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SESQUITERPENE LACTONES FROM MICHELIA RAJANIANA STEM BARK AND GRANGEA MADERASPATANA

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ศรีรัตน์ กลิ่นวงศ์ : เซสควิเทอร์ปีนแลคโทนจากเปลือกต้นจำปีหลวง และต้นพญามุตดี (SESQUITERPENE LACTONES FROM MICHELIA RAJANIANA STEM BARK AND GRANGEA MADERASPATANA) อ. ที่ปรึกษา : รศ. นิจศิริ เรืองรังษี, ๑๔๙ หน้า.

สามารถพบสารเซสควิเทอร์ปีนแลคโทนกลุ่มอีปอกซีเยอร์มาคราโนไลด์ ๕ ชนิด จากสารสกัดเปลือกต้นจำปีหลวง คือ parthenolide, bisparthenolidine, paramicholide, N-acetylparthenolidine และ N-acetyl-8α-hydroxyparthenolidine สาร ๓ ชนิดหลังเป็นสารใหม่ยังไม่เคยมีรายงานมาก่อน สำหรับสาร ๒ ชนิดแรกเคยมีการทดลองให้ผลต้านเนื้องอก ส่วนสารชนิดที่ ๖ เป็นสารกลุ่มออกโซอปอร์ฟีนอยด์อัลคาลอยด์ ชื่อ Liriodenine

จากการสกัดต้นพญามุตดีพบสารเซสควิเทอร์ปีนแลคโทน กลุ่มยูเดสมาโนไลด์ ๓ ชนิด คือ frullanolide, 7α-hydroxyfrullanolide และ 3α, 7α-dihydroxydihydrofrullanolide สารชนิดแรกเคยมีรายงานว่าเป็นสารที่ทำให้เกิดอาการแพ้ ส่วนสาร ๒ ชนิดหลังเป็นสารใหม่ยังไม่เคยมีรายงานมาก่อน

การกำหนดสูตรโครงสร้าง ใช้เครื่องมือโปรตอน และคาร์บอน ๑๓ นิวเคลียร์ แมกเนติกเรโซแนนซ์ ที่มีประสิทธิภาพสูง และมีการทดลองแบบ ๒ มิติ ทำให้ทำนายสูตรโครงสร้างได้แม่นยำมากขึ้น

ภาควิชา เภสัชเวท ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชเวท

ปีการศึกษา ๒๕๓๑ ลายมือชื่ออาจารย์ที่ปรึกษา .....

SRIRAT KASIWONG : SESQUITERPENE LACTONES FROM MICHELIA RAJANIANA STEM BARK AND GRANGEA MADERASPATANA. THESIS ADVISOR : ASSO. PROF. NIJSIRI RUANGRUNGSI, Ed. D. 149 PP.

Examination of the stem bark of *Michelia rajaniana* Craib. (Magnoliaceae) revealed the presence of five epoxy germacranolides, parthenolide, bisparthenolidine, paramicholide, N-acetylparthenolidine and N-acetyl-8α-hydroxy-parthenolidine. The latter three components were found to be unusual germacranolides which have not been reported previously while the former two were demonstrated to possess antitumor activity. In addition, the sixth component was oxoaporphinoid alkaloid liriodenine.

The present investigation was also undertaken to further study of sesquiterpene lactones from *Grangea maderaspatana* Poir. (Compositae). Three eudesmanolides were isolated and their structures were determined. The first component was allergenic lactone named frullanolide whilst the other two were unusual 7-hydroxy eudesmanolide named 7α-hydroxy-frullanolide and 3α, 7α-dihydroxydihydrofrullanolide

^{1}H-NMR ^{13}Structural elucidations have been established through high field C-NMR spectroscopy and 2D-COSY experiment. A detailed discussion on the elucidation of chemical structures is included.

ภาควิชา เภสัชเวท ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชเวท

ปีการศึกษา ๒๕๓๑ ลายมือชื่ออาจารย์ที่ปรึกษา .....

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LIST OF STRUCTURES

structure

1 isoprene unit

2 α-methylene γ-lactone

3 germacrolides

4 melampolides

5 heleangolides

6 *cis,cis*-germacranolides

7 costunolide

8 costunolide cation

9 α-cyclocostunolide

10 β-cyclocostunolide

11 epitulipinolide

12 tanacin

13 herbolide B

14 eriofertin

15 dihydroparthenolide 4,5-epoxide

16 cation of 15

17 guaianolides

18 parthenolide

19 lanuginolide

20 peroxyferolide

21 peroxycostunolide

22 peroxyparthenolide

23 (-)-frullanolide

24 (+)-frullanolide

25 eriolanin

26 eriolangin

structure

27 ivangulin

28 lumisantonin

29 vernodesmin

30 alantolactone

31 isoalantolactone

32 eudalene

33 saussurea lactone

34 1,2-epoxide of 33

35 3,4-epoxide of 33

36 dihydrosantamarin

37 dihydroreynosin

38 alcohol derivative

39 ambrosanolide

40 helenanolide

41 acetyl CoA

42 acetoacetyl CoA

43 3-hydroxy methyl glutaryl CoA

44 mevalonic acid

45 mevalonic acid pyrophosphate

46 isopentenylpyrophosphate

47 3,3-dimethylallylpyrophosphate

48 geranylpyrophosphate

49 *trans,trans*-farnesylpyrophosphate

50 *trans,trans*-germacradiene

51 germacrene

52 epoxide intermediate

53 hydroperoxide

54 alcohol

structure

55 aldehyde

56 acid derivative

57 inunolide

58 costunolide 1,10-epoxide

59 reynosin

60 santamarin

61 intermediate cation

62 eudesmanolides hydroperoxide

63 aldehyde derivative

64 germacrolide-4,5-epoxide

65 guaianolide cation

66 guaianolide diol

67 xanthanolide

68 guaianolide dienol

69 cyclopropane guaianolide

70 ivaxillarin

71 tamaulipin acetate

72 chair-like transition state

73 divinylcyclohexane

74 guaianolide type cation

75 ambrosanolide derivatives

76 damsin

77,77' melampolide-4,5-epoxide

78 intermediate cation

79 eudesmanolides

80 eremophilanolides

81 furanoeremophilane

structure

82 intermediate of 83 and 84

83 furan derivative

84 eremophilenolide

85 furanolactone

86 furanodilactone

87 fukinone

88 α,β-epoxyketone

89 ring contraction product

90 bakkenolide A

ABBREVIATION

AcOH	= acetic acid
br	= broad
°C	= degree Celsius
C	= carbon
^{13}C-NMR	= carbon-13 nuclear magnetic resonance
cm	= centimetre
CoA	= coenzyme A
d	= doublet
d.b.	= double bond
EIMS	= electron impact mass spectrometry
ent	= enantiomer
EtOH	= ethanol
g	= gram
^{1}H-NMR	= proton nuclear magnetic resonance
hRf	= $\frac{\text{distance of spot centre from start point}}{\text{distance of solvent front from start point}} \times 100$
$h\upsilon$	= photon
Hz	= Hertz
IR	= infrared
kg	= kilogram
L	= litre
lactoniz.	= lactonization
m	= metre
m	= multiplet
M^+	= molecular ion
m/z	= mass to charge ratio

continued

mg	= milligram
MHz	= megahertz
ml	= millilitre
mm	= millimetre
m.p.	= melting point
N	= normality
nm	= nanometre
OAc	= acetyl
OAng	= angelate
OH	= hydroxy
OPP	= pyrophosphate
oxidat.	= oxidation
p.	= page
PD-C	= palladium-carbon
ppm	= part per million
Py	= pyridine
q	= quartet
s	= singlet
Se	= selenium
sp.(spp.)	= species
t	= triplet
TLC	= thin-layer chromatogram
UV-visible	= ultraviolet-visible

CHEMICAL FORMULAE

BF_3	=borontrifluoride
CCl_4	=carbontetrachloride
$CDCl_3$	=deuterated-chloroform
CH_2N_2	=diazine
$CrCl_2$	=chromium dichloride
HCl	=hydrochloric acid
$HClO_4$	=perchloric acid
H_2O	=water
H_2O_2	=hydrogen peroxide
K_2CO_3	=potassium carbonate
MeOH	=methanol
MnO_2	=manganese dioxide
$NaBH_4$	=sodium borohydride
RCO_3O	=peroxy acid
SeO_2	=selenium dioxide
$SOCl_2$	=thionyl chloride

CHAPTER I

INTRODUCTION

Michelia rajaniana Craib

Michelia Linn., is a small genus of trees and shrubs of the family Magnoliaceae. Over forty-five species of *Michelia* are distributed widely in the tropical and subtropical Asia from India to China, Japan and Malaysia: four species are native to Thailand and more two other species are commonly cultivated. About 14 species are found in India (1, 2, 3).

According to the Index Kewensis, the 83 species of this genus are shown below :-

Michelia aenea Dandy

M. alba DC. *(M. longifolia* Blume)*

M. baillonii Finet & Gagnep.

M. balansae Dandy

M. baviensis Finet & Gagnep.

M. bodinieri Finet & Gagnep.

M. caerulea DC.

M. calcuttensis Parmentier

M. cathcartii Hook & Thoms.

M. cavaleriei Finet & Gagnep. *(M. cavaleriei* Leveille, *M. leveilleana* Dandy)

M. champaca Linn. (*M. aurantiaca* Wall., *M. blumei* Steud., *M. evonymoides* Burm., *M. pubinervia* Blume., *M. rheedii* Wight., *M. champaca* Lour. ex Gomez)*

M. chapensis Dandy

M. chingii Cheng

M. coerulea Steud.

M. compressa Sarg.

M. compressa var. *formosana* Kanchira

M. compressa var. *macrantha* Hatusima

M. constricta Dandy

M. cris Ruiz & Lopez

M. cumingii Merrill & Rolfe

M. dandyi Hu

M. doltsopa Buch.-Ham. ex DC.

M. ecicatrisata Miq.

M. excelsa Blume

M. fallax Dandy

M. figo Spreng.

M. floribunda Finet & Gagnep. (*M. kerii* Craib, *M. manipurensis* Craib)*

M. farbesii Baker

M. foveolata Merrill ex Dandy

M. fulgens Dandy

M. fuscata Blume

M. glaba Parmentier

M. gracilis Kostel

M. gravis Dandy ex Gagnep.

M. griffithii Finet & Gagnep.

M. gustavi King

M. hypolampra Dandy

M. kachirachirai Kanchira & Yamamoto

M. kingii Dandy

M. kisopa Buch.-Ham. ex DC.

M. lacei Smith

M. lanuginosa Wall. (*M. velutina* DC.)

M. macclurei Dandy

M. macrophylla Don

M. magnifica Hu

M. manipurensis Watt ex Blandis

M. mannii King

M. martinii Dandy (*M. martinii* Leveille)

M. masticata Dandy

M. maudiae Dunn.

M. mediocris Dandy

M. microtricha Hand.-Mazz.

M. mollis (Dandy) McLaughl.

M. montana Blume

M. nilagirica Zenker (*M. glauca* Wight, *M. ovalifolia* Wight. *M. pulneyensis* Wight, *M. walkeri* Wight)

M. oblonga Wall.(*M. lactea* Buch.-Ham. ex Wall.)

M. parviflora Merrill (*M. parviflora* Rumph. ex DC.)

M. parvifolia Blume (*M. parvifolia* DC.)

M. pealiana Finet & Gagnep. (*M. pealiana* King)

M. phellocarpa Finet & Gagnep.

M. philippinensis Dandy

M. pilifera Bakh. *(M. velutina* Blume)

M. platyphylla Merrill

M. platypetala Hand.-Mazz.

M. punauana Hook & Thoms.

M. rajaniana Craib*

M. scortechinii Dandy

M. sinensis Hemsl. & Wils.

M. skinneriana Dunn.

M. subulifera Dandy

M. sumatrae Dandy

M. szechuanica Dandy

M. tignifera Dandy

M. tila Buch.-Ham.

M. tonkinensis Linn.

M. tsiampaca Linn. *(M. velutina* Blume)

M. tsoi Dandy

M. uniflora Dandy

M. wardii Dandy

M. wilsonii Finet & Gagnep.

M. yulan Kostel

M. yunnanensis Franch ex Finet & Gagnep.

(The asterisked names are endemic species in Thailand)

Utilization of this genus has been reported in many countries. The wood is used excellently for boxes, furniture, building, decorative fittings, carving and carriage. The flowers, exceedingly fragrant are used for perfume (2, 3). The bitter substances in the plants make them medicinal such as the bark of *M. champaca* Linn. is used as stimulant,

diuretic and febrifuge; dried root and root bark are purgative and emmenagogue; the juice of the leaves is used in colic; flowers and fruits are stimulant, antispasmodic, stomachic and diuretic and are considered useful in dyspepsia, fever and in renal diseases; the flower oil is used as an application in cephalagia, ophthalmia, gout and rheumatism; fruits and seeds are considered useful for healing cracks in feet (2, 3, 5). The bark of *M. montana* Bl. is used as a bitter tonic in fevers (3). The bark and leaves of *M. nilagirica* Zenker are used as febrifuge. The bitter bark of *M. baillonii* (Pierre) Finet & Gagnep. is considered useful for stimulant and febrifuge (5). The flower buds of *M. alba* DC. are put into an infusion given to women for sapremia, following a miscarriage (5).

Michelia rajaniana Craib is known in various local names in Thailand as Champee luang จำปีหลวง (Chiang Mai), Cha-Kae ชะแก (Karen-Mae Hong Son) (4).

M. rajaniana Craib is a medium-sized tree, to 25 m tall. Leaves elliptic to broadly ovate-oblong, 17-26 (-30) by 11-12 cm; apex obtuse; base rounded or truncate; lateral veins 15-22 pairs, almost parallel; glabrous above, densely greyish-tomentose beneath; petiole 3-3.5 cm long, tomentose, with stipular scars in lower $\frac{1}{2}$- $\frac{2}{3}$. Flower-buds fusiform, 2.5-3 cm long; spathaceous bract densely sericeous; peduncle 1.2-1.5 cm long. Tepals 12 white to pale yellow, outer ones narrowly obvate, 3 by 1.4 cm; inner ones much narrower. Stamens 5-6 mm long, shortly appendaged. Gynoecium about 1 cm long; carpels 25-28, covered with golden hairs, the stigma black, beaked. Fruiting carpels 3-5 or more in a cluster; individual carpels globose or oblong-ovoid, about 1 cm long.

M. rajaniana Craib is found especially in the northern part of Thailand, in lower montane forest, or found at the edge of hill slope, at medium altitudes (1000-1300 m) (1).

In Thailand, there is no report about the medicinal uses of this plant..

Chemical studies of many other species of *Michelia* have been reported the presence of sesquiterpene lactones, the germacranolide group, for example micheliolide, compressanolide, parthenolide, costunolide, lanuginolide, santamarine and michelenolide from the root bark of *M. compressa* Sarg. (6); parthenolide, lanugilide, 11, 13-dehydrolanuginolide and dihydroparthenolide from the stem bark of *M. lanuginosa* Wall. (7) and parthenolide from the root of *M. champaca* Linn. (8). Some of the above mentioned sesquiterpene lactones such as micheldiolide, michelenolide, parthenolide, costunolide, santamarine, dehydrolanuginolide and epitulipinolide diepoxide are cytotoxic agents (9).

M. rajaniana Craib is found especially in the northern part of Thailand. Previously, there have been no reports on phytochemical studies of this plant. On phytochemical and biological screening, it was found that crude extract of the stem bark exhibits positive results with the mixture of 2 % resorcin in methanol and 2 % sulphuric acid (1:1) on TLC and the KB cytotoxicity assay. Hence it is indicated the presence of cytotoxic sesquiterpene lactones.

Accordingly, this present investigation deals with extraction, isolation and elucidation of sesquiterpene lactones in the stem bark, in order to contribute our knowledge of the constituents containing in this species and to search for compounds which might exert biological activities.

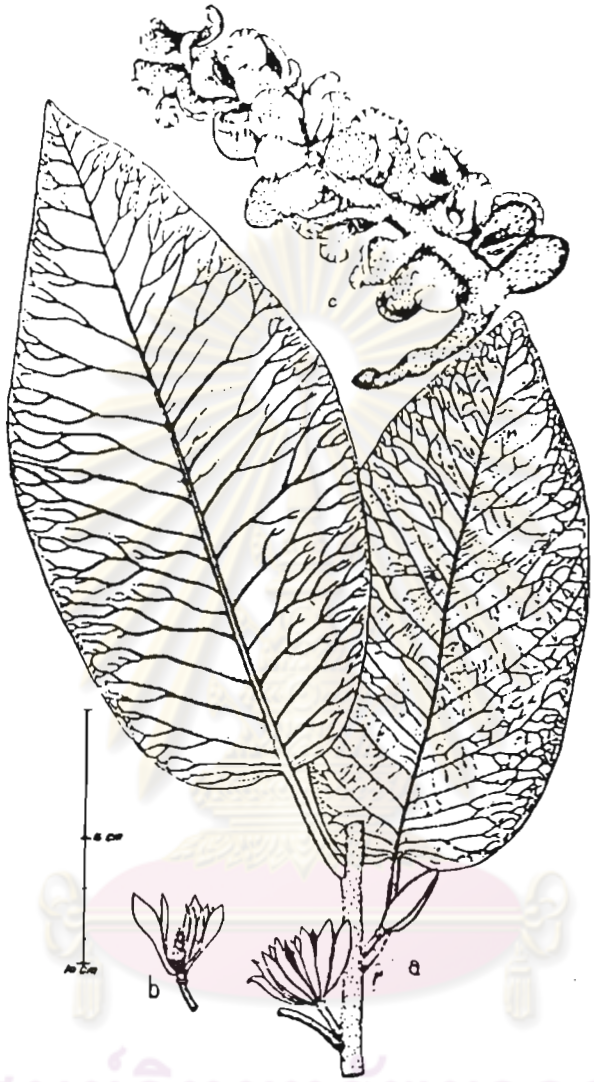


Figure 1 *Michelia rajaniana* Craib :a,twig and flower b,flower c,fruit (1)

Chemical Constituents of *Michelia* spp.

Members of the genus *Michelia* are found to contain a wide range of chemical constituents; sesquiterpene lactones, alkaloids, volatile oil, fixed oil, lignan and steroid.

A list of compounds found in various species of *Michelia* genus is shown in table 1 (page 9)

Table 1 Chemical investigations of *Michelia* spp.

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia alba DC.	–	Michelalbine	Alkaloid	10
		Oxoushinsunine	Alkaloid	10
		Salicifoline	Alkaloid	10,12
		Ushinsunine	Alkaloid	10
		Micheline	Alkaloid	11
		Normicheline	Alkaloid	11
	flowers	-	Essential oil	13
M. cathcartii Hook.f.	leaves,	Lanuginoside	Sesquiterpene lactone	14
& Thoms. Fl.	trunk &	Liriodenine	Alkaloid	14
	root bark	Sitosterol	Steroid	14
M. champaca Linn.	flowers,	-	Fixed oil	15
	oil	-	Volatile oil	15
	bark	Michepressine	Alkaloid	16
		Oxyacanthine	Alkaloid	16

Table 1 (Continued)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
		Liriodenine	Alkaloid	17
		Oxoushinsunine	Alkaloid	17
		Ushinsunine	Alkaloid	17
		Magnoflorine	Alkaloid	18
	root	Liriodenine	Alkaloid	19
		Parthenolide	Sesquiterpene lactone	19
M. compressa Sarg.	bark	Magnoflorine	Alkaloid	20
		Oxyacanthine	Alkaloid	20
		Tetrahydroberberine	Alkaloid	20
		Tetrahydrojatrorrhizine	Alkaloid	20
	wood	Oxoushinsunine	Alkaloid	21
		Ushinsunine	Alkaloid	21
	root bark	Michelenolide	Sesquiterpene lactone	2
		Micheliolide	Sesquiterpene lactone	2

Table 1 (Continued)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
		Parthenolide	Sesquiterpene lactone	2
		Costunolide	Sesquiterpene lactone	2
		Santamarine	Sesquiterpene lactone	2
		Lanuginolide	Sesquiterpene lactone	2
		Dihydroparthenolide	Sesquiterpene lactone	2
		Compressenolide	Sesquiterpene lactone	2
		Liriodenine	Alkaloid	2
M. compressa Maxim.	bark	Ushinsunine	Alkaloid	21
var. *formosana* Kanchira		Liriodenine	Alkaloid	21
		Magnoflorine	Alkaloid	21
M. doltsopa Buch.-	fruit	Dihydroparthenolide	Sesquiterpene lactone	22
Ham. ex DC.		Parthenolide	Sesquiterpene lactone	22
		Lanuginolide	Sesquiterpene lactone	22

Table 1 (Continued)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
M. excelsa Blume	trunk & root bark	Sitosterol	Steroid	14
M. figo Spreng.	leaves	Magnolamine	Alkaloid	23
M. fuscata Blume	leaves	Magnolamine	Alkaloid	24,25
		Picrolanate	Alkaloid	24
		d-Laudanosine	Alkaloid	24
		d-Armepavine	Alkaloid	24
		Magnocurarine	Alkaloid	25
		Magnoflorine	Alkaloid	25
	bark	Dehydrolanuginolide	Sesquiterpene lactone	25
		Lipiferolide	Sesquiterpene lactone	26
		Deacetylanuginolide	Sesquiterpene lactone	26
		Michefuscalide	Sesquiterpene lactone	26
		Syringaresinol	Lignan	26

Table 1 (Continued)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
M. hedyosperma Law.	-	-	Essential oil	27
		-	Volatile oil	27
M. lanuginosa Wall.	trunk bark	Lanuginolide	Sesquiterpene lactone	28
		Dihydroparthenolide	Sesquiterpene lactone	28
		Michelanugine	Alkaloid	14,29
		Liriodenine	Alkaloid	14,29
		Parthenolide	Sesquiterpene lactone	7
		11,13 Dehydroparthenolide	Sesquiterpene lactone	7
		Lanuginosine	Alkaloid	29
	Leaves	Sitosterol	Steroid	14
M. paniculata Linn.	flowers	-	Essential oil	13

Grangea maderaspatana Poir.

Grangea Adanson, a genus in the subtribe Grangeinae, the tribe Astereae, the family Compositae, is a genus of suberect or prostrate annual herbs. The occurence of fourteen species of *Grangea* is found in tropical and subtropical Asia and Africa, all seasons (30, 31, 32, 33).

According to the Index Kewensis, the 14 species of this genus are shown below :-

Grangea anthemoides O. Hoffm.

G. chinensis Lound. (*G. cuneifolia* Poir., *G. minima* Poir., *G. minuta* Poir.)

G. cinera Link.

G. dissecta Boj. (*G. latifolia* Lam., *G. sonchifolia* Lound.)

G. domingensis (Cass.) Gomez.

G. hispida Humbert.

G. hippiodes Merxm.

G. lanata Humbert.

G. lanceolata Poir.

G. madagacariensis Batke.

G. maderaspatana (Linn.) Poir. (*Artemisia maderaspatana* Roxb., *Cotula maderaspatana* Willd., *C. sphaeranthus* Link., *Grangea sphaeranthus* Koch. (33))

G. minima Lipp.

G. mucronata Buch.

G. strigosa Gandoger

Grangea maderaspatana Poir. is known in various local names such as Phayaa mutti พญามุตตี (Suphan Buri); Yaa chaam luang หญ้าจามหลวง (Chiang Mai) (4, 33).

G. maderaspatana Poir. is prostrate or sometimes erect annual herb, stems spreading from the root prostrate, upto 25 cm long, whitish pubescent and glandular. Leaves alternate, lyrate-pinnatifid or almost bipinnatifid, upto 10 cm long, bearing 3-5 lobed or pinnatifid lateral segments 0.5-1.5 cm long, 2-10 mm broad, segments obtused or subacute at the apex, coarsely pubescent on both surfaces. Capitula (0.5) 0.75-1 cm diameter, phyllaries obvate, spathulate, 4.5 mm long, 2 mm broad, coarsely pubescent, margins scarious. Flowers yellow, glandular; female corollas 2 mm long, hermaphrodite corollas 2 mm long; anther-bases obtuses; styles-arms of disk florets flattened, cuneate, obtuse or with triangular points. Achenes greenish 1.25-1.5 mm long, minutely puberulent and glandular; stipes 0.5 mm long, pappus whitish, 0.5 mm long (31, 32, 34, 35).

Utilization of *Grangea maderaspatana* Poir. has been reported in many countries. The leaves are used as a stomachic, a sedative, a carminative, an antiflatulence and an emmenagogue or to facilitate the return of the menses after parturition if the delay is accompanied by abdominal and kidney pain; further the leaves are used as a bechic and in antiseptic fomentations (5, 35, 36).

In Thailand, all parts of *G. maderaspatana* Poir. have been used in folk-loric medicine as bitter tonic, carminative, antiflatulence and antidiarrhoea (37).

Grangea maderaspatana Poir. is only one species which is found on moist ground and paddy field in Thailand. Previously there have been three reports on phytochemical study of this plant, they found the presence of furanoditerpene, acetylene and steroid but no report on sesquiterpene lactones (38, 39, 40). On phytochemical and biological screening, it was found that the crude extract from the whole part of this plant exhibits positive results with the mixture of 2 % resorcin in methanol and 2 % sulphuric acid (1:1) on TLC and the the KB cytotoxicity assay. Hence, it is indicated the presence of cytotoxic sesquiterpene lactone.

Accordingly, this present investigation deals with the extraction, isolation and elucidation of sesquiterpene lactones in the whole parts of this plant, in order to contribute our knowledge of the constituents containing in this species and to search for compounds which might exert biological activities.

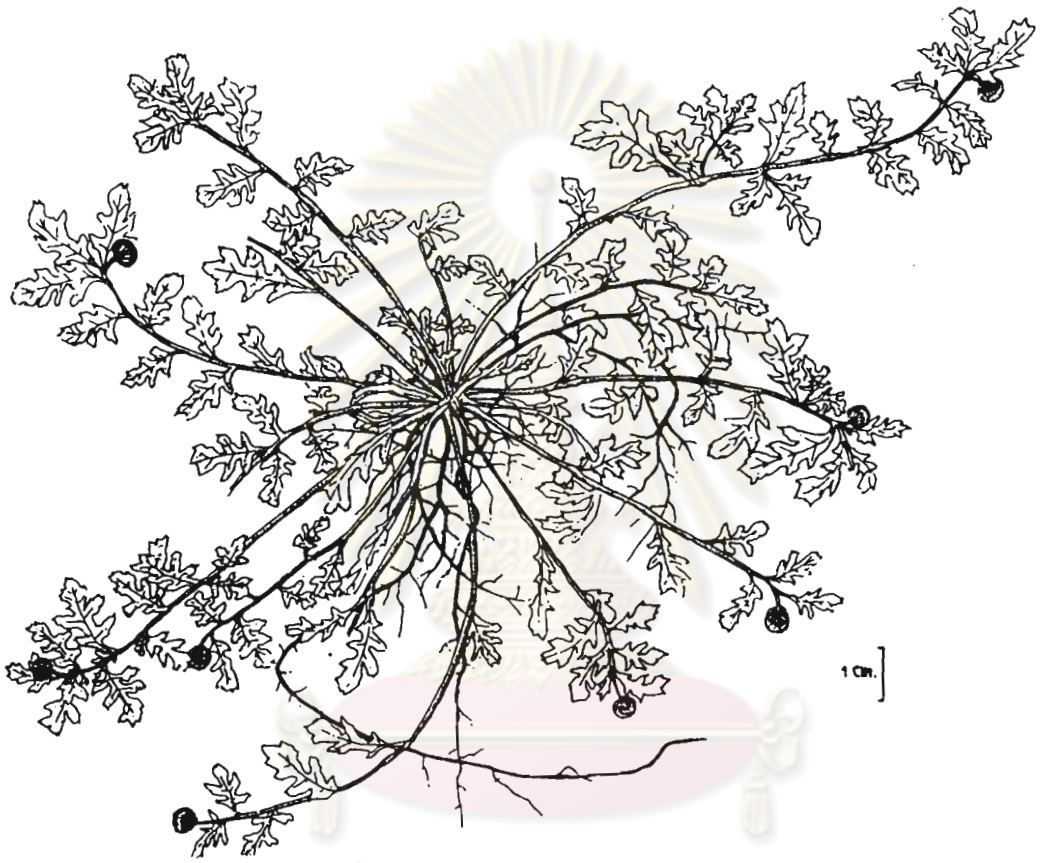


Figure 2 *Grangea maderaspatana* (Linn.)Poir.(35)

Chemical Constituents of *Grangea* sp.

Only one species, *Grangea maderaspatana* Poir., was studied on phytochemical studied and found to contain three groups of chemical constituents, furanoditerpene, acetylene and steroid.

A list of compounds found in *G. maderaspatana* Poir is shown in table 2 (page 18)

Table 2 Chemical constituents found in *Grangea* sp.

Botanical Origin	Plant Part	Compound	Reference
Grangea maderaspatana Poir	all parts	(-)-Hardwiekii acid	38
		ent-2 β-Hydroxy-15, 16-epoxy-3, 13(16), 14-cleroclatrien-18-oic acid	38
		Strictic acid	38, 40
		3-Hydroxy-8-acetoxypentadeca-1, 9, 14-trien-4,6-diyne	38
		Chondrillasterol	39
		Chondrillasterone	39

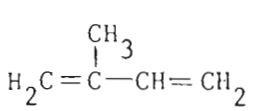
CHAPTER II

HISTORICAL

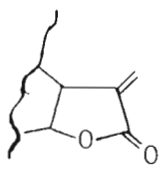
1. Chemistry of Sesquiterpene Lactones

Sesquiterpene lactones, the derivatives of sesquiterpene, belong to a group of terpenoid or isoprenoid compounds (41, 42). Terpenoids are formed by the polymerization of isoprene units 1 (42). The term sesquiterpene lactone refers to a group of natural products containing 15 carbon atoms which are derived from three isoprene units to form various types of carbocyclic skeleton and the γ-lactone ring. Numbering of the basic carbocyclic ring systems is generally found to be consistent in this thesis with the exception of C-14 and C-15 which are frequently interchanged as shown in chart 9 p. 37 (43).

Most sesquiterpene lactones have the lactone ring either *cis*- or *trans*-fused to the C_6-C_7 or C_8-C_7 position of the carbocyclic skeleton. If the lactone ring is the exocyclic α, β-unsaturated lactone or α-methylene γ-lactone or "active functional group" 2, it can react with thiols *via* Michael addition reaction, and shows biological activities (44, 45). The structural modifications of the basic terpene skeleton involve the incorporation of an epoxide ring, hydroxyl groups, O-acyl groups, side chain esters and a conjugated cyclopentenone (44, 45).



1 isoprene unit



2 α-methylene γ-lactone

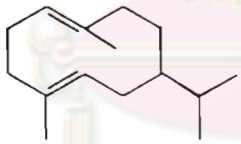
2. Classification of Sesquiterpene lactones

The classification of sesquiterpene lactones is based on their carbocyclic skeletons as germacranolides, eudesmanolides, guaianolides, xanthanolides, elemanolides, pseudoguaianolides, eremophilanolides and bakkenolides. The suffix "olide" refers to the lactone group (43, 44).

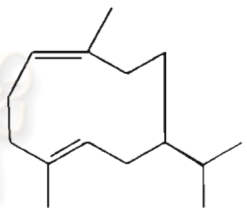
2.1 Germacranolides

2.1.1 Structural Types of Germacranolides

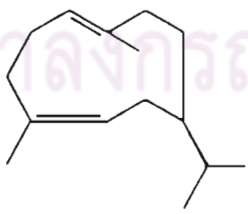
The germacranolides represent the largest group of sesquiterpene lactones with nearly 300 known naturally occuring members (44). The structural skeleton, a 1(10), 4-cyclodecadiene, has four basic configurational isomers as shown in chart 1 3-6 (p. 20).



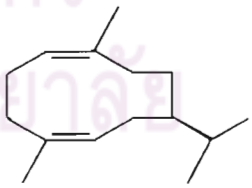
3 germacrolide



4 melampolide



5 heliangolide



6 *cis,cis*-germacranolide

Chart 1. Configurational types of germacranolides

The majority of the germacranolide subgroup represents germacrolides 3, although an increasing number of melampolides 4 and heliangolides 5 have been isolated, and the smallest group is *cis, cis*-germacranolides 6 (43). Most of germacranolides have a *trans*-7, 6 or *trans*-7, 8 lactone ring fusion and the C_6-and C_8-oxygens are alpha configuration (43).

2.1.2 Chemical Transformations of Germacranolides

a. Hydrolysis and Relactonization

The various ester side chains attached to a germacranolide can be hydrolysed by alkaline with a distinct difference in ease (46). For example solution of K_2CO_3 in methanol-water at room temperature hydrolyzes 3-hydroxy-2-methylbut-2-enoate in 20 to 30 minutes whereas the isobutyrate, α-methyl-n-butyrate, tiglate and angelate give no reaction under the same conditions (43).

A general relactonization rule for germacranolides containing C-6 and C-8 α-oxygen functions depends upon strong alkaline treatment followed by acidification; this type of germacranolide always relactonizes to C-8 (43, 47).

b. Reduction and Oxidation

Generally, catalytic hydrogenation of sesquiterpene lactones with Pd-C as a catalyst as well as chemical reduction with $NaBH_4$ in methanol proceed with ease under saturation of the lactonic exocyclic methylene group to form the 11, 13-dihydro derivatives (43).

Reductive transformations of epoxides to alkenes have been used either zinc-copper couple (49) or $CrCl_2$ (43) as reducing agents.

Oxidation reactions have been frequently applied in structural elucidations of various types of germacranolides. MnO_2 oxidations generally transform primary allylic alcohols into α ,β-unsaturated aldehydes, a reaction that can be of considerable use for making configurational assignments to double bonds in the cyclodecadiene skeleton as well as for siting OH groups at C_{14} and/or C_{15} (43).

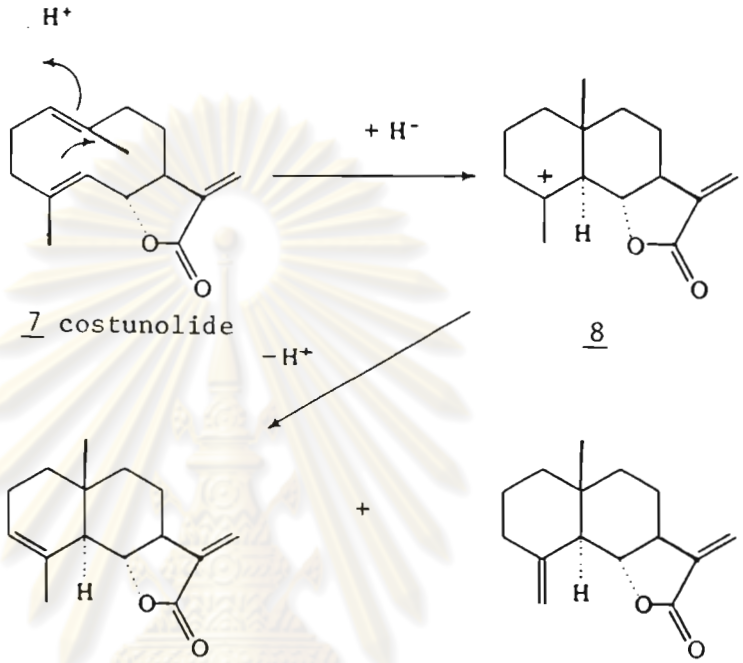
c. Cyclization Reaction of Germacranolides

Many lewis acid-catalyzed cyclization reactions of the cyclodecadiene or of 1, 10-and 4,5-epoxide derivatives have been studied. In general, cyclization of germacra-1,5-dienes provides eudesmanolides. For example the costunolide 7, when treated with a cation exchange resin (51) or $HClO_4$/AcOH (52), undergoes an acid-initiated cyclization through cation 8 to give a mixture of the eudesmanolides 9 and 10 (chart 2 p.23)

Further examples of the conversion of 1, 10-epoxygermacranolides into eudesmanolides include the derivatives of costunolide 7 (52), epitulipinolide 11 (53), tanacin 12 (54), herbolide B 13 (55) and eriofertin 14 (56).

In contrast, BF_3-catalysed reaction of the 4,5-epoxide dihydroparthenolide 15 gives cation 16 which after the loss of a proton from C-1 now forms the guaianolides 17 (57) (chart 3 p.24).

Further examples of cyclization reaction of 4,5-epoxides which result in formation of guaianolides include parthenolide 18, lanuginolide 19 and derivatives (58).



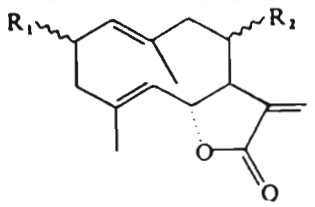
7 costunolide

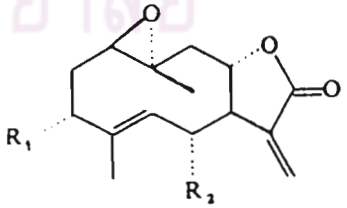
8

9 α-cyclocostunolide

10 β-cyclocostunolide

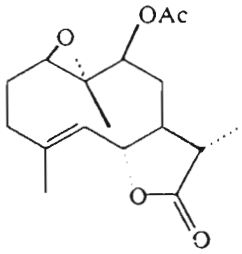
Chart 2. Cyclization of costunolide



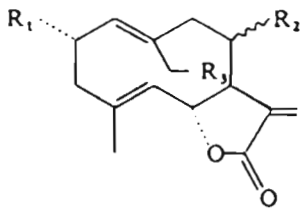


11 Tulipinolide, epi; R_1=H,R_2=β-OAc

12 Tanacin; R_1=H,R_2=OAng



13 Herbolide B



14 Eriofertin; R_1=OH, R_2=χ-OAng, R_3=OH

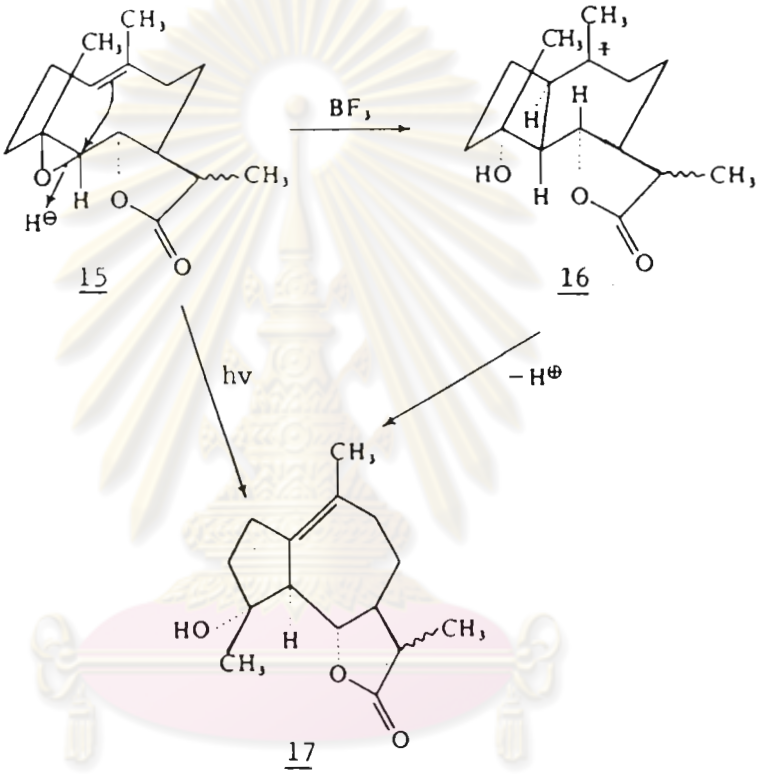
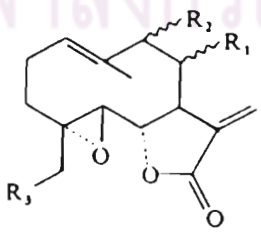
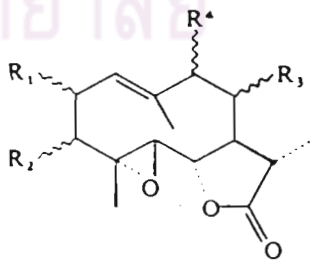


Chart 3. Cyclization of dihydroparthenolides



18 Parthenolide; R_1=R_2=R_3=H



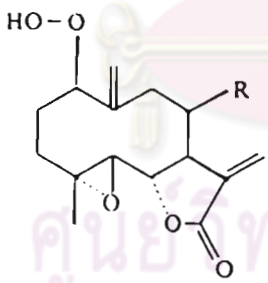
19 Lanuginolide; R_1=R_2=R_4=H, R_3= α-OAc

d. Cope Rearrangements of Germacranolides

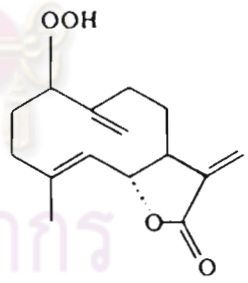
In general, thermal rearrangements of *trans,trans*-cyclodeca 1,5-dienic sesquiterpenes proceed in a highly stereospecific manner through a chair-like transition state resulting in a divinylcyclohexane skeleton, elemadiene skeleton.

e. Photochemical Reactions of Germacranolides

Doskotch and coworkers (59) isolated the first hydroperoxide-containing sesquiterpene lactones peroxyferolide 20, peroxycostunolide 21 and peroxyparthenolide 22, compounds of unexpected high thermal stability. Fischer and coworkers (43) were able to prepare the hydroxyperoxides 20 and 21 by a common photooxygenation procedure involving singlet oxygen generated by methylene blue sensitized oxygenation.



