Chemical Constituents from Seeds, Roots and Heartwood of *Caesalpinia sappan*

Orapun Yodsaoue

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Organic Chemistry
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Thesis Title Chemical Constituents from Seeds, Roots and Heartwood of *Caesalpinia sappan*
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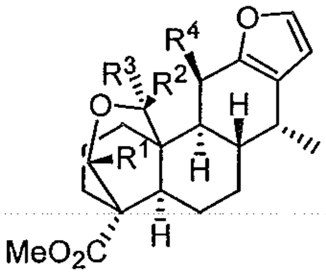
ชื่อวิทยานิพนธ์ องค์ประกอบทางเคมีจากเมล็ด รากและแก่นของฝาง
ผู้เขียน นางสาวอรพรรณ ยอดสะอึ
สาขาวิชา เคมีอินทรีย์
ปีการศึกษา 2550

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีจากเมล็ด รากและแก่นของฝาง สามารถแยกสารใหม่เป็นสารประกอบไดเทอร์พีนชนิดคาสเซนได้ 11 สาร คือ phanginin A-K (**DS1- DS11**) ซึ่งแยกได้จากส่วนของเมล็ด นอกจากนี้ยังสามารถแยกสารประกอบที่มีการรายงานแล้ว 9 สาร ซึ่งแบ่งเป็นสารประกอบโฮโมไอโซฟลาโวน 7 สาร [3-(3',4'-dihydroxybenzyl)-7-hydroxychroman-4-one (**DH1**), 4-*O*-methylepisappanol (**DH2**), 4-*O*-methylsappanol (**DH3**), 3,4-isopropylidene sappanol (**DH4**), brazilin (**DH5**), protosappannin A (**DH6**) และ episappanol (**MR1**)] และสารประกอบชาลโคน 2 สาร [3-deoxysappanchalcone (**MR2**) และ sappanchalcone (**MR3**)] ซึ่งแยกได้จากส่วนของรากและแก่นของฝาง จากการศึกษาฤทธิ์ทางชีวภาพพบว่า สารประกอบ **MR3** แสดงฤทธิ์ต้านโรคภูมิแพ้ใน RBL-2H3 เซลล์โมเดลดีที่สุด ด้วยค่า IC_{50} = 7.6 μM รองลงมาคือสารประกอบ **MR2** (IC_{50} = 15.3 μM) สารประกอบ **MR3** เพียงสารเดียวที่มีฤทธิ์ยับยั้งความเป็นพิษต่อเซลล์มะเร็งเต้านม (MCF-7) มะเร็งปากมดลูก (HeLa) มะเร็งลำไส้ (HT-29) และ มะเร็งในช่องปาก (KB) ที่ค่า IC_{50} 0.64, 0.72, 0.80 และ 0.75 $\mu g/ml$ ตามลำดับ โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี สำหรับสารประกอบ **DS1** มีข้อมูลทางเอกซ์เรย์ในการพิสูจน์โครงสร้างด้วย



DS1: $R_1 = R_3 = R_4 = H$, $R_2 = OH$; Phanginin A

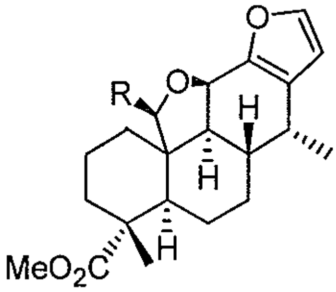
DS2: $R_1 = OH$, $R_2 = R_3 = R_4 = H$; Phanginin B

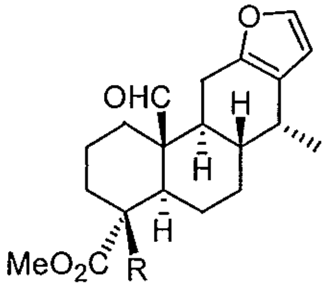
DS3: $R_1 = R_2 = R_4 = H$, $R_3 = OMe$; Phanginin C

DS4: $R_1 = OMe$, $R_2 = R_3 = R_4 = H$; Phanginin D

DS5: $R_1 = {=}O$, $R_2 = R_3 = R_4 = H$; Phanginin E

DS6: $R_1 = R_2 = H$, $R_3 = R_4 = OH$; Phanginin F





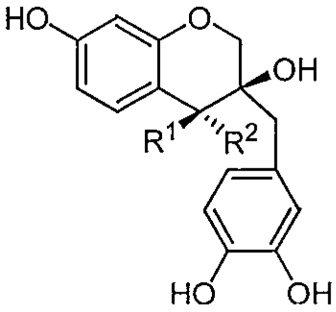
DS7: R = OH; Phanginin G

DS8: R = H; Phanginin H

DS9: R = Me; Phanginin I

DS10: R = CHO; Phanginin J

DS11: R = CO_2Me; Phanginin K

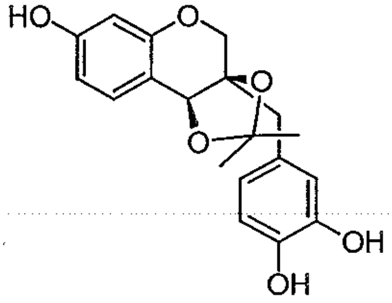


DH1: $R_1 = R_2 = {=}O$; 3-(3',4'-Dihydroxybenzyl)-7-hydroxychroman-4-one

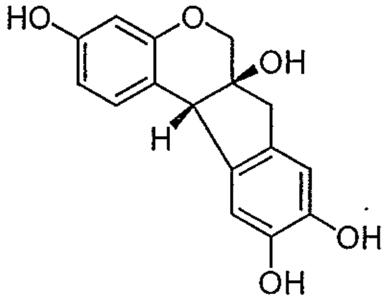
MR1: $R_1 = H$, $R_2 = OH$; Episappanol

DH2: $R_1 = H$, $R_2 = OMe$; 4-*O*-Methylepisappanol

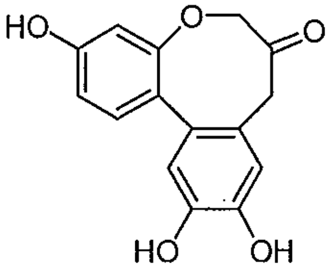
DH3: $R_1 = OMe$, $R_2 = H$; 4-*O*-Methylsappanol



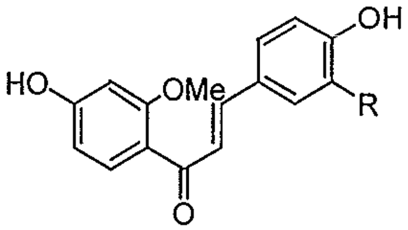
DH4: 3,4-Isopropylidene sappanol



DH5: Brazilin



DH6: Protosappannin A



MR2: R = H; 3-Deoxysappanchalcone

MR3: R = OH; Sappanchalcone

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Author	Miss Orapun Yodsaoue
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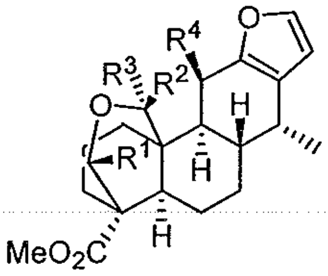
Academic Year	2007

ABSTRACT

Investigation of the chemical constituents from seeds, roots and heartwood of *Caesalpinia sappan* has led to the isolation of eleven new cassane-type diterpenes phanginin A-K (**DS1**- **DS11**) which were obtained from the seeds, together with nine previously reported compounds: seven homoisoflavones [3-(3',4'-dihydroxybenzyl)-7-hydroxychroman-4-one (**DH1**), 4-*O*-methylepisappanol (**DH2**), 4-*O*-methylsappanol (**DH3**), 3,4-isopropylidene sappanol (**DH4**), brazilin (**DH5**), protosappannin A (**DH6**) and episappanol (**MR1**)] and two chalcones [3-deoxysappanchalcone (**MR2**) and sappanchalcone (**MR3**)] were isolated from the roots and heartwood. It was found that **MR3** possessed the most potent effect against allergic reaction in RBL-2H3 cells with an IC_{50} value of 7.6 μM, followed by **MR2** (IC_{50} = 15.3 μM). Only one compound (**MR3**) demonstrated significant inhibitory activity against MCF-7 (breast adenocarcinoma), KB (human oral cancer), HeLa (Human cervical cancer) and HT-29 (colon cancer) cell lines with IC_{50} values of 0.64, 0.72, 0.80 and 0.75 μg/ml, respectively. Their structures were elucidated by spectroscopic methods. The structure of **DS1** was additionally confirmed by X-ray diffraction technique.



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DS4: $R_1 = OMe$, $R_2 = R_3 = R_4 = H$; Phanginin D
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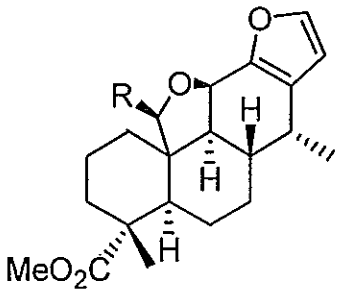
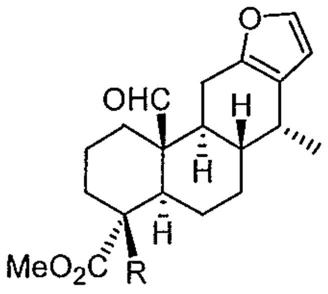
DS6: $R_1 = R_2 = H$, $R_3 = R_4 = OH$; Phanginin F





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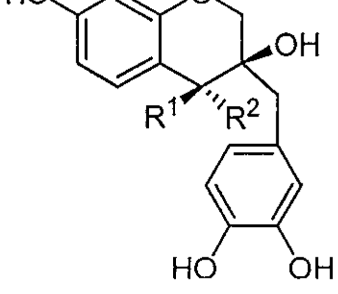

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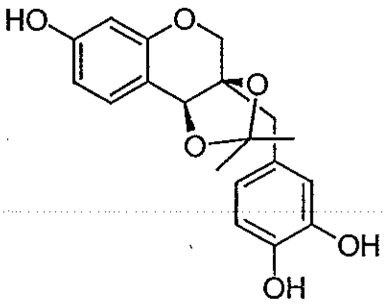
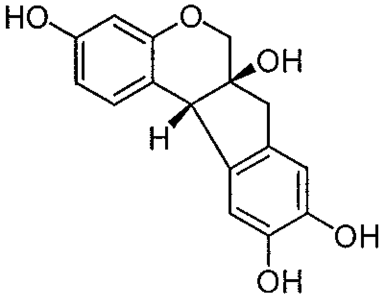
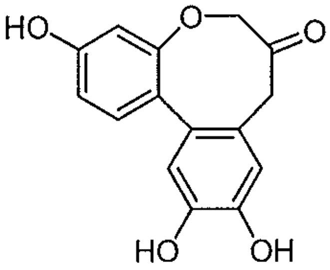


DH4: 3,4-Isopropylidene sappanol



DH5: Brazilin



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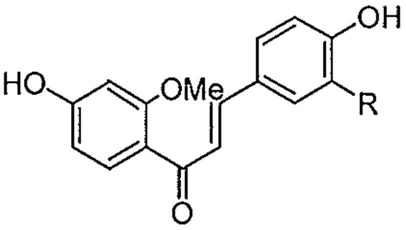

MR2: R = H; 3-Deoxysappanchalcone

MR3: R = OH; Sappanchalcone

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Orapun Yodsaoue

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents of *C. sappan*. It is a part of the basic research on the utilization of the Thai medicinal plants. Chemical investigation of constituents from seeds, roots and heartwood of *Caesalpinia sappan* has led to isolation of eleven new compounds together with nine known compounds. Some of the compounds exhibited anti-allergic and cytotoxic activities. Two pure compounds (**MR2** and **MR3**) possessed the most potent effect against allergic reaction in RBL-2H3 cells and only **MR3** exhibited significant cytotoxic activity. Hence they have potential to be developed into drugs from natural product resources.

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LISTS OF SCHEMES

ABBREVIATIONS AND SYMBOLS

s = *singlet*
d = *doublet*
t = *triplet*
q = *quartet*
m = *multiplet*
dd = *doublet of doublet*
dt = *doublet of triplet*
br s = *broad singlet*
R_f = Retention factor
g = gram
nm = nanometer
mp = melting point
cm^{-1} = reciprocol centimeter (wave number)
δ = chemical shift relative to TMS
J = coupling constant
$[\alpha]_D$ = specific rotation
λ_{max} = maximum wavelength
ν = absorption frequencies
ε = molar extinction coefficient
m/z = a value of mass divided by charge
°C = degree celcius
MHz = Megahertz
ppm = part per million
c = concentration
IR = Infrared
UV-VIS = Ultraviolet-Visible
MS = Mass Spectroscopy
NMR = Nuclear Magnetic Resonance

ABBREVIATIONS AND SYMBOLS (continued)

2D NMR = Two Dimensional Nuclear Magnetic Resonance
COSY = Correlation Spectroscopy
DEPT = Distortionless Enhancement by Polarization Transfer
HMBC = Heteronuclear Multiple Bond Correlation
HMQC = Heteronuclear Multiple Quantum Coherence
NOE = Nuclear Overhauser Effect Spectroscopy
CC = Column Chromatography
QCC = Quick Column Chromatography
PLC = Preparative Thin Layer Chromatography
TMS = tetramethylsilane
$CDCl_3$ = deuterochloroform
CD_3OD = deuteromethanol
DMSO-d_6 = deuterodimethylsulfoxide

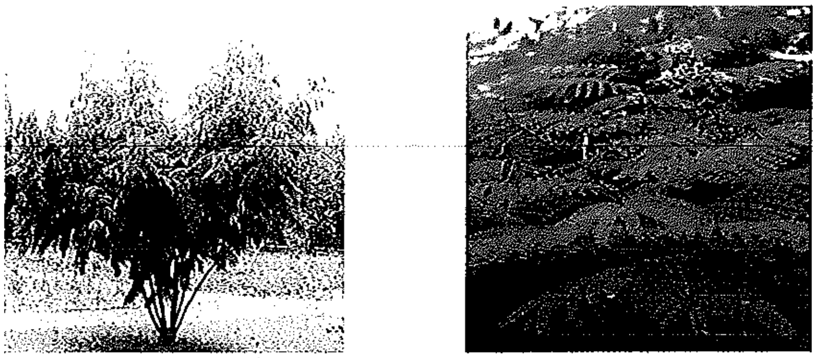
CHAPTER 1
INTRODUCTION

1.1 Introduction

Caesalpinia sappan Linn., known locally as "Phang (ฝาง)" in Thai belonged to the Leguminosae-Caesalpinioideae family. The origin of *C. sappan* is not certain, but is thought to be in the region from central and southern India through Indo-China to Peninsular Malaysia. It is cultivated and naturalized in many parts of Malaysia and also in central India to Hawaii. The Leguminosae-Caesalpinioideae family contains about 150 genera with 2,200 species. In Thailand only 20 genera with 113 species are found, from *Caesalpinia* genus only 18 species are found. *C. sappan* has been found in the northeastern and the northern parts of Thailand.

Caesalpinia sappan is a small to medium-sized, shrubby tree, 4-10 m tall. The bark is distinct ridges and many prickles that is greyish brown colour. Leaves stipulate are bipinnate and alternate, 20-50 cm long, 10-20 cm broad, which has 8-16 pairs of up to 20 cm long pinnace. The pinnace has prickle at the base and comprises of 10-20 pairs of oblong, 10-20 mm × 6-10 mm long. Flowers are panicles pendulous 30-40 cm long, which are racemes pubescent. The bracts are ovate-acuminate about 6 mm long. Petals pubescent are yellow. Fruit is a dehiscence pod, glabrous, thick, flattened, obliquely oblong, prominently beaked, woody, polished-brown, 7-10 cm × 3-4 cm, 2-5 seeded. Seeds are ellipsoid flattened, brown.

Several members of *Caesalpinia* genus are being used traditionally for a wide variety of ethnomedical properties (Uphof, 1968). The heartwood of this plant has long been used as blood tonic, expectorant and emmenagougue (Wutthithamavet, 1997) and possesses interesting biological activities such as antioxidation (Safitri *et al.*, 2003; Badami *et al.*, 2003), anticomplementary activity (Oh *et al.*, 1998), antimicrobial activity (Lim *et al.*, 2007) and vasorelaxation (Hu *et al.*, 2003). Previous studies undertaken on *C. sappan* in view of its medicinal significance have led to the isolation of several homoisoflavonoids (Xie *et al.*, 2000).



a. Tree **b.** Leaves

c. Stem **d.** Heartwood **e.** Flowers

f. Fruits **g.** Seeds

Figure 1 Parts of *Caesalpinia sappan*

1.2 Review of literatures

Chemical constituents isolated from 18 species of the genus *Caesalpinia* were summarized by Sarot Cheenpracha in 2007 (Cheenpracha, 2007). Information from NAPRALERT database developed by University of Illinois at Chicago and Chemical Abstracts of the year 2007 reported additional constituents from three new species of the *Caesalpinia* genus and they could be classified into groups, such as benzenoids, coumarins, diterpenes, flavonoids, flavonols, flavones, flavonones, sesquiterpenes, steroids and triterpenes. These compounds are presented in Table 1.

Table 1 Compounds from plants of *Caesalpinia* genus

a : Benzenoids **b** : Coumarins **c** : Diterpenes
d : Flavonoids **e** : Phenylpropanoids
f : Sesquiterpenes **g** : Steroids **h** : Triterpenes

Scientific name	Investigated part	Compound	Bibliography
C. benthamiana	Root bark	Benthaminin 1, **8c** Benthaminin 2, **9c** Deoxycaesaldekarin C, **70c**	Dickson *et al.*, 2007
C. bonduc	Part not specified	Caesalpinolide A, **66c** Caesalpinolide B, **67c**	Yadav *et al.*, 2007
	Bark	Caesaldekarin J, **19c** 17-Hydroxy-campesta-4,6-dien-3-one, **134g** 13,14-*seco*-Stigmasta-5,14-dien-3α-ol, **135g** 13,14-*seco*-Stigmasta-9(11), 14-Dien-3α-ol, **136g** Pipataline, **131e**	Udenigwe *et al.*, 2007

Table 1 (Continued)

Scientific name	**Investigated part**	**Compound**	**Bibliography**
C. bonduc	Kernels	2-Acetoxycaesaldekarin E, **6c** Bonducellpin A, **10c** Bonducellpin B, **14c** Bonducellpin C, **11c** Bonducellpin E, **15c** Bonducellpin F, **16c** Bonducellpin G, **17c** α-Caesalpin, **27c** γ-Caesalpin, **29c** Caesalmin B, **20c** Caesalmin D, **25c** Caesalmin E, **26c** Caesalpinin C, **31c** Caesalpinin I, **40c** Caesalpinin K, **45c** Caesalpinin P, **49c** 14(17)-Dehydrocaesalpin F, **36c**	Pudhom *et al.*, 2007
C. crista	Seeds	Taepeenin J, **92c** Taepeenin K, **93c** Taepeenin L, **94c** (5α)-Vouacapa-(14),9(11)-diene, **95c** (5α,8β)-Vouacapane, **96c** (5α,6β,8β)-Vouacapan-6-ol, **97c**	Cheenpracha *et al.*, 2006

Table 1 (Continued)

Scientific name	Investigated part	Compound	Bibliography
C. crista	Root	Taepeenin E, **85c** Taepeenin H, **88c** Taepeenin I, **89c** Vinhaticoic acid, **90c**	Cheenpracha *et al.*, 2005
	Stems	*ent*-11β-Hydroxy-rosa-5,15-diene, **71c** Methyl vinhaticoate, **91c** Nortaepeenin A, **79c** Nortaepeenin B, **80c** Taepeenin A, **81c** Taepeenin B, **82c** Taepeenin C, **83c** Taepeenin D, **84c** Taepeenin F, **86c** Taepeenin G, **87c**	Cheenpracha *et al.*, 2005
	Kernels	7-Acetoxybonducellpin C, **38c** 2-Acetoxycaesaldekarin E, **6c** 2-Acetoxy-3-deacetoxy caesaldekarin E, **4c** 6-Acetoxy-3-deacetoxy caesaldekarin E, **7c** Caesaldekarin E, **5c** Caesalmin B, **20c** Caesalmin C, **23c** Caesalmin E, **24c**	Kalauni *et al.*, 2004

Table 1 (Continued)

Scientific name	**Investigated part**	**Compound**	**Bibliography**
C. crista	Kernels	14(17)-Dehydrocaesalpin F, **36c**	Kalauni *et al.*, 2004
		Caesalpinin C, **31c**	
		Caesalpinin E, **37c**	
		Caesalpinin MA, **50c**	
		Caesalpinin MB, **51c**	
		Caesalpinin MC, **52c**	
		Caesalpinin ME, **54c**	
		Norcaesalpinin B, **35c**	
		Norcaesalpinin MA, **75c**	
		Norcaesalpinin MB, **76c**	
		Norcaesalpinin MC, **77c**	
		7-Acetoxybonducellpin C, **38c**	Kalauni *et al.*, 2005a
		2-Acetoxycaesaldekarin E, **6c**	
		Caesaldekarin E, **5c**	
		Caesalmin C, **23c**	
		Caesalmin G, **21c**	
		β-Caesalpin, **28c**	
		Caesalpinin C, **31c**	
		Caesalpinin D, **22c**	
		Caesalpinin E, **37c**	
		Caesalpinin F, **39c**	
		Caesalpinin H, **43c**	
		Caesalpinin I, **40c**	
		Caesalpinin J, **41c**	
		Caesalpinin K, **45c**	
		Caesalpinin MF, **55c**	

Table 1 (Continued)

Scientific name	**Investigated part**	**Compound**	**Bibliography**
C. crista	Kernels	Caesalpinin MG, **56c** Caesalpinin MH, **57c** Caesalpinin MI, **58c** Caesalpinin MJ, **59c** Caesalpinin MK, **60c** Caesalpinin ML, **63c** Caesalpinin M, **48c** Caesalpinin N, **47c** Caesalpinin O, **44c** Norcaesalpinin MD, **78c**	Kalauni *et al.*, 2005a
		2-Acetoxycaesaldekarin E, **6c** Bonducellpin C, **11c** Caesaldekarin E, **5c** Caesalmin C, **23c** Caesalpinin MM, **61c** Caesalpinin MN, **62c** Caesalpinin MO, **64c** Caesalpinin MP, **65c** 1-Deacetoxy-1-oxocaesalmin C, **68c** 1-Deacetylcaesalmin C, **69c** Norcaesalpinin E, **33c**	Kalauni *et al.*, 2005b
		2-Acetoxycaesaldekarin E, **6c** 7-Acetoxybonducellpin, **38c** 2-Acetoxy-3-deacetoxycaesal dekarin E, **4c**	Linn *et al.*, 2005

Table 1 (Continued)

Scientific name	Investigated part	Compound	Bibliography
C. crista	Kernels	Caesaldekarin E, **5c** Caesalmin B, **20c** Caesalmin G, **42c** Caesalpin F, **30c** Caesalpinin D, **22c** Caesalpinin E, **37c** Caesalpinin F, **39c** Caesalpinin G, **42c** 14(17)-Dehydrocaesalpin F, **36c** Norcaesalpinin A, **34c** Norcaesalpinin B, **35c** Norcaesalpinin D, **32c** Norcaesalpinin E, **33c**	Linn *et al.*, 2005
		2-Acetoxyl-3-deacetoxycaesaldekarin E, **4c** 6-Acetoxy-3-deacetoxycaesaldekarin E, **7c** Bonducellpin A, **41c** Bonducellpin B, **11c** Bonducellpin C, **14c** Caesaldekarin E, **5c** Caesalmin E, **24c** α-Caesalpin, **27c** Caesalpinin C, **31c** Caesalpinin D, **32c** Caesalpinin H, **43c**	Awale *et al.*, 2006

Table 1 (Continued)

Scientific name	**Investigated part**	**Compound**	**Bibliography**
C. crista	Kernels	Caesalpinin I, **40c** Caesalpinin J, **41c** Caesalpinin K, **45c** Caesalpinin L, **46c** Caesalpinin M, **48c** Caesalpinin N, **47c** Caesalpinin O, **44c** Caesalpinin P, **49c** 1-Deacetoxy-1-oxocaesalmin C, **68c** Norcaesalpinin E, **33c** Norcaesalpinin F, **74c**	Awale *et al.*, 2006
	Leaves	Neocaesalpin H, **72c** Neocaesalpin I, **73c**	Kinoshita *et al.*, 2005
C. decapetala	Leaves	Caesaldecan, **18c** 4,5-Epoxy-8(14)-caryophyllene, **132f** Spathulenol, **133f** Lupeol, **137h** Squalene, **138h** *trans*-Resveratrol, **2a** Quercetin, **126d** Astragalin, **98d**	Kiem *et al.*, 2005
C. digyna	Twigs and stems roots	Bonducellin, **99d** Bergenin, **3b**	Boonsri *et a*l., 2005 Srinivasan *et al.*, 2007

Table 1 (Continued)

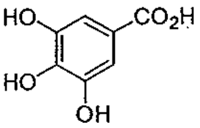
Scientific name	**Investigated part**	**Compound**	**Bibliography**
C. millettii HOOK. *et* ARN	Stems	Bonducellin, **99d** Eucomin, **109d** Intricatinol, **111d** 8-Methoxybonducellin, **110d** 8-Methoxyisobonducellin, **117d** Tamarixetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-alactopyranoside, **130d**	Chen and Yang, 2007
C. mimosoides Lamk	Part not specified	Gallic acid, **1a**	Chanwitheesuk *et al.*, 2007
C. pulcherrima	Part not specified	Bonducellin, **99d** Dihydrobonducellin, **102d** Isobonducellin, **115d** 20-Methoxybonducellin, **116d** 20-Methoxydihydrobonducellin, **103d**	Zhao *et al.*, 2004
		Isobonducellin, **115d** (*E*)-7-Hydroxy-3-(4'-methoxybenzylidene)chroman-4-one, **113d** (E)-7-Hydroxy-3-(3',4',5'-trimethoxybenzylidene)chroman-4-one, **114d**	Maheswara *et al.*,2006

Table 1 (Continued)

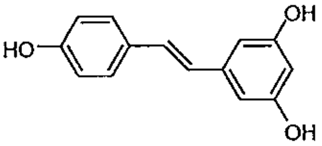
Scientific name	Investigated part	Compound	Bibliography
C. pulcherrima	Part not specified	(*E*)-7-Methoxy-3-(4'-methoxy benzylidene)chroman-4-one, **112d**	Maheswara *et al.*, 2006
C. sappan	Heartwood	Bonducellin, **99d** Neoprotosappanin, **119d** Brazilin, **100d** 3'-Deoxysappanol, **106d** 3'-Deoxy-4-*O*-methylsappanol, **105d** 3′-Deoxysappanone B, **128d** 3'-*O*-Methylbrazilin, **101d** 4-*O*-Methylepisappanol, **118d** 4-*O*-Methylsappanol, **107d** Neosappanone A, **120d** Protosappanin A, **121d** Protosappanin A dimethyl acetal, **123d** Protosappanin B, **124d** Protosappanin C dimethyl acetal, **125d** Protosappanin E-2, **122d** Sappanol, **104d** Sappanone B, **129d** Sappanchalcone, **127d**	Nguyen *et al.*, 2004 Nguyen *et al.*, 2005

Structures

a: Benzenoids

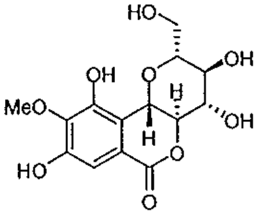


1a: R = H; Gallic acid



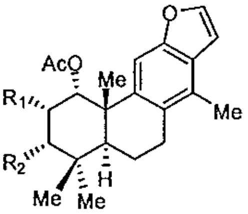
2a: *trans*-Resveratrol

b: Coumarins



3b: Bergenin

c: Diterpenes

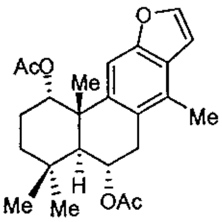


4c: R_1 = OAc, R_2 = H;

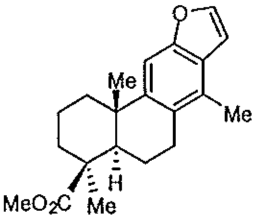
2-Acetoxy-3-deacetoxycaesaldekarin E

5c: R_1 = H, R_2 = OAc; Caesaldekarin E

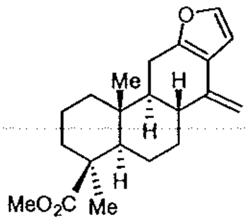
6c: R_1 = R_2 = OAc; 2-Acetoxycaesaldekarin E



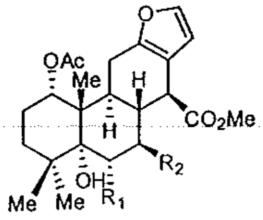
7c: 6-Acetoxy-3-deacetoxycaesaldekarin E



8c: Benthaminin 1



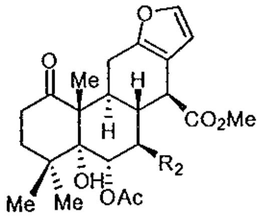
9c: Benthaminin 2

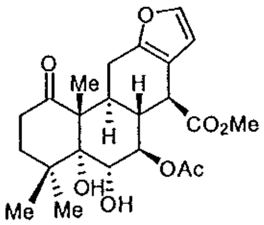


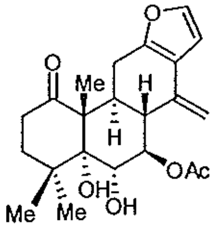
10c: R_1 = OAc, R_2 = OH; Bonducellpin A

11c: R_1 = H, R_2 = OH; Bonducellpin C

12c: R_1 = OH, R_2 = OAc; Caesalpinin M

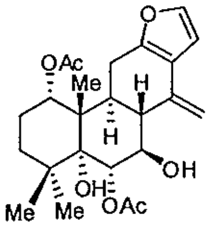


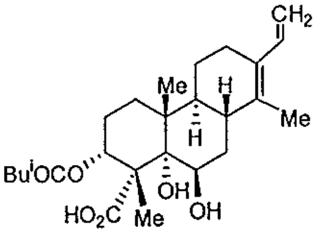


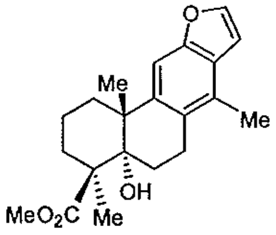


13c: R = OAc; Caesalpinin J **15c**: Bonducellpin E **16c**: Bonducellpin F

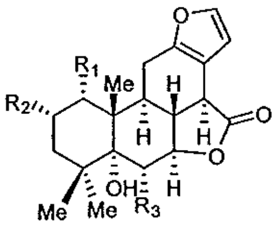
14c: R = OH; Bonducellpin B







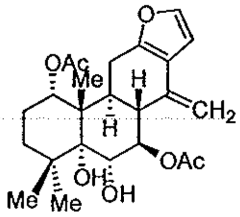
17c: Bonducellpin G **18c**: Caesaldecan **19c**: Caesaldekarin J



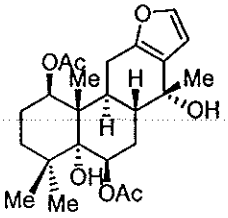
20c: R_1 = OAc, R_2 = R_3 = H; Caesalmin B

21c: R_1 = OH, R_2 = R_3 = H; Caesalmin G

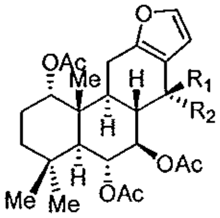
22c: R_1 = R_3 = OAc, R_2 = H; Caesalpinin D



23c: Caesalmin C

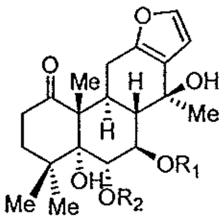


24c: Caesalmin E



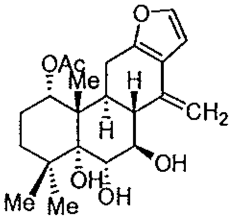
25c: R_1 = Me, R_2 = OH; Caesalmin D

26c: R_1 = OH, R_2 = Me; Caesalmin E

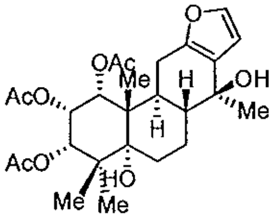


27c: R = Ac; *α*-Caesalpin

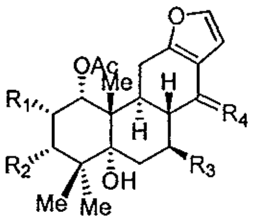
28c: R = H; *β*-Caesalpin



29c: *γ*-Caesalpin



30c: Caesalpin F



31c: R_1 = R_3 = OH, R_2 = OAc, R_4 = CH_2; Caesalpinin C

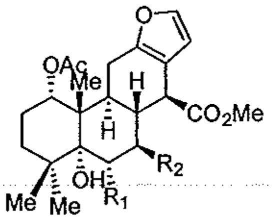
32c: R_1 = R_2 = OAc, R_3 = H, R_4 = O; Norcaesalpinin D

33c: R_1 = R_2 = H, R_3 = OH, R_4 = O; Norcaesalpinin E

34c: R_1 = OAc, R_2 = R_3 = H, R_4 = O; Norcaesalpinin A

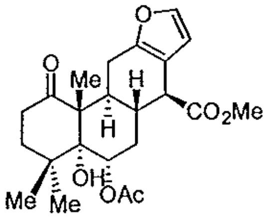
35c: R_1 = R_3 = H, R_2 = OAc, R_4 = O; Norcaesalpinin B

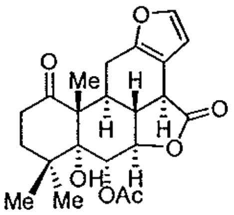
36c: R_1 = R_2 = OAc, R_3 = H, R_4 = CH_2;
14(17)-Dehydrocaesalpin F

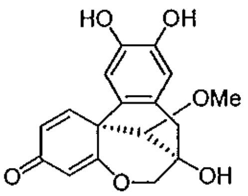


37c: R_1 = OAc, R_2 = H; Caesalpinin E

38c: R_1 = H, R_2 = OAc; 7-Acetoxybonducellpin C



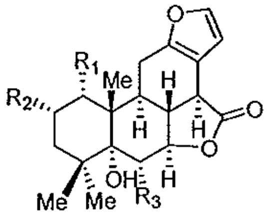




39c: Caesalpinin F

40c: Caesalpinin I

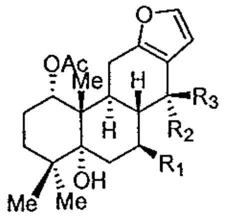
41c: Caesalpinin J



42c: $R_1 = R_2$ = OAc, R_3 = H; Caesalpinin G

43c: R_1 = OH, R_2 = H, R_3 = OAc; Caesalpinin H

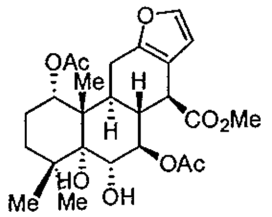
44c: R_1 = OAc, R_2 = H, R_3 = OH; Caesalpinin O

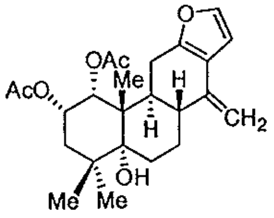


45c: R_1 = OH, R_2 = Me, R_3 = H; Caesalpinin K

46c: R_1 = OAc, R_2 = OH, R_3 = Me; Caesalpinin L

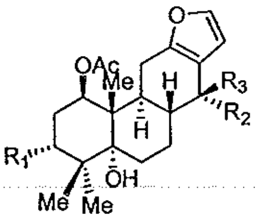
47c: R_1 = OH, R_2 = H, R_3 = CHO; Caesalpinin N





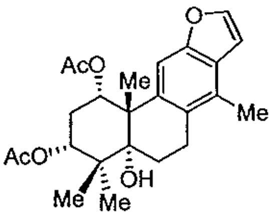
48c: Caesalpinin M

49c: Caesalpinin P

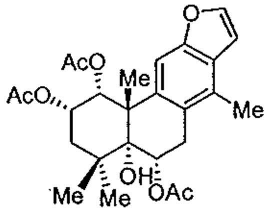


50c: R_1 = OAc, R_2 = Me, R_3 = H; Caesalpinin MA

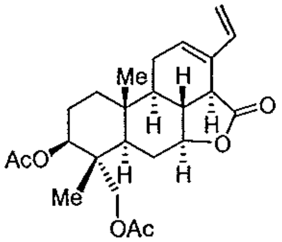
51c: R_1 = R_2 = H, R_3 = CO_2Me; Caesalpinin MB



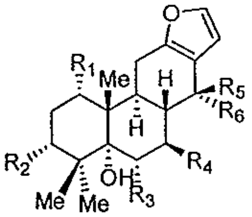
52c: Caesalpinin MC



53c: Caesalpinin MD



54c: Caesalpinin ME

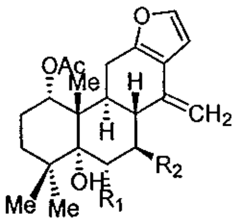


55c: R_1 = R_2 = OAc, R_3 = R_4 = R_6 = H, R_5 = CO_2Me; Caesalpinin MF

56c: R_1 = R_3 = R_4 = OAc, R_2= R_6 = H, R_5 = CO_2Me; Caesalpinin MG

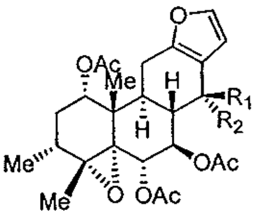
57c: R_1 = R_3 = OAc, R_2 = R_6 = H, R_4 = OH, R_5 = CO_2H; Caesalpinin MH

58c: R_1 = R_2 = R_3 = R_5 = H, R_4 = OH, R_6 = Me; Caesalpinin MI



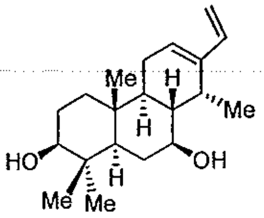
59c: R_1 = H, R_2 = OAc; Caesalpinin MJ

60c: R_1 = OAc, R_2 = H; Caesalpinin MK

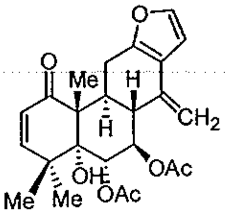


61c: R_1 = Me, R_2 = OH; Caesalpinin MM

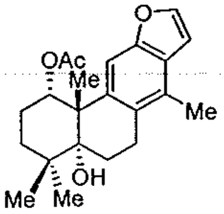
62c: R_1 = OH, R_2 = Me; Caesalpinin MN



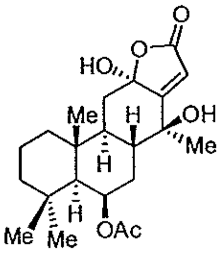
63c: Caesalpinin ML



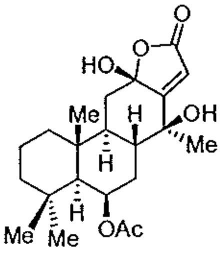
64c: Caesalpinin MO



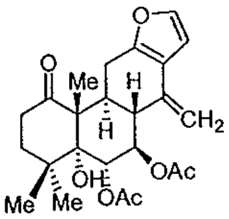
65c: Caesalpinin MP



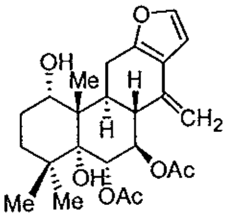
66c: Caesalpinolide A



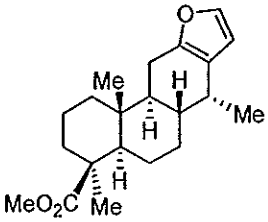
67c: Caesalpinolide B



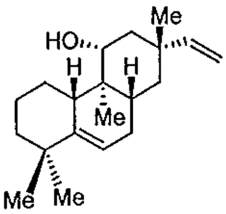
68c: 1-Deacetoxy-1-oxocaesalmin C



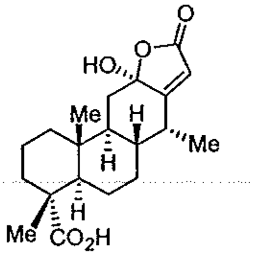
69c: 1-Deacetylcaesalmin C



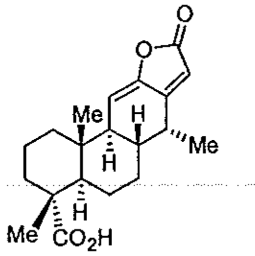
70c: Deoxycaesaldekarin C



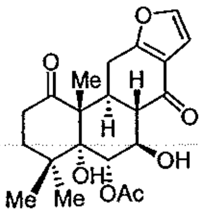
71c: *ent*-11β-Hydroxy-rosa-5,15-diene



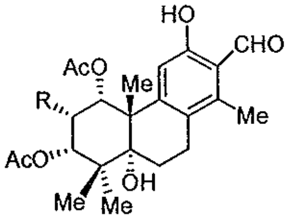
72c: Neocaesalpin H



73c: Neocaesalpin I

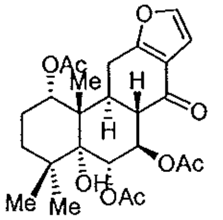


74c: Norcaesalpinin F

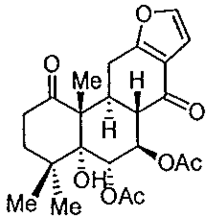


75c: R = H; Norcaesalpinin MA

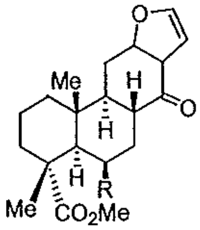
76c: R = OAc; Norcaesalpinin MB



77c: Norcaesalpinin MC

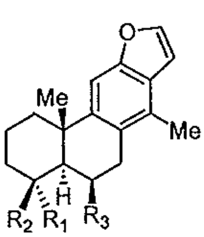


78c: Norcaesalpinin MD



79c: R = H; Nortaepeenin A

80c: R = OH; Nortaepeenin B



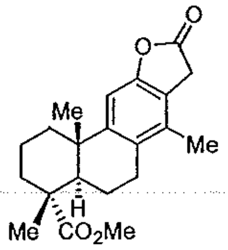
81c: $R_1 = CO_2Me$, $R_2 = Me$, $R_3 = H$; Taepeenin A

82c: $R_1 = CO_2H$, $R_2 = Me$, $R_3 = H$; Taepeenin B

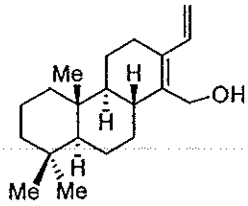
83c: $R_1 = CO_2Me$, $R_2 = Me$, $R_3 = OH$; Taepeenin C

84c: $R_1 = CO_2Me$, $R_2 = Me$, $R_3 = OAc$; Taepeenin D

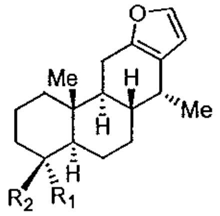
85c: $R_1 = CO_2Me$, $R_2 = CHO$, $R_3 = H$; Taepeenin E



86c: Taepeenin F



87c: Taepeenin G

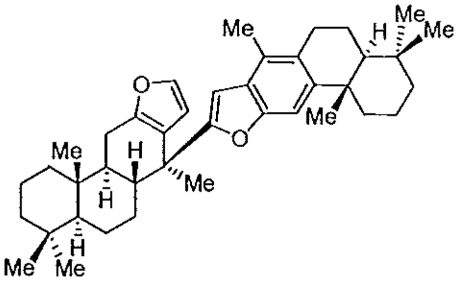


88c: $R_1 = CO_2Me$, $R_2 = CHO$; Taepeenin H

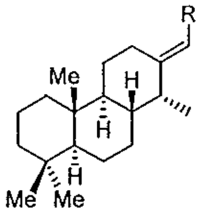
89c: $R_1 = CO_2Me$, $R_2 = CH_2OH$; Taepeenin I

90c: $R_1 = CO_2H$, $R_2 = Me$; Vinhaticoic acid

91c: $R_1 = CO_2Me$, $R_2 = Me$; Methyl vinhaticoate

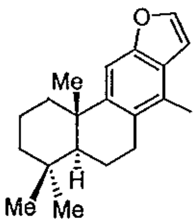


92c: Taepeenin J

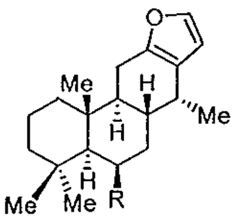


93c: R = CHO; Taepeenin K

94c: R = CH_2OH; Taepeenin L



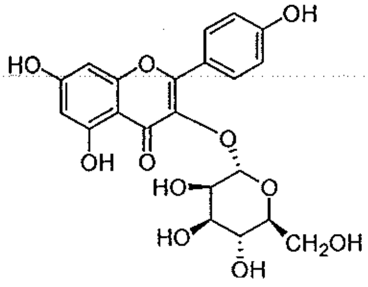
95c: (5α)-Vouacapa-8(14),9(11)-diene



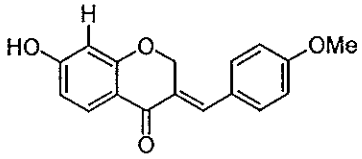
96c: R = H; (5α,8β)-Vouacapane

97c: R = OH; (5α,6β,8β)-Vouacapan-6-ol

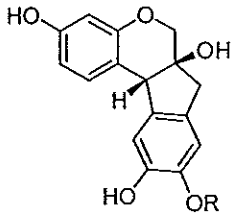
d : Flavonoids



98d: Astragalin

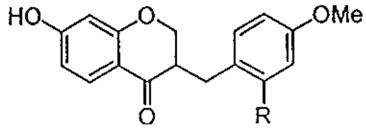


99d: R = H; Bonducellin



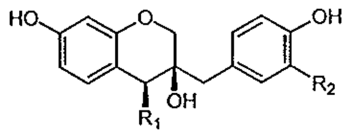
100d: R = H; Brazilin

101d: R = CH_3; 3′-*O*-Methylbrazilin



102d: R = H; Dihydrobonducellin

103d: R = CH_3; 20-Methoxydihydrobonducellin



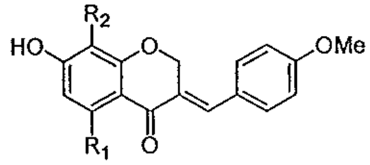
104d: $R_1 = R_2 =$ OH; Sappanol

105d: R_1 = OMe, R_2 = H; 3′-Deoxy-4-*O*-methylsappanol

106d: R_1 = OH, R_2 = H; 3′-Deoxysappanol

107d: R_1 = OMe, R_2 = OH; 4-*O*-Methylsappanol

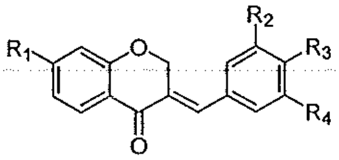
108d: R_1 = OH, R_2 = OMe; 3′-*O*-Methylsappanol



109d: R_1 = OH, R_2 = H; Eucomin

110d: R_1 = H, R_2 = OMe; 8-Methoxybonducellin

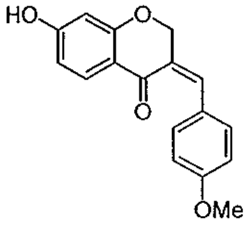
111d: R_1 = H, R_2 = OH; Intricatinol



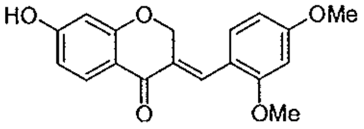
112d: R_1 = OMe, R_2 = R_4 = H, R_3 = OMe; (*E*)-7-Methoxy-3-(4'-methoxybenzylidene)chroman-4-one

113d: R_1 = OH, R_2 = R_4 = H, R_3 = OMe; (*E*)-7-Hydroxy-3-(4'-methoxybenzylidene)chroman-4-one

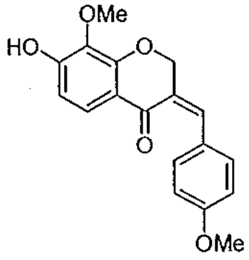
114d: R_1 = OH, R_2 = R_4 = R_3 = OMe; (*E*)-7-Hydroxy-3-(3',4',5'-trimethoxybenzylidene)chroman-4-one



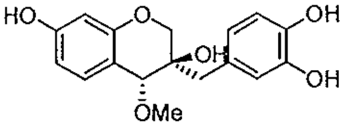
115d: Isobonducellin



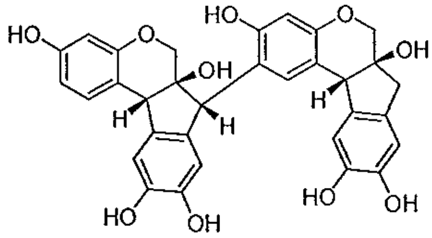
116d: 20-Methoxybonducellin



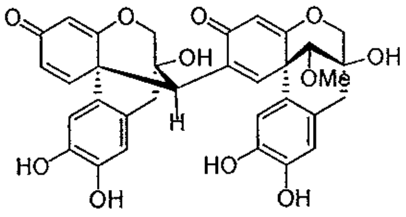
117d: 8-Methoxyisobonducellin



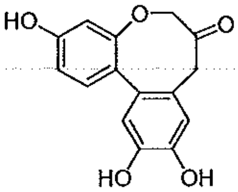
118d: 4-*O*-Methylepisappanol



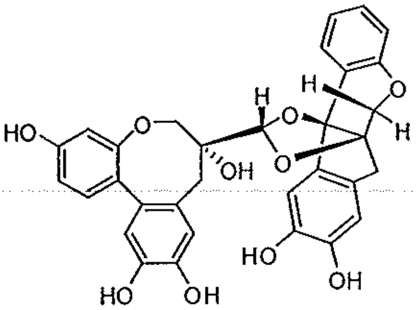
119d: Neoprotosappanin



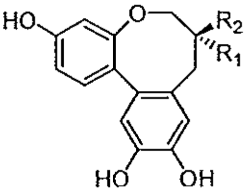
120d: Neosappanone A



121d: Protosappanin A



122d: Protosappanin E-2

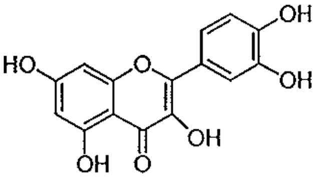


123d: $R_1 = R_2 =$ OMe; Protosappanin A dimethyl acetal

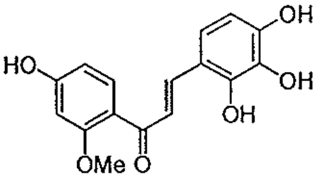
124d: $R_1 =$ OH, $R_2 = CH_2OH$; Protosappanin B

125d: $R_1 =$ OH, $R_2 = CH(OCH_3)_2$;

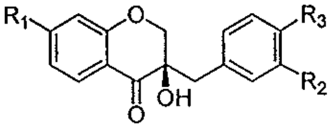
Protosappanin C dimethyl acetal



126d: $R_1 = R_2 = R_3 = R_4 =$ OH; Quercetin

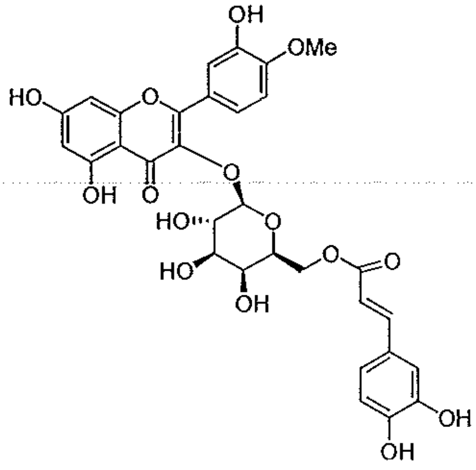


127d: $R_1 =$ OMe, $R_2 =$ H, $R_3 =$ OH; Sappanchalcone



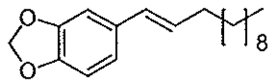
128d: $R_1 = R_3 =$ OMe, $R_2 =$ H; 3′-Deoxysappanone B

129d: $R_1 = R_2 = R_3 =$ OH; Sappanone B



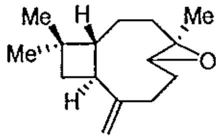
130d: Tamarixetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-alactopyranoside

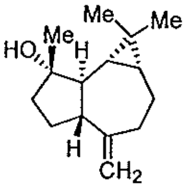
e: Phenylpropanoids



131e: Pipataline

f: Sesquiterpene

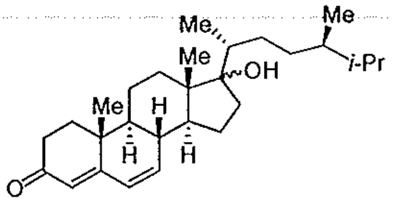




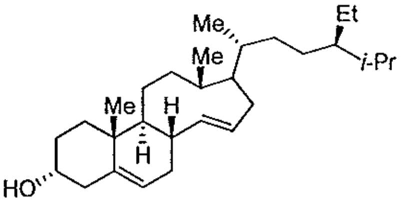
132f: 4,5-Epoxy-8(14)-caryophyllene

133f: Spathulenol

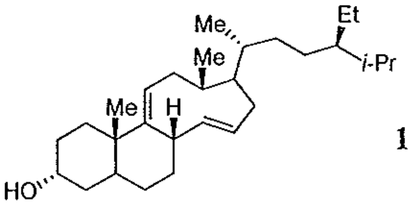
g: Steroids



134g: 17-Hydroxy-campesta-4,6-dien-3-one

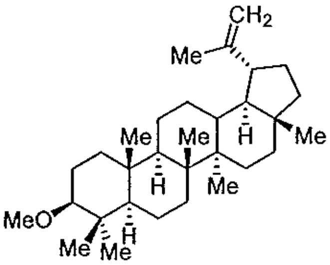


135g: 13,14-*seco*-Stigmasta-5,14-dien-3α-ol

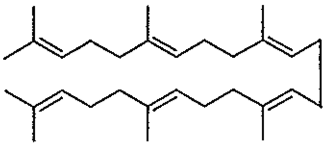


136g: 13,14-*seco*-Stigmasta-9(11),14-dien-3α-ol

h: Triterpenes



137h: Lupeol



138h: Squalene

1.3 Objective

Up to the present, the chemical constituents and biological activities of these plants are interested. This research part involved isolation, purification and structure elucidation of chemical constituents isolation from the seeds, roots and heartwood of *Caesalpinia sappan* and also evaluation of pure compounds for anti-allergic and cytotoxic activities.

