Chemical Constituents from the Stem and the Roots of *Ellipanthus tomentosus* Kurz var. *tomentosus*

Jarinthon Sonprasit

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
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Thesis Title Chemical Constituents from the Stem and the Roots of *Ellipanthus tomentosus* Kurz var.*tomentosus*

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(Prof. Dr. Amornrat Phongdara)

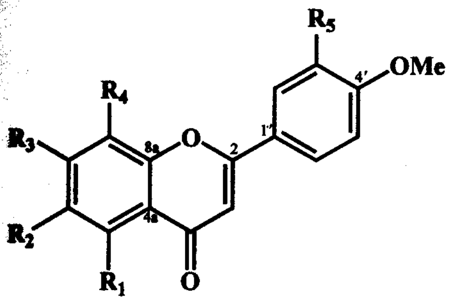
Dean of Graduate School

ชื่อวิทยานิพนธ์ องค์ประกอบทางเคมีจากลำต้นและรากของตานกกด
ผู้เขียน นางสาวจรินทร ศรประสิทธิ์
สาขาวิชา เคมีศึกษา
ปีการศึกษา 2554

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมี ของส่วนสกัดหยาบอะซีโตนจากลำต้นตานกกด สามารถแยกสารที่มีการรายงานแล้ว 3 สารเป็นสารในกลุ่มไตรเทอร์พีน คือ lupeol (**TA1**), ไบฟลาโวนอยด์ คือ lophirone C (**TA2**) และ ไอโซฟลาโวน คือ gerontoisoflavone (**TA3**)

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบเมทิลีนคลอไรด์จากรากของต้นตานกกดสามารถแยกสารที่มีการรายงานแล้ว 12 สาร เป็นสารในกลุ่มชาลโคน 1 สาร คือ flavokawain A (**RD1**), ฟลาโวน 4 สาร คือ 4′,5,6,7,8-pentamethoxyflavone (**RD2**), 3′,4′,5,6,7,8-hexamethoxyflavone (**RD3**), 5-demethyl nobiletin (**RD4**) และ 5,7,8,3′,4′-pentamethoxyflavone (**RD5**), สารประเภทเฟอรูริกแอซิดเอสเทอร์ 1 สาร คือ (*E*)-ferulic acid tetracosyl ester (**RD6**), สารประเภทไอโซฟลาโวน 2 สาร คือ 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (**RD7**) และ 5,4′-dimethoxy-6,7-methylenedioxyisoflavone (**RD8**) สารผสมประเภทสเตอรอยด์ 2 สาร คือ β-sitosterol (**RD9**) และ stigmasterol (**RD10**), สารประเภท อนุพันธ์ของกรดเบนโซอิก 2 สาร คือ 4-hydroxybenzaldehyde (**RD11**) และ vanillin (**RD12**) และจากส่วนสกัดหยาบอะซีโตน จากรากของต้นตานกกดสามารถแยกสารที่มีการรายงานแล้ว 5 สาร เป็นสารประเภทไบฟลาโวนอยด์ 4 สารคือ lophirone A (**RA1**), calodenone (**RA2**), 6‴-hydroxylophirone B (**RA3**) และ calodenin B (**RA4**) และสารประเภทฟลาโวน 1 สารคือ (2*R*,3*R*)-2,3-*trans*-4′,5,7-trimethoxydihydroflavonol (**RA5**) โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี และเปรียบเทียบกับสารที่มีรายงานการวิจัยแล้ว

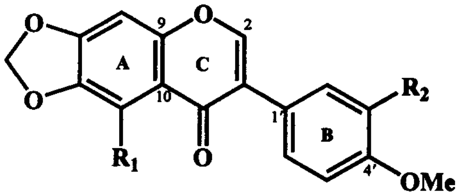


RD2 $R_1 = R_2 = R_3 = R_4 = OMe, R_5 = H$

RD3 $R_1 = R_2 = R_3 = R_4 = R_5 = OMe$

RD4 $R_1 = OH, R_2 = R_3 = R_4 = R_5 = OMe$

RD5 $R_1 = R_3 = R_4 = R_5 = OMe, R_2 = H$



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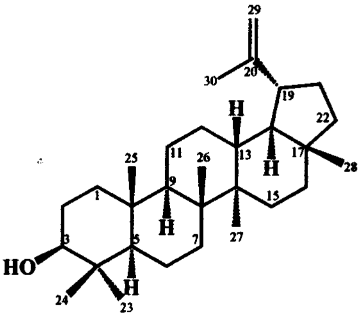
RD8 $R_1 = OMe, R_2 = H$



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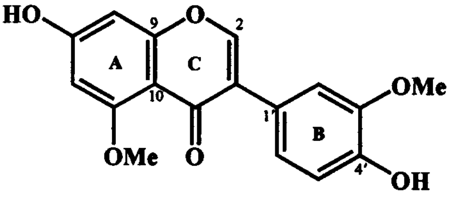

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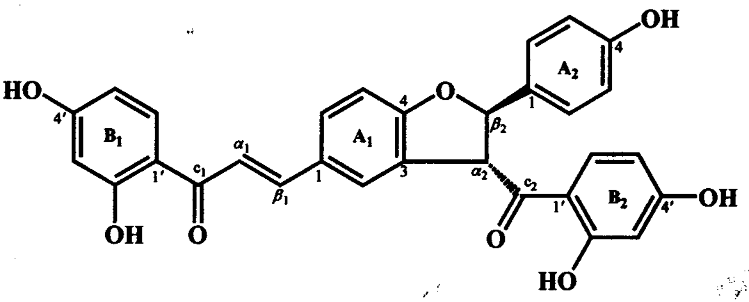

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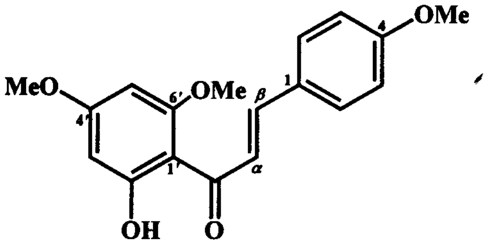

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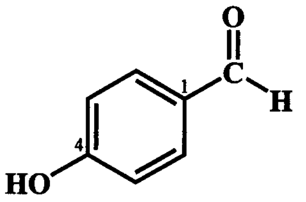

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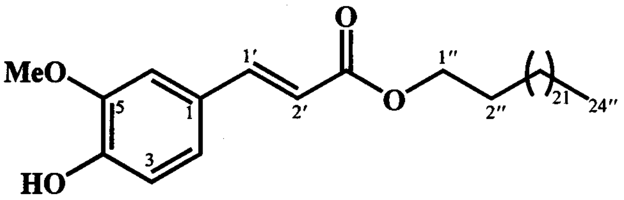

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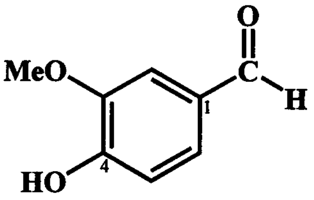

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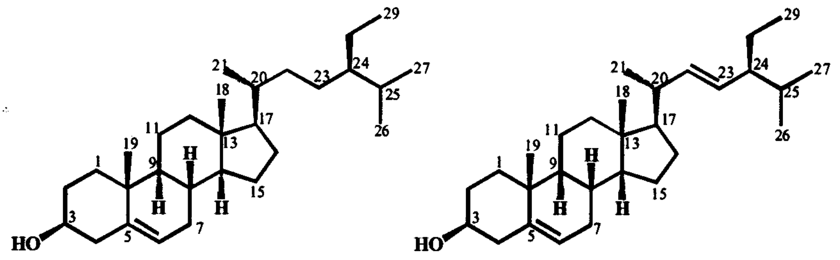
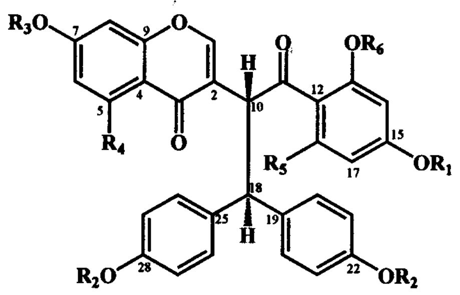
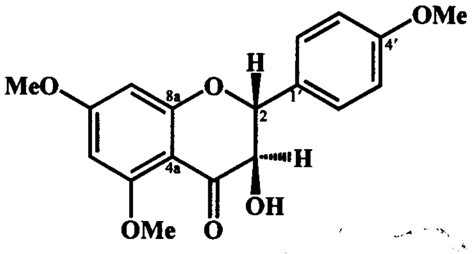


RD9 and RD10





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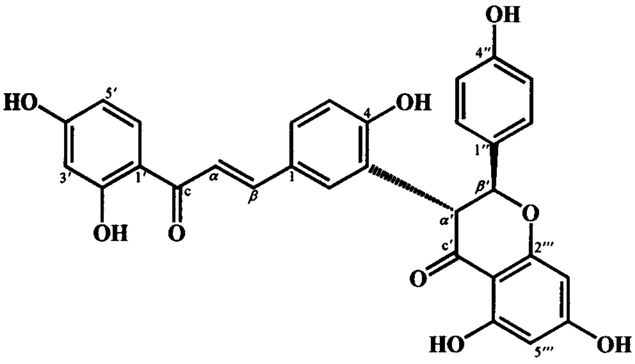
RA2 $R_1 = Me, R_2 = R_3 = R_4 = R_5 = R_6 = H$

RA5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1′
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β
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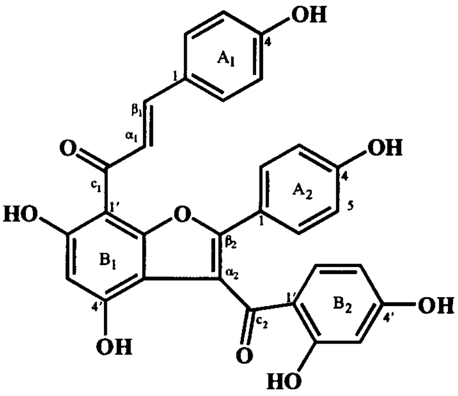
1″
β′
α′
c′
2‴
5‴

RA3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α1
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c1
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O
OH
A2
4
5
1
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HO
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α2
4′
OH
1′
B2
OH
4′
c2
O
HO

RA4

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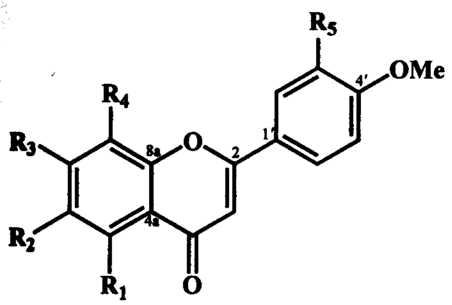
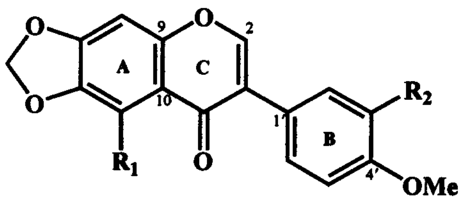
Author Miss Jarinthon Sonprasit
Major Program Chemical Studies
Academic Year 2011

ABSTRACT

Investigation of the crude acetone extract of the stem of *Ellipanthus tomentosus* Kurz var. *tomentosus* yielded three known compounds; triterpenoid: lupeol (**TA1**), biflavonoid: lophirone C (**TA2**) and isoflavone: gerontoisoflavone (**TA3**).

Investigation of the crude methylene chloride extract of the roots of *Ellipanthus tomentosus* Kurz var. *tomentosus* yielded thirteen known compounds; one chalcone: flavokawain A (**RD1**), four flavones: 4′,5,6,7,8-pentamethoxyflavone (**RD2**), 3′,4′,5,6,7,8-hexamethoxyflavone (**RD3**), 5-demethylnobiletin (**RD4**) and 5,7,8,3′,4′-pentamethoxyflavone (**RD5**), one ferulic acid ester: (*E*)-ferulic acid tetracosyl ester (**RD6**), two isoflavones: 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (**RD7**) and 5,4′-dimethoxy-6,7-methylenedioxyisoflavone (**RD8**), a mixture of two steroids: *β*-sitosterol (**RD9**) and stigmasterol (**RD10**), two benzoic acid derivatives: 4-hydroxybenzaldehyde (**RD11**) and vanillin (**RD12**). Investigation of the crude acetone extract of the roots of *Ellipanthus tomentosus* Kurz var. *tomentosus* yielded five known compounds; four biflavonoids: lophirone A (**RA1**), calodenone (**RA2**), 6‴-hydroxylophirone B (**RA3**) and calodenin B (**RA4**) and one flavones: (2*R*,3*R*)-2,3-*trans*-4′,5,7-trimethoxydihydroflavonol (**RA5**). Their structures were determined on the basis of spectroscopic data and comparison with those reported.





RD2 $R_1 = R_2 = R_3 = R_4 = OMe, R_5 = H$

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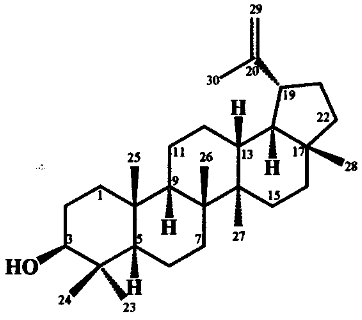

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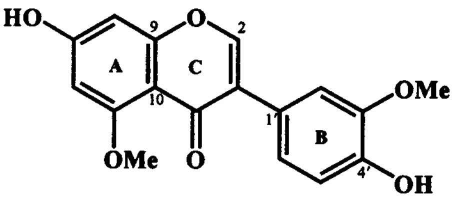

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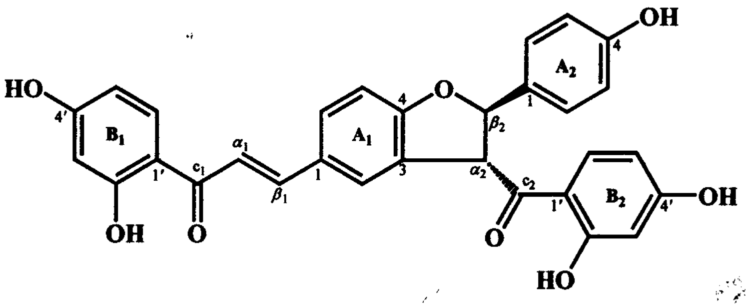

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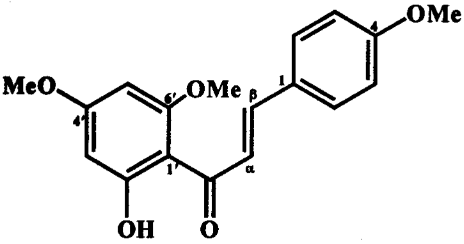

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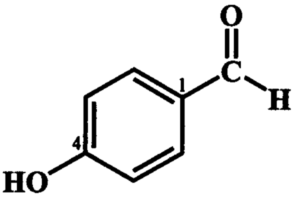
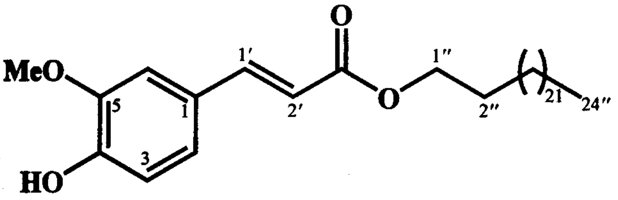
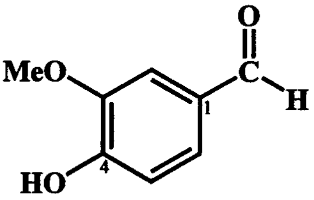


RD11



RD6



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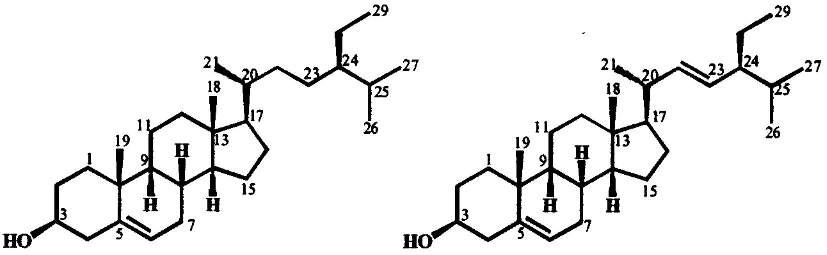
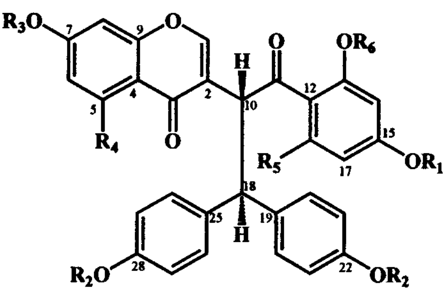
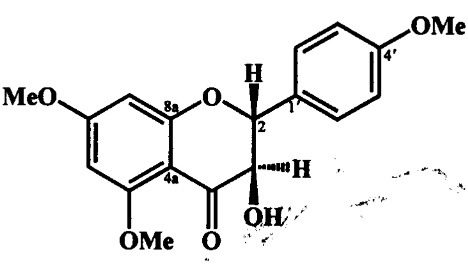


RD9 and RD10

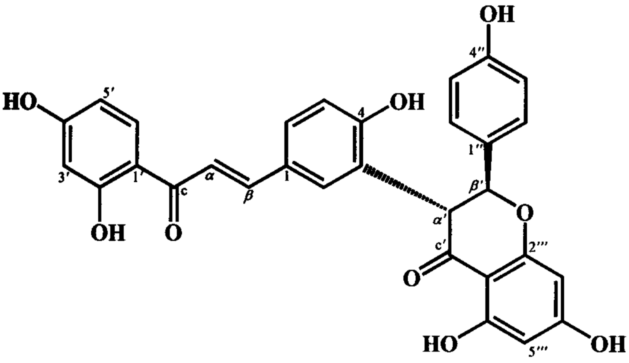


RA1 $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$

RA2 $R_1 = Me, R_2 = R_3 = R_4 = R_5 = R_6 = H$



RA5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5′
4
OH
1‴
3′
1′
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β
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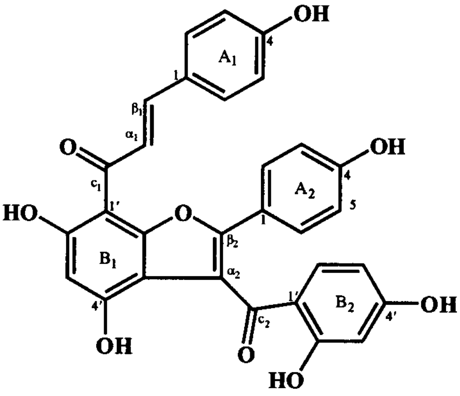
β′
O
α′
c′
2‴
OH
O
O
HO
5‴
OH

RA3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A₂
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HO
1′
O
1
5
B₁
β₂
α₂
B₂
1′
OH
4′
4′
c₂
OH
O
HO

RA4

ACKNOWLEDGEMENT

I wish to express my deepest and sincere gratitude to my supervisor, Associate Professor Dr. Chatchanok Karalai, for his valuable instructions, expert guidance, excellent suggestions and kindness which are more than I can describe here. Everything will always be in my mind.

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I am very grateful to Associate Professor Dr. Supinya Tewtrakul for bioactivity testing and valuable advices.

I would like to express my appreciation to the staffs of the Department of Chemistry, Faculty of Science, Prince of Songkla University for making this thesis possible.

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Jarinthon Sonprasit

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents from the stem and the roots of *Ellipanthus tomentosus* Kurz var. *tomentosus*. They are a part of the basic research on the Thai medicinal plants. Twenty known compounds have been isolated from the stem and the roots of *Ellipanthus tomentosus* Kurz var. *tomentosus*.

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LIST OF ABBREVIATIONS AND SYMBOLS

s = singlet

d = doublet

t = triplet

m = multiplet

dd = doublet of doublets

dt = doublet of triplets

g = gram

nm = nanometer

mp = melting point

cm^{-1} = reciprocal 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

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

UV = Ultraviolet

MS = Mass Spectroscopy

EIMS = Electron Impact Mass Spectroscopy

NMR = Nuclear Magnetic Resonance

1D NMR = One Dimensional Nuclear Magnetic Resonance

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

NOESY = Nuclear Overhauser Effect Spectrosopy

CC = Column Chromatography

QCC = Quick Column Chromatography

PLC = Preparative Thin Layer Chromatography

TLC = Thin Layer Chromatography

TMS = tetramethylsilane

$CDCl_3$ = deuterochloroform

DMSO-d_6 = dimethylsulfoxide-d_6

Acetone-d_6 = deuteroacetone

CHAPTER 1

INTRODOCTION

1.1 Introtroduction

Ellipanthus tomentosus **Kurz var.** ***tomentosus***, locally known as "Tanokkod (ตานกกด)", belongs to the family Connaraceae and is widely grown in West Africa, Ghana and Thailand. *E. tomentosus* is the only one species found in Thailand.

