การศึกษาทางพฤกษเคมีของใบและเปลือกต้นตีนเป็ดฝรั่ง

ว่าที่ร้อยเอกหญิง อัญชิษฐา วิยาภรณ์

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PHYTOCHEMICAL STUDY OF LEAVES AND BARK OF *CRESCENTIA ALATA* HBK.

Capt. Aunchita Wiyaporn

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmaceutical Botany

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BY	Capt. Aunchita Wiyaporn
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…….. Dean of Faculty of Pharmaceutical Sciences

(Associate Professor Boonyong Tantisira, Ph.D.)

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……Chairman

(Associate Professor Ekarin Saifah, Ph.D.)

……..Thesis Advisor

(Assistant Professor Rutt Suttisri, Ph.D.)

……Member

(Associate Professor Rapepol Bavovada, Ph.D.)

……Member

(Associate Professor Chaiyo Chaichantipyuth, M.Sc. in Pharm.)

……..Member

(Witchuda Thanakijcharoenpath, Ph.D.)

อัญชิษฐา วิยาภรณ์ : การศึกษาทางพฤกษเคมีของใบและเปลือกต้นตีนเป็ดฝรั่ง (PHYTOCHEMICAL STUDY OF LEAVES AND BARK OF *CRESCENTIA ALATA* HBK.) อ. ที่ปรึกษา : ผศ. ดร. รุทธ์ สุทธิศรี, 144 หน้า. ISBN 974-17-9798-2

การศึกษาทางพฤกษเคมีของใบและเปลือกต้นตีนเป็ดฝรั่ง (*Crescentia alata* HBK.) วงศ์ Bignoniaceae ด้วยเทคนิคโครมาโทกราฟี สามารถแยกองค์ประกอบทางเคมีจากสิ่งสกัดได้สารบริสุทธิ์ จากเปลือกต้น 3 กลุ่ม เป็นสารในกลุ่มเฟนิลโพรพานอยด์ 1 ชนิด คือ 4-hydroxy-3-methoxybenzoic acid (vanillic acid) สารกลุ่มไตรเทอร์ปีนอยด์ 1 ชนิด คือ lupeol สารกลุ่มฟลาโวนอลไกลโคไซด์ 1 ชนิด คือ quercetin-3-rutinoside (rutin) และสารจากใบ 1 ชนิด คือ สารในกลุ่มสเตอรอล คือ 24-ethyl-5,22-cholestadien-3-β-ol (stigmasterol) การพิสูจน์เอกลักษณ์ของสารเหล่านี้ ทำโดยการวิเคราะห์ข้อมูล UV, IR, MS, ^{1}H-NMR และ ^{13}C-NMR โดยเฉพาะอย่างยิ่ง 1D-NMR และ 2D-NMR ร่วมกับการเปรียบเทียบข้อมูลที่ได้มีรายงานไว้แล้ว เมื่อทำการทดสอบเบื้องต้นหาฤทธิ์ต้านออกซิเดชั่น ของ สิ่งสกัดหยาบ และสารบริสุทธิ์จากพืชชนิดนี้ โดยใช้ DPPH พบว่า มีฤทธิ์เทียบได้กับวิตามินอี

ภาควิชา.....เภสัชพฤกษศาสตร์..... ลายมือชื่อนิสิต.....
สาขาวิชา...เภสัชพฤกษศาสตร์.....ลายมือชื่ออาจารย์ที่ปรึกษา.....
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AUNCHITA WIYAPORN : PHYTOCHEMICAL STUDY OF LEAVES AND BARK OF *CRESCENTIA ALATA* HBK. THESIS ADVISOR : ASSISTANT PROFESSOR RUTT SUTTISRI, Ph.D. 144 pp. ISBN 974-17-9798-2

Phytochemical study of the leaves and bark of *Crescentia alata* HBK. (family Bignoniaceae) using chromatographic techniques yielded three compounds isolated from the bark: a phenylpropanoid, 4-hydroxy-3-methoxybenzoic acid (vanillic acid); a triterpenoid, lupeol; and a flavonol glycoside, quercetin-3-rutinoside (rutin), and one compound from the leaves: a sterol, 24-ethyl-5,22-cholestadien-3-β-ol (stigmasterol). The structure elucidation of these compounds were accomplished through the analysis of their UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR, especially the 1D-NMR and 2D-NMR, data, together with comparison with reported values. Screening test of crude extracts and pure compounds from this plant for antioxidant activity using DPPH indicated their activity to be comparable with vitamin E.

Department…….Pharmaceutical Botany……Student's signature……
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LIST OF SCHEMES

LIST OF ABBREVIATIONS

A	= Absorbance
br	= broad (for NMR spectra)
br d	= broad doublet (for NMR spectra)
br s	= broad singlet (for NMR spectra)
°C	= Degree Celsius
CC	= Conventional Column Chromatography
$CDCl_3$	= Deuterated chloroform
$CHCl_3$	= Chloroform
cm	= Centimeter
^{13}C-NMR	= Carbon-13 nuclear magnetic resonance
COSY	= Correlation spectroscopy
1D	= One dimensional
2D	= Two dimensional
d	= doublet (for NMR spectra)
dd	= doublet of doublets (for NMR spectra)
DEPT	= Distortionless Enhancement by Polarization Transfer
DMSO-d_6	= deuterated dimethylsulfoxide
DPPH	= α,α-diphenyl-β-picrylhydrazyl-hydrate
δ	= Chemical shift
ε	= Molar absorptivity
EIMS	= Electron Impact Mass Spectrum

$Fecl_3$ = ferric chloride

g = Gram

gal = galactose

glc = glucose

glcA = glucuronic acid

^{1}H-NMR = Proton nuclear magnetic resonance

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

HETCOR = Heteronuclear Chemical Shift Correlation

Hz = Hertz

IR = Infrared

J = *Coupling Constant*

KBr = Potassium Bromide

Kg = Kilogram

L = Liter

λ_{max} = Wavelength at maximal absorption

M^+ = Molecular ion

m = multiplet (for NMR spectra)

MeOH = Methanol

mg = Milligram

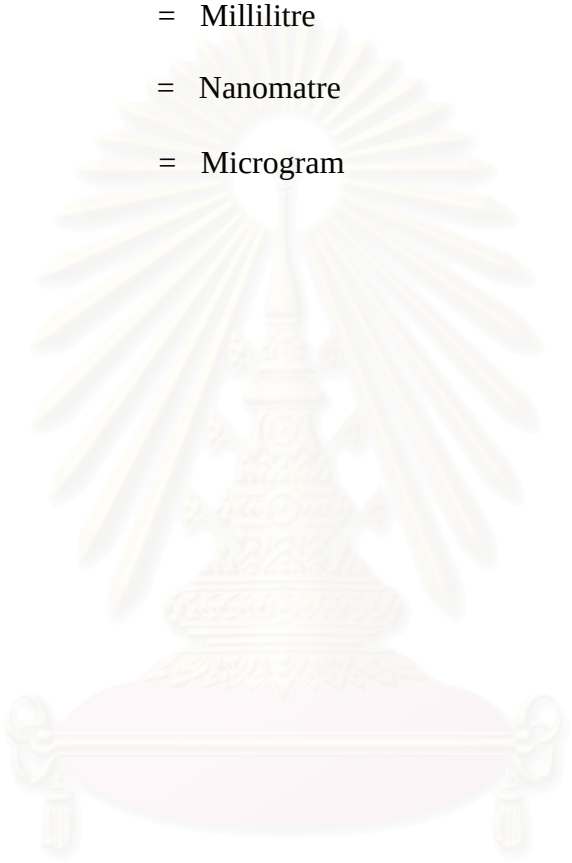
MHz = Megahertz

Mm = Millimole

m.p. = Melting points

MIC = Minimum Inhibitory Concentration

m/z	=	mass-to-charge ratio
ppm	=	part per million
rha	=	rhamnose
UV/ VIS	=	Ultraviolet / visible
TLC	=	Thin-layer Chromatography
ml	=	Millilitre
nm	=	Nanomatre
μg	=	Microgram



CHAPTER I

INTRODUCTION

The genus *Crescentia* belongs to the family Bignoniaceae. This genus is native to tropical regions such as Central America and Africa. In Thailand, two species of *Crescentia*, *C. alata* and *C. cujete*, are cultivated as ornamental plants (Santisuk, 1987).

The characteristics of plants in the genus *Crescentia* are as follows. They are usually small trees (3-5 meter high) or vine-like shrubs. Their leaves are simple or 3-foliolate, born on thick twigs in alternate fascicles resulting from the condensation of short shoots. The inflorescence consists of 1 or 2 cauliflorous flowers arising from nodes on the trunk and older branches. The calyx is bilabiately splitted to base, whereas the corolla is campanulate with transverse fold in the throat and curved forward. The corolla lobes are 5, recurved. Their tips are broad and acute. The number of the stamens is 4. The ovary is 1-celled. The fruit is either a large, spherical, indehiscent pepo or a calabash with a hard woody shell. The inside is pulpy with small seeds (Backer and Van den Brink, 1965).

According to Index Kewensis (Jackson, 1895; 1927; 1942), seven species of *Crescentia* have been recorded, as follows:

1. *Crescentia alata* HBK.

Synonyms : *C. aculeata* Sesse., *C. edulis* Desv., *C. musaecarp* Zaldivar., *C. trifolia* Blanco., *Permentiera alata* (Kunth) Miers.

Thai name : teenpet farang

Common names : calabash, gourd tree, cirian (Mexico), morrito (Mexico)

2. *Crescentia amazonica* Ducke.

Common names : cayadi, tapara, taparito, totumo

3. *Crescentia cujete* Linn.

Synonyms : *C. acuminata* HBK., *C. angustifolia* Wild., *C. arborea* Raf., *C. cucurbitifera* Houtt., *C. cucurbitina* Linn., *C. cuneifolia* Gard., *C. fasciculata* Miers., *C. latifolia* Mill., *C. obovata* Benth., *C. ovata* Burm., *C. plectantha* Miers., *C. spatulata* Miers.

Thai names : namtao yipun, namtao ton

Common name : calabash tree

4. *Crescentia elongata* Miers.

5. *Crescentia linearifolia* Miers.

Common name : higuerito

6. *Crescentia mirabilis* EK.

7. *Crescentia portoricensis* Britt.

Synonyms : *C. microcarpa* Bello.

Common name : higuero de sierra (Puerto Rico)

In Thailand, *Crescentia alata* HBK. is an exotic plant known as "teenpet farang". It is popular as ornamental tree and can be found distributed throughout the country. The plant is native to Central America (from Mexico to Costa Rica). It is a crooked tree, 4-10 meter high. Its leaves are 3-foliolate, with winged petiole, fasciculate on old branches and stem. The sessile leaflets are lanceolate, narrowly obovate or spathulate, 1-4 by 0.4–1.2 cm. The winged petiole is 2.5-10.5 by 0.3-1.4 cm. The flowers are single or in pairs, with a rancid odour. The calyx is bi-lobed nearly to the base. The corolla is thick, fleshy, 4-6 cm in length. Its color is yellowish green on the upside and deep purple to brownish near the base. Purple venation can be observed on the corolla lobes. The fruit are spherical, 5-10 cm in diameter, indehiscent, with hard shell and fleshy pulp (Santisuk, 1987).

In Mexico, the plant is used traditionally to treat respiratory infections. Its fruit extract was demonstrated as having strong antimicrobial activity (Rojas *et al*., 2001). A decoction of the leaves or the pulp is prescribed for diseases of the kidney, diarrhoea, to promote hair growth and prevent its falling (Quisumbing, 1951). In Guatemala, the plant is used in folk medicine as an anti-inflammatory remedy. Its leaves have been used for the treatment of pathological processes such as ulcers, boils, skin lesions, rheumatism and as a febrifuge (Autore, Rastrelli and Navarro , 2001). In the Philippines, decoction of the leaves of *C. alata* is used as astringent and antihemorrhagic. It is also frequently used in the treatment of spitting of blood and dysentery (Perry, 1980).

No traditional medical use of this plant in Thailand has been recorded, probably because it is grown chiefly for ornamental purpose.

Although Bignoniaceae is a relatively large family, only a limited number of its species have been studied chemically. Plants in this family were found to contain a wide range of chemical constituents, such as triterpenoids, alkaloids, saponins, steroids, lignans, phenyl propanoids, flavonoids, naphthoquinones, and miscellaneous substances. The aim of this study is to study the chemical constituents of the leaves and bark of *Crescentia alata*. The result from this study would contribute to the knowledge on chemical profile of this plant and, hence, provide useful information on both chemotaxonomic and phytochemical aspects of a member of the family Bignoniaceae.

Figure 1 *Crescentia Alata* HBK

CHAPTER II

HISTORICAL

1. Chemical Constituents of Plants in the Family Bignoniaceae

The family Bignoniaceae of the order Lamiales (Dahlgren, 1989) consists of about 120 genera and 650 species which are mostly tropical and subtropical, with a few genera found in warm temperate regions. It is a family of trees, shrubs, or lianas. Twelve genera and twenty-three species of this family can be found in Thailand (Santisuk, 1987).

Many bignoniaceous species have been reported to have medicinal properties, especially in South America where several species are widely used as folk medicines (Jamenez *et al*.,1987). So far, major types of phytochemicals which have been linked to traditional uses of plants in this family, such as treatment of tumours and antiseptic effects, are napthoquinones and iridoids. The antitumour activity of Bignoniaceae is probably due mainly to its napthoquinones, of which an example is lapachol, a compound considered as candidate for clinical use. *Kigelia pinnata*, a bignoniaceous plant reputed to be antimicrobial, has been shown to contain iridoids possessing this activity (Rasadah and Houghton, 1998).

Two types of the chemical constituents of the family Bignoniaceae are reviewed here, namely the flavonoids and the triterpenoids.

1.1 Flavonoids of the Family Bignoniaceae

The flavonoids are a large group of natural products which are widespread in higher plants and can also be found in some lower plants, including algae. The anthocyanidins are responsible for flower colour in the majority of angiosperms, but colourless flavonoids are also widespread and abundant. A variety of biological functions is fulfilled by various members of the series, but many metabolic and extracellular roles doubtless remain to be discovered.

Flavonoids occur in a variety of structure forms. All contain fifteen carbon atoms in their basic nucleus and these are arranged in a C_6–C_3-C_6 configuration, that is, two aromatic rings linked by a three carbon unit which may or may not form a third ring. For convenience the rings are labeled A, B and C and the individual carbon atoms are referred to by a numbering system which utilizes ordinary numerals for the A- and C-rings and "primed" numerals for the B-ring (Harborne and Mabry, 1982; Markham, 1982), as shown in **Figure 2**.

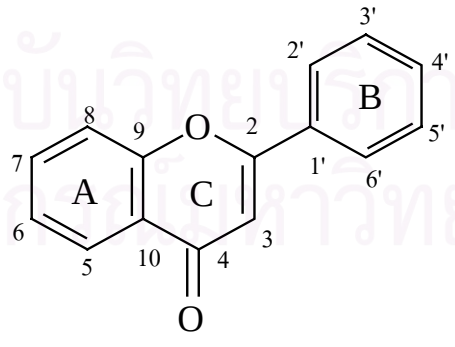


Figure 2 Basic structure of flavonoids

Flavonoids fall into two major categories according to whether the central heterocyclic ring is unsaturated or not. When unsaturation is present, as in anthocyanins, flavones and flavonols, the molecule is planar (occasionally distorted, e.g. by the substitution of the 2′-hydroxyl group in a 3-O-methyl flavonol). Saturated flavonoids (flavanones, flavans) have one or more chiral centers and can thus exist in more than one optically active forms. Optical activity may also present in flavonoids due to the presence of glycosidic substituents.

The flavonoids are all related by common biosynthetic pathway which incorporates precursors from both the “shikimate” and “acetate-malonate” pathways (Hahlbrock and Grisebach, 1975).

The biological properties of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds. One of the undisputed functions of flavonoids is their role in protecting plants against microbial invasion, including their accumulation as phytoalexins in response to microbial attack . There is an ever increasing interest in plant flavonoids for treating human diseases as antifungal, antibacterial, and antiviral (Harborne and Williams, 2000). Several recent research articles have reported the regular presence of antibacterial activity among flavonoids. For example, 5,7-dihydroxy-3,8-dimethoxyflavone has a minimum growth inhibitory concentration (MIC) of 50 μg/ml towards *Staphylococcus edidermis* (Iniesta-Sanmartin *et al*., 1990).

One very interesting property of flavonoids that has been discovered recently has been the antiviral activity, most notably against the human immunodeficiency virus (HIV), the causative agent of AIDS. Some flavonoids appear to have direct inhibitory activity on the virus. This is apparently true of baicalin (5,6,7-trihydroxyflavone-7-glucuronide) from *Scutellaria baicalensis* (Li *et al*., 1997). It is not yet clear what range of flavonoids have anti-HIV activity.

However, a study of the inhibition of tomato ringspot virus by flavonoids revealed that a range of common flavonols and an aurone were all strongly active. Quercetin and other flavonoids appear to interfere with an early event in virus life cycle (Malhora *et al*., 1996).

Several flavonoids have their role in antioxidant properties of the plant extracts, of which an example is rutin, a flavonol glycoside that showed antioxidant activity in an assay system measuring thiobarbituric acid production (Yoshikawa *et al*., 1993). In a different assay, the flavonoids quercetin, kaempferol, catechin and taxifolin were shown to suppress the cytotoxicity of oxygen radical (O_2^-) and hydrogen peroxide (H_2O_2) on Chinese hamster V79 cells in a protective manner, that is, by preventing the decrease in the number of colonies at concentrations at which the compounds themselves were not toxic (Nakayama *et al*., 1993).

Flavonoids may act in a number of different ways on various components of blood such as platelets, monocytes, low density lipoprotein (LDL) and smooth muscles. Platelets are key participants in atherogenesis and pro-inflammatory mediators such as thromboxane A_2, platelet-activating factor (PAF) and serotonin are produced from them. Flavonoids may inhibit platelet adhesion, aggregation and secretion (Beretz and Cazenave, 1988). For example, quercetin, kaempferol derivatives and apigenin have been demonstrated to inhibit the aggregation of rabbit platelets caused by various inducers (Harborne and Williams, 2000). Moreover, flavonoids are reported to affect the inflammatory process of the mammalian system and possess anti-inflammatory activity both *in vitro* and *in vivo* (Autore *et al*., 2001). For example, carajurin from *Arrabidae chica* showed anti-inflammatory activity through inhibition of Nuclear Factor-κB (NF-κB), a central mediator of the human immune response that regulates the transcription of genes encoding various inflammatory cytokines, chemokines, adhesion molecules and inflammatory enzymes such as nitric oxide synthase

(iNOS), cyclooxygenase (COX-2), 5-lipoxygenase (5-LOX) and cytosolic phospholipase A2 (Bettina *et al*., 2001). The flavanone hesperitin also has been shown to reduce carrageenin-induced paw edema in rats (Emim, Oliveira and Lapa, 1994). Other simple flavonoids which have been shown to exhibit useful anti-inflammatory activity include apigenin and quercetin. Apigenin showed significant inhibition of fibroblast growth in the concentration range of 0.01 to 100 mg/ml (Koganov, Dues, and Tsorin, 1999).

Although the flavonoids can be found abundantly in the plants of the family Bignoniaceae (they have been isolated from all parts of bignoniaceous plants i.e. from the fruits, seeds, root, bark, heartwood and leaves), the family is rather understudied from the flavonoids' point of view. A limited number of this compound type have been reported as constituents of plants in this family and there appears to be a large predominance of flavones glycosides over its aglycone, anthocyanins, flavonols, flavonol glycosides and flavanone glycosides.

The flavonoids of the family Bignoniaceae are summarized in **Table 1.**

Table 1 Distribution of flavonoids in the family Bignoniacea

Compounds	Sources	References
1. Anthocyanins		
Carajurin (**1**)	*Arrabidaea chica*	Bettina *et al.*, 2001
	Bignonia chica	Chapman, Perkin and Robinsor, 1927
Carajurone (**2**)	*Arrabidaea chica*	Bettina *et al.*, 2001
	Bignonia chica	Chapman *et al* ., 1927
Cyanidin-3,5-diglucoside (**3**)	*Clytostoma callistegioides*	Scogin, 1985
	Jacaranda acutifolia	Harborne, 1967
	Mansoa verrucifera	Scogin, 1985
Cyanidin-3-glucoside (**4**)	*Arrabidaea brachypoda*	Scogin, 1985
	Arrabidaea chica	Scogin, 1985
	Incarvillea olgae	Harborne, 1967
	Jacaranda acutifolia	Harborne, 1967
	Macfadyena unguis-cati	Duarte *et al.*, 2000
	Paragonia pyramidata	Scogin, 1985
Cyanidin-3-(*p* -coumaroyl)-5-glucoside (**5**)	*Clytostoma callistegioides*	Scogin, 1985
	Mansoa verrucifera	Scogin, 1985

Table 1 Distribution of flavonoids in the family Bignoniaceae (continuec

Compounds	Sources	References
Cyanidin-3-rutinoside (**6**)	*Arrabidaea brachypoda*	Scogin, 1985
	Arrabidaea chica	Scogin, 1985
	Campsis radicans	Harborne, 1967
	Catalpa bignonoides	Harborne, 1967
	Crescentia alata	Scogin, 1980
	Eccremocarpus scaber	Harborne, 1967
	Incarvillea delavayi	Scogin, 1985
	Incarvillea marei	Harborne, 1967
	Incarvillea olgae	Scogin, 1985
	Mansoa verrucifera	Scogin, 1985
	Paragonia pyramidata	Scogin, 1985
	Podranea ricasoliana	Scogin, 1985
	Spathodea campanulata	Scogin, 1980
	Tecoma garrocha	Harborne, 1967

Table 1 Distribution of flavonoids in the family Bignoniaceae (continuec

Compounds	Sources	References
Peonidin-3-cinnamoylsophoroside (**7**)	*Tabebuia impetiginosa*	Pamilio, 1973
Peonidin-3-rutinoside (**8**)	*Tabebuia impetiginosa*	Pamilio, 1973
6,7,3',4'- Tetrahydroxy-5-methoxy-flavylium (**9**)	*Arrabidaea chica*	Bettina *et al.,* 2001
6,7,3'-Trihydroxy-5,4'-dimethoxy-flavylium (**10**)	*Arrabidaea chica*	Bettina *et al.,* 2001
2. Flavones		
Acacetin (**11**)	*Arrabidaea chica*	Bettina *et al.,* 2001
	Clytostoma callestigioides	Harborne, 1967
Apigenin (**12**)	*Arrabidaea brachypoda*	Blatt, Santos and Salatino, 1998
	Chilopsis saligna	Harborne, 1967
	Oroxylum indicum	Tomimori *et al.,* 1988
Baicalein (**13**)	*Oroxylum indicum*	Harborne, 1967
Carajuflavone (**14**)	*Arrabidaea chica f. cuprea*	Takemura *et al.,* 1995
Chrysin (**15**)	*Oroxylym indium*	Subramanian and Nair, 1972
Cirsilineol (**16**)	*Arrabidaea brachypoda*	Alcerito *et al.,* 2002
	Tecomella undulata	Azam and Ghanim, 2000

Table 1 Distribution of flavonoids in the family Bignoniaceae (continuec

Compounds	Sources	References
Cirsiliol (**17**)	*Arrabidaea brachypoda*	Alcerito *et al.,* 2002
Cirsimaritin (**18**)	*Arrabidaea brachypoda*	Alcerito *et al.,* 2002
	Arrabidaea brachypoda	Hase *et al.,* 1995
	Tecomella undulata	Azam and Ghanim, 2000
5,6-Dihydroxy-7-methoxyflavone (**19**)	*Oroxylum indicum*	Tomimori *et al.,* 1988
6,7-Dihydroxy-3-methoxyflavone (**20**)	*Oroxylum indicum*	Grampurohit, Baichwan and Jolly, 1994
3',4'-Dihydroxy-5,6,7-trimethoxyflavone (**21**)	*Arrabidaea brachypoda*	Alcerito *et al.,* 2002
7,4'-Dimethoxyflavone (**22**)	*Catalpa bignonoides*	Kutney and Hanssen, 1971
Hispidulin (**23**)	*Arrabidaea brachypoda*	Alcerito *et al.,* 2002
	Millingtonia hortensis	Hase *et al.,* 1995
	Oroxylum indicum	Tomimori *et al.,* 1988
	Stereospermum xylocarpum	Subramanian, Nagarajan and Sulochana, 1972
Hortensin (24)	*Millingtonia hortensis*	Chulasiri, Bunyapraphatsara and Moongkarnd, 1992
5-Hydroxy-6,7-dimethoxyflavone (**25**)	*Oroxylum indicum*	Tomimori *et al.,* 1988

Table 1 Distribution of flavonoids in the family Bignoniaceae (continued)

Compounds	Sources	References
6-Hydroxyluteolin (**26**)	*Catalpa bignonioides*	Harborne, 1967
	Catalpa bungei	Harborne, 1967
	Catalpa speciosa	Harborne, 1967
	Kigelia africana	El-Sayyad, 1981
	Tecoma australis	Harborne, 1967
	Tabebuia caraiba	Harborne, 1967
5-Hydroxy-6,7,4'-trimethoxyflavone (**27**)	*Phyllarthron madagascariensis*	Tillequin, Henri and Paris, 1977
Ladanein (**28**)	*Catalpa bignonoides*	Okuda, Yoshida and Ono, 1975
	Catalpa ovata	Savone, 1984
Luteolin (**29**)	*Arrabidaea brachypoda*	Blatt *et al* ., 1998
	Campsis radicans	Harborne, 1967
	Catalpa bignonioides	Harborne, 1967
	Catalpa bungei	Harborne, 1967
	Catalpa speciosa	Harborne, 1967
	Kigelia africana	El-Sayyad, 1981
	Tecoma australis	Harborne, 1967
	Tecoma garrocha	Harborne, 1967

Table 1 Distribution of flavonoids in the family Bignoniaceae (continued)

Compounds	Sources	References
6-Methyl etherflavone (**30**)	*Oroxylum indicum*	Harborne, 1967
Oroxylin A (**31**)	*Oroxylum indicum*	Tomimori *et al.,* 1988
Pectolinarigenin (**32**)	*Millingtonia hortensis*	Hase *et al.,* 1995
Salvigenin (**33**)	*Phyllarthorn madagascariensis*	Hase *et al.,* 1995
Scutellarein (**34**)	*Millingtonia hortensis*	Subramanian, Nagaraja and Nair, 1971
	Oroxylum indicum	Subramanian and Nair, 1972
5,6,7,8-Tetramethoxyflavone (**35**)	*Zeyhera tuberculosa*	Kutney and Hanssen, 1971
Thevetiaflavone (**36**)	*Arrabidaea chica f. cuprea*	Takemura *et al.,* 1995
5,6,7-Trihydroxyflavone (**37**)	*Oroxylum indicum*	Harborne, 1967
5,6,7-Trimethoxyflavone (**38**)	*Zeyhera tuberculosa*	Kutney and Hassen, 1971
3. Flavone glycosides		
Apigenin-7-O-β–glucuronide (**39**)	*Fernandoa adenophylla*	Hase *et al.,* 1995
	Millingtonia hortensis	Hase *et al.,* 1995
Baicalein-7-O-β-gentibioside (**40**)	*Oroxylum indicum*	Tomimori *et al.,* 1988
Baicalein –6-glucoside (**41**)	*Oroxylum indicum*	Subramanian and Nair, 1972

Table 1 Distribution of flavonoids in the family Bignoniaceae (continued)

Compounds	Sources	References
Baicalein–7-glucoside (**42**)	*Oroxylum indicum*	Subramanian and Nair, 1972
Baicalein–6-O-β-D-glucuronoside (**43**)	*Oroxylum indicum*	Subramanian and Nair, 1972
Baicalin (**44**)	*Oroxylum indicum*	Tomimori *et al.*, 1988
Bignonoside (**45**)	*Catalpa bignonioides*	Markham and Chari, 1982
Chrysin-7-O-β-gentibioside (**46**)	*Oroxylum indicum*	Tomimori *et al.*, 1988
Chrysin-7-O-β-D-glucoside (**47**)	*Oroxylum indicum*	Tomimori *et al.*, 1988
Chrysin–7-O-β-D-glucuronide (**48**)	*Oroxylum indicum*	Tomimori *et al.*, 1988
Chrysin–7-O-rutinoside (**49**)	*Dolichandrone falcata*	Subramanian *et al* ., 1972
	Dolichandrone platycalyx	Subramanian *et al* ., 1972
5,6-Dihydroxy-7,4'-dimethoxyflavone-6-O-glucoside (**50**)	*Catalpa ovata*	Okuda, Yoshida and Ono, 1975
5,6-Dihydroxy-7,4'-dimethoxyflavone-6-O-sophoroside (**51**)	*Catalpa ovata*	Okuda, Yoshida and Ono, 1975
5,7-Dihydroxy-6-methoxyflavone-7-O-α–L-rhamno-pyranoside (**52**)	*Tecomella undulata*	Prakash and Rao, 1999
Diosmetin-7-glucuronide (**53**)	*Stereospermum chelonoides*	Subramanian *et al* ., 1972

Table 1 Distribution of flavonoids in the family Bignoniaceae (continued)

Compounds	Sources	References
Hispidulin-7-O-β-glucoside (**54**)	*Fernandoa adenophylla*	Hase *et al.*, 1995
	Millingtonia hortensis	Hase *et al.*, 1995
Hispidulin-7-O-β-glucuronide (**55**)	*Fernandoa adenophylla*	Subramanian and Nair, 1972
	Stereospermum chelonoides	Subramanian *et al* ., 1972
Hispidulin-7-O-glucuronide-methyl ester (**56**)	*Millingtonia hortensis*	Hase *et al.*, 1995
Hispidulin-7-O–β–rutinoside (**57**)	*Millingtonia hortensis*	Mangayarkarasi and Nagarajan, 1984
6-Hydroxyluteolin-7-O-β-D-galactopyranoside (**58**)	*Stereospermum suaveolens*	Harborne, 1967
6-Hydroxyluteolin-7-O-β-D-glucopyranoside (**59**)	*Catalpa bignonioides*	Harborne, 1967
	Kigelia africana	El-Sayyad, 1981
Ladanein-6-O-β-D-glucopyranoside (**60**)	*Catalpa ovata*	Okuda *et al* ., 1975
Ladanein-6-O-sophoroside (**61**)	*Catalpa ovata*	Okuda *et al* ., 1975
Luteolin-7-O-glucoside (**62**)	*Campsis radicans*	Harborne, 1967
	Catalpa bignonioides	Harborne, 1967
	Kigelia africana	El-Sayyad, 1981
	Tabebuia caraiba	Blatt, Salatino and Salatino, 1996
	Tecomella undulata	Azam and Ghanim, 2000

Table 1 Distribution of flavonoids in the family Bignoniaceae (continued)

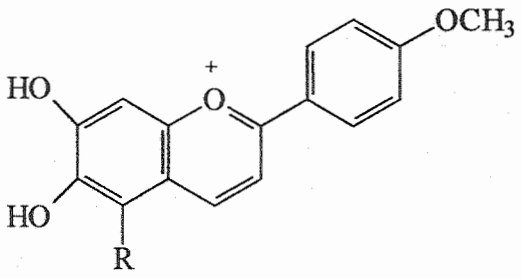
Compounds	Sources	References
Scutellarein-5-galactoside (**63**)	*Millingtonia hortensis*	Sharma, Zaman and Kidwai, 1968
Scutellarein–5-O-β-D-glucuronide (**64**)	*Millingtonia hortensis*	Kar, Satyawan and Khanna, 1976
Scutellarein-7-O-glucuronide (**65**)	*Jacaranda mimosaefolia*	Subramanian *et al* ., 1972
	Millingtonia hortensis	Subramanian, Nagarajan and Sulochana, 1971
	Oroxylum indicum	Subramanian and Nair, 1972
Scutellarein-7-O-rutinoside (**66**)	*Oroxylum indicum*	Markham and Chari, 1982
Stereolensin (**67**)	*Stereospermum suaveolens*	Harborne, 1967
7,2',3',4'-Tetrahydroxyflavone-3-O-neohesperidoside (**68**)	*Jacaranda acutifolia*	Ferguson and Lien, 1982
Tetuin (**69**)	*Oroxylum indicum*	Subramanian *et al* ., 1972
Vicenin-2 (**70**)	*Macfadyena unguis-cati*	Duarte *et al.*, 2000
4. Flavonols		
4'-Hydroxy-6,7-dimethoxyflavonol (**71**)	*Millingtonia hortensis*	Hase *et al.*, 1995
Kaempferol (**72**)	*Crescentia alata*	Autore *et al.*, 2001
	Incarvillea mairei	Harborne, 1967
	Pajanelia longifolia	Subramanian and Nair, 1972
	Tecoma stans	Harborne, 1967

Table 1 Distribution of flavonoids in the family Bignoniaceae (continued)

Compounds	Sources	References
Quercetin (**73**)	*Cydista aequinoctialis*	Harborne, 1967
	Incarvillea mairei	Harborne, 1967
	Kigelia africana	El-Sayyad, 1981
	Macfadyena unguis cati	Duarte *et al.,* 2001
	Pajanelia longifolia	Subramanian and Nair, 1972
	Spathodea campanulata	Subramanian *et al* ., 1972
	Tecoma stans	Harborne, 1967
	Tecomella undulata	Azam and Ghanim, 2000
5. Flavonol glycosides		
Dihydrokaempferol-7-glucoside (**74**)	*Pajanelia longifolia*	Subramanian and Nair, 1972
Kaempferol-3-O-rutinoside (**75**)	*Crescentia alata*	Autore *et al.,* 2001
Kaempferol-3-sophoroside (**76**)	*Pajanelia longifolia*	Subramanian and Nair, 1972
Quercetin-3-O-diglucoside (**77**)	*Tabebuia sp.*	Subramanian *et al* ., 1972
Quercetin-3-O-galactoside (**78**)	*Tabebuia caraiba*	Blatt *et al* ., 1996

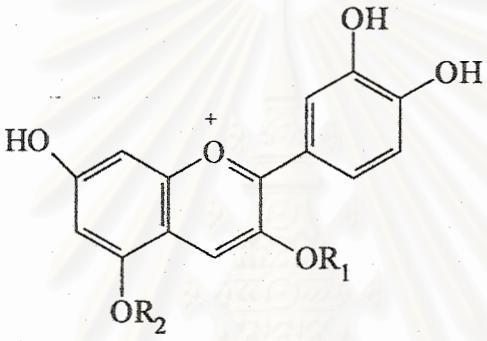
Table 1 Distribution of flavonoids in the family Bignoniaceae (continued)

Compounds	Sources	References
Quercetin-3-O-galactoside (**78**)	*Bignonia megapotanica*	Subramanian *et al* ., 1972
Quercetin-3-O-glucoside (**79**)	*Tabebuia caraiba*	Blatt *et al* ., 1996
Quercetin-3-O-α-L-rhamnoside (**80**)	*Pajanelia longifolia*	Harborne, 1967
Rutin (**81**)	*Crescentia alata*	Autore *et al.*, 2001
	Tecomella undulata	Azam and Ghanim, 2000
6. Flavanone glycosides		
Dihydrokaempferol-3-O-α-rhamnoside-5-O-β-D-glucoside (**82**)	*Tecomella grandiflora*	Harborne, 1967
Hesperidin (**83**)	*Pyrostegia venusta*	Ferreira *et al.*, 2000
Naringenin-7-O-α-L-rhamnosyl rhamnoside (**84**)	*Pyrostegia venusta*	Ferreira *et al.*, 2000



Carajurin (**1**) : R = OCH_3

Carajurone (**2**) : R = OH



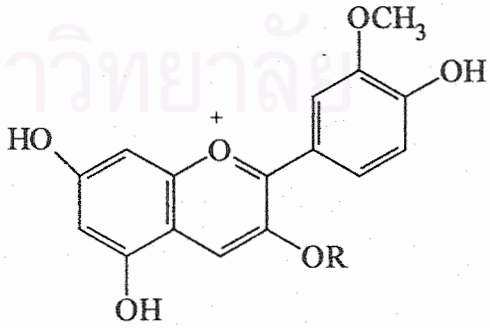
Cyanidin 3, 5-diglucoside (**3**) : R_1 = β-glc, R_2 = β-glc

Cyanidin-3-glucoside (**4**) : R_1 = β-glc, R_2 = H

Cyanidin-3-(*p*-coumaroyl)-glucoside-5-glucoside (**5**) :

R_1 = *p*-coumaroyl-glc, R_2 = glc

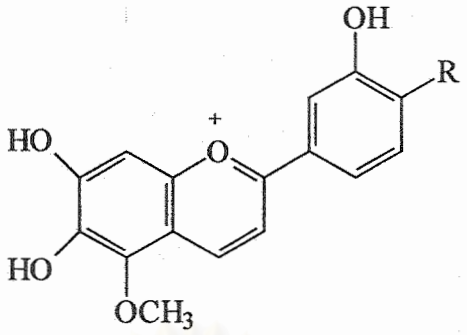
Cyanidin-3-rutinoside (**6**) : R_1 = β-glc^6-α-rha, R_2 = H