CHAPTER 2
EXPERIMENTAL

2.1 Instruments and chemicals

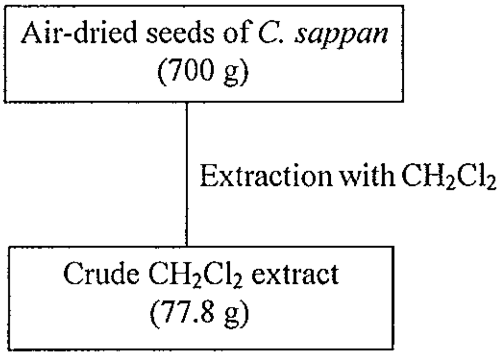
Melting point was recorded in °C on a Fisher-Johns melting point apparatus. Infrared spectra were recorded using FTS FT-IR spectrophotometer and major bands (ν) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using a SPECORD S 100 (Analytikjena) and UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in chloroform and methanol solution. Nuclear magnetic resonance spectra were recorded using 300 MHz Bruker FTNMR Ultra Shield^{TM}. Spectra were recorded in deuterochloroform, deuteroacetone deuteromethanol and deuterodimethyl sulphoxide solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). The EI-MS and ESI-TOF-MS were performed using a MAT 95 XL and Micromass LCT mass spectrometer, respectively. Single-crystal X-ray diffraction measurements were collected using SMART 1-K CCD diffractometer with monochromated Mo-Kα radiation (λ = 0.71073 Å) using ω-scan mode and SHELXTL for structure solution and refinement. Optical rotation was measured in chloroform solution with sodium D line (590 nm) on an AUTOPOL^{R} II automatic polarimeter. Solvent for extraction and chromatography were distilled at their boiling point ranges prior to use except diethyl ether was analytical grade reagent. Quick column chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (0.063 – 0.200). Precoated plates of silica gel 60 GF_{254} or reversed-phase C_{18} were used for analytical purposes.

2.2 Plant material

Seeds, roots and heartwood of *Caesalpinia sappan* were collected from Khonkaen province, Thailand in October 2005. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. SC07) deposited at Prince of Songkla University Herbarium.

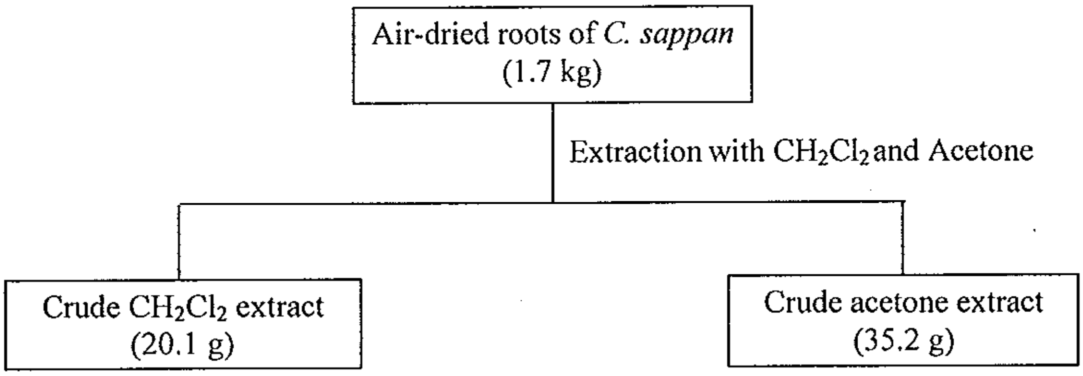
2.3 Extraction

The air-dried seeds (700 g) of *C. sappan* were extracted with methylene chloride (each 2 x 2.5 L, for 5 days) at room temp. The crude extracts were evaporated under reduced pressure to afford brownish methylene chloride as a yellow oil extract (77.8 g). The process of extraction was shown in Scheme 1.



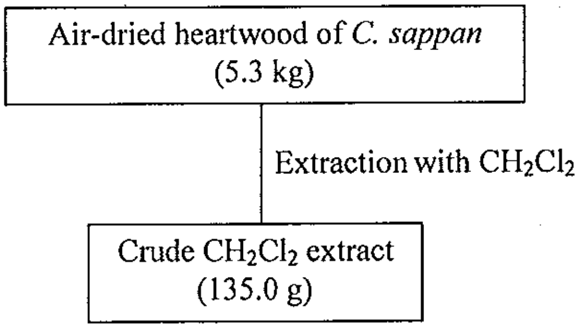
Scheme 1 Extraction of the seeds of *C. sappan*

The air-dried roots (1.7 kg) of *C. sappan* were extracted with methylene chloride and acetone, successively (each 2 x 2 L, for 5 days) at room temp. The crude extracts were evaporated under reduced pressure to afford a brownish methylene chloride (20.1 g) and acetone extracts (35.2 g), respectively.



Scheme 2 Extraction of the roots of *C. sappan*

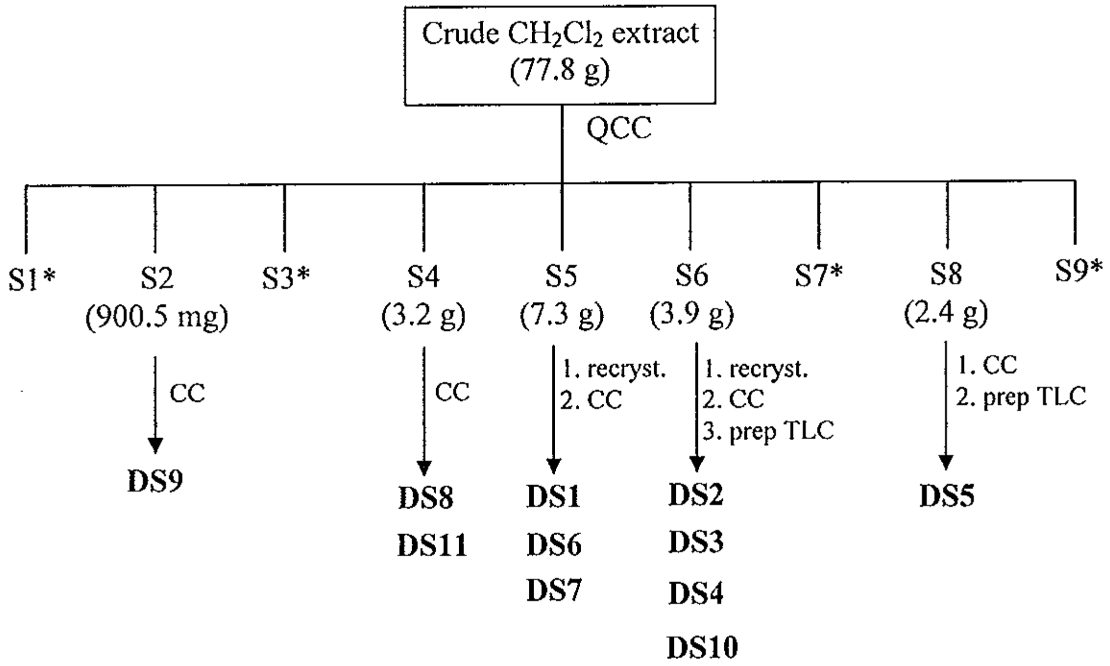
Chopped-dried heartwood (5.3 kg) of *C. sappan* was extracted with methylene chloride (each 2 x 5 L, for 5 days) at room temp. and evaporated under reduced pressure to afford a brownish methylene chloride extract (135.0 g).



Scheme 3 Extraction of the heartwood of *C. sappan*

2.4 Isolation and chemical investigation

2.4.1 Investigation of the crude methylene chloride extract from the seeds of *C. sappan*



* Not further investigated

Scheme 4 Isolation of compounds **DS1-DS11** from the seeds of *C. sappan*

The crude methylene chloride extract (77.8 g) was further purified by QCC using hexane as eluent and increasing polarity with ethyl acetate to give nine fractions (S1-S9, Scheme 4).

Fraction S2 (900.5 mg) was further purified by CC with EtOAc-hexane (1:9, v/v) to give **DS9** (307.5 mg, $R_f = 0.27$ (1:9, EtOAc-hexane)).

Fraction S4 (3.2 g) was purified by CC with EtOAc-hexane (3:17, v/v) as eluent to afford nine subfractions (S4a-S4i). Subfraction S4b (84.5 mg) was separated by CC with EtOAc-CH_2Cl_2-hexane (1:1:8, v/v) to give **DS8** (26.3 mg, $R_f =$ 0.19 (CH_2Cl_2)). Subfraction S4d (60.8 mg) was purified by CC with CH_2Cl_2-hexane (7:3, v/v) to give **DS11** (10.4 mg, $R_f = 0.29$ (7:3, CH_2Cl_2-hexane)).

Fraction S5 (7.3 g) was recrystallized from CH_2Cl_2 to give **DS1** (832.3 mg, R_f = 0.44 (3:7, EtOAc-hexane)) and the mother liquor (6.4 g) was further subjected to QCC with CH_2Cl_2–hexane (8:2, v/v) to afford seven subfractions (S5a-S5g). Subfraction S5c (117.2 mg) was purified by CC with CH_2Cl_2 to give **DS6** (6.6 mg, R_f = 0.24 (CH_2Cl_2)) and **DS7** (8.2 mg, R_f = 0.14 (1:49, EtOAc-CH_2Cl_2)).

Fraction S6 (3.9 g) was separated by CC with EtOAc-CH_2Cl_2 (1:19, v/v) to afford ten subfractions (S6a-S6j). Subfraction S6b (211.5 mg) was recrystallized from CH_2Cl_2 to give **DS10** (156.3 mg, R_f = 0.42 (CH_2Cl_2)). Subfraction S6h (345.4 mg) was purified by CC with acetone-hexane (1:9, v/v) and followed by prep TLC with acetone-hexane (1:9, v/v) to give **DS2** (3.2 mg, R_f = 0.37 (1:9, acetone-hexane)), **DS3** (6.0 mg, R_f = 0.38 (1:9, acetone-hexane)) and **DS4** (31.7 mg, R_f = 0.47 (1:9, acetone-hexane)).

Fraction S8 (2.4 g) was separated by CC with CH_2Cl_2 and followed by prep TLC with CH_2Cl_2-hexane (4:1, v/v) to give **DS5** (10.5 mg, R_f = 0.27 (CH_2Cl_2)).

Compound DS1: White solid; mp: 110-112 °C; $[\alpha]^{30}_D$: - 41.4° (c = 0.76, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 218 (3.85) nm; IR (neat) υ_{max}: 3433, 1726, 755 cm^{-1}; HREIMS: *m/z* $[M]^+$ 360.1949 (calcd for $C_{21}H_{28}O_5$, 360.1937); ^1H ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2.

Compound DS2: Viscous oil; $[\alpha]^{30}_D$: + 48.7° (c = 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 214 (3.84) nm; IR (neat) υ_{max}: 3394, 1730, 762 cm^{-1}; HREIMS: *m/z* $[M]^+$ 360.1927 (calcd for $C_{21}H_{28}O_5$, 360.1937); ^1H NMR ($CDCl_3$, 300 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

Compound DS3: Viscous oil; $[\alpha]^{30}_D$: - 40.4° (c = 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 216 (3.88) nm; IR (neat) υ_{max}: 1728, 757 cm^{-1}; HREIMS: *m/z* $[M]^+$ 374.2116 (calcd for $C_{22}H_{30}O_5$, 374.2093); ^1H NMR ($CDCl_3$, 300 MHz), see Table 4; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 4.

Compound DS4: Viscous oil; $[\alpha]^{30}_D$: + 44.8° (c = 0.61, $CHCl_3$); UV

(MeOH) λ_{max} (log ε): 215 (2.78) nm; IR (neat) υ_{max}: 1731, 755 cm^{-1}; HREIMS: *m/z* [M]$^+$ 374.2110 (calcd for $C_{22}H_{30}O_5$, 374.2093); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 5; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 5.

Compound DS5: Viscous oil; $[\alpha]^{30}{}_D$: - 24.8° (c = 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 215 (3.26) nm; IR (neat) υ_{max}: 1726, 824 cm^{-1}; HREIMS: *m/z* [M]$^+$ 358.1744 (calcd for $C_{21}H_{26}O_5$, 358.1780); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 6; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 6.

Compound DS6: White solid; mp: 105-107 °C; $[\alpha]^{30}{}_D$: - 1.8° (c = 0.52, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 215 (4.05) nm; IR (neat) υ_{max}: 3436, 1725, 756 cm^{-1}; HREIMS: *m/z* [M-H_2O]$^+$ 358.1765 (calcd for $C_{21}H_{28}O_6$, 376.1886); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 7; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 7.

Compound DS7: Viscous oil; $[\alpha]^{30}{}_D$: + 31.1° (c = 0.61, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 219 (3.94) nm; IR (neat) υ_{max}: 3395, 1721, 755 cm^{-1}; HREIMS: *m/z* [M]$^+$ 360.1916 (calcd for $C_{21}H_{28}O_5$, 360.1937); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 9; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 9.

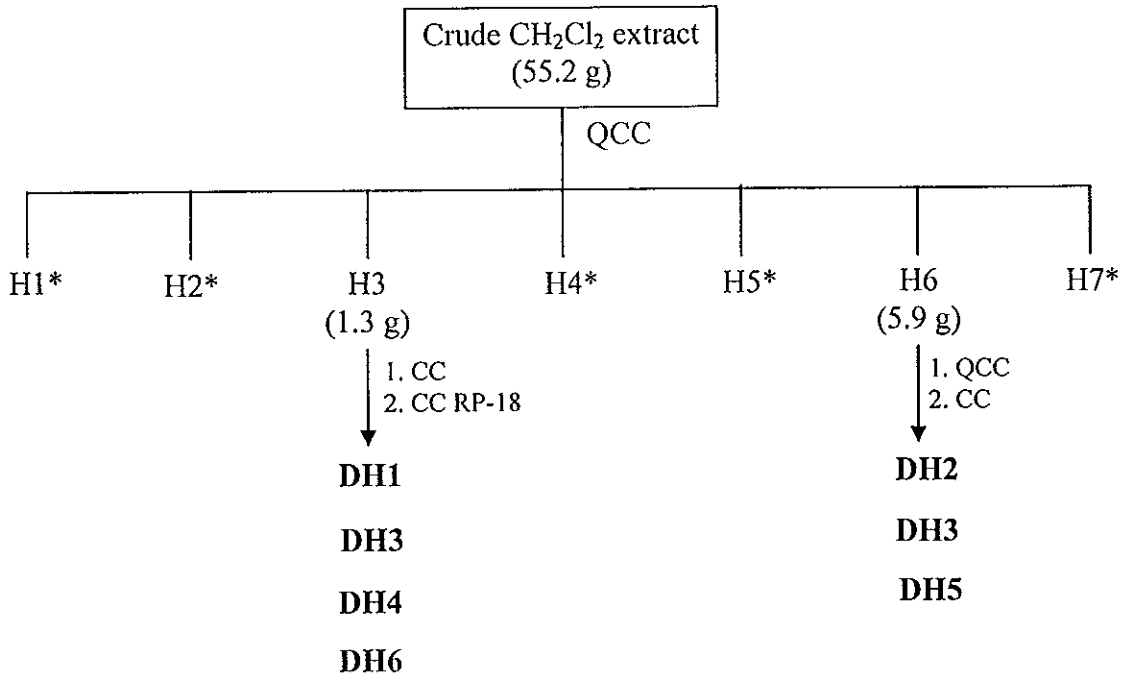
Compound DS8: Viscous oil; $[\alpha]^{30}{}_D$: + 3.6° (c = 0.39, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 216 (3.72) nm; IR (neat) υ_{max}: 1726, 755 cm^{-1}; HREIMS: *m/z* [M]$^+$ 344.1996 (calcd for $C_{21}H_{28}O_4$, 344.1988); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 10; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table Table 10.

Compound DS9: White solid; mp: 121-122 °C; $[\alpha]^{30}{}_D$: + 76.9° (c = 0.91, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 220 (3.80) nm; IR (neat) υ_{max}: 2930, 1725, 737 cm^{-1}; HREIMS: *m/z* [M]$^+$ 344.1983 (calcd for $C_{21}H_{28}O_4$, 344.1988); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 12; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 12.

Compound DS10: White solid; mp: 148-150 °C; $[\alpha]^{30}{}_D$: + 41.5° (c = 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 219 (3.93) nm; IR (neat) υ_{max}: 2865, 1717, 824 cm^{-1}; HREIMS: *m/z* [M]$^+$ 358.1779 (calcd for $C_{21}H_{26}O_5$, 358.1780) ; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 13; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 13.

Compound DS11: Viscous oil; $[\alpha]^{30}_D$: + 1.8° (*c* = 0.55, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 215, (3.87) nm; IR (neat) υ_{max}: 2929, 1727, 755 cm^{-1}; HREIMS: *m/z* [M]$^+$ 388.1881 (calcd for $C_{22}H_{28}O_6$, 388.1886); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 14; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 14.

2.4.2 Investigation of the crude methylene chloride extract from the heartwood of *C. sappan*



* Not further investigated

Scheme 5 Isolation of compounds **DH1-DH6** from the heartwood of *C. sappan*

A portion of crude CH_2Cl_2 extract (55.2 g) was further purified by QCC using CH_2Cl_2 as eluent and increasing polarity with EtOAc and acetone to give seven fractions (H1-H7, Scheme 5).

Fraction H3 (1.3 g) was further purified by CC with EtOAc-CH_2Cl_2 (3:17, v/v) to afford ten subfractions (H3a-H3h) and to give **DH3** (50.0 mg, $R_f = 0.47$ (3:7, acetone-CH_2Cl_2)) and **DH4** (10.0 mg, $R_f = 0.59$ (3:2, EtOAc-hexane)). Subfraction H3e (164.3 mg) was separated by CC with EtOAc-hexane (2:3, v/v) and followed by CC RP-18 with MeOH-H_2O (2:3, v/v) to give **DH1** (8.0 mg, $R_f = 0.42$ (1:1, acetone-hexane)) and **DH6** (15.0 mg, $R_f = 0.25$ (1:1, acetone-hexane)).

Fraction H6 (5.9 g) was separated by QCC with acetone-CH_2Cl_2 (1:4, v/v). The major component of this fraction, **DH5** (170.0 mg, $R_f = 0.27$ (3:2, EtOAc-hexane)) and the minor component (483.2 mg) was purified by CC with EtOAc-hexane (1:1, v/v) to give **DH2** (15.0 mg, $R_f = 0.21$ (3:7, acetone-CH_2Cl_2)) and **DH3** (20.0 mg).

Compound DH1: White solid, mp: 194-195 °C; $[\alpha]^{27}_{D}$: - 12.6° (c = 0.62, MeOH); UV (MeOH) λ_{max} (log ε): 228 (4.19), 277 (4.19), 312 (3.92) nm; IR (neat) υ_{max}: 3364, 1657, 1606 cm^{-1}; ^1H NMR (CD_3OD + $CDCl_3$, 300 MHz), see Table 16; ^{13}C NMR (CD_3OD + $CDCl_3$, 75 MHz), see Table 16.

Compound DH2: Viscous oil; $[\alpha]^{27}_{D}$: - 24.2° (c = 0.75, MeOH); UV (MeOH) λ_{max} (log ε): 221 (4.90), 280 (4.49), 285 (4.47) nm; IR (neat) υ_{max}: 3380, 1619 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz), see Table 19; ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 75 MHz), see Table 19.

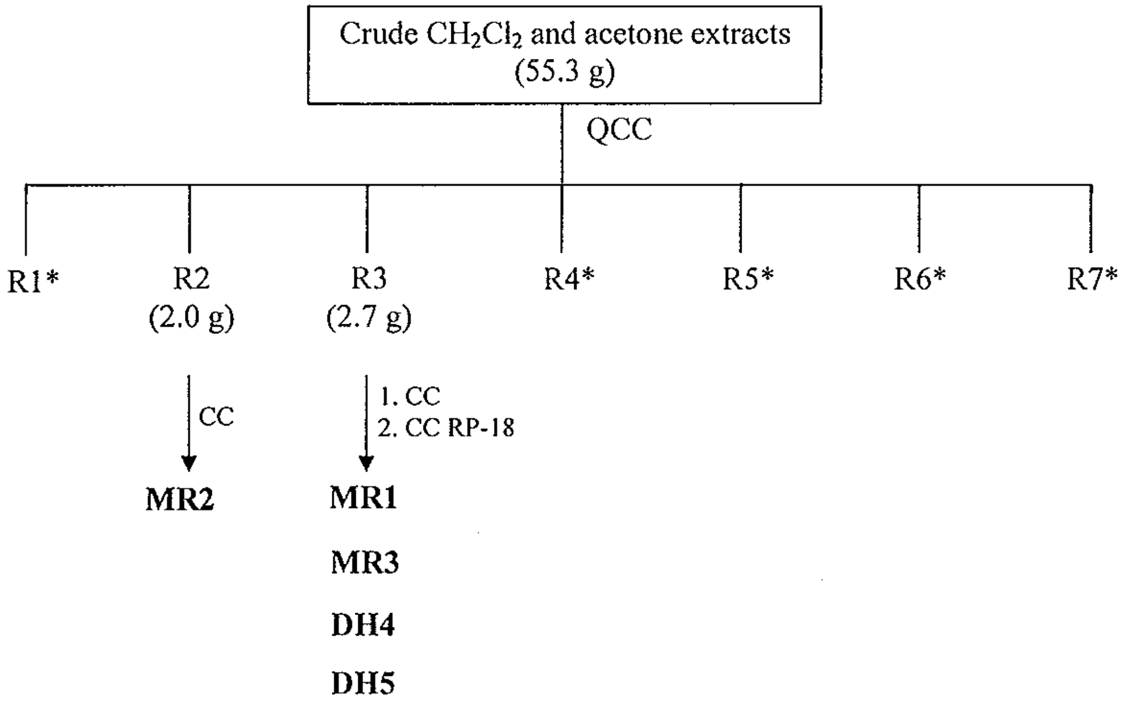
Compound DH3: Viscous oil; $[\alpha]^{27}_{D}$: + 13.8° (c = 5.23, MeOH); UV (MeOH) λ_{max} (log ε): 280 (2.44), 284 (2.43) nm; IR (neat) υ_{max}: 3390, 1622 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz), see Table 22; ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 75 MHz), see Table 22.

Compound DH4: Viscous oil; $[\alpha]^{27}_{D}$: – 13.4° (c = 2.65, MeOH); UV (MeOH) λ_{max} (log ε): 221 (4.18), 278 (3.82), 284 (3.82) nm; IR (neat) υ_{max}: 3386, 1622 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz), see Table 25; ^{13}C NMR (acetone-d_6, 75 MHz), see Table 25.

Compound DH5: Viscous oil; $[\alpha]^{27}_{D}$: + 70.0° (c = 3.64, MeOH); UV (MeOH) λ_{max} (log ε): 284 (3.78), 288 (3.76) nm; IR (neat) υ_{max}: 3360, 1618 cm^{-1}; ^{1}H NMR (acetone-d_6, 300 MHz), see Table 27; ^{13}C NMR (acetone-d_6, 75 MHz), see Table 27.

Compound DH6: Viscous oil; UV (MeOH) λ_{max} (log ε): 246 (3.85), 280 (3.70) nm; IR (neat) υ_{max}: 3393, 1655, 1604 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz), see Table 31; ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 75 MHz), see Table 31.

2.4.3 Investigation of the crude methylene chloride and acetone extracts from the roots of *C. sappan*



* Not further investigated

Scheme 6 Isolation of compounds **MR1-MR3, DH4, and DH5** from the roots of *C. sappan*

The Crude CH_2Cl_2 and acetone extracts gave similar chromatogram so they were combined and further purified by QCC using CH_2Cl_2 as eluent and increasing polarity with acetone to give seven fractions (R1-R7, Scheme 6).

Fraction R2 (2.0 g) was further purified by CC with EtOAc-CH_2Cl_2 (1:4, v/v) to give five subfractions (R2a-R2e). Subfraction R2b (140.2 mg) was separated by CC with acetone-CH_2Cl_2 (1:19 v/v) to give **MR2** (8.0 mg, $R_f = 0.53$ (1:1, Acetone-hexane)).

Fraction R3 (2.7 g) was separated by CC with acetone-hexane (2:3, v/v) to afford seven subfractions (R3a-R3g). Subfraction R3b (200.2 mg) was purified by CC with EtOAc-hexane (2:3, v/v) to give **DH4** (16.0 mg). Subfraction R3e (838.4 mg) was separated by CC RP-18 with MeOH-H_2O (2:3, v/v) to give **DH5** (15.0 mg), **MR1** (6.0 mg, $R_f = 0.16$ (3:2, EtOAc-hexane)) and **MR3** (34.0 mg, $R_f = 0.39$ (1:1, Acetone-hexane)).

Compound MR1: Viscous oil; $[\alpha]^{27}_{D}$: – 24.3° (c = 0.61, MeOH); UV (MeOH) λ_{max} (log ε): 282 (3.73), 284 (3.70) nm; IR (neat) υ_{max}: 3375, 1607 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz), see Table 34; ^{13}C NMR (acetone-d_6, 75 MHz), see Table 34.

Compound MR2: Yellow solid, mp: 228-230 °C; UV (MeOH) λ_{max} (log ε): 237 (4.08), 351 (4.36) nm; IR (neat) υ_{max}: 3390, 1601 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz), see Table 37; ^{13}C NMR (acetone-d_6, 75 MHz), see Table 37.

Compound MR3: Viscous oil; UV (MeOH) λ_{max} (log ε): 231 (3.87), 266 (3.87), 312 (3.86), 361 (3.86) nm; IR (neat) υ_{max}: 3375, 1603 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz), see Table 39; ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 75 MHz), see Table 39.

2.5 Bioassay

2.5.1 Anti-allergic activity assay

2.5.1.1 Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells

Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells (purchased from ATCC) were evaluated by the following method (Matsuda *et al.*, 2004) with some modifications. Briefly, RBL-2H3 cells were dispensed in 24-well plates at a concentration of 2×10^5 cells/well using Minimum Essential Medium Eagle (MEM) containing 10% fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 unit/ml) and anti-dinitrophenyl-immunoglobulin E (anti-DNP IgE) (0.45 μg/ml), then incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. The cells were washed twice with 500 μl of Siraganian buffer [119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM piperazine-*N*,*N'*-bis(2-ethanesulfonic acid) (PIPES), 0.1 % bovine serum albumin (BSA) and 40 mM NaOH, pH 7.2] and then incubated in 160 μl of Siraganian buffer for an additional 10 min at 37 °C. After that, 20 μl of test sample solution was added to each well and incubated for 10 min, followed by addition of 20 μl of antigen (DNP-BSA, final concentration is 10 μg/ml) at 37 °C for 20 min to stimulate the cells to degranulate. The supernatant was transferred into a 96-well plate and incubated with 50 μl of substrate (1mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 2 h. The reaction was stopped by adding 200 μl of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to Siraganian buffer (final DMSO concentration was 0.1 %). The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated by the following equation, and IC_{50} values were determined graphically:

Inhibition % = [1– (T–B–N)/(C–N)] × 100

Control (C): DNP-BSA (+), Test sample (–); Test (T): DNP-BSA (+), Test sample (+); Blank (B): DNP-BSA (–), Test sample (+); Normal (N): DNP-BSA (–), Test sample (–)

2.5.1.2 β-Hexosaminidase inhibitory activity

The following assay was carried out in order to clarify that the anti-allergic effects of samples were due to the inhibition of β-hexosaminidase release, and not from the inhibition of β-hexosaminidase activity.

The cell suspension (5×10^6 cells) in 10 ml of PBS (phosphate buffer saline) was sonicated. The solution was then centrifuged; and the supernatant was diluted with Siraganian buffer and adjusted to equalize the enzyme activity of the degranulation tested above. The enzyme solution (45 μl) and test sample solution (5 μl) were transferred into a 96-well microplate and incubated with 50 μl of the substrate solution at 37 °C for 2 h. The reaction was stopped by adding 200 μl of the stop solution and the absorbance was measured using a microplate reader at 405 nm. The results were expressed as mean ± S.E.M of four determinations at each concentration for each sample. The IC_{50} values were calculated using the Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

2.5.2 Cytotoxic assay

The procedure for cytotoxic assay was performed by the sulphorhodamine B (SRB) assay as descried by Skehan *et al.*, (Skehan *et al.*, 1990). In this study, four cancer cell lines obtained from National Cancer Institute, Bangkok, Thailand, were used including MCF-7 (breast adenocarcinoma), KB (human oral cancer), HeLa (Human cervical cancer) and HT-29 (colon cancer). Camptothecin which were used as a standard showed cytotoxic activity in the range of 0.2-2.0 μg/ml.

2.6 X-ray crystallographic analysis of phanginin A (DS1)

$C_{21}H_{28}O_5$, $M = 360.43$, orthorhombic, $P2_12_12_1$, $a = 7.5063(5)$ Å, $b = 8.1013(5)$ Å, $c = 30.554(2)$ Å, $\alpha = \beta = \gamma = 90$ °, $V = 1858.0(2)$ Å^3, $Z = 4$, $T = 100.0(1)$ K. Two thousand five hundred and ninety one independent reflections (2591 independent, $R_{int} = 0.057$) were collected. Largest electron density residue: 0.39 eÅ^{-3}, R_1(for $I > 2\sigma(I) = 0.0560$ and $wR_2 = 0.1854$ (all data) with $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $wR_2 = (\Sigma w(F^2_o - F^2_c)^2 / \Sigma w(F^2_o)^2)^{0.5}$. Data were collected on a Bruker SMART APEX2 CCD area detector diffractometer with MoK$_\alpha$ radiation ($\lambda = 0.71073$ Å) equipped with an Oxford Cryosystem Cobra low-temperature attachment. Cell refinement: *APEX2*; data reduction: *SAINT* (Bruker, 2005); programs used to solve structures: *SHELXTL* (Sheldrick, 1998); molecular graphics: *SHELXTL*; softwares used to prepare material for publication: *SHELXTL* and *PLATON* (Spek, 2003). The structure was solved by direct methods (*SHELXTL*, (Sheldrick, 1998)) and all non-hydrogen atoms were refined anisotropically using the least-squares method on F^2 (*SHELXTL*, (Sheldrick, 1998)). Hydroxyl H and H atom attached to C9 were located from the difference map and isotropically refined. The remaining H atoms were positioned geometrically and allowed to ride on their parent atoms, with C-H distances in the range 0.93-0.97 Å. The U_{iso} values were constrained to be $1.5U_{eq}$ of the carrier atom for methyl H atoms and $1.2U_{eq}$ for the remaining H atoms.

The crystallographic data for the structure of **DS1** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication (Deposition No.CCDC 650908). This data can be obtained free of charge, by request to the Director, via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; email deposit@ccdc.cam.ac.uk).

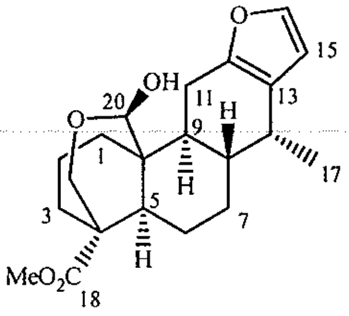
CHAPTER 3

RESULT AND DISCUSSION

3.1 Structural elucidation of compounds from the seeds of *C. sappan*

The crude methylene chloride extract from the seeds of *C. sappan* was subjected to chromatography and/or crystallization to give new cassane-type diterpenes, **DS1-DS11**. The basic skeleton of all compounds was identified to be a furanoditerpene on the basis of UV λ_{max} 211-225 nm (Cheenpracha *et al.*, 2005) and a positive Ehrlich test (Kuroda *et al.*, 2004). In addition, the IR spectrum of these compounds displayed carbonyl ester (1717-1731 cm^{-1}) functionality. The ^{1}H NMR spectral data of all compounds showed characteristic of 1,2-disubstituted furan ring as signals between δ 6.15 and 7.35 (each d) with coupling constants between 1.2 and 1.8 Hz. Also, the proton signals of singlets between δ 3.64 – 3.77 and doublets between δ 0.94 – 1.01 were assigned as methyl ester groups of 18-OMe and aliphatic methyl groups at C-17, respectively. Their structures were determined using 1D and 2D NMR spectroscopic data. All carbons were assigned by ^{13}C NMR, HMQC and HMBC data. In addition, the structure of **DS1** was confirmed by X-ray diffraction technique.

3.1.1 Compound DS1



Compound **DS1** was obtained as a white powder which was further recrystallized from CH_2Cl_2 to yield colorless crystals. The subsequent X-ray structure (Figure 2) confirms a pentacyclic furano cassane-type diterpene framework with a molecular formula $C_{21}H_{28}O_5$ ($[M]^+$ *m/z* 360.1949 obtained from HREIMS). This structure was in agreement with the ^1H and ^{13}C NMR spectral data (Table 2, Figures 5 and 6). The ^{13}C NMR spectral data displayed 21 carbons including those of an ester carbonyl carbon at δ 175.8 (C-18), and a 1,2-disubstituted furan ring moiety at δ 109.8 (C-15), 122.4 (C-13), 140.3 (C-16) and 149.5 (C-12). The ^1H NMR spectral data displayed the presence of two AB systems. One of them was shown at δ 2.60 (dd, J = 15.9, 11.7 Hz, H-11α) and 2.72 (dd, J = 15.9, 5.7 Hz, H-11β), and the other as the oxymethylene protons at δ 3.69 and 4.37 (each d, J = 11.7 Hz, 2H-19). The lower field signal at δ 5.01 (s) was deduced to be a dioxymethine proton H-20 whose HMBC spectrum showed correlations with carbons at δ 38.6 (C-10), 45.2 (C-5) and 61.6 (C-19). The oxymethylene protons at δ 3.69 and 4.37 (2H-19) showed correlations with carbons at δ 35.5 (C-3), 45.2 (C-5) and 97.2 (C-20). These data suggested an ether bridge between C-19 and C-20. The relative stereochemistry of a pyran ring was determined on the basis of X-ray diffraction analysis and NOESY experiments whose cross-peaks of a dioxymethine proton at δ 5.01 (H-20) with protons at δ 2.02 (H-1β) and 2.72 (H-11β) suggested β-orientation of a dioxymethine proton H-20 and in an equatorial position. From these data, **DS1** was concluded to be phanginin A, a new compound (Yodsaoue *et al.*, 2008).

Table 2 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS1**

Position	δ_H (mult, J, Hz)	δ_C	DEPT	HMBC
1*α*	1.24 (m)	37.8	CH_2	3, 5
β	2.02 (m)			
2	1.52 (m), 1.62 (m)	20.9	CH_2	3, 4
3*α*	1.87 (m)	35.5	CH_2	1, 4, 5, 6, 19
β	1.98 (m)			
4		45.6	C	
5	1.62 (m)	45.2	CH	1, 10, 19, 20
6*α*	1.19 (m)	23.5	CH_2	4
β	2.23 (m)			
7	1.36 (m), 1.70 (m)	29.6	CH_2	5, 6
8	2.38 (m)	36.6	CH	9, 14, 17
9	1.45 (m)	42.6	CH	1, 8, 10, 14, 20
10		38.6	C	
11*α*	2.60 (dd, J = 15.9, 11.7)	22.5	CH_2	8, 9, 12, 13
β	2.72 (dd, J = 15.9, 5.7)			
12		149.5	C	
13		122.4	C	
14	2.58 (m)	31.5	CH	8, 9, 12, 13, 15, 17
15	6.17 (br s)	109.8	CH	12, 13, 14, 16
16	7.19 (br s)	140.3	CH	12, 13, 15
17	0.96 (d, J = 6.9)	16.8	CH_3	8, 13, 14
18		175.8	C	
19*ax*	4.37 (d, J = 11.7)	61.6	CH_2	3, 5, 20
eq	3.69 (d, J = 11.7)			
20	5.01 (s)	97.2	CH	5, 10, 19
18-OMe	3.65 (s)	51.5	CH_3	18

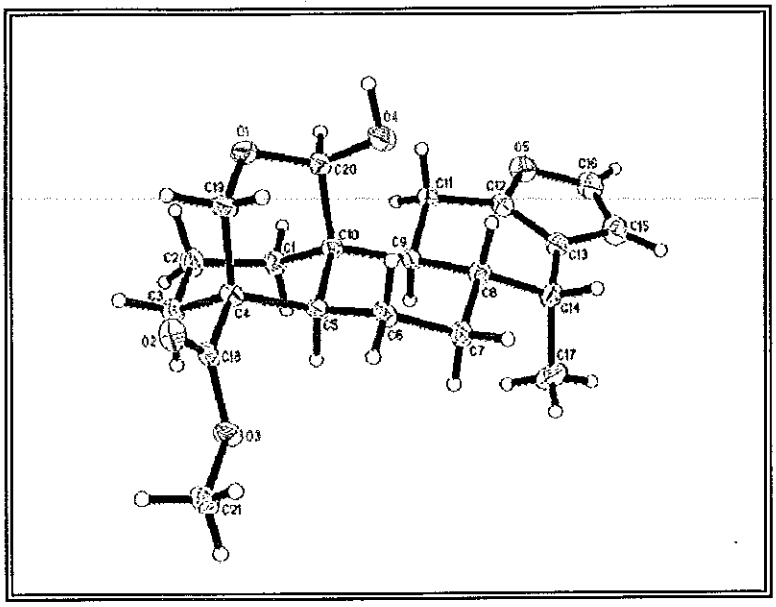
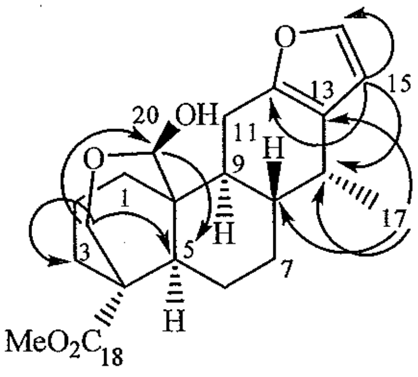
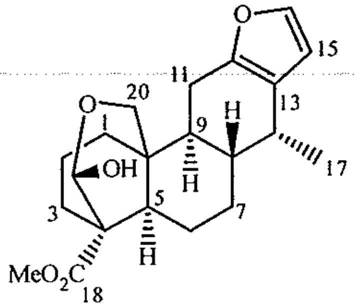


Figure 2 ORTEP drawing of compound **DS1**

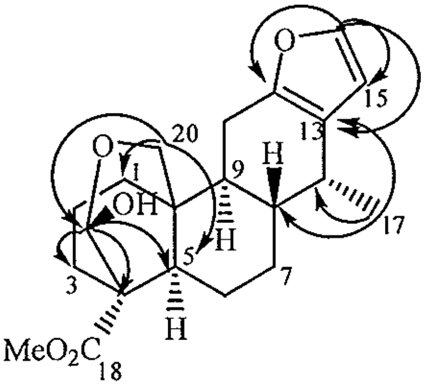


Selective HMBC correlation of **DS1**

3.1.2 Compound DS2



Compound **DS2** $[M]^+$ *m/z* 360.1927 ($C_{21}H_{28}O_5$) by HREIMS, exhibited a hydroxyl functionality at 3394 cm^{-1} in IR spectrum. The ^1H and ^{13}C NMR spectral data (Table 3, Figures 12 and 13) were closely related to those of **DS1**. The difference was in the position of OH which was placed at C-20 in **DS1** (H-20: δ_H 5.01 (s), δ_C 97.2) but C-19 in 2 (H-19: δ_H 5.33 (s), δ_C 94.8). The HMBC correlations of an oxymethine proton at δ 5.33 (H-19) with carbons at δ 36.9 (C-3), 44.0 (C-5), 49.1 (C-4) and 63.5 (C-20) confirmed the location of OH at C-19. The relative stereochemistry of a pyran ring was analyzed by NOESY correlations, the dioxymethine proton at δ 5.33 (H-19) showed a cross-peak with the proton at δ 1.96 (H-3*β*) and no cross-peak with 2H-20 suggesting equatorial orientation of H-19. Therefore, **DS2** was determined to be phanginin B, a new compound (Yodsaoue *et al.*, 2008).

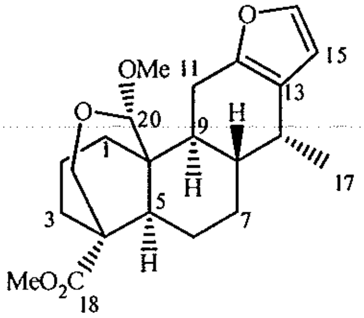


Selective HMBC correlation of **DS2**

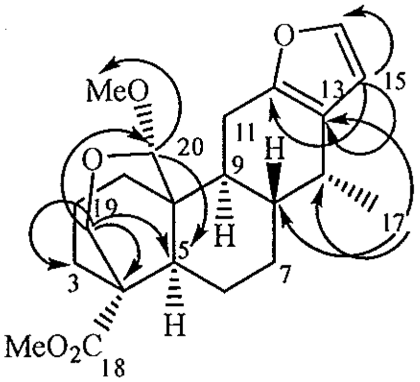
Table 3 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS2**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
1*α*	1.23 (m)	38.4	CH_2	5, 10
β	2.19 (m)			
2	1.64 (m)	21.1	CH_2	
3*α*	1.62 (m)	36.9	CH_2	4, 5
β	1.96 (m)			
4		49.1	C	
5	1.70 (m)	44.0	CH	4, 19
6	1.84 (m)	23.3	CH_2	
7	1.65 (m)	30.8	CH_2	
8	1.62 (m)	37.3	CH	10
9	1.52 (m)	42.0	CH	10, 11, 14, 20
10		36.0	C	
11*α*	2.27 (m)	22.6	CH_2	8, 9, 13
β	2.72 (dd, *J* = 16.2, 5.7)			
12		149.2	C	
13		122.2	C	
14	2.62 (br q, *J* = 7.2)	31.7	CH	8, 9, 12, 13, 17
15	6.17 (d, *J* = 1.5)	109.6	CH	12, 13
16	7.22 (d, *J* = 1.5)	140.6	CH	12, 13, 15
17	0.97 (d, *J* = 7.2)	17.2	CH_3	8, 13, 14
18		174.7	C	
19	5.33 (s)	94.8	CH	3, 4, 5, 20
20*ax*	4.16 (dd, *J* = 11.4, 2.4)	63.5	CH_2	1, 5, 10, 19
eq	3.56 (d, *J* = 11.4)			
18-OMe	3.64 (s)	51.7	CH_3	18

3.1.3 Compound DS3



Compound **DS3** had a molecular formula $C_{22}H_{30}O_5$, ($[M]^+$ *m/z* 374.2116), based on HREIMS which was 14 mass units more than that of **DS1**, suggesting the addition of a Me group. The ^1H and ^{13}C NMR spectra (Table 4, Figures 14 and 15) of **DS3** displayed characteristics similar to those of **DS1**, except for the presence of an additional methoxyl group at δ_H 3.53 (s) in **DS3**. This signal correlated with carbon at δ 101.1 (C-20) in the HMQC experiment, suggesting the OMe group at C-20. In the NOESY spectrum, the dioxymethine proton at δ 5.02 (H-20) displayed a cross-peak with protons at δ 1.76 (H-6*β*), 3.53 (20-OMe) and 4.03 (H-19*ax*), indicating that this dioxymethine proton was in axial orientation. Thus the structure of phanginin C was concluded to be **DS3**, a new compound (Yodsaoue *et al.*, 2008).

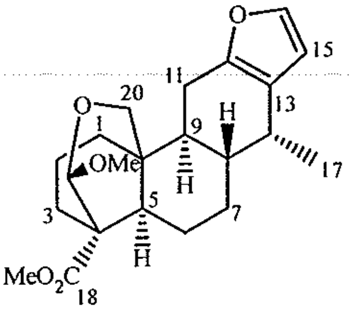


Selective HMBC correlation of **DS3**

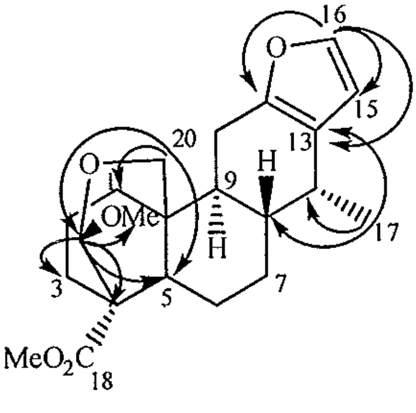
Table 4 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS3**

Position	δ_H (mult, *J*, Hz)	δ_C	DEPT	HMBC
1*α*	1.88 (ddd, *J* = 13.8, 6.3, 1.2)	29.0	CH_2	2, 9, 5, 10, 20
β	2.22 (m)			
2	1.22 (m), 1.62 (m)	21.0	CH_2	4, 10
3*α*	1.31 (m)	38.4	CH_2	2, 4, 5, 18, 19
β	2.20 (m)			
4		49.4	C	
5	1.71 (m)	47.9	CH	4, 7, 9, 10
6*α*	1.35 (m)	23.1	CH_2	4, 7, 8, 10
β	1.76 (m)			
7	1.35 (m), 1.73 (m)	30.0	CH_2	5, 9
8	1.70 (m)	36.3	CH	9, 10, 11, 17
9	1.57 (m)	41.3	CH	8, 10, 11, 14
10		35.4	C	
11*α*	2.25 (m)	22.5	CH_2	8, 9, 13
β	2.73 (dd, *J* = 16.2, 6.0)			
12		148.9	C	
13		122.1	C	
14	2.64 (br q, *J* = 6.9)	31.5	CH	8, 9, 12, 13, 17
15	6.17 (d, *J* = 1.8)	109.5	CH	12, 13, 16
16	7.22 (d, *J* = 1.8)	140.6	CH	12, 13, 15
17	0.95 (d, *J* = 6.9)	17.1	CH_3	8, 13, 14
18		176.5	C	
19*ax*	4.03 (dd, *J* = 11.7, 2.7)	66.9	CH_2	3, 4, 5, 20
eq	3.81 (d, *J* = 11.7)			
20	5.02 (s)	101.1	CH	1, 10, 20-OMe
18-OMe	3.69 (s)	51.6	CH_3	18
20-OMe	3.53 (s)	57.4	CH_3	20

3.1.4 Compound DS4



Compound **DS4** was found to have a molecular formula $C_{22}H_{30}O_5$, ($[M]^+$ *m/z* 374.2110), by HREIMS. The ^1H and ^{13}C NMR spectral data (Table 5, Figures 16 and 17) of **DS4** were similar to those of **DS2** with an additional OMe signal at δ_H 3.35 which showed HMBC correlation with C-19 (δ 102.6). The relative stereochemistry of a pyran ring was determined from the results of NOESY experiments and comparison with **DS2**. The dioxymethine proton at δ 4.76 (H-19) showed a cross-peak with protons at δ 1.92 (H-3*β*) and 3.35 (19-OMe) suggesting an equatorial orientation of H-19. Therefore, the structure of phanginin D was assigned as **DS4**, a new compound (Yodsaoue *et al.*, 2008).