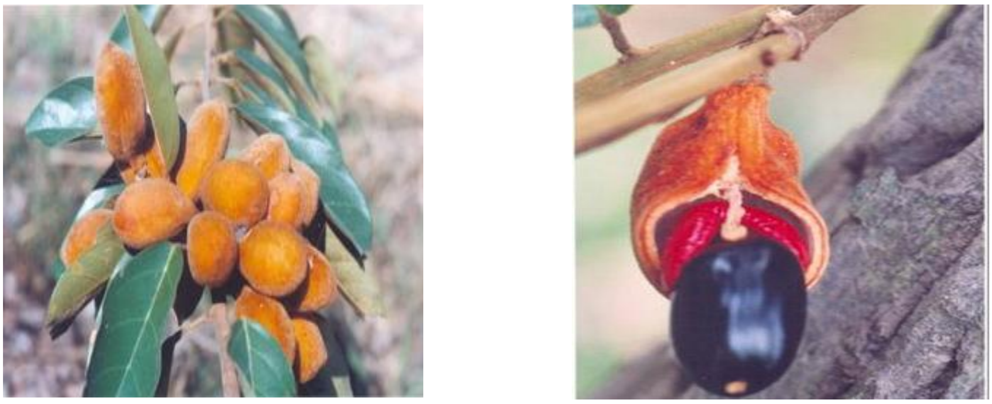
E. tomentosus may be a shrub of 3-4 m in height. Its branches are densely rusty brown pubescent. Leaves are indeciduous, 4-16-jugate with petiole 2-10 cm, rachis 12-27 cm long and densely rusty brown pubescent. Lateral leaflets are more or less alternate or sometime opposite, ovate to narrowly oblong, slightly or not unequal at base, and terminal leaflet is elliptic or narrowly ovate, cuneate at base. All leaflets acuminate or obtuse are densely pilose beneath; petiolules 1.5-3 mm long are also densely brown pilose (Berhaut, 1954; Jongkind and Lemmens, 1989). Inflorescences panicles are 1-10 per leaf axil, 5-20 cm long, up to 100-flowered by little white flowers almost homostylous. Often the supporting leaves are reduced resulting in a compound pseudoterminal inflorescence. Follicles are 1-5 in fruit, often united at base, ovoid, more or less oblique (2-4.5) x (1-2.5) cm with abeak blunt and broad often indistinctly separated. Pericarp has short red hairs outside and long brownish hairs inside. Each follicle contains one seed ovoid (12-20) x (5-10) mm, surrounded by a sarcotesta 3-7 mm long.

Tree

Stem

Leaves

Flowers



Fruits

Seeds

Figure 1 Parts of *Ellipanthus tomentosus* Kurz var.*tomentosus*

According to Smitinand (2001), there are six genus of family Connaraceae found in Thailand as follows.

1. Agelaea
2. Byrsocarpus
3. Connarus
4. Cnestic
5. Ellipanthus
6. Rourea

1.2 Review of literatures

Chemical constituents isolated from family Connaraceae were summarized in **Table 1**. Information obtained from SciFinder Scholar copyright in 2010 will be presented and classified into groups: acids, flavonoids and glycoside (Ramiah *et al*., **1976**; Jiang *et al*., **1990**).

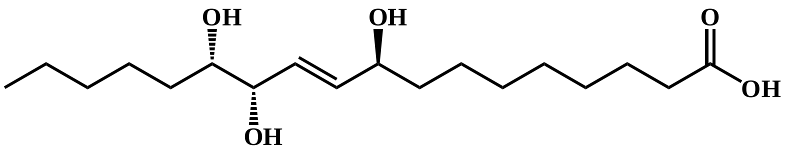
Table 1 Compounds from plants of Connaraceae family

a : Acids
b : Flavonoids
c : Glycoside

Scientific name	**Part**	**Compounds**	**Bibliography**
Rorrea minor	Stem	Rourinoside, **c1** Rouremin, **c2** 1-(26-Hydroxyhexacosanoyl)-glycerol, **c3** 1-O-β-D-glucopyranosyl-(2*S*, 3*R*, 4*E*-8*Z*)-2-*N*-(2′-hydroxypalmitoyl)-octadecasphinga-4,8-dienine, **c4** 9*S*, 12*S*, 13*S*-Trihydroxy-10*E*-octadecenoic acid, **a1** Dihydrovomifoliol-9-β-D-glucopyranoside, **c5**	He *et al*., 2006
Byrsocarpus coccineu	Leaves	Quercetin 3-o-α-arabinoside, **b1** Quercetin, **b2** Quercetin 3-β-D-glucoside, **b3**	Ahmadu *et al*., 2007

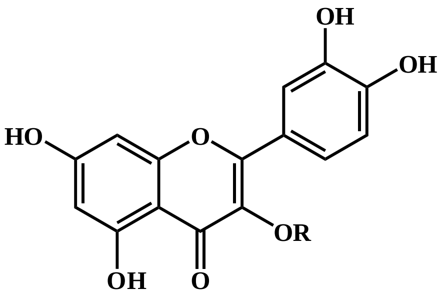
Structures

a: acids



9*S*, 12*S*, 13*S*-trihydroxy-10*E*-octadecenoic acid, **a1**

b: flavonoids

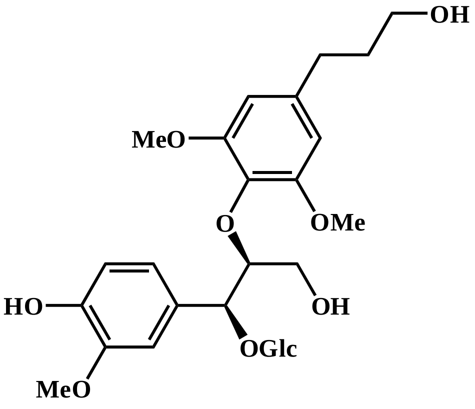


R = *α*–arabinose; quercetin 3-o-*α*-arabinoside, **b1**

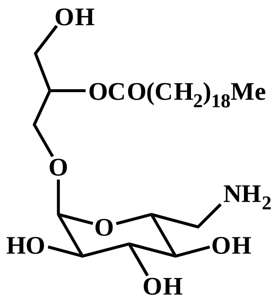
R = H; quercetin, **b2**

R = *β*-D–glucose; quercetin 3-*β*-D–glucoside, **b3**

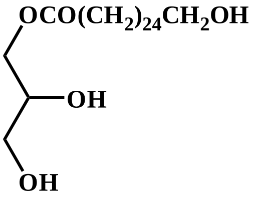
c: glycoside



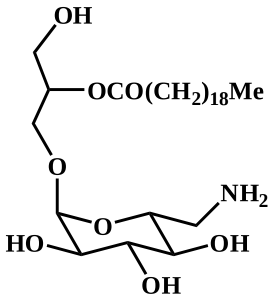
Rourinoside, **c1**



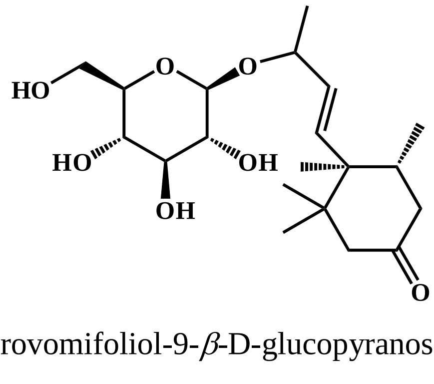
Rouremin, **c2**



1-(26-Hydroxyhexacosanoyl)-glycerol, **c3**



1-O-β-D-glucopyranosyl-(2*S*, 3*R*, 4*E*-8*Z*)-2-*N*-(2′-hydroxypalmitoyl)-octadecasphinga-4,8-dienine**, c4**



Dihydrovomifoliol-9-β-D-glucopyranoside, **c5**

1.3 Objective

The purpose of this research work is to investigate the chemical constituents from the stem and roots of *Ellipanthus tomentosus* Kurz var.*tomentosus*. It involves isolation, purification and structure elucidation.

CHAPTER 2

EXPERIMENTAL

2.1 Instruments and Chemicals

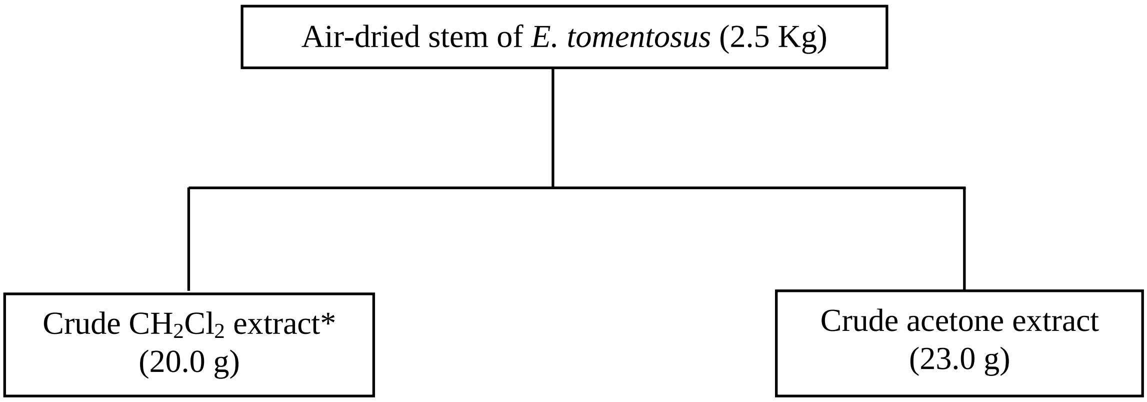
Melting point was recorded in ^{o}C on a digital Electrothermal 9100 Melting Point Apparatus. Ultraviolet spectra were measured with a UV-160A spectrophotometer (SHIMADZU) and principle bands ($_{\text{max}}$) were recorded as wavelengths (nm) and log   in methanol solution. The optical rotation $[\alpha]_D$ was measured in chloroform and methanol solution with Sodium D line (590 nm) on a JASCO P-1020 digital polarimeter. The IR spectra were measured with a Perkin-Elmer 783 FTS165 FT-IR spectrophotometer. ^{1}H and ^{13}C – Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield^{TM} 300 and 500 MHz spectrometer. Spectra were recorded in deuterochloroform, dimethylsulfoxide-d_6 and acetone-d_6 as   value in ppm downfield from TMS (internal standard   0.00) and coupling constant (*J*) are expressed in hertz. EI mass spectra was measured on MAT 95 XL Mass spectrometer. Quick column chromatography (QCC) and column chromatography (cc) was performed by using silica gel 60 H (Merck) and silica gel 100 (70-230 Mesh ASTM, Merck), respectively. For thin-layer chromatography (TLC), aluminum sheets of silica gel 60 F_{254} (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light. Solvents for extraction and chromatography were distilled at their boiling ranges prior to use except chloroform was analytical grade reagent.

2.2 Plant Material

Stem and roots of *Ellipanthus tomentosus* Kurs var. *tomentosus* were collected from Khon Kaen in the Northeast region of Thailand, in September 2009. The plant was identified by Associate Professor Dr. Kittichate Sridith and a voucher specimen (No. S. Jarinthon) has been deposited in the herbarium of Department of Biology, Faculty of Science, Prince of Songkla University.

2.3 Extraction and Isolation

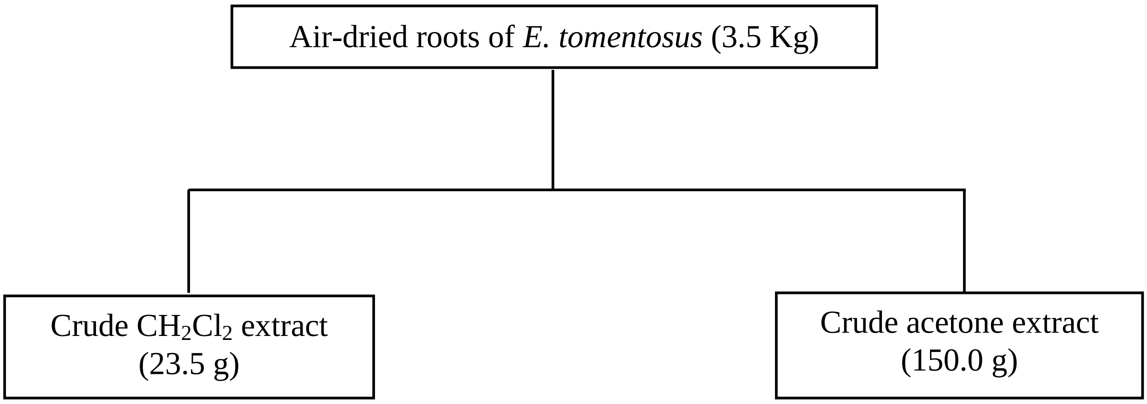
The small pieces of air-dried stem (2.5 Kg) of *E. tomentosus* were extracted with methylene chloride and acetone successively (each 2 x 10.5 L, for 5 days) at room temperature. The crude extracts were evaporated under reduced pressure to afford brownish methylene chloride (20.0 g) and acetone extracts (23.0 g), respectively. The process of extraction was shown in **Scheme 1**.



*****Not further investigated

Scheme 1 Extraction of the stem of *E. tomentosus*.

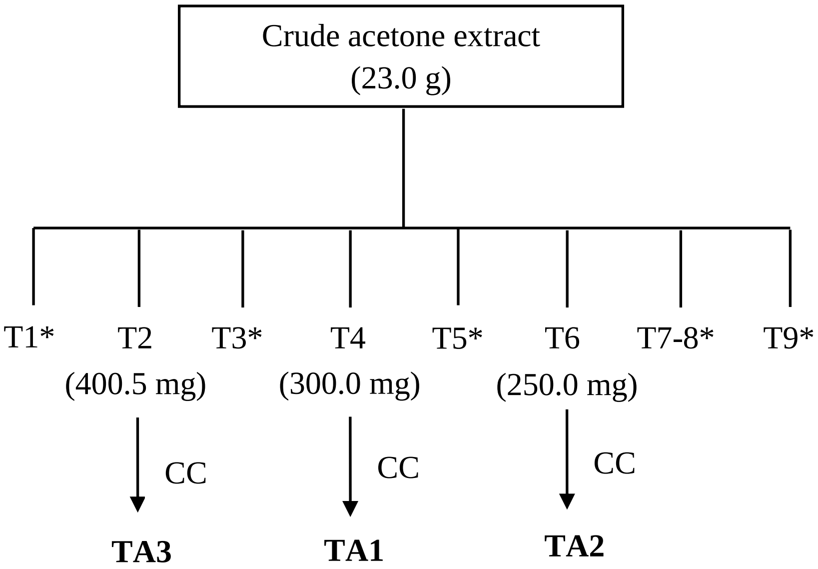
The air-dried roots (3.5 Kg) of *E. tomentosus* were extracted with methylene chloride and acetone successively (each 2 x 21 L, for 5 days) at room temperature. The crude extracts were evaporated under reduced pressure to afford brownish methylene chloride (23.5 g) and acetone extracts (150.0 g), respectively.



Scheme 2 Extraction of the roots of *E. tomentosus*.

2.4 Isolation and Chemical Investigation

2.4.1 Investigation of the crude acetone extract from the stem of *E. tomentosus*



* Not further investigated

Scheme 3 Isolation of compounds **TA1-TA3** from the stem of *E. tomentosus*.

The crude acetone extract (23.0 g) was further purified by QCC using hexane as eluent and increasing polarity with acetone to give ten fractions (T1-T9, **Scheme 3**).

Fraction T2 (400.5 mg) was further purified by CC with CH_2Cl_2-hexane (2:8, v/v) to give **TA3** (30.0 mg, R_f = 0.29 (7:3, CH_2Cl_2 – hexane).

Fraction T4 (300.0 mg) was further purified by CC with acetone-hexane (3:7, v/v) to give **TA1** (4.0 mg, R_f = 0.27 (7:3, acetone – hexane).

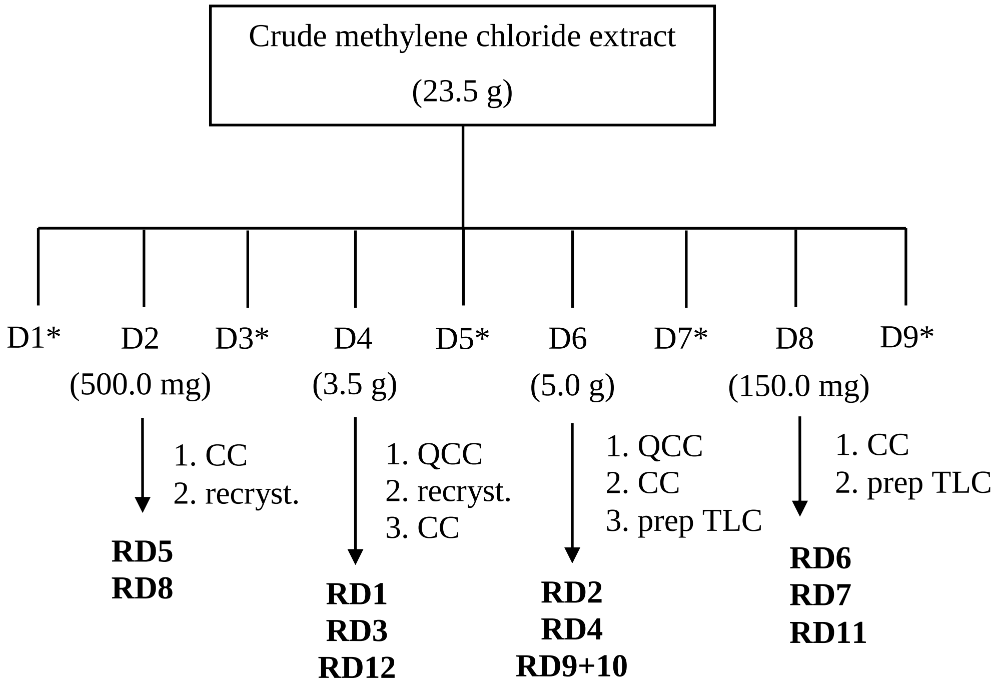
Fraction T6 (250.0 mg) was further purified by CC with acetone-hexane (5:5, v/v) to afford nine subfractions (T6a-T6i). Subfraction T6d (190.0 mg) was recrystallized from acetone-hexane (5:5, acetone – hexane) to give **TA2** (15.0 mg, R_f = 0.27 (5:5, acetone – hexane).

Compound TA1, 3β-lupeol: White solid; mp: 193 C; [ ]$^{24}_{D}$: +21 (*c* 0.20, $CHCl_3$); IR (neat) $_{max}$ (cm^{-1}) : 3245 and 1636; ^{1}H ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2.

Compound TA2, lophirone C: Yellow solid; mp: 191-192 C; [ ]$^{24}_{D}$: -10 (*c* 0.50, MeOH); UV (MeOH) $_{max}$ (log): 285 (3.05) and 305 (3.26) nm; IR (neat) $_{max}$ (cm^{-1}): 3245, 1698 and 1630; ^{1}H (Acetone-d_6, 300 MHz), see Table 3; ^{13}C NMR (Acetone-d_6, 75 MHz), see Table 3.

Compound TA3, gerontoisoflavone: white solid; mp: 283-284 C; UV (MeOH) $_{max}$ (log): 259 (3.05) and 375 (2.50) nm; IR (neat) $_{max}$ (cm^{-1}): 3551 and 1683; ^{1}H (Acetone-d_6, 500 MHz), see Table 5; ^{13}C NMR (Acetone-d_6, 125 MHz), see Table 5.

2.4.2 Investigation of the crude methylene chloride extract from the roots of *E. tomentosus*



* Not further investigated

Scheme 4 Isolation of compounds **RD1-RD12** from the roots of *E.tomentosus*.

The crude methylene chloride extract (23.5 g) was further purified by QCC using hexane as eluent and increasing polarity with acetone to give nine fractions (D1-D9, **Scheme 4**).

Fraction D2 (500.0 mg) was separated by CC with acetone-hexane (1:9, v/v) to afford twelve subfractions (D2a-D2l). Subfraction D2c (280.0 mg) was recrystallized from CH_2Cl_2 to give **RD5** (40.0 mg, R_f = 0.44 (CH_2Cl_2)) and **RD8** (4.0 mg, R_f = 0.67 (CH_2Cl_2)).

Fraction D4 (3.5 g) was purified by QCC with acetone- hexane (1:9, v/v) as eluent to afford thirteen subfractions (D4a-D4m). Subfraction D4e was recrystallized from CH_2Cl_2 to give **RD1** (30.0 mg, R_f = 0.42 (CH_2Cl_2)) and mother liquor (2.6 g) was further subjected to QCC with acetone-hexane (3:7, v/v) to afford eight subfractions (D4a1-D4a8). Subfraction D4a7 (350.0 mg) was purified by CC with acetone-hexane (3:7, v/v) to give **RD3** (5.0 mg, R$_f$ = 0.47 (4:6, acetone – hexane)) and **RD12** (3.0 mg, R$_f$ = 0.62 (4:6, acetone – hexane)).

Fraction D6 (5.0 g) was purified by QCC with acetone- hexane (2:8, v/v) as eluent to afford fourteen subfractions (D6a-D6n). Subfraction D6f (900.0 mg) was purified by CC with acetone-hexane (3:7, v/v) to give **RD2** (20.0 mg, R$_f$ = 0.44 (4:6, acetone-hexane)). Subfraction D6h (300.0 mg) was purified by CC with CH$_2$Cl$_2$ -hexane (8:2, v/v) and followed by prep TLC with acetone-hexane (3:7, v/v) to give **RD4** (11.0 mg, R$_f$ = 0.29 (CH$_2$Cl$_2$)) and **RD (9+10)**.

