ส่วนประกอบทางเคมีและการยับยั้งไซคลิกเอเอ็มพีฟอสฟอไดเอสเทอเรส ของเหง้าว่านร่อนทอง (*Globba malaccensis* Ridl.)

นายสถาพร อ่อนอนงค์

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สาขาวิชาเคมี ภาควิชาเคมี
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CHEMICAL CONSTITUENTS AND THEIR INHIBITIONS OF CYCLIC AMP PHOSPHODIESTERASE OF THE RHIZOMES OF *Globba malaccensis* Ridl.

Mr. Sathaporn Onanong

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Thesis Title	CHEMICAL CONSTITUENTS AND THEIR INHIBITIONS OF CYCLIC AMP PHOSPHODIESTERASE OF THE RHIZOMES OF *Globba malaccensis* Ridl.
By	Mr. Sathaporn Onanong
Field of Study	Chemistry
Thesis Advisor	Associate Professor Amorn Petsom, Ph.D.
Thesis Co-Advisor	Nattaya Ngamrojnavanich, Ph.D.

Accepted By the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master's Degree

..... Dean of Faculty of Science

(Associate Professor Wanchai Phothiphichitr, Ph.D.)

Thesis Committee

..... Chairman

(Associate Professor Sophon Roengsumran, Ph.D.)

..... Thesis Advisor

(Associate Professor Amorn Petsom, Ph.D.)

..... Thesis Co-Advisor

(Nattaya Ngamrojnavanich, Ph.D.)

..... Member

(Associate Professor Pipat Karntiang, Ph.D.)

..... Member

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

สถาพร อ่อนอนงค์ : ส่วนประกอบทางเคมีและการยับยั้งไซคลิกเอเอ็มพีฟอสฟอไดเอสเทอเรสของเหง้าว่านร่อนทอง (CHEMICAL CONSTITUENTS AND THEIR INHIBITIONS OF CYCLIC AMP PHOSPHODIESTERASE OF THE RHIZOMES OF *Globba malaccensis* Ridl.)

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นำเหง้าว่านร่อนทอง *Globba malaccensis* Ridl. แห้งและบดละเอียดไปสกัดด้วยตัวทำละลายอินทรีย์ที่เหมาะสม 3 ชนิด ได้แก่ เอธานอล คลอโรฟอร์ม และ เฮกเซน ตามลำดับ จากสิ่งสกัดคลอโรฟอร์มสามารถทำการแยกสารบริสุทธิ์ได้ 4 ชนิดโดยอาศัยเทคนิคทางคอลัมน์โครมาโตกราฟี ดำเนินการหาสูตรโครงสร้างของสารที่แยกได้แต่ละชนิด โดยอาศัยคุณสมบัติทางกายภาพทางเคมี และ ข้อมูลทางสเปกโตรสโกปี สามารถพิสูจน์โครงสร้างได้ คือ สารประกอบจำพวก Sesquiterpenoids ได้แก่ Isocurcumenol (**1**) จำนวน $1.2x10^{-3}$% Curcumenol (**2**) จำนวน $5.5x10^{-2}$ % และ Zedoarondiol (**4**) จำนวน $1.4x10^{-3}$% และสารประกอบจำพวก Curcumenoid ได้แก่ Curcumin (**3**) จำนวน $1.3x10^{-1}$% (เปอร์เซ็นต์ที่คำนวณได้เทียบจากน้ำหนักแห้งของเหง้าว่านร่อนทอง 4 กิโลกรัม) จากงานวิจัยนี้พบว่าเป็นครั้งแรกที่สามารถวิเคราะห์โครงสร้างของ Curcumenol ได้โดยเทคนิคทางเอ็กซ์เรย์ คริสตอลโลกราฟี นำสารที่แยกได้ทั้ง4 ชนิดมาทดสอบกับเอนไซม์ไซคลิกเอเอ็มพีฟอสฟอไดเอสเทอเรสพบว่ามีฤทธิ์ยับยั้งเอนไซม์ดังกล่าวได้ปานกลางถึงมาก

ภาควิชา.....เคมี..... ลายมือชื่อนิสิต.....

สาขาวิชา.....เคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2543..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

SATHAPORN ONANONG: CHEMICAL CONSTITUENTS AND THEIR INHIBITIONS OF CYCLIC AMP PHOSPHODIESTERASE OF THE RHIZOMES OF *Globba malaccensis* Ridl. THESIS ADVISOR : ASSOC. PROF. AMORN PETSOM, Ph.D. THESIS Co-ADVISOR : NATTAYA NGAMROJNAVANICH, Ph.D. 105 pp. ISBN 974-13-0495-1.

The ground-dried rhizomes of *Globba malaccensis* Ridl. were extracted subsequently with suitable organic solvents including ethanol, chloroform and hexane. The chloroform crude extract was separated and purified by column chromatography, and gave four known compounds. The structures of these compounds were established on the basis of physical and chemical properties and spectroscopic evidences. The four structures were proved to be sesquiterpenoids; Isocurcumenol (**1**, $1.2x10^{-3}$ % wt.by wt. of the dry plant 4 kg.), Curcumenol (**2**, $5.5x10^{-2}$ % wt.by wt. of the dry plant 4 kg.) and Zedoarondiol (**4**, $1.4x10^{-3}$ % wt.by wt. of the dry plant 4 kg), and curcumenoid; Curcumin (**3**, $1.3x10^{-1}$ % wt.by wt. of the dry plant 4 kg). It was the first report of the x-ray crystallographic analysis of curcumenol. These four isolated compounds were tested for their inhibitory activities against cyclic AMP phosphodiesterase. The result indicated that most of tested compounds exhibited moderate to high inhibitory activities.

Department.....Chemistry..... Student's signature. Sathaporn Onanong

Field of study.....Chemistry..... Advisor's signature. .....

Academic year.....2000..... Co- advisor's signature. Nattaya Ngamrojnavanich

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Finally, the author would like to thank his parents for their inexhaustible love and support and his friends for their friendship and helps throughout the entire course of study.

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LIST OF ABBREVIATIONS

TMS	tetramethylsilane
Hz	Hertz
ppm	part per million
δ	Chemical shift
s	singlet (NMS)
d	doublet (MR)
t	triplet (NMR)
q	quartet (NMR)
dd	double doublet
ddd	double doublet doublet
dt	double triplet
cm^{-1}	unit of wave number
M^+	molecular ion
m/z	mass to charge ratio
M.W.	molecular weight
ν_{max}	the wavelength at maximum absorption
br	broad
s	strong
m	medium
w	weak
%	percent
m.p.	melting point
Fig.	Figure
°C	degree celsius
ml	milliliter (s)
mg	milligram
g	gram (s)

TLC	Thin Layer Chromatography
wt	weight
DEPT	Distortionless Enhancement by Polarisation Transfer
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
COSY	Correlated Spectroscopy
NOESY	Nuclear Overhauser Enhancement Spectroscopy

CHAPTER I
Introduction

It has been a long time that human can obtain immensely valuable benefits from many natural resources. Fortunately, as a tropical country, Thailand has a large abundant kind of plants, especially herbs which are used as medicinal plants and are natural products that can be used as therapeutic agent. In the past, indigenous people have learnt to use medicinal plants, without systematic methodology, accidentally as their primary treatment in the health care system, so utilization of plants were still limited back then. Nowadays, they are not only widely studied by several modern techniques in a more scientific and systematic investigation, but also considerably accepted as a replacement option for synthetic drugs. The popularity of utilizing plants might be due to their less side effects, reasonable cost and greater availability. In addition, consuming local medicinal plants can reduce the import of synthetic drugs from foreign countries as well.

Natural product chemistry is one of the approaches in searching for chemical constituents from natural plants and involves seeking their activities by bioassay tests and therapeutic applications. The criteria traditionally used to select plant to study might be the history of that plant in treating diseases, preliminary screening bioactivity tests, or literature surveys about chemical constituents that may have the potential as drugs. This thesis is focused on searching for bioactive compounds which could inhibit the cyclic AMP phosphodiesterase from the tropical Thai plant, botanically named *Globba malaccensis* Ridl. This technique is widely used as a screening method to detect biologically active compounds in natural plants.

Figure 1. Leaves and rhizomes of *G. malaccensis* Ridl.

The scope of research

This research began by extracting specimens of the plant with suitable solvents, then isolating compounds from those crude extracts by chromatrographical techniques. Structural elucidation of the isolated compounds was deduced from spectroscopic evidences. Finally, inhibitory activity tests of the isolated compounds were conducted on the inhibition of cyclic AMP phosphodiesterase.

The purpose of research

1. To isolate chemical constituents from the rhizomes of *G. malaccensis* Ridl.
2. To elucidate the structures of isolated compounds.
3. To add chemical information on the plants in genus *Globba*
4. To study the cyclic AMP phosphodiesterase inhibitory activities of crude extracts and isolated compounds.

CHAPTER II
Literature Reviews

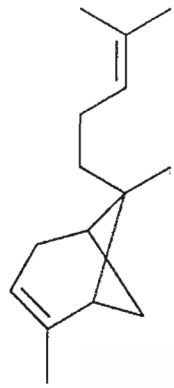
Chemical constituents of plants found in Globba genus.

No detailed information about *G. malaccensis* Ridl. has been reported, but some reports have been made of *G. obscura* K. Larsen which are shown in Table 1 and some of their structures in Figure 2.

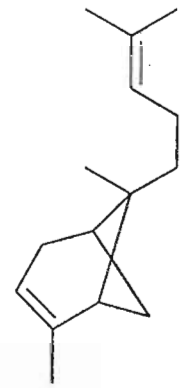
Table 1. The chemical constituents and pharmacological activities of *G. obscura* K.Larsen.

Scientific name	Activities[1,10]	Chemical constituents	Ref. No.
G. obscura K. Larsen	Cardiotonic CNS stimulant Antipyretic Anti-inflammatory	α-bergamotene β-bisabolene β-caryophyllene chavicol α-copaene p-cymene δ-elemene,γ-elemene eugenol (E)-β-farnesene farnesol myrcene α-pinene,β-pinene sabinene β-sesquiphellandrene α-terpineol terpinen-4-ol cineol	11

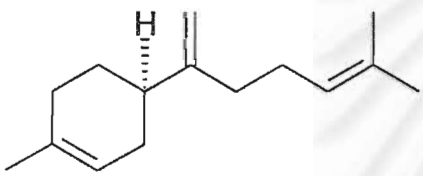
		Chemical constituents	Ref. No.
		galangin, kaemferide 1,7-diphenyl-5-hydroxy -3-heptanone 5-hydroxy- 7- (4-hydro xy-3-methoxyphenyl)- 1-phenyl-3-heptanone 7-(4-hydroxy-3-metho xyphenyl)-1-phenylhept -4-en-3-one)	12



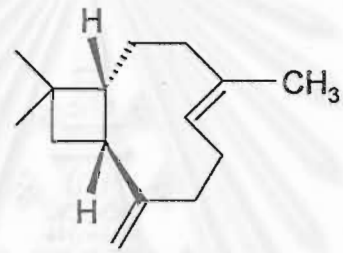
cis-α-bergamotene



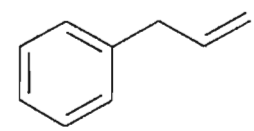
trans-α-bergamotene



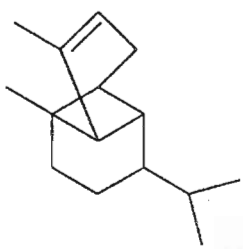
β-bisabolene



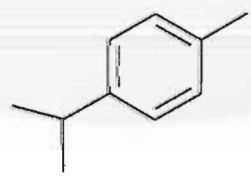
β-caryophyllene



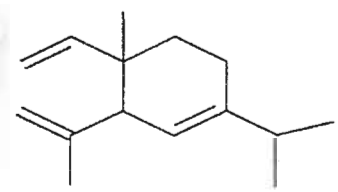
chavicol



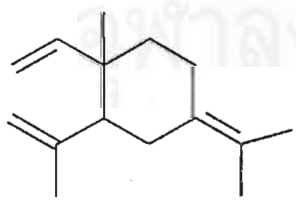
α-copaene



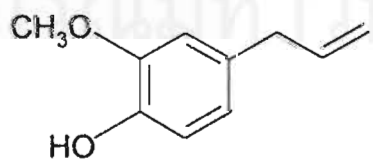
p-cymene



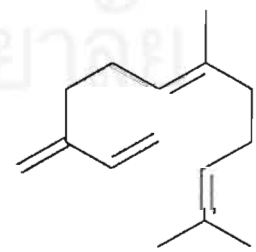
δ-elemene



γ-elemene

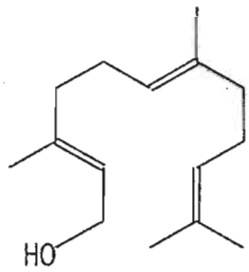


eugenol

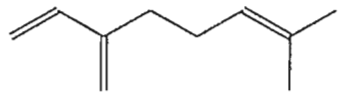


(E)-β-farnesene

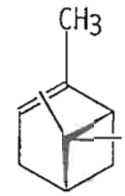
Figure 2. Chemical constituents of *G.obscura* K. Larsen



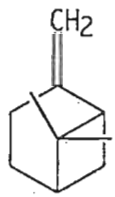
farnesol



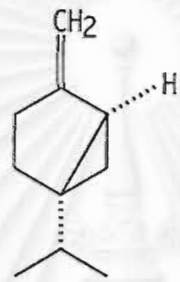
myrcene



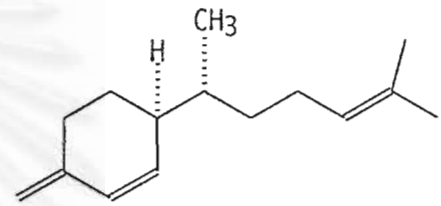
α-pinene



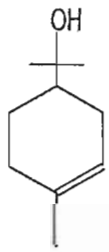
β-pinene



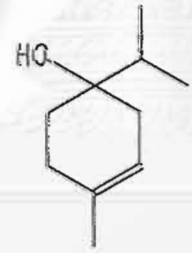
sabinene



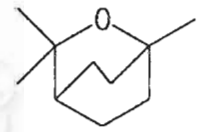
β-sesquiphellandrene



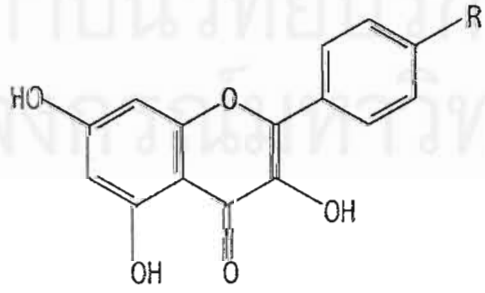
α-terpineol



terpinen-4-ol

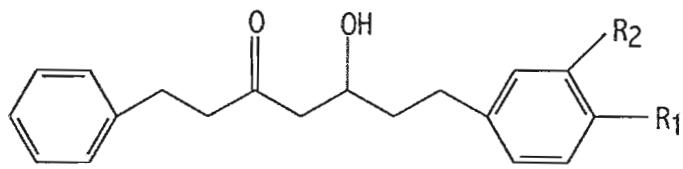


cineol

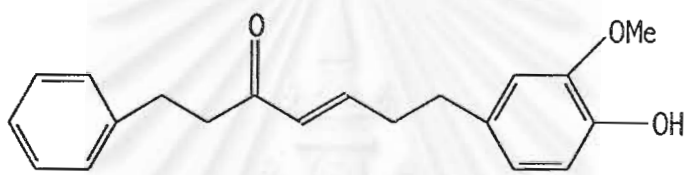


galangin ; R=H
kaemferide ; R=OCH_3

Figure 2.(cont.) Chemical constituents of *G.obscura* K. Larsen



$R_1 = R_2 = H$: 1,7-diphenyl-5-hydroxy-3-heptanone
$R_1 = OH$, $R_2 = OMe$: 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone



7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one)

Figure 2.(cont.) Chemical constituents of *G.obscura* K. Larsen

PDE Assay

In 1960, Sutherland and Rall[13] first described the role of cyclic AMP as a "Second messenger", mediating the response of cells to a variety of hormones and neurotransmitters. Many water soluble hormones do not cross the cell membrane by themselves, but instead cause effects within the cell via a second messenger (Fig.3). Sutherland and his contemporaries subsequently demonstrated the importance of cyclic AMP in the regulation of a variety of metabolic processes, including cardiac and smooth muscle contractility, glycogenolysis, platelet aggregation, secretion and lipolysis. The physiological importance of cyclic GMP, on the other hand, remains largely a mystery, and early suggestions that cyclic GMP acts in opposition to cyclic AMP (The "Yin-and-Yang"hypothesis) have been discounted. Moreover, they also reported the presence of a large and diverse family of enzymes called cyclic nucleotide phosphodiesterases (PDEs) which are responsible for the cyclic nucleotide hydrolyzing activity in extracts of a variety of tissues. This enzymatic activity terminates the biological actions of cyclic AMP and cyclic GMP, ubiquitous second messengers that regulate countless biological processes. PDEs have been classified into seven structurally, biochemically, and pharmacologically distinct families [14,15]. Among them, cyclic AMP phosphodiesterase type IV (PDE IV), is characterized by selective, high-affinity hydrolysis of cAMP[16]. Inhibition of PDE IV results in the increase in cellular levels of cAMP, which contributes to both the relaxation of airway smooth muscle and the prevention of pro-inflammatory cell activation [17].

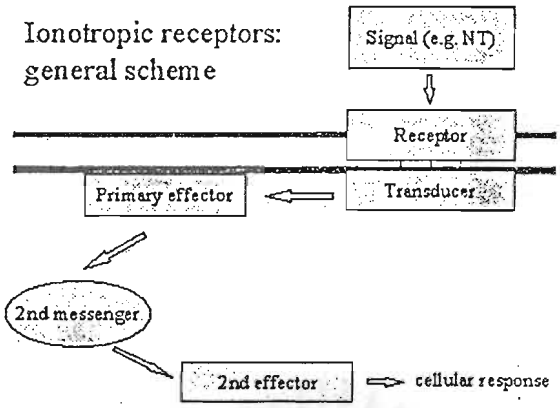


Figure 3. The roles of secondary messenger in general cell

Briefly, cyclic AMP is produced from ATP by the catalytic action of adenylyl cyclase (Fig.4). This enzyme is activated either directly (e.g. forskolin) or indirectly through cell-surface receptor, e.g. β-adrenocepter agonists and prostaglandin E_2 . As the intracellular concentration of the cyclic nucleotide rises, it binds to and activates its target enzyme, PKA. This protein kinase phosphorylate substrates (e.g. ion channels, contractile proteins, transcription factors) that regulate key cellular functions. Phosphorylation alters the activity of these substrates, and thus, changes cellular activity. As cyclic nucleotide is inactivated by PDEs there is a corresponding decrease in protein kinase activity. Phosphoprotein phosphatases dephosphorylate substrates, and cellular activity returns to normal. Obviously, altering the rate of cyclic nucleotide formation or degradation will change the activation state of these pathways[18].

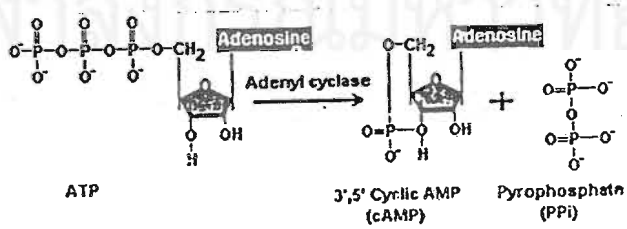
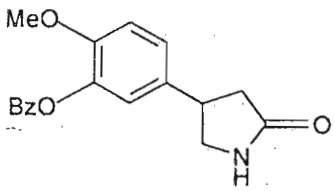


Figure 4. Formation of cyclic AMP

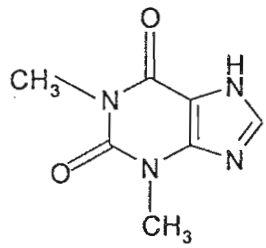
In some situations, as in hypertension, hepatomas, diabetes and obesity, increased activity of the high affinity phosphodiesterase may be an important part of the etiology of the disease state, although this may not always be the case. In these conditions, the use of appropriate phosphodiesterase inhibitors could alleviate the deficiency in intracellular cAMP by the most desired mechanism for any drug, namely, by correcting the basic biological defect. On the other hand, there is also evidence for decreased phosphodiesterase activity in diabetes and hepatomas[19]. The most feasible approach to correct the cAMP deficiency under these circumstances is the inhibition of phosphodiesterase in summary.

There are both activators and inhibitors for PDEs. Compounds which reported to stimulate phosphodiesterase are not as numerous as those that appeared to inhibit it. Since the most common abnormalities in cyclic nucleotide metabolism usually result in lower levels of these important mediators, inhibition of their degradation is the more desirable therapeutic target. Nevertheless, activators are of interest in diseases associated with elevated cyclic nucleotide levels, e.g. cAMP in diabetes, cholera, alcoholism and mania. Examples of these compounds are introglycerine, papaverine, hydralazine, imidazole and sulfhydryl compounds[19].

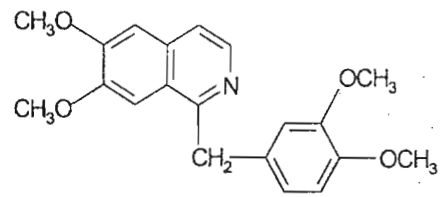
Many compounds of widely differing structures show phosphodiesterase inhibition[19]. Some of their structures were shown in Figure5.



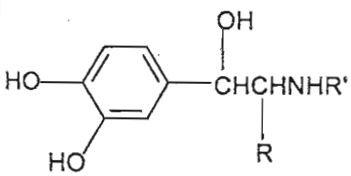
Rolipram



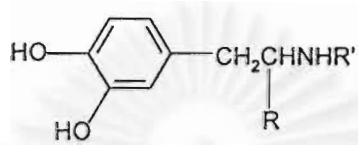
Theophylline



papaverine



phenethanolamine



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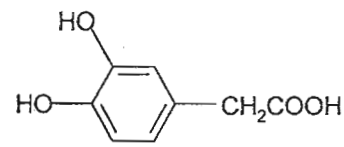
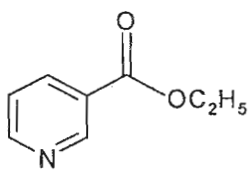
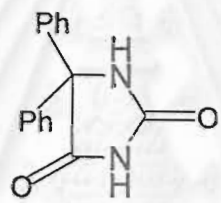


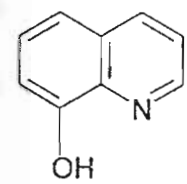
Catechol acid



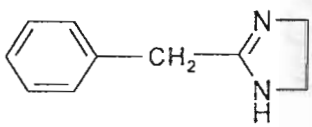
ethylnicotinate



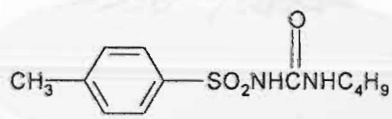
Diphenylhydantoin



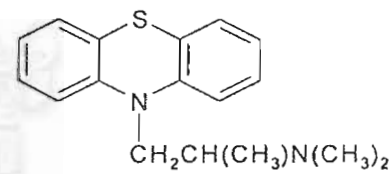
8-hydroxyquinoline



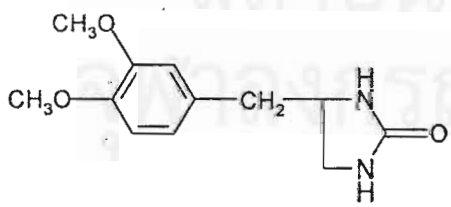
tolazoline



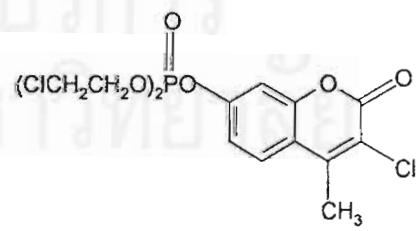
Tolbutamide



promethazine



4-(3,4-dialkoxybenzyl)-2-imidazolidinone



Acenocoumarol (Anticoagulants)

Figure 5. cAMP phosphodiesterase inhibitors

Phosphodiesterase inhibition among various drug classes is perhaps not unexpected since cyclic nucleotides appear to be involved in the control of nearly all facets of cellular activity and metabolism in both healthy and disease states. The cyclic nucleotides, therefore, understandably present a worthy target for the action of drugs. The association between drugs and cyclic nucleotides is strong and vital to the successful mission of both in maintaining health.

In this research, *G. mallaccensis* Ridl. was preliminary screened. It was found that ethanol and chloroform crude extracts exhibited highly inhibitory activity against PDE. Therefore, it was of interest to further investigate their chemical constituents.

CHAPTER III
Experimental

Plant material

The plant material of *G. malaccensis* Ridl. used in this study were purchased in Bangkok, Thailand. Botanical identification was achieved through comparison with the herbarium specimen plant in the Royal Forest Department of Thailand.

Instruments and Equipments

1. Rotary Evaporator

The Eyela rotary evaporator model N-1 was used for the rapid removal of large amounts of volatile solvents.

2. Melting point recorder

The melting points were recorded on a Fisher-Johns melting point apparatus

3. Fourier Transform-Infrared Spectrophotometer (FT-IR)

The FT-IR spectra were recorded on a Nicolet Impact 410 Spectrophotometer. Spectra of solid samples were recorded as KBr pellets. Liquid samples were recorded as thin film on NaCl cell.

4. Ultraviolet-visible Spectrophotometer (UV-VIS)

The UV-VIS spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer in chloroform.

5. Nuclear Magnetic Resonance Spectrometer (NMR)

The ^{1}H and ^{13}C Nuclear magnetic resonance spectra were recorded at 200.13 and 50.32 MHz. respectively, on a Bruker Model AC-F200 Spectrometer, and at 500.00 and 125.65 MHz. on a JEOL JNM-A500 Spectrometer in deuterated chloroform. Chemical shifts are given in parts per million using residual protonated solvent as reference. HMQC, HMBC, COSY and NOESY experiments were performed on the JEOL JNM-A500 Spectrometer.

6. Mass Spectrometer (MS)

Low resolution mass spectra were acquired by a Fison Instruments Mass Spectrometer Model Trio 2000 in EI mode at 70 eV.

7. Elemental Analysis (EA)

The EAs values were measured by a Perkin Elmer PE 2400 Series II (CHN/O Analyzer)

8. Optical Rotation

The optical rotation values were measured by a Perkin-Elmer 341 polarimeter in chloroform.

9. X-ray Diffractrometer

The x-ray diffractrometer were obtained on a SIEMEN SMART diffractrometer at Department of Physics, Faculty of Science and Technology, Thammasat University.

Chemicals

1. Solvents

All commercial grade solvents used in this research such as hexane, chloroform, ethyl acetate and ethanol were purified by distillation prior to use

2. Chromatographic Media

2.1 Merck's silica gel 60 G Art. 7734 (70-230 mesh ASTM) and 9385 (230-400 mesh ASTM) were used as adsorbents for normal column chromatography and flash column chromatography.

2.2 Merck's TLC Aluminum sheets, silica gel 60 F_{254} precoated 25 sheets, 20x20 cm^2, layer thickness 0.2 mm were used for TLC analysis.

3. For PDE Assay

3.1 Malachite green or Brilliant green($C_{27}H_{34}N_2O_4S$) from Tokyo Kasei Kogyo, Japan.

3.2 Polyvinyl alcohol from Metro Company Limited.

3.3 Ammonium molybdate from E. Merck Company, Germany.

3.4 cAMP Phosphodiesterase (bovine heart) from Sigma Chemical Company, United States.

3.5 5'-nucleotidase (*Crotualus atrox* venon) from Sigma Chemical Company, United States.

3.6 Adenosine 3',5'-cyclicmonophosphate(c-AMP)sodium salt. from Sigma Chemical Company, United States.

3.7 Magnesium chloride hexahydrate from J.T.Barker Inc., United States.

3.8 Sodium citrate dihydrate from E.Merck, Germany.

3.9 Sodium dihydrogen phosphate monohydrate from E.Merck, Germany.

3.10 Tris (hydroxymethyl)-aminomethane from E.Merck, Germany.

3.11 Theophylline (1,3-dimethylxanthine) anhydrous from Sigma Chemical Company, United States.

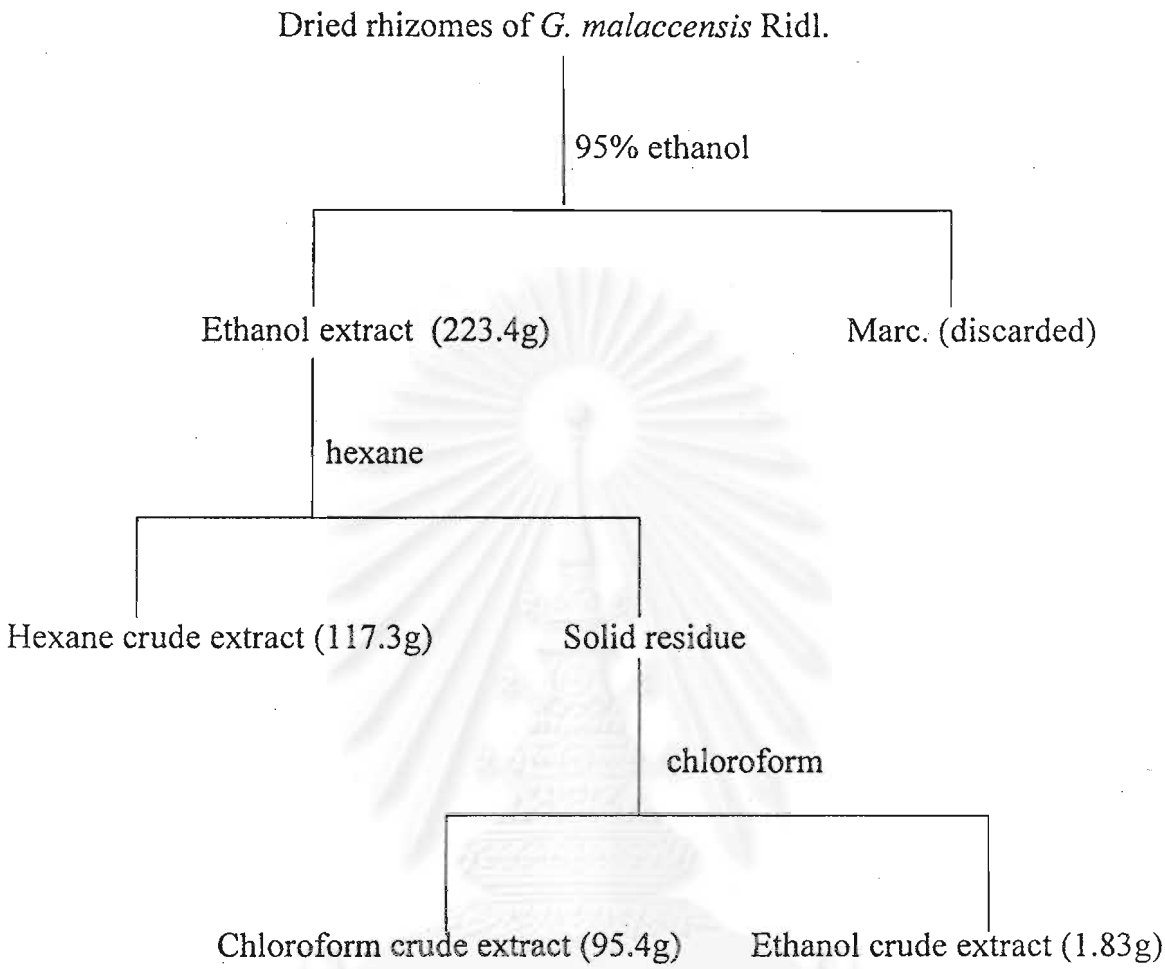
3.12 Caffeine (1,3,7-trimethylxanthine) andydrous from Sigma Chemical Company, United States.

