องค์ประกอบทางเคมีของรากและเมล็ดลำดวน

นาย ทวีศักดิ์ จึงวัฒนตระกูล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวท
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2543

ISBN 974-13-1254-7

CHEMICAL CONSTITUENTS
OF
MELODORUM FRUTICOSUM ROOT AND SEEDS

Mr. Thaweesak Juengwatanatrakul

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy
Department of Pharmacognosy
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2000
ISBN 974-13-1254-7

Thesis Title	CHEMICAL CONSTITUENTS OF *MELODORUM FRUTICOSUM* ROOT AND SEEDS
By	Mr. Thaweesak Juengwatanatrakul
Field of study	Pharmacognosy
Thesis Advisor	Associate Professor Chaiyo Chaichantipyuth

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

……Dean of Faculty of Pharmaceutical Sciences

(Associate Professor Sunibhond Pummangura, Ph.D.)

THESIS COMMITTEE

……Chairman

(Associate Professor Kittisak Likhitwitayawuid, Ph.D.)

……Thesis Advisor

(Associate Professor Chaiyo Chaichantipyuth, M. Sc.)

……Member

(Assistant Professor Rutt Suttisri, Ph.D.)

……Member

(Associate Professor Amorn Petsom, Ph.D.)

ทวีศักดิ์ จึงวัฒนตระกูล : องค์ประกอบทางเคมีของรากและเมล็ดลำดวน (CHEMICAL CONSTITUENTS OF *MELODORUM FRUTICOSUM* ROOT AND SEEDS) อาจารย์ที่ปรึกษา : รศ. ชัยโย ชัยชาญทิพยุทธ, 124 หน้า. ISBN 974-13-1254-7

การศึกษาองค์ประกอบทางเคมีของรากและเมล็ดลำดวน (วงศ์ Annonaceae) สามารถแยกสารบริสุทธิ์ได้ 5 ชนิด แบ่งเป็น 2 กลุ่มคือ สารในกลุ่มเฮปทีน 3 ชนิด และ สารกลุ่มไตรเทอปีนอยด์ 2 ชนิด และสามารถพิสูจน์สูตรโครงสร้างทางเคมีของสารบริสุทธิ์ที่แยกได้โดยวิธีการทางสเปคโตรสโคปีชนิดต่าง ๆ และเปรียบเทียบข้อมูลที่ได้กับสารที่มีการรายงานในอดีต พบว่าสารบริสุทธิ์ที่แยกได้จากราก 1 ชนิด เป็นอนุพันธ์ใหม่ของสารกลุ่มไตรเทอปีนอยด์ที่ยังไม่มีการรายงาน มีชื่อทางเคมีว่า lanosta-7,9(11),24-trien-3β-acetoxy-15α-ol และได้ให้ชื่อสามัญแก่สารที่แยกได้นี้ว่า acetylpolycarpol สำหรับสารบริสุทธิ์ที่แยกได้อีก 4 ชนิด เมื่อพิสูจน์สูตรโครงสร้างทางเคมีแล้ว พบว่าเป็นสารที่มีการรายงานมาก่อนแล้ว มีชื่อสามัญว่า (4*E*)-7-benzoyloxy-6-hydroxy-2,4-heptadien-4-olide, (4*Z*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide (acetylmelodorinol), (4*Z*)-7-benzoyloxy-6-hydroxy-2,4-heptadien-4-olide (melodorinol) และ lanosta-7,9(11),24-trien-3β,15α-ol (polycarpol)

ภาควิชาเภสัชเวท ลายมือชื่อนิสิต.....

สาขาวิชาเภสัชเวท ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2543

4276564533 PHARMACOGNOSY
KEY WORD: TRITERPENOIDS/ LANOSTANE/ ACETYLPOLYCARPOL/ HEPTENES/ HEPTADIENES/ *Melodorum fruticosum*/ CYTOTOXICITY. THESIS ADVISOR: ASSOCIATE PROFESSOR CHAIYO CHAICHANTIPYUTH, M.Sc. in Pharm. 124 pp. ISBN 974-13-1254-7

This research on the chemical constituents of *Melodorum fruticosum* Lour. root and seeds (Annonaceae) led to the isolation of three heptene derivatives and two triterpenoid compounds. The structure identification and structure elucidation of these compounds were based on the data from various spectroscopic techniques and comparison with reported data. Novel triterpene was assigned the chemical structure lanosta-7,9(11),24-trien-3β-acetoxy-15α-ol and was given the trivial name acetylpolycarpol, isolated from its root. The other four are the known compounds (4*E*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide, (4*Z*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide (acetylmelodorinol), (4*Z*)-7-benzoyloxy-6-hydroxy-2,4-heptadien-4-olide (melodorinol), and lanosta-7,9(11),24-trien-3β,15α-diol (polycarpol) .

Department of Pharmacognosy Student's……
Field of study Pharmacognosy Adviser's……
Academic year 2000

ACKNOWLEDGEMENT

The author wishes to express his deepest gratitude to his thesis advisor, Associate Professor Chaiyo Chaichantipyuth, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his valuable advice, guidance, suggestion and encouragement throughout the course of this study.

The author would like to acknowledge his sincere thanks to his thesis committee, for valuable suggestion and encouragement.

The author wishes to express his sincere thanks to Associate Professor Dr. Amorn Petsom, Department of Chemistry, Faculty of Science, Chulalongkorn University, for his meaningful concern and encouragement, and helpful spectroscopic discussion.

The author would like to acknowledge his grateful thanks to Dr. Khanit Suwanborirux, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his valuable suggestion and helpful spectroscopic discussion.

The author would like to thank the University Development Commission (UDC) and the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, for granting the scholarship throughout the course of his study.

The author would also like to thank the Graduate School of Chulalongkorn University for granting partial financial support to conduct this investigation.

The author would like to thank all his teachers and his friends for their contribution and friendship.

Finally, the author wishes to express his infinite gratitude to his family for their love, concern and encouragement.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

Figure **Page**

LIST OF ABBREVIATIONS

$[\alpha]^{25}_{D}$	=	Specific Rotation at 25°C and Sodium D line (589 nm)
br	=	Broad (for NMR spectral data)
c	=	Concentration
calcd.	=	Calculated
°C	=	Degree Celsius
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
cm	=	Centimeter
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
^{1}H-^{1}H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
2D	=	Two Dimensional
d	=	doublet (for NMR spectral data)
dd	=	doublet of doublets (for NMR spectral data)
ddd	=	doublet of doublets of doublets (for NMR spectral data)
dt	=	doublet of triplets (for NMR spectral data)
DEPT	=	Distortionless Enhancement by Polarization Transfer
ε	=	Molar Absorptivity
δ	=	Chemical Shift
EIMS	=	Electron Impact Mass Specrtroscopy
EtOAc	=	Ethyl acetate
enh.	=	Enhanced (for NOE difference spectral data)
g	=	Gram
^{1}H NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^{1}H-detected Heteronuclear Multiple Bond Coherence
HMQC	=	^{1}H-detected Heteronuclear Multiple Quantum Coherence
Hz	=	Hertz
IR	=	Infrared
irr.	=	Irradiated (for NOE difference spectral data)
J	=	Coupling Constant
KBr	=	Potassium bromide
kg	=	Kilogram
L	=	Liter

λ_{max}	=	Wavelength at Maximal Absorption
m	=	Multiplet (for NMR spectral data)
MeOH	=	Methanol
mg	=	Milligram
ml	=	Milliliter
mm	=	Milimeter
MS	=	Mass Spectroscopy
m/z	=	mass-to-charge ratio
M^+	=	Molecular Ion
No.	=	Number
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOE	=	Nuclear Overhauser Effect
ppm	=	part per million
ν_{max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectral data)
t	=	Triplet (for NMR spectral data)
td	=	Triplet of doublets (for NMR spectral data)
tt	=	Triplet of Triplets (for NMR spectral data)
TLC	=	Thin Layer Chromatography
UV-VIS	=	Ultraviolet and Visible Spectrophotometry

CHAPTER I
INTRODUCTION

The genus *Melodorum* belongs to the family Annonaceae distributed in Indo-China and Australia. In 1790 the first manifestation of the type of this genus had been proposed by Loureiro (Loureiro, 1790). The generic name *Melodorum* had two authorities, Dunal and Loureiro (Merrill, 1919). Thereafter, Griff had converted Dunal's *Melodorum* into the genus *Fissistigma* Griff. According to the Index Kewensis, ninety-three species of the genus *Melodorum* have been identified (Taylor, 1959). Nowadays, only fourteen species remain in this generic name while the rest have switched to the genera *Fissistigma, Sphaerocoryne, Mitrella, Mitrephora, Xylopia, Anaxagorea, Melogyne, Papualthia, Cyathocalyx, Richella,* and *Pyramidanthe* (Kessler, 1994). In Thailand, two species of the genus *Melodorum* have been recorded (Smitinand, 1980; Kessler, 1994). They are as follows:

***Melodorum fruticosum* Lour.**, Fl. Cochinchinensis 351 (1790)

Common name: ลำดวน Lamduan (Central), หอมนวล Hom nuan (Northern)

Synonym: *Melodorum schefferi* Finet & Gagnep.,Bull. Soc. Fr. 53:134 (1906)

Uvaria godefroyana var. *nervosa* Finet & Gagnep., Lecomte Fl. Gen. Indochine 1:56 (1907)

Rauwenhoffia siamensis auct non Scheffer, Hembert Suppl. Fl. Gen. Indochine 1:104 (1938)

***Melodorum siamensis* (Scheff.) Tien Ban**, Bot. Zhurn. 59:241 (1974)

Common name: นมแมว Nom maeo

Synonym: *Rauwenhoffia siamensis* Scheffer, Ann. Jard. Bot. Buitenz. 2:23 (1885)

?*Uvaria godefroyana* Finet & Gagnep., Bull. Soc. Bot. Fr. 53:71 (1906)

Melodorum fruticosum Lour. is a shrub reaching 3-6 m in height. Young twigs slender, glabrous, black, finely striate. Leaves membranous, oblong-lanceolate, acute or acuminate, base slightly cuneata, grabrous, shining above, glaucous beneath; main nerves 14-18 pairs, fine, the secondary quite as well as marked; reticulations rather lax, fine on both surfaces; length 9-12 cm; breadth 3.5-4.5 cm; petiole 5-7 mm long; pale brown beneath in herbarium material. Flower solitary, axillary or terminal. Pedicels 2 cm long, thickened below calyx, 3-3.5 cm long, lengthening in fruit, bearing 2-3 minute bracts at base and another slightly below the middle. Sepals broadly triangular, connate 3-4 mm long, puberulous or glabrous outside, glabrous inside. Petals coriaceous, nearly orbicular, acute with broad base, tomentose outside, puberulous inside except the base, concave inside; outer about 1 cm long and 1.1 cm broad, the inner slightly smaller, thicker and more concave. Stamens 2 mm long, connectives flat-topped, pollen grains large, visible under a lens. Torus depressed in centre. Ovaries 2 mm long, elongate, tomentose, with short style, grooved on the inner side from the stigmatic portion downwards, stigma small, not thickened, expanded or extinct from style. Fruits violet, ripe carpels ovoid, slightly apiculate, glabrous, 8 mm long and 7 mm in diameter; stalks slender, glabrous, 1.8-2.5 cm long. Seeds 1,7 cm long and 5 mm in diameter, occasionally 2, pale brown, smooth, and shining. Distribution in South-East Asia (Merrill, 1919).

With regard to ethnopharmacology of *Melodorum fruticosum* Lour., the flowers have been used as a component of the Thai traditional medicine recipe' known as " Gaesorn Thung Gao" (เกสรทั้งเก้า) which are used as tonic stimulant and mild cardiotonic. Furthermore, its flowers are used as hematinic, and its heartwood is used as dye (Saralamp, 1992).

Previous phytochemical works on *Melodorum fruticosum* Lour. showed that the abundant metabolites of this plant were heptene derivatives (heptadienes). These Heptadienes (Heptenes) have a benzoyl moiety conjugated with a seven-carbon dienone or lactone terminal which appears to arise from a heptose or the equivalents. Heptadiene derivatives were first described by Jung *et al.* (1990a). Up to the present, thirteen heptenes have been obtained from the bark, leaves, branches, and flowers of this plant. Some of these compounds have shown cytotoxic activity against human

tumor cell lines (Jung *et al.*, 1990a; Jung *et al.*, 1990b; Jung *et al.*, 1991; Tuchinda *et al.*, 1991; Tiyaworanan, 1998). As for the root and seeds of this plant, no phytochemical study has been reported.

The main objectives of this investigation are as follows:

1. To isolate and purify compounds from the root and seeds of *Melodorum fruticosum* Lour.
2. To determine the chemical structure of each isolated compound.

Figure 1. *Melodorum fruticosum* Lour.

CHAPTER II
HISTORICAL

Phytochemical studies on the genus *Melodorum* have been done, only on *M. fruticosum* Lour. The chemical constituents can be classified as flavonoids, triterpenoids, aromatic compounds, and heptenes (heptadienes), which are presented in Table 1.

Table 1. Chemical constituents of *Melodorum fruticosum* Lour.

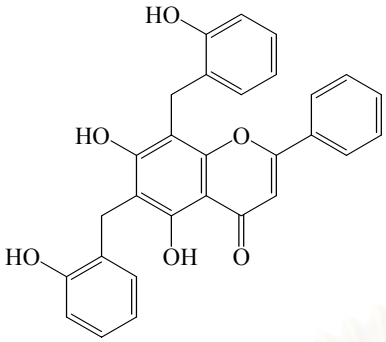
Chemical Compound	Plant Part	Reference
Flavonoids		
• Dichamanetin [**1**]	bark	Jung *et al*., 1990
• Pinocembrin [**2**]	bark leaves, branches	Jung *et al*., 1990 Tuchinda *et al*., 1991
• Chrysin [**3**]	leaves, branches flowers	Jung *et al*., 1990 Tiyaworanan, 1998
• 5,7-Dimethoxyflavone [**4**]	flowers	Tiyaworanan, 1998
Triterpenoids		
• Lanosta-7,9(11),24-trien-3β,15α-ol (polycarpol) [**5**]	bark	Jung *et al*., 1990
• Stigmasterol [**6**]	bark	Jung *et al*., 1990
• β-Sitosterol [**7**]	bark	Jung *et al*., 1990
Aromatic compounds		
• Benzoic acid [**8**]	leaves, branches	Tuchinda *et al*., 1991
• Benzylbenzoate [**9**]	bark	Jung *et al*., 1990

Table 1. Chemical constituents of *Melodorum fruticosum* Lour. (continued)

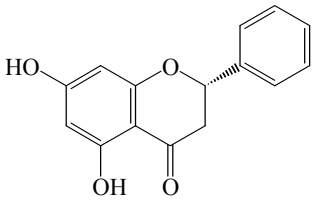
Chemical Compounds	Plant Part	Reference
Heptenes (Heptadienes)		
• (4*Z*)-7-Benzoyloxy-6-hydroxy-2,4-heptadien-4-olide (melodorinol) [**10**]	bark leaves, branches	Tuchinda *et al*., 1991 Jung *et al*., 1991
• (4*E*)-7-Benzoyloxy-6-hydroxy-2,4-heptadien-4-olide [**11**]	leaves, branches	Tuchinda *et al*., 1991
• (4*Z*)-7-Benzoyloxy-6-acetoxy-2,4-heptadien-4-olide (acetylmelodorinol) [**12**]	bark leaves, branches, flowers	Jung *et al*., 1990 Tuchinda *et al*., 1991 Tiyaworanan, 1998
• (4*E*)-7-Benzoyloxy-6-acetoxy-2,4-heptadien-4-olide [**13**]	leaves, branches	Tuchinda *et al*., 1991
• (4*Z*)-6-Benzoyloxy-7-hydroxy-2,4-heptadien-4-olide [**14**]	leaves, branches	Tuchinda *et al*., 1991
• (2*E*,5*E*)-7-Benzoyloxy-1-methoxy-2,5-heptadien-1,4-dione (melodienone) [**15**]	bark	Jung *et al*., 1990
• (2*E*,5*E*)-7-Benzoyloxy-1-ethoxy-2,5-heptadien-1,4-dione (homomelodienone) [**16**]	bark	Jung *et al*., 1991
• (2*Z*,5*E*)-7-Benzoyloxy-1-methoxy-2,5-heptadien-1,4-dione (isomelodienone) [**17**]	bark	Jung *et al*., 1990
• (2*Z*,5*E*)-7-Benzoyloxy-1-ethoxy-2,5-heptadien-1,4-dione (homoisomelodienone) [**18**]	bark	Jung *et al*., 1991
• (*E*)-7-Benzoyloxy-6-hydroxy-1-methoxy-2-heptadien-1,4-dione (6-hydroxy-5-hydromelodienone) [**19**]	bark	Jung *et al*., 1991

Table 1. : Chemical constituents of *Melodorum fruticosum* Lour. (continued)

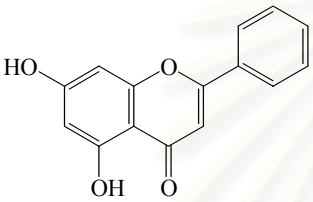
Chemical Compounds	Plant Part	Reference
Heptenes (Heptadienes) • (4*Z*)-7-Benzoyloxy-2,4-heptadien-6-one-4-olide (melodorinone A) [**20**]	flowers	Tiyaworanan, 1998
• (4*E*)-7-Benzoyloxy-2,4-heptadien-6-one-4-olide (melodorinone B) [**21**]	flowers	Tiyaworanan, 1998
• (*E*)-7-Benzoyloxy-4-hydroxy-1-methoxy-2,4-heptadien-1,6-dione (tautomelodorinone) [**22**]	flowers	Tiyaworanan, 1998



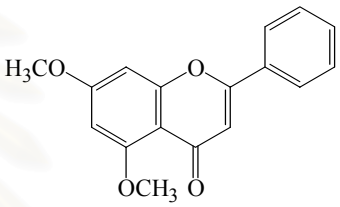
Dichamanetin [**1**]



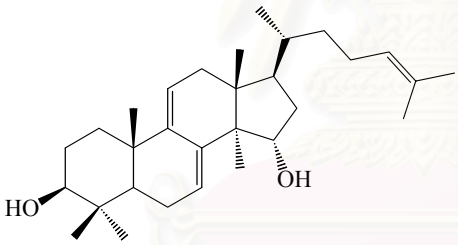
Pinocembrin [**2**]



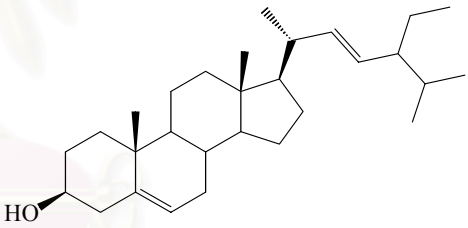
Chrysin [**3**]



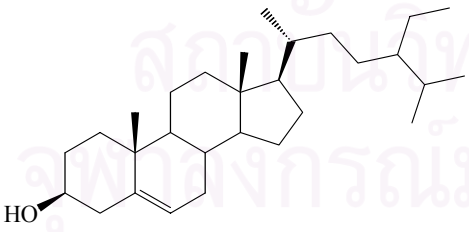
5,7-Dimethoxyflavone [**4**]



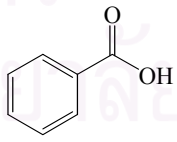
Polycarpol [**5**]



Stigmasterol [**6**]

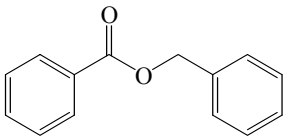


β-Sitosterol [**7**]

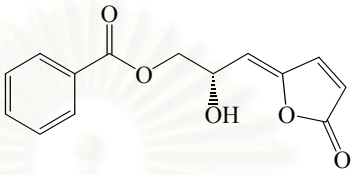


Benzoic acid [**8**]

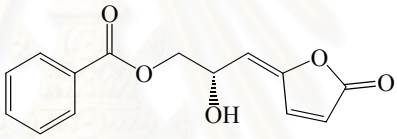
Figure 2. : Structures of chemical components of *Melodorum fruticosum*



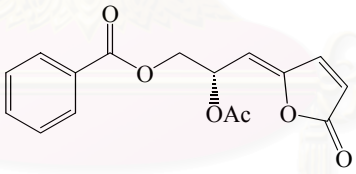
Benzylbenzoate [**9**]



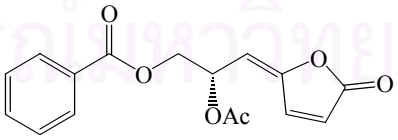
(4*Z*)-7-Benzoyloxy-6-hydroxy-2,4-heptadien-4-olide
(melodorinol) [**10**]



(4*E*)-7-Benzoyloxy-6-hydroxy-2,4-heptadien-4-olide [**11**]

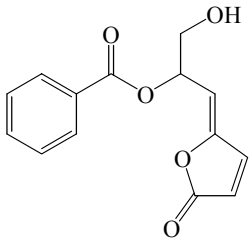


(4*Z*)-7-Benzoyloxy-6-acetoxy-2,4-heptadien-4-olide
(acetylmelodorinol) [**12**]

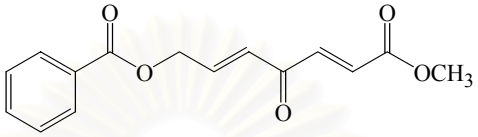


(4*E*)-7-Benzoyloxy-6-acetoxy-2,4-heptadien-4-olide [**13**]

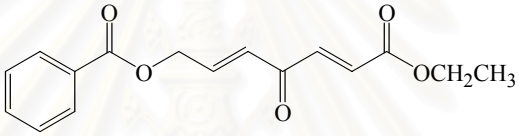
Figure 2. : Structures of chemical components of *Melodorum fruticosum* (continued)



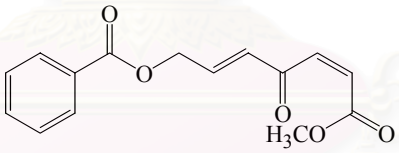
(4*Z*)-6-Benzoyloxy-7-hydroxy-2,4-heptadien-4-olide [**14**]



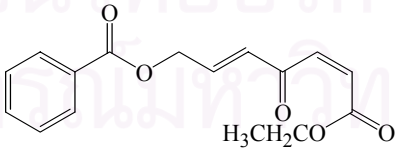
(2*E*,5*E*)-7-Benzoyloxy-1-methoxy-2,5-heptadien-1,4-dione (melodienone) [**15**]



(2*E*,5*E*)-7-Benzoyloxy-1-ethoxy-2,5-heptadien-1,4-dione (homomelodienone) [**16**]

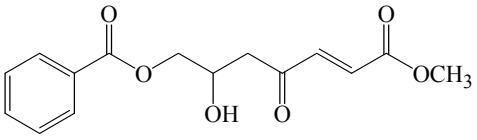


(2*Z*,5*E*)-7-Benzoyloxy-1-methoxy-2,5-heptadien-1,4-dione (isomelodienone) [**17**]

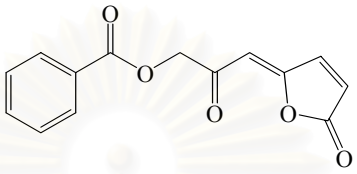


(2*Z*,5*E*)-7-Benzoyloxy-1-ethoxy-2,5-heptadien-1,4-dione (homoisomelodienone) [**18**]

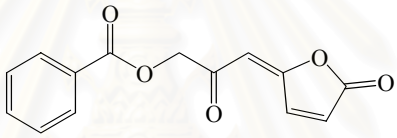
Figure 2. : Structures of chemical components of *Melodorum fruticosum* (continued)



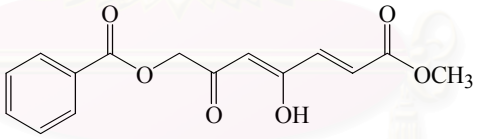
(*E*)-7-Benzoyloxy-6-hydroxy-1-methoxy-2-heptadien-1,4-dione
(6-hydroxy-5-hydromelodienone) [**19**]



(4*Z*)-7-Benzoyloxy-2,4-heptadien-6-one-4-olide
(melodorinone A) [**20**]



(4*E*)-7-Benzoyloxy-2,4-heptadien-6-one-4-olide
(melodorinone B) [**21**]



(*E*)-7-Benzoyloxy-4-hydroxy-1-methoxy-2,4-heptadien-1,6-dione
(tautomelodorinone) [**22**]

Figure 2. : Structures of chemical components of *Melodorum fruticosum* (continued)

Table 2. Bioactivity of chemical components of *Melodorum fruticosum*

Compound	Brine shrimp LC_{50} in ppm[1]	Potato disc[2]	Cytotoxicities (ED_{50} in µg/ml)										
			A-549	KB	HT-29	MCF-7	9-PS	SK-MEL-5	Melme-3M	Mel2	P-388	HT	Vero
• **Dichamanetin [1]**	3 (1/6)[a]	50/70[a]	>10[a]	5.38[a]	5.1[a]	-	-	-	-	-	-	-	-
• **Pinocembrin [2]**	12 (8/20)[a]	20/90[a]	>10[a]	>10[a]	>10[a]	-	7.53[a]	-	-	-	-	-	-
• **polycarpol [5]**	254 (153/448)[a]	-180/-70[a]	0.545[a]	-	0.127[a]	0.222[a]	-	-	-	-	-	-	-
• **Stigmasterol [6] & β-Sitosterol [7]**	110 (51/181)[a]	-10[a]	15.13[a]	-	8.1[a]	14.84[a]	-	-	-	-	-	-	-
• **Benzylbenzoate [9]**	2 (1/3)[a]	-110/30[a]	>10[a]	>10[a]	>10[a]	-	>10[a]	-	-	-	-	-	-
• **(4*Z*)- Melodorinol [10]**	-	-	5.89[c] 7.9[d]	2.3[d]	2.87[c] 2.6[d]	1.99[c] 2.4[d]	-	3.75[c]	3.32[c]	0.75[d]	0.35[d]	2.2[d]	-
• **(4*E*)- Melodorinol [11]**	-	-	2.6[d]	2.0[d]	1.7[d]	2.7[d]	-	-	-	0.92[d]	0.36[d]	3.7[d]	-
• **(4*Z*)- Acetylmelodorinol [12]**	246 (163/383)[b]	-	2.89[c] 2.74[b] 2.2[d]	1.2[d]	1.96[c] 0.304[b] 1.3[d]	2.38[c] 0.408[b] 2.2[d]	-	4.21[c]	2.74[c]	-	0.08[d]	1.8[d]	-
• **(4*E*)- Acetylmelodorinol [13]**	-	-	1.1[d]	0.49[d]	2.0[d]	2.2[d]	-	-	-	1.6[d]	0.14[d]	1.7[d]	-
• **(4*Z*)-6-Benzoyloxy-7-hydroxy-2,4-heptadien-4-olide [14]**	-	-	2.5[d]	2.6[d]	2.4[d]	3.9[d]	-	-	-	0.58[d]	0.33[d]	2.0[d]	-
• **Melodienone[15]**	24 (15/37)[b]	-	8.92[b]	-	3.85[b]	4.43[b]	-	-	-	-	-	-	-
• **Homomelodienone [16]**	-	-	36.91[c]	-	25.61[c]	37.88[c]	-	-	-	-	-	-	-
• **Isomelodienone [17]**	10 (3/19)[b]	-	1.69[b]	-	0.514[b]	0.171[b]	-	-	-	-	-	-	-
• **Homoisomelodienone [18]**	-	-	6.70[c]	-	4.26[c]	36.32[c]	-	-	-	-	-	-	-
• **6-Hydroxy-5-hydromelodienone[19]**	-	-	3.28[c]	-	1.92[c]	0.253[c]	-	1.04[c]	1.10[c]	-	-	-	-
• **Melodorinone A [20]**	-	-	-	>20[e]	-	5.0[e]	-	-	-	-	4.54[e]	-	15[e]
• **Melodorinone B [21]**	-	-	-	>20[e]	-	>20[e]	-	-	-	-	-	-	5[e]
• **Tautomelodorinone [22]**	-	-	-	>20[e]	-	7.5[e]	-	-	-	-	0.60[e]	-	7.5[e]

[1] 95% confidence interval in parentheses.

[2] % inhibition, (-) values indicate tumour promoting effects.

[a] Jung *et al.*, 1990b

[b] Jung *et al.*, 1990a

[c] Jung *et al.*, 1991

[d] Tuchinda *et al.*, 1991

[e] Tiyawornan, 1998

- The test was not carried out.

Tumor Cell lines:

A-549[*] (Lu1[**])	=	Human lung carcinoma
KB[**] (KBMRI[*])	=	Human nasopharyngeal carcinoma
HT-29[*] (Col2[**])	=	Human colon adrenocarcinoma
MCF-7[*] (BC1[**])	=	Human breast carcinoma
9-PS	=	A chemical-induced murine lymphatic leukemia
SK-MEL-5	=	Human melanoma, metastasis of axillary node
Melme-3M	=	Human melanoma, metastasis of lung
Mel2	=	Human melanoma, not specific
P-388	=	Murine lymphatic leukemia
HT	=	Human fibrosacoma (HT-1080)
Vero	=	African green monkey kidney cell line

[*] Jung *et al.*, 1990a and 1990b; Jung *et al.*, 1991

[**] Tuchinda *et al.*, 1991

CHAPTER III
EXPERIMENTAL

1. Source of Plant Material

The root of *Melodorum fruticosum* Lour. was collected from Khao Hin Son Botanic Garden, Amphur Phanomsarakham, Chachoengsao Province, Thailand in October 1999, whereas the seeds were collected in April 2000. This plant was identified by comparison with the herbarium specimen at The Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

2. General Techniques

2.1. Analytical Thin Layer Chromatography (TLC)

Technique : One dimension, ascending
Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness : 0.2 mm
Distance : 6 cm
Temperature : Laboratory temperature (30-35°C)
Detection : 1. Ultraviolet light at wavelengths of 254 and 365 nm
2. 10% Sulfuric acid in ethanol and heated at 105°C for 10 minutes

2.2. Preparative Centrifugal Thin Layer Chromatography

Instrument model : 1. Chromatotron Model 7924T
2. Lab Pump Model RH
Adsorbent : Silica gel 60 PF_{254} with Calcium Sulfate (E. Merck)
Layer thickness : 2 mm
Flow rate : 6 ml/min
Temperature : Laboratory temperature (30-35°C)
Detection : Ultraviolet light at wavelengths of 254 and 365 nm employed to detect bands of UV absorbing compounds on the adsorbent layer of the rotor.

2.3. Column Chromatography (CC)

2.3.1. Quick Column Chromatography

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

Packing method : Dry packing

Sample loading : The sample was dissolved in a small amount of organic solvent, mixed with kieselguhr, triturated, dried and then placed gently on top of the column

Detection : Fractions were examined by TLC observing under UV light at the wavelengths of 254 and 365 nm

2.3.2. Flash Column Chromatography

Adsorbent : Silica gel 60 (No. 7734)
particle size 0.063-0.200 nm
Silica gel 60 (No. 9385)
particle size 0.040-0.063 nm

Packing method : Wet and dry packing

Sample loading : For wet packing, the sample was dissolved in a small amount of eluant, then applied gently on the top of the column

For dry packing, the sample was dissolved in a small volume of the optimal organic solvent, mixed with a small amount of adsorbent, triturated, dried, and then gently placed on top of the column.

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

2.3.3. Gel Filtration Chromatography

Gel filter : Sephadex LH-20 (Pharmacia)
MCI gel CHP20P (75~150μ) high porous polymer (Mitsubishi Chemical Corporation)

Packing method : The gel filter was dispersed in the eluant and left standing for gel initiation for about 24 hours before use.

Sample loading : The sample was dissolved in a small amount of eluant, then applied gently on top of the column

Detection : 1. Ultraviolet light at wavelengths of 254 and 365 nm employed to detect bands of UV absorbing compounds
2. Fractions were examined in the same manner as described in section 2.3.1

2.4. Spectroscopic Techniques

2.4.1. Ultraviolet (UV) Absorption Spectra

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

2.4.2. Infrared (IR) Absorption Spectra

IR spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrophotometer in the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Samples were prepared as KBr pellets

2.4.3. Mass Spectra (MS)

Electron Impact Mass Spectra (EIMS) were obtained on a FISONS VG TRIO 2000 mass spectrometer at the Department of Chemistry, Faculty of Sciences, Chulalongkorn University, at 70 eV.

2.4.4. Nuclear Magnetic Resonance (NMR) Spectra

^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker AVANCE DPX-300 FT-NMR spectrometer at the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University. ^{1}H NMR spectra were recorded at 500 MHz spectrometer, on a JEOL JMN-A500 (Alpha series) at The Scientific and Technology Research Equipment Center, Chulalongkorn University. Deuterated chloroform was used as the NMR solvent in this study. Spectral data were reported in ppm scale using the solvent chemical shift as the reference frequency.

2.5. Physical Property Measurement Apparatus

2.5.1. Melting Points

Melting points were determined on a Gallenkamp Melting Point Apparatus at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2.5.2. Optical Rotations

Optical rotations were measured on a Perkin-Elmer Polarimeter model 341 at the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2.5.3. Elemental Components

Elemental components of the compounds were analyzed on a Perkin-Elmer PE2400 Series II CHNS/O Analyzer (option CHN) at the Scientific and Technological Research Equipment Center, Chulalongkorn University, by the method of pyrolysis in high-purity oxygen (static-state oxidation) and quantitatively detected by thermal conductivity detector.

2.6. Solvents

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

3. Bioassay

Bioassay of cytotoxic activity against Human breast cancer (BT 474), Human lung cancer (CHAGO), Human gastric cancer (KATO-3), Human hepatoma (HEP-G2), Human skin fibrobrast (Hs27), and Human colon cancer (SW620) cell cultures in vitro were performed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method (Twentyman, 1987; Carmicheal, 1987).

4. Extraction and Isolation

4.1. Extraction of the root and seeds of *Melodorum fruticosum* Lour.

4.1.1. Extraction of the root

The dried-powdered root of *Melodorum fruticosum* (3 kg) was macerated with ethyl acetate (3 x 3 L), and methanol (3 x 3 L) successively. The obtained extract was evaporated under reduced pressure at temperature about 50 ^{o} C to give 79.58 g of ethyl acetate extract (2.65% of dry weight of the root) and 50.84 g of methanol extract (1.69% of dry weight of the root).

4.1.2. Extraction of the seeds

The dried-powdered seeds of *Melodorum fruticosum* (1.2 kg) were macerated with hexane (3 x 3 L), ethyl acetate (3 x 3 L), and methanol (3 x 3 L), successively. The filtrates were evaporated under reduced pressure at temperature about 50 ^{o} C by rotary evaporator to give 88.81 g of hexane extract (7.42% w/w of the seeds), 93.93 g of ethyl acetate extract (7.85% w/w), and 52.56 g of methanol extract (4.39% w/w) .

4.2. Isolation

4.2.1. Isolation of compound L-1

The ethyl acetate extract of the root (79.58 g) was dissolved in a small amount of chloroform, triturated with kieselguhr and dried under reduced pressure. It

was then fractionated by quick column chromatography using a sintered glass filter column of silica gel 60 (No. 7734) 600 g. Elution was performed in polarity gradient manner with mixtures of hexane and ethyl acetate as the solvents by increasing ethyl acetate from 10% ethyl acetate in hexane to 100% ethyl acetate (approximately 500 ml per fraction). The eluants were examined by TLC using 40% ethyl acetate in hexane as developing solvent. Fractions with similar chromatographic pattern were combined to yield six fractions, as shown in Table 2.

Table 3. Combination of fractions from quick column chromatography of the ethyl acetate extract (79.58 g) of the root.

Fraction code	Number of fraction	Weight (g)
RE1	1-2	3.69
RE2	3-7	4.41
RE3	8-9	10.23
RE4	10-16	27.93
RE5	17-25	21.80
RE6	26-30	6.95

Fraction RE3 (10.23g) yielded 6.85 g of a residue. The mother liquor was removed and the residue was recrystallized in chloroform to give 5.94 g of compound L-1 as a colorless needle crystals (7.46% of the ethyl acetate extract of the root).

4.2.2. Isolation of compound L-2

Fraction RE5 (21.7964 g) was further chromatographed by column chromatography using silica gel 60 (No. 9385) as adsorbent. Gradient elution from 10% ethyl acetate in hexane to 100% ethyl acetate was performed (approximately 50 ml per fraction). Eleven fractions (RE501-RE511) were obtained, as shown in Table 3.

Table 4. Fractionation of the fraction RE5 (21.7964 g) by column chromatography

Fraction code	Number of fraction	Weight (g)
RE501	1-10	0.4280
RE502	11-20	0.0738
RE503	21-24	0.2200
RE504	25-36	1.2358
RE505	37-44	0.3194
RE506	45-56	1.0052
RE507	57-64	1.8208
RE508	65-75	2.2780
RE509	76-90	1.1592
RE510	91-113	2.0995
RE511	114-130	8.7428

Fraction RE502 (0.0738 g) was recrystallized in a mixture of chloroform and ethyl acetate, to furnish compound L-2 as colorless needle crystals, (45.3 mg, 0.0569% of the ethyl acetate extract of the root).

4.2.3. Isolation of compound L-3

Fraction RE4 (27.9324 g) yield crystals in ethyl acetate at room temperature. The mother liquor was thereafter removed and the crystals were recrystallized in a mixture of ethyl acetate and hexane to obtain compound L-3 (15.15 g) as colorless prism-like crystals (19.04% of the ethyl acetate extract of the root).

In addition, compound L-3 could be obtained from the ethyl acetate extract of the seeds (93.93 g). After evaporating under vacuum, 47.91 g of the solid matter and 46.02 g of mother liquor were obtained. The solid matter was recrystallized in a mixture of acetone and hexane to yield 46.43 g of a similar crystal (49.43% of the ethyl acetate extract of the seeds).

4.2.4. Isolation of compound L-4

The mother liquor from the ethyl acetate extract of the seeds was combined and evaporated under reduced pressure to yield 46.02 g of ethyl acetate extract of the seeds. It was then separated by a column of silica gel 60 (No.7734) 600 g as the stationary adsorbent, using hexane and ethyl acetate as a mobile solvent performed in a polarity gradient manner by increasing ethyl acetate from from 10% ethyl acetate in hexane to 100% ethyl acetate (approximately 100 ml per fraction). Eight fractions (SE1-SE8) were obtained, as shown in Table 4.

Table 5. Isolation of the mother liquor of ethyl acetate extract of the seeds by column chromatography

Fraction code	Number of fraction	Weight (g)
SE1	1-10	5.27
SE2	11-23	3.15
SE3	24-33	10.63
SE4	34-45	5.26
SE5	46-71	10.68
SE6	72-95	3.10
SE7	96-115	1.91
SE8	116-130	3.49

Fraction SE6 (3.10 g) was further fractionated on a column chromatography using a silica gel 60 (No. 9385) 150 g as the adsorbent. Gradient elution from 10% ethyl acetate in hexane to 100% ethyl acetate was performed (approximately 50 ml per fraction). The eluants were combined to yield six fractions, as shown in Table 5.

Table 6. Isolation of fraction SE6 (3.1003 g) by column chromatography

Fraction code	Number of fraction	Weight (g)
SE61	1-8	0.0568
SE62	9-13	0.0843
SE63	14-18	0.0684
SE64	19-26	0.5746
SE65	27-40	1.9782
SE66	41-70	0.0345

Fraction SE65 (1.9782 g) was then purified by gel filtration chromatography using a column of Sephadex-LH 20 (100 g, 2.5 x 80 cm) with 10% methanol in ethyl acetate as the solvent. Four fractions were collected by UV light employed to detect bands and also examined by TLC using hexane: ethyl acetate (1 : 1) as developing solvent. From the third fraction (SE653) 1.5842 g of a yellow oil of compound L-4 (1.67% of ethyl acetate of the seeds) was obtained.

Furthermore, compound L-4 could be isolated from fraction RE508 (2.2780 g) of the root by preparative centrifugal thin layer chromatography (Chromatotron). Isocratic elution by hexane : ethyl acetate (3 : 2) was performed. The eluants were collected by employing UV light at wavelength of 254 and 365 nm to detect bands of UV absorbing compounds on the adsorbent layer, and also analysed by TLC technique. The fourth of five fractions (RE5084) gave compound L-4 at 2.45% of the weight ethyl acetate extract of the root (1.9520 g).

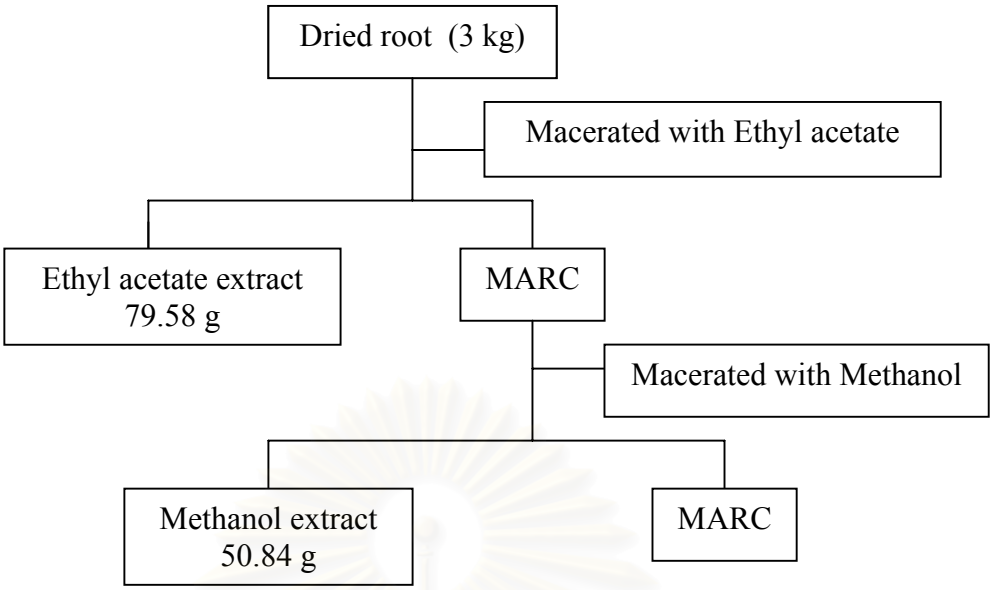
4.2.5. Isolation of compound L-5

The hexane extract (88.81 g) of the seeds was divided into two portions: A (42.53 g) and B (46.28 g), which then were separated by two column of silica gel 60 (No. 7734) 600 g as a stationary adsorbent, using hexane and ethyl acetate as a mobile solvent performed in a polarity gradient manner by increasing ethyl acetate from from 10% ethyl acetate in hexane to 100% ethyl acetate (approximately 100 ml per fraction). Each eluate obtained from the column were analysed by TLC using hexane: ethyl acetate (3:2) as the developing system. Fractions with similar chromatographic pattern were combined to give as eight fractions, as shown in Table 6.