Fraction D8 (150.0 mg) was separated by CC with acetone-hexane (3:7, v/v) to afford seven subfractions (D8a-D8g). Subfraction D8c (50.0 mg) was recrystallized from CH$_2$Cl$_2$ to give **RD6** (15.0 mg, R$_f$ = 0.37 (2:8, acetone-hexane)). Subfraction D8e (60.0 mg) was purified by CC with acetone-hexane (3:7, v/v) and followed by prep TLC with acetone-hexane (4:6, v/v) to give **RD7** (5.0 mg, R$_f$ = 0.44 (4:7, acetone-hexane)). Subfraction D8f (22.0 mg) was purified by prep TLC with acetone-hexane (5:5, v/v) to give **RD11** (2.0 mg, R$_f$ = 0.34 (6:4, acetone-hexane)).

Compound RD1, flavokawain A: Yellow solid; mp: 113 C; UV (MeOH) $_{max}$ (log): 249 (2.75) and 363 (2.80) nm; IR (neat) $_{max}$ (cm^{-1}): 3423 (O-H stretching) and 1622 (C=O stretching); ^{1}H (CDCl$_3$, 300 MHz), see Table 7; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 7.

Compound RD2, 4′,5,6,7,8-pentamethoxyflavone: White solid; mp: 155-156 C; UV (MeOH) $_{max}$ (log): 271 (2.87) and 323 (2.97) nm; IR (neat) $_{max}$ (cm^{-1}): 2940, 1641 and 1273; ^{1}H (CDCl$_3$, 300 MHz), see Table 9.; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 9.

Compound RD3, 3′,4′,5,6,7,8-hexamethoxyflavone: White solid; mp: 136-137 C; UV (MeOH) $_{max}$ (log): 277 (2.86) and 325 (2.87) nm; IR (neat) $_{max}$ (cm^{-1}): 2939, 1641 and 1272; ^{1}H (CDCl$_3$, 300 MHz), see Table 11; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 11.

Compound RD4, 5-demethylnobiletin: White solid; mp: 137-139 C; UV (MeOH) $_{max}$ (log): 275 (2.56) and 329 (2.77) nm; IR (neat) $_{max}$ (cm^{-1}): 3425 and 1638; ^{1}H (CDCl$_3$, 300 MHz), see Table 13; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 13.

Compound RD5, 5,7,8,3′,4′-pentamethoxyflavone: White solid; mp: 138 C; UV (MeOH) $_{max}$ (log): 280 (2.87) and 320 (2.77) nm; IR (neat) $_{max}$ (cm^{-1}): 2939, 1641 and 1272; ^{1}H (CDCl$_3$, 300 MHz), see Table 15; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 15.

Compound RD6, (*E*)-ferulic acid tetracosyl ester: White solid; mp: 64 C; UV (MeOH) $_{max}$ (log): 234 (4.33), 291 (4.12) and 325 (4.42) nm; IR (neat) $_{max}$ (cm^{-1}): 3551 and 1682; EIMS: *m/z* [M-1]$^{+}$ 530 (100) and 193.8 (60); ^{1}H (CDCl$_3$, 300 MHz), see Table 17; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 17.

Compound RD7, 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone: White solid; mp: 178 C; [ ]$^{24}_{D}$: +12 (*c* 0.55 , MeOH); UV (MeOH) $_{max}$ (log): 265 (2.99) and 325 (2.46) nm; IR (neat) $_{max}$ (cm^{-1}): 1643; ^{1}H (CDCl$_3$, 300 MHz), see Table 19; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 19.

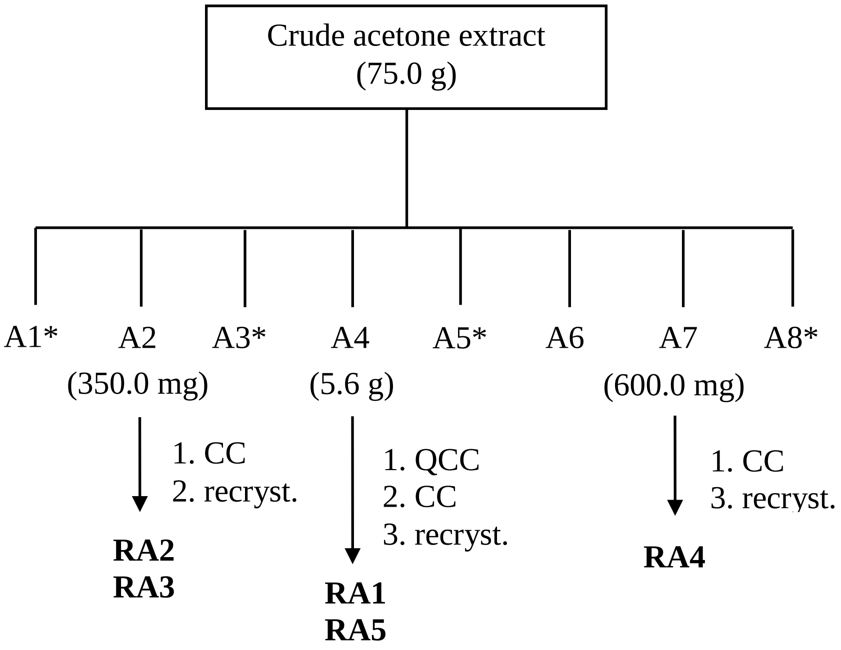
Compound RD8, 5,4′-dimethoxy-6,7-methylenedioxyisoflavone: White solid; mp: 175 C; UV (MeOH) $_{max}$ (log): 262 (2.98) and 328 (2.42) nm; IR (neat) $_{max}$ (cm^{-1}): 1646^{1}H (CDCl$_3$, 300 MHz), see Table 21; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 21.

Compound RD9, *β*-sitosterol and ***RD10***, stigmasterol: White solid; IR (neat) $_{max}$ (cm^{-1}): 3425 and 1642.

Compound RD11, 4-hydroxybenzaldehyde: Colorless oil; UV (MeOH) $_{max}$ (log): 237 (2.70), 293 (3.01) and 306 (3.05) nm; IR (neat) $_{max}$ (cm^{-1}): 3367 and 1684; ^{1}H (CDCl$_3$, 300 MHz), see Table 23; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 23.

Compound RD12, vanillin: Colorless oil; UV (MeOH) $_{max}$ (log): 233 (2.69), 291 (3.02) and 306 (3.03) nm; IR (neat) $_{max}$ (cm^{-1}): 3384 and 1648; ^{1}H (CDCl$_3$, 300 MHz), see Table 24; ^{13}C NMR (CDCl$_3$, 75 MHz), see Table 24.

2.4.3 Investigation of the acetone extract from the roots of *E. tomentosus*



* Not further investigated

Scheme 5 Isolation of compounds **RA1-RA5** from the roots of *E. tomentosus*.

The acetone extract (75.0 g) was further purified by QCC using CH_2Cl_2 as eluent and increasing polarity with MeOH to give eight fractions (A1-A8, **Scheme 5**).

Fraction A2 (350.0 mg) was further purified by CC with MeOH-CH_2Cl_2 (1:9, v/v) to afford nine subfractions (A2a-A2i). Subfraction A2c (125.0 mg) was recrystallized from CH_2Cl_2 to give **RA2** (20.0 mg, R_f = 0.47 (1:9, MeOH-CH_2Cl_2)). Subfraction A2e (115.0 mg) was purified by CC with MeOH-CH_2Cl_2 (1:9, v/v) to give **RA3** (8.0 mg, R_f = 0.37 (1:9, MeOH-CH_2Cl_2)).

Fraction A4 (5.6 g) was separated by QCC with MeOH-CH_2Cl_2 (1:9, v/v) to afford fourteen subfractions (A4a-A4n). Subfractions A4d (190.5 mg) was purified by CC with MeOH-CH_2Cl_2 (1:9, v/v) to give **RA1** (30.0 mg, R_f = 0.24 (1:49, MeOH-CH_2Cl_2)) and **RA5** (4.0 mg, R_f = 0.27 (1:49, MeOH-CH_2Cl_2)).

Fraction A7 (600.0 mg) was further purified by CC with MeOH-CH_2Cl_2 (1:49, v/v) to afford eleventh subfractions (A7a-A7k). Subfraction A7d (230.0 mg) was recrystallized from CH_2Cl_2 to give **RA4** (15.0 mg, R_f = 0.44 (1:24, MeOH-CH_2Cl_2)).

Compound RA1, lophirone A: White solid; mp: 189-190 C; [ ]$^{24}_{D}$: +12 (*c* 0.55, MeOH); UV (MeOH) $_{max}$ (log): 285 (2.96) and 367 (3.20) nm; IR (neat) $_{max}$ (cm^{-1}): 3382, 1700 and 1632; ^{1}H (Acetone-d_6, 300 MHz), see Table 25; ^{13}C NMR (Acetone-d_6, 75 MHz), see Table 25.

Compound RA2, calodenone: White solid; mp: 192 C; [ ]$^{23.8}_{D}$: +30 (*c* 0.55, MeOH)); UV (MeOH) $_{max}$ (log): 286 (3.00) and 369 (3.10) nm; IR (neat) $_{max}$ (cm^{-1}) : 3383, 1700 and 1630; ^{1}H (Acetone-d_6, 300 MHz), see Table 27; ^{13}C NMR (Acetone-d_6, 75 MHz), see Table 27.

Compound RA3, 6′′′-hydroxylophirone B: Yellow solid; mp: 181-182 C; [ ]$^{23.8}_{D}$: -48.8 (*c* 0.34, MeOH); UV (MeOH) $_{max}$ (log): 287 (2.81) and 374 (2.76) nm; IR (neat) $_{max}$ (cm^{-1}): 3407, 1629, 1229, 832; ^{1}H (Acetone-d_6, 300 MHz), see Table 29; ^{13}C NMR (Acetone-d_6, 75 MHz), see Table 29.

Compound RA4, calodenin B: Orange needles; mp: 249-250 C; [ ]$^{23.8}_{D}$: -49.0 (*c* 0.36, MeOH); UV (MeOH) $_{max}$ (log): 304 (3.00) and 361 (3.10) nm; IR (neat) $_{max}$ (cm^{-1}): 3211, 1738 and 1611; ^{1}H (Acetone-d_6, 300 MHz), see Table 31; ^{13}C NMR (Acetone-d_6, 75 MHz), see Table 31.

Compound RA5: (*2R,3R*)-2,3-*trans*-4´,5,7-trimethoxydihydroflavonol Viscous oil; [ ]$^{23.8}_{D}$: -7.5 (*c* 0.35, $CHCl_3$); UV (MeOH) $_{max}$ (log): 275 (3.05) and 312 (2.99) nm; IR (neat) $_{max}$ (cm^{-1}) : 3321 and 1682; ^{1}H ($CDCl_3$, 300 MHz), see Table 33; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 33.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Structural elucidation of compounds from the stem of *E. tomentosus*

The crude acetone extract from the stem of *Ellipanthus tomentosus* was subjected to chromatography and/or crystallization to give three known compounds of lupeol (**TA1**), lophirone C (**TA2**) and gerontoisoflavone (**TA3**).

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C, DEPT 135 , DEPT 90 , HMQC, HMBC and COSY. The physical data of the known compounds were also compared with the reported values.

3.1.1 Compound TA1

Compound **TA1** was isolated as a white solid, mp 193 C, $_{D}^{24}$ +21 (*c* 0.20, $CHCl_3$). The IR spectrum showed absorption bands of hydroxyl group (3245 cm^{-1}) and double bond (1636 cm^{-1}).

The ^{13}C NMR spectral data (Table 2, Figure 4) exhibited 30 signals, which comprised of seven methyl (14.6, 15.4, 16.0, 16.1, 18.0, 19.3 and 28.0), eleven methylene (18.3, 20.9, 25.2, 27.4, 27.5, 29.9, 34.3, 35.6, 38.7, 40.0 and 109.3), six methine (38.1, 48.0, 48.3, 50.5, 55.3 and 79.0) and six quaternary carbons (37.2, 38.9, 40.8, 42.8, 43.0 and 151.0) .

The ^{1}H NMR spectrum of **TA1**, displayed signals for a characteristic of lupane triterpenoid as seven methyl singlet signals at 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03 including one vinylic methyl at 1.68, two protons of an isopropenyl moiety at 4.69 (1H, *d*, J = 2.4 Hz) and 4.56 (1H, *m*) and a typical lupane H_{β}-19 proton at 2.38 (*dt*, J = 11.1, 5.7 Hz). An oxymethine proton was shown at 3.19 (1H, *dd*, J = 10.8, 5.4 Hz, H-3). The doublet splitting pattern together with a large coupling constant of H-3 with *Jax-ax* = 10.8 Hz and *Jax-eq* = 5.4 Hz indicated an axial (α) orientation of H-3. The position of the hydroxyl group at C-3 was determined through an HMBC experiment (Table 2) in which the oxymethine proton at 3.19 (H-3) showed correlations with C-1 (38.7), C-4 (38.9), C-23 (28.0) and C-24 (15.4). The position of a methine proton at C-19 was determined from HMBC correlation of H-19 (2.38) with C-18 (48.3), C-20 (151.0), C-21 (29.9) and C-30 (19.3). Thus on the basis of its spectroscopic data and comparison with the previous report [Reyolds *et al.*, 1986, $[\alpha]^{25}_{D}$: +23.0 (*c* 0.50, EtOH); Thongdeeying 2005], therefore compound **TA1** was identified as 3*β*-lupeol.

Selected HMBC correlations for compound **TA1**

Table 2 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **TA1** ($CDCl_3$) and 3*β*-lupeol (**R**, $CDCl_3$)

Position	**$_{H}$ (mult, *J*, Hz)**	**$_{C}$**	**R**	**DEPT**	**HMBC**
1	0.19	38.7	38.7	CH_2	-
2	1.56 (m)	27.4	27.4	CH_2	-
3	3.19 (dd, *J* = 10.8, 5.4)	79.0	79.0	CH	-
4	-	38.9	38.8	C	1, 4, 23, 24
5	0.68 (m)	55.3	55.3	CH	-
6	1.40 (m), 1.55 (m)	18.3	18.3	CH_2	-
7	1.40 (m)	34.3	34.2	CH_2	-
8	-	40.8	40.8	C	-
9	1.28 (m)	50.5	50.4	CH	-

Table 2 (continued)

Position	**$_{\text{H}}$ (mult, *J*, Hz)**	**$_{\text{C}}$**	**R**	**DEPT**	**HMBC**
10	-	37.2	37.1	C	-
11	1.22 (m), 1.45 (m)	20.9	20.9	CH_2	-
12	1.08 (m)	25.2	25.1	CH_2	-
13	1.67 (m)	38.1	38.0	CH	-
14	-	42.8	42.8	C	-
15	1.56 (m)	27.5	27.4	CH_2	-
16	1.51 (m)	35.6	35.5	CH_2	-
17	-	43.0	43.0	C	-
18	1.39 (m)	48.3	48.2	CH	-
19	2.38 (dt, *J* = 11.7, 5.7)	48.0	47.9	CH	13, 18, 20, 21, 29, 30
20	-	151.0	150.9	C	-
21	1.92 (m)	29.9	29.8	CH_2	-
22	1.20 (m), 1.40 (m)	40.0	40.0	CH_2	-
23	0.97 (s)	28.0	28.0	CH_3	3, 4, 5, 24
24	0.76 (s)	15.4	15.4	CH_3	3, 4, 5, 23
25	0.83 (s)	16.1	16.1	CH_3	1, 5, 9, 10
26	1.03 (s)	16.0	16.0	CH_3	7, 8, 9, 14
27	0.94 (s)	14.6	14.5	CH_3	8, 14, 15
28	0.79 (s)	18.0	18.0	CH_3	16, 17, 18, 22
29	4.56 (m), 4.69 (d, *J* = 2.4)	109.3	109.3	CH_2	19, 30
30	1.68	19.3	19.3	CH_3	19

3.1.2 Compound TA2

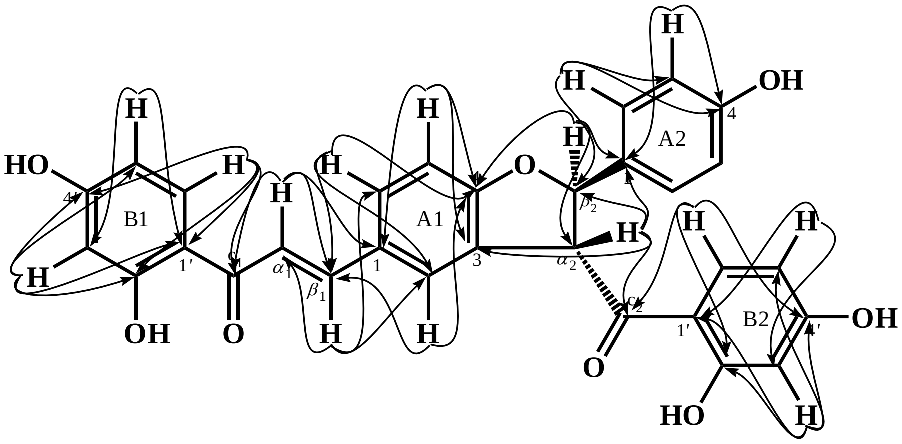
Compound **TA2** was isolated as a yellow solid, mp 191-192 C, $_{D}^{24}$ -10 (*c* 0.50, MeOH) T d a d a o on a $_{max}$ 285 and 305 nm suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of hydroxyl group (3245 cm^{-1}) and conjugated carbonyl (1630 cm^{-1}).

The ^{13}C NMR spectral data (Table 3, Figure 14) exhibited 30 signals, which comprised of two carbonyls (191.8, 200.4), two aliphatic methines (56.4, 87.7) and 26 olefinic and aromatic carbons (102.9, 103.0, 107.9, 108.8, 110.1, 112.6, 113.5, 115.5 (2C), 118.1, 126.1, 127.8 (3C), 128.8, 131.0, 131.1, 132.3, 133.8, 135.9, 143.9, 158.0, 162.2, 164.9, 166.4, 166.7).

The ^{1}H NMR spectrum of **TA2**, displayed signals for a chalcone skeleton: the typical *trans* olefinic protons at 7.70 (*d*, *J* = 15.3 Hz, H-α_1) and 7.80 (*d*, *J* = 15.3 Hz, H-β_1) and two 1,2,4-trisubstituted benzene rings at 7.00 (*d*, *J* = 8.4 Hz, H-5 (A1)), 7.62 (*br s*, H-2 (A1)) and 7.81 (*br d*, *J* = 8.4 Hz, H-6 (A1)) and at 6.36 (*d*, *J* = 2.1 Hz, H-3′), 6.44 (*dd*, *J* = 8.7, 2.1 Hz, H-5′ (B1)) and 7.99 (*d*, *J* = 8.7 Hz, H-6′ (B1)), including the chelated hydroxyl group at 13.57. The signal of a *p*–disubstituted benzene ring were evident at 6.87 (2H, *d*, *J* = 8.7 Hz, H-3, 5 (A2)) and 7.32 (2H, *d*, *J* = 8.4 Hz, H-2, 6 (A2)), together with those of a 1,2,4-trisubstituted benzene ring at 6.42 (*d*, *J* = 2.1 Hz, H-3′ (B2)), 6.55 (*dd*, *J* = 8.7, 2.1 Hz, H-5′ (B2)) and 7.97 (*d*, *J* = 8.7 Hz, H-6′ (B2)). Additional ^{1}H NMR signal of a dihydrobenzofuran ring were suggested from the resonances of two aliphatic protons at 5.49 (*d*, *J* = 6.9 Hz, H-α_2) and 6.20 (*d*, *J* = 6.9 Hz, H-β_2). The HMBC correlations of the proton signals at 5.49

with the carbons at 87.7 (C-β_2), 126.1 (C-2 (A1)), 131.0 (C-1 (A2)), 162.2 (C-4 (A1)) and 200.4 (C=O, (c$_2$)) and the proton signal at 6.20 (H-β_2) with the carbons at 56.4 (C- $_2$), 127.8 (C-3 (A1)), 131.0 (C-1 (A2)), 162.2 (C-4 (A1)) and 200.4 (c$_2$), indicated that the dihydrofuran ring was fused to the chalcone nucleus at C-3 and C-4 to form a dihydrobenzofuran ring.

The structure of **TA2** was confirmed by HMBC experiment. The proton signal at 7.99 (H-6′ (B1)) showed correlations with the carbons at 103.0 (C-3′ (B1)), 113.5 (C-1′ (B1)), 164.9 (C-4′ (B1)), 166.7 (C-2′ (B1)) and 191.8 (C=O, (c$_1$)), suggesting the connection of a 1,2,4-trisubstituted benzene ring (B1) at C=O (c$_1$). The o a on o o on na a -β_1) with carbons at 118.1 (C-α_1), 126.1 (C-2 (A1)) and 131.1 (C-6 (A1)) suggested that a 1,2,4-trisubstituted benzene ring (A1) was connected to C-β_1. The correlations of the proton signals at 7.97 (H-6′ (B2)) with the carbons at 135.9 (C-4′ (B2)), 166.4 (C-2′ (B2)) and 200.4 (c$_2$) suggested that a 1,2,4-trisubstituted benzene ring (B2) was connected to c$_2$. In addition the proton signals at 7.32 (H-2/6 (A2)) showed correlations with the carbons at 87.7 (C-β_2), 115.5 (C-3 (A2)) and 158.0 (C-4 (A2)), suggesting the connection of a *p*–disubstituted benzene ring at C-β_2. From the spectral data and comparison with those of lophirone C, therefore compound **TA2** was identified as lophirone C (Messanga *et al.*, 1994).