20 1-peroxycostunolide



21 peroxyferolide

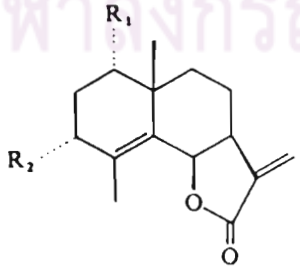
2.2 Eudesmanolides and Biogenetic Derivatives

2.2.1 Structure of Eudesmanolides and Biogenetic Derivatives

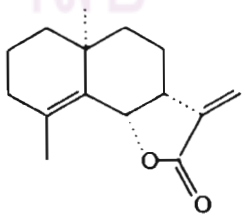
The eudesmanolides (selinanolides) are based on the eudesmane (selinane) skeleton, most of members containing *trans*-7,6-α ,β-unsaturated γ-lactone. Compounds with 7,8-γ-lactone group may occur as *cis*-and *trans*-γ-lactones. Many members contain 3,4-, 4,5-and 4,15 double bonds as well as epoxide derivatives and hydroxyl and/or ketonic oxygen functions predominantly at C_1, C_3 and C_8 (43).

It is interesting that various taxa of the Hepaticae produce the eudesmanolides. The liverworts *Frullania tamarisci* (60) and *F. nisqualensis* contain eudesmanolides such as (-)-frullanolide 23, which are of the skeletal type commonly found in higher plants. However, other liverworts (61) produce the *ent*-eudesmanolides (+)-frullanolide 24 and related compounds.

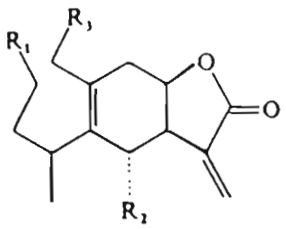
The biogenetic derivatives of eudesmanolides are three 1,10-secoeudesmanolides eriolanin 25, eriolangin 26 and ivangulin 27; lumisantonin 28 and the phenyl containing vernodesmine 29 represent structural exceptions.



23 (-) Frullania lactone; $R_1=R_2=H$



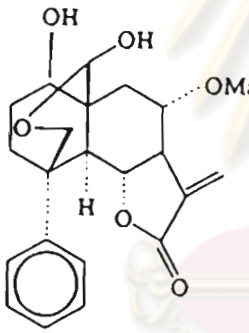
24 (+) Frullanolide



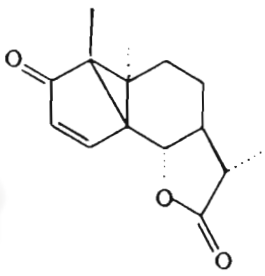
25 Eriolanin; $R_1=CH_2OH, R_2=OMac, R_3=OH$

26 Eriolangin; $R_1=CH_2OH, R_2=OAng, R_3=OH$

27 Ivangulin; $R_1=CO_2CH_3, R_2=R_3=H$



29 Vernodesmin



28 Lumisantonin

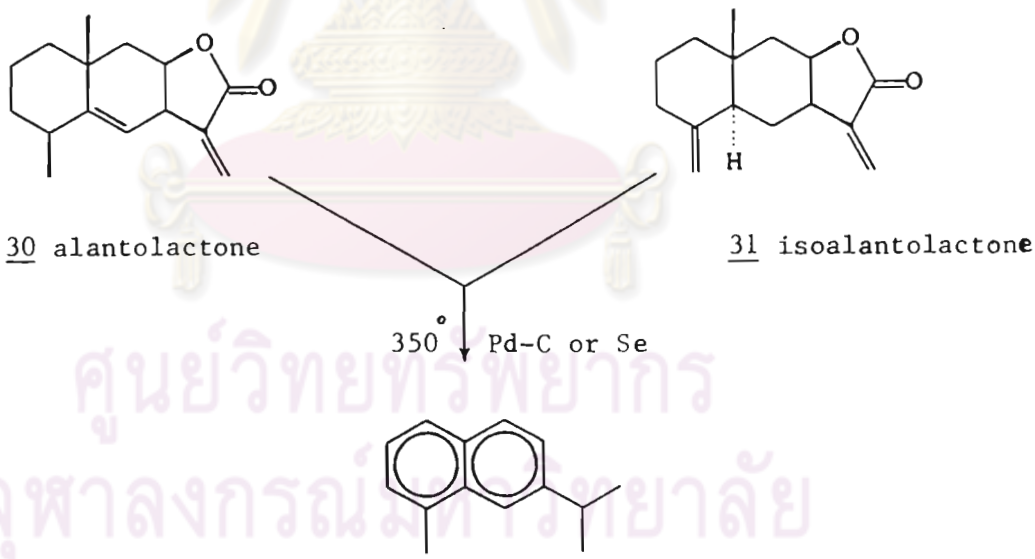
2.2.2 Chemical Transformation of Eudesmanolides

a. Dehydrogenation and Hydrogenation

Pyrolysis of alantolactone 30 and isoalantolactone 31 at 350° C in the presence of Pd-C or Se results in the naphthalene derivative eudalene 32 with loss of the C_{10} methyl group (62) (chart 4 p. 28)

Grieco (63) used the dehydrogenation process involving the conversion of α-methyl γ-lactones into α-methylene-γ-lactones.

Catalytic hydrogenation of eudesmanolides results hydroderivatives (43).



32 eudalene

Chart 4. Dehydrogenation of alantolactone and isoalantolactone

b. Selected Chemical and Photochemical Modifications and Transformations of Eudesmanolides

Eudesmanolides have been frequently used as starting material for chemical and photochemical rearrangement processes which lead to other skeletal types of sesquiterpenes guaianolides, pseudoguaianolides, germacranolides and their derivatives (43).

2.3 Guaianolides and Seco-guaianolides (Xanthanolides)

2.3.1 Structural types

The guaianolides together with their seco-derivatives, the xanthanolides, represent one of the largest groups of sesquiterpene lactones with over 200 known naturally occuring compounds (43). The structural skeleton of guaianolides contains the 5,7-ring system and xanthanolides opened ring at C_4-C_5. Most of members contain *trans* 7,6-α,β-unsaturated γ-lactones or their 11,13-dihydroderivatives, Compounds with 7,8 lactone groups may occur as *cis*-and *trans*-γ-lactones.

2.3.2 Selected Chemical Transformations of Guaianolides and Xanthanolides

The simple chemical modifications have been frequently used in structure elucidation and making stereochemical assignment (43). Examples are epoxidation, catalytic hydrogenation, relactonization, oxidation, reduction and hydrolysis (43).

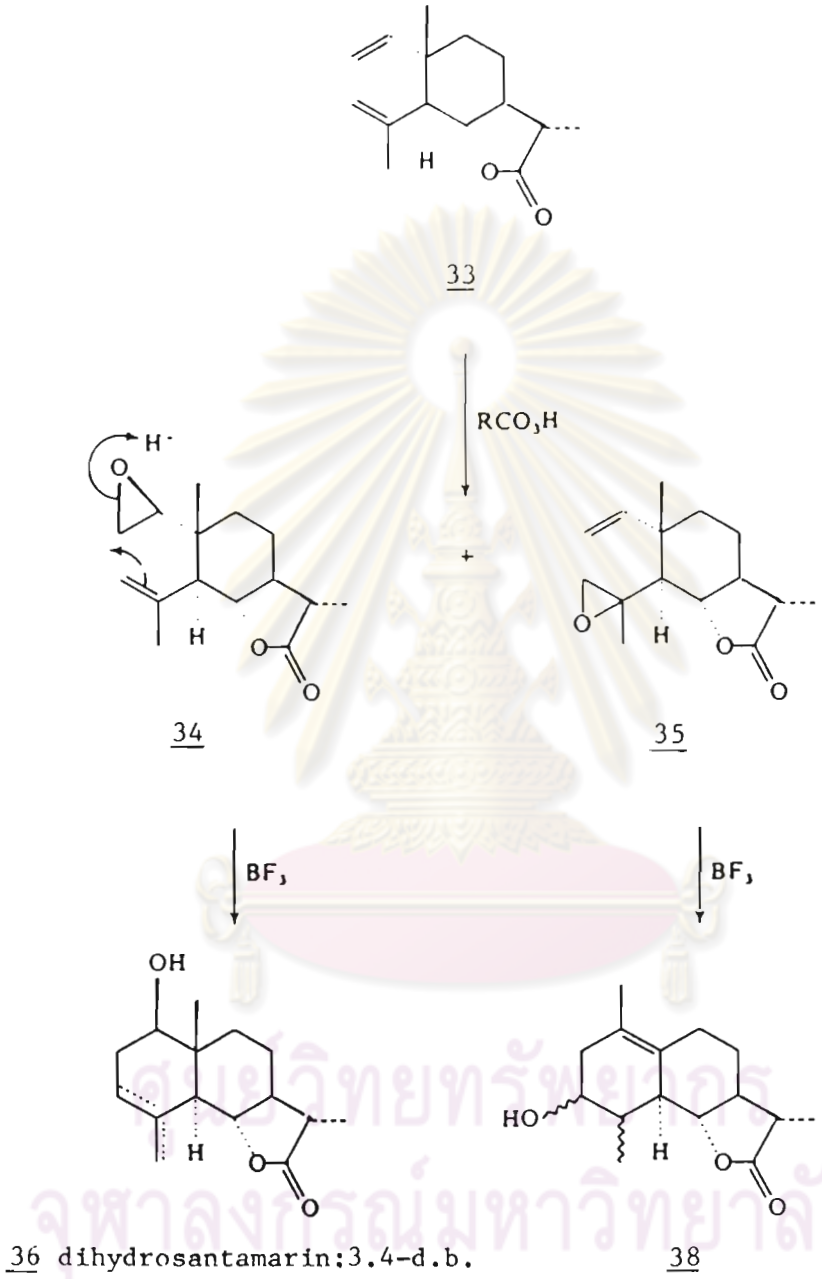
2.4 Elemanolides

2.4.1 Structure of Elemanolides

Elemanolides most likely involve Cope rearrangements of germacranolides which occur under laboratory reactions. It has been suggested that elemanolides isolated from plants are the artifact which formed from the germacranolides during the isolation procedure (43). The structural skeleton is divinylcyclohexane.

2.4.2 Selected Chemical Transformations of Elemanolides

Banker and Kulkarni (64) described monoepoxidations of saussurea lactone 33 and the Lewis acid catalyzed cyclization of the resulting monoepoxides 34, 35. Treatment of the 1,2-epoxide 34 with BF_3 results in the formation of the eudesmanolide dihydrosantamarin 36 and its 4, 15-double bond isomer, dihydroreynosin 37. The cyclization of the 3,4-epoxide 35 is more involved. Most likely, the epoxide ring is isomerized first with formation of an aldehyde at C-3 which is subsequently attacked by C-2 of the 1,2-double bond. Methyl shift from C-10 to C-1 and 1,10-double bond formation finally provide alcohol 38 (chart 5 p. 31)



33

34 35

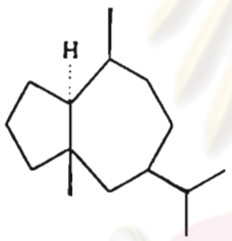
36 dihydrosantamarin:3.4-d.b. 38

37 dihydrosantamarin:4.15-d.b.

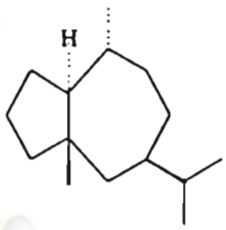
Chart 5. Lewis acid catalysed cyclization of elemanolide monoepoxides

2.5 Pseudoguaianolides and Biogenetic Derivatives

The pseudoguaianolides are based on the 5,7-ring skeleton which typically contain a methyl group at the C-5 ring junction. Formulae 39 and 40 illustrate the two major types of pseudoguaianolides both of which posses a *trans*-fusion of the 5,7-ring system. In the ambrosanolides 39, which usually occur in the subtribe Ambrosiinae and the genus *Parthenium* of the Compositae, the lactone ring is closed predominantly toward 6-β-oxygen. On the other hand, in the helenanolides, the type usually formed in the tribe Heleniae, the lactone ring is closed toward C-8 with the C-8-oxygen bond oriented either α or β(43).



39 ambrosanolide



40 helenanolide

2.6 Eremophilanolides and Bakkenolides

Eremophilanolides are eudesmanolides with methyl migration from C-10 to C-5 (43). Bakkenolides are being considered as derivatives of the eremophilanolides which result from ring contraction of ring B of the eremophilanolane skeleton followed by biomodification (43, 65).

3. Biosynthesis (Biogenesis) of Sesquiterpene Lactones

3.1 Biosynthesis of Germacranolides

The formation of germacranolides derived from *trans*, *trans*-farnesylpyrophosphate occurs through three principles stages :

a. *trans, trans*-Farnesylpyrophosphate Biosynthesis

The formation of *trans, trans*-farnesylpyrophosphate is through the pathway of acetyl CoA 41, acetoacetyl CoA 42, 3-hydroxy methyl glutaryl CoA 43, mevalonic acid 44, mevalonic acid pyrophosphate 45, isopentenylpyrophosphate 46, 3,3-dimethylallylpyrophosphate 47, geranylpyrophosphate 48 and *trans, trans*-farnesylpyrophosphate 49 (chart 6 p. 34)

b. Germacrene Cyclization

The elimination of the pyrophosphate group of the *trans, trans*-farnesylpyrophosphate 49 directly forms *trans, trans*-germacradiene cation 50. The intermediate 50 may be stabilized by elimination of a proton to form germacrene 51 (43, 66) (chart 7, p 35)

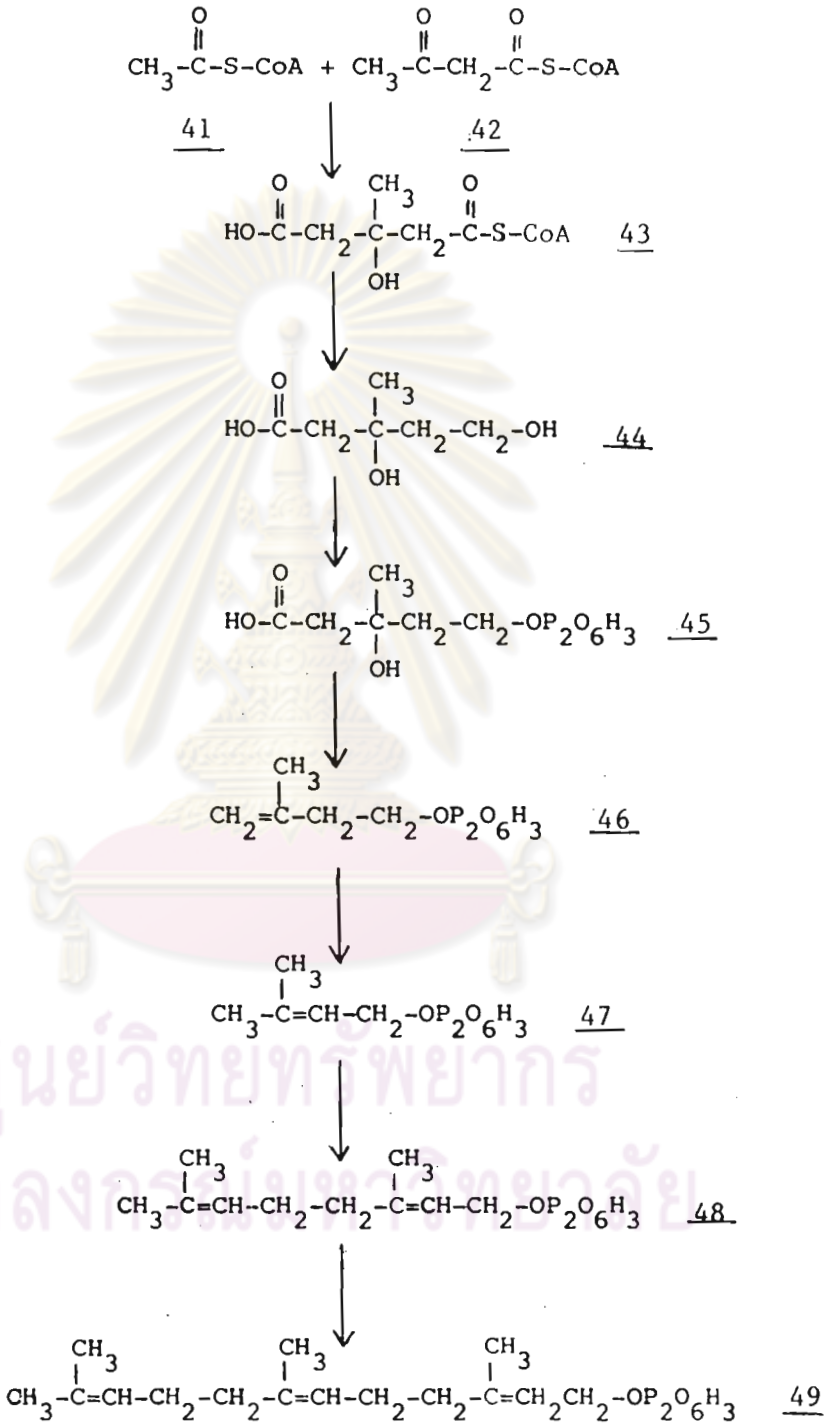


Chart 6 *trans,trans*-farnesylpyrophosphate biosynthesis

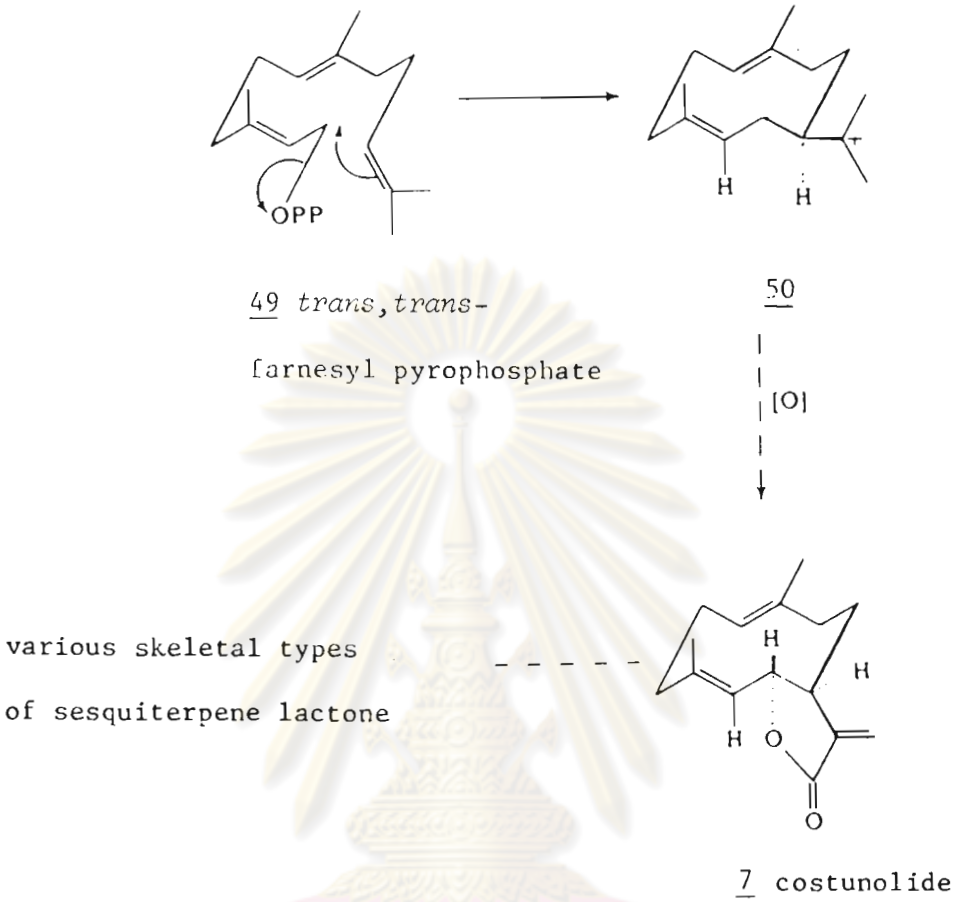


Chart 7. Biogenesis of the germacranolide skeleton

c. Lactone Ring Formation

The possible biogenetic route has been suggested for the formation of the lactone ring of germacranolides (67,68,69). Introduction of an oxygen function at C-12 in 51 to give alcohol 54 could either proceed through epoxide intermediate 52 or could involve the hydroperoxide 53, the latter being formed by an enzymatically-mediated reaction mimicking the reaction of singlet oxygen with olefins. In either case the process involves migration of a double bond from what was originally C-11, C-13 to C-11, C-12. Further oxidative modifications of 54 through aldehyde 55 and 56 and

hydroxylations at C-6 or C-8 would after lactonization give costunolide 7 inunolide 57 respectively (chart 8 p. 36)

Other skeletal types of sesquiterpene lactones different from the germacradienes are shown in chart 9 (p. 37)

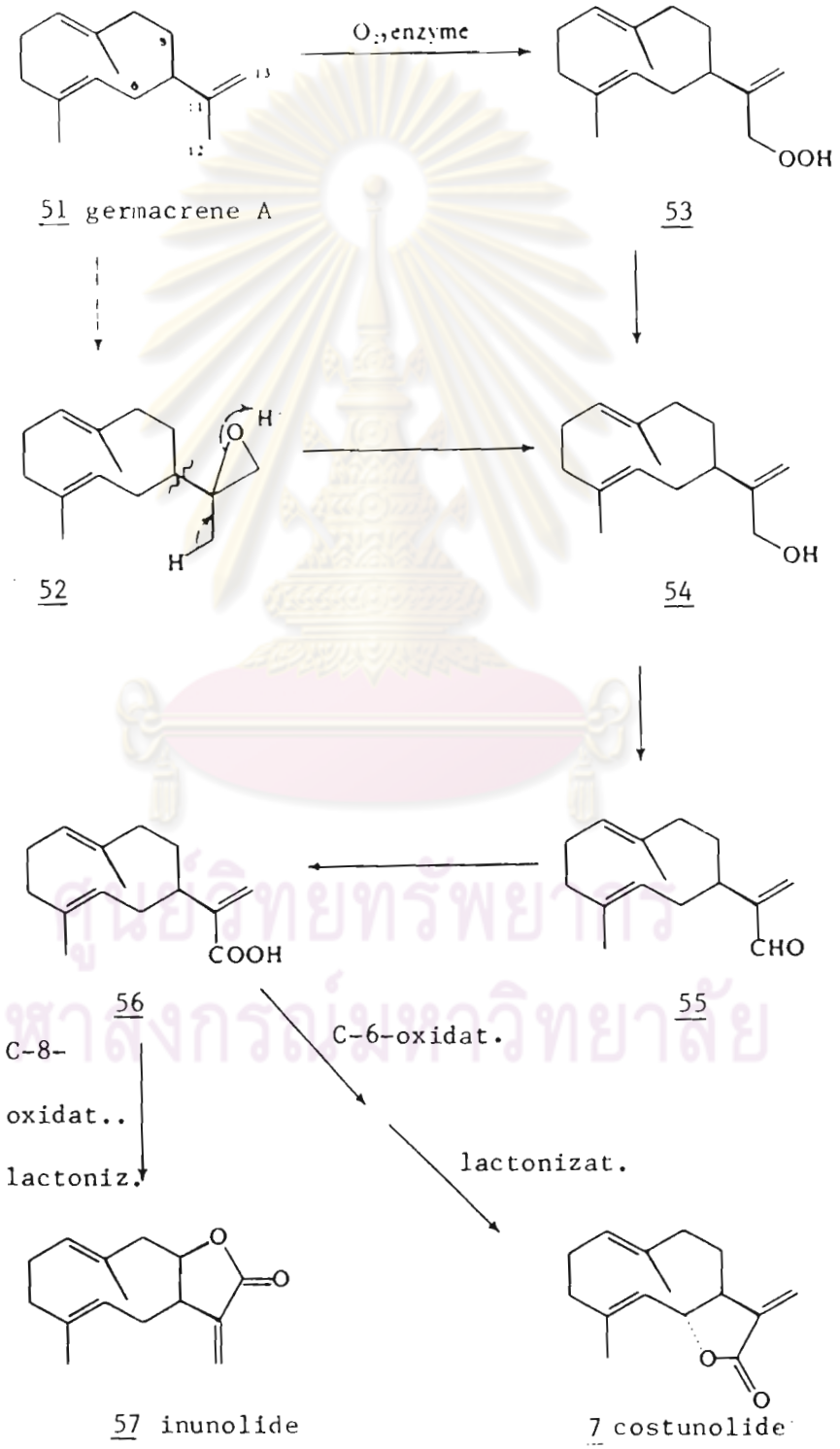


Chart 8. Biogenesis of the lactone ring

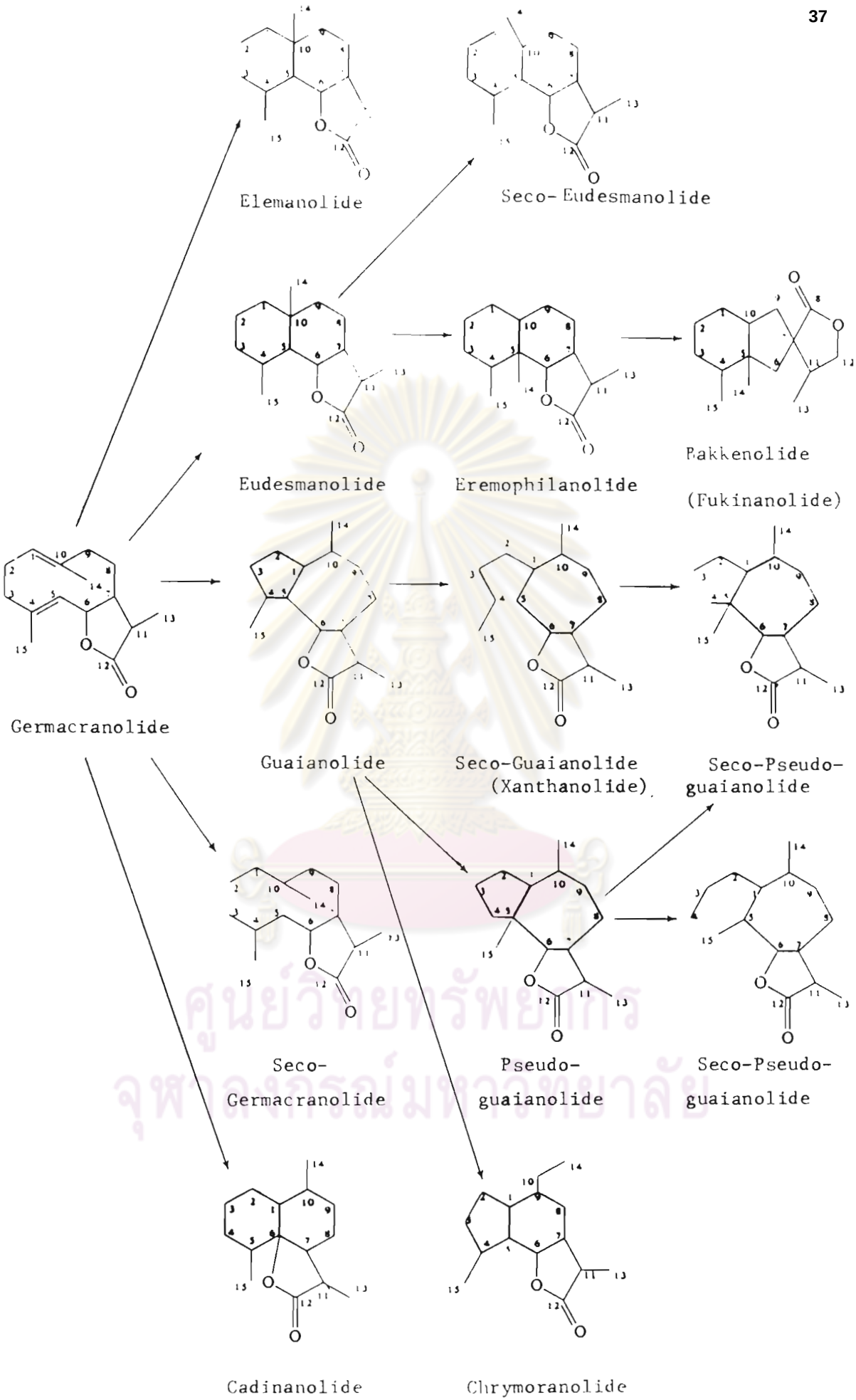


Chart 9. Types and biogenetic relationships of germacranolide-derived sesquiterpene lactones (after W. Herz)

3.2 Biogenesis of Eudesmanolides

The biogenesis of eudesmanolides undergoes cyclization of germacranolides (chart 2 p. 23) and their epoxide derivatives (50). The acid-catalyzed cyclization of costunolide-1,10-epoxide 58 gives the eudesmanolides reynosin 59 and santamarin 60 through the intermediate cation 61 (70). Ivangulin 27, 1,10-seco-eudesmanolides, could be derived from a eudesmanolides hydroperoxide 62 which by a fragmentation reaction, would provide the aldehyde 63. Further oxidative biomodification followed by methyl ester formation would result in ivangulin 27 (chart 10 p. 39).

3.3 Biogenesis of Guaianolides and Xanthanolides

The Markovnikov type cyclization of germacrolide-4,5-epoxide 64 in a chair-like transition state would lead to the cis-fused guaianolide cation 65 from which the guaianolide skeleton 66 would be formed by an uptake of water. Fragmentation of the 4,5-bond and H-12 to C-10α shift from cation 65, would give the xanthanolide skeleton 67. The diol 66 after water elimination and oxidation at C-8 would provide the dienol 68 which upon intramolecular substitution, C-5 to C-1 hydride shift and uptake of water could provide the cyclopropane guaianolide skeleton 69 from which ivaxillarin 70 might be derived (chart 11 p. 40)

3.4 Biogenesis of Elemanolides

The biogenesis of elemanolides most likely involves Cope rearrangements of germacranolides which occur under laboratory with great ease. The thermal rearrangements of *trans, trans*-cyclodeca-1,5-dienic sesquiterpenes proceed in a highly stereospecific manner

through a chair-like transition state resulting in a divinylcyclohexane skeleton. Short term thermolysis of dihydrotamaulipin A acetate 71 at 220° C gives an 2 : 3 equilibrium mixture of starting material 71 and the divinylcyclohexane derivative 73, the reaction proceeding with high stereospecific through the chair-like transition state 72 (71) (chart 12 p. 41)

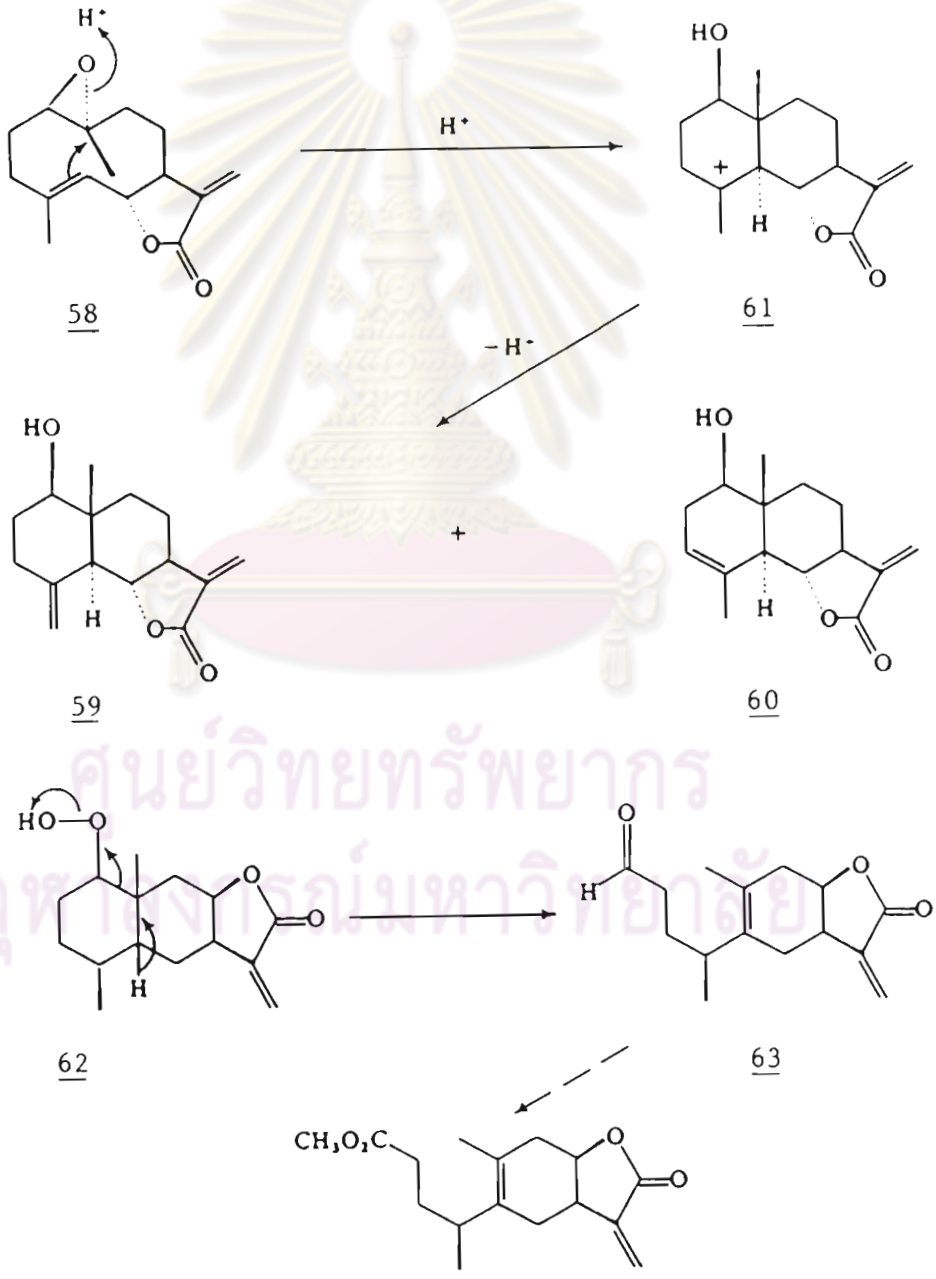


Chart 10. Biogenesis of eudesmanolides and 1,10-seco-Eudesmanolides

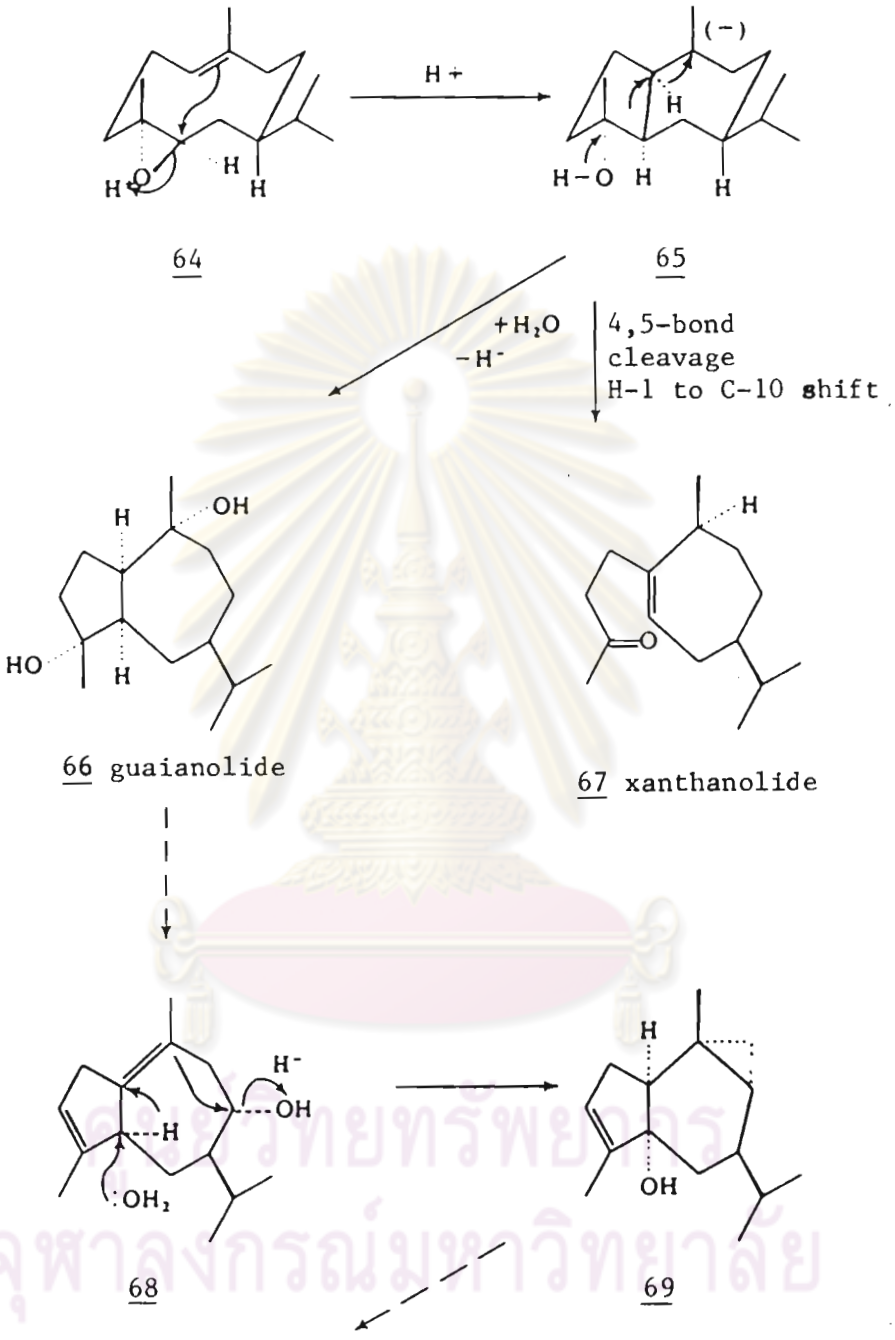
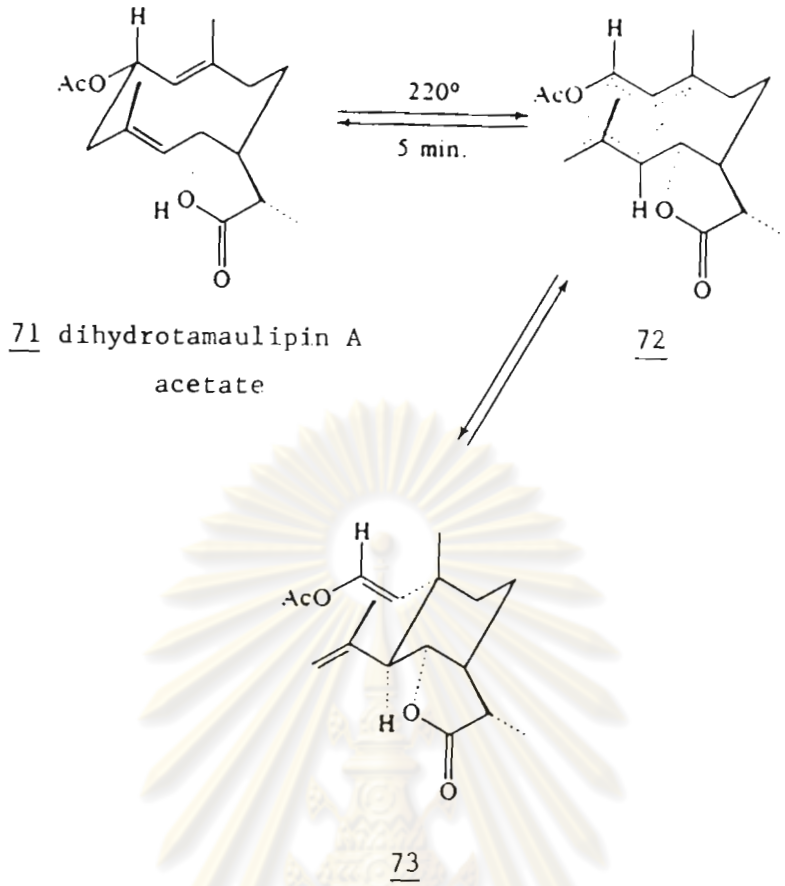