Peonidine-3-cinnamoylsophoroside (**7**) :

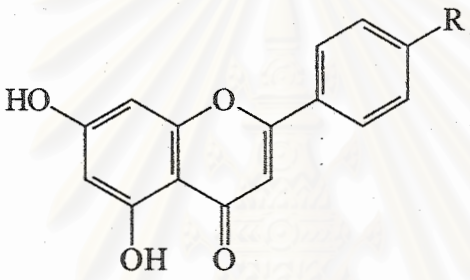
R = cinnamoyl-α-glc^2-β-glc

Peonidine-3-rutinoside (**8**) : R = β-glc^6-α-rha



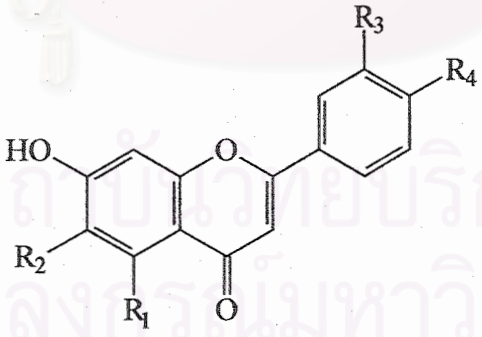
6,7,3′,4′-Tetrahydroxy-5-methoxy-flavylium (**9**) : R = OH

6,7,3′-Trihydroxy-5,4′-dimethoxy-flavylium (**10**) : R = OCH_3



Acacetin (**11**) : R = OCH_3

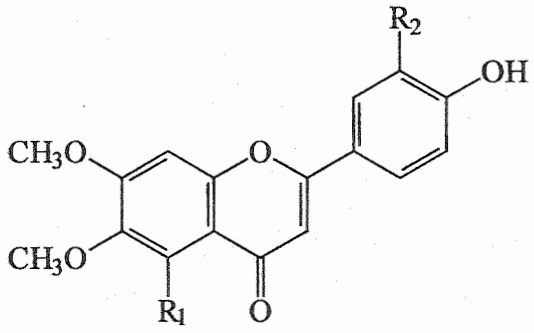
Apigenin (**12**) : R = OH



Baicalein (**13**) : R_1 = OH, R_2 = OH, R_3 = H, R_4 = H

Carajuflavone (**14**) : R_1 = OCH_3, R_2 = OH, R_3 = OH, R_4 = OH

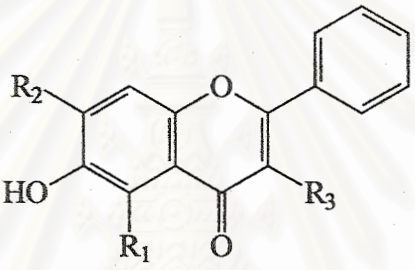
Chrysin (**15**) : R_1 = OH, R_2 = H, R_3 = H, R_4 = H



Cirsilineol (**16**) : $R_1 = OCH_3$, $R_2 = OCH_3$

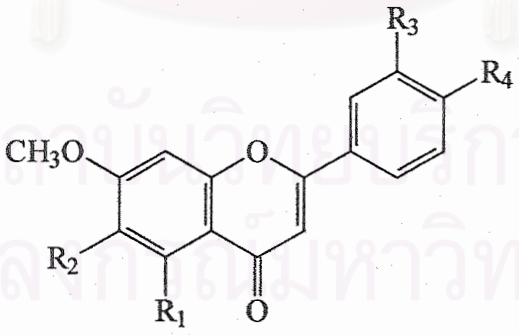
Cirsiliol (**17**) : $R_1 = OCH_3$, $R_2 = OH$

Cirsimaritin (**18**) : $R_1 = OH$, $R_2 = H$



5,6- Dihydroxy-7-methoxyflavone (**19**) : $R_1 = OH$, $R_2 = OCH_3$, $R_3 = OH$

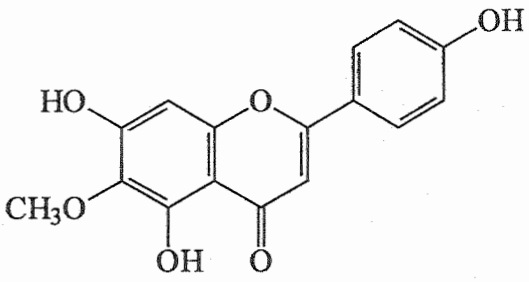
6, 7- Dihydroxy-3-methoxyflavone (**20**) : $R_1 = H$, $R_2 = OH$, $R_3 = OCH_3$



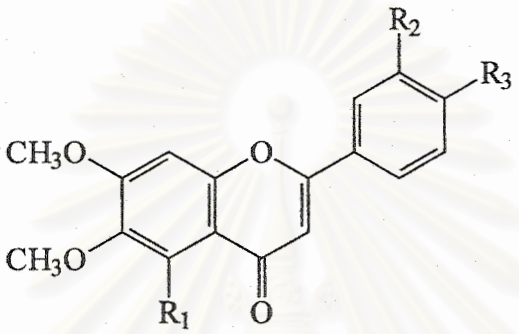
3′,4′-Dihydroxy-5,6,7-trimethoxyflavone (**21**) :

$R_1 = OCH_3$, $R_2 = OCH_3$, $R_3 = OH$, $R_4 = OH$

7, 4′- Dimethoxyflavone (**22**) : $R_1 = H$, $R_2 = H$, $R_3 = H$, $R_4 = OCH_3$

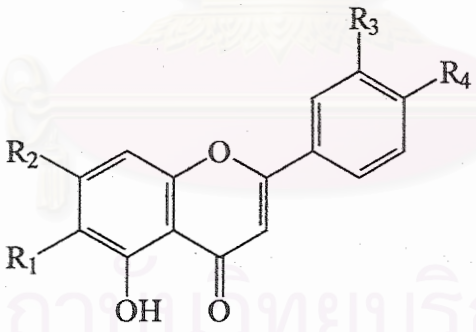


Hispidulin (**23**)



Hortensin (**24**) : $R_1 = OCH_3$, $R_2 = OH$, $R_3 = OH$

5-Hydroxy-6,7-dimethoxyflavone (**25**) : $R_1 = OH$, $R_2 = H$, $R_3 = H$



6-Hydroxyluteolin (**26**) : $R_1 = OH$, $R_2 = OH$, $R_3 = OH$, $R_4 = OH$

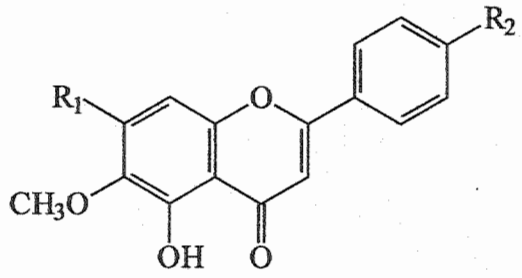
5-Hydroxy-6,7,4′- trimethoxyflavone (**27**) :

$R_1 = OCH_3$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = OCH_3$

Ladanein (**28**) : $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = OCH_3$

Luteolin (**29**) : $R_1 = H$, $R_2 = OH$, $R_3 = OH$, $R_4 = OH$

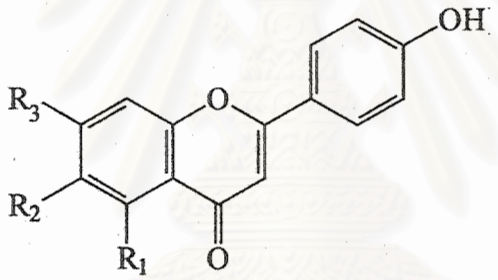
6-Methyl etherflavone (**30**) : $R_1 = OCH_3$, $R_2 = H$, $R_3 = H$, $R_4 = H$



Oroxylin A (**31**) : R_1 = OH, R_2 = H

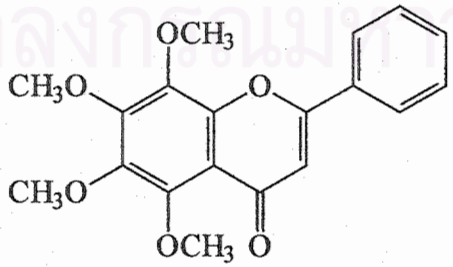
Pectolinarigenin (**32**) : R_1 = OH, R_2 = OCH_3

Salvigenin (**33**) : R_1 = OCH_3, R_2 = OCH_3

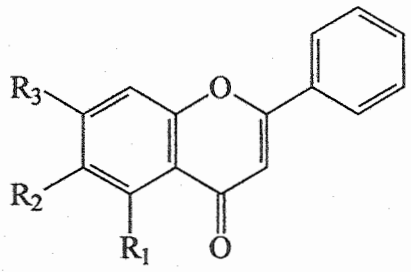


Scutellarein (**34**) : R_1 = OH, R_2 = OH, R_3 = OH

Thevetiaflavone (**36**) : R_1 = OCH_3, R_2 = H, R_3 = H

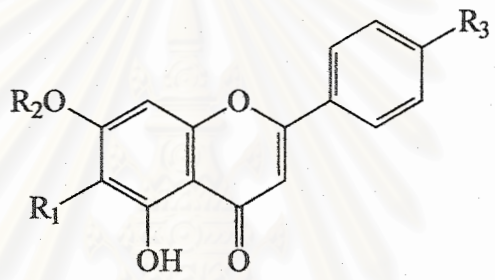


5,6,7,8-Tetramethoxyflavone (**35**)



5,6,7-Trihydroxyflavone (**37**) : R_1 = OH, R_2 = OH, R_3 = OH

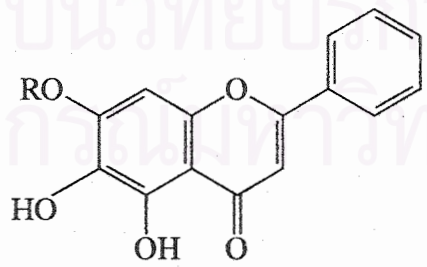
5,6,7-Trimethoxyflavone (**38**) : R_1 = OCH_3, R_2 = OCH_3 , R_3 = OCH_3



Apigenin-7-O-β-glucuronide (**39**) : R_1 = H, R_2 = β-glcA, R_3 = OH

Baicalein-6-O-glucoside (**41**) : R_1 = O-glc, R_2 = H, R_3 = H

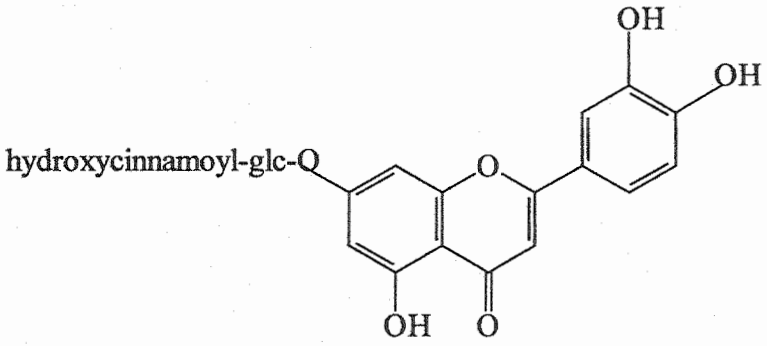
Baicalein-6-O-β-D-glucuronoside (**43**) : R_1 = O-β-glcA, R_1 = H, R_3 = H



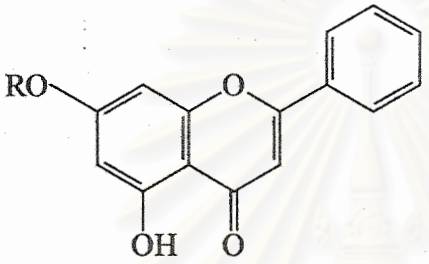
Baicalein-7-O-β-gentibioside (**40**) : R = β-glc^6-glc

Baicalein-7-glucoside (**42**) : R = glc

Baicalin (**44**) : R = β-glcA



Bignonoside (**45**)

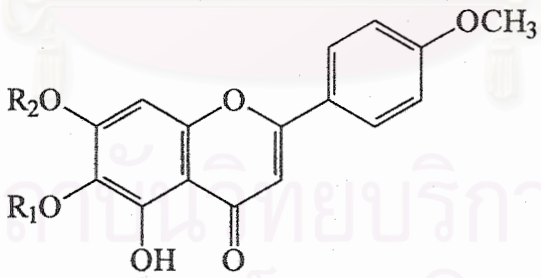


Chrysin-7-O-β-gentibioside (**46**) : R = β-glc^{6}-glc

Chrysin-7-O-β-D-glucoside (**47**) : R = β-glc

Chrysin-7-O-β-D-glucuronide (**48**) : R = β-glcA

Chrysin-7-O-rutinoside (**49**) : R = glc^{6}-rha

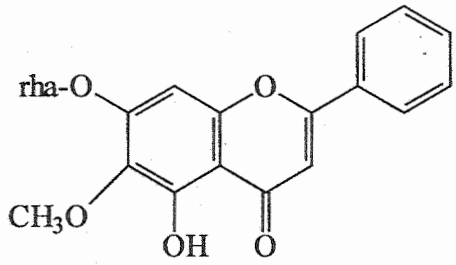


5,6-Dihydroxy-7,4′-dimethoxyflavone-6-O-glucoside (**50**) :

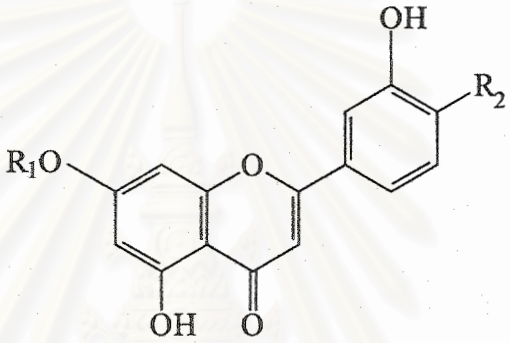
R_1 = glc, R_2 = CH_3

5,6-Dihydroxy-7,4′-dimethoxyflavone-6-O-sophoroside (**51**) :

R_1 = glc^{2}-glc, R_2 = CH_3

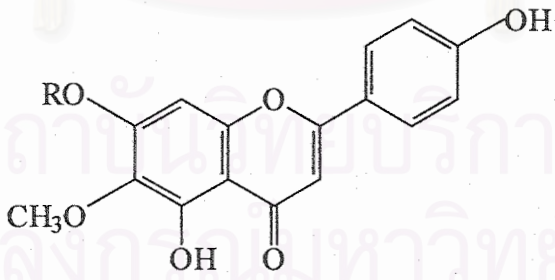


5,7-Dihydroxy-6-methoxyflavone-7-O-α-L-rhamnopyranoside (**52**)



Diosmetin-7-glucuronide (**53**) : R_1 = α-glcA , R_2 = OCH_3

Luteolin-7-O-glucoside (**62**) : R_1 = glc, R_2 = OH

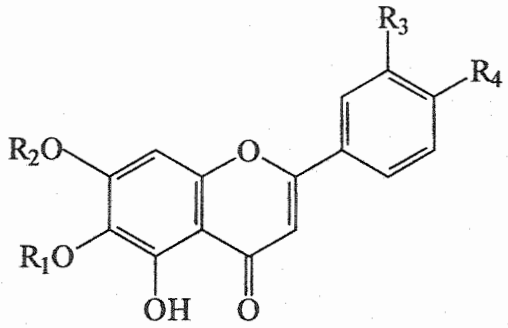


Hispidulin-7-O-β-glucoside (**54**) : R = β-glc

Hispidulin-7-O-β-glucuronide (**55**) : R = β-glcA

Hispidulin-7-O-glucuronide-methyl ester (**56**) : R = β-glcA methyl ester

Hispidulin-7-O-β-rutinoside (**57**) : R = β-glc^6-rha

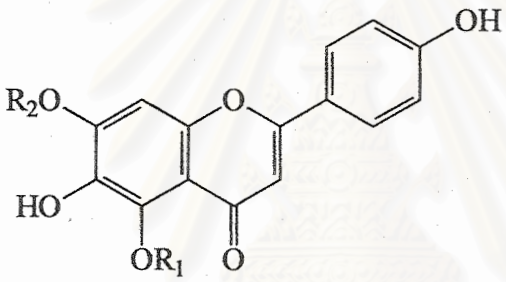


6-Hydroxyluteoin-7-O-β-D-galactopyranoside (**58**) : R_1 = H, R_2 = β-gal, R_3 = OH, R_4 = OH

6-Hydroxyluteoin-7-O-β-D-glucopyranoside (**59**) : R_1 = H, R_2 = β-glc, R_3 = OH, R_4 = OH

Ladanein-6-O-β-D-glucopyranoside (**60**) : R_1 = β-glc, R_2 = OCH_3, R_3 = H, R_4 = OCH_3

Ladanein-6-O-sophoroside (**61**) : R_1 = glc^2-glc, R_2 = OCH_3, R_3 = H, R_4 = OCH_3

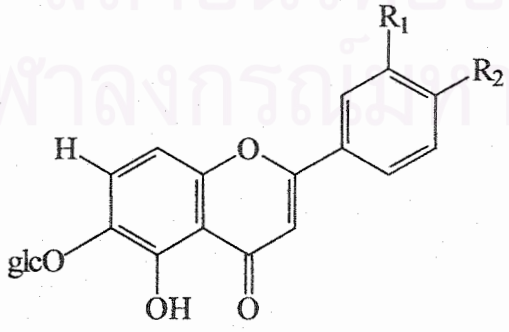


Scutellarein-5-galactoside (**63**) : R_1 = β-gal, R_2 = H

Scutellarein-5-O-β-D-glucuronide (**64**) : R_1 = β-glcA, R_2 = H

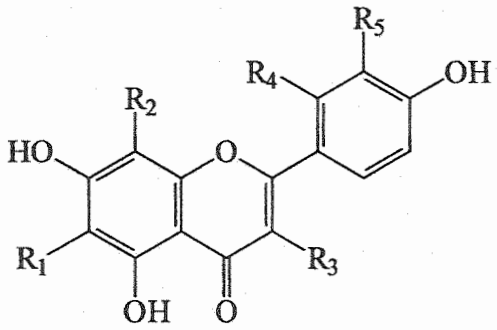
Scutellarein-7-O-glucuronide (**65**) : R_1 = H, R_2 = β-glcA

Scutellarein-7-O-rutinoside (**66**) : R_1 = H, R_2 = $\beta\text{-gal}^6\text{-rha}$



Stereolensin (**67**) : R_1 = OH, R_2 = OH

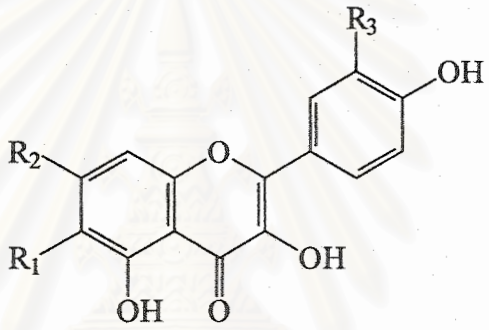
Tetuin (**69**) : R_1 = H, R_2 = H



7,2′,3′,4′-Tetrahydroxyflavone-3-O-neohesperidoside (**68**) :

R_1 = H, R_2 = H, R_3 = O-glc^2-rha, R_4 = OH, R_5 = OH

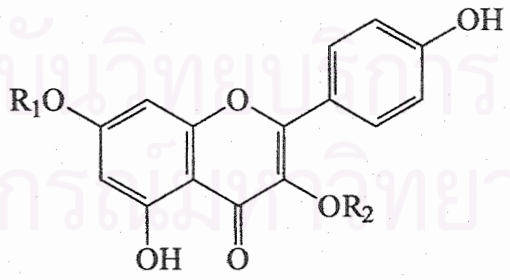
Vicenin-2 (**70**) : R_1 = O-β-glc, R_2 = O-β-glc, R_3 = H, R_4 = H, R_5 = H



Hortensin (**71**) : R_1 = OCH_3, R_2 = OCH_3 , R_3 = H

Kaempferol (**72**) : R_1 = H, R_2 = OH , R_3 = H

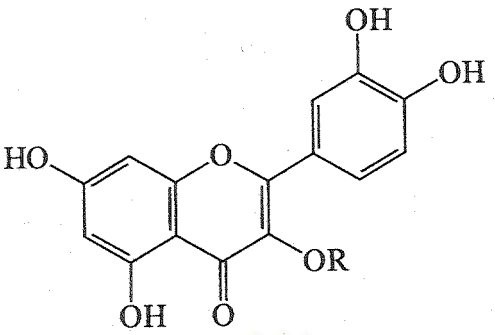
Quercetin (**73**) : R_1 = H, R_2 = OH , R_3 = OH



Dihydrokaempferol-7-glucoside (**74**) : R_1 = β-glc, R_2 = H

Kaempferol-3-O-rutinoside (**75**) : R_1 = H, R_2 = glc^6-rha

Kaempferol-3-sophoroside (**76**) : R_1 = H, R_2 = glc^2-glc

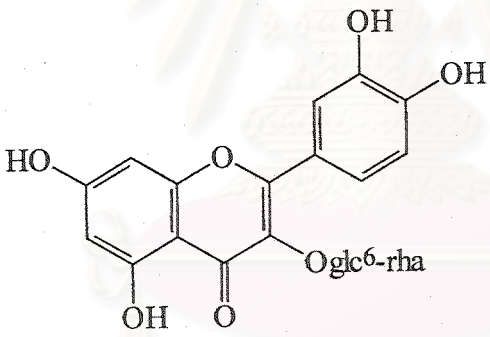


Quercetin-3-O-diglucoside (**77**) : R = glc^6-glc

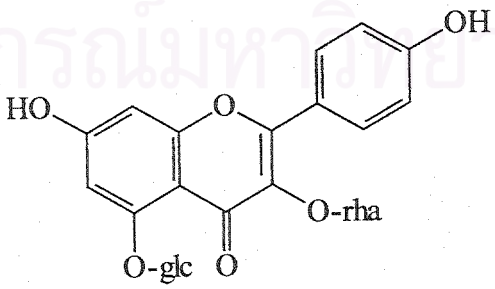
Quercetin-3-O-galactoside (**78**) : R = gal

Quercetin-3-O-glucoside (**79**) : R = β-glc

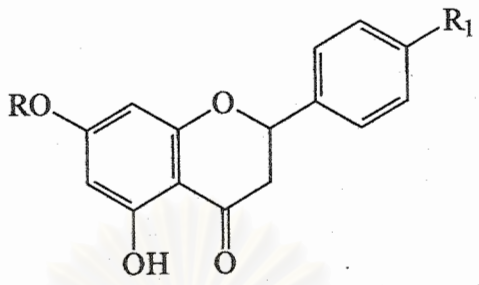
Quercetin-3-O-α-L-rhamnoside (**80**) : R = α-rha



Rutin (**81**)



Dihydrokaempferol-3-O-α-rhamnoside-5-O-β-D-glucoside (**82**)



Hesperidin (**83**) : R = β-glc^{6}-rha, R_1 = H

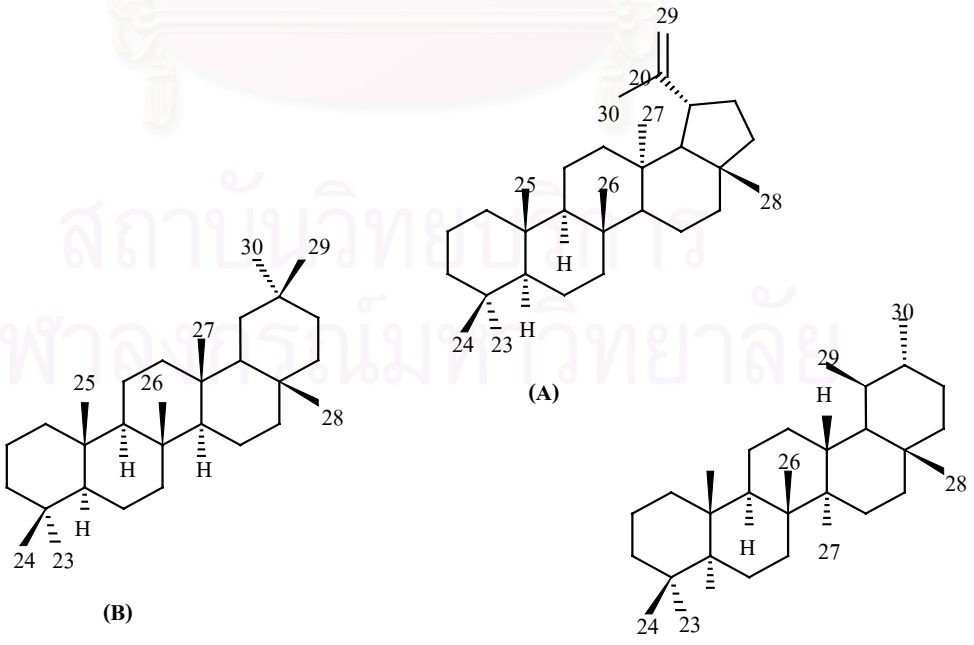
Naringenin-7-O-α-L-rhamnosyl rhamnoside (**84**) : R = α-rha^{4}-rha, R_1 = OH

1.2 Triterpenoids of the Family Bignoniaceae

Previous phytochemical studies have indicated that terpenoids are abundant metabolites found from plants of the family Bignoniaceae. The triterpenes constitute a large, diverse group of natural products derived from mevalonic acid through squalene and, in most cases, *via* 2,3-epoxy squalene.

Their various structures, of which there are about 20 skeletal types, depend on the tendency of squalene, with its six double bonds, to undergo multiple cyclizations mediated by cyclase enzymes capable of exerting rigorous stereochemical controls (Manitto, 1981).

The triterpenoids isolated so far from this plant family all have pentacyclic core belonging to lupane (A), oleanane (B) or ursane (C) skeleton. Several of these pentacyclic triterpenoids have been reported as possessing useful pharmacological activities, such as cytotoxic, antimalarial and anti-inflammatory activity.



Betulinic acid, a lupane-type triterpene, has been considered as a promising anticancer agent. The compound was also active as antibacterial (Pisha *et al*., 1995) and antitumor against P-388 lymphocytic leukemia cell line (Ogura, Cordell, and Farnsworth, 1976).

Oleanolic acid, another common triterpenoid, was also reported as having biological effects, including hypoglycemic, anti-inflammatory and hepatoprotective effects (Liu, 1995).

Ursolic acid, another common triterpenoid, was also reported as having hypoglycemic, anti-inflammatory and hepatoprotective effects. It was also reported as capable of protecting lipid peroxidation of liver microsomes induced by free radicals (Hung and Yen, 2000).

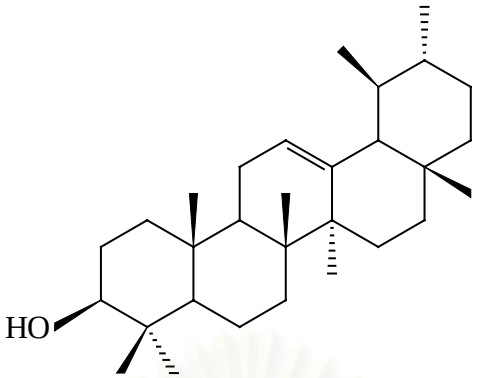
The triterpenoids of the family Bignoniaceae are summarized in **Table 2.**

Table 2 Distribution of triterpenoids in the family Bignoniaceae

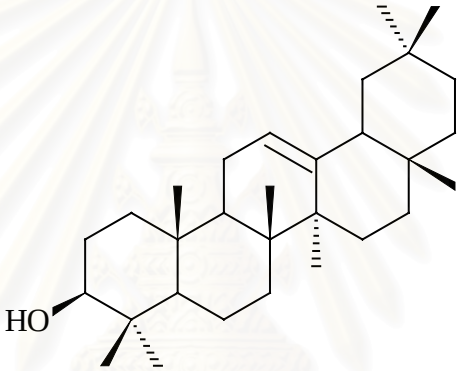
Compounds	Sources	References
α–Amyrin (**85**)	*Crescentia cujete*	Agarwal and Popli, 1992
β–Amyrin (**86**)	*Crescentia cujete*	Agarwal and Popli, 1992
	Jacaranda caucana	Ogura *et al* ., 1977
Betulinic acid (**87**)	*Tecomella undulata*	Azam and Ghanim, 2000
3β,19α-Dihydroxy-24-*trans* -ferulyloxyurs-12-en-28-oic-acid (**88**)	*Stizophyllum riparium*	Duh *et al.*, 1987
3β,20β-Dihydroxyurs-12-en-28-oic-acid (**89**)	*Spathodea campanulata*	Amusan, 1996
Glycyrrhetol (**90**)	*Adenocalymma alliaceum*	Pandey, 1992
3β-hydroxy-24-*cis* -ferulyloxyurs-12-en-28-oic-acid (**91**)	*Stizophyllum riparium*	Duh *et al.*, 1987
3β-hydroxy-24-*trans* -ferulyloxyurs-12-en-28-oic-acid (**92**)	*Stizophyllum riparium*	Duh *et al.*, 1987
2α-Hydroxyursolic acid (**93**)	*Jacaranda caucana*	Ogura *et al* ., 1977
Jacoumaric acid (**94**)	*Jacaranda caucana*	Ogura *et al* ., 1977
Lupeol (**95**)	*Macfadyena ungis-cati*	Duh *et al.*, 1987

Table 2 Distribution of triterpenoids in the family Bignoniaceae (continued)

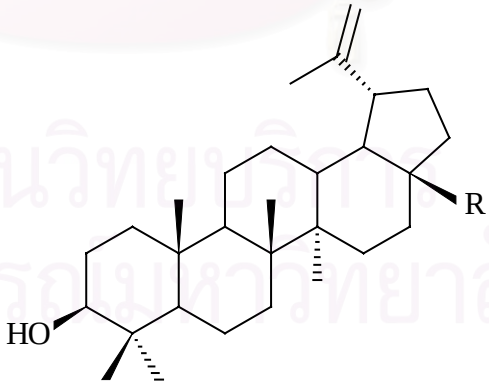
Compounds	Sources	References
Methyl-3β,23-diacetoxyurs-12-en-28-oate (**96**)	*Stizophyllum riparium*	Duh *et al.*, 1987
Methyl-3β,24-diacetoxyurs-12-en-28-oate (**97**)	*Stizophyllum riparium*	Duh *et al.*, 1987
Oleanolic acid (**98**)	*Arrabidaea chica*	Zorn *et al.*, 2001
	Spathodea campanulata	Ngouela, Tsamo and Sondengam, 1991
	Tecomella undulata	Azam and Ghanim, 2000
β-Peltoboykinolic acid (**99**)	*Adenocalymma alliaceum*	Pandey, 1992
Spathodic acid (**100**)	*Spathodea campanulata*	Ngouela *et al* ., 1990
Ursolic acid (**101**)	*Crescentia cujete*	Binutu, 1997
	Jacaranda caucana	Ogura *et al.* , 1977
	Spathodea campanulata	Ngouela *et al* ., 1990
	Stizophyllum riparium	Duh *et al.*, 1987



α-Amyrin (**85**)

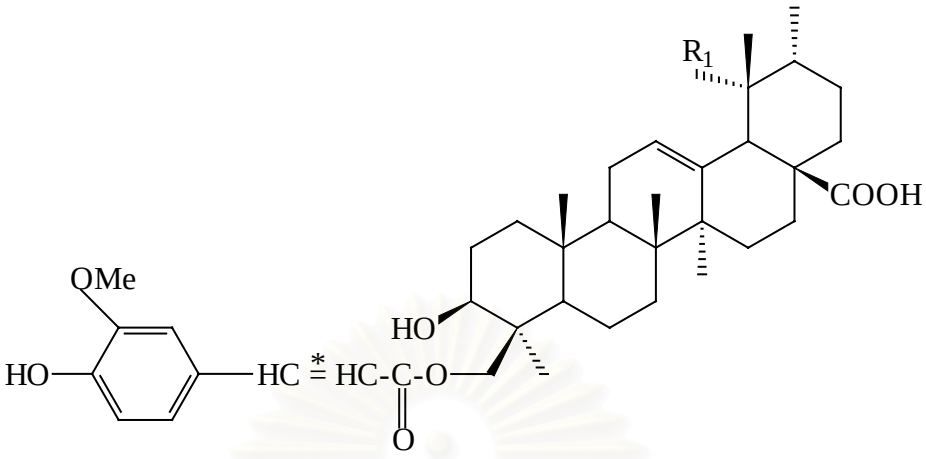


β-Amyrin (**86**)



Betulinic acid (**87**) : R = COOH

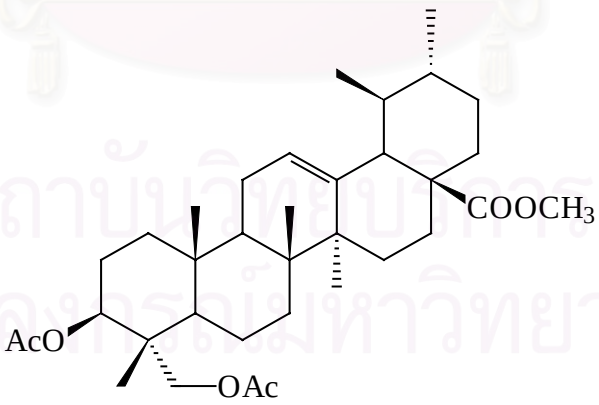
Lupeol (**95**) : R = CH_3



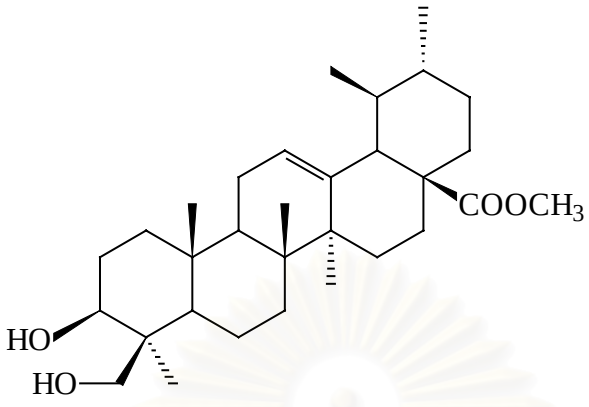
3β,19-Dihydroxy-24-*trans*-ferulyloxyurs-12-en-28-oic acid (**88**) : R_1 = OH, * = *trans*

3β-Hydroxy-24-*cis*-ferulyloxyurs-12-en-28-oic acid (**91**) : R_1 = H, * = *cis*

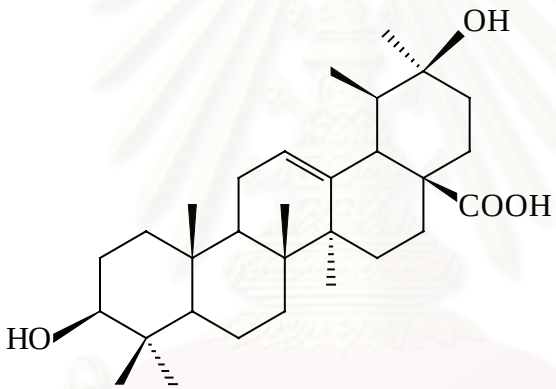
3β-Hydroxy-24-*trans*-ferulyloxyurs-12-en-28-oic acid (**92**) : R_1 = H, * = *trans*



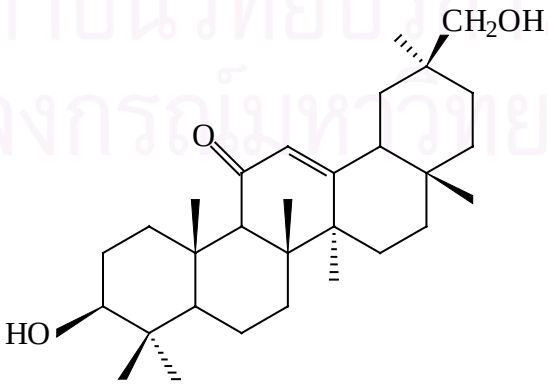
Methyl-3β,23-diacetoxyurs-12-en-28-oate (**96**)



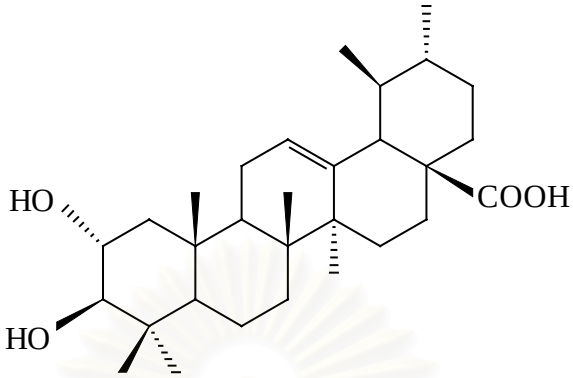
Methyl-3β,24-dihydroxyurs-12-en-28-oate (**97**)



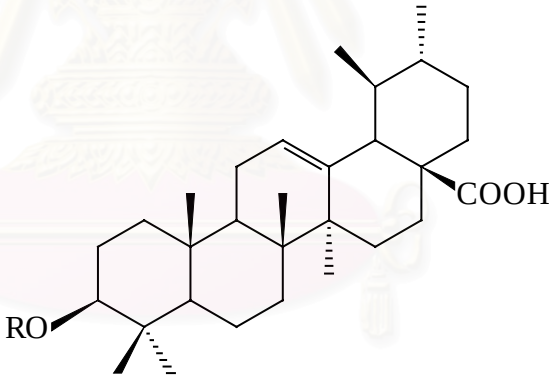
3β, 20β-Dihydroxyurs-12-en-28-oic acid (**89**)



Glycyrrhetol (**90**)

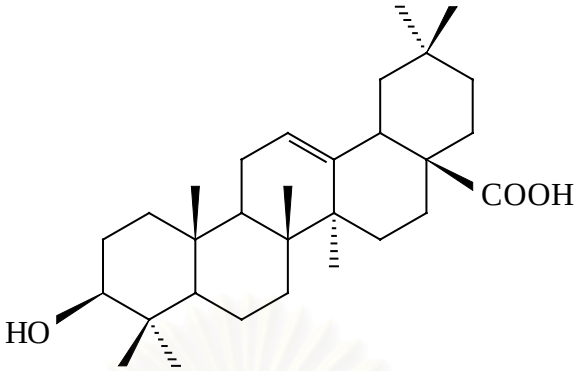


2α-Hydroxyursolic acid (**93**)

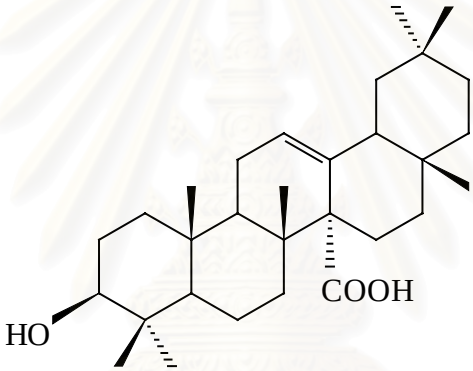


Jacoumaric acid (**94**) : R = 4-hydroxycinnamoyl

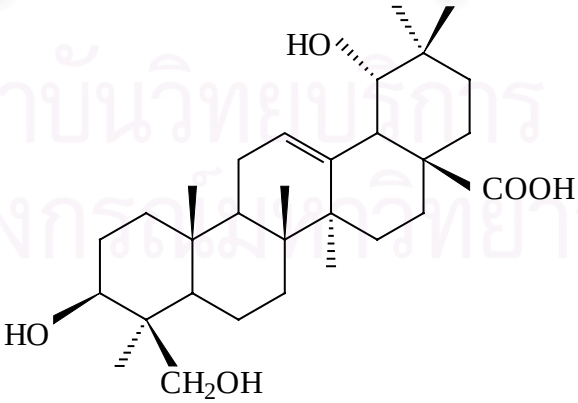
Ursolic acid (**101**) : R = H



Oleanolic acid (**98**)



β-Peltoboykinolic acid (**99**)



Spathodic acid (**100**)

2. Traditional Uses and Biological Activities of Plants in the Family Bignoniaceae

Plants of the family Bignoniaceae have been used medicinally in several countries, especially in Asia, Africa and South America.

