การศึกษาทางพฤกษเคมีของใบหอมไกลดง

นางสาวรัชนี ภูวพัฒนชาติ

วิทยาพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตร์มหาบัณฑิต
สาขาวิชาเภสัชพฤกษศาสตร์ ภาควิชาเภสัชพฤกษศาสตร์
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2546

ISBN 974-17-4397-1

PHYTOCHEMICAL STUDY OF *HARPULLIA ARBOREA* LEAVES

Miss Ratchanee Poovapatthanachart

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy in Pharmaceutical Botany

Department of Pharmaceutical Botany

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2003

ISBN 974-17-4397-1

Thesis Title	Phytochemical study of *Harpullia arborea* leaves
By	Miss Ratchanee Poovapatthanachart
Field of study	Pharmaceutical Botany
Thesis Advisor	Witchuda Thanakijcharoenpath, Ph.D.

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

..... Dean of the Faculty of Pharmaceutical Sciences

(Associate Professor Boonyong Tantisira, Ph.D.)

THESIS COMMITTEE

..... Chairman

(Associate Professor Ekarin Saifah, Ph.D.)

..... Thesis Advisor

(Witchuda Thanakijcharoenpath, Ph.D.)

..... Member

(Associate Professor Rapepol Bavovada, Ph.D.)

..... Member

(Assistant Professor Rutt Suttisri, Ph.D.)

..... Member

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

รัชนี ภูวพัฒนชาติ : การศึกษาทางพฤกษเคมีของใบหอมไกลดง (PHYTOCHEMICAL STUDY OF *HARPULLIA ARBOREA* LEAVES) อาจารย์ที่ปรึกษา : อ.ดร. วิชชุดา ธนกิจ-เจริญพัฒน์, 183 หน้า. ISBN 974-17-4397-1.

จากส่วนใบของหอมไกลดง (วงศ์ Sapindaceae) สามารถแยกได้สารในกลุ่มไตรเทอร์ปีนอยด์ชนิดใหม่ 1 ชนิด คือ 3β-eicosanoyl-6β-hydroxy-21αH-24-norhopa-4(23),22(29)-diene ร่วมกับสารในกลุ่มไตรเทอร์ปีนอยด์ประเภท lupane 1 ชนิด คือ lupeol, สารในกลุ่ม sterol 1 ชนิด คือ (α-spinasterol) และอนุพันธ์ของ inositol 1 ชนิด คือ quebrachitol การพิสูจน์เอกลักษณ์ของสารเหล่านี้ทำโดยการวิเคราะห์ข้อมูล MS, IR, ^{1}H-NMR และ ^{13}C-NMR โดยเฉพาะอย่างยิ่ง 1D-NMR และ 2D-NMR ร่วมกับการเปรียบเทียบข้อมูลที่ได้มีรายงานไว้แล้ว ในการศึกษาเพื่อตรวจสอบฤทธิ์ที่มีต่อการเพิ่มจำนวนของ lymphocyte ในหลอดทดลอง ของสารสกัดจากพืช และ quebrachitol พบว่า สารสกัดในชั้นเมทธานอลและ quebrachitol แสดงฤทธิ์กระตุ้นการเพิ่มจำนวนของ lymphocyte

ภาควิชา เภสัชพฤกษศาสตร์ ลายมือชื่อนิสิต.....

สาขาวิชา เภสัชพฤกษศาสตร์ ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2546 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4476602933 : MAJOR PHARMACEUTICAL BOTANY

KEY WORD : *HARPULLIA ARBOREA* / Sapindaceae / triterpenoid / inositol derivative / sterol / lymphocyte proliferation

RATCHANEE POOVAPATTHANACHART : PHYTOCHEMICAL STUDY OF *HARPULLIA ARBOREA* LEAVES. THESIS ADVISOR : WITCHUDA THANAKIJCHAROENPATH Ph.D., 183 pp. ISBN 974-17-4397-1

From the leaves of *Harpullia arborea* (Sapindaceae), a new triterpenoid, 3β-eicosanoyl-6β-hydroxy-21αH-24-norhopa-4(23),22(29)-diene, together with a lupane-type triterpenoid, lupeol; a sterol ,α-spinasterol and an inositol derivative , quebrachitol, has been isolated. The structure elucidation of these compounds were accomplished through the analysis of their MS, IR, ^{1}H-NMR and ^{13}C-NMR, especially the 1D-NMR and 2D-NMR data as well as comparison with reported values. In the investigation of the effects on *in vitro* lymphocyte proliferation of extracts from the plant and the isolated compound quebrachitol, methanol extract and quebrachitol have been found to exhibited stimulatory effect on lymphocyte proliferation.

Department Pharmaceutical Botany Student's signature……..

Field of study Pharmaceutical Botany Advisor's signature……..

Academic year 2003 Co-advisor's signature…….

ACKNOWLEDGMENTS

The author wishes to express her deep indebtedness and sincere gratitude to her advisor, Dr. Witchuda Thanakijcharoenpath of the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for her helpful guidance, suggestion, continual encouragement and kindness throughout the course of this study.

The author would like to acknowledge her grateful thanks to Associate Professor Dr. Ekarin Saifah, Associate Professor Dr. Rapepol Bavovada and Assistant Professor Dr. Rutt Suttisri of the Department of Pharmaceutical Botany, and Associate Professor Chaiyo Chaichantipyuth of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their valuable critical comment.

The author would like to express her thanks to Miss Chompunuch Boonarkart of the Department of Pharmaceutical Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University , for biological test on lymphocyte proliferation.

The author would like to express her thanks to all staff members of the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their kindness and help.

The author is also indebted to all her friends in the Department for their useful contribution and friendship.

The author is also greatefully indebted to her family for their love, understanding, encouragement and financial support.

Finally, the author's thanks are due to Chulalongkorn University Graduate School, for granting partial financial support to conduct this investigation.

CONTENTS

Page

CONTENTS (continued)

Page

LIST OF FIGURES

Page

Figure

LIST OF FIGURES (continued)

Page

Figure

LIST OF FIGURES (continued)

Page

Figure

LIST OF FIGURES (continued)

Page

Figure

LIST OF TABLES

Page

Table

LIST OF TABLES (continued)

Page

Table

LIST OF SCHEMES

Page

Scheme

LIST OF ABBREVIATIONS

Api	=	apiose
Ara	=	arabinose
br	=	broad
°C	=	Degree celsius
CC	=	Column Chromatography
CCs_{50}	=	50% cytotoxic concentration
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
cm	=	Centimeter
^{13}C-NMR	=	Carbon-13 Nuclear Magnetic Resonance
CO_2	=	Carbon dioxide
Con-A	=	Concanavalin A
COSY	=	Correlated spectroscopy
1D	=	one dimensional
2D	=	two dimensional
δ	=	Chemical shift
d	=	doublet
dd	=	doublet of doublets
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO	=	Dimethylsulfoxide
DMSO-d_6	=	Deuterated dimethylsulfoxide
EIMS	=	Electron Impact Mass Spectrum
EtOH	=	Ethanol
EtOAc	=	Ethyl acetate

LIST OF ABBREVIATIONS (continued)

eV	=	electron Volt
Fuc	=	fucose
Gal	=	galactose
Glc	=	glucose
Glc A	=	glucuronic acid
gm	=	gram
^{1}H-NMR	=	Proton Nuclear Magnetic Resonance
HETCOR	=	Heteronuclear Correlation Spectroscopy
HMBC	=	^{1}H –detected Heteronuclear Multiple Bond Coherence
Hz	=	Hertz
IR	=	Infrared
J	=	Coupling constant
KBr	=	Potassium bromide
L	=	Liter
LPS	=	Lipopolysaccharide
m	=	multiplet
M^{+}	=	Molecular ion
mcg	=	microgram
mcl	=	microliter
MeOH	=	Methanol
mg	=	milligram
mp	=	melting point
MHz	=	Megahertz
ml	=	milliliter

LIST OF ABBREVIATIONS (continued)

MS	=	Mass Spectrum
m/z	=	mass-to-charge ratio
nm	=	nanometer
NMR	=	Nuclear Magnetic Resonance
ppm	=	part per million
rpm	=	round per minute
Rha	=	rhamnose
s	=	singlet
S.E.M.	=	Standard Error Mean
sp.	=	species
t	=	triplet
TLC	=	Thin-Layer Chromatography
Xyl	=	xylose

CHAPTER I

INTRODUCTION

Harpullia is one of the genera of the Sapindaceae, a family which consists of about 140 genera and 1,350 species worldwide (Van Welzen, 1999). Plants in this genus are found from Sri Lanka and India through Southeast China and Southeast Asia to Northeast and East Australia, New Caledonia, Tonga (Van Welzen, 1999) and also in Madagascar (Chopra, Badhwar, and Ghosh, 1965). The characteristic features of the plants are as follows.

Shrubs to trees, dioecious. *Indumentum* of simple and stellately bundled hairs. *Leaves* paripinnate. *Leaflets* smooth underneath; base symmetrical to oblique; margin entire; domatia absent; nerves looped and joined near the margin, venation coarsely reticulate. *Inflorescences* axillary to terminal and solitary or tufted and rami/ cauliflorous, thyrsoid, at most laxly branching, pilose. *Flowers* unisexual, actinomorphic. *Sepals* 5, free, imbricate, outer 2 usually smaller. *Petals* 5, larger than sepals, without ornamentation (then fleshy) or with 2 auricles (thin). *Disc* annular, pilose (Thailand). *Stamens* 5 – 8, glabrous. *Ovary* sessile or short–stalked, 2-locular (Thailand), hairy; stigma not lobed, often bent and hooked; ovules 1 or 2 per locule. *Fruits* capsular, loculicidal, usually short–stipitate, smooth, lobed; lobes spreading, inflated, rounded; wall parchment–like to woody, glabrous to hairy on both sides. *Seeds* ellipsoid, with a basal sarcotestal ring or arilloid completely covering seed, but lower part sarcotestal and upper part free; hilum small (Van Welzen, 1999).

According to the Index Kewensis, 48 species of *Harpullia* have been recorded as follows.

1. *Harpullia aeruginosa* Radlk.
2. *H. alata* F. Muell.
3. *H. angustialata* C. T.
4. *H. angustifolia* Radlk.
5. *H. arborea* Radlk.
6. *H. austrocaledonica* Baill.
7. *H. camptoneura* Radlk.
8. *H. cauliflora* K. Schum. & Lauterb.
9. *H. cochinchinensis* Pierre.
10. *H. condorensis* Pierre.
11. *H. crustacea* Radlk.
12. *H. cupanioides* Roxb.
13. *H. divaricata* Radlk.
14. *H. fraxinifolia* Blume
15. *H.* frutescens F. M. Bailey
16. *H. fruticosa* Blume
17. *H. fosteri* Sprague
18. *H. glanduligera* Radlk.
19. *H. hillii* F. Muell.
20. *H. hirsuta* Radlk.
21. *H. holoptera* Radlk.
22. *H. largifolia* Radlk.
23. *H. leichhardtii* F. Muell ex Benth.
24. *H. leptococca* Radlk.
25. *H. longithyrsifera* Kanehira & Hatusima
26. *H. macrocalyx* Radlk.

27. *H. madagascariensis* Radlk.
28. *H. marginata* Radlk.
29. *H. mellea* Lauterb.
30. *H. multijuga* Radlk.
31. *H. myrmecophila* Merrill & Perry
32. *H. obscura* Radlk.
33. *H. oococca* Radlk.
34. *H. parviflora* Lecomte
35. *H. pedicellaris* Radlk.
36. *H. peekeliana* Melch.
37. *H. pendula* Planch.
38. *H. petiolaris* Radlk.
39. *H. ramiflora* Radlk.
40. *H. reticulata* Radlk.
41. *H. rhachiptera* Radlk.
42. *H. rhyticarpa* C. T.
43. *H. sphaeroloba* Radlk.
44. *H. thanatophora* Blume
45. *H. tomentosa* Ridley
46. *H. vaga* Merrill & Perry
47. *H. weinlandii* K. Schum.
48. *H. zanguebarica* Radlk.

In Thailand, only two species of *Harpullia* could be found as listed below (กรมป่าไม้, สำนักงานวิชาการป่าไม้, ส่วนพฤกษศาสตร์ป่าไม้, 2544).

1. *Harpullia arborea* (Blanco) Radlk.

กระโปกลิง kra pok ling (Saraburi), หมังขะอุย Mang kha ui,
หอมไกลดง Hom klai dong, ฮางแกน Hang kaen (Sukhothai)

2. *Harpullia cupanioides* Roxb.

ขางขาว khang khao (Phrae), พริกป่า Phrik pa (Chon Buri),
หงอนไก่ดง Ngon kai dong (Surat Thani)

Harpullia arborea (Blanco) Radlk. is an evergreen tree which can grow up to 33 m. The stem bark is cream or grey, smooth or slightly wrinkled. The leaves are pinnate with 2 – 6 pairs of leaflets, which are 3.0 – 5.5 × 2 – 10 cm., narrowly ovate or elliptic with tapering or slightly pointed tips and asymmetric base. Young shoots are densely golden - brown hairy and the mature leaves are nearly smooth to densely hairy, usually with scattered brown hairs at least on midvein above. The leaflet stalks are 0.3 – 0.8 cm. and the main stalk 7 – 12 cm. The inflorescences are up to 35 cm. long, in leaf axils or behind leaves on old twigs. The flowers are white to yellow green, up to more than 1 cm. wide, with red – brown hairy stalk. The sepals are ovate to obovate, 5 – 10.5 × 3 – 5 mm., light green. The petals are free, obovate, membranous; 5 mm. long or longer, white to light yellow, clawed; with auricles, usually hairy especially along margin. The number of stamens is 5(- 7). The filaments are 10 – 17 mm. long, light green; the anthers are 2 – 2.5 mm. long, light orange. The pistil has 2 – 4 styles with minute stigmas and hairy disc. The fruits are bright orange – red, leathery, deeply lobed (lobes are broader than high), usually without calyx, splitting into 2 sections each containing 2(1) glossy black seeds with fleshy orange ring at base (Van Welzen, 1999; Gardner, Sidisunthorn, and Anusamsunthorn, 2000).

The plant can be found in Sri Lanka, India; from Thailand-Vietnam to Java, the Philippines, Australia and the Pacific (Tonga and Samoa) (Quisumbing, 1951; Backer and Van der brink Jr, 1965; Van Welzen, 1999). It is usually found growing in well-drained evergreen to deciduous forest; also found on ridges, slopes and plains, in rarities and sometimes along or in swamps, on river banks or coasts (Van Welzen, 1999).

Traditional uses of this plant have been recorded. The watery exudate of the bark, and sometimes the fruits, is used for washing, to keep away leeches, or is drunk to allay pain. The bark is also used as fish poison. The oil from the seeds has been used as anti-rheumatic (Van Welzen, 1999).

Of 48 *Harpullia* species, six species have been phytochemically investigated and a variety of compounds, including triterpenoids, flavonoids, steroids and miscellaneous substances have been found as their constituents. Some extracts of these plants have also been subjected to biological investigation and found to exert interesting bioactivities.

As two *Harpullia* species found in Thailand, *H. cupanioides* is one of those six species with previous reports on phytochemical study while such study of the other species, *H. arborea*, has never been documented. This study deals with the isolation and identification of chemical constituents from the leaves of *H. arborea*. The result obtained would contribute to the knowledge on chemical nature of the genus *Harpullia*, and provide useful information in the field of phytochemistry and chemotaxonomy.

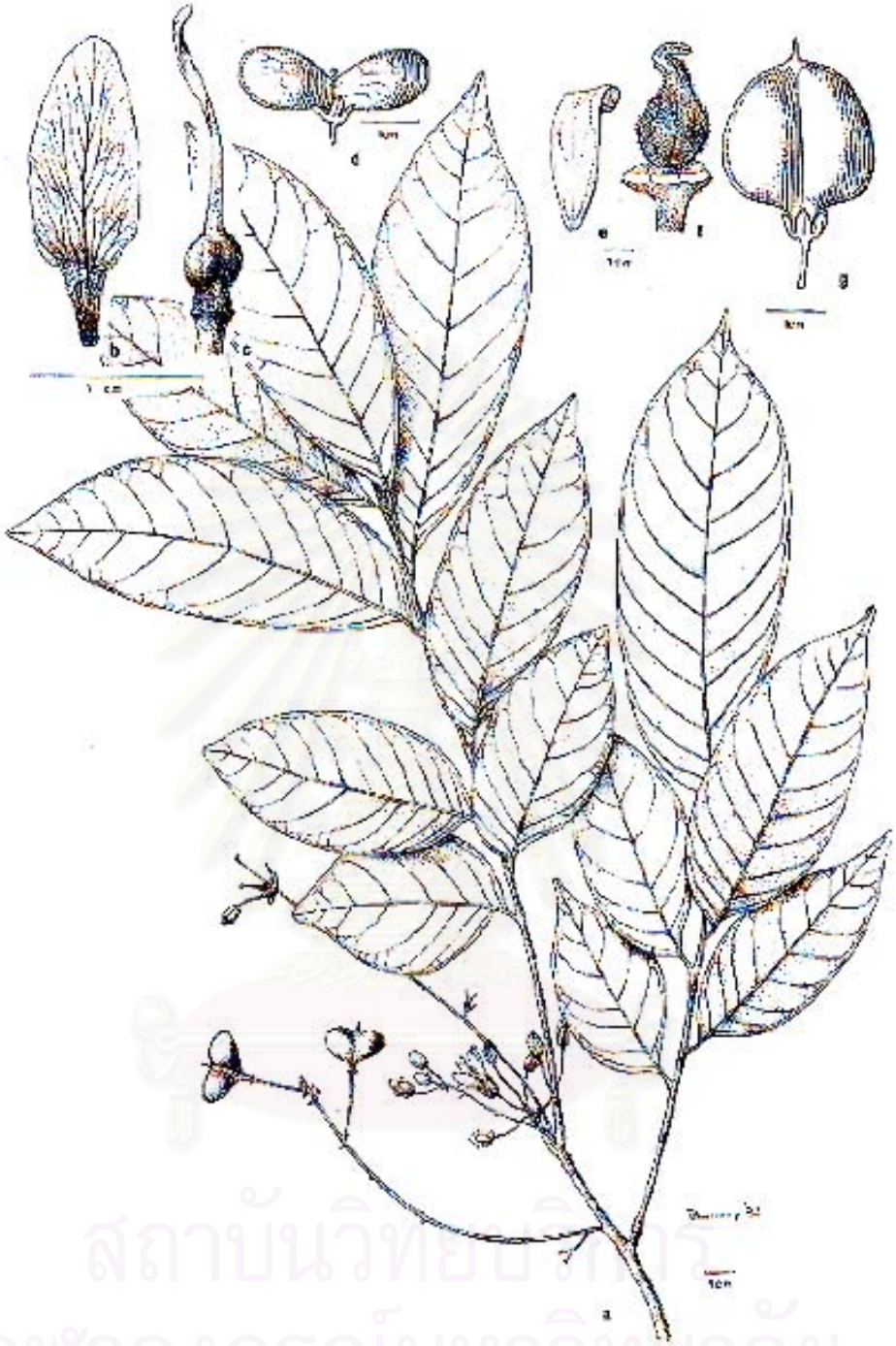


Figure 1. *Harpullia arborea* (Van Welzen, 1999).

CHAPTER II

HISTORICAL

1. The Family Sapindaceae and Its Species in Thailand.

The family Sapindaceae, the name of which refers to the traditional use as soap of several of its species, is a large family of about 140 genera and 1,350 species found widespread in all tropical and subtropical regions of the world. The family belongs to the order Sapindales and is subdivided into 2 subfamilies, which are Sapindoideae (with 8 tribes) and Dodonoideae (with 5 tribes) (Van Welzen, 1999).

Plants in this family are shrubs or trees, occasionally lianas. The leaves are alternate or spirally arranged, usually paripinnate, estipulate (except in *Cardiospermum*). The leaflets are usually with asymmetric base and entire margin; the lower surface is often with papillae, domatia or glands. The inflorescences are usually axillary, thyrsoid. The flowers are usually zygomorphic, inconspicious, typically with 5 sepals and 5 free petals (rarely name); the petals are often smaller than sepals and usually with 1 or 2 having scales at base. The disc is usually an entire ring, sometimes interrupted or divided into separate glands. The number of stamens is 5 – 8; the filaments are free. The ovary is superior, usually 2- or 3-locular, smooth or tuberculate, with 1 or sometimes 2 ovules per locule; the stigma may or may not be lobed. Many species have both bisexual and male only flowers, either in the same cluster or on different trees. The fruits are very variable, but usually leathery capsules or drupes, lobes or not, smooth, warty or with short to long spines. The seeds are often surrounded by an arilloid (Van Welzen, 1999).

In Thailand, 23 genera and 45 species of sapindaceous plants can be found as follows (Van Welzen,1999; กรมป่าไม้, สำนักวิชาการป่าไม้, ส่วนพฤกษศาสตร์ป่าไม้, 2544).

1. *Allophylus*

   A. cobbe (L.) Raeusch. : ต่อไส้

2. *Amesiodendron*

   A. chinense (Merr.) : ไม้ขัน

3. *Arfeuillea*

   A. arborescens Pierre : คงคาเดือด

4. *Arytera*

   A. litoralis Blume : สีฟัน

5. *Cardiospermum*

   C. halicacabum L. : โคกกระออม

6. *Dimocarpus*

   D. fumatus (Blume) subsp. *fumatus* Leenh. : เงาะลำไย

   subsp. *indochinensis* Leenh. : เงาะลำไย

   D. longan Lour. subsp. *longan* var. *longan* Leenh. : ลำไย

   subsp. *longan* var. *obtusus* (Pierre) Leenh. : ลำไยเครือ

   subsp. *malesianus* Leenh. var. *malesianus* Leenh. : เงาะดิเรก

7. *Dodonaea*

   D. viscosa Jacq. : ชุมเห็ดเล

8. *Filicium*

   F. decipiens (W.& A.) Thwaites & Hooker : ตานเสี้ยน

9. *Ganophyllum*

   G. falcatum Blume : สะเดาแดง

10. *Glenniea*

    G. philippinensis (Radlk.) Leenh. : ลำไยฟิลิปปินส์

11. *Guioa*

    G. bijuga (Hiern) Radlk. : ส้มลิงแกนปีก

    G. diplopetala (Hassk.) Radlk. : ส้มลิงแกนเกลี้ยง

12. *Harpullia*

H. arborea (Blanco) Radlk. : หอมไกลดง

H. cupanioides Roxb. : หงอนไก่ดง

13. *Lepisanthes*

L. ferruginea (Radlk.) Leenh.

L. fruticosa (Roxb.) Leenh. : ชำมะเลียง

L. rubiginosa (Roxb.) Leenh. : มะหวด

L. senegalensis (Poir.) Leenh. : หมากอ้อ

L. tetraphylla (Vahl) Radlk. : มะเฟืองช้าง

14. *Lichi*

L. chinensis Sonn. subsp. *chinensis* Leenh. : ลิ้นจี่

15. *Mischocarpus*

M. pentapetalus (Roxb.) Radlk. : มะป่าง

M. sundaicus Blume : เขากวาง

16. *Nephelium*

N. cuspidatum Blume : เงาะป่า

N. hypoleucum Kurz : คอแลน

N. lappaceum L. : เงาะ

N. laurinum Blume

N. maingayi Hien in Hook. F. : เงาะป่า

N. melliferum Gagnep. : คอแลน

N. ramboutan-ake (Labill.) Leenh. : เงาะขนสั้น

17. *Paranephelium*

P. macrophyllum King : ขัน

P. spirei Lec.

P. xestophyllum Miq. : ลำไยป่า

18. *Pometia*

P. pinnata J. R. & G. : แดงน้ำ

P. ridleyi King ex Radlk. : สาย

19. *Sapindus*

S. rarak DC. : มะคำดีควาย

20. *Schleichera*

S. oleosa (Lour.) Oken : ตะคร้อ

21. *Sisyrolepis*

S. muricata (Pierre) Leenh. : ตะคร้อหนาม

22. *Xerospermum*

X. laevigatum Radlk. subsp. *laevigatum* Leenh. : คอแลน

X. noronhianum (Blume) Blume : คอแลน

23. *Zollingeria*

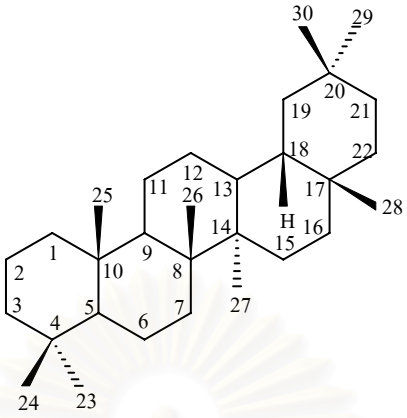
Z. dongnaiensis Pierre : ขี้หนอน

2. Chemical Constituents of Plants in the Family Sapindaceae

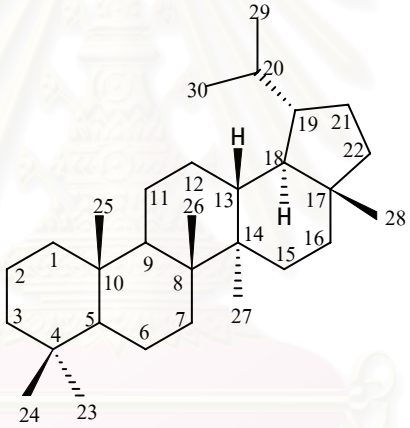
The family Sapindaceae is a well-known source of triterpenoid saponins which are linked to the traditional use as soap of plants in the family. Besides triterpenoids, other chemical constituents found in this family include flavonoids, sesquiterpenoids, diterpenoids, steroids and miscellaneous substances. The most prevalent group of compounds are the triterpenoids, of which occurrence in Sapindaceae is reviewed here.

Triterpenoids of the Sapindaceae

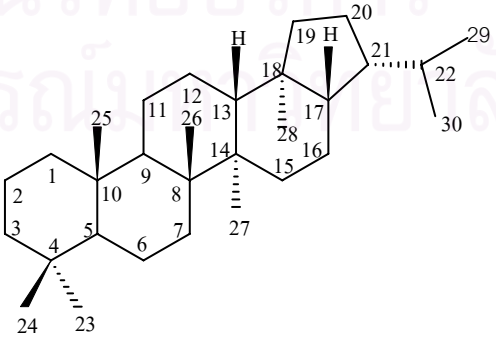
Triterpenoids can be found widespread in plants of the family Sapindaceae. These compounds isolated so far belong to the pentacyclic oleanane, lupane and hopane types, together with the tetracyclic cycloartane and tirucallane types. The most abundant type is the oleananes which include more than 90% of all triterpenoids isolated from the plants. The distribution of triterpenoids in the family Sapindaceae is summerized in Table 1.



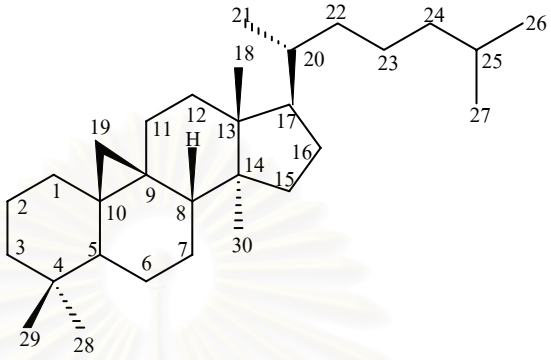
Oleanane



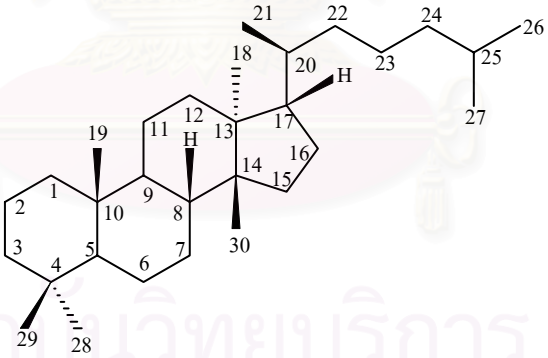
Lupane



Hopane



Cycloartane



Tirucallane

Table 1. Distribution of triterpenoids in the family Sapindaceae

Compounds	Sources	References
1. Oleanane type		
Oleanolic acid (**1**)	*Xanthoceras sorbifolia*	Ma *et al.*, 2000
Emarginatoside B (**2**)	*Sapindus emarginatus*	Gupta and Ahmed, 1990
Emarginatoside C (**3**)	*Sapindus emarginatus*	Gupta and Ahmed, 1990
Hishoushisaponin A (**4**)	*Sapindus delavayi* *Sapindus mukurossi*	Nakayama *et al.*, 1986
3-*O*-β-D-Xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)- α-L-arabinopyranosyl oleanolic acid (**5**)	*Sapindus delavayi* *Sapindus mukurossi*	Nakayama *et al.*, 1986
3-*O*-[β-D-Glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]- α-L-arabinopyranosyl] olenolic acid (**6**)	*Serjania salzmanniana*	Ekabo and Farnsworth., 1996

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
Hederagenin (**7**)	*Pometia eximia* *Sapindus delavayi* *Sapindus emarginatus* *Sapindus mukurossi*	Nakayama *et al*., 1986; Jayasinghe *et l*., 1995; Kanchanapoom, Kasai, and Yamasaki, 2001
3-*O*-α-L-Arabinopyranosyl hederagenin (**8**)	*Lepisanthes rubiginosa* *Pometia eximia* *Sapindus emarginatus* *Thinouia coriacea*	Schenkel, Werner, and Schulte 1991; Jayasinghe *et al*., 1995; Adesanya *et al.,* 1999; Kanchanapoom *et al*., 2001
3-*O*-β-D-Xlopyranosyl-(1$\rightarrow$3)-α-L-arabinopyranosyl hederagenin (**9**)	*Pometia eximia*	Jayasinghe *et al*., 1995
3-*O*-β-D-Gucopyranosyl-(1$\rightarrow$4)-α-L-arabinopyranosyl hederagenin (**10**)	*Thinouia coriacea*	Schenkel *et al*., 1991

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
Sapindoside A (**11**)	*Elattostachys apetala* *Lecaniodiscus cupanioides* *Lepisanthes rubiginosa* *Sapindus delavayi* *Sapindus mukurossi* *Sapindus trifoliatus* *Thinouia coriacea*	Encarnacion *et al*., 1981; Nakayama *et al*., 1986; Tamura, Mizutani, and Yamamota, 1990; Schenkel *et al*., 1991; Adesanya *et al*., 1999; Lavaud *et al*., 2001
Saponin A (**12**)	*Elattostachys apetala* *Lecaniodiscus cupanioides* *Lepisanthes rubiginosa* *Sapindus delavayi* *Sapindus mukurossi* *Sapindus trifoliatus*	Encarnacion *et al.,*1981; Kasai *et al*., 1986; Nakayama *et al*., 1986; Kasai *et al*., 1988; Tamura *et al*., 1990; Tamura *et al*., 1990; Adesanya *et al*., 1999; Lavaud *et al*., 2001

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
Saponin C (**13**)	*Lecaniodiscus cupanioides* *Sapindus delavayi* *Sapindus mukurossi* *Sapindus trifoliatus*	Encanacion *et al*., 1981; Kasai *et al*., 1986; Nakayama *et al*., 1986; Kasai *et al*., 1988; Tamura *et al*., 1990
3-*O*-β-D-Glucopyranosyl-(1$\rightarrow$3)-α-L-rhamnopyranosyl-(1$\rightarrow$2)- α-L-arabinopyranosyl hederagenin (**14**)	*Lepisanthes rubiginosa* *Thinouia coriacea*	Schenkel *et al*., 1991; Adesanya *et al*., 1999
Sapinoside B (**15**)	*Elattostachys apetala* *Lecaniodiscus cupanioides* *Sapindus emarginatus* *Sapindus delavayi* *Sapindus mukurossi* *Sapindus trifolia*	Encarnacion *et al*., 1981; Nakayama *et al*., 1986; Kasai *et al*., 1986; Kasai *et al*., 1988; Tamura *et al*., 1990; Kanchanapoom *et al*., 2001; Lavaud *et al*., 2001

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
Pulsatilla saponin D (**16**)	*Serjania salzmanniana* *Thinouia coriacea*	Schenkel *et al*., 1991; Ekabo and Farnsworth., 1996
Hishoushi-saponin Ee (**17**)	*Sapindus delavayi* *Sapindus mukurossi*	Nakayama *et al*., 1986
Mukurozi-saponin E_1 (**18**)	*Sapindus emarginatus* *Sapindus delavayi* *Sapindus mukurossi*	Nakayama *et al*., 1986; Kanchanapoom *et al*., 2001
Mukurozi-saponin G (**19**)	*Sapindus delavayi* *Sapindus mukurossi*	Nakayama *et al*., 1986
3-*O*-[α-L-Arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranosyl] hederagenin (**20**)	*Pometia eximia*	Jayasinghe *et al*., 1995

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
3-*O*-[β-D-Xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- α-L-Arabinopyranosyl] hederagenin (**21**)	*Pometia eximia*	Jayasinghe *et al*., 1995
3-*O*-[β-D-Galactopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-gluconopyranosyl] hederagenin (**22**)	*Pometia eximia*	Jayasinghe *et al*., 1995
3-*O*-[β-D-Xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-glucopyranosyl] hederagenin (**23**)	*Pometia eximia*	Jayasinghe *et al*., 1995
3-*O*-[β-D-Apiosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-glucopyranosyl] hederagenin (**24**)	*Pometia eximia*	Jayasinghe *et al*., 1995
3-*O*-[α-L-Arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-xylopyranosyl] hederagenin (**25**)	*Pometia eximia*	Jayasinghe *et al*., 1995
3-*O*-[β-D-Xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)- [β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl] hederagenin (**26**)	*Thinouia coriacea*	Schenkel *et al*., 1991

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
3-*O*-[β-D-Glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)- [β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl] hederagenin (**27**)	*Thinouia coriacea*	Schenkel *et al*., 1991
Salzmannianoside B (**28**)	*Serjania salzmanniana*	Ekabo and Farnsworth, 1996
28-*O*-β-D-Apiosyl-(1→2)-β-D-glucopyranosyl hederagenin (**29**)	*Pometia eximia*	Jayasinghe *et al*., 1995
3-*O*-α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl ester (**30**)	*Lepisanthes rubiginosa*	Adesanya *et al*., 1999
3-*O*-[α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]- 3β,23-dihydroxyolean-12-en-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→2)-β -D-glucopyranosyl ester (**31**)	*Elattostachys apetala*	Lavaud *et al*., 2001

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
Mukurozi-saponin X (**32**)	*Sapindus delavayi* *Sapindus mukurossi*	Kasai *et al*., 1986; Nakayama *et al*., 1986
Mukurozi-saponin Y_1 (**33**)	*Lepisanthes rubiginosa* *Sapindus delavayi* *Sapindus mukurossi*	Nakayama *et al*., 1986; Adesanya *et al*., 1999
Mukurozi-saponin Y_2 (**34**)	*Sapindus delavayi* *Sapindus mukurossi*	Nakayama *et al*., 1986
3-*O*-[β-D-Xylopyranosyl-(1$\rightarrow$3)-α-L-rhamnopyranosyl]-(1$\rightarrow$2)-α-L-arabinopyranosyl]-3β,23-dihydroxyolean-12-en-28-oic acid-28-*O*-[α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl] ester (**35**)	*Elattostachys apetala*	Lavaud *et al.,* 2001

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
3-*O*-[β-D-Xylopyranosyl-(1$\rightarrow$3)-α-L-rhamnopyranosyl]-(1$\rightarrow$2)-α-L-arabinopyranosyl]-3β,23-dihydroxyolean-12-en-28-oic acid-28-*O*-[α-L-rhamnopyranosyl-(1$\rightarrow$6)-β-D-glucopyranosyl]-β-D-glucopyranosyl ester (**36**)	*Elattostachys apetala*	Lavaud *et al.*, 2001
Sapindoside E (**37**)	*Sapindus mukurossi*	Ahmad and Rahman, 1994
3-*O*-[α-L-Rhamnopyranosyl-(1$\rightarrow$2)-α-L-arabinopyranosyl]- 3β,23-29-trihydroxyolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1$\rightarrow$2)-{β-D-glucopyranosyl-(1$\rightarrow$6)]- β-D-glucopyranosyl] ester (**38**)	*Elattostachys apetala*	Lavaud *et al.*, 2001
3-*O*-[β-D-Xylopyranosyl-(1$\rightarrow$3)-α-L-rhamnopyranosyl]-(1$\rightarrow$2)-α-L-arabinopyranosyl]-3β,23-dihydroxyolean-12-en-28-oic acid-28-*O*-[α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl] ester (**39**)	*Elattostachys apetala*	Lavaud *et al.*, 2001

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
21β,22α-*O*-diangeloyl-camelliagenin D (**40**)	*Harpullia austro-caledonica*	Ito *et al*., 1967; Voutquenne *et al*., 2002
Gypsogenin (**41**)	*Harpullia cupanioides*	Gedeon and Kincl, 1957
Salzmannianoside A (**42**)	*Serjania salzmanniana*	Ekabo and Farnsworth., 1996
22α-Hydroxyerythrodiol (**43**)	*Harpullia pendula*	Tori *et al.,* 1974; Khong and Lewis, 1976
Camelliagenin A (**44**)	*Harpullia cupanioides* *Harpullia pendula* *Majidea fosteri*	Khong and Lewis, 1976; Asmahan *et al*., 1989
16-*O*-(3-Methyl-2-butenoyl)-camelliagenin A (**45**)	*Harpullia cupanioides*	Khong and Lewis, 1976

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
16-β,β-D-dimethylacryloyl camelliagenin A (**46**)	*Harpullia cupanioides*	Dimbi *et al*., 1983
16-*O*-Angeloyl camelliagenin A (**47**)	*Harpullia pendula*	Khong and Lewis, 1976
22-(3-Methyl-2-butenoyl) camelliagenin A (**48**)	*Harpullia cupanioides*	Khong and Lewis, 1976
22-β,β-Dimethylacryloyl camelliagenin A (**49**)	*Harpullia cupanioides*	Dimbi *et al*., 1983
22-*O*-Angeloyl camelliagenin A (**50**)	*Harpullia cupanioides* *Harpullia pendula*	Sandoval *et al*., 1957; Khong and Lewis, 1976
3-*O*-β-D-Galactopyranosyl-(1$\rightarrow$2)-[α-L-rhamnopyranosyl-(1$\rightarrow$3)]-β-D-glucuronopyranosyl-16-*O*-β,β-dimethylacryloyl-camelliagenin A (**51**)	*Harpullia cupanioides*	Voutquenne *et al*., 1998

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
21β,22α-*O*-Diangeloyl barringenol C (**52**)	*Dodonaea viscosa* *Harpullia austrocaledonica*	Ahmad and Rahman, 1994; Tuntiwachwuttikul *et al*., 1997; Voutquenne *et al*., 2002
A1-Barringenol (**53**)	*Harpullia cupanioides* *Harpullia pendula* *Majidea fosteri*	Khong and Lewis, 1976; Cherry, Khong, and Lewis, 1977; Asmahan, Jacques, and Clement, 1989
22-*O*-Angeloyl A1-barringenol (**54**)	*Harpullia pendula*	Shamma, Glick, and Mumma, 1962; Ito *et al*., 1967
21,22-Di-*O*-angeloyl-barringenol (**55**)	*Dodonaea viscose* *Xanthoceras sorbifolia*	Chen, Takeda, and Ogihara, 1985; Ahmad and Rahman, 1994
22β,β-Dimethyl acryloyl barringenol (**56**)	*Harpullia cupanioides*	Dimbi *et al*., 1983

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
3-*O*-β-D-Glucopyranosyl-(1$\rightarrow$2)-[α-L-rhamnopyranosyl-(1$\rightarrow$3)]-β-D-glucuronopyranosyl-22-*O*-angeloyl-A1-barringenol (**57**)	*Harpullia cupanioides*	Voutquenne *et al*., 1998
3-*O*-β-D-Glucopyranosyl-(1$\rightarrow$2)-[α-L-rhamnopyranosyl-(1$\rightarrow$3)]-β-D-glucopyranosyl]-28-*O*-angeloyl-camelliagenin A (**58**)	*Harpullia cupanioides*	Voutquenne *et al*., 1998
3-*O*-β-D-Glucopyranosyl-(1$\rightarrow$2)-[α-L-rhamnopyranosyl-(1$\rightarrow$3)]-β-D-glucopyranosyl]-28-*O*-angeloyl-A1-barringenol (**59**)	*Harpullia cupanioides*	Voutquenne *et al*., 1998
3-*O*-β-D-Galactopyranosyl-(1$\rightarrow$2)-[α-L-rhamnopyranosyl-(1$\rightarrow$3)]-β-D-glucopyranosyl]-28-*O*-angeloyl-A1-barringenol (**60**)	*Harpullia cupanioides*	Voutquenne *et al*., 1998
Jegosapogenol (**61**)	*Harpullia cupanioides* *Majidea fosteri*	Asmahan *et al*., 1989
Harpullone (**62**)	*Harpullia pendula*	Cherry *et al*., 1977

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
Protoescigenin (**63**)	*Xanthoceras sorbifolia*	Chen *et al*., 1984; Chen *et al*., 1985
21β,22α-*O*-Diangeloyl-protoaescigenin (**64**)	*Harpullia austro-caledonica* *Harpullia ramiflora*	Dizes *et al*., 1998; Voutquenne *et al*., 2002
Harpulloside (**65**)	*Harpullia ramiflora*	Dizes *et al*., 1998
Napoleogenin B (**66**)	*Xanthoceras sorbifolia*	Chen *et al*., 1984; Chen *et al*., 1985
21-*O*-(4-*O*-Acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl-22-*O*-acetyl protoaescigenin (**67**)	*Xanthoceras sorbifolia*	Chen *et al*., 1984; Chen *et al*., 1985
21-*O*-(3,4-Di-*O*-angeloyl)-β-D-fucopyranosyl theasapogenol B (**68**)	*Xanthoceras sorbifolia*	Chen *et al*., 1984; Chen *et al*., 1985

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

compounds	Sources	References
21-*O*-(4-*O*-Acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl thesapogenol B (**69**)	*Xanthoceras sorbifolia*	Chen *et al*., 1984; Chen *et al*., 1985
24-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-protoaescigenin (**70**)	*Harpullia austrocaledonica*	Voutquenne *et al*., 2002
24-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-16 – desoxyprotoaescigenin (**71**)	*Harpullia austrocaledonica*	Voutquenne *et al*., 2002
24-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-24-oxo-camelliagenin D (**72**)	*Harpullia austrocaledonica*	Voutquenne *et al*., 2002
Zanhic acid (**73**)	*Zanha golungensis* *Ganophyllum giganteum*	Cuellar *et al*., 1997a; Cuellar *et al*., 1997b

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
Zanhasaponin A (**74**)	*Zanha africana*	Cuellar *et al*., 1997a; Cuellar *et al*., 1997b
Zanhasaponin B (**75**)	*Zanha africana*	Cuellar *et al*., 1997a; Cuellar *et al*., 1997b
Zanhasaponin C (**76**)	*Zanha africana*	Cuellar *et al*., 1997a; Cuellar *et al*., 1997b
3-*O*-β-D-Glucopyranosyl-28-*O*-{[α-L-arabinopyranosyl-(1$\rightarrow$2)]- [β-D-xylopyranosyl-(1$\rightarrow$4)]-α-L-rhamnopyranosyl-(1$\rightarrow$2)-4-*O*-[(3'-hydroxy-2'-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} zanhic acid (**77**)	*Filicium decipiens*	Lavaud *et al.,* 1998
3-*O*-{β-D-Glucopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl}-28-*O*-{(α-L-arabinopyranosyl-(1$\rightarrow$2)]- [β-D-xylopyranosyl-(1$\rightarrow$6)]-[4-*O*-angeloxy-α-L-arabinopyranosyl-(1$\rightarrow$2)-α-L-rhamnopyranosyl-(1$\rightarrow$	*Filicium decipiens*	Lavaud *et al*., 1998

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

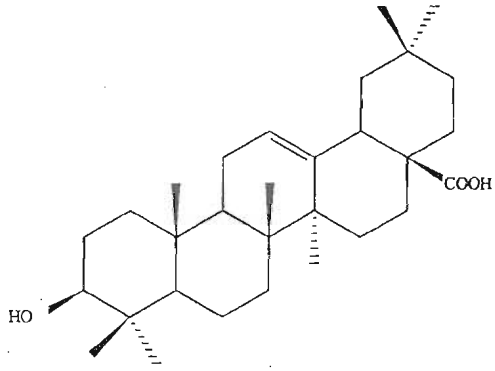
Compounds	Sources	References
2)]-β-D- glucopyranosyl medicagenic acid (**78**)		
3-*O*-{β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl-(1→2)]- [β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-4-*O*-[(3'-hydroxy-2'-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} medicagenic acid (**79**)	*Filicium decipiens*	Lavaud *et al.,* 1998
3-*O*-{β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl-(1→6)]- [4-*O*-angeloyloxy-α-L-arabinopyranosyl-(1→ 2)-α-L-rhamnopyranosyl-(1→2)]}-β-D-glucopyranosyl-gypsogenic acid (**80**)	*Filicium decipiens*	Lavaud *et al.,* 1998
Majideagenin (**81**)	*Majidea fosteri*	Asmahan *et al*., 1989
Zanhagenic acid (**82**)	*Zanha africana*	Cuellar *et al*., 1997a

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

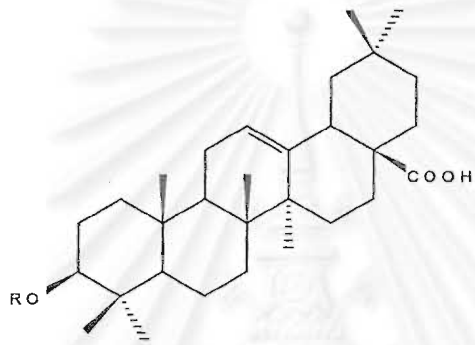
Compounds	Sources	References
Sapindic acid (**83**)	*Sapindus laurifolius*	Ahmad and Rahman, 1994
3β,17β-Dihydroxy-28-norolean-12-ene (**84**)	*Sapindus mukurossi*	Ahmad and Rahman, 1994
3β,15α,21β,22α,28-Pentahydroxy-16α-angeloyloxy-12-oleanene(**85**)	*Dodonaea viscosa*	Linnazam, 1993
2. Lupane type		
Lupeol (**86**)	*Schleichera oleosa*	Dan and Dan, 1987
Betulin (**87**)	*Schleichera oleosa*	Dan and Dan, 1987
Betulinic acid (**88**)	*Schleichera oleosa*	Dan and Dan, 1987
3,11-Dihydroxy-20(29)-lupene (**89**)	*Schleichera oleosa*	Dan and Dan, 1987

Table 1. Distribution of triterpenoids in the family Sapindaceae (continued)

Compounds	Sources	References
3. Hopane type		
3β,6β-Dihydroxy-21-αH-24-norhopa-4(23),22(29)-diene (**90**)	*Diatenopteryx sorbifolia*	Chavez *et al.,* 1997
3β,5β-Dihydroxy-6β-[(4-hydroxybenzoyl)oxy]-21-αH-24-norhopa-4 (23),22(29)-diene (**91**)	*Diatenopteryx sorbifolia*	Chavez *et al.,* 1997
4. Tirucallane type		
3-Oxotirucalla-7,24-dien-21-oic acid (**92**)	*Xanthoceras sorbifolia*	Ma *et al*., 2000
Xanthocerasic acid (**93**)	*Xanthoceras sorbifolia*	Ma *et al*., 2000
5. Cycloartane type		
24-Methylene cycloartan-3-ol (**94**)	*Xanthoceras sorbifolia*	Ma *et al*., 2000



Oleanolic acid (1)



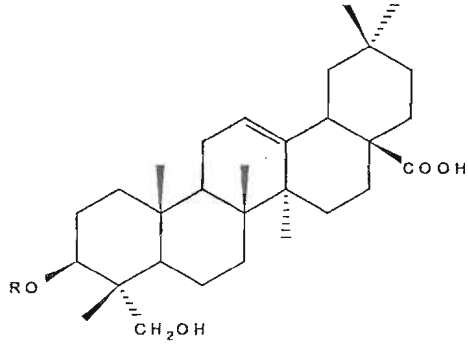
Emarginatoside B (2) R = Gal(1→2)Glc—

Emarginatoside C (3) R = Gal(1→2)Gal—

Hishoushisaponin A (4) R = Ara(1→3)Rha(1→2)Ara—

3-*O*-*β*-D-Xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)- *α*-L-arabinopyranosyl olenolic acid (5) R = Xyl(1→3)Rha(1→2)Ara—

3-*O*-[*β*-D-Glucopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]- *α*-L-arabinopyranosyl] olenolic acid (6) R = Rha(1→2)[Glc(1→4)]Ara—



Hederagenin (7) R = H

3-*O*-α-L-Arabinopyranosyl hederagenin (8) R = Ara—

3-*O*-β-D-Xlopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin (9) R = Xyl(3)—Ara—

3-*O*-β-D-Gucopyranosyl-(1→4)-α-L-arabinopyranosyl hederagenin (10) R = Glc(4)—Ara

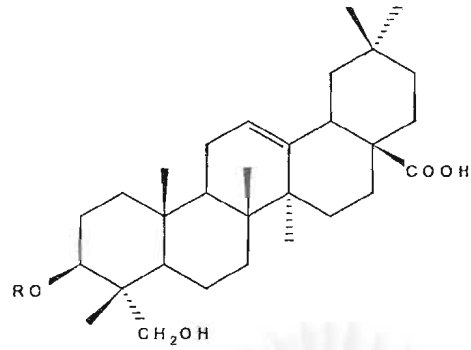
Sapindoside A (11) R = Rha(2)—Ara—

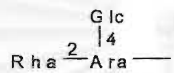
Saponin A (12) R = Ara(3)—Rha(2)—Ara—

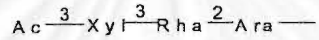
Saponin C (13) R = (f) Ara(3)—Rha(2)—Ara—

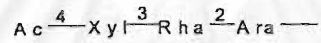
3-*O*-β-D-Glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)- α-L-arabinopyranosyl hederagenin (14) R = Glc(3)—Rha(2)—Ara—

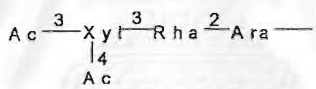
Sapinoside B (15) R = Xyl(3)—Rha(2)—Ara—



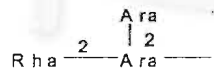
Pulsatilla saponin D (16) R = 

Hishoushi-saponin Ee (17) R = 

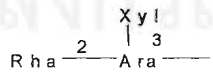
Mukurozi-saponin E_1 (18) R = 

Mukurozi-saponin G (19) R = 

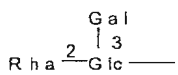
3-*O*-[α-L-Arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β- L-arabinopyranosyl]

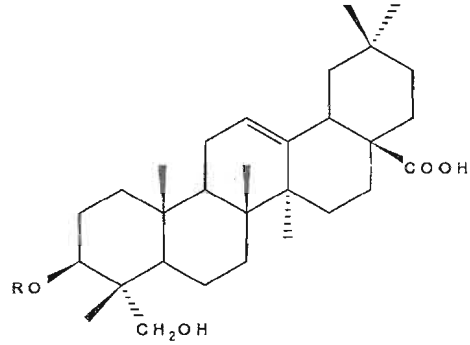
hederagenin (20) R = 

3-*O*-[β-D-Xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- α-L-Arabinopyranosyl]

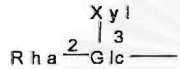
hederagenin (21) R = 

3-*O*-[β-D-Galactopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-gluconopyranosyl]

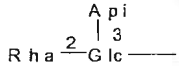
hederagenin (22) R = 



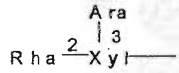
3-*O*-[β-D-Xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-glucopyranosyl]

hederagenin (**23**) R = 

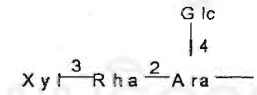
3-*O*-[β-D-Apiosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-glucopyranosyl] hederagenin

(**24**) R = 

3-*O*-[α-L-Arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]- β-D-xylopyranosyl]

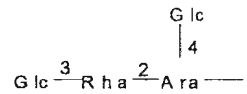
hederagenin (**25**) R = 

3-*O*-[β-D-Xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)- [β-D-glucopyranosyl-(1→4)]-

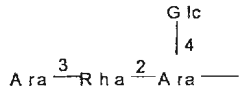


α-L-arabinopyranosyl] hederagenin (**26**) R =

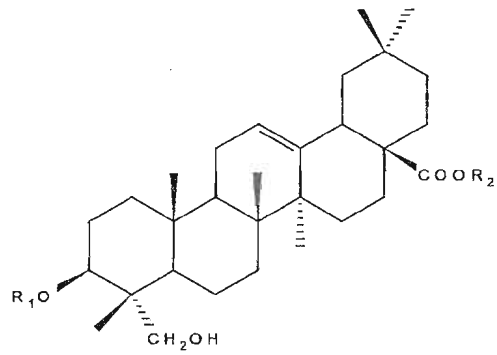
3-*O*-[β-D-Glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)- [β-D-glucopyranosyl-(1→



4)]-α-L-arabinopyranosyl] hederagenin (**27**) R =



Salzmannianoside B (**28**) R =



28-*O*-β-D-Apiosyl-(1→2)-β-D-glucopyranosyl hederagenin (29)

R_1 = H R_2 = —Glc(2→)Api

3-*O*-α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl ester (30)

R_1 = Rha(2→)Ara— R_2 = —Glc(2→)Glc

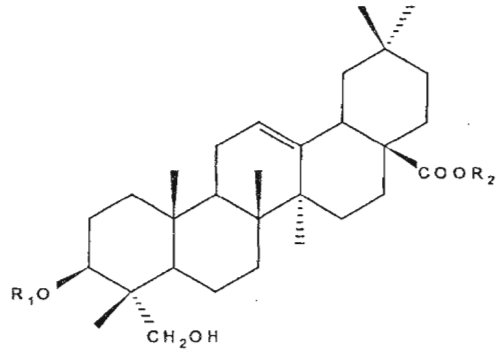
3-*O*-[α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]- 3β,23-dihydroxyolean-12-en-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→2)-β -D-glucopyranosyl ester (31)

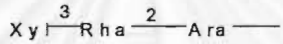
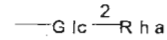
R_1 = Rha(2→)Ara— R_2 = —Glc(2→)Rha

Mukurozi-saponin X (32) R_1 = Rha(2→)Ara— R_2 = —Glc(2→)Glc

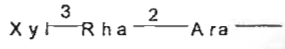
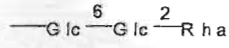
Mukurozi-saponin Y_1 (33)) R_1 = Xyl(3→)Rha(2→)Ara— R_2 = —Glc(2→)Glc

Mukurozi-saponin Y_2 (34) R_1 = Ara(3→)Rha(2→)Ara— R_2 = —Glc(2→)Glc

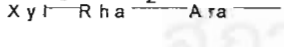
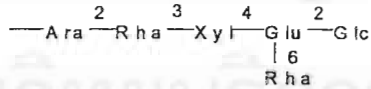


