องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของผักจ๋อนแจ๋นและเจตพังคี

นางสาว มยุรี กัลยาวัฒนกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *PTEROCAULON REDOLENS* AND *CLADOGYNOS ORIENTALIS*

Miss Mayuree Kanlayavattanakul

A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *PTEROCAULON REDOLENS* AND *CLADOGYNOS ORIENTALIS*
By	Miss Mayuree Kanlayavattanakul
Field of Study	Pharmaceutical Chemistry and Natural Products
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Thesis Co-advisor	Professor Tsutomu Ishikawa, Ph.D.

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มยุรี กัลยาวัฒนกุล: องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของผักจ๋อนแจ๋นและเจตพังคี (CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *PTEROCAULON REDOLENS* AND *CLADOGYNOS ORIENTALIS*) อาจารย์ที่ปรึกษา: รศ. ดร. นิจศิริ เรืองรังษี, อาจารย์ที่ปรึกษาร่วม: Prof. Dr. Tsutomu Ishikawa, 212 หน้า. ISBN: 974-53-1766-7

การศึกษาองค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของผักจ๋อนแจ๋นและเจตพังคี สามารถแยกสารในกลุ่มคูมารินได้ 7 ชนิด ฟลาโวนอยด์ 3 ชนิด และ เทอร์ปีน 11 ชนิด ซึ่งประกอบด้วย เซสควิเทอร์ปีน 3 ชนิด ไดเทอร์ปีน 6 ชนิด และไตรเทอร์ปีน 2 ชนิด การพิสูจน์โครงสร้างของสารทั้งหมดที่แยกได้โดยอาศัยการวิเคราะห์เชิงสเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลกับสารที่ทราบโครงสร้างแล้ว พบว่าสารที่แยกได้จากส่วนเหนือดินของผักจ๋อนแจ๋นประกอบด้วยสารในกลุ่มคูมาริน ที่พบครั้งแรกในธรรมชาติ 1 ชนิด คือ 2′,3′-dihydroxypuberulin [**52**] สารกลุ่มคูมารินที่เคยมีรายงานมาแล้วอีก 6 ชนิด คือ 5-methoxy-6,7-methylenedioxycomarin [**9**], ayapin [**10**], sabandinol [**23**], puberulin [**50**], 5-methoxyscopoletin [**51**] และ isofraxidin [**53**] นอกจากนี้ยังพบสารกลุ่มฟลาโวนอยด์ที่เคยมีรายงานมาแล้วอีก 3 ชนิด คือ chrysosplenol C [**35**], luteolin [**54**] และ tomentin [**55**] ส่วนสารที่แยกได้จากรากเจตพังคีประกอบด้วยกลุ่มเซสควิเทอร์ปีนชนิดใหม่ 1 ชนิด คือ (*4S*,7R*,8R*,10S**)-8-hydroxy-α-guaiene [**56**] และที่เคยมีรายงานมาแล้วอีก 2 ชนิด คือ spathulenol [**57**] และ cyperenoic acid [**64**] กลุ่มไดเทอร์ปีนชนิดใหม่ 4 ชนิด คือ 5-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-1-carboxylic acid [**58**], methyl-9-(furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo [5.3.3.$0^{1,6}$] trideca-5,8-diene-2-carboxylate [**59**], 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**] และ 6-[2-(furan-3-yl)oxoethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**63**] และที่เคยรายงานมาแล้วอีก 2 ชนิด คือ chettaphanin I [**48**] และ chettaphanin II [**49**] สารในกลุ่มไตรเทอร์ปีนที่เคยมีรายงานมาแล้ว 2 ชนิด คือ acetoxyaleuritolate [**60**] และ taraxerol [**61**] สารที่แยกได้ทั้งหมด 21 ชนิด ถูกนำไปทดสอบฤทธิ์ทางชีวภาพ ได้แก่ ฤทธิ์ความเป็นพิษต่อเซลล์ และฤทธิ์ต้านเชื้อวัณโรค พบว่า chrysosplenol C [**35**], chettaphanin II [**49**], taraxerol [**61**] และ 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxotricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**] มีฤทธิ์ความเป็นพิษต่อเซลล์ระดับอ่อนถึงปานกลางและพบว่าสารที่แยกได้เกือบทุกชนิดมีฤทธิ์ต้านเชื้อวัณโรคอย่างอ่อน ยกเว้น chrysosplenol C [**35**], 2′,3′-dihydroxypuberulin [**52**] และ acetoxyaleuritolate [**60**] ไม่มีฤทธิ์ต้านเชื้อวัณโรค

สาขาวิชา เภสัชเคมีและผลิตภัณฑ์ธรรมชาติ ลายมือชื่อนิสิต.....

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORDS: COUMARINS/ FLAVONOIDS/ SESQUITERPENES/ DITERPENES/ TRITERPENES/ ANTIMYCOBACTERIAL ACTIVITY/ CYTOTOXICITY/ *PTEROCAULON REDOLRNS*/ *CLADOGYNOS ORIENTALIS*

MAYUREE KANLAYAVATTANAKUL: CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *PTEROCAULON REDOLENS* AND *CLADOGYNOS ORIENTALIS* THESIS ADVISOR: ASSOCIATE PROFESSOR NIJSIRI RUANGRUNGSI, Ph.D., THESIS CO-ADVISOR: PROFESSOR TSUTOMU ISHIKAWA, Ph.D., 212 pp. ISBN: 974-53-1766-7

Chemical investigation of *Pterocaulon redolens* (Forst. f) F. Vill. and *Cladogynos orientalis* Zipp. ex Span. led to the isolation of seven coumarins, three flavonoids and eleven terpenes including three sesquiterpenes, six diterpenes and two triterpenes. The structure determination of these compounds was extensively accomplished by spectroscopic analyses (UV, IR, MS and NMR properties) and by comparison with previously reported data of known compounds. The aerial parts of *Pterocaulon redolens* provided one new natural coumarin, namely, 2′,3′-dihydroxypuberulin [**52**], six known coumarins identified as 5-methoxy-6,7-methylenedioxycoumarin [**9**], ayapin [**10**], sabandinol [**23**], puberulin [**50**], 5-methoxyscopoletin [**51**] and isofraxidin [**53**] and also gave three known flavonoids, chrysosplenol C [**35**], luteolin [**54**] and tomentin [**55**]. The roots of *Cladogynos orientalis* yielded a new sesquiterpene, (*4S*,7R*,8R*,10S**)-8-hydroxy-α-guaiene [**56**], together with two known sesquiterpenes, spathulenol [**57**] and cyperenoic acid [**64**]. In addition, four new diterpenes, namely, 5-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-1-carboxylic acid [**58**], methyl 9-(furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo[5.3.3.$0^{1,6}$]trideca-5,8-diene-2-carboxylate [**59**], 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**], 6-[2-(furan-3-yl)-2-oxoethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]-dodec-2(7)-en-11-one [**63**], two known diterpenes, chettaphanin I [**48**] and chettaphanin II [**49**] and two known triterpenes, acetoxyaleuritolate [**60**] and taraxerol [**61**] were afforded. All isolated compounds were evaluated for their cytotoxicity and antimycobacterial activity. It was found that chrysosplenol C [**35**], chettaphanin II [**49**], taraxerol [**61**], 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo- [7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**] were mild to moderate cytotoxic activity. All of them showed weak antimycobacterial activity except chrysosplenol C [**35**], 2′,3′-dihydroxypuberulin [**52**] and acetoxyaleuritolate [**60**], which showed no antimycobacterial activity.

Field of Study; Pharmaceutical Chemistry and Natural Products — Student's signature…….....

Advisor's signature……..

Academic year 2004 — Co-advisor's signature…….

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CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

Figure **Page**

Figure **Page**

Figure **Page**

Figure **Page**

Figure **Page**

LIST OF SCHEMES

LIST OF ABBREVEATIONS AND SYMBOLS

α	=	Alpha
$[\alpha]^t_D$	=	Specific rotation at t °C and sodium D line (589 nm)
β	=	Beta
°C	=	Degree Celsius
calcd.	=	Calculated
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
CH_2Cl_2	=	Dichloromethane
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
^{13}C NMR	=	Carbon-13 Nuclear Megnetic Resonance
CO_2	=	Carbon dioxide
2-D NMR	=	Two Dimensional Nuclear Magnetic resonance
d	=	Doublet (for NMR spectra)
dd	=	Doublet of Doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO	=	Dimethyl sulfoxide
δ	=	Chemical Shift
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
FABMS	=	Fast Atom Bombardment Mass spectrometry
g	=	Gram
GC	=	Gas Chromatography
hr	=	Hour
^1H NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^1H-detected Heteronuclear Multiple Bond Coherence
HMQC	=	^1H-detected Heteronuclear Multiple Quantum Coherence
HRFABMS	=	High Resolution Fast Atom Bombardment Mass spectrometry
Hz	=	Hertz
IC_{50}	=	Inhibition Concentration at 50%
IR	=	Infrared Spectrum

J	=	Coupling constant
Kg	=	Kilogram
L	=	Liter
μg	=	Microgram
μL	=	Microliter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
M^+	=	Molecular ion
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
$[M+H]^+$	=	Protonated molecular ion
MHz	=	Megahertz
min	=	minute
mL	=	Milliliter
MW	=	Molecular weight
m/z	=	Mass to charge ratio
MS	=	Mass Spectrometry
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOE	=	Nuclear Overhauser Effect
ppm	=	Part per million
spp.	=	Species
ν_{max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
TLC	=	Thin Layer Chromatography
TMS	=	Tetramethylsilane
UV-VIS	=	Ultraviolet and Visible Spectrophotometry

CHAPTER I

INTRODUCTION

The genus *Pterocaulon* belongs to the family Asteraceae. This genus consists of about 25-30 species distributed in tropical America, Madagascar, tropical Asia and Australia (Koyama, 1984).

According to the Acta Phytotaxonamica Et Geobotanica (Koyama, 1984), there is only one species of the genus *Pterocaulon* found in Thailand as followed.

Pterocaulon redolens (Forst. f) F. Vill.	ผักจ๋อนแจ๋น	Pahk jawn jan;
(*Pterocaulon cylindrostachyum* Cl.)		nobcheese

Pterocaulon redolens (Forst. f) F. Vill. is distributed in India, Southern China, Thailand, Laos, Vietnam, Philippines and Australia. It is an annual herb up to 1.5 m tall. It is erect, branching, pleasantly scented and tap root. Stems and branches are terete, white floccose, glabrescent, light green and ageing brown, oldest parts to 12.0 mm thick, continuous, light green to green wings 2.0-2.5 mm wide which are less conspicuous than the oldest parts. Leaves are blades thin, lanceolate to somewhat spathulate, tip rounded, base narrowed and winged to the insertion, margins shallowly and sharply double serrate less than in younger blades, venation pinnate, midnerve distinct, other venation obscure, youngest blades densely white villous-floccose on both sides, upper surface in mature blades pilose and dull green, lower side villous-floccose and light green, 5.5-12.5 × 1.2-4.0 cm. Inflorescence terminate on each branch, numerous on each plant, speciform, 2.0-4.0 cm long, consisting of numerous spirally arranged, confluent, sessile heads, each 4.0-5.0 mm long and concealed in white floccose indumentum. Several involucral bracts in 2-3 series are thin, all similar, spathulate, tip acute with a sharp mucro and upper half green, the tips often pink to dark violet, lower half light green, densely white floccose, 2.5 × 0.3 mm. Flowers are several in each head, all tubular, glabrous, 3.0-3.5 mm long, outer ones are female and inner ones are bisexual, regular and 5-merous. Pappus is a single whorl of erect, white, glabrous hair as long as the corolla. Female flowers are slender, tube pale light green in the lower part, dark violet in upper part. Two stigmas are spreading, dark violet, 0.5 mm long, style as long as the corolla. Bisexual flowers are more prominent than pigmented and similar size as the female flowers. Five lobes are

ovate-oblong, tip obtuse, 0.5 mm long. Five Stamens are slightly shorter than the corolla, glabrous. Anthers are linear, marginally connate, 2-locular, tip with a thin, rounded extension of the connective which is as wide as and 1/3 as long as the locules; base with a thin, 0.2 mm long appendage on each side, light pink, 1.25 mm long. Filaments are free, inserted on the lower 1/3 of the corolla, pale light greenish, 1 mm long. One style is pale light greenish, 2.0 mm long. Ovary is inferior, cylindric, glabrous, 1-locular with 1 basal ovule, 0.75 mm long. Achenes are cylindric, striate, glabrous, 0.75 mm long, crowned by the pappus (**Figure 1**) (Radanachaless, 1994).

Although *P. redolens* has not been recorded in Thai Plant Names (Smitinand, 2001), however the herbarium specimen of this species has been kept at the National Park, Wildlife and Plant Coservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

The genus *Cladogynos* (Family Euphorbiaceae) consists of only one species distributed in China, Indo-China, Thailand and Philippines. Plants in genus *Cladogynos* are freshy shrubs with copious white latex in all parts. Leaves are spiral. Monoecious. Twigs are densely hairly at least at tip. Male flowers are sepals 2-4, not overlapping, no disc, usually 4 stamens, slender pistillate and female flowers are sepals 5-7, big and leafy, ovary 3-chambered, styles joined at base and above several times forked. Fruits are capsules and splitting into bivalved parts leaving central column (Whitemore, 1973).

According to Smitinand (2001), the species of the genus *Cladogynos* found in Thailand are as *Cladogynos orientalis* Zipp. ex Span (*Adenocleana siamensis* Ridl.). It has a local name as Chettaphangkhi (เจตพังคี), Plao num-ngeon (เปล้าน้ำเงิน) and Bai Lung Kaw (ใบหลังขาว). It is a shrubly tree, 90-150 cm high, common in dry evergreen or moist mixed deciduous forest or scrub up to 450 m, frequently on limestone. Leaves are conspicuously white-tomentellous below, coarsely repand-denatate or lobulate. The ovate-elliptic leaves are 10 cm long, stalk 7.5 cm long. Male flowers are small, dense, stalk slender piltillate, not overlapping, no disc, stellate hairy. Female flowers are 5-7 sepals and leafy, ovary 3-chambered, styles joined at base, flower head; cernuous in bug stage. Fruits are capsule, splitting in to bilvalved part leaving a central column. Yellow root-bark is rigid and smell (**Figure 2**) (Shaw, 1972).

During our preliminary evaluation for biological activities. Both plant extracts exhibited cytotoxic and antimycobacterial activities (see Results and Discussion section). Therefore, the following objectives are put forwards:

1. Isolation and purification of compounds from the aerial parts of *P. redolens* and the roots of *C. orientalis*
2. Determination of the chemical structure of each isolated compound
3. Evaluation of each isolate for its cytotoxic and antimycobacterial acitivities

Flowers

Aerial parts

Figure1 *Pterocaulon redolens* (Forst. f) F. Vill.

Fruits

Flowers

Dried roots

Figure2 *Cladogynos orientalis* Zipp. ex Span.

CHAPTER II

HISTORICAL

1. Chemical Constituents of *Pterocaulon* spp.

Chemical investigations of a number of *Pterocaulon* spp. have shown them to be a good source of coumarins (**Table 1**). In additional, other classes of natural compounds such as flavonoids, polyacetylenes and terpenes have been found (**Table 2-4**). As *Pterocaulon redolens* (Forst. f) F. Vill., no phytochemical study has been reported.

Table 1 Distribution of coumarins in *Pterocaulon* spp.

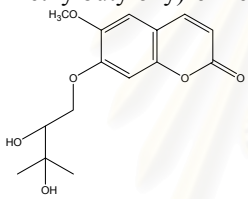
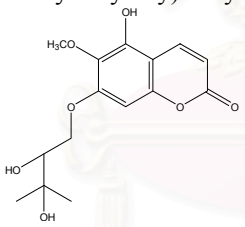
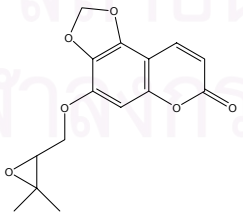
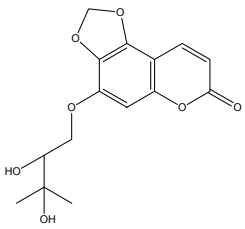
Plant and chemical compounds	Plant part	References
Pterocaulon alopeculoides		
7-(2,3-Dihydroxy-3-methylbutyloxy)-6-methoxycoumarin [**1**] 	Aerial part	Vilegas *et al*., 1995
7-(2,3-Dihydroxy-3-methylbutyloxy)-5-hydroxy-6-methoxycoumarin [**2**] 	Aerial part	Vilegas *et al*., 1995
P. balansae		
7-(2,3-Epoxy-3-methylbutyloxy)-5,6-methylenedioxycoumarin [**3**] 	Aerial part	Magalhaes *et al*., 1981
7-(2,3-Dihydroxy-3-methylbutyloxy)-5,6-methylenedioxycoumarin [**4**] 	Aerial part	Magalhaes *et al*., 1981

Table 1 (continued)

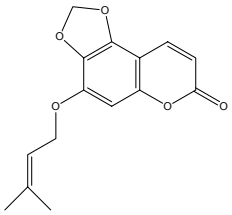
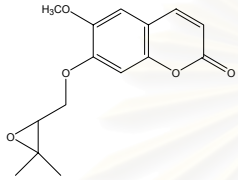
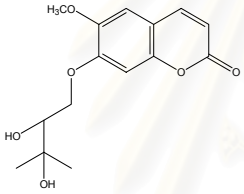
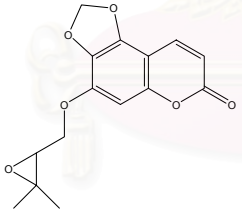
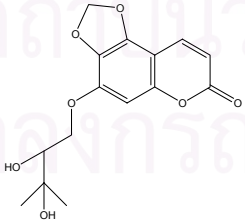
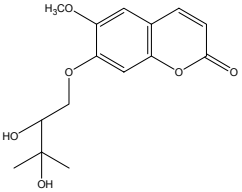
Plant and chemical compounds	Plant part	References
P. balansae		
7-(3-Methyl-2-butenyloxy)-5,6-methylenedioxycoumarin [**5**] 	Aerial part	Magalhaes *et al*., 1981
7-(2,3-Epoxy-3-methylbutyloxy)-6-methoxycoumarin [**6**] 	Aerial part	Magalhaes *et al*., 1981
7-(2,3-Dihydroxy-3-methylbutyloxy)-6-methoxycoumarin [**1**] 	Aerial part	Magalhaes *et al*., 1981
P. lanatum		
7-(2,3-Epoxy-3-methylbutyloxy)-5,6-methylenedioxycoumarin [**3**] 	Aerial part	Magalhaes *et al*., 1981
7-(2,3-Dihydroxy-3-methylbutyloxy)-5,6-methylenedioxycoumarin [**4**] 	Aerial part	Magalhaes *et al*., 1981
7-(2,3-Dihydroxy-3-methylbutyloxy)-6-methoxycoumarin [**1**] 	Aerial part	Magalhaes *et al*., 1981

Table 1 (continued)

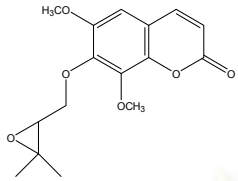
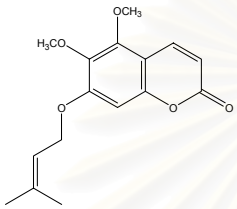
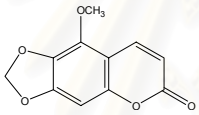
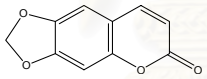
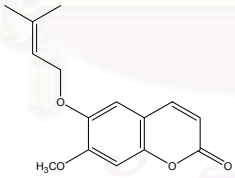
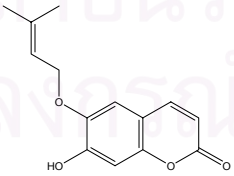
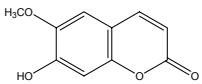
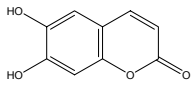
Plant and chemical compounds	Plant part	References
P. lanatum		
2′,3′-Epoxypuberulin [**7**] 	Aerial part	Magalhaes *et al*., 1981
7-(3-Methyl-2-butenyloxy)-5,6-methoxycoumarin [**8**] 	Aerial part	Magalhaes *et al*., 1981
P. polystachium		
5-Methoxy-6,7-methylenedioxycoumarin [**9**] 	Aerial part	Palacios *et al*., 1999
Ayapin [**10**] 	Aerial part	Palacios *et al*., 1999
7-(3-Methyl-2-butenyloxy)-6-methoxycoumarin [**11**] 	Aerial part	Palacios *et al*., 1999
Prenylatin [**12**] 	Aerial part	Palacios *et al*., 1999
Scopoletin [**13**] 	Aerial part	Palacios *et al*., 1999
Aesculetin [**14**] 	Aerial part	Palacios *et al*., 1999

Table 1 (continued)

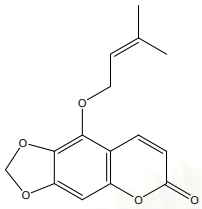
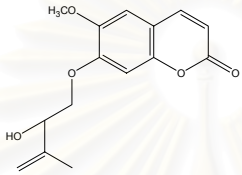
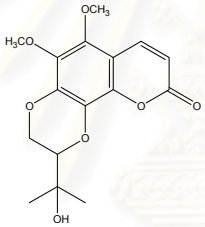
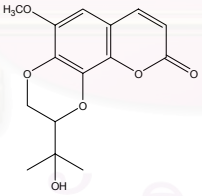
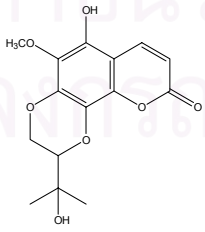
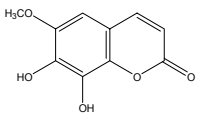
Plant and chemical compounds	Plant part	References
P. polystachium		
5-(3-Methyl-2-butenyloxy)-6,7-methylenedioxycoumarin [**15**] 	Aerial part	Palacios *et al*., 1999
Virgatenol [**16**] 	Aerial part	Palacios *et al*., 1999
P. purpurescens		
Purpurenol [**17**] 	Aerial part	Debenedetti *et al*., 1991
Purpurasol [**18**] 	Aerial part	Debenedetti *et al*., 1992
Purpurasolol [**19**] 	Aerial part	Debenedetti *et al*., 1996
Fraxetin [**20**] 	Aerial part	Debenedetti *et al*., 1996

Table 1 (continued)

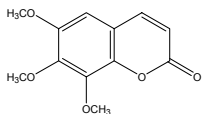
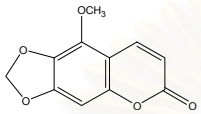
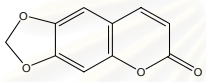
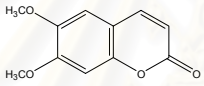
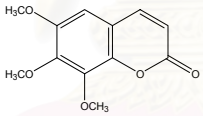
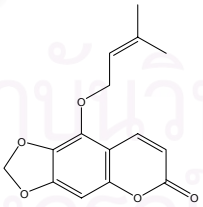
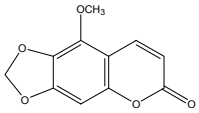
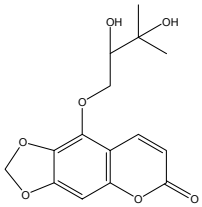
Plant and chemical compounds	Plant part	References
P. serrulatum		
6,7,8-Trimethoxycoumarin [**21**] 	Aerial part	Macleod and Rasmussen, 1999
5-Methoxy-6,7-methylenedioxycoumarin [**9**] 	Aerial part	Macleod and Rasmussen, 1999
Ayapin [**10**] 	Aerial part	Macleod and Rasmussen, 1999
P. sphacelatum		
6,7-Dimethoxycoumarin [**22**] 	Aerial part	Johns *et al*, 1968
6,7,8-Trimethoxycoumarin [**21**] 	Aerial part	Semple *et al*, 1999
P. virgatum		
5-(3-Methyl-2-butenyloxy)-6,7-methylenedioxycoumarin [**15**] 	Aerial part	Debenedetti *et al*., 1994
5-Methoxy-6,7-methylenedioxycoumarin [**9**] 	Aerial part	Debenedetti *et al*., 1994
Sabandinol [**23**] 	Aerial part	Debenedetti *et al*., 1997

Table 1 (continued)

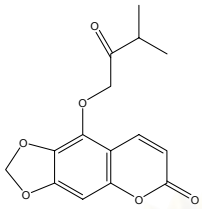
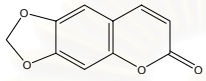
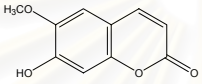
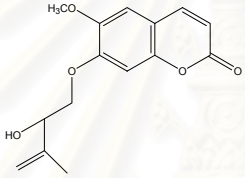
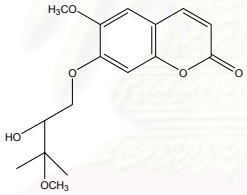
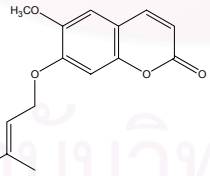
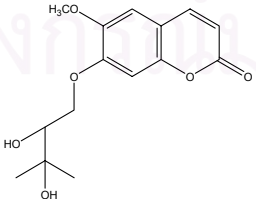
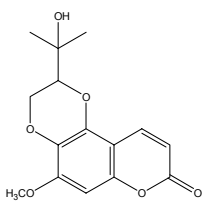
Plant and chemical compounds	Plant part	References
P. virgatum		
Sabandinone [**24**] 	Aerial part	Debenedetti *et al*., 1997
Ayapin [**10**] 	Aerial part	Debenedetti *et al*., 1998
Scopoletin [**13**] 	Aerial part	Debenedetti *et al*., 1998
Virgatenol [**16**] 	Aerial part	Debenedetti *et al*., 1998
Virgatol [**25**] 	Aerial part	Debenedetti *et al*., 1998
7-(3-Methyl-2-butenyloxy)-6-methoxycoumarin [**26**] 	Aerial part	Debenedetti *et al*., 1998
7-(2,3-Dihydroxy-3-methylbutyloxy)-6-methoxycoumarin [**1**] 	Aerial part	Debenedetti *et al*., 1999
Isopurpurasol [**27**] 	Aerial part	Debenedetti *et al*., 1999

Table 2 Distribution of flavonoids in *Pterocaulon* spp.

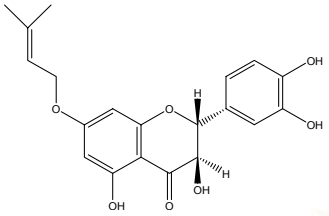
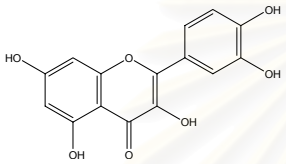
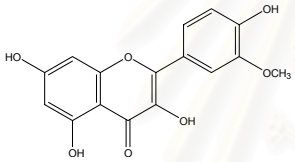
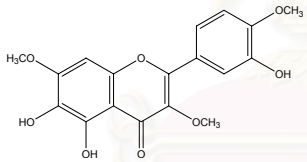
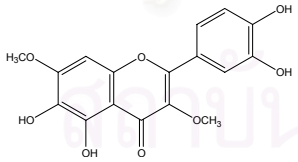
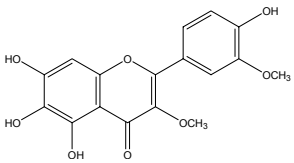
Plant and chemical compounds	Plant part	References
P. alopeculoides		
7-(3-Methylbut-2-enyloxy)-3,5,3′,4′-tetrahydroxy-2,3-dihydroflavonol [**28**] 	Aerial part	Vilegas *et al*., 1995
P. purpurascens		
Quercetin [**29**] 	Aerial part	Debenedetti *et al*., 1987
Isorhamnetin [**30**] 	Aerial part	Debenedetti *et al*., 1987
Quercetagetin 3,7,4′-trimethylether [**31**] 	Aerial part	Debenedetti *et al*., 1987
Quercetagetin 3,7-dimethylether [**32**] 	Aerial part	Debenedetti *et al*., 1987
Quercetagetin 3,3′-dimethylether [**33**] 	Aerial part	Debenedetti *et al*., 1987

Table 2 (continued)

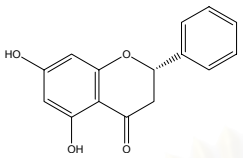
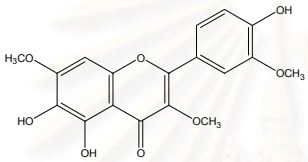
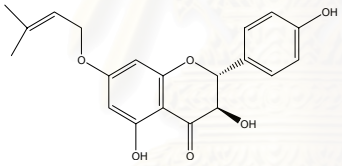
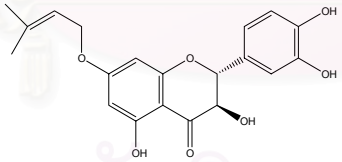
Plant and chemical compounds	Plant part	References
P. serrulatum		
Pinocembrin [**34**] 	Aerial part	Macleod and Rasmussen, 1999
P. sphacelatum		
Chrysosplenol C [**35**] 	Aerial part	Semple *et al*., 1999
P. virgatum		
7-*O*-(2,2-Dimethylallyl)aromadendrin [**36**] 	Aerial part	Bohlmann *et al*., 1981
7-*O*-Prenyltaxifolin [**37**] 	Aerial part	Bohlmann *et al*., 1981

Table 3 Distribution of terpenes in *Pterocaulon* spp.

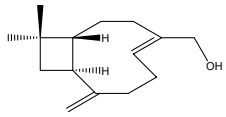
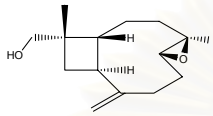
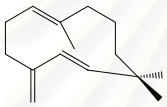
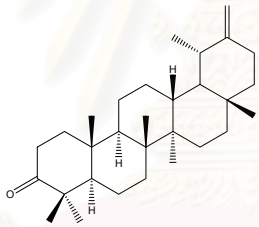
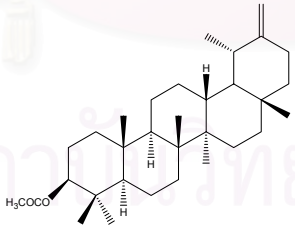
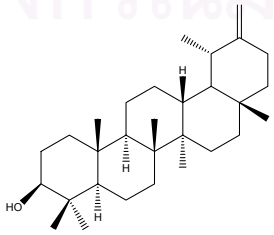
Plant and chemical compounds	Plant part	References
P. serrulatum		
14-Hydroxy-β-caryophyllene [**38**] 	Aerial part	Macleod and Rasmussen, 1999
4,5-Epoxy-13-hydroxy-β-caryophyllene [**39**] 	Aerial part	Macleod and Rasmussen, 1999
P. virgatum		
Humulene [**40**] 	Root	Bohlmann *et al*., 1981
Taraxasterone [**41**] 	Root	Bohlmann *et al*., 1981
Acetyltaraxasterate [**42**] 	Root	Bohlmann *et al*., 1981
Taraxasterol [**43**] 	Root	Bohlmann *et al*., 1981

Table 4 Distribution of polyacetylenes in *Pterocaulon* spp.

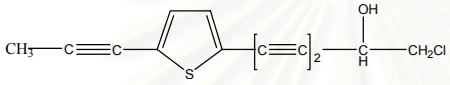
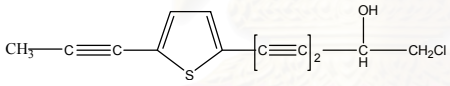
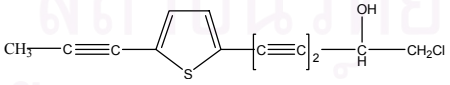
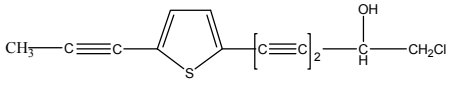
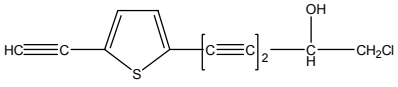
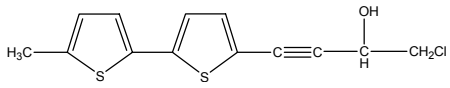
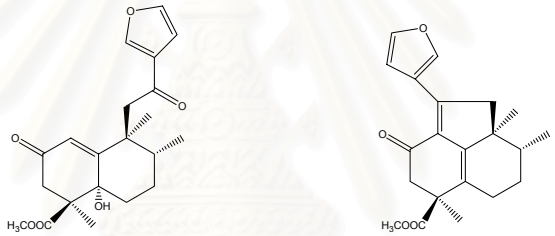
Plant and chemical compounds	Plant part	References
P. alopeculoides		
Pentayne-ene [**44**] $CH_3–[C≡C]_5–CH=CH_2$	Root	Magalhaes *et al*., 1989
P. balansae		
Pentayne-ene [**44**] $CH_3–[C≡C]_5–CH=CH_2$	Root	Magalhaes *et al*., 1989
Tridec-1,2-dimethoxy-3,5,7,9,11-pentyne [**45**] $CH_3–[C≡C]_5–CH(OCH_3)–CH_2OCH_3$	Root	Magalhaes *et al*., 1989
5-[Prop-1″-inylthienyl-(1)]-6′-chloro-5′hydroxyhexa-3′,5-diyene [**46**] 	Root	Bohlmann *et al*., 1981
P. lanatum		
Pentayne-ene [**44**] $CH_3–[C≡C]_5–CH=CH_2$	Root	Magalhaes *et al*., 1989
5-[Prop-1″-inylthienyl-(1)]-6′-chloro-5′hydroxyhexa-3′,5-diyene [**45**] 	Root	Bohlmann *et al*., 1981
P. rugasum		
Pentayne-ene [**44**] $CH_3–[C≡C]_5–CH=CH_2$	Root	Magalhaes *et al*., 1989
5-[Prop-1″-inylthienyl-(1)]-6′-chloro-5′hydroxyhexa-3′,5-diyene [**45**] 	Root	Bohlmann *et al*., 1981
P. virgatum		
5-[Prop-1″-inylthienyl-(1)]-6′-chloro-5′hydroxyhexa-3′,5-diyene [**45**] 	Root	Bohlmann *et al*., 1981
5-Ethinylthienyl-6′-chloro-5′-hydroxyhexa-3′,5′-diyene [**46**] 	Root	Bohlmann *et al*., 1981

Table 4 (continued)

Plant and chemical compounds	Plant part	References
P. virgatum 5′-Methyl-2-[4′-chloro-3′-hydroxybut-1-ynyl]-dithiophene [**47**] 	Root	Bohlmann *et al*., 1981

2. Chemical Constituents of *Cladogynos orientalis*

A number of compounds have been isolated from the only one species, *Cladogynos orientalis*. On the literature research, up to the present time only two studies were reported on this plant until now. Chettaphanin I [**48**] (Sato *et al*., 1970) and Chettaphanin II [**49**] (Sato *et al*., 1971) were isolated from the root of this plant. However, their biological activities have not been reported.



[**48**] [**49**]

Figure 3 Structures of compounds isolated from *Cladogynos orientalis* Zipp. ex Span.

3. Traditional Uses and Biological Activities of *Pterocaulon* spp.

Pterocaulon plants have been used in traditional medicine in many countries with several proposes. In Australia, many parts of *P. sphacelatum* are used eg. aerial parts for treatment of infection, colds, blocked sinuses, sores, wounds, inflamed or infected eyes (Semple *et al*., 1998), crushed leaves for the relief of congestion and as an antiseptic wash, leaves and twigs for treatment of skin disorders such as scabies and ringworm as well as sores and cuts (Macleod and Rasmussen, 1999). In Argentina, aerial parts of *P. polystachium* have been used against flies, fleas and sunstroke (Mongelli *et al*., 2000). Aerial parts of *P. purpurascens* are used as a digestive and as an insecticide. In southern Brazil and Paraguay, aerial parts of *P. virgatum* are used in traditional medicine as an insecticide and an agent against snake bites (Debenedetti *et al*., 1998).

A number of biological investigations of *Pterocaulon* species have been reported. Ethanol extract of aerial parts of *P. sphacelatum* showed inhibition of poliovirus-induced cytopathic effect more than 75% in the crystal violet assay at a non-cytotoxic concentration (Semple *et al*., 1998). The CH_2Cl_2 extract of aerial parts of *P. polystachium* inhibited crown gall tumor at 30% (Mongelli *et al*., 2000).

4. Traditional Uses and Biological Activities of *Cladogynos orientalis*

Cladogynos orientalis has been used in traditional medicine as roborant and carminative properties. A decoction of the root of this plant combined with the roots of *Styrax benzoides* had been used as the cardiac or tonic drugs and the trunk had been used as antidiarrhea and flatulence (Pongboonrod, 1976). The ethanol extract of the roots of *C. orientalis* showed 14% inhibition of HIV-I RT activity at 200 μg/mL (Tan, Pezzuto and Kinghorn, 1991).

CHAPTER III

EXPERIMENTAL

1. Sources of Plant Materials

The aerial parts of *Pterocaulon redolen* (Forst. f) F. Vill. were collected from Kanchanaburi province, Thailand in August 2000. Authentication of the plant materials was done by comparison with the herbarium specimen (BKF No. 1482) at the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

The roots of *Cladogynos orientalis* Zipp. ex Span. were collected from the World Biosphere Reserve, Sakaeraj Environmental Research Station, Nakorn-Rachasima province, Thailand in October 2002. Authentication was achieved by comparison with the herbarium specimen (BKF No. 28024) at the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Voucher specimens were deposited at the Museum of Natural Medicine, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2. General Techniques

2.1 Analytical Thin-Layer Chromatography (TLC)

Technique	:	One Dimension, ascending
Adsorbent	:	Silica gel 60G F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	5.0 cm
Temperature	:	Laboratory temperature (25-35 °C)
Detection	:	1. Ultraviolet light at wavelengths at 254 and 365 nm 2. Anisaldehyde-H_2SO_4 reagent and heating at 105 °C for 10 min

2.2 Column Chromatography

2.2.1 Vacuum Liquid Column Chromatography

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

(E. Merck)

Packing method : Dry packing

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

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

2.2.2 Flash Column Chromatography

Adsorbent : 1. Silica gel 60 (No. 9385) paricle size 0.040-0.063 nm (E. Merck)

2. Silica gel FL100D (Fuji Silysia Chemical Ltd.)

Packing method : Wet Packing

Sample loading : The sample was dissolved in a small volumn of eluent and then applied gently on the top of the column.

Detection : Fractions were examined in the same way as described in section 2.2.1

2.2.3 Gel Filtration Chromatography

Gel filter : Sephadex LH 20 (Pharmacia)

Packing method : Gel filter was suspended in the eluent and left standing to swell for 4 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample Loading : The sample was dissolved in a small volumn of eluent and applied on the top of the column.

2.2.4 Gas Chromatography

Instrument model : Varian Saturn III

Column : Fused silica capillary column (30 m × 0.25 mm i.d., coated with DB-5 (J&W) film thickness 0.25 μm

Detector type : F.I.D. (Flame Ionization Detector)

Column programming : 60 – 240 °C (rate 3 °C/min)

Injector temperature : 240 °C

Helium carrier gas : 1 mL/min

Split ratio	:	100 : 1
Accelerating voltage	:	1700 volts
Sample size	:	1 μL
Slovent	:	HPLC grade methanol

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) Absorption Spectra

UV (in MeOH) spectra were obtained on a Shimadzu UV-160A spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand) and a JASCO V-560 UV Spectrophotometer (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

2.3.2 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and film) were recorded on a JASCO FT/IR-300E spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand and Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

2.3.3 Mass Spectra

Molecular ion were measured on a JEOL JMS-AM20 mass spectrometer and high-resolution fast atom bombardment mass spectrometry (HRFABMS) on a JEOL JMS-HX110 spectrometer (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

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

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP400 spectrometer, and ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained on a JEOL JNM-GSX500A spectrometer (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

Solvents for NMR spectra were deuterated chloroform ($CDCl_3$) and deuterated dimethyl sulfoxide (DMSO-d_6). Chemical shifts were reported in ppm scale using the chemical shift of the solvent and internal standard (TMS) as the reference signals.

2.4 Physical Properties

2.4.1 Melting Points

Melting points were measured on a micro melting point hot-stage apparatus (Yanagimoto) (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

2.4.2 Optical rotations

Optical rotations were obtained on a JASCO P-1020 polarimeter (Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan).

2.4.3 X-ray crystallography

X-ray crystallographic data were measured at -100 °C on a Bruker/SMART 1000 CCD (Chemical Analytical Center, Chiba University, Chiba, Japan).

2.5 Solvents

All organic solvents employed throughout this work were of commercial grade and were redistilled prior to use.

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from *Pterocaulon redolens*

3.1.1 Extraction

Essential oil was determined by the method described in the Association of Official Analytical Chemist (method 962.17, AOAC, 1990). The aerial parts was hydrodistillated in Clevenger type apparatus. The exactly weight was put into a 1000 ml round bottom flask and distilled water was added into the flask to around half-full. This flask was then connected to the apparatus for determination of essential oil. The content in this flask was distilled until two consecutive reading taken at one hour interval showed no change in oil content (around four to six hours). After cooling, the essential oil was diluted to 1:100 in methanol and then analysed for its chemical constituents by Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS condition was described in 2.2.4 and the spectrum was recorded and compared with the terpenes library (Adam, 1995).

The dried aerial parts of *Pterocaulon redolens* (1.5 kg) were chopped, ground and then extracted with hexane (3 × 4.5 L), chloroform ($CHCl_3$, 5 × 4.5 L), and then 95% methanol (MeOH, 4 × 4.5 L) to give, after removal of the organic solvent, a hexane extract (20.1 g), a chloroform extract (30.8 g) and a methanol extract (12.4 g), respectively.

The methanol extract (12.4 g) was then partition between butanol and water. The butanol layer was dried to yield 4.5 g of a BuOH extract while 7.0 g of an aqueous extract was obtained.

3.1.2 Isolation of Compounds from $CHCl_3$ Extract

The $CHCl_3$ extract (30.8 g) was dissolved in a small amount of $CHCl_3$, triturated with silica gel 60 (No. 7734) and dried under room temperature. It was then fractionated by vacumn liquid column chromatography using sintered glass filter column of silica gel (No. 7734). Elution was completed in a polarity gredient manner with mixture of hexane, $CHCl_3$ and MeOH. The eluate was collected 200 mL per fraction and examined by TLC (Silica gel, 40% hexane in $CHCl_3$). Fractions (42 fractions) with similar chromatographic pattern were combined to yield 8 fractions: Fractions PC1 (1.2 g), PC2 (5.3 g), PC3 (3.9 g), PC4 (6.8 g), PC5 (1.4 g), PC6 (3.1 g), PC7 (4.7 g) and PC8 (3.4 g).

3.1.2.1 Isolation of Compound PRC1 (5-Methoxy-6,7-methylenedi-oxycoumarin)

Fraction PC2 (5.3 g) was further purified on a silica gel column chromatography (40% hexane in $CHCl_3$). The eluates were examined by TLC using 30% hexane in $CHCl_3$, as developing solvent. Fractions with similar chromatographic pattern were combined to yield 6 fractions (P21-P26). Fraction PC22 (720.0 mg) was recrystallized from $CHCl_3$-MeOH mixture to afford white crystals of compound **PRC1** (60.0 mg). This compound was eventually identified as 5-methoxy-6,7-methylenedioxycoumarin [**9**].

3.1.2.2 Isolation of Compound PRC2 (Ayapin)

Fraction PC24 (680.0 mg) was fractionated on a silica gel column using isocratic elution with 35% hexane in $CHCl_3$ to give white crystals of compound **PRC2** (30.8 mg). This compound was later identified as ayapin [**10**].

3.1.2.3 Isolation of Compound PRC3 (Puberulin)

Fraction PC3 (3.9 g) was separated on a silica gel column chromatography (30% hexane in $CHCl_3$). Fractions (35 fractions) with similar chromatographic pattern were combined by TLC, to give 8 fractions (PC31 to PC38). Fraction PC34 (600.0 mg) was chromatographed on Sephadex LH20 (50% acetone in MeOH) column and repurified on sephadex LH20 using acetone as eluent to obtain compound **PRC3** (18.0 mg). It was subsequently identified as puberulin [**50**].

3.1.2.4 Isolation of Compound PRC4 (5-Methoxyscopoletin)

Compound **PRC4** (20.0 mg) was obtained as white crystal from fraction PC36 (900.0 mg) by separation on sephadex LH20 (50% $CHCl_3$ in MeOH) column. It was identified as 5-methoxyscopoletin [**51**].

3.1.2.5 Isolation of Compound PRC5 (2′,3′-Dihydroxypuberulin)

Fraction PC4 (6.8 g) was rechromatographed on a silica gel column chromatography. Gradient elution (30% hexane in $CHCl_3$) was performed to give 10 fractions (PC41 to PC410). Fraction PC48 (0.9 g) was further fractionated by repeated column chromatography (30% hexane in EtOAc gradient elution) to furnish compound **PRC5** (40.1 mg). This compound was identified as 2′,3′-dihydroxypuberulin [**52**]. Here is the first time to isolate this compound from natural source.

3.1.2.6 Isolation of Compound PRC6 (Isofraxidin)

Fraction PC49 (720.0 mg) was purified on a silica gel column chromatography (20% hexane in EtOAc) to afford compound **PRC6** (10.1 mg). This compound was identified as isofraxidin [**53**].

3.1.2.7 Isolation of Compound PRC7 (Sabandinol)

Fraction PC5 (1.4 g) was repeated a silica gel column chromatography (10% hexane in $CHCl_3$) to give compound **PRC7** (20.3 mg) as white crystals. It was identified as sabandinol [**23**].

3.1.3 Isolation of Compounds from BuOH Extract

The BuOH extract (4.5 g) was separated on sephadex LH20 (MeOH) to obtain 6 fractions (fraction PB1 to PB6)

3.1.3.1 Isolation of Compound PRB8 (Luteolin)

Fraction PB2 (900.0 mg) was rechromatographed on sephadex LH20 (acetone) to afford 5 fractions (PB21 to PB25). Fraction PB22 (250.0 mg) was futher

purified on sephadex LH20 (70% acetone in MeOH) to give compound **PRB8** as yellow crystals (20.9 mg). This compound was eventually identified as luteolin [**54**].

3.1.3.2 Isolation of Compound PRB9 (Tomentin) and Compound PRB10 (Chrysosplenol C)

Fraction PB5 (490.0 mg) was separated on sephadex LH20 (50% $CHCl_3$ in MeOH) to acquire 5 fractions (PB51to PB55). Compound **PRB9** (tomentin [**55**], 12.0 mg) was obtained as yellow crystals on sephadex LH20 (50% $CHCl_3$ in MeOH) from fraction PB51. Fraction PB54 (120.0 mg) was futher purified on sephadex LH20 (50% acetone in MeOH) to furnish compound **PRB10** (25.0 mg) as yellow crystals. It was identified as chrysosplenol C [**35**].

3.2 Extraction and Isolation of Compounds from *Cladogynos orientalis*

3.2.1 Extraction

The roots of *Cladogynos orientalis* (4.5 kg) were minced and extracted successively with $CHCl_3$ (5 × 20.0 L) and then with MeOH (3 × 20.0 L). Removal of the solvent from the extract under reduced pressure gave a $CHCl_3$ extract (208.6 g) and a MeOH extract (227.3 g), respectively.

3.2.2 Isolation of Compounds from $CHCl_3$ Extract

The $CHCl_3$ extract (208.6 g) was dissolved a small amount of $CHCl_3$, triturated with silica gel 60 (No. 7734) and dried under room temperature. It was then fractionated by vacumn liquid column chromatography using sintered glass filter column of silica gel (No. 7734). Elution was completed in a polarity gradient manner with mixture of hexane, $CHCl_3$, and MeOH. The eluated was collected 500 mL per fraction and examined by TLC (Silica gel, 30% hexane in $CHCl_3$). Fractions (83 fractions) with similar chromatographic pattern were combined to yield 8 fractions: Fraction CC1-CC8.

3.2.2.1 Isolation of Compound COC1 (8-Hydroxy-*α*-guaiene)

Fraction CC2 (18.2 g) was fractionated on a silica gel column using gradient elution with 90% hexane in $CHCl_3$ to give 5 fractions (CC21 to CC25). Fraction CC23 (3.8 g) was futher purified with 50% hexane in EtOAc to furnish compound **COC1** (50.7 mg). This compound was later identified as a new guaiene sesquiterpene, namely, (4*S**,7*R**,8*R**,10*S**)-8-hydroxy-α-guaiene [**56**].

3.2.2.2 Isolation of Compound COC2 (Spathulenol)

Fraction CC3 (6.8 g) was rechromatographed on a silica gel column chromatography. Gradient elution (5% EtOAc in hexane) was performed to give 7 fractions (CC31 to CC37). Fraction CC32 (1.0 g) was repeated a silica gel column chromatography (5% ether in hexane) to give compound **COC2** (62.5 mg). It was identified as spathulenol [**57**].

3.2.2.3 Isolation of Compound COC3 (5-[2-(Furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-1-carboxylic Acid)

Fraction CC33 (192.0 mg) was further purified on a silica gel column chromatography (2.5% CH_2Cl_2 in hexane) to obtain compound **COC3** (3.2 mg) as a new 5-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-1-carboxylic acid [**58**].

3.2.2.4 Isolation of Compound COC4 (Methyl 9-(Furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo[5.3.3.$0^{1,6}$]trideca-5,8-diene-2-carboxylate)

Fraction CC35 (1.3 g) was further purified on a silica gel column chromatography (10% CH_2Cl_2 in hexane) to obtain compound **COC4** (32.7 mg) as methyl 9-(furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo[5.3.3.$0^{1,6}$]trideca-5,8-diene-2-carboxylate [**59**].

3.2.2.5 Isolation of Compound COC5 (Acetoxyaleuritolate)

Fraction CC4 (16.6 g) was chromatographed on a silica gel column chromatography. Gradient elution (20% ether in hexane) was performed to give 9 fractions (CC41 to CC49). Fraction CC42 (2.1 g) was crystallized from a hexane-$CHCl_3$ mixture to give compound **COC5** (62.5 mg). It was identified as acetoxyaleuritolate [**60**].

3.2.2.6 Isolation of Compound COC6 (Taraxerol) and compound COC7 (Chettaphanin II)

Fraction CC47 (1.4 g) was crystallized from a hexane-$CHCl_3$ mixture to afford compound **COC6** (79.0 mg). It was identified as taraxerol [**61**]. The mother liquid of fraction CC47 was futher purified on silica gel column (50% CH_2Cl_2 in hexane) to obtain compound **COC7** (25.2 mg) as chettaphanin II [**49**].

3.2.2.7 Isolation of Compound COC8 (6-[2-(Furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one)

Fraction CC5 (20.2 g) was chromatographed on a silica gel column chromatography. Gradient elution (40% hexane in $CHCl_3$) was performed to give 6 fractions (CC51 to CC56). Fraction CC52 (75.0 mg) was further fractionated by repeated column chromatography (80% $CHCl_3$ in EtOAc gradient elution) to furnish compound **COC8** (4.1 mg). This compound was newly identified as 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one) [**62**].

3.2.2.8 Isolation of Compound COC9 (6-[2-(Furan-3-yl)oxoethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one)

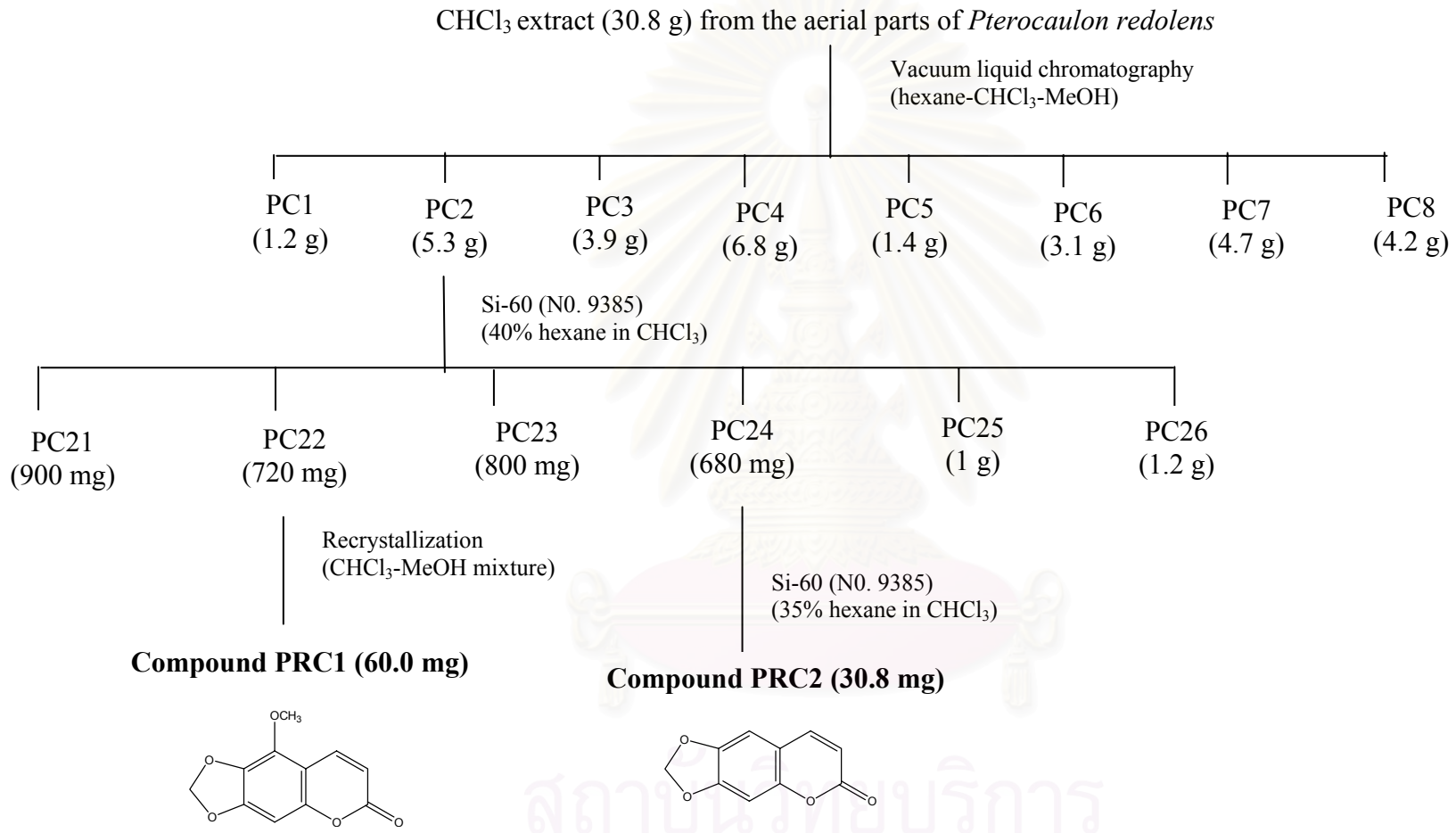
Fraction CC56 (10.0 g) was further fractionated by repeated column chromatography (CH_2Cl_2) to furnish compound **COC9** (33.4 mg). This compound was newly identified as 6-[2-(furan-3-yl)oxo]ethyl]-1,5-6-trimethyl-10-oxatricyclo [7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one) [**63**].

3.2.2.9 Isolation of Compound COC10 (Chettaphanin I)

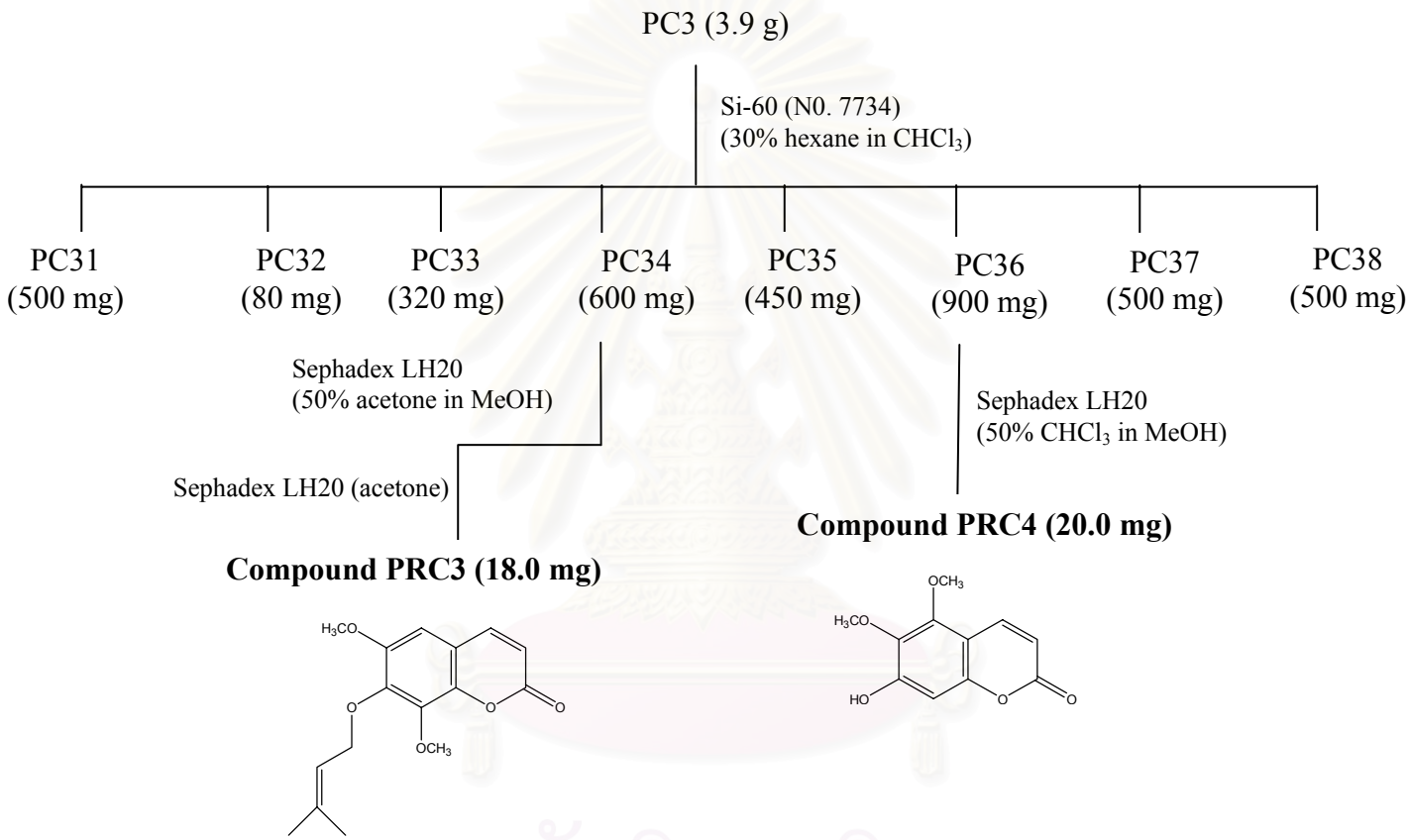
Fraction CC6 (9.8 g) was further purified on a silica gel column chromatography (30% hexane in EtOAc gradient elution) to obtain 4 fractions (CC61 to CC64). Fraction CC62 (1.2 g) was repeated a silica gel column chromatography (15% EtOAc in CH_2Cl_2) to give compound **COC10** (258.0 mg). It was identified as chettaphanin I [**48**].

3.2.2.10 Isolation of Compound COC11 (Cyperenoic Acid)

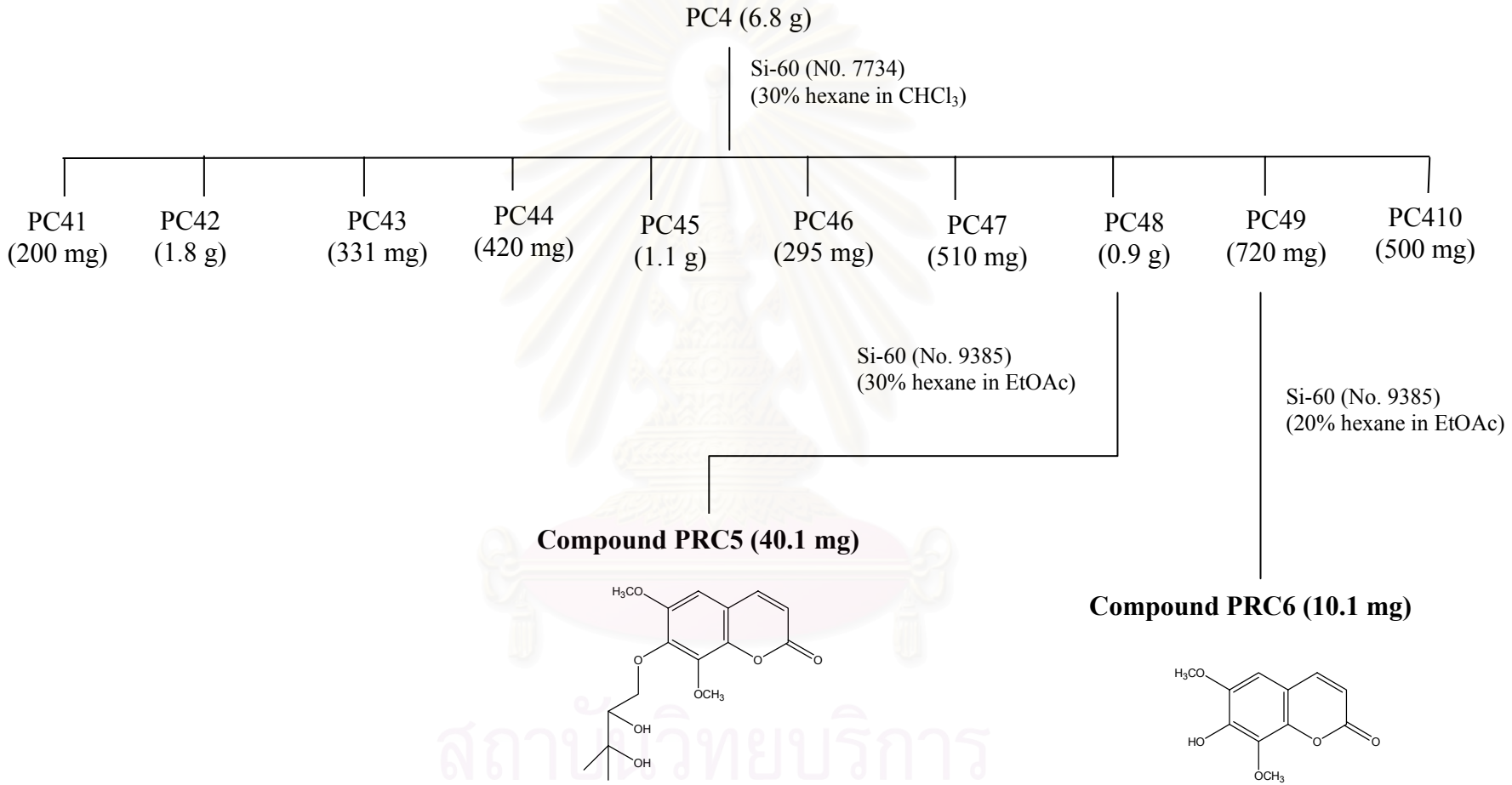
Fraction CC7 (113.9 g) was crystallized with hexane-$CHCl_3$ mixture to give compound **COC11** (295.9 mg) as cyperenoic acid [**64**].



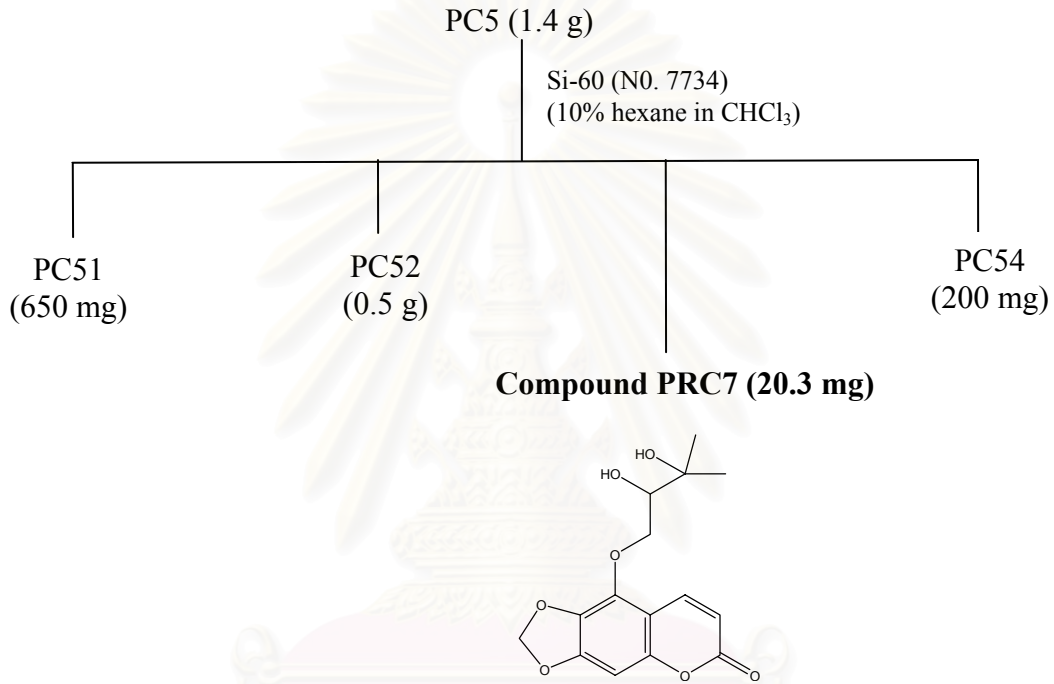
Scheme1 Seperation of $CHCl_3$ extract of the aerial parts of *Pterocaulon redolens*



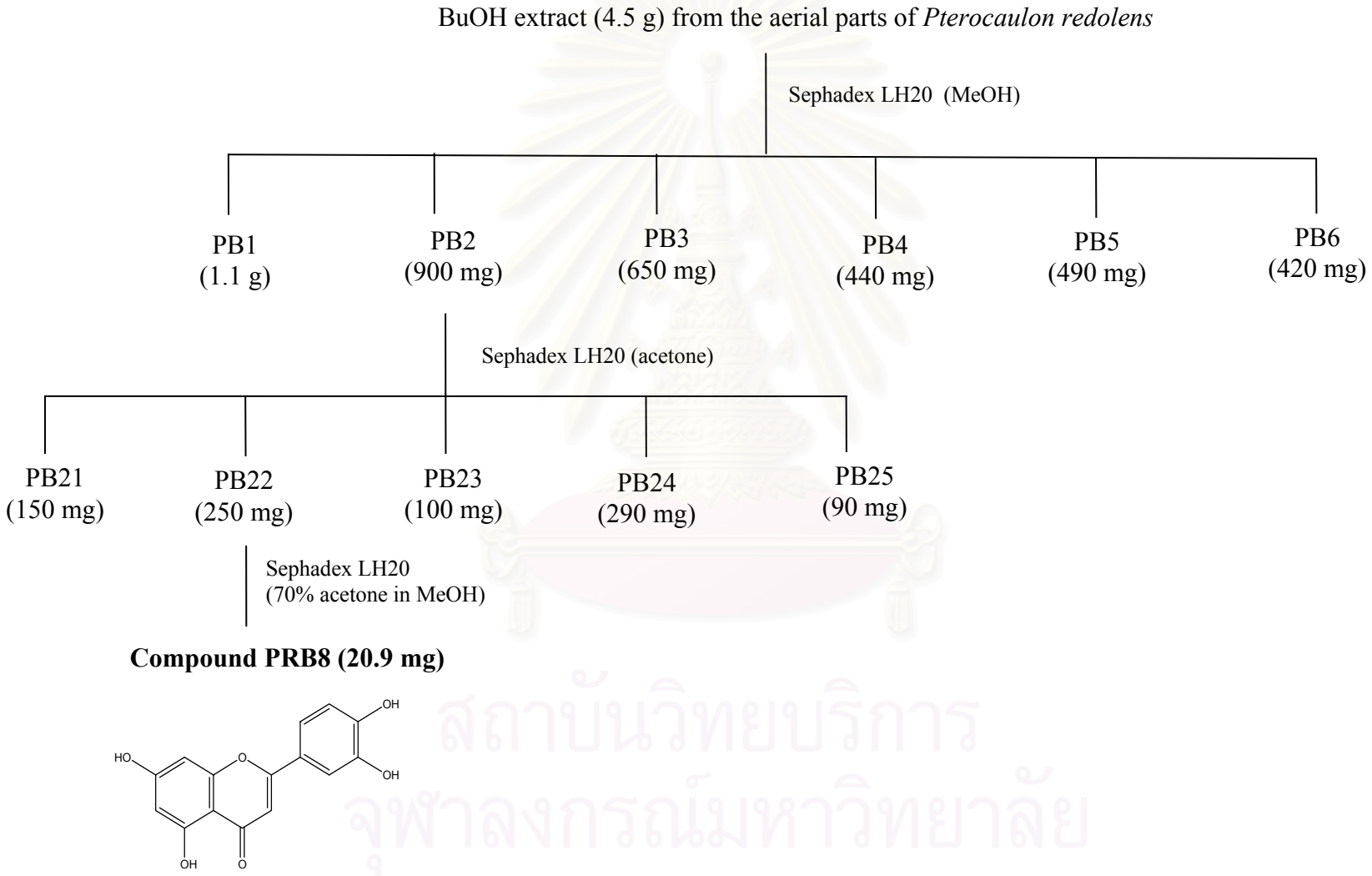
Scheme 2 Seperation of fraction PC3 from the $CHCl_3$ extract of the aerial parts of *Pterocaulon redolens*



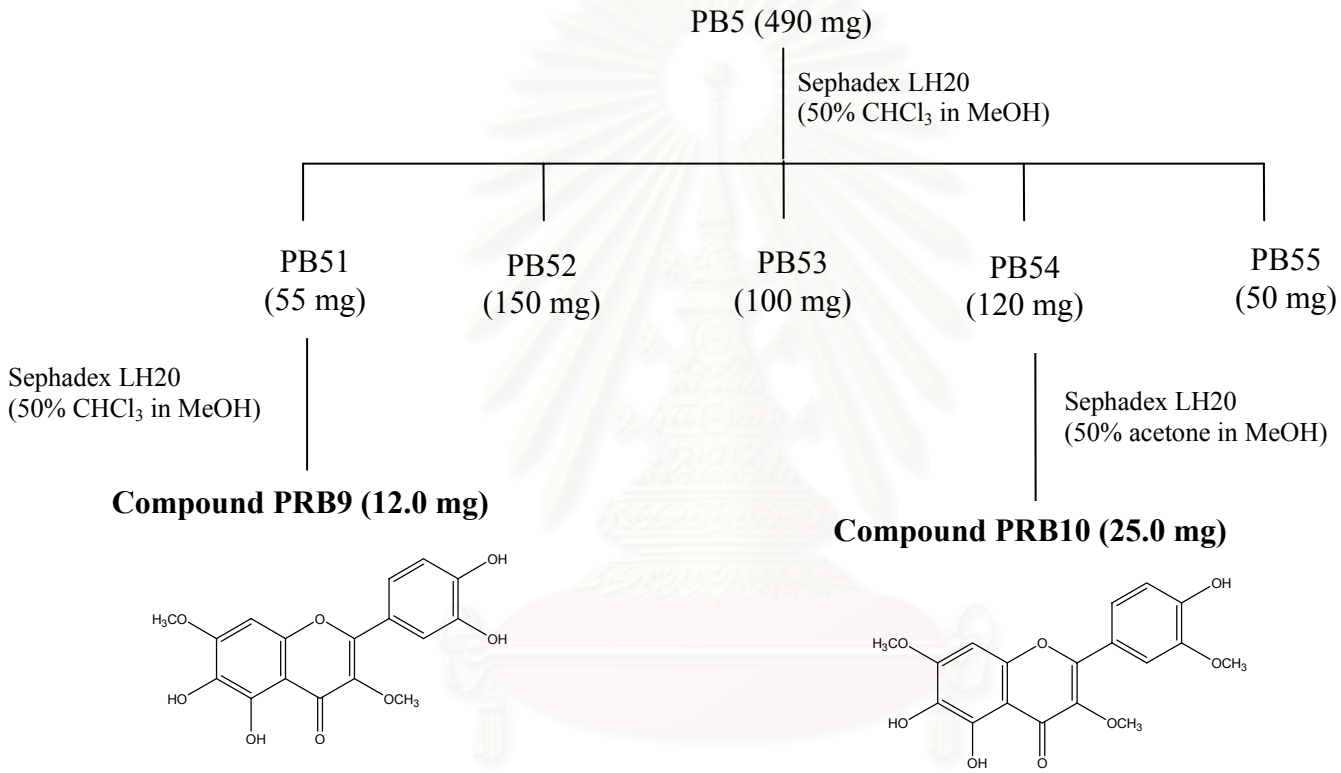
Scheme 3 Seperation of fraction PC4 from the $CHCl_3$ extract of the aerial parts of *Pterocaulon redolens*



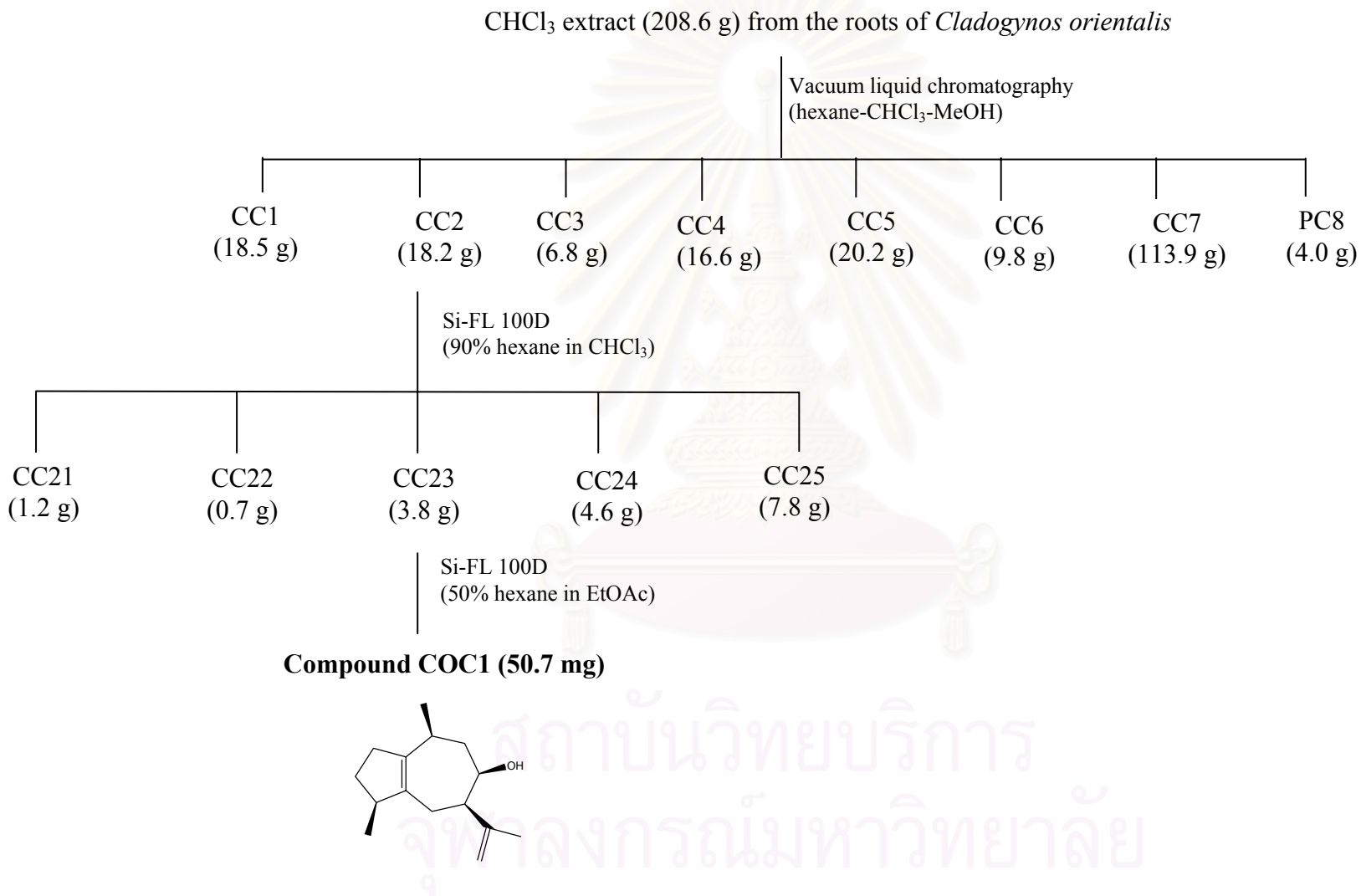
Scheme 4 Seperation of fraction PC5 from the $CHCl_3$ extract of the aerial parts of *Pterocaulon redolens*



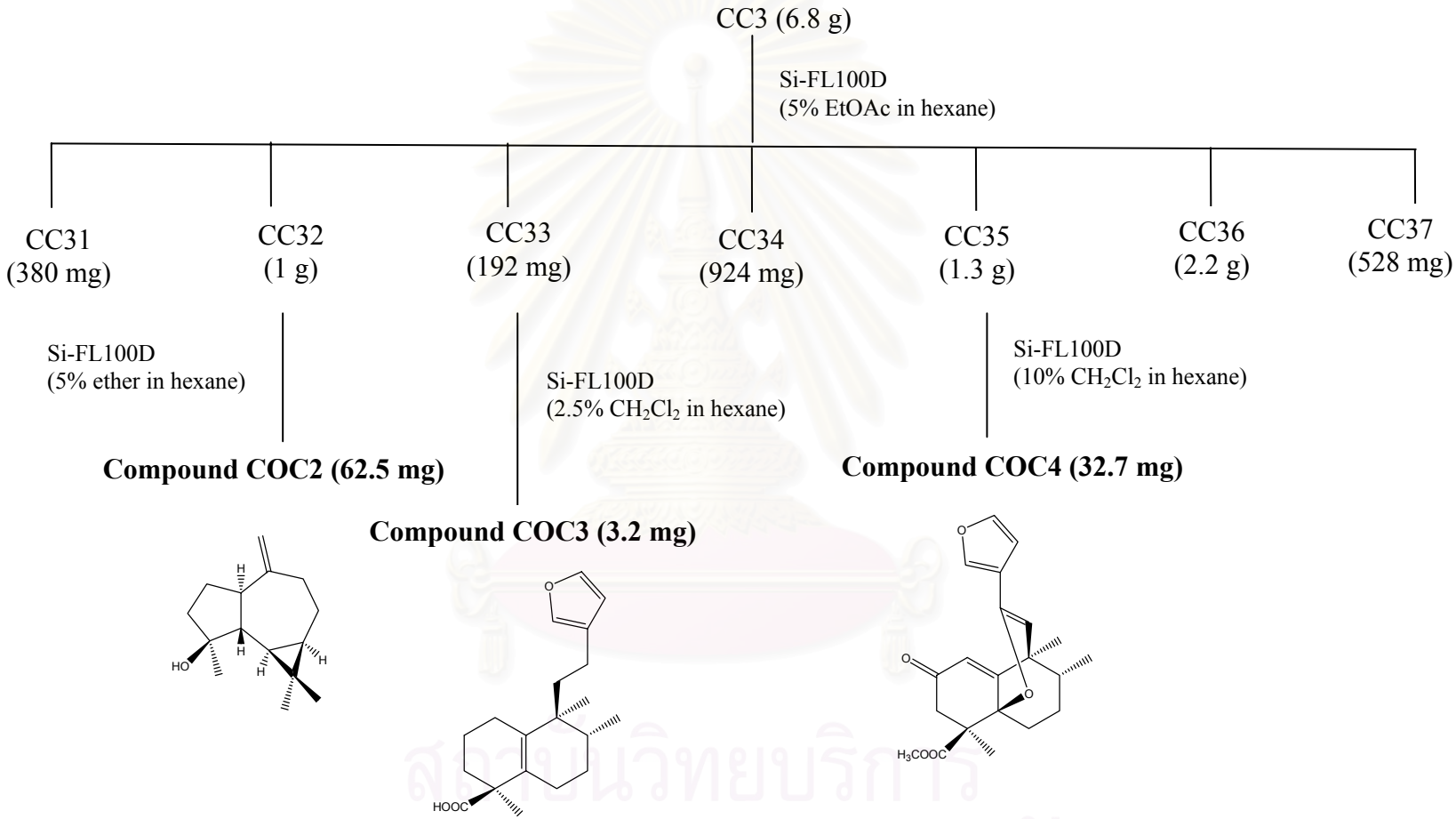
Scheme 5 Seperation of the BuOH extract of the aerial parts of *Pterocaulon redolens*



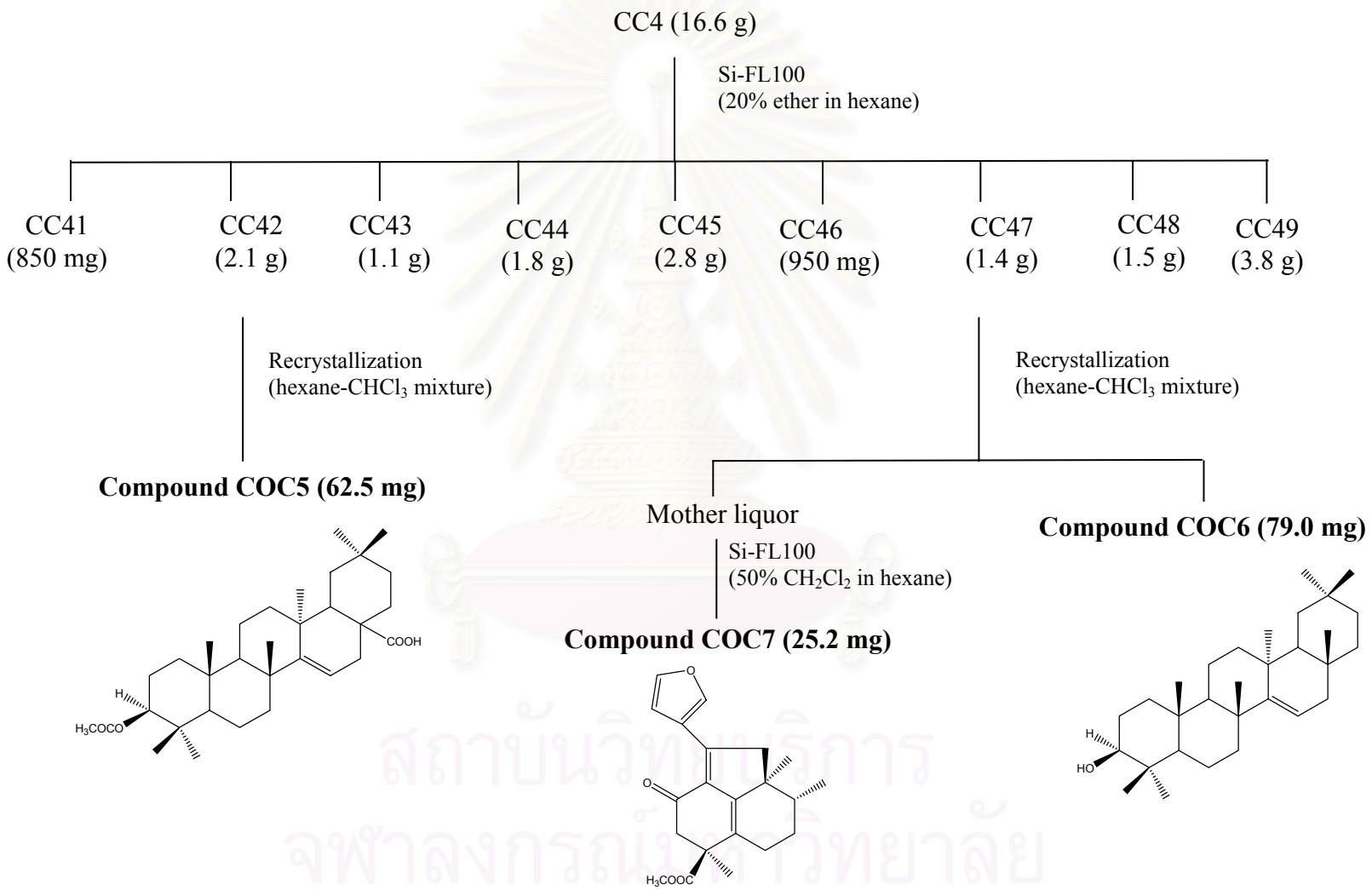
Scheme 6 Seperation of the fraction PB5 from the BuOH extract of the aerial parts of *Pterocaulon redolens*



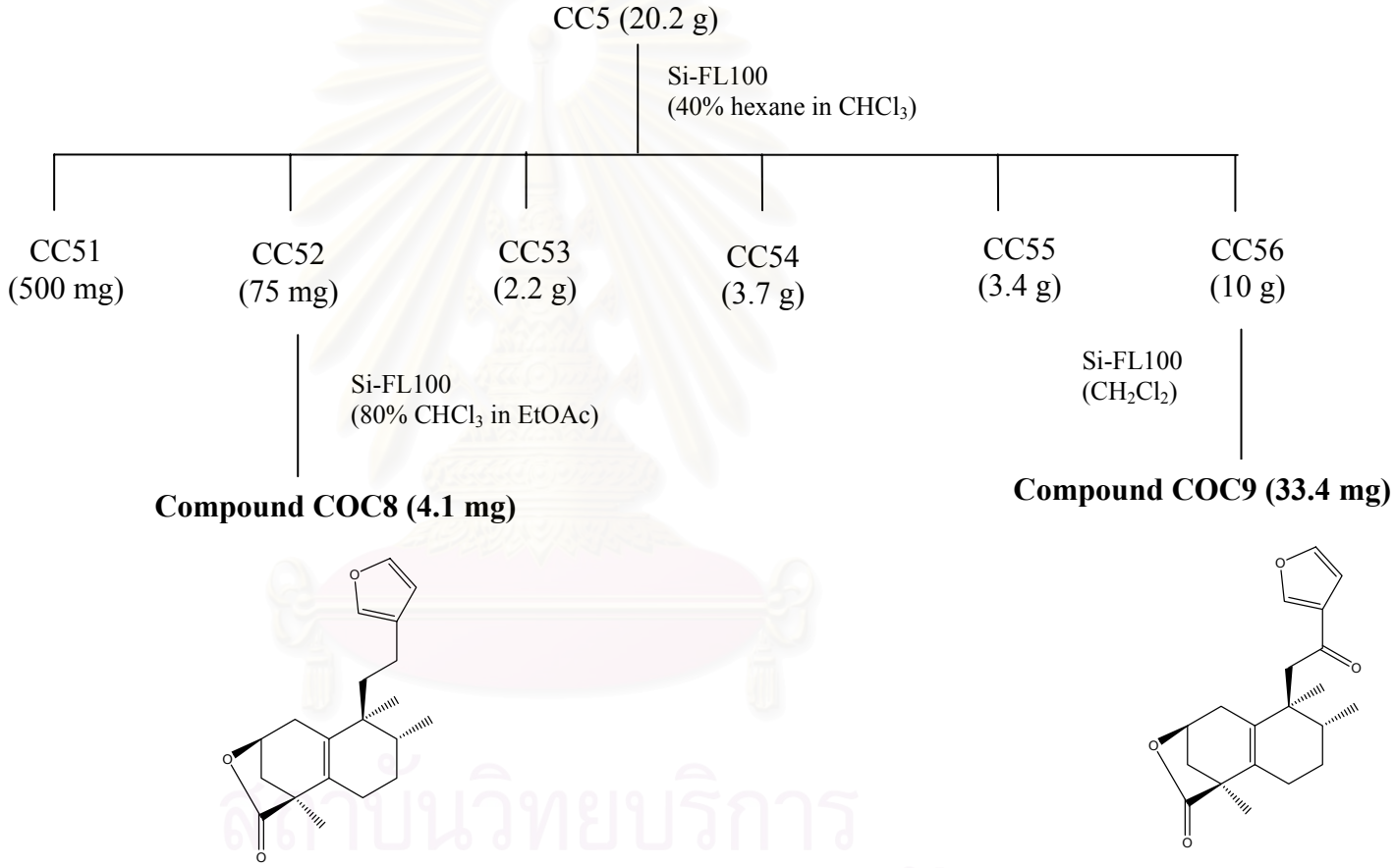
Scheme 7 Seperation of the $CHCl_3$ extract of the roots of *Cladogynos orientalis*



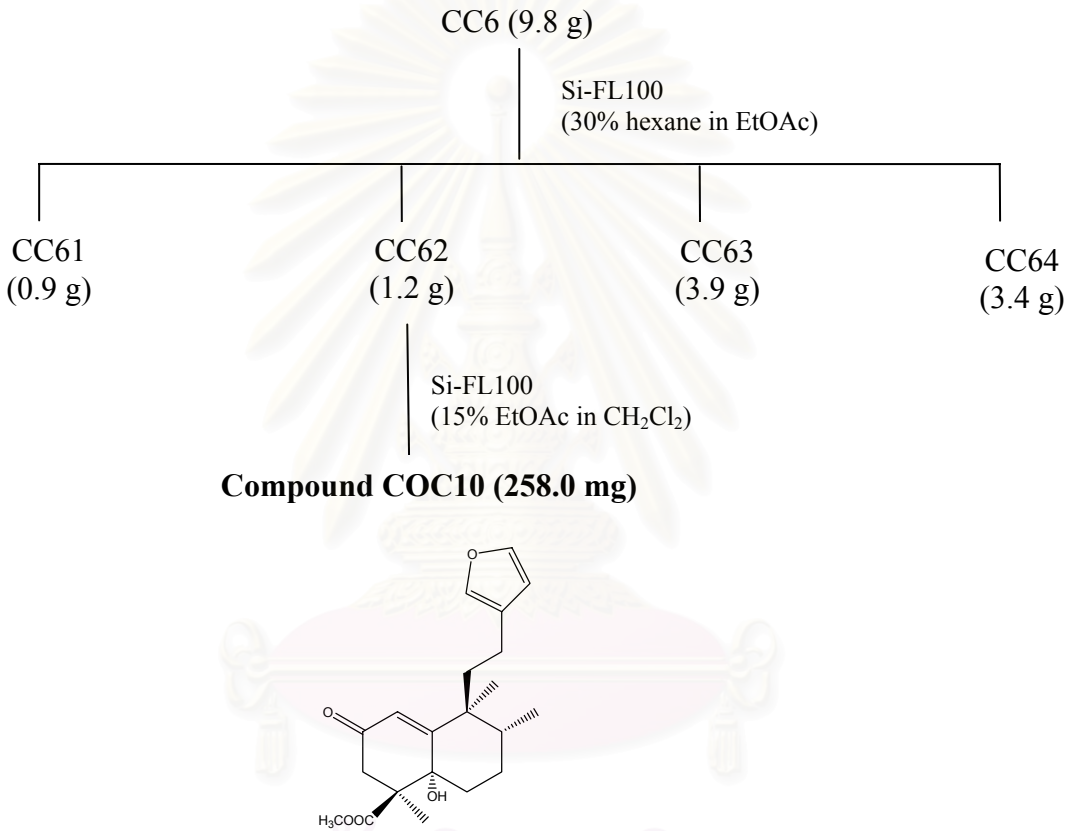
Scheme 8 Seperation of fraction CC3 from the $CHCl_3$ extract of the roots of *Cladogynos orientalis*



Scheme 9 Seperation of fraction CC4 from the $CHCl_3$ extract of the roots of *Cladogynos orientalis*



Scheme 10 Seperation of fraction CC5 from the $CHCl_3$ extract of the roots of *Cladogynos orientalis*



Scheme 11 Seperation of fraction CC6 from the $CHCl_3$ extract of the roots of *Cladogynos orientalis*

4. Physical and Spectral Data of Isolated Compounds

4.1 Compound PRC1 (5-Methoxy-6,7-methylenedioxycoumarin)

Compound **PRC1** was obtained as white crystals, soluble in $CHCl_3$ (60.0 mg, 4.0×10^{-3} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 221, **Figure 6**

UV : λ_{max} nm (log ε), in MeOH; 238 (3.25), 269 (4.12), 316 (4.13), **Figure7**

IR : ν_{max} cm^{-1}, KBr; 3077, 2920, 1737, 1628, 1481, 1248, 1046, **Figure 8**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 5** and **Figure 9**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 5** and **Figure 10**

4.2 Compound PRC2 (Ayapin)

Compound **PRC2** was obtained as colourless needles, soluble in $CHCl_3$ (30.8 mg, 2.0×10^{-3} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 191, **Figure 13**

UV : λ_{max} nm (log ε), in MeOH; 234 (4.34), 294 (3.79), 346 (4.20), **Figure 14**

IR : ν_{max} cm^{-1}, KBr; 3079, 2919, 1702, 1630, 1453, 1256, 940, 888, **Figure 15**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 6** and **Figure 16**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 6** and **Figure 17**

4.3 Compound PRC3 (Puberulin)

Compound **PRC3** was obtained as white crystals, soluble in $CHCl_3$ (18.0 mg, 1.2×10^{-3} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 291, **Figure 20**

UV : λ_{max} nm (log ε), in MeOH; 227 (4.13), 260 (3.46), 297(4.05), **Figure 21**

IR : ν_{max} cm^{-1}, KBr; 3020, 2941, 1729, 1605, 1565, 1459, 1120, 976, **Figure 22**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 7** and **Figure 23**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 7** and **Figure 24**

4.4 Compound PRC4 (5-Methoxyscopoletin)

Compound **PRC4** was obtained as white solid, soluble in $CHCl_3$ (20.0 mg, 1.3×10^{-3} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 223, **Figure 28**

UV : λ_{max} nm (log ε), in MeOH; 222 (4.13), 266 (3.06), 328 (4.13), **Figure 29**

IR : ν_{max} cm^{-1}, KBr; 3413**,** 3085, 2952, 1722, 1608, 1468, 1140, 824**, Figure 30**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, see **Table 8** and **Figure 31**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 8** and **Figure 32**

4.5 Compound PRC5 (2′,3′-Dihydroxypuberulin)

Compound **PRC5** was obtained as white crystals, soluble in $CHCl_3$ (40.1 mg, 2.7×10^{-3} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 325, **Figure 35**

$[\alpha]^{23}_D$: + 25° (*c* 0.9, $CHCl_3$)

UV : λ_{max} nm (log ε), in MeOH; 228 (4.32), 296 (4.06), 343(3.90), **Figure 36**

IR : ν_{max} cm^{-1}, KBr; 3474, 1716, 1605, 1566, 1459, 1125, 984, **Figure 37**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 9** and **Figure 38**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 9** and **Figure 39**

4.6 Compound PRC6 (Isofraxidin)

Compound **PRC6** was obtained as yellow crystals, soluble in $CHCl_3$ (10.1 mg, 6.7×10^{-4} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 223, **Figure 42**

UV : λ_{max} nm (log ε), in MeOH; 228 (4.39), 268 (3.36), 345(4.28), **Figure 43**

IR : ν_{max} cm^{-1}, KBr; 3369, 1706, 1600, 1575, 1456, 1120, 1084, **Figure 44**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 10** and **Figure 45**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 10** and **Figure 46**

4.7 Compound PRC7 (Sabandinol)

Compound **PRC7** was obtained as white crystals, soluble in MeOH (20.3 mg, 1.4×10^{-3} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 309, **Figure 50**

$[\alpha]^{25}_D$: + 30.9° (*c* 0.65, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 220 (4.12), 239 (2.94), 320 (3.84), **Figure 51**

IR : ν_{max} cm^{-1}, KBr; 3438, 1715, 1638, 1579, 1473, 1249, 1131, 938, **Figure 52**

^{1}H NMR : δ_H ppm, 500 MHz, in DMSO-*d_6*, **Table 11** and **Figure 53**

^{13}C NMR : δ_C ppm, 125 MHz, in DMSO-*d_6*, **Table 11** and **Figure 54**

4.8 Compound PRB8 (Luteolin)

Compound **PRB8** was obtained as yellow crystals, soluble in MeOH (20.9 mg, 1.4×10^{-3} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 287, **Figure 58**

UV : λ_{max} nm (log ε), in MeOH; 212 (4.49), 270 (3.22), 317 (4.11), **Figure 59**

IR : ν_{max} cm^{-1}, KBr; 3347, 1654, 1609, 1490, 1260, 1032, 840, **Figure 60**

^{1}H NMR : δ_H ppm, 500 MHz, in DMSO-*d*$_6$, **Table 12** and **Figure 61**

^{13}C NMR : δ_C ppm, 125 MHz, in DMSO-*d*$_6$, **Table 12** and **Figure 62**

4.9 Compound PRB9 (Tomentin)

Compound **PRB9** was obtained as yellow crystals, soluble in MeOH (12.0 mg, 8.0×10^{-4} % based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 347, **Figure 65**

UV : λ_{max} nm (log ε), in MeOH; 213 (4.54), 303 (3.92), 347 (4.37), **Figure 66**

IR : ν_{max} cm^{-1}, KBr; 3369, 1655, 1609, 1560, 1491, 1290, 796, **Figure 67**

^{1}H NMR : δ_H ppm, 500 MHz, in DMSO-*d*$_6$, **Table 13** and **Figure 68**

^{13}C NMR : δ_C ppm, 125 MHz, in DMSO-*d*$_6$, **Table 13** and **Figure 69**

4.10 Compound PRB10 (Chrysosplenol C)

Compound **PRB10** was obtained as yellow solid, soluble in MeOH (25.0 mg, 1.7×10^{-3}% based on dried weight of the aerial parts).