Selected HMBC correlations for compound **TA2**

Table 3 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **TA2** (Acetone-d_6)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	$_{\mathrm{C}}$	**DEPT**	**HMBC**
1-A1	-	128.8	C	-
2-A1	7.62 (br s)	126.1	CH	*β*1, 5-A1, 4-A1
3-A1	-	127.8	C	-
4-A1	-	162.2	C	-
5-A1	7.00 (d, *J* = 8.4)	110.1	CH	1-A1, 4-A1, 3-A1
6-A1	7.81 (br d, *J* = 1.5)	131.1	CH	*β*1, 2-A1, 4-A1
*β*1	7.80 (d, *J* = 15.3)	143.9	CH	2-A1, 6-A1, C1
α1	7.70 (d, *J* = 15.3)	118.1	CH	C1, 1-A1, 4′-B1
C1	-	191.8	C=O	-
1′-B1	-	113.5	C	-
2′-B1	-	166.7	C	-
3′-B1	6.36 (d, *J* = 2.1)	103.0	CH	2′-B1, 4′-B1
4′-B1	-	164.9	C	-
5′-B1	6.44 (dd, *J* = 8.7, 2.1)	107.9	CH	1′-B1, 3′-B1
6′-B1	7.99 (d, *J* = 8.7)	132.3	CH	4′-B1, 3′-B1

Table 3 (continued)

Position	**$_H$ (mult, *J*, Hz)**	**$_C$**	**DEPT**	**HMBC**
1-A2	-	131.0	C	-
2-A2	7.32 (d, *J* = 8.7)	127.8	CH	3-A2, 4-A2, *β*2
3-A2	6.87 (d, *J* = 8.7)	115.5	CH	1-A2, 4-A2
4-A2	-	158.0	C	-
5-A2	6.87 (d, *J* = 8.7)	115.5	CH	1-A2, 4-A2
6-A2	7.32 (d, *J* = 8.7)	127.8	CH	2-A2, 4-A2, *β*2
*β*2	6.20 (d, *J* = 6.9)	87.7	CH	-
*α*2	5.49 (d, *J* = 6.9)	56.4	CH	-
C2	-	200.4	C=O	-
1′-B2	-	112.6	C	-
2′-B2	-	166.4	C	-
3′-B2	6.42 (d, *J* = 2.1)	102.9	CH	2′-B2, 5′-B2
4′-B2	-	135.9	C	-
5′-B2	6.55 (dd, *J* = 8.7, 2.1)	108.8	CH	1′-B2, 3′-B2
6′-B2	7.97 (d, *J* = 8.7)	133.8	CH	2′-B2, 4′-2, C2
2′-B1(OH)	13.57 (s)	-	-	1′-B1, 2′-B1, 3′-B1

Table 4 Comparison of ^{1}H NMR and ^{13}C NMR spectral data of **TA2** (Acetone-d_6) and lophirone C (**R**, Acetone-d_6)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathrm{C}}$	**R**
1-A1	-	-	128.8	129.3
2-A1	7.62 (br s)	7.56 (d)	126.1	126.9
3-A1	-	-	127.8	132.1
4-A1	-	-	162.2	158.9
5-A1	7.00 (d, *J* = 8.4)	6.99 (d, *J* = 8.4)	110.1	111.0
6-A1	7.81 (br d, *J* = 1.5)	7.81 (dd, *J* = 8.7, 1.8)	131.1	132.1
β1	7.80 (d, *J* = 15.3)	7.79 (d, *J* = 15.4)	143.9	144.8
α1	7.70 (d, *J* = 15.3)	7.68 (d, *J* = 15.4)	118.1	119.3
C1	-	-	191.8	192.8
1′-B1	-	-	113.5	114.7
2′-B1	-	-	166.7	167.7
3′-B1	6.36 (d, *J* = 2.1)	6.35 (d, *J* = 2.4)	103.0	103.9
4′-B1	-	-	164.9	166.7
5′-B1	6.44 (dd, *J* = 8.7, 2.1)	6.43 (dd, *J* = 9.0, 2.4)	107.9	108.8
6′-B1	7.99 (d, *J* = 8.7)	7.97 (d, *J* = 9.0)	132.3	133.2
1-A2	-	-	131.0	129.3
2-A2	7.32 (d, *J* = 8.7)	7.31 (m)	127.8	128.7
3-A2	6.87 (d, *J* = 8.7)	6.86 (m)	115.5	116.5
4-A2	-	-	158.0	158.9
5-A2	6.87 (d, *J* = 8.7)	6.86 (m)	115.5	116.5
6-A2	7.32 (d, *J* = 8.7)	7.31 (m)	127.8	128.7
β2	6.20 (d, *J* = 6.9)	6.20 (d, *J* = 6.8)	87.7	88.6
α2	5.49 (d, *J* = 6.9)	5.46 (d, *J* = 6.8)	56.4	57.6
C2	-	-	200.4	201.4
1′-B2	-	-	112.6	113.8
2′-B2	-	-	166.4	165.6

Table 4 (continued)

Position	**$_{H}$ (mult, *J*, Hz)**	**R**	**$_{C}$**	**R**
3′-B2	6.42 (d, *J* = 2.1)	6.41 (d, *J* = 2.4)	102.9	104.0
4′-B2	-	-	135.9	163.2
5′-B2	6.55 (dd, *J* = 8.7, 2.1)	6.55 (dd, *J* = 9.0, 2.4)	108.8	109.7
6′-B2	7.97 (d, *J* = 8.7)	7.96 (d, *J* = 9.0)	133.8	134.7
2′B1(OH)	13.57 (s)	13.52 (s)	-	-

3.1.3 Compound TA3

Compound **TA3** was isolated as a pale-yellow solid, mp 283-284 C. The UV spectrum displayed maximum absorption bands at 259 and 375 nm suggesting an isoflavone skeleton. The IR spectrum showed absorption bands of hydroxyl group (3551 cm^{-1}) and C=O stretching (1683 cm^{-1}).

The ^{13}C NMR spectral data (Table 5, Figure 24) exhibited 19 carbons, of two methyl (55.4 (2C)), six methine (94.9, 96.3, 113.5, 114.6, 121.7, 150.3) and nine quaternary carbons (110.1, 127.0, 127.5, 146.0, 147.8, 148.5, 150.3, 162.6, 175.4).

The ^{1}H NMR spectral data (Table 5, Figure 23) displayed a characteristic isoflavone signals for H-2 as a singlet at 7.98 together with a *meta*-coupled aromatic signals at 6.46 (2H, *s*, H-6, 8), whose signal of the latter indicated a tetrasubstituted A-ring. The signals of a B-ring at 6.85 (*d*, *J* = 8.0 Hz, H-5′), 6.99 (*dd*, *J* = 8.0, 2.0 Hz, H-6′) and 7.23 (*d*, *J* = 2.0 Hz, H-2′) indicated a 1,2,4-trisubstituted B ring. The spectrum also revealed the presence of two methoxyl groups (3.87 and 3.88) in the compound.

The structure of **TA3** was confirmed by HMBC correlation. The proton signal at 7.98 (H-2) showed correlations with the carbons at 127.0 (C-3), 127.5 (C-1′), 161.5 (C-9) and 175.4 (C-4), suggesting the connection of a 1,2,4-trisubstituted benzene ring at 127.0 (C-3). The proton signal of H-8 at 6.46 showed correlations with the carbons at 96.3 (C-6), 110.1 (C-10), 146.0 (C-7) and 161.5 (C-9) and the proton signal of H-6 showed correlations with the carbons at 94.9 (C-8), 146.0 (C-7) and 162.6 (C-5). The methoxyl groups at 3.87 and 3.88 showed correlations with the carbons at 162.6 (C-5) and 147.8 (C-3′), respectively, confirming their locations at C-5 and C-3′, respectively. Therefore, compound **TA3** was identified as gerontoisoflavone (Chang *et al*., 1995).

Selected HMBC correlations for compound **TA3**

Table 5 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **TA3** (Acetone-d_6)

Position	**$_{H}$ (mult, *J*, Hz)**	**$_{C}$**	**DEPT**	**HMBC**
2	7.98 (s)	150.3	CH	3, 4, 9, 1′
3	-	127.0	C	-
4	-	175.4	C=O	-
5	-	162.6	C	-
6	6.46 (s)	96.3	CH	5, 7, 8
7	-	146.0	C	-
8	6.46 (s)	94.9	CH	6, 7, 9, 10
9	-	161.5	C	-
10	-	110.1	C	-
1′	-	127.5	C	-
2′	7.23 (d, *J* = 2.0)	113.5	CH	3, 4′, 6′
3′	-	147.8	C	-
4′	-	148.5	C	-
5′	6.85 (d, *J* = 8.0)	114.6	CH	1′, 4′
6′	6.99 (dd, *J* = 8.0, 2.0)	121.7	CH	2′, 3′
5-OMe	3.87 (s)	55.4	CH_3	5
3′-OMe	3.88 (s)	55.4	CH_3	3′

Table 6 Comparison of ^{1}H NMR spectral data of **TA3** (Acetone-d_6) and gerontoisoflavone (**R**, DMSO-d_6)

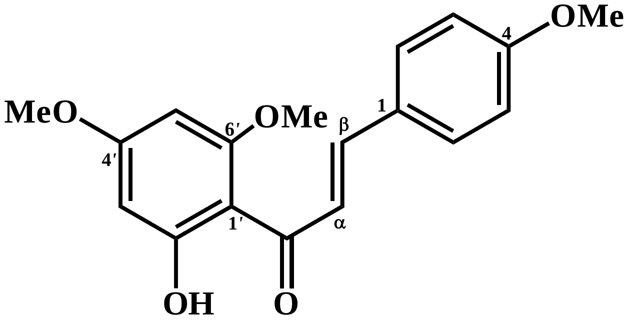
Position	$_{\text{H}}$ **(mult, *J*, Hz)**	**R**	$_{\text{C}}$	**R**
2	7.98 (s)	8.07 (s)	150.3	150.7
3	-	-	127.0	124.8
4	-	-	175.4	173.9
5	-	-	162.6	161.4
6	6.46 (s)	6.38 (s)	96.3	96.7
7	-	-	146.0	162.5
8	6.46 (s)	6.38 (s)	94.9	94.9
9	-	-	161.5	159.2
10	-	-	110.1	108.1
1′	-	-	127.5	123.5
2′	7.23 (d, *J* = 2.0)	7.08 (d, *J* = 2.0)	113.5	113.7
3′	-	-	147.8	147.2
4′	-	-	148.5	146.5
5′	6.85 (d, *J* = 8.0)	6.77 (d, *J* = 8.0)	114.6	115.2
6′	6.99 (dd, *J* = 8.0, 2.0)	6.88 (dd, *J* = 8.0, 2.0)	121.7	121.7
5-OMe	3.87 (s)	3.73 (s)	55.4	56.0
3′-OMe	3.88 (s)	3.78 (s)	55.4	55.8

3.2 Structural elucidation of compounds from the roots of *E. tomentosus*

The air-dried roots of *E. tomentosus* were extracted with methylene chloride and acetone successively. The crude methylene chloride extract was subjected to chromatography and/or crystallization to give twelve known compounds of flavokawain A (**RD1**), 4′,5,6,7,8-pentamethoxyflavone (**RD2**), 3′,4′,5,6,7,8-hexamethoxyflavone (**RD3**), 5-demethylnobiletin (**RD4**), 5,7,8,3′,4′-pentamethoxyflavone (**RD5**), (*E*)-ferulic acid tetracosyl ester (**RD6**), 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (**RD7**), 5,4′-dimethoxy-6,7-methylenedioxyisoflavone (**RD8**), *β*-sitosterol (**RD9**) and stigmasterol (**RD10**), 4-hydroxybenzaldehyde (**RD11**) and vanillin (**RD12**). The crude acetone extract was subjected to chromatography and/or crystallization to give five known compounds: lophirone A (**RA1**), calodenone (**RA2**), 6′′′-hydroxylophirone B (**RA3**), calodenin B (**RA4**) and (2*R*,3*R*)-2,3-*trans*-4´,5,7-trimethoxydihydroflavonol (**RA5**).

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C, DEPT 135 , DEPT 90 , HMQC, HMBC and COSY. The physical data of the known compounds were also compared with the reported values.

3.2.1 Compound RD1



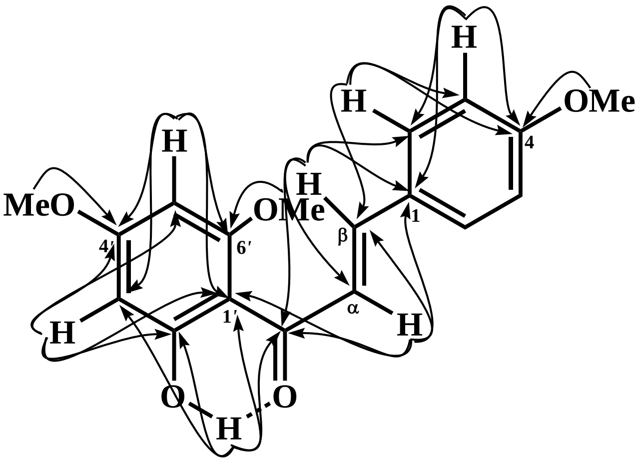
Compound **RD1** was isolated as a yellow solid, mp 113-114 C. The UV spectrum displayed maximum absorptions a $_{max}$ 249 and 363 nm, suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of hydroxyl group (3423 cm^{-1}) and C=O stretching (1622 cm^{-1}).

The ^{13}C NMR spectral data (Table 7, Figure 34) exhibited 18 carbons, of three methyl (55.4, 55.6, 55.8), eight methines (91.2, 93.8, 114.4 (2C), 125.1, 130.1 (2C), 142.5) and seven quaternary carbons (106.4, 128.3, 161.4, 162.5, 166.0, 168.4, 192.6).

The ^{1}H NMR spectral data (Table 7, Figure 33) displayed the presence of *p*-disubstituted benzene ring at 6.85 and 7.48 (each 2H, *d*, *J* = 8.7 Hz), and 1,2,3,5-tetrasubstituted benzene ring at 5.88 and 6.02 (*d*, *J* = 2.1 Hz). The three singlet proton signals at 3.75, 3.77 and 3.83 were assigned as methoxyl groups at C-4′, C-4 and C-6′, respectively and the proton signals at 7.68 and 7.74 (each 1H, *d*, *J* = 15.9 Hz) were deduced as a *trans* double bond at C-β and C-α, respectively. The chelated hydroxyl group was evident at 14.35. These data indicated a chalcone skeleton.

The structure of **RD1** was confirmed by HMBC correlation. The proton signal at 7.48 (H-2/H-6) showed correlations with the carbons at 114.4 (C-3/C-5), 125.1(C-α), 142.5 (C-β) and 161.4 (C-4), suggesting the connection of a *p*-

disubstituted benzene ring at C-*β*. The correlation of the proton signals at 14.35 (2′-OH) with the carbons at 93.8 (C-3′), 106.4 (C-1′), 168.4 (C-2′) and 192.6 (C=O), suggested that a tetrasubstituted benzene ring was connected to C=O. The methoxyl groups at 3.75, 3.77 and 3.83 showed correlations with the carbons at 166.0 (C-4′), 161.4 (C-4) and 162.5 (C-6′), respectively confirming the locations of the methoxyl group at C-4′, C-4 and C-6′, respectively. Therefore, compound **RD1** was identified as flavokawain A (Seidel *et al*., 2000).



Selective HMBC correlations of **RD1**

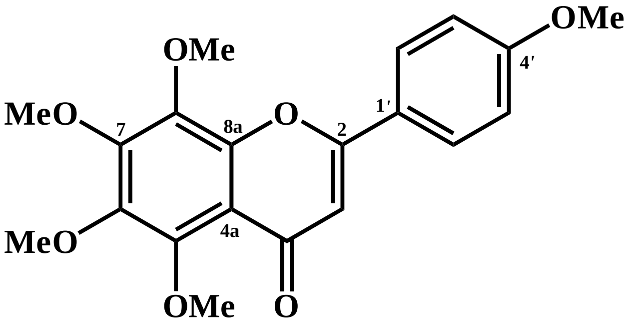
Table 7 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD1** (CDCl$_3$)

Position	$_{\mathbf{H}}$ **(mult, *J*, Hz)**	$_{\mathbf{C}}$	**DEPT**	**HMBC**
1	-	128.3	C	-
2, 6	7.48 (d, *J* = 8.7)	130.1	CH	3, 4, 5, *α*, *β*
3, 5	6.85 (d, *J* = 8.7)	114.4	CH	1, 2, 4
4	-	161.4	C	-
1′	-	106.4	C	-
2′	-	168.4	C	-
3′	6.02 (d, *J* = 2.1)	93.8	CH	1′, 2′, 4′, 5′
4′	-	166.0	C	-
5′	5.88 (d, *J* = 2.1)	91.2	CH	1′, 3′, 4′, 6′
6′	-	162.5	C	-
α	7.74 (d, *J* = 15.9)	125.1	CH	1, C=O, *β*, 1′
β	7.68 (d, *J* = 15.9)	142.5	CH	1, 2, C=O, *α*
4-OMe	3.77 (s)	55.4	CH$_3$	4
4′-OMe	3.75 (s)	55.6	CH$_3$	4′
6′-OMe	3.83 (s)	55.8	CH$_3$	6
C=O	-	192.6	C=O	-
2′-OH	14.35 (s)	-	-	1′, 2′, 3′, C=O

Table 8 Comparison of ^{1}H NMR and ^{13}C spectral data of **RD1** ($CDCl_3$) and flavokawain A (**R**, $CDCl_3$)

Position	$_{\mathbf{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathbf{C}}$	**R**
1	-	-	128.3	128.6
2, 6	7.48 (d, *J* = 8.7)	7.57 (d, *J* = 8.8)	130.1	130.3
3, 5	6.85 (d, *J* = 8.7)	6.94 (d, *J* = 8.8)	114.4	114.6
4	-	-	161.4	161.6
1′	-	-	106.4	106.6
2′	-	-	168.4	168.6
3′	6.02 (d, *J* = 2.1)	6.12 (d, *J* = 2.4)	93.8	94.0
4′	-	-	166.0	166.2
5′	5.88 (d, *J* = 2.1)	5.97 (d, *J* = 2.4)	91.2	91.5
6′	-	-	162.5	162.7
α	7.74 (d, *J* = 15.9)	7.82 (d, *J* = 15.6)	125.1	125.3
β	7.68 (d, *J* = 15.9)	7.78 (d, *J* = 15.6)	142.5	142.7
4-OMe	3.77 (s)	3.86 (s)	55.4	55.6
4′-OMe	3.75 (s)	3.84 (s)	55.6	55.8
6′-OMe	3.83 (s)	3.92 (s)	55.8	56.0
C=O	-	-	192.6	192.8
2′-OH	14.35 (s)	14.40 (s)	-	-

3.2.2 Compound RD2

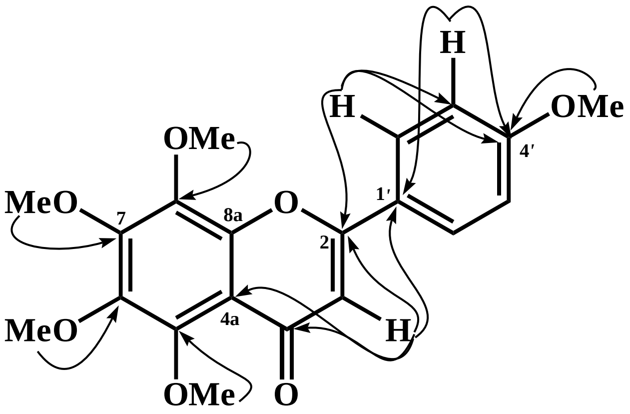


Compound **RD2** was isolated as a pale-yellow solid, mp 155-156 C. The UV spectrum displayed absorptions a $_{\text{max}}$ 271 and 323 nm, suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of C=O stretching (1641 cm^{-1}).

The ^{13}C NMR spectral data (Table 9, Figure 43) exhibited 18 carbons, of five methyl (55.0, 61.7, 61.8, 62.0, 62.3), five methines (106.6, 114.5 (2C), 127.7 (2C)) and ten quaternary carbons (115.0, 123.8, 138.1, 144.1, 147.7, 148.4, 151.4, 161.2, 162.3, 177.4).

The ^{1}H NMR spectrum (Table 9, Figure 42) indicated a flavone nucleus by the appearance of a singlet of a methine proton (H-3) at   6.01. The signals for *p*-disubstituted benzene ring were shown at   7.03 (2H, *d*, *J* = 9.0 Hz, H-3′, 5′) and 7.88 (2H, *d*, *J* = 9.0 Hz, H-2′, 6′). The signals for five singlet methoxyl groups at   3.89, 3.96 and 3.96, 4.00 and 4.10 were assigned at C-4′, C-5, C-6, C-7 and C-8 due to their HMBC correlations to the carbons at   162.3, 148.4, 138.1, 151.4 and 144.1, respectively.