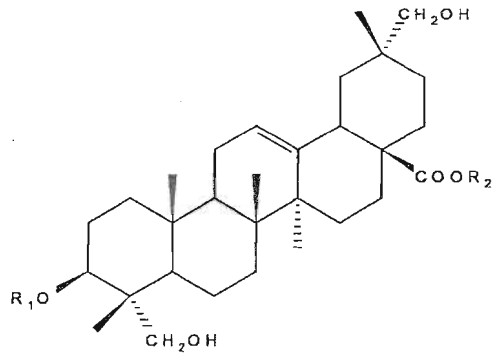
3-*O*-[β-D-Xylopyranosyl-(1→3)-α-L-rhamnopyranosyl]-(1→2)-α-L-arabinopyranosyl]-3β,23-dihydroxyolean-12-en-28-oic acid-28-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester (**35**) R_1 =  R_2 = 

3-*O*-[β-D-Xylopyranosyl-(1→3)-α-L-rhamnopyranosyl]-(1→2)-α-L-arabinopyranosyl]-3β,23-dihydroxyolean-12-en-28-oic acid-28-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-β-D-glucopyranosyl ester (**36**)

R_1 =  R_2 = 

Sapindoside E (**37**)

R_1 =  R_2 = 



3-*O*-[α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]- 3β,23-29-trihydroxyolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1→2)-{β-D-glucopyranosyl-(1→6)]- β-D-glucopyranosyl] ester (38)

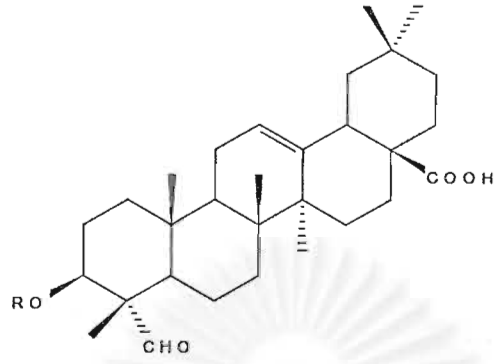
R_1 = Rha(2→)Ara— R_2 = Glc(2→)Glc, Glc(6→)Glc

3-*O*-[β-D-Xylopyranosyl-(1→3)-α-L-rhamnopyranosyl]-(1→2)-α-L-arabinopyranosyl]-3β,23-dihydroxyolean-12-en-28-oic acid-28-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester (39)

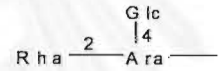
R_1 = Ara(3→)Rha(2→)Ara— R_2 = Glc(2→)Glc, Glc(6→)Glc

21β,22α-*O*-diangeloyl-camelliagenin D (40)

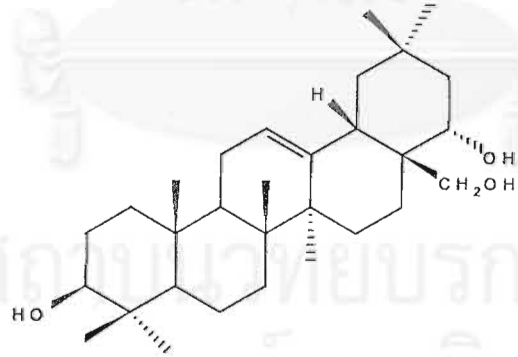
R_1 = Xyl(3→)Rha(2→)Ara— R_2 = Glc(2→)Glc, Glc(6→)Glc



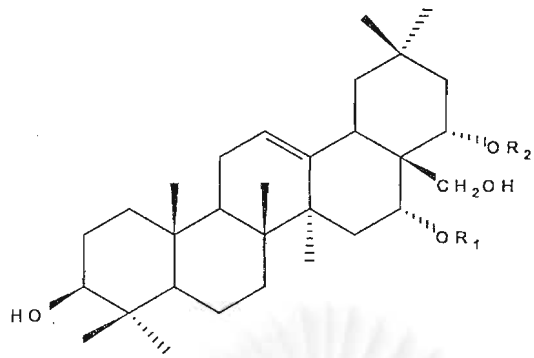
Gypsogenin (41) R = H



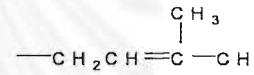
Salzmannianoside A (42) R =

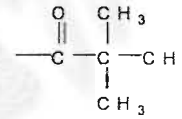


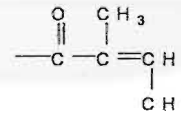
22α-Hydroxyerythrodiol (43)



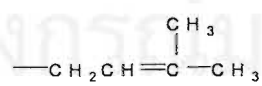
Camelliagenin A (**44**) $R_1 = R_2 = H$

16-*O*-(3-Methyl-2-butenoyl)-camelliagenin A (**45**) R_1 =  $R_2 = H$

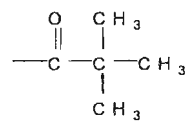
16-*β*,*β*-D-dimethylacryloyl camelliagenin A (**46**) R_1 =  $R_2 = H$

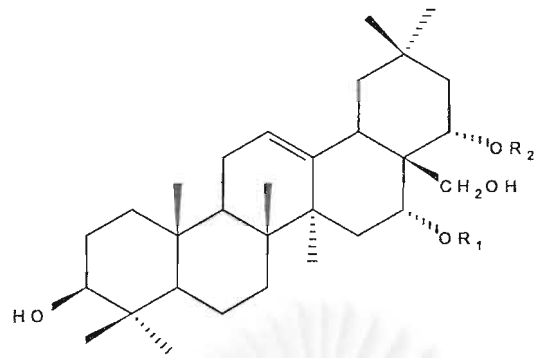
16-*O*-Angeloyl camelliagenin A (**47**) R_1 =  $R_2 = H$

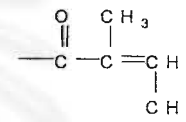
22-(3-Methyl-2-butenoyl) camelliagenin A (**48**)

$R_1 = H$ R_2 = 

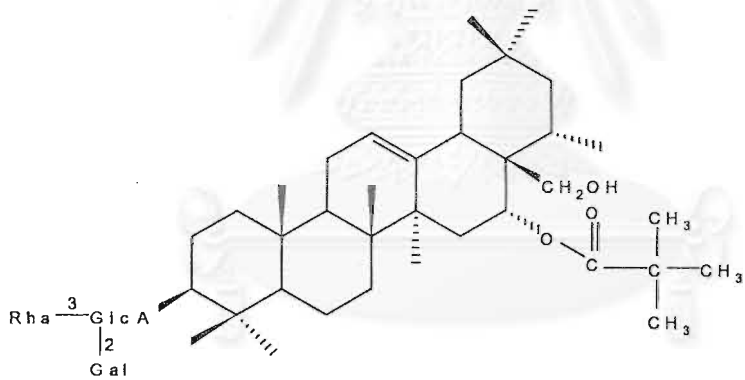
22-*β*,*β*-Dimethylacryloyl camelliagenin A (**49**)

$R_1 = H$ R_2 = 

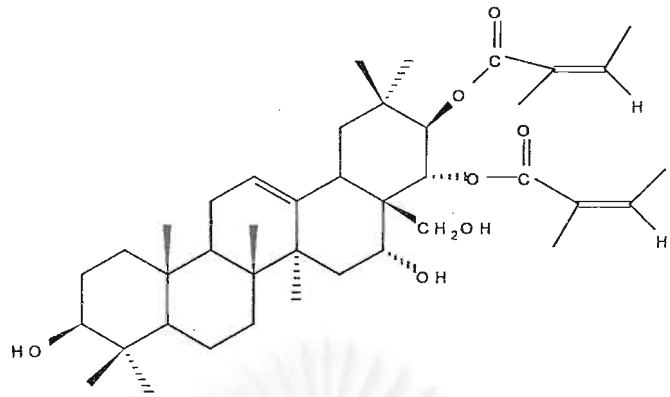




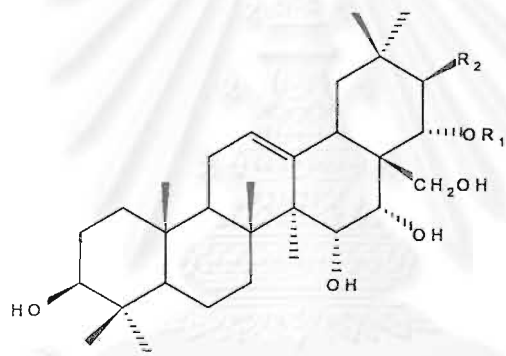
22-*O*-Angeloyl camelliagenin A (**50**) R_1 = H R_2 =



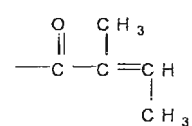
3-*O*-β-D-Galactopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucuronopyranosyl-16-*O*-β,β-dimethylacryloyl-camelliagenin A (**51**)



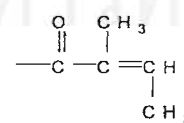
21β,22α-*O*-Diangeloyl barringenol C (**52**)



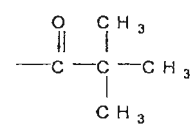
A1-Barringenol (**53**) $R_1 = R_2 = H$



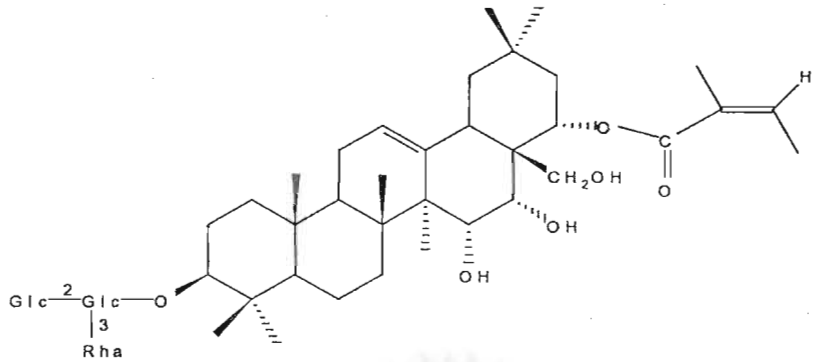
22-*O*-Angeloyl A1-barringenol (**54**) R_1 = $R_2 = H$



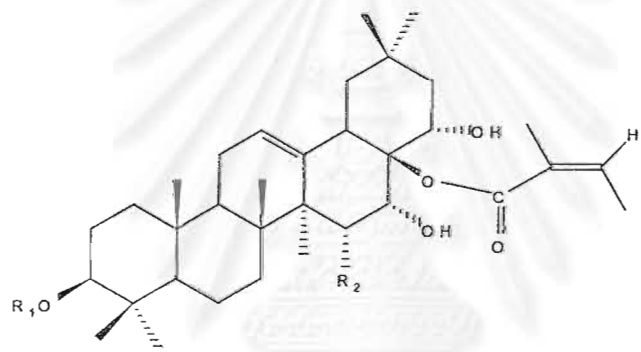
21,22-Di-*O*-angeloyl-barringenol (**55**) $R_1 = R_2$ =



22β,β-Dimethyl acryloyl barringenol (**56**) R_1 = $R_2 = H$



3-*O*-β-D-Glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucuronopyranosyl-22-*O*-angeloyl-A1-barringenol (**57**)



3-*O*-β-D-Glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranosyl]-28-*O*-angeloyl-camelliagenin A (**58**)

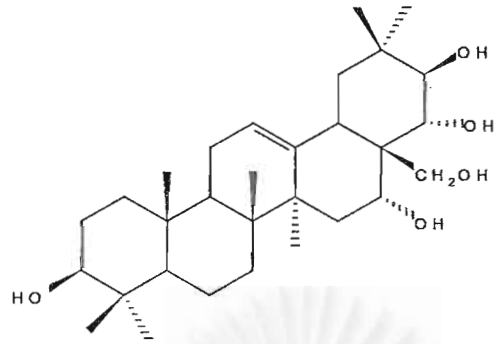
R_1 = Rha(3)-Glc(2)-Glc— R_2 = H

3-*O*-β-D-Glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranosyl]-28-*O*-angeloyl-A1-barringenol (**59**)

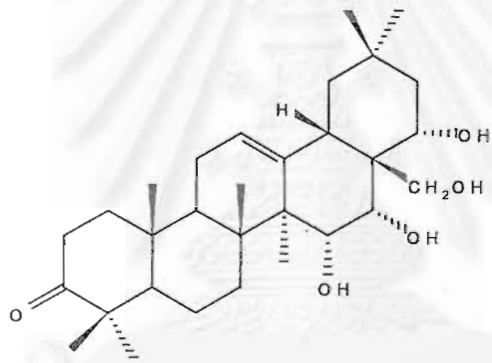
R_1 = Rha(3)-Glc(2)-Glc— R_2 = OH

3-*O*-β-D-Galactopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranosyl]-28-*O*-angeloyl-A1-barringenol (**60**)

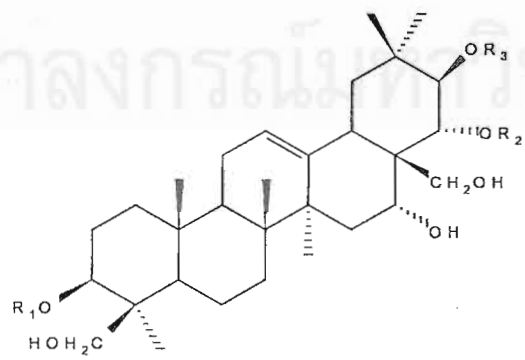
R_1 = Rha(3)-Glc(2)-Gal— R_2 = OH



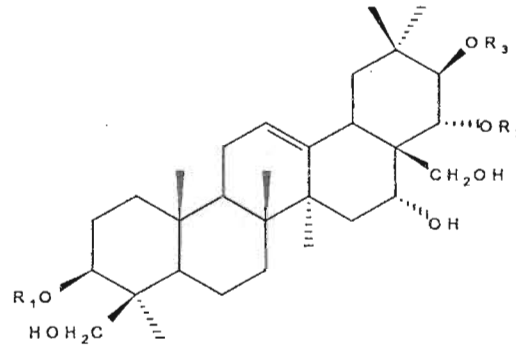
Jegosapogenol (61)



Harpullone (62)

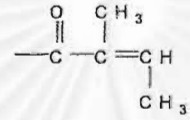


Protoescigenin (63) $R_1 = R_2 = R_3 = H$

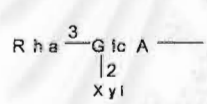


21β,22α-*O*-Diangeloyl-protoaescigenin (**64**)

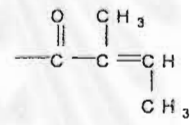
R_1 = H R_2 = R_3 =



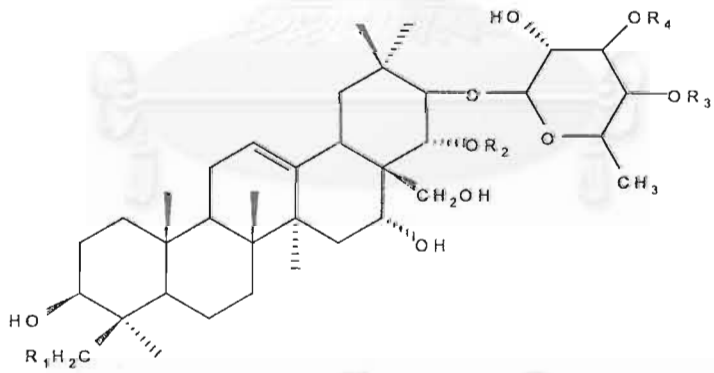
Harpulloside (**65**) R_1 =



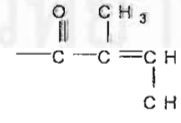
R_2 =



R_3 = H

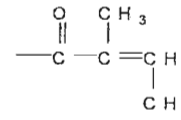


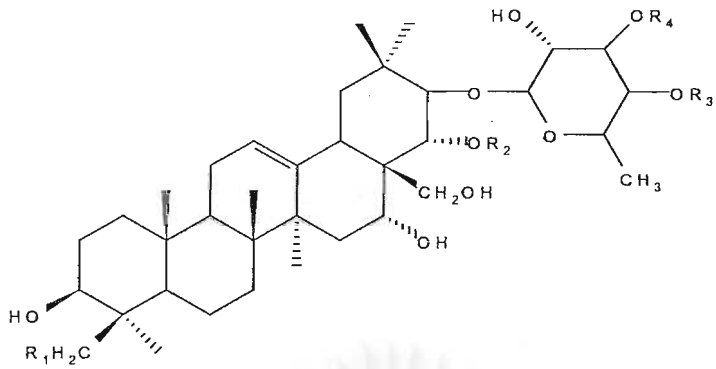
Napoleogenin B (**66**) R_1 = OH R_2 = H R_3 = R_4 =



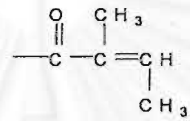
21-*O*-(4-*O*-Acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl-22-*O*-acetyl protoaescigenin (**67**)

R_1 = OH R_2 = R_3 = Ac R_4 =



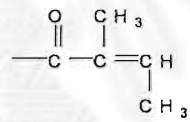


21-*O*-(3,4-Di-*O*-angeloyl)-β-D-fucopyranosyl theasapogenol B (**68**)

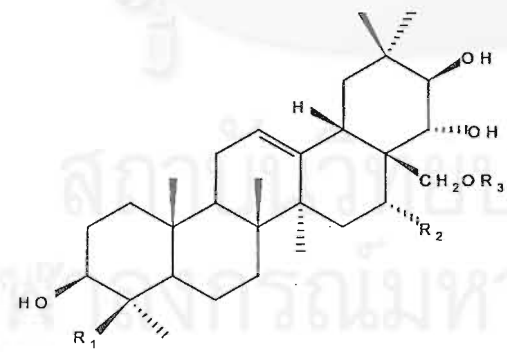


$R_1 = R_2 = H$ $R_3 = R_4 =$

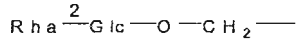
21-*O*-(4-*O*-Acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl thesapogenol B (**69**)

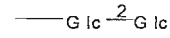


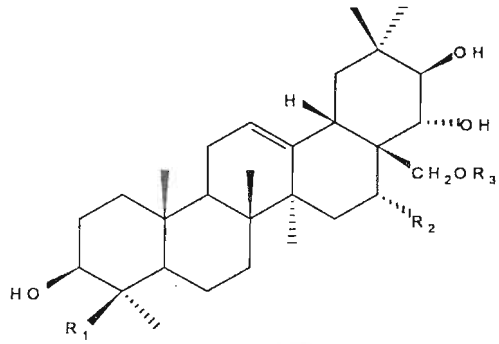
$R_1 = R_2 = H$ $R_3 = Ac$ $R_4 =$



24-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-protoaescigenin (**70**)



$R_1 =$ $R_2 = OH$ $R_3 =$ 

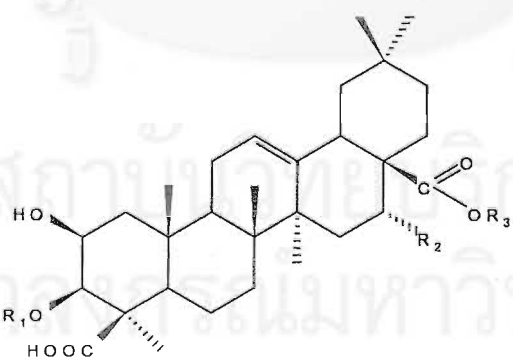


24-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-16 –desoxyprotoaescigenin (71)

R_1 = Rha(2→)Glc—O—CH_2— R_2 = H R_3 = —Glc(2→)Glc

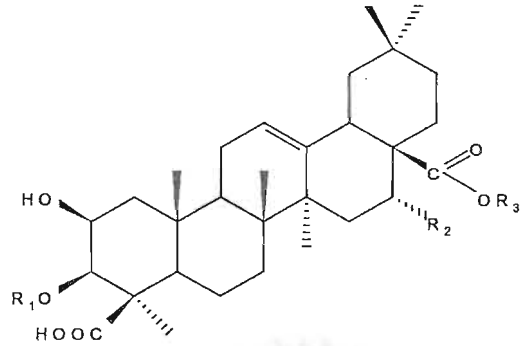
24-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-24-oxo-camelliagenin D (72)

R_1 = Rha(2→)Glc—O—C(=O)— R_2 = H R_3 = —Glc(2→)Glc



Zanhic acid (73) R_1 = H R_2 = OH R_3 = H

Zanhasaponin A (74) R_1 = Glc— R_2 = OH R_3 = —Rha(2→)Rha

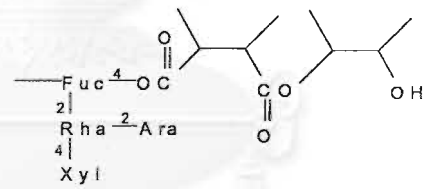


Zanhasaponin B (**75**) R_1 = Glc— R_2 = OH R_3 = —Rha(2→)Rha(2→)Xyl

Zanhasaponin C (**76**) R_1 = Glc— R_2 = OH R_3 = —Rha(2→)Rha(2→)Xyl(3→)Xyl

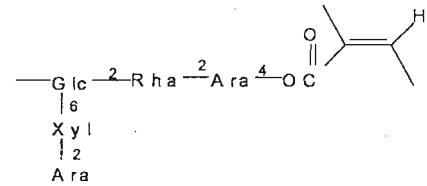
3-*O*-β-D-Glucopyranosyl-28-*O*-{[α-L-arabinopyranosyl-(1→2)]- [β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-4-*O*-[(3'-hydroxy-2'-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} zanhic acid (**77**)

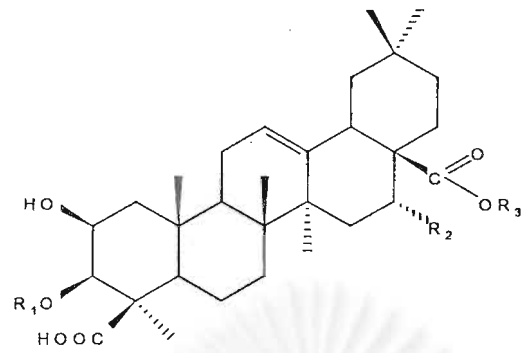
R_1 = Glc— R_2 = OH R_3 =



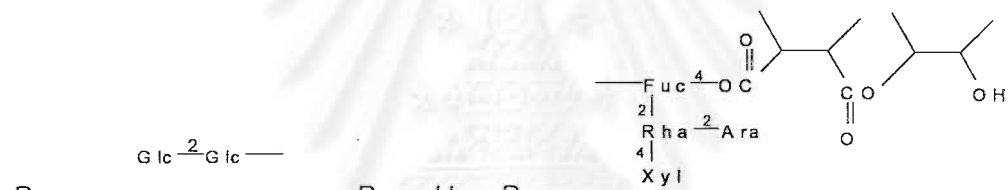
3-*O*-{β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl}-28-*O*-{(α-L-arabinopyranosyl-(1→2)]- [β-D-xylopyranosyl-(1→6)]-[4-*O*-angeloxy-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)]-β-D- glucopyranosyl medicagenic acid (**78**)

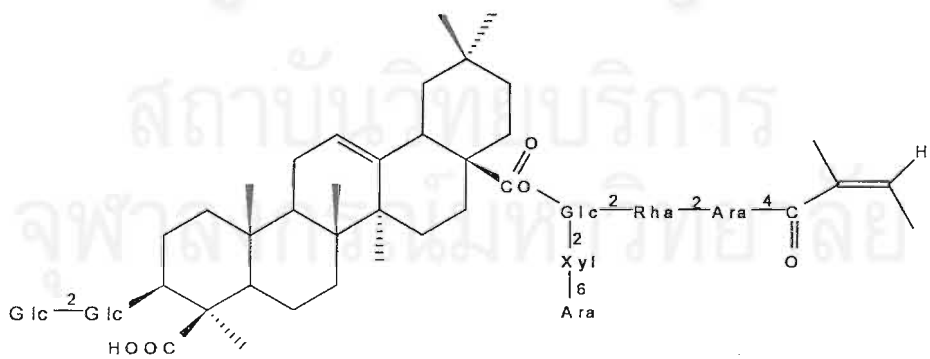
R_1 = Glc(2→)Glc— R_2 = H R_3 =



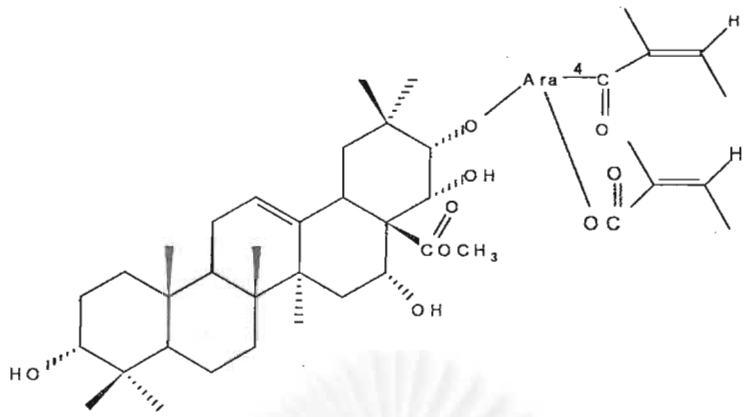


3-*O*-{β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl-(1→2)]- [β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-4-*O*-[(3'-hydroxy-2'-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} medicagenic acid (79)

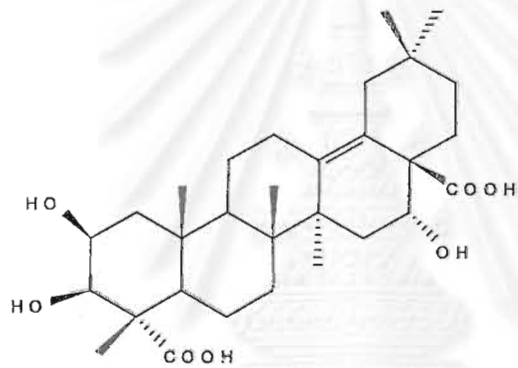




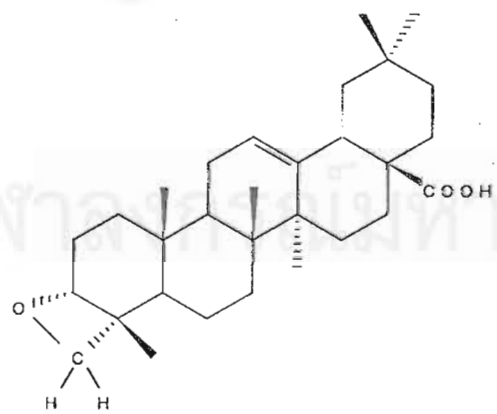
3-*O*-{β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1→6)]-[4-*O*-angeloyloxy-α-L-arabinopyranosyl-(1→ 2)-α-L-rhamnopyranosyl-(1→2)]}-β-D-glucopyranosyl-gypsogenic acid (80)



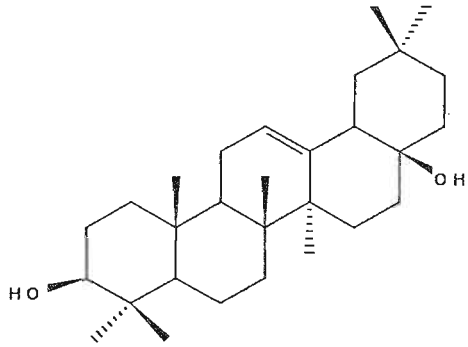
Majideagenin (81)



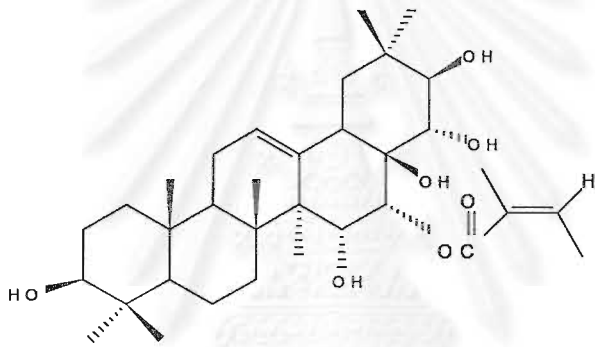
Zanhagenic acid (82)



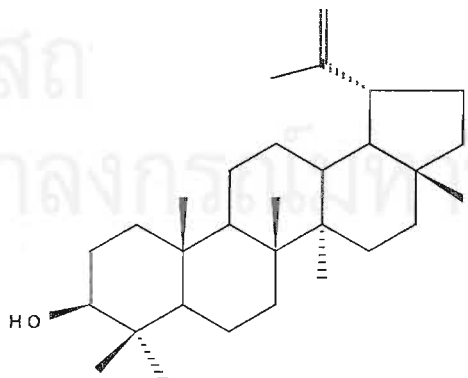
Sapindic acid (83)



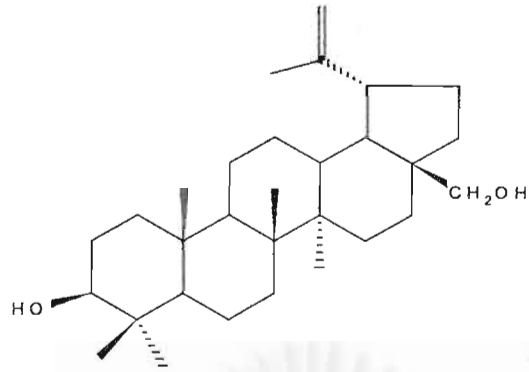
3β,17β-Dihydroxy-28-norolean-12-ene (84)



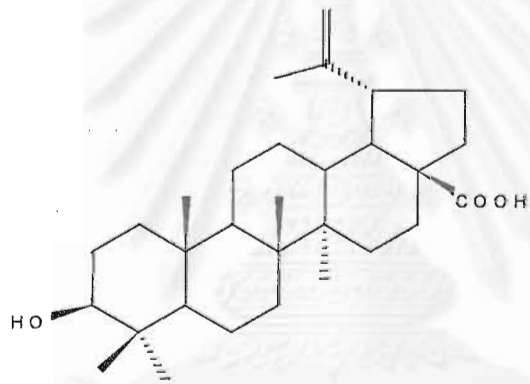
3β,15α,21β,22α,28-Pentahydroxy-16α-angeloyloxy-12-oleanene(85)



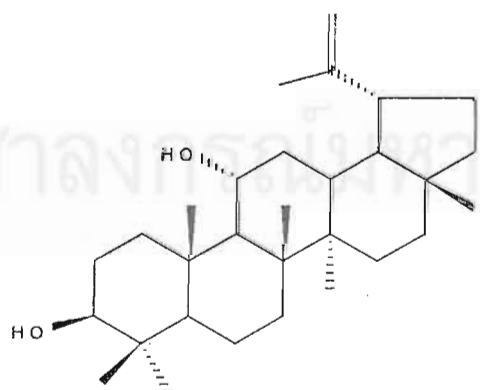
Lupeol (86)



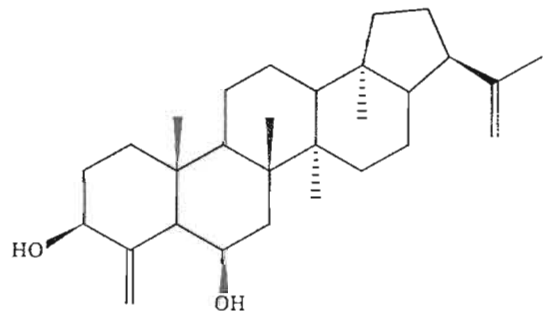
Betulin (87)



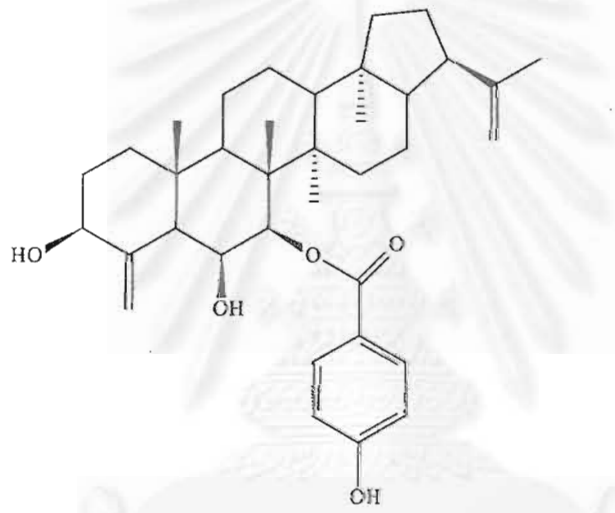
Betulinic acid (88)



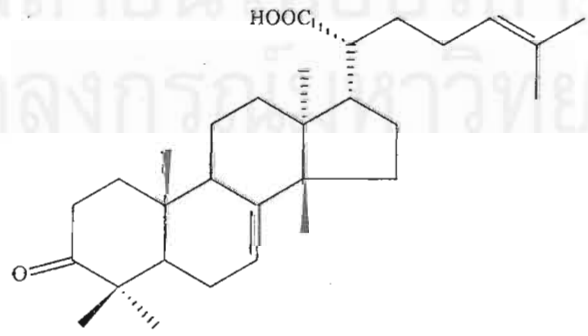
3,11-Dihydroxy-20(29)-lupene (89)



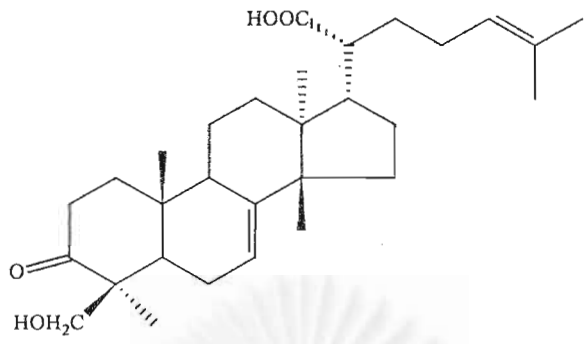
3β,6β-Dihydroxy-21-αH-24-norhopa-4(23),22(29)-diene (**90**)



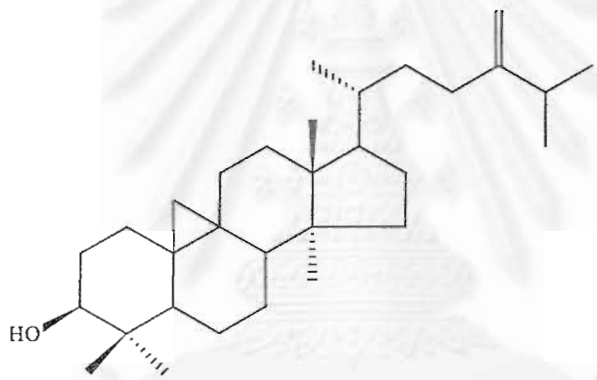
3β,5β-Dihydroxy-6β-[(4-hydroxybenzoyl)oxy]-21-αH-24-norhopa-4 (23),22(29)-diene (**91**)



3-Oxotirucalla-7,24-dien-21-oic acid (**92**)



Xanthocerasic acid (93)



24-Methylene cycloartan-3-ol (94)

3. Chemical Constituents of Plants in the Genus *Harpullia*.

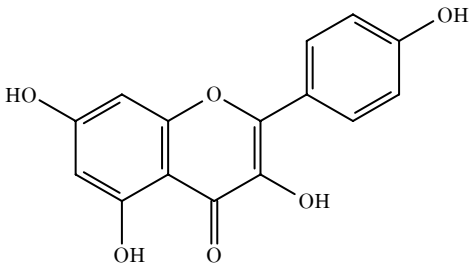
Previous chemical studies on the genus *Harpullia*, dealing with 6 species including *H. austrocaledonica, H. cupanioides, H. pendula, H. petiolaris, H. ramiflora* and *H. thanatophora*, resulted in the isolation of triterpenoids, flavonoids, steroids and miscellaneous substances. The majority of the compounds isolated are triterpenoids, all of which belong to the oleanane type. The distribution of these compounds in *Harpullia* species is already given in Table 1. Other isolated compounds and their distribution in the genus *Harpullia* are summarized in Table 2.

Table 2. Distribution of compounds in the genus *Harpullia*

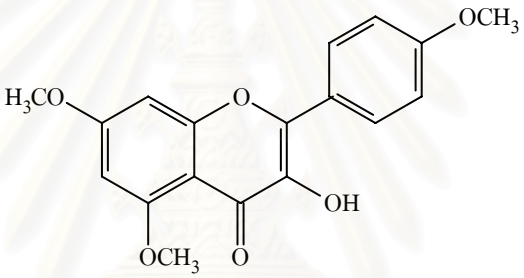
Compounds	Sources	References
1. Flavonoids		
Kaempferol (**95**)	*Harpullia pendula*	El Sayed *et al*., 1989
3-Hydroxy-4',5,7-trimethoxyflavone (**96**)	*Harpullia cupanioides*	Suttisri *et al*., 1999
Flavokavain A (**97**)	*Harpullia cupanioides*	Sandoval *et al*., 1957; Suttisri *et al*., 1999
Quercetin (**98**)	*Harpullia pendula*	El Sayed *et al*., 1989
2. Tannins		
Gallic acid (**99**)	*Harpullia austrocaledonica* *Harpullia pendula*	Sati and Rana, 1987; El Sayed *et al*., 1989; Tuntiwachwuttikul *et al*., 1997
Penta-*O*-galloyl-D-glucose (**100**)	*Harpullia austrocaledonica* *Harpullia pendula*	Ito *et al*., 1967; El Sayed *et al*., 1989; Voutquenne *et al*., 2002

Table 2. Distribution of compounds in the genus *Harpullia* (continued)

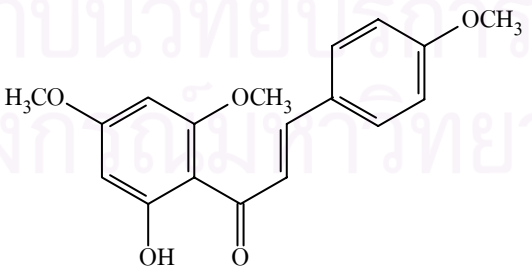
Compounds	Sources	References
Chinese gallotannin (**101**)	*Harpullia austro-caledonica* *Harpullia pendula* *Harpullia ramiflora*	El Sayed *et al*., 1989; Dizes *et al*., 1998; Voutquenne *et al*., 2002
Tetra-*O*-galloylglucose (**102**)	*Harpullia pendula*	El Sayed *et al*., 1989
3. Steroids		
Stigmasterol (**103**)	*Harpullia cupanioides*	Sandoval *et al*., 1988; Suttisri *et al*., 1999
4. Miscellaneous		
Chlorogenic acid (**104**)	*Harpullia pendula*	Khong and Lewis, 1976; El Sayed *et al*., 1989
p-Coumaric acid methyl ester (**105**)	*Harpullia pendula*	Khong and Lewis, 1976
Quebrachitol (**106**)	*Harpullia pendula*	Khong and Lewis, 1976; Cherry, 1977



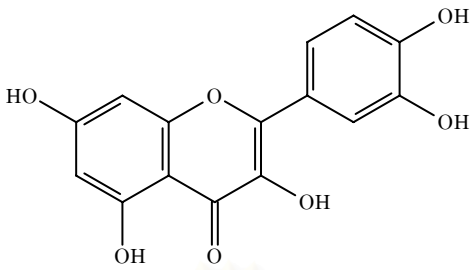
Kaempferol (95)



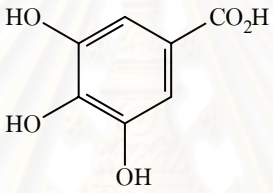
3-Hydroxy-4',5,7-trimethoxyflavone (96)



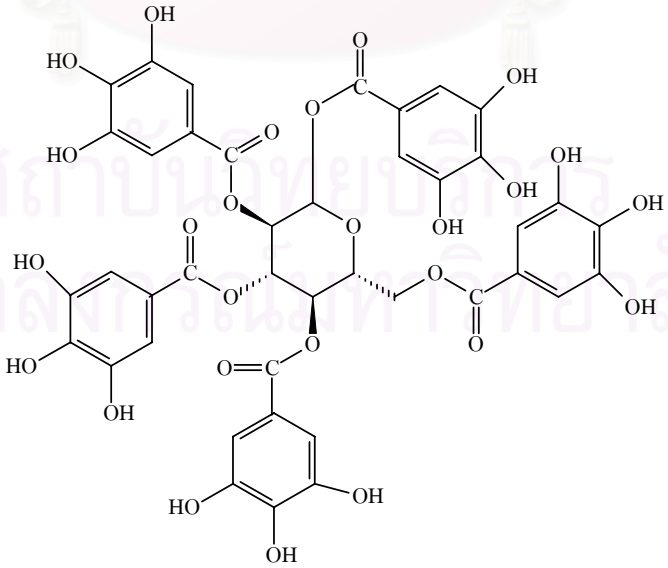
Flavokavain A (97)



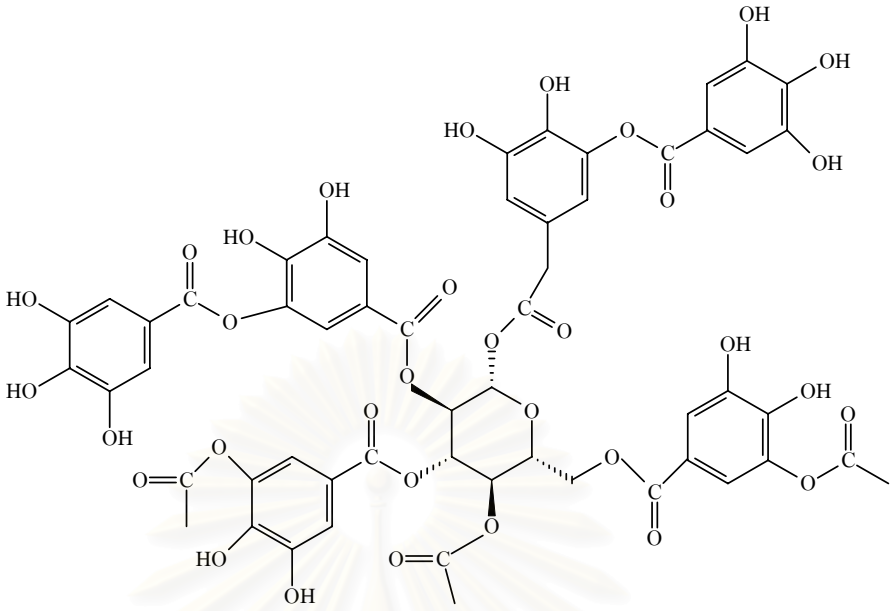
Quercetin (**98**)



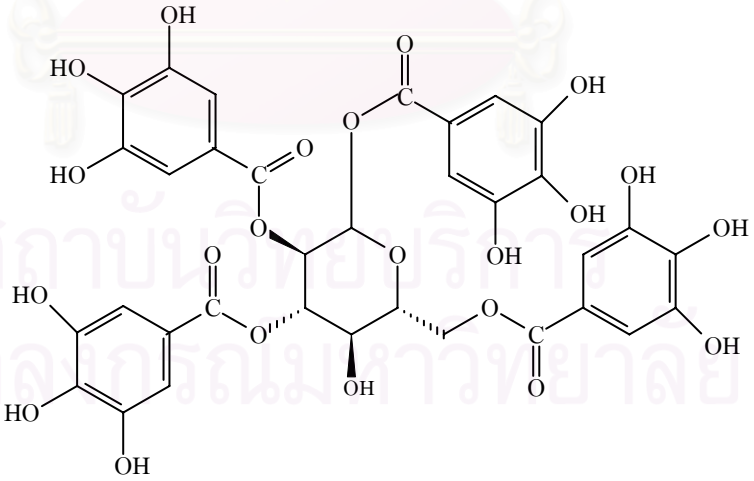
Gallic acid (**99**)



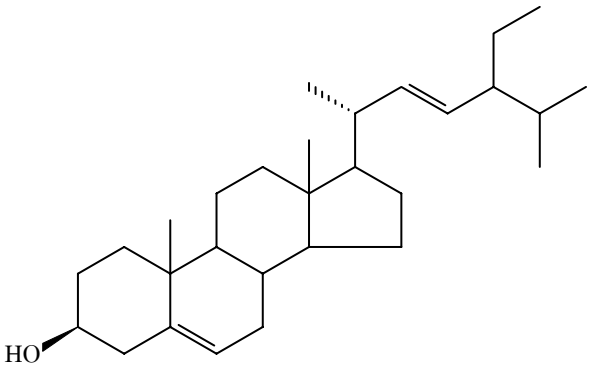
Penta-*O*-galloyl-D-glucose (**100**)



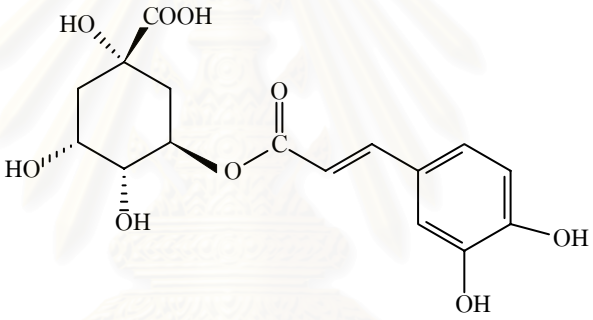
Chinese gallotannin (**101**)



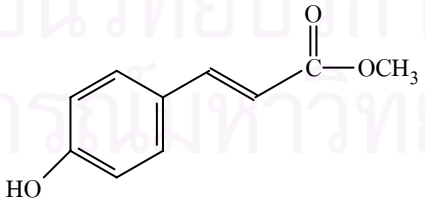
Tetra-*O*-galloylglucose (**102**)



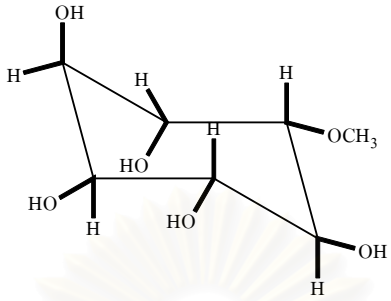
Stigmasterol (**103**)



Chlorogenic acid (**104**)



p-Coumaric acid methyl ester (**105**)



Quebrachitol (**106**)

4. Traditional Uses of *Harpullia* Species.

Traditional uses of several species of *Harpullia* have been recorded. In Philipines, the bark and fruit of *H. arborea* are used to prevent leech bites and they are also a fish poison. Oil from the seeds is sometimes used as antirheumatic (Perry and Metzger, 1895). In Sri Lanka, the fruits of *H. cupanioides* have been used for washing (Burkill, 1935).; the plant is also recorded as a fish poison (Burkill, 1935). The bark of *H. pendula* is used as a fish poison by the Aborigines in Australia (Khong and Lewis, 1976). The stem bark of *H. ramiflora* is used, by the traditional healers in Papua New-Guinea, as a topical medicine against skin disease (Dizes *et al*., 1998). *H. thanatophora* is reported to be poisonous by the natives in New Guinea (Perry and Metzger, 1895).

5. Biological Activities of *Harpullia* species.

Some plants of the genus *Harpullia* have been reported as exhibiting bioactivity. The ethanolic extract of the stem bark of *H. austro-caledonica* exhibited *in vitro* cytotoxic activity against KB cell (90% at 10 μg/ml) (Voutquenne *et al*., 2002). The methanol extract of the leaves of *H. cupanioides* displayed activity against both Herpes simplex virus types 1 and 2. Its hexane-soluble fraction of this extract also exhibited cytotoxicity against KB and BC cell lines with ED_{50} of 5.9 and 5.0 μg/ml, respectively (Suttisri *et al*., 1999). Saponins extracted from *H. cupanioides* were reported to be active in releasing hormone from cultured rat hypophyseal cells (Asmahan *et al*., 1989). The seed extract of this plant was found to strongly inhibit the growth of some fungal plant pathogens (Bharathimatha, Doraiswamy, and Velazhahan, 2002). Methanol extracts of the leaves, the flowers, the stem and the root bark of *H. ramiflora* demonstrated broad spectrum and high levels of antibacterial activity (Khan, Kihara, and Omoloso, 2001). Methanol extracts of the leaves, the stem, the root bark and the heartwood of *H. petiolaris* displayed antibacterial activity (Khan and Omoloso, 2002).

CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The leaves of *Harpullia arborea* (Blanco) Radlk. (Sapindaceae) were collected from Mae Wong National Park, Kampangpet Province, Thailand in May, 2001. The plant material was identified by comparison with the herbarium specimens (BKF no. 075890) at the Royal Forest Department, Bangkok, Thailand.

2. General Techniques

2.1 Chromatographic Techniques

2.1.1 Thin – Layer Chromatography (TLC)

Technique : One dimension, ascending

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

Layer thickness : 0.2 mm

Solvent system : Various solvent systems depending on materials

Distance : 6 cm

Temperature : Laboratory temperature (28 – 35 °C)

Detection : 1. Ultraviolet light (254 and 365 nm)

2. Anisaldehyde - sulfuric acid reagent, heating at 100 -105°C for 5 – 10 minutes

3. 10% Sulfuric acid in ethanol, heating at 100 – 105 °C for 5 – 10 minutes

4. Libermann-Burchard reagent

5. Iodine vapor

2.1.2 Column Chromatography (CC)

Column : Flat bottom glass column (various diameters)

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

Packing method : Wet and dry packing

Sample loading : The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on the top of the column. Or the sample was dissolved in a smal volume of the eluent, then loaded directly on the top of the column.

Solvent system : Various solvent systems depending on materials.

Detection : Fractions were examined by TLC observed under UV light at the wavelengths of 254 and 365 nm. The TLC plate was then sprayed with anisaldehyde – sulfuric acid reagent and heated at 100°C for 5 – 10 minutes. Fractions of similar chromatographic pattern were combined.

2.2 Spectroscopy

2.2.1 Infrared (IR) Absorption Spectra

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

2.2.2 Mass Spectra (MS)

Electron Impact Mass Spectra (EIMS) were obtained on a Polaris Q Finnigan Gas Chromatography - Mass Spectrometer (Department of Chemistry, Facultry of Science, Mahidol University), operating at 12 and 70 eV.

2.2.3 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).

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

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

2.3 Physical constants

2.3.1 Melting Point

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

2.3.2 Optical Rotation

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

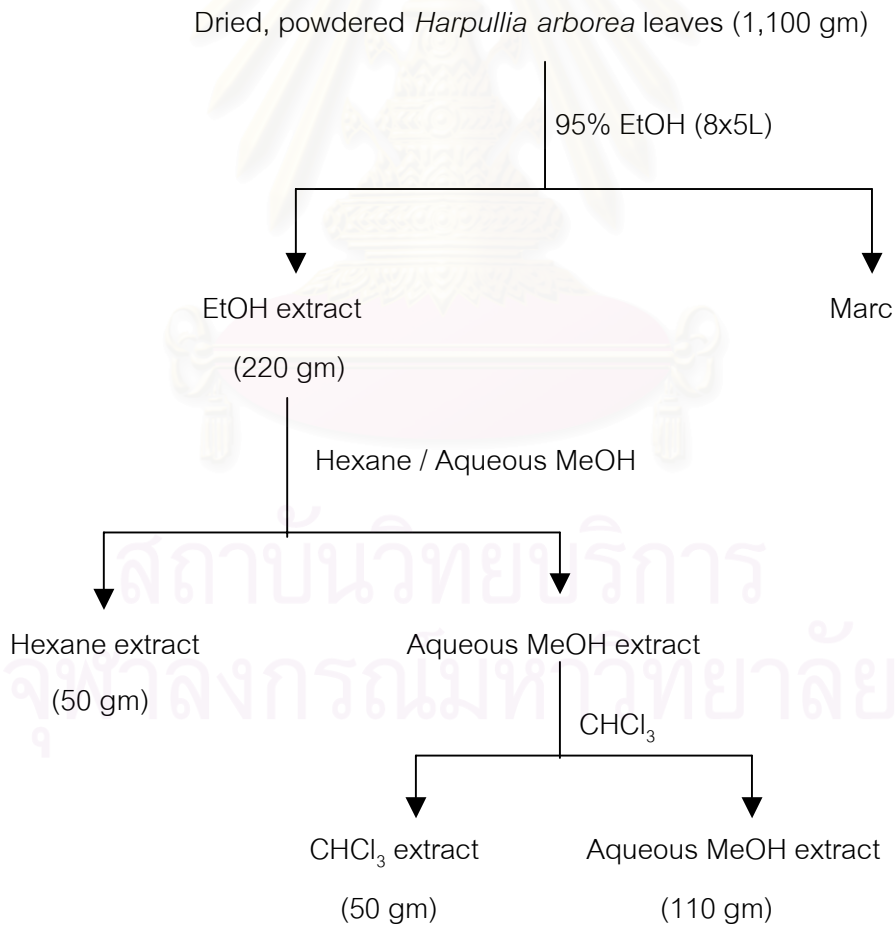
2.4 Solvents

All organic solvents used in the extraction and isolation procedure were of commercial grade and were redistilled prior to use.

3. Extraction and Isolation

3.1 Extraction

The dried, coarsely powdered leaves of *Harpullia arborea* Radlk. (1,100 gm) were macerated with 95% ethanol (8x5 L) and filtered. The filtrates were pooled and evaporated under reduced pressure to yield 220 gm of dried crude extract (20% of dry weight). The ethanol extract was diluted with aqueous methanol, and partitioned with hexane (6x4L). The combined hexane extract was evaporated to dryness under reduced pressure to give hexane extract (50 gm, 4.55% of dry weight). The aqueous methanol layer was then partitioned with chloroform (6x4L). The combined chloroform extract was similarity dried to give chloroform extract (50 gm, 4.55% of dry weight) and 110 gm of aqueous methanol extract (10% of dry weight).



Scheme 1. Extraction scheme of *Harpullia arborea* leaves

3.2 Isolation

3.2.1 Fractionation of hexane extract

A portion of the hexane extract (25 gm) was subjected to a silica gel column (300 gm, 5 × 60 cm) eluted with chloroform. One hundred and twenty-seven 30-ml fractions were collected and combined according to their TLC patterns into six major fractions (A_1-F_1) as shown in Table 3. The column was then washed down with methanol.

Table 3. Combined fractions from the hexane extract.

Fraction	Number of eluates	Weight of fraction (gm)
A_1	1 – 7	3.49
B_1	8 – 17	6.78
C_1	18 – 20	3.98
D_1	21 – 63	4.09
E_1	64 – 110	1.23
F_1	111 – 127	3.17
Methnol eluate		1.95

3.2.1.1 Isolation of compound HA-1

Fraction D_1 (4.09 gm) was subjected to a silica gel column (200 gm, 5×60 cm) using hexane – ethyl acetate mixture of increasing polarity (from 95:5 to 70:30) as the eluent. One hundred and fifty fractions (20 ml each) were collected and combined according to their TLC patterns into nine major fractions (D_1A - D_1I) as shown in Table 4.

Table 4. Combined fractions from D_1

Fraction	Solvent Ratio (hexane:EtOAc)	Number of eluates	Weight of fraction (mg)
D_1A	95 : 5	1 – 18	73.5
D_1B	95 : 5	19 – 30	126.2
D_1C	95 : 5	31 – 50	710.6
D_1D	90 : 10	51 – 54	41.0
D_1E	90 : 10	55 – 85	1512.4
D_1F	90 : 10	86 – 92	15.2
D_1G	90 : 10	93 – 102	156.8
D_1H	80 : 20	103 – 112	195.1
D_1I	70 : 30	113 – 150	345.7
Methanol eluate			718.2

Fraction D_1C (710.6 mg) was further separated on a silica gel column (300 gm, 2×50 cm) eluted with hexane - acetone (97:3). Fifty - three fractions (10 ml each) were collected and pooled according to their TLC patherns into eight major fractions (D_1C_1 - D_1C_8) as shown in Table 5.

