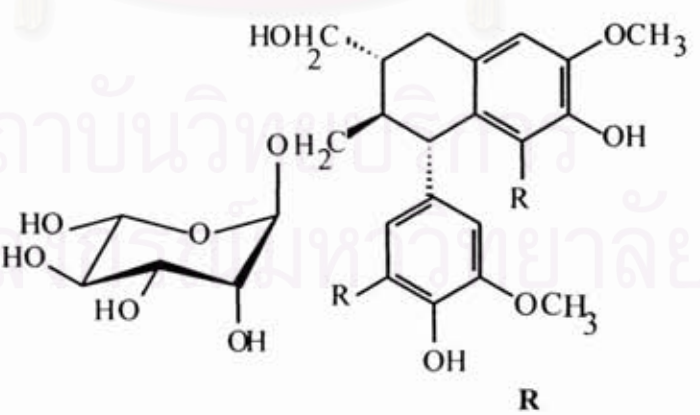
Vicenin **[32]**



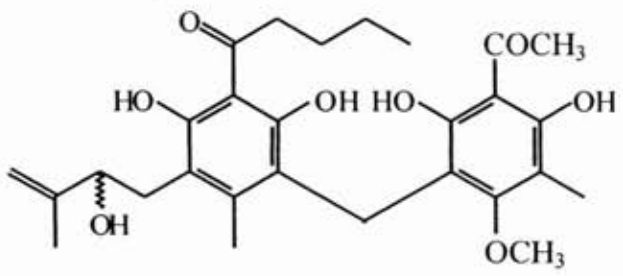
Mallophenol A **[33]**



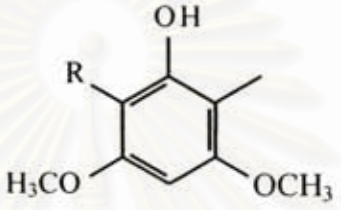
| | **R** |
|---|---|
| Mallophenol B **[34]** | galloyl |
| (6*S*, 9*R*)-Roseoside **[35]** | H |



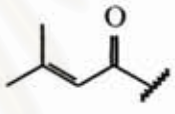
| | **R** |
|---|---|
| Aviculin **[36]** | H |
| (+)-Lyoniresinol-3-$\alpha$-o-L-rhampyranoside **[37]** | $OCH_3$ |



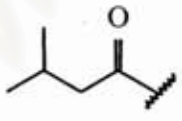

Butyrylmallotolerin **[38]**



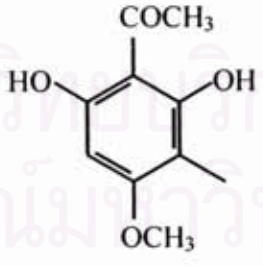

**R**



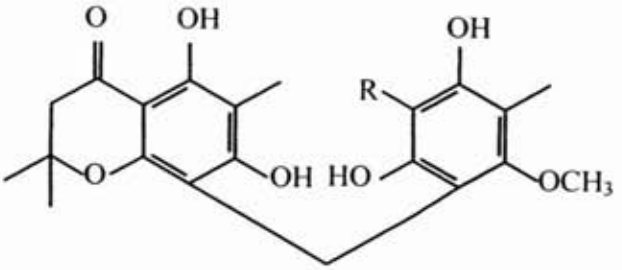

Dehydropallidusol **[39]**

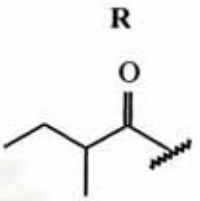



Pallidusol **[57]**

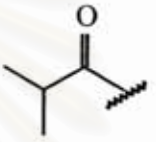



2,6-Dihydroxy-3-methyl-4-methoxy acetophenone **[40]**

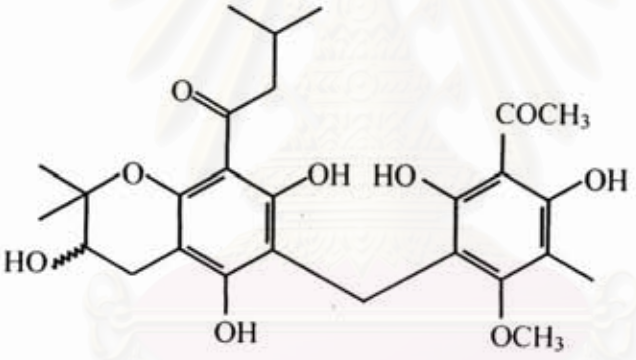


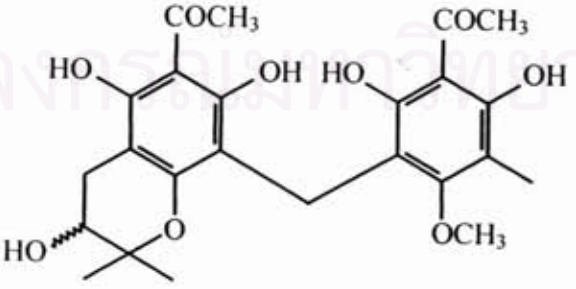

Homopallidol **[41]**



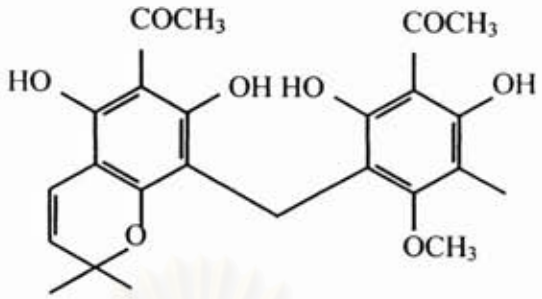

Mallopallidol **[45]**



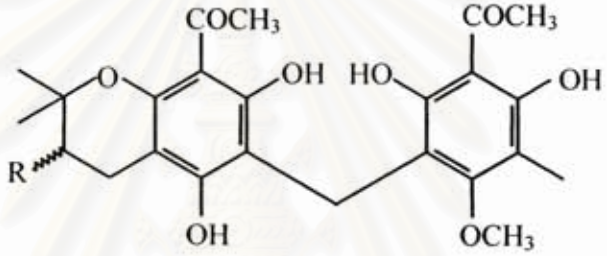

Isobutyrylmallotochromanol **[42]**



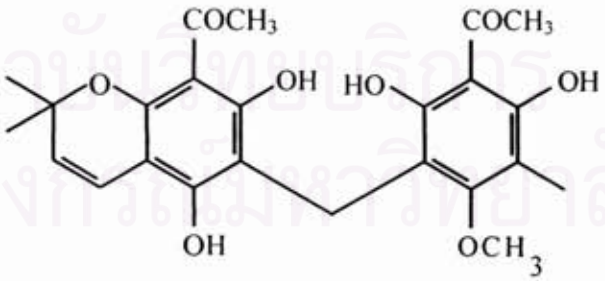

Isomallotochromanol **[43]**



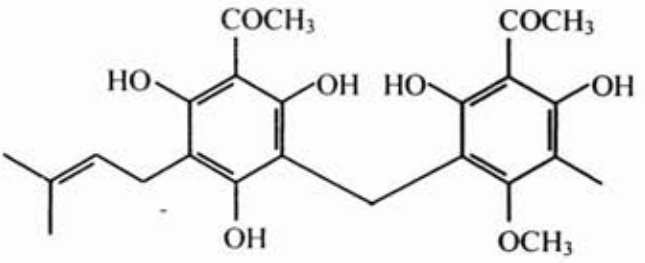

Isomallotochromene **[48]**



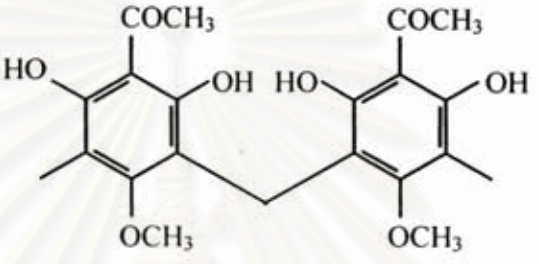

| | **R** |
|---|---|
| Mallotochroman **[46]** | H |
| Mallotochromanol **[47]** | OH |



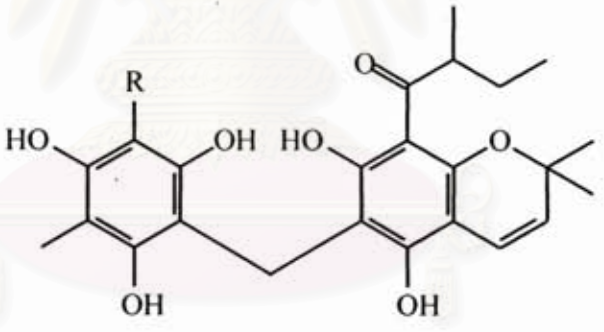

Mallotochromene **[48]**




Mallotojaponin **[49]**

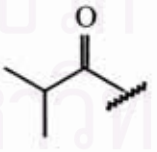



Mallotophenone **[50]**

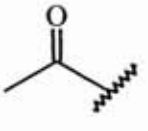


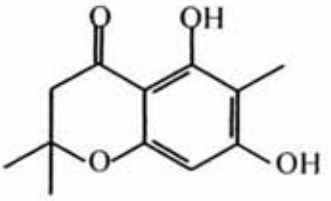

**R**

Mallotophilippen A **[51]**

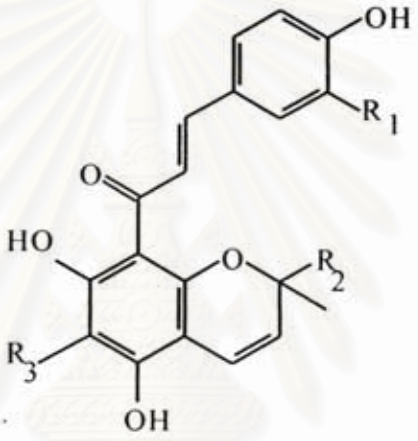


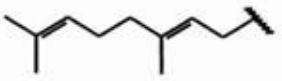
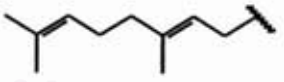
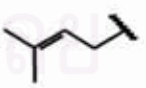

Mallotophilippen B **[52]**

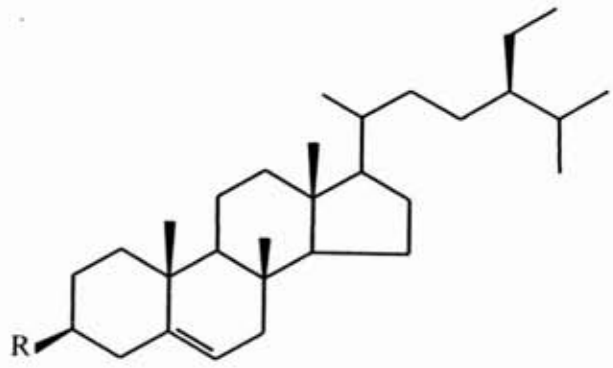




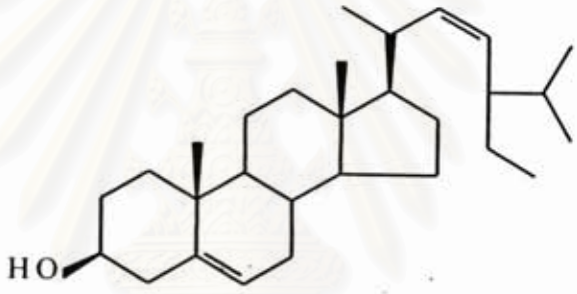
Pallidol **[56]**



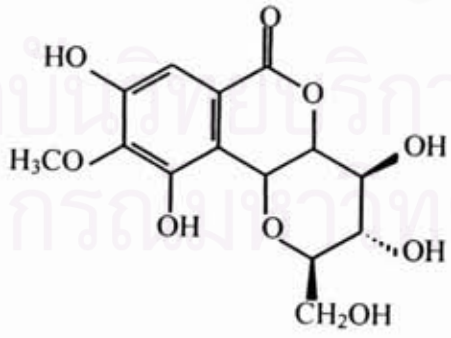
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Mallotophilippen C **[53]** | H | $CH_3$ |  |
| Mallotophilippen D **[54]** | OH | $CH_3$ |  |
| Mallotophilippen E **[55]** | OH |  |  |



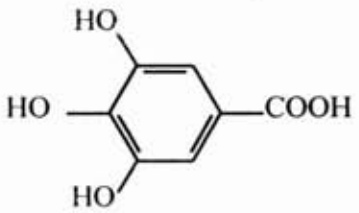

| | R |
|---|---|
| β-Sitosterol **[58]** | H |
| β-Sitosterol-β-D-glucoside **[59]** | Glu |



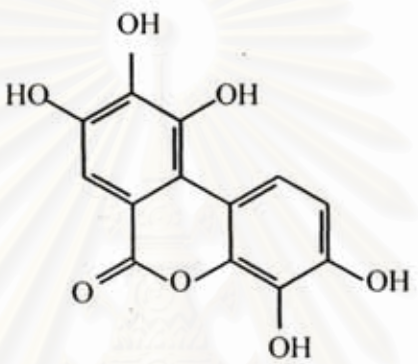

Stigmasterol **[60]**



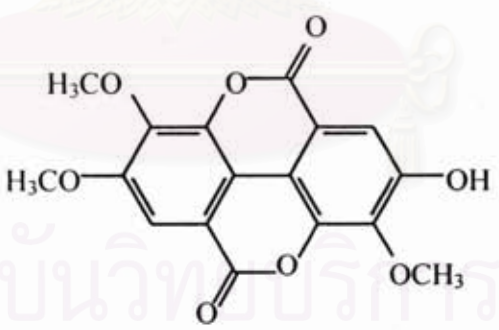

Bergenin **[61]**

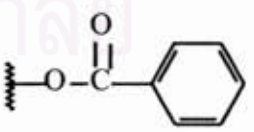


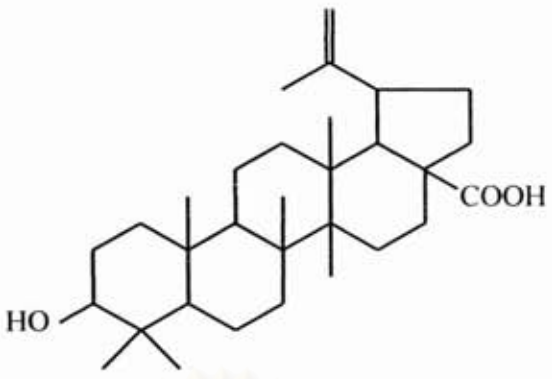
Gallic acid **[62]**



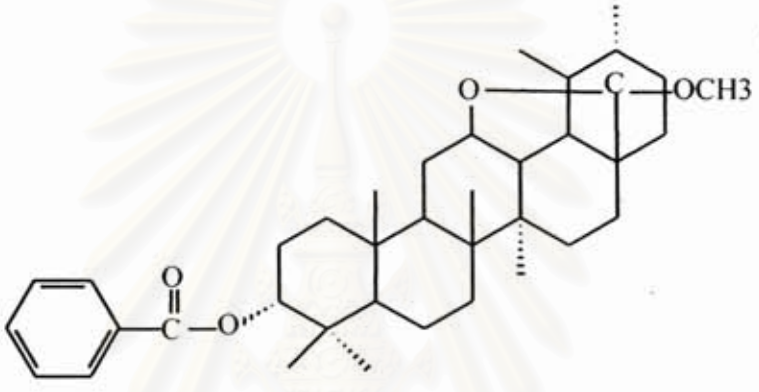
2,3,8,9,10-Pentahydroxydibenzo[b,d]pyran-6-one **[63]**



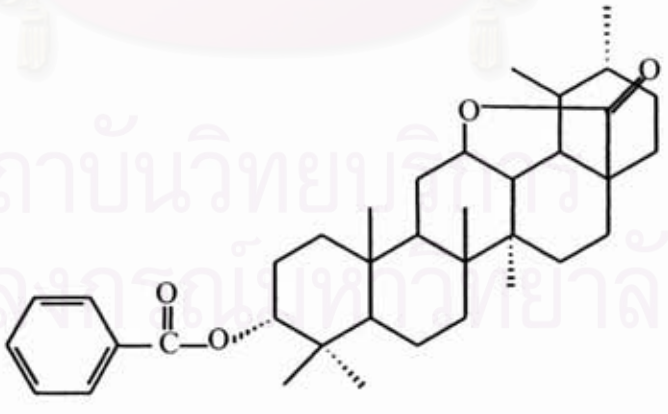
| | $R_1$ | $R_2$ |
|---|---|---|
| 3$\beta$-Benzoyl-D:A-friedo-oleanan-27,16$\alpha$-lactone **[65]** | H |  |
| 3$\beta$-Hydroxy-D:A-friedo-oleanan-27,16$\alpha$-lactone **[67]** | H | OH |
| 3-Oxo-D:A-friedo-oleanan-27,16$\alpha$-lactone **[71]** | $R_1$=$R_2$ = O | |



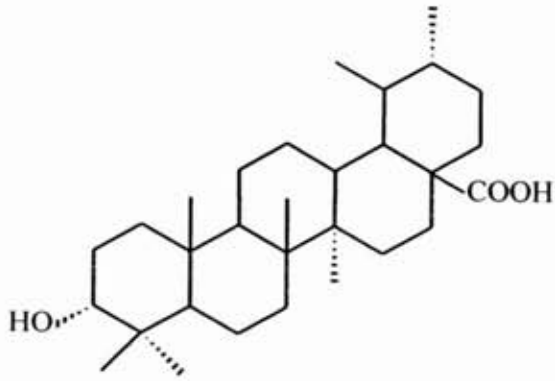

Betulinic acid **[66]**



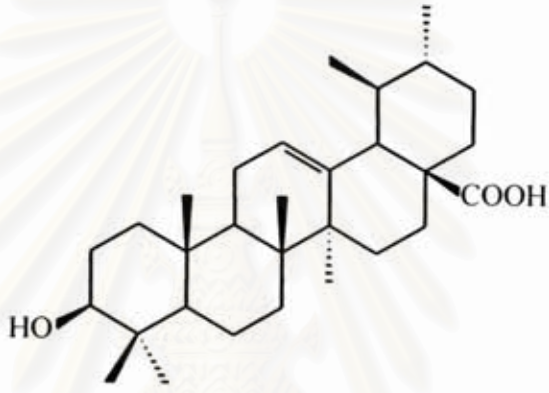

3α- Hydroxy-28β-methoxy-13α-ursan-28,12β-epoxide -3-benzoate **[68]**



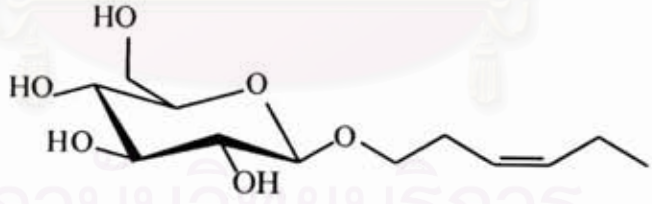

3α-Hydroxy-13α-ursan-28,12β- olide-3-benzoate **[70]**



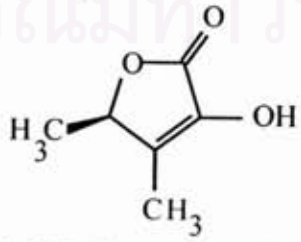

3α- Hydroxy-13α-ursan-28-oic acid **[69]**




Ursolic acid **[72]**




(*Z*)-3-Hexenyl-*β*-D-glucopyranoside **[73]**




3-Hydroxy-4, 5(*R*)-dimethyl-2(5H)-furanone **[71]**

**4. Biological Activities of *Mallotus* species**

Several species of the genus *Mallotus* have been investigated pharmacologically and were shown to exhibit interesting bioactivities. The activities of extracts and compounds isolated from these plants are summarized in Table 2 and Table 3.

**Table 2.** Biological activities of extract of *Mallotus* species

| Species | Plant part | Extract | Activity | Reference |
|---|---|---|---|---|
| *M. apelta* | root | ethyl acetate | anti-HIV, hepatitis, antioxidant | Chen, Meng and Chen, 1998; Xu *et al.*, 2006; Zhaou *et al.*, 2002 |
| *M. japonicus* | pericarps, leaves | acetone | inhibit NO, anti-tumor, anti-viral, cytotoxic activity | Arisawa *et al.*, 1986; 1990; 1991 |
| *M. nepalensis* | - | ethanol | CNS depressant | Rastogi, Mehrotra and Kulshreshtha, 2004 |
| *M. oppositifolium* | - | aqueous, ethanol | antifungal | Adekunle and Ikumapayi, 2006 |
| | leaves | hexane | antimicrobial | Ogundipe *et al.*, 2000 |
| | leaves | aqueous | antidiarrhoeal | Kamgang *et al.*, 2006 |
| | leaves, root | hexane, methanol | antioxidant, anti-inflammatory | Farombi, Ogundipe and Moody, 2001 |

**Table 2.** Biological activities of extract of *Mallotus* species (continued)

| Species | Plant part | Extract | Activity | Reference |
|---|---|---|---|---|
| *M. philippinensis* | leaves, bark, stem | - | antimicrobial | Taylor *et al.*, 1996; Singh, Singhal and Khan, 1997; Moorthy *et al.*, 2007 |
| | capsules | - | purgative, antihelmintic | Srivastava, Singh and Tewari, 1967; Gupta, Verma and Hishi Kar, 1984 |
| | fruits, barks | acetone | antioxidant | Arfan *et al.*, 2007 |
| *M. roxburghianus* | leaves | $CHCl_3$ | antioxidant | Rana *et al.*, 2005 |
| *M. spodocarpus* | root | $CHCl_3$ | anti-inflammatory | Intahphuak *et al.*, 2004 |

**Table 3.** Biological activities of compound isolated *Mallotus* species

| Species | Plant part | Compound isolated | Activity | Reference |
|---|---|---|---|---|
| *M. apelta* | leaves | 4-Hydroxy-2,6-dimethyl-6-(3,7-dimethyl-2,6-octadienyl)-8-(3-methyl-2-butenyl)-2H-1-benzopyran-5,7(3H,6H)-dione | antibacterial | An *et al.*, 2001 |
| | root | 4,5,4′-Trimethoxyl-ellagic acid, malloapeltine | anti-HIV | Cheng, Meng and Chen, 1998<br>Cheng *et al.*, 1998 |
| *M. japonicas* | cortex | Bergenin | antihepatotoxic | Lim *et al.*, 2000; 2001; Kim *et al.*, 2000; Rana *et al.*, 2005 |
| | pericarps | 2,6-Dihydroxy-3-methyl-4-methoxyacetophenone<br>Mallotophenone, Mallotojaponin, Butyrylmallotolerin, Mallotochromene, Mallotochroman, Mallotochromanol, Isobutyrylmallotochromanol, Isomallotochromene | anti-inflammatory | Ishii *et al.*, 2001 |
| *M. millietii* | cane | Bergenin | antihepatotoxic | Li *et al.*, 2007 |

**Table 3.** Biological activities of *Mallotus* species (continued)

| Species | Plant part | Compound isolated | Activity | Reference |
|---|---|---|---|---|
| *M. pallidus* | leaves | Dehydropallidusol, Homomallopallidol, Pallidol, Pallidusol, Mallopallidol | antiviral | Likithiwitayawuid *et al.*, 2005 |
| *M. philippinensis* | pericarps | Mallotojaponin | anti-tumor | Satomi *et al.*, 1994 |
| | fruits | Mallotophilippen A&B | anti-allergic | Daikonya *et al.*, 2002a; 2002b |
| | fruits | Mallotophilippen C-E | anti-inflammatory | Daikonya, Katsuki and Kitanaka, 2004 |
| *M. resinosus* | root | scopoletin | DNA cleavage activity | Ma *et al.*, 2004 |

# CHAPTER III

# EXPERIMENTAL

## 1. Source of Plant Material

The root parts of *Mallotus spodocarpus* Airy Shaw were collected from Saraburi Province, Thailand, in May 2005. The plant was identified by comparison with herbarium specimen (BKF 118006) at the Royal Forest Department, Bangkok, Thailand.

## 2. Phytochemical Techniques

### 2.1 Chromatographic Techniques

#### 2.1.1 Thin Layer Chromatography (TLC)

| | | |
|---|---|---|
| Technique | : | One way ascending |
| Stationary phase | : | TLC aluminium sheet silica gel $60F_{254}$, Layer thickness 0.2 mm |
| Distance | : | 5 cm. |
| Temperature | : | Laboratory temperature (28-35 °C) |
| Detection | : | 1) Ultraviolet light (254 and 356 nm)<br>2) 10% Sulfuric acid in ethanol<br>3) Anisaldehyde reagent |
| Solvent system | : | Various solvent systems depending on materials |

#### 2.1.2 Column Chromatography (CC)

| | | |
|---|---|---|
| Column | : | Flat bottom glass column (various diameter) |
| Stationary phase | : | Silica gel 60 (No. 9385, E. Merck) particle size 0.040-0.063 mm (230-400 mesh ASTM) |
| Packing method | : | Wet packing |
| Sample loading | : | 1) Dry method: The sample was dissolved in a small amount of suitable organic solvent, mixed with a small quantity of adsorbent, triturated, |

dried and loaded on top of the column.

2) Wet method: The sample was dissolved in a small amount of the eluent, then loaded on top of the column.

Solvent system : Various solvent systems depending on materials

Detection : Fractions were examined by TLC observing under UV light at the wavelenghts of 254 and 365 nm, then the TLC plate was sprayed with 10% sulfuric acid in ethanol or anisaldehyde reagent and heat at 110°C. Fractions with similar chromatographic pattern were combined.

### 2.1.3 Gel Filtration Chromatography

Gel filter : Sephadex$^{TM}$ LH-20

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

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

Solvent : MeOH

MeOH-$CH_2Cl_2$ (1:1)

## 2.2 Spectroscopy

### 2.2.1 Ultraviolet (UV) Absorption Spectra

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

### 2.2.2 Infared (IR) Absorption Spectra

IR spectra (KBr disc) were obtained on a Perkin Elmer FT-IR Spectrophotometer Model 1760X (Scientific and Technological Research Equipment Center, Chulalongkorn University).

### 2.2.3 Mass Spectra (MS)

Electron impact mass spectra (EIMS) were recorded on a Polaris Q serial number MS 210179 mass spectrometer (Department of Chemistry, Faculty of Science, Mahidol University) operating at 15 eV.

### 2.2.4 Proton and Carbon-13 Nuclear Magnetic Resonance spectra ($^{1}$H and $^{13}$C-NMR) Spectra

$^{1}$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were obtained either on a JEOL JNM-A500 (Alpha series) NMR spectrometer (Science and Technology Research Equipment Center, Chulalongkorn University) or a Bruker-AV 500 MHz (National Science and Technology Development Agency) $^{1}$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) a Bruker Avance DPX-300 300 MHz NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

NMR solvents used in this study were deuterated dimethylsulfoxide (DMSO-$d_6$), and deuterated chloroform ($CDCl_3$). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

### 2.3 Melting Point

Melting points were obtained on a Fisher-John melting point apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University) and were uncorrected.