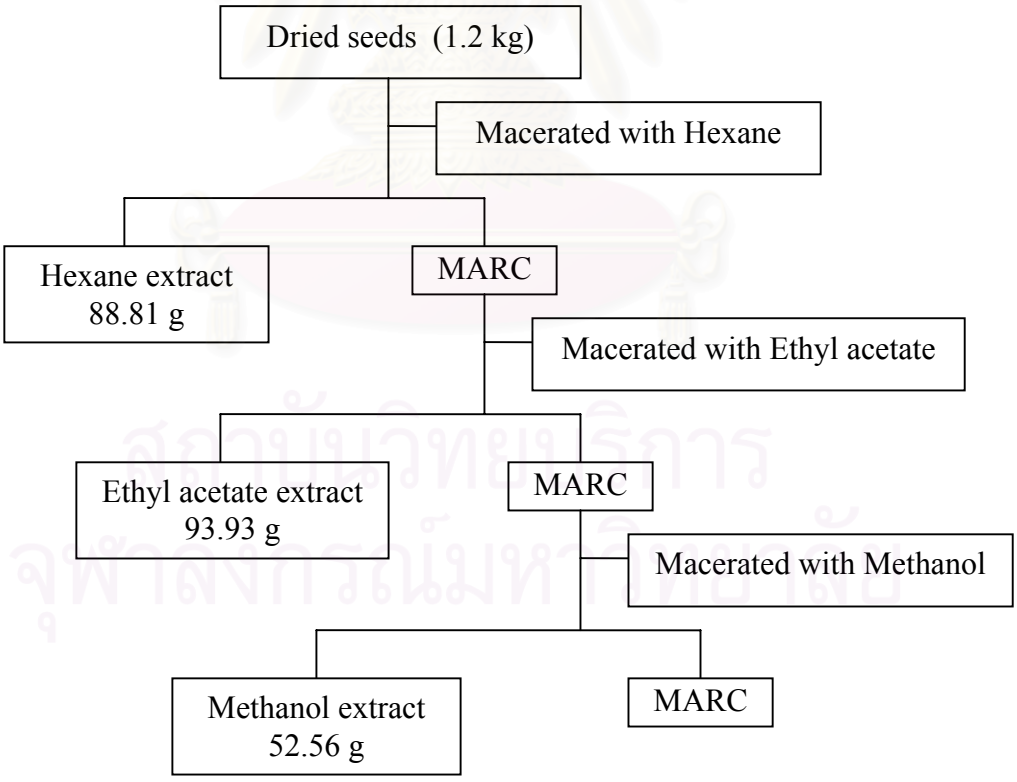
Table 7. Combination of fractions from column chromatography of hexane extract (88.81 g) of the seeds

Fraction code	Number of fraction	Total weight (g)
SH1	1-13A 1-11B	2.1976
SH2	14-20A 12-24B	9.9831
SH3	21-37A 25-40B	50.1587
SH4	38-45A 41-47B	4.3724
SH5	46-64A 48-66B	6.7082
SH6	65-74A 67-75B	0.9625
SH7	75-85A 76-92B	7.6381
SH8	86-120A 93-120B	3.1594

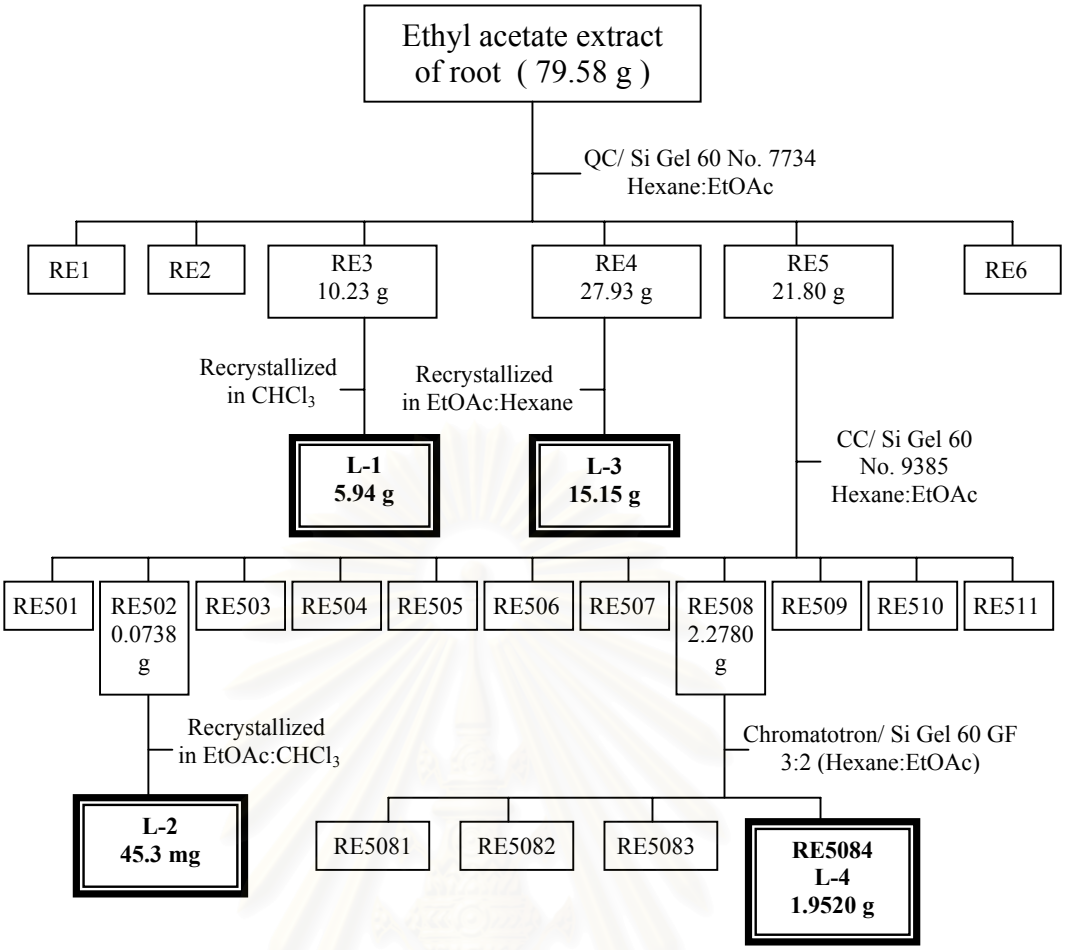
Fraction SH6 (0.9625 g) was further separated by chromatotron using silica gel 60 GF as the adsorbent and hexane: ethyl acetate (3:2) as the isocratic eluant. The eluates were collected by employing ultraviolet light to detect bands of compounds and also examined by TLC pattern. The fractions were combined to yield five fractions (SH61-SH65). The second fraction (SH62) gave 624 mg of yellow oil, compound L-5 (0.70% of hexane extract of the seeds).



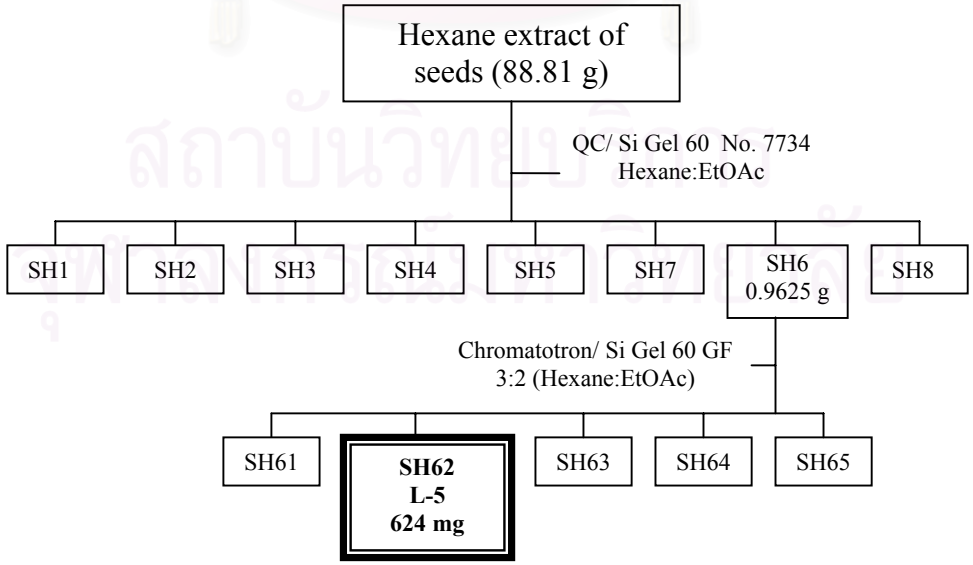
Scheme 1. Extraction scheme of *Melodorum fruticosum* root



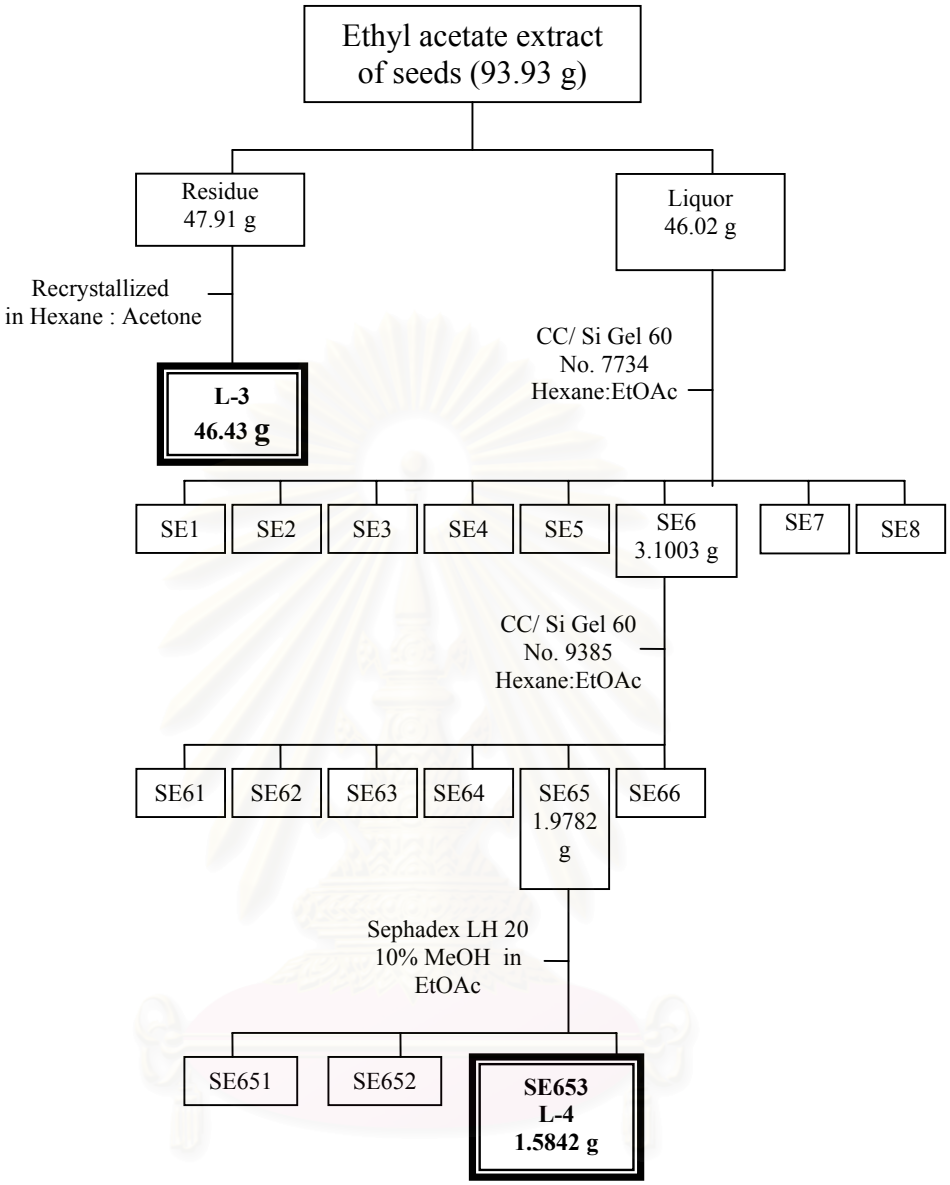
Scheme 2. Extraction scheme of *Melodorum fruticosum* seeds



Scheme 3. Isolation scheme of ethyl acetate extract of root



Scheme 4. Isolation scheme of hexane extract of seeds



Scheme 5. Isolation scheme of ethyl acetate extract of seeds

Physical and Spectral Data of Isolated Compounds

1. Compound L-1

The compound L-1 was obtained as colorless needle crystals.

UV : λ_{max} nm (log ε), in MeOH; Figure 8
243 (3.12)

IR : ν_{max} cm^{-1}, KBr disc; Figure 9
3377, 2933, 1640, 1445, 1374 , 1037

EIMS : *m/z* (% relative intensity); Figure 10
440 [M^+] (100), 425 (7), 407 (20), 379 (6), 356 (8), 353(10), 329 (7), 328 (8), 327 (13), 311 (11), 272 (15), 239 (17), 227 (25), 119 (35), 105 (24), 69 (56), 55 (61)

^{1}H-NMR : δ in ppm (multiplicity, *J* in Hz), 500 MHz, in $CDCl_3$; Figure 11
0.72-2.40 (overlapping), 0.54 (s), 0.81 (s), 0.82 (d, *J* = 5.2 Hz), 0.87 (s), 0.91 (s), 0.94 (s), 0.98 (s), 1.53 (s), 1.62 (s), 3.18 (dd, *J* = 4.3, 11.6 Hz), 4.20 (dd, *J* = 10.0, 6.0 Hz), 5.02 (tt, *J* = 7.0, 1.5 Hz), 5.24 (d, *J* = 6.1 Hz), 5.78 (d, *J* = 6.7 Hz)

^{13}C-NMR : δ in ppm, 75 MHz, in $CDCl_3$; Figure 12
15.8, 15.9, 17.1, 17.6, 18.4, 22.8, 22.9, 24.9, 25.7, 27.8, 28.1, 35.7, 35.8, 36.2, 37.4, 38.5, 38.7, 40.1, 44.3, 48.9, 48.9, 51.9, 74.8, 78.9, 116.1, 121.3, 124.9, 131.2, 140.9, 146.1

2. Compound L-2

The compound L-2 was obtained as colorless needle crystals.

Elemental Components : C : H: O ; 79.69 : 10.40 : 9.94

Melting Point : 177-180 ^{o} C

$[\alpha]^{25}_{D}$: + 98 ($CHCl_3$; c 0.1)

UV : λ_{max} nm (log ε), in MeOH; Figure 18
243 (3.12)

IR : ν_{max} cm^{-1}, KBr disc; Figure 19
3436, 2929, 1723, 1629, 1374, 1248, 1029

EIMS : *m/z* (% relative intensity); Figure 20
482 [M^+] (100), 449 (10), 407 (20), 389 (10), 369 (20), 353 (20), 315 (11), 272 (14), 239 (12), 227 (23), 119 (32), 105 (26), 69 (61), 55 (48)

^{1}H-NMR : δ in ppm (multiplicity, *J* in Hz), 500 MHz, in $CDCl_3$; Figure 21
1.00-2.40 (overlapping), 0.59 (s), 0.85 (d, *J* = 6.4 Hz), 0.86 (s), 0.91 (s), 0.93 (s), 0.98 (s), 1.58 (s), 1.66 (s), 2.04 (s), 4.24 dd, *J* = 9.6, 5.7 Hz), 4.49 (dd, *J* = 4.4, 11.4 Hz), 5.06 (tt, *J* = 7.2, 1.4 Hz), 5.28 (d, *J* = 6.1 Hz), 5.82 (d, *J* = 6.4 Hz)

^{13}C-NMR : δ in ppm, 75 MHz, in $CDCl_3$; Figure 22
17.6, 18.4, 21.3, 22.8, 22.9, 24.2, 24.9, 25.7, 28.1, 35.4, 35.8, 36.2, 37.3, 37.6, 38.5, 40.1, 44.3, 48.7, 49.1, 51.9, 74.8, 80.8, 116.3, 121.0, 124.9, 131.2, 140.9, 145.8, 170.9

3. Compound L-3

The compound L-3 was obtained as colorless prism-like crystals.

UV : λ_{max} nm (log ε), in MeOH; Figure 28
267 (3.15)

IR : ν_{max} cm^{-1}, KBr disc; Figure 29
3070, 2944, 1775, 1738, 1719, 1679, 1561, 1450, 1365, 1285, 1266, 1229

EIMS : *m/z* (% relative intensity); Figure 30
302 [M^+] (20), 272 (10), 243 (70), 242 (21), 230 (6), 215 (7), 180 (30), 138 (63), 125 (31), 110 (14), 105 (100), 97 (9), 77 (77), 51 (23)

^{1}H-NMR : δ in ppm (multiplicity, *J* in Hz), 300 MHz, in $CDCl_3$; Figure 31
2.02 (s), 4.43 (dd, *J* = 11.8, 6.1 Hz), 4.50 (dd, *J* = 11.8, 4.1 Hz), 5.31 (d, *J* = 8.1 Hz), 6.08 (ddd, *J* = 8.1, 6.1, 4.1 Hz), 6.20 (d, *J* = 5.4 Hz), 7.36 (d, *J* = 5.4 Hz), 7.37 (m), 7.50 (m), 7.95 (m)

^{13}C-NMR : δ in ppm, 75 MHz, in $CDCl_3$; Figure 32
20.6, 64.4, 66.9, 108.6, 121.3, 128.2, 129.2, 129.4, 133.0, 143.3, 150.5, 165.7, 168.3, 169.5

4. Compound L-4

The compound L-4 was obtained as yellow oil.

UV : λ_{max} nm (log ε), in MeOH; Figure 38
270 (3.06)

IR : ν_{max} cm^{-1}, KBr disc; Figure 39
3457, 3075, 2954, 1779, 1750, 1718, 1274, 1114, 1070

EIMS : *m/z* (% relative intensity); Figure 40
260 [M^+](4), 243 (33), 230 (83), 215 (6), 138 (79), 125(60), 110(24), 105(100), 97(48), 77(82), 51(65)

^{1}H-NMR : δ in ppm (multiplicity, *J* in Hz), 300 MHz, in $CDCl_3$; Figure 41
3.42 (br), 4.39 (d, *J* = 5.1 Hz), 5.12 (dq, *J* = 8.4, 5.1 Hz), 5.39 (d, *J* = 8.4 Hz), 6.16 (d, *J* = 5.4 Hz), 7.33 (d, *J* = 5.4 Hz), 7.36 (t, *J* = 7.2 Hz), 7.50 (t, *J* = 7.2 Hz), 7.97 (t, *J* = 7.2 Hz)

^{13}C-NMR : δ in ppm, 75 MHz, in $CDCl_3$; Figure 42
65.3, 67.3, 113.4, 120.7, 128.3, 129.3, 129.6, 133.2, 143.8, 149.8, 166.5, 169.1

5. Compound L-5

The compound L-2 was obtained as pale-yellow oil.

UV : λ_{max} nm (log ε), in MeOH; Figure 48
269 (2.92)

IR : ν_{max} cm^{-1}, KBr disc; Figure 49
3076, 2957, 1790, 1765, 1735, 1724, 1274, 1228, 1113

EIMS : *m/z* (% relative intensity); Figure 50
302 [M^+] (20), 272 (18), 243 (70), 242 (21), 230 (6), 215 (7), 180 (45), 138 (51), 125 (29), 110 (14), 105 (100), 97 (9), 77 (77), 51 (33)

^{1}H-NMR : δ in ppm (multiplicity, *J* in Hz), 300 MHz, in $CDCl_3$; Figure 51
2.07 (s), 4.46 (dd, *J* = 11.7, 6.6 Hz), 4.48 (dd, *J* = 11.7, 4.2 Hz), 5.70 (d, *J* = 10.2 Hz), 5.94 (ddd, *J* = 10.2, 6.6, 4.2 Hz), 6.31

(dd, J = 5.4, 1.5 Hz), 7.43 (t, J = 7.5 Hz), 7.56 (t, J = 7.5 Hz), 7.87 (d, J = 5.4 Hz), 7.98 (d, J = 7.5 Hz)

^{13}C-NMR : δ in ppm, 75 MHz, in $CDCl_3$; Figure 52

20.9, 65.0, 66.8, 107.5, 122.5, 128.5, 129.2, 129.6, 133.4, 140.2, 153.4, 165.9, 168.6, 170.0

CHAPTER IV
RESULTS AND DISCUSSION

The dried root of *Melodorum fruticosum* Lour. (3 kg) was extracted with ethyl acetate. The ethyl acetate extract was then separated by several chromatographic techniques to afford four compounds (L-1, L-2, L-3, and L-4).

The dried seeds of this plant (1.2 kg) were successively extracted with hexane, ethyl acetate, and methanol. The ethyl acetate extract was then fractionated by extensive repeated chromatography to afford two compounds (L-3 and L-4). The hexane extract was further separated by various chromatographic techniques to afford one compound (L-5).

The structure determinations of all of the isolated compounds were performed by interpretation of their spectroscopic data (UV-VIS, IR, MS, and NMR), and subsequently confirmed by comparison with reported values.

Structure Determination of Isolated Compounds

1. Structure Determination of Compound L-1

Compound L-1 was obtained a colorless needle crystals. The compound gave a violet color to anisaldehyde-sulfuric acid reagent and a pink color to Libermann-Burchard test. The UV of compound L-1 (Figure 8) displayed maximum absorption at 243 (log $\varepsilon = 3.12$) nm, indicating the presence of a transoid heteroannular diene group in the molecule. The IR spectrum of compound L-1 (Figure 9) revealed absorption bands at 3377 (O-H stretching), 2933 (=C-H stretching, aliphatic), 1445 (C-O stretching), 1440 (C-H stretching, methylene), and 1374 (C-H stretching, methyl) cm^{-1}. The EIMS of compound L-1 (Figure 10) exhibited a molecular ion $[M]^+$ at *m/z* 440 consistent with the molecular formula $C_{30}H_{48}O_2$. The degree of unsaturation calculated for the molecular formula $C_{30}H_{48}O_2$ is seven.

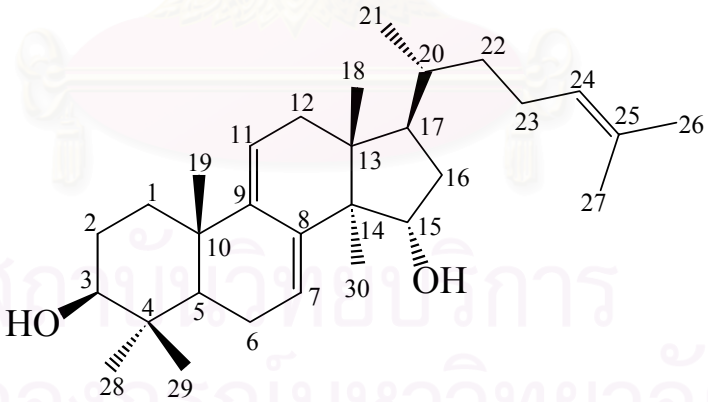
The ^{1}H NMR spectrum of compound L-1 (Figure 11) indicated the presence of three olefinic protons at δ 5.78 (1H, d, *J* = 6.7 Hz; H-7), 5.24 (1H, d, *J* = 6.1 Hz; H-11), and 5.02 (1H, tt, *J* = 7.0, 1.5 Hz; H-24) ppm; two oxygen-bearing methine protons at δ 4.20 (1H, dd, *J* = 10.0, 6.0 Hz; H-15β), and 3.18 (1H, dd, *J* = 11.6, 4.3 Hz; H-3α) ppm; two geminal olefinic methyl groups at δ 1.62 (3H, s; H-26) and 1.53 (3H, s; H-27) ppm; five tertiary methyl groups at δ 0.94 (3H, s; H-28), 0.81 (3H, s; H-29), 0.91 (3H, s; H-19), 0.87 (3H, s; H-30), and 0.54 (3H, s; H-18) ppm; a secondary methyl group at δ 0.82 (3H, d, *J* = 5.2 Hz; H-21) ppm. The signals at δ 0.72-2.30 ppm were the signals of methylene and methine protons.

The ^{13}C NMR spectrum (Figure 12) and DEPT (Figure 13) exhibited thirty carbon resonances, which revealed the presence of eight methyl carbons, seven methylene carbons, eight methine carbons, and seven quaternary carbons.

The compound L-1 gave violet color to anisaldehyde-sulfuric acid reagent, pink color to Libermann-Burchard test, and its ^{13}C NMR spectrum exhibited thirty carbon signals, therefore suggested that it possessed a triterpenoid nucleus. The fragment ion peak in the EIMS spectrum at *m/z* 329 represented the loss of an eight carbons unit (C_8H_{15}) due to the cleavage of the bond between C-17 and C-20, which was rather characteristic of a lanostane-type triterpene (Tanaka *et al.*, 1991). The assignments of the carbon resonances were achieved by the HMQC experiment (Figure 16). The ^{13}C NMR signals at δ_C 121.3, 140.9, 146.1, 116.1 ppm and the ^{1}H NMR signals at δ_H 5.78 , 5.24 ppm indicated that the two double bonds were positioned between C-7 and C-8 and between C-9 and C-11, such as two endo-double bonds. In the HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-1 (Figure 3 and 17), correlations between H-19 to C-9, H-12 to C-9, H-12 to C-11, and H-30 to C-8 also supported this suggestion. Thus, compound L-1 was a 7,9(11) diene lanostane-type triterpenoid (Li *et al.*, 1993; Tai *et al.*, 1995; Tripathi *et al.*, 1996). The resonance of one hydrogen at δ_H 3.43 ppm corresponded to H-3. An NOE effect (Figure 14) between H-28 (δ_H 0.94 ppm, irr.) and H-3 (δ_H 3.18 ppm, enh. 1.31 %) indicated that H-3 was α-oriented and therefore the 3-OH was a β-OH. An additional hydroxyl was positioned on ring D (possible position either 15-OH or 16-OH), since the MS peak at *m/z* 273 which included only one hydroxyl (3-OH) was due to the cleavage of ring D (Lue *et al.*, 1998). In the ^{1}H-^{1}H COSY spectrum of compound

L-1 (Figure 15), the correlation between H-20 (δ_H 1.30 ppm) and a methine proton (H-17 at δ_H 1.57 ppm) was determined, then the corresponding carbon (C-17) was assigned at δ_C 48.9 ppm through the HMQC experiment, thus inferring that the hydroxyl was not positioned at C-16, but at C-15. This suggestion was also supported by the ^{1}H-^{1}H COSY spectrum in which H-17 correlated with the protons of a methylene (at δ_H 1.64 and 1.89 ppm; H-16). Based on the above analysis, the other hydroxyl has been placed at C-15 and the NOE effect between H-18 (δ_H 0.54 ppm, irr.) and H-15 (δ_H 4.20 ppm, enh. 2.33%) indicated that the 15-OH was α-oriented. Furthermore, observation of the NOE between H-18 (δ_H 0.54 ppm, irr.) and H-20 (δ_H 1.30 ppm, enh. 2.98%) confirmed the configuration of the side chain at C-17 as β-oriented.

By comparison with previous report, compound L-1 was identified as polycarpol (lanosta-7,9(11),24-trien-3β,5α-diol) **[5]** (Jung *et al*., 1990). Polycarpol has been isolated from several plants in the family Annonaceae. So far, it has never been isolated from plants belonging to families other than Annonaceae, including neighbouring families such as Lauraceae, Monimiaceae, and Menispermaceae, therefore this triterpene may be a useful chemotaxonomic marker (Leboeuf *et al.*, 1982).



[5]

Table 8. ^{1}H-NMR (500 MHz in $CDCl_3$) and ^{13}C-NMR (75 MHz in $CDCl_3$) spectral data of compound L-1 and ^{1}H-NMR (200 MHz in $CDCl_3$) and ^{13}C-NMR (50.2 MHz in $CDCl_3$) spectral data of polycarpol (Jung *et al.*, 1990)

Position	Compound L-1		Polycarpol	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1 α β	1.37 (dt, 13.4, 3.97) 1.94 (m)	35.7	1.39 (dt, 13, 4) 1.99 (td, 13, 3)	35.7
2 α β	1.67 (m) 1.59 (m)	27.8	1. 70 (ddd, 14, 8, 4) 1.60 (ddd, 14, 11, 3)	27.8
3α	3.18 (dd, 11.6, 4.3)	78.9	3.21 (dd, 11, 4)	78.9
4	-	38.7	-	38.7
5	1.02 (dd, 11.9, 3.9)	48.9	1.02 (dd, 12, 4)	48.9
6 α β	2.07 (dd, 6.7, 3.7) 2.09 (dd, 6.7, 3.7)	22.9	*ca.* 2.0 *ca.* 2.0	22.9
7	5.78 (d, 6.7)	121.3	5.81 (d, 6)	121.3
8	-	140.9	-	140.8
9	-	146.1	-	146.1
10	-	37.4	-	37.4
11	5.24 (d, 6.1)	116.1	5.27 (d, 6)	116.0
12 α β	2.22 (br.d, 18.0) 1.99 (dd, 18.0, 6.2)	38.5	2.20 (d, 17) 2.00 (dd, 17, 6)	38.5
13	-	44.4	-	44.3
14	-	51.9	-	51.9
15β	4.20 (dd, 10.0, 6.0)	74.8	4.24 (dd, 9, 6)	74.7
16 α β	1.64 (m) 1.89 (m)	40.1	1.65 (d, 13) 1.88 (dd, 13, 8)	40.0
17	1.57 (m)	48.9	*ca.* 1.55	48.9
18	0.54 (s)	15.8	0.58 (s)	15.8
19	0.91 (s)	22.9	0.91 (s)	22.9
20	1.30 (m)	35.8	1.32 (td, 11, 4)	35.8
21	0.82 (d, 5.2)	18.4	0.85 (d, 5)	18.4
22	0.98 (m)	36.2	0.98 (m)	36.2
23	1.79 (m)	24.9	1.78 (m)	24.9
24	5.02 (tt, 7.0, 1.5)	124.9	5.06 (t, 7)	124.9
25	-	131.2	-	131.1
26	1.62 (s)	25.7	1.65 (s)	25.7
27	1.53 (s)	17.6	1.57 (s)	17.6
28	0.94 (s)	28.1	0.97 (s)	28.1
29	0.81 (s)	15.9	0.85 (s)	15.9
30	0.87 (s)	17.1	0.91 (s)	17.1

Table 9. ^{1}H-NMR (500 MHz in $CDCl_3$), ^{13}C-NMR (75 MHz in $CDCl_3$), ^{1}H-^{1}H COSY and HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-1

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	^{1}H, ^{1}H-COSY	HMBC ($^{n}J_{CH}$ = 8 Hz)
1 α β	1.37 (dt, 13.4, 3.97) 1.94 (m)	35.7	H-2	C-3
2 α β	1.59 (m) 1.67 (m)	27.8	H-1, H-3α	-
3α	3.18 (dd 11.6, 4.3)	78.9	H-2	C-2
4	-	38.7	-	-
5	1.02 (dd 11.9, 3.9)	48.9	H-6	-
6 α β	2.07 (dd, 6.7, 3.7) 2.09 (dd, 6.7, 3.7)	22.9	H-5, H-6	-
7	5.78 (d, 6.7)	121.3	H-6	-
8	-	140.9	-	-
9	-	146.1	-	-
10	-	37.4	-	-
11	5.24 (d, 6.1)	116.1	H-12	-
12 α β	2.22 (br.d, 18.0) 1.99 (dd, 18.0, 6.2)	38.5	H-11	C-11, C-9
13	-	44.4	-	-
14	-	51.9	-	-
15β	4.20 (dd, 10.0, 6.0)	74.8	H-16	-
16 α β	1.64 (m) 1.89 (m)	40.1	H-15β, H-17	-
17	1.57 (m)	48.9	H-16, H-20	-
18	0.54 (s)	15.8	-	C-12, C-13, C-14, C-17
19	0.91 (s)	22.9	-	C-1, C-5, C-9, C-10
20	1.30 (m)	35.8	H-21, H-22, H-17	-
21	0.82 (d, 5.2)	18.4	H-20	C-17, C-20, C-22
22	0.98 (m)	36.2	H-20, H-23	-
23	1.79 (m)	24.9	H-22, H-24	-
24	5.02 (tt, 7.0, 1.5)	124.9	H-23	-
25	-	131.2	-	-
26	1.62 (s)	25.7	-	C-25, C-24, C-27
27	1.53 (s)	17.6	-	C-25, C-24, C-26
28	0.94 (s)	28.1	-	C-3, C-4, C-5, C-29
29	0.81 (s)	15.9	-	C-3, C-4, C-5, C-28
30	0.87 (s)	17.1	-	C-8, C-13, C-14, C-15

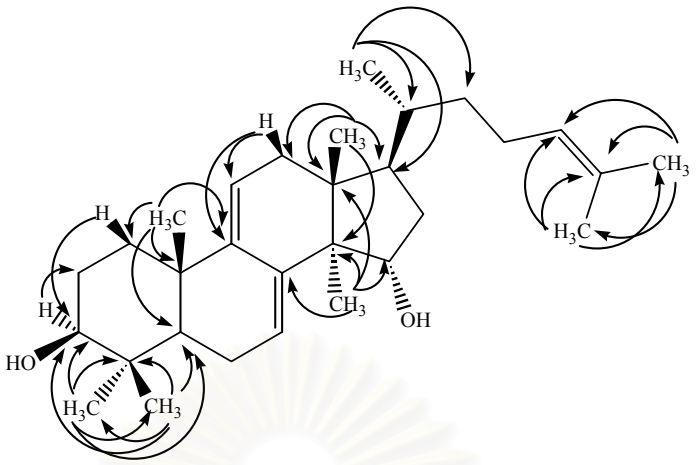


Figure 3. Long-range correlations from HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-1

2. Structure Determination of Compound L-2

Compound L-2 was obtained as colorless needles from chloroform. The compound gave violet color to anisaldehyde-sulfuric acid reagent and a pink color to Libermann-Burchard test which suggested that it was a triterpenoid nucleus. The UV spectrum of compound L-2 (Figure 18) revealed characteristic absorption at 243 (log ε = 3.12) nm, indicating the presence of a transoid heteroannular diene group in the molecule. The IR spectrum of compound L-2 (Figure 19) showed absorption bands at 3436 (O-H stretching), 2929 (=C-H stretching, aliphatic), 1723 (C=O stretching, ester), 1442 (C-H stretching, methylene), 1374 (C-H stretching, methyl), and 1248 (C-O stretching) cm^{-1}. In the EIMS, compound L-2 (Figure 20) gave a molecular ion peak $[M]^{+}$ at m/z 482 consistent with the molecular formula $C_{32}H_{50}O_3$. The degree of unsaturation calculated for the molecular formula $C_{32}H_{50}O_3$ is eight.

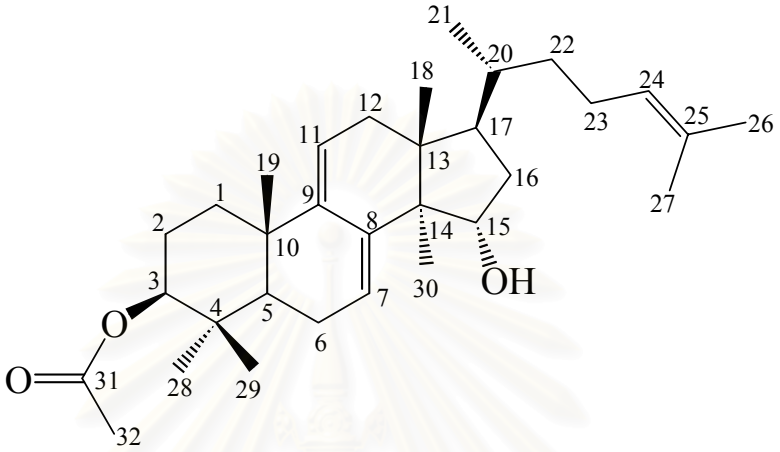
The ^{1}H-NMR spectral data of compound L-2 (Figure 21) exhibited the presence of five tertiary methyl groups at δ 0.98 (3H, s; H-19), 0.93 (3H, s; H-29), 0.91 (3H, s; H-30), 0.86 (3H, s; H-28), 0.59 (3H, s; H-18) ppm; two geminal olefinic methyl groups at δ 1.66 (3H, s; H-26), 1.58 (3H, s; H-27) ppm; one secondary methyl group at δ 0.85 (3H, d, *J* = 6.41 Hz; H-21) ppm; one acetate methyl group at δ 2.04 (3H, s; H-32) ppm. The lowfield region also showed two oxygen-bearing methine signals at δ 4.49 (1H, dd, *J*

= 11.4, 4.4 Hz; H-3α) and 4.24 (1H, dd, J = 9.6, 5.7 Hz; H-15β) ppm, and three olefinic signals at δ 5.82 (1H, d, J = 6.4 Hz; H-7), 5.28 (1H, d, J = 6.1 Hz; H-11), and 5.06 (1H, tt, J = 7.2, 1.4 Hz; H-24) ppm. The signals at δ 1.00-2.40 ppm were the signals of methylene and methine proton.

The ^{13}C-NMR (Figure 22) and DEPT (Figure 23) of compound L-2 exhibited thirty-two carbon resonances, showing the existence of nine methyl carbons, two oxygen-bearing methine carbons, three tri-substituted double bond carbons, three tertiary methine carbons, seven methylene carbons, seven quaternary carbons and an ester carbonyl carbons. The assignments of the carbon resonances were achieved by the HMQC experiment (Figure 26).

Most of the ^{1}H and ^{13}C NMR data of compound L-2 were similar to those of compound L-1(polycarpol, **[5]**), except that the chemical shift of position 3 (δ_H 4.49, δ_C 80.1 ppm) of compound L-2 was more downfield than those of compound L-1 (δ_H 3.22, δ_C 78.9 ppm), and an adition methyl signal at δ_H 2.04 ppm was presented. The ^{13}C NMR of compound L-2 also exhibited two additional signals (δ_C 21.3 and 170.9 ppm) which were assigned to C-32 (methyl) and C-31 (ester carbonyl), respectively. The HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-2 (Figure 27) showed the correlations of this methyl protons (δ_H 2.04 ppm) and ester carbon (δ_C 170.9 ppm), whereas the IR spectrum showed absorption bands at 1723 (C=O stretching, ester) cm^{-1}, which confirmed the presence of an additional acetoxy group in this molecule. Thus, the structure of compound L-2 could possibly be proposed as having an acetate group instead of a hydroxy proton at C-3 position. The long range HMBC correlation ($^{n}J_{CH}$ = 8 Hz) between H-3α (δ_H 4.49 ppm) to the acetate carbonyl carbon at δ_C 170.9 ppm (C-31) also confirmed the position of the additional acetoxy group at C-3. The β-configuration of C-3 acetoxy groups was confirmed by the large coupling constant (J = 11.44 Hz) between H-3α (δ_H 4.49 ppm) and H-2β (δ_H 1.72 ppm), and by observation of the NOE enhancement (Figure 24) between H-28 (δ_H 0.86 ppm, irr.) and H-3 (δ_H 4.49 ppm, enh. 1.16%). Furthermore, the NOE observed between H-18 (δ_H 0.59 ppm, irr.) and H-15 (δ_H 4.24 ppm, enh. 2.25%), confirmed the configuration of OH group at C-15 as α-oriented. The NOE was also observed between H-18 (δ_H 0.59 ppm, irr.) and H-20 (δ_H 1.35 ppm, enh. 3.48%), indicating the configuration of the side chain at C-17 as β-oriented.

According to its spectroscopic data and using the same approach as compound L-1, compound L-2 was assigned the structure lanosta-7,9(11),24-trien-3β-acetoxy-15α-ol **[23]**, which is a novel triterpene derivative and was trivially named acetylpolycarpol.



[23]

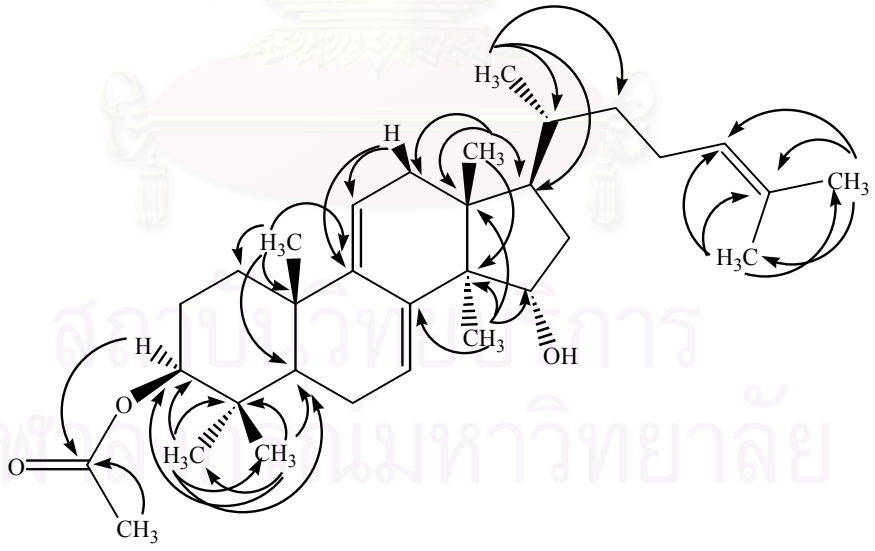


Figure 4. Long-range correlations from HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-2

Table 10. ^{1}H-NMR (500 MHz in $CDCl_3$), ^{13}C-NMR (75 MHz in $CDCl_3$), ^{1}H-^{1}H COSY and HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-2

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	^{1}H, ^{1}H-COSY	HMBC ($^{n}J_{CH}$ = 8 Hz)
1 α β	1.45 (dd, 13.43, 3.96) 1.96 (dt, 13.43, 3.36)	35.4	H-2	-
2 α β	1.70 (ddd, 13.12, 4.42, 3.96) 1.72 (ddd, 13.12, 11.44, 3.36)	24.2	H-1, H-3α	-
3 α	4.49 (dd, 4.42, 11.44)	80.1	H-2	C-31
4	-	37.6	-	-
5	1.17 (dd, 11.9, 3.96)	49.1	H-6	C-4, C-19, C-6
6 α β	2.11 (dd, 6.41, 3.96) 2.14 (dd, 6.41, 3.96)	22.6	H-5	-
7	5.82 (d, 6.41)	121.0	H-6	-
8	-	140.9	-	-
9	-	145.8	-	-
10	-	37.3	-	-
11	5.28 (d, 6.11)	116.3	H-12α, H-12β	-
12 α β	2.28 (br.d, 17.49) 2.04(dd, 17.49, 6.11)	38.5	H-11, H-12β H-11, H-12α	-
13	-	44.3	-	-
14	-	51.9	-	-
15 β	4.24 (dd, 9.62, 5.65)	74.8	H-16β, H-16α	-
16 α β	1.75 (m) 1.95 (m)	40.1	H-15β, H-17	-
17	~1.62	48.9	H-16, H-20	-
18	0.59 (s)	15.9	-	C-12, C-13, C-14, C-17
19	0.98 (s)	28.4	-	C-1, C-5, C-9, C-10
20	1.35 (m)	35.8	H-21, H-22, H-17	-
21	0.85 (d, 6.41)	18.4	H-20	C-17, C-20, C-22
22	1.03 (ddd, 9.77, 8.54, 4.88)	36.2	H-20, H-23	-
23	1.84 (m)	24.2	H-22, H-24	-
24	5.06 (tt, 7.18, 1.3)	124.9	H-23	-
25	-	131.1	-	-
26	1.66 (s)	25.7	-	C-25, C-24, C-27
27	1.58 (s)	17.6	-	C-25, C-24, C-26
28	0.86 (s)	28.1	-	C-3, C-4, C-5, C-29
29	0.93 (s)	16.8	-	C-3, C-4, C-5, C-28
30	0.91 (s)	16.9	-	C-8, C-13, C-14, C-15.
31	-	170.9	-	-
32	2.04 (s)	21.3	-	C-31

3. Structure Determination of Compound L-3

Compound L-3 was obtained as colorless crystals from ethyl acetate. The UV spectrum of compound L-3 (Figure 28) showed maximum absorption at 267 (log ε = 3.15) nm. The IR spectrum of compound L-3 (Figure 29) exhibited absorption bands at 1775 (C=O stretching, lactone carbonyl), 1738 (C=O stretching, acetate carbonyl), 1719 (C=O stretching, benzoate carbonyl), 3070 (=C-H stretching, aromatic), 2944 (=C-H stretching, aliphatic), 1561 (C=C stretching), 1450 (C-H stretching, methylene), 1365 (C-H stretching, methyl), and 1229 (C-O stretching, ester) cm^{-1}. The EIMS of compound L-3 (Figure 30) revealed a molecular ion peak $[M]^+$ at m/z 302 , corresponding to the molecular formula $C_{16}H_{14}O_6$. The degree of unsaturation calculated for the molecular formula $C_{16}H_{14}O_6$ is ten.

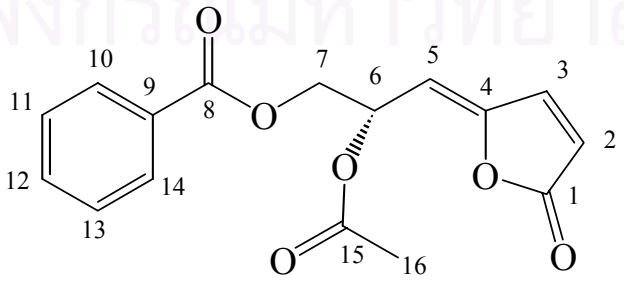
The ^{1}H-NMR spectrum (Figure 31) of compound L-3 indicated the presence of three olefinic protons at δ 5.30 (1H, d, *J* = 8.1 Hz; H-5), 6.20 (1H, d, *J* = 5.4 Hz; H-2), and 7.36 (1H, d, *J* = 5.4 Hz; H-3) ppm; one oxygen-bearing methine proton at δ 6.07 (1H, ddd, *J* = 8.1, 6.1, 4.1 Hz; H-6) ppm; one oxygen-bearing methylene proton at δ 4.43 (dd, *J* = 11.8, 6.1 Hz; H-7α) and 4.50 (dd, *J* = 11.8, 4.1 Hz; H-7β) ppm; one acetate methyl groups at δ 2.02 (3H, s; H-16) ppm; monosubstituted aromatic protons at δ 7.37 (2H, t; *meta*-H), 7.50 (1H, d; *para*-H), 7.95 (1H, d; *ortho*-H) ppm.

The ^{13}C NMR spectrum (Figure 32) and DEPT (Figure 33) exhibited sixteen carbon resonances, revealing the presence of one acetate methyl carbon, one oxygen-bearing methylene carbon, three olefinic methine carbons, one oxygen-bearing methine carbon, one oxygen-bearing quaternary carbon, three ester carbonyl carbons, five aromatic methine carbon, and one aromatic quaternary carbon.

The ^{1}H-NMR and ^{13}C-NMR of compound L-3 showed fourteen protons and sixteen carbon signals at fourteen different frequencies, suggesting the molecular formula $C_{16}H_{14}O_6$. The IR spectrum showed three carbonyl absorptions in the ester carbonyl regions, corresponding to the ^{13}C-NMR at δ_C 168.2, 169.5, 165.7 ppm, therefore the presence of one unsaturated lactone ring (1775 cm^{-1}) and two ester linkages (1738 and 1719 cm^{-1}) was assumed. The HMBC ($^nJ_{CH}$ = 8 Hz) spectrum of compound L-3 (Figure

37) represented long range coupling between methylene proton (δ_H 4.50 and 4.43 ppm) and *ortho*-proton (δ_H 7.95 ppm) to ester carbonyl at δ_C 165.7 ppm, suggesting this carbonyl was benzoyl carbonyl. The ester carbonyl at δ_C 169.5 ppm exhibited long range coupling with methine proton at δ_H 6.08 ppm, showing the connection between this ester carbonyl to the methine proton. The ^{1}H-^{1}H COSY spectrum of compound L-3 (Figure 35) established the proton connections of H-6 (δ_H 6.08 ppm) to H-7 (δ_H 4.50 and 4.43 ppm) and H-6 (δ_H 6.08 ppm) to H-5 (δ_H 5.31 ppm), thus connecting the fragment to the benzoyl moiety. Two olefinic protons (δ_H 6.20 and 7.36 ppm) coupled to each other with an unusually small coupling constant of 5.4 Hz, indicating that this double bond is part of an α,β-unsaturated-γ-lactone ring. Both the ester carbonyl (δ_C 168.3 ppm) and the quaternary carbon (δ_C 150.5 ppm), showing coupling in the HMBC ($^nJ_{CH}$ = 8 Hz) experiment with the two olefinic protons (δ_H 6.20 and 7.36 ppm), were assigned to constitute the lactone ring as C-1 and C-4, respectively. The connection of α,β-unsaturated-γ-lactone ring with the olefinic proton at δ_H 5.31 ppm was confirmed by HMBC experiment which established long range coupling between H-5 (δ_H 5.31 ppm) to C-4 (δ_C 150.3 ppm) and C-3 (δ_C 143.2 ppm). The configuration of these connection was cisoid, as confirmed by NOE enhancement (Figure 34) in which irradiation of the H-3 signal (δ_H 7.36 ppm) resulted in the enhancement of the H-5 (δ_H 5.31 ppm)(7.42%) and H-2 (δ_H 6.20 ppm)(9.19%) signal. The assignments of the carbon resonances were achieved by the DEPT and the HMQC experiment (Figure 36).