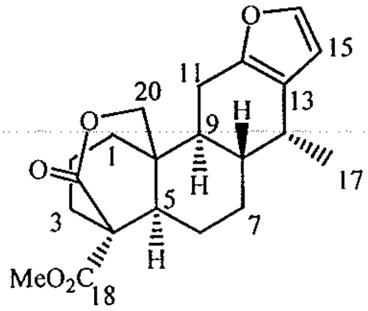


Selective HMBC correlation of **DS4**

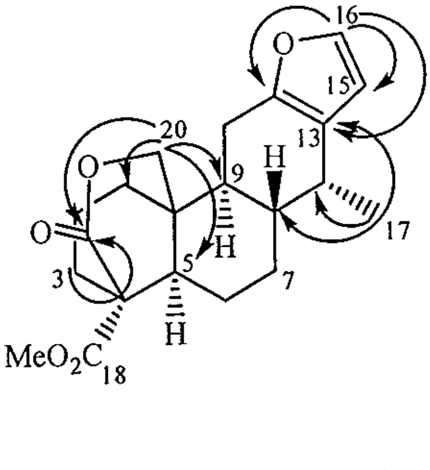
Table 5 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS4**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
1*α*	1.21 (m)	38.4	CH_2	3, 20
β	2.18 (m)			
2	1.57 (m)	21.1	CH_2	4, 10
3*α*	1.62 (m)	36.2	CH_2	1, 5, 19, 18
β	1.92 (m)			
4		49.7	C	
5	1.73 (dd, *J* = 12.3, 3.3)	44.2	CH	3, 4, 7, 9, 18, 19, 20
6*α*	1.88 (m)	23.8	CH_2	4, 5, 8, 9
β	2.07 (m)			
7	1.33 (m), 1.67 (m)	30.7	CH_2	5, 8, 14
8	1.73 (m)	37.0	CH	6, 11, 14, 17
9	1.52 (dt, *J* = 11.7, 5.7)	42.0	CH	1, 5, 8, 10, 14, 20
10		36.0	C	
11*α*	2.24 (m)	22.5	CH_2	8, 9, 12, 13
β	2.71 (dd, *J* = 16.2, 5.7)			
12		149.0	C	
13		122.2	C	
14	2.62 (m)	31.6	CH	8, 9, 12, 13, 15, 17
15	6.17 (d, *J* = 1.8)	109.6	CH	12, 13, 16
16	7.21 (d, *J* = 1.8)	140.5	CH	12, 13, 15
17	0.96 (d, *J* = 6.9)	17.2	CH_3	8, 13, 14
18		174.9	C	
19	4.76 (s)	102.6	CH	3, 4, 5, 20, 19-OMe
20*ax*	4.04 (dd, *J* = 11.1, 2.4)	62.7	CH_2	1, 5, 10, 19
eq	3.54 (d, *J* = 11.1)			
18-OMe	3.70 (s)	51.5	CH_3	18
19-OMe	3.35 (s)	55.5	CH_3	19

3.1.5 Compound DS5



Compound **DS5** $C_{21}H_{26}O_5$ $[M]^+$ at *m/z* 358.1744 by HREIMS, was 2 mass units less than that of **DS2**. The ^{1}H and ^{13}C NMR spectral data (Table 6, Figures 18 and 19) resembled those of **DS2**, except that the signal of the dioxymethine proton of **DS2** at δ_H 5.33 (δ_C 94.8, C-19) was replaced by a carbonyl carbon at δ_C 170.5. This finding was supported by HMBC spectrum, in which the oxymethylene protons of 2H-20 at δ 4.24 (dd, J = 11.7, 0.9 Hz) and 4.67 (dd, J = 11.7, 2.4 Hz) were correlated with carbons at δ 35.4 (C-10), 38.1 (C-1), 40.9 (C-9), 46.7 (C-5) and 170.5 (C-19). Thus, the structure of **DS5** was assigned as phanginin E, a new compound (Yodsaoue *et al.*, 2008).

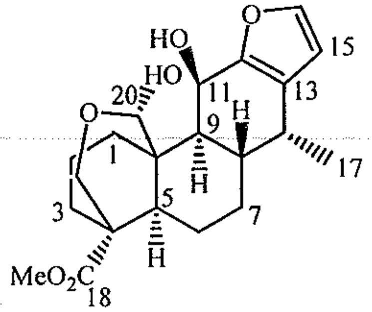


Selective HMBC correlation of **DS5**

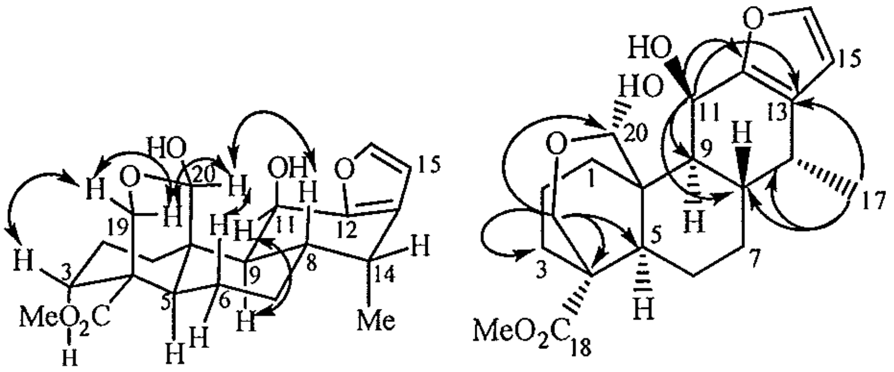
Table 6 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS5**

Position	δ_H (mult, *J*, Hz)	δ_C	DEPT	HMBC
1*α*	1.40 (m)	38.1	CH_2	
β	2.20 (m)			
2	1.92 (m)	20.0	CH_2	
3	2.14 (m)	34.8	CH_2	1, 4, 5, 19
4		55.9	C	
5	1.73 (m)	46.7	CH	1, 4, 10, 20
6*α*	1.43 (m)	25.2	CH_2	
β	1.62 (m)			
7	1.80 (m)	29.1	CH_2	
8	1.90 (m)	36.0	CH	9, 11
9	1.70 (m)	40.9	CH	1, 10, 11, 14, 20
10		35.4	C	
11*α*	2.07 (m)	22.6	CH_2	8, 9, 13, 12
β	2.78 (dd, *J* = 16.2, 5.7)			
12		147.9	C	
13		122.5	C	
14	2.71 (br q, *J* = 7.2)	31.3	CH	8, 9, 12, 13, 17
15	6.18 (d, *J* = 1.8)	109.5	CH	12, 13
16	7.23 (d, *J* = 1.8)	141.0	CH	12, 13, 15
17	0.99 (d, *J* = 7.2)	17.2	CH_3	8, 13, 14
18		172.0	C	
19		170.5	C	
20*ax*	4.67 (dd, *J* = 11.7, 2.4)	73.6	CH	1, 5, 9, 10, 19
eq	4.24 (dd, *J* = 11.7, 0.9)			
18-OMe	3.77 (s)	52.3	CH_3	18

3.1.6 Compound DS6



Compound **DS6** with molecular formula $C_{21}H_{26}O_5$ $[M-H_2O]^+$ whose molecular weight was assigned at *m/z* 358.1765 by HREIMS. The NMR data (Table 7, Figures 20 and 21) of **DS6** displayed similarities with **DS1**. The ^{13}C NMR spectra exhibited a couple of oxymethine carbons at δ 71.1 and 104.5 assigning to C-11 and C-20, respectively. The ^{1}H NMR signal of H-11 was displayed as a doublet at δ 5.19 (J = 4.5 Hz) whose HMBC spectrum showed correlations to carbons at δ 38.5 (C-8), 43.4 (C-9), 129.0 (C-13) and 148.0 (C-12). The relative stereochemistry of **DS6** was determined on the basis of coupling constants and the results of NOESY experiments. The small J values for H-9 and H-11 (J = 4.5 Hz) indicated that H-11 should be an equatorial proton. In addition, the oxymethine proton at δ 5.19 (H-11) showed a cross-peak with the protons at δ 1.85 (H-9*α*) and 2.28 (H-1*β*) and the dioxymethine proton at δ 5.47 (H-20) with protons at δ 1.70 (H-6*β*), 2.07 (H-8*β*) and 4.22 (H-19*ax*) in NOESY experiment confirming *α*-orientation of H-11 and axial position of H-20. Thus phanginin F was characterized as **DS6**, a new compound (Yodsaoue *et al.*, 2008).



Selected NOESY cross-peak and HMBC correlation for compound **DS6**

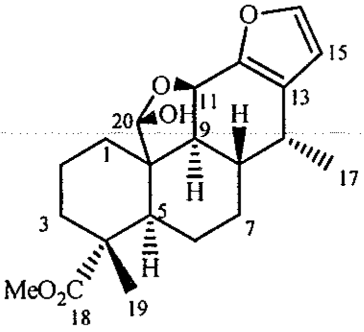
Table 7 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS6**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
1*α*	1.37 (m)	33.9	CH$_2$	2, 3, 5, 10, 20
β	2.28 (m)			
2	1.68 (m), 2.60 (m)	20.4	CH$_2$	1, 3, 10
3*α*	2.06 (m)	37.7	CH$_2$	1, 2, 5, 19
β	2.18 (m)			
4		45.0	C	
5	2.18 (dd, *J* = 11.4, 6.3)	46.3	CH	1, 3, 4, 10, 19, 20
6*α*	1.60 (m)	23.8	CH$_2$	4, 5, 8, 10
β	1.70 (m)			
7	1.32 (m), 1.72 (m)	28.2	CH$_2$	5, 8, 9
8	2.07 (m)	38.5	CH	10, 11, 17
9	1.85 (dd, *J* = 12.3, 4.5)	43.4	CH	1, 8, 10, 11, 14, 20
10		40.9	C	
11	5.19 (d, *J* = 4.5)	71.1	CH$_2$	8, 9, 12, 13
12		148.0	C	
13		129.0	C	
14	2.71 (qd, *J* = 7.2, 4.2)	31.8	CH	8, 9, 12, 13, 15, 17
15	6.22 (d, *J* = 1.8)	109.2	CH	12, 13
16	7.35 (d, *J* = 1.8)	143.2	CH	12, 13, 15
17	0.96 (d, *J* = 7.2)	14.0	CH$_3$	8, 13, 14
18		174.4	C	
19*ax*	4.22 (dd, *J* = 12.6, 3.0)	70.0	CH$_2$	3, 4, 5, 20
eq	4.07 (d, *J* = 12.6)			
20	5.47 (s)	104.5	CH	1, 5, 10
18-OMe	3.68 (s)	51.6	CH$_3$	18

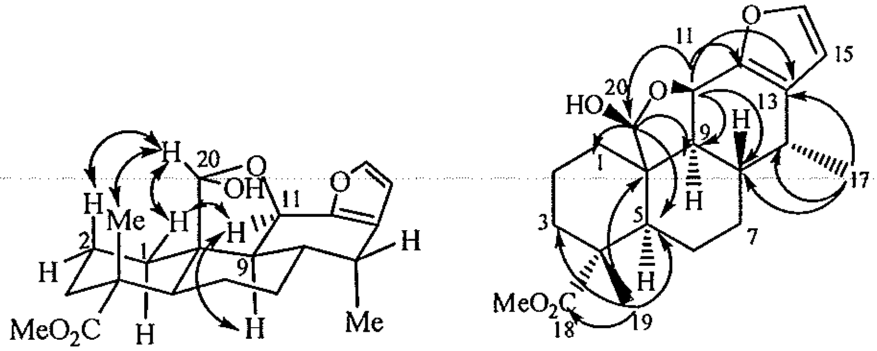
Table 8 Comparison of ^{13}C NMR spectral data of compounds **DS1-DS6**

Position	DS1	DS2	DS3	DS4	DS5	DS6
1	37.8	38.4	29.0	38.4	38.1	33.9
2	20.9	21.1	21.0	21.1	20.0	20.4
3	35.5	36.9	38.4	36.2	34.8	37.7
4	45.6	49.1	49.4	49.7	55.9	45.0
5	45.2	44.0	47.9	44.2	46.7	46.3
6	23.5	23.3	23.1	23.8	25.2	23.8
7	29.6	30.8	30.0	30.7	29.1	28.2
8	36.6	37.3	36.3	37.0	36.0	38.5
9	42.6	42.0	41.3	42.0	40.9	43.4
10	38.6	36.0	35.4	36.0	35.4	40.9
11	22.5	22.6	22.5	22.5	22.6	71.1
12	149.5	149.2	148.9	149.0	147.9	148.0
13	122.4	122.2	122.1	122.2	122.5	129.0
14	31.5	31.7	31.5	31.6	31.3	31.8
15	109.8	109.6	109.5	109.6	109.5	109.2
16	140.3	140.6	140.6	140.5	141.0	143.2
17	16.8	17.2	17.1	17.2	17.2	14.0
18	175.8	174.7	176.5	174.9	172.0	174.4
19	61.6	94.8	66.9	102.6	170.5	70.0
20	97.2	63.5	101.1	62.7	73.6	104.5
18-OMe	51.5	51.7	51.6	51.5	52.3	51.6
19-OMe				55.5		
20-OMe			57.4			

3.1.7 Compound DS7



Compound **DS7** had the molecular formula $C_{21}H_{28}O_5$, ($[M]^+$ *m/z* 360.1916), based on HREIMS. The IR absorption exhibited hydroxyl functionality at 3395 cm^{-1}. The ^1H and ^{13}C NMR spectral data (Table 9, Figures 22 and 23) of **DS7** and **DS6** were comparable, except that the methylene proton signals of 2H-19 at δ 4.22 (dd, *J* = 12.6, 3.0 Hz) and 4.07 (d, *J* = 12.6 Hz) in **DS6** were replaced by a singlet methyl proton signal at δ 1.25 (Me-19). The latter protons showed HMBC correlations with carbons at δ 37.1 (C-3), 48.1 (C-4), 48.5 (C-5) and 179.0 (C-18), suggesting its location at C-4. The ^1H NMR signal of a dioxymethine proton H-20 at δ 5.52 (s); δ_C 103.3 showed HMBC correlations with carbons at δ 36.0 (C-1), 47.9 (C-10), 48.5 (C-5), 48.7 (C-9) and 69.3 (C-11). The oxymethine proton H-11 (δ_H 4.85, d, *J* = 4.8 Hz; δ_C 69.3) showed HMBC correlations with carbons at δ 36.2 (C-8), 48.7 (C-9), 103.3 (C-20), 128.0 (C-13) and 147.0 (C-12). These data suggested an ether bridge between C-20 and C-11 (forming a tetrahydrofuran skeleton). The small *J* value for H-11 (*J* = 4.8 Hz) indicated that H-11 should be an equatorial proton. In the NOESY spectrum, the dioxymethine proton at δ 5.52 (H-20) correlate with the methyl protons at δ 1.25 (3H-19) and protons at δ 1.70 (H-2*β*) and 1.92 (H-1*β*), and the oxymethine proton at δ 4.85 (H-11) displayed a cross-peak with protons at δ 1.79 (H-9*α*) and 1.92 (H-1*β*) confirming that protons H-20 and H-11 of a tetrahydrofuran ring were in *cis* configuration. Thus, phanginin G was concluded to be **DS7**, a new compound (Yodsaoue *et al.*, 2008).



Selected NOESY cross-peak and HMBC correlation for compound **DS7**

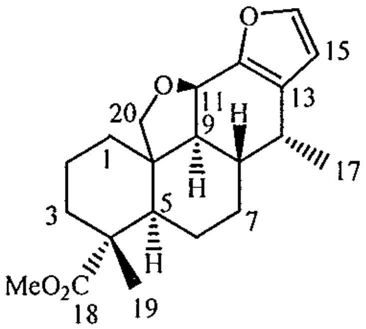
Table 9 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS7**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
1*α*	1.22 (m)	36.0	CH$_2$	2, 5, 9, 10, 20
β	1.92 (m)			
2	1.70 (m)	19.0	CH$_2$	1, 4
3	1.64 (m)	37.1	CH$_2$	
4		48.1	C	
5	2.20 (br d, *J* = 12.0)	48.5	CH	3, 4, 5, 7, 10, 19
6*α*	1.14 (m)	24.9	CH$_2$	5
β	2.10 (m)			
7	1.39 (m), 1.68 (m)	29.9	CH$_2$	5
8	2.69 (m)	36.2	CH	7, 9, 13, 14, 17
9	1.79 (dd, *J* = 12.0, 4.8)	48.7	CH	1, 5, 8, 14, 20
10		47.9	C	
11*α*	4.85 (d, *J* = 4.8)	69.3	CH	8, 9, 12, 13, 20
12		147.0	C	
13		128.0	C	
14	2.62 (m)	32.2	CH	8, 9, 12, 13, 17
15	6.23 (d, *J* = 1.5)	109.3	CH	12, 13
16	7.33 (d, *J* = 1.5)	142.8	CH	12, 13, 15
17	0.94 (d, *J* = 6.6)	14.4	CH$_3$	8, 13, 14
18		179.0	C	

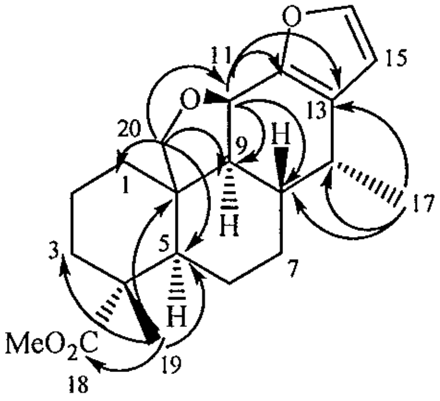
Table 9 (continued)

Position	δ_H (mult, J, Hz)	δ_C	DEPT	HMBC
19	1.25 (s)	17.3	CH_3	3, 4, 5, 18
20	5.52 (s)	103.3	CH	1, 5, 9, 10, 11
18-OMe	3.69 (s)	51.9	CH_3	18

3.1.8 Compound DS8



Compound **DS8** was found to have the molecular formula $C_{21}H_{28}O_4$ ($[M]^+$ *m/z* 344.1996) by HREIMS. The ^1H and ^{13}C NMR spectral data (Table 10, Figures 24 and 25) of **DS8** were similar to those of **DS7**, except that the signal of a dioxymethine proton at δ 5.52 (s, H-20); δ_C 103.3 in **DS7** was replaced by those of the oxymethylene protons at δ 3.92 (d, J = 8.1 Hz) and 3.97 (br dd, J = 8.1, 3.0 Hz). Both oxymethylene protons showed HMBC correlations with the carbons at δ 35.9 (C-1), 45.8 (C-10), 47.4 (C-5), 49.4 (C-9) and 68.7 (C-11). Therefore, phanginin H was assigned as **DS8**, a new compound (Yodsaoue *et al.*, 2008).



Selected HMBC correlation for compound **DS8**

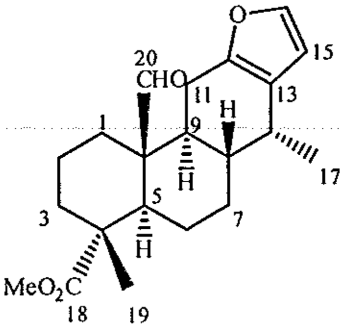
Table 10 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS8**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
1*α*	1.22 (m)	35.9	CH_2	3, 5, 10, 20
β	2.08 (m)			
2	1.65 (m)	18.3	CH_2	1, 3, 4, 10
3*α*	1.60 (m)	36.8	CH_2	1, 2, 5, 18, 19
β	1.86 (m)			
4		47.6	C	
5	2.20 (d, *J* = 11.7, 3.0)	47.4	CH	1, 3, 4, 6, 7, 10, 18, 19, 20
6*α*	1.29 (m)	25.0	CH_2	4, 7, 8, 10
β	1.43 (m)			
7	1.46 (m), 1.71 (m)	30.1	CH_2	5, 6, 8
8	1.93 (m)	37.1	CH	6, 7, 11, 14, 17
9	1.80 (dd, *J* = 12.0, 3.9)	49.4	CH	1, 5, 8, 10, 11, 14, 20
10		45.8	C	
11	4.83 (d, *J* = 3.9)	68.7	CH	8, 9, 12, 13
12		148.3	C	
13		127.3	C	
14	2.64 (qd, *J* = 7.2, 4.2)	32.1	CH	8, 9, 12, 13, 15, 17
15	6.22 (d, *J* = 1.8)	109.1	CH	12, 13, 14, 16
16	7.31 (d, *J* = 1.8)	142.8	CH	12, 13, 15
17	0.97 (d, *J* = 7.2)	14.7	CH_3	8, 14, 13
18		178.5	C	
19	0.99 (s)	15.8	CH_3	3, 4, 5, 18
20*ax*	3.97 (br dd, *J* = 8.1, 3.0),	70.3	CH_2	1, 5, 9, 10, 11
eq	3.92 (d, *J* = 8.1)			
18-OMe	3.69 (s)	52.0	CH_3	18

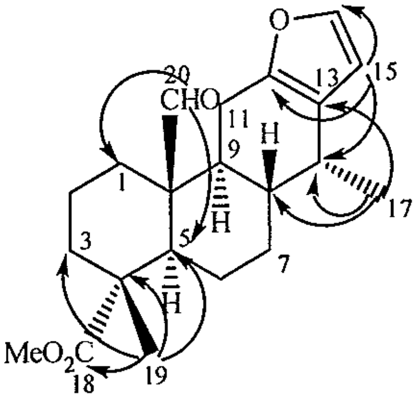
Table 11 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **DS7** and **DS8**

Position	**DS7**		**DS8**	
	δ_H (mult, *J*, Hz)	**δ_C**	**δ_H (mult, *J*, Hz)**	**δ_C**
1*α*	1.22 (m)	36.0	1.22 (m)	35.9
β	1.92 (m)		2.08 (m)	
2	1.70 (m)	19.0	1.65 (m)	18.3
3*α*	1.64 (m)	37.1	1.60 (m)	36.8
β			1.86 (m)	
4		48.1		47.6
5	2.20 (br d, *J* = 12.0)	48.5	2.20 (d, *J* = 11.7, 3.0)	47.4
6*α*	1.14 (m)	24.9	1.29 (m)	25.0
β	2.10 (m)		1.43 (m)	
7	1.39 (m), 1.68 (m)	29.9	1.46 (m), 1.71 (m)	30.1
8	2.69 (m)	36.2	1.93 (m)	37.1
9	1.79 (dd, *J* = 12.0, 4.8)	48.7	1.80 (dd, *J* = 12.0, 3.9)	49.4
10		47.9		45.8
11*α*	4.85 (d, *J* = 4.8)	69.3	4.83 (d, *J* = 3.9)	68.7
12		147.0		148.3
13		128.0		127.3
14	2.62 (m)	32.2	2.64 (qd, *J* = 7.2, 4.2)	32.1
15	6.23 (d, *J* = 1.5)	109.3	6.22 (d, *J* = 1.8)	109.1
16	7.33 (d, *J* = 1.5)	142.8	7.31 (d, *J* = 1.8)	142.8
17	0.94 (d, *J* = 6.6)	14.4	0.97 (d, *J* = 7.2)	14.7
18		179.0		178.5
19	1.25 (s)	17.3	0.99 (s)	15.8
20*ax*	5.52 (s)	103.3	3.97 (br dd, *J* = 8.1, 3.0),	70.3
eq			3.92 (d, *J* = 8.1)	
18-OMe	3.69 (s)	51.9	3.69 (s)	52.0

3.1.9 Compound DS9



Compound **DS9** was found to have the molecular formula $C_{21}H_{28}O_4$, $[M]^+$ *m/z* 344.1983, by HREIMS. The ^1H and ^{13}C NMR spectra (Table 12, Figures 26 and 27) showed similarity with **DS8** and taepeenin H previously isolated from *C. crista* (Cheenpracha *et al.*, 2005). The ^1H NMR spectral data exhibited a signal of an aldehydic proton at δ 10.21 (d, J = 1.2 Hz, H-20); δ_C 208.4 whose HMBC spectrum showed correlations with carbons at δ 31.9 (C-1) and 49.9 (C-5), confirming its location at C-20. The relative stereochemistry of **DS9** was assigned by NOESY experiments in which the aldehydic proton H-20 (δ 10.21) showed a cross-peak with δ 1.04 (Me-19) and 2.06 (H-8). Thus the structure of phanginin I was identified to be **DS9**, a new compound (Yodsaoue *et al.*, 2008).

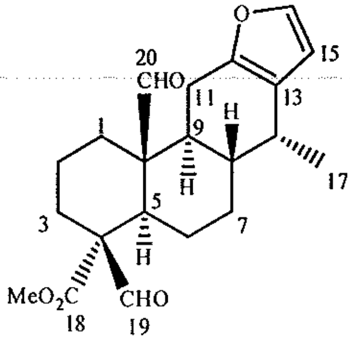


Selected HMBC correlation for compound **DS9**

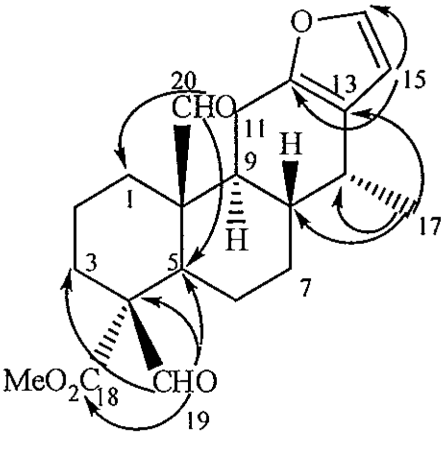
Table 12 ^1H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS9**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
1*α*	0.90 (m)	31.9	CH_2	5, 9, 20
β	2.46 (br d, *J* = 12)			
2	1.61 (m), 1.80 (m)	18.3	CH_2	10
3*α*	1.59 (m)	36.6	CH_2	2, 10, 18, 19
β	1.77 (m)			
4		51.4	C	
5	2.18 (dd, *J* = 12.9, 2.1)	49.9	CH	3, 4, 6, 7, 9, 10, 18, 19, 20
6*α*	1.40 (m)	23.1	CH_2	4, 5, 7, 8
β	2.17 (m)			
7	1.69 (m), 1.94 (m)	30.8	CH_2	6, 8, 14
8	2.06 (m)	36.7	CH	6, 9, 11, 14, 17
9	1.84 (m)	44.3	CH	1, 5, 7, 8, 11, 14, 20
10		47.3	C	
11*α*	2.69 (dd, *J* = 17.1, 6.6)	23.0	CH_2	8, 9, 12, 13
β	2.18 (m)			
12		148.2	C	
13		122.3	C	
14	2.65 (br q, *J* = 7.2)	31.5	CH	8, 9, 12, 13, 15, 17
15	6.15 (d, *J* = 1.8)	109.4	CH	12, 13, 14, 16
16	7.19 (d, *J* = 1.8)	140.7	CH	12, 13, 15
17	1.00 (d, *J* = 7.2)	16.9	CH_3	8, 14, 13
18		178.2	C	
19	1.04 (s)	15.8	CH_3	3, 4, 5, 18
20	10.21 (d, *J* = 1.2)	208.4	CH	1, 5
18-OMe	3.70 (s)	52.0	CH_3	18

3.1.10 Compound DS10



Compound **DS10** was deduced as $C_{21}H_{26}O_5$ from an exact mass measurement ($[M]^+$ *m/z* 358.1779) by HREIMS. The ^1H and ^{13}C NMR spectra (Table 13, Figures 33 and 34) of **DS10** were comparable with those of **DS9**. The difference was shown as the disappearance of a singlet at δ 1.04 (Me-19) in **DS9** and the appearance of an additional aldehydic proton signal at δ 9.68 (s, H-19) in **DS10** whose HMBC spectrum showed correlations with carbons at δ 30.6 (C-3), 48.1 (C-5), 60.7 (C-4) and 173.1 (C-18), confirming the attachment of an aldehyde group at C-4. Therefore, phanginin J was assigned as **DS10**, a new compound (Yodsaoue *et al.*, 2008).

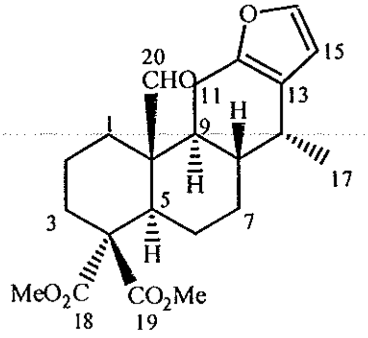


Selected HMBC correlation for compound **DS10**

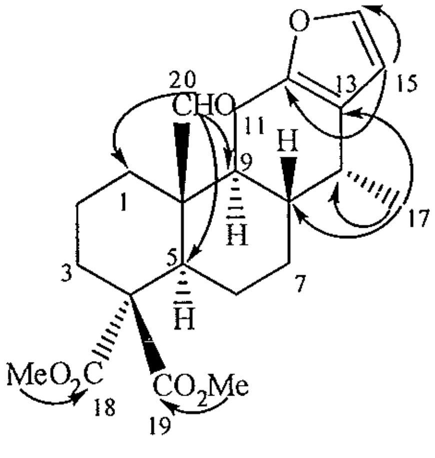
Table 13 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS10**

Position	δ_H (mult, *J*, Hz)	δ_C	DEPT	HMBC
1*α*	1.07 (dd, *J* = 13.2, 3.3)	31.8	CH_2	2, 3, 5, 9, 20
β	2.44 (m)			
2	1.69 (m), 1.85 (m)	19.0	CH_2	
3*α*	1.65 (m)	30.6	CH_2	1, 2, 4, 5, 18, 19
β	2.32 (m)			
4		60.7	C	
5	2.18 (br d, *J* = 11.7)	48.1	CH	1, 4, 9, 10, 18, 19, 20
6*α*	1.60 (m)	23.0	CH_2	4, 10
β	2.50 (m)			
7	1.52 (m), 1.87 (m)	31.1	CH_2	5, 9, 8, 14
8	1.97 (m)	36.8	CH	6, 9, 11, 14, 17
9	1.83 (m)	44.1	CH	1, 5, 8, 10, 11, 14, 20
10		50.8	C	
11*α*	2.77 (dd, *J* = 16.8, 6.6)	23.2	CH_2	8, 9, 12, 13, 15
β	2.31 (dd, *J* = 16.8, 10.5)			
12		147.8	C	
13		122.4	C	
14	2.67 (br q, *J* = 7.2)	31.4	CH	8, 9, 12, 13, 15, 17
15	6.16 (d, *J* = 1.5)	109.4	CH	12, 13, 16
16	7.19 (d, *J* = 1.5)	140.8	CH	12, 13, 15
17	1.00 (d, *J* = 7.2)	17.1	CH_3	8, 13, 14
18		173.1	C	
19	9.68 (s)	198.5	CH	3, 4, 5, 18
20	9.98 (s)	206.2	CH	1, 5
18-OMe	3.77 (s)	52.7	CH_3	18

3.1.11 Compound DS11



Compound **DS11**, [M]$^+$ *m/z* 388.1881 ($C_{22}H_{28}O_6$) by HREIMS, exhibited similar ^{1}H and ^{13}C NMR spectral data (Table 14, Figures 35 and 36) to those of **DS10**, except that an aldehydic proton signal at δ 9.68 in **DS10** was replaced by the methoxyl protons at δ 3.74 (s). The ^{13}C NMR spectra indicated the presence of two ester carbonyl carbons at δ 171.2 (C-18) and 172.8 (C-19). The location of 19-OMe was confirmed by its HMBC correlation with C-19 (δ 172.8). In the NOESY spectrum, correlations between the methoxyl protons at δ 3.74 (OMe-19) with the aldehydic proton signal at δ 10.04 (H-20) indicated that this ester carbonyl group was *β*-oriented. Thus, the structure of phanginin K was deduced to be **DS11**, a new compound (Yodsaoue *et al.*, 2008).



Selected HMBC correlation for compound **DS11**

Table 14 ^{1}H and ^{13}C NMR, DEPT and HMBC spectral data of compound **DS11**

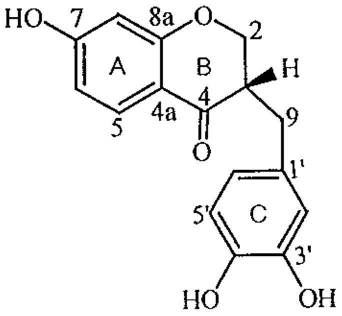
Position	δ_H (mult, J, Hz)	δ_C	DEPT	HMBC
1α	0.98 (m)	32.3	CH_2	2, 10
β	2.42 (m)			
2	1.67 (m)	20.0	CH_2	-
3α	1.66 (m)	34.1	CH_2	4, 5, 19
β	2.40 (m)			
4	-	58.7	C	-
5	2.16 (dd, J = 12.6, 2.4)	50.1	CH	4, 7, 9, 10, 18, 20
6α	1.65 (m)	24.2	CH_2	5
β	2.61 (m)			
7	1.60 (m), 1.92 (m)	31.2	CH_2	5, 8
8	2.20 (m)	36.9	CH	6, 9, 10, 11, 13, 17
9	1.82 (dt, J = 11.4, 6.6)	44.2	CH	1, 8, 10, 11, 14, 20
10	-	51.4	C	-
11α	2.72 (dd, J = 16.8, 6.6)	23.5	CH_2	8, 9, 10, 13
β	2.17 (m)			
12	-	148.1	C	-
13	-	122.4	C	-
14	2.69 (m)	31.5	CH	8, 9, 12, 13, 15, 17
15	6.17 (d, J = 1.8)	109.4	CH	12, 13
16	7.21 (d, J = 1.8)	140.8	CH	12, 13, 15
17	1.01 (d, J = 7.2)	17.0	CH_3	8, 13, 14
18	-	171.2	C	-
19	-	172.8	C	-
20	10.04 (d, J = 1.2)	206.4	CH	1
18-OMe	3.70 (s)	52.1	CH_3	18
19-OMe	3.74 (s)	52.7	CH_3	19

Table 15 Comparison of ^{13}C NMR spectral data of compounds **DS9-DS11**

Position	**DS9**	**DS10**	**DS11**
1	31.9	31.8	32.3
2	18.3	19.0	20.0
3	36.6	30.6	34.1
4	51.4	60.7	58.7
5	49.9	48.1	50.1
6	23.1	23.0	24.2
7	30.8	31.1	31.2
8	36.7	36.8	36.9
9	44.3	44.1	44.2
10	47.3	50.8	51.4
11	23.0	23.2	23.5
12	148.2	147.8	148.1
13	122.3	122.4	122.4
14	31.5	31.4	31.5
15	109.4	109.4	109.4
16	140.7	140.8	140.8
17	16.9	17.1	17.0
18	178.2	173.1	171.2
19	15.8	198.5	172.8
20	208.4	206.2	206.4
18-OMe	52.0	52.7	52.1
19-OMe	-	-	52.7

3.2 Structural elucidation of compounds from the heartwood of *C. sappan*

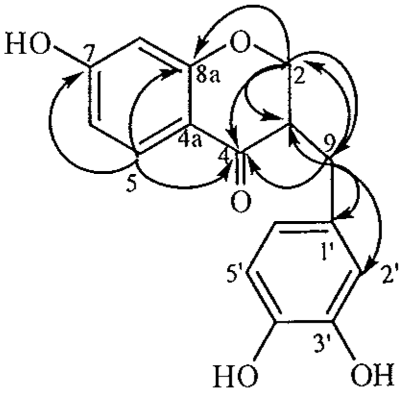
3.2.1 Compound DH1



Compound **DH1** was obtained as a yellow solid, mp: 194-195 °C, $[\alpha]^{27}_{D}$: - 12.6° (*c* = 0.62, MeOH). The UV absorption bands at λ_{max} 228, 277 and 312 nm supported the presence of conjugated chromophore in the structure. The IR spectrum showed absorption bands of hydroxyl group (3364 cm^{-1}), C=O (1657 cm^{-1}) and C=C stretching (1606 cm^{-1}).

The ^{13}C NMR and DEPT spectral data (Tables 16 and 18, Figure 40) exhibited 16 carbons of two methylenes (δ 31.9, 69.3), seven methines (δ 48.6, 102.2, 110.5, 115.1, 115.8, 120.1, 128.9) and seven quaternary carbons (δ 113.2, 129.8, 143.6, 145.0, 164.0, 165.1, 193.8).

The ^{1}H NMR spectral data (Tables 16 and 17, Figure 39) displayed the presence of two ABX system at δ 2.62 (m, H-3), 2.46 (dd, *J* = 13.8, 10.5 Hz, H_A-9), 2.90 (dd, *J* = 13.8, 4.5 Hz, H_B-9), 4.03 (dd, *J* = 11.4, 7.5 Hz, H-2*ax*) and 4.19 (dd, *J* = 11.4, 4.2 Hz, H-2*eq*) and two units of 1,2,4-trisubstituted benzene ring (six aromatic protons) at δ 6.21 (d, *J* = 2.1 Hz, H-8), 6.39 (dd, *J* = 8.7, 2.1 Hz, H-6) and 7.62 (d, *J* = 8.7 Hz, H-5) and one unit at δ 6.44 (dd, *J* = 8.1, 2.1 Hz, H-6′), 6.57 (d, *J* = 2.1 Hz, H-2′) and 6.61 (d, *J* = 8.1 Hz, H-5′). The structure of **DH1** was confirmed by HMBC correlations. The oxymethylene protons at δ 4.03 and 4.19 (2H-2) showed correlation with carbons at δ 31.9 (C-9), 48.6 (C-3), 164.0 (C-8a) and 193.8 (C-4) and an aromatic proton at δ 7.62 (H-5) showed correlation with carbons at δ 102.2 (C-8), 110.5 (C-6), 164.0 (C-8a), 165.1 (C-7) and 193.8 (C-4). The correlation of methylene protons at δ 2.46 and 2.90 (2H-9) with carbons at δ 48.6 (C-3), 69.3 (C-2), 115.1 (C-2′), 129.8 (C-1′) and 193.8 (C-4) confirmed the location of the dihydroxybenzyl moiety (unit C) at C-9. Therefore, compound **DH1** was identified as 3-(3',4'-dihydroxybenzyl)-7-hydroxychroman-4-one (Saitoh *et al.*, 1986).



Selected HMBC correlation for compound **DH1**

Table 16 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DH1**

Position	δ_H (mult, *J*, Hz)	δ_C	DEPT	HMBC
2	4.03 (dd, *J* = 11.4, 7.5), 4.19 (dd, *J* = 11.4, 4.2)	69.3	CH_2	3, 4, 8a, 9
3	2.62 (m)	48.6	CH	2, 4, 1′
4		193.8	C	
4a		113.2	C	
5	7.62 (d, *J* = 8.7)	128.9	CH	4, 6, 7, 8, 8a
6	6.39 (dd, *J* = 8.7, 2.1)	110.5	CH	4a, 7, 8
7		165.1	C	
8	6.21 (d, *J* = 2.1)	102.2	CH	4, 4a, 6, 7, 8a
8a		164.0	C	
9	2.46 (dd, *J* = 13.8, 10.5), 2.90 (dd, *J* = 13.8, 4.5)	31.9	CH_2	2, 3, 4, 1′, 2′, 6′
1′		129.8	C	
2′	6.57 (d, *J* = 2.1)	115.1	CH	9, 1′, 3′, 4′, 6′
3′		145.0	C	
4′		143.6	C	
5′	6.61 (d, *J* = 8.1)	115.8	CH	1′, 3′, 4′
6′	6.44 (dd, *J* = 8.1, 2.1)	120.1	CH	9, 2′, 4′

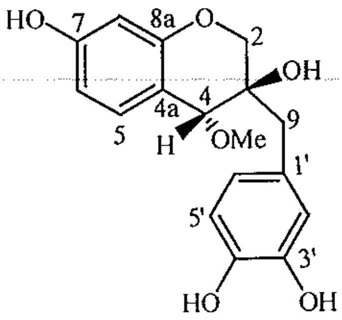
Table 17 Comparison of ^{1}H NMR spectral data between compound **DH1** and 3-(3',4'-dihydroxybenzyl)-7-hydroxychroman-4-one (**R**, recorded in DMSO-d_6)

Position	**DH1** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
2	4.03 (dd, *J* = 11.4, 7.5), 4.19 (dd, *J* = 11.4, 4.2)	4.09 (dd, *J* = 11, 8), 4.29 (dd, *J* =11, 4)
3	2.62 (m)	2.79 (m)
4		
4a		
5	7.62 (d, *J* = 8.7)	7.65 (d, *J* = 9)
6	6.39 (dd, *J* = 8.7, 2.1)	6.51 (dd, *J* = 9, 2)
7		
8	6.21 (d, *J* = 2.1)	6.32 9 (d, *J* = 2)
8a		
9	2.46 (dd, *J* = 13.8, 10.5), 2.90 (dd, *J* = 13.8, 4.5)	2.47 (dd, *J* = 13, 9), 2.90 (dd, *J* = 13,5)
1′		
2′	6.57 (d, *J* = 2.1)	6.62 (d, *J* = 2)
3′		
4′		
5′	6.61 (d, *J* = 8.1)	6.66 (d, *J* = 8)
6′	6.44 (dd, *J* = 8.1, 2.1)	6.47 (dd, *J* =8, 2)

Table 18 Comparison of ^{13}C NMR spectral data between compound **DH1** and 3-(3',4'-dihydroxybenzyl)-7-hydroxychroman-4-one (**R** , recorded in DMSO-d_6)

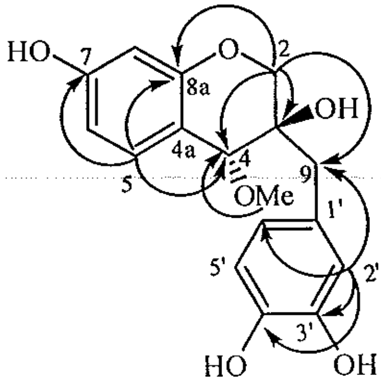
Position	**DH1**	**R**
2	69.3	69.4
3	48.6	46.5
4	193.8	191.4
4a	113.2	113.1
5	128.9	129.1
6	110.5	110.5
7	165.1	164.3
8	102.2	102.2
8a	164.0	163.0
9	31.9	31.2
1′	129.8	128.7
2′	115.1	115.5
3′	145.0	145.0
4′	143.6	143.6
5′	115.8	116.2
6′	120.1	119.5

3.2.2 Compound DH2



Compound **DH2** was isolated as a yellow viscous oil; $[\alpha]^{27}_{D}$: - 24.2° (c = 0.75, MeOH). The UV absorption band at λ_{max} 221, 280 and 285 nm supported the presence of conjugated chromophore in the structure. The IR spectrum showed absorption bands of hydroxyl group (3380 cm^{-1}) and C=C stretching (1619 cm^{-1}).

The ^{1}H and ^{13}C (Tables 19-21, Figures 46 and 47) NMR spectral data of **DH2** were similar to those of **DH1**, except that the methine proton signal of H-3 at δ_H 2.62 (m) and δ_C 48.6 in **DH1** disappeared but in the ^{13}C NMR spectrum a signal at δ 70.1 of oxycarbon was displayed. In addition, the ^{1}H NMR signals of methoxyl and oxymethine protons at δ_H 3.32 (s): δ_C 55.8 and δ_H 3.59 (s): δ_C 76.8 (C-4), respectively in **DH2** replaced the carbonyl carbon signal at δ 193.8 (C-4) in **DH1**. The connectivity of the methoxyl group was assigned at C-4 from HMBC correlation of methoxyl protons at δ 3.32 with the carbon at δ 76.8 (C-4). The ^{1}H NMR spectral data of **DH2** were similar to 4-*O*-methylepisappanol (Namikoshi *et al.*, 1987b). The optical rotation of compound **DH2** is levorotatory ($[\alpha]^{27}_{D}$: - 24.2°), the same as 4-*O*-methylepisappanol (lit. $[\alpha]^{25}_{D}$: - 36.2°) (Namikoshi *et al.*, 1987b) suggesting the same configuration at C-4. Thus compound **DH2** was assigned as 4-*O*-methylepisappanol.



Selected HMBC correlation for compound **DH2**

Table 19 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DH2**

Position	δ_H (mult, *J*, Hz)	δ_C	DEPT	HMBC
2	3.82 (d, *J* = 11.1), 4.10 (d, *J* = 11.1)	69.1	CH_2	3, 4, 8a, 9
3		70.1	C	
4	3.59 (s)	76.8	CH	2, 3, 4a, 5, 8a, 9, OMe
4a		111.2	C	
5	6.90 (d, *J* = 8.1)	132.2	CH	4, 7, 8, 8a
6	6.35 (dd, *J* = 8.1, 2.4)	107.7	CH	4a, 7, 8
7		158.5	C	
8	6.34 (d, *J* = 2.4)	103.0	CH	4, 4a, 6, 7, 8a
8a		154.8	C	
9	2.68 (d, *J* = 13.8), 2.88 (d, *J* = 13.8)	38.6	CH_2	2, 3, 4, 1′, 2′, 6′
1′		127.4	C	
2′	6.84 (d, *J* = 1.8)	118.1	CH	9, 3′, 4′, 6′
3′		144.4	C	
4′		143.5	C	
5′	6.78 (d, *J* = 8.1)	115.2	CH	1′, 3′
6′	6.63 (dd, *J* = 8.1, 1.8)	122.3	CH	2′, 4′
4-OMe	3.32 (s)	55.8	CH_3	4

Table 20 Comparison of ^{1}H NMR spectral data of compounds **DH1, DH2** and 4-*O*-methylepisappanol (**R**, recorded in acetone-d_6)

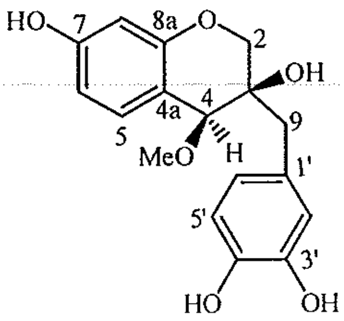
Position	**DH1** **δ_H (mult, *J*, Hz)**	**DH2** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
2	4.03 (dd, *J* = 11.4, 7.5), 4.19 (dd, *J* = 11.4, 4.2)	3.82 (d, *J* = 11.1), 4.10 (d, *J* = 11.1)	3.80 (dd, *J* = 11.0, 1.6), 4.10 (d, *J* = 11.0)
3	2.62 (m)		
4		3.59 (s)	3.66 (br s)
4a			
5	7.62 (d, *J* = 8.7)	6.90 (d, *J* = 8.1)	6.96 (d, *J* = 8.2)
6	6.39 (dd, *J* = 8.7, 2.1)	6.35 (dd, *J* = 8.1, 2.4)	6.34 (dd, *J* = 8.2, 2.4)
7			
8	6.21 (d, *J* = 2.1)	6.34 (d, *J* = 2.4)	6.28 (d, *J* = 2.4)
8a			
9	2.46 (dd, *J* = 13.8, 10.5), 2.90 (dd, *J* = 13.8, 4.5)	2.68 (d, *J* = 13.8), 2.88 (d, *J* = 13.8)	2.63 (d, *J* = 14.0), 2.89 (d, *J* = 14.0)
1′			
2′	6.57 (d, *J* = 2.1)	6.84 (d, *J* = 1.8)	6.85 (d, *J* = 1.8)
3′			
4′			
5′	6.61 (d, *J* = 8.1)	6.78 (d, *J* = 8.1)	6.76 (d, *J* = 8.0)
6′	6.44 (dd, *J* = 8.1, 2.1)	6.63 (dd, *J* = 8.1, 1.8)	6.64 (dd, *J* = 8.0, 1.8)
4-OMe		3.32 (s)	3.31 (s)

Table 21 Comparison of ^{13}C NMR spectral data of compounds **DH1, DH2** and 4-*O*-methylepisappanol (**R**, recorded in DMSO-d_6)

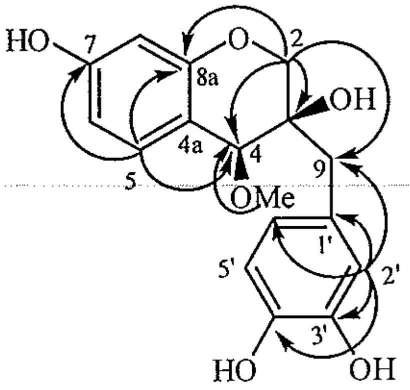
Position	**DH1**	**DH2**	**R**
2	69.3	69.1	68.9
3	48.6	70.1	69.4
4	193.8	76.8	76.2
4a	113.2	111.2	111.6
5	128.9	132.2	132.1
6	110.5	107.7	106.9
7	165.1	158.5	158.4
8	102.2	103.0	102.4
8a	164.0	154.8	154.7
9	31.9	38.6	a
1′	129.8	127.4	127.2
2′	115.1	118.1	115.0
3′	145.0	144.4	144.5
4′	143.6	143.5	143.6
5′	115.8	115.2	118.4
6′	120.1	122.3	121.7
4-OMe		55.8	55.3

a = overlap with DMSO-d_6

3.2.3 Compound DH3



Compound **DH3** was isolated as a yellow viscous oil; $[\alpha]^{27}_{D}$: + 13.8° (c = 5.23, MeOH). The absorption band for UV and IR spectrum were identical to **DH2**. The ^{1}H and ^{13}C NMR spectral data (Tables 22-24, Figures 48 and 49) of **DH3** and **DH2** showed structural similarity, except the comfiguration at C-4 position was determined to be (*S*). Namikoshi *et al.*, 1987a suggested that the 3,4-*cis*- and 3,4-*trans-di-O-* substituted homoisoflavans could be characterized through the ^{1}H NMR signal at C-9. The signal of 3,4-*cis* compound was observed as a singlet, while signal of 3,4-*trans* compound appeared as geminal-coupled AA′-type doublet. **DH3** could be deduced to have 3,4-*cis* configuration, because the signal due to the methyene protons at C-9 was observed as a singlet at δ 2.44, while **DH2** was observed as a pair of doublet (J = 13.8 Hz) at δ 2.68 and 2.88. The ^{1}H NMR spectral data of **DH3** were similar to 4-*O*-methylsappanol (Namikoshi *et al.*, 1987b). The optical rotation of compound **DH2** is a dextrorotatory ($[\alpha]^{27}_{D}$: + 13.8°), the same as 4-*O*-methylepisappanol (lit. $[\alpha]^{25}_{D}$: + 54.4°) (Namikoshi *et al.*, 1987b), suggesting that the absolute configuration at C-4 of **DH3** should be (*S*), the same as 4-*O*-methylsappanol. Thus compound **DH3** was assigned as 4-*O*-methylsappanol.