Extraction and Isolation

The dried rhizomes of *G. malaccensis* Ridl. (4kg.) were coarsely ground and then soaked in 95% ethanol (1 x 9liters) for 4-5 days at room temperature for two times. The combined filtrate was evaporated under reduced pressure to dryness yielding the ethanol extract. Then the ethanol extract was re-extracted with hexane (3 x 1liters) until the solution was colorless. The filtered hexane solution was evaporated to afford the hexane crude extract as a dark-brown oil.

The hexane insoluble part was re-extracted with chloroform (3 x 1.5liters) repeatedly until the solution was clear. The combined chloroform solution was concentrated on a rotary evaporator under reduced pressure to give the chloroform crude extract as a viscous dark-brown residue and the final insoluble residue as a dark brown gummy. The procedure and results of the extraction are shown in Scheme 1

Scheme 1. The procedure of extraction of the dried rhizomes of *G. malaccensis* Ridl.



The crude extracts of the rhizomes of *G. malaccensis* Ridl. with various solvents were shown in Table 2.

Table 2. The crude extracts of the rhizomes of *G. malaccensis* Ridl with various solvents.

Solvent extract	Appearance	Weight (g)	% wt.by wt. of the dried rhizome
Hexane	dark-brown oil	117.3	2.93
Chloroform	dark-brown oil	95.4	2.39
Ethanol	dark-brown gummy	1.83	0.05

Isolation of Crude Extracts of *G. malaccensis* Ridl.

-Separation of hexane crude extract

The nonbioactive hexane crude extract was obtained as a dark-brown oil (117.3 g) after evaporation. The hexane crude extract was fractionated by open column chromatography using Merck's silica gel Art. 7734.1000 (70-230 mesh ASTM) as an adsorbent. The column was eluted with hexane-ethylacetate gradient in a stepwise fashion to give similar compounds found in chloroform except for Compound 3 and Compound 4.

-Separation of chloroform crude extract.

The chloroform crude extract (95.4g) was preadsorbed on silica gel 70-230 mesh ASTM (2x20g) prior to application to the top of the column. The column was eluted in order of increasing polarity to give compounds 1,2,3 and 4, respectively.

-Separation of ethanol crude extract.

The ethanol crude extract was obtained as a dark-brown gummy residue (1.83g). This extract was not selected for further separation, even though it exhibited highly inhibitory activity to PDE since it was insoluble in all solvents and ,moreover, investigation by using TLC analysis revealed that separation by silica gel column was not possible.

Purification and physical properties of isolated compounds from chloroform crude extract.

Purification and properties of Compound 1

Compound 1 was eluted with 30 % ethylacetate in hexane and similar fractions were combined. The solvent was removed under reduced pressure by rotary evaporator. It was purified repeatedly by column chromatography (Merck's silica gel Art. 1.09385.1000) with 25% ethylacetate-hexane solvent system. This compound is UV inactive and soluble in chloroform, ethylacetate and ethanol.

Re-crystallization from hexane and ethylacetate (2:1) gave colourless-needle crystals of Compound 1 (48 mg, 0.05 % wt.by wt. of chloroform crude extract and $1.2x10^{-3}$ % wt.by wt. of the dry plant) R_f = 0.75 (50% Ethylacetate-hexane) mp. 145-146 °C, $[\alpha]_D^{20}$ +36.5° ($CHCl_3$; c 0.2)

This compound has no absorption peak in UV absorption.

FT-IR spectrum (Fig.25, Table 4) $\nu_{max}(cm^{-1})$: 3400-3300 (br), 2954 (s), 2904(s), 1694 (s), 1645 (s), 1446 (m), 1391 (m), 1107 (s) and 883 (s)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz.)(Fig.26)

δ (ppm) : 0.9 (3H, d, J=6.4 Hz), 1.4-1.5 (2H, m), 1.61 (3H, s), 1.65-1.74 (2H, m), 1.77 (3H, m), 1.9-2.0 (2H, m), 2.02 (1H, s), 2.25 (1H, dd, J=10.1,10.0 Hz), 2.43 (1H, d, J=11.8 Hz), 2.5-2.6 (2H, ddd, J=10.4,1.5,1.0 Hz), 4.68 (1H, dd, J=2.1,2.1 Hz), 4.76 (1H, dd, J=2.2,2.2 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 500 MHz.)(Fig.27, Table 5)

δ (ppm) : 146.8 (s), 134.7 (s), 127.1 (s), 112.4 (t), 105.0 (s), 87.9 (s), 54.1 (d), 43.1 (t), 40.0 (d), 37.3 (t), 31.9 (t), 29.3 (t), 22.5 (q), 19.2 (q) and 12.8 (q)

m/z (EI) (Fig.29) : 234[M^+] (5.5), 219 (5.5), 201 (4.2), 191 (50), 173 (15), 147 (27), 133 (21), 121 (73), 105 (100), 95(26), 93(30), 91(29) and 67 (42)

Purification and properties of Compound 2

Compound 2 was eluted with 30 % ethylacetate in hexane. Similar fractions were combined and the solvent was removed by rotary evaporation. It was further purified by column chromatography (Merck's silica gel Art 7734.1000, 70-230 mesh ASTM) and then re-crystallization with hexane and ethylacetate (1:2) to give colourless-prism crystals of Compound 2 (2.2 g, 2.30 % wt.by wt. of chloroform crude extract and $5.5x10^{-2}$ % wt.by wt. of the dry plant) It is soluble in chloroform, ethylacetate and ethanol.

R_f = 0.71 (50% Ethylacetate-hexane) mp. 114-116 °C, $[\alpha]_D^{20}$ +36.2° ($CHCl_3$; c 0.2) UV $\lambda_{max}^{CHCl_3}$ nm (log ε) : 256 (1.07)

FT-IR spectrum (Fig.30, Table 6) $\nu_{max}(cm^{-1})$: 3400-3300 (br), 2874-2954 (s), 1655 (s), 1620 (s), 1445 (m), 1376 (s), 1306 (s), 1276 (s), 1216 (s), 953 (m) and 753 (s)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz.)(Fig.31, Table 7)

δ (ppm) : 1.0 (3H, d, J=6.7 Hz), 1.50 (1H, m), 1.58 (3H, s), 1.64 (3H, s), 1.77 (3H, s), 1.8-2.0 (6H, m), 2.13 (1H, d, J=15.5 Hz), 2.65 (1H, d, J=15.5 Hz), 4.84 (1H, s) and 5.71 (1H, d, J= 1.9 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 500 MHz.)(Fig.32, Table 9)

δ (ppm) : 139.3 (s), 138.1 (s), 127.5 (s), 122.4 (d), 102.6 (s), 86.6 (s), 52.3 (d), 41.4 (d), 38.1 (t), 32.3 (t), 28.7 (t), 22.4 (q), 21.0 (q), 19.1 (q) and 12.2 (q)

m/z (EI) (Fig.40) : 234[M^+] (32), 191 (23), 189 (53), 173 (23), 165 (19), 149 (19), 147 (48), 145(32), 133 (59), 121(27), 119(30), 105 (100) and 91 (36)

Purification and properties of Compound 3

Compound 3 was eluted with 65 % ethylacetate in hexane. Similar fractions were combined and the solvent was removed by rotary evaporation. It was further purified by column chromatography (Merck's silica gel Art 7734.1000, 70-230 mesh ASTM) and then re-crystallization with ethylacetate and methanol (3:1) to give orange-needle crystals of Compound 3 (5.4 g, 5.66 % wt.by wt. of chloroform crude extract and $1.3x10^{-1}$ % wt.by wt. of the dry plant) It is soluble in chloroform, ethylacetate and ethanol.

R_f = 0.28 (50% Ethylacetate-hexane) mp. 182-183 °C UV $\lambda_{max}^{CHCl_3}$ nm (log ε) : 262 (1.26)

FT-IR spectrum (Fig.41, Table 14) $\nu_{max}(cm^{-1})$: 3600-3200 (br), 1625 (s), 1585 (m), 1510 (s), 1428 (m), 1280 (m) and 1137 (m)

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.)(Fig.42)

δ (ppm) : 1.56 (2H,s), 3.93 (6H,s), 5.82 (2H,d, J=12.8 Hz), 5.77 (2H,s), 6.46 (2H,d, J=12.8 Hz), 7.06 (4H,m) and 7.58 (1H, d, J=10.0 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 50 MHz.)(Fig.43, Table 15)

δ (ppm) : 183.6 (s), 148.0 (d), 146.9 (s), 140.8 (s), 127.7 (d), 122.8 (s), 121.7 (d), 114.9 (d), 109.7 (d), 101.2 (t) and 56.1 (q)

m/z (EI) (Fig.45) : 368 [M^+](52), 350(50), 320(16), 272(20), 232(35), 226 (27), 217(55), 191(76), 190(96), 177(100), 175(39), 147(35), 145(55), 137(56), 131(35), 117(30), 91(37), 89(52) and 77(44)

Purification and properties of Compound 4

Compound 4 was eluted with 80 % ethylacetate in hexane and similar fractions were combined. The solvent was removed under reduced pressure by rotary evaporator. It was further purified by column chromatography (Merck's silica gel Art. 1.09385.1000) and then re-crystallization with hexane and ethylacetate (1:3) to give colourless-needle crystals of Compound 4 (90 mg, 0.09 % wt.by wt. of chloroform crude extract and $1.4x10^{-3}$ % wt.by wt. of the dry plant). It is soluble in chloroform, ethylacetate and ethanol.

R_f = 0.39 (50% Ethylacetate-hexane) mp. 134-135 °C, $[\alpha]_D^{20}$ –41° ($CHCl_3$; c 0.2) UV $\lambda_{max}^{CHCl_3}$ nm (log ε) : 254 (2.60)

FT-IR spectrum (Fig.46, Table 22) $\nu_{max}(cm^{-1})$: 3400-3300 (br), 2962-2876 (m), 2352 (m), 1711 (s), 1667(s), 1366 (s), 1223 (s) and 820 (s)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz.)(Fig.47, Table 23)

δ (ppm) : 1.17 (3H,s), 1.19 (3H,s), 1.37 (1H,dt ,J=1.5,12.0 Hz), 1.66 (2Hm), 1.71 (2H,m), 1.78 (2H,m), 1.81 (3H,s), 1.92 (3H,s), 1.96 (1H,m), 2.58 (1H,d, J=12.8 Hz) and 2.94 (1H,d, J=13.0 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 500 MHz.)(Fig.48, Table 25)

δ (ppm) : 202.7 (s), 142.1 (s), 134.6 (s), 79.9 (s), 72.7 (s), 59.8 (t), 55.9 (d), 52.0 (d), 39.7 (t), 28.5 (t), 22.8 (t), 22.7 (q), 22.1 (q), 21.8 (q) and 20.6 (q)

m/z (EI) (Fig.56) : 252[M^+] (0.5), 234 (13), 219 (8.2), 206 (9.9), 191 (23), 173 (12), 149 (23), 133 (21), 122 (22), 107 (27), 83(25), 81 (100) and 67(27)

X-ray Diffraction

Compound 2 and 3 were identified by x-ray diffraction analysis. All data were collected at room temperature using graphite monochromated Mo Kα Radiation (lamda = 0.71069 A°) on Siemens SAMART CCD diffractrometer. The data were corrected for Lorentz and polarization effects. The crystal experiment data of Compound 2 and 3 were given in Table 10 and 16 respectively.

The structure were solved by direct methods using SHELXS-97 and refined by full matrix least-square on F^2 using SHELXS-97 .with anisotropic thermal parameters for all the non-hydrogen atoms. All the hydrogen atoms were found in difference Fourier maps and were included in refinement. The fraction coordinates of non-hydrogen atom and selected bond distances and angels of Compound 2 and 3 were listed in Table 11,12,13 and 17,18,19,20,21 respectively.

Assay Method for Inhibition of PDE

Samples were tested for inhibition of cAMP phosphodiesterase activity in duplicate by following experimental of Nattaya Chairungsrilerd and co-workers[20].

Bioassay. Phosphodiesterase activity was determined from the amount of inorganic phosphate liberated from the reaction of adenosine 5'-monophosphate and 5'-nucleotidase. It was determined by the malachite green method which is highly sensitive to inorganic phosphate. Phosphodiesterase assay solutions were as follows: (a) The enzyme solution contains phosphodiesterase (0.3 units / ml.), 5'-nucleotidase (13.4 units / ml.), $MgCl_2$ (50 mM.) and Tris-HCl (500 mM.) (b) The reaction mixture A contains malachite green (1.68 mM.), polyvinyl alcohol (23.2 g/l) and ammonium molybdate in 6N HCl (57.2 g/l). Sample was dissolved in 1.5 % dimethyl sulfoxide. The reaction was started by the addition of cyclic AMP (10mM, 100µl.) to the enzyme solution (400µl.) at 30°C. After that, sample solution (500µl.), the reagent mixture A (1.0ml.) and 25% sodium citrate (200µl.)

were added to above solution successively every 5 minutes. The absorbance of the coloured complex was measured at 630 nm. using a UV spectrophotometer. A mixed reagent blank was used as reference, obtained by this procedure using potassium dehydrophosphate solutions of known concentrations, was used to determine the amount of phosphorus present in the assay. (Fig.6, Table 3) In the control experiment, dimethyl sulfoxide was added instead of the solution of sample to minimize the effect of the solvent. Biological activity of samples was compared with theophyllene (reference compound for phosphodiesterase assay.) All reagents were prepared freshly and de-ionizing water was used in making these reagents.

Table 3. Concentration and Absorbance of standard phosphate solution (NaH_2PO_4 aqueous solution).

Concentration of Phosphate (μM.)	Absorbance at 630 nm.
0.0	0.0
10.0	0.15
20.0	0.42
30.0	0.60
40.0	0.78

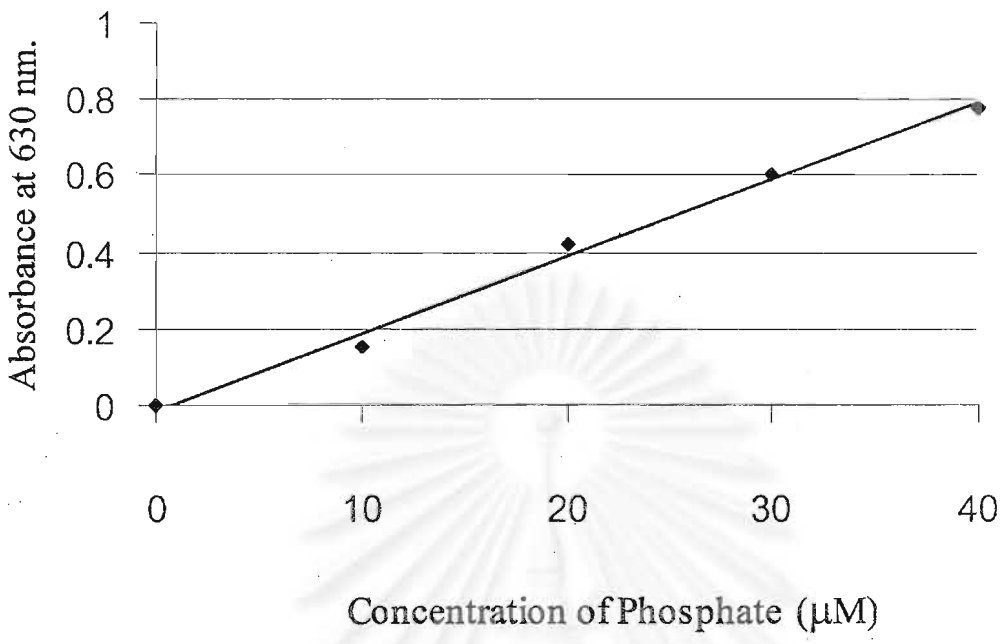


Figure 6. Standard curve of standard phosphate solution.

CHAPTER IV
Results and Discussion

The chloroform crude extract extracted from the rhizomes of *G. malaccensis* Ridl. was separated by chromatography on a silica gel column using a hexane-ethylacetate gradient system. Compound 1,2,3 and 4 were isolated.

Structural elucidation of Compound 1

The IR spectrum of Compound 1(Fig.25) displayed broad band in the range of 3400-3300 cm^{-1} belonging to O-H stretching and the absorption peak of C-O stretching vibration at 1107 cm^{-1}. The additional bands of olefinic moiety were also observed at 1694 and 1645cm^{-1}. The IR spectrum of Compound 1 was summarized in Table 4.

Table 4. The IR absorption bands assignment of Compound 1

Wave number (cm^{-1})	Peak intensity	Tentative assignment
3400-3300	Broad	O-H stretching vibration of alcohol
2954,2904	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1694,1645	Strong	C=C stretching vibration
1442	Medium	C-H bending vibration of $-CH_2$
1107	Strong	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig.26) exhibited the important signal at δ 4.68 ppm(1H, J=2.1 Hz) and δ 4.76 (1H, J=2.2 Hz) ppm.which compatible with a terminal methylene proton (CH_2=CR_2). A proton of hydroxy group show the signal at δ 2.02 ppm. Other signals in the region of δ 0.9-2.6 ppm were attributed to methyl, methylene and methine protons.

The ^{13}C-NMR spectrum (Fig.27, Table 5) displayed a total 15 signals of carbons. It showed the olefinic carbon signals at δ 112.4, 127.0, 134.7 and 114.8 ppm. The two carbons bearing one atom and two atoms of oxygen showed the signals at δ 88.0 and 105.0 ppm respectively. The signals of methyl, methylene and methine carbons were also evidenced at around δ 54.1 to 12.8 ppm

DEPT 90 experiments (Fig.28) indicated the presence of two saturated methine carbons at δ 54.1 and 40.0 ppm.

DEPT 135 spectrum (Fig.28) showed five methylene carbons at δ 112.4, 43.1, 37.3, 31.9 and 29.3 ppm and three methyl carbons at δ 22.5, 19.2 and 12.8 ppm, which indicated that the carbon signals at δ 146.8, 134.7, 127.1, 105.0 and 88.0 ppm were quaternary carbons.

The EI mass spectrum (Fig.29) of this compound gave a molecular ion at m/z 234 in accordance with a sesquiterpenoid containing one hydroxy group which was corresponding to the molecular formula $C_{15}H_{22}O_2$ indicating the DBE of 5. These data precluded the possibility of unsaturation and therefore required Compound 1 to have an oxygenated pentacyclic skeleton. Its structure could be deduced as isocurcumenol. This deduction was established by comparing the ^{13}C-NMR spectrum of this compound with those of the known isocurcumenol [21]. The result was displayed in Table 5.

Table 5. The ^{13}C-NMR chemical assignment of Compound 1 [21].

Carbon position	Chemical shift (ppm)	
	Compound 1	Isocurcumenol
1	54.1 (d)	52.7 (d)
2	29.3 (t)	28.3 (t)
3	31.9 (t)	30.8 (t)
4	40.0 (d)	41.7 (d)
5	87.9 (s)	86.9 (s)
6	43.1 (t)	38.9 (t)
7	134.7 (s)	133.0 (s)
8	105.0 (s)	103.8 (s)
9	37.3 (t)	36.2 (t)
10	146.8 (s)	144.8 (s)
11	127.1 (s)	126.5 (s)
12	22.5 (q)	22.4 (q)
13	19.2 (q)	18.9 (q)
14	12.8 (q)	12.4 (q)
15	112.4 (t)	111.9 (t)

It could be concluded that Compound 1 exhibited the ^{13}C-NMR chemical shifts similarly to those of isocurcumenol. Therefore, Compound 1 was assigned as isocurcumenol which was previously isolated from *C. Zedoaria* Roscoe. and *C. Heyneana*.[22] In 1999, Jan W. Bats and collaboraters had reported the absolute configuration of isocurcumenol. The C=C double bonds in the 5-isopropylidene and 8-methylene groups were confirmed. The molecules are arranged by intermolecular hydrogen bonds between the OH groups to form helices about 3/2 screw axes[23].

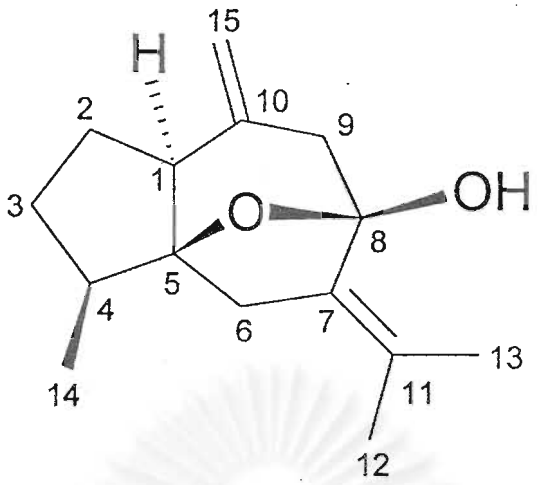


Figure 7. The structure of Compound 1

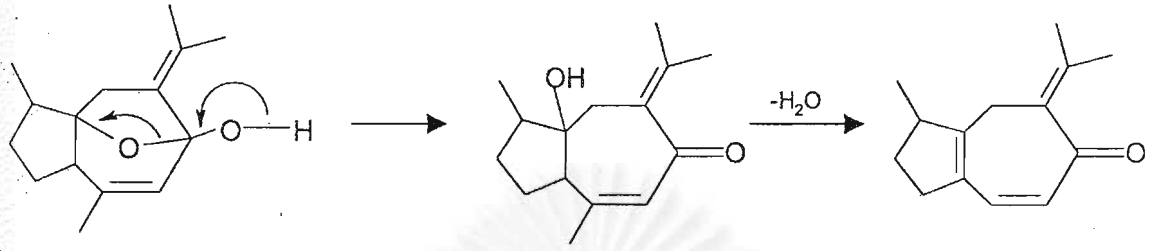
Structural elucidation of Compound 2

The IR spectrum (Fig.30) exhibited an absorption band due to O-H stretching vibration of hydroxy group at 3400-3300 cm^{-1}. The absorption bands at 1694 and 1645 cm^{-1} which correspond to the C=C stretching vibration were observed. The C-H stretching and bending vibrations of $-CH_3$ and $-CH_2$ were also observed at 2954-2874 cm^{-1} and 1445 cm^{-1} respectively. The IR spectrum of Compound 2 was summarized in Table 6.

Table 6. The IR absorption bands assignment of Compound 2

Wave number (cm^{-1})	Peak intensity	Tentative assignment
3400-3300	Broad	O-H stretching vibration of alcohol
2954-2874	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1655,1620	Strong	C=C stretching vibration
1445	Medium	C-H bending vibration of $-CH_2$

During NMR operations, it was investigated from the ^{13}C-NMR spectrum (Fig.32) that Compound 2 was unstable in CD_3OD and decomposed to be another molecule which contains the carbonyl group in its structure. This led to the proposed mechanism shown below.



The ^{1}H-NMR spectrum (Fig.31, Table 7) displayed the important signal at δ 5.71 ppm (1H,t,J=1.9) of methylene proton. The spectrum also showed the signals at δ 1.00, 1.58, 1.64, 1.77 ppm of protons belonging to four methyl groups.

The ^{13}C-NMR (Fig.32) and DEPT spectra (Fig.33) revealed 15 significant signals of carbon. The signals of quaternary carbons at δ 139.3, 138.1, 122.4, 102.6 and 86.6 ppm , three methine carbons at δ 127.5, 52.3 and 41.4 ppm, three methylene carbons at δ 38.7, 32.3 and 28.7 ppm and four methyl carbons at 22.4, 21.0, 19.1 and 12.2 ppm were visualized.

The EI mass spectrum (Fig.40) showed a molecular ion with m/z 234 ($C_{20}H_{22}O_2$) that indicated DBE of 5 and also supported the proposed structure of Compound 2. The information from 2D-NMR techniques; HMQC correlation (Fig.34, Table 7), HMBC correlation (Fig.35, Table 8), COSY correlation (Fig.36-37, Table 8) and NOESY correlation (Fig.38-39) were used to assist the interpretation of the structure of Compound 2.

Table 7. The HMQC spectral data of Compound 2.

^{13}C-NMR (ppm)	^{1}H-NMR (ppm), coupling constant (Hz)
12.2(q)	1.00d (J = 6.7)
19.1(q)	1.58s
21.0(q)	1.77 (J = 2.0)
22.4(q)	1.64s
28.7(t)	1.50m, 1.99m
32.3(t)	1.92m
38.1(t)	2.13dt (J = 2.3, 15.5), 2.65dt (J = 2.2, 15.5)
41.1(d)	1.87m
52.3(d)	1.93m
86.6(s)	-
102.6(s)	-
122.4(s)	-
127.5(d)	5.71t (J = 1.9)
138.1(s)	-
139.3(s)	-

Table 8. The HMQC, HMBC and COSY spectral data of Compound 2.

Position	δ_C	δ_H	HMBC (H to C)	COSY
1	52.3(d)	1.93m	C-2,C-3,C-5,C-10, C-15	H-2(1.50)
2	28.7(t)	1.50m 1.99m	C-3,C-4 C-1,C-5,C-10	H-1(1.93),H-3(1.92)
3	32.3(t)	1.92m	C-4,C-5	H-2(1.50),H-4(1.87),H-14(1.00)
4	41.1(d)	1.87m 2.16d	C-2,C-3,C-5 C-18	H-3(1.92),H-14(1.00)
5	86.6(s)	-	-	-
6	38.1(t)	2.13dt (J=2.3,15.5)	C-7,C-11	H-6(2.65),H-13(1.58), H-15(1.77)
		2.65dt (J=2.2,15.5)	C-1,C-5,C-7	H-6(2.13),H-13(1.58), H-15(1.77)
7	139.3(s)	-	-	-
8	102.6(s)	-	-	-
9	127.5(d)	5.71t (J=1.9)	C-1,C-8,C-15	H-13(1.58)
10	138.1(s)	-	-	-
11	122.4(s)	-	-	-
12	22.4(q)	1.64s	C-1,C-7,C-9,C-11	-
13	19.1(q)	1.58s	C-7,C-11	H-6(2.13,2.65),H-5.71
14	12.2(q)	1.00d (J=6.7)	C-3,C-4,C-5	H-3(1.92),H-4(1.87)
15	21.0(d)	1.77t (J=2.0)	C-10	H-6(2.13,2.65)

Compound 2 showed spectral data identical to that of curcumenol reported in the literature.[21] The signal of ^{13}C-NMR spectra of this compound was compared as shown in Table 9.

Table 9. the ^{13}C-NMR chemical assignment of Compound 2 [21].