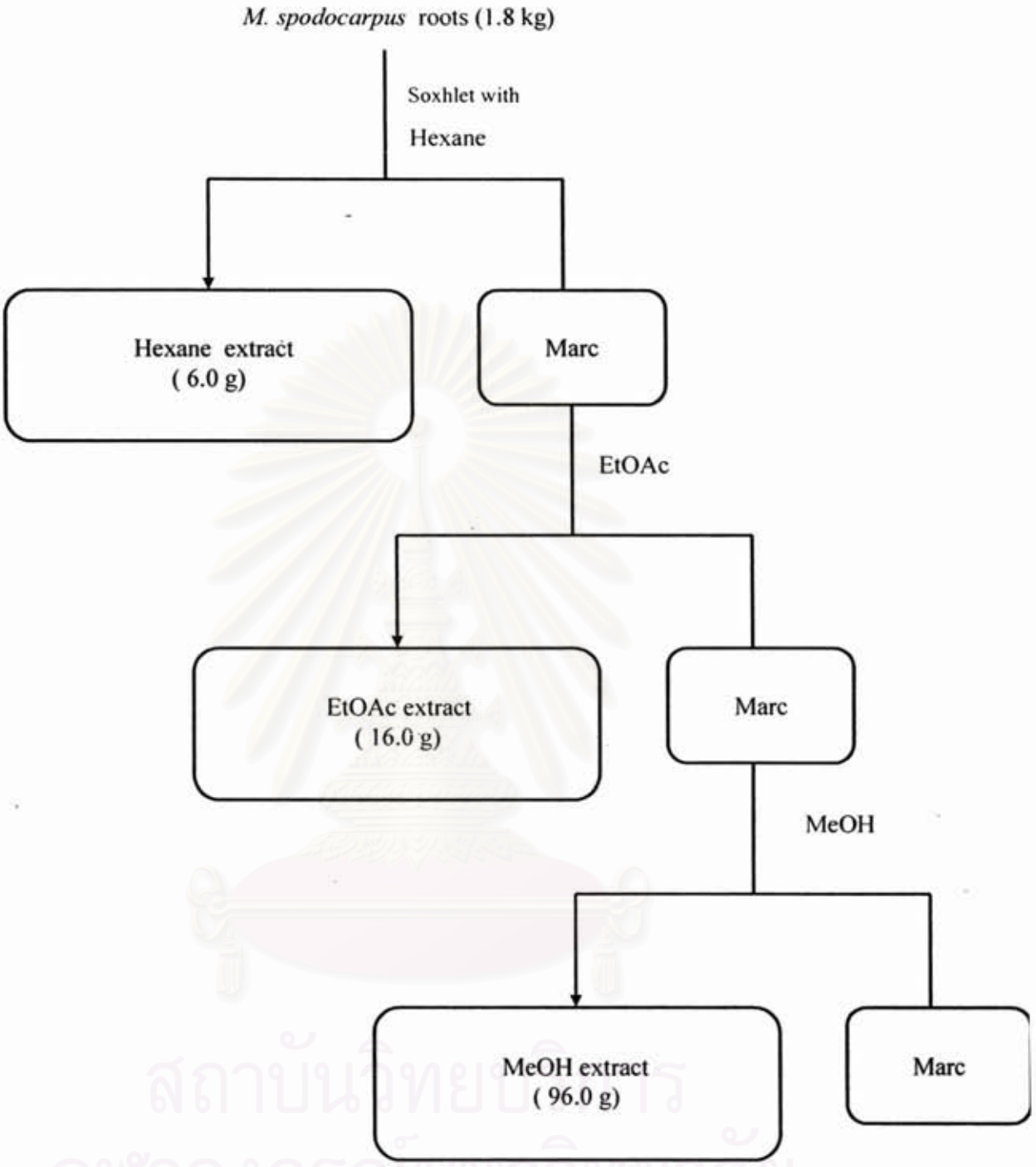
### 2.4 Solvent

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

## 3. Extraction and Isolation of Compounds from the Roots of *M. spodocarpus*

### 3.1 Extraction

Dried, coarsely powdered roots (1.8 kg) were extracted with hexane, EtOAc and MeOH, respectively, by soxhlet apparatus to afford 6 g of crude hexane extract (0.3% of dry weight), 16 g of crude EtOAc extract (0.8% of dry weight) and 96 g of crude MeOH extract (5.3% of dry weight), respectively.




**Scheme 1.** Extraction of *M. spodocarpus* roots

### 3.2 Isolation

**Fractionation of hexane extract**

The hexane extract (6.0 g) was first fractionated on a silica gel column (200 g, 5x25 cm) eluted with hexane-EtOAc (60:1) to give one hundred fractions of 30 ml each, then washed down with MeOH. The fractions were combined according to their TLC pattern into seven portions as shown in Table 3.

**Table 4.** Combined fractions from the hexane extract

| Portion | Number of fraction | Weight (g) |
|---|---|---|
| H01 | 1-20 | 0.71 |
| H02 | 21-36 | 0.10 |
| H03 | 37-45 | 0.43 |
| H04 | 46-76 | 1.26 |
| H05 | 77-85 | 0.12 |
| H06 | 86-100 | 0.03 |
| H07 | MeOH eluate | 3.08 |

#### 3.2.1 Isolation of compound H1

Portion H04 was chromatographed on a Sephadex LH-20 column (1x40 cm), using MeOH-$CH_2Cl_2$ (1:1) to give thirty fractions of 5 ml each. The fractions were then combined according to their TLC pattern into three portions as shown in Table 4.

**Table 5.** Combined fractions from portion H04

| Portion | Number of fraction | Weight (mg) |
|---|---|---|
| H8 | 1-8 | 300 |
| H9 | 9-18 | 410 |
| H10 | 19-30 | 450 |

Portion H09, which gave one major orange-red spot on TLC upon detection with anisaldehyde reagent, was purified by partition with MeOH to give compound H1 as pale yellow oil (410.0 mg).

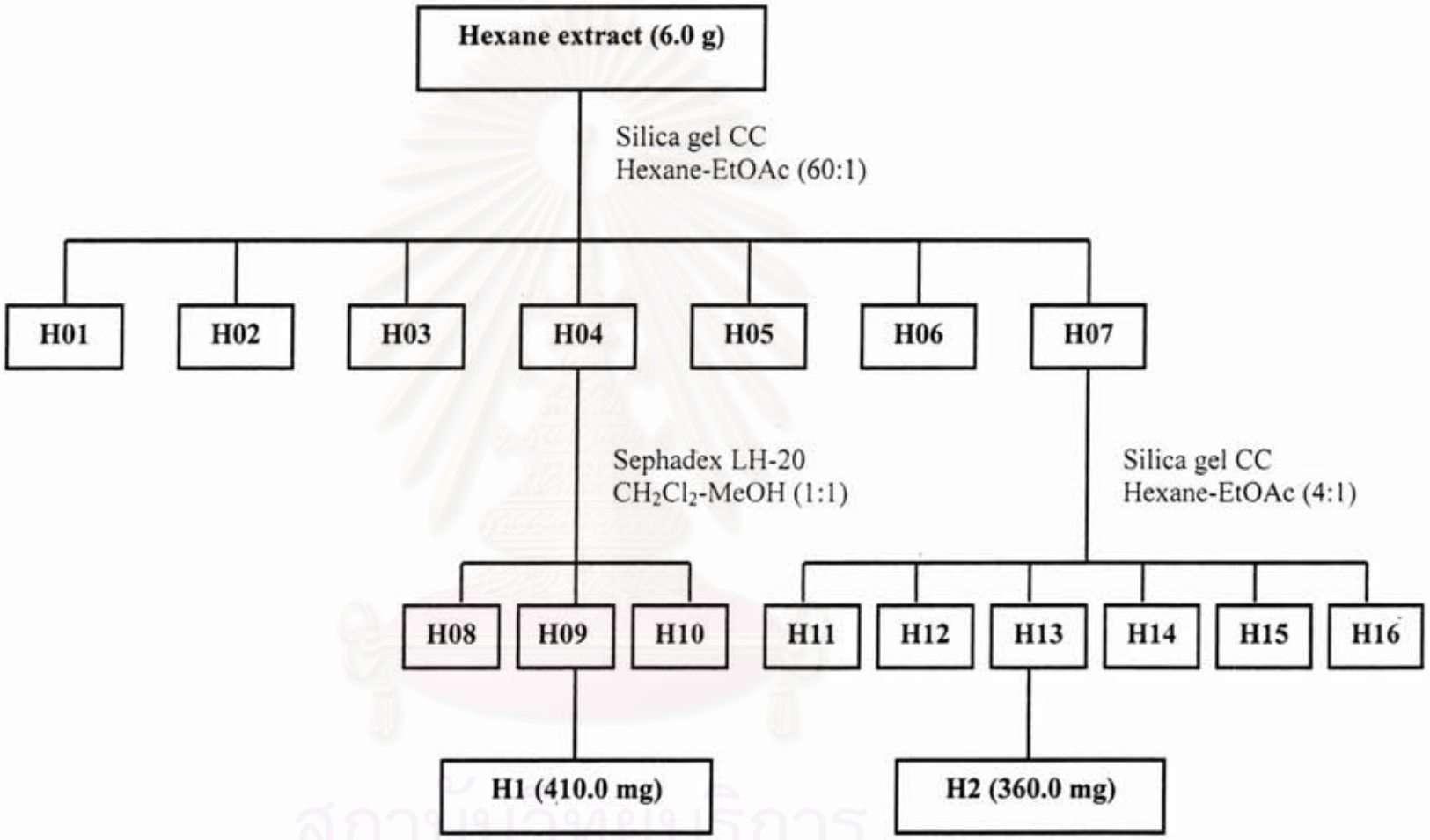
### 3.2.2 Isolation of compound H2

Portion H07 (3.08 g) was subjected to a silica gel column chromatography (100 g, 3x50 cm), eluted with hexane-EtOAc (4:1) to give one hundred fractions of 30 ml each and then washed down with MeOH. The fractions were then combined according to their TLC pattern into six portions as shown in Table 5.

**Table 6.** Combined fractions from portion H07

| Portion | Number of fraction | Weight (mg) |
|---|---|---|
| H11 | 1-10 | 320 |
| H12 | 11-22 | 583 |
| H13 | 23-46 | 457 |
| H14 | 47-83 | 327 |
| H15 | 84-100 | 496 |
| H16 | MeOH eluate | 717 |

Portion H13, which gaved one major pink-violet spot on TLC upon detection with 10% sulfuric acid, was crystallized in MeOH to give compound H2 as colorless needles (360.0 mg).




**Scheme 2.** Isolation of compounds H1 and H2 from the hexane extract

**Fractionation of the EtOAc extract**

The EtOAc extract (16.0 g) was first fractionated on a silica gel column (480 g, 5x60 cm) eluted with $CH_2Cl_2$-MeOH (4:1) to give one hundred and eighteen fractions of 30 ml each and then washed down with MeOH. The fractions were combined according to their TLC pattern into seven portions as shown in Table 6.

**Table 7.** Combined fractions from the EtOAc extract

| Portion | Number of fraction | Weight (g) |
|---|---|---|
| E01 | 1-11 | 0.13 |
| E02 | 12-30 | 2.23 |
| E03 | 31-35 | 1.79 |
| E04 | 36-52 | 0.30 |
| E05 | 53-64 | 1.76 |
| E06 | 65-118 | 3.23 |
| E07 | MeOH eluate | 5.36 |

**3.23 Isolation of compound EA1**

Portion E02, which gaved one major greenish spot on TLC upon detection with anisaldehyde reagent, were crystallized in methanol to give 1.1 g of compound EA1 as colorless crystals.

**3.24 Isolation of compound EA2**

Portion E05 was further separated on a silica gel column chromatography (30 g, 2.5x15 cm), eluted with hexane-acetone (2:1) to give seventy fractions of 30 ml each and then washed down with MeOH. These fraction were combined according to their TLC pattern into six portions as shown in Table 7.

**Table 8.** Combined fractions from portion E05

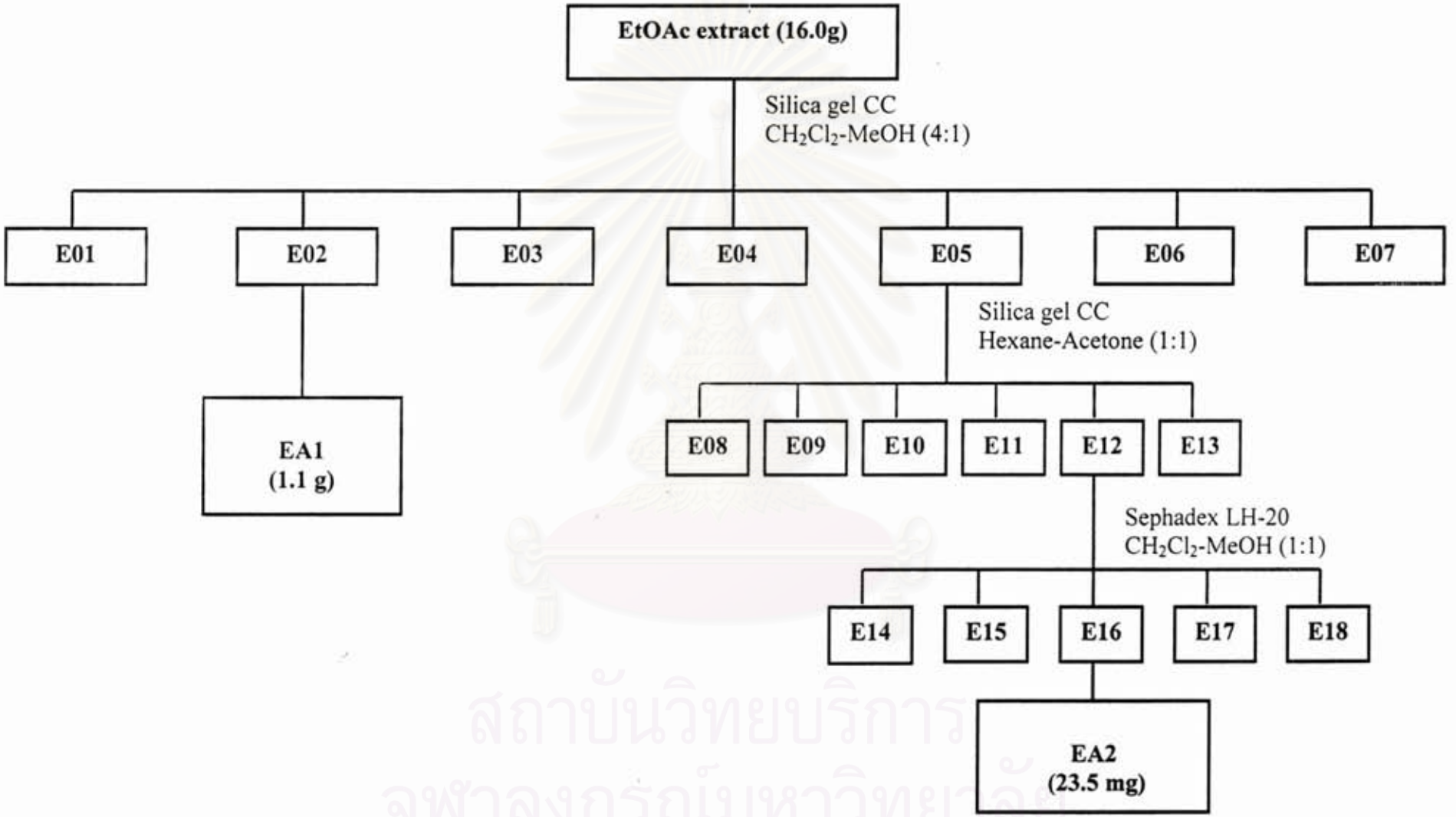
| Portion | Number of fraction | Weight (mg) |
|---|---|---|
| E08 | 1-10 | 167.4 |
| E09 | 11-35 | 318.5 |
| E10 | 36-46 | 220.2 |
| E11 | 47-56 | 270.7 |
| E12 | 57-70 | 285.9 |
| E13 | MeOH eluate | 197.3 |

Portion E12 was further separated on a Sephadex LH-20 column (1x40 cm), eluted with MeOH to give forty-one fractions of 10 ml each. The fractions were then combined according to their TLC pattern in to five portions as shown in Table 8.

**Table 9.** Combined fractions from portion E12

| Portion | Number of fraction | Weight (mg) |
|---|---|---|
| E14 | 1-5 | 73.8 |
| E15 | 6-11 | 23.5 |
| E16 | 12-23 | 69.7 |
| E17 | 24-33 | 33.4 |
| E18 | 34-41 | 63.1 |

Portion E15 gaved one major purple spot on TLC upon detection with anisaldehyde reagent and was purified by crystallization in MeOH to give 23.5 mg of compound EA2 as white amorphous powder.




**Scheme 3.** Isolation of compounds EA1 and EA2 from the EtOAc extract

**Characterization of isolated compounds**

**1. Compound H1**

Compound H1 was obtained as a pale yellow oil (410 mg, 0.02% based on dried weight of roots), soluble in $CH_2Cl_2$

**IR** : $\nu_{max}$ $cm^{-1}$, (KBr) ; 2925, 2854, 1745, 1464, 1239, 1164, 1116, 972, 723; see **Figure 2**

**EIMS** : *m/z* ; 716 $[M]^+$; see **Figure 3**

**$^1$H-NMR** : δ ppm, 500 MHz, in $CDCl_3$; **Figure 4**

**$^{13}$C-NMR** : δ ppm, 125 MHz, in $CDCl_3$; **Figure 5, Table 10**

**2. Compound H2**

Compound H2 was obtained as colorless needles (360 mg, 0.02% based on dried weight of roots), soluble in $CH_2Cl_2$

**$^1$H-NMR** : δ ppm, 300 MHz, in $CDCl_3$; **Figure 10**

**$^{13}$C-NMR** : δ ppm, 75 MHz, in $CDCl_3$; **Figure 11, Table 11**

**3. Compound EA1**

Compound E1 was obtained as colorless crystals (1.1 g, 0.06% base on dried weight of roots), soluble in MeOH

**m.p.** : 152-153°C

**IR** : $\nu_{max}$ $^{cm-1}$, (KBr) ; 3389, 2959, 1702; see **Figure 13**

**EIMS** : *m/z* ; 328 $[M]^+$ ; see **Figure 14**

**$^1$H-NMR** : δ ppm, 500 MHz, in DMSO-$d_6$ ; **Figure 15**

**$^{13}$C-NMR** : δ ppm, 125 MHz, in DMSO-$d_6$ ; **Figure 16, Table 12**

**4. Compound EA2**

Compound E2 was obtained as colorless white powder, soluble in MeOH (33.4 mg, 0.01% base on dried weight of roots)

| | | |
|---|---|---|
| **m.p.** | : | 304-306 °C |
| **IR** | : | $\nu_{max}$ $cm^{-1}$. (KBr) ; 3334, 2923, 1464, 1648, 1254, 1166, 1024, 1073, 1024; see **Figure 21** |
| **$^{1}$H-NMR** | : | δ ppm, 500 MHz, in DMSO-$d_6$ ; **Figure 22** |
| **$^{13}$C-NMR** | : | δ ppm, 125 MHz, in DMSO-$d_6$ ; **Figure 23 Table 13** |

# CHAPTER IV

# RESULTS AND DISCUSSION

Investigation of chemical constituents of *Mallotus spodocarpus* roots by chromatographic techniques led to the isolation of four compounds. The identification and structure elucidation of these compounds were based on spectroscopic evidences (UV, IR, MS, and NMR spectral data) and comparison with previously reported data in the literature. The details are as follows.

## 1. Structure elucidation of compound H1

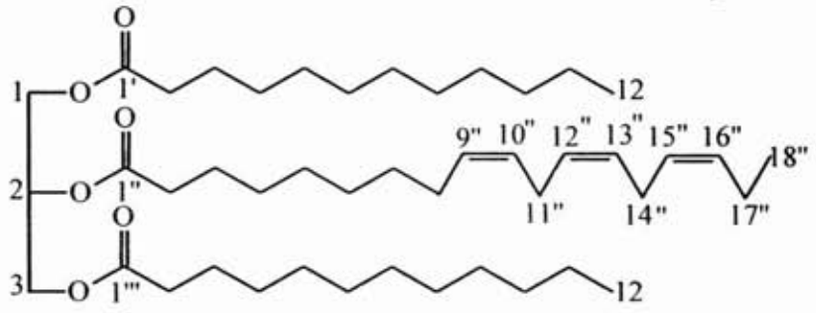
Compound H1 was obtained as pale yellow oil, showing violet spot upon spraying with anisaldehyde reagent. Its IR spectrum (Figure 2) exhibited ester absorption bands at 1745 (C=O stretching of ester), 1239 (C-O stretching of ester), 1164 (C-O stretching) and 1116 (C-O stretching). The EI mass spectrum (Figure 3) showed the molecular ion peak at *m/z* 716 $[M]^+$, in agreement with $C_{45}H_{80}O_6$ as the molecular formula.

The $^1H$ NMR spectrum (Figure 4) exhibited overlapped signals due to three methyl group in the region of δ 0.87 - 0.91 ppm and saturated methylene group in the region of δ 1.30 - 1.62 ppm. Three sites of esterification in the molecule of compound H1 were inferred from one triplet at δ 2.32 ppm (6H, *m*, H-2', H-2", H-2"'). A pair of doublets of doublets at δ 4.16 ppm (2H, *dd*, *J* =6, 12 Hz, H-1a, H-3a) and 4.31 ppm (2H, *dd*, *J* = 6, 12 Hz, H-1b, H-3b) and the signal at δ 5.27 ppm (H, *m*, H-2) was attributable to oxymethine protons of the glycerol backbone. The most downfield signal at δ 5.35 ppm (6H, *m*, H-9", H-10", H-12", H-13", H-15", H-16",) implied the presence of three olefinic groups. As shown in the COSY spectrum (Figures 7a-7c), this olefenic groups were coupled to an allylic methylene protons at position H-8",H-17" (δ 2.03) and diallylic methylene protons at position H-11", H-14"(δ 2.77) ppm.

The $^{13}C$ NMR spectrum (Figures 5) exhibited a number of signals in the region of 14.1-34.2 ppm and ten signals in the more down field region at δ 62.1, 68.9, 127.8, 128.1, 129.6, 129.7, 130.0, 130.2, 172.8 and 173.2 ppm. The DEPT 135 experiments

(Figures 6) and HMQC spectrum (Figures 8) were useful in differentiating these signals. The signals at δ 127.8(C-15"), 128.1(C-10"), 129.6(C-13"), 129.7(C-12"), 130.0(C-9"), and 130.2 (C-16"), represented the unsaturation due to olefenic groups, while those at δ 62.1 (C-1, C-3) and δ 68.9 (C-2) were attributed to methylene and methine carbons connected to the ester oxygen, respectively. The most downfield signal of the carbonyl carbon appeared at δ 172.8 and 173.2 suggested the presence of three ester carbonyls. The proton signals at δ 4.16 ppm (2H, *dd*, *J* =6, 12 Hz, H-1a, H-3a), δ 4.31 ppm (2H, *dd*, *J* = 6, 12 Hz, H-1b, H-3b), δ 5.27 ppm (H, *m*, H-2) and the carbon signals at δ 62.1 (C-1, C-3) and δ 68.8 (C-2) along with mass fragments at *m/z* 262 $(C_{18}H_{29}O+H)^+$ and *m/z* 200 $(CH_3(CH_2)_{10}COOH)$ confirmed that compound H1 is a triglyceride with $C_{18}$ and $C_{12}$ fatty acid ester. In HMBC spectrum (Figures 9a-9b), the carbonyl carbon signals at δ 172.8 (C-2) and 173.2 (C-1, C-3) showed correlation with proton signal at δ 2.32 (H-2, H-2', H-2") and δ 1.62 (H-3, H-3', H-3"). The proton signals at δ 2.03 (H-8", H-17") and δ 2.77 (H-11", H-14") showed correlation with carbon signals at δ 127.8(C-15"), 128.1(C-10"), 129.6(C-13"), 129.7(C-12"), 130.0(C-9"), and 130.2 (C-16"), also confirmed the presence of three olefenic bond in compound H1. The proton signal at δ 2.77 (H-11", H-14") correlated with carbon signals at δ 127.8(C-15"), 128.1(C-10"), 129.6(C-13"), 129.7(C-12"), 130.0(C-9"), and 130.2 (C-16")along with the peak at at *m/z* 262 $(C_{18}H_{29}O+H)^+$ in the EI Mass spectrum confirmed the presence of linolenic acid moiety in compound H1. The position of linolenic acid moiety was confirmed on the basis of $^{13}$C-NMR. The presence of only two carbon signals for three ester carbonyl at δ 172.8 (C-1") and 173.2 (C-1, C-1'") suggested compound H1 as a symmetrical triglyceride (Chandra and Nair, 1993; Kelm and Nair, 1998; Ramsewak *et al.*, 2001), hence confirming position of linolenic moiety at C-2 and lauric acid moiety at C-1 and C-3.

From all of above spectroscopic data, compound H1 was elucidated as new triglyceryl esters, namely 1,3-dilauroyl-2-linolenoylglycerol.



1,3-dilauroyl-2-linolenoylglycerol

**Table 10.** Comparison of the $^{13}C$ NMR spectral data of Linolenic acid*, Lauric acid** and compound H1 ($CDCl_3$, 125 MHz)

| Position | Linolenic acid | H1 | Position | Lauric acid | H1 |
|---|---|---|---|---|---|
| 1" | 172.8 | 172.8 | 1",1"' | 173.7 | 173.2 |
| 2" | 34.2 | 34.2 | 2",2"' | 34.4 | 34.0 |
| 3" | 24.8 | 24.8 | 3",3"' | 25.3 | 24.9 |
| 4",5",6",7",8" | 27.2-29.0 | 27.2-29.8 | 4",4"',",5"', 6",6"',7",7"', 8",8"',9",9"', | 29.5-30.0 | 27.2-29.8 |
| 9" | 130.2 | 130.0 | 10",10"' | 32.3 | 31.5, 31.9 |
| 10" | 127.8 | 128.1 | 11",11"' | 23.1 | 22.7 |
| 11" | 25.6 | 25.6 | 12",12"' | 14.5 | 14.1 |
| 12" | 128.3 | 129.7 | CHO | 62.4 | 62.1 |
| 13" | 128.2 | 129.6 | | | |
| 14" | 25.5 | 25.5 | | | |
| 15" | 127.1 | 127.8 | | | |
| 16" | 131.9 | 130.2 | | | |
| 17" | 20.5 | 22.5 | | | |
| 18" | 14.2 | 14.1 | | | |
| CHO | 68.9 | 68.9 | | | |

* Fauconnot *et al.*, 2005 ($CDCl_3$, 90 MHz)

** Mannina *et al.*, 1999 ($CDCl_3$ + TMS, 150.9 MHz)

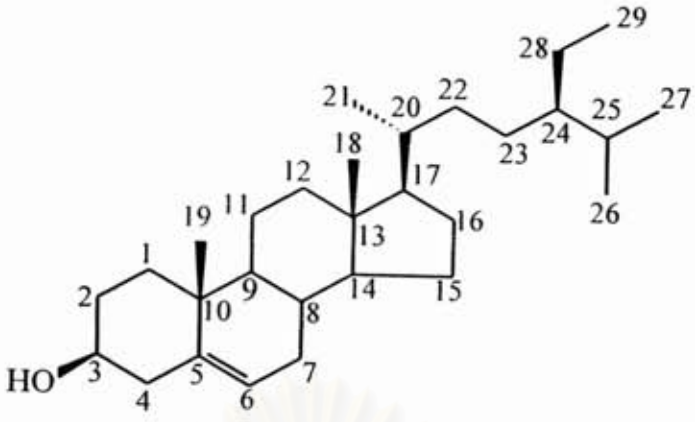
**2. Identification of compound H2**

Compound H2 was obtained as colorless needles. It gave purple color upon spraying with 10% $H_2SO_4$ in 95% ethanol and heated. Liebermann-Burchard test of this compound gave positive green color, suggesting the presence of a steroidal skeleton.