Selected HMBC correlation for compound **DH3**

Table 22 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DH3**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
2	3.55 (dd, *J* = 10.2, 1.5), 3.83 (d, *J* = 10.2)	66.7	CH_2	3, 4, 8a, 9
3	-	69.8	C	-
4	3.60 (brs)	78.4	CH	2, 3, 4a, 5, 8a, 9
4a	-	111.7	C	-
5	6.90 (d, *J* = 8.1)	132.1	CH	4, 4a, 6, 7, 8
6	6.31 (dd, *J* = 8.1, 2.4)	107.6	CH	4a, 7, 8
7	-	159.0	C	-
8	6.21 (d, *J* = 2.4)	102.5	CH	4a, 6, 7, 8a
8a	-	154.5	C	-
9	2.44 (s)	39.6	CH_2	2, 3, 4, 1′, 2′, 6′
1′	-	127.6	C	-
2′	6.54 (d, *J* = 2.1)	117.7	CH	9, 1′, 3′, 4′, 6′
3′	-	143.5	C	-
4′	-	144.2	C	-
5′	6.54 (d, *J* = 7.8)	114.4	CH	1′, 3′, 4′, 6′
6′	6.33 (dd, *J* = 7.8, 2.1)	121.9	CH	9, 2′, 4′
4-OMe	3.26 (s)	55.6	CH_3	4

Table 23 Comparison of ^{1}H NMR spectral data of compounds **DH2, DH3** and 4-*O*-methylsappanol (**R**, recorded in acetone-d_6)

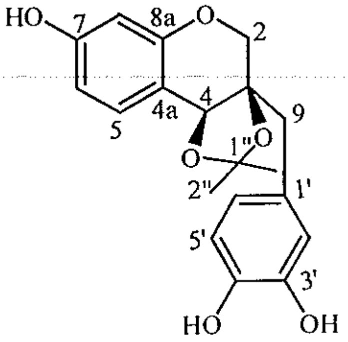
Position	**DH2** **δ_H (mult, *J*, Hz)**	**DH3** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
2	3.82 (d, *J* = 11.1), 4.10 (d, *J* = 11.1)	3.55 (dd, *J* = 10.2, 1.5), 3.83 (d, *J* = 10.2)	3.62 (br d, *J* = 10.2), 3.89 (d, *J* = 10.2)
3			
4	3.59 (s)	3.60 (br s)	3.69 (br s)
4a			
5	6.90 (d, *J* = 8.1)	6.90 (d, *J* = 8.1)	7.04 (d, *J* = 8.2)
6	6.35 (dd, *J* = 8.1, 2.4)	6.31 (dd, *J* = 8.1, 2.4)	6.44 (dd, *J* = 8.2, 2.2)
7			
8	6.34 (d, *J* = 2.4)	6.21 (d, *J* = 2.4)	6.35 (d, *J* = 2.2)
8a			
9	2.68 (d, *J* = 13.8), 2.88 (d, *J* = 13.8)	2.44 (s)	2.56 (s)
1′			
2′	6.84 (d, *J* = 1.8)	6.54 (d, *J* = 2.1)	6.70 (d, *J* = 2.0)
3′			
4′			
5′	6.78 (d, *J* = 8.1)	6.54 (d, *J* = 7.8)	6.68 (d, *J* = 8.0)
6′	6.63 (dd, *J* = 8.1, 1.8)	6.33 (dd, *J* = 7.8, 2.1)	6.44 (dd, *J* = 8.0, 2.0)
4-OMe	3.32 (s)	3.26 (s)	3.33 (s)

Table 24 Comparison of ^{13}C NMR spectral data of compounds **DH2, DH3** and 4-*O*-methylepisappanol (**R**, recorded in DMSO-d_6)

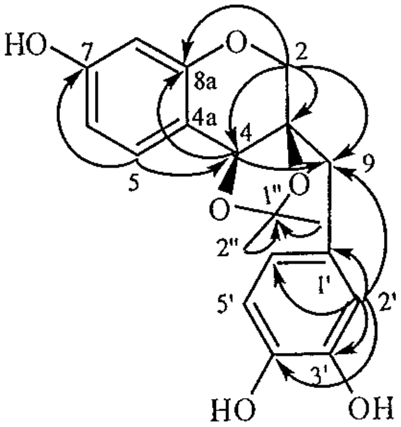
Position	**DH2**	**DH3**	**R**
2	69.1	66.7	66.7
3	70.1	69.8	69.5
4	76.8	78.4	78.3
4a	111.2	111.7	112.1
5	132.2	132.1	132.2
6	107.7	107.6	107.9
7	158.5	159.0	158.9
8	103.0	102.5	102.5
8a	154.8	154.5	154.1
9	38.6	39.6	a
1′	127.4	127.6	127.2
2′	118.1	117.7	114.9
3′	144.4	143.5	144.7
4′	143.5	144.2	143.7
5′	115.2	114.4	118.2
6′	122.3	121.9	121.4
4-OMe	55.8	55.6	56.6

a = overlap with DMSO-d_6

3.2.4 Compound DH4



Compound **DH4** was isolated as a yellow viscous oil; $[\alpha]^{27}_{D}$: – 13.4° (*c* = 2.65, MeOH). The absorption bands for UV and IR spectrum were similar to **DH2**. The ^{1}H and ^{13}C NMR spectra (Tables 25-26, Figures 50 and 51) of **DH4** were comparable to those of **DH2**. The difference was shown as the disappearance of a singlet at δ 3.32 (OMe-4) in **DH2** but appearance of the signals of the dioxyquaternary carbon at δ 108.0 (C-1″) and two additional singlets of the gem-dimethyl protons at δ 1.22 (Me-2″) and 1.33 (Me-3″) whose HMBC spectrum showed correlations with carbons at δ 108.0 (C-1″), confirming the attachment of a gem-dimethyl group at C-1″ (forming a dioxolane skeleton). Therefore, compound **DH4** was identified as 3,4-isopropylidene sappanol (Namikoshi *et al.*, 1987c).



Selected HMBC correlation for compound **DH4**

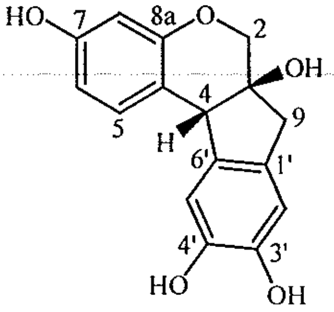
Table 25 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DH4**

Position	**δ_H (mult, *J*, Hz)**	**δ_C**	**DEPT**	**HMBC**
2	3.59 (d, *J* = 10.5), 3.76 (dd, *J* = 10.5, 0.9)	67.6	CH_2	3, 4, 8a, 9
3		77.3	C	
4	4.63 (br s)	74.0	CH	2, 3, 4a, 5, 8a, 9
4a		111.1	C	
5	7.17 (d, *J* = 8.4)	132.6	CH	4, 6, 7, 8
6	6.53 (dd, *J* = 8.4, 2.4)	109.5	CH	4a, 7, 8
7		158.9	C	
8	6.39 (d, *J* = 2.4)	102.9	CH	4, 4a, 6, 7, 8a
8a		155.9	C	
9	2.71 (d, *J* = 14.4), 2.77 (d, *J* = 14.4)	38.2	CH_2	2, 3, 4, 1′, 2′, 6′
1′		127.7	C	
2′	6.75 (d, *J* = 2.4)	117.0	CH	9, 1′, 3′, 4′, 6′
3′		143.8	C	
4′		144.5	C	
5′	6.76 (d, *J* = 8.4)	114.9	CH	1′, 3′, 4′, 6′
6′	6.53 (dd, *J* = 8.1, 2.4)	122.1	CH	9, 2′, 4′, 5′
1″		108.0	C	
2″	1. 22 (s)	26.3	CH_3	1″
3″	1.33 (s)	28.2	CH_3	1″

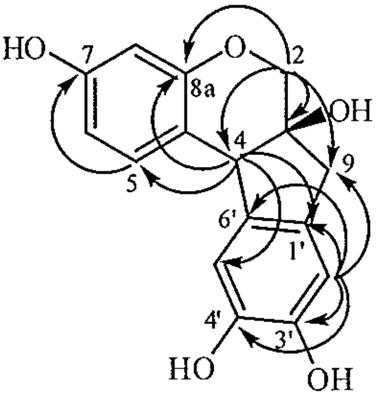
Table 26 Comparison of ^{1}H NMR spectral data of compounds **DH2, DH4** and 3,4-isopropylidene sappanol (**R**, recorded in acetone-d_6)

	DH2	**DH4**	**R**
Position	**δ_H (mult, *J*, Hz)**	**δ_H (mult, *J*, Hz)**	**δ_H (mult, *J*, Hz)**
2	3.82 (d, *J* = 11.1), 4.10 (d, *J* = 11.1)	3.59 (d, *J* = 10.5), 3.76 (dd, *J* = 10.5, 0.9)	3.61 (d, *J* = 10.5), 3.78 (dd, *J* = 10.5, 1.3)
3			
4	3.59 (s)	4.63 (br s)	4.61 (br s)
4a			
5	6.90 (d, *J* = 8.1)	7.17 (d, *J* = 8.4)	7.12 (d, *J* = 8.5)
6	6.35 (dd, *J* = 8.1, 2.4)	6.53 (dd, *J* = 8.4, 2.4)	6.46 (dd, *J* = 8.5, 2.2)
7			
8	6.34 (d, *J* = 2.4)	6.39 (d, *J* = 2.4)	6.33 (d, *J* = 2.2)
8a			
9	2.68 (d, *J* = 13.8), 2.88 (d, *J* = 13.8)	2.71 (d, *J* = 14.4), 2.77 (d, *J* = 14.4)	2.70 (d, *J* = 14.5), 2.76 (d, *J* = 14.5)
1′			
2′	6.84 (d, *J* = 1.8)	6.75 (d, *J* = 2.4)	6.66 (d, *J* = 2.0)
3′			
4′			
5′	6.78 (d, *J* = 8.1)	6.76 (d, *J* = 8.1)	6.67 (d, *J* = 8.0)
6′	6.63 (dd, *J* = 8.1, 1.8)	6.53 (dd, *J* = 8.1, 2.4)	6.48 (dd, *J* = 8.0, 2.0)
1″			
2″		1.22 (s)	1.24 (s)
3″		1.33 (s)	1.35 (s)
4-OMe	3.32 (s)		

3.2.5 Compound DH5



Compound **DH5** was isolated as a yellow viscous oil; $[\alpha]^{27}{}_{D}$: + 70.0° (c = 3.64, MeOH). The absorption band for UV and IR spectrum were similar to **DH2**. The NMR data of **DH5** displayed similarities with **DH2**. The ^{13}C NMR spectra (Table Tables 27, 29 and 30, Figure 53) exhibited a methine carbon at δ 49.9 and an aromatic carbon at δ 130.2 assigning to C-4 and C-6′, respectively. The ^{1}H NMR (Tables 27-28, Figure 52) signal of H-4 was displayed as a singlet at *δ* 3.84 whose HMBC spectrum showed correlations to carbons at δ 42.3 (C-9), 70.0 (C-3), 76.8 (C-2), 112.1 (C-5′), 114.7 (C-4a), 130.2 (C-6′) 131.3 (C-5), 136.0 (C-1′) and 154.5 (C-8a), suggesting a connection between C-4 and C-6′ (forming a pentacyclic). Therefore, compound **DH5** was assigned as brazilin (Namikoshi *et al.*, 1987b, c and Xie *et al.*, 2000).



Selected HMBC correlation for compound **DH5**

Table 27 ^1H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DH5**

Position	δ_H (mult, *J*, Hz)	δ_C	DEPT	HMBC
2	3.57 (d, *J* = 11.4), 3.84 (d, *J* = 11.4)	76.8	CH_2	3, 4, 8a, 9
3		70.0	C	
4	3.84 (s)	49.9	CH	2, 3, 4a, 5, 8a, 9, 1′, 5′, 6′
4a		114.7	C	
5	7.13 (d, *J* = 8.4)	131.3	CH	4, 7, 8
6	6.41 (dd, *J* = 8.4, 2.4)	109.2	CH	4a, 7, 8
7		156.9	C	
8	6.20 (d, *J* = 2.4)	103.2	CH	4a, 6, 7, 8a
8a		154.5	C	
9	2.69 (d, *J* = 15.6), 2.87 (d, *J* = 15.6)	42.3	CH_2	2, 3, 4, 1′, 2′, 3′, 6′
1′		136.0	C	
2′	6.63 (s)	112.5	CH	9, 1′, 3′, 4′, 6′
3′		144.7	C	
4′		144.4	C	
5′	6.54 (s)	112.1	CH	9, 1′, 3′, 4′
6′		130.2	C	

Table 28 Comparison of ^1H NMR spectral data of compounds **DH2, DH5** and brazilin (**R**, recorded in acetone-d_6)

Position	**DH2** **δ_H (mult, J, Hz)**	**DH5** **δ_H (mult, J, Hz)**	**R** **δ_H (mult, J, Hz)**
2	3.82 (d, J = 11.1), 4.10 (d, J = 11.1)	3.57 (d, J = 11.4), 3.84 (d, J = 11.4)	3.39 (d, J = 11.1), 3.62 (d, J = 11.1)
3			
4	3.59 (s)	3.84 (s)	3.65 (s)
4a			
5	6.90 (d, J = 8.1)	7.13 (d, J = 8.4)	6.90 (d, J = 8.4)
6	6.35 (dd, J = 8.1, 2.4)	6.41 (dd, J = 8.4, 2.4)	6.17 (dd, J = 8.4, 1.8)
7			
8	6.34 (d, J = 2.4)	6.20 (d, J = 2.4)	5.98 (d, J = 1.8)
8a			
9	2.68 (d, J = 13.8), 2.88 (d, J = 13.8)	2.69 (d, J = 15.6), 2.87 (d, J = 15.6)	2.48 (d, J = 15.9), 2.73 (d, J = 15.9)
1′			
2′	6.84 (d, J = 1.8)	6.63 (s)	6.42 (s)
3′			
4′			
5′	6.78 (d, J = 8.1)	6.54 (s)	6.35 (s)
6′	6.63 (dd, J = 8.1, 1.8)		
4-OMe	3.32 (s)		

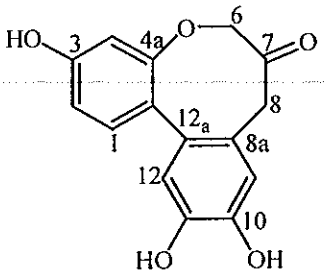
Table 29 Comparison of ^{13}C NMR spectral data of compounds **DH2, DH5** and brazilin (**R**, recorded in acetone-d_6)

Position	**DH2**	**DH5**	**R**
2	69.1	76.8	78.1
3	70.1	70.0	70.8
4	76.8	49.9	51.0
4a	111.2	114.7	115.8
5	132.2	131.3	132.7
6	107.7	109.2	109.9
7	158.5	156.9	155.7
8	103.0	103.2	104.2
8a	154.8	154.5	157.8
9	38.6	42.3	42.8
1′	127.4	136.0	131.3
2′	118.1	112.5	112.9
3′	144.4	144.7	145.3
4′	143.5	144.4	145.0
5′	115.2	112.1	112.4
6′	122.3	130.2	137.4
4-OMe	55.8		

Table 30 Comparison of ^{13}C NMR spectral data of compounds **DH1- DH5**

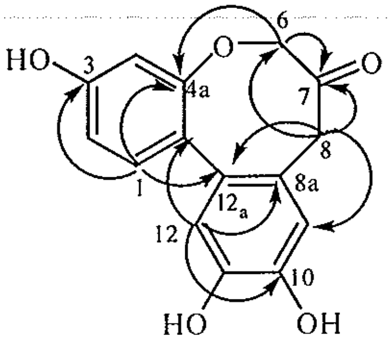
Position	**DH1**	**DH2**	**DH3**	**DH4**	**DH5**
2	69.3	69.1	66.7	67.6	76.8
3	48.6	70.1	69.8	77.3	70.0
4	193.8	76.8	78.4	74.0	49.9
4a	113.2	111.2	111.7	111.1	114.7
5	128.9	132.2	132.1	132.6	131.3
6	110.5	107.7	107.6	109.5	109.2
7	165.1	158.5	159.0	158.9	156.9
8	102.2	103.0	102.5	102.9	103.2
8a	164.0	154.8	154.5	155.9	154.5
9	31.9	38.6	39.6	38.2	42.3
1′	129.8	127.4	127.6	127.7	136.0
2′	115.1	118.1	117.7	117.0	112.5
3′	145.0	144.4	143.5	143.8	144.7
4′	143.6	143.5	144.2	144.5	144.4
5′	115.8	115.2	114.4	114.9	112.1
6′	120.1	122.3	121.9	122.1	130.2
4-OMe		55.8	55.6		
1″				108.0	
2″				26.3	
3″				28.2	

3.2.6 Compound DH6



Compound **DH6** was isolated as a yellow viscous oil. The UV spectrum displayed maximum absorptions at λ_{max} 246 and 280 nm, suggesting the presence of conjugation chromophore in the molecule. The IR spectrum showed absorption bands of hydroxyl group (3393 cm^{-1}), C=O (1655 cm^{-1}) and C=C stretching (1604 cm^{-1}).

The ^{13}C NMR spectral data (Tables 31 and 33, Figure 57) showed a total of 15 carbons including one carbonyl carbon at δ 205.8. The assignment of all carbons were achieved by ^{13}C, DEPT, HMQC, and HMBC experiments. The ^{13}C NMR and DEPT spectral data suggested two methylenes (δ 48.5 and 77.8) and twelve aromatic carbons identified as five protonated (δ 107.8, 112.6, 116.7, 116.8, 130.0), and seven non-protonated, of which four oxygenated (δ 144.1, 144.5, 157.4, 158.5) and three non-oxygenated (δ 123.2, 125.5, 130.4) carbons were observed. The ^{1}H NMR spectral data (Tables 31-32, Figure 56) displayed the presence of two overlapped singlets at δ 6.79 (H-9, H-12) from 1,2,4,5-tetrasubstituted benzene ring. The 1,2,4-trisubstituted benzene ring displayed the ^{1}H NMR signals at δ 6.67 (d, J = 2.4 Hz, H-4), 6.73 (dd, J = 8.1, 2.4 Hz, H-2) and 7.10 (d, J = 8.1 Hz, H-1). Two methylene proton signals were evidenced at δ 3.44 (2H-8) and 4.46 (2H-6) (each, s). The HMBC correlation of oxymethylene protons at δ 4.46 (2H-6) with carbons at δ 48.5 (C-8), 158.5 (C-4a) and 205.8 (C-7) confirmed an ether linkage between C-6 and C-4a of the 1,2,4-trisubstituted benzene ring. The ^{1}H NMR signal of methylene protons 2H-8 at δ 3.44 showed HMBC correlations with carbons at δ 130.4 (C-12a), 116.8 (C-9), 123.2 (C-8a) and 205.8 (C-7), of H-1 at δ 7.10 with carbon at δ 130.4 (C-12a) and of H-12 at δ 6.79 with carbons at δ 125.5 (C-12b) and 123.2 (C-8a), suggesting a bond formation C-12a and 12b. This data also suggested a carbonyl at C-7. Thus compound **DH6** was identified as protosappannin A (Nagai *et al.*, 1986 and 1987).



Selected HMBC correlation for compound **DH6**

Table 31 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DH6**

Position	δ_H (mult, J, Hz)	δ_C	DEPT	HMBC
1	7.10 (d, J = 8.1)	130.0	CH	2, 4, 4a, 12a
2	6.73 (dd, J = 8.1, 2.4)	112.6	CH	4, 12b
3		157.4	C	
4	6.67 (d, J = 2.4)	107.8	CH	2, 3, 4a, 12b
4a		158.5	C	
6	4.46 (s)	77.8	CH_2	4a, 7, 8
7		205.8	C	
8	3.44 (s)	48.5	CH_2	7, 8a, 9, 12a
8a		123.2	C	
9	6.79 (s)	116.8	CH	7, 8, 8a, 10, 11, 12, 12a
10		144.5	C	
11		144.1	C	
12	6.79 (s)	116.7	CH	8a, 9, 10, 11, 12a, 12b
12a		130.4	C	
12b		125.5	C	
3-OH	9.35 (s)			2, 3, 4
10-OH	8.41 (s)			9, 10, 11, 12
11-OH	8.33 (s)			9, 10, 11, 12

Table 32 Comparison of ^{1}H NMR spectral data between compound **DH6** and protosappannin A (**R**, recorded in acetone-d_6)

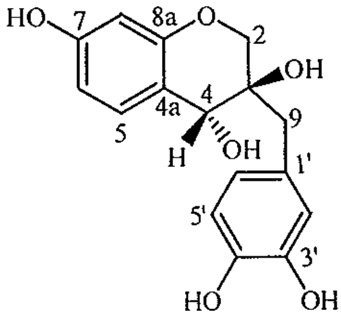
Position	**DH6** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
1	7.10 (d, *J* = 8.1)	7.16 (d, *J* = 9)
2	6.73 (dd, *J* = 8.1, 2.4)	6.65-6.90
3		
4	6.67 (d, *J* = 2.4)	6.65-6.90
4a		
6	4.46 (s)	4.49 (s)
7		
8	3.44 (s)	3.44 (s)
8a		
9	6.79 (s)	6.65-6.90
10		
11		
12	6.79 (s)	6.65-6.90
12a		
12b		
3-OH	9.35 (s)	
10-OH	8.41 (s)	
11-OH	8.33 (s)	

Table 33 Comparison of ^{13}C NMR spectral data between compound **DH6** and protosappannin A (**R**, recorded in acetone-d_6)

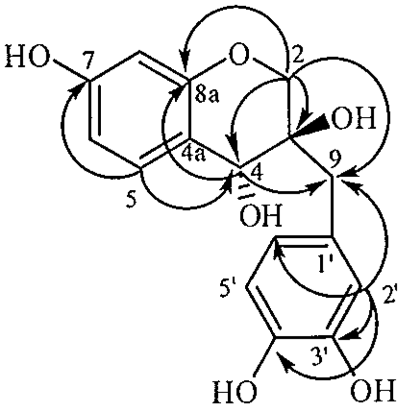
Position	**DH6**	**R**
1	130.0	131.1
2	112.6	113.5
3	157.4	159.2
4	107.8	109.2
4a	158.5	159.8
6	77.8	78.9
7	205.8	206.0
8	48.5	49.4
8a	123.2	125.1
9	116.8	117.7
10	144.5	145.7
11	144.1	145.4
12	116.7	117.5
12a	130.4	131.9
12b	125.5	127.2

3.3 Structural elucidation of compounds from the roots of *C. sappan*

3.3.1 Compound MR1



Compound **MR1** was isolated as a yellow viscous oil; $[\alpha]^{27}_{D}$: + 24.3° (c = 0.61, MeOH). The absorption bands for UV and IR spectrum were similar to **DH2**. The ^{1}H NMR spectral data (Tables 34-35, Figure 65) of **MR1** and **DH2** showed structural similarity, except that the signal of the methoxyl protons of **DH2** at δ_H 3.32 (δ_C 55.8, 4-OMe) disappeared in **MR1**. The ^{13}C NMR (Tables 34 and 36, Figure 66) chemical shift of C-4 at δ 69.2 suggested its connection to a hydroxyl group. Therefore, compound **MR1** was identified as episappanol (Namikoshi *et al.*, 1987c).



Selected HMBC correlation for compound **MR1**

Table 34 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **MR1**

Position	δ_H (mult, *J*, Hz)	δ_C	DEPT	HMBC
2	3.76 (dd, *J* = 10.8, 1.2), 4.06 (d, *J* = 10.8)	68.3	CH_2	3, 4, 8a, 9
3	-	70.4	C	-
4	4.24 (br s)	69.2	CH	2, 3, 4a, 5, 8a, 9
4a	-	116.0	C	-
5	7.06 (d, *J* = 8.1)	131.3	CH	4, 7, 8
6	6.38 (dd, *J* = 8.1, 2.4)	108.0	CH	4a, 7, 8
7	-	158.0	C	-
8	6.24 (d, *J* = 2.4)	102.1	CH	4a, 6, 7
8a	-	154.8	C	-
9	2.64 (d, *J* = 13.8), 2.91 (d, *J* = 13.8)	38.1	CH_2	2, 3, 4, 1′, 2′, 6′
1′	-	128.1	C	-
2′	6.90 (d, *J* = 1.8)	118.1	CH	9, 3′, 4′, 6′
3′	-	144.4	C	-
4′	-	143.5	C	-
5′	6.74 (d, *J* = 8.1)	114.6	CH	1′, 9, 3′
6′	6.69 (dd, *J* = 8.1, 1.8)	122.3	CH	9, 2′, 4′, 5′

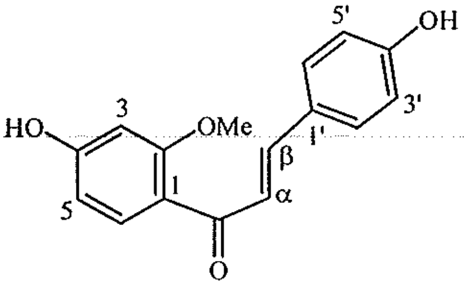
Table 35 Comparison of ^{1}H NMR spectral data of compounds **DH2, MR1** and episappanol (**R**, recorded in CD_3OD)

Position	**DH2** **δ_H (mult, *J*, Hz)**	**MR1** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
2	3.82 (d, *J* = 11.1), 4.10 (d, *J* = 11.1)	3.76 (dd, *J* = 10.8, 1.2), 4.06 (d, *J* = 10.8)	3.74 (dd, *J* = 11.2, 1.6), 4.01 (d, *J* = 11.2)
3		-	-
4	3.59 (s)	4.24 (br s)	4.19 (br s)
4a		-	-
5	6.90 (d, *J* = 8.1)	7.06 (d, *J* = 8.1)	7.07 (d, *J* = 8.2)
6	6.35 (dd, *J* = 8.1, 2.4)	6.38 (dd, *J* = 8.1, 2.4)	6.37 (dd, *J* = 8.2, 2.4)
7		-	-
8	6.34 (d, *J* = 2.4)	6.24 (d, *J* = 2.4)	6.23 (d, *J* = 2.4)
8a		-	-
9	2.68 (d, *J* = 13.8), 2.88 (d, *J* = 13.8)	2.64 (d, *J* = 13.8), 2.91 (d, *J* = 13.8)	2.67 (d, *J* = 14.4), 2.84 (d, *J* = 14.4)
1′		-	-
2′	6.84 (d, *J* = 1.8)	6.90 (d, *J* = 1.8)	6.81 (d, *J* = 1.9)
3′		-	-
4′		-	-
5′	6.78 (d, *J* = 8.1)	6.74 (d, *J* = 8.1)	6.70 (d, *J* = 8.0)
6′	6.63 (dd, *J* = 8.1, 1.8)	6.69 (dd, *J* = 8.1, 1.8)	6.65 (dd, *J* = 8.0, 1.9)
4-OMe	3.32 (s)		

Table 36 Comparison of ^{13}C NMR spectral data of compounds **DH2** and **MR1**

Position	**DH2**	**DH5**
2	69.1	68.3
3	70.1	70.4
4	76.8	69.2
4a	111.2	116.0
5	132.2	131.3
6	107.7	108.0
7	158.5	158.0
8	103.0	102.1
8a	154.8	154.8
9	38.6	38.1
1′	127.4	128.1
2′	118.1	118.1
3′	144.4	144.4
4′	143.5	143.5
5′	115.2	114.6
6′	122.3	122.3
4-OMe	55.8	

3.3.2 Compound MR2

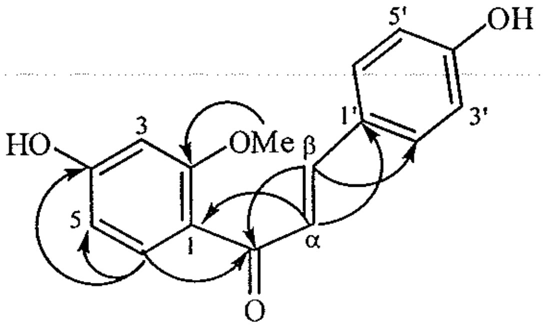


Compound **MR2** was isolated as yellow solid, mp: 228-230 °C. The UV spectrum displayed maximum absorptions at λ_{max} 237 and 351 nm, suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of hydroxyl group (3390 cm^{-1}) and C=C stretching (1601 cm^{-1}).

The ^{13}C NMR spectral data (Table 37, Figure 75) exhibited 16 carbons, of one methyl (δ 55.2), nine methines (δ 99.1, 107.8, 115.8 (2C), 124.5, 130.0 (2C), 132.5, 141.1) and six quarternary carbons (δ 121.2, 127.1, 159.5, 160.8, 162.5, 189.0).

The ^{1}H NMR spectral data (Tables 37-38, Figure 74) displayed the presence of *p*-disubstituted benzene ring at δ 6.91 and 7.58 (each 2H, dd, J = 8.4, 1.8 Hz), and 1,2,4-trisubstituted benzene ring at δ 6.53 (dd, J = 8.4, 2.1 Hz), 6.59 (d, J = 2.1 Hz) and 7.61 (d, J = 8.4 Hz). The singlet proton signal at δ 3.92 was assigned as a methoxyl group at C-2. In addition, the proton signals at δ 7.48 and 7.57 (each 1H, d, J = 15.9 Hz) were deduced as a *trans* double bond at C-α and C-β, respectively.

The structure of **MR2** was confirmed by HMBC correlations. The proton signal at δ 7.58 (H-2'/H-6') showed correlation with carbons at δ 115.8 (C-3'/C-5'), 132.5 (C-β) and 159.5 (C-4'), suggesting the connection of *p*-disubstituted benzene ring at C-β. The correlations of proton signals at δ 7.61 (H-6) with carbons at δ 99.1 (C-3), 107.8 (C-5), 162.5 (C-4) and 189.0 (C=O) suggested that 1,2,4-trisubstituted benzene ring was connected to C=O. The proton at δ 6.59 (H-3) showed correlations with carbons at δ 107.8 (C-5), 121.2 (C-1), 160.8 (C-2) and 162.5 (C-4), and the methoxyl group at δ 3.92 with carbon at δ 99.1 (C-3) and 160.8 (C-2) confirming the location of the methoxyl group at C-2. Therefore, compound **MR2** was identified as 3-deoxysappanchalcone (Namikoshi *et al.*, 1987a, b).



Selected HMBC correlation for compound **MR2**

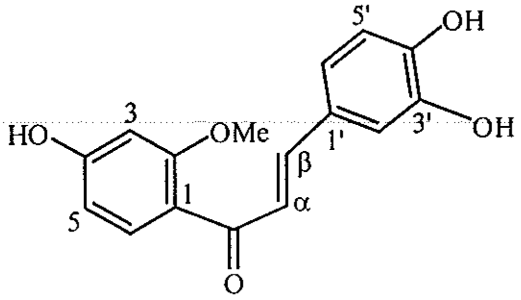
Table 37 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **MR2**

Position	δ_H **(mult, *J*, Hz)**	δ_C	**DEPT**	**HMBC**
α	7.48 (d, *J* = 15.9)	124.5	CH	1′, 1
β	7.57 (d, *J* = 15.9)	132.5	CH	α, C=O, 1′, 2′, 6′
1		121.2	C	
2		160.8	C	
3	6.59 (d, *J* = 2.1)	99.1	CH	1, 2, 4, 5
4		162.5	C	
5	6.53 (dd, *J* = 8.4, 2.1)	107.8	CH	1, 2, 3, 4
6	7.61 (d, *J* = 8.4)	141.1	CH	C=O, 3, 4, 5
1′		127.1	C	
2′	7.58 (dd, *J* = 8.4, 1.8)	130.0	CH	β, 3′, 4′, 6′
3′	6.91 (dd, *J* = 8.4, 1.8)	115.8	CH	1′, 2′, 4′, 5′, 6′
4′		159.5	C	
5′	6.91 (dd, *J* = 8.4, 1.8)	115.8	CH	1′, 2′, 3′, 4′, 6′
6′	7.58 (dd, *J* = 8.4, 1.8)	130.0	CH	β, 2′, 4′ ,5′
C=O		189.0	C	
2-OMe	3.92 (s)	55.2	CH_3	2, 3

Table 38 Comparison of ^{1}H NMR spectral data of compounds **MR2** and 3-deoxysappanchalcone (**R**, recorded in acetone-d_6)

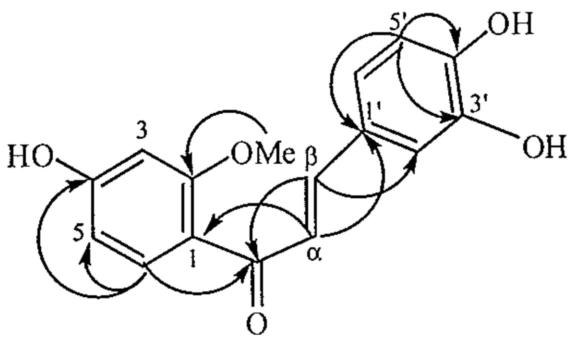
Position	**MR2** **δ_H (mult, *J*, Hz)**	**R** **δ_H (mult, *J*, Hz)**
α	7.48 (d, *J* = 15.9)	7.44 (d, *J* = 16.2)
β	7.57 (d, *J* = 15.9)	7.62 (d, *J* = 16.2)
1		
2		
3	6.59 (d, *J* = 2.1)	6.61 (d, *J* = 2.2)
4		
5	6.53 (dd, *J* = 8.4, 2.1)	6.54 (dd, *J* = 8.5, 2.2)
6	7.61 (d, *J* = 8.4)	7.63 (d, *J* = 8.5)
1′		
2′	7.58 (dd, *J* = 8.4, 1.8)	7.58 (d, *J* = 8.8)
3′	6.91 (dd, *J* = 8.4, 1.8)	6.92 (d, *J* = 8.8)
4′		
5′	6.91 (dd, *J* = 8.4, 1.8)	6.92 (d, *J* = 8.8)
6′	7.58 (dd, *J* = 8.4, 1.8)	7.58 (d, *J* = 8.8)
C=O		
2-OMe	3.92 (s)	3.92 (s)

3.3.3 Compound MR3



Compound **MR3** was isolated as yellow viscous oil. The UV and IR spectra closely resembled those of compound **MR2**.

The ^{1}H and ^{13}C NMR spectra (Tables 39-40, Figures 81 and 82) of **MR3** were comparable with those of **MR2**. The difference was shown as the disappearance of the signals of *p*-disubstituted benzene ring in **MR2** and the appearance of 1,2,4-trisubstituted benzene ring at δ 6.86 (1H, d, J = 8.1 Hz), 6.98 (1H, dd, J = 8.1, 2.1 Hz) and 7.15 (1H, d, J = 2.1 Hz) assigning to H-5', H-6' and H-2', respectively. The HMBC spectrum showed correlations of proton at δ 7.53 (H-*β*) with carbons at δ 114.7 (C-2'), 121.1 (C-6'), 124.4 (C-*α*), 127.5 (C-1'), and 190.6 (C=O) and the proton at 6.86 (H-5') showed correlations with carbons at δ 127.5 (C-1'), 145.0 (C-3') and 147.4 (C-4'), confirming the location of 1,2,4-trisubstituted benzene ring at C-*β*. Thus, **MR3** was characterized to be sappanchalcone (Namikoshi *et al.*, 1987b).



Selected HMBC correlation for compound **MR3**

Table 39 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **MR3**

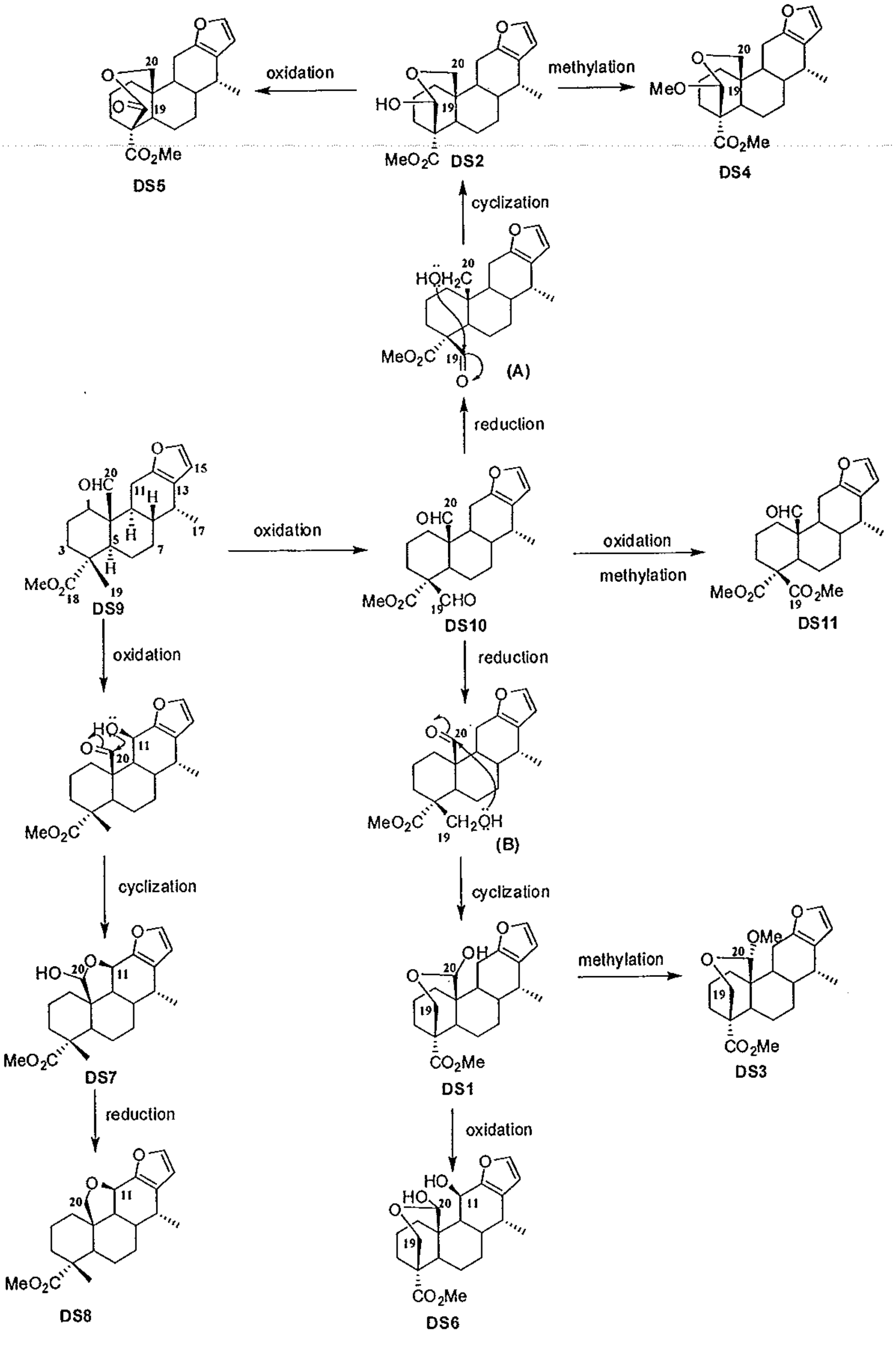
Position	δ_H (mult, J, Hz)	δ_C	DEPT	HMBC
α	7.34 (d, J = 15.6)	124.4	CH	β, C=O, 1′
β	7.53 (d, J = 15.6)	142.5	CH	α, C=O, 1′, 2′, 6′
1		120.7	C	
2		160.6	C	
3	6.49 (br s)	99.2	CH	C=O, 1, 2, 4, 5
4		162.5	C	
5	6.50 (dd, J = 8.1, 2.1)	108.0	CH	1, 3, 4
6	7.61 (d, J = 8.1)	132.5	CH	α, C=O, 2, 3
1′		127.5	C	
2′	7.15 (d, J = 2.1)	114.7	CH	β, 3′, 4′, 6′
3′		145.0	C	
4′		147.4	C	
5′	6.86 (d, J = 8.1)	115.6	CH	1′, 3′, 4′
6′	6.98 (dd, J = 8.1, 2.1)	121.8	CH	β, 2′, 4′
C=O		190.6	C	
OMe	3.78 (s)	55.1	CH_3	2, 3

Table 40 Comparison of ^{1}H NMR spectral data of compounds **MR2**, **MR3** and sappanchalcone (**R**, recorded in acetone-d_6)

Position	**MR2** **δ_H (mult, J, Hz)**	**MR3** **δ_H (mult, J, Hz)**	**R** **δ_H (mult, J, Hz)**
α	7.48 (d, J = 15.9)	7.34 (d, J = 15.6)	7.48 (d, J = 16.2)
β	7.57 (d, J = 15.9)	7.53 (d, J = 15.6)	7.56 (d, J = 16.2)
1			
2			
3	6.59 (d, J = 2.1)	6.49 (br s)	6.61 (d, J = 2.2)
4			
5	6.53 (dd, J = 8.4, 2.1)	6.50 (dd, J = 8.1, 2.1)	6.56 (dd, J = 8.5, 2.2)
6	7.61 (d, J = 8.4)	7.61 (d, J = 8.1)	7.67 (d, J = 8.5)
1′			
2′	7.58 (dd, J = 8.4, 1.8)	7.15 (d, J = 2.1)	7.26 (d, J = 2.0)
3′	6.91 (dd, J = 8.4, 1.8)		
4′			
5′	6.91 (dd, J = 8.4, 1.8)	6.86 (d, J = 8.1)	6.92 (d, J = 8.2)
6′	7.58 (dd, J = 8.4, 1.8)	6.98 (dd, J = 8.1, 2.1)	7.12 (dd, J = 8.2, 2.0)
C=O			
OMe	3.92 (s)	3.78 (s)	3.92 (s)

3.4 Proposed biogenesis of compounds from the seeds of *C. sappan*

Investigation of the seeds of *C. sappan* has revealed that cassane-type diterpenes are major components of this plant. The biosynthetic pathway (Devon and Scott, 1972) and partial synthetic approach (Johnston and Overton, 1973) of cassane-type diterpenes were proposed to be derived from the pimarane skeleton via 1,2-methyl migration from C-13 to C-14. The occurrence of furanoditerpene derivatives isolated can be discussed as follow: compound **DS9** is a precursor for biogenesis of compounds **DS1-DS8**, **DS10** and **DS1** (Scheme 7). Oxidation of Me-19 of **DS9** afforded **DS10** which was further oxidized and esterified to give **DS11**. Reduction of aldehydic C-19 of **DS10** to alcohol followed by cyclization resulted in **DS1** which upon further methylation gave **DS3**, whereas **DS6** was obtained upon oxidation at C-11 of **DS1**. On the other hand, reduction of aldehydic C-20 to alcohol followed by cyclization afforded **DS2** which was further methylated to give **DS4**, whereas **DS5** was obtained from oxidation of **DS2**. Oxidation at C-11 of **DS9** to alcohol, followed by cyclization resulted in **DS7** which was further reduced to **DS8**.



Scheme 7 Proposed biogenesis of **DS1-8, 10, 11** from **DS9** precursor

3.5 Bioactivities of isolated compounds from the heartwood and roots of *C. sappan*

In this research, we have isolated several compounds from *C. sappan* including diterpene and homoisoflavone groups. This plant has been reported to exhibit several biological activities. However, only anti-allergic and cytotoxic activities were chosen according to positive activity of the crude extracts.

3.5.1 Anti-allergic activity

The results are shown in **Table 41**. Of all metabolites evaluated, sappanchalcone (**MR3**) possessed the most potent anti-allergic activity against cell degranulation in RBL-2H3 cells with an IC_{50} value of 7.6 μM, followed by 3-deoxysappanchalcone (**MR2**, IC_{50} = 15.3 μM), whereas other compounds displayed moderate effects (IC_{50} = 32.4-58.8 μM) or inactive (IC_{50} >100 μM). Sappanchalcone (**MR3**, IC_{50} = 7.6 μM) displayed six-fold higher effect than ketotifen fumarate (IC_{50} = 47.5 μM), a clinically used drug. The compounds were also tested on β-hexosaminidase activity to clarify whether their effects were due to the inhibition of enzyme activity or of degranulation. As a result, these isolated compounds were inactive against the enzyme activity of β-hexosaminidase (Table 41).

Table 41 Anti-allergic activities of compounds (**DH1- DH6** and **MR1-MR3**) from heartwood and roots[a] of *C. sappan*

Compounds	**IC_{50} (μM)**	**Enzyme inhibition at 100 μM**
3-(3',4'-Dihydroxybenzyl)-7-hydroxychroman-4-one (**DH1**)	47.7	20.5
4-*O*-Methylepisappanol (**DH2**)	58.8	21.9
4-*O*-Methylsappanol (**DH3**)	62.8	21.7
3,4-Isopropylidene sappanol (**DH4**)	32.4	23.0
Brazilin (**DH5**)	>100	-
Protosappanin A (**DH6**)	47.4	19.0
Episappanol (**MR1**)	>100	-
3-Deoxysappanchalcone (**MR2**)	15.3	18.5
Sappanchalcone (**MR3**)	7.6	20.6
Ketotifen fumarate	47.5	15.8

[a]Each value represents mean ± S.E.M. of four determinations.

3.5.2 Cytotoxic activity

All isolated compounds were evaluated for their cytotoxicity against the human MCF-7 (breast adenocarcinoma), HeLa (human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer) cell lines by the sulphorhodamine B (SRB) assay (Skehan *et al.*, 1990). The results suggested that chalcone exhibited higher activity than homoisoflavone and diterpenes. Sappanchalcone (**MR3**) demonstrated significant inhibitory activity against MCF-7, HeLa, HT-29 and KB cell lines with IC_{50} value of 0.64, 0.72, 0.80 and 0.75 μg/ml, respectively. The rest of the compounds were inactive.

CHAPTER 4

CONCLUSION

Eleven new cassane-type diterpenes, named phanginin A-K (**DS1-DS11**) and nine known compounds of seven homoisoflavones: 3-(3',4'-dihydroxybenzyl)-7-hydroxychroman-4-one (**DH1**), 4-*O*-methylepisappanol (**DH2**), 4-*O*-methylsappanol (**DH3**), 3,4-isopropylidene sappanol (**DH4**), brazilin (**DH5**), protosappanin A (**DH6**) episappanol (**MR1**) and and two chalcones: 3-deoxysappanchalcone (**MR2**) and sappanchalcone (**MR3**) were isolated from the *C. sappan*. Their structures were elucidated on the basis of spectroscopic techniques. In addition, the X-ray structure of phanginin A (**DS1**) was also reported. It was found that chalcone groups especially sappanchalcone (**MR3**) possessed the most potent anti-allergic activity against cell degranulation in RBL-2H3 cells with an IC_{50} value of 7.6 μM, followed by 3-deoxysappanchalcone (**MR2**, IC_{50} = 15.3 μM), whereas other compounds displayed moderate effects (IC_{50} = 32.4-58.8 μM) or inactive (IC_{50} >100 μM). Moreover, sappanchalcone (**MR3**) demonstrated significant inhibitory activity against MCF-7, HeLa, HT-29 and KB cell lines with IC_{50} values of 0.64, 0.72, 0.80 and 0.75 μg/ml, respectively. The rest of the compounds were inactive.