Table 5. Combined fractions from D_1C

Fraction	Number of eluates	Weight of fraction (mg)
D_1C_1	1 – 6	21.0
D_1C_2	7 – 9	4.9
D_1C_3	10 – 18	104.8
D_1C_4	19 – 22	5.2
D_1C_5	23 – 27	19.8
D_1C_6	28 – 29	3.2
D_1C_7	30 – 31	2.7
D_1C_8	32 – 53	112.5
Methanol eluate		294.0

Fraction D_1C_3, which gave single blue spot on TLC under detection with 10% sulfuric acid in ethanol, was purified by recrystallization from methanol to give compound HA-1 as colorless crystals (56 mg). The compound gave red-violet color with Libermann-Burchard reagent.

3.2.1.2 Isolation of compound HA-2

Fraction E_1 (1.23 gm) was separated on a silica gel column (90 gm, 2.5×50 cm) eluted with chloroform. Forty, 20-ml fractions were collected and combined according to their TLC patterns into three fractions (E_1A – E_1C) as shown in Table 6.

Table 6. Combined fractions from E_1

Fraction	Number of eluates	Weight of fraction (mg)
E_1A	1 – 15	191.0
E_1B	16 – 31	387.0
E_1C	32 – 40	159.0
Methanol eluate		309.1

Fraction E_1B, which gave a red-violet spot on TLC under detection with 10% sulfuric acid in ethanol, was purified by recrystallization in methanol to give compound HA-2 as white amorphous powder (63 mg). The compound gave deep green color with Libermann-Burchard reagent.

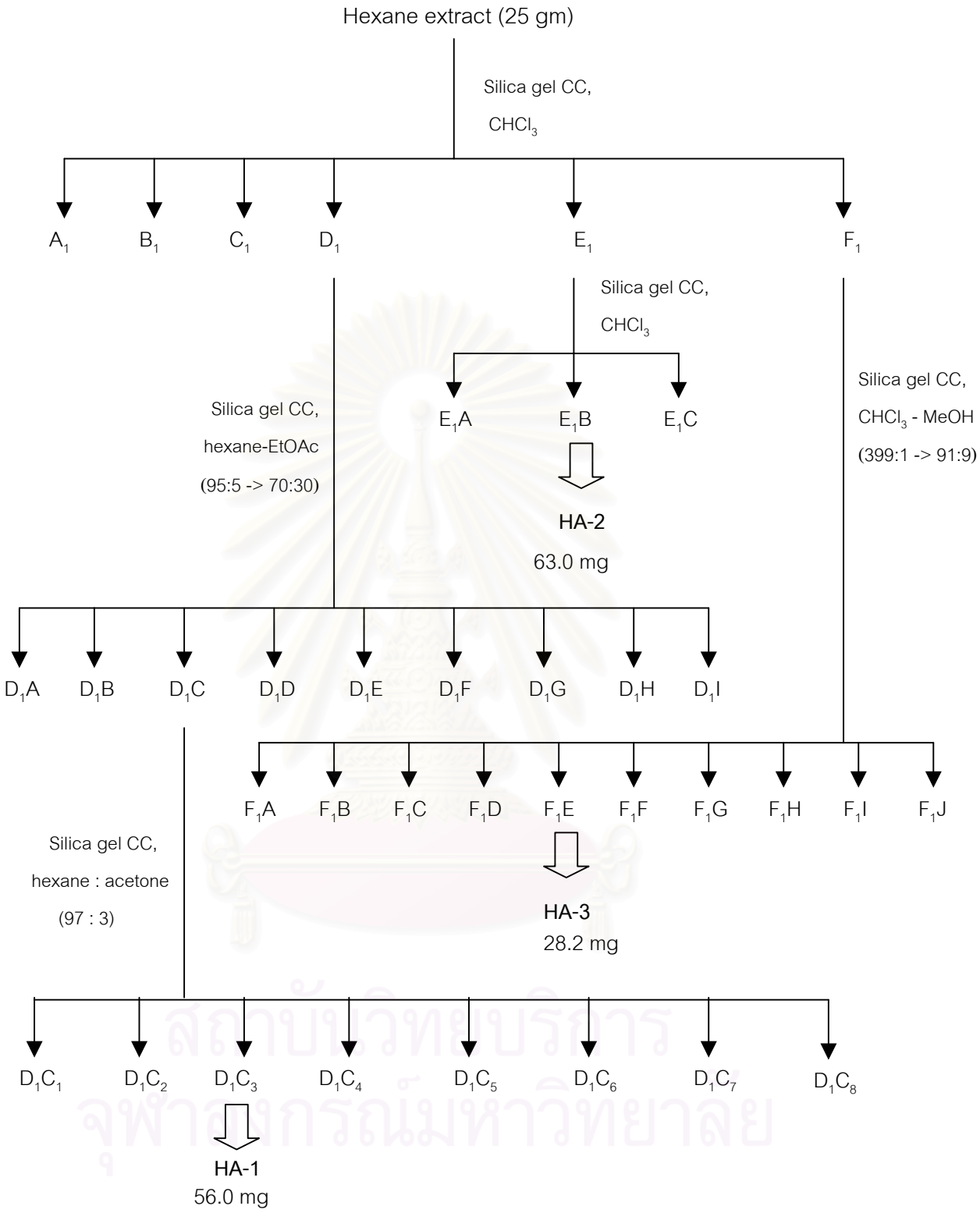
3.2.1.3 Isolation of compound HA-3

Fraction F_1 (3.17 gm) was submitted for further purification on silica gel column (90 gm, 2.5×50 cm) using chloroform – methanol of increasing polarity (from 399:1 to 91:9) as the eluent. One hundred and seventy-one, 20-ml fractions were collected and combined according to their TLC patterns into ten major fractions, (F_1A – F_1J), as shown in Table 7.

Table 7. Combined fractions from F_1

Fraction	Solvent ratio ($CHCl_3$: MeOH)	Number of eluates	Weight of fraction (mg)
F_1A	399 : 1	1 – 7	32.8
F_1B	399 : 1	8 – 11	14.9
F_1C	399 : 1	12 – 17	27.1
F_1D	399 : 1	18 – 28	54.9
F_1E	399 : 1	29 – 52	130.7
F_1F	399 : 1	53 – 55	13.5
F_1G	98 : 2	56 – 79	137.2
F_1H	98 : 2	80 – 82	10.9
F_1I	98 : 2	83 – 99	120.8
F_1J	91 : 9	100 – 171	580.7
Methanol eluate			1198.0

Fraction F_1E, which gave a red-violet spot on TLC under detection with 10% sulfuric acid in ethanol, was purified by recrystallization in methanol to give compound HA-3 as white amorphous powder (28.2 mg). The compound gave red-violet color with Libermann-Burchard reagent.



Scheme 2. Fractionation of hexane extract

3.2.2 Fractionation of methanol extract

The methanol extract (28 gm) was separated on a silica gel column (400 gm, 5×60 cm) using mixture of hexane – ethyl acetate – methanol as the eluent. Four hundred and twenty-six (30 ml) fractions were collected and combined according to their TLC patterns into eight major fractions (A_2- H_2) as shown in Table 8.

Table 8. Combined fractions from methanol extract.

Fraction	Solvent ratio (hexane-EtOAc-MeOH)	Number of eluates	Weight of fraction (gm)
A_2	1 : 6 : 1	1 – 6	1.47
B_2	1 : 6 : 1	7	0.53
C_2	1 : 6 : 1	8 – 12	1.22
D_2	1 : 6 : 1	13 – 19	1.36
E_2	1 : 7 : 3	20 – 84	5.45
F_2	1 : 8 : 8	85 – 185	8.76
G_2	1 : 6 : 13	186 – 259	3.47
H_2	1 : 7 : 41	260 – 426	3.11
Methanol eluate			1.42

3.2.2.1 Isolation of compound HA-4

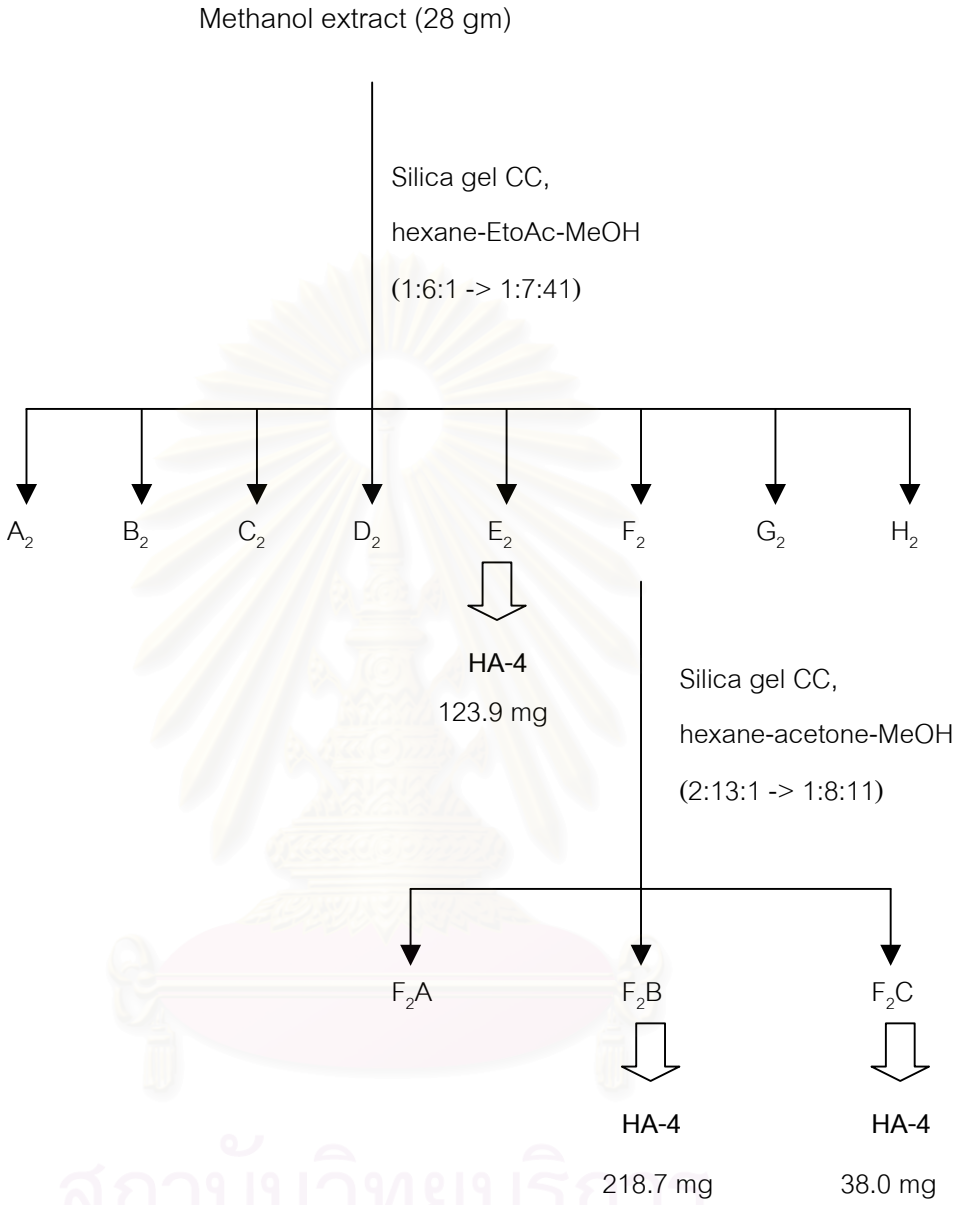
Fraction E_2 (5.45 gm), which gave single brown spot with iodine vapor, was further purified by recrystallization in methanol to give compound HA-4 as colorless crystals (123.9 mg)

Fraction F_2 (8.76 gm), was separated on a silica gel column (250 gm, 5X60 cm), eluted with hexane-acetone- methanol. One hundred and ten (30 ml) fractions were collected and combined according to their TLC pattern into three major fractions (F_2A – F_2C) as shown in Table 9.

Table 9. Combined fractions from F_2

Fraction	Solvent ratio (hexane-acetone-MeOH)	Number of eluates	Weight of fraction (gm)
F_2A	2 : 13 : 1	1 – 5	0.72
F_2B	1 : 6 : 1	6 – 65	2.25
F_2C	1 : 8 : 11	66 – 110	3.13
Methanol eluate			1.64

Fractions F_2B and F_2C, both of which were appeared to contain compound HA-4 by TLC when investigated, were separately purified by recrystallization in methanol to give compound HA-4 (218.7 mg and 38.0 mg, respectively).



Scheme 3. Fractionation of methanol extract

4. Characterization of Isolated Compounds

4.1 Compound HA-1

Appearance	:	colorless crystals (methanol)
Solubility	:	Soluble in chloroform
Melting Point	:	76 °C
EIMS *m/z* (% relative intensity)	:	720(28), 718(49), 408(35), 191(25), 190(53), 189(100), 175(27), 171(45), 95 (36) and 91 (35) (Figure 2, page 93)
IR ν_{max}(KBr disc) cm^{-1}	:	3576, 3077, 1734, 1649 and 885 (Figure 3, page 94)
^{1}H-NMR (δ ppm, 500 MHz, $CDCl_3$)	:	0.67 (3H,*s*), 0.86 (3H,*t*, *J*=7.0 Hz), 0.90 (3H, *s*), 1.02 (3H, *s*), 1.37 (3H, *s*), 1.66 (3H,*s*), 1.74 (1H,*br s*), 1.83 (2H,*m*), 2.23 (1H,*m*), 2.36 (2H,*t*,*J*=7.6 Hz), 4.42 (1H,*m*), 4.66 (1H,*br s*), 4.68 (1H,*br s*), 5.00 (1H, *br s*), 5.12 (1H,*dd*,*J*=11.9,5.2 Hz), and 5.22 (1H,*br s*) (Figure 4, page 95)
^{13}C-NMR (δ ppm, 125 MHz, $CDCl_3$)	:	14.1, 15.0, 16.2, 16.8, 17.8, 19.6, 20.9, 21.5, 22.7, 23.9, 25.1, 27.3, 28.5, 29.2 - 29.7, 31.9, 32.5, 34.6, 38.2, 39.1, 40.0, 40.2, 41.3, 42.7, 44.2, 47.5, 47.9, 48.7, 52.3, 53.9, 70.0, 74.1, 105.2,109.5, 146.0, 148.1, and 173.0 (Figure 5, page 98)

4.2 Compound HA-2

Appearance	:	Colorless needles (methanol)
Solubility	:	Soluble in chloroform
Melting Point	:	165 °C
EIMS *m/z* (% relative intensity)	:	426(28), 411(23), 393(13), 218(74), 207(46), 204(53), 203(51), 190(46), 189 (100) and 175 (45) (Figure 11, page 126)
IR ν_{max}(KBr disc) cm^{-1}	:	3300, 2900, 1470, 1390, 1020 and 820 (Figure 12, page 127)
^{1}H-NMR (δ ppm, 300 MHz, $CDCl_3$)	:	0.73 (3H,*s*), 0.76 (3H,*s*), 0.80 (3H,*s*), 0.91 (3H,*s*), 0.94 (3H,*s*), 1.00 (3H,*s*), 1.66 (3H,*s*), 2.35 (*td*,*J*=11.4,11.4,5.7 Hz), 3.17 (1H,*dd*,*J*=10.8,5.1 Hz), 4.54(1H,*br s*), and 4.66(1H,*br s*) (Figure 13, page 128)
^{13}C-NMR (δ ppm, 75 MHz, $CDCl_3$)	:	14.5, 15.4, 16.0, 16.1, 18.0, 18.3, 19.3, 20.9, 25.2, 27.4, 27.5, 28.0, 29.7, 34.3, 35.6, 37.2, 38.1, 38.7, 38.9, 40.0, 40.8, 42.8, 43.0, 48.0, 48.3, 50.4, 55.30, 79.0 109.3 and 151.0 (Figures 14, page 130)

4.3 Compound HA-3

Appearance	:	White powder (methanol)
Solubility	:	Soluble in chloroform
Melting Point	:	162 °C
EIMS *m/z* (% relative intensity)	:	412(17), 397(10), 300(13), 299(10), 273(18), 272(27), 271(100), 255(22), 253 (21) and 213 (12) (Figure 16, page 142)
IR ν_{max}(KBr disc) cm^{-1}	:	2950, 1450, 1380, 1050 and 980 (Figure 17, page 143)
^{1}H-NMR (δ ppm, 500 MHz, $CDCl_3$)	:	0.53 (3H,*s*), 0.78 (3H,*s*), 0.78 (3H,*d*,*J*=6.1 Hz), (3H,*d*,*J*=6.1 Hz), 0.79 (3H,*t*,*J*=7.5 Hz), 0.83 (3H,*d*,*J*=6.4 Hz) and 1.01 (3H,*d*,*J*=6.7 Hz), 3.58 (1H,*tt*,*J*=8.8,4.6 Hz), and 5.01 (1H,*dd*,*J*=15.3,8.5 Hz),5.1(1H,*dd*,*J*=15.1,8.7 Hz) (Figure 18, page 144)
^{13}C-NMR (δ ppm, 125 MHz, $CDCl_3$)	:	12.1, 12.3, 13.0, 19.0, 21.1, 21.4, 21.5, 23.0, 25.4, 28.5, 29.6, 31.5, 31.9, 34.2, 37.1, 38.0, 39.5, 40.3, 40.8, 43.3, 49.4, 51.2, 55.1, 55.9, 71.1, 117.5, 129.4, 138.2, and 139.6 (Figure 19, page 147)

4.4 Compound HA-4

Appearance	:	Colorless crystals (methanol)
Solubility	:	Soluble in methanol
Melting Point	:	185 °C
Optical Rotation	:	$[\alpha]^{20}_{D}$ -94.04° (c, 0.06 in methanol)
EIMS *m/z* (% relative intensity)	:	116(21), 102(20), 87(100), 85(48), 74(20), 73(81), 71(20), 60(20), 59(18), and 57(44) (Figure 24, page 159)
IR ν_{max}(KBr disc) cm^{-1}	:	3350, 1050 and 1010 (Figure 25, page 160)
^{1}H-NMR (δ ppm, 500 MHz, DMSO-d_6)	:	3.09 (1H, *dd*, *J*=,9.5,3.1 Hz), 3.28 (1H,*ddd*, *J* = 9.5, 9.2, 4.3 Hz), 3.30 (3H, *s*), 3.36 (1H,*ddd*,*J* = 9.5,9.2,4.6 Hz), 3.42 (1H,*ddd*, *J*=9.5,5.8,3.4 Hz), 3.67 (1H,*ddd*, *J* = 3.7,3.4, 3.4 Hz), 3.85 (1H,*ddd*,*J*=3.7,3.4,3.1 HZ), 4.26 (1H,*d*,*J*=5.8 Hz), 4.39 (1H,d,*J*=4.3Hz), 4.42 (1H,*d*,*J*=4.6 Hz), 4.6 (1H,*d*, *J*=3.7 Hz), 4.62 (1H,*d*,*J* =3.7 Hz) (Figure 26,page 161)
^{13}C-NMR (δ ppm, 75 MHz, DMSO-d_6)	:	57.2, 68.2, 70.6, 72.2, 72.4, 73.5, and 81.2 (Figure 27, page 163)

5. Determination on *In Vitro* Stimulation of Lymphocyte Proliferation

Extracts of the leaves of *Harpullia arborea* including hexane, chloroform and methanol extracts, together with the isolated compound HA-4, were subjected to preliminary screening for immunostimulatory activity by determining the ability to stimulate the proliferation of lymphocytes *in vitro*. The extent of lymphocyte proliferation was measured by colorimetric method (Alamar Blue Assay) and the result was reported as % stimulation (in case the degree of proliferation was increased) or % cytotoxicity (in case the degree of proliferation was decreased). The tested substances were also tested for cytotoxicity against a human monocytic cell line, U-937. All tests were performed in triplicate and repeated three times.

Experimental methods

1. *In vitro* lymphocyte proliferation assay

1.1 Isolation of Wistar rat lymphocytes

Spleens were obtained from Wistar rat under sterile condition and submerged in RPMI 1640 with 1% antibiotic-antimycotic and stored at 4°C until use. Spleens were grinded with syringe and gently teased on nylon mesh to obtain single cell suspension in a sterile disposable 60 mm petri dish containing 5 ml of RPMI 1640. The cell suspensions were then layered onto histopaque-1077 and were centrifuged at 5500 rpm for 20 min at 25°C to isolate mononuclear cells. The buffy coat containing lymphocytes was removed and suspended in RPMI 1640 and centrifuged at 5500 rpm for 10 min. The supernatant was discarded, and the pellet was resuspended in complete RPMI 1640 medium supplement with 10% heat inactivated-fetal bovine serum. The viability of splenic lymphocytes, determined by trypan blue exclusion test, was >95%.

1.2 Determination of lymphocyte proliferation by Alamar Blue method

The splenic cell suspension was adjusted to 2.5×10^6 cells/ml in complete RPMI 1640 medium. One hundred microlitres of the suspension were placed in 96-well sterile culture plate containing 10 mcl of two-fold dilution of 1.6-200 mcg/ml of plant extract or compound or 0.5% DMSO as vehicle control or 10 mcg/ml of concanavalin A (Con-A) or 50 mcg/ml of lipopolysaccharide (LPS) as positive control. Then, 90 mcl of

complete RPMI 1640 medium were added. The plate was incubated in 5% CO_2 under humidified conditions at 37 °C for 48 hrs.

After an incubation period of 48 hrs, 20 mcl/well Alamar Blue were added and the plate was re-incubated for 24 hrs. Since Alamar Blue contained an oxidation-reduction (redox) indicator, and cellular proliferation induced chemical reduction of the media which resulted of a change in color from blue to red. The intensity of red color reflected the extent of cellular proliferation. The absorbance was then measured at the wavelengths of 570 nm (reduced form) and 600 nm (oxidized form) using microplate reader. Specific absorbance (specific OD), obtained by subtraction of the absorbance at 600 nm from that at 570 nm, was used in the calculation for % stimulation and % cytotoxicity

$$\% \text{ Stimulation} = \left[\frac{\text{specific OD (sample)} - \text{specific OD (control)}}{\text{specific OD (control)}} \right] \times 100$$

$$\% \text{ Cytotoxicity} = \left[\frac{\text{specific OD (control)} - \text{specific OD (sample)}}{\text{specific OD (control)}} \right] \times 100$$

2. *In vitro* cytotoxicity assay

2.1 U-937 monocytic cell line culture

The U-937 cells were maintained in 25 cm^2 plastic culture flasks in complete RPMI 1640 medium containing 10% heat inactivated-fetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cells harvested by centrifugation at 5500 rpm for 5 min at 25°C were passaged every 3 – 4 days and viability was assessed by trypan blue exclusion test.

2.2 Determination of cytotoxicity by Alamar Blue Method

Monocytes were adjusted to 5×10^5 cells/ml in complete RPMI 1640 medium. One hundred microlitres of the suspension were placed in 96-well sterile culture plate containing 10 mcl of two-fold dilution of 1.6-200 mcg/ml of plant extract or compound or 0.5% DMSO as vehicle control or 50 mcg/ml of ellipticine as positive control, and then 90 mcg of complete RPMI 1640 medium were added. The plate was incubated in 5% CO_2 under humidified conditions at 37°C for 48 hrs.

After an incubation period of 48 hrs, 20 mcl/well of Alamar Blue were added and the plate was re-incubated for 24 hrs. The absorbance was then measured at wavelengths of 570 nm and 600 nm using microplate reader. Specific absorbance (specific OD), obtained by subtracting the absorbance at 600 nm from that of 570 nm, was used in the calculation for % cytotoxicity. The % cytotoxicity – log concentration curve was then established for evaluating CC_{50} of the samples.

CHAPTER IV

RESULTS AND DISCUSSION

The leaves of *Harpullia arborea* Radlk. were extracted with 95% ethanol. The ethanol extract was then extracted respectively with hexane, chloroform and methanol to yield hexane (4.55% yield), chloroform (4.55% yield) and methanol extracts (10.00% yield). Chromatographic separation of the hexane extract afforded three pure compounds, while one compound was obtained from the methanol extract. The identification and structure elucidation of the isolated compounds were based on the interpretation of their spectral data and further confirmed by comparison with those values reported in the literature. Preliminary screening for the immunostimulatory effect of all extracts and one pure compound were also performed. The details can be discussed as follows.

Identification and Structure elucidation of Isolated Compounds

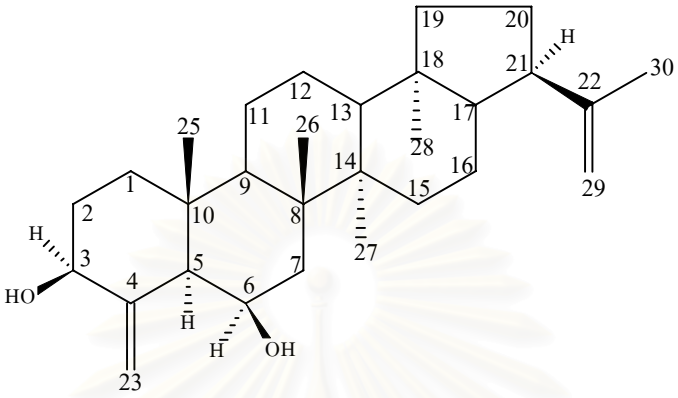
1. Structure elucidation of HA-1

Compound HA-1 was recrystallized from methanol as colorless crystals (56.0 mg, 0.005% yield), The compound was suggested to be a triterpenoid through a positive Libermann-Burchard's test. The EIMS of HA-1 (Figure 2) showed the molecular ion peak at *m/z* 720 corresponding to the molecular formula of $C_{49}H_{84}O_3$. The base peak at *m/z* 189 was indicative of a triterpenoid with lupane/hopane skeleton containing an isopropenyl group in ring E (Ogunkoya, 1981). The IR spectrum of HA-1 (Figure 3) displayed the OH and carbonyl absorption bands at 3576 and 1734 cm^{-1}, respectively. The IR absorption at 3077, 1649 and 885 cm^{-1} suggested the presence of terminal double bond in the molecule.

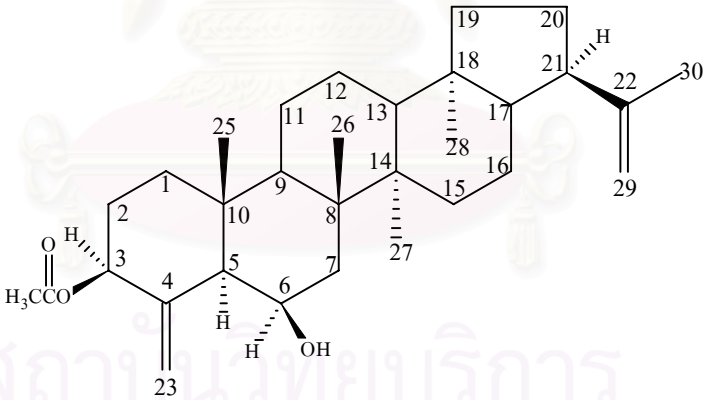
The ^{1}H-NMR spectrum (Figures 4a-4c) exhibited five singlets of tertiary methyls at δ 0.67, 0.90, 1.02, 1.37 and 1.66, one triplet of primary methyl at δ 0.86 and signals due to two hydroxymethine protons at δ 4.42 and 5.12. The number of methyl singlets indicated the absence of two methyls in the basic lupane/hopane skeleton. The methyl singlet at δ 1.66 showed correlation with a pair of signals at δ 4.66 and 4.68 in the ^{1}H-^{1}H COSY spectrum (Figures 8a-8c), demonstrating the presence of an isopropenyl group. Another pair of correlated signals at δ 5.00 and 5.22 was suggestive of one additional terminal vinyl group.

The ^{13}C-NMR spectrum of HA-1 (Figures 5a-5b) showed more than 40 carbon signals, indicating the presence of a conjugated moiety in the molecule of triterpenoid. Analysis of the DEPT and HETCOR experiments (Figures 6a-6b and 7a-7d) suggested that the compound contained six methyl carbons (δ 14.1, 15.0, 16.2, 16.8, 17.8 and 19.6), seven methine carbons (δ 47.5, 47.9, 48.7, 52.3, 53.9, 70.0 and 74.1), seven quaternary carbons (δ 38.2, 41.3, 42.7, 44.2, 145.9, 148.1 and 173.0), and more than 20 methylene carbons (δ 20.9, 21.5, 22.7, 23.9, 25.1, 27.3, 28.5, 29.2-29.7, 31.9, 32.5, 34.6, 39.1, 40.0, 40.2, 105.2 and 109.5).

From the above information, HA-1 was proposed to be a long-chain fatty acid ester of a triterpenoid having the lupane or hopane skeleton with two absence methyl groups. The triterpenoid part of HA-1 was deduced to be 3*β*,6*β*-dihydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**90**) by comparison of its ^{1}H and ^{13}C-NMR data with those of **90** and 3*β*-acetoxy-6*β*-hydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**107**) (Chavez *et al.,* 1997) as shown in Tables 10 and 11, respectively.



3β,6β-dihydroxy-21α*H*-24-norhopa-4(23), 22(29)-diene (**90**)



3β-Acetoxy-6β-hydroxy-21α*H*-24-norhopa-4(23), 22(29)-diene (**107**)

Table 10. ^{1}H-NMR assignments of compound HA-1 (triterpenoid part) and reported data of 3*β*,6*β*-dihydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**90**) and 3*β*-acetoxy-6*β*-hydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**107**) (in $CDCl_3$).

Position	δ H		
	Compound HA-1	**90**	**107**
2	1.60, 1.85 (*m*)	1.60, 1.90	-
3	5.12 (*dd*,*J*=11.9, 5.2 Hz)	3.99 (*dd*; *J*=11.0, 5.6 Hz)	5.09 (*dd*; *J*=12.2. 5.2 Hz)
5	1.74 (*br s*)	1.70 (*m*)	-
6	4.42 (*m*)	4.46 (*d*; *J*=1.0 Hz)	4.42 (*d*; *J*=1.0 Hz)
7	-	1.50, 1.60	-
9	-	1.40	-
13	-	1.50	-
15	-	1.19	-
17	-	1.00 (*m*)	-
20	-	1.10, 2.00	-
21	2.23 (*m*)	2.30 (*m*)	-
23	5.00, 5.22 (*br s*)	5.29, 5.30 (*br s*)	4.99, 5.24 (*br s*)
25	1.02 (*s*)	1.03 (*s*)	1.01 (*s*)
26	1.37 (*s*)	1.42 (*s*)	1.37 (*s*)
27	0.90 (*s*)	0.95 (*s*)	0.90 (*s*)
28	0.67 (*s*)	0.71 (*s*)	0.70 (*s*)
29	4.66, 4.68 (*br s*)	4.70, 4.72 (*br s*)	4.66, 4.68 (*br s*)
30	1.66 (*s*)	1.70 (*br s*)	1.65 (*br s*)

Table 11. ^{13}C-NMR assignments of compound HA-1 (triterpenoid part) and reported data of 3*β*,6*β*-dihydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**90**) and 3*β*-acetoxy-6*β*-hydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**107**) (in $CDCl_3$).

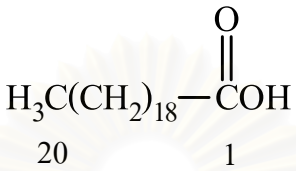
Position	δ C		
	Compound HA-1	**90**	**107**
1	40.0	40.1	39.9
2	28.5	32.1	28.5
3	74.1	73.1	74.5
4	146.0	150.9	145.8
5	52.3	52.2	52.3
6	70.0	70.2	70.0
7	39.1	39.0	39.1
8	41.3	41.3	41.3
9	48.7	48.7	48.7
10	38.2	38.3	38.2
11	20.9	20.9	20.9
12	23.9	23.9	23.9
13	47.5	47.5	47.5
14	42.7	42.7	42.7
15	32.5	32.5	32.5
16	21.5	21.5	21.5
17	53.9	53.9	53.9
18	44.2	44.2	44.2
19	40.2	40.2	40.2
20	27.3	27.3	27.3

Table 11. ^{13}C-NMR assignments of compound HA-1 (triterpenoid part) and reported data of 3*β*,6*β*-dihydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**90**) and 3*β*-acetoxy-6*β*-hydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**107**) (in $CDCl_3$) (continued).

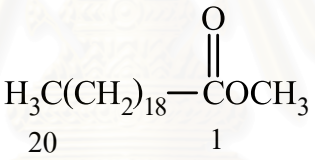
Position	δ C		
	Compound HA-1	**90**	**107**
21	47.9	47.9	47.9
22	148.1	148.1	148.1
23	105.2	104.5	105.4
25	16.2	16.3	16.2
26	17.8	17.8	17.8
27	16.8	16.9	16.8
28	15.0	15.0	15.0
29	109.5	109.6	109.6
30	19.6	19.7	19.7

The site of ester formation could be determined on the basis of chemical shift analysis. Significant differences between the chemical shifts of HA-1 and **90** were due to C-2, C-3, C-4, and H-3. On the other hand, these carbon and proton chemical shifts of HA-1 were found to be proximate to those of **107**. Therefore, this indicated the fatty acyl moiety as attaching to C-3 of the triterpenoid skeleton. As the molecular formula of $C_{49}H_{84}O_3$ was established for HA-1 on the basis of its EIMS (M^+ at *m/z* 720) and there were no other evidences of the presence of any additional double bond other than those in the triterpenoid part, of the compound, the fatty acid part of HA-1 was thus deduced to be a saturated C-20, eicosanoic acid ($C_{20}H_{40}O_2$). Comparison of ^{13}C-NMR data of the fatty acyl moiety of HA-1 with those of methyl eicosanoate

(Gunstone, Pollard, and Scrimgeour, 1976), as presented in Table 12, gave supportive evidence for this deduction.



Eicosanoic acid



Methyl eicosanoate

Table 12. ^{1}H and ^{13}C-NMR assignments of compound HA-1 (fatty acid part) and reported ^{13}C-NMR data of methyl eicosanoate (in $CDCl_3$).

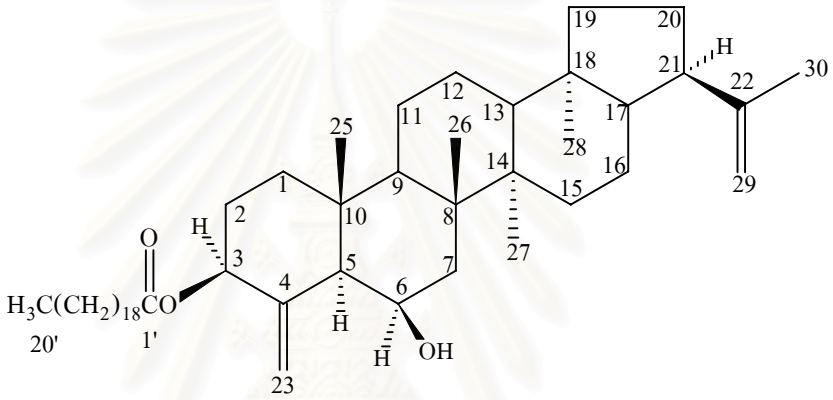
Compound HA-1			Methyl eicosanoate	
Position	δ H	δ C	Position	δ C
1'	-	173.0	1	174.3
2'	2.36 (*t*; *J*=7.6 Hz)	34.6	2	34.2
3'	-	25.1	3	25.1
4' – 17'	-	29.2 – 29.7	4 - 17	29.4 – 29.8
18'	-	31.9	18	32.1
19'	-	22.7	19	22.8
20'	0.86 (*t*; *J*=7.0 Hz)	14.1	20	14.1

The proposed structure of HA-1 was confirmed by HMBC experiment (Figures 10a-10i). Correlations observed for Me-28 (δ 15.0) with C-13 (δ 47.5), C-17 (δ 53.9), C-18 (δ 44.2) and C-19 (δ 40.2) were important in showing the hopane nature of the compound. The vinylic protons (δ 5.00, 5.22) displayed correlations with C-3 (δ 74.1) and C-5 (δ 52.3), demonstrating the location of an exo-methylene group at C-4. Correlations observed for the hydroxymethine proton (δ 4.42) with C-8 (δ 41.3) and C-10 (δ 38.2) indicated the attachment of a hydroxyl substituent at C-6. The location of fatty acyl moiety was supported by correlations between H-3 (δ 5.12) and C-1' (δ 173.0).

The NOESY experiment (Figures 9a-9c) was also employed to confirm the structure of HA-1. Correlations displayed between Me-25 (δ 16.2) and Me-26 (δ 17.8); Me-27 (δ 16.8) and Me-28 (δ 15.0); Me-28 and H-21 (δ 2.23) were in agreement with configurations of the methyl and isopropenyl groups in the typical hopane skeleton. The β-orientation of the hydroxyl group attached at C-6 was supported by correlations observed for H-5 (δ 1.74) with H-6 (δ 4.42)

and H-3 (δ 5.12). The correlation between one of the vinylic H-23 protons (δ 5.22) and H-6 was also observed, indicating the close proximity of the vinyl and hydroxyl groups in the molecule.

Therefore, HA-1 was elucidated as 3*β*-eicosanoyl-6*β*-hydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene, the structure of which is shown below.



3*β*-Eicosanoyl-6*β*-hydroxy-21*αH*-24-norhopa-4(23),22(29)-diene

This is the first report of a fatty acid ester of the 24-norhopene triterpenoid in nature. Two other compounds with this rare triterpenoid skeletal type, 3*β*,6*β*-dihydroxy-21*αH*-24-norhopa-4(23), 22(29)-diene (**90**) and 3*β*,5*β*-dihydroxy-6*β*-[(4-hydroxybenzoyl)oxy]-21*αH*-24-norhopa-4(23),22(29)-diene (**91**), have been previously isolated from *Diatenopteryx sorbifolia* (Sapindaceae) (Chavez *et al.*, 1997).

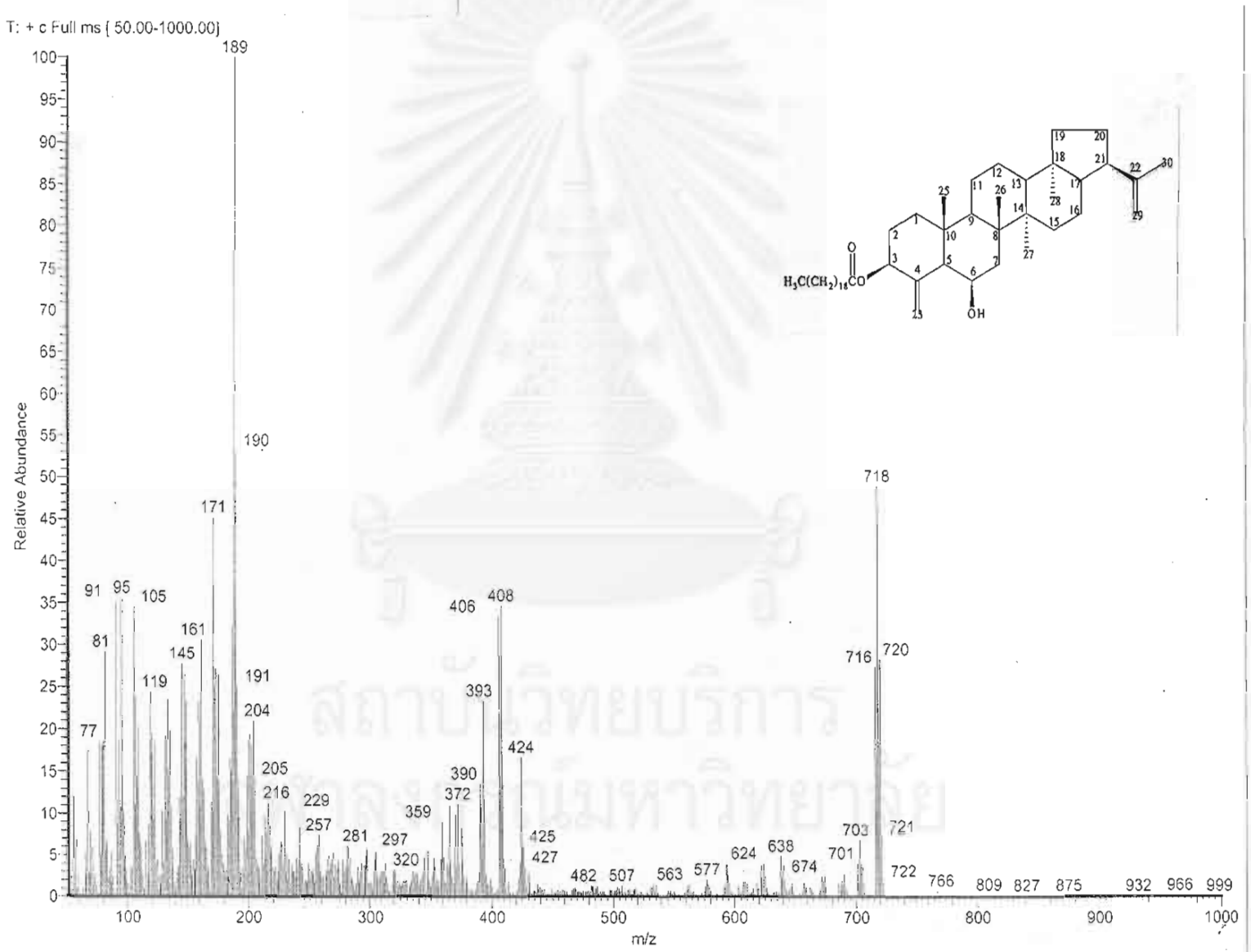


Figure 2. EIMS of compound HA-1

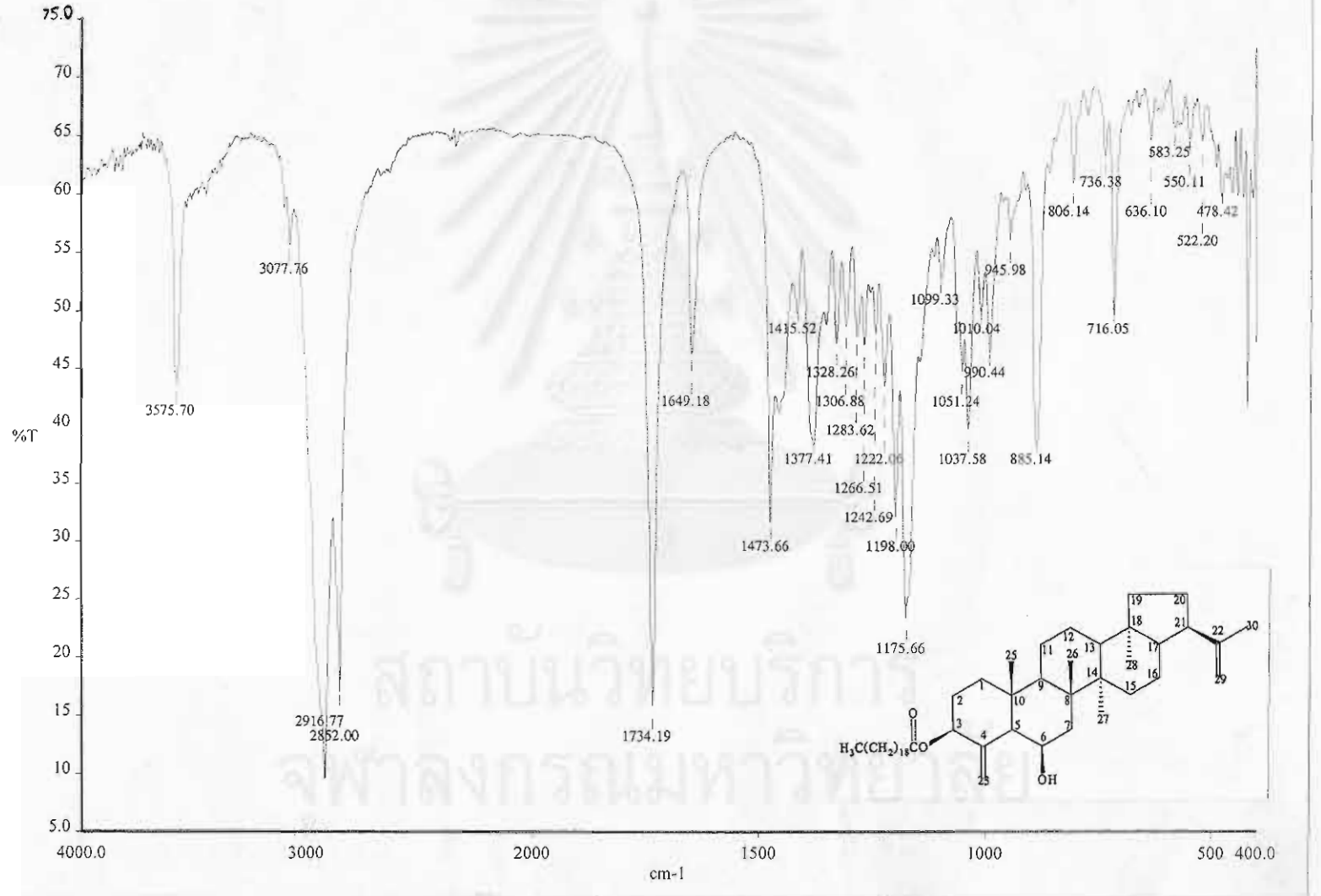


Figure 3. IR spectrum of compound HA-1

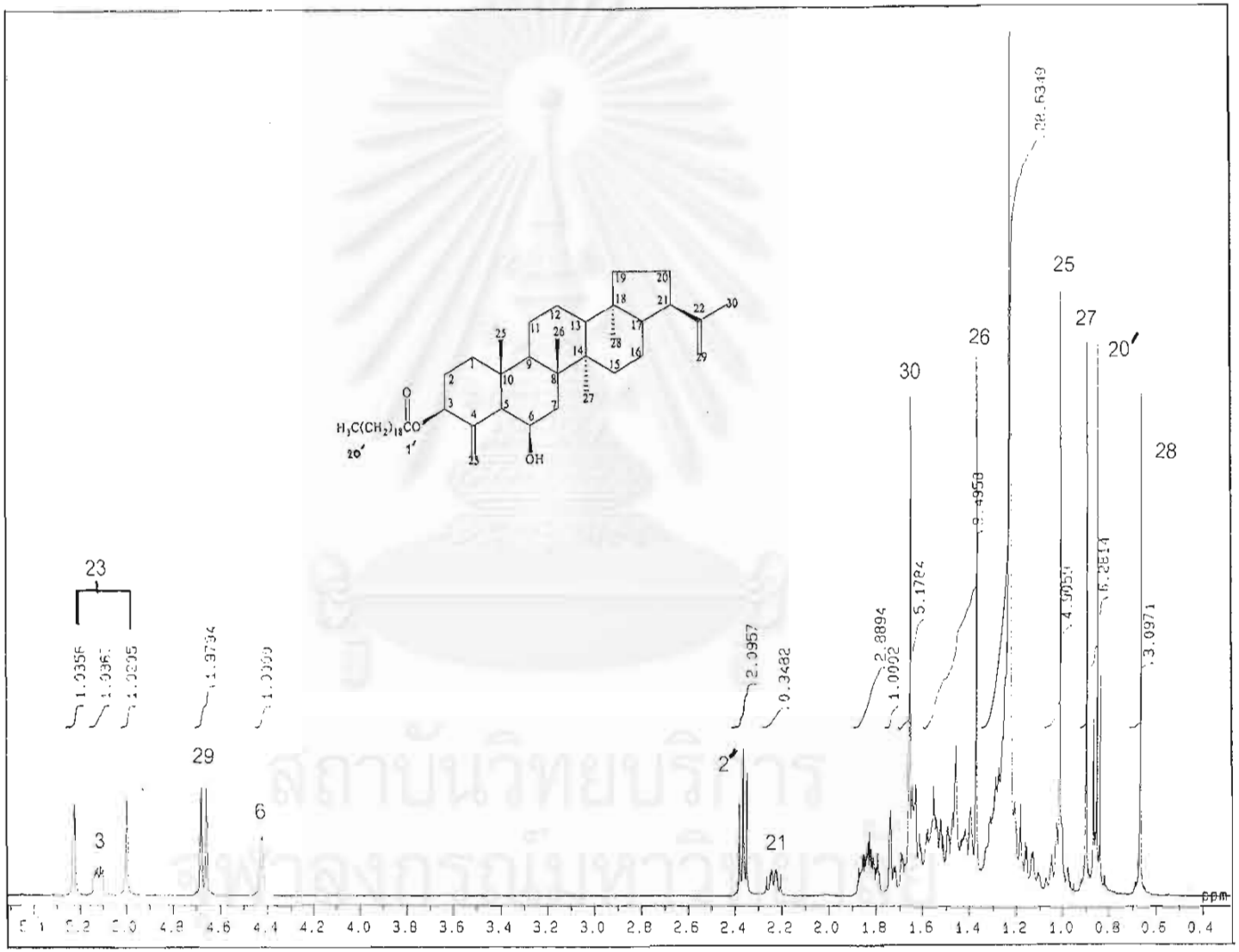


Figure 4a. The 500 MHz ^{1}H-NMR spectrum of compound HA-1 (in $CDCl_3$)

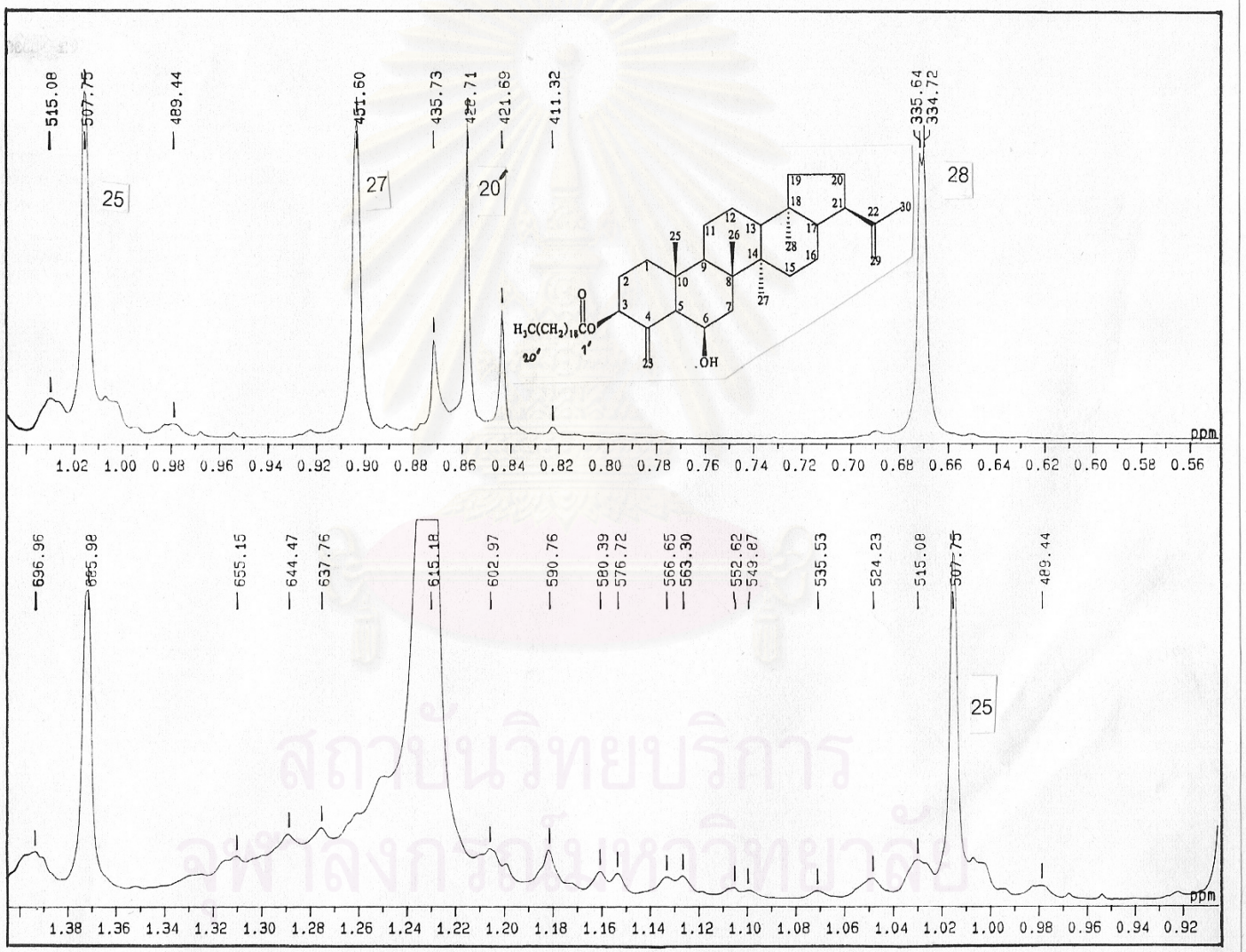


Figure 4b. The 500 MHz ^{1}H-NMR spectrum of compound HA-1 (expanded)