The $^1$H NMR spectrum (Figure 10) showed the signal at δ 5.35 which could be assigned to the vinyl proton H-6, while a another one-proton multiplet at δ 3.50 was attributable to the proton geminal to the 3-OH group. The signals in the region of δ 0.66-0.99 ppm are those of methyl protons, including the signals at δ 0.66 (H-18), 0.78 (H-27), 0.80 (H-26), 0.83 (H-29), 0.90 (H-21) and 0.99 ppm (H-19). The signals which appeared at δ 1.1-2.3 ppm were those of methylene and methine protons.

Its $^{13}$C NMR spectrum (Figures 11) showed the signals of 29 carbon atoms, supporting the assignment of this compound as a steroid derivative. The DEPT experiments (Figure 12) were performed to differentiate these 29 signals into those of six methyl carbons at δ 12.0 (C-18), 12.1 (C-29), 18.9 (C-21), 19.2 (C-27), 19.5 (C-19) and 20.0 (C-26), eleven methylene carbons at δ 21.2 (C-11), 23.2 (C-28), 24.4 (C-15), 26.3 (C-23), 28.4 (C-16), 31.8 (C-2), 32.0 (C-7), 34.1 (C-22), 37.4 (C-1),39.9 (C-12) and 42.4 (C-4), nine methine carbons at δ 29.3 (C-25), 32.0 (C-8), 36.3 (C-20), 46.0 (C-24), 50.2 (C-9), 56.2 (C-17), 56.8 (C-14), 71.8 (C-3) and 121.6 (C-6), and three quaternary carbons at δ 36.6 (C-10), 42.4 (C-13) and 140.6 (C-5). The two most downfield signals at δ 140.6 and 121.6 could be assigned to the olefenic C-5 and C-6, respectively. The carbon signal at δ 71.8 ppm represented the hydroxyl substituted C-3.

Comparison of the $^{13}$C-NMR data of compound H2 with those values previously reported for β-Sitosterol (De-Eknamkul and Potduang, 2003) revealed them to be fully in agreement, as summarized in Table 10. Therefore, compound H2 was identified as β-Sitosterol.




*β*-Sitosterol

**Table 11**. Comparison of the $^{13}C$ NMR spectral data of $\beta$-Sitosterol and compound H2 ($CDCl_3$, 75 MHz)

| Position | $\beta$-Sitosterol* | Compound H2 |
|---|---|---|
| 1 | 37.2 | 37.4 |
| 2 | 31.6 | 31.8 |
| 3 | 71.8 | 71.8 |
| 4 | 42.2 | 42.4 |
| 5 | 140.7 | 140.6 |
| 6 | 121.7 | 121.6 |
| 7 | 31.9 | 32.0 |
| 8 | 31.9 | 32.0 |
| 9 | 50.1 | 50.2 |
| 10 | 36.5 | 36.6 |
| 11 | 21.1 | 21.2 |
| 12 | 39.7 | 39.9 |
| 13 | 42.3 | 42.4 |
| 14 | 56.7 | 56.8 |
| 15 | 24.3 | 24.4 |
| 16 | 28.2 | 28.4 |
| 17 | 56.0 | 56.2 |
| 18 | 11.8 | 12.0 |
| 19 | 19.4 | 19.5 |
| 20 | 36.1 | 36.3 |
| 21 | 18.8 | 18.9 |
| 22 | 33.9 | 34.1 |
| 23 | 26.0 | 26.3 |
| 24 | 45.8 | 46.0 |
| 25 | 29.1 | 29.3 |
| 26 | 19.8 | 20.0 |
| 27 | 19.0 | 19.2 |
| 28 | 23.0 | 23.2 |
| 29 | 12.0 | 12.1 |

* De-Eknamkul and Potduang, 2003 (in $CDCl_3$, 125 MHz)

### 3. Structure elucidation of compound EA1

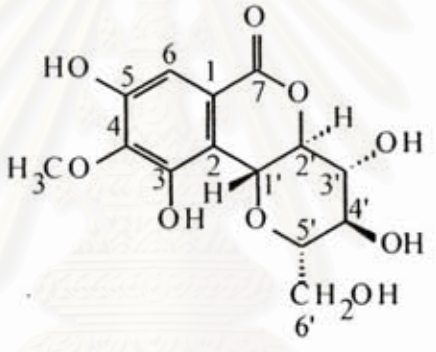
Compound EA1 was obtained as colorless crystal from MeOH. It gave greenish color with anisaldehyde reagent. Its molecular formula, $C_{14}H_{18}O_6$ was determined from the [$M^+$] ion peak at *m/z* 328 (Figure 14). The IR spectrum (Figure 13) showed bands at 3424 (O-H stretching), 1702 (C=O stretching) and 1463 (aromatic ring) $cm^{-1}$.

The $^1H$ NMR spectrum (Figure15) displayed an aromatic proton signal at δ 6.98 (1H, *s*, H-6). A three-proton singlet at δ 3.76 (3H, *s*, H-5) indicated the presence of one methoxy group. Five methine protons connected to heteroatom resonated at δ 4.96 (1H, *d*, *J* = 10.4 Hz, H-1'), δ 3.98 (1H, *dd*, *J* = 10.4, 9.2 Hz, H-2'), δ 3.63 (1H, *br t*, *J* = 9.2 Hz, H-3'), δ 3.18 (1H, *t*, *J* = 9.0 Hz, H-4'), δ 3.55 (1H, *ddd*, *J* = 9.8, 7.6,2.0 Hz, H-5') and one methylene resonated at δ 3.40 (1H, *dd*, H-6'a), and δ 3.83 (2H, *dd*, *J* = 11.9, 2.0 Hz, H-6'b), suggesting the presence of sugar moiety in the molecule. Connectivity of glucose moiety could also be observed in the $^1H$-$^1H$ COSY spectrum (Figure 18).

The $^{13}C$ NMR spectrum (Figure 16 ) showed the signals of 14 carbon signals. The DEPT 135 experiment (Figure 17) and HMQC experiment (Figure 19a-19b), were performed to differentiate these 14 signals into those of one methoxy carbon at δ 59.9 (5-$OCH_3$) whereas one methylene carbon resonated at δ 61.1(C-6'). Six methine carbons exhibited their signals at δ 70.7(C-4'), 72.1(C-1'), 73.7(C-3'), 79.8(C-2'), 81.8(C-5') and 109.5 (C-6), five quaternary signals at δ 115.9 (C-2), 118.1 (C-1), 140.6 (C-5), 148.1 (C-3) and 151.0 (C-4) and the most downfield signal of the carbonyl carbon at C-7.

The two-bond correlation, observed in the HMBC spectrum (Figure 20a-20h), of the anomeric H-1′ with C-2 (δ 115.9) indicated that the glucose unit was connected carbon atom to aglycone at position 2, which confirmed by the three bond correlation of H-1′ with C-1 (δ118.1, C-C=O) and C-3 (δ148.1, C-OH). The three-bond correlation, observed in the HMBC spectrum (Figure 21a-21i) of an aromatic proton at δ 6.98 (1H, *s*) with C-2 (δ 115.9) and C-7 (δ 163.4) indicated the aromatic proton and the carboxylic group were placed at position 6 and 7, respectively, which

confirmed by two bond correlation of aromatic proton with C-5 (δ 115.9). Therefore methoxy group was assigned to the position C-4, confirmed by HMBC correlation of methoxyl protons (δ 3.76) with C-4. The remaining two hydroxyl groups could placed nicely at C-3 and C-5. Compound EA1 was identified as Bergenin by analysis of the above spectral data and confirmed by comparison with previous published data (Taneyama *et al.*, 1982). Bergenin has been used as a folk medicine for gastrointestinal disease and constipation (Okada *et al.*, 1973; Abe *et al.*, 1980). In addition, it has been reported anti-inflammatory (Swarnalakshmi *et al.*, 1984), and hypolipidaemic (Jahromi *et al.*, 1992).




Bergenin

**Table 12.** Comparison of the $^{13}C$ NMR spectral data of Bergenin and compound EA1 (DMSO-$d_6$, 125 MHz)

| Position | Bergenin | EA2 |
|---|---|---|
| 1 | 118.0 | 118.1 |
| 2 | 115.9 | 115.9 |
| 3 | 148.0 | 148.1 |
| 4 | 140.6 | 140.6 |
| 5 | 150.9 | 151.1 |
| 6 | 109.5 | 109.5 |
| 7 | 163.3 | 163.4 |
| $OCH_3$ | 59.8 | 59.9 |
| 1' | 72.2 | 72.1 |
| 2' | 73.7 | 73.7 |
| 3' | 79.8 | 79.8 |
| 4' | 70.7 | 70.7 |
| 5' | 81.8 | 81.8 |
| 6' | 61.2 | 61.1 |

* Taneyama *et al.*, 1982 (TMS, 21.15 MHz)

**4. Identification of compound EA2**

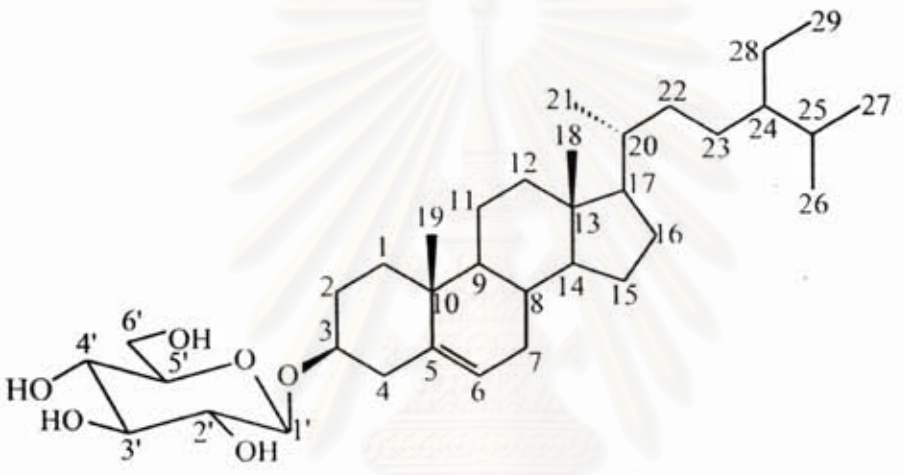
Compound EA2 was obtained as white amorphous powder. This compound gave purple coloror with anisaldehyde reagent. Liebermann-Burchard test of this compound gave positive green color, suggesting the presence of a steroidal skeleton. The IR spectrum (Figure 21) exhibited an O-H absorption at 3334 $cm^{-1}$ as well as (C-O stretching) at 1020-1170 $cm^{-1}$.

In the $^{1}H$ NMR spectrum (Figure 22), the signal at δ 5.33 (2H, *d, J*=5.2 Hz) which could be assigned to the vinylic proton H-6, whereas another one-proton multiplet at δ 3.42 was attributable to the proton at position 3. The signals of methyl protons, which appeared at δ 0.65 (3H, *s,* H-18), 0.80 (3H, *d, J*=7.0 Hz, H-27), 0.80 (3H, *d, J=7.0* Hz, H-26), 0.82 (3H, *t, J=7.0* Hz, H-29), 0.90 (3H, *d, J=6.4* Hz, H-21) and 0.95 (3H, *s,* H-19). The signals at δ 1.1-2.3 were those of methylene and methane protons. The multiplet signals at δ 2.80-3.20 were those of protons resonances due to the sugar moiety (H-2', H-3', H-4' and H-5'). The signal at δ 3.40 and 3.64 were those of methylene proton at position 6' and the doublet at δ 4.22 (1H, *J*=7.8 Hz), was assignable to anomeric proton at position 1' of the sugar moiety. The sugar component in compound EA2 was concluded to be glucose.

The $^{13}C$ NMR spectrum (Figures 23) showed the signals of 35 carbons atoms, supporting the assignment of this compound as a steroid glucoside. The DEPT experiment (Figures 24) were performed to differentiate these 35 signals in to those of six methyl carbons at δ 11.7 (C-18), 11.8 (C-29), 18.6 (C-21), 18.9 (C-27), 19.1 (C-19) and 19.7 (C-26), twelve methylene carbons at δ 20.6 (C-11), 22.1 (C-28), 23.9 (C-15), 25.5 (C-23), 27.8 (C-16), 29.3 (C-2), 31.4 (C-7), 31.4 (C-22), 36.8 (C-1), 38.3 (C-12), 41.9 (C-4) and 61.0 (C-6'), fourteen methine carbons at δ 28.7 (C-25), 31.4 (C-8), 35.5 (C-20), 45.2 (C-24), 49.6 (C-9), 55.4 (C-17), 56.2 (C-14), 70.1 (C-4'), 73.5 (C-2'), 76.7 (C-3), 76.9 (C-5'), 79.2 (C-3'), 100.8 (C-1') and 121.2 (C-6), and three quaternary carbons at δ 36.2 (C-10), 41.9 (C-13) and 140.5 (C-5). The two most downfield signals at δ 140.5 and 121.2 could be assigned to the olefinic C-5 and C-6, respectively. The carbon signal at δ 76.7 represented the oxygenated carbon at C-3.

The carbon signal at δ 100.8 corresponding to the anomeric carbon (C-1') confirming that compound EA2 should be a monoglycoside.

Comparison of the $^{13}C$ NMR data of compound EA2 with previously reported data for β-Sitosterol glucoside (Kojima *et al.*, 1990; Mizushina *et al.*, 2006) revealed them to be fully in agreement, as shown in Table 12. Therefore, compound EA2 was identified as β-Sitosterol glucoside. This compound is the common sterol in higher plants.




β-Sitosterol glucoside

**Table 13.** Comparison of the $^{13}$C-NMR spectral data of $\beta$-Sitosterol glucoside and compound EA2 (DMSO-$d_6$ , 500 MHz)

| Position | $\beta$-Sitosterol glucoside* | EA2 |
|---|---|---|
| 1 | 37.5 | 36.8 |
| 2 | 30.3 | 29.3 |
| 3 | 78.2 | 76.7 |
| 4 | 39.4 | 41.9 |
| 5 | 141.0 | 140.5 |
| 6 | 122.0 | 121.2 |
| 7 | 32.2 | 31.4 |
| 8 | 32.1 | 31.4 |
| 9 | 50.4 | 49.6 |
| 10 | 37.0 | 36.2 |
| 11 | 21.3 | 20.6 |
| 12 | 40.0 | 38.3 |
| 13 | 42.5 | 41.9 |
| 14 | 56.9 | 56.2 |
| 15 | 24.6 | 23.9 |
| 16 | 28.6 | 27.8 |
| 17 | 56.3 | 55.4 |
| 18 | 12.0 | 11.7 |
| 19 | 19.3 | 19.1 |
| 20 | 36.4 | 35.5 |
| 21 | 19.0 | 18.6 |
| 22 | 34.3 | 31.4 |
| 23 | 26.4 | 25.5 |
| 24 | 46.1 | 45.2 |
| 25 | 29.5 | 28.7 |
| 26 | 19.5 | 19.7 |
| 27 | 20.0 | 18.9 |
| 28 | 23.4 | 22.1 |
| 29 | 12.2 | 11.8 |
| 1' | 102.6 | 100.8 |
| 2' | 75.4 | 73.5 |
| 3' | 78.7 | 79.2 |
| 4' | 71.8 | 70.1 |
| 5' | 78.6 | 76.9 |
| 6' | 62.9 | 61.0 |

* Mizushina *et al.*, 2006 (in pyridine-$d_5$, 100 MHz)

# CHAPTER V

# CONCLUSION

Investigation of chemical constituents of the roots of *Mallotus spodocarpus* (family Euphorbiaceae) led to the isolation of one new triglyceryl esters named 1,3-dilauroyl-2-linolenoylglycerol and other three known compounds, named $\beta$-Sitosterol, $\beta$-Sitosterol glucoside and Bergenin.

This is the first chemical investigation of *Mallotus* species and the data obtained would be valuable in the chemotaxonomic and phytochemical studies of this plant genus.

# REFERENCES


Thai

กรมป่าไม้, สำนักวิชากรมป่าไม้, ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาป่าไม้. 2544. ชื่อพรรณไม้แห่งประเทศไทย กรุงเทพมหานคร:ประชาชน.

English

Abe, K., Sakai, K. and Uchida, M. 1980. Effect of bergenin on experimental ulcers-prevention of stress of induced ulcers in rats. Gen. Pharmacol. 11: 361-368.

Adekunle, A. A. and Ikumapayi, A. M. 2006. Antifungal property and phytochemical screening of the crude extracts of Funtumia elastic and *Mallotus oppositifolium*. West Indian Medical Journal 55: 219-223.

Akhtar, M. S. and Ahmed, L. 1992. Comparative efficacy of *Mallotus philippinensis* fruit (kamala) or Nilzan drug against gastrointestinal cestodes in beetal goats. Small Rumin. Res. 8: 121-128.

An, T.-Y., Hu, L.-H., Cheng, X.-F. and Chen Z.-L. 2001. Benzopyran derivatives from *Mallotus apelta*. Phytochemistry 57: 273-278.

Arfan, M., Amin, H., Karamac, M., Kosinska, A., Shahidi, F., Wickzkowski, W. and Amarowicz, R. 2007. Antioxidant activity of extracts of *Mallotus philippinensis* fruit and bark. J. Food Lipids. 14: 280-297.

Arisawa, M. 2003. Constituents of the pericarps of *Mallotus japonicus* (Euphorbiaceae). Yakugaku Zasshi 123: 217-224.

Arisawa, M., Fujita, A., Bai, H., Nagasaki, A., Morikoshi, K. and Morita, N. 1990. Studies on cytotoxic constituents in leaves of *Mallotus japonicus* (Euphorbiaceae). Japanese Journal of Pharmacognosy 44: 179-182.

Arisawa, M., Fujita, A. and Morita, N. 1991. Inhibition of tumor-promoter enhanced $^{3}$H-choline incorporation into cellular phospholipids by phloroglucinol derivatives from *Mallotus japonicus*. J. Nat. Prod. 54: 1409-1412.

Arisawa, M., Fujita, A., Morita, N. and Koshimura, S. 1990. Cytotoxic and antitumor constituents in pericarps of *Mallotus japonicus*. Planta Med. 56: 377-379.

Arisawa, M., Fujita, A., Saga, M., Hayashi, T. and Morita, N. 1986. Studies on cytotoxic constituents in pericarps of *Mallotus japonicus* Part II. J. Nat. Prod. 49: 298-302.

Bai, Y., Yang, Y-P. and Ye, Y. 2006. Hookerianolides A-C : Three novel casbane-type diterpenoid lactones from *Mallotus hookerianus*. Tetrahedron Lett. 47: 6637-6640.

Chandra A. and Nair M.G. 1993. Characterization of pit oil from Montmorency (*Prunus coresus* L.) cherry. J. Agric. Food Chem. 41: 879-881.

Chattopadhyay, D., Arunachalam, G., Mandal, A.B. and Mandal, S. C. 2002. Evaluation of antipyretic of leaf extracts of *Mallotus peltatus* (Geist) Muell. Arg. var *acuminatus*: A folk medicine. Phytomedicine 9: 727-730.

Cheng, X.F. and Chen, Z.L. 2000. Coumarinolignoids of *Mallotus apelta*. Fitoterapia 71: 341-342.

Cheng, X.-F., Meng, Z.-M. and Chen Z.-L. 1998. A pyridine alkaloid from *Mallotus apelta*. Phytochemistry 49: 2193-2194.

Daikonya, A., Katsuki, S. and Kitanaka, S. 2004. Inhibition of nitric oxide production by novel chalcone derivatives from *Mallotus philippinensis* (Euphorbiaceae). Chem. Pharm. Bull. 52: 1326-1329.

Daikonya, A., Katsuki, S., Wu, J.B. and Kitanaka, S. 2002a. Anti-allergic agents from natural sources(4): anti-allergic activity of new phloroglucinol derivatives from *Mallotus philippinensis*(Euphorbiaceae). Chem Pharm Bull. 50: 1566-1569.

Daikonya, A., Katsuki, S., Wu J.-B. and Kitanaka, S. 2002b. Anti-allergic activity of new phloroglucinol derivatives from *Mallotus philippinensis*. Chem. Pharm. Bull. 50: 1566-1659.

De-Eknamkul, W. and Potduang, B. 2003. Biosynthesis of $\beta$-sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. Phytochemistry 62: 389-398.

Dennis, W. 2000. Contemporary Plant Systematics. USA : Andrews University Press.

Farombi, E.O., Ogundipe, O. and Moody J. O. 2001. Antioxidant and anti-inflammatory activities of *Mallotus oppositifolium* in model systems. African journal of medicine and medical sciences 30: 213-215.

Fauconnot, L., Robert, F., Villard, R. and Dionisi, F. 2005. Chemical synthesis and NMR characterization of structured polyunsaturated triaglycerols. Chem. Phys. Lipids. 139: 125-136.

Furasawa, M., Yoshiyuhi, I., Tanaka, T., Ito, T., Nakaya, K.-I., Ibrahim, I., Ohyama, M., Iinuma, M., Shirataka, Y. and Takahashi, Y. 2005. Novel, complex flavonoids from *Mallotus philippensis* (Kamala tree). Helv. Chim. Acta. 88: 1048-1058.

Gupta, SS., Verma, P. and Hishi, K.K. 1984. Purgative and anthelmintic effect of *Mallotus philippinensis* in rats against tapeworm. Indian J. Physiol. Pharmacol. 28: 63-66.

Huang, P-L., Wang, L-W. and Lin, C.-N. 1999. New triterpenoids of *Mallotus repandus*. J. Nat. Prod. 62: 891-892.

Intahphuak, S., Panthong, A., Kanjannapothi, D., Taesotikul, T., Krachangchaeng, C. and Reutrakul, V. 2004. Anti-inflammatory and analgesic activities of *Mallotus spodocarpus* Airy Shaw. J. Ethnopharmacol. 90: 69-72.

Ishii, R., Horie, M., Saito, K., Arisawa, M. and Kitanaka, S. 2001. Inhibitory effect of phloroglucinol derivatives from *Mallotus japonicus* on nitric oxide production by murine macrophage-like cell line, RAW 264.7, activated by lipopolysaccharide and interferon-γ. Biochim. Biophys. Acta 1568: 74-82.

Jahromi, M. A. F., Chansouria, J. P. N. and Ray, A. B. 1992. Hypolipididaemic activity in rats of bergenin, the major constituent of *Flueggea microcarpa*. Phytotherapy Research 6: 180-183.

Kamgang, R., Vidal Pouokam Kamgne, E., Fonkoua, M. C., Penlap N Beng, V. and Biwole' Sida, M. 2006. Activities of aqueous extracts of *Mallotus oppositifolium* on Shigella dysenteriae A1-induced diarrhea in rats. Clin. Exp. Pharmacol. Physiol. 33: 89-94.

Kelm M. A. and Nair M.G. 1998. Mosquitocidal comounds and a triglyceride, 1, 3-dilinoleneoyl-2-palmitin from *Ocimum santum*. J. Agric. Food Chem. 46: 3092-3094.

Kiem, P. V., Dang, N. H., Bao, H. V., Huong, H. T., Minh, C. V., Huong, L. M., Lee, J. J. and Kim, Y. K. 2005. New Cytotoxic Benzopyrans from the Leaves of *Mallotus apelta*. Arch. Pharm Res. 28: 1131-1134.

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

Li, G.-P., Yang, L.-J., Zhao, J.-F., Yang, X.-D. and Li, L. 2007. Chemical constituents in cane of *Mallotus milliettii*. Chin. Trad. Herbal Drugs. 38: 804-806.

Likhitwitayawuid, K., Supudompol, B., Sritularak, B., Lipipun, V., Rapp, K. and Schinazi, R. F. 2005. Phenolics with Anti-HSV and Anti-HIV Activities from *Artocarpus gomezianus*, *Mallotus pallidus*, and *Triphasiatrifolia*. Pharma. Biol. 43: 651-657.

Lim, H.-K., Kim, H.-S., Choi, H.-S., Oh, S. and Choi, J. 2000. Hepatoprotective effects of bergenin, a major constituent of *Mallotus japonicus*, on carbon tetrachloride-intoxicated rats. J. Ethnopharmacol. 72: 469-474.

Lim, H.-K., Kim, H.-S., Choi, H.-S., Choi, J., Kim, S.-H. and Chang, M.-J. 2001. Effects of Bergenin, the Major Constituent of *Mallotus japonicus* against D-Galactosamine-Induced Hepatotoxicity in Rats. Pharmacology 63: 71-75.

Ma, J., Jones, S. H. and Hecht, S. M. 2004. A coumarin from *Mallotus resinosus* that mediate DNA cleavage. J. Nat. Prod. 67 : 1614-1616.

Mannina, L., Luchinat, C., Emanuele, M. C. and Segre, A. 1999. Acyl position distribution of glycerol tri-esters in vegetable oils: a $^{13}C$ NMR study. Chem. Phys. Lipids. 103: 47-55.

Mizushina, Y., Nakanishi, R., Kuriyama, I., Kamiya, K., Satake, T., Shimazaki, N., Koiwai, O., Uchiyama, Y., Yonezawa, Y., Takemura, M., Sakaguchi, K. and Yoshida, H. 2006. $\beta$-Sitosterol-3-O-$\beta$-D-glucoside: A eukaryotic DNA polymerase $\lambda$ inhibitor. J. Steroid Biochem. Mol. Biol. 99: 100-107.

Moorthy, K., Srinivasan, K., Subramanian, C., Mohanasundari, C. and Palaniswamy, M. 2007. Phytochemical screening and antibacterial evaluation of stem bark of *Mallotus philippinensis* var. *Tomentosus*. Af. J. of Biotechnol. 6: 1521-1523.

Okada, T., Suzuki, T., Hasobe, S. and Kisara, K. 1973. Bergenin : antiulcerogenic activities of bergenin. Nippon Yakurigaku Zasshi-Folia Pharmacological Japonica 69: 369-378.

Ogundipe, O. O., Moody, J. O., Fakeye, T. O. and Ladipo, O. B. 2000. Antimicrobial activity of *Mallotus oppositifolium* extractives. African journal of medicine and medical sciences 29: 281-283.