FABMS : $[M+H]^+$ *m/z* 361, **Figure 72**

UV : λ_{max} nm (log ε), in MeOH; 214 (4.68), 281 (4.37), 349 (4.53), **Figure 73**

IR : ν_{max} cm^{-1}, KBr; 3392, 1668, 1608, 1491, 1285, 940, 880, **Figure 74**

^{1}H NMR : δ_H ppm, 500 MHz, in DMSO-*d*$_6$, **Table 14** and **Figure 75**

^{13}C NMR : δ_C ppm, 125 MHz, in DMSO-*d*$_6$, **Table 14** and **Figure 76**

4.11 Compound COC1 ((4*S, 7*R**, 8*R**, 10*S**)-8-Hydroxy-α-guaiene)**

Compound **COC1** was obtained as pale yellow oil, soluble in $CHCl_3$ (50.7 mg, 1.1×10^{-3} % based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 220, **Figure 79**

HRFABMS : $[M+K]^+$ *m/z* 259.1487 (calcd. for $C_{15}H_{24}OK$ = 259.1464)

$[\alpha]^{23}_D$: - 65.1° (*c* 0.03, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 206 (3.85), 263 (2.99), **Figure 80**

IR : ν_{max} cm^{-1}, Neat; 3448, 3100, 2926,1457, 1023, **Figure 81**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 15** and **Figure 82-83**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 15** and **Figure 84**

4.12 Compound COC2 (Spathulenol)

Compound **COC2** was obtained as pale yellow oil, soluble in $CHCl_3$ (62.5 mg, 1.4×10^{-3}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 221, **Figure 90**

IR : ν_{max} cm^{-1}, Neat; 3384, 3080, 2926,1458, 1375, 889, **Figure 91**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 16** and **Figure 92**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 16** and **Figure 93**

4.13 Compound COC3 (5-[2-(Furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6, -7,8-octahydronaphthalene-1-carboxylic acid)

Compound **COC3** was obtained as pale yellow oil, soluble in $CHCl_3$ (3.2 mg, 0.7×10^{-4}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 317, **Figure 98**

HRFABMS : $[M+H]^+$ *m/z* 317.2108 (calcd. for $C_{20}H_{29}O_3$ = 317.2117)

$[\alpha]^{23}_D$: - 23.2° (*c* 0.0013, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 299 (3.23) , **Figure 99**

IR : ν_{max} cm^{-1}, Neat; 3600-2400, 2929, 1699, 1458, 1190, 938, **Figure 100**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 17** and **Figure 101-102**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 17** and **Figure 103**

4.14 Compound COC4 (Methyl-9-(furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo [5.3.3.0^{1,6}] trideca-5,8-diene-2-carboxylate)

Compound **COC4** was obtained as pale yellow oil, soluble in $CHCl_3$ (32.7 mg, 7.2×10^{-4}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 357, **Figure 109**

HRFABMS : $[M+H]^+$ *m/z* 357.1685 (calcd. for $C_{21}H_{25}O_5$ = 357.1702)

$[\alpha]^{23}_D$: + 56.1° (*c* 0.015, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 239 (4.19) , **Figure 110**

IR : ν_{max} cm^{-1}, Neat; 3150, 1736, 1676, 1456, 1227 ,1020, 920, **Figure 111**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 18** and **Figure 112-113**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 18** and **Figure 114**

4.15 Compound COC5 (Acetoxyaleuritolate)

Compound **COC5** was obtained as white solid, soluble in $CHCl_3$ (162.5 mg, 3.6×10^{-3}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 499, **Figure 122**

IR : ν_{max} cm^{-1}, KBr; 3423, 2937, 2856, 1736, 1687, 1458, 1365, 1244, **Figure 123**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 19** and **Figure 124**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 19** and **Figure 125**

4.16 Compound COC6 (Taraxerol)

Compound **COC6** was obtained as white solid, soluble in $CHCl_3$ (79.0 mg, 1.8×10^{-3}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 427, **Figure 127**

IR : ν_{max} cm^{-1}, KBr; 3483, 2933, 2852, 1473, 1385, 816, **Figure 128**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 20** and **Figure 129**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 20** and **Figure 130**

4.17 Compound COC7 (Chettaphanin II)

Compound **COC7** was obtained as yellow solid, soluble in $CHCl_3$ (25.2 mg, 5.6×10^{-3}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 341, **Figure 132**

UV : λ_{max} nm (log ε), in EtOH; 242 (3.68), 294 (3.48), **Figure 133**

IR : ν_{max} cm^{-1}, KBr; 3167, 2964, 1722, 1682, 1576, 1149, 1280, 816, **Figure 134**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 21** and **Figure 135**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 21** and **Figure 136**

4.18 Compound COC8 (6-[2-(Furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatri-cyclo[7.2.1.0^{2,7}]dodec-2(7)-en-11-one)

Compound **COC8** was obtained as pale yellow oil, soluble in $CHCl_3$ (4.1 mg, 0.9×10^{-4}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 315, **Figure 140**

HRFABMS : $[M+H]^+$ *m/z* 315.1990 (calcd. for $C_{20}H_{27}O_3$ = 315.1960)

$[\alpha]^{23}_D$: - 88.6° (*c* 0.0017, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 204 (4.19) , **Figure 141**

IR : ν_{max} cm^{-1}, Neat; 3124, 1773, 1459, 1290, 1024, 873, **Figure 142**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 22** and **Figure 143-144**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 22** and **Figure 145**

4.19 Compound COC9 (6-[2-(Furan-3-yl)-2-oxoethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.0^{2,7}]dodec-2(7)-en-11-one)

Compound **COC9** was obtained as yellow solid, soluble in $CHCl_3$ (33.4 mg, 7.4×10^{-4}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 329, **Figure 151**

HRFABMS : $[M+H]^+$ *m/z* 329.1727 (calcd. for $C_{20}H_{25}O_4$ = 329.1753)

$[\alpha]^{23}_D$: - 151.5° (*c* 0.017, $CHCl_3$)

UV : λ_{max} nm (log ε), in MeOH; 230 (4.13) , **Figure 152**

IR : ν_{max} cm^{-1}, KBr; 3122, 1757, 1671, 1509, 1276, 872, **Figure 153**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 23** and **Figure 154-155**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 23** and **Figure 156**

4.20 Compound COC10 (Chettaphanin I)

Compound **COC10** was obtained as white crystals, soluble in $CHCl_3$ (258.0 mg, 5.8×10^{-3}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 375, **Figure 163**

UV : λ_{max} nm (log ε), in EtOH; 248 (4.07) , **Figure 164**

IR : ν_{max} cm^{-1}, KBr; 3423, 3140, 2956, 1731, 1653, 1462, 1281, 1153, 997, **Figure 165**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 24** and **Figure 166-167**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 24** and **Figure 168**

4.21 Compound COC11 (Cyperenoic acid)

Compound **COC11** was obtained as white solid, soluble in $CHCl_3$ (259.9 mg, 5.8×10^{-3}% based on dried weight of the roots).

FABMS : $[M+H]^+$ *m/z* 235, **Figure 174**

UV : λ_{max} nm (log ε), in MeOH; 241 (4.01) , **Figure 175**

$[\alpha]^{23}_D$: - 7.8° (*c* 0.08, $CHCl_3$)

IR : ν_{max} cm^{-1}, Neat; 3200-2400, 1672, 1435, 1286, 951, **Figure 176**

^{1}H NMR : δ_H ppm, 500 MHz, in $CDCl_3$, **Table 31** and **Figure 177**

^{13}C NMR : δ_C ppm, 125 MHz, in $CDCl_3$, **Table 31** and **Figure 178**

5. Evaluation of biological Activities

5.1 Cytotoxic Activity

In vitro cytotoxicity test **(**Skehan *et al*., 1990**)** was assessed using the sulforhodamine B (SRB)-assay using human tumor cell lines of KB (human oral epidermoid carcinoma of nasopharynx), BC (human breast cancer) and NCI-H 187 (human small cell lung cancer). The cell lines were incubated at 37 $^{\circ}$C for 72 hr, at which time the SRB was added. The results were expressed as IC_{50} of tested compounds.

5.2 Antimycobacterial Activity

In vitro antimycobacterial activity was performed by a Microplate Alamar Blue Assay (Collins and Franzblau, 1997). *Mycobacterium tuberculosis* H37Ra was used as the tested microorganism. The Minimum Inhibitory Concentrations (MICs) of the test compounds were measured in μg/mL.

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary bioactivity screening revealed that *Pterocaulon redolens* and *Cladogynos orientalis* exhibited cytotoxic and antimycobacterial activities. These results of bioactivities are summarized as shown below.

Crude extact	Cytotoxicity IC_{50} (μg/mL) *			Antimycobacterial activity MIC (μg/mL) [d]
	KB [a]	BC [b]	NCI-H 187 [c]	
P. redolens				
The hexane extract	> 20	> 20	> 20	inactive
The $CHCl_3$ extract	> 20	5.0	> 20	50
The BuOH extract	> 20	4.2	5.7	50
C. orientalis				
The $CHCl_3$ extract	> 20	4.4	0.7	12.5
The MeOH extract	> 20	> 20	> 20	inactive

[a] KB; Oral human epidermoid carcinoma cell lines of nasopharynx

[b] BC; Human breast cancer cell lines

[c] NCI-H 187; Human small cell lung cancer cell lines

[d] Antimycobacterial activity toward *Mycobacterium tuberculosis* H37Ra

IC_{50}; Inhibition Concentration at 50%

* IC_{50} (μg/mL) > 20; inactive
10-20; weakly active
5-10; moderately active
< 5; strongly active

MIC; Minimun Inhibition Concentration

The dried aerial parts of *P. redolens* were extracted with $CHCl_3$ and MeOH to give a $CHCl_3$ extract (30.8 g) and a MeOH extract (10.2 g), respectively. The MeOH extract was then partitioned with BuOH and water to obtain the BuOH extract (4.5 g). The $CHCl_3$ extract was further purified using several chromatography techniques to

yield 7 pure compounds (compound **PRC1** to compound **PRC7**). By the repetitive chromatography, 3 compounds (compound **PRB8** to compound **PRB10**) were obtained from the BuOH extract.

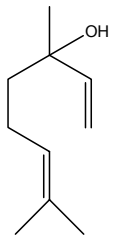
The $CHCl_3$ extract (208.6 g) from the roots of *C. orientalis* were separated using several chromatographic techniques to afford 11 pure compounds (compound **COC1** to compound **COC11**).

The structures of all isolates were determined by interpletation of their UV, IR, NMR and MS data and further confirmed by comparison with literature values. Additionally, their cytotoxic and antimycobacterial activities were also discussed.

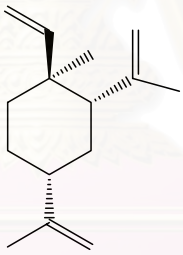
1. Determination of Volatile Oil Compositions from *Pterocaulon redolens*

The volatile oil obtained from the aerial parts of *P. redolen* is yellow. After analysis by GC-MS, the percentages of normal terpenes were determined and reported in the following table. GC-MS chromatogram (**Figure 5**) is demonstrated in Appendices part.

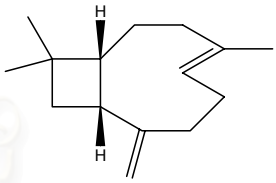
Peak number	Component name	% Area
1	linalool [**65**]	6.27
2	*β*-elemene [**66**]	2.76
3	9-*epi*-*β*-caryophyllene [**67**]	59.93
4	*α*-humulene [**68**]	8.22
5	*β*-selinene [**69**]	1.57
6	*α*-selinene [**70**]	0.92
7	germacrene A [**71**]	6.94
8	*Z*-nerolidol [**72**]	0.83
9	caryophyllene oxide [**73**]	12.56



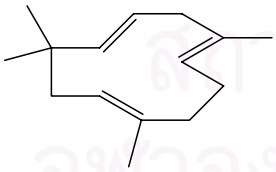
[**65**]



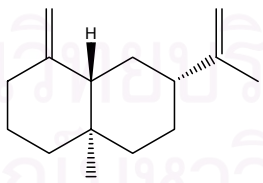
[**66**]



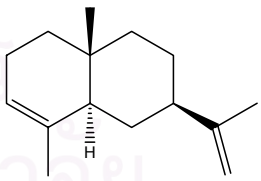
[**67**]



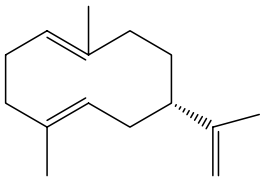
[**68**]



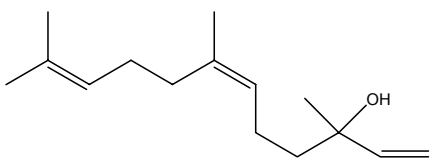
[**69**]



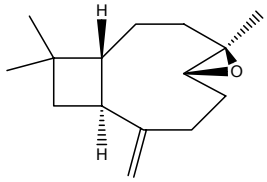
[**70**]



[**71**]



[**72**]



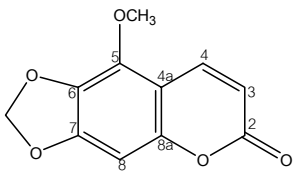
[**73**]

Figure 4 Structures of volatile oil compositions from *Pterocaulon redolens*

2. Structure Determination of Compounds Isolated from *Pterocaulon redolens*

2.1 Structure Determination of Compound PRC1

Compound **PRC1** was obtained as white crystals with m.p. 200-202 °C. The FAB mass spectrum (**Figure 6**) showed the protonated molecular ion peak $[M+H]^+$ at *m/z* 221, consistent with its molecular formula $C_{11}H_8O_5$. The UV spectrum (**Figure 7**) showed absorption maxima at 238, 269 and 316 nm. The IR spectrum (**Figure 8**) displayed absorption bands at 1737 (conjugated C=O stretching) and 1628 and 1481 (aromatic ring) cm^{-1}. The ^{1}H-NMR spectrum of compound **PRC1** (**Figure 9**) showed three singlet signals at δ_H 4.14 (3H, *s*), 6.01 (2H, *s*) and 6.53 (1H, *s*) attributed to methoxy, methylenedioxy and methine groups, respectively, and two doublet signals at δ_H 6.20 (H-3, *d*, *J* = 9.5 Hz) and 7.94 (H-4, *d*, *J* = 9.5 Hz) assigned to vinyl protons. The latter doublet proton signal at C-4 suggested that there is an oxygen substituent at C-5 (Steck and Mazurek, 1972). The HMBC spectrum of compound **PRC1** (**Figure 12**) showed the correlation from δ_H 7.94 (H-4) and 4.14 (OCH_3-5) to δ_C 138.0 (C-5), suggesting the presence of methoxy group at C-5. The singlet signal at δ_H 6.53 was assigned to H-8, confirmed by the HMBC correlations from δ_H 6.53 (H-8) to δ_C 106.6 (C-4a), 151.5 (C-8a), 131.7 (C-6) and 152.6 (C-7). The ^{1}H-NMR data exhibited close similarity to those in the literature (Maldonado, Hernandez and Ortega, 1992). The ^{13}C-NMR spectrum of compound **PRC1** (**Figure 10**) showed signals of the carbon 7 and 8a at δ_C 152.6 and 151.5 ppm, respectively. These signals had been conversely assigned in the literature. Based on the above spectral evidence and the assignment by the HMQC (**Figure 11**), HMBC (**Figure 12** and **Table 5**) experiments, compound **PRC1** was identified as 5-methoxy-6,7-methylenedioxycoumarin [**9**]. This compound was previously found in *Simsia cronquistii* (Maldonado, Hernandez and Ortega, 1992).



[**9**]

Table 5 NMR spectral data of compound **PRC1** and 5-methoxy-6,7-methylenedioxy-coumarin ($CDCl_3$)

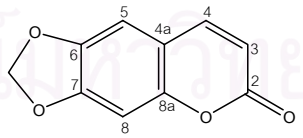
position	Compound **PRC1**			5-Methoxy-6,7-methylenedioxycoumarin	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC correlation	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	161.3	H-3*, H-4	-	161.4
3	6.20 (1H, *d*, 9.5)	111.7	-	6.17 (1H, *d*, 10.0)	111.7
4	7.94 (1H, *d*, 9.5)	138.8	-	7.89 (1H, *d*, 10.0, 1.0)	138.7
4a	-	106.6	H-3, H-8	-	106.6
5	-	138.0	H-4, OCH_3-5	-	138.0
6	-	131.7	OCH_2O, H-8	-	131.7
7	-	**152.6**	OCH_2O, H-8*	-	**151.5**
8	6.53 (1H, *s*)	92.3	-	6.46 (1H, *d*, 1.0)	92.4
8a	-	**151.5**	H-4, H-8*	-	**152.6**
OCH_2O	6.01 (2H, *s*)	101.8	-	5.97 (2H, *s*)	101.8
OCH_3	4.14 (3H, *s*)	59.9	-	4.11 (3H, *s*)	59.9

The bold values are revised assignments.

* Two bond coupling

2.2 Structure Determination of Compound PRC2

Compound **PRC2,** $[M+H]^+$ at *m/z* 191 in FAB mass spectrum (**Figure 13**) agreeing with the molecular formula $C_{10}H_6O_4$, was isolated as colourless needles with m.p. 220-221 °C. The UV spectrum (**Figure 14**) provided at 234, 294 and 346 nm. The IR spectrum (**Figure 15**) displayed the presence of a lactone carbonyl, typical of coumarin, at 1702 cm^{-1} together with the bands of an aromatic ring at 1630 and 1453 cm^{-1}. The ^{1}H-NMR spectrum of compound **PRC2** (**Figure 16**) showed a typical pair of doublets at δ_H 6.28 and 7.58 (1H each, *d*, $J = 9.7$ Hz) for H-3 and H-4, respectively. The relatively high field position of H-4 in compound **PRC2** suggested the lack of an oxygen substituent at C-5 (Steck and Mazurek, 1972). The presence of 6,7-dioxygenated aromatic ring was suggested by two singlet signals at δ_H 6.828 and 6.833 (each 1H, s), referring to H-5 and H-8. The presence of two protons signal at δ_H 6.07 as a singlet is characteristic of a methylenedioxy group. All signals of ^{1}H-NMR and ^{13}C-NMR spectra of compound **PRC2** (**Figure 17**) were corresponding to those of the literature (Debenedetti *et al*., 1998). The literature noted that 3 signals were observed at δ_C 143.4 (overlapped), 144.9 and 151.3 due to the carbon 4, 6, 8a and 7. Precise examination of the ^{13}C-NMR spectrum showed that the corresponding signals were separatedly observed at δ_C 143.4, 144.9, 151.2 and 151.3, assignable to carbons 4, 6, 7 and 8a. This present study completely assigned the ^{1}H- and ^{13}C-NMR data of this compound by HMQC (**Figure 18**), HMBC (**Figure 19** and **Table 6**) experiments and compound **PRC2** was identified as ayapin [**10**]. This compound has been reported to be present widely in plants such as *Pterocaulon virgatum* (Debenedetti *et al*., 1998) and *P. polystachium* (Palacios *et al*., 1999).



[**10**]

Table 6 NMR spectral data of compound **PRC2** and ayapin ($CDCl_3$)

position	Compound **PRC2**			Ayapin	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC correlation	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	161.2	H-3*, H-4	-	161.2
3	6.28 (1H, *d*, 9.7)	113.4	-	6.28 (1H, *d*, 9.5)	113.4
4	7.58 (1H, *d*, 9.7)	143.4	H-5	7.58 (1H, *d*, 9.5)	143.4
4a	-	112.7	H-3, H-4*, H-5*, H-8	-	112.7
5	6.828 (1H, *s*) [a]	105.0	H-4	6.82 (1H, *s*)	105.0
6	-	**144.9**	OCH_2O, H-5*, H-8	-	**143.4**
7	-	151.2 [a]	OCH_2O, H-5, H-8*	-	151.3
8	6.833 (1H, *s*) [a]	98.4	-	6.82 (1H, *s*)	98.4
8a	-	**151.3** [a]	H-4, H-5, H-8*	-	**144.9**
OCH_2O	6.07 (2H, *s*)	102.3	-	6.10 (2H, *s*)	102.3

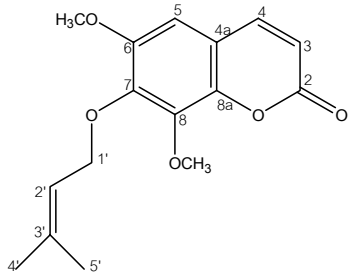
The bold values are revised assignments.

[a] Assignment may be interchanged.

* Two bond coupling

2.3 Structure Determination of Compound PRC3

Compound **PRC3** was obtained as white crystals with m.p. 92-93 °C. The FAB mass spectrum (**Figure 20**) showed $[M+H]^+$ at *m/z* 291, corresponding to the molecular formula $C_{16}H_{18}O_5$. The UV spectrum (**Figure 21**) showed 227, 260 and 297 nm. The IR spectrum (**Figure 22**) displayed absorption bands at 1729 cm^{-1} (a coumaryl lactone group) and 1605 and 1459 cm^{-1} (aromatic ring). The ^{1}H-NMR spectrum of compound **PRC3** (**Figure 23**) defined eighteen protons. The two doublets at δ_H 6.34 and 7.61 (each 1H, *d*, *J* = 9.5 Hz) were due to H-3 and H-4. The chemical shift of the latter showed that C-5 must contain no oxygen function otherwise it would appear at δ_H 7.8-8.2 (Steck and Mazurek, 1972), accordingly, the one proton singlet at δ_H 6.66 is assigned to H-5. The presence of two methoxy signals at δ_H 3.89 and 4.09 and a prenyl substituent [a methylene doublet at δ_H 4.64 (H_2-1′, *d*, *J* = 7.0 Hz), a coupled olefinic triplet like at δ_H 5.57 (H-2′, *t* like, *J* = 7.0 Hz) and two non-equivalent methyl resonances at δ_H 1.71 and 1.77 (H_3-4′ and H_3-5′, *s*)] confirmed that these three substituents should accupy the remaining vacant positions. The relative positions of these substituents were confirmly established by HMQC (**Figure 25**), HMBC (**Figure 26** and **Table 7**) and NOE (**Figure 27**) experiments that observed from H-5 (δ_H 6.66) to H-4 (δ_H 7.61) by 12.1% and H_3-6 (δ_H 3.89) by 10.0%, from OCH_3-6 (δ_H 3.89) to H-5 (δ_H 6.66) by 18.1% and H-2′ (δ_H 5.56) by 12.5% and from OCH_3-8 (δ_H 4.09) to H-1′ (δ_H 4.64) by 8.5% and H-2′ (δ_H 5.56) by 2.1%. Its ^{1}H-NMR properties were in agreement with previously published values (Jackson, Campbell and Davidowitz, 1990). Additionally, the ^{13}C-NMR spectrum (**Figure 24**) showed signals at δ_C 141.7, 143.0, 144.9 and 150.6 which had been previously assigned to C-8a, C-8, C-6 and C-7. They should be revised to C-8, C-8a, C-7 and C-6, respectively. Compound **PRC3** was identified as puberulin [**50**] based on the above spectral data, a coumarin first isolated from the aerial parts of *Agathosma puberula* (Finkelstein and Rivett, 1976)



[**50**]

Table 7 NMR spectral data of compound **PRC3** and puberulin ($CDCl_3$)

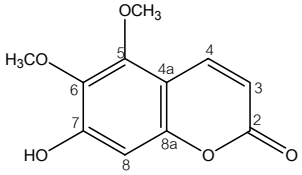
position	Compound **PRB3**			Puberulin	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC correlation	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	160.6	H-3*, H-4	-	160.6
3	6.34. (1H, *d*, 9.5)	115.1	-	6.31 (1H, *d*, 9.6)	115.1
4	7.61 (1H, *d*, 9.5)	143.4	-	7.58 (1H, *d*, 9.4)	143.5
4a	-	114.4	H-3, H-4*	-	114.4
5	6.66 (1H, *s*)	103.6	H-4	6.63 (1H, *s*)	103.6
6	-	**150.6**	H-5*, OCH_3-6	-	**144.9**
7	-	**144.9**	H-5, H_2-1′	-	**150.7**
8	-	**141.7**	OCH_3-8	-	**143.0**
8a	-	**143.0**	H-4, H-5	-	**141.8**
1′	4 .64 (2H, *d*, 7.0)	70.2	-	4.61 (2H, *d*, 8.0)	70.3
2′	5.56 (1H, *t* like, 7.0)	119.1	H_2-1′*, H_3-4′, H_3-5′	5.55 (1H, *t*, 8.0)	120.0
3′	-	139.3	H_2-1′, H_3-4′*, H_3-5′*	-	139.3
4′	1.71 (3H, *s*)	17.9	H-4′	1.68 (3H, *s*)	17.9
5′	1.77 (3H, *s*)	25.8	H-5′	1.74 (3H, *s*)	25.8
OCH_3-6	3.89 (3H, *s*)	56.3	-	3.91 (3H, *s*)	56.3
OCH_3-8	4.09 (3H, *s*)	61.7	-	4.00 (3H, *s*)	-

The bold values are revised assignments.

* Two bond coupling

2.4 Structure Determination of Compound PRC4

Compound **PRC4**, white solid with m.p. 147-148 °C, showed $[M+H]^+$ at *m/*z 223 in the FABMS (**Figure 28**), suggesting the molecular formula $C_{11}H_{10}O_5$. The UV spectrum (**Figure 29**) showed absorptions at 222, 266 and 328 nm. The IR spectrum (**Figure 30**) revealed absorption at λ_{max} 3413 (OH stretching), 1722 (conjugated C=O stretching) and 1608 and 1468 (aromatic ring) cm^{-1}. The ^{1}H-NMR spectrum of compound **PRC4** (**Figure 31**) showed two signals at δ_H 6.23 (*d*, *J* = 9.8 Hz) and 7.91 (*d*, *J* = 9.8 Hz) assigned to the vinylic protons H-3 and H-4. The deshielded nature of the H-4 suggested that there was an oxygen function at the C-5 (Steck and Mazurek, 1972). The presence of only one aromatic proton at δ_H 6.70, clearly indicated a trisubstituted aromatic moiety. The ^{1}H-NMR of compound **PRC4** showed two aromatic methoxy signals at δ_H 3.94 and 3.99 and one aromatic hydroxyl signal at δ_H 6.43. Detection of HMBC correlations, from δ_H 6.23 (H-3) and 6.70 (H-8) to δ_C 107.2 (C-4a), from δ_H 7.91 (H-4) to δ_C 148.4 (C-5), 151.6 (C-8a) and 161.4 (C-2), from δ_H 3.99 (H_3-5) to δ_C 148.4 (C-5), from δ_H 3.94 (H_3-6), 6.43 (OH-7) and 6.70 (H-8) to δ_C 136.3 (C-6) and from δ_H 6.43 (OH-7) to δ_C 98.8 (C-8), confirmed that compound **PRC4** was 5,6,7-substitution of coumarin system and also defined location of the methoxy groups at C-5 and C-6 and the hydroxy group at C-7. The ^{1}H-NMR (**Figure 31**) showed the signal at 3.94 and 3.99 due to the protons OCH_3-6 and OCH_3-5, respectively. These were revised from previous report (Wagner and Bladt, 1975). From the above ^{1}H-NMR and ^{13}C-NMR spectral data (**Figure 32**), together with the information from the HMQC (**Figure 33**) and HMBC (**Figure 34** and **Table 8**) experiments, compound **PRC4** was identified as 5-methoxyscopoletin [**51**]. This compound was firstly isolated from the roots of *Pelargonium reniforme* (Wagner and Bladt, 1975).



[**51**]

Table 8 NMR spectral data of compound **PRC4** and 5-methoxyscopoletin ($CDCl_3$)

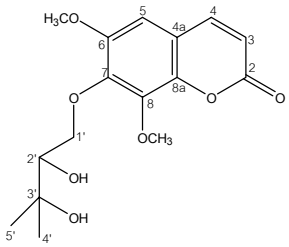
position	Compound **PRC4**			5-Methoxyscopoletin
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC correlation	δ_H (ppm), *J* (Hz)
2	-	161.4	H-3*, H-4	-
3	6.23 (1H, *d*, 9.8)	112.4	-	6.23 (1H, *d*, 9.5)
4	7.91 (1H, *d*, 9.8)	138.6	-	7.94 (1H, *d*, 9.5)
4a	-	107.2	H-3, H-8	-
5	-	148.4	H-4, OCH_3-5	-
6	-	136.3	OCH_3-6, OH-7, H-8	-
7	-	153.3	H-8	-
8	6.70 (1H, *s*)	98.8	OH-7	6.72 (1H, *s*)
8a	-	151.6	H-4, H-8	-
OCH_3-5	**3.99** (3H, *s*)	61.5	-	**3.95** (3H, *s*)
OCH_3-6	**3.94** (3H, *s*)	61.2	-	**4.03** (3H, *s*)
OH-7	6.43 (1H, *br s*)	-	-	6.78 (1H, *br s*)

The bold values are revised assignments.

* Two bond coupling

2.5 Structure Determination of Compound PRC5

Compound **PRC5** was isolated as white crystals, with m.p. 78-80 °C. The FAB mass spectrum (**Figure 35**) showed $[M+H]^+$ at *m/z* 325, corresponding to $C_{16}H_{20}O_7$. The UV absorptions were observed at 228, 296 and 343 nm (**Figure 36**). The IR spectrum (**Figure 37**) exhibited absorption bands due to the presence of hydroxyl group (3474 cm^{-1}), conjugated carbonyl group (1716 cm^{-1}) and aromatic ring (1605 and 1459 cm^{-1}). The ^{1}H-NMR spectrum of compound **PRC5** (**Figure 38**) showed a typical pair of doublets at δ_H 6.37 and 7.62 (1H each, *d*, *J* = 9.4 Hz) for H-3 and H-4, respectively. The relatively high field position of H-4 suggested the lack of an oxygen substituent at C-5 (Steck and Mazurek, 1972) and the presence of only one singlet aromatic proton at δ_H 6.70 confirmed a trisubstituent aromatic moiety. The singlet signals at δ_H 3.92 and 4.07 (3H each) were assigned as two methoxy group on aromatic nucleus. More characteristically, two pairs of doublet of doublets at δ_H 4.00 (Ha-1′, *dd*, *J* = 10.4, 7.8 Hz), 4.54 (Hb-1′, *dd*, *J* = 10.4, 2.6 Hz) and a doublet of doublet of doublet at δ_H 3.67 (H-2′, *ddd*, *J* = 7.8, 3.6, 2.6 Hz) corresponded to methylene and methine in -O-CH_2-CH-OH fragment. The singlet signals at δ_H 1.23 and 1.28 (3H each) corresponded to a *gem*-dimethyl group and the hydroxy groups were assigned at δ_H 2.71 (OH-3′, *s*) and 3.87 (OH-2′, *d*, *J* = 3.6 Hz). The positions of a trisubstituent aromatic moiety were analyzed by the HMBC correlations from δ_H 6.70 (H-5) to δ_C 142.4 (C-8a), 143.3 (C-4), 144.6 (C-7) and 149.7 (C-6), from δ_H 3.92 (OCH_3-6) to δ_C 149.7 (C-6), from δ_H 4.00 (Ha-1′) to δ_C 144.6 (C-7) and from δ_H 4.07 (OCH_3-8) to δ_C 141.0 (C-8). The ^{1}H-NMR data of compound **PRC5** showed all signals corresponding to the literature (Magalhaes *et al*., 1981) and also confirmed by the optical rotation; $[\alpha]^{23}_D$ +25° (*c* 0.9 in $CHCl_3$), which was related to that of 2′,3′-dihydroxypuberulin [**52**]. It should be noted that the isolation of this compound from a natural source is the first time. This compound was known, however, the ^{13}C-NMR (**Figure 39**), HMQC (**Figure 40**) and HMBC (**Figure 41** and **Table 9**) were presented at the first time in this study.



[**52**]

Table 9 The ^{1}H- and ^{13}C-NMR data of compound **PRC5** in $CDCl_3$

position	Compound **PRC5**			2′,3′-Dihydropuberulin
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC correlation	δ_H (ppm), *J* (Hz)
2	-	160.1	H-3*, H-4	-
3	6.37 (1H, *d*, 9.4)	115.5	-	6.45 (1H, *d*)
4	7.62 (1H, *d*, 9.4)	143.3	H-5	7.75 (1H, *d*)
4a	-	114.8	H-3	-
5	6.70 (1H, *s*)	103.8	H-4	6.81 (1H, *s*)
6	-	149.7	H-5*, OCH_3-6	-
7	-	144.6	H-5, Ha-1′	-
8	-	141.0	OCH_3-8	-
8a	-	142.4	H-4, H-5	-
1′	4.00 (1Ha, *dd*, 10.4, 7.8)**	76.3	OH-2′	4.70 (2H, *m*)
	4.54 (1Hb, *dd*, 10.4, 2.6)**			
2′	3.67 (1H, *ddd*, 7.8, 3.6, 2.6)**	75.7	Hb-1′*, OH-3′, H_3-4′, H_3-5′	3.80 (1H, *m*)
3′	-	71.3	OH-2′, OH-3′*, H_3-4′*, H_3-5′*	-
OCH_3-4′	1.23 (*s*)	25.1	OH-3′, H_3-5′	1.26 (*s*)
OCH_3-5′	1.28 (*s*)	26.7	OH-3′, H_3-4′	1.30 (*s*)
OCH_3-6	3.92 (3H, *s*)	56.3	-	3.98 (3H, *s*)
OCH_3-8	4.07 (3H, *s*)	62.0	-	4.03 (3H, *s*)
OH-2′	3.87 (1H, *d*, 3.6)**	-	-	-
OH-3′	2.71 (1H, *s*)	-	-	-

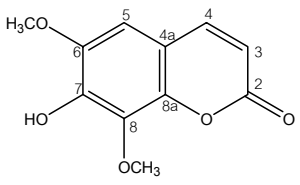
* Two bond coupling

** Precise assignment of coupling constants, see; δ_H 3.67 (*ddd*, $J_{H\text{-}2', Ha\text{-}1'}$ = 7.8 Hz, $J_{H\text{-}2', OH\text{-}2'}$ = 3.6 Hz, $J_{H\text{-}2', Hb\text{-}1'}$ = 2.6 Hz), 3.87 (*d*, $J_{OH\text{-}2', H\text{-}2'}$ = 3.6 Hz), 4.00 (*dd*, $J_{Ha\text{-}1', Hb\text{-}2'}$ = 10.4 Hz, $J_{Ha\text{-}1', H\text{-}2'}$ = 7.8 Hz), 4.54 (*dd*, $J_{Hb\text{-}1', Ha\text{-}1'}$ = 10.4 Hz, $J_{Hb\text{-}1', H\text{-}2'}$ = 2.6 Hz)

2.6 Structure Determination of Compound PRC6

Compound **PRC6** was characterized as yellow crystals, with m.p. 149-150 °C. The FAB mass spectrum (**Figure 42**) demonstrated the molecular ion peak $[M+H]^+$ at *m/z* 223, harmonizing with the molecular formular $C_{11}H_{10}O_5$. The UV spectrum (**Figure 43**) revealed absorptions at λ_{max} 228, 268 and 345 nm. The IR spectrum (**Figure 44**) exhibited absorption bands at 3369 (hydroxy stretching), 1706 (conjugated carbonyl group) and 1600 and 1456 (aromatic ring) cm^{-1}. The ^{1}H-NMR spectrum of compound **PRC6** exhibited the diagnostic H-3 and H-4 olefinic doublets in the aromatic region (δ_H 6.28 and 7.60, *d*, *J* = 9.5 Hz). The relatively high field position of H-4 suggested the lack of an oxygen substituent at C-5 (Steck and Mazurek, 1972). The aromatic region in the spectrum additionally displayed a one-proton singlet at δ_H 6.66, consistent with a trisubstitution pattern on the aromatic ring in each instance. The ^{1}H-NMR spectrum (**Figure 45**) showed all signals corresponding to those in the literature and the ^{13}C-NMR spectrum (**Figure 46**) has been reported already (Panichayupakaranant *et al*., 1995) but some positions should be revised as C-3 (δ_C 113.6), C-5 (δ_C 103.2), C-7 (δ_C 142.4), C-8 (δ_C 134.5) and C-8a (δ_C 143.1). This assignment was determined by HMQC (**Figure 47**) and HMBC (**Figure 48** and **Table 10**) experiments. The NOE difference spectra (**Figure 49**) confirmed the position of the methoxy group at C-6 and C-8 of the coumarin nucleus. Thus, irradiation of the H-5 at δ_H 6.66 caused an enhancement of the methoxy signal at δ_H 3.95 (OCH_3-6) and olefinic proton at δ_H 7.60 (H-4). Futhermore, NOEs were observed between the methoxy signal at δ_H 3.95 (OCH_3-6) and the methine signal at δ_H 6.66 (H-5) and between the methoxy signal at δ_H 4.10 (OCH_3-8) and the hydroxy signal at δ_H 6.13 (OH-7).

Based on the above spectral evidence, compound **PRC6** was analyzed as isofraxedin [**53**], previously characterized from *Carduus tenuiflorus* (Cardona *et al*., 1992)



[**53**]

Table 10 NMR spectral data of compound **PRC6** and isofraxidin ($CDCl_3$)

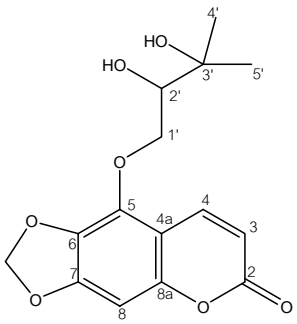
position	Compound **PRC6**			Isofraxidin	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC correlation	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	160.6	H-3*, H-4	-	160.6
3	6.28 (1H, *d*, 9.5)	**113.6**	-	6.28 (1H, *d*, 10.0)	**103.2**
4	7.60 (1H, *d*, 9.5)	143.8	-	7.60 (1H, *d*, 10.0, 1.0)	143.8
4a	-	111.2	H-3, H-4*, H-5*	-	111.2
5	6.66 (1H, s)	**103.2**	H-4	6.66 (1H, s)	**113.5**
6	-	144.6	OCH_3-6	-	144.6
7	-	**142.4**	-	-	**134.5**
8	-	**134.5**	OCH_3-8	-	**143.1**
8a	-	**143.1**	H-4, H-5	-	**142.5**
OCH_3-6	3.95 (3H, *s*)	56.5	-	3.94 (3H, *s*)	56.5
OCH_3-8	4.10 (3H, *s*)	61.6	-	4.09 (3H, *s*)	61.6
OH-7	6.13 (1H, *br s*)	-	-	-	-

The bold values are revised assignments.

* Two bond coupling

2.7 Structure Determination of Compound PRC7

Compound **PRC7**, white crystals with m.p. 150-151°C, showed its protonated molecular ion $[M+H]^+$at m/z 309 in FAB mass spectrum (**Figure 50**), indicating a molecular of $C_{15}H_{16}O_7$. The UV spectrum (**Figure 51**) showed maximum absorption at 220, 239 and 320 nm. The IR spectrum (**Figure 52**) displayed 3438 (hydroxyl group), 1715 (conjugated carbonyl group) and 1638 and 1473 (aromatic ring) cm^{-1}. The ^{1}H-NMR spectrum of compound **PRC7** (**Figure 53**) showed the characteristic coumarin C-3/C-4 doublet pair appearing at δ_H 6.27 and 8.15 (each 1H, *d*, *J* = 9.8 Hz). The latter, corresponding to H-4, suggested that there was an oxygen substitution at C-5 (Steck and Mazurek, 1972). Additionally, the ^{1}H-NMR indicated the presence of an aromatic proton at δ_H 6.80 (H-8, *s*), consistent with a trisubstitution pattern on the aromatic ring. The signal at δ_H 6.11 was assigned to a methylenedioxyphenyl group. The remaining signals at δ_H 1.02 (H_3-4′, *s*), 1.22 (H_3-5′, *s*), 3.53 (H-2′, *ddd*, *J* = 8.6, 5.8, 2.5 Hz), 4.11 (Ha-1′, *dd*, *J* = 10.1, 8.6 Hz), 4.40 (OH-3′, *s*), 4.60 (Hb-1′, *dd*, *J* = 10.1, 2.5 Hz) and 5.13 (OH-2′, *d*, *J* = 5.8 Hz) were attributed to a 2′,3′-dihydroxy-3′-methylbutyloxy substituent, which could be placed at δ_C 137.1 (C-5). In HMBC experiments, these were confirmed by the three-bond correlations from δ_H 4.11 (Ha-1′), 4.60 (Hb-1′) and 8.15 (H-4) to δ_C 137.1 (C-5) and from δ_H 6.80 (H-8) to δ_C 106.6 (C-4a) and 132.2 (C-6), from δ_H 5.13 (OH-2′) to δ_C 74.4 (C-1′) and 70.6 (C-3′), from 1.02 (H_3-4′), 1.22 (H_3-5′) and 4.40 (OH-3′) to δ_C 76.1 (C-2′). The $[\alpha]^{24}_D$ + 30.9° (*c* 0.65 in MeOH) and the ^{1}H-NMR spectrum data exhibited close similarity to those in the literature (Debenedetti *et al*., 1997). The ^{13}C-NMR spectrum (**Figure 54**) showed the signals of the C-2′ and C-3′ at δ_C 76.1 and 70.6 ppm, respectively. These were revised from previous report (Debenedetti *et al*., 1997). This assignment was confirmed by the application of HMQC (**Figure 55**), HMBC (**Figure 56** and **Table 11**) and ^{1}H-^{1}H COSY (**Figure 57**) experiments and compound **PRC7** was identified as 5-(2′,3′-dihydroxy-3′-methylbutyl-oxo-6,7-methylenedioxycoumarin (sabandinol) [**23**]. This compound has been reported to be present wildly in plants such as *Ruta pinnata* (Gonzalez *et al*., 1973) and *Pterocaulon virgatum* (Debenedetti *et al*., 1997).



[**23**]

Table 11 NMR spectral data of compound **PRC7** (DMSO-d_6) and sabandinol ($CDCl_3$)

position	Compound **PRC7**			Sabandinol [**23**]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC correlation	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	160.3	H-3*, H-4	-	161.2
3	6.27 (1H, *d*, 9.8)	111.2	-	6.23 (1H, *d*, 9.7)	112.1
4	8.15 (1H, *d*, 9.8)	139.6	-	7.96 (1H, *d*, 9.7)	138.6
4a	-	106.6	H-3, H-8	-	107.0
5	-	137.1	Ha-1′, Hb-1′, H-4	-	136.8
6	-	132.2	-OCH_2O-, H-8	-	132.3
7	-	152.4	-OCH_2O-, H-8*	-	151.5
8	6.80 (1H, *s*)	92.2	-	6.57 (1H, *s*)	93.1
8a	-	150.9	H-8*	-	152.4
1′	4.11 (1Ha, *dd*, 10.1, 8.6)**	74.4	H-2′*, OH-2′	4.37 (1Ha, *dd*, 10.4, 8.1)	73.8
	4.60 (1Hb, *dd*, 10.1, 2.5)**			4.51 (1Hb, *dd*, 10.4, 2.9)	
2′	3.53 (1H, *ddd*, 8.6, 5.8, 2.5)**	**76.1**	H_3-4′, H_3-5′, OH-2′, OH-3′	3.80 (1H, *m*)	**71.6**
3′	-	**70.6**	H-2′, H_3-4′, H_3-5′, OH-2′, OH-3′	-	**76.5**
CH_3-4′	1.02 (3H, *s*)	24.3	H_3-5′	1.33 (3H, *s*)	24.8
CH_3-5′	1.22 (3H, *s*)	27.6	H_3-4′, OH-3′	1.33 (3H, *s*)	24.8
OCH_2O	6.11 (2H, *s*)	102.3	-	6.06 (2H, *s*)	102.1
OH-2′	5.13 (1H, *d*, 5.8)	-	-	-	-
OH-3′	4.40 (1H, *s*)	-	-	-	-

The bold values are revised assignments.

* Two bond coupling

** Precise assignment of coupling constant, see; δ_H 3.53 (*ddd*, $J_{H\text{-}2', Ha\text{-}1'}$ = 8.6 Hz, $J_{H\text{-}2', OH\text{-}2'}$ = 5.8 Hz, $J_{H\text{-}2', Hb\text{-}1'}$ = 2.5 Hz), 4.11 (*dd*, $J_{Ha\text{-}1', Hb\text{-}2'}$ = 10.1 Hz, $J_{Ha\text{-}1', H\text{-}2'}$ = 8.6 Hz), 4.60 (*dd*, $J_{Hb\text{-}1', Ha\text{-}1'}$ = 10.1 Hz, $J_{Hb\text{-}1', H\text{-}2'}$ = 2.5 Hz), 5.13 (*d*, $J_{OH\text{-}2', H\text{-}2'}$ = 5.8 Hz)

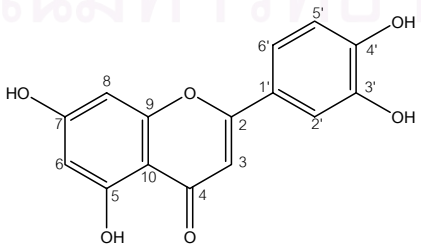
2.8 Structure Determination of Compound PRB8

Compound **PRB8** was obtained yellow crystals with m.p. 351-352 °C and observed a molecular formula as $C_{15}H_{10}O_6$. The FAB mass spectrum (**Figure 58**) exhibited a $[M+H]^+$ at *m/z* 287. The UV spectrum (**Figure 59**) showed maxima absorption bands at 212, 270 and 317 nm. The IR absorption spectrum (**Figure 60**) displayed ν_{max} at 3347 (hydroxyl stretching), 1654 (carbonyl stretching) and 1609 and 1490 (aromatic ring) cm^{-1}.

The ^{1}H-NMR spectrum of compound **PRB8** (**Figure 61**) showed a H-bonded phenolic proton at δ_H 12.97 (OH-5), indicating a 5-hydroxyflavone structure. The protons in B-ring (H-2′, H-5′ and H-6′) formed a characteristic ABX pattern at δ_H 6.87 (H-5′, *d*, *J* = 8.5 Hz), 7.39 (H-2′, *d*, *J* = 2.0 Hz) and 7.41 (H-6′, *dd*, *J* = 2.0, 8.5 Hz) while the signals of H-6 and H-8 in A-ring appeared as a pair of doublets at δ_H 6.18 (H-6, *d*, *J* = 2.0 Hz) and 6.43 (H-8, *d*, *J* = 2.0 Hz), respectively. An olefinic singlet proton at δ_H 6.66 was assigned to H-3 by its HMBC correlations with C-10 (δ_C 157.3) and C-1′ (δ_C 121.5).

The ^{13}C-NMR spectrum of compound **PRB8** (**Figure 62**) showed fifteen signals for carbon atoms. The types of carbons are classified by analysis of the DEPT135 experiment (**Table 12**).

Based on the above spectral evidences, and comparison of the spectral data of compound **PRB8** with those previously reported (Agrawal, 1989), together with the information from the HMQC (**Figure 63**) and HMBC experiments (**Figure 64** and **Table 12**), compound **PRB8** was identified as luteolin [**54**]. This compound occurred in many plants of the family Leguminosae, Umbelliferae, Asteraceae and Cistaceae (Buckingham, 2001).



[**54**]

Table 12 NMR spectral data of compound **PRB8** and luteolin (DMSO-d_6)

position	Compound **PRB8**			Luteolin
	δ_H (ppm), *J* (Hz)	δ_C (ppm)[#]	HMBC correlation	δ_C (ppm)
2	-	163.9 (C)	H-3*, H-2′, H-6′	164.5
3	6.66 (1H, *s*)	102.8 (CH)	-	103.3
4	-	181.6 (C)	H-3*	182.2
5	-	161.5 (C)	-	162.1
6	6.18 (1H, *d*, 2.0)	98.8 (CH)	OH-5, H-8	99.2
7	-	164.1 (C)	H-6*, H-8*	164.7
8	6.43 (1H, *d*, 2.0)	93.8 (CH)	H-6	94.2
9	-	103.7 (C)	-	104.2
10	-	157.3 (C)	H-3, H-6, H-8, OH-5	157.9
1′	-	121.5 (C)	H-3, H-5′	122.1
2′	7.39 (1H, *d*, 2.0)	113.3 (CH)	H-6′	113.8
3′	-	145.7 (C)	H-2′*, H-5′	146.2
4′	-	149.7 (C)	H-2′, H-5′*, H-6′	150.1
5′	6.87 (1H, *d*, 8.5)	116.0 (CH)	-	116.4
6′	7.41 (1H, *dd*, 2.0, 8.5)	119.0 (CH)	H-2′	119.3
OH-5	12.97 (1H, *s*)	-	-	-
	10.68 (1H, *s*)[a]	-	-	-
	9.69 (1H, *s*)[a]	-	-	-

[a] Assignment may be interchanged.

* Two bond coupling

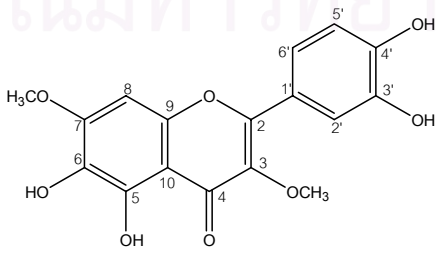
[#] Carbon types were deduced from DEPT135 experiment.

2.9 Structure Determination of Compound PRB9

Compound **PRB9** was obtained as yellow crystals with m.p. 183-185 °C. The FAB mass spectrum (**Figure 65**) exhibited a $[M+H]^+$ at *m/z* 347, indicating molecular formula as $C_{17}H_{14}O_8$. The UV spectrum (**Figure 66**) showed maxima absorption bands at 213, 303 and 347 nm. The IR absorption spectrum (**Figure 67**) displayed ν_{max} at 3369 (hydroxyl stretching), 1655 (carbonyl stretching) and 1609 and 1491 (aromatic ring) cm^{-1}.

The ^{1}H-NMR spectrum of compound **PRB9** (**Figure 68**) showed a H-bonded phenolic proton at δ_H 12.36 (OH-5), indicating a 5-hydroxyflavone structure. The protons in B-ring (H-2′, H-5′ and H-6′) formed a characteristic ABX pattern at δ_H 6.90 (H-5′, *d*, *J* = 8.5 Hz), 7.47 (H-6′, *dd*, *J* = 2.0, 8.5 Hz) and 7.58 (H-2′, *d*, *J* = 2.0 Hz) while the signal of H-8 in A-ring appeared as a singlet at δ_H 6.83.

The ^{13}C-NMR spectrum of compound **PRB9** (**Figure 69**) showed seventeen signals for carbon atoms. The types of carbons are classified by analysis of the DEPT135 experiment (**Table 13**), including two methoxy carbons at δ_C 56.3 (OCH_3-7) and 59.6 (OCH_3-3), four aromatic methine carbons at δ_C 90.8 (C-8), 115.5 (C-2′), 115.7 (C-5′) and 120.5 (C-6′) and eleven aromatic quaternary carbons at δ_C 105.5 (C-10), 121.0 (C-1′), 129.6 (C-6), 137.5 (C-3), 145.2 (C-3′), 148.6 (C-4′), 148.8 (C-9), 146.7 (C-5), 154.5 (C-7), 155.6 (C-2) and 178.1 (C-4). Based on the careful analysis of the above spectra, 2D technique such as HMQC (**Figure 70**) and HMBC (**Figure 71** and **Table 13**) and comparison with those previously reported (Ulubelen, Kerr and Mabry, 1980), compound **PRB9** was identified as tomentin [**55**]. This compound has been isolated from many plants such as *Neurolaena oaxacana* (Ulubelen, Kerr and Mabry, 1980) and *Parthenium hysterophorus* (Shen *et al*., 1976).



[**55**]

Table 13 NMR spectral data of compound **PRB9** and tomentin (DMSO-d_6)

position	Compound **PRB9**			Tomentin
	δ_H (ppm), *J* (Hz)	δ_C(ppm)[#]	HMBC correlation	δ_H (ppm), *J* (Hz)
2	-	155.6 (C)	H-2′, H-6′	-
3	-	137.5 (C)	OCH_3-3	-
4	-	178.1 (C)	-	-
5	-	146.7 (C)	-	-
6	-	129.6 (C)	H-8, OH-5	-
7	-	154.5 (C)	H-8*, OCH_3-7	-
8	6.83 (1H, *s*)	90.8 (CH)	-	6.50 (1H, *s*)
9	-	148.8 (C)	H-8*	-
10	-	105.5 (C)	H-8, OH-5	-
1′	-	121.0 (C)	H-5′	-
2′	7.58 (1H, *d*, 2.0)	115.5 (CH)	H-6′	7.60 (1H, *d*, 2.5)
3′	-	145.2 (CH)	H-2′*, H-5′	-
4′	-	148.6 (C)	H-2′, H-6′, H-5′*	-
5′	6.90 (1H, *d*, 8.5)	115.7 (C)	-	6.38 (1H, *d*, 9.0)
6′	7.47 (1H, *dd*, 2.0, 8.5)	120.5 (CH)	H-2′	7.55 (1H, *dd*, 2.5, 9.0)
OCH_3-3	3.78 (3H, *s*)	59.6 (CH_3)	-	-
OCH_3-7	3.90 (3H, *s*)	56.3 (CH_3)	-	-
OH-5	12.36 (1H, *s*)	-	-	-
OH-6	9.77 (1H, *s*)[a]	-	-	-
OH-3′	9.35 (1H, *s*)[a]	-	-	-
OH-4′	8.70 (1H, *s*)[a]	-	-	-

[a] Assignment may be interchanged.

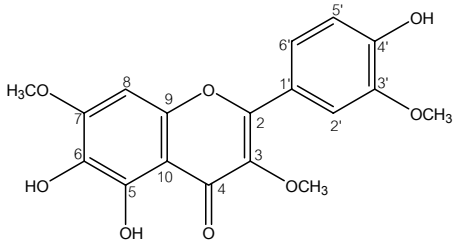
* Two bond coupling

[#] Carbon types were deduced from DEPT135 experiment.

2.10 Structure Determination of Compound PRB10

Compound **PRB10**, a yellow solid with m.p. 218-220 °C, was analyzed for $C_{18}H_{16}O_8$ from its $[M+H]^+$ at *m/z* 361 in FABMS spectrum (**Figure 72**). The UV spectrum displayed absorption bands at 214, 281 and 349 nm (**Figure 73**). The IR spectrum exhibited absorption bands at 3392 (OH stretching), 1668 (C=O stretching) and 1608 and 1491 (C=C stretching) cm^{-1} (**Figure 74**). The ^{1}H-NMR spectrum of compound **PRB10** (**Figure 75**) showed a H-bond phenolic proton at δ_H 12.35, indicating a 5-hydroxy flavone structure. The protons in B-ring ring showed a characteristic ABX pattern at δ_H 6.95 (H-5′, *d*, *J* = 8.5 Hz), 7.61 (H-6′, *dd*, *J* = 2.5, 8.5 Hz) and 7.66 (H-2′, *d*, *J* = 2.5 Hz), while the signals of H-8 in A-ring appeared as a singlet at δ_H 6.89. The ^{13}C-NMR spectrum of compound **PRB10** (**Figure 76**) displayed resonances for eighteen carbons. The two signals at δ_C 55.8 and 56.3 were within the range typical for the carbon of an aromatic methoxy group with at least one free ortho position (55.0-57.0 ppm) and the signal at δ_C 59.7 was characteristic of the carbon of a methoxy group attached to C-3 on a flavone (Agrawal, 1989). The remaining fifteen signals occurred within the δ_C 90.0-200.0 range typical of the nucleus of a 2,3-unsaturated flavonoid; six signals consistent with non-oxygenated aromatic carbons (δ_C 91.0, 105.5, 112.0, 115.6, 121.0 and 122.2), eight signals consistent with oxyaryl carbons (δ_C 129.6, 137.6, 145.6, 147.5, 148.8, 149.7, 154.5 and 155.5) and one signal at δ_C 178.1, within the range typical for the carbon of the 4-keto function of a flavone (Agrawal, 1989). The ^{1}H-NMR and ^{13}C-NMR assignments were performed using the HMQC (**Figure 77**) and HMBC (**Figure 78** and **Table 14**) experiments. Thus, compound **PRB10** possessed the 3,7,3′-trimethoxy-5,6,4′-trihydroxyflavone.

Compound **PRB10** was identified as chrysosplenol C [**35**] based on the above spectral data and comparison of those previously reported (Semple *et al*., 1999). This compound has been isolated from *Pterocaulon sphacelatum* (Semple *et al*., 1999) and the other plant species including *Tanacetum parathenium* (William *et al*., 1995).



[**35**]

Table 14 NMR spectral data of compound **PRB10** and chrysosplenol C (DMSO-d_6)

position	Compound **PRB10**			Chrysosplenol C	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]	HMBC correlation	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	155.5 (C)	H-2′, H-6′	-	155.5
3	-	137.6 (C)	OCH_3-3	-	137.6
4	-	178.1 (C)	-	-	178.1
5	-	145.6 (C)	OH-5*	-	145.7
6	-	129.6 (C)	OH-5, H-8	-	129.6
7	-	154.5 (C)	OCH_3-7, H-8*	-	154.5
8	6.89 (1H, *s*)	91.0 (CH)	-	6.87 (1H)	91.0
9	-	148.8 (C)	H-8*	-	148.8
10	-	105.5 (C)	H-8, OH-5	-	105.5
1′	-	121.0 (C)	H-2′*, H-5′	-	121.0
2′	7.66 (1H, *d*, 2.5)	112.0 (CH)	-	7.65 (1H)	112.0
3′	-	147.5 (C)	H-2′*, H-5′, OCH_3-3′	-	147.5
4′	-	149.7 (C)	H-2′, H-5′*, H-6′	-	149.7
5′	6.95 (1H, *d*, 8.5)	115.6 (CH)	-	6.95 (1H)	115.6
6′	7.61 (1H, *dd*, 2.5, 8.5)	122.2 (CH)	H-2′	7.60 (1H)	122.2
OCH_3-3	3.80 (3H, *s*)	59.7 (CH_3)	-	3.80 (3H, *s*)	59.7
OCH_3-7	3.87 (3H, *s*)	56.3 (CH_3)	-	3.87 (3H, *s*)	56.3
OCH_3-3′	3.90 (3H, *s*)	55.8 (CH_3)	-	3.90 (3H, *s*)	55.8
OH-5	12.35 (1H, *s*)	-	-	12.34 (1H)	-
OH-6	9.91 (1H, *s*) [a]	-	-	8.69 (1H)	-
OH-4′	8.71 (1H, *s*) [a]	-	-	9.88 (1H)	-

[a] Assignment may be interchanged.

* Two bond coupling

[#] Carbon types were deduced from DEPT135 experiment.

3. Structure Determination of Compounds Isolated from *Cladogynos orientalis*

3.1 Structure Determination of Compound COC1

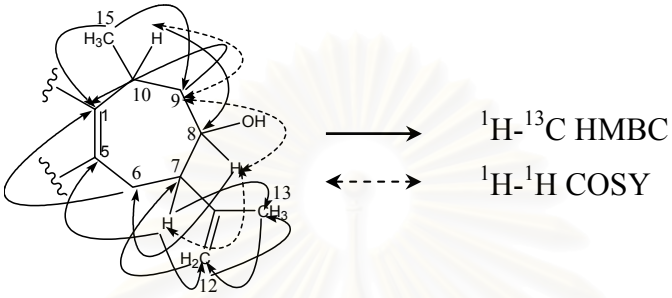
Compound **COC1**, pale yellow oil, possessed a quasimolecular ion $[M+K]^+$ at *m/z* 259.1487 (calcd. 259.1464) in the HRFABMS, corresponding to the molecular formula $C_{15}H_{24}O$. The UV spectrum (**Figure 80**) showed λ_{max} at 206 and 263 nm. The IR spectrum (**Figure 81**) showed ν_{max} 3448 cm^{-1}(OH streching). The optical rotation of compound **COC1** was negative, $[\alpha]^{23}_D$ -65.1° (*c* 0.03, MeOH).

The ^{1}H-NMR spectra (**Figure 82-83**) of compound **COC1** showed signals of one methyl singlet proton at δ_H 1.80 (H_3-13, *s*), two methyl doublet protons at δ_H 0.98 (H_3-14, *d*, *J* = 7.0 Hz) and 1.06 (H_3-15, *d*, *J* = 7.5 Hz), two singlet signals of exocyclic methylene proton at δ_H 4.78 (Ha-12, *s*) and 5.02 (Hb-12, *s*), four methylene multiplet protons at δ_H 1.26-1.32 (Ha-3, *m*), 1.67-1.77 (Ha-6, *m* and Ha-9, *m*), 1.93-2.01 (Hb-3, *m* and Hb-9, *m*), 2.10-2.17 (Ha-2, *m*), 2.43-2.56 (Hb-2, *m* and Hb-6, *m*), three methine multiplet protons at δ_H 2.43-2.56 (H-4, *m* and H-7, *m*) and 3.97-4.01 (H-8, *m*), one methine broad singlet proton at δ_H 2.32 (H-10, *br s*) and a hydroxyl proton at δ_H 1.57 (OH-8, *s*).

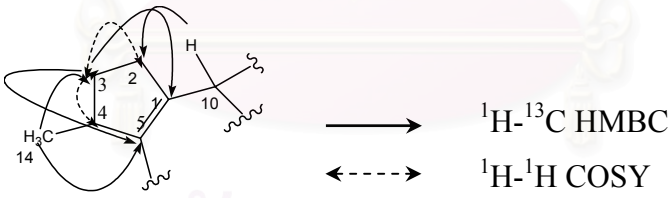
The ^{13}C NMR (**Figure 84**) and DEPT135 (**Figure 85** and **Table 15**) spectra of compound **COC1** revealed the presence of three methyl carbons at δ_C 23.0 (C-13), 20.0 (C-14) and 21.6 (C-15), five methylenes at δ_C 33.8 (C-2), 30.8 (C-3), 26.0 (C-6), 42.0 (C-9) and 112.3 (C-12), four methine carbons at δ_C 46.1 (C-4), 49.7 (C-7), 68.3 (C-8) and 29.2 (C-10) and three quaternary carbons at δ_C 139.6 (C-1), 140.9 (C-5) and 148.0 (C-11).

The ^{1}H-NMR spectral data exhibited resonances of an oxygenated methine proton at δ_H 3.97-4.01 (H-8, *m*) and exocyclic methylene proton at δ_H 4.78 (Ha-12, *s*) and 5.02 (Hb-12, *s*). The HMBC spectrum (**Figure 87**) showed correlations from δ_H 4.78 (Ha-12) and 5.02 (Hb-12) to δ_C 49.7 (C-7) and 23.0 (C-13), from δ_H 2.43-2.56 (H-7) to δ_C 112.3 (C-12), 23.0 (C-13) and 140.9 (C-5), from δ_H 3.97-4.01 (H-8) to δ_C 26.0 (C-6), from δ_H 2.32 (H-10) to δ_C 68.3 (C-8), from δ_H 1.67-1.77 (Ha-6) to δ_C 139.6 (C-1), from δ_H 1.80 (H_3-13) to δ_C 112.3 (C-12) and from δ_H 1.06 (H_3-15) to δ_C 139.6 (C-1) and 42.0 (C-9). The ^{1}H-^{1}H COSY spectrum (**Figure 88**) demonstrated

the cross peaks of methine protons from δ_H 2.43-2.56 (Hb-7) to 3.97-4.01 (H-8), from δ_H 3.97-4.01 (H-8) to 1.67-1.77 (Ha-7) and 1.93-2.01 (Hb-9) and from δ_H 1.67-1.77 (Ha-9) to 2.32 (H-10). Based on these spectral data the first fragment of compound **COC1** is proposed as shown below.

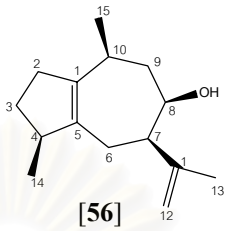


The ^{1}H-^{1}H COSY spectrum (**Figure 88**) of compound **COC1** displayed the correlations between δ_H 1.26-1.32 (Ha-3) to 2.10-2.17 (Ha-2) and 2.43-2.56 (H-4), while the HMBC spectrum (**Figure 87**) of compound **COC1** showed the correlations from δ_H 0.98 (H_3-14) to δ_C 140.9 (C-5) and 30.8 (C-3), from δ_H 2.32 (H-10) to δ_C 33.8 (C-2) and from δ_H 1.26-1.32 (H-3) to δ_C 139.6 (C-1) and 140.9 (C-5), therefore the second fragment of compound **COC1** is assembled as shown.



Combination of the first and the second fragments established a gross structure of compound **COC1**. The relative stereochemistry of compound **COC1** was proven by NOE experiments (**Figure 89**). On irradiation at δ_H 3.97-4.01 (H-8), NOE spectrum was observed on the methine protons resonance at δ_H 2.43-2.56 (H-7), 2.32 (H-10) and the methyl proton at δ_H 1.80 (H_3-13). Additionally, on irradiation at δ_H 0.98 (H_3-14), NOE was observed on the methylene protons resonated at δ_H 2.10-2.17 (H-2a) and 1.26-1.32 (H-3a) and at δ_H 1.06 (H_3-15), NOE was also observed at δ_H 2.10-2.17 (H-2a). The basis of these spectral data, biosynthesis consideration and the literature indicated that the substitutions at those positions were situated in *cis*

orientation to each other. Thus, compound **COC1** was assigned as a hydroxylated derivative of the known α-guaiene (Rakotonirainy *et al*.,1997) and identified as a new compound namely, (4*S**,7*R**,8*R**,10*S**)-8-hydroxy-α-guaiene [**56**].



[**56**]

Table 15 NMR spectral data of compound **COC1** ($CDCl_3$)

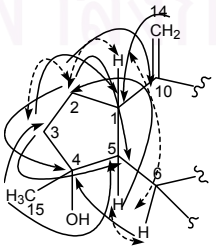
position	Compound **COC1**	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]
1	-	139.6 (C)
2	2.10-2.17 (1Ha, *m*)	33.8 (CH_2)
	2.43-2.56 (1Hb, *m*)	
3	1.26-1.32 (1Ha, *m*)	30.8 (CH_2)
	1.93-2.01 (1Hb, *m*)	
4	2.43-2.56 (1H, *m*)	46.1 (CH)
5	-	140.9 (C)
6	1.67-1.77 (1Ha, *m*)	26.0 (CH_2)
	2.43-2.56 (1Hb, *m*)	
7	2.43-2.56 (1H, *m*)	49.7 (CH)
8	3.97-4.01 (1H, *m*)	68.3 (CH)
9	1.67-1.77 (1Ha, *m*)	42.0 (CH_2)
	1.93-2.01 (1Hb, *m*)	
10	2.32 (1H, *br s*)	29.2 (CH)
11	-	148.0 (C)
12	4.78 (1Ha, *s*)	112.3 (CH_2)
	5.02 (1Hb, *s*)	
13	1.80 (3H, *s*)	23.0 (CH_3)
14	0.98 (3H, *d*, 7.0)	20.0 (CH_3)
15	1.06 (3H, *d*, 7.5)	21.6 (CH_3)
OH-8	1.57 (1H, *s*)	-

[#] Carbon types were deduced from DEPT135 experiment.

3.2 Structure Determination of Compound COC2

Compound **COC2** was obtained as pale yellow oil. The FAB mass spectrum (**Figure 90**) displayed $[M+H]^+$ at *m/z* 221, consistent with $C_{15}H_{24}O$. The IR spectrum (**Figure 91**) showed absorptions at ν_{max} 3384 cm^{-1} (hydroxyl group), 3080 cm^{-1} (CH stretching) and 1458 and 1375 cm^{-1} (CH bending). The ^{1}H-NMR spectrum (**Figure 92**) of compound **COC2** exhibited three singlet protons at δ_H 1.02 (H_3-13), 1.03 (H_3-12) and 1.25 (H_3-15), one exocyclic methylene proton at δ_H 4.64 and 4.67 (1H each, Ha-14 and Hb-14, *s*), four methylene protons at δ_H 0.95-1.00 (Ha-8, *m*), 1.50-1.63 (Ha-2 and Ha-3, *m*), 1.75 (Hb-3, *dd*, *J* = 7.0, 12.5 Hz), 1.84-1.90 (Hb-2, *m*), 1.93-1.99 (Hb-8, *m*), 2.02 (Ha-9, *dd*, *J* = 13.0, 13.0 Hz) and 2.40 (Hb-9, *dd*, *J* = 6.3, 13.0 Hz) and four methine protons at 0.44 (H-6, *dd*, *J* = 9.5, 11.3 Hz), 0.69 (H-7, *ddd*, *J* = 6.0, 9.5, 11.0 Hz), 1.25-1.31 (H-5, *m*) and 2.17 (H-1, *ddd*, *J* = 6.2, 10.6, 10.6 Hz). The ^{13}C-NMR (**Figure 93**) and DEPT135 (**Table 16**) spectra of compound **COC2** showed three methyl, five methylene, four methine and three quaternary carbons, one of which carried a hydroxyl group.

The HMBC spectrum of compound **COC2** (**Figure 95**) showed the correlations from δ_H 1.75 (Hb-3), 4.64 (Ha-14), 4.67 (Hb-14) to δ_C 53.4 (C-1), from δ_H 1.25-1.31 (H-5) to δ_C 26.7 (C-2), from δ_H 1.25 (H_3-15) to δ_C 41.7 (C-3), from δ_H 0.44 (H-6) and 1.84-1.90 (Hb-2) to δ_C 80.9 (C-4), from δ_H 1.75 (Hb-3), 1.50-1.63 (Ha-2) and 1.25 (H_3-15) to δ_C 54.2 (C-5) and from δ_H 1.84-1.90 (Hb-2) to δ_C 153.3 (C-10), while ^{1}H-^{1}H COSY spectrum of compound **COC2** (**Figure 96**) showed cross peaks from δ_H 1.84-1.90 (Hb-2) to 1.75 (Hb-3) and 2.17 (H-1) and from δ_H 1.25-1.31 (H-5) to 0.44 (H-6) and 2.17 (H-1). Assignment of the first substructure was constructed as shown.

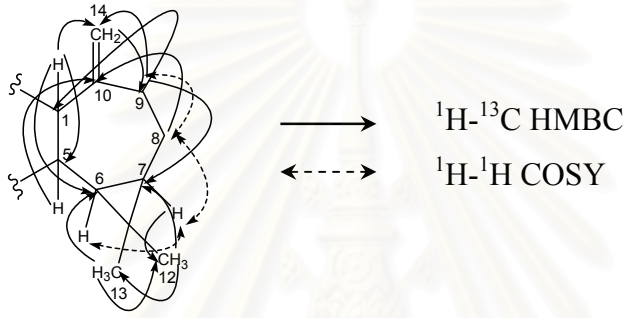


^{1}H-^{13}C HMBC

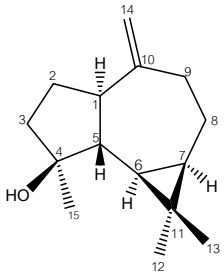
^{1}H-^{1}H COSY

The HMBC spectrum (**Figure 95**) were observed from δ_H 2.40 (Hb-9) to δ_C 53.4 (C-1), from δ_H 2.17 (H-1) to δ_C 54.2 (C-5) and 29.9 (C-6), from δ_H 1.02 (H_3-

13) to δ_C 28.6 (C-12), from 1.03 (H_3-12), 2.02 (Ha-9) and 2.40 (Hb-9) to δ_C. 27.4 (C-7), from δ_H 2.17 (H-1) and 2.02 (Ha-9) to δ_C 106.2 (C-14), from δ_H 4.64 (Ha-14) and 4.67 (Hb-14) to δ_C 38.8 (C-9), from δ_H 0.95-1.00 (Ha-8), 1.25-1.31 (H-5) and 1.93-1.99 (Hb-8) to δ_C 153.3 (C-10), while ^{1}H-^{1}H COSY spectrum (**Figure 96**) exhibited the correlation of δ_H 0.69 (H-7) to 0.44 (H-6), δ_H 0.95-1.00 (Ha-8), 1.93-1.99 (Hb-8) and δ_H 0.95-1.00 (Ha-8) to 2.02 (Ha-9) and 2.40 (Hb-9). Based upon these spectral data, the second partial structure of compound **COC2** was established as shown.



The relative stereochemistry of compound **COC2** was detected by NOE difference technique (**Figure 97**). On irradiation at the methine proton resonance H-6 (δ_H 0.44), NOE was observed on the H-1 (δ_H 2.17), H-7 (δ_H 0.69), H_3-12 (δ_H 1.03) and H_3-15 (δ_H 1.25). When the methyl proton resonance H_3-12 (δ_H 1.03) was irradiated, NOE was observed on the methine proton resonance H-1 (δ_H 2.17), H-6 (δ_H 0.44) and H-7 (δ_H 0.69). Moreover, the methyl proton resonance H_3-13 (δ_H 1.02) was irradiated, NOE was observed on the methine proton resonance H-5 (δ_H 1.25-1.31). Thus, the configuration at the junction between the five and seven-membered rings was deduced to be *trans* configuration. By analysis of the above spectroscopic data and comparison with reported data (Inagaki and Abe, 1985), compound **COC2** was determined as spathulenol [**57**], an aromadendrane sesquiterpene previously isolated from several plants eg. *Panax ginseng* (Iwabuchi, Yoshikura and Kamisako, 1989) and *Citrus junos* (Inagaki and Abe, 1985)



[**57**]

Table 16 NMR spectral data of compound **COC2** and spathulenol ($CDCl_3$)

position	Compound **COC2**		Spathulenol	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	2.17 (1H, *ddd*, 6.2, 10.6, 10.6)	53.4 (CH)	2.20 (1H)	53.4
2	1.50-1.63 (1Ha, *m*)	26.7 (CH_2)	1.64 (1Ha)	26.7
	1.84-1.90 (1Hb, *m*)		1.91 (1Hb)	
3	1.50-1.63 (1Ha, *m*)	41.7 (CH_2)	1.54 (1Ha)	41.8
	1.75 (1Hb, *dd*, 7.0, 12.5)		1.78 (1Hb)	
4	-	80.9 (C)	-	80.9
5	1.25-1.31 (1H, *m*)	54.2 (CH)	1.31 (1H)	53.4
6	0.44 (1H, *dd*, 9.5, 11.3)	29.9 (CH)	0.46 (1H)	30.0
7	0.69 (1H, *ddd*, 6.0, 9.5, 11.0)	27.4 (CH)	0.71 (1H)	27.7
8	0.95-1.00 (1Ha, *m*)	24.7 (CH_2)	1.01 (1Ha)	24.9
	1.93-1.99 (1Hb, *m*)		1.96 (1Hb)	
9	2.02 (1Ha, *dd*, 13.0, 13.0)	38.8 (CH_2)	2.04 (1Ha, *m*)	39.0
	2.40 (1Hb, *dd*, 6.3, 13.0)		2.42 (1Hb, *m*)	
10	-	153.3 (C)	-	153.5
11	-	20.2 (C)	-	20.3
12	1.03 (3H, *s*)	28.6 (CH_3)	1.05 (3H)	28.7
13	1.02 (3H, *s*)	16.3 (CH_3)	1.04 (3H)	16.4
14	4.64 (1Ha, *s*)	106.2 (CH_2)	4.66 (1Ha, *s*)	106.3
	4.67 (1Hb, *s*)		4.69 (1Hb, *s*)	
15	1.25 (3H, *s*)	26.0 (CH_3)	1.28 (3H, *s*)	26.1
OH-8	1.41 (1H, *brs*)	-	-	-

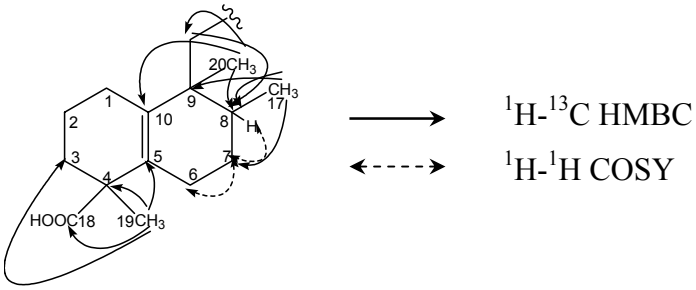
[#] Carbon types were deduced from DEPT135 experiment.

3.3 Structure Determination of Compound COC3

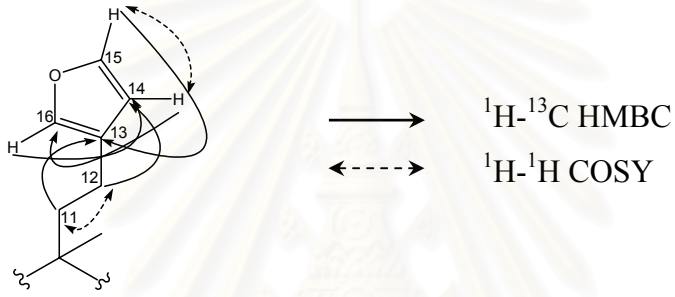
Compound **COC3** was obtained as pale yellow oil. The HRFABMS spectrum displayed the protonated molecular ion $[M+H]^+$ at *m/z* 317.2108 (calcd. 317.2117), consistent with $C_{20}H_{28}O_3$. The UV absorption bands (**Figure 99**) appeared at λ_{max} 299 nm. The IR absorption spectrum (**Figure 100**) showed ν_{max} at 3600-2400 and 1699 (carboxylic acid) cm^{-1}. The optical rotation provided negative, $[\alpha]^{23}_D$ -23.2° (*c* 0.0013, MeOH).

The ^{1}H-NMR spectra (**Figure 101-102**) of compound **COC3** displayed signals of two methyl singlets at δ_H 1.30 (3H, *s*) and 0.86 (3H, *s*), one methyl doublet at δ_H 0.87 (3H, *d*, *J* = 7.0 Hz), seven methylene multiplets at δ_H 1.34-1.44 (Ha-6, *m*), δ_H 1.50-1.56 (H_2-7, *m*), 1.64-1.69 (Ha-3, *m* and H_2-11, *m*), 1.74-1.81 (H_2-2, *m*), 1.89-2.02 (Ha-1, *m*, Hb-3, *m* and Hb-6, *m*), 2.07-2.17 (Hb-1, *m* and Ha-12, *s*) and 2.33-2.40 (Hb-12, *s*), one methine multiplet at δ_H 1.78-1.81 (1H, *m*) and three olefenic protons at δ_H 6.36, 7.20 and 7.34 (each 1H, H-14, H-16 and H-15)

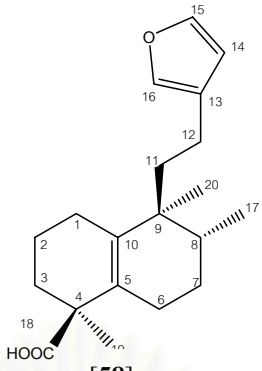
The ^{13}C-NMR (**Figure 103**) and DEPT135 (**Figure 104** and **Table 17**) spectral data of compound **COC3** revealed 20 signals as three methyl carbons , seven methylene carbons, four methine carbons and six quaternary carbons. A carbonyl group was found in **COC3**, as one singlet resonance at δ_C 181.3 (C-18). In addition, HMBC spectral data (**Figure 106-107**) demonstrated the correlations from δ_H 1.30 (H_3-19) to δ_C 35.4 (C-3), 47.4 (C-4), δ_C 131.0 (C-5) and 183.1 (C-18), from δ_H 0.86 (H_3-20) to δ_C 33.3 (C-8), 36.5 (C-11) and 136.0 (C-10), from δ_H 0.87 (H_3-17) to δ_C 26.8 (C-7) and 40.9 (C-9) and from δ_H 1.64-1.69 (H_2-11) to δ_C 33.3 (C-8). The ^{1}H-^{1}H COSY spectrum (**Figure 108**) showed the cross peaks of H_2-7 (δ_H 1.50-1.56) to Ha-6 (δ_H 1.34-1.44) and H-8 (δ_H 1.74-1.81). Based on these data the first substructure of compound **COC3** was assembled as shown.



The HMBC spectra (**Figure 106-107**) displayed the correlations from H_2-11 (δ_H 1.64-1.69) to C-13 (δ_C 125.8), from Hb-12 (δ_H 2.33-2.40) to C-14 (δ_C 111.0) and C-16 (δ_C 138.4), from H-14 (δ_H 6.26) to C-16 (δ_C 138.4), from H-15 (δ_H 7.34) to C-13 (δ_C 125.8) and from H-16 (δ_H 7.20) to C-14 (δ_C 111.0), while ^{1}H-^{1}H COSY spectrum (**Figure 108**) of compound **COC3** revealed the correlations between Hb-12 (δ_H 2.33-2.40) and H_2-11 (δ_H 1.64-1.69) and between H-14 (δ_H 6.26) and H-15 (δ_H 7.34). The construction of the second partial structure was analysed by the above spectral data.



Combination of the first and the second fragments established a gross structure of **COC3**. The relative stereochemistry of compound **COC3** could not be completely established by application of NOE experiments. However, it would be reasonable deduced that three methyl groups at C-17 (δ_C 16.0), C-19 (δ_C 22.9) and C-20 (δ_C 20.8) in *cis* orientation because of the biogenesis considerations and the agreement of the crystal structure of compound **COC10**, chettaphanin I [**48**]. Therefore, compound **COC3** was identified as a new compound, 5-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-1-carboxylic acid [**58**] and has been given the trivial name as chettaphanin III. The structurally related crotohalimaneic acid, a 4-epimer of compound **COC10**, had been isolated as a natural product from *Croton oblongifolius* (Roengsumran *et al*., 2004)



[**58**]

Table 17 NMR spectral data of compound **COC3** ($CDCl_3$)

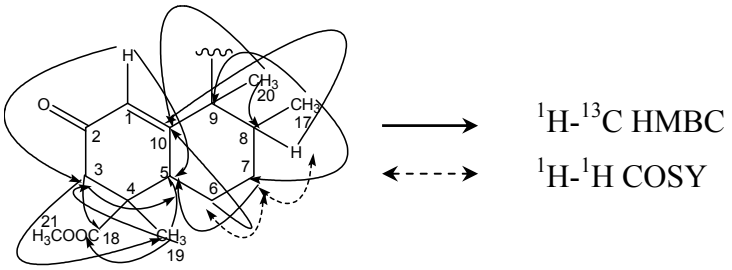
position	Compound **COC3**	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]
1	1.89-2.02 (1Ha, *m*)	25.1 (CH_2)
	2.07-2.17 (1Hb, *m*)	
2	1.74-1.81 (2H, *m*)	19.5 (CH_2)
3	1.64-1.69 (1Ha, *m*)	35.4 (CH_2)
	1.89-2.02 (1Hb, *m*)	
4	-	47.4 (C)
5	-	131.0 (C)
6	1.34-1.44 (1Ha, *m*)	25.9 (CH_2)
	1.89-2.02 (1Hb, *m*)	
7	1.50-1.56 (2H, *m*)	26.8 (CH_2)
8	1.74-1.81 (1H, *m*)	33.3 (CH)
9	-	40.9 (C)
10	-	136.0 (C)
11	1.64-1.69 (2H, *m*)	36.5 (CH_2)
12	2.07-2.17 (1Ha, *s*)	19.5 (CH)
	2.33-2.40 (1Hb, *s*)	
13	-	125.8 (C)
14	6.26 (1H, *dd*, 0.8, 0.8)	111.0 (CH)
15	7.34 (1H, *dd*, 1.5, 1.5)	142.6 (CH)
16	7.20 (1H, *s*)	138.4 (CH)
17	0.87 (3H, *d*, 7.0)	16.0 (CH_3)
18	-	183.1 (C)
19	1.30 (3H, *s*)	22.9 (CH_3)
20	0.86 (3H, s)	20.8 (CH_3)

[#] Carbon types were deduced from DEPT135 experiment.

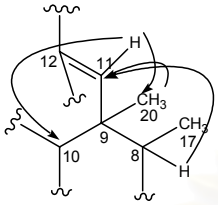
3.4 Structure Determination of Compound COC4

Compound **COC4** was obtained pale yellow oil. The molecular formula was determined as $C_{21}H_{24}O_5$ by HRFABMS spectrum of its $[M+H]^+$ at *m/z* 357.1685 (calcd 357.1702). The IR spectrum (**Figure 111**) showed absorption bands due to a keto carbonyl group (1676 cm^{-1}), an ester carbonyl (1736 and 1277 cm^{-1}) and a furan ring (3150, 1458, 920 cm^{-1}) and the UV absorption at 239 nm (**Figure 110**). The optical rotation was positive, $[\alpha]^{23}_D$ + 56.1° (*c* 0.015, MeOH).

The ^{1}H-NMR spectra (**Figure 112-113**) showed signals for four methyl protons at δ_H 0.88 (H_3-17, *d*, *J* = 7 Hz), 1.17 (H_3-20, *s*), 1.42 (H_3-19, *s*) and 3.54 (H_3-21, *s*), three methylene protons at δ_H 1.37-1.41 (Ha-7, *m*), 1.89 (Ha-6, *dd*, *J* = 4.8, 13.3 Hz), 2.12-2.20 (Hb-7, *m*), 2.34 (Hb-6, *dd*, *J* = 4.8, 13.3 Hz), 2.38 (Ha-3, *d*, *J* = 16.3 Hz) and 2.39 (Hb-3, *d*, *J* = 16.3 Hz), six methine protons, three protons of which at δ_H 6.40, 7.33 and 7.47 (1H each, H-14, H-15 and H-16) were characteristic of furan proton and the other protons at δ_H 1.92-1.97 (H-8, *m*), 4.80 (H-11, *s*) and 5.90 (H-1, *s*). The ^{13}C-NMR (**Figure 114**) and DEPT135 (**Figure 115** and **Table 18**) spectra showed four methyl carbons, three methylene carbons, six methine carbons and eight quaternary carbons. The HMBC spectra (**Figure 117-118**) showed the correlations from δ_H 2.12-2.20 (Hb-7) to δ_C 79.5 (C-5), from δ_H 5.90 (H-1) to δ_C 45.5 (C-3), 79.5 (C-5), from δ_H 2.38 (Ha-3) and 2.39 (Hb-3) to δ_C 20.1 (C-19), 79.5 (C-5) and 173.9 (C-18), from δ_H 1.42 (H_3-19) to δ_C 45.5 (C-3), 79.5 (C-5) and 173.9 (C-18), from δ_H 1.17 (H_3-20), 1.95 (H-8) and 2.34 (H-6) to δ_C 157.7 (C-10) and from δ_H 0.88 (H_3-17) to δ_C 26.5 (C-7) and 41.4 (C-9). The ^{1}H-^{1}H COSY spectrum (**Figure 119**) displayed the correlations from δ_H 2.12-2.20 (Hb-7) to 1.92-1.97 (H-8) and 1.89 (Ha-6). Based on these spectral data the first substructure of compound **COC4** is proposed as shown below.

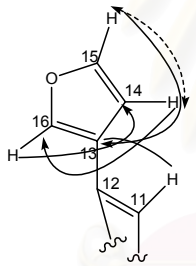


The HMBC spectra (**Figure 117-118**) showed the correlations from δ_H 4.80 (H-11) to δ_C 157.7 (C-10) and 22.3 (C-20) and from δ_H 1.17 (H_3-20) and 1.95 (H-8) to δ_C 103.2 (C-11), therefore the second fragment of compound **COC4** is assembled as shown.



⟶ ^{1}H-^{13}C HMBC

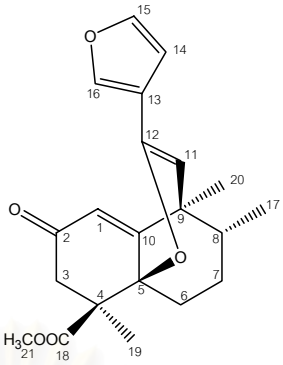
The ^{1}H-^{1}H COSY spectrum of compound **COC4** (**Figure 119**) displayed a correlation between δ_H 6.40 (H-14) and 7.33 (H-15), while the HMBC spectra (**Figure 117-118**) showed the correlations from δ_H 6.40 (H-14) to δ_C 139.5 (C-16), from δ_H 7.33 (H-15) and 4.80 (H-11) to δ_C 121.3 (C-13) and from δ_H 7.47 (H-16) to δ_C 107.2 (C-14). Therefore the third partial structure is created as shown.



⟶ ^{1}H-^{13}C HMBC

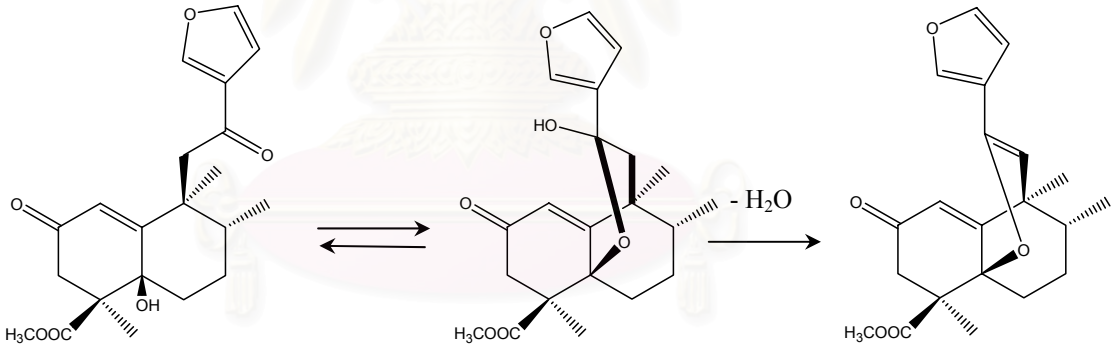
⟷ ^{1}H-^{1}H COSY

The combination of the three fragments established a gross structure of compound **COC4**. The NOE experiments (**Figure 120**) indicated interactions of H_3-17 with H_3-20 and H-1, H_3-21 with H-16. The agreement of the spectroscopic data and NOE interactions and biogenesis consideration led us to assign the structure of compound **COC4** including the relative configuration. This is a new compound, methyl 9-(furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo[5.3.3.$0^{1,6}$]trideca-5,8-di-ene-2-carboxylate [**59**] and has been named chettaphanin IV.



[**59**]

To our knowledge, it is reasonable to suppose that the ether bridge between C-5 (δ_C 79.5) and C-12 (δ_C 146.2) in compound **COC4** could be built up by intramolecular hemiacetal formation of the 12-keto group with a *cis*-oriented OH-5 group, as in compound A, the C-5 epimer of compound **COC10** (chettaphanin I), followed by dehydration as shown in **Figure 121**. However, compound A has not been isolated until now.



compound A compound **COC4**

Figure 121 Possible formation of compound **COC4** from compound A, the C-5 epimer of chettaphanin I.

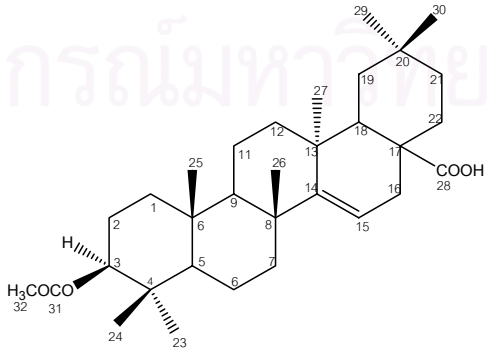
Table 18 NMR spectral data of compound **COC4** ($CDCl_3$)

position	Compound **COC4**	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]
1	5.90 (1H, *s*)	121.8 (CH)
2	-	196.4 (C)
3	2.38 (1Ha, *d*, 16.3)	45.5 (CH_2)
	2.39 (1Hb, *d*, 16.3)	
4	-	51.4 (C)
5	-	79.5 (C)
6	1.89 (1Ha, *dd*, 4.8, 13.3)	31.7 (CH_2)
	2.34 (1Hb, *dd*, 4.8, 13.3)	
7	1.37-1.41 (1Ha, *m*)	26.5 (CH_2)
	2.12-2.20 (1Hb, *m*)	
8	1.92-1.97 (1H, *m*)	42.5 (CH)
9	-	41.4 (C)
10	-	157.7 (C)
11	4.80 (1H, *s*)	103.2 (CH)
12	-	146.2 (C)
13	-	121.3 (C)
14	6.40 (1H, *dd*, 0.8, 0.8)	107.2 (CH)
15	7.33 (1H, *dd*, 1.8, 1.8)	143.2 (CH)
16	7.47 (1H, *d*, 1.0)	139.5 (CH)
17	0.88 (3H, *d*, 7.0)	14.3 (CH_3)
18	-	173.9 (C)
19	1.42 (3H, *s*)	20.1 (CH_3)
20	1.17 (3H, s)	22.3 (CH_3)
21	3.54 (3H, *s*)	52.1 (CH_3)

[#] Carbon types were deduced from DEPT135 experiment.

3.5 Structure Determination of Compound COC5

Compound **COC5**, white solid with m.p. 297-299 °C, showed a protonated molecular ion $[M+H]^+$ at *m/z* 499 in FAB mass spectrum (**Figure 122**), corresponding to molecular formula $C_{32}H_{50}O_4$. The IR spectrum showed absorption bands of carboxylic group (1687, 3423 cm^{-1}), ester carbonyl group (1736, 1244 cm^{-1}) and hydrocarbon group (2937, 2856, 1458, 1365 cm^{-1}) (**Figure 123**). The ^{1}H-NMR spetrum (**Figure 124**) showed signals for a vinyl proton at δ_H 5.43 (H-15) and a methine proton at 4.46 (H-3). Signals for seven methyl protons at δ_H 0.85-0.96, one carbomethyl proton at δ_H 2.04, ten methylene and three methine protons at δ_H 1.03-1.99 were observed. The ^{13}C-NMR (**Figure 125**) and DEPT135 (**Figure 126** and **Table 19**) spectra displayed 32 carbon signals, including eight methyl carbons at δ_C 15.6, 16.6, 21.3, 22.4, 26.2, 27.9, 28.6 and 31.8, ten methylene carbons at δ_C 17.3, 18.7, 23.5, 30.7, 31.3, 33.3, 33.7, 35.3, 37.4 and 40.7, five methine carbons at δ_C 41.4, 49.1, 55.6, 80.9 and 116.9, nine quaternary carbons at δ_C 29.3, 37.3, 37.7, 37.9, 39.0, 51.5, 160.5, 171.0 and 184.3. The ^{13}C-NMR chemical shifts of C-22 and C-29 of this compound were assigned as δ_C 33.3 and 31.8, respectively, since these appeared as methylene and methyl carbon in DEPT135 experiment. In previous report (Carpenter *et al*., 1980), the ^{13}C-NMR chemical shifts of C-22 and C-29 of acetoxyaleuritolate were assigned as δ_C 31.8 and 33.3, respectively, these assignments were transposed. From all of the above spectroscopic data in comparison with reported data, compound **COC5** was assigned as acetoxyaleuritolate [**60**]. This compound had been isolated from other Euphorbiaceae family such as *Panadenia thwaitesii* (Carpenter *et al*., 1980) and *Sapium baccatum* (Ray, Misra and Khastgir, 1975).