In tropical America, from Mexico to Rio de la Plata, and also in Western India, *Macfadyena unguis-cati* is used in folk medicine against snakebite, dysentery, inflammation and rheumatism. There are also reports on its use in the treatment of venereal disease and as a quinine substitute for malaria (Duarte *et al*., 2000). In South America, *Tabebuia* species are used in folk medicine as astringent, anti-inflammatory, antibacterial, antifungal, antiallergic, and as treatment for gastrointestinal problems (Bernardes, 1984; Sousa, Matos and Matos, 1991). In tropical America, the roots of *Stenolobium stans* are reported to be a powerful diuretic, antisyphilitic, tonic and vermifuge. In Veracruz, a decoction of its flowers and bark is administered to treat pains in the stomach, while in some parts of Mexico the plant has the reputation of alleviating and even curing diabetes (Quisumbing, 1951). The leaves of *Arrabidaea chica* are used as anti-inflammatory, astringent and as a remedy for intestinal colic, sanguine diarrhoea, leucorrhoea, anaemia and leukaemia in traditional medicine of tropical America (Bettina *et al.,* 2001). In the northeastern part of Brazil, the inner bark of *Tabebuia avellanedae* is used as analgesic, anti-inflammatory, anti-neoplastic and diuretic (Antoniolli *et al*., 2001). Brazilians also use the leaves of *Tecoma chrysantha* to treat disease of the throat and against stomatitis (Bianco *et al*., 1982), whereas *Pseudocalymma elegans* is well-known in this country due to its poisonous character against animals (Muhammad *et al*., 2000). In Venezuela, consumption of fresh *Arrabidaea bilabiata* leaves produces a paraplegic syndrome in cattle (Gonzalez *et al*., 2000). In Guatemala, the aqueous extract of *Tecoma stans* is used in the treatment of blood disorder and parasitic infections (Villar *et al.,*1997). In Mexico, water extract of the fruits of *Parmetiera edulis* has long been used for treating diabetes mellitus (Perez *et al.,* 2000). In the northwest region of Colombia, another extract of a bignoniaceous plant, the ethanolic extract of the stem barks of *Tabebuia rosea* is used by traditional healers

for snake bites (Otero *et al*., 2000).

In South Africa, the unripe fruit of *Kigelia africana* is an ingredient of the Gbaya arrow poison, while in South Zambia it is used against syphilis. In Benin, the root or stem bark extract is used for dysentery. A decoction of the leaves is drunk as a treatment for jaundice, and is also diuretic. In Senegal, the fresh ripe fruit boiled in some water is rubbed into the breasts of young girls to enlarge them. In Uganda, the bark decoction is used to treat liver and spleen problems. In Nigeria, the bark is cooked with soda and flour and used for syphilis, gonorrhea and rheumatism. In Ghana, it is used as a medication against dysentery, rheumatism and tapeworms. In Tanzania, the stem bark decoction is drunk for abdominal pain, swellings and epilepsy (Thomas, 1989). In northern Nigeria, the fruit of this plant is sold as medicine in markets, to be used as a purgative and cure for dysentery (Quisumbing, 1951). The bark and fruit extracts of another *Kigelia* species, *Kigelia pinnata*, are used traditionally in South Africa to treat diseases caused by micro-organisms and as a remedy for skin cancer (Houghton *et al*., 1994).

In South Cameroon, the dried stem bark powder of *Spathodea campanulata* is applied to wounds to hasten wound healing. In Togo, decoction of the stem bark is used to treat irregular menstruation and chronic wounds (Sandberg and Cronlund, 1982), while in Rwanda, it is used to treat diabetes. In Nigeria, its bark, leaves and flowers are used to heal ulcers, edemas and skin diseases. The leaf decoction is used for urethral inflammation and the stem bark decoction for dysentery and diarrhoea. In Senegal, the bruised leaves and flowers are put on wounds. In the Gold Coast, a decoction of the bark is taken for constipation, gastro-intestinal troubles and dysentery (Quisumbing, 1951).

In northern China, the dried whole plant of *Incarvillea sinensis* has been traditionally used in treating rheumatism and relieving pain as an ancient Chinese crude drug called “Jiaohao (Kakko)” (Nakamura *et al*., 2001). In China, the fruit pulp of *Crescentia cujete* is used as emollient and pectoral (Perry, 1980). The leaves of *Catalpa bungei* are used as dressing on sores of pigs. The seeds are applied on boils and scabies. The bark are used as stomachic, anthelmintic and as

an ingredient in lotions for sores. The bark of *Catalpa ovata* is used as a remedy for kidney disease and dropsy. The fruit is an effective diuretic, administered in kidney diseases, peritonitis, dropsy, and beri-beri (Perry, 1980). In Japan, it is also used as a diuretic (Okuda *et al*., 1975).

In Japan, the flowers of *Campsis grandiflora* are used as a treatment for various menstrual disorders, hemorrhage after labor, and leucorrhea (Perry, 1980).

In India, the fruit of *Tyranthus schumannianus* is used against diarrhoea (Munoz *et al*., 2000). The fruit distillate of the African plant *Kigelia africana* is also used in Indian traditional medicine to remove kidney stones (Sharma and Kaul, 1993). The bark of *Tecomella undulata* is used in folk medicine as a remedy for syphilis. In Bolan, it is used as a cure for liver diseases (Perry, 1980).

India people use the root bark of *Oroxylum indicum* is used traditionally as astringent to the intestinal tract, cooling, aphrodisiac, tonic, appetizer, as treatment for fevers, bronchitis, asthma, dysentery and inflammation. The tender fruits are described as carminative and stomachic. The seeds are purgative (Blatter, Laius and Mhaskar, 1980). They are also used internally to treat stomach and liver trouble and externally applied to carbuncles, ulcers and furuncles. In Burma, Indo-China and the Philippines, its root bark and stem bark are used as an astringent, tonic and as treatment of dysentery, diarrhea and rheumatism. In Indonesia, its bitter bark is a remedy for stomach complaints, a tonic and appetizer. The bark is chewed as a depurative, especially after parturition. The flowers are used as a remedy for inflammation of the eyes. The soft part between the bark and the wood is used as astringent. The extract of the leaves is used as a remedy for fever. In Malay Peninsula, its bark is used in dysentery. A decoction of its leaves is drunk for stomach disorders, rheumatism and wounds (Perry, 1980).

In India, the seeds of *Dolichandrone spathacea* with ginger or the root of this plant are administered in spasmodic conditions, while in the Philipines its powdered seeds are administered to treat nervous conditions. A poultice of fresh leaves and bark is applied after parturition to relieve flatulence

(Quisumbing, 1951). A decoction of *Dolichandrone falcata* fruit is used to cause abortion and the bark is used as a fish poison (Perry, 1980). The wood extract of *Heterophragma roxburghii* is used in skin disease and the root is prescribed for snake bites. The roots, leaves and flowers of *Stereospermum tetragonus* are used in decoction as antipyretic, while the root of *Stereospermum sauveolens* is useful in India folk medicine as treatment for inflammations, flatulence, vomiting, asthma, fevers, disease of the blood, thirst and loss of taste. The flowers of the latter are useful in diarhea and burning sensation, and the fruit is useful in hiccup and leprosy. In Burma and Indo-China (except Vietnam), the roots, leaves, and flowers of *Stereospermum chelonoides* are used as antipyretic. In Malay Peninsula, the juice of the leaves of *Stereospermum fimbriatum* is used to treat earache. A decoction of the roots is given as protective medicine after childbirth (Perry, 1980). The oil from the wood of *Radermachera xylocarpa* is used in skin infections. In Kashmir, plants in the genus *Amphicome* are used as antipyretic. The root of *Tecoma stans* is considered in the Satara district an effective remedy for snake and rat bites and for scorpion-sting. The pulp of the fruit of *Crescentia cujete* is used as emollient and pectoral. In Indonesia, a decoction of its bark is used to clean wounds. The pounded leaves may be made into a poultice for headache (Perry, 1980).

In the Philippines, a decoction of the leaves of *Crescentia alata* is considered to be astringent and antihemorrhagic. It is often used in treating hemoptysis and dysentery. In Burma, *Markhamia stipulata* is used as a cure for psoriasis (Perry, 1980). In the southwest of Burma, the leaves or bark can be used as a hot poultice to guard against yeast infection and athlete's foot or a soothing tea for coughing. The tea (from the flowers) produces a natural anti-oxidant, which promotes cardiovascular health and regulates glucose metabolism (Moore, 1989).

In Thailand, several plants in this family are employed in traditional Thai medicine. The leaves of *Fernandoa adenophylla* are used externally as a treatment of skin disease (Kanchanapoom, Kasai and Yamasaki, 2001). The leaves of *Oroxylum indicum* are used for stomach ache and rheumatism. The bark is used as astringent, diaphoretic, antidiarrheal and as treatment for

rheumatism and stomach ache. The roots are used as antidiarrheal but the seeds are laxative (นันทวัน บุณยะประภัศร และ อรนุช โชคชัย เจริญพร, 2542). The decoction of the leaves of *Dolichandrone spathacea* are used as mouth wash, whereas its bark is used to relieve flatulence after parturition. The roots are used as expectorant, antiflatulence and antipyretic. Its flowers are also used as antipyretic.

The uses of *Millingtonia hortensis* in traditional Thai medicine are well-known. Its leaves are substituted for opium, while the roots are used to enrich the lung and cure tuberculosis. The flowers are smoked as a treatment for asthma. The leaves and flowers of *Spathodea campanulata* are applied to wounds to promote healing. Its bark is used to treat skin disease and dysentery. The leaf juice of *Stereospermum fimbriatum* is put in the ear to relieve earache. A decoction of the roots is given as protective medicine after childbirth. Another tree of the same genus, *Stereospermum personatum*, is used as antipyretic in the form of a decoction of its leaves, roots and flowers. The flowers and fruits are also used as a remedy for scorpion-sting (ลีนา ผู้พัฒนพงศ์, 2525).

A number of extracts from plants in the family Bignoniaceae have been shown to possess biological activities, including anticancer, antifungal, analgesic, antiplasmodial, anti-inflammatory, anti-edema, diuretic, anti-platelet aggregating and hypoglycemic activities. Examples are as follows.

The epicuticular wax of the leaves of *Arrabidaea brachypoda* showed antifungal activity against *Cladosporium sphaerospermum* (Alcerito *et al*., 2002). The methanol extract from the aerial parts of *Arrabidaea bilabiata* showed *in vitro* antifungal activity against *Candida albicans* (Gonzalez *et al*., 2000). The dichloromethane extracts of *Tabebuia chrysantha, Oroxylum indicum, Fernandoa adenophylla* and *Jacaranda filicifolia* showed antifungal activity against dermatophytic and filamentous fungi (Ali, Houghton and Hoo, 1998). A methanol extract of *Tecoma stans* leaves was found to be effective against *Candida albicans* (Binutu and Lajabutu, 1994). The crude aqueous extract of *Kigelia africana* stem bark showed *in vitro* antifungal activity against *Candida albicans* (Akunyili, Houghton and Raman, 1991).

An aqueous ethanol extract of *Jacaranda caucana* showed *in vitro* antitumor activity against the P-388 lymphocytic leukemia system (Ogura *et al*., 1977). The chloroform extract of the roots of *Ekmanianthe longiflora* showed significant cytotoxicity in a panel of human cancer cells (Peraza-Sanchez *et al*., 2000). The aqueous and alcoholic extracts of pods and flowers of *Tecoma sambucifolia* showed cytotoxicity *in vitro* in Chinese hamster ovary cells, human hepatoma cells and human epidermal carcinoma cells of the larynx (Alguacil *et al.,* 2000). A chloroform extract of the entire plant of *Stizophyllum riparium* was found to display significant activity against the P-388 lymphocytic leukemia cell system (Duh *et al*., 1987). A methanolic extract of *Oroxylum indicum* strongly inhibited the mutagenicity of 3-amino-1,4-dimethyl-5H-pyrido (4,3-6) indole (Trp-P-1) in Ames test (Nakamura *et al*., 2001).

A lipophilic extract of the root bark of *Stereospermum kunthianum* revealed antiplasmodial activity *in vitro* against the endothelial cell line ECV-304 (Onegi *et al*., 2002). A leaf extract of *Spathodea campanulata* showed schizontocidal activity on *Plasmodium berghei* in mice (Makinde, Adesogan and Amusan, 1987).

A decoction of the stem bark of *Spathodea campanulata* (Niyonzima *et al*., 1991) and the chloroform extract of the dried fruits of *Parmentiera edulis* (Perez *et al*., 2000) showed hypoglycemic activity in mice.

The methanol extract of whole plants of *Tecomella undulata* showed analgesic potential *in vivo* in mice and rat, when compared with aspirin (Ahmad, Khan and Rasheed, 1994).

The aqueous inner bark extract of *Tabebuia avellanedae* showed antinociceptive activity in mice (Antoniolli *et al*., 2001). The alcoholic extracts of pods and flowers of *Tecoma sambucifolia* showed similar activity when evaluated in acetic acid writhing test (Alguacil *et al.,* 2000).

The alcoholic extracts of pods and flowers of *Tecoma sambucifolia* showed anti-inflammatory activity *in vivo* in carrageenan-induced edema test using Chinese hamster ovary cells (Alguacil *et al.,* 2000). The lipophilic extract of *Arrabidaea chica* completely inhibited NF-κB, a factor involved in inflammation process, at a concentration of 200 μg/ml (Bettina *et al*., 2001).

The aqueous inner bark extract of *Tabebuia avellanedae* showed *in vivo* antiedematogenic effect in a rat paw edema test induced by carrageenan (Antoniolli *et al*., 2001).

An aqueous extract of *Tecoma stans* showed anti-platelet aggregating activity against aggregation induced by thrombin (Villar *et al*., 1997).

The extract of *Tabebuia spectabilis* was found to be the most active against the gram-positive bacteria *Bacillus subtilis* (Rasadah and Houghton, 1998). The methanolic extract of *Kigella africana* stem bark exhibited antibacterial activity against *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* (Almagboul *et al*., 1985), whereas its aqueous crude extract of the same plant part is used by native healers against venereal disease and as antimicrobial against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* (Akunyili *et al*., 1991). The methanolic extract of *Crescentia alata* possesed strong antimicrobial activity against *Staphylococcus aureus, Enterococcus faecalis, Staphylococcus pyogenes, Escherichia coli and Candida albicans* (Rojas *et al*., 2001).

The methanolic stem bark extract of *Spathodea campanulata* caused urination in rats (Sandberg and Cronlund, 1982), whereas its hexane and chloroform extracts exhibited blood schizontocidal action on *Plasmodium berghei* in mice (Makinde *et al*., 1987).

Several pure compounds isolated from bignoniaceous plants were shown to be biologically active. These compounds are naphthoquinones, iridoid, flavonoids, anthraquinones, triterpenoids, alkaloids or sesquiterpene lactones. Examples are as follows.

Lapachol from the liana extract of *Macfadyena unguis-cati* showed antitumor activity when studied using the potato disc assay (Duarte *et al*., 2000). The same compound from *Tecomella undulata* the bark and wood of plant in the genus *Tabebuia* (Rao and Black, 1968) also demonstrated significant activity against cancerous tumours. β-Lapachone from the dichloromethane extract of *Tabebuia chrysantha, Oroxylum indicum, Fernandoa adenophylla* and *Jacaranda*

filicifolia showed antifungal activity by inhibiting the development of filamentous fungi (Ali *et al.*, 1998). α-Lapachone from the root of *Ekmanianthe longiflora* showed significant cytotoxicity in a panel of human cancer cells (Peraza-Sanchez *et al*., 2000).

Aucubin from *Tecoma chrysantha* showed significant antibiotic activity (Bianco *et al*., 1982), whereas analogs of furanonaphthoquinone (FNQ) from *Tecoma ipe* showed antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and *Helicobacter pylori* (Nagata *et al*., 1998). The napthoquinone pinnatal of the root bark of *Stereospermum kunthianum* showed antiplasmodial activity *in vitro* (Onegi *et al*., 2002). 2-(1-Hydroxyethyl) naphtho [2,3-b] furan-4,9-quinone, 2-acetylnaphthol [2,3-b] furan-4,9-quinone and dehydro-iso-α-lapachone from the roots of *Ekmanianthe longiflora* displayed significant cytotoxicity in a panel of human cancer cells (Peraza-Sanchez *et al*., 2000).

Lupeol from the liana extract of *Macfadyena unguis-cati* showed *in vitro* antimalarial activity against *Plasmodium falciparum* (Duarte *et al*., 2000) and *in vitro* anti-inflammatory and antipyretic effects against the enzymes 5-lipoxygenase and cyclooxygenase (Kuhl *et al*., 1984; Davis *et al*., 1994). Ursolic acid, isolated from the stem bark of *Spathodea campanulata*, also showed antimalarial activity (Amusan, 1996). A triterpene ester, 3β-hydroxy-24-*trans*-ferulyloxyurs-12-en-28-oic acid, from *Stizophyllum riparium* showed cytotoxic activity against the P-388 lymphocytic leukemia cell line (Duh *et al*., 1987).

Allantoin from isopropanol extract of the aerial part of *Arrabidae bilabiata* produced significant increase in motor activity which produces a paraplegic syndrome in cattle (Gonzalez *et al*., 2000).

The flavonoid compounds, cirsiliol, cirsimaritin, hispidulin and 3′,4′-dihydroxy-5,6,7-trimethoxyflavone of *Arrabidaea brachypoda* showed antifungal activity against *Cladosporium sphaerospermum* (Alcerito *et al*., 2002). Hortensin, a methoxylated flavone possessing anticancer property, was isolated from the flower of *Millingtonia hortensis* (Hase *et al*., 1995). Hispidulin, another flavonoid from *Millingtonia hortensis*, showed antimutagenicity when assayed by the method

of *Salmonella*/microsome test (Chulasiri, Bunyapraphatsara and Moongkarndi, 1992). Scutellarein-5-O-β-D-glucuronide from the same plant showed diuretic activity (Kar *et al*., 1976). Baicalein from *Oroxylum indicum* was demonstrated to be a cancer metastasis inhibitor (Umezawa *et al*., 1995). Quercetin from *Spathodea campanulata* and *Kigelia africana* showed relatively strong *in vitro* inhibitory activity against the malaria organism *Plasmodium falciparum* (Khalid *et al*., 1986). Quercitrin from *Spathodea campanulata* exhibited antidiarrheal effect (Galvez *et al*., 1993).

Incarvillateine, a new monoterpene alkaloid from *Incarvillea sinensis*, showed significant antinociceptive activity in a formalin-induced pain model in mice (Nakamura *et al*., 2001).

A sesquiterpene lactone, lactucin-8-O-methylacrylate, found in *Parmentiera edulis* fruit, exhibited hypoglycemic activity when tested in mice (Perez *et al*., 2000).

3. Traditional Uses and Biological Activities of *Crescentia* Species.

Plants of the genus *Crescentia* are cultivated for ornamental purpose and are also used therapeutically as traditional medicine in a number of developing countries.

In Brazil, the pulp of unripe fruit of *Crescentia cujete* is sweetened with sugar and used as an antipyretic, while the ripe fruit is made into a poultice and applied topically for headache. In South Africa, the burnt and powdered seeds are taken internally and applied in case of snake-bite (Blatter, Caius and Mhaskar, 1988). In the West Indies, a syrup made from the pulp is often used in dysentery and also as a treatment for respiratory diseases (Quisumbing, 1951).

In western Africa, the bark of *Crescentia cujete* is employed to treat dysentery and the pulp of the fruit is used as laxative and expectorant. In the West Indies, the fruit macerated in water is considered depurative, cooling and febrifuge. It is employed to treat headache and burns. In Venezuela, a decoction of the bark of this plant is given for diarrhoea, similar to its use in western Africa (Quisumbing, 1951).

In Bombay, the fruit of *Crescentia cujete* is used by herbalists as a respiratory aid in the form of poultice of the pulp applied to the chest (Quisumbing, 1951). In Sumatra, a decoction of the bark is used to clean wounds, and the pounded leaves are used as a poultice for headache. In Indo-China, the pulp of the fruit is used as emollient and as a treatment for respiratory diseases (Perry, 1980). In Vietnam, the dried fruit, locally named “Dao Tien”, is used in folk medicine as an expectorant, antitussive, laxative and stomachic (Kaneko *et al.*, 1997).

In Thailand the leaves of *Crescentia cujete* is used in the treatment of bruise (เทพพนม เมืองแมน และ คณะ, 2533).

Investigations on biological activities of plants in this genus have been performed on both *Crescentia alata* and *Crescentia cujete.* There are experimental data on the extract from the fruit of *Crescentia alata* (as a component of Mexican traditional medicine for the treatment for respiratory diseases) which exhibited strong antimicrobial activity against the pathogens tested (*Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*) (Rojas *et al*., 2001).

Furanonaphthoquinones from *Crescentia cujete* showed selective DNA-damaging activity against yeast (Heltzel *et al*., 1993). Kaempferol, which is the flavonoid isolated from *Crescentia alata,* showed *in vivo* anti-inflammatory activity on carrageenin-induced paw edema in rats and *in vitro* on *Escherichia coli* lipopolysaccharide-(LPS)-induced nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression in J774.A1 macrophage cell line. This property could be important in the prevention of inflammation and may contribute to its anti-inflammatory and immunoregulatory effects (Aotore *et al.,* 2001).

4. Chemical Constituents of *Crescentia alata.*

There are only a limited number of reports on the phytochemical study of this plant. In 1980, Gomez-Brenes and his co-workers have analyzed the composition of the fruit as consisting of 17% crude fat, 11% crude fiber and 18% crude protein (Gomez-Brenes *et al*., 1980). In the same year, Scogin reported the isolation of an anthocyanin glycoside, cyanidin-3-O-β-D-rutinoside, from the fruit of this plant (Scogin, 1980). Recently, in 2001, two flavonol glycosides, quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (rutin), kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (kaempferol-3-O-rutinoside), and a flavonol aglycone, kaempferol, were isolated from the methanolic extract of its leaves (Autore *et al.*, 2001).

CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The leaves and bark of *Crescentia alata* H.B.K. were collected from Nakhon Nayok province, Thailand in May, 2000. The plant was identified by comparison with herbarium specimens at the Museum of Natural Medicine, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. Phytochemical Techniques

2.1 Chromatographic Techniques

2.1.1 Analytical Thin-layer Chromatography (TLC)

Technique:	One dimension, ascending
Adsorbent:	Silica gel 60F 254 (E. Merck) precoated plate
Layer thickness:	0.2 mm
Distance:	6 cm
Temperature:	Laboratory temperature (30-35 ºC)
Detection:	1) UV light at the wavelengths of 254 and 365 nm 2) Spraying with 10% ethanolic sulphuric acid reagent and heating at 110 ºC for 5-10 minutes
Solvent system:	Various solvent systems depending on materials

2.1.2 Conventional Column Chromatography (CC)

Column sizes : Glass columns of 1-4 inches in diameter were used depending on the quantity of sample to be separated

Absorbent :	Silica gel 60 (E. Merck, particle size 0.040-0.063 nm)
Packing method :	Wet packing
Sample loading :	The sample was dissolved in a small volume of organic solvent, then applied gently on the top of the column.
Solvent system :	Various solvent systems depending on materials.

2.1.3 Gel Filtration Chromatography

Gel filter :	Sephadex ® LH –20 (Pharmacia)
Packing method :	The gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into column and allowed to set tightly.
Sample loading :	The sample was dissolved in a small volume of the eluent and applied onto the top of the column

2.2 Spectroscopy

2.2.1 Ultraviolet (UV) Absorption Spectra

UV spectra (in methanol) were obtained on a Shimadzu UV-160A UV/VIS spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.2.2 Infrared (IR) Absorption Spectra

IR spectra were recorded on a Perkin Elmer FT-IR 1760X spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University). The samples were prepared as KBr pellets.

2.2.3 Mass Spectra (MS)

Electron impact mass spectra (EIMS) were recorded on a Fison Micromass VG Platform II mass spectrometer (Faculty of Sciences, Mahidol University).

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

^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University), whereas ^{1} H-NMR (500 MHZ) and ^{13}C-NMR (125 MHz) spectra were obtained with a JEOL JMN-A 500 NMR spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University). Deuterated chloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6) were used as NMR solvents in this study. Spectral data were reported in ppm scale, using the solvent chemical shifts as the reference signal.

2.3 Physical Properties

2.3.1 Melting Points

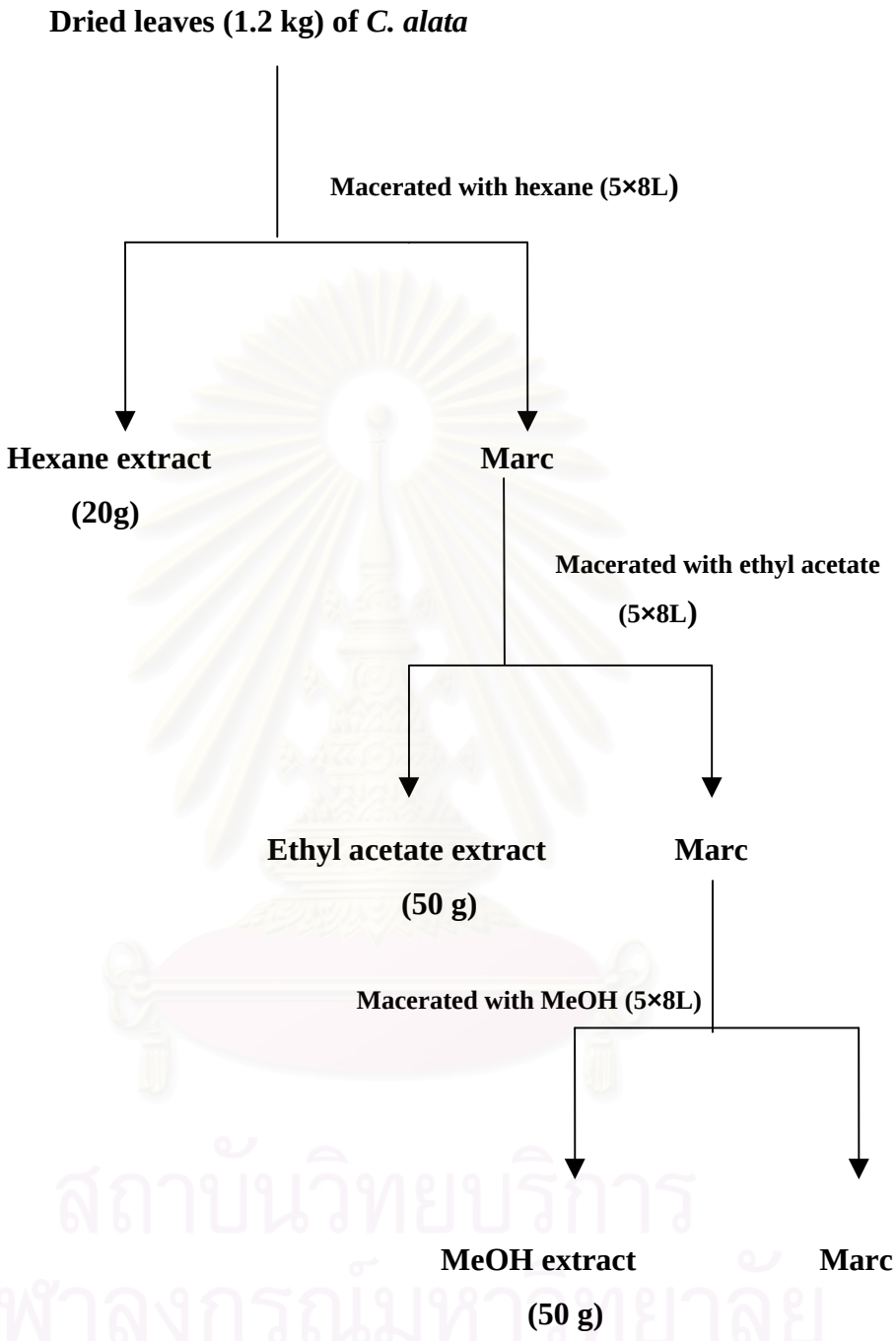
Melting points were obtained on a Fisher/Johns melting point apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4 Solvents

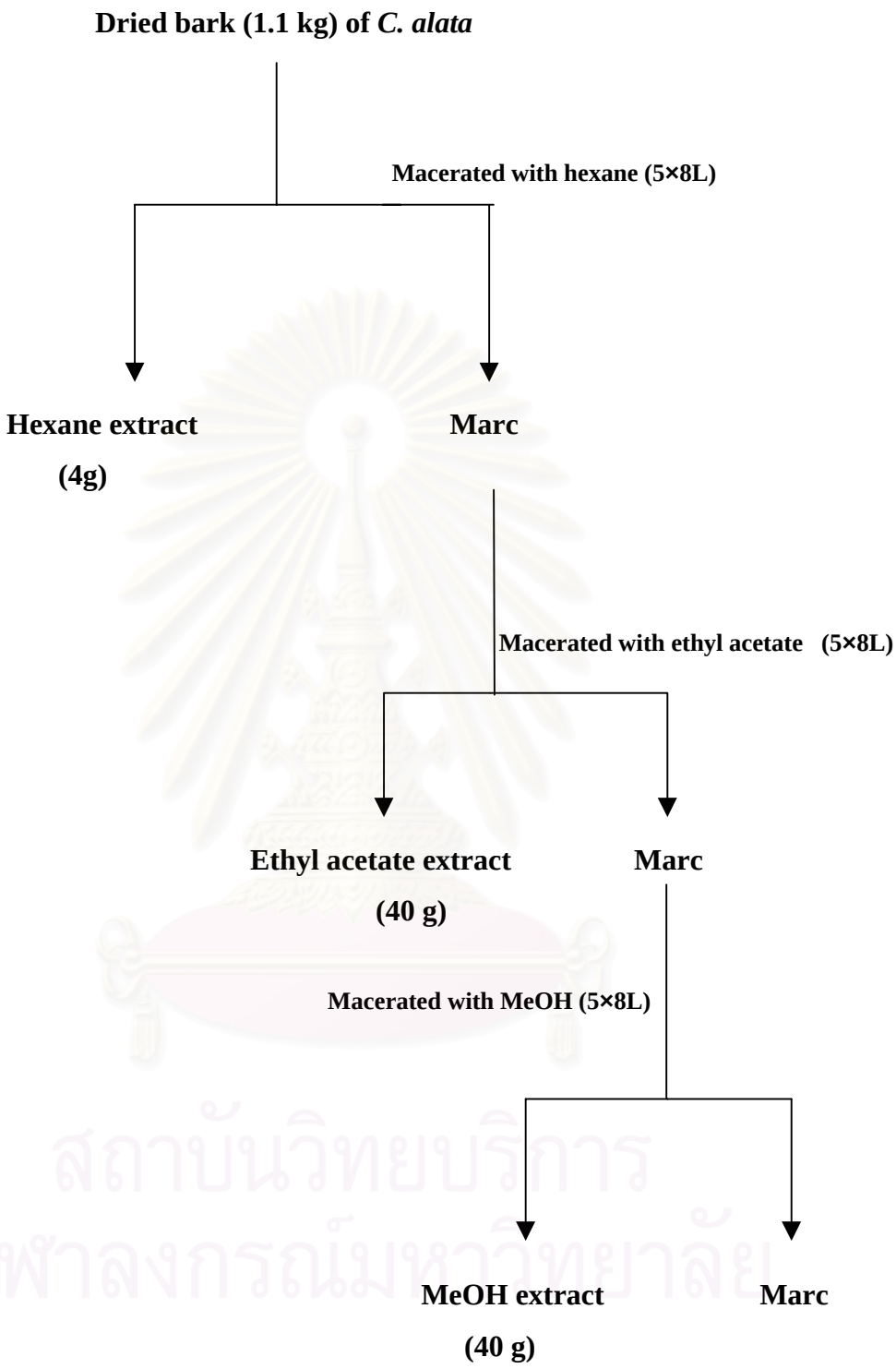
Solvents of commercial grade were redistilled before used.

3. Extraction and Isolation

The dried, powdered leaves (1.2 kg) and bark (1.1 kg) of *Crescentia alata* were macerated with hexane (5×8 L), ethyl acetate (5×8 L), and MeOH (5×8 L), respectively. The leaf extracts were filtered and evaporated under reduced pressure to yield hexane (20 g), ethyl acetate (50 g) and methanol extracts (50 g), respectively, whereas the bark yielded 4 g of hexane extract, 40 g of ethyl acetate extract and 40 g of methanol extract (**Schemes 1** and **2**).



Scheme 1 Extraction of *Crescentia alata* leaves



Scheme 2 Extraction of *Crescentia alata* bark

4. Isolation Procedure

4.1 Isolation of Chemical Constituents from the Ethyl Acetate Extract of *C. alata* Bark

A portion (20 g) of the ethyl acetate extract of *C. alata* bark was divided into 2 equal parts and subjected to two successive silica gel column chromatography, both using the same solvent mixture of chloroform-methanol (from 20:1 to 1:1) as the eluent. The extract was dissolved in a small volume of the eluent and applied to the top of a glass column (4.5×60 cm) already packed with a slurry of silica gel (300 g) in the eluting solvent. Fractions (40 ml each) were monitored by TLC, using chloroform-methanol (15:1) as the developing solvent. Two hundred collected fractions were combined according to their TLC profiles into 14 major fractions (F001-F014) as shown in **Table 3.** Both columns were then washed down with methanol and the eluate combined with fraction F014.