Ramsewak, R. S., Nair, M. G., Muruggesan, S., Mattson W. J. and Zasada J. 2001. Antifeedant compounds and triglycerides from *Dirca palustris*. J. Agric. Food Chem. 49: 5852-5856.

Rana, V. S., Rawat, M. S. M., Pant, G. and Nagatsu, A. 2005. Chemical constituent and antioxidant activity of *Mallotus roxburghianus* leaves. Chem. Biodiversity. 2: 792-798.

Rastogi, S., Mehrotra, B. N. and Kulshreshtha, D. K. 2004. Constituents of *Mallotus nepalensis* muell. Arg. : A mild CNS depressant. Nat. Prod. Sci. 10: 237-239.

Satomi, Y., Arisawa, M., Nishino, H. and Iwashima, A. 1994. Antitumor-promoting activity of mallotojaponin, a major constituent of pericarps of *Mallotus japonicus*. Oncology. 2: 215-219.

Singh, R., Singhal, K.C. and Khan, N. U. 1997. Antifilarial activity of *Mallotus philippinensis* Lam on *Setaria cervie* (Nematoda: Filarioida) in vitro. Indian J. Physiol. Pharmacol. 41: 397-403.

Srivastava, M. C., Singh, S. W. and Tewari J. P. 1967. Anthelmintic activity in *Mallotus philippinensis* (Kampila) powder. Indian J. Med. Res. 55: 746-748.

Supudompol, B., Likhitwitayawuid, K. and Houghton, P. J. 2004. Phloroglucinol derivatives from *Mallotus pallidus*. Phytochemistry 65 : 2589-2594.

Sutthivaiyakit, S., Thongtan, J., Pisutjaroenpong, S., Jiaranantanont, K. and Kongsaeree, P. 2001. D:A Friedo-oleanane lactones from the stems of *Mallotus repandus*. J. Nat. Prod. 64 : 569-571.

Swarnalakshmi, T., Sethuraman, M. G., Sulochana, N. and Arivudainambi, R. 1984. A note on the anti-inflammatory activity of bergenin. Curr. Sci. 53: 917.

Taneyama, M., Yoshida, M., Kobayashi, M. and Hasegawa, M. 1983. Isolation of norbergenin from *Saxifraga stolonifera*. Phytochemistry 22: 1053-1054.

Taylor, R. S., Edel, F., Manandhar, N.P. and Towers, G. H. 1996. Antimicrobial activities of southern Nepalese medicinal plants. J. Ethnopharmacol. 50 : 97-102.

Thakur, S. C., Thakur, S. S., Chaube, S. K. and Singh, S. P. 2005. An ethereal extract of Kamala (*Mallotus philippinensis (Moll.Arg) Lam.)* seed induce adverse effect on reproductive parameters of female rats. Reprod Toxicol. 20 : 149-156.

Wei, K., Li, W., Koike, K., Liu, L., Fu, X., Lin, L., Chen, Y. and Nikaido, T. 2004. Two New Galloylglucosides from the Leaves of *Mallotus furetianus*. Chem. Pharm. Bull. 52: 776-779.

Welzen, P. C. V., Haegens, R. M. A. P., Slik, J. W. F., Bollenbor, S.M., Dressler, S. and Esser, H. J., 2000. Checklist of the genera of Thai euphorbiaceae-I. Thai Forest Bulletin (B. O. T.) 28: 109.

Wu, G.-F., Wei, S., Lan, S.-B. and Xu, X.-J. 2006. Chin. Trad. Herbal Drugs 37: 1126-1128.

Xu, S., Lu, Z.-P., Cai, H.-B., Zhang, X.-G., Liu, Q. and Tan, Y. 2006. Inhibiting effects of root of *Mallotus apelta* on duck hepatitis B virus. J. of Chin. Integr. Med. 4: 285-288.

Zhao, J., Lu, Z., Wang, X. and Zhang, X. 2002. The study on the anti-oxidation effect of the root of Mallotus apelta in the rat model of liver fibrosis. J. of Chin. Med. Mater. 25: 185-187.

Zhu, B., Bai, G.-C., Jiang, S.-J., Wei, F. and Un, R.-C. 2007. Studies on chemical constituent and quantitative determination of *Mallotus apelta*. Zhongguo Zhongyao Zashi 32: 932-934.