Chart 11. Biogenesis of guaianolides and xanthanolides



71 dihydrotamaulipin A acetate

72

73

Chart 12. Cope rearrangement of dihydrotamaulipin A acetate

3.5 Biogenesis of Pseudoguaianolides

The biogenesis of ambrosanolide skeleton 75 from the germacrolide-4,5-epoxide 64 will initially undergo Markovnikov cyclization to the guaianolide type cation 74 which upon double hydride and methyl shift, gives 75 from which the 7,6-*cis*-lactone damsin 76 is formed (chart 13 p.42)

The biogenesis of helenanolides skeleton 40, acid-induced cyclization of the melampolide-4,5-epoxide 77 and 77' would give cation 78 from which by the indicated shifts the skeleton 40 results, showing a stereochemical arrangement typical for most naturally occuring helenanolides (chart 14 p. 42)

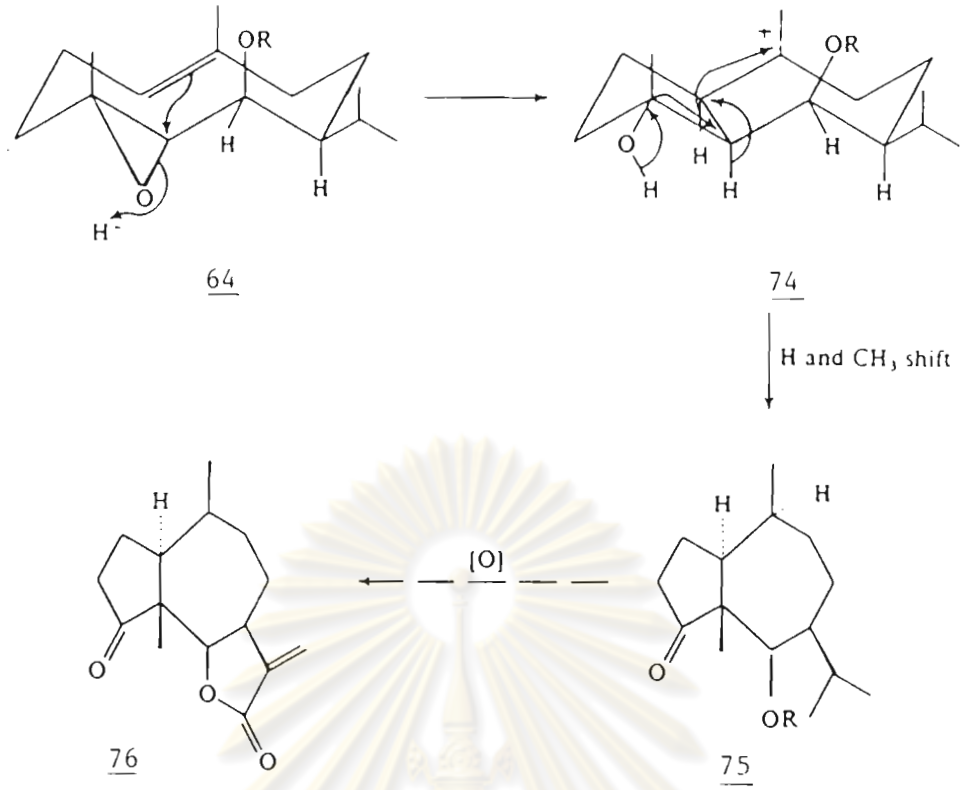


Chart 13. Biogenesis of ambrosanolides and psilostachyanolides

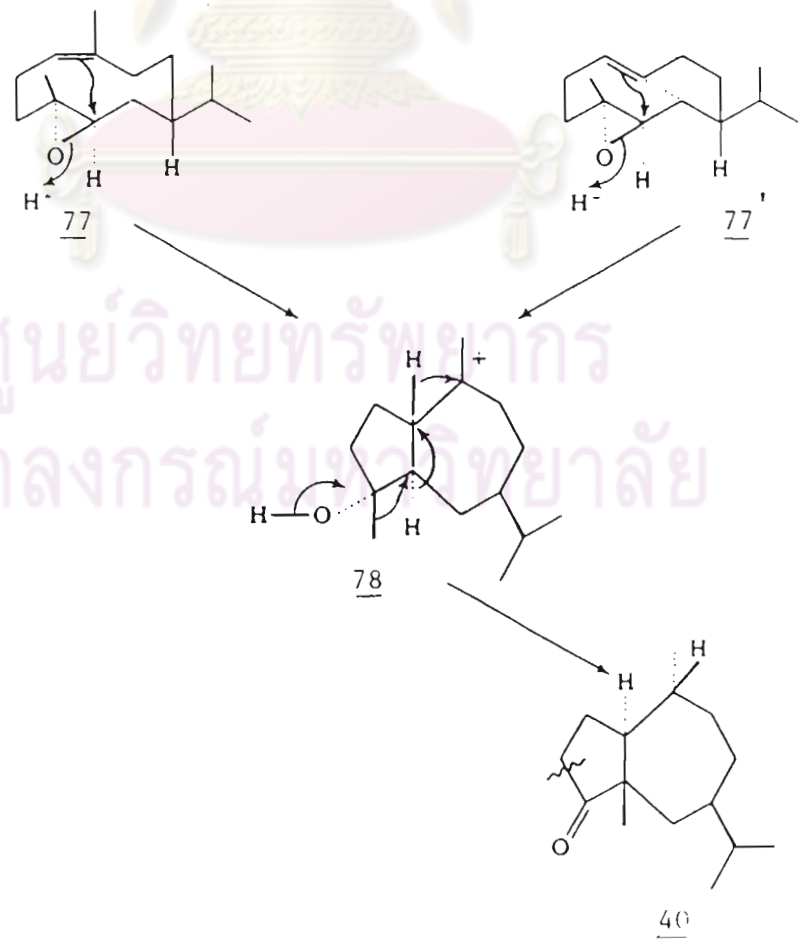


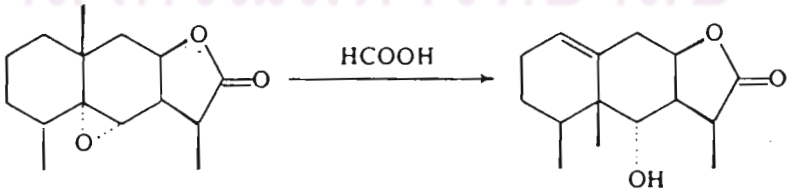
Chart 14. Biogenesis of helenanolides

3.6 Biogenesis of Eremophilanolides and Bakkenolides

Reactions mimicking the postulated methyl migration from C-10 to C-5 of a eudesmanolides 79 lead to an eremophilanolide 80 (65). For example, in a biogenetic-type conversion dihydroalantolactone epoxide 79 was transformed to the eremophilanolide 80 upon treatment with formic acid in acetone (72) (chart 15 p. 43)

Oxidative biogenetic conversion of furanoeremophilane 81 would provide the naturally occuring furan derivative 83 which can be transformed in vitro to eremophilenolide 84 through the intermediate 82 (73). The photosensitized autoxidation of the furanolactone 85 leads to the dilactone 86, naturally occuring lactones (chart 16 p. 44)

Bakkenolides are being considered as derivatives of the eremophilanolides which result from ring contraction of ring B of the eremophilane skeleton followed by biomodifications (65). Epoxidation of fukinone 87 gave the α ,β-epoxiketone 88 which upon treatment with base underwent a Favorskii-type rearrangement forming after methylation the ring contraction product 89. Subsequent elimination, SeO_2-oxidation and spontaneous lactonization yeilded bakkenolide A 90 (43) (chart 17 p. 44)



79 80

Chart 15. Transformation of a eudesmanolide to an eremophilanolide

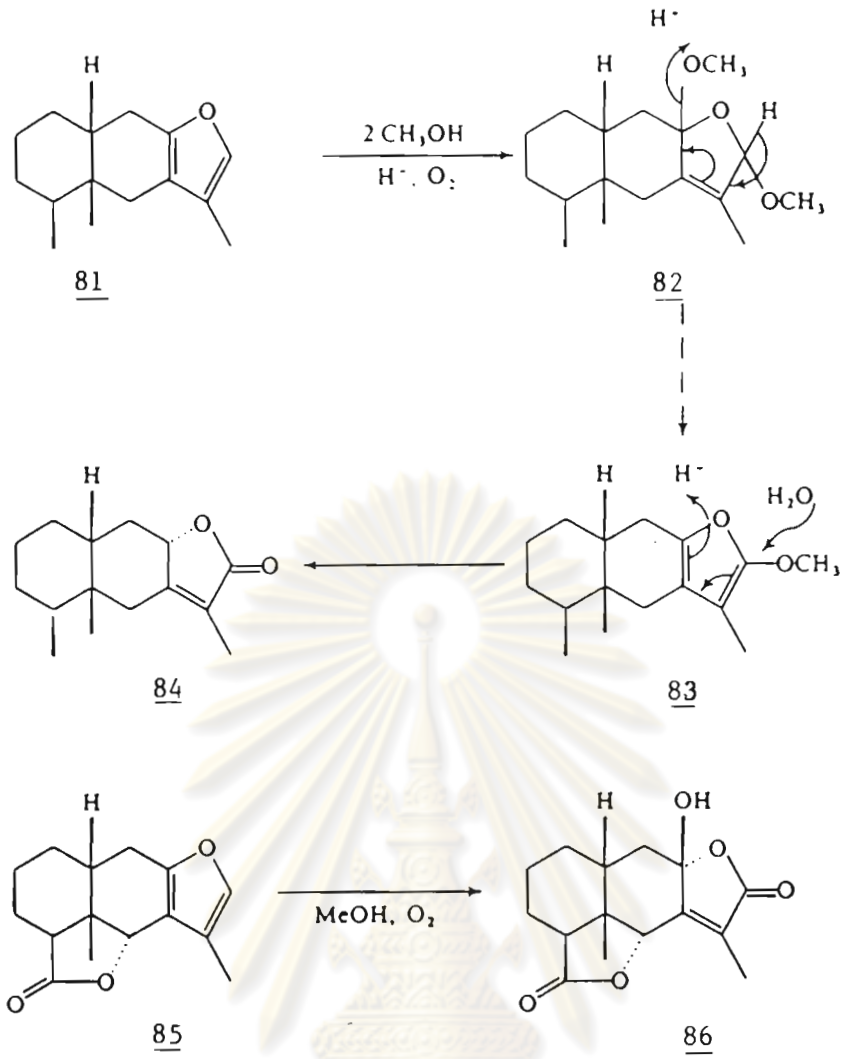


Chart 16. Conversion of eremophilanofurans to eremophilanolides

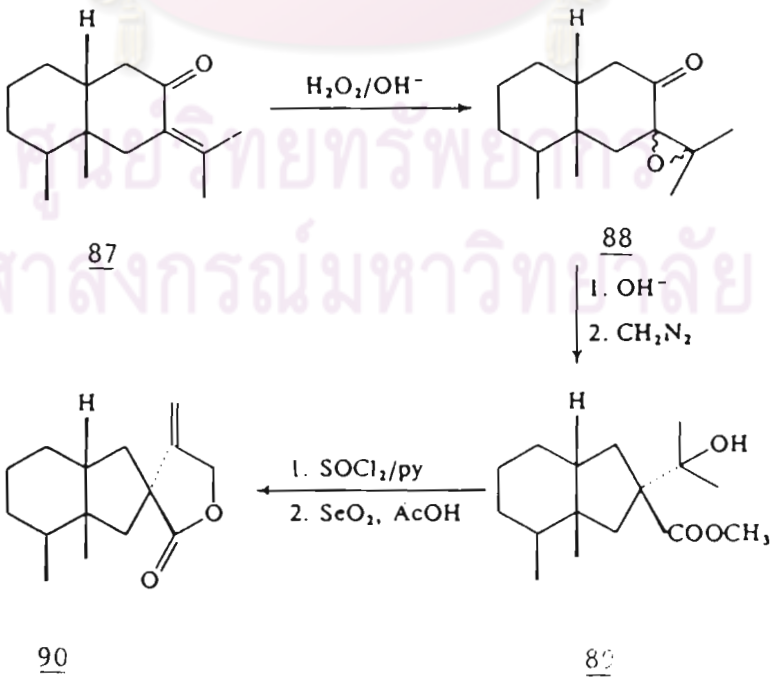


Chart 17. Synthesis of bakkenolide A from fukinone

CHAPTER III

EXPERIMENTAL

1. Source of Plant Materials

1.1 *Michelia rajaniana* Craib

The stem bark of *Michelia rajaniana* Craib was collected from Doi Suthep-Doi Pui National Park, ChiangMai Province, Thailand, in July, 1985 and authenticated by comparison with herbarium specimens at Royal Forest Department, Ministry of Agriculture and Cooperatives, Thailand. A voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

1.2 *Grangea maderaspatana* Poir.

The whole parts of *Grangea maderaspatana* Poir. were collected from Suphan Buri Province, Thailand, in May, 1986. Authentication was achieved by comparison with herbarium specimens at Botany Section, Technical Division, Ministry of Agriculture and Co-orperatives,Thailand. A voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2. General Techniques

2.1 Thin-layer Chromatography (TLC)

Analytical

Technique : one way, ascending

Absorbent : sillica gel GF_{254} (E. Merck) 30 gm/60 ml of distilled water

Plate size : 5 x 20 cm, 10 x 20 cm, and 20 x 20 cm

Layer thickness

: 250 μ

Activation : air dried for 15 minutes and then at 110 °C for 1 hour.

Solvent systems

: a) benzene : acetone (1:1)

b) benzene : acetone (4:1)

c) benzene : ethyl acetate (4:1)

d) benzene : ethyl acetate (1:2)

e) chloroform : acetone (5:1)

f) ethyl acetate : acetone (1:1)

g) benzene : acetone (1:1)

h) benzene : acetone (4:1)

i) benzene : ethyl acetate (4:1)

j) benzene : ethyl acetate (1:2)

k) chloroform : acetone (5:1)

Distance : 15 cm

Temperature : 24-30 °C

Detection on chromatographic plate

: a) Ultraviolet light at wavelength 254 and 366 nm

b) Chromogenic spray reagents

- Dragendorff's spray reagent

Solution A : bismuth subnitrate (850 mg), distilled water (40 ml) and acetic acid (10 ml)

Solution B : potassium iodide (8 gm) and distilled water (20 ml)

Solution A and B, each of 5 ml, were mixed. Then 20 ml of glacial acetic acid and 70 ml of distilled water were added and used as spray reagent.

The alkaloids give orange spots as positive test.

- Mixture of 2 % resorcinol in methanol and 2 % sulphuric acid (1:1). Plate after spraying, was warmed in hot air oven. The colours developed are indicated the presence of various types of were sesquiterpene lactone.

2.2 Column Chromatography (CC)

Adsorbent : silica 0.040-0.063 mm (E. Merck)

Packing of column

: dry packing

Sample loading

: the portion of crude extract was dissolved in a small amount of volatile solvent, mixed with small a quantity of adsorbent, air dried, triturated and added onto the top of a dry column.

Examination of eluate

: fractions were examined by thin-layer chromatography using the chromogenic spray reagents and UV-light. Those fractions of similar pattern were combined.

2.3 Physical Constants

Optical Rotation

: Optical rotations were determined by Bendix-NPL automatic polarimeter.

Melting Points

: Melting points were determined by Gallenkamp melting point apparatus.

2.4 Spectroscopy.

Ultraviolet-visible (UV) Absorption Spectra

Ultraviolet-visible absorption spectra were obtained on a Varian DMS 90 spectrophotometer.

Infrared (IR) Absorption spectra

IR absorption spectra were obtained on a Perkin-Elmer Model 1330 or 180 spectrophotometer.

Nuclear Magnetic Resonance (NMR) Spectra

Proton (^{1}H) and Carbon-13 (^{13}C) NMR spectra were taken on a Bruker WH 400 spectrometer with TMS (=0) as internal standard and with solvents as indicated.

Mass Spectra (MS)

Mass spectra were recorded on a Varian MAT CH 7 or VG Micromass 7070 F spectrometer.

2.5 Authentic Samples

Liriodenine, parthenolide and bisparthenolidine obtained from *Paramichelia baillonii* HU were kindly supplied by Mr. Arthorn Rivepiboon, graduate student of Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3. Extraction and Purification

3.1 *Michelia rajaniana* Craib.

The fresh bark of *M. rajaniana* Craib. (3 kg) was with blended with 95 % ethanol, macerated twice for 3 day-periods (10 L and 8 L) and filtered by suction. The combined filtrate was evaporated under reduced pressure to dryness. The residue was suspended in water (1 L) and extracted withe chloroform (8 x 500 ml). The combined chloroform fractions, after drying (Na_2SO_4 anhydrous) and evaporation, yielded 12.5 gm of syrupy mass (crude MR).

The crude MR was divided into 12 equal portion and each one was treated in the same manner. Each portion was chromatographed on a silica gel column (2.5 x 15 cm) using benzene : ethyl acetate (1:1) as eluent. Twenty five millilitre of each fraction was collected and compared by TLC. Those fractions of similar pattern were combined and ebaporated to dryness. Fraction 1-13, 17-39 and 41-53 after evaporation, afforded residued A, B and C respectively.

a) Residue A was rechromatographed on silica gel (2.5 x 15 cm) column using benzene : acetone 4 : 1 as eluent to furnish 138 mg (0.0046 %) of MR-1 and 79 mg (0.00263 %) of MR-3.

b) Residue B was rechromatographed on silica gel (2.5 x 15 cm) column using chloroform : acetone 5 : 1 to afford 84 mg (0.0028 %) of MR-4 and 80 mg (0.00266 %) of MR-6

c) Residue C was rechromatographed on silica gel (2.5 x 15 cm) column using chloroform to give 344 (0.0115 %) of MR-7 and 150 mg (0.005 %) of MR-8

3.2 *Granagea maderaspatana* Poir.

The dried powdered plant material (2 kg) was macerated with 95 % ethanol (2 x 5 L) for 3 day-periods and filtered by suction. The combined filtrate was evaporated under reduced pressure until dryness to give syrupy mass (250 gm). The residue was suspended in water (500 ml), extracted with chloroform (6 x 300 ml). The combined chloroform fraction, after drying (Na_2SO_4 anhydrous) and evaporation, yielded 45 gm of syrupy mass (crude GM).

The crude GM was divided into 15 equal portions and each one was treated in the same manner. Each portion was chromatographed on a silica gel column (5 X 15 cm) using chloroform : acetone 5 : 1 as eluent. One hundred millilitre of each fraction was collected, evaporated and compared by TLC. Those fractions of similar pattern were combined and evaporated to dryness.

a) fractions 3-4 were designated as GM-1 (24 mg, 0.0012 %)

b) fractions 7-11 were designated as GM-2 (115 mg, 0.00775 %)

c) fractions 21-28 were designated as GM-3 (117 mg, 0.00585 %)

4. Identification of the Isolated Compounds

The isolated compounds were identified by comparison of hRf values, melting points, optical rotation, ultra-violet absorption spectra, infrared absorption spectra, nuclear magnetic resonance spectra and mass spectra with authentic samples and data published previously.

4.1 Identification of MR-1

MR-1 was crystallized from methanol as white needle crystals. It is soluble in benzene, chloroform, ethylacetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

a) benzene : acetone (1:1) = 85

b) benzene : acetone (4:1) = 67

c) benzene : ethyl acetate (4:1) = 62

d) benzene : ethyl acetate (1:2) = 70

e) chloroform : acetone (5:1) = 78

The thin-layer chromatograms of MR-1 are shown in Figures 3-7 (pp. 102-106)

Optical Rotation (in $CHCl_3$)

$(\alpha)_D^{20} = -78^\circ$

Melting Point

112-115° C

Molecular Weight

248

Infrared Absorption Spectrum (in CCl_4) (Figure 14, p. 113)

$\nu_{max}(cm^{-1})$

3020, 2920, 1770, 1650, 1281, 1260, 1130 and 940

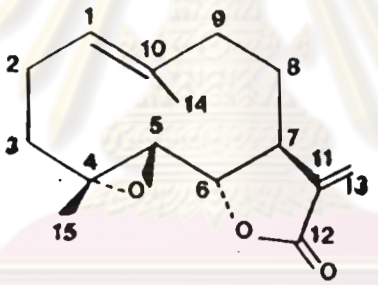
Proton NMR Spectrum (in $CDCl_3$, 400 MHz) (Table 3, p. 67 and Figure 15, p. 114).

Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 16, p. 115)

248 (M^+, 2), 230 (9), 191 (25), 190 (61), 177 (16.4), 175 (34.4), 155 (65), 119 (100), 91 (87.7), 67 (73.7), 43 (100) and 41 (77.2)

From the above data, MR-1 was in complete agreement with the structure of (-)-parthenolide (77). It is therefore concluded that MR-1 is (-)-parthenolide, the structure of which is shown below.



(-)- parthenolide

4.2 Identification of MR-3

MR-3 was crystallized from ethyl acetate as white amorphous solid. It is soluble in benzene, chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given obtained from the following systems :-

a) benzene : acetone (1:1) = 76

b) benzene : acetone (4:1) = 54

c) benzene : ethyl acetate (4:1) = 51

d) benzene : ethyl acetate (1:2) = 56

e) chloroform : acetone (5:1) = 61

The thin-layer chromatograms of MR-3 are shown in Figure 3-7 (pp. 102-106)

Optical Rotation (in $CHCl_3$)

$(\alpha)_D^{20} = -112^\circ$

Melting Point

100-102° C

Molecular weight

513

Infrared Absorption Spectrum (in CCl_4) (Figure 17, p. 116)

$\nu_{max}(cm^{-1})$

3365, 3020, 2920, 1770, 1450, 1215, 1175, 1000 and 940

Proton NMR Spectrum (in $CDCl_3$, 400 MHz) (Table 3, p. 67 and Figure18, p.117)

Carbon-13 NMR Spectrum (in $CDCl_3$, 100 MHz) (Table 4, p. 68and Figure 19, p.118)

Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 20, p.119)

513 (M^+, 1.74), 495 (5.9), 278 (100), 264 (13.61), 260 (15), 231 (12), 220 (19) and 43 (51)

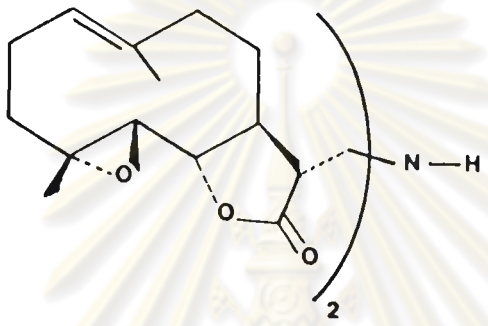
HRMS (composition interprete, calculated millimass)

513.3071 ($C_{30}H_{43}NO_6$, M^+, 513.3090)

278.1752 ($C_{16}H_{24}NO_3$, $M-C_{14}H_{19}O_3$, 278.1756)

264.1600 ($C_{16}H_{22}NO_3$, $M-C_{15}H_{21}O_3$, 264.1594)

From the above data, MR-3 was in complete agreement with the structure of (-)-bisparthenolidine (77). It is therefore concluded the MR-3 is (-)-bisparthenolidine, the structure of which is shown below.



(-)-bisparthenolidine

4.3 Identification of MR-4

MR-4 was crystallized from ethyl acetate as white needle crystals. It is soluble in chloroform, benzene and acetone.

hRf Value

The hRf values given are obtained with the following systems :-

a) silica gel GF_{254}/benzene : acetone (1:1) = 73

b) silica gel GF_{254}/benzene : acetone (4:1) = 45

c) silica gel GF_{254}/benzene : ethyl acetate(4:1) = 40

d) silica gel GF_{254}/benzene : ethyl acetate(1:2) =51

e) silica gel GF_{254}/chloroform : acetone (5:1) = 54

The thin-layer chromatograms of MR-4 shown in Figure 3-7 (pp. 102-106)

Molecular Weight

324

Infrared Absorption Spectrum (in $CHCl_3$)

$\nu_{max}(cm^{-1})$

3620-3240 (br), 2932, 1777, 1734, 1262, 1099, 1069, 1020 and 1006.9

(Figure 21, p. 120)

Proton NMR Spectrum (in $CDCl_3$, 400 MHz) (Figure 22, p. 121)

Proton	
1	5.30 (d, 10.2)[a]
2_β	4.70 (ddd, 10.3, 6.1)
3_α	1.23 (dd, 11.4)
3_β	2.55-2.62 (dd)
5	2.73 (d, 8.8)
6	3.95 (dd,8.8)
7	2.34-2.43 (ddd, 8.8, 12.1)
8_β	4.92 (ddd, 8.2, 3.6)
9_α,9_β	2.43-2.45 (m)
11	2.55-2.62 (dq, 6.7)
13	1.45 (d, 6.5)
14	1.87 (s)
15	1.30 (s)
$2'$	2.12 (s)

[a]Chemical shifts are in ppm from TMS, multiplicity and coupling constants are in parenthesis in Hertz.

Carbon-13 NMR Spectrum (in $CDCl_3$, 100 MHz) (Table 4, p. 68 , Figure 23, p. 122)

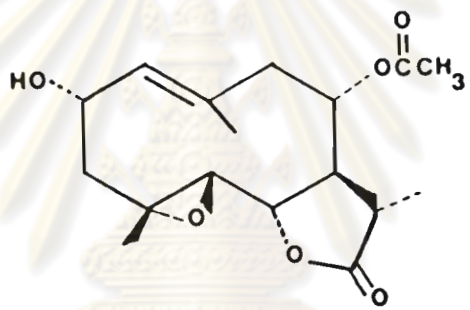
Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 24, p. 123)

264 (6.2),137 (12), 118 (17), 95 (50), 43 (100) and 29 (22)

From EIMS data, it dose not show parent peak (324) but shows fragmentation peak (324-60 =264) which loss hydroxyl and acetyl groups. These groups show in IR spectrum ^{1}H-and ^{13}C-NMR spectrum.

The results were concluded for the structure of MR-4 as a novel sesquiterpene lactone and the chemical structure was assigned as $C_{17}H_{23}O_7$, 8α-acetoxy-2α-hydroxy-dihydroparthenolide and named paramicholide, the structure of which is shown below.



paramicholide

4.4 Identification of MR-6

MR-6 was crystallized from ethyl acetate as white needle crystals. It is soluble in chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

a) benzene : acetone (1:1) = 60

b) benzene : acetone (4:1) = 30

c) benzene : ethyl acetate (4:1) = 25

d) benzene : ethyl acetate (1:2) = 34.6

e) chloroform : acetone (5:1) = 33

The thin-layer chromatograms of MR-6 are shown in Figure 3-7 (pp. 102-106)

Molecular Weight

307

Infrared Absorption Spectrum (in $CHCl_3$) (Figure 25, p. 124)

ν_{max} (cm^{-1})

3020, 2955.8, 2928.05, 2855.26, 1769.79, 1670.26, 908.55 and 864.17

Proton NMR Spectrum (in $CDCl_3$, 400 MHz) (Figure 26, p. 125)

Proton	
1	5.17 (dd, br, 9.7, 1.5)
2_α	2.35-2.45 (m)
2_β	2.35-2.45 (m)
3_α	1.21 (ddd, 13.0, 6.0)
3_β	2.05-2.15 (m)
5	2.70 (d, 8.9)
6	3.87 (dd, 8.9)
7	2.4-2.45 (m)
8_α	2.35-2.45 (m)
8_β	2.05-2.15 (m)

9_{α}	2.05-2.15 (m)
9_{β}	2.2-2.3 (m)
11	2.4-2.45 (m)
13_{a}	3.64-3.69 (ddd)
13_{b}	3.50-3.56 (ddd)
14	1.69 (s)
15	1.30 (s)
2'	2.02 (s)
N-H	6.31 (br.)

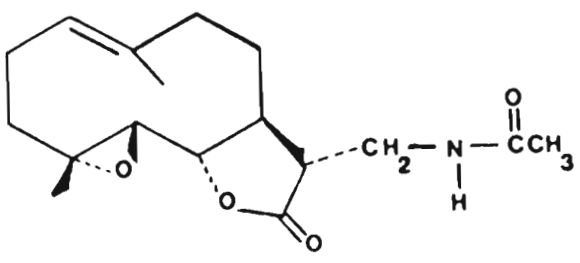
Carbon-13 NMR Spectrum (in $CDCl_3$, 100 MHz) (Table 4, p. 68 , Figure 27 , p 126)

Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 28, p. 127)

307 (M^+, 26), 235 (39), 230 (35), 218 (43)
217 (40.5), 208 (64.6), 205 (31), 191 (35)
190 (63), 117 (83), 131 (100), 95 (72)
72 (100), 43 (100)

The results were concluded for the structure of MR-6 as a novel sesquiterpene lactone and chemical structure was assigned as $C_{17}H_{25}NO_4$ and named N-acetylparthenolidine, the structure of which is shown below.



N-acetylparthenolidine

4.5 Identification of MR-7

MR-7 was crystallized from ethyl acetate as white needle crystals. It is soluble in acetone and alcohol.

hRf Value

The hRf values given are obtained from the following systems :-

a) benzene : acetone (1:1) = 52

b) benzene : acetone (4:1) = 26

c) benzene : ethyl acetate (4:1) = 20

d) benzene : ethyl acetate (1:2) = 28

e) chloroform : acetone (5:1) = 30

The thin-layer chromatograms of MR-7 are shown in Figure 3-7 (pp. 102-106)

Molecular Weight

323

Infrared Absorption Spectrum (in $CDCl_3$) (Figure 29 p. 128)

$\nu_{max}(cm^{-1})$

2932.47, 1768.28, 1656.14, 1079.48, 979.64, and 908.47

Proton NMR Spectrum (in $CDCl_3$, 400 MHz) (Figure 29, p. 129)

Proton	
1	5.2 (dd)
2_α	2.2-2.4 (m)
2_β	2.2-2.4 (m)
3_α	1.25 (m)
3_β	2.05-2.15 (m)
5	2.7 (d)
6	3.92 (dd)
7	2.65 (ddd)
8	4.1 (ddd)
9_α	2.35-2.45 (m)
9_β	2.35-2.45 (m)
11	3.57 (ddd)
13_a	3.0 (ddd)
13_b	3.77 (ddd)
14	1.75 (s)
15	1.3 (s)
2'	2.1 (s)
N-M	6.95 (dd)
O-H	4.95 (d)

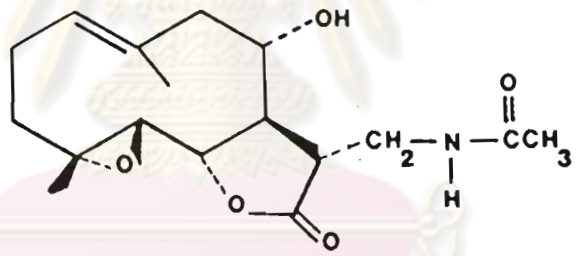
Carbon-13 NMR Spectrum (in $CDCl_3$, 100 MHz) (Table 4, p. 68, Figure 31, p.130)

Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 32, p.131)

256 (100), 241 (19.5), 228 (16), 216 (12.3),
214 (39), 212 (16.3), 196 (16), 188 (16.3),
184 (31), 179 (37.8), 176 (13.5), 175 (13.5),
156 (63.6),68 (100), 43 (100)

The results were concluded for the structure of MR-7 as a novel sesquiterpene lactone and chemical structure was assigned as $C_{17}H_{25}NO_5$ and named N-acetyl-8α-hydroxyparthenolidine, the structure of which is shown below.



N-acetyl-8α-hydroxyparthenolidine

4.6 Identification of MR-8

MR-8 was crystallized from chloroform as yellow needle crystals. It is soluble in chloroform, acetone, ethyl acetate and alcohol. It gives orange colour with the dragendorff's reagent. This reaction is indicated that MR-8 might be alkaloid.

hRf Value

The hRf values given are obtained with the following systems :-

a) benzene : acetone (1:1) = 43

b) benzene : acetone (4:1) = 18

c) benzene : ethyl acetate (4:1) = 14

d) benzene : ethyl acetate (1:2) = 15

e) chloroform : acetone (5:1) = 20

The thin layer chromatograms of MR-8 are shown in Figure 3-7 (pp. 102-106)

Melting Point

278-281 °C

Ultraviolet-Visible Absorption Spectra (Figure 33 and 34, pp. 132-133)

λ_{max}(nm) (in 95 % ethanol)

250, 270, 310, 400 (SH) and 416 nm

λ_{max}(nm) (in 0.1 N HCl in ethanol)

260, 282, 320, 396 and 452

Infrared Absorption Spectrum (in CH_2Cl_2) (Figure 35 p. 134)

$\nu_{max}(cm^{-1})$

3040, 2920, 1655, 1590, 1480, 1462, 1438, 1410, 1300, 1220, 1200, 1050, 1010, 965, 890 and 865

Proton NMR Spectrum (Figure 36, p.135)

Proton	10 % DMSO-d_6:$CDCl_3$	$CDCl_3$
3	7.21 (s)	7.17 (s)
4	7.83 (br s)	7.74 (br s)
5	8.90 (br s)	8.80 (br s)
8	8.57 (d, J=8.0 Hz)	8.58 (d, J=8.1)
9	7.58 (t, J=8.0 Hz)	7.57 (t, J=8.0)
10	7.77 (t, J=8.0 Hz)	7.73 (t, J=8.0)
11	8.72 (d, J=8.0 Hz)	8.61 (d, J=8.1)
OCH_2O	6.41 (s)	6.37 (s)

Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 36, p. 136)

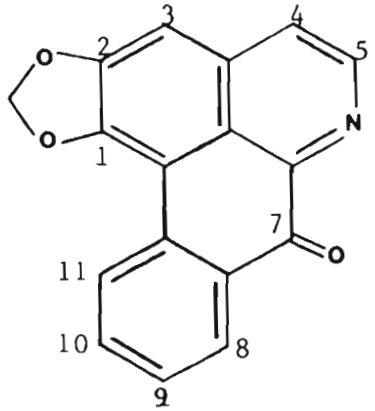
275 (M^+, 80),247 (14), 246 (10), 224 (62.9)

178 (19.5), 149 (36.8), 143 (66.5), 125 (20.6),

123 (22.8)

111 (30.9), 99 (100), 98 (70), 97 (48) and 49 (100)

This data are in agreement with the published values of liriodenine (77). It is therefore concluded that MR-8 is liriodenine, the structure of which is shown below.



liriodenine

Table 3 ^{1}H-NMR Spectra of MR-1 and MR-3[a]

Proton	MR-1[c]	MR-3
1	5.21 (dd, br, 4.0,12.2)	5.27(dd, 2.2, 9.8)
2^{b}_{α}	2.38 (dd, 5.1, 13.1)	2.26(dd, 6.0, 12.1)
2_{β}	2.46 (ddd, 13.8, 12.2, 12.5)	2.40 (m)
3_{α}	1.25 (m)	1.23 (dt, 5.9, 13.9)
3^{b}_{β}	2.17 (m)	1.88 (dd, 5.9,14.6)
5	2.79 (d, 8.8)	2.74 (d, 8.8)
6	3.86 (dd, 8.8, 8.3)	3.86 (dd, 8.8)
7	2.78 (m)	2.40 (m)
8^{b}_{α}	1.72 (m)	2.18 (m)
8_{β}	1.73 (m)	1.70 (m)
9_{α}	2.09-2.24 (m)	2.10-2.18 (m)
9_{β}	2.38 (m)	2.10-2.18 (m)
11_{β}	-	2.40 (m)
13_{a}	6.33 (d, 3.6)	3.15 (dd, 2.8, 13.1)
13_{b}	5.62 (d, 3.1)	2.92 (dd, 2.8, 13.1)
14	1.72 (s)	1.67 (s)
15	1.31 (s)	1.30 (s)

[a]Chemical shifts are in ppm from TMS, multiplicity, coupling constants (Hz) are in parenthesis and the samples were dissolved in $CDCl_3$.

[b]previously assigned 200 MHz spectrum from Badesinsky *et al.* $H_{2\alpha}$, 2.09-2.24 (m); $H_{3\beta}$, 2.09-2.24 (m) and $H_{8\alpha}$, 2.09-2.24 (m) (78).

[c]Specific assignments possible at 400 MHz with 2D-COSY and decoupling experiments.