Table 3 Combined fractions from the ethyl acetate extract of *Crescentia alata* bark

Fraction	Solvent Ratio Chloroform : Methanol	Number of Eluates	Weight of Fraction (g)
F001	20:1	1-10	2.20
F002	20:1	11-14	1.60
F003	20:1	15-23	2.20
F004	10:2	24	0.40
F005	10:2	25-33	1.00
F006	10:2	34-92	0.90
F007	10:2	93-95	0.20
F008	9:2	96-122	1.40
F009	9:2	123-139	1.00
F010	4:1	140-143	0.50
F011	4:1	144-158	2.00
F012	3:1	159-169	1.20
F013	2:1	170-179	1.60
F014	1:1	180-200	1.90

4.1.1 Isolation of Compound CA1

Fraction F005 (1.00 g) displayed interesting violet spot on TLC and therefore was subjected to a silica gel column chromatography (50 g, 2.5×60 cm), using solvent mixture of chloroform-acetone (from 10:1 to 1:1) as the mobile phase. Twenty-three fractions of about 20 ml each were collected and combined according to their TLC profiles in chloroform-acetone (10: 1). Methanol was used to wash down the column, then combined with the last fraction. Five major fractions (F015-F019) were collected as shown in **Table 4.** Recrystallization of

fraction F015 in methanol afforded compound CA1 as colorless needles (70 mg, 0.012% yield).

Table 4 Combined fractions from F005

Fraction	Solvent Ratio Chloroform : Acetone	Number of Eluates	Weight of Fraction (g)
F015	10:1	1-3	0.08
F016	10:1	4-9	0.08
F017	8:1	10-13	0.07
F018	5:1	14-22	0.12
F019	1:1	23	0.10

4.1.2 Isolation of Compound CA2

Fraction F011 (2.00 g) was subjected to a silica gel column chromatography (80 g, 2.5×60 cm), using solvent mixture of chloroform-acetone (from 50:1 to 1:1) as the mobile phase. One hundred and fifteen fractions of about 30 ml each were collected and combined according to their TLC profiles in chloroform-methanol (90:1) into 10 major fractions (F020-F029), as shown in **Table 5.** Methanol used to wash down the column was combined with the last fraction. The sixth fraction (F025) displayed interesting TLC pattern and it was subjected to further study.

Table 5 Combined fractions from F011

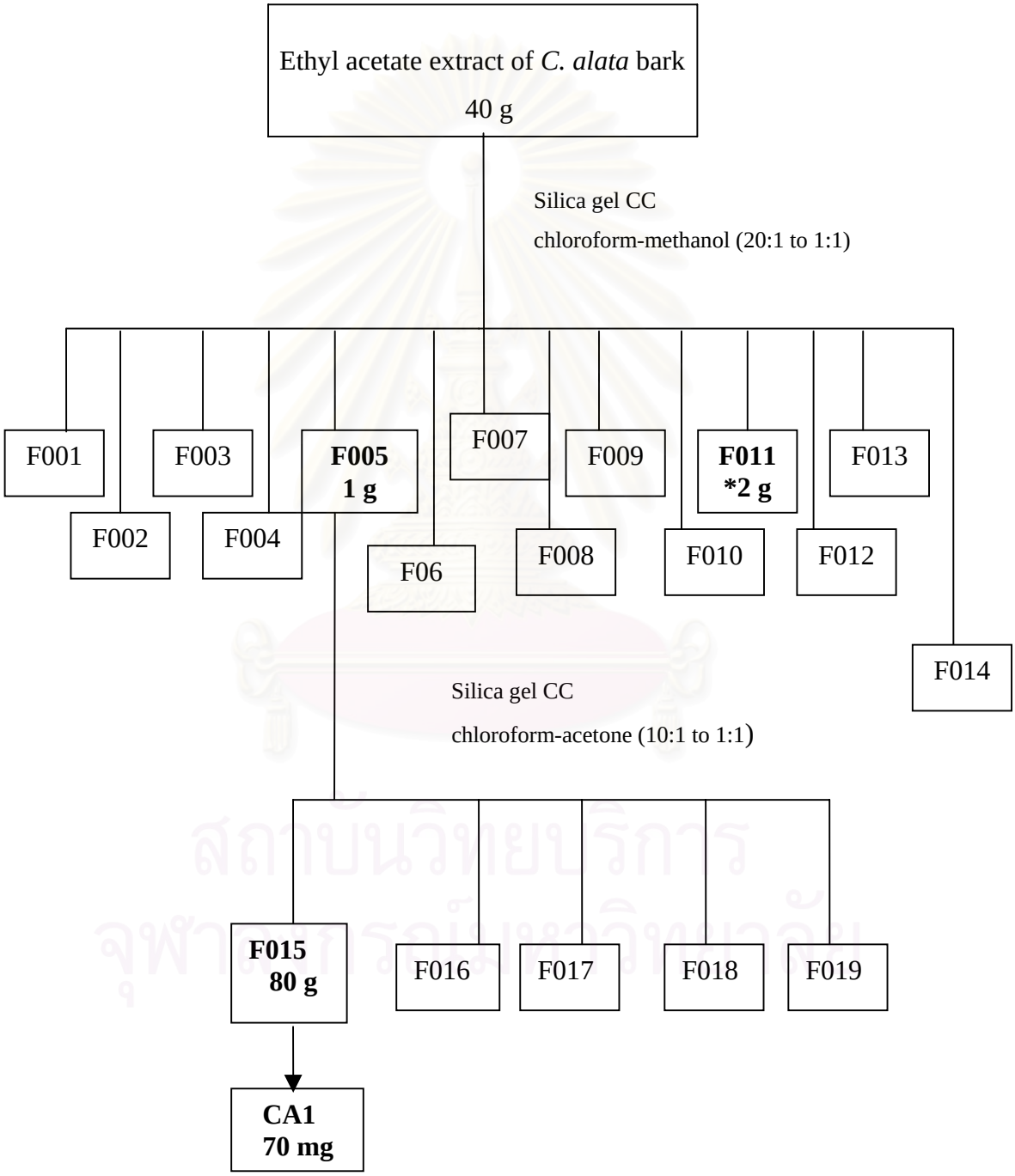
Fraction	Solvent Ratio Chloroform : Acetone	Number of Eluates	Weight of Fraction (g)
F020	50:1	1-5	0.10
F021	40:1	6-11	0.10
F022	30:1	12-16	0.20
F023	25:1	17-28	0.20
F024	20:1	29-33	0.05
F025	15:1	34-45	0.30
F026	10:1	46-64	0.20
F027	5:1	65-72	0.05
F028	3:1	73-86	0.20
F029	1:1	87-115	1.10

Fraction F025 (0.30 g) was subjected to size exclusion column chromatography using Sephadex LH-20 and chloroform-methanol (1:1) as the eluent to give twenty 5ml fractions. The fractions were then combined according to their TLC pattern using chloroform-methanol (25:1) as the developing system to give 4 fractions (F030-F033) as summarized in **Table 6.**

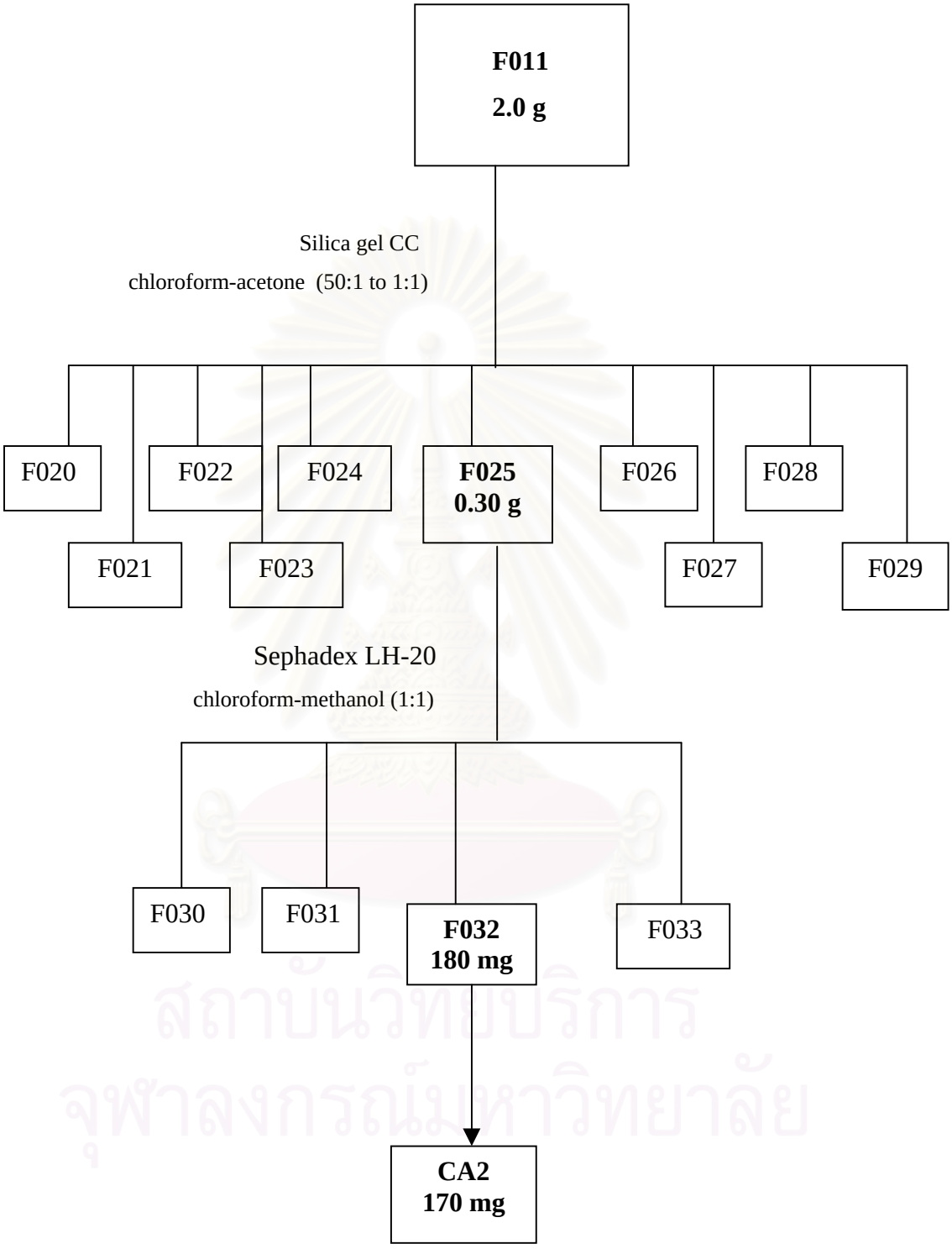
Table 6 Combined fractions from F025

Fraction	Number of Eluates	Weight of Fraction (g)
F030	1-4	0.02
F031	5-11	0.04
F032	12-14	0.18
F033	15-20	0.03

Fraction F032 was further purified by recrystallization in hexane to afford compound CA2 as white needles (170 mg, 0.03% yield).



Scheme 3 Isolation of chemical constituents from the ethyl acetate extract of *C. alata* bark



Scheme 4 Isolation of chemical constituents from the ethyl acetate extract of *C. alata* bark (continued)

4.2 Isolation of Chemical Constituents from the Methanol Extract of *C. alata* Bark

The methanol extract of *C. alata* bark (5 g) was subjected to silica gel column chromatography (150 g, 2.5×60 cm), eluted with chloroform-methanol (from 5:1 to 1:1), to give 163 fractions of 30 ml each. The fractions were then combined according to their TLC patterns, using chloroform-methanol (18:1) as the developing system, to give 8 major fractions (M001-M008) as shown in Table 7. The column was then washed down with methanol and the eluate combined with fraction M008. The third fraction (M003) displayed a major spot on TLC and, therefore, it was subjected to further purification.

Table 7 Combined fractions from the methanol extract of *Crescentia alata* bark

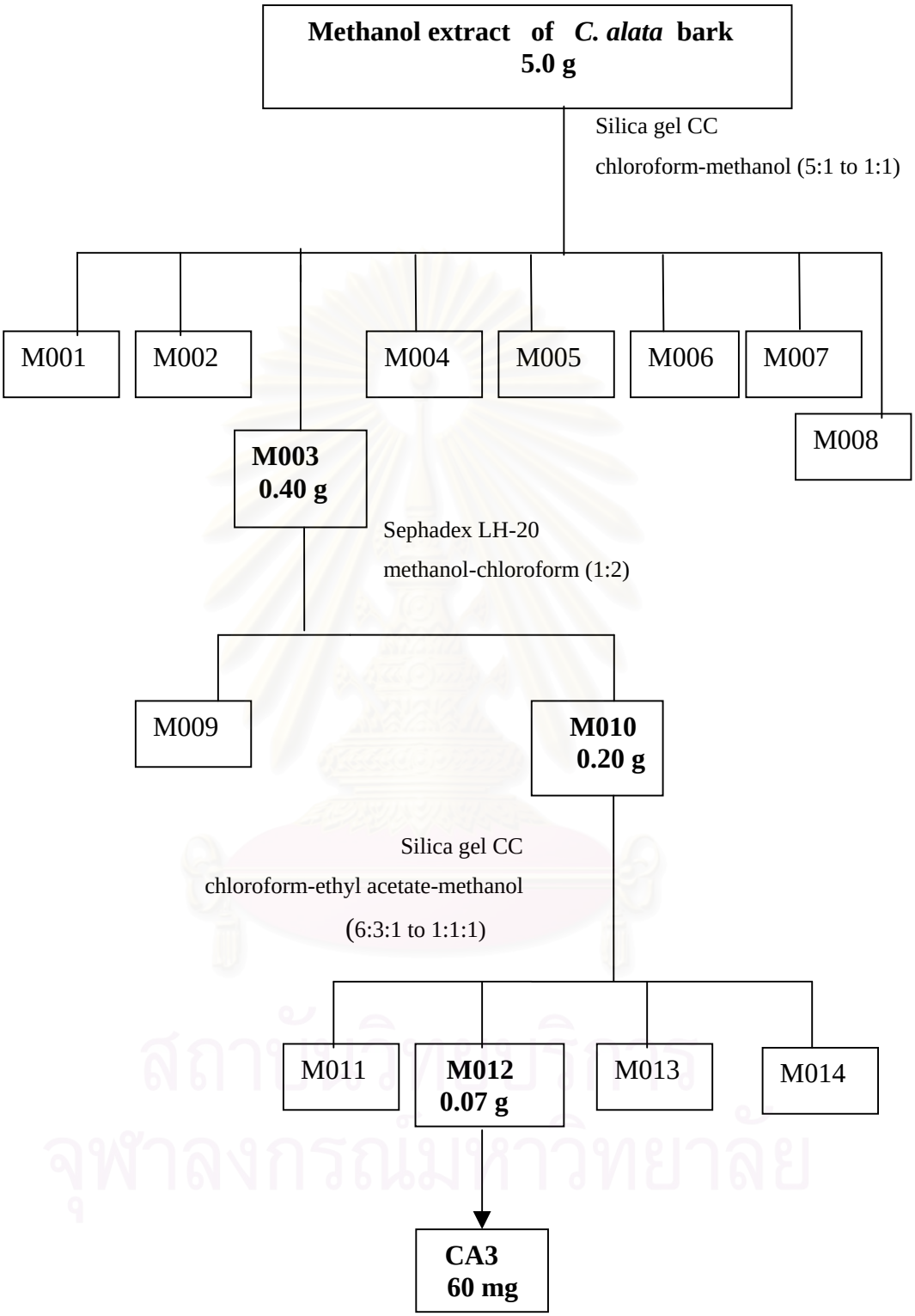
Fraction	Solvent Ratio Chloroform : Methanol	Number of Eluates	Weight of Fraction (g)
M001	5:1	1-12	0.50
M002	5:1	13-15	0.70
M003	5:1	16-19	0.40
M004	4:1	20-45	0.60
M005	4:1	46-75	0.50
M006	3:1	76-99	0.50
M007	2:1	100-135	0.40
M008	1:1	136-163	0.90

4.2.1 Isolation of Compound CA3

Fraction M003 (0.40 g) were subjected to Sephadex LH-20 gel filtration chromatography using a solvent system of methanol-chloroform (1:2) to give twelve 5-ml fractions, which were later combined into 2 major fractions (M009-M010) according to TLC monitoring with chloroform-methanol (47:3) as the developing solvent. Fraction M010 (0.20 g) was further purified through a silica gel column (30 g, 2×30 cm) eluted with chloroform- ethyl acetate-methanol (from 6:3:1 to 1:1:1) to give 17 fractions of 10 ml each. The fractions were combined according to their TLC pattern in chloroform-ethyl acetate-methanol (7:2:1) into 4 major fractions (M011-M014) as shown in **Table 8**. Methanol used to wash down the column was combined with the last fraction (M014). Recrystallization of fraction M012 in hexane afforded compound CA3 as pale yellow amorphous powder (60 mg, 0.044% yield).

Table 8 Combined fractions from M010

Fraction	Solvent Ratio Chloroform : Ethyl acetate : Methanol	Number of Eluates	Weight of Fraction (g)
M011	6:3:1	1-4	0.03
M012	5:4:1	5-6	0.07
M013	1:2:1	7-10	0.03
M014	1:1:1	11-17	0.05



Scheme 5 **Isolation of compound CA3 from the methanol extract of *C. alata* bark**

4.3 Isolation of Chemical Constituents from the Hexane Extract of *C. alata* Leaves

The hexane extract of the leaves of *C. alata* (10 g) was subjected to a silica gel column (300 g, 4.5×60 cm), eluted with hexane-chloroform (from 5:1 to 1:1) to give 160 fractions of 30 ml each. These fractions were monitored by TLC in chloroform-ethyl acetate (10:1) and then combined into 11 major fractions (B001-B011) as shown in **Table 9**. Methanol used to wash down the column was combined with fraction B011.

Table 9 Combined fractions from the hexane extract of *Crescentia alata* leaves

Fraction	Solvent Ratio Hexane : Chloroform	Number of Eluates	Weight of Fraction (g)
B001	5:1	1-13	1.70
B002	5:1	14-19	1.00
B003	5:1	20-31	0.30
B004	4:1	32-39	0.30
B005	4:1	40-58	0.80
B006	4:1	59-69	0.80
B007	3:1	70-95	1.80
B008	3:1	96-105	0.30
B009	3:1	106-154	0.40
B010	2:1	155-158	0.30
B011	1:1	59-160	0.60

4.3.1 Isolation of Compound CA4

Fraction B007 was subjected to silica gel column chromatography. The fraction (1.80 g) was dissolved in a small volume of chloroform-acetone (97:3)

and applied to the top of a glass column (2.5×60 cm) already packed with slurry of silica gel (80 g). This same solvent system was employed as the eluent to give seventy-three 25-ml fractions. These fractions were then combined according to their TLC pattern using chloroform-acetone (97:3) to give 5 major fractions (B012-B016), as summarized in **Table 10**. The fourth fraction (B015) exhibited interesting TLC pattern and therefore was subjected to further study.

Table 10 Combined fractions from B007

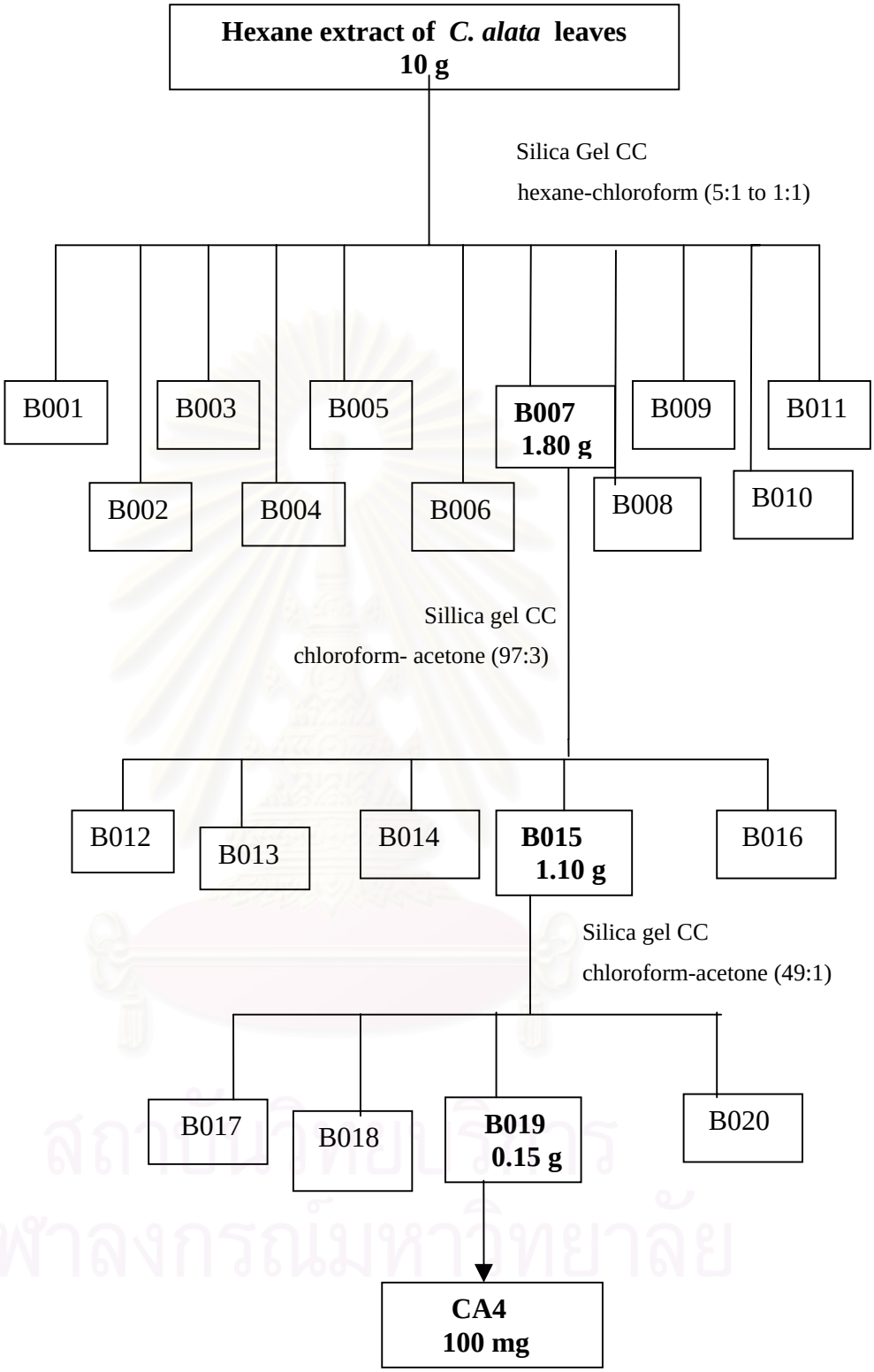
Fraction	Solvent Ratio Chloroform : Acetone	Number of Eluates	Weight of Fraction (g)
B012	97:3	1-4	0.10
B013	20:1	5-9	0.10
B014	15:1	7-14	0.10
B015	7:1	15-34	1.10
B016	1:1	35-73	0.40

Fraction B015 (1.10 g) was subjected to another silica gel column (60 g, 2.5×60 cm) eluted with chloroform-acetone (49:1) to give 43 fractions of 15 ml each. The fractions were combined according to their TLC profile in chloroform-acetone (49:1) into 4 major fractions (B017-B020), as summarized in **Table 11**. Methanol used to wash down the column was combined with fraction B020.

Table 11 Combined fraction from B015

Fraction	Solvent Ratio Chloroform : Acetone	Number of Eluates	Weight of Fraction (g)
B017	49:1	1-2	0.11
B018	20:1	3-9	0.25
B019	7:1	10-19	0.15
B020	1:1	20-43	0.35

When fraction B019 was recrystallized in methanol, it yielded compound CA4 as colorless needles (100 mg, 0.017% yield).



Scheme 6 Isolation of compound CA4 from the hexane extract of *C. alata* leaves

5. Determination of AntioxidantActivity

The antioxidant activities of *C. alata* crude extracts and pure compounds were determined using 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) radical scavenging assay (Yen and Hsieh, 1997). DPPH is a relatively stable paramagnetic free radical having purple color that accepts electrons or hydrogen radical to become a stable diamagnetic molecule (Brand-Williams, Cuvelier and Breset, 1995). The antioxidant activity is estimated by the ability of a compound to scavenge free DPPH radical. The reduction of DPPH is measured spectrophotometrically by the discoloration of the typical purple color of free DPPH radical at 517 nm.

A solution of 0.2 mM DPPH in methanol was prepared, then 1 ml of this solution was mixed with 0.5 ml of a methanolic solution of 100 ppm plant extract or pure compound. The extracts and chemical constituents of *Crescentia alata* tested were: methanol leaf extract, methanol bark extract, ethyl acetate leaf extract, ethyl acetate bark extract, vanillic acid and rutin. This method was validated with α-tocopherol (vitamin E), a well-known antioxidants, dissolved in methanol to a final concentration of 12 mg/ml. The mixture was shaken vigorously and allowed to stand in the dark for 30 min at room temperature. Absorbance measurements commenced immediately at 517 nm with a Shimadzu UV-160A UV/VIS spectrophotometer. A blank was run with DPPH solution. All determinations were performed in triplicate and the results were averaged.

The antioxidant activity is given as percentage of radical scavenging calculated according to the formula:

$$\text{radical scavenging (\%)} = (1-A_{sample}/A_{control}) \times 100$$

when A_{sample} = absorbance of the sample solution + DPPH

$A_{control}$ = absorbance of the control (DPPH)

6. Physical and Spectra Data of Isolated Compounds

6.1 Compound CA1

Colorless needle from methanol. Soluble in chloroform

m.p. : 215-217ºC

EIMS m/z (% relative intensity):

426 (26), 411 (22), 393 (8), 313 (19), 297 (7), 272 (10), 257 (17), 243 (7), 219 (30), 218 (93), 204 (82), 191 (40), 189 (100), 175 (50), 161 (52), 147 (55), 135 (52), 121 (59), 107 (69), 95 (70), 93 (55), 81 (47), 67 (51), and 55 (18)
(Figure 4, page 84)

IR ν_{max}, KBr disc, cm^{-1}:

3485, 2933, 2853, 1640, 1473, 1384, 1084, 1056, 999, and 863 (Figure 3, page 83)

^{1}H-NMR (δ ppm, 300 MHz, $CDCl_3$) :

4.66 (1H, s), 4.54 (1H, s), 3.16 (1H, dd, *J*= 6, 10 Hz), 2.35 (1H, m), 1.90 (1H, m), 1.55 (3H, s),1.01 (1H, s), 0.95 (6H, s), 0.92 (3H, s), 0.81 (3H, s), 0.74 (3H, s), 0.77 (3H,s), 0.67 (1H, d) (Figures 5a-5b, pages 85-86)

^{13}C-NMR (δ ppm, 75 MHz, $CDCl_3$) :

150.8 (s), 109.3 (t), 79.0 (d), 55.3 (d), 50.5 (d), 48.3 (d), 48.0 (q), 43.0 (s), 42.8 (s), 40.8 (s), 40.0 (t), 39.0 (s), 38.7 (t), 38.1 (d), 37.2 (s), 35.6 (t), 34.3 (t), 30.0 (t), 28.0 (q), 27.5 (t), 27.5 (t), 25.2 (t), 21.0 (t), 19.4 (q), 18.3 (t), 18.0 (q), 16.1 (q), 16.0 (q), 15.4 (q), 14.6 (q) (Figure 6, page 87)

6.2 Compound CA2

White needles from chloroform. Soluble in methanol
m.p: 209-212°C

EIMS m/z (% relative intensity):

168 (100), 169 (23), 170 (2.5), 153 (55), 125 (22), 123 (3), 97 (23), 63 (5), 51 (11) (Figure 8, page 93)

IR ν_{max} , KBr disc, cm^{-1}:

3488, 2952, 1682, 1599, 1526, 1436, 1302, 1286, 1240, 1030 (Figure 9, page 94)

UV χ_{max} (MeOH), nm (ε):

223 (ε 39110), 260 (ε 36136), 290 (ε 19370) nm (Figure 10, page 95)

^{1}H-NMR (δ ppm, 300 MHz, DMSO-d_6) :

7.44 (1H, d, *J* = 7.5 Hz), 7.43 (1H, br s), 6.84 (1H, d, *J* = 7.5 Hz), 3.80 (3H, s) (Figure 11, page 96)

^{13}C-NMR (δ ppm, 75 MHz, DMSO-d_6) :

167.3 (s), 151.2 (s), 147.3 (s), 151.1 (d), 123.6 (d), 121.8 (s), 112.9 (d), 55.7 (q) (Figure 12, page 97)

6.3 Compound CA3

Pale yellow crystals from choloform. Soluble in methanol

m.p. : 202- 204 °C

EIMS m/z (% relative intensity):

610 (2), 302 (1), 153 (4), 77 (24) (Figure 19, page 112)

IR ν_{max}, KBr disc, cm^{-1}:

3418, 1660, 1600, 1500, 1060, 1015, 85 (Figure 17, page 110)

UV χ_{max} (MeOH), nm (ε):

256, 357 mm (ε 116713, 73544) (Figure 18, page 111)

^{1}H-NMR (δ ppm, 300 MHz, DMSO-d_6) :

7.52 (1H, d, *J*= 7.8 Hz), 7.52 (1H, br s), 6.84 (1H, d, *J*= 7.8 Hz), 6.38 (1H, br s), 6.18 (1H, br s), 5.33 (1H, d, *J*= 6.0 Hz), 4.37 (s), 0.98 (1H, d, *J* = 6.0 Hz) (Figures 20a-20b, pages 113-114)

^{13}C-NMR (δ ppm, 75 MHz, DMSO-d_6) :

177.4 (s), 164.8 (s), 161.1 (s), 156.9 (s), 156.8 (s), 148.8 (s), 145.1 (s), 133.7 (s) 121.8 (d), 121.3 (d), 116.0 (d), 115.0 (d), 104.3 (s), 101.8 (d), 101.1 (d), 99.1 (d), 94.3 (d), 76.3 (s), 76.1 (s), 74.3 (s), 72.1 (d), 70.8 (s), 70.7 (d), 70.2 (s), 68.5 (s), 67.3 (t), 18.1 (q) (Figure 22, page 116)

6.4 Compound CA4

Colorless needle from methanol. Soluble in chloroform
m.p. : 135-138°C

EIMS m/z (% relative intensity):

412 (100), 396 (8), 351 (29), 300 (42), 273 (27), 95 (40), 81(60), 69 (9)
(Figure 26, page 124)

IR ν_{max}, KBr disc, cm^{-1}:

3463, 2928, 1654, 1560, 1542, 1509 and 1459 (Figure 25, page 123)

^{1}H-NMR (δ ppm, 300 MHz, $CDCl_3$) :

5.32 (1H, br s), 5.17 (H, dd, *J*= 15.0, 8.4 Hz), 5.00 (1H, dd, *J*= 15.0, 8.4 Hz), 3.49 (m, 1 H), 1.00 (3H, d, *J*= 6.5 Hz), 0.99 (3H, s), 0.82 (3H, s), 0.81 (3H, t, *J*= 7.1 Hz), 0.80 (3H, br s), 0.68 (3H, s) (Figures 27a-27b, pages 125-126)

^{12}C-NMR (δ ppm, 75 MHz, $CDCl_3$) :

140.7, 138.3, 129.3, 121.7, 71.8, 56.9, 56.0, 51.2, 50.2, 42.3, 42.3, 40.5, 39.7, 37.3, 36.5, 31.9, 31.9, 31.9, 31.6, 28.9, 25.4 , 24.4, 22.7, 21.2, 21.1, 19.4, 19.0, 12.2, 12.0
(Figures 28a-28b, pages 127-128)

CHAPTER IV

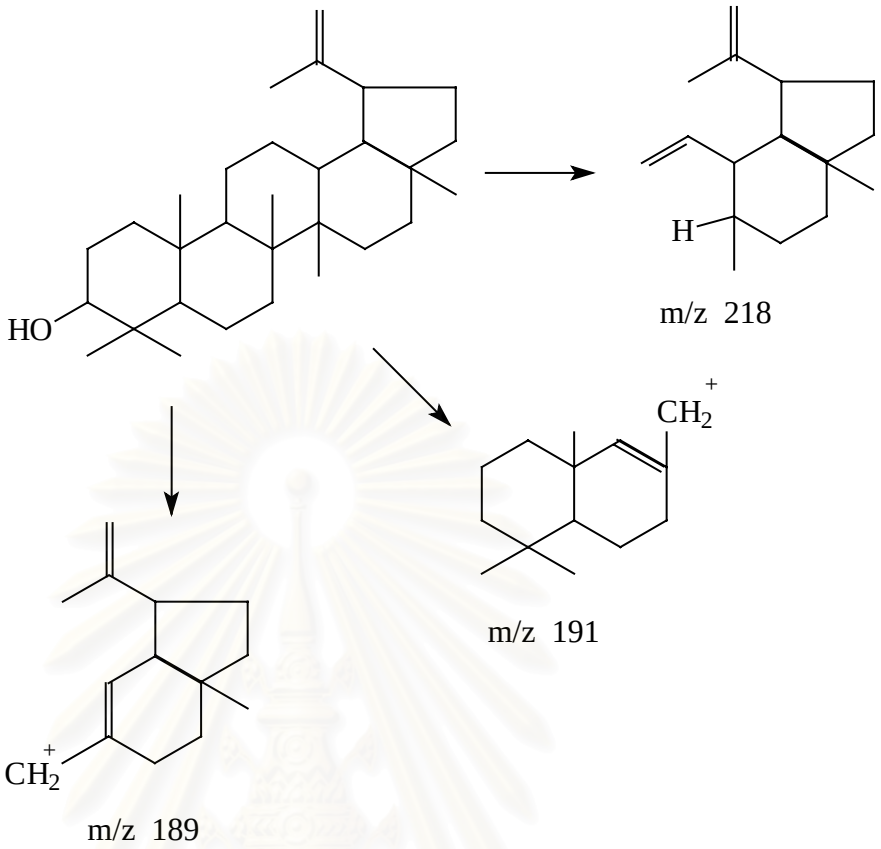
RESULTS AND DISCUSSION

Investigation of the chemical constituents of the leaves and bark of *Crescentia alata* HBK. by column chromatography afforded three compounds from the bark and one from the leaves. Identification of these compounds were based on the interpretation of their spectral data and comparison with those reported in the literature.

1. Identification of Compound CA1

Compound CA1 was recrystallized as colorless needle from methanol. It gave violet color when sprayed with 10 % ethanolic sulphuric acid and heated, suggesting the presence of a triterpenoid nucleus. The IR spectum of CA1 (Figure 3) exhibited a broad band at 3485 cm^{-1} (O-H streching), indicating the presence of hydroxyl group (s) in the molecule.

The EIMS of CA1 (Figure 4) displayed a prominent molecular ion peak at *m/z* 426 ($C_{30}H_{50}O$), 411 ($[M - CH_3]^+$) and 393 ($[M - CH_3\text{-}H_2O]^+$). Intense EIMS fragment peaks at *m/z* 189, 191 and 218 were important in showing CA1 as having a skeletal structure of the lupane-type triterpenoid (Budzikiewicz, Djerassi and Williams, 1964).



Mass fragmentation of CA1

The ^{1}H NMR spectrum of CA1 (Figures 5a-5b) displayed the signals of 7 methyl protons as singlets at δ 0.74, 0.77, 0.81, 0.92, 0.95, 1.01 and 1.55 ppm. The presence of two exomethylene singlets (H_2-30) could be observed at δ 4.54 and 4.66 ppm, and a doublet of doublets attributable to the proton of the hydroxy-substituted position 3 at δ 3.16 ppm.

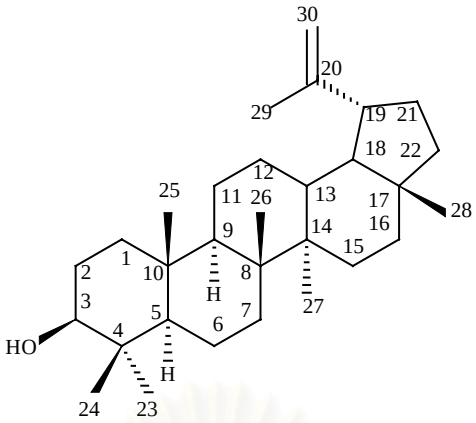
The ^{13}C-NMR spectrum (Figure 6) showed the signals of 30 carbon atoms, supporting the assignment of CA1 as a triterpenoid derivative. The DEPT-90 and DEPT-135 experiments (Figure 7) were performed to differentiate these 30 signals into those of seven methyl carbons at δ 14.6, 15.4, 16.0, 16.1, 18.0, 19.4 and

28.0 ppm, which could then be assigned as those of C-27, C-26, C-25, C-28, C-29 and C-23, respectively, whereas the eleven methylene carbons at δ 18.3, 21.0, 25.2, 27.5, 27.5, 30.0, 34.3, 35.6, 38.7, 40.0 and 109.3 ppm were assigned as those of C-6, C-11, C-12, C-2, C-15, C-21, C-7, C-1, C-22, and C-30, respectively, while the six methine carbons at δ 38.1, 48.0, 48.3, 50.5, 55.3 and 79.0 ppm were assigned as those of C-13, C-18, C-19, C-9, C-5 and C-3, respectively. Similarly, the six quaternary carbon signals at δ 37.2, 39.0, 40.8, 42.8, 43.0 and 150.8 ppm were assigned as those of C-10, C-4, C-8, C-14, C-17 and C-20, respectively.