# Appendix

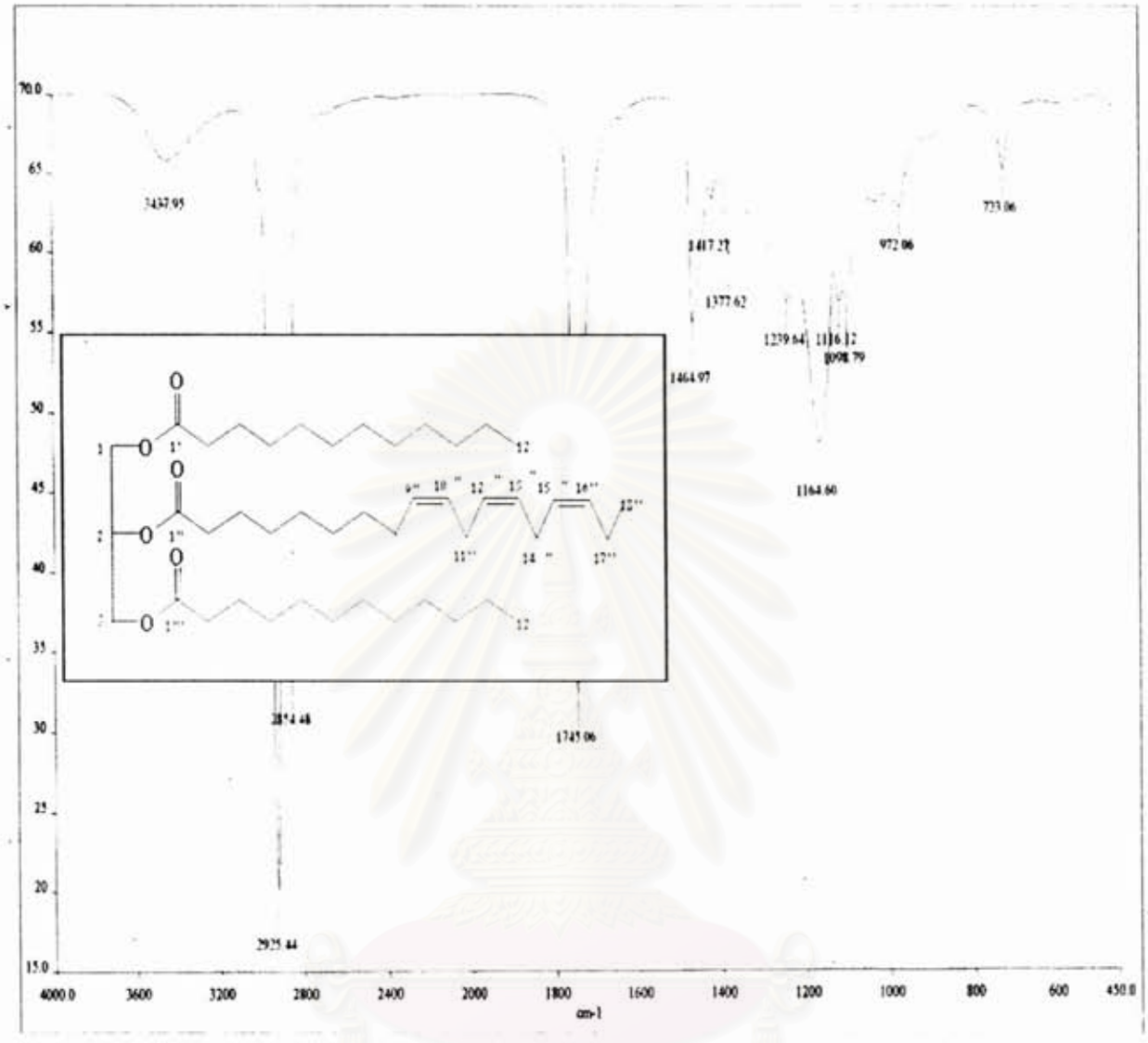



**Figure 2.** IR Spectrum of compound H1 (KBr disc)

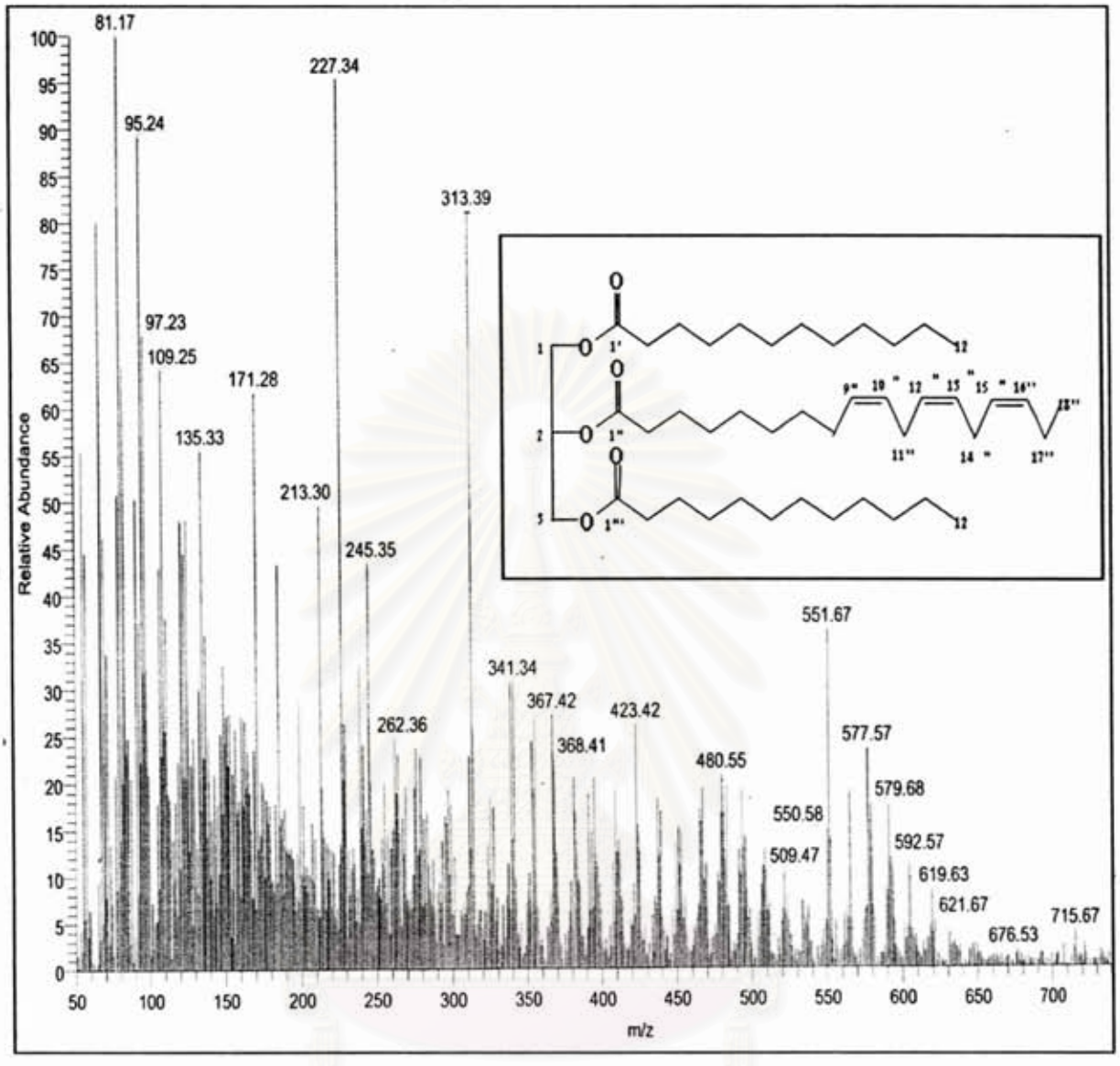



**Figure 3.** EI Mass spectrum of compound H1

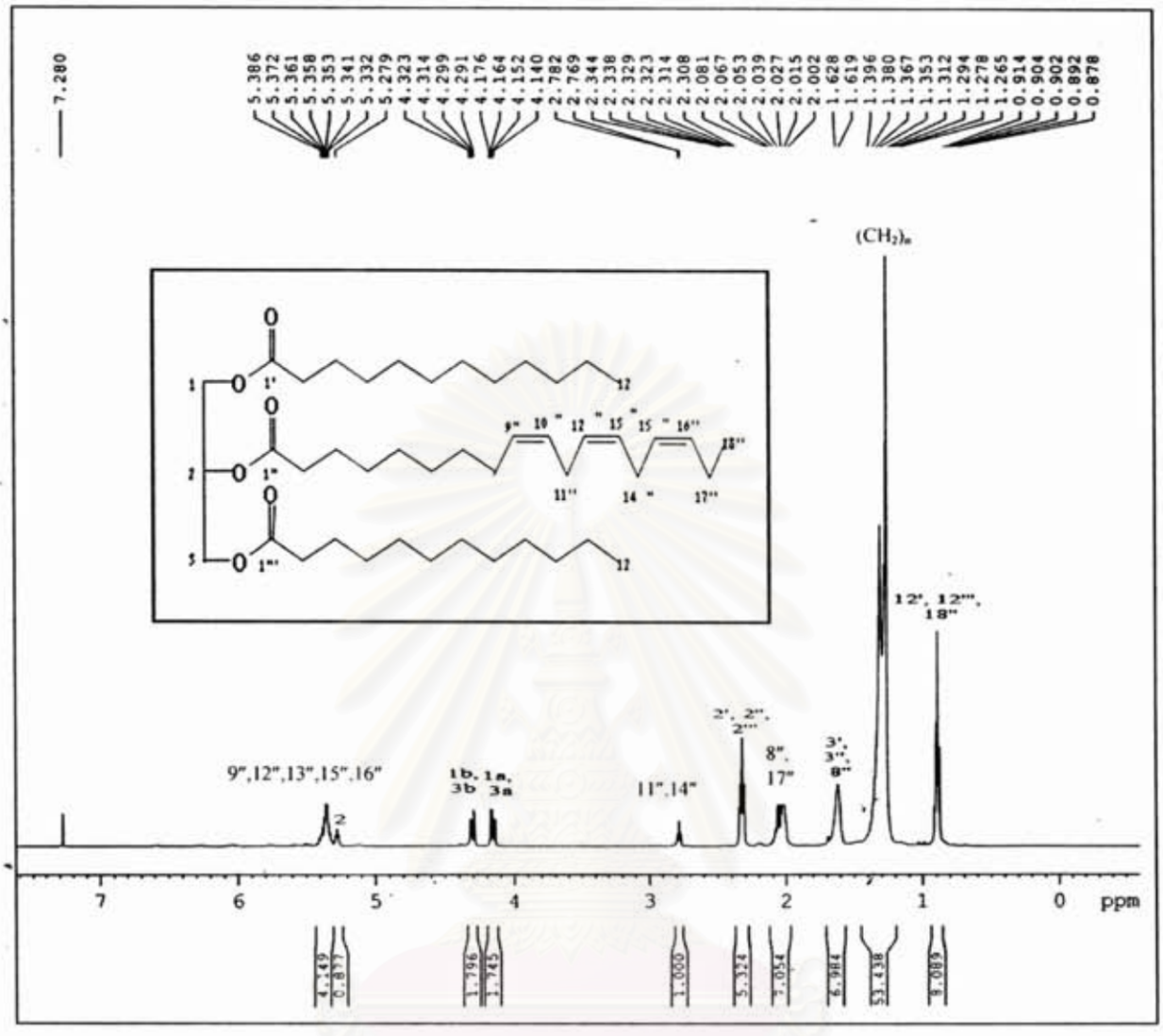



**Figure 4.** $^{1}$H NMR (500 MHz) Spectrum of compound H1

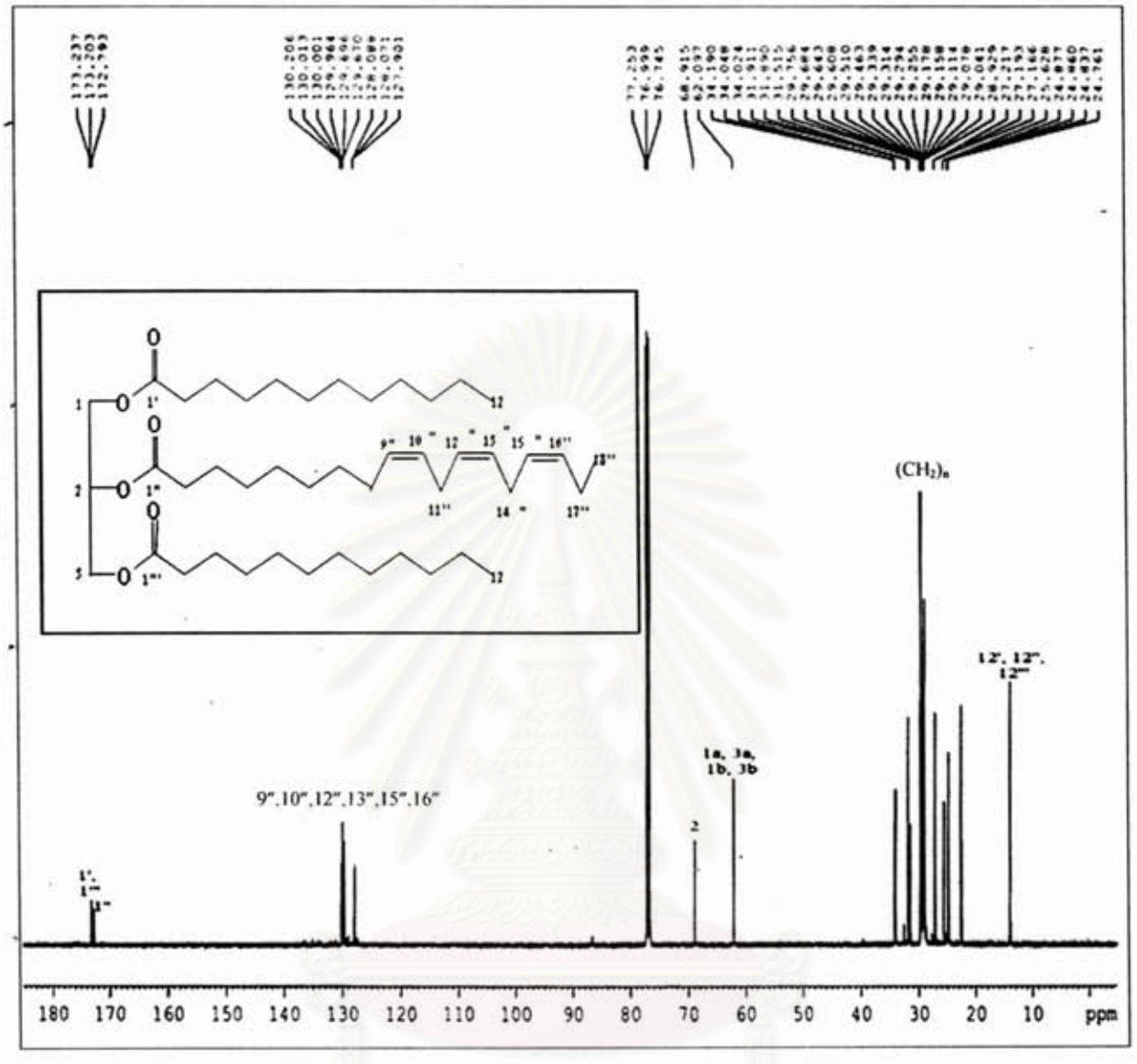



**Figure 5.** $^{13}$C NMR (125 MHz) Spectrum of compound H1

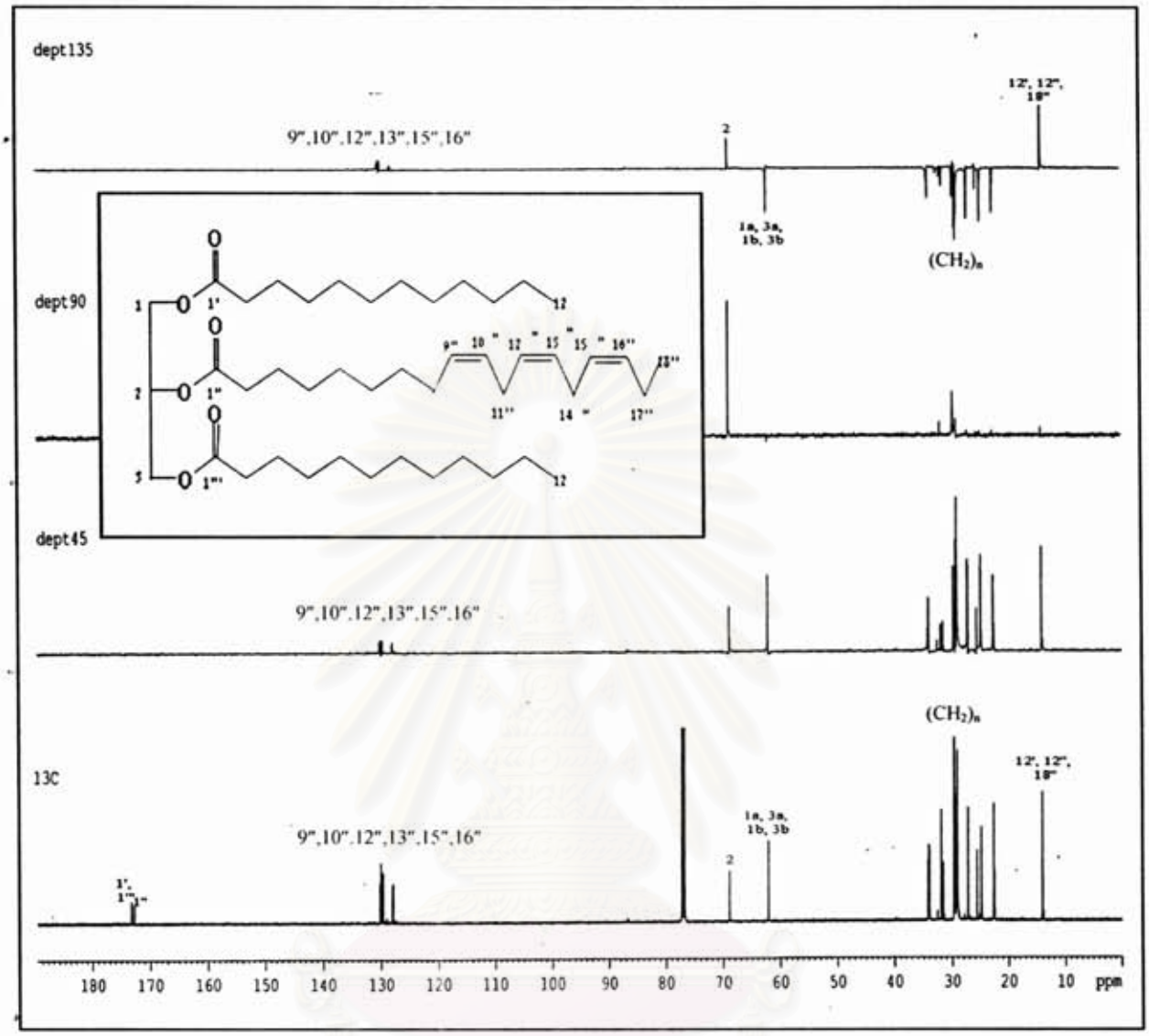



**Figure 6.** DEPT 135 Spectrum of compound H1

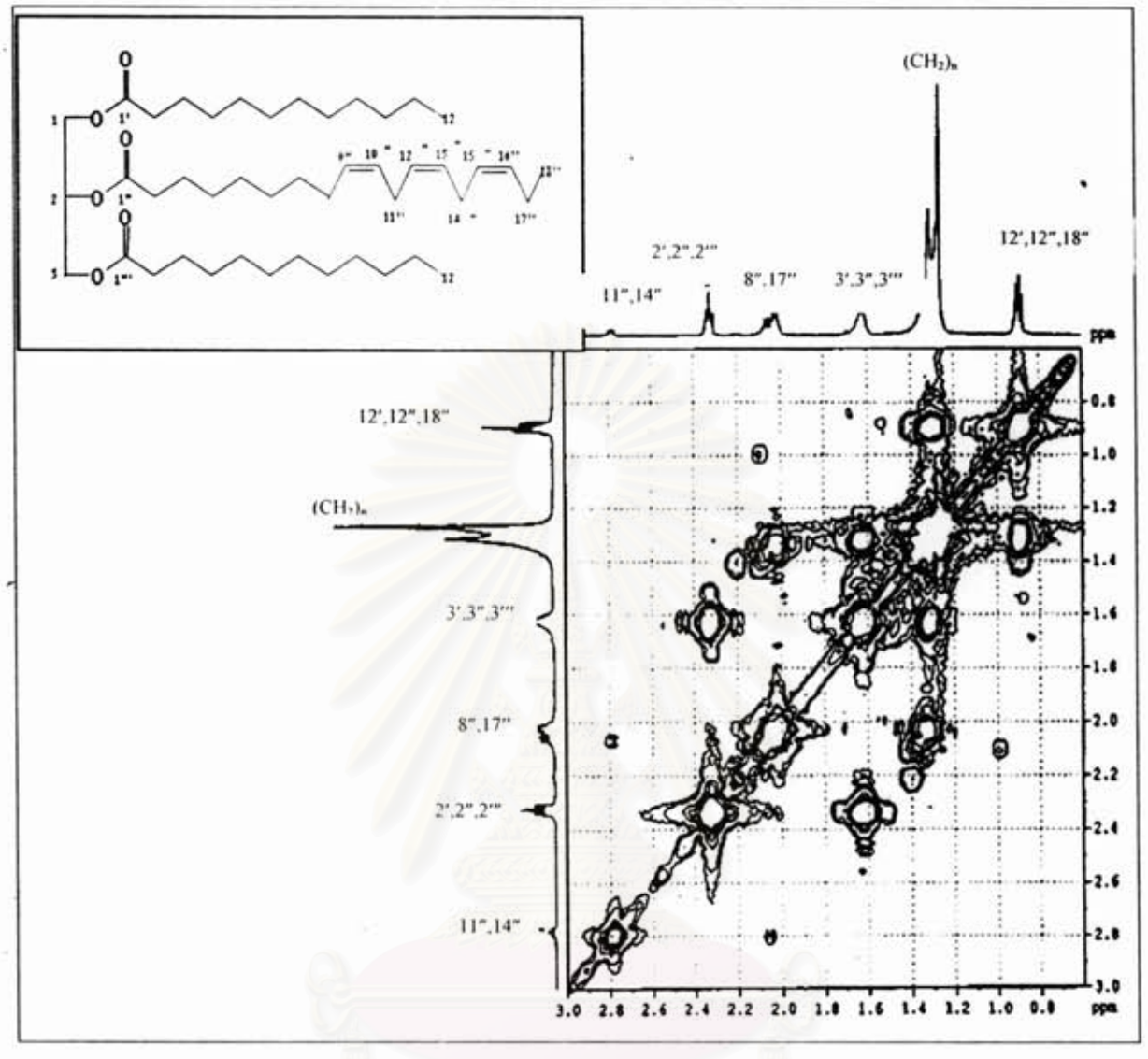



**Figure 7a.** $^{1}H$-$^{1}H$ COSY Spectrum of compound H1

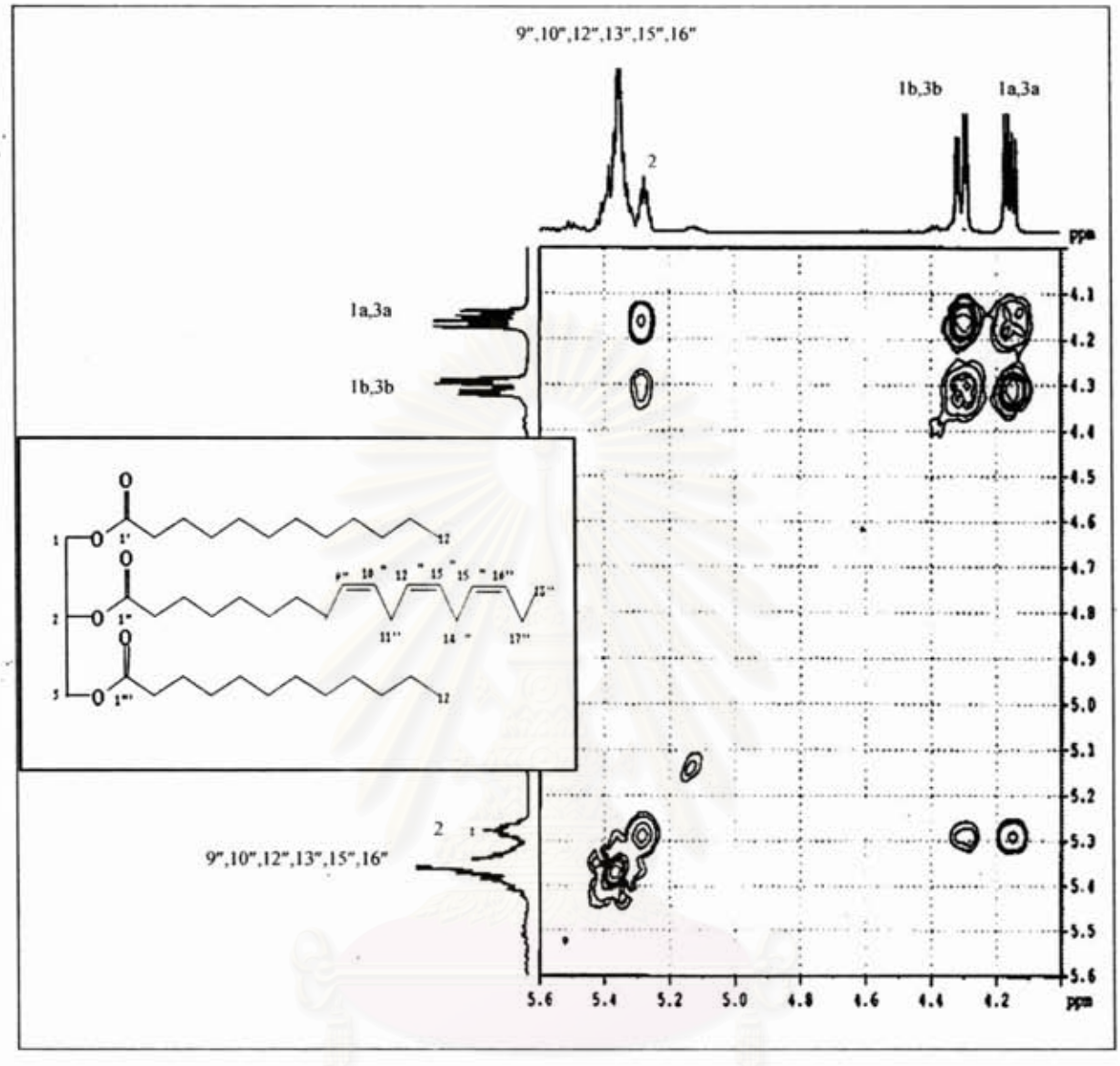



**Figure 7b.** $^{1}H$-$^{1}H$ COSY Spectrum of compound H1

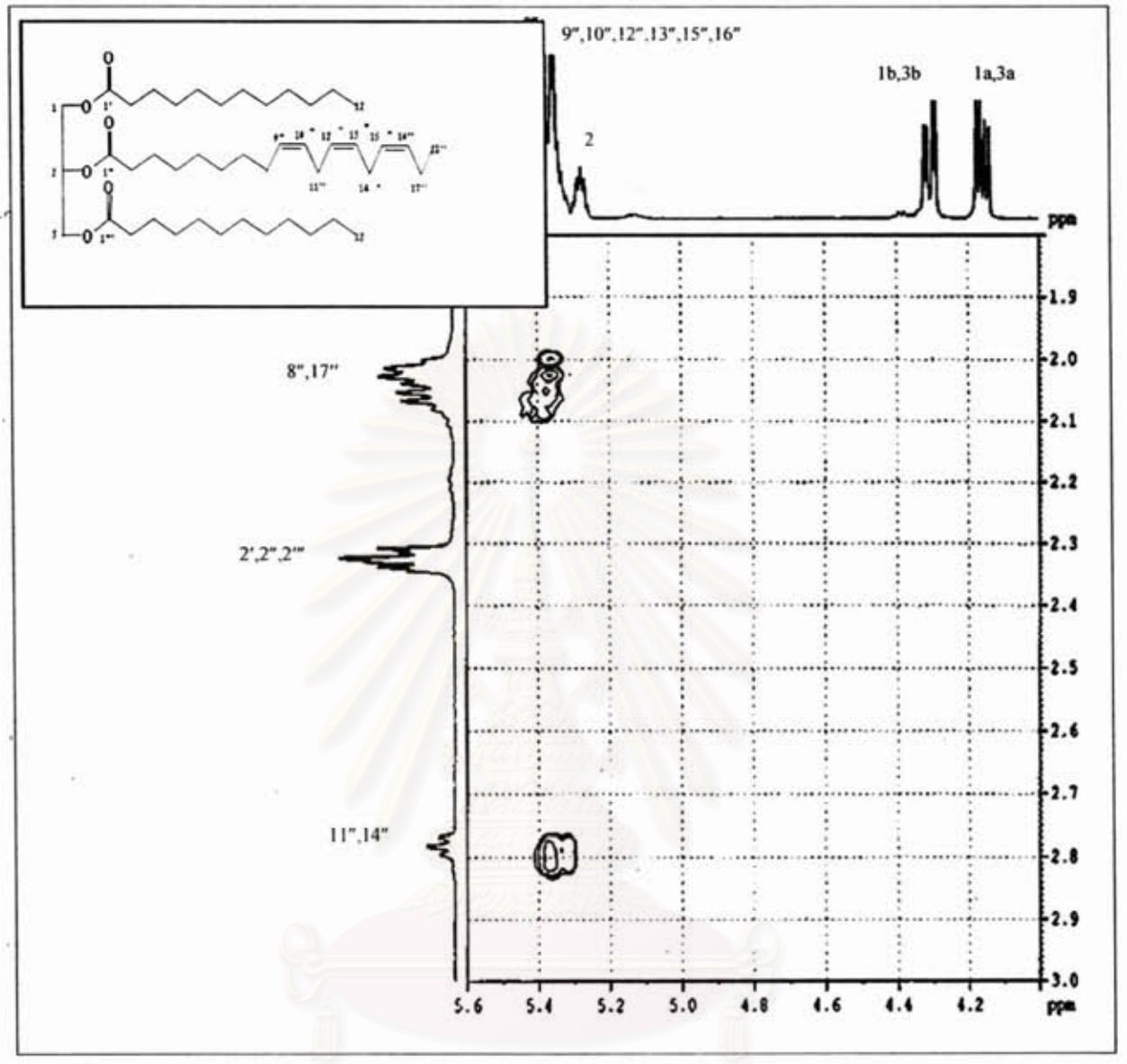



**Figure 7c.** $^{1}H$-$^{1}H$ COSY Spectrum of compound H1

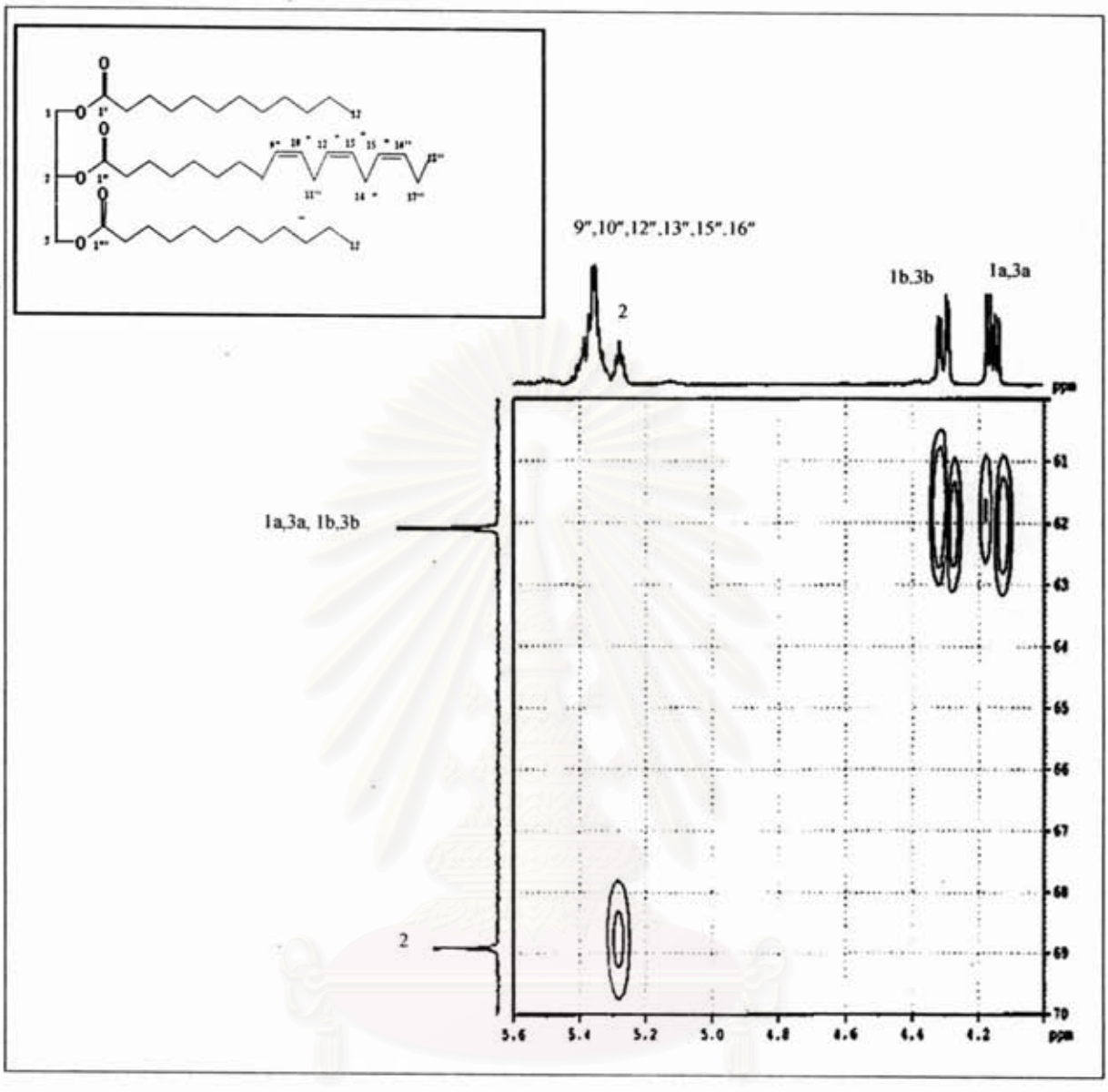