The structure of **RD2** was confirmed by HMBC correlation. The proton signal at   6.01 (H-3) showed correlations with the carbons at   115.0 (C-4a), 123.8 (C-1′), 161.2 (C-2) and 177.4 (C-4), in turn the proton signals at   7.88 (H-2′, 6′) correlated with the carbons at 114.5 (C-3′, 5′), 161.2 (C-2) and 162.3 (C-4′) suggesting the connection of *p*-disubstituted benzene ring at 161.2 (C-2). Therefore, compound **RD2** was identified as 4′,5,6,7,8-pentamethoxyflavone (Machida *et al*., 1989).



Selective HMBC correlations of **RD2**

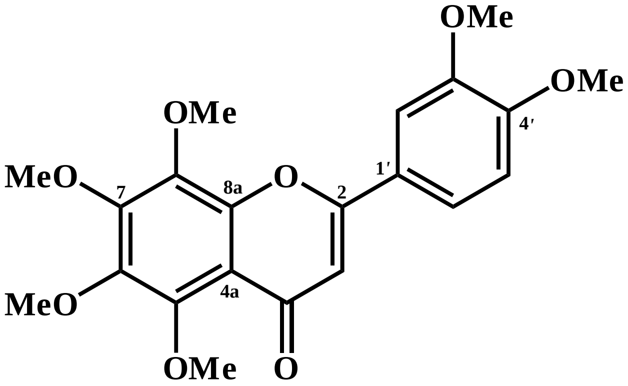
Table 9 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD2** (CDCl$_3$)

Position	**$_{\text{H}}$ (mult, *J*, Hz)**	**$_{\text{C}}$**	**DEPT**	**HMBC**
2	-	161.2	C	-
3	6.01 (s)	106.6	CH	2, 4, 1′, 4a
4	-	177.4	C=O	-
4a	-	115.0	C	-
5	-	148.4	C	-
6	-	138.1	C	-
7	-	151.4	C	-
8	-	144.1	C	-
8a	-	147.7	C	-
1′	-	123.8	C	-
2′, 6′	7.88 (d, *J* = 9.0)	127.7	CH	2, 4′, 5′
3′, 5′	7.03 (d, *J* = 9.0)	114.5	CH	1′, 4′
4′	-	162.3	C	-
5-OMe	3.96 (s)	61.7	CH$_3$	5
6-OMe	3.96 (s)	61.8	CH$_3$	6
7-OMe	4.00 (s)	62.0	CH$_3$	7
8-OMe	4.10 (s)	62.3	CH$_3$	8
4′-OMe	3.89 (s)	55.0	CH$_3$	4′

Table 10 Comparison of ^{1}H NMR spectral data of **RD2** ($CDCl_3$) and 4′,5,6,7,8-pentamethoxyflavone (**R**, $CDCl_3$)

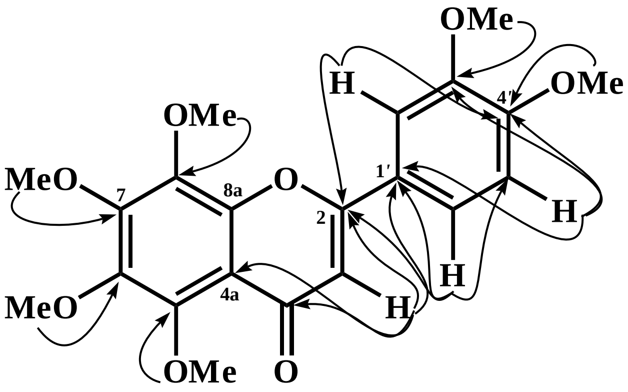
Position	$_H$ **(mult, *J*, Hz)**	**R**	$_C$	**R**
2	-	-	161.2	162.4
3	6.01 (s)	6.59 (s)	106.6	106.7
4	-	-	177.4	177.3
4a	-	-	115.0	144.0
5	-	-	148.4	138.1
6	-	-	138.1	151.4
7	-	-	151.4	138.1
8	-	-	144.1	147.7
8a	-	-	147.7	114.7
1′	-	-	123.8	123.9
2′, 6′	7.88 (d, *J* = 9.0)	7.88 (d, *J* = 9.0)	127.7	127.8
3′, 5′	7.03 (d, *J* = 9.0)	7.02 (d, *J* = 9.0)	114.5	114.3
4′	-	-	162.3	161.2
5-OMe	3.96 (s)	3.94 (s)	61.7	61.7
6-OMe	3.96 (s)	3.94 (s)	61.8	61.9
7-OMe	4.00 (s)	4.02 (s)	62.0	62.0
8-OMe	4.10 (s)	4.10 (s)	62.3	62.3
4′-OMe	3.89 (s)	3.89 (s)	55.0	55.5

3.2.3 Compound RD3



Compound **RD3** was isolated as a pale-yellow solid, mp 136-137 C. The UV and IR spectra closely resembled those of compound **RD2**.

The ^{1}H NMR and ^{13}C NMR spectral data (Table 11, Figure 51 and 52) of **RD3** were comparable with those of **RD2**. The difference was shown as the disappearance of the signals of a *p*-disubstituted benzene ring in **RD2** and the appearance of a 1,2,4–trisubstituted benzene ring at 7.00 (d, *J* = 8.4 Hz, H-5′), 7.42 (d, *J* = 2.1 Hz, H-2′) and 7.58 (dd, *J* = 8.4, 2.1 Hz, H-6′). Additional ^{1}H NMR signals of a methoxyl group was displayed at 3.98 (s): $_{\mathrm{C}}$ 56.1 in **RD3**, whose position was assigned at C-3′ from HMBC correlation with the carbon at 149.3 (C-3′). The HMBC spectrum showed correlations of the proton at 6.23 (H-3) with the carbons at 114.8 (C-4a), 124.0 (C-1′), 161.0 (C-2) and 177.3 (C-4) and the proton at 7.58 (H-6′) showed correlations with the carbons at 108.6 (C-2′), 151.9 (C-4′) and 161.0 (C-2) confirming the location of a 1,2, 4–trisubstituted benzene ring at C-2. Therefore, compound **RD3** was identified as 3′,4′,5,6,7,8-hexamethoxyflavone (Machida *et al*., 1989).



Selective HMBC correlations of **RD3**

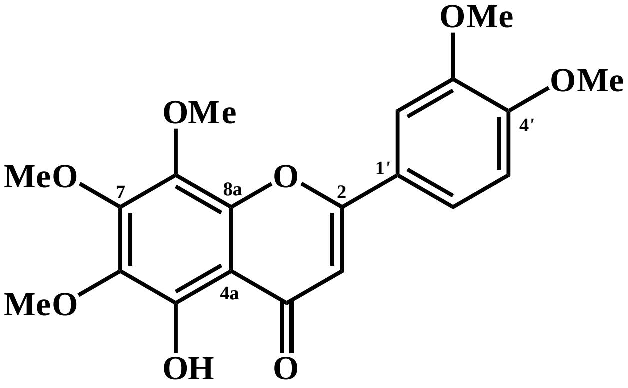
Table 11 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD3** ($CDCl_3$)

Position	$_{H}$ **(mult, *J*, Hz)**	$_{C}$	**DEPT**	**HMBC**
2	-	161.0	C	-
3	6.63 (s)	106.8	CH	2, 4, 4a, 1′
4	-	177.3	C=O	-
4a	-	114.8	C	-
5	-	148.4	C	-
6	-	138.0	C	-
7	-	151.4	C	-
8	-	144.1	C	-
8a	-	147.7	C	-
1′	-	124.0	C	-
2′	7.42 (d, *J* = 2.1)	108.6	CH	2, 1′, 6′, 4′, 3′
3′	-	149.3	C	-
4′	-	151.9	C	-
5′	7.00 (d, *J* = 8.4)	111.2	CH	1′, 4′, 3′
6′	7.58 (dd, *J* = 8.4, 2.1)	119.6	CH	2, 4′, 2′
5-OMe	3.96 (s)	61.6	CH_3	5
6-OMe	3.96 (s)	62.2	CH_3	6
7-OMe	4.04 (s)	61.8	CH_3	7
8-OMe	4.12 (s)	61.9	CH_3	8
3′-OMe	3.98 (s)	56.1	CH_3	3′
4′-OMe	3.97 (s)	55.9	CH_3	4′

Table 12 Comparison of ^{1}H NMR and ^{13}C spectral data of **RD3** ($CDCl_3$) and 3′,4′,5,6,7,8-hexamethoxyflavone (**R**, $CDCl_3$)

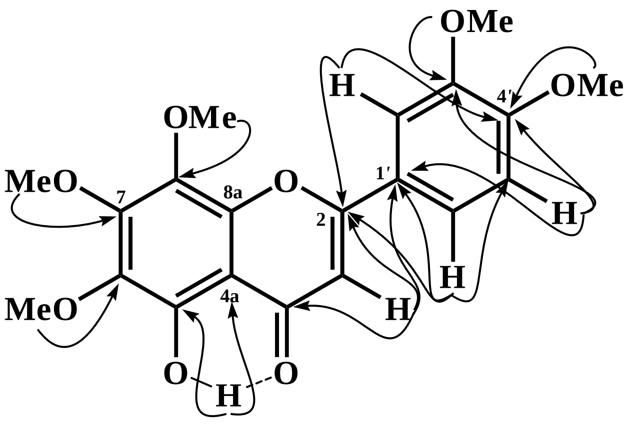
Position	**$_{\text{H}}$ (mult, *J*, Hz)**	**R**	**$_{\text{C}}$**	**R**
2	-	-	161.0	160.0
3	6.63 (s)	6.73 (s)	106.8	106.7
4	-	-	177.3	177.4
4a	-	-	114.8	144.0
5	-	-	148.4	138.0
6	-	-	138.0	151.4
7	-	-	151.4	138.0
8	-	-	144.1	147.7
8a	-	-	147.7	114.8
1′	-	-	124.0	124.0
2′	7.42 (d, *J* = 2.1)	7.42 (d, *J* = 1.8)	108.6	108.7
3′	-	-	149.3	149.3
4′	-	-	151.9	151.9
5′	7.00 (d, *J* = 8.4)	7.00 (d, *J* = 8.7)	111.2	111.0
6′	7.58 (dd, *J* = 8.4, 2.1)	7.59 (dd, *J* = 8.7, 1.8)	119.6	119.6
5-OMe	3.96 (s)	3.96 (s)	61.6	55.6
6-OMe	3.96 (s)	3.97 (s)	61.8	55.7
7-OMe	4.04 (s)	4.03 (s)	61.9	61.8
8-OMe	4.12 (s)	4.11 (s)	62.2	61.9
3′-OMe	3.98 (s)	4.00 (s)	56.1	61.5
4′-OMe	3.97 (s)	3.98 (s)	55.9	61.4

3.2.4 Compound RD4



Compound **RD4** was isolated as a pale-yellow solid, mp 137-138 C. The UV spectra closely resembled those of compound **RD3**. The IR spectrum showed absorption bands of hydroxyl group (3452 cm^{-1}) and C=O stretching (1638 cm^{-1}).

The ^{1}H NMR and ^{13}C NMR spectral data (Table 13, Figure 61 and 62) of **RD4** were comparable with those of **RD3**. The difference was shown as the disappearance of the signal of one methoxyl group in **RD3** and the appearance of a chelated hydroxyl group at   12.57 (*s*) in **RD4**. The chelated hydroxyl group was assigned at C-5 from the HMBC correlation with the carbons at   107.1 (C-4a) and 149.6 (C-5). Therefore, compound **RD4** was identified as 5-demethylnobiletin (Li *et al*., 2006).



Selective HMBC correlations of **RD4**

Table 13 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD4** ($CDCl_3$)

Position	**$_\mathrm{H}$ (mult, *J*, Hz)**	**$_\mathrm{C}$**	**DEPT**	**HMBC**
2	-	164.0	C	-
3	6.64 (s)	104.1	CH	2, 4, 1′
4	-	183.0	C=O	-
4a	-	107.1	C	-
5	-	149.6	C	-
6	-	153.1	C	-
7	-	152.6	C	-
8	-	133.0	C	-
8a	-	145.8	C	-
1′	-	123.8	C	-
2′	7.62 (dd, *J* = 8.7, 2.1)	120.2	CH	2, 4′
3′	-	149.5	C	-
4′	-	152.6	C	-
5′	7.03 (d, *J* = 8.7)	111.3	CH	1′, 4′, 3′
6′	7.45 (d, *J* = 2.1)	108.8	CH	2, 4′, 3′
6-OMe	3.96 (s)	61.8	CH_3	6
7-OMe	3.96 (s)	62.0	CH_3	7
8-OMe	4.10 (s)	62.3	CH_3	8
3′-OMe	4.01 (s)	56.0	CH_3	3′
4′-OMe	3.89 (s)	61.1	CH_3	4′
5-OH	12.57 (s)	-	-	4a, 5

Table 14 Comparison of ^{1}H NMR spectral data of **RD4** ($CDCl_3$) and 5-demethylnobiletin (**R**, DMSO-d_6)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathrm{C}}$	**R**
2	-	-	164.0	163.5
3	6.64 (s)	6.99 (s)	104.1	103.5
4	-	-	183.0	182.5
4a	-	-	107.1	106.2
5	-	-	149.6	148.5
6	-	-	153.1	135.8
7	-	-	152.6	152.4
8	-	-	133.0	132.5
8a	-	-	145.8	145.2
1′	-	-	123.8	122.6
2′	7.62 (dd, *J* = 8.7, 2.1)	7.63 (dd, *J* = 9.0, 2.0)	120.2	111.7
3′	-	-	149.5	148.9
4′	-	-	152.6	152.3
5′	7.03 (d, *J* = 8.7)	7.11 (d, *J* = 9.0)	111.3	109.0
6′	7.45 (d, *J* = 2.1)	7.51 (d, *J* = 2.0)	108.8	119.9
6-OMe	3.96 (s)	3.82 (s)	62.0	60.5
7-OMe	4.10 (s)	3.92 (s)	61.8	61.7
8-OMe	3.96 (s)	3.86 (s)	62.3	61.4
3′-OMe	4.01 (s)	4.02 (s)	56.0	55.7
4′-OMe	3.89 (s)	3.85 (s)	61.1	55.6
5-OH	12.57 (s)	12.72 (s)	-	-

3.2.5 Compound RD5

Compound **RD5** was isolated as a pale-yellow solid, mp 138-139 C. The UV and IR spectra closely resembled those of compound **RD3**.

The ^{1}H NMR and ^{13}C NMR spectral data (Table 15, Figure 71 and 72) of **RD5** were comparable with those of **RD4**. The difference was shown as the disappearance of the signal of a chelated hydroxyl group in **RD4** and the appearance of a methine proton at 6.44 (*s*). The methine proton was assigned at C-6 from its HMBC correlation with the carbons at 109.1 (C-4a) and 156.4 (C-7). Therefore, compound **RD5** was identified as 5,7,8,3′,4′-pentamethoxyflavone (Chen *et al*., 1997).

Selective HMBC correlations of **RD5**

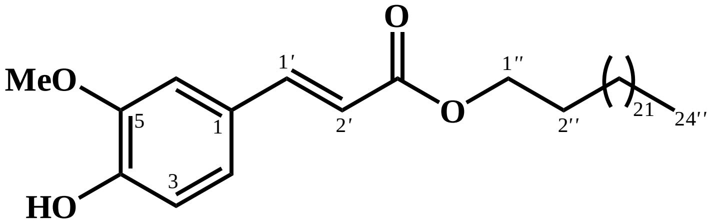
Table 15 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD5** ($CDCl_3$)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	$_{\mathrm{C}}$	**DEPT**	**HMBC**
2	-	160.6	C	-
3	6.62 (s)	107.2	CH	2, 4, 4a, 1′
4	-	177.9	C=O	-
4a	-	109.1	C	-
5	-	152.0	C	-
6	6.44 (s)	92.7	CH	4a, 6, 7
7	-	156.4	C	-
8	-	156.6	C	-
8a	-	130.9	C	-
1′	-	124.1	C	-
2′	7.43 (d, *J* = 3.0)	108.7	CH	2, 1′, 6′, 3′
3′	-	149.4	C	-
4′	-	151.9	-	-
5′	6.99 (d, *J* = 9.0)	111.3	CH	1′, 3′
6′	7.59 (dd, *J* = 9.0, 3.0)	119.6	CH	2, 2′
5-OMe	3.96 (s)	61.5	CH_3	5
7-OMe	3.99 (s)	56.1	CH_3	7
8-OMe	4.02 (s)	56.4	CH_3	8
3′-OMe	3.98 (s)	56.6	CH_3	3′
4′-OMe	3.96 (s)	56.0	CH_3	4′

Table 16 Comparison of ^{1}H NMR and ^{13}C spectral data of **RD5** ($CDCl_3$) and 5,7,8,3′,4′-pentamethoxyflavone (**R**, $CDCl_3$)

Position	**$_H$ (mult, *J*, Hz)**	**R**	**$_C$**	**R**
2	-	-	160.6	160.5
3	6.62 (s)	6.61 (s)	107.2	107.2
4	-	-	177.9	177.9
4a	-	-	109.1	109.1
5	-	-	152.0	152.0
6	6.44 (s)	6.44 (s)	92.7	92.6
7	-	-	156.4	156.3
8	-	-	156.6	156.3
8a	-	-	130.9	130.7
1′	-	-	124.1	124.1
2′	7.43 (d, *J* = 3.0)	7.42 (d, *J* = 2.4)	108.7	108.7
3′	-	-	149.4	149.2
4′	-	-	151.9	151.5
5′	6.99 (d, *J* = 9.0)	6.98 (d, *J* = 8.4)	111.3	111.0
6′	7.59 (dd, *J* = 9.0, 3.0)	7.58 (dd, *J* = 8.4, 2.4)	119.6	119.6
5-OMe	3.96 (s)	3.96 (s)	61.5	61.5
7-OMe	3.99 (s)	3.99 (s)	56.1	56.1
8-OMe	4.02 (s)	4.01 (s)	56.4	56.5
3′-OMe	3.98 (s)	3.97 (s)	56.6	56.0
4′-OMe	3.96 (s)	3.96 (s)	56.0	56.0

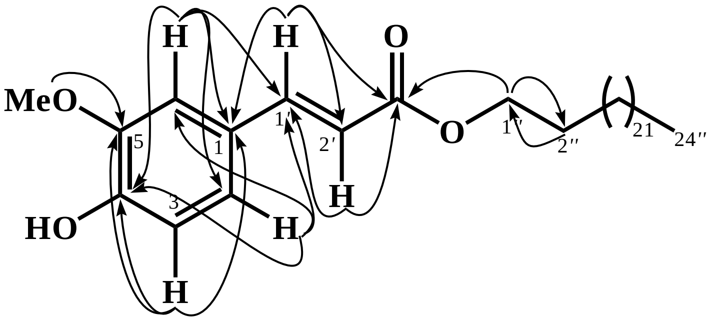
3.2.6 Compound RD6



Compound **RD6** was isolated as a white solid, mp 64-65 C. The UV spectrum displayed maximum absorptions at 234, 291 and 325 nm, suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of hydroxyl group (3551 cm^{-1}) and C=O stretching (1682 cm^{-1}).

The ^{1}H NMR spectral data (Table 17, Figure 78) of **RD6**, showed the presence of a *trans* double bond as evidenced by two doublet signals at 6.30 and 7.62 ppm with a coupling constant of 15.9 Hz. The ^{1}H NMR signals at 6.92 (*d*, *J* = 8.1 Hz, H-3), 7.07 (*dd*, *J* = 8.1, 1.8 Hz, H-2) and 7.03 (*d*, *J* = 1.8 Hz, H-6) established the presence of three aromatic protons with *ortho*, *ortho*/*meta* and *meta* coupling, respectively. The presence of one methoxyl group was also shown by a three-proton singlet at 3.92 ppm. Furthermore, the calculated MW of 529.6 was in agreement with the molecular formula, $C_{33}H_{56}O_{4}$ as deduced by EI mass spectrum. The ^{1}H NMR spectrum showed signals of methylene protons at 4.20 (*t*, *J* = 6.6 Hz, H-1″), a triplet at 0.89 (H-24″), and a broad signal at 1.15-1.39 which could be deduced from molecular formula to be those of 42H. Therefore, compound **RD6** should be a long chain ester of ferulic acid. The ^{13}C NMR spectral data of **RD6** showed signals at 167.4 (C-3′) due to the carbonyl group of an ester function and 144.7 (C-1′) and 115.6 (C-2′) due to a side chain C-C double bond. Further confirmation of this skeleton came from the mass spectrum of **RD6** which showed, besides the molecular ion, significant fragment peak at *m/z* ion 530.0, both being characteristic of a methoxy and hydroxyl substituted cinnamic moiety. HMBC correlations were summarized in

Table 17. Therefore, compound **RD6** was identified as (*E*)-ferulic acid tetracosyl ester (Mensah *et al*., 1992).