Comparison of the carbon chemical shifts of compound CA1 with those of the previously reported lupeol (Sholichin *et al*., 1980), a lupane-type triterpenoid, revealed them to be fully in agreement (Table 12).

Therefore, compound CA1 was identified as the known triterpenoid lupeol, the structure of which is shown below.

Although lupeol is a ubiquitous natural compound, it was rarely reported as a constituent of bignoniaceous plants. Previously, there was only one report of these compound from *Macfadyena unguis-cati* (Duarte *et al*., 2000). To some extent, the presence of this compound explains the uses of *Macfadyena unguis-cati* in folk medicine as anti-inflammatory, antimalarial and antivenereal (Duarte *et al*., 2000), since lupeol has been shown as possessing *in vitro* activity against *Plasmodium falciparum* (Alves *et al*., 1997), and also exhibiting antifungal and germination inhibitory activities (Higa *et al*., 1998), antitumor and anti-inflammatory activities (Davis *et al*., 1994; Kuhl *et al*., 1984). An abundance source of lupeol in nature might prove to be useful in the development of medicinal agents.



Lupeol (CA1)

Table 12 Comparison of ^{13}C-NMR chemical shifts in $CDCl_3$ of CA1 and lupeol (Sholichin *et al.*, 1980)

Position	CA1 (ppm)	lupeol (ppm)	Position	CA1 (ppm)	lupeol (ppm)
1	38.7	38.7	16	35.6	35.6
2	27.5	27.5	17	43.0	43.0
3	79.0	79.0	18	48.0	48.0
4	39.0	38.9	19	48.3	48.3
5	55.3	55.3	20	150.8	150.9
6	18.3	18.3	21	30.0	29.9
7	34.3	34.3	22	40.0	40.0
8	40.8	40.9	23	28.0	28.0
9	50.5	50.5	24	15.4	15.3
10	37.2	37.2	25	16.1	16.1
11	21.0	21.0	26	16.0	16.0
12	25.2	25.2	27	14.6	14.6
13	38.1	38.1	28	18.0	18.0
14	42.8	42.9	29	19.4	19.3
15	27.5	27.5	30	109.3	109.3

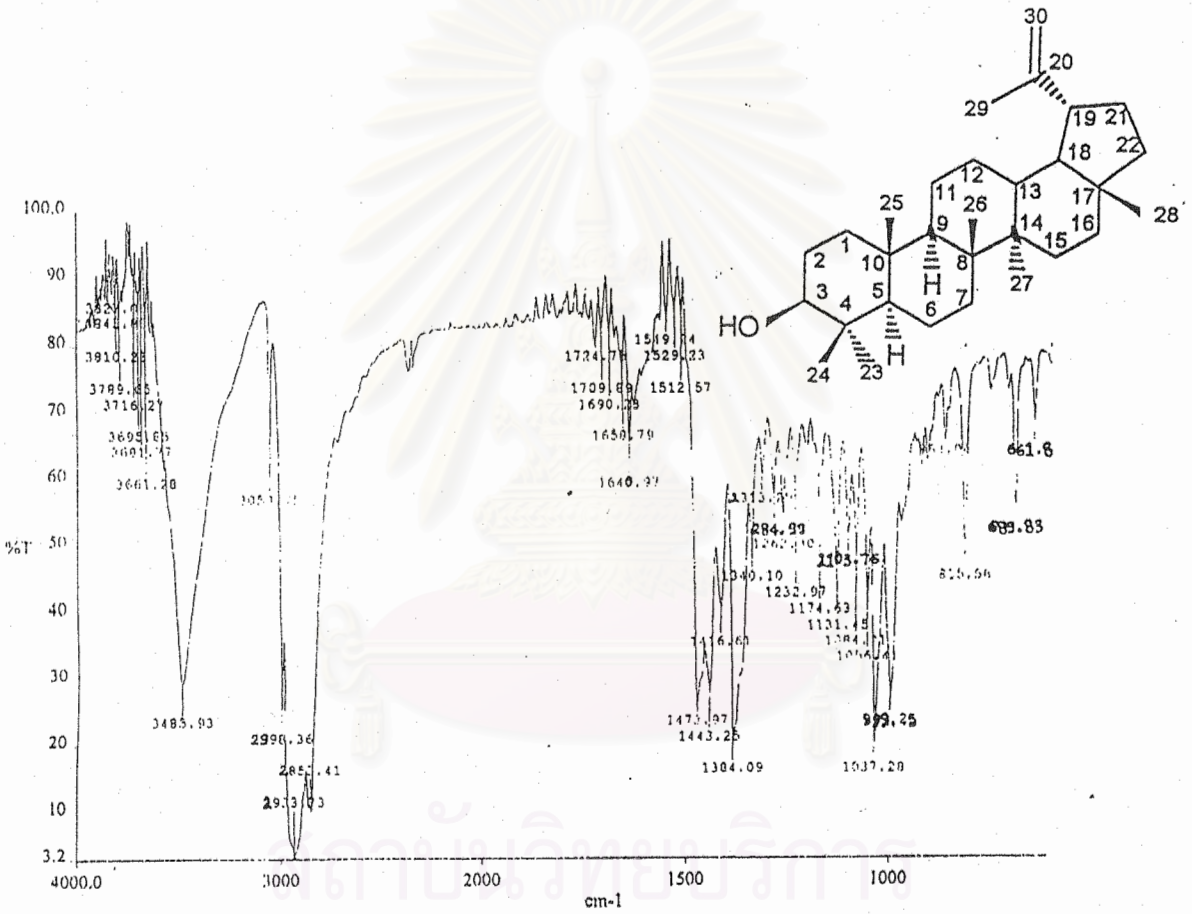


Figure 3 IR spectrum of compound CA1

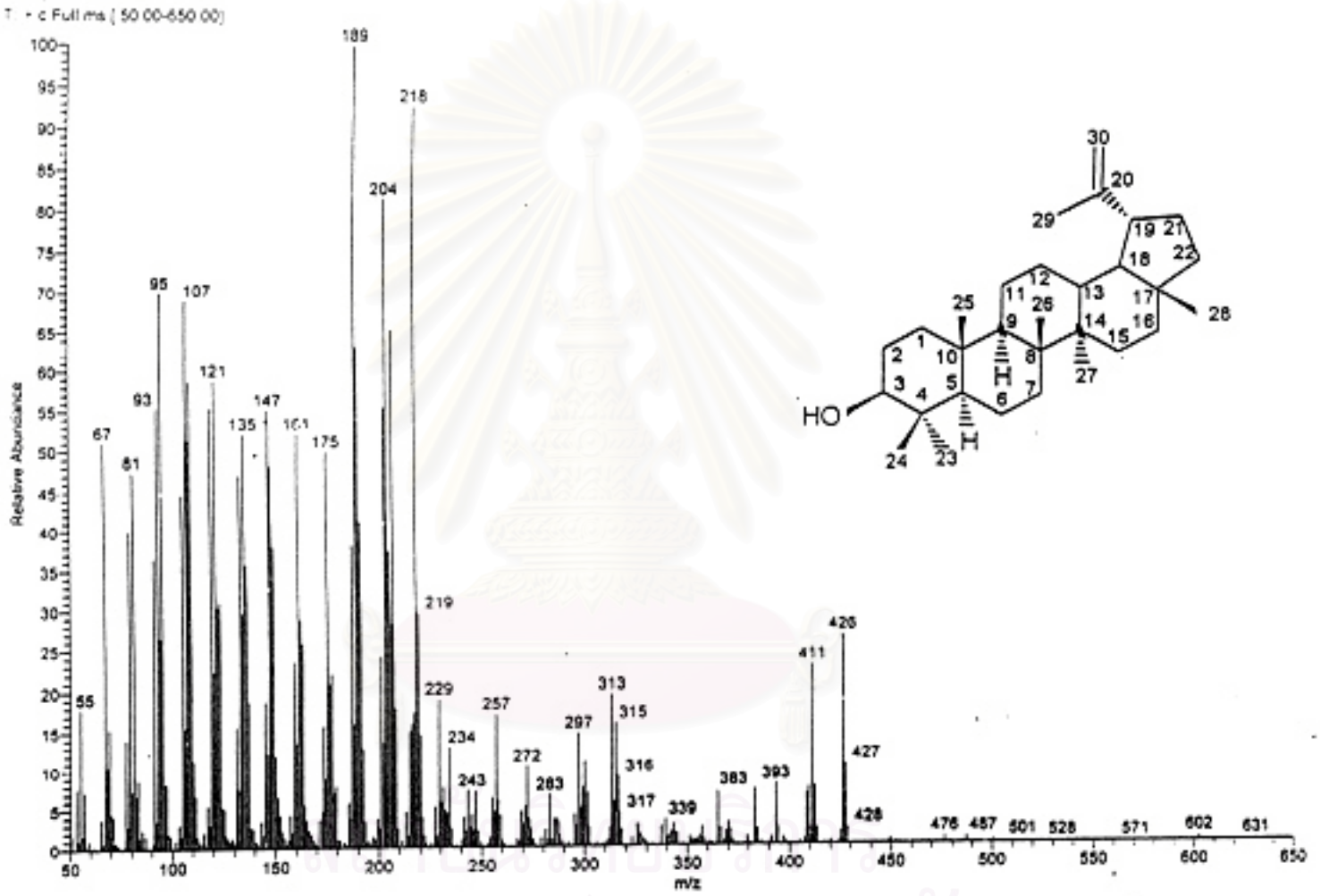


Figure 4 EI mass spectrum of compound CA1

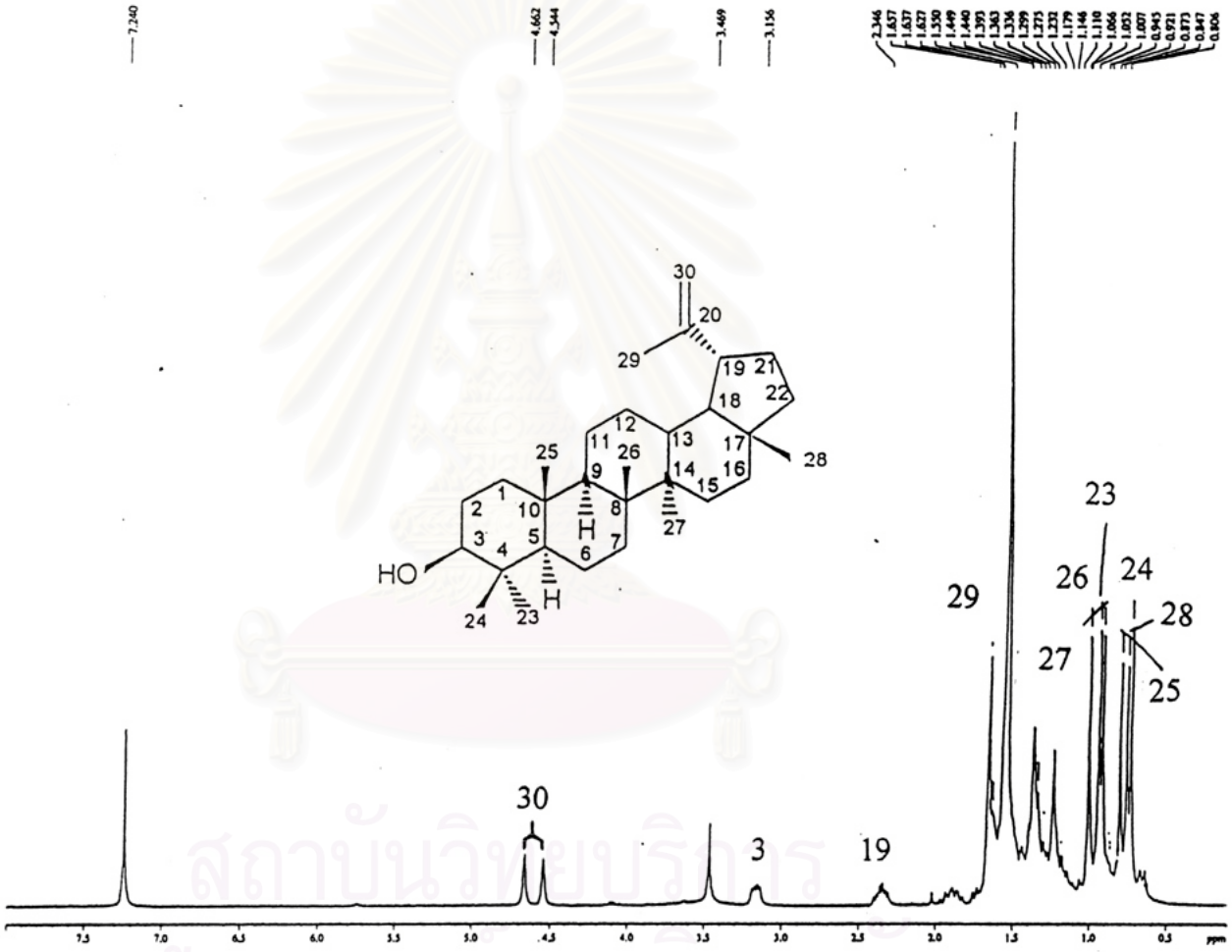


Figure 5a The 300 MHz ^{1}H NMR spectrum of compound CA1

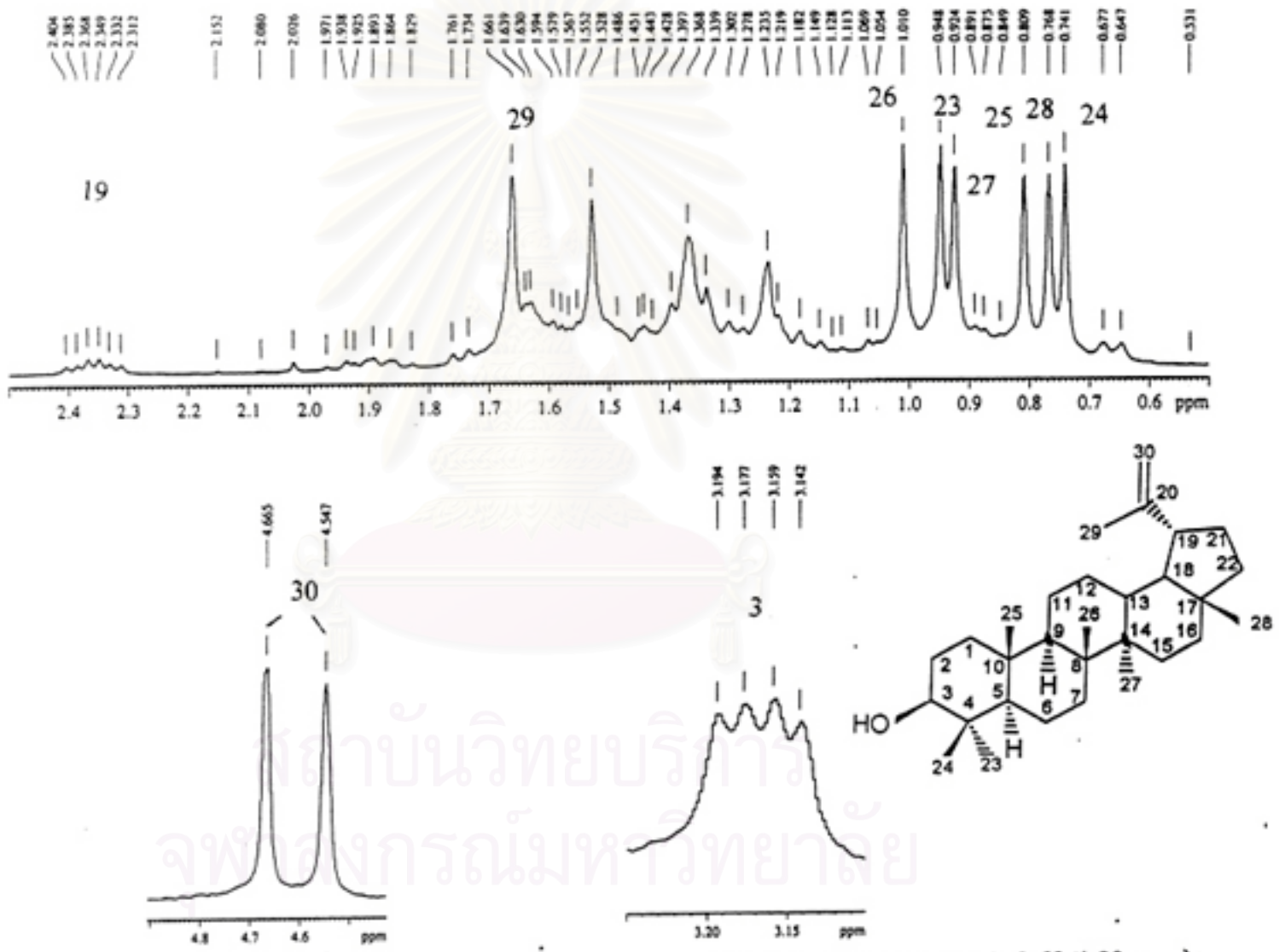


Figure 5b The 300 MHz ^{1}H NMR spectrum of compound CA1 (expanded from δ 0.60-4.80 ppm)

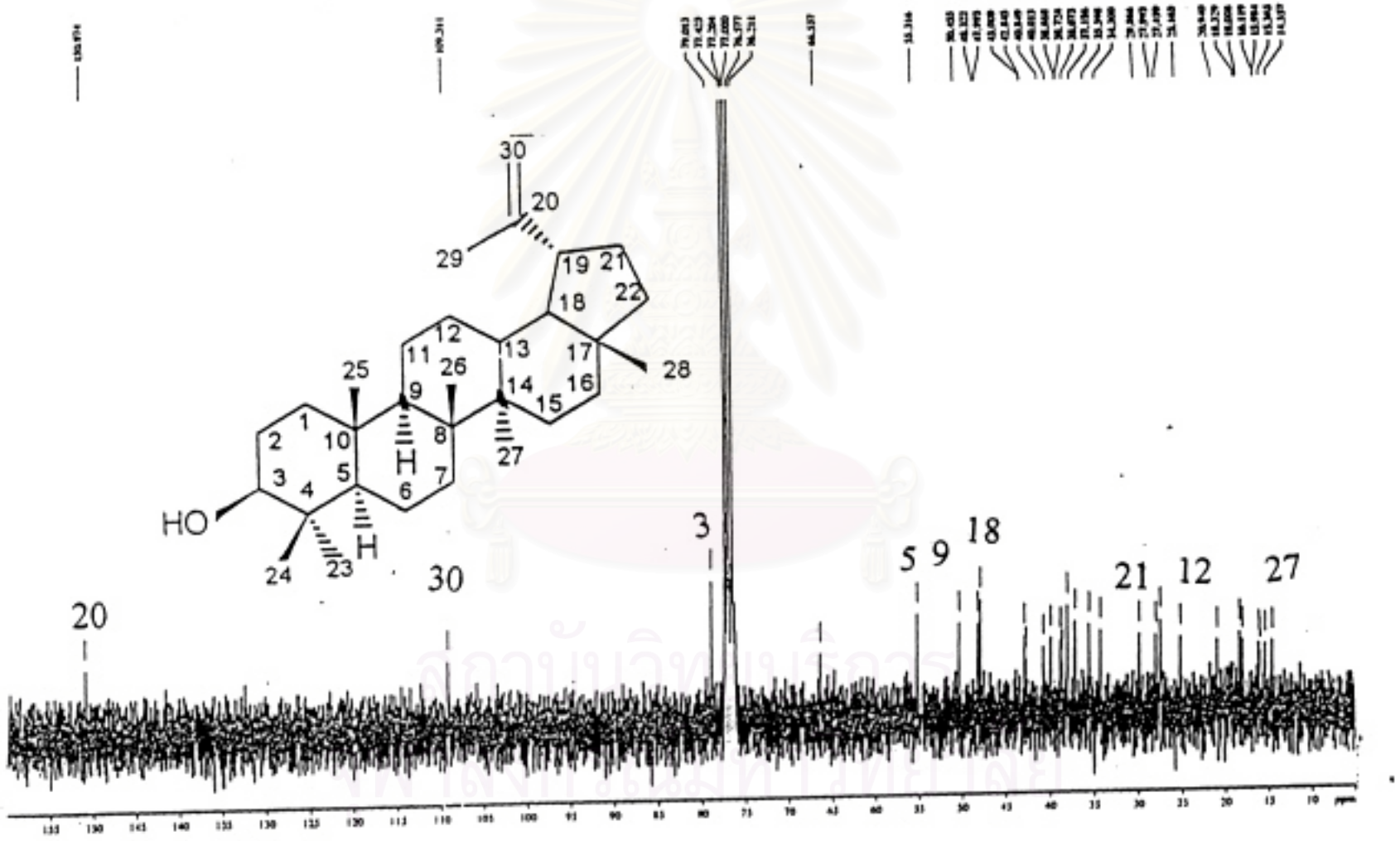


Figure 6 The 75 MHz ^{13}C NMR spectrum of compound CA1

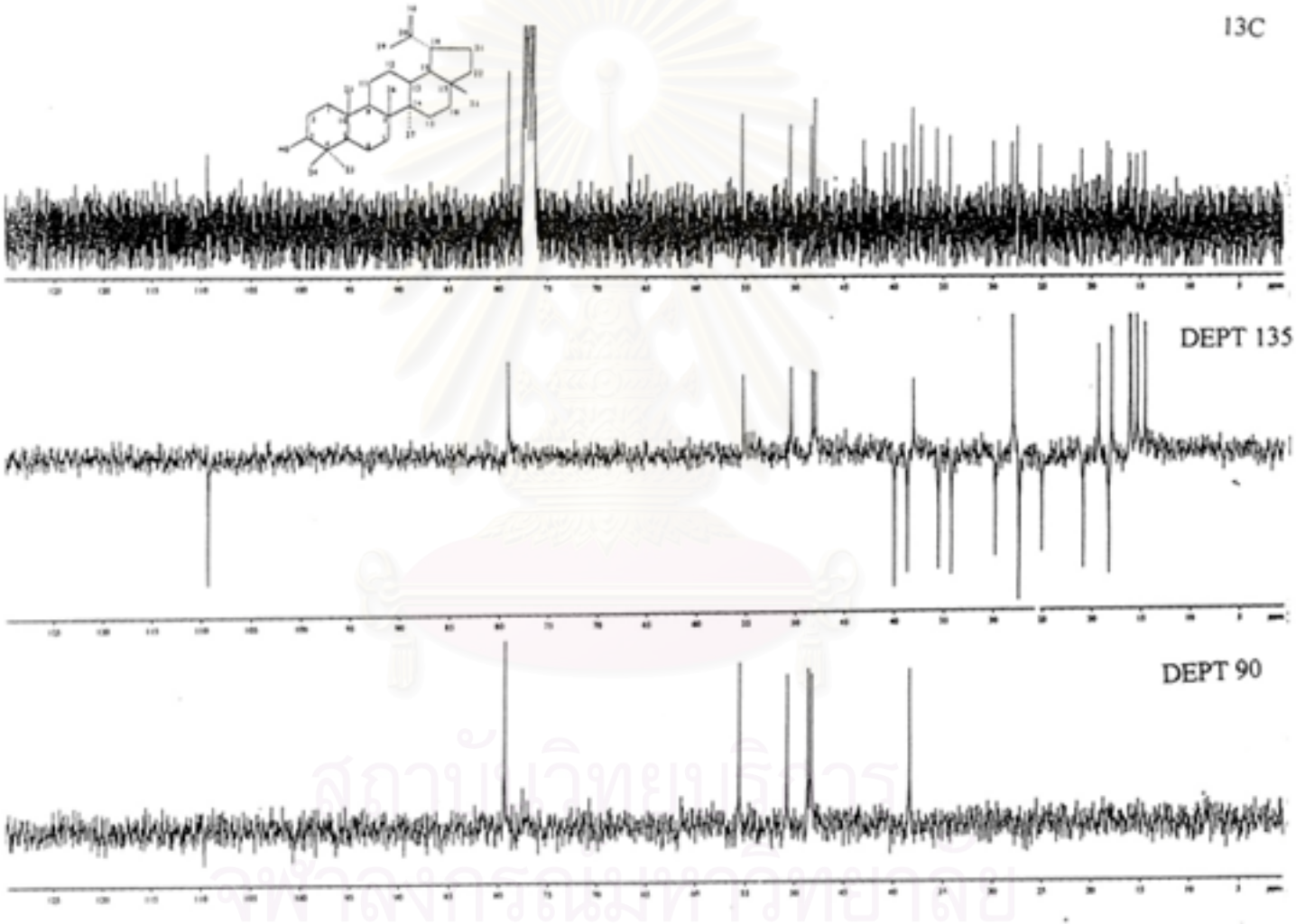


Figure 7 The 75 MHz ^{13}C-DEPT NMR spectrum of compound CA1

2. Identification of Compound CA2

Compound CA2 was recrystallized as white needles from hexane and gave a yellow color when visualized with 10% ethanolic sulphuric acid reagent. Its UV spectrum (Figure 10) showed three absorption maxima at λ_{max} 223, 260 and 290 nm, typical of benzoic acid derivatives (Kamath, Mehta and Bafna, 1975), while the IR spectrum (Figure 9) showed absorption bands at 2952 cm^{-1} (C-H stretching), 1599 (aromatic C=C), 1526 (aromatic C=C), 1682 (C=O), 1286, 1240, 1030 (C-O), the hydroxy absorption band at 3488 cm^{-1} and the broad carboxylic absorption band (broad band acid) at 3300-2400 cm^{-1}.

The EIMS of CA2 (Figure 8) displayed a prominent molecular ion peak (M^+) at *m/z* 168, corresponded to the molecular formula of $C_8H_8O_4$. Intense EIMS fragment peaks at *m/z* 153 ($[M - CH_3]^+$), 125 ($[M - COCH_3]^+$), 123 ($[M - COOH]^+$), 97 ($[M - CH=CH-COOH]^+$) were important in showing compound CA2 as having a skeletal structure of phenylpropanoid acid.

The ^1H NMR spectrum of CA2 (Figure 11) displayed an aromatic proton signal at δ 6.84 ppm (d, *J* = 7.5 Hz, H-5) ortho-coupled to another signal at δ 7.44 ppm (d, *J* = 7.5 Hz, H-6). The third aromatic signal appeared as a broad singlet at δ 7.43 ppm. A three-proton singlet at δ 3.80 ppm indicated the presence of one methoxy group on the aromatic ring.

The ^{13}C NMR spectrum (Figure 12) of CA2 gave 8 carbon signals. The DEPT-90 and DEPT-135 spectra (Figure 13), together with the ^1H-^{13}C HMQC experiment (Figure 15), helped in classifying these signals into those of a carbonyl carbon at δ 167.3 ppm, three quaternary carbons at δ 151.2 (C-4), 147.3

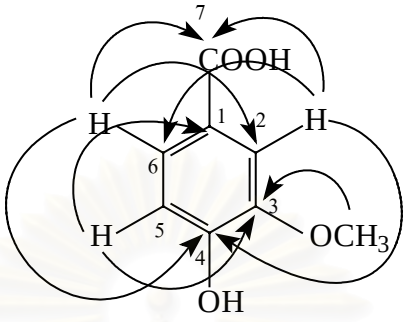
(C-3) and 121.8 (C-1)ppm, three methine carbons at δ 123.6 (C-6), 115.1 (C-5) and 112.9 (C-2) ppm, and one methoxy carbon at δ 55.7 ppm.

The structure of compound CA2 consists of an aromatic ring substituted with 3 functional groups: hydroxyl, methoxyl and carboxyl group. The positions of these moieties on the benzene ring were confirmed by the HMBC experiment (Figure 16). A three-bond correlation between the methoxyl proton at δ 3.80 ppm to C-3 signal (δ 147.3 ppm) placed this group at position 3. The downfield, hydroxy-substituted carbon signal of position 4 (δ 151.2 ppm) gave cross-peaks with both H-2 (δ 7.43 ppm, br s) and H-6 (δ 7.44 ppm, d, J = 7.5 Hz). These two proton signals also displayed three-bond correlation with the carbonyl carbon (δ 167.3 ppm) of the carboxyl group (C-7), therefore establishing the position of this substituent at C-1. Major HMBC correlations in the structure of compound CA2 are summarized in Table 13.

Compound CA2 was thus identified as vanillic acid. Comparison of the ^{13}C-NMR chemical shifts of CA2 and reported values for vanillic acid was also done, as shown in Table 14.

Although vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a well-known phenolic compounds, it was rarely reported as a constituent of bignoniaceous plants. Previously, there were only two reports of this compound from *Spathodea campanulata* (Niyonzima *et al*., 1991) and *Crescentia cujete* (Binutu, 1997). Vanillic acid has been reported as possessing antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) radical scavenging assays (Sakushima, Coskun, and Maoka, 1995). To some extent, vanillic acid has been shown in the literature to exert antibacterial activity (Fernandez, Garcia and Saenz, 1996). It possesses antibacterial activity against the growth of *Escherichia coli, Klebsiella pneumoniae* and *Bacillus cereus* and also exhibiting antifungal

activity by inhibiting the growth and aflatoxin production by both *Aspergillus flavus* and *Aspergillus parasiticus* (Aziz *et al*., 1998).



HMBC correlations of CA2

Vanillic Acid (CA2)

Table 13 ^{1}H , ^{13}C NMR assignment and HMBC correlations of compound CA2

Position	δ_C (ppm)	δ_H (ppm)	HMBC correlations
1	121.8	-	-
2	112.9	7.43, br s	C-4, C-6, C-7
3	147.3	-	-
4	151.2	-	-
5	115.1	6.84, d, *J* = 7.5 Hz	C-1, C-3
6	123.6	7.44, d, *J* = 7.5 Hz	C-2, C-4, C-7
7	167.3	-	-
3-OCH_3	55.7	3.80, s	C-3

Table 14 Comparison of ^{13}C-NMR chemical shifts in DMSO-d_6 of CA2 and vanillic acid (Scott, 1972)

Position	CA2 (ppm)	vanillic acid (ppm)
1	121.8	122.8
2	112.9	113.5
3	147.3	148.0
4	151.2	152.0
5	115.1	115.5
6	123.6	124.9
7	167.3	167.9
OCH_3	55.7	56.4