**Figure 8.** HMQC Spectrum of compound H1

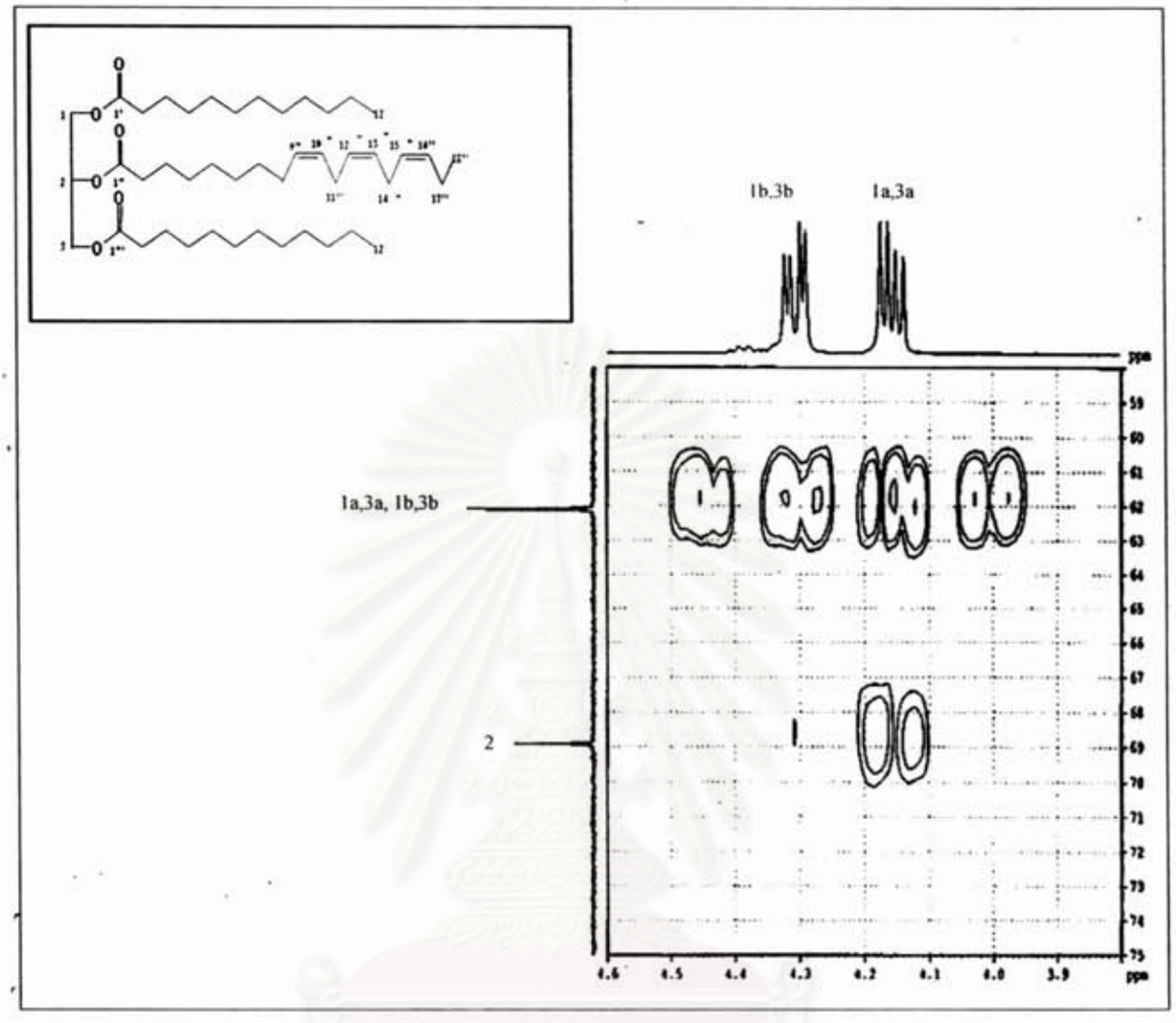



**Figure 9a.** HMBC Spectrum of compound H1

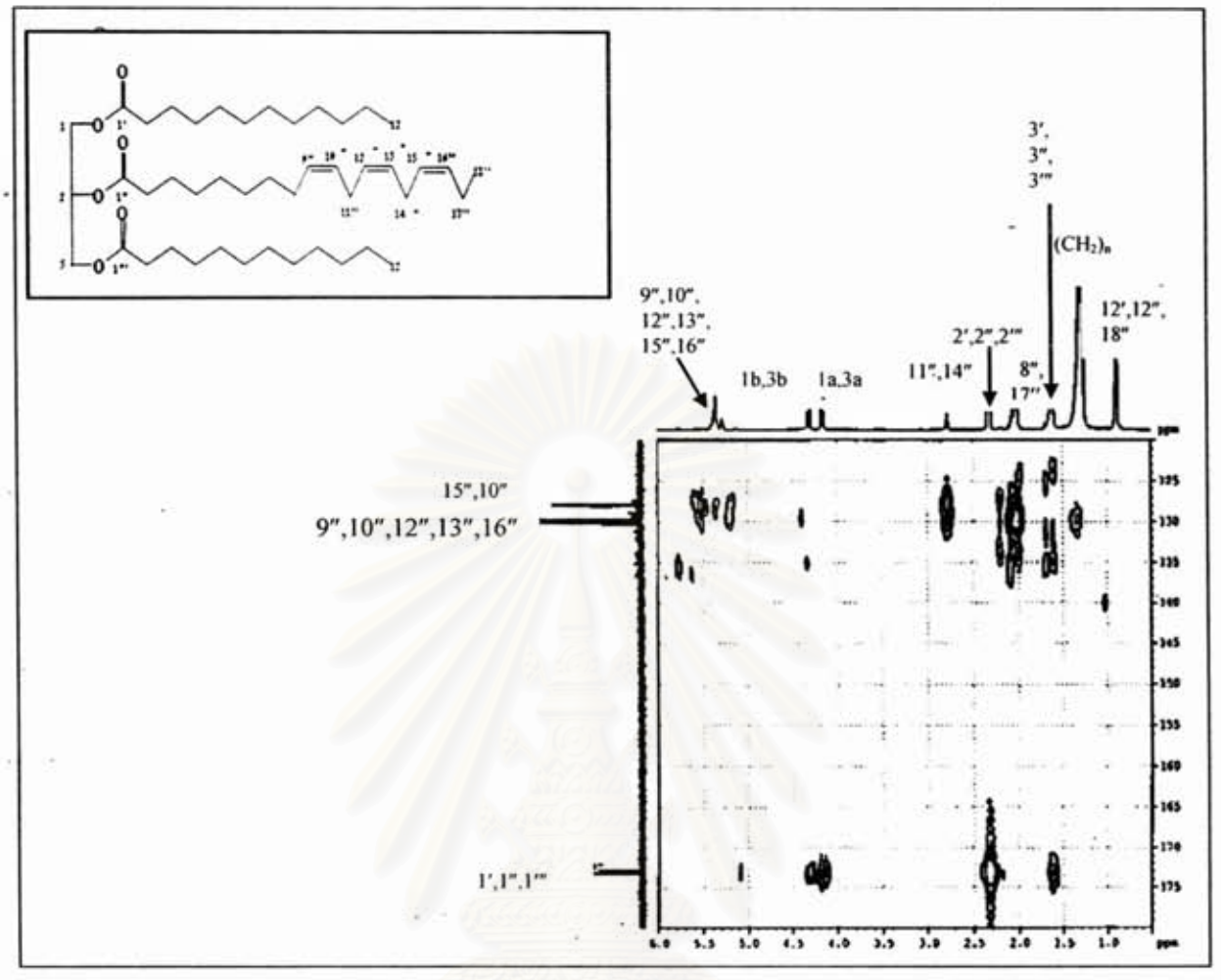



**Figure 9b.** HMBC Spectrum of compound H1

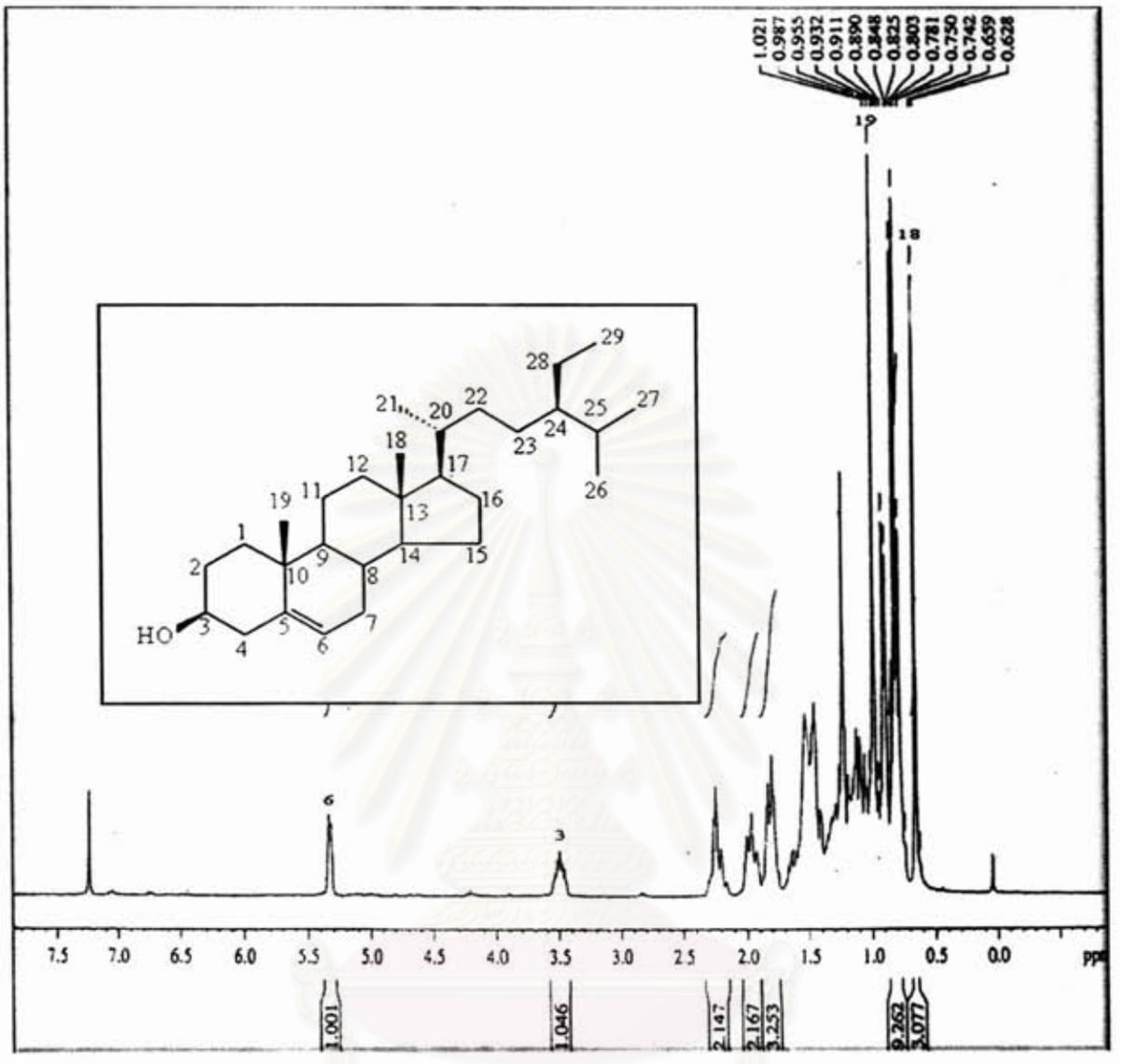



**Figure 10.** $^{1}$H NMR (125 MHz) Spectrum of compound H2

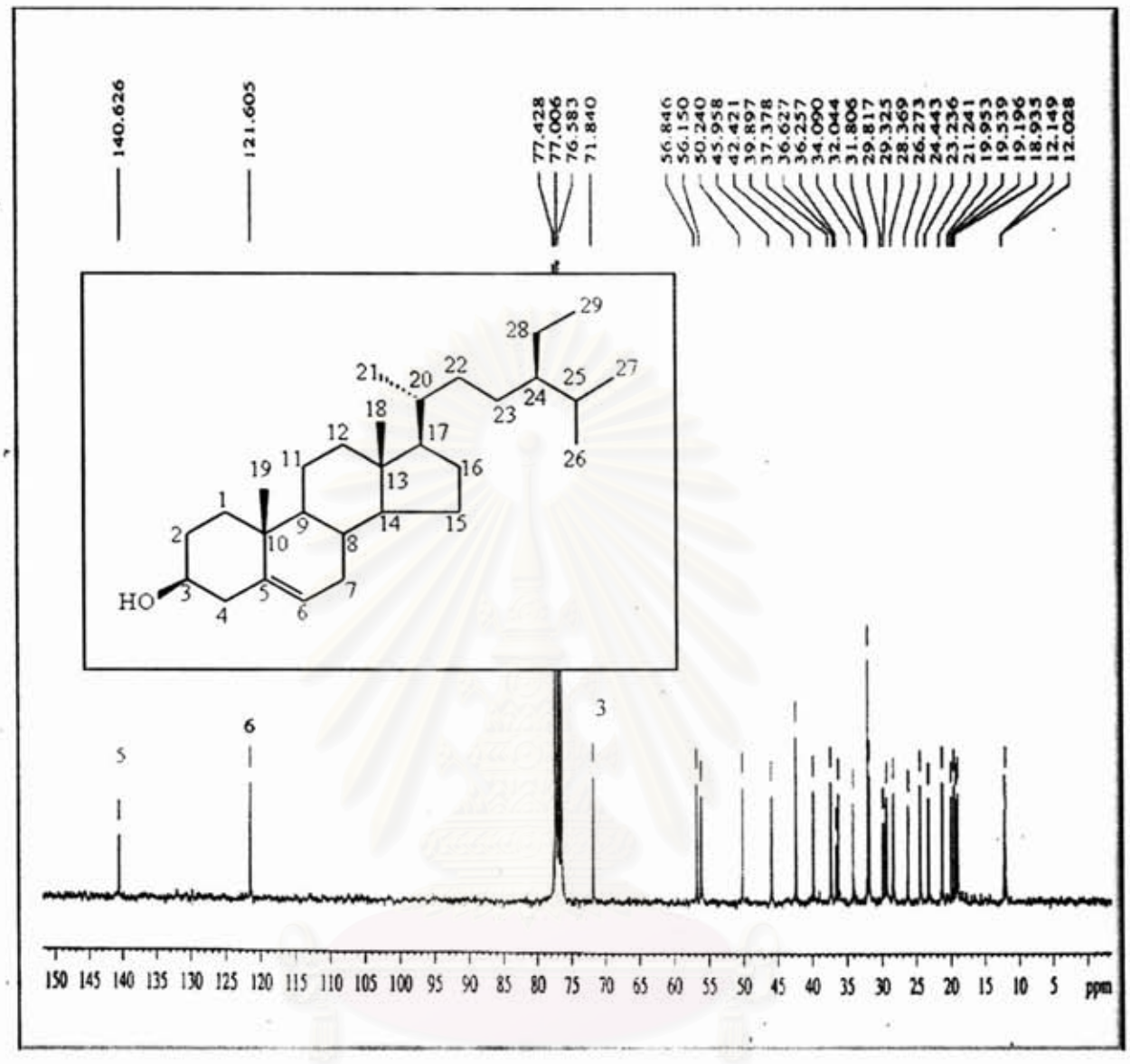



**Figure 11.** $^{13}C$ NMR (75 MHz) Spectrum of compound H2

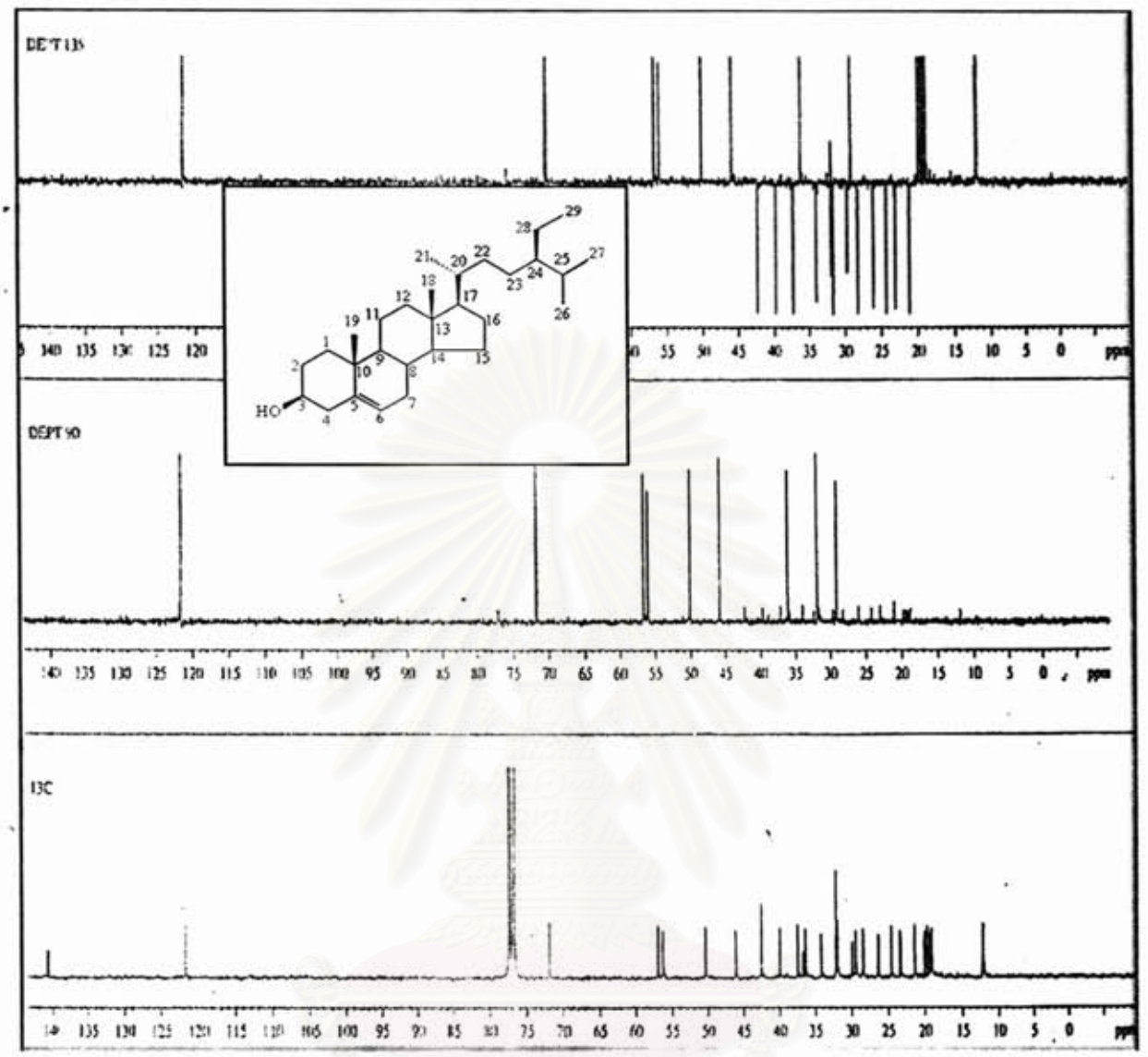



**Figure 12.** DEPT 135 Spectrum of compound H2

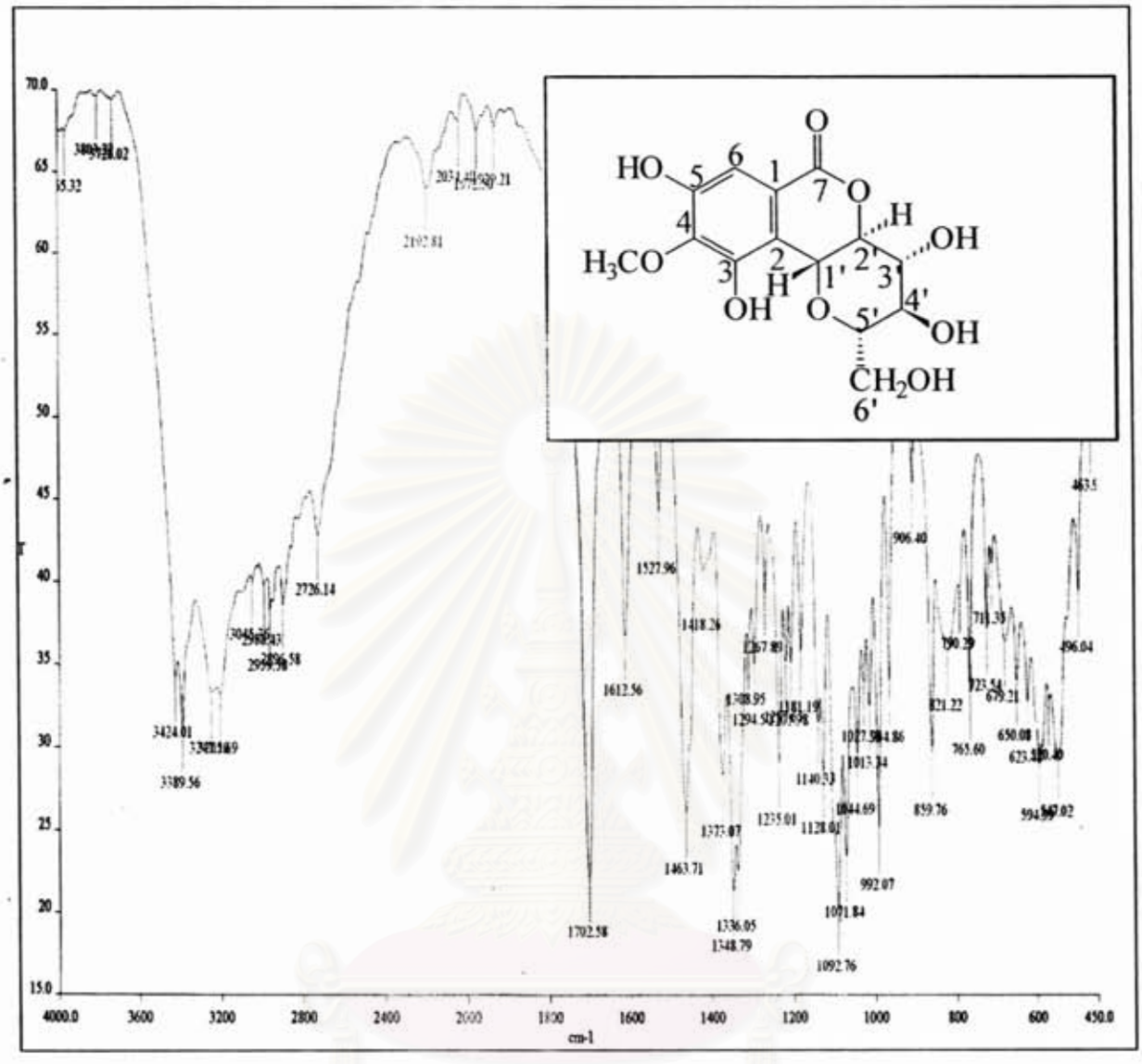



**Figure 13.** IR Spectrum of compound EA1 (KBr disc)

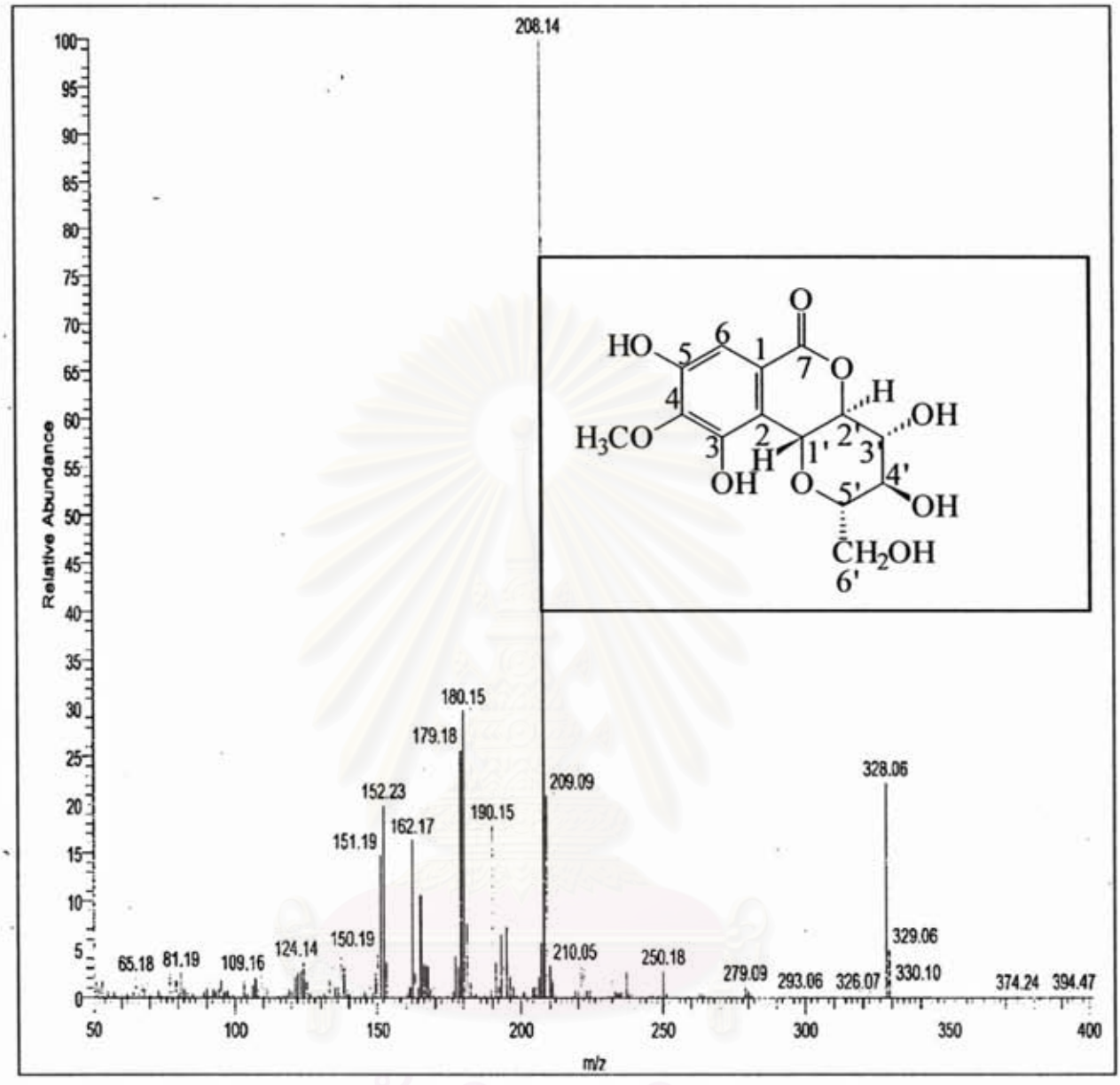



**Figure 14.** EI Mass spectrum of compound EA1

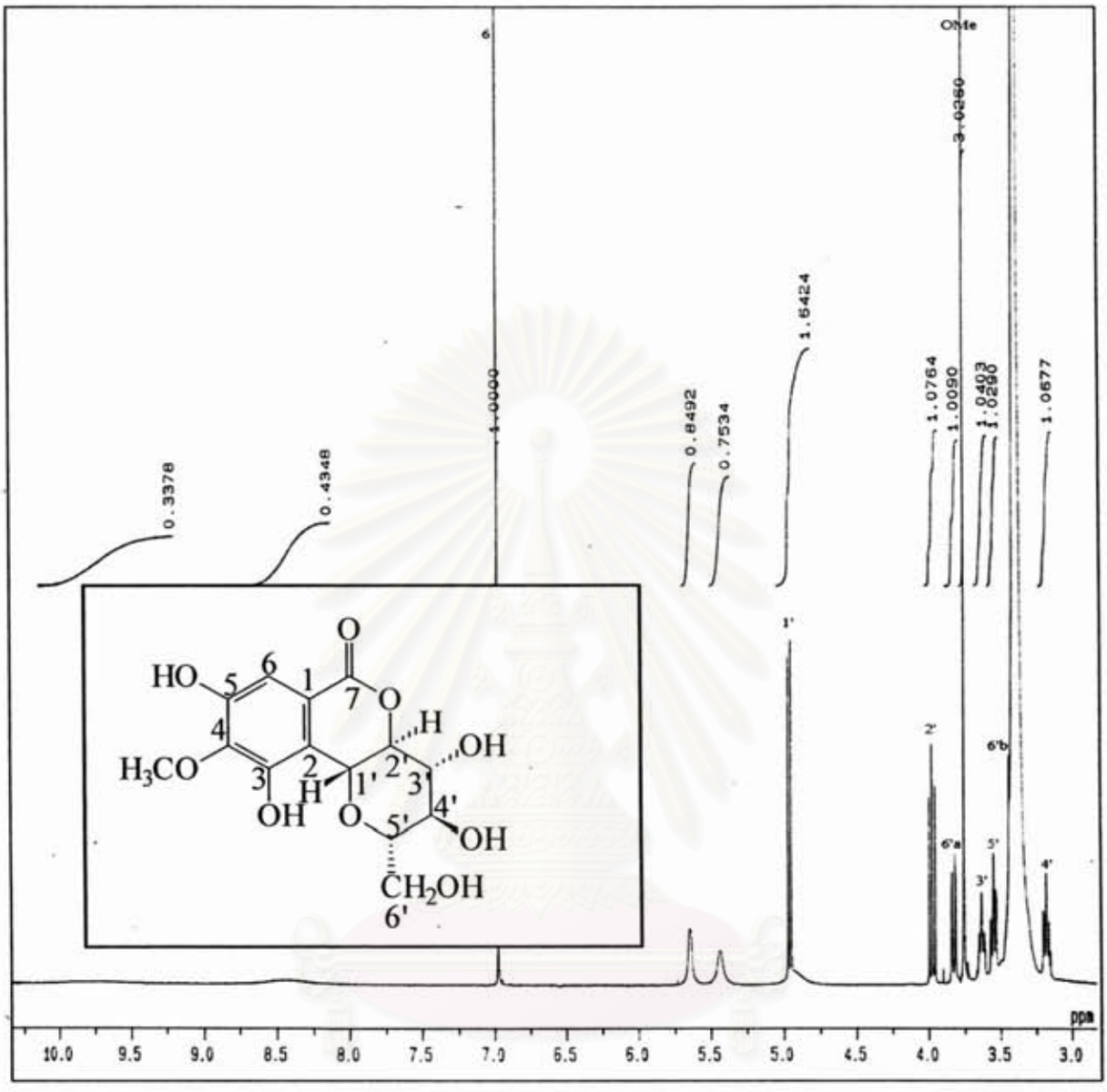



**Figure 15.** $^{1}$H NMR (500 MHz) Spectrum of compound EA1

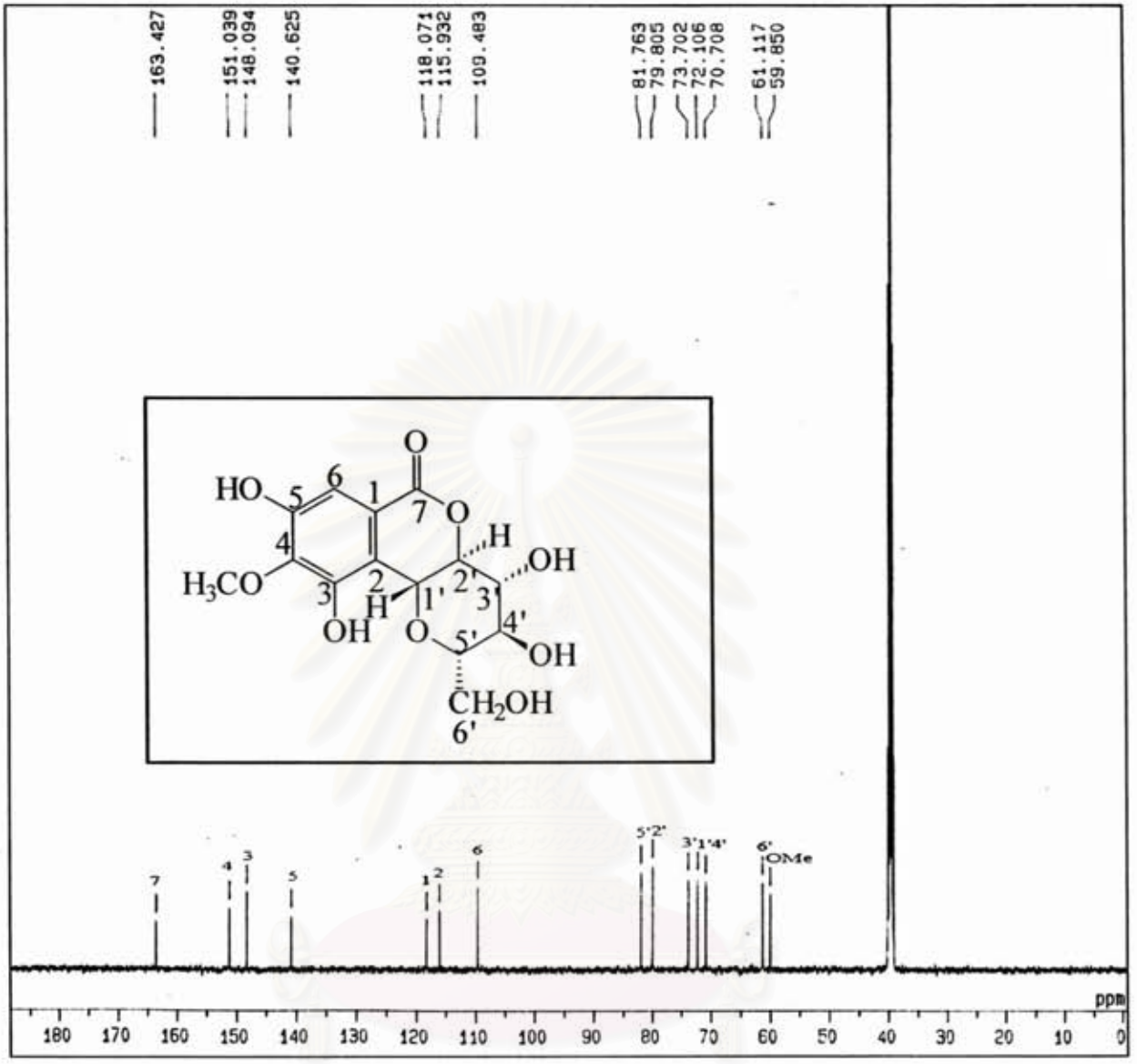