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APPENDIX

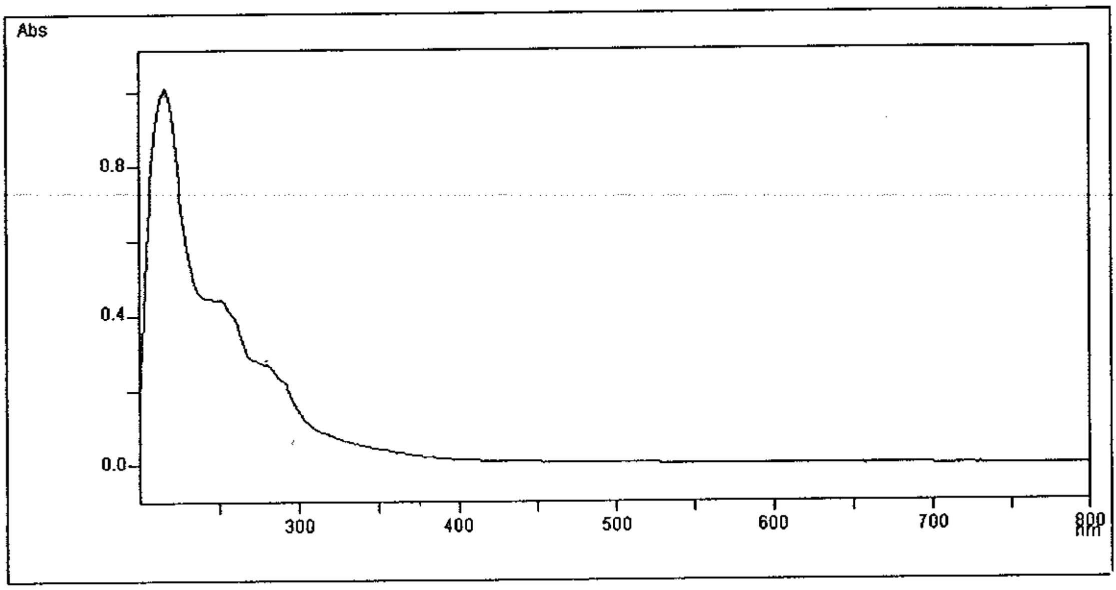

Figure 3 UV (MeOH) spectrum of compound **DS1**

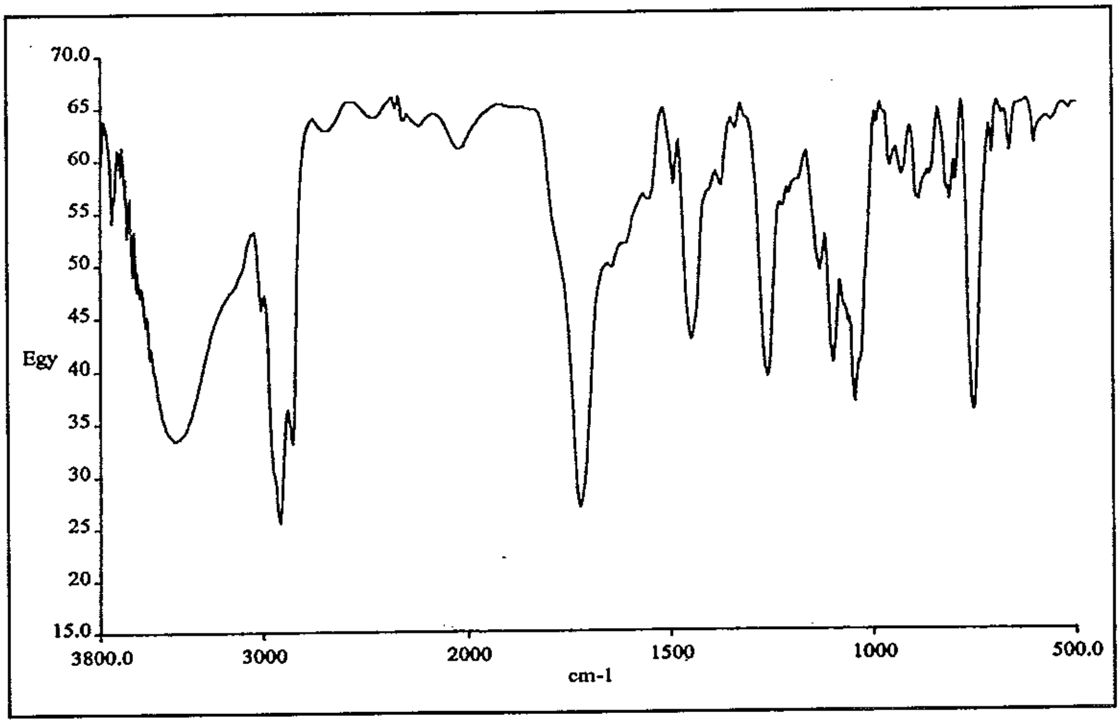

Figure 4 IR (neat) spectrum of compound **DS1**

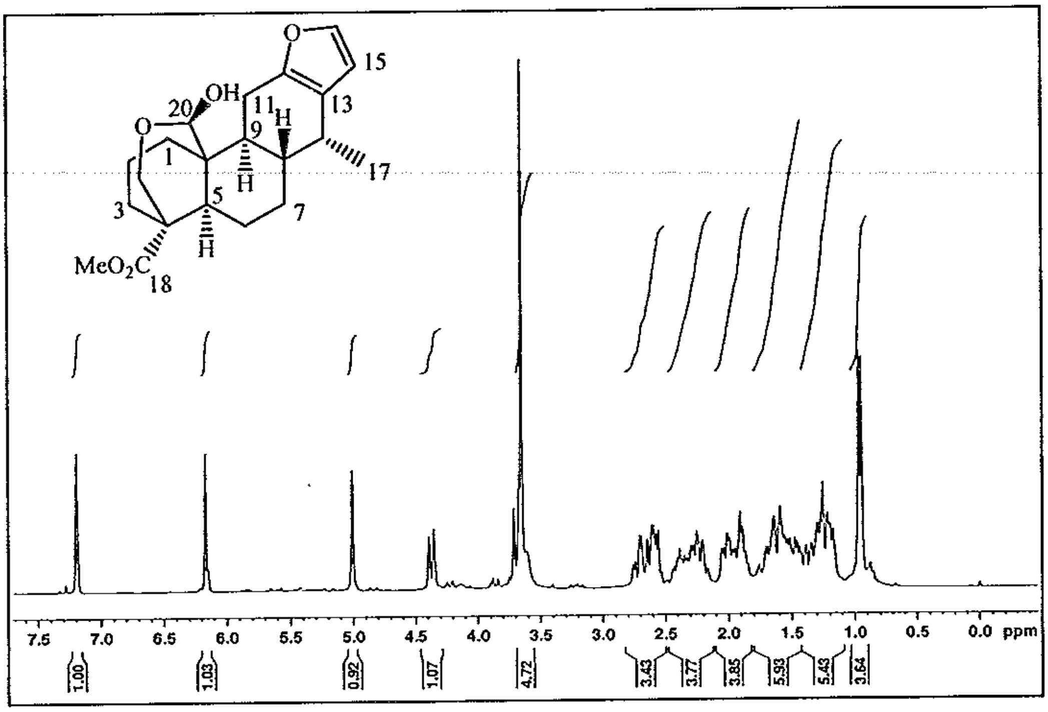

Figure 5 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS1**

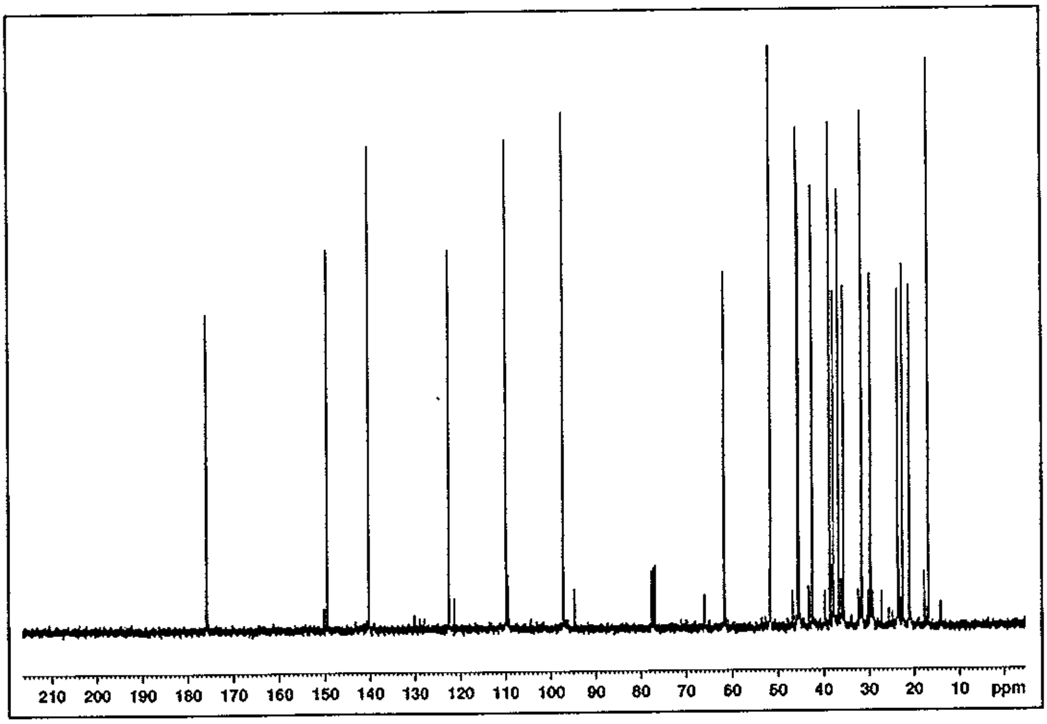

Figure 6 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS1**

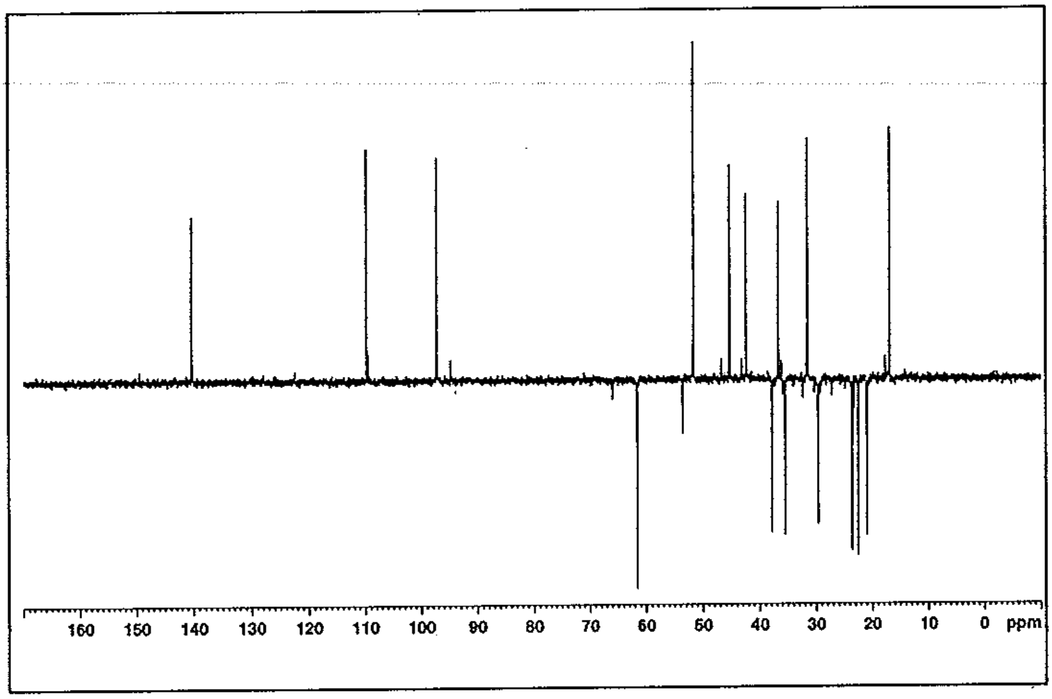

Figure 7 DEPT 135° ($CDCl_3$) spectrum of compound **DS1**

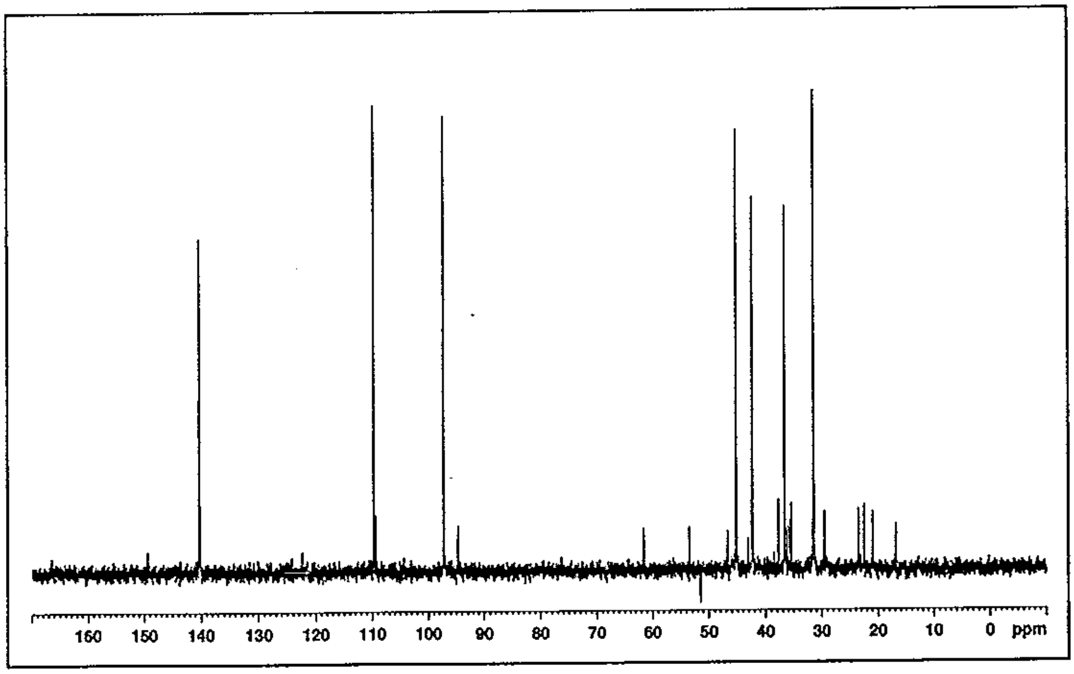

Figure 8 DEPT 90° ($CDCl_3$) spectrum of compound **DS1**

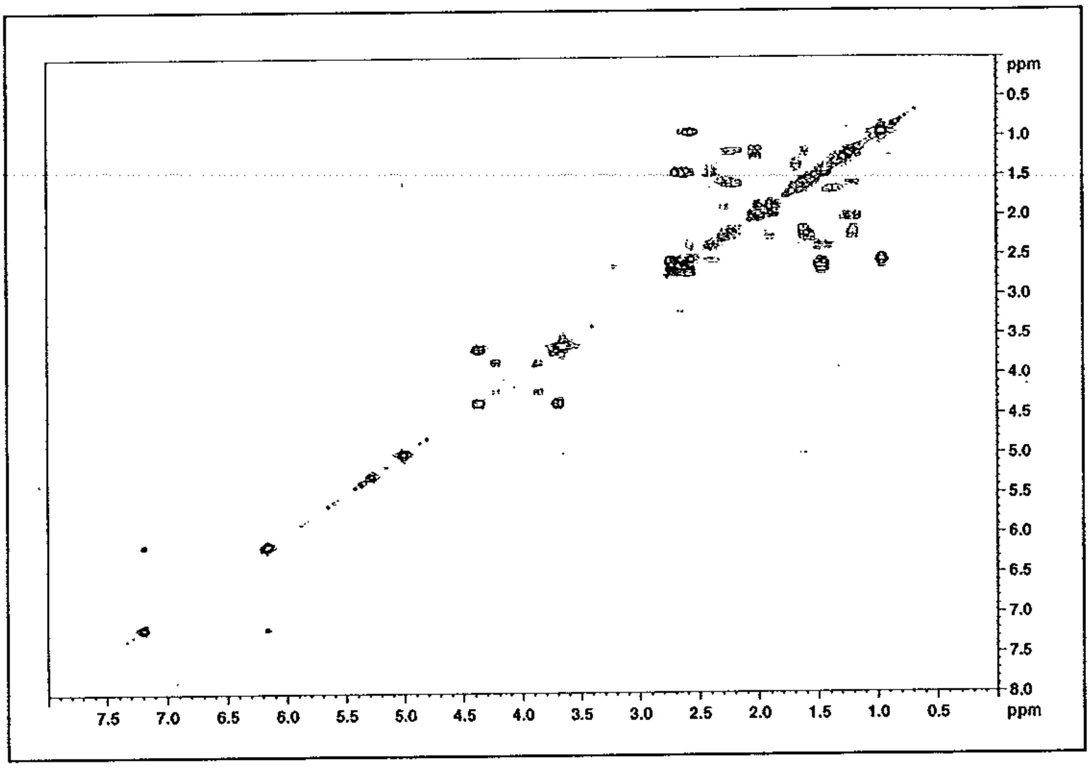

Figure 9 2D COSY ($CDCl_3$) spectrum of compound **DS1**

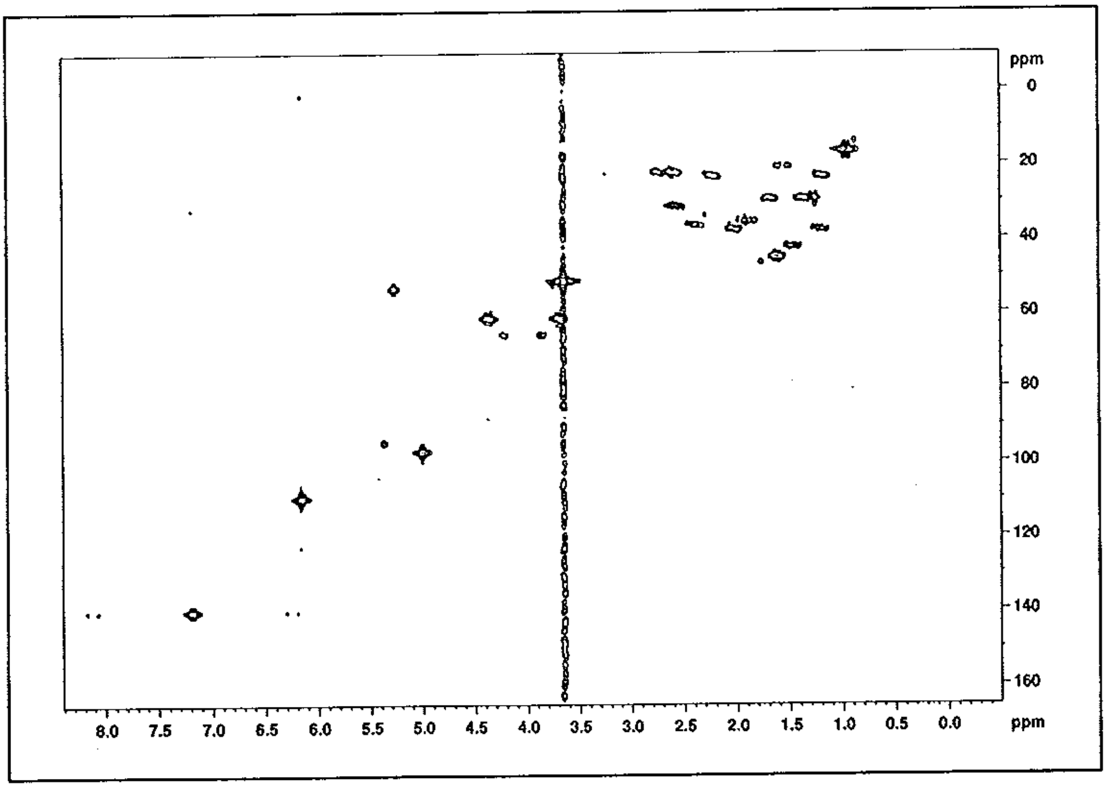

Figure 10 2D HMQC ($CDCl_3$) spectrum of compound **DS1**

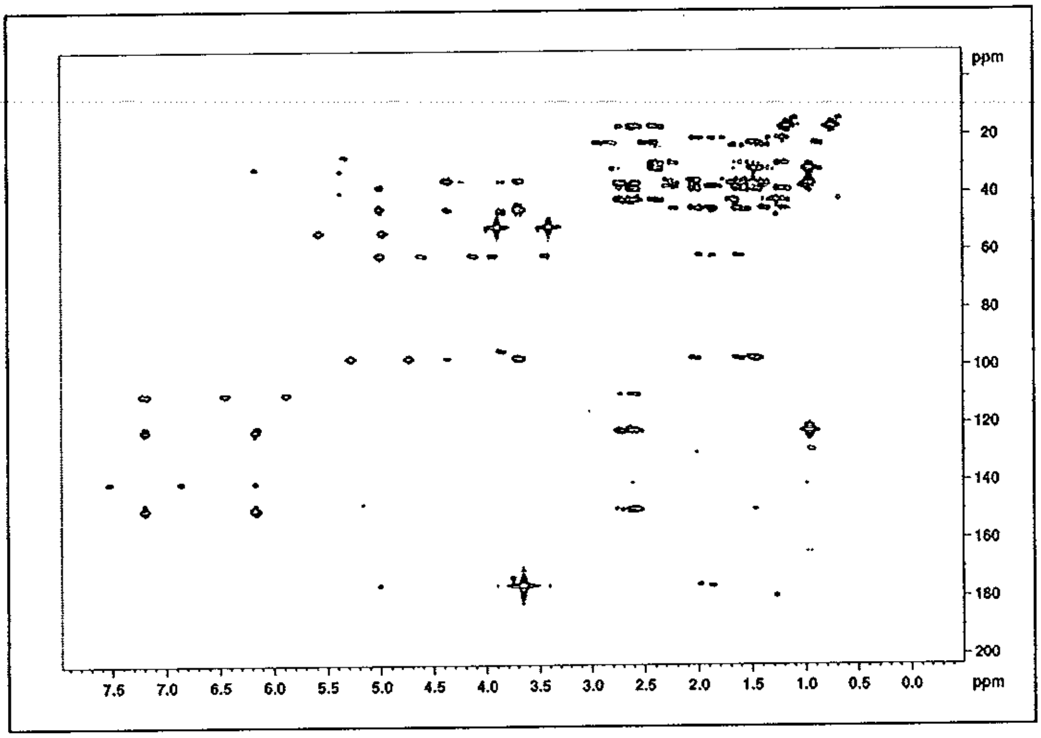

Figure 11 2D HMBC (CDCl$_3$) spectrum of compound **DS1**

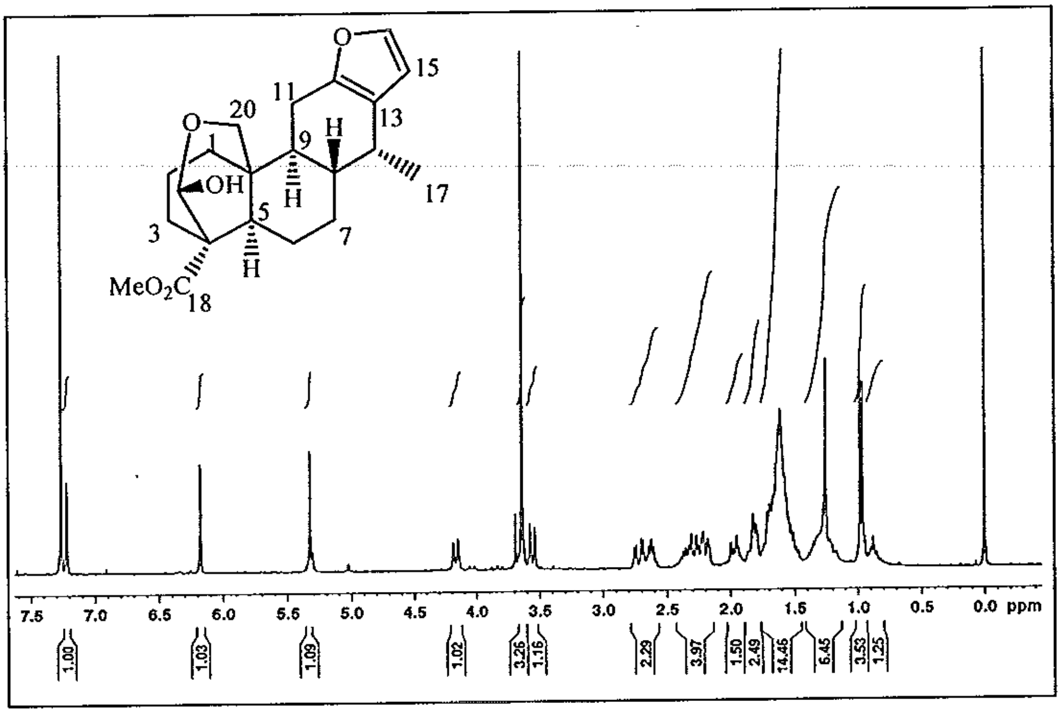

Figure 12 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS2**

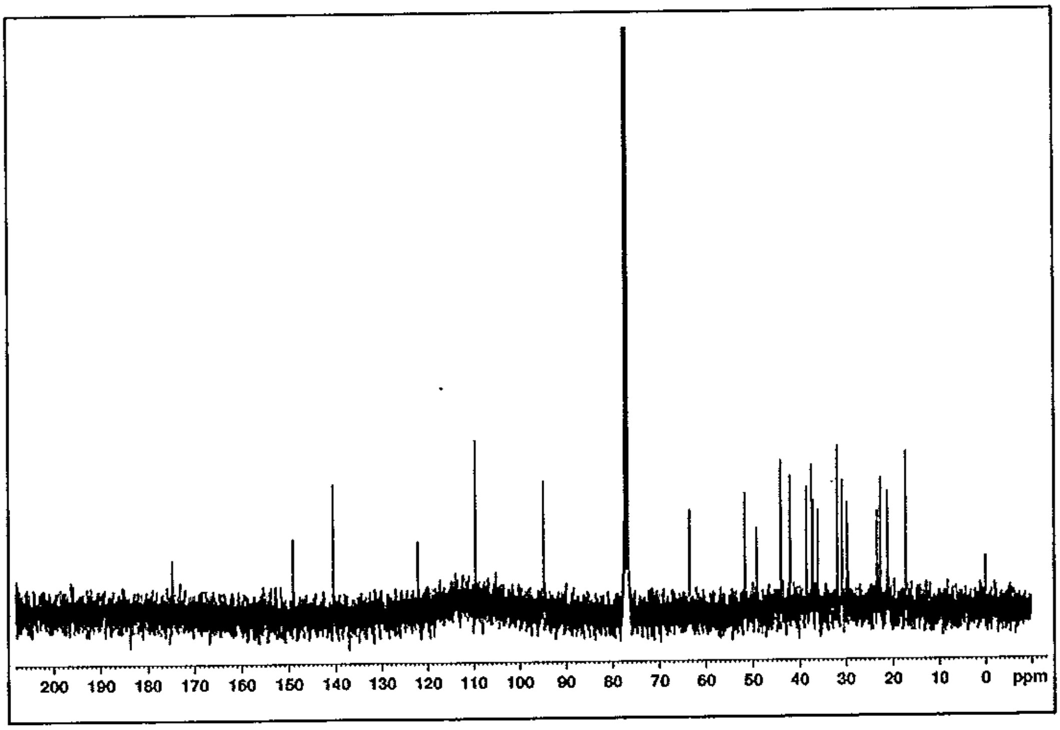

Figure 13 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS2**

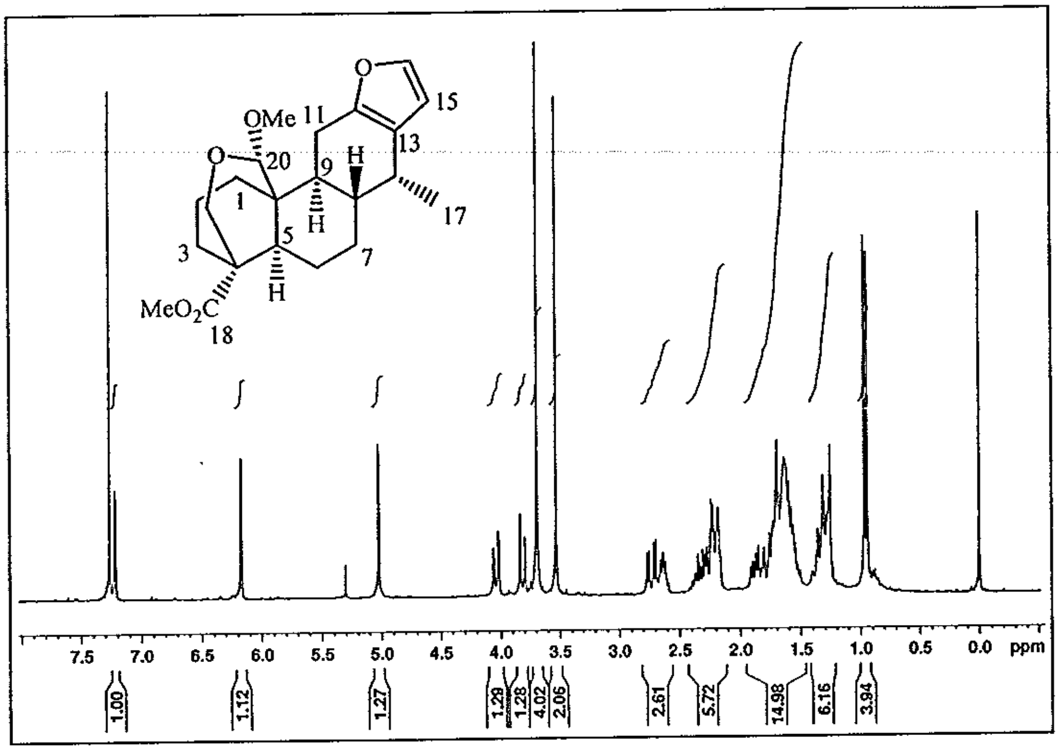

Figure 14 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS3**

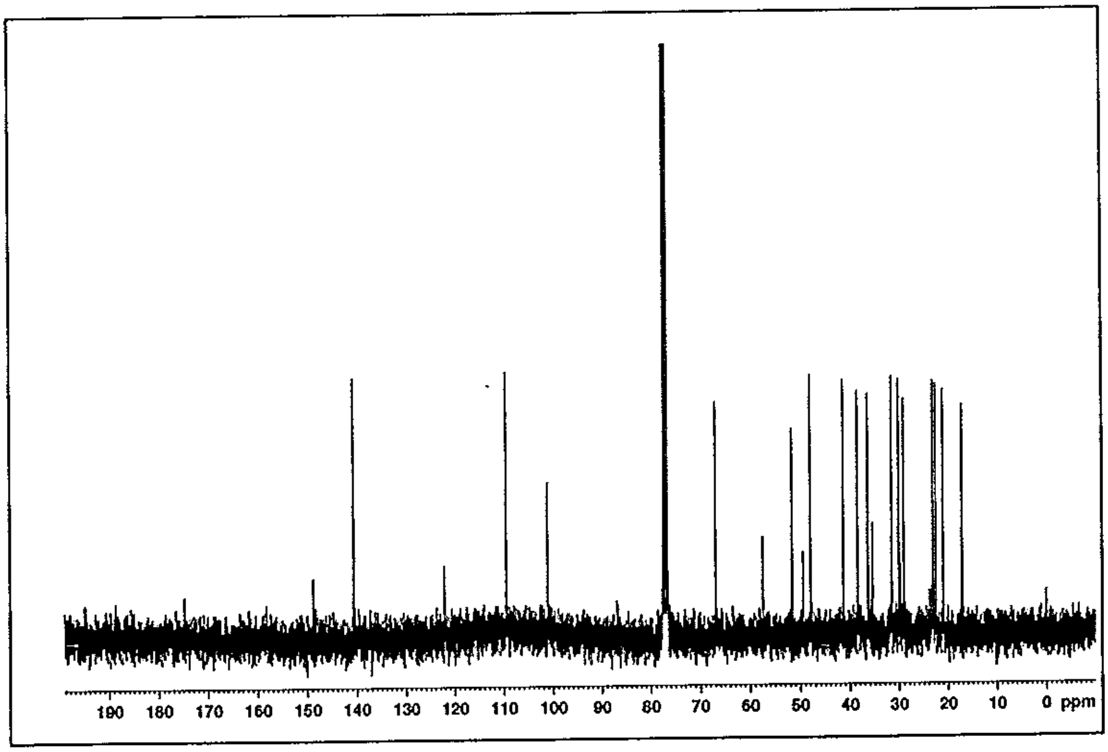

Figure 15 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS3**

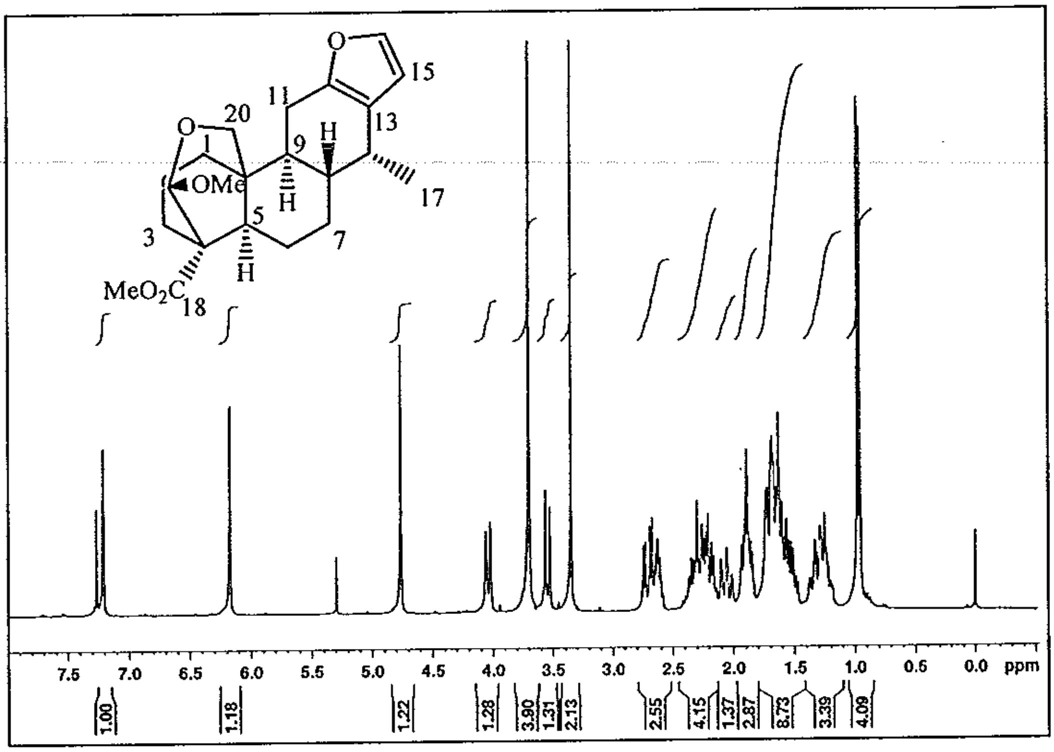

Figure 16 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS4**

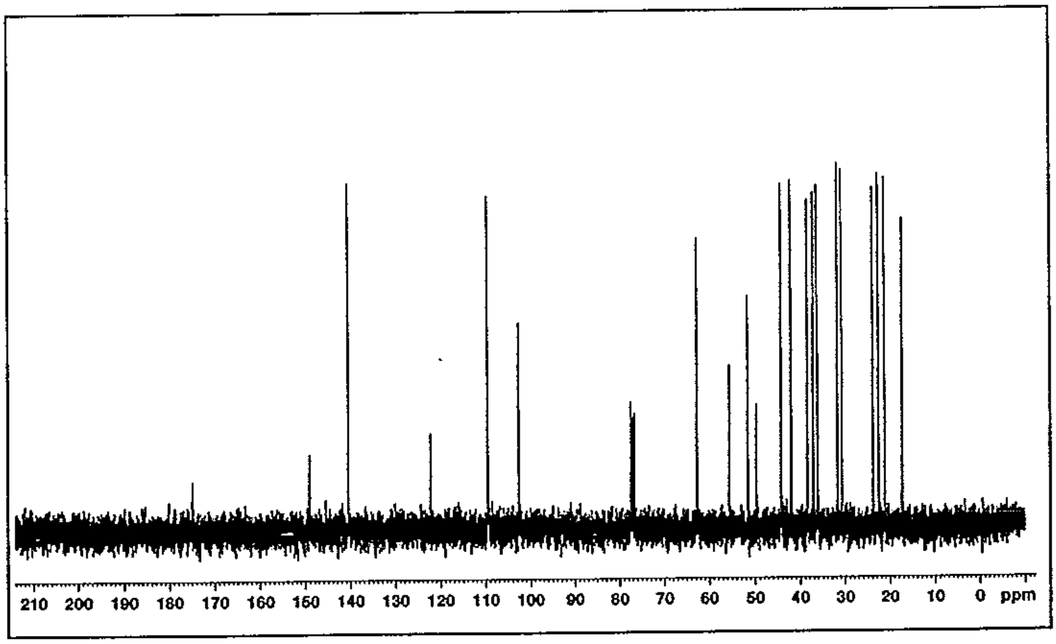

Figure 17 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS4**

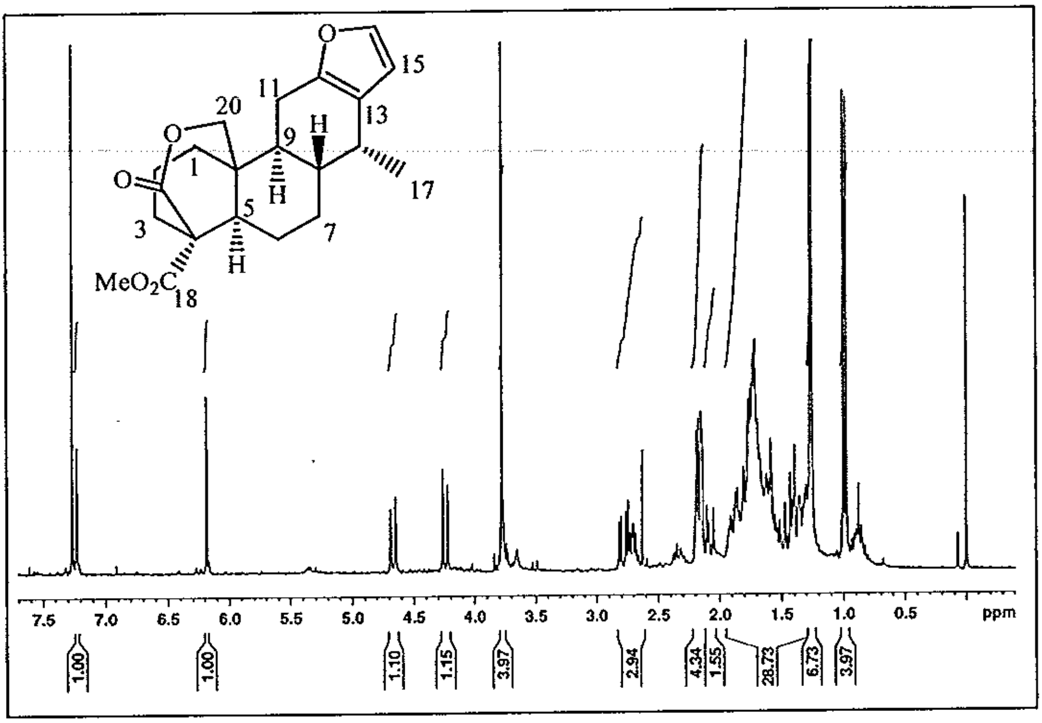

Figure 18 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS5**

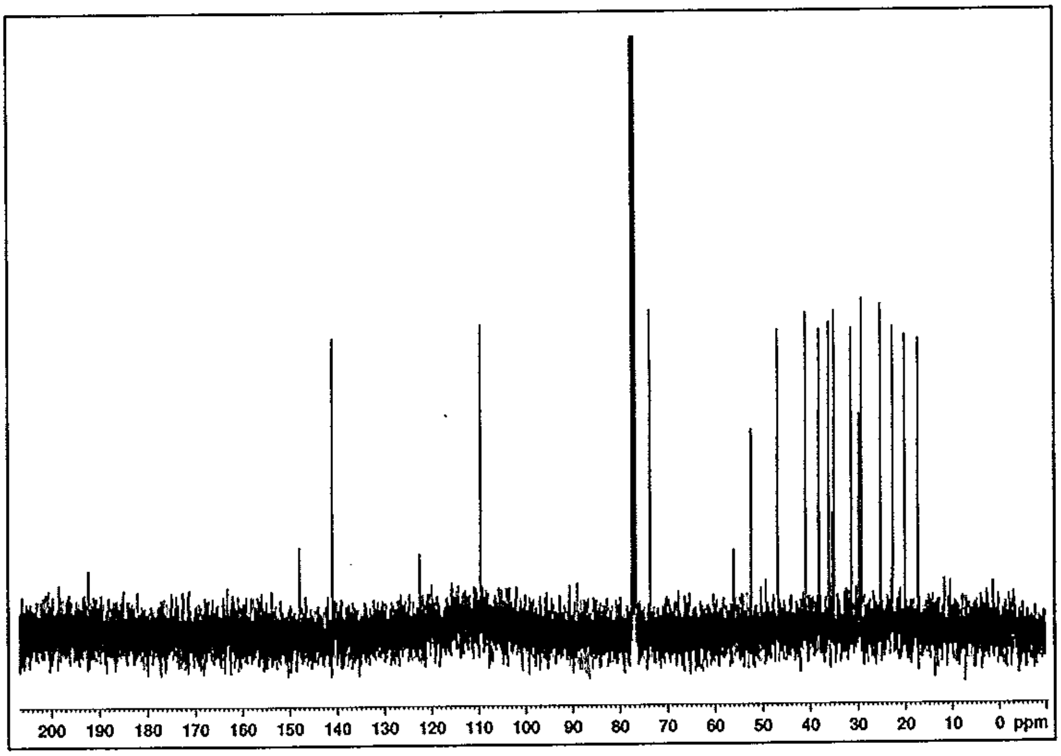

Figure 19 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS5**

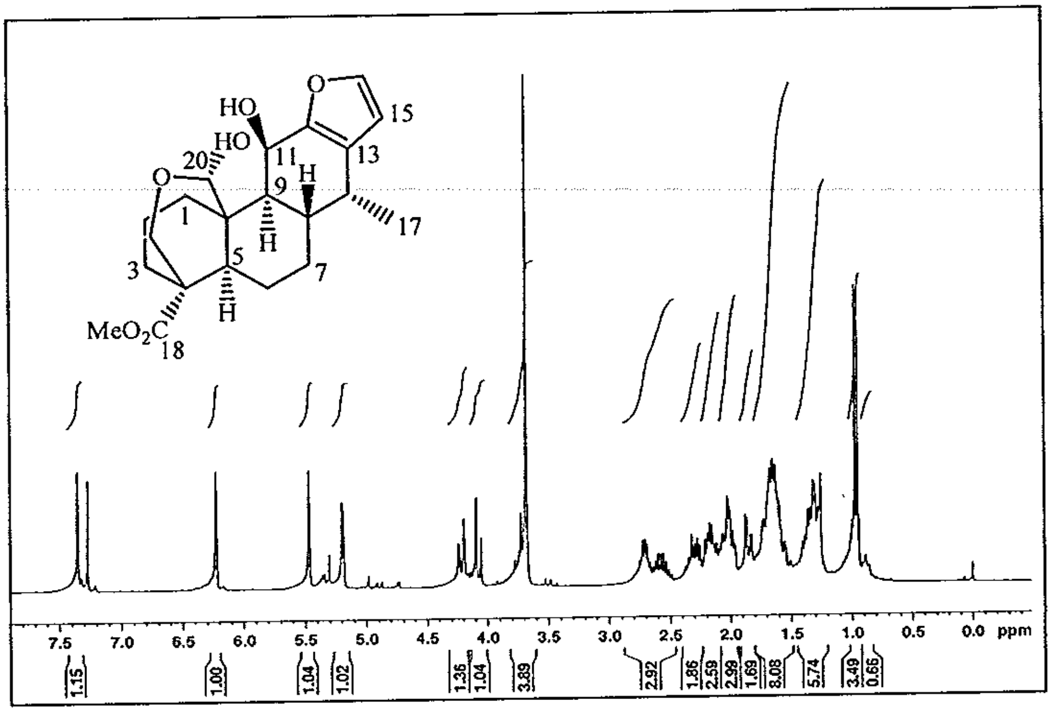

Figure 20 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DS6**

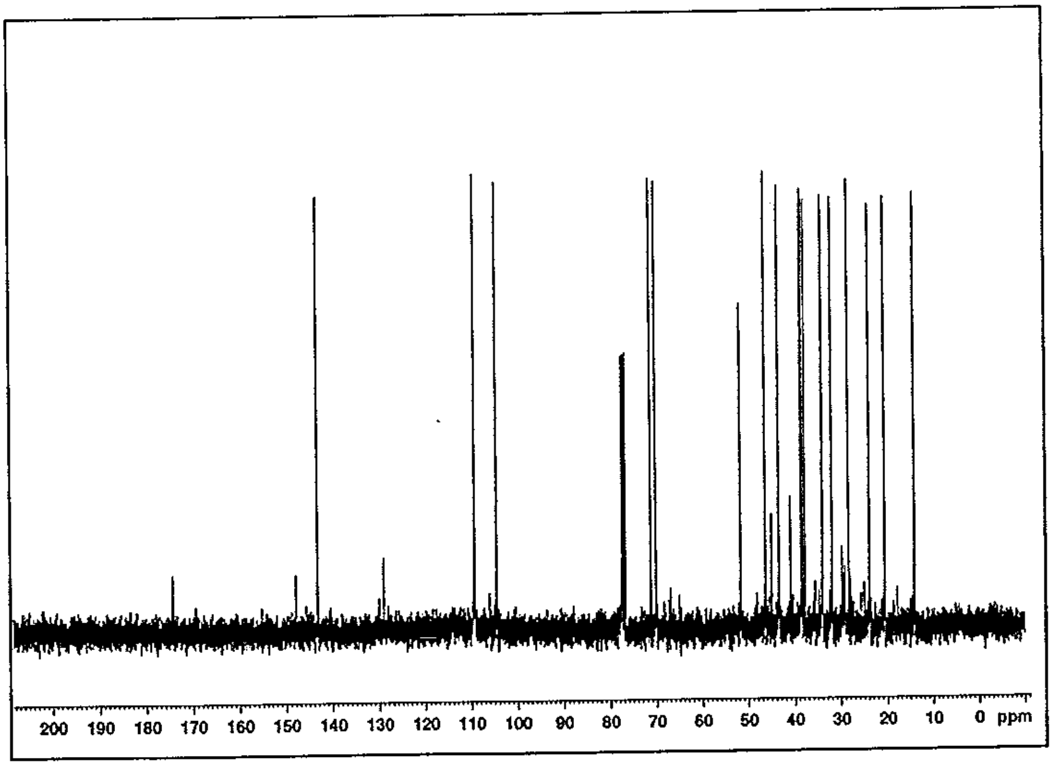

Figure 21 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DS6**

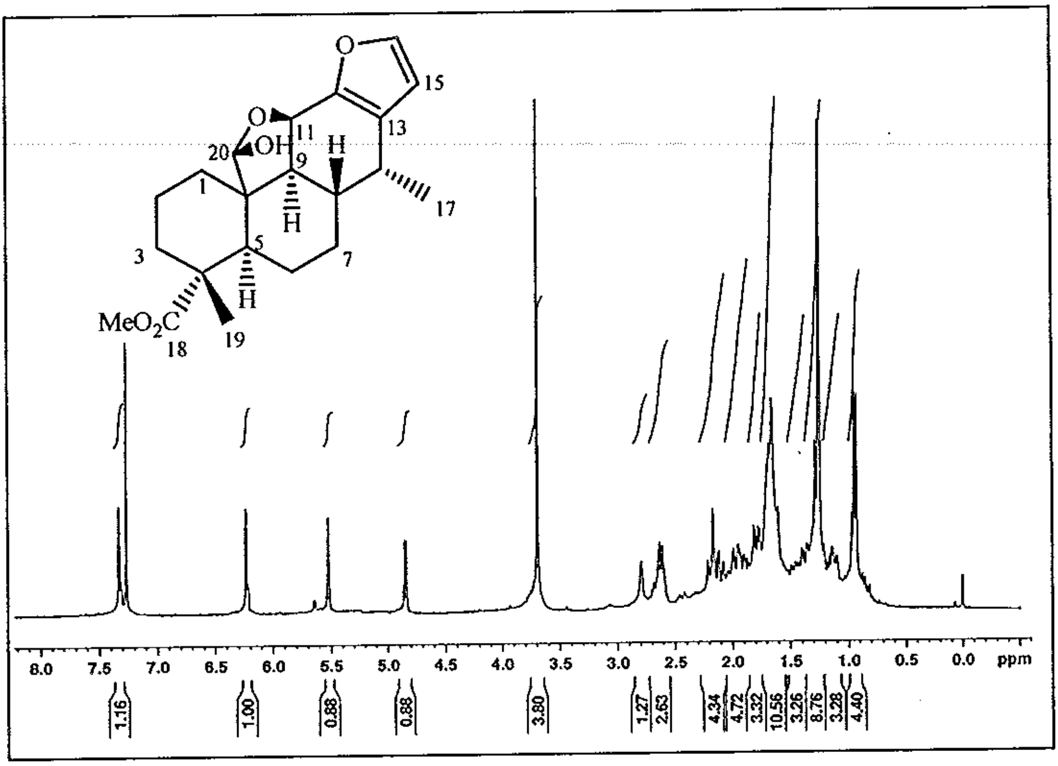

Figure 22 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS7**

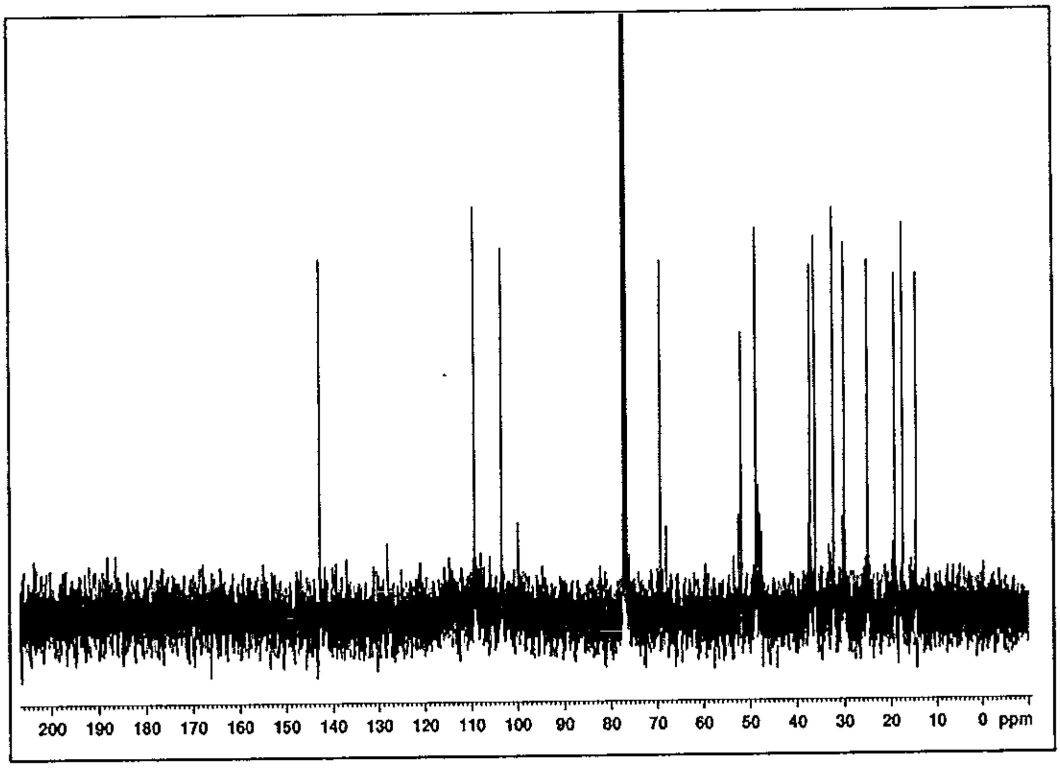

Figure 23 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS7**

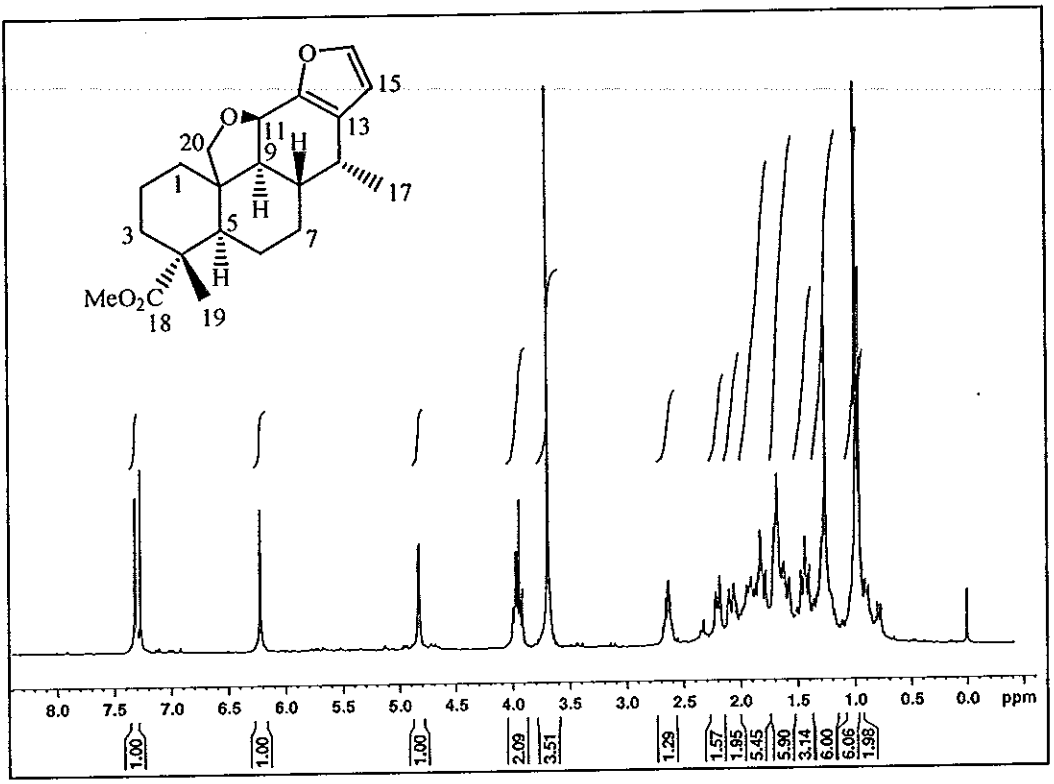

Figure 24 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS8**