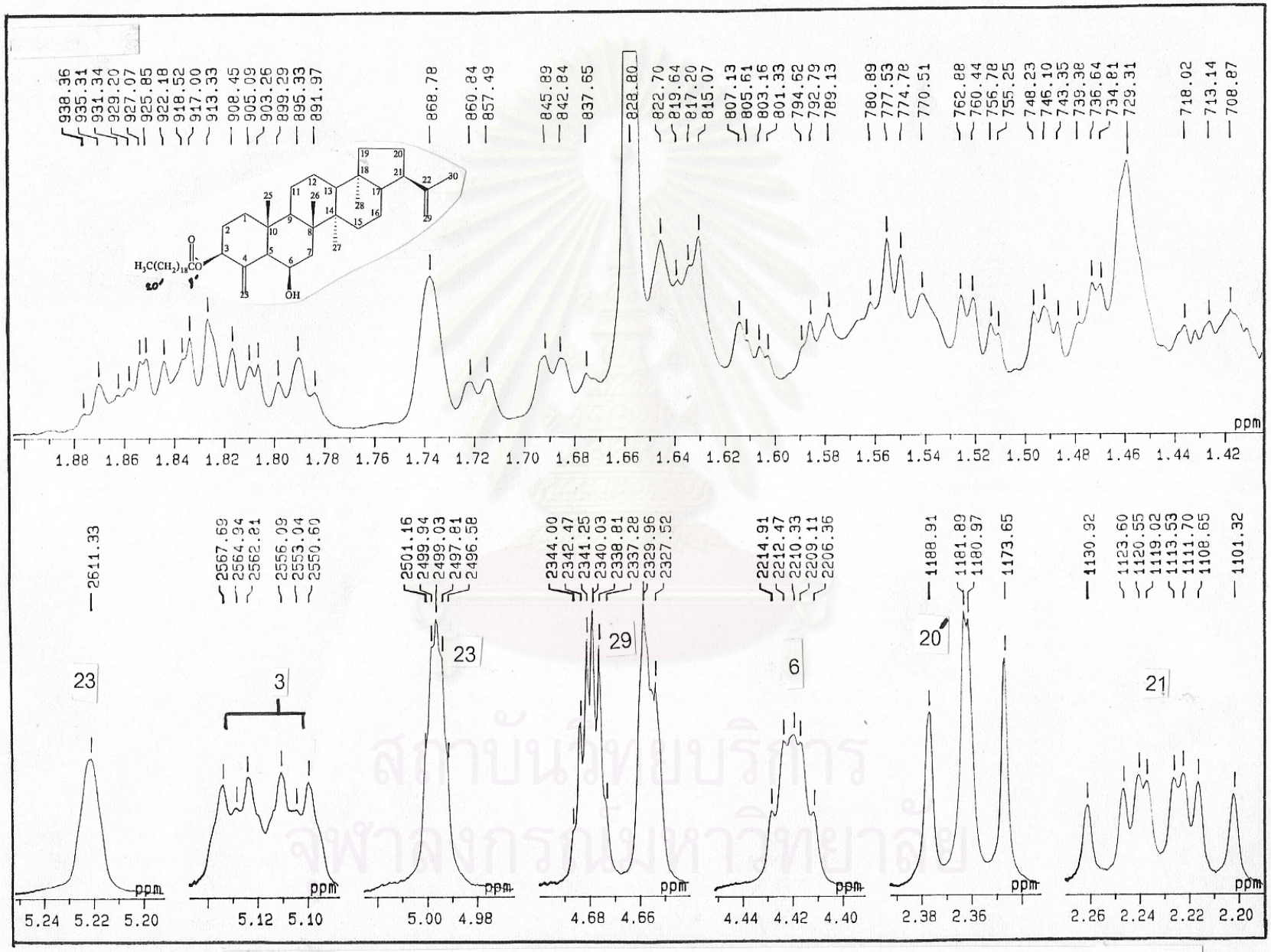


Figure 4c. The 500 MHz ^{1}H-NMR spectrum of compound HA-1 (expanded)

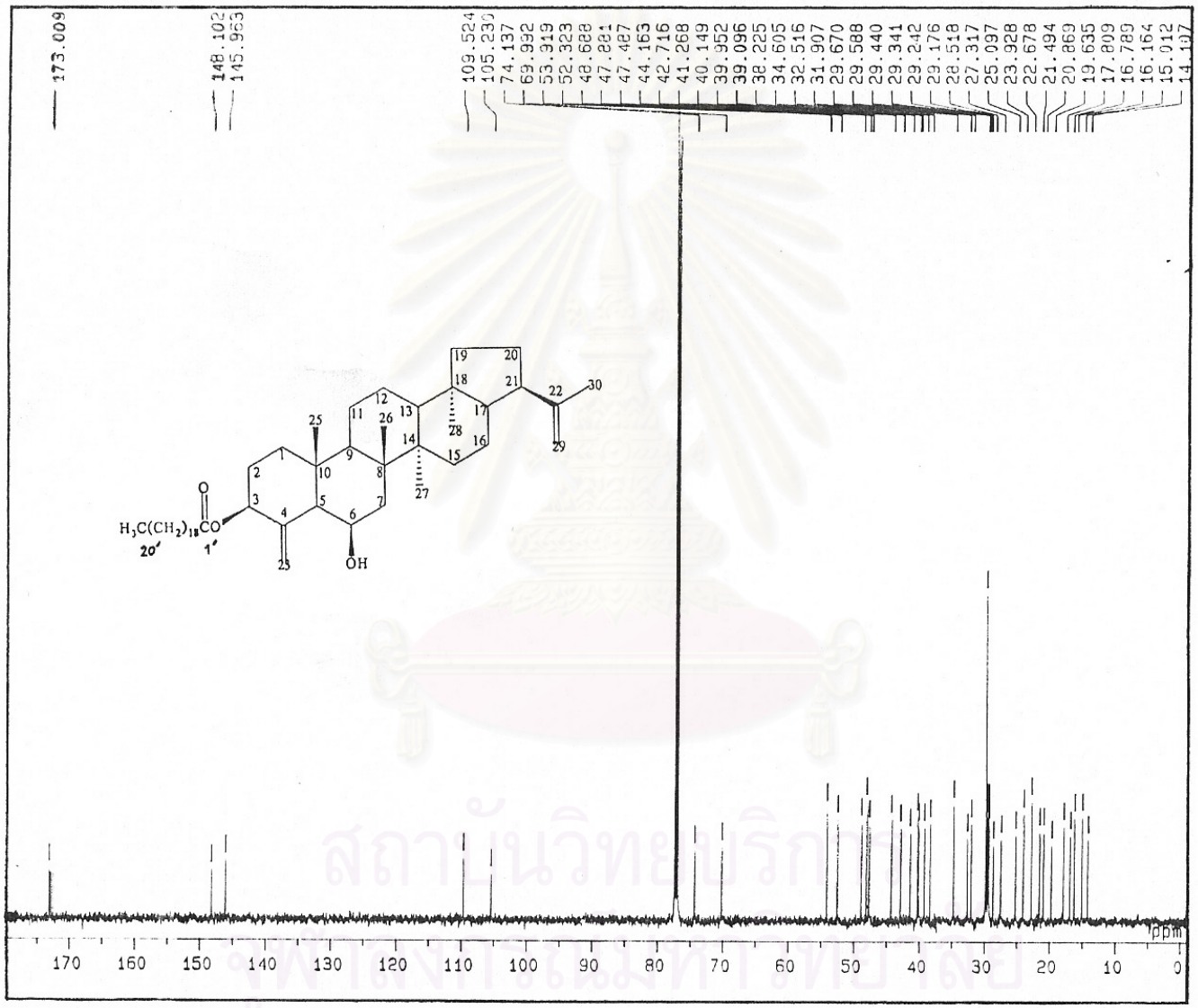


Figure 5a. The 125 MHz ^{13}C-NMR spectrum of compound HA-1 (in $CDCl_3$)

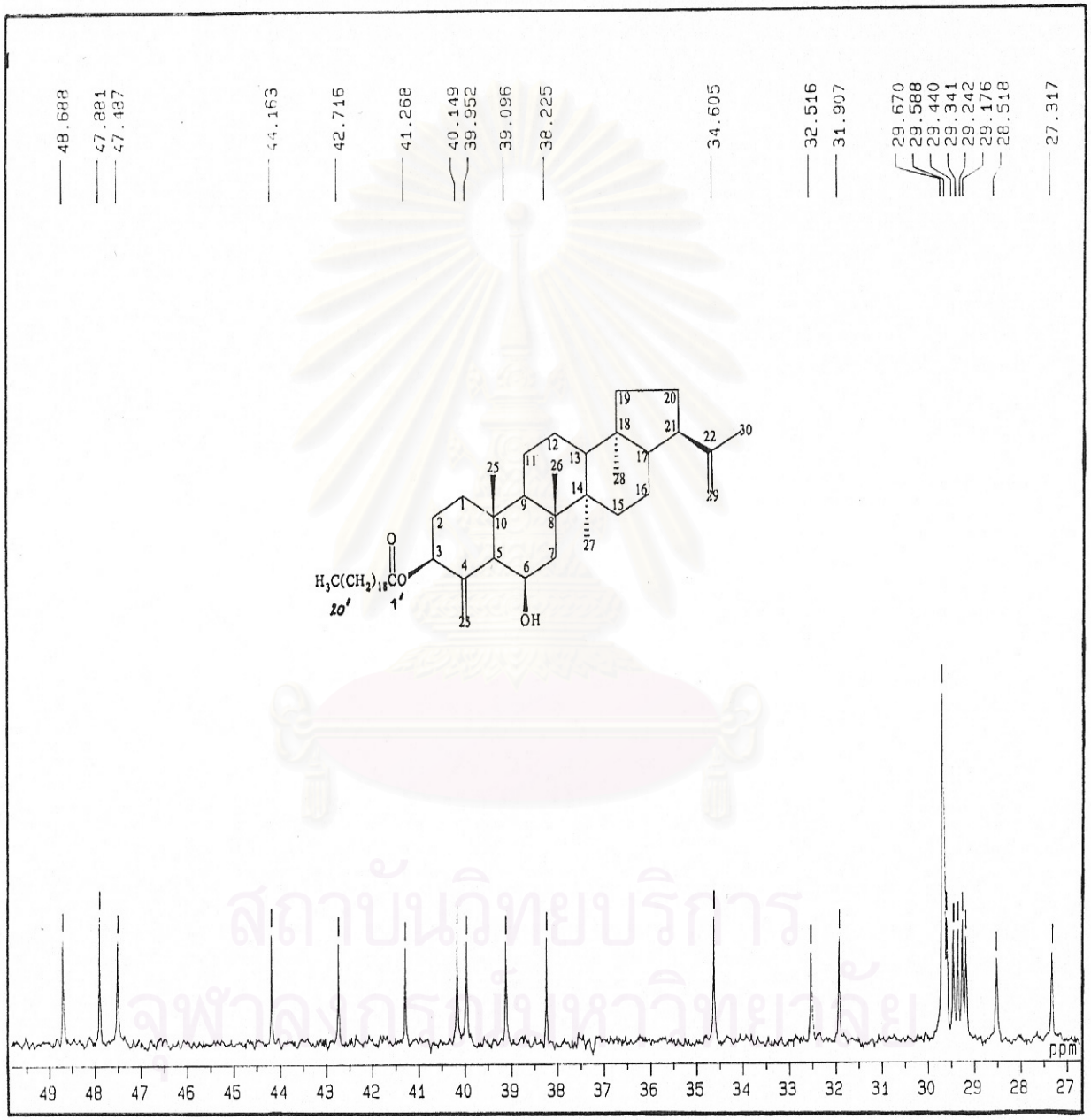


Figure 5b. The 125 MHz ^{13}C-NMR spectrum of compound HA-1 (expanded)

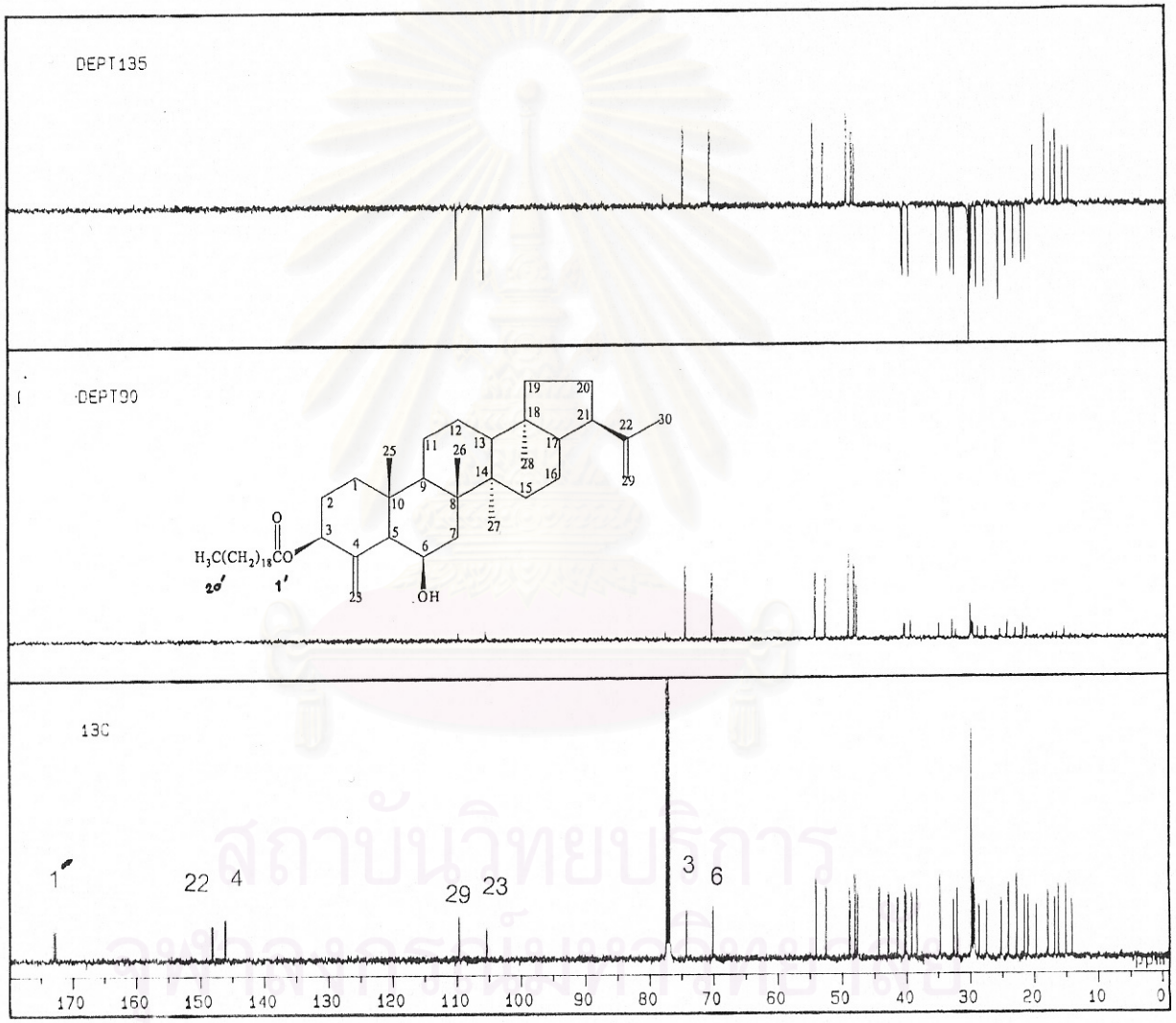


Figure 6a. The 125 MHz ^{13}C-DEPT NMR spectrum of compound HA-1 (in $CDCl_3$)

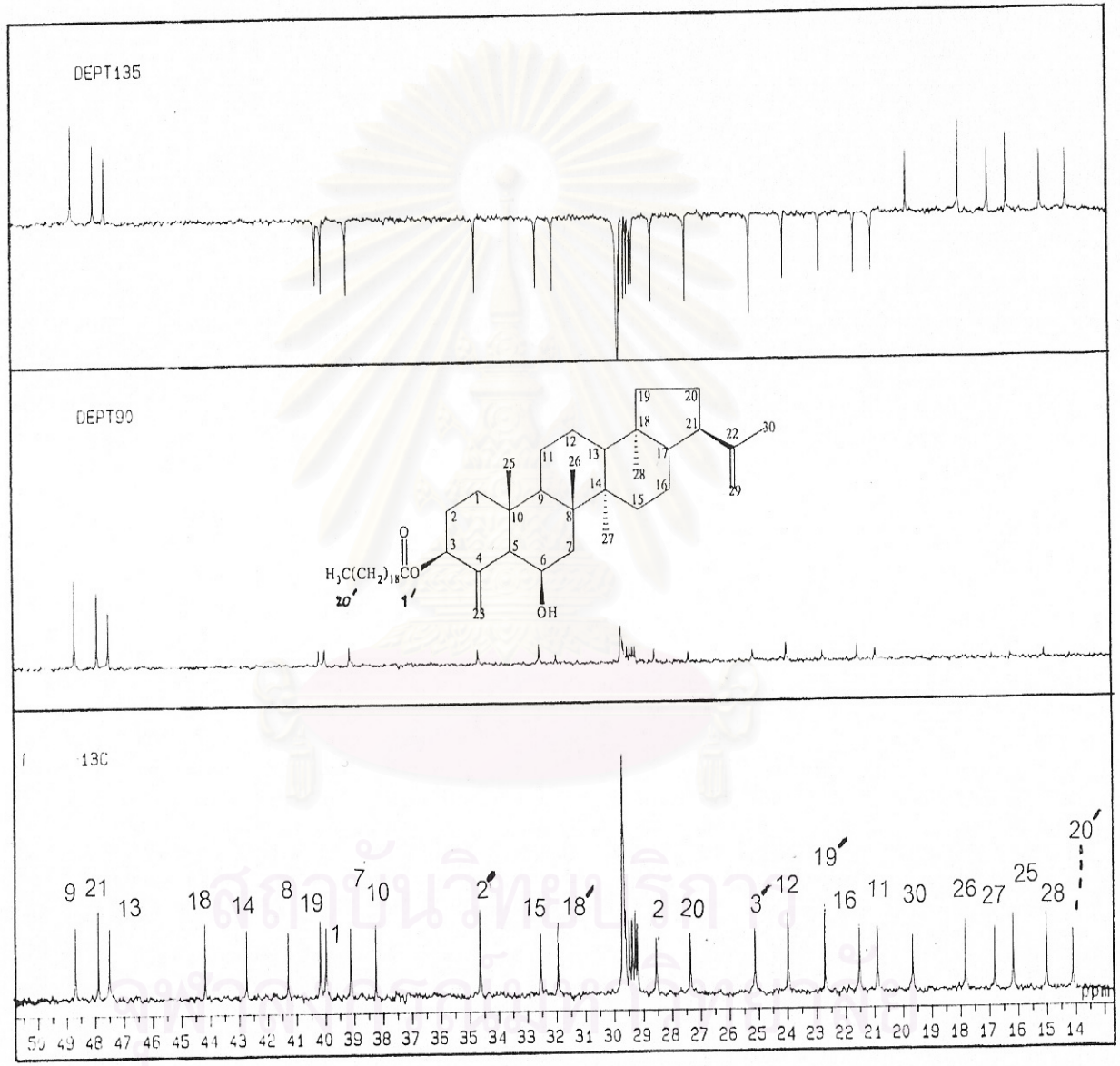
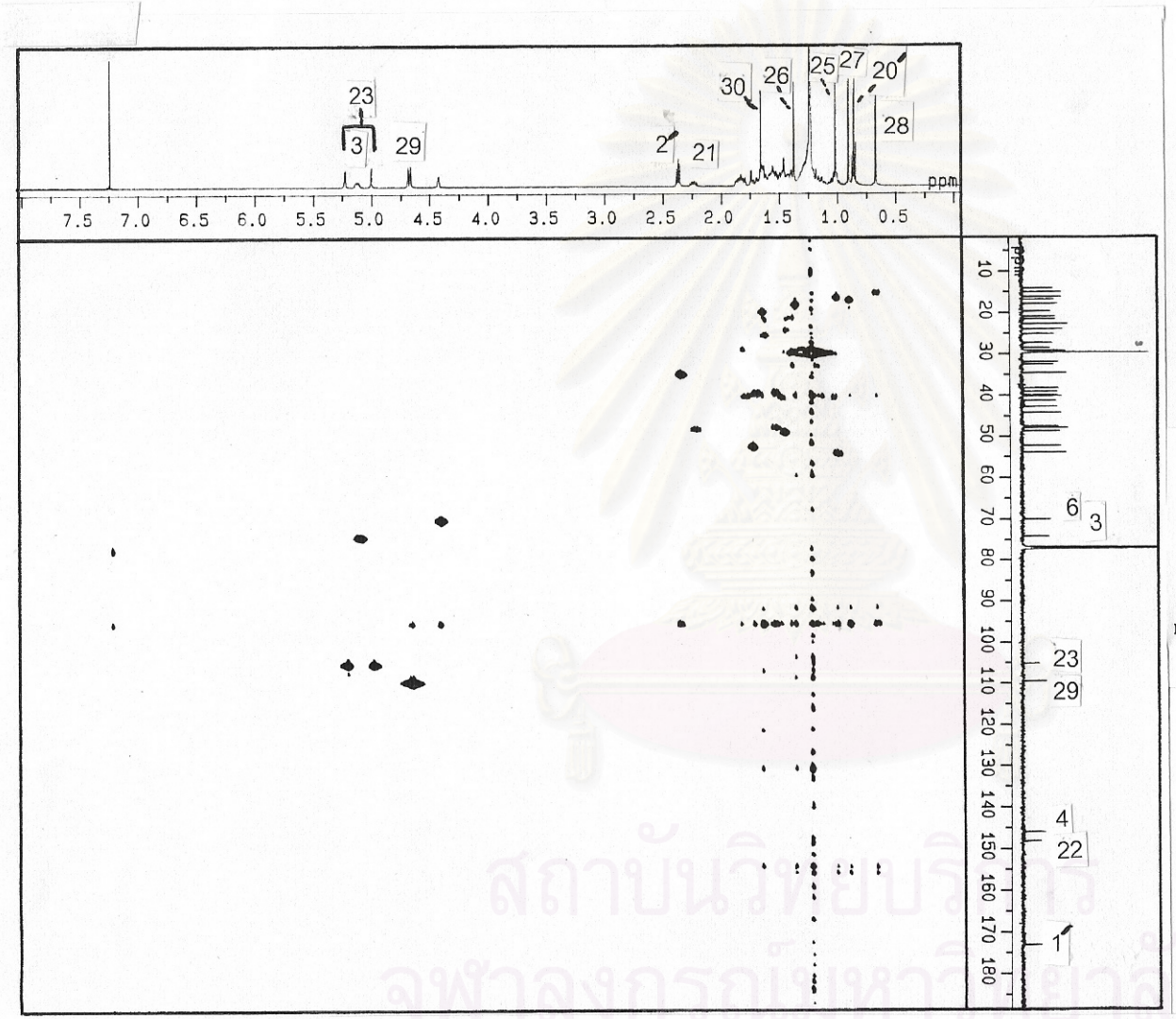


Figure 6b. The 125 MHz ^{13}C-DEPT NMR spectrum of compound HA-1 (expanded)



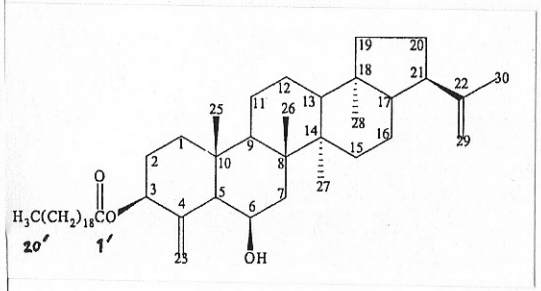
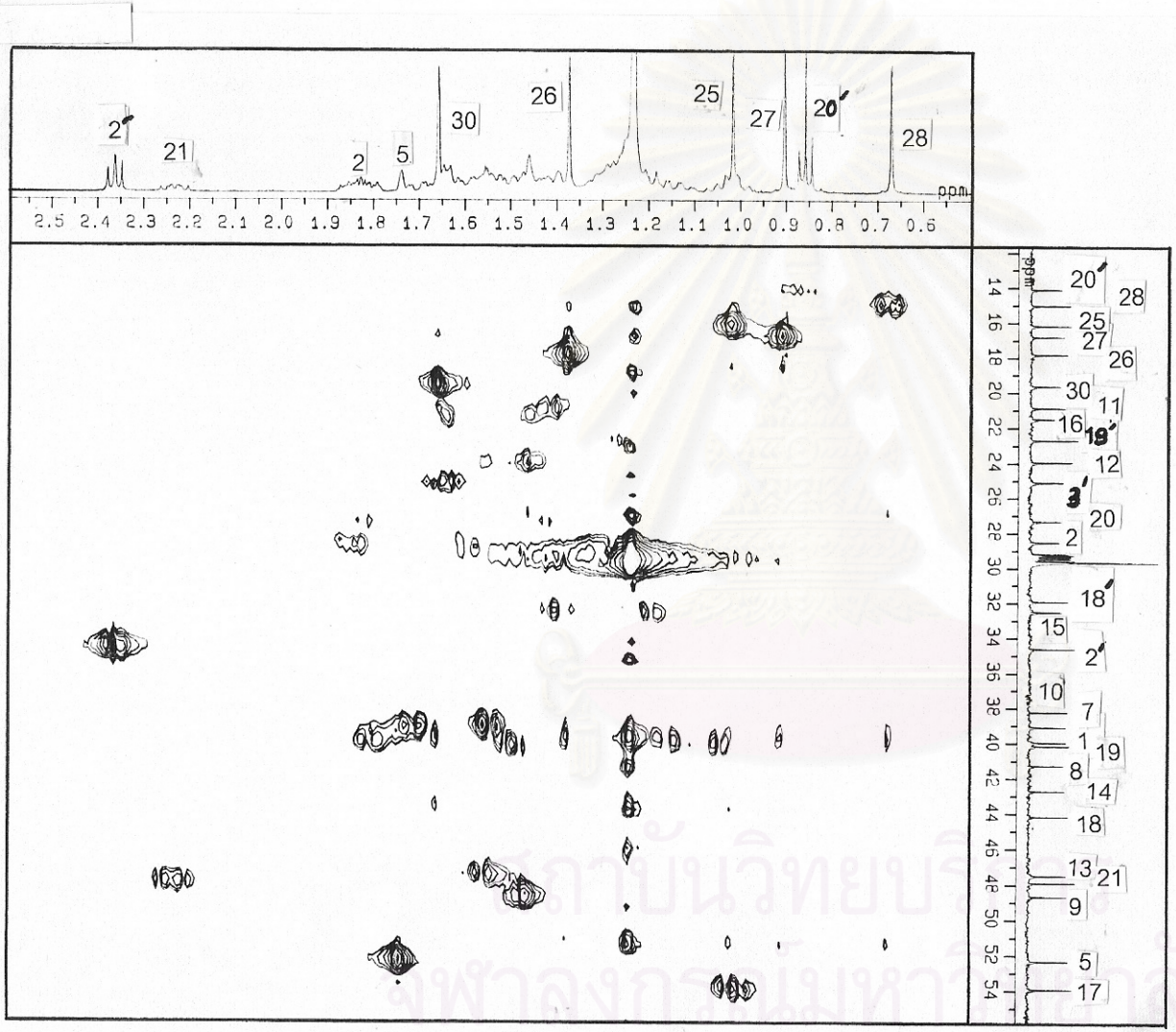


Figure 7a. The 125 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-1 (in $CDCl_3$)



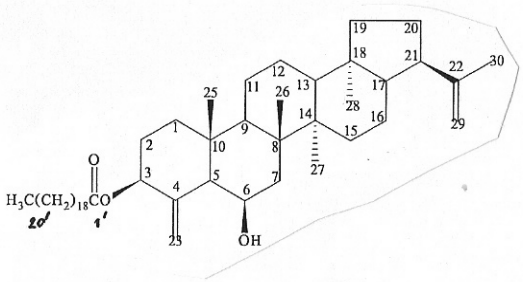


Figure 7b. The 125 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-1 (expanded)

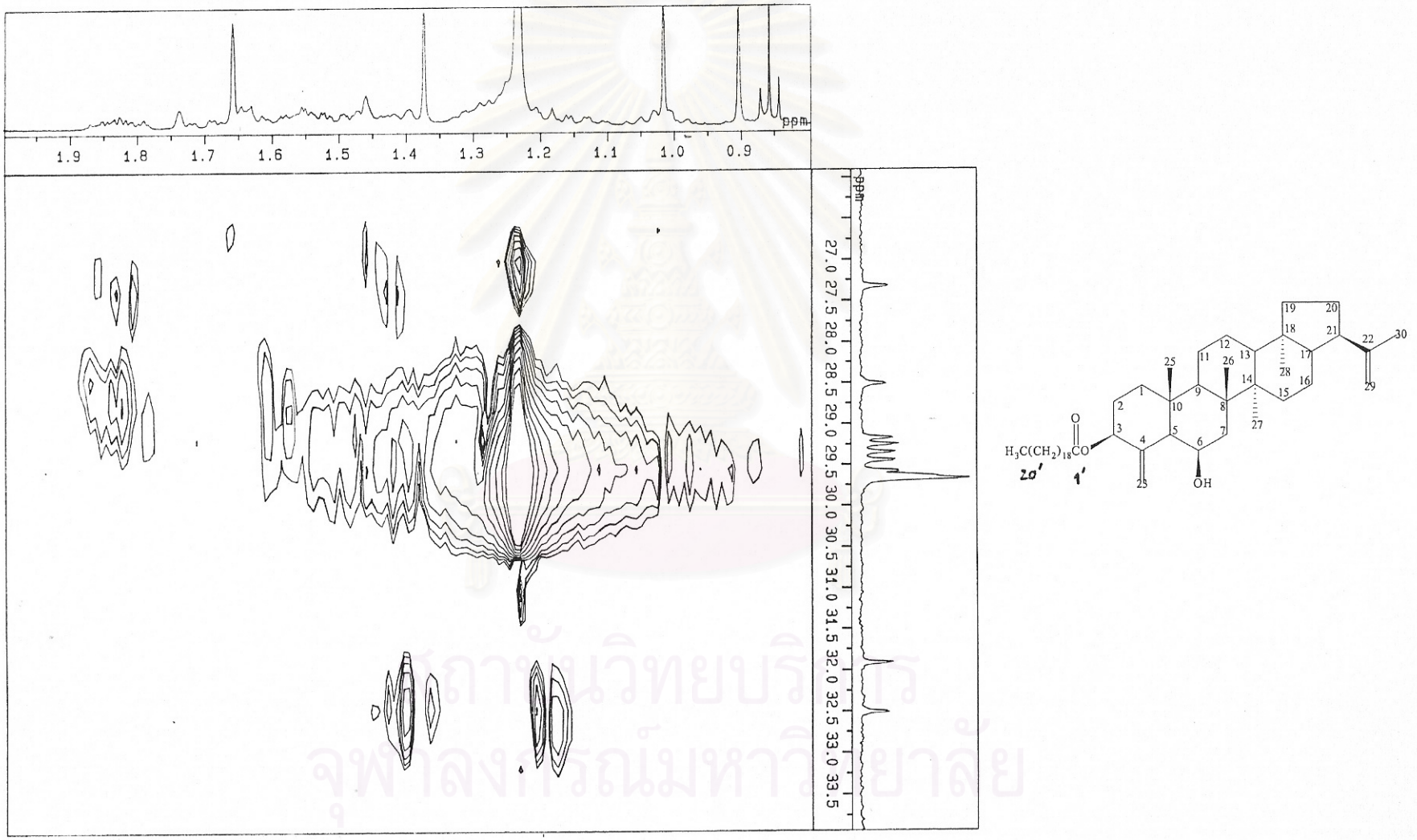
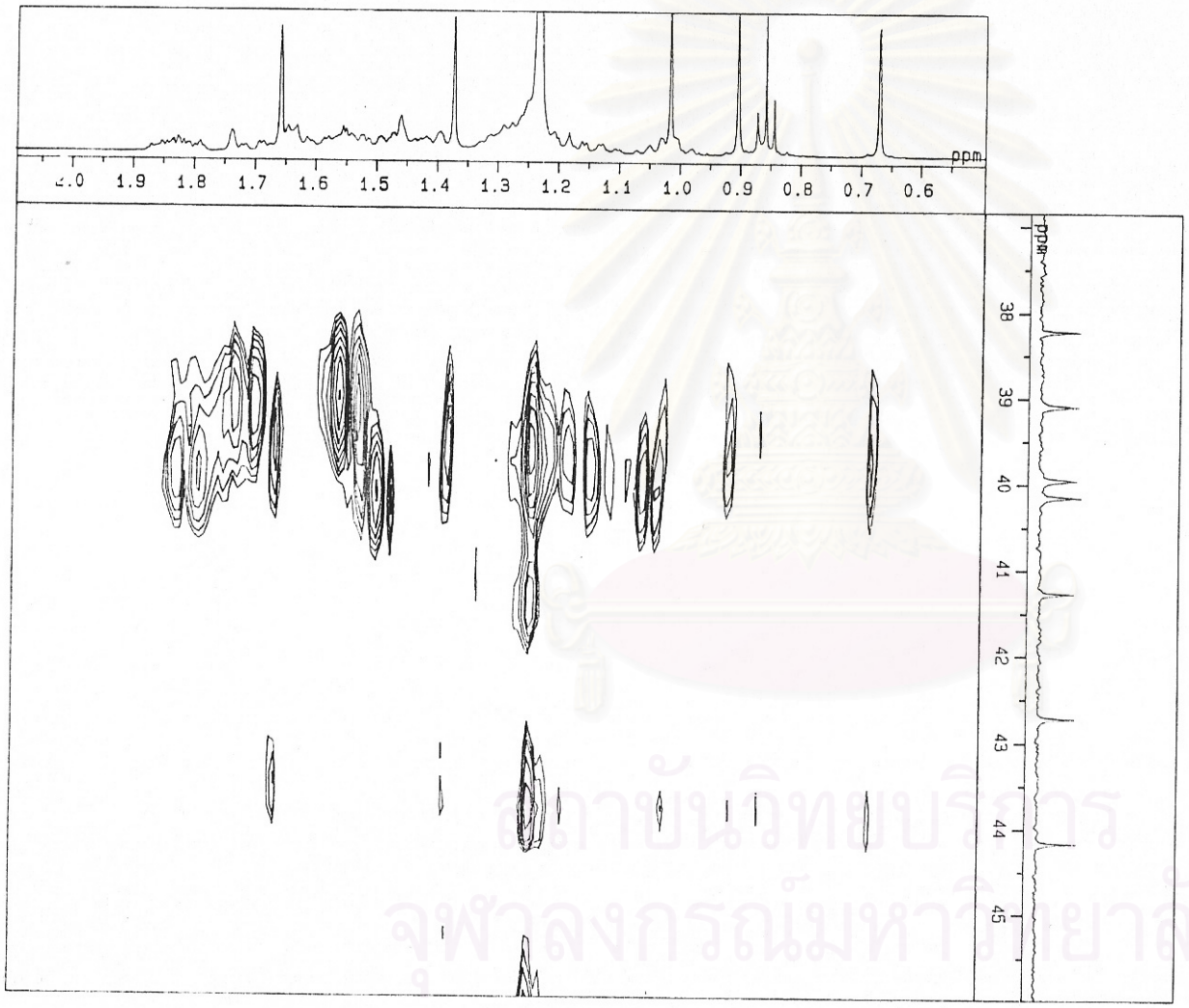


Figure 7c. The 125 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-1 (expanded)



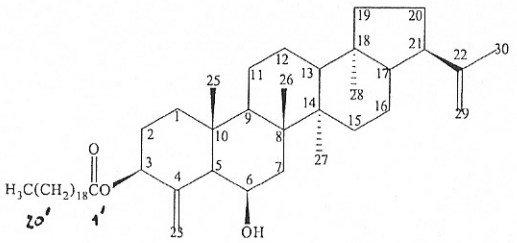


Figure 7d. The 125 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-1 (expanded)

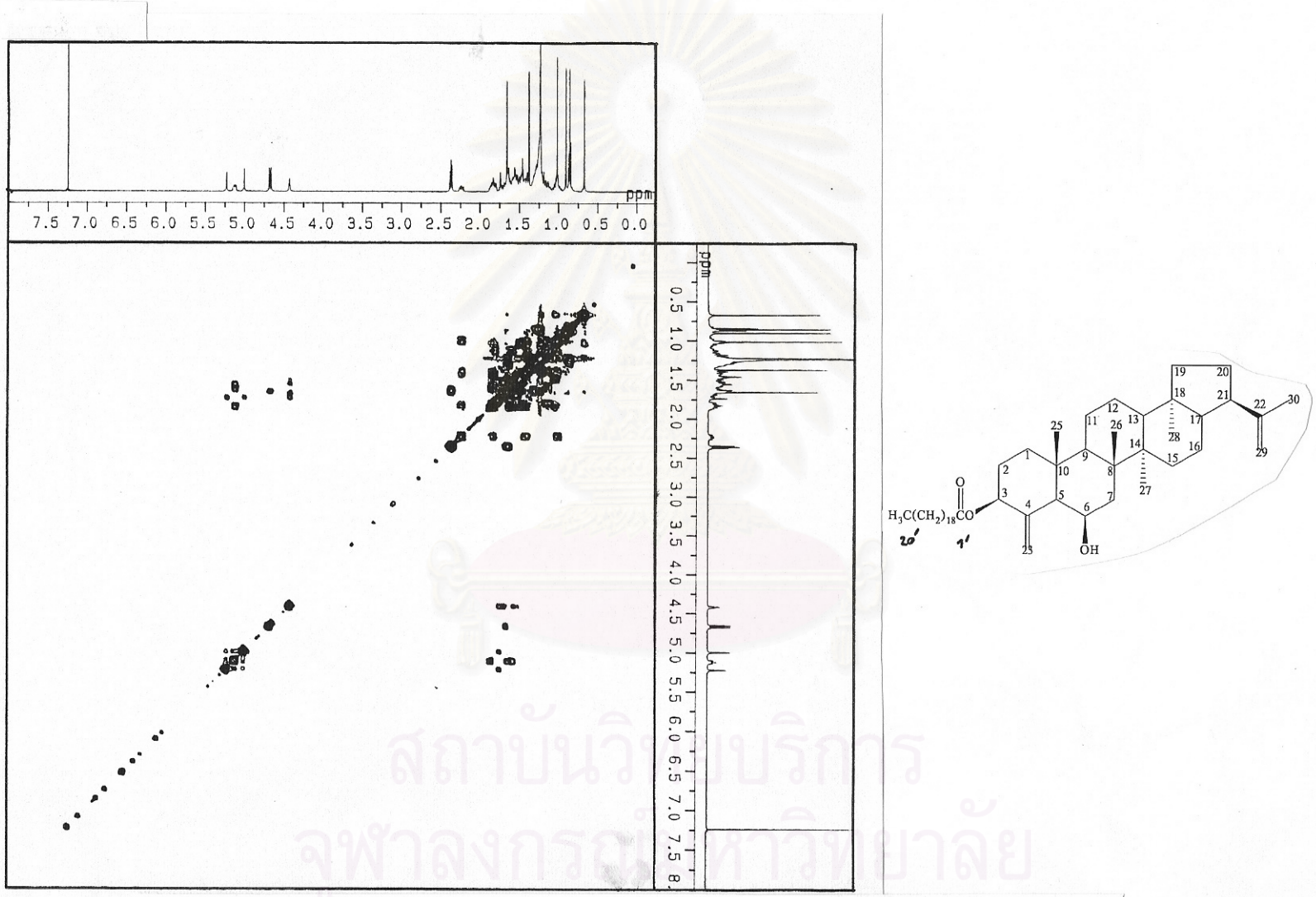


Figure 8a. The 500 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-1 (in $CDCl_3$)

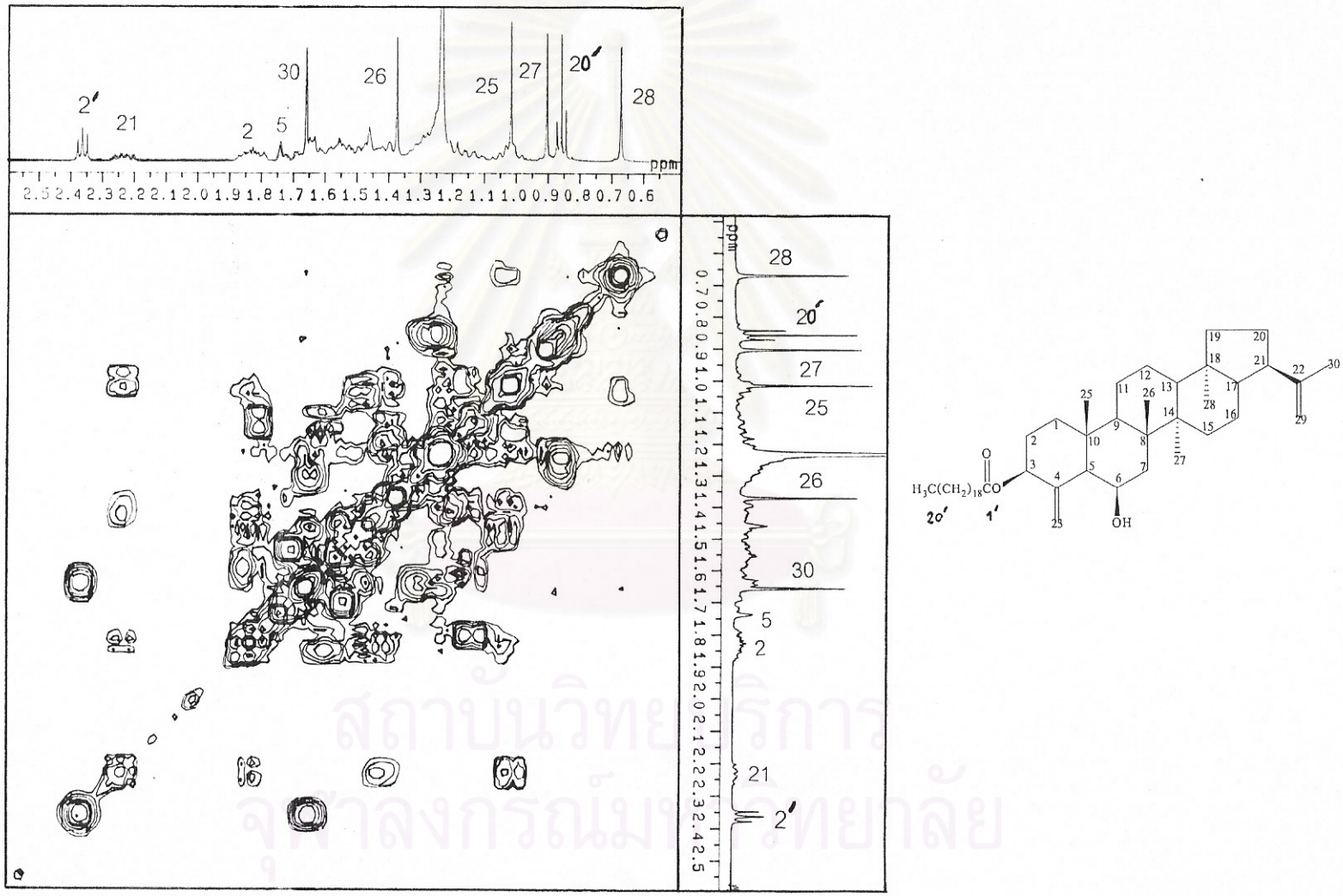
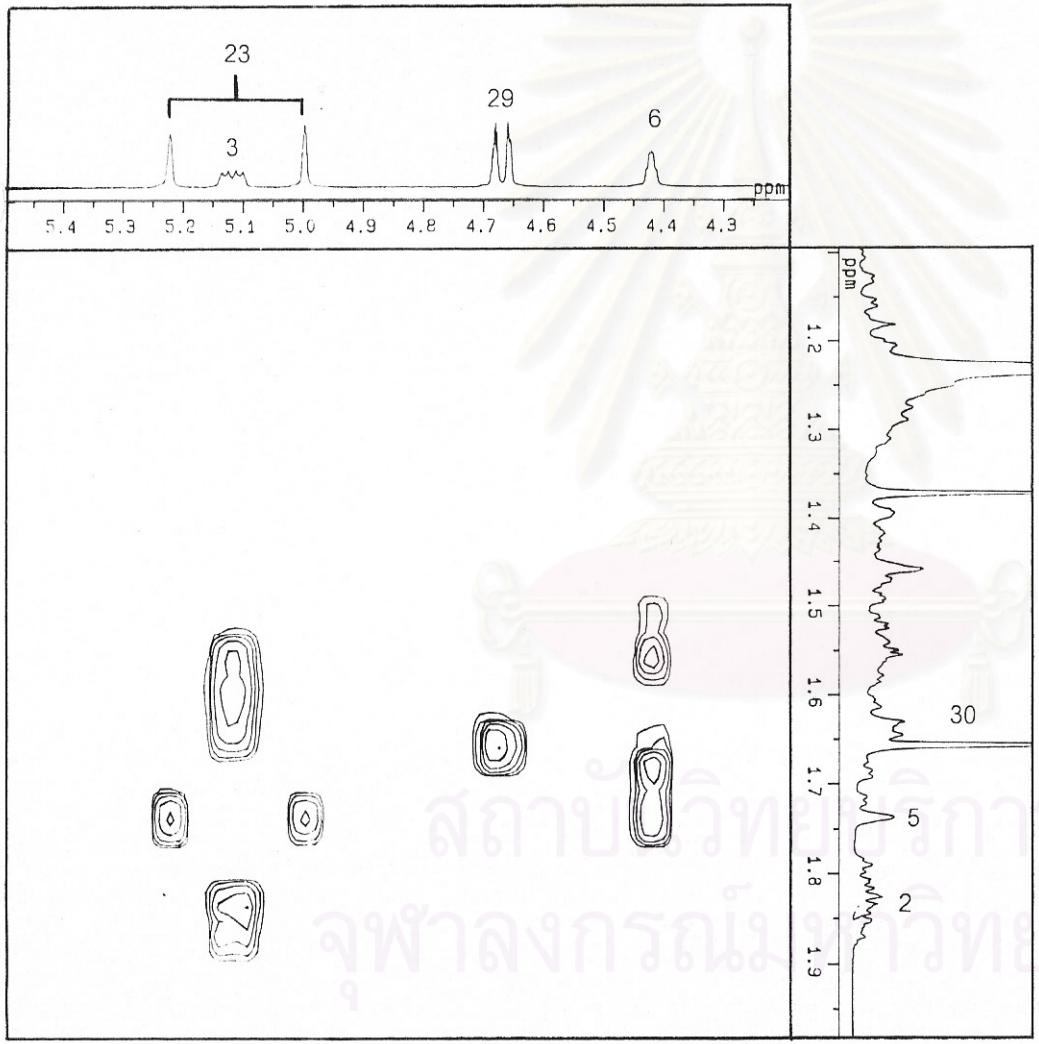


Figure 8b. The 500 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-1 (expanded)



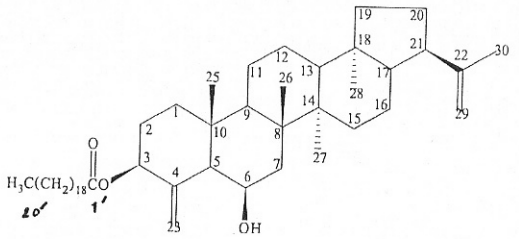
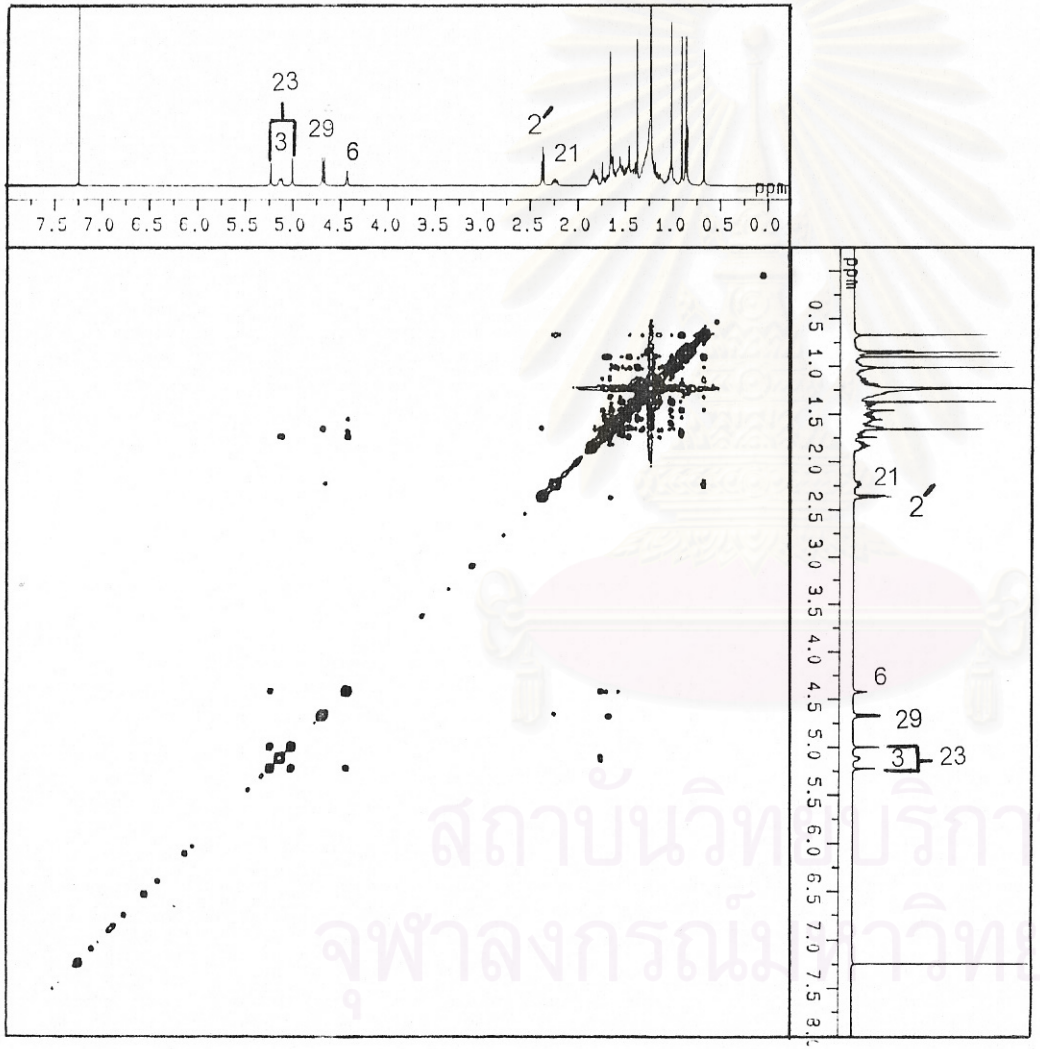


Figure 8c. The 500 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-1 (expanded)



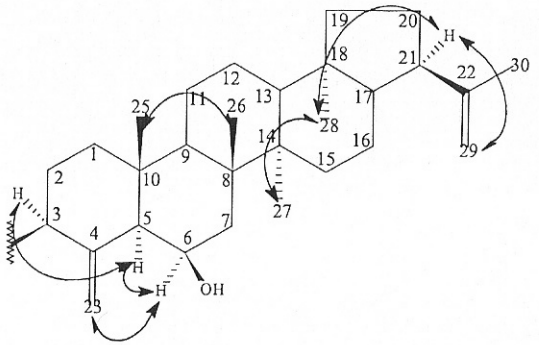
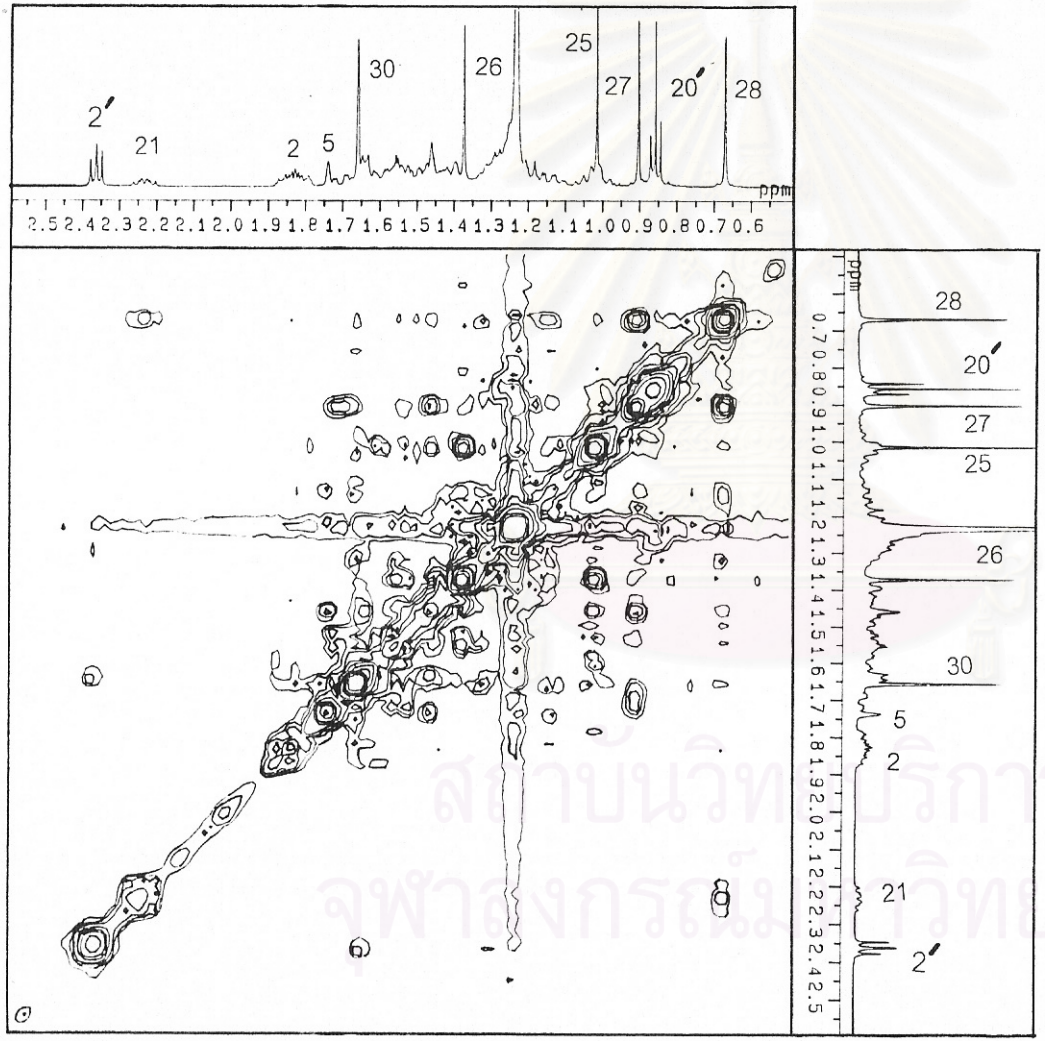


Figure 9a. The 500 MHz ^{1}H-^{1}H NOESY NMR spectrum of compound HA-1 (in $CDCl_3$)