**Figure 16.** $^{13}C$ NMR (125 MHz) Spectrum of compound EA1

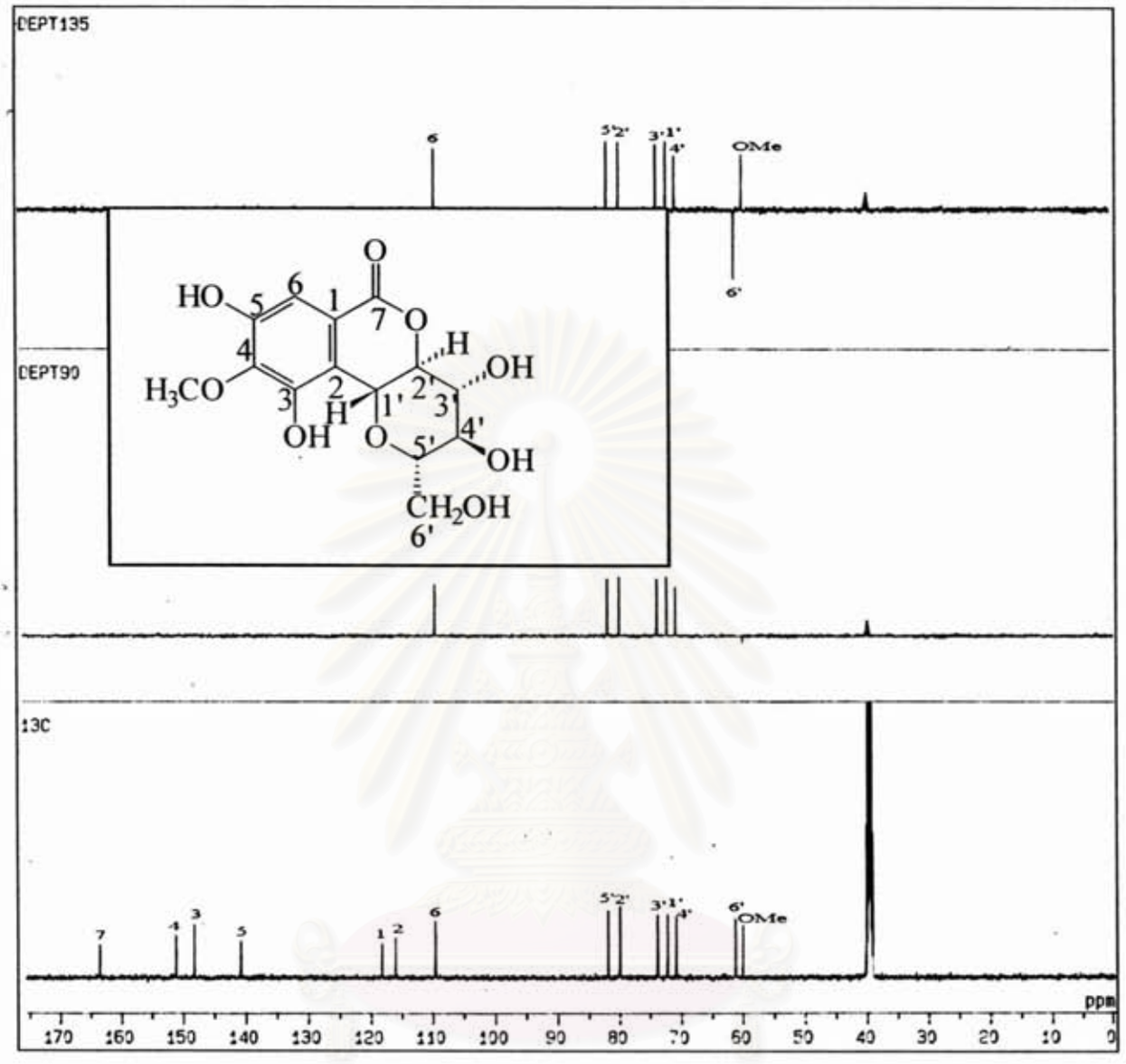



**Figure 17.** DEPT 135 Spectrum of compound EA1

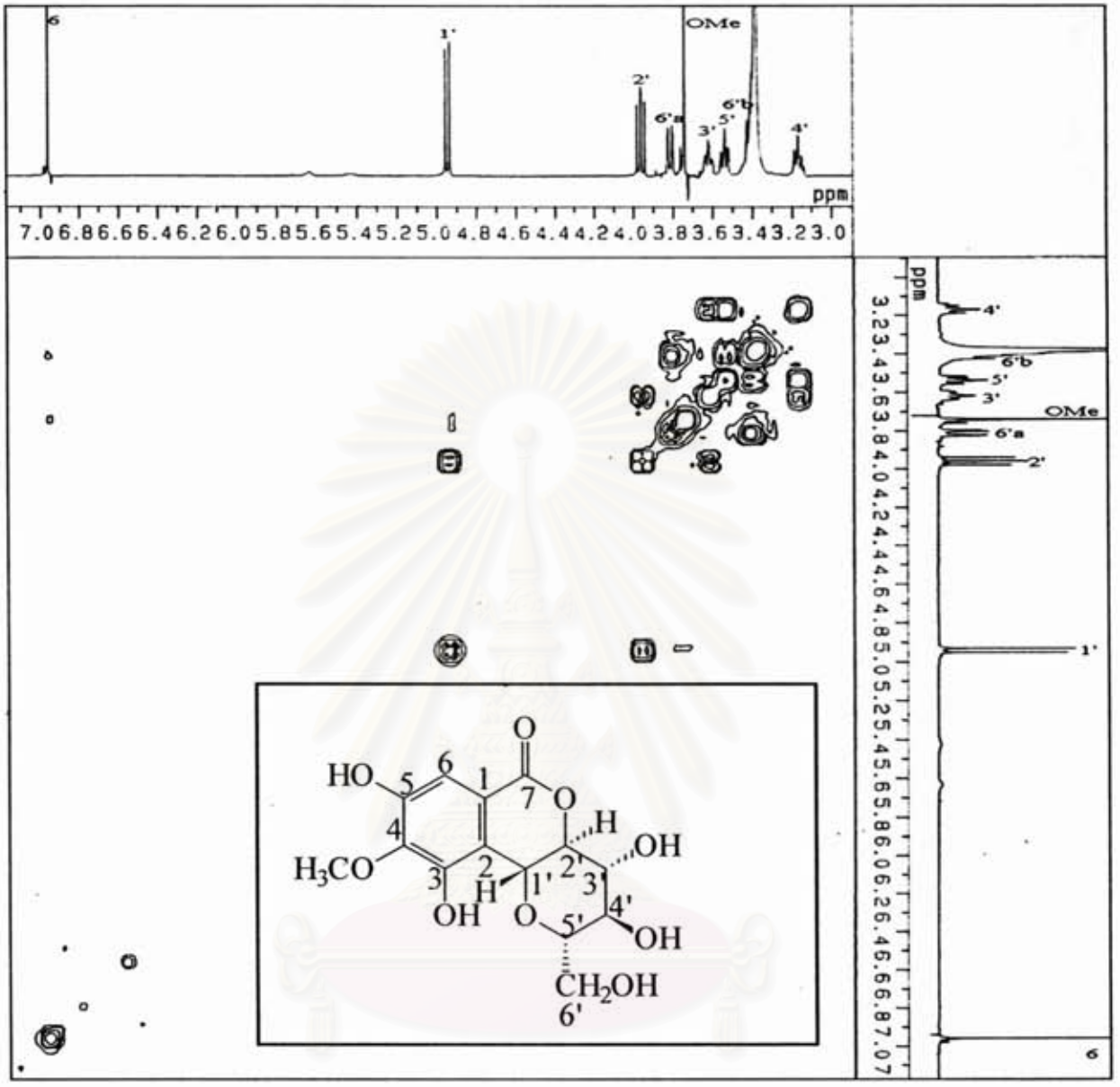



**Figure 18.** $^{1}H$-$^{1}H$ COSY Spectrum of compound EA1

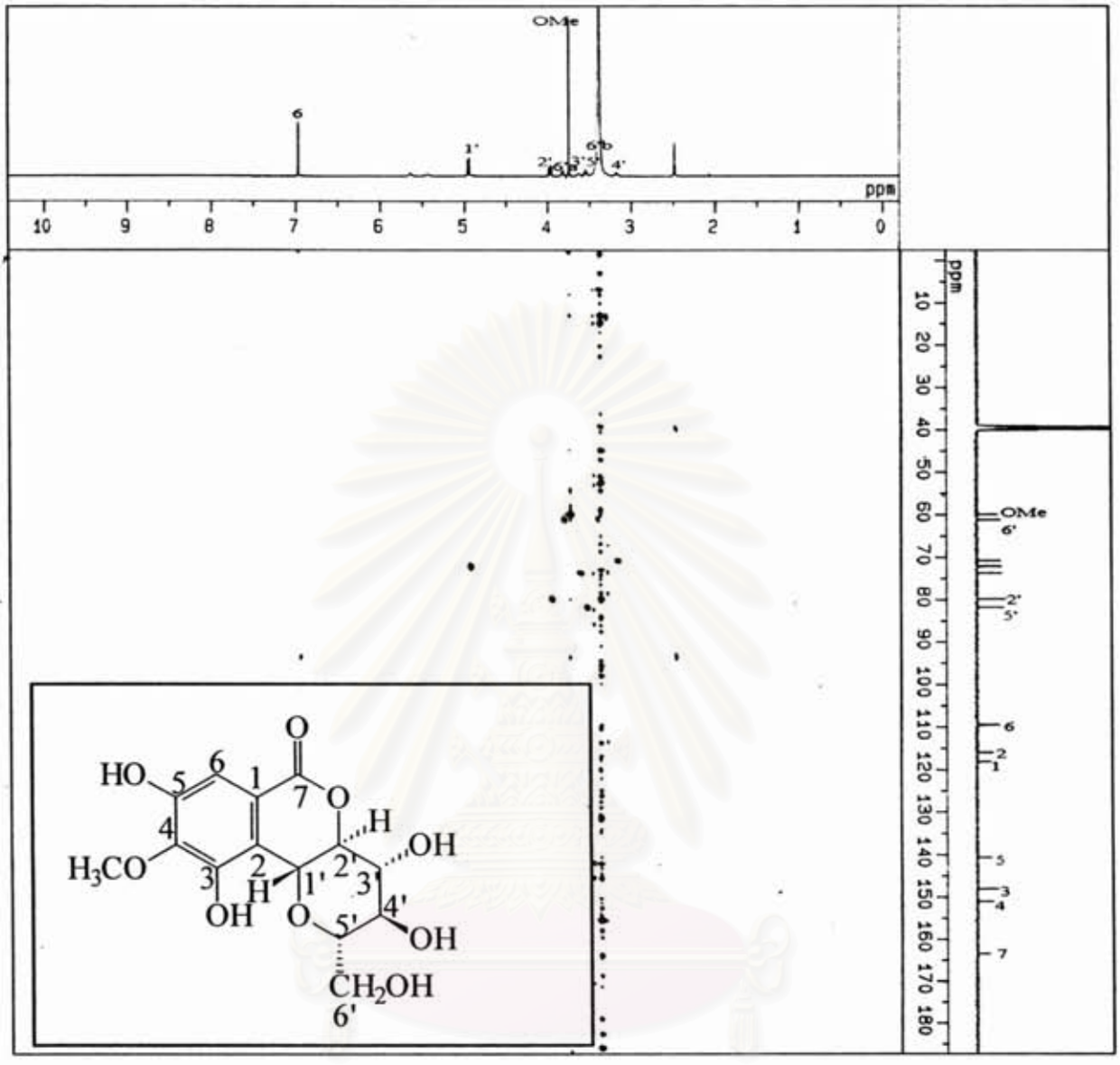



**Figure 19a.** HMQC Spectrum of compound EA1

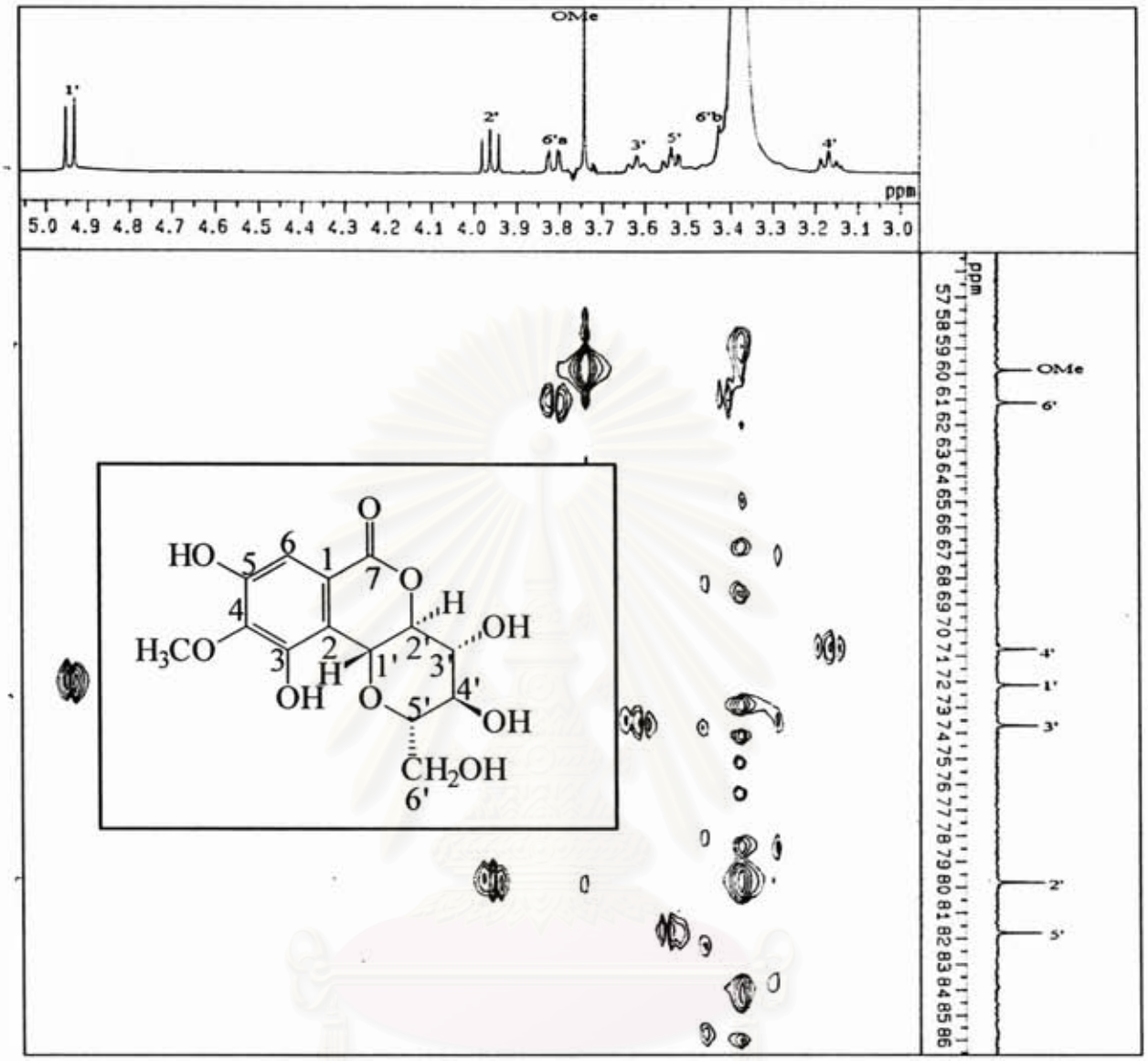



**Figure 19b.** HMQC Spectrum of compound EA1

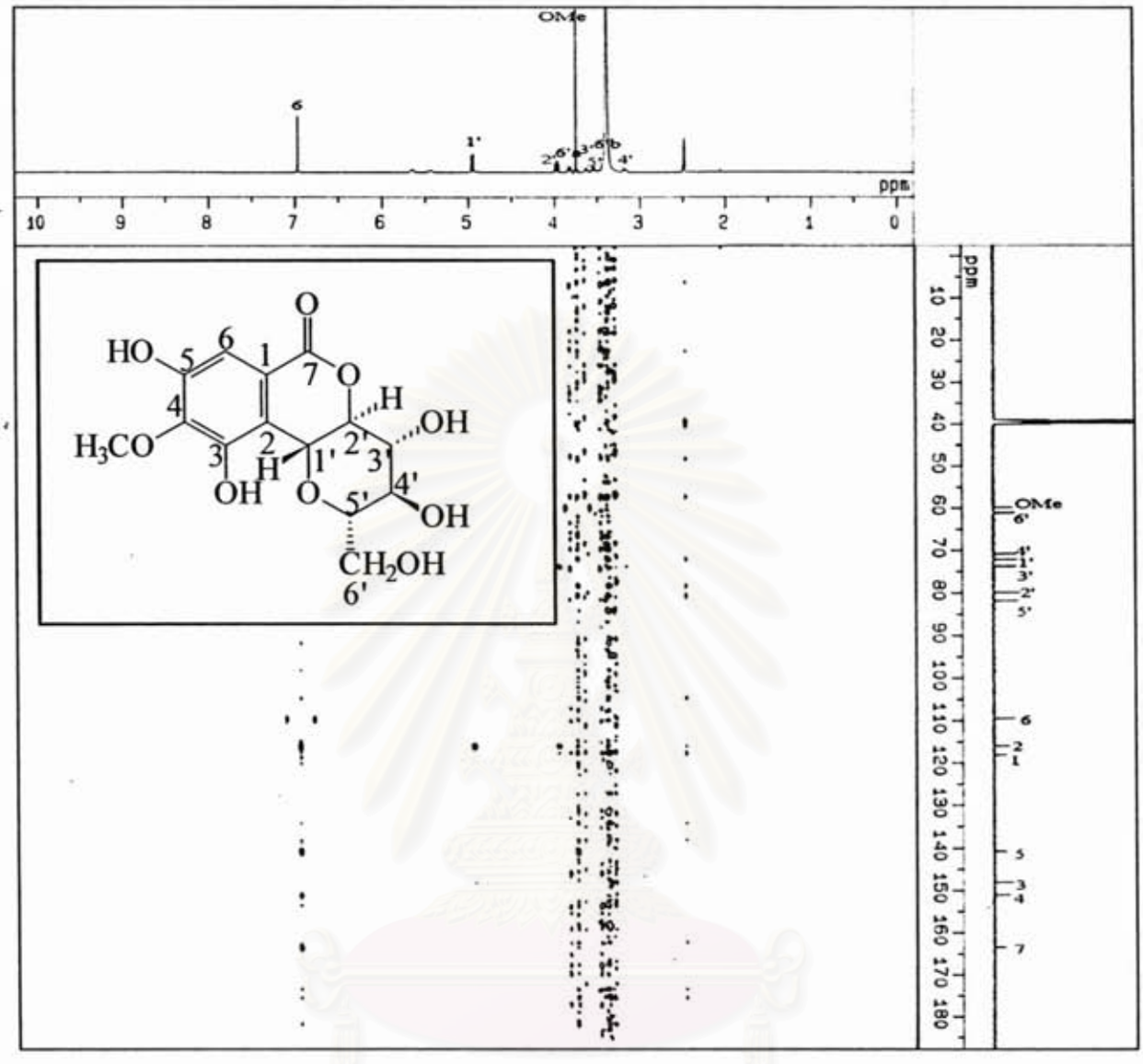



**Figure 20a.** HMBC Spectrum of compound EA1

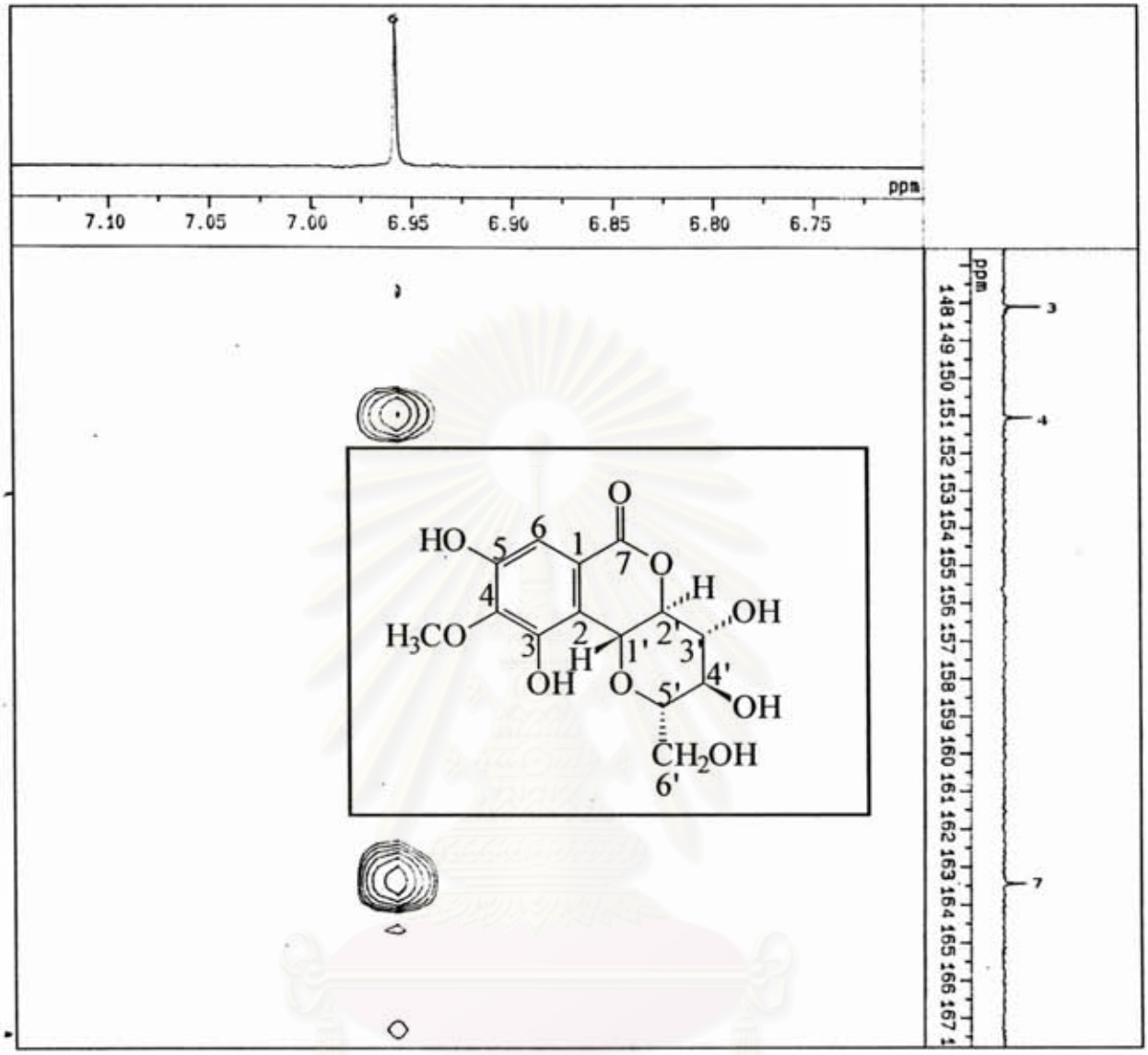



**Figure 20b.** HMBC Spectrum of compound EA1

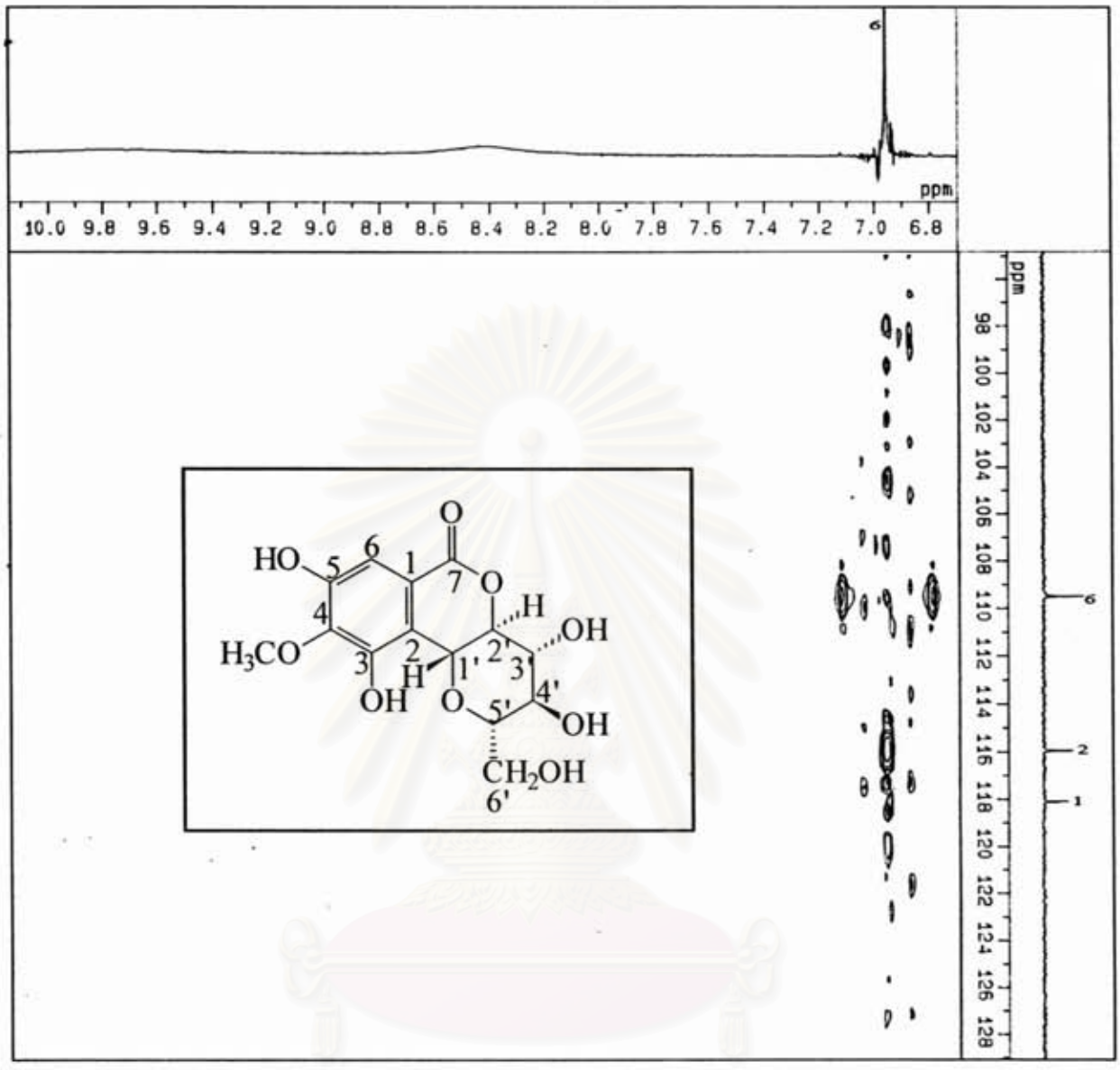



**Figure 20c.** HMBC Spectrum of compound EA1

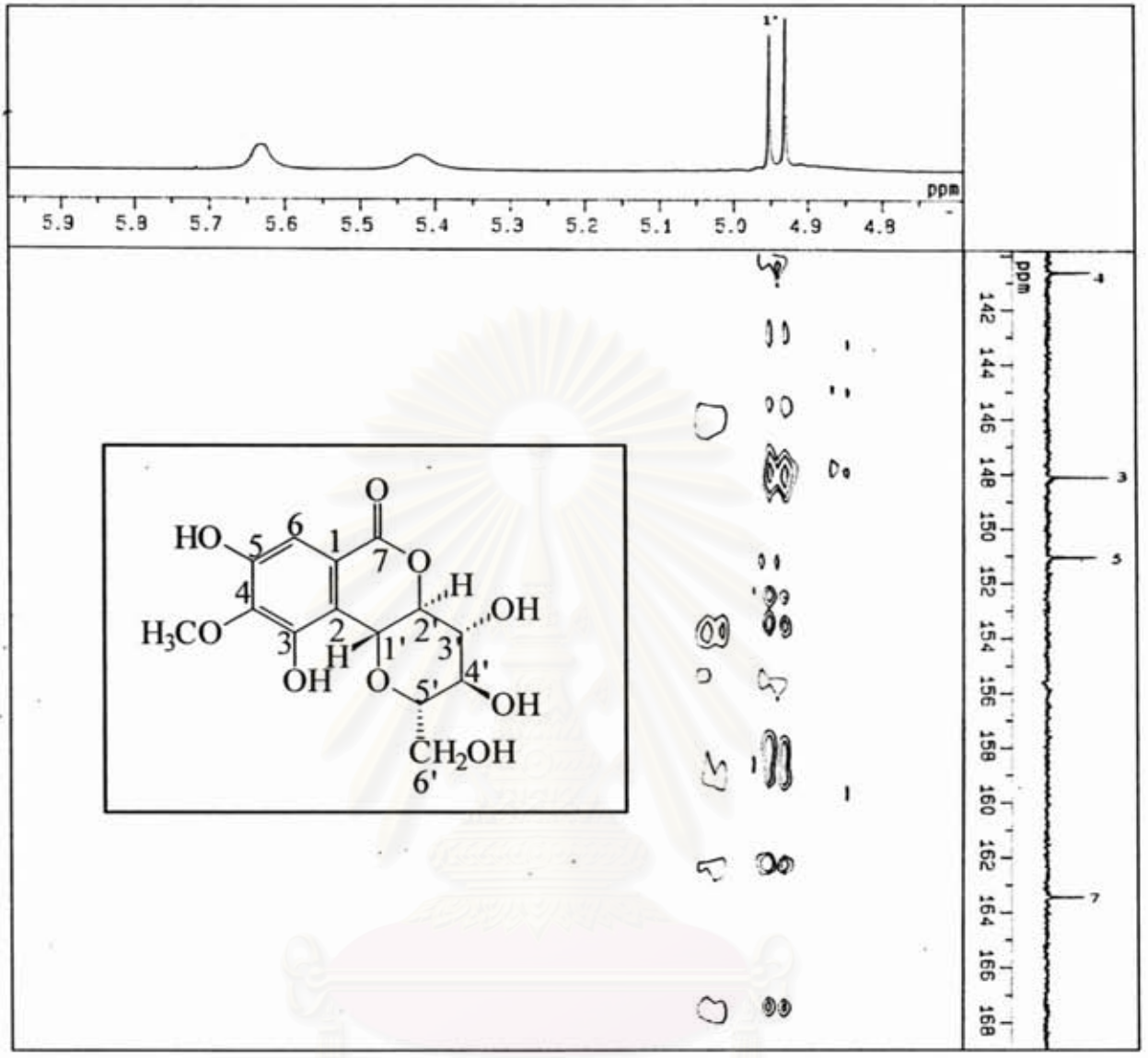