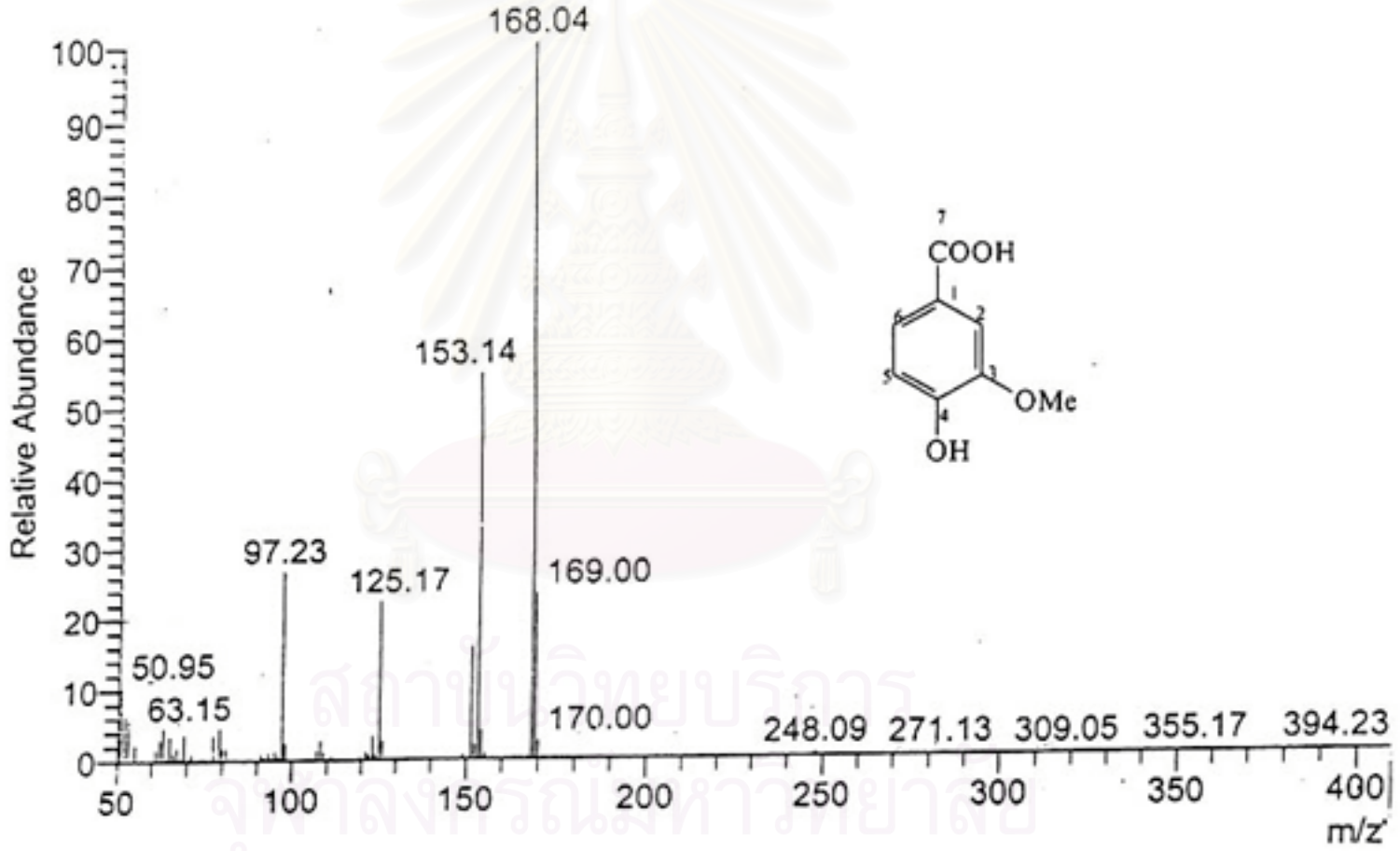


Figure 8 EI mass spectrum of compound CA2

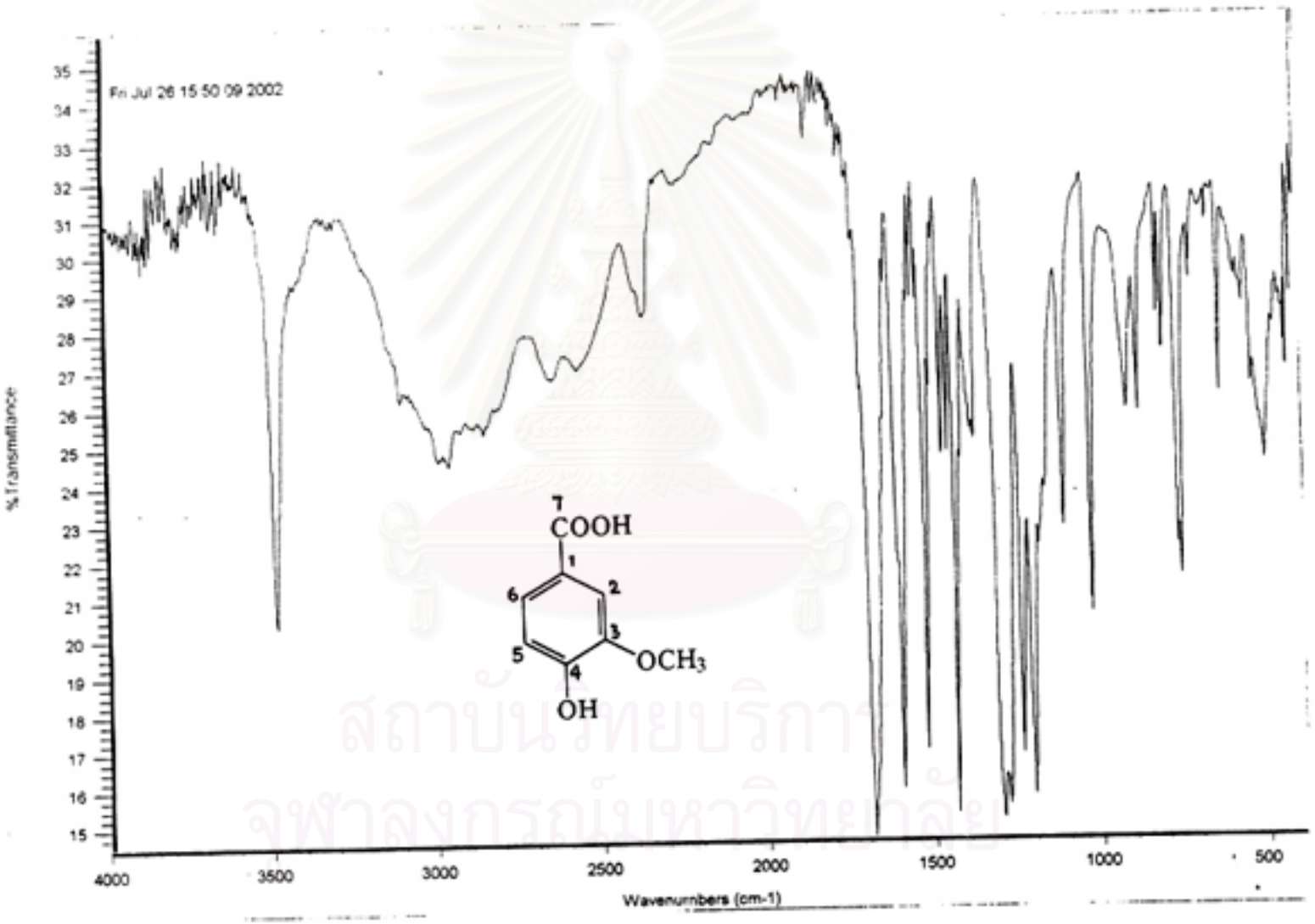


Figure 9 IR spectrum of compound CA2

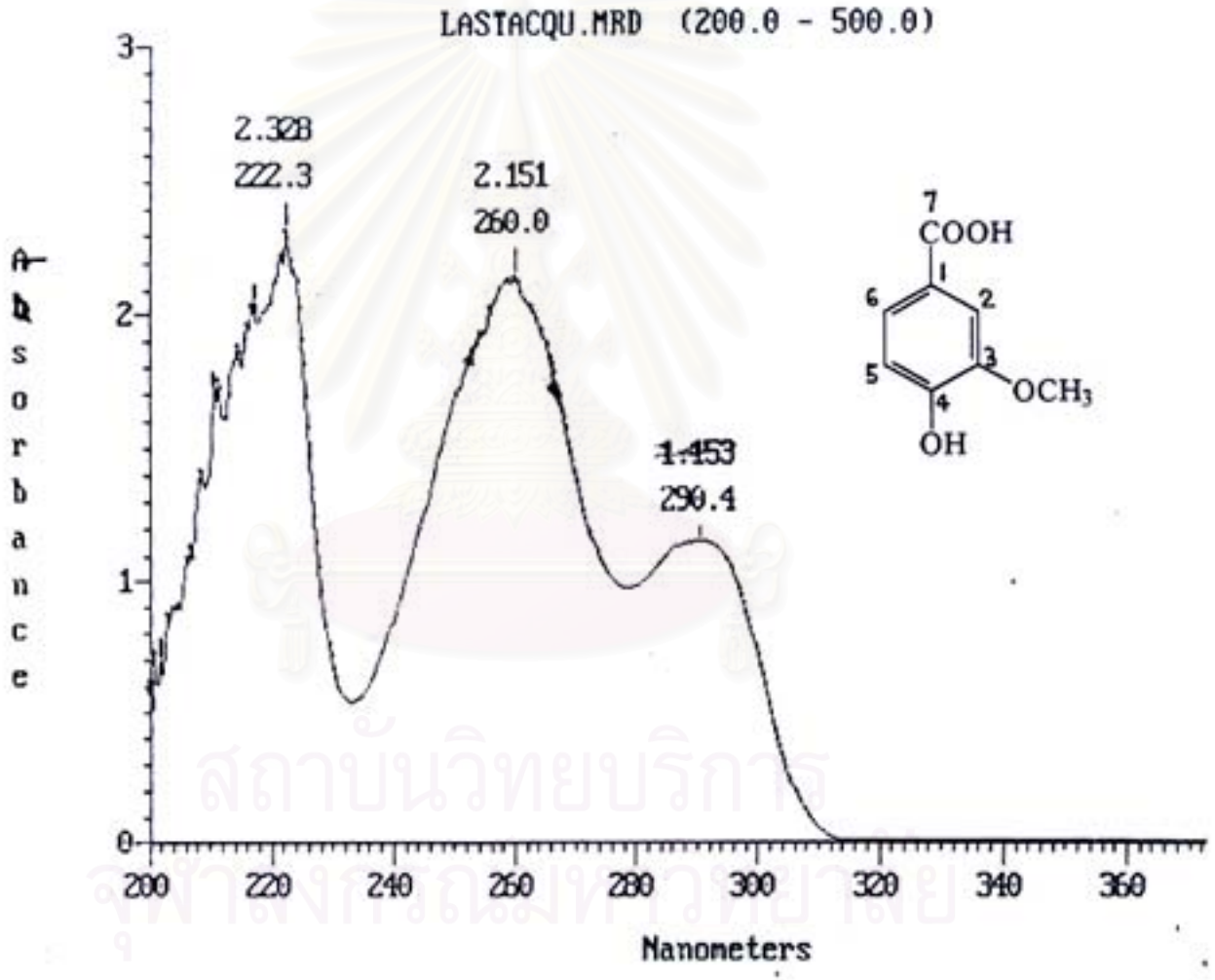


Figure 10 UV spectrum of compound CA2

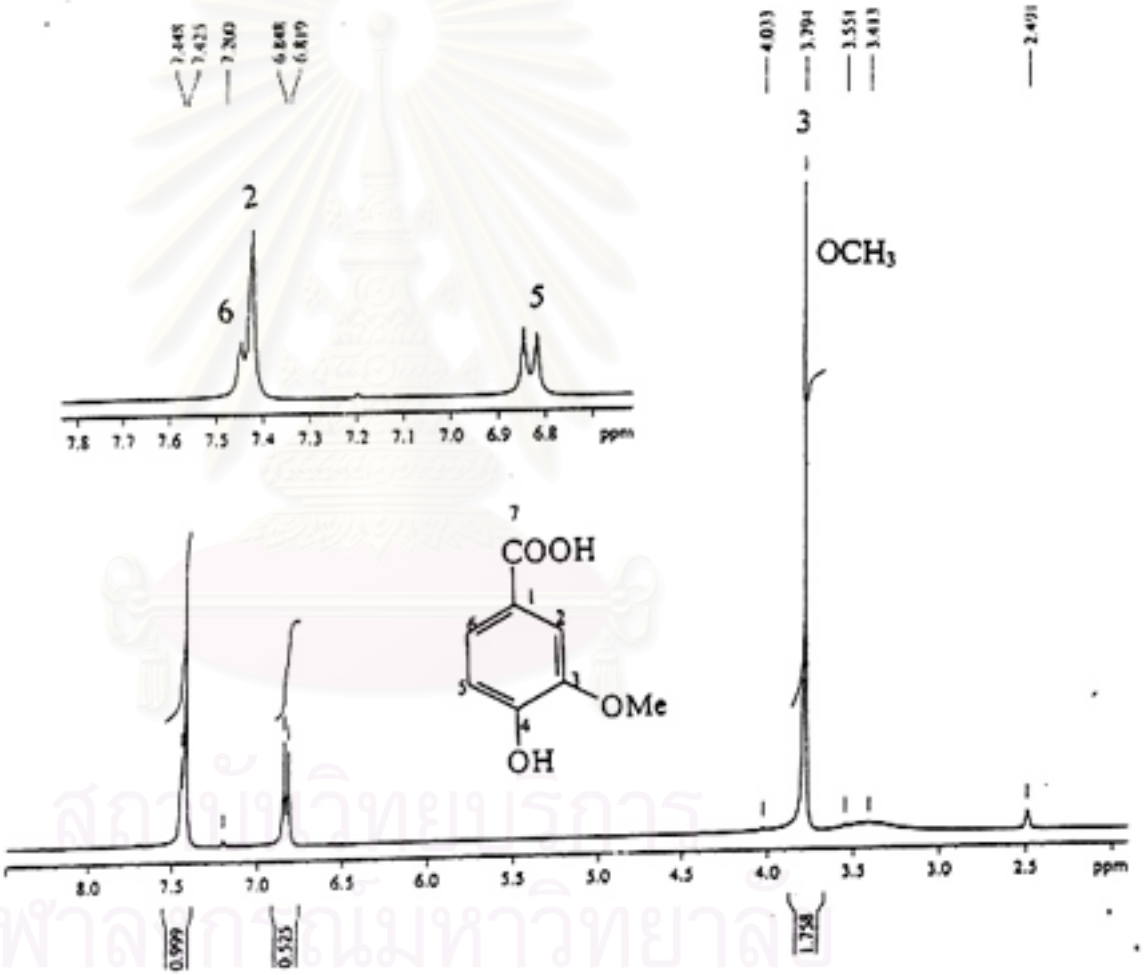


Figure 11 The 300 MHZ ^{1}H NMR spectrum of compound CA2

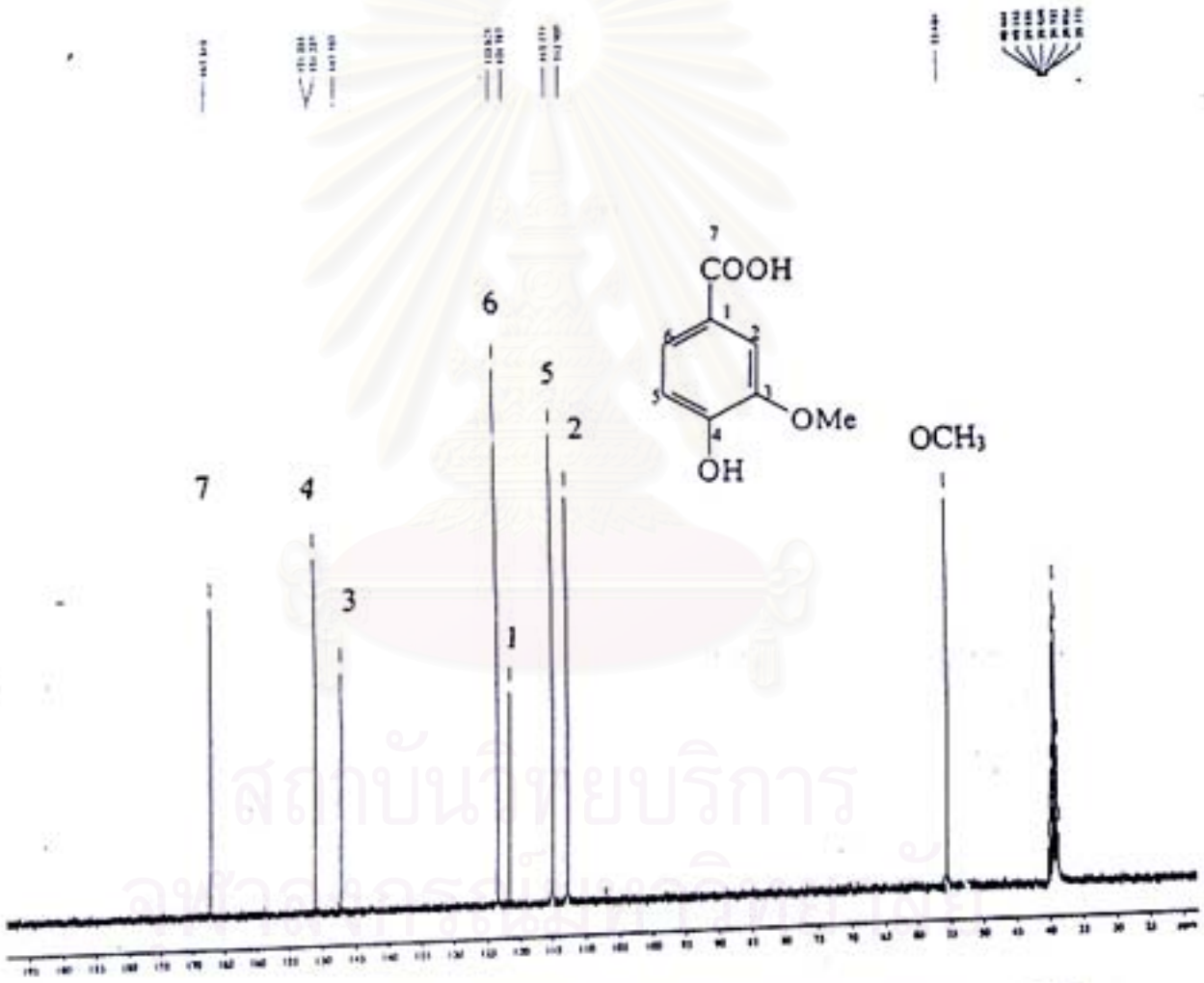
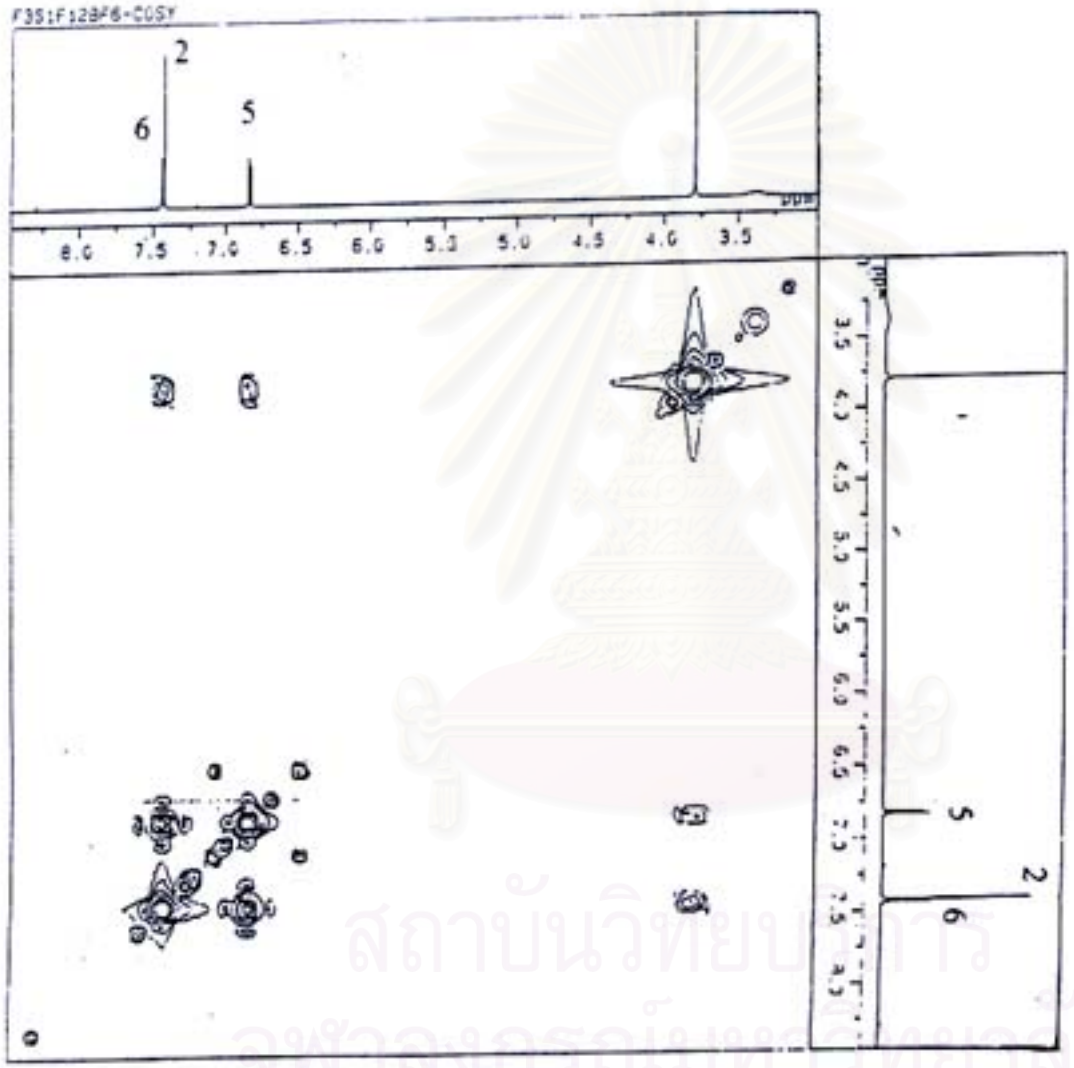


Figure 12 The 75 MHz ^{13}C NMR spectrum of compound CA2

Figure 13 The 75 MHz ^{13}C-DEPT NMR spectrum of compound CA2



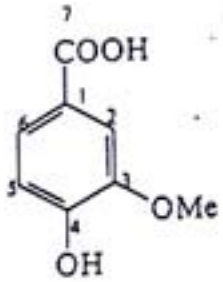


Figure 14 ^{1}H-^{1}H COSY spectrum of compound CA2

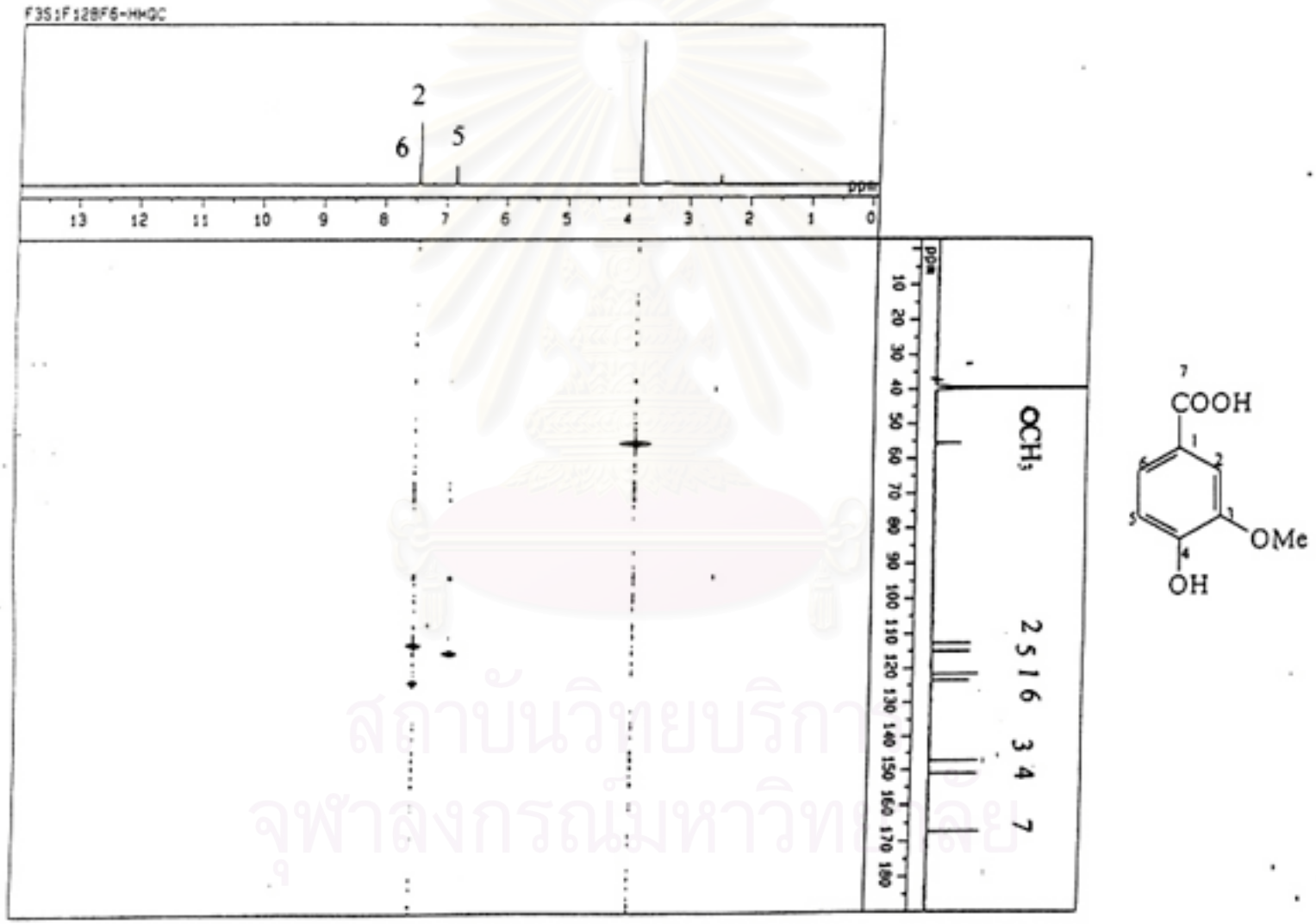


Figure 15a The HETCOR spectrum of compound CA2

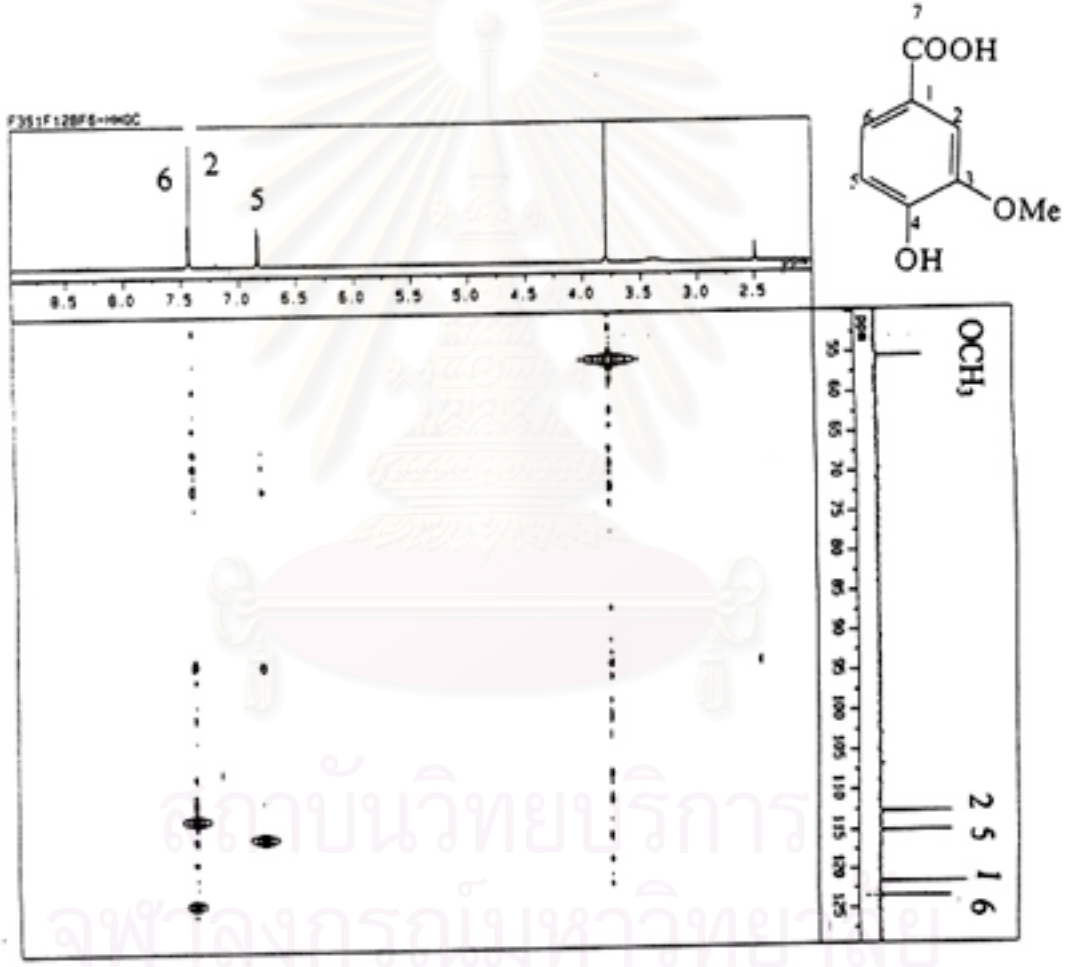


Figure 15b The HETCOR spectrum of compound CA2 (expanded)

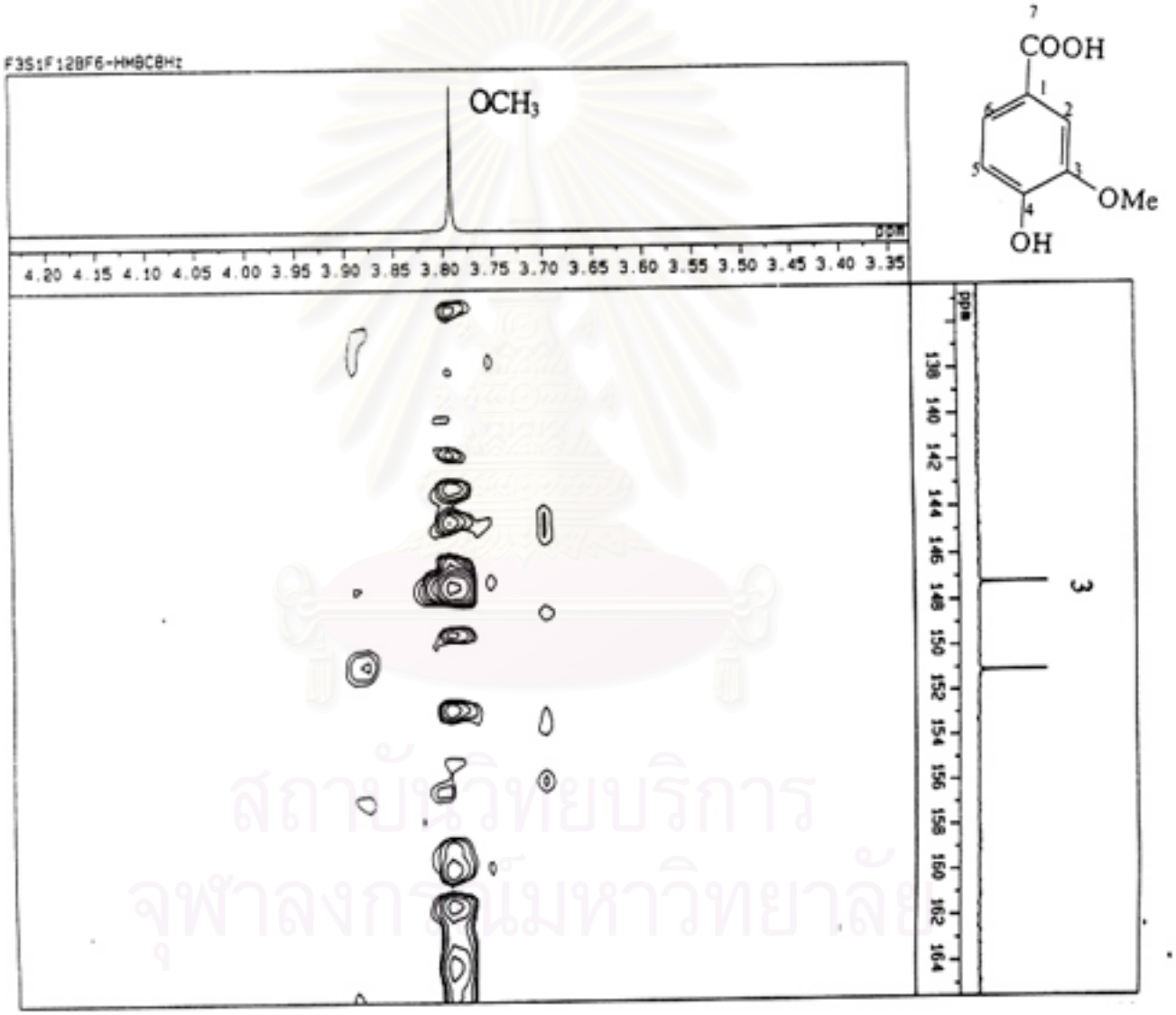


Figure 16a ^{1}H-^{13}C HMBC spectrum of compound CA2

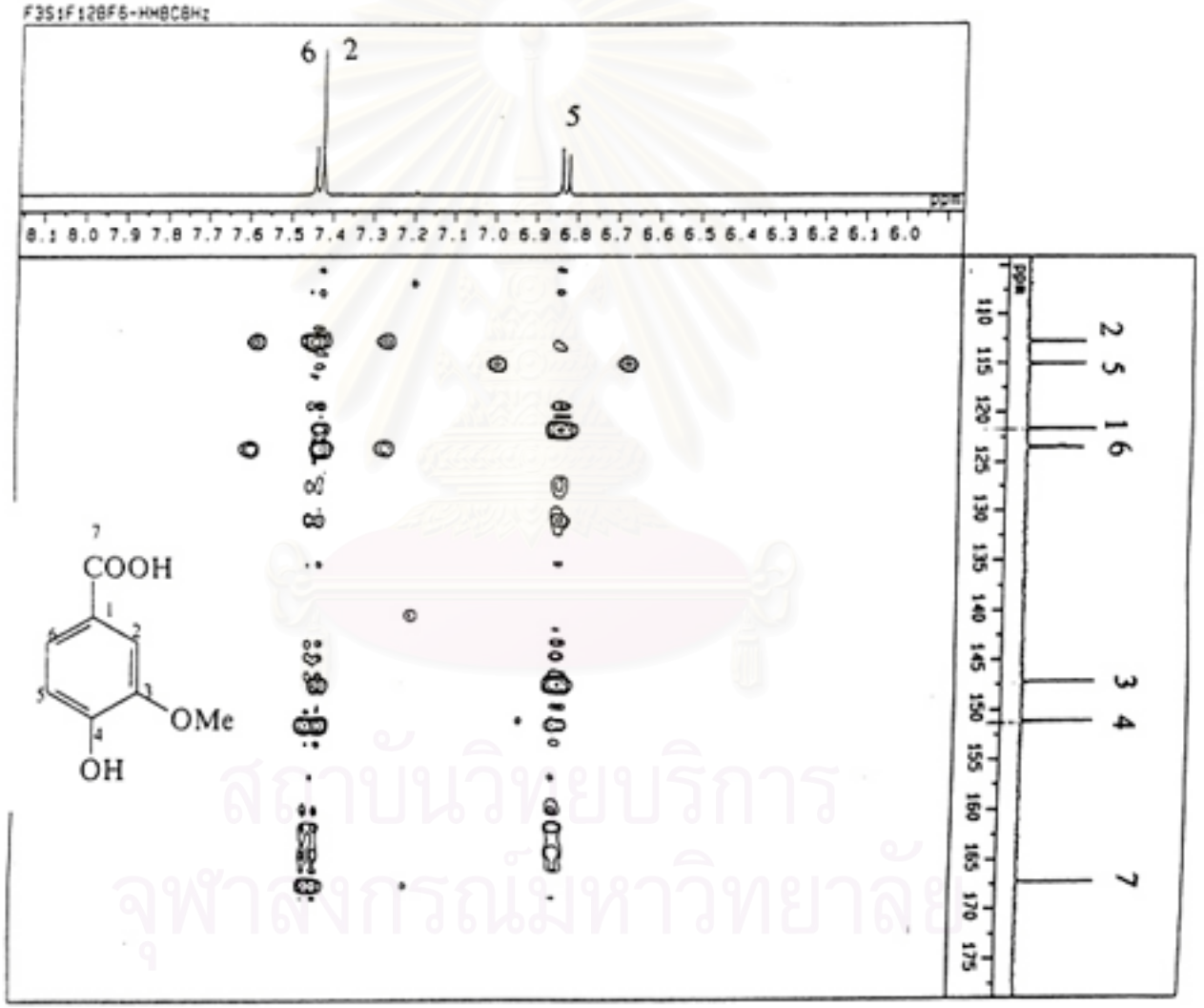


Figure 16b ^{1}H-^{13}C HMBC spectrum of compound CA2 (continued)

3. Identification of Compound CA3

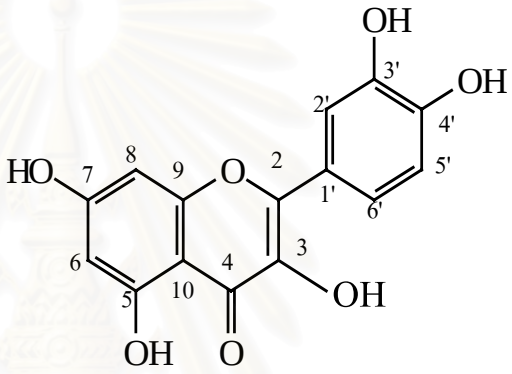
Compound CA3 was recrystallized as pale yellow needles from chloroform. It gave pale yellow color when sprayed with 10% ethanolic sulphuric acid and heated. The compound produced a crimson color when subjected to Shinoda test and gave greenish-brown color with $FeCl_3$, suggesting the presence of a flavonoid nucleus. The IR spectrum of CA3 (Figure 17) revealed absorption bands at 3418, 1660, 1600 and 1500 cm^{-1}, suggesting the presence of hydroxyl groups (3418 cm^{-1}), chelated carbonyl (1660 cm^{-1}) and aromatic ring (1600, 1500 cm^{-1}) , respectively, whereas the UV spectrum (Figure 18) exhibited absorption maxima at 256 and 357 nm, characteristic of a flavonol skeleton (Markham, 1982).

The EIMS of CA3 (Figure 19) showed the molecular ion (M^+) peak at *m/z* 610, corresponding to the molecular formula $C_{27}H_{30}O_{16}$. The presence of a fragment ion at *m/z* 449 indicated the loss of a rhamnose moiety from the molecule, whereas a fragment peak at *m/z* 269 represents the loss of two sugar moieties and two additional hydroxy groups from the flavonoid aglycone. The *m/z* 153 peak in the mass spectrum suggested ring A plus the carbonyl group of the flavonoid structure and the *m/z* 77 fragment ion confirmed the presence of aromatic ring (s) in the structure.

The ^{1}H-NMR spectrum of CA3 (Figures 20a-20b) showed the substitution pattern of the aromatic A and B-rings. The substitution pattern of ring A (5-OH and 7-OH) was substantiated by the *meta* coupling of the two aromatic proton signals at δ 6.19 and δ 6.38 ppm, which could be assigned to H-6 and H-8, respectively. Both signal appeared as broad singlets that gave cross-peak in the ^{1}H - ^{1}H COSY spectrum (Figure 21). The substitution pattern of ring B was deduced from the *ortho* coupling of aromatic proton at δ 7.52 ppm (br d, J = 7.8 Hz, H-6′) to the signal at 6.84 ppm (d, J = 7.8 Hz, H-5′) and the meta

coupling of the same proton to another signal at δ 7.52 ppm (br s, H-2′). Therefore, two hydroxy groups could be assigned to the 3′ and 4′ position of ring B.

From these evidences, the aglycone of this flavonoid glycoside was identified as quercetin (3, 5, 7, 3′,4′-pentahydroxyflavone) a well-known and widely distributed flavonol.

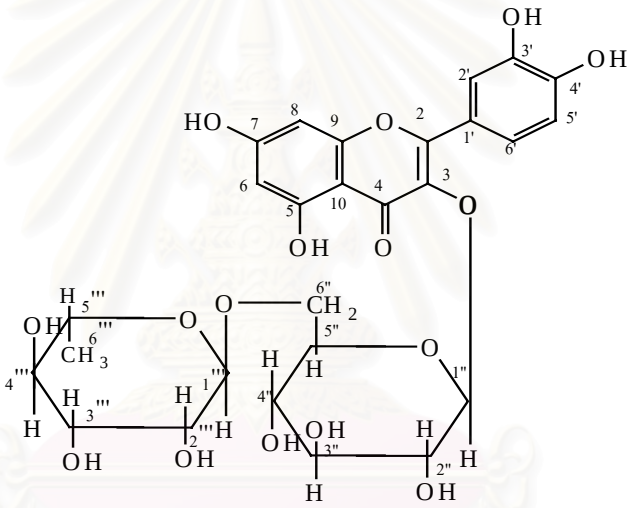


The ^{13}C NMR spectrum of CA3 (Figure 22) exhibited 27 signals. DEPT (Figures 23a-23b) and ^{1}H-^{13}C HMQC experiment (Figure 24) were useful in classifying these signals into those of one carbonyl carbon at δ 177.4 ppm (C-4), nine quaternary carbons at δ 164.8 (C-7), 161.1 (C-9), 157.0 (C-5), 156.8 (C-2), 148.8 (C-4′), 145.1 (C-3′), 133.7 (C-3), 121.3 (C-1′), and 104.3 (C-10), and five methine carbons at δ 121.8 (C-6′), 116.4 (C-5′), 115.9 (C-2′), 99.4 (C-6), and 94.3 (C-8) ppm. The other twelve carbon signals were of the rutinose moiety connected at the 3-OH of the aglycone. Six carbon signals at δ 101.8, 76.6, 76.1, 74.3, 70.7 and 67.3 ppm were those of a glucose unit, connected at position 6 to position 1 of a rhamnose unit which gave six more signals at δ 101.1, 72.1, 70.8,

70.2, 68.5 and 18.1 ppm reminiscent of a rhamnosyl unit.

Therefore, compound CA3 was identified as rutin (quercetin-3-rutinoside). Comparison of both proton and carbon chemical shifts of CA3 with those of rutin previously reported (Lin *et al.,* 2000) revealed them to be fully in agreement.

The structure of CA3 is shown below.