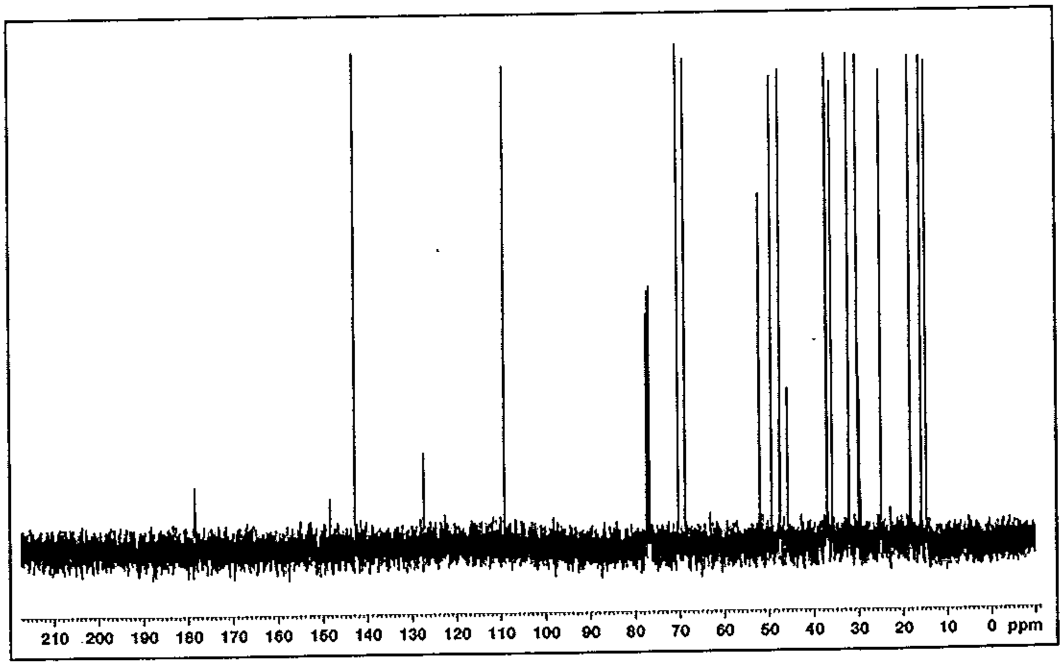

Figure 25 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS8**

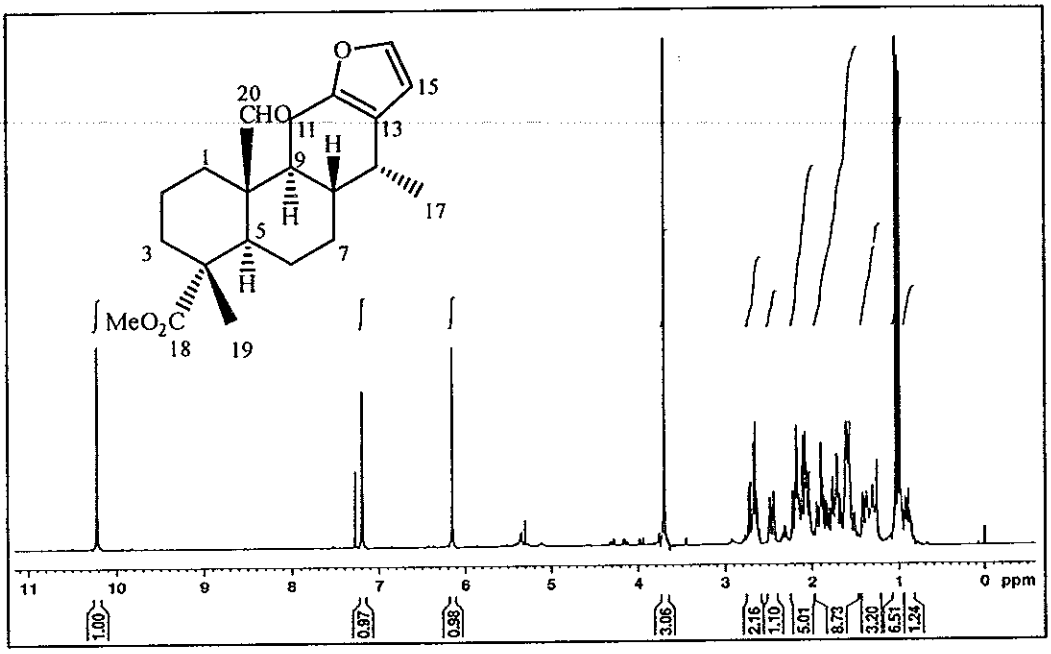

Figure 26 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS9**

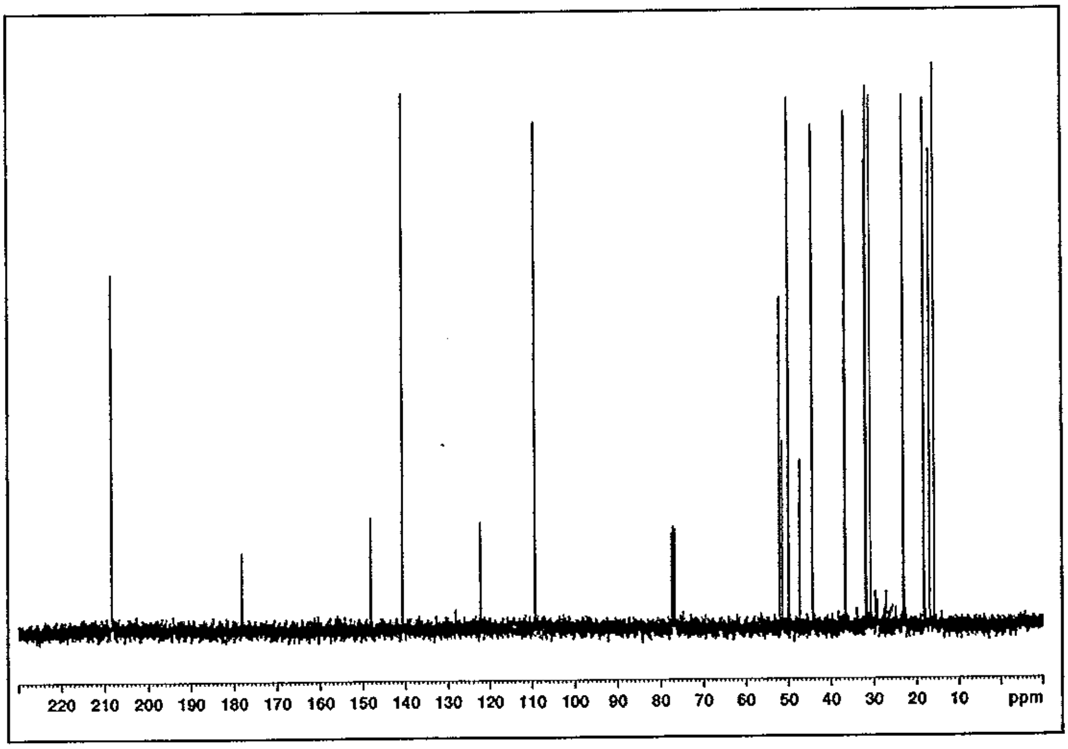

Figure 27 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS9**

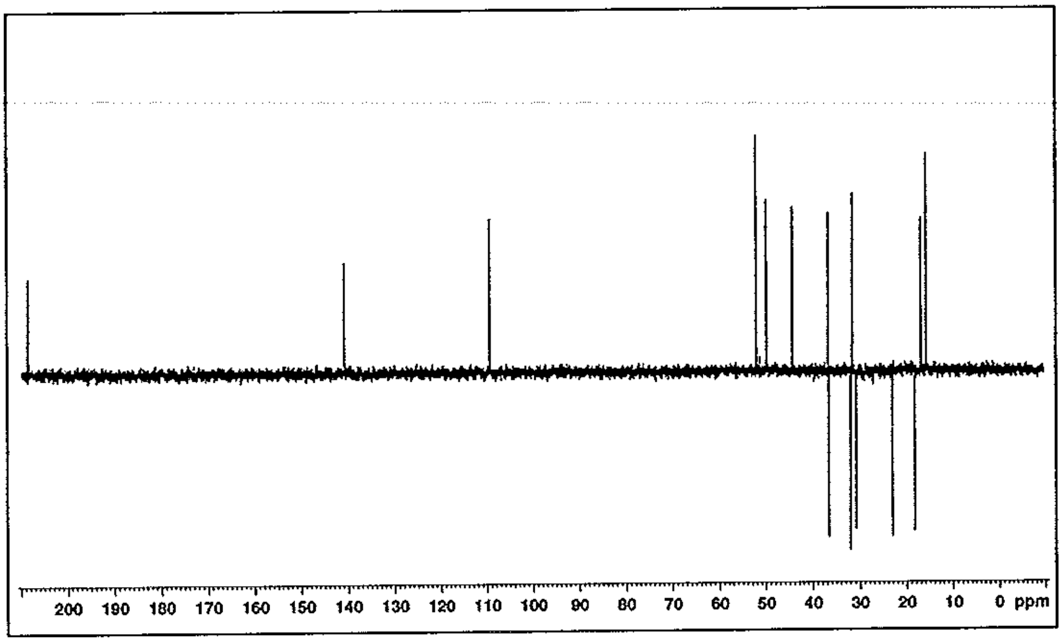

Figure 28 DEPT 135° ($CDCl_3$) spectrum of compound **DS9**

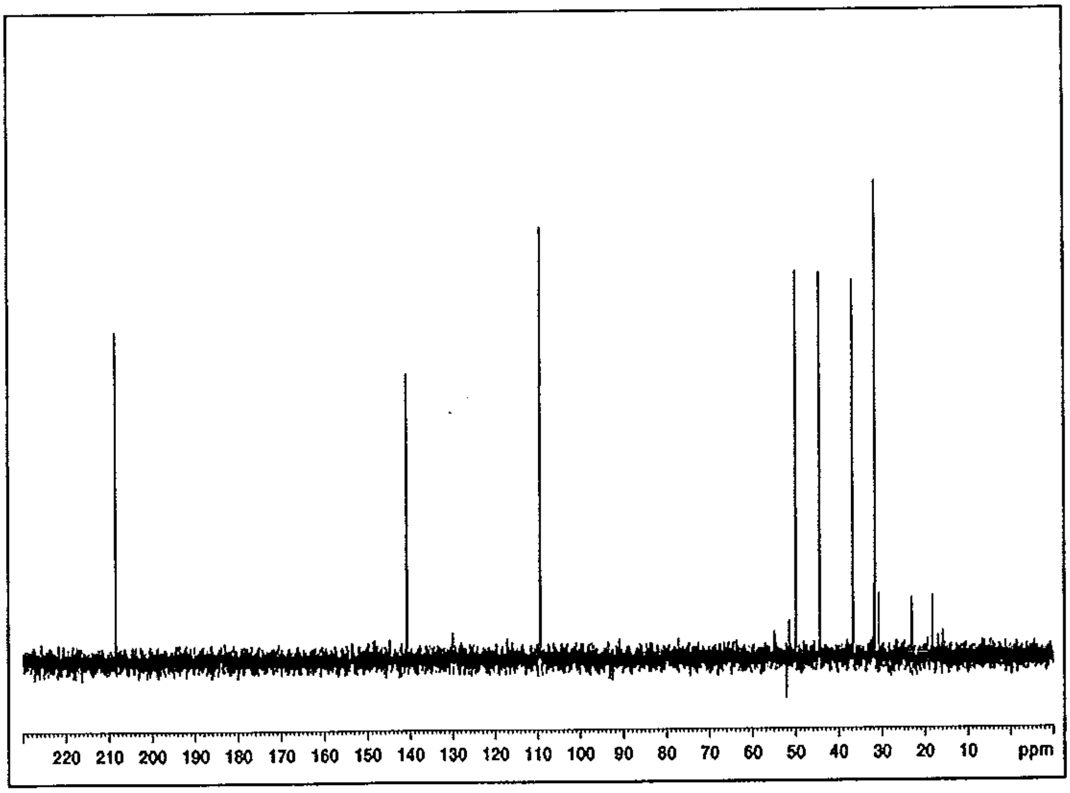

Figure 29 DEPT 90° ($CDCl_3$) spectrum of compound **DS9**

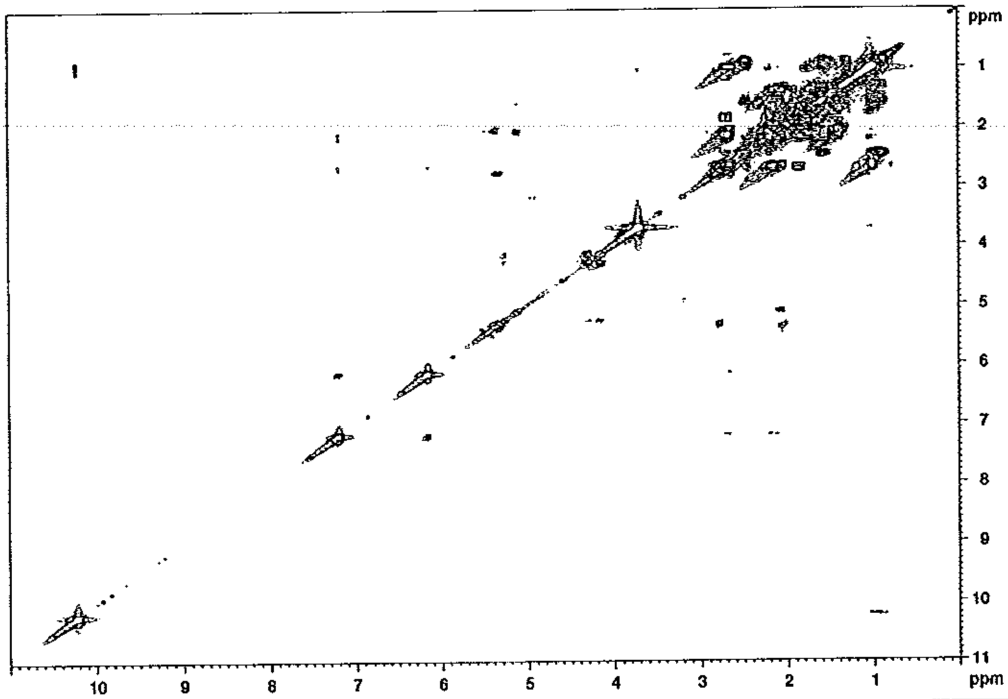

Figure 30 2D COSY ($CDCl_3$) spectrum of compound **DS9**

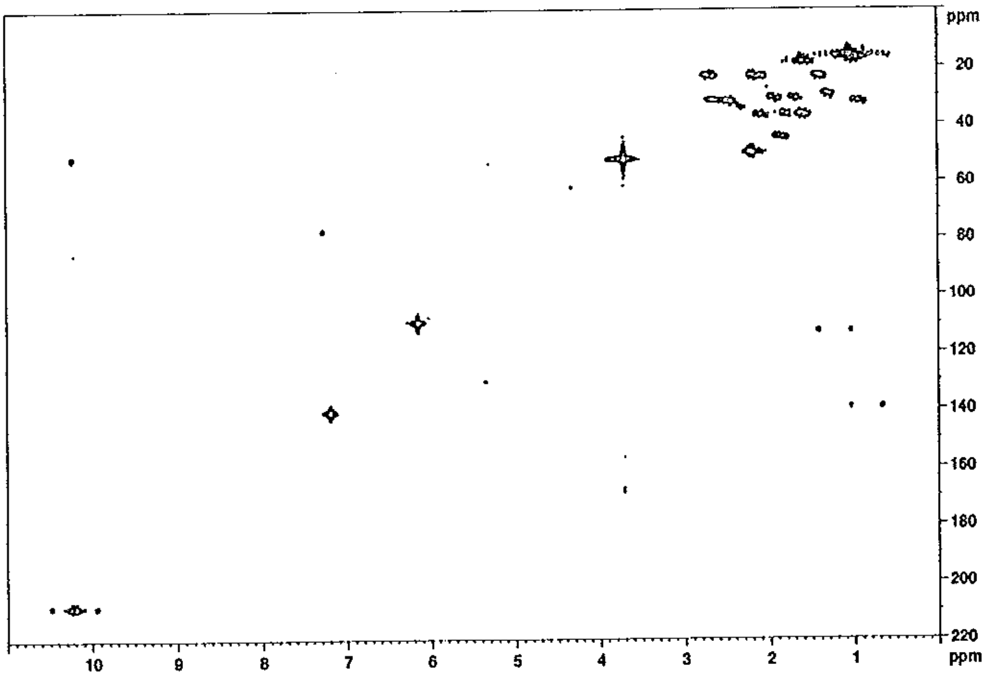

Figure 31 2D HMQC ($CDCl_3$) spectrum of compound **DS9**

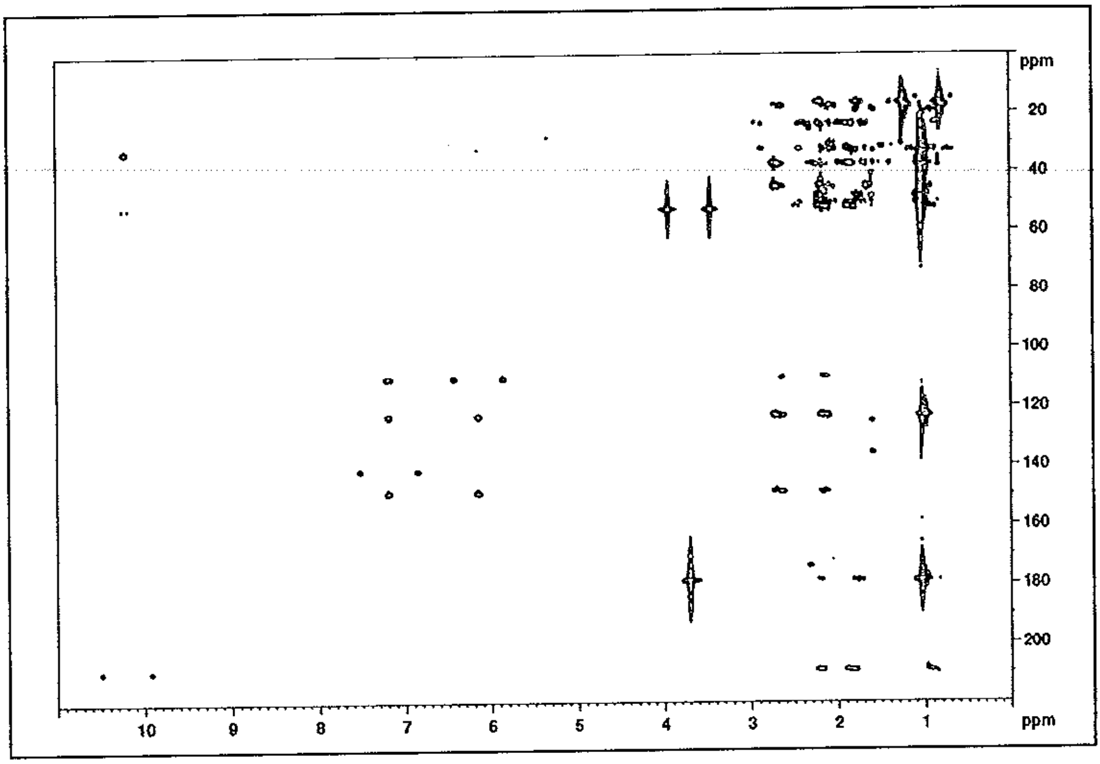

Figure 32 2D HMBC ($CDCl_3$) spectrum of compound **DS9**

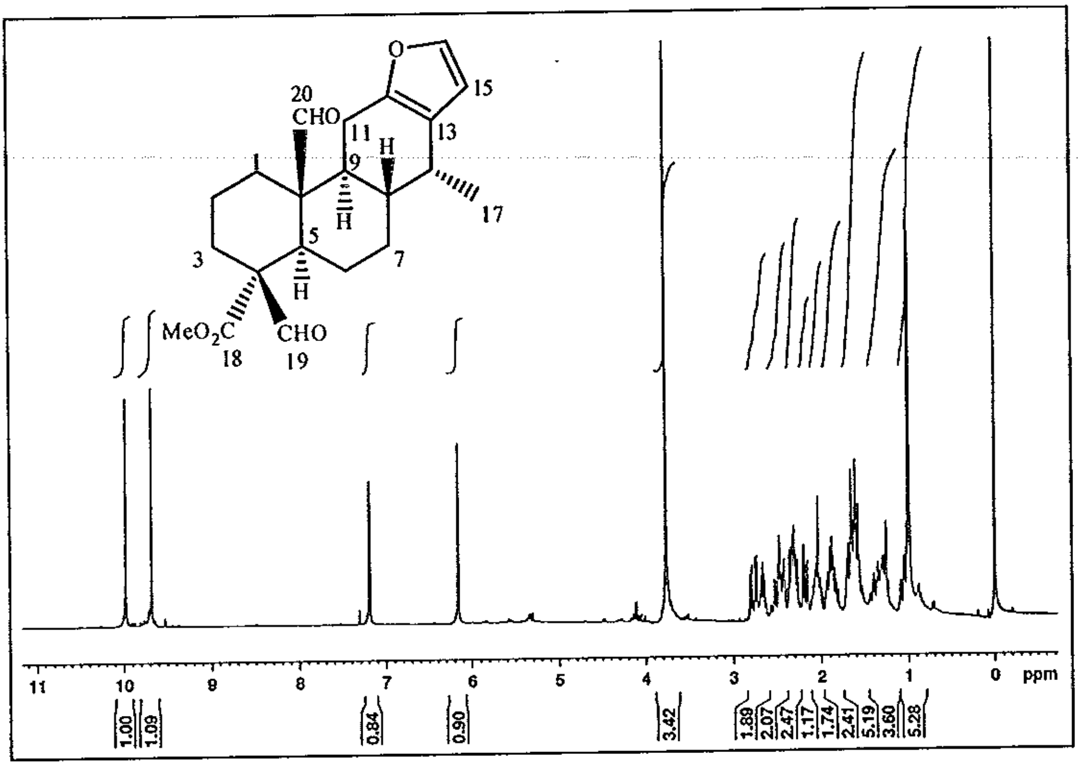

Figure 33 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS10**

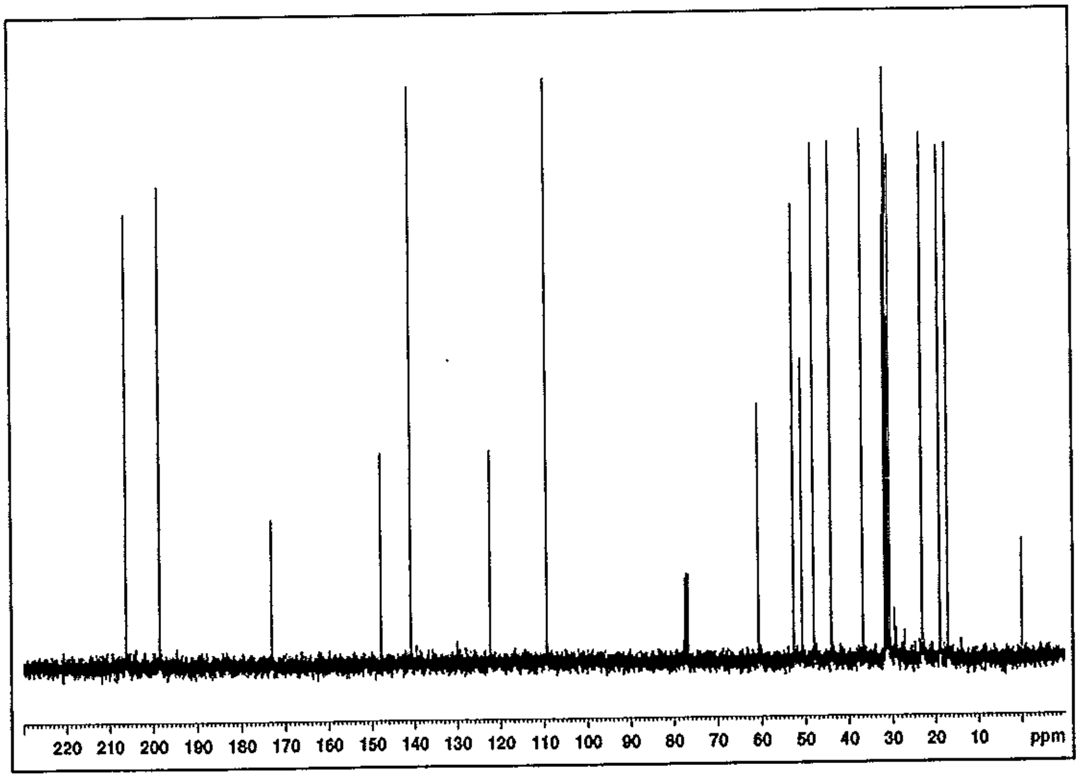

Figure 34 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS10**

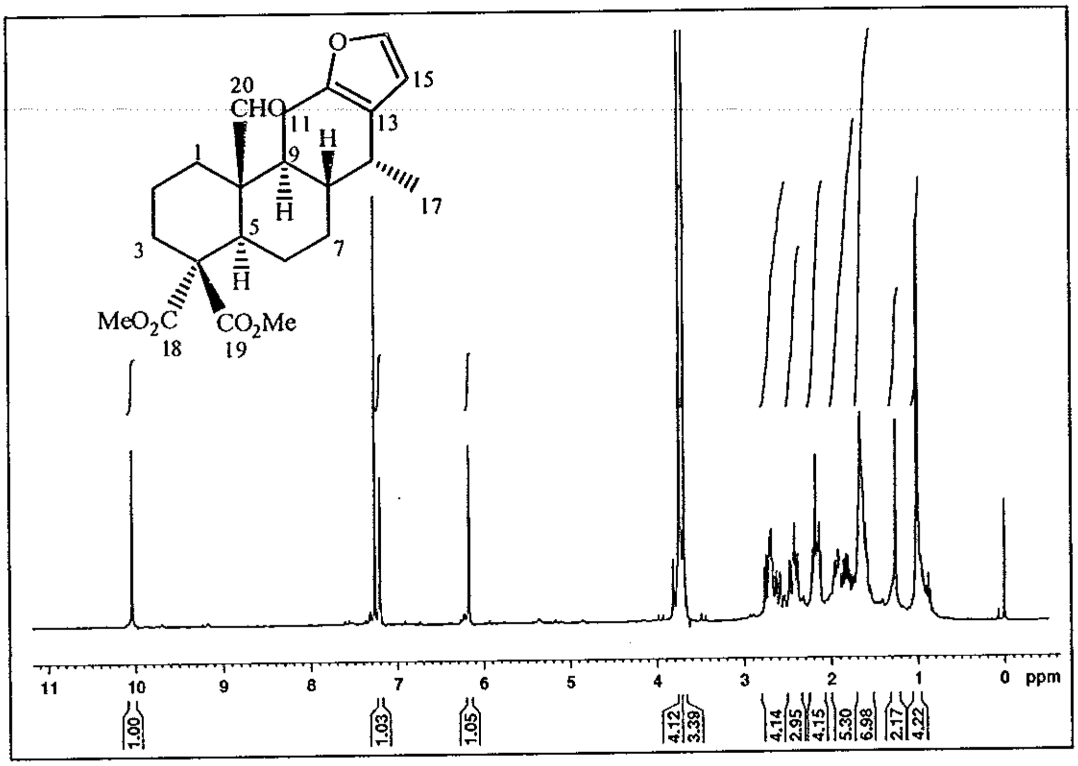

Figure 35 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **DS11**

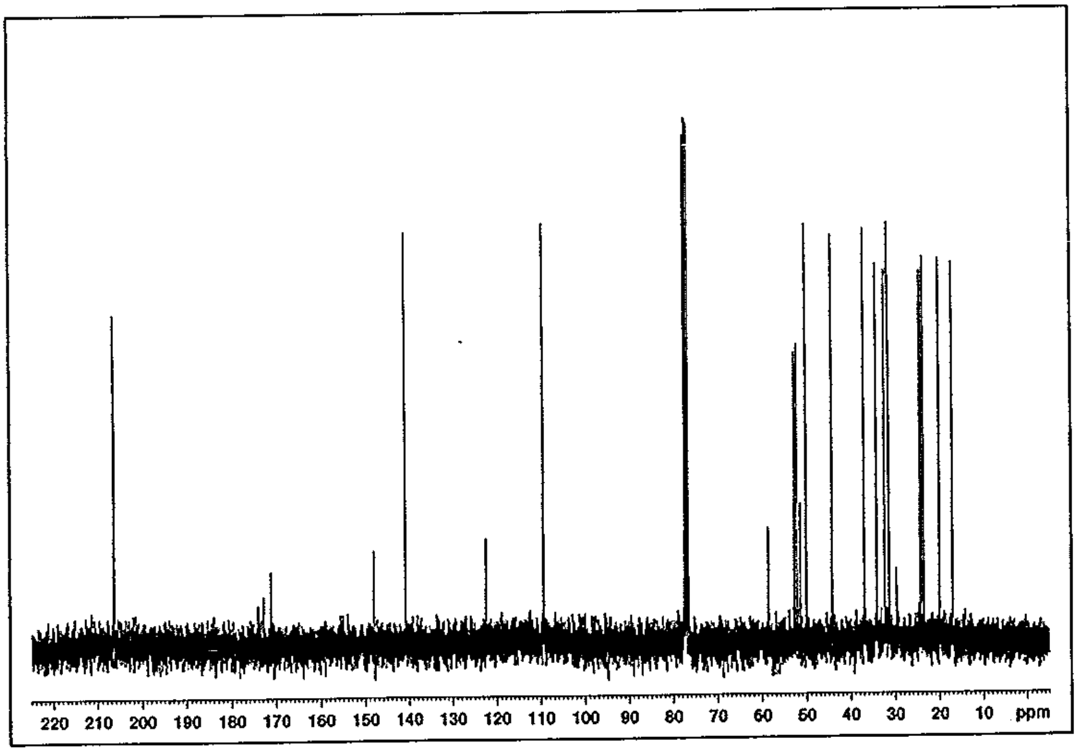

Figure 36 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **DS11**

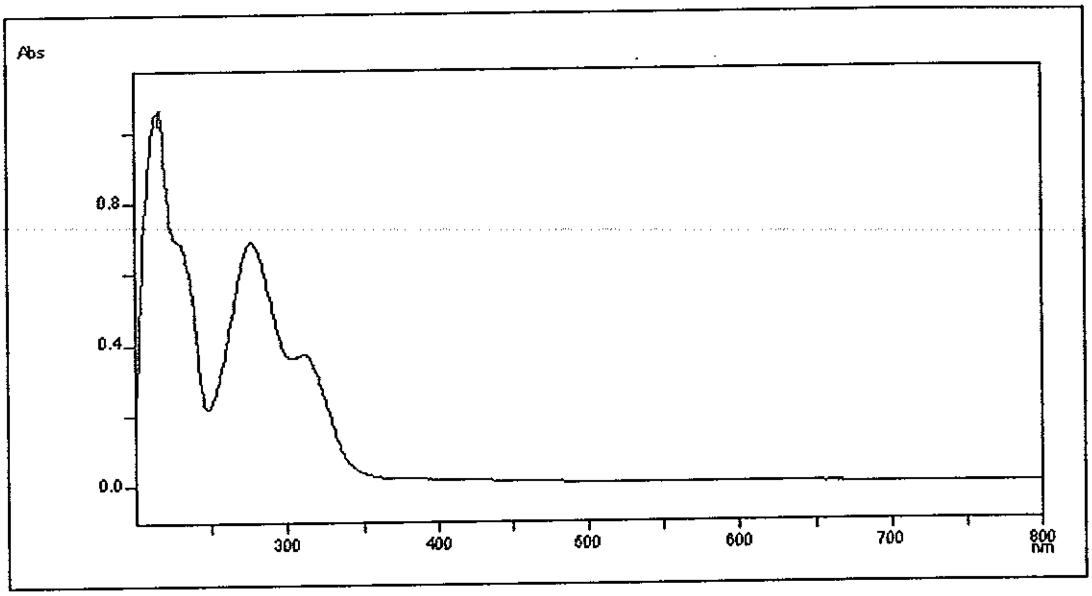

Figure 37 UV (MeOH) spectrum of compound **DH1**

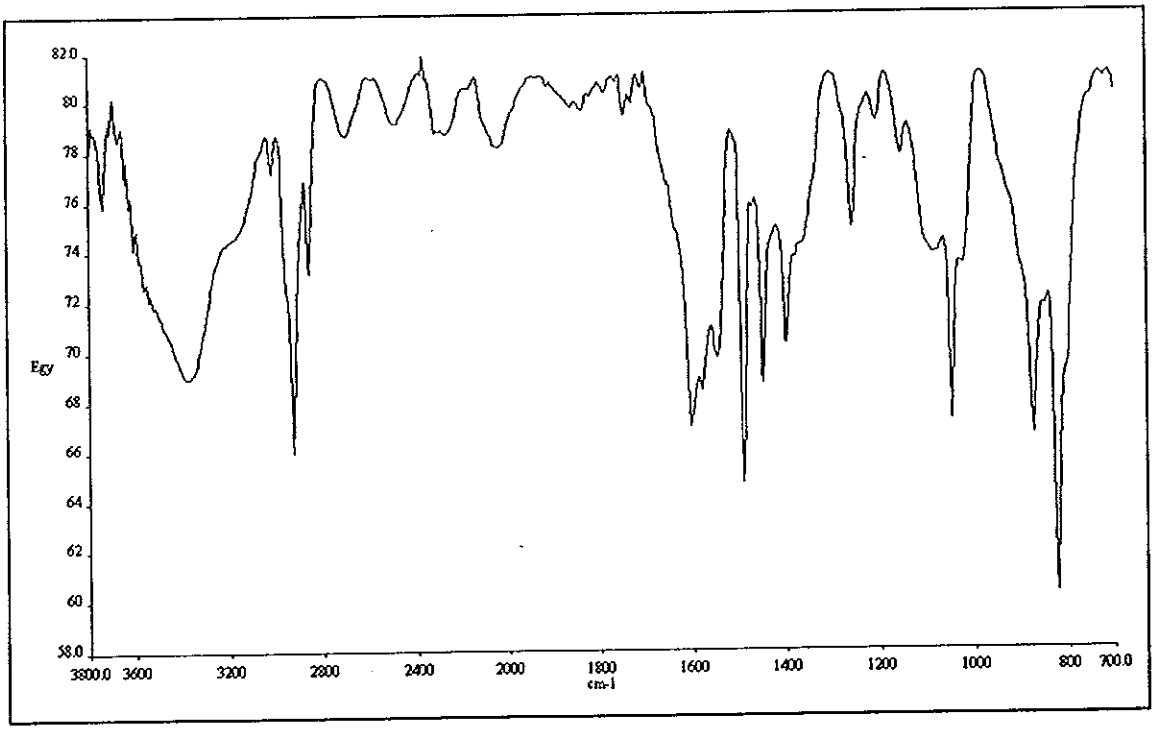

Figure 38 IR (neat) spectrum of compound **DH1**

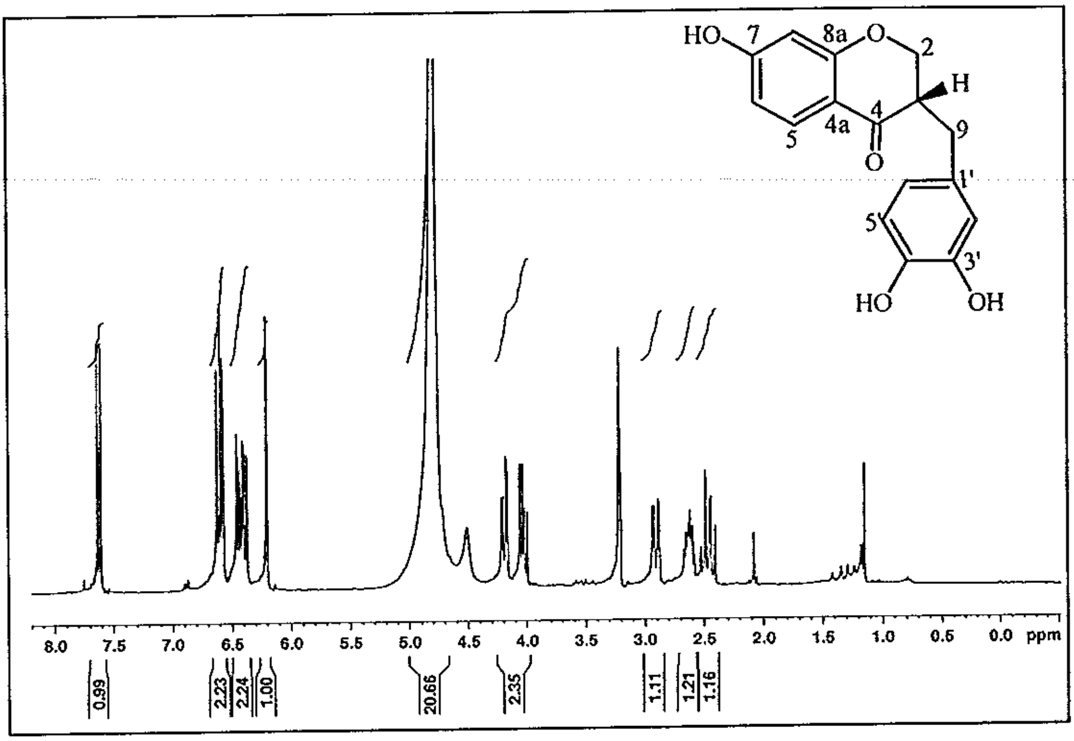

Figure 39 ^{1}H NMR (300 MHz) ($CD_3OD + CDCl_3$) spectrum of compound **DH1**

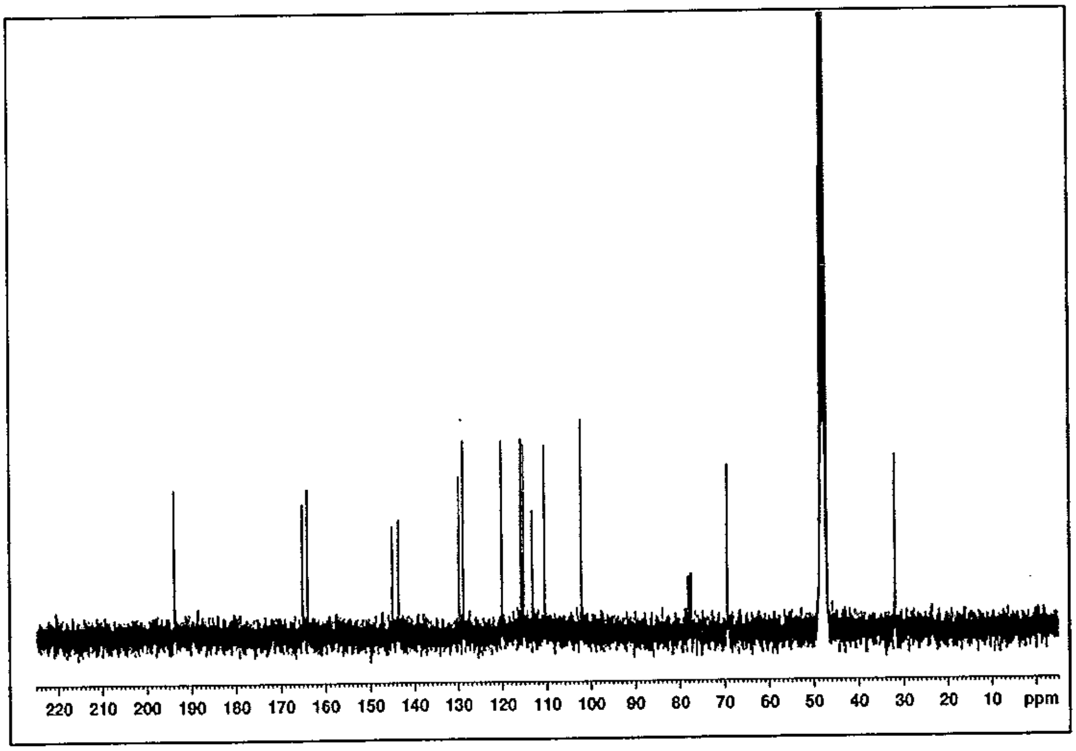

Figure 40 ^{13}C NMR (75 MHz) ($CD_3OD + CDCl_3$) spectrum of compound **DH1**

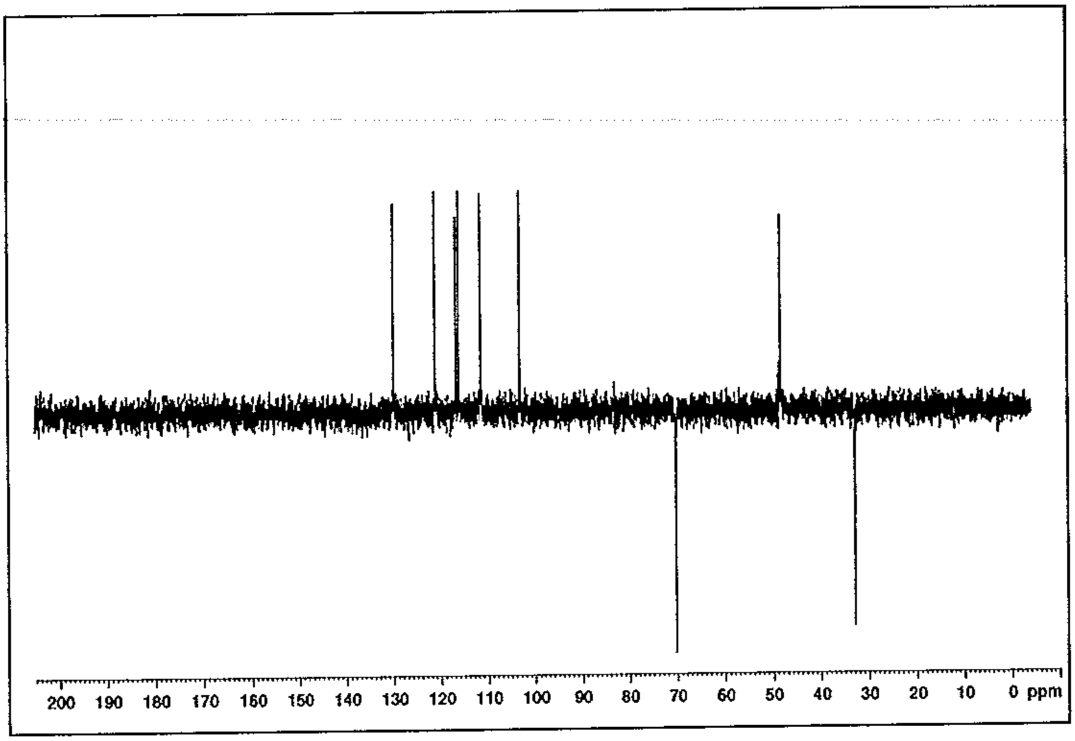

Figure 41 DEPT 135° ($CD_3OD + CDCl_3$) spectrum of compound **DH1**

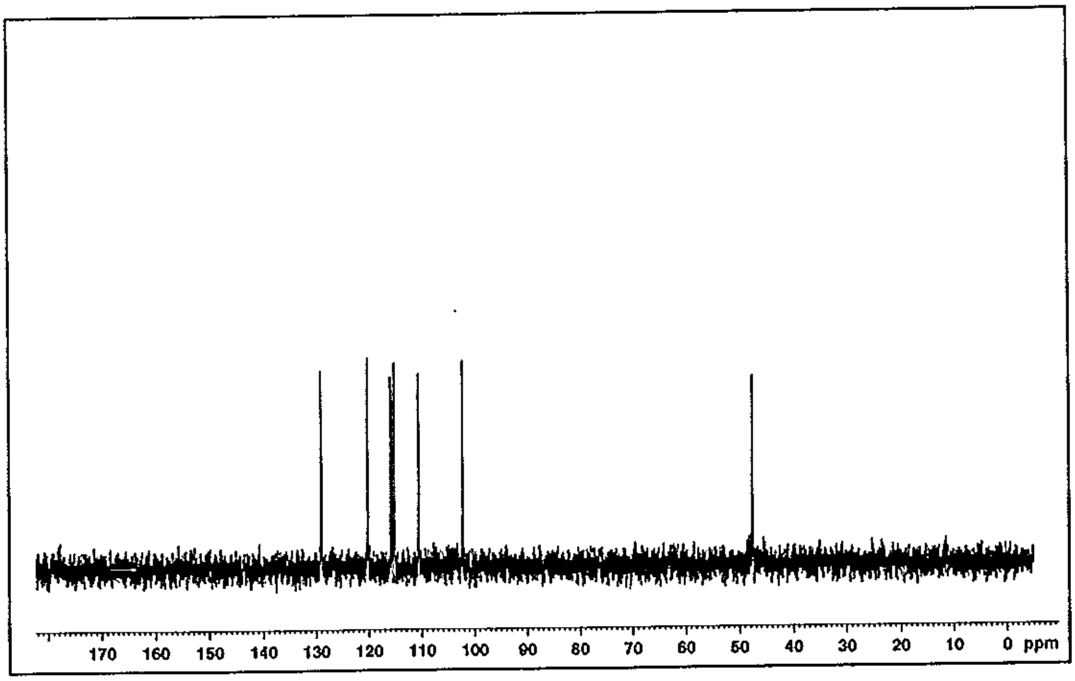

Figure 42 DEPT 90° ($CD_3OD + CDCl_3$) spectrum of compound **DH1**

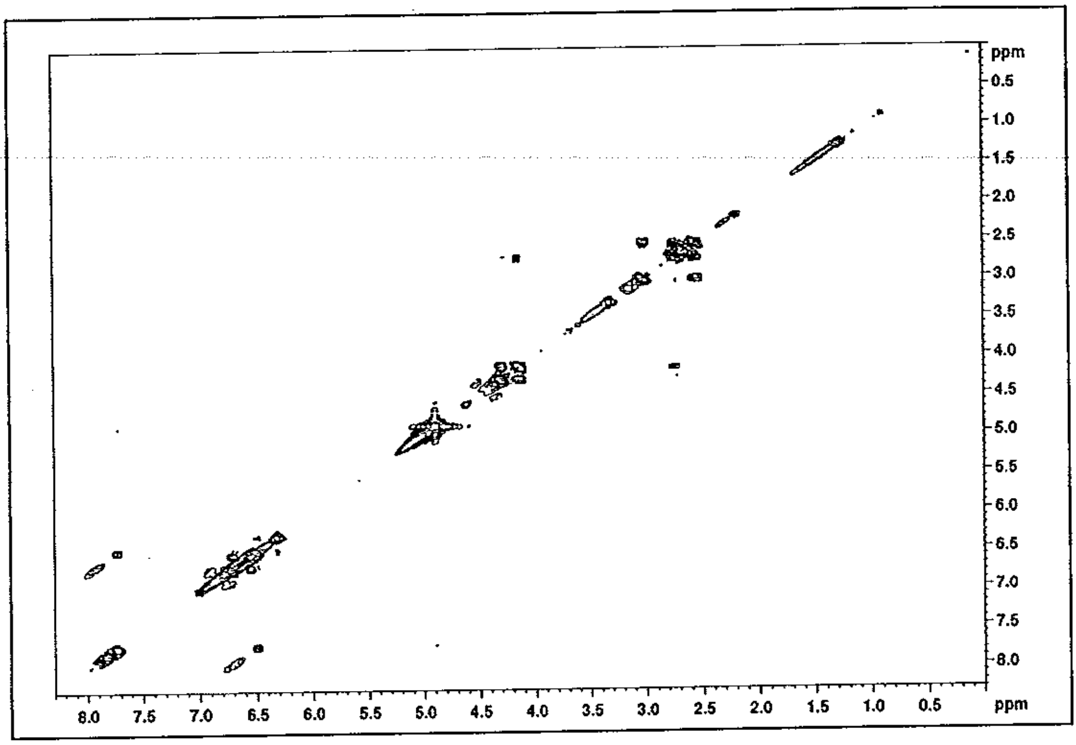

Figure 43 2D COSY ($CD_3OD + CDCl_3$) spectrum of compound **DH1**

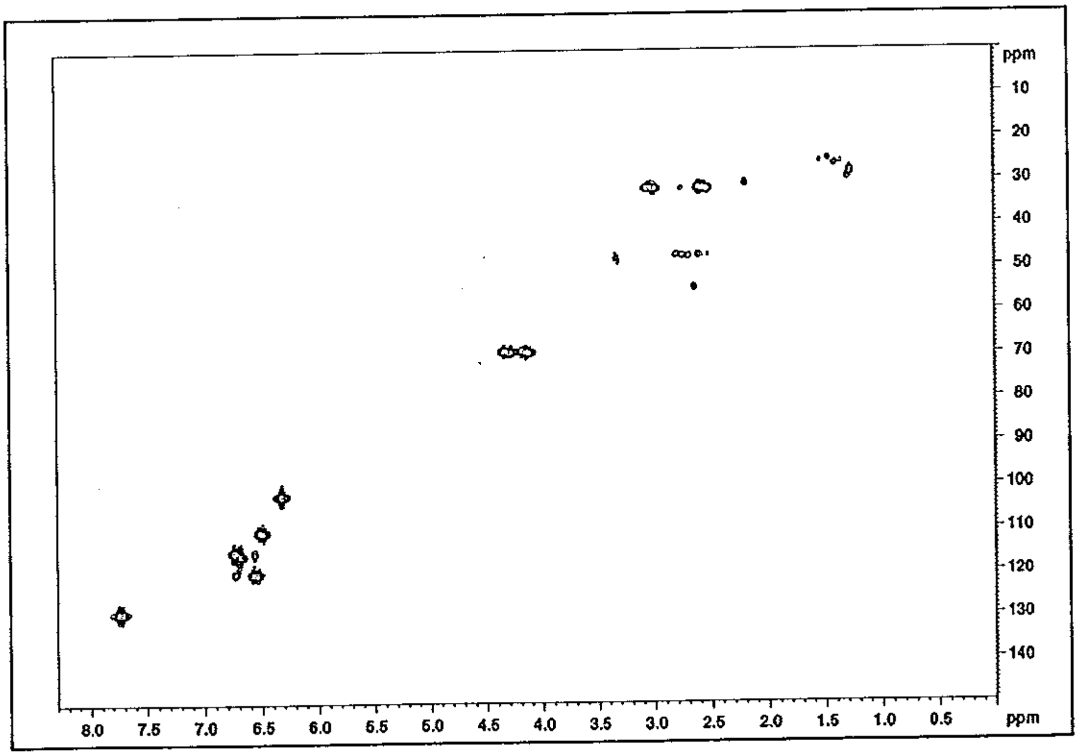

Figure 44 2D HMQC ($CD_3OD + CDCl_3$) spectrum of compound **DH1**

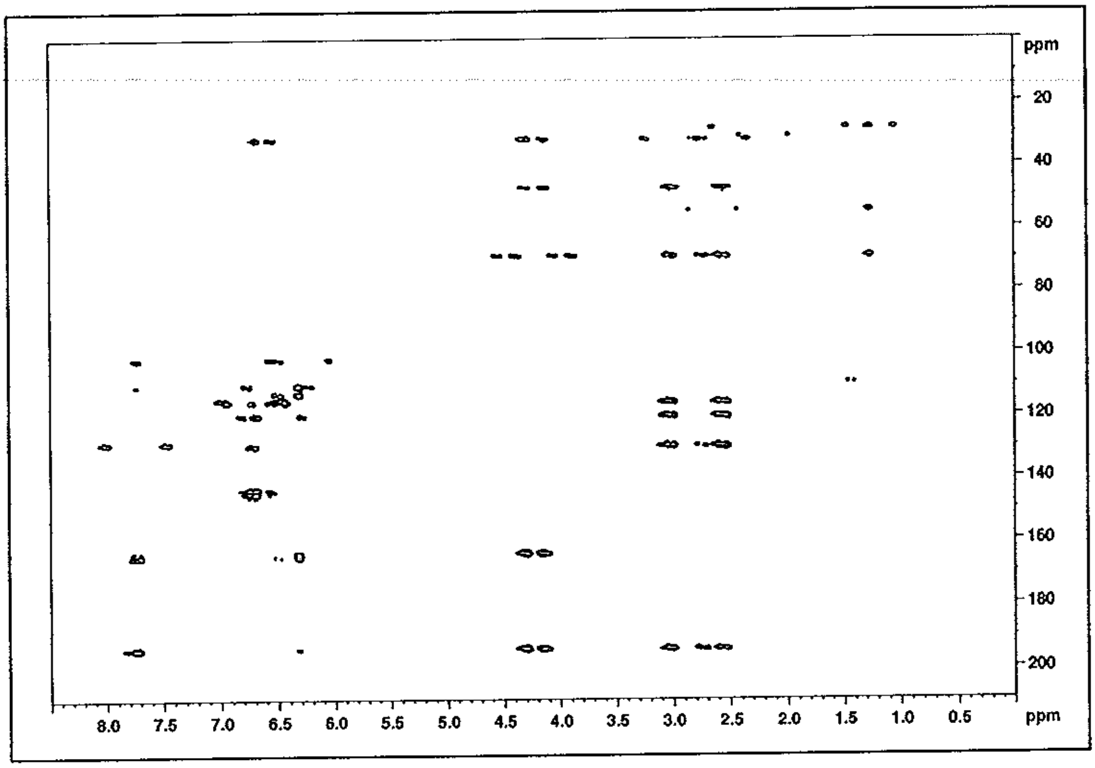

Figure 45 2D HMBC ($CD_3OD + CDCl_3$) spectrum of compound **DH1**

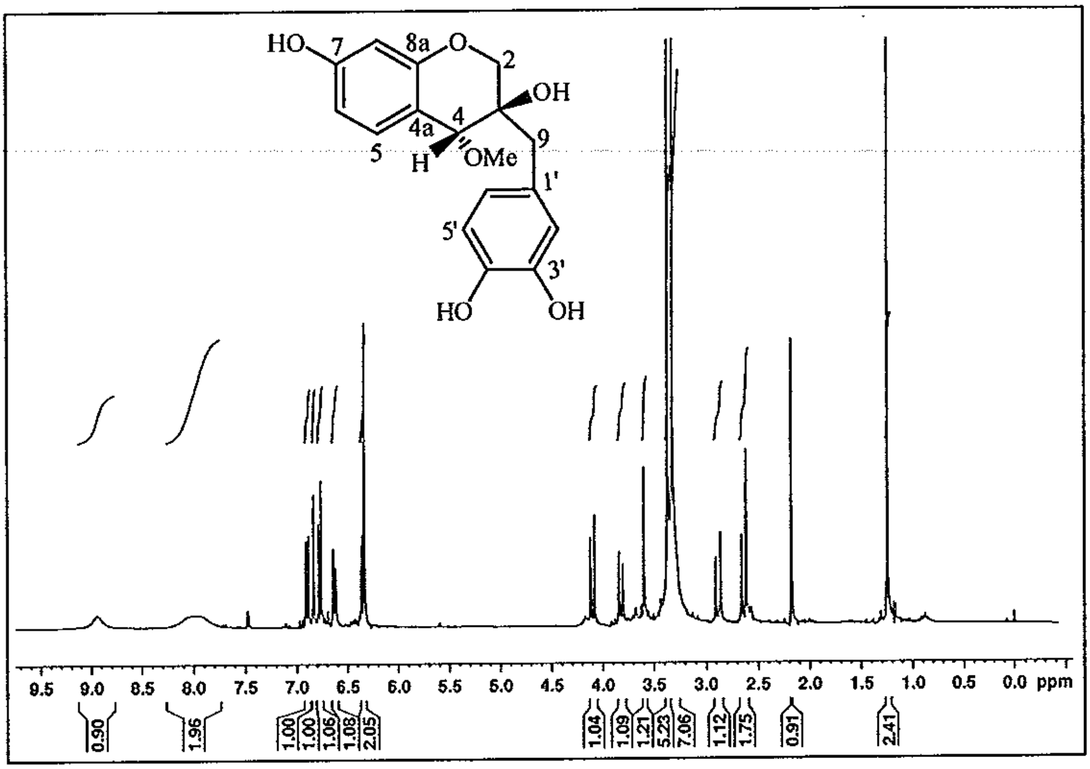

Figure 46 ^{1}H NMR (300 MHz) ($CDCl_3$ + DMSO-*d*$_6$) spectrum of compound **DH2**