Selective HMBC correlations of **RD6**

Table 17 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD6** ($CDCl_3$)

Position	**$_{\text{H}}$ (mult, *J*, Hz)**	**$_{\text{C}}$**	**DEPT**	**HMBC**
1	-	127.0	C	-
2	7.07 (dd, *J* = 8.1, 1.8)	123.0	CH	4, 6, 1′
3	6.92 (d, *J* = 8.1)	114.8	CH	1, 2, 4, 5
4	-	148.0	C	-
5	-	146.8	C	-
6	7.03 (d, *J* = 1.8)	109.4	CH	2, 4, 5, 1′
1′	7.62 (d, *J* = 15.9)	144.7	CH	1, 2, 6, 2′, 3′
2′	6.30 (d, *J* = 15.9)	115.6	CH	1′, 3′
3′	-	167.4	C=O	-
1′′	4.20 (t, *J* = 6.6)	64.6	CH_2	2′′
2′′	1.71 (m)	28.8	CH_2	1′′
3′′′-23′′′	1.15-1.39 (m)	-	-	-
24′′	0.89 (t, *J* = 6.3)	14.1	CH_3	-
5-OMe	3.92 (s)	55.9	CH_3	5

Table 18 Comparison of ^{1}H NMR and ^{13}C spectral data of **RD6** ($CDCl_3$) and (*E*)-ferulic acid tetracosyl ester (**R**, $CDCl_3$)

Position	$_{\mathbf{H}}$ **(mult, *J*, Hz)**	**R**
1	-	
2	7.07 (dd, *J* = 8.1, 1.8)	7.07 (dd, *J* = 8.5, 2.0)
3	6.92 (d, *J* = 8.1)	6.91 (d, *J* = 8.5)
4	-	-
5	-	-
6	7.03 (d, *J* = 1.8)	7.04 (d, *J* = 2.0)
1′	7.62 (d, *J* = 15.9)	7.62 (d, *J* = 16.5)
2′	6.30 (d, *J* = 15.9)	6.28 (d, *J* = 16.5)
3′	-	-
1″	4.20 (t, *J* = 6.6)	4.18 (t, *J* = 7.0)
2″	1.71 (m)	1.69 (m)
3‴-23‴	1.15-1.39 (m)	1.25 (m)
24″	0.89 (t, *J* = 6.3)	0.89 (t, *J* = 7.0)
5-OMe	3.92 (s)	3.93 (s)

3.2.7 Compound RD7

Compound **RD7** was isolated as a pale-yellow solid, mp 178-179 C, $_D^{24}$ +12 (*c* 0.55, MeOH). The UV spectrum displayed maximum absorption bands at 265, 325 nm suggesting an isoflavone skeleton. The IR spectrum showed absorption bands for a carbonyl group (1643 cm^{-1}).

The ^{13}C NMR spectral data (Table 19, figure 90) exhibited 19 carbons, of three methyl (55.9, 56.0, 62.2), one methylene (102.1) five methines (93.1, 111.1, 112.9, 121.3, 150.4) and ten quaternary carbons (113.8, 124.6, 125.4, 135.5, 141.7, 148.7, 149.1, 152.8, 154.7, 175.4).

The ^{1}H NMR spectral data (Table 19, Figure 89) displayed a characteristic isoflavone signals for H-2 as a singlet at 7.79 together with a singlet of an aromatic proton signal at 6.64 (H-8), whose signal of the latter indicated a pentasubstituted A-ring. The signals of a B-ring at 6.70 (*d*, *J* = 8.4 Hz), 7.00 (*dd*, *J* = 8.4, 2.1 Hz) and 7.19 (*d*, *J* = 2.1 Hz) indicated a 1,2,4-trisubstituted B ring. The spectrum also revealed the presence of three methoxyl groups (3.90, 3.92, and 4.09) and a methylenedioxy (6.07) in the compound.

The structure of **RD7** was confirmed by HMBC correlation. The proton signal at 7.79 (H-2) showed correlations with the carbons at 124.6 (C-1′), 125.4 (C-3), 154.7 (C-9) and 175.4 (C-4), suggesting the connection of a 1,2,4-trisubstituted benzene ring at 125.4 (C-3). The proton signal of H-8 at 6.64 showed correlations with the carbons at 113.8 (C-10), 135.5 (C-6), 141.7 (C-5), 152.8 (C-7) and 154.7

(C-9). The correlation of the proton signal at 6.07 (CH_2O_2) with the carbons at 135.5 (C-6) and 152.8 (C-7) suggested that the methylenedioxy group must be located between C-6 and C-7 of ring A. The methoxyl group at 3.90, 3.92 and 4.09 showed correlations with the carbons at 148.7 (C-4′), 149.1 (C-3′) and 141.7 (C-5), respectively confirming their locations at C-4′, C-3′ and C-5, respectively. Therefore, compound **RD7** was identified as 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (Veitch *et al*., 2002).

Selected HMBC correlations for compound **RD7**

Table 19 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD7** ($CDCl_3$)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	$_{\mathrm{C}}$	**DEPT**	**HMBC**
2	7.79 (s)	150.4	CH	3, 4, 9, 1′
3	-	125.4	C	-
4	-	175.4	C=O	-
5	-	141.7	C	-
6		135.5	C	-
7	-	152.8	C	-
8	6.64 (s)	93.1	CH	5, 6, 7, 9, 10
9	-	154.7	C	-
10	-	113.8	C	-
1′	-	124.6	C	-
2′	7.19 (d, *J* = 2.1)	112.9	CH	3, 1′, 3′, 6′
3′	-	149.1	C	-
4′	-	148.7	C	-
5′	6.70 (d, *J* = 8.4)	111.1	CH	1′, 3′, 4′, 6′
6′	7.00 (dd, *J* = 8.4, 2.1)	121.3	CH	3, 5′
CH_2O_2	6.07 (s)	102.1	CH_2	6, 7
5-OMe	4.09 (s)	62.2	CH_3	5
3′-OMe	3.92 (s)	56.0	CH_3	3′
4′-OMe	3.90 (s)	55.9	CH_3	4′

Table 20 Comparison of ^{1}H NMR spectral data of **RD7** ($CDCl_3$) and 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (**R**, $CDCl_3$)

Position	**$_{H}$ (mult, *J*, Hz)**	**R**	**$_{C}$**	**R**
2	7.79 (s)	7.78 (s)	150.4	150.4
3	-	-	125.4	125.4
4	-	-	175.4	175.4
5	-	-	141.7	141.8
6		-	135.5	135.5
7	-	-	152.8	152.8
8	6.64 (s)	6.62 (s)	93.1	93.2
9	-	-	154.7	154.7
10	-	-	113.8	113.9
1′	-	-	124.6	124.7
2′	7.19 (d, *J* = 2.1)	7.18 (d, *J* = 2.0)	112.9	113.0
3′	-	-	149.1	148.8
4′	-	-	148.7	149.1
5′	6.70 (d, *J* = 8.4)	6.89 (d, *J* = 8.3)	111.1	111.2
6′	7.00 (dd, *J* = 8.4, 2.1)	7.00 (dd, *J* = 8.3, 2.0)	121.3	121.4
CH_2O_2	6.07 (s)	6.05 (s)	102.1	102.2
5-OMe	4.09 (s)	4.08 (s)	62.2	61.2
3′-OMe	3.92 (s)	3.91 (s)	56.0	56.1
4′-OMe	3.90 (s)	3.89 (s)	55.9	56.0

3.2.8 Compound RD8

Compound **RD8** was isolated as a pale-yellow solid, mp 175-176 C. The UV and IR spectra were closely resembled those of compound **RD7**.

The ^{1}H NMR and ^{13}C NMR spectral data (Table 21, Figure 98 and 99) of **RD8** were comparable with those of **RD7**. The difference was shown in the signals of the B ring, of which those of **RD7** was a 1,2,4-trisubstituted whereas those of **RD8** was a 1,4-disubstituted B ring which appeared as signals at 6.95 (*d*, *J* = 8.7 Hz, H-3′, 5′) and 7.47 (*d*, *J* = 8.7 Hz, H-2′, 6′). In the NOESY spectrum, the methoxyl protons at 3.83 (4′-OMe) displayed a cross-peak with the protons at 6.95 (H-3′, 5′), indicating their substitution on B-ring. Therefore compound **RD8** was identified as 5,4′-dimethoxy-6,7-methylenedioxyisoflavone (Veitch *et al*., 2002).

Selected NOESY cross-peak for compound **RD8**

Table 21 ^{1}H, ^{13}C NMR, DEPT and NOSEY spectral data of **RD8** ($CDCl_3$)

Position	**$_{H}$ (mult, *J*, Hz)**	**$_{C}$**	**DEPT**	**HMBC**
2	7.78 (s)	150.3	CH	-
3	-	125.4	C	-
4	-	175.3	C=O	-
5	-	141.6	C	-
6		134.3	C	-
7	-	152.5	C	-
8	6.64 (s)	93.2	CH	-
9	-	154.8	C	-
10	-	113.9	C	-
1′	-	124.1	C	-
2′, 6′	7.47 (2H, d, *J* = 8.7)	130.4	CH	3′, 5′
3′, 5′	6.95 (2H, d, *J* = 8.7)	113.9	CH	2′, 6′, 4′
4′	-	159.5	C	-
CH_2O_2	6.07 (2H, s)	102.3	CH_2	-
5-OMe	4.09 (s)	61.2	CH_3	-
4′-OMe	3.83 (s)	55.3	CH_3	3′ ′

Table 22 Comparison of ^{1}H NMR spectral data of **RD8** ($CDCl_3$) and 5,4′-dimethoxy-6,7-methylenedioxyisoflavone (**R**, $CDCl_3$)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathrm{C}}$	**R**
2	7.78 (s)	7.77 (s)	150.3	150.2
3	-	-	125.4	125.4
4	-	-	175.3	175.4
5	-	-	141.6	141.8
6		-	134.3	135.6
7	-	-	152.5	152.8
8	6.64 (s)	6.63 (s)	93.2	93.3
9	-	-	154.8	154.8
10	-	-	113.9	114.0
1′	-	-	124.1	124.2
2′, 6′	7.47 (2H, d, *J* = 8.7)	7.47 (2H, d, *J* = 8.8)	130.4	130.4
3′, 5′	6.95 (2H, d, *J* = 8.7)	6.94 (2H, d, *J* = 8.8)	113.9	113.9
4′	-	-	159.5	159.6
CH_2O_2	6.07 (2H, s)	6.06 (2H, s)	102.3	102.2
5-OMe	4.09 (s)	4.08 (s)	61.2	61.3
4′-OMe	3.83 (s)	3.83 (s)	55.3	55.4

3.2.9 Compound RD9 and RD10

The mixture of **RD9** and **RD10** was isolated as a white solid. Its IR spectrum showed absorption bands at 3425 (hydroxyl) and 1642 cm^{-1} (double bond). The ^{1}H NMR spectral data contained an oxymethine protons at 5.36 (*d*, J = 5.1 Hz), 5.16 (*dd*, J = 15.1, 8.4 Hz). The ^{1}H NMR (Cheenpracha, 2004) data was corresponded to a previous reported data of β-sitosterol and stigmasterol. Thus, this mixture was identified as β-sitosterol (**RD9**) and stigmasterol (**RD10**).

3.2.10 Compound RD11

Compound **RD11** was obtained as colorless oil. The UV spectrum showed absorption bands at $_{max}$ 237, 293 and 306 nm, indicating the presence of a benzene chromophore. The IR spectrum exhibited absorption bands at 3367 and 1684 cm^{-1} for hydroxyl and carbonyl groups, respectively.

The ^{1}H NMR spectrum displayed characteristic signals of a 1,4-disubstituted benzene at 7.80 (2H, *d*, *J* = 9.0 Hz) and 6.87 (2H, *d*, *J* = 9.0 Hz) and appearance of a singlet of an aldehydic group at 9.89 (1H, *s*, CHO). The presence of a carbonyl carbon at 190.6 in the ^{13}C NMR spectrum was in agreement with the IR data. The complete HMBC data were summarized in Table 23. Accordingly, the structure of **RD11** was assigned as 4-hydroxybenzaldehyde.

Figure Selected HMBC correlations of **RD11**

Table 23 ^{1}H, ^{13}C NMR and HMBC spectral data of **RD11** ($CDCl_3$)

Position	**$_{\text{H}}$ (mult, *J*, Hz)**	**$_{\text{C}}$**	**DEPT**	**HMBC**
1	-	130.0	C	-
2/6	7.80 (d, *J* = 9.0)	132.5	CH	3, 4, 7
3/5	6.87 (d, *J* = 9.0)	116.1	CH	1, 2
4	-	161.0	C	-
7	9.89 (s)	190.6	C=O	-
4-OH	-	-	-	-

3.2.11 Compound RD12

Compound **RD12** was obtained as colorless oil. The UV spectrum showed absorption bands at $_{max}$ 233, 291 and 306 nm, indicating the presence of a benzene chromophore. The IR spectrum exhibited absorption bands at 3384 and 1648 cm^{-1} for hydroxyl and carbonyl groups, respectively.

The ^{1}H NMR spectral data (Table 24), displayed characteristic signals of a 1,2,4-trisubstituted benzene at 7.43 (*dd*, *J* = 9.0, 3.0 Hz, H-6), 7.42 (*d*, *J* = 3.0 Hz, H-2) and 7.04 (*d*, *J* = 9.0 Hz, H-5) and appearance of a singlet of an aldehydic group at 9.80 (*s*, CHO). A singlet signal of a methoxyl group was evident at 3.91 (3H, *s*, 3-OMe). The location of a methoxyl group at C-3 was confirmed by HMBC correlation of OMe-3 (3.91) with $_{C}$ 147.4 (C-3).

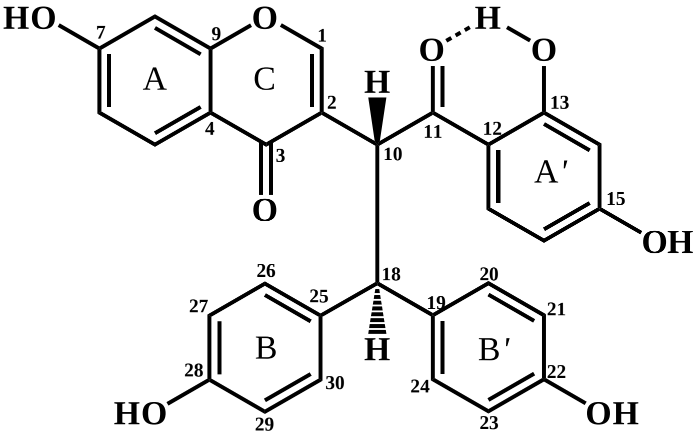
The structure of **RD12** was confirmed by HMBC correlation. The proton signal at 9.80 (H-7) showed correlations with the carbons at 109.1 (C-2), 127.6 (C-6), 129.6 (C-1) and 147.4 (C-3), suggesting the connection of an aldehylic group at C-1. The proton signals at 7.43 (H-6) showed correlations with the carbons at 109.1 (C-2), 114.7 (C-5), 129.6 (C-1), 152.1 (C-4) and 191.3 (C=O). The complete HMBC data were summarized in Table 24. Therefore, compound **RD12** was identified as vanillin.

Selective HMBC correlations of **RD12**

Table 24 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RD12** (CDCl$_3$)

Position	**$_H$ (mult, *J*, Hz)**	**$_C$**	**DEPT**	**HMBC**
1	-	129.6	C	-
2	7.42 (d, *J* = 3.0)	109.1	CH	1, 3, 4, 5, 6
3	-	148.5	C	-
4	-	152.1	C	-
5	7.04 (d, *J* = 9.0)	114.7	CH	1, 3, 4
6	7.43 (dd, *J* = 9.0, 3.0)	127.6	CH	1, 2, 4, 5, 7
7	9.80 (s)	191.3	C=O	1, 2, 3, 6
3-OMe	3.91 (s)	56.0	CH$_3$	3
4-OH	7.15 (s)	-	-	2, 6

3.2.12 Compound RA1

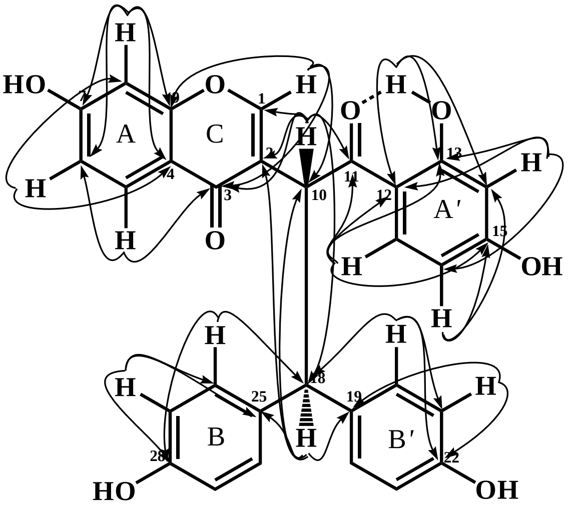


Compound **RA1** was isolated as a white solid, mp 189-190 C, $_{\text{D}}^{24}$ +12 (*c* 0.55, MeOH). The UV spectrum displayed maximum absorptions a $_{\text{max}}$ 285 and 367 nm supporting the presence of conjugated chromophore in the structure. The IR spectrum showed absorption bands of hydroxyl group (3382 cm^{-1}) and C=O stretching (1700 and 1632 cm^{-1}).

The ^{13}C NMR and DEPT spectra data (Tables 25, Figure 123) exhibited 30 carbons of seventeen methines (43.1, 52.7, 102.4, 102.6, 108.2, 115.0 (2C), 115.2 (3C), 127.4, 128.6 (2C), 129.2 (2C), 133.6, 155.5 and thirteen quaternary carbons (113.3, 116.4, 121.4, 133.8, 134.8, 155.6, 155.7, 157.7, 162.7, 165.4, 166.0, 174.6, 203.8).

The ^{1}H NMR spectral data (Tables 25, Figure 122), assigned by COSY spectrum, enabled the assignment of two 1,4-disubstituted benzene rings at 6.66 (4H, *m*, H-21, 23 / 27, 29) and 7.28 (4H, *m*, H-20, 24 / 26, 30) assigned on ring B and B′ and two 1,2,4-trisubstituted benzene rings at 6.79 (*d*, *J* = 3.0 Hz, H-8), 6.93 (*dd*, *J* = 9.0, 3.0 Hz, H-6) and 7.96 (*d*, *J* = 9.0 Hz, H-5) assigned on ring A and at 6.24 (*d*, *J* = 3.0 Hz, H-14), 6.47 (*dd*, *J* = 9.0, 3.0 Hz, H-16) and 8.37 (*d*, *J* = 9.0 Hz, H-17) assigned on ring A′. Two doublets were displayed at 4.82 (C-18) and 6.18 (H-10) (each 1H, J = 12.0 Hz) whose large coupling constants indicated *trans* conformation. In addition two singlets of an aromatic methine proton (H-1) and a chelated hydroxyl group (13-OH) were evident at 8.31 and 12.70, respectively.

The structure of **RA1** was confirmed by HMBC correlations. The proton signal at 4.82 (H-18) showed correlations with the carbons at 43.1 (C-10), 121.4 (C-2), 133.8 (C-19) and 134.8 (C-25), suggesting the connection of two *p*-disubstituted benzene rings at C-18. The correlations of the proton signal at 6.12 (H-10) with the carbons at 52.7 (C-18), 121.4 (C-2), 174.6 (C-3) and 203.8 (C-11), suggested that a methine carbon (C-10) was connected to C-2. The proton signal at 7.96 (H-5) showed correlations with the carbons at 116.4 (C-4), 162.7 (C-7) and 174.6 (C-3), in turn the proton signal at 8.31 (H-1) correlated with the carbons at 43.1 (C-10), 121.4 (C-2), 157.7 (C-9) and 174.6 (C-3), suggesting the connection of ring A with the ring C. The correlation of the proton signals at 12.70 (13-OH) with the carbons at 102.6 (C-14), 113.3 (C-12) and 166.0 (C-13), in turn the proton signal at 8.37 (H-17) correlated with the carbons at 113.3 (C-12), 165.4 (C-15), 166.0 (C-13) and 203.8 (C-11), suggesting the connection of a 1,2,4-trisubstituted benzene ring (ring A′) at C-11. Therefore, compound **RA1** was identified as lophirone A (Ghogomu *et al*., 1987).