[**60**]

Table 19 NMR spectral data of compound **COC5** and acetoxyaleuritolate ($CDCl_3$)

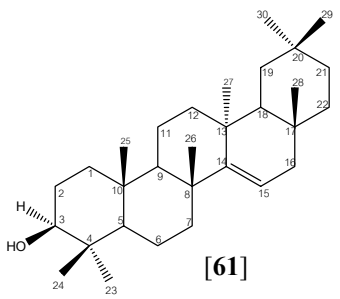
position	Compound **COC5**		Acetoxyaleuritolate
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]	δ_C (ppm)
1	1.55-1.85 (2H, *m*)	37.4 (CH_2)	37.4
2	1.55-1.85 (2H, *m*)	23.5 (CH_2)	23.4
3	4.46 (1H, *dd*, *J* = 5.5, 10.0)	80.9 (CH)	80.8
4	-	37.7 (C)	37.6
5	0.85-0.95 (1H, *m*)	55.6 (CH)	55.6
6	1.55-1.85 (2H, *m*)	18.7 (CH_2)	18.7
7	1.00-1.35 (2H, *m*)	35.3 (CH_2)	35.3
8	-	39.0 (C)	39.0
9	1.40-1.55 (1H, *m*)	49.1 (CH)	49.0
10	-	37.3 (C)	37.3
11	1.40-1.55 (2H, *m*)	17.3 (CH_2)	17.3
12	1.90-2.00 (1Ha, *m*) 2.37 (1Hb, *m*)	31.3 (CH_2)	31.2
13	-	37.9 (C)	37.9
14	-	160.5 (C)	160.5
15	5.54 (1H, *dd*, *J* = 3.3, 7.8)	116.9 (CH)	116.8
16	1.40-1.85 (2H, *m*)	30.7 (CH_2)	30.6
17	-	51.5 (C)	51.5
18	2.27 (1H, *dd*, 3.3, 14.3)	41.4 (CH)	41.3
19	1.90-2.00 (2H, *m*)	40.7 (CH_2)	40.7
20	-	29.3 (C)	29.3
21	1.00-1.35 (2H, *m*)	33.7 (CH_2)	33.6
22	1.55-1.85 (2H, *m*)	**33.3** (CH_2)	**31.8**
23	0.85 (3H, *s*)	27.9 (CH_3)	27.9
24	0.89 (3H, *s*)	16.6 (CH_3)	16.6
25	0.96 (3H, *s*)	15.6 (CH_3)	15.7
26	0.91 (3H, *s*)	28.6 (CH_3)	28.6
27	0.96 (3H, *s*)	26.2 (CH_3)	26.2
28	-	184.3 (C)	184.4
29	0.94 (3H, *s*)	**31.8** (CH_3)	**33.3**
30	0.92 (3H, *s*)	22.4 (CH_3)	22.4
31	-	171.0 (C)	-
32	2.04 (3H, *s*)	21.3 (CH_3)	-

The bold values are revised assignments.

[#] Carbon types were deduced from DEPT135 experiment.

3.6 Structure Determination of Compound COC6

Compound **COC6**, white solid with m.p. 281-282 °C, showed a protonated molecular ion $[M+H]^+$ at *m/z* 427 in FAB mass spectrum (**Figure 127**), corresponding to the molecular formula $C_{30}H_{50}O$. The IR spectrum (**Figure 128**) showed absorption at ν_{max} 3483 (hydroxy group) and 2933, 2852, 1473 and 1385 (hydrocarbon system) cm^{-1}. The ^{1}H-NMR spectrum (**Figure 129**) displayed signals for a vinyl proton at δ_H 5.55 (H-15) and a carbinol proton at δ_H 3.20 (H-3). Signals for eight methyl protons between δ 0.79-1.11, ten methylene and three methine protons at δ_H 0.93-2.06. The ^{13}C-NMR (**Figure 130**) and DEPT135 (**Figure 131** and **Table 20**) spectra showed 30 carbon signals, corresponding to eight methyl carbons at δ_C 15.4, 15.5, 21.4, 25.9, 28.1, 29.9, 30.0 and 33.4, ten methylene carbons at δ_C 17.6, 18.9, 27.3, 33.3, 33.9, 35.4, 36.8, 37.8, 37.9 and 41.5, five methine carbons at δ_C 49.1, 49.5, 55.7, 79.1 and 117.0 and seven quaternary carbons at δ_C 28.9, 35.8, 37.7, 38.1, 38.9, 39.1 and 158.3. The ^{1}H-NMR spectra of taraxerol and isotaraxerol showed the expected differences in the carbinol proton region. The H-3 in isotaraxerol appeared as a well defined triplet center at δ_H 3.38 (J = 3.0 Hz), typical of an equatorial proton associated with 3α-hydroxy group in ring A of triterpene, whereas the H-3 in taraxerol appeared as ill-defined quartet (δ_H 3.22), typical of the axial proton associated with a 3β-hydroxy group. The melting point of taraxerol was 282-283 °C whereas that of isotaraxerol was 267-269 °C (Corbett and Cumming, 1972). The ^{13}C-NMR chemical shifts of C-10 and C-12 of compound **COC6** were assigned as δ_C 35.8 and 37.8, respectively, since these appeared as quaternary and methylene carbons in DEPT135 experiment. In previous report (Sakurai, Yaguchi and Inoue, 1987), the ^{13}C-NMR chemical shifts of C-10 and C-12 of taraxerol were assigned as δ_C 37.9 and 35.9, respectively. Thus, these assignments were transposed. Compound **COC6** was identified as taraxerol [**61**] by analysis of the above spectra data and confirmed by comparison with an authentic sample. This compound was obtained previously from *Myrica rubra* (Sakurai, Yaguchi and Inoue, 1987).



[**61**]

Table 20 NMR spectral data of compound **COC6** and taraxerol ($CDCl_3$)

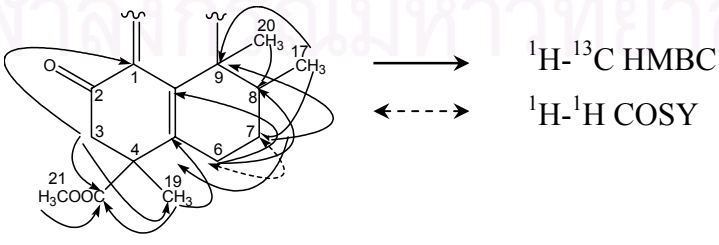
position	Compound **COC6**		Taraxerol
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]	δ_C (ppm)
1	0.93-1.10 (1Ha, *m*)	37.9 (CH_2)	38.1
	1.25-2.06 (1Hb, *m*)		
2	1.25-1.67 (2H, *m*)	27.3 (CH_2)	27.3
3	3.20 (1H, *q*, *J* = 5.5)	79.1 (CH)	79.2
4	-	39.1 (C)	39.1
5	0.79-0.84 (1H, *m*)	55.7 (CH)	55.7
6	1.25-1.67 (2H, *m*)	18.9 (CH_2)	19.0
7	0.93-1.10 (1Ha, *m*)	35.4 (CH_2)	35.3
	1.25-1.67 (1Hb, *m*)		
8	-	38.9 (C)	38.3
9	0.93-1.10 (1H, *m*)	49.5 (CH)	48.9
10	-	**35.8** (C)	**37.9**
11	1.25-1.67 (2H, *m*)	17.6 (CH_2)	17.7
12	1.25-1.67 (1Ha, *m*)	**37.8** (CH_2)	**35.9**
	1.93 (1Hb, *brd*, *J* = 14.5)		
13	-	37.7 (C)	37.9
14	-	158.3 (C)	158.1
15	5.55 (1H, *dd*, *J* = 3.0, 8.0)	117.0 (CH)	117.0
16	0.93-1.10 (1Ha, *m*)	36.8 (CH_2)	36.9
	1.25-1.67 (1Hb, *m*)		
17	-	38.8 (C)	38.1
18	1.25-1.67 (1H, *m*)	49.5 (CH)	49.4
19	1.25-1.67 (1Ha, *m*)	41.5 (CH_2)	41.4
	2.05 (1Hb, *brd*, *J* = 12.5)		
20	-	28.9 (C)	29.0
21	1.25-1.67 (2H, *m*)	33.9 (CH_2)	33.9
22	1.25-1.67 (2H, *m*)	33.3 (CH_2)	32.2
23	0.93-1.10 (3H)	28.1 (CH_3)	28.1
24	0.79-0.84 (3H)	15.5 (CH_3) [a]	15.6
25	0.93-1.10 (3H)	15.4 (CH_3) [a]	15.6
26	0.79-0.84 (3H)	29.9 (CH_3) [b]	30.1
27	1.11 (3H)	25.9 (CH_3)	26.0
28	0.93-1.10 (3H)	30.0 (CH_3) [b]	30.1
29	0.93-1.10 (3H)	33.4 (CH_3)	33.5
30	0.93-1.10 (3H)	21.4 (CH_3)	21.5

[a,b] Assignment may be interchanged. The bold values are revised assignments.

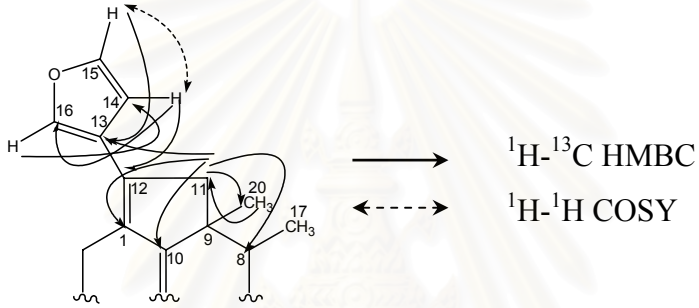
[#] Carbon types were deduced from DEPT135 experiment.

3.7 Structure Determination of Compound COC7

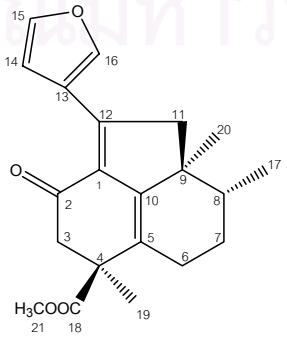
Compound **COC7** was characterized as yellow solid, with m.p. 127-128 °C. The FAB mass spectrum (**Figure 132**) demonstrated $[M+H]^+$ at *m/z* 341, harmonizing with the molecular formula $C_{21}H_{24}O_4$. The UV spectrum (**Figure 133**) showed absorption maxima at 242 and 294 nm. The IR absorption spectrum (**Figure 134**) displayed ν_{max} at 1722 and 1280 (ester group), 1682 (carbonyl group) and 3167, 1576 and 816 (furan ring) cm^{-1}. The ^{1}H-NMR spectrum (**Figure 135**) showed signals for β-substituted of furan ring proton at δ_H 7.00, 7.42 and 8.57 (H-14, H-15 and H-16). The ester methyl proton gave rise to a singlet at δ_H 3.57 (H-21, *s*). Singlets at δ_H 0.99 (H_3-20, *s*) and 1.38 (H_3-19, *s*) and doublet at δ_H 0.97 (H_3-17, *d*, J = 6.0 Hz) demonstrated the presence of three methyl groups. The ^{13}C-NMR (**Figure 136**) and DEPT135 (**Table 21**) spectra exhibited 21 carbon signals, corresponding to four methyl carbons, four methylene carbons, four methine carbons and nine quaternary carbons that included a keto carbonyl (δ_C 195.1, C-2) and an ester carbonyl carbons (δ_C 174.6, C-18). The HMBC spectrum (**Figure 138**) showed the correlations from δ_H 2.45 (Ha-3) and 2.82 (Hb-3) to δ_C 22.3 (C-19), 128.0 (C-1) and 174.6 (C-18), from δ_H 3.57 (H_3-21) to δ_C 174.6 (C-18), from δ_H 1.38 (H_3-19) to δ_C 125.1 (C-5) and δ_C 174.6 (C-18), from δ_H 2.20-2.38 (H_2-6) to δ_C 37.1 (C-8) and 150.4 (C-10), from δ_H 1.50-1.73 (H_2-7) to δ_C 125.1 (C-5) and 42.4 (C-9), from δ_H 0.97 (H_3-17) to δ_C 27.1 (C-7) and 42.4 (C-9) and from δ_H 0.99 (H_3-20) to δ_C 37.1 (C-8). The ^{1}H-^{1}H COSY spectrum (**Figure 138**) exhibited the correlation between H_2-6 (δ_H 2.20-2.38) and H_2-7 (δ_H 1.50-1.73). These spectral data assisted the construction of the first partial structure of compound **COC7** as shown.



The HMBC spectrum of compound **COC7** (**Figure 138**) revealed the correlations from δ_H 2.65 (Ha-11) and 2.72 (Hb-11) to δ_C 20.3 (C-20), 37.1 (C-8), 121.9 (C-13), 128.0 (C-1) and 150.4 (C-10), from δ_H 0.99 (H_3-20) to δ_C 50.3 (C-11), from δ_H 7.00 (H-14) to δ_C 139.7 (C-12) and 146.3 (C-16), from δ_H 7.42 (H-15) to δ_C 121.9 (C-13) and from δ_H 8.57 (H-16) to δ_C 111.0 (C-14), while the ^{1}H-^{1}H COSY (**Figure 139**) showed a correlation between δ_H 7.00 (H-14) and 7.42 (H-15). Combination of these fragments established a gross structure of compound **COC7** as shown below.



The stereochemistry of compound **COC7** had been presumed by biosynthesis considerations, the X-ray crystallography of its derivative (Sato *et al*., 1971). The absolute configuration had been established by its chemical correlation to *ent*-halimic acid, a bicyclic diterpene with a known absolute configuration (Marcos *et al*., 2002). Based on the spectroscopic data, stereochemical information and comparison with the previous report (Marcos *et al*., 2002), compound **COC7** was identified as chettaphanin II [**49**], which is a known compound previously isolated from the root of *Adenocleana siamensis* (*Cladogynos orientalis*) (Sato *et al*., 1971).



[**49**]

Table 21 NMR spectral data of compound **COC7** and chettaphanin II [**49**] ($CDCl_3$)

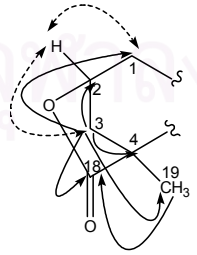
position	Compound **COC7**		Chettaphanin II [**49**]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)[#]	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	-	128.0 (C)	-	127.9
2	-	195.1 (C)	-	195.1
3	2.82 (1Ha, *d*, 15.8)	52.1 (CH_2)	2.81 (1Ha, *d*, 15.7)	52.1
	2.45 (1Hb, *d*, 15.8)		2.45 (1Hb, *d*, 15.7)	
4	-	48.5 (C)	-	48.4
5	-	125.1 (C)	-	125.1
6	2.20-2.38 (2H, *m*)	23.7 (CH_2)	2.37 (1Ha, *ddd*, 6.2, 10, 18)	23.7
			2.25 (1Hb, *ddd*, 9.2, 1.0, 18)	
7	1.50-1.73 (2H, *m*)	27.1 (CH_2)	1.58-1.66 (2H, *m*)	27.0
8	1.50-1.73 (1H, *m*)	37.1 (CH)	1.56-1.59 (1H, *m*)	37.1
9	-	42.4 (C)	-	42.4
10	-	150.4 (C)	-	150.3
11	2.65 (1Ha, *d*, 16.8)	50.3 (CH_2)	2.66 (1Ha, *d*, 16.9)	50.3
	2.72 (1Hb, *d*, 16.8)		2.71 (1Hb, *d*, 16.9)	
12	-	139.7 (C)	-	139.7
13	-	121.9 (C)	-	121.8
14	7.00 (1H, *d*, 1.5)	111.0 (CH)	7.00 (1H, *s*)	111.0
15	7.42 (1H, *dd*, 0.75, 1.5)	142.7 (CH)	7.43 (1H, *s*)	142.7
16	8.57 (1H, *s*)	146.3 (CH)	8.57 (1H, *s*)	146.3
17	0.97 (3H, *d*, 6.0)	16.4 (CH_3)	0.97 (3H, *d*, 6.2)	16.4
18	-	174.6 (C)	-	174.6
19	1.38 (3H, *s*)	22.3 (CH_3)	1.38 (3H, *s*)	22.3
20	0.99 (3H, *s*)	20.3 (CH_3)	0.99 (3H, *s*)	20.3
21	3.57 (3H, *s*)	52.3 (CH_3)	3.57 (3H, *s*)	52.3

[#] Carbon types were deduced from DEPT135 experiment.

3.8 Structure Determination of Compound COC8

Compound **COC8** was isolated as pale yellow oil. The HRFABMS spectrum exhibited $[M+H]^+$ at *m/z* 315.1990 (calcd. 315.1690), indicating a molecular formula of $C_{20}H_{26}O_3$. The optical rotation was negative, $[\alpha]^{23}_D$ – 88.6° (*c* 0.0017, MeOH). The IR absorption spectrum (**Figure 142**) revealed at ν_{max} 3124, 1459 and 873 (furan ring), 1773 and 1290 (ester carbonyl group) cm^{-1} and the UV absorption at 204 nm (**Figure 141**). The ^{1}H-NMR spectra (**Figure 143-144**) were showed one secondary methyl protons at δ_H 0.88 (H_3-17, *d*, *J* = 7.0 Hz) and two tertiary methyl protons at δ_H 0.90 (H_3-20, *s*) and 1.31 (H_3-19, *s*), six methylene protons at δ_H 1.38-1.46 (Ha-7, *m*), 1.58-1.59 (Ha-11, *m*), 1.61-1.65 (Hb-7, *m*), 1.67-1.76 (Hb-11, *m*), δ_H 1.96 (Ha-3, *d*, 11.0 Hz), 1.99-2.21 (H_2-6, *m* and Ha-12, *m*), 2.13 (Hb-3, *dd*, 5.5, 11.0 Hz), 2.27-2.35 (Hb-12, *m*) and 2.39-2.45 (H_2-1, *m*) and two methine protons at δ_H 4.81 (H-2, *ddd*, 2.5, 2.8, 5.5 Hz) and 1.67-1.76 (H-8, *m*). The signals at δ_H 6.40 (H-14, *s*), 7.33 (H-15, *dd*, *J* = 1.5, 1.5 Hz) and 7.19 (H-16, *d*, *J* = 1.0 Hz) were characteristic of a *β*-substitued furan ring.

The ^{13}C-NMR (**Figure 145**) and DEPT135 (**Figure 146** and **Table 22**) spetra showed three methyl carbons, six methylene carbons, five methine carbons and six quaternary carbons. The HMBC spectra (**Figure 148-149**) demonstrated the correlations from δ_H 1.31 (H_3-19) and 1.96 (Ha-3) to δ_C 178.8 (C-18) and from δ_H 2.13 (Hb-3) to δ_C 31.2(C-1), 74.4 (C-2) and 43.5 (C-4), while the ^{1}H-^{1}H COSY spectrum (**Figure 150**) showed cross peaks from δ_H 4.81 (H-2) to δ_H 2.13 (Hb-3) and 2.39-2.45 (H_2-1), establishing the first substructure of compound **COC8** as shown below.

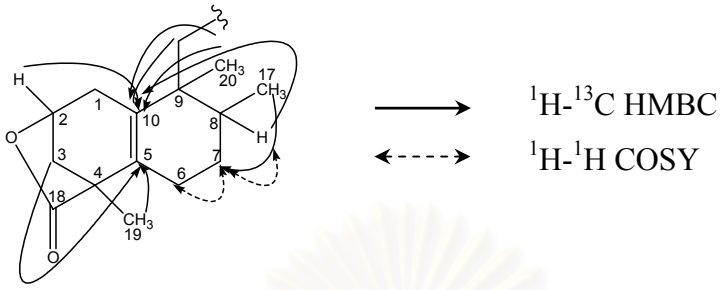


^{1}H-^{13}C HMBC

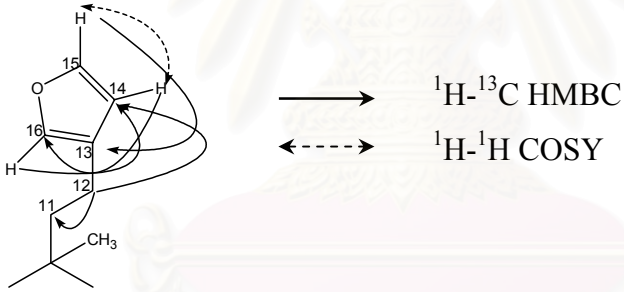
^{1}H-^{1}H COSY

The HMBC spectra (**Figure 148** and **149**) revealed the correlations from δ_H 1.31 (H_3-19) and 1.96 (Ha-3) to δ_C 133.5 (C-5), from δ_H 0.90 (H_3-20), 1.58-1.59 (Ha-11), 1.67-1.76 (H-8) and 4.81 (H-2) to δ_C 133.9 (C-10) and from δ_H 0.88 (H_3-17) to δ_C 26.2 (C-7), while the ^{1}H-^{1}H COSY spectrum (**Figure 150**) showed cross peaks from

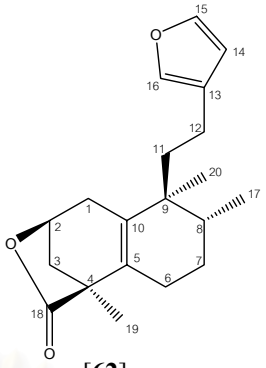
δ_H 1.38-1.46 (Ha-7) to 1.67-1.76 (H-8) and 1.99-2.21 (H_2-6), establishing the second substructure of compound **COC8** as shown.



The HMBC spectra (**Figure 148** and **149**) of compound **COC8** showed the correlations from δ_H 2.27-2.35 (Hb-12) to δ_C 37.9 (C-11) and 110.9 (C-14), from δ_H 6.24 (H-14) to δ_C 138.5 (C-16), from δ_H 7.33 (H-15) to 125.3 (C-13) and from δ_H 7.19 (H-16) to 110.9 (C-14), while the ^{1}H-^{1}H COSY spectrum (**Figure 150**) showed cross peak from δ_H 6.24 (H-14) to 7.33 (H-15). The construction of the third partial structure was by analyses of the above spectral data.



Combination of these fragments allowed us to deduced compound **COC8** as shown below. The relative stereochemistry of compound **COC8** could not be completely established by application of NOE experiments. We supposed the stereochemistry at δ_C 0.88 (C-17), 0.90 (C-20) and 1.31 (C-19) were *cis* orientation as same as the other diterpene isolates in this plant. Thus, the structure of compound **COC8** was newly assigned as 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo [7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**] and has been given the trivial name as chettaphanin V.



[**62**]

Table 22 NMR spectral data of compound **COC8** ($CDCl_3$)

position	Compound **COC8**	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]
1	2.39-2.45 (2H, *m*)	31.2 (CH_2)
2	4.81 (1H, *ddd*, 2.5, 2.8, 5.5) **	74.4 (CH)
3	1.96 (1Ha, *d*, 11.0) **	41.2 (CH_2)
	2.13 (1Hb, *dd*, 5.5, 11.0) **	
4	-	43.5 (C)
5	-	133.5 (C)
6	1.99-2.21 (2H, *m*)	24.5 (CH_2)
7	1.38-1.46 (1Ha, *m*)	26.2 (CH_2)
	1.61-1.65 (1Hb, *m*)	
8	1.67-1.76 (1H, *m*)	32.4 (CH)
9	-	39.9 (C)
10	-	133.9 (C)
11	1.58-1.59 (1Ha, *m*)	37.9 (CH_2)
	1.67-1.76 (1Hb, *m*)	
12	1.99-2.21 (1Ha, *m*)	19.1 (CH_2)
	2.27-2.35 (1Hb, *m*)	
13	-	125.3 (C)
14	6.24 (1H, *s*)	110.9 (CH)
15	7.33 (1H, *dd*, 1.5, 1.5)	142.7 (CH)
16	7.19 (1H, *d*, 1.0)	138.5 (CH)
17	0.88 (3H, *d*, 7.0)	15.7 (CH_3)
18	-	178.8 (C)
19	1.31 (3H, *s*)	17.0 (CH_3)
20	0.90 (3H, *s*)	21.4 (CH_3)

[#] Carbon types were deduced from DEPT135 experiment.

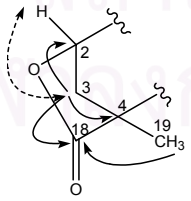
** Precise assignment of coupling constant, see; δ_H 1.96 (*d*, $J_{Ha\text{-}3,\ Hb\text{-}3}$ = 11.0 Hz), 2.13 (*dd*, $J_{Hb\text{-}3,\ Ha\text{-}3}$ = 11.0 Hz, $J_{Hb\text{-}3,\ H\text{-}2}$ = 5.5 Hz), 4.81 (*ddd*, $J_{H\text{-}2,\ Hb\text{-}3}$ = 5.5 Hz, $J_{H\text{-}2,\ Ha\text{-}1\ and\ Hb\text{-}1}$ = 2.5 and 2.8 Hz)

3.9 Structure Determination of Compound COC9

Compound **COC9** was obtained as yellow solid with m.p. 103-105 °C. It showed $[M+H]^+$ ion at *m/z* 329.1727 (calcd. 329.1753) in HRFABMS, corresponding to the molecular formula $C_{20}H_{24}O_4$. The IR spectrum (**Figure 153**) showed absorption bands due to a keto carbonyl (1671 cm^{-1}), a lactone ring (1757, 1276 cm^{-1}) and a furan ring (3122, 1509, 872 cm^{-1}) and the UV absorption at 230 nm (**Figure 152**). The optical rotation was negative, $[\alpha]^{23}_D$ – 151.5° (*c* 0.017, $CHCl_3$).

The ^1H NMR spectra (**Figure 154-155**) showed one secondary methyl proton at δ_H 0.86 (H_3-17, *d*, *J* = 7.0 Hz), two tertiary methyl protons at δ_H 1.07 (H_3-20, *s*) and 1.32 (H_3-19, *s*), five methylene proton at δ_H 1.42-1.49 (Ha-7, *m*), 1.74-1.81 (Hb-7, *m*), 1.93 (Ha-3, *d*, *J* = 11.0 Hz), 2.10-2.19 (H_2-6, *m*), 2.13 (Hb-3, *dd*, *J* = 6.0, 11.0 Hz), 2.33 (Ha-1, *dd*, *J* = 2.7, 17.9 Hz), 2.40 (Hb-1, *dddd*, *J* = 2.5, 2.8, 2.8, 17.9 Hz), 2.74 (Ha-11, *d*, *J* = 15.5 Hz) and 2.85 (Hb-11, *d*, *J* = 15.5 Hz) and five methine protons, three of which at δ_H 6.73, 7.41 and 7.95 were assigned to be a furan ring signals for H-14, H-15 and H-16, respectively and the other at δ_H 2.01-2.08 (H-8, *m*) and 4.76 (H-2, *ddd*, 2.7, 2.8, 6.0 Hz).

The ^{13}C-NMR spectrum (**Figure 156**) and DEPT135 experiments (**Figure 157** and **Table 23**) showed three methyl carbons, five methylene carbons, five methine carbons, and seven quaternary carbons. The HMBC spectra (**Figure 159-160**) demonstrated the correlations from H_3-19 (δ_H 1.32) and Ha-3 (δ_H 1.93) to C-18 (δ_C 178.3) and from Hb-3 (δ_H 2.13) to C-2 (δ_C 74.0) and C-4 (δ_C 43.6), while the ^1H-^1H COSY spectrum (**Figure 161**) displayed a cross peak from H-2 (δ_H 4.76) to Hb-3 (δ_H 1.93), establishing the first substructure of compound **COC9** as shown below.

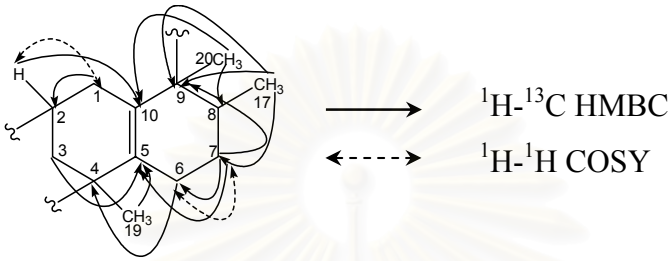


^1H-^{13}C HMBC

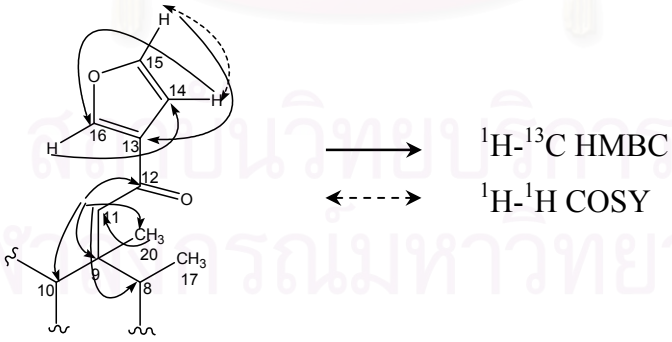
^1H-^1H COSY

The HMBC spectra (**Figure 159-160**) revealed correlations from H_3-20 (δ_H 1.07) to C-10 (δ_C 132.4) and C-8 (δ_C 33.2), from H_3-17 (δ_H 0.86) to C-7 (δ_C 25.5) and C-9 (δ_C 40.3), from Ha-7 (δ_H 1.42-1.49) to C-9 (δ_C 40.3), C-6 (δ_C 22.2) and C-5 (δ_C 132.1), from H_2-6 (δ_H 2.10-2.19) to C-4 (δ_C 43.6), from H_3-19 (δ_H 1.32), Ha-3 (δ_H

1.93) and Hb-3 (δ_H 2.13) to C-5 (δ_C 132.1), from H-2 (δ_H 4.76) to C-10 (δ_C 132.4) and from Ha-1 (δ_H 2.33) to C-2 (δ_C 74.0), while ^{1}H-^{1}H COSY spectrum (**Figure 161**) revealed the correlation from Ha-1 (δ_H 2.33) to H-2 (δ_H 4.76) and from H_2-6 (δ_H 2.10-2.19) to Ha-7 (δ_H 1.42-1.49). Therefore, the second substructure of compound **COC9** was assembled as shown.



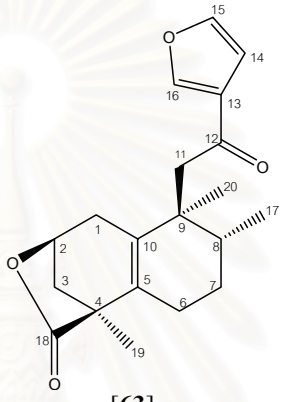
The HMBC spectra (**Figure 159-160**) displayed the correlations from H_3-20 (δ_H 1.07) to C-11 (δ_C 47.7), from Ha-11 (δ_H 2.74) and Hb-11 (δ_H 2.85) to C-8 (δ_C 33.2), C-9 (δ_C 40.3), C-10 (δ_C 132.4), C-12 (δ_C 193.6) and C-20 (δ_C 21.9). A typical of furan ring was found in compound **COC9**, exhibiting three olefinic protons at δ_H 6.73, 7.41 and 7.95 (H-14, H-15 and H-16), the HMBC spectra revealed the correlations from H-14 (δ_H 6.73) to C-16 (δ_C 147.6), from H-15 (δ_H 7.41) to C-13 (δ_C 129.3) and from H-16 (δ_H 7.95) to C-14 (δ_C 108.7), while ^{1}H-^{1}H COSY spectrum (**Figure 161**) showed cross peak between H-14 (δ_H 6.73) and H-15 (δ_H 7.41). Based on these spectral data, the third substructure was created as shown.



The NOE experiments (**Figure 162**) indicated interactions of H_3-17 (δ_H 0.86) with H_3-20 (δ_H 1.07), Ha-7 (δ_H 1.42-1.49) and Hb-11 (δ_H 2.85); from these interactions, the absence of the other significant interactions, biosynthetic considerations and the agreement of the crystal structure of compound **COC10**, which

was also isolated in this study, we assigned the relative configuration of three methyl groups as *cis* orientation.

A gross structure of compound **COC9** was assembled by the combination of these substructures, leading to newly identify as 6-[2-(furan-3-yl)-2-oxoethyl-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**63**] and has been given name as chettaphanin VI.



[**63**]

Table 23 NMR spectral data of compound **COC9** ($CDCl_3$)

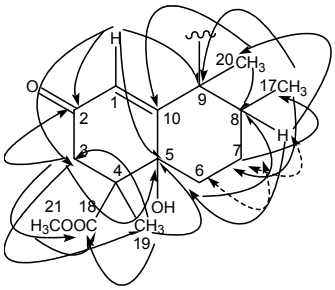
position	Compound **COC9**	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]
1	2.33 (1Ha, *dd*, 2.7, 17.9) **	31.6 (CH_2)
	2.40 (1Hb, *dddd*, 2.5, 2.8, 2.8, 17.9) **	
2	4.76 (1H, *ddd*, 2.7, 2.8, 6.0) **	74.0 (CH)
3	1.93 (1Ha, *d*, 11.0) **	41.1 (CH_2)
	2.13 (1Hb, *dd*, 6.0, 11.0) **	
4	-	43.6 (C)
5	-	132.1 (C)
6	2.10-2.19 (2H, *m*)	22.2 (CH_2)
7	1.42-1.49 (1Ha, *m*)	25.5 (CH_2)
	1.74-1.81 (1Hb, *m*)	
8	2.01-2.08 (1H, *m*)	33.2 (CH)
9	-	40.3 (C)
10	-	132.4 (C)
11	2.74 (1Ha, *d*, 15.5)	47.7 (CH_2)
	2.85 (1Hb, *d*, 15.5)	
12	-	193.6 (C)
13	-	129.3 (C)
14	6.73 (1H, *dd*, 1.0, 2.0)	108.7 (CH)
15	7.41 (1H, *dd*, 1.5, 2.0)	144.2 (CH)
16	7.95 (1H, *dd*, 0.5, 1.5)	147.6 (CH)
17	0.86 (3H, *d*, 7.0)	15.2 (CH_3)
18	-	178.3 (C)
19	1.32 (3H, *s*)	16.5 (CH_3)
20	1.07 (3H, s)	21.9 (CH_3)

[#] Carbon types were deduced from DEPT135 experiment.

** Precise assignment of coupling constant, see; δ_H 1.93 (*d*, $J_{Ha\text{-}3,\ Hb\text{-}3}$ = 11.0 Hz), 2.13 (*dd*, $J_{Hb\text{-}3,\ Ha\text{-}3}$ = 11.0 Hz, $J_{Hb\text{-}3,\ H\text{-}2}$ = 6.0 Hz), 2.33 (*dd*, $J_{Ha\text{-}1,\ Hb\text{-}1}$ = 17.9 Hz, $J_{Ha\text{-}1,\ H\text{-}2}$ = 2.7 Hz), 2.40 (*dddd*, $J_{Hb\text{-}1,\ Ha\text{-}1}$ = 17.9 Hz, $J_{Hb\text{-}1,\ H\text{-}2}$ = 2.8 Hz, $J_{Hb\text{-}1,\ Hb\text{-}3}$ = 2.8 Hz, $J_{Hb\text{-}1,\ H\text{-}6}$ = 2.5 Hz), 4.76 (*ddd*, $J_{H\text{-}2,\ Hb\text{-}3}$ = 6.0 Hz, $J_{H\text{-}2,\ Ha\text{-}1}$ = 2.7 Hz, $J_{H\text{-}2,\ Hb\text{-}1}$ = 2.8 Hz)

3.10 Structure Determination of Compound COC10

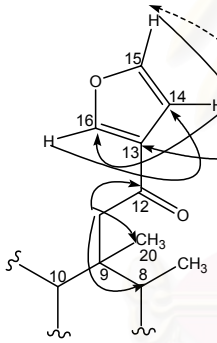
Compound **COC10**, white crystals with m.p. 157-159 °C, showed a protonated molecular ion $[M+H]^+$ at *m/z* 375 in FAB mass spectrum (**Figure 163**), corresponding to the molecular formular $C_{21}H_{26}O_6$. The UV spectrum (**Figure 164**) showed absorption at λ_{max} 248 nm. The IR bands (**Figure 165**) of a hydroxyl group (3423 cm^{-1}), a keto carbonyl groups (1653 cm^{-1}), an ester carbonyl group (1731 and 1281 cm^{-1}) and a furan ring (3140, 1462 and 997 cm^{-1}) were observed. The ^{1}H-NMR spectra (**Figure 166-167**) revealed four methyl protons at δ_H 0.84 (H_3-17, *d*, *J* = 6.5 Hz), 1.14 (H_3-20, *s*), 1.32 (H_3-19, *s*) and 3.66 (H_3-21, *s*), four methylene protons at δ_H 1.45 (Ha-7, *dddd*, *J* = 3.0, 3.5, 4.0, 14.0 Hz), 1.72 (Hb-7, *dddd*, *J* = 3.0, 14.0, 14.0, 14.0 Hz), 1.96 (Ha-6, *ddd*, *J* = 3.0, 3.0, 14.0 Hz), 2.31 (Hb-6, *ddd*, *J* = 4.0, 14.0, 14.0 Hz), 2.47 (Ha-3, *d*, *J* = 17.0 Hz), 2.67 (Hb-3, *d*, *J* = 17.0 Hz), 3.09 (Ha-11, *d*, *J* = 19.0 Hz) and 3.23 (Hb-11, *d*, *J* = 19.0 Hz), five methine protons; three protons of which showed a characteristic furan protons at δ_H 6.61, 7.37 and 7.97 (1H each, H-14, H-15 and H-16), the other signals at δ_H 2.15 (H-8, *m*) and 5.76 (H-1, *s*) and one hydroxyl proton at δ_H 2.44. The ^{13}C-NMR (**Figure 168**) and DEPT135 (**Table 24**) spectra showed four methyl carbons, four methylene carbons, five methine carbons and eight quaternary carbons. The HMBC spectrum of compound **COC10** (**Figure 170**) demonstrated the correlations from δ_H 5.76 (H-1) to δ_C 41.1 (C-9), 43.2 (C-3), 72.4 (C-5) and 198.1 (C-2), from δ_H 2.47 (Ha-3) and 2.67 (Hb-3) to δ_C 19.4 (C-19), 72.4 (C-5), 174.6 (C-18) and 198.1 (C-2), from δ_H 1.32 (H_3-19) to δ_C 43.2 (C-3), 72.4 (C-5) and 174.6 (C-18), from δ_H 1.96 (Ha-6), 2.31 (Hb-6) and 1.14 (H_3-20) to δ_C 35.1 (C-8), from δ_H 1.45 (Ha-7) to δ_C 16.7 (C-17), from δ_H 0.84 (H_3-17) to δ_C 25.0 (C-7) and 35.1 (C-9) and from δ_H 2.15 (H-8) to δ_C 31.6 (C-6) and 25.8 (C-20), while the ^{1}H-^{1}H COSY spectrum (**Figure 171**) showed cross peaks from δ_H 1.45 (Ha-7) and 1.72 (Hb-7) to δ_H 1.96 (Ha-6), 2.31 (Hb-6) and 2.15 (H-8). These spectral data assisted in the construction of the first partial structure of compound **COC10** as shown.



^{1}H-^{13}C HMBC

^{1}H-^{1}H COSY

The HMBC spectrum of compound **COC10** (**Figure 170**) appeared the correlations from δ_H 3.09 (Ha-11) and 3.23 (Hb-11) to δ_C 25.8 (C-20), 35.1 (C-8) and 190.9 (C-12), from δ_H 3.09 (Ha-11) to δ_C 167.8 (C-10), from δ_H 6.61 (H-14) to δ_C 146.3 (C-16), from δ_H 7.37 (H-15) to δ_C 127.9 (C-13) and from δ_H 7.97 (H-16) to δ_C 108.3 (C-14), along with the ^{1}H-^{1}H COSY correlation (**Figure 171**) between δ_H 6.61 (H-14) and 7.37 (H-15). The construction of the second partial structure was by analyses of the above spectral data.



^{1}H-^{13}C HMBC

^{1}H-^{1}H COSY

A gross structure of compound **COC10** was assembled by combination of the two partial structures and comparison with the previous report (Marcos *et al*., 2003). Thus, compound **COC10** was identified as chettaphanin I [**48**], which was previously isolated from *Adenochleana siamensis* (Sato *et al*., 1970) and *Croton crassifolius* (Boonyarathanakornkit *et al*., 1988).

Although the structure of this compound was determined spectroscopically and chemically, the stereochemistry was unknown even by X-ray crystallography (Marcos *et al*., 2003). In this study, NOE experiments (**Figure 172**) indicated interactions from H_3-20 (δ_H 1.14) with H-1 (δ_H 5.76), OH-5 (δ_H 2.44) and H_3-17 (δ_H 0.84) and from H_3-19 (δ_H 1.32) with OH-5 (δ_H 2.44). Additionally, we succeeded in preparing a single crystal of compound **COC10** carrying $CHCl_3$ in its molecule by recrystallization from hexane-$CHCl_3$. The X-ray crystallographic analysis (**Figure**

173 and **Table 25-30**) of the $CHCl_3$-contained crystal indicated that the reported stereochemistry of compound **COC10** including absolute configurations at C-4, C-5, C-8 and C-9 is in the *S*, *S*, *R*, *S* configuration.

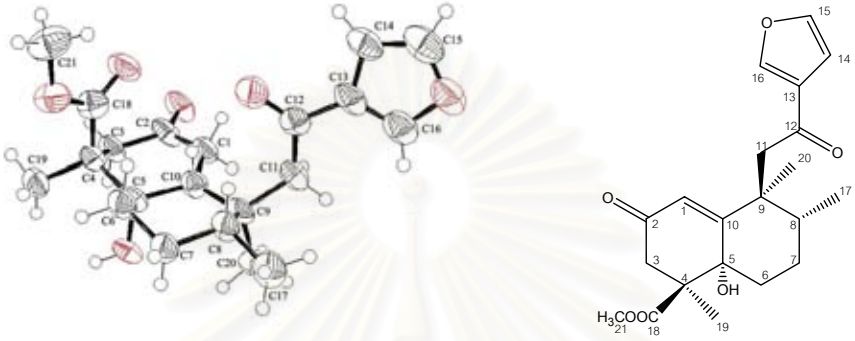


Figure 173 ORTEP drawing of compound **COC10**. The chloroform molecule is omitted for clarity.

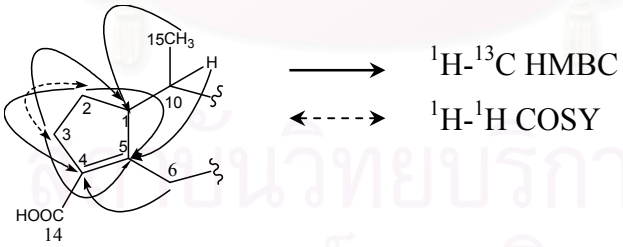
Table 24 NMR spectral data of compound **COC10** and chettaphanin I ($CDCl_3$)

position	Compound **COC10**		Chettaphanin I [**48**]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	5.76 (1H, *s*)	125.4 (CH)	5.84 (1H, *s*)	125.6
2	-	198.1 (C)	-	197.6
3	2.47 (1Ha, *d*, 17.0)	43.2 (CH_2)	2.57 (1Ha, *d*, 17.4)	43.1
	2.67 (1Hb, *d*, 17.0)		2.69 (1Hb, *d*, 17.4)	
4	-	53.0 (C)	-	52.9
5	-	72.4 (C)	-	72.7
6	1.96 (1Ha, *ddd*, 3.0, 3.0, 14.0)	31.6 (CH_2)	2.00 (1Ha, *ddd*, 3.0, 3.0, 14.0)	31.8
	2.31 (1Hb, *ddd*, 4.0, 14.0, 14.0)		2.40 (1Hb, *ddd*, 3.6, 14.0, 14.0)	
7	1.45 (1Ha, *dddd*, 3.0, 3.5, 4.0, 14.0)	25.0 (CH_2)	1.2-1.3 (1H, *m*)	25.0
	1.72 (1Hb, *dddd*, 3.0, 14.0, 14.0, 14.0)		1.5-1.8 (1H, *m*)	
8	2.15 (1H, *m*)	35.1 (CH)	2.40 (1H, *m*)	35.2
9	-	41.1 (C)	-	41.2
10	-	167.8 (C)	-	167.4
11	3.09 (1Ha, *d*, 19.0)	47.7 (CH_2)	3.14 (1Ha, *d*, 18.0)	47.8
	3.23 (1Hb, *d*, 19.0)		3.25 (1Hb, *d*, 18.0)	
12	-	190.9 (C)	-	190.8
13	-	127.9 (C)	-	127.6
14	6.61 (1H, *dd*, 0.5, 2.0)	108.3 (CH)	6.64 (1H, *s*)	108.3
15	7.37 (1H, *dd*, 1.5, 1.5)	144.0 (CH)	7.41 (1H, *s*)	144.1
16	7.97 (1H, *s*)	146.3 (CH)	7.98 (1H, *s*)	146.3
17	0.84 (3H, *d*, 6.5)	16.7 (CH_3)	0.89 (3H, *d*, 6.8)	16.7
18	-	174.6 (C)	-	174.5
19	1.32 (3H, *s*)	19.4 (CH_3)	1.38 (3H, *s*)	19.4
20	1.14 (3H, s)	25.8 (CH_3)	1.20 (3H, *s*)	25.9
21	3.66 (3H, *s*)	52.3 (CH_3)	3.71 (3H, *s*)	52.4
OH-5	2.44 (1H, *s*)		-	

[#] Carbon types were deduced from DEPT135 experiment.

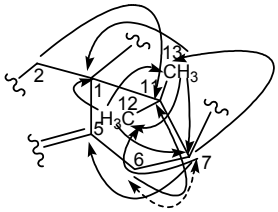
3.11 Structure Determination of Compound COC11

Compound **COC11** was isolated as white solid with m.p. 161-163 °C. The FABMS spectrum (**Figure 174**) showed $[M+H]^+$ at *m/z* 235, harmonizing with the molecular formula $C_{15}H_{22}O_2$. The UV absorptions at 241 nm suggested the presence of such a conjugated chromophore in compound **COC11** (**Figure 175**). The IR absorption peaks (**Figure 176**) at 3200-2400 cm^{-1} and 1672 cm^{-1} revealed a carboxylic acid group. The ^{1}H-NMR spectrum (**Figure 177**) showed signals for methyl, methylene and methine of alicyclic at δ_H 0.82-2.84. The ^{13}C-NMR (**Figure 178**) and DEPT135 (**Table 31**) spectra displayed 15 signals; three methyl carbons (δ_C 18.0, 19.3 and 26.2), five methylene carbons (δ_C 25.7, 26.9, 27.9, 31.3 and 36.3), two methine carbons (δ_C 36.0 and 48.1) and two quaternary carbons (δ_C 41.7 and 68.2), in addition to two olefinic carbons (δ_C 123.1 and 173.1) and a carboxylic acid moiety (δ_C 170.9). The HMBC spectrum of compound **COC11** (**Figure 180**) demonstrated the correlations from δ_H 0.89 (H_3-15) and 2.67-2.84 (H_2-3) to δ_C 68.2 (C-1), from δ_H 1.49-1.56 (Ha-2) and 2.24 (Ha-6) to δ_C 123.1 (C-4), from δ_H 1.76 (Hb-2), 2.67-2.84 (H_2-3) and 2.06 (H-10) to δ_C 173.1 (C-5) and from δ_H 2.67-2.84 (H_2-3) to δ_C 170.9 (C-14), in addition to the ^{1}H-^{1}H COSY spectrum of compound **COC11** (**Figure 181**), displaying a correlation between δ_H 1.76 (Hb-2) to δ_H 2.67-2.84 (H_2-3). Therefore the first fragment of compound **COC11** is assembled as shown.



The ^{1}H-^{1}H COSY spectrum of compound **COC11** (**Figure 181**) demonstrated for the cross peak of methylene proton from δ_H 2.67-2.84 (Hb-6) to 1.96 (H-7). The HMBC spectrum of compound **COC11** (**Figure 180**) showed the correlations from δ_H 0.82 (H_3-13) and 0.99 (H_3-12) to δ_C 68.2 (C-1), from δ_H 1.76 (Hb-2) and 2.24 (Ha-6) to δ_C 41.7 (C-11), from δ_H 0.99 (H_3-12) and 0.82 (H_3-13) to δ_C 48.1 (C-7), from δ_H 0.99 (H_3-12) and 1.96 (H-7) to δ_C 26.2 (C-13), from δ_H 0.82 (H_3-13) and 1.96 (H-7) to δ_C 19.3 (C-12) and from δ_H 1.96 (H-7) to δ_C 173.1 (C-5).

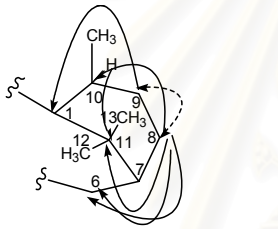
Based on these spectral data the second substructure of compound **COC11** is proposed as shown below.



^{1}H-^{13}C HMBC

^{1}H-^{1}H COSY

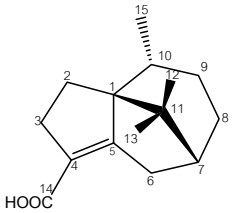
The ^{1}H-^{1}H COSY spectrum of compound **COC11** (**Figure 181**) displayed a cross peak from δ_H 1.36 (Ha-8) to 1.12 (Ha-9), while the HMBC correlations from δ_H 1.12 (Ha-9) to δ_C 68.2 (C-1), from δ_H 2.06 (H-10) to δ_C 41.7 (C-11), from δ_H 1.89 (Hb-8) to δ_C 31.3 (C-6), 41.7 (C-11) and 36.0 (C-10) and from δ_H 2.24 (Ha-6) to δ_C 26.9 (C-8). Therefore the third partial structure is created as shown below.



^{1}H-^{13}C HMBC

^{1}H-^{1}H COSY

Combination of the first, the second, and the third fragments established a gross structure of compound **COC11**. The relative configuration of compound **COC11** was assumed to be the same as that previously reported (Jacobs *et al*., 1987) due to the same negative rotations $\{[\alpha]^{23}_{D} - 7.8^{\circ}$ (*c* 0.08, $CHCl_3)\}$ observed. In addition, this assumption was confirmed by NOE experiments (**Figure 182**) on irradiation at δ_H 2.06 (H-10), in which an enhancement was observed at δ_H 0.99 (H_3-12). When methyl proton at δ_H 0.99 (H_3-12) was irradiated, the enhancement was observed at δ_H 0.82 (H_3-13) and 2.06 (H-10). By analysis of the above spectroscopic data and comparison of its ^{1}H- and ^{13}C-NMR data with the previous report. Compound **COC11** was identified as patchoulane type sesquiterpene, namely cyperenoic acid [**64**] which is a known substance previously isolated from *Sandwithia guyanensis* (Jacobs, Lachmansing and Ramdayal, 1987) and *Croton crassifolius* (Boonyaratavej and Roengsumran, 1988)



[**64**]

Table 31 NMR spectral data of compound **COC11** and cyperenoic acid ($CDCl_3$)

position	Compound **COC11**		Cyperenoic acid	
	δ_H (ppm), *J* (Hz)	δ_C (ppm) [#]	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	-	68.2 (C)	-	68.2
2	1.49-1.56 (1Ha, *m*)	25.7 (CH_2)	1.53 (1H)	25.7
	1.76 (1Hb, *ddd*, 9.8, 9.8, 13.3)		1.75 (1H)	
3	2.67-2.84 (2H, *m*)	36.3 (CH_2)	2.69 (1H)	36.3
			2.79 (1H)	
4	-	123.1 (C)	-	123.1
5	-	173.1 (C)	-	173.2
6	2.24 (1Ha, *brd*, 20.0)	31.3 (CH_2)	2.25 (1H)	31.3
	2.67-2.84 (1Hb, *m*)		2.76 (1H)	
7	1.96 (1H, *dd*, 3.0, 3.5)	48.1 (CH)	1.95 (1H)	48.1
8	1.36 (1Ha, *dddd*, 3.0, 6.0, 6.0, 6.0)	26.9 (CH_2)	1.30 (1H)	26.9
	1.89 (1Hb, *m*)		1.88 (1H)	
9	1.12 (1Ha, *m*)	27.9 (CH_2)	1.11 (1H)	27.9
	1.49-1.56 (1Hb, *m*)		1.51 (1H)	
10	2.06 (1H, *m*)	36.0 (CH)	2.07 (1H)	36.0
11	-	41.7 (C)	-	41.7
12	0.99 (3H, *s*)	19.3 (CH_3)	0.99 (3H)	19.3
13	0.82 (3H, *s*)	26.2 (CH_3)	0.82 (3H)	26.2
14	-	170.9 (C)	-	170.9
15	0.89 (3H, *d*, 6.5)	18.0 (CH_3)	0.82 (3H)	18.0

[#] Carbon types were deduced from DEPT135 experiment.

4. Biological Activities of Isolated Compounds

The results of biological activities including cytotoxic and antimycobacterial activities are shown in **Tables 32** and **33**.

4.1 Biological Activities of the Compounds from *Pterocaulon redolens*

Compounds **PRC1, 2, 3, 4, 6** and **7** and **PRB 8** and **9** have displayed mild antimycobacterial activity toward *Mycobacterium tuberculosis* H37Ra and compound **PRB10** exhibited moderate cytotoxicity to BC and NCI-H187 cell line. These results are shown in **Table 32**.

4.2. Biological Activities of the Compounds from *Cladogynos orientalis*

Compounds **COC6**, **7** and **8** possessed weak to moderate cytotoxicity, while all isolates showed mild antimycobacterial activity toward *Mycobacterium tuberculosis* H37Ra except compound **COC5**. These results are demonstrated in **Table 33**.

Table 32 Biological activities of isolated compounds of *Pterocaulon redolens*.

compounds	Cytotoxicity IC_{50}(μg/mL) *				Antimycobacterial activity [d] MIC (μg/mL)
	Vero cell	KB [a]	BC [b]	NCI-H 187 [c]	
PRC1	> 50	> 20	> 20	> 20	200
PRC2	> 50	> 20	> 20	> 20	200
PRC3	> 50	> 20	> 20	> 20	100
PRC4	> 50	> 20	> 20	> 20	100
PRC5	> 50	> 20	> 20	> 20	inactive
PRC6	> 50	> 20	> 20	> 20	200
PRC7	> 50	> 20	> 20	> 20	200
PRC8	> 50	> 20	> 20	> 20	100
PRC9	> 50	> 20	> 20	> 20	100
PRC10	> 50	> 20	5.5	9.3	inactive

[a] KB; Human epidermoid carcinoma cell lines of nasopharynx

[b] BC; Human breast cancer cell lines

[c] NCI-H 187; Human small cell lung cancer cell lines

[d] Antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra

IC_{50}; Inhibition Concentration at 50%

* IC_{50} (μg/mL) > 20; inactive
10-20; weakly active
5-10; moderately active
< 5; strongly active

MIC; Minimun Inhibition Concentration

Table 33 Biological activities of isolated compounds of *Cladogynos orientalis*.

compounds	Cytotoxicity IC_{50} (μg/mL)*				Antimycobacterial activity [d] MIC (μg/mL)
	Vero cell	KB [a]	BC [b]	NCI-H 187 [c]	
COC1	> 50	> 20	> 20	> 20	200
COC2	> 50	> 20	> 20	> 20	50
COC3	> 50	> 20	> 20	> 20	50
COC4	> 50	> 20	> 20	> 20	200
COC5	> 50	> 20	> 20	> 20	inactive
COC6	> 50	> 20	> 20	12.2	100
COC7	> 50	> 20	> 20	17.4	100
COC8	> 50	17.1	15.8	8.3	100
COC9	> 50	> 20	> 20	> 20	200
COC10	> 50	> 20	> 20	> 20	200
COC11	> 50	> 20	> 20	> 20	100

[a] KB; Human epidermoid carcinoma cell lines of nasopharynx

[b] BC; Human breast cancer cell lines

[c] NCI-H 187; Human small cell lung cancer cell lines

[d] Antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra

IC_{50}; Inhibition Concentration at 50%

* IC_{50} (μg/mL) > 20; inactive
10-20; weakly active
5-10; moderately active
< 5; strongly active

MIC; Minimun Inhibition Concentration

CHAPTER V

CONCLUSION

In this investigation, from the aerial parts of *Pterocaulon redolens* (Forst. f) F. Vill, a new natural product, namely 2′,3′-dihydroxypuberulin [**52**], was isolated along with 9 known compounds. These known compounds are 5-methoxy-6,7-methylenedioxycoumarin [**9**], ayapin [**10**], puberulin [**50**], 5-methoxyscopoletin [**51**], isofraxidin [**53**], sabandinol [**23**], luteolin [**54**], tomentin [**55**] and chrysosplenol C [**35**]. Chrysosplenol C [**35**] possessed moderate cytotoxicity against human breast cancer (BC) and human small cell lung cancer (NCI-H187) cell lines with IC_{50} 5.5 and 9.3 μg/mL, respectively.

Chemical examination of the roots of *Cladogynos orientalis* Zipp. ex Span. led to isolation of 5 new compounds, namely (*4S*, 7R*, 8R*, 10S**)-8-hydroxy-α-guaiene [**56**], 5-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-otahydronaphthalene-1-carboxylic acid [**58**], methyl 9-(furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo [5.3.3.$0^{1,6}$]trideca-5,8-diene-2-carboxylate [**59**], 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**] and 6-[2-(furan-3-yl)-2-oxoethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**63**] along with 6 known compounds. These known compounds are chettaphanin I [**48**], chettaphanin II [**49**], spathulenol [**57**], acetoxyaleuritolate [**60**], taraxerol [**61**] and cyperenoic acid [**64**]. Chettaphanin II [**49**], taraxerol [**61**] and 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**] showed mild to moderate cytotoxicity to NCI-H187 cell line with IC_{50} 17.4, 12.2 and 8.3 μg/mL, respectively. Additionally, 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one [**62**] possessed mild cytotoxicity to KB and BC cell lines with IC_{50} 17.1 and 15.8 μg/mL, respectively. All of 21 isolated compounds showed mild antimycobacterial activity toward *Microbacterium tuberculosis* H37Ra (MIC 50-200 μg/mL) except chrysosplenol C [**35**], 2′,3′-dihydroxypuberulin [**52**] and acetoxyaleuritolate [**60**]. The structures of some isolated compounds were revised and completed by ^{1}H- and ^{13}C-NMR assignments.

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APPENDICES

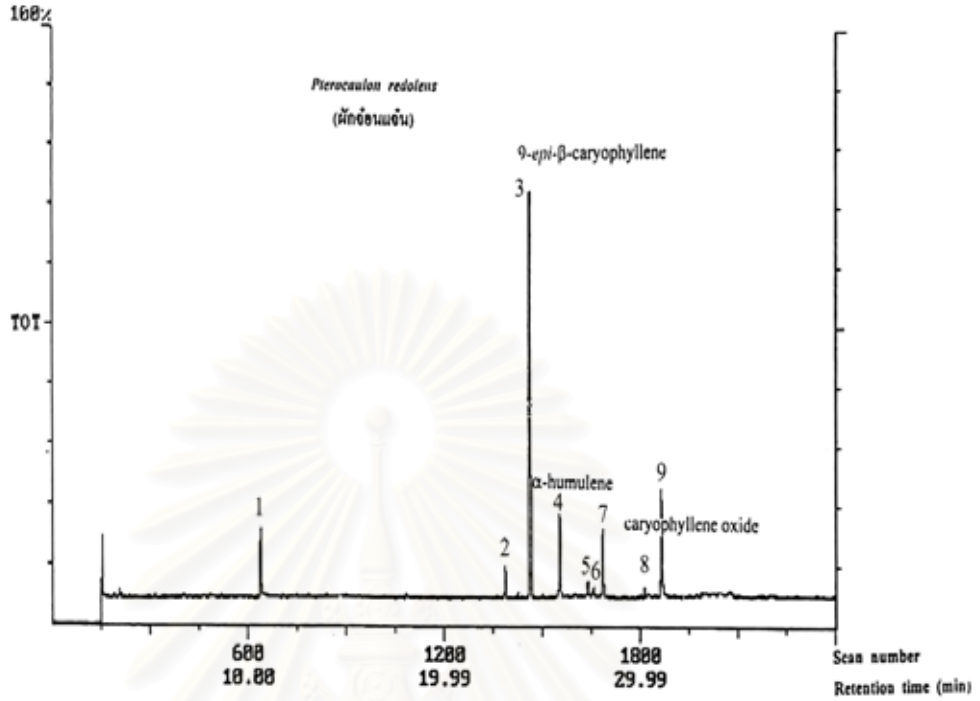

Figure 5 GC Chromatogram of the oil of *Pterocaulon redolen*s aerial parts.

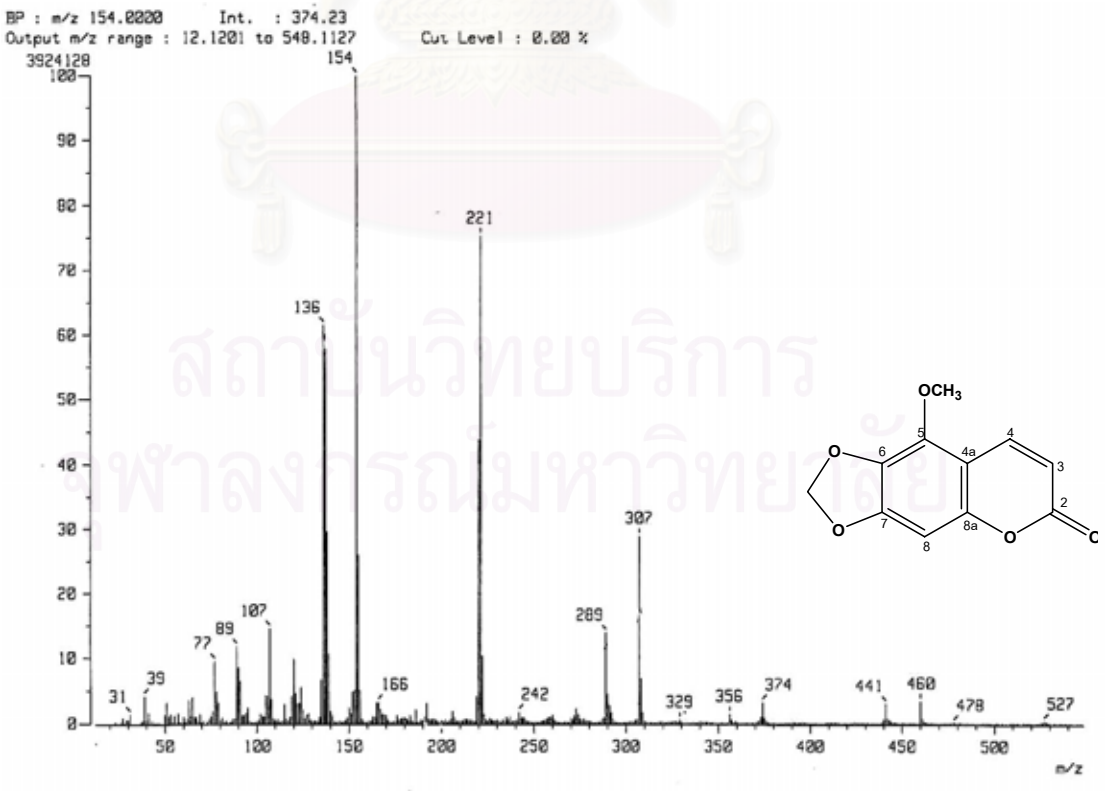

Figure 6 FAB Mass spectrum of compound **PRC1**.

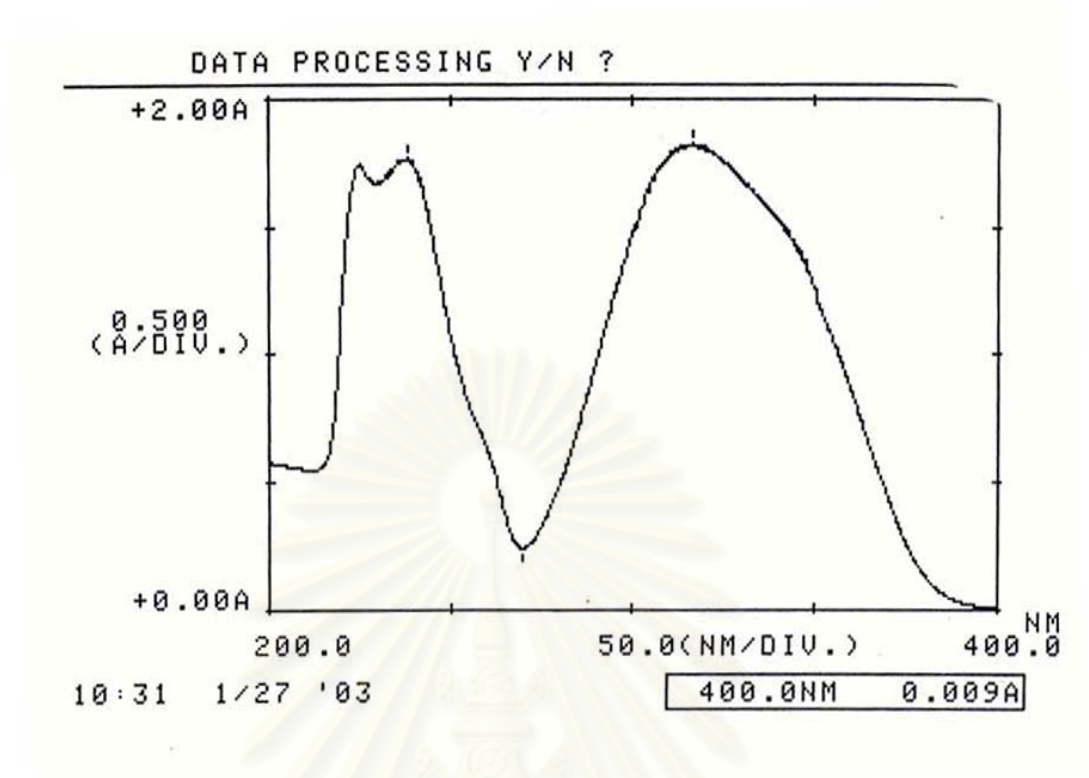

Figure 7 UV spectrum of compound **PRC1** (MeOH).

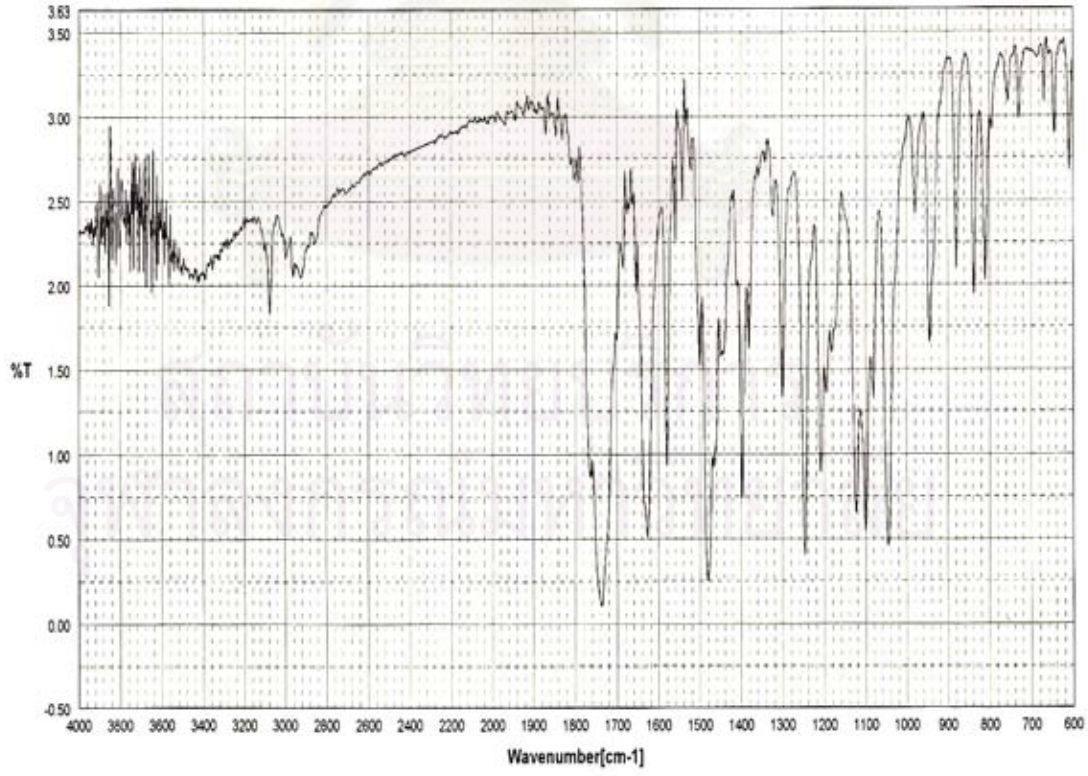

Figure 8 IR spectrum of compound **PRC1** (KBr disc).

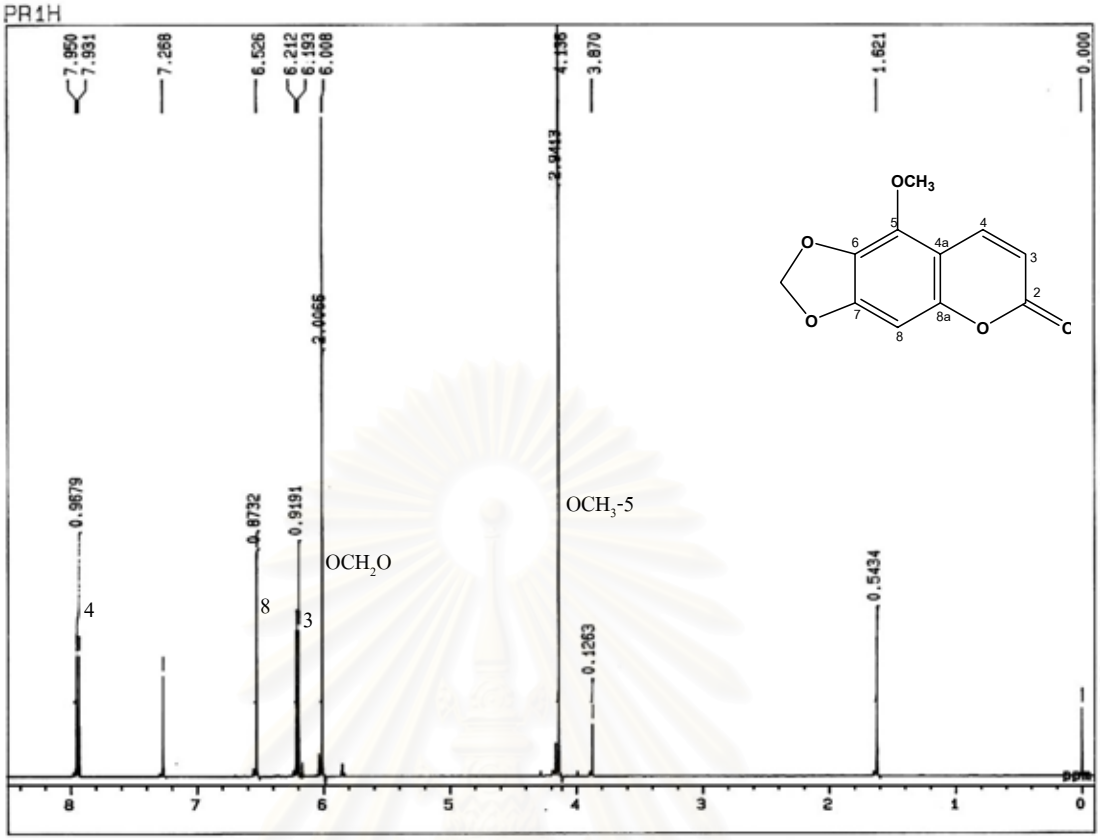

Figure 9 ^{1}H-NMR (500 MHz) spectrum of compound **PRC1** ($CDCl_3$).

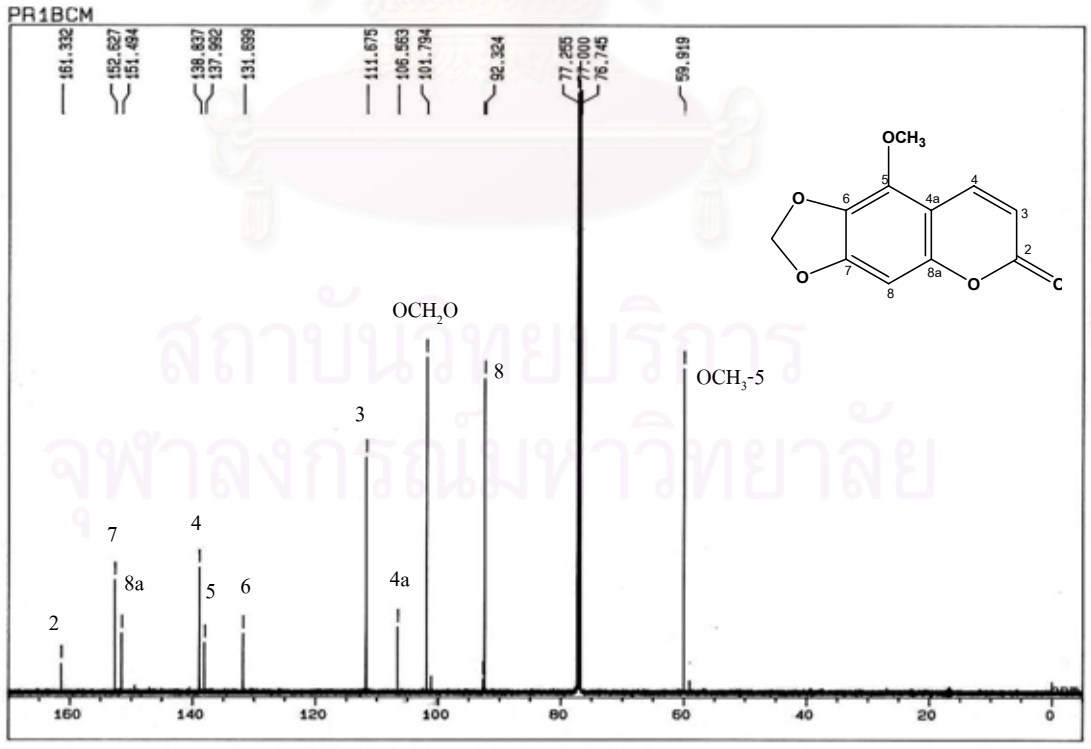

Figure 10 ^{13}C-NMR (125 MHz) spectrum of compound **PRC1** ($CDCl_3$).

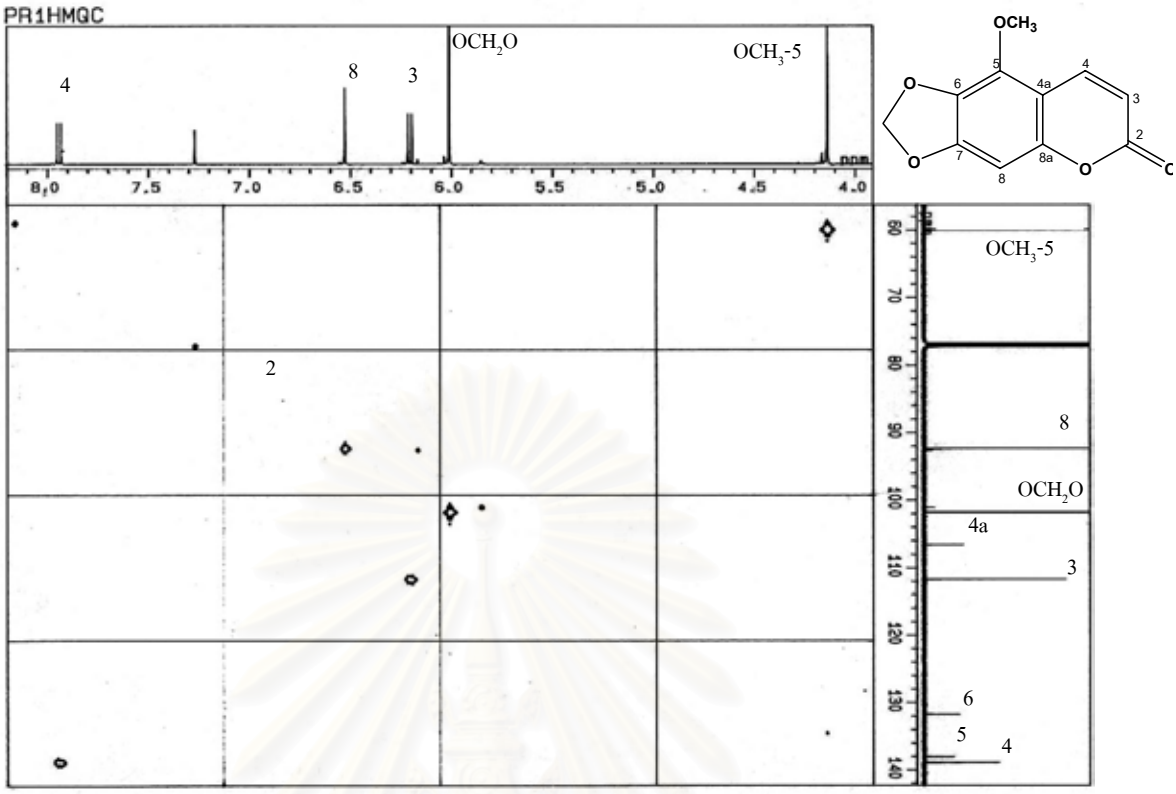

Figure 11 HMQC spectrum of compound **PRC1** ($CDCl_3$).

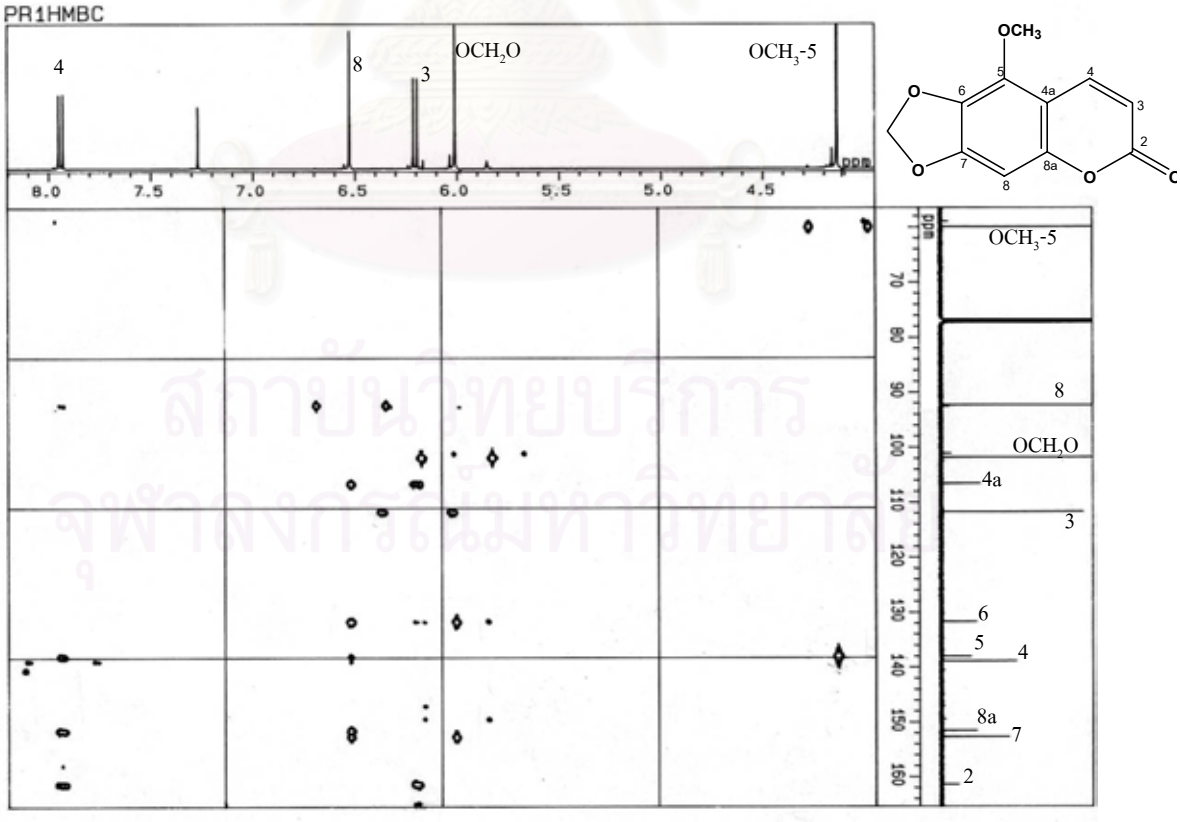

Figure 12 HMBC spectrum of compound **PRC1** ($CDCl_3$).

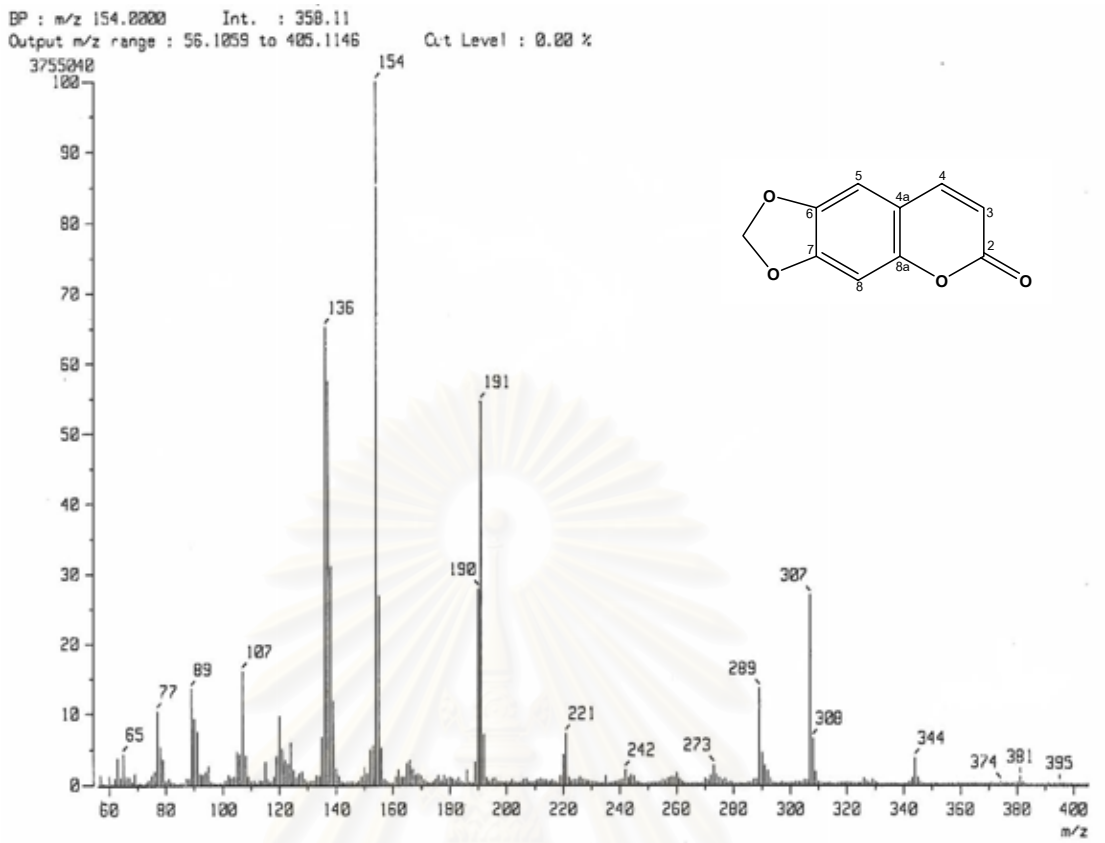

Figure 13 FAB Mass spectrum of compound **PRC2**.

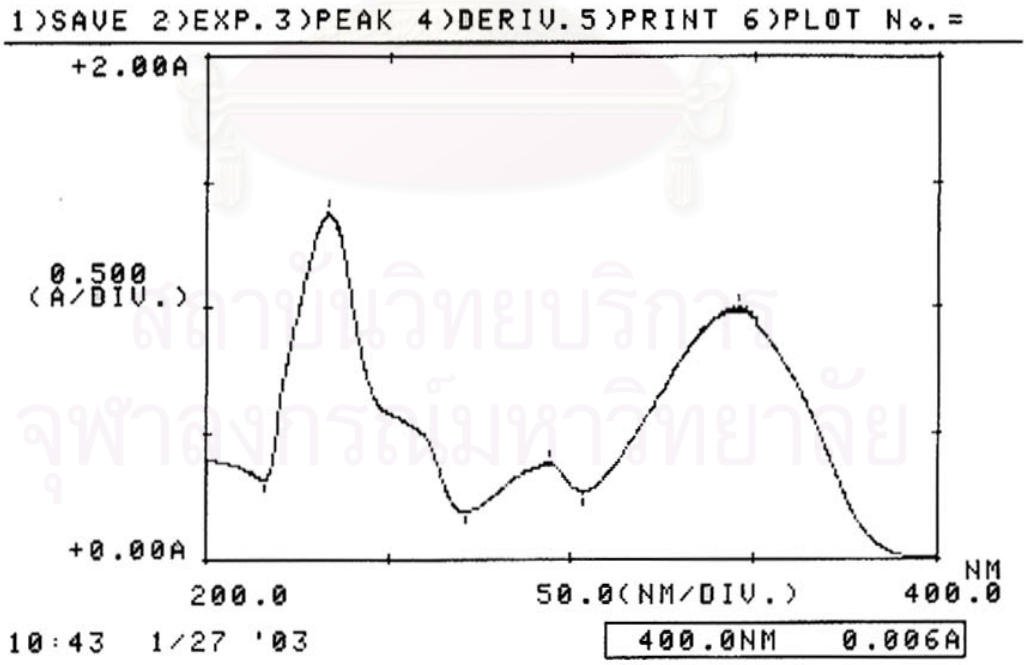

Figure 14 UV spectrum of compound **PRC2**.

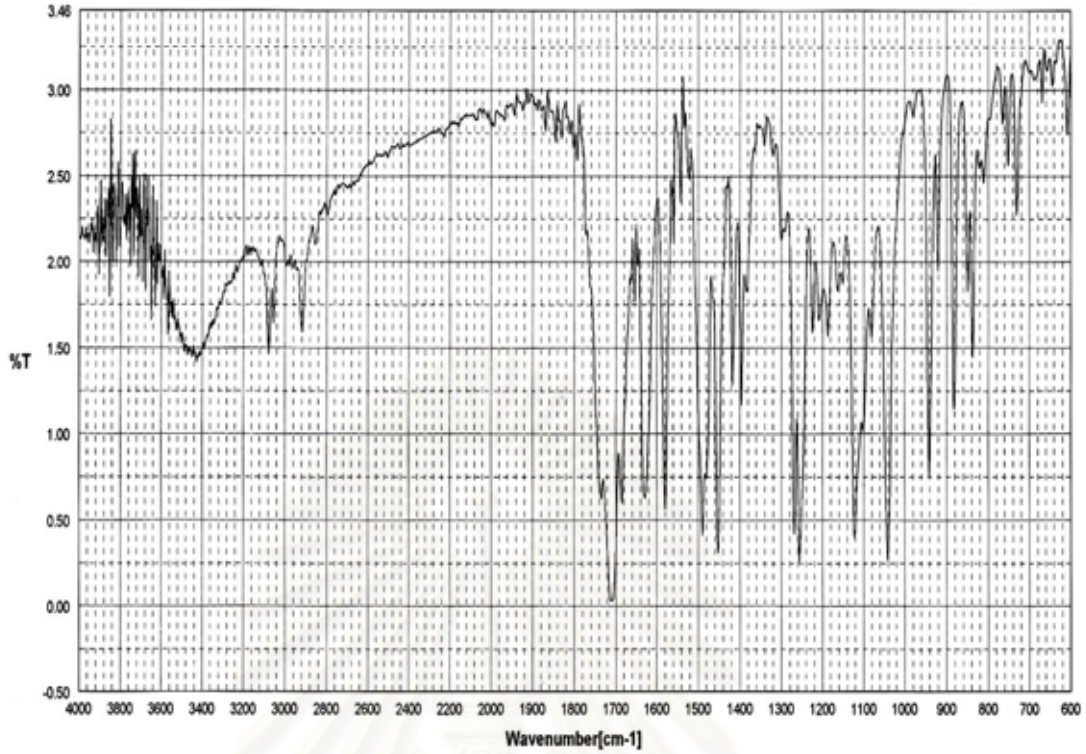

Figure 15 IR spectrum of compound **PRC2** (KBr disc).

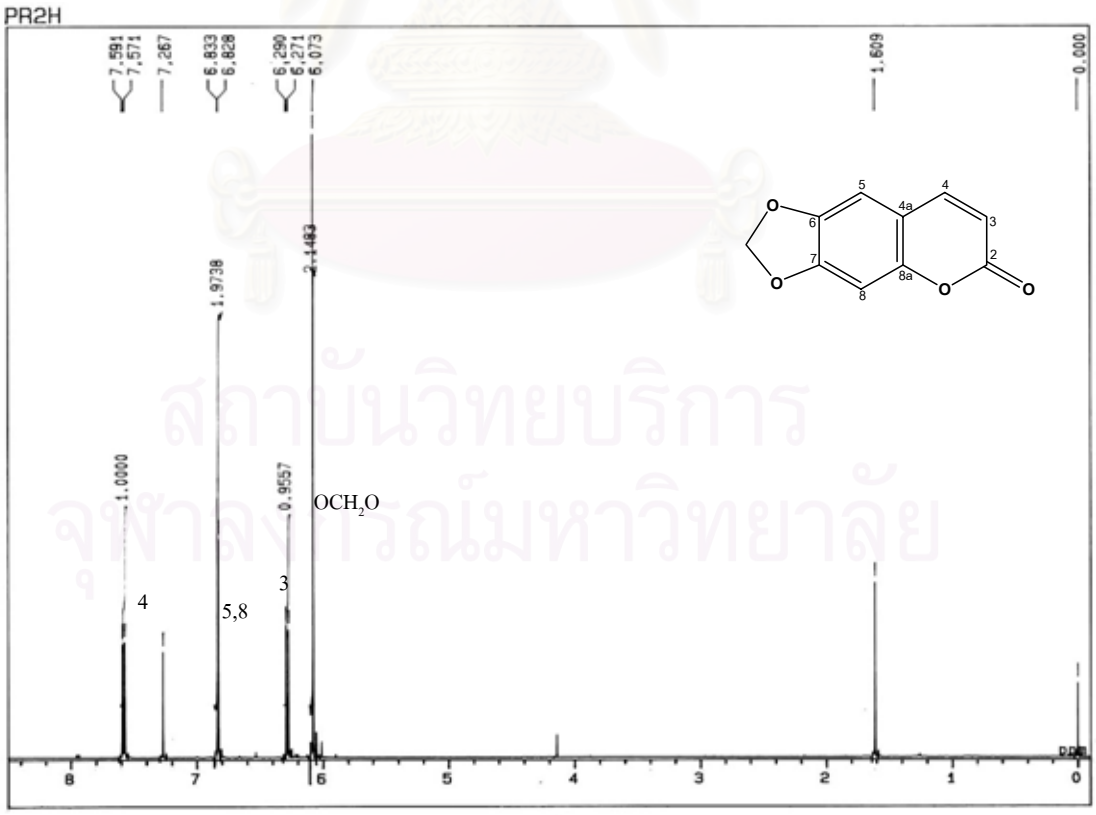

Figure 16 ^{1}H-NMR (500 MHz) spectrum of compound **PRC2** ($CDCl_3$).

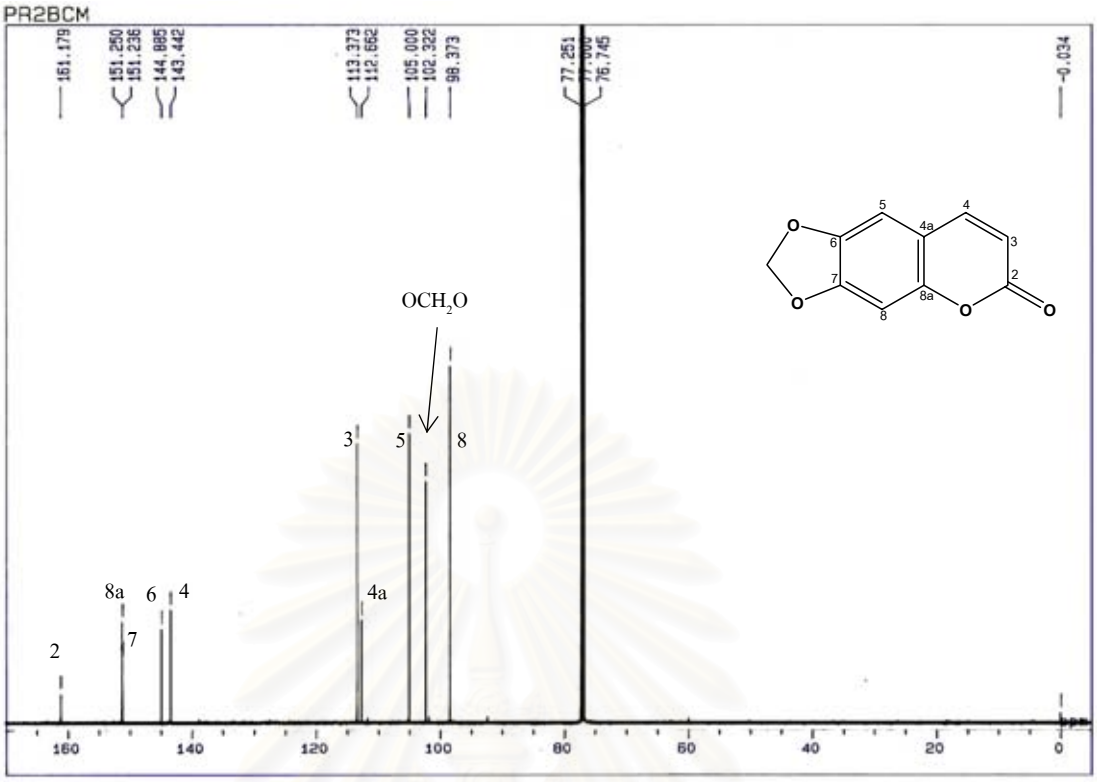

Figure 17 ^{13}C-NMR (125 MHz) spectrum of compound **PRC2** ($CDCl_3$).