By analyses of ^{1}H-NMR and ^{13}C-NMR data of compound L-3 in comparison with previously reported (Jung *et al.*, 1991; Jung *et al.*, 1990a; Tuchinda *et al.*, 1991; Tiyawornan, 1998), compound L-3 was identified as (4*Z*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide (acetylmelodorinol) **[12]**.



[12]

Table 11. ^{1}H-NMR (300 MHz in $CDCl_3$) and ^{13}C-NMR (75 MHz in $CDCl_3$) spectral data of compound L-3 and ^{1}H-NMR (400 MHz in $CDCl_3$) and ^{13}C-NMR (50 MHz in $CDCl_3$) spectral data of acetylmelodorinol (Tuchinda *et al.*, 1991)

Position	Compound L-3		Acetylmelodorinol	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1	-	168.3	-	168.5
2	6.20 (d, 5.4)	121.3	6.28 (dd, 5.6, 0.9)	121.5
3	7.36 (d, 5.4)	143.4	7.38 (d, 5.6)	143.3
4	-	150.5	-	150.7
5	5.31 (d, 8.1)	108.6	5.33 (dd, 8.2 0.9)	108.8
6	6.08 (ddd, 8.1, 6.1, 4.1)	67.0	6.15 (ddd, 6.2, 4.2, 8.2)	67.4
7 α β	4.43 (dd, 11.8, 6.1) 4.50 (dd, 11.8, 4.1)	64.4	4.52 (dd, 12.0, 6.2) 4.58 (dd, 12.0, 4.2)	64.4
8	-	165.7	-	166.1
9	-	129.3	-	129.5
10, 14	7.95 (m)	129.4	8.03 (m)	129.7
11, 13	7.37 (m)	128.2	7.45 (m)	128.5
12	7.50 (m)	133.1	7.58 (m)	133.3
15	-	169.5	-	169.8
16	2.02 (s)	20.6	2.11 (s)	20.8

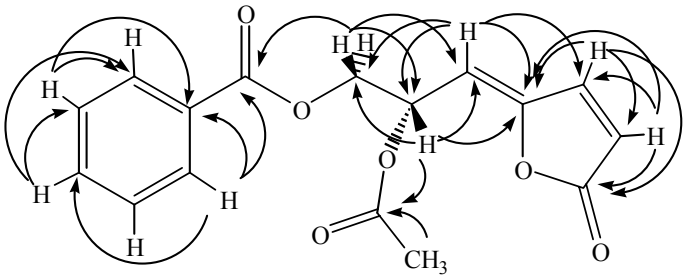


Figure 5. Long-range correlations from HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-3

Table 12. ^{1}H NMR (300 MHz in $CDCl_3$), ^{13}C NMR (75 MHz in $CDCl_3$), ^{1}H-^{1}H COSY and HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-3

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	^{1}H, ^{1}H-COSY	HMBC ($^{n}J_{CH}$ = 8 Hz)
1	-	168.3	-	-
2	6.20 (d, 5.4)	121.3	H-3	C-1, C-3, C-4
3	7.36 (d, 5.4)	143.4	H-2	C-1, C-2, C-4
4	-	150.5	-	-
5	5.31 (d, 8.1)	108.6	H-6	C-3, C-4, C-6
6	6.08 (ddd, 8.1, 6.1, 4.1)	67.0	H-5, H-7	C-4, C-5, C-7, C-15
7 α β	4.43 (dd, 11.8, 6.1) 4.50 (dd, 11.8, 4.1)	64.4	H-6	C-5, C-6, C-8
8	-	165.7	-	-
9	-	129.3	-	-
10, 14	7.95 (m)	129.4	H-11/13	C-8, C-9, C-12
11, 13	7.37 (m)	128.2	H-10/14, H-12	C-9, C-10/14
12	7.50 (m)	133.1	H-11/13	C-10/14, C-11/13
15	-	169.5	-	-
16	2.02 (s)	20.6	-	C-15

3. Structure Determination of Compound L-4

Compound L-4 was obtained as a yellow liquid. The UV spectrum of compound L-4 (Figure 38) revealed characteristic absorption at 270 (log ε = 3.06) nm. The IR spectrum of compound L-4 (Figure 39) showed absorption bands at 3457 (O-H stretching), 3075 (=C-H stretching, aromatic), 2954 (=C-H stretching, aliphatic), 1779 and 1750 (C=O stretching, lactone carbonyl), 1718 (C=O stretching, benzoate carbonyl), 1560 (C=C stretching), 1452 (C-H stretching, methylene), 1274 (C-O stretching, benzoate), 1114 (C-O stretching, benzoate), and 1070 (C-O stretching, alcohol) cm^{-1}. In the EIMS, compound L-4 (Figure 40) gave a molecular ion peak $[M]^+$ at m/z 260 consistent with the molecular formula $C_{14}H_{12}O_5$ having degree of unsaturation as nine.

The ^{1}H-NMR spectrum (Figure 41) of compound L-4 exhibited the presence of three olefinic protons at δ 5.39 (1H, d, *J* = 8.4 Hz; H-5), 6.16 (1H, d, *J* = 5.4 Hz; H-2), and 7.33 (1H, d, *J* = 5.4 Hz; H-3) ppm; one oxygen-bearing methine proton at δ 5.12 (1H, dq, *J* = 8.4, 5.1 Hz; H-6) ppm; one oxygen-bearing methylene proton at δ 4.39 (2H, d, *J* = 5.1 Hz; H-7) ppm; monosubstituted aromatic protons at δ 7.36 (2H, t, *J* = 7.2 Hz; *meta*-H), 7.50 (1H,d, *J* = 7.2 Hz; *para*-H), 7.95 (1H, d, *J* = 7.2 Hz; *ortho*-H) ppm.

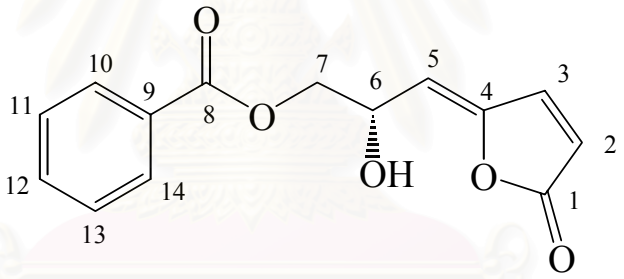
The ^{13}C NMR spectrum (Figure 42) and DEPT (Figure 43) exhibited fourteen carbon resonances, revealing the presence of one oxygen-bearing methylene carbon, three olefinic methine carbons, one oxygen-bearing methine carbon, one oxygen-bearing quaternary carbon, two ester carbonyl carbons, five aromatic methine carbon, and one aromatic quaternary carbon. The assignments of the carbon resonances were achieved by the HMQC experiment (Figure 46).

The ^{1}H-^{1}H COSY spectrum of compound L-4 (Figure 47) established the proton connections of the following: H-2 to H-3, H-5 to H-6, H-6 to H-7, H-10 and H-14 to H-11 and H-13, H-11 and H-13 to H-12.

The ^{1}H-NMR spectral data of compound L-4 are close to those of compound L-3 (acetylmelodorinol) **[12]**. The major difference in the IR spectrum was the presence of a broad OH absorption (3457 cm^{-1}). The acetate proton which were observed in compound

L-3 had disappeared from the ^{1}H-NMR spectrum of compound L-4, while an additional hydroxy proton signal was observed (broad, δ_H 3.42 ppm). Moreover, the H-6 signal had shifted significantly upfield (δ_H 5.11ppm) and the H-7 signal had shifted slightly upfield (δ_H 4.39 ppm). The acetate methyl carbon (δ_C 20.6 ppm) and the carbonyl carbon (δ_C 169.5 ppm) signals had disapeared from the ^{13}C-NMR (Figure 42) and DEPT (Figure 43) of compound L-4. Thus, compound L-4 could be assigned as having a hydroxy instead of an acetate group at C-6 position. The NOE difference data (Figure 44) in which irradiation of H-3 signal (δ_H 7.33 ppm) enhanced the signals of H-5 (δ_H 5.39 ppm) (10.41%) and H-2 (δ_H 6.16 ppm)(11.53%), confirmed the cisoid configuration.

On the basis of these spectral data and comparison with previously reported data (Jung *et al.*, 1991; Jung *et al.*, 1990a; Tuchinda *et al.*, 1991), compound L-4 was identified as (4*Z*)-7-benzoyloxy-6-hydroxy-2,4-heptadien-4-olide (melodorinol) [**10**]



[10]

Table 13. ^{1}H-NMR (300 MHz in $CDCl_3$) and ^{13}C-NMR (75 MHz in $CDCl_3$) spectral data of compound L-4 and ^{1}H-NMR (400 MHz in $CDCl_3$) and ^{13}C-NMR (50 MHz in $CDCl_3$) spectral data of melodorinol (Tuchinda *et al.*, 1991)

Position	Compound L-3		Melodorinol	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1	-	169.1	-	169.1
2	6.16 (d, 5.4)	120.7	6.23 (dd, 5.4, 0.8)	120.9
3	7.33 (d, 5.4)	143.8	7.39 (d, 5.4)	143.7
4	-	149.8	-	150.0
5	5.39 (d, 8.4)	113.4	5.42 (dd, 8.2, 0.8)	113.0
6	5.12 (dq, 8.4, 5.1)	65.3	5.18 (ddd, 5.7, 4.7, 8.2)	65.7
7	4.39 (d, 5.1)	67.3	α 4.44 (dd, 11.4, 5.7) β 4.47 (dd, 11.4, 4.7)	67.4
8	-	166.5	-	166.7
9	-	129.3	-	129.4
10, 14	7.97 (d, 7.2)	129.6	8.03 (m)	129.7
11, 13	7.36 (t, 7.2)	128.3	7.42 (m)	128.4
12	7.50 (t, 7.2)	133.2	7.56 (m)	133.3
OH	3.42 (br)	-	2.96 (br)	-

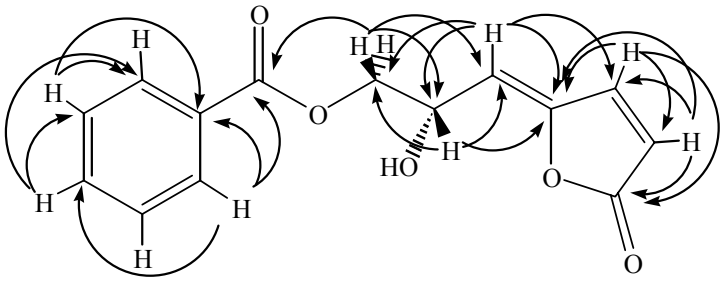


Figure 6. Long-range correlations from HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-4

Table 14. ^{1}H NMR (300 MHz in $CDCl_3$), ^{13}C NMR (75 MHz in $CDCl_3$), ^{1}H-^{1}H COSY and HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-4

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	^{1}H, ^{1}H-COSY	HMBC ($^{n}J_{CH}$ = 8 Hz)
1	-	169.1	-	-
2	6.16 (d, 5.4)	120.7	H-3	C-1, C-3, C-4
3	7.33 (d, 5.4)	143.8	H-2	C-1, C-2, C-4
4	-	149.8	-	-
5	5.39 (d, 8.4)	113.4	H-6	C-3, C-4, C-7
6	5.12 (dq, 8.4, 5.1)	65.3	H-5, H-7	C-4, C-7
7	4.39 (d, 5.1)	67.3	H-6	C-8
8	-	166.5	-	-
9	-	129.3	-	-
10, 14	7.97 (t, 7.2)	129.6	H-11/13	C-8, C-9, C-12
11, 13	7.36 (t, 7.2)	128.3	H-10/14, H-12	C-9, C-10/14
12	7.50 (t, 7.2)	133.2	H-11/13	C-10/14, C-11/13
OH	3.42 (br)	-	-	-

4. Structure Determination of Compound L-5

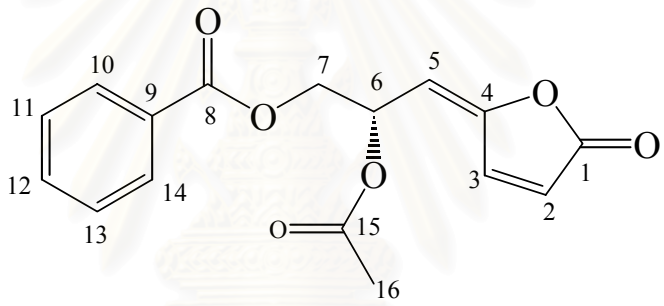
Compound L-5 was obtained as yellow liquid. The UV spectrum of compound L-5 (Figure 48) revealed characteristic absorption at 269 (log ε = 2.92) nm. The IR spectrum of compound L-5 (Figure 49) showed absorption bands at 3076 (=C-H stretching, aromatic), 2957 (=C-H stretching, aliphatic), 1790 and 1765 (C=O stretching, lactone carbonyl), 1735 (C=O stretching, acetate carbonyl), 1724 (C=O stretching, benzoate carbonyl), 1560 (C=C stretching), 1451 (C-H stretching, methylene), 1371 (C-H stretching, methyl), 1274 (C-O stretching, benzoate), and 1113 (C-O stretching, benzoate) cm^{-1}. In the EIMS, compound L-5 (Figure 50) gave a molecular ion peak $[M]^+$ at m/z 302 consistent with the molecular formula $C_{16}H_{14}O_6$ of which the degree of unsaturation is ten.

The ^{1}H-NMR spectrum (Figure 51) of compound L-5 exhibited the presence of three olefinic protons at δ 5.70 (1H, d, *J* = 10.2 Hz; H-5), 6.31 (1H, dd, *J* = 5.4, 1.5 Hz; H-2), and 7.87 (1H, d, *J* = 5.4 Hz; H-3) ppm; one oxygen-bearing methine proton at δ 5.94 (1H, ddd, *J* = 10.2, 6.6, 4.2 Hz; H-6) ppm; one oxygen-bearing methylene proton at δ 4.46 (dd, *J* = 11.7, 6.6 Hz; H-7α) and 4.48 (dd, *J* = 11.7, 4.2 Hz; H-7β) ppm; one acetate methyl groups at δ 2.07 (3H, s; H-16) ppm; monosubstituted aromatic protons at δ 7.43 (2H, t, *J* = 7.5 Hz; *meta*-H), 7.56 (1H, t, *J* = 7.5 Hz; *para*-H), 7.98 (1H, d, *J* = 7.5 Hz; *ortho*-H) ppm.

The ^{13}C NMR spectrum (Figure 52) and DEPT (Figure 53) exhibited sixteen carbon resonances, revealing the presence of one acetate methyl carbon, one oxygen-bearing methylene carbon, three olefinic methine carbons, one oxygen-bearing methine carbon, one oxygen-bearing quaternary carbon, three ester carbonyl carbons, five aromatic methine carbon, and one aromatic quaternary carbon.

The compound L-5 exhibited similar IR and EIMS spectral data to those of compound L-3 (acetylmelodorinol) **[12]**, suggesting the similarity in functional groups. The ^{1}H-NMR and ^{13}C-NMR spectral data of compound L-5 are very close to those of compound L-3 (acetylmelodorinol) **[12]**, except that the chemical shifts of H-3 (δ_H 7.87 ppm), H-5 (δ_H 5.70 ppm), and C-4 (δ_C 153.4 ppm) of this compound were more

downfield than those of compound L-3. Thus, the structure of compound L-5 could possibly be proposed as having transoid configuration, which was confirmed by NOE enhancements (Figure 54) in which irradiation of H-3 signal (δ_H 7.87 ppm) enhanced the signals of H-2 (δ_H 6.31ppm)(10.10%) and H-6 (δ_H 5.94 ppm)(11.28%), but not H-5 (δ_H 5.70 ppm). The ^{1}H-^{1}H COSY spectrum of compound L-5 (Figure 55) established the proton connections of the following: H-2 to H-3, H-5 to H-6, H-6 to H-7, H-10 and H-14 to H-11 and H-13, H-11 and H-13 to H-12. The assignments of the carbon resonances were achieved by the DEPT and the HMQC experiment (Figure 56), and were confirmed by the HMBC ($^nJ_{CH}$ = 8 Hz) experiment (Figure 57). By analyses of these available spectral data and comparison with reported data, compound L-5 was thus identified as a known compound, (4*E*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide **[13]** (Jung *et al.*, 1991; Tuchinda *et al.*, 1991).



[13]

Table 15. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectral data of compound L-5 (in $CDCl_3$) and ^{1}H-NMR (400 MHz) and ^{13}C-NMR (50 MHz) spectral data of (4*E*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide (in $CDCl_3$) (Tuchinda *et al.*, 1991)

Position	Compound L-5		(4*E*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1	-	168.0	-	168.7
2	6.31 (dd, 5.4, 1.5)	122.5	6.34 (dd, 5.7, 1.8)	122.5
3	7.87 (d, 5.4)	140.2	7.91 (dd, 5.7, 0.8)	140.2
4	-	153.4	-	151.5
5	5.70 (d, 10.2)	107.5	5.73 (ddd, 10.1, 1.8, 0.8)	107.5
6	5.94 (ddd, 10.2, 6.6, 4.2)	67.7	5.98 (ddd, 6.7, 4.4, 10.1)	66.8
7 α β	4.46 (dd, 11.7, 6.6) 4.48 (dd, 11.7, 4.2)	65.0	4.47 (dd, 11.8, 6.7) 4.54 (dd, 11.8, 4.4)	65.0
8	-	165.9	-	166.0
9	-	129.2	-	129.2
10, 14	7.98 (d, 7.5)	129.6	8.01 (m)	129.6
11, 13	7.43 (t, 7.5)	128.5	7.46 (m)	128.5
12	7.56 (t, 7.5)	133.4	7.59 (m)	133.5
15	-	170.0	-	170.7
16	2.07 (s)	20.9	2.09 (s)	20.9

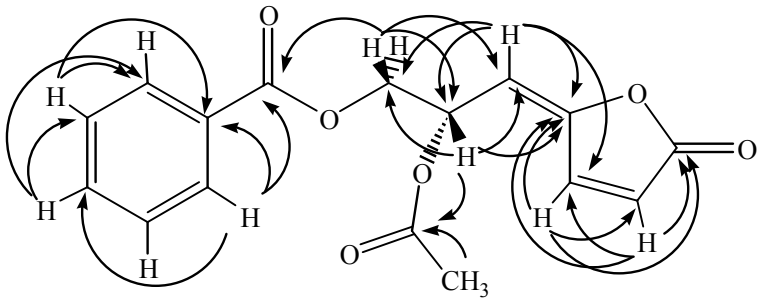


Figure 7. Long-range correlations from HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-5

Table 16. ^{1}H NMR (300 MHz in $CDCl_3$), ^{13}C NMR (75 MHz in $CDCl_3$), ^{1}H-^{1}H COSY and HMBC ($^{n}J_{CH}$ = 8 Hz) spectral data of compound L-5

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	^{1}H, ^{1}H-COSY	HMBC ($^{n}J_{CH}$ = 8 Hz)
1	-	168.0	-	-
2	6.31 (dd, 5.4, 1.5)	122.5	H-3	C-1, C-3, C-4
3	7.87 (d, 5.4)	140.2	H-2	C-1, C-2, C-4
4	-	153.4	-	-
5	5.70 (d, 10.2)	107.5	H-6	C-3, C-4, C-6, C-7
6	5.94 (ddd, 10.2, 6.6, 4.2)	67.7	H-5, H-7	C-4, C-5, C-7, C-15
7 α β	4.46 (dd, 11.7, 6.6) 4.48 (dd, 11.7, 4.2)	65.0	H-6	C-5, C-6, C-8
8	-	165.9	-	-
9	-	129.2	-	-
10, 14	7.98 (d, 7.5)	129.6	H-11/13	C-8, C-9, C-12
11, 13	7.43 (t, 7.5)	128.5	H-10/14, H-12	C-9, C-10/14
12	7.56 (t, 7.5)	133.4	H-11/13	C-10/14, C-11/13
15	-	170.0	-	-
16	2.07 (s)	20.9		C-15

All isolated compounds have been tested for their *in vitro* cytotoxicity against Human breast cancer (BT 474), Human lung cancer (CHAGO), Human gastric cancer (KATO-3), Human hepatoma (HEP-G2), Human skin fibrobrast (Hs27), and Human colon cancer (SW620) cell cultures. Their cytotoxicity data were tabulated in Table 16.

Table 17. Cytotoxicity data of isolated compounds

Compound	Cytotoxicity IC_{50} (μg/ml)					
	BT 474	CHAGO	KATO-3	HEP-G2	Hs27	SW620
L-1	10	9.7	8.3	7.3	>10	5.5
L-2	>10	>10	>10	>10	>10	>10
L-3	>10	8.7	8.4	4.2	>10	5.7
L-4	10	7.7	7.2	5.2	>10	5.8
L-5	8.6	>10	>10	6.9	9.4	5.7

All of the isolated compounds showed weak to no activity against BT 474, CHAGO, KATO-3, HEP-G2, Hs27, and SW620 cell cultures. For the triterpenoid derivatives, compound L-1 exhibited greater cytotoxicity against BT 474, CHAGO, KATO-3, HEP-G2, and SW620 cell cultures than compound L-2. It should be noted that the position 3 of compound L-2 possesses a acetoxy functionality while at the same position of compound L-1 a hydroxy group is present. It appears that the substitution at position 3 of these compounds is important for cytotoxic activity. For the heptadiene derivatives, compound L-3 was more cytotoxic against CHAGO, KATO-3, and HEP-G2 cell cultures than compound L-5. Whereas L-3 and L-5 contain an acetoxy substitute at position 6, L-4 which has a hydroxy at this position demonstrated greater cytotoxic activity than both L-3 and L-5. Thus, the configuration of the lactone moiety and the substitution at position 6 of the heptene derivatives are important for cytotoxic activity .

CHAPTER V
CONCLUSION

Melodorum fruticosum Lour. (family Annonaceae) is one of the medicinal plants used in Thai traditional medicine. Chemical constituents of the bark, leaves, branches and flowers of this plant have previously been studied. In the present investigation, five compounds were isolated from the root and seeds of *M. fruticosum*. Two of these are compounds in the lanostane-type triterpenoid, of which one is a novel and the other is a known compound. The novel triterpenoid (L-2) was assigned the structure lanosta-7,9(11),24-trien-3β-acetoxy-15α-ol and given the trivial name as acetylpolycarpol, whereas the other was assigned the structure lanosta-7,9(11),24-trien-3β,15α-diol (polycarpol, L-1). Three other compounds isolated: (4*Z*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide (acetylmelodorinol, L-3), (4*E*)-7-benzoyloxy-6-acetoxy-2,4-heptadien-4-olide (L-5), and (4*Z*)-7-benzoyloxy-6-hydroxy-2,4-heptadien-4-olide (melodorinol, L-4) are known heptene derivatives. Compounds L-1, L-2, L-3 and L-4 were obtained from the root, while compounds L-3, L-4, and L-5 were obtained from the seeds of this plant. The structure elucidations of these compounds were based on various spectroscopic data. Compounds L-1, L-3, L-4,and L-5 showed weak activity against BT 474, CHAGO, KATO-3, HEP-G2, Hs27, and SW620 cell cultures. This study has offered additional information concerning the phytochemical constituents of *Melodorum fruticosum* Lour. The fruit and heartwood are recommended to investigate further for the complementary of the phytochemical study of this plant.

REFERENCES

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D. and Mitchell, B. 1987. Cancer Research. 47: 936-942.

Connolly, J. D., and Hill, R. A. 2000. Triterpenoids. Natural Product Report. 17: 463-482.

Jackson, B. D. 1946. Index Kewensis. Vol. II. 2nd ed. London: Clarenson Press.

Jung, J. H., Chang, C.-J., Smith, D. L., McLaughlin, J. L., Pummangura, S., Chaichantipyuth, C., and Patarapanich, C. 1991. Additional bioactive heptenes from *Melodorum fruticosum*. Journal of Natural Products 54 (2): 500-505.

Jung, J. H., Pummangura, S., Chaichantipyuth, C., Patarapanich, C., Fanwick, P. E., Chang, C.-J., and McLaughlin, J. L. 1990a. New bioactive heptenes from *Melodorum fruticosum* (Annonaceae). Tetrahedron 46 (15): 5043-5054.

Jung, J. H., Pummangura, S., Chaichantipyuth, C., Patarapanich, C., and McLaughlin, J. L. 1990b. Bioactive constituents of *Melodorum fruticosum*. Phytochemistry 29 (5): 1667-1670.

Kessler, P. J. A., Jessup, L. W., and Kruijer, J. D. 1994. Provisional Checklist of The Asiatic-Australian Species of Annonaceae. The herbarium Department of Biology, University Pertanian Malaysia and UNESCO BOTANY 2000 ASIA.

Lebouf, M., Cave, A., Bhaumik, P. K., Mukherjee, B., and Mukherjee, R. 1982. The phytochemistry of the Annonceae. Phytochemistry. 21 (12): 2783-2813.

Li, H. Y., Sun, N. J., Kashiwada, Y., and Sun, L. 1993. Anti-AIDS agent, 9.[1] Suberosol, a new C_{31} lanostane-type triterpene and anti-HIV priciple from *Polyathia suberosa*. Journal of Natural Products. 56(7): 1130-1133.

Lue, Y. P., Mu, Q., Zheng, H. L., and Li C. M. 1998. 24-Methylene tetracyclic triterpenes from *Polyalthia lancilimba*. Phytochemistry. 49(7): 2053-2056.

Mahato, S. B., and Kunda, A. P. 1994. ^{13}C NMR spectra of pentacyclic triterpenoids – A compilation and some salient features. Phytochemistry. 37(6): 1517-1575.

Mahato, S. B., Nandy, A. K., and Roy, G. 1992. Triterpenoids. Phytochemistry. 31 (7): 2199-2249.

Mahato, S. B., and Sen, S. 1997. Advances in triterpenoid research, 1990-1994. Phytochemistry. 44(7): 1185-1236.

Merrill, E. D. 1919. On the application of the generic name *Melodorum* of Loureiro. The Philippine Journal of Science 15: 125-137.

Saralamp, P. ed. 1992. Medicinal plants in Siri Ruckhachati Garden. Bangkok: Amarin Printing Group.

Silverstein, R. M., Bassler, G. C., and Morrill, T. C. 1997. Spectrometric identification of organic compounds. 6th ed. New York: John Wiley & Sons.

Sinclair, J. 1955. A revision of the Malayan Annonaceae. The Gardens' Bulletin 14: 149-516.

Smitinand T. 1980. Thai Plant Names (Botanical names-Vernacular names). Funny Publising Limited Partnership. Second printing. 379 p.

Tai, T., Shingu, T., Kikushi, T., Tezuka, Y., and Akahori, A. 1995. Isolation of lanostane-type triterpene acids having an acetoxy group from sclerotia of *Posia cocos*. Phytochemistry. 40(1): 225-231.

Tanaka, R. and Matsunaga, S. 1991. Journol of Natural product. 54: 1337.

Taylor, G. 1959. Index Kewensis Supplement. Vol. XII. London: Clarenson Press.

Tripathi, M., Jain, L., Pandey, V. B., and Rucker, G. 1996. Pindrolactone, a lanostane derivative from the leaves of *Abies pindrow*. Phytochemistry. 43 (4): 853-855.

Tiyawornan, S. 1998. Chemical Constituents of *Melodorum fruticosum* Flowers. (Master' s thesis, Department of Pharmacognosy, Graduate School, Chulalongkorn University).

Tuchinda, P., Udchachon, J., Reutrakul, V., Santisuk, T., Taylor, W. C., Farnsworth, N. R., Pezzuto, J. M., and Kinghorn, A. D. 1991. Bioactive butenolides from *Melodorum fruticosum*. Phytochemistry. 30 (8): 2685-2689.

Twentyman, P. R. and Luscombe, M. 1987. British Journal of Cancer. 56: 279-285.

Ueda, K. 1986. Comment on the uncertain application of *Melodorum* Lour. (Annonaceae). Taxon. 35(2): 347-349.

APPENDIX

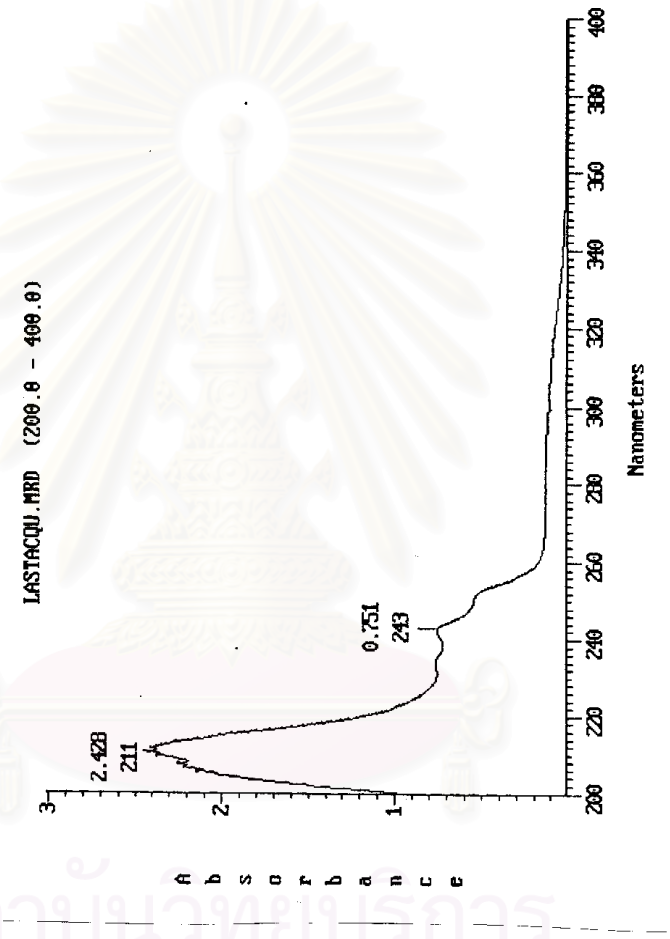


Figure 8. The UV spectrum of compound L-1 (in MeOH)

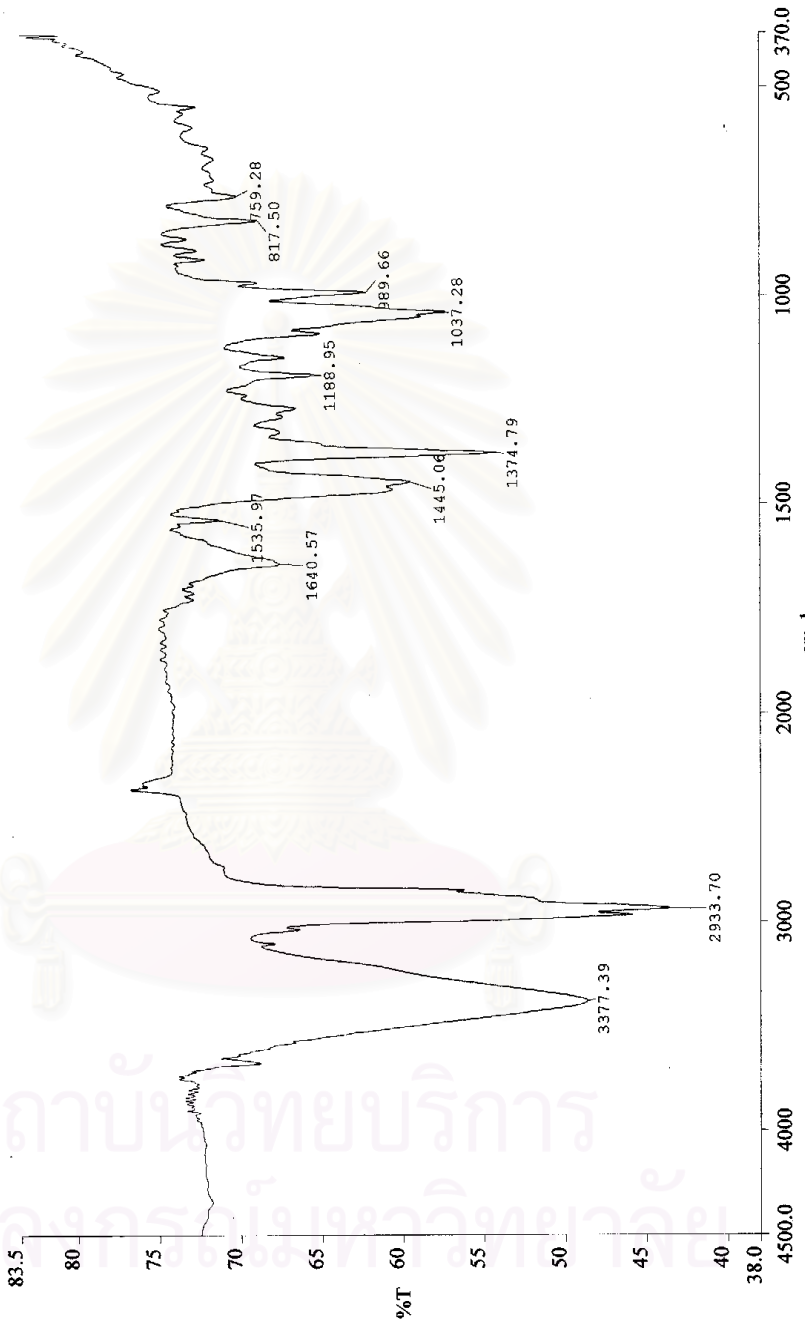


Figure 9. The IR spectrum of compound L-1 (KBr disc)

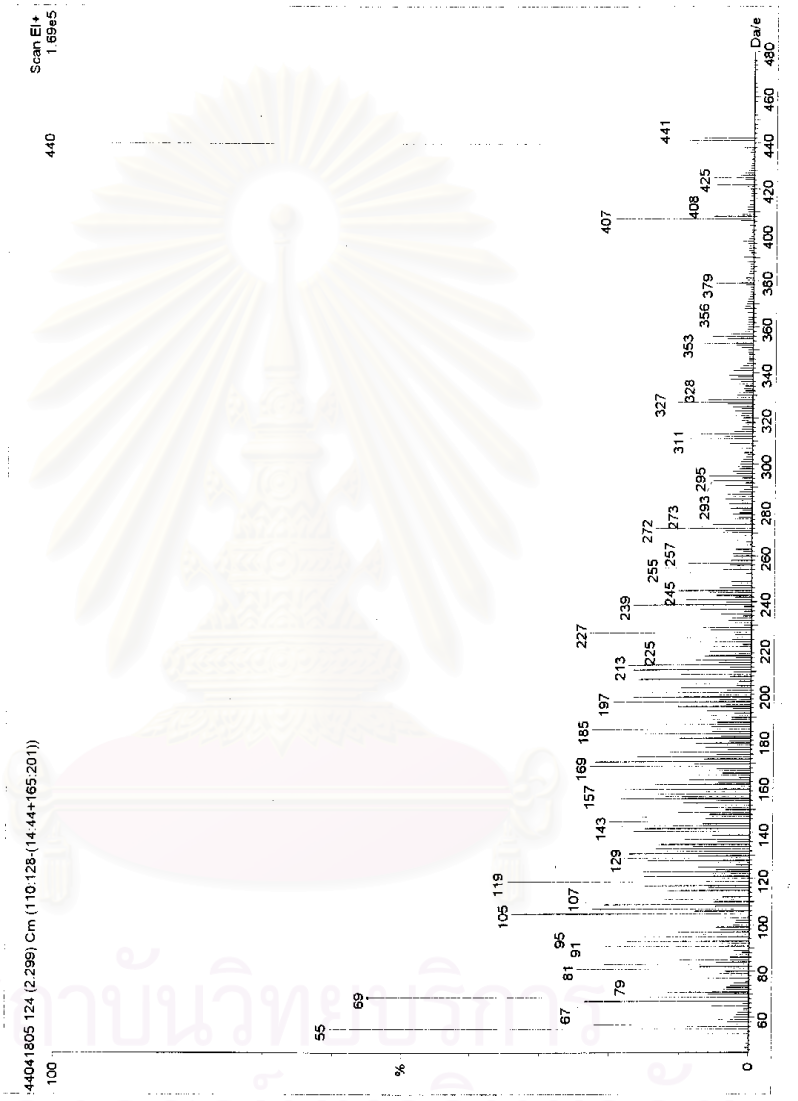


Figure 10. The EIMS spectrum of compound L-1

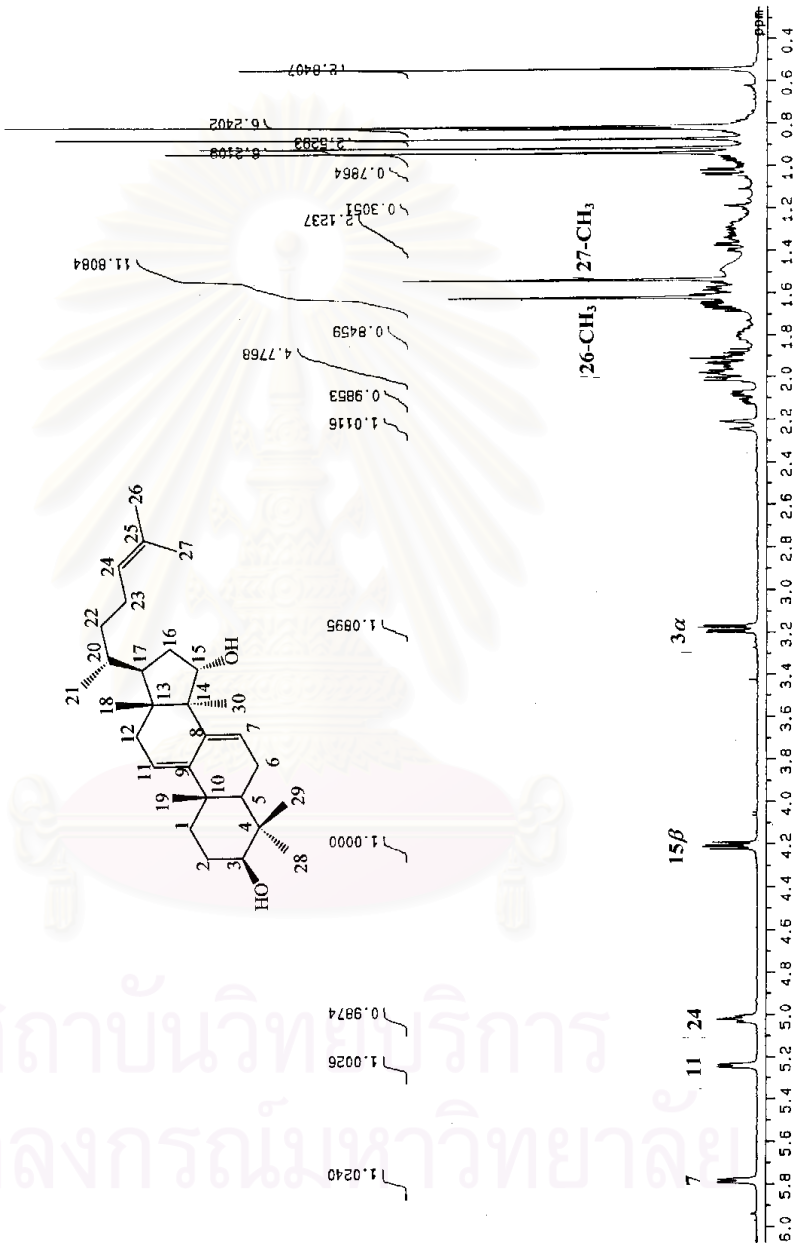


Figure 11a. The 500 MHz ^{1}H NMR spectrum of compound L-1 (in $CDCl_3$)

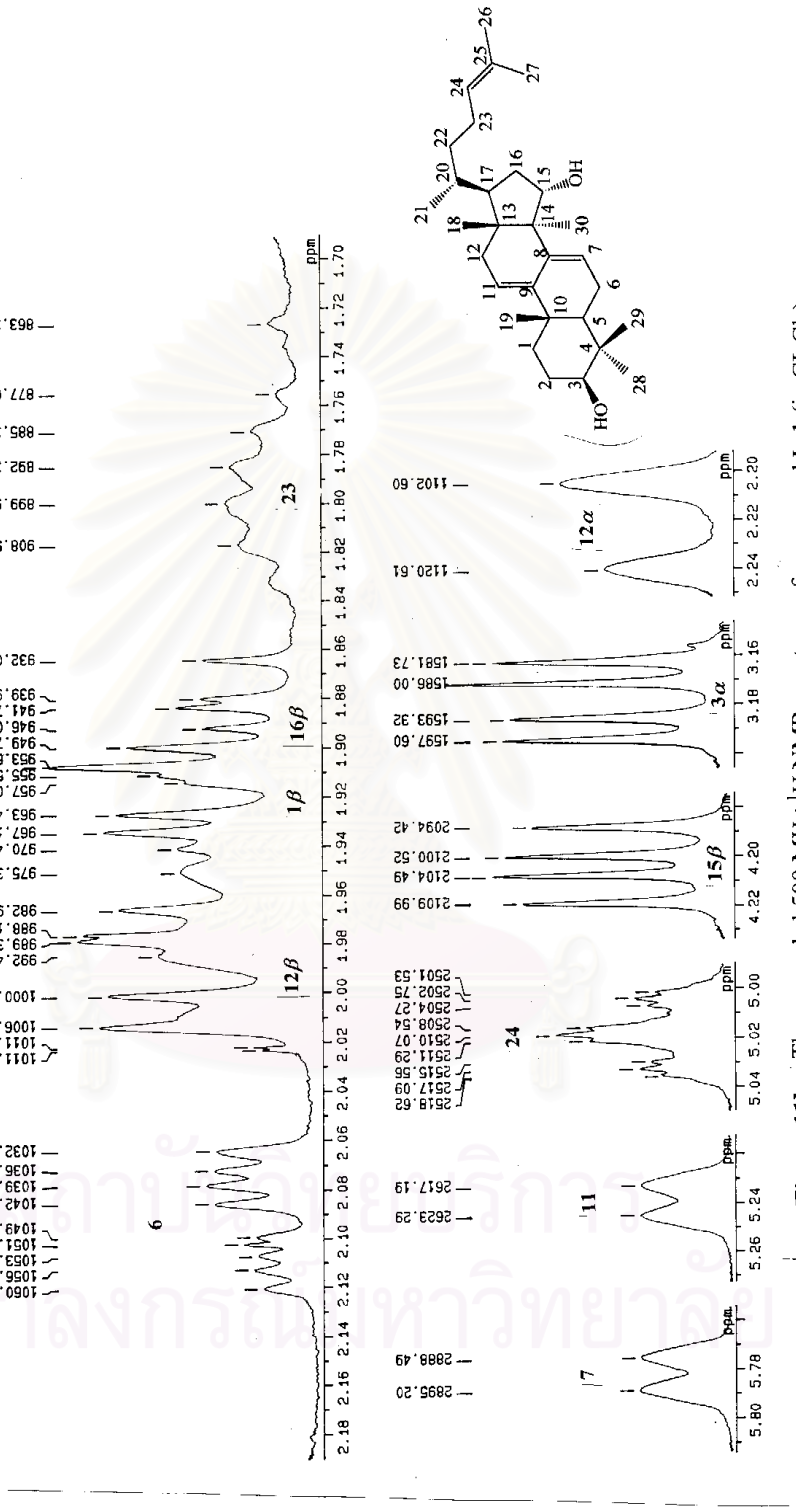


Figure 11b. The expanded 500 MHz ^{1}H NMR spectrum of compound **L-1** (in CL_3Cl_3) (δ_H 1.70-5.90 ppm)