Rutin (CA3)

Previously, rutin has already been reported from *Crescentia alata* (Autore *et al*., 2001) and another bignoniaceous plants, *Tecomella undulata* (Azam and Ghanim, 2000). Rutin has been used medicinally in the treatment of many different ailments. For example, it is an antioxidant with potential use for strengthening the immune system. One study showed rutin to be effective in

reducing oxidative damage to red blood cells (Grinberg, Rachmilewitz and Newmark, 1994). It may also contain anti-inflammatory and vasoactive properties (Casa *et al*., 2000). It possesses beneficial protective effects against reflux oesophagitis by the inhibition of gastric acid secretion, oxidative stress, inflammatory cytokine production (i.e. interleukin-1-β (IL-1-β), and intracellular calcium mobilization in polymorphonucleocytes (PMNs) in rats (Shin *et al.*, 2002). To some extent, Rutin enhanced antibacterial activities of flavonoids against *Bacillus cereus* and *Salmonella enteritidis* (Arima, Ashida and Danno, 2002) and it exhihited hepatoprotective effect against paracetamol and CCl_4-induced hepatotoxicity in rodents (Janbaz, Saeed and Gilani, 2002). Furthermore, it has been found to show significant analgesic activity (Harborne and Williams, 2000) and also to exhibit hypoglycemic activity in rat (Onunkwo, Akah and Udeala, 1998). Therefore, rutin might be useful in the treatment of clinical disorders.

Table 15 Comparison of ^{1}H NMR chemical shift in DMSO-d_6 of CA3 and rutin (Lin *et al.*, 2000)

Position	CA3 (ppm)	rutin (ppm)
6	6.19 br s	6.18 d (J = 2.0 Hz)
8	6.38 br s	6.37 d (J = 2.0 Hz)
2′	7.52 br s	7.52 d (J = 2.0 Hz)
5′	6.84 d (J = 7.8 Hz)	6.83 d (J = 9.0 Hz)
6′	7.52 br d (J = 7.8 Hz)	7.53 dd (J = 9.0, 2.0 Hz)
1″	5.33 d (J = 6.0 Hz)	5.33 d (J = 6.9 Hz)
1‴	4.37 s	4.39 br s
CH_3	0.98 d (J = 6.0 Hz)	0.99 d (J = 6.0 Hz)

Table 16 Comparison of ^{13}C NMR chemical shift in DMSO-d_6 of CA3 and rutin (Lin *et al*., 2000)

Position	CA3 (ppm)	Rutin (ppm)
2	156.8	156.8
3	133.7	133.6
4	177.4	177.4
5	157.0	156.6
6	99.4	98.8
7	164.8	165.8
8	94.3	93.6
9	161.1	161.2
10	104.3	105.2
1′	121.3	121.6
2′	115.9	115.3
3′	145.1	144.6
4′	148.8	148.3
5′	116.4	116.5
6′	121.8	121.6
1″	101.8	101.8
2″	74.3	74.3
3″	76.6	76.6
4″	70.7	70.7
5″	76.1	76.1
6″	67.3	67.3
1‴	101.1	101.1
2‴	70.2	70.2
3‴	70.8	70.3
4‴	72.1	72.1
5‴	68.5	68.5
6‴	18.1	17.9

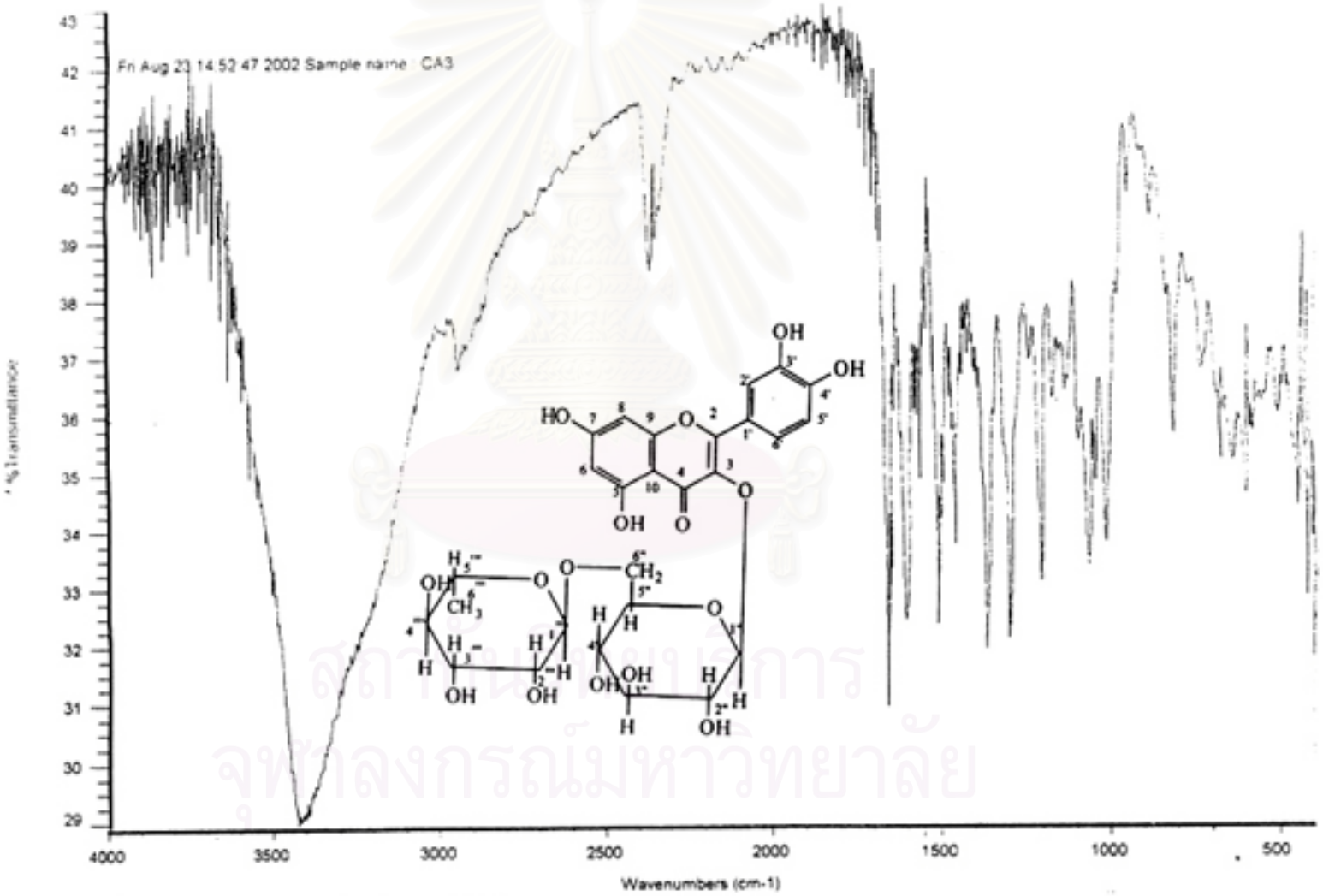


Figure 17 IR spectrum of compound CA3

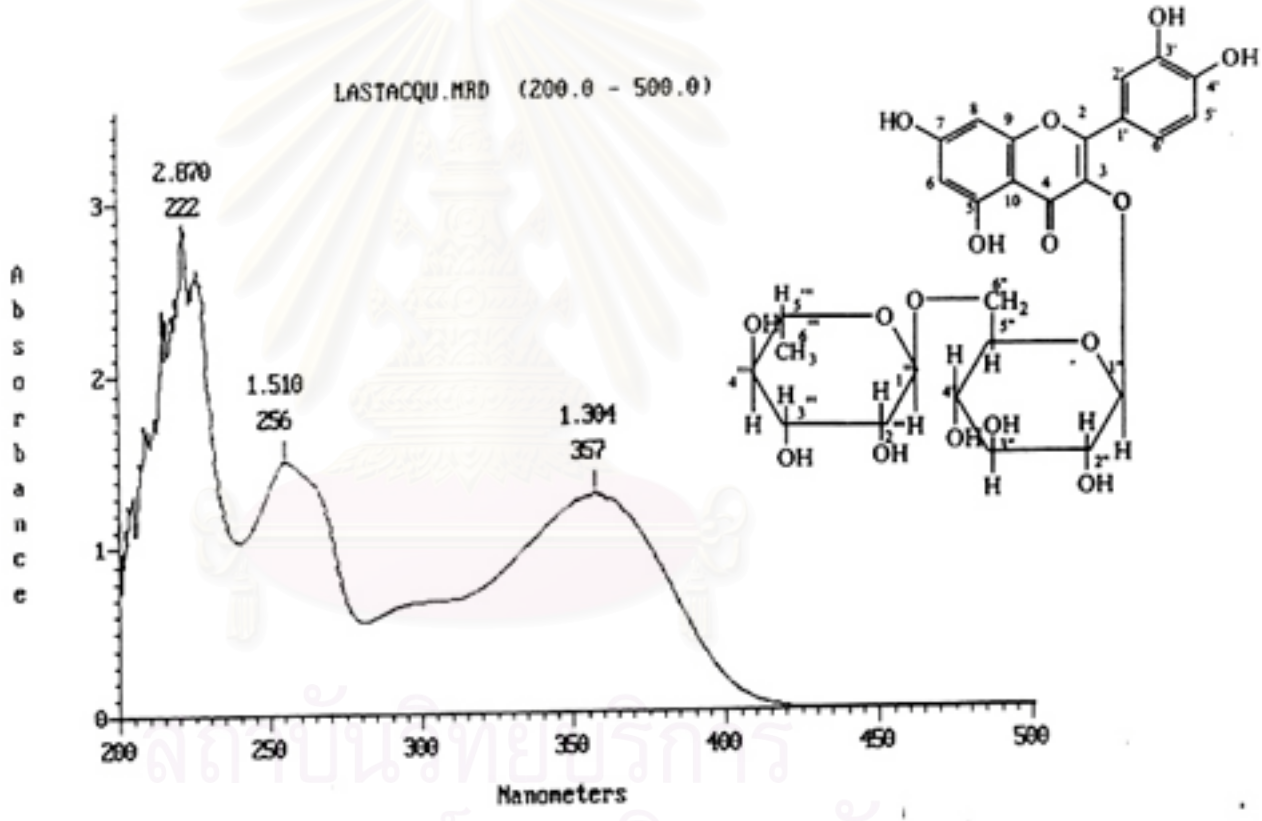


Figure 18 UV spectrum of compound CA3

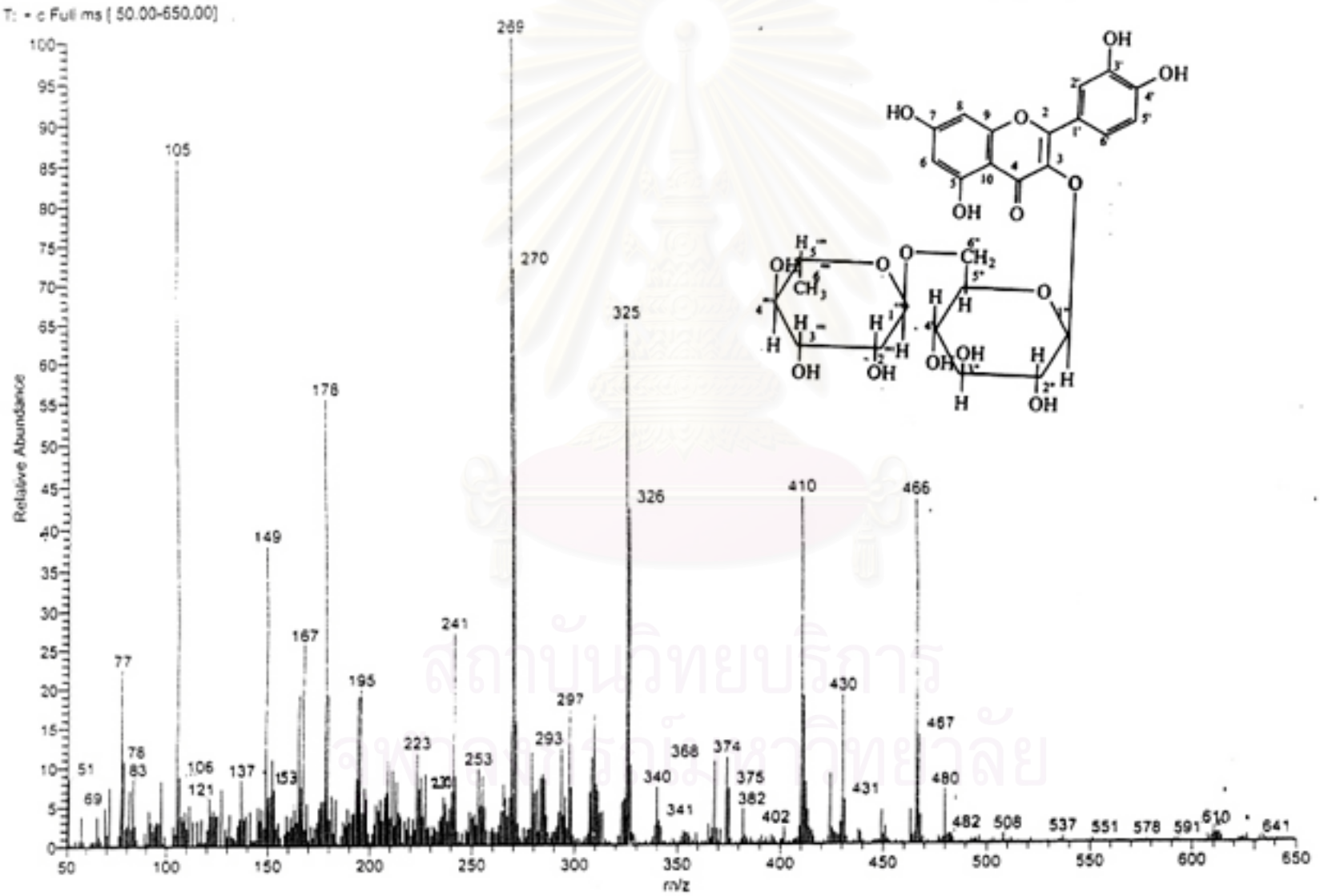


Figure 19 EI mass spectrum of compound CA3

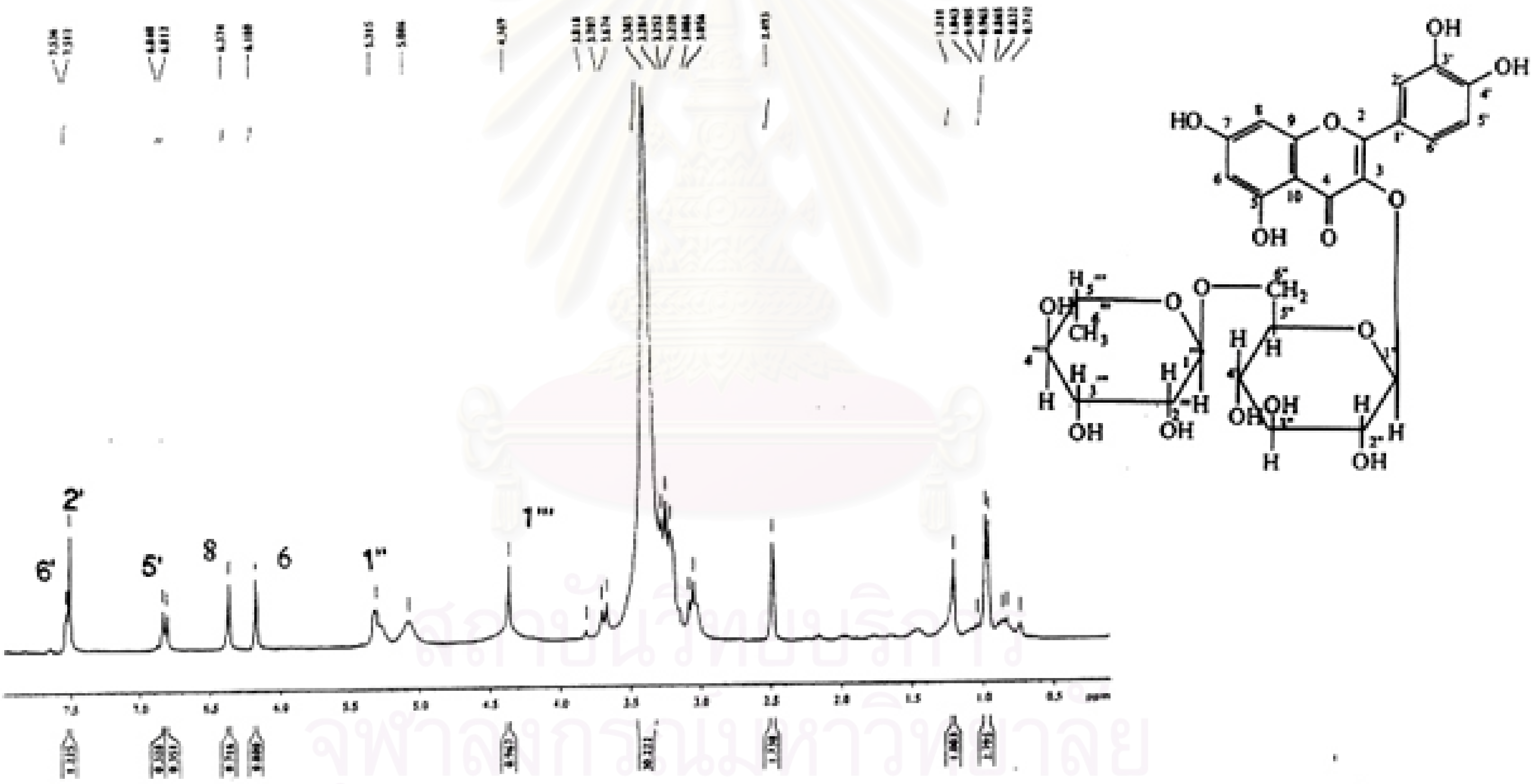


Figure 20a The 300 MHz ^{1}H NMR spectrum of compound CA3

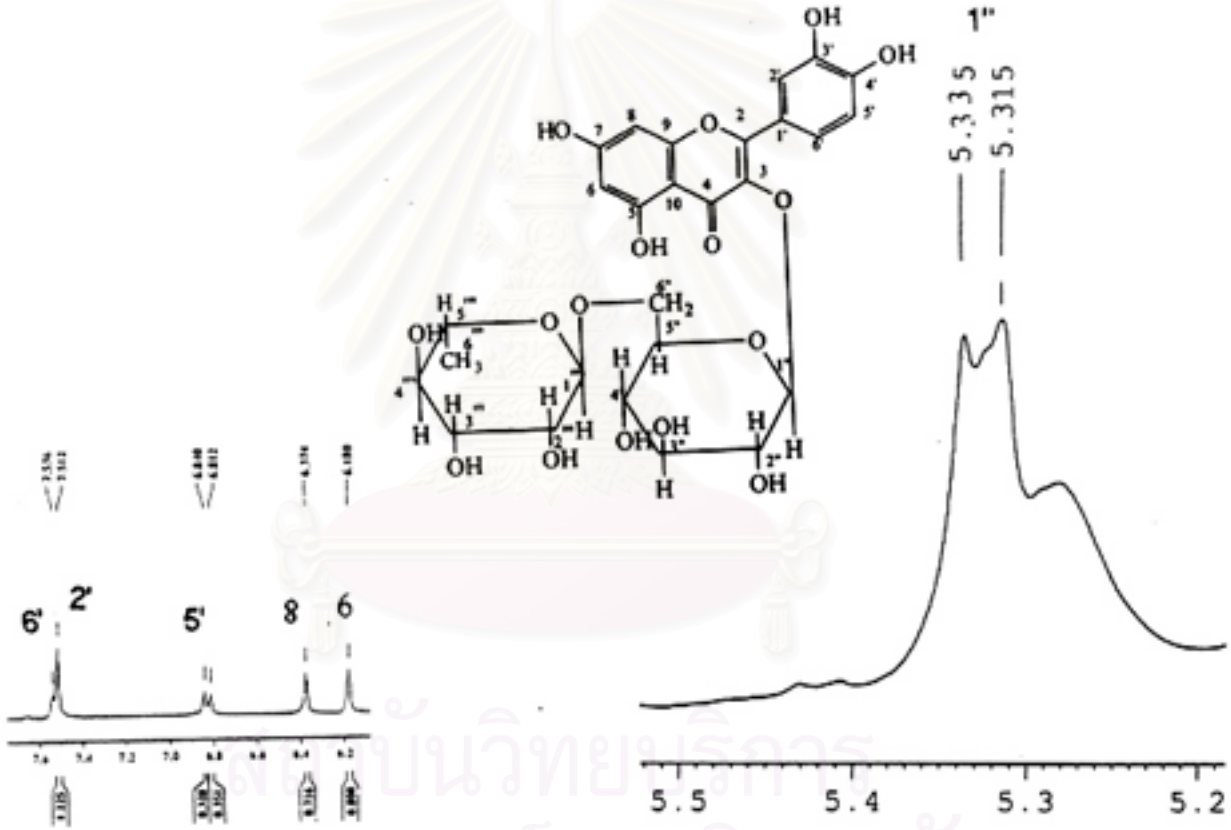


Figure 20b The 300 MHZ ^{1}H NMR spectrum of compound CA3 (expanded)

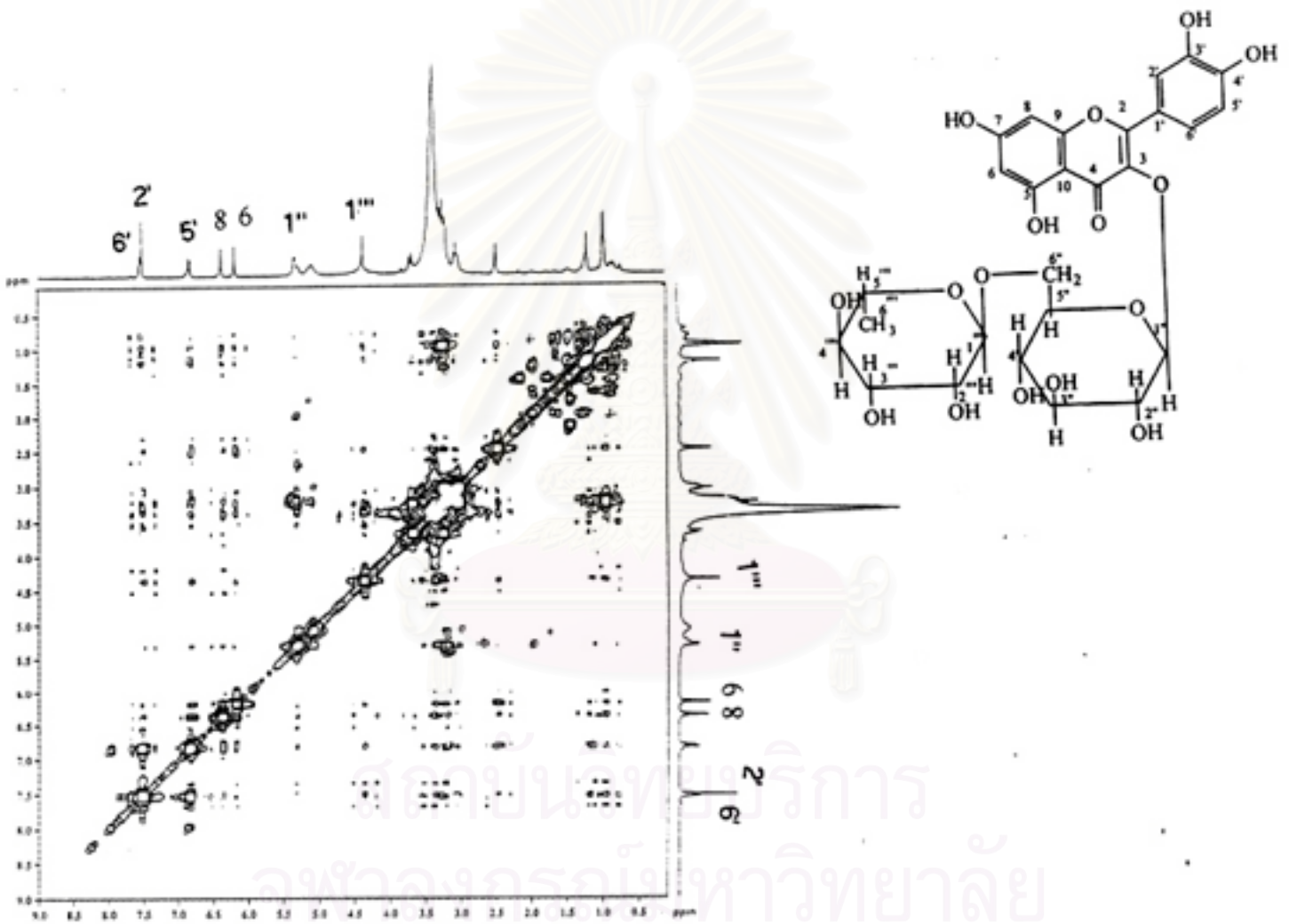


Figure 21 ^{1}H - ^{1}H COSY spectrum of compound CA3

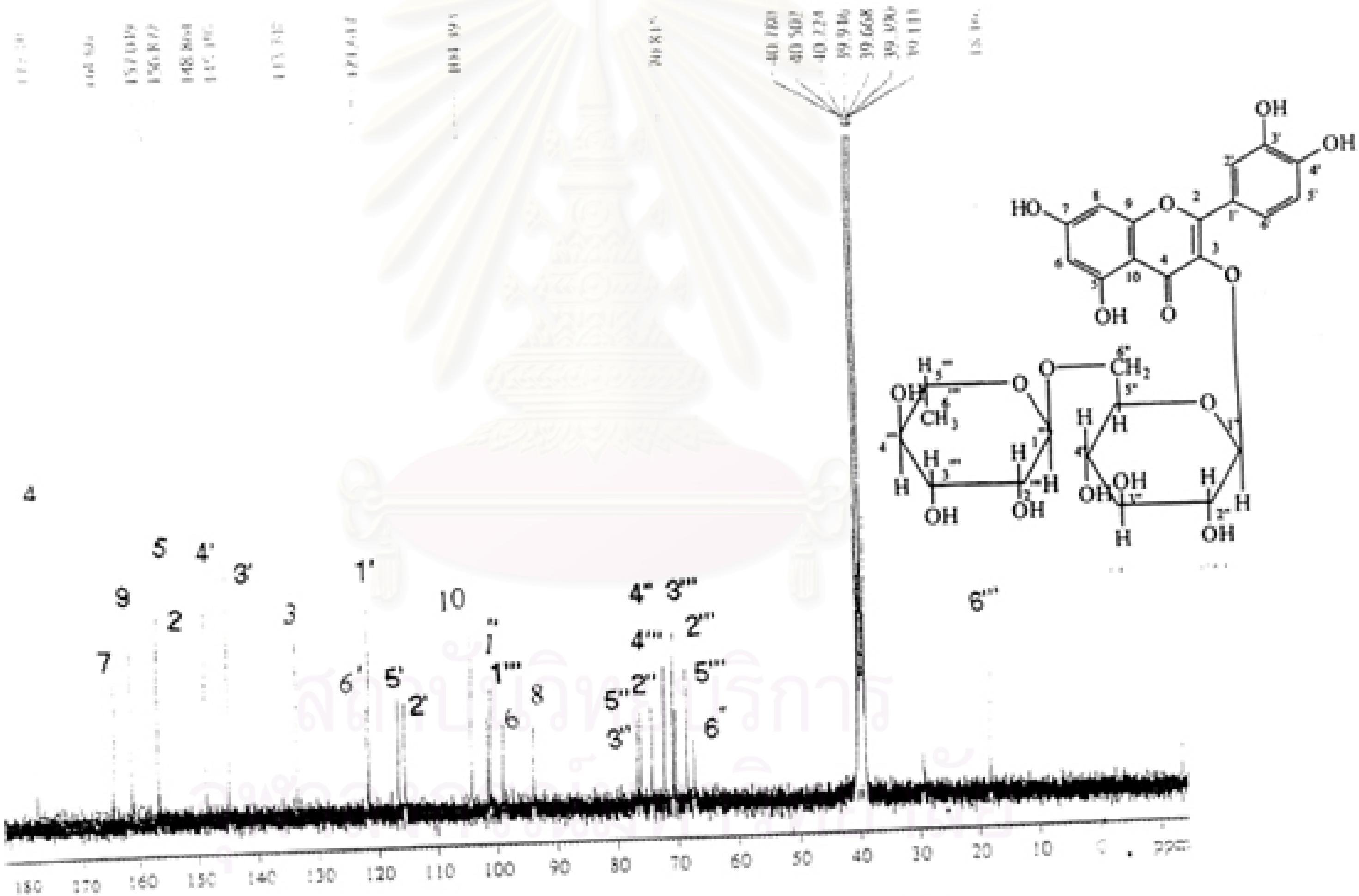


Figure 22 The 75 MHz ^{13}C NMR spectrum of compound CA3

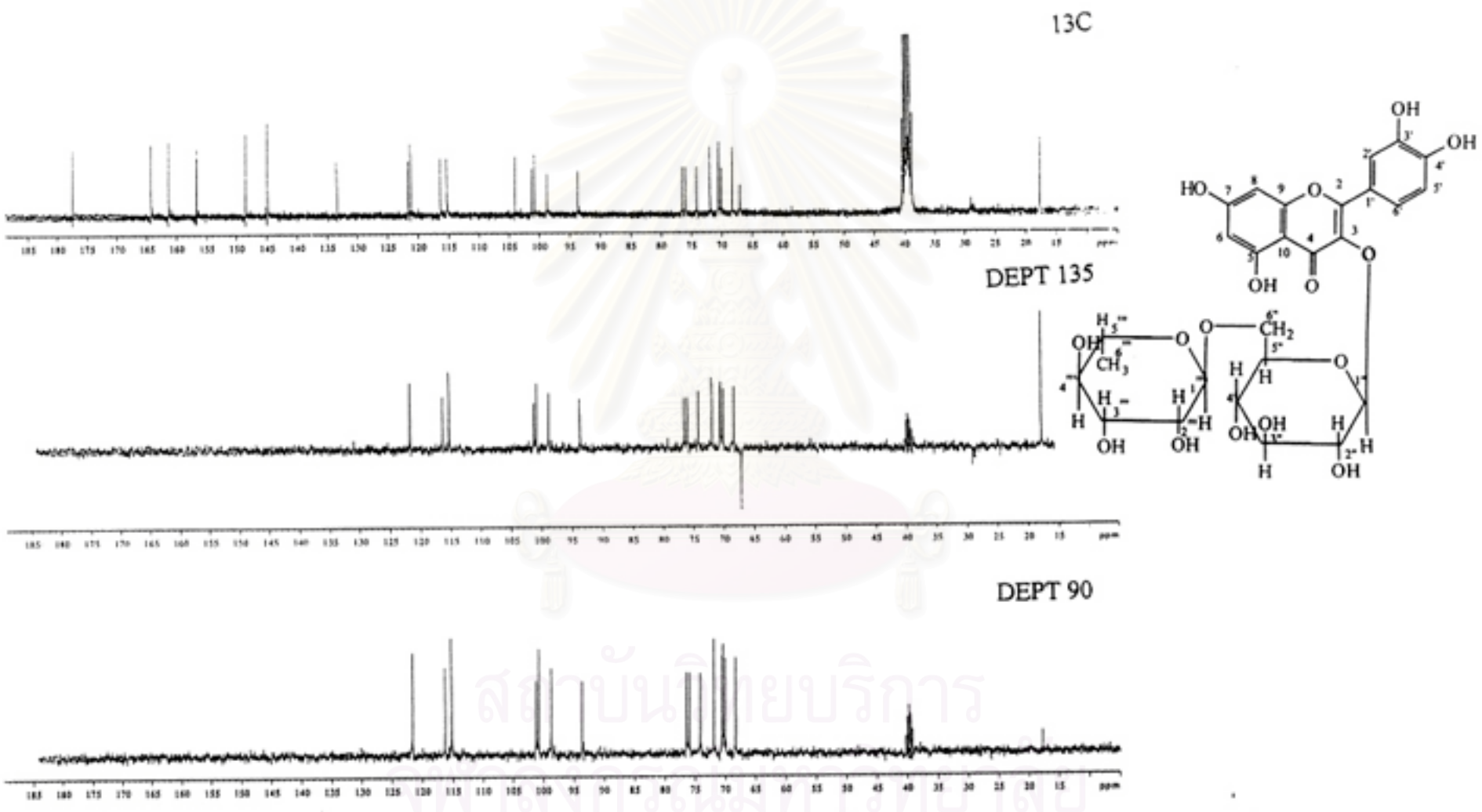


Figure 23a The 75 MHz ^{13}C-DEPT NMR spectrum of compound CA3

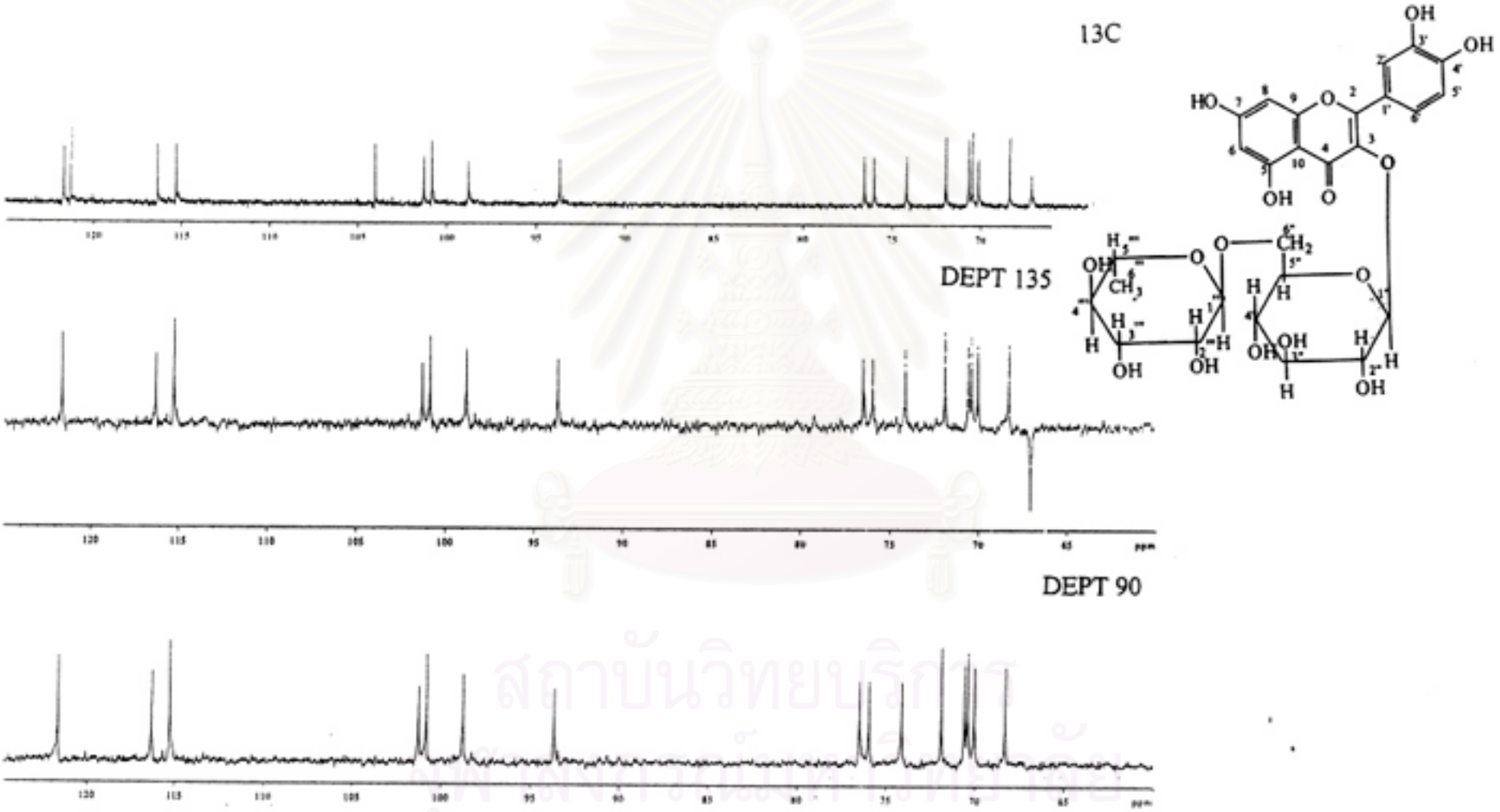


Figure 23b The 75 MHz ^{13}C-DEPT NMR spectrum of compound CA3 (continued)

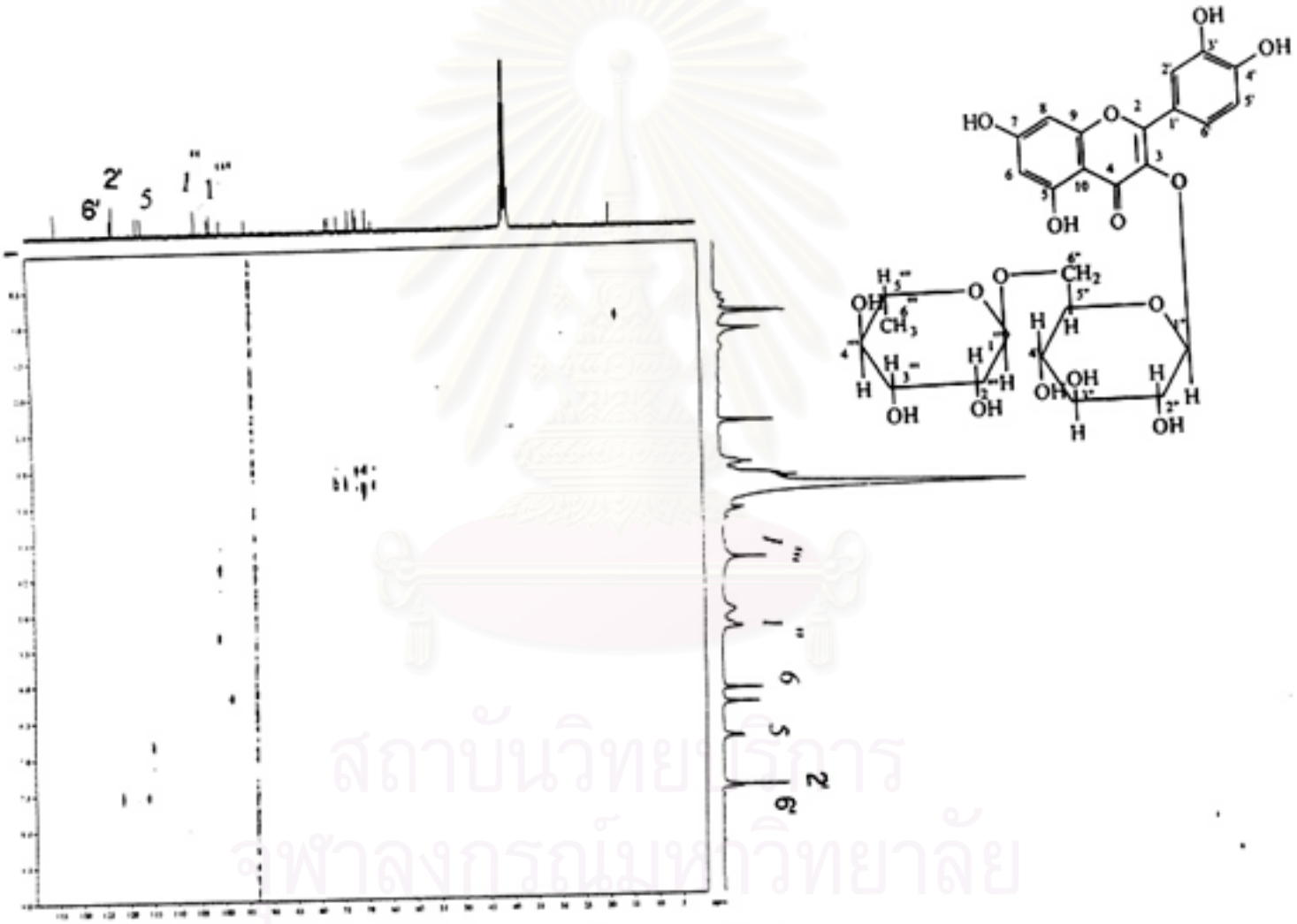


Figure 24 · The HETCOR spectrum of compound CA3

4. Identification of Compound CA4

Compound CA4 was recrystallized as colorless needles from methanol. It gave violet color when sprayed with 10 % ethanolic sulphuric acid and heated. Libermann Burchard test of this compound gave positive green color, suggesting the presence of a steroidal skeleton. The IR spectrum of CA4 (Figure 25) exhibited a broad band at 3463 cm^{-1} (O-H stretching), indicating the presence of hydroxyl group (s) in the molecule.