Table 4 ^{13}C-NMR Spectra of MR-1, MR-3, MR-4, MR-6 and MR-7 [a]

carbon	MR-1[c]	MR-3	MR-4	MR-6	MR-7
1	125.3(-)	125.3(-)	131.0(-)	125.1 (+)	127.4(-)
2	24.2[b](+)	24.2(+)[b]	66.6(-)	24.1(+)	24.4(+)
3	36.2[b](+)	36.5(+)[b]	45.1(+)	63.3(+)	35.6(+)
4	61.5(+)	61.6(+)	60.6(+)	61.7(+)	61.9(+)
5	66.4(-)	66.1(-)	66.6(-)	62.2(-)	66.0(+)
6	82.5(-)	82.3(-)	78.6(-)	82.9(-)	78.9(-)
7	47.7(-)	49.0(-)	55.4(-)	48.4(-)	51.5(-)
8	41.2[b](+)	30.2(+)[b]	72.1(-)	29.8(+)	72.3(-)
9	30.2[b](+)	40.9(+)	49.3(+)	40.9(+)	39.6(+)
10	134.7(+)	134.3(+)	131.0(+)	134.6(+)	130.3(+)
11	139.5(-)	45.5(-)	39.6(-)	46.6(-)	46.8(-)
12	169.3(+)	176.7(+)	176.8(+)	176.6(+)	177.0(+)
13	121.0(+)	46.2(+)	18.3(-)	36.6(+)	52.4(+)
14	17.3(-)	17.2(-)	8.2(-)	17.2(-)	17.5(-)
15	17.0(-)	16.8(-)	17.6(-)	16.7(-)	17.3(-)
1'	-	-	169.8(+)	170.7(+)	173.2(+)
2'	-	-	21.0(-)	23.2(-)	22.9(-)

[a] Chemical shifts are in ppm from TMS, solvent was $CDCl_3$, (+) and(-) are signs from the attached proton test (APT).

[b] Assignments may be interchanged.

[c] Data taken from El-Feraly *et al.* (79).

4.7 Identification of GM-1

GM-1 was crystallized from n-hexane as rod crystals. It is soluble in chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

f) ethyl acetate : acetone (1:1) = 80

g) benzene : acetone (1:1) = 70

h) benzene : acetone (4:1) = 60

i) benzene : ethyl acetate (4:1) = 52

j) benzene : ethyl acetate (1:2) = 75

k) chloroform : acetone (5:1) = 80

The thin layer chromatograms of GM-1 are shown in Figure 8-13, pp. 107-112.

Molecular Weight

232

Infrared Absorption Spectrum (in CCl_4) (Figure 38, p. 137)

ν_{max} (cm-1)

3020, 2940, 1767, 1264, 1142.9 and 940

Proton NMR Spectrum (in $CDCl_3$, 400 MHz) (Figure 39, p. 138)

Proton	
1_α	0.9-1.9(m)[a]
1_β	
2_α	
2_β	
3_α	2.08
3_β	2.1
6	5.26 (d, 5.9)
7	2.95 (m)
8_α	0.9-1.9(m)[a]
8_β	
9_α	
9_β	
13_a	6.14 (d, 1)
13_b	5.57 (d, 1)
14	1.70 (s)
15	1.08 (s)

[a] No specific assignments with two dimension and decoupling experiments.

Carbon-13 NMR Spectrum (in $CDCl_3$, 100 MHz) (Table 5, p. 77, Figure 40, p. 139)

Mass Spectrum (EIMS)

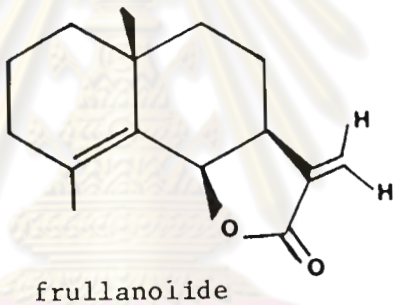
m/z (%, relative intensity) (Figure 41, p.140)

232 (M^+, 15.6), 218 (15.5), 217 (100), 161 (10.6)

146 (16.6), 119 (9), 105 (14), 93 (12)

91 (19), 81 (12), 79 (13), 55 (13) and 40 (18)

From the above data, GM-1 was in complete agreement with structure of frullanolide (43, 81, 82). It is therefore concluded that GM-1 is frullanolide, the structure of which is shown below.



frullanolide

4.8 Identification of GM-2

GM-2 was crystallized from benzene : ethyl acetate (4:1) as white needle crystals. It is soluble in chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

f) ethyl acetate : acetone (1:1) = 68

g) benzene : acetone (1:1) = 60

h) benzene : acetone (4:1) = 42

i) benzene : ethyl acetate (4:1) = 39

j) benzene : ethyl acetate (1:2) = 61

k) chloroform : acetone (5:1) = 71

The thin layer chromatograms of GM-2 are shown in Figure 8-13. pp. 107-112

Melting Point

69.5-70.0

Molecular Weight

248

Infrared Absorption Spectrum (in CCl_4)

$\nu_{max}(cm^{-1})$

3020, 3010, 2933, 1771.8, 1751.5, 1142.4 and 956

(Figure 42, p. 141)

Proton NMR Spectrum (in $CDCl_3$, 400 MHz) (Figure 43, p. 142)

Proton	
1_α, 1_β, 2_α, 2_β, 3_α, 3_β	1.35-2.2(m)[a]
6	5.0 (s)

Proton	
8_{α}, 8_{β}, 9_{α}, 9_{β}	1.35-2.2(m)[a]
13_a	6.27 (d)
13_b	5.81 (s)
14	1.01 (s)
15	1.78 (s)

[a] No specific assignments with two dimension and decoupling experiments.

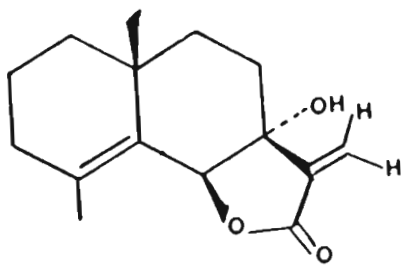
Carbon-13 NMR Spectrum (in acetone-d_6, 100 Hz)(Table 5,p.77, Figure 44, p. 143)

Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 45, p.144)

248 (M^+, 18), 233 (100), 215 (29.7), 187 (25) 178 (17) and 169 (30.5)

The results were concluded for the structure of GM-2 as a novel sesquiterpene lactone and the chemical structure was assigned as $C_{15}H_{20}O_3$ and named 7α-hydroxyfrullanolide, the structure of which is shown below.



7 α-hydroxyfrullanolide

4.9 Identification of GM-3

GM-3 was crystallized from n-hexane : acetone (1:1) as needle crystals. It is soluble in chloroform, ethyl acetate and acetone,

hRf Value

The hRf values given are obtained from the following systems systems :-

f) ethyl acetate : acetone (1:1) = 40

g) benzene : acetone (1:1) = 36.6

h) benzene : acetone (4:1) = 25

i) benzene : ethyl acetate (4:1) = 20

j) benzene : ethyl acetate (1:2) = 34

k) chloroform : acetone (5:1) = 42

The thin layer chromatograms of GM-3 are shown in Figure 8-13, pp. 107-112.

Melting Point

135.0-139.0

Molecular Weight

266

Infrared Absorption Spectrum (in $CHCl_3$) (Figure 46, p. 145)

ν_{max}(cm-1)

3600-3500, 2920, 1770.9, 1006.5 and 971

Proton NMR Spectrum (in $CDCl_3$, 100 MHz) (Figure 47, p. 146)

Proton	
1_α, 1_β, 2_α, 2_β	1.3-2.1(m)[a]
3	4.0 (dd)
6	4.89 (s)
8_α, 8_β, 9_α, 9_β	1.3-2.1 (m)[a]
11	2.8 (q, 7.2)
13	1.21 (d, 7.2)
14	1.04 (s)
15	1.96 (s)
O-H	2.6 (br)

[a] No specific assignments with two dimension and decoupling experiments.

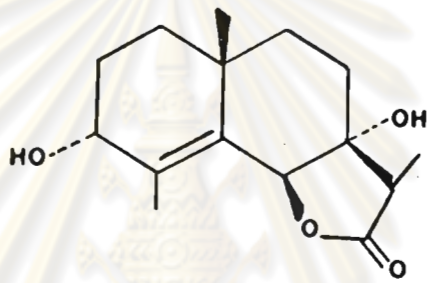
Carbon-13 NMR Spectrum (in $CDCl_3$, 100 MHz) (Table 5, p. 77 , Figure 48, p. 147)

Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 49, p. 148)

266 (M^+, 52), 251 (25), 248 (61.7), 215 (40.3), 204 (36), 192 (33), 187 (47), 177 (37), 123 (100)

The results were concluded for the structure of GM-3 as a novel sesquiterpene lactone and the chemical structure was assigned as $C_{15}H_{22}O_4$ and named 7α, 3α-dihydroxy dihydrofrullanolide, the structure of which is shown below.



7α, 3α-dihydroxy dihydrofrullanolide

Table 5 Carbon-13 Spectra of GM-1, GM-2 and GM-3

Carbon	GM-1	GM-2	GM-3
1	18.2 (+)	18.2 (+)	24.9 (+)
2	33.1 (+)	33.1 (+)	33.8 (+)
3	39.1 (+)	38.8 (+)	69.6 (-)
4	138.5 (+)	140.5 (+)	140.5 (+)
5	128.5 (+)	126.8 (+)	131.5 (+)
6	75.9 (-)	81.4 (-)	79.9 (-)
7	41.2 (-)	76.0 (+)	77.4 (+)
8	25.0 (+)	31.5 (+)	27.4 (+)
9	37.9 (+)	34.9 (+)	34.7 (+)
10	32.6 (+)	32.7 (+)	32.7 (+)
11	142.3 (+)	144.7 (+)	47.7 (-)
12	170.9 (+)	169.1 (+)	177.0 (+)
13	120.1 (+)	121 (+)	7.2 (-)
14	19.3 (-)	19.4 (-)	17.9 (-)
15	25.8 (-)	26.1 (-)	24.0 (-)

CHAPTER IV

DISCUSSION

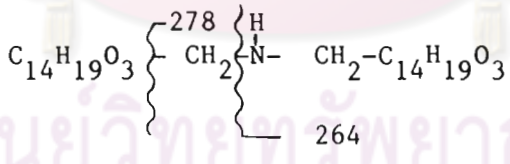
Phytochemical study of the genus *Michelia* (Magnoliaceae) has previously led to the isolation of a variety of alkaloids, sesquiterpene lactones, essential oils and steroids. Most of the sesquiterpene lactones found in *Michelia* spp. are germacranolides having α,β-unsaturated lactone which is *trans*-fused to the C_6-C_7 or C_8-C_7 positions of the carbocyclic skeleton. The presence of α-methylene-γ-lactone in sesquiterpenoid skeleton can enhance the cytotoxic activity of such compounds eg. micheliolide, michelenolide, parthenolide costunolide, santamarine, dehydrolanuginolide and epitulipinolide diepoxide. Review of chemical constituents in *Michelia* spp. is shown in Table 1.

Fractionation by column chromatography from chloroform extract of the fresh bark of *M. rajaniana* Craib led to obtain alkaloid and several sesquiterpene lactones, four of which are found to contain unusual substituents of germacranolide skeleton.

The white needle crystal (MR-1), was isolated as described in the Experimental section and was found to be the germacranolide epoxide on the basis of the data reported below. MR-1 exhibited IR absorption at 1770 and 1650 cm^{-1} indicating the presence of α-methylene-γ-lactone functionality. Its mass spectrum showed a weak parent ion at *m/z* 248 which was corresponding to the molecular formula $C_{15}H_{20}O_3$. The 400 MHz ^{1}H-NMR spectrum of this component as shown in Table 3 was

in excellent agreement with that of previously reported for the germacranolide epoxide, parthenolide. With the aid of a ^{1}H-^{1}H 2D-COSY spectrum ($CDCl_3$), assignment of all the protons in MR-1 was possible (Table 3). The structure and conformation of MR-1 were previously established unambiguously by single crystal X-ray analysis (82). Determination of the optical rotation of this component established that it was (-)-parthenolide of (6S) absolute configuration as depicted on page 81.

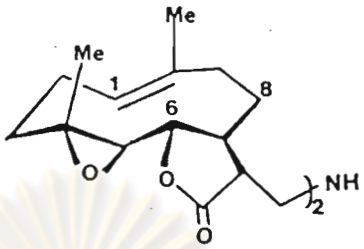
The second component (MR-3), was an amorphous solid and its EIMS (parent peak *m/z* 513, base peak 278) was consistent with a compound containing two sesquiterpenoid units and one nitrogen atom. Accurate mass determinations of the parent peak and two fragments (*m/z* 278 and 264, see Experimental) further supported the presence of a nitrogen atom and fragmentations as indicated below. The IR spectrum displayed a strong absorption at 1770 (γ-lactone) and a weak band at 3365 cm^{-1}(N-H). Both the ^{1}H-and the ^{13}C-NMR spectra (Tables 3 and 4),



respectively showed many similarities to the spectra of parthenolide, (6, 19, 79) with the only significant differences being in the region of C(11) and C(13). In particular, C(11) in MR-3 was a methine carbon (C-H) as determined by the ^{13}C attached proton test (APT) and the two protons on C(13) appeared as an AB pattern (J = 13.1 Hz) at about 3 ppm with additional splitting (J = 2.8 Hz). The chemical shifts of C(13) (46.2 ppm) and of the attached protons were consistent with the presence of a nitrogen atom on that carbon. Assignments for the

protons (2D-COSY experiment) of MR-3 were given in Table 3. On the basis of this spectroscopic information, it was proposed that the second component isolated from *M. rajaniana* is the sesquiterpenoid alkaloid, formed by Michael addition of ammonia to two molecules of parthenolide. The aminomethyl group at C(11) is tentatively assigned the α-configuration because in the ^{1}H-NMR spectrum of MR-3 in deuterated benzene solvent, H(7) appears as a well-resolved quartet (J = 8.8 Hz) as a result of *trans* coupling with H(6), H(8β) and H(11). Presumably MR-3 is derived from (-)-parthenolide, which has a (6S)-configuration (83), so the same absolute configuration is assigned to this new alkaloid, which the authors have chosen to call bisparthenolidine.

As MR-3 is a new germacranolide derivative, it was interested in determining its conformation in solution using ^{1}H-NOE (Nuclear Overhauser Effect) experiments. Low-intensity irradiation of a degassed $CDCl_3$ solution of MR-3 at 1.67 ppm (the resonance for the C(14) methyl group) caused an increase in the intensity of the signals for H(2β), H(8β) and H(9β) of 43, 43 and 10 %, respectively. In addition, irradiation of the H(6) resonance at δ 3.86 ppm resulted in a 47 and 9 % enhancement of the H(8β) and C(15) methyl signals, respectively. Since there was interaction between the C(15) methyl group and H(6) but none between H(5) and H(6), the *trans* configuration of the C(4)-C(5) epoxide was confirmed. A 2D-NOE experiment (NOESY) confirmed the interactions mentioned above but also revealed a weak cross-peak correlation between C(14) and C(15) methyl signals, thus indicating the *syn* relationship between these two groups. The NOE results clearly indicate a conformation for this parthenolide derivative as shown below. A similar conformation has been reported for parthenolide (82).



As MR-3 is an unusual natural product, it deserves some comment. From literature citation, MR-3 is the first reported example of a naturally-occurring germacranolide alkaloid, although a piperidine adduct of a pseudoguaianolide (84) and a tertiary amine derived from ammonia and three molecules of α-methylenebutyrolactone (85) have been isolated previously from natural sources. A secondary amine related to MR-3 has been synthesized from ammonia and two molecules of the eudesmanolide, alantolactone, and there have been numerous reports of reactions of secondary amines with the α-methylene group of germacranolides (9, 45, 86)

The third component (MR-4) was isolated as a white crystalline solid. The IR spectrum exhibited absorptions at 3620 (hydroxyl), 1777 (γ-lactone) and 1734 cm^{-1}(ester). The parent peak at *m/z* 324 was appropriate for the molecular formula $C_{17}H_{24}O_6$, and the fragment at *m/z* 264, $(M-HOAc)^+$, suggested the presence of an acetoxyl group. The ^{1}H-NMR spectrum (Experimental) and ^{13}C-NMR spectrum (Table 4), along with the information above, suggested that MR-4 was a dihydroparthenolide containing hydroxyl and acetoxyl substituents. In the ^{1}H-NMR spectrum, H-1 appears as a sharp doublet because of coupling with H-2β (J = 10.2 Hz). Of the two resonances at 4.92 and 4.70 ppm, the more deshielded proton should be attached to the carbon bearing the acetoxyl group, and, consequently, the authors would like to propose that the hydroxyl group is at the 2α position and the H-2β resonance is at δ4.70 ppm. Placement of the acetoxy group at C-8 is consistent with the ^{1}H-NMR spectrum, but, more conclusively, this location results in the anticipated shifts in the ^{13}C-NMR spectrum relative to that of dihydroparthenolide (87). The C-8 resonance is down-field by 42.4 ppm (α effect), the C-7 and C-9 resonances are downfield by 4.8 ppm (β effect), and the C-6 resonance is shifted upfield by 3.5 ppm (γ effect). The 8-acetoxyl group is placed in the alpha position by analogy to the ^{1}H-NMR spectrum of the closely related 8α-acetoxydihydroparthenolide (26). In the latter compound the H-8β resonance appears at 4.90, while in MR-4 it is found at 4.92 ppm. In 8-acetoxygermacranolides in which H-8 is alpha, this resonance appears much further downfield at about 5.7 ppm (88). The very

significant deshielding of the C-13 methyl group in MR-4 relative to its position in dihydroparthenolide (δ 18.3 VS δ 13.2, respectively) suggests a *syn*-orientation of this methyl and the acetoxyl group (i.e., both groups alpha). Thus, it is proposed component MR-4 is 8α-acetoxy-2α-hydroxydihydroparthenolide, for which the author suggested the name paramicholide. For a related discussion of these assignments, see Jakupovic *et al* (89).

In previous reports on the ^{13}C-NMR spectra of germacranolides, the resonances assigned to C-2, C-3, C-8 and C-9 are usually indicated as being interchangeable (90). Comparison of the spectrum of dihydroparthenolide with those of the oxygenated derivative MR-4 enabled to assign each of these resonances for this family of compounds. Clearly, the resonances at δ 24.0 and δ 36.6 ppm in dihydroparthenolide must be assigned to C-2 and C-3 because introduction of a hydroxyl group at C-9 has little effect on the chemical shifts of these two carbons. The resonance at 36.6 ppm is to C-3 because of the deshielding effect of the neighboring epoxide function. The resonance at 41.1 ppm in dihydroparthenolide is attributed to C-9 because a methylene carbon adjacent to a methyl group on a methyl group on a trisubstituted *trans* double bond, *trans*-(-CH = C(CH_3) CH_2-), has been shown to be significantly deshielded relative to the other allylic carbon. Also, the chemical shifts predicted for these four carbons in MR-4 based on the α ,β and γ effects of oxygen substituents on the model system dihydroparthenolide are in excellent agreement with the actual values.

The fourth compound (MR-6) was isolated as white needle crystal. Its EIMS spectrum exhibited a molecular ion at *m/z* 307 corresponding

to the molecular formula $C_{17}H_{25}NO_4$. The IR spectrum exhibited absorption bands at 3400, 1769 and 1670 cm^{-1}, suggested the presence of N-H, γ-lactone and amide respectively. The ^{1}H-NMR (400 MHz) spectrum of MR-6 indicated similar pattern to that of parthenolide. A broad signal at δ 6.31 and singlet at δ 2.02 were assigned to N-H and $\overset{O}{\overset{\|}{C}}-CH_3$ respectively. This was confirmed by ^{13}C-NMR that two carbon signals indicated the N-acetyl of parthenolide nucleus. Confirmation of the chemical shift of the C_1' and C_2' resonances by using APT technique showed that an oxygenated sp^2 carbon at δ 170.7 ppm indicated C_1' carbonyl and δ 23.9 ppm indicated C_2' methyl. Differentiations of C_{11} and C_{13} with those of parthenolide were remarkable. It was shown for C_{11} and C_{12} resonances in MR-6 as sp^3 carbon while in parthenolide was a sp^2 carbon. Thus, it is concluded that MR-6 is N-acetylparthenolidine, a new germacranolide.

The fifth component (MR-7) was isolated as colourless crystalline solid. The IR spectrum suggested the presence of a γ-lactone (1968), a hydroxyl group (3420-3240), a N-H group (3500) and an amide (1658 cm^{-1}). The molecular weight was 323 corresponding to the molecular formula $C_{17}H_{25}NO_5$. The ^{1}H-NMR (400 MHz) spectral data was similar to those obtained from MR-6 except for a few points as indicated in the following. Hydroxyl signal appeared as doublet while NH appeared as a doublet of doublet (coupling with H_8 and H_{13a}, H_{13b} respectively). Study on ^{13}C-NMR spectrum showed significant chemical shift of C-8 to lower field at 72.3 ppm indicating a hydroxyl group attached to this carbon. Thus, it is concluded that MR-7 is N-acetyl-8α-hydroxyparthenolidine, also a new germacranolide.

The sixth component (MR-8) and most polar component was a high-melting, yellow, crystalline solid. Its EIMS exhibited a strong

molecular ion at *m/z* 217 ($C_{17}H_9NO_3$) and its fragmentation pattern was similar to that reported for the alkaloid liriodenine. Also, the IR and UV spectra (91) for this component were the same as those previously reported for liriodenine. The ^{1}H-NMR (DMSO-d_6 or TFA (92)) and ^{13}C-NMR spectra (93) of MR-8 have been reported previously and are in agreement with these spectra. Liriodenine has limited solubility in chloroform but in experimental the author reported the 400 MHz ^{1}H-NMR spectra of MR-8 in very dilute solution of $CDCl_3$ and in 10% DMSO-d_6/$CDCl_3$ solution as previous NMR reports only assigned some of the protons. To assign all the aromatic protons a 2D-COSY experiment(10% DMSO/$CDCl_3$) was performed. It showed clearly that the doublet for H(11) at 8.72 was coupled to the triplet for H(10) at 7.77 and that this latter proton was also coupled with H(9) at 7.58 ppm. The remaining downfield doublet for H(8) at 8.57 ppm was also coupled with H(9). This spectroscopic data unambiguously established that the sixth component was the oxoaporphinoid alkaloid liriodenine. Liriodenine has previously been reported to be present in a number of different genera of Magnoliaceae(see table 1 p.9).

Sesquiterpene lactones are common constituents of most genera of the Compositae, with the exception of the evolutionary "advanced" tribe, the Tagetae. Eudesmanolides, similar to those found in the genus *Inula* (Compositae), are also present in the liverworts (Hepaticae), *Frullania tamarisci* (80, 81). This present investigation has led to isolation and characterization of eudesmanolides occurring in *Grangea maderaspatana* (Compositae), a well known indigenous drug of Thailnad. Three eudesmanolides were found to form *cis*-lactone ring junction.

The first component (GM-1) was isolated as a crystalline solid which exhibited IR spectrum at 1767cm^{-1} (γ-lactone). Its EIMS showed a molecular ion at *m/z* 232 corresponding to the molecular formula $C_{15}H_{20}O_2$. Evidences from the ^{1}H-NMR spectrum (400 MHz) have shown that GM-1 was *cis*-lactone. The major differences from *trans*-lactone were in the coupling of the exomethylene protons (δ6.14, 5.57, both d, *J* 1Hz in *cis*-lactone, δ 6.13, 5.43, both d, *J* 3 Hz (81) in *trans*-lactone) and in the signal for H-6 which appeared as a doublet at δ 5.26 *J* 8.9 Hz for GM-1 while a diffuse doublet at higher field (δ 4.54 for *trans*-lactone (81)). Irradiation at δ 5.26 (H-6) changed the signal at δ 2.95 (H-7) to a broadened triplet consistent with the presence of an adjacent methylene group. The reverse experiment caused H-6 to collapse to a broad singlet. The residual broadenting of H-6 is due to homoallylic coupling with the vinyl methyl group and this was demonstrated by irradiating H-6 whereupon the intensity of the vinyl methyl signal increased. From the magnitude of this residue coupling the stereochemistry of the lactone ring junction was deduced to be *cis* (81). All these data are consistent with GM-1 being frullanolide (81).

The second component (GM-2) was isolated as crystalline solid. The EIMS showed a molecular ion at *m/z* 248 corresponding to the molecular formula $C_{15}H_{20}O_3$. Infrared absorption bands at 1771 and 3580 cm^{-1} suggested the presence of α-methylene-γ-lactone moiety and hydroxyl group. Both ^{1}H and ^{13}C-NMR spectra showed many similarities to those of frullanolide, with the only significant differences being in the region of C(7). In particular, C(7) in GM-1 was a methine carbon (C-H) (-41.2 ppm) as determined by the ^{13}C attached proton test (APT). The chemical shift of C(7) to lower field (76.0) in GM-2 was consistent with the

presence of a hydroxyl group on that carbon. ^{1}H-NMR signal for the C-6 methine proton appeared as sirglet which suggested the preserce of substituent on C-7. Thus, it is concluded that structure of GM-2 is a new and unusual 7-hydroxy eudesmanolide called 7-hydroxyfrullanolide.

The third component (GM-3) obtained as crystalline solid, its EIMS exhibited molecular ion at *m/z* 266 corresponding to molecular formula $C_{15}H_{22}O_4$. The IR spectrum exhibited absorption bands at 3750 and 1770 cm^{-1} suggested the presence of hydroxyl and γ-lactone respectively. ^{1}H-NMR spectrum was similar to that obtained from GM-2, with the only few points should be discussed. H-13 (3H) appeared to be doublet while H-11 appeared as quartet. This evident was confirmed that at C-11 and C-13 region was dihydrofrullanolide. Methine proton at C-3 appeared as doublet of doublet at δ 4.0 because of the two protons coupling ($H_{2\alpha}$ and $H_{2\beta}$) while carbon resonance at-69.6 ppm suggested the introduction of hydroxyl group on this carbon. Thus, it is concluded that GM-3 was 3α-, 7α-dihydroxy dihydrofrullanolide, a new and unusual eudesmanolide.

CHAPTER V

CONCLUSION AND RECOMMENDATION

The present investigation was performed on two species of cytotoxic indigenous plants of Thailand; *Michelia rajaniana* Craib and *Grangea maderaspatana* Poir. Results have shown that the isolated sesquiterpene lactones were characterized as germacranolides in the former species and eudesmanolides in the latter one, most of which were new natural products. This is the first report of germacranolide occurring amide and eudesmanolide having unusual 7-OH from natural sources. From chemotaxonomic and biogenetic point of view, it is worth to infer that these isolates are appropriate models for further studies.

Although this work has offered some insight into the occurrence of various sesquiterpene lactones in *Michelia rajaniana* and *Grangea maderaspatana*, further work is required with much larger scale of plant materials in order to obtain sufficient quantities of components which are considered important for biological test.

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APPENDIX

a) silica gel GF_{245}/benzene : acetone (1:1)

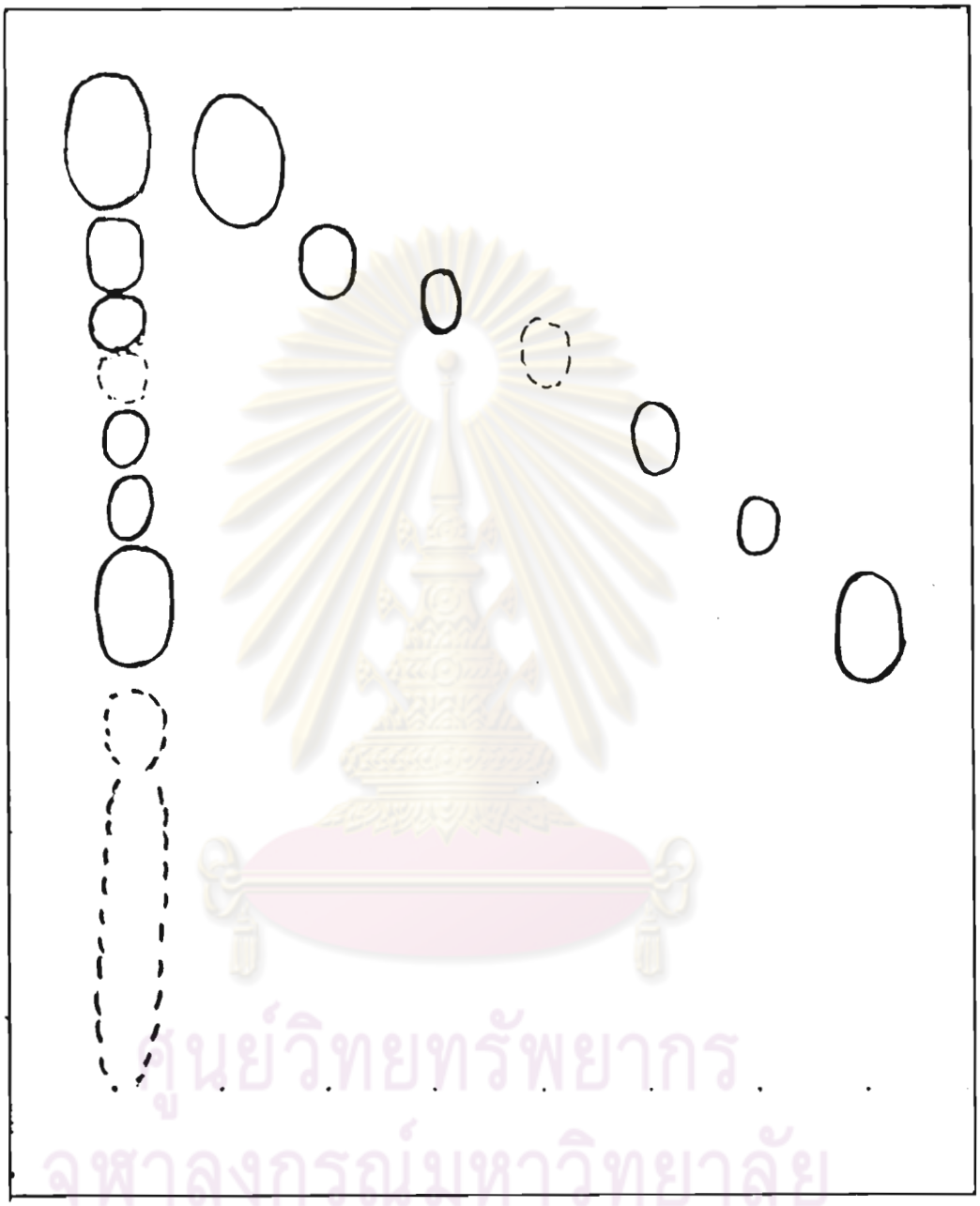


Figure 3 Thin-layer chromatogram of isolated compounds from *Michelia rajaniana* Craib. stem bark.

Note: After warmed, MR-1 - MR-6 gave yellow color ,MR-7 gave pale pink colour. These colours are indicated a germacranolide group of sesquiterpene lactone.

(the mixture of 2% resorcinol in methanol and 2% sulphuric acid as spraying reagent)

b) silica gel GF_{254}/benzene : acetone (4:1)

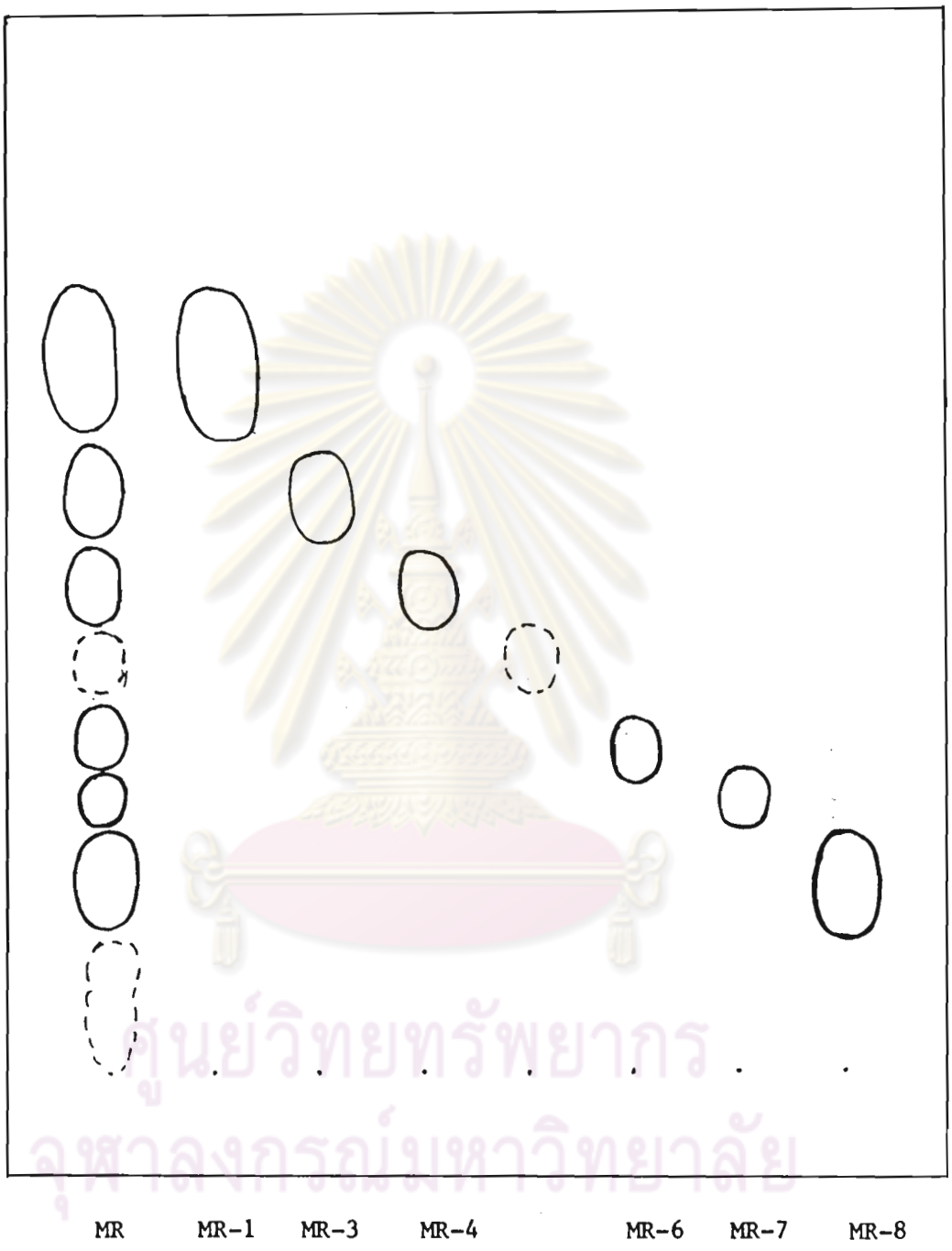


Figure 4 Thin-layer chromatogram of isolated compounds from *Michelia rajaniana* Craib. stem bark.

c) silica gel GF_{254}/ benzene : ethylacetate (4:1)

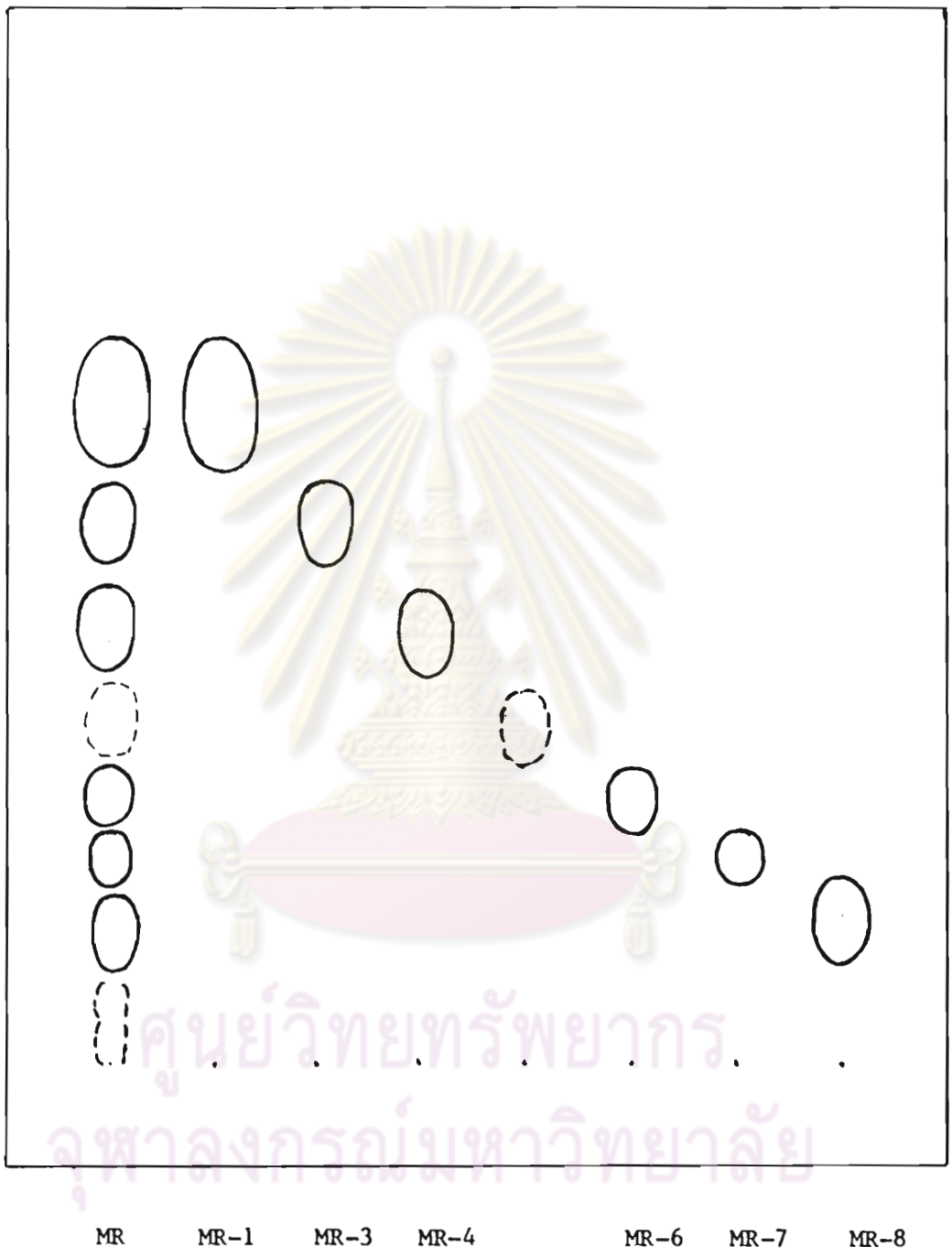


Figure 5 Thin-layer chromatogram of isolated compounds from *Michelia rajaniana* Craib.stem bark.

d) silica gel GF_{254}/benzene : ethylacetate (1:2)