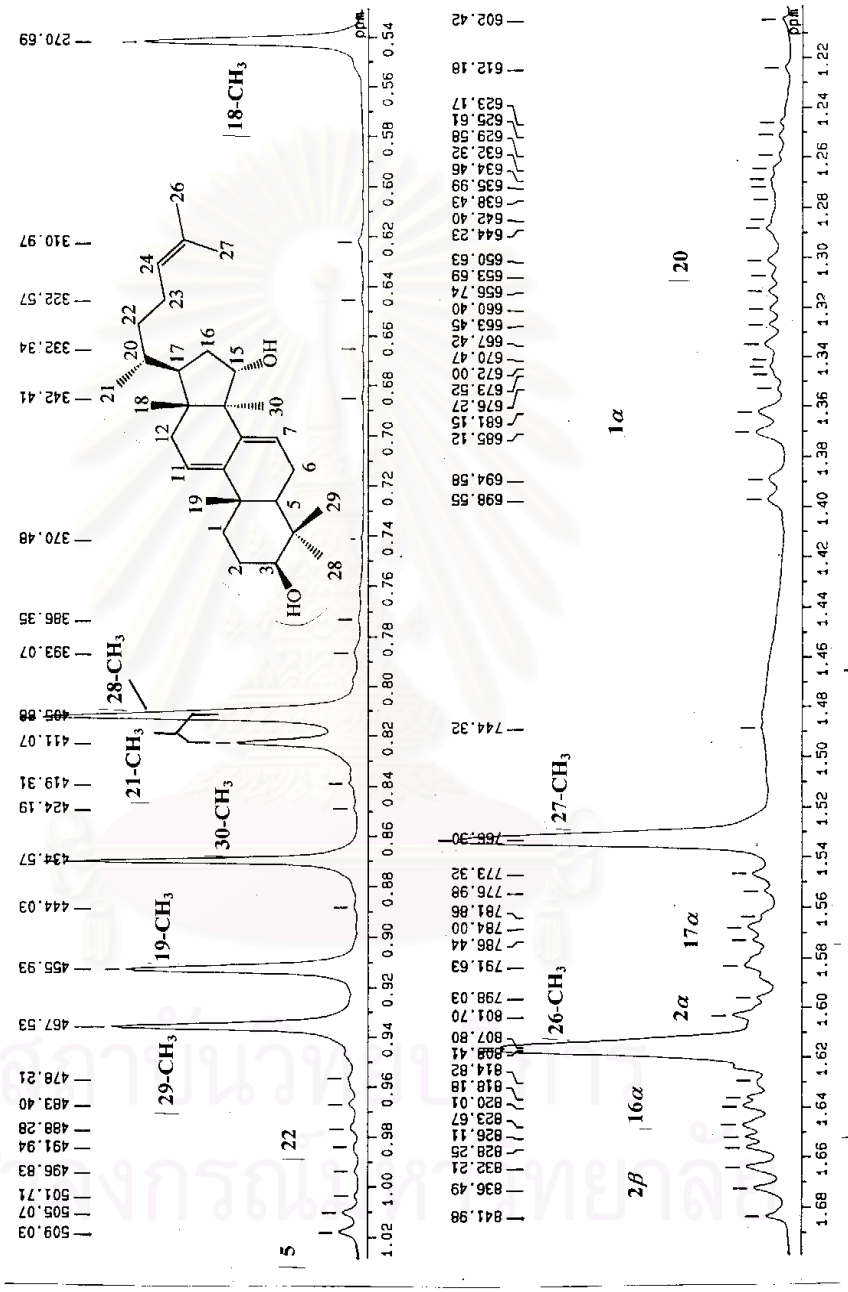


Figure 11c. The expanded 500 MHz ^{1}H NMR spectrum of compound L-1 (in $CDCl_3$)

(δ_H 0.53-1.69 ppm)

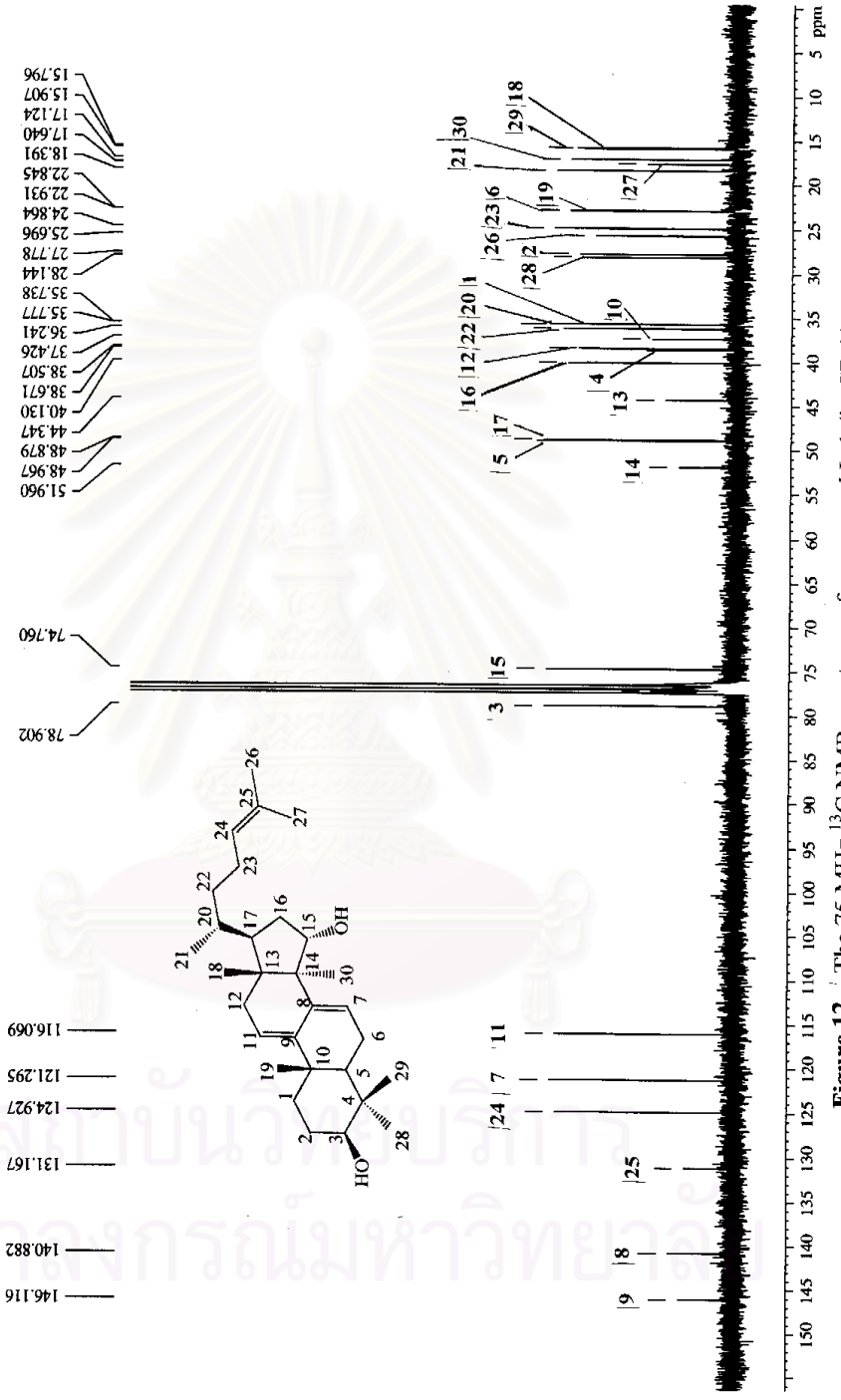


Figure 12. The 75 MHz ^{13}C NMR spectrum of compound L-1 (in $CDCl_3$)

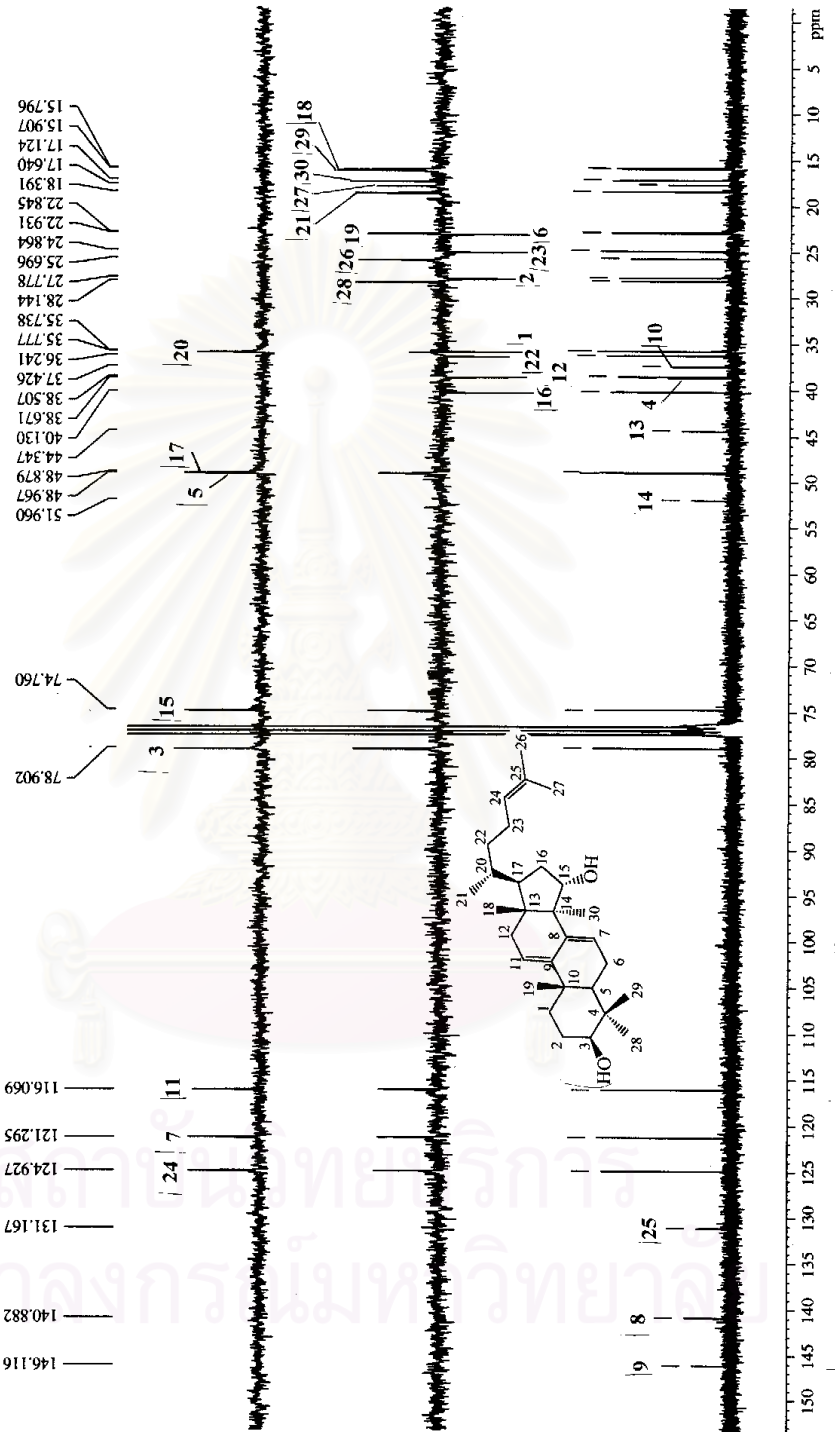


Figure 13. The 75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound L-1 (in $CDCl_3$)

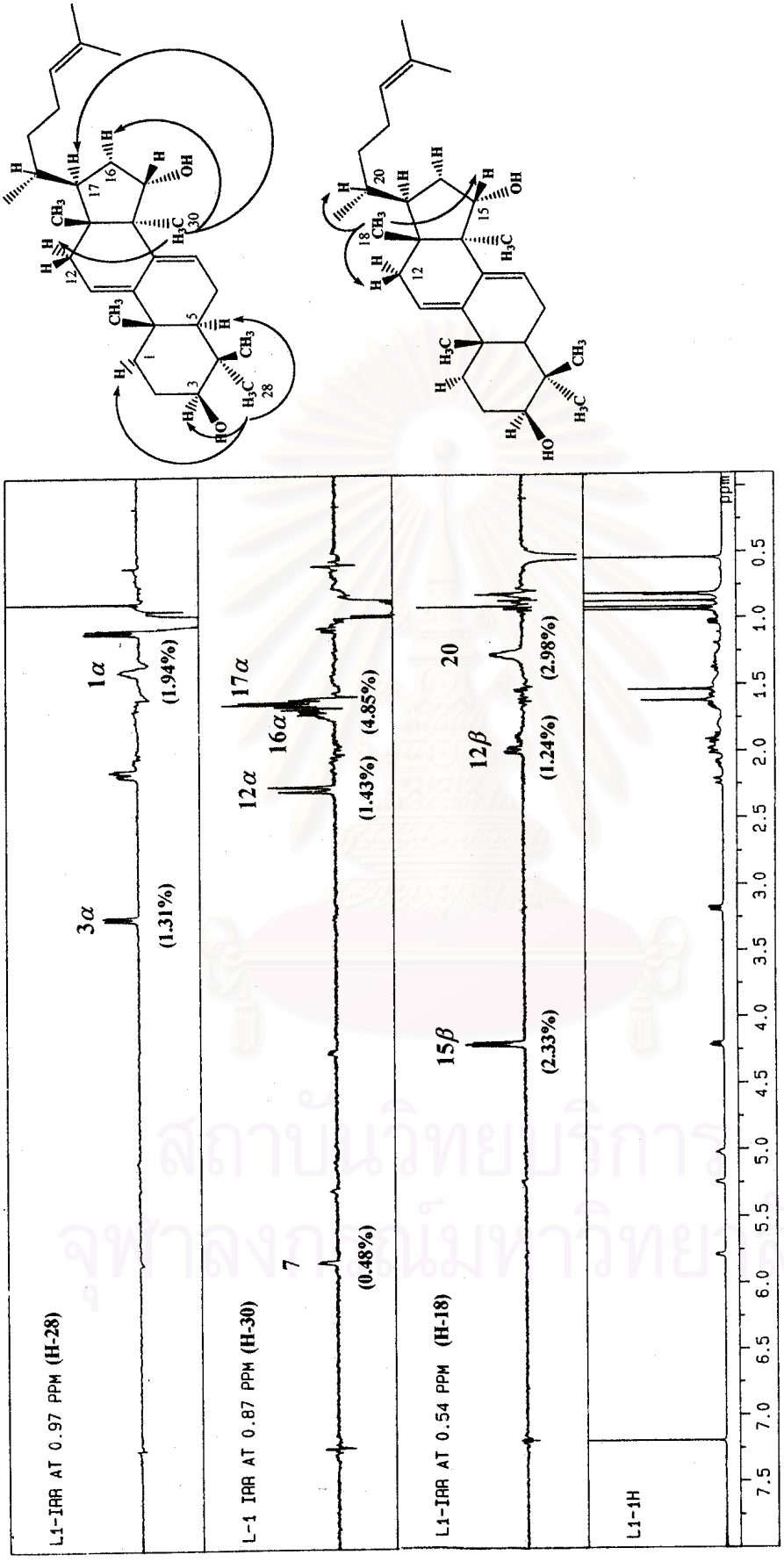


Figure 14. NOE Difference spectra of compound L-1(in $CDCl_3$)

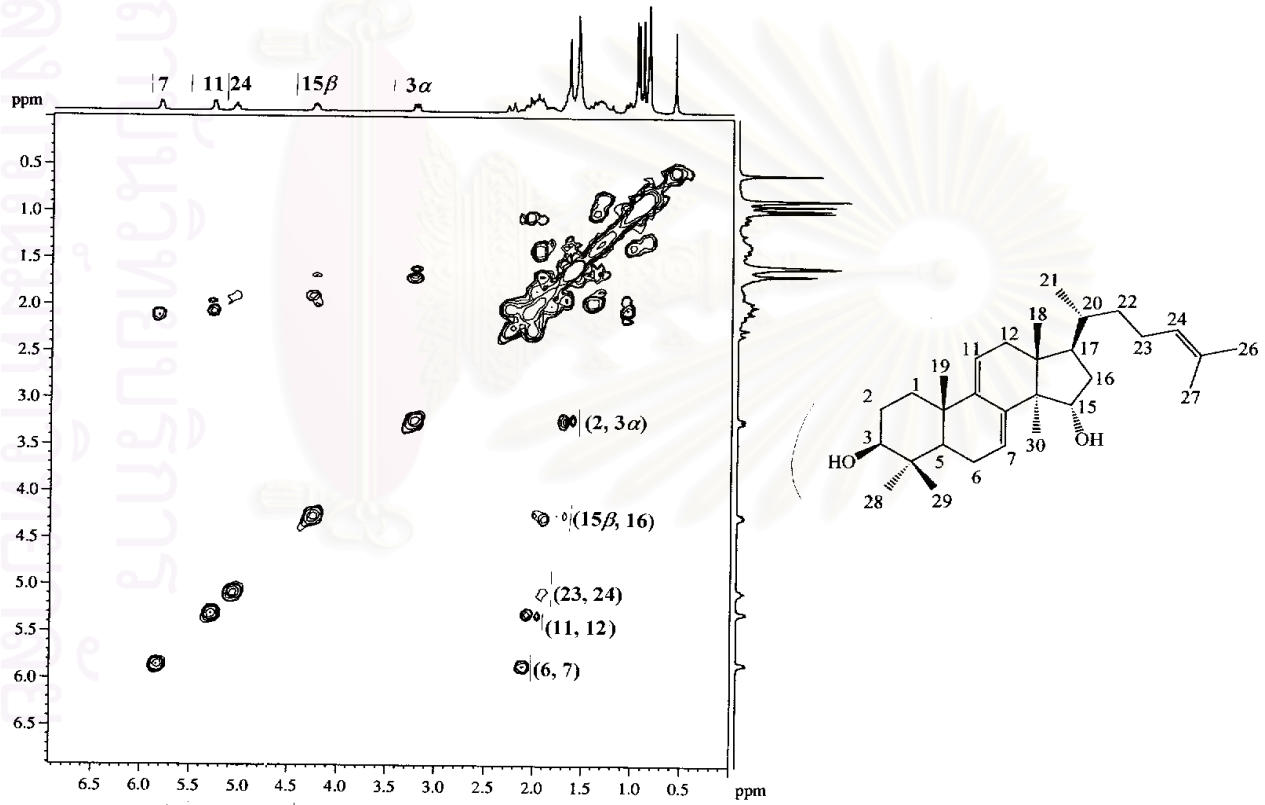


Figure 15a. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound L-1 (in $CDCl_3$)

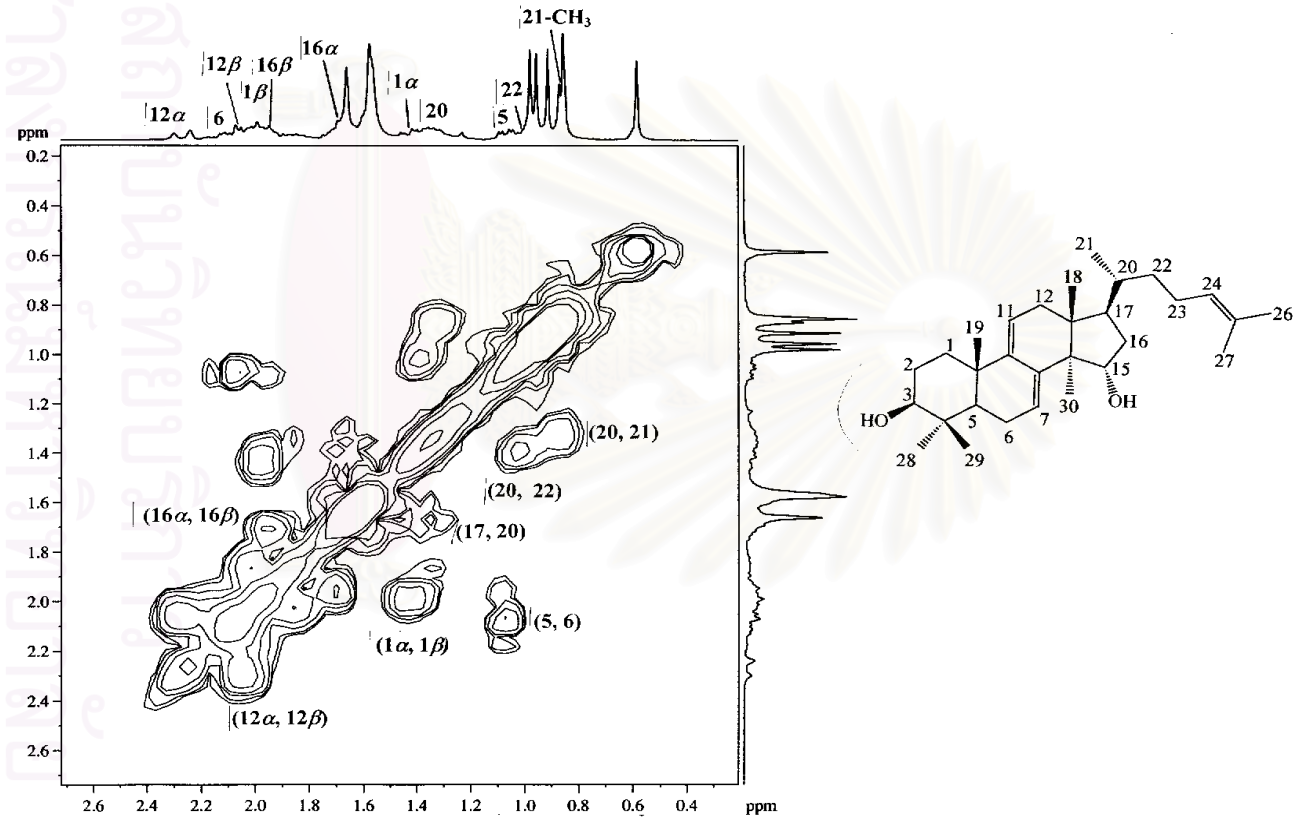


Figure 15b. The expanded 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound L-1 (in $CDCl_3$) (δ_H 0.5-2.5 ppm)

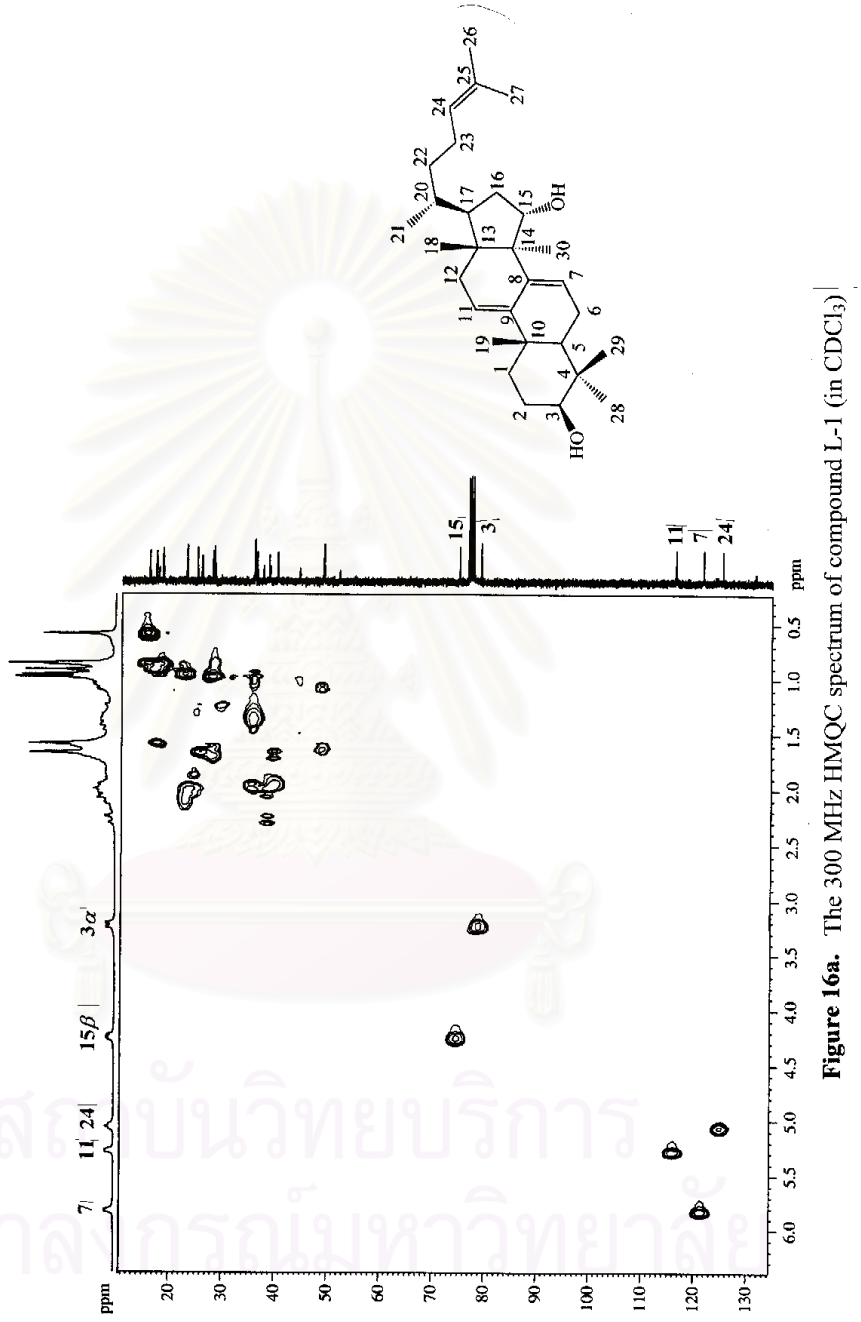


Figure 16a. The 300 MHz HMQC spectrum of compound L-1 (in $CDCl_3$)

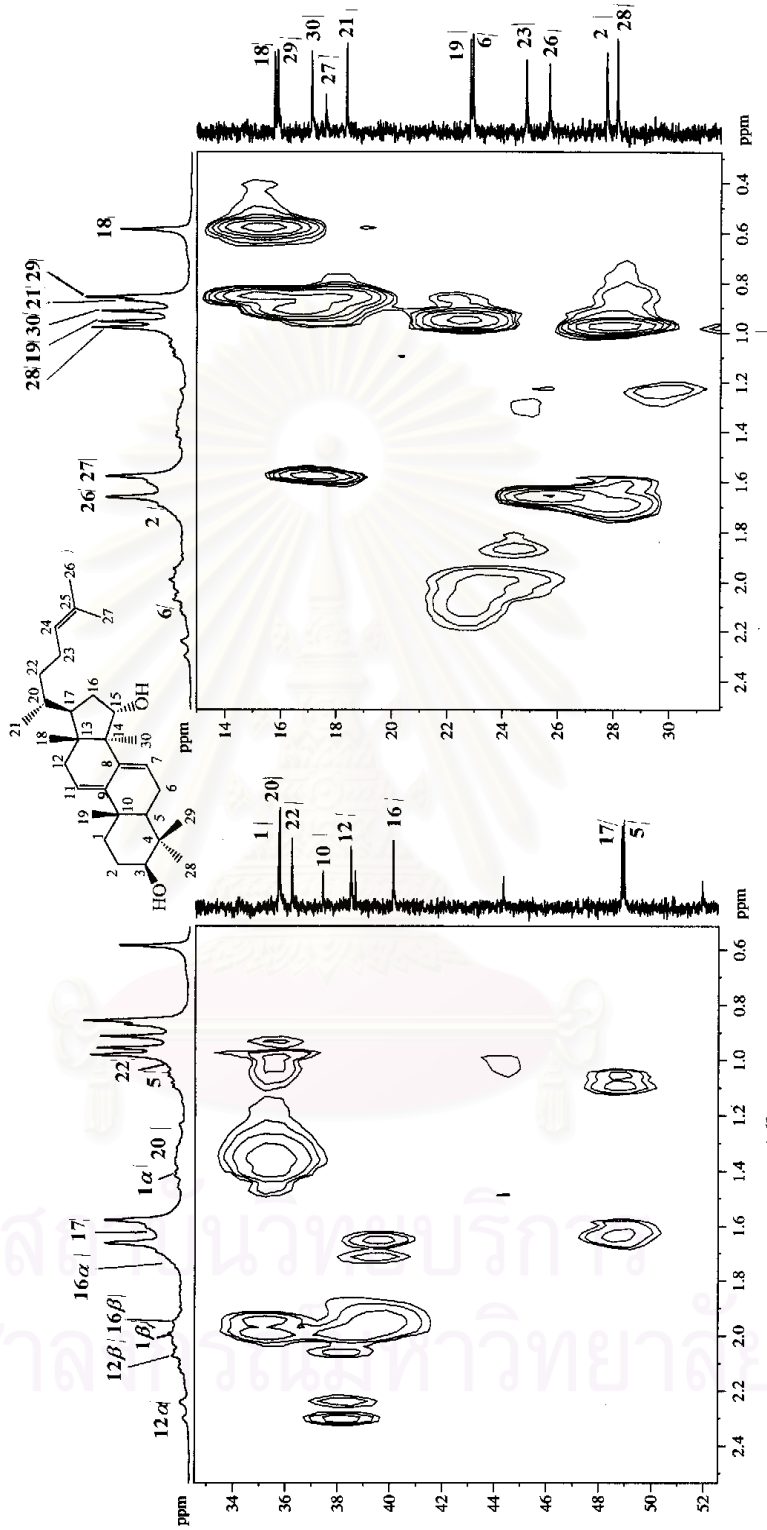


Figure 16b. The expanded 300 MHz HMQC spectrum of compound L-1 (in $CDCl_3$)
(δ_H 0.5-2.5 ppm, δ_C 33.2-52.3 ppm and δ_H 0.5-2.5 ppm, δ_C 15.0-40.0 ppm)

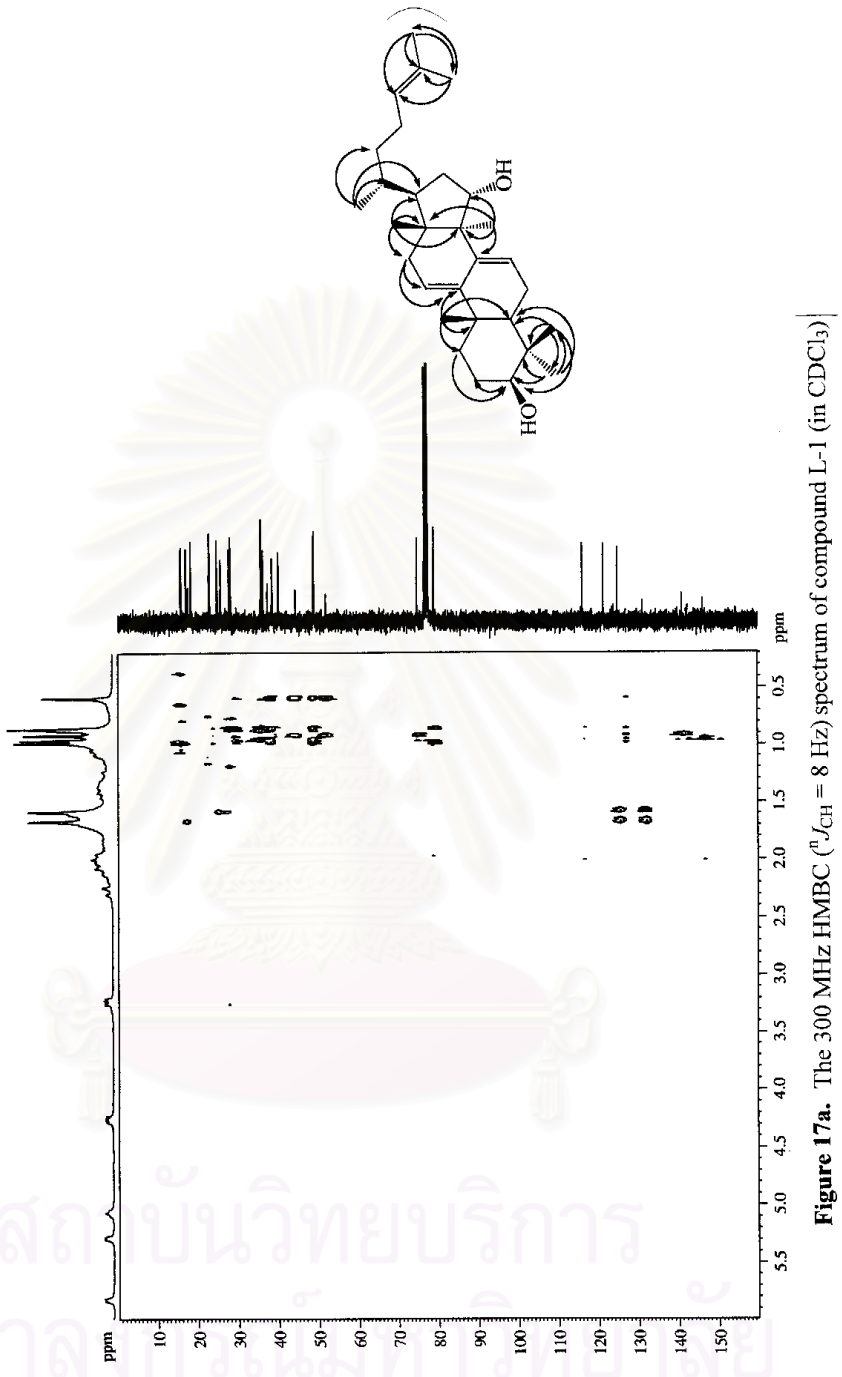


Figure 17a. The 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-1 (in $CDCl_3$)

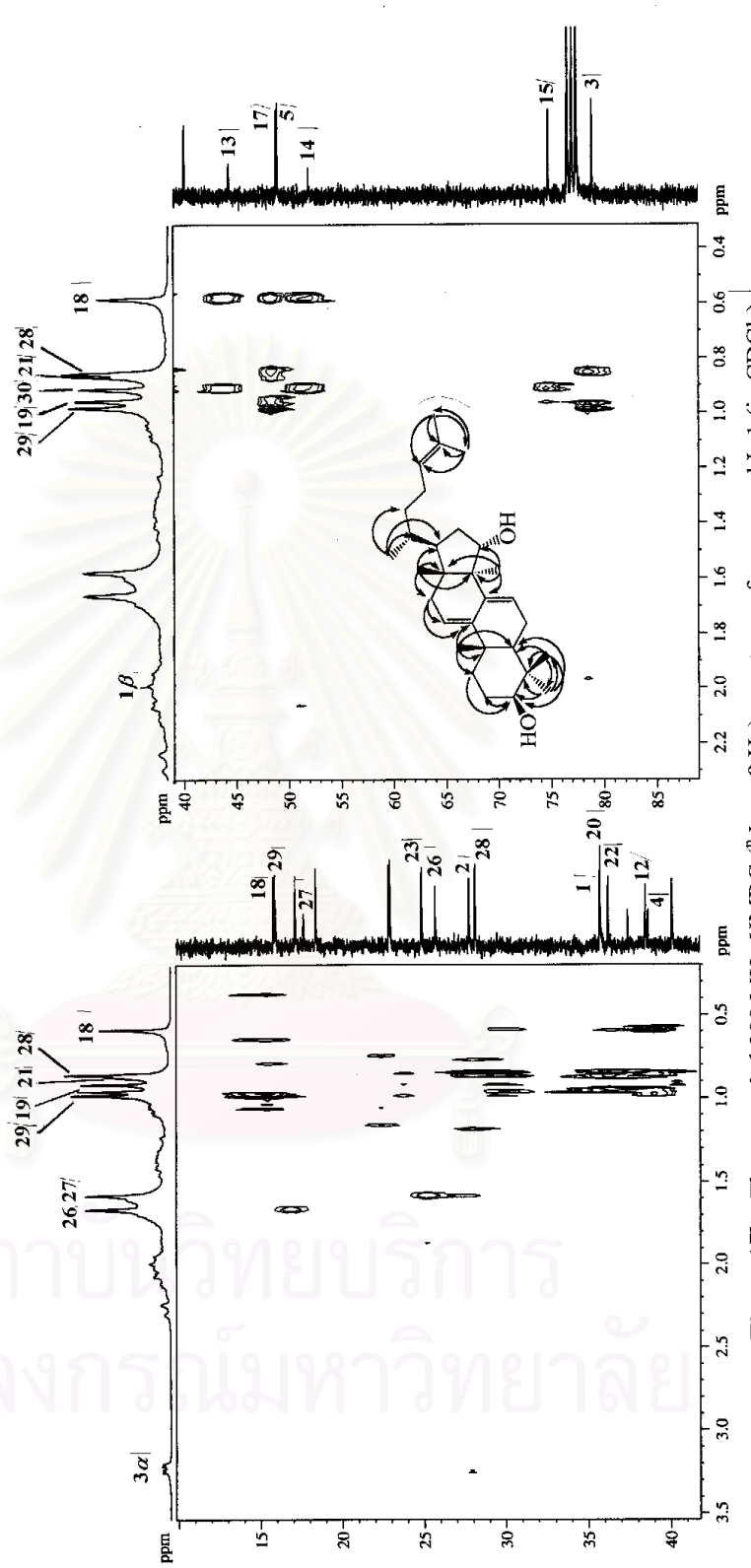


Figure 17b. The expanded 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-1 (in $CDCl_3$) (δ_H 0.2-3.5 ppm, δ_C 10.0-42.0 ppm and δ_H 0.5-2.3 ppm, δ_C 39.0-89.0 ppm)

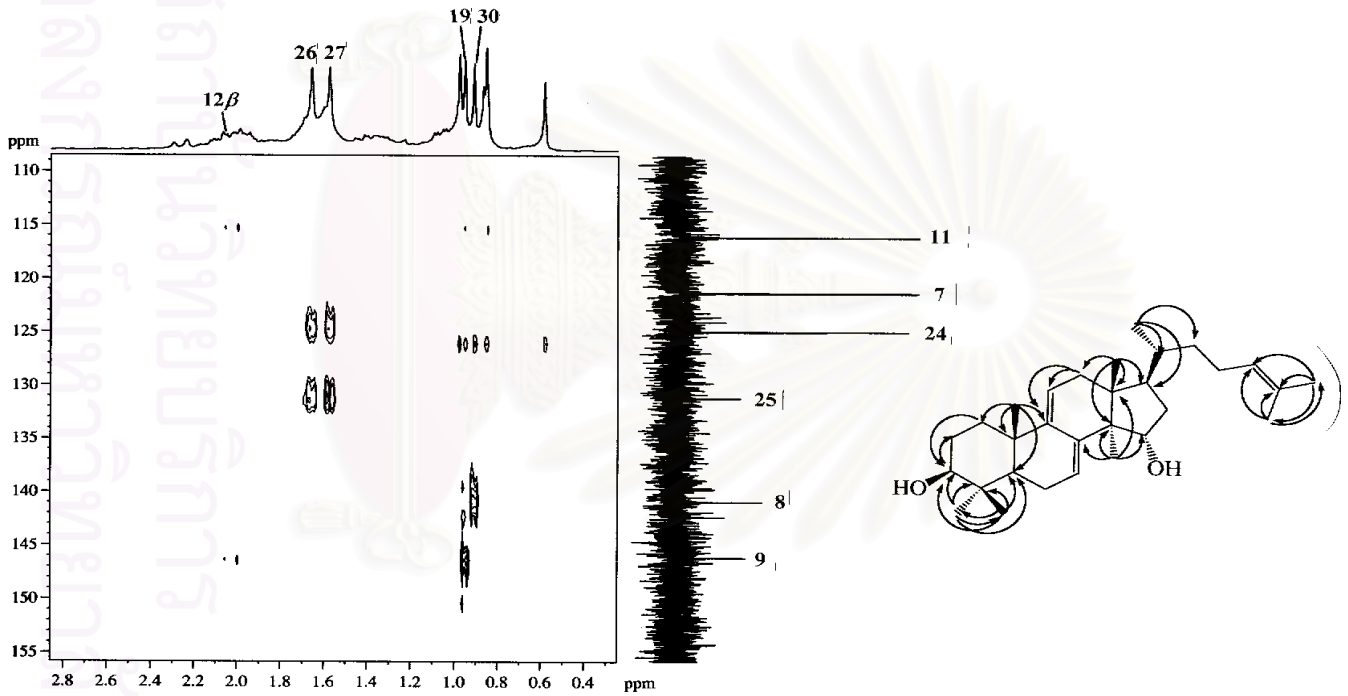


Figure 17c. The expanded 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-1 (in $CDCl_3$) (δ_H 0.3-2.9 ppm, δ_C 109.0-156.0 ppm)

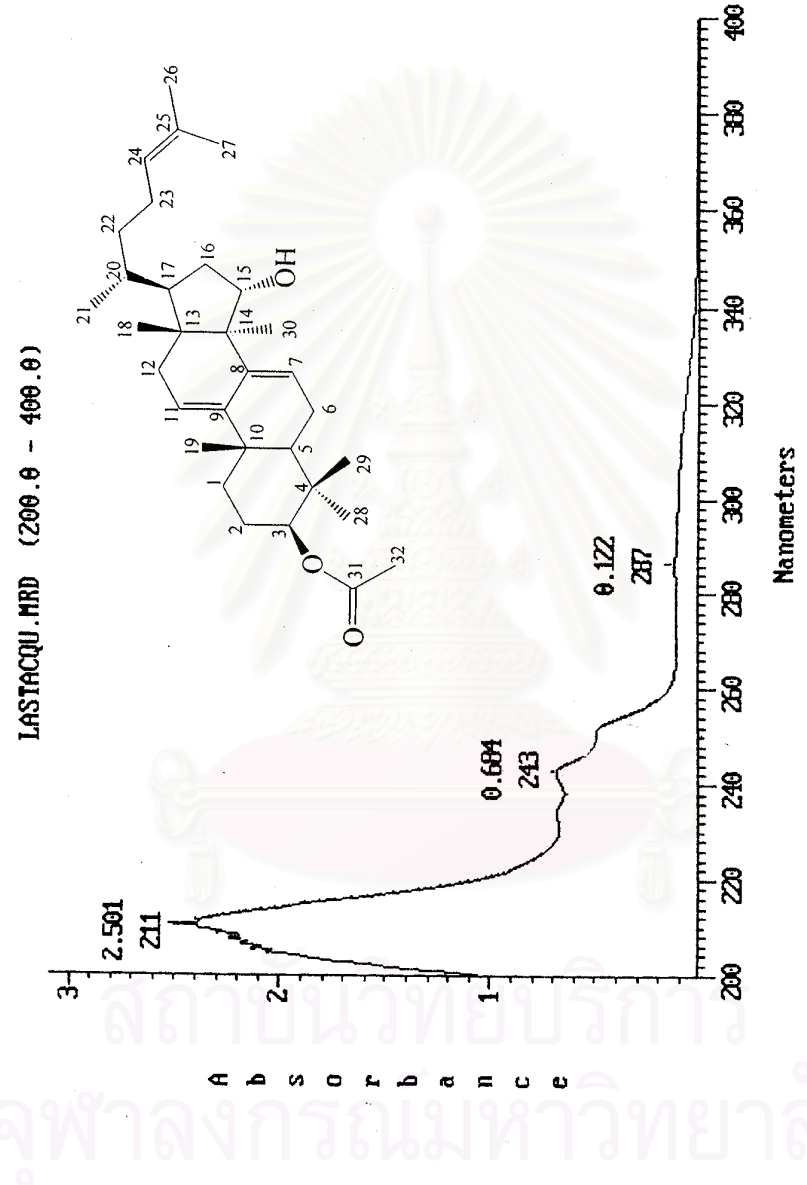


Figure 18. The UV spectrum of compound L-2 (in MeOH)

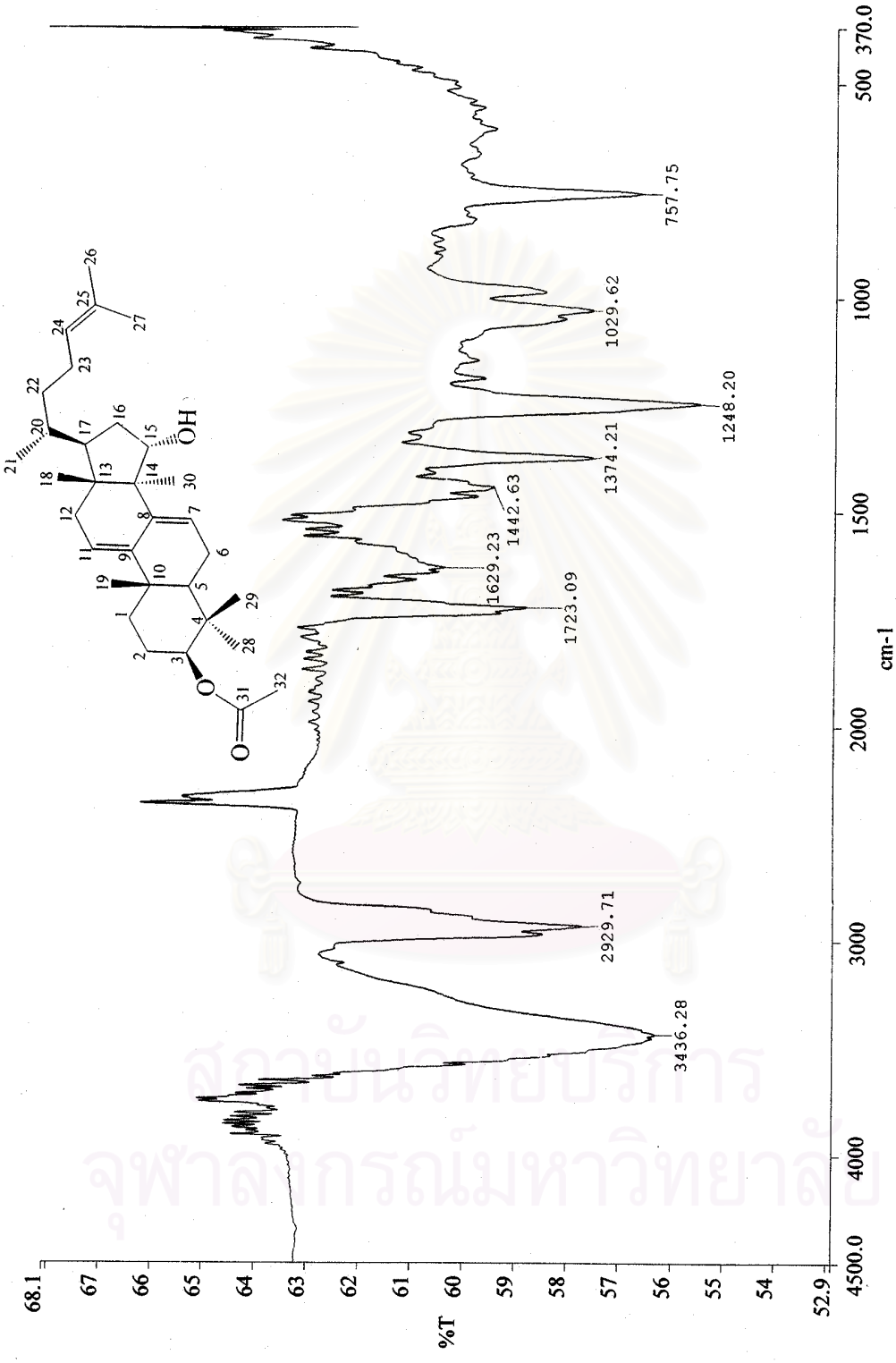


Figure 19. The IR spectrum of compound L-2 (KBr disc)