Carbon position	Chemical shift (ppm)	
	Compound 2	curcumenol
1	52.3 (d)	52.1 (d)
2	28.7 (t)	28.4 (t)
3	32.3 (t)	32.3 (t)
4	41.4 (d)	41.3 (d)
5	86.6 (s)	86.2 (s)
6	38.1 (t)	38.1 (t)
7	139.3 (s)	138.2 (s)
8	102.6 (s)	102.4 (s)
9	127.5 (d)	128.0 (d)
10	138.1 (s)	137.9 (s)
11	122.4 (s)	121.7 (s)
12	22.4 (q)	22.9 (q)
13	19.1 (q)	19.7 (q)
14	12.2 (q)	12.8 (q)
15	21.0 (q)	21.4 (q)

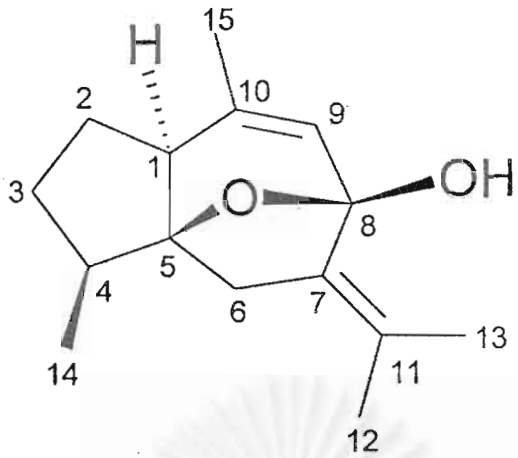


Figure 8. The structure of Compound 2

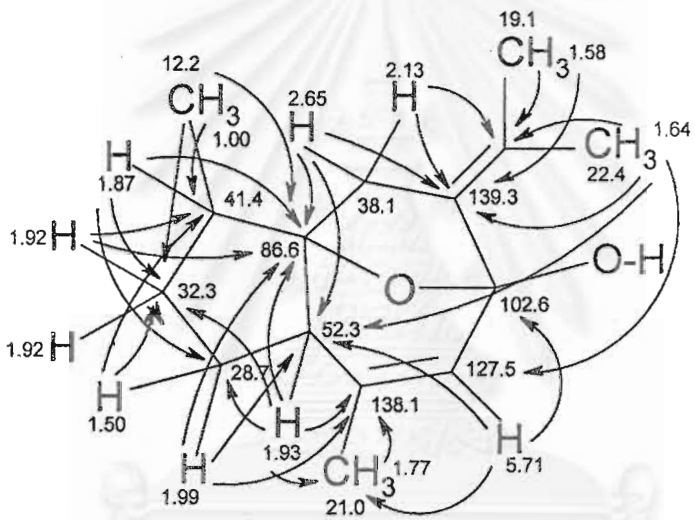


Figure 9. The HMBC correlation of Compound 2

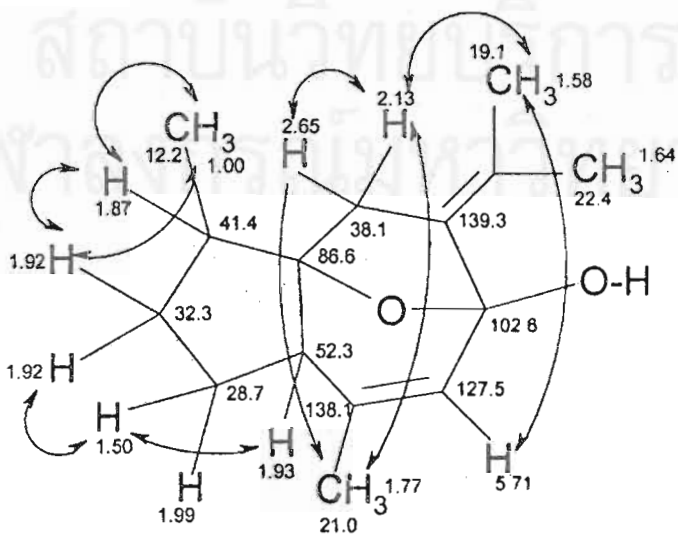


Figure 10. The COSY correlation of Compound 2

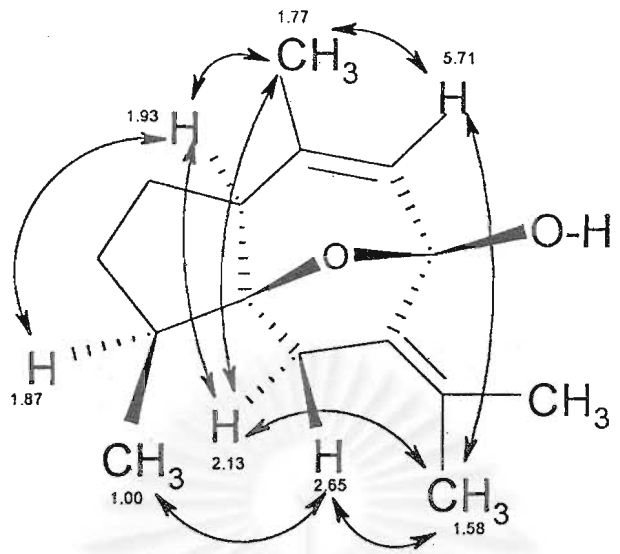


Figure 11. The NOESY correlation of Compound 2

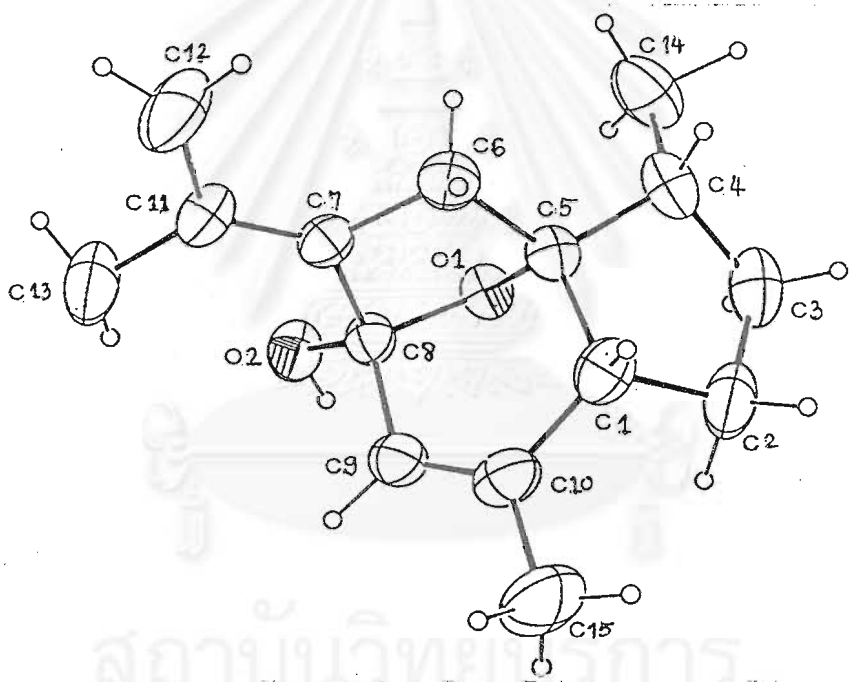


Figure 12. The ORTEP structure of Compound 2.

Furthermore, the structure of Compound 2 was unequivocally confirmed by x-ray diffraction analysis of a crystal obtained using hexane-ethylacetate (1:1) as the solvent system.It was the first report of the x-ray crystallographic analysis of curcumenol but some report have been made of its derivative which is oxycurcumenol[21]. The molecular structure was shown in Figure 8. The x-ray diffraction information was given in Table 10,11,12 and 13.

Table 10. Crystal data and structure refinement for Compound 2.

Empirical formula	$C_{15}H_{22}O_2$
Formula weight	234.33
Temperature	293(2) K
Wavelength	0.71073 A°
Crystal system, space group	Monoclinic, C2
Unit cell dimensions	a = 16.8467(4)A° alpha = 90 deg.
	b = 7.6799(2)A° beta = 115.9970(10) deg.
	c = 11.86130(10)A° gamma = 90 deg.
Volume	1379.35(5)$A°^3$
Z, Calculated density	4, 1.128 Mg/m^3
Absorption coefficient	0.073 mm^{-1}
F(000)	512
Theta range for data collection	1.91 to 30.56 deg.
Limiting indices	$-20 \leq h \leq 23$, $-10 \leq k \leq 10$, $-16 \leq l \leq 12$
Reflections collected / unique	5199 / 3276 [R(int) = 0.0218]
Completeness to theta = 30.56	95.2 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3276 / 1 / 230
Goodness-of-fit on F^2	1.039
Final R indices [I > 2sigma(I)]	R1 = 0.0491, wR2 = 0.1202
R indices (all data)	R1 = 0.0604, wR2 = 0.1286
Absolute structure parameter	-0.9(11)
Largest diff. peak and hole	0.210 and –0.288 $e.A^{-3}$

Table 11. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($A^2 \times 10^3$) for Compound 2.

	X	Y	Z	U(eq)*
C(1)	9655(1)	-898(3)	7251(2)	44(1)
C(2)	8699(2)	-1306(4)	6255(2)	58(1)
C(3)	8171(1)	352(4)	6125(2)	59(1)
C(4)	8750(1)	1560(3)	7211(2)	49(1)
C(5)	9683(1)	1086(2)	7422(2)	35(1)
C(6)	10452(1)	1757(3)	8621(2)	42(1)
C(7)	11191(1)	2029(2)	8239(1)	34(1)
C(8)	10793(1)	1540(2)	6853(1)	33(1)
C(9)	10895(1)	-401(3)	6724(2)	40(1)
C(10)	10371(1)	-1538(3)	6906(2)	47(1)
C(11)	12019(1)	2492(3)	8986(2)	44(1)
C(12)	12296(2)	2853(5)	10354(2)	68(1)
C(13)	12738(2)	2644(5)	8572(3)	65(1)
C(14)	8520(2)	3473(4)	6998(3)	68(1)
C(15)	10465(2)	-3476(3)	6837(4)	79(1)
O(1)	9860(1)	1871(2)	6443(1)	34(1)
O(2)	11090(1)	2557(2)	6155(1)	43(1)

*U(eq) is defined as one third of the trace of the orthogonalized.

Table 12. Bond distances (A°) for Compound 2.

Bond Distances	Distances(A°)	Bond Distances	Distances(A°)
C(1)-C(10)	1.299(3)	C(6)-C(7)	1.514(2)
C(1)-C(5)	1.292(4)	C(7)-C(8)	1.526(2)
C(1)-C(2)	1.359(4)	C(8)-O(2)	1.381(2)
C(2)-C(3)	1.352(4)	C(8)-O(1)	1.4496(19)
C(3)-C(4)	1.426(5)	C(8)-C(9)	1.516(3)
C(4)-C(14)	1.512(4)	C(9)-C(10)	1.326(3)
C(4)-C(5)	1.524(3)	C(10)-C(15)	1.503(3)
C(5)-O(1)	1.4495(18)	C(11)-C(13)	1.499(3)
C(5)-C(6)	1.534(2)	C(11)-C(12)	1.504(3)

Table 13. Bond Angels (deg.) for Compound 2.

Angels	deg.	Angels	deg.
C(10)-C(1)-C(5)	112.07(16)	C(11)-C(7)-C(8)	128.33(15)
C(10)-C(1)-C(2)	114.35(18)	C(6)-C(7)-C(8)	104.93(13)
C(5)-C(1)-C(2)	105.16(18)	O(2)-C(8)-O(1)	108.94(13)
C(3)-C(2)-C(1)	105.87(19)	O(2)-C(8)-C(9)	114.12(14)
C(2)-C(3)-C(4)	106.96(18)	O(1)-C(8)-C(9)	107.05(14)
C(14)-C(4)-C(5)	115.45(19)	O(2)-C(8)-C(7)	113.74(14)
C(14)-C(4)-C(3)	115.10(2)	O(1)-C(8)-C(7)	102.57(12)
C(5)-C(4)-C(3)	102.85(17)	C(9)-C(8)-C(7)	109.57(14)
O(1)-C(5)-C(4)	109.17(14)	C(10)-C(9)-C(8)	121.19(17)
O(1)-C(5)-C(6)	102.50(13)	C(9)-C(10)-C(15)	123.30(2)
C(4)-C(5)-C(6)	117.57(16)	C(9)-C(10)-C(1)	119.76(17)
O(1)-C(5)-C(1)	108.19(14)	C(15)-C(10)-C(1)	116.90(2)
C(4)-C(5)-C(1)	104.23(16)	C(7)-C(11)-C(13)	124.38(18)
C(6)-C(5)-C(1)	114.89(15)	C(7)-C(11)-C(12)	120.45(19)
C(7)-C(6)-C(5)	103.88(13)	C(13)-C(11)-C(12)	115.10(2)
C(11)-C(7)-C(6)	126.63(15)	C(5)-O(1)-C(8)	103.41(11)

From the literature survey, isocurcumenol and curcumenol were only found to show potent protective effect on D-galactosamine (D-GalN)/lipopolysaccharide (LPS)- induced acute liver injury in mice[24].

Biogenetic of Isocurcumenol and Curcumenol [22].

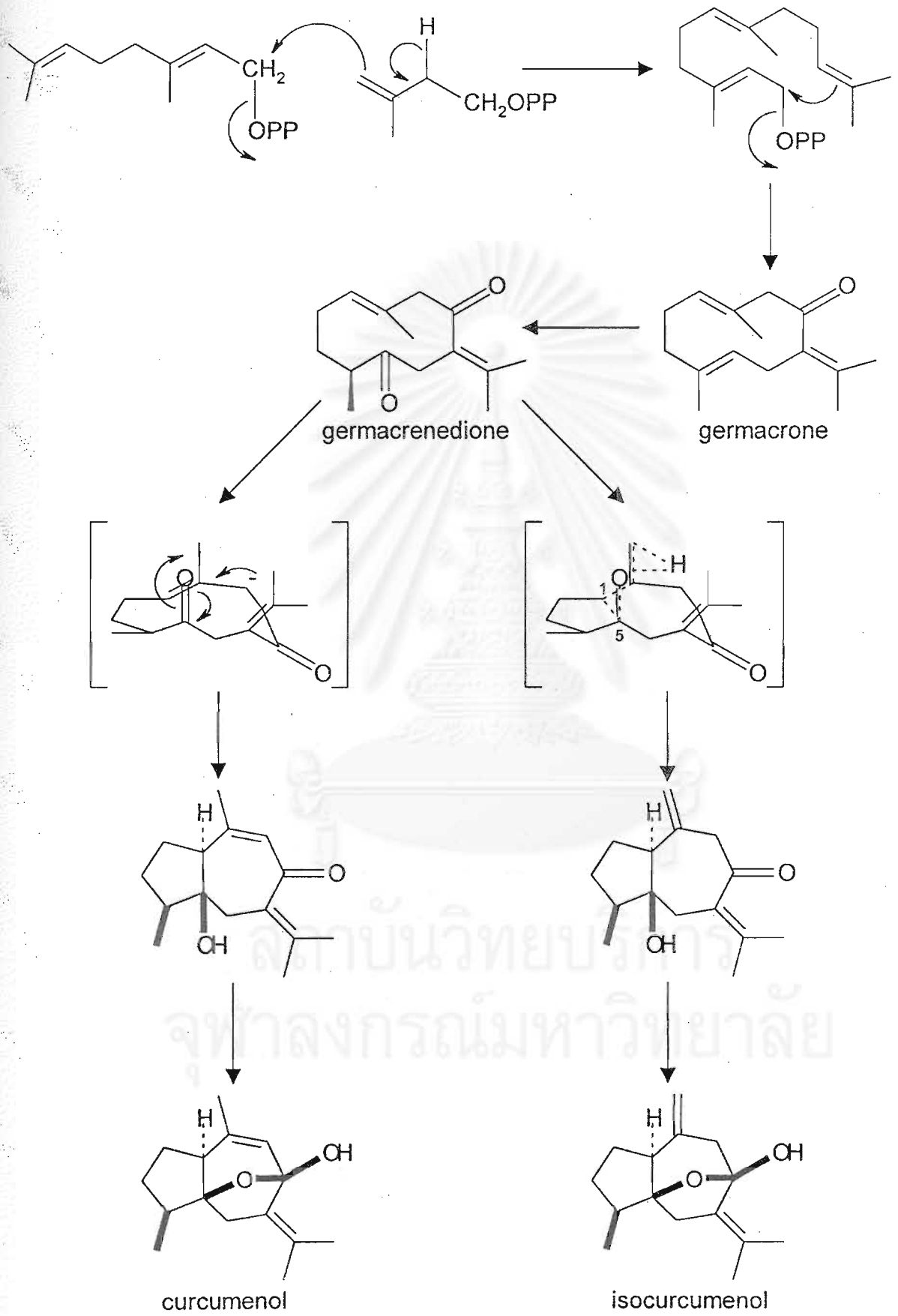


Figure 13. Biosynthesis of sesquiterpenoids found in *G.malaccensis* Ridl.

Structural elucidation of Compound 3

The IR spectrum of Compound 3 (Fig.41) clearly revealed the presence of phonolic hydroxy group according to the broad absorption band between 3600-3200 cm^{-1}. The strong absorption band at 1625 cm^{-1} revealed the presence of α,β-unsaturated carbonyl moiety and the characteristic absorption peak due to an aromatic moiety was observed at 1585, 1510 and 1428 cm^{-1}. The IR spectrum of Compound 3 was summarized in Table 14.

Table 14. The IR absorption bands assignment of Compound 3

Wave number (cm^{-1})	Peak intensity	Tentative assignment
3600-3200	Broad	O-H stretching vibration of phenol
1625	Strong	C=O stretching vibration of conjugated ketone
1585,1510,1428	Strong	C=C stretching vibration of aromatic

The ^{1}H-NMR spectrum (Fig.42) displayed the doublet signal at δ 7.58 ppm with 2H integration which could be assigned for an aromatic proton. Another aromatic signal was observed at δ 7.06 ppm (4H) and the six-proton singlet signal at δ 3.93 ppm was compatible to a methoxy group. Olefinic protons were detected at δ 6.46 ppm (2H,d) and δ 5.82 ppm (2H,d), which regarded to be tran-form due to the great value of coupling constant (J=12.8 Hz)

The ^{13}C-NMR spectrum (Fig.43, Table 15) showed only 11 lines, due to its symmetrical structure, which the carbonyl group of α,β- unsaturated ketone corresponding to the signal at δ 183.6 ppm. The six signals of aromatic group appeared at δ 146.9, 140.8, 122.8, 121.7, 114.9 and 109.7 ppm. Olefinic carbon appeared at δ 148.0 and 127.7 ppm. while the methoxy and methylene carbon appeared at δ 101.2 and 56.1 ppm ,respectively.

DEPT 90 experiments (Fig.44) indicated the presence of five sp^2 methine carbons at δ 148.0, 127.7, 121.7, 114.9 and 109.7 ppm.

DEPT 135 experiments (Fig.44) showed only one methylene carbon at 101.2 ppm and two methyl carbons at 56.1 ppm, which led to the indication that the carbon signals at 183.6, 146.9, 140.8 and 122.8 were quaternary carbons. The ^{13}C-NMR chemical shifts of Compound 3 were found to be very close to those of the known polyphenolic aromatic compound namely curcumin. The ^{13}C-NMR chemical shifts assignment of Compound 3 is shown in Table 15.

Table 15. The ^{13}C-NMR chemical assignment of Compound 3 [25].

Carbon position	Chemical shift (ppm)	
	Compound 3	Curcumin
1	56.1	56.3
2	101.2	101.6
3	109.7	111.6
4	114.9	116.2
5	121.7	122.3
6	122.8	123.8
7	127.7	128.2
8	140.8	141.4
9	146.9	148.8
10	148.0	150.0
11	183.6	184.5

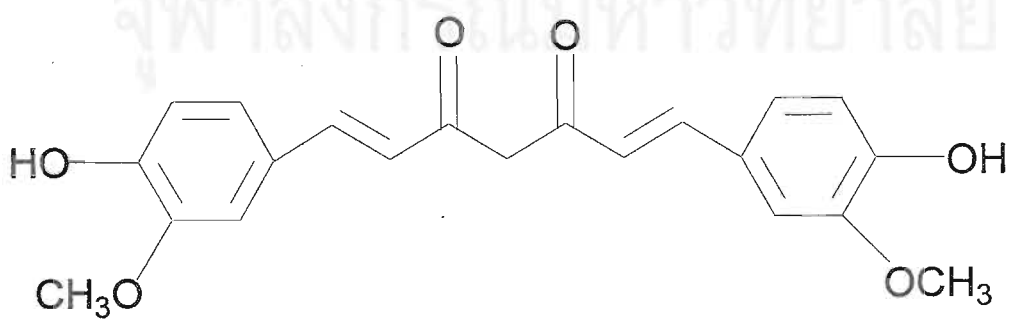


Figure 14. The structure of Compound 3.

The information obtained from mass spectrum (Fig.45) gave good agreement with those from IR, ^{1}H-NMR and ^{13}C-NMR spectra. It gave the expected molecular ion at m/z 368 which was corresponded to the molecular formula $C_{21}H_{20}O_6$ together with the series of fragmentation at m/z 177 and 137 which suggested that this compound should be a member of curcuminoid compound[26]. The possible mass fragmentation pattern of Compound 3 is presented in Figure 14.

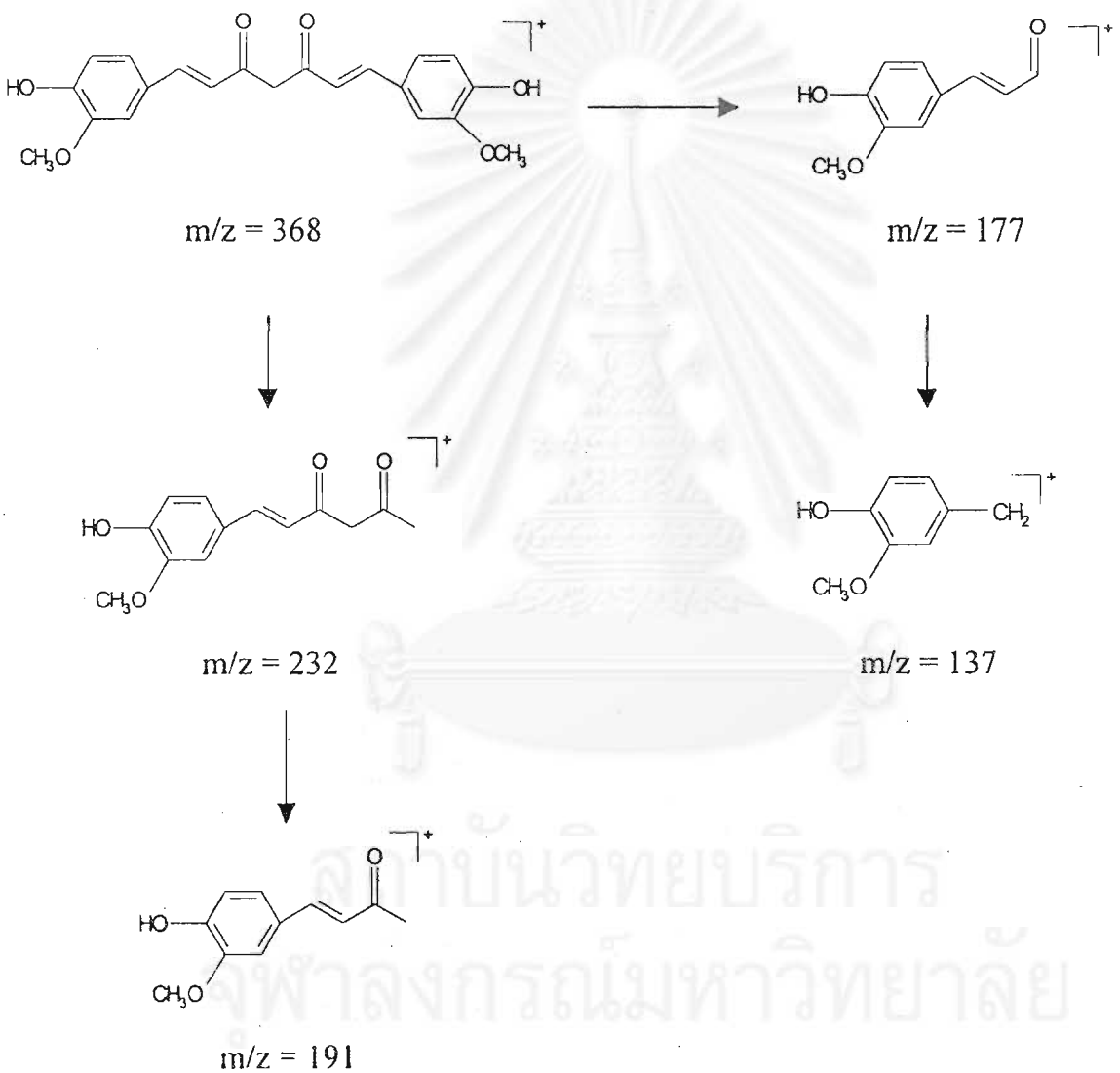


Figure 15. Mass fragmentation of curcumin

Recrystallization of compound 3 with hexane-ethylacetate (2:1) solvent system gave suitable crystal for x-ray diffraction analysis, which established the molecular structure as shown in Figure15. The obtained ORTEP structure was similar to that reported by Ishigami Yutaka et.al.[27]. The x-ray diffraction information is given in Table 16,17,18,19,20 and 21.

Table 16. Crystal data and structure refinement for Compound 3.

Empirical formula	$C_{21}H_{19}O_6$
Formula weight	367.36
Temperature	293(2) K
Wavelength	0.71073 A°
Crystal system, space group	Monoclinic, P2/n
Unit cell dimensions	a = 12.6987(6)A° alpha = 90 deg.
	b = 7.2140(3)A° beta = 95.498(2) deg.
	c = 19.8448(8)A° gamma = 90 deg.
Volume	1809.59(14)$A°^3$
Z, Calculated density	4, 1.348 Mg/m^3
Absorption coefficient	0.099 mm^{-1}
F(000)	772
Theta range for data collection	1.83 to 30.52 deg.
Limiting indices	$-18 \leq h \leq 11$, $-10 \leq k \leq 10$, $-24 \leq l \leq 28$
Reflections collected / unique	12650 / 5227 [R(int) = 0.0604]
Completeness to theta = 30.52	94.4 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5227 / 0 / 324
Goodness-of-fit on F^2	1.021
Final R indices [I > 2sigma(I)]	R1 = 0.0793, wR2 = 0.1691
R indices (all data)	R1 = 0.2013, wR2 = 0.2255
Largest diff. peak and hole	0.436 and –0.317 $e.A^{-3}$

Table 17. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($A^2 \times 10^3$) for Compound 3.

	X	Y	Z	U(eq)*
O(1)	7995(2)	1434(3)	4150(1)	60(1)
O(2)	6327(2)	1084(4)	4668(1)	67(1)
O(3)	3942(2)	1736(5)	8611(1)	70(1)
O(4)	3807(2)	-1025(4)	7690(1)	84(1)
O(5)	14465(2)	3377(4)	4027(1)	65(1)
O(6)	13755(2)	3840(3)	5237(1)	63(1)
C(3)	13430(3)	3840(7)	5900(2)	68(1)
C(5)	13009(2)	3377(4)	4721(2)	47(1)
C(6)	13410(2)	3164(4)	4092(2)	48(1)
C(7)	12748(3)	2732(5)	3535(2)	53(1)
C(8)	11679(3)	2495(5)	3586(2)	51(1)
C(9)	11262(2)	2676(4)	4207(2)	43(1)
C(10)	11946(2)	3119(4)	4774(2)	47(1)
C(11)	10135(3)	2351(5)	4244(2)	49(1)
C(12)	9590(2)	2322(4)	4778(2)	48(1)
C(13)	8467(2)	1899(4)	4736(2)	46(1)
C(14)	7899(3)	1962(4)	5298(2)	47(1)
C(15)	6815(2)	1510(4)	5249(2)	50(1)
C(16)	6163(3)	1422(5)	5816(2)	56(1)
C(17)	6432(3)	2147(5)	6411(2)	59(1)
C(18)	5769(3)	1985(5)	6993(2)	55(1)
C(19)	5851(3)	3281(5)	7496(2)	64(1)
C(20)	5233(3)	3189(5)	8026(2)	60(1)
C(21)	4527(3)	1773(5)	8072(2)	52(1)
C(22)	4451(3)	407(5)	7574(2)	53(1)
C(23)	5060(3)	540(5)	7033(2)	55(1)
C(25)	3810(4)	-2615(7)	7262(2)	87(1)

*U(eq) is defined as one third of the trace of the orthogonalized.

Table 18. Bond distances (A°) for Compound 3.

Bond Distances	Distances(A°)	Bond Distances	Distances(A°)
O(1)-C(13)	1.299(3)	C(9)-C(11)	1.459(4)
O(2)-C(15)	1.292(4)	C(11)-C(12)	1.320(5)
O(3)-C(21)	1.359(4)	C(12)-C(13)	1.453(4)
O(4)-C(22)	1.352(4)	C(13)-C(14)	1.386(4)
O(4)-C(25)	1.426(5)	C(14)-C(15)	1.410(4)
O(5)-C(6)	1.367(4)	C(15)-C(16)	1.460(4)
C(3)-O(6)	1.417(4)	C(16)-C(17)	1.308(5)
O(6)-C(5)	1.368(3)	C(17)-C(18)	1.498(5)
C(5)-C(10)	1.377(4)	C(18)-C(19)	1.365(5)
C(5)-C(6)	1.400(4)	C(18)-C(23)	1.385(5)
C(6)-C(7)	1.360(4)	C(19)-C(20)	1.373(5)
C(7)-C(8)	1.382(4)	C(20)-C(21)	1.368(5)
C(8)-C(9)	1.393(4)	C(21)-C(22)	1.393(4)
C(9)-C(10)	1.391(4)	C(22)-C(23)	1.385(4)

Table 19. Hydrogen bonds for Compound 3. [A and deg.]

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(10)...O(2)	1.24(5)	1.27(5)	2.455(3)	155(4)
O(5)-H(50)...O(6)	0.84(3)	2.10(3)	2.664(3)	124(3)
O(3)-H(30)...O(4)	0.76(4)	2.33(4)	2.698(4)	111(3)
O(3)-H(30)...O(1)$^{\#1}$	0.76(4)	2.13(4)	2.842(4)	156(4)

Symmetry transformation used to generate equivalent atoms :

$^{\#1}$ x-1/2, -y, z+1/2

Table 20. Bond Angels (deg.) for Compound 3.

Angels	deg.	Angels	deg.
C(22)-O(4)-C(25)	118.5(3)	C(13)-C(14)-C(15)	121.1(3)
C(5)-O(6)-C(3)	117.0(3)	O(2)-C(15)-C(14)	119.9(3)
O(6)-C(5)-C(10)	126.0(3)	O(2)-C(15)-C(16)	114.7(3)
O(6)-C(5)-C(6)	114.2(3)	C(14)-C(15)-C(16)	125.4(3)
C(10)-C(5)-C(6)	119.8(3)	C(17)-C(16)-C(15)	124.0(3)
C(7)-C(6)-O(5)	119.0(3)	C(16)-C(17)-C(18)	123.2(3)
C(7)-C(6)-C(5)	120.1(3)	C(19)-C(18)-C(23)	118.6(3)
O(5)-C(6)-C(5)	120.9(3)	C(19)-C(18)-C(17)	120.1(3)
C(6)-C(7)-C(8)	120.2(3)	C(23)-C(18)-C(17)	121.3(3)
C(7)-C(8)-C(9)	120.9(3)	C(18)-C(19)-C(20)	121.2(4)
C(10)-C(9)-C(8)	118.5(3)	C(21)-C(20)-C(19)	120.9(3)
C(10)-C(9)-C(11)	122.3(3)	O(3)-C(21)-C(20)	118.6(3)
C(8)-C(9)-C(11)	119.1(3)	O(3)-C(21)-C(22)	122.6(3)
C(5)-C(10)-C(9)	120.6(3)	C(20)-C(21)-C(22)	118.8(3)
C(12)-C(11)-C(9)	129.4(3)	O(4)-C(22)-C(23)	125.3(3)
C(11)-C(12)-C(13)	122.9(3)	O(4)-C(22)-C(21)	114.9(3)
O(1)-C(13)-C(14)	119.8(3)	C(23)-C(22)-C(21)	119.7(3)
O(1)-C(13)-C(12)	118.2(3)	C(18)-C(23)-C(22)	120.7(3)
C(14)-C(13)-C(12)	122.0(3)		

Table 21. Torsion angels (deg.) for Compound 3.