The EIMS of CA4 (Figure 26) displayed a prominent molecular ion peak at *m/z* 412 ($C_{29}H_{48}O$). Intense EIMS fragment peak at *m/z* 271 ($[M - C_{10}H_{21}]^+$) was also important in showing CA4 as having a steroid skeleton (Rubinstein *et al*., 1976).

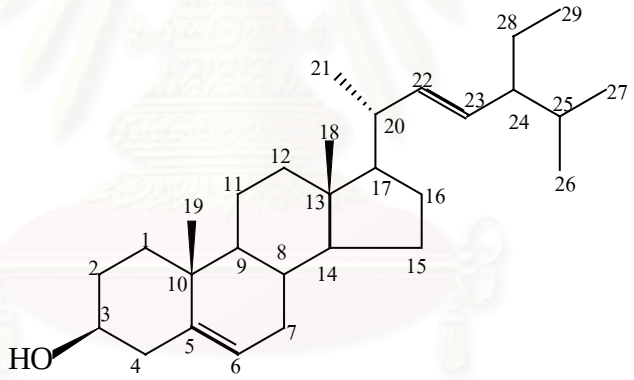
The ^1H NMR spectrum of CA4 (Figures 27a-27b) displayed the signals at δ 5.17 (1H, dd, *J* = 15.0, 8.4 Hz) and 5.00 ppm (1H, dd, *J* = 15.0, 8.4 Hz) assignable to H-23 and H-22 of the disubstituted double bond in the side chain of stigmasterol. Another vinylic proton appeared as a multiplet at δ 5.32 ppm (H-6). Another multiplet at δ 3.49 ppm was attributable to the proton geminal to the 3-OH group. The singlets at δ 0.68 and 0.99 ppm represented the angular methyl groups at H-18 and H-19, respectively.

The ^{13}C-NMR spectrum (Figures 28a-28b) showed the signals of 29 carbon atoms, supporting the assignment of CA4 as a steroid derivative. The DEPT-90 and DEPT-135 experiments (Figure 29) were performed to differentiate these 29 signals into those of the six methyl carbons at δ 22.7, 21.1, 19.4, 19.0, 12.2 and 12.0 ppm, nine methylene carbons at δ 42.3, 39.7, 37.3, 31.9, 31.6, 28.9, 25.4, 24.4 and 21.2ppm, eleven methine carbons at δ 138.3, 129.3, 121.7, 71.8, 56.9, 56.0, 51.2, 50.2, 40.5, 31.9 and 31.9 ppm, and three quaternary carbons at δ 140.7, 42.3 and 36.5 ppm. The three most downfield methine signals at δ 138.3,

129.3 and 121.7 ppm. and the quaternary signal at δ 140.7 could be assigned to C-22 and C-23 of the disubstituted double bond and C-6 and C-5 of the trisubstituted double bond, respectively. The signal at δ 71.8 ppm represented the hydroxy-substituted C-3.

Comparision of the carbon and proton chemical shifts of compound CA4 with those of the previously reported stigmasterol (Rubinstein *et al*., 1976) revealed them to be fully in agreement (Table 17).

Therefore, compound CA4 was identified as the steroid stigmasterol, the structure of which is shown below.



Stigmasterol (CA4)

Stigmasterol (24-ethyl-5,22-cholestadiene-3β-ol) was previously isolated from several bignoniaceous species, i.e. *Kigelia africana* (El-Sayyad, 1981), *K. pinnata* (Govindachari, Patankar and Viswanathan, 1971) and *Crescentia cujete* (Agarwal and Popli, 1992). The compound was demonstrated as possesing significant topical anti-inflammatory activity (Garcia *et al*., 1999; Gomez *et al*., 1999), antioxidant (Hung and Yen, 2001) and antihypercholesterolemic effects (Chandle, Hooper and Ismail, 1979).

Table 17 Comparison of ^{13}C-NMR chemical shifts in $CDCl_3$ of CA4 and stigmasterol (Rubinstein *et al.*, 1976)

Position	CA4 (ppm)	Stigmasterol (ppm)	Position	CA4 (ppm)	Stigmasterol (ppm)
1	37.3	37.3	16	28.9	29.0
2	31.6	31.7	17	56.0	56.0
3	71.8	71.8	18	12.0	12.0
4	42.3	42.4	19	19.4	19.4
5	140.7	140.8	20	39.7	40.5
6	121.7	121.7	21	21.1	21.1
7	31.9	31.9	22	138.3	138.4
8	31.9	31.9	23	129.3	129.3
9	50.2	50.2	24	51.2	51.3
10	36.5	36.6	25	31.9	31.9
11	21.2	21.1	26	22.7	22.3
12	39.7	39.7	27	19.0	19.0
13	42.3	42.4	28	25.4	25.4
14	56.9	56.9	29	12.2	12.3
15	24.4	24.4			

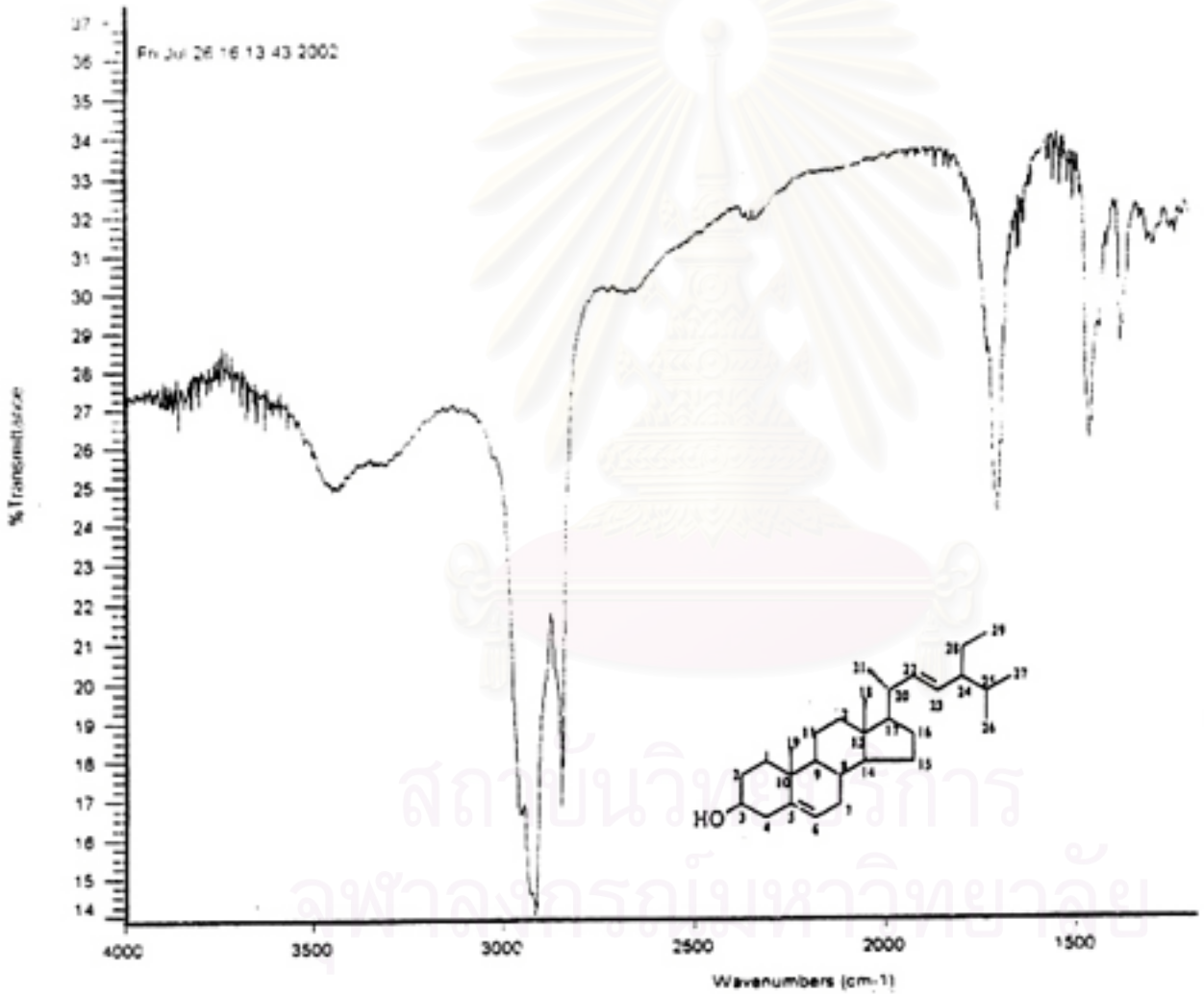


Figure 25 IR spectrum of compound CA4

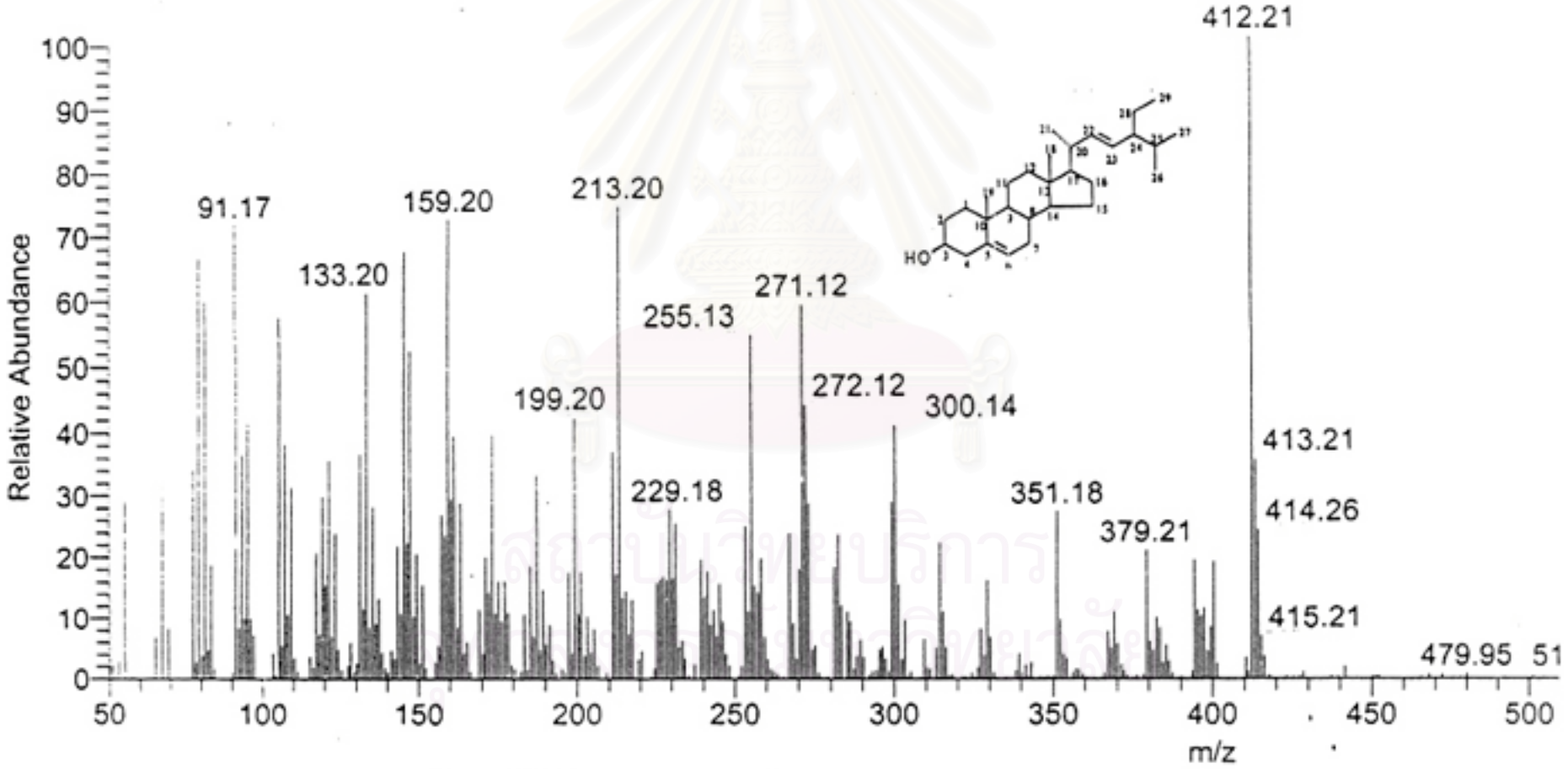


Figure 26 EI mass spectrum of compound CA4

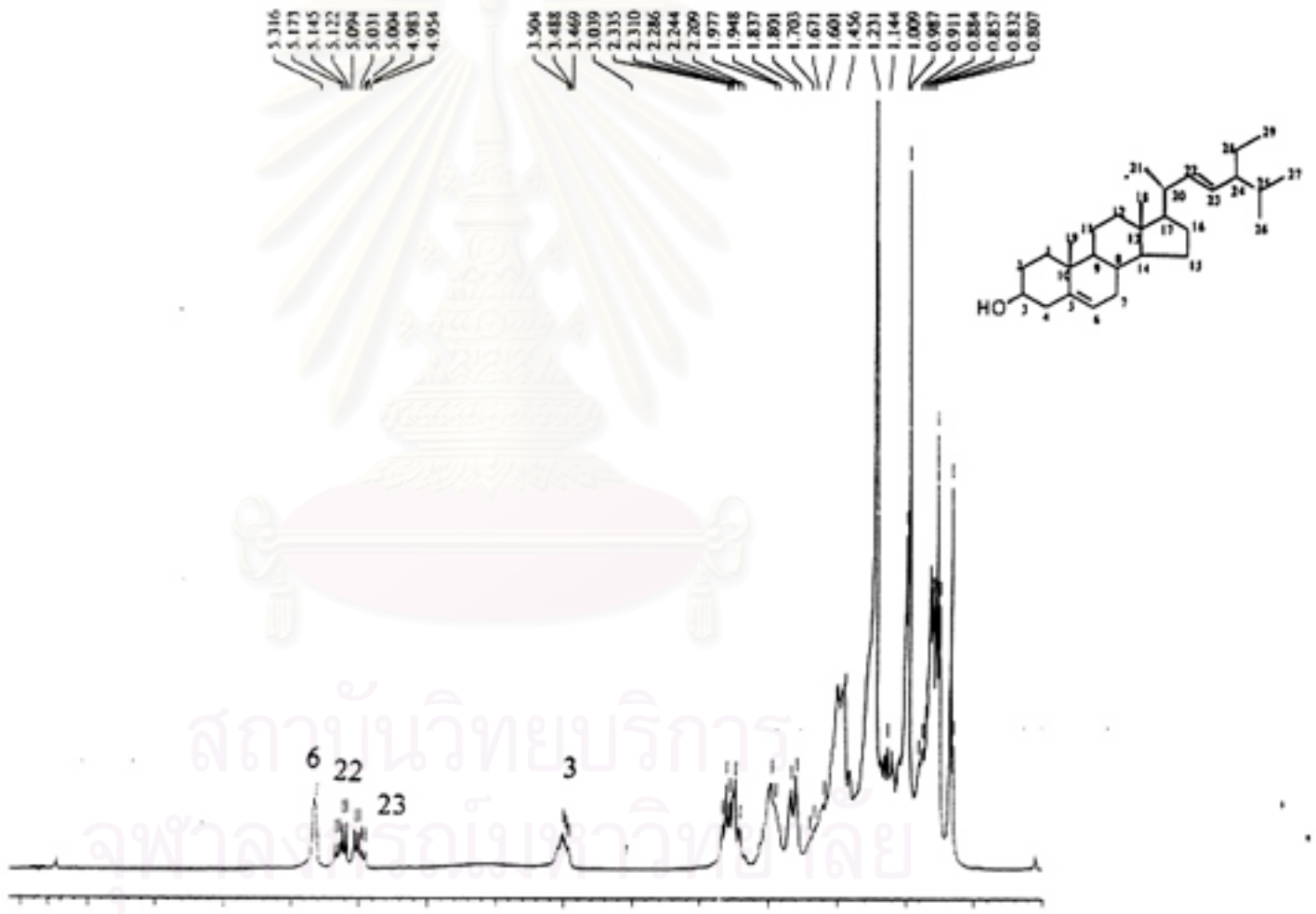


Figure 27a The 300 MHz ^{1}H NMR spectrum of compound CA4

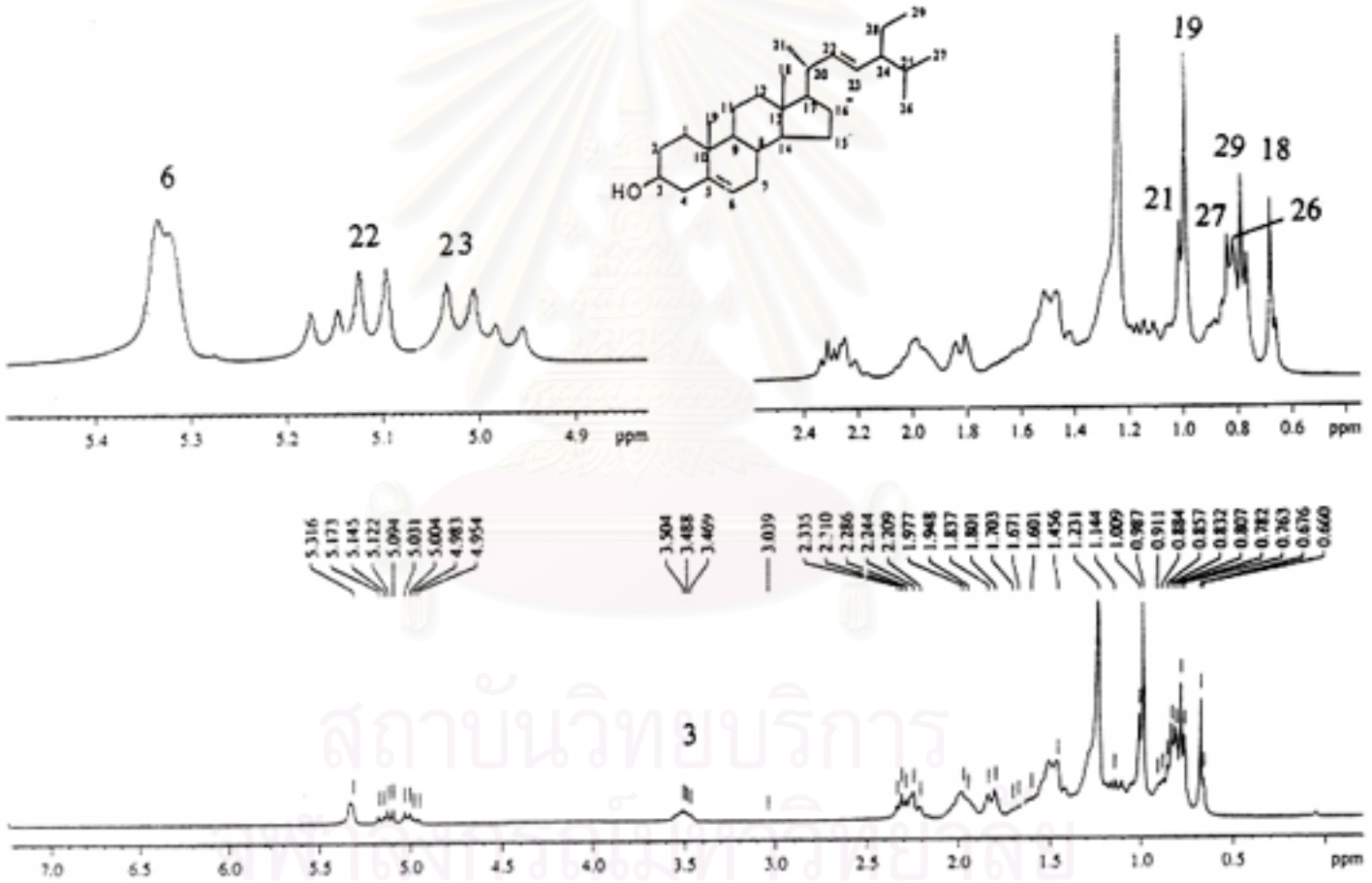


Figure 27b The 300 MHZ ^{1}H NMR spectrum of compound CA4 (expanded)

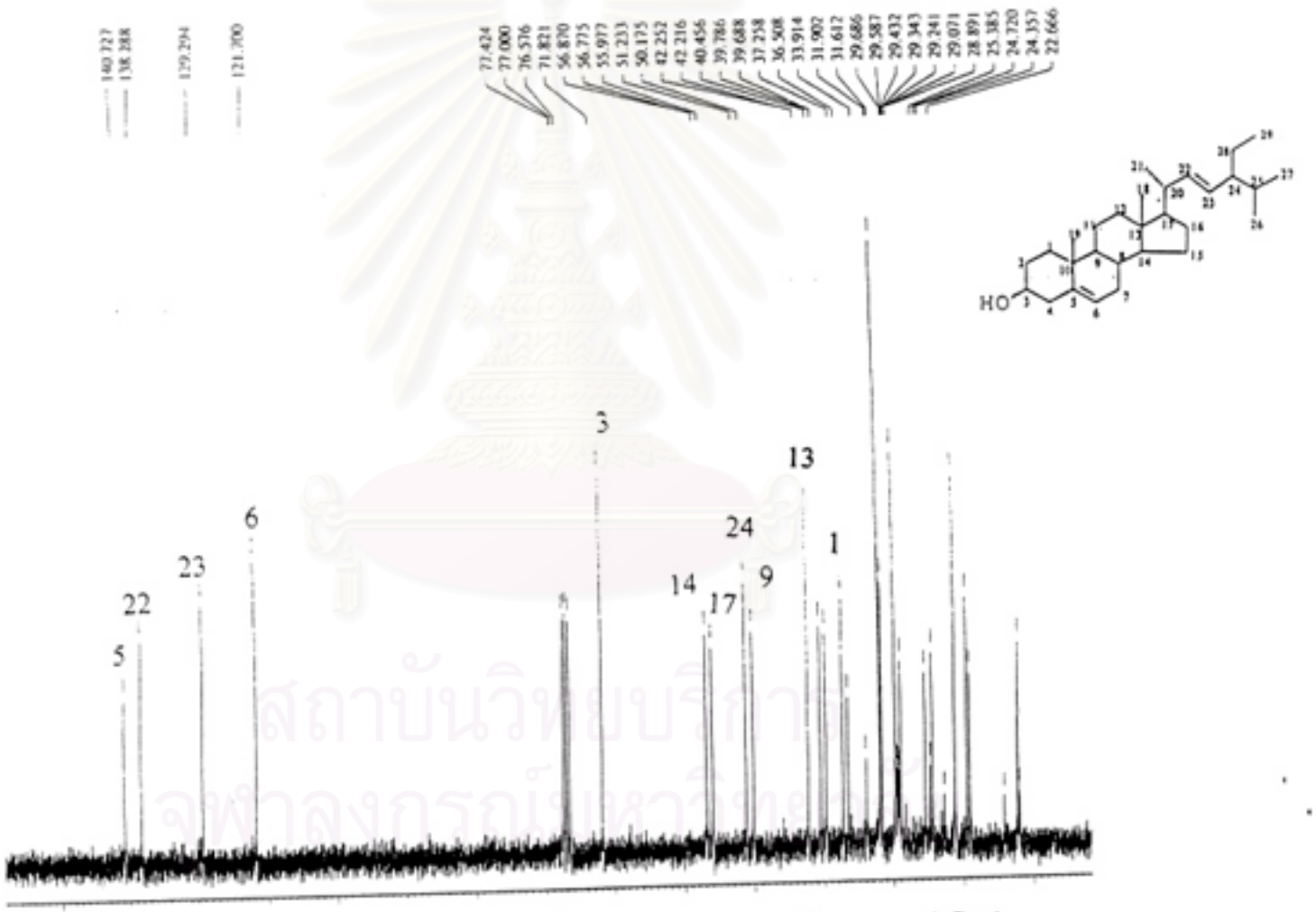


Figure 28a The 75 MHz ^{13}C NMR spectrum of compound CA4

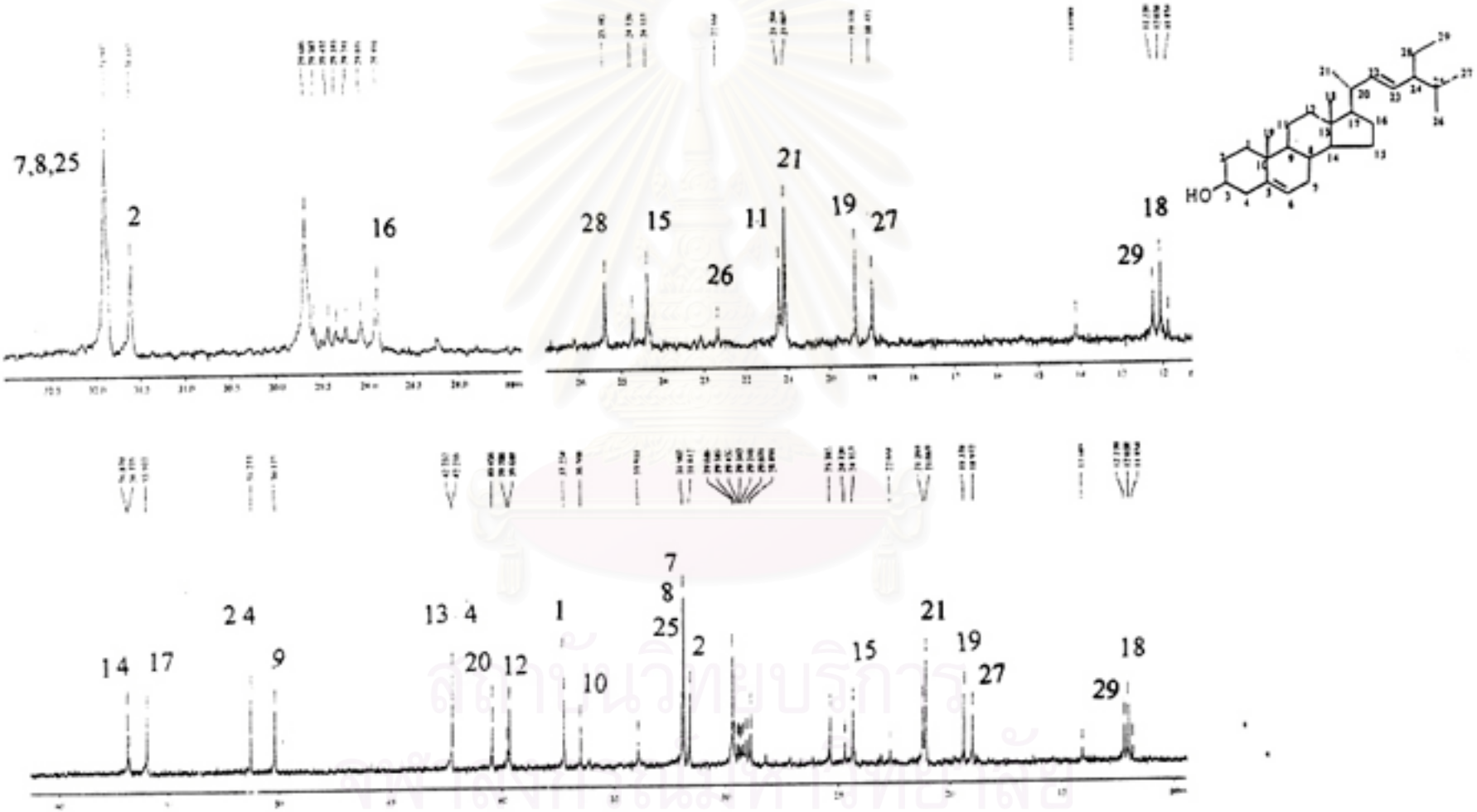


Figure 28b The 75 MHz ^{13}C NMR spectrum of compound CA4 (expanded)

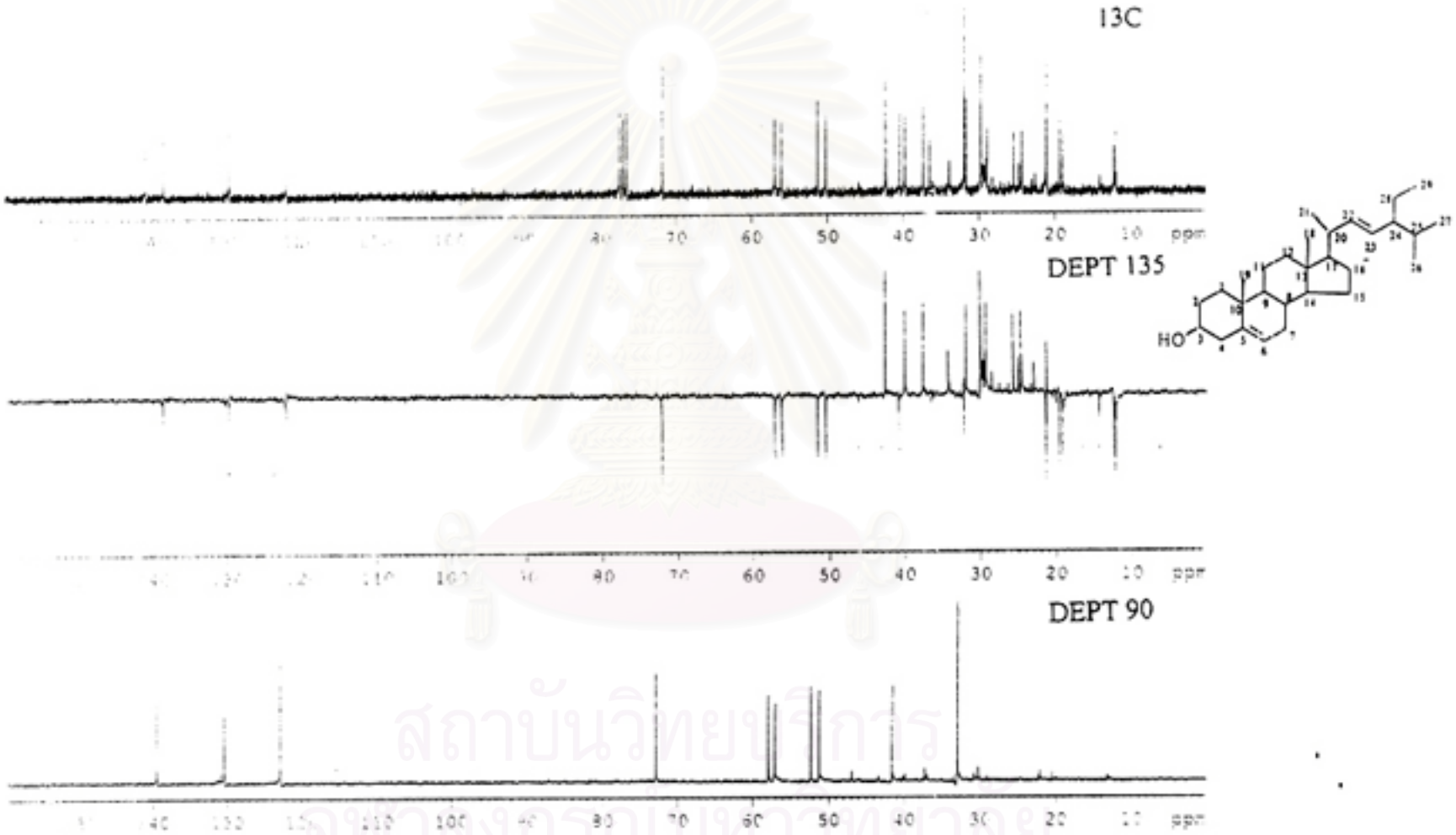


Figure **29** The 75 MHz ^{13}C-DEPT NMR spectrum of compound CA4

5. Screening Test for Antioxidant Activity of Crude Extracts and Pure Compounds.

The ethyl acetate and methanol extracts of the bark and leaves of *C. alata* exhibited antioxidant activity as percent DPPH radical scavenging similar to that of the widely-used α-tocopherol (Table 18). When subjected to the same assay system, two chemical constitutes isolated from this plant, i.e. vanillic acid (CA2)and rutin (CA3), were shown to have slightly weaker activity than the extracts, suggesting there might be other compounds which possess strong antioxidant activity and still have not been isolated in this study.

Table 18 Antioxidant activity of extracts and chemical constituents of *C. alata* measured by DPPH radical scavenging assay.

Samples	% DPPH scavenging
methanol bark extracts	98
ethyl acetate bark extracts	94
methanol leaf extracts	96
ethyl acetate leaf extracts	88
Rutin (CA3)	81
Vanillic acid (CA2)	80
∝-tocopherol	94

CHAPTER V

Conclusion

In the present investigation of *Crescentia alata* HBK., triterpernoid, phenyl propanoid, flavonoid and sterol were isolated from the bark and the leaves of this plant collected from Nakhon Nayok, Thailand.

Screening test of the anti-oxidant acitivity using DPPH showed interesting radical-scavenging activity of the ethyl acetate and methanol extracts of the leaves and bark of *C. alata.* These extracts appeared to be even more active than vitamin E, a common natural anti-oxidant.

Three compounds, lupeol, 4-hydroxy-3-methoxybenzoic acid (vanillic acid), quercetin-3-rutinoside (rutin), were isolated from the bark, whereas another compound, 24-ethyl-5,22-cholestadien-3-β-ol (stigmasterol), was isolated from the leaves of *C. alata* by chromatographic technique. Their chemical structures were identified by spectroscopic techniques. Both vanillic acid and rutin have been demonstrated as exhibiting antioxidant activity. However, it is possible that there might be other constituents of *C. alata* with similar activity. Further study on the chemical constituents from different parts of this plant should be performed.

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VITA

Capt. Aunchita Wiyaporn was born on December 20, 1974 in Bangkok, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1997 from the Faculty of Pharmaceutical Sciences, Silpakorn University, Thailand. She is now working as a hospital pharmacist at Chulachomklao Royal Military Academy Hospital, Nakhon Nayok, Thailand.