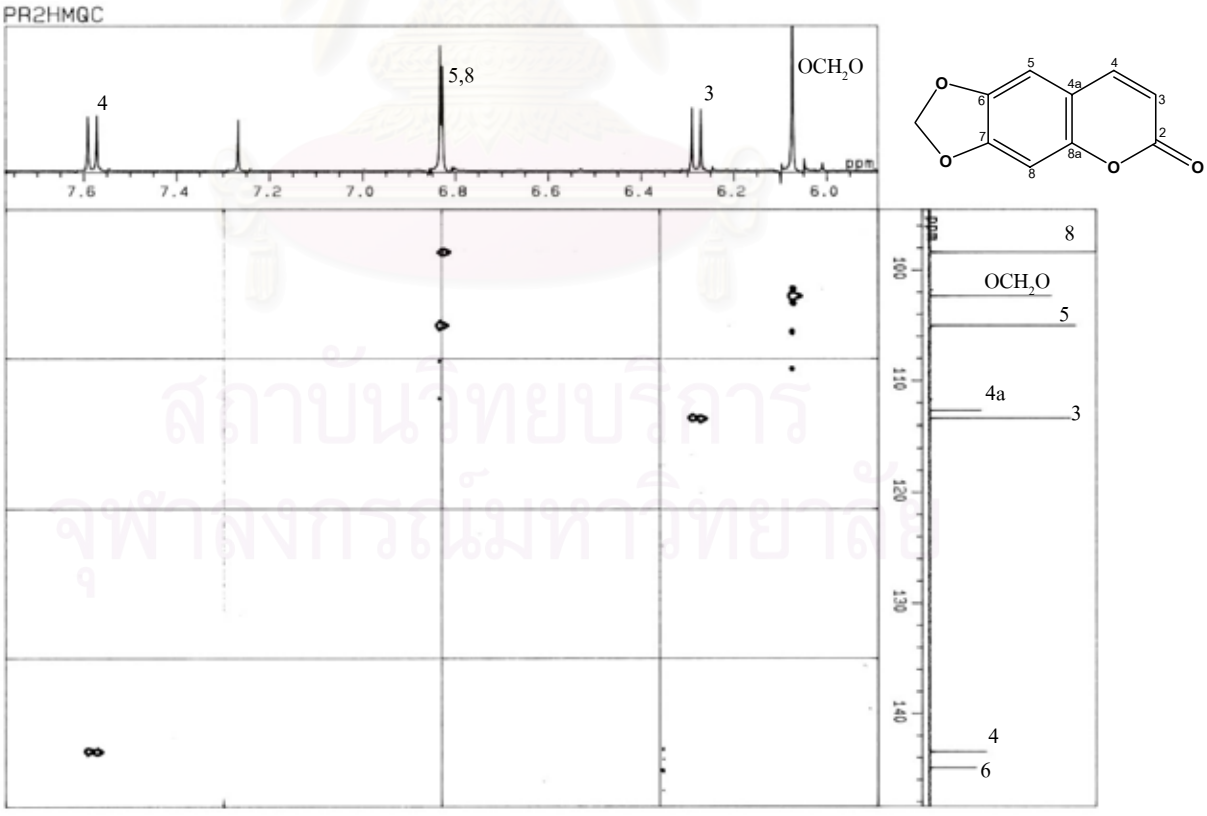

Figure 18 HMQC spectrum of compound **PRC2** ($CDCl_3$).

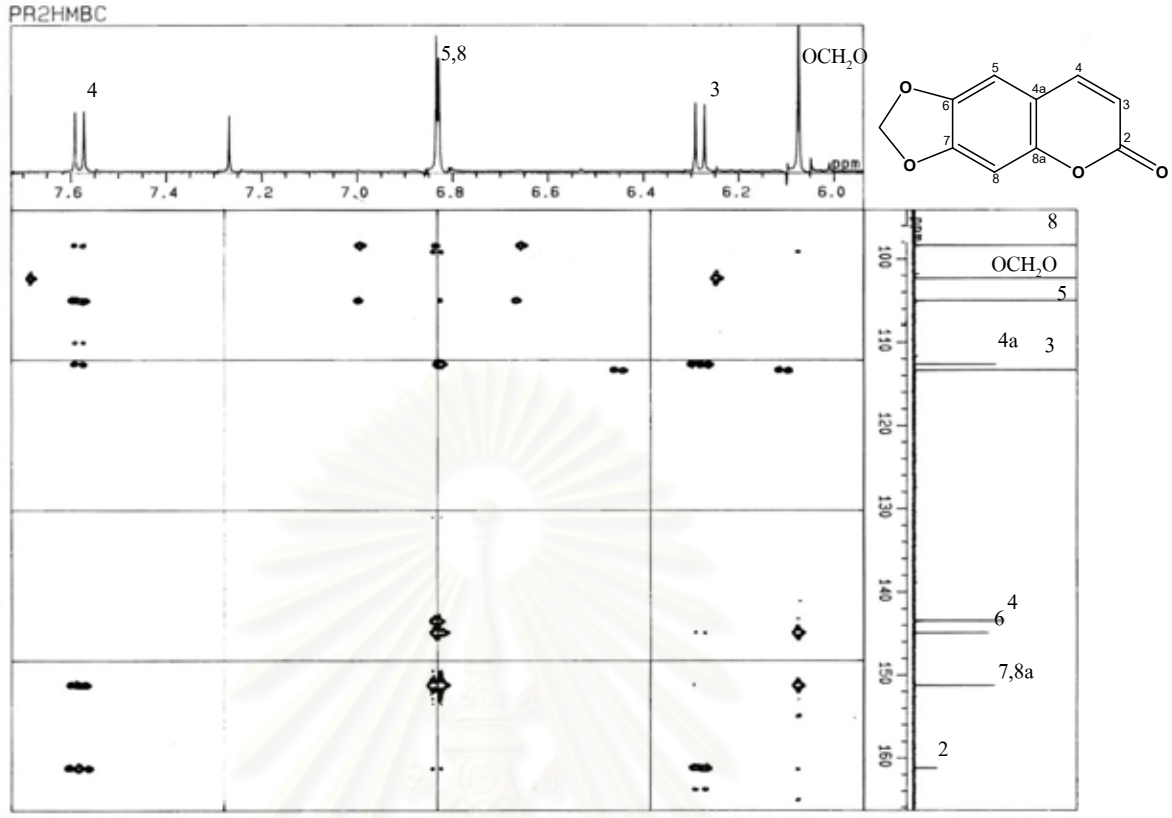

Figure 19 HMBC spectrum of compound **PRC2** ($CDCl_3$).

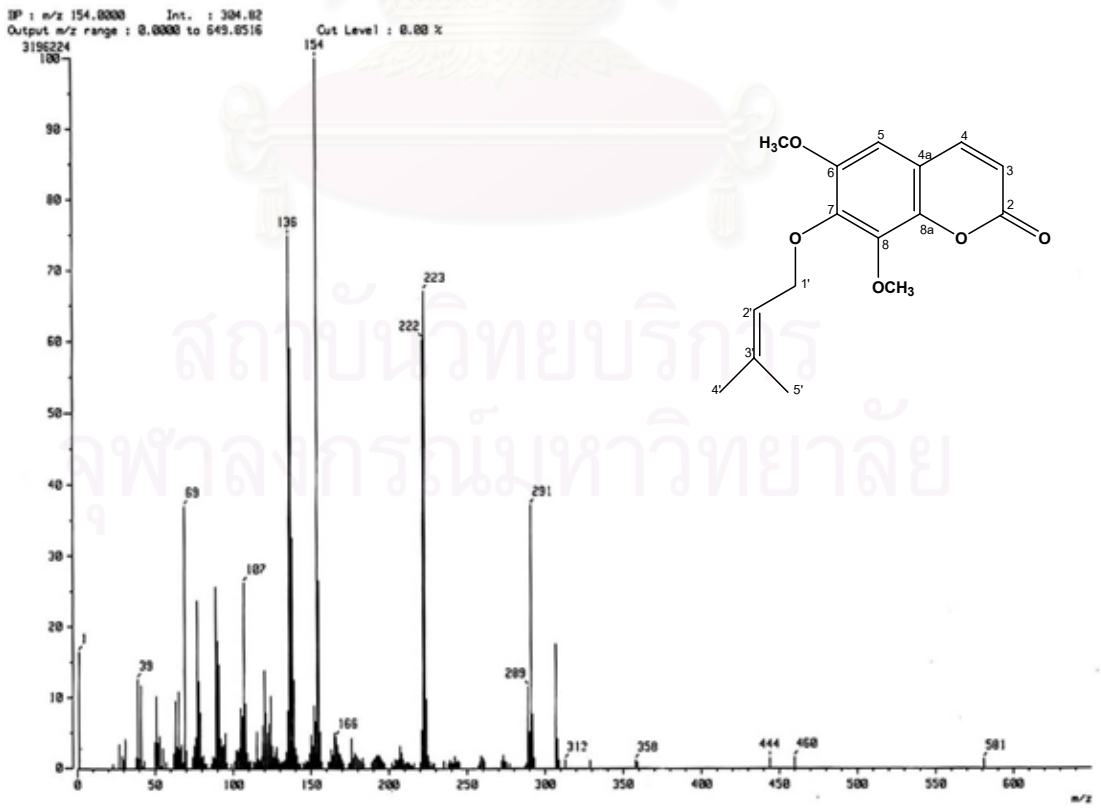

Figure 20 FAB Mass spectrum of compound **PRC3**.

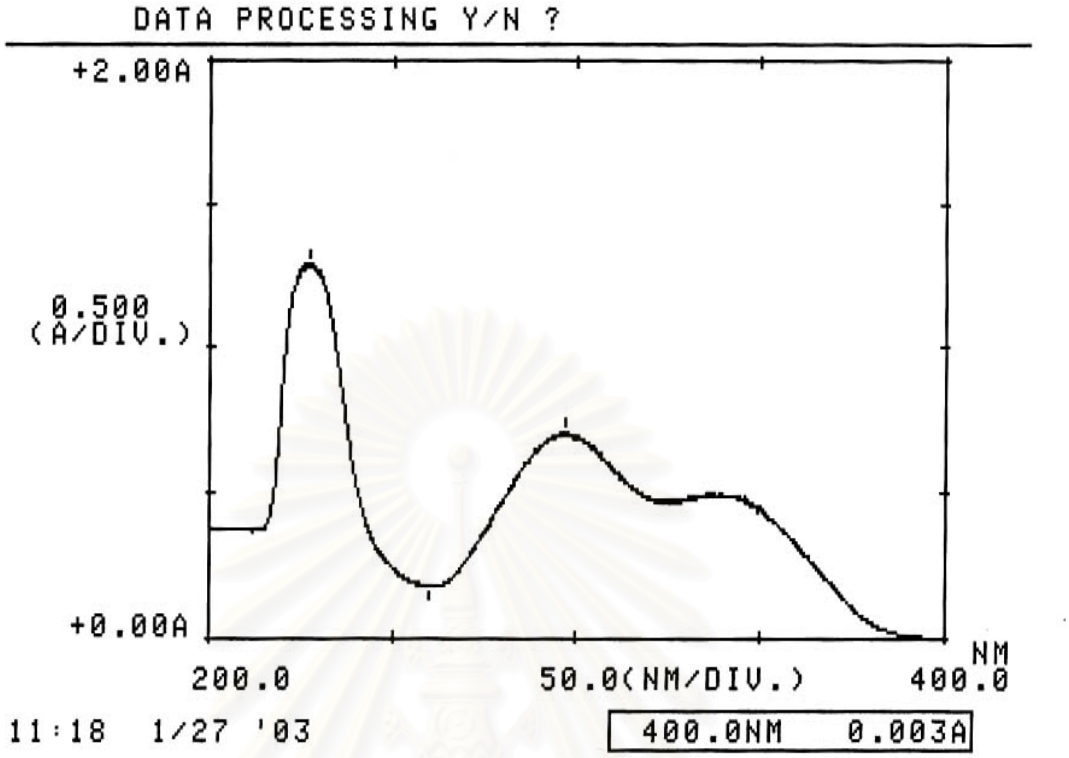

Figure 21 UV spectrum of compound **PRC3** (MeOH).

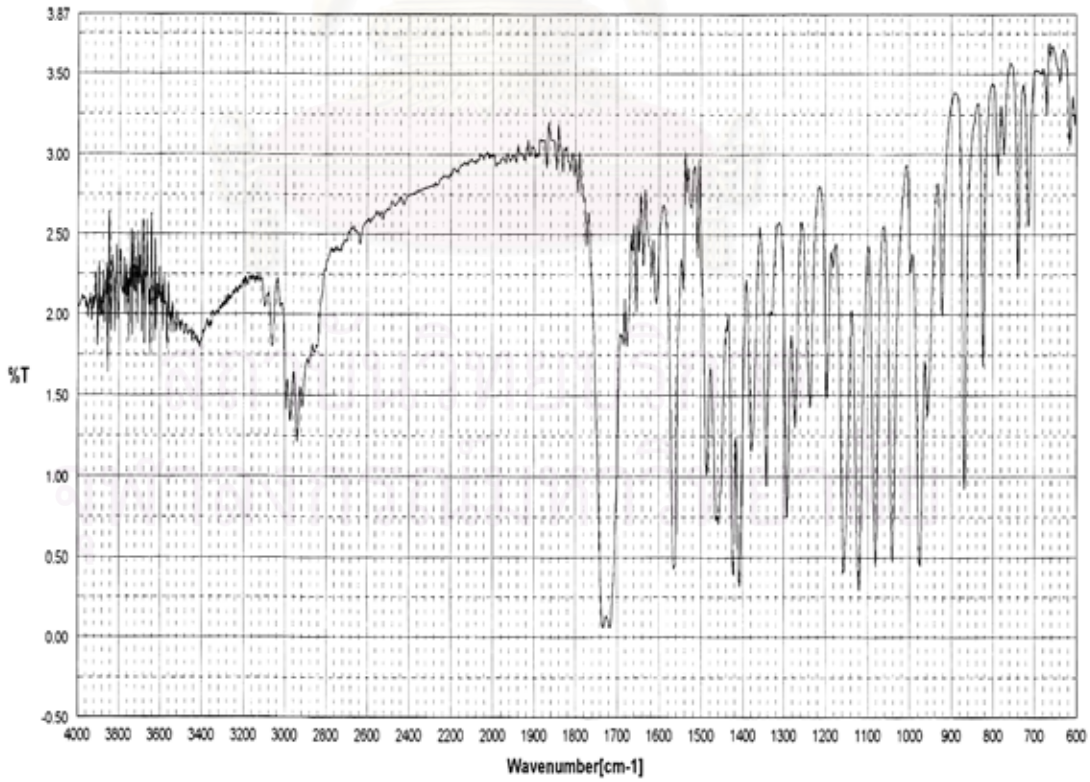

Figure 22 IR spectrum of compound **PRC3** (KBr disc).

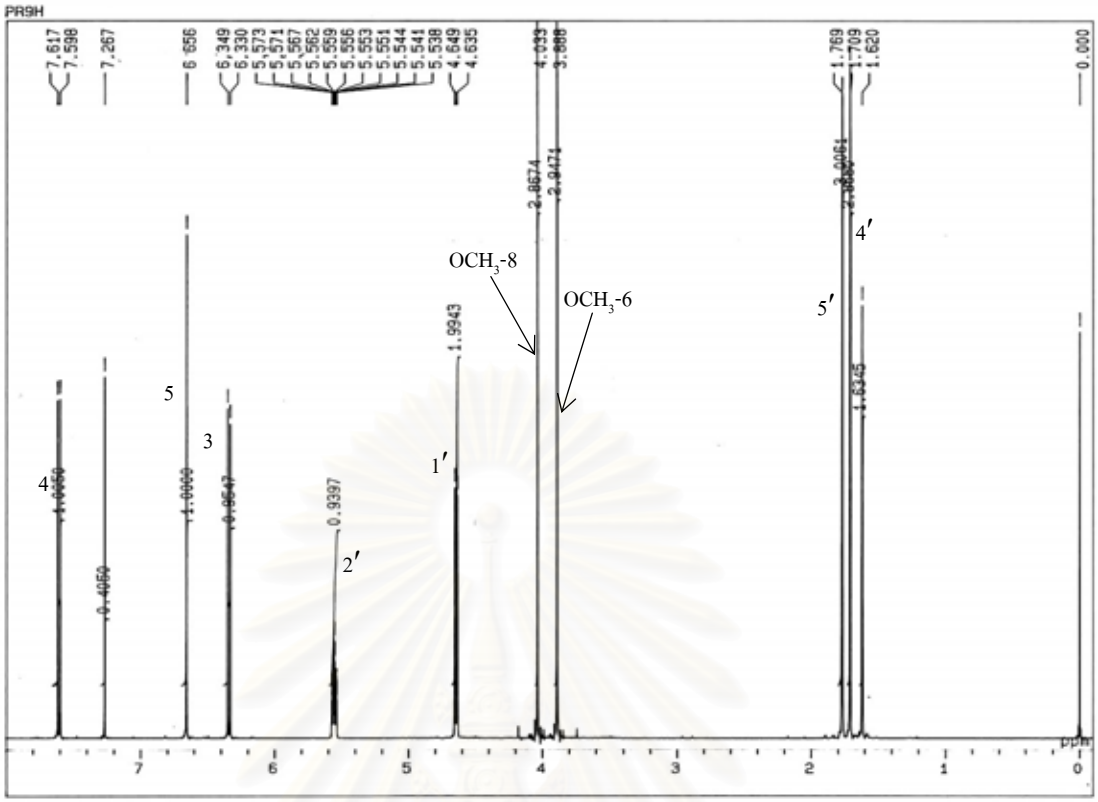

Figure 23 ^{1}H-NMR (500 MHz) spectrum of compound **PRC3** ($CDCl_3$).

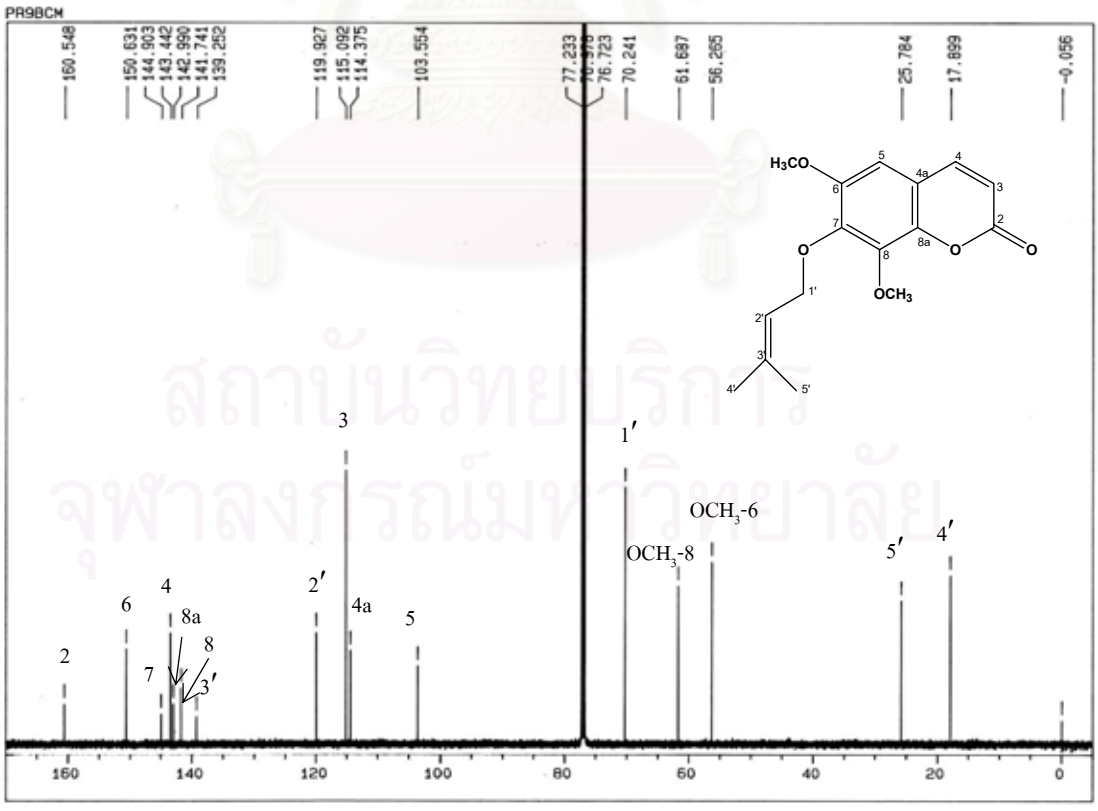

Figure 24 ^{13}C-NMR (125 MHz) spectrum of compound **PRC3** ($CDCl_3$).

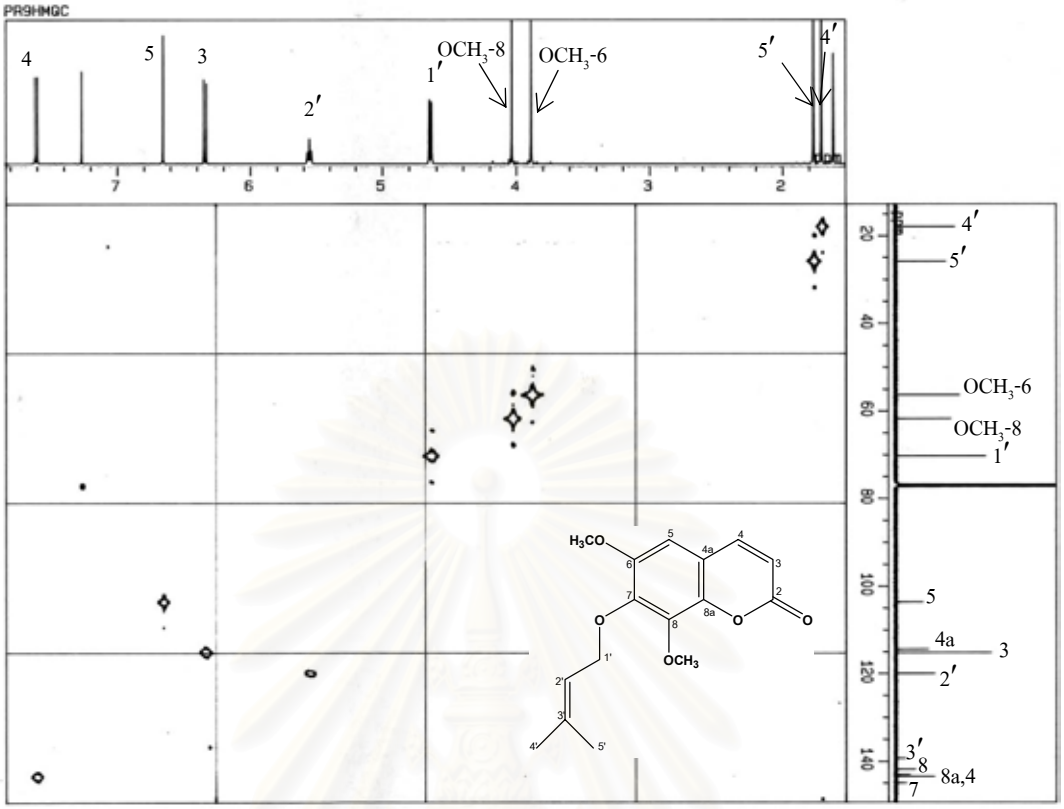

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Figure 25 HMQC spectrum of compound **PRC3** ($CDCl_3$).

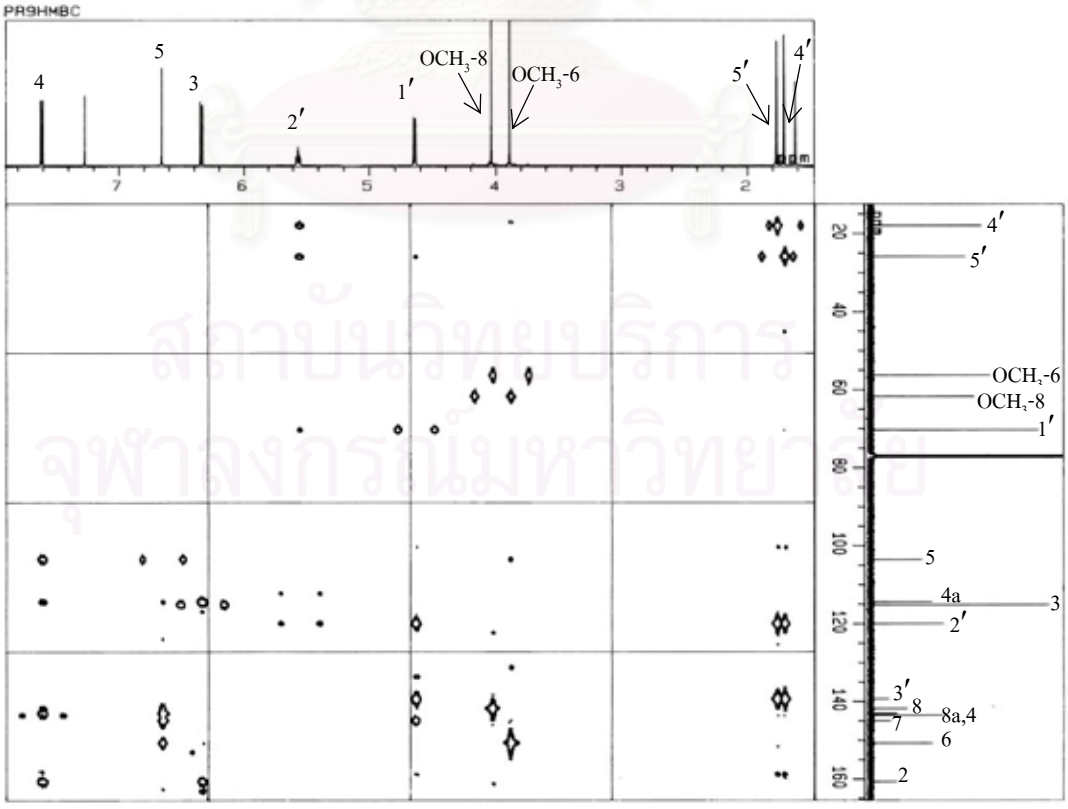

Figure 26 HMBC spectrum of compound **PRC3** ($CDCl_3$).

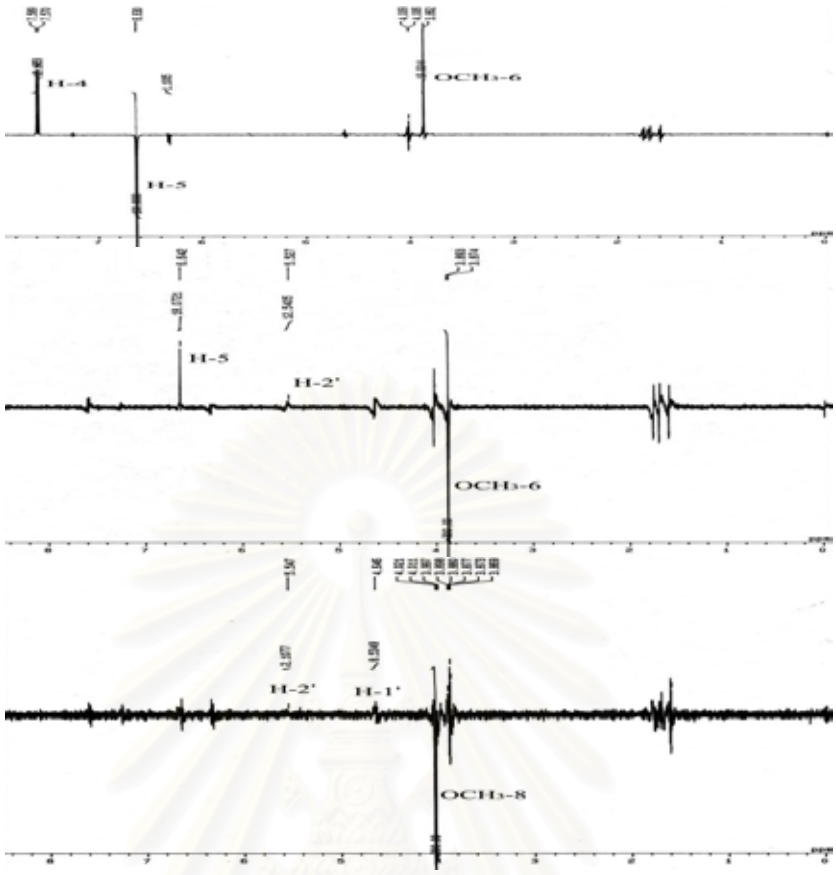

Figure 27 NOE spectra of compound **PRC3** ($CDCl_3$).

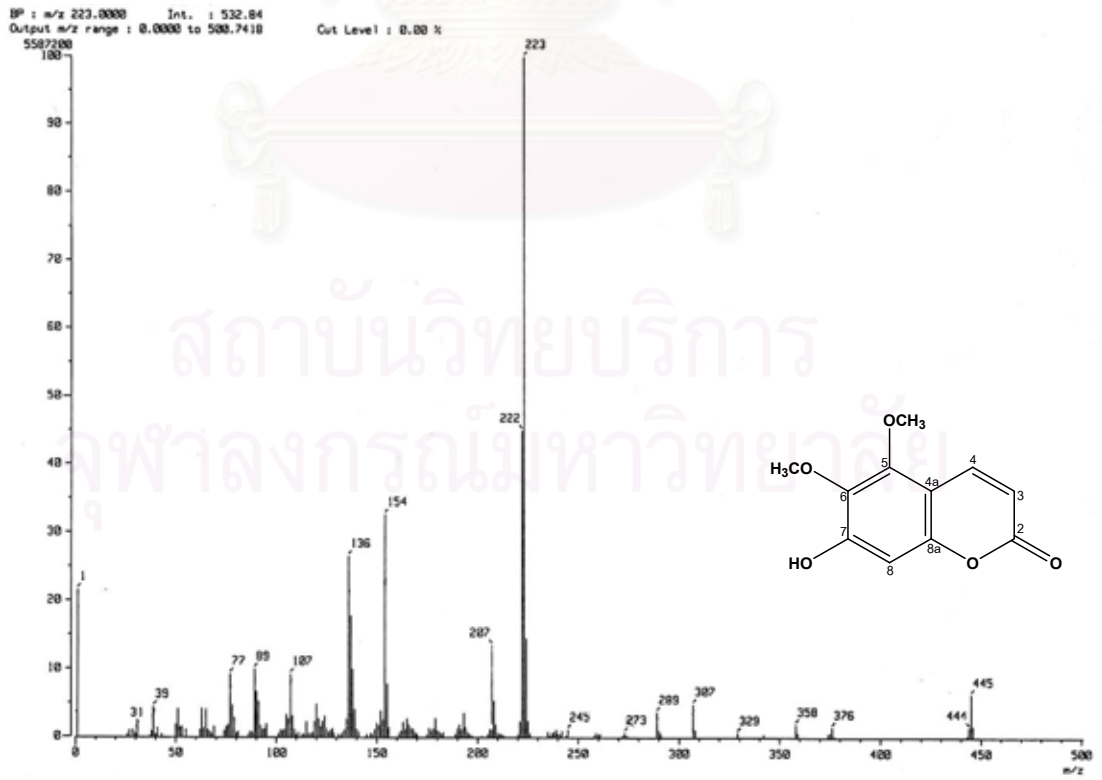

Figure 28 FAB Mass spectrum of compound **PRC4**.

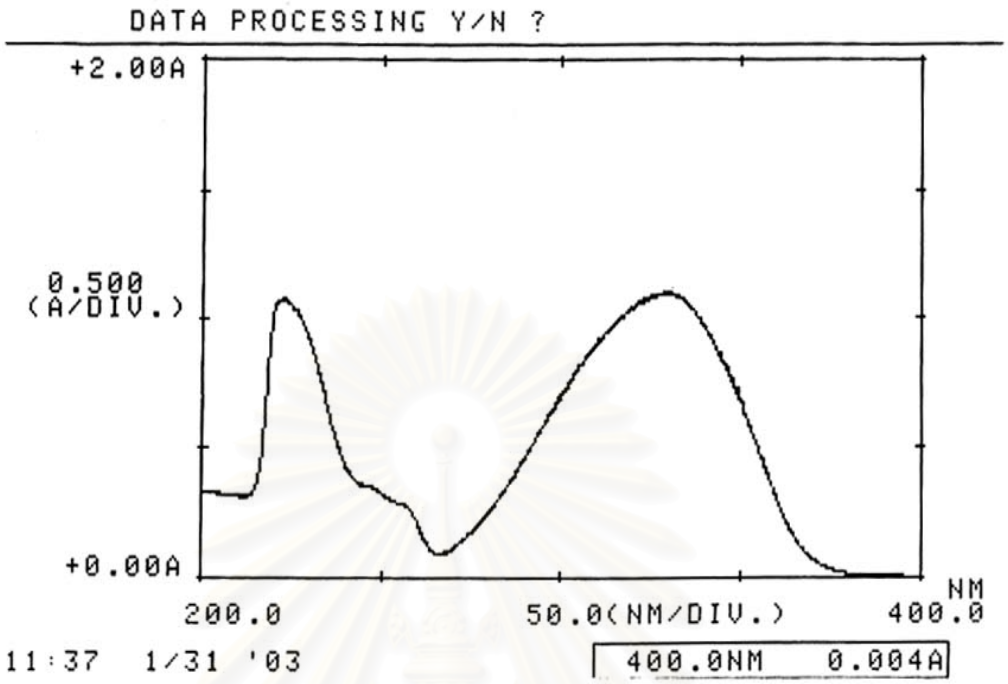

Figure 29 UV spectrum of compound **PRC4** (MeOH).

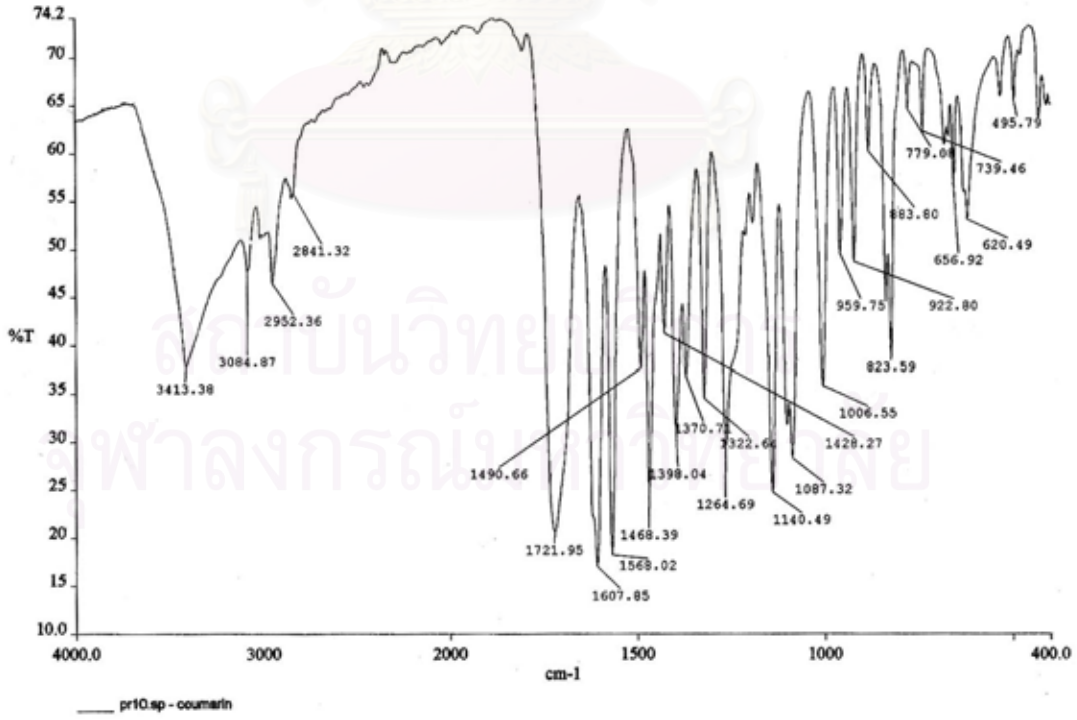

Figure 30 IR spectrum of compound **PRC4** (KBr disc).

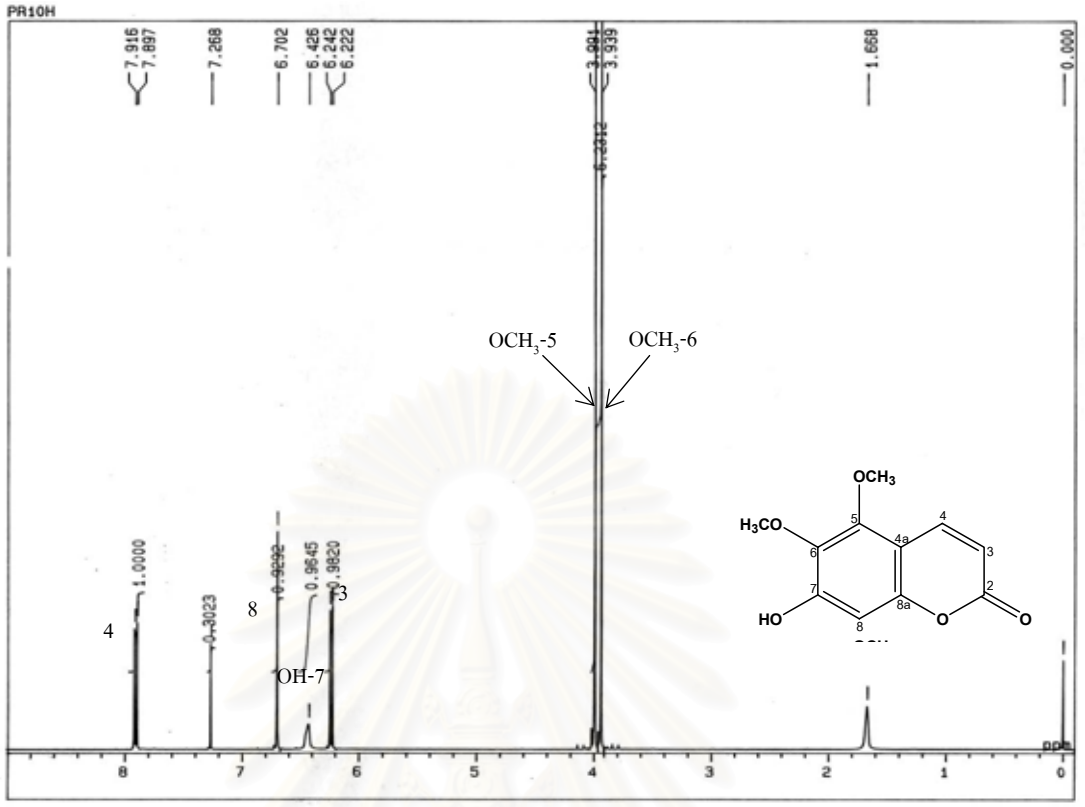

Figure 31 ^{1}H-NMR (500 MHz) spectrum of compound **PRC4** ($CDCl_3$).

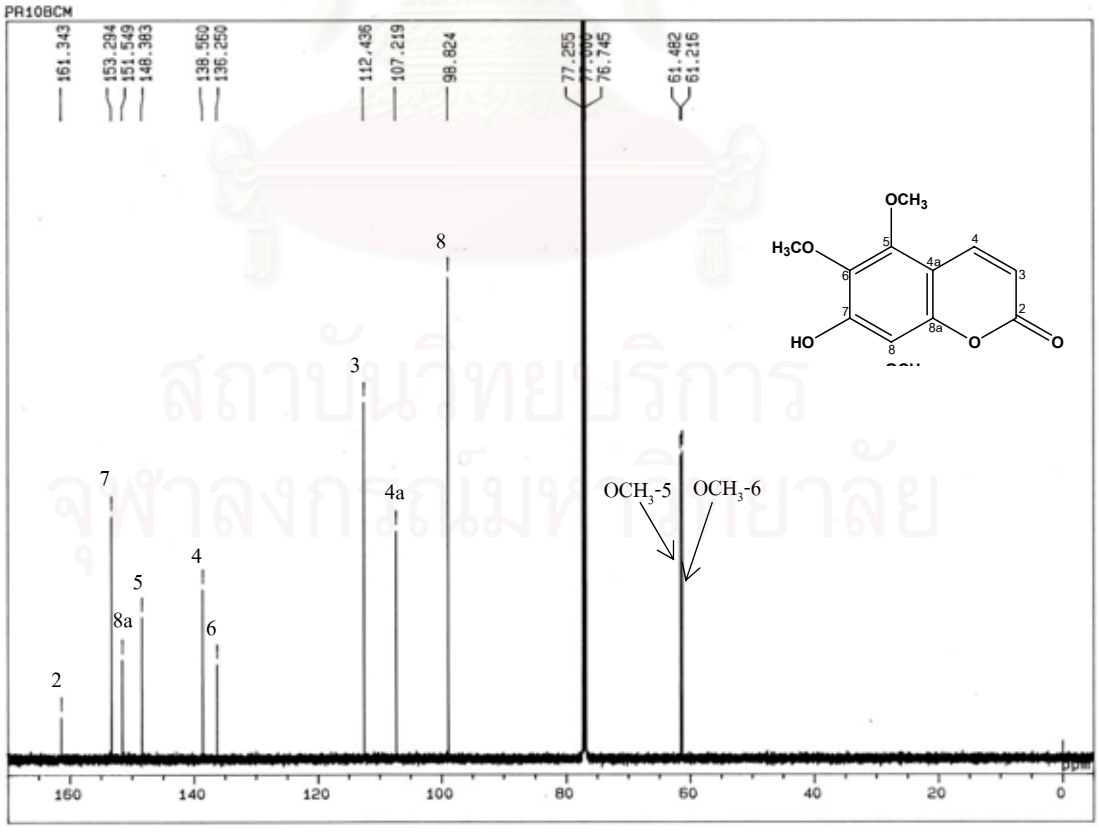

Figure 32 ^{13}C-NMR (125 MHz) spectrum of compound **PRC4** ($CDCl_3$).

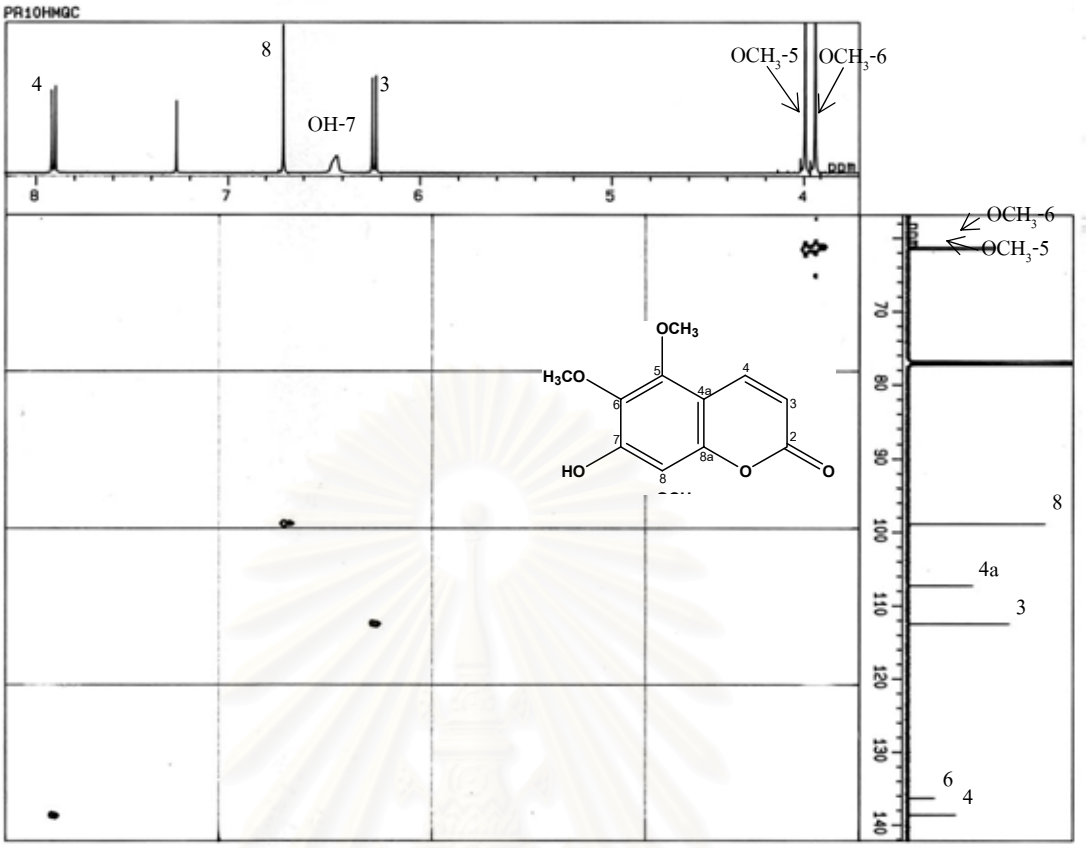

Figure 33 HMQC spectrum of compound **PRC4** ($CDCl_3$)**.**

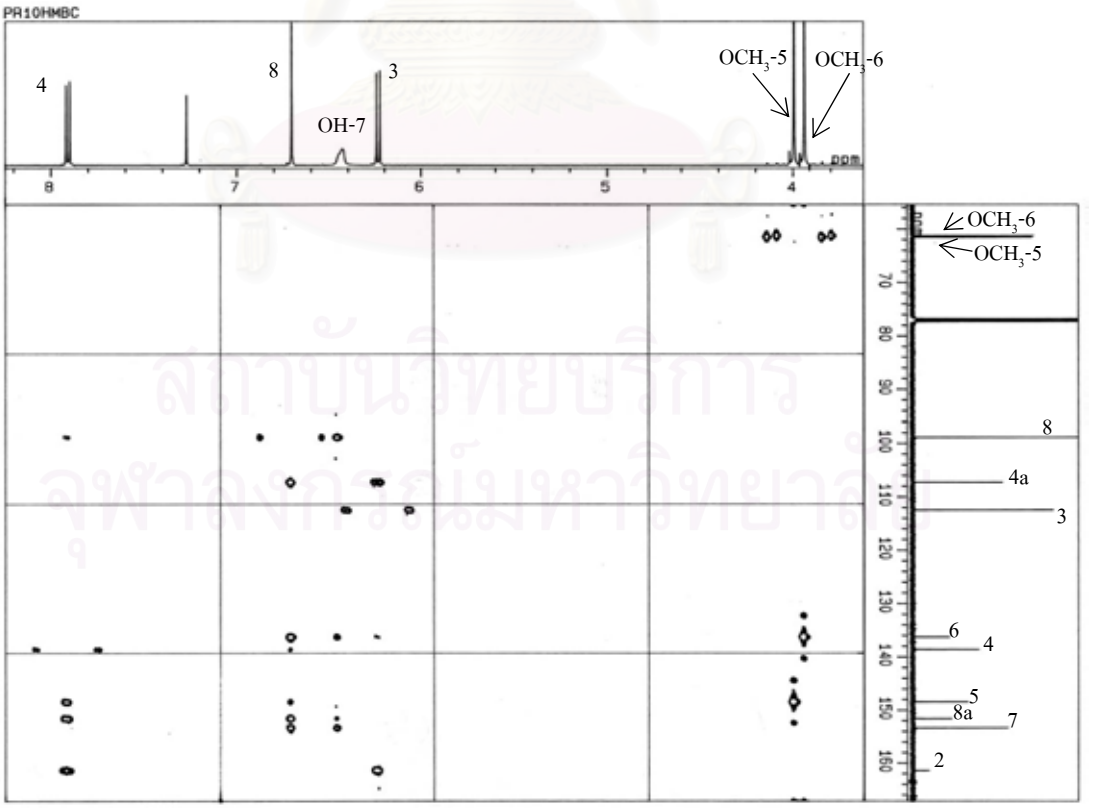

Figure 34 HMBC spectrum of compound **PRC4** ($CDCl_3$).

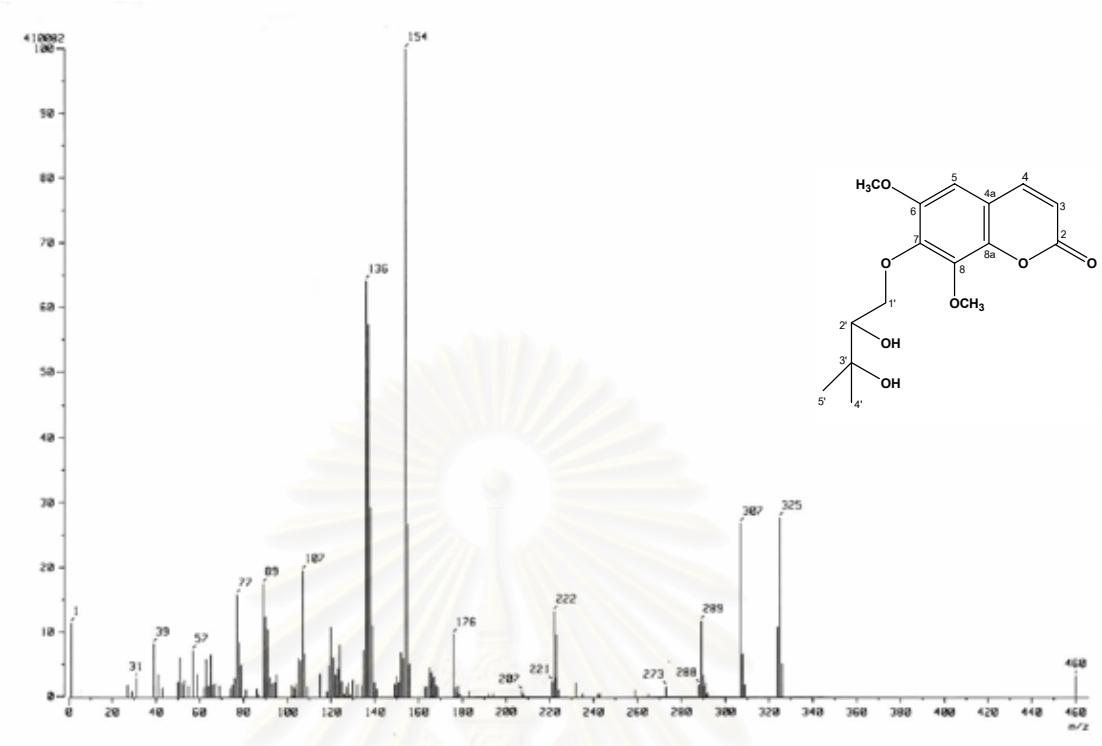

Figure 35 FAB Mass spectrum of compound **PRC5**.

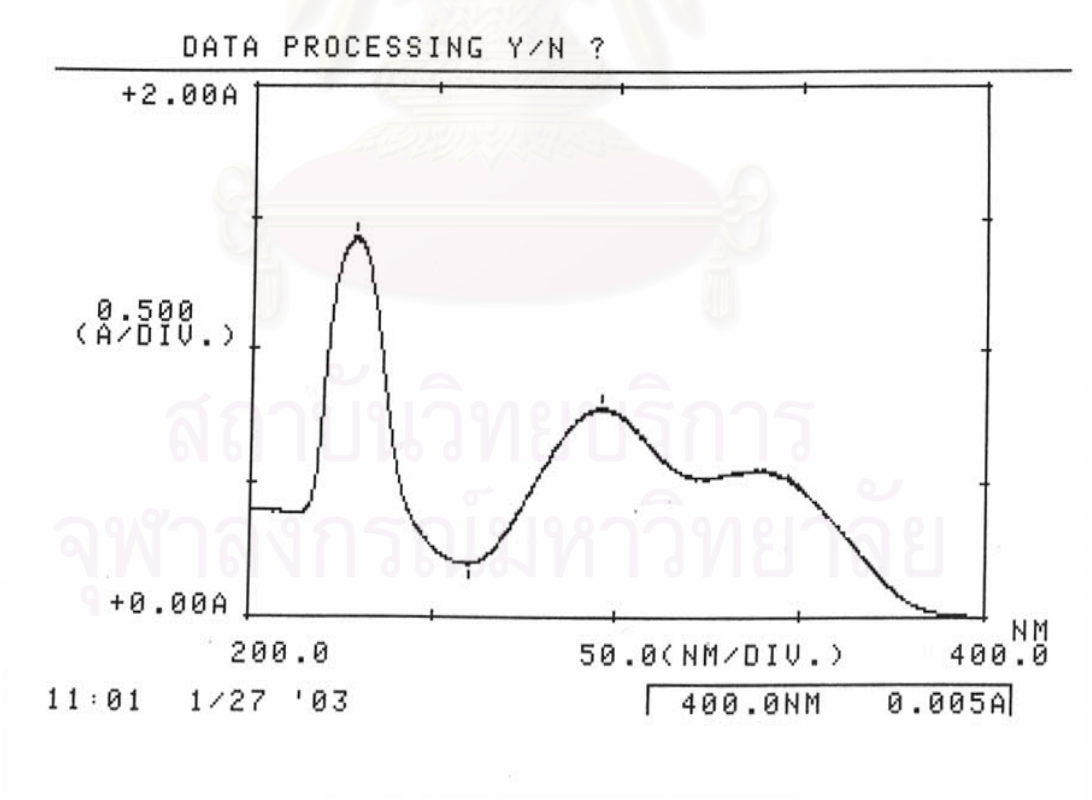

Figure 36 UV spectrum of compound **PRC5** (MeOH).

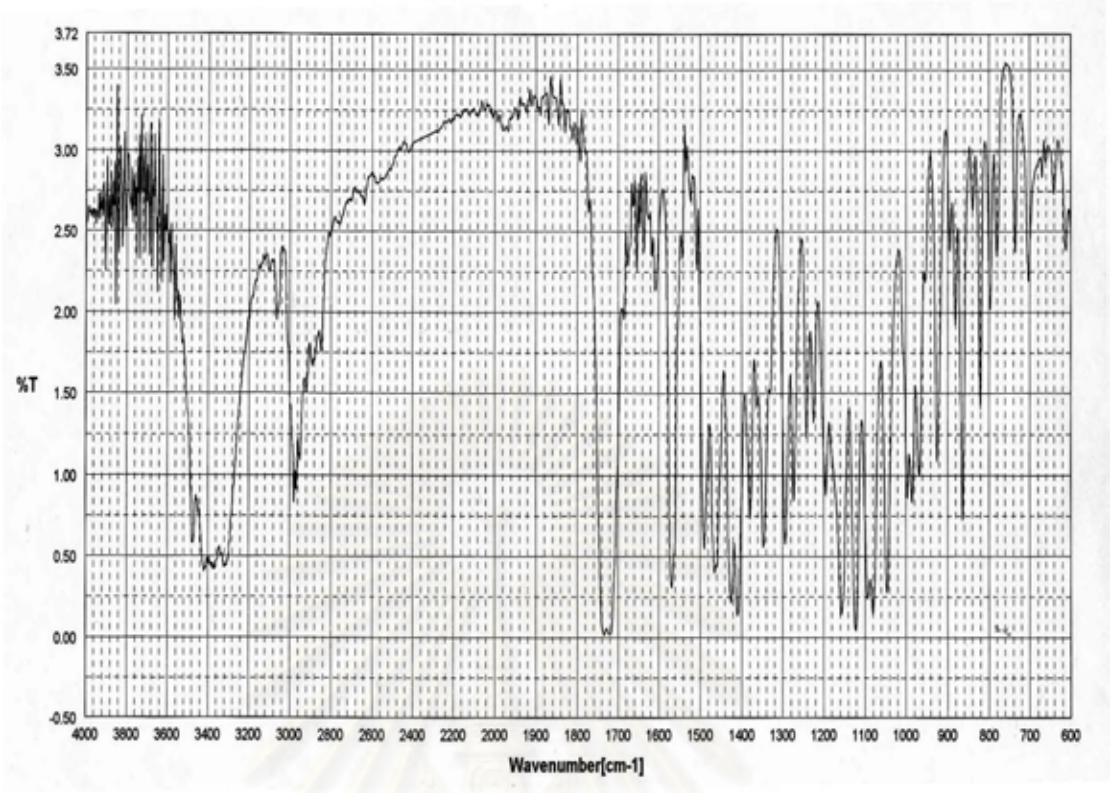

Figure 37 IR spectrum of compound **PRC5** (KBr disc).

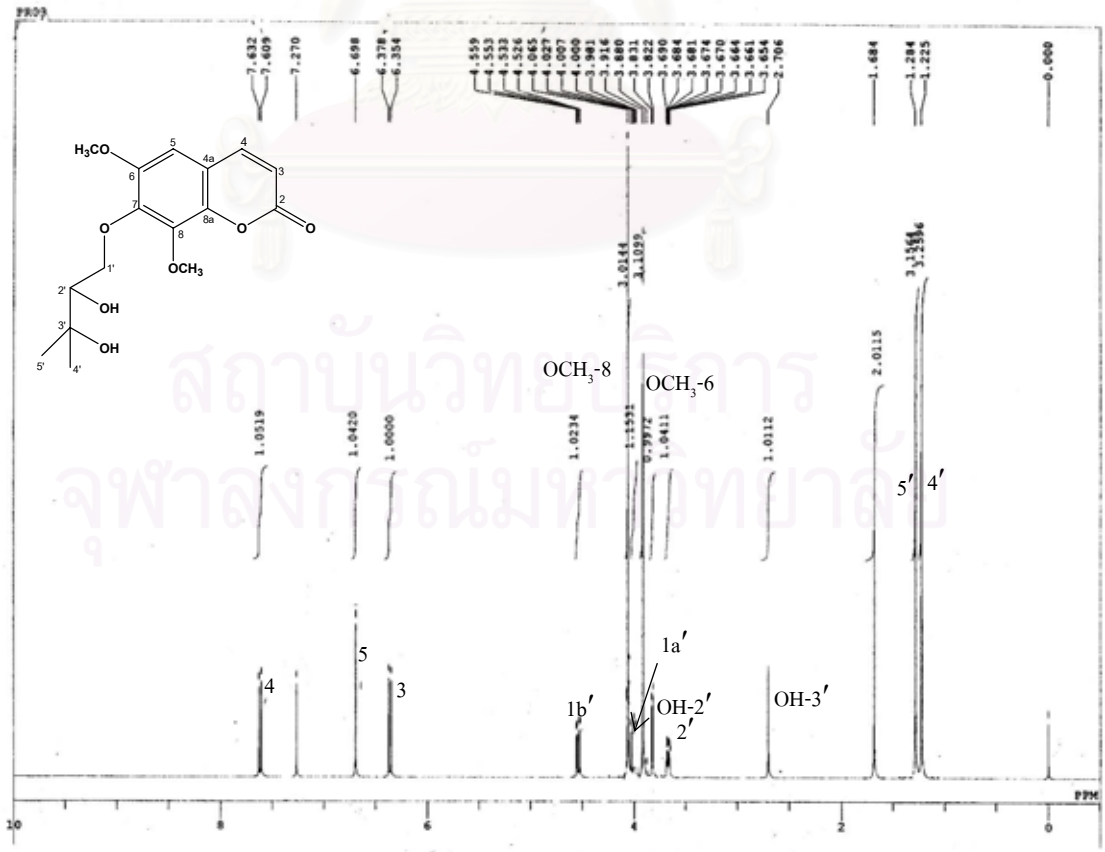

Figure 38 ^{1}H-NMR (500 MHz) spectrum of compound **PRC5** ($CDCl_3$).

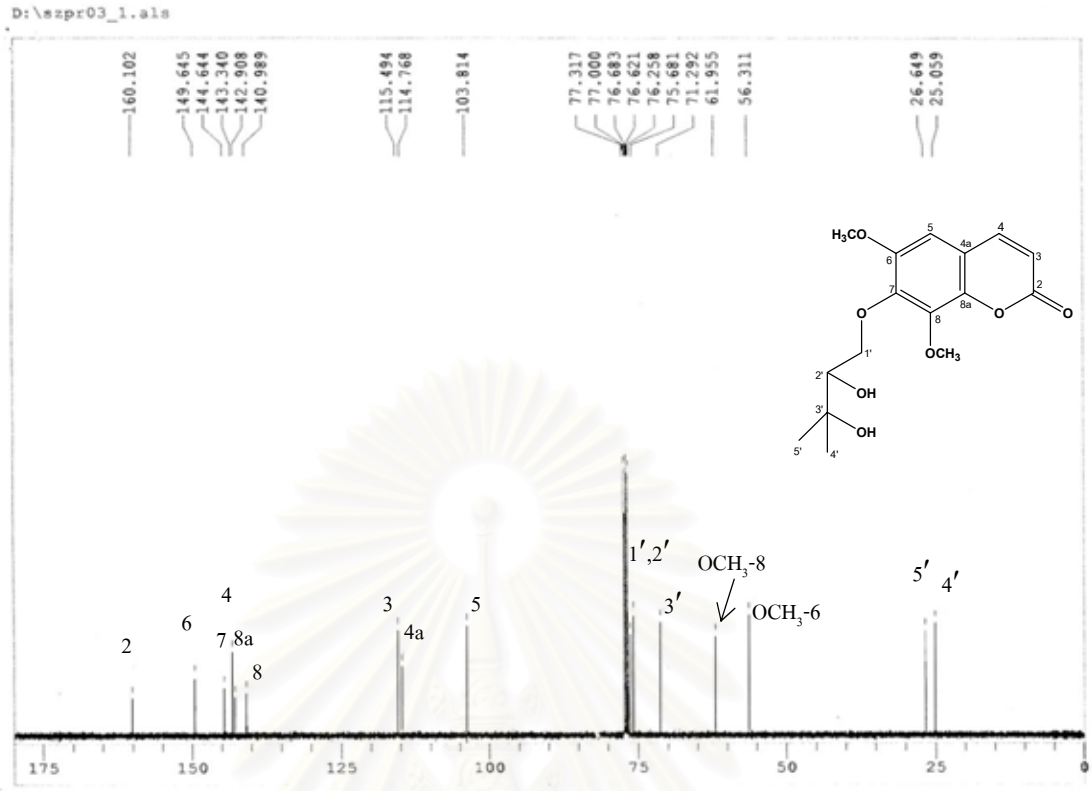

Figure 39 ^{13}C-NMR (125 MHz) spectrum of compound **PRC5** ($CDCl_3$).

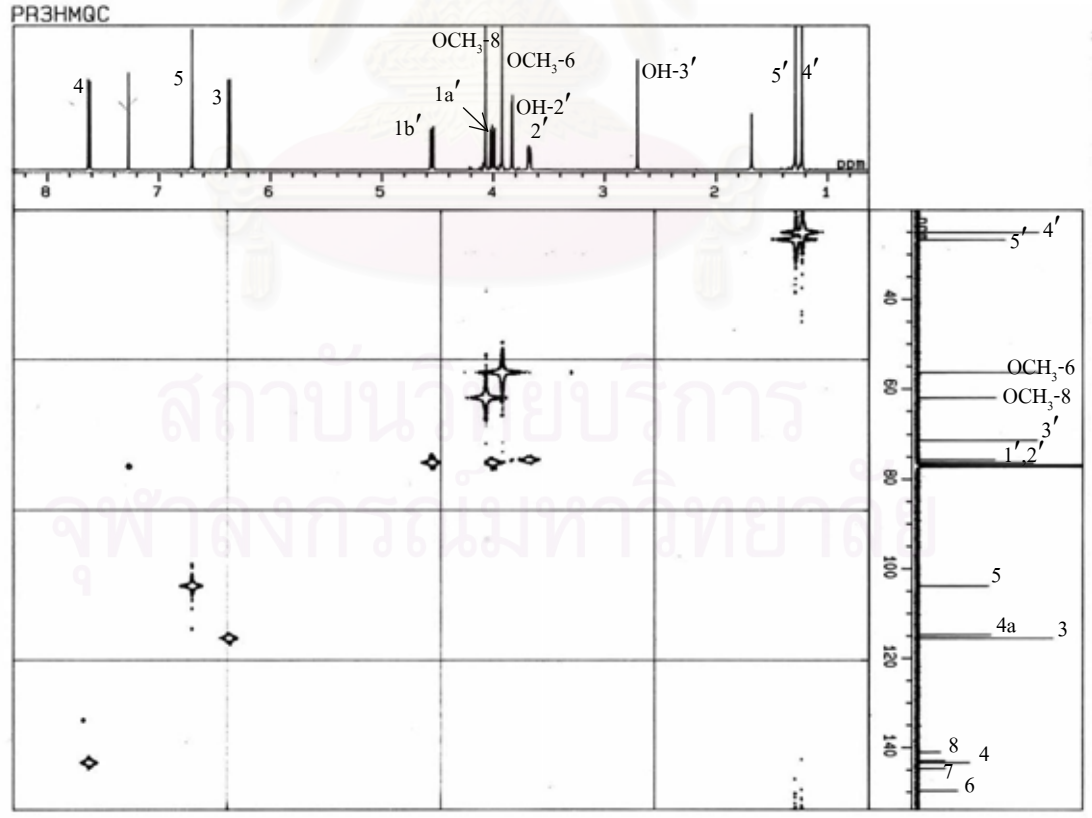

Figure 40 HMQC spectrum of compound **PRC5** ($CDCl_3$).

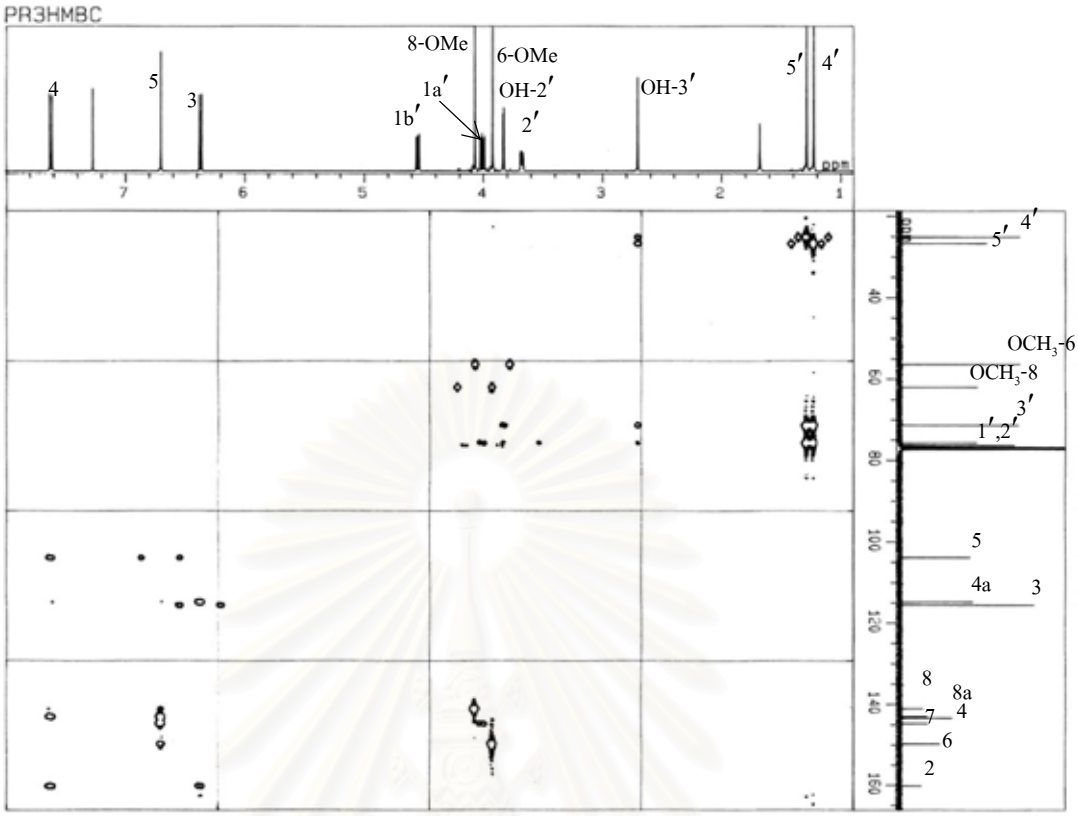

Figure 41 HMBC spectrum of compound **PRC5** ($CDCl_3$).

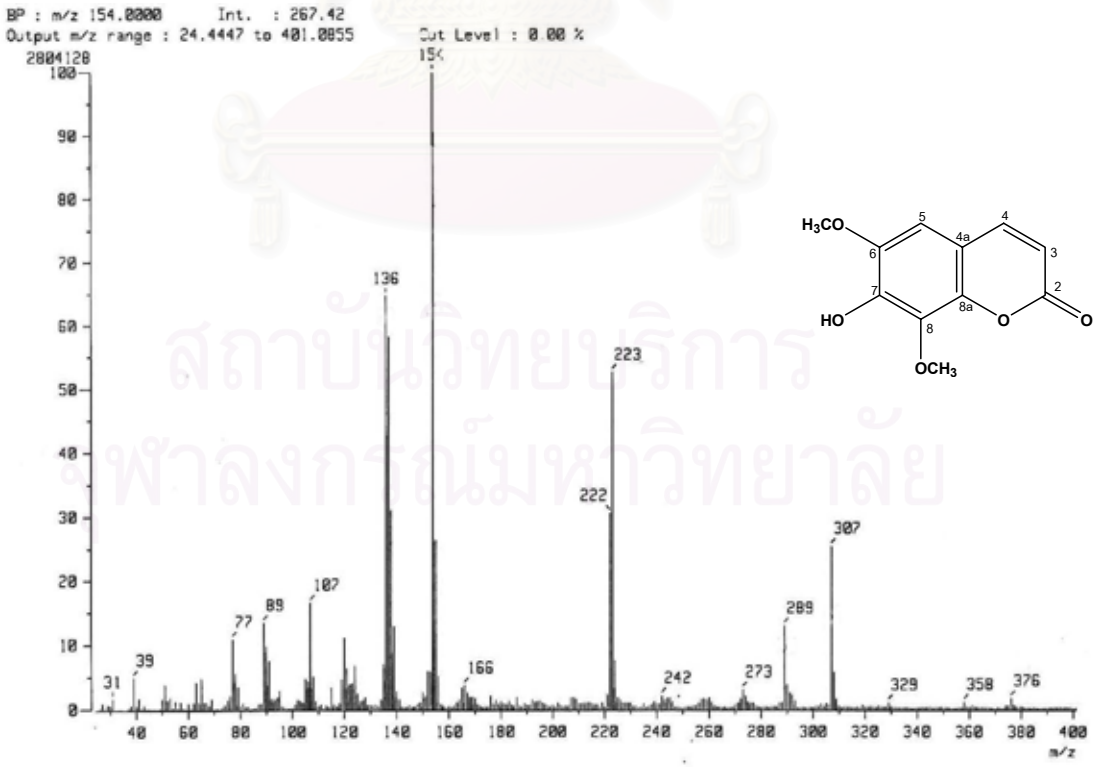

Figure 42 FAB Mass spectrum of compound **PRC6**.

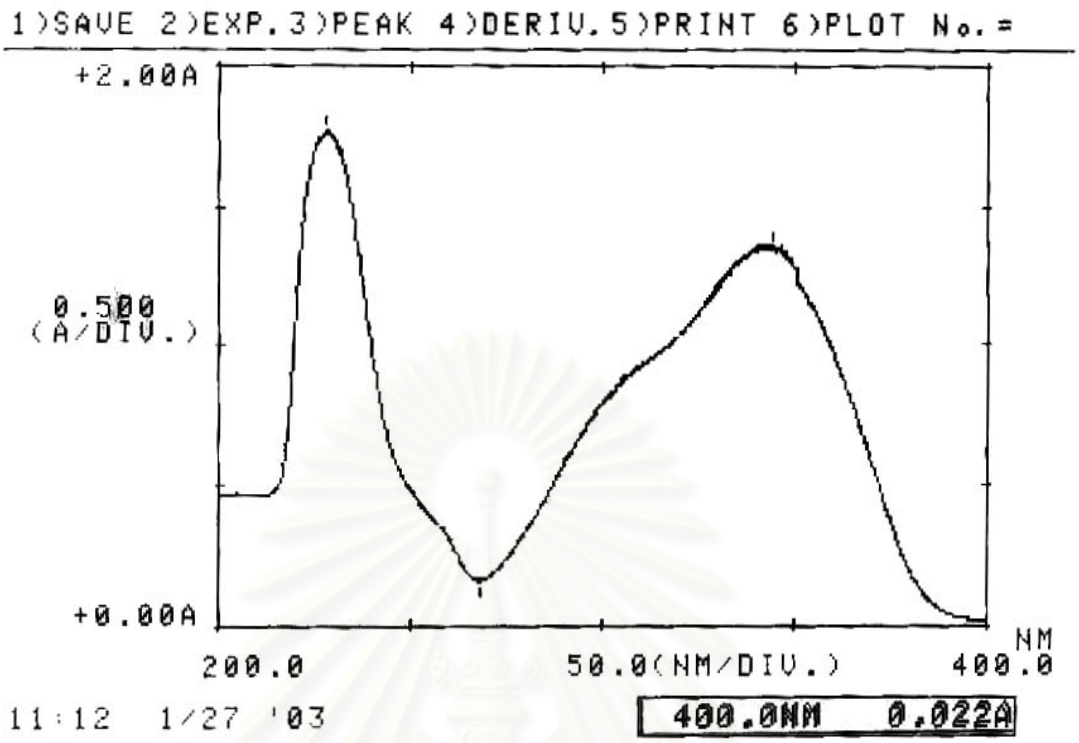

Figure 43 UV spectrum of compound **PRC6** (MeOH).

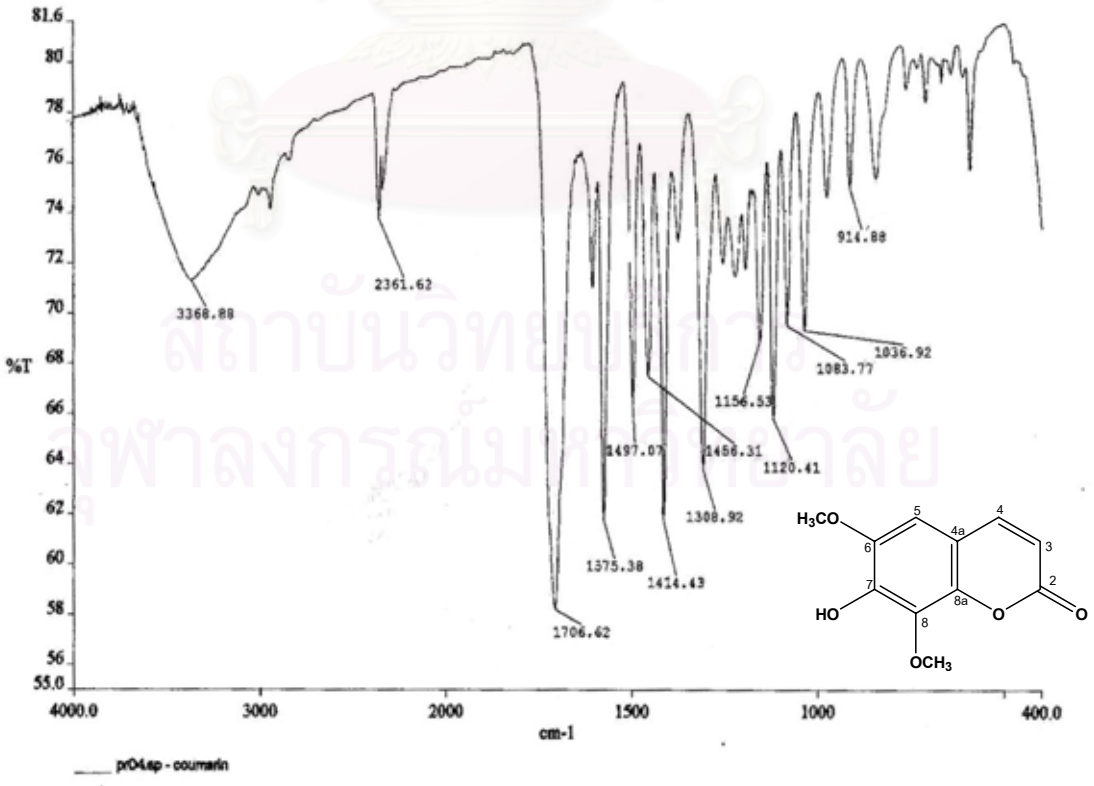

Figure 44 IR spectrum of compound **PRC6** (KBr disc).

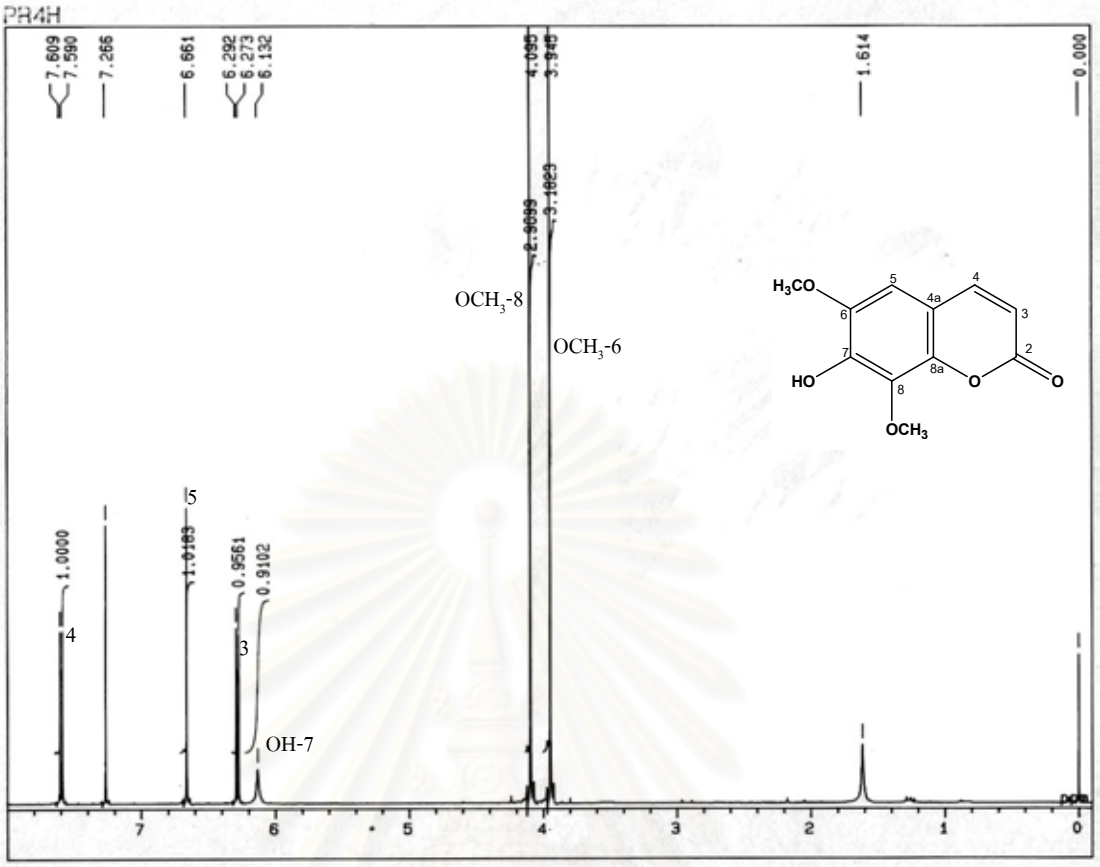

Figure 45 ^{1}H-NMR (500 MHz) spectrum of compound **PRC6** ($CDCl_3$).

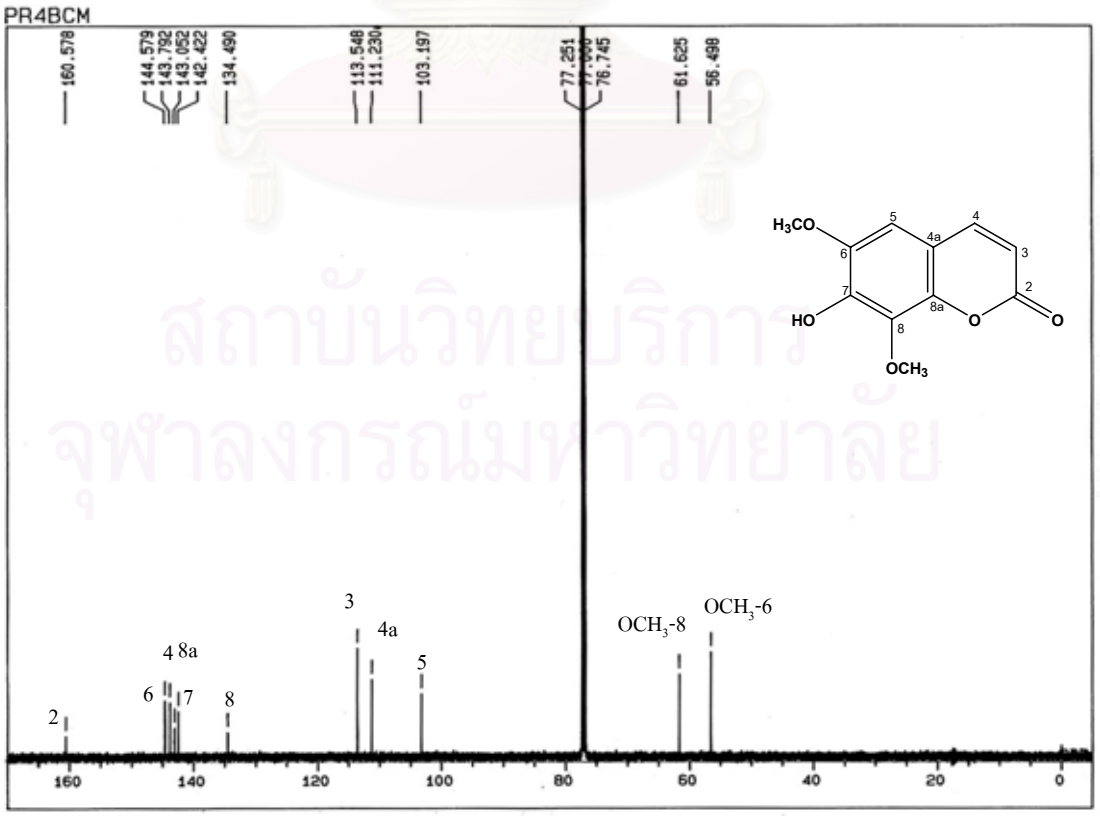

Figure 46 ^{13}C-NMR (125 MHz) spectrum of compound **PRC6** ($CDCl_3$).

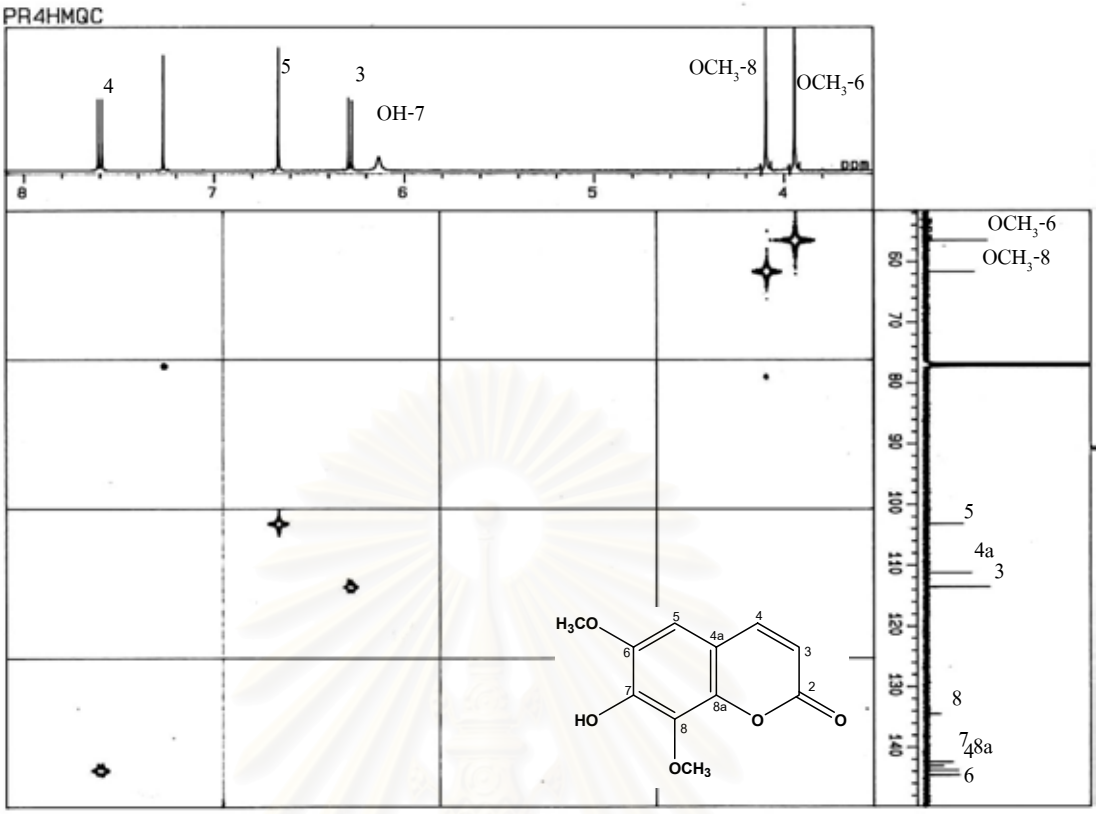

Figure 47 HMQC spectrum of compound **PRC6** ($CDCl_3$).

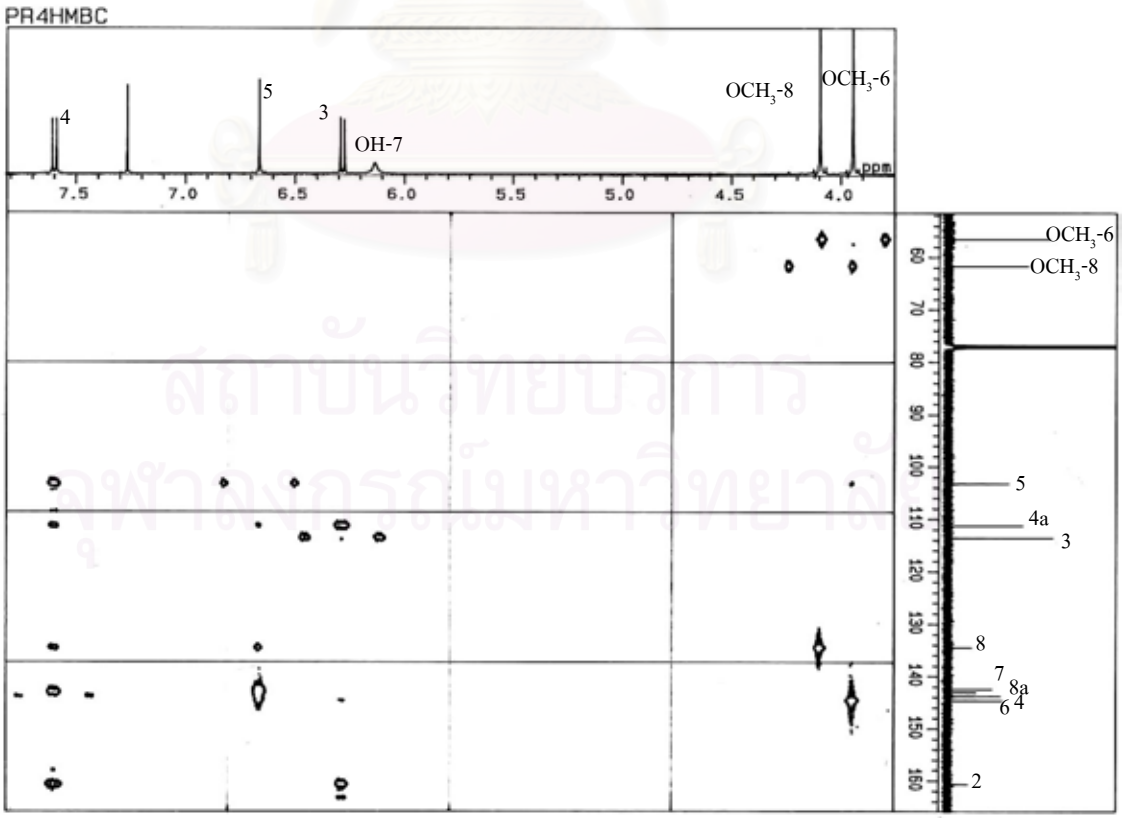

Figure 48 HMBC spectrum of compound **PRC6** ($CDCl_3$).

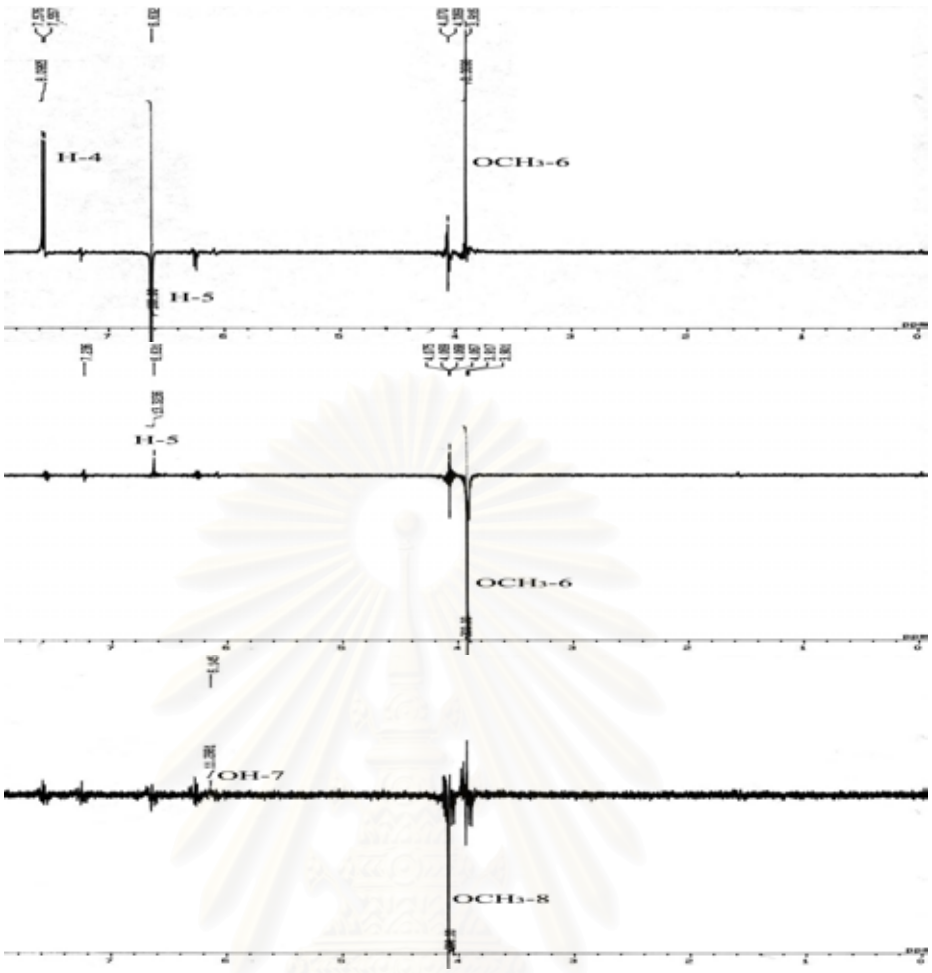

Figure 49 NOE spectra of compound **PRC6** ($CDCl_3$).

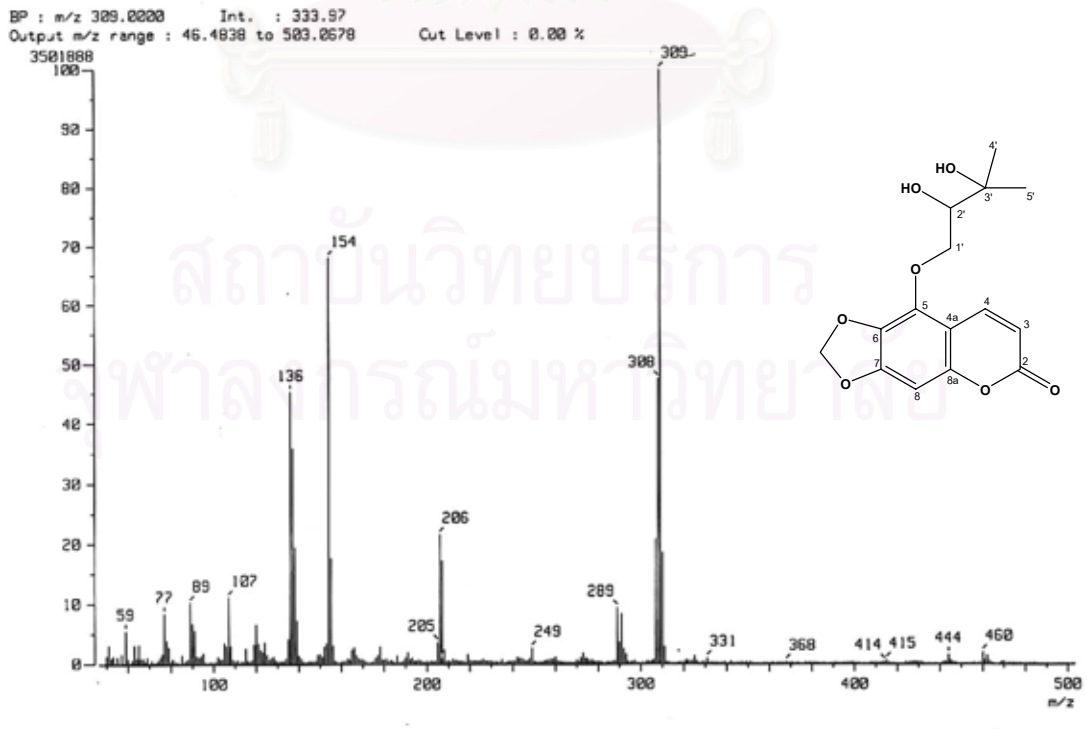

Figure 50 FAB Mass spectrum of compound **PRC7**.

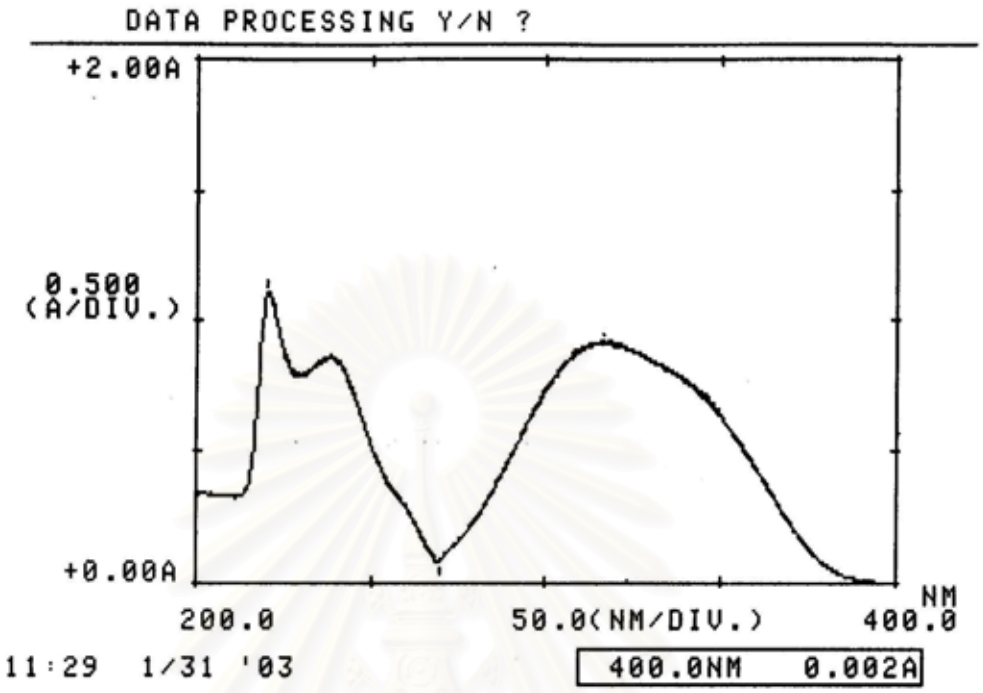

Figure 51 UV spectrum of compound **PRC7** (MeOH).