Torsion angles	(deg.)	Torsion angels	(deg.)
C(3)-O(6)-C(5)-C(10)	8.0(5)	C(13)-C(14)-C(15)-O(2)	-2.2(5)
C(3)-O(6)-C(5)-C(6)	-172.0(3)	C(13)-C(14)-C(15)-C(16)	176.0(3)
O(6)-C(5)-C(6)-C(7)	-179.0(3)	O(2)-C(15)-C(16)-C(17)	-165.5(3)
C(10)-C(5)-C(6)-C(7)	1.0(4)	C(14)-C(15)-C(16)-C(17)	16.2(5)
O(6)-C(5)-C(6)-O(5)	1.3(4)	C(15)-C(16)-C(17)-C(18)	-177.8(3)
C(10)-C(5)-C(6)-O(5)	-178.7(3)	C(16)-C(17)-C(18)-C(19)	-154.9(4)
O(5)-C(6)-C(7)-C(8)	179.5(3)	C(16)-C(17)-C(18)-C(23)	24.6(5)
C(5)-C(6)-C(7)-C(8)	-0.2(5)	C(23)-C(18)-C(19)-C(20)	-1.2(5)
C(6)-C(7)-C(8)-C(9)	-0.6(5)	C(17)-C(18)-C(19)-C(20)	178.3(3)
C(7)-C(8)-C(9)-C(10)	0.6(4)	C(18)-C(19)-C(20)-C(21)	1.2(5)
C(7)-C(8)-C(9)-C(11)	-177.9(3)	C(19)-C(20)-C(21)-O(3)	179.3(3)
O(6)-C(5)-C(10)-C(9)	179.0(3)	C(19)-C(20)-C(21)-C(22)	0.6(5)
C(6)-C(5)-C(10)-C(9)	-1.0(4)	C(25)-O(4)-C(22)-C(23)	5.8(5)
C(8)-C(9)-C(10)-C(5)	0.2(4)	C(25)-O(4)-C(22)-C(21)	-170.4(3)
C(11)-C(9)-C(10)-C(5)	178.6(3)	O(3)-C(21)-C(22)-O(4)	-4.4(5)
C(10)-C(9)-C(11)-C(12)	-3.5(5)	C(20)-C(21)-C(22)-O(4)	174.3(3)
C(8)-C(9)-C(11)-C(12)	175.0(3)	O(3)-C(21)-C(22)-C(23)	179.2(3)
C(9)-C(11)-C(12)-C(13)	-177.4(3)	C(20)-C(21)-C(22)-C(23)	-2.2(5)
C(11)-C(12)-C(13)-O(1)	3.8(5)	C(19)-C(18)-C(23)-C(22)	-0.4(5)
C(11)-C(12)-C(13)-C(14)	-176.9(3)	C(17)-C(18)-C(23)-C(22)	-179.9(3)
O(1)-C(13)-C(14)-C(15)	0.7(5)	O(4)-C(22)-C(23)-C(18)	-173.9(3)
C(12)-C(13)-C(14)-C(15)	-178.7(3)	C(21)-C(22)-C(23)-C(18)	2.1(5)

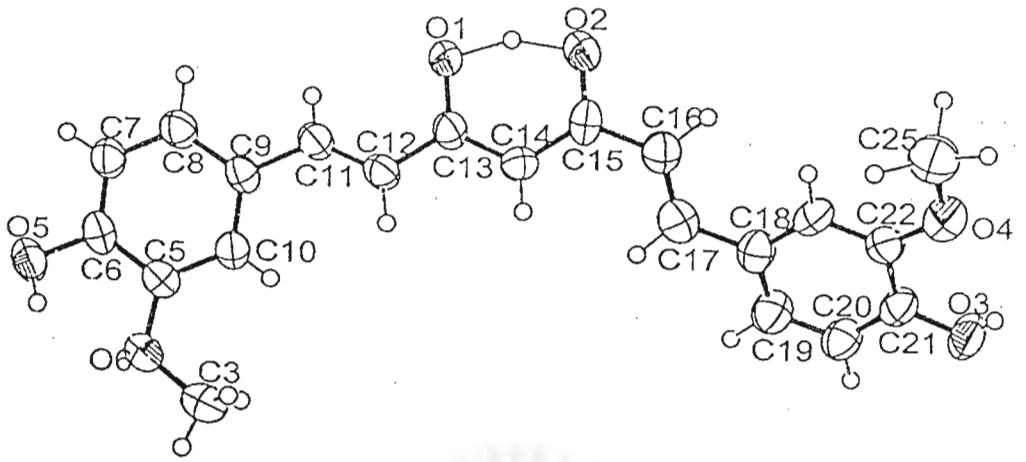


Figure 16. The ORTEP structure of Compound 3.

Curcumin is the main yellow pigment of turmeric and has been used as a food coloring reagent and preservative in a food industry. Curcumin has received much attention with its interesting biological activities [28]. Curcumin exhibited a variety of pharmacological effects including antitumor, anti-inflammatory, antioxidant (against the peroxidation of lipid),and anti-infectious activities and is currently in clinical trials for AIDS patients. The effects of curcumin have been determined on purified human immunodeficiency virus type 1 (HIV-1) integrase. Curcumin has an inhibitory concentration (IC_{50}) for strand transfer of 40 muM (that could be due to an intramolecular stacking of two phenyl rings that brings the hydroxy groups into close proximity and may contribute to the antiviral activity of curcumin.)[29].

Also, most of its biological activities may be related to its antioxidant activity. It has been previously found to have antipromotor activity against carcinogenesis and extensively studied as a chemopreventive agent against cancers in various models. It was found to be cytotoxic to human HCT-15 and HT-29 colon cells, erb B_2 oncogene-transformed NIH 3T3 cells , mouse sarcoma cells, human 293 kidney cancer cells, OVCAR-3 ovarian cancer cells and human heptacellular carcinoma Hep G_2 cells, but not to several primary fibroblast cells [25]. Moreover, it was found to possess an anti-inflammatory activity as well.

The biosynthesis of curcumin was studied by means of feeding experiments but was not clear whether curcumin was biosynthesized either from two C_6-C_3 units and an acetate or from a C_6-C_3 as the starter and five acetates[30].

Structural elucidation of Compound 4

The IR spectrum of Compound 4 (Fig.46) displayed broad peak in the range of 3400-3300 cm^{-1} belonging to O-H stretching and the absorption peak of α,β- unsaturated carbonyl stretching vibration at 1711 cm^{-1}. The C-H stretching and bending vibrations of $-CH_3$ and $-CH_2$ were also observed at 2962-2876 cm^{-1} and 1366 cm^{-1} respectively. The IR spectrum of Compound 4 was summarized in Table 22.

Table 22. The IR absorption bands assignment of Compound 4.

Wave number (cm^{-1})	Peak intensity	Tentative assignment
3400-3300	Broad	O-H stretching vibration of alcohol
2962-2876	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1711	Strong	C=O stretching vibration
1366	Strong	C-H bending vibration of $-CH_2$

The ^{1}H-NMR spectrum (Fig.47, Table 23) exhibited the important signals at δ 2.58 ppm (1H, d, J = 12.8 Hz) and δ 2.94 ppm (1H, d, J = 13.0 Hz) of 9α and 9β protons respectively. The spectrum also showed the signals at δ 1.17, 1.19, 1.81 and 1.92 ppm of protons belonging to four methyl groups.

The ^{13}C-NMR (Fig.48, Table 23) displayed a total 15 signals of carbons. The carbonyl group of α,β- unsaturated ketone showed the signal at δ 202.7 ppm and the olefinic carbons showed the signals at δ 134.6 and 142.1 ppm. The signals of two carbons bearing each single hydroxy group appeared at δ 72.7 and 79.9 ppm.

DEPT 90 experiments (Fig.49) indicated the presence of two saturated methine carbons at δ 52.0 and 55.9 ppm.

DEPT 135 spectrum (Fig.49) showed four methylene carbons at δ 59.8, 39.7, 28.5 and 22.8 ppm and four methyl carbons at δ 22.7, 22.1, 21.8 and 20.6 ppm. which indicated that the carbon signals at δ 202.7, 142.1, 134.6, 79.9 and 72.7 ppm were quaternary.

The EI mass spectrum (Fig.56) of this compound gave a molecular ion at m/z 252 in accordance with a sesquiterpenoid containing two hydroxy group. which was corresponding to the molecular formula $C_{15}H_{24}O_3$ indicating the DBE of 4. The information from 2D-NMR techniques; HMQC correlation (Fig.50, Table 23), HMBC correlation (Fig.51, Table 24), COSY correlation (Fig.52-53, Table 24) and NOESY correlation (Fig.54-55) were used to determine the structure of Compound 4.

Table 23. The HMQC spectral data of Compound 4.

^{13}C-NMR (ppm)	^{1}H-NMR (ppm), coupling constant (Hz)
20.6(q)	1.17s
21.8(q)	1.81s
22.1(q)	1.92s
22.7(q)	1.19s
22.8(t)	1.66m
28.5(t)	1.71m
39.7(t)	1.78m
52.0(d)	1.37dt (J = 1.5, 12.0)
55.9(d)	1.96m
59.8(t)	2.58d (J = 12.8), 2.94d (J = 13.0)
72.7(s)	-
79.9(s)	-
134.6(s)	-
142.1(s)	-
202.7(s)	-

Table 24. The HMQC, HMBC and COSY spectral data of Compound 4.

Position	δ_C	δ_H	HMBC (H to C)	COSY
1	55.9(d)	1.96m	C-2,C-5,C-9,C-10, C-15	H-2(1.66),H-3(1.71),H-5(1.37)
2	22.8(t)	1.66m	C-1,C-3,C-4,C-5	H-3(1.71),H-5(1.37),H-15(1.18s)
3	28.5(t)	1.71m	C-2,C-4	H-2(1.66),H-5(1.37),H-14(1.20)
4	79.9(s)	-	-	-
5	52.0(d)	1.37dt (J=1.5,12.0)	C-1,C-2,C-4,C-6,C-7, C-10,C-14	H-1(1.96)
6	39.7(t)	1.78m	C-4,C-5,C-14	H-12(1.84),H-14(1.20)
7	134.6(s)	-	-	-
8	202.7(s)	-	-	-
9	59.8(t)	2.58d (J=12.8)	C-1,C-7,C-8,C-10	H-9(2.94),H-15(1.18)
		2.94d (J=13.0)	C-1,C-8,C-10,C-15	H-9(2.58)
10	72.7(s)	-	-	-
11	142.1(s)	-	-	-
12	21.8(q)	1.84s	C-7,C-11,C-13	H-6(1.78),H-13(1.94)
13	22.1(q)	1.94s	C-7,C-11,C-12	H-12(1.84)
14	22.7(q)	1.20s	C-4,C-5,C-6	H-3(1.71),H-6(1.78)
15	24.6(q)	1.18s	C-1,C-9,C-10	H-2(1.66),H-9(2.58)

The ^{13}C-NMR chemical shifts of Compound 4 were found to be closely matched with those of Zedoarondiol isolated from *Curcuma aromatica* SALISB. by Kuroyanagi [31]. The signals of ^{13}C-NMR spectrum of this compound were compared as shown in Table 25.

Table 25. the ^{13}C-NMR chemical assignment of Compound 4 [31].

Carbon position	Chemical shift (ppm)	
	Compound 4	Zedoarondiol
1	55.9 (d)	55.9 (d)
2	22.8 (t)	22.9 (t)
3	28.5 (t)	28.5 (t)
4	79.9 (s)	79.9 (s)
5	52.0 (d)	52.0 (d)
6	39.7 (t)	39.7 (t)
7	134.6(s)	134.6(s)
8	202.7(s)	202.9(s)
9	59.8 (t)	59.8 (t)
10	72.7 (s)	72.7 (s)
11	142.1(s)	142.1(s)
12	21.8 (q)	21.9 (q)
13	22.1 (q)	22.2 (q)
14	22.7 (q)	22.7 (q)
15	20.6 (q)	20.6 (q)

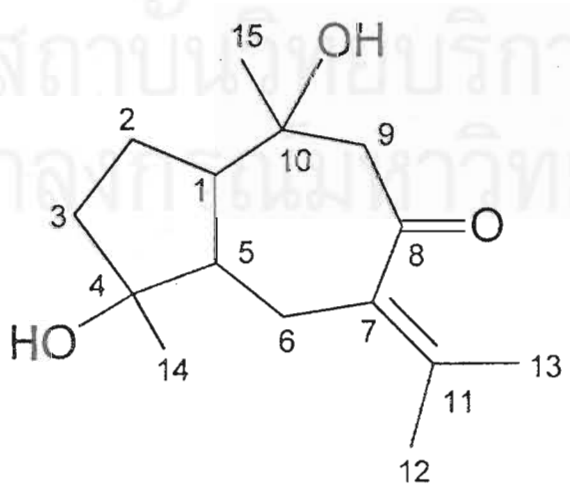


Figure 17. The structure of Compound 4.

The biogenetic pathway of Compound 4 was considered to be as shown in Figure 18.[31]

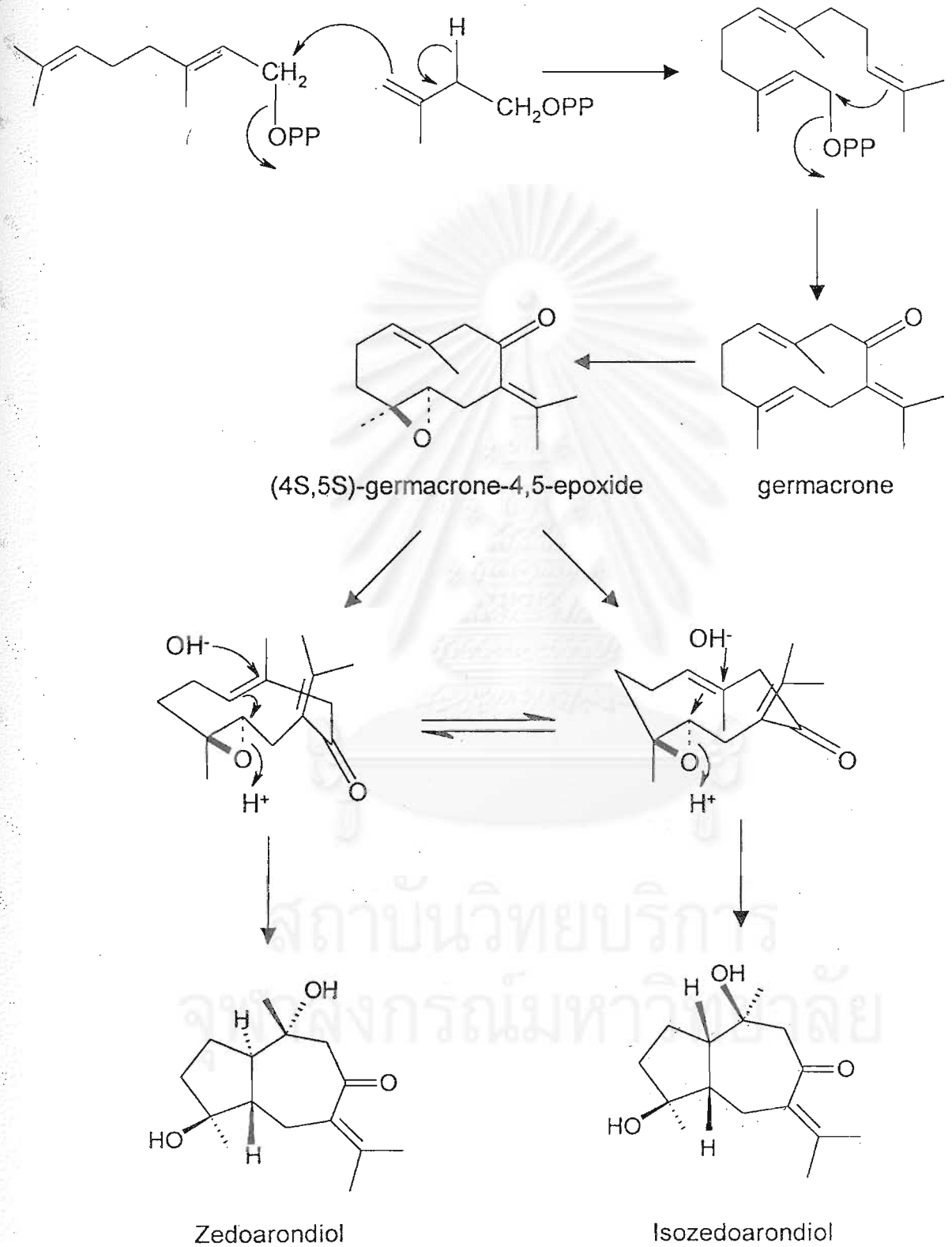


Figure 18. The biogenetic of Zedoarondiol and Isozedoarondiol.

Kuroyanagi suggested that (4s,5s)-germacrone-4,5-epoxide, which acted as a key intermediate in the pathway, was transformed by transannular cyclization via a cationic intermediate to give zedoarondiol and its diastereomer that was isozedoarondiol. Considerably, the information obtained from UV, IR and NMR spectra along with physical properties, such as melting point and optical rotation led to the deduction that Compound 4 should be Zedoarondiol as shown below.

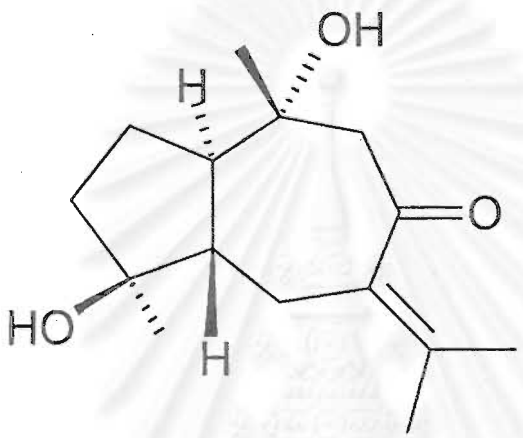


Figure 19. The structure of Zedoarondiol.

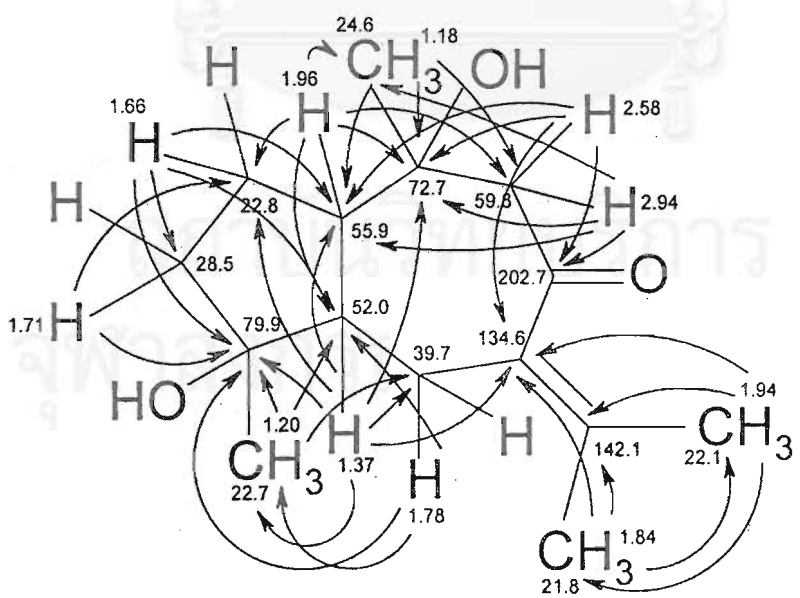


Figure 20. The HMBC correlation of Compound 4.

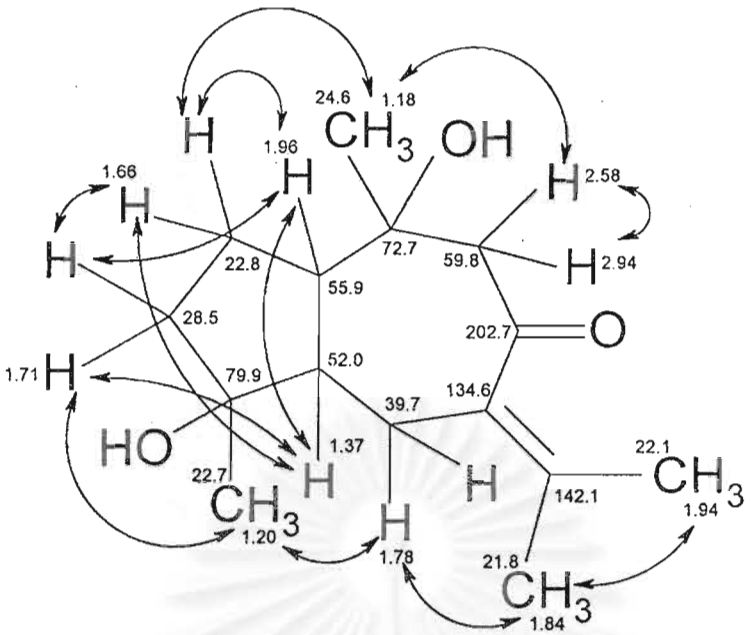


Figure 21. The COSY correlation of Compound 4.

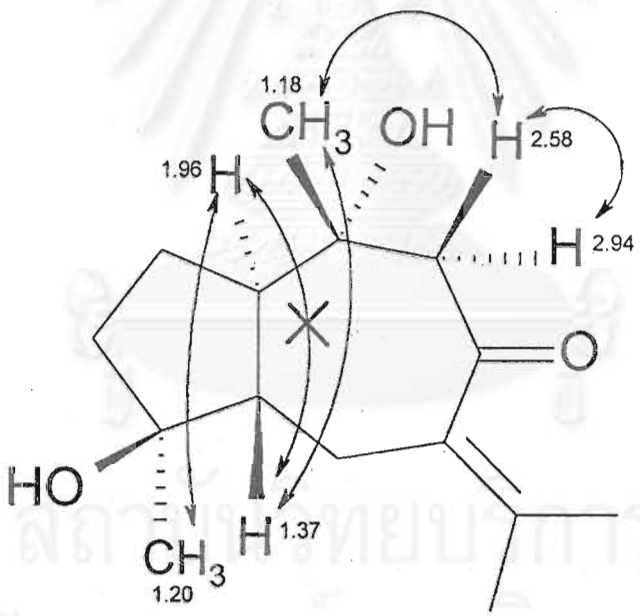


Figure 22. The NOESY correlation of Compound 4

Octahydro-1,4-dihydroxy-1,4-dimethyl-7-(1-methylethylidene) azulenone or zedoarondiol has been reported previously in the rhizomes of C. *zedoaria* spp. And C. *aromatica* SALISB. and was found to inhibit acetyl transferase, which is a key enzyme in the biosynthesis of platelet-activating factor (PAF)[32].

Inhibitory Activities of Crude Extracts on c-AMP Phosphodiesterase

In order to study and investigate the biological activity of the rhizomes of *Globba malaccensis* Ridl. The three crude extracts were at first tested for their Cyclic AMP Phosphodiesterase inhibitory activity by using malachite green method. The results were summarized in Table 26.

Table 26 Percentage inhibition of crude extract from the rhizomes of *Globba malaccensis* Ridl.

Samples	Final Concentration (μg/ml)	% inhibition
Hexane crude extract	200	18.5
Chloroform crude extract	200	63.1
Ethanol crude extract	200	68.6
Thyophylline (+ve)	200	56.8
DMSO (-ve)	-	0.0

From the data in Table 26, it showed that the hexane crude extract of *Globba malaccensis* Ridl. did not show inhibitory activity on cyclic AMP phosphodiesterase as compared with positive control. In spite of that, the chloroform and ethanol crude extracts have more inhibitory activities than the positive control. Unfortunately, the ethanol crude extract was insoluble in all solvents and inappropriate to separate and purify. Therefore, only the chloroform crude extract was selected and then purified by using silica-gel column chromatography with hexane-ethylacetate gradient solvent system to give four compounds.

Cyclic AMP Phosphodiesterase inhibitory activities of Compound 1-4

Table 27. Percentage inhibition of each isolated compounds.

Samples	Concentration (μg/ml)	% inhibition				
		I	II	III	IV	Average
Compound 1	100	12.1	13.4	11.7	12.9	12.5
	300	28.2	29.0	28.8	27.4	28.4
	500	49.2	50.1	48.6	50.7	49.7
	1,000	66.1	68.0	66.7	67.3	67.0
Compound 2	100	14.5	15.1	14.1	13.6	14.3
	300	30.7	31.4	32.6	30.0	31.2
	500	52.4	55.7	52.9	54.3	53.8
	1,000	70.4	72.8	69.7	70.1	70.8
Compound 3	100	0.0	0.0	0.0	0.0	0.0
	300	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0
	1,000	1.8	2.3	1.5	1.1	1.7
Compound 4	100	24.2	25.6	22.7	22.9	23.9
	300	48.6	48.1	49.7	47.8	48.6
	500	57.5	56.4	56.7	57.1	56.9
	1,000	73.0	73.9	73.5	72.7	73.3
Theophyllene (+ve)	100	34.9	35.6	34.1	33.7	34.6
	300	79.5	80.6	78.1	77.4	78.9
	500	87.0	87.6	85.4	88.3	87.1
	1,000	95.3	97.1	96.5	95.7	96.2
DMSO (-ve)	-	0.0	0.0	0.0	0.0	0.0

From the results in Table 27. The IC_{50} values of Compound 1,2,4 and theophyllene were determined by graph plotting between Concentration of Sample (x-axis) and % inhibition (y-axis). To determine IC_{50} values, a perpendicular line was drown from the y-axis at the % inhibition value of 50 to the x-axis as shown in Figure 23.

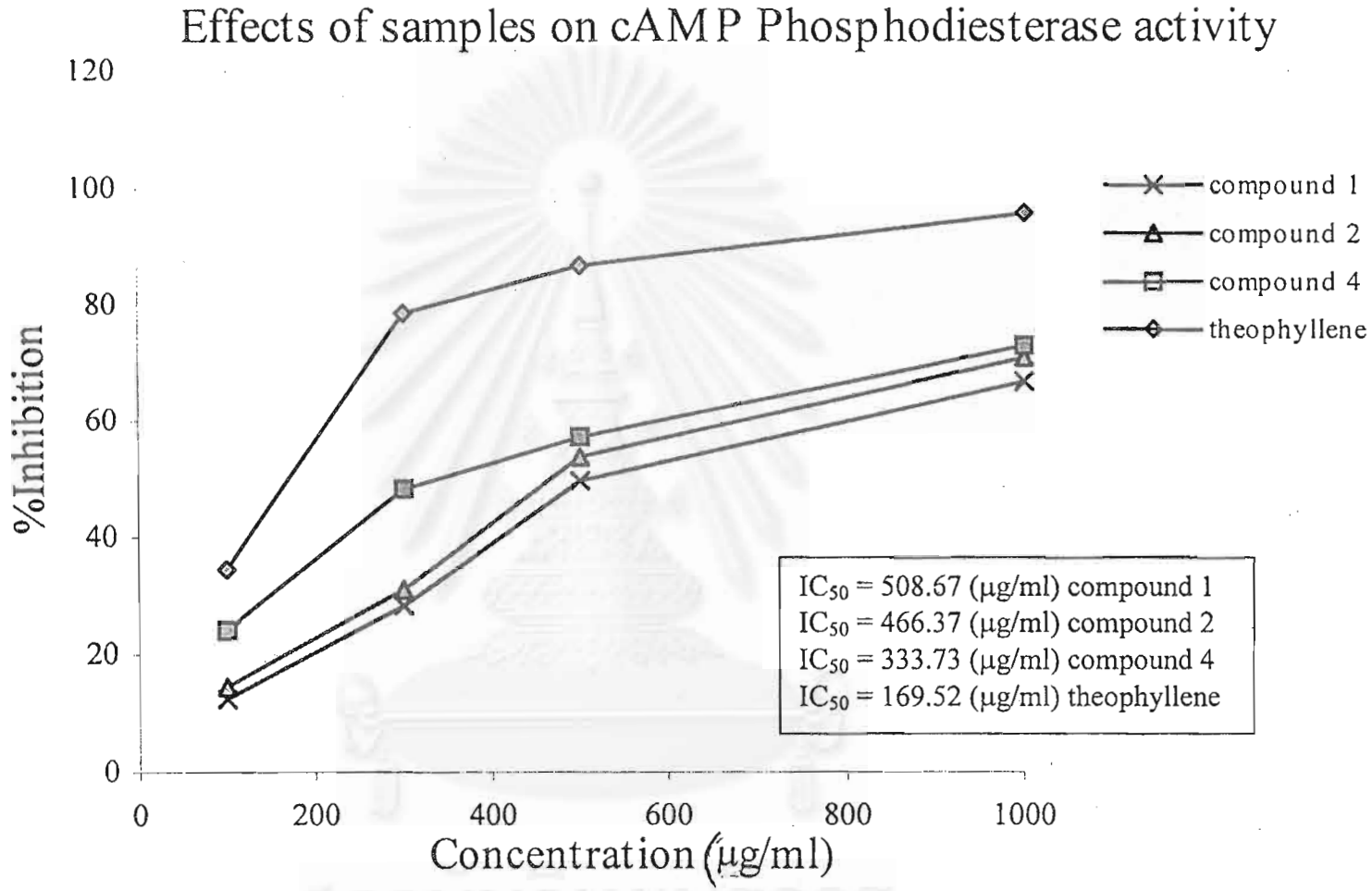


Figure 23. Effects of Compound 1,2,4 and theophyllene on cyclic AMP Phosphodiesterase.

From Figure 23. The IC_{50} values of theophyllene and isolated compounds were summarized in Table 28.

Table 28. IC_{50} values of theophyllene and each isolated compounds.

Samples	IC_{50} (μg/ml)	IC_{50} (mM)
Theophyllene	508.67	2.82
Compound 1	466.37	1.99
Compound 2	333.73	1.42
Compound 4	169.52	0.67

IC_{50} is the concentration of compound required to give 50%inhibition of phosphodiesterase activity.

Table 28 indicated that compound 1,2 and 4 which exhibited high inhibitory activity against cyclic AMP phosphodiesterase, showed IC_{50} of 1.99, 1.42 and 0.67 mM, respectively. These results indicated that compound 1,2 and 4 can stimulate central nervous system (CNS) activities higher than the way theophyllene did. Therefore these compounds might be developed and then utilized as new drugs in the future.

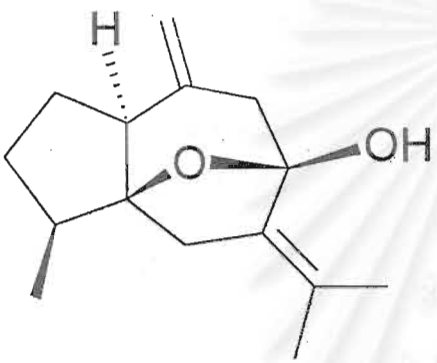
CHAPTER V
Conclusion

In our research, the powdered, sun-dried rhizomes (4kg) of *Globba malaccensis* Ridl. were extracted with ethanol, chloroform and hexane, respectively and obtained three different crude extracts; hexane crude extract (117.3 g, 2.93 % wt.by wt. of the dried rhizome), chloroform crude extract (95.4 g, 2.39 % wt.by wt. of the dried rhizome) and ethanol crude extract (1.83 g, 0.05 % wt.by wt. of the dried rhizome). The chemical constituents of the rhizomes of *G. malaccensis* Ridl. were investigated. Three substances were isolated from the rhizome using traditional chromatoghaphic techniques. The structures were determined from physical and chemical properties and various spectroscopic methods including 2D-NMR techniques and also by comparison with the spectral data which previously reported. Their structures were established as: Isocurcumenol (1), Curcumenol (2), Curcumin (3) and Zedoarondiol (4). The crystal structures of Curcumenol and Curcumin were determined by using x-ray diffraction analysis and their ORTEP structure were shown in Figure 12 and 16, respectively. In this research, it is the first report of crystal structure of Curcumenol.