**Figure 20d.** HMBC Spectrum of compound EA1

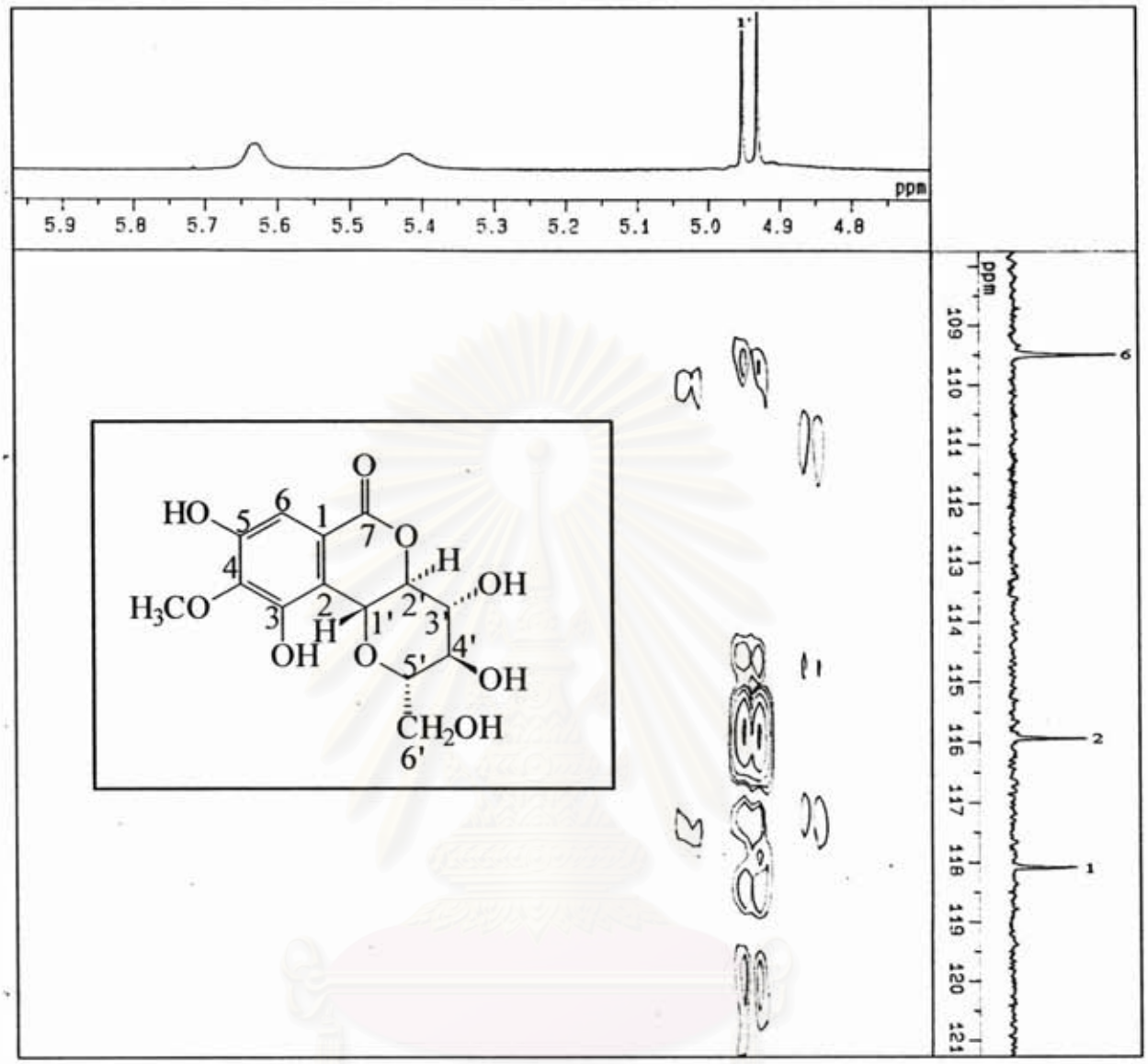



**Figure 20e.** HMBC Spectrum of compound EA1

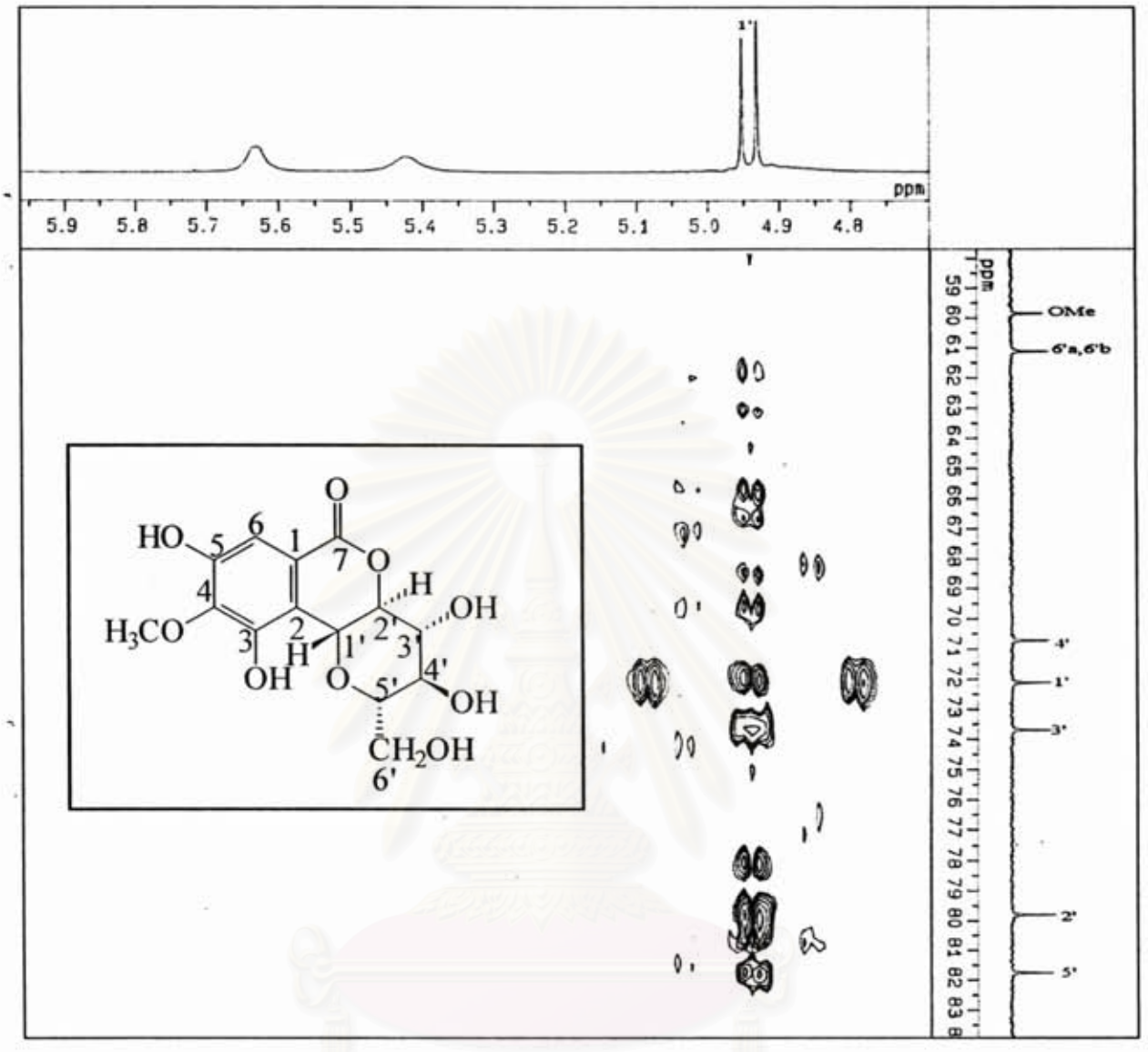



**Figure 20f.** HMBC Spectrum of compound EA1

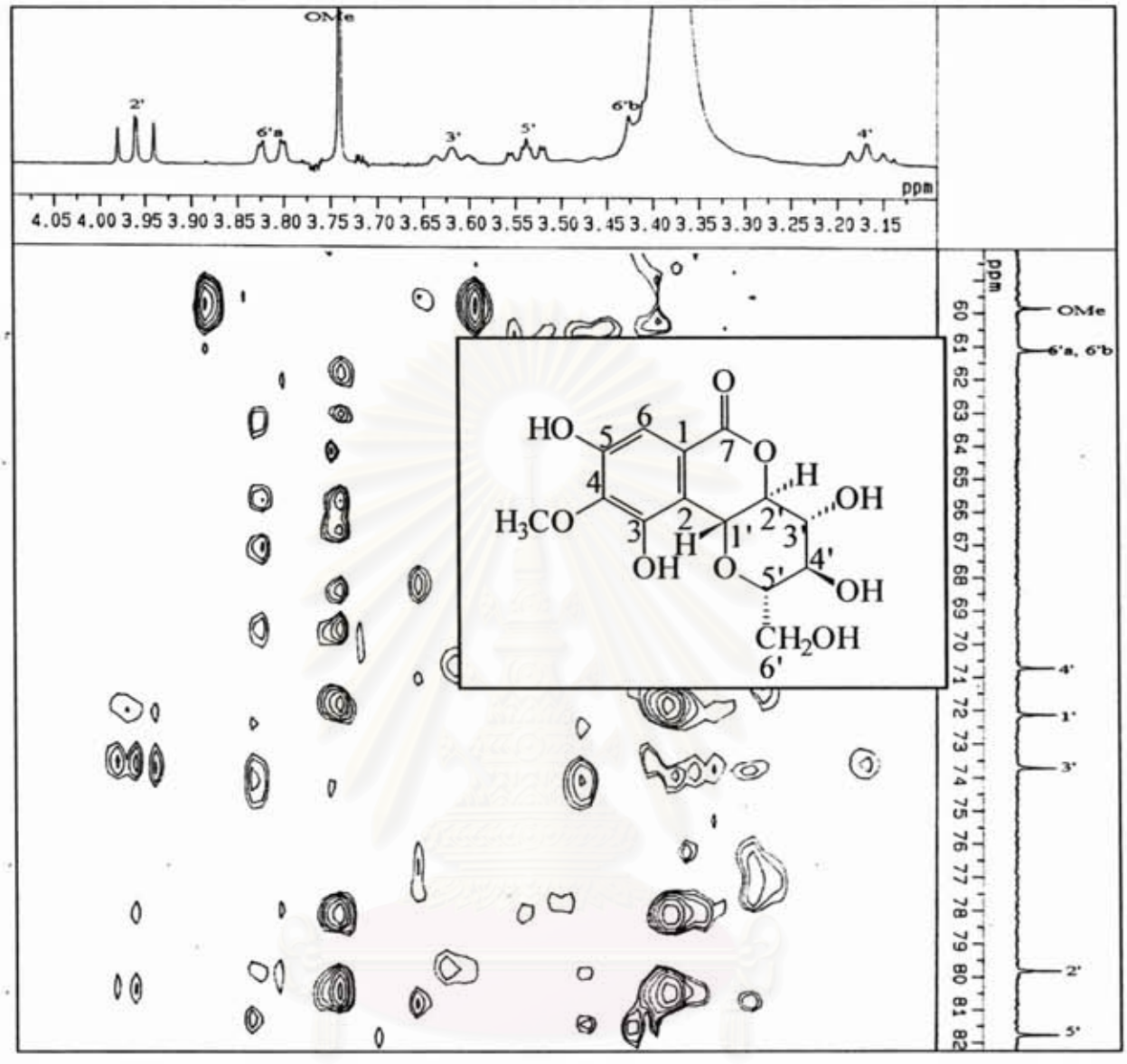



**Figure 20g.** HMBC Spectrum of compound EA1

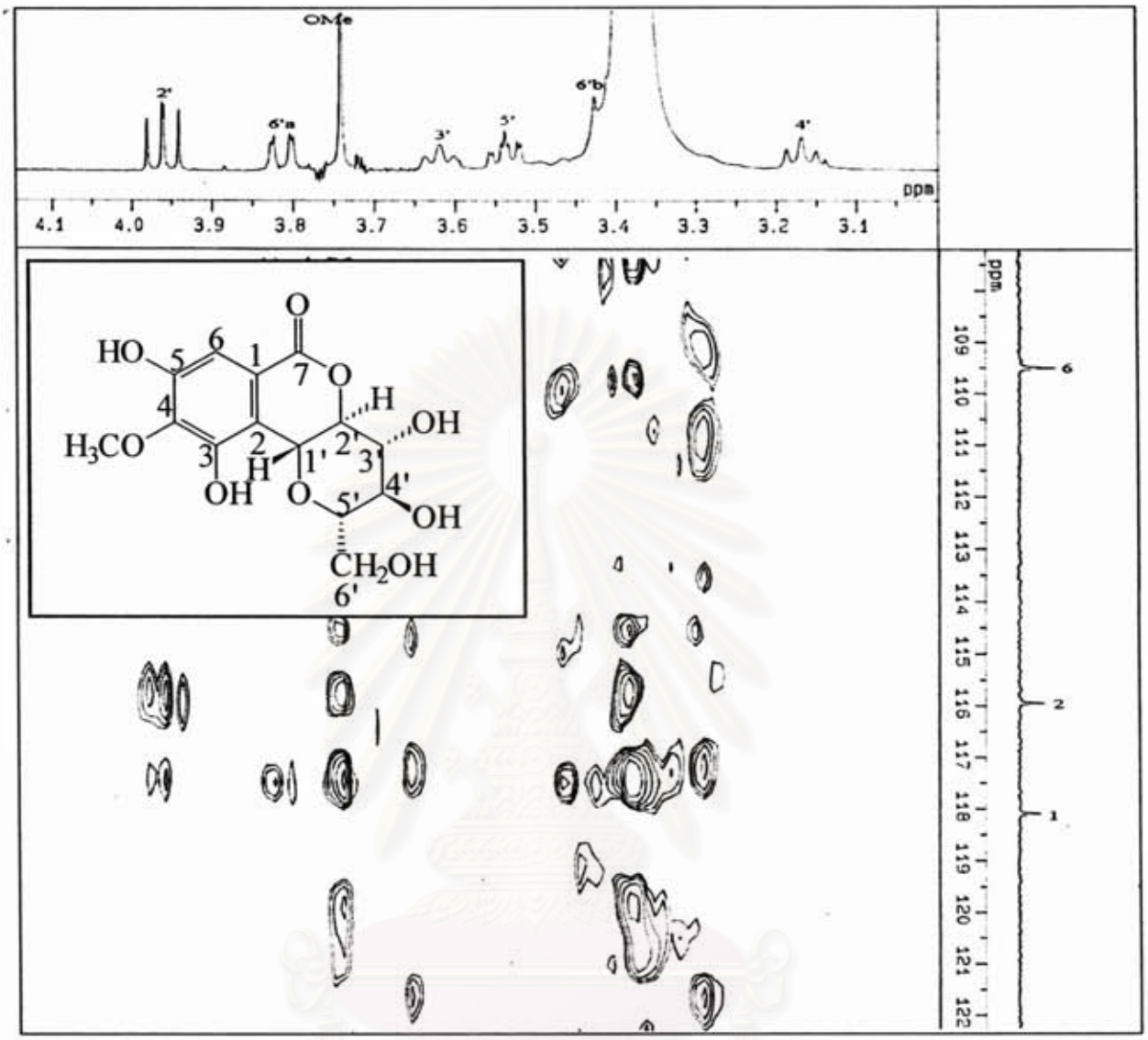



**Figure 20h.** HMBC Spectrum of compound EA1

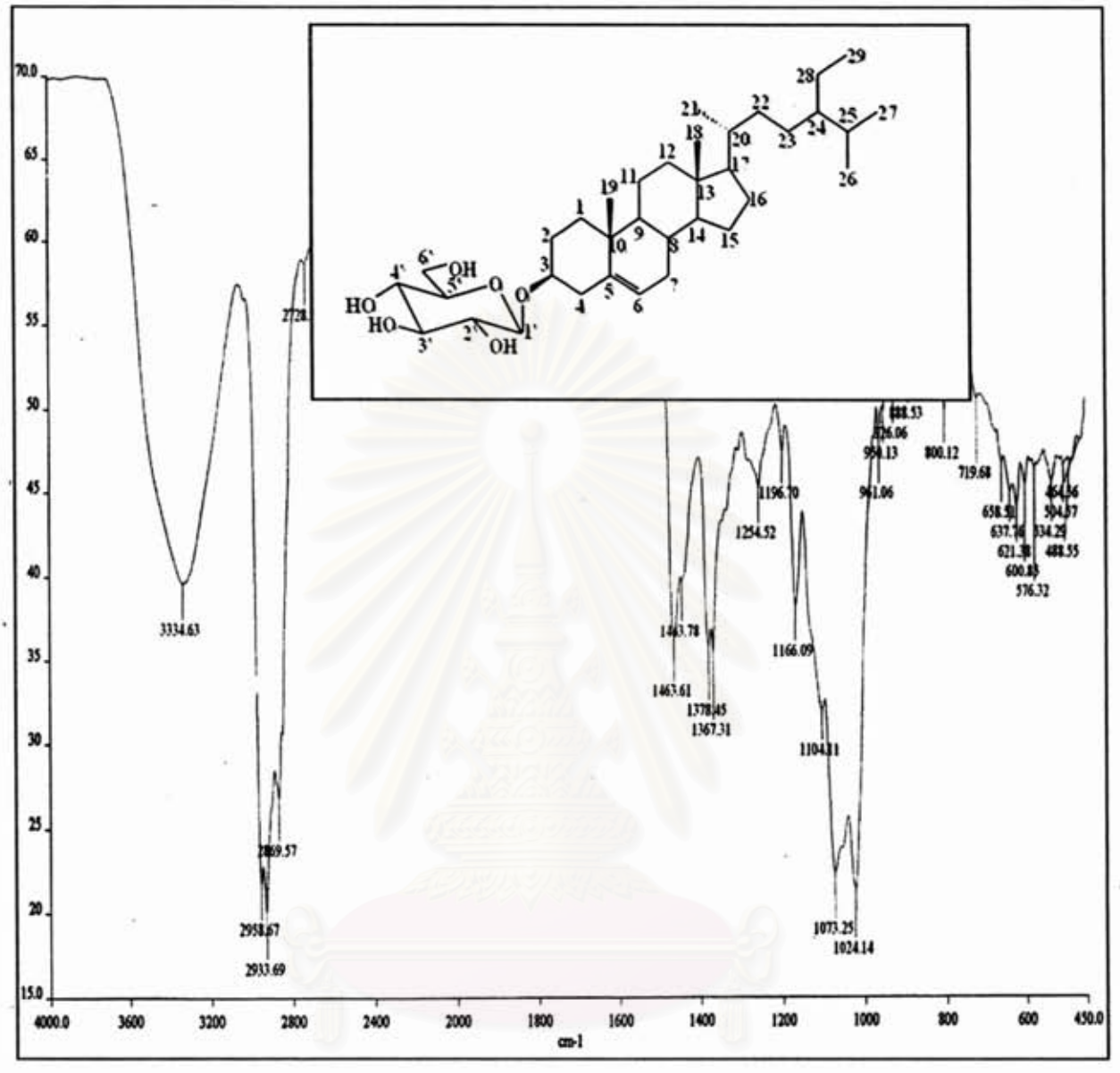



**Figure 21.** IR Spectrum of compound EA2 (KBr disc)

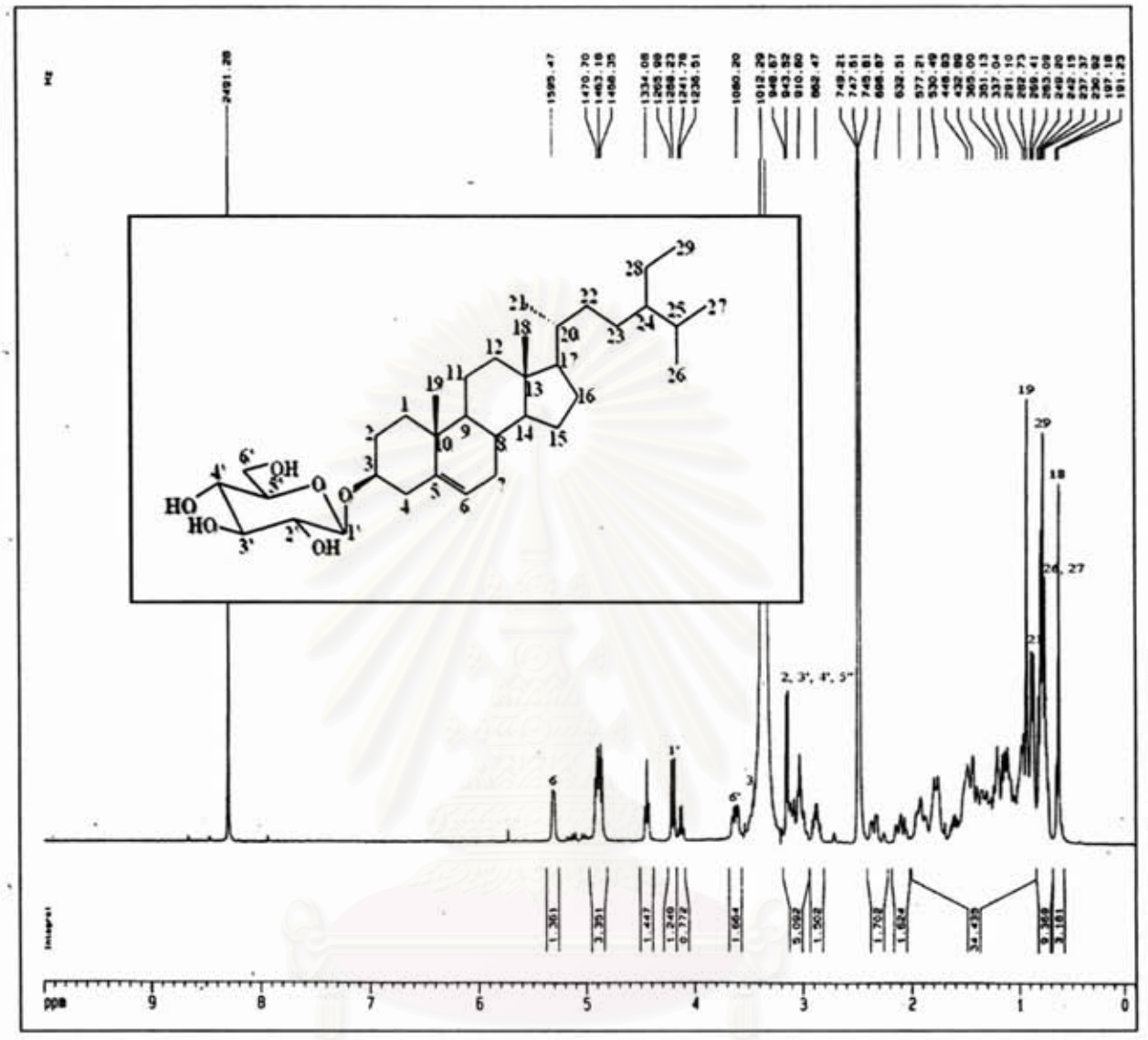



**Figure 22.** $^{1}$H NMR (500 MHz) Spectrum of compound EA2

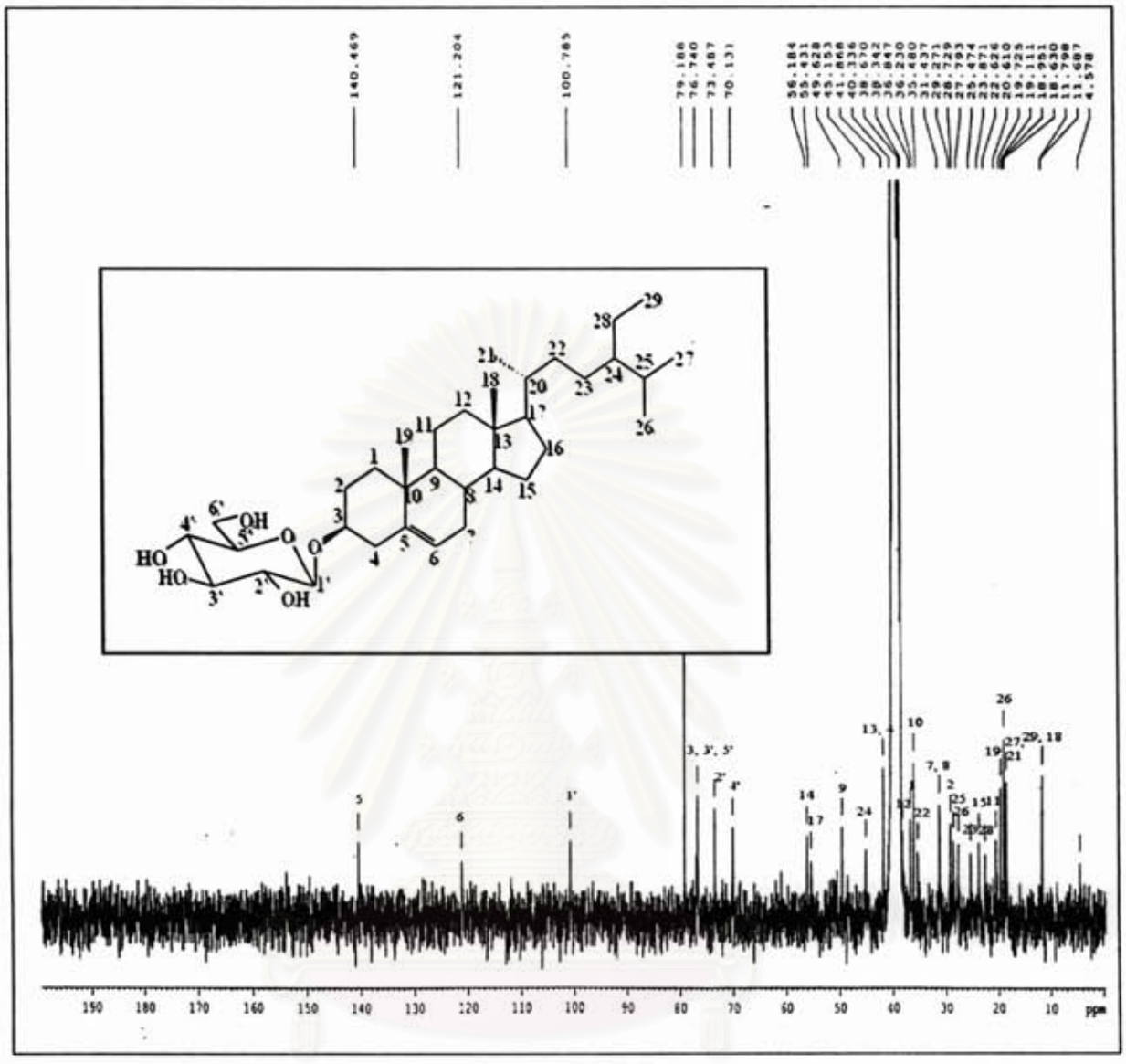



**Figure 23.** $^{13}C$ NMR (125 MHz) Spectrum of compound EA2

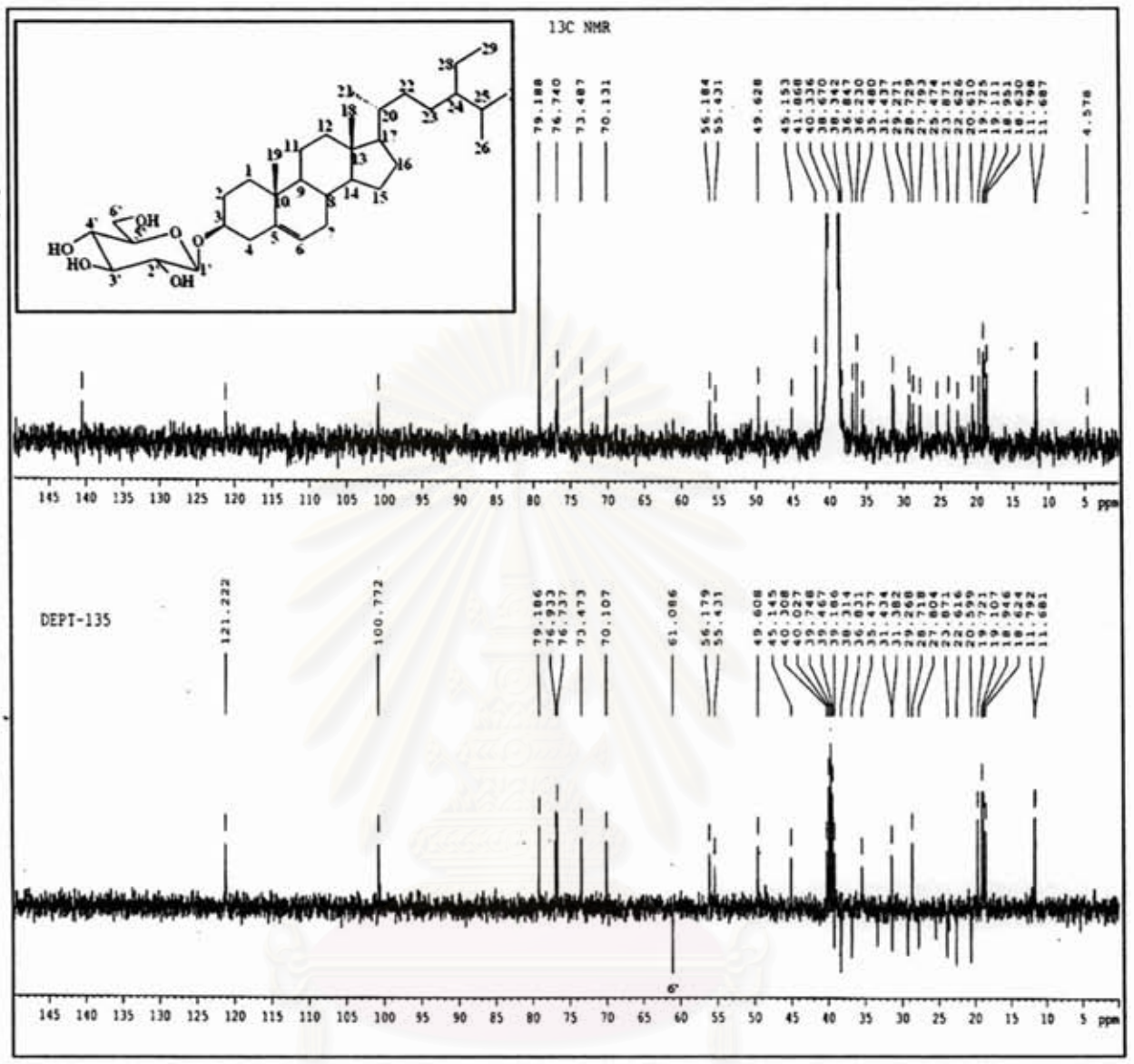



**Figure 24.** DEPT 135 Spectrum of compound EA2

# VITA

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