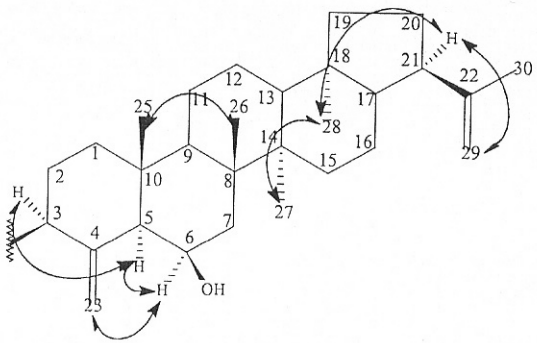
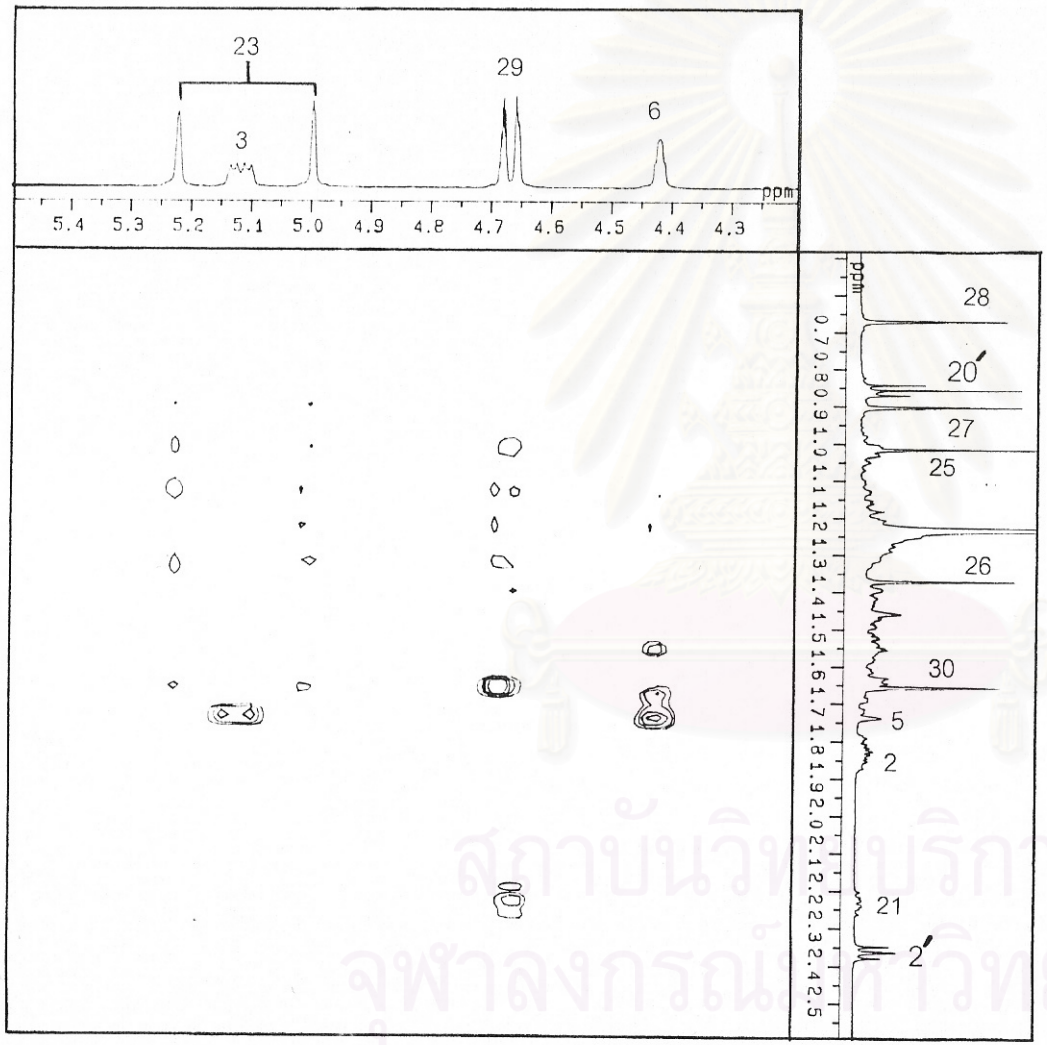


Figure 9b. The 500 MHz ^{1}H-^{1}H NOESY NMR spectrum of compound HA-1 (expanded)



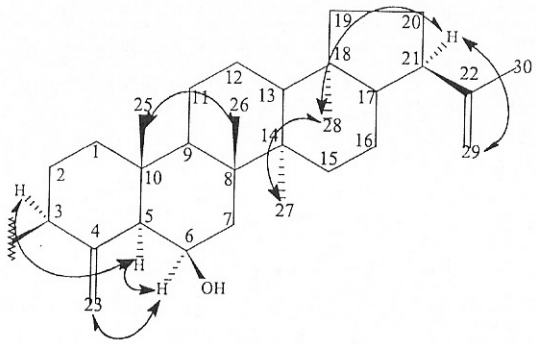


Figure 9c. The 500 MHz ^{1}H-^{1}H NOESY NMR spectrum of compound HA-1 (expanded)

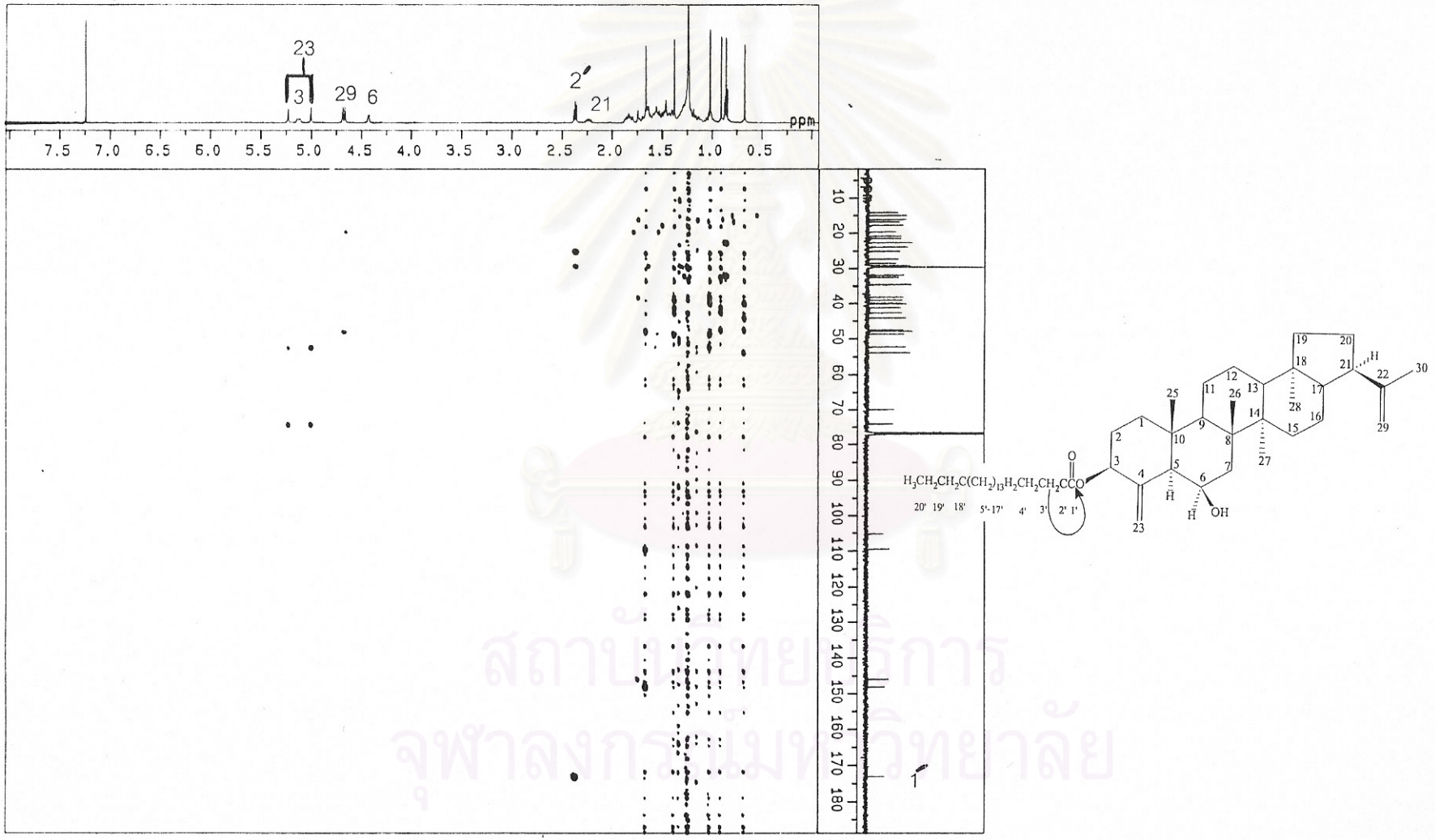


Figure 10a. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (in $CDCl_3$)

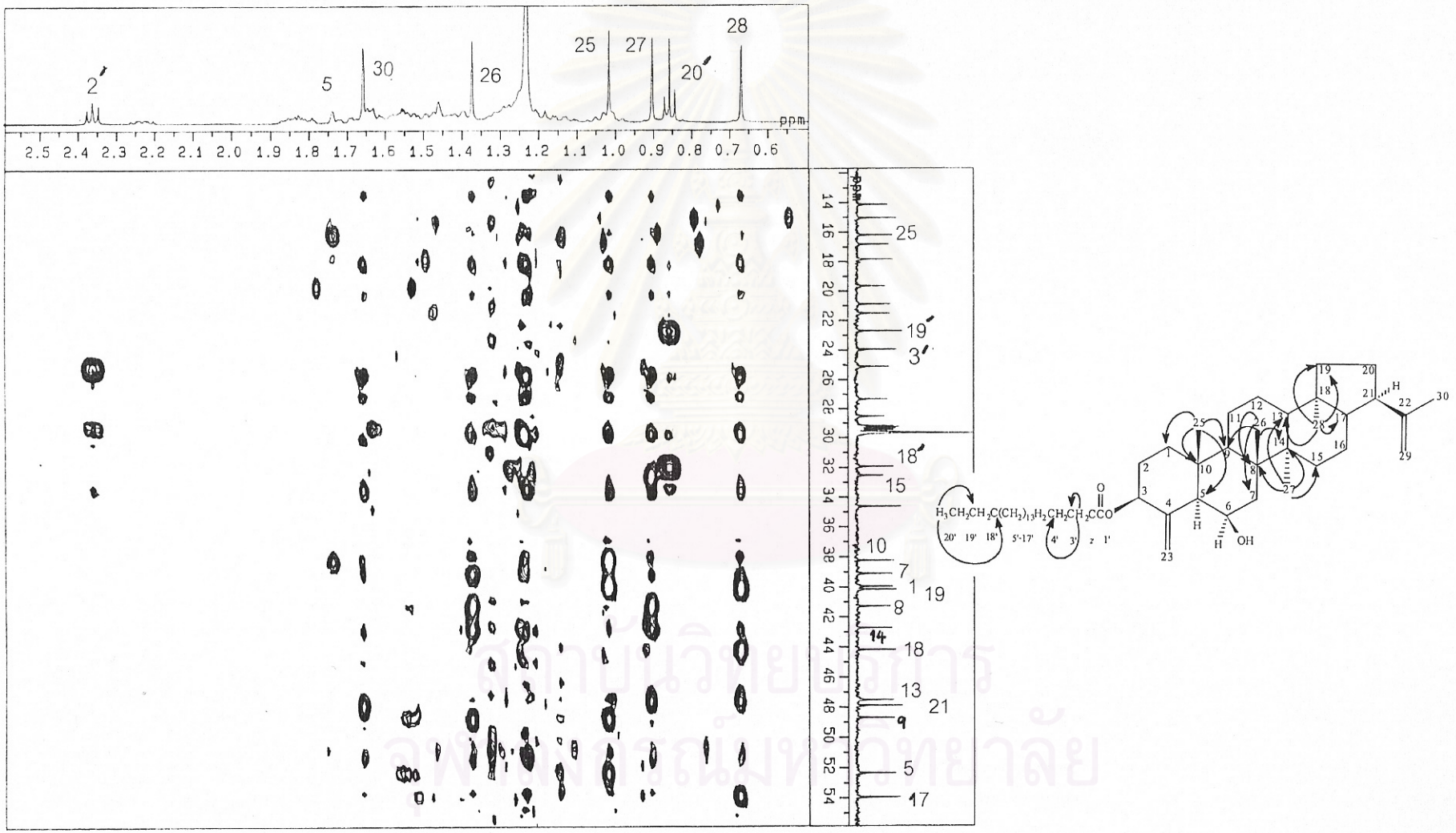


Figure 10b. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

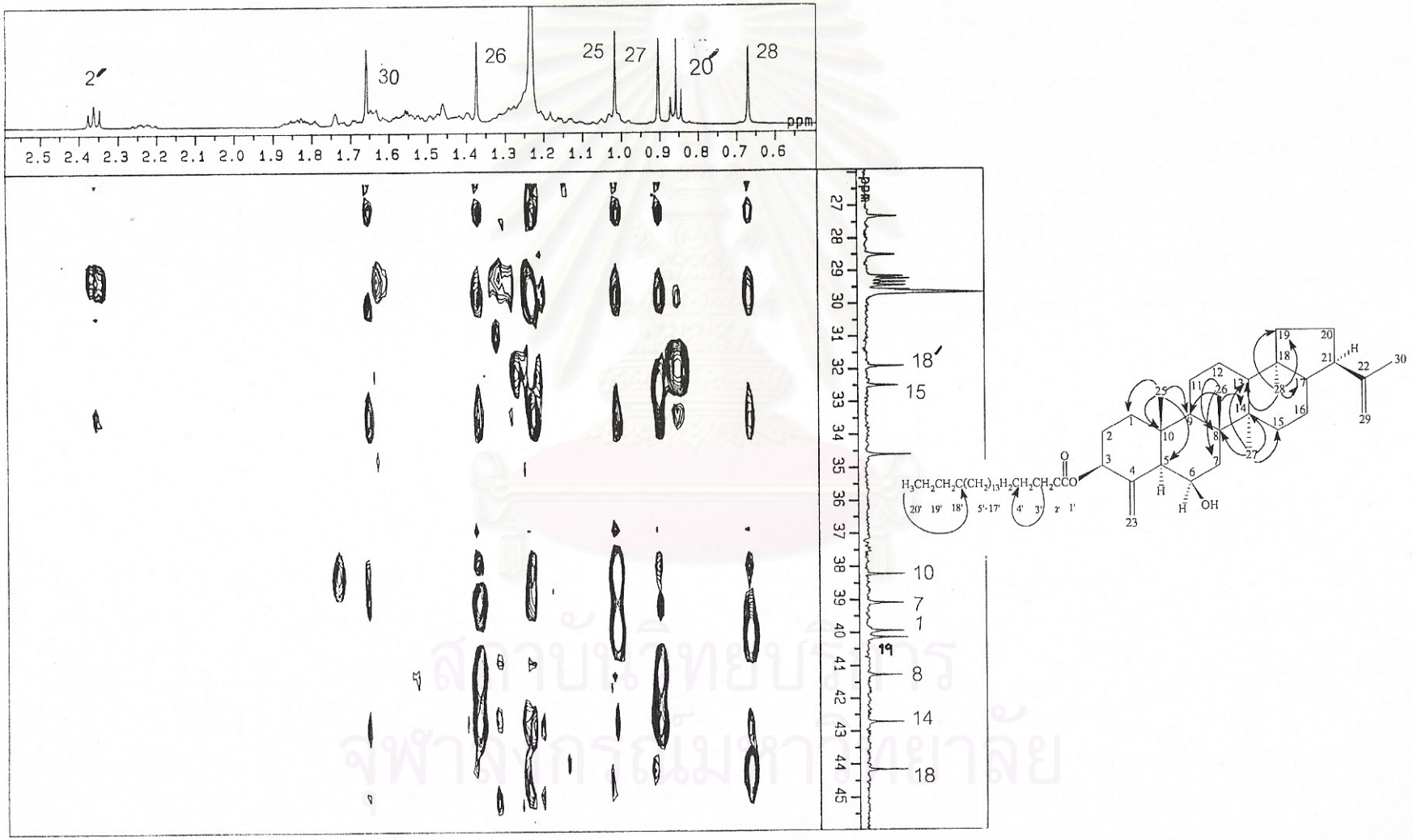


Figure 10c. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

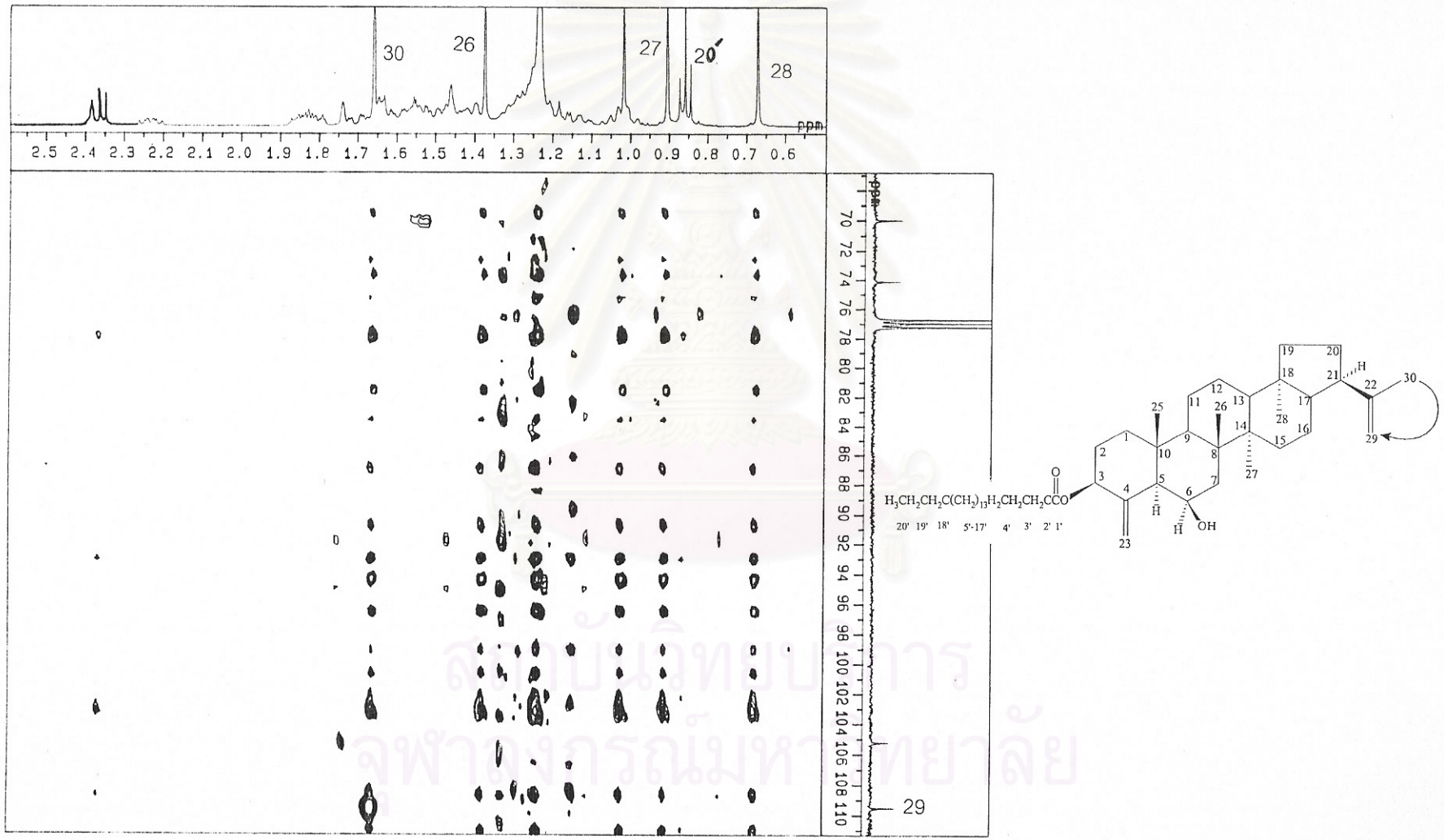


Figure 10d. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

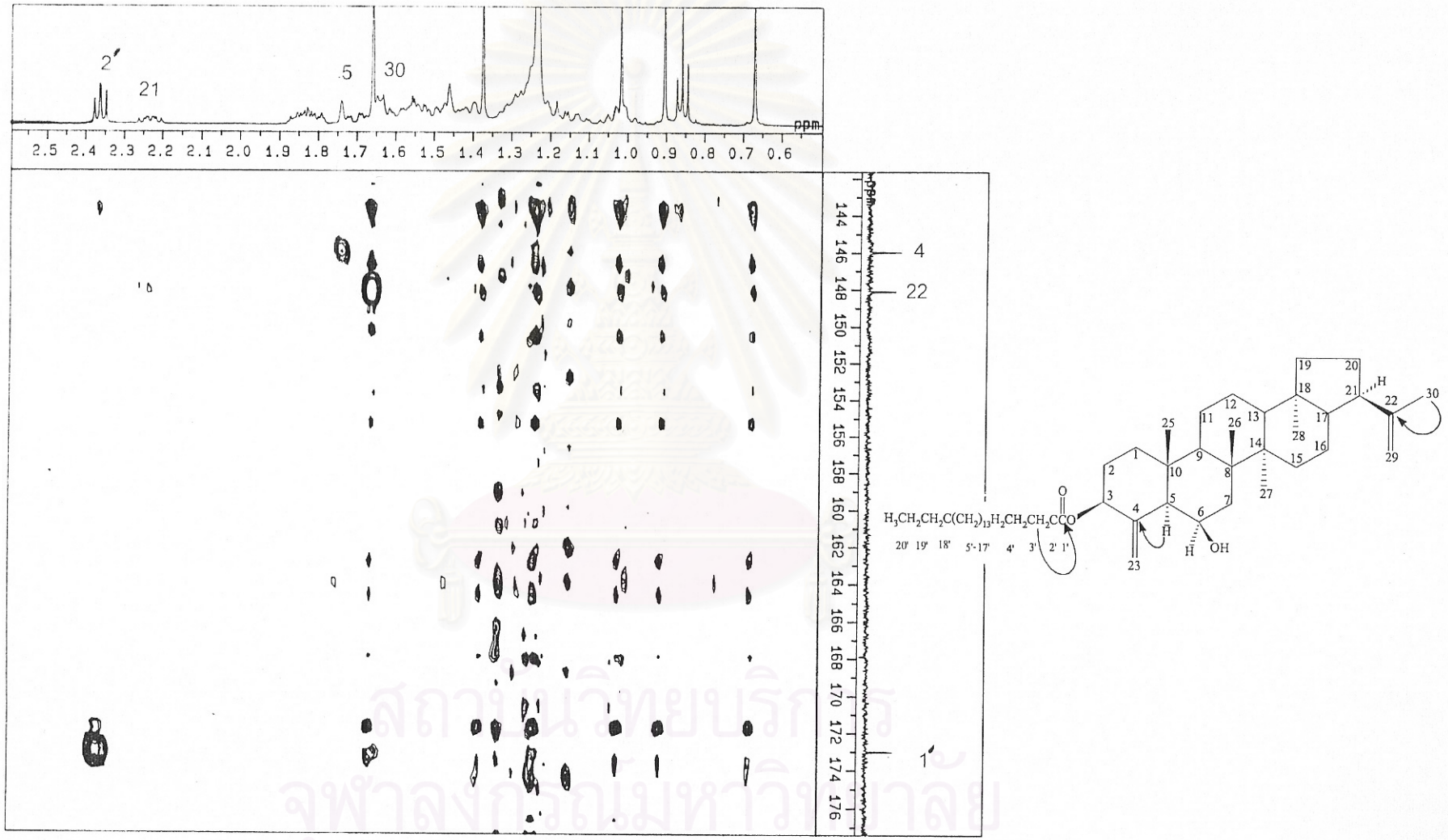


Figure 10e. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

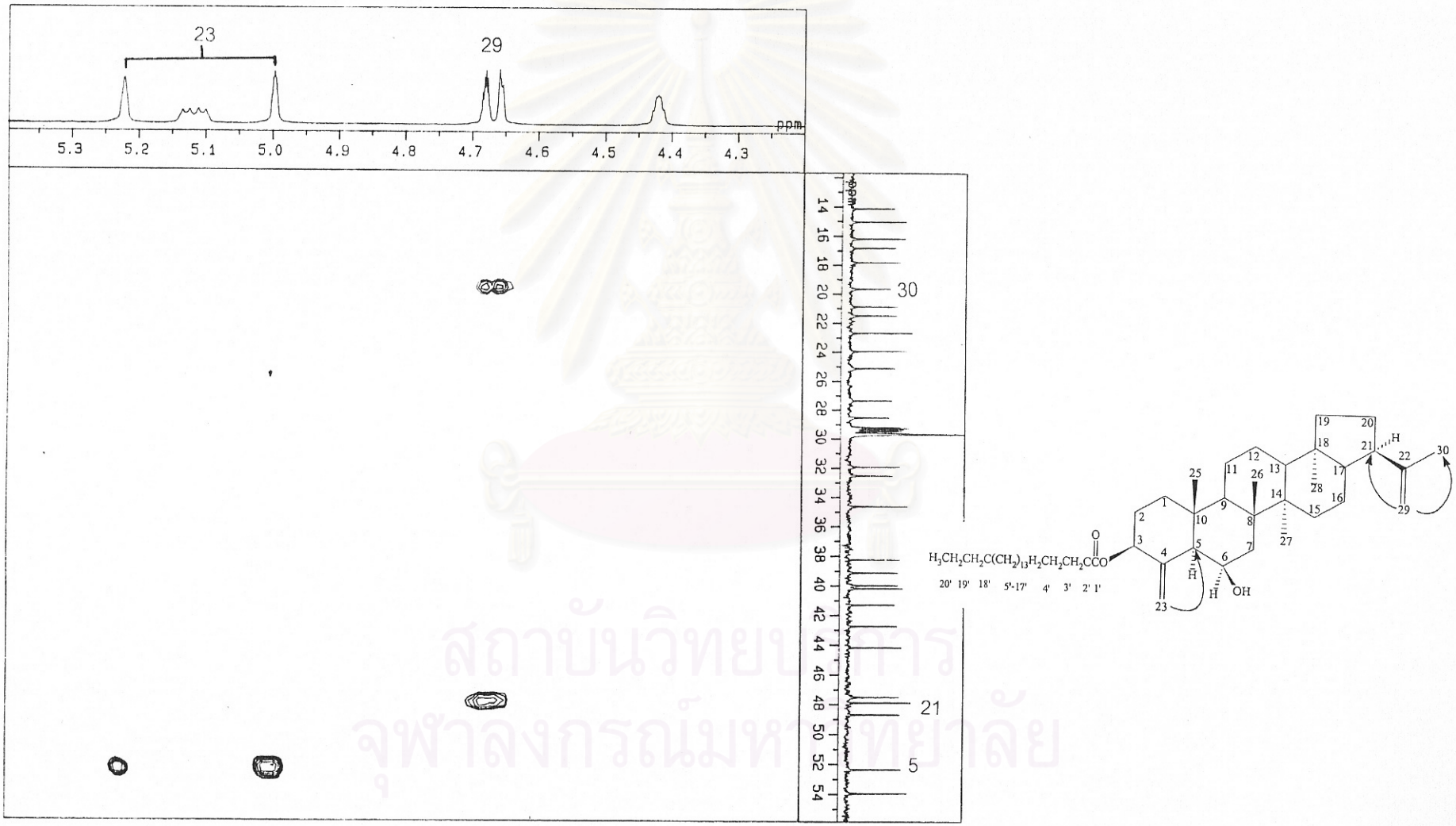


Figure 10f. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

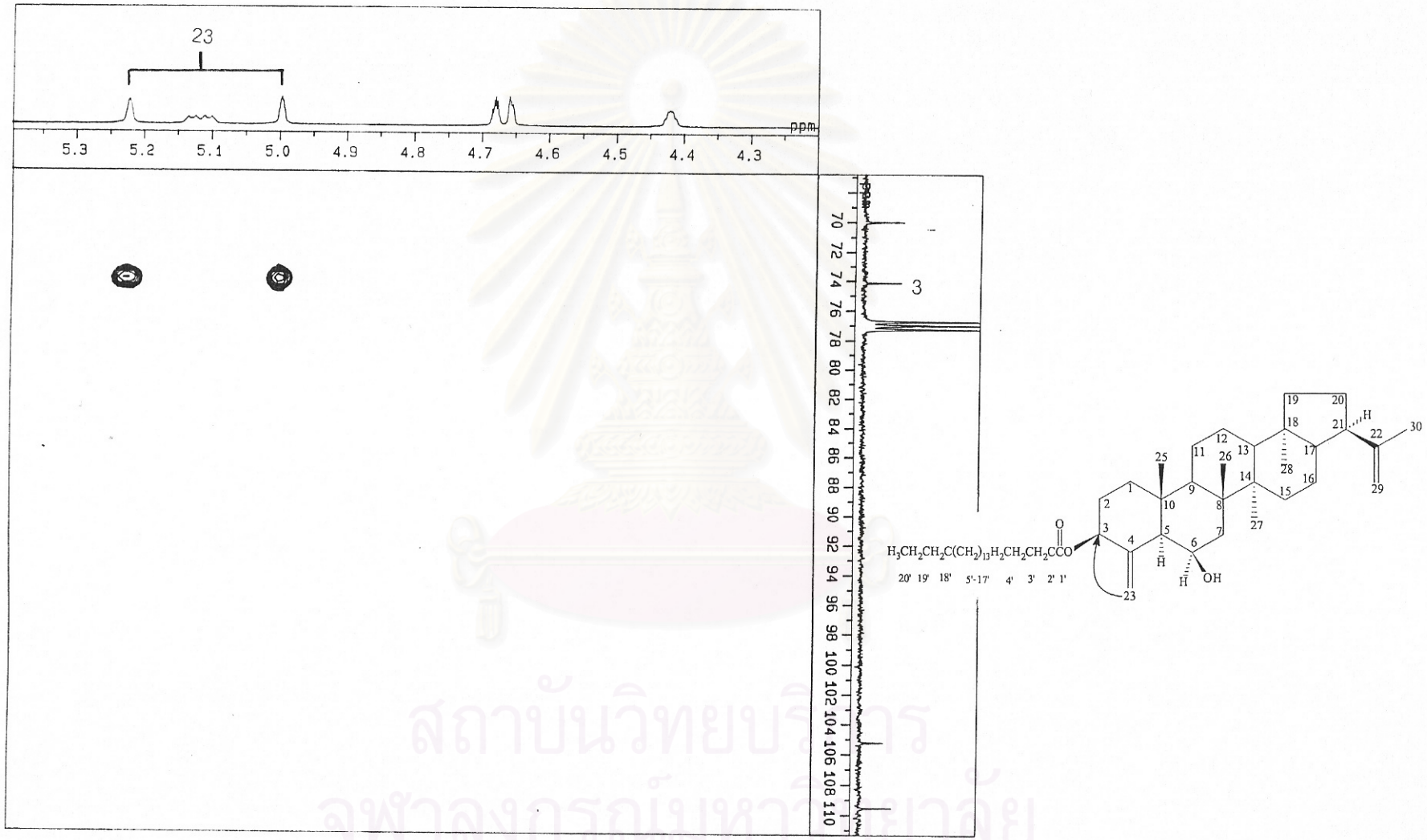


Figure 10g. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

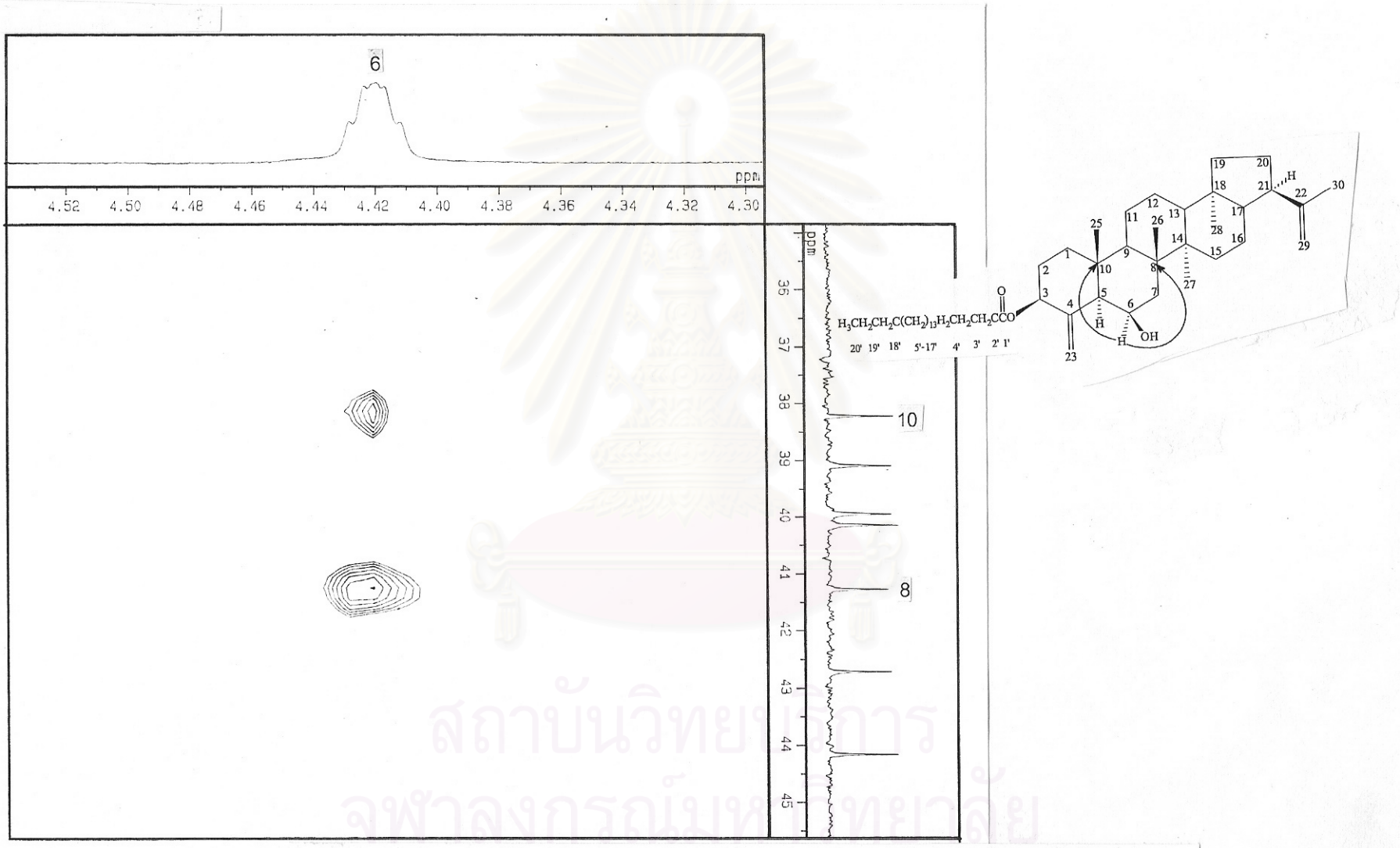


Figure 10h. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

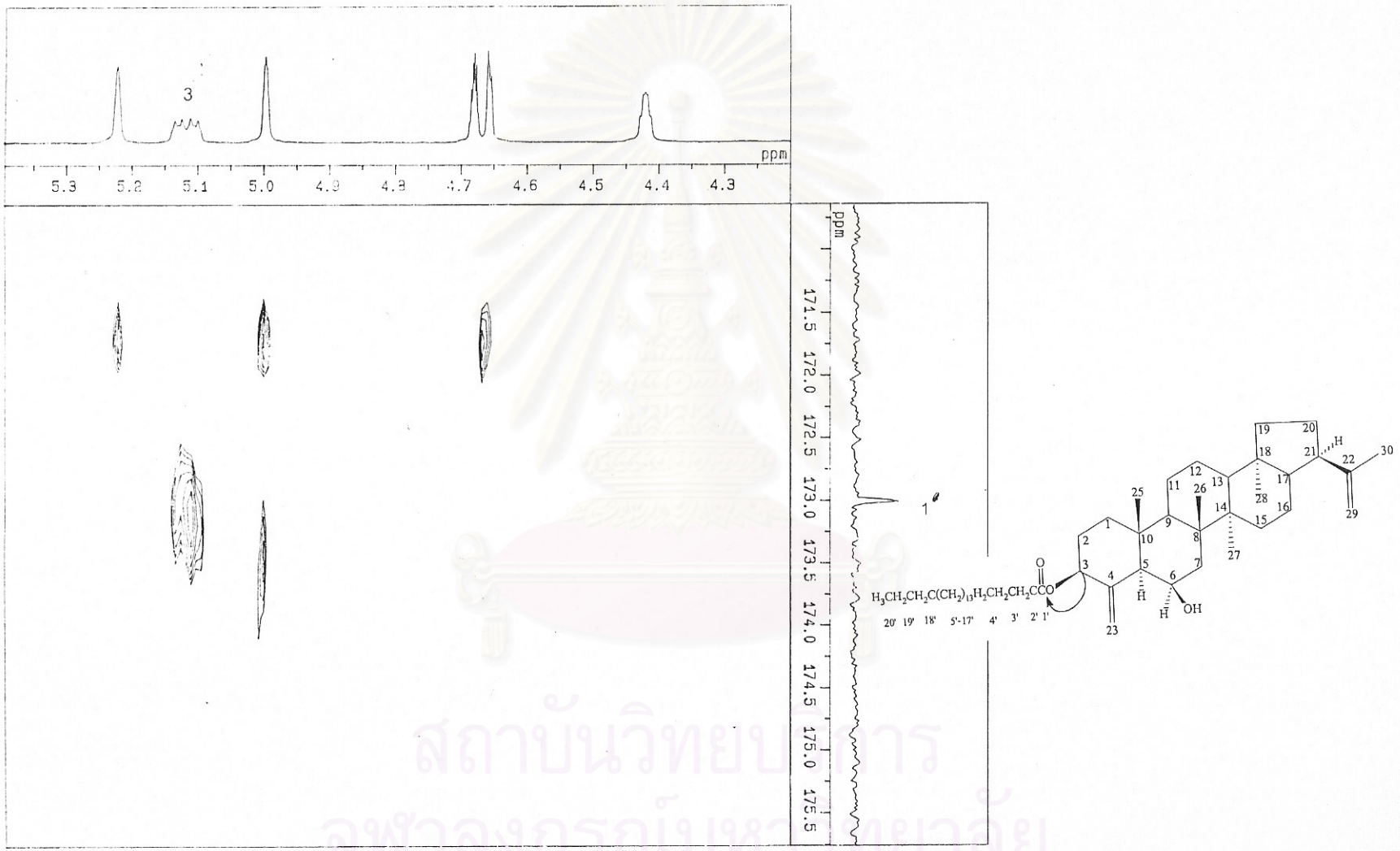


Figure 10i. The 125 MHz ^{1}H-^{13}C HMBC NMR spectrum of compound HA-1 (expanded)

2. Structure elucidation of HA-2

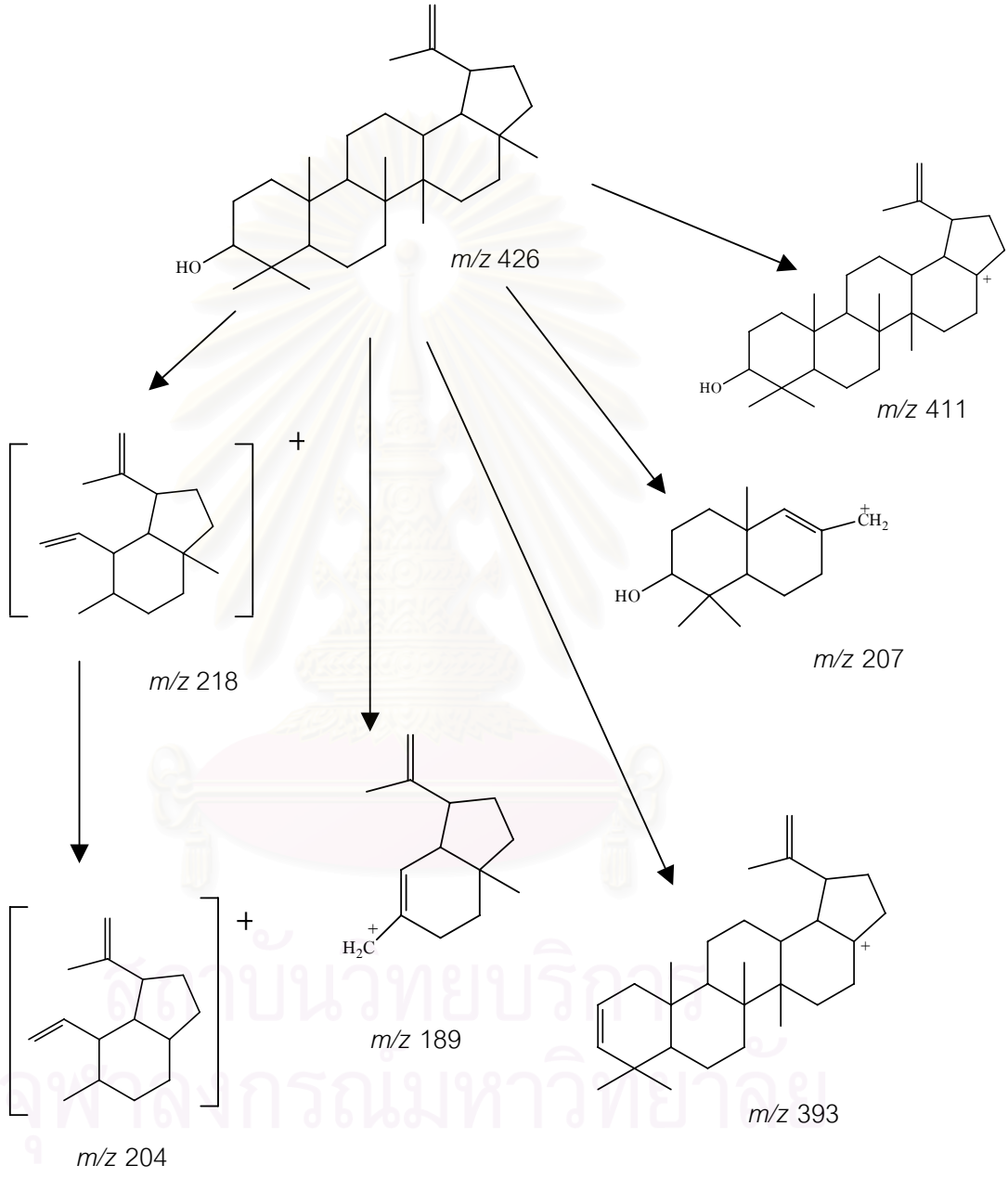
Compound HA-2 was recrystallized as colorless needles from methanol (63 mg, 0.006% yield). The compound gave purple color to Libermann-Burchard's reagent, suggesting that it is a triterpenoid. The EIMS of HA-2 (Figure 11) displayed a molecular ion peak at *m/z* 426, corresponding to the molecular formula of $C_{30}H_{50}O$. The base peak at *m/z* 189 were important in showing that HA-2 has the skeletal structure of a lupane-type triterpenoid (Ogunkoya, 1981). The prominent peak at *m/z* 218 was the result of cleavage across the C ring of the lupane skeleton, and successive loss of a methyl group produced the fragment peak at *m/z* 204. The peak at *m/z* 411 ($M-CH_3$) and *m/z* 393 ($M–CH_3–H_2O$) were also observed (Scheme 4). The IR spectrum of HA-2 (Figure 12) showed a broad band at 3300 cm^{-1} (OH streching), indicating the presence of a hydroxyl group in the molecule.

The ^{1}H-NMR spectrum (Figures 13a–13b) displayed the signals of seven methyl groups as singlets at δ 0.74, 0.76, 0.81, 0.92, 0.94, 1.01, and 1.62. The presence of exomethylene protons (H-29) in the isopropenyl group of a lupane-type triterpenoid could be observed as a pair of broad singlets at δ 4.66 and 4.54. A doublet of doublets (1H, *J*= 10.8 Hz) at δ 3.17 was assignable to the hydroxymethine proton at position 3.

The ^{13}C-NMR spectrum (Figures 14a–14b) showed the signals of 30 carbon atoms, supporting the assignment of HA-2 as a triterpenoid. The DEPT-90 and DEPT-135 experiments (Figures 15a–15b) helped in identifying the signals of seven methyl carbons at δ 14.5, 15.3, 15.9, 16.1, 18.0, 19.3 and 28.0, eleven methylene carbons at δ 18.3, 20.9, 25.1, 27.4, 27.4, 29.8, 34.2, 35.5, 38.7, 39.9 and 109.3, six methine carbons at δ 38.0, 47.9, 48.3, 50.4, 55.3 and 78.9, and six quaternary carbons at δ 37.1, 38.8, 40.8, 42.8, 42.9 and 150.9.

^{1}H and ^{13}C-NMR data of HA-2 was found to be in agreement with those of previously reported for lupeol (Ahmad, Bano, and Mohammad,1985). Comparison of ^{13}C-NMR

assignment of HA-2 and lupeol together with ^{1}H-NMR assignment of HA-2 are shown in Table 13.



Scheme 4. Mass fragmentation of compound HA-2

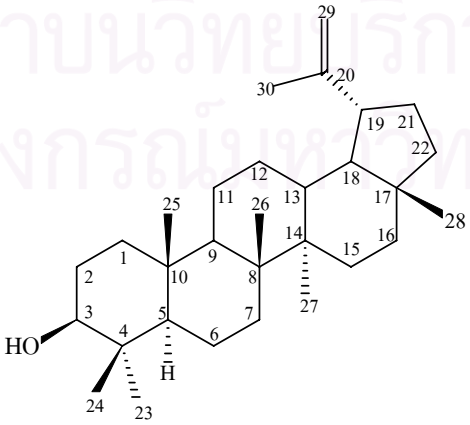
Table 13. ^{1}H and ^{13}C-NMR assignments of compound HA-2 and reported data of lupeol (in $CDCl_3$).

Position	Compound HA-2		Lupeol	
	δH	δC	δH	δC
1		38.7		38.7
2		27.5		27.4
3	3.17 (*dd*, *J*=10.8,5.1 Hz)	79.0	3.20 (*dd*, *J*=10.6 Hz)	78.8
4		38.9		38.8
5		55.3		55.2
6		18.3		18.3
7		34.3		34.2
8		40.8		40.8
9		50.4		50.4
10		37.2		37.1
11		20.9		20.9
12		25.2		25.1
13		38.1		38.0
14		42.8		42.8
15		27.4		27.4
16		35.6		35.5
17		43.0		42.9
18		48.3		48.2
19	2.35(*td*;*J*=11.4,11.4,5.7 Hz)	48.0	2.38(*ddd*;*J*=10.6,10.6,5.3 Hz)	47.9
20		151.0		150.6

Table 13. ^{1}H and ^{13}C-NMR assignments of compound HA-2 and reported data of lupeol (in $CDCl_3$) (continued).

Position	Compound HA-2		Lupeol	
	δH	δ C	δH	δ C
21		29.7		29.8
22		40.0		39.9
23	0.94 (*s*)	28.0	0.94 (*s*)	28.0
24	0.73 (*s*)	15.4	0.76 (*s*)	15.4
25	0.80 (*s*)	16.1	0.83 (*s*)	16.1
26	1.00 (*s*)	16.0	1.03 (*s*)	15.9
27	0.91 (*s*)	14.5	0.96 (*s*)	14.5
28	0.76 (*s*)	18.0	0.79 (*s*)	18.0
29	4.54, 4.66 (*br s*)	109.3	4.57,4.68(*dd; J*=1 Hz)	109.2
30	1.66 (*s*)	19.3	1.67 (*br s*)	19.3

Therefore, it was concluded that compound HA-2 is the triterpenoid lupeol, having the structure as shown below :



Lupeol

The lupane triterpenoid lupeol can be found in a variety of plant sources (Hasmeda *et al*., 1999). In Sapindaceae, the compound has been isolated from the bark of *Schleichera oleosa* (Dan and Dan, 1987). The toxicity of lupeol is very low (Patocka, 2003), and it has been found to exhibit some interesting bioactivities including anti-inflammatory (Akihisa *et al*., 1996; Rajic *et al*., 2000; Fernandez *et al*., 2001; Mitaine-Offer *et al*., 2002), antiarthritic (Kweifiookai and Carroll, 1993; Kweifiookai *et al*., 1995; Geetha and Varalakshmi, 1998), antibacterial (Woldemichael *et al*., 2003), and cytotoxic activities (Moriarity *et al*., 1998; Wada, Iida, and Tanaka, 2001), as well as *in vitro* inhibitory activity against *Plasmodium falciparum* (Alves *et al*., 1997 ; Ziegler *et al*., 2002).