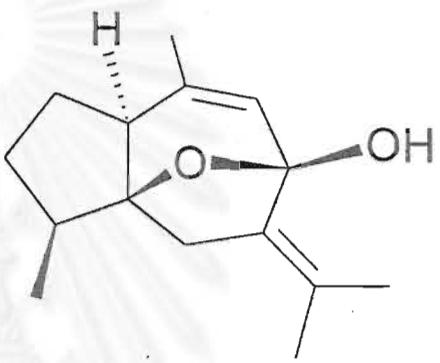
All isolated compounds from chloroform crude extract (95.4 g) of the rhizomes of *G. malaccensis* Ridl. could be summarized as shown in Table 29. The structures of isolated compounds were illustrated in Figure 24. Compound 1-4 were tested for inhibition of adenosine 3',5'-cyclic monophosphate phosphodiesterase (PDE) which can be used as a screening method to detect biologically active compound. The IC_{50} values of these compounds were summarized in Table 29.

Table 29. Isolated compounds from the rhizomes of *G. malaccensis* Ridl.

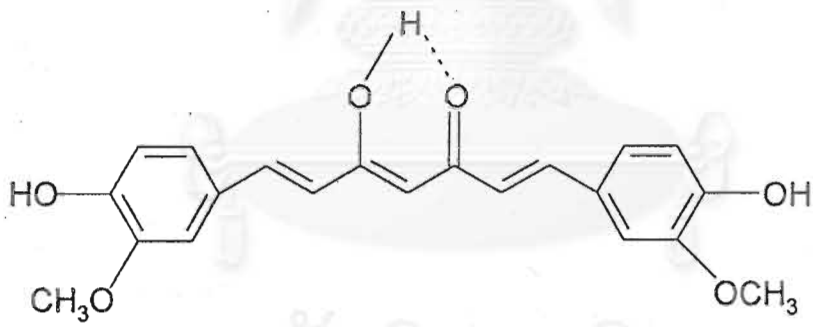
Compound	Name of compound	Weight (g)	%wt.by wt.of the starting material	IC_{50} (mM)
1	Isocurcumenol	0.048	$1.2x10^{-3}$	1.99
2	Curcumenol	2.2	$5.5x10^{-2}$	1.42
3	Curcumin	5.4	$1.3x10^{-1}$	-
4	Zedoarondiol	0.09	$1.4x10^{-3}$	0.67



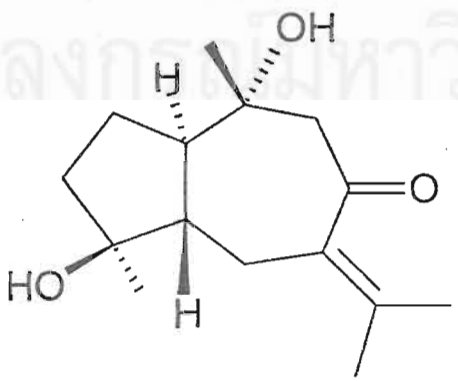
Isocurcumenol



Curcumenol



Curcumin



Zedoarondiol

Figure 24. The structures of Compound 1-4.

Proposal for the future work

The discovery of compounds belonging to *G. malaccensis* Ridl. firstly reported in this research would be interesting for future investigation. It could be clearly seen that various biologically active compounds could be isolated from the active fraction of the rhizomes of *G. malaccensis* Ridl. Therefore, the bioassay directed fractionation is quite vital for discovery of biologically active compounds. Even though, the use of cyclic AMP Phosphodiesterase assay in this research is the first preliminary screening indication among well known bioassays, its result was firmly reliable and showed the good tendency towards studying on more sophisticated bioassays such as anticancer, antitumor in pharmaceutical aspects or insecticidal activity in agricultural aspects. Therefore, further studies on those mentioned biossays should be conducted.

The study of the relation between the structure of isolated compounds by modification of functional group and inhibitory activity against cyclic AMP Phosphodiesterase or Structure-Activity Relationship(SAR) should be covered. Moreover, the chemical constituents and biological activity study of other parts of *G. malaccensis* Ridl. should also be investigated. The results from that study would reveal the similarity or difference and might lead to understanding of biosynthesis pathway of some major components.

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APPENDIX

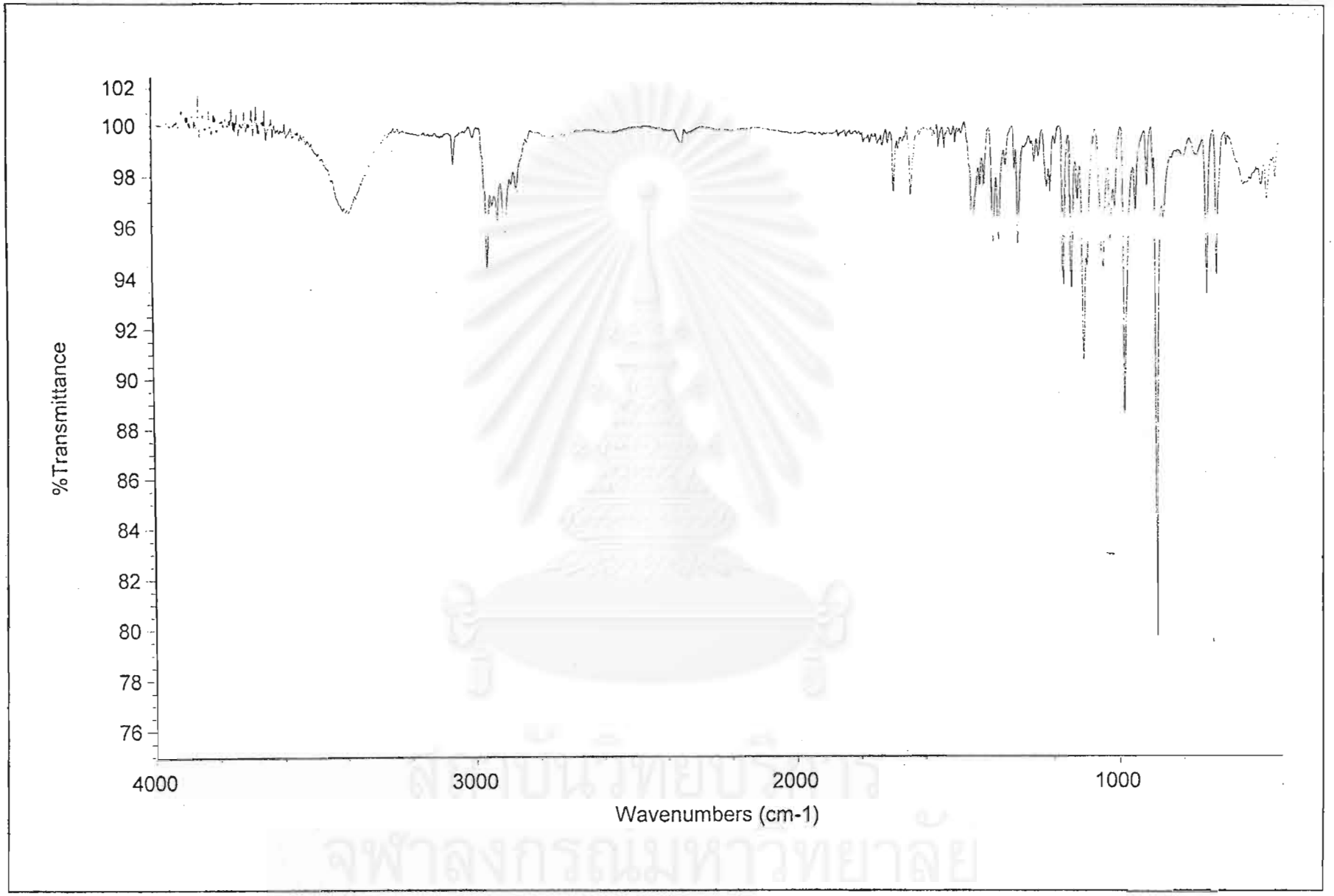


Figure 25. The IR spectrum of Isocurcumenol.

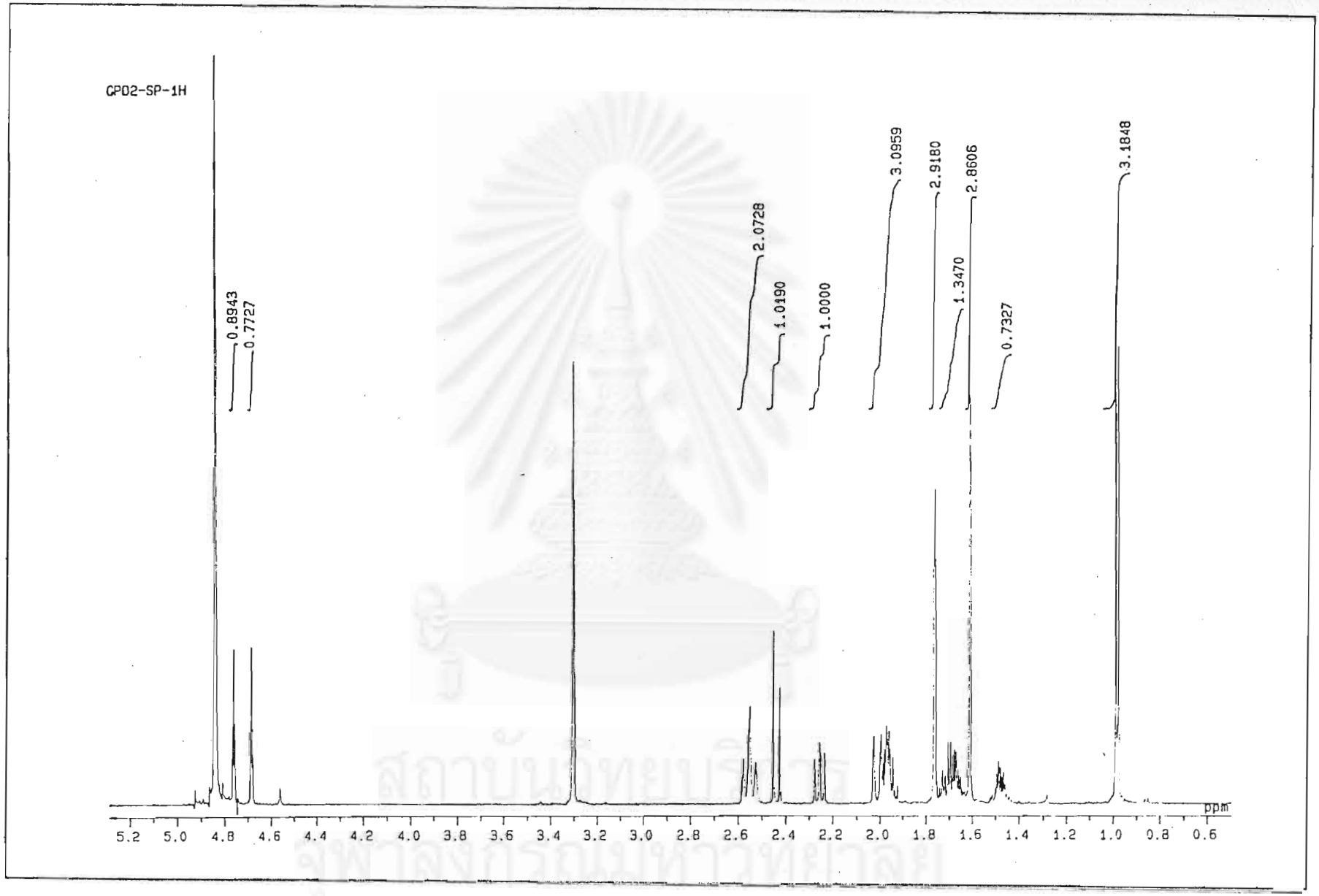


Figure 26. The ^{1}H-NMR spectrum of Isocurcumenol.

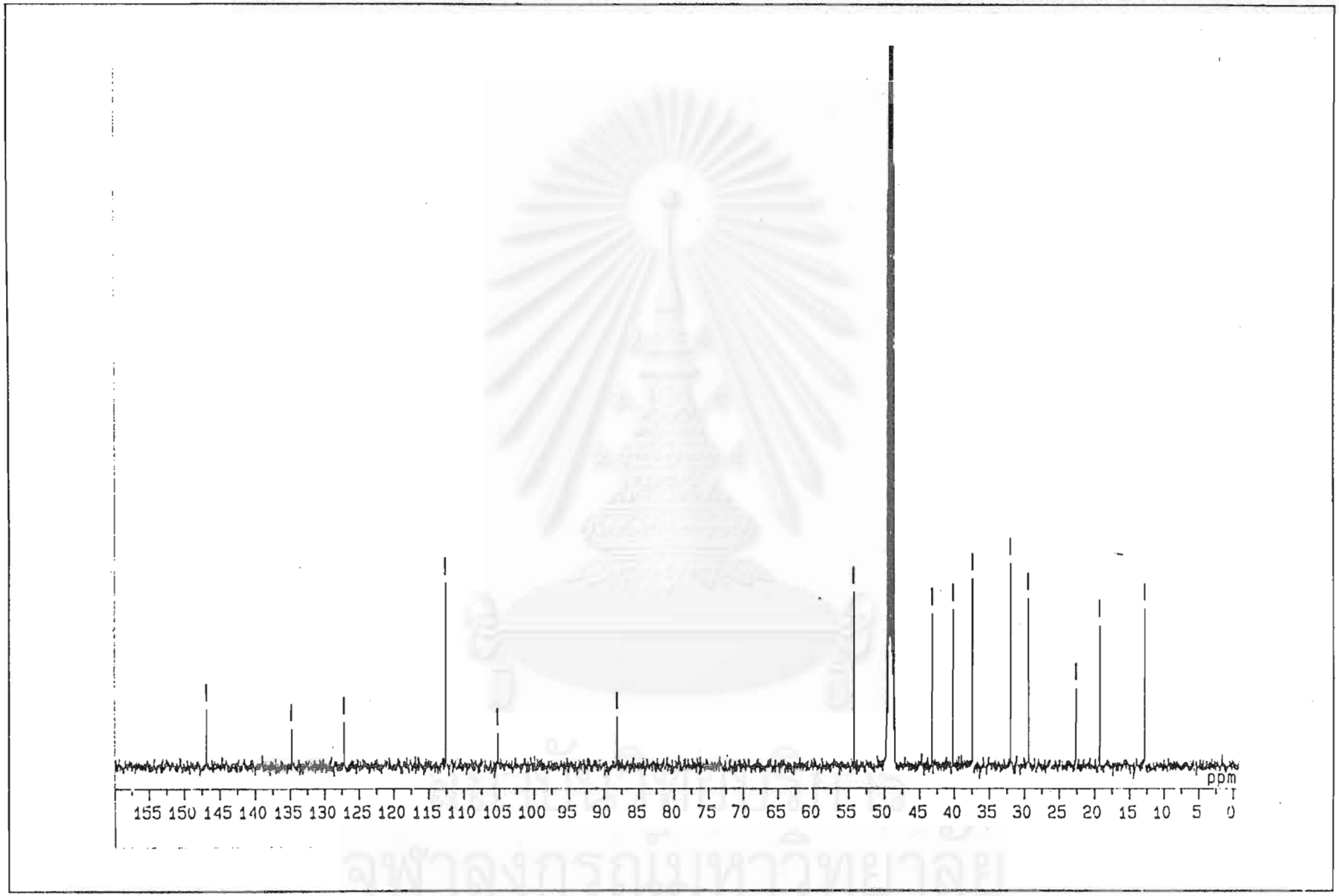


Figure 27. The ^{13}C-NMR spectrum of Isocurcumenol.

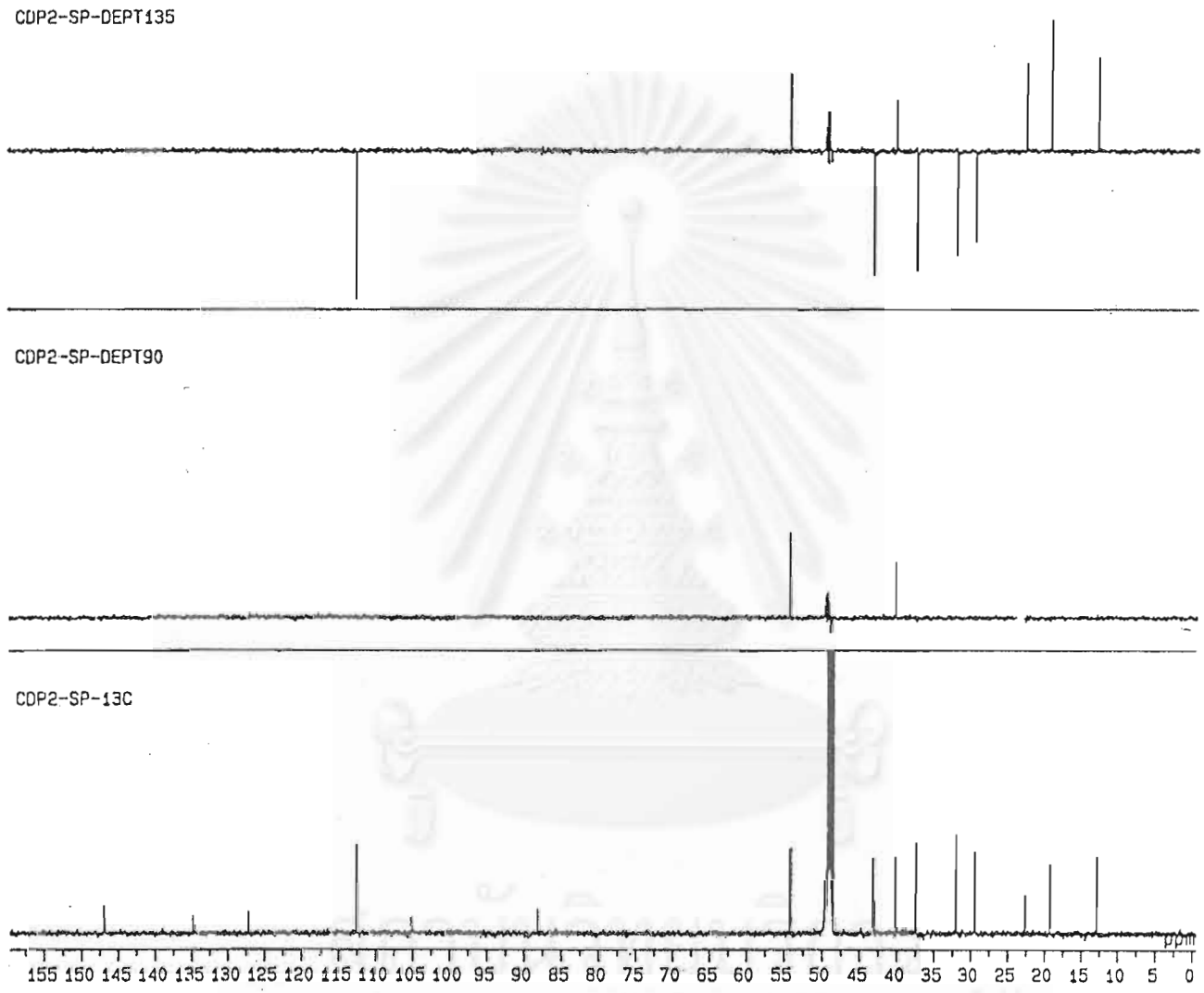


Figure 28. DEPT 90, DEPT 135 and ^{13}C-NMR spectrum of Isocurcumenol.

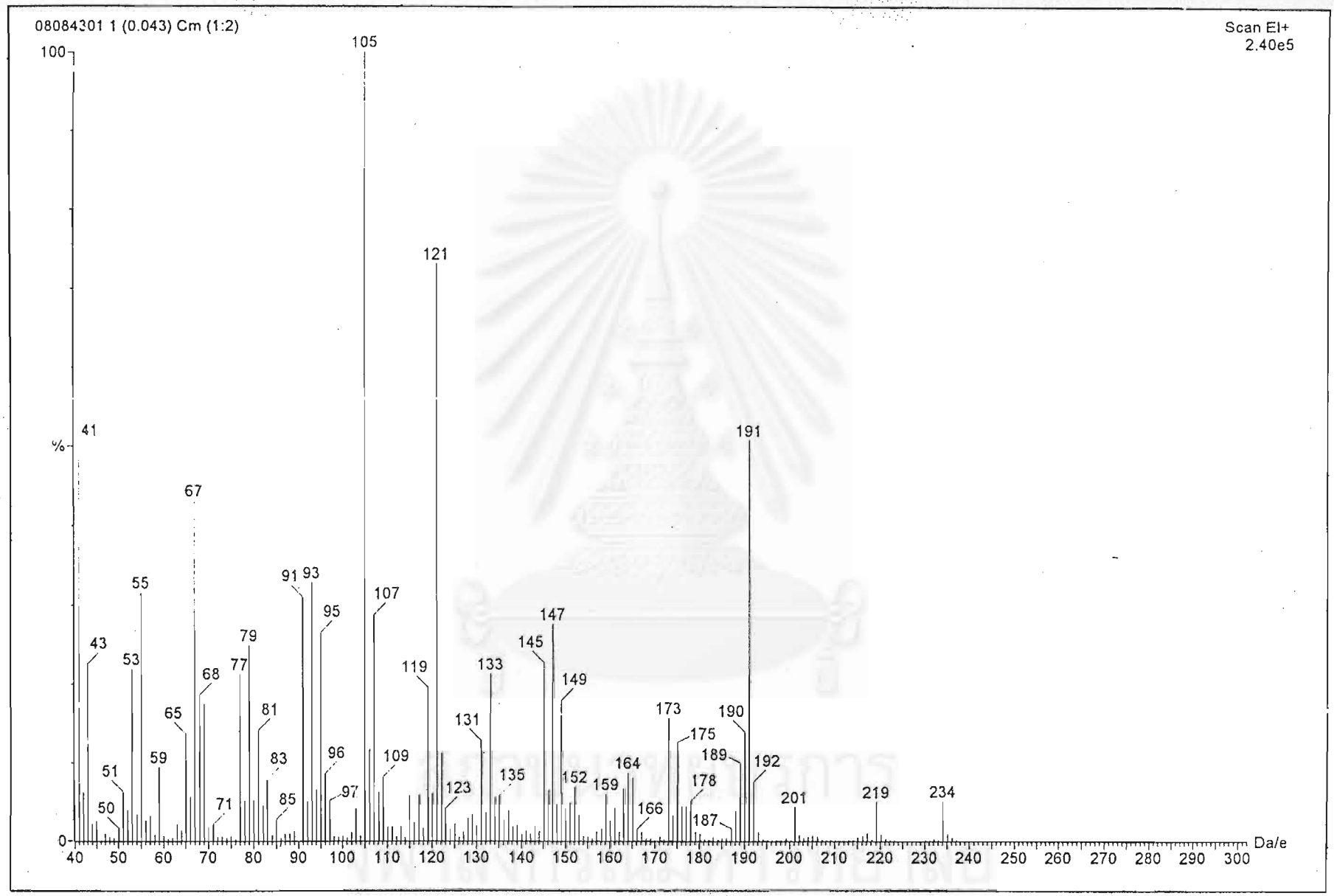


Figure 29. The EI mass spectrum of Isocurcumenol.

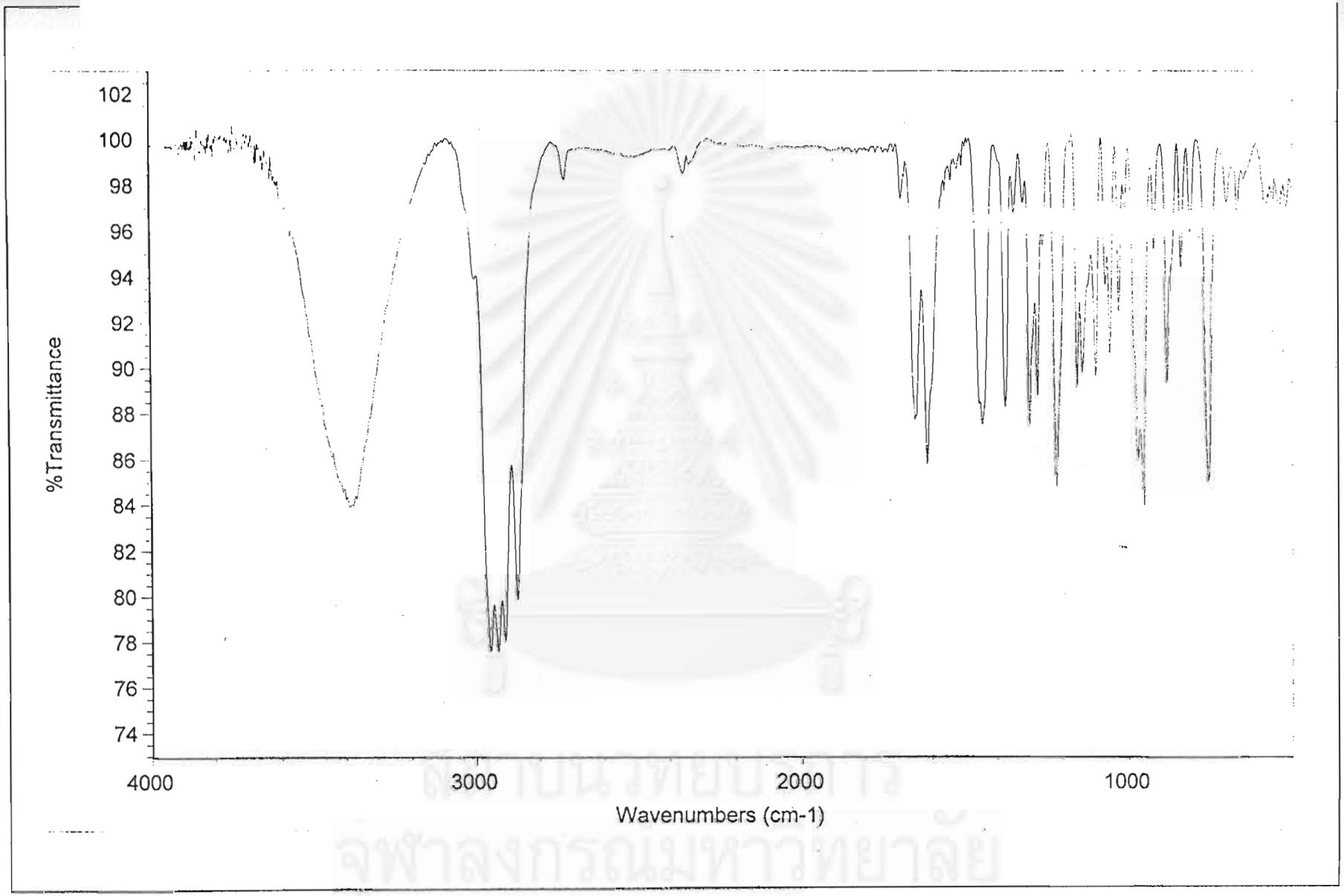


Figure 30. The IR spectrum of Curcumenol.

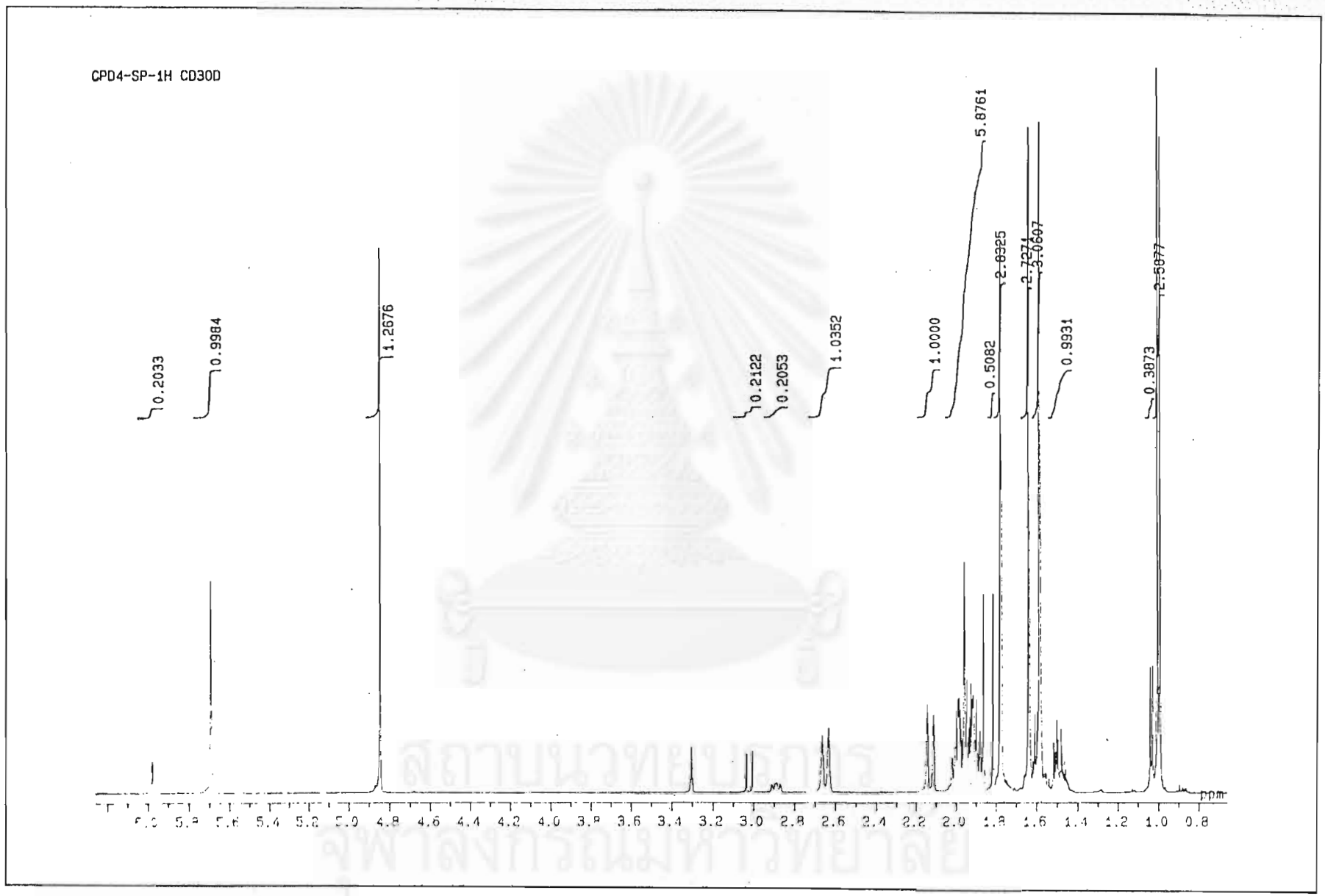


Figure 31. The ^{1}H-NMR spectrum of Curcumenol.