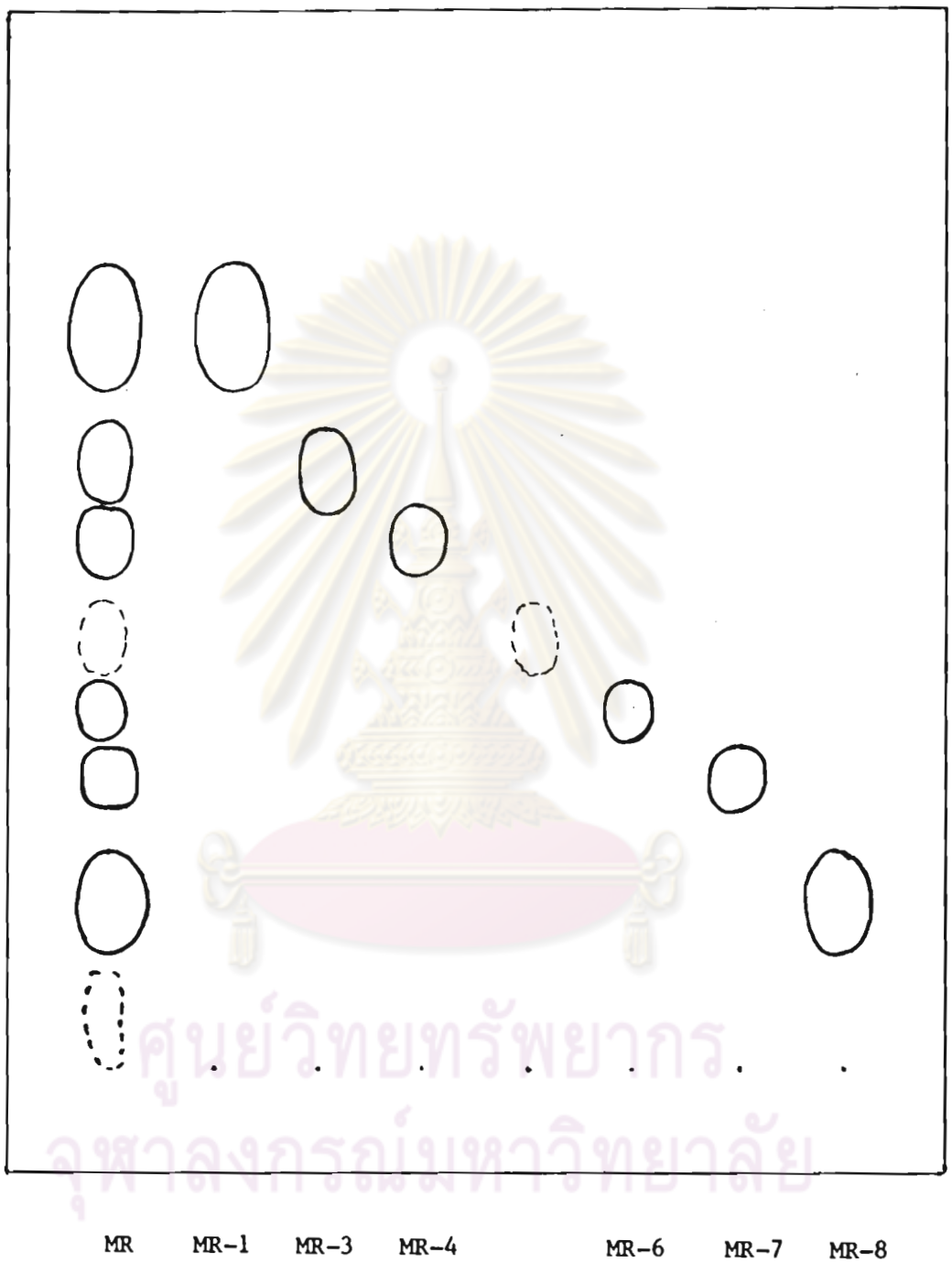


Figure 6 Thin-layer chromatogram of isolated compounds from *Michelia rajaniana* Craib.stem bark.

e) silica gel GF_{254}/chloroform : acetone (5:1)

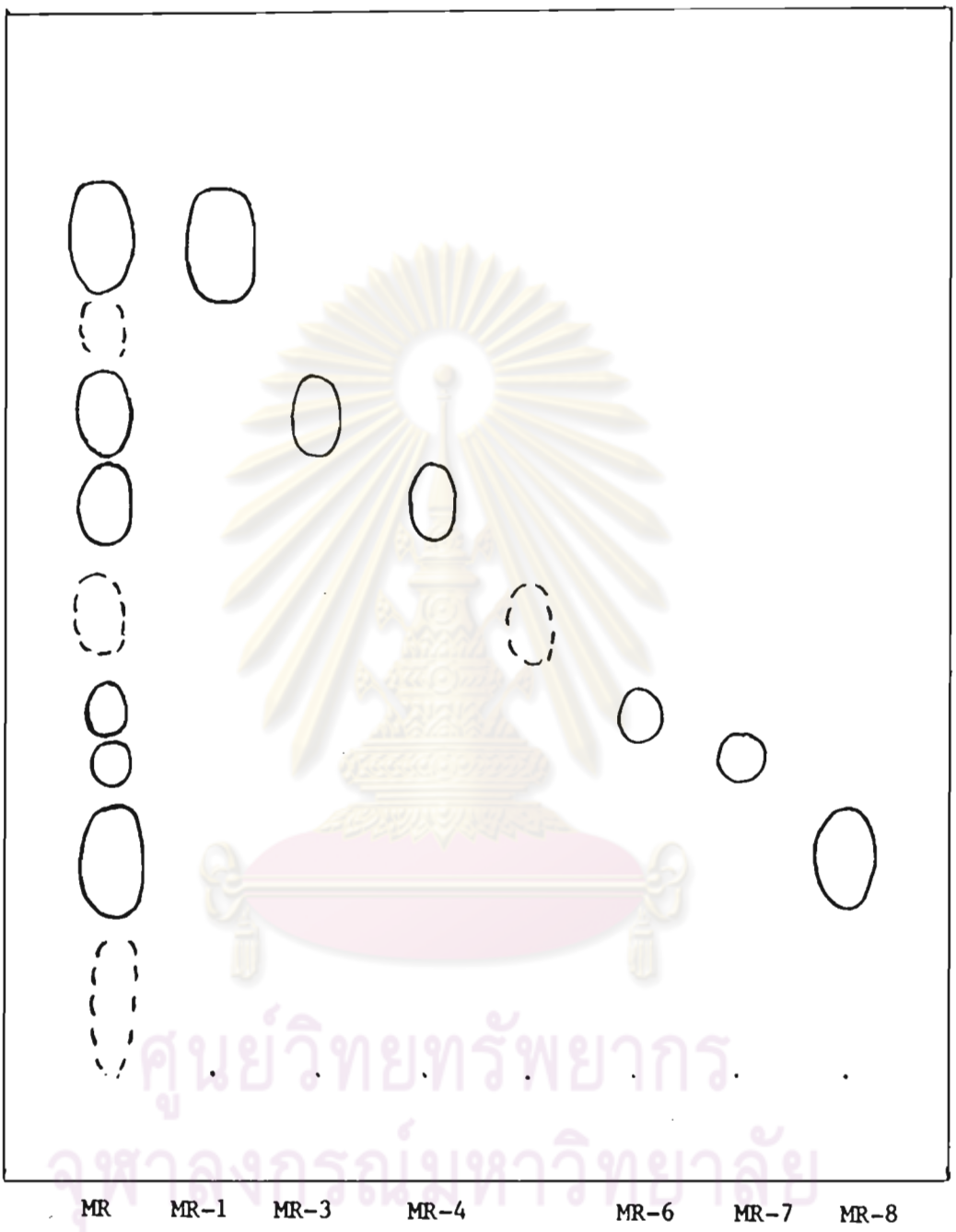


Figure 7 Thin-layer chromatogram of isolated compounds from *Michelia rajaniana* Craib.stem bark.

f) silica gel GF_{254}/ethylacetate : acetone (1:1)

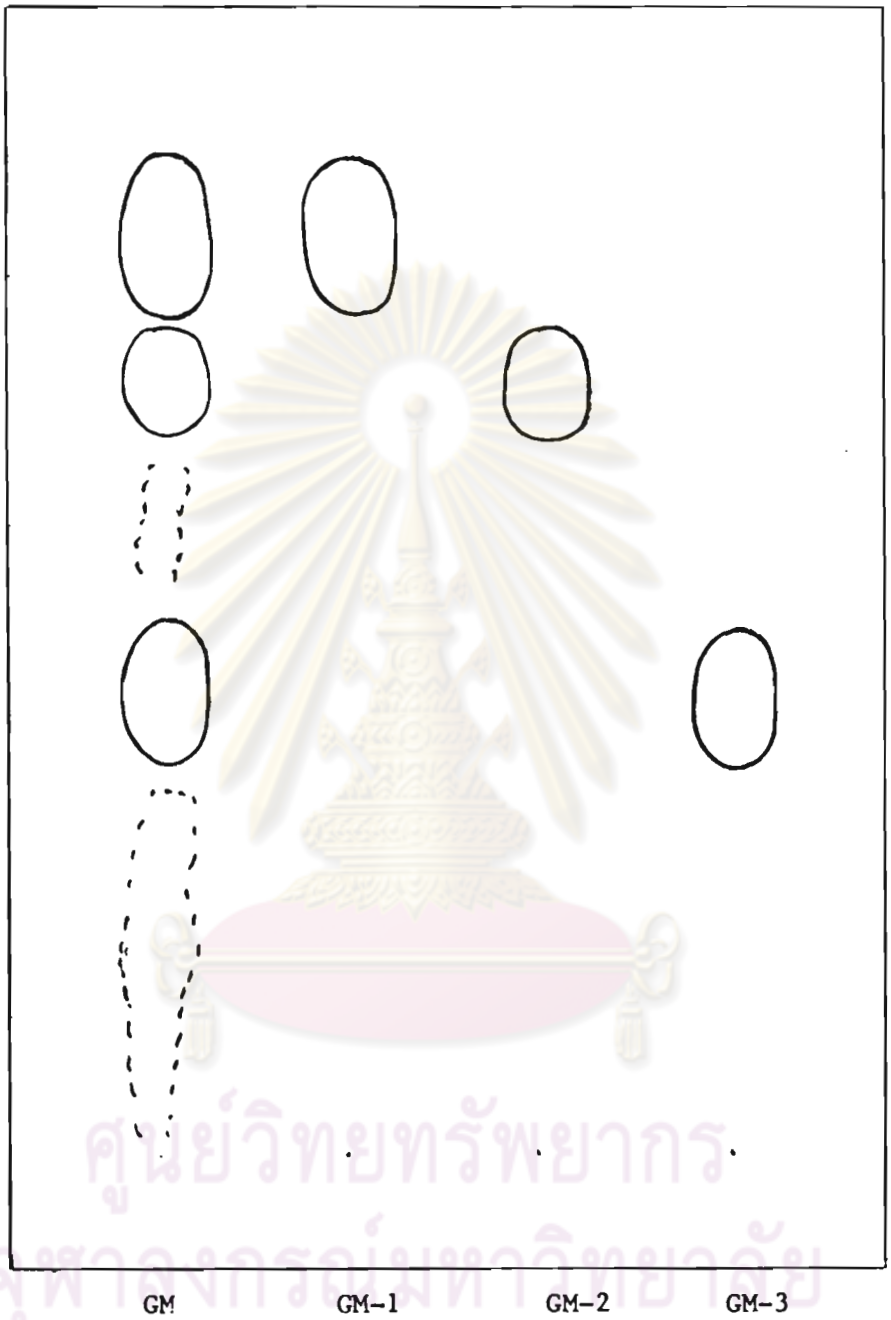


Figure 8 Thin-layer chromatogram of isolated compounds from *Grangea maderaspatana* Poir.

Note: After warmed, GM-1 gave purple colour, GM-2 and GM-3 gave violet colour. These colour are indicated a eudesmanolide group of sesquiterpene lactone, (the mixture of 2% resorcinol in methanol and 2% sulphuric acid as spraying reagent)

g) silica gel GF_{254}/benzene : acetone (1:1)

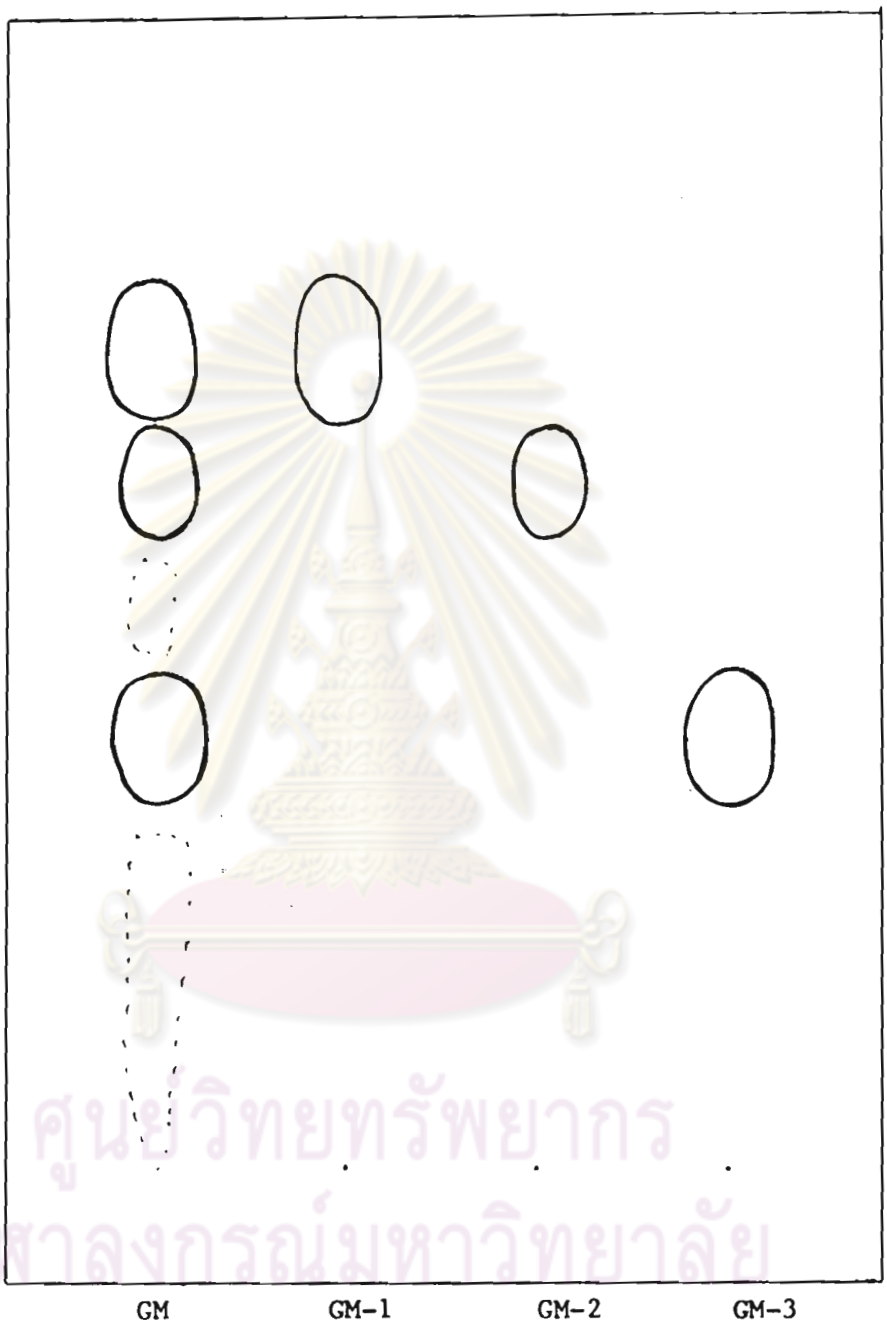


Figure 9 Thin-layer chromatogram of isolated compounds from *Grangea maderaspatana* Poir.

h) silica gel GF_{254}/benzene : acetone (4:1)

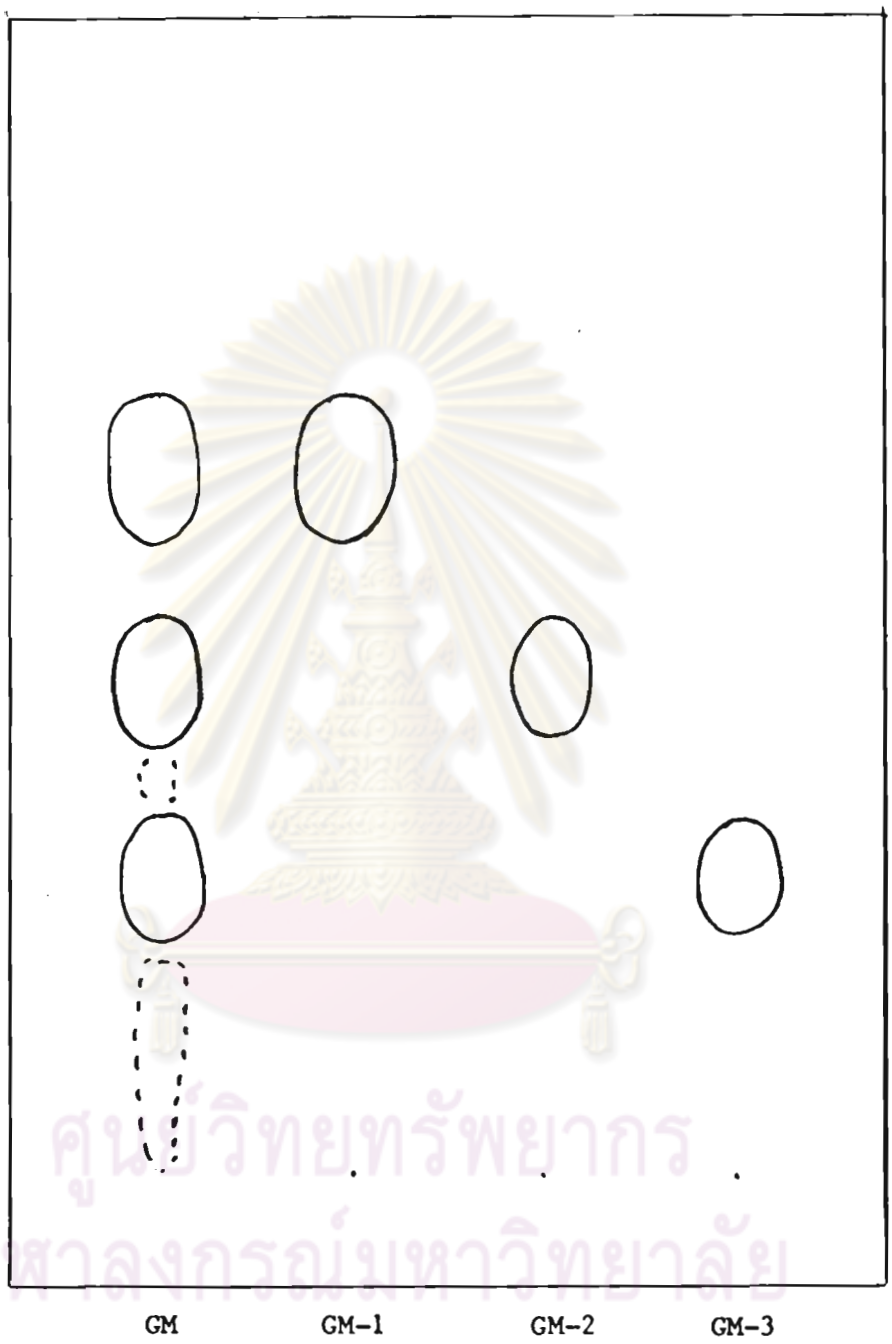


Figure 10 Thin-layer chromatogram of isolated compounds from *Grangea maderaspatana* Poir.

i) silica gel GF_{254}/benzene : ethylacetate (4:1)

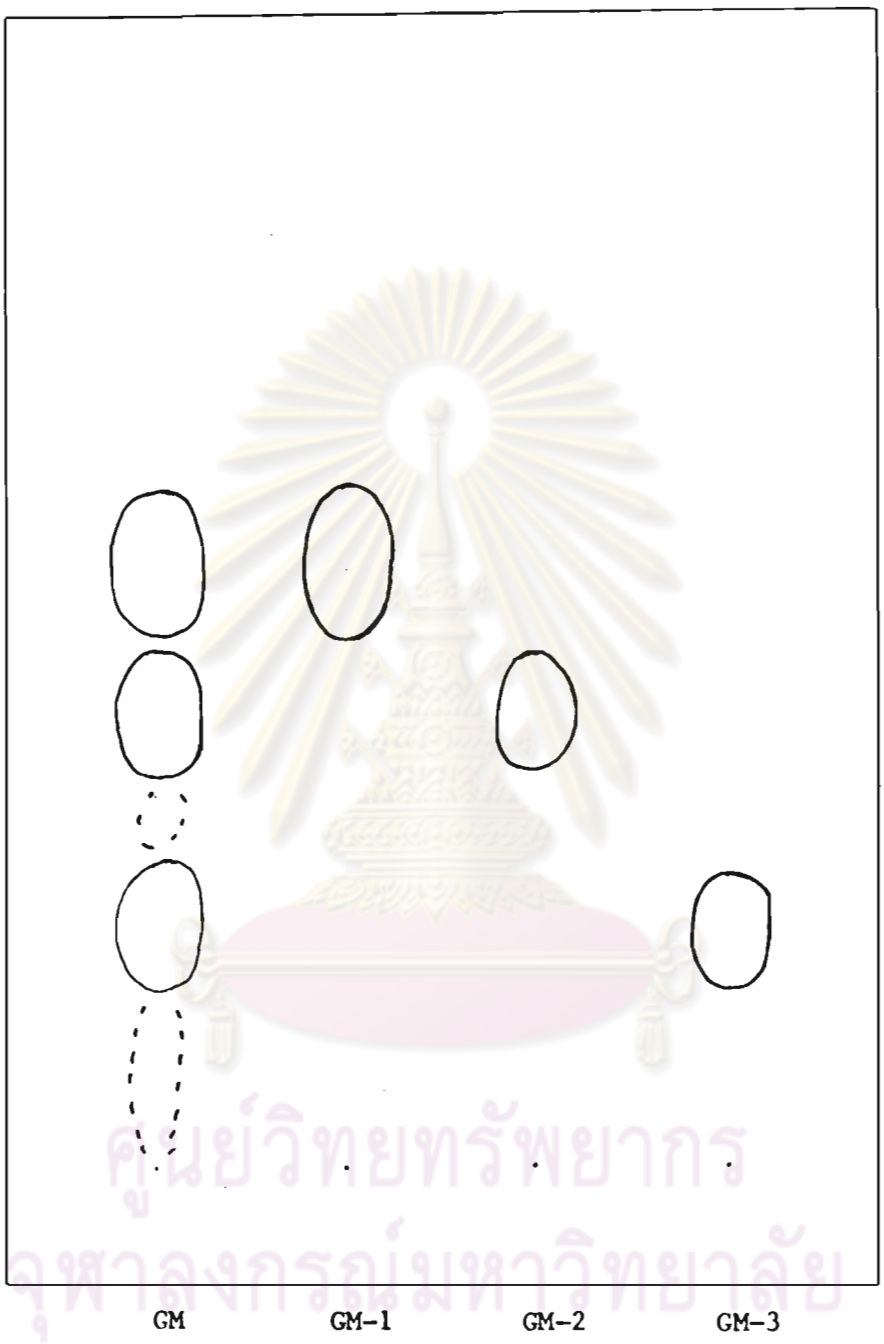


Figure 11 Thin-layer chromatogram of isolated compounds from *Grangea maderaspatana* Poir.

j) silica gel GF_{254}/benzene : ethylacetate (1:2)

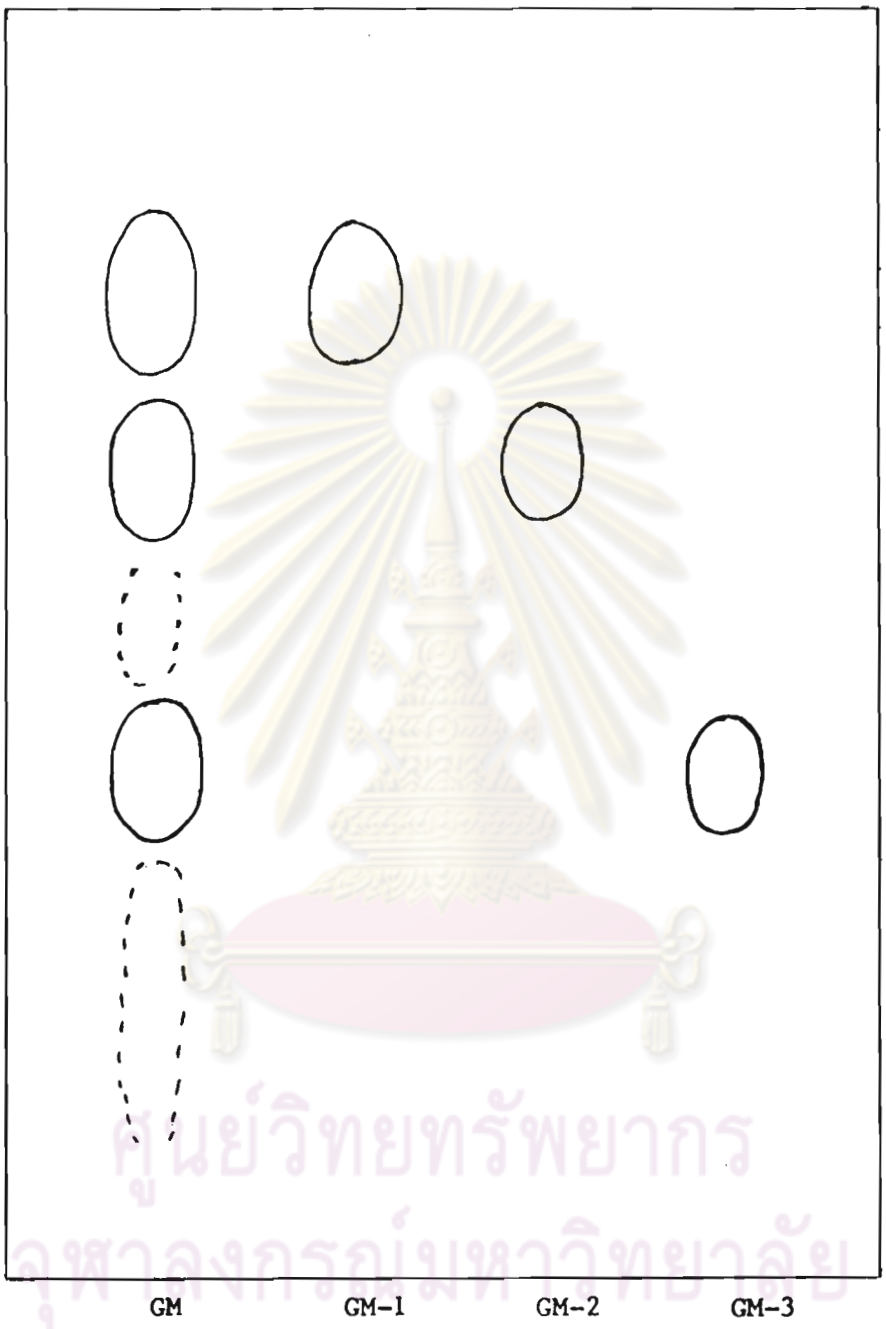


Figure 12 Thin-layer chromatogram of isolated compounds from *Grangea maderaspatana* Poir.

k) silica gel GF_{254}/chloroform : acetone (5:1)

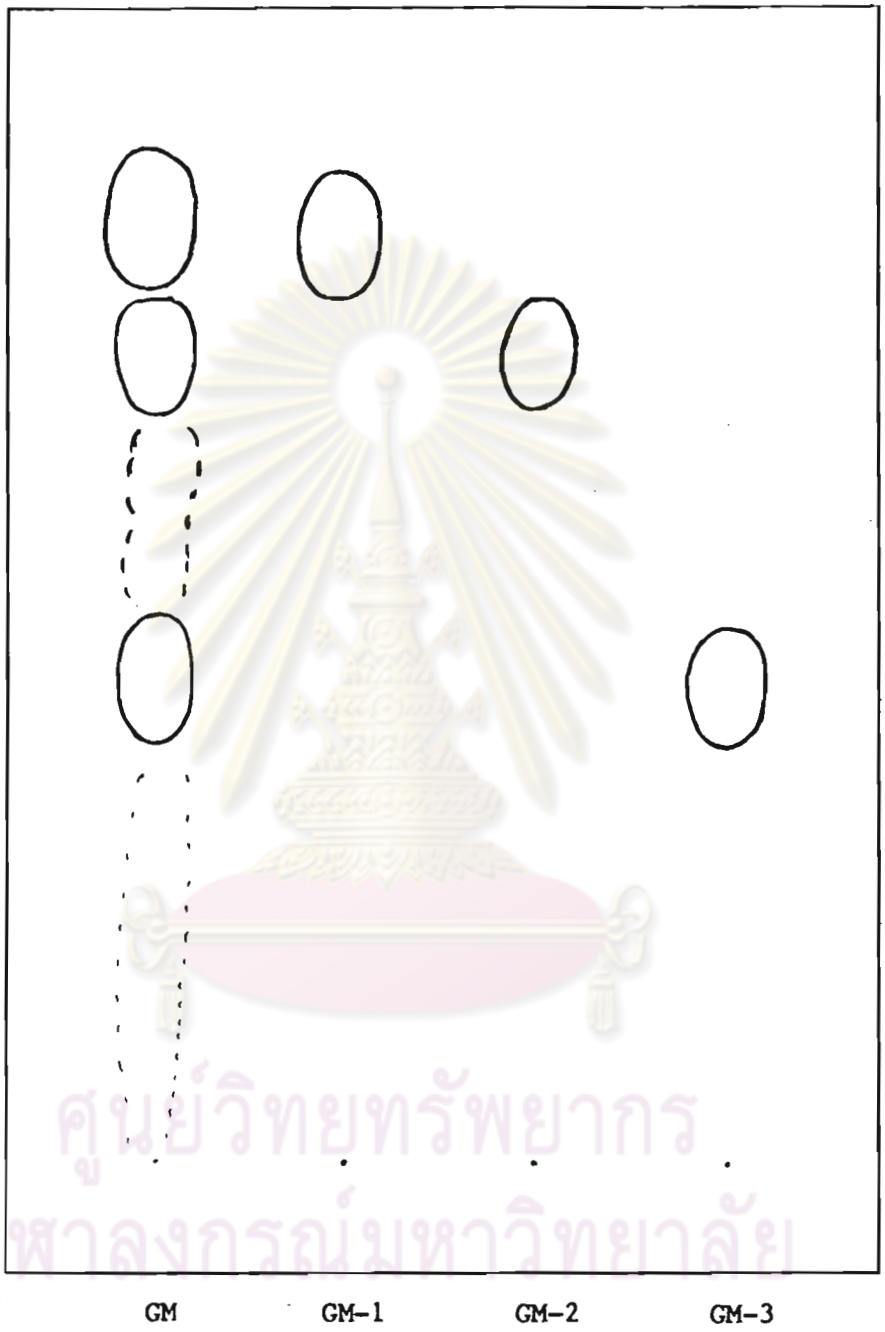


Figure 13 Thin-layer chromatogram of isolated compounds from *Grangea maderaspatana* Poir.

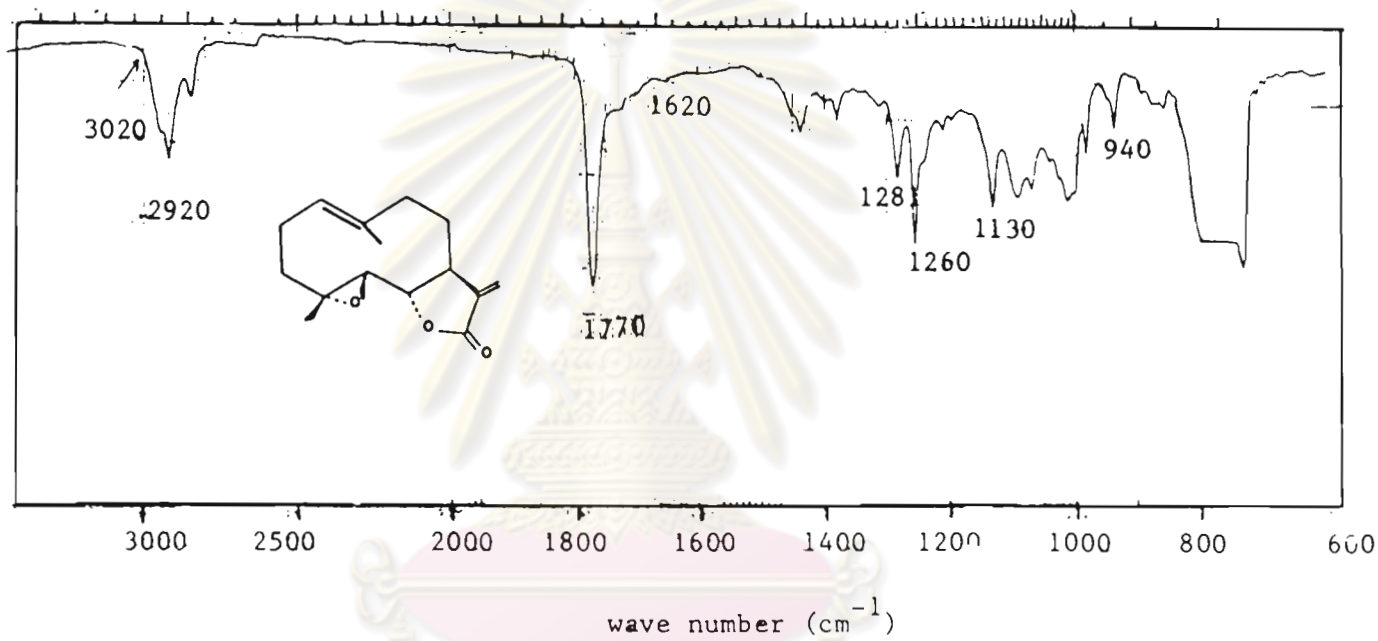


Figure 14 Infrared absorption spectrum of MR-1 from *Michelia rajaniana* Craib.stem bark in CCl_4.

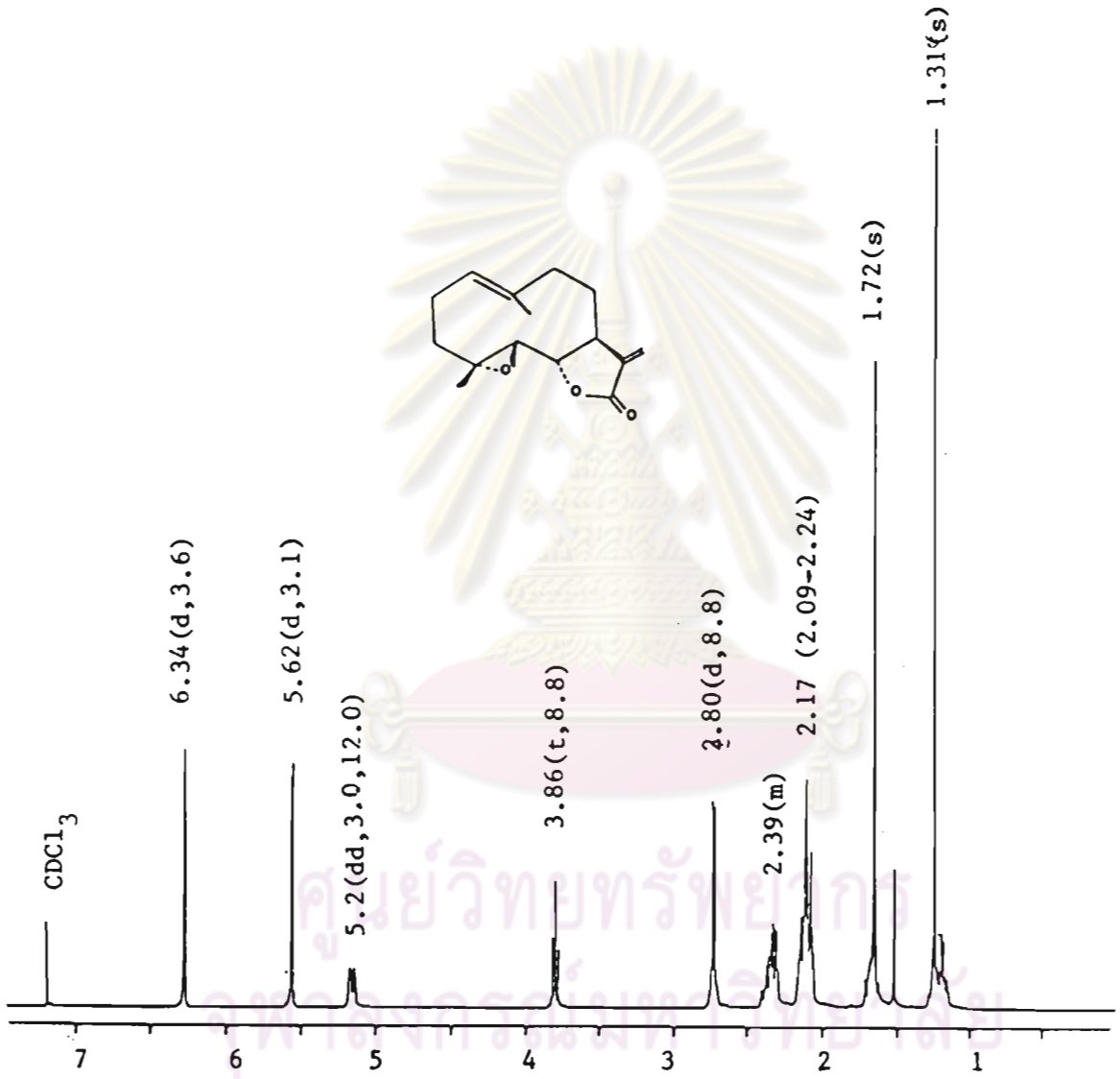


Figure 15 Proton NMR spectrum of MR-1 from *Michelia rajaniana* Craib, stem bark in $CDCl_3$

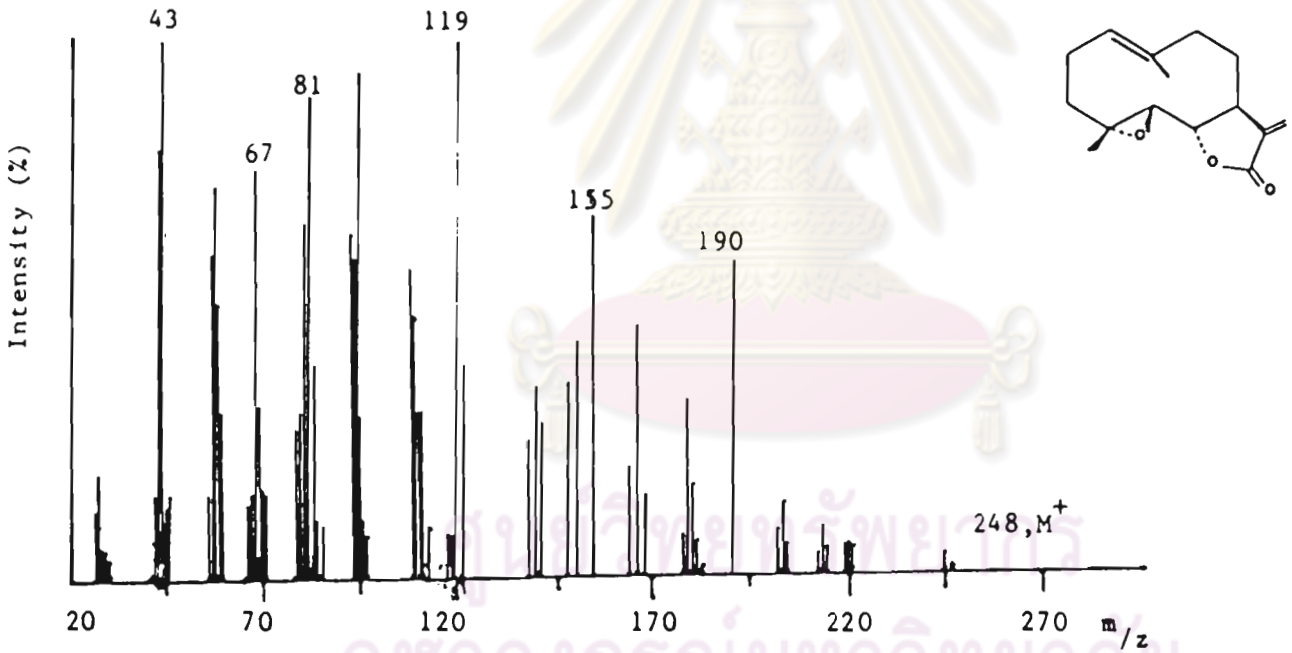


Figure 16 Mass spectrum of MR-1 from *Michelia rajaniana* Craib.stem bark.