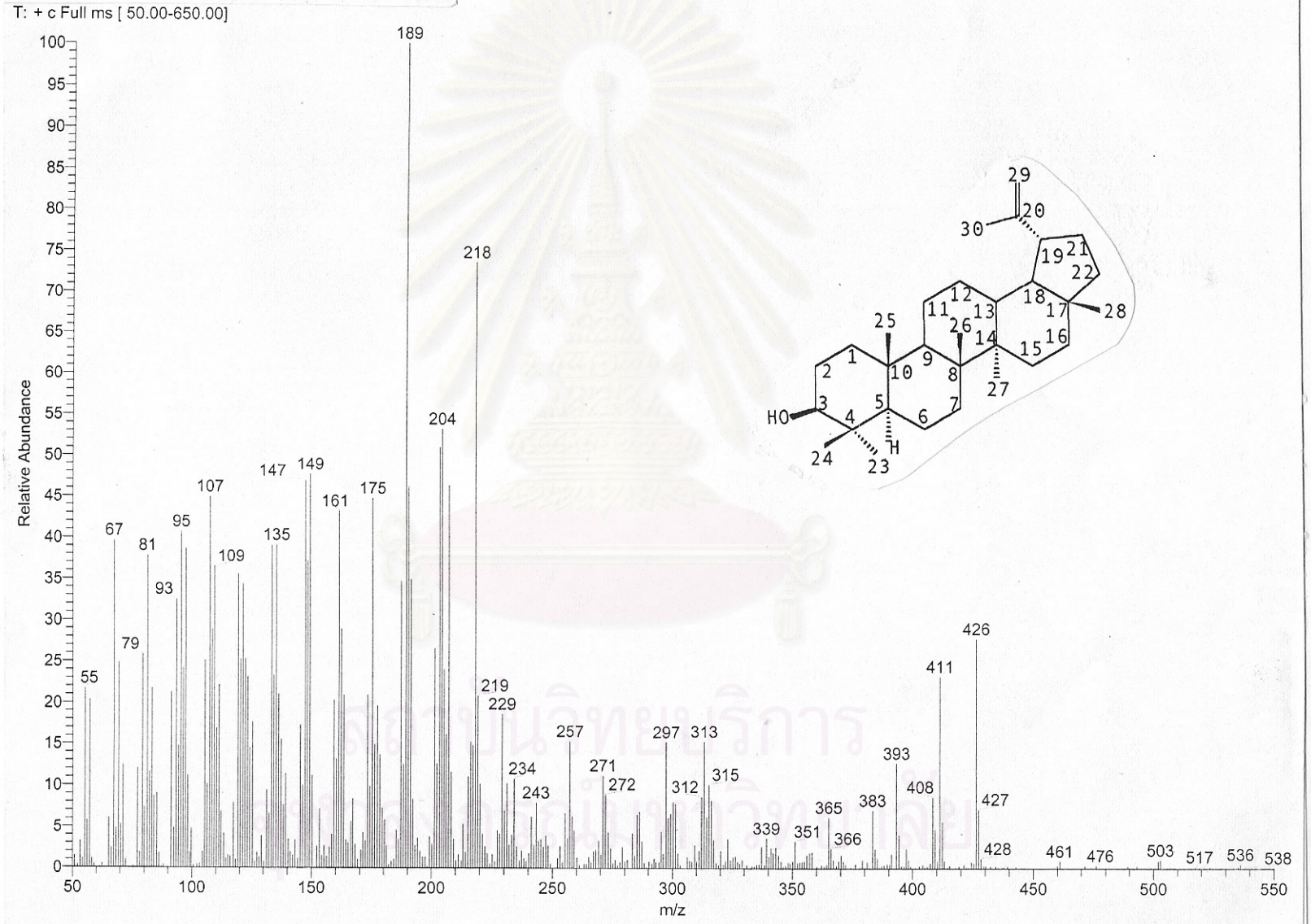


Figure 11. EIMS of compound HA-2

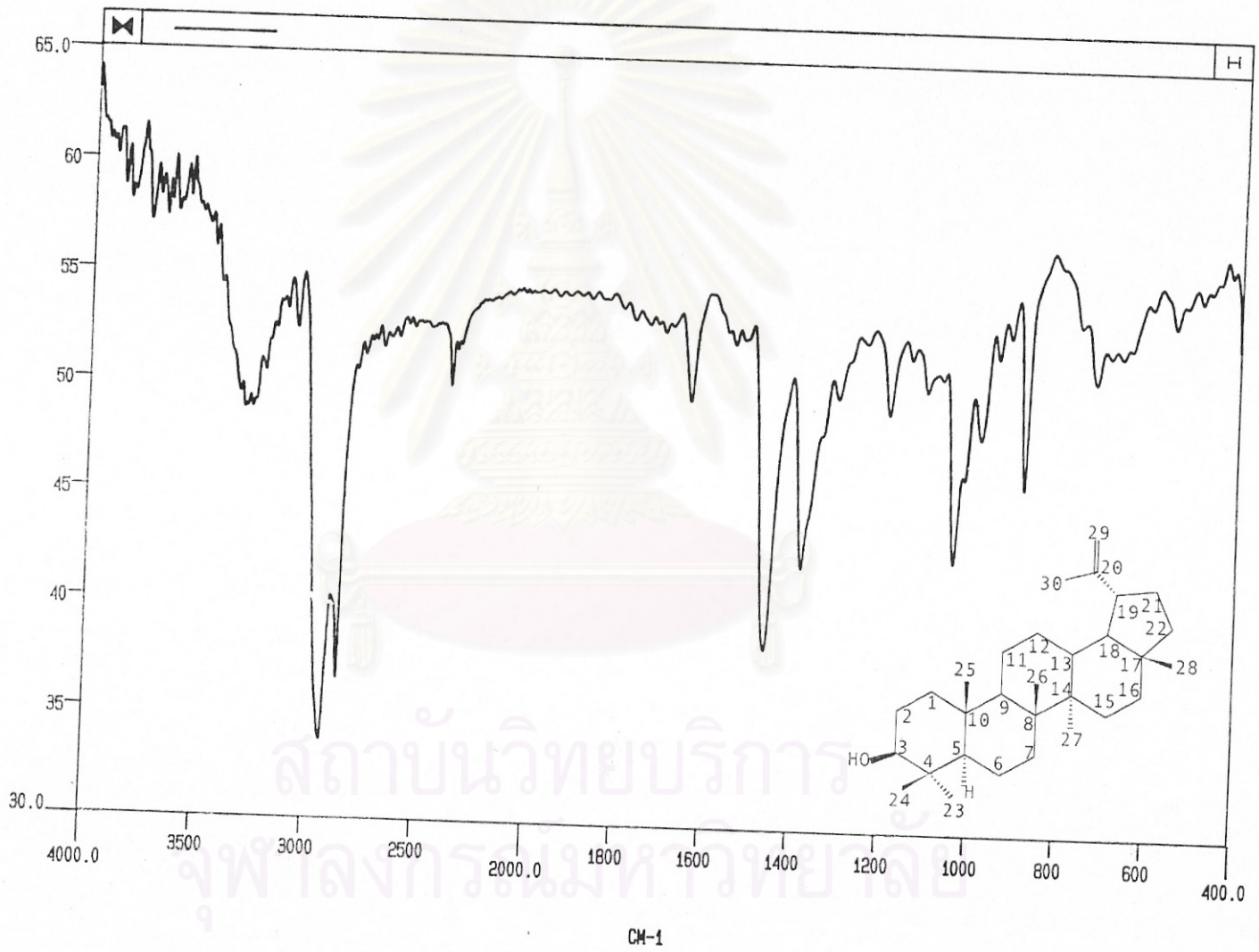


Figure 12. IR spectrum of compound HA-2

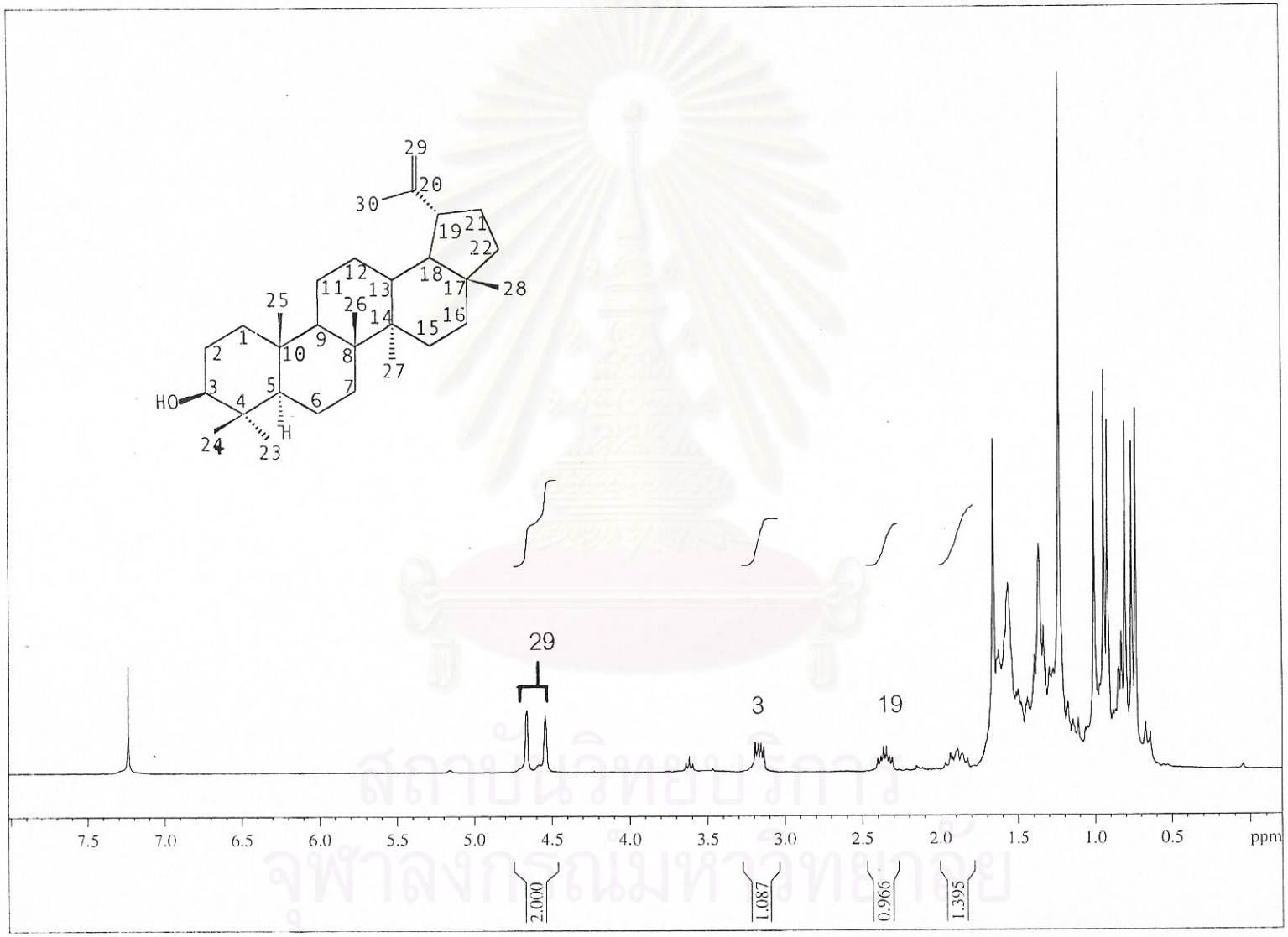


Figure 13a. The 300 MHz ^{1}H-NMR spectrum of compound HA-2 (in $CDCl_3$)

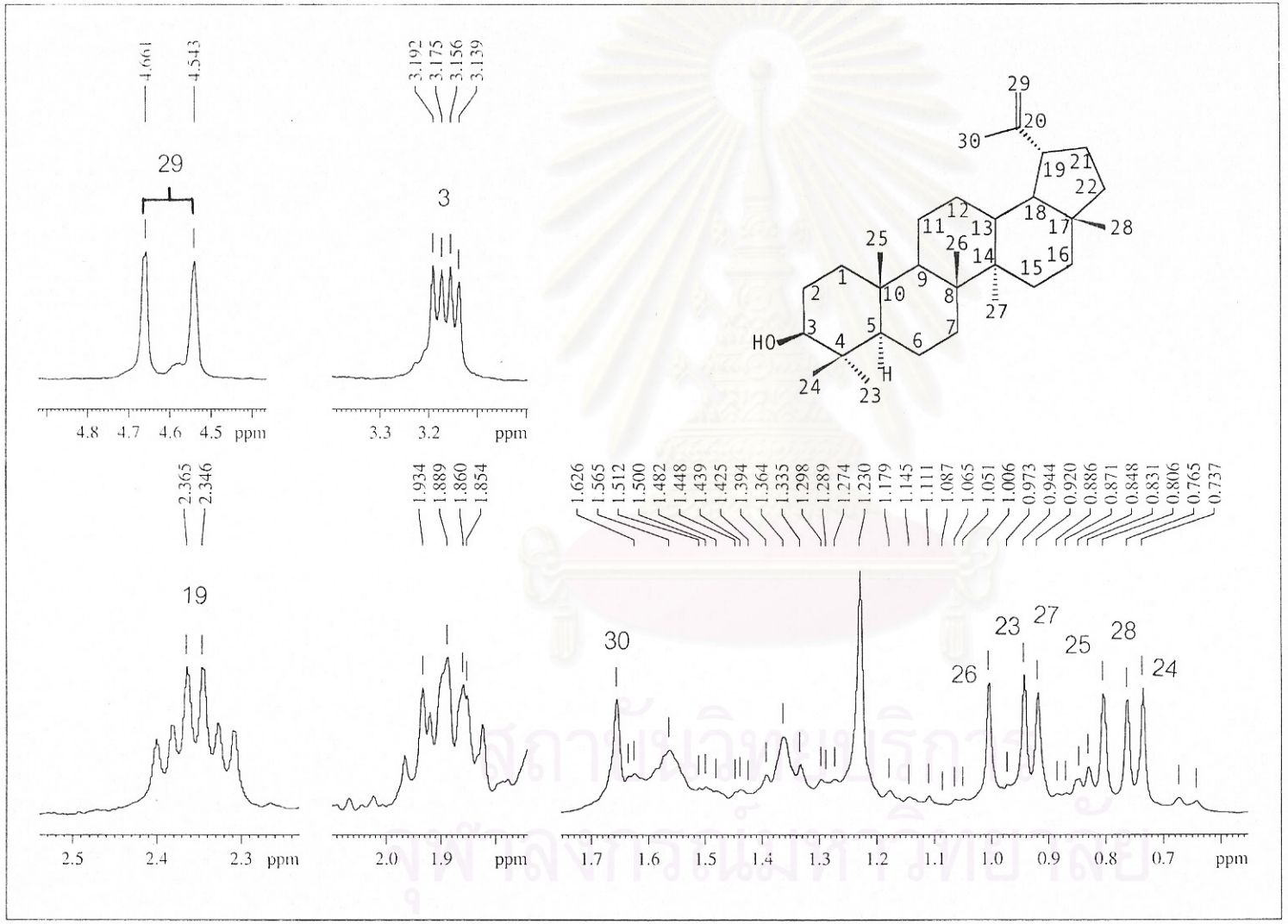


Figure 13b. The 300 MHz ^{1}H-NMR spectrum of compound HA-2 (expanded)

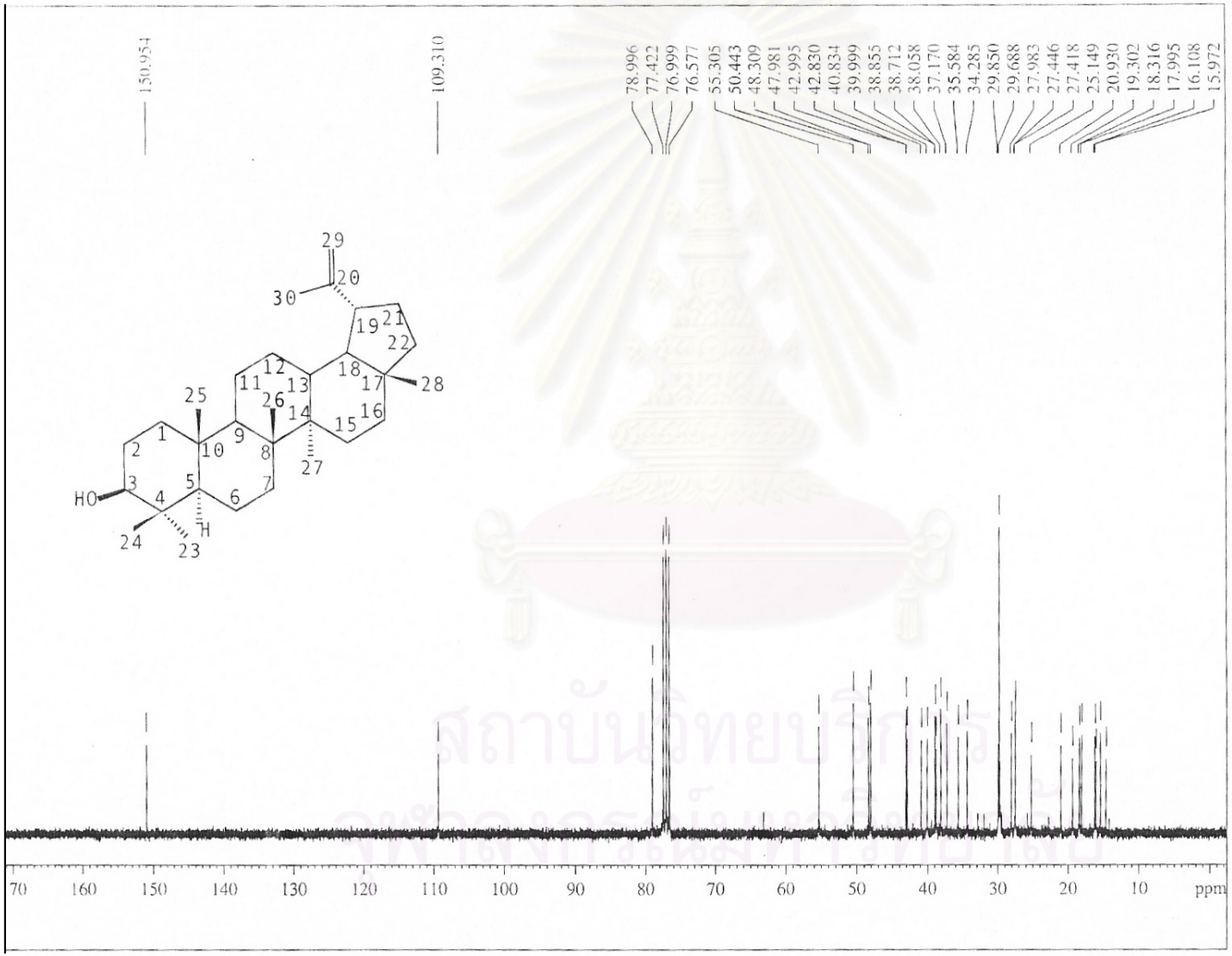


Figure 14a. The 75 MHz ^{13}C-NMR spectrum of compound HA-2 (in $CDCl_3$)

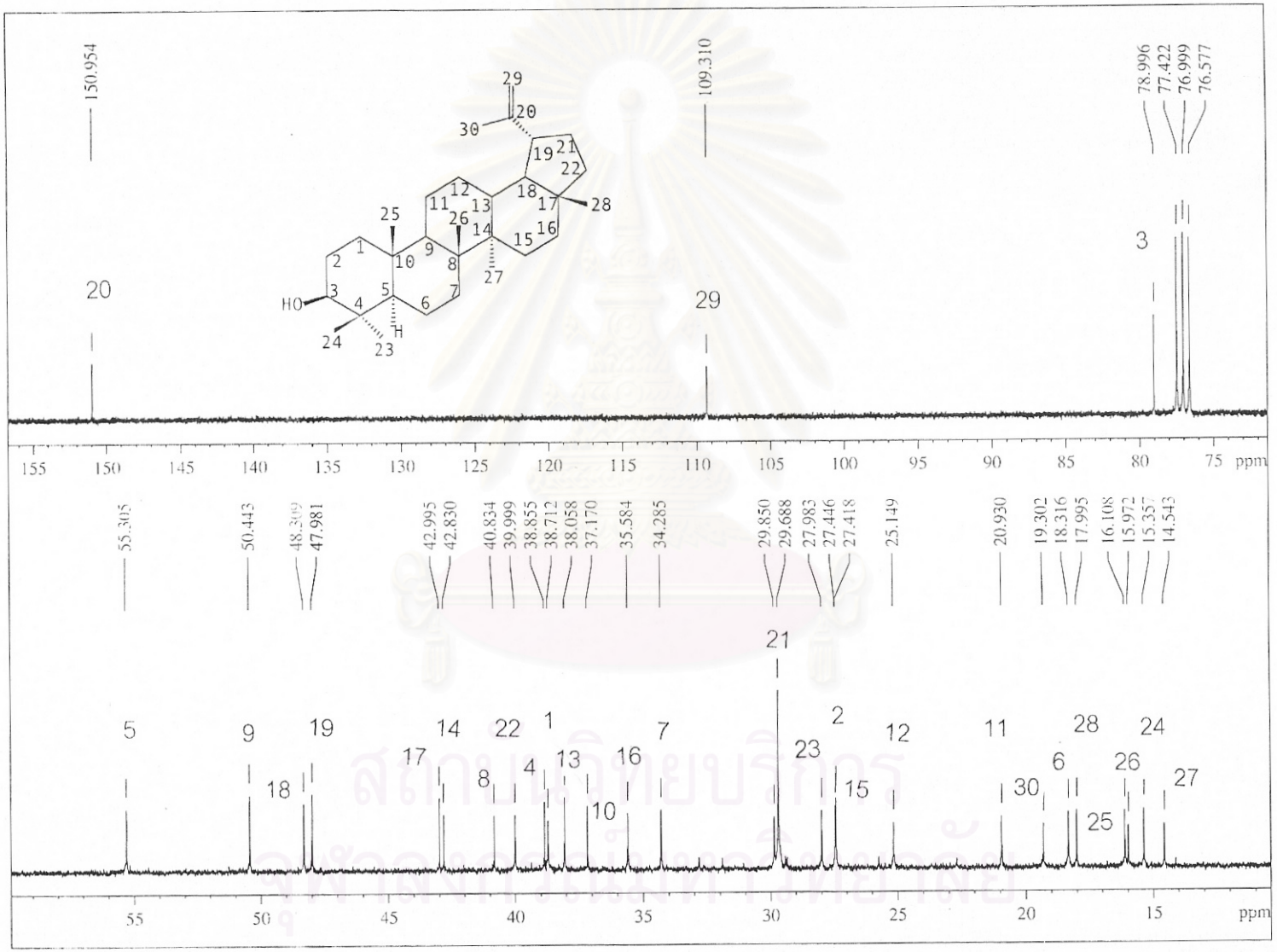


Figure 14b. The 75 MHz ^{13}C-NMR spectrum of compound HA-2 (expanded)

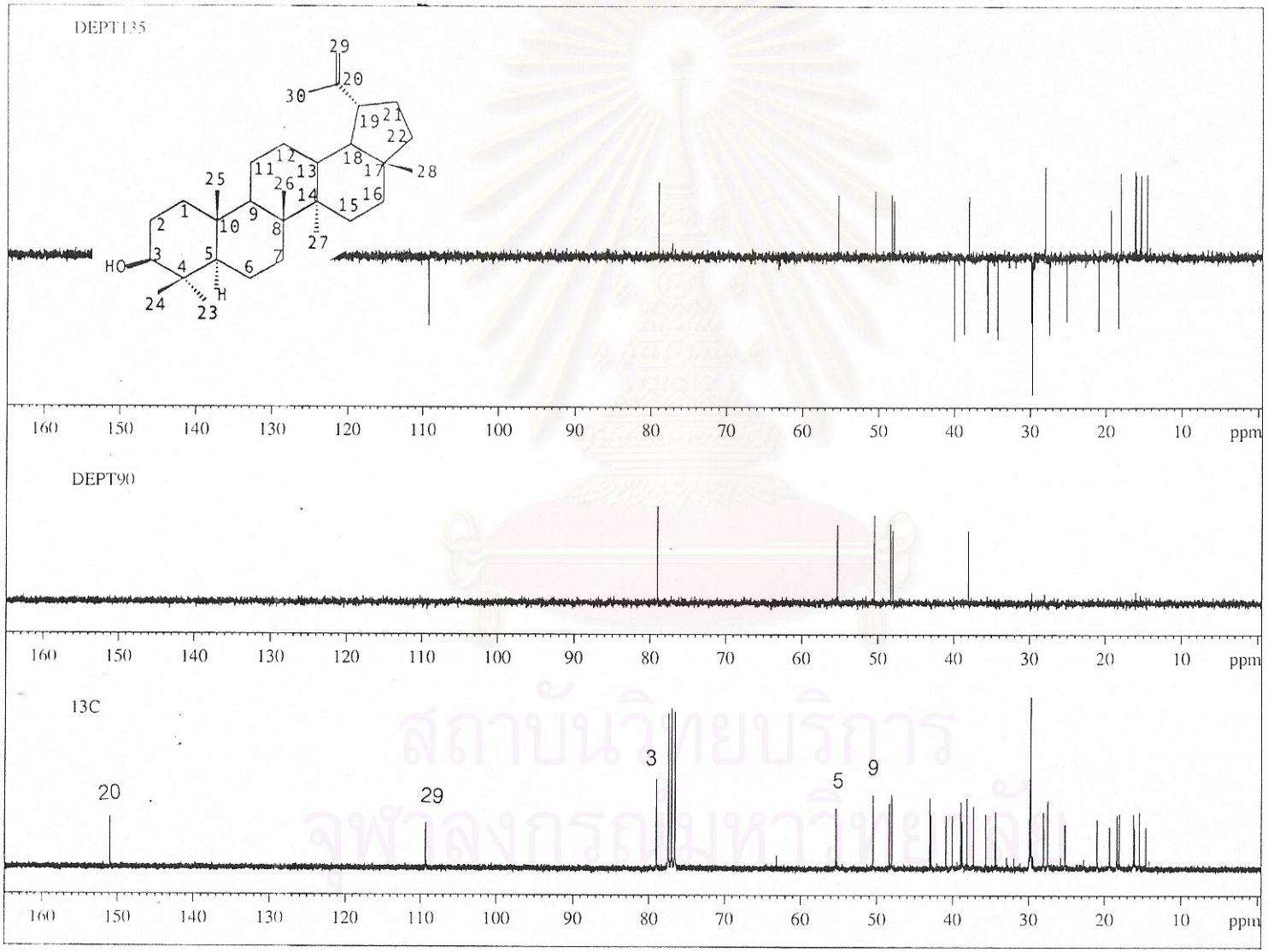


Figure 15a. The 75 MHz ^{13}C-DEPT NMR spectrum of compound HA-2 (in $CDCl_3$)

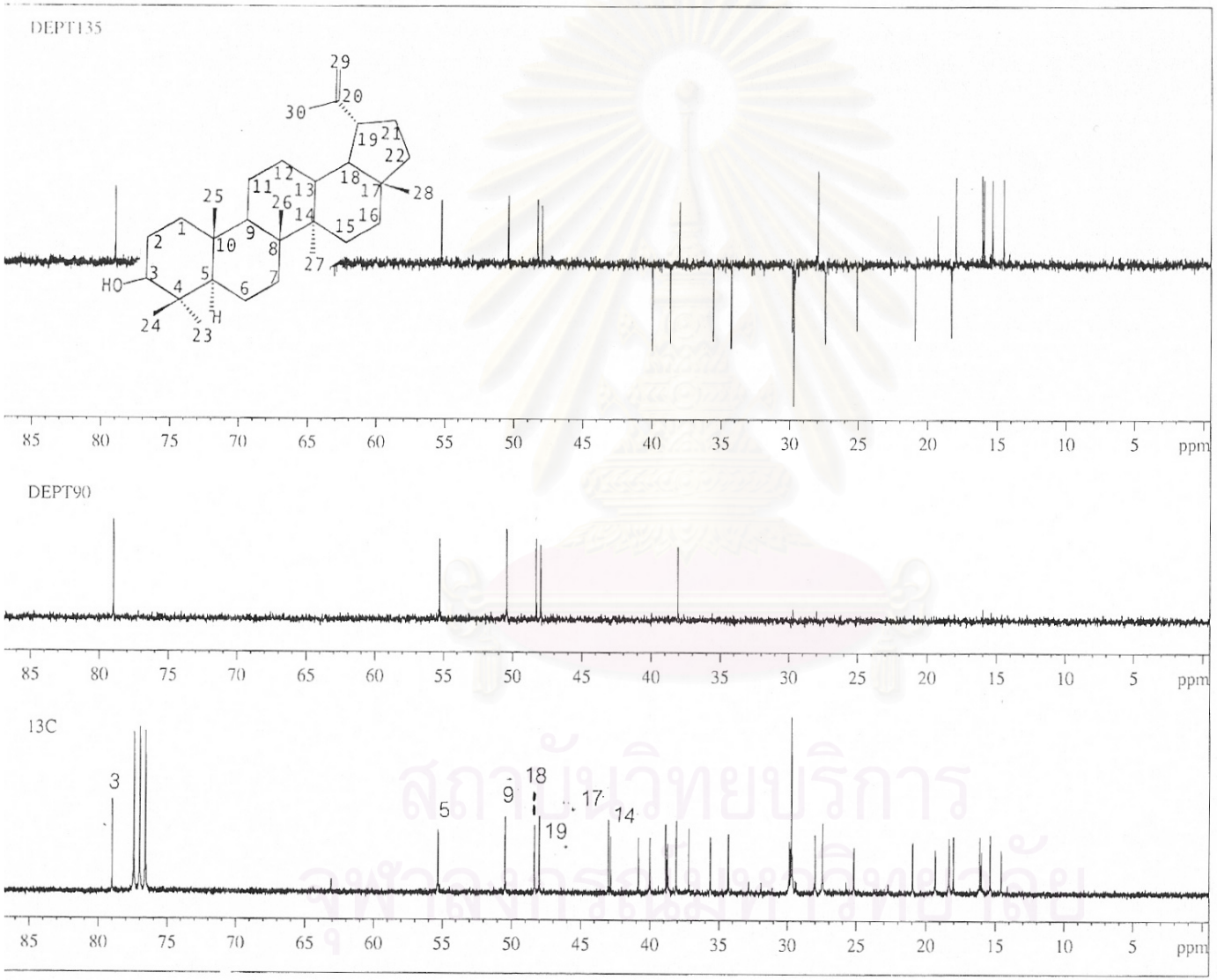


Figure 15b. The 75 MHz ^{13}C-DEPT NMR spectrum of compound HA-2 (expanded)

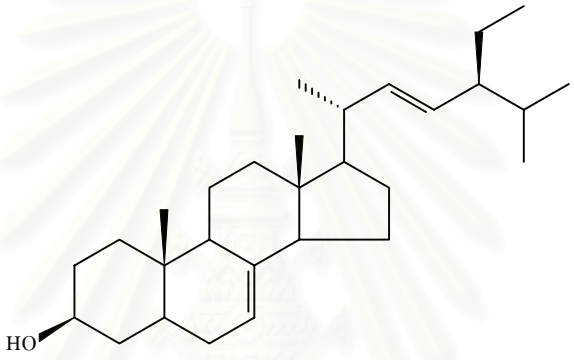
3. Structure elucidatin of HA-3

Compound HA-3 was purified by recrystallization from methanol (28.2 mg, 0.003 % yield). The compound gave a deep green color with the Libermann-Burchard test, suggesting that it is a steroid. The EIMS of HA-3 (Figure 16) showed the molecular ion (M^+) peak at *m/z* 412, corresponding to the molecular formula of $C_{29}H_{42}O$. Two fragment ions at *m/z* 273 and 300 implied the structure of a Δ^{22} sterol with a nuclear double bond (Nes *et al*, 1976; Ikram *et al.*,1987). These fragments could be explained as resulting from cleavage of the side chain at C-17 and C-20, respectively. The base peak at *m/z* 271 was produced by the loss of 2H from the fragment ion at *m/z* 273 while the peak at *m/z* 255 from the loss of water (Scheme 5).

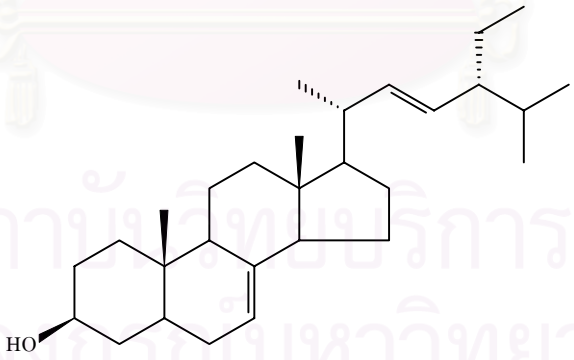
The ^{1}H-NMR spectrum of HA-3 (Figures 18a-18c) displayed a singlet of one tertiary methyl at δ 0.53, doublets of two secondary methyls at δ 0.83 and 1.01 and overlapping signals in the range of δ 0.76 – 0.81 due to three methyls (9H), including one primary (δ 0.79; *t*; *J*=7.5 Hz), one secondary (δ 0.78; *d*; *J*=6.1 Hz) and one tertiary (δ 0.78; *s*) methyl. The signal of one hydroxymethine proton could be observed at δ 3.58 (*tt*; *J*=8.8,4.6 Hz). The signals due to three vinylic protons appearing of δ 5.01 (1H; *dd*; *J*=15.3,8.5 Hz) and 5.14 (2H; *m*), which showed intercorrelation in the COSY spectrum (Figures 22a-22c), supported the presence of the disubstituted Δ^{22} double bond and indicated that the other double bond in the molecule is tribsubstituted.

The ^{13}C-NMR spectrum (Figure 19) showed signals of 29 carbon atoms, supporting the assignment of HA-3 as a steroid derivative. The DEPT and HETCOR experiments (Figures 20a-20b and 21a-21b) displayed signals for six methyl carbons at δ 12.1, 12.3, 13.0, 19.0, 21.1 and 21.4, nine methylene carbons at δ 21.5, 23.0, 25.4, 28.5, 29.6, 31.5, 37.1, 38.0 and 39.5, eleven methine carbons at δ 31.9, 40.3, 40.8, 49.4, 51.2, 55.1, 55.9, 71.1, 117.5, 129.4 and 138.2, and three quaternary carbons at δ 34.2, 43.3 and 139.6.

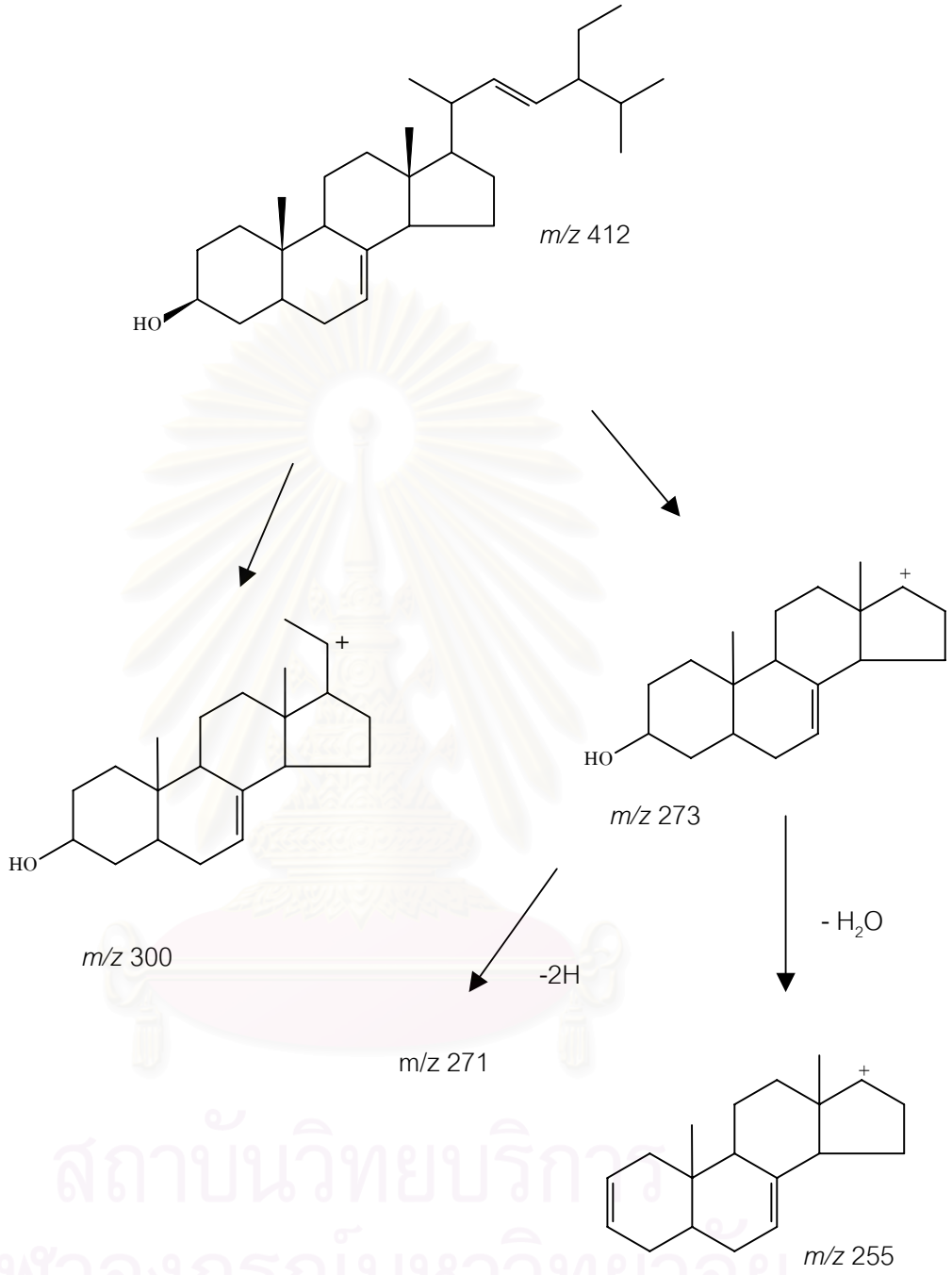
From the above information, HA-3 was proposed to be a C_{29}-Δ^{22} sterol with a trisubstituted double bond in the steroid nucleus. By comparing the NMR data of the compound with those of literature values, HA-3 was suggested to be either α-spinasterol (24S-ethylcholesta-7,22-dien-3β-ol) or its 24R epimer, chondrillasterol. Comparison of ^{1}H-NMR and ^{13}C-NMR data of HA-3 with those of α-spinasterol and chondrillasterol (Iida *et al*., 1979; Akihisa *et al*., 1986) is shown in Tables 14 and 15, respectively.



α- Spinasterol (**108**)



Chondrillasterol (**109**)



Scheme 5. Mass fragmentation of compound HA-3

Table 14. ^{1}H-NMR assignments of compound HA-3 (500 MHz; in $CDCl_3$) and reported data of α-spinasterol (270 MHz; in $CDCl_3$) and chondrillasterol (270 MHz; in $CDCl_3$).

Position	δH		
	Compound HA-3	α-Spinasterol	Chondrillasterol
3	3.58 (*tt*, *J*=8.8,4.6 Hz)		
7	5.14 (*t*, *J*=11.9 Hz)		
18	0.53 (*s*)	0.55 (*s*)	0.54 (*s*)
19	0.78 (*s*)	0.81 (*s*)	0.81 (*s*)
21	1.01 (*d*,*J*=6.7 Hz)	1.03 (*d*,*J*=6.6 Hz)	1.03 (*d*,*J*=6.5 Hz)
22	5.14 (*dd*,*J*=15.2,8.7 Hz)		
23	5.01 (*dd*,*J*=15.2,8.5 Hz)		
26	0.83 (*d*,*J*=6.4 Hz)	0.85 (*d*,*J*=6.5 Hz)	0.84 (*d*,*J*=6.5 Hz)
27	0.78 (*d*,*J*=6.1 Hz)	0.80 (*d*,*J*=7.0 Hz)	0.79 (*d*,*J*=7.0 Hz)
29	0.79 (*t*,*J*=7.5 Hz)	0.81 (*t*,*J*=6.5 Hz)	0.81 (*t*,*J*=7.2 Hz)

Table 15. ^{13}C-NMR assingments of compound HA-3 (500 MHz; in $CDCl_3$) and reported data of α-spinasterol (270 MHz; in $CDCl_3$) and chondrillasterol (270 MHz; in $CDCl_3$).

Position	δC		
	Compound HA-3	α-Spinasterol	Chondrillasterol
1	37.1	37.2	37.2
2	31.5	31.6	31.7
3	71.1	71.1	71.1

Table 15. ^{13}C-NMR assingments of compound HA-3 (500 MHz; in $CDCl_3$) and reported data of α-spinasterol (270 MHz; in $CDCl_3$) and chondrillasterol (270 MHz; in $CDCl_3$).

Position	δC		
	Compound HA-3	α-Spinasterol	Chondrillasterol
4	38.0	38.1	38.1
5	40.3	40.4	40.4
6	29.6	29.7	29.7
7	117.5	117.5	117.5
8	139.6	139.6	139.6
9	49.4	49.6	49.6
10	34.2	34.3	34.3
11	21.5	21.6	21.6
12	39.5	39.6	39.6
13	43.3	43.3	43.3
14	55.1	55.2	55.2
15	23.0	23.1	23.1
16	28.5	28.5	28.4
17	55.9	56.0	56.0
18	12.1	12.1	12.1
19	13.0	13.1	13.1
20	40.8	40.8	40.8
21	21.1	21.3	19.0
22	138.2	138.2	138.1
23	129.4	129.5	129.6
24	51.2	51.3	51.3
25	31.9	32.0	32.0

Table 15. ^{13}C-NMR assingments of compound HA-3 (500 MHz; in $CDCl_3$) and reported data of α-spinasterol (270 MHz; in $CDCl_3$) and chondrillasterol (270 MHz; in $CDCl_3$).

Position	δC		
	Compound HA-3	α-Spinasterol	Chondrillasterol
26	21.4	21.1	20.9
27	19.0	19.0	21.3
28	25.4	25.5	25.5
29	12.3	12.3	12.5

The NMR data of α-spinasterol and chondrillasterol were very similar; however, significant differences in the ^{1}H-NMR spectra could be observed in the C-26, C-27 and C-29 methyl proton region (ca δ 0.75 – 0.90) when the spectra were measured at 270 MHz (Iida *et al*., 1979). The doublets due to the C-26 and C-27 methyl protons of α-spinasterol appeared at higher field while the triplet due to the C-29 methyl proton at lower field than the corresponding methyl signals of chondrillasterol, causing different spectral patterns which can be used as diagnostic feature in identifying the two isomers. Comparison of such spectral pattern of HA-3, measured at 300 MHz, with those of α-spinasterol and chondrillasterol (Iida *et al*., 1999), as shown in Figure 23, indicated the agreement between HA-3 and α-spinasterol.

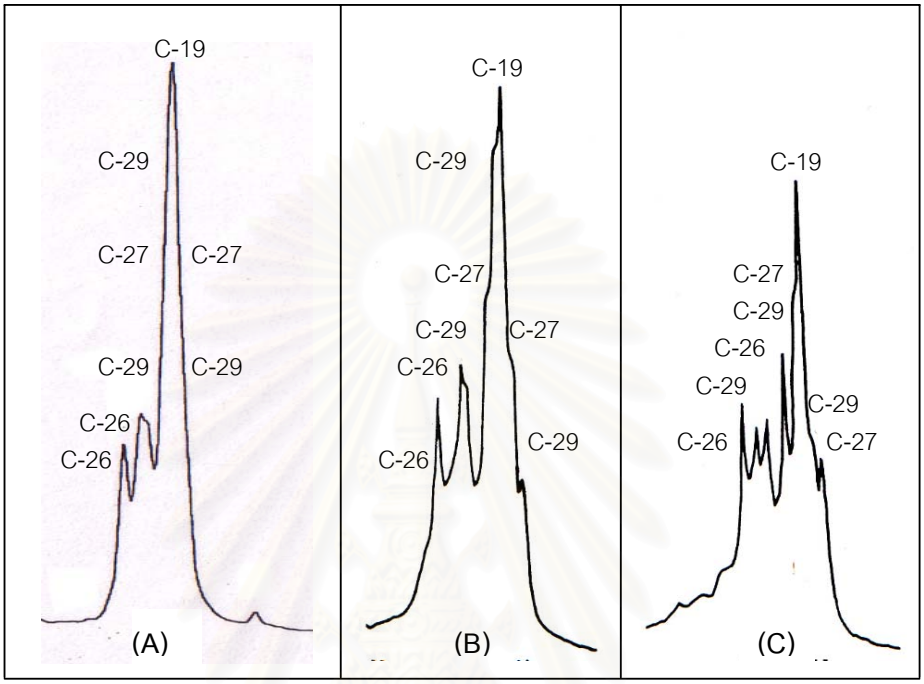
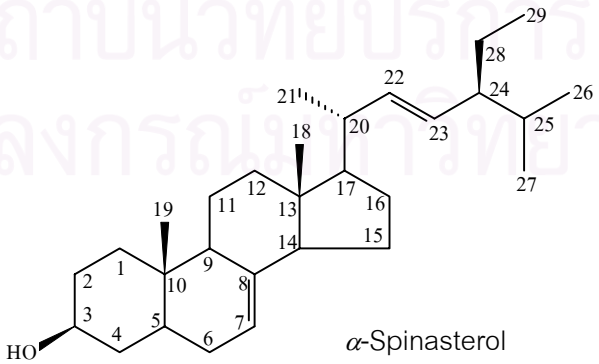


Figure 23. ^{1}H-NMR spectral patterns of the C-26, C-27 and C-29 methyl signals of (A) HA-3 (B) α-spinasterol and (C) chondrillasterol.

Therefore, it was concluded that HA-3 is the known Δ^{22} –24 ethyl steroid with 24 S configuration, α-spinasterol.



α-Spinasterol

α-Spinasterol is widely found in higher plants (Iida *et al*, 1979), including *Saponaria officinalis* (Henry and Chantalat-Dublanche, 1985), *Lagenaria leucantha* var. *gourda*, *Citrullus battich* (Itoh *et al*., 1981), *Phytolacca americana* (Woo, 1974). This compound has never been previously reported as a constituent of plants in the family Sapindaceae.

α-Spinasterol has been reported as possessing anti-inflammatory (Frotan *et al*, 1983; Zhou *et al*., 1985; EI-Sawu, Hashem, and Biuomy, 2003), antipyretic (Ikram *et al*., 1987) and antigenotoxic (Irene *et al*., 1996) activities. It induced necrosis of primary roots and resulted the death of the plant and also inhibited growth of *Mucor racemosus*, and it showed weak cytotoxicity to two animal cancer cell lines (L 1210, K 562) (Mi, Hwan, and Sam, 1996).

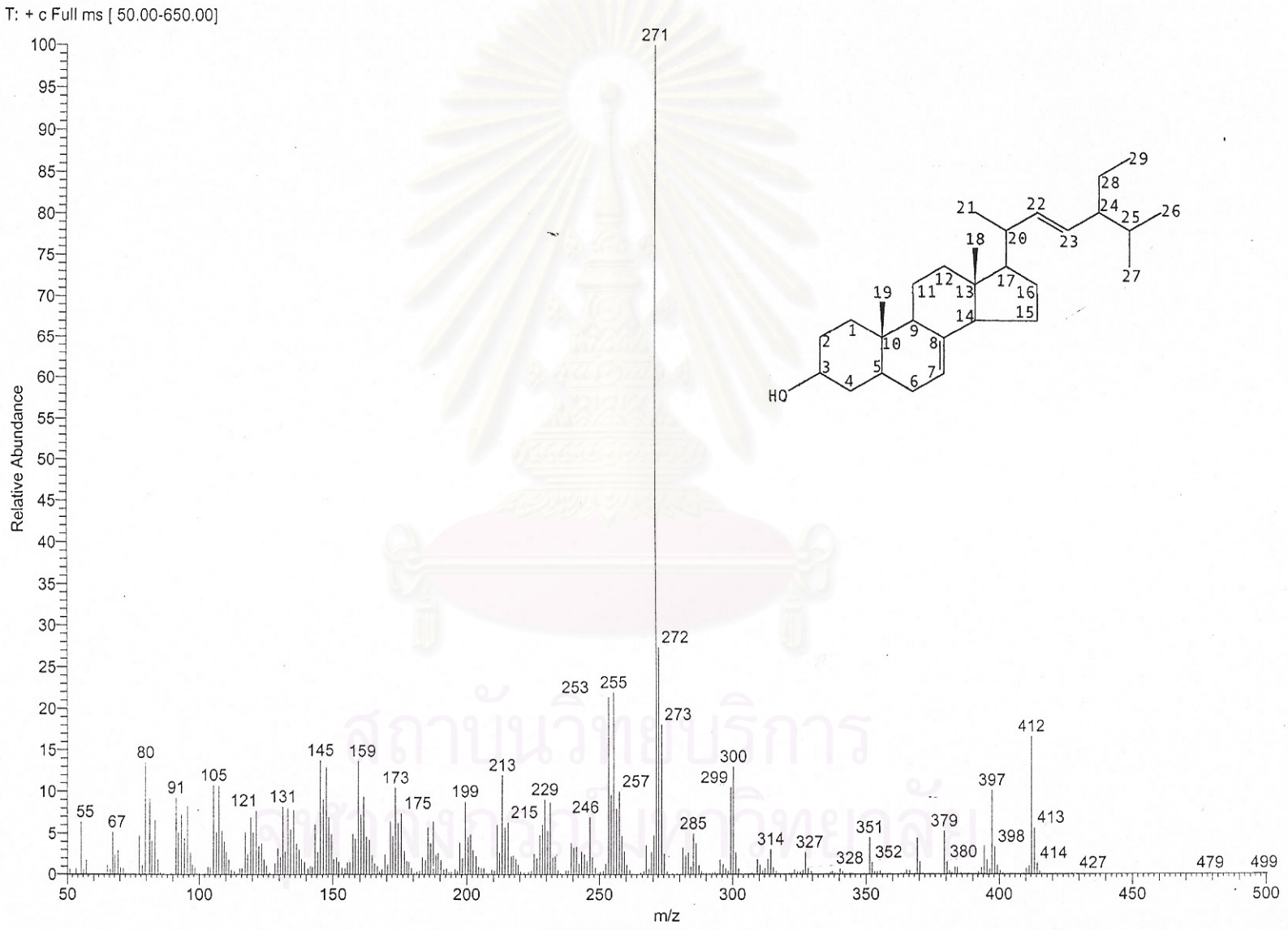


Figure 16. EIMS of compound HA-3

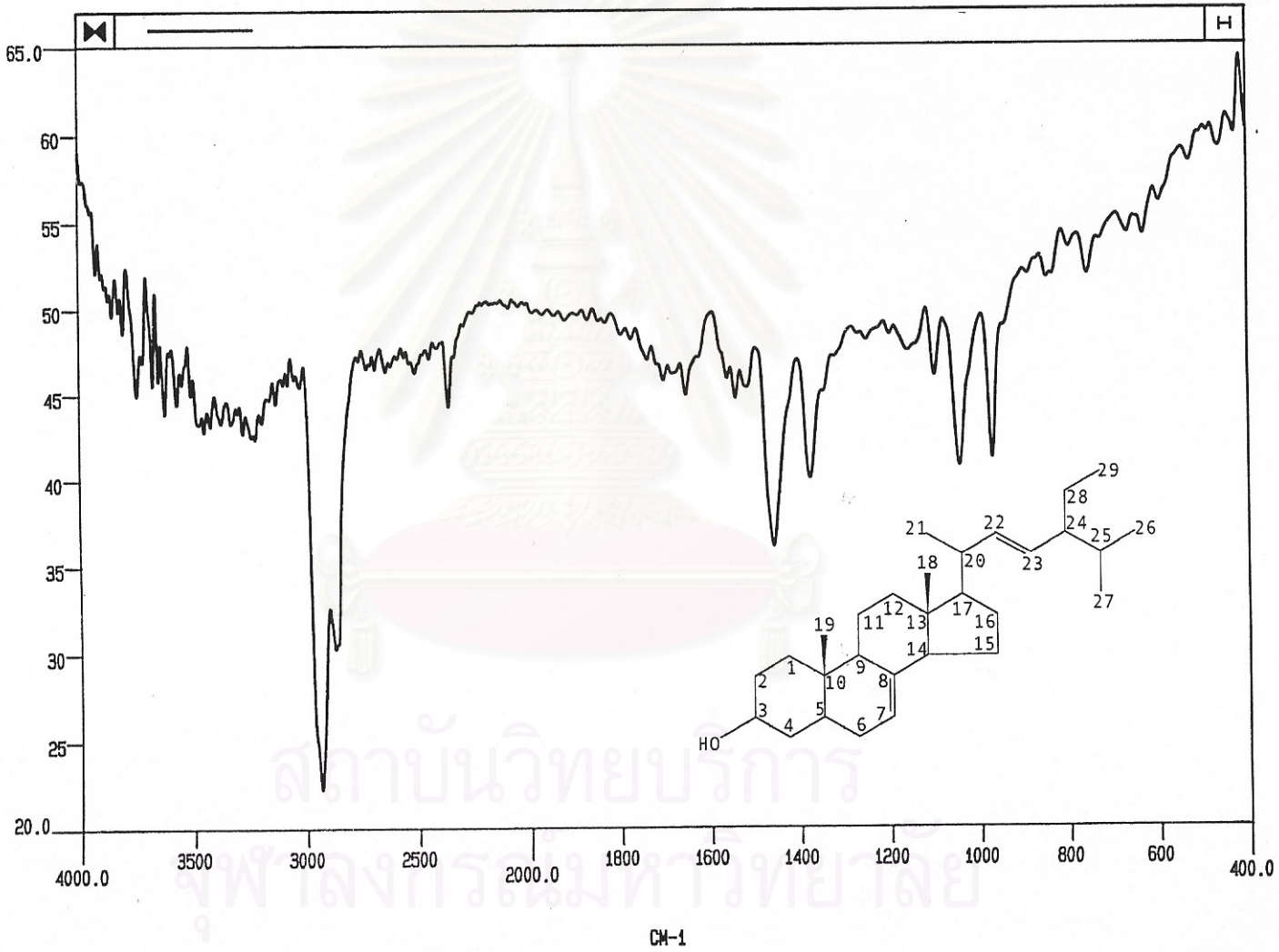


Figure 17. IR spectrum of compound HA-3

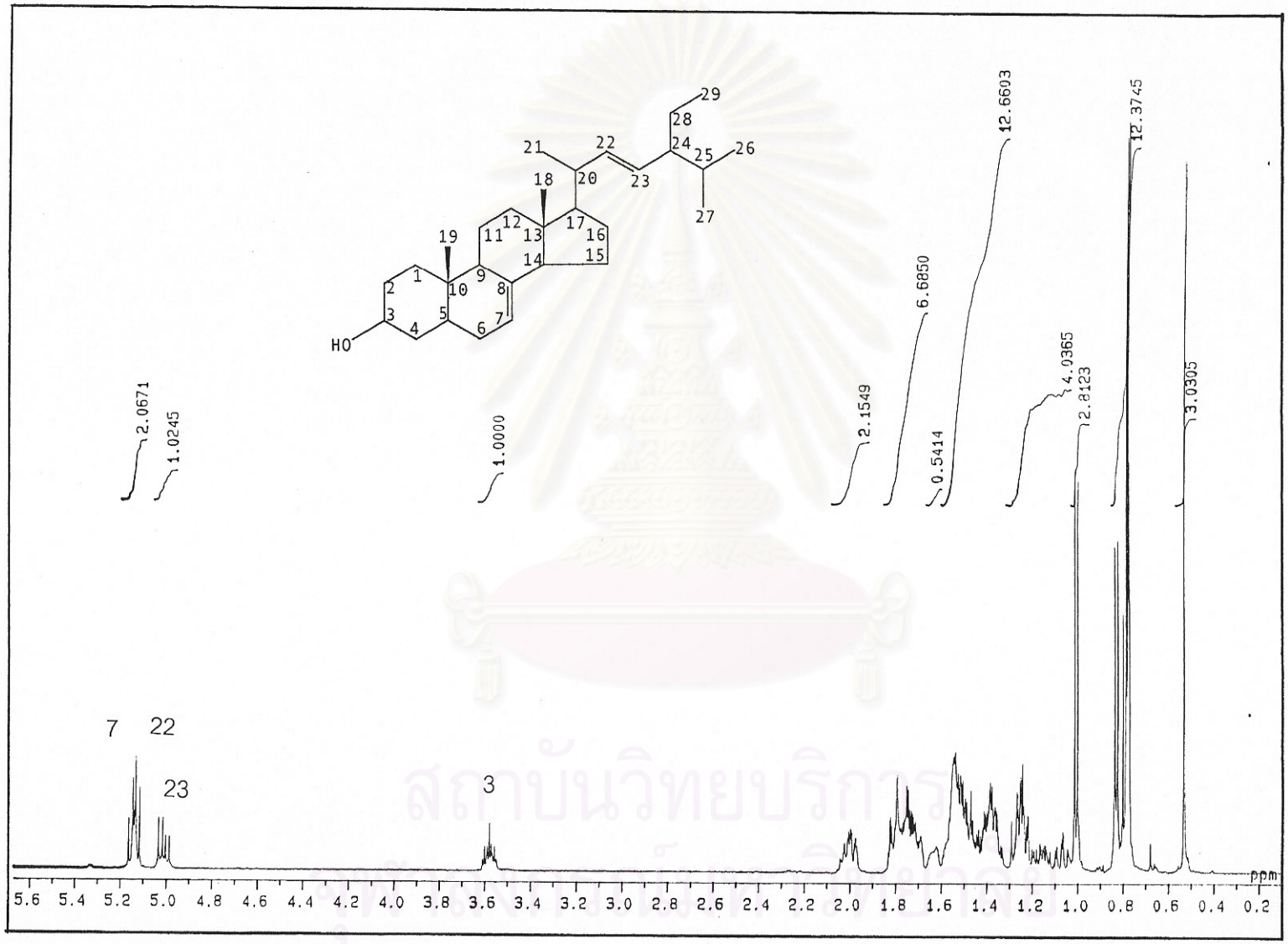


Figure 18a. The 500 MHz ^{1}H-NMR spectrum of compound HA-3 (in $CDCl_3$)

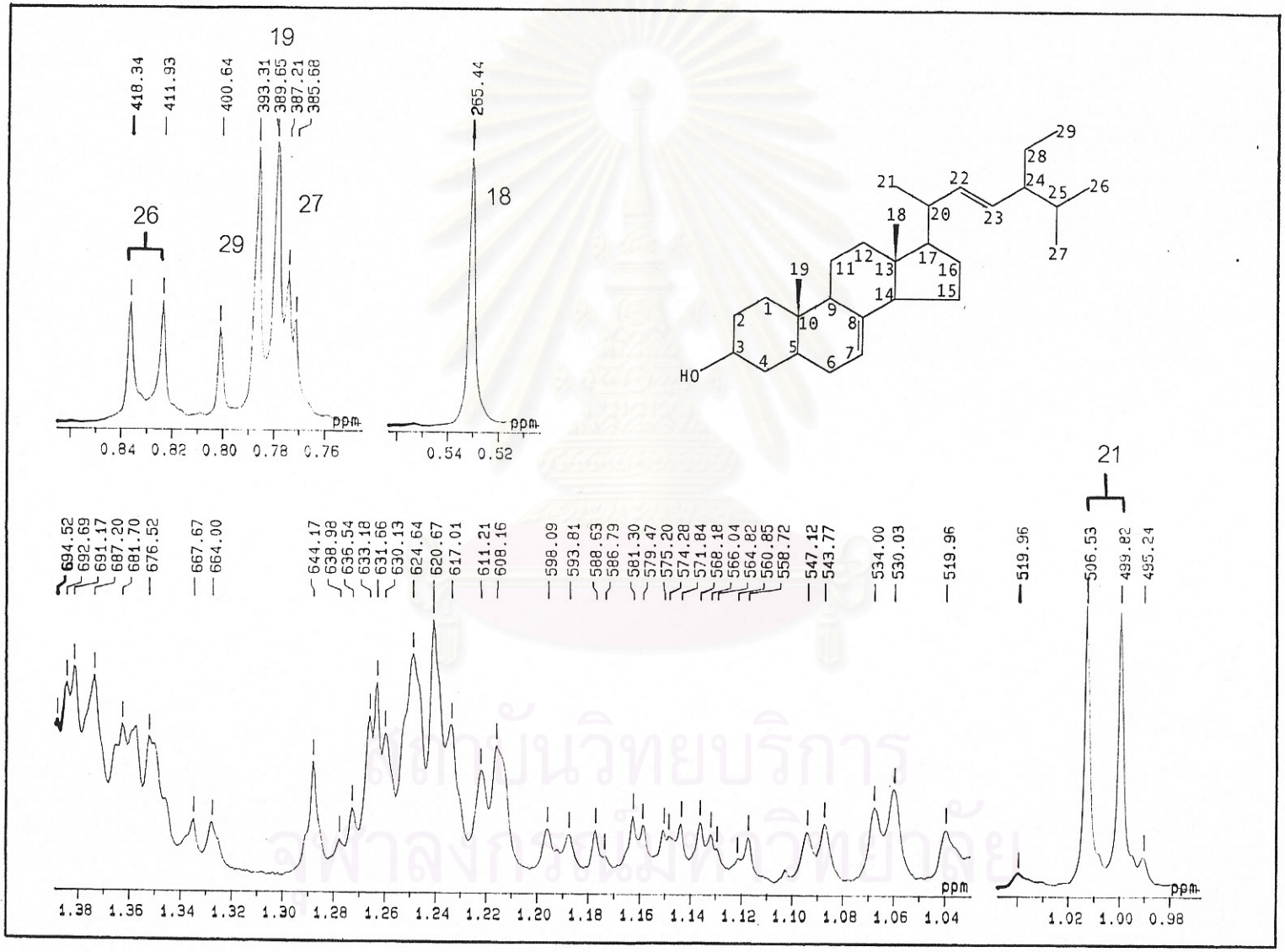


Figure 18b. The 500 MHz ^{1}H-NMR spectrum of compound HA-3 (expanded)