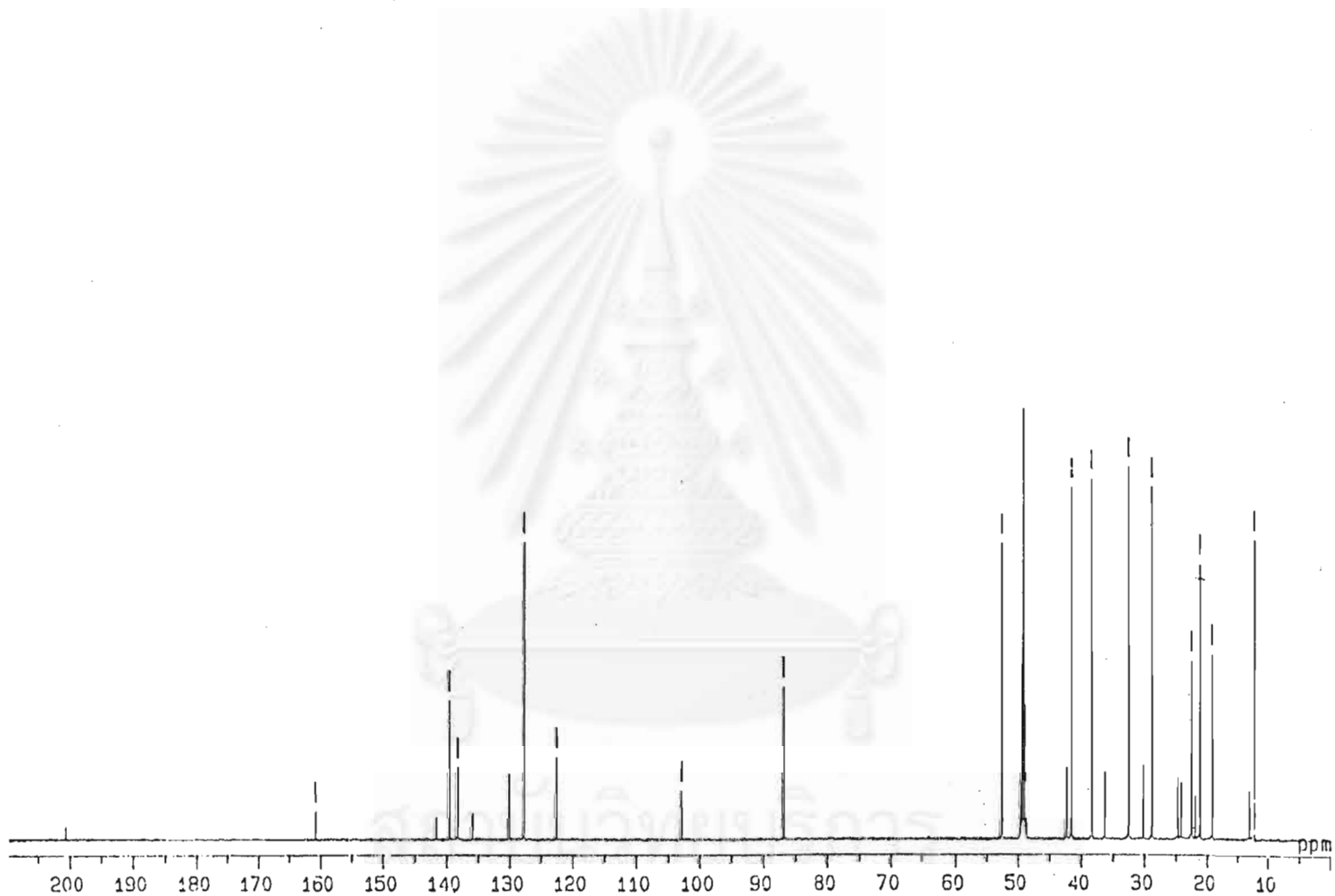


Figure 32. The ^{13}C-NMR spectrum of Curcumenol.

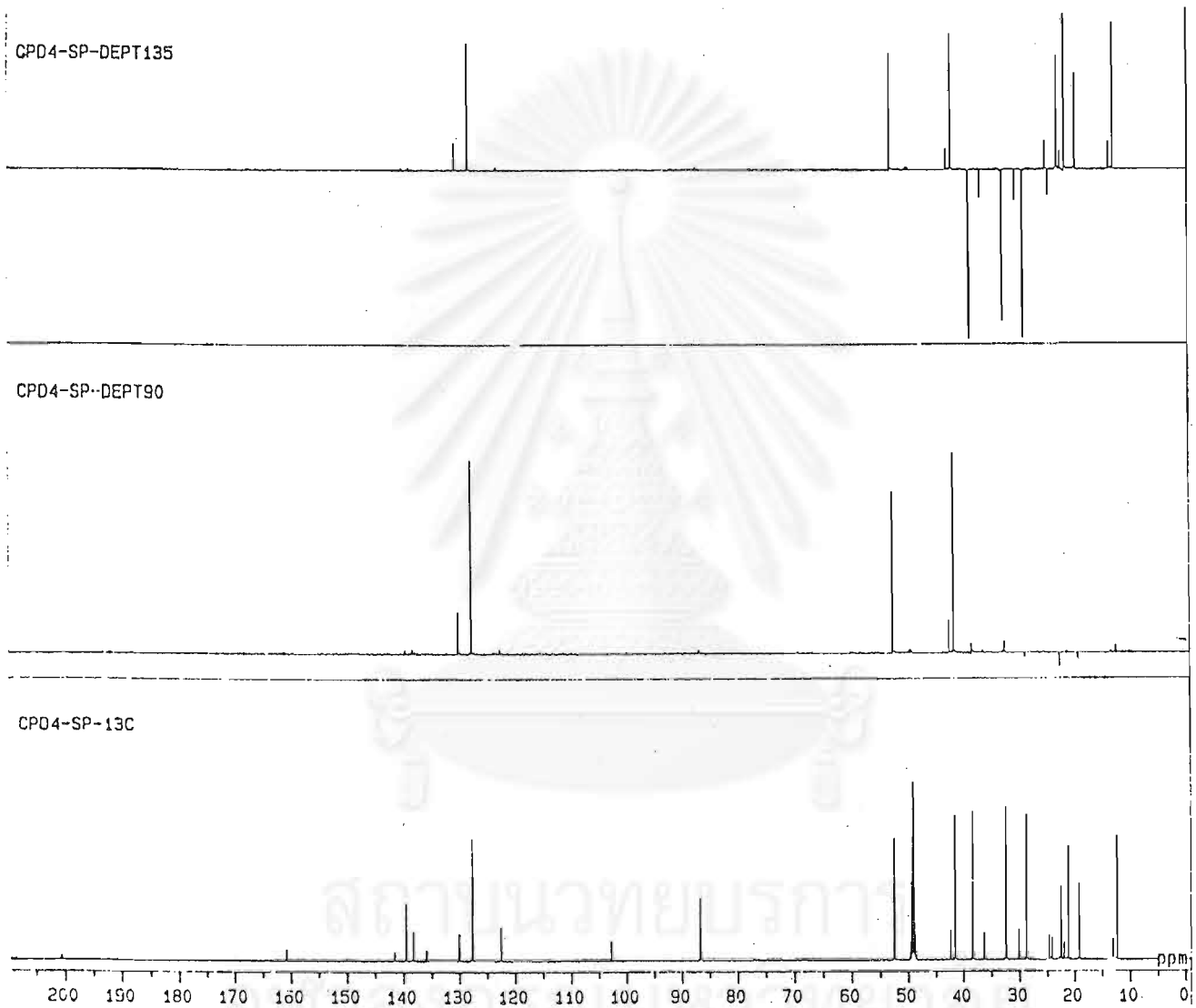


Figure 33. DEPT 90, DEPT 135 and ^{13}C-NMR spectrum of Curcumenol.

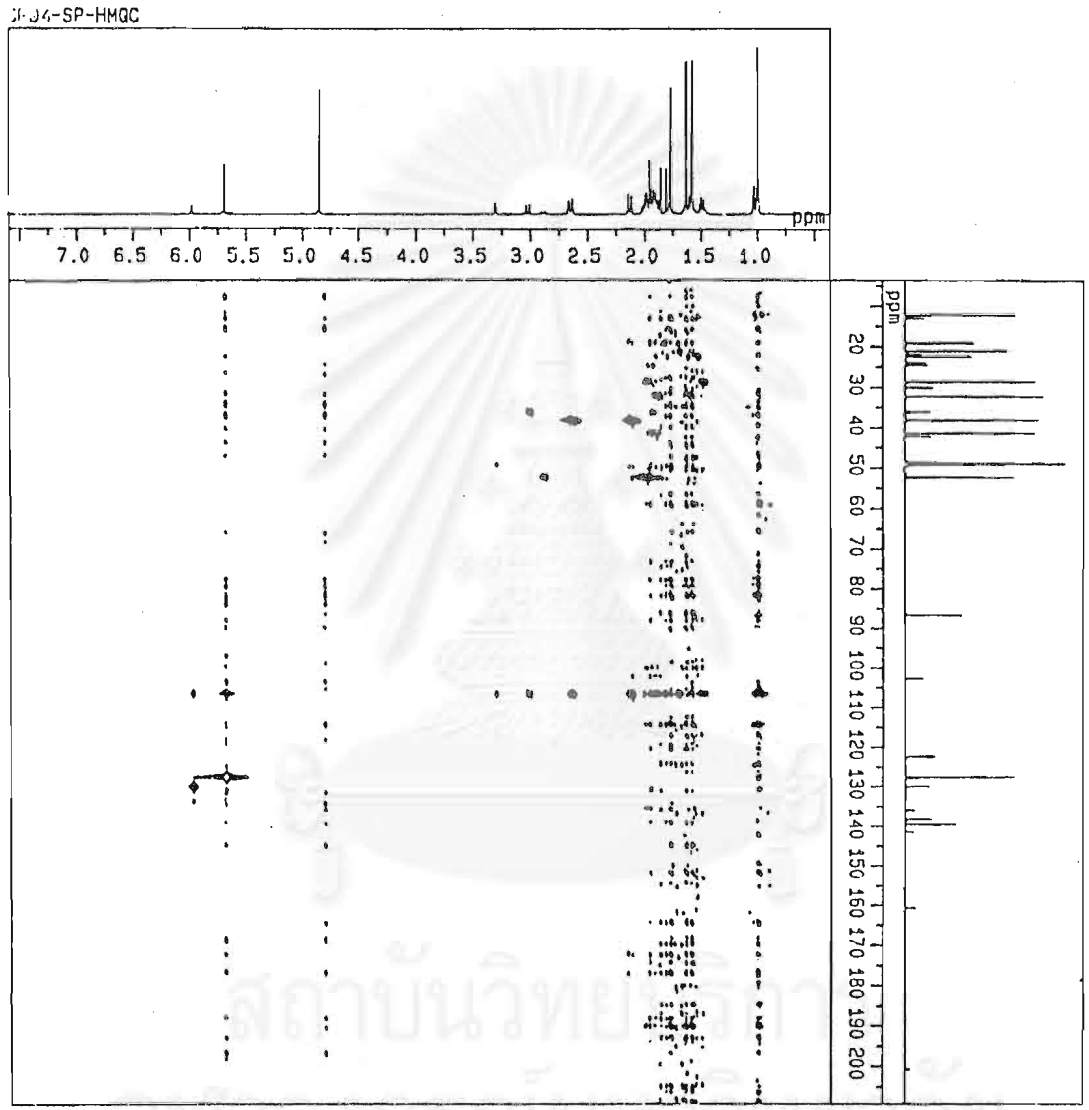


Figure 34. HMQC spectrum of Curcumenol.

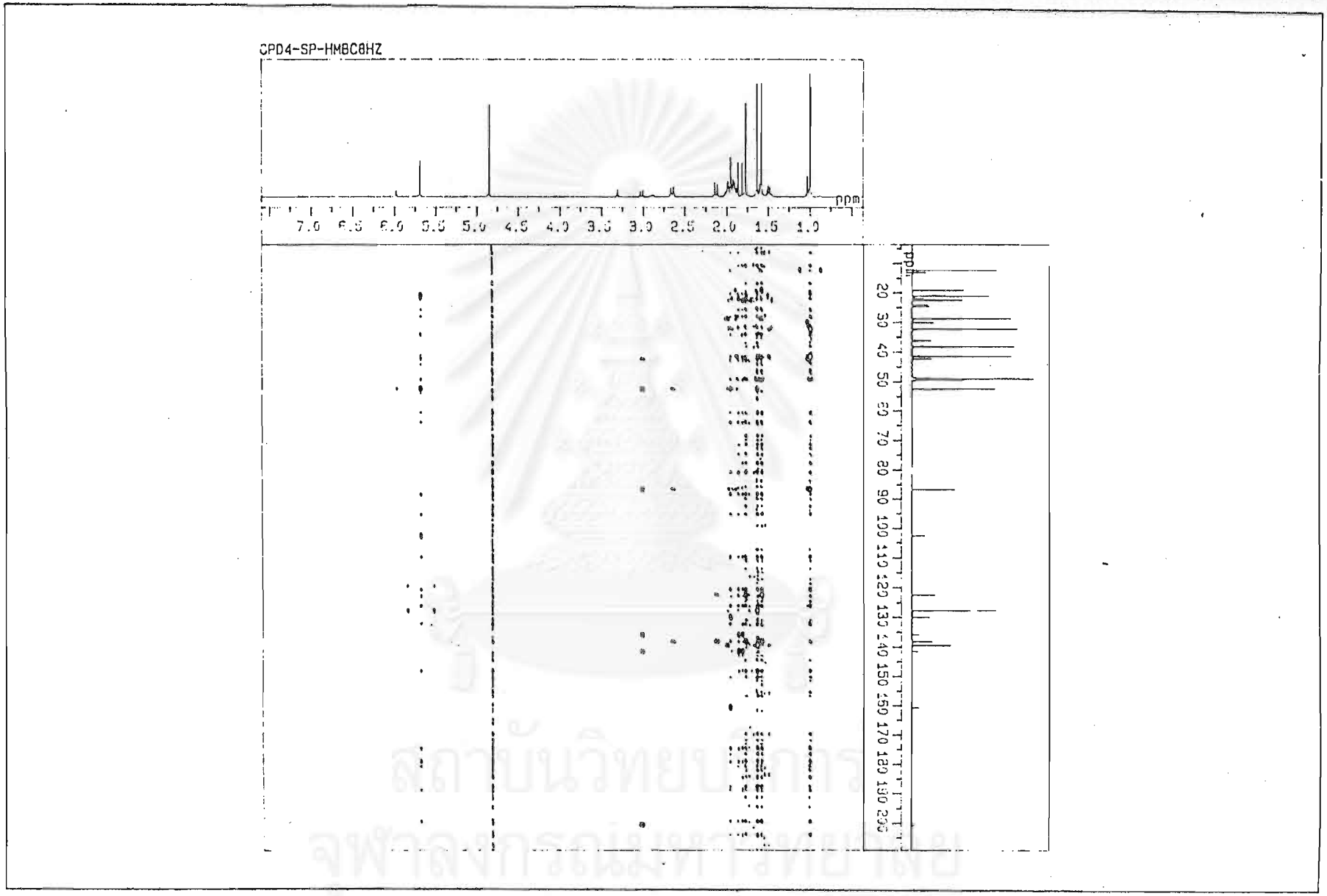


Figure 35. HMBC spectrum of Curcumenol.

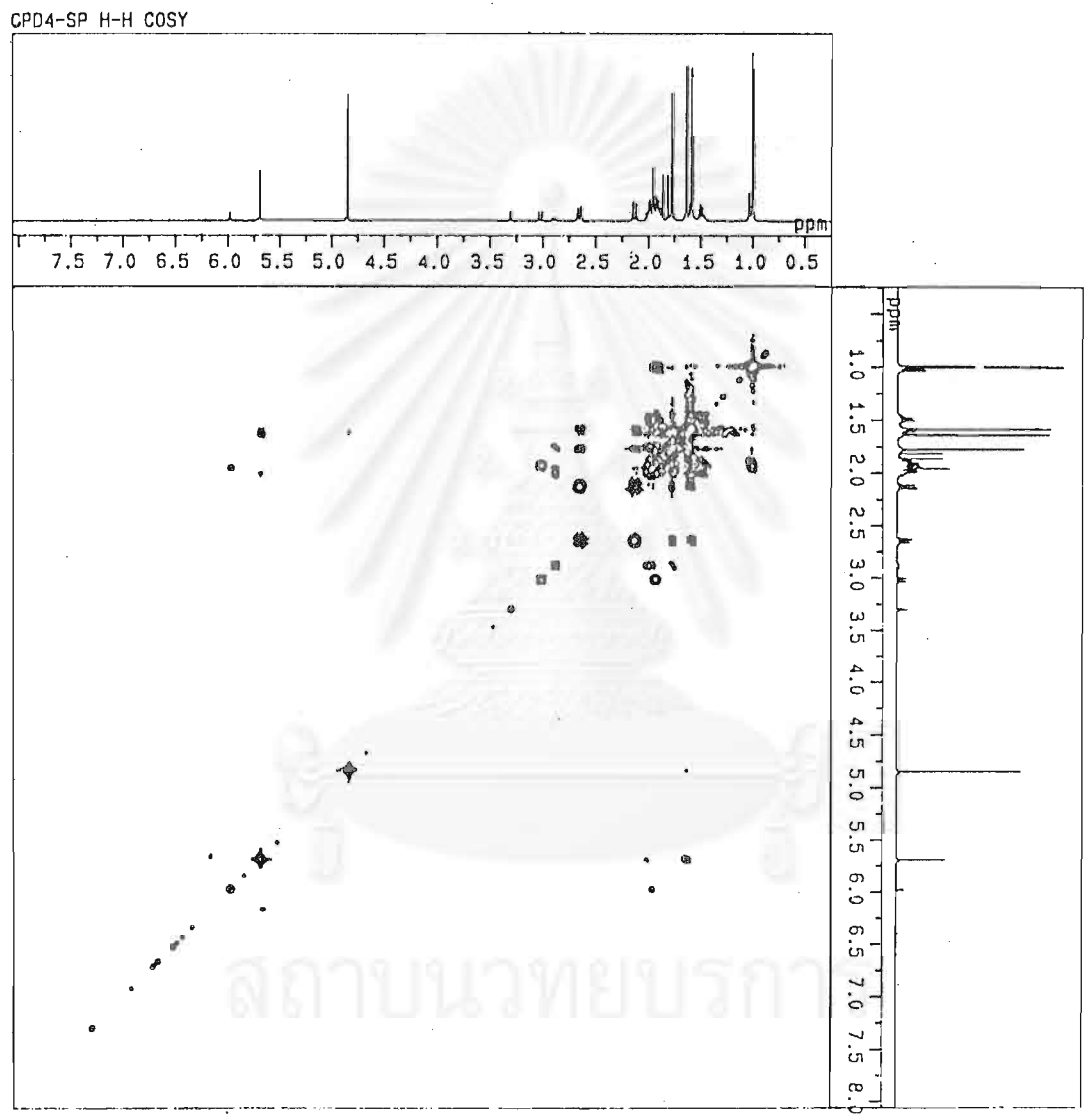


Figure 36. ^{1}H-^{1}H COSY spectrum of Curcumenol.

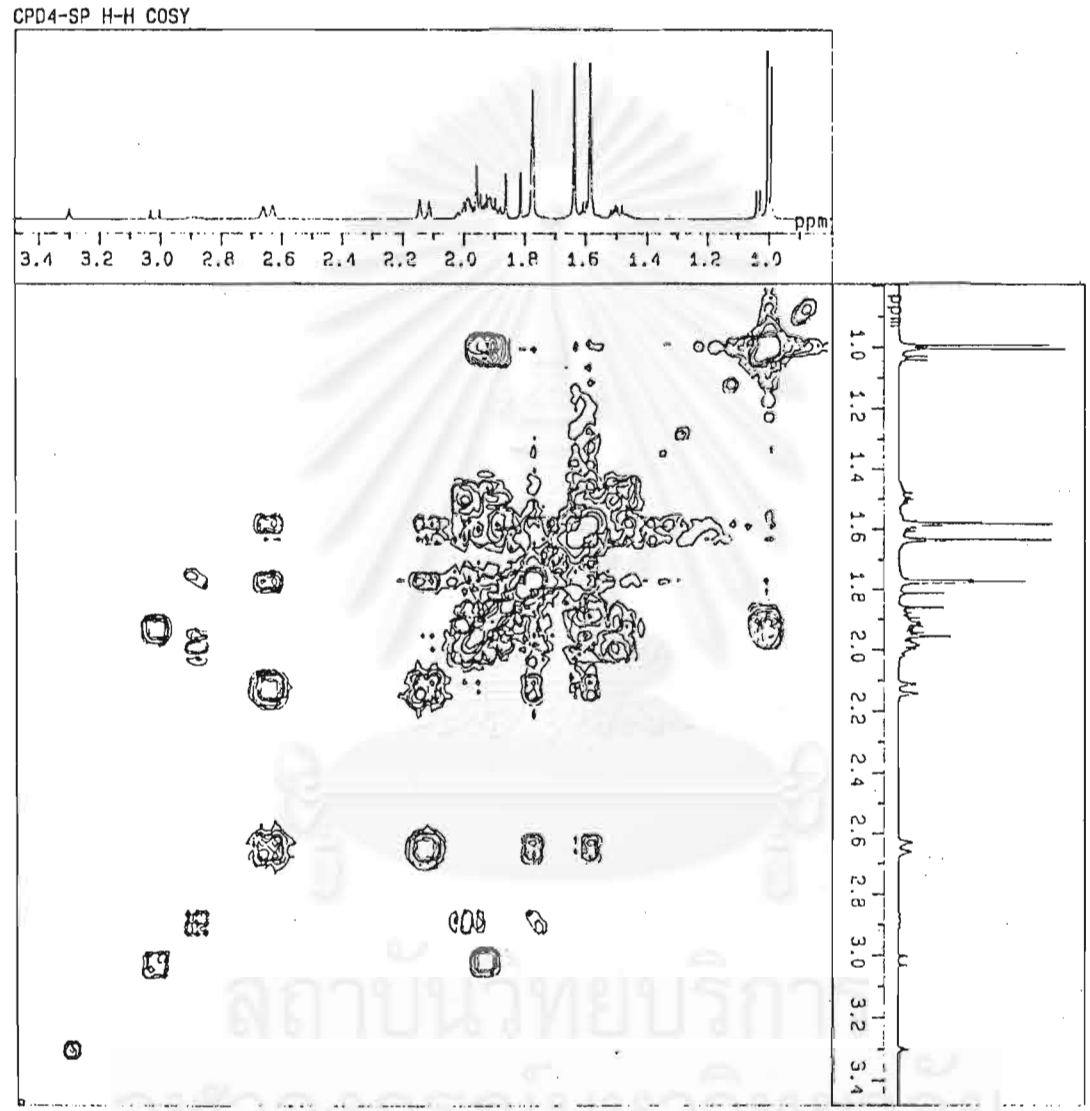


Figure 37. ^{1}H-^{1}H COSY spectrum of Curcumenol.

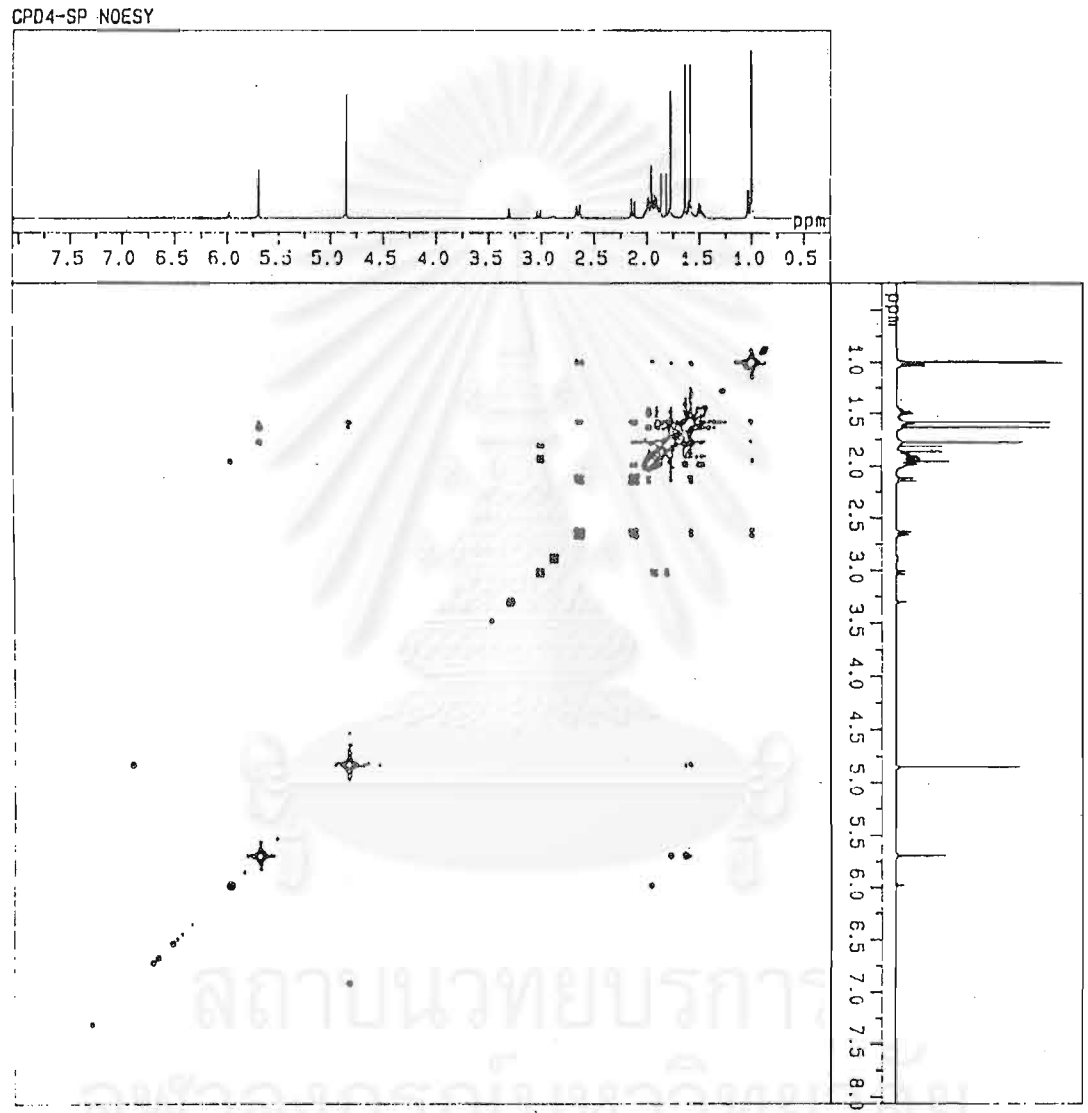


Figure 38. NOESY spectrum of Curcumenol.

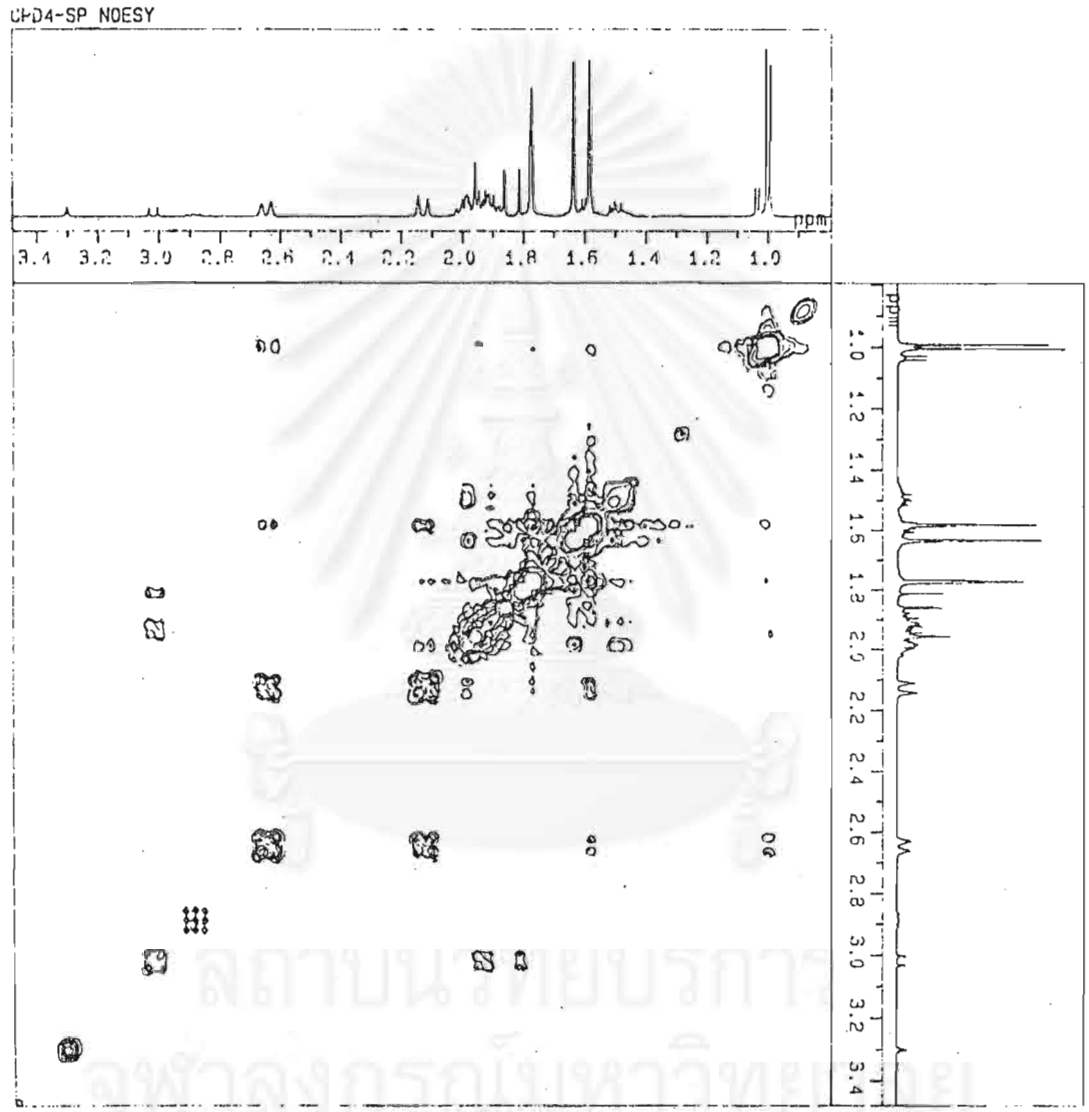


Figure 39. NOESY spectrum of Curcumenol.