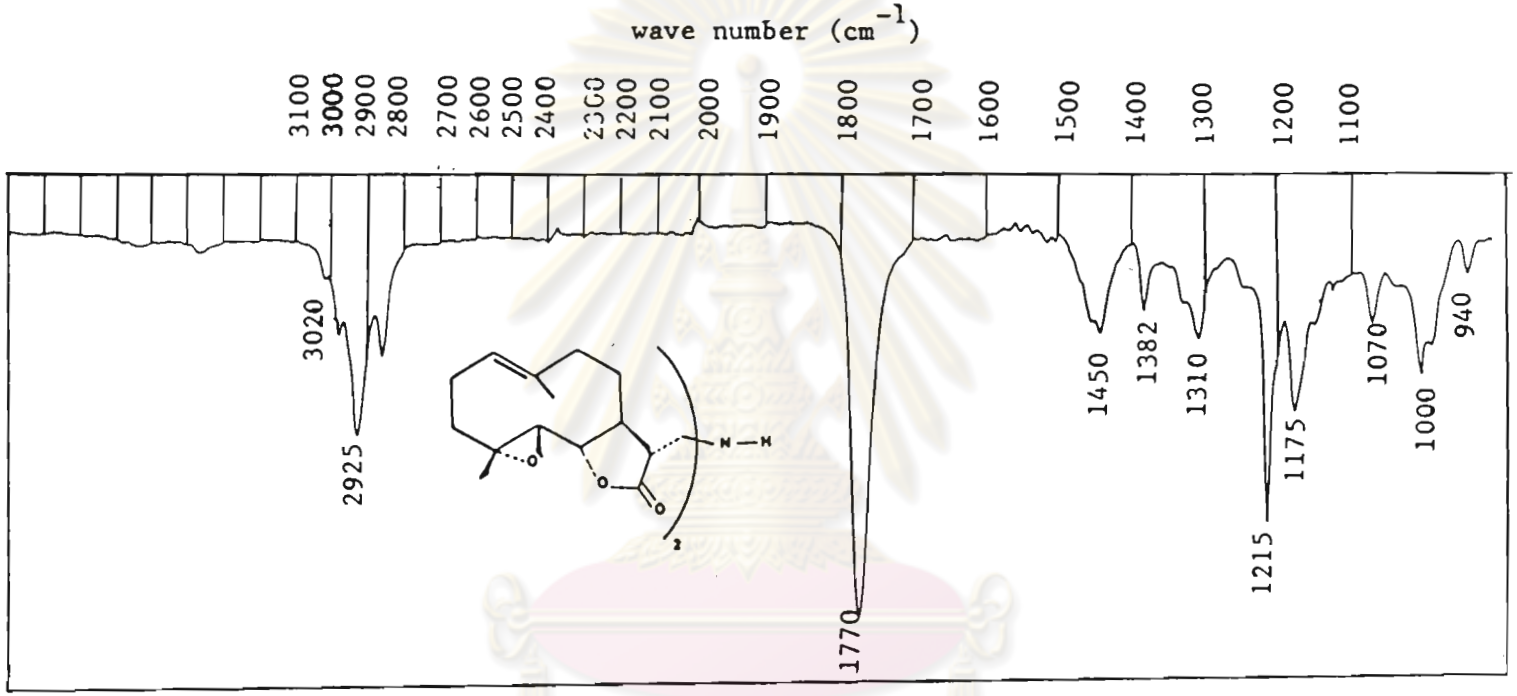


Figure 17 Infrared absorption spectrum of MR-3 from *Michlia rajaniana* Craib.stem bark in CCl_4.

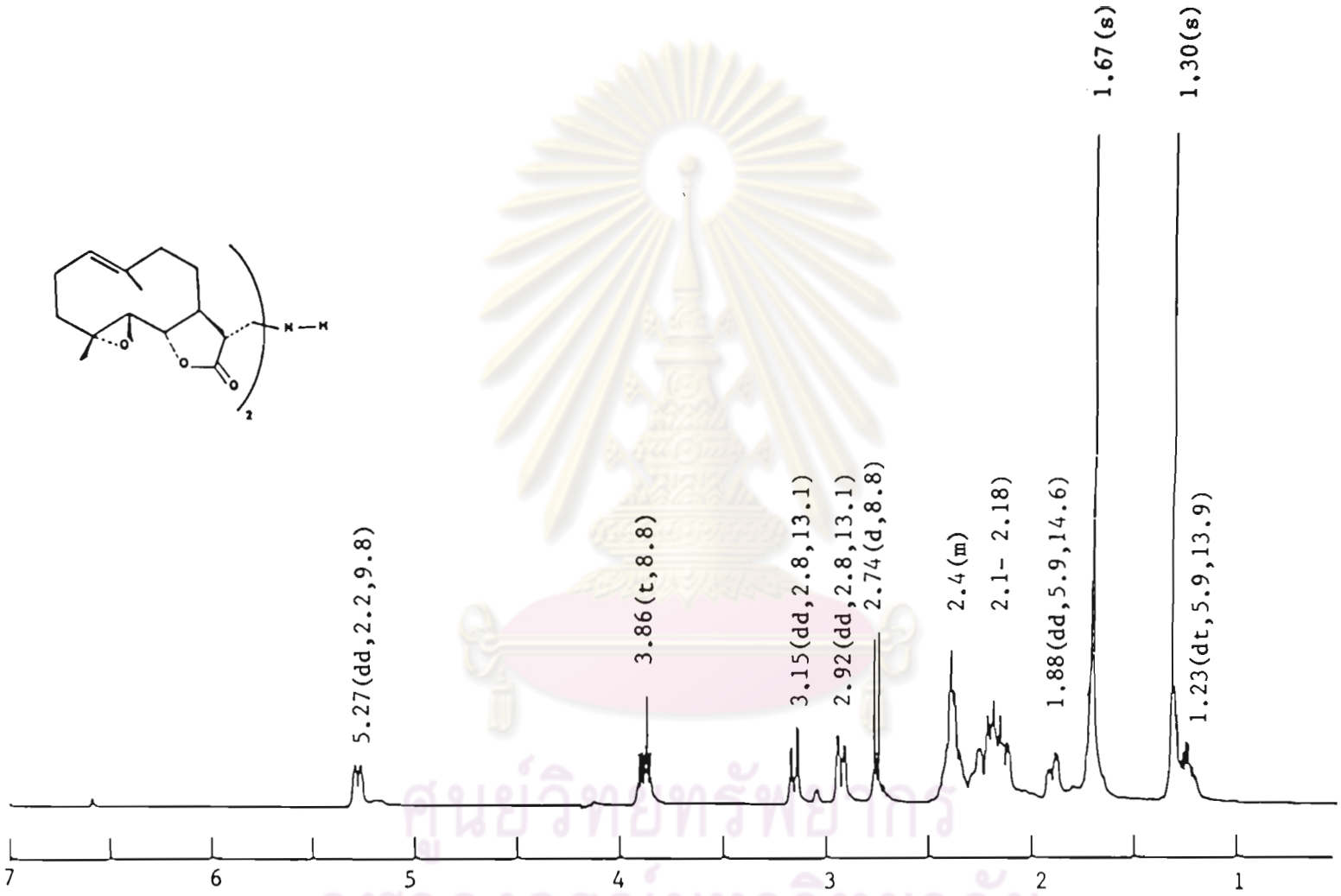


Figure 18 Proton NMR spectrum of MR-3 from *Michelia rajaniana* Craib,stem bark in $CDCl_3$.

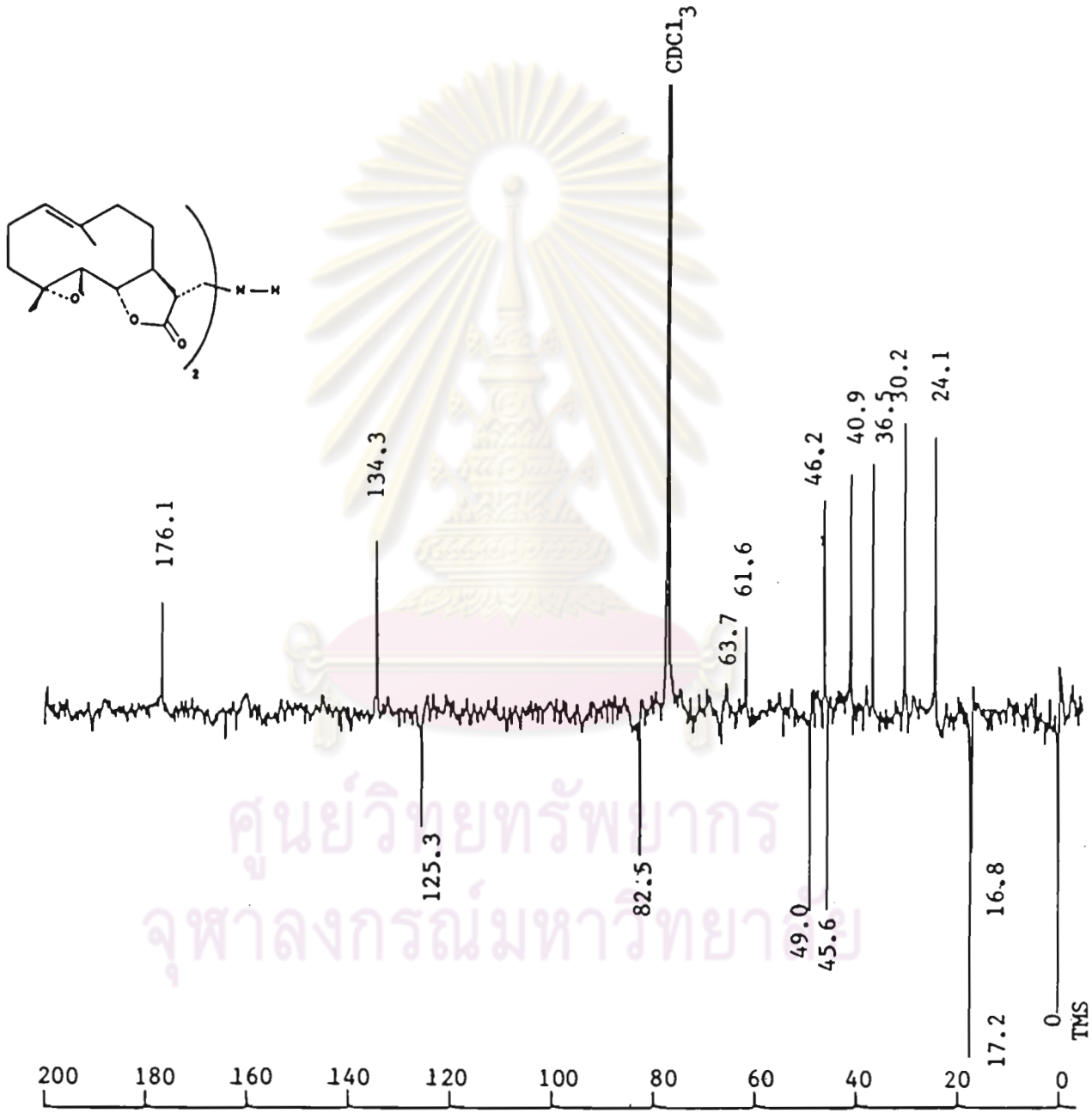


Figure 19 Carbon-13 spectrum of MR-3 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

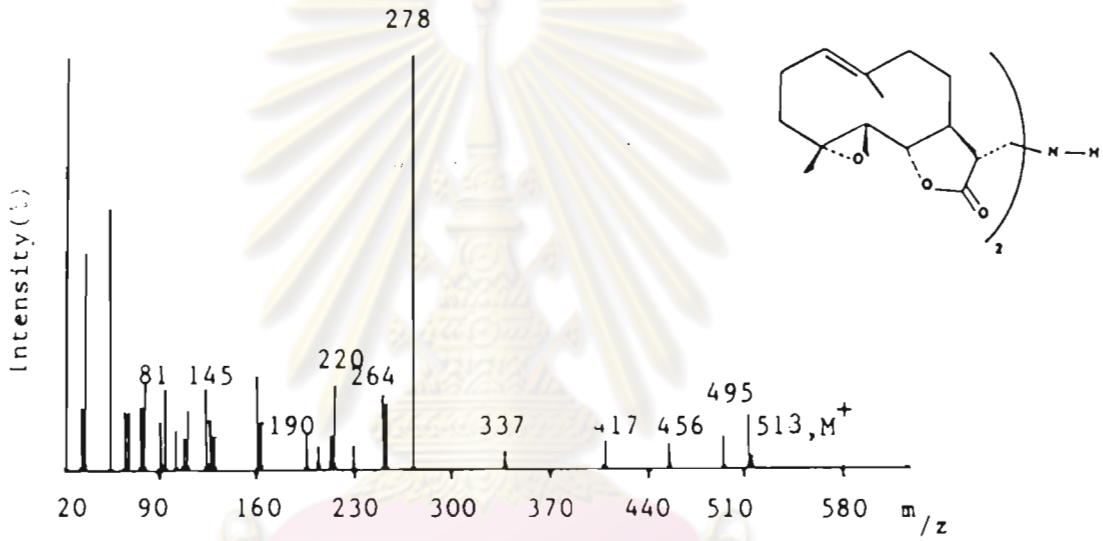


Figure 20 Mass spectrum of MR-3 from *Michelia rajaniana* Craib.stem bark.

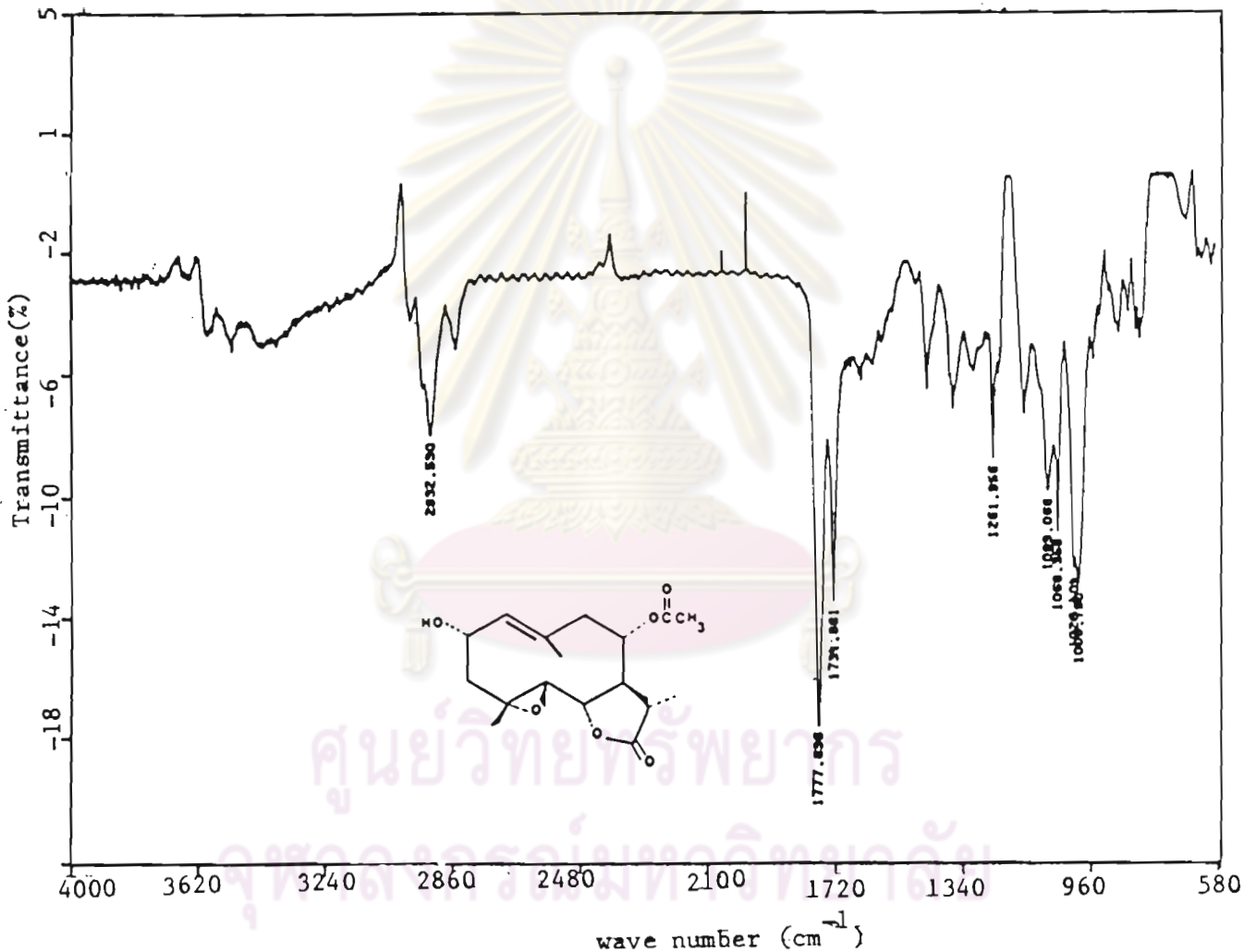


Figure 21 Infrared spectrum of MR-4 from *Michelia rajaniana* Craib.stem bark in $CHCl_3$.

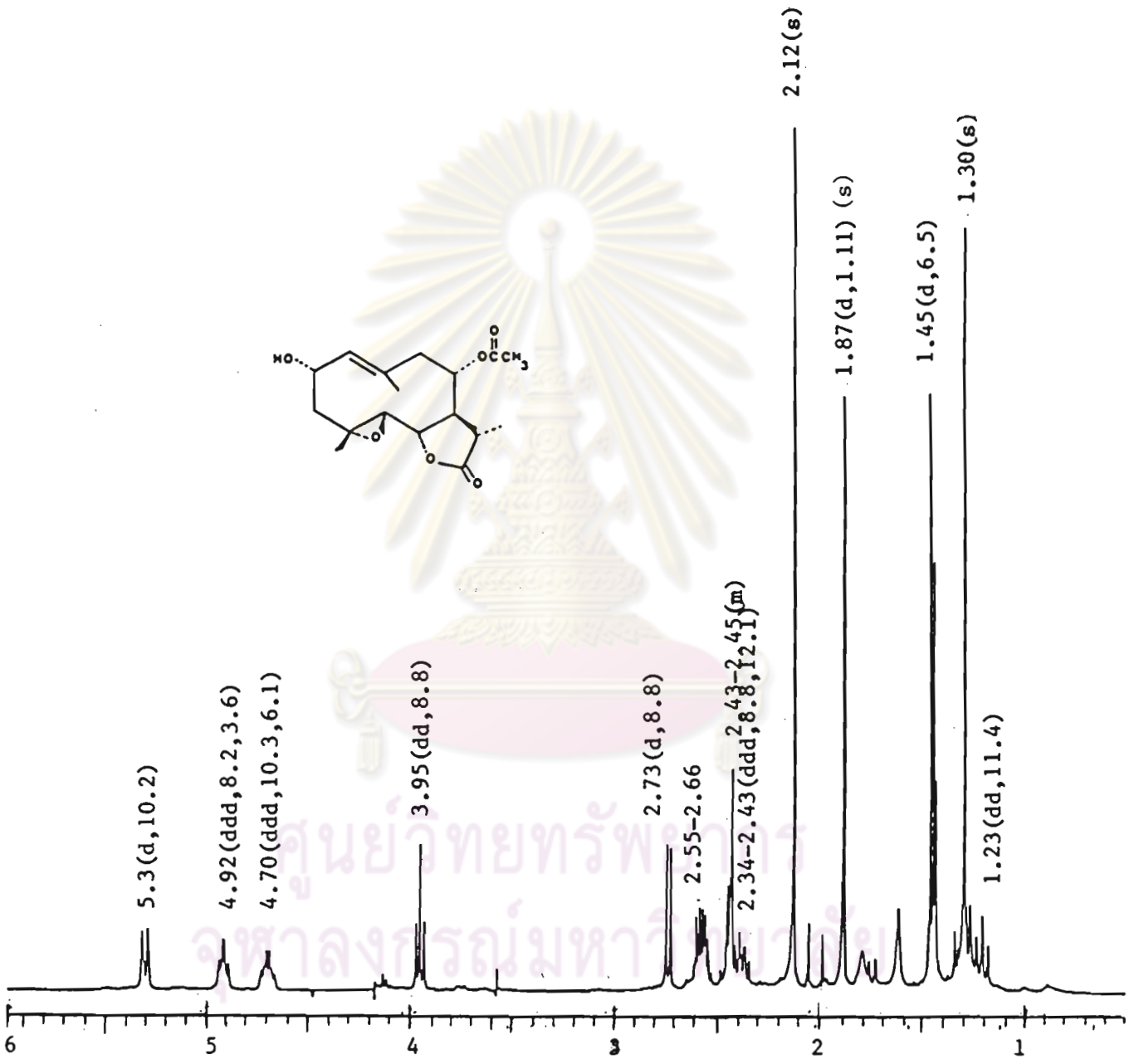


Figure 22 Proton NMR spectrum of MR-4 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

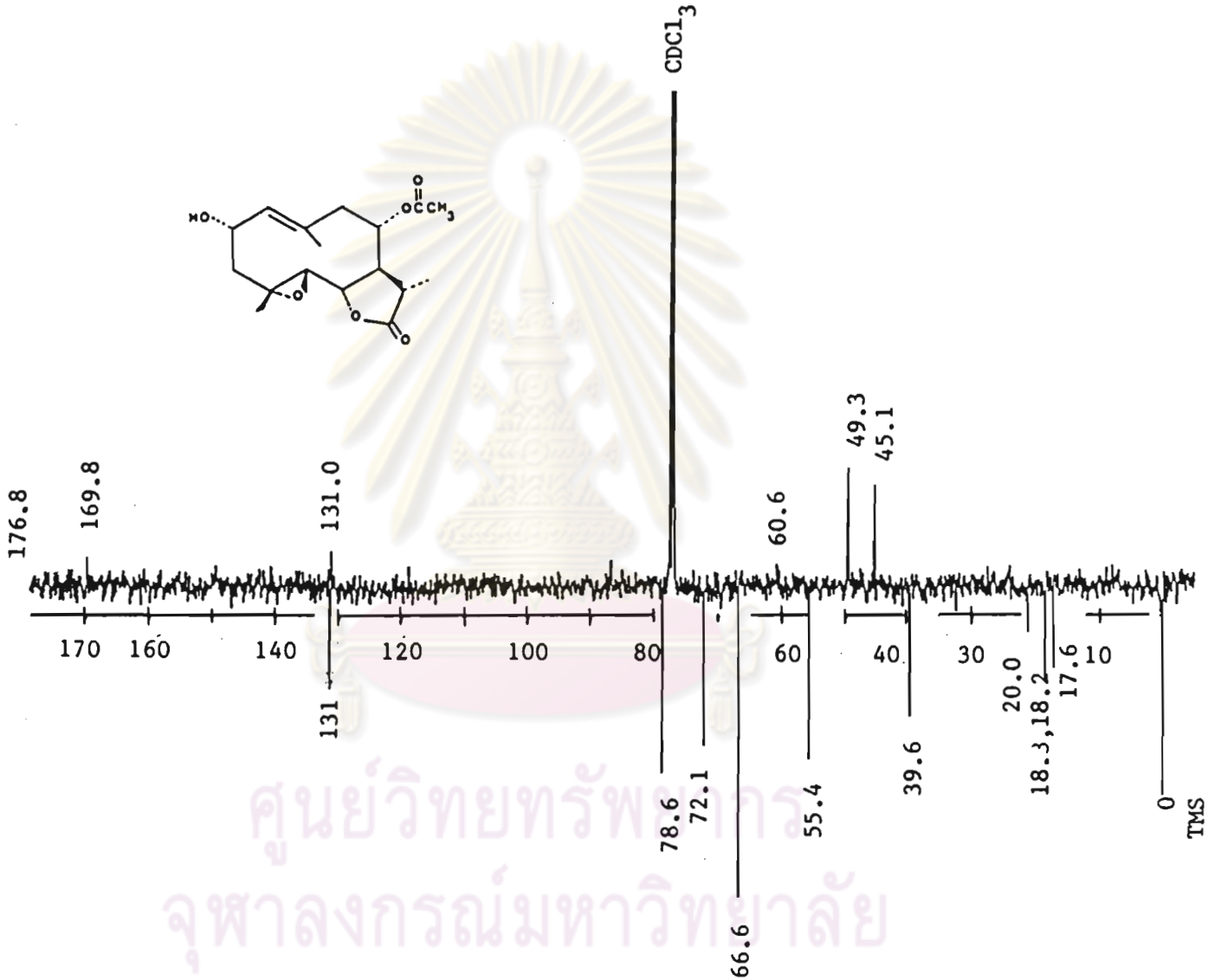


Figure 23 Carbon-13 NMR spectrum of MR-4 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

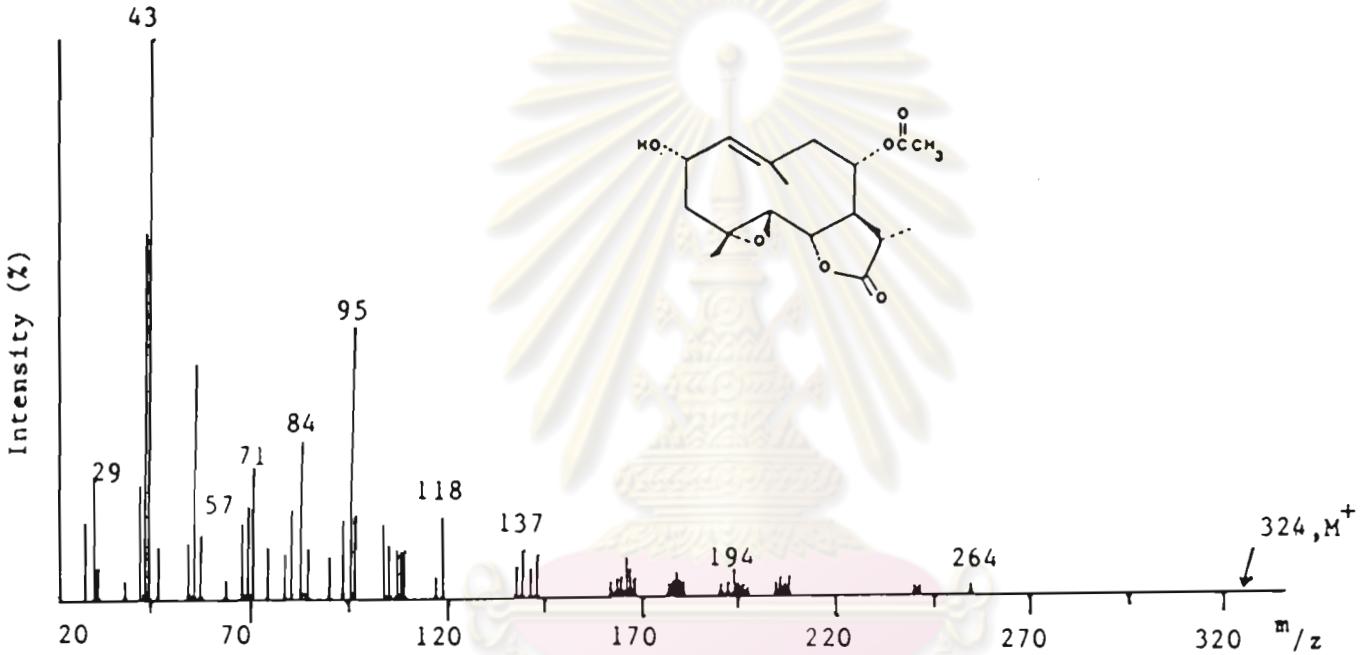


Figure 24 Mass spectrum of MR-4 from *Michelia rajaniana* Craib.stem bark.

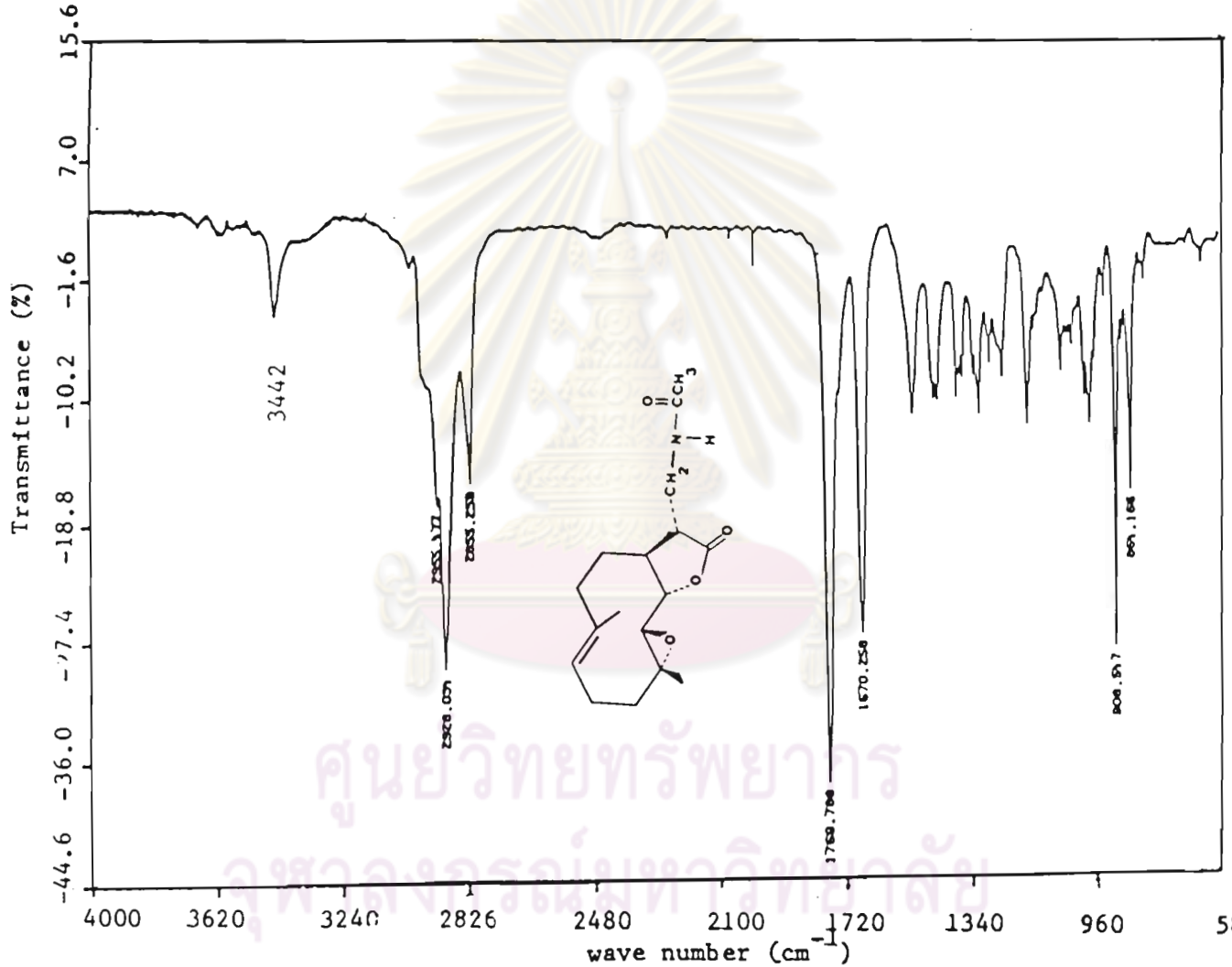


Figure 25 Infrared spectrum of MR-6 from *Michelia rajaniana* Craib. stem bark in $CHCl_3$.

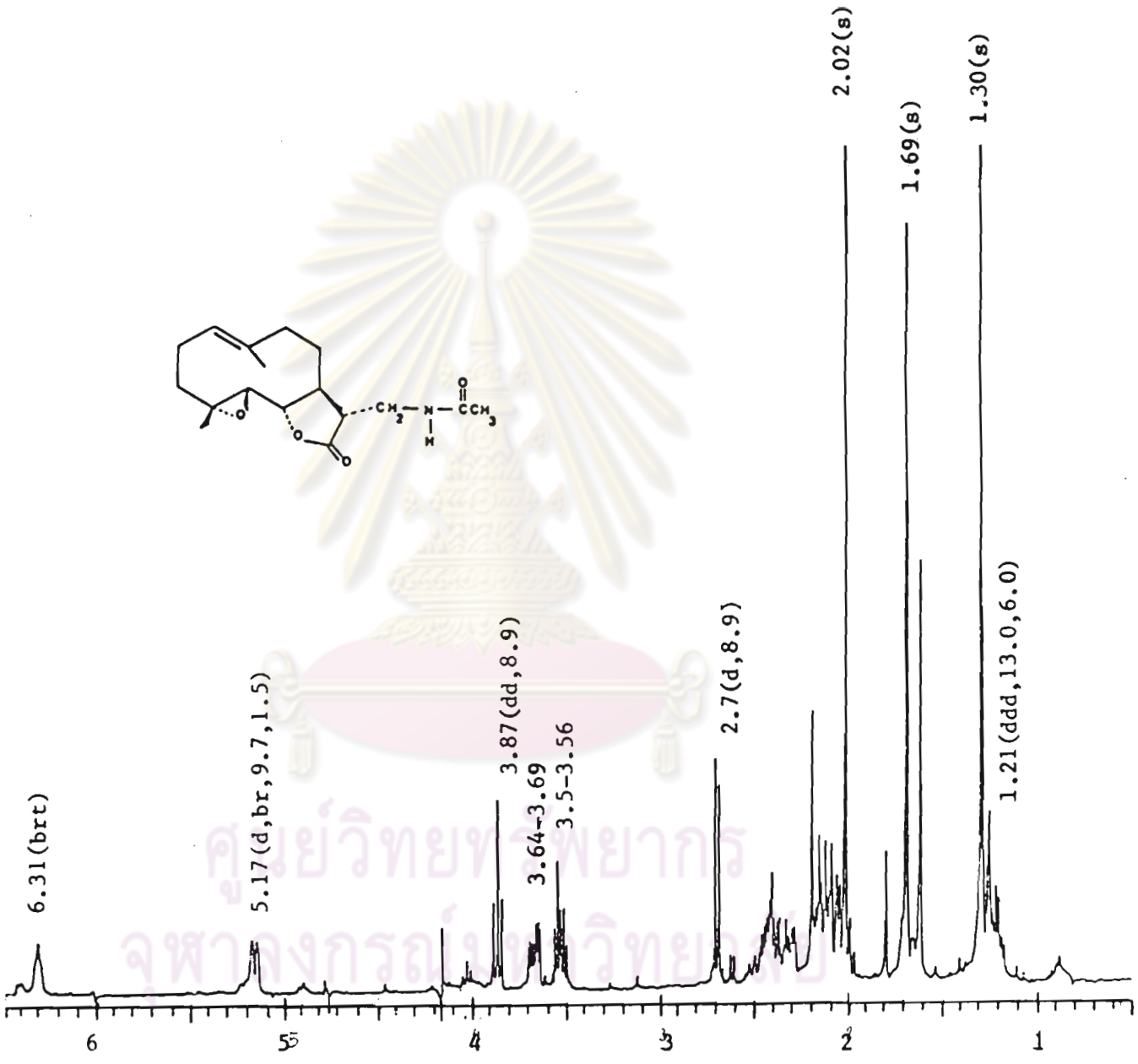


Figure 26 Proton NMR spectrum of MR-6 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

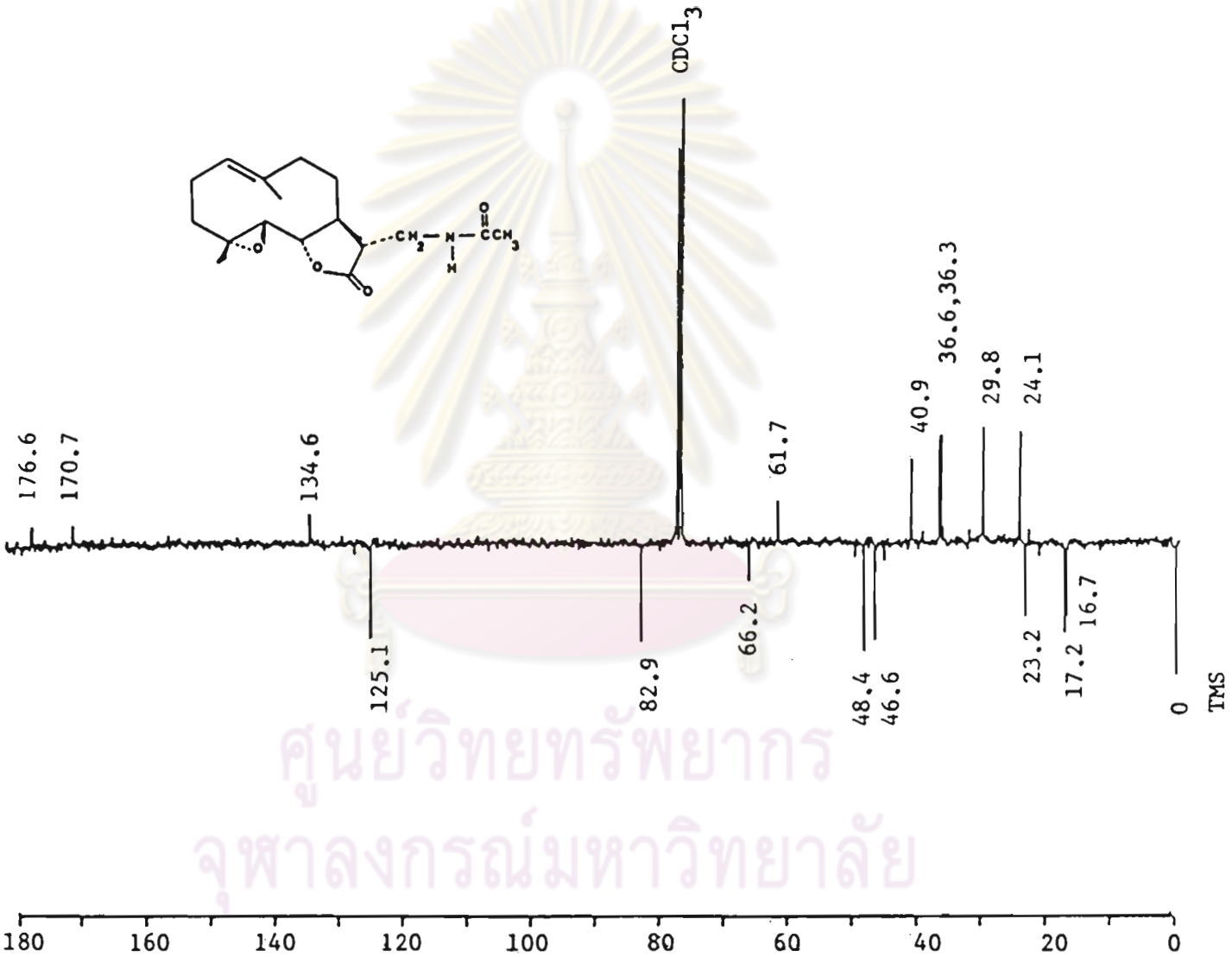


Figure 27 Carbon-13 NMR spectrum of MR-6 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

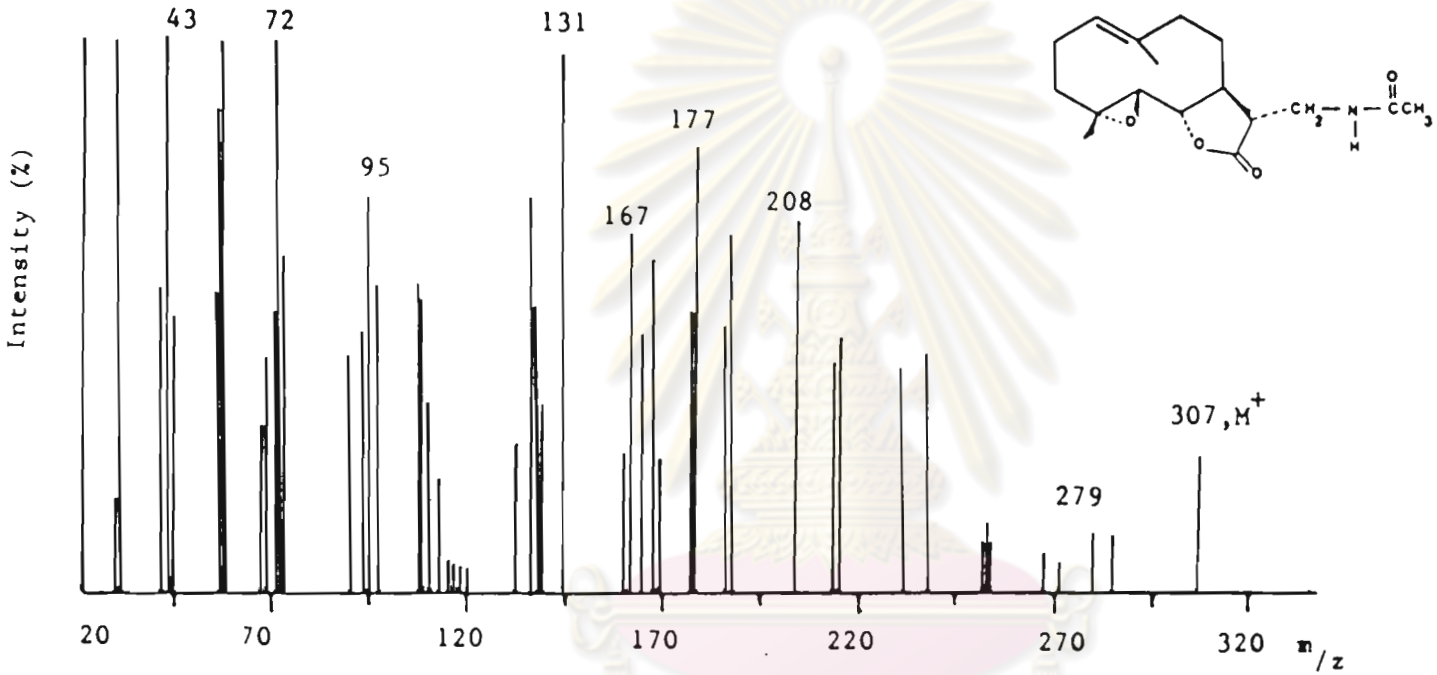


Figure 28 Mass spectrum of MR-6 from *Michelia rajaœiœna* Craib.stem bark.