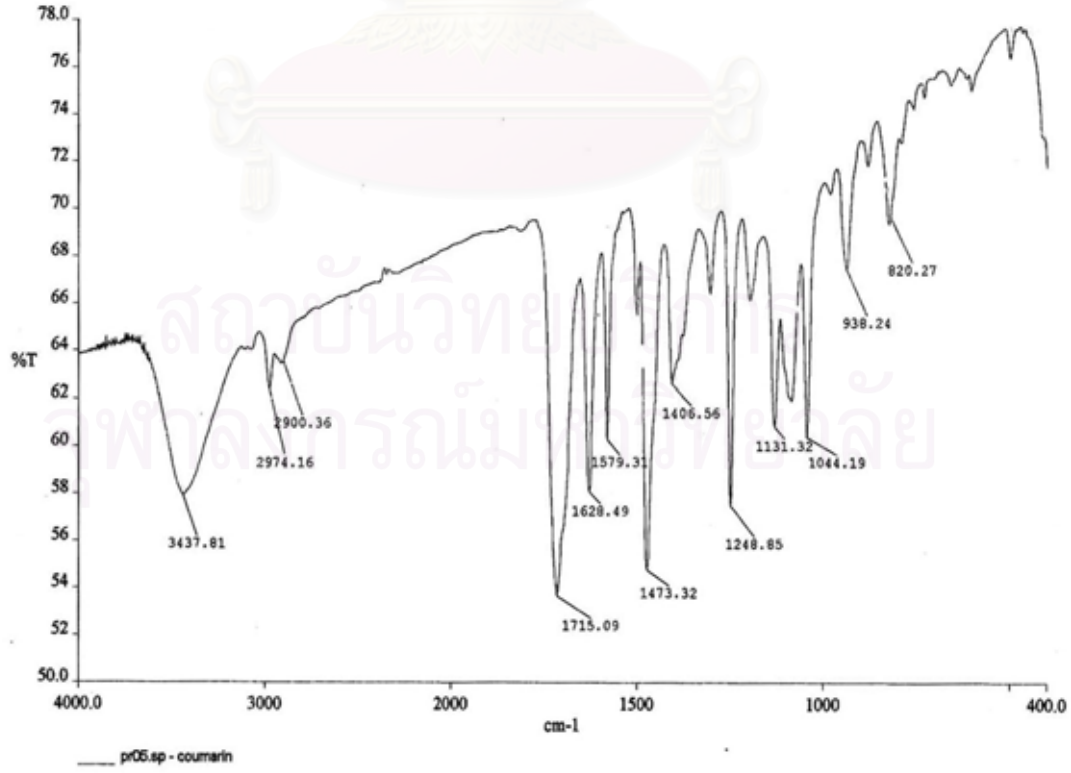

Figure 52 IR spectrum of compound **PRC7** (KBr disc).

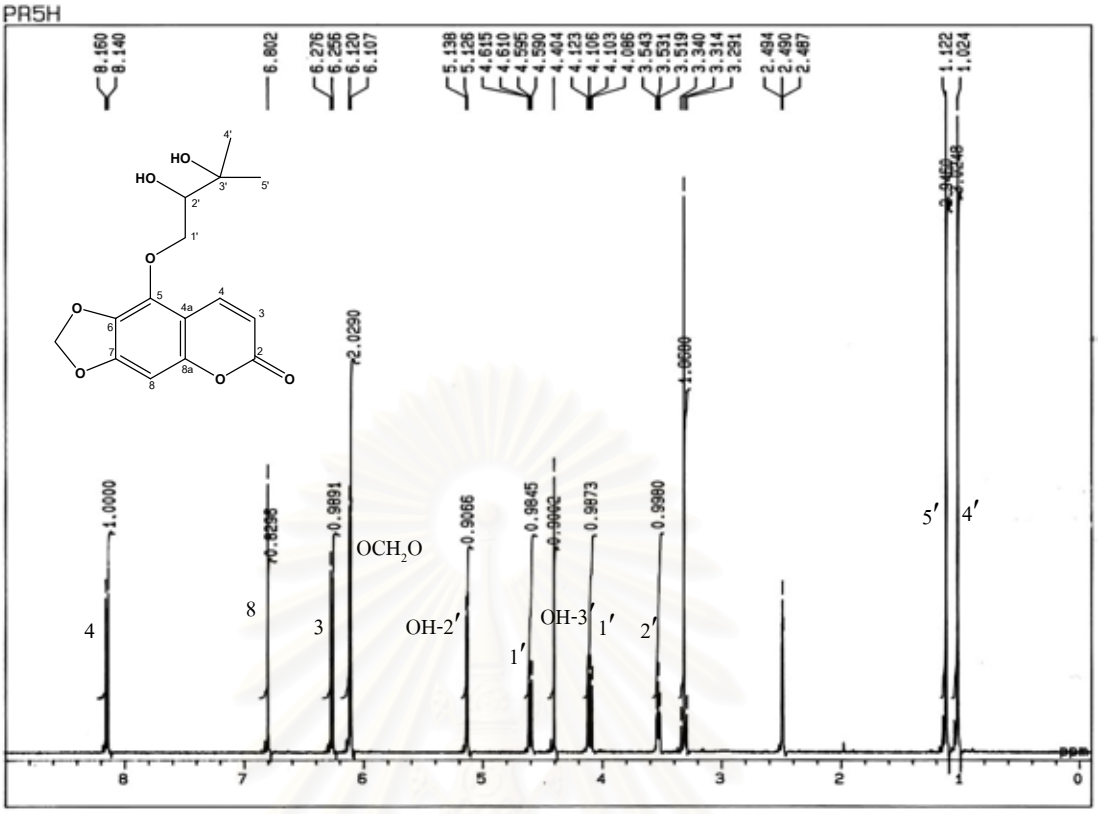

Figure 53 ^{1}H-NMR (500 MHz) spectrum of compound **PRC7** (DMSO-d_6).

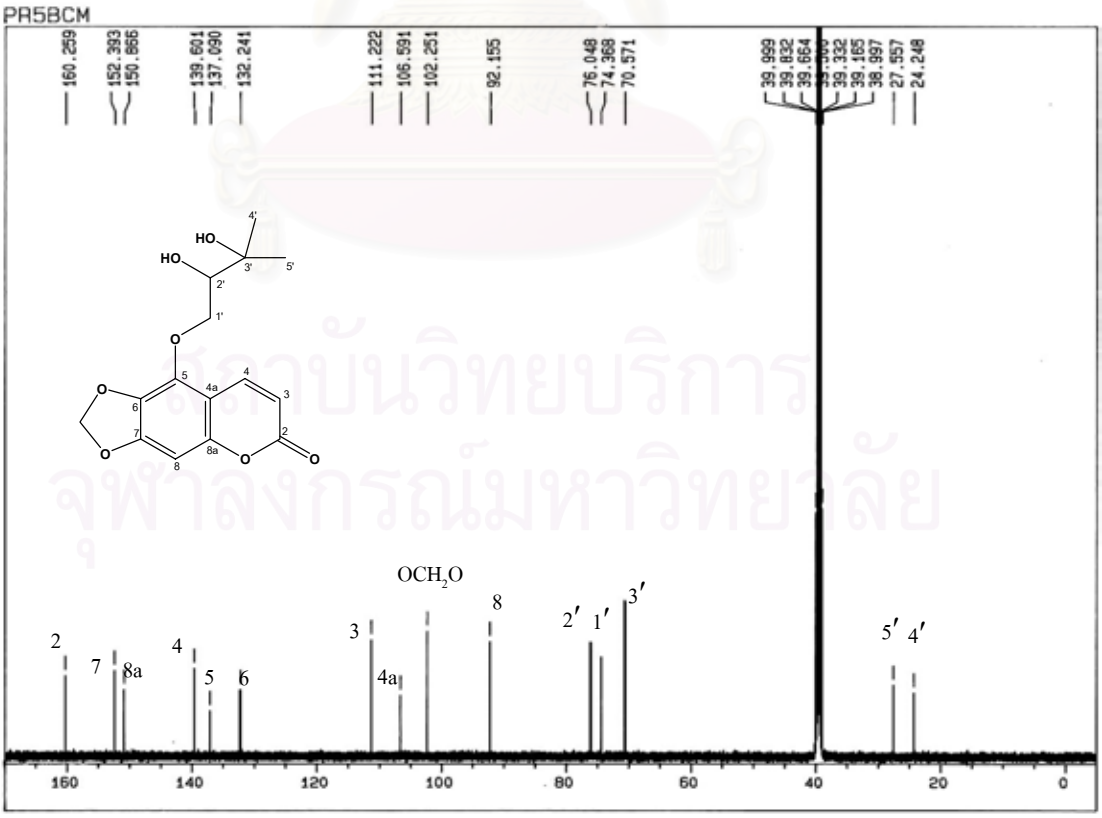

Figure 54 ^{13}C-NMR (125 MHz) spectrum of compound **PRC7** (DMSO-d_6).

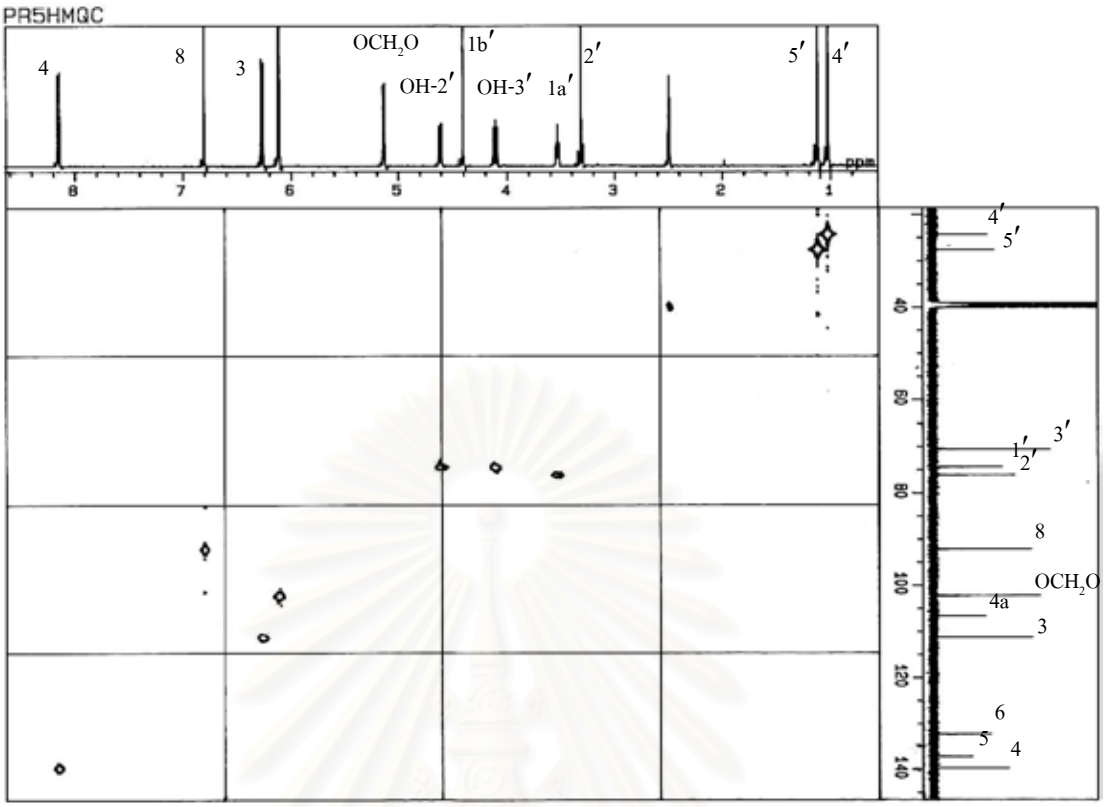

Figure 55 HMQC spectrum of compound **PRC7** (DMSO-d_6).

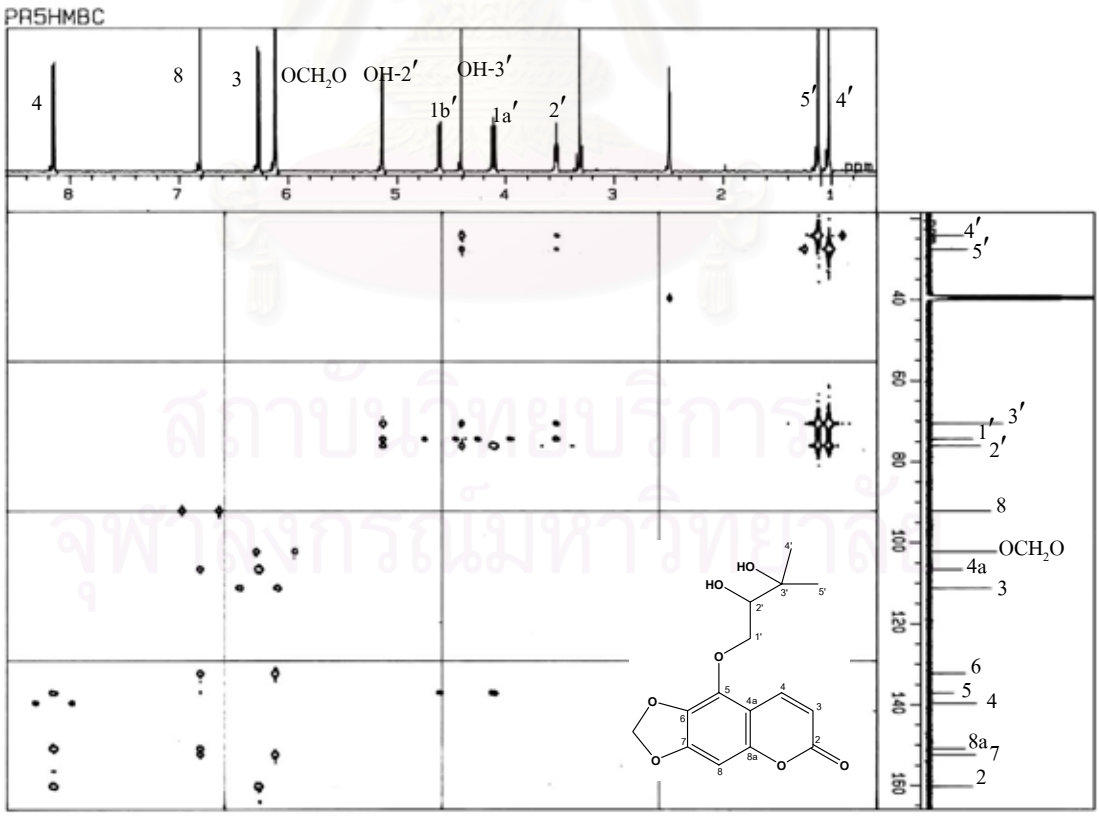

Figure 56 HMBC spectrum of compound **PRC7** (DMSO-d_6).

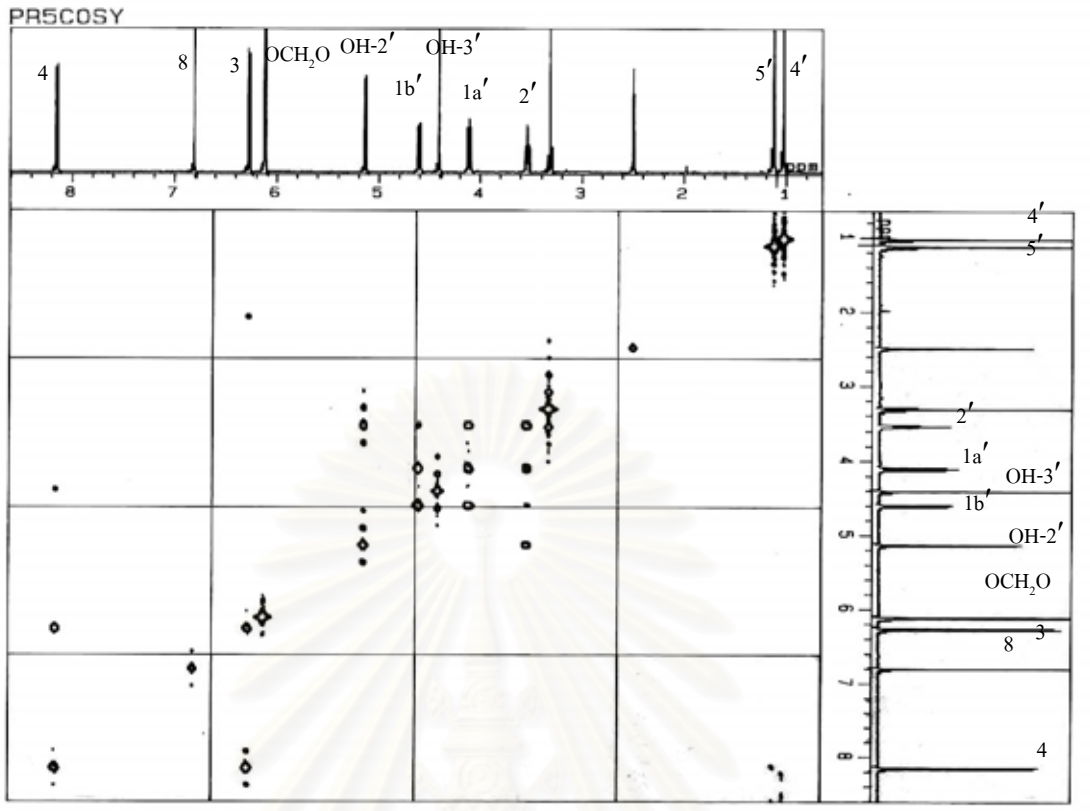

Figure 57 ^{1}H-^{1}H COSY spectrum of compound **PRC7** (DMSO-d_6).

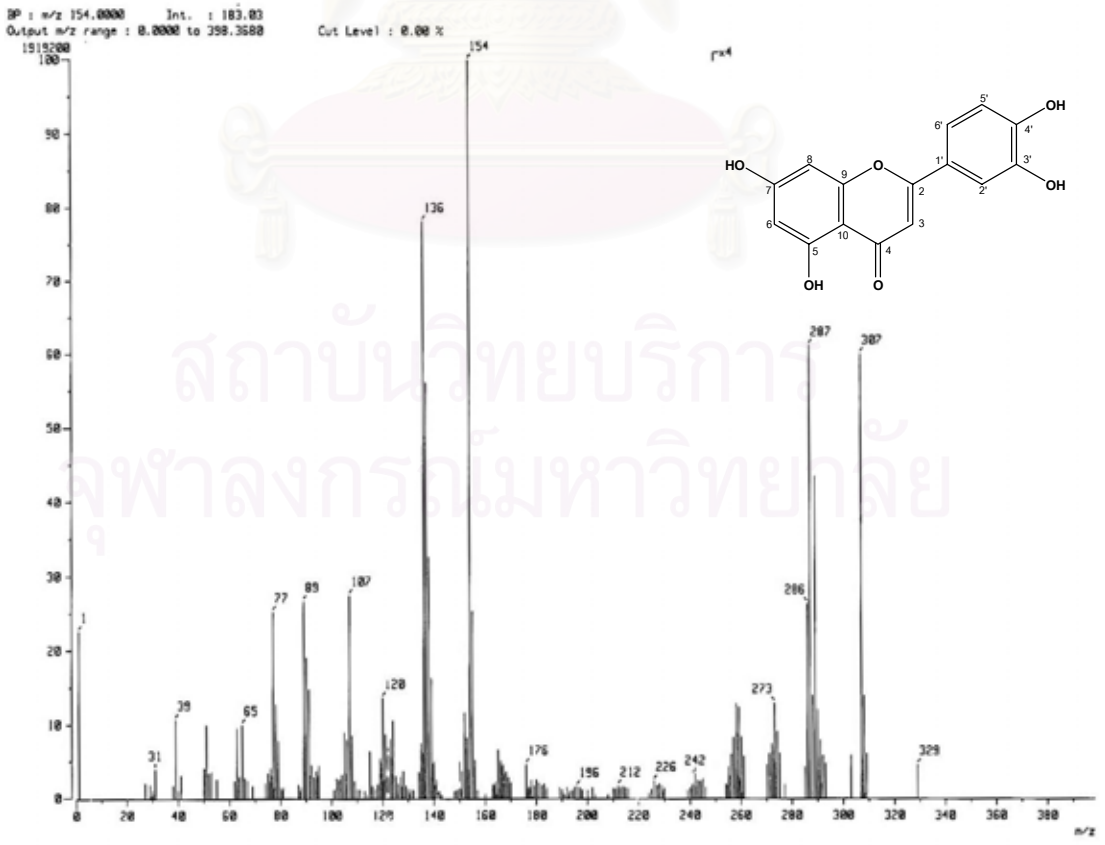

Figure 58 FAB Mass spectrum of compound **PRB8**.

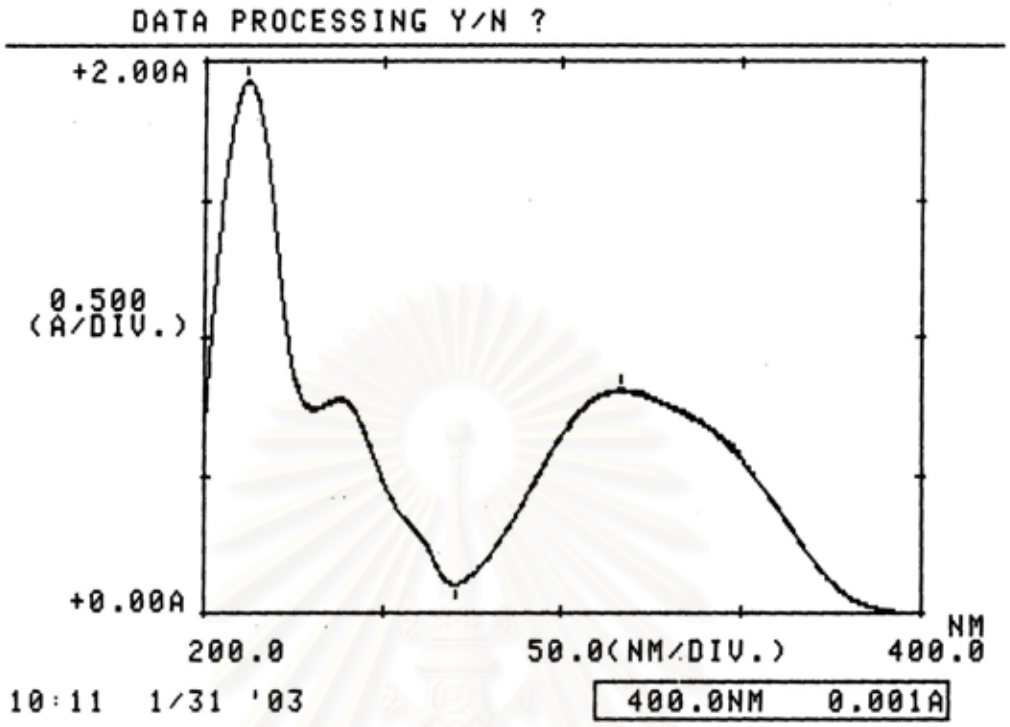

Figure 59 UV spectrum of compound **PRB8** (MeOH).

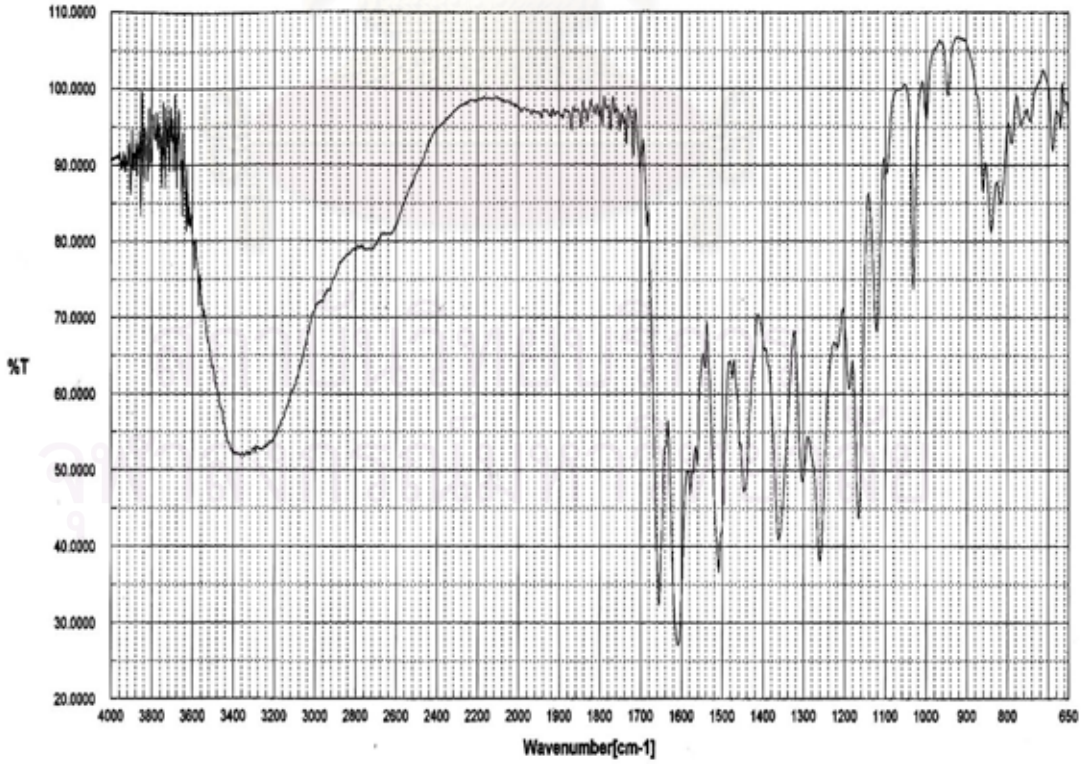

Figure 60 IR spectrum of compound **PRB8** (KBr disc).

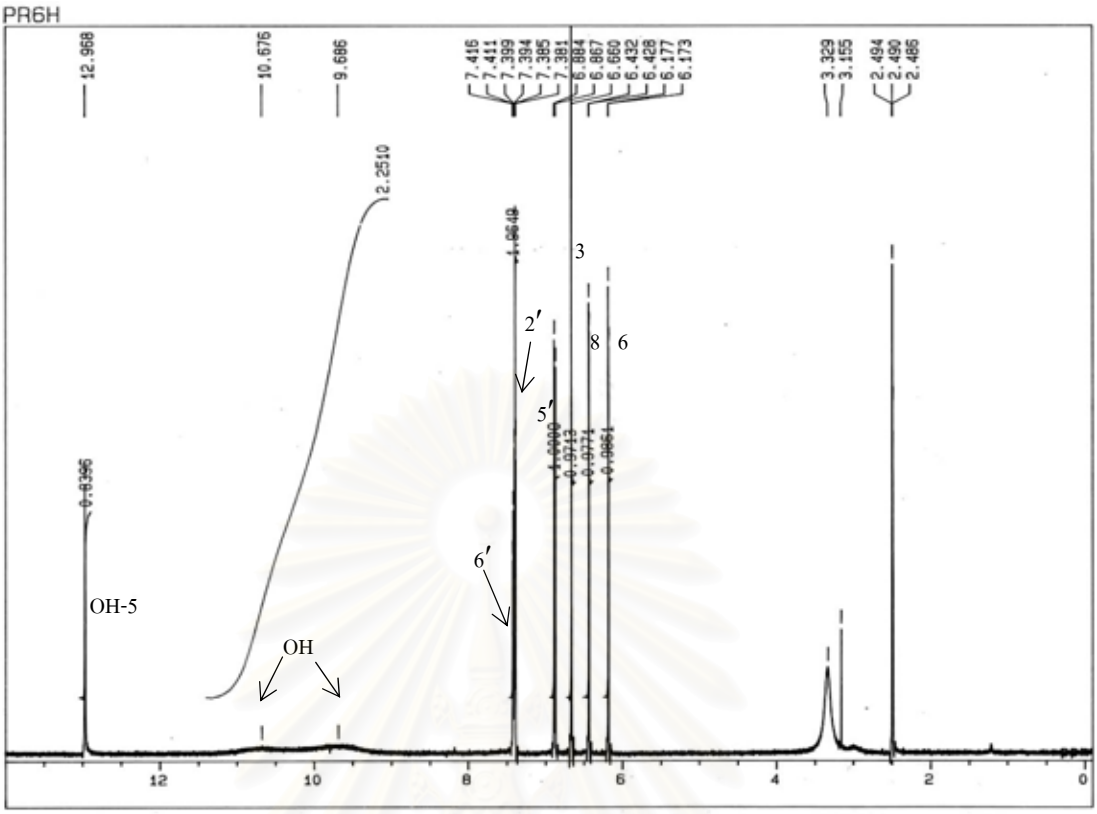

Figure 61 ^{1}H-NMR (500 MHz) spectrum of compound **PRB8** (DMSO-d_6).

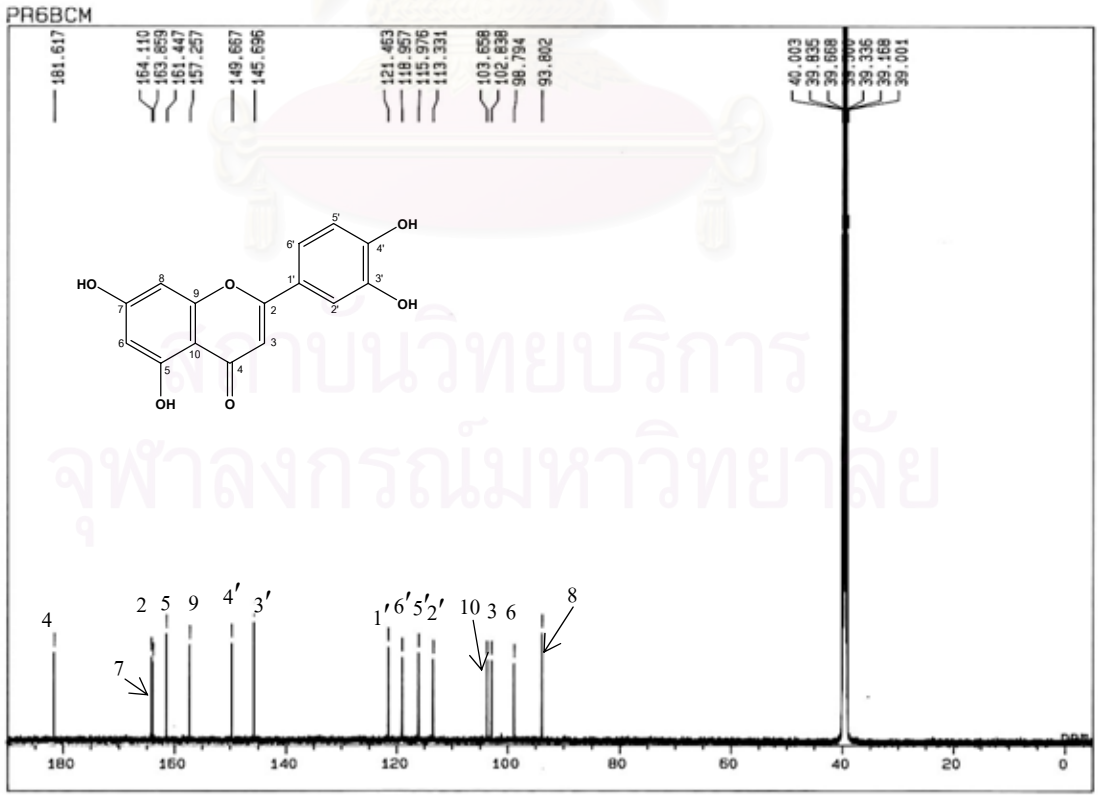

Figure 62 ^{13}C-NMR (125 MHz) spectrum of compound **PRB8** (DMSO-d_6).

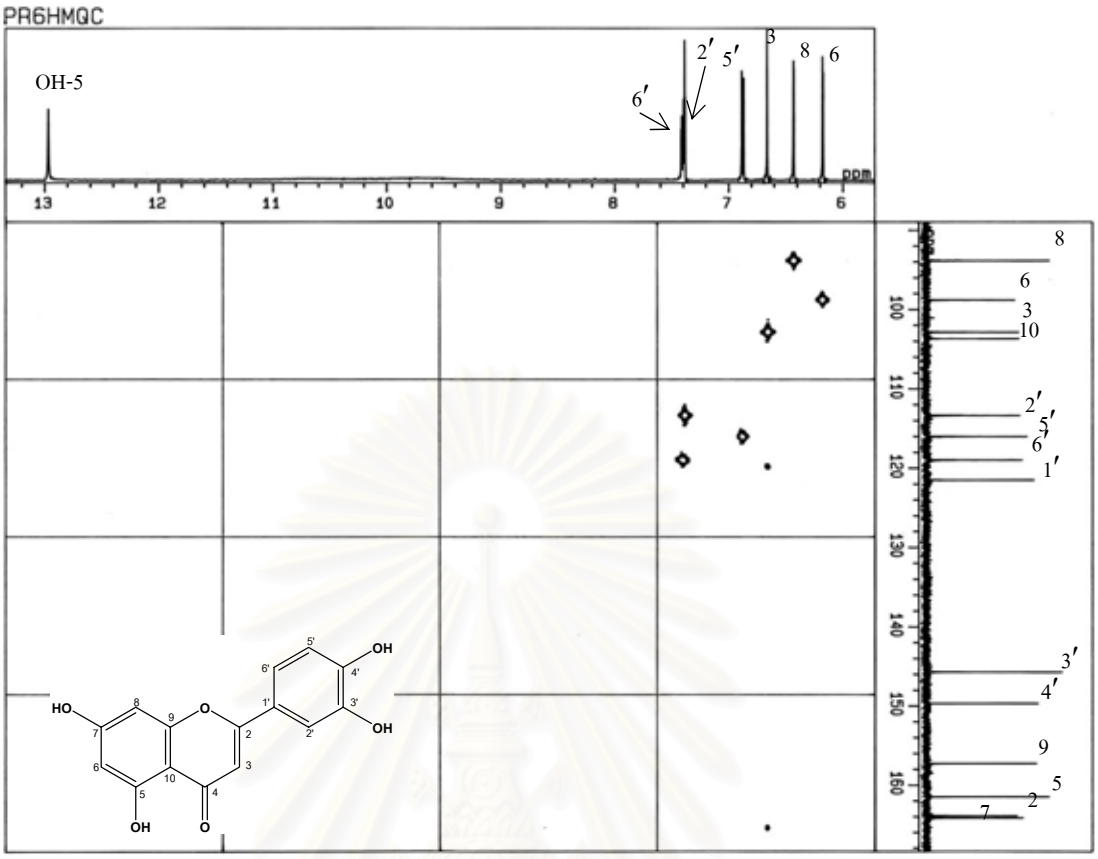

Figure 63 HMQC spectrum of compound **PRB8** (DMSO-d_6).

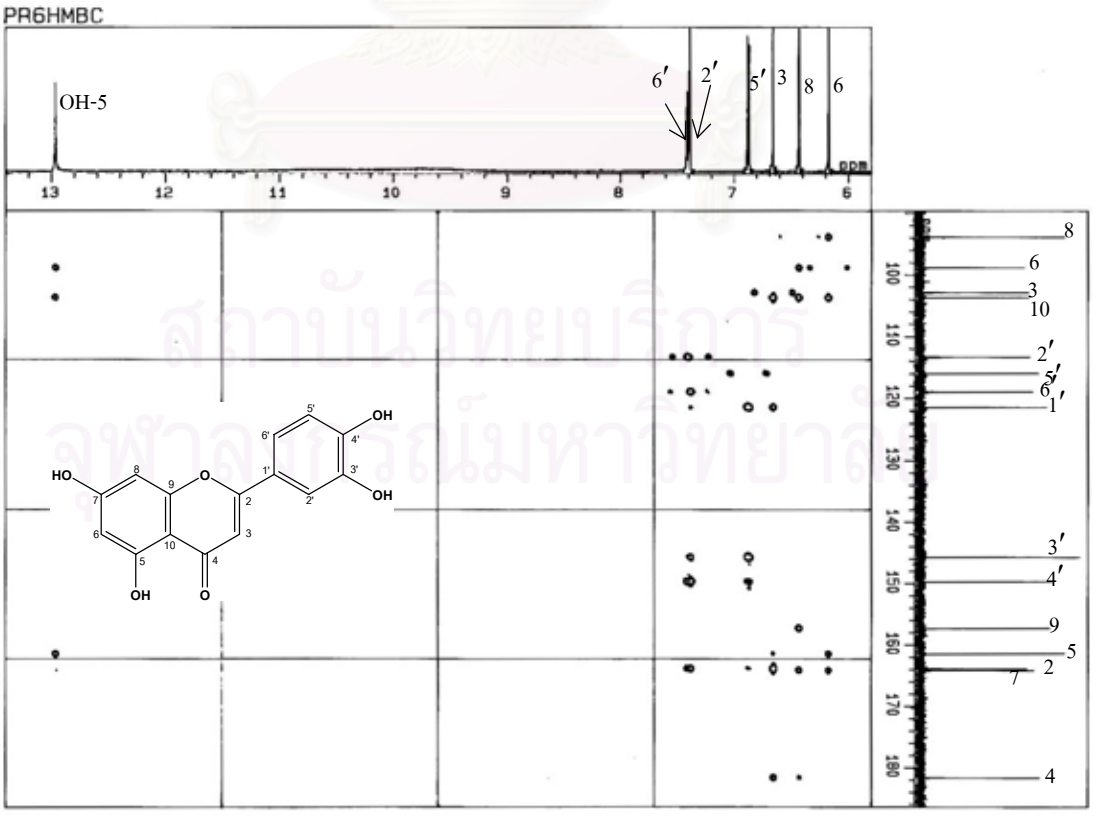

Figure 64 HMBC spectrum of compound **PRB8** (DMSO-d_6).

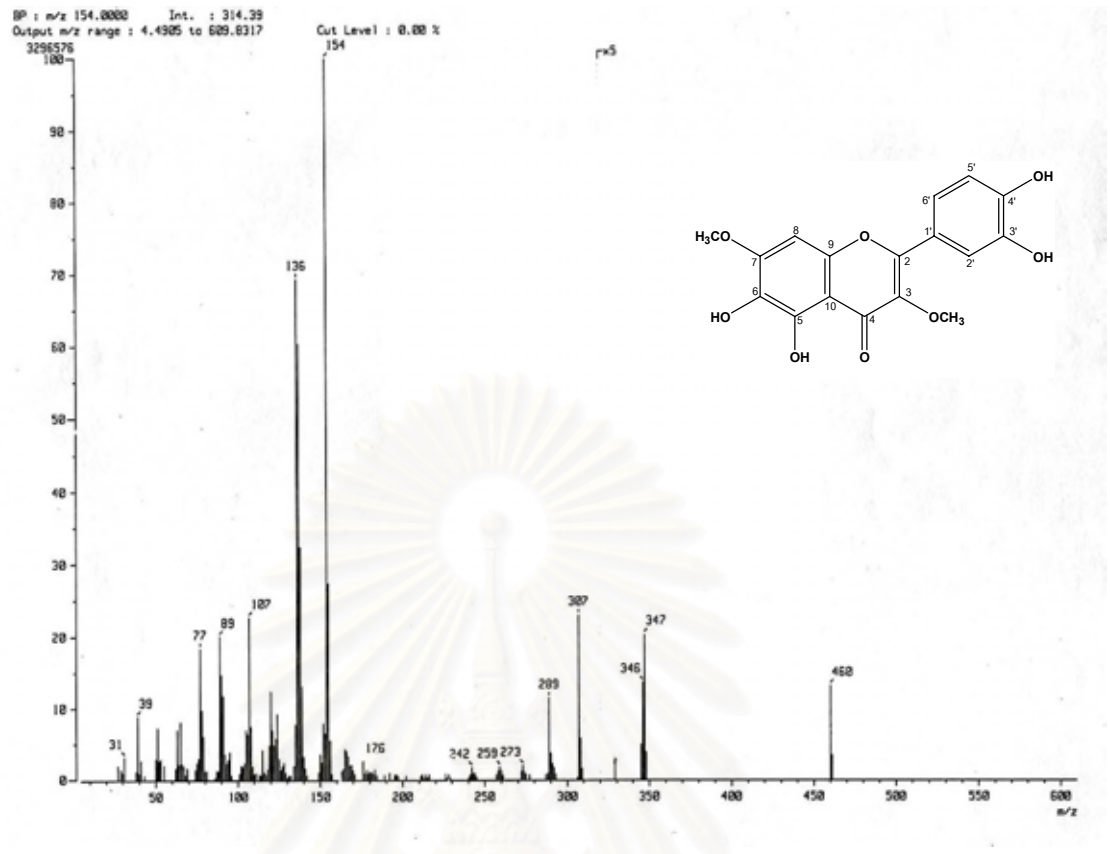

Figure 65 FAB Mass spectrum of compound **PRB9**.

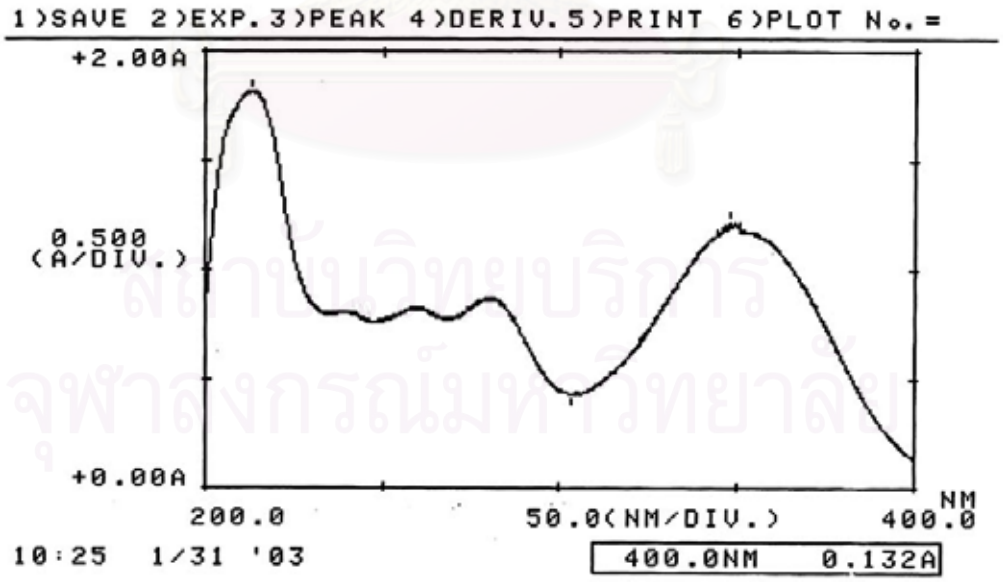

Figure 66 UV spectrum of compound **PRB9** (MeOH).

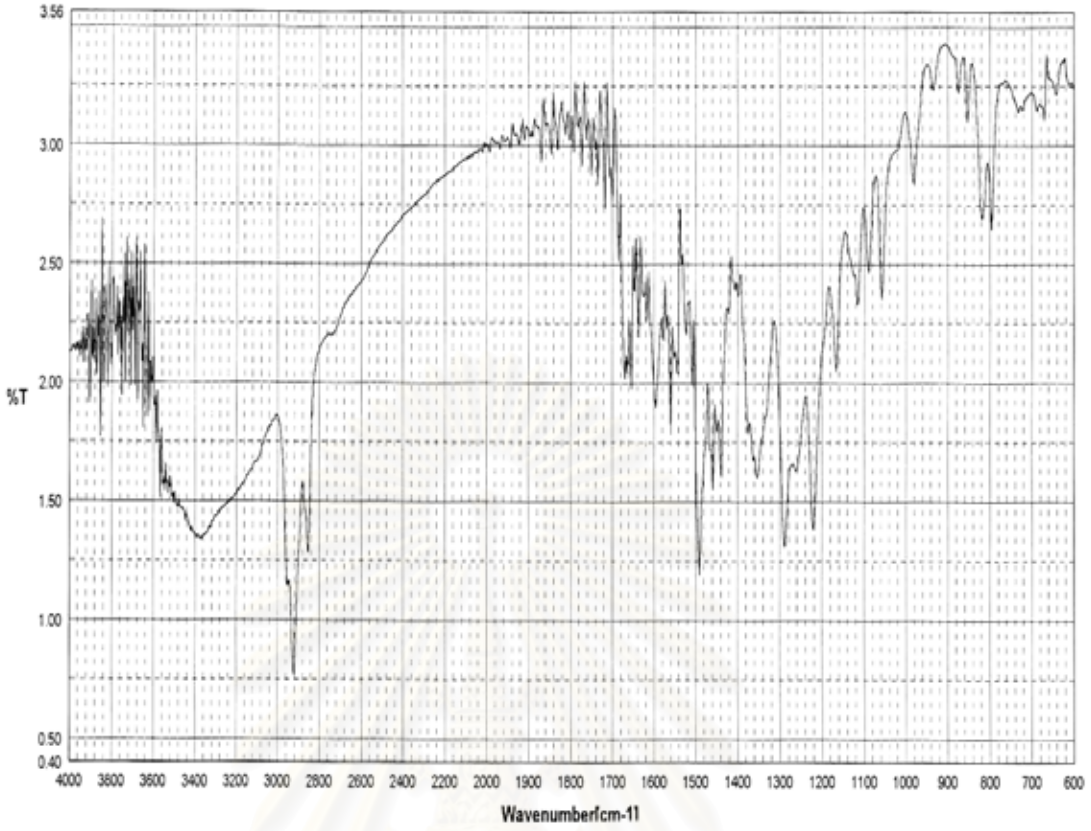

Figure 67 IR spectrum of compound **PRB9** (KBr disc).

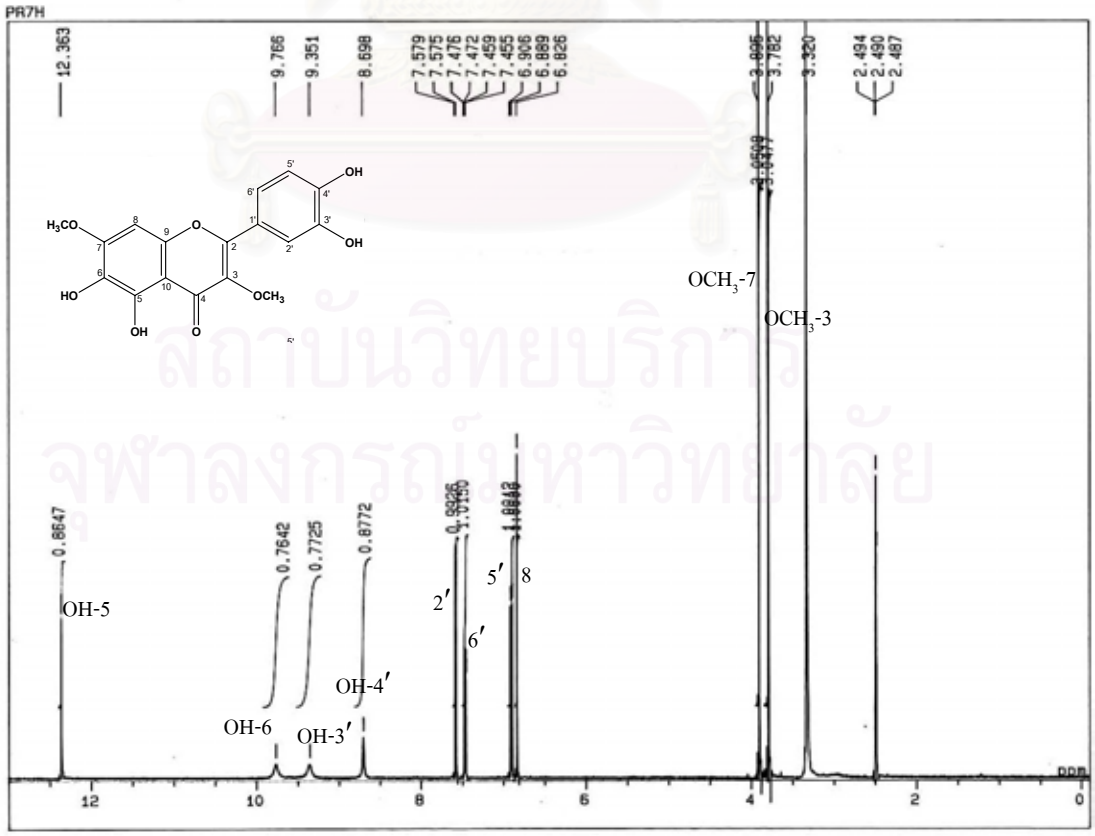

Figure 68 ^{1}H-NMR (500 MHz) spectrum of compound **PRB9** (DMSO-d_6).

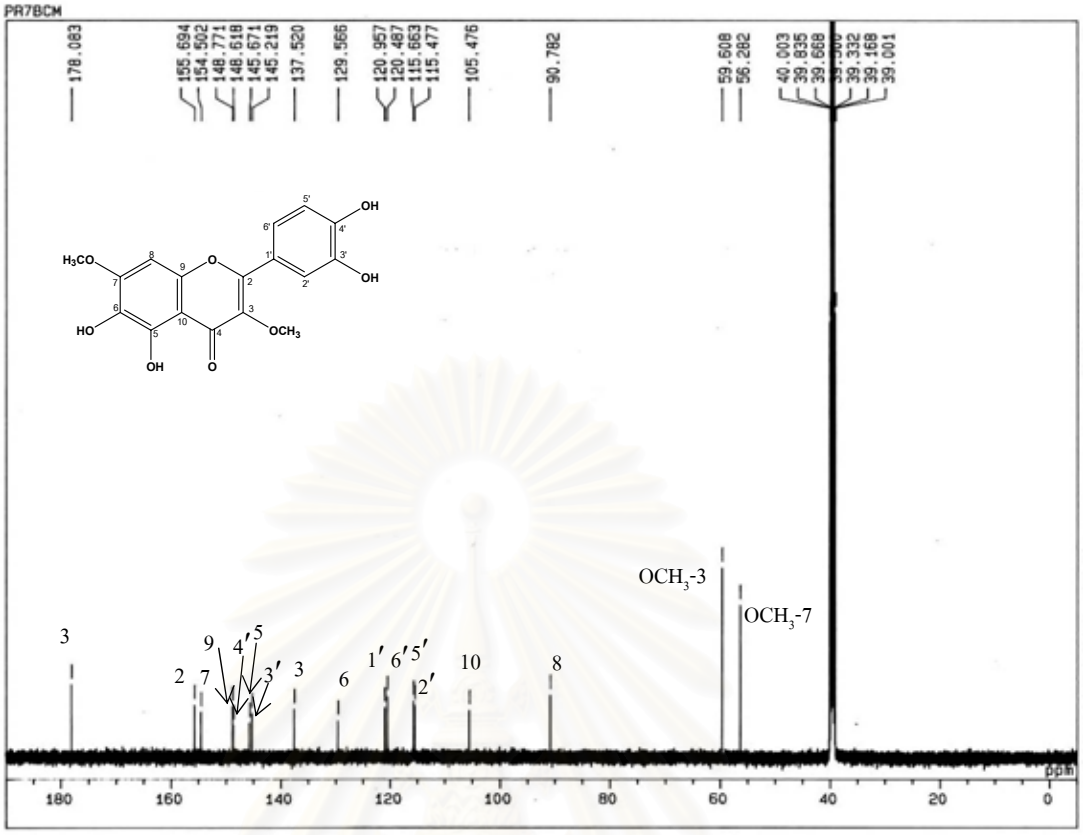

Figure 69 ^{13}C-NMR (125 MHz) spectrum of compound **PRB9** (DMSO-d_6).

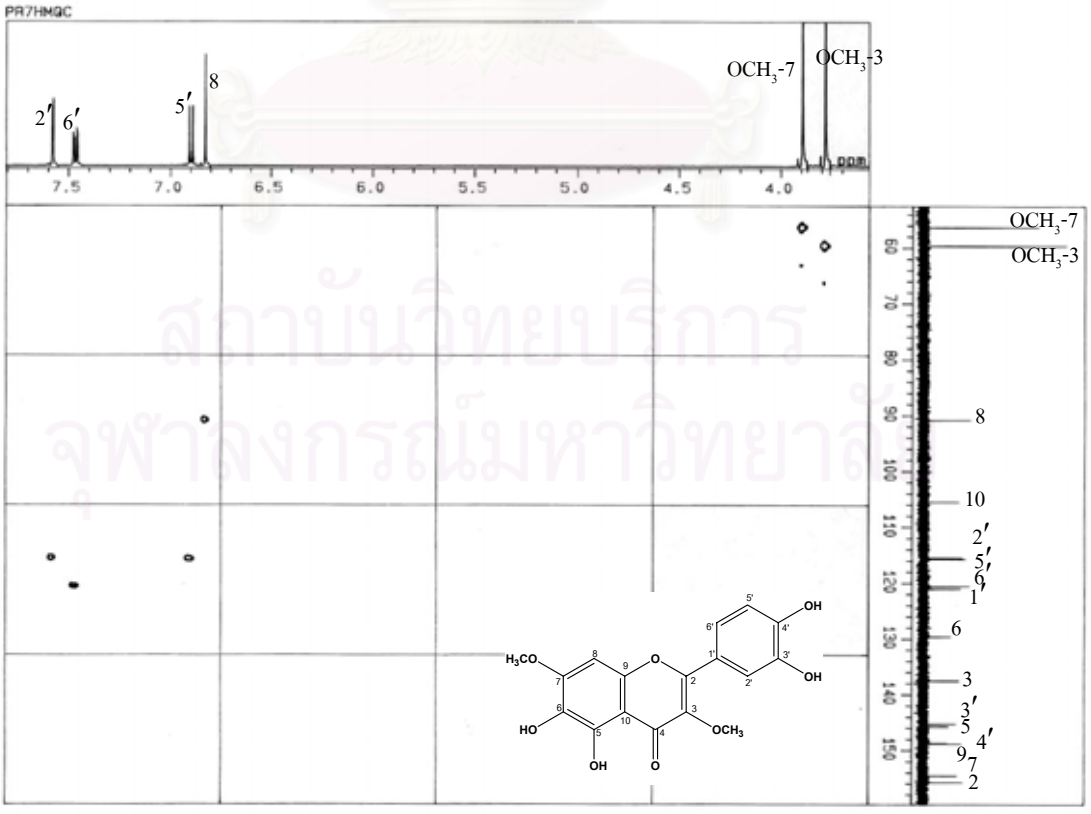

Figure 70 HMQC spectrum of compound **PRB9** (DMSO-d_6).

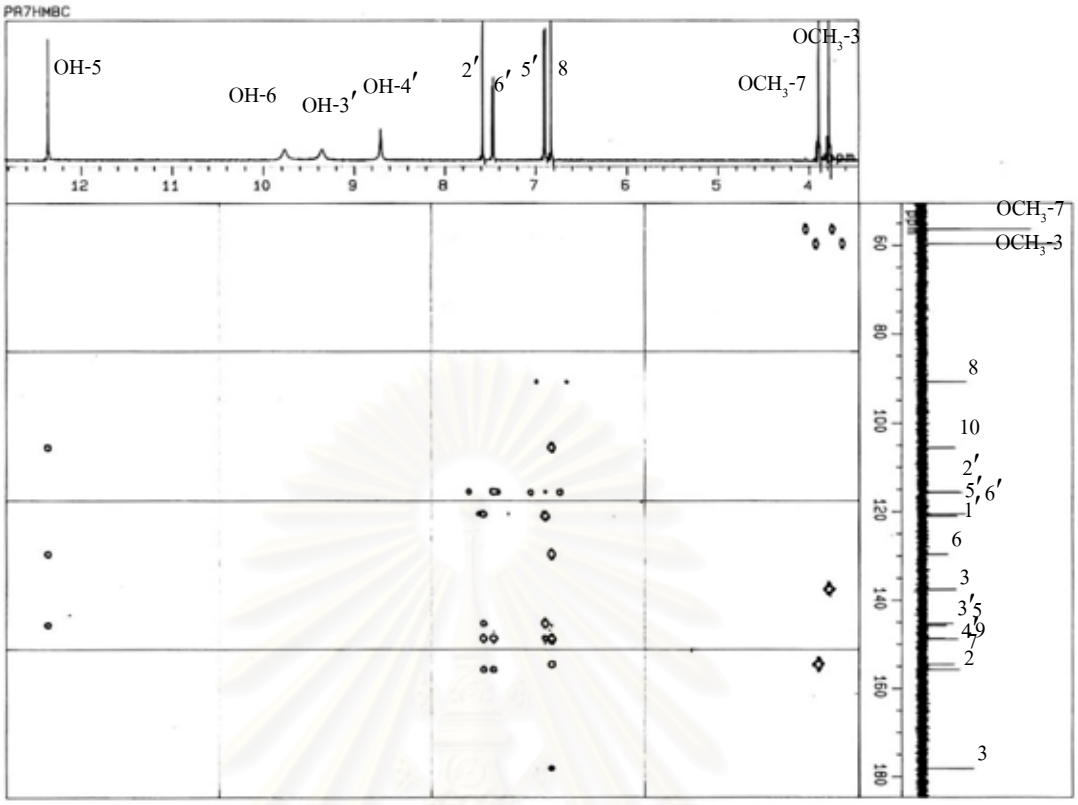

Figure 71 HMBC spectrum of compound **PRB9** (DMSO-d_6).

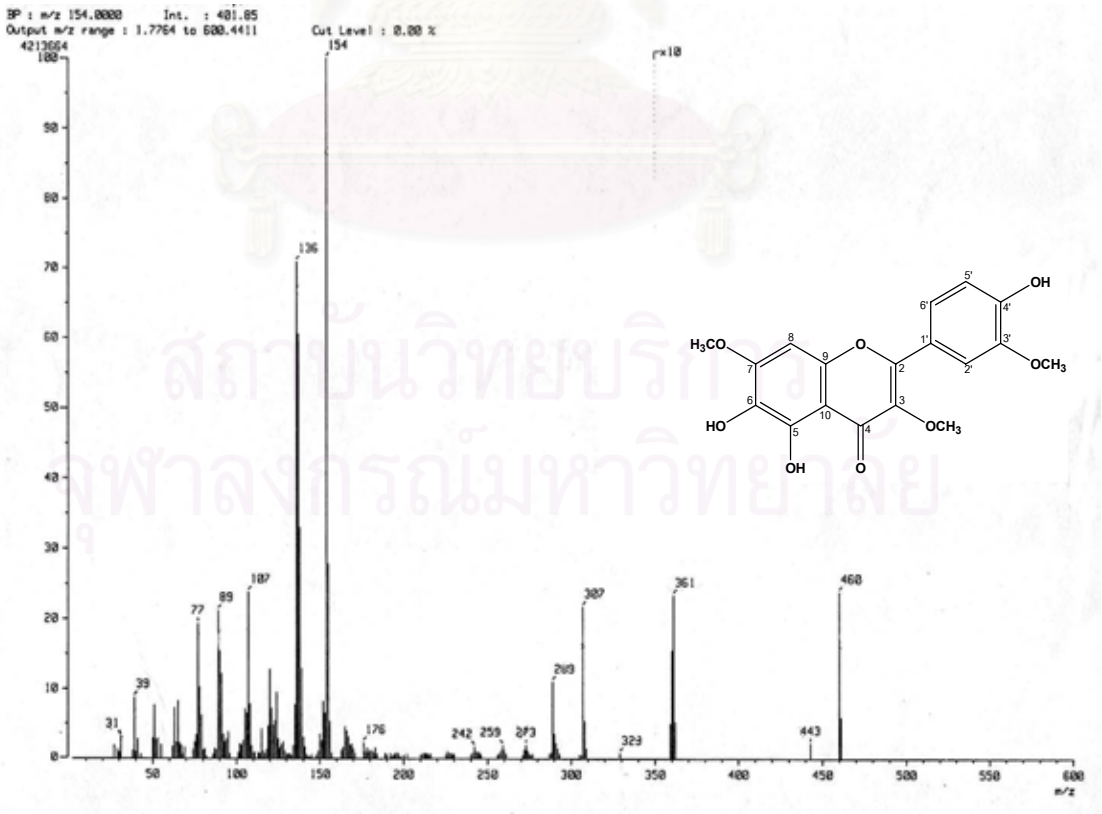

Figure 72 FAB Mass spectrum of Compound **PRB10**.

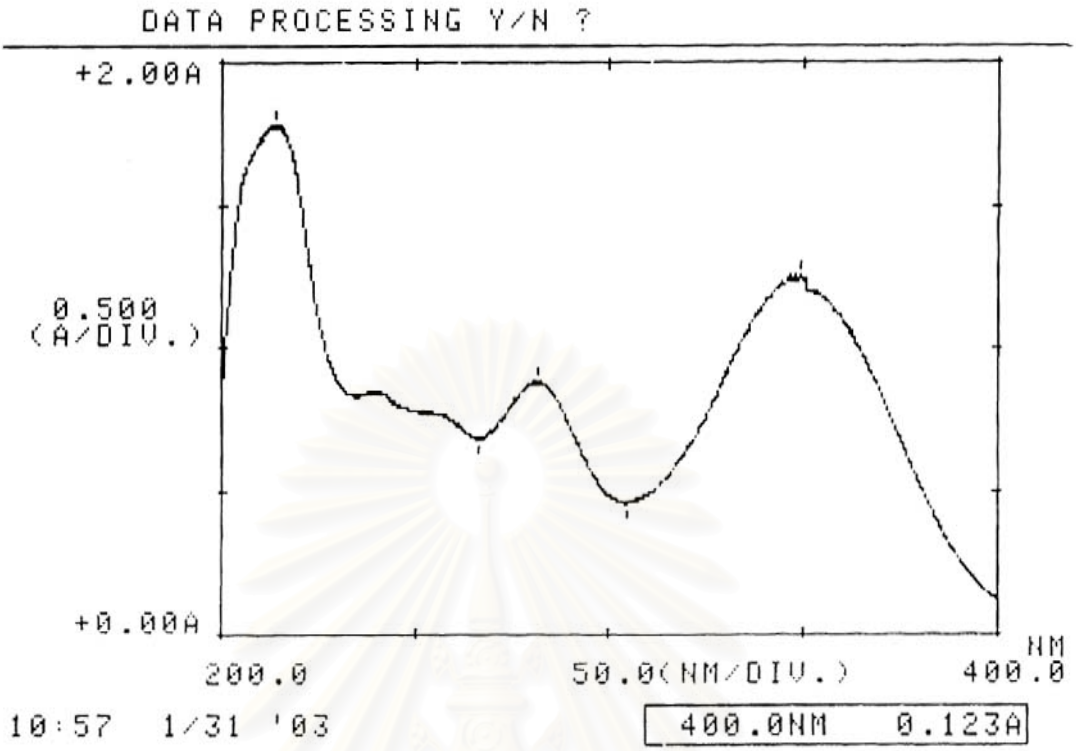

Figure 73 UV spectrum of Compound **PRB10** (MeOH).

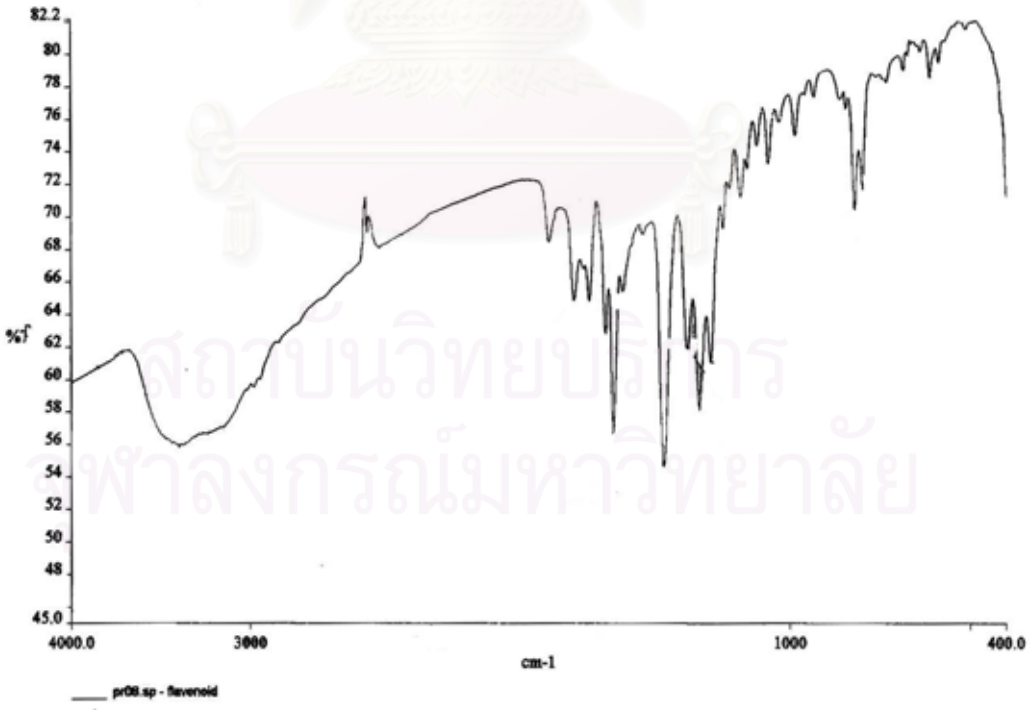

Figure 74 IR spectrum of Compound **PRB10** (KBr disc).

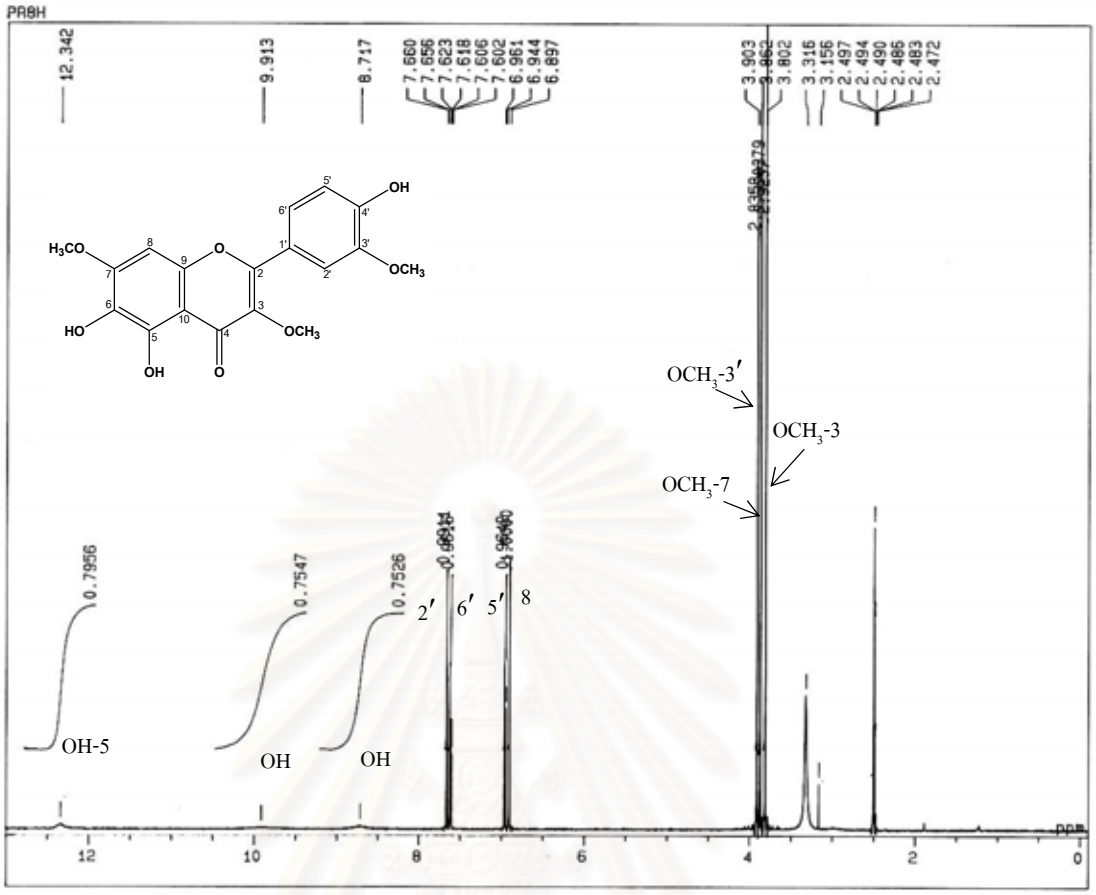

Figure 75 ^{1}H-NMR (500 MHz) spectrum of Compound **PRB10** (DMSO-d_6).

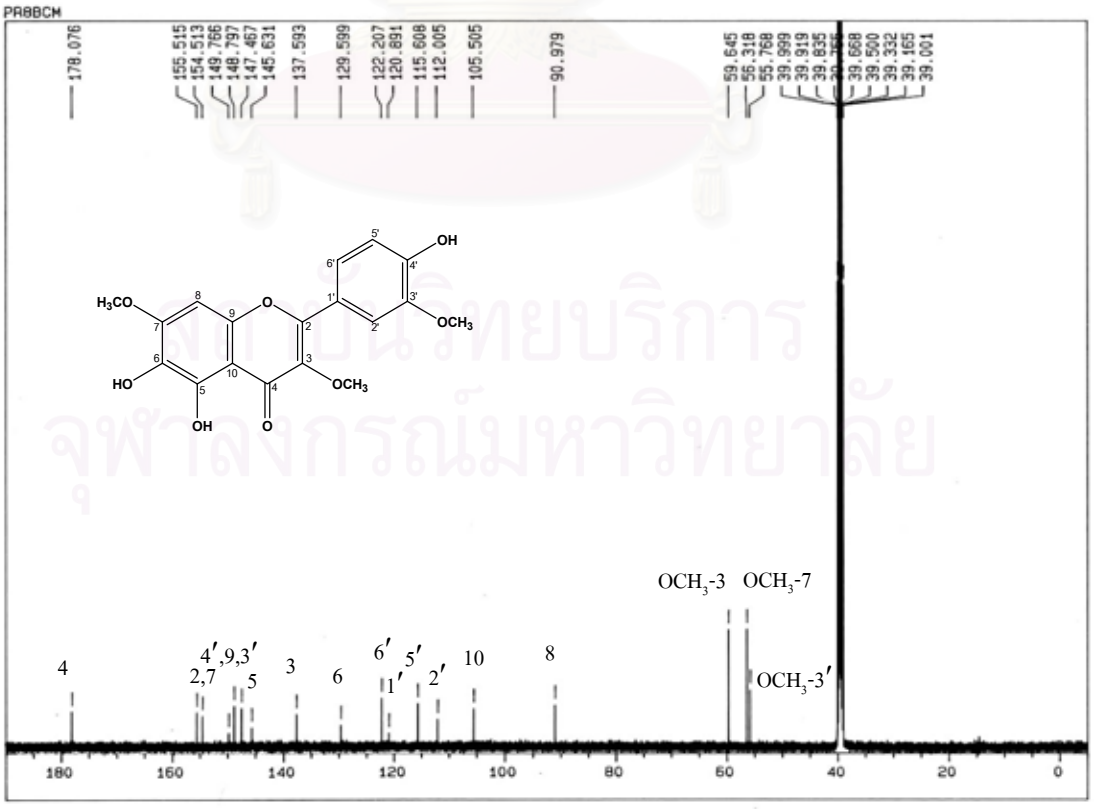

Figure 76 ^{13}C-NMR (125 MHz) spectrum of Compound **PRB10** (DMSO-d_6).

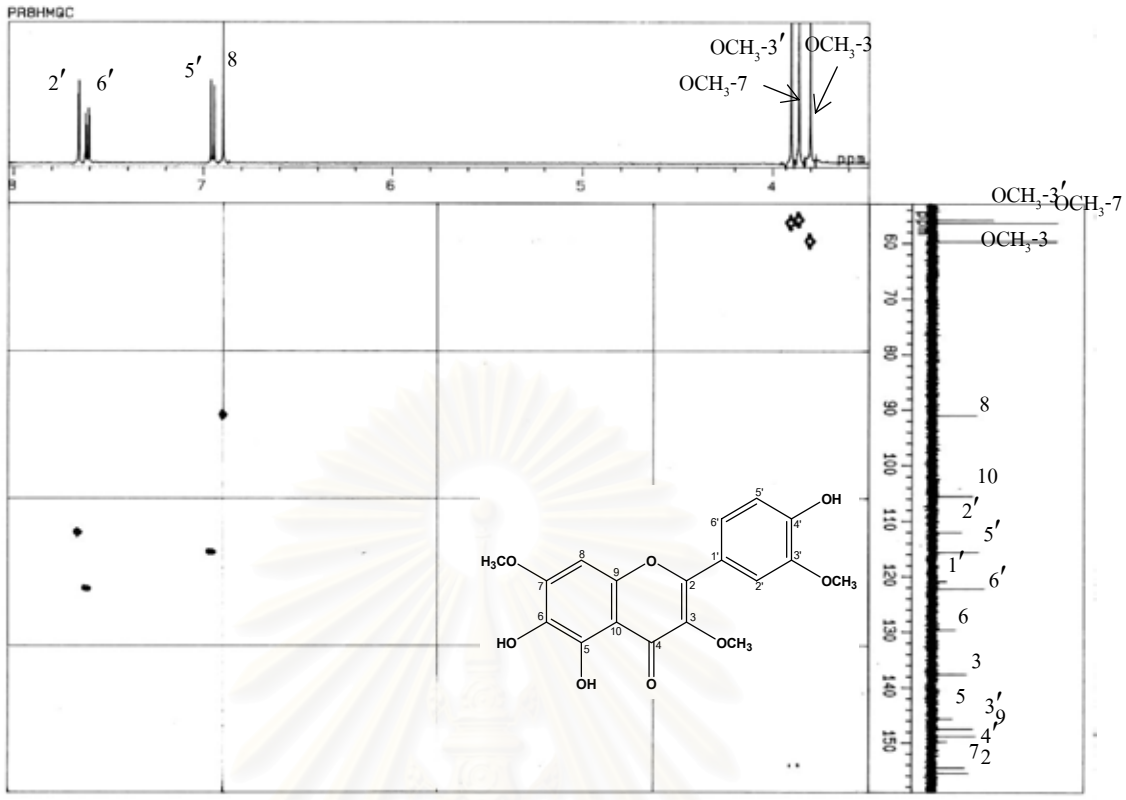

Figure 77 HMQC spectrum of Compound **PRB10** (DMSO-d_6).

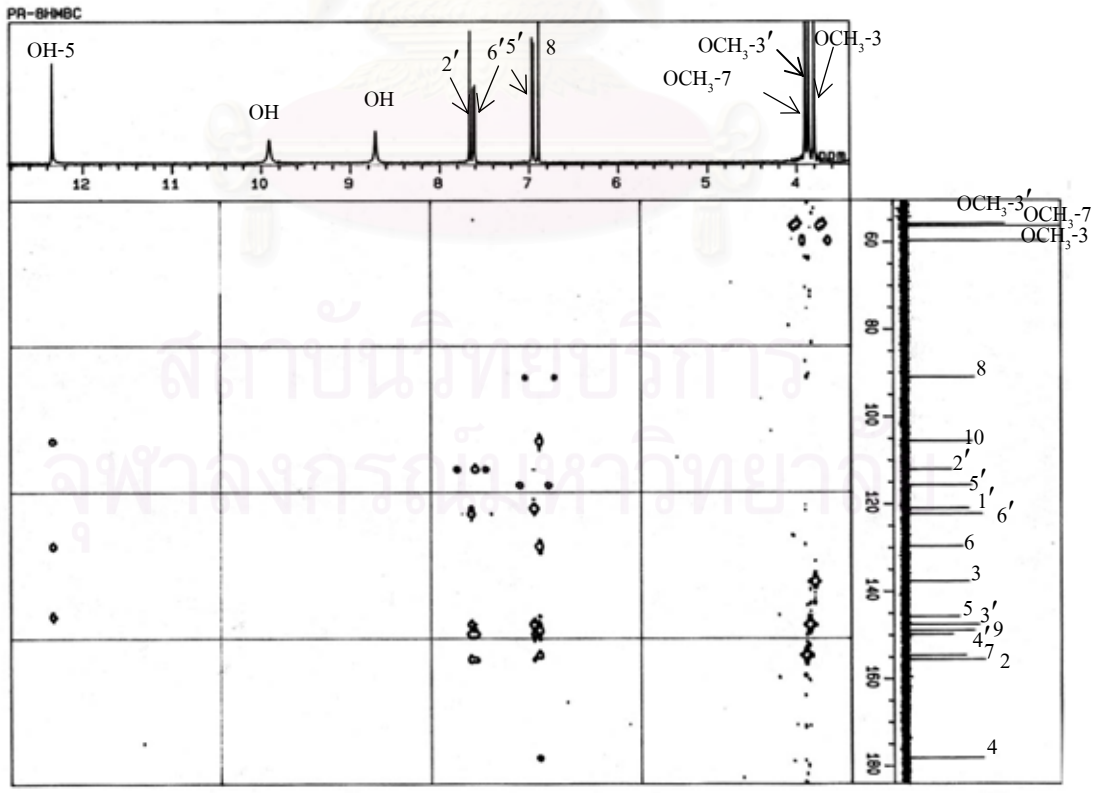

Figure 78 HMBC spectrum of Compound **PRB10** (DMSO-d_6).

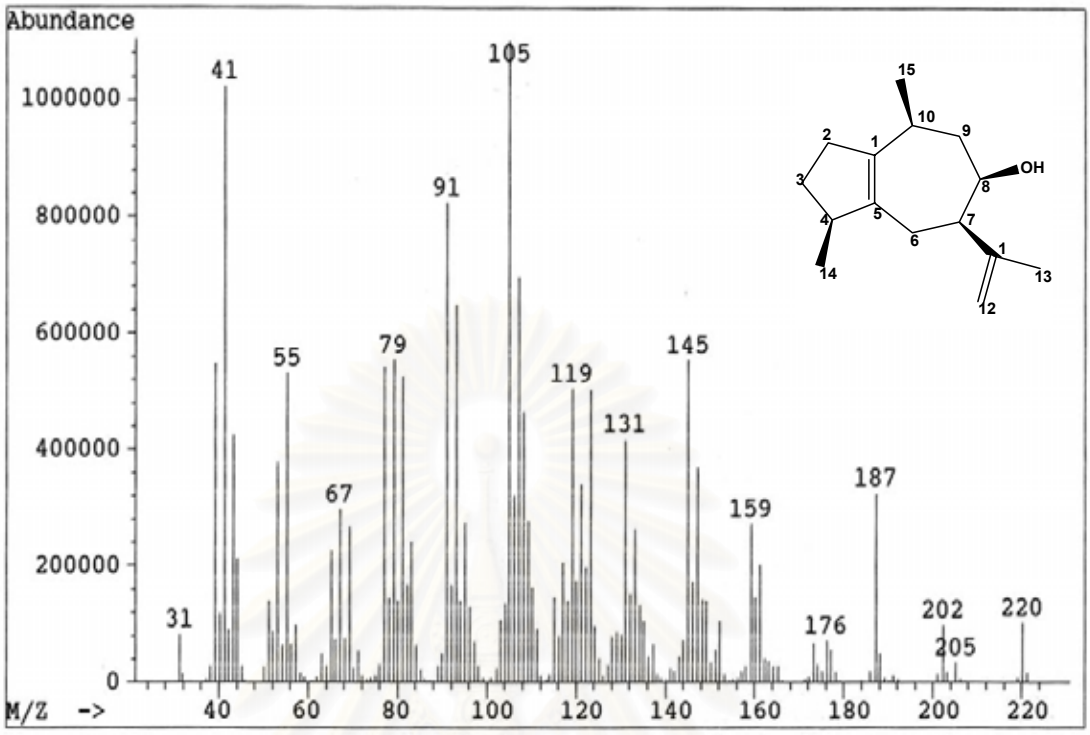

Figure 79 GC Mass spectrum of compound **COC1**.

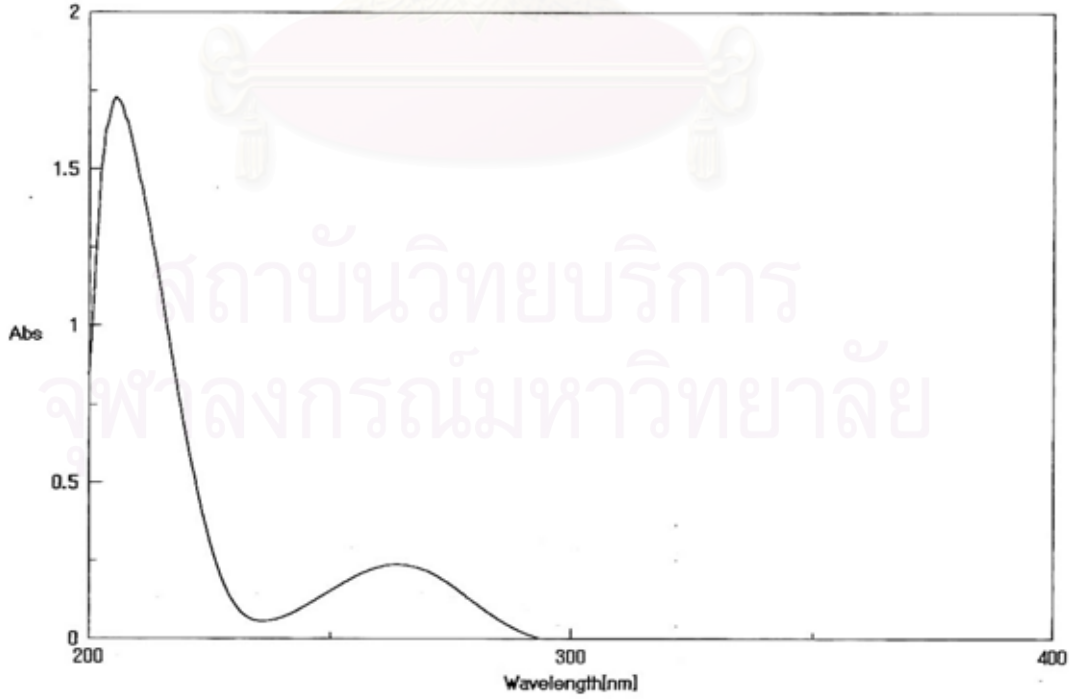

Figure 80 UV spectrum of compound **COC1** (MeOH).

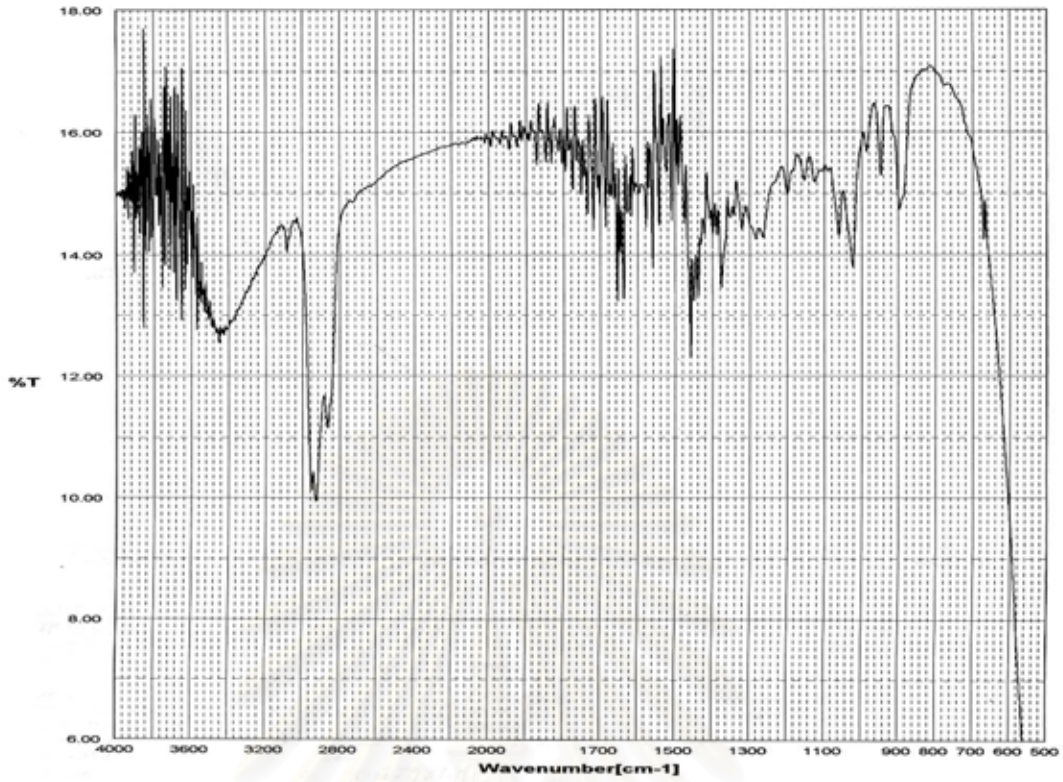

Figure 81 IR spectrum of compound **COC1** (Neat).

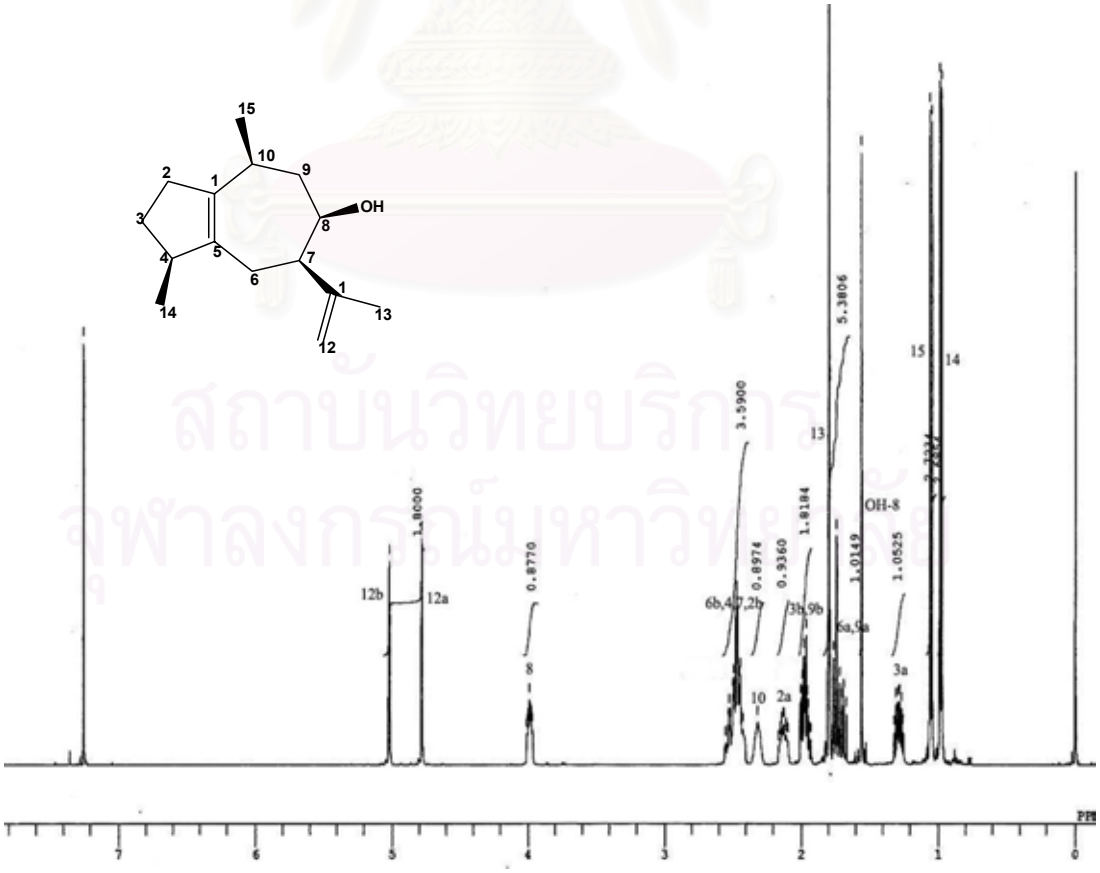

Figure 82 ^{1}H-NMR (500 MHz) spectrum of compound **COC1** ($CDCl_3$).

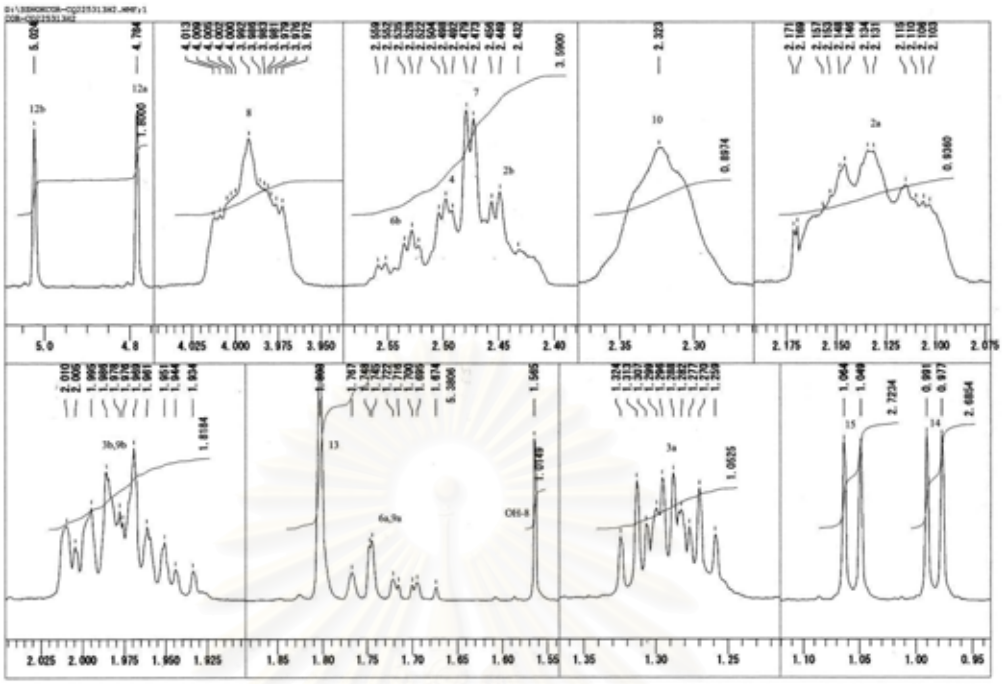

Figure 83 Expanded ^{1}H-NMR (500 MHz) spectrum of compound **COC1** ($CDCl_3$).

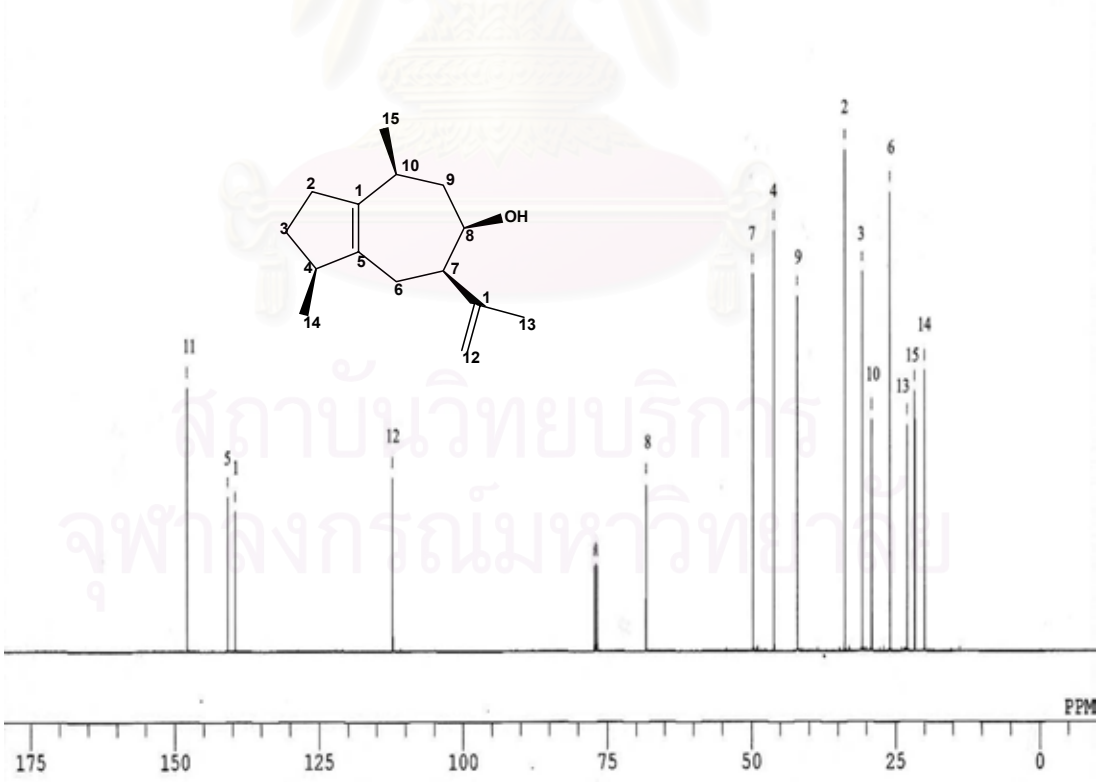

Figure 84 ^{13}C-NMR (125 MHz) spectrum of compound **COC1** ($CDCl_3$).

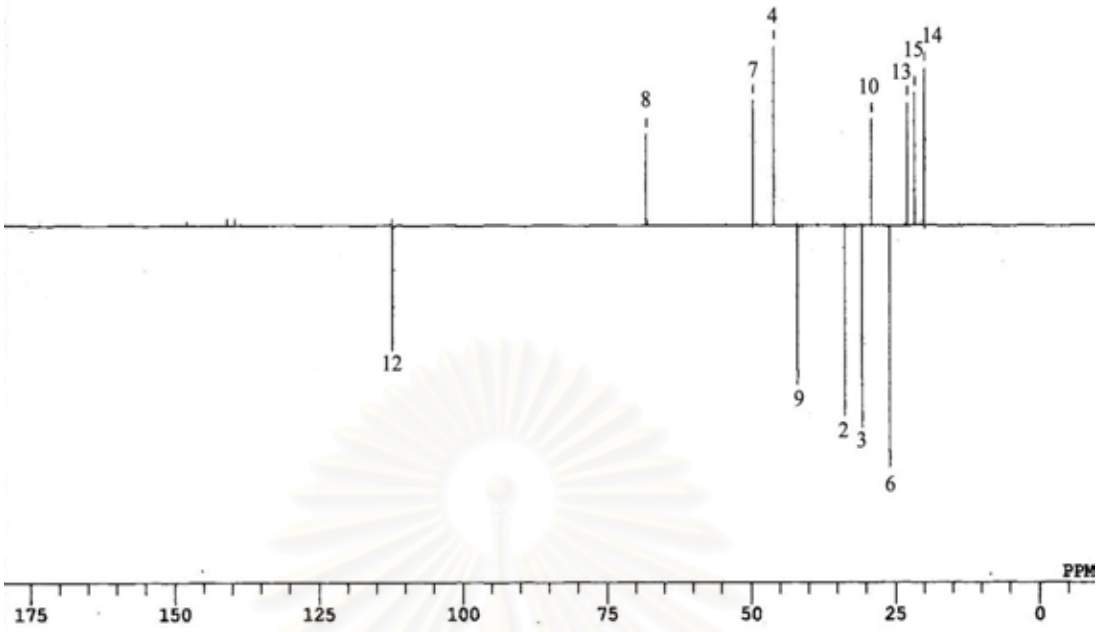

Figure 85 DEPT135 spectrum of compound **COC1** ($CDCl_3$).

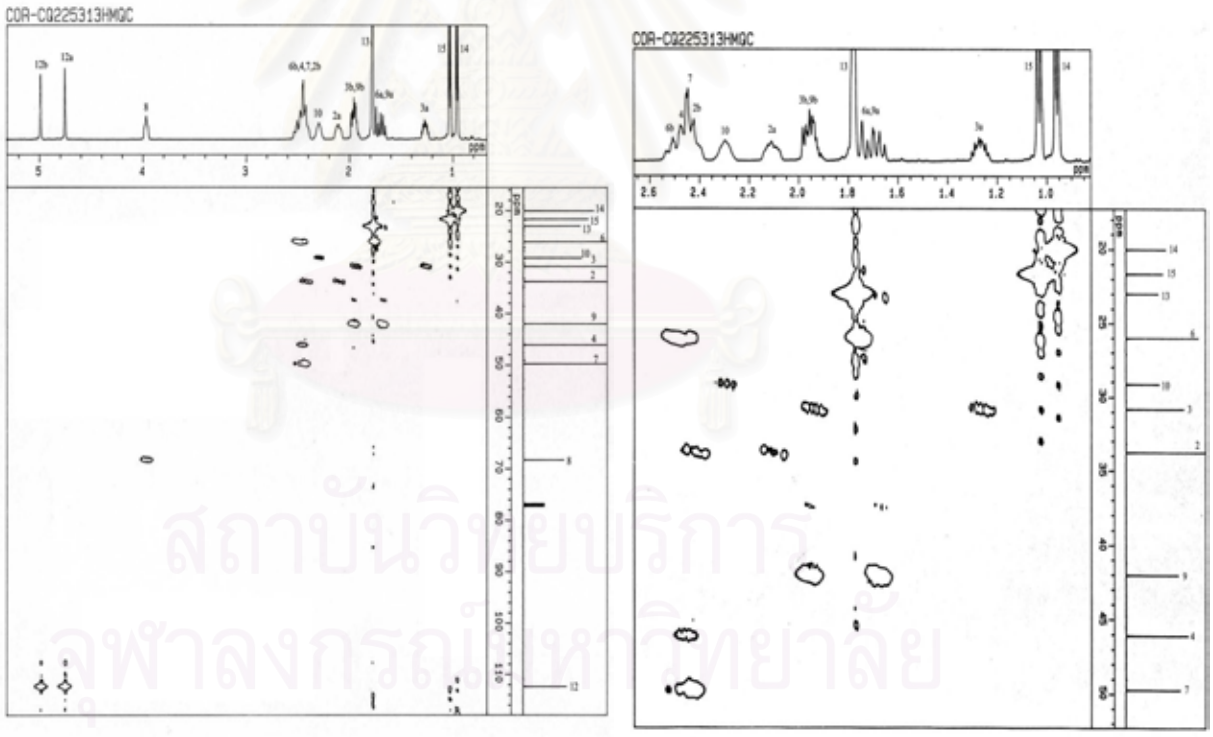

Figure 86 HMQC spectra of compound **COC1** ($CDCl_3$).