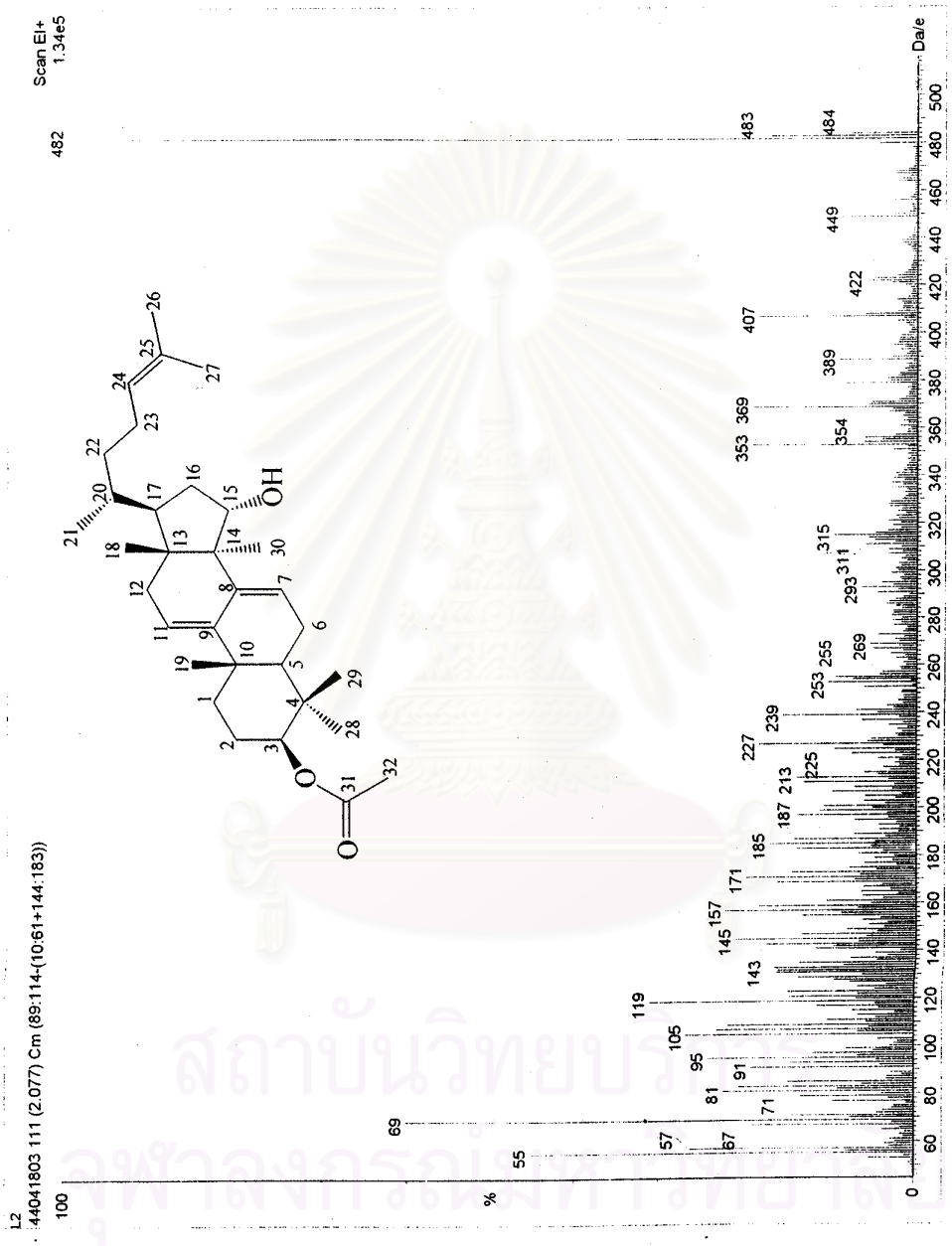


Figure 20. The EIMS spectrum of compound L-2

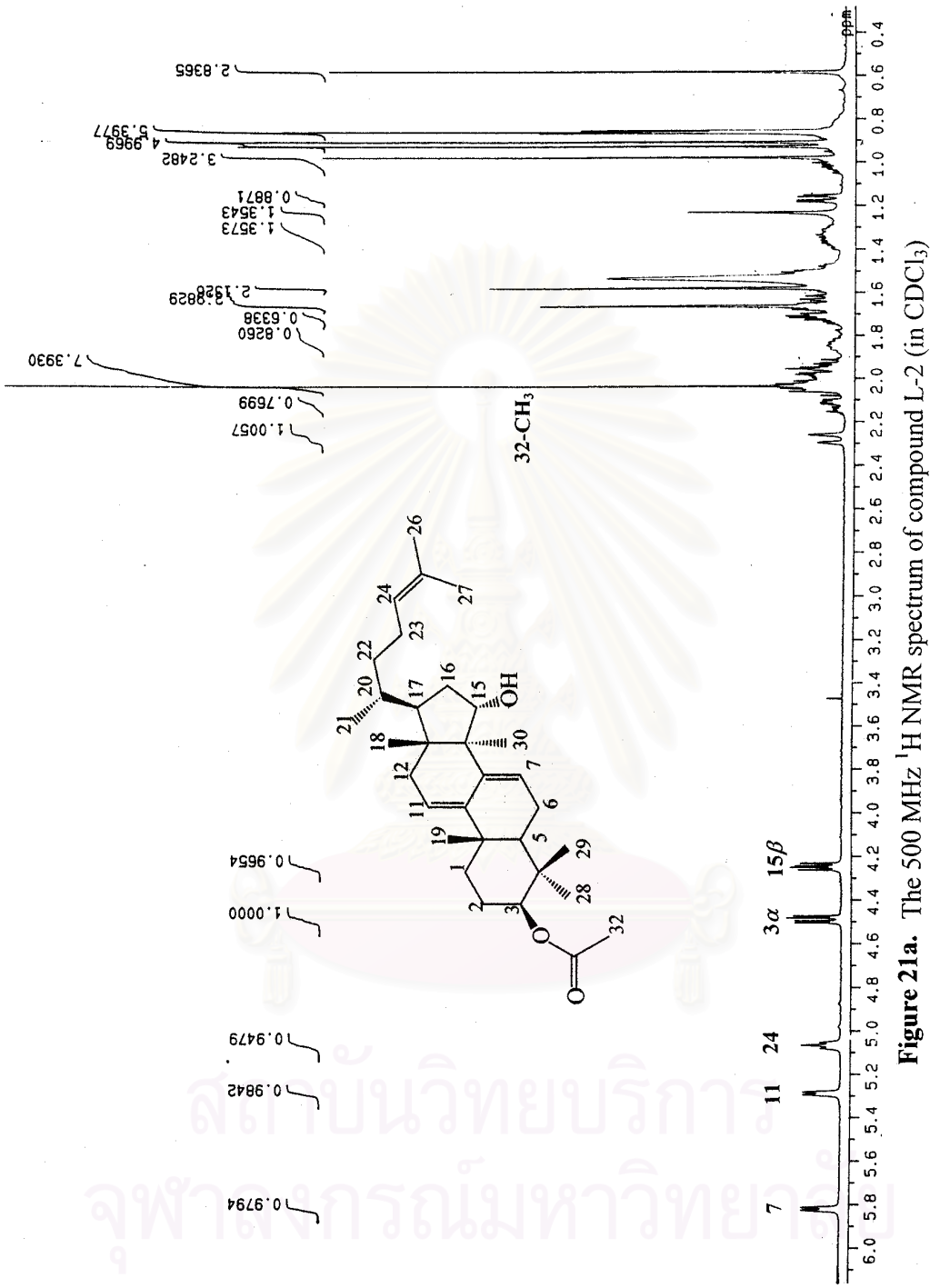


Figure 21a. The 500 MHz ^{1}H NMR spectrum of compound L-2 (in $CDCl_3$)

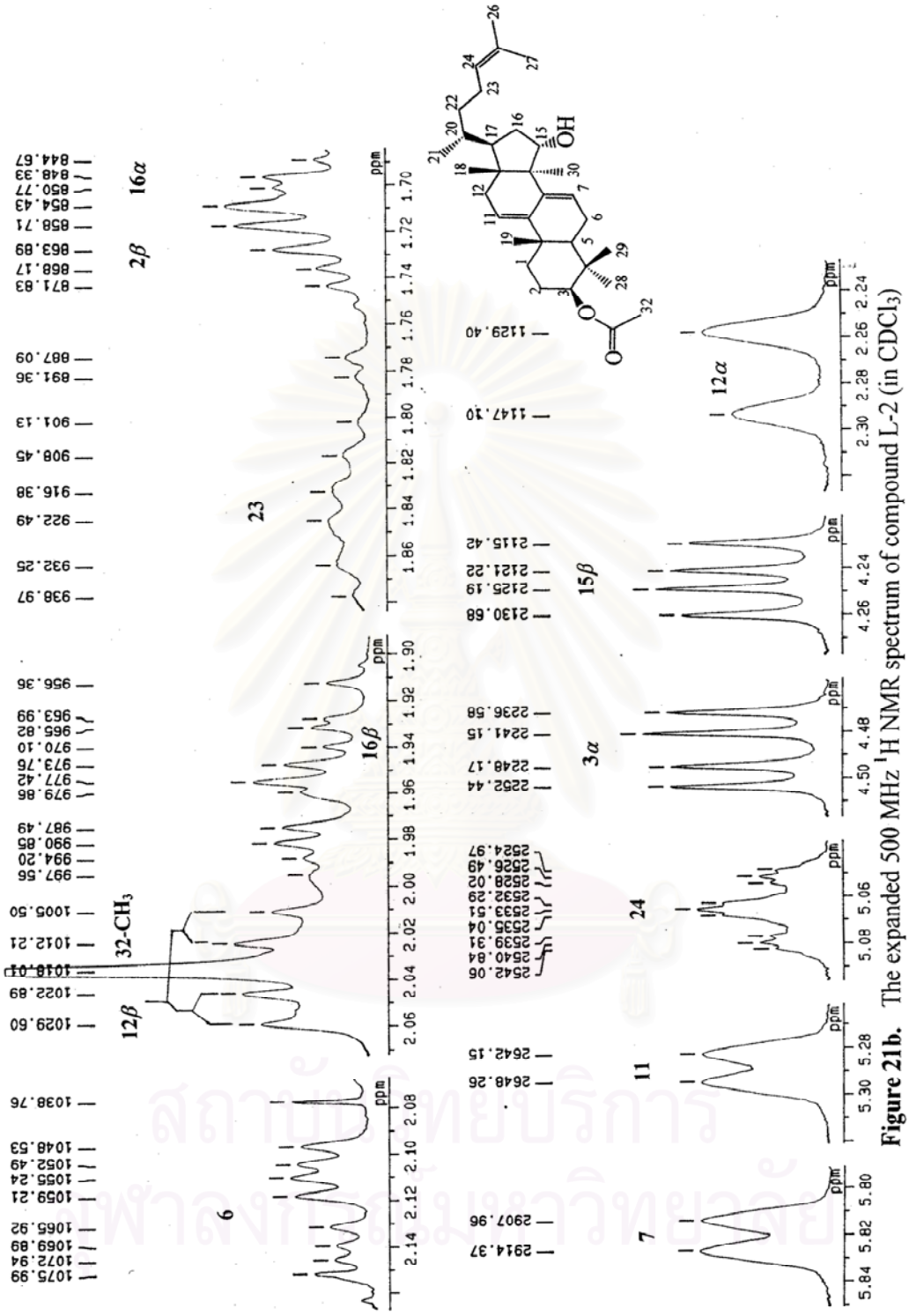


Figure 21b. The expanded 500 MHz ^{1}H NMR spectrum of compound L-2 (in $CDCl_3$) (δ_H 1.71-5.85 ppm)

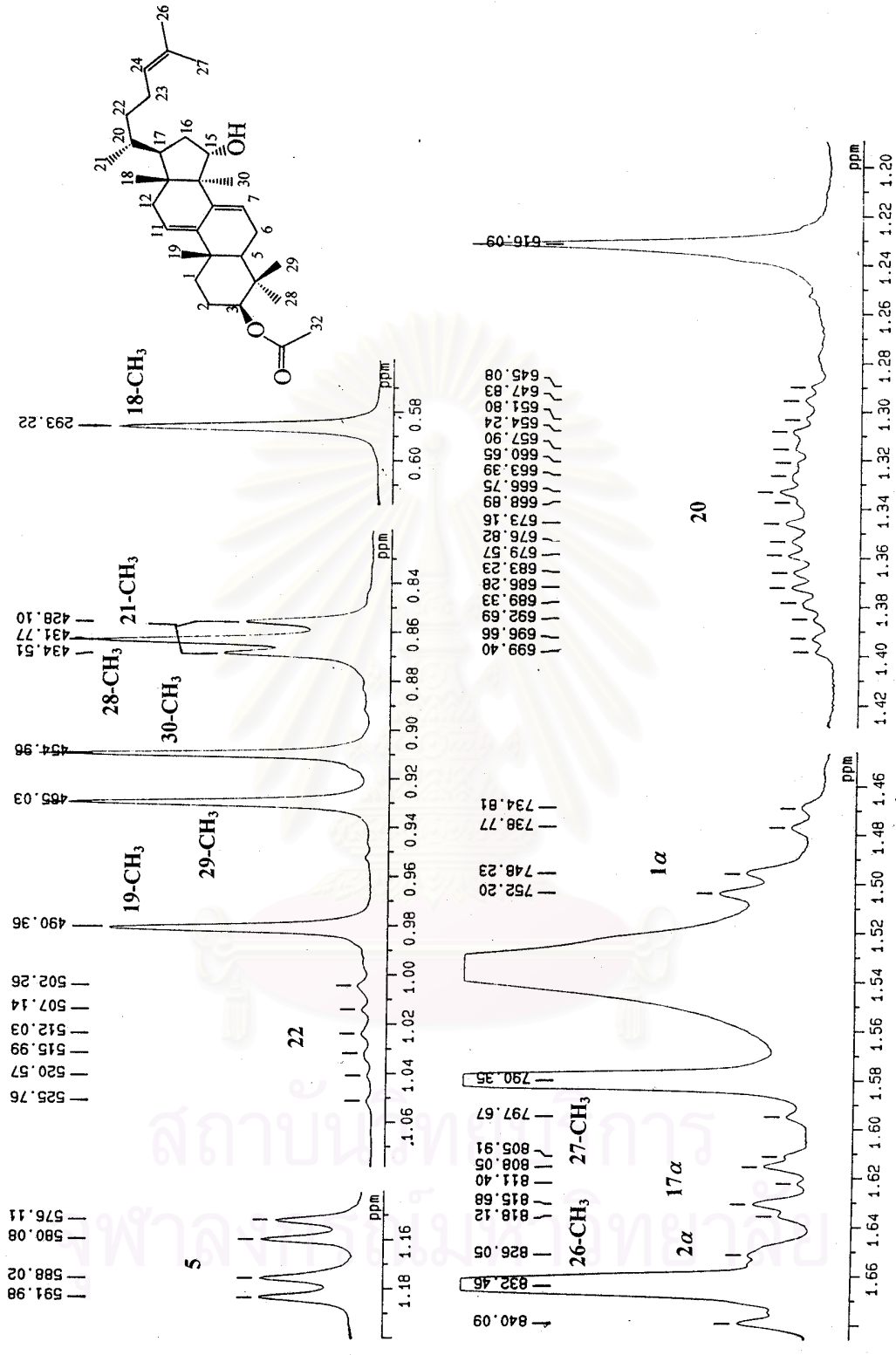


Figure 21c. The expanded 500 MHz ^{1}H NMR spectrum of compound L-2 (in $CDCl_3$)

(δ_H 0.57-1.68 ppm)

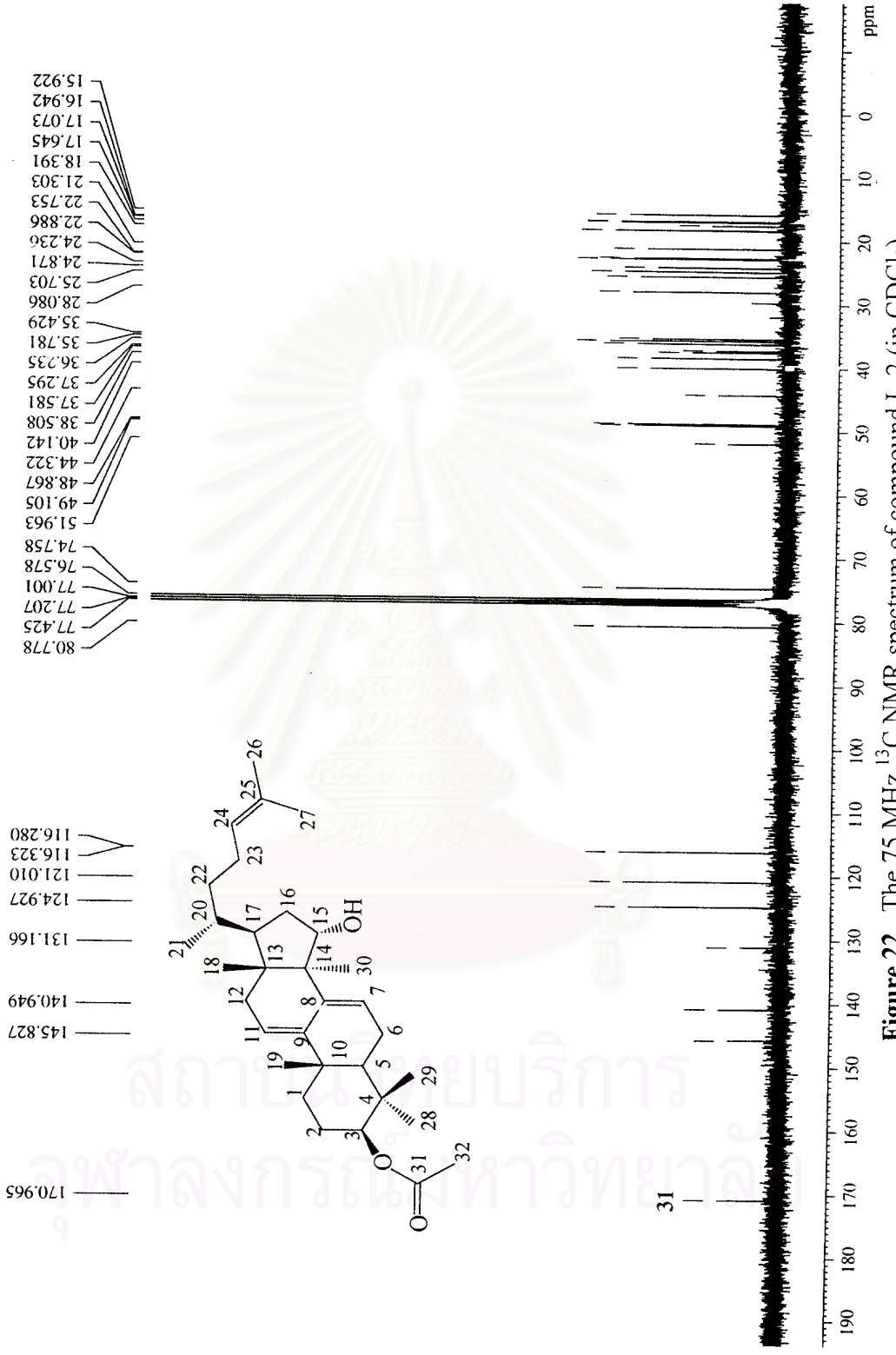


Figure 22. The 75 MHz ^{13}C NMR spectrum of compound L-2 (in $CDCl_3$)

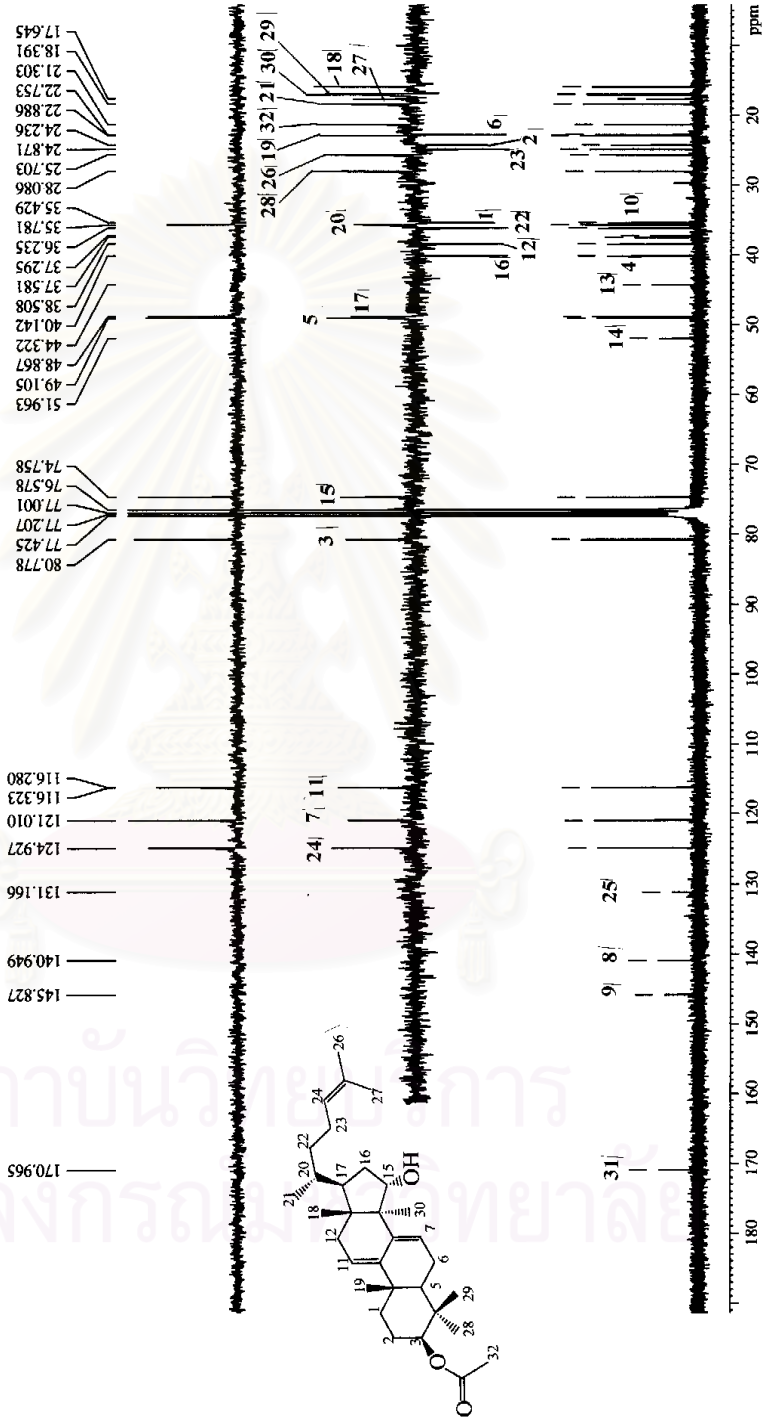


Figure 23. The 75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound L-2 (in $CDCl_3$)

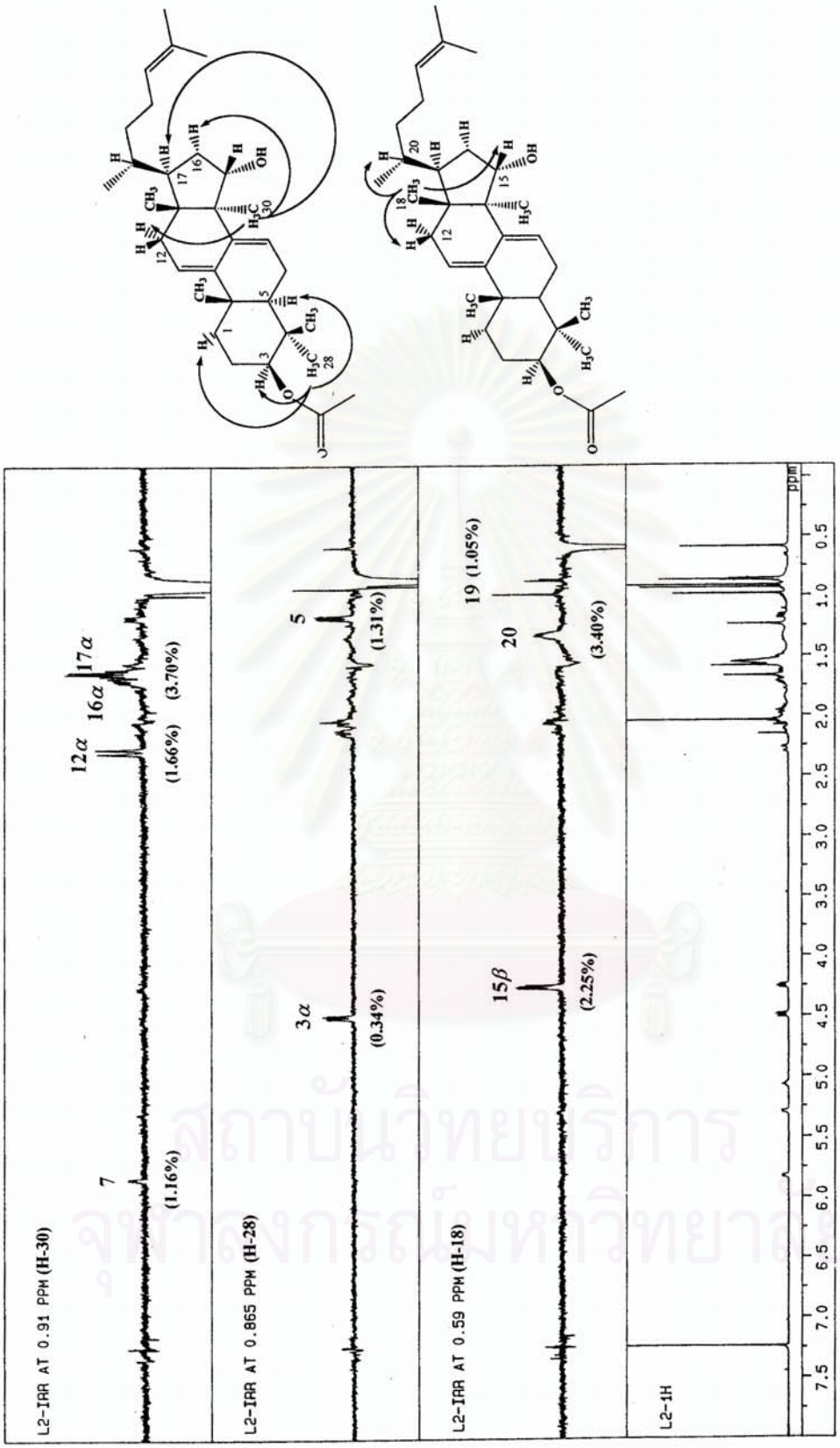


Figure 24. NOE Difference spectra of compound L-2 (in $CDCl_3$)

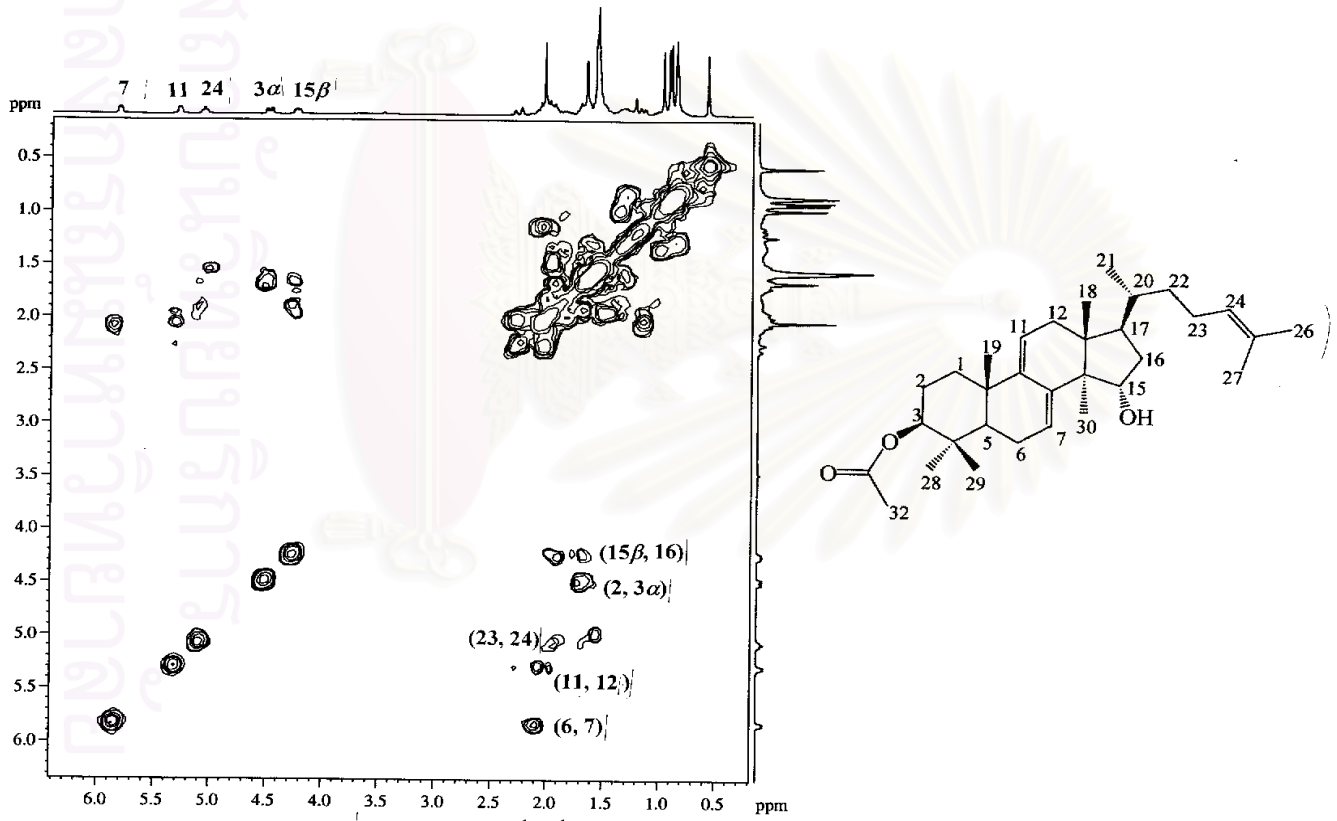


Figure 25a. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound

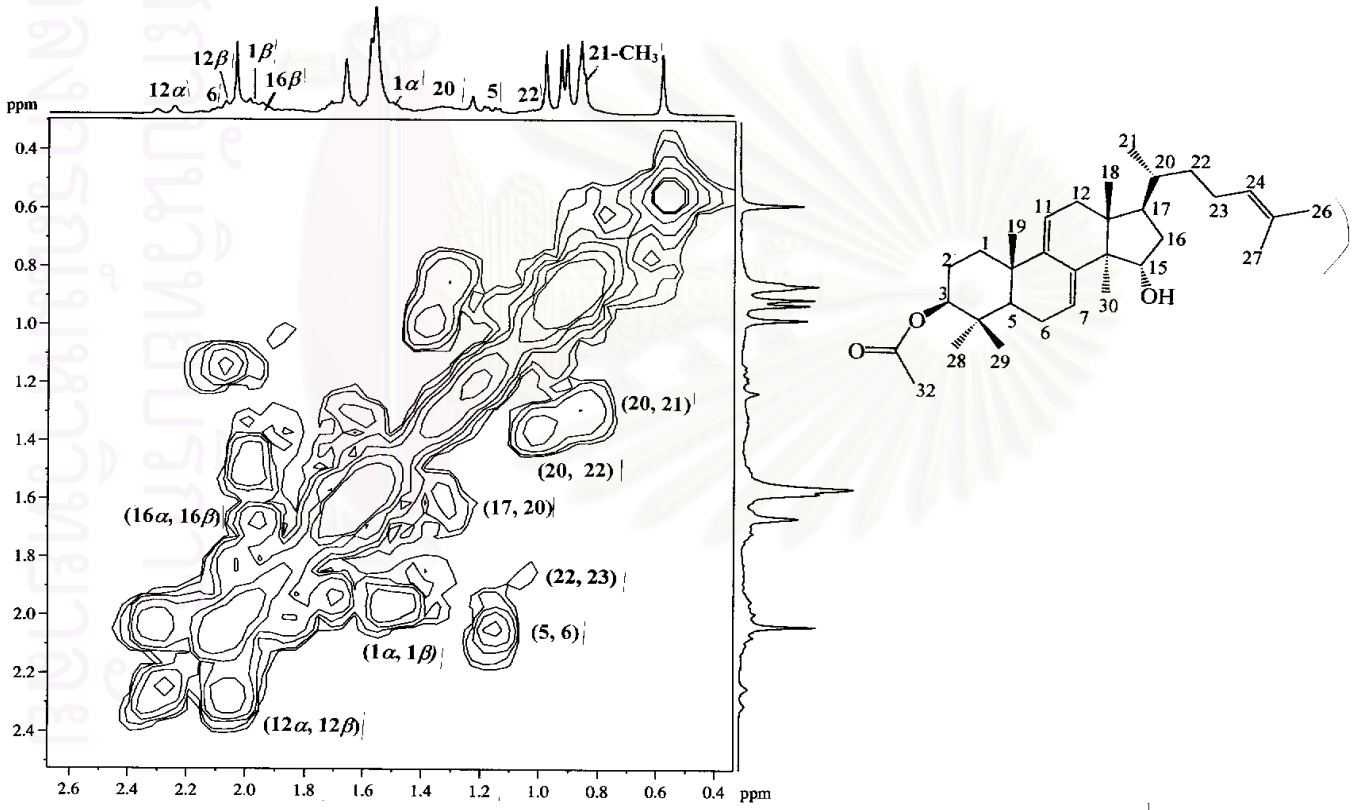


Figure 25b. The expanded 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound L-2 (in $CDCl_3$) (δ_H 0.3-2.7 ppm)

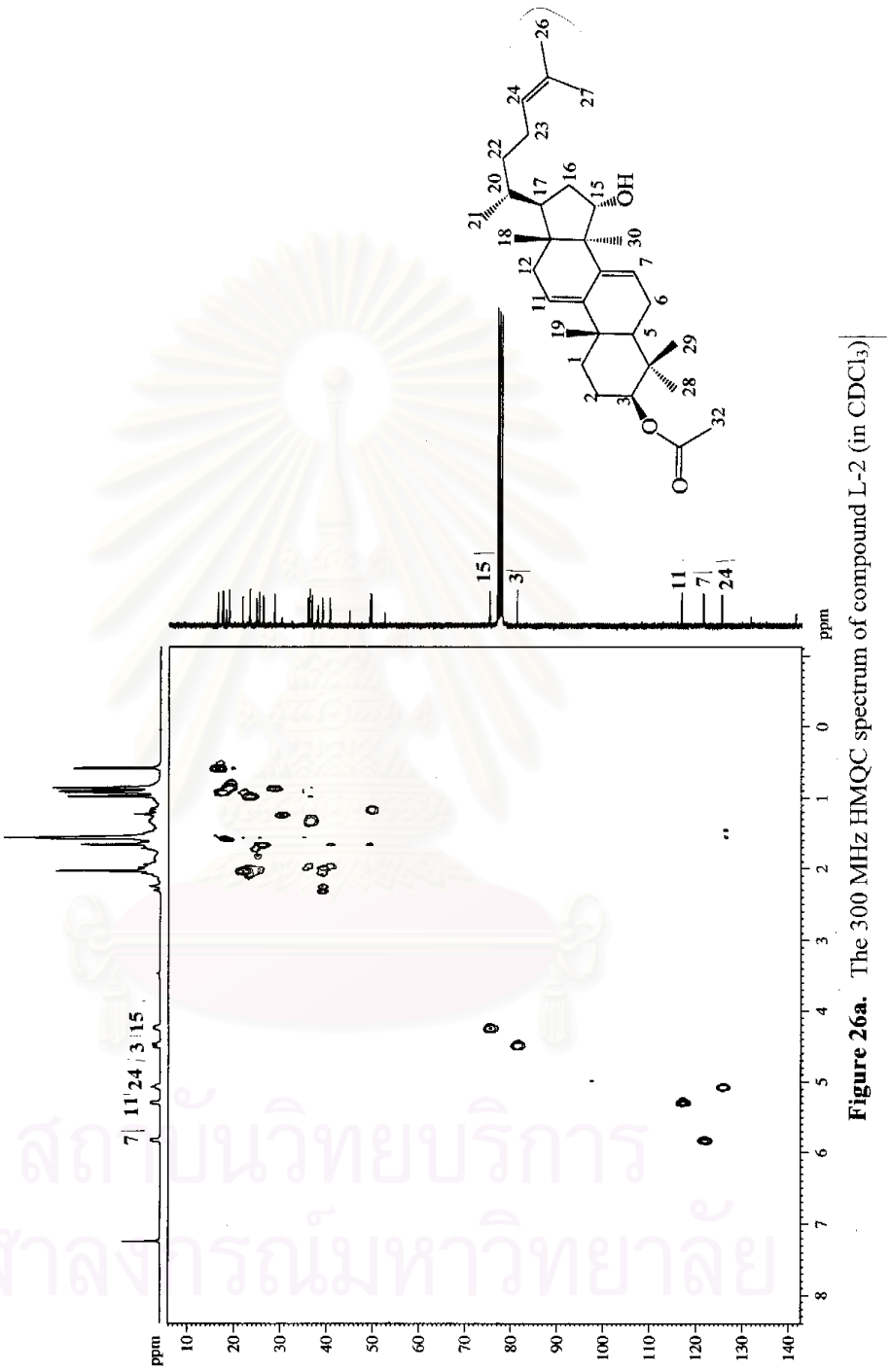


Figure 26a. The 300 MHz HMQC spectrum of compound L-2 (in $CDCl_3$)

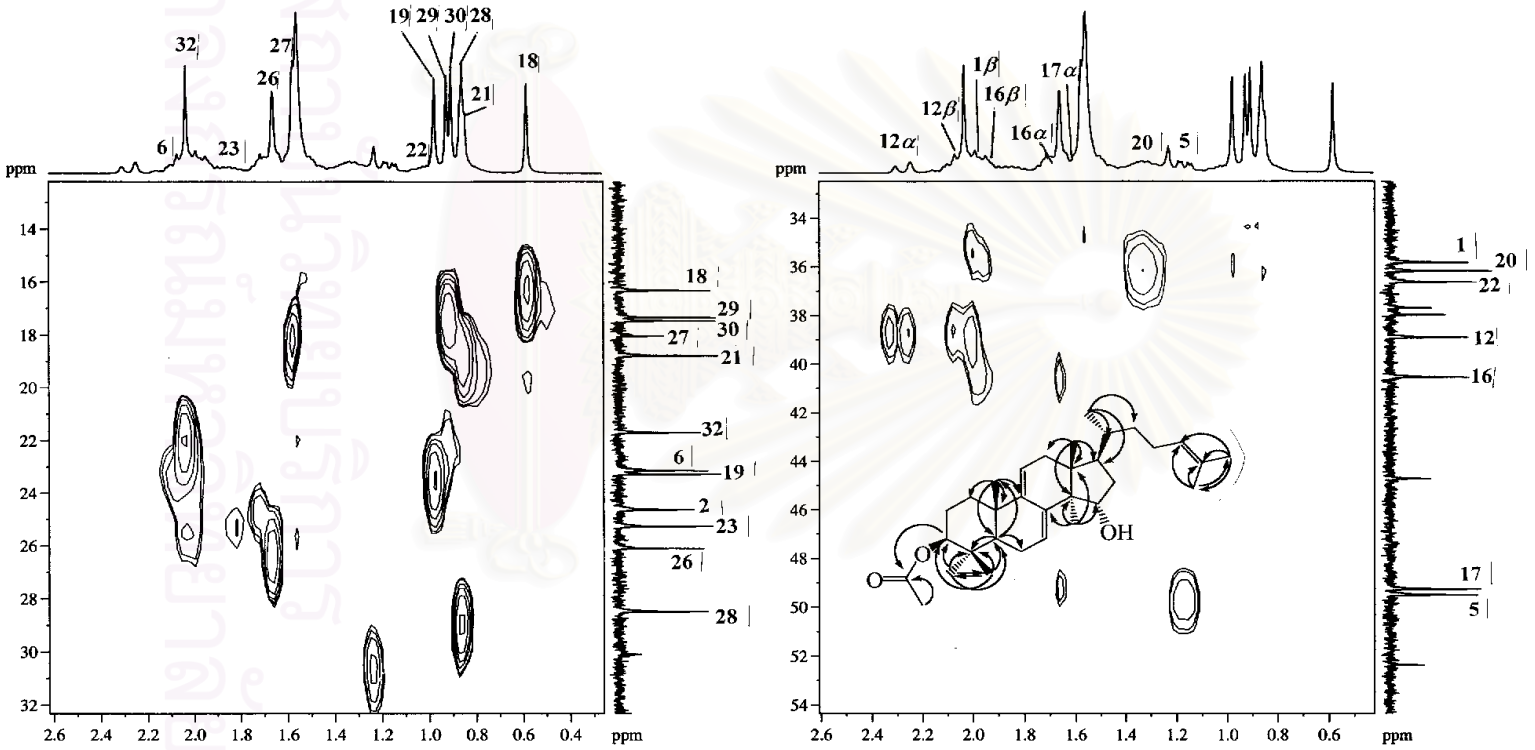


Figure 26b. The expanded 300 MHz HMQC spectrum of compound L-2 (in $CDCl_3$)

(δ_H 0.3-2.6 ppm, δ_C 12.0-32.5 ppm and δ_H 0.4-2.6 ppm, δ_C 32.5-54.5ppm)

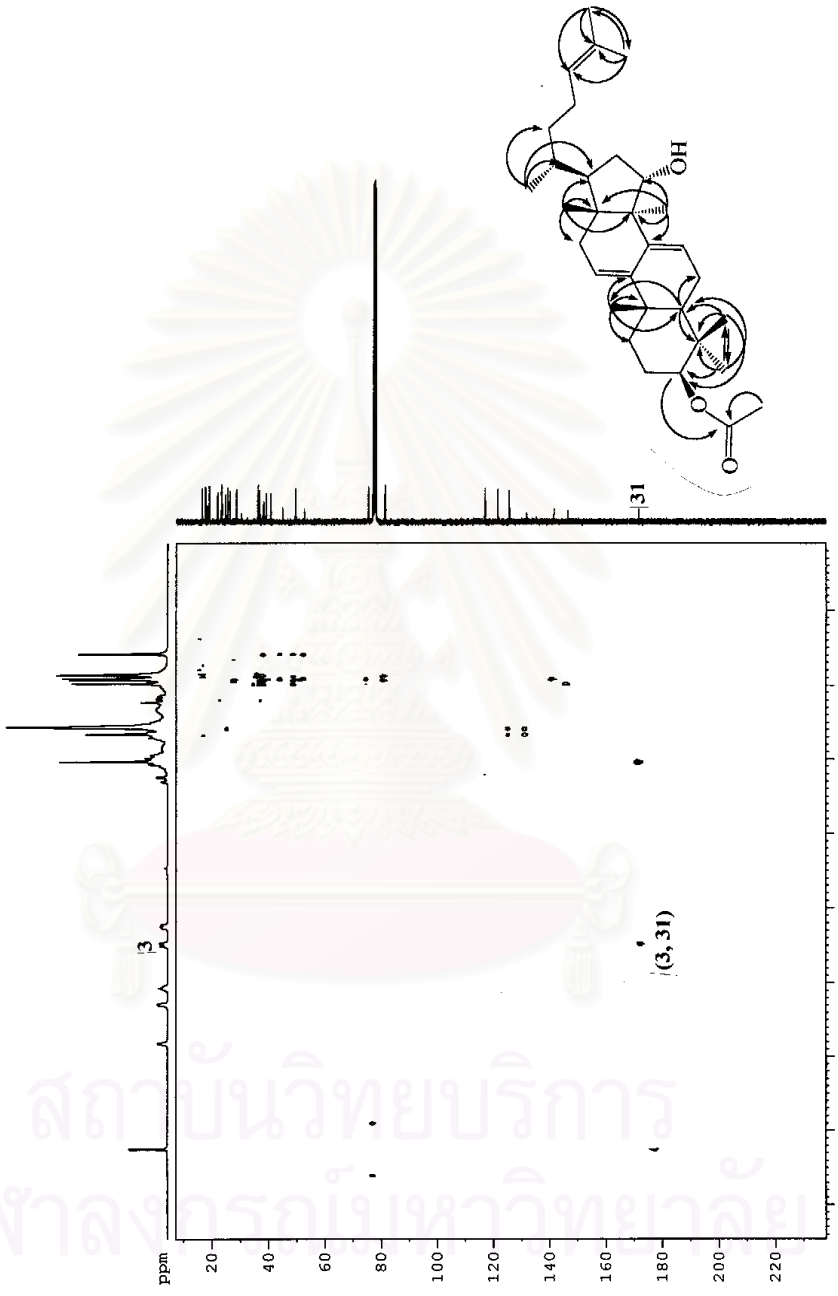


Figure 27a. The 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-2 (in $CDCl_3$)