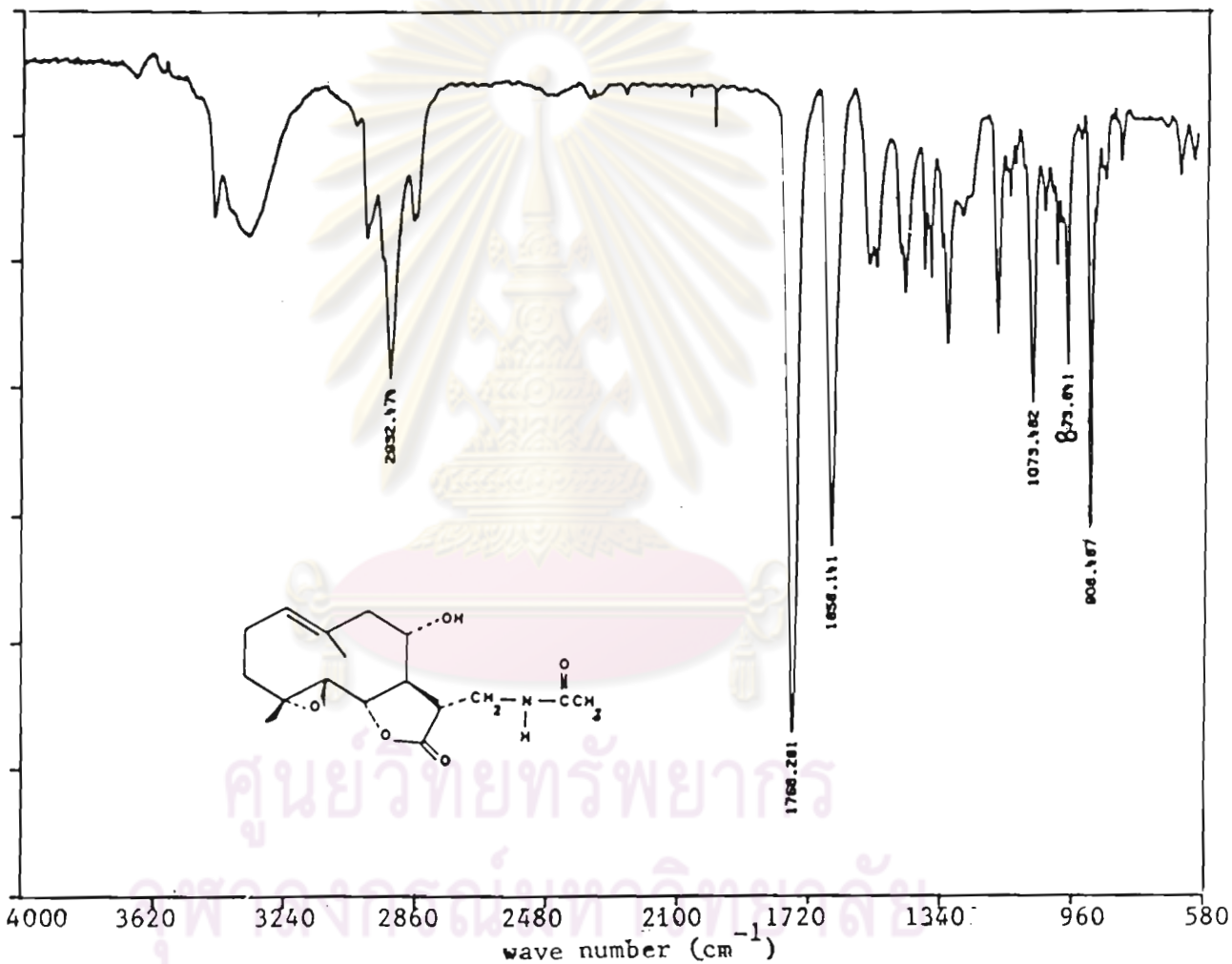


Figure 29 Infrared spectrum of MR-7 from *Michelia rajaniana* Craib.stem bark in $CHCl_3$.

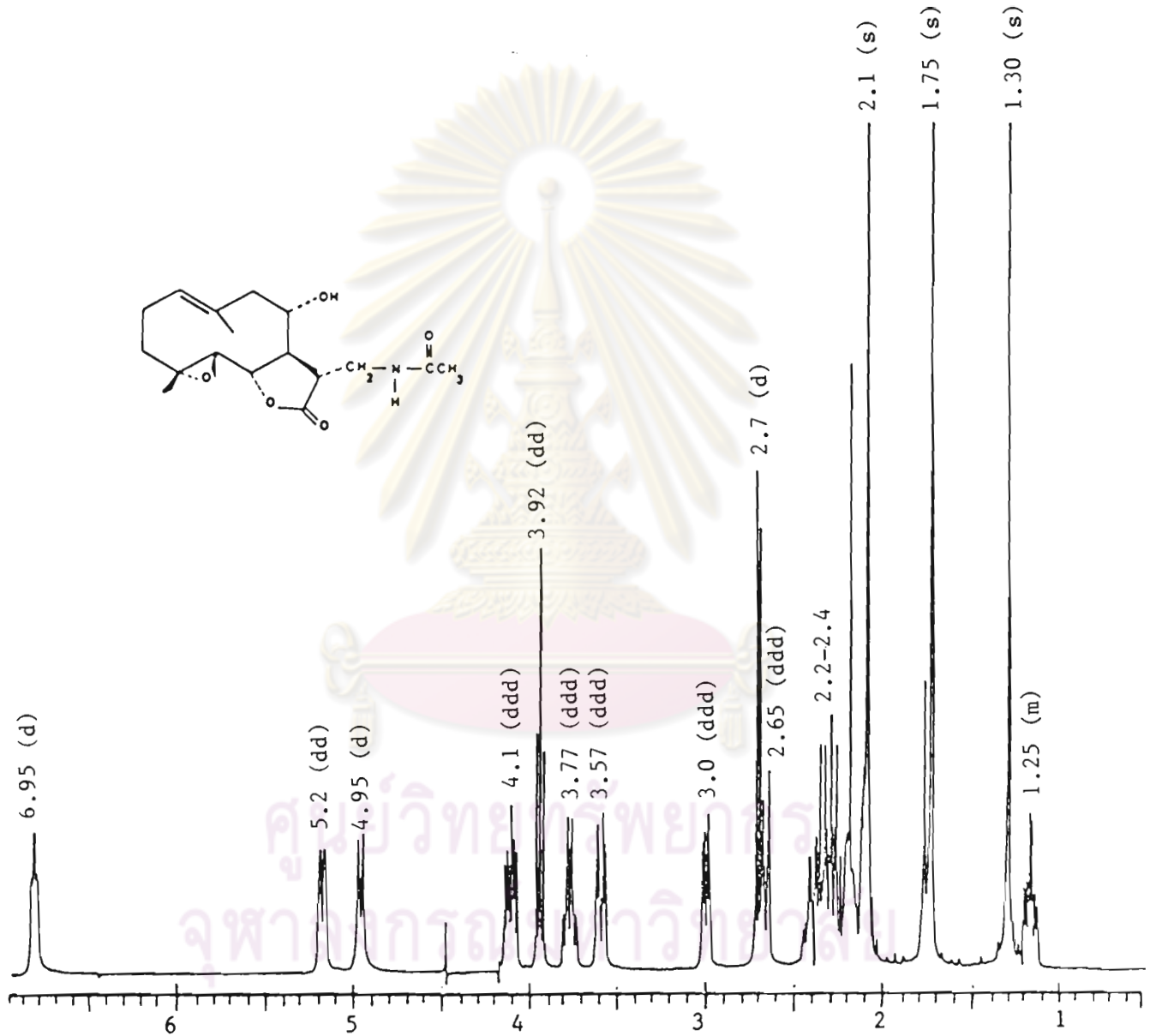


Figure 30 Proton NMR spectrum of MR-7 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

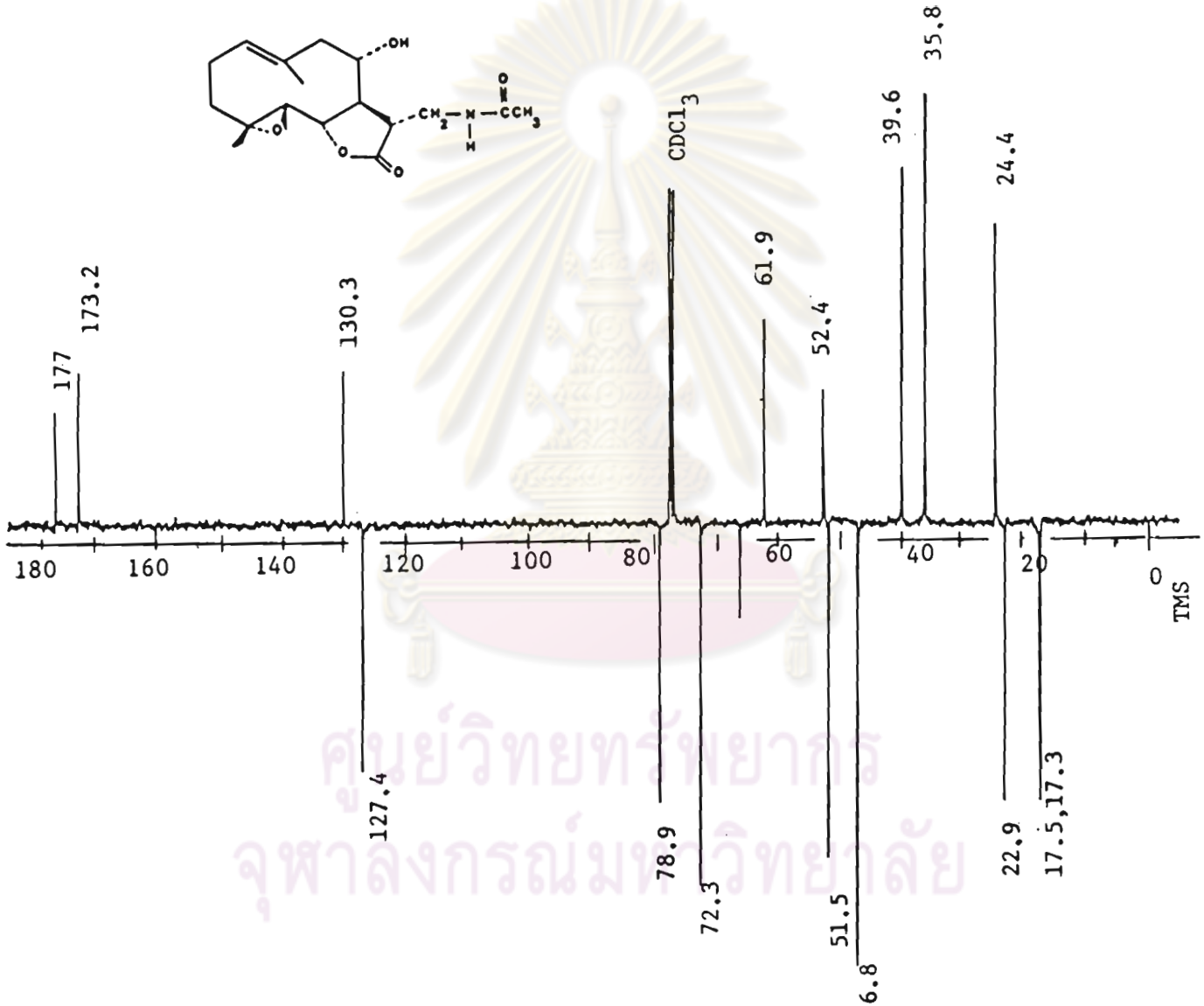


Figure 31 Carbon-13 spectrum of MR-7 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

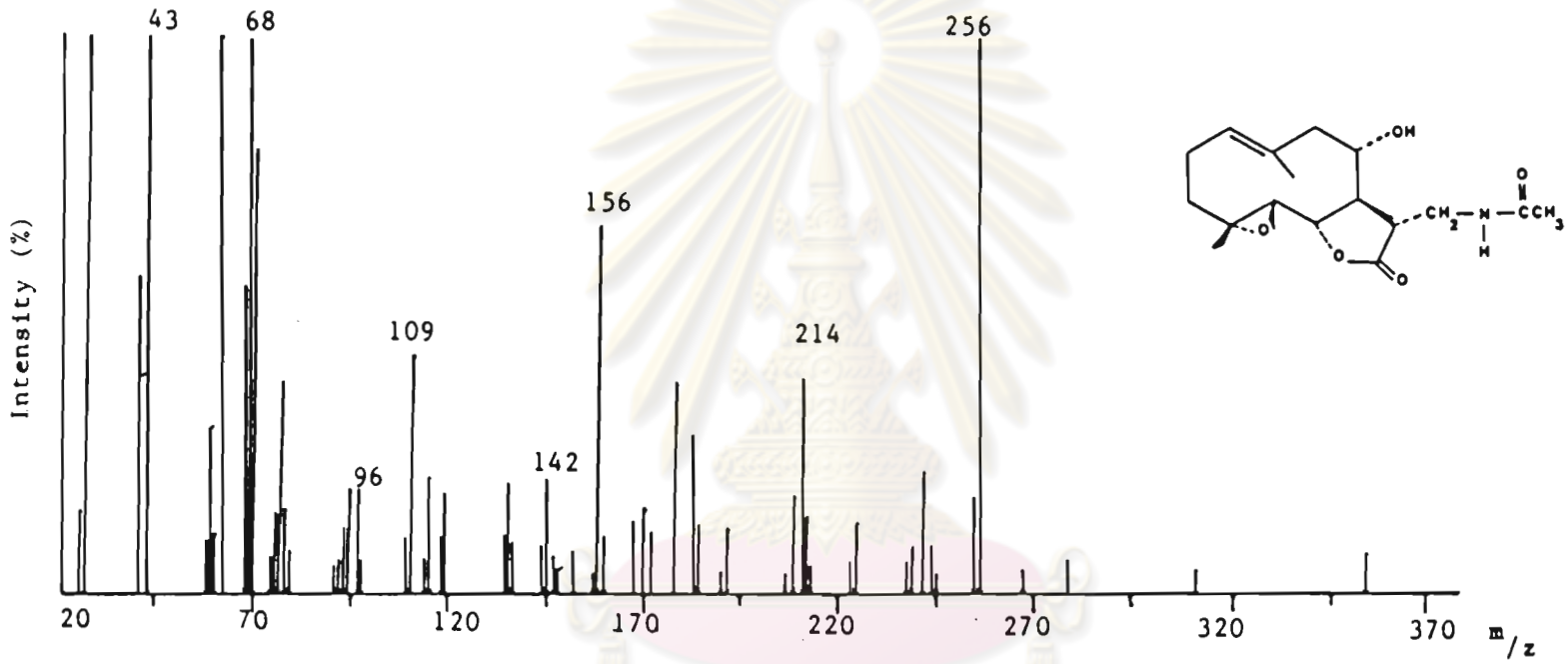


Figure 32 Mass spectrum of MR-7 from *Michelia rajaniana* Craib, stem bark.

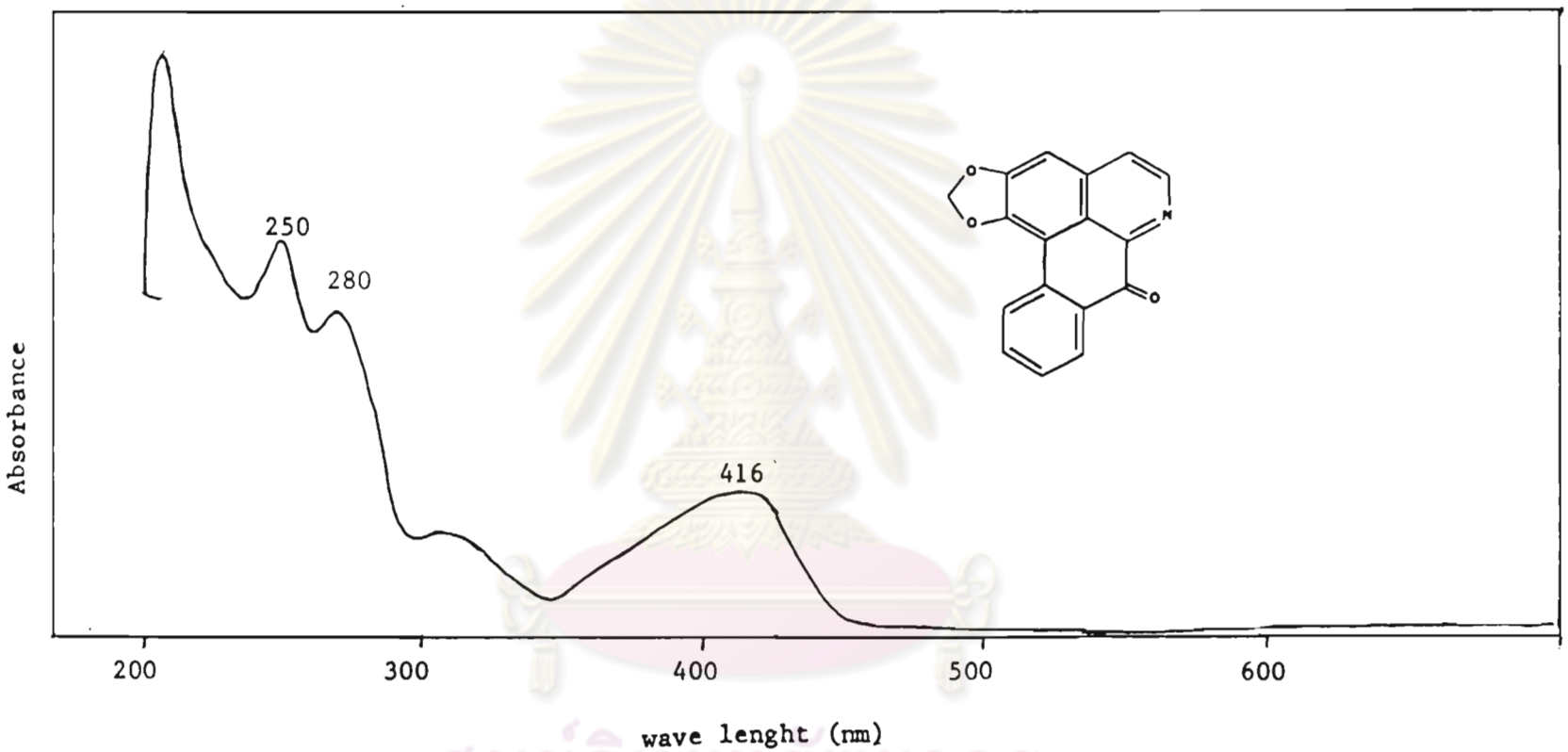


Figure 33 Ultraviolet-visible spectrum of MR-8 from *Michelia rajaniana* Craib.stem bark in 95% ethanol.

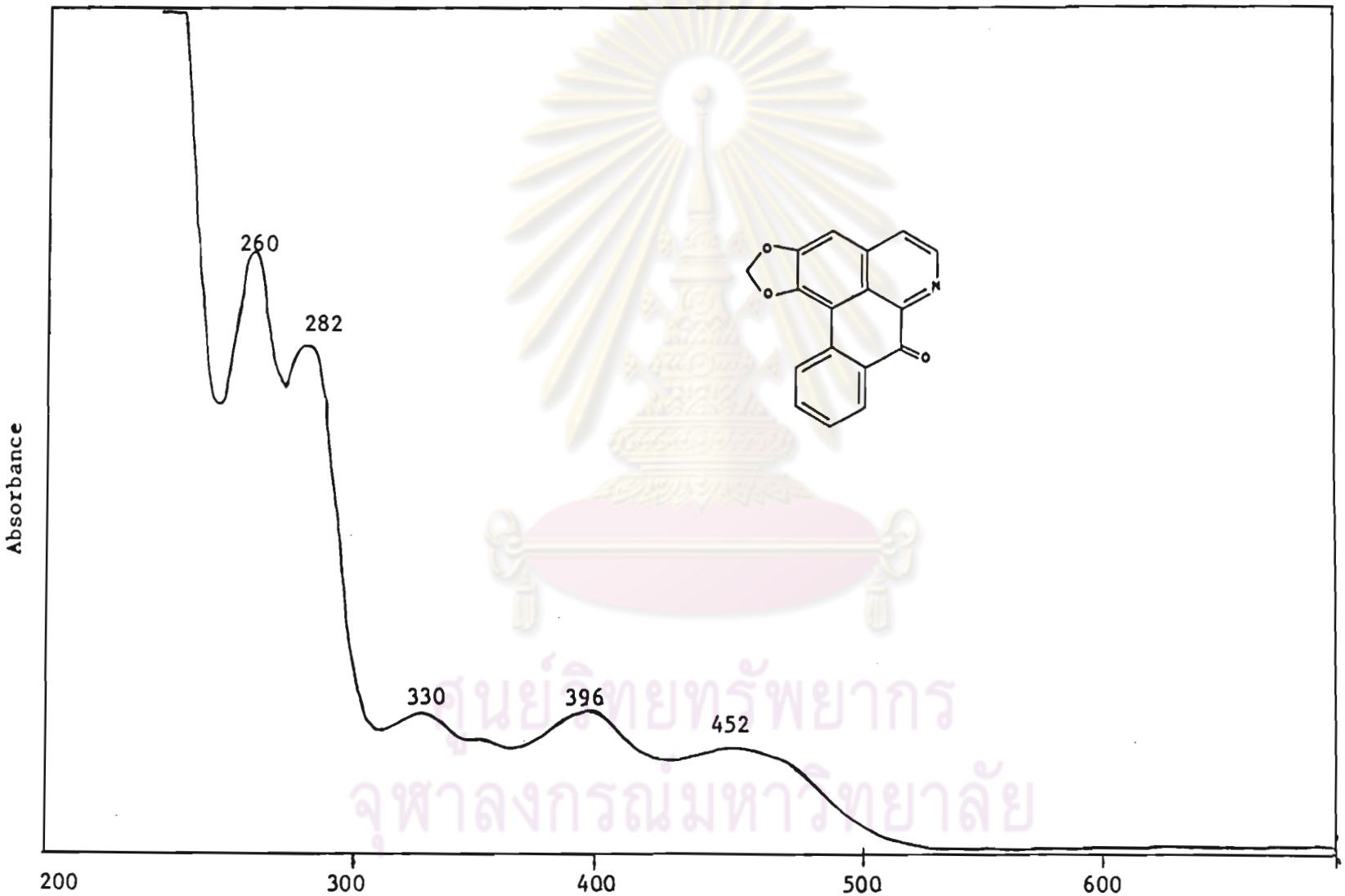


Figure 34 ultraviolet-visible spectrum of MR-8 from *Michlia rajaniana* Craib.stem bark in 0.1N HCl in ethanol

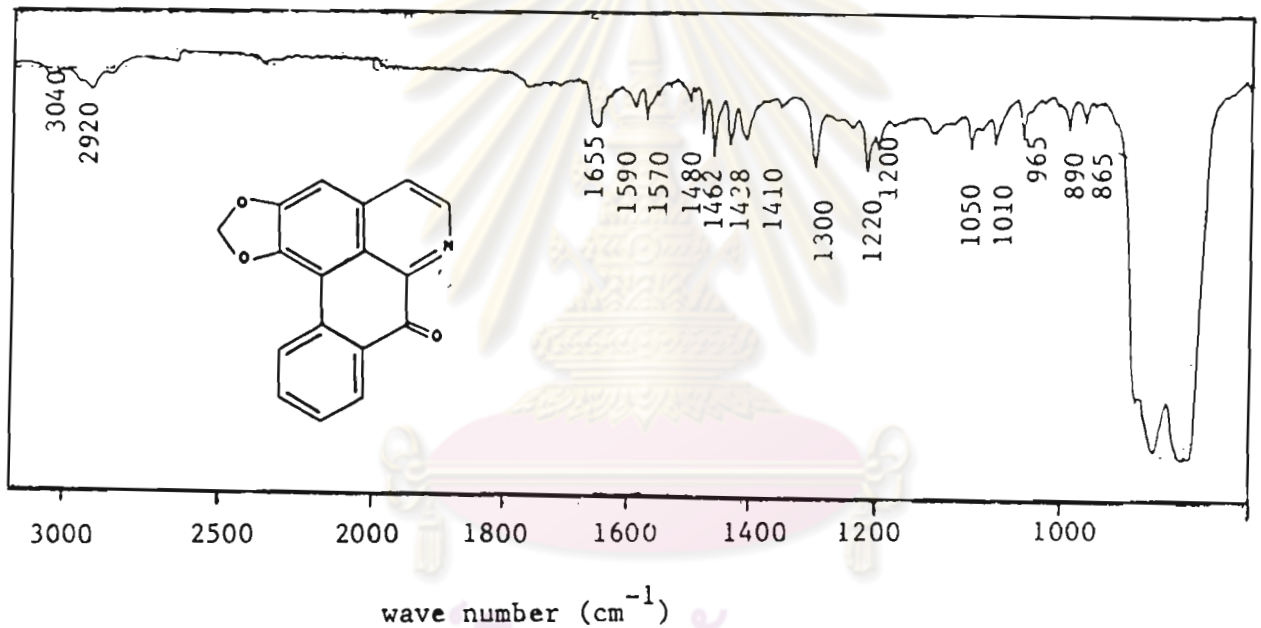


Figure 35 Infrared spectrum of MR-8 from *Michelia rajaniana* Craib.stem bark in dichloromethane

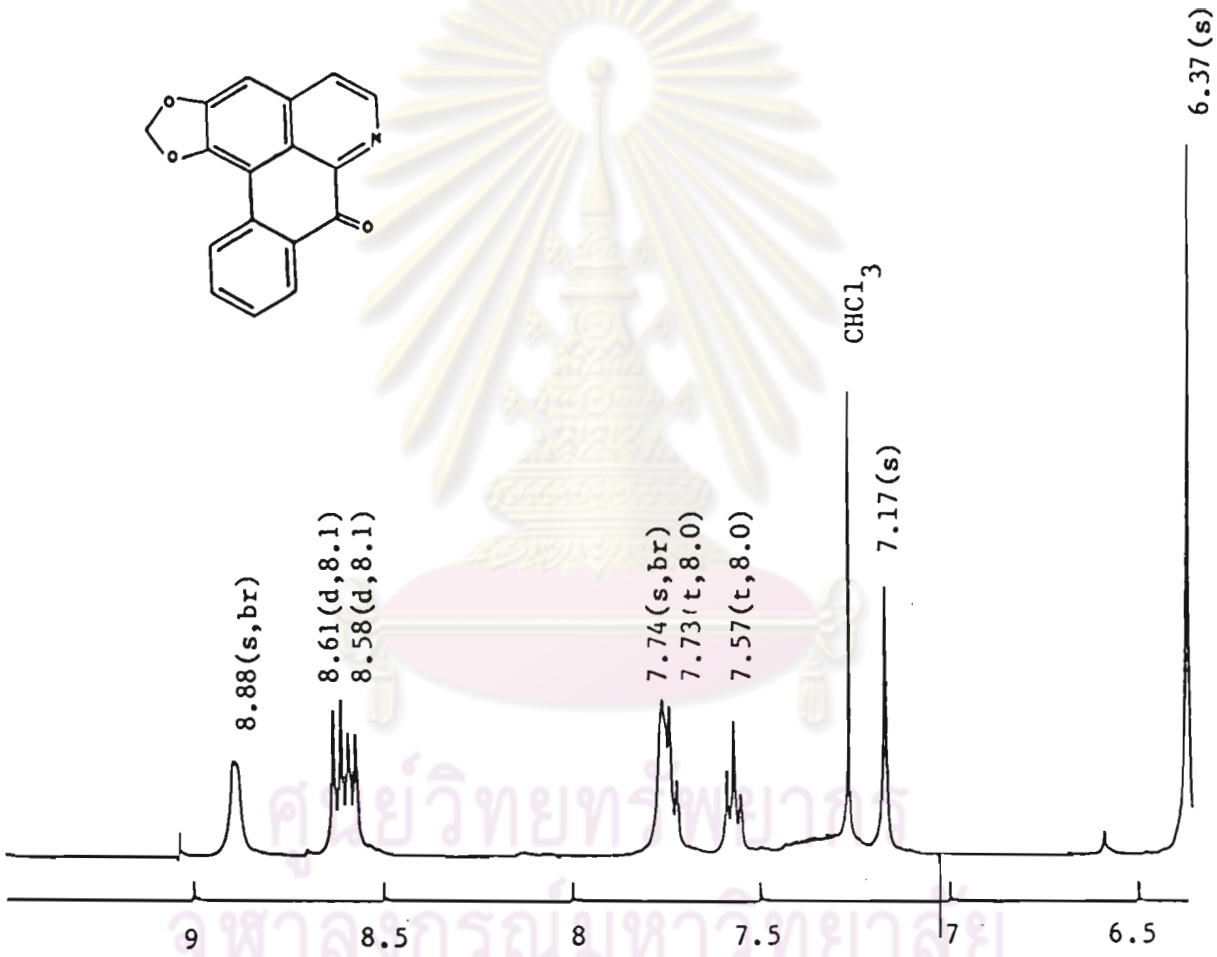


Figure 36 Proton NMR spectrum of MR-8 from *Michelia rajaniana* Craib.stem bark in $CDCl_3$.

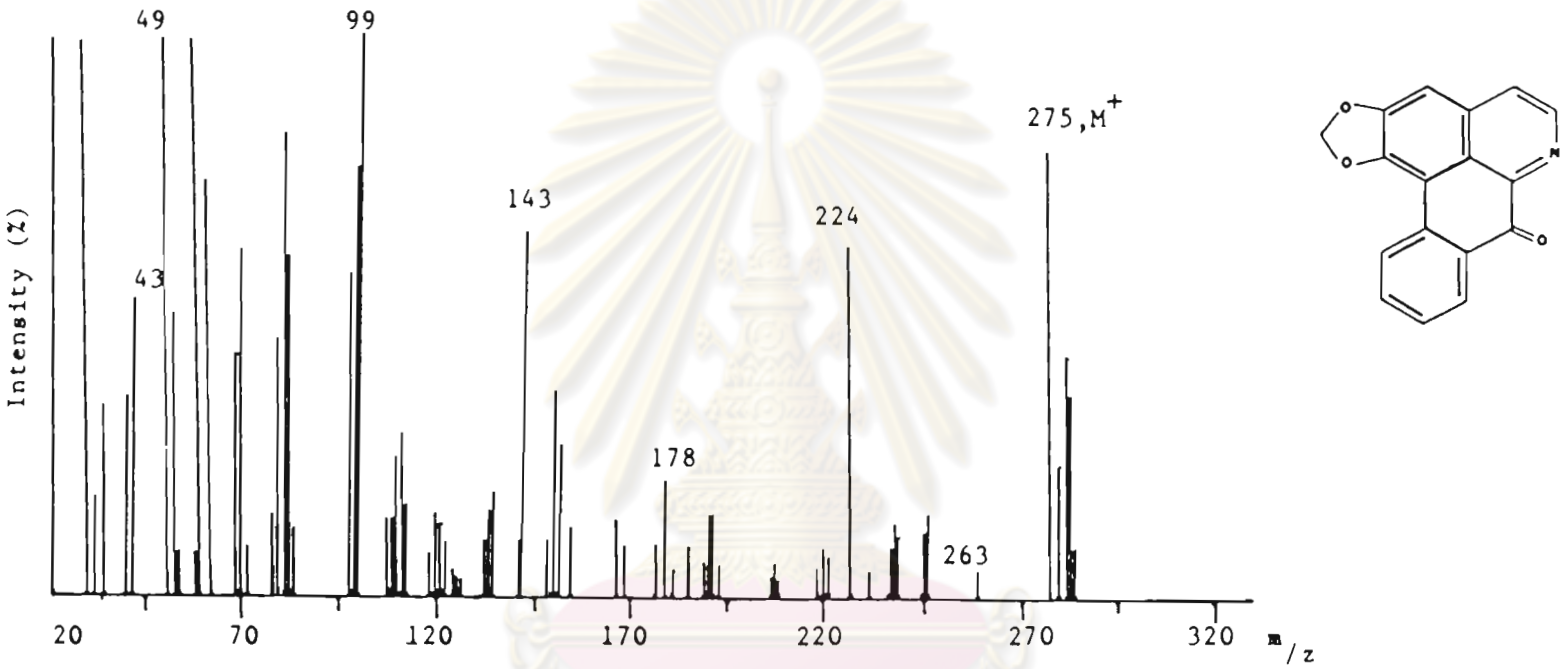


Figure 37 Mass spectrum of MR-8 from *Michelia rajaniana* Craib.stem bark.

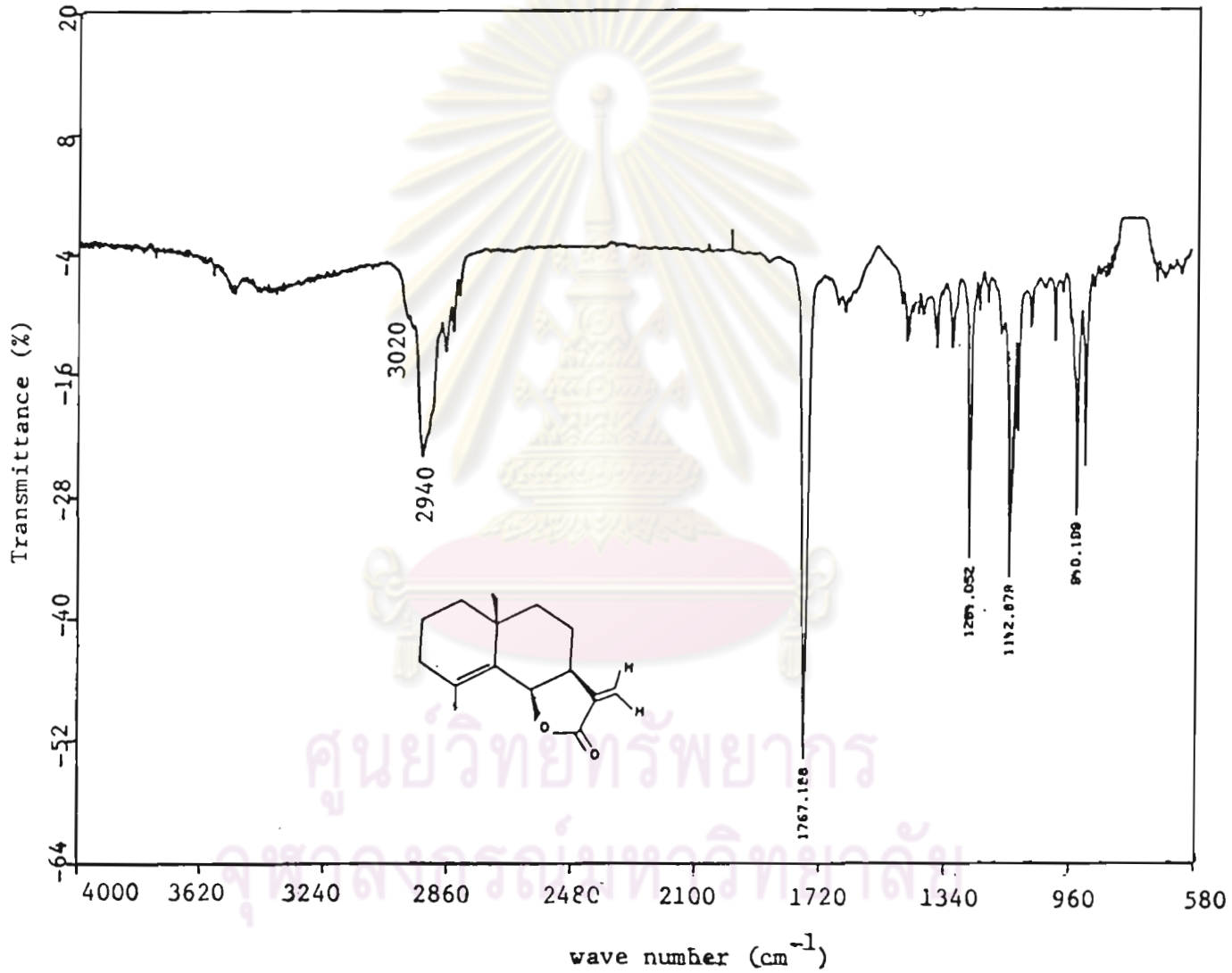


Figure 38 Infrared spectrum of GM-1 from *Grangea maderaspatana* . Poir. in CCl_4.