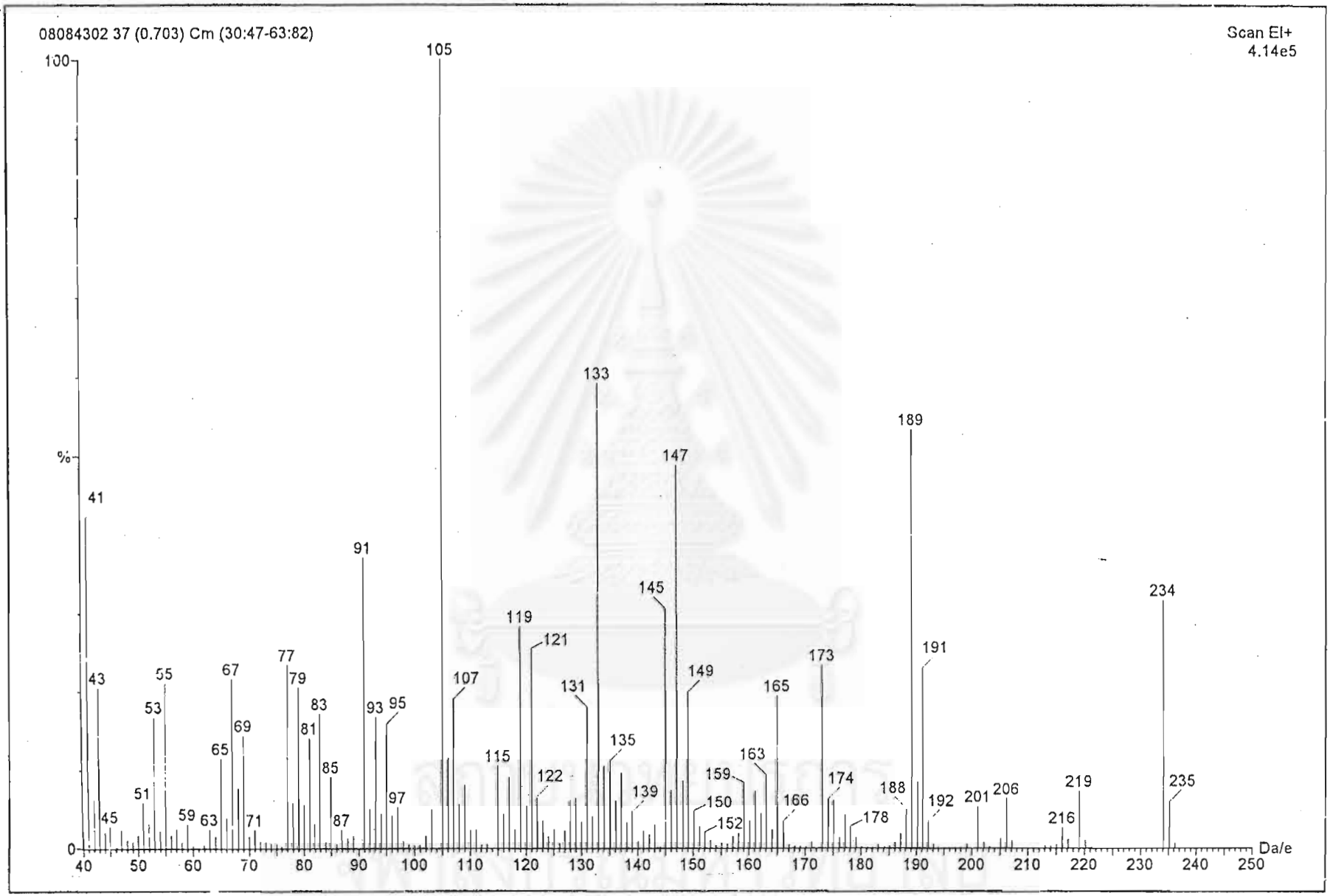


Figure 40. The EI mass spectrum of Curcumenol

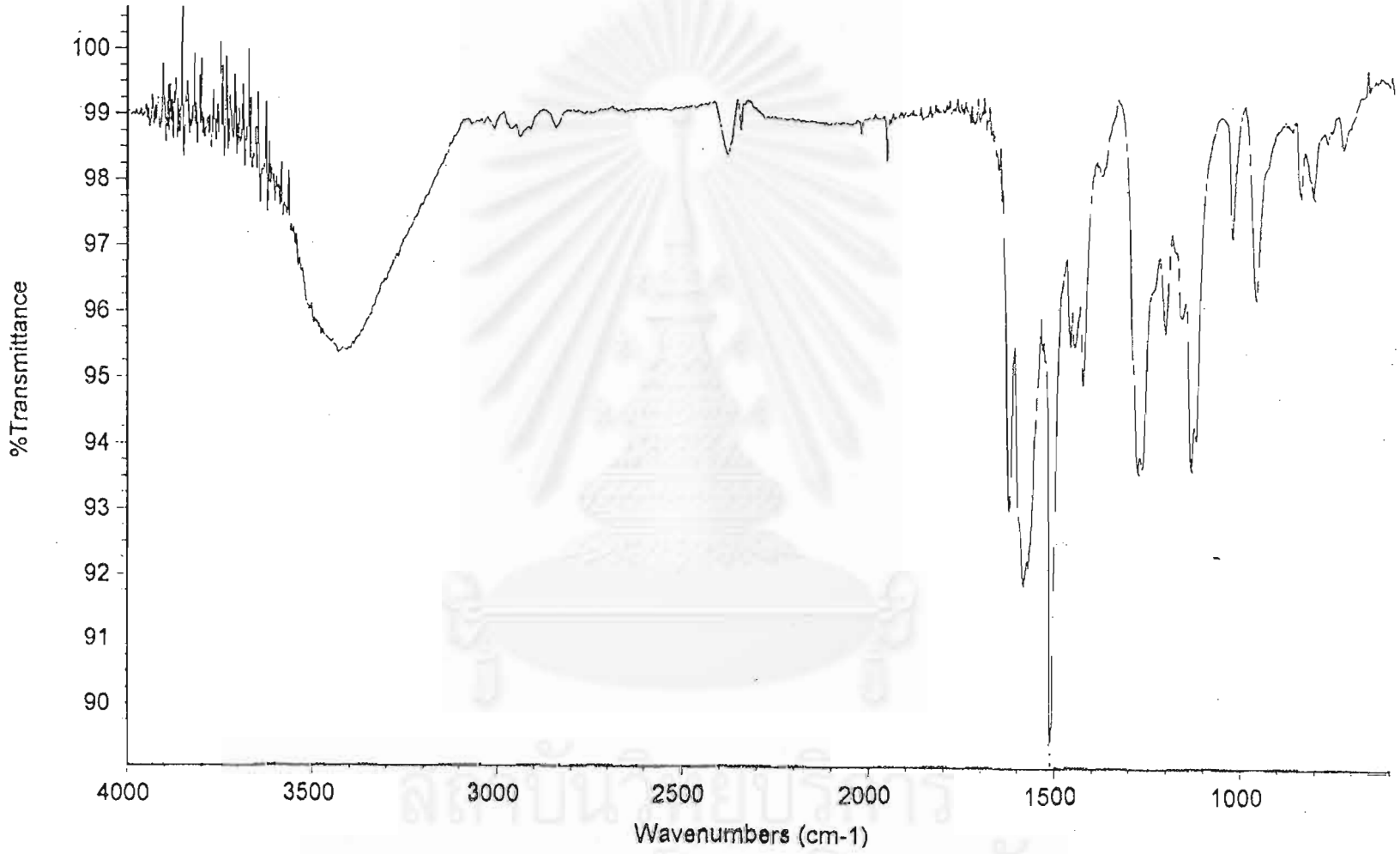


Figure 41. The IR spectrum of Curcumin.

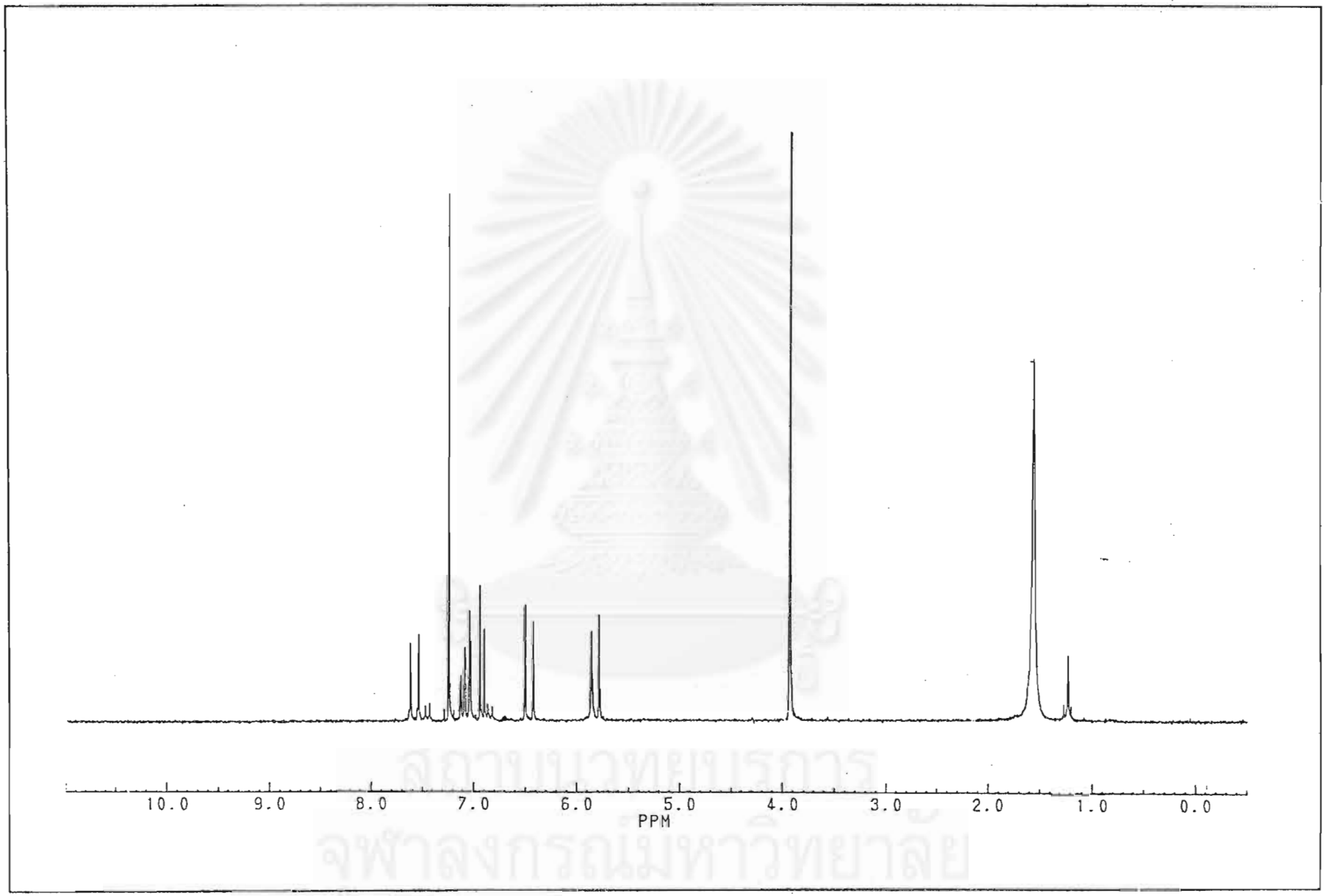


Figure 42. The ^{1}H-NMR spectrum of Curcumin.

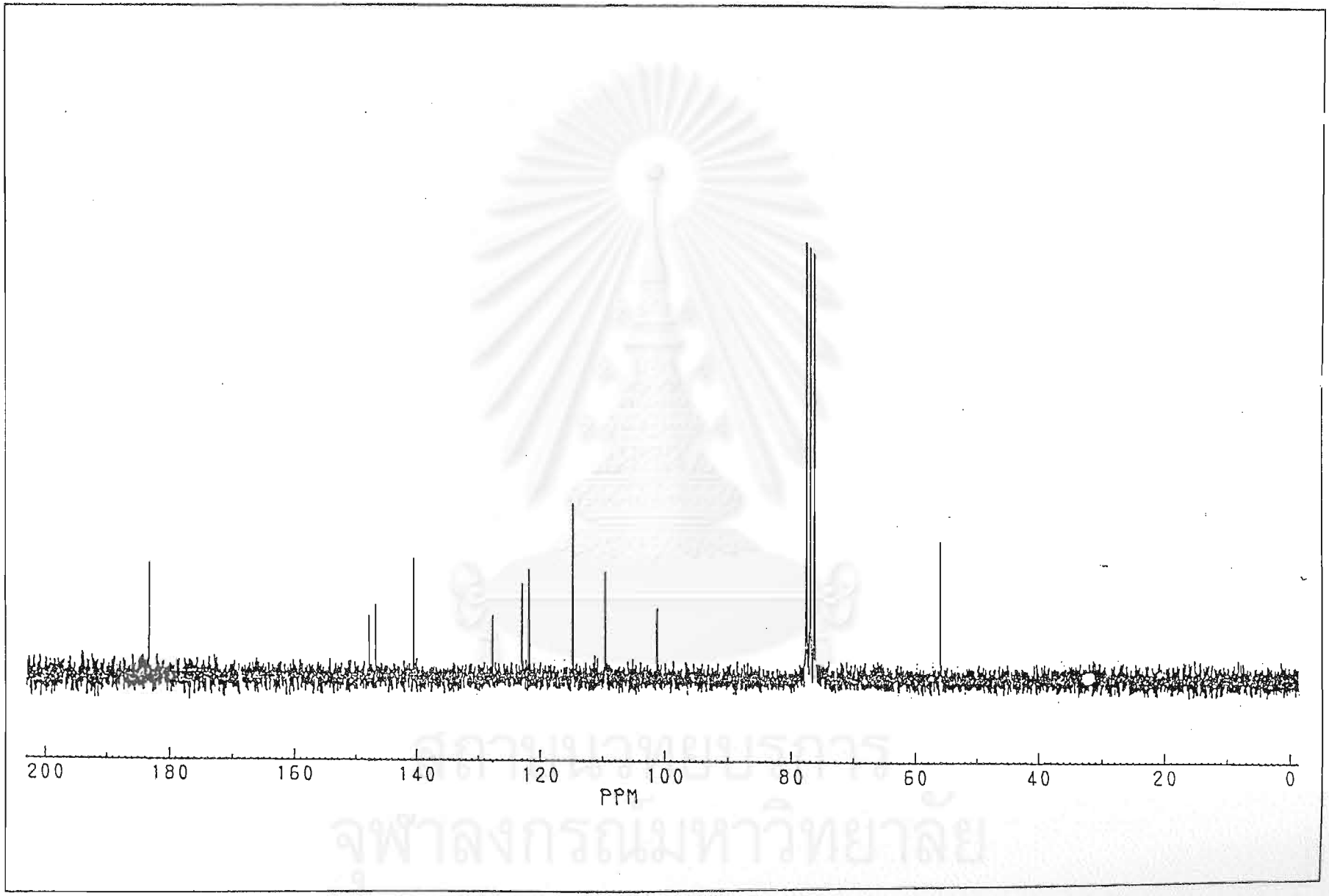


Figure 43. The ^{13}C-NMR spectrum of Curcumin.

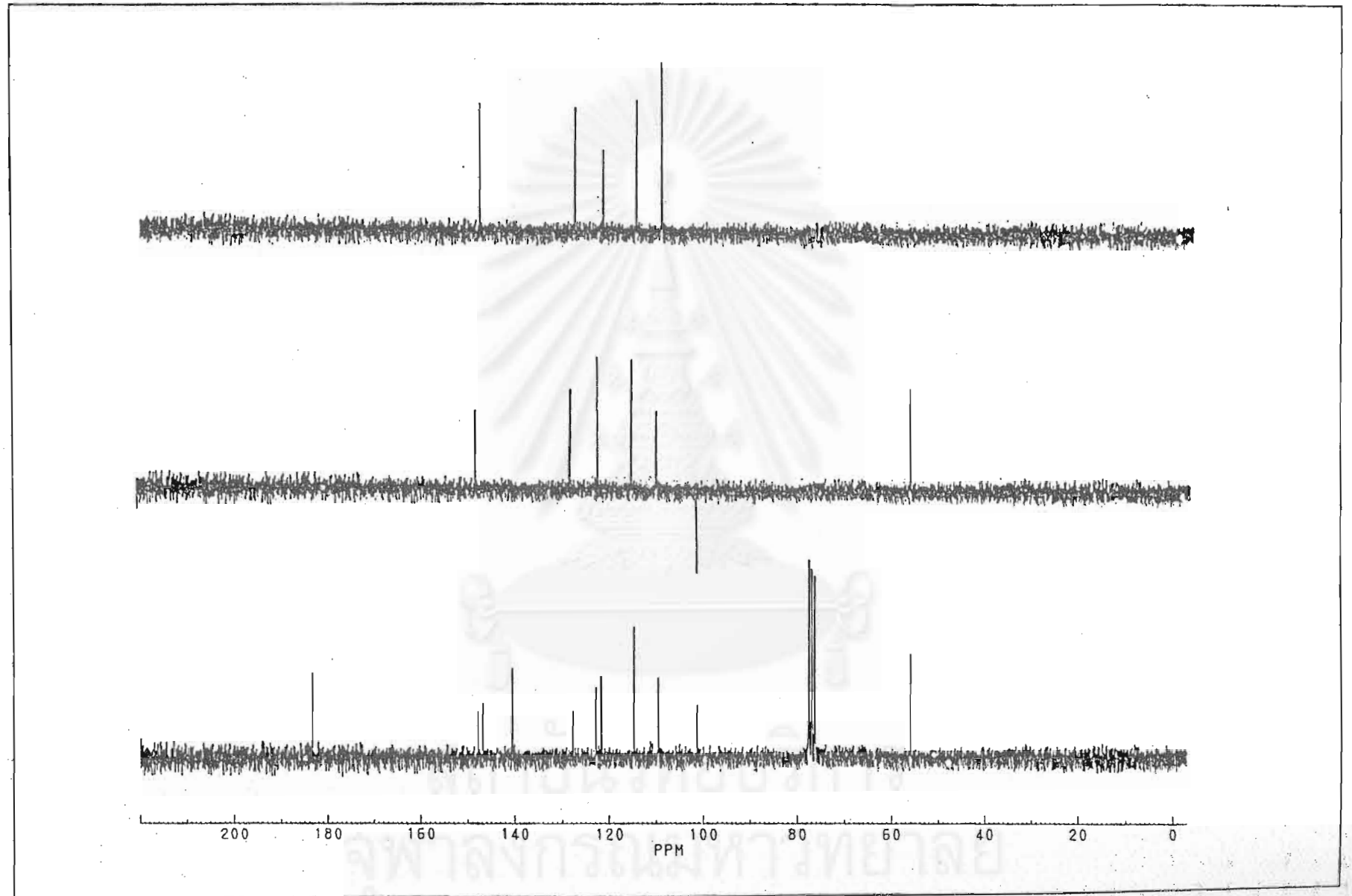


Figure 44. DEPT 90, DEPT 135 and ^{13}C-NMR spectrum of Curcumin.

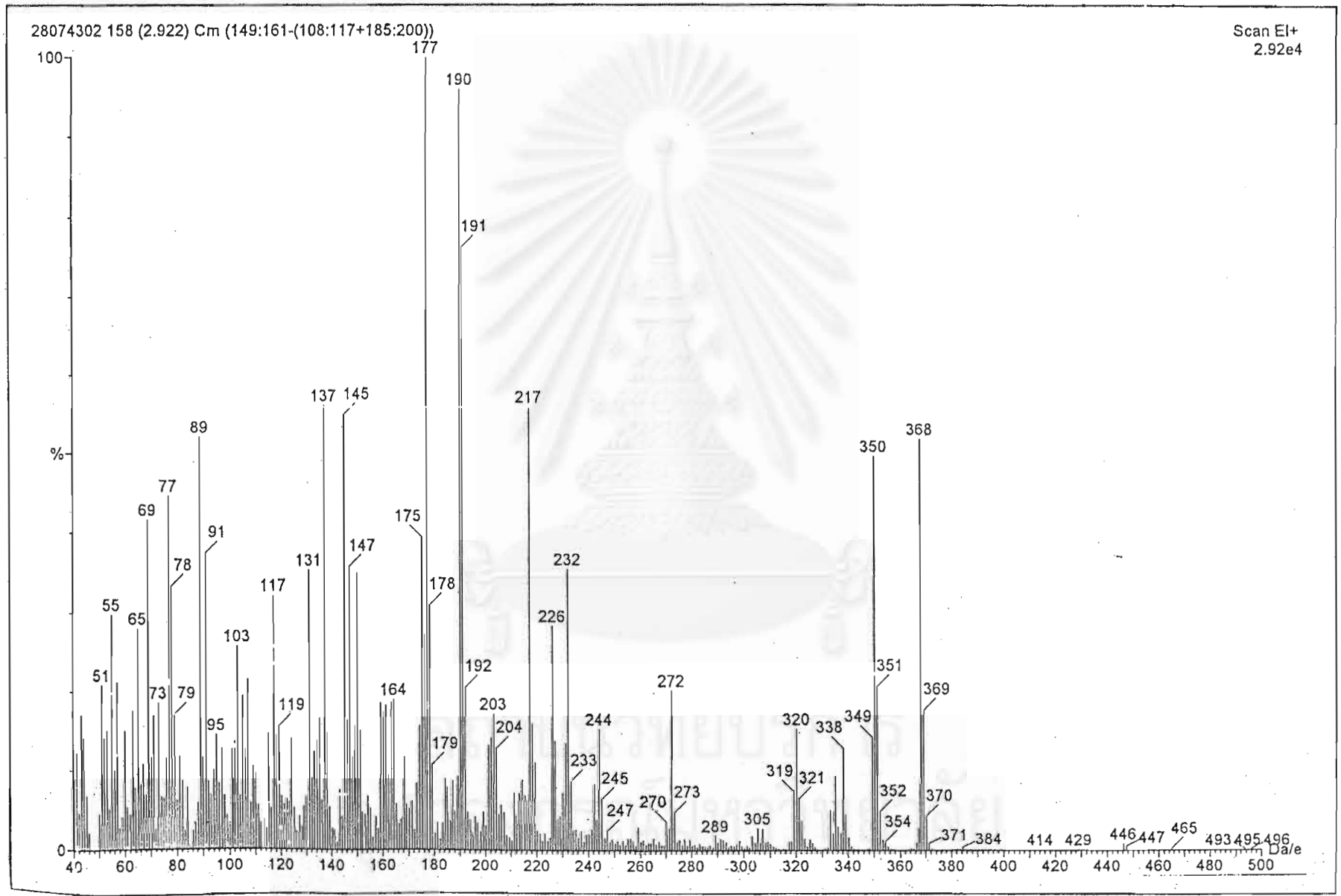


Figure 45. The EI mass spectrum of Curcumin.

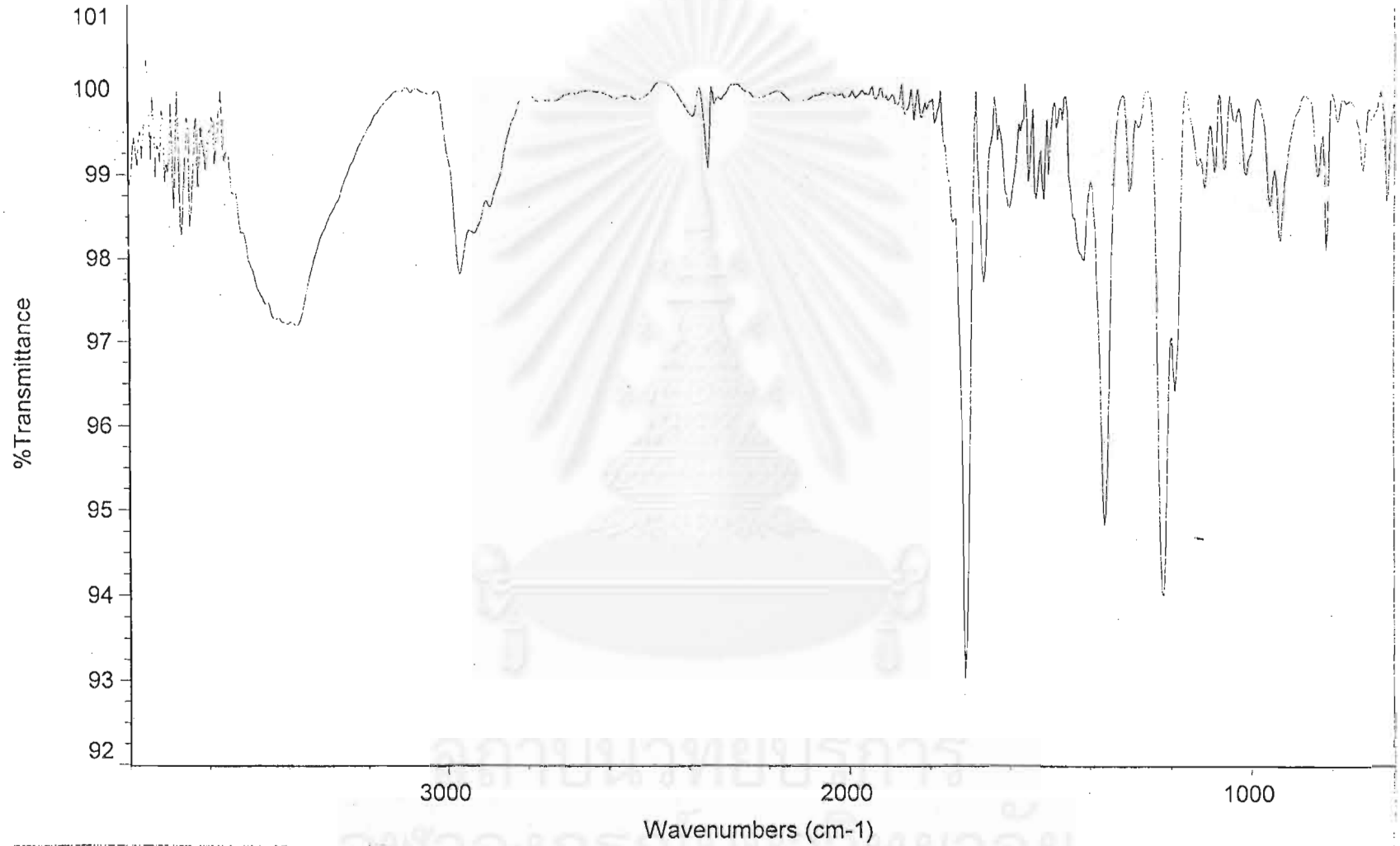
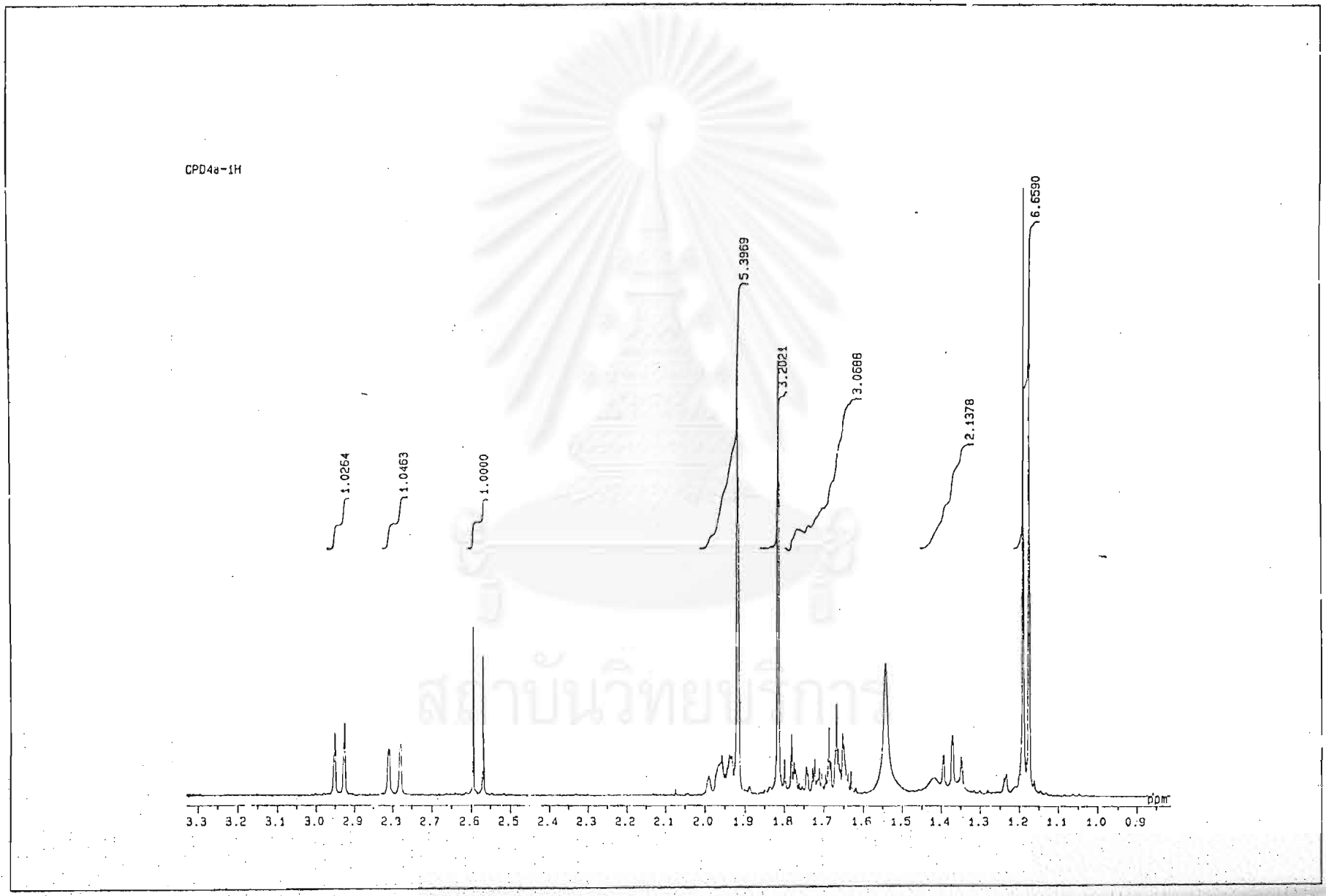


Figure 46. The IR spectrum of Zedoarondiol.