Selected HMBC correlations for compound **RA1**

Table 25 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RA1** (Acetone-d_6)

Position	**$_{\mathrm{H}}$ (mult, *J*, Hz)**	**$_{\mathrm{C}}$**	**DEPT**	**HMBC**
1	8.31 (s)	155.5	CH	2, 3, 9, 10
2	-	121.4	C	-
3	-	174.6	C=O	-
4	-	116.4	C	-
5	7.96 (d, *J* = 9.0)	127.4	CH	3, 6, 7, 9
6	6.93 (dd, *J* = 9.0, 3.0)	115.2	CH	4, 7, 8
7	-	162.7	C	-
8	6.79 (d, *J* = 3.0)	102.4	CH	3, 4, 6, 7, 9
9	-	157.7	C	-
10	6.12 (d, *J* = 12.0)	43.1	CH	1, 2, 3, 18, 19, 25
11	-	203.8	C	-
12	-	113.3	C	-
13	-	166.0	C	-
14	6.24 (d, *J* = 3.0)	102.6	CH	12, 13, 16
15	-	165.4	C	-
16	6.47 (dd, *J* = 9.0, 3.0)	108.2	CH	14, 15
17	8.37 (d, *J* = 9.0)	133.6	CH	12, 13, 15
18	4.82 (d, *J* = 12.0)	52.7	CH	2, 10, 20, 24, 25
19	-	133.8	C	-
20	7.28 (m)	129.2	CH	18, 21, 22, 24
21	6.66 (m)	115.0	CH	19, 22, 23
22	-	155.6	C	-
23	6.66 (m)	115.0	CH	19, 22, 27, 29
24	7.28 (m)	129.2	CH	18, 21, 22
25	-	134.8	C	-
26	7.28 (m)	128.6	CH	18, 28, 30
27	6.66 (m)	115.2	CH	25, 28, 30
28	-	155.7	C	-

Table 25 (continued)

Position	**$_H$ (mult, *J*, Hz)**	**$_C$**	**DEPT**	**HMBC**
29	6.66 (m)	115.2	CH	25, 28, 30
30	7.28 (m)	128.6	CH	18, 28, 29
13-OH	12.70 (s)	-	-	12, 13, 14

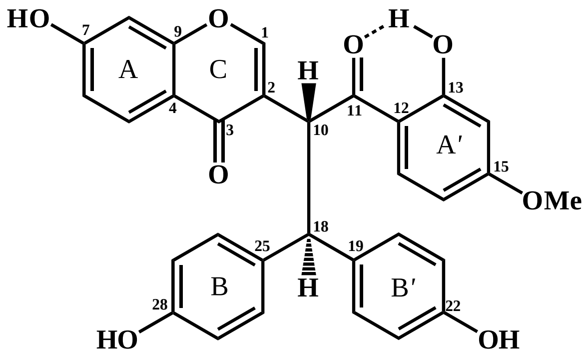
Table 26 Comparison of ^{1}H NMR and ^{13}C spectral data of **RA1** (Acetone-d_6) and lophirone A (**R**, Acetone-d_6)

Position	**$_H$ (mult, *J*, Hz)**	**R**	**$_C$**	**R**
1	8.31 (s)	8.27 (s)	155.5	156.4
2	-	-	121.4	122.1
3	-	-	174.6	175.4
4	-	-	116.4	117.2
5	7.96 (d, *J* = 9.0)	7.94 (d, *J* = 8.8)	127.4	128.2
6	6.93 (dd, *J* = 9.0, 3.0)	6.91 (dd, *J* = 8.8, 2.3)	115.2	115.9
7	-	-	162.7	163.4
8	6.79 (d, *J* = 3.0)	6.77 (d, *J* = 2.3)	102.4	103.2
9	-	-	157.7	158.5
10	6.18 (d, *J* = 12.0)	6.14 (d, *J* = 12.3)	43.1	43.9
11	-	-	203.8	204.5
12	-	-	113.3	114.1
13	-	-	166.0	166.8
14	6.24 (d, *J* = 3.0)	6.20 (d, *J* = 2.4)	102.6	103.3
15	-	-	165.4	166.1
16	6.47 (dd, *J* = 9.0, 3.0)	6.44 (dd, *J* = 9.0, 2.4)	108.2	109.0
17	8.37 (d, *J* = 9.0)	8.34 (d, *J* = 9.0)	133.6	134.4
18	4.82 (d, *J* = 12.0)	4.79 (d, *J* = 12.3)	52.7	53.4
19	-	-	133.8	134.6
20	7.28 (m)	7.26 (m)	129.2	130.0

Table 26 (continued)

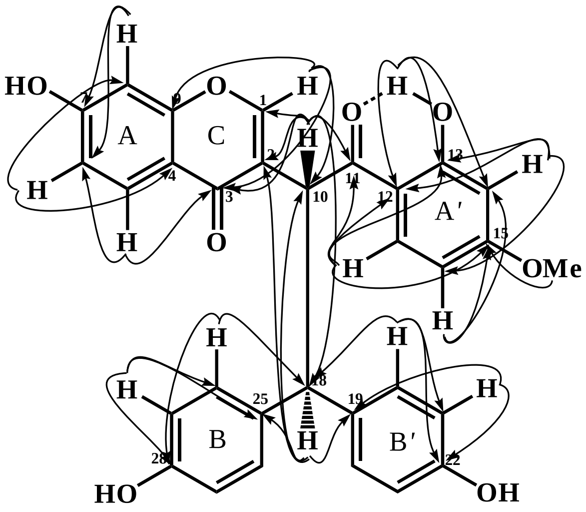
Position	$_{\mathbf{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathbf{C}}$	**R**
21	6.66 (m)	6.61 (m)	115.0	115.8
22	-	-	155.6	156.4
23	6.66 (m)	6.61 (m)	115.0	115.8
24	7.28 (m)	7.26 (m)	129.2	130.0
25	-	-	134.8	135.6
26	7.28 (m)	7.26 (m)	128.6	129.4
27	6.66 (m)	6.61 (m)	115.2	115.9
28	-	-	155.7	156.5
29	6.66 (m)	6.61 (m)	115.2	115.9
30	7.28 (m)	7.26 (m)	128.6	129.4
13-OH	12.70 (s)	12.60 (s)	-	-

3.2.13 Compound RA2



Compound **RA2** was isolated as a white solid, mp 191-192 C, $_{\mathrm{D}}^{24}$ +30 (*c* 0.55, MeOH). The UV and IR spectra were closely resembled those of compound **RA1**.

The ^{1}H and ^{13}C NMR spectral data (Table 27, Figures 132 and 133) of **RA2** were similar to those of **RA1**, except that **RA2** had an additional singlet signal of methoxyl protons at 3.79 (3H, s) ($_{\mathrm{C}}$ 55.2). The position of the methoxyl group at C-15 was determined through HMBC correlation of $_{\mathrm{H}}$ 3.79 (15-OMe) with the signal at $_{\mathrm{C}}$ 166.7 (C-15). Therefore, compound **RA2** was identified as calodenone (Messanga *et al.*, 1992).



Selected HMBC correlations for compound **RA2**

Table 27 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RA2** (Acetone-d_6)

Position	$_{\text{H}}$ **(mult, *J*, Hz)**	$_{\text{C}}$	**DEPT**	**HMBC**
1	8.31 (s)	155.5	CH	2, 3
2	-	121.2	C	-
3	-	174.4	C=O	-
4	-	116.3	C	-
5	7.95 (d, *J* = 8.7)	127.4	CH	3, 6, 7
6	6.93 (d, *J* = 8.7, 2.1)	115.1	CH	4,7, 8
7	-	162.7	C	-
8	6.79 (d, *J* = 2.1)	102.3	CH	7, 9
9	-	157.7	C	-
10	6.19 (d, *J* = 12.3)	43.3	CH	2, 3, 18
11	-	204.1	C	-
12	-	113.7	C	-
13	-	165.9	C	-
14	6.30 (d, *J* = 2.1)	100.6	CH	12, 13, 16
15	-	166.7	C	-
16	6.50 (d, *J* = 9.0, 2.1)	107.4	CH	12, 14, 15
17	8.42 (d, *J* = 9.0)	133.1	CH	12, 13, 15
18	4.83 (d, *J* = 12.3)	52.6	CH	10, 19, 24, 25
19	-	133.7	C	-
20	7.30 (m)	129.2	CH	18, 21, 22, 24
21	6.66 (m)	115.0	CH	19, 22, 23
22	-	155.6	C	-
23	6.66 (m)	115.0	CH	19, 22
24	7.30 (m)	129.2	CH	18, 21, 22, 24
25	-	134.8	C	-
26	7.30 (m)	128.6	CH	18, 28, 30
27	6.66 (m)	115.1	CH	25, 28, 30
28	-	155.7	C	-

Table 27 (continued)

Position	**$_{H}$ (mult, *J*, Hz)**	**$_{C}$**	**DEPT**	**HMBC**
29	6.66 (m)	115.1	CH	25, 28, 30
30	7.30 (m)	128.6	CH	18, 28, 29
15-OMe	3.79 (s)	55.2	CH_3	15
13-OH	12.73 (s)	-	-	12, 13, 14

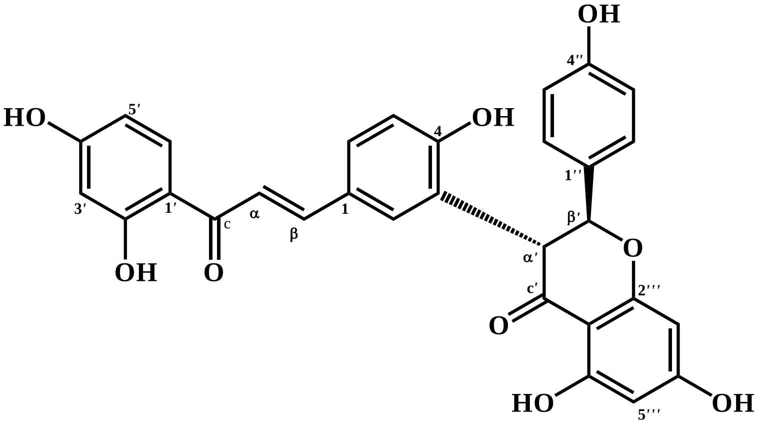
Table 28 Comparison of ^{1}H NMR spectral data of **RA2** (Acetone-d_6) and calodenone (**R**, Acetone-d_6)

Position	**$_{H}$ (mult, *J*, Hz)**	**R**
1	8.31 (s)	8.26 (s)
2	-	-
3	-	-
4	-	-
5	7.95 (d, *J* = 8.7)	7.91 (d, *J* = 8.8)
6	6.93 (dd, *J* = 8.7, 2.1)	6.89 (dd, *J* = 8.8, 2.3)
7	-	-
8	6.79 (d, *J* = 2.1)	6.75 (d, *J* = 2.3)
9	-	-
10	6.19 (d, *J* = 12.3)	6.15 (d, *J* = 12.3)
11	-	-
12	-	-
13	-	-
14	6.30 (d, *J* = 2.1)	6.29 (d, *J* = 2.4)
15	-	-
16	6.50 (dd, *J* = 9.0, 2.1)	6.49 (dd, *J* = 8.1, 2.4)
17	8.42 (d, *J* = 9.0)	8.37 (d, *J* = 8.1)
18	4.83 (d, *J* = 12.3)	4.79 (d, *J* = 12.3)
19	-	-

Table 28 (continued)

Position	$_{H}$ **(mult, *J*, Hz)**	**R**
20	7.30 (m)	7.25 (m)
21	6.66 (m)	6.60 (m)
22	-	-
23	6.66 (m)	6.60 (m)
24	7.30 (m)	7.25 (m)
25	-	-
26	7.30 (m)	7.25 (m)
27	6.66 (m)	6.60 (m)
28	-	-
29	6.66 (m)	6.60 (m)
30	7.30 (m)	7.25 (m)
15-OMe	3.79 (s)	3.78 (s)
13-OH	12.73 (s)	12.69 (s)

3.2.14 Compound RA3

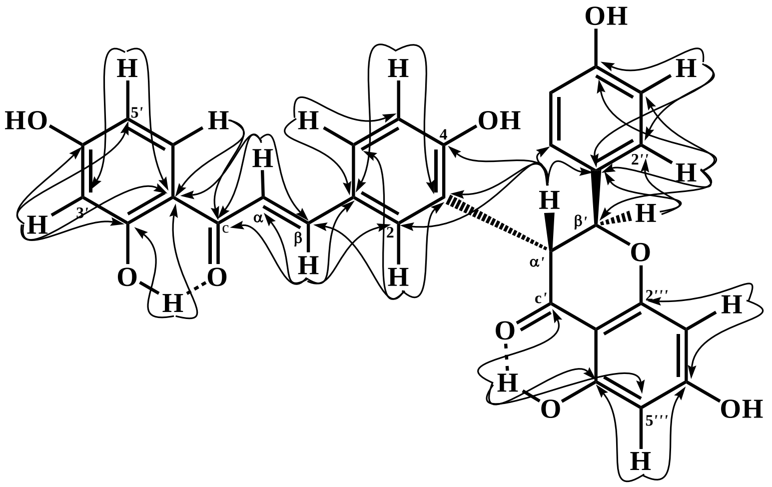


Compound **RA3** was isolated as a yellow solid, mp 181-182 C, The UV spectrum displayed maximum absorptions at 287 and 374 nm suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of hydroxyl group (3407 cm^{-1}) and carbonyl (1629 cm^{-1}).

The ^{13}C NMR spectral data (Table 29, Figure 143) exhibited 30 signals, which were comprised by two carbonyls (191.8, 196.9), two aliphatic methines (54.3, 82.5) and 26 olefinic and aromatic carbons (94.9, 96.1, 102.1, 102.9, 107.8, 113.6, 114.9 (2C), 115.8, 117.6, 123.0, 126.7, 129.0 (2C), 129.7, 132.2 (2C), 133.2, 144.1, 157.7, 157.9, 163.4, 164.6, 164.7, 166.4, 166.7).

The ^{1}H NMR spectrum of **RA3**, displayed the typical *trans* olefinic protons of a chalcone structure at 7.67 (*d*, *J* = 15.3 Hz, H-*α*) and 7.73 (*d*, *J* = 15.3 Hz, H-*β*) and two 1,2,4-trisubstituted benzene rings at 6.36 (*d*, *J* = 2.4 Hz, H-3′), 6.46 (*dd*, *J* = 9.0, 2.4 Hz, H-5′) and 8.01 (*d*, *J* = 9.0 Hz, H-6′) and at 6.88 (*d*, *J* = 8.7 Hz, H-5), 7.56 (*m*, H-2) and 7.57 (*m*, H-6). The chelated hydroxyl group was evident at 13.61. Additional ^{1}H NMR signal of characteristic aliphatic protons of a flavanone skeleton was displayed at 4.67 (*d*, *J* = 12.3 Hz, H-*α*′) and 5.90 (*d*, *J* = 12.3 Hz, H-*β*′). The following signals were shown: a *p*–disubstituted benzene ring at 6.74 (2H, *d*, *J* = 8.4 Hz, H-3″, 5″) and 7.30 (2H, *d*, *J* = 8.4 Hz, H-2″, 6″), a 1,2,3,5-tetrasubstituted benzene ring at 6.00 (*d*, *J* = 2.1 Hz, H-3′′′) and 6.01 (*d*, *J* = 2.1 Hz, H-5′′′). The chelated hydroxyl group was evident at 12.28. These spectral data indicated a chalcone-flavanone type for **RA3**.

The structure of **RA3** was confirmed by HMBC experiment. For a chalcone skeleton, the proton signal at 7.67 (H-*α*) showed correlations with the carbons at 113.6 (C-1′), 144.1(C-*β*) and 191.8 (C=O), suggesting the connection of a 1,2,4-trisubstituted benzene ring at C=O. The correlation of the proton signals at 7.71 (H-*β*) with the carbons at 117.6 (C-*α*), 126.7 (C-1), 129.7 (C-2), 133.2 (C-6) and 191.8 (C=O) suggested a connection of another 1,2,4-trisubstituted benzene ring to C-*β*. For a flavanone skeleton, the proton signal at 4.67 (H-*α*′) showed correlations with the carbons at 82.5 (C-*β*′), 123.0 (C-3), 129.0 (C-2′′/C-6′′), 129.7 (C-2), 132.2 (C-1′′) and 157.9 (C-4), suggesting the connection of the 1,2,4-trisubstituted benzene ring to C-3. The correlation of the proton signals at 5.90 (H-*β*′) with the carbons at 129.0 (C-2′′/C-6′′) and 132.2 (C-1′′) suggested that a *p*-disubstituted benzene ring was connected to C-*β*′. In addition the chelated proton signals at 12.28 (OH-6′′′) showed correlations with the carbons at 102.1 (C-1′′′), 166.4 (C-6′′′) and 196.9 (C-c′). With regard to the sterreochemistry of H-*α*′ and H-*β*′on the pyrone ring of the flavanone unit, the large vicinal coupling constant (*J* = 12.3 Hz) suggested a *trans* relative configuration. By comparison of the spectral data of **RA3** with those of 6′′′-hydroxylophirone B, therefore compound **RA3** was identified as 6′′′-hydroxylophirone B (Kaewamatawong *et al*., 2002).



Selective HMBC correlations of **RA3**

Table 29 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RA3** (Acetone-d_6)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	$_{\mathrm{C}}$	**DEPT**	**HMBC**
1′	-	113.6	C	-
2′	-	166.7	C	-
3′	6.36 (d, *J* = 2.4)	102.9	CH	1′, 2′, 4′, 5′
4′	-	164.7	C	-
5′	6.46 (dd, *J* = 9.0, 2.4)	107.8	CH	1′, 3′
6′	8.01 (d, *J* = 9.0)	132.2	CH	2′, 4′, c
CO (c)	-	191.8	C=O	-
α	7.67 (d, *J* = 15.3)	117.6	CH	1, c, β
β	7.73 (d, *J* = 15.3)	144.1	CH	1, 2, 6, α, c
1	-	126.7	C	-
2	7.56 (m)	129.7	CH	4, 6, β
3	-	123.0	C	-
4	-	157.9	C	-
5	6.88 (d, *J* = 8.7)	115.8	CH	1, 3
6	7.57 (m)	133.2	CH	2, 6

Table 29 (continued)

Position	**$_{\mathrm{H}}$ (mult, *J*, Hz)**	**$_{\mathrm{C}}$**	**DEPT**	**HMBC**
α′	4.67 (d, *J* = 12.3)	54.3	CH	2, 3, 4, 1″, 2″, 6″
β′	5.90 (d, *J* = 12.3)	82.5	CH	1″, 2″, 6″
CO (c′)	-	196.9	C=O	-
1″	-	132.2	C	-
2″, 6″	7.30 (d, *J* = 8.4)	129.0	CH	1″, 4″, *β*′
3″, 5″	6.74 (d, *J* = 8.4)	114.9	CH	1″, 2″, 4″, 5″, 6″
4″	-	157.7	C	-
1‴	-	102.1	C	-
2‴	-	163.4	C	-
3‴	6.00 (d, *J* = 2.1)	94.9	CH	2‴, 4‴
4‴	-	164.6	C	
5‴	6.01 (d, *J* = 2.1)	96.1	CH	4‴, 6‴
6‴	-	166.4	C	-
2′-OH	13.61 (s)	-	-	1′, 2′, 3′
6‴-OH	12.28 (s)	-	-	4‴, 5‴, 6‴

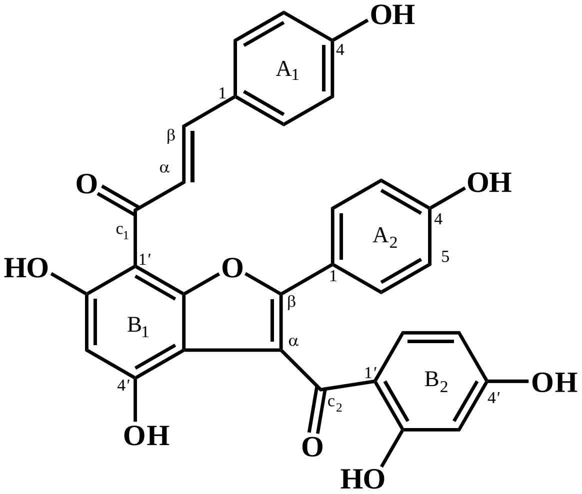
Table 30 Comparison of ^{1}H NMR and ^{13}C NMR spectral data of **RA3** (Acetone-d_6) and 6′′′-hydroxylophirone B (**R**, Acetone-d_6)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathrm{C}}$	**R**
1′	-	-	113.6	113.7
2′	-	-	166.7	166.8
3′	6.36 (d, *J* = 2.4)	6.36 (d, *J* = 2.3)	102.9	103.0
4′	-	-	164.7	164.8
5′	6.46 (dd, *J* = 9.0, 2.4)	6.45 (dd, *J* = 8.9, 2.3)	107.8	107.9
6′	8.01(d, *J* = 9.0)	8.01(d, *J* = 8.9)	132.2	132.4
CO (c)	-	-	191.8	191.9
α	7.67 (d, *J* = 15.3)	7.67 (d, *J* = 15.2)	117.6	117.6
β	7.73 (d, *J* = 15.3)	7.72 (d, *J* = 15.2)	144.1	144.2
1	-	-	126.7	126.8
2	7.56 (br s)	7.55 (br s)	133.2	133.4
3	-	-	123.0	123.1
4	-	-	157.9	157.9
5	6.88 (d, *J* = 8.7)	6.86 (d, *J* = 8.9)	115.8	115.8
6	7.57 (m)	7.56 (m)	129.7	129.8
α'	4.67 (d, *J* = 12.3)	4.66 (d, *J* = 12.0)	54.3	54.3

Table 30 (continued)

Position	$_{\mathbf{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathbf{C}}$	**R**
β′	5.90 (d, *J* = 12.3)	5.90 (d, *J* = 12.0)	82.5	82.5
CO (c′)	-	-	196.9	197.0
1″	-	-	132.2	132.2
2″, 6″	7.30 (d, *J* = 8.4)	7.29 (dd, *J* = 6.6, 2.0)	129.0	129.2
3″, 5″	6.74 (d, *J* = 8.4)	6.74 (dd, *J* = 6.6, 2.0)	114.9	115.0
4″	-	-	157.7	157.7
1‴	-	-	102.1	102.1
2‴	-	-	163.4	163.5
3‴	6.00 (d, *J* = 2.1)	6.00 (d, *J* = 2.0)	94.9	95.0
4‴	-	-	164.6	166.4
5‴	6.01 (d, *J* = 2.1)	6.01 (d, *J* = 2.0)	96.1	96.2
6‴	-	-	166.4	166.8
2′-OH	13.61 (s)	13.60 (s)	-	-
6‴OH	12.28 (s)	12.27 (s)	-	-

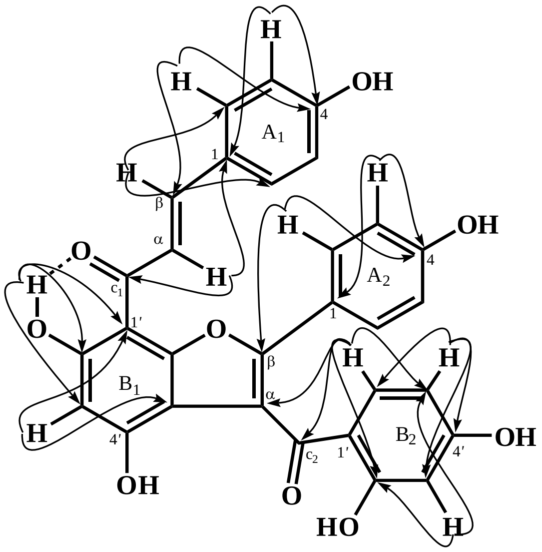
3.2.15 Compound RA4



Compound **RA4** was isolated as orange needles, mp: 249-250 C. The UV and IR spectra were closely resembled those of compound **TA2**.