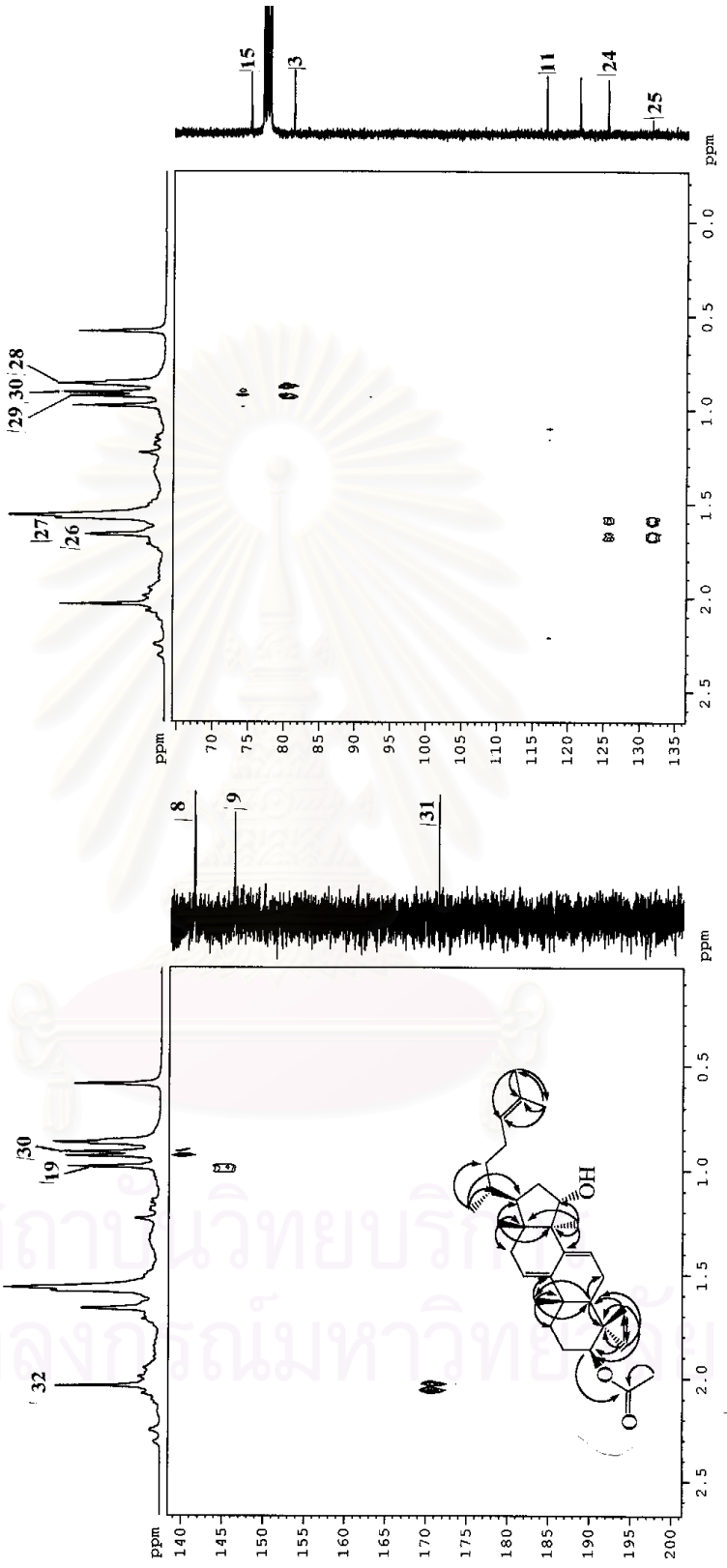


Figure 27b. The expanded 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-2 (in $CDCl_3$)
(δ_H 0.0-2.7 ppm, δ_C 139.0-201.0 ppm and δ_H –0.2-2.7 ppm, δ_C 65.0-136.0 ppm)

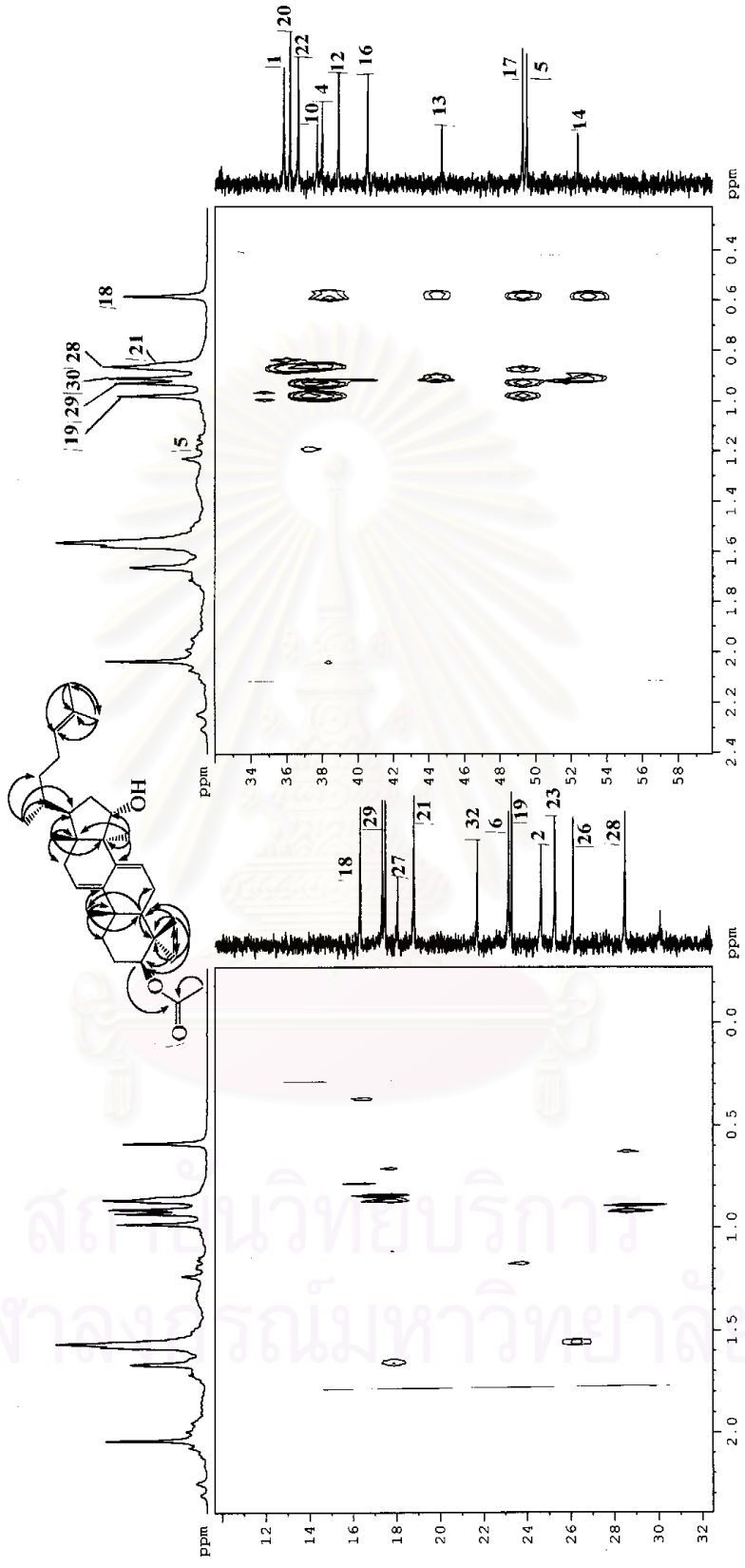


Figure 27c. The expanded 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-2 (in $CDCl_3$)
(δ_H –0.2-2.4 ppm, δ_C 10.0-33.0 ppm and δ_H –0.2-2.4 ppm, δ_C 32.0-60.0 ppm)

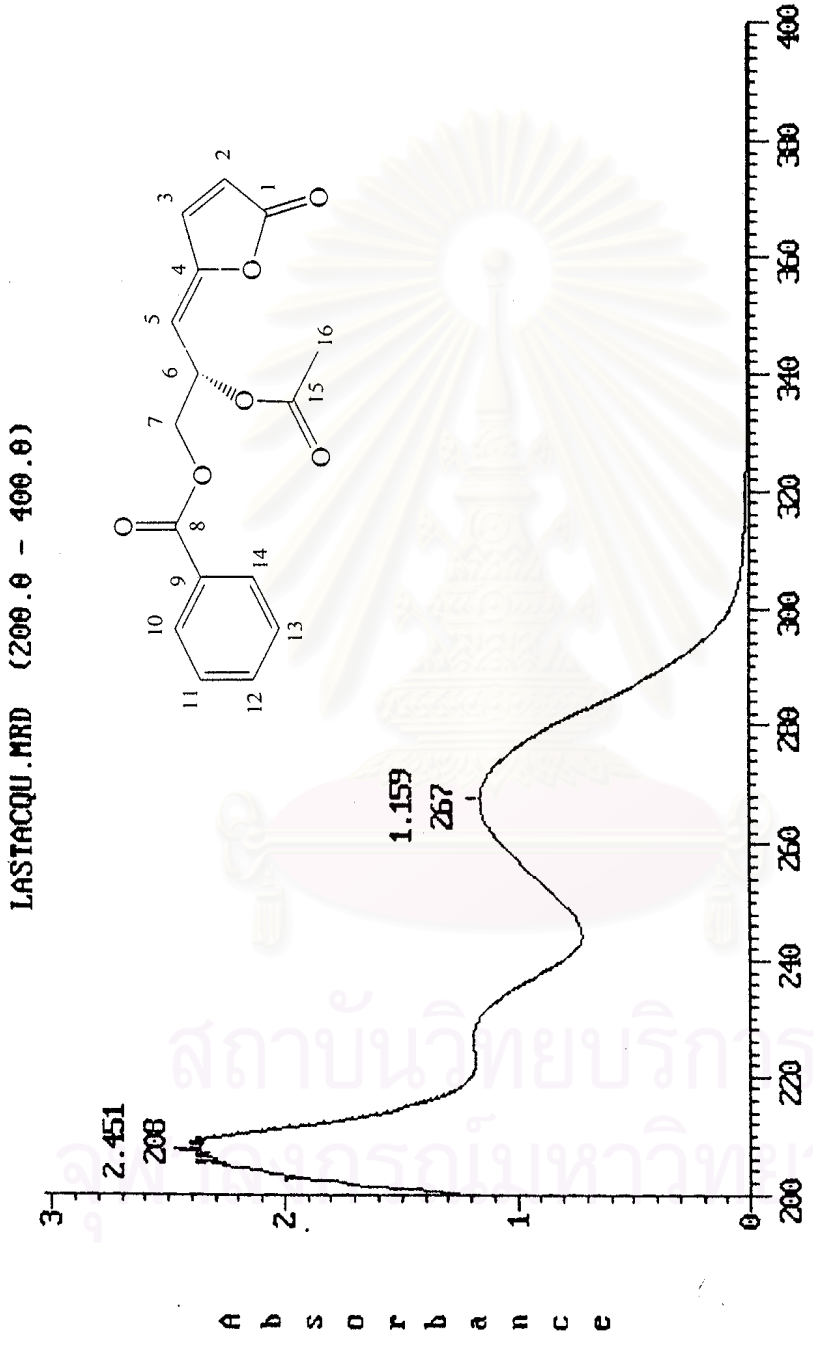


Figure 28. The UV spectrum of compound L-3 (in MeOH)

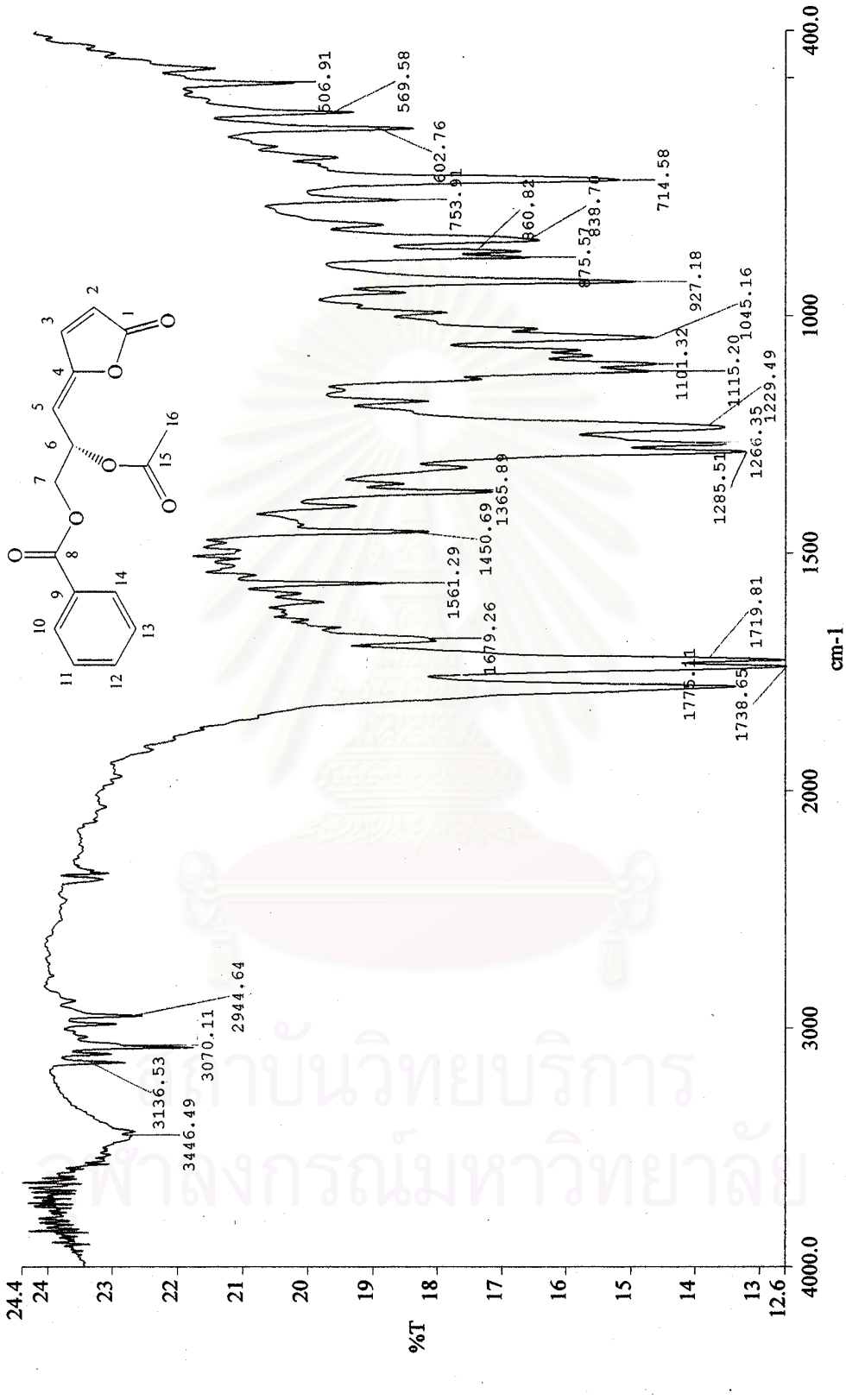


Figure 29. The IR spectrum of compound L-3 (KBr disc)

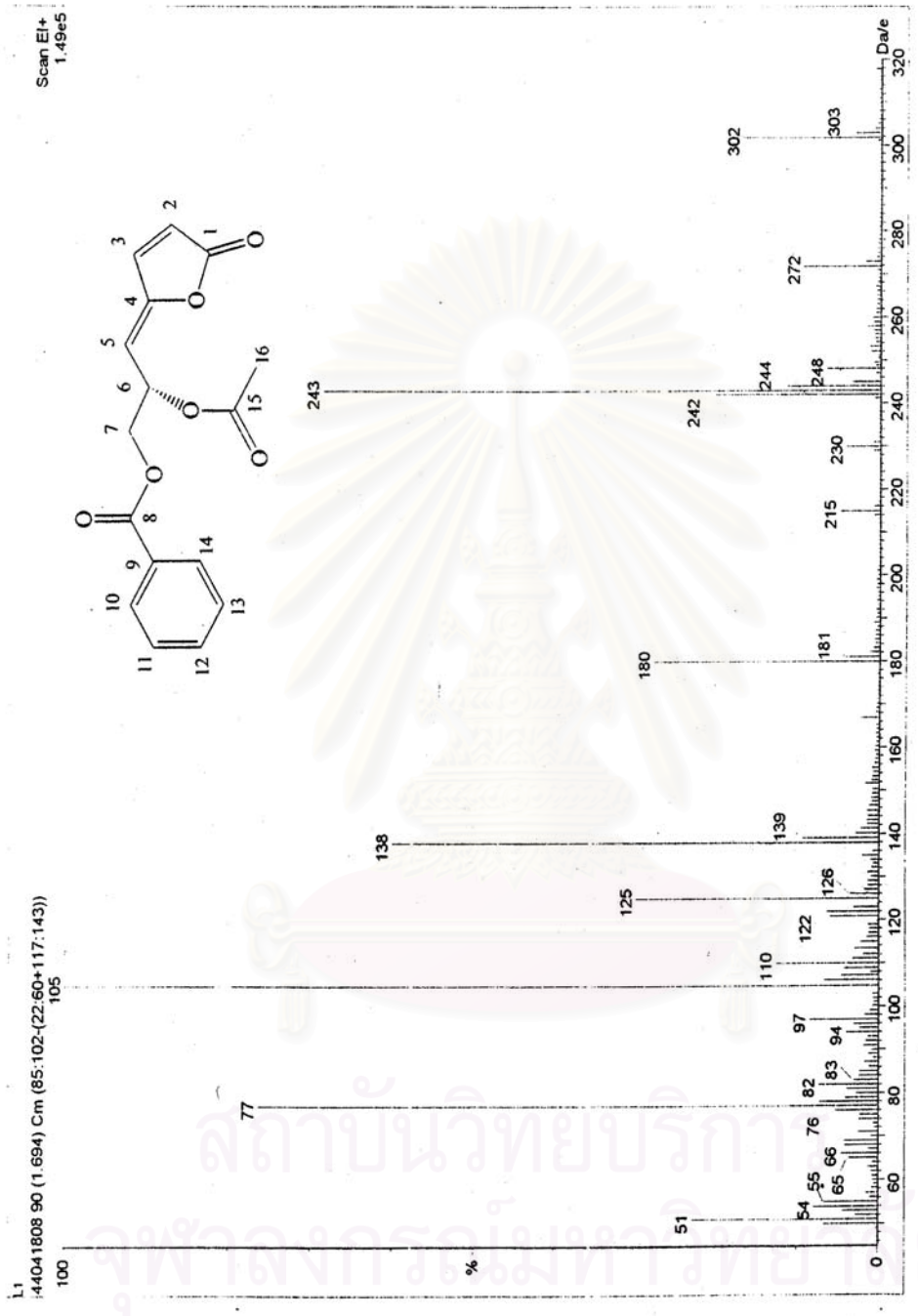


Figure 30. The EIMS spectrum of compound L-3

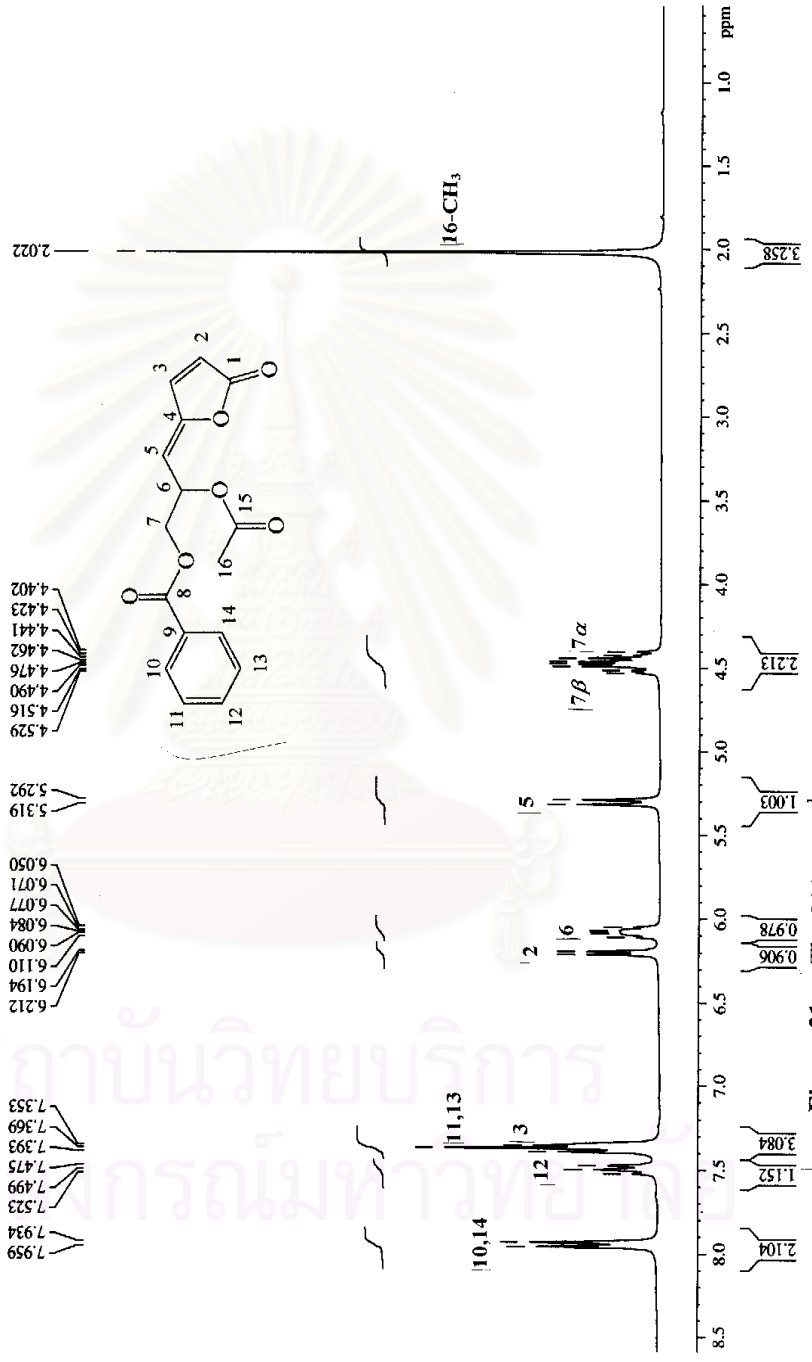


Figure 31a. The 300 MHz ^{1}H NMR spectrum of compound L-3 (in $CDCl_3$)

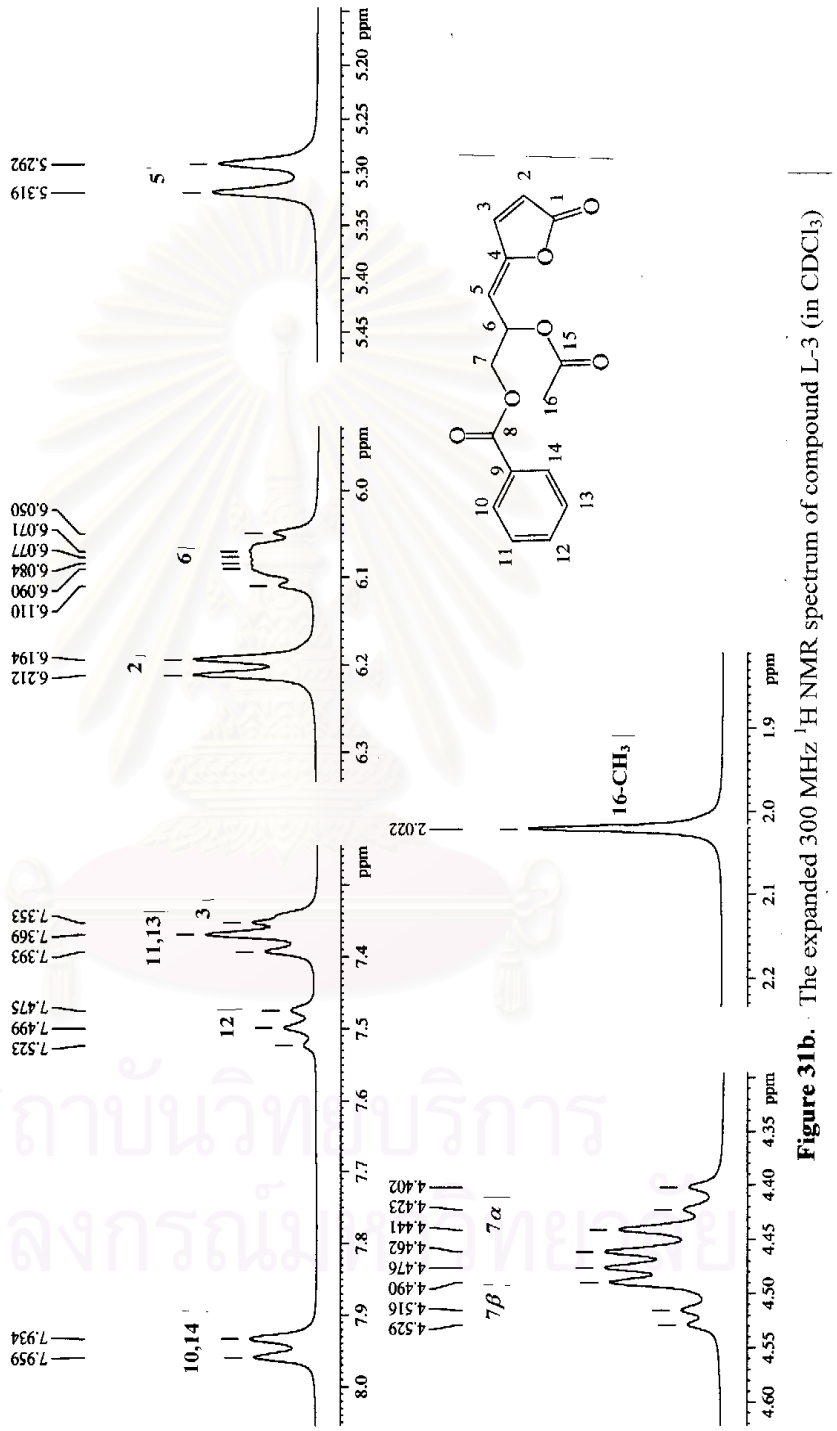


Figure 31b. The expanded 300 MHz ^{1}H NMR spectrum of compound L-3 (in $CDCl_3$)

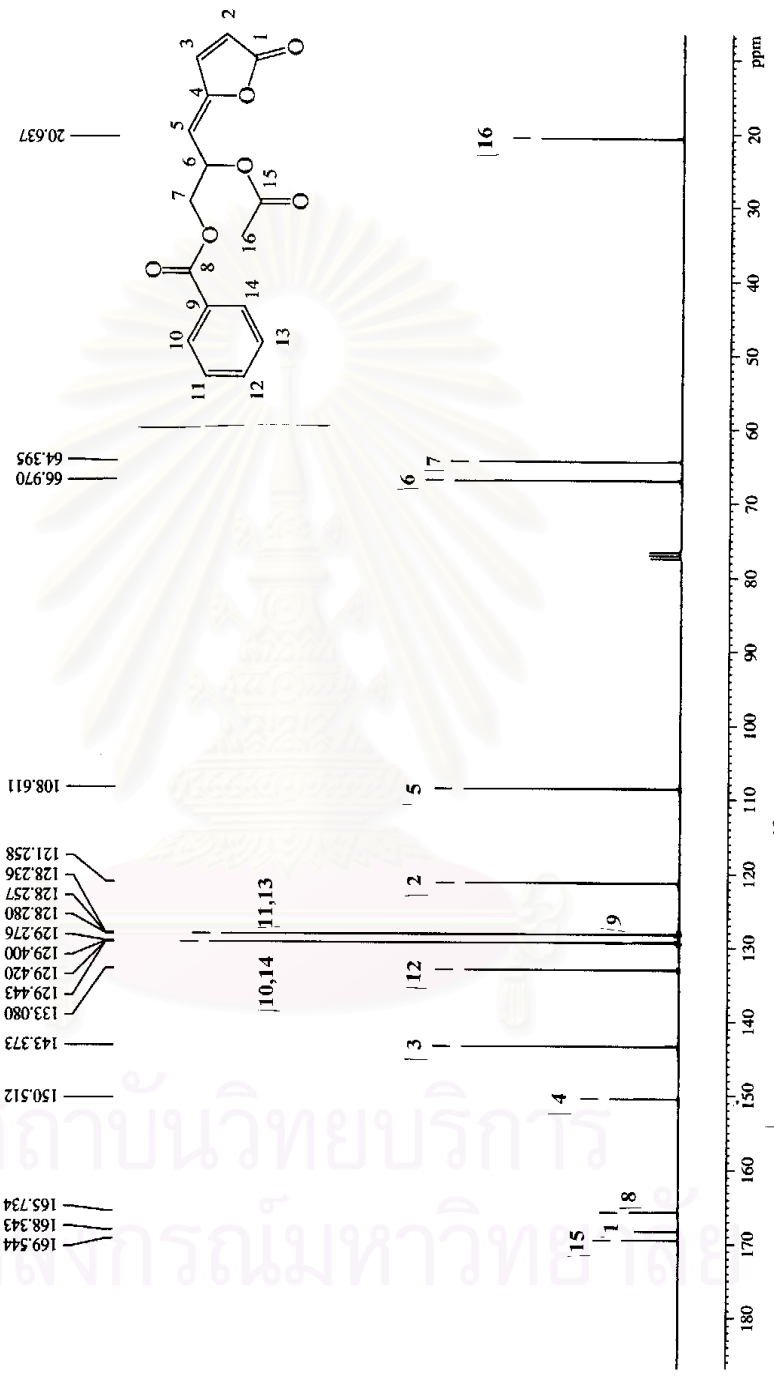


Figure 32. The 75 MHz ^{13}C NMR spectrum of compound L-3 (in $CDCl_3$)

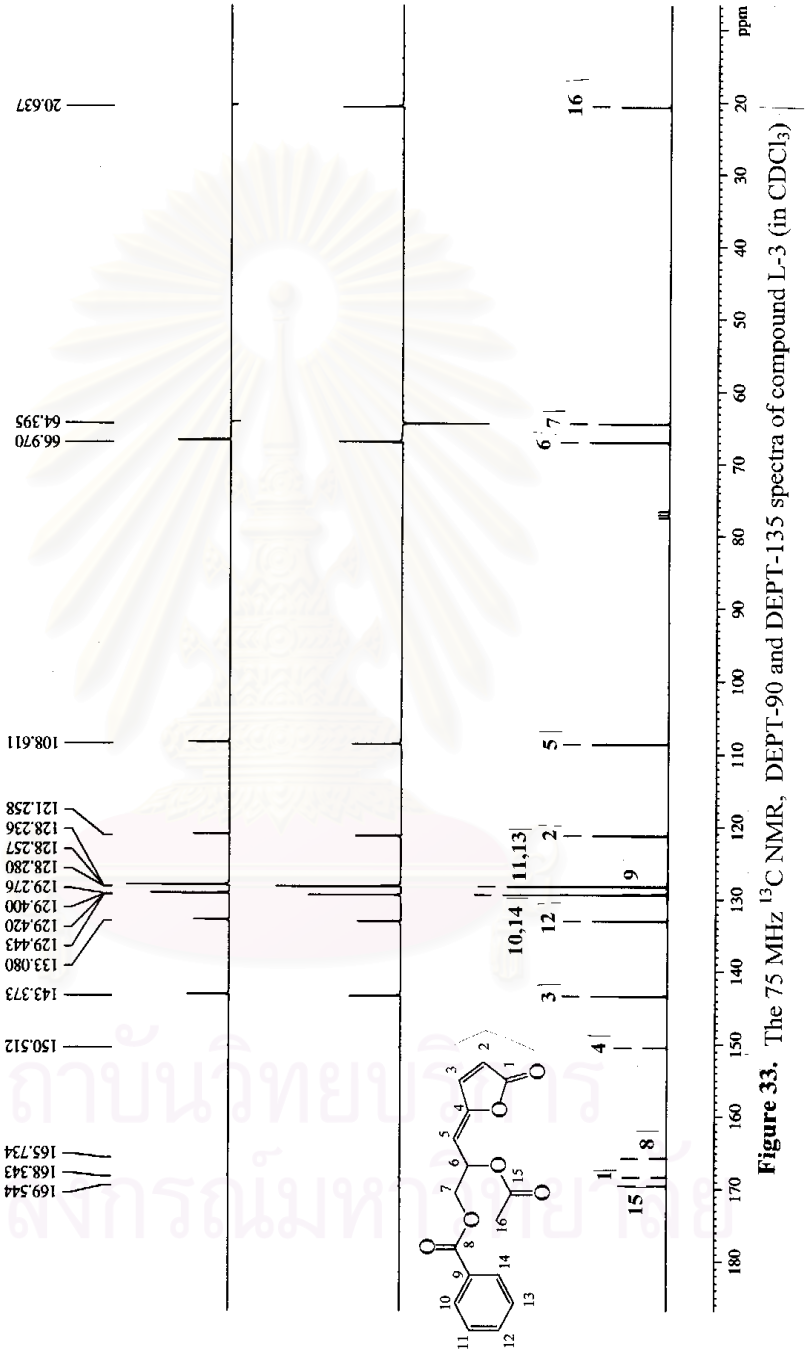


Figure 33. The 75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound L-3 (in $CDCl_3$)

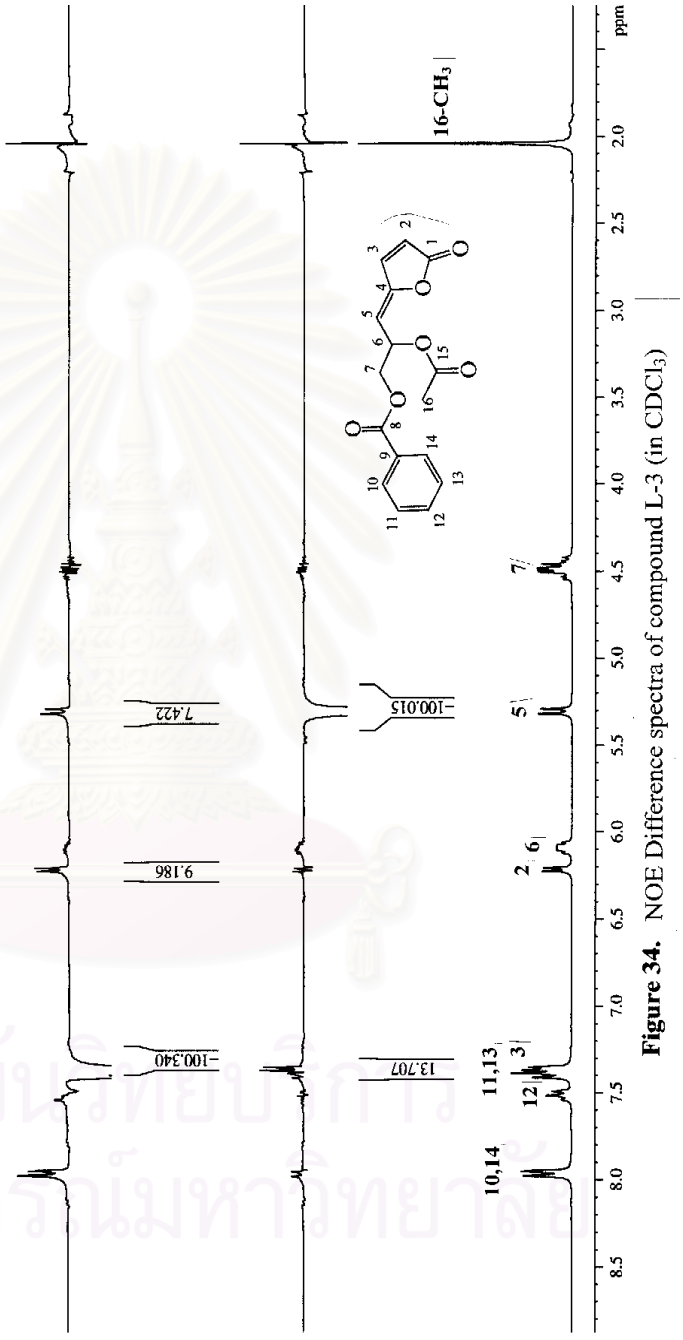


Figure 34. NOE Difference spectra of compound L-3 (in $CDCl_3$)

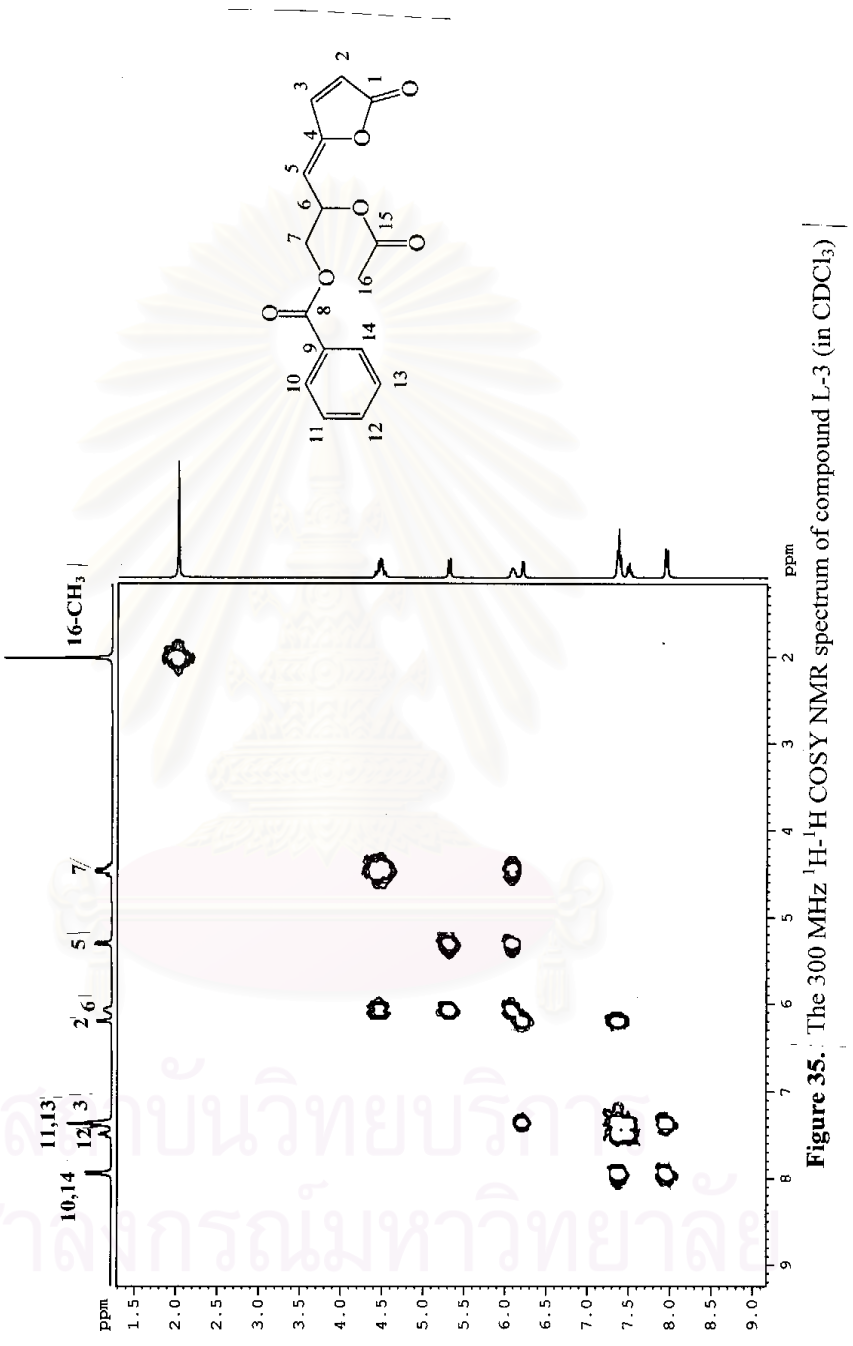


Figure 35. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound L-3 (in $CDCl_3$)

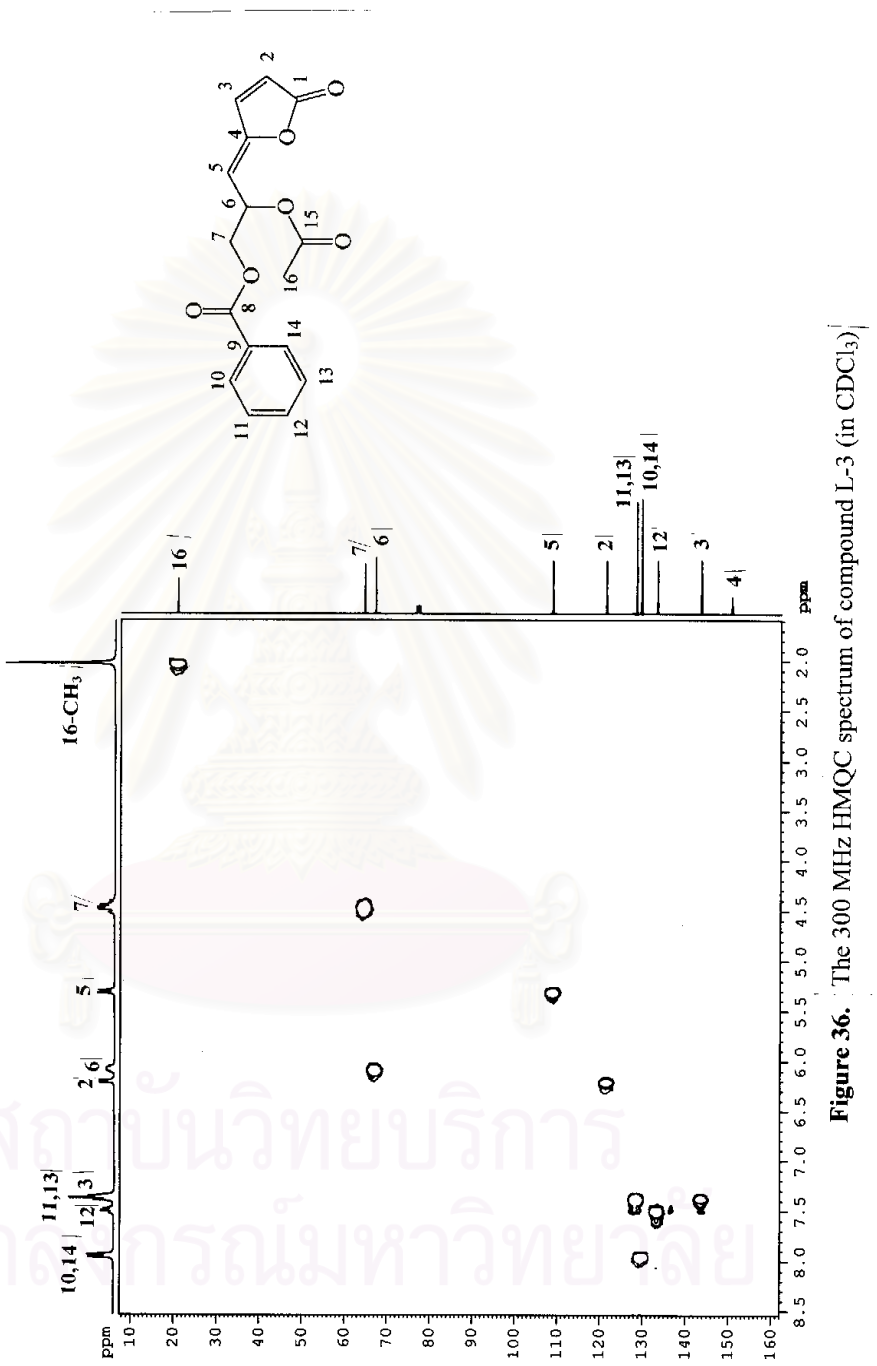


Figure 36. The 300 MHz HMQC spectrum of compound L-3 (in $CDCl_3$)

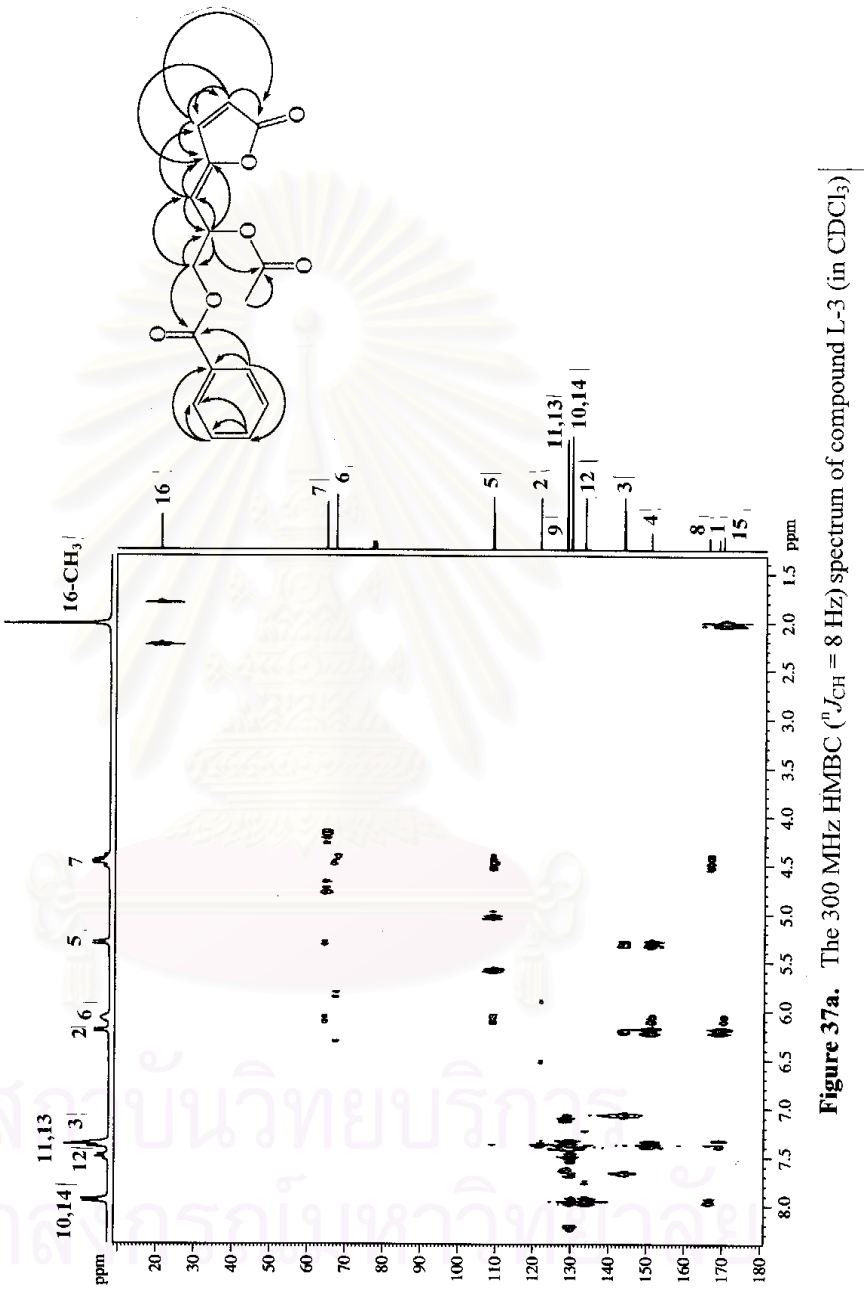


Figure 37a. The 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-3 (in $CDCl_3$)