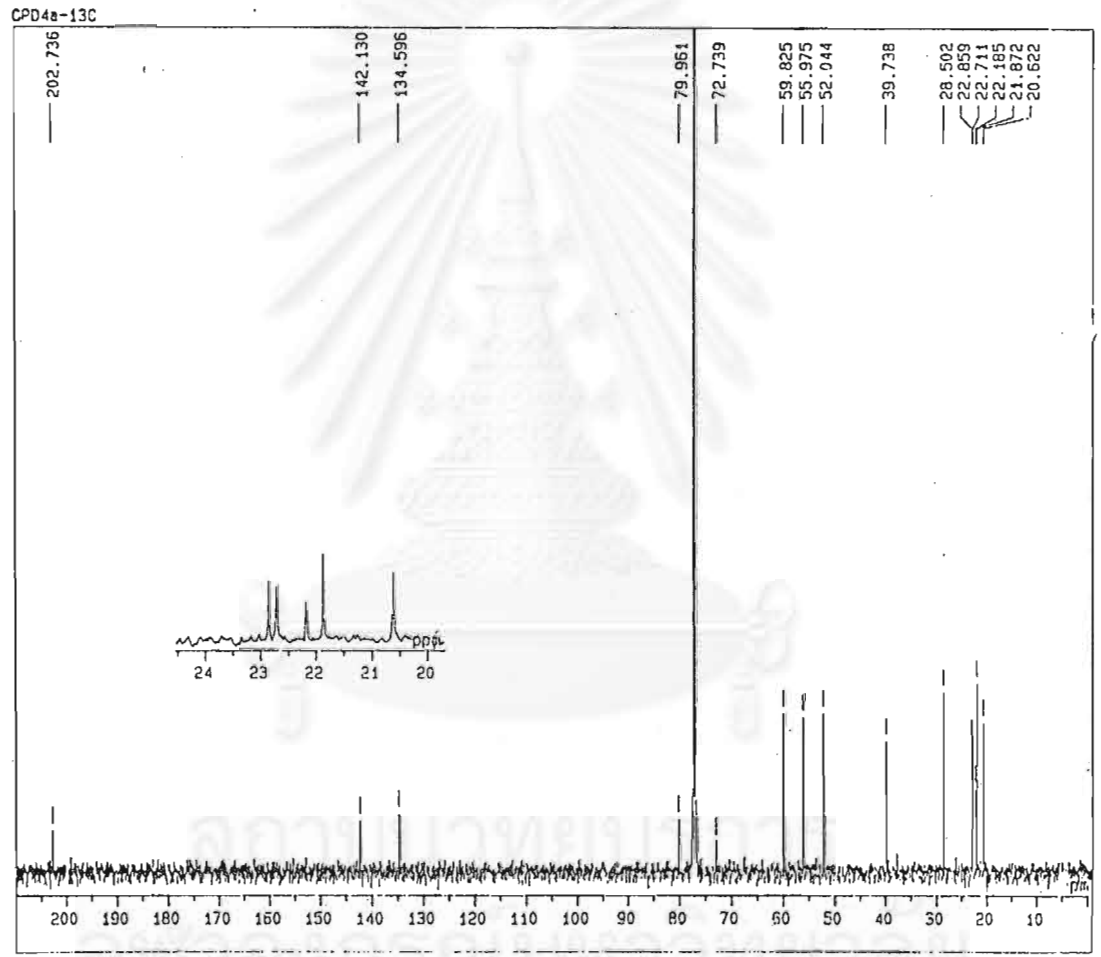


Figure 48. The ^{13}C-NMR spectrum of Zedoarondiol.

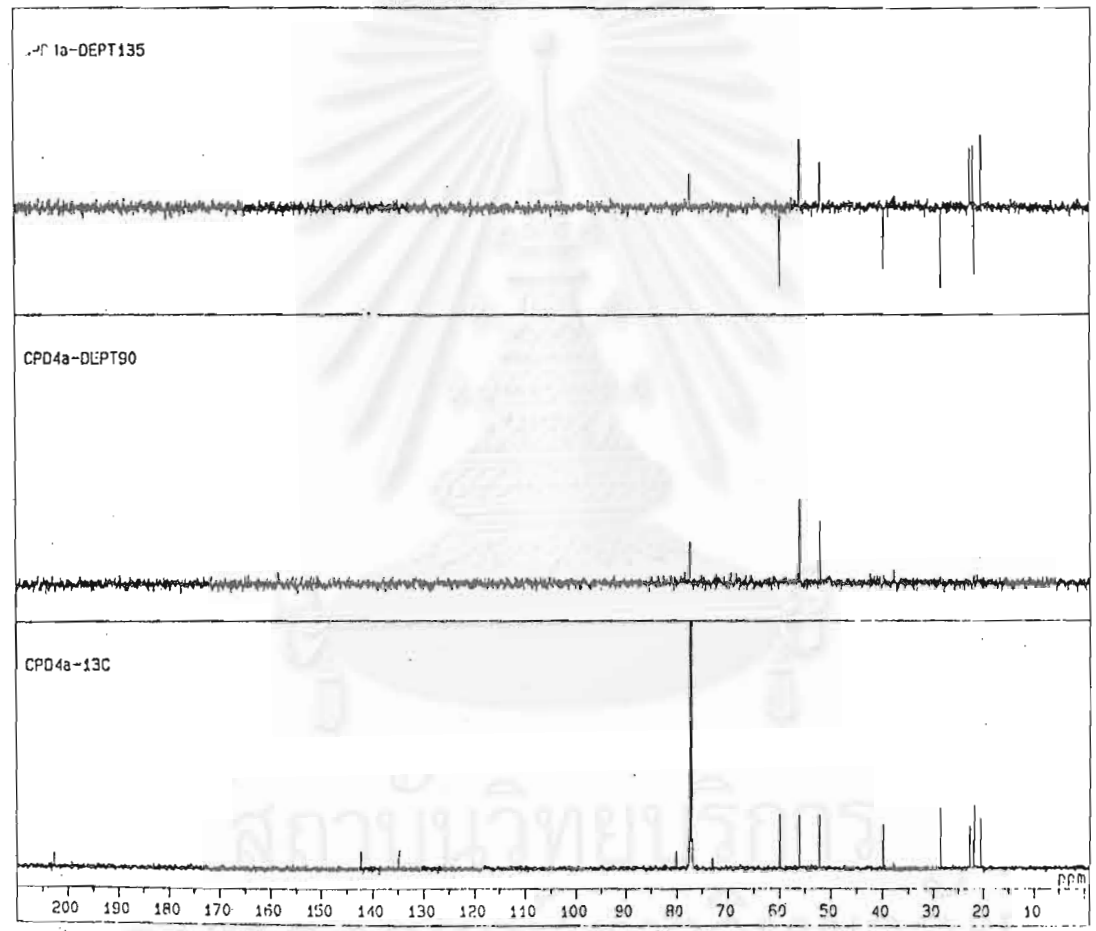


Figure 49. DEPT 90, DEPT 135 and ^{13}C-NMR spectrum of Zedoarondiol.

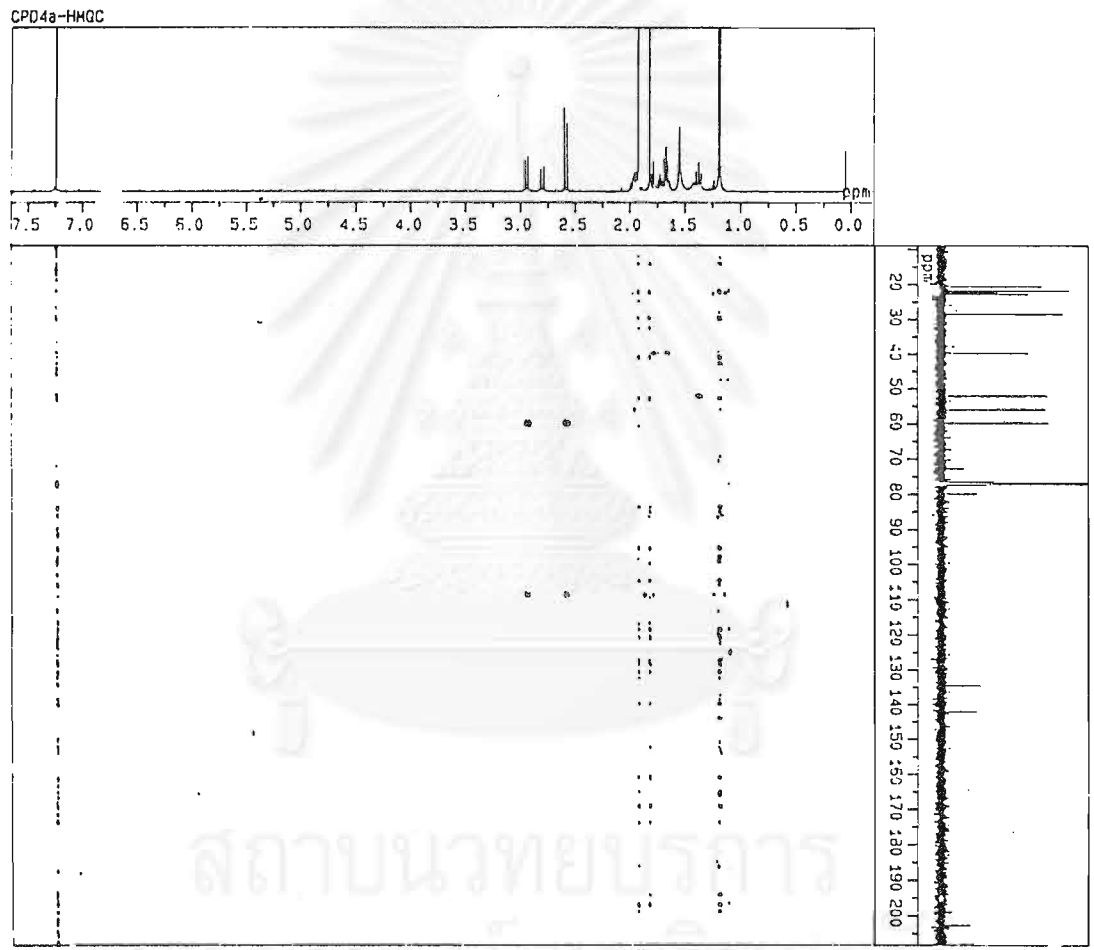


Figure 50. HMQC spectrum of Zedoarondiol.

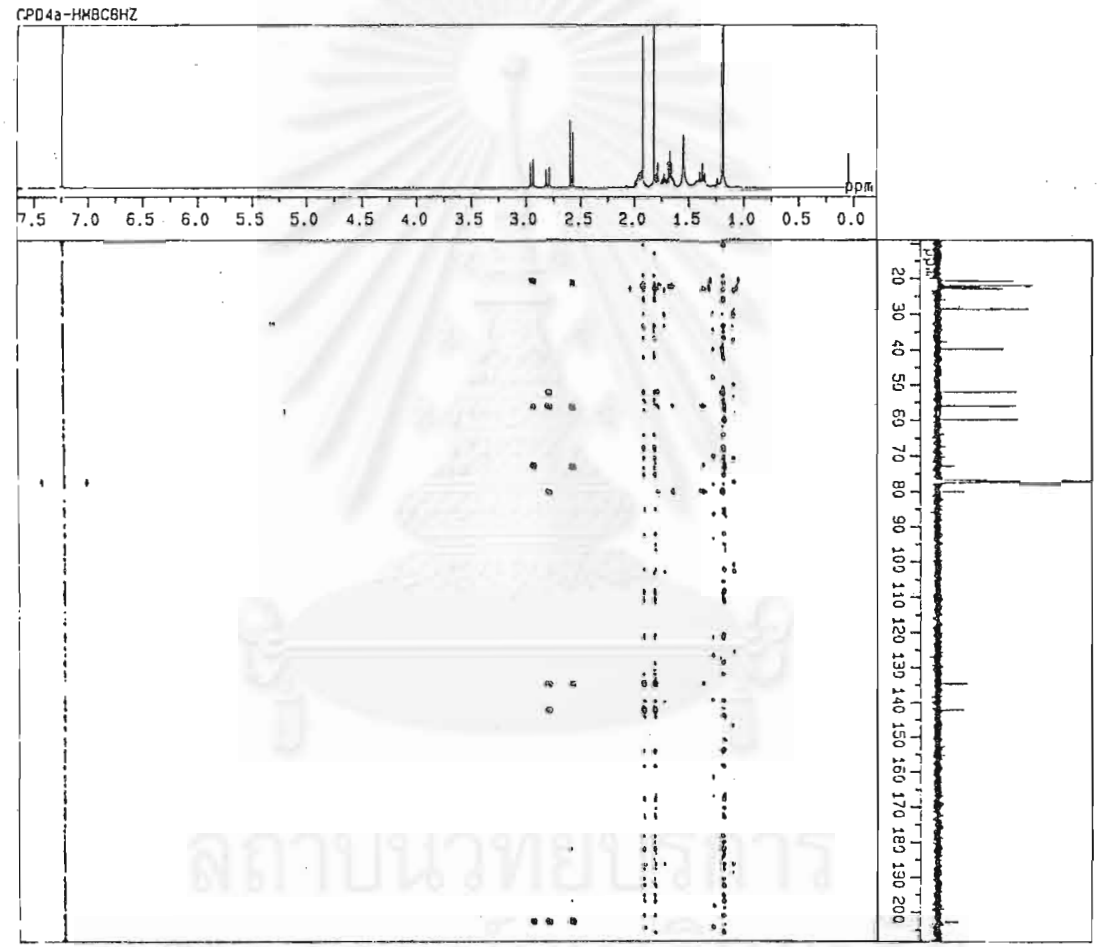


Figure 51. HMBC spectrum of Zedoarondiol.

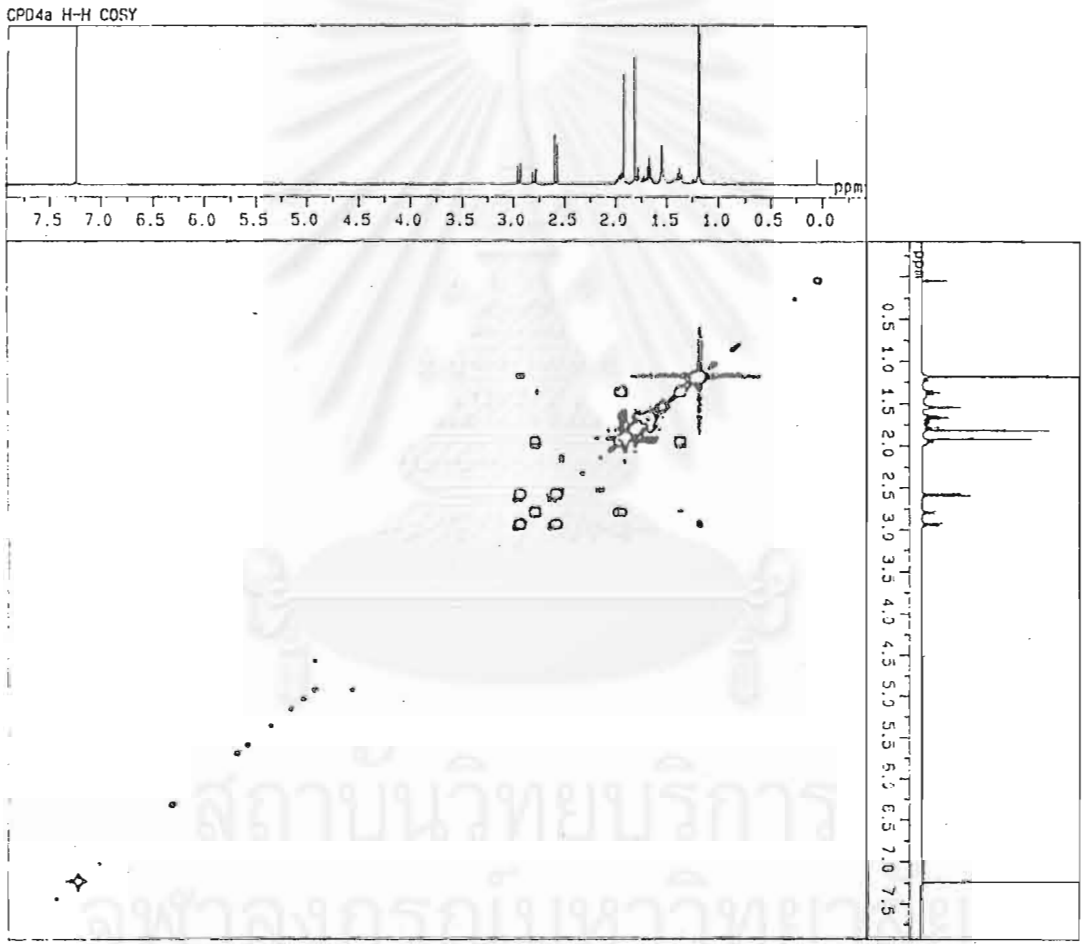


Figure 52. ^{1}H-^{1}H COSY spectrum of Zedoarondiol.

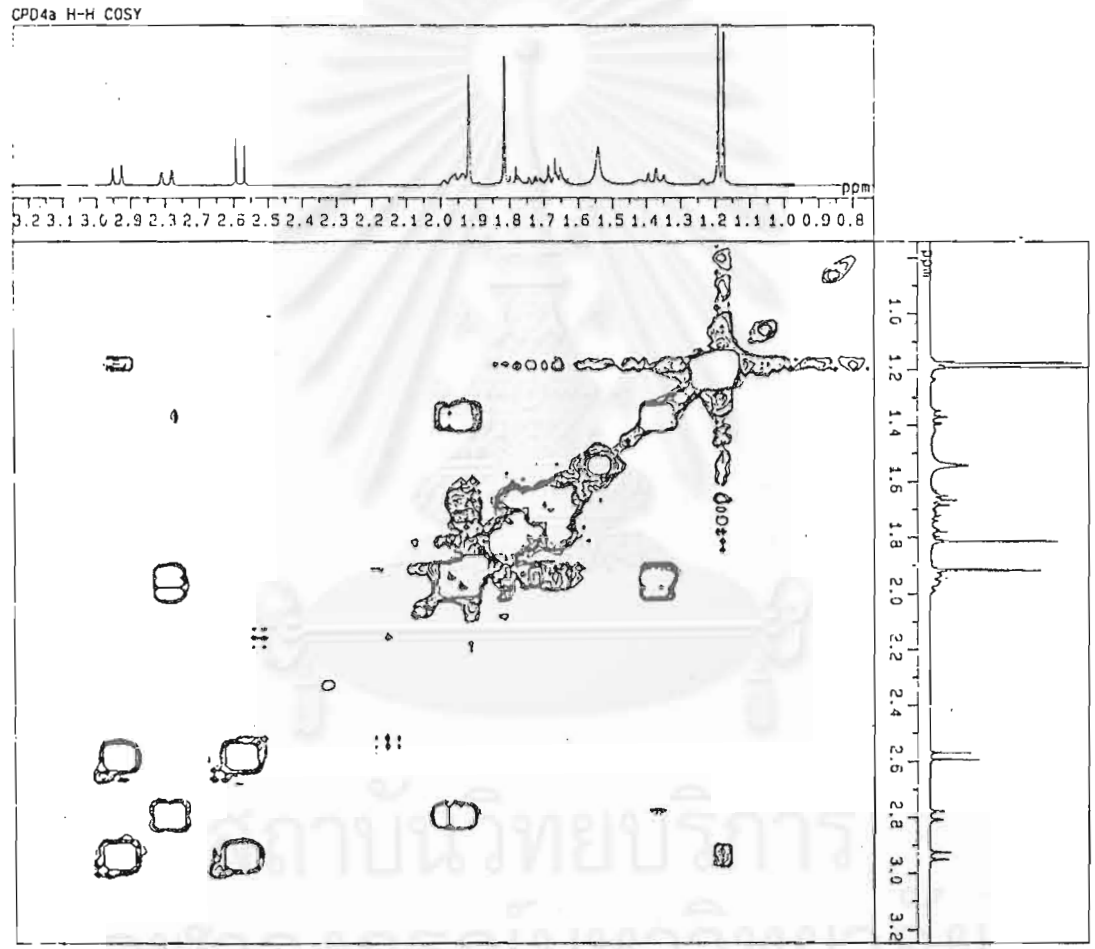


Figure 53. ^{1}H-^{1}H COSY spectrum of Zedoarondiol.

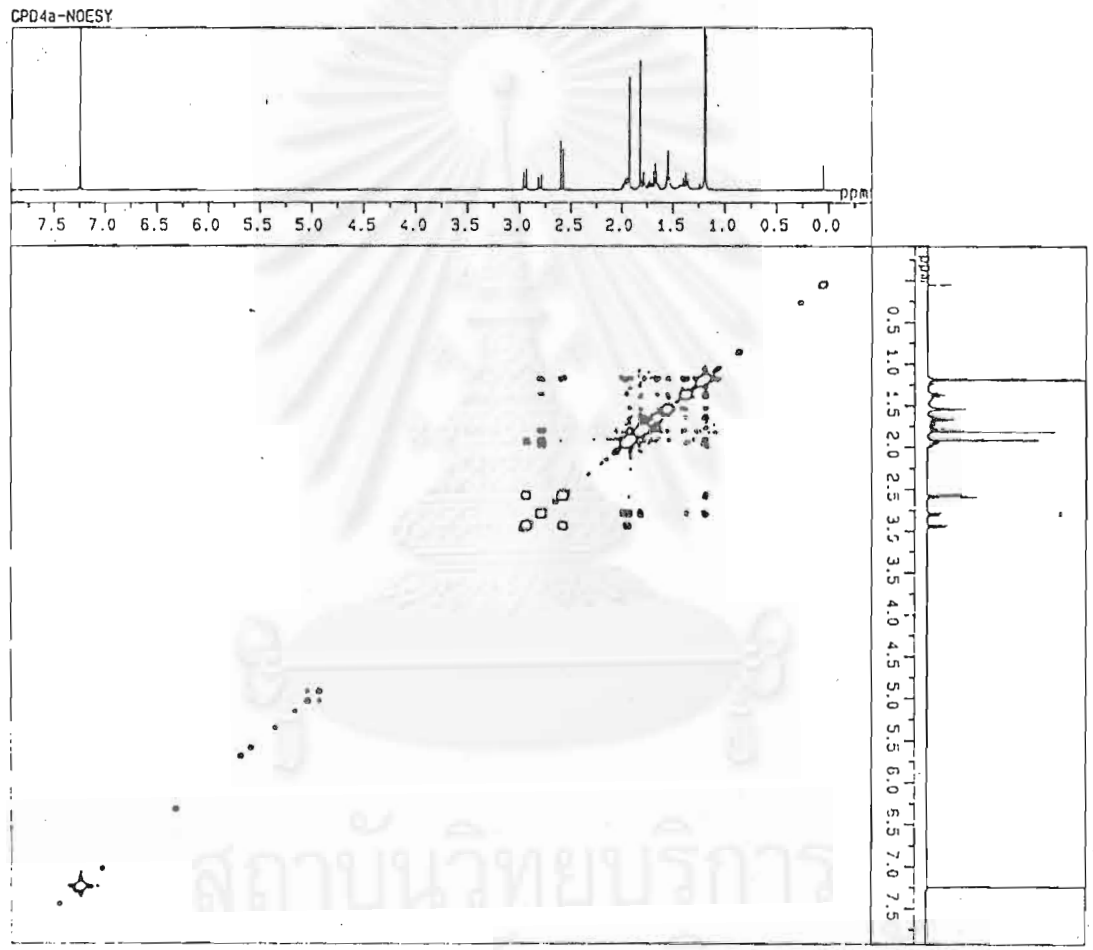


Figure 54. NOESY spectrum of Zedoarondiol.

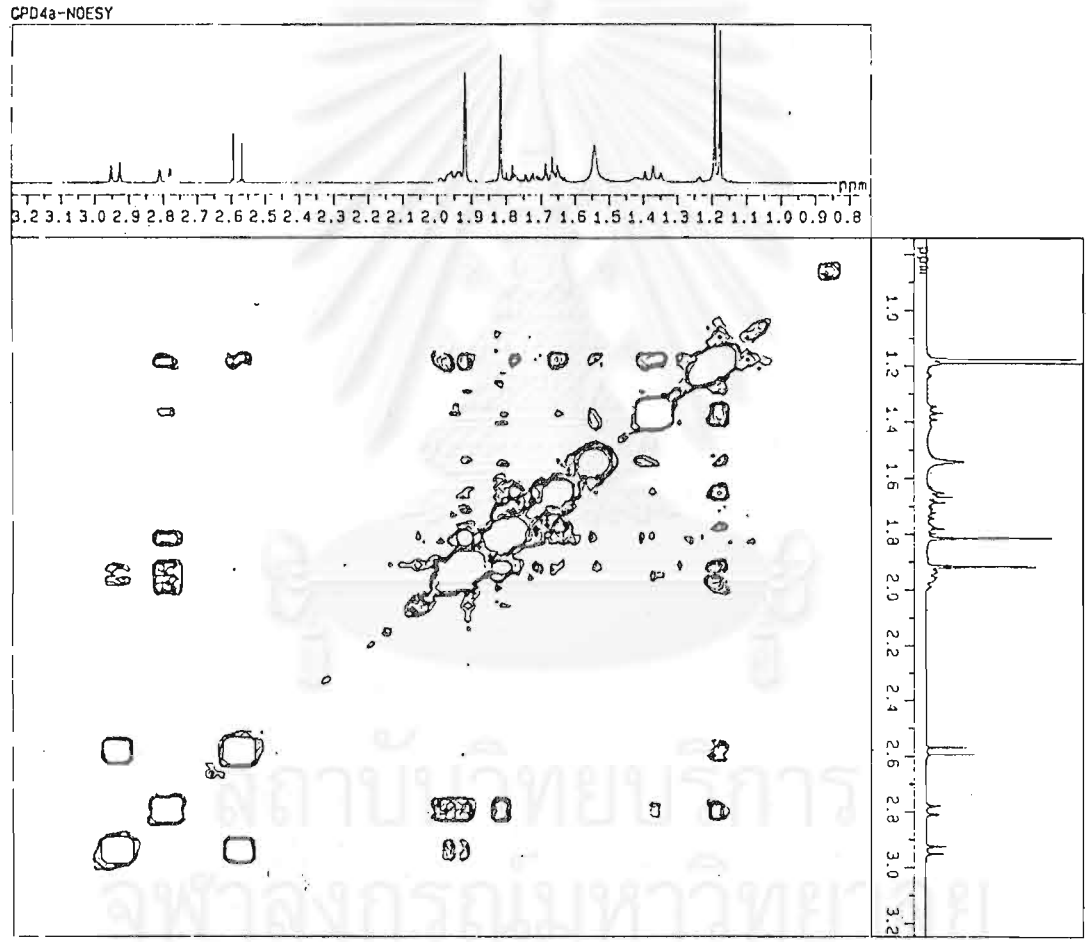
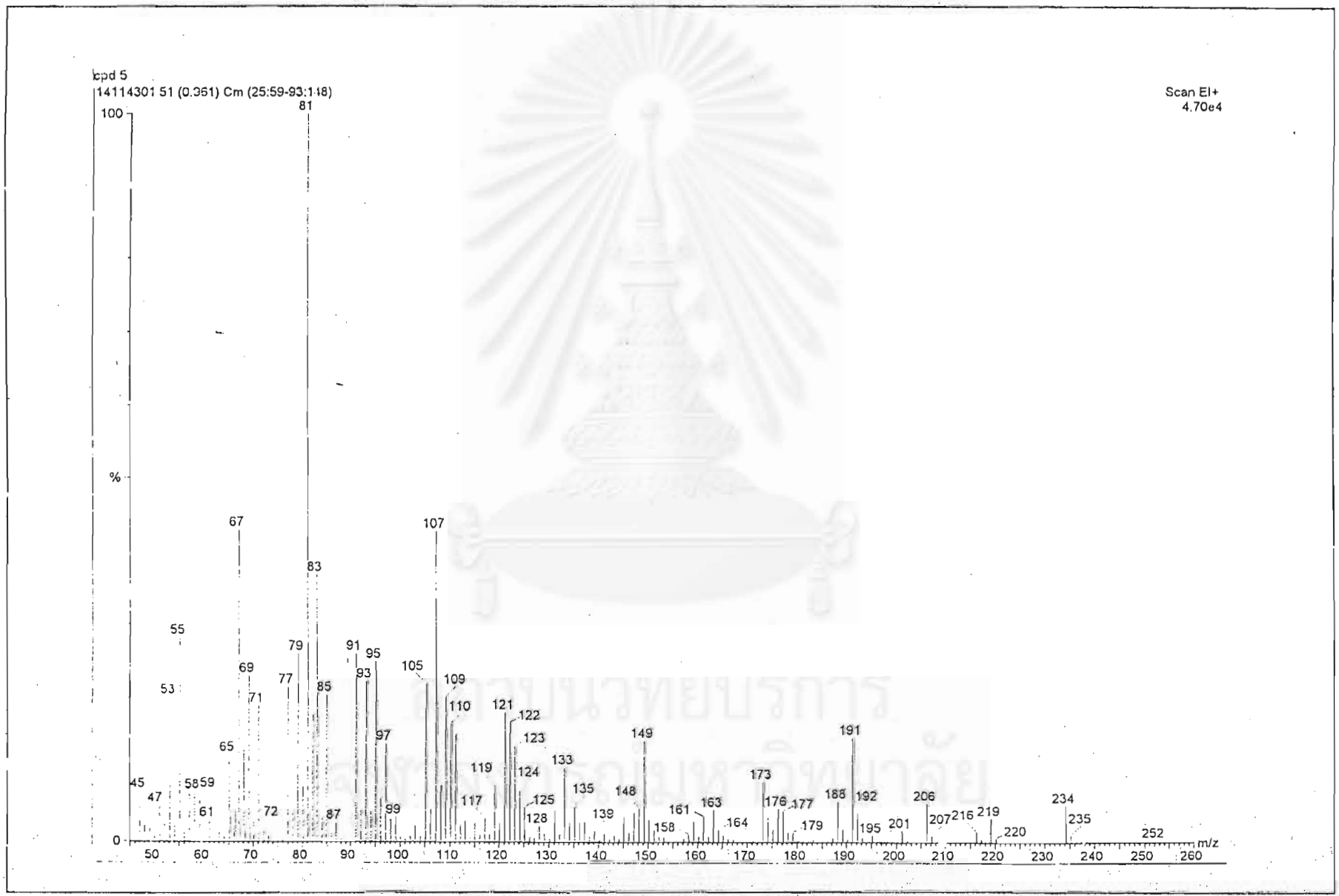


Figure 55 NOESY spectrum of Zedoarondiol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14114301 51 (0.951) Cm (25:59-93:118)
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252
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VITA

Mr.Sathaporn Onanong was born on October 11, 1977 in Bangkok. He accomplished the Bachelor Degree of Science in 1998 form Department of Chemistry, Faculty of Science, Kasetsart University. In the same year, he was admitted into a Master Degree Program in Organic Chemistry at Chulalongkorn University. During his study towards the Master's degree, he received a teaching assistantship by the Faculty of Science in 1998 and was supported by a research subsidy from The Graduate School, Chulalongkorn University in 2000.