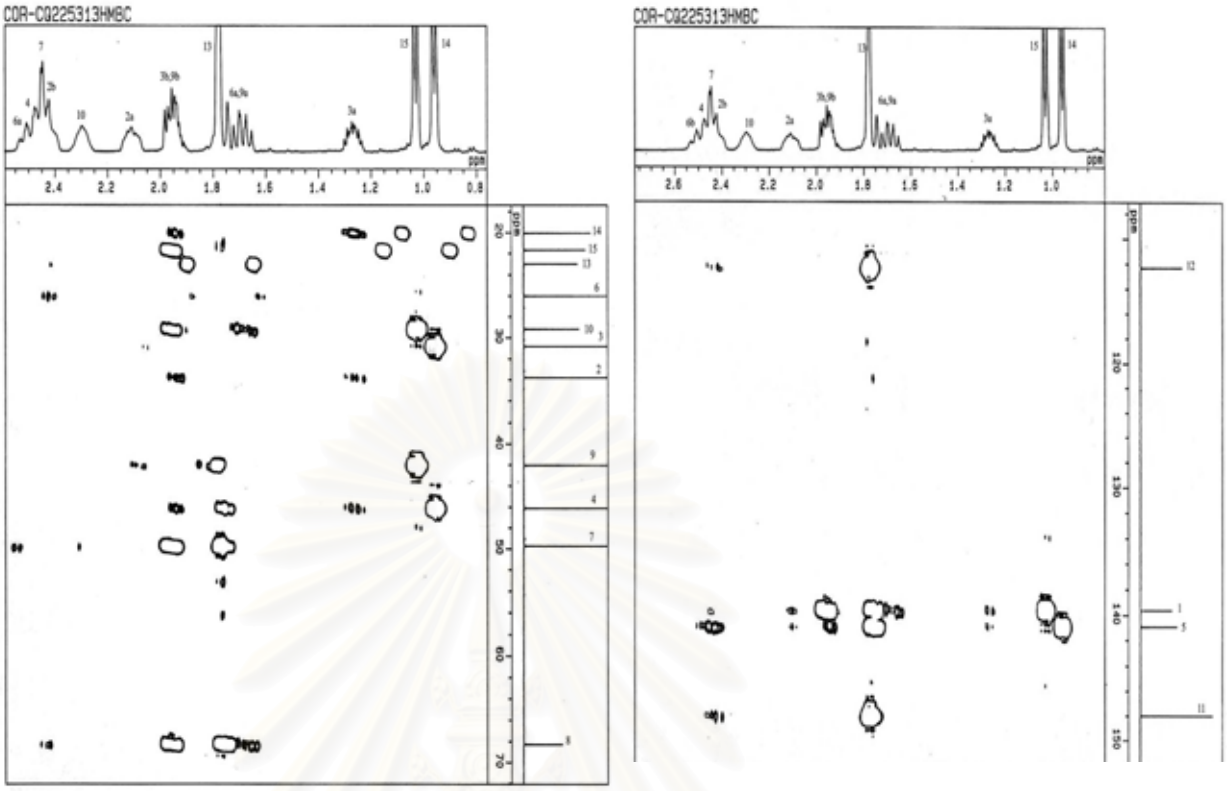

Figure 87 HMBC spectra of compound **COC1** ($CDCl_3$).

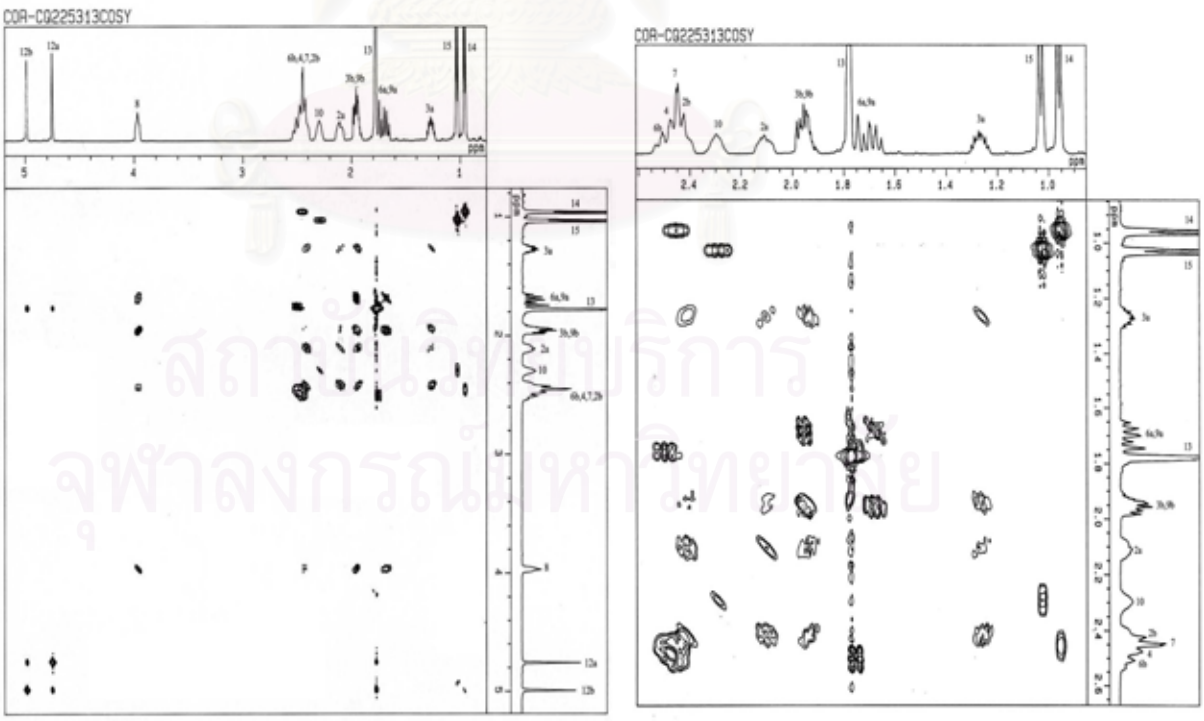

Figure 88 ^{1}H-^{1}H COSY spectra of compound **COC1** ($CDCl_3$).

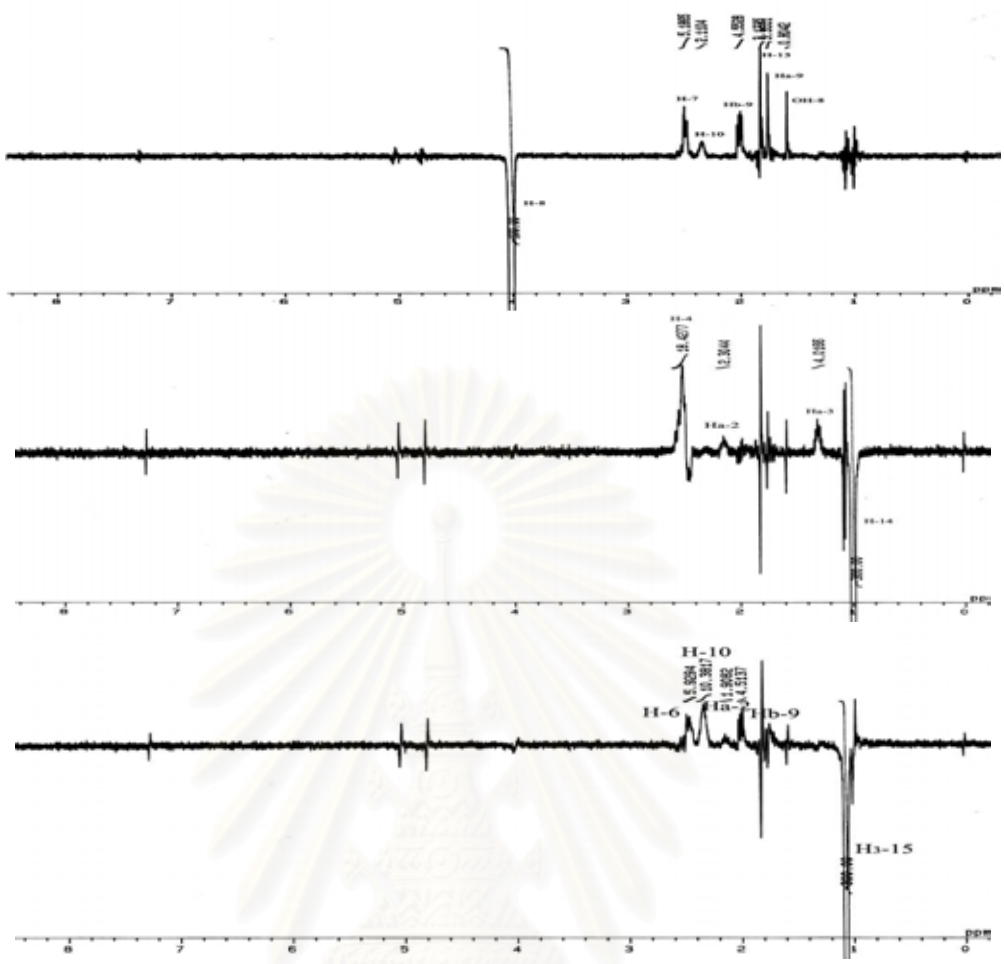

Figure 89 NOE spectrum of compound **COC1** ($CDCl_3$).

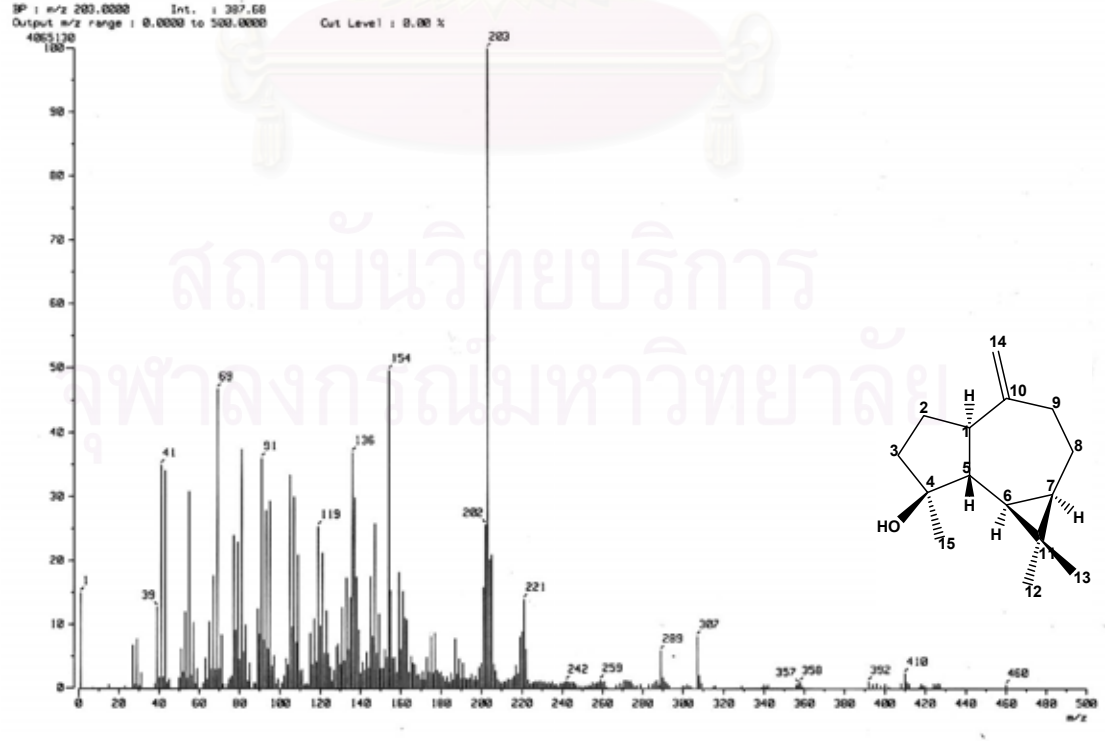

Figure 90 FAB Mass spectrum of compound **COC2**.

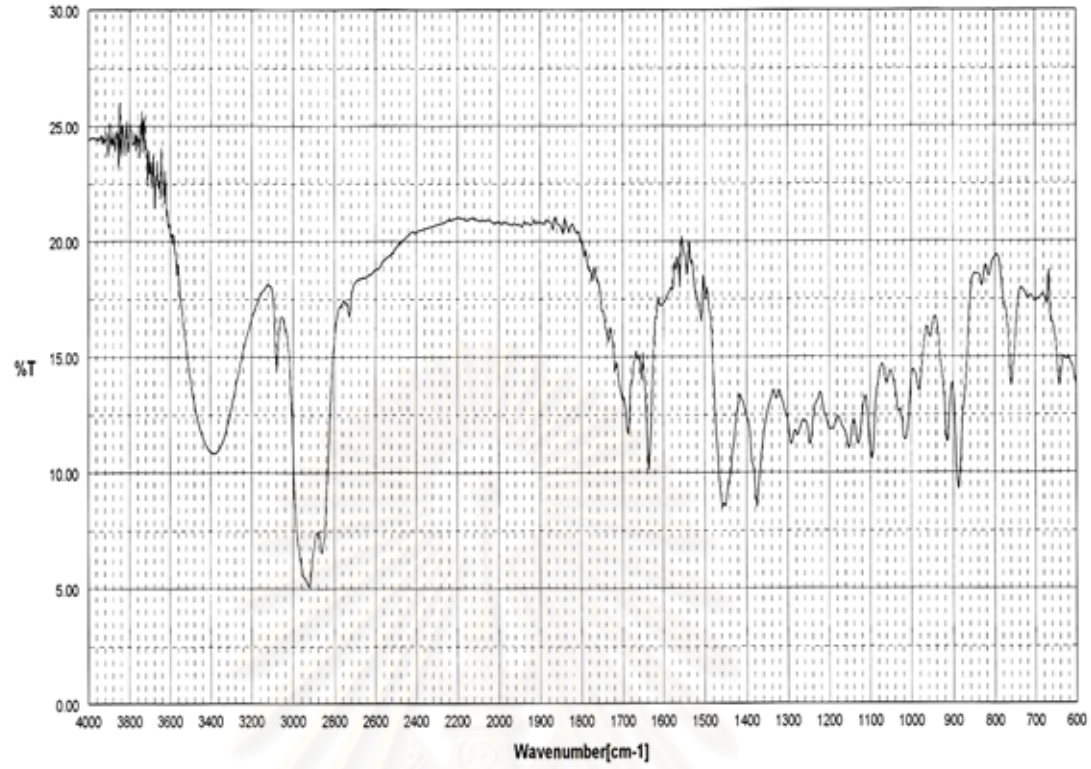

Figure 91 IR spectrum of compound **COC2** (Neat).

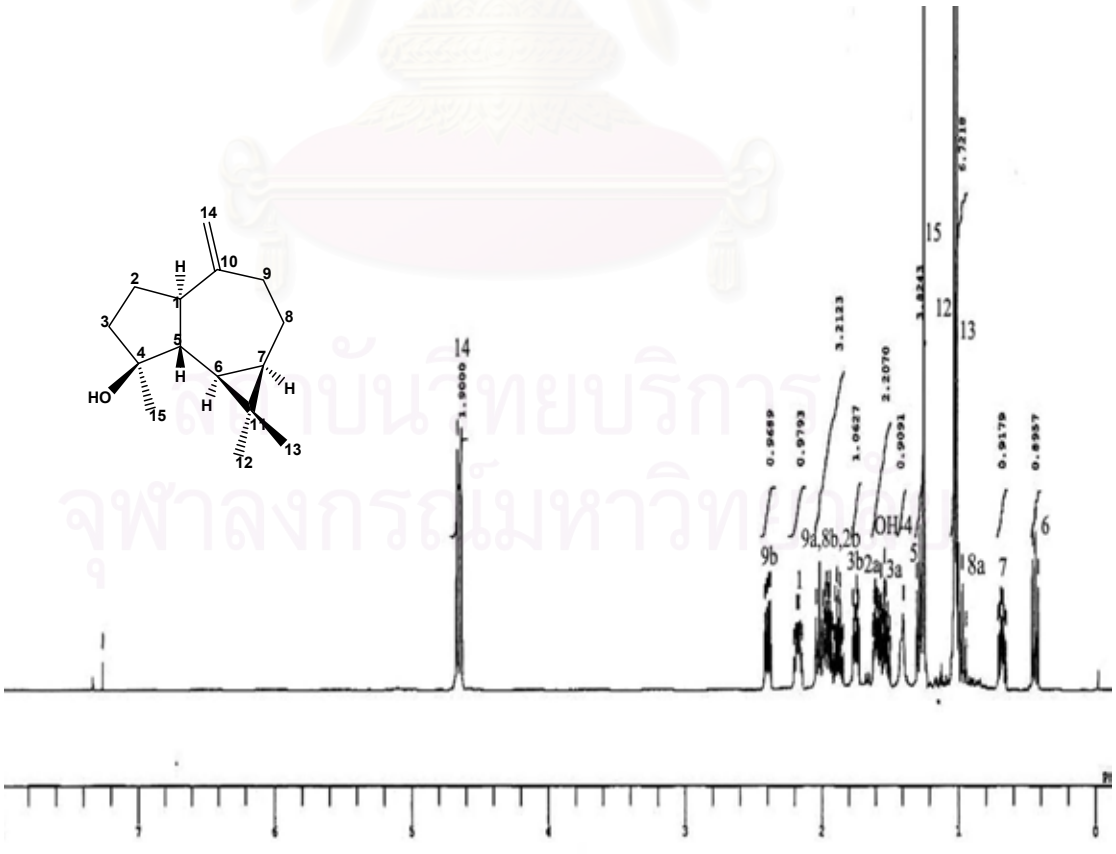

Figure 92 ^{1}H-NMR (500 MHz) spectrum of compound **COC2** ($CDCl_3$)

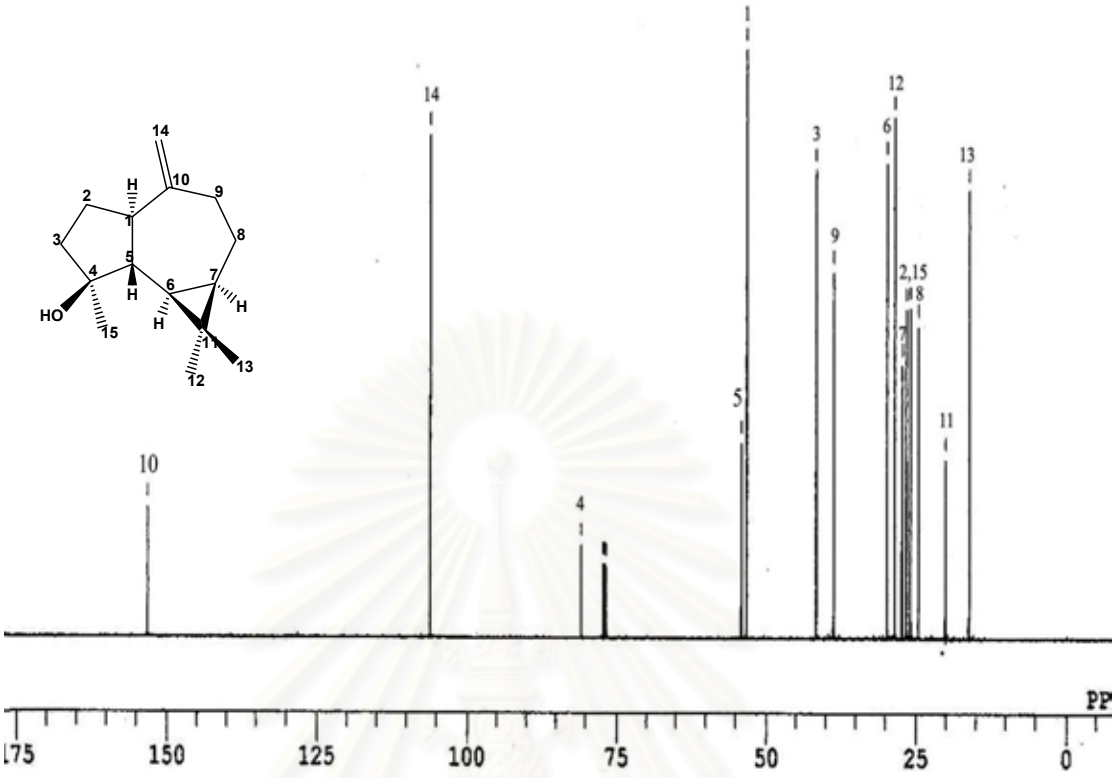

Figure 93 ^{13}C-NMR (125 MHz) spectrum of compound **COC2** ($CDCl_3$)

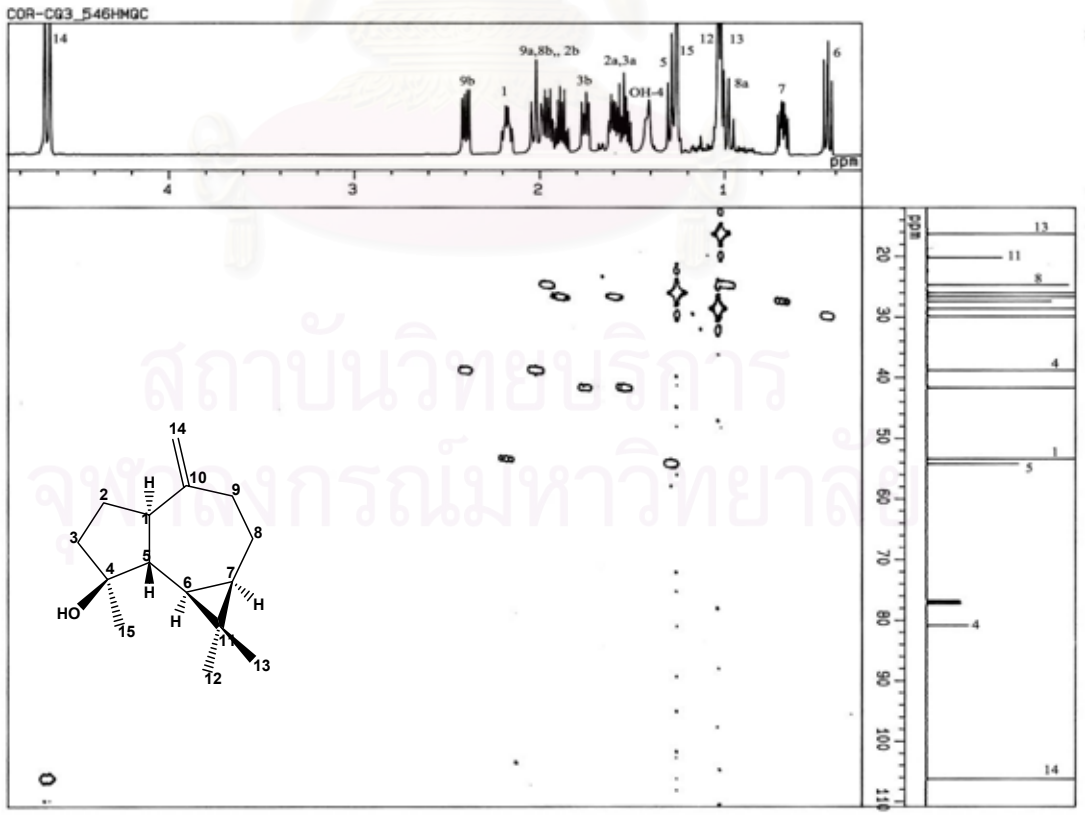

Figure 94 HMQC spectrum of compound **COC2** ($CDCl_3$).

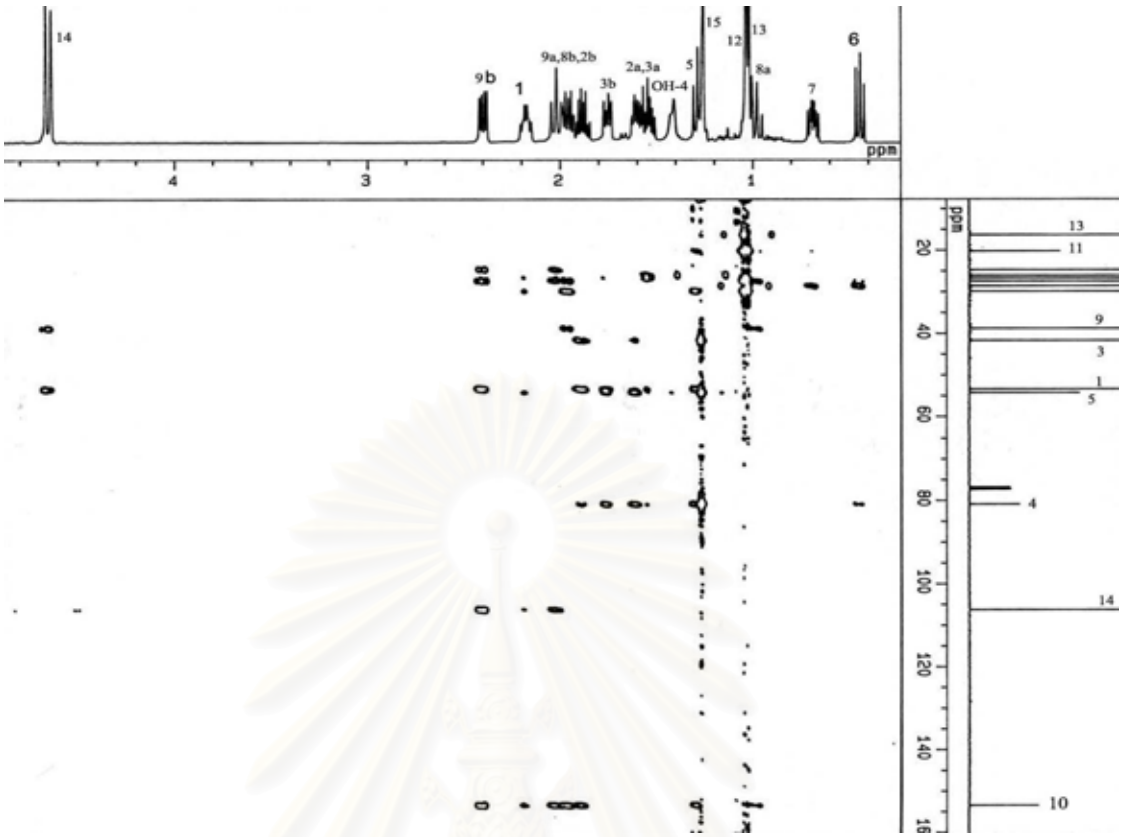

Figure 95 HMBC spectrum of compound **COC2** ($CDCl_3$).

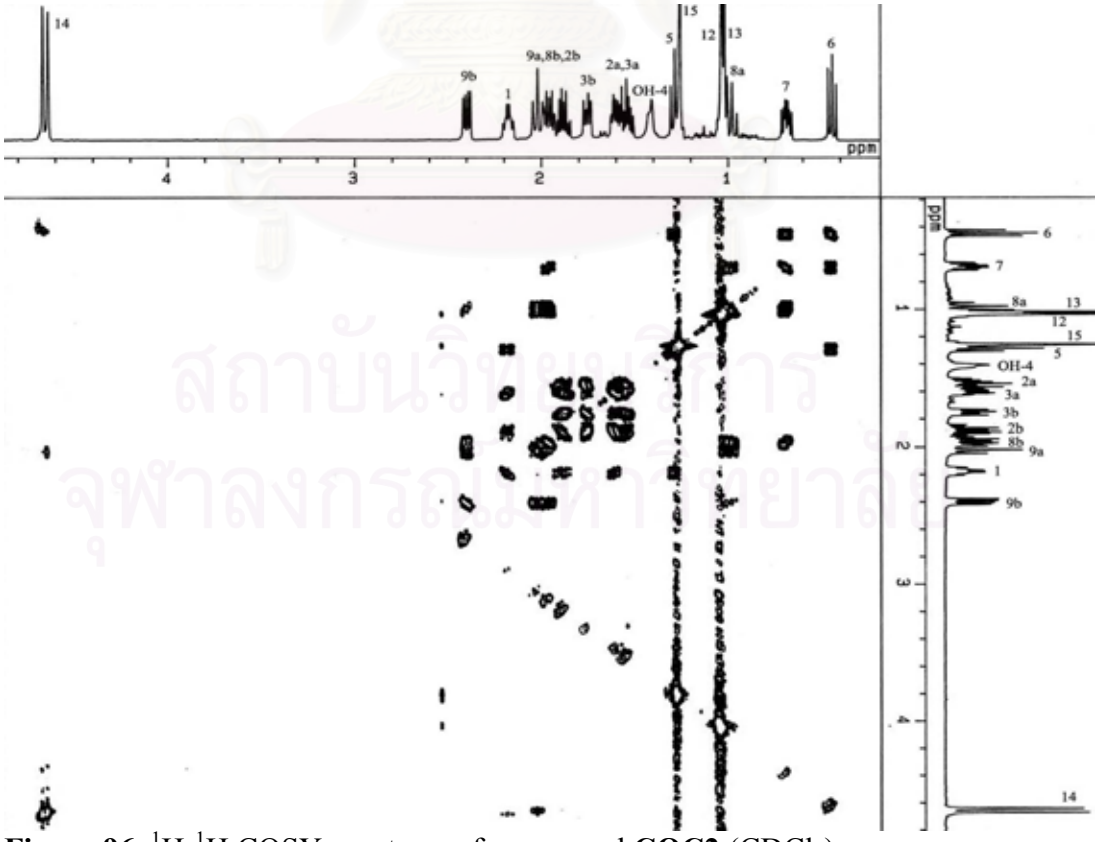

Figure 96 ^{1}H-^{1}H COSY spectrum of compound **COC2** ($CDCl_3$).

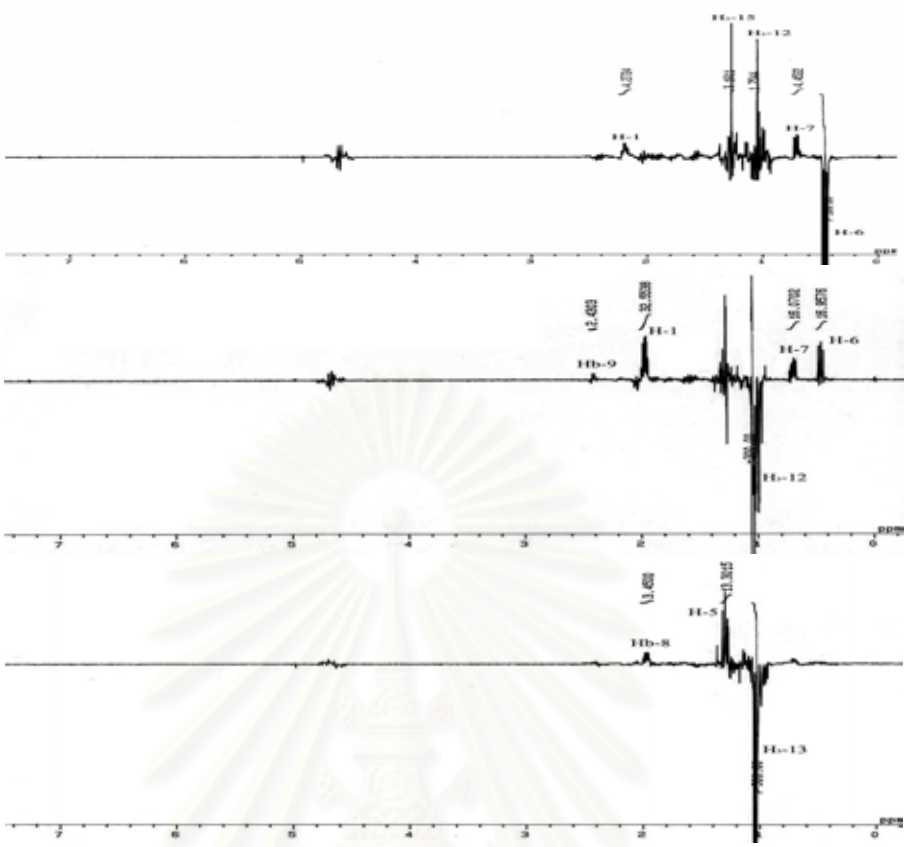

Figure 97 NOE spectra of compound **COC2** ($CDCl_3$).

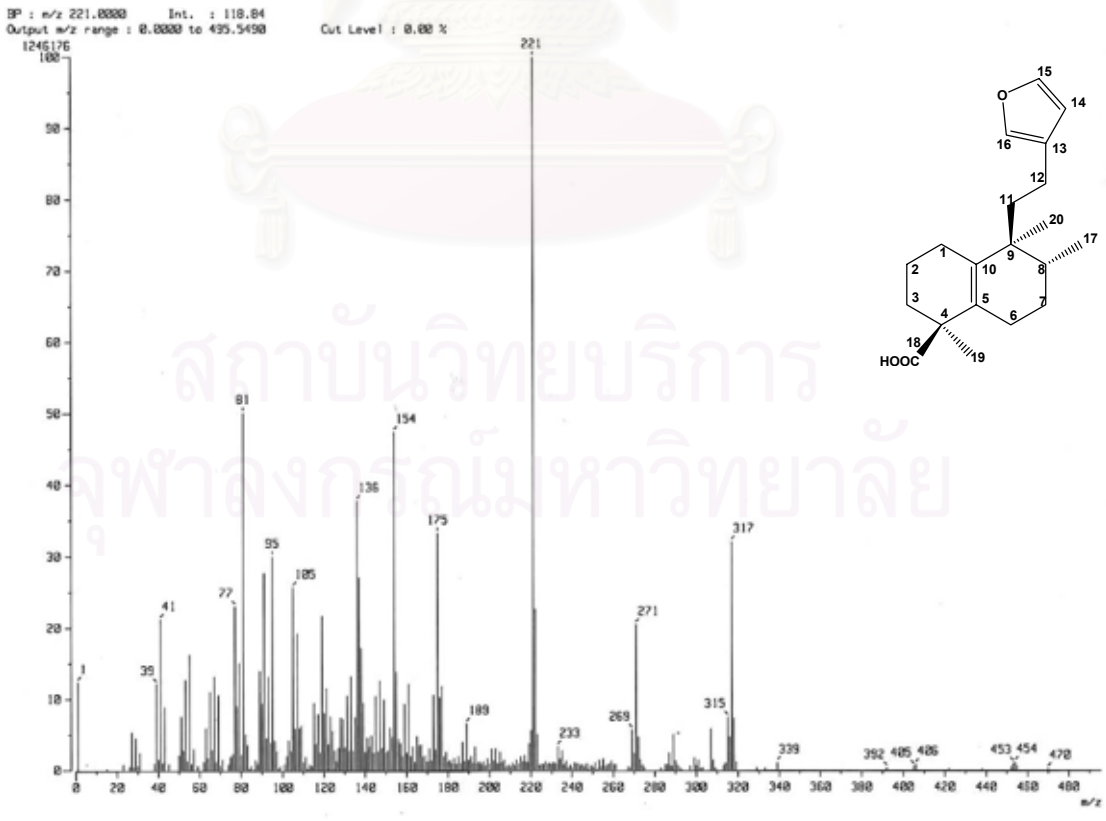

Figure 98 FAB Mass spectrum of compound **COC3**.

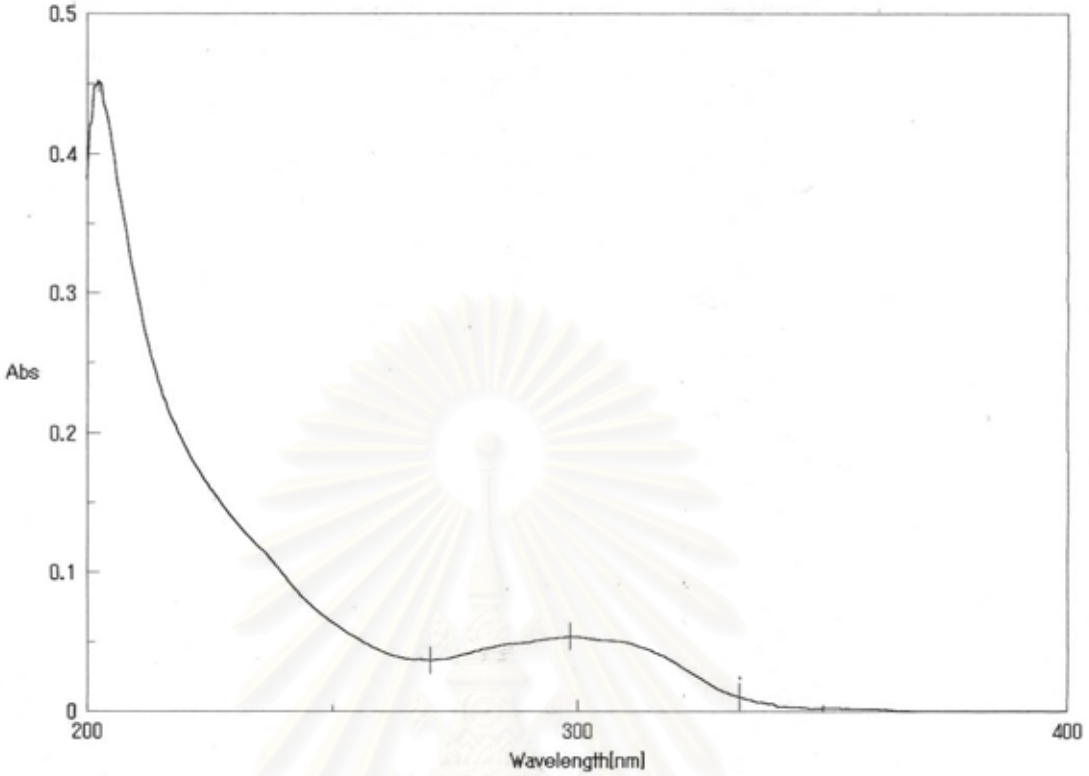

Figure 99 UV spectrum of compound **COC3** (MeOH).

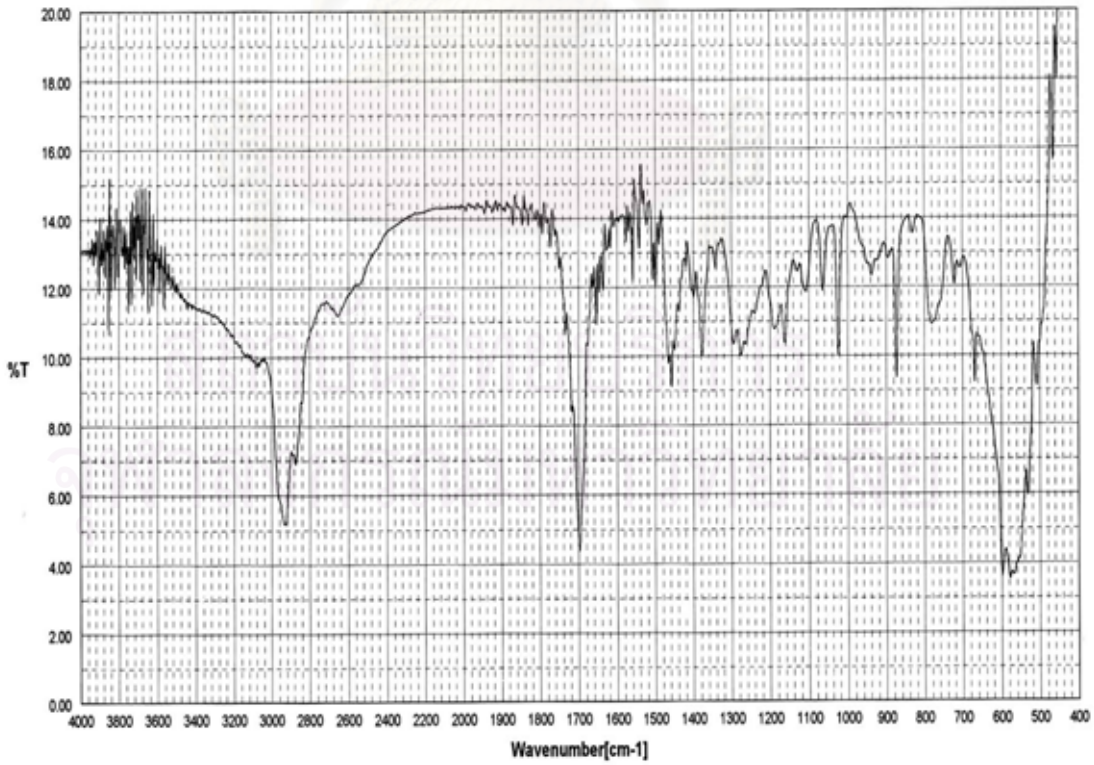

Figure 100 IR spectrum of compound **COC3** (Neat).

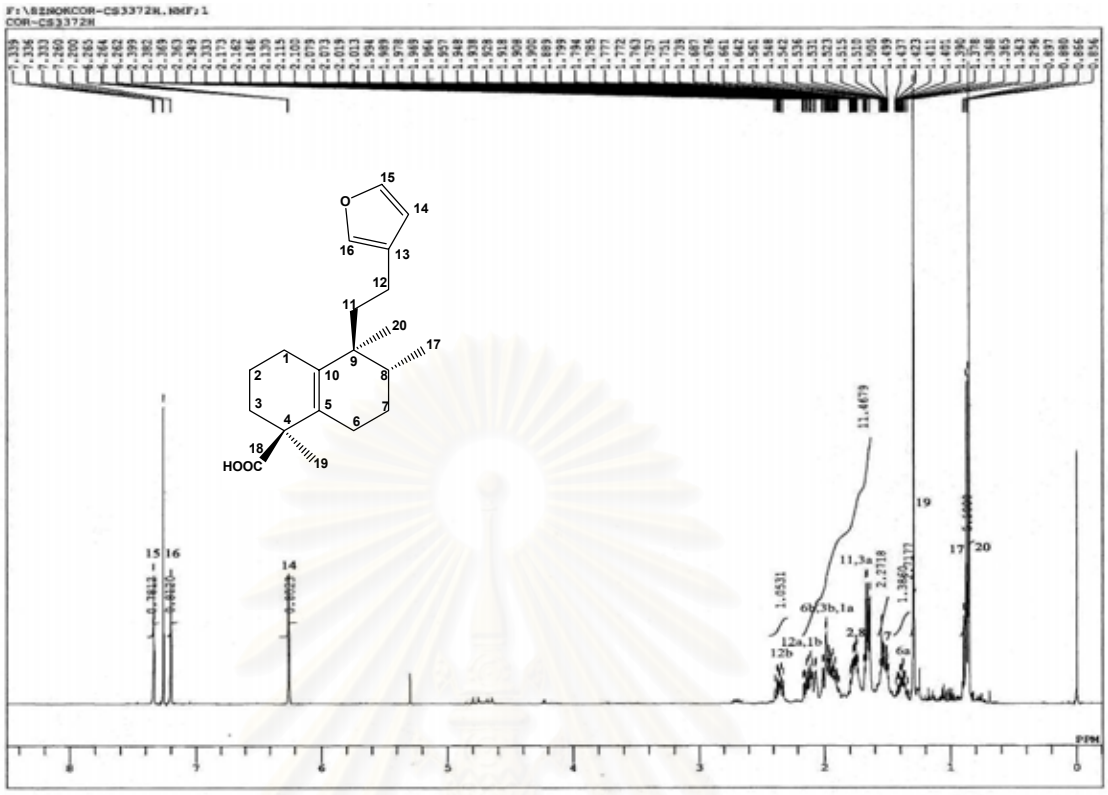

Figure 101 ^{1}H-NMR (500 MHz) spectrum of compound **COC3** ($CDCl_3$).

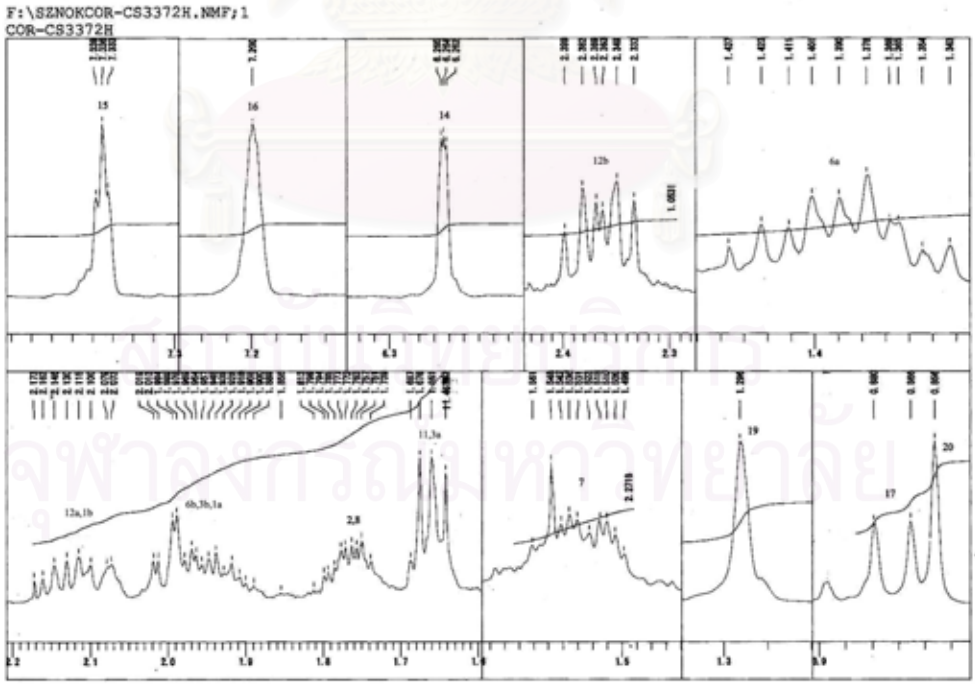

Figure 102 Expanded ^{1}H-NMR (500 MHz) spectrum of compound **COC3** ($CDCl_3$).

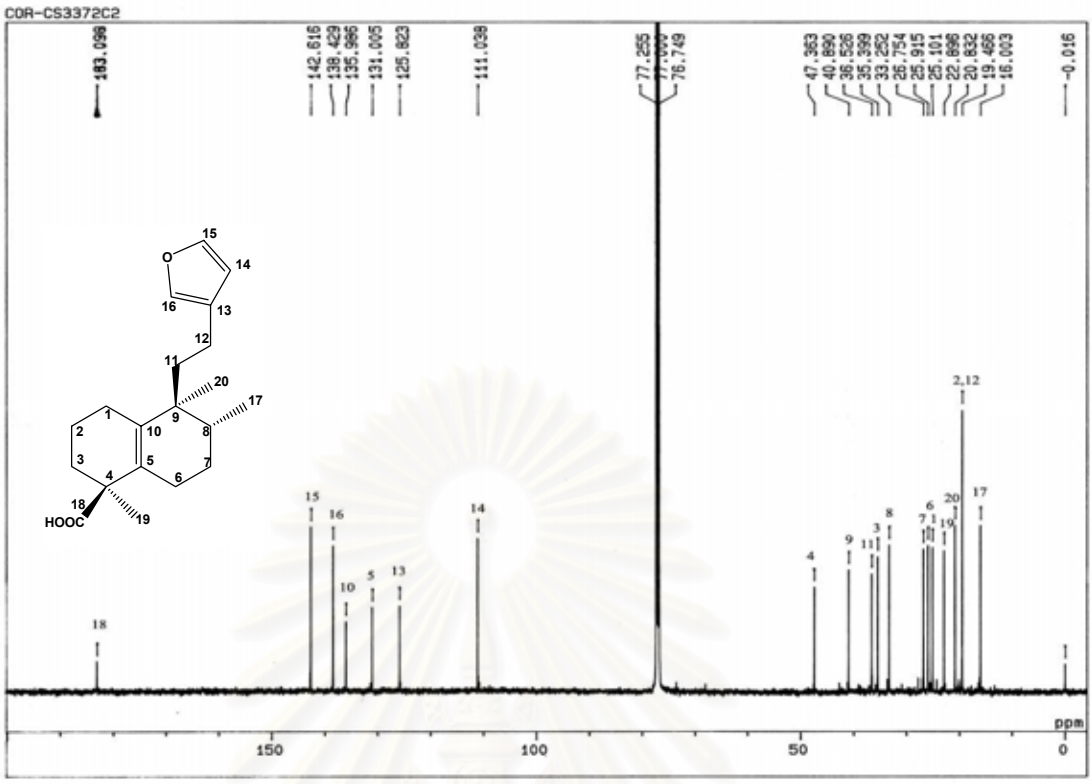

Figure 103 ^{13}C-NMR (125 MHz) spectrum of compound **COC3** ($CDCl_3$).

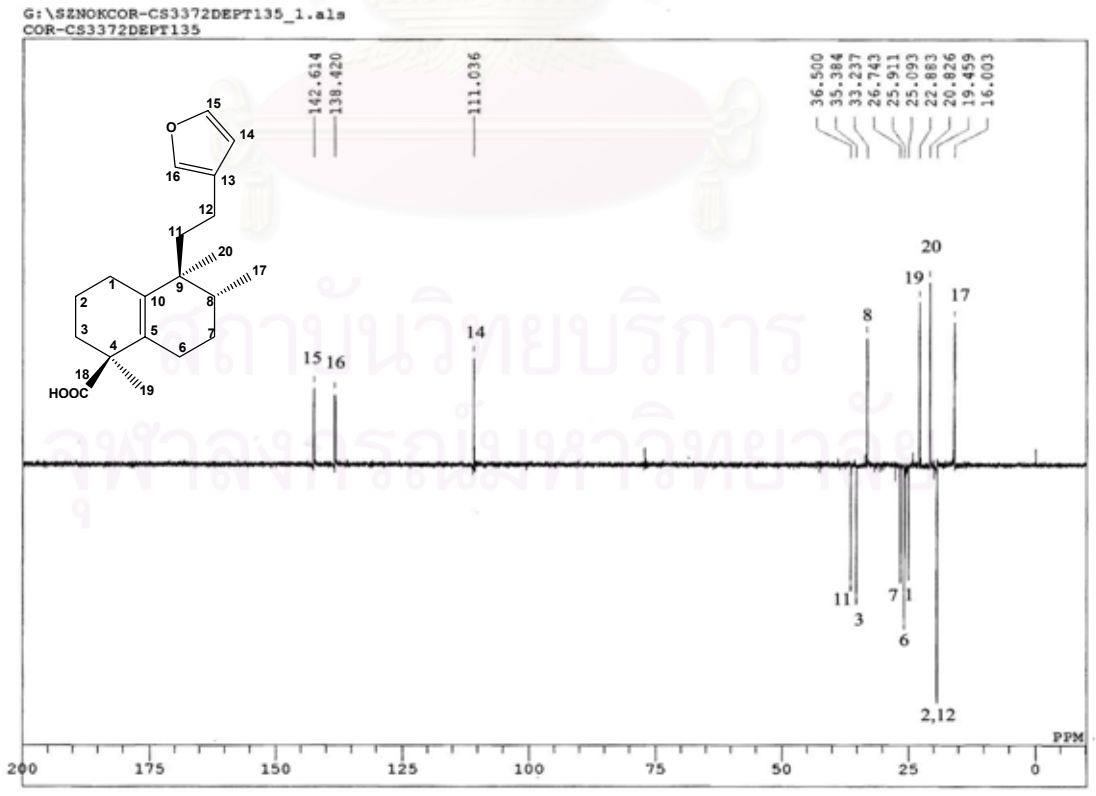

Figure 104 DEPT135 spectrum of compound **COC3** ($CDCl_3$).

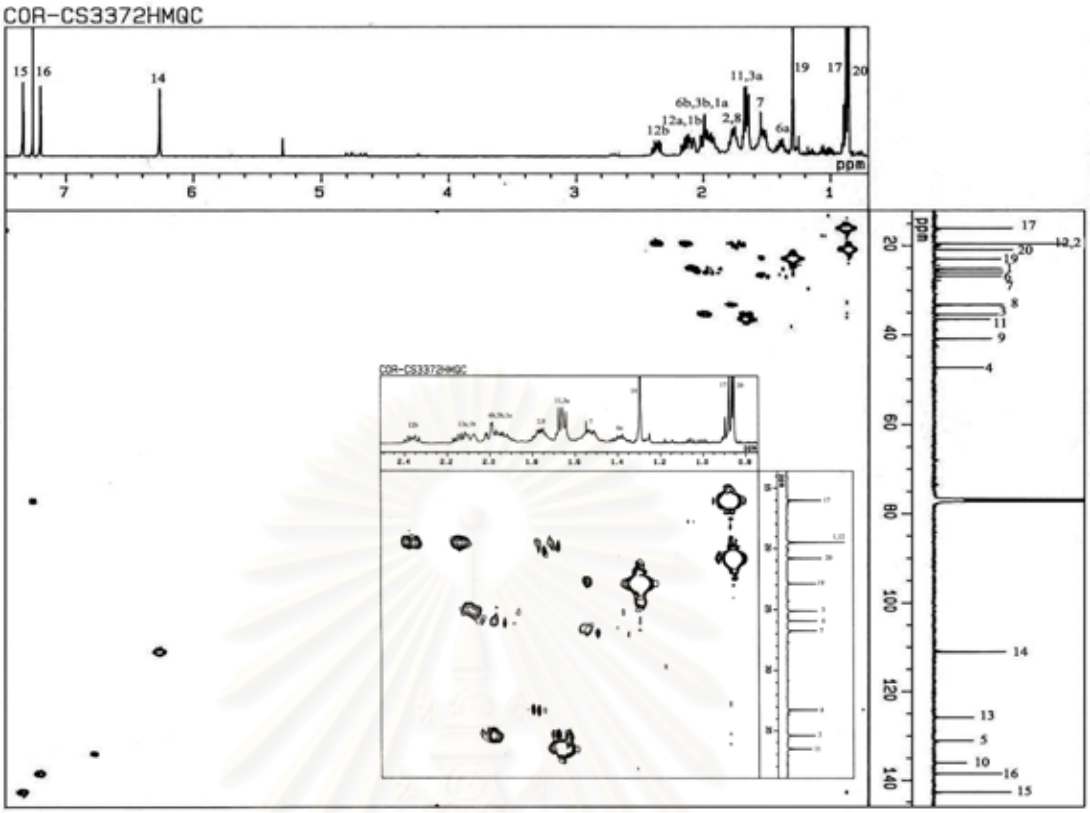

Figure 105 HMQC spectrum of compound **COC3** ($CDCl_3$).

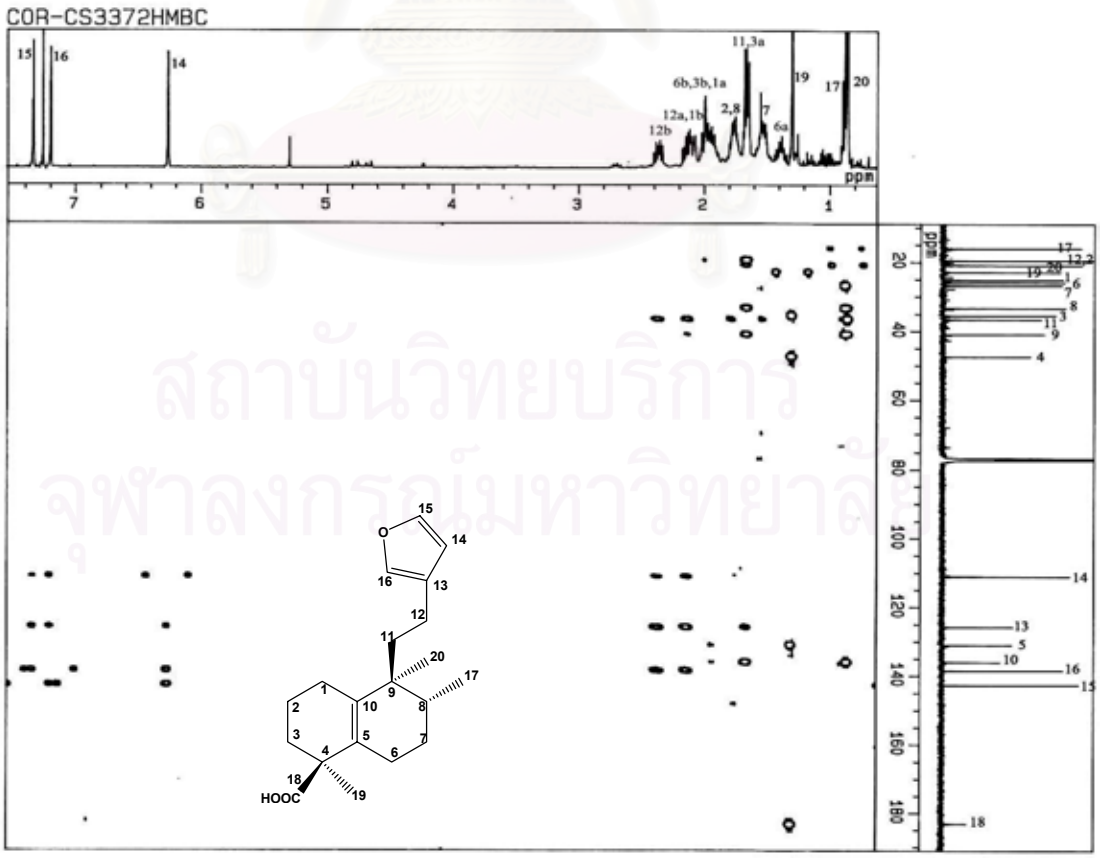

Figure 106 HMBC spectrum of compound **COC3** ($CDCl_3$).

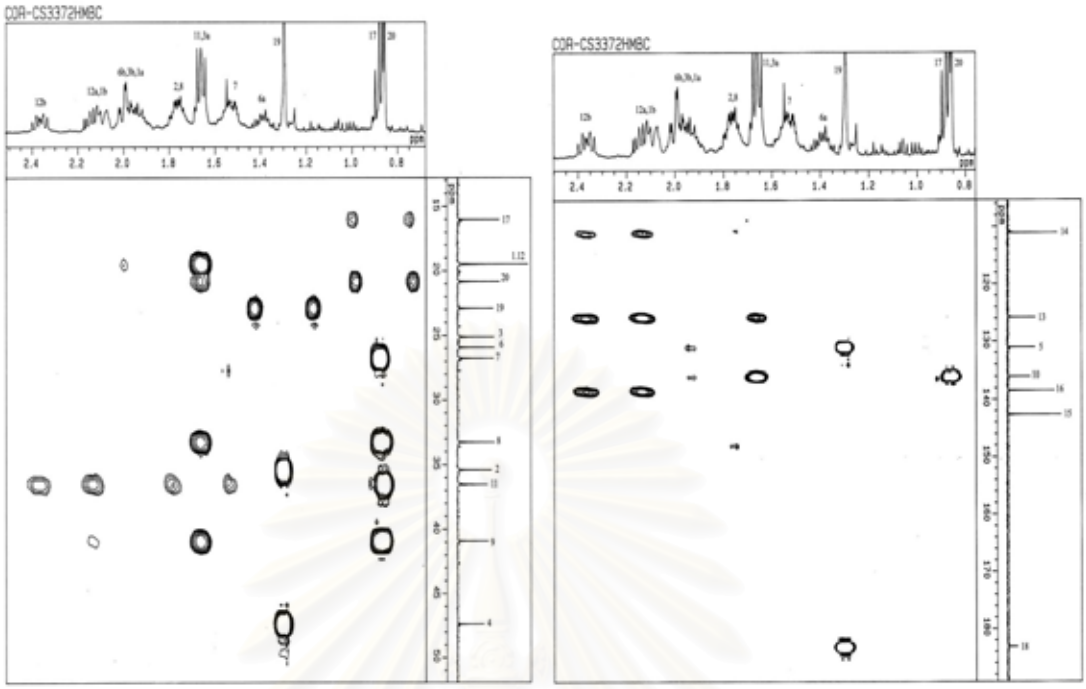

Figure 107 Expanded HMBC spectra of compound **COC3** ($CDCl_3$).

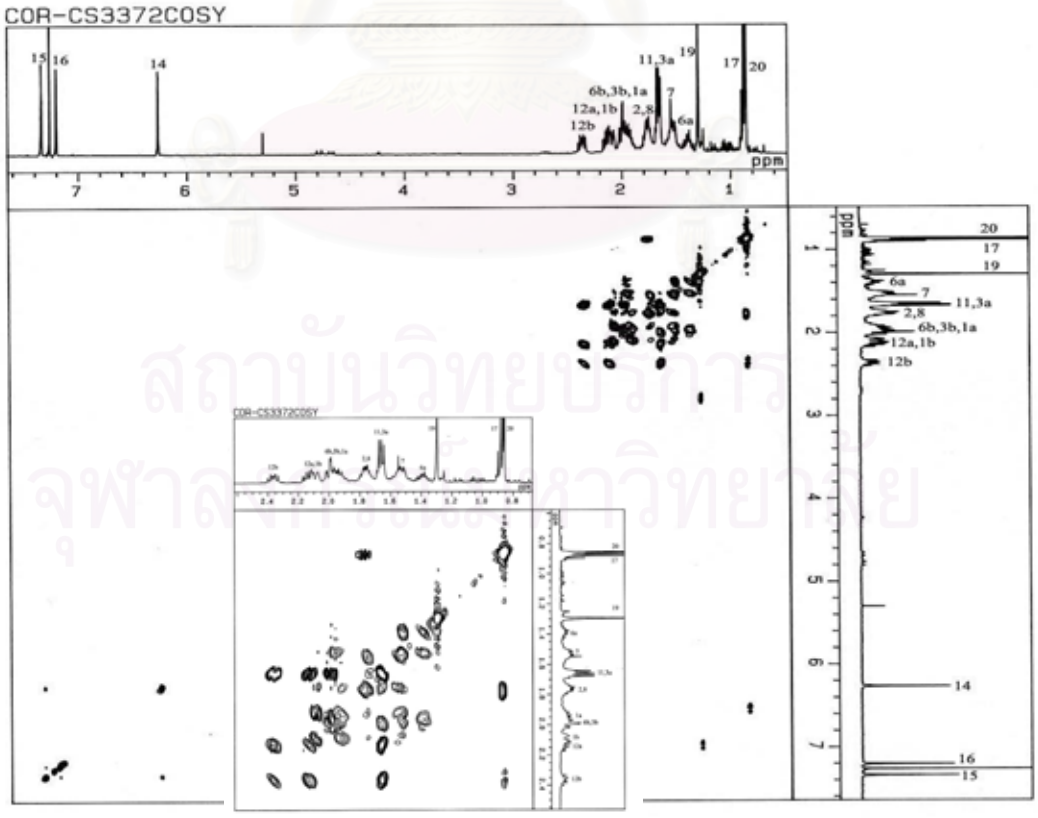

Figure 108 ^{1}H-^{1}H COSY spectrum of compound **COC3** ($CDCl_3$).

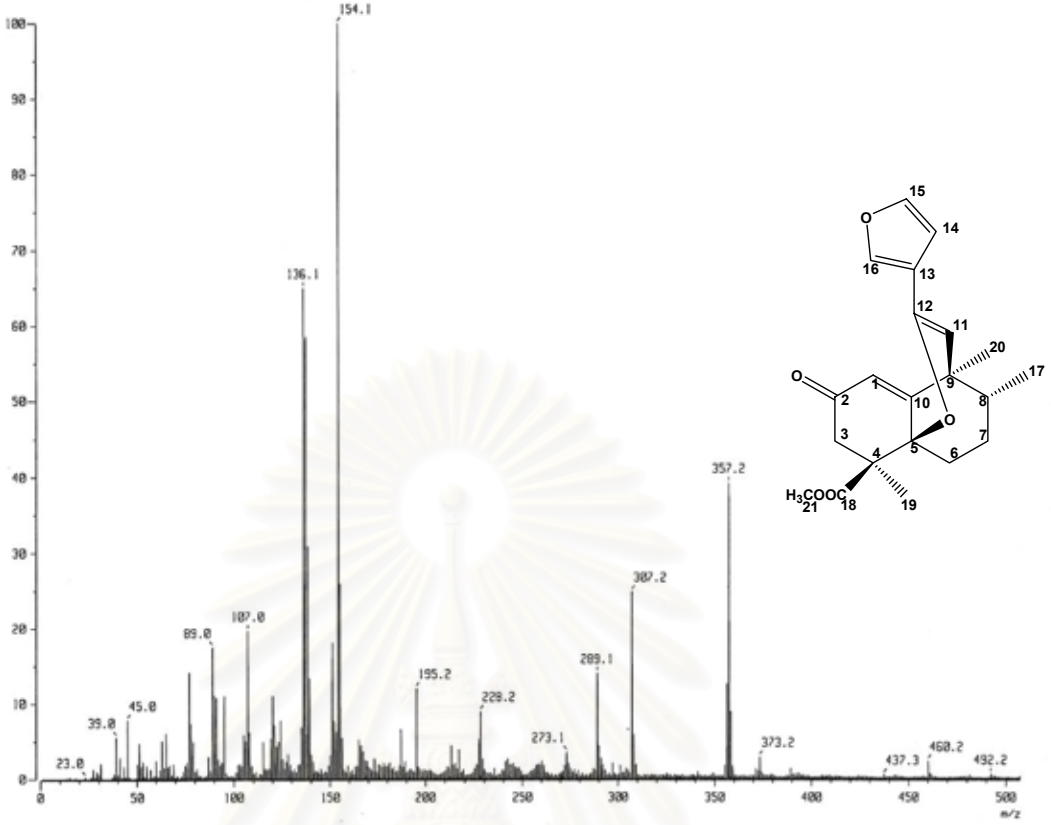

Figure 109 FAB Mass spectrum of compound **COC4**.

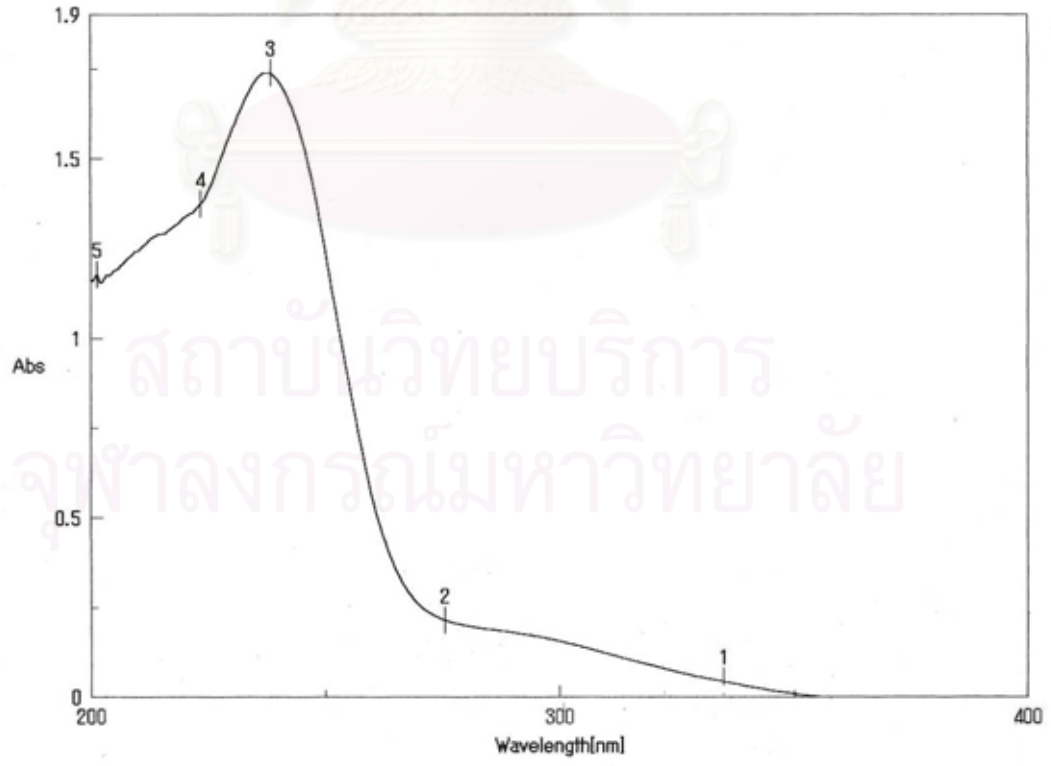

Figure 110 UV spectrum of compound **COC4** (MeOH).

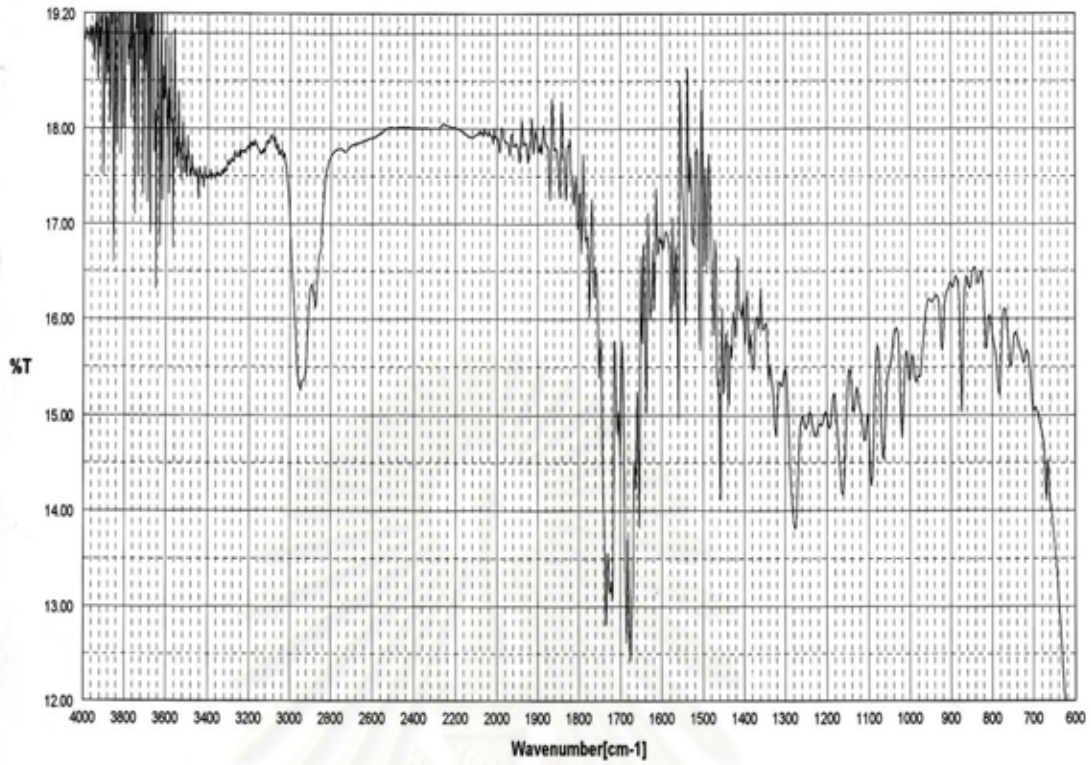

Figure 111 IR spectrum of compound **COC4** (Neat).

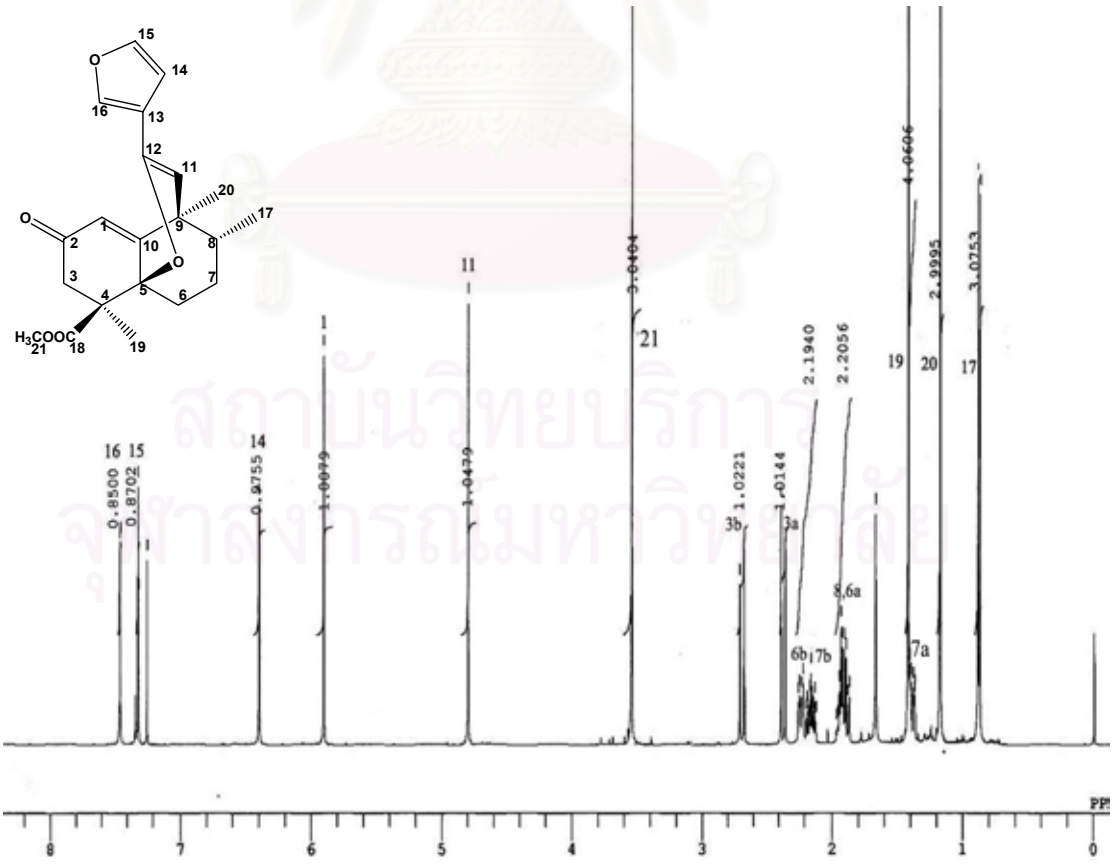

Figure 112 ^{1}H-NMR (500 MHz) spectrum of compound **COC4** ($CDCl_3$).

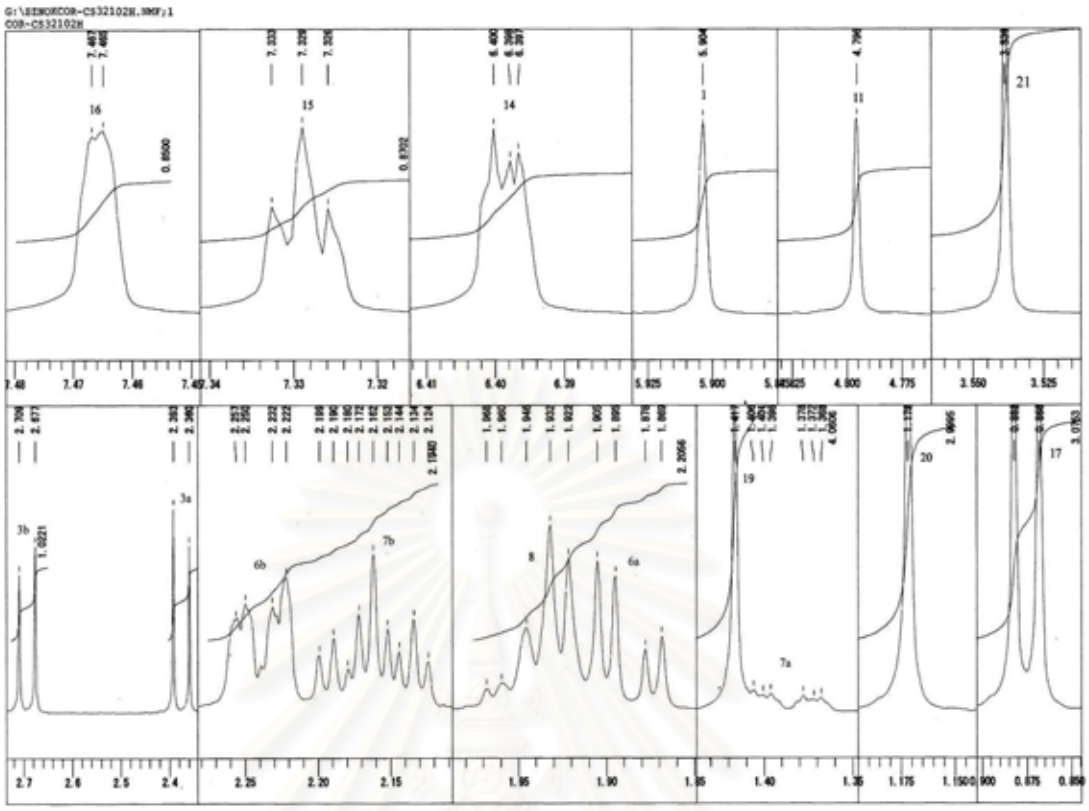

Figure 113 Expanded ^{1}H-NMR (500 MHz) spectrum of compound **COC4** ($CDCl_3$).

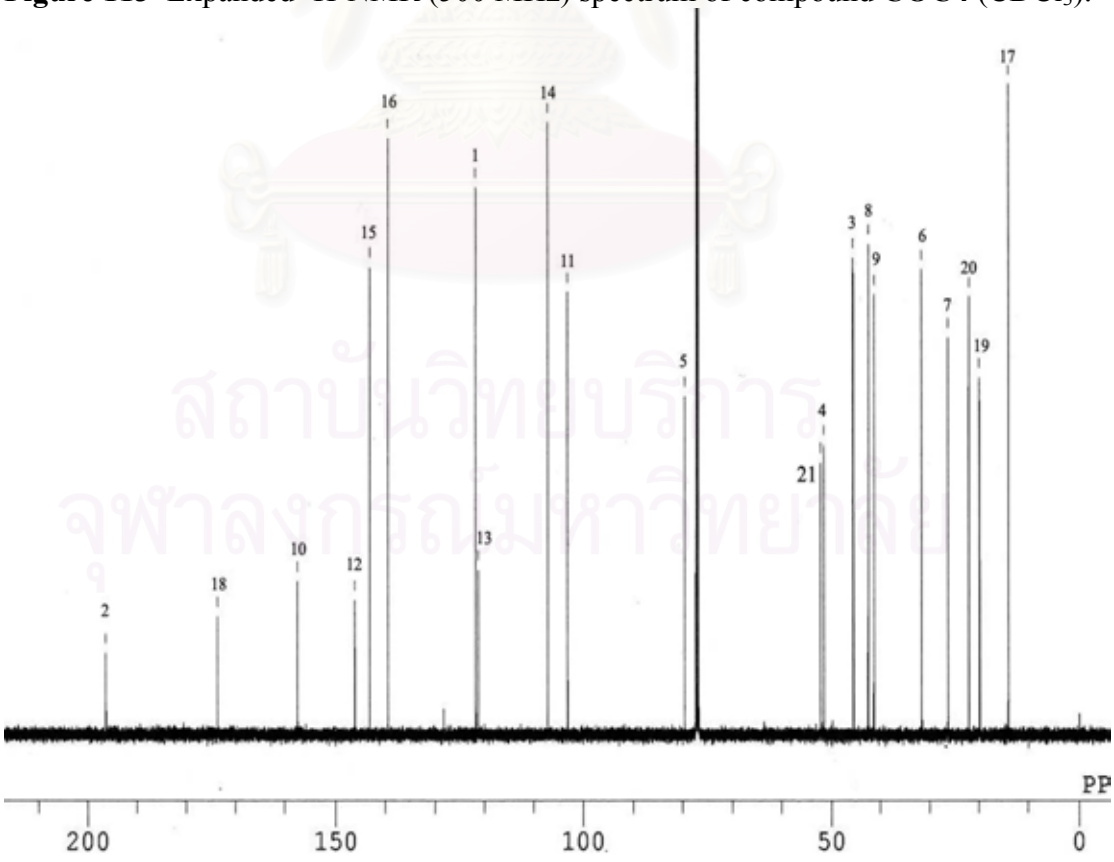

Figure 114 ^{13}C-NMR (125 MHz) spectrum of compound **COC4** ($CDCl_3$).

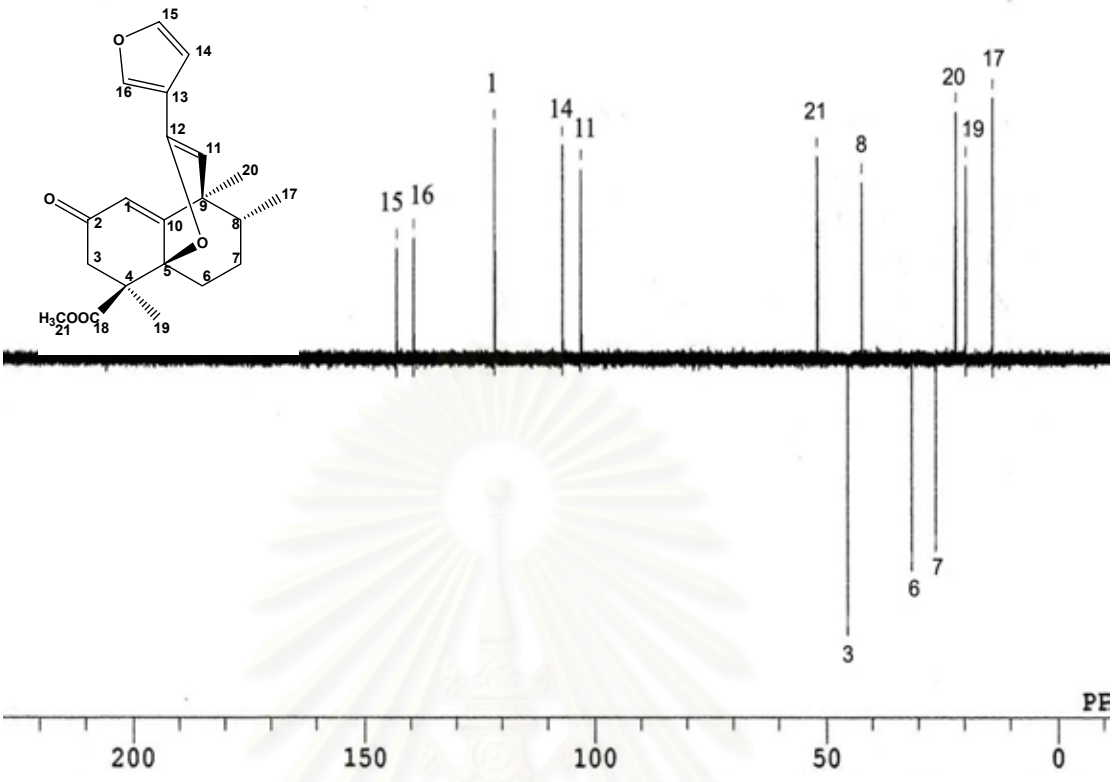

Figure 115 DEPT135 spectrum of compound **COC4** ($CDCl_3$).

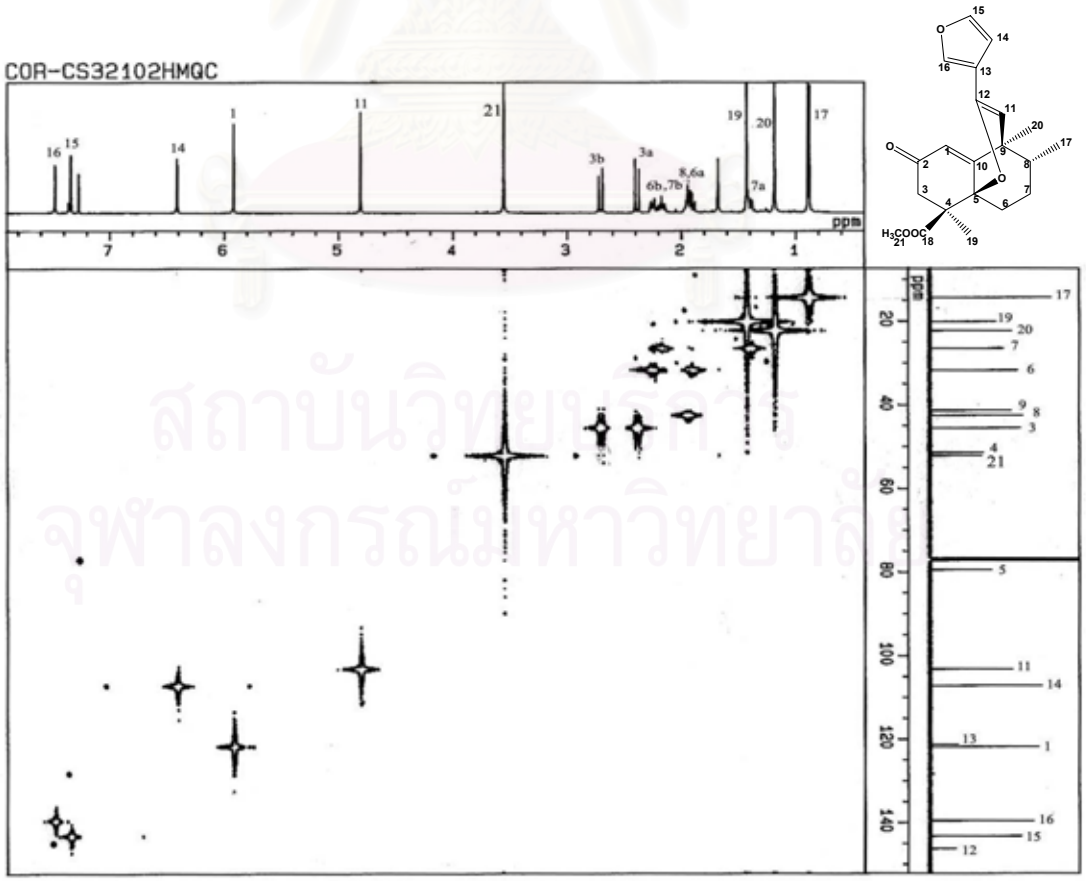

Figure 116 HMQC spectrum of compound **COC4** ($CDCl_3$).

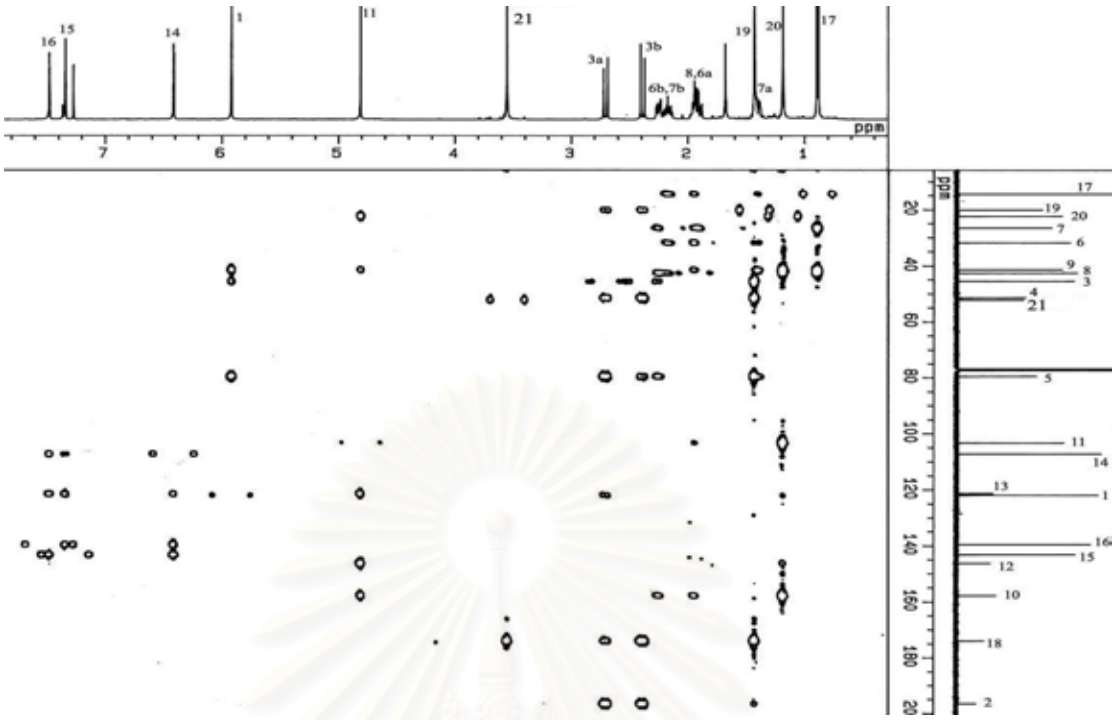

Figure 117 HMBC spectrum of compound **COC4** ($CDCl_3$).

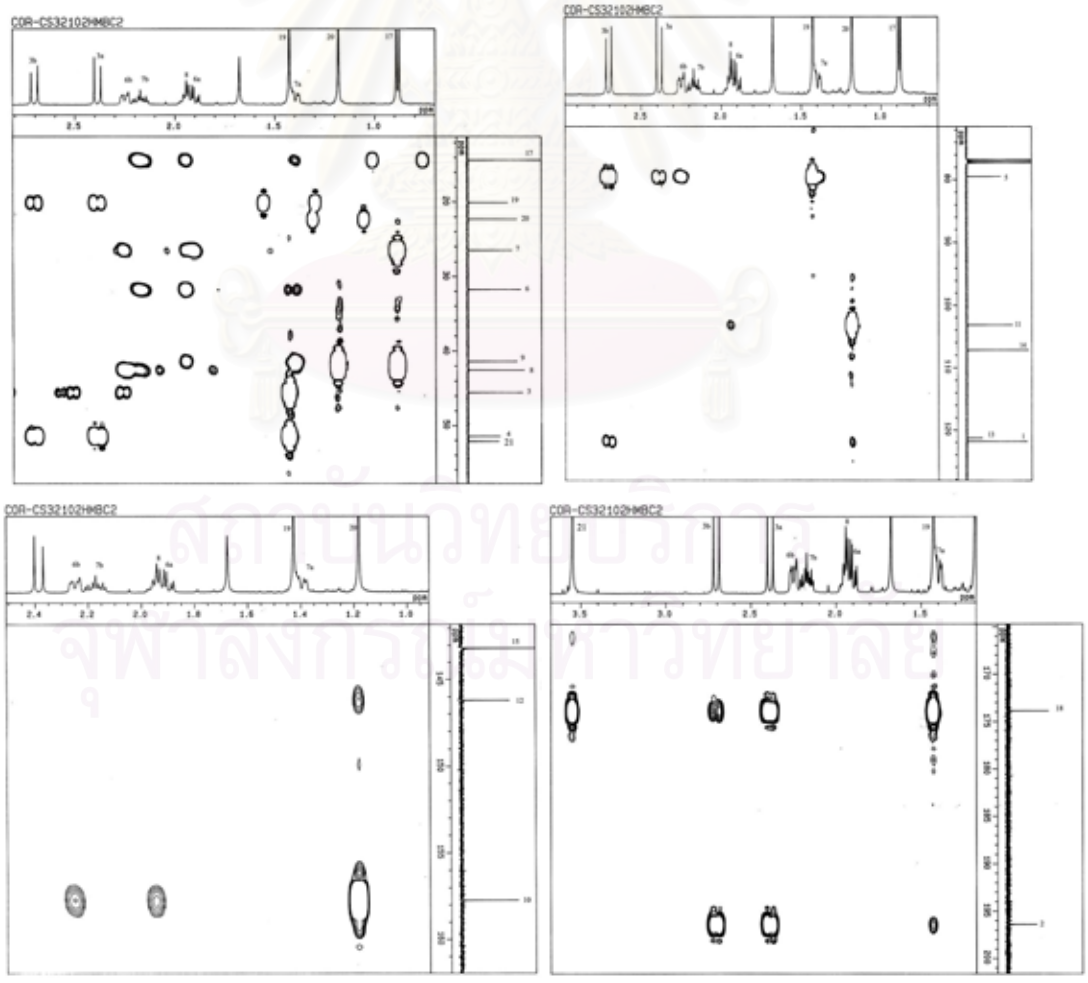

Figure 118 Expanded HMBC spectra of compound **COC4** ($CDCl_3$).

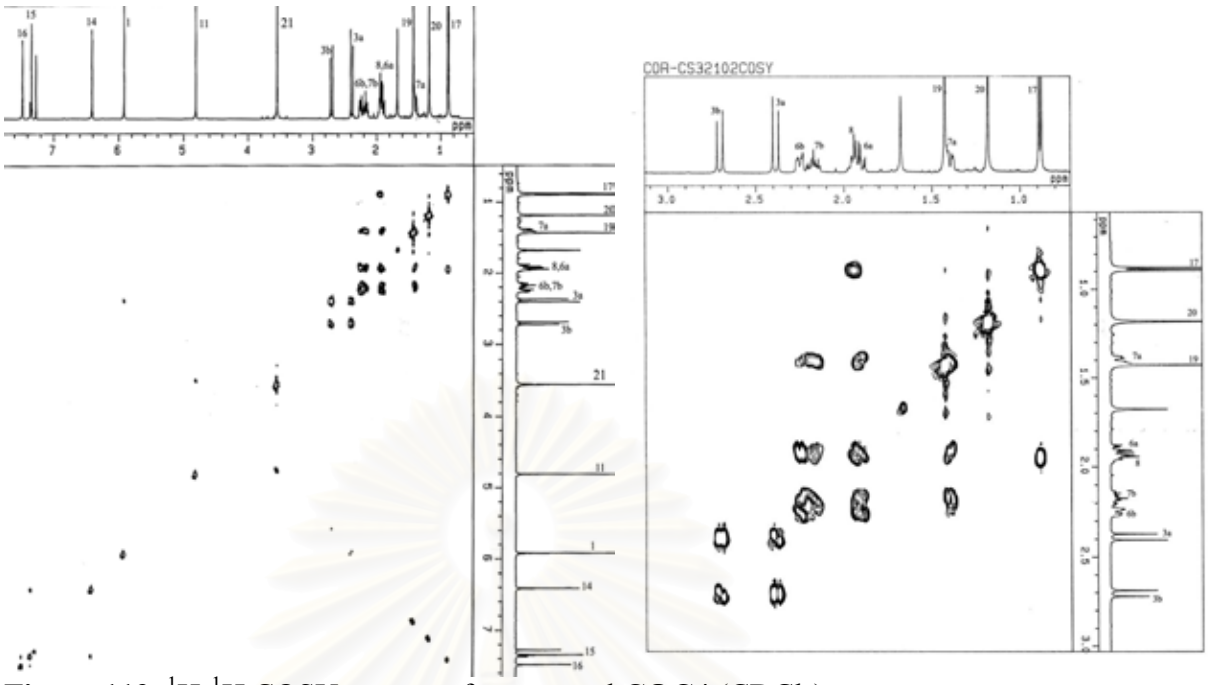

Figure 119 ^{1}H-^{1}H COSY spectra of compound **COC4** ($CDCl_3$).