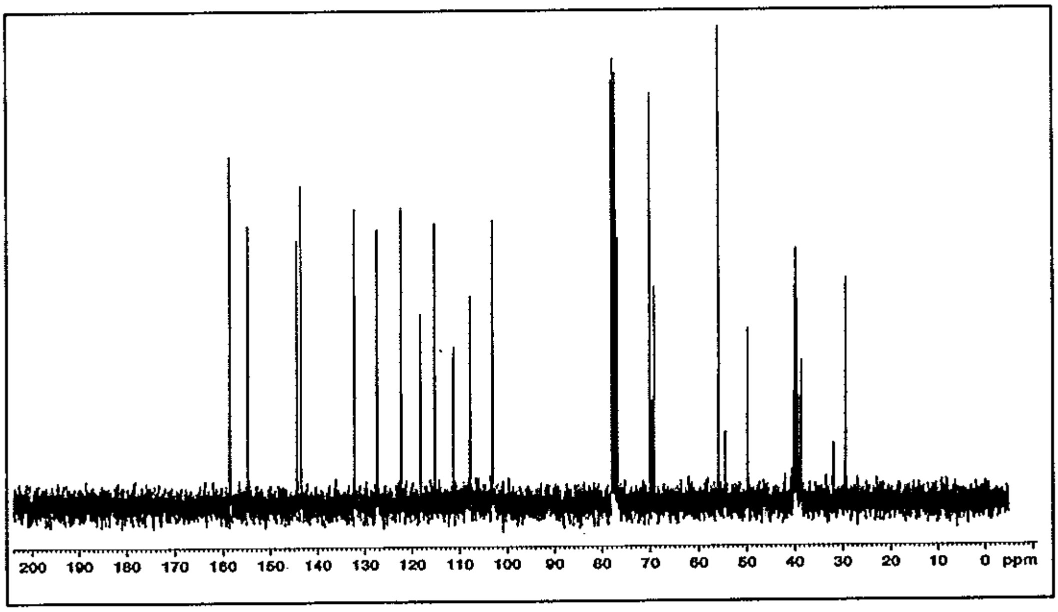

Figure 47 ^{13}C NMR (75 MHz) ($CDCl_3$ + DMSO-*d*$_6$) spectrum of compound **DH2**

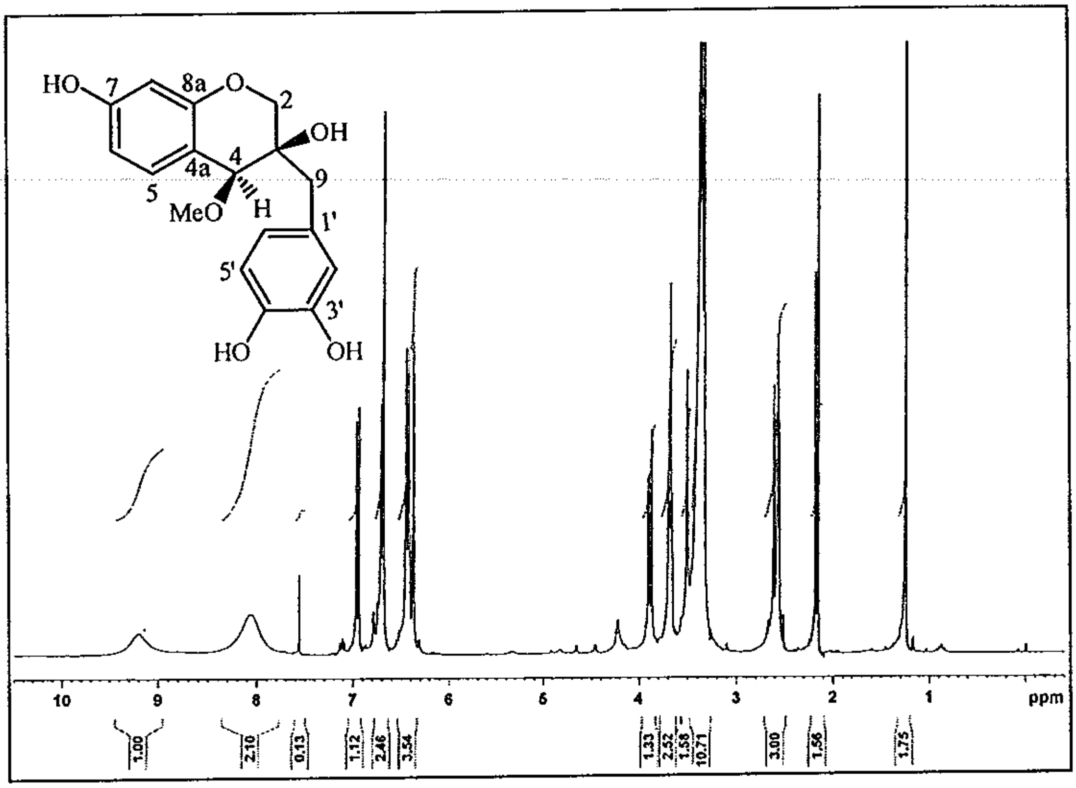

Figure 48 ^{1}H NMR (300 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH3**

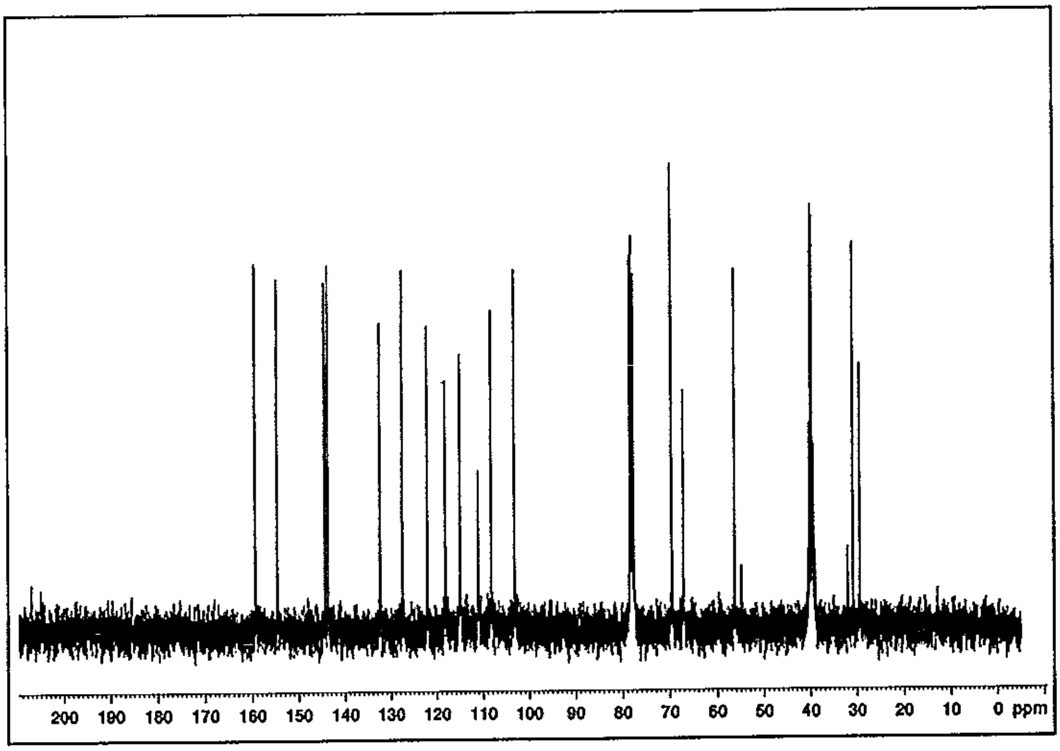

Figure 49 ^{13}C NMR (75 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH3**

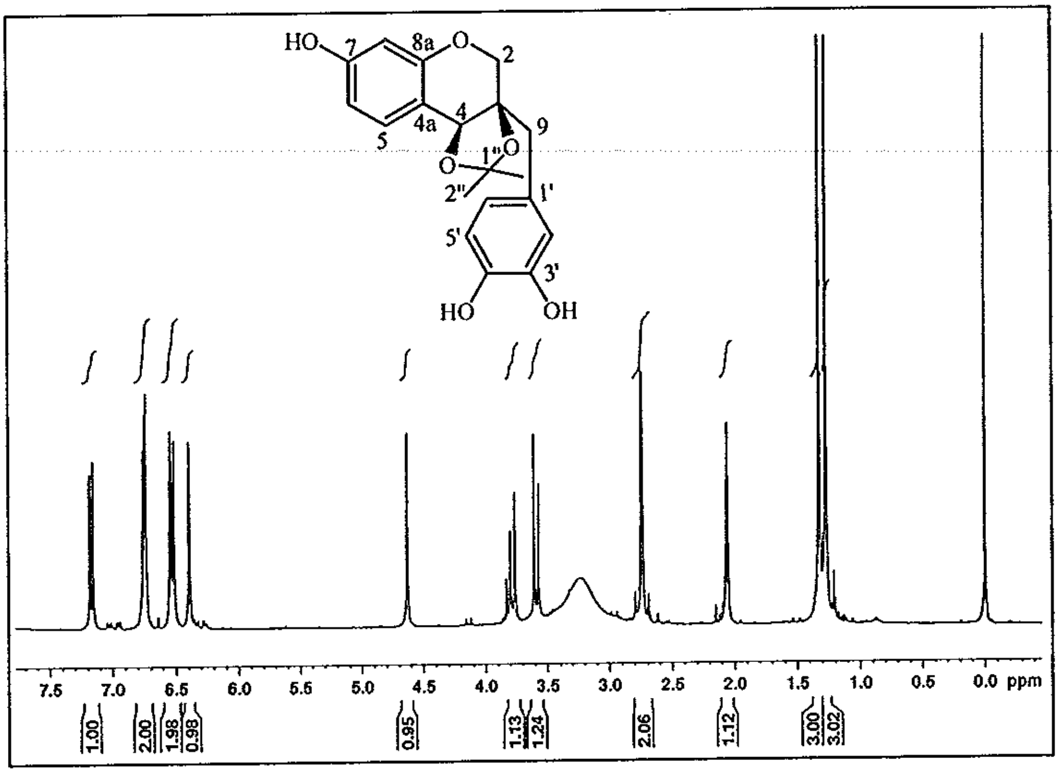

Figure 50 ¹H NMR (300 MHz) (acetone-d_6) spectrum of compound **DH4**

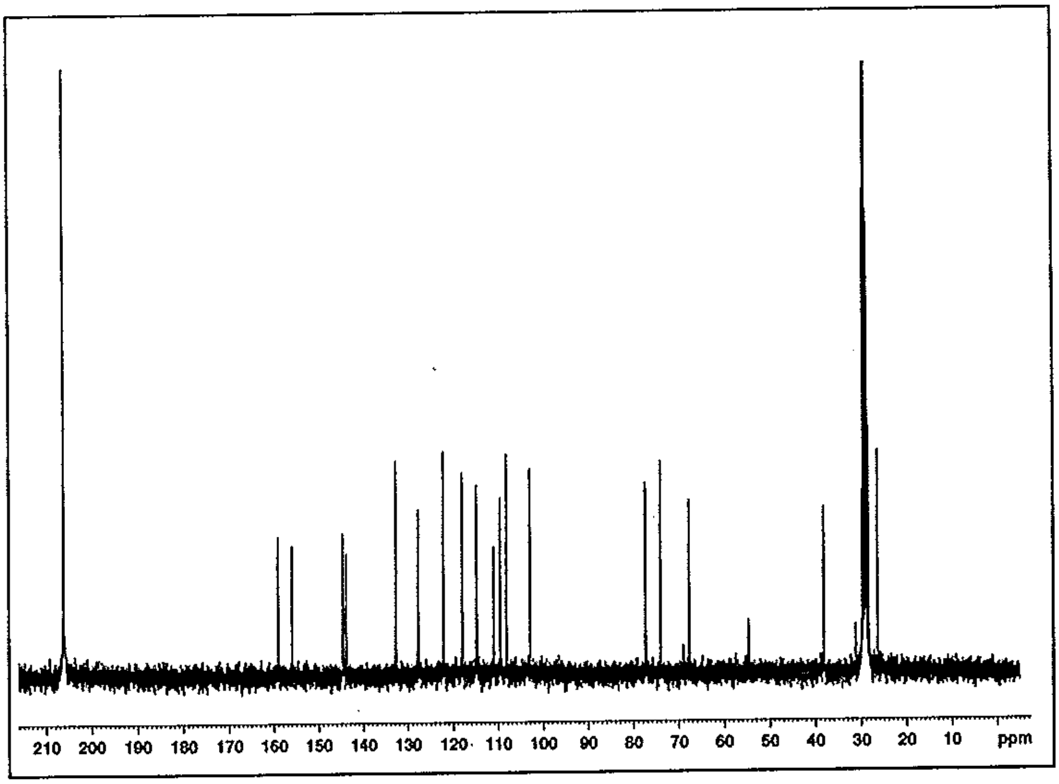

Figure 51 ¹³C NMR (75 MHz) (acetone-d_6) spectrum of compound **DH4**

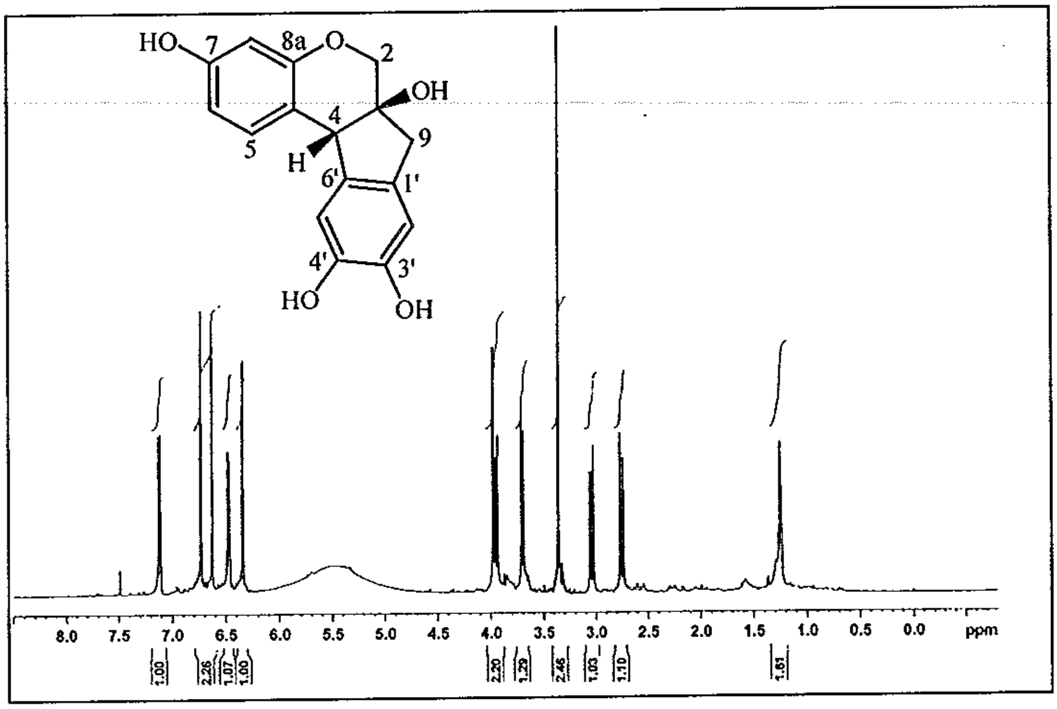

Figure 52 ^{1}H NMR (300 MHz) (DMSO-d_6) spectrum of compound **DH5**

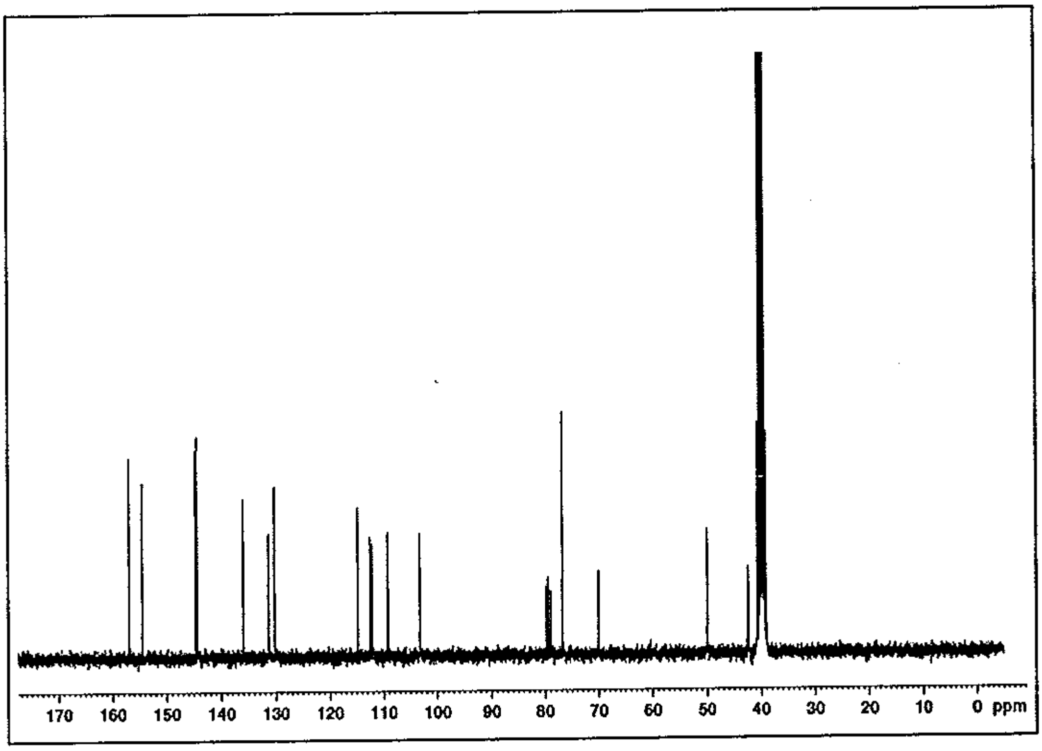

Figure 53 ^{13}C NMR (75 MHz) (DMSO-d_6) spectrum of compound **DH5**

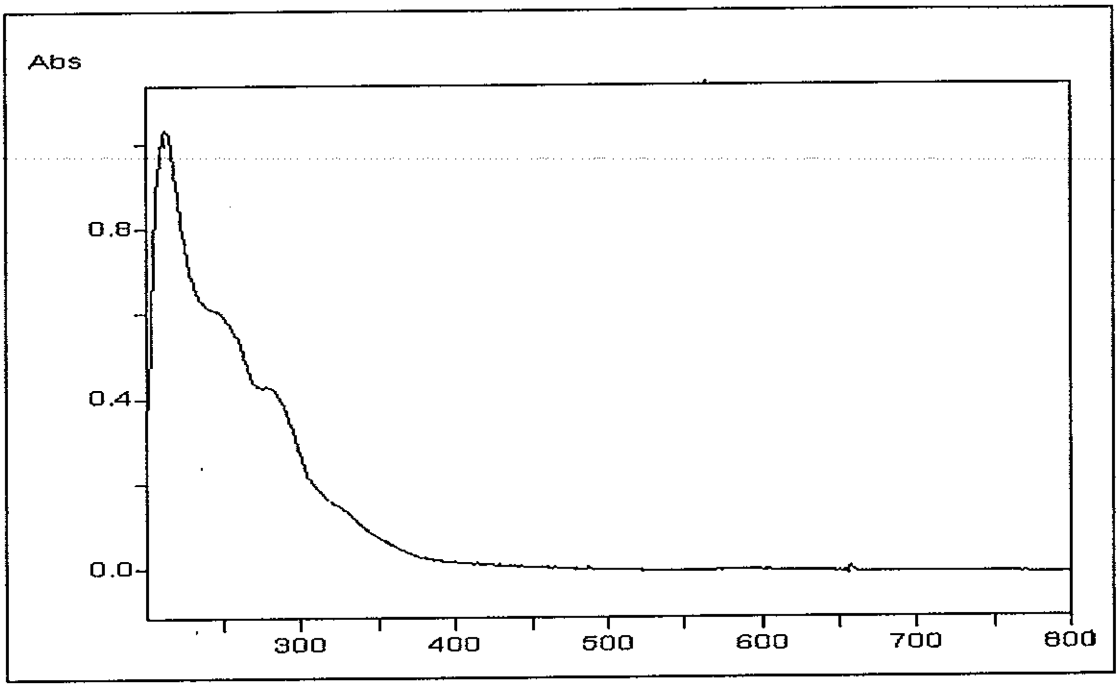

Figure 54 UV (MeOH) spectrum of compound **DH6**

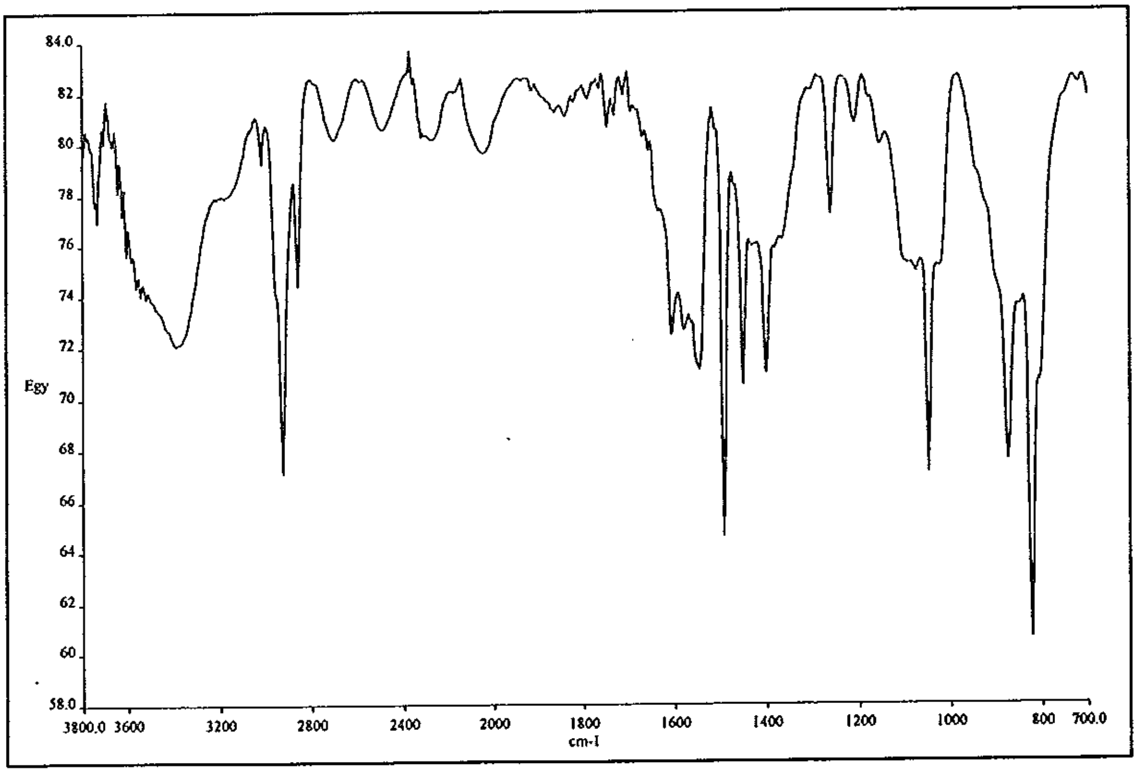

Figure 55 IR (neat) spectrum of compound **DH6**

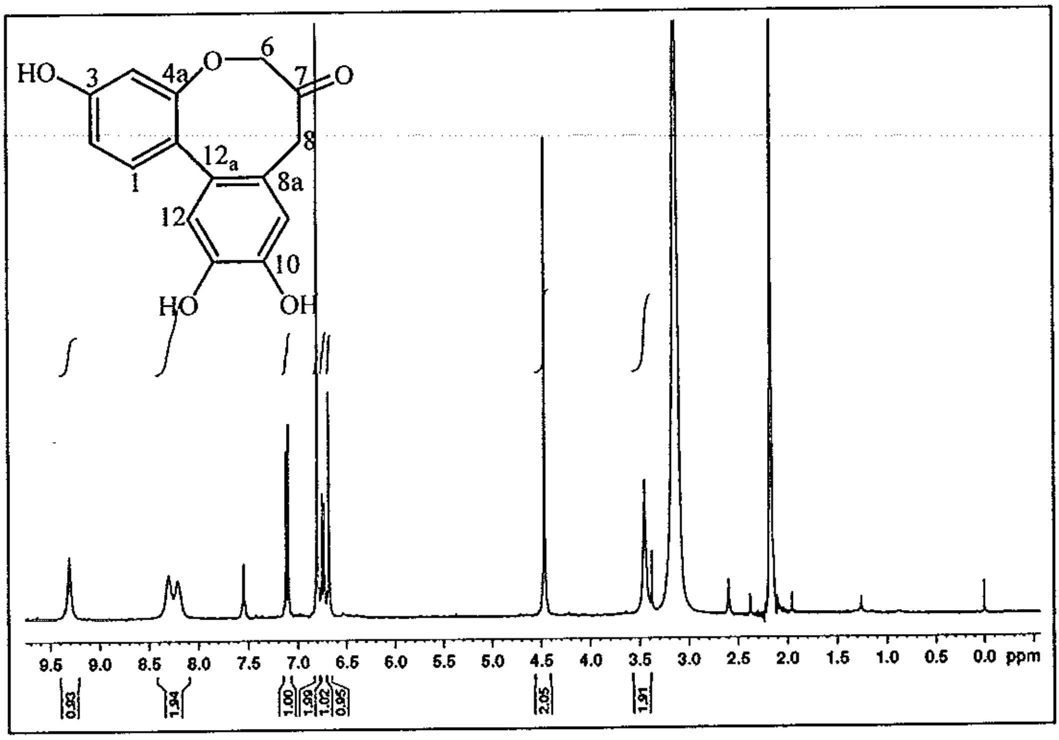

Figure 56 ^{1}H NMR (300 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH6**

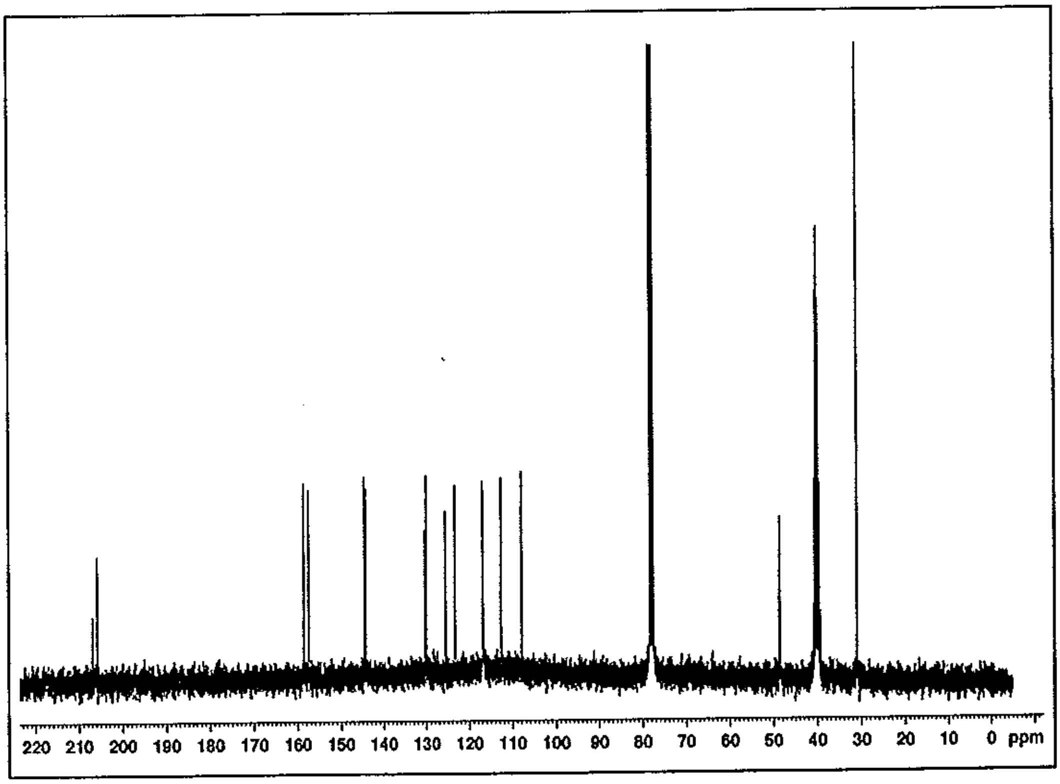

Figure 57 ^{13}C NMR (75 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH6**