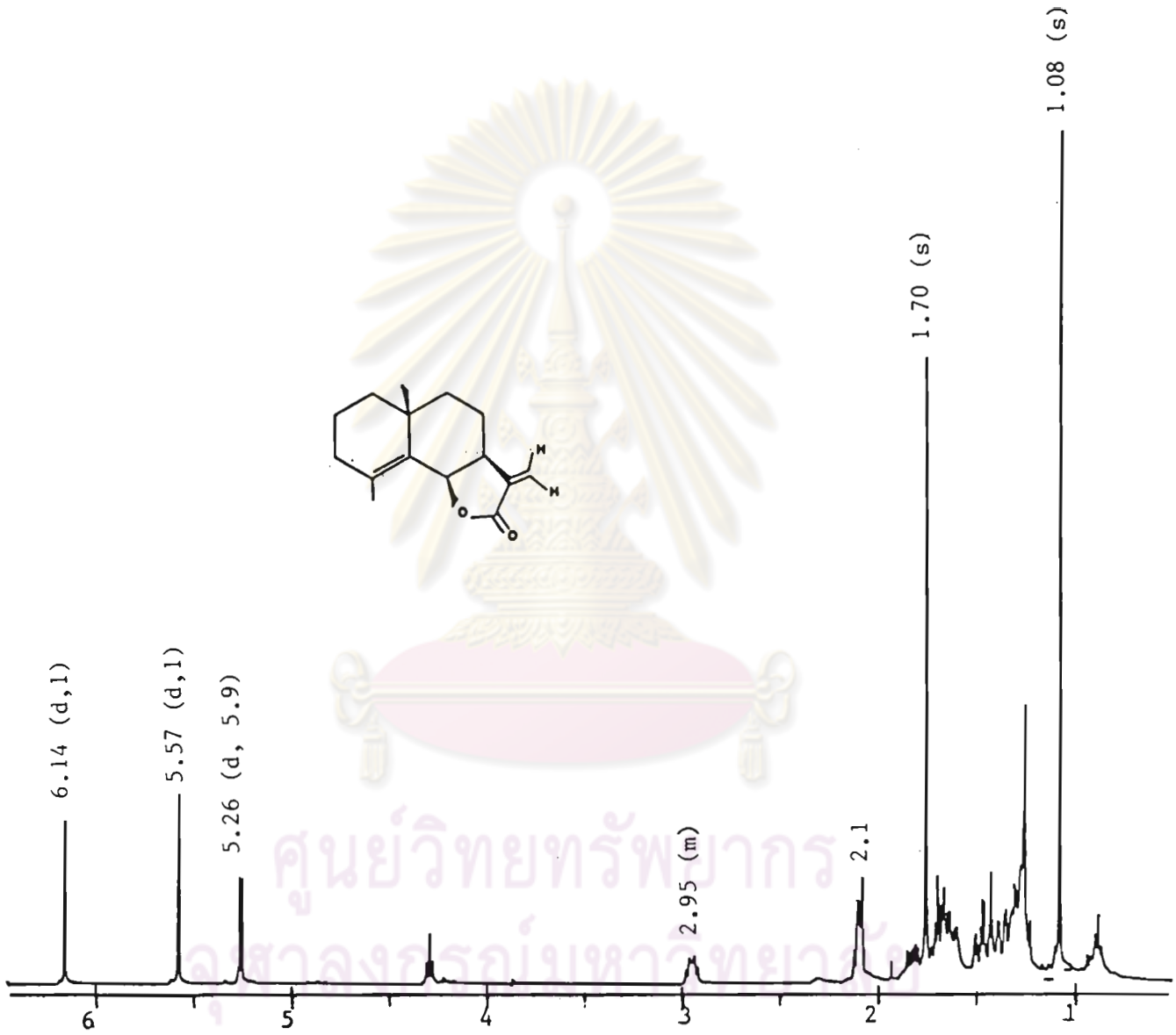


Figure 39 Proton NMR spectrum of GM-1 from *Grangea maderaspatana* Poir.in$CDCl_3$.

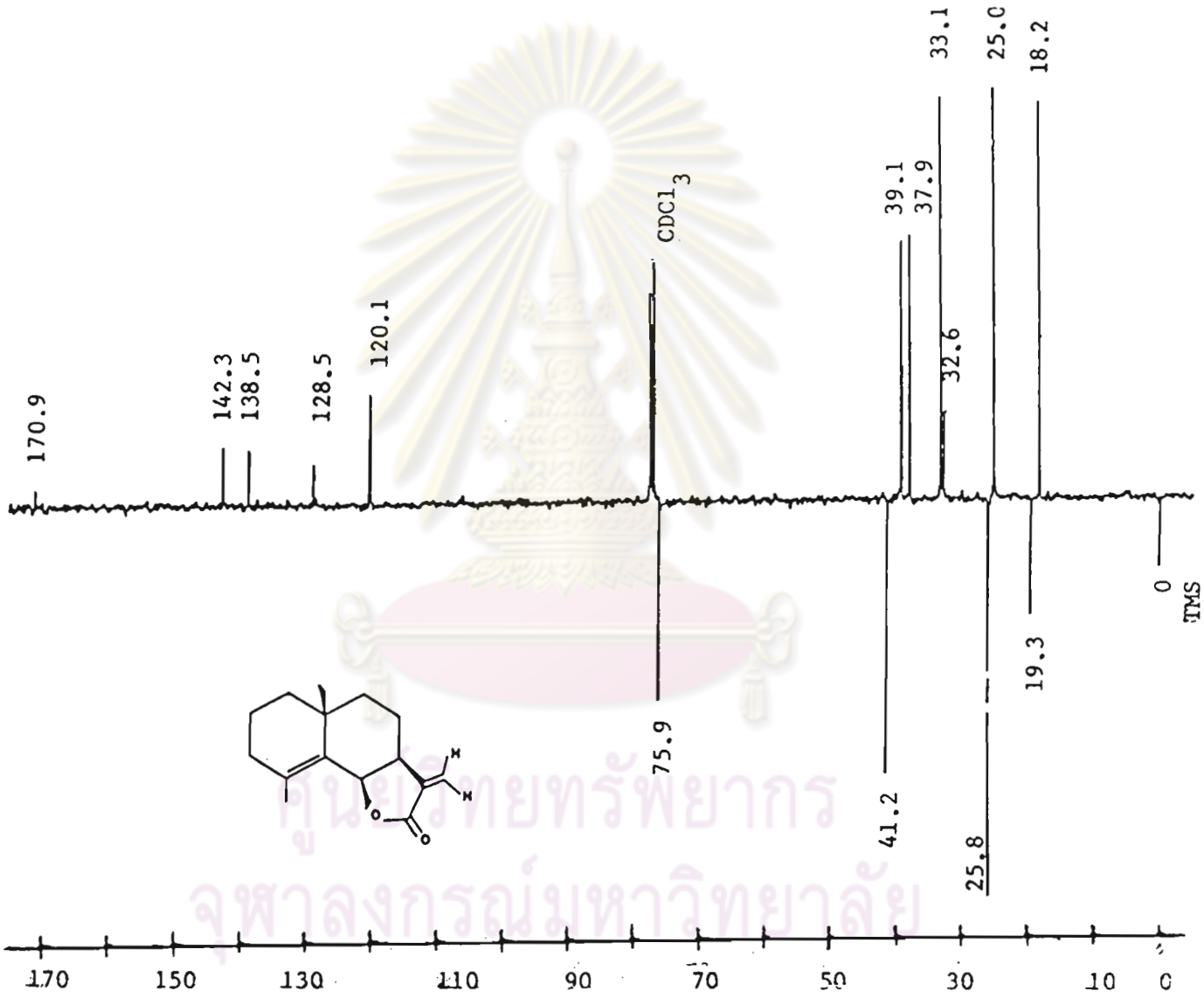


Figure 40 Carbon-13 NMR spectrum of GM-1 from *Grangea maderaspatana* Poir.in$CDCl_3$.

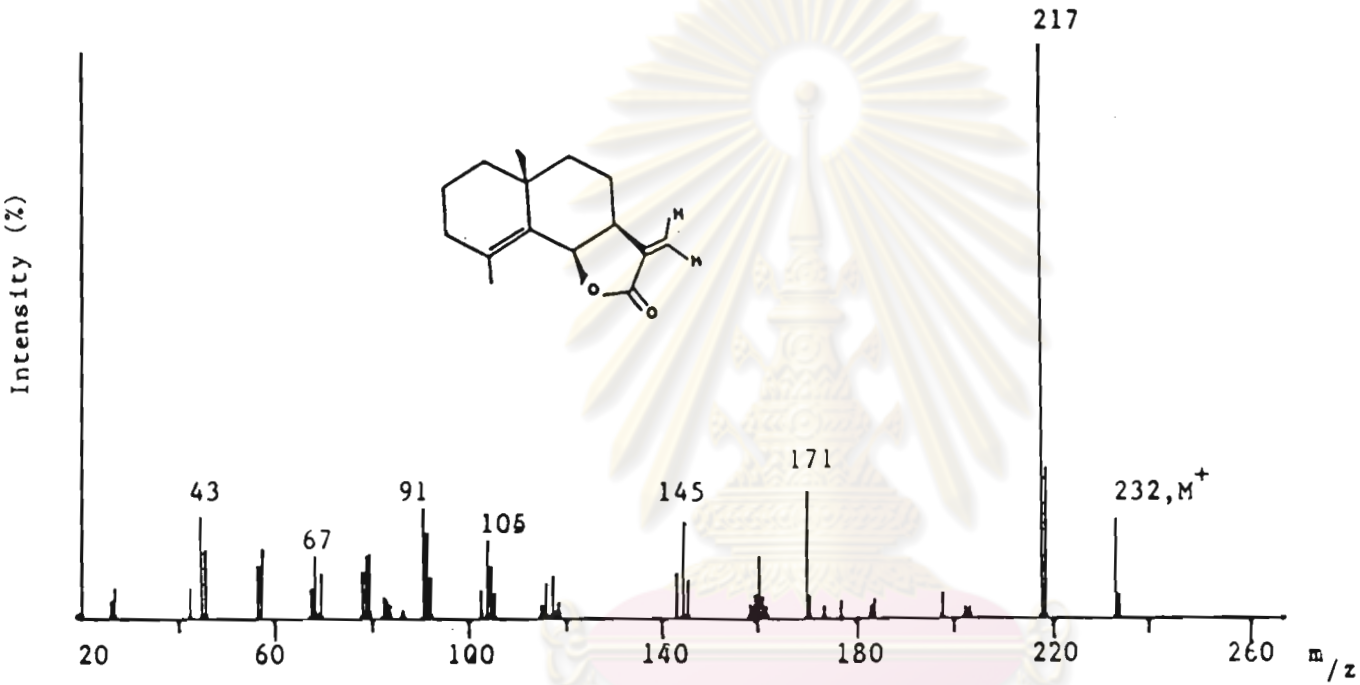
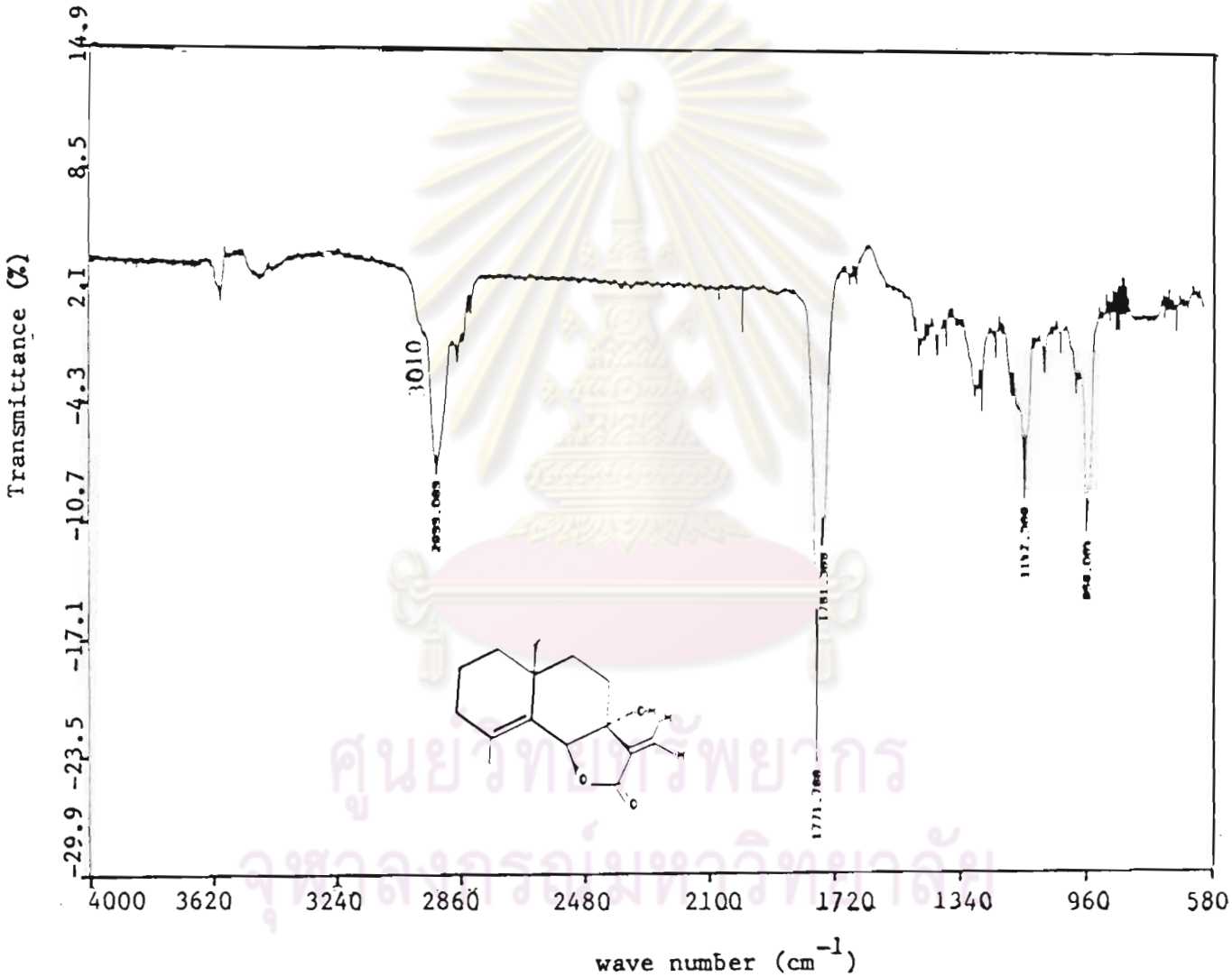


Figure 41 Mass spectrum of GM-1 from *Grangea maderaspatana* Poir.



Figer 42 Infrared spectrum of GM-2 from *Grangea maderaspatana* .Poir.inCCl_4.

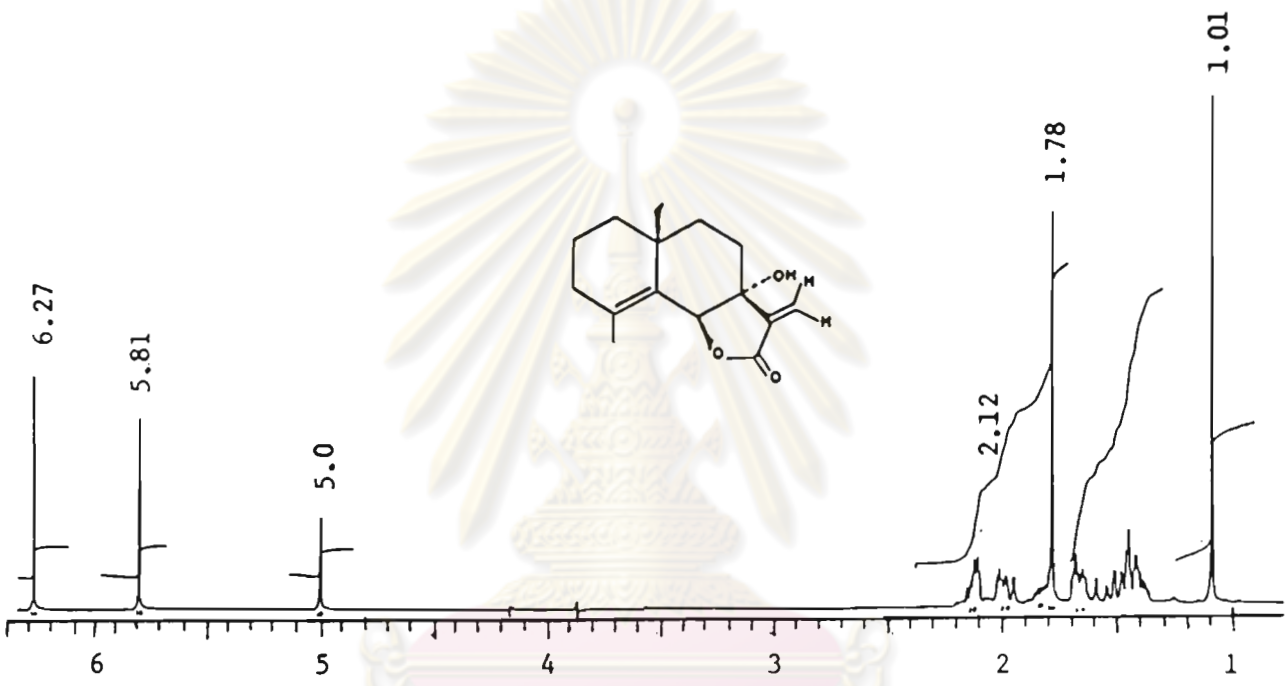


Figure 43 Proton NMR spectrum of GM-2 from *Grangea maderaspatana* Poir.in $CDCl_3$.

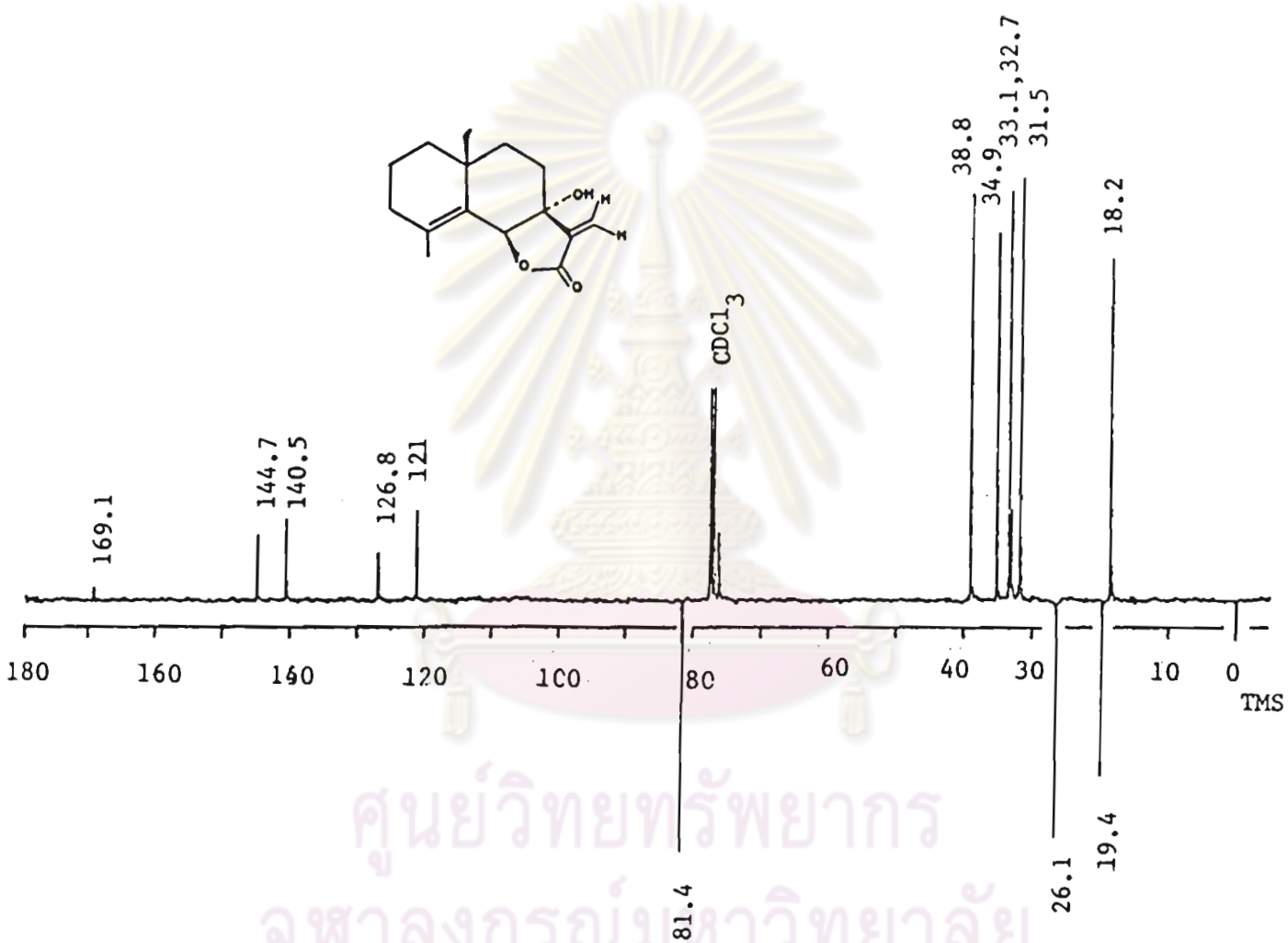


Figure 44 Carbon-13 NMR spectrum of GM-2 from *Grangea maderaspatana* Poir.in $CDCl_3$.

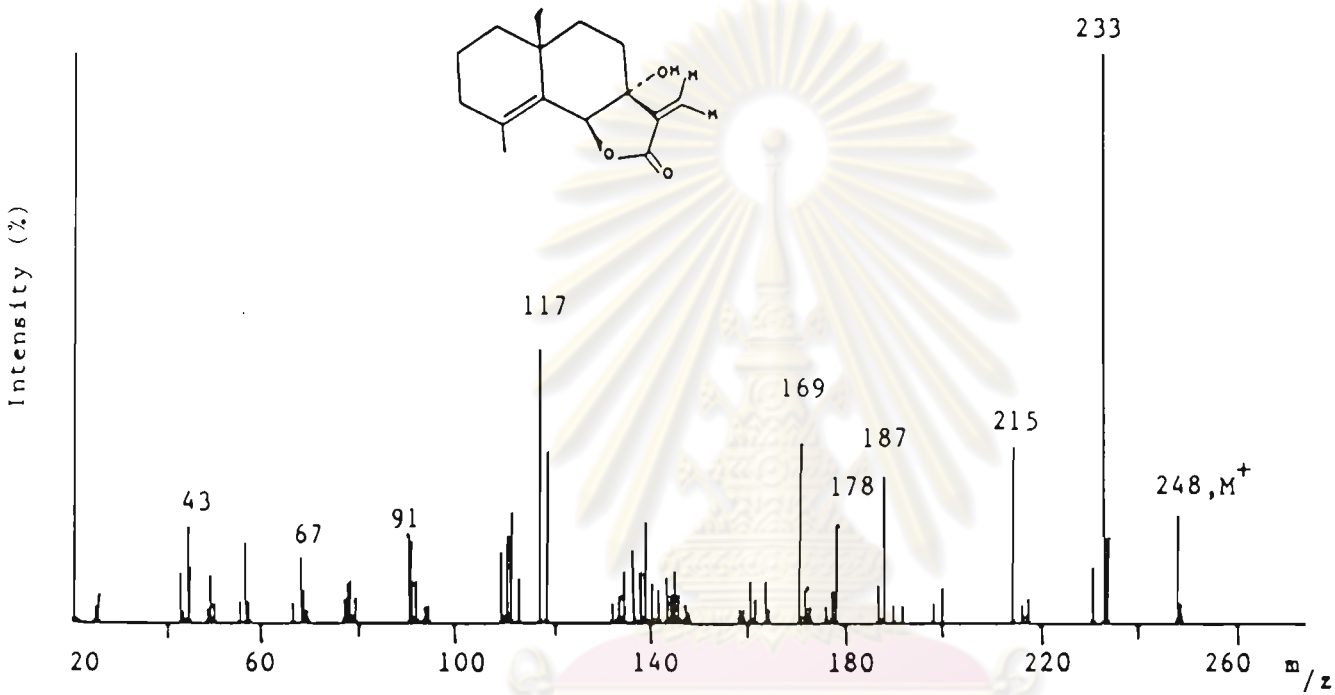


Figure 45 Mass spectrum of GM-2 from *Grangea maderaspatana* Poir.

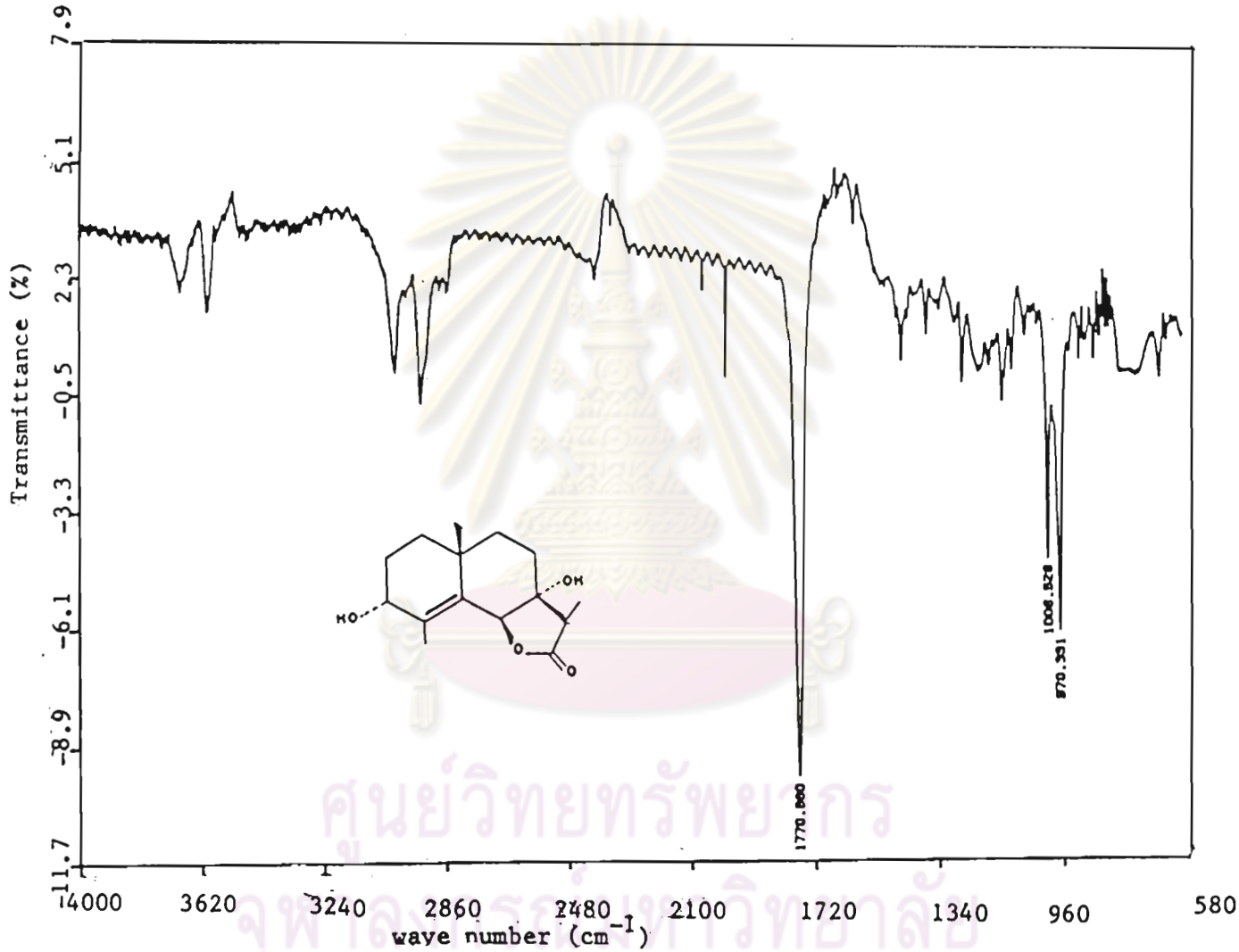


Figure 46 Infrared spectrum of GM-3 from *Grangea maderaspatana* Poir.in $CHCl_3$.

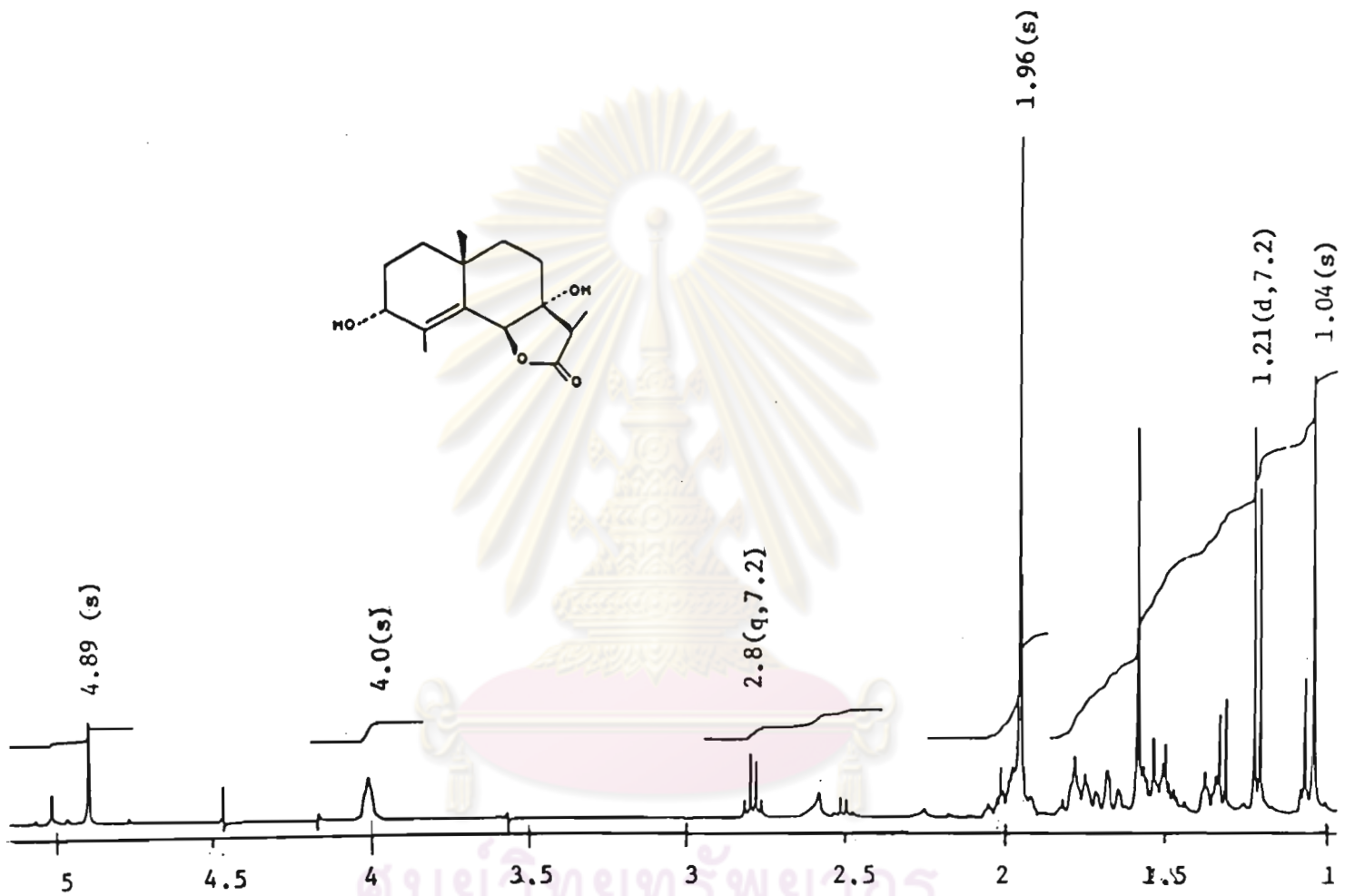


Figure 47 Proton NMR spectrum of GM-3 from *Grangea maderaspatana* Poir.in$CDCl_3$.

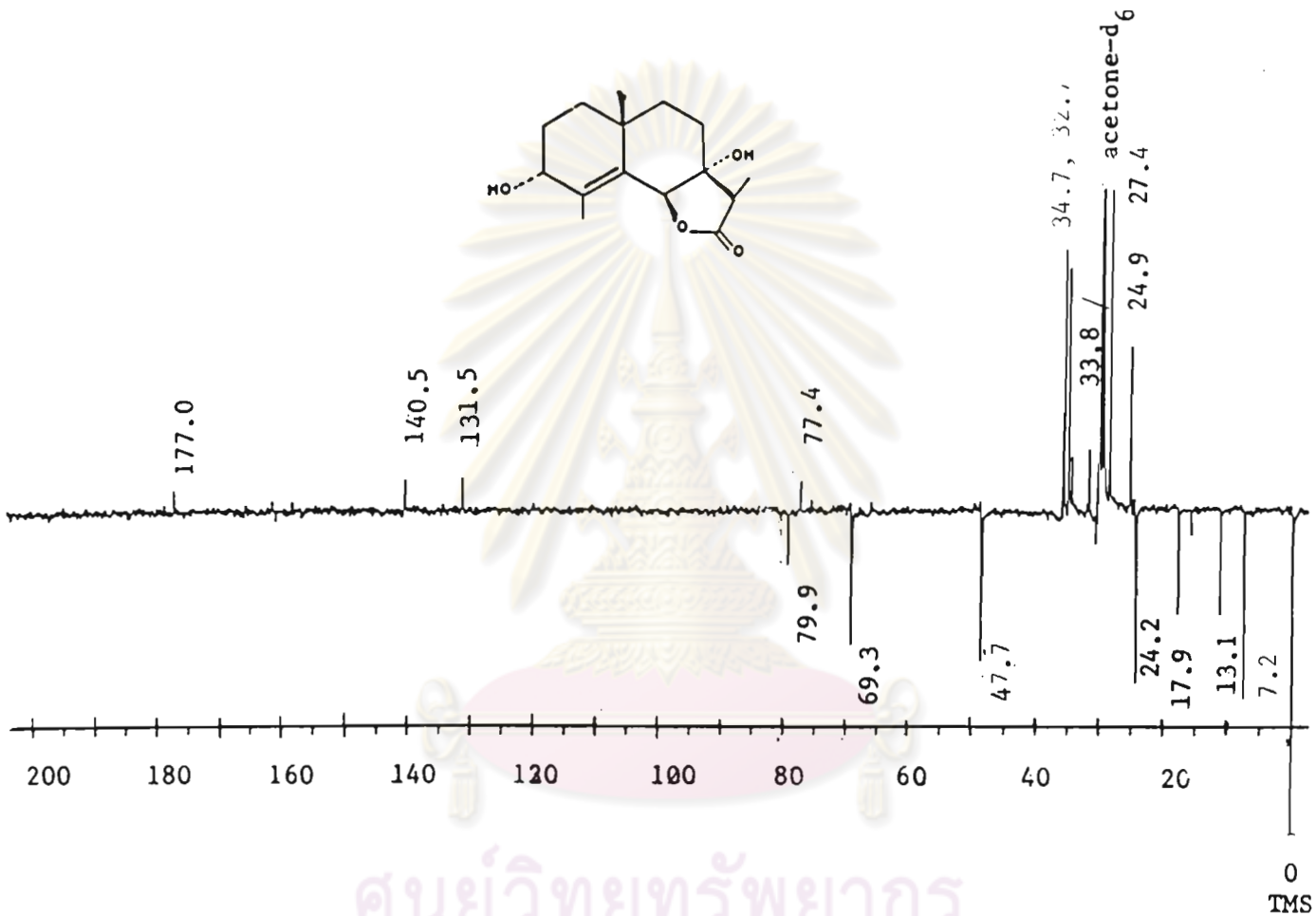


Figure 48 Carbon-13 NMR spectrum of GM-3 from *Grangea maderaspatana* Poir.in acetone-d_6.

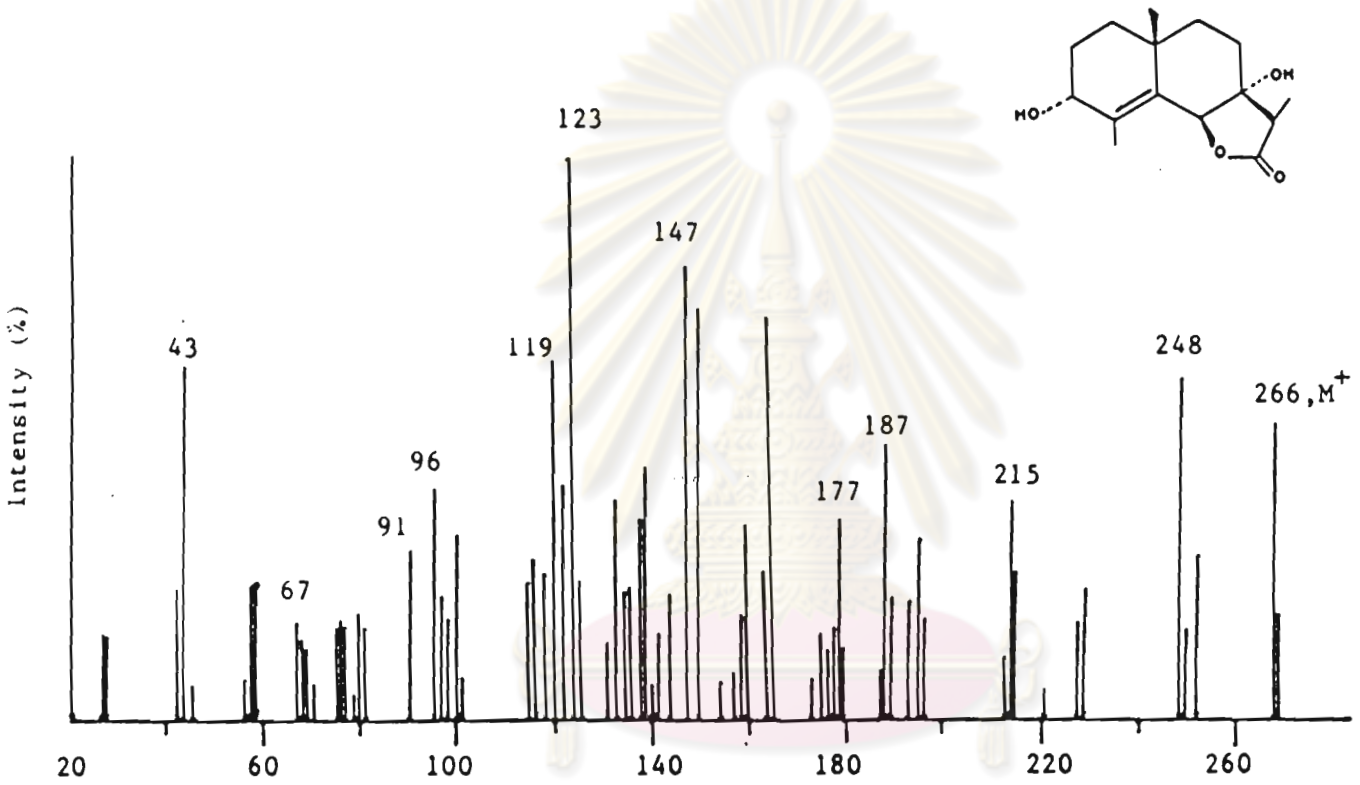


Figure 49 Mass spectrum of GM-3 from *Grangea maderaspatana* Poir.

VITA

Miss Srirat Kasiwong was born on February 2, 1964 in Patthalung, Thailand. She received her Bachelor of Science in Pharmacy (Second Class Honor) in 1986 from the Faculty of Pharmacy, Prince of Songkla University, Thailand.