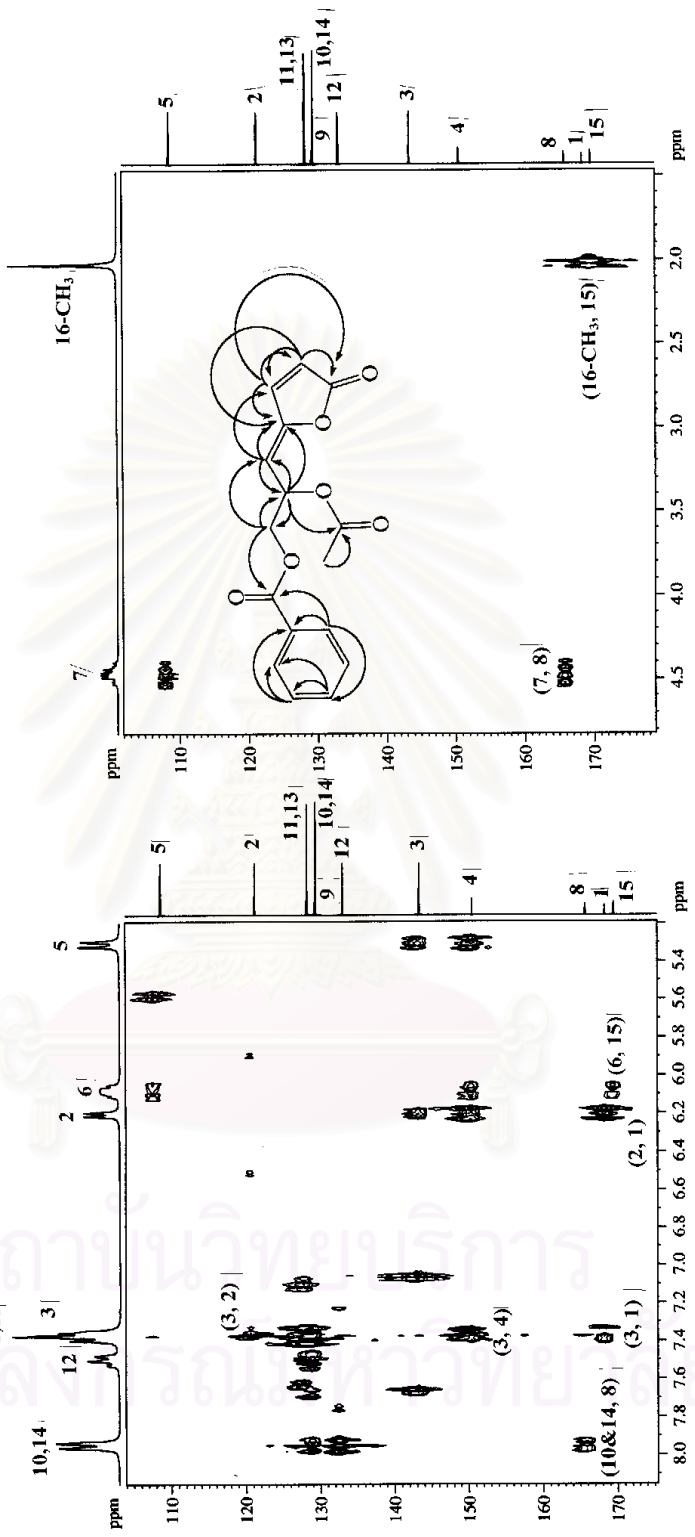


Figure 37b. The expanded 300 MHz HMBC ($^nJ_{CH}$ = 8 Hz) spectrum of compound L-3 (in $CDCl_3$)
(δ_H 5.2-8.1 ppm, δ_C 104.0-175.0 ppm and δ_H 1.5-4.8 ppm, δ_C 101.0-179.0 ppm)

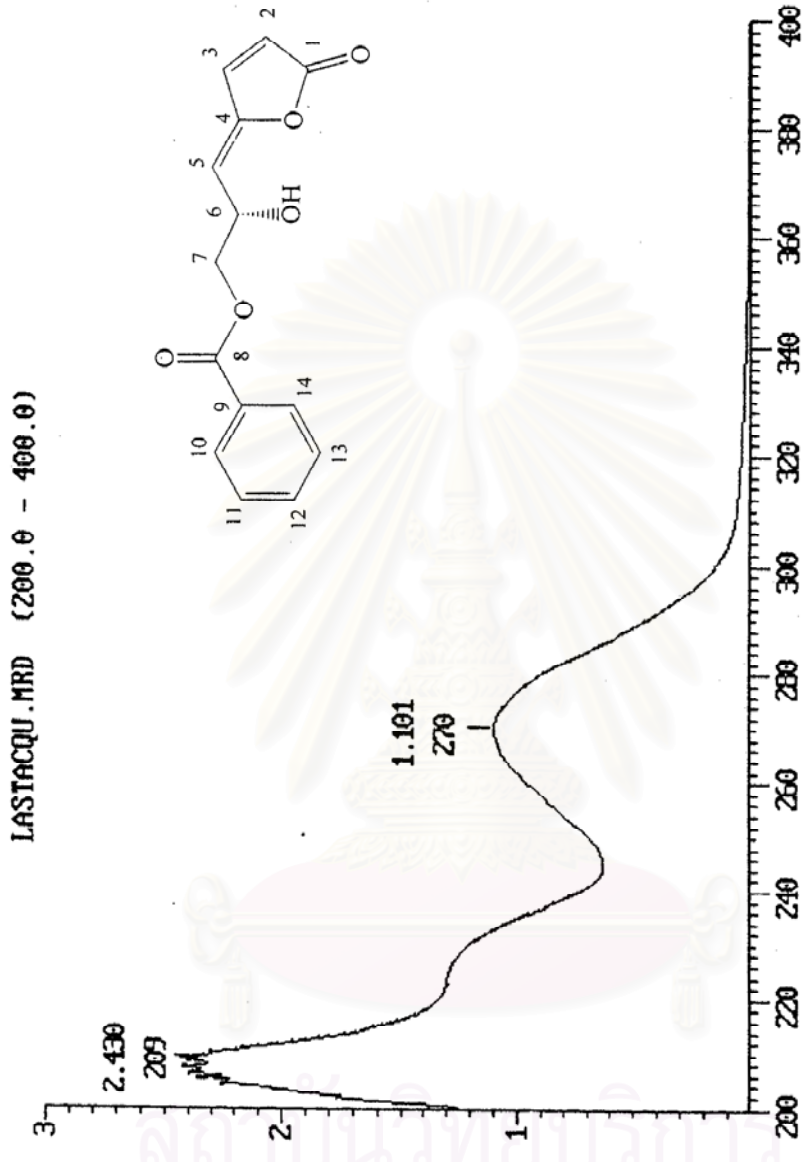


Figure 38. The UV spectrum of compound L-4 (in MeOH)

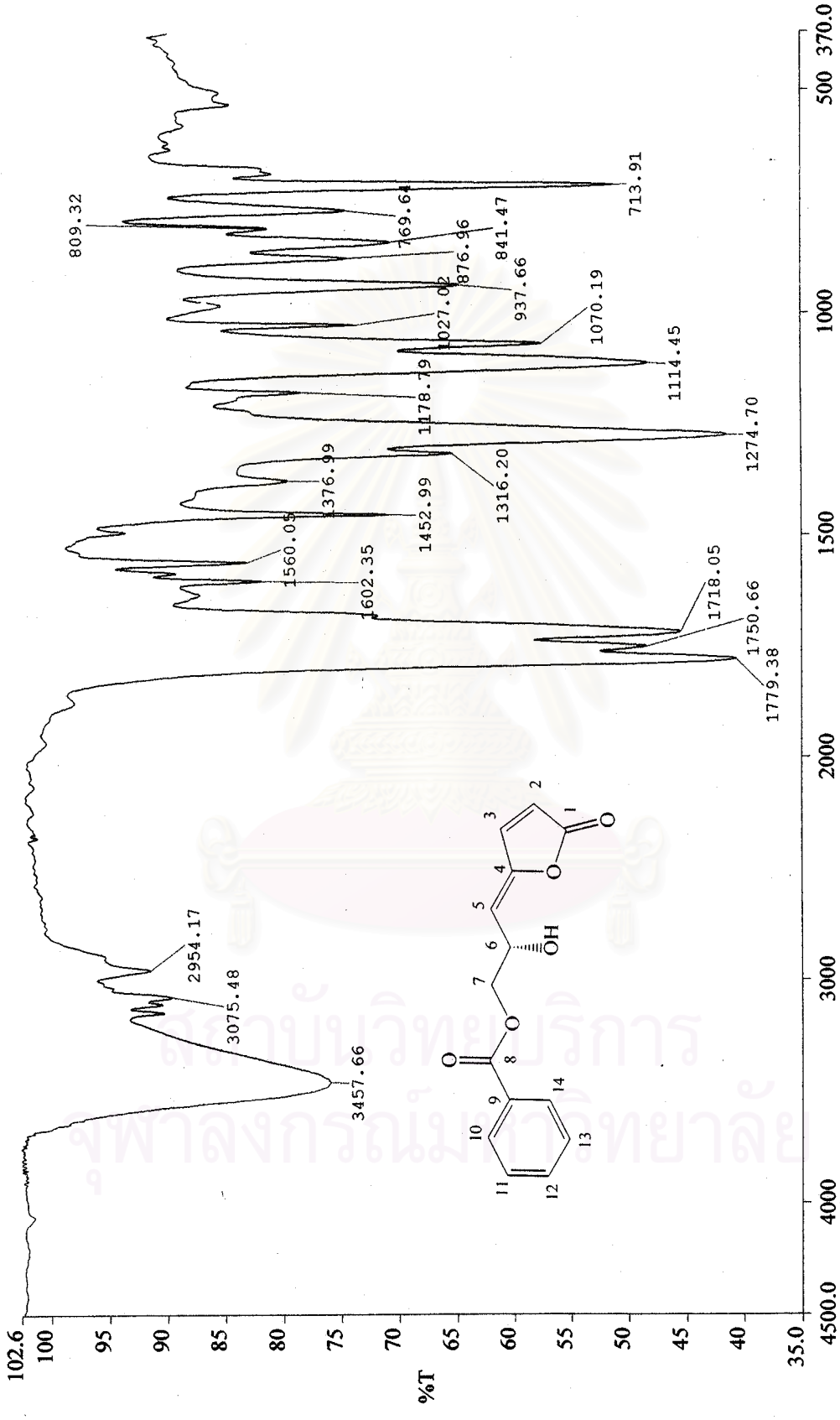


Figure 39. The IR spectrum of compound L-4 (KBr disc)

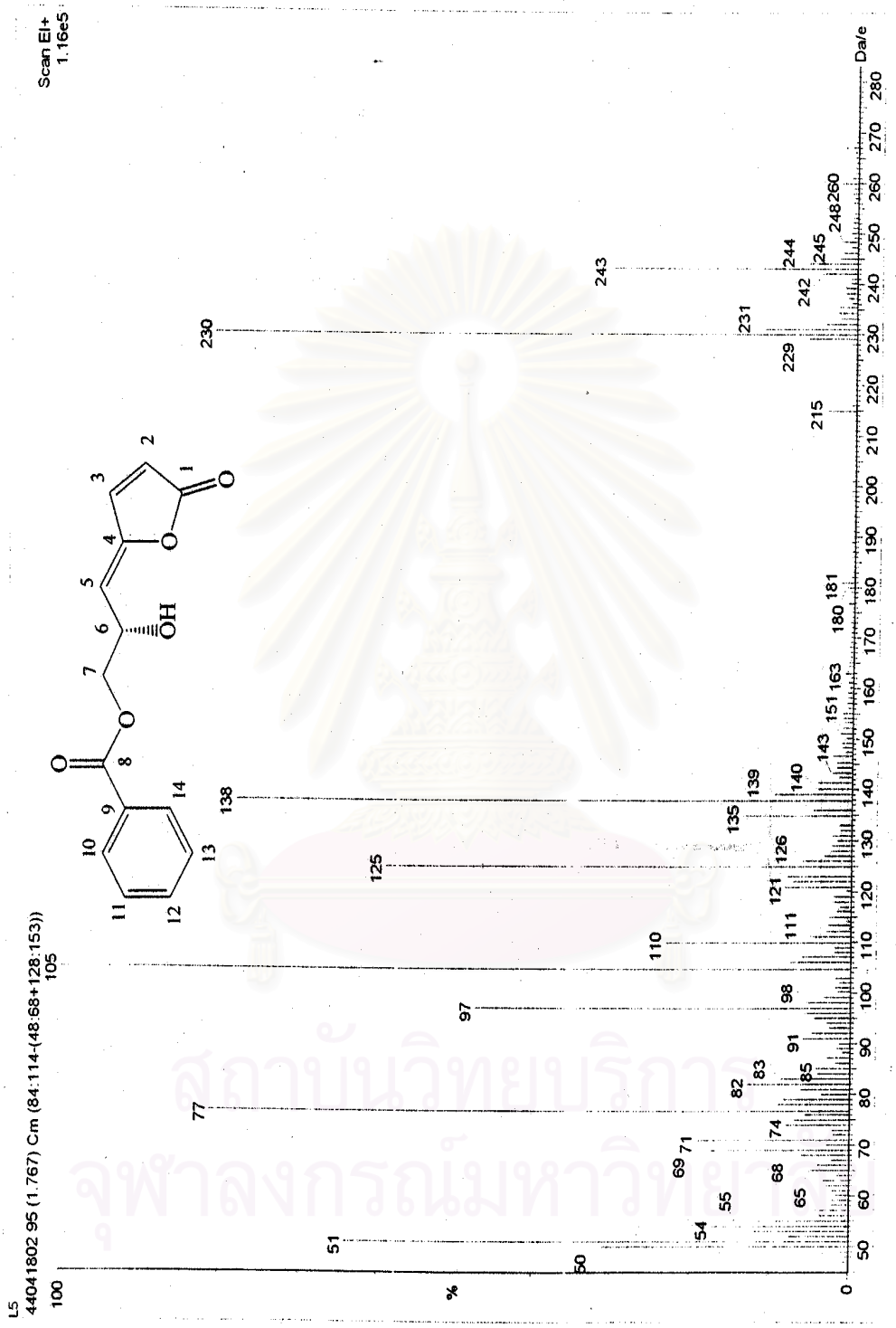


Figure 40. The EIMS spectrum of compound L-4

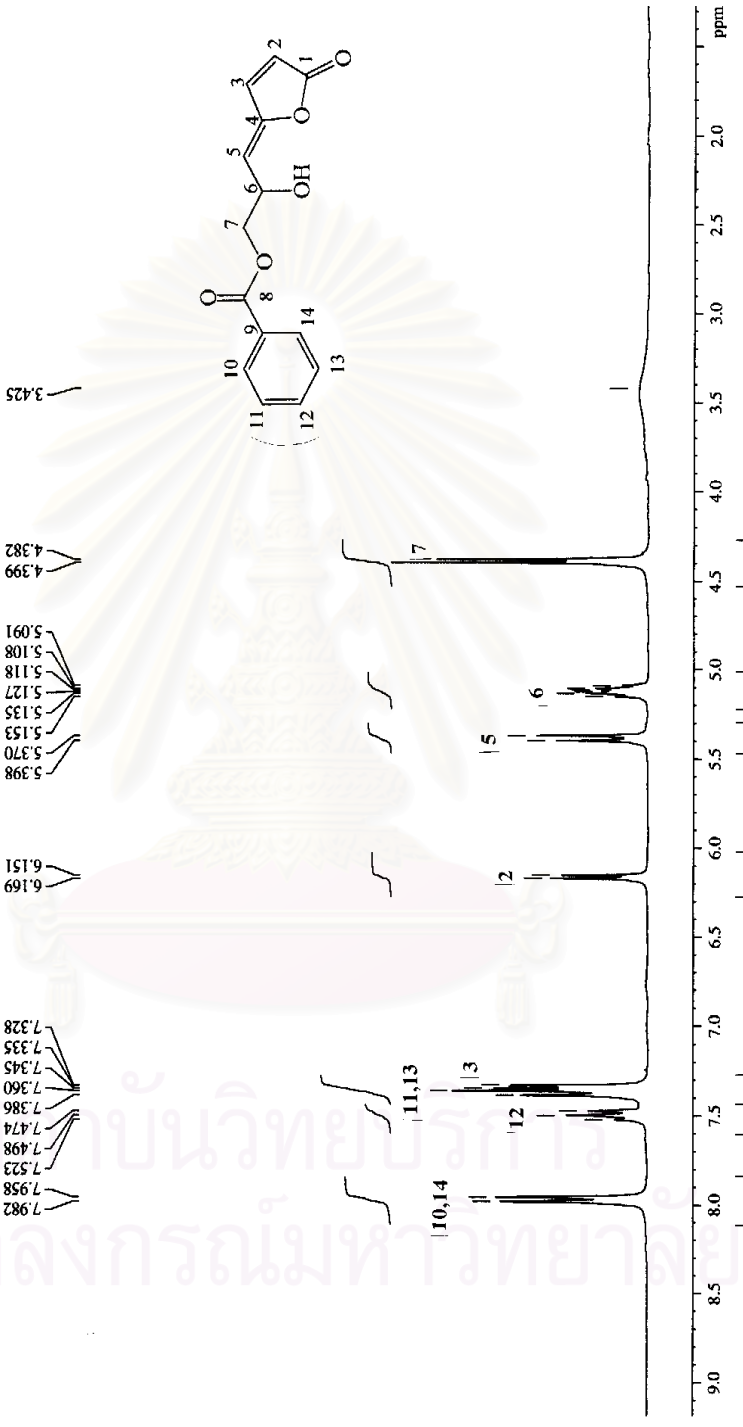


Figure 41a. The 300 MHz ^{1}H NMR spectrum of compound **L-4** (in $CDCl_3$)

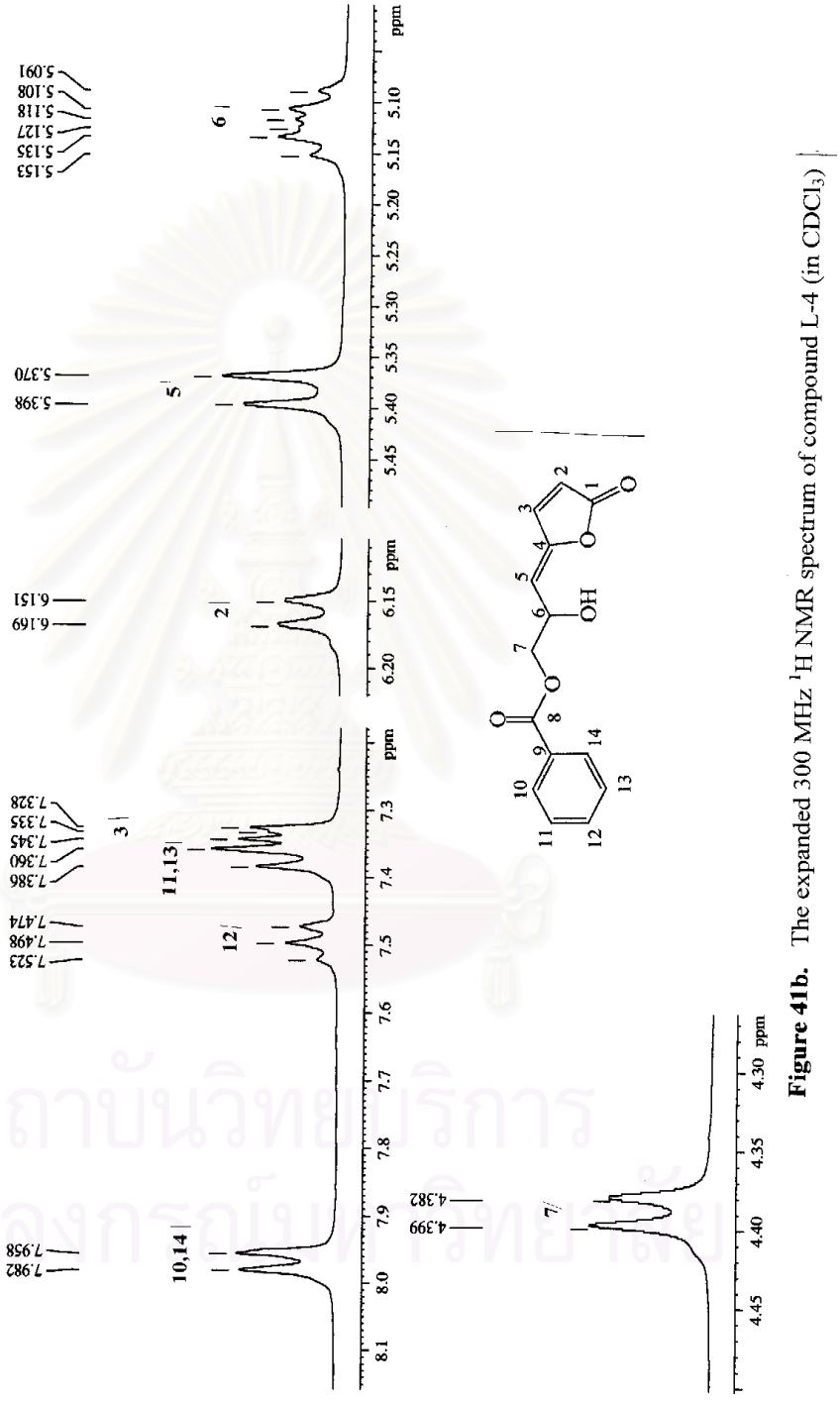


Figure 41b. The expanded 300 MHz ^{1}H NMR spectrum of compound L-4 (in $CDCl_3$)

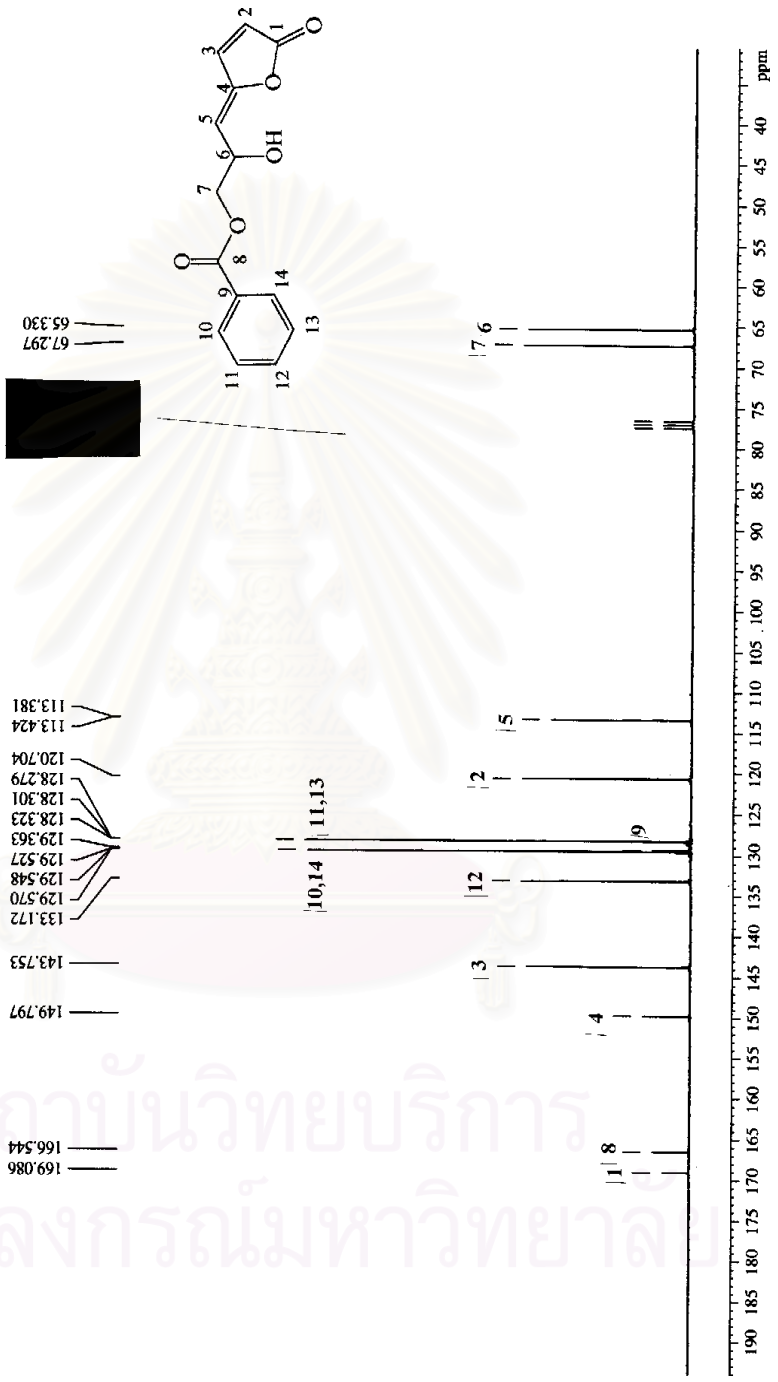


Figure 42. The 75 MHz ^{13}C NMR spectrum of compound L-4 (in $CDCl_3$)

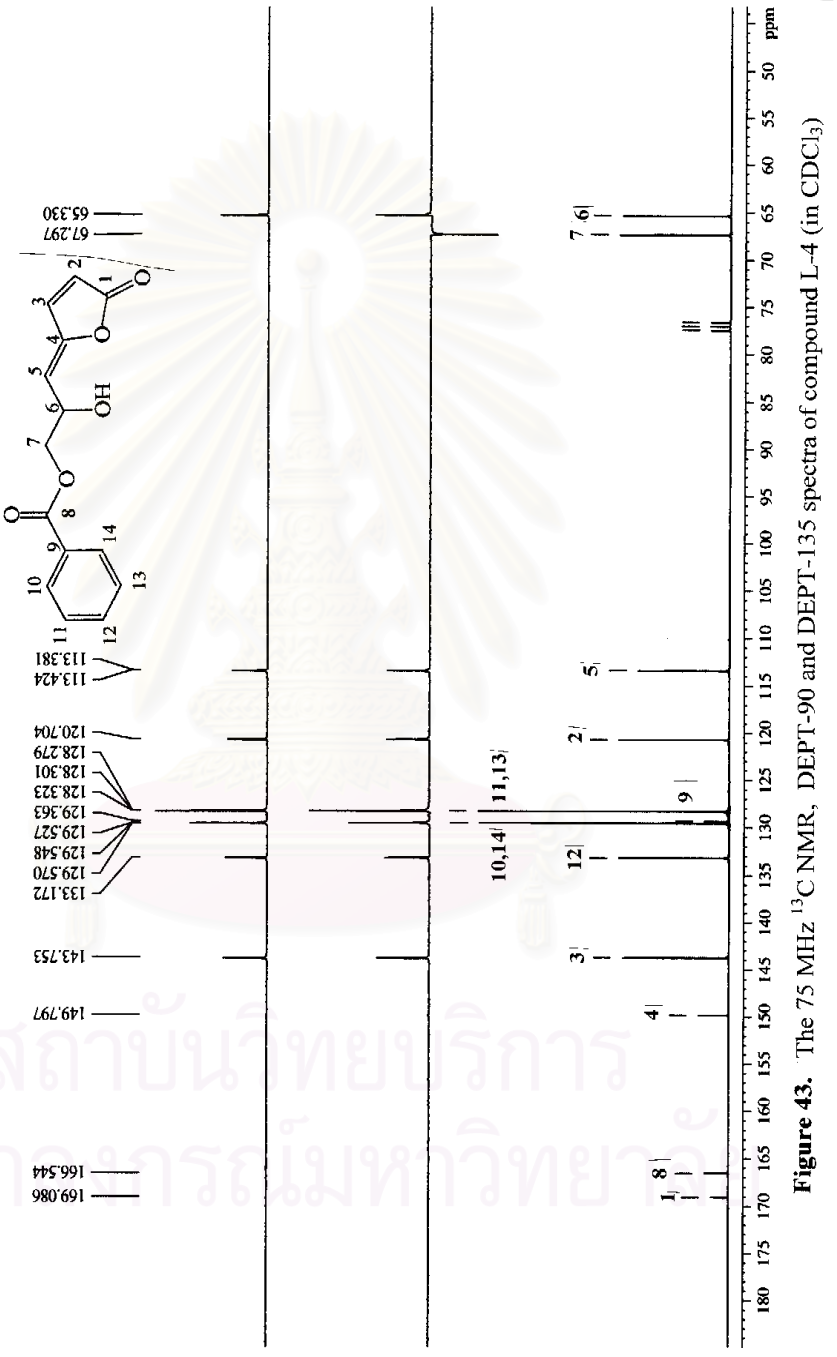


Figure 43. The 75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound L-4 (in $CDCl_3$)

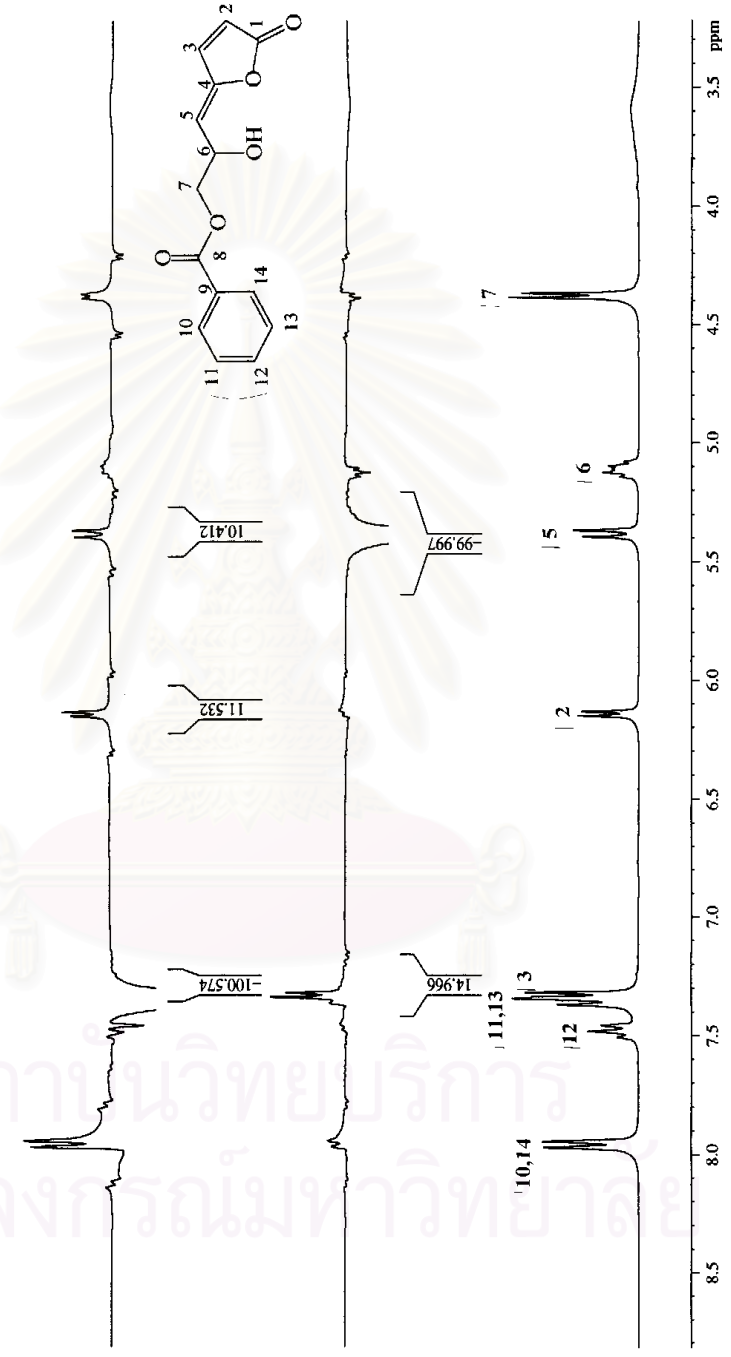


Figure 44. NOE Difference spectra of compound L-4 (in $CDCl_3$)

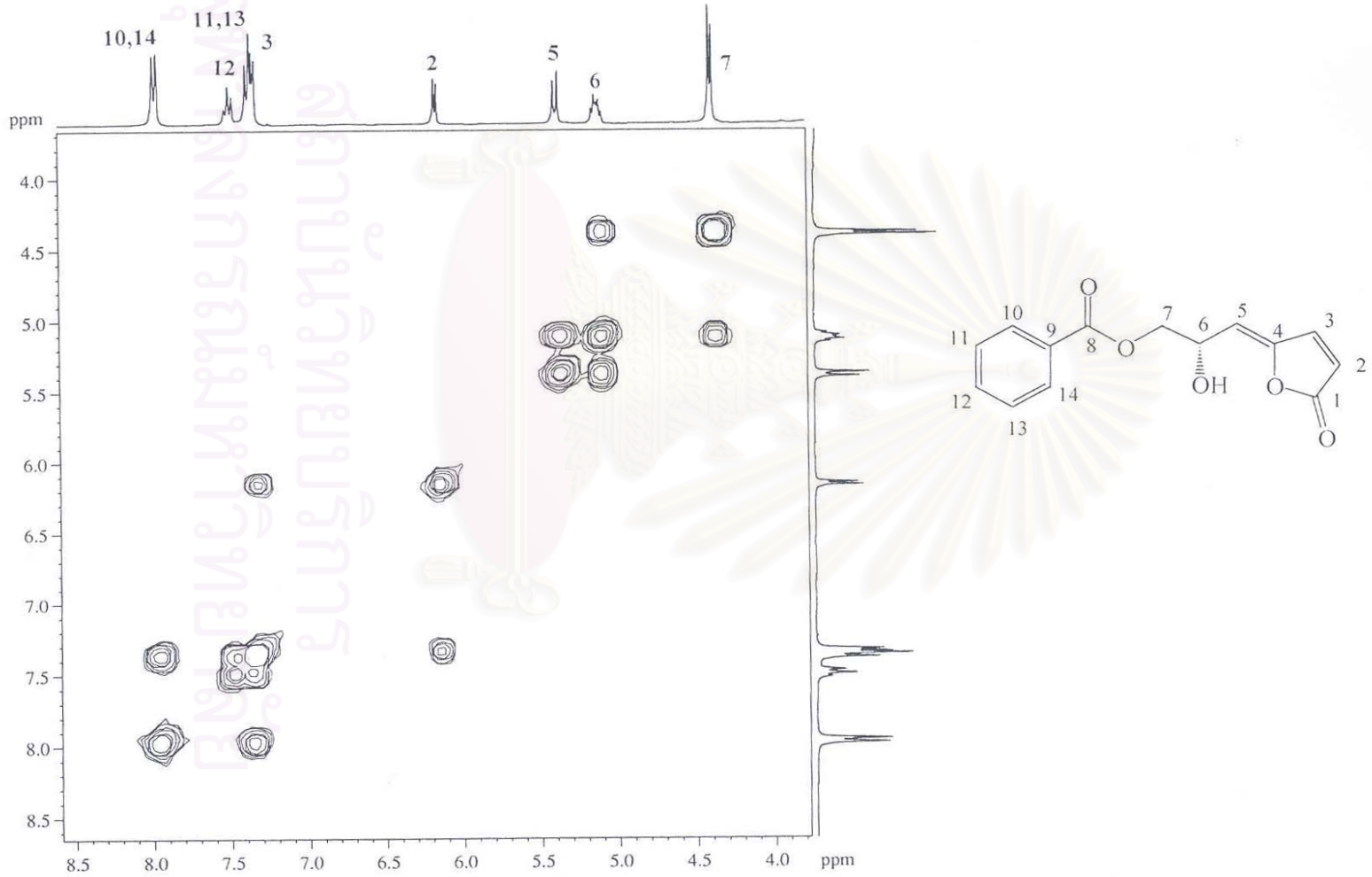


Figure 45. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound L-4 (in $CDCl_3$)

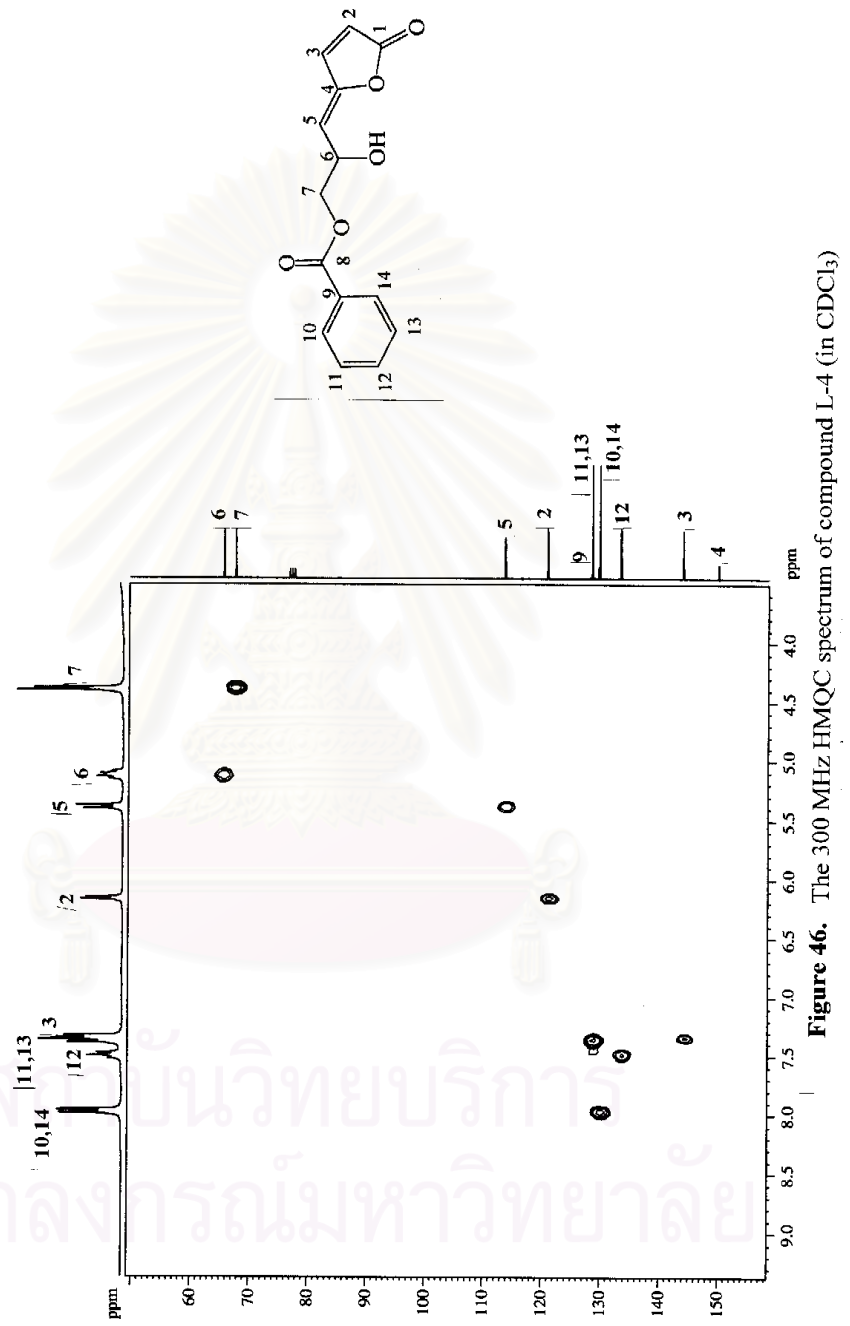


Figure 46. The 300 MHz HMQC spectrum of compound L-4 (in $CDCl_3$)

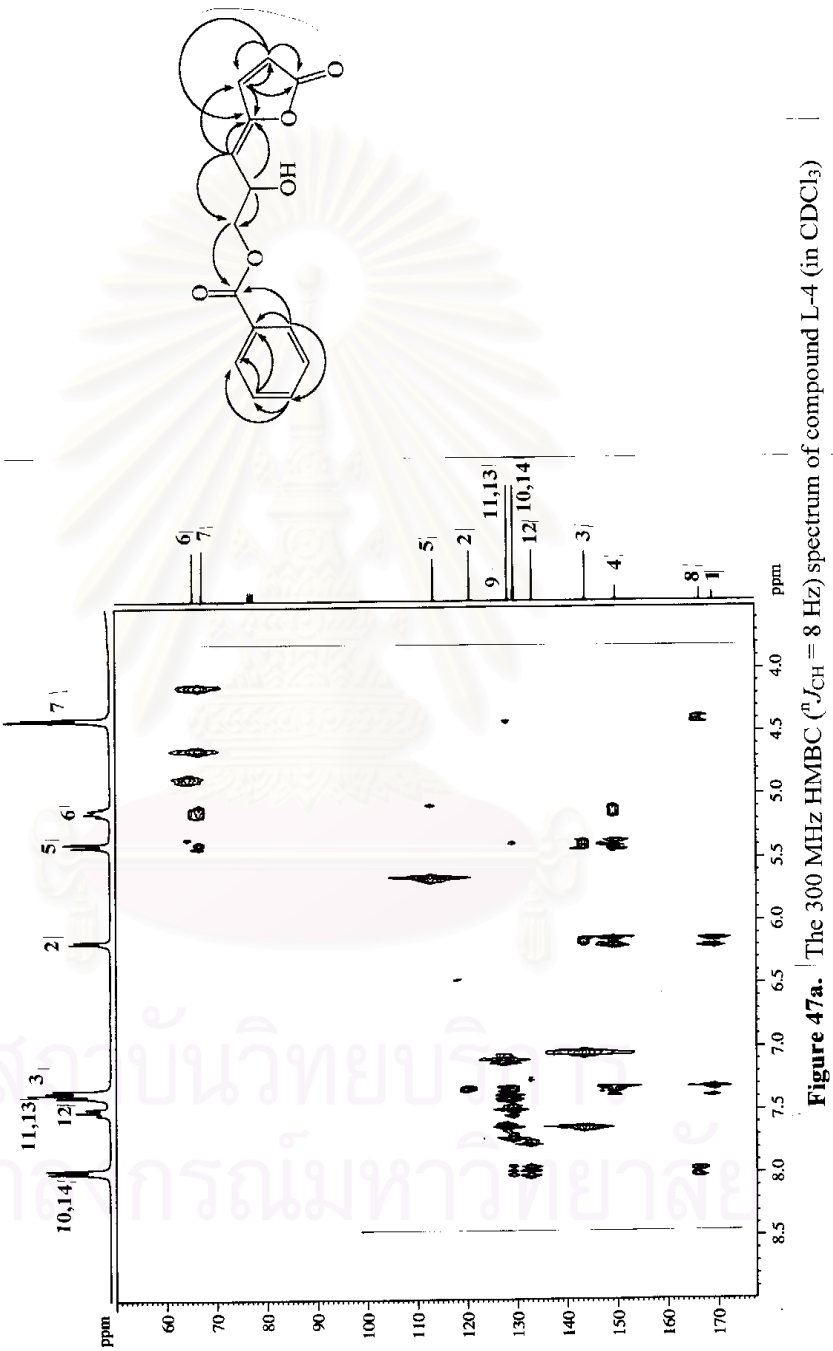


Figure 47a. The 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-4 (in $CDCl_3$)