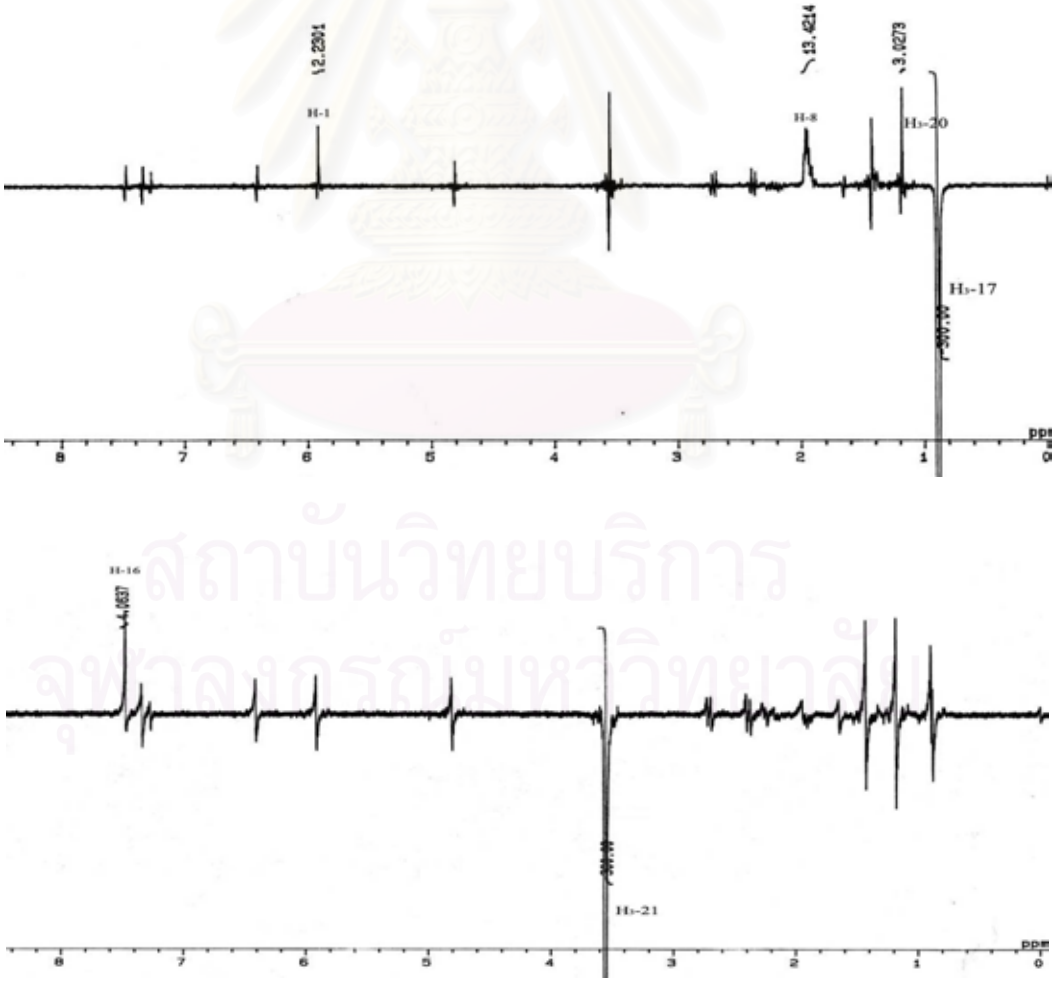

Figure 120 NOE spectra of compound **COC4** ($CDCl_3$).

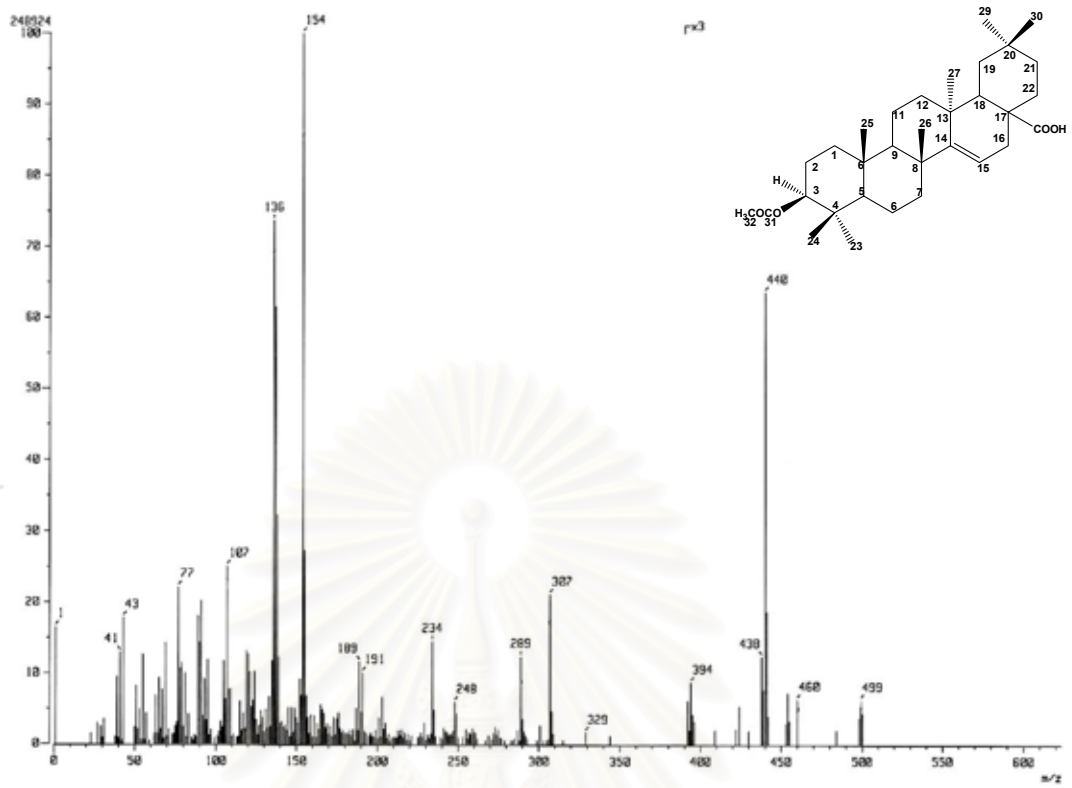

Figure 122 FAB Mass spectrum of compound **COC5**.

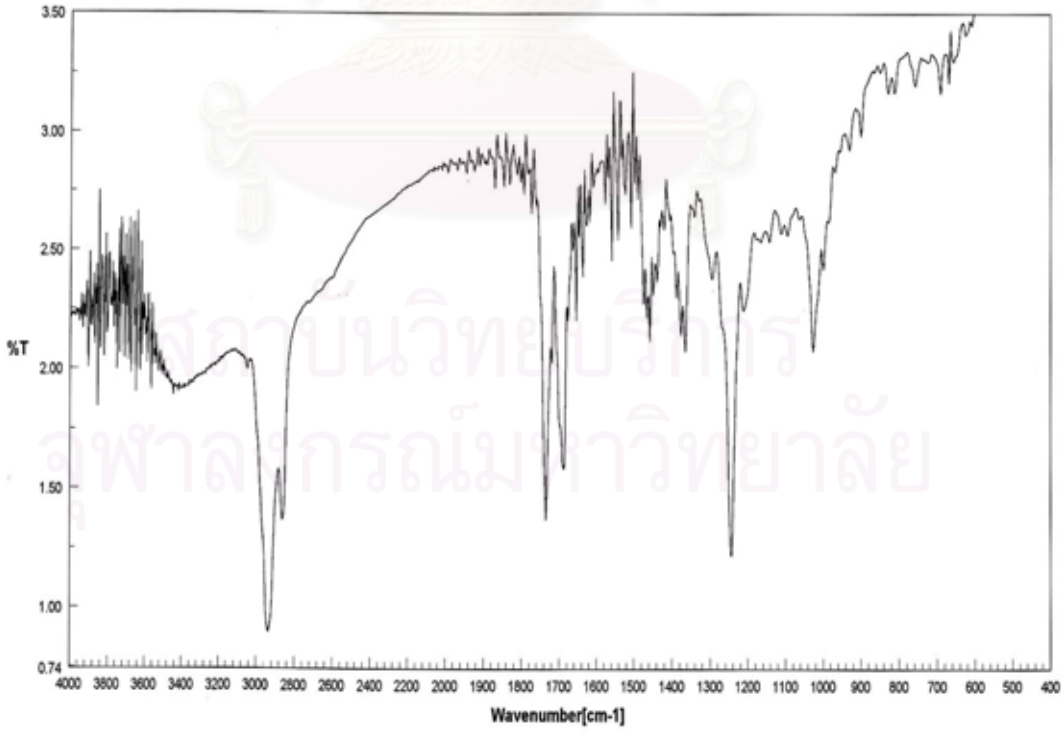

Figure 123 IR spectrum of compound **COC5** (KBr disc).

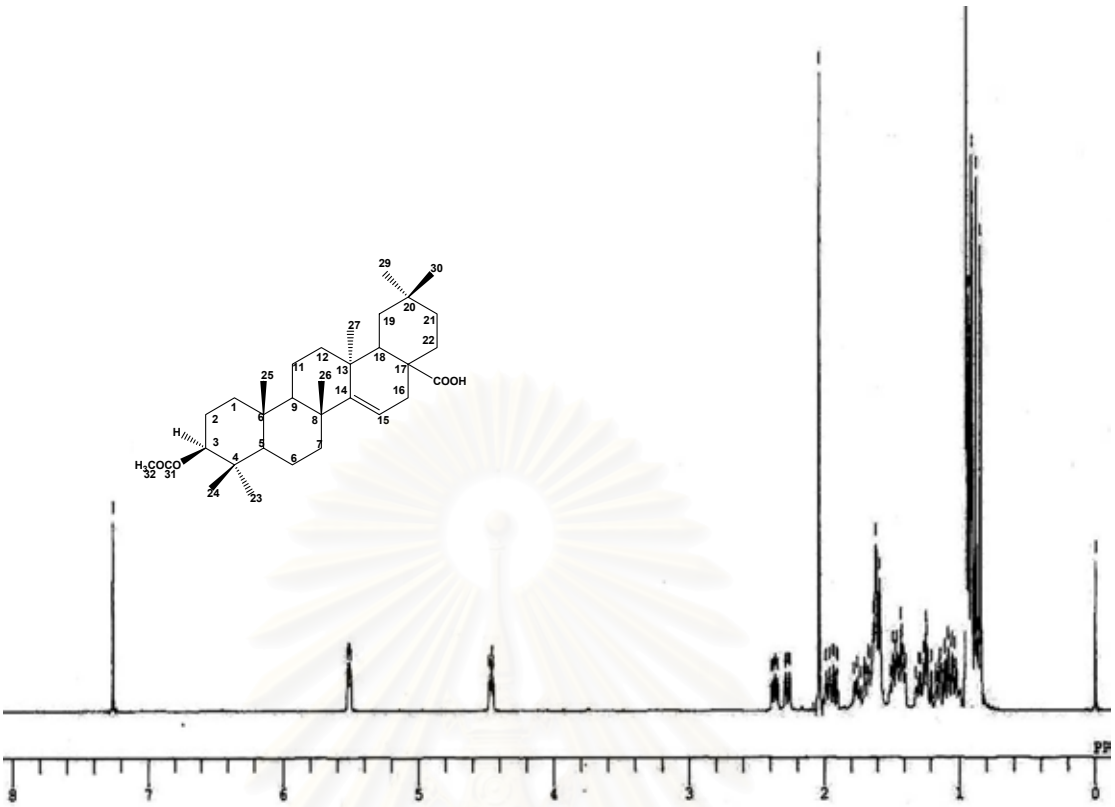

Figure 124 ^{1}H-NMR (500 MHz) spectrum of compound **COC5** ($CDCl_3$).

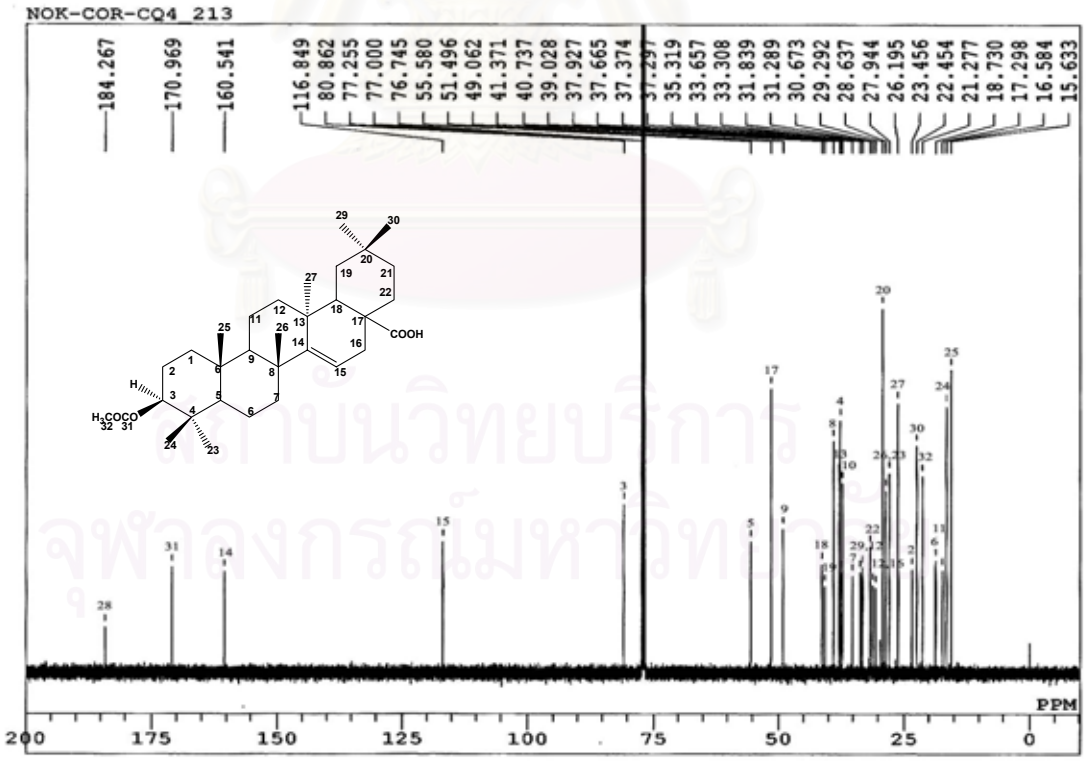

Figure 125 ^{13}C-NMR (125MHz) spectrum of compound **COC5** ($CDCl_3$).

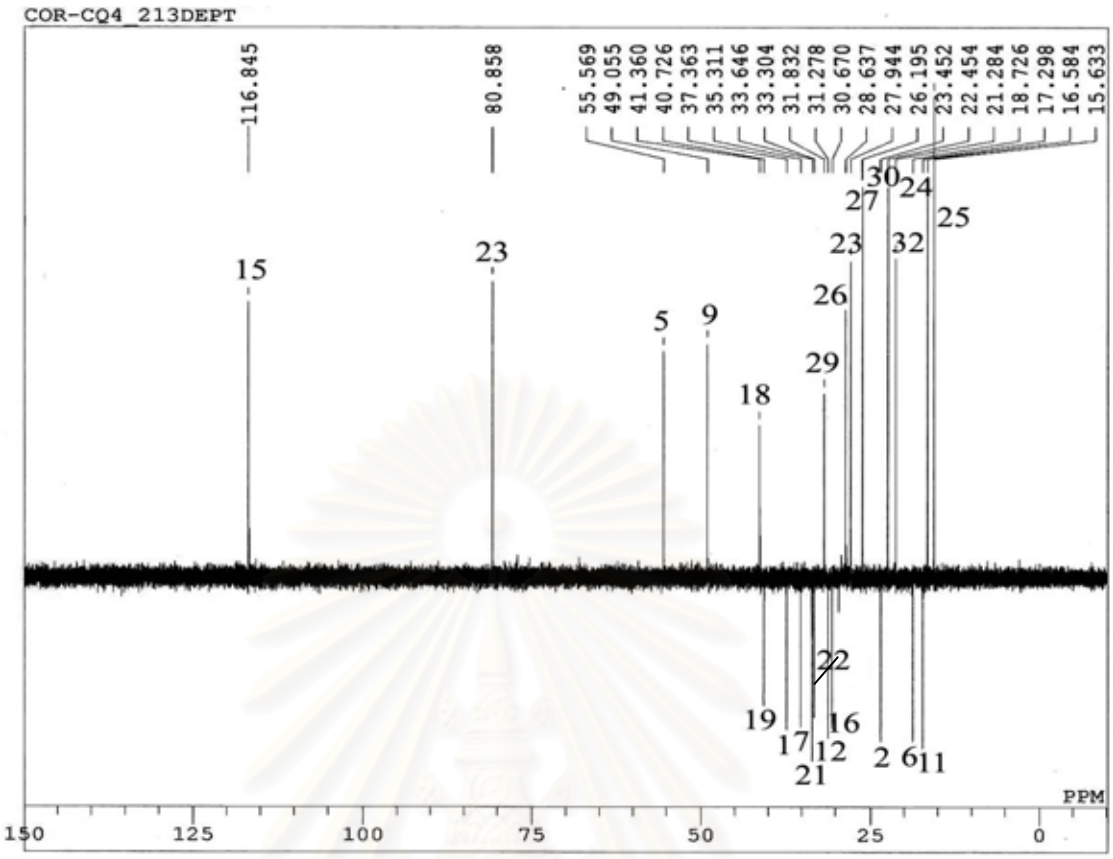

Figure 126 DEPT135 spectrum of compound **COC5** ($CDCl_3$).

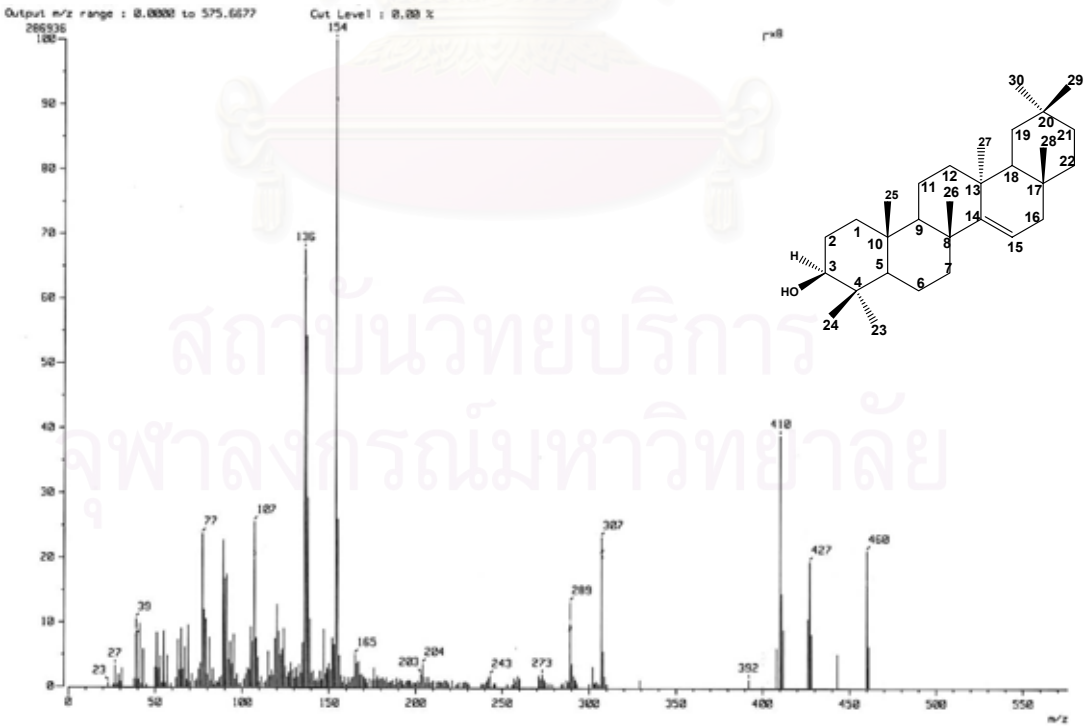

Figure 127 FAB Mass spectrum of compound **COC6**.

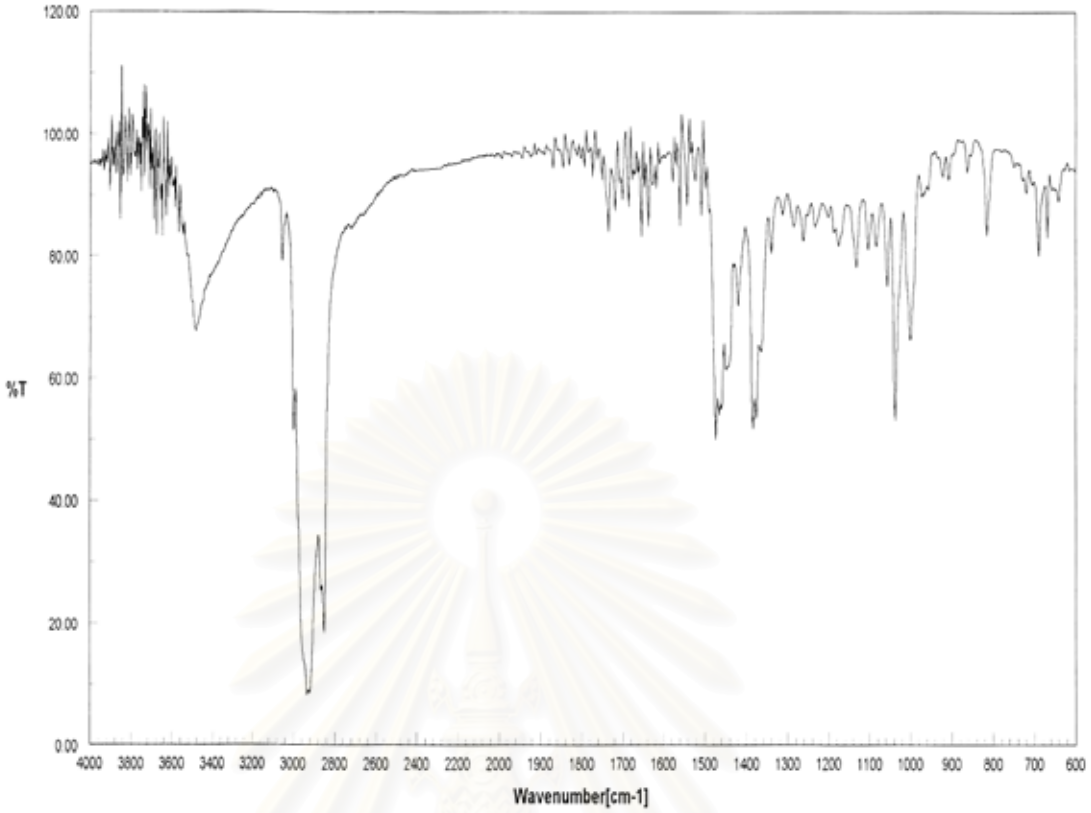

Figure 128 IR spectrum of compound **COC6** (KBr disc).

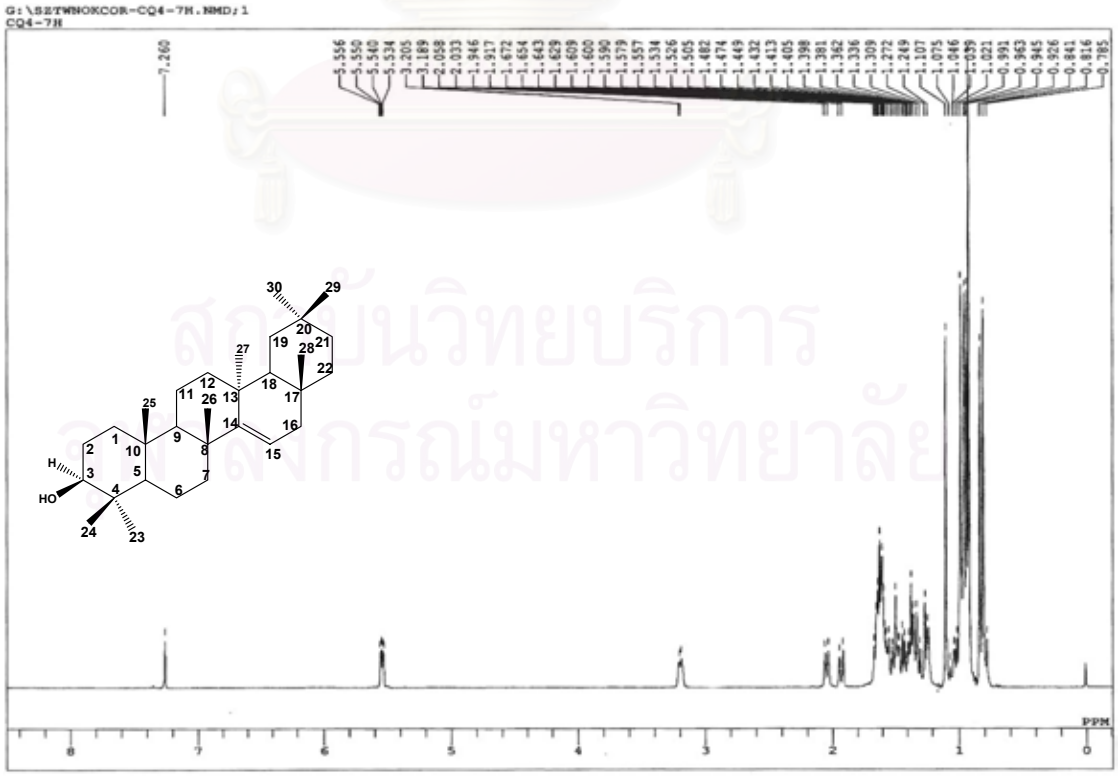

Figure 129 ^{1}H-NMR (500 MHz) spectrum of compound **COC6** ($CDCl_3$).

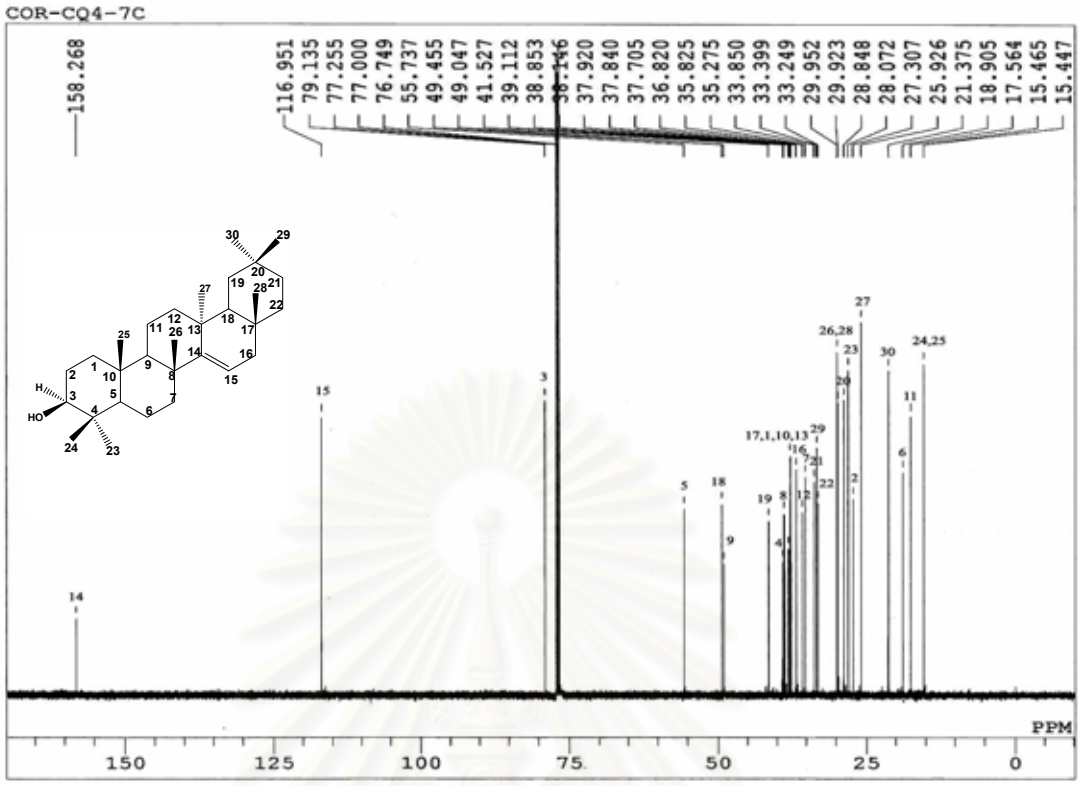

Figure 130 ^{13}C-NMR (125 MHz) spectrum of compound **COC6** ($CDCl_3$).

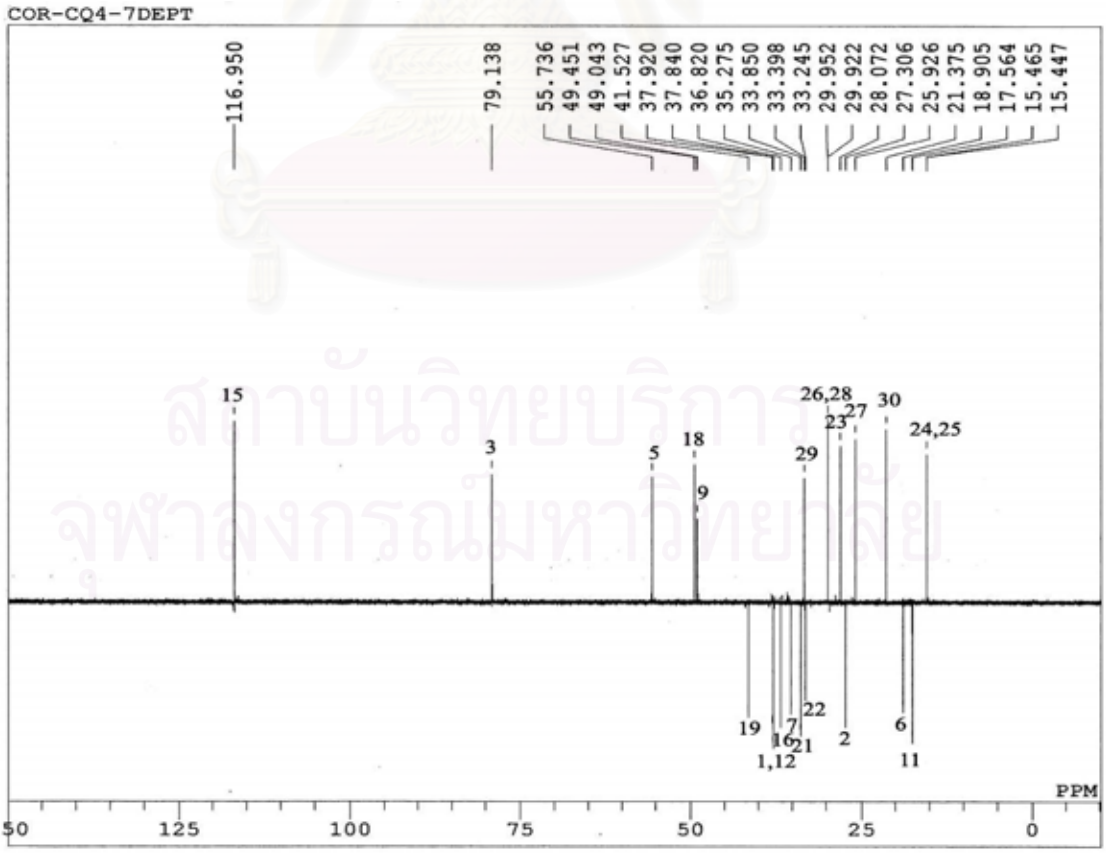

Figure 131 DEPT135 spectrum of compound **COC6** ($CDCl_3$).

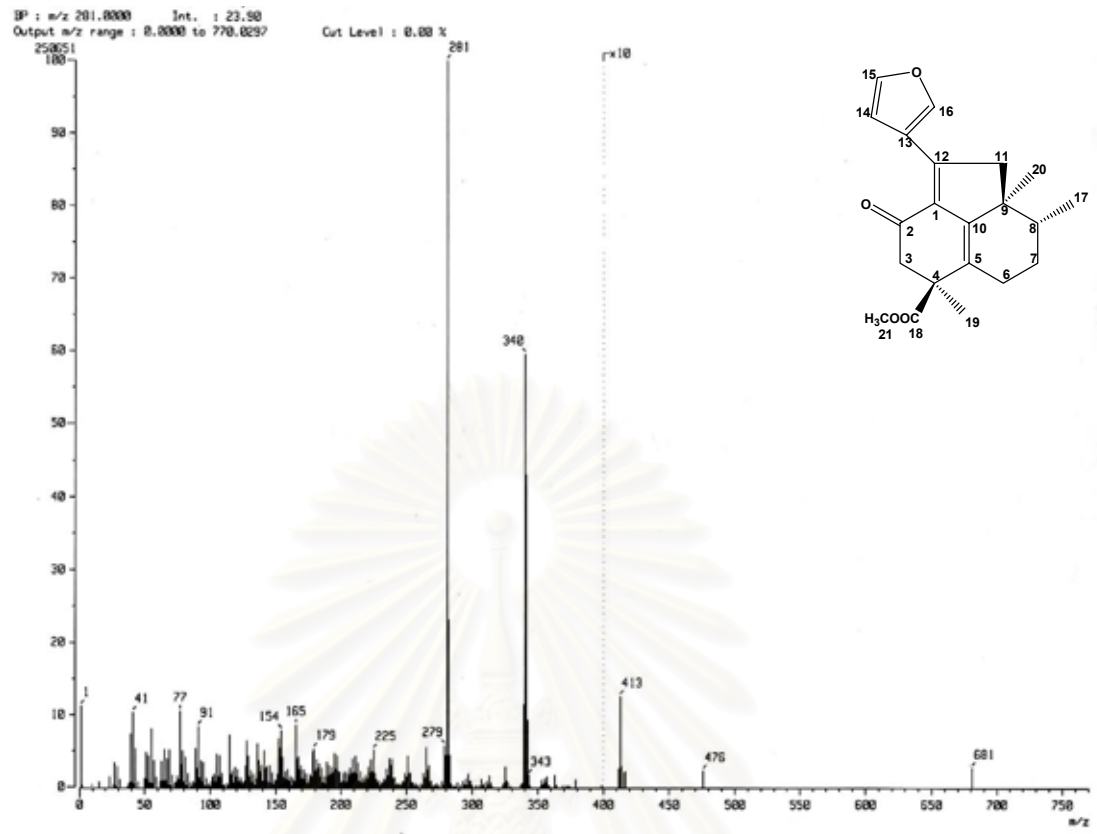

Figure 132 FAB Mass spectrum of compound **COC7**.

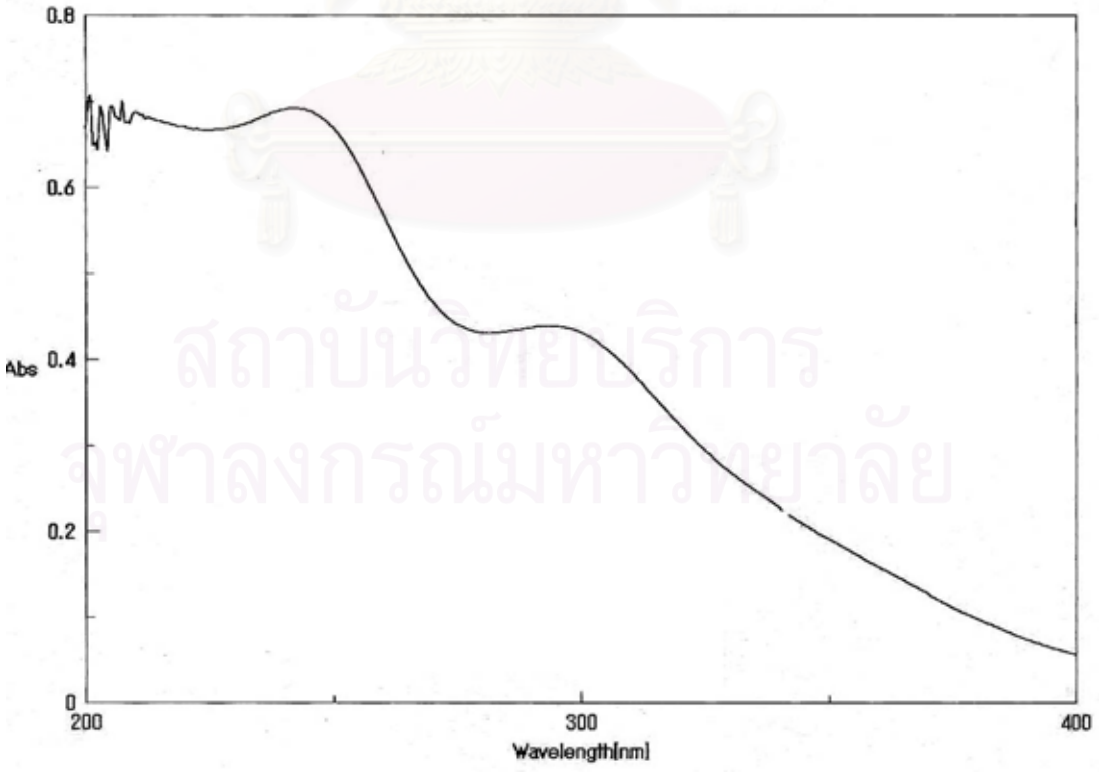

Figure 133 UV spectrum of compound **COC7** (EtOH).

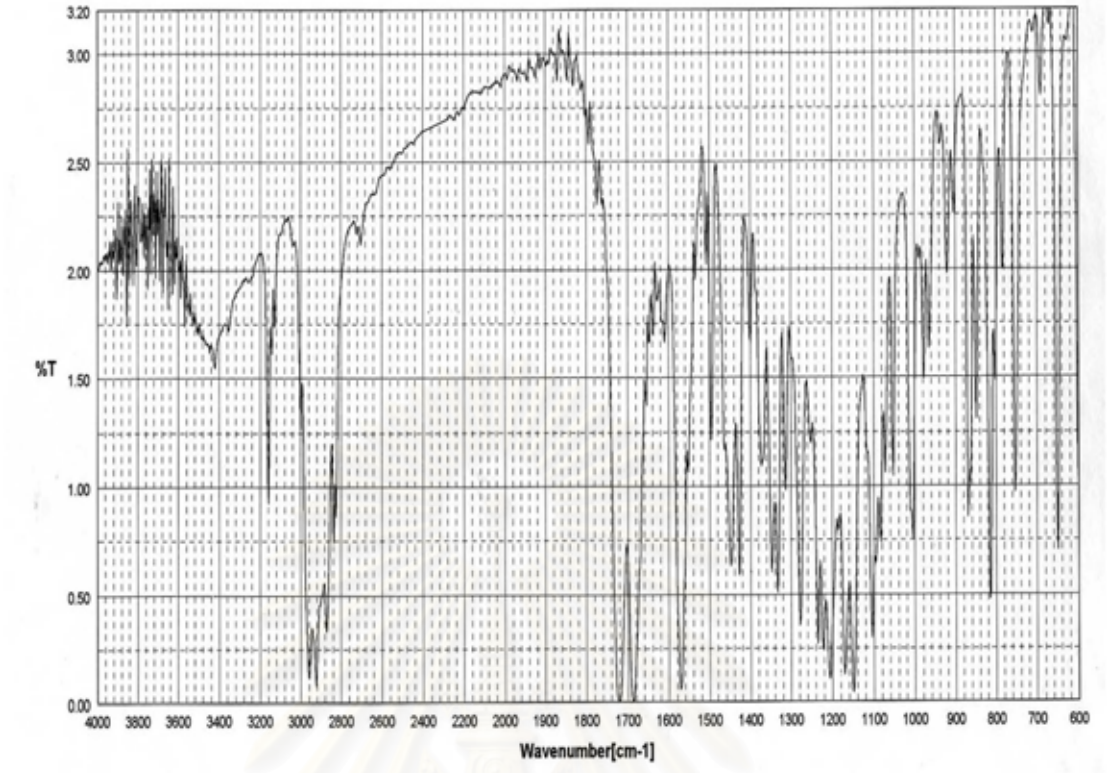

Figure 134 IR spectrum of compound **COC7** (KBr disc).

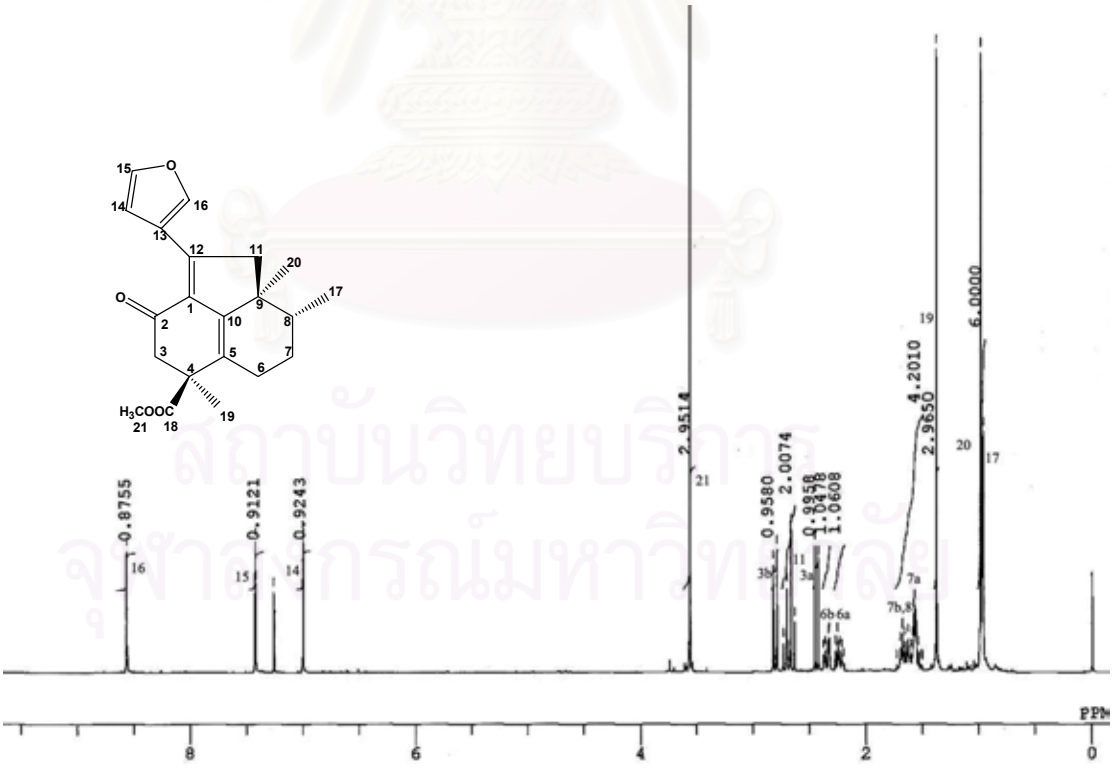

Figure 135 ^{1}H-NMR (500 MHz) spectrum of compound **COC7** ($CDCl_3$).

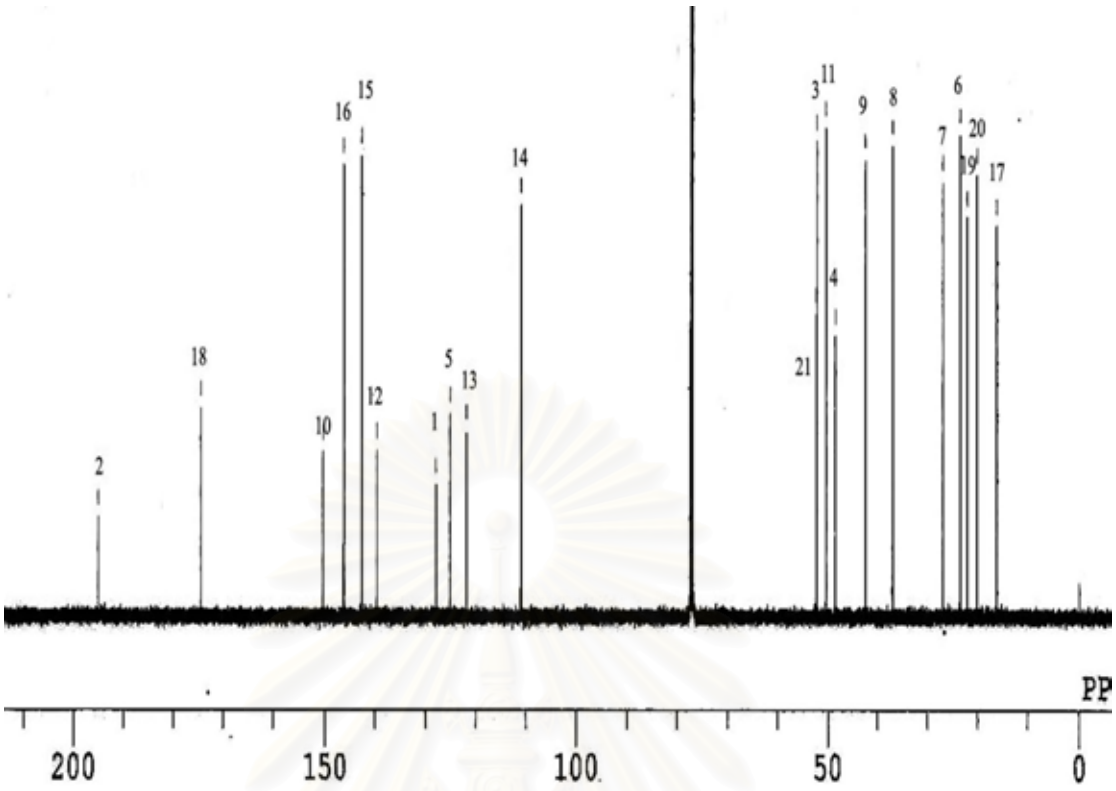

Figure 136 ^{13}C-NMR (125 MHz) spectrum of compound **COC7** ($CDCl_3$).

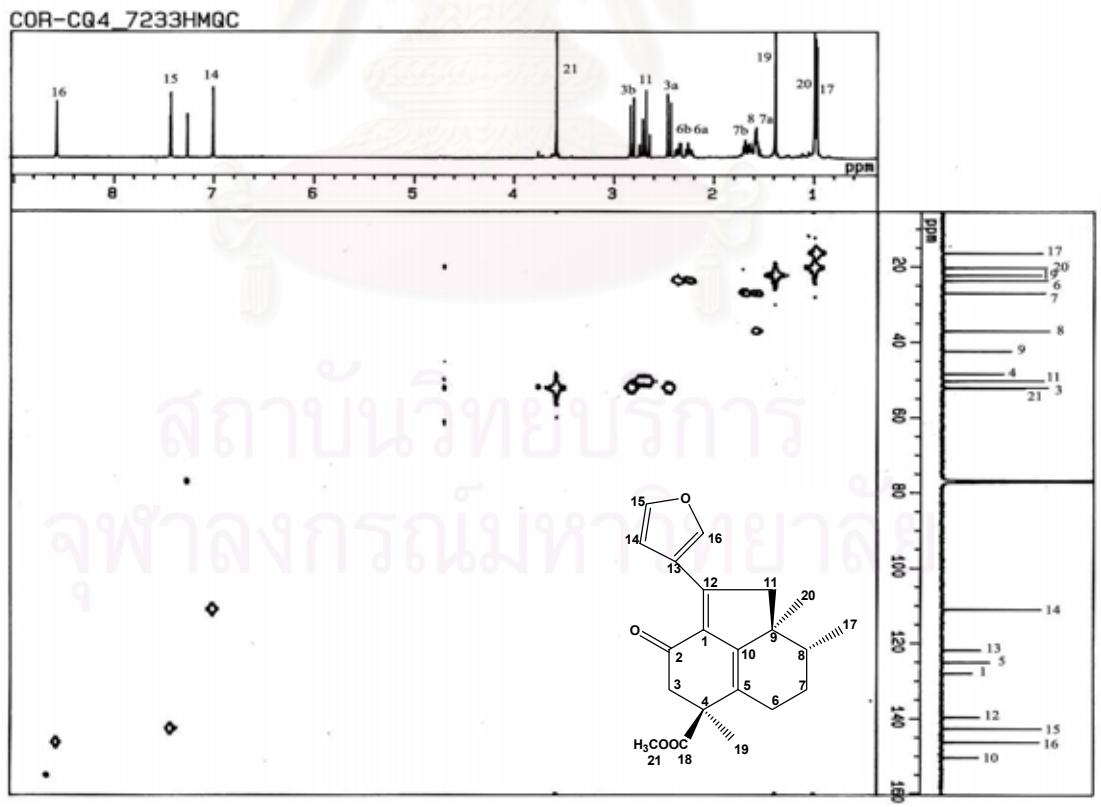

Figure 137 HMQC spectrum of compound **COC7** ($CDCl_3$).

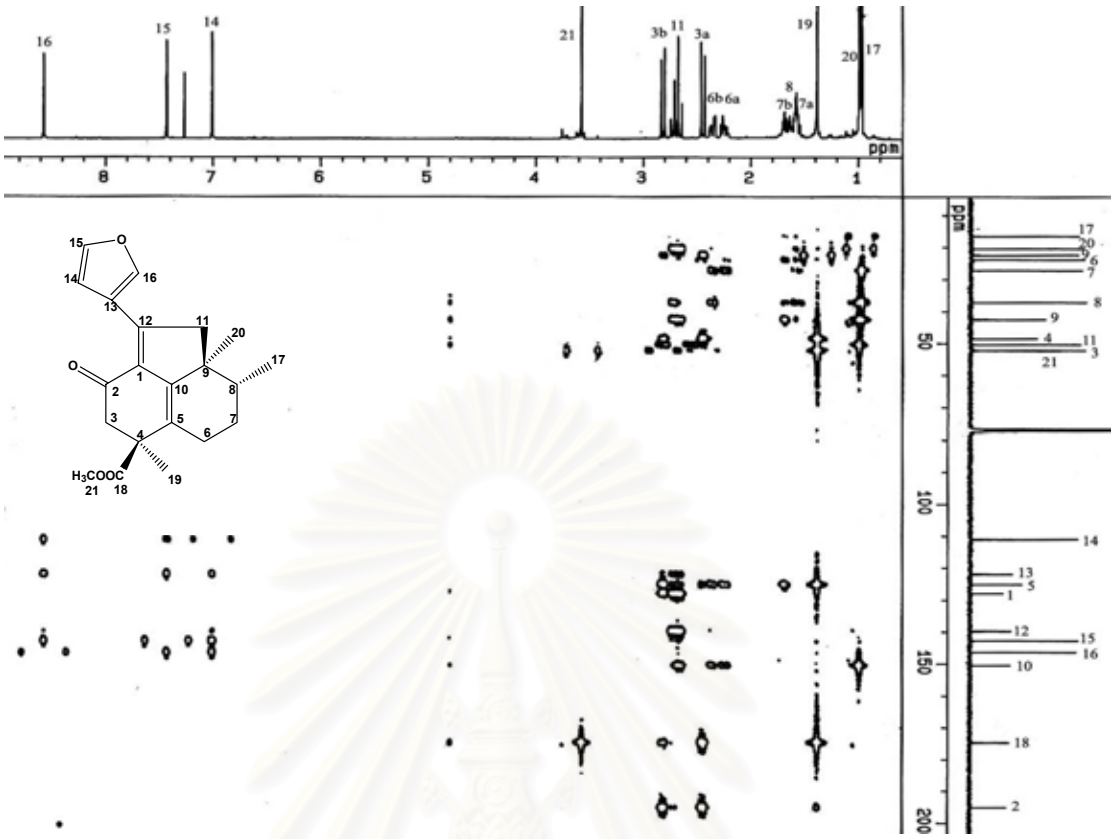

Figure 138 HMBC spectrum of compound **COC7** ($CDCl_3$).

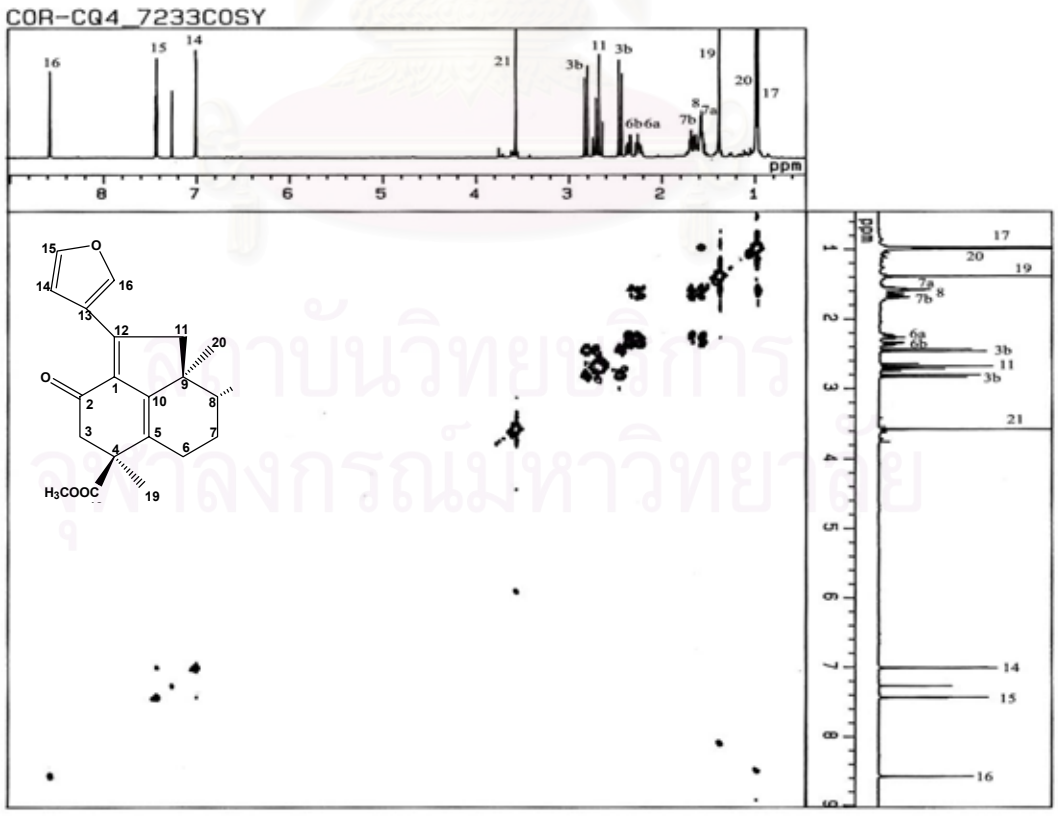

Figure 139 ^{1}H-^{1}H COSY spectrum of compound **COC7** ($CDCl_3$).

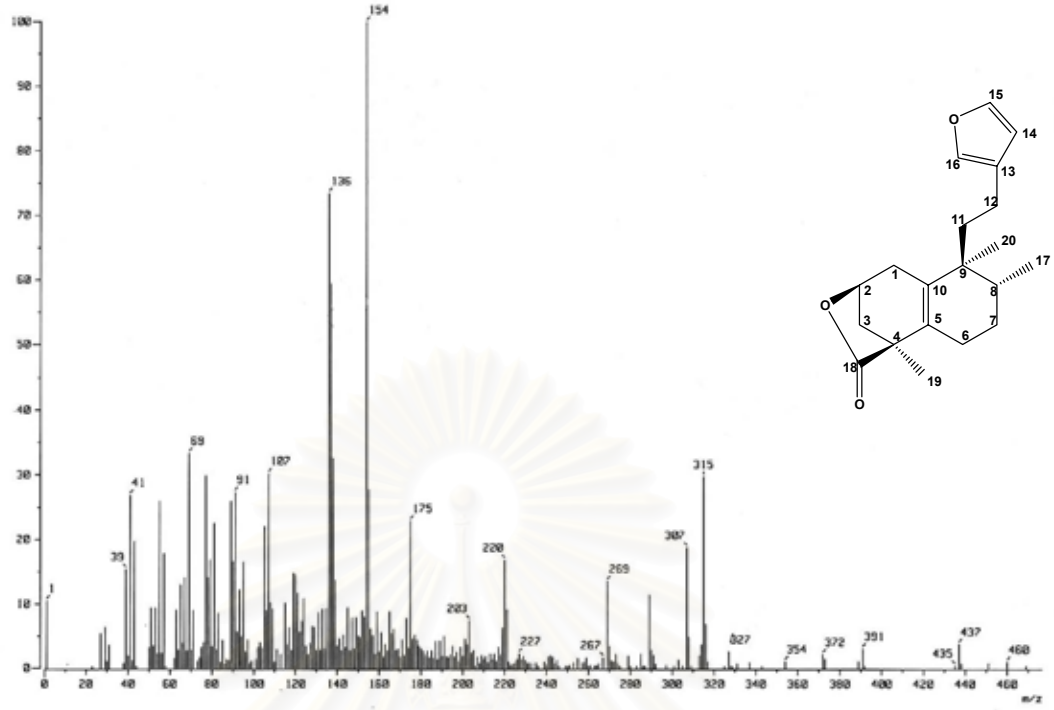

Figure 140 FAB Mass spectrum of compound **COC8**.

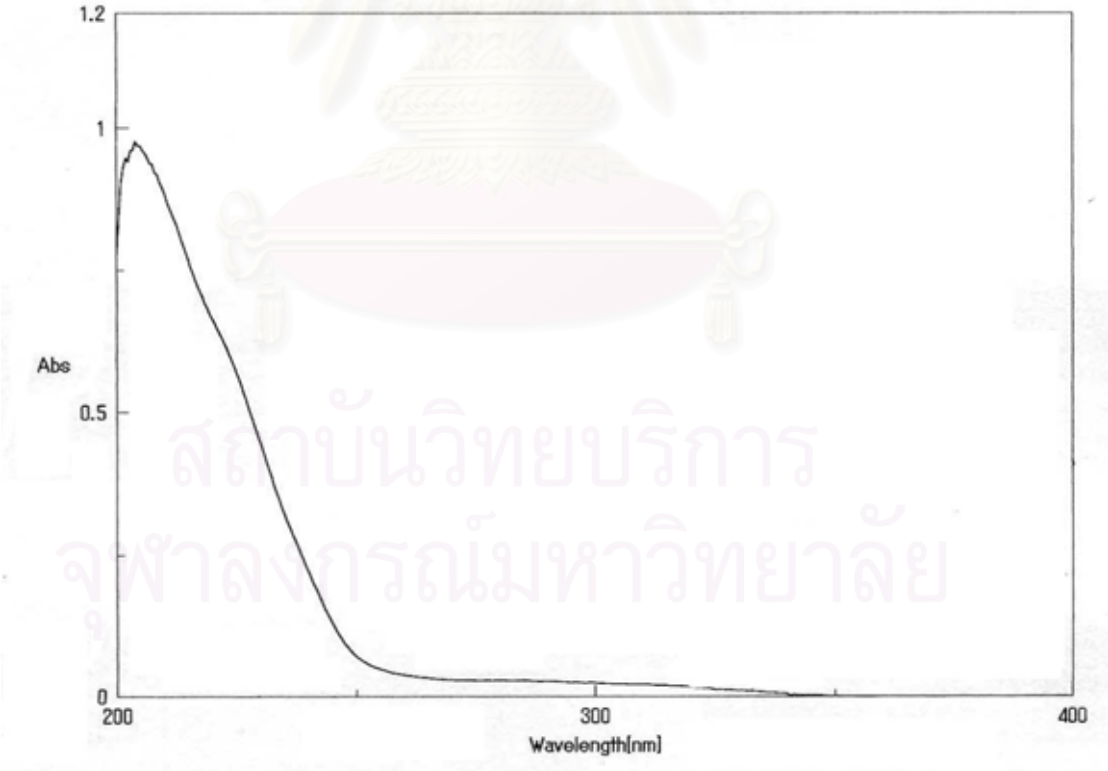

Figure 141 UV spectrum of compound **COC8** (MeOH).

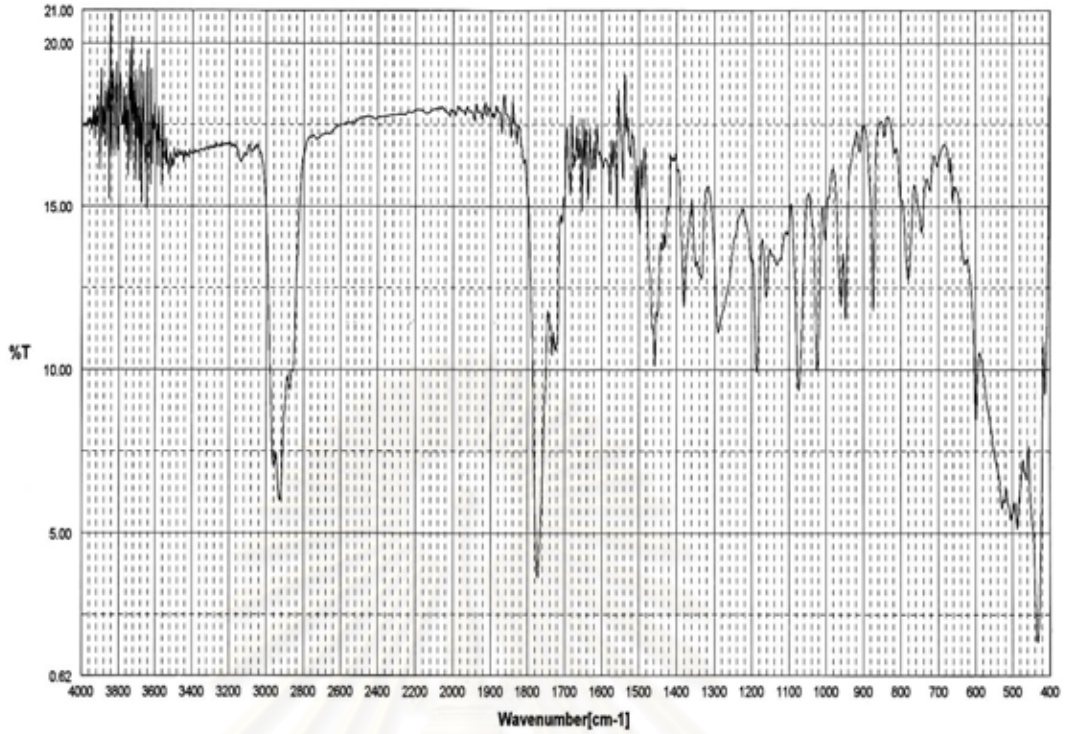

Figure 142 IR spectrum of compound **COC8** (Neat).

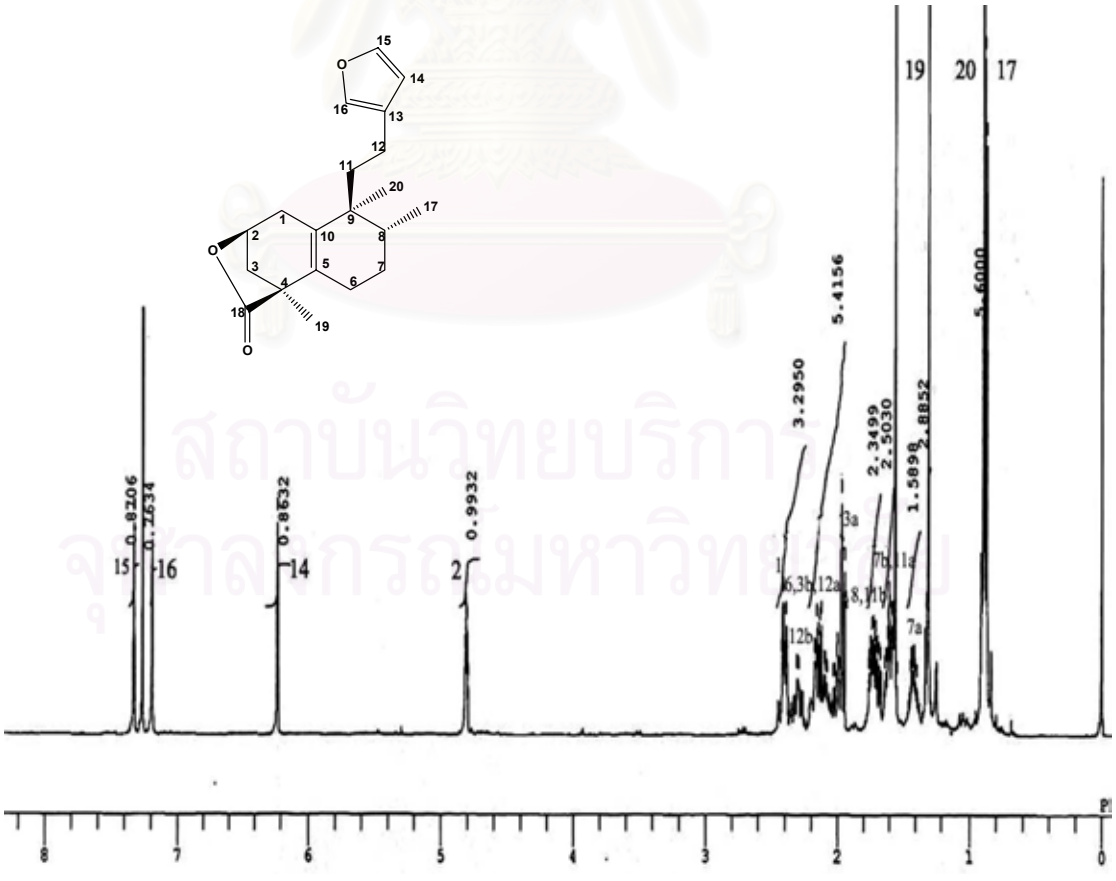

Figure 143 ^{1}H-NMR (500 MHz) spectrum of compound **COC8** ($CDCl_3$).

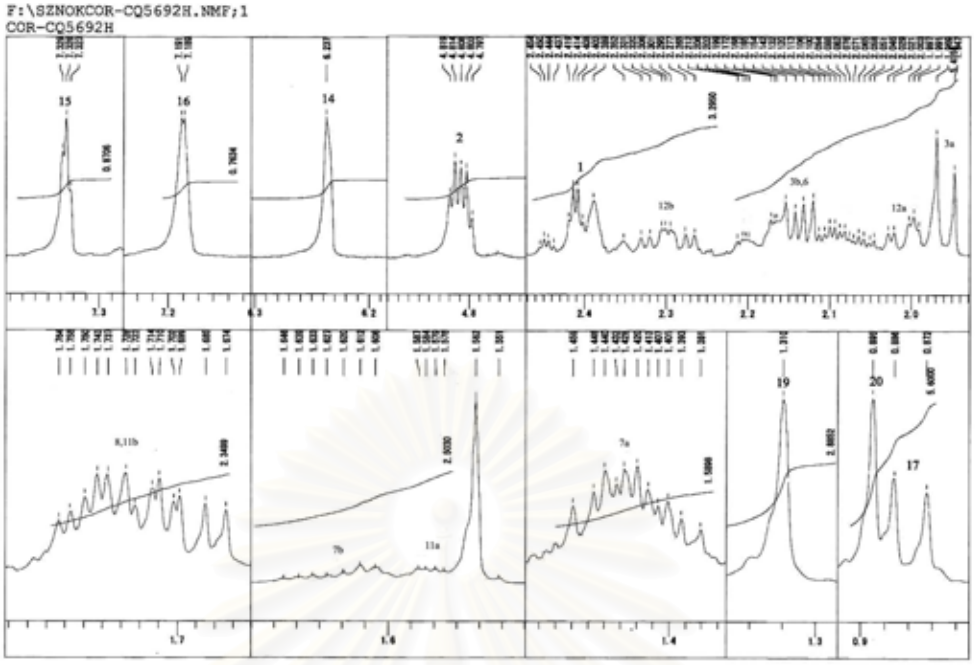

Figure 144 Expanded ^{1}H-NMR (500 MHz) spectrum of compound **COC8** ($CDCl_3$).

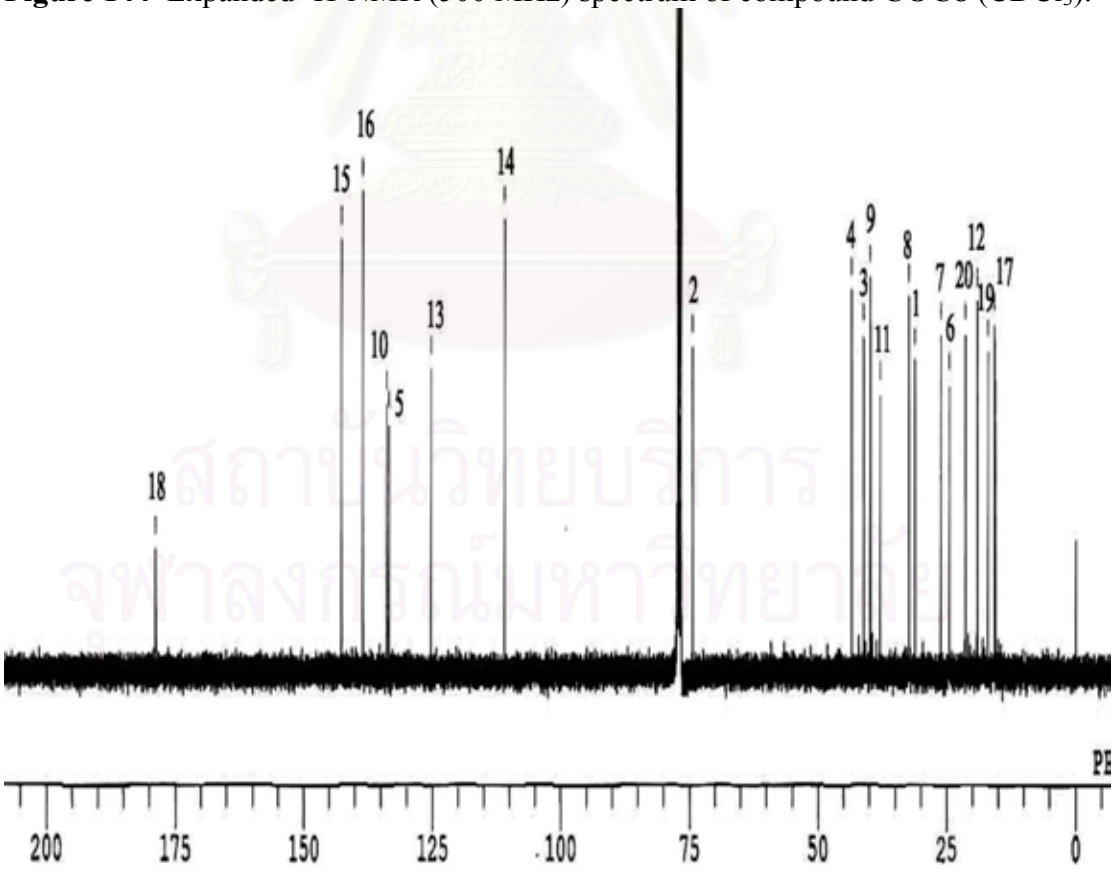

Figure 145 ^{13}C-NMR (125 MHz) spectrum of compound **COC8** ($CDCl_3$).

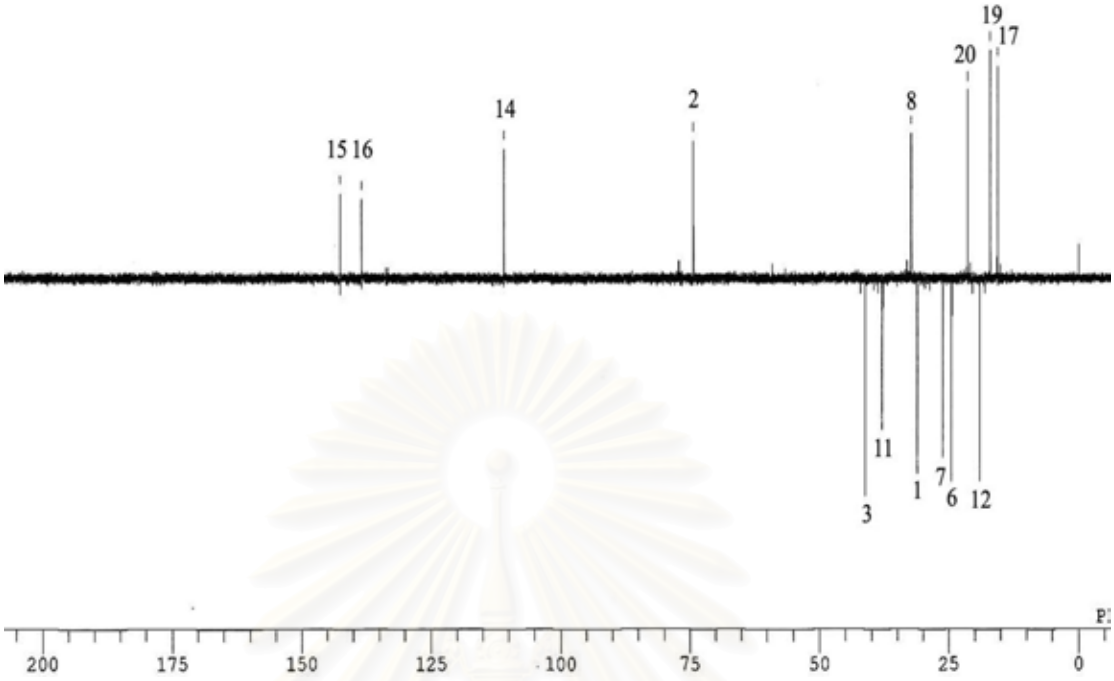

Figure 146 DEPT135 spectrum of compound **COC8** ($CDCl_3$).

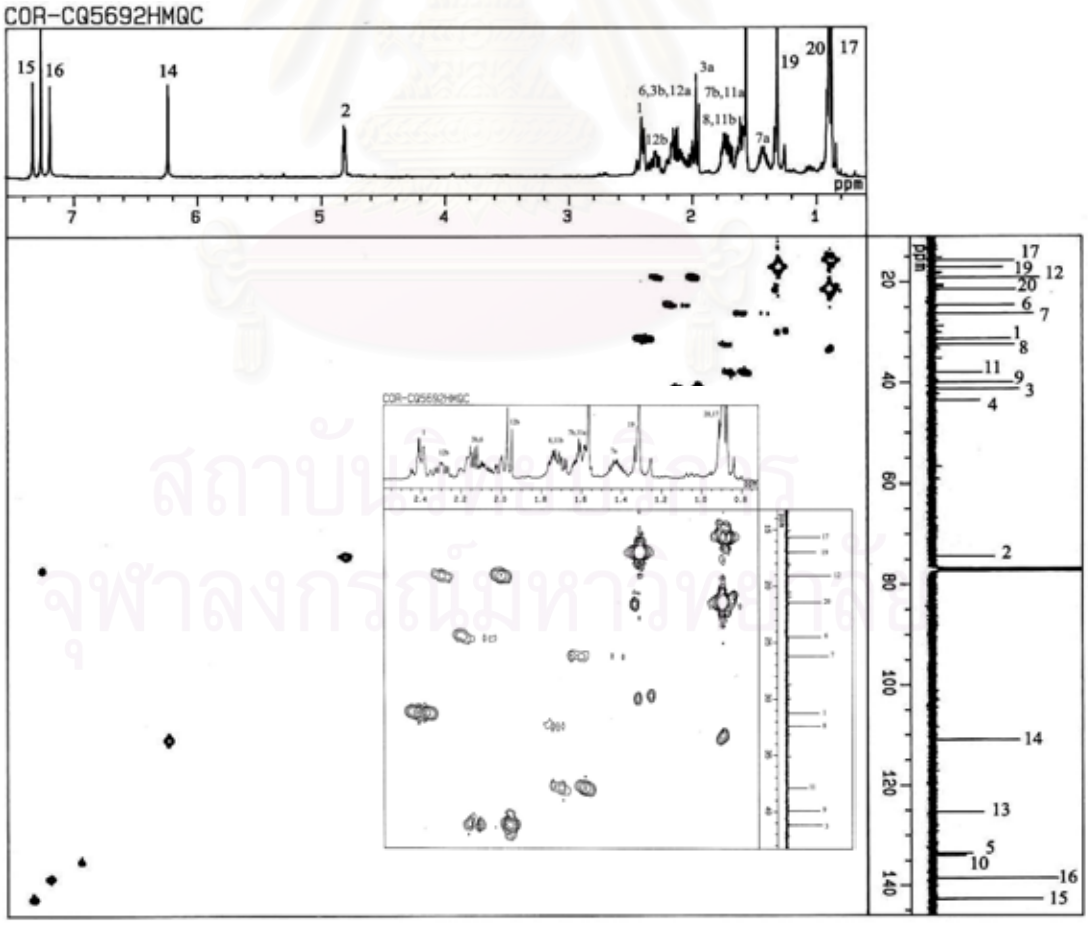

Figure 147 HMQC spectrum of compound **COC8** ($CDCl_3$).

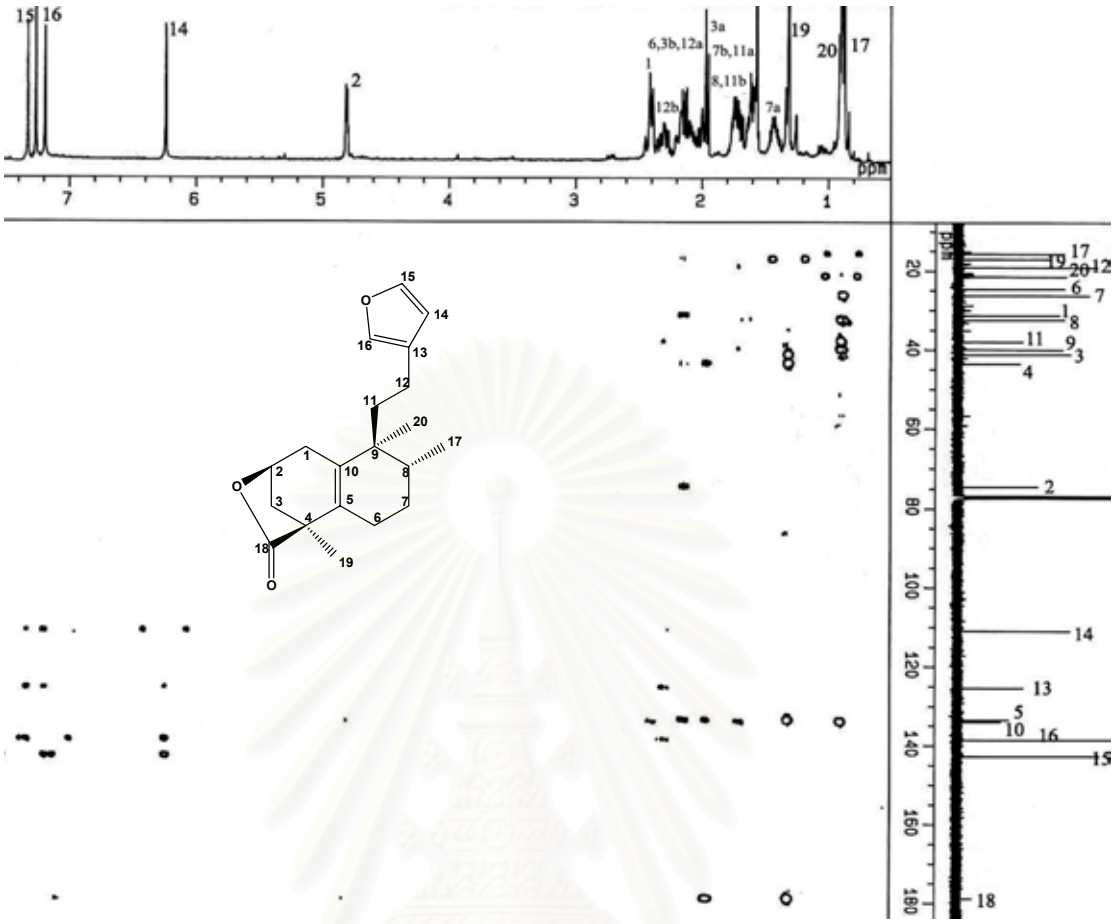

Figure 148 HMBC spectrum of compound **COC8** ($CDCl_3$).

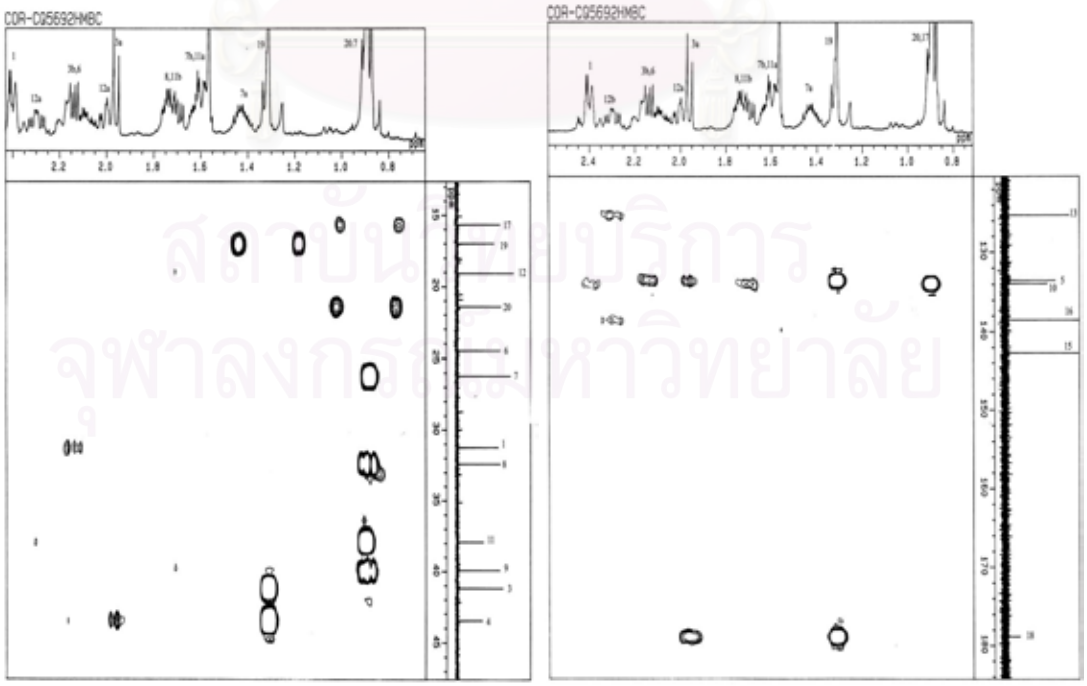

Figure 149 Expanded HMBC spectra of compound **COC8** ($CDCl_3$).

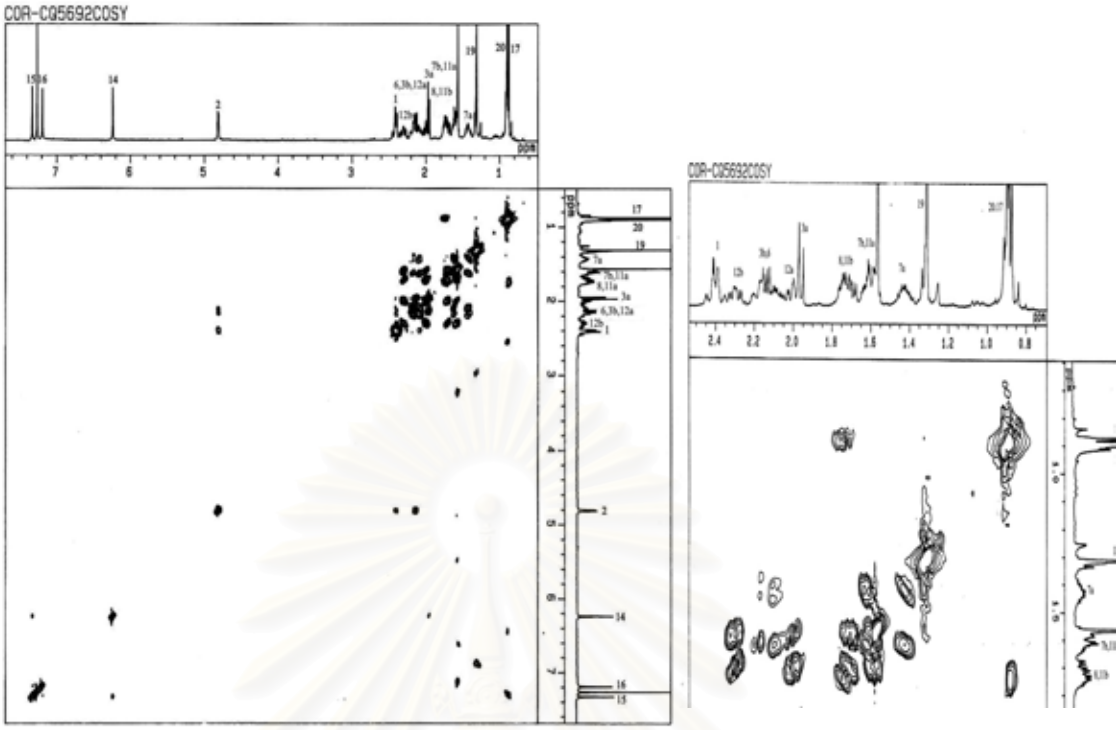

Figure 150 ^{1}H-^{1}H COSY spectra of compound **COC8** ($CDCl_3$).

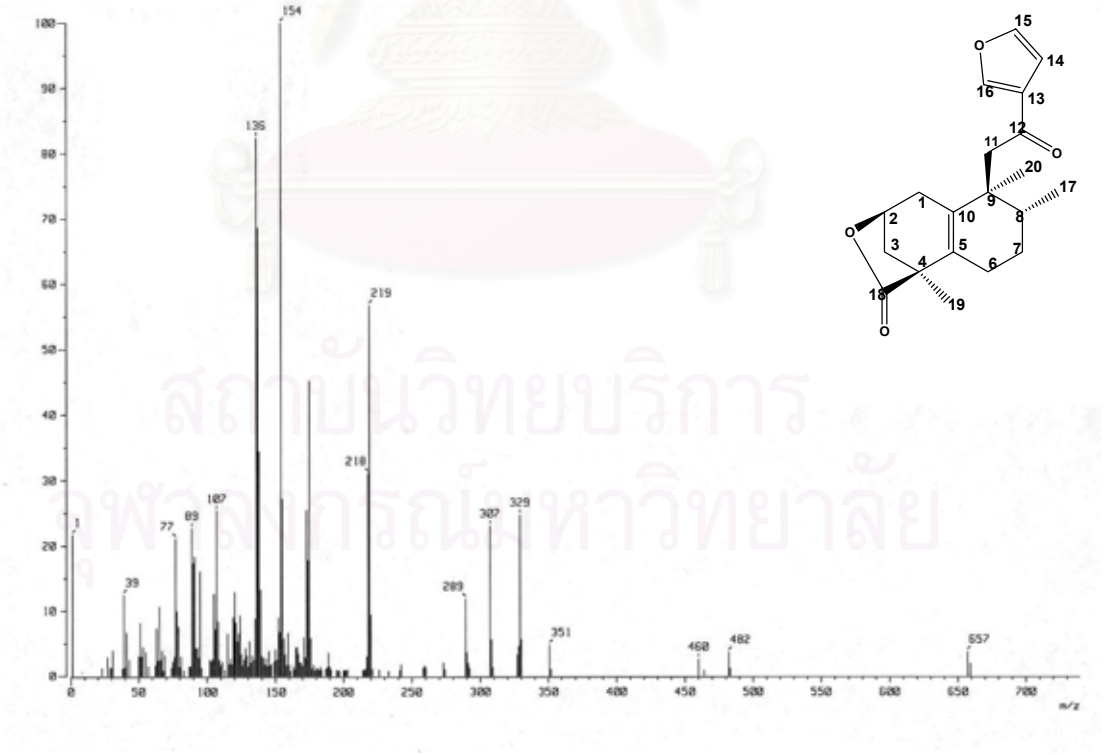

Figure 151 FAB Mass spectrum of compound **COC9**.

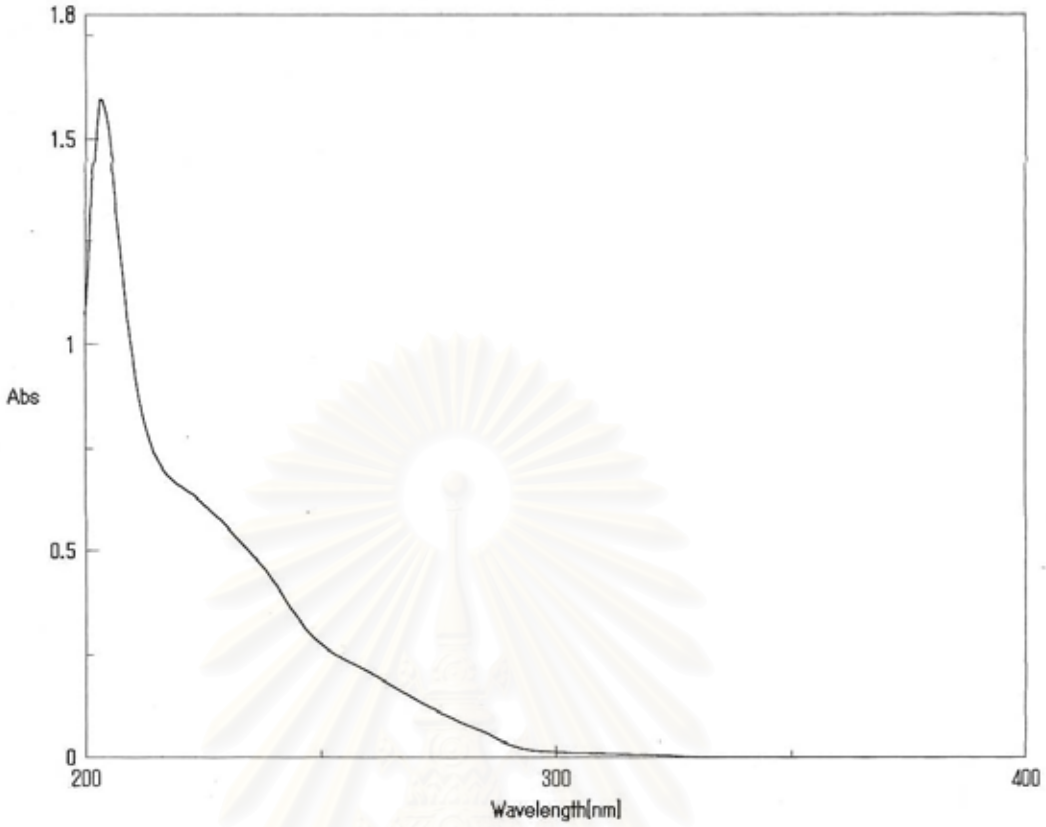

Figure 152 UV spectrum of compound **COC9** (MeOH).

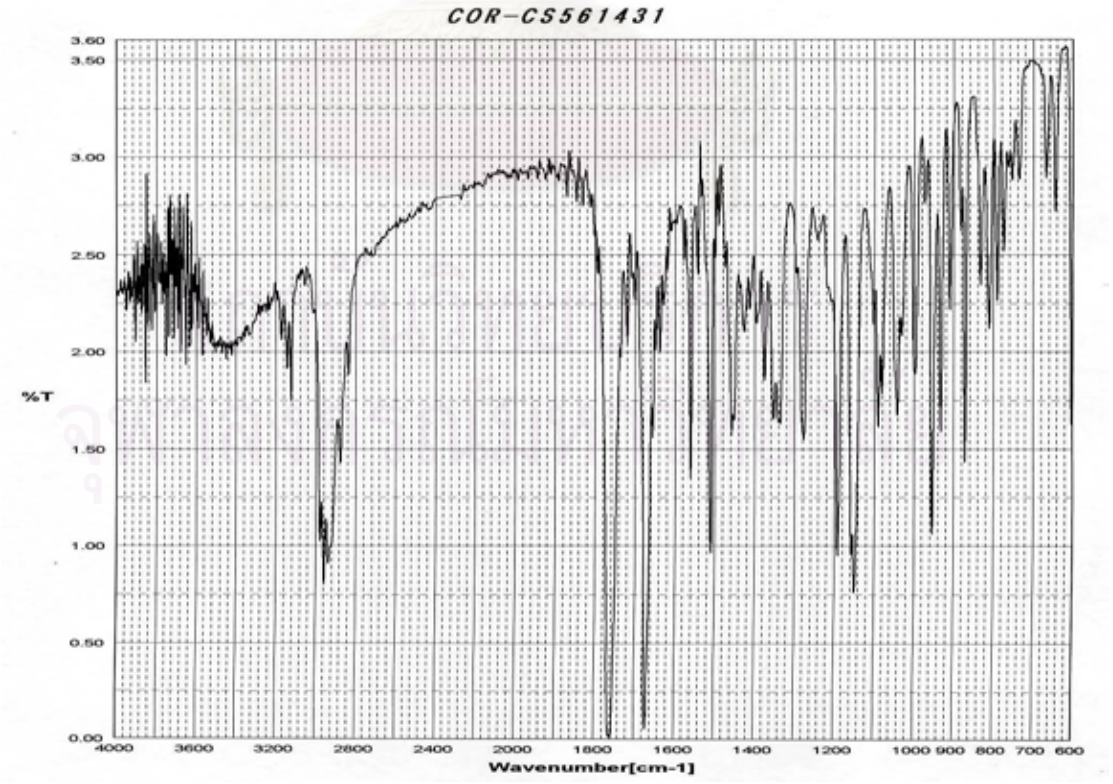

Figure 153 IR spectrum of compound **COC9** (KBr disc).

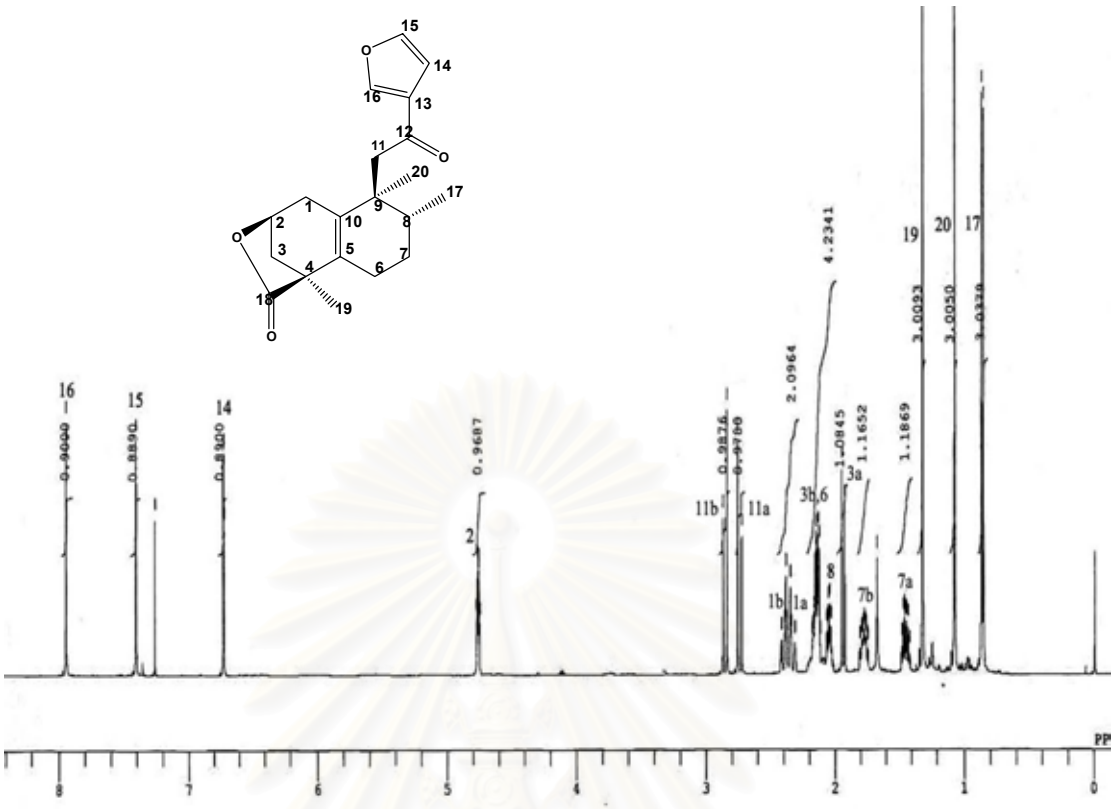

Figure 154 ^{1}H-NMR (500 MHz) spectrum of compound **COC9** ($CDCl_3$).