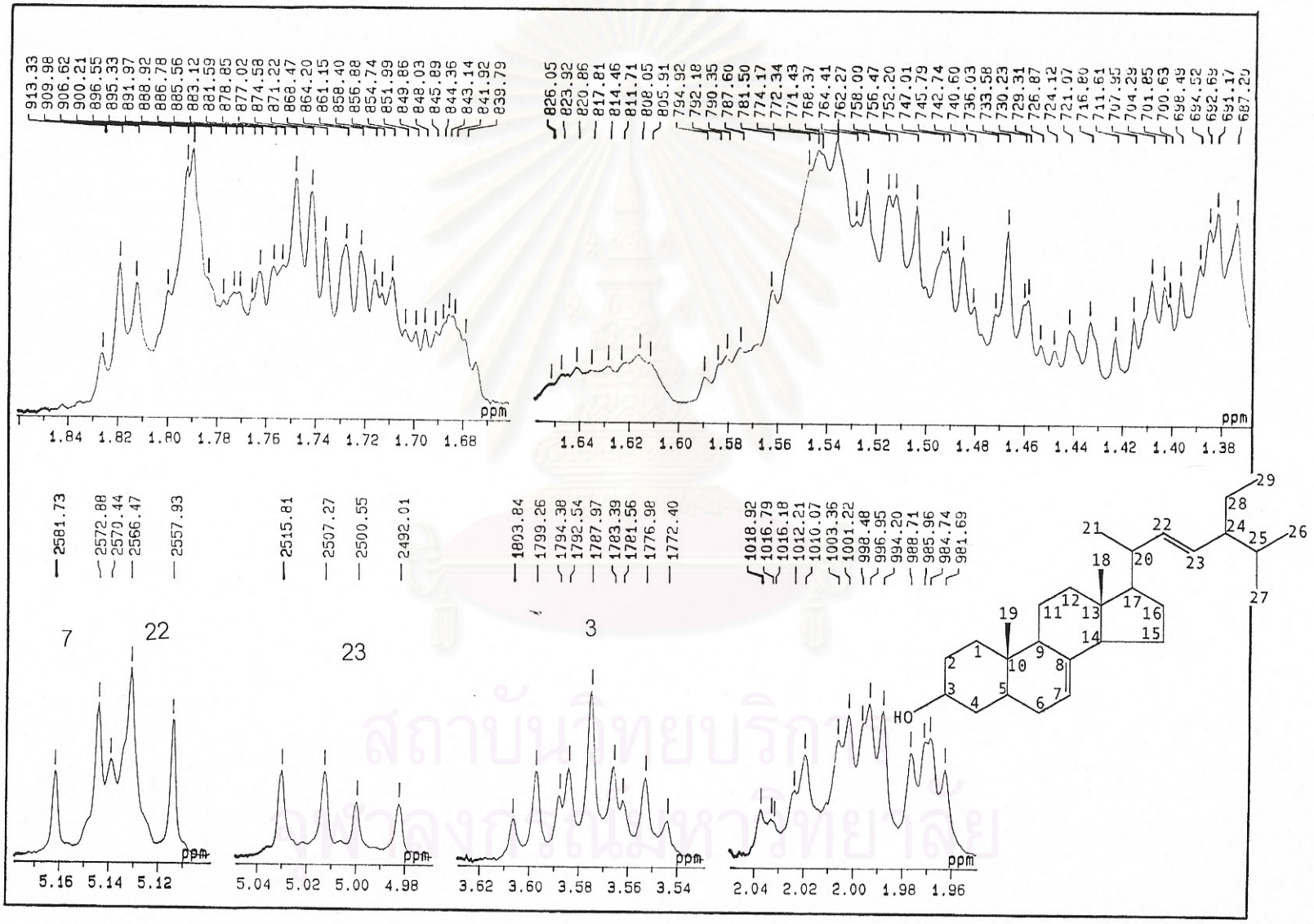


Figure 18c. The 500 MHz ^{1}H-NMR spectrum of compound HA-3 (expanded)

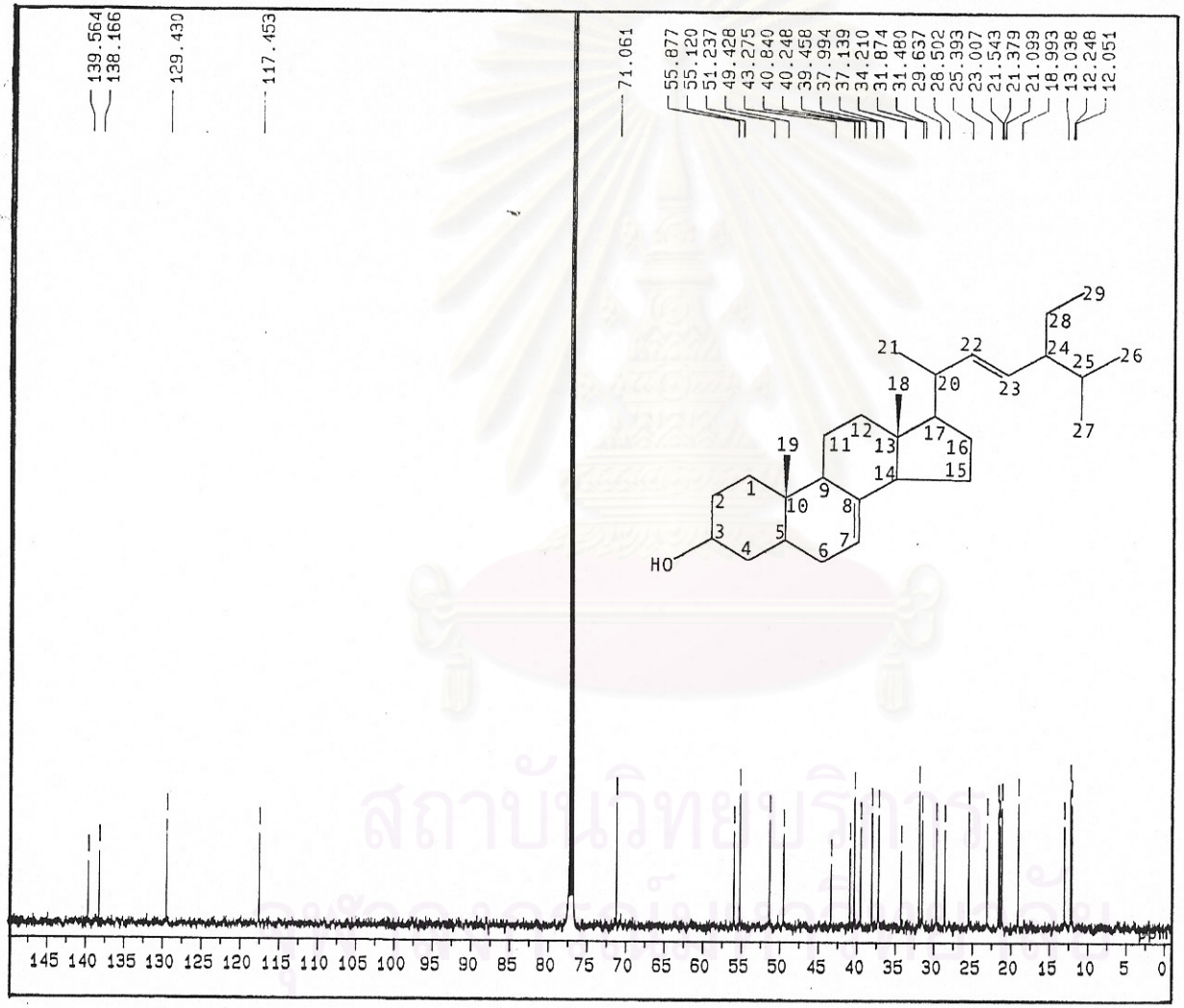


Figure 19. The 125 MHz ^{13}C-NMR spectrum of compound HA-3 (in $CDCl_3$)

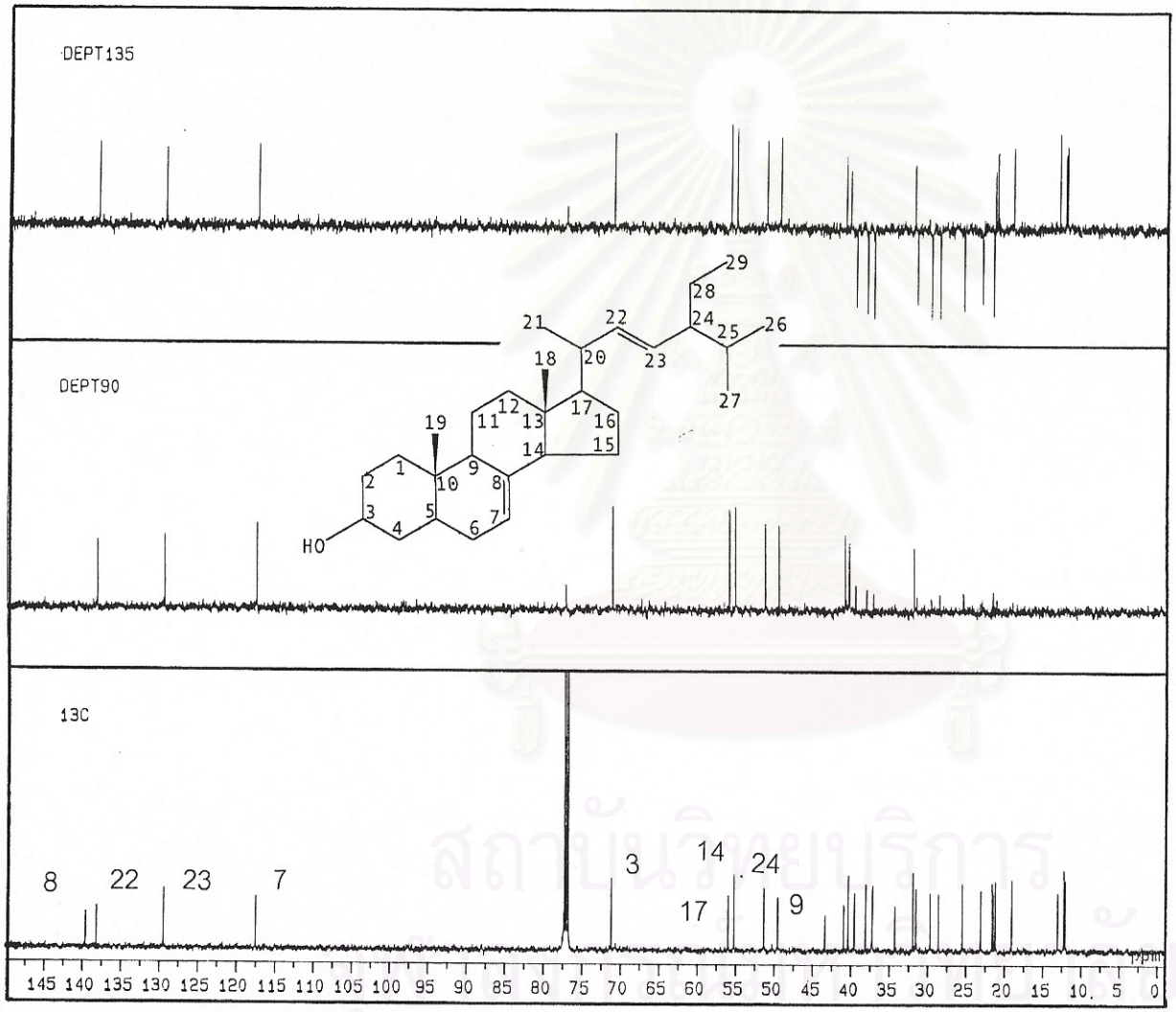


Figure 20a. The 125 MHz ^{13}C-DEPT NMR spectrum of compound HA-3 (in $CDCl_3$)

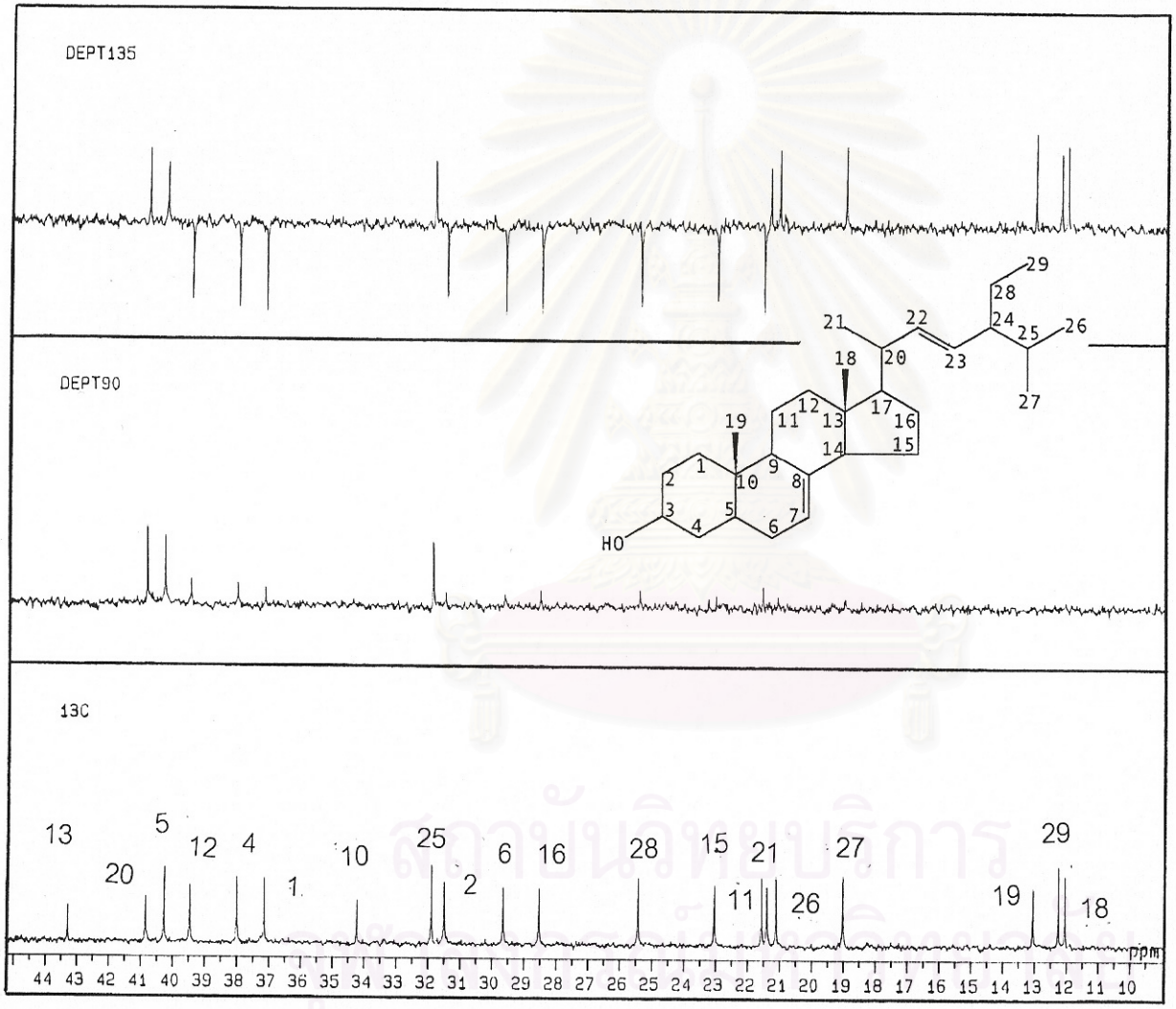


Figure 20b. The 125 MHz ^{13}C-DEPT NMR spectrum of compound HA-3 (expanded)

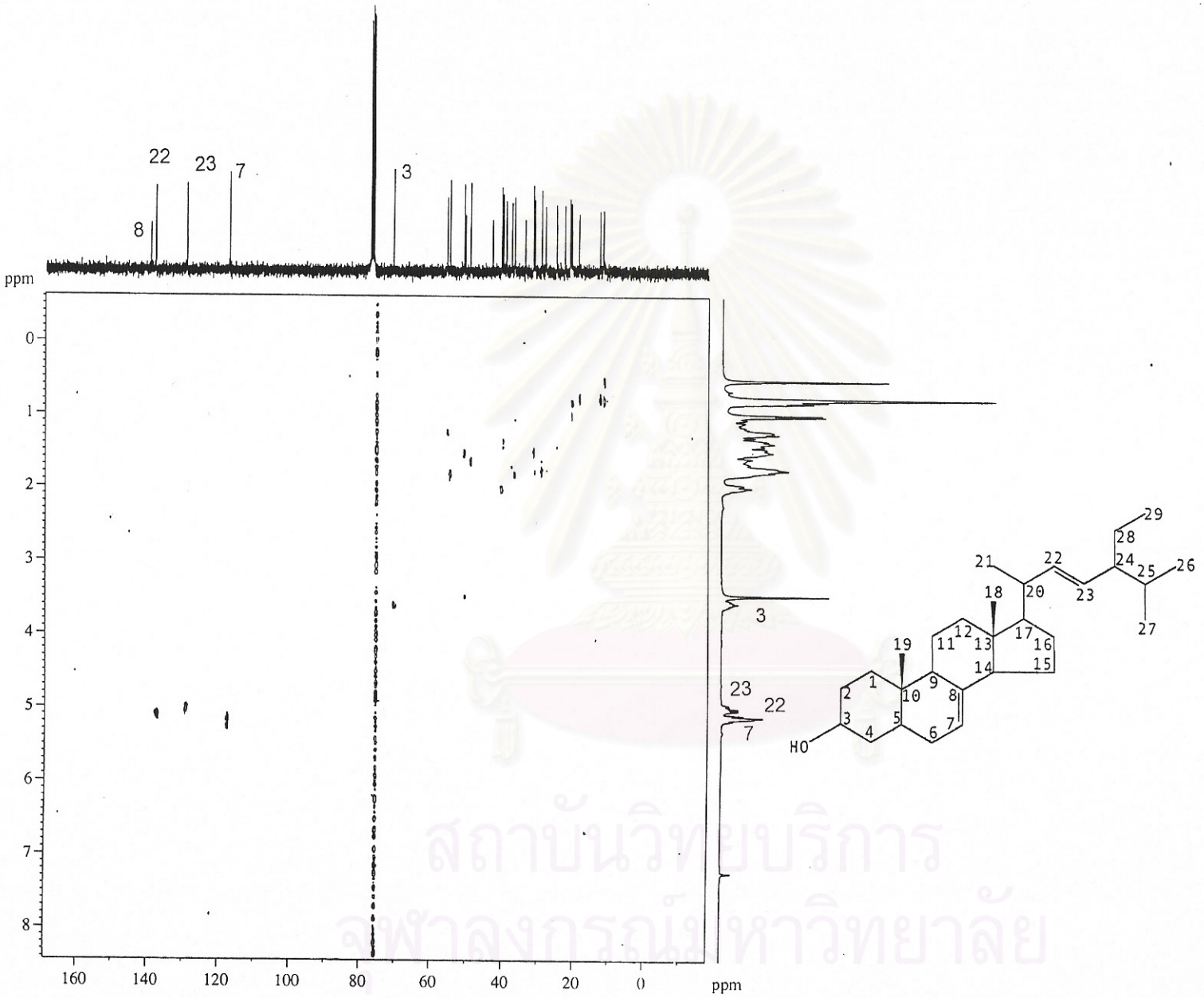


Figure 21a. The 75 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-3 (in $CDCl_3$)

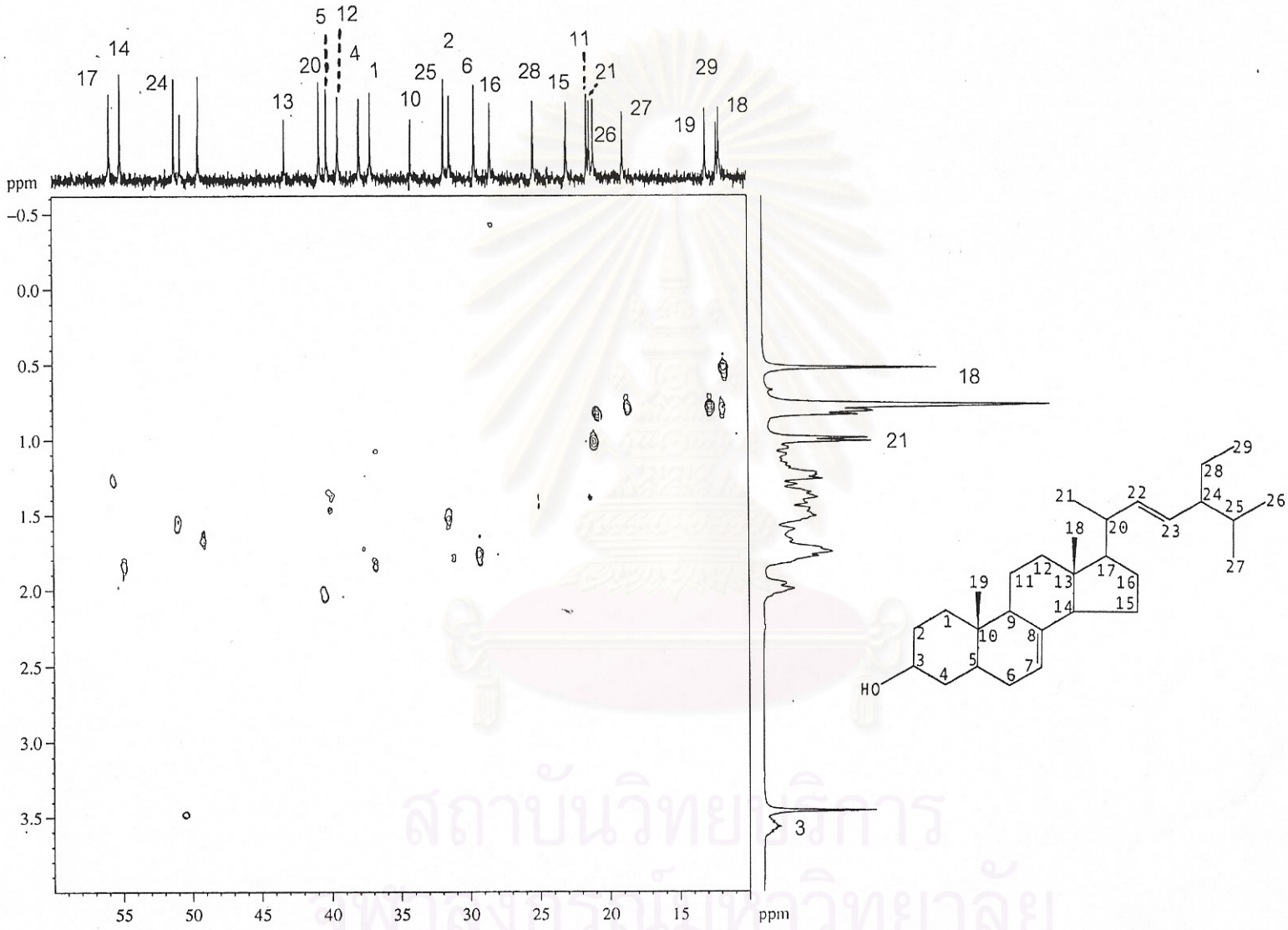


Figure 21b. The 75 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-3 (expanded)

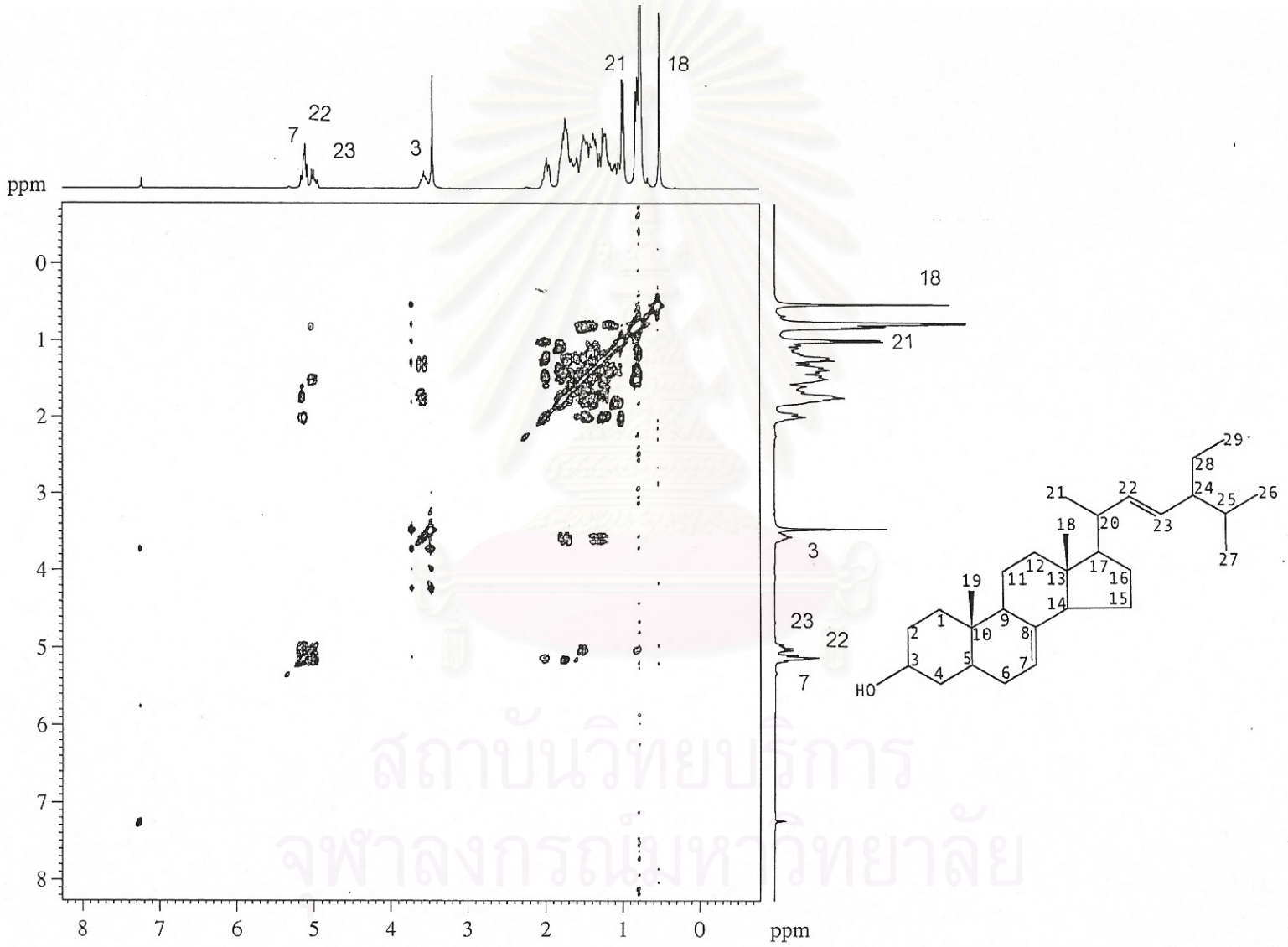


Figure 22a. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-3 (in $CDCl_3$)

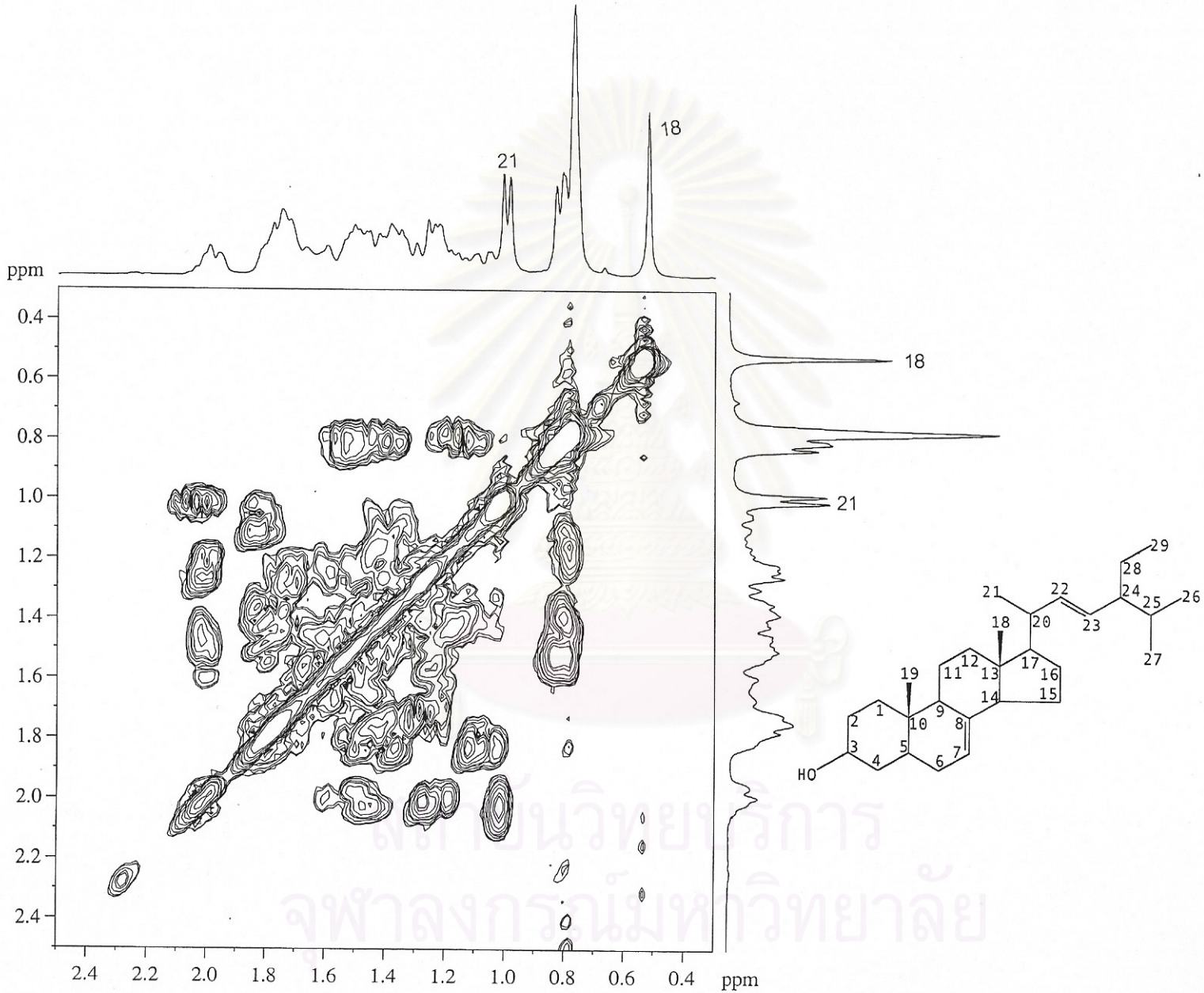


Figure 22b. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-3 (expanded)

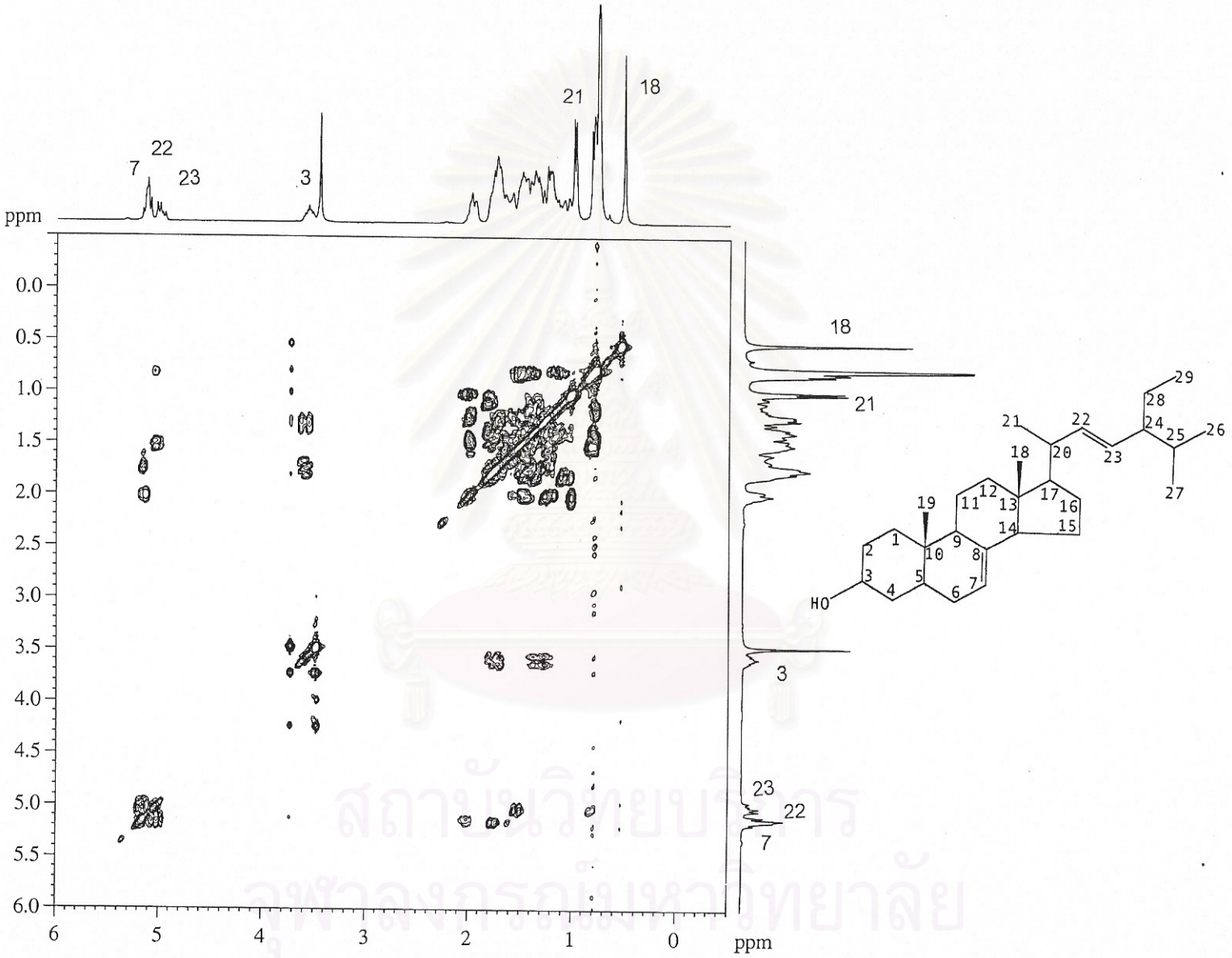


Figure 22c. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-3 (expanded)

4. Structure elucidation of HA-4

Compound HA-4 was recrystallized from methanol as colorless crystals (380.6 mg, 0.035% yield). The IR spectrum of HA-4 (Figure 25) exhibited OH band at 3350 cm^{-1}. Its ^{1}H-NMR spectrum (Figures 26a–26b) displayed a three-proton singlet for a methoxy group at δ 3.30, a double doublet at δ 3.09 (*J*=9.5,3.1 Hz), a pair of quartet-like signals at δ 3.67 (*J*=3.7, 3.4, 3.4 Hz) and 3.85 (*J*=3.7, 3.4, 3.1 Hz), and a group of three one-proton signals at δ 3.25 – 3.45. Five doublets for hydroxyl groups were also observed at δ 4.26 (*J*=5.8 Hz), 4.39 (*J*= 4.3 Hz), 4.42 (*J*=4.6 Hz), 4.60 (*J*=3.7 Hz) and 4.62 (*J*=3.7 Hz). The ^{13}C-NMR spectrum (Figure 27) showed seven carbon signals, indicated by the DEPT (Figure 28) and HETCOR (Figures 29a-29b) experiments as those of six methine carbons at δ 68.2, 70.6, 72.2, 72.4, 73.5, 81.2 and one methyl carbon at δ 57.2. All this information suggested the structure of a pentahydroxylated monomethoxylated cyclohexane for HA-4. The molecular formula $C_7H_{14}O_6$ of the suggested structure was also supported by its EIMS (Figure 24) which showed $[M+1]^+$ at *m/z* 195.

Assignments of all the proton signals were achieved by the analysis of the ^{1}H-^{1}H COSY spectrum (Figures 30a-30c). The double doublet at δ 3.09, which was due to the methoxyl substituted methine proton as indicated by its correlation with the carbon resonance at δ 81.2 in the HETCOR spectrum, was used as the starting point. This proton signal displayed cross peaks with two signals at δ 3.85 and 3.36. The former at δ 3.85 was correlated to a signal at δ 3.67 which was moreover correlated to a signal at δ 3.42. The latter at δ 3.36 exhibited cross peak with a signal a δ 3.28 which was in turn correlated to the signal at δ 3.42. All these cross peak correlations led to the assignments of all the ring protons. The signals for hydroxyl groups could be readily assigned through their correlations with the ring proton signals.

The orientation of hydroxyl groups and a methoxyl group on the cyclohexane ring was determined on the basis of coupling constant analysis, and the result led to the

establishment of 2-*O*-methyl-chiro-inositol (quebrachitol) as the structure for HA-4. ^{1}H and ^{13}C NMR data (in DMSO-d_6) of HA-4 together with the reported data (in D_2O) of L-quebrachitol (Agrawal and Singh, 1994) are shown in Tables 16 and 17, respectively.

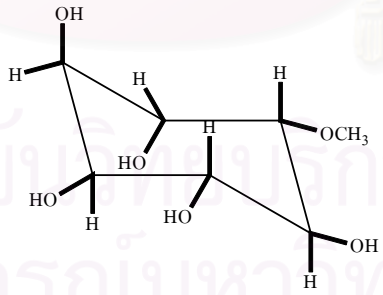
Table 16. ^{1}H-NMR data of compound HA-4 (in DMSO-d_6) and L-quebrachitol (in D_2O).

Position	δ H	
	Compound HA-4	L-quebrachitol
1	3.85 (*ddd, J*=3.7, 3.4, 3.1 Hz)	4.25 (*t*,*J*=3.2 Hz)
2	3.09 (*dd, J*=9.5,3.1 Hz)	3.39 (*dd*,*J*=9.5, 3.2 Hz)
3	3.36 (*ddd, J*=9.5, 9.2, 4.6 Hz)	3.62 (*t*,*J*=9.5 Hz)
4	3.28 (*ddd, J*=9.5, 9.2, 4.3 Hz)	3.60 (*t*,*J*=9.5 Hz)
5	3.42 (*ddd, J*=9.5, 5.8, 3.4 Hz)	3.74 (*dd*,*J*=9.5, 3.2 Hz)
6	3.67 (*ddd, J*=3.7, 3.4, 3.4 Hz)	4.05 (*t*,*J*=3.2, 3.0 Hz)
OCH_3	3.30 (*s*)	3.45 (*s*)
1-OH	4.60 (*d, J*=3.7 Hz)	-
3-OH	4.42 (*d, J*=4.6 Hz)	-
4-OH	4.39 (*d, J*=4.3 Hz)	-
5-OH	4.26 (*d, J*=5.8 Hz)	-
6-OH	4.62 (*d, J*=3.7 Hz)	-

Table 17. ^{13}C-NMR data of compound HA-4 (in DMSO-d_6) and L-quebrachitol (in D_2O).

Position	δ C	
	Compound HA-4	L-quebrachitol
1	68.2	67.8
2	81.2	80.7
3	72.4	71.9
4	73.5	73.0
5	70.6	70.1
6	72.2	71.8
OCH_3	57.2	56.9

Therefore, HA-4 was identified as quebrachitol, the structure of which is shown below.



Quebrachitol

Quebrachitol is a natural inositol methyl ether which can be found in a number of plants eg. *Aspidosperma quebracho* of the Apocynaceae (Schilling, Dittrich, and Kandler, 1972), *Hevea braziliensis* of the Euphorbiaceae (Schmatz *et al*., 1988), *Artemisia nilagirica* of the Compositae (Agrawal and Singh, 1994) and *Sapindus rarak* (Chung *et al*., 1997) as well as *Harpullia pendula* (Cherry *et al*., 1977) of the Sapindaceae. It was also detected in the protozoa *Eimeria tenella* (Schmatz *et al*., 1988). This compound was found to exhibit hypoglycemic effect in hyperglycemic situations and therefore may be useful in the treatment of diabetes mellitus type 2 (Musalmah *et al*., 2001).

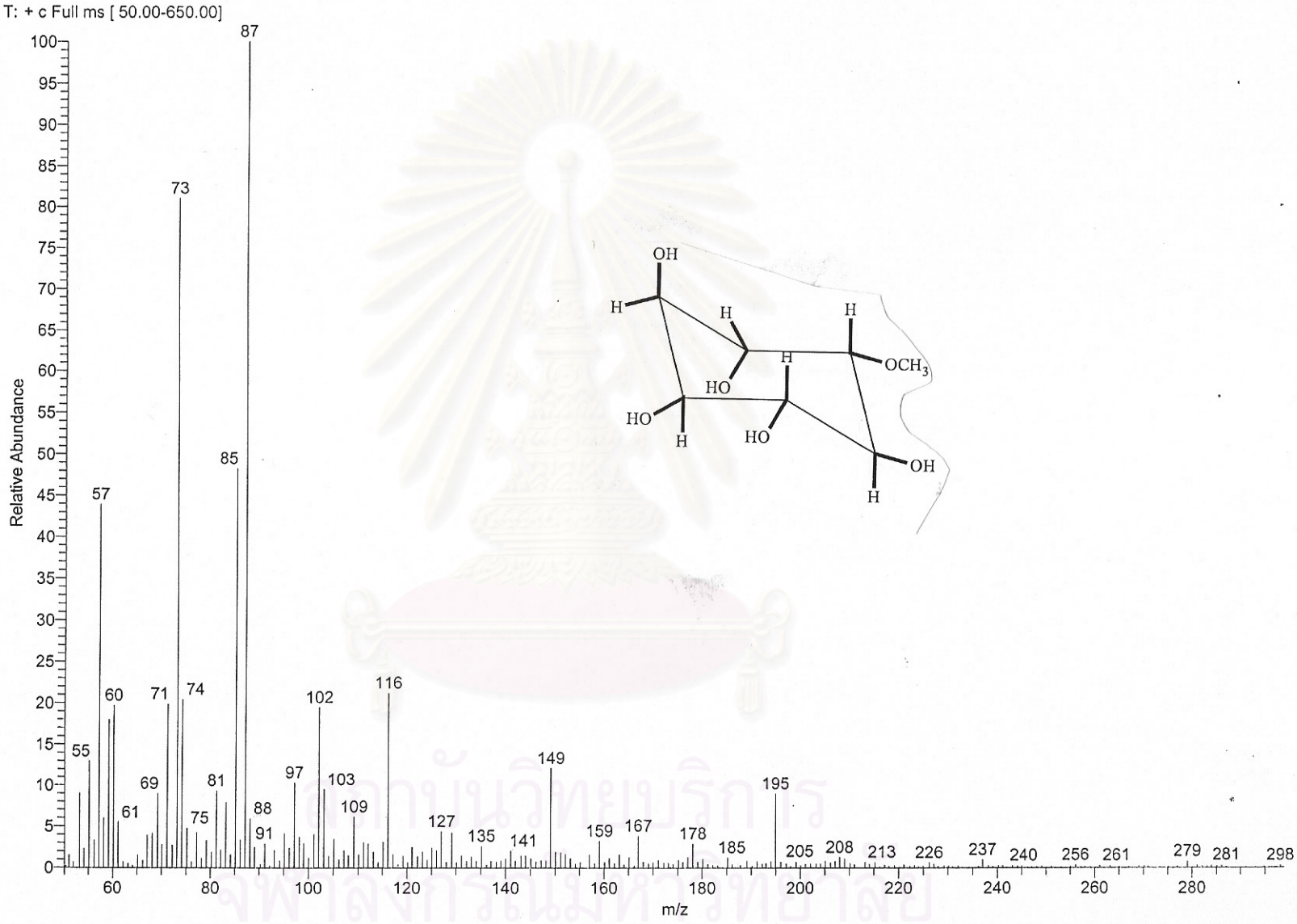


Figure 24. EIMS of compound HA-4

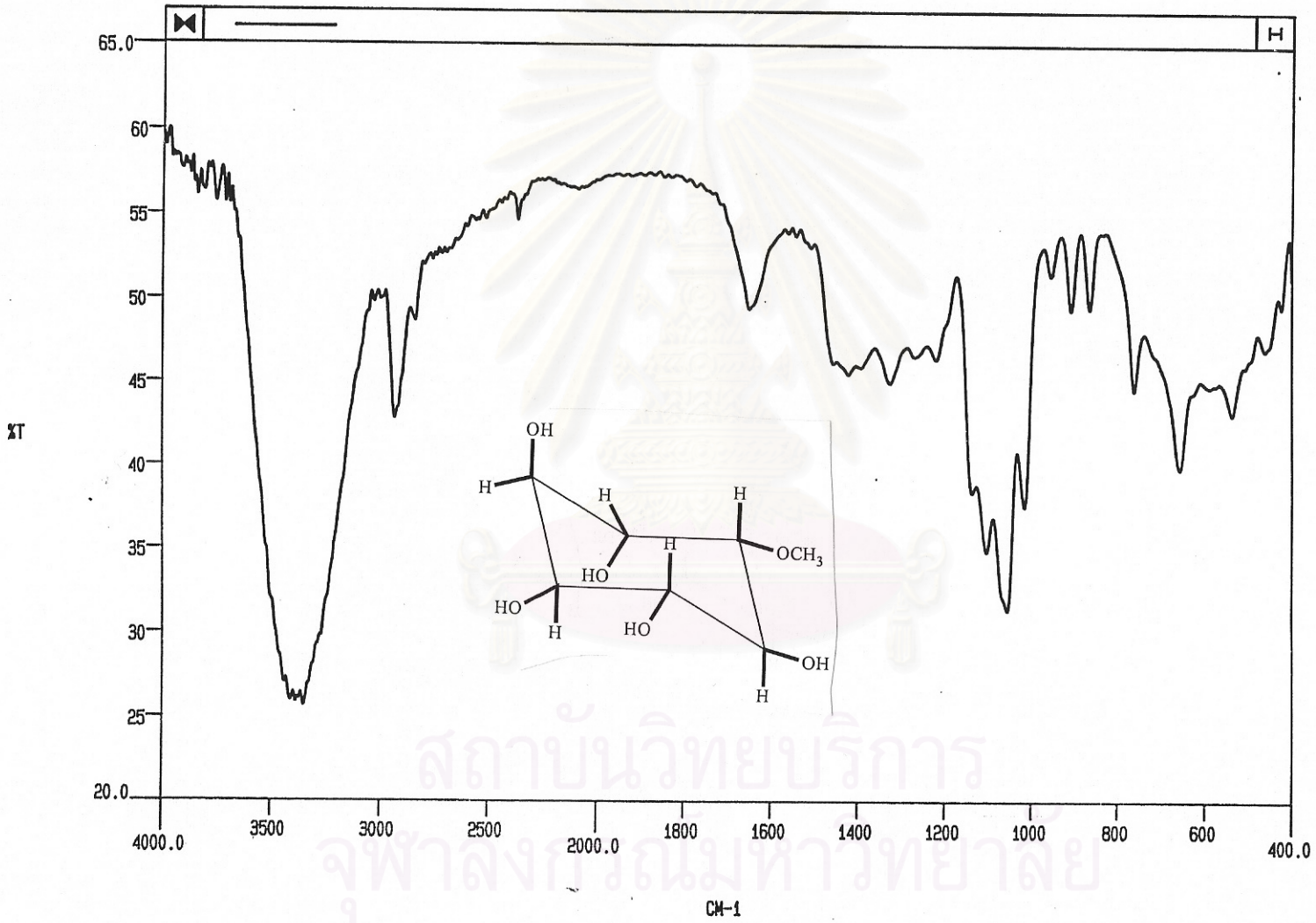


Figure 25. IR spectrum of compound HA-4

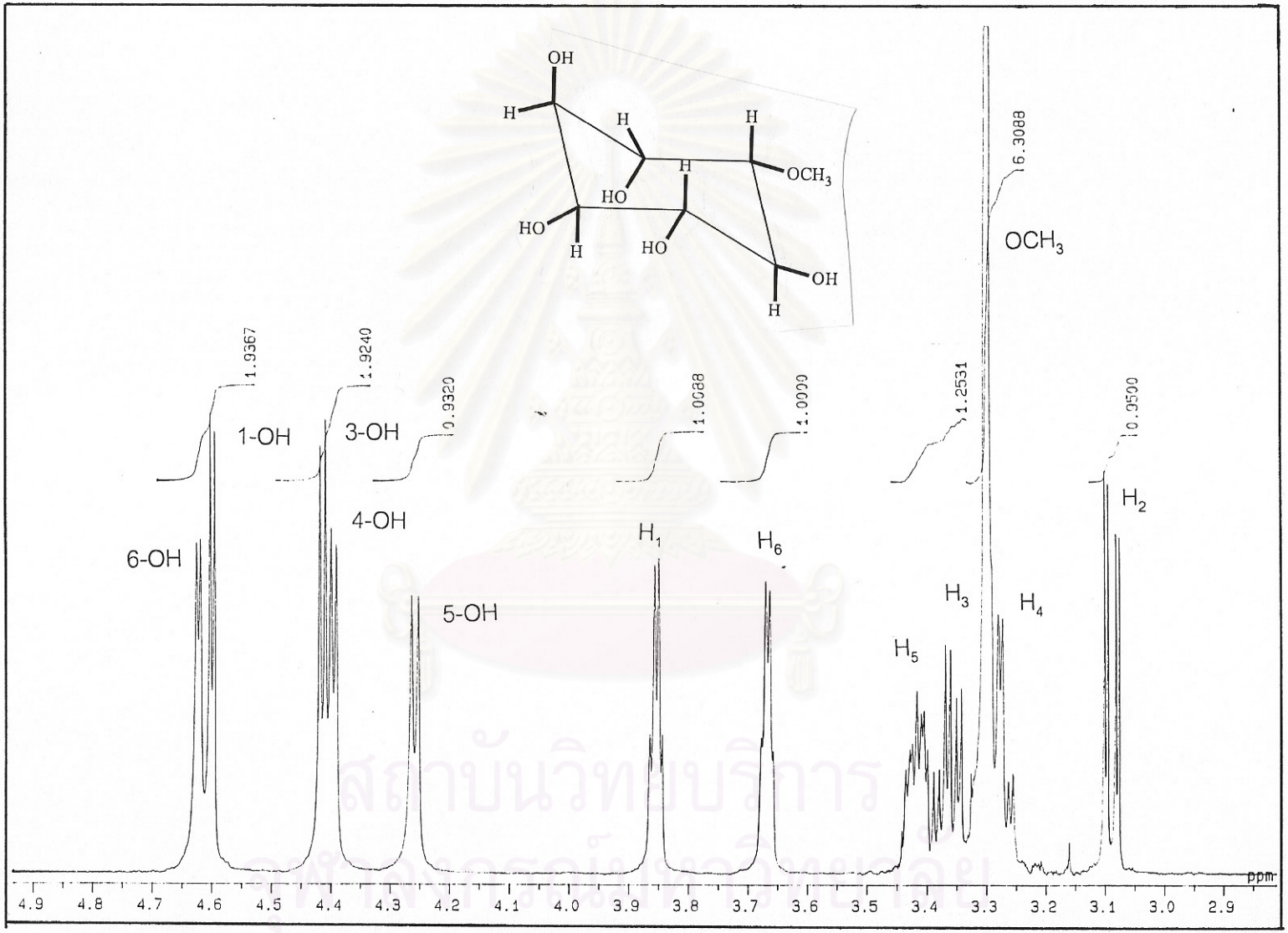


Figure 26a. The 500 MHz ^{1}H-NMR spectrum of compound HA-4 (in DMSO-d_6)

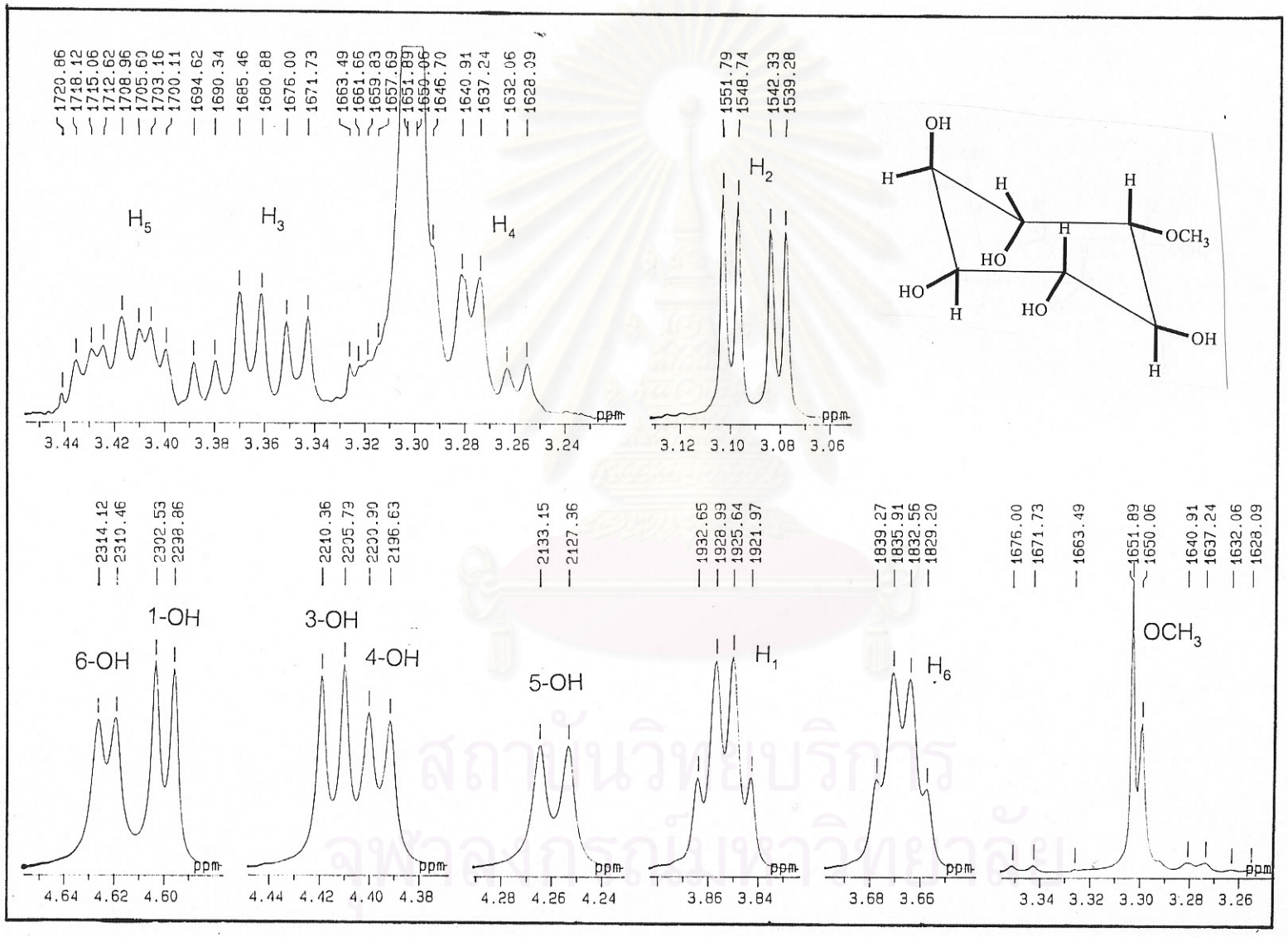


Figure 26b. The 500 MHz ^{1}H-NMR spectrum of compound HA-4 (expanded)