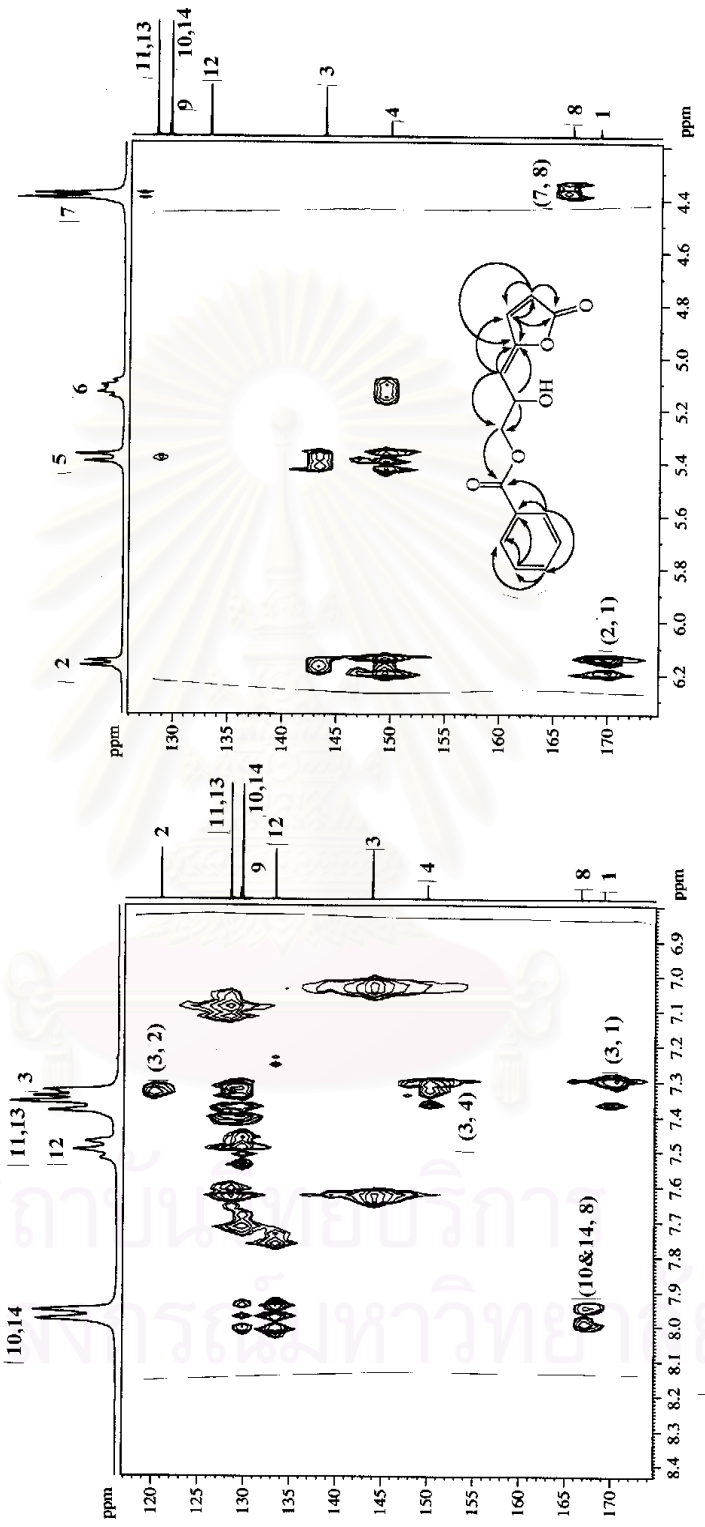


Figure 47b. The expanded 300 MHz HMBC ($^nJ_{CH}$ = 8 Hz) spectrum of compound L-4 (in $CDCl_3$)
(δ_H 6.8-8.4 ppm, δ_C 117.0-175.0 ppm and δ_H 4.2-6.3 ppm, δ_C 125.0-174.0 ppm)

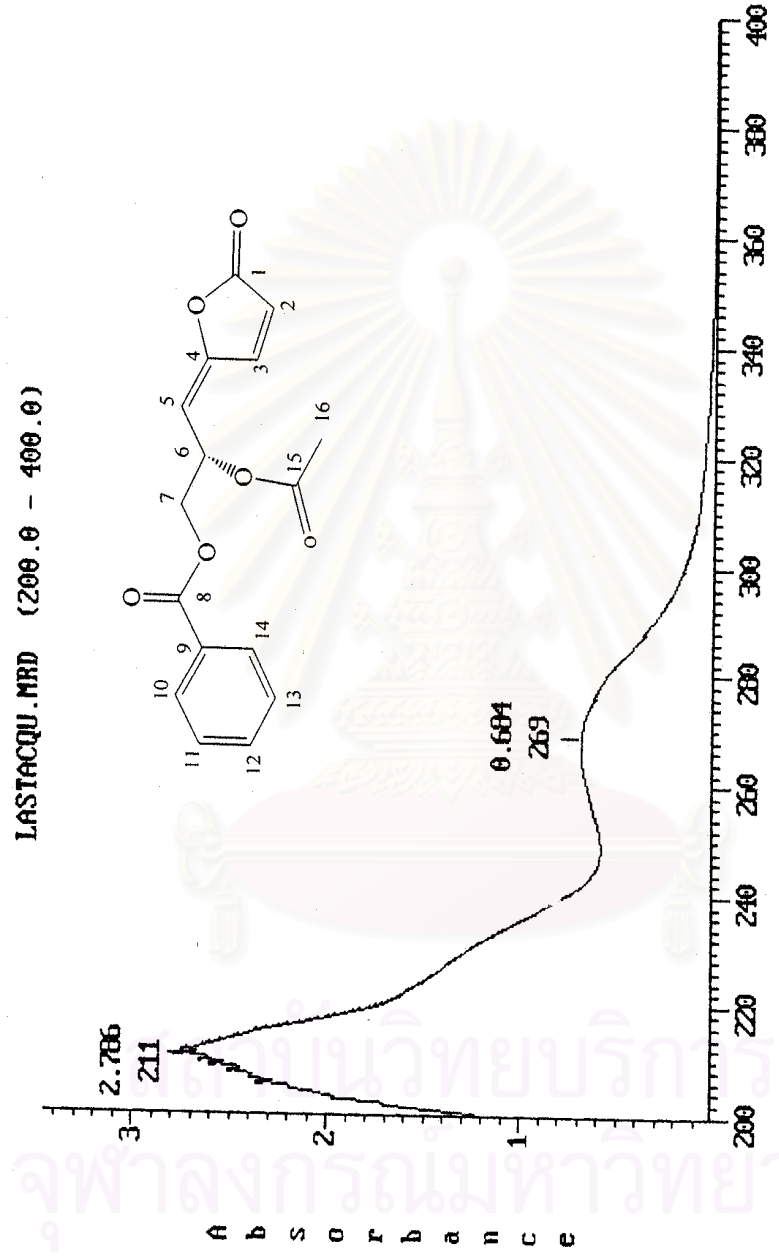


Figure 48. The UV spectrum of compound L-5 (in MeOH)

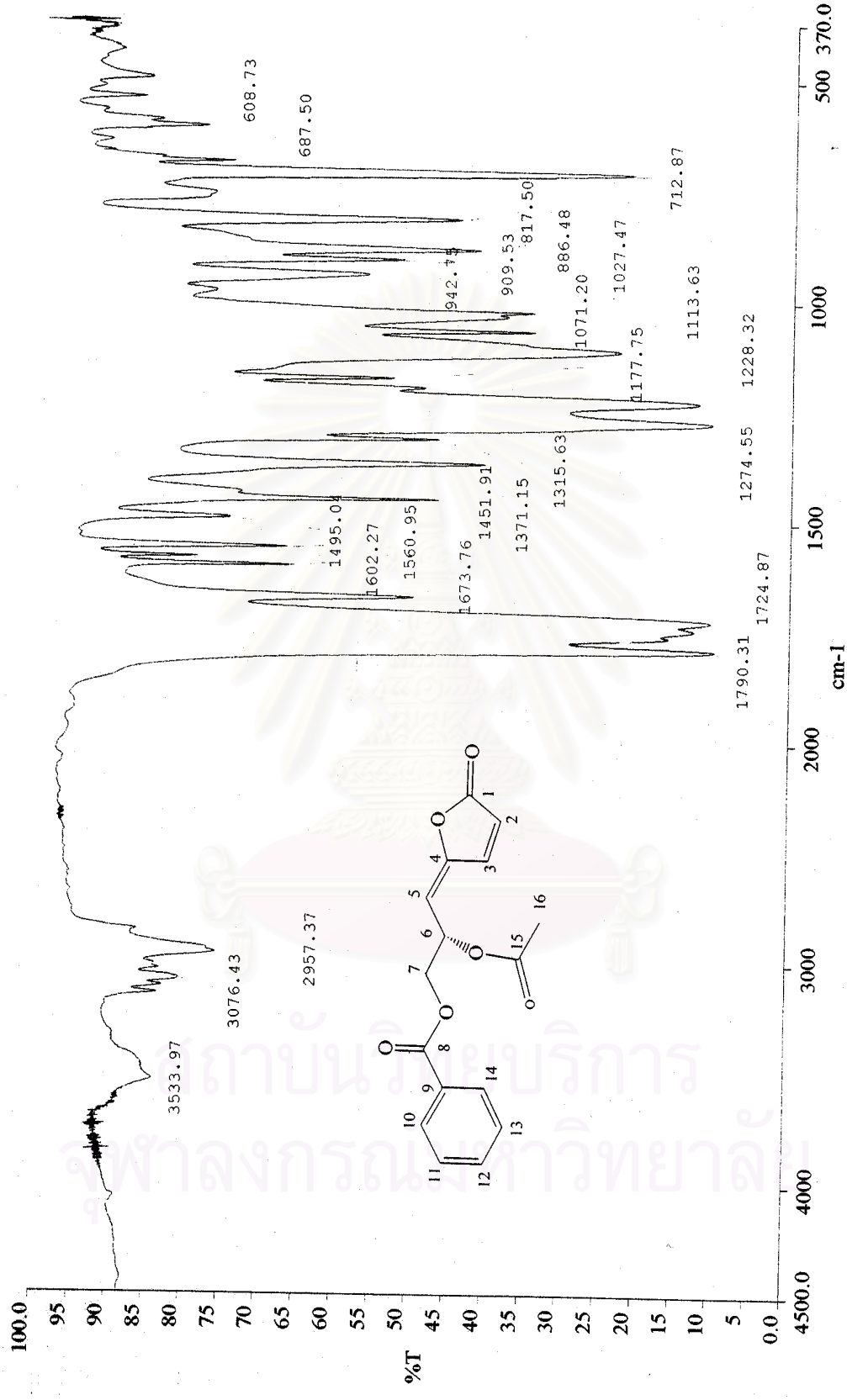


Figure 49. The IR spectrum of compound L-5 (KBr disc)

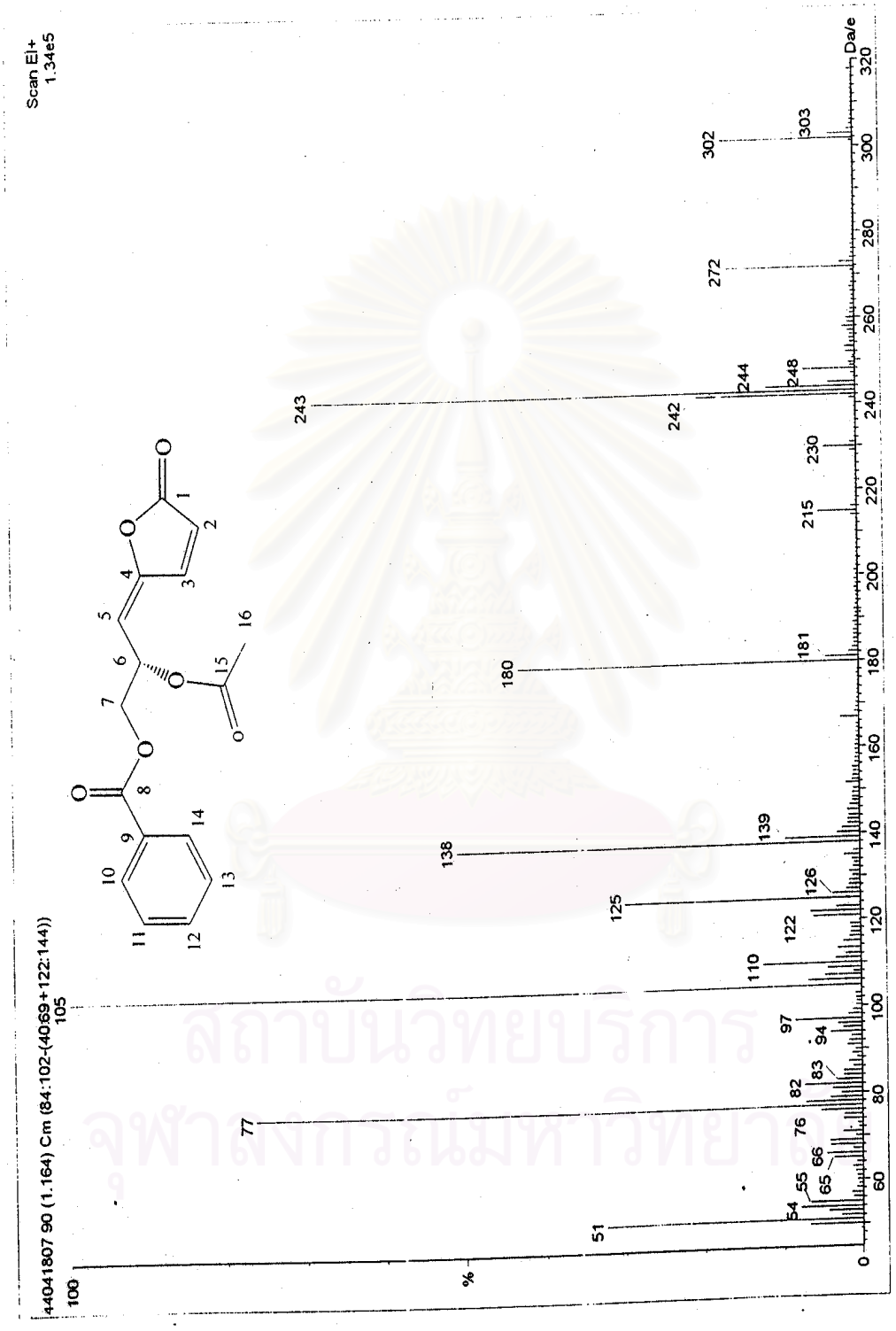


Figure 50. The EIMS spectrum of compound L-5

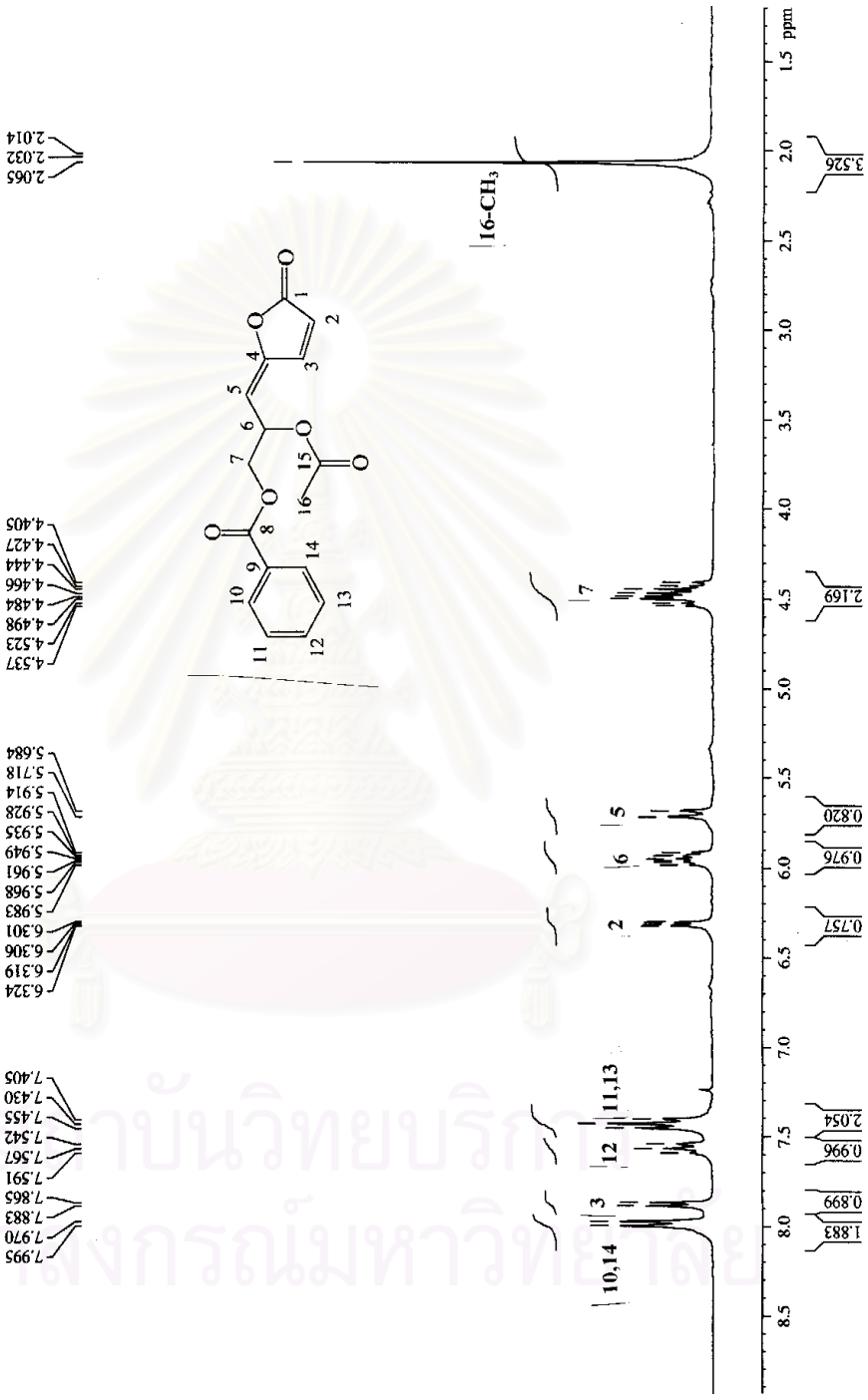


Figure 51a. The 300 MHz ^{1}H NMR spectrum of compound L-5 (in $CDCl_3$)

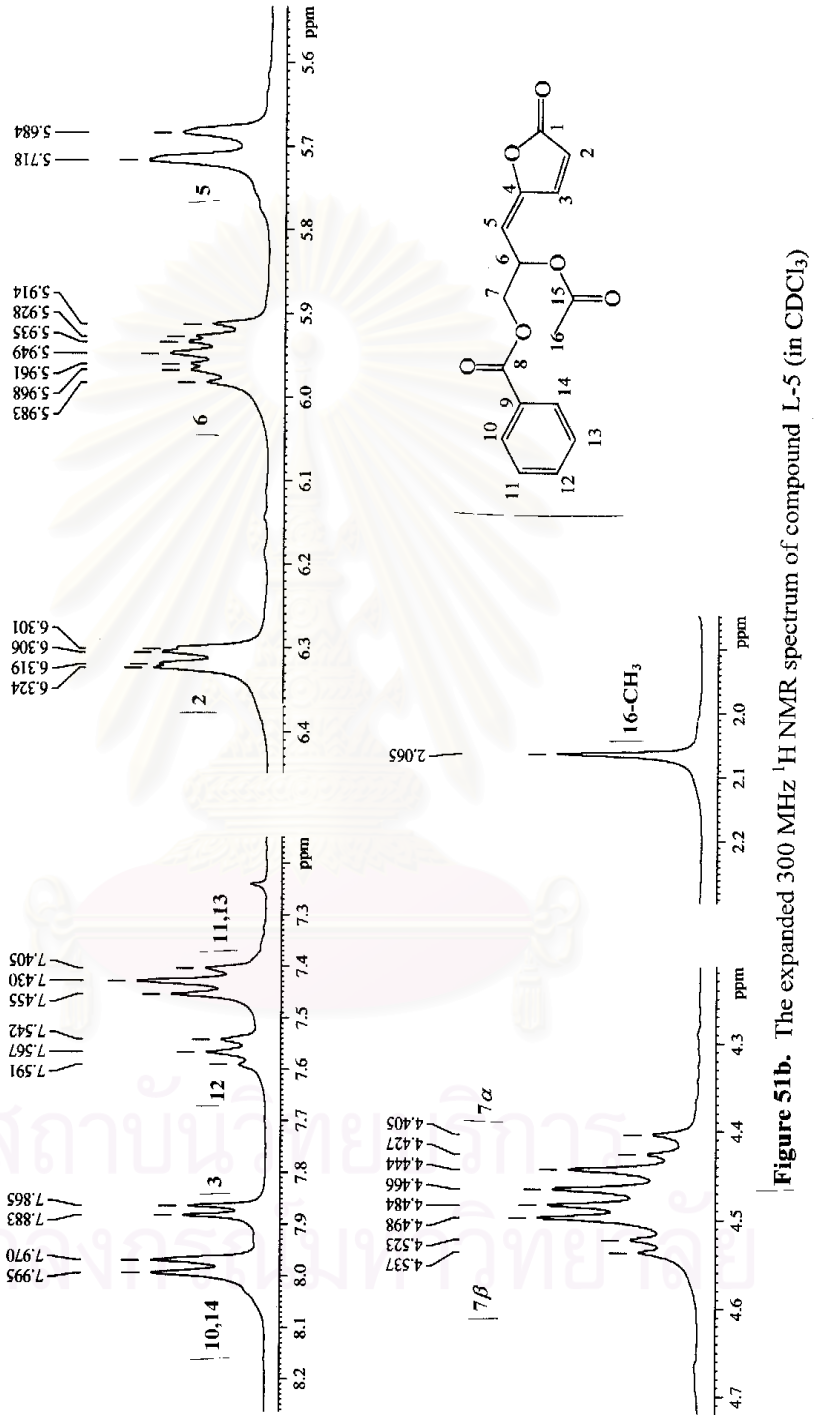


Figure 51b. The expanded 300 MHz ^{1}H NMR spectrum of compound L-5 (in $CDCl_3$)

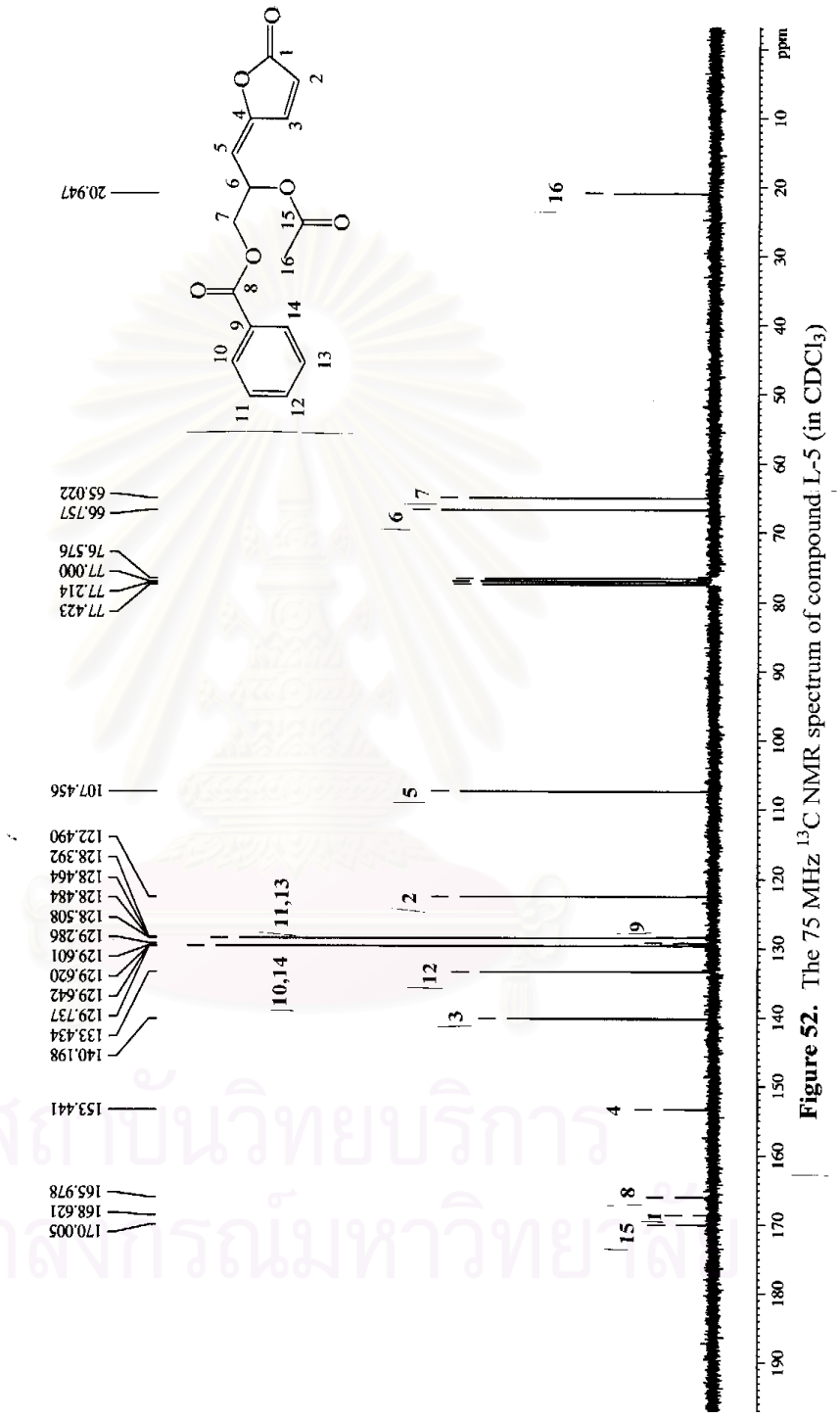


Figure 52. The 75 MHz ^{13}C NMR spectrum of compound L-5 (in $CDCl_3$)

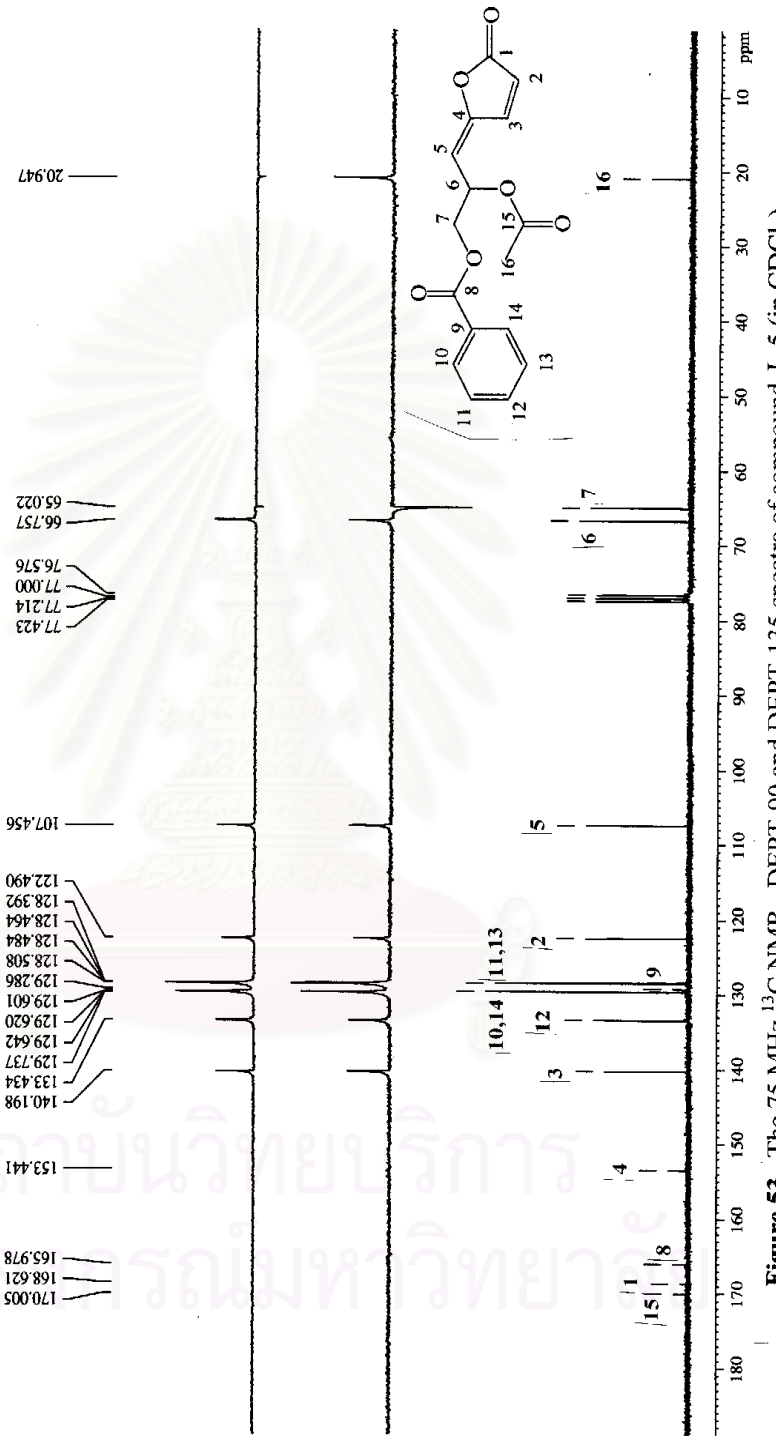


Figure 53. The 75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound L-5 (in $CDCl_3$)

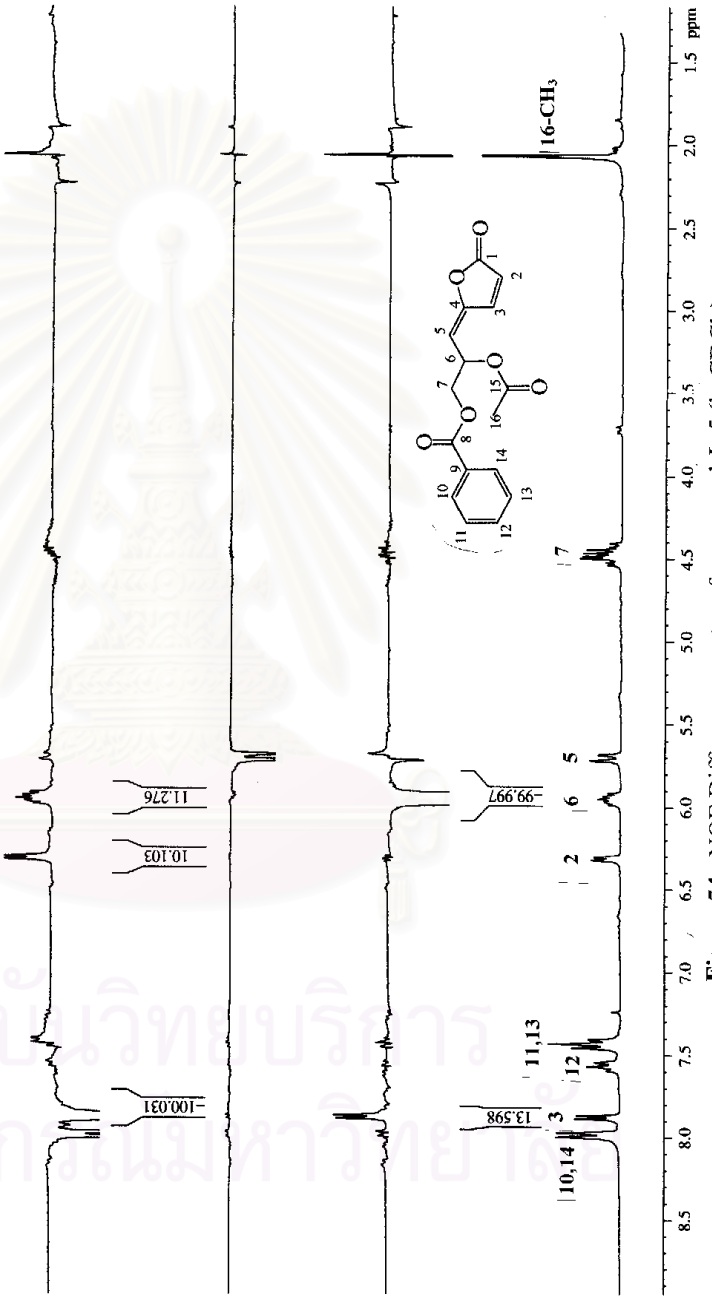


Figure 54. NOE Difference spectra of compound L-5 (in $CDCl_3$)

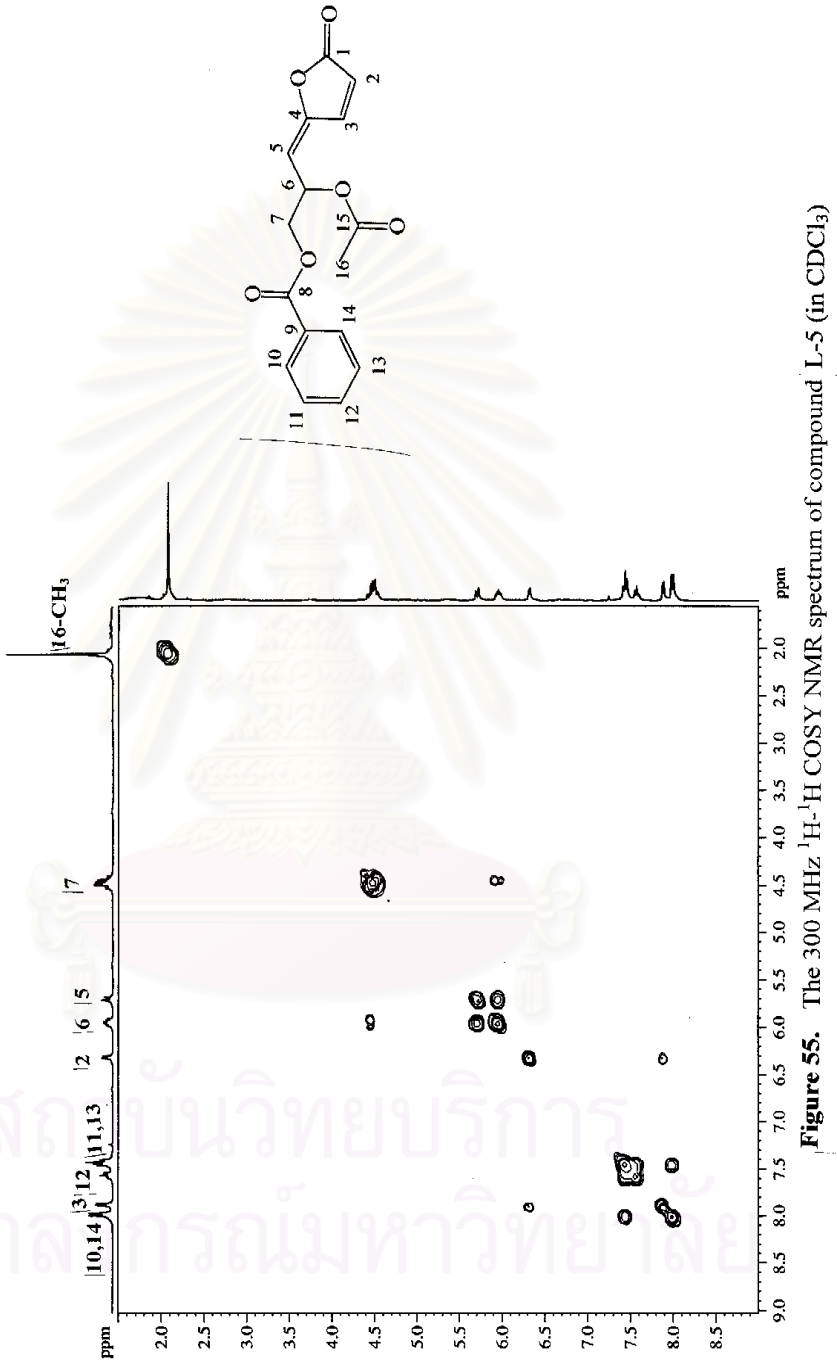


Figure 55. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound L-5 (in $CDCl_3$)

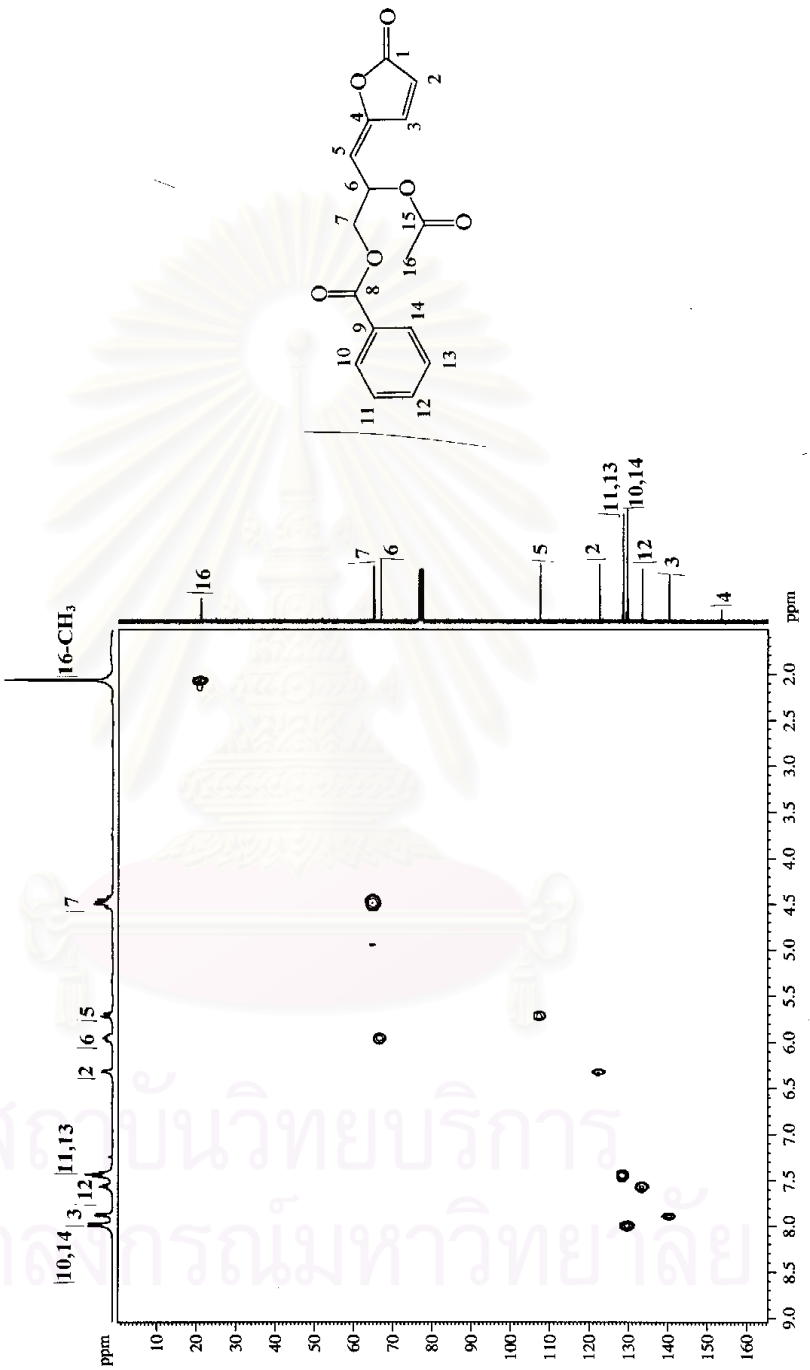


Figure 56. The 300 MHz HMQC spectrum of compound L-5 (in $CDCl_3$)

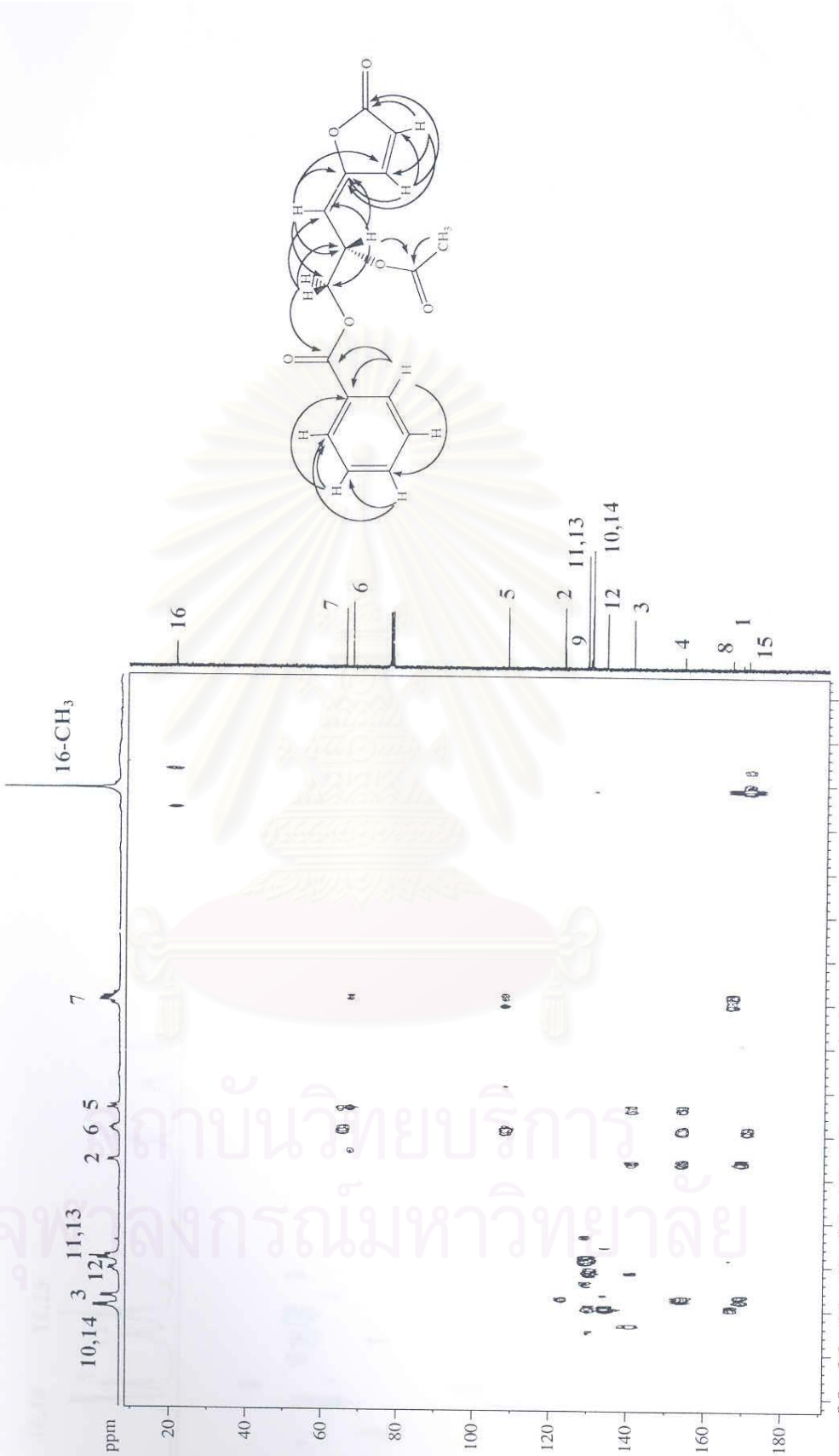


Figure 57a. The 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-5 (in $CDCl_3$)

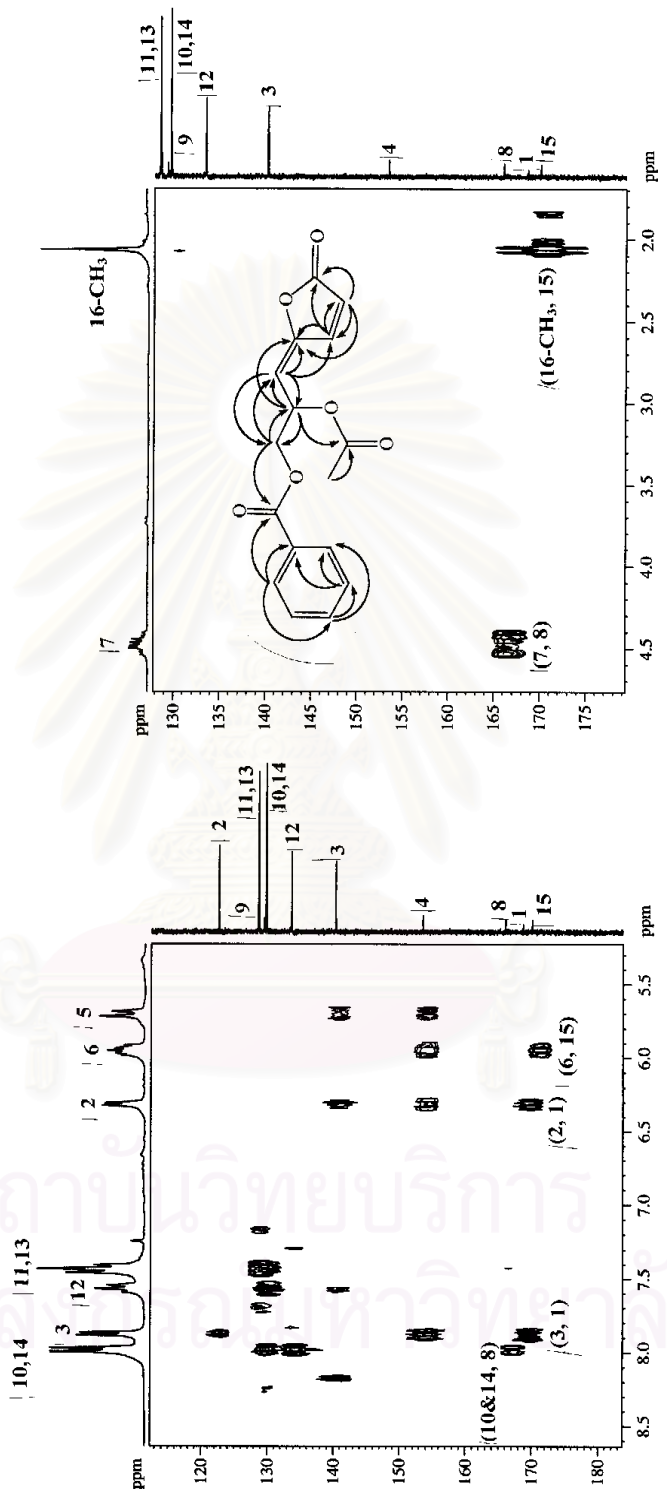


Figure 57b. The expanded 300 MHz HMBC ($^{n}J_{CH}$ = 8 Hz) spectrum of compound L-5 (in $CDCl_3$)
(δ_H 5.2-8.6 ppm δ_C 112.0-184.0 ppm and δ_H 0.7-4.8 ppm, δ_C 128.0-179.0 ppm)

VITA

Mr. Thaweesak Juengwatanatrakul was born on October 2, 1976 in Udornthani, Thailand. He received his Bachelor's degree of Science in Pharmacy in 1998 from the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. He was awarded a University Development Commission (UDC) scholarship with an obligation to serve in the same year and is now a lecturer at the Department of Pharmaceutical Chemistry and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University.