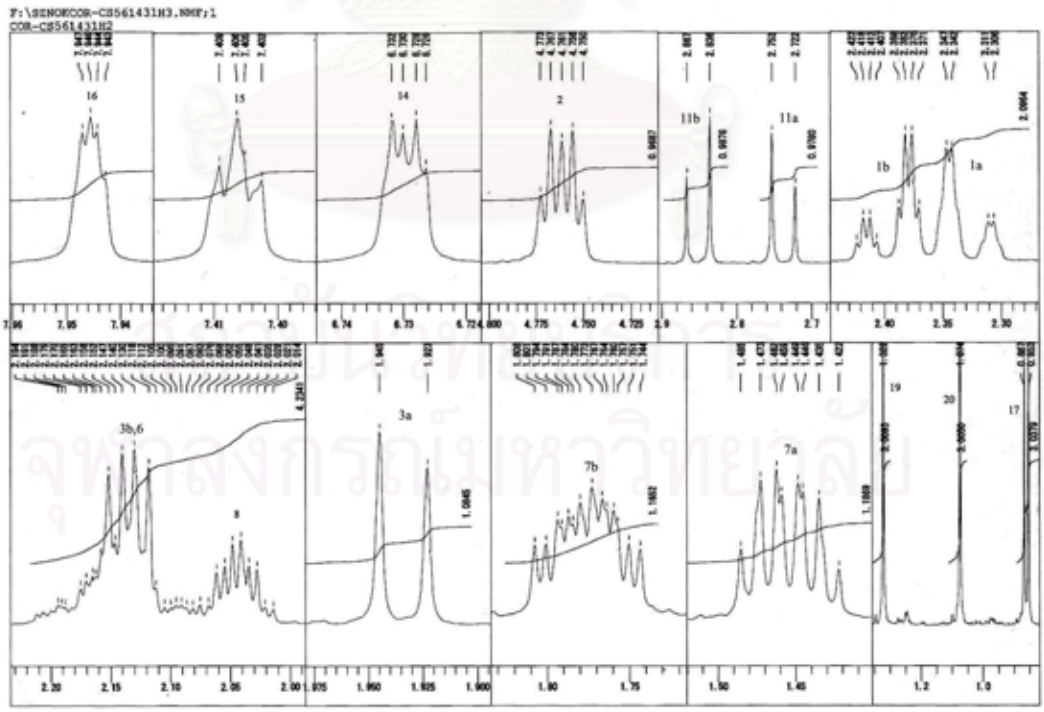

Figure 155 Expanded ^{1}H-NMR (500 MHz) spectrum of compound **COC9** ($CDCl_3$).

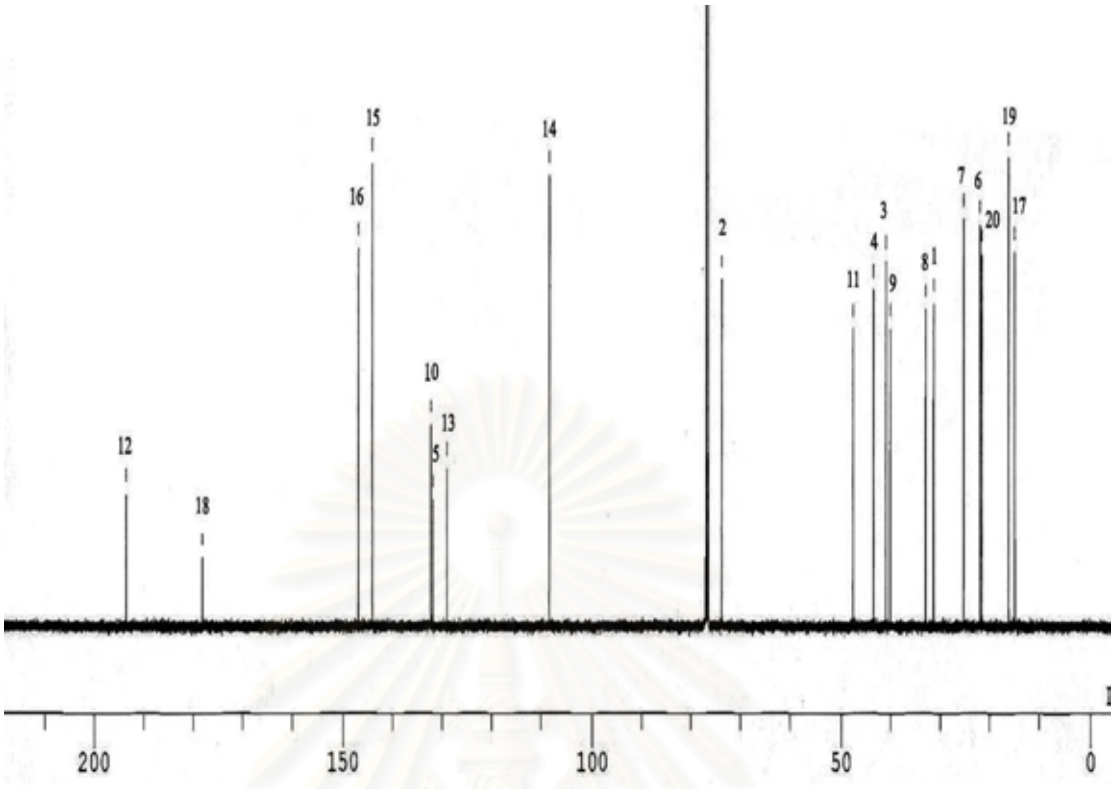

Figure 156 ^{13}C-NMR (125 MHz) spectrum of compound **COC9** ($CDCl_3$).

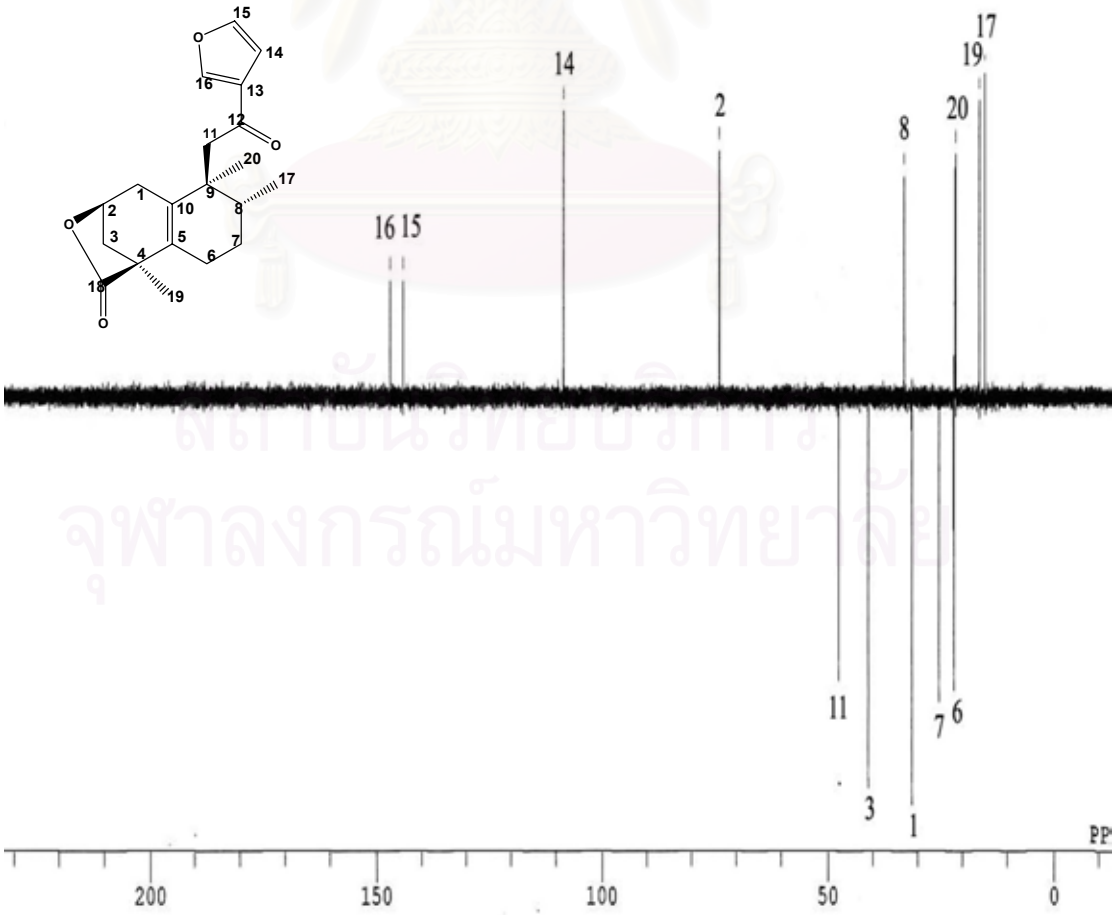

Figure 157 DEPT135 spectrum of compound **COC9** ($CDCl_3$).

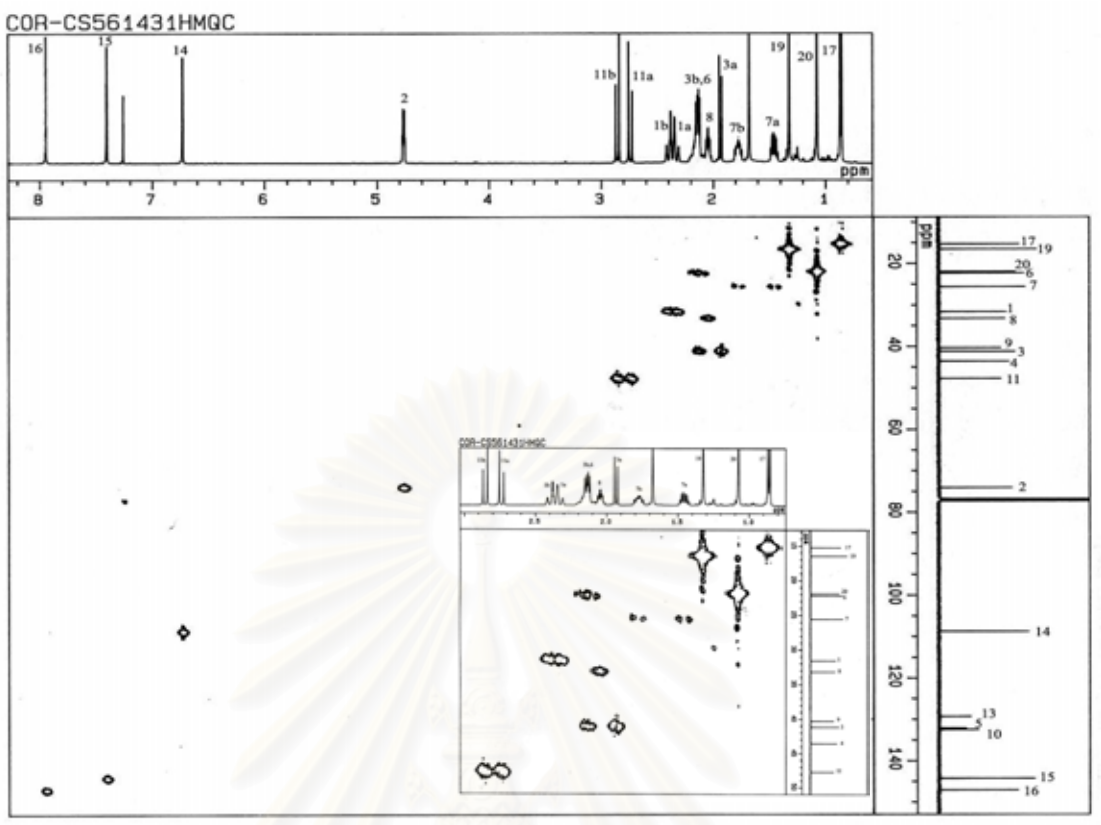

Figure 158 HMQC spectrum of compound **COC9** ($CDCl_3$).

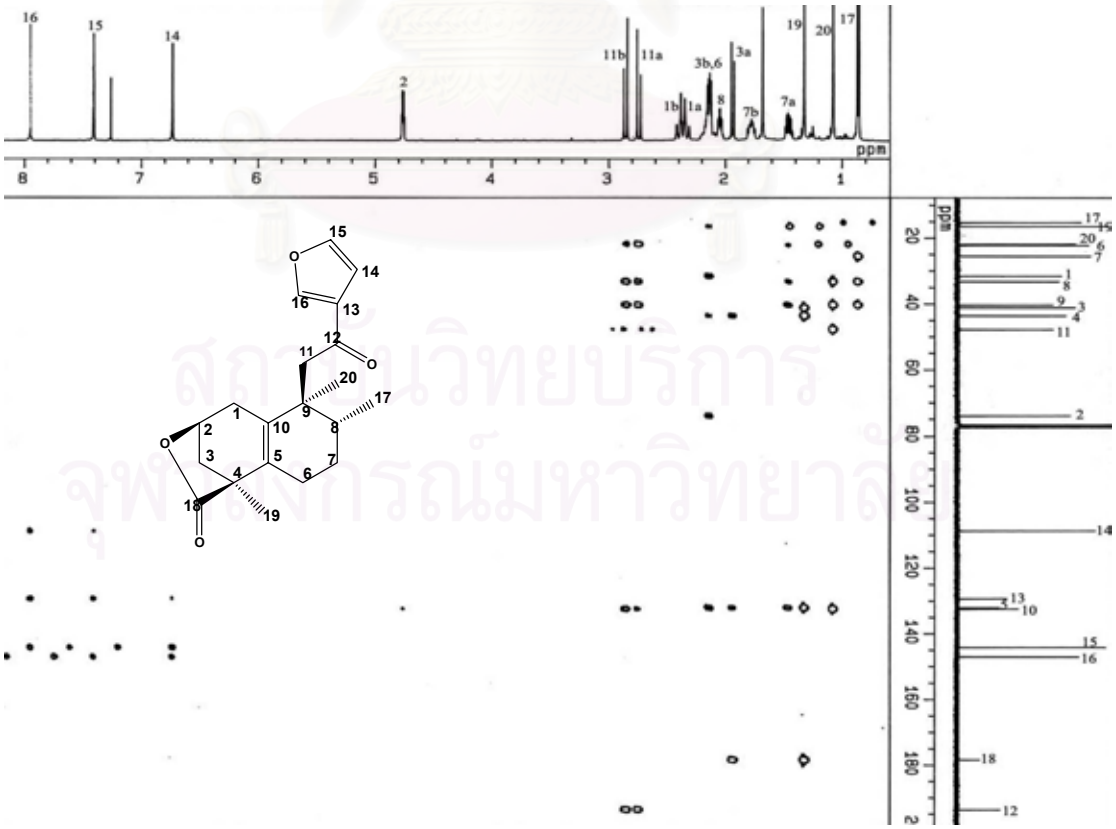

Figure 159 HMBC spectrum of compound **COC9** ($CDCl_3$).

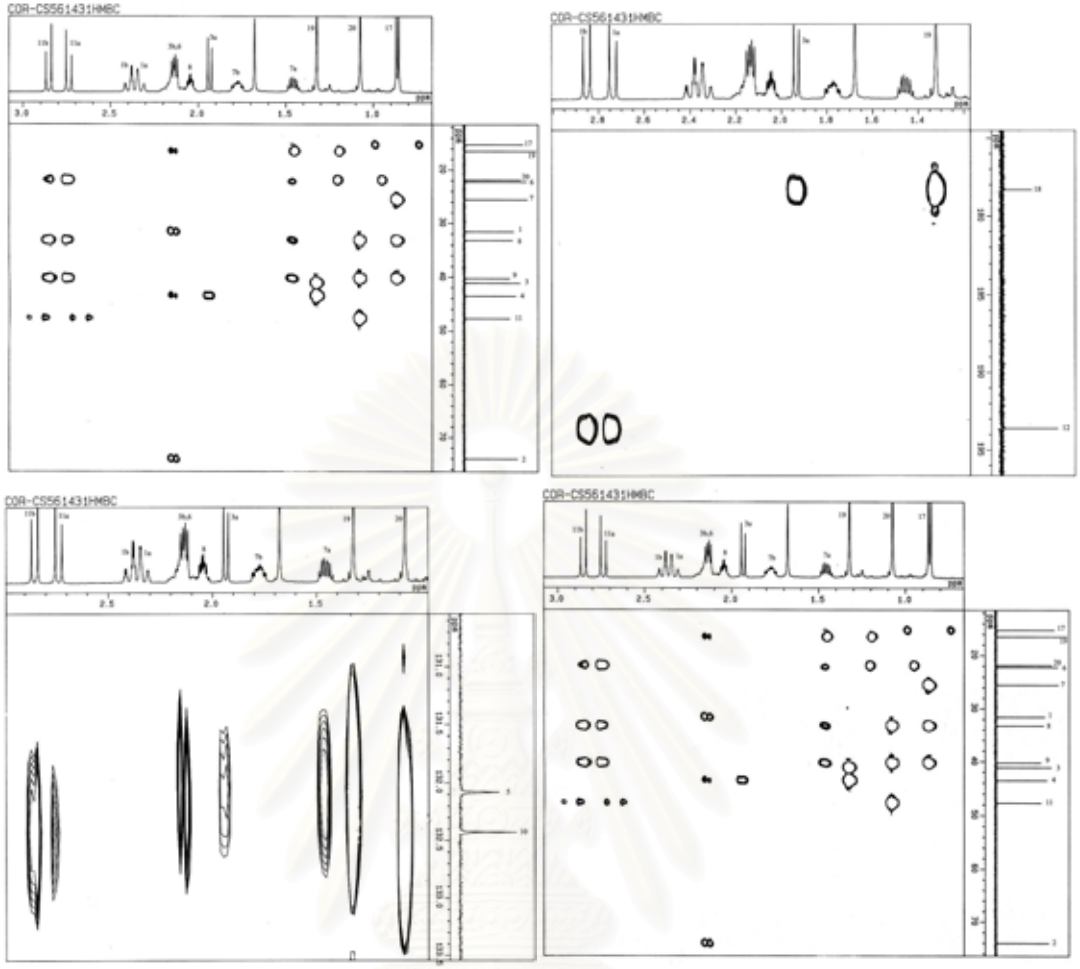

Figure 160 Expanded HMBC spectra of compound **COC9** ($CDCl_3$).

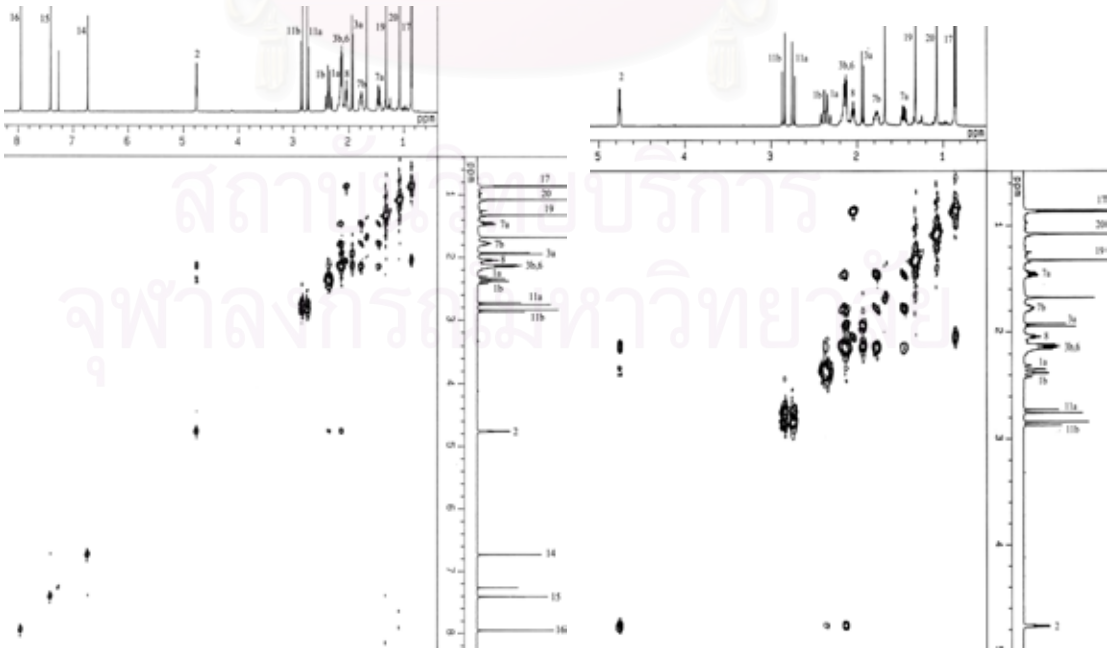

Figure 161 ^{1}H-^{1}H COSY spectra of compound **COC9** ($CDCl_3$).

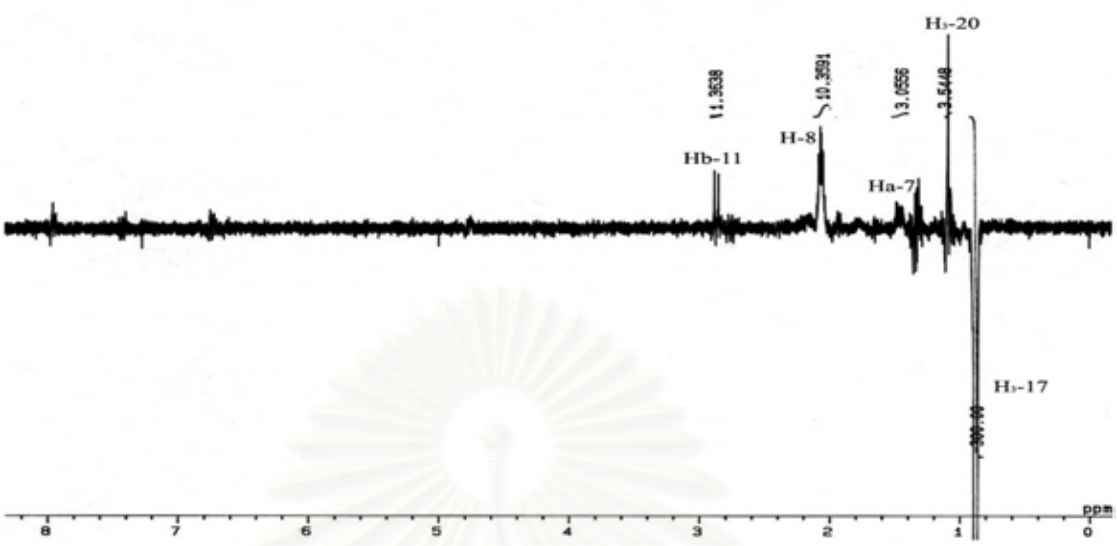

Figure 162 NOE spectrum of compound **COC9** ($CDCl_3$).

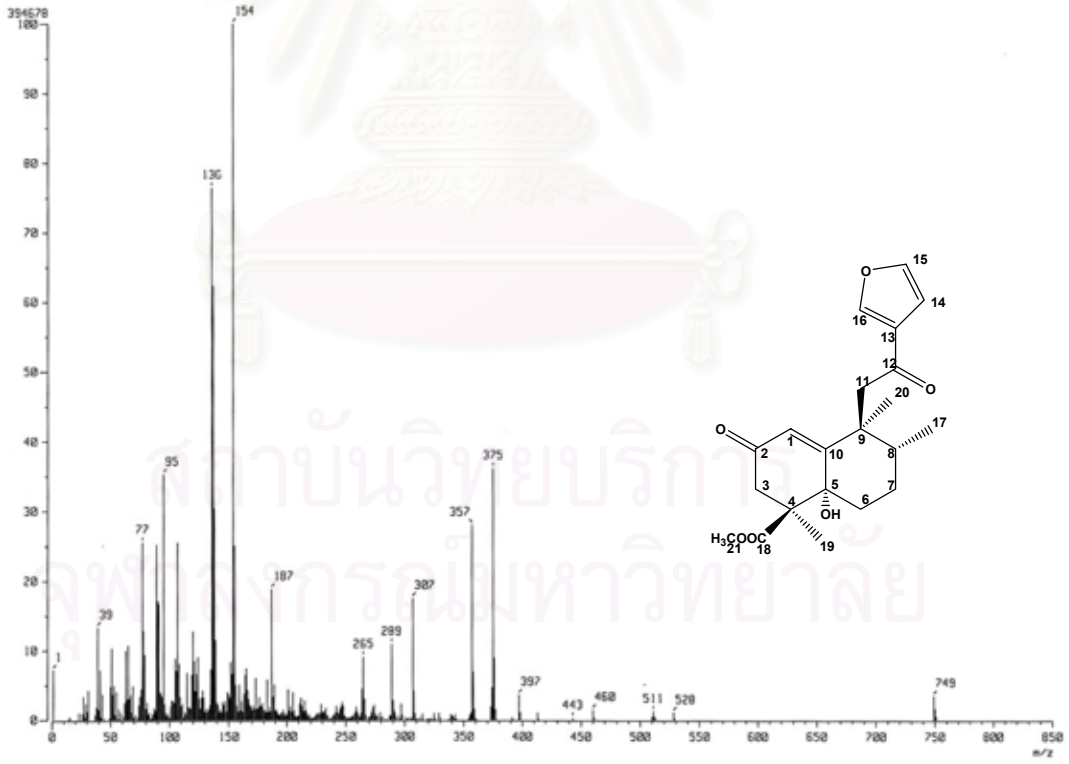

Figure 163 FAB Mass spectrum of compound **COC10**.

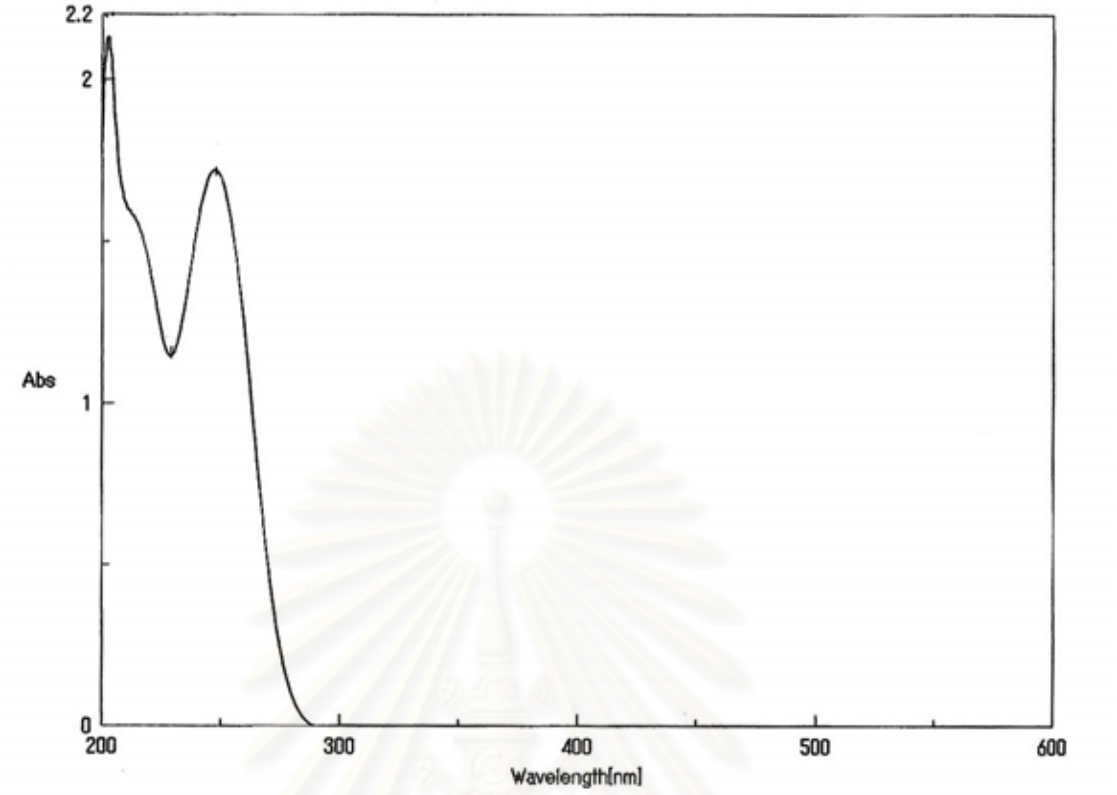

Figure 164 UV spectrum of compound **COC10** (EtOH).

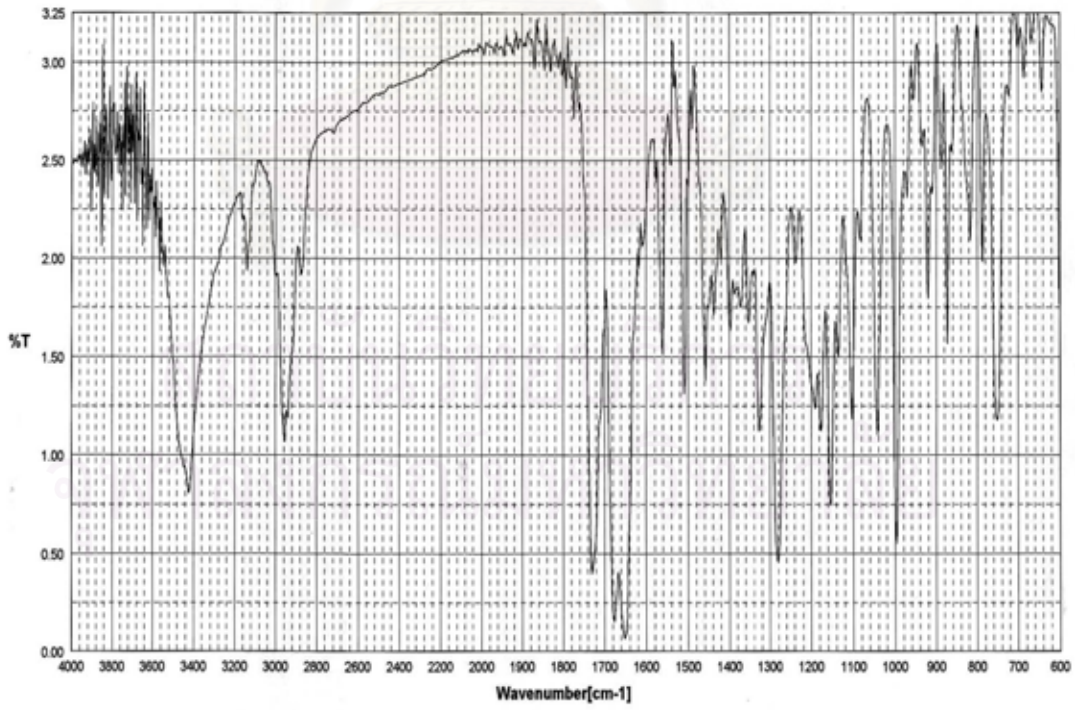

Figure 165 IR spectrum of compound **COC10** (KBr disc).

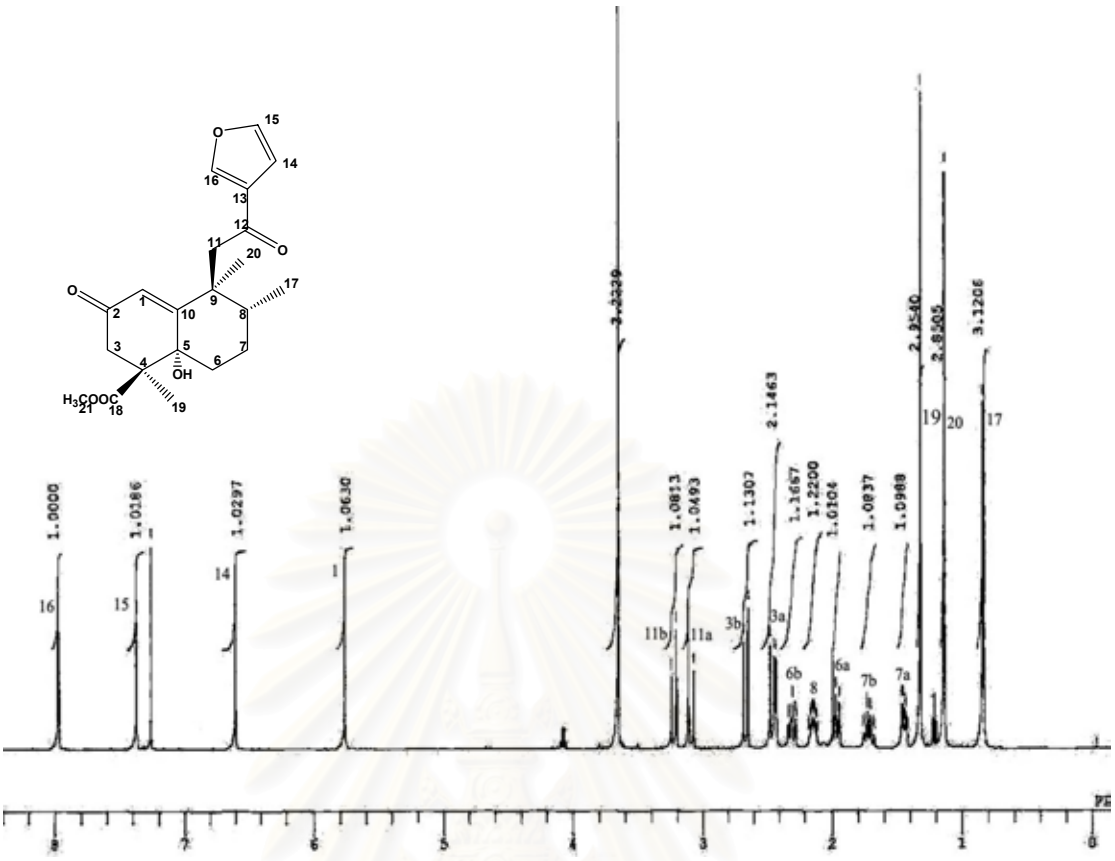

Figure 166 ^{1}H-NMR (500 MHz) spectrum of compound **COC10** ($CDCl_3$).

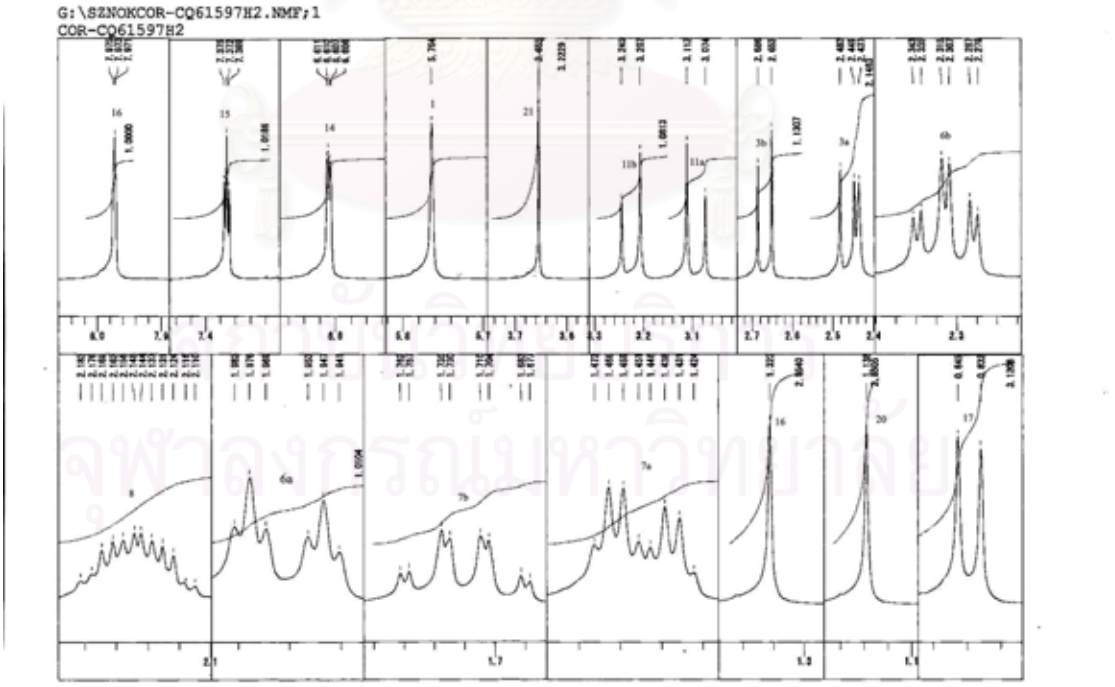

Figure 167 Expanded ^{1}H-NMR (500 MHz) spectrum of compound **COC10** ($CDCl_3$).

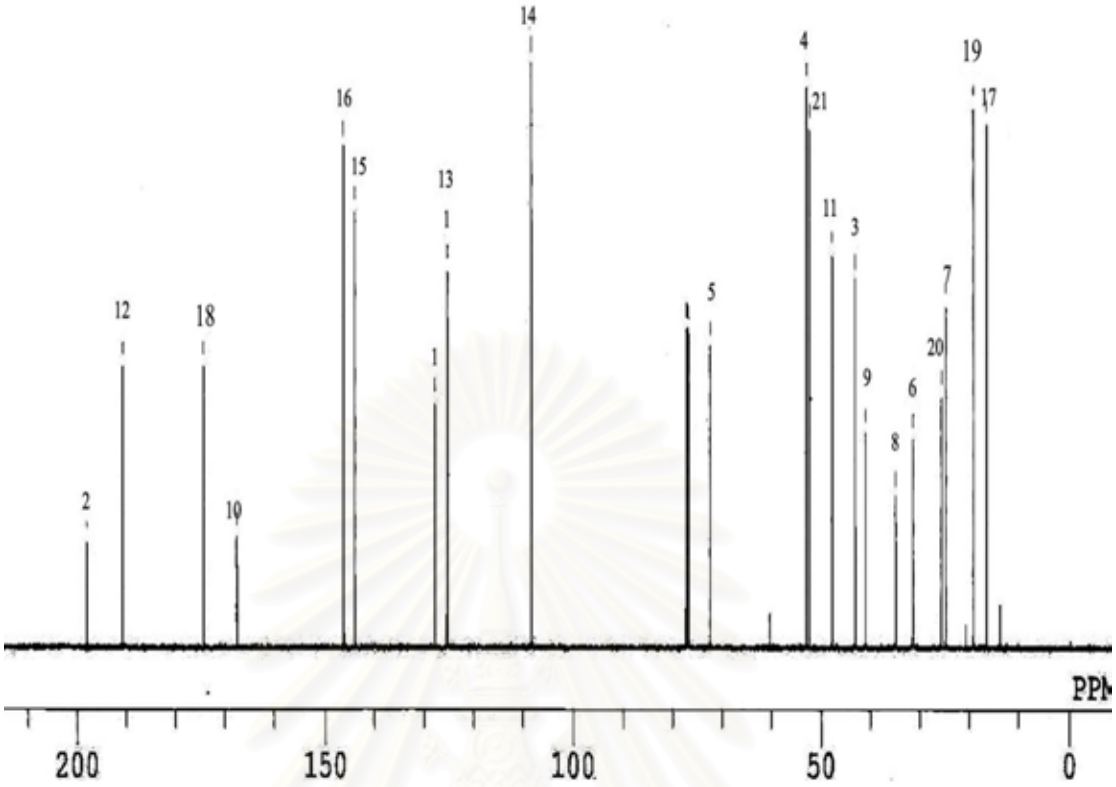

Figure 168 ^{13}C-NMR (125 MHz) spectrum of compound **COC10** ($CDCl_3$).

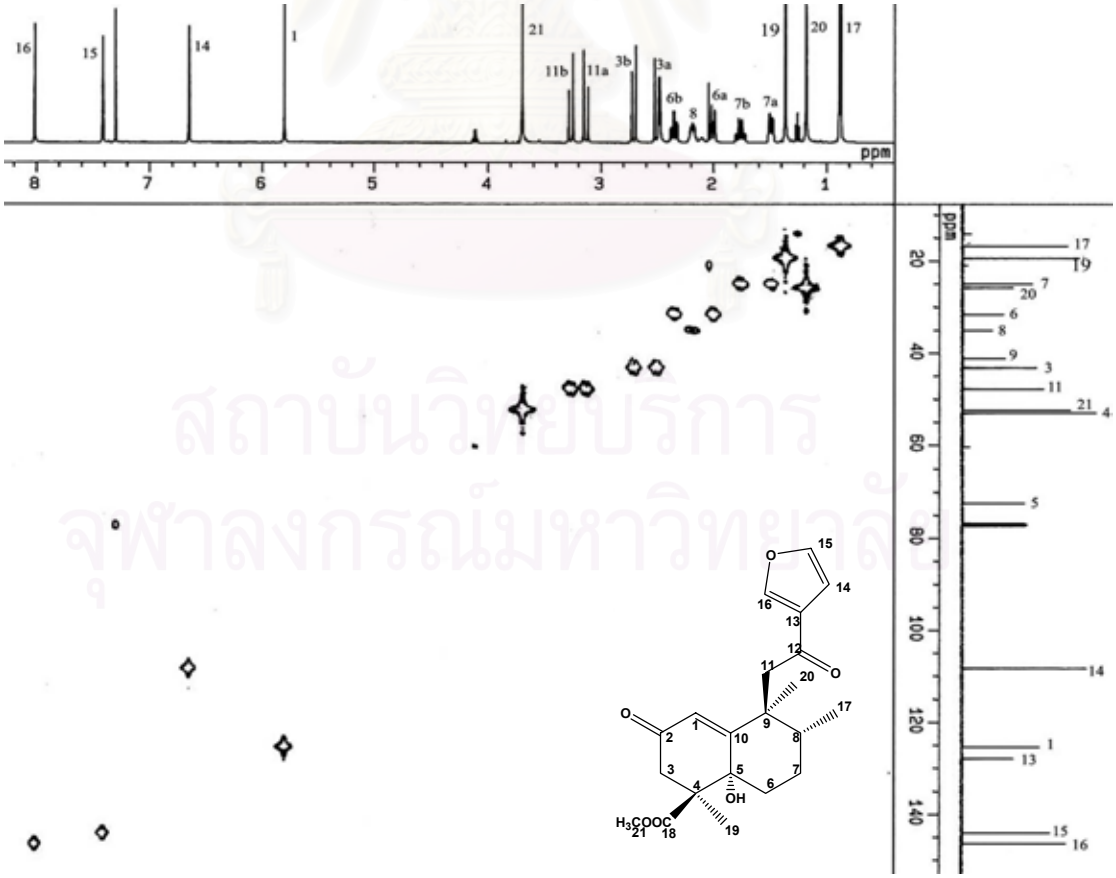

Figure 169 HMQC spectrum of compound **COC10** ($CDCl_3$).

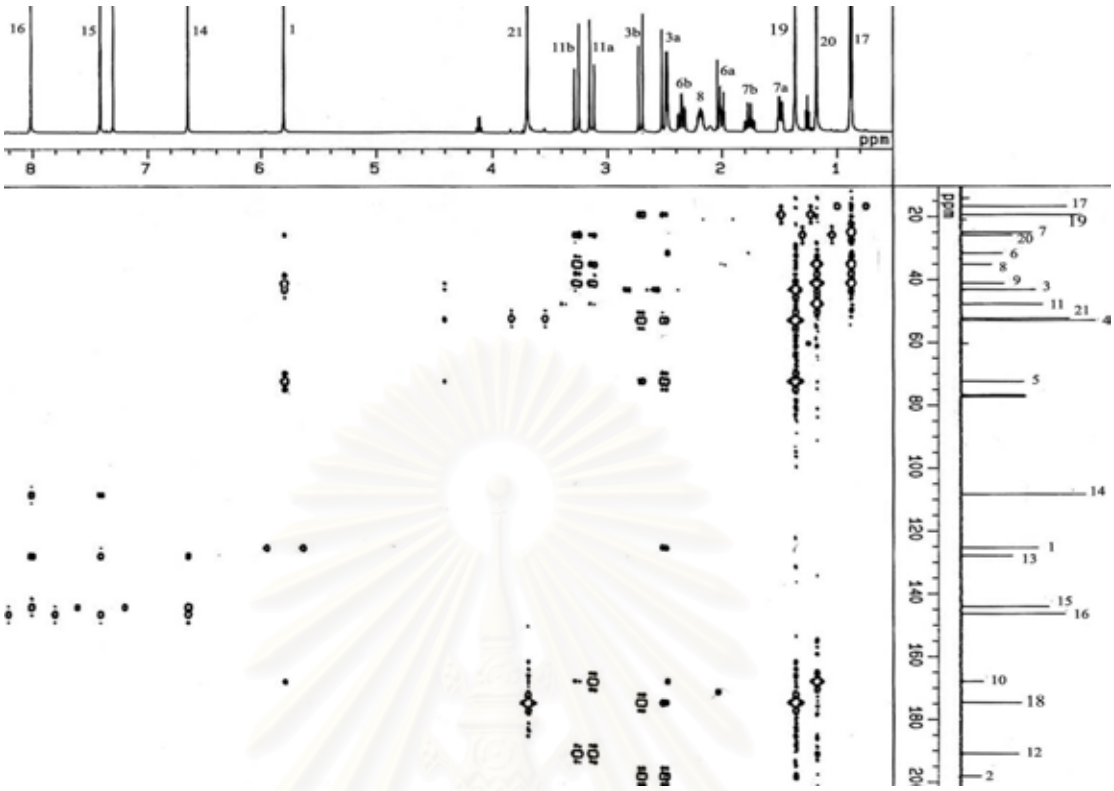

Figure 170 HMBC spectrum of compound **COC10** ($CDCl_3$).

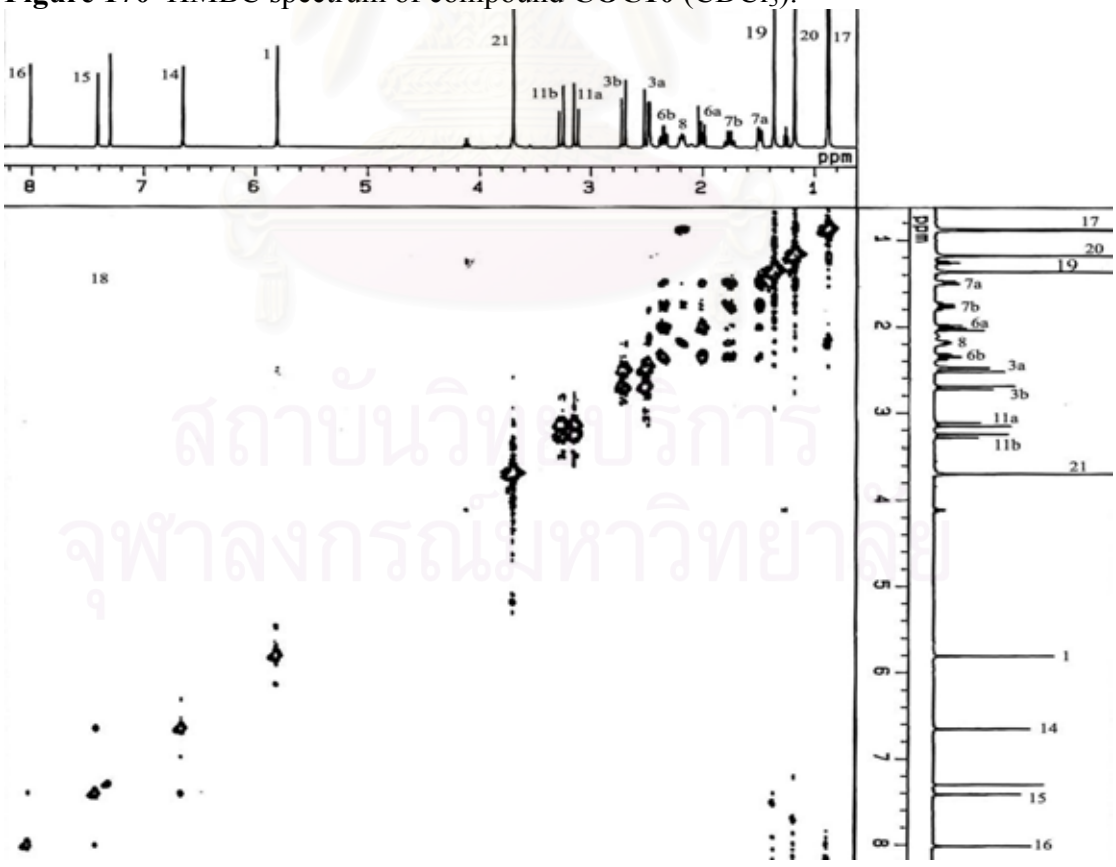

Figure 171 ^{1}H-^{1}H COSY spectrum of compound **COC10** ($CDCl_3$).

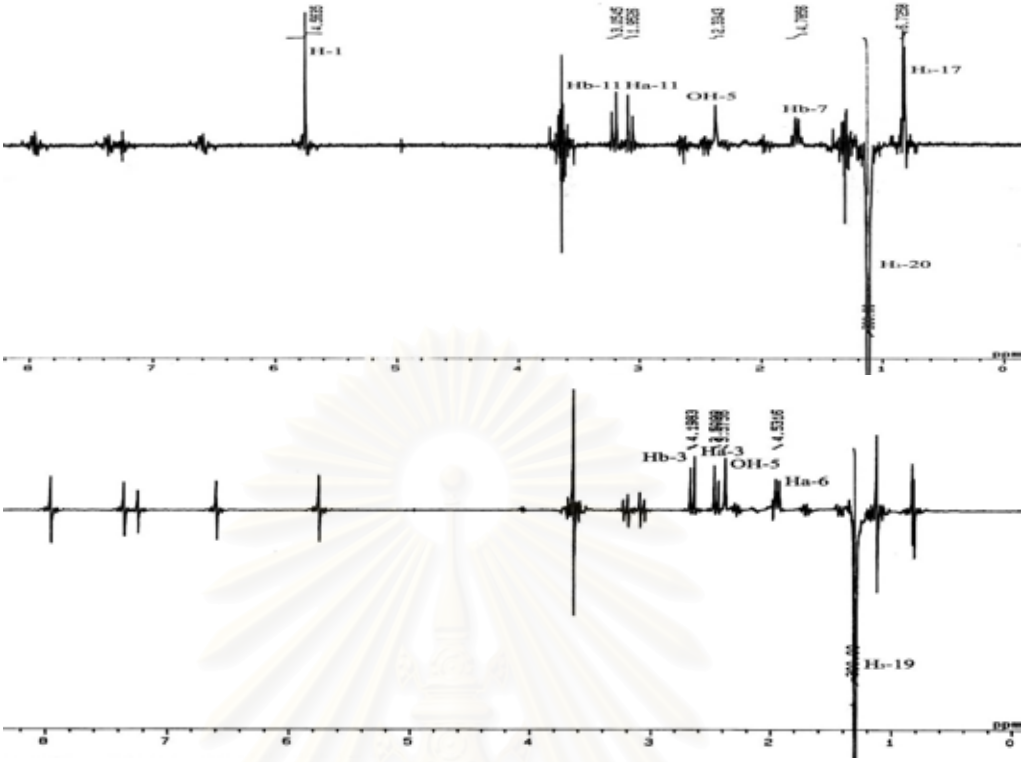

Figure 172 NOE spectra of compound **COC10** ($CDCl_3$).

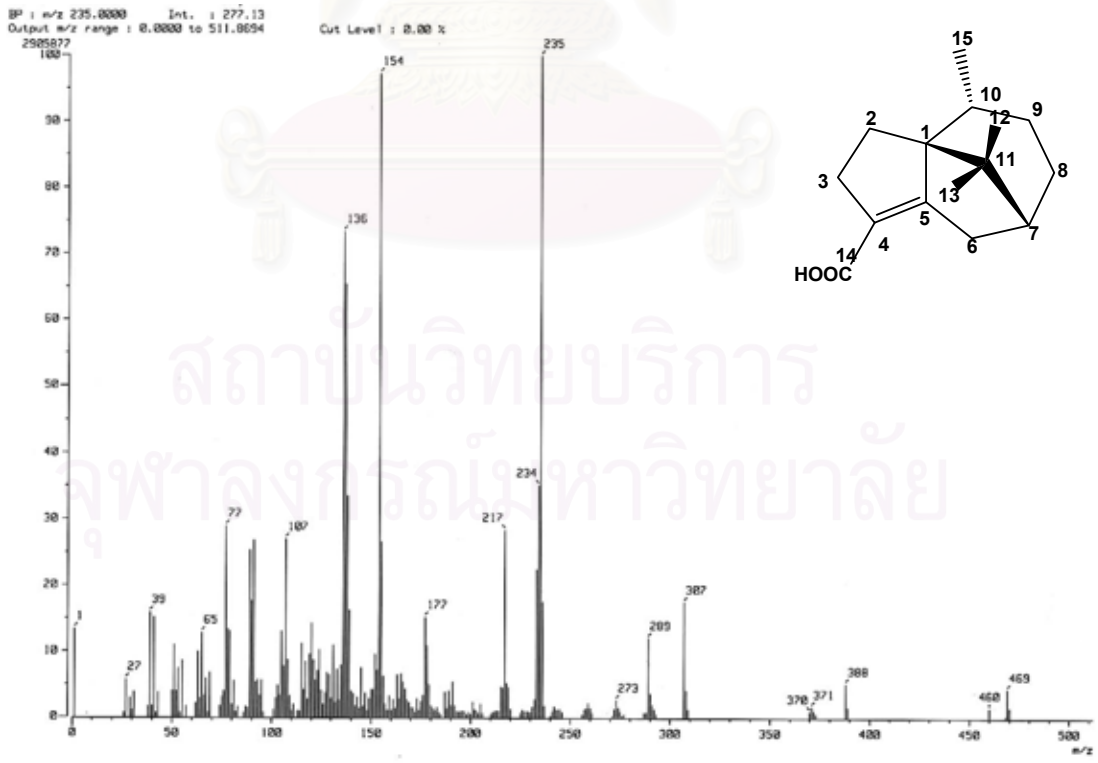

Figure 174 FAB Mass spectrum of compound **COC11**.

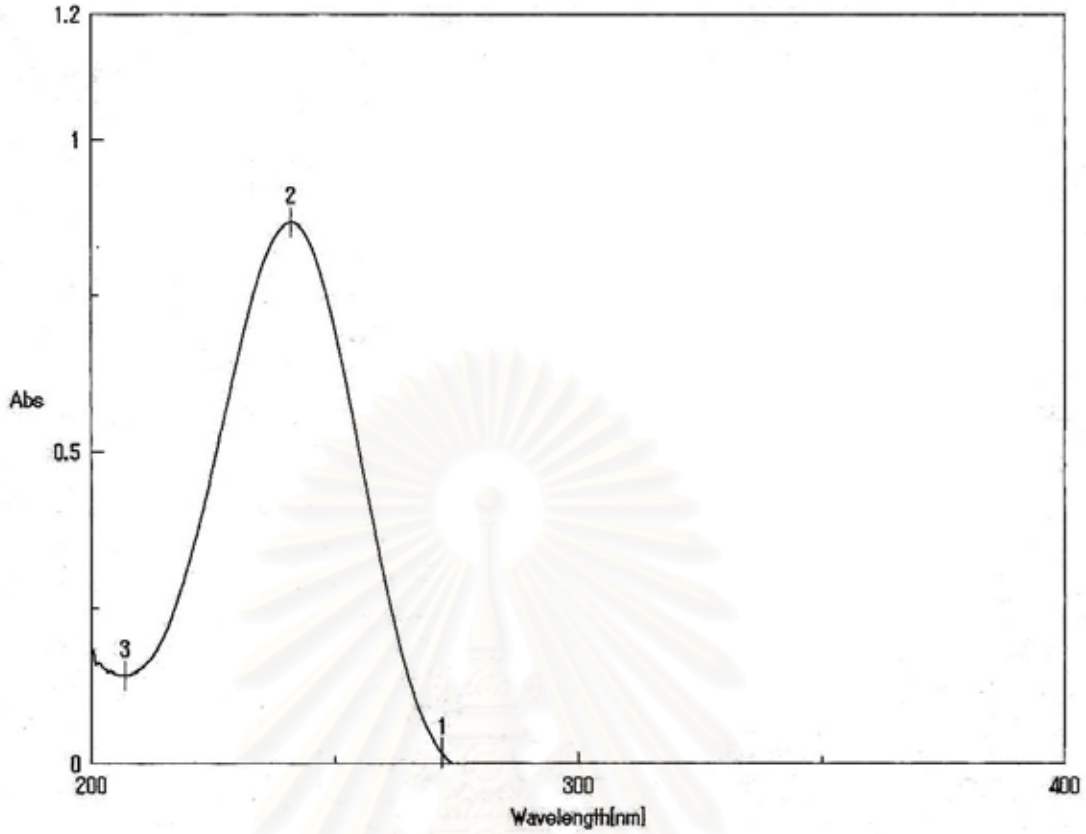

Figure 175 UV spectrum of compound **COC11**(MeOH).

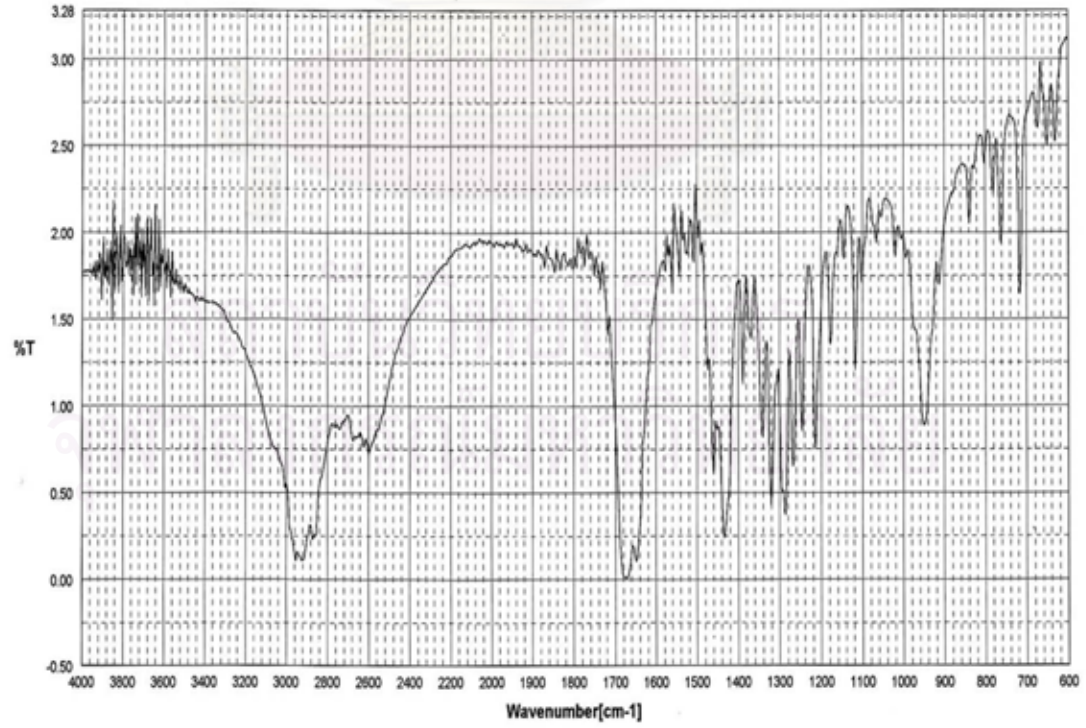

Figure 176 IR spectrum of compound **COC11** (KBr disc).

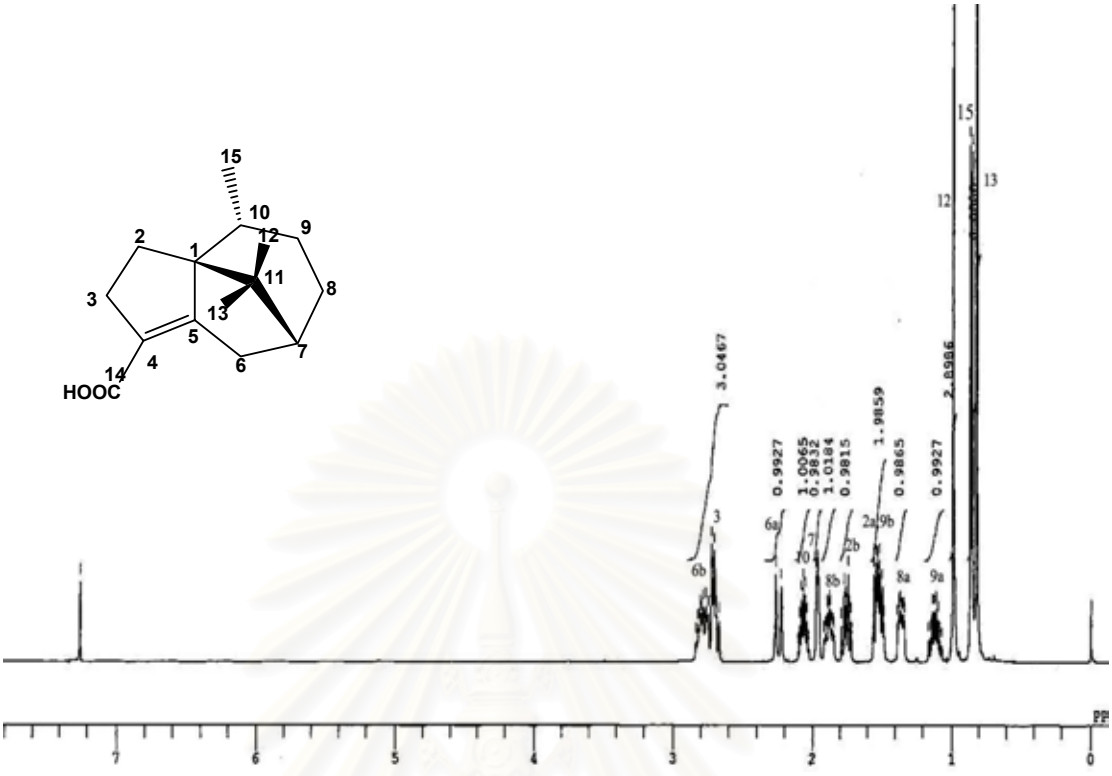

Figure 177 ^{1}H-NMR (500 MHz) spectrum of compound **COC11** ($CDCl_3$).

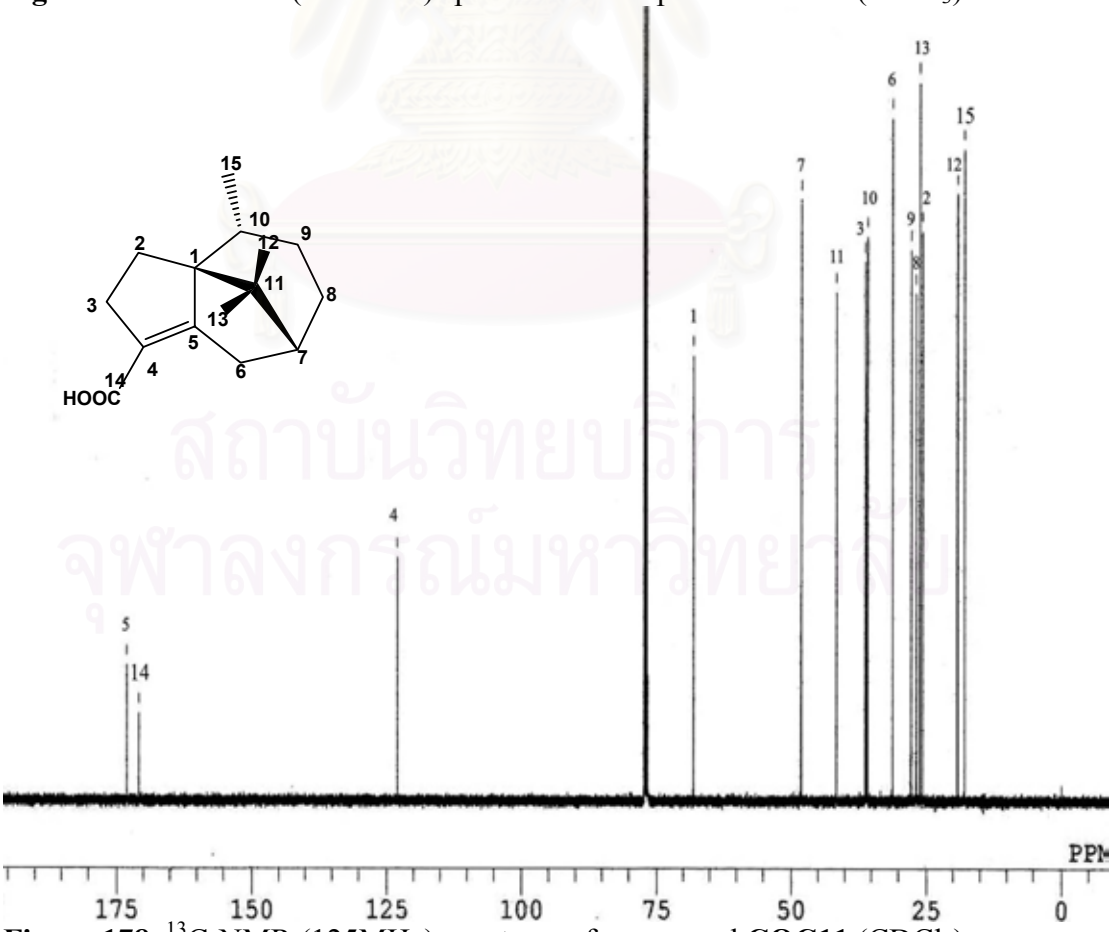

Figure 178 ^{13}C-NMR (125MHz) spectrum of compound **COC11** ($CDCl_3$).

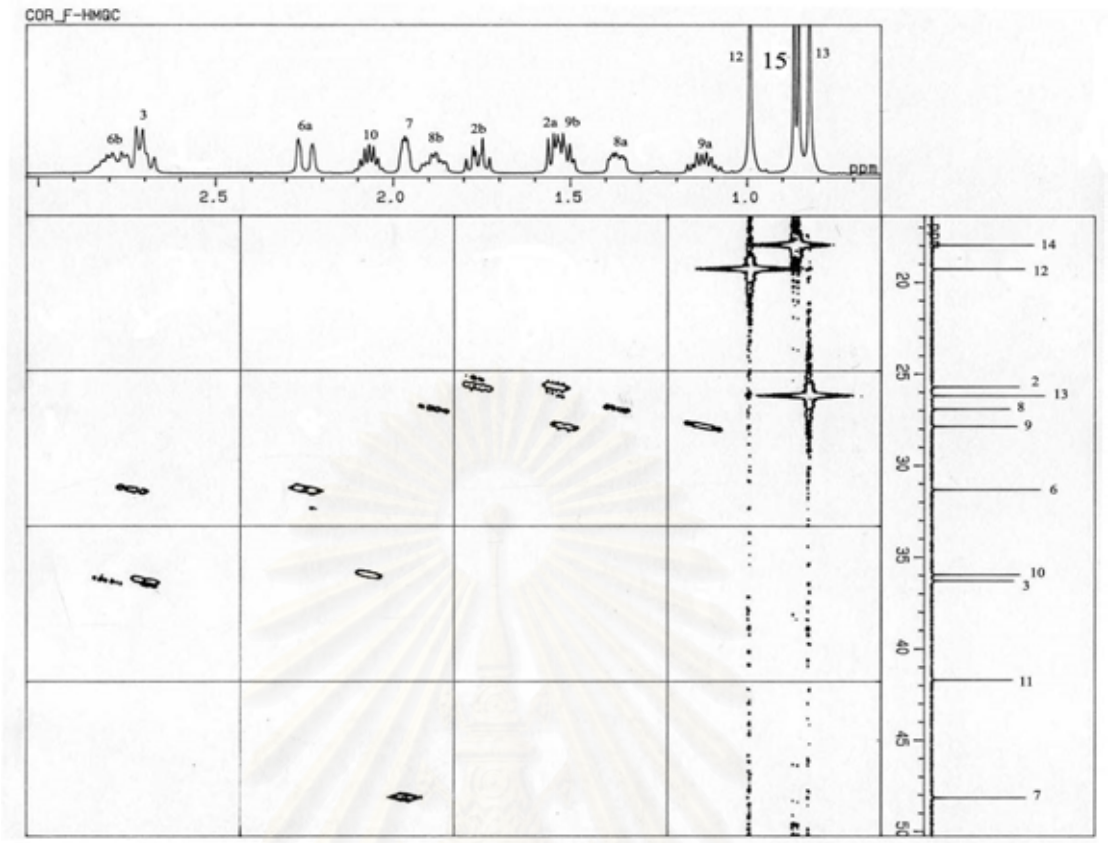

Figure 179 HMQC spectrum of compound **COC11** ($CDCl_3$).

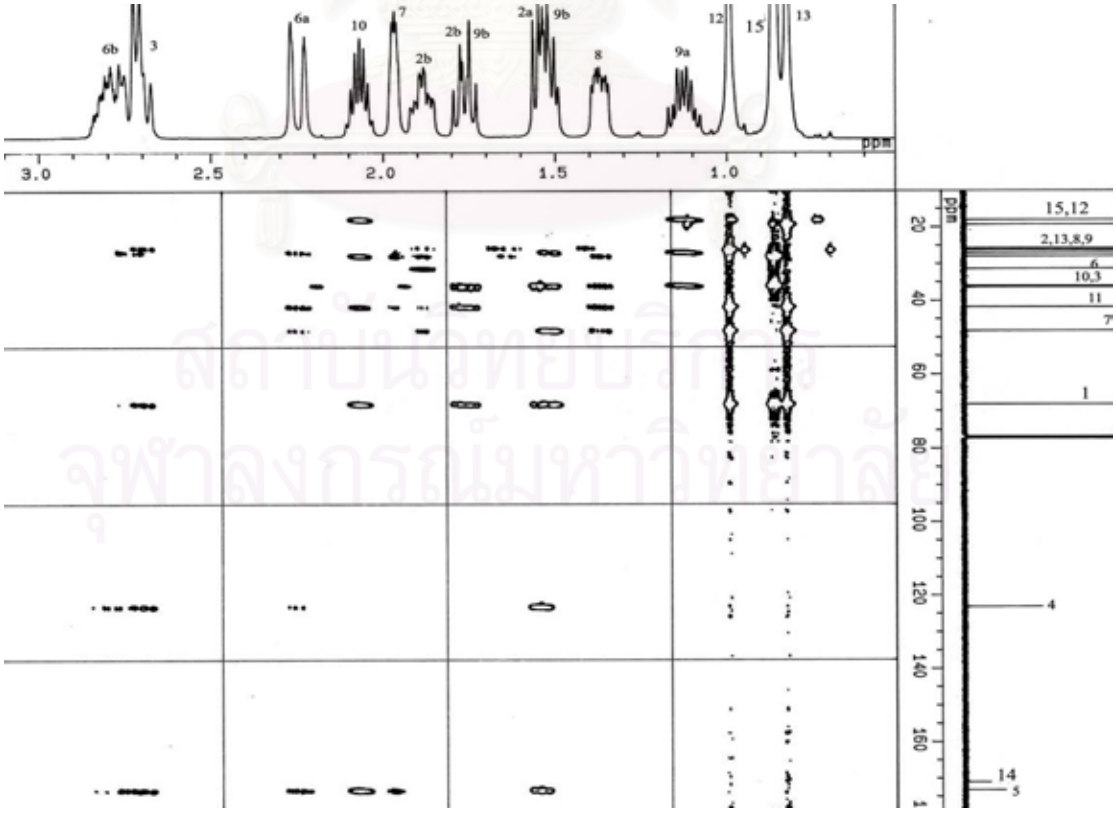

Figure 180 HMBC spectrum of compound **COC11** ($CDCl_3$).

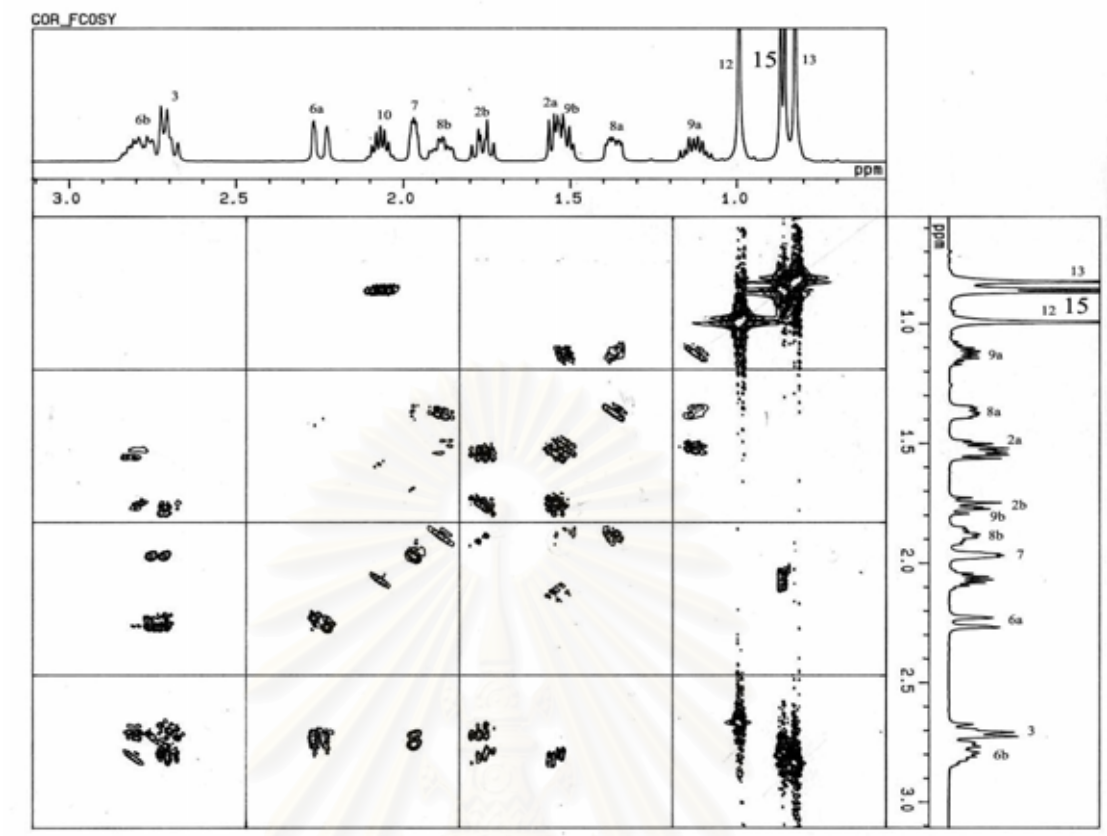

Figure 181 ^{1}H-^{1}H COSY spectrum of compound **COC11** ($CDCl_3$).

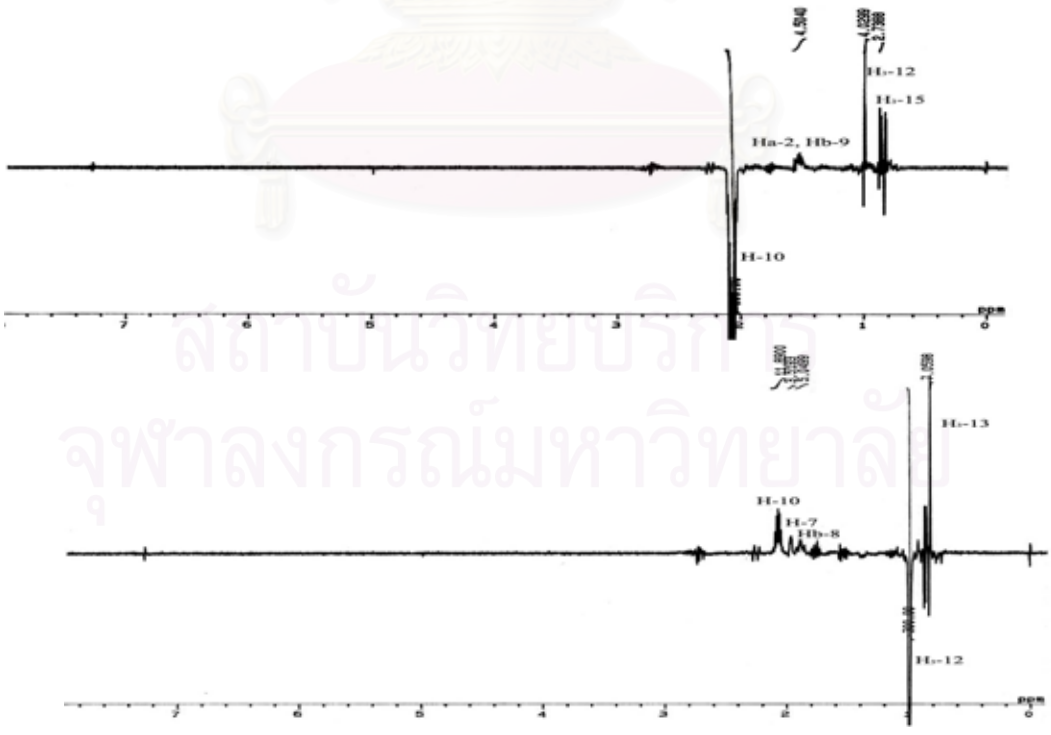

Figure 182 NOE spectra of compound **COC11** ($CDCl_3$).

Table 25 Crystal data and structure refinement for compound **COC10**.

Chemical formula	$C_{22}H_{29}Cl_3O_6$
Formula weight	495.80
Crystal system	Orthorhombic
Space group	P 2_1 2_1 2_1
Crystal colour and shape	colourless block
Crystal size	0.20 x 0.20 x 0.10
a (Å)	7.338(3)
b (Å)	11.777(5)
c (Å)	26.354(12)
V (Å^3)	2277.5(18)
Z	4
T (K)	173(2)
D_c (g·cm^{-3})	1.446
μ (mm^{-1})	0.439
Scan range (°)	1.55 < θ < 28.67
Unique reflections	5438
Reflections used [I>2σ(I)]	2094
Absolute structure parameters	0.15(19)
R_{int}	0.1894
Final *R* indices [I>2σ(I)]	0.1195, wR_2 0.2671
R indices (all data)	0.2263, wR_2 0.3330
Goodness-of-fit	0.905
Max, Min Δρ/e (Å^{-3})	1.048, -0.669

Table 26 Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for 4101. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U (eq)
C(1)	683(11)	- 481(7)	- 1085(3)	45(2)
C(2)	2206(11)	139(8)	-1339(3)	52(2)
C(3)	3640(11)	-581(7)	-1565(4)	54(2)
C(4)	3092(11)	-1696(7)	-1753(3)	49(2)
C(5)	1376(11)	-2093(7)	-1742(3)	46(2)
C(6)	862(10)	-3173(7)	-2025(3)	46(2)
C(7)	-757(10)	-3826(8)	-1780(3)	51(2)
C(8)	-2220(11)	-3062(7)	-1579(3)	50(2)
C(9)	-1447(11)	-2220(8)	-1193(3)	50(2)
C(10)	-120(10)	-1399(7)	-1472(3)	46(2)
C(11)	-813(12)	347(8)	-922(3)	57(2)
C(12)	1472(13)	-1061(8)	-639(3)	55(2)
C(13)	963(15)	-1767(10)	193(3)	78(3)
C(14)	2458(11)	-4023(7)	-2081(3)	50(2)
C(15)	3148(11)	-4577(7)	-1622(3)	48(2)
C(16)	3982(11)	-5696(8)	-1669(4)	58(2)
C(17)	4874(12)	-6359(8)	-1298(4)	63(2)
C(18)	5311(13)	-7332(10)	-1520(4)	73(3)
C(19)	4026(12)	-6314(8)	-2092(4)	65(3)
C(20)	373(11)	-2789(8)	-2569(3)	51(2)
C(21)	-1591(13)	-4725(9)	-2133(4)	70(3)
C(22)	6194(13)	1054(9)	470(4)	64(3)
O(1)	3063(8)	-1403(6)	-607(2)	64(2)
O(2)	287(8)	-1209(6)	-246(2)	67(2)
O(3)	5249(7)	-244(5)	-1612(2)	63(2)
O(4)	-1073(7)	-814(5)	-1866(2)	52(2)
O(5)	3017(8)	-4175(6)	-1186(2)	61(2)
O(6)	4825(9)	-7359(6)	-2019(3)	71(2)
Cl(1)	7211(4)	2204(3)	169(1)	81(1)
Cl(2)	5054(4)	194(3)	35(1)	86(1)
Cl(3)	4754(4)	1493(3)	952(1)	86(1)

Table 27 Bond lengths [Å] and angles [°] for compound **COC10**.

C(1)-C(12)	1.477(12)
C(1)-C(2)	1.494(11)
C(1)-C(10)	1.599(11)
C(1)-C(11)	1.530(11)
C(2)-C(3)	1.477(11)
C(2)-H(2A)	0.9900
C(2)-H(2B)	0.9900
C(3)-O(3)	1.252(10)
C(3)-C(4)	1.461(12)
C(4)-C(5)	1.343(11)
C(4)-H(4A)	0.9900
C(4)-H(4B)	0.9900
C(5)-C(6)	1.522(11)
C(5)-C(10)	1.543(11)
C(5)-H(5)	1.0000
C(6)-C(20)	1.546(11)
C(6)-C(14)	1.548(11)
C(6)-C(7)	1.555(11)
C(7)-C(8)	1.498(11)
C(7)-C(21)	1.537(12)
C(7)-H(7)	1.0000
C(8)-C(9)	1.530(11)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
C(9)-C(10)	1.556(11)
C(9)-H(9A)	0.9900
C(9)-H(9B)	0.9900
C(10)-O(4)	1.430(9)
C(11)-H(11A)	0.9800
C(11)-H(11B)	0.9800
C(11)-H(11C)	0.9800
C(12)-O(1)	1.237(11)
C(12)-O(2)	1.362(10)
C(13)-O(2)	1.421(11)
C(13)-H(13A)	0.9800

C(13)-H(13B)	0.9800
C(13)-H(13C)	0.9800
C(14)-C(15)	1.465(12)
C(14)-H(14A)	0.9900
C(14)-H(14B)	0.9900
C(15)-O(5)	1.244(10)
C(15)-C(16)	1.458(12)
C(16)-C(19)	1.333(13)
C(16)-C(17)	1.413(13)
C(17)-C(18)	1.327(14)
C(17)-H(17)	0.9500
C(18)-O(6)	1.363(12)
C(18)-H(18)	0.9500
C(19)-O(6)	1.377(11)
C(19)-H(19)	0.9500
C(20)-H(20A)	0.9800
C(20)-H(20B)	0.9800
C(20)-H(20C)	0.9800
C(21)-H(21A)	0.9800
C(21)-H(21B)	0.9800
C(21)-H(21C)	0.9800
C(22)-Cl(3)	1.732(10)
C(22)-Cl(1)	1.737(10)
C(22)-Cl(2)	1.744(10)
C(22)-H(22)	1.0000
O(4)-H(4)	0.8400

C(12)-C(1)-C(2)	106.8(7)
C(12)-C(1)C(11)	110.7(7)
C(2)-C(1)-C(11)	110.5(7)
C(12)-C(1)C(10)	109.8(7)
C(2)-C(1)-C(10)	108.7(6)
C(11)-C(1)C(10)	110.2(6)
C(3)-C(2)-C(1)	115.7(7)
C(3)-C(2)H(2A)	108.4
C(1)-C(2)H(2A)	108.4
C(3)-C(2)-H(2B)	108.4
C(1)-C(2)-H(2B)	108.4
H(2A)-C(2)-H(2B)	107.4

O(3)-C(3)-C(4)	120.8(8)
O(3)-C(3)-C(2)	122.0(8)
C(4)-C(3)-C(2)	117.2(7)
C(5)-C(4)-C(3)	124.3(7)
C(5)-C(4)-H(4A)	106.3
C(3)-C(4)-H(4A)	106.3
C(5)-C(4)-H(4B)	106.3
C(3)-C(4)-H(4B)	106.3
H(4A)-C(4)-H(4B)	106.4
C(4)-C(5)-C(6)	120.8(7)
C(4)-C(5)-C(10)	119.6(7)
C(6)-C(5)-C(10)	119.5(7)
C(4)-C(5)-H(5)	91.3
C(6)-C(5)-H(5)	91.3
C(10)-C(5)-H(5)	91.3
C(5)-C(6)-C(20)	105.5(7)
C(5)-C(6)-C(14)	113.6(6)
C(20)-C(6)-C(14)	106.1(6)
C(5)-C(6)-C(7)	113.6(7)
C(20)-C(6)-C(7)	110.6(7)
C(14)-C(6)-C(7)	107.3(6)
C(8)-C(7)-C(21)	110.1(7)
C(8)-C(7)-C(6)	113.4(7)
C(21)-C(7)-C(6)	113.2(7)
C(8)-C(7)-H(7)	106.5
C(21)-C(7)-H(7)	106.5
C(6)-C(7)-H(7)	106.5
C(7)-C(8)-C(9)	111.1(7)
C(7)-C(8)-H(8A)	109.4
C(9)-C(8)-H(8A)	109.4
C(7)-C(8)-H(8B)	109.4
C(9)-C(8)-H(8B)	109.4
H(8A)-C(8)-H(8B)	108.0
C(8)-C(9)-C(10)	108.7(6)
C(8)-C(9)-H(9A)	110.0
C(10)-C(9)-H(9A)	110.0
C(8)-C(9)-H(9B)	110.0
C(10)-C(9)-H(9B)	110.0
H(9A)-C(9)-H(9B)	108.3

O(4)-C(10)-C(5)	105.5(6)
O(4)-C(10)-C(9)	109.7(6)
C(5)-C(10)-C(9)	109.5(7)
O(4)-C(10)-C(1)	108.5(6)
C(5)-C(10)-C(1)	112.9(6)
C(9)-C(10)-C(1)	110.4(6)
C(1)-C(11)-H(11A)	109.5
C(1)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(1)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
O(1)-C(12)-O(2)	120.7(8)
O(1)-C(12)-C(1)	124.9(8)
O(2)-C(12)-C(1)	114.4(8)
O(2)-C(13)-H(13A)	109.5
O(2)-C(13)-H(13B)	109.5
H(13A)-C(13)-H(13B)	109.5
O(2)-C(13)-H(13C)	109.5
H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5
C(15)-C(14)-C(6)	118.1(7)
C(15)-C(14)-H(14A)	107.8
C(6)-C(14)-H(14A)	107.8
C(15)-C(14)-H(14B)	107.8
C(6)-C(14)-H(14B)	107.8
H(14A)-C(14)-H(14B)	107.1
O(5)-C(15)-C(16)	117.1(8)
O(5)-C(15)-C(14)	124.4(8)
C(16)-C(15)-C(14)	118.5(8)
C(19)-C(16)-C(17)	105.5(8)
C(19)-C(16)-C(15)	125.2(9)
C(17)-C(16)-C(15)	129.4(9)
C(18)-C(17)-C(16)	106.5(9)
C(18)-C(17)-H(17)	126.8
C(16)-C(17)-H(17)	126.8
C(17)-C(18)-O(6)	112.5(9)
C(17)-C(18)-H(18)	123.8
O(6)-C(18)-H(18)	123.8

C(16)-C(19)-O(6)	112.4(9)
C(16)-C(19)-H(19)	123.8
O(6)-C(19)-H(19)	123.8
C(6)-C(20)-H(20A)	109.5
C(6)-C(20)-H(20B)	109.5
H(20A)-C(20)-H(20B)	109.5
C(6)-C(20)-H(20C)	109.5
H(20A)-C(20)-H(20C)	109.5
H(20B)-C(20)-H(20C)	109.5
C(7)-C(21)-H(21A)	109.5
C(7)-C(21)-H(21B)	109.5
H(21A)-C(21)-H(21B)	109.5
C(7)-C(21)-H(21C)	109.5
H(21A)-C(21)-H(21C)	109.5
H(21B)-C(21)-H(21C)	109.5
Cl(3)-C(22)-Cl(1)	111.3(6)
Cl(3)-C(22)-Cl(2)	111.3(5)
Cl(1)-C(22)-Cl(2)	111.0(5)
Cl(3)-C(22)-H(22)	107.7
Cl(1)-C(22)-H(22)	107.7
Cl(2)-C(22)-H(22)	107.7
C(12)-O(2)-C(13)	117.0(7)
C(10)-O(4)-H(4)	109.5
C(18)-O(6)-C(19)	103.1(8)

Table 28 Anisotropic displacement parameters ($Å^2 \times 10^3$) for compound **COC10**.

The anisotropic displacement factor exponent takes the form:

$-2\pi^2 [h^2 a^{*2} U_{11} + ... + 2 h k a^* b^* U_{12}]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(1)	30(4)	54(5)	50(5)	-1(4)	1(3)	4(3)
C(2)	24(4)	60(5)	74(6)	-4(4)	2(4)	4(4)
C(3)	25(4)	50(5)	87(6)	8(4)	0(4)	4(3)
C(4)	30(4)	53(5)	63(5)	-6(4)	-1(4)	5(3)
C(5)	34(4)	49(5)	55(5)	-2(4)	5(3)	-1(4)
C(6)	17(4)	58(5)	62(5)	-3(4)	3(3)	3(3)
C(7)	27(4)	61(5)	64(5)	11(4)	9(3)	-5(4)
C(8)	30(4)	59(5)	61(5)	12(4)	5(4)	-4(4)
C(9)	32(4)	55(5)	63(5)	-8(4)	3(4)	-3(4)
C(10)	24(4)	61(5)	54(4)	-3(4)	2(3)	2(4)
C(11)	36(4)	67(5)	68(5)	12(5)	4(4)	7(4)
C(12)	49(5)	62(5)	54(5)	4(4)	0(4)	-8(4)
C(13)	78(7)	103(8)	52(5)	16(6)	0(5)	25(6)
C(14)	30(4)	57(5)	64(5)	3(4)	6(4)	6(4)
C(15)	25(4)	58(5)	91(7)	0(5)	-1(4)	2(4)
C(17)	35(5)	76(6)	78(6)	13(5)	4(4)	12(5)
C(18)	37(5)	87(7)	94(8)	18(6)	14(5)	10(5)
C(19)	39(5)	65(6)	93(7)	-1(6)	-2(5)	2(5)
C(20)	37(5)	59(5)	56(5)	4(4)	0(3)	-4(4)
C(21)	37(5)	74(6)	98(7)	23(6)	8(5)	11(5)
C(22)	40(5)	74(6)	79(6)	9(5)	2(4)	1(5)
O(1)	35(3)	84(5)	74(4)	6(3)	-4(3)	9(3)
O(2)	51(4)	99(5)	52(3)	11(3)	1(3)	-9(4)
O(3)	22(3)	63(4)	103(5)	-2(3)	5(3)	-4(3)
O(4)	26(3)	60(3)	68(4)	5(3)	-5(2)	5(3)
O(5)	42(4)	70(4)	72(4)	-7(3)	-2(3)	-2(3)
O(6)	47(4)	64(4)	103(5)	-1(4)	0(4)	9(3)
Cl(1)	55(2)	84(2)	104(2)	12(2)	9(1)	-9(1)
Cl(2)	53(2)	95(2)	109(2)	-5(2)	-10(1)	-11(2)
Cl(3)	72(2)	85(2)	102(2)	16(2)	21(2)	14(2)

Table 29 Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($Å^2 \times 10^3$) for compound **COC10**.

	x	y	z	U(eq)
H(2A)	1685	622	-1610	63
H(2B)	2782	649	-1087	63
H(4A)	3483	-1729	-2113	59
H(4B)	3845	-2258	-1569	59
H(5)	1632	-2532	-1425	56
H(7)	-249	-4244	-1482	61
H(8A)	-3180	-3526	-1415	60
H(8B)	-2783	-2640	-1863	60
H(9A)	-789	-2634	-922	60
H(9B)	-2450	-1783	-1034	60
H(11A)	-1874	-80	-802	85
H(11B)	-1167	818	-1213	85
H(11C)	-356	834	-649	85
H(13A)	1443	-2514	98	116
H(13B)	-26	-1861	439	116
H(13C)	1939	-1311	344	116
H(14A)	2068	-4626	-2319	60
H(14B)	3487	-3617	-2242	60
H(17)	5114	-6149	-956	76
H(18)	5898	-7942	-1350	87
H(19)	3558	-6062	-2409	79
H(20A)	-690	-2285	-2557	76
H(20B)	90	-3456	-2777	76
H(20C)	1409	-2382	-2717	76
H(21A)	-2379	-5232	-1935	105
H(21B)	-614	-5168	-2291	105
H(21C)	-2313	-4349	-2397	105
H(22)	7185	589	627	77
H(4)	-1915	-423	-1739	77

Table 30 Torsion angles [deg] for compound **COC10**.

C(12)-C(1)-C(2)-C(3)	66.1(10)
C(11)-C(1)-C(2)-C(3)	-173.3(7)
C(10)-C(1)-C(2)-C(3)	-52.3(10)
C(1)-C(2)-C(3)-O(3)	-152.1(8)
C(1)-C(2)-C(3)-C(4)	30.4(12)
O(3)-C(3)-C(4)-C(5)	-176.3(9)
C(2)-C(3)-C(4)-C(5)	1.3(14)
C(3)-C(4)-C(5)-C(6)	169.6(8)
C(3)-C(4)-C(5)-C(10)	-5.9(14)
C(4)-C(5)-C(6)-C(20)	-87.1(9)
C(10)-C(5)-C(6)-C(20)	88.4(8)
C(4)-C(5)-C(6)-C(14)	28.6(11)
C(10)-C(5)-C(6)-C(14)	-155.9(7)
C(4)-C(5)-C(6)-C(7)	151.6(8)
C(10)-C(5)-C(6)-C(7)	-32.9(10)
C(5)-C(6)-C(7)-C(8)	38.6(10)
C(20)-C(6)-C(7)-C(8)	-79.7(9)
C(14)-C(6)-C(7)-C(8)	165.0(7)
C(5)-C(6)-C(7)-C(21)	165.0(8)
C(20)-C(6)-C(7)-C(21)	46.6(10)
C(14)-C(6)-C(7)-C(21)	-68.6(10)
C(21)-C(7)-C(8)-C(9)	175.4(7)
C(6)-C(7)-C(8)-C(9)	-56.6(10)
C(7)-C(8)-C(9)-C(10)	66.1(9)
C(4)-C(5)-C(10)-O(4)	99.8(9)
C(6)-C(5)-C(10)-O(4)	-75.8(9)
C(4)-C(5)-C(10)-C(9)	-142.2(8)
C(6)-C(5)-C(10)-C(9)	42.2(9)
C(4)-C(5)-C(10)-C(1)	-18.7(11)
C(6)-C(5)-C(10)-C(1)	165.8(7)
C(8)-C(9)-C(10)-O(4)	59.0(9)
C(8)-C(9)-C(10)-C(5)	-56.5(8)
C(8)-C(9)-C(10)-C(1)	178.5(6)
C(12)-C(1)-C(10)-O(4)	172.8(6)
C(2)-C(1)-C(10)-O(4)	-70.6(7)

C(11)-C(1)-C(10)-O(4)	50.6(8)
C(12)-C(1)-C(10)-C(5)	-70.5(9)
C(2)-C(1)-C(10)-C(5)	46.0(9)
C(11)-C(1)-C(10)-C(5)	167.2(7)
C(12)-C(1)-C(10)-C(9)	52.5(9)
C(2)-C(1)-C(10)-C(9)	169.1(7)
C(11)-C(1)-C(10)-C(9)	-69.7(8)
C(2)-C(1)-C(12)-O(1)	-29.5(12)
C(11)-C(1)-C(12)-O(1)	-149.9(9)
C(10)-C(1)-C(12)-O(1)	88.3(11)
C(2)-C(1)-C(12)-O(2)	150.8(7)
C(11)-C(1)-C(12)-O(2)	30.4(10)
C(10)-C(1)-C(12)-O(2)	-91.5(9)
C(5)-C(6)-C(14)-C(15)	67.7(10)
C(20)-C(6)-C(14)-C(15)	-176.9(7)
C(7)-C(6)-C(14)-C(15)	-58.6(10)
C(6)-C(14)-C(15)-O(5)	-25.2(12)
C(6)-C(14)-C(15)-C(16)	152.2(7)
O(5)-C(15)-C(16)-C(19)	171.0(9)
C(14)-C(15)-C(16)-C(19)	-6.5(13)
O(5)-C(15)-C(16)-C(17)	-8.3(13)
C(14)-C(15)-C(16)-C(17)	174.2(8)
C(19)-C(16)-C(17)-C(18)	-2.5(10)
C(15)-C(16)-C(17)-C(18)	176.9(9)
C(16)-C(17)-C(18)-O(6)	1.8(11)
C(17)-C(16)-C(19)-O(6)	2.4(10)
C(15)-C(16)-C(19)-O(6)	-177.1(8)
O(1)-C(12)-O(2)-C(13)	0.0(13)
C(1)-C(12)-O(2)-C(13)	179.7(8)
C(17)-C(18)-O(6)-C(19)	-0.4(10)
C(16)-C(19)-O(6)-C(18)	-1.3(10)

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Poster Presentations

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