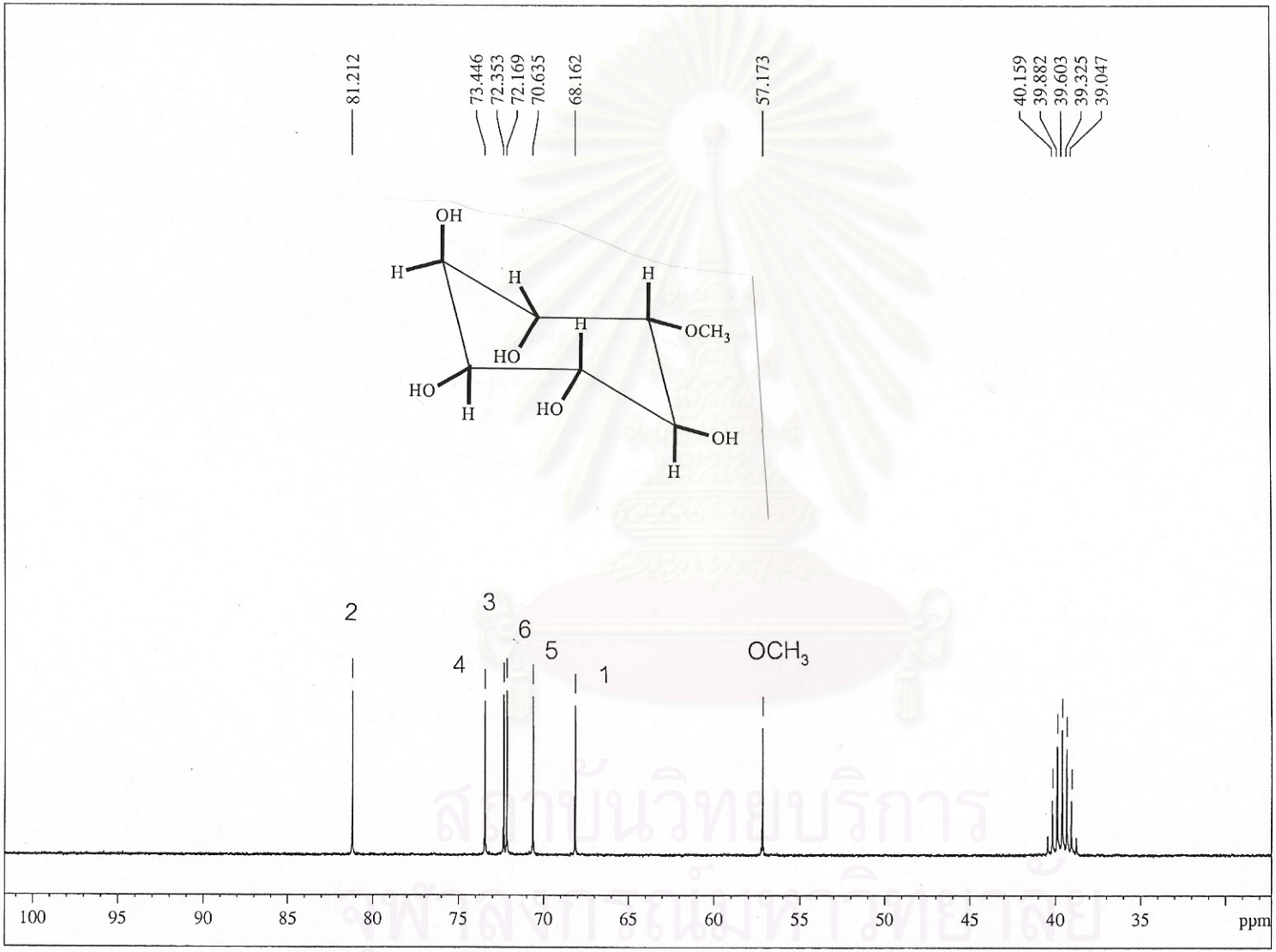


Figure 27. The 75 MHz ^{13}C-NMR spectrum of compound HA-4 (in DMSO-d_6)

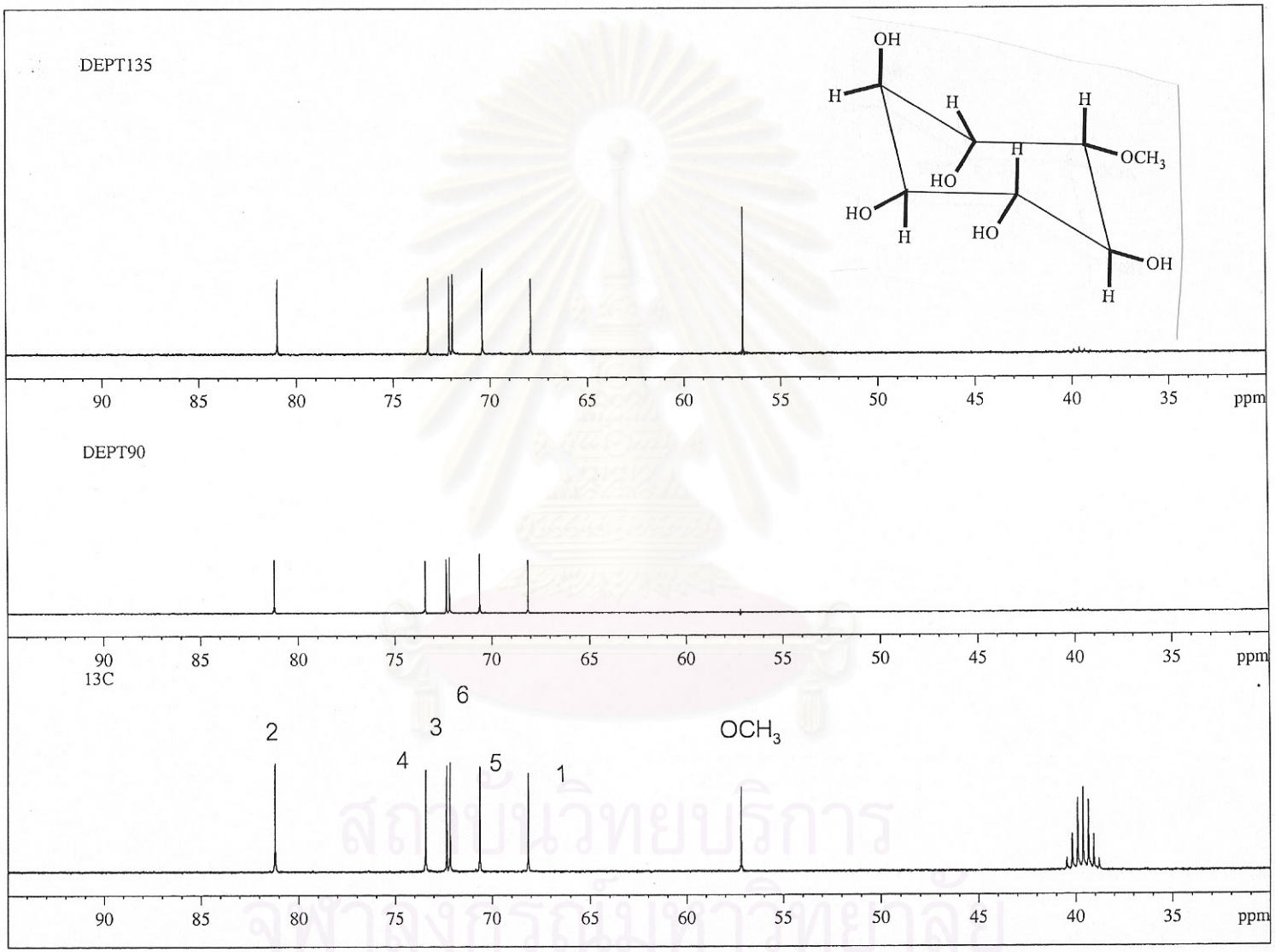


Figure 28. The 75 MHz ^{13}C-DEPT NMR spectrum of compound HA-4 (in DMSO-d_6)

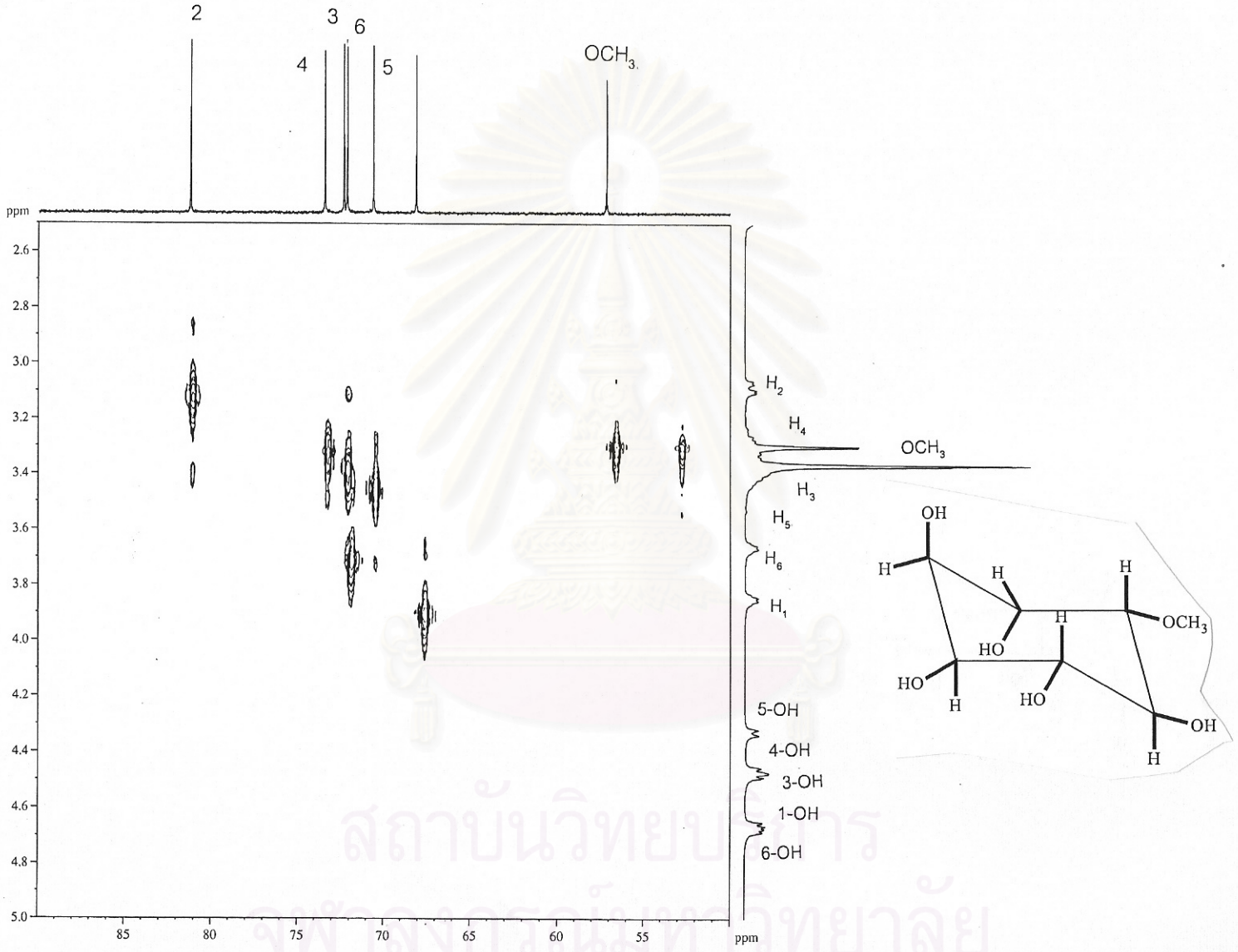


Figure 29a. The 75 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-4 (in DMSO-d_6)

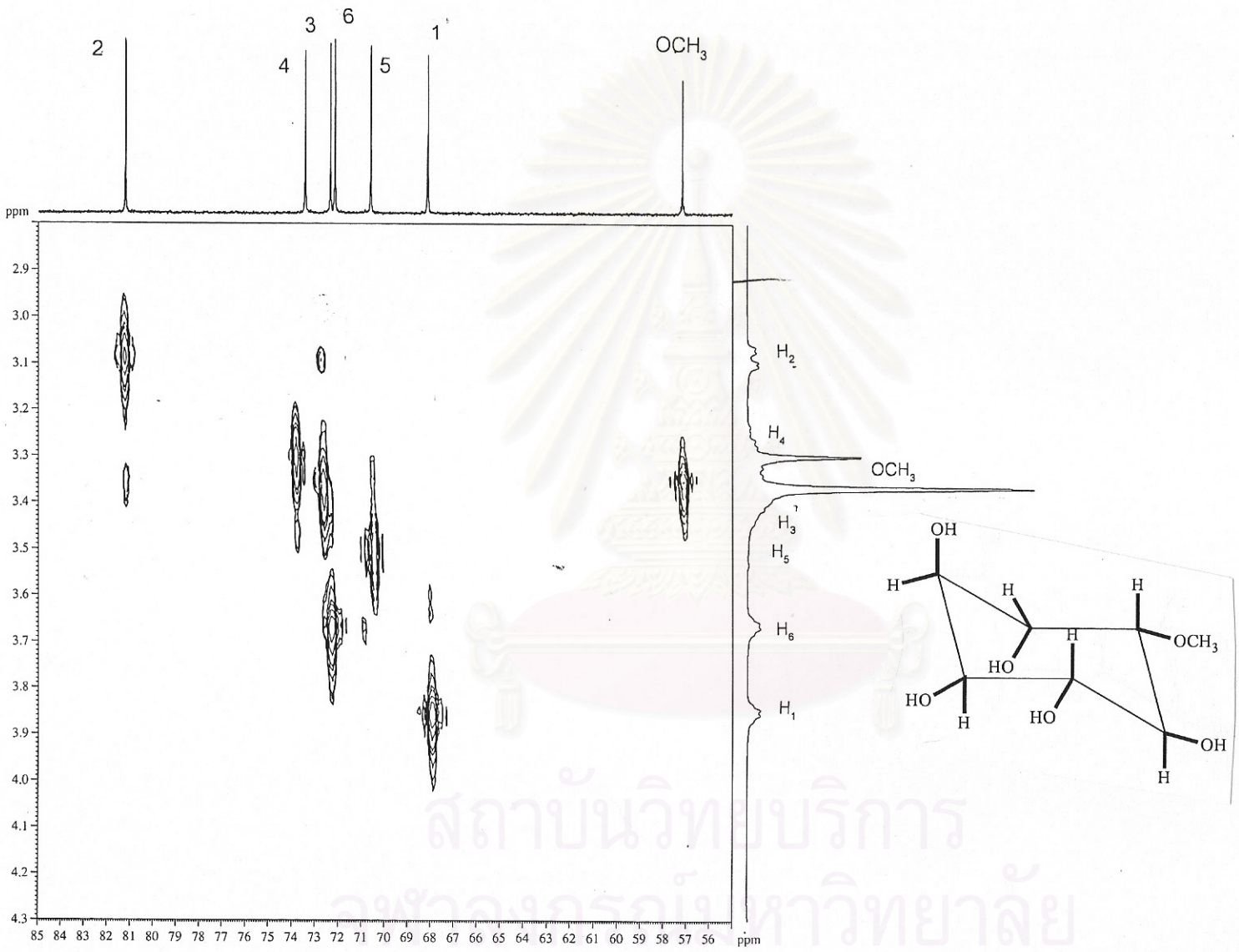
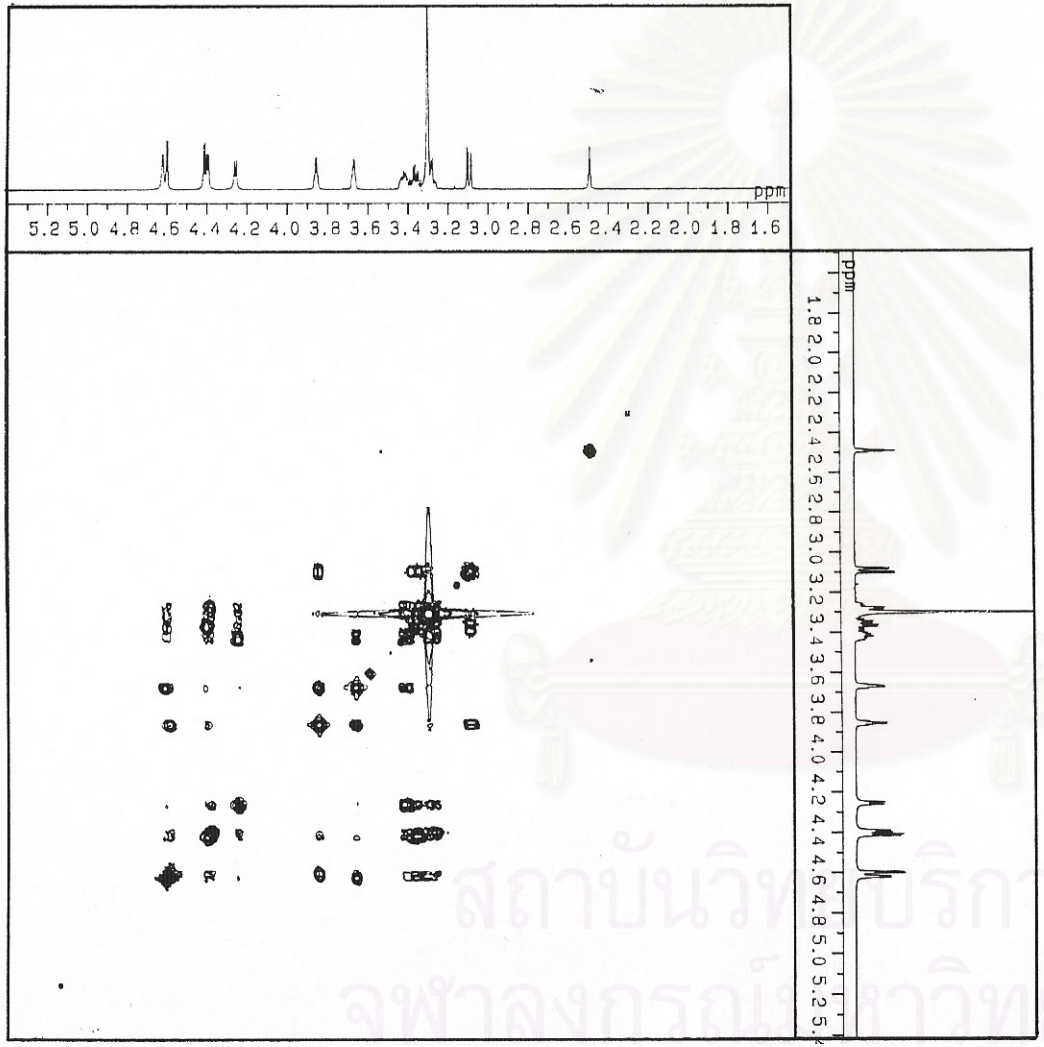


Figure 29b. The 75 MHz ^{1}H-^{13}C HETCOR NMR spectrum of compound HA-4 (expanded)



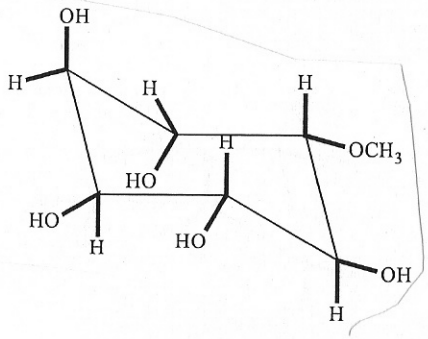
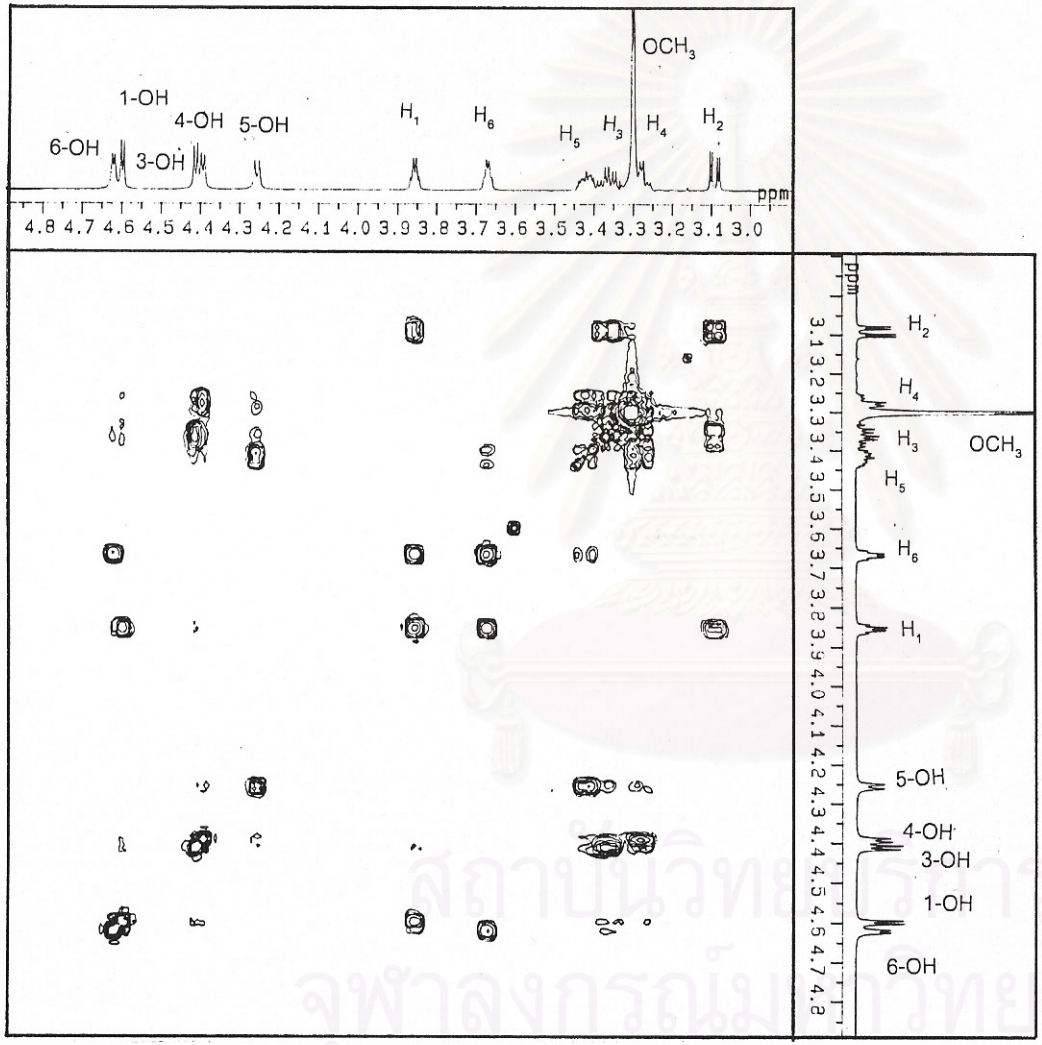


Figure 30a. The 500 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-4 (in DMSO-d_6)



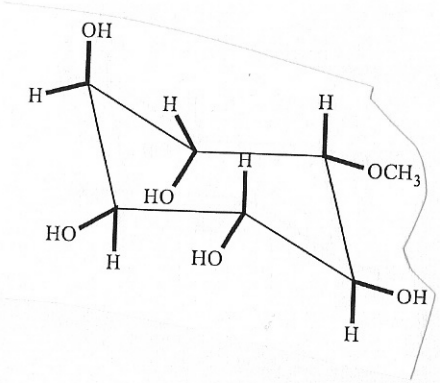
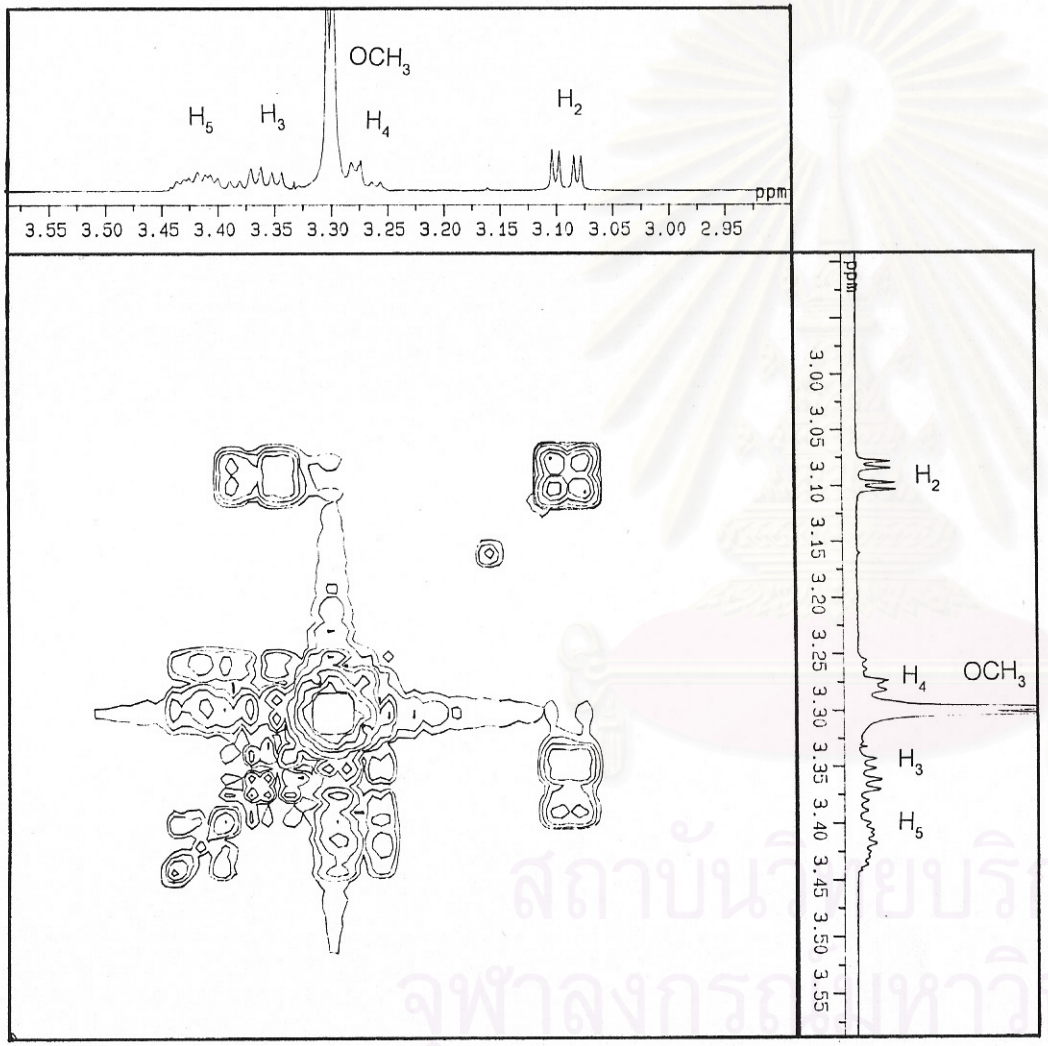


Figure 30b. The 500 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-4 (expanded)



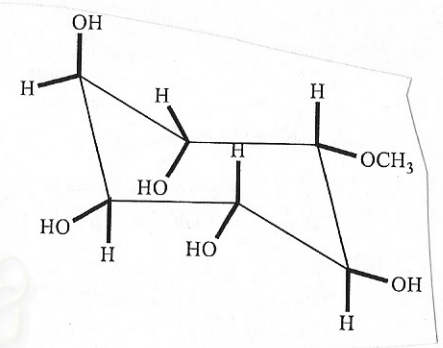


Figure 30c. The 500 MHz ^{1}H-^{1}H COSY NMR spectrum of compound HA-4 (expanded)

Determination on *In Vitro* Stimulation of Lymphocyte Proliferation

The results obtained from lymphocyte proliferation assay are shown in Tables 22-26 and Figures 34-36. % Stimulation and % Cytotoxicity reported in the tables are expressed as the mean ± S.E.M. . Statistical differences were assessed by the student's t-test with $P < 0.05$ considered significant. The values with $P > 0.05$ are marked with the symbol †.

The crude ethanolic extract of the leaves of *Harpullia arborea* exhibited both stimulatory and cytotoxic (inhibitory) effects on lymphocyte proliferation (Table 18, Figures 31a-31b). The extract enhanced the proliferation at concentrations up to 12.5 mcg/ml; at higher concentrations, the proliferation was inhibited. Both the hexane and chloroform extracts showed cytotoxic effect (Table 19, Figure 32) while the methanol extract and quebrachitol (compound HA-4) showed stimulatory effect (Table 20, Figure 33) at all tested concentrations. The minimal concentration used in testing the hexane and chloroform extracts was 12.5 mcg/ml, since in this experiment if a sample did not show the stimulatory effect at this concentration, it was considered as not interesting for further tests at lower concentrations.

Table 18. Effects of crude ethanolic extract on lymphocyte proliferation

Concentration (mcg/ml)	% Stimulation	% Cytotoxicity
1.6	30.57 ± 6.72	-
3.1	49.01 ± 5.12	-
6.3	36.15 ± 5.33	-
12.5	20.58 ± 4.49	-
25	-	17.60 ± 2.47 †
50	-	44.44 ± 4.41
100	-	83.31 ± 3.54
200	-	93.53 ± 7.50

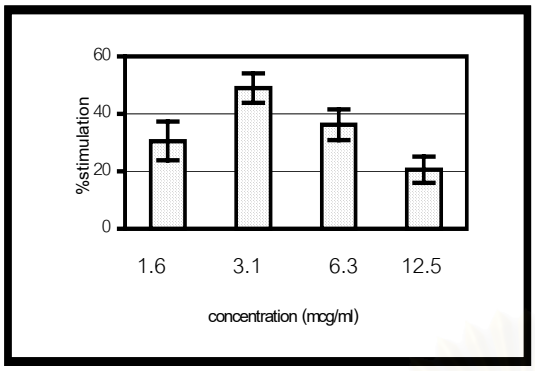


Figure 31a. % Stimulation of crude ethanolic extract on lymphocyte proliferation

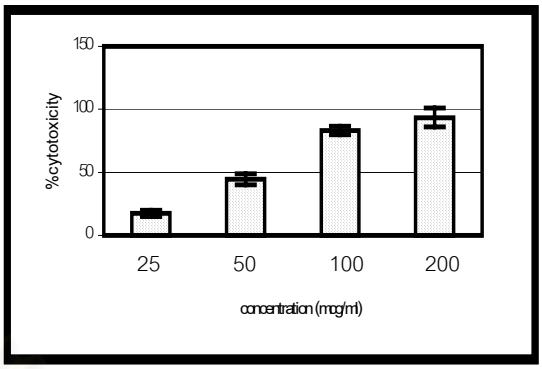


Figure 31b. % Cytotoxicity of crude ethanolic extract on lymphocyte proliferation

Table19. Effects of hexane and chloroform extracts on lymphocyte proliferation.

Concentration (mcg/ml)	% Cytotoxicity	
	hexane extract	chloroform extract
12.5	9.53 ± 5.67	7.83 ± 1.68
25	101.88 ± 0.69	20.79 ± 6.25
50	104.10 ± 1.26	50.99 ± 4.75
100	103.78 ± 1.18	86.48 ± 3.03
200	103.74 ± 1.18	96.83 ± 1.66

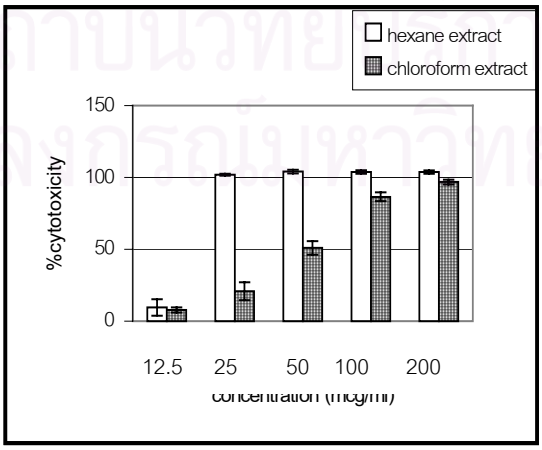


Figure 32. % Cytotoxicity of hexane and chloroform extract on lymphocyte proliferation.

Table 20. Effects of methanol extract and quebrachitol (compound HA-4) on lymphocyte proliferation.

Concentration (mcg/ml)	% Stimulation	
	Methanol extract	Quebrachitol
1.6	17.10 ± 0.33	17.19 ± 5.50
3.1	18.80 ± 1.83	13.29 ± 1.06
6.3	25.97 ± 3.45	15.63 ± 2.77
12.5	25.34 ± 1.63	18.11 ± 1.77
25	40.16 ± 3.85	36.44 ± 5.35
50	43.41 ± 3.15	45.68 ± 1.48
100	44.66 ± 4.25	43.10 ± 6.85
200	9.83 ± 5.77 †	37.49 ± 4.64

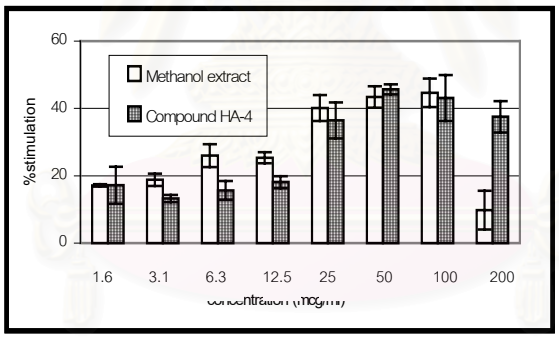


Figure 33. % Stimulation of methanol extract and the quebrachitol on lymphocyte proliferation.

The stimulatory effects of the methanol extract and quebrachitol were shown to be dose dependent. The methanol extract exhibited remarkable effects at the concentrations of 25-100 mcg/ml and, in case of HA-4, 25-200 mcg/ml. The effects of these two samples were considered as interesting, compared with those of concanavalin A (Con-A) and lipopolysaccharide (LPS) which gave 52.93 ± 3.71 % stimulation at the concentration of 10

mcg/ml and 58.61 ± 12.87 % stimulation at the concentration of 50 mcg/ml, respectively (Table 21).

Table 21. Effects of concanavalin A (Con-A) and lipopolysaccharide (LPS) on lymphocyte proliferation.

Compounds	Concentration (mcg/ml)	% Stimulation
con-A	10	52.93 ± 3.71
LPS	50	58.61 ± 12.87

In determining the cytotoxic effect on U-937 monocytic cell line, the CCs_{50} of the tested substances were evaluated as shown in Table 22. The CC_{50} due to the effect on splenic lymphocytes were also given in the same Table. For the methanol extract and HA-4, the results demonstrated that both the extract and pure compound enhanced the lymphocyte proliferation at non-cytotoxic concentrations.

Table 22. CCs_{50} of the extracts of *Harpullia arborea* and quebrachitol (compound HA-4)

Tested substance	CCs_{50} (mcg/ml)	
	U-937	Splenic lymphocytes
Crude ethanolic extract	51.48	92.06
Hexane extract	62.58	< 12.5
Chloroform extract	> 200	50.93
Methanol extract	> 200	> 200
L-quebrachitol	> 100	> 200

CHAPTER V

CONCLUSION

Investigation of the leaves of *Harpullia arborea* led to the isolation of four compounds: two triterpenoids, one of which is a new 24-norhopene type triterpenoid identified as 3β-eicosanoyl-6β-hydroxy-21α*H*-24-norhopa-4(23),22(29)-diene and the other is lupeol; a sterol, α - spinasterol (24S-ethylcholesta-7,22-dien-3β-ol); and an inositol derivative, quebrachitol. The chemical structures were identified and elucidated by spectroscopic techniques. The methanol extract and quebrachitol were demonstrated *in vitro* to have Stimulatory effect on lymphocyte proliferation. Further study on the chemical constituents from different parts of this plant should be performed.

REFERENCES

กรมป่าไม้, สำนักวิชาการป่าไม้, ส่วนพฤกษศาสตร์ป่าไม้, รายชื่อพรรณไม้ในประเทศไทย (ชื่อพฤกษศาสตร์ - ชื่อพื้นเมือง). กรุงเทพมหานคร, 2544.

Adesanya, S. A.; Martin, M. T.; Hill, B.; Dumontet, V.; Tri, M. V.; Sevenet, T.; and Pais, M. Rubiginoside, a farnesyl glycoside from *Lepisanthes rubiginosa*. Phytochemistry 51 (1999): 1039-1041.

Agrawal, P. K.; and Singh, A. K. Structure determination of an inositol from *Artemisia nilagirica* by high-field NMR spectroscopic methods. Indian. J. Chem. 33B (1994): 803-805.

Ahmad, V. U.; Bano, S.; and Mohammad, F. V. Nepehinol – a new triterpene from *Nepeta hindostana*. Planta Med. (1985): 521 – 523.

Ahmad, V. U.; and Rahman, A. U. Handbook of natural products data, Volume 2. Pentacyclic Triterpenoids. Netherlands: Elsevier Science, 1994.

Akihisa, T.; Thakur, S.; Rosenstein, F. U.; and Matsumoto, T. Sterols of Cucurbitaceae : The configurations at C-24 of 24-alkyl-Δ-^{5},Δ-^{7}and Δ-^{8}sterols. Lipids 21 (1986): 39 – 47.

Akihisa, T.; Yasukawa, K.; Oinuma, H.; Kasahara, Y.; Yamanouchi, S.; Takido, M.; Kumaki, K.; and Tamura, T. Triterpene alcohols from the flowers of Compositae and their anti-inflammatory effects. Phytochemistry 43 (1996): 1255-1260.

Alves, T. M. D.; Nagem, T. J.; de Carvalho, L. H.; Krettli, A. U.; and Zani, C. L. Antiplsmodial triterpene from *Vernonia brasiliana*. Planta Med. 63 (1997): 554-555.

Asmahan, El. I.; Jacques, D.; and Clement, D. Effect of a series of saponins extracted from tropical African plants on the release of luteinizing hormone by hypophyseal cells in culture. Bull. Soc. R. Sci. Liege. 58 (1989): 53-56. Through Chemi. Abstr. 111 (1989): 187270p.

Backer, C. C.; and Van der brink Jr, R. C. B. Flora of Java. (Vol.1). Netherlands: N. V. F. Noord Hoff, 1965.

Bharathimatha, C.; Doraiswamy, S.; and Velazhahan, R. Inhibition of fungal plant pathogens by seed proteins of *Harpullia cupanioides* (Roxb.). Acta. Phytopathol. Hun. 37 (2002): 75-82.

Burkill, I. H. A dictionary of the economic products of the Malay Peninsula, Vol I, p. 1127. London:Crown Agents, 1935.

Chavez, J. P.; David, J. M.; Yang, S. W.; and Cordell, G. A. 24-Norhopene derivatives from *Diatenopteryx sorbifolia*. J. Nat. Prod. 60 (1997): 909 – 911.

Chen, Y.; Takeda, T.; and Ogihara, Y. Studies on the constituents of *Xanthoceras sorbifolia* Bunge. III. Minor prosapodenins from the fruits of *Xanthoceras sorbifolia* Bunge. Chem. Pharm. Bull. 33 (1985): 127-134.

Chen, Y.; Takeda, T.; Ogihara, Y.; and Iitaka, Y. Studies on the constituents of *Xanthoceras sorbifolia* Bunge. II. Major sapogenol and prosapogenin from the fruits of *Xanthoceras sorbifolia* Bunge. Chem. Pharm. Bull. 32 (1984): 3378-3383.

Cherry, R. F.; Khong, P. W.; and Lewis, K. G. Chemical constituents of *Harpullia pendula*. II. Further constituents of the bark and leaves. Aust. J. Chem. 30 (1977): 1397-1400.

Chopra, R. N.; Badhwar, R. L.; and Ghosh, S. *Harpullia.* In, Poisonous plant of India, p. 263. New delhi: National Printing, 1965.

Chung, M. S.; Kim, N. C.; Long, L.; Shmon, L.; Ahmad, W. Y.; Sagrero, N. L.; Kardono, L. B. S.; Kennelly, E. J.; Pezzuto, J. M.; Soejarto, D. D.; and Kinghorn, A. D. Dereplication of saccharide and polyol constituents of candidate sweet-tasting plants : Isolation of the sesquiterpene glycoside mukurozioside IIb as a sweet principle of *Sapindus rarak*. Phytochem. Anal. 8 (1997): 49-54.

Cuellar, M. J.; Giner, R. M.; Recio, M. C.; Just, M. J.; Manez, S.; Cerda, M.; Hostettman, K.; and Rios, J. L. Zanhasaponins A and B, antiphospholipase A_2 saponins from an antiinflammatory extract of *Zanha africana* root bark. J. Nat. Prod. 60 (1997a): 1158-1160.

Cuellar, M. J.; Giner, R. M.; Recio, M. C.; Just, M. J.; Manez, S.; and Rios, J. L. Three new oleanane saponins from *Zanha africana* root bark. J. Nat. Prod. 60 (1997b): 191-194.

Dan, S.; and Dan, S. S. Phytochemical study of *Adansonia digitata*, *Coccoloba excoriata*, *Psychotria adenophylla* and *Schleichera oleosa*. Fitoterapia 57 (1986): 445-446. Through Chemi. Abst. 107 (1987): 172422h.

Dimbi, M. Z.; Warin, R.; Delaude, C.; Huls, R.; and Mpuza, K. Triterpenoids of *Harpullia cupanioides*. Bull. Soc. Chim. Belg. 92 (1983): 473 – 484. Through Chemi. Abstr. 99 (1983): 155180b.

Dizes, C.; Gerald, F.; Levaud, C.; Elias, R.; Faure, R.; Massiot, F.; and Balansard, G. Harpuloside A triterpenoid saponin from *Harpullia ramiflora*. Phytochemistry 48 (1998): 1229 – 1232.

El-Sawu, S. A.; Hashem, F. A.; and Biuomy, A. R. Investigation of lipid and volatile oil contents from the aerial parts of *Acacia saligna* Wendl and its anti-inflammatory activity. Bulletin of the Nation Research Centre (Egypt) 28 (2003): 21-33.

El Sayed, N. H.; El Ansari, M. A.; Sahlaby, A. M.; and Mabry, Tom J. Phenolics and flavonoids of the pericarp of *Harpullia pendula*. Rev. Latinoam. Quim. 19 (1988): 66. Through Chemi. Abstr. 110 (1989): 111748j.

Ekabo, O. A.; and Farnsworth, N. R. Antifungal and molluscicidal saponins from *Serjania salzmanniana*. J. Nat. Prod. 59 (1996): 431-435.

Encarnacion, R.; Kenne, L.; Samuelsson, G.; and Sandberg, F. Structural studies on some saponins from *Lecaniodiscus dupanioides*. Phytochemistry 20 (1981): 1939-1942.

Fernandez, M. A.; De las, H. B.; Garcia, M. D.; Saenz, M. T.; and Villar, A. New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. J. Pharm. Pharmacol. 53 (2001): 1533-1539.

Frotan, M. H.; Acharya, S. B.; Frotan, R.; Pathak, N. K. R.; and Biswas, M. Pharmacological investigations on α-spinasterol isolated from *Symplocos spicata*. Indian J. Pharmacol. 15 (1983): 197-201.

Gardner, S.; Sidisunthorn, P.; and Anusamsunthorn, V. A field guide to forest trees of northern Thailand. Bangkok: Kobfai Publishing, 2000.

Gedeon, J.; and Kincl, F. A. Saponins and sapogenins II. Arch. Pharm. 289 (1956): 162 – 165. Through Chemi. Abstr. 51 (1957): 382e.

Geetha,T.; and Varalakshmi, P. Anti-inflammatory activity of lupeol and lupeol linoleate in adjuvant-induced arthritis. Fitoterapia 69 (1998): 13-19.

Gunstone, F. D.; Pollard, M. R.; and Scrimgeour, C. M. Fatty acid. Part 48. ^{13}C Nuclear magnetic resonance studies of acetylenic fatty acids. Chem. Phys. Lipids. 17 (1976): 1 –13.

Gupta, D. R.; and Ahmed, B. Emarginatosides B and C: Two new saponins from *Sapindus emarginatus* fruits. Indian J. Chem., Sect. B 29 (1990): 268-270.

Han, S. M.; Bae, K. H.; and Choi, K. S. Biological activities of α-spinasterol isolated from root of *Phytolacca americana* L. Nongop Kwahak Yongu (Chunganam Taehakkyo) 23 (1996): 177-181.

Hasmeda, M.; Kweifir-Okai, G.; Macrides, T.; and Polya, G. M. Selective Inhibition of eukaryote protein kinases by anti-inflammatory triterpenoids. Planta Med. 65 (1999): 14-18.

Henry, M.; and Chantalat-Dublanche, I. Isolation of spinasterol and its glucoside from cell suspension cultures of *Saponaria officinalis* : ^{13}C-NMR spectral data and batch culture production. Planta Med. 51 (1985): 322-325.

IiDA, T.; Jeong, T. M.; Tamura, T.; and Matsumoto, T. Identification of Chondrillasterol in two Cucurbitaceae seed oils by proton nuclear magnetic resonance spectroscopy. Lipids 15 (1979): 66 – 73.

Ikram, M.; Shaifi, N.; Mir, I.; Do, M. N.; Nguyen, P.; and Le quesne, P. W. 24ξ-Ethylcholesta-7,22-dien-3β-ol : A possibly antipyrotic constituent of *Artemisia absinthium*. Planta Med. 53 (1987): 389.

Ito, S.; Ogino, T.; Sugiyama, H.; and Kodama, M. Structures of A_1-barrigenol and R_1-barrigenol. Tetrahedron Lett. 24 (1967): 2289 – 2294.

Itoh, T.; Kikuchi, T.; Tamura, T.; and Matsumoto, T. Co-occurrence of chondrillasterol and spinasterol in two Cucurbitaceae seeds as shown by ^{13}C-NMR. Phytochemistry 20 (1981): 761-764.

Jayasinghe, L.; Shimada, H.; Hara, N.; and Fujimoto, Y. Hederagenin glycosides from *Pometia exima*. Phytochemistry 40 (1995): 891-897.

Kanchanapoom, T.; Kasai, R.; and Yamasaki, K. Acetylated triterpene saponins from the Thai medicinal plants, *Sapindus emarginatus*. Chem. Pharm. Bull. 49 (2001): 1195-1197.

Kasai, R.; Fujino, H.; Kuzuki, T.; Wong, W. H.; Goto, C.; Yata, N.; Tanaka, O.; Yasuhara, F.; and Yamaguchi, S. Acyclic sesquiterpene oligoglycosides from pericarps of *Sapindus mukurossi*. Phytochemistry 25 (1986): 871-876.

Kasai, R.; Nishi, M; Mizutani, K.; Miyahara, I.; Moriya, T.; Miyahara, K.; and Tanaka, O. Trifolioside II, an acyclic sesquiterpene oligoglycoside from pericarps of *Sapindus trifoliatus*. Phytochemistry 27 (1988): 2209-2211.

Khan, M. R.; Kihara, M.; and Omoloso, A. D. Antimicrobial activity of *Harpullia ramiflora*. Fitoterapia 72 (2001): 298-300.

Khan, M. R.; and Omoloso, A. D. Antibacterial, antifungal activity of *Harpullia petiolaris*. Fitoterapia 73 (2002): 331-335.

Khong, P. W.; and Lewis, K. G. Chemical constituents of *Harpullia pendula*. Aust. J. Chem. 29 (1976): 1351 – 1364.

Tamura, K.; Mizutani, K.; and Yamamoto, S. Isolation of monodesmoside saponins from rind of *sapindus trifoliatus* or mukorossii fruits as skin fungicides. Jpn. Kokai Tokkyo koho JP 88 (1990). Through Chemi. Abstr. 113 (1990): 218226b.

Kweifiookai, G.; and Carroll, A. R. Antiarthritic effect of lupeol acetate. Phytother. Res. 7 (1993): 213-215.

Kweifiookai, G.; Field, B.; Rumble, B. A.; Macrides, T. A.; and Demunk, F. Esterification improves antiarthritic effectiveness of lupeol. Drug Develop. Res. 35 (1995): 137-141

Lavaud, C.; Crublet, M. L.; Pouny, I.; Litaudon, M.; and Sevenet, T. Triterpenoid saponins from the stem bark of *Elattostachys apetala*. Phytochemistry 57 (2001): 469-478.

Lavaud, C.; Voutquenne, L.; Massiot, G.; Oliver, L. M.; Das, B. C.; Laprevote, O.; Serani, L.; Delaude, C.; and Becchi, M. Saponins from the stem bark of *Filicium decipiens*. Phytochemistry 47 (1998): 441-449.

Linnazam, A. A triterpenoidal sapogenin from the seeds of *Dodonaea viscosa*. Indian J. Chem. B. 32 (1993): 513-514.

Ma, C. M.; Nakamura, N.; Hattori, M.; Kakuda, H.; Qiao, J. C.; and Yu, H. L. Inhibitory effects on HIV-1 protease of constituents from the wood of *Xanthoceras sorbifolia*. J. Nat. Prod. 63 (2000): 238-242.

Mitaine-Offer, A. C.; Hornebeck, W.; Sauvain, M.; and Zeches-Hanrot, M. Triterpenes and phytosterols as human leucocyte elastase inhibitors. Planta Med. 68 (2002): 930-932.

Moriarity, D. M.; Huang, J.; Yancey, C. A.; Zhang, P.; Setzer, W. N.; Lawton, R. O.; Bates, R. B.; and Caldera, S. Lupeol is the cytotoxic principle in the leaf extract of *Dendropanax cf. querceti*. Planta Med. 64 (1998): 370-372.

Musalmah, M.; Elkhairee, M. R.; Lau, C. M.; Wan, N. W. Z. Effect on blood glucose levels in normal and alloxan-induced diabetic rats. Malaysian Journal of Biochemistry and Molecular Biology 6 (2001): 7-11.

Nakayama, K.; Fujino, H.; Kasai, R.; Tanaka, O.; and Zhou, J. Saponins of pericarps of Chinese *Sapindus delavayi* (pyi-shiau-tzu), a source of natural surfactants. Chem. Pharm. Bull. 34 (1986): 2209-2213.

Nes, W. R.; Krevitz, K.; Joseph, J.; Nes, W. D.; Harris, B.; and Gibbons, F. G. The phylogenetic distribution of sterols in tracheophytes. Lipids 12 (1976): 51–527.

Ogunkoya, L. Application of mass spectrometry in structural problems in triterpenoids. Phytochemistry 20 (1981): 121 – 126.

Patocka, J. Biologically active pentacyclic triterpenes and their current medicine signification. Journal of Applied Biomedicine 1 (2003): 7-12.

Perry, L. M.; and Metzger, J. Medicinal plants of East and Southeast Asia, Vol. 1. Netherlands: N. V. F. Noordhoff, 1895.

Rajic, A.; Kweifio-Okai, G.; Macrides, T.; Sandeman, R. M.; Chandler, D. S.; and Polya, G. M. Inhibition of serine proteases by anti-inflammatory triterpenoids. Planta Med. 66 (2000): 206-210.

Quisumbing, E. Medicinal plants of the Phillippines. Phillippines: Manila bureau, 1951.

Sandoval, A.; Manjarrez, A.; Leeming, P. R.; Thomas, G. H.; and Djerassi, C. Terpenoids. XXX. The structure of the cactus triterpene chichipegenin. J. Am. Chem. Soc. 79 (1957): 4468 – 4472.

Sati, O. P.; and Rana, U. Triterpenoids of *Aesculeu indica*. Pharmazie 42 (1987): 141.

Schenkel, E. P.; Werner, W.; and Schulte, K. E. Die saoponine aus *Thinouia coriacea*. Planta Med. 57 (1991): 463-467.

Schilling, N.; Dittrich, P.; and Kandler, O. Formation of L-quebrachitol from D- bornesitol in leaves of *Acer pseudoplatanus*. Phytochemistry 11 (1972): 1401-1404.

Schmatz, D. M.; Arison, B. H.; Dashkevicz, M. P.; Liesch, J. M.; and Turner, M. J. Identification and possible role of D-mannitol and 2-O-methyl-chiro-inositol (quebrachitol) in *Eimeria tenella*. Mol. Biochem. Parasit. 29 (1988): 29 – 36.

Shamma, M.; Glick, R. E.; and Mumm, R. O. The nuclear magnetic resonance spectra of pentacyclic triterpenoid. J. Org. Chem. 27 (1962): 4512 – 4517.

Suttisri, R.; Homhaun, A.; Srangpol, A.; Yamchamuang, P.; Pengsuparp, T.; and Saifah, E. Chemical constituents of the leaves of *Harpullia cupanioides*. Thai. J. Pharm. Sci. 23 (1999): 29 – 33.

Tori, K.; Seo, S.; Shimaoka, A.; and Tomita, Y. Carbon-13 NMR spectra of olean-12-enes. Full signal assignments including quaternary carbon signals assigned by use of indirect $^{13}C,^{1}H$ spin couplings. Tetrahedron Lett. 48 (1974): 4227 – 4230.

Tuntiwachwuttikul, P.; Pancharoen, O.; Mahabusarakam, W.; Wiriya, C. P.; Taylor, W. C.; Bubb, W. A.; and Towers, G. H. N. A triterpenoid saponin from *Maesa ramentacea*. Phytochemistry 44 (1997): 491 – 495.

Van Welzen, P. C. Sapindaceae. In Santisuk, T.; and Larsen, K. (eds.), Flora of Thailand Vol. 7 Part 1, pp. 169-250. Bangkok: Diamond Printing, 1999.

Irene,M. V.; Pauline, L.; Allan, P.; and John, B. B. Antigenotoxic spinasterol from *Cucurbita maxima* flowers. Mutat. Res. 360 (1996): 89-93.

Voutquenne, L.; Kokougan, C.; Lavaud, C.; Pouny, I.; and Litaudon, M. Triterpenoid saponins and acylated prosapogenins from *Harpullia austro-caledonica*. Phytochemistry 59 (2002): 825 – 832.

Voutquenne, L.; Lavaud, C.; Massiot, G.; and Delaude, C. Saponins from *Harpullia cupanioides*. Phytochemistry 49 (1998): 2081 – 2085.

Wada, S. I.; Iida, A.; and Tanaka, R. Screening of triterpenoids isolated from *Phyllanthus flexuosus* for DNA topoisomerase inhibitory activity. J. Nat. Prod. 64 (2000): 1545-1547.

Woldemichael, G. M.; Singh, M. P.; Maiese, W. M.; and Timmernann, B. N. Constituents of antibacterial extract of *Caesalpinia paraguariensis* Burk. Z.Naturforsch. C. 58 (2003): 70-75.

Woo, W. S. Steroids and pentacyclic triterpenoids from *Phytolacca americana*. Phytochemistry 13 (1974): 2887-2889.

Zhou, C.; Sun, X.; Liu, J.; Luo, S.; and Lu, C. Antiinflammatory effect of α-spinasterol. Yaoxue Xuebao 20 (1985): 257-261.

Ziegler, H. L.; Staerk, D.; Christensen, J.; Hviid, L.; Hagerstrand, H.; and Jaroszewski, J. W. In vitro *Plasmodium falciparum* drug sensitivity assay: Inhibition of parasite growth by incorporation of stomatocytogenic amphiphiles into the erythrocyte membrane. Antimicrob. Agents Ch. 46 (2002): 1441-1446.

VITA

Miss Ratchanee Poovapatthanachart was born on December 1st, 1976 in Ayuttaya, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1999 from the Faculty of Pharmacy, Rangsit University, Phathum Thani, Thailand.