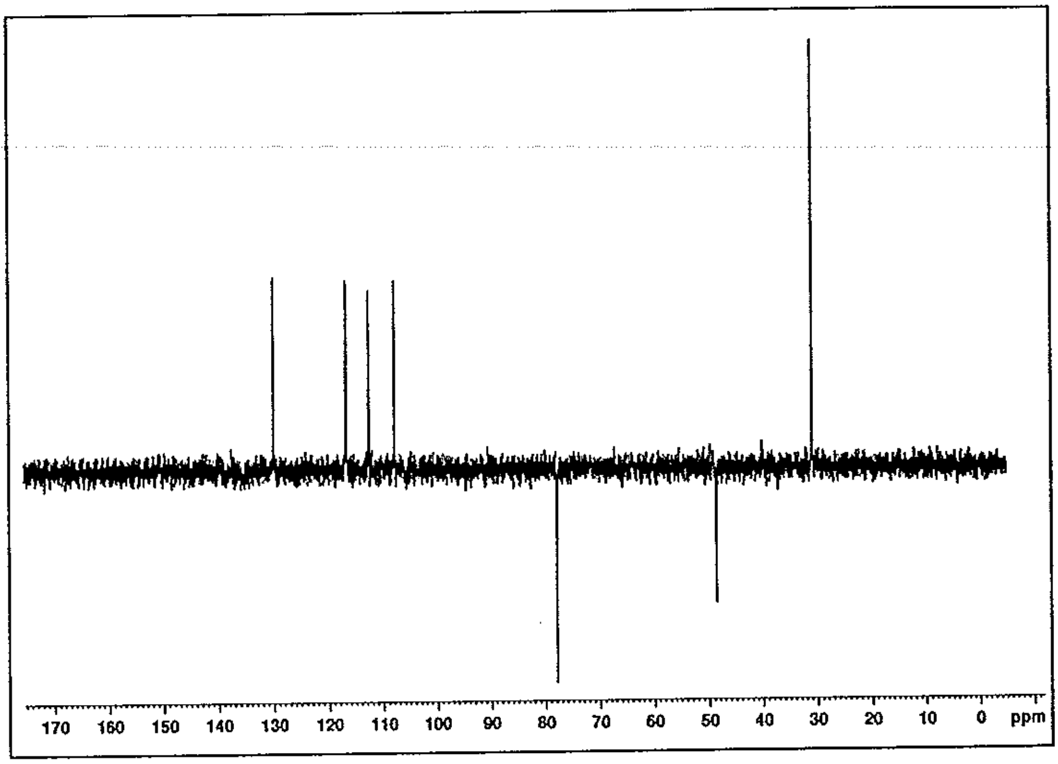

Figure 58 DEPT 135° ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH6**

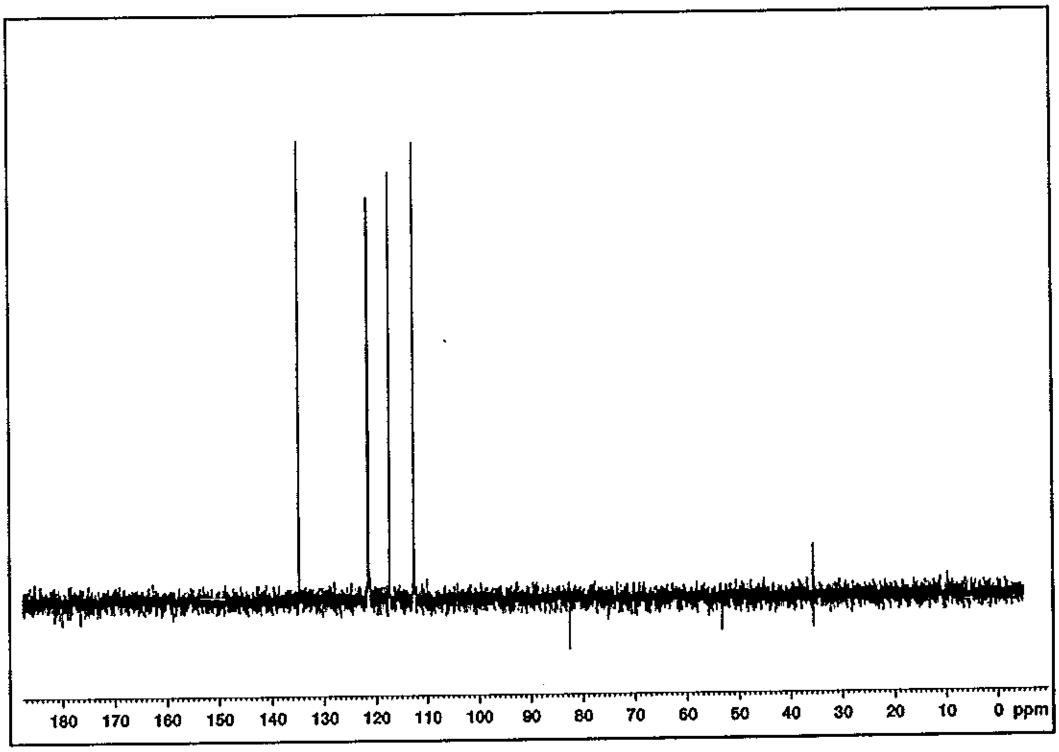

Figure 59 DEPT 90° ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH6**

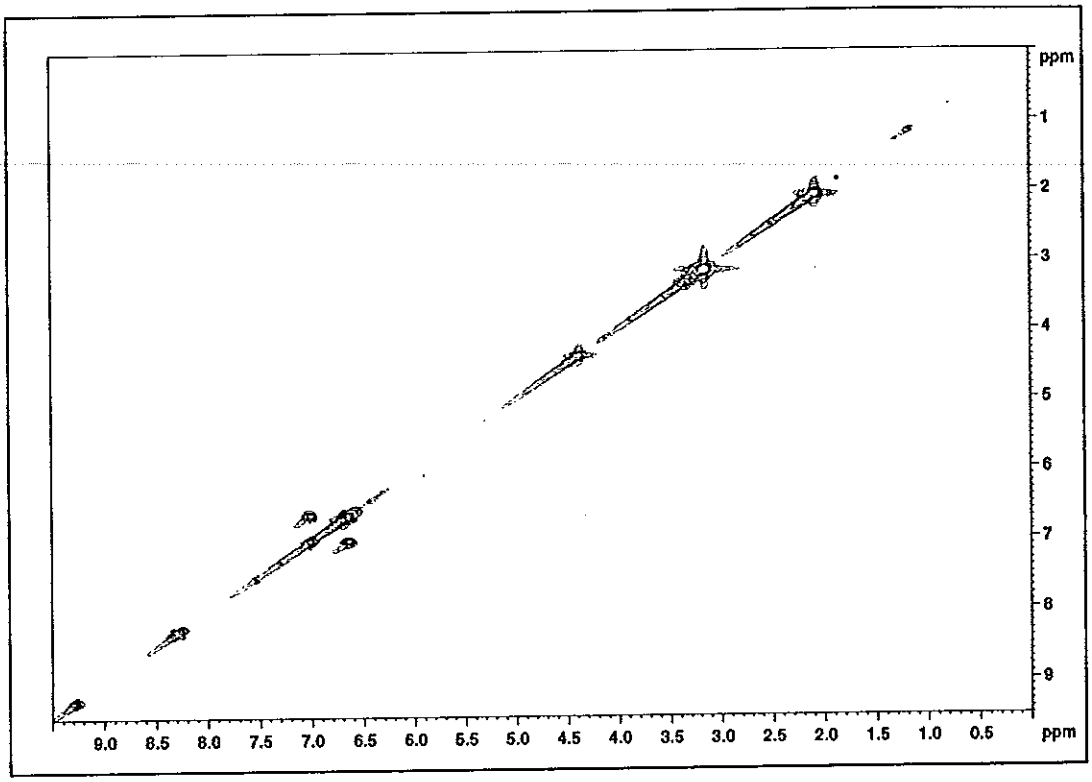

Figure 60 2D COSY ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH6**

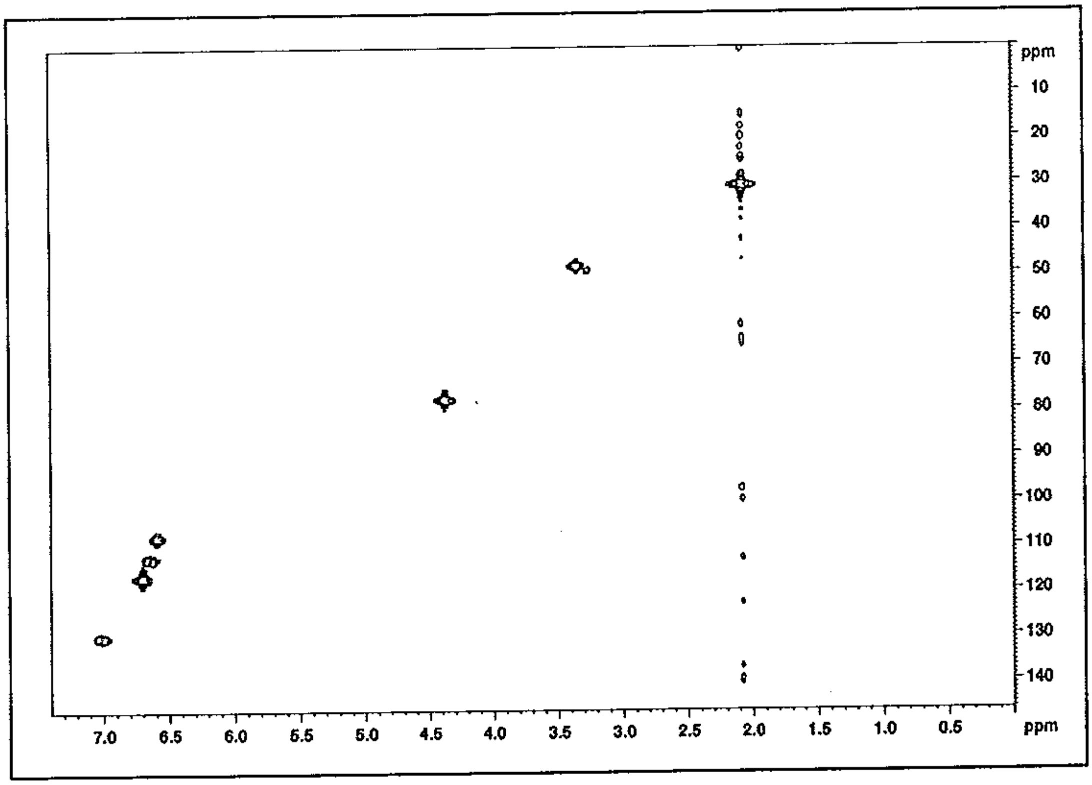

Figure 61 2D HMQC ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH6**

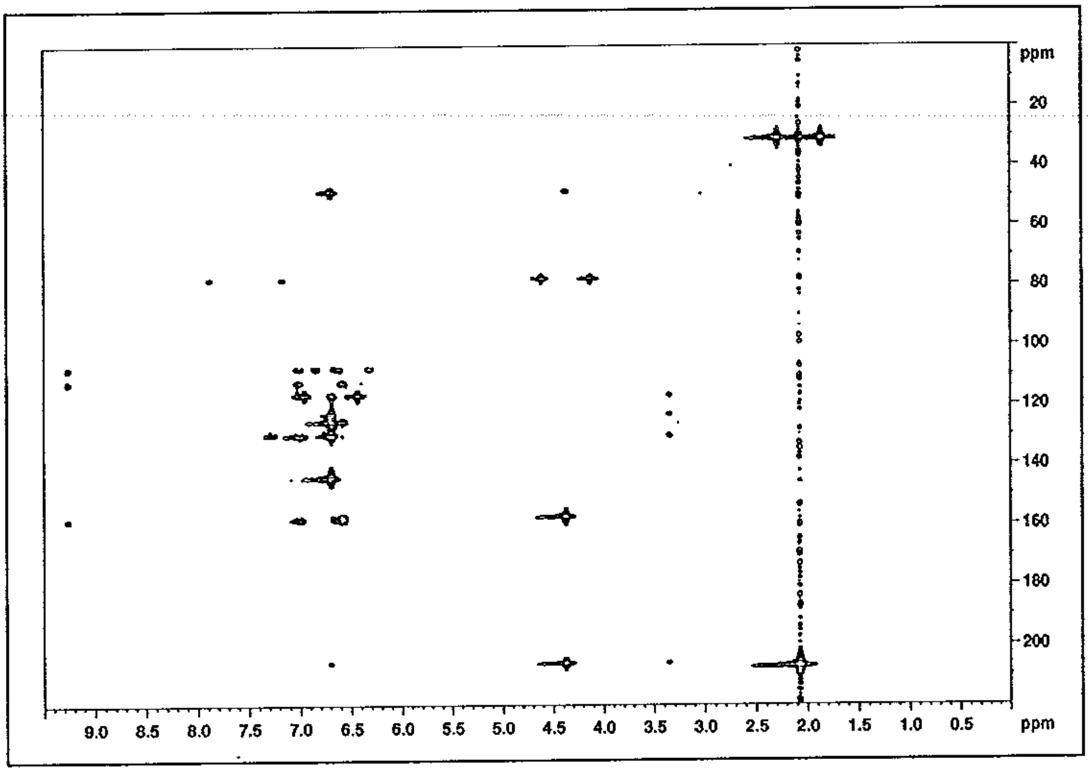

Figure 62 2D HMBC ($CDCl_3$ + DMSO-d_6) spectrum of compound **DH6**

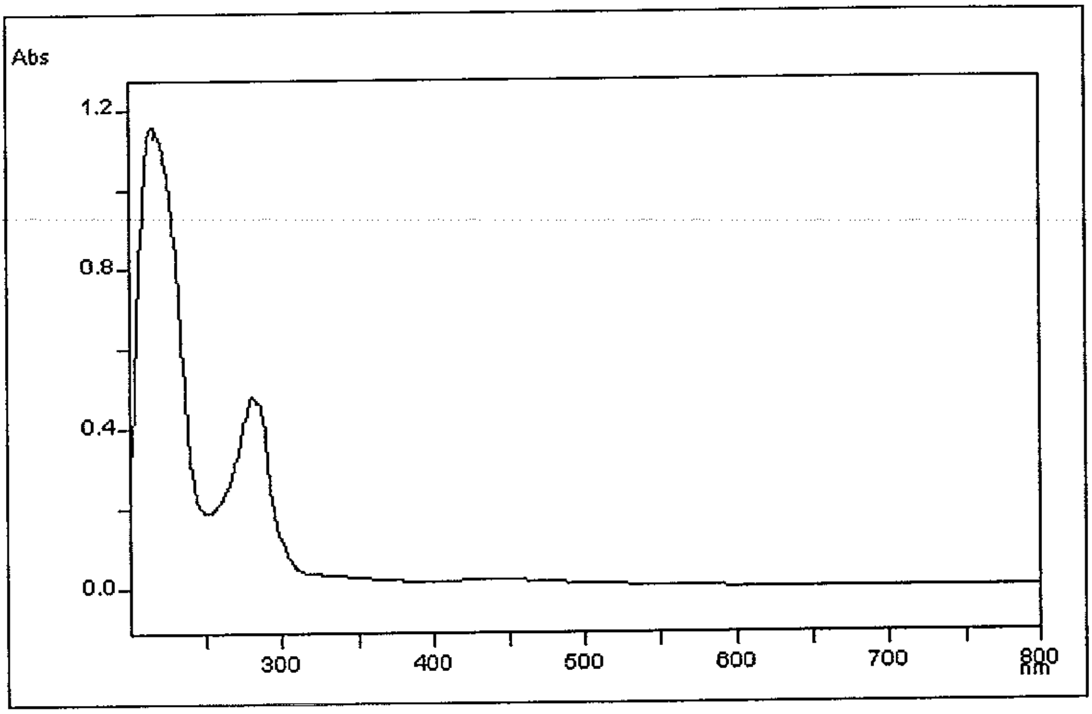

Figure 63 UV (MeOH) spectrum of compound **MR1**

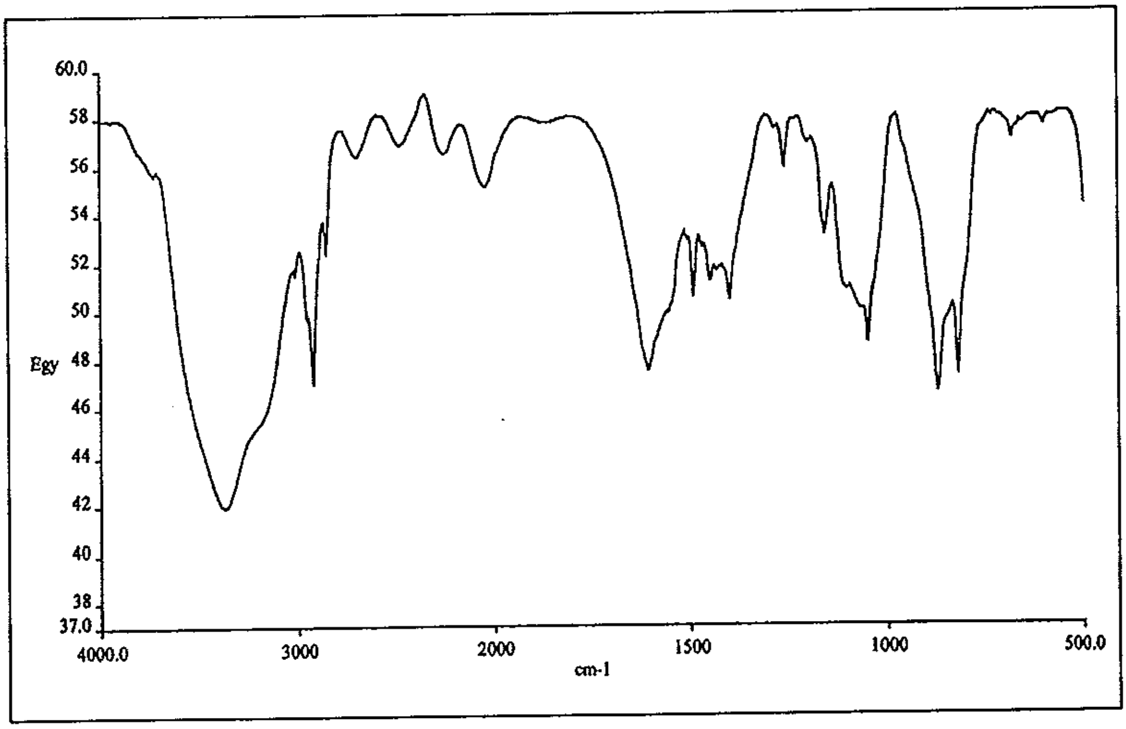

Figure 64 IR (neat) spectrum of compound **MR1**

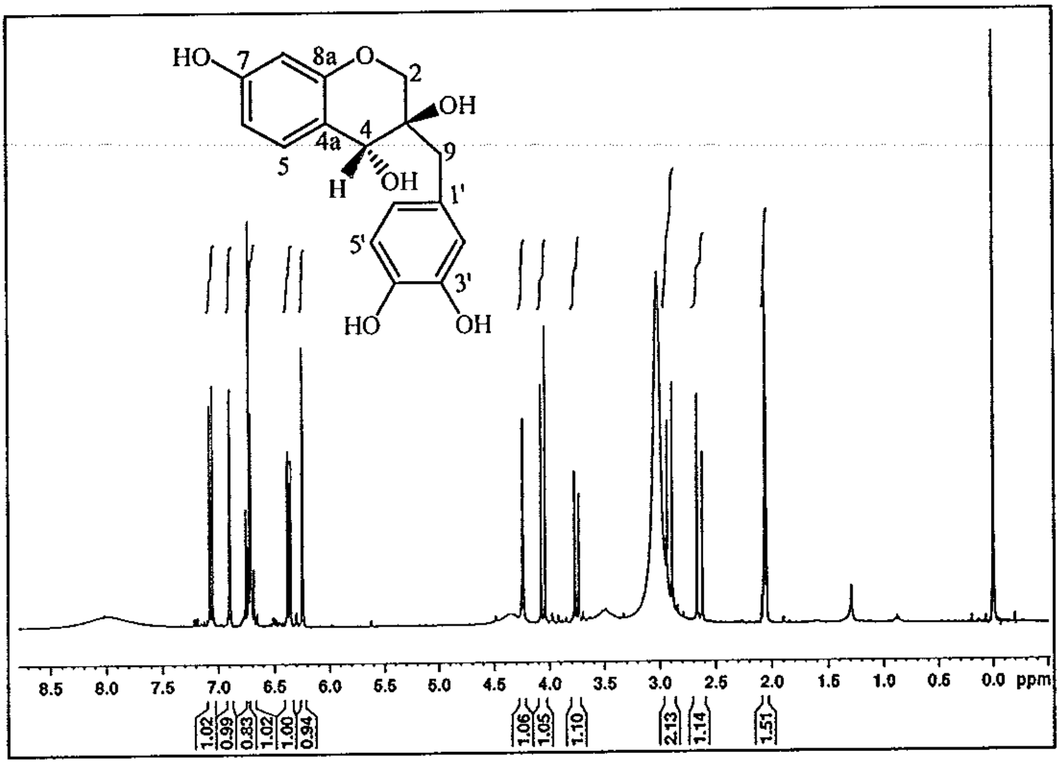

Figure 65 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **MR1**

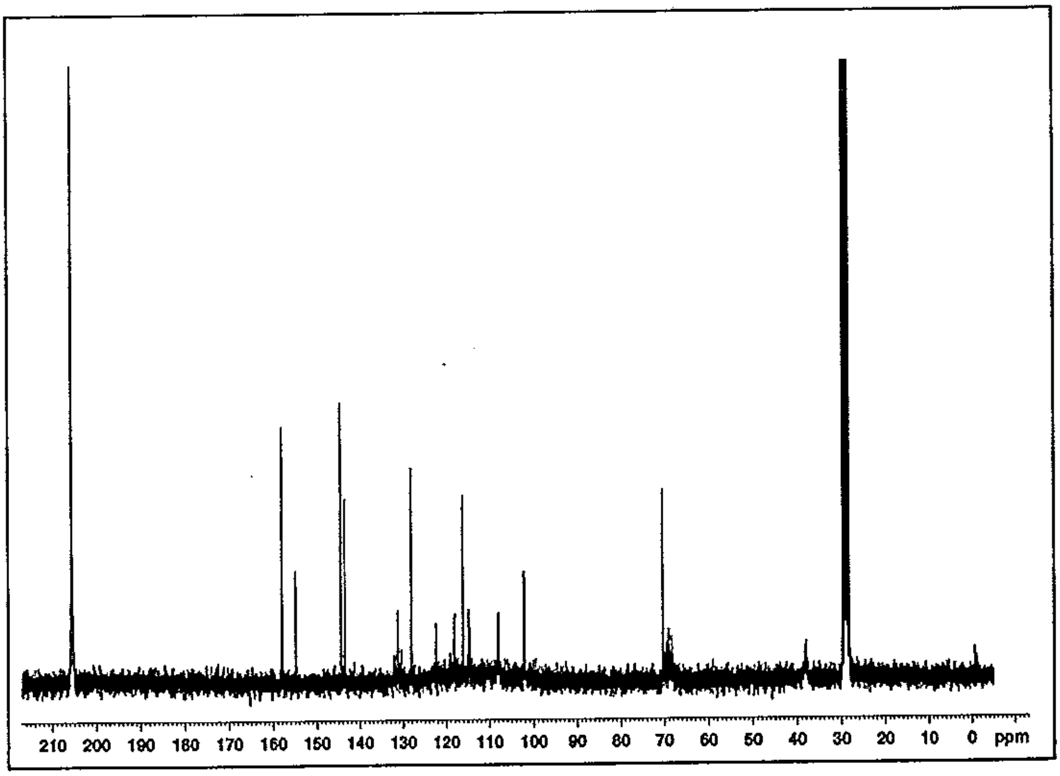

Figure 66 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **MR1**

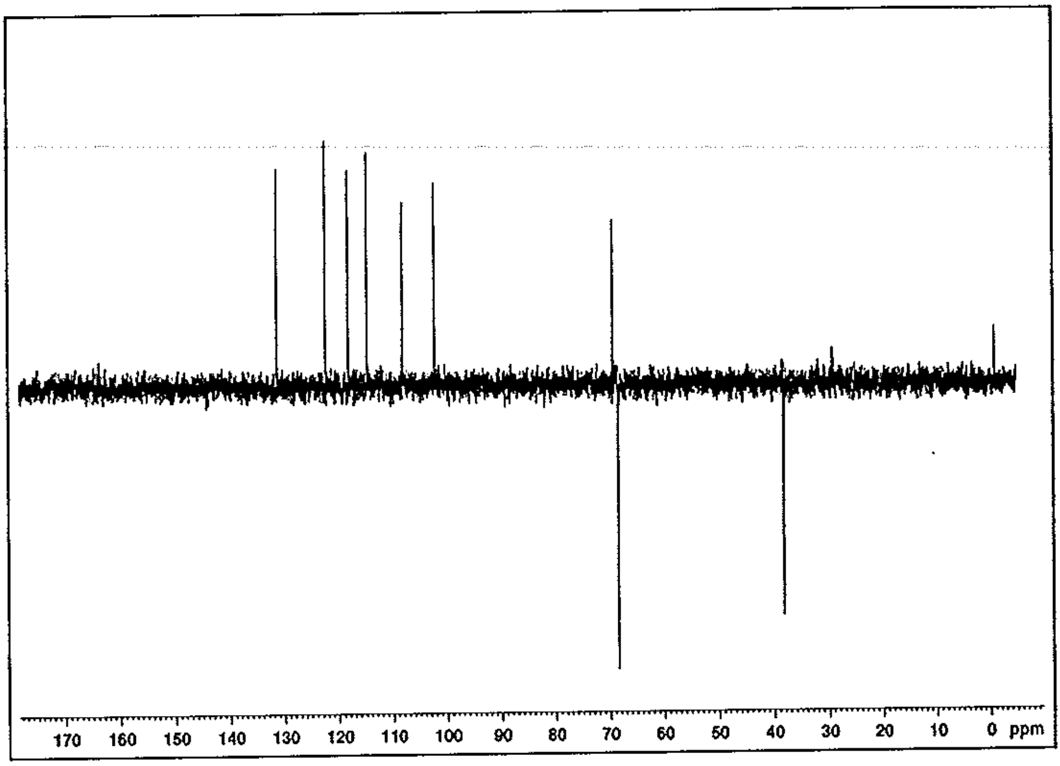

Figure 67 DEPT 135° (acetone-d_6) spectrum of compound **MR1**

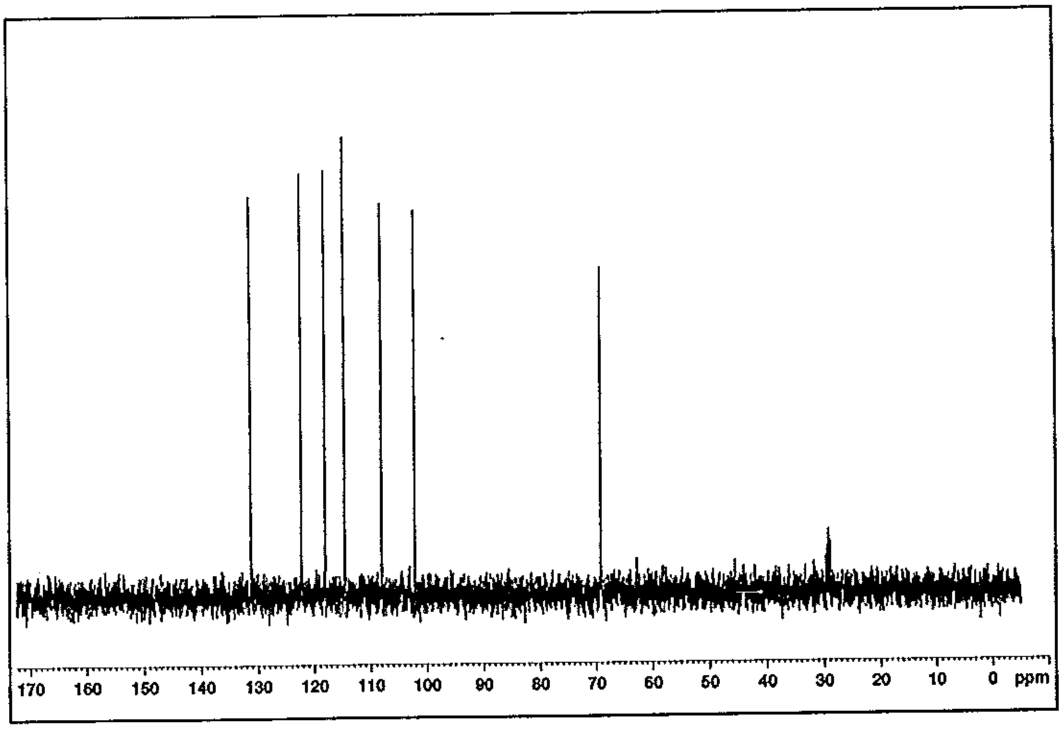

Figure 68 DEPT 90° (acetone-d_6) spectrum of compound **MR1**

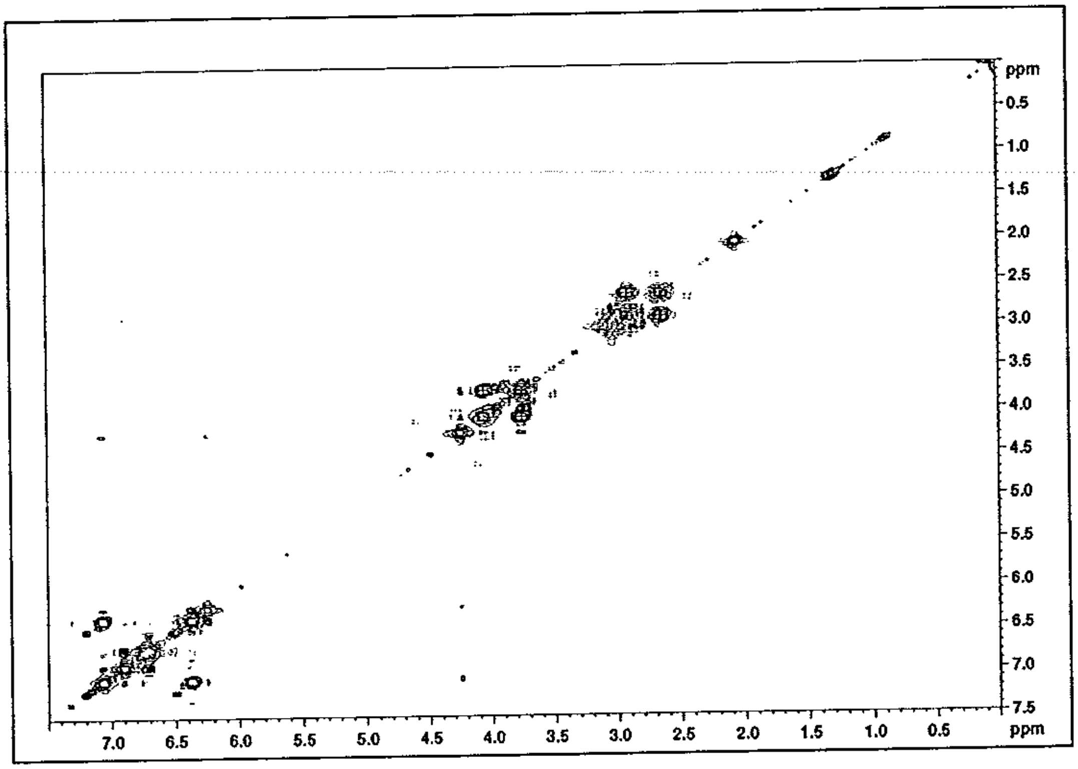

Figure 69 2D COSY (acetone-d_6) spectrum of compound **MR1**

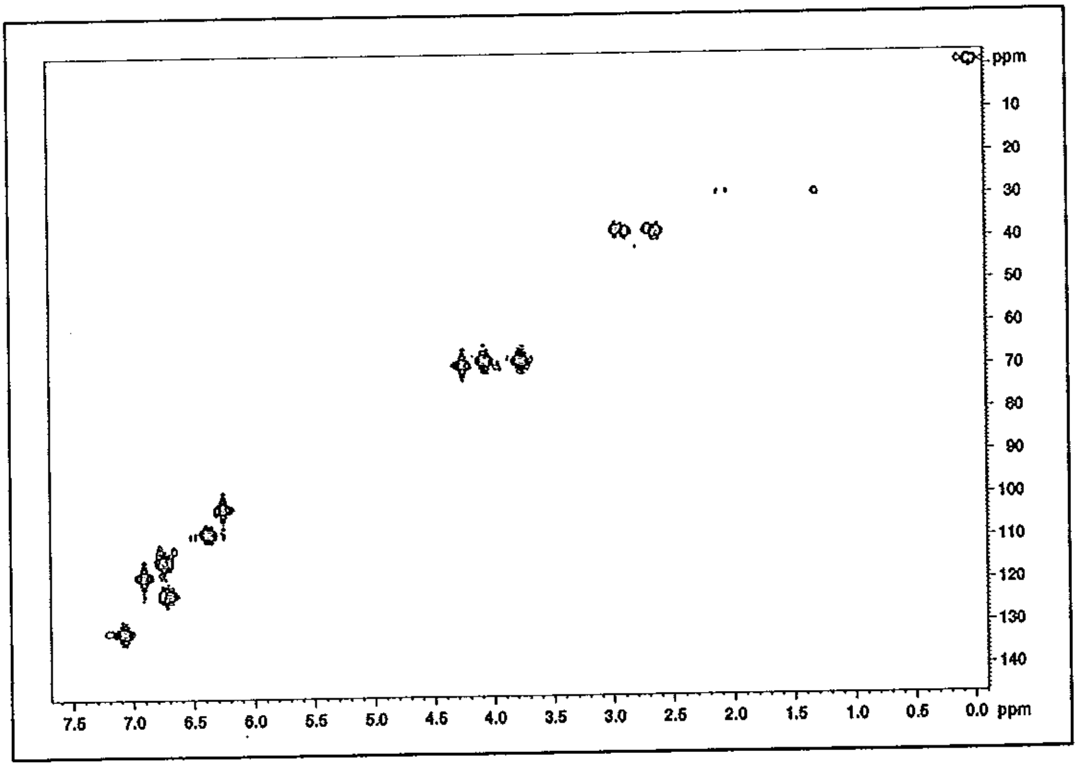

Figure 70 2D HMQC (acetone-d_6) spectrum of compound **MR1**

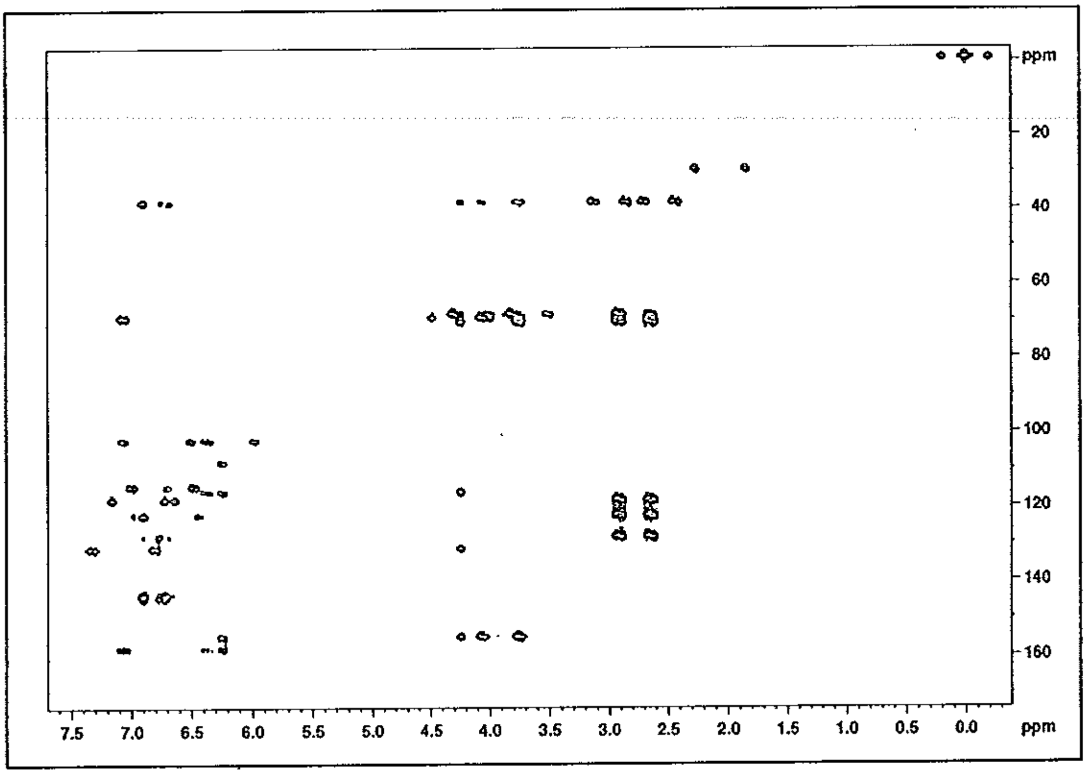

Figure 71 2D HMBC (acetone-d_6) spectrum of compound **MR1**

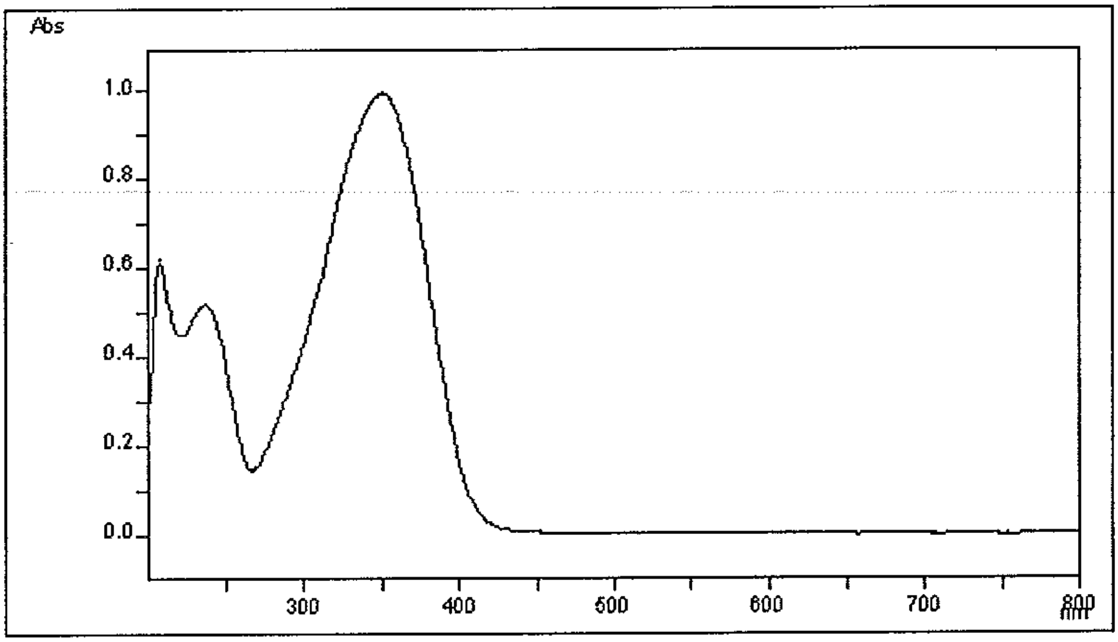

Figure 72 UV (MeOH) spectrum of compound **MR2**

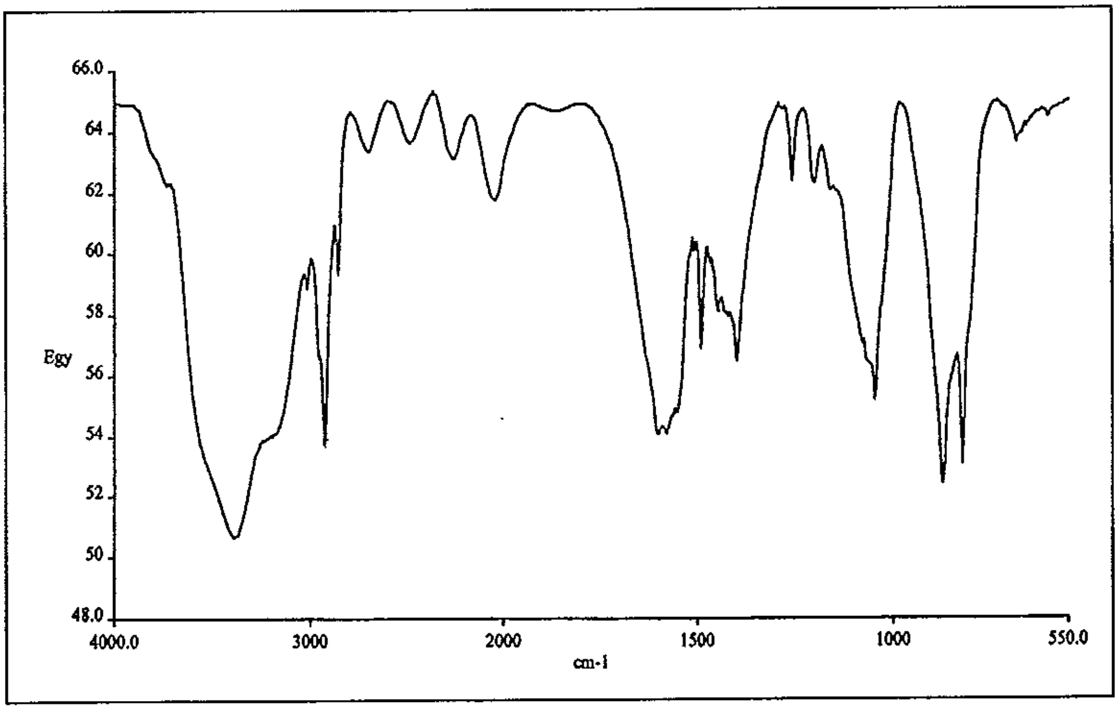

Figure 73 IR (neat) spectrum of compound **MR2**

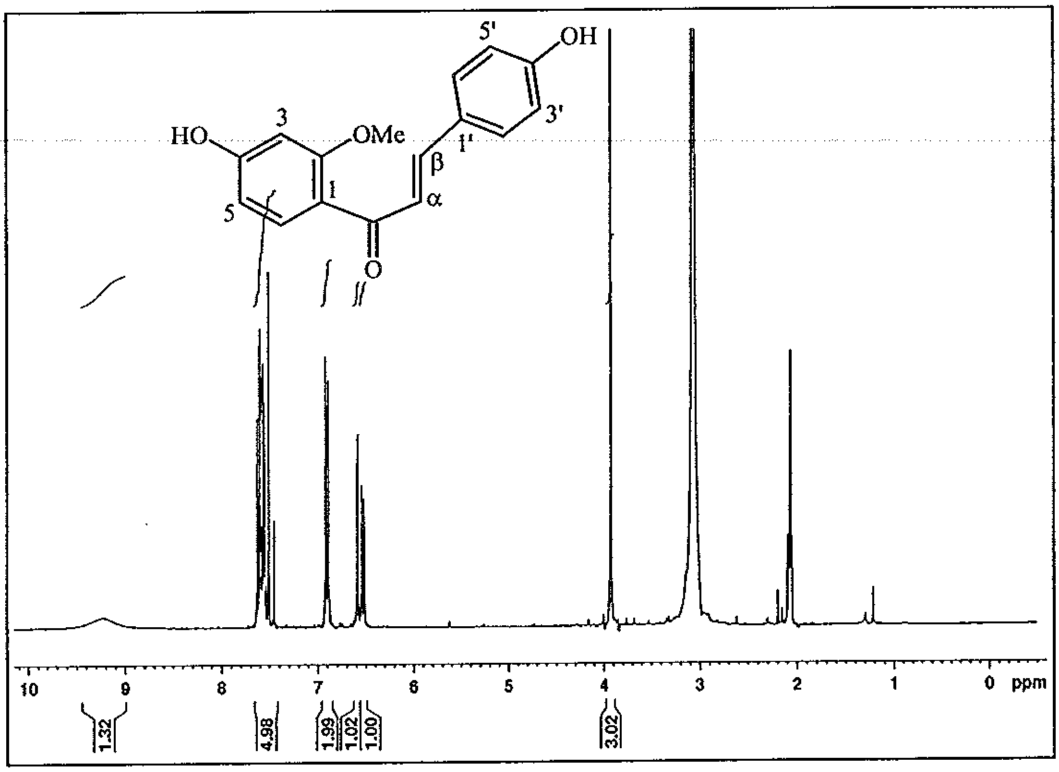

Figure 74 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **MR2**

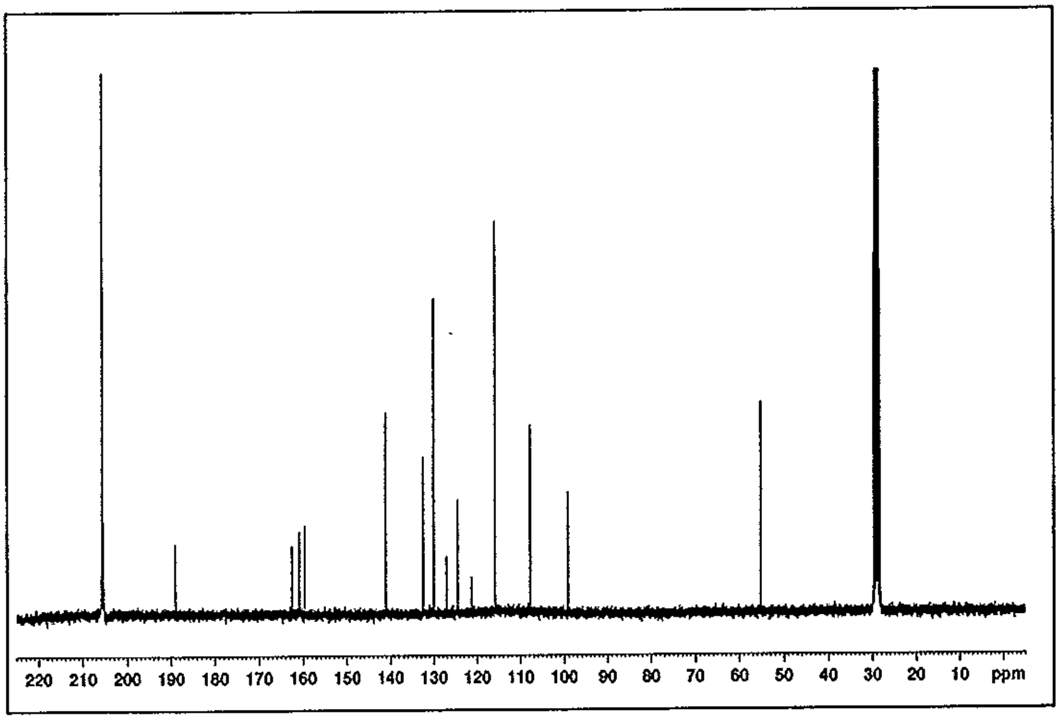

Figure 75 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **MR2**

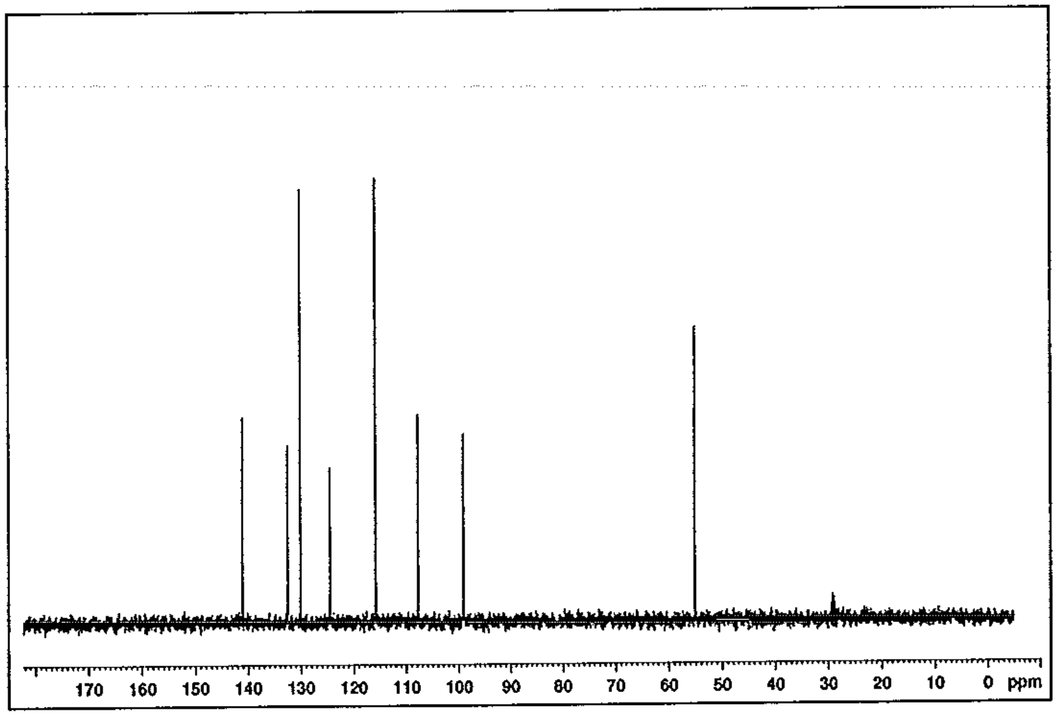

Figure 76 DEPT 135° (acetone-d_6) spectrum of compound **MR2**

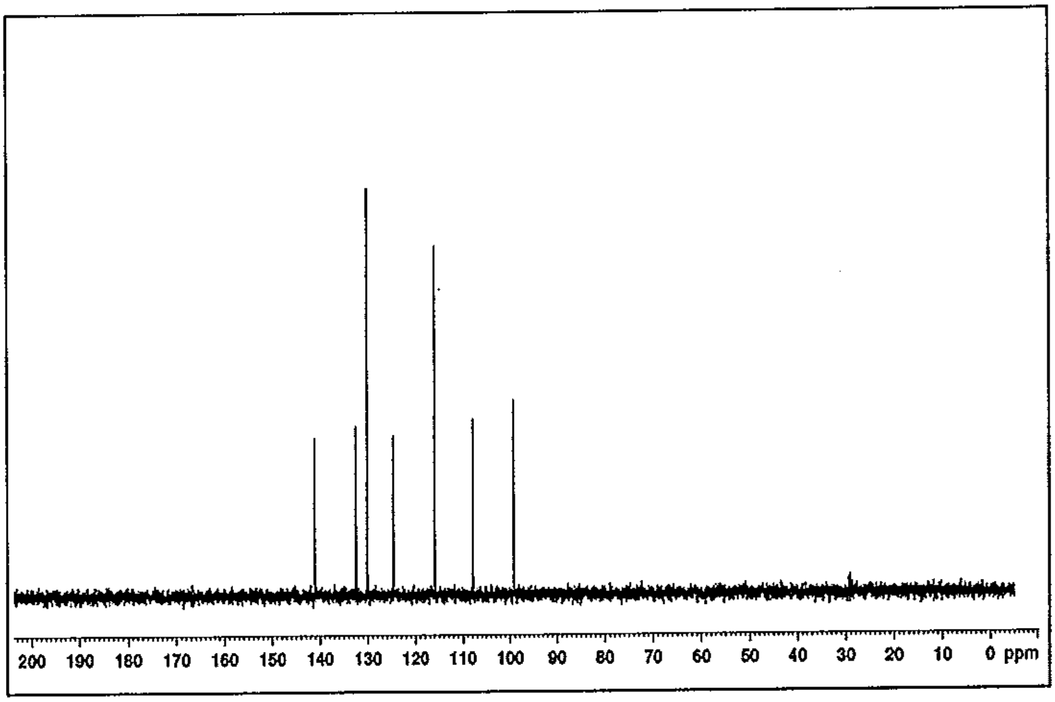

Figure 77 DEPT 135° (acetone-d_6) spectrum of compound **MR2**

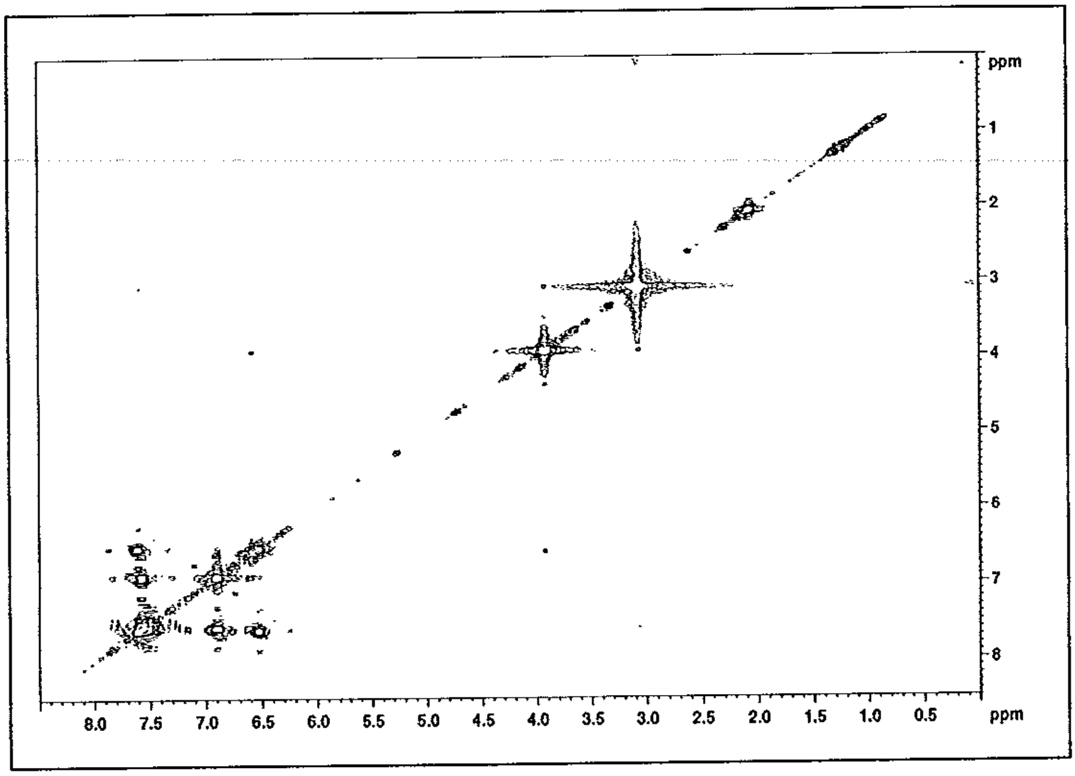

Figure 78 2D COSY (acetone-d_6) spectrum of compound **MR2**

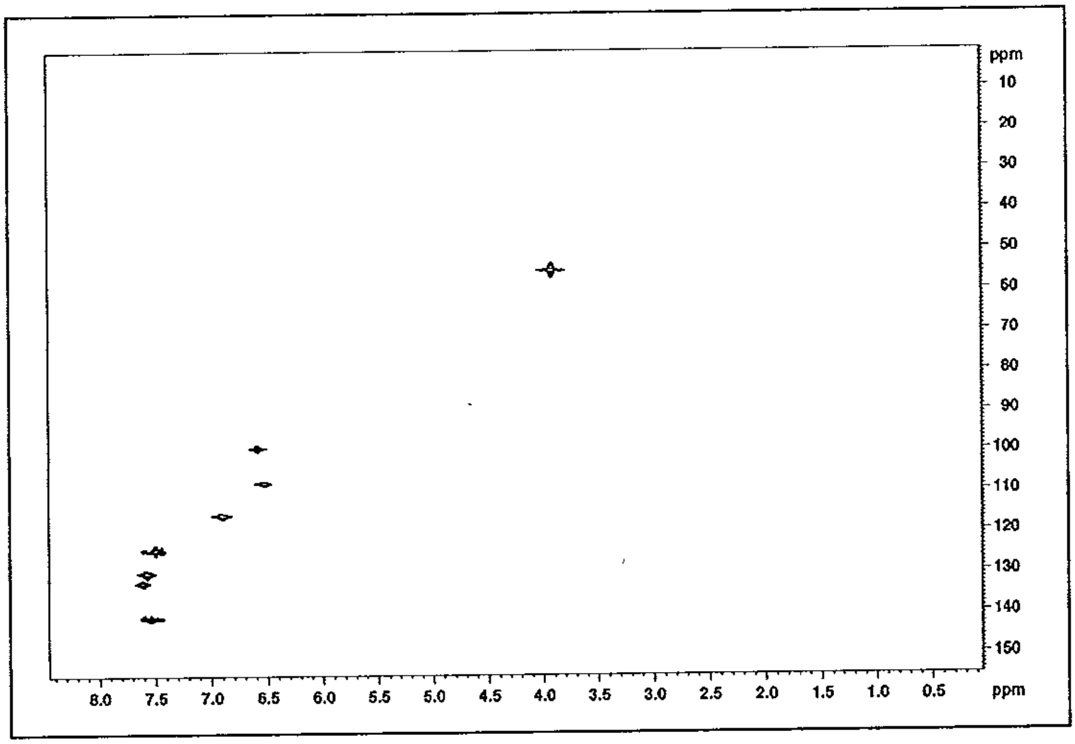

Figure 79 2D HMQC (acetone-d_6) spectrum of compound **MR2**

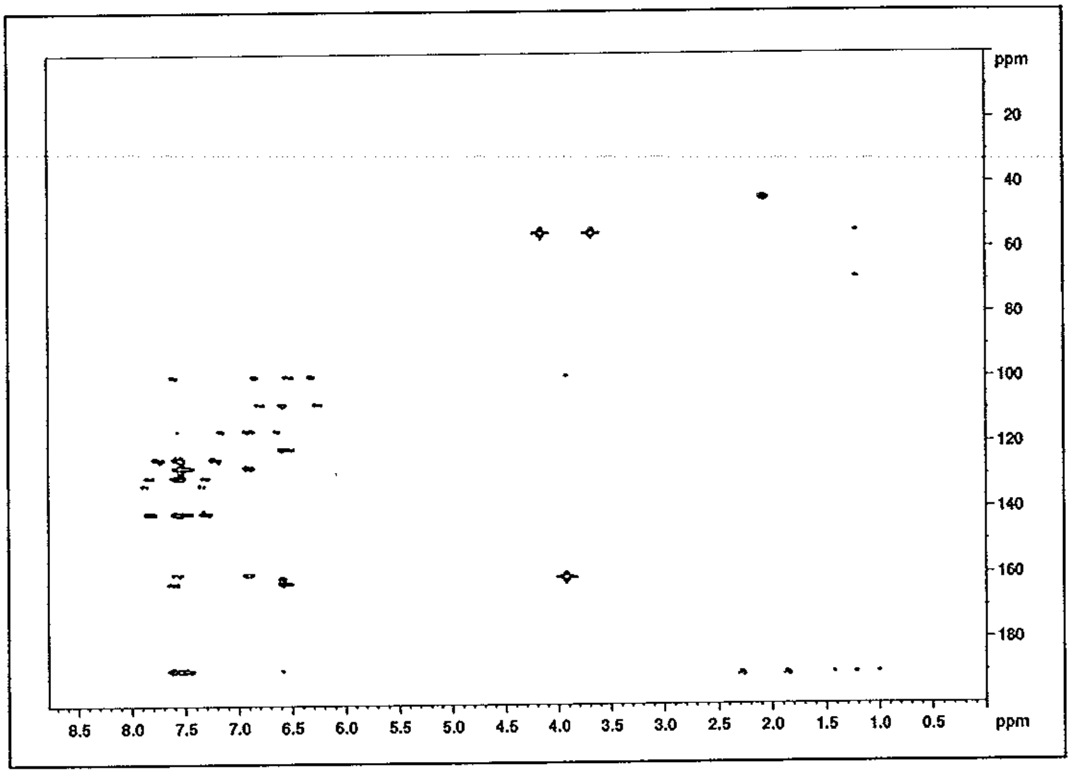

Figure 80 2D HMQC (acetone-d_6) spectrum of compound **MR2**

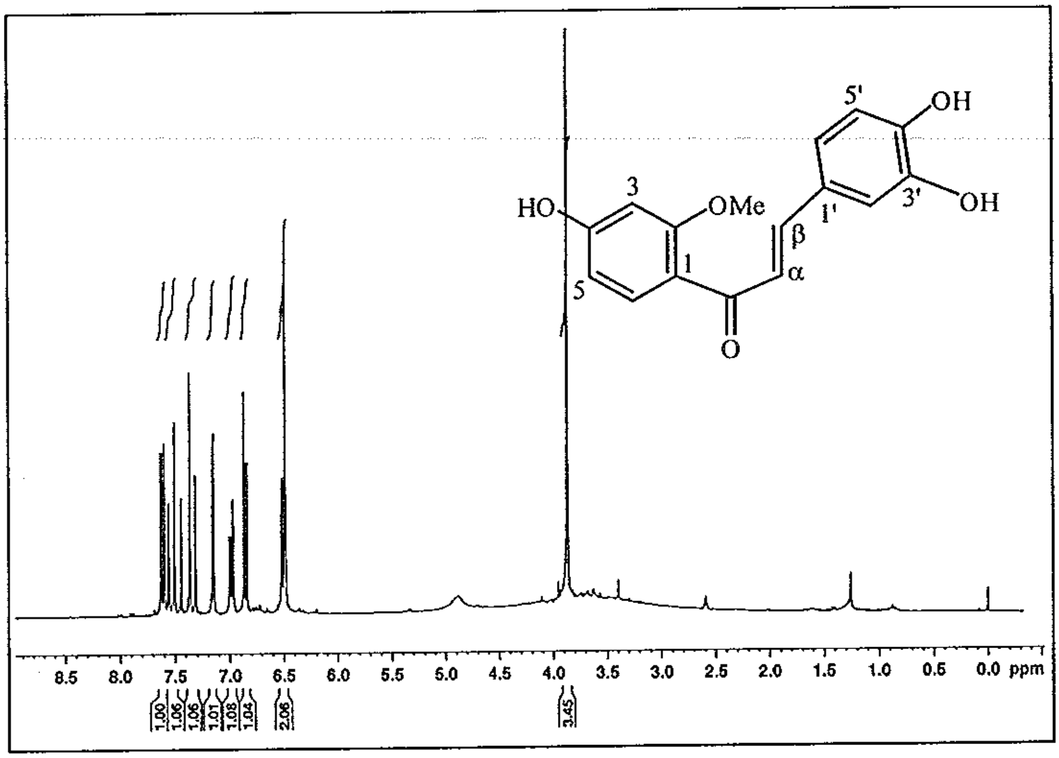

Figure 81 ^{1}H NMR (300 MHz) ($CDCl_3$ + DMSO-*d*$_6$) spectrum of compound **MR3**

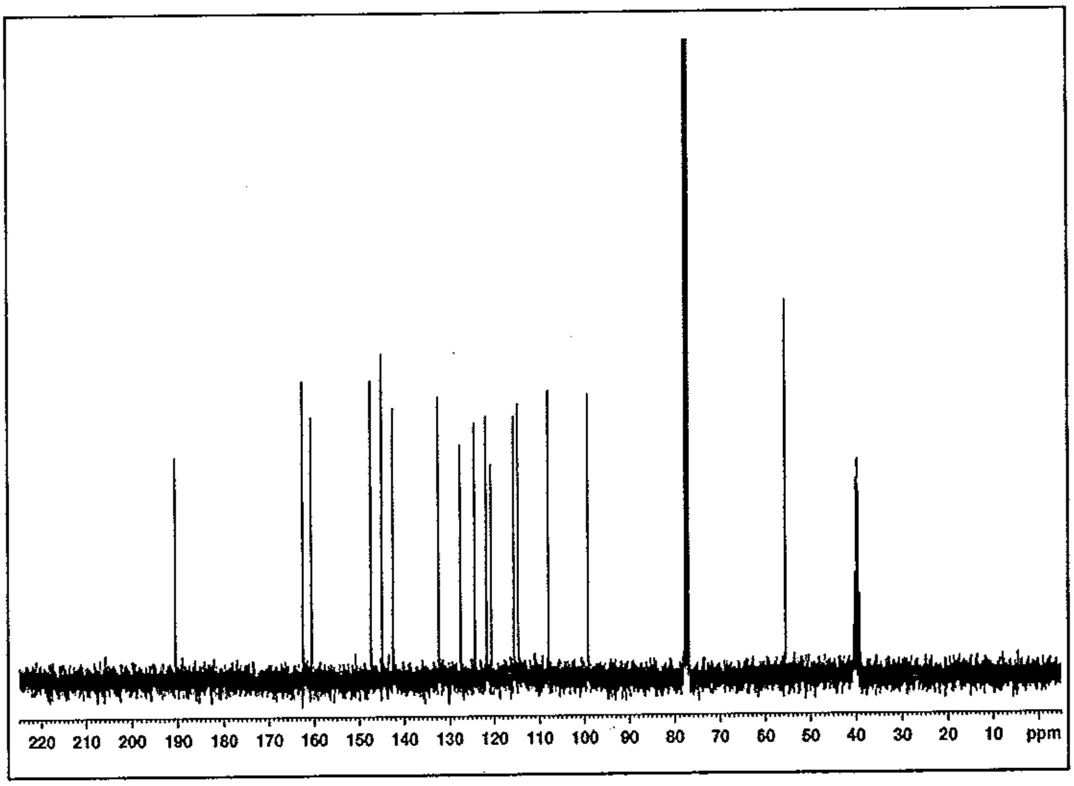

Figure 82 ^{13}C NMR (75 MHz) ($CDCl_3$ + DMSO-*d*$_6$) spectrum of compound **MR3**

VITAE

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Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Chemistry)	Prince of Songkla University	2005

Scholarship Awards during Enrolment

Scholarship was awarded by the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), the Natural Products from Mangrove Plants and Synthetic Materials Research Unit (NSU) and the Prince of Songkla University.

List of Publication and Proceeding

Publication

Yodsaoue, O., Cheenpracha, S., Ponglimanont, C., Chantrapromma, S., Fun, H.-K. and Kanjana-opas, A. (2008). Phanginin A-K, diterpenoids from the seeds of *Caesalpinia sappan* Linn.: Phytochemisty 69, 1242-1249.

Proceeding

1. **Yodsaoue, O.**, Karalai, C. and Ponglimanont, C. Chemical constituents from the heartwood and roots of *Caesalpinia sappan.*: PERCH-CIC Congress V. Jomtein Palm Beach, Pattaya, Chonburi, Thailand. 6-9 May 2007. (Poster)
2. **Yodsaoue, O.**, Karalai, C. and Ponglimanont, C. Chemical constituents from the heartwood of *Caesalpinia sappan.* The 33rd Congress on Science and Technology of Thailand, Walailak University, Nakhon si thammarat, Thailand. 18-20 October 2007. (Poster).