The ^{1}H NMR and ^{13}C NMR spectral data (Table 31, Figure 152 and 153) of **RA4** were comparable with those of **TA2**. The difference was shown in the part of a chalcone skeleton as the disappearance of the signals of two 1,2,4–trisubstituted benzene ring in **TA2** and the appearance of a *p*-disubstituted benzene ring at 7.01 (2H, *d*, J = 9.0 Hz, H-3, 5 (A1)) and 7.75 (2H, *d*, J = 9.0 Hz, H-2, 6 (A1)) and pentasubstituted benzene ring at 6.29 (*s*, H-5′ (B1). Additional difference was shown as the disappearance of the signal of dihydrobenzofuran ring in **TA2** and the appearance of a benzofuran ring in **RA4**. The HMBC spectrum showed correlations of the proton at 7.75 (H-2, 6 (A1)) with the carbons at 144.2 (C-β1) and 160.5 (C-4 (A1)), suggesting the connection of a *p*-disubstituted benzene ring (A1) at C-β1. The correlations of the proton signals at 8.29 (H-α1) with the carbons at 126.8 (C-1 (A1)) and 189.7 (C-c$_1$), in turn the chelated proton signal at 14.30 correlated with the carbons at 98.8 (H-5′ (B1)) and 166.7 (H-4′ (B1)), suggested the connection of a pentasubstituted benzene ring (ring B1) at C-c$_1$. Furthermore an aromatic methine proton at 7.64 (H-6 (A-2)) showed correlations with the carbons at 158.8 (C-4

(A2)) and 154.1 (C-β2). By comparison of the spectral data of **RA4** with those of calodenin B, therefore compound **RA4** was identified as calodenin B (Messanga *et al.*, 1994).



Selective HMBC correlations of **RA4**

Table 31 ^{1}H and ^{13}C, DEPT and HMBC spectral data of **RA4** (Acetone-*d*$_{6}$)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	$_{\mathrm{C}}$	**DEPT**	**HMBC**
1-A1	-	126.8	C	-
2-A1	7.75 (d, *J* = 9.0)	130.7	CH	β1, 4A1, 4A6
3-A1	7.01 (d, *J* = 9.0)	116.2	CH	1A1, 4A1, 5A1
4-A1	-	160.5	C	-
5-A1	7.01 (d, *J* = 9.0)	116.2	CH	1A1, 4A1, 3A1
6-A1	7.75 (d, *J* = 9.0)	130.7	CH	β1, 4A1
β1	7.94 (d, *J* = 15.3)	144.2	CH	2A1, 6A1, C1
α*1*	8.29 (d, *J* = 15.3)	122.0	CH	C1, 1A1
C1	-	189.7	C	-
1′-B1	-	101.6	C	-
2′-B1	-	152.2	C	-
3′-B1	-	111.0	C	-

Table 31 (continued)

Position	**$_H$ (mult, *J*, Hz)**	**$_C$**	**DEPT**	**HMBC**
4′-B1	-	166.7	C	-
5′-B1	6.29 (s)	98.8	CH	1′B1, 3′B1,4′B1
6′-B1	-	166.7	C	-
1-A2	-	120.8	C	-
2-A2	7.64 (dd, *J* = 9.0, 3.0)	128.2	CH	6A2, 4A2, *β*2
3-A2	6.94 (m)	115.9	CH	1A2, 4A2, 5A2
4-A2	-	158.8	C	-
5-A2	6.94 (m)	115.9	CH	1A2, 4A2
6-A2	7.64 (dd, *J* = 9.0, 3.0)	128.2	CH	2A2, 4A2, *β*2
*β*2	-	154.1	C	-
*α*2	-	114.5	C	-
C2	-	195.9	C	-
1′-B2	-	102.6	C	-
2′-B2	-	165.7	C	-
3′-B2	6.39 (d, *J* = 3.0)	102.6	CH	2′B2, 5′B2
4′-B2	-	158.3	C	-
5′-B2	6.25 (dd, *J* = 9.0, 3.0)	108.4	CH	1′B2
6′-B2	7.45 (d, *J* = 9.0)	135.8	CH	2′B2, C2
2′-B1(OH)	14.30 (s)	-	-	1′B1, 5′B1, 6′B1

Table 32 Comparison of ^{1}H NMR and ^{13}C spectral data of **RA4** (Acetone-d_6) and calodenin B (**A**cetone-d_6)

Position	$_{\mathbf{H}}$ **(mult, *J*, Hz)**	**R**	$_{\mathbf{C}}$	**R**
1-A1	-	-	126.8	127.7
2-A1	7.75 (d, *J* = 9.0)	7.74 (m)	130.7	131.6
3-A1	7.01 (d, *J* = 9.0)	7.00 (m)	116.2	116.9
4-A1	-	-	160.5	161.1
5-A1	7.01 (d, *J* = 9.0)	7.00 (m)	116.2	116.9
6-A1	7.75 (d, *J* = 9.0)	7.74 (m)	130.7	131.6
*β*1	7.94 (d, *J* = 15.3)	7.95 (d, *J* = 15.4)	144.2	145.1
α1	8.29 (d, *J* = 15.3)	8.29 (d, *J* = 15.4)	122.0	123.0
C1	-	-	189.7	190.6
1′-B1	-	-	101.6	102.5
2′-B1	-	-	152.2	155.0
3′-B1	-	-	111.0	115.4
4′-B1	-	-	166.7	167.1
5′-B1	6.29 (s)	6.28 (s)	98.8	99.7
6′-B1	-	-	166.7	158.6
1-A2	-	-	120.8	121.7
2-A2	7.64 (dd, *J* = 9.0, 3.0)	7.64 (m)	128.2	129.3
3-A2	6.94 (m)	6.94 (m)	115.9	116.7
4-A2	-	-	158.8	159.5
5-A2	6.94 (m)	6.94 (m)	115.9	116.7
6-A2	7.64 (dd, *J* = 9.0, 3.0)	7.64 (m)	128.2	129.3
*β*2	-	-	154.1	153.5
*α*2	-	-	114.5	113.7
C2	-	-	195.9	196.7
1′-B2	-	-	102.6	115.4
2′-B2	-	-	165.7	166.2

Table 32 (continued)

Position	**$_{H}$ (mult, *J*, Hz)**	**R**	**$_{C}$**	**R**
3′-B2	6.39 (d, *J* = 3.0)	6.39 (d, *J* = 2.2)	102.6	103.3
4′-B2	-	-	158.3	166.3
5′-B2	6.25 (dd, *J* = 9.0, 3.0)	6.24 (dd, *J* = 8.9, 2.2)	108.4	109.1
6′-B2	7.45 (d, *J* = 9.0)	7.46 (d, *J* = 8.9)	135.8	136.8
2′-B1(OH)	14.30 (s)	13.30 (s)	-	-

3.2.16 Compound RA5

Compound **RA5** was isolated as a viscous oil, $_D^{23}$ -7.5 (*c* 0.35, $CDCl_3$). The UV spectrum displayed maximum absorption bands at 275 and 312 nm suggesting a flavone skeleton. The IR spectrum showed absorption bands of hydroxyl group (3321 cm^{-1}) and C=O stretching (1682 cm^{-1}).

The ^1H NMR and ^{13}C NMR spectral data (Table 33, Figure 162 and 163) of **RA5** were comparable with those of **RD2**. The difference was shown as the disappearance of the signals of two methoxyl groups in **RD2** and the appearance of *meta*-coupled aromatic signals at 6.12 (2H, *d*, *J* = 1.8, H-6, 8) in **RA5**. The oxymethine protons were displayed at 4.45 (*d*, *J* = 12.0, H-3) and 4.99 (*d*, *J* = 12.0, H-2) whose large vicinal coupling constant suggested a *trans* relative configuration. Additionnal ^1H NMR signal of hydroxyl proton was displayed at 4.05 (*s*) in **RA5**, whose position was assigned at C-3 from HMBC correlations with the carbons at 72.7 (C-3), 83.0 (C-2) and 190.9 (C-4). The HMBC spectrum showed correlations of the proton at 4.45 (H-3) with the carbons at 83.0 (C-2) and 190.9 (C-4) and the proton at 4.99 (H-2) showed correlations with the carbons at 72.7 (C-3), 128.9 (C-2′, 6′), 165.0 (C-8a) and 190.9 (C-4) confirming the location of oxymethine protons at C-3 and C-2, respectively. Therefore, compound **RA5** was identified as (2*R*,3*R*)-2,3-*trans*-4´,5,7-trimethoxydihydroflavonol (Rensburg *et al*., 1997).

Selected HMBC correlations for compound **RA5**

Table 33 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of **RA5** (CDCl$_3$)

Position	$_{\mathrm{H}}$ **(mult, *J*, Hz)**	$_{\mathrm{C}}$	**DEPT**	**HMBC**
2	4.99 (d, *J* = 12.0)	83.0	CH	3, 4, 8a, 2′, 6′
3	4.45 (d, *J* = 12.0)	72.7	CH	2, 4a
4	-	190.9	C=O	-
4a	-	102.9	C	-
5	-	162.2	C	-
6	6.12 (d, *J* = 1.8)	93.3	CH	5, 8, 4a
7	-	167.0	C	-
8	6.12 (d, *J* = 1.8)	93.3	CH	6, 4a, 8a
8a	-	165.0	C	-
1′	-	128.6	C	-
2′, 6′	7.49 (d, *J* = 8.7)	128.9	CH	2, 3′, 5′
3′, 5′	6.99 (d, *J* = 8.7)	114.2	CH	2′, 6′
4′	-	160.3	C	-
5-OMe	3.93 (s)	56.2	CH$_3$	5
7-OMe	3.82 (s)	62.4	CH$_3$	7
4′-OMe	3.84 (s)	55.7	CH$_3$	4′
3-OH	4.05 (s)	-	-	-

Table 34 Comparison of ^{1}H NMR spectral data of **RA5** ($CDCl_3$) and (2*R*,3*R*)-2,3-*trans*-4´,5,7-trimethoxydihydroflavonol (**R**, $CDCl_3$)

Position	**$_H$ (mult, *J*, Hz)**	**R**
2	4.99 (d, *J* = 12.0)	5.67 (d, *J* = 12.0)
3	4.45 (d, *J* = 12.0)	5.28 (d, *J* = 12.0)
4	-	-
4a	-	-
5	-	-
6	6.12 (d, *J* = 1.8)	6.11 (d, *J* = 2.0)
7	-	-
8	6.12 (d, *J* = 1.8)	6.09 (d, *J* = 2.0)
8a	-	-
1′	-	-
2′, 6′	7.49 (d, *J* = 8.7)	7.38 (d, *J* = 8.8)
3′, 5′	6.99 (d, *J* = 8.7)	6.93 (d, *J* = 8.8)
4′	-	-
5-OMe	3.93 (s)	3.93 (s)
7-OMe	3.82 (s)	3.82 (s)
4′-OMe	3.84 (s)	3.84 (s)
3-OH	4.05 (s)	2.01 (s)

CHAPTER 4

CONCLUSION

Three known compounds of lupeol (**TA1**), lophirone C (**TA2**) and gerontoisoflavone (**TA3**) were isolated from the stem of *Ellipanthus tomentosus*. Their structures were elucidated by spectroscopic methods. Compound **TA2** was a major component.

Twelve known compounds were isolated from the methylene chloride extract of the roots of *Ellipanthus tomentosus*: flavokawain A (**RD1**), 4′,5,6,7,8-pentamethoxyflavone (**RD2**), 3′,4′,5,6,7,8-hexamethoxyflavone (**RD3**), 5-demethyl nobiletin (**RD4**), 5,7,8,3′,4′-pentamethoxyflavone (**RD5**), (*E*)-ferulic acid tetracosyl ester (**RD6**), 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (**RD7**), 5,4′-dimethoxy-6,7-methylenedioxyisoflavone (**RD8**), a mixture of *β*-sitosterol (**RD9**) and stigmasterol (**RD10**), 4-hydroxybenzaldehyde (**RD11**) and vanillin (**RD12**). The crude acetone extract was subjected to chromatography and/or crystallization to give five known compounds of lophirone A (**RA1**), calodenone (**RA2**), 6′′′-hydroxylophirone B (**RA3**), calodenin B (**RA4**) and (2*R*,3*R*)-2,3-*trans*-4´,5,7-trimethoxydihydroflavonol (**RA5**). Their structures were elucidated by spectroscopic methods. Compounds **RD1, RD5, RA1** and **RA2** were major components.

REFERENCES

Ahmadu, A. A., Hassan, H. S., Abubakar, M. U. and Akpulu, I. N. **2007**. Flavonoid glycosides from *Byrsocarpus coccineus* leaves. Schum and Thonn (Connaraceae). Afr. J. Trad. 4(3), 257-260.

Chang, C-H., Lin, C-C., Kadota, S., Hattori, M. and Namba, T. **1995**. Flavonoids and a prenylated xanthone from *Cudrania Cochinchinensis* Var. *Gerontogea*. Phytochemistry 40, 945-947.

Cheenpracha, S. **2004**. Chemical constituents from the seeds of *Cerbera manghas* and the stems of *Derris trifoliate*. Master of Science Thesis in Organic Chemistry, Prince of Songkla University, 137 pp.

Chen, J., Montanari, A. M. and Widmer, W. W. **1997**. Two new polymethoxylated flavones, a class of compounds with potential anticancer activity, isolated from cold pressed *Dancy tangerine* peel oil solids. J. Agric Food Chem. 45, 364-368.

Ghogomu, R., Sondengam, B. L., Martin, M. T. and Bodo, B. **1987**. Lophirone A, a biflavonoid with unusual skeleton from *Lophira lanceolata*. Tetrahedron Lett. 28, 2967-2968.

He, Z.-D., Ma, C.-Y., Tan, G. T., Sydara, K., Tamez, P., Southavong, B., Bouamanivong, S., Soejarto, D. D., Pezzuto, J. M., Fong, H. S. and Zhang, H.-J. **2006**. Rourinoside and rouremin, antimalarial constituents from *Rourea minor*. Phytochemistry 67, 1378-1384.

Jiang, J. Q., Fang, S. D., Xu, C. F. and Luo, J. T. **1990**. Chemical constituents of *Rourea microphylla* (Hock. Et Arn) planch. Zhiwu Xuebao 32, 376-379.

Kaewamatawong, R., Likhitwitayawuid, K., Ruangrungsi, N., Kitajima, T. M. and Aimi, N. **2002**. Novel biflavonoids from the stem bark of *Ochna integerrima*. J. Nat. Prod. 65, 1027-1029.

Li, S., Lo, C.-Y. and Ho, C.-T. **2006**. Hydroxylated polymethoxyflavones and methylated flavonoids in sweet orange (*Citrus sinensis*) peel. J. Agric food Chem. 54, 4176-85.

Machida, K. and Osawa, K. **1989**. On the flavonoid constituents from the peels of *Citrus hassaku* HORT. *ex* TANAKA. Chem. Pharm. Bull. 37, 1092-1094.

Mensah, I. A., Achenbach, H., Thoithi, G. N., Waibel, R. and Mwangi, J. W. **1992**. Epoxychiromodine and other constituents of *Corton megalocarpus*. Phytochemistry 31, 2055-2058.

Messanga, B. B., Tih, G. R., Kimbu, S. F. and Sondengam, B. L. **1992**. Calodenone, a new isobiflavonoid from *Ochna calodendron*. J. Nat. Prod. 55, 245-248.

Messanga, B., Tih, G. R., Sondengam, B. L., Martin, M-T. and Bodo, B. **1994**. Biflavonoids from *Ochna calodendron*. Phytochemistry 35, 791-794.

Rensburg, H., Heerden, P., Bezuidenhoudt, B. and Ferreira, D. **1997**. Stereoselective synthesis of flavonoids. Part 4. *Trans*- and *cis*-dihydroflavonols. Tetrahedron 53, 14141-14152.

Ramiah, N., Prasad, N. B. R. and Abraham, K. **1976**. Rapanone and leucopelargonidin from the roots of *Rourea santaloides*. Journal of Institute of Chemists (India) 48 (Pt. 4), 196-197.

Reynolds, W. F., Mclean, S., Poplawski, J., Enriquez, R. G., Escobar, L. I. and Lenon, I. **1986**. Total assignment of ^{13}C and ^{1}H spectra of three isomeric triterpenol derivative by 2D NMR: an investigations of the potential utility of ^{1}H chemical shifts in structural investigations of complex natural products, Tetrahedron 42, 3419-3428.

Seidel, V., Bailleul, F. and Waterman, P. G. **2000**. (Rel)-1β, 2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β, 4a-di-(4-methoxyphenyl)-cyclobutane and other flavonoids from the aerial parts of *Goniothalamus gardneri* and *Goniothalamus thwaitesii*. Phytochemistry 55, 439-446.

Smitinand, T. **2004**. Thai plant names. Bangkok: The Forest Herbarium, Royal Forest Department, p 810.

Thongdeeying, P. **2005**. Chemical constituents from the leaves of *Ceriops decandra* (Giff.) Ding Hou. Master of Science Thesis in Organic Chemistry, Prince of Songkla University, p 54-55.

Veitch, N. C., Sutton, P. S. E., Kite, G. C. and Ireland, H. E. **2003**. Six new isoflavones and a 5-deoxyflavonol glycoside from the leaves of *Ateleia herbert-smithii*. J. Nat. Prod. 66, 210-216.

APPENDIX

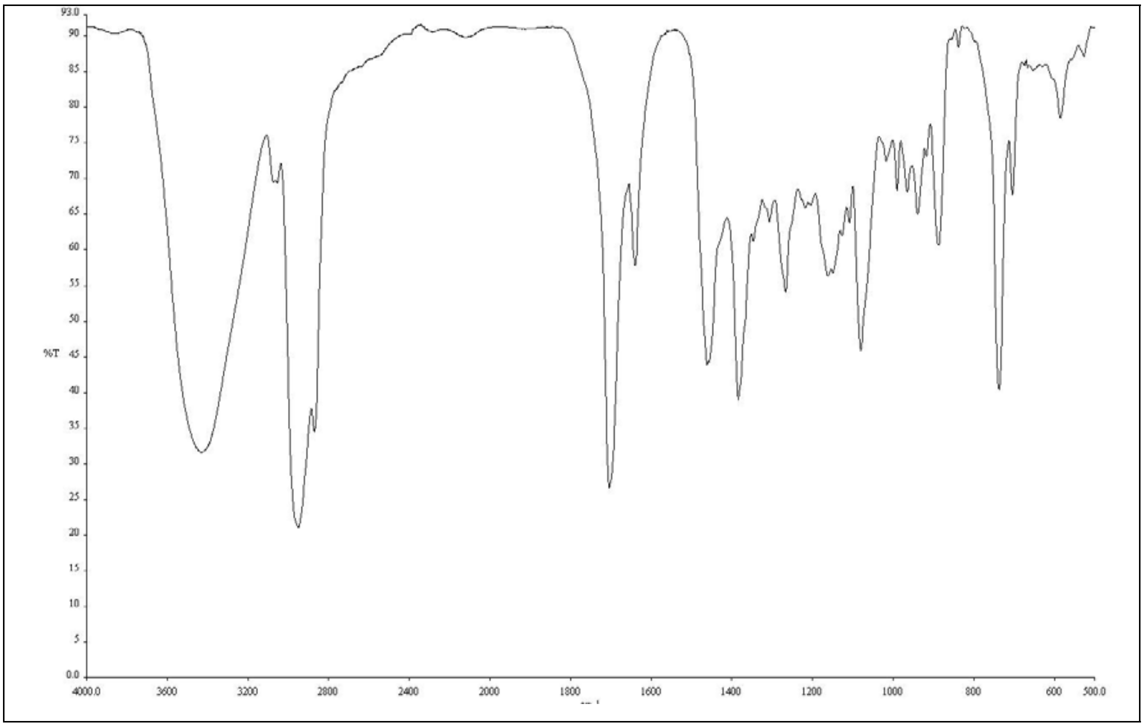


Figure 2 IR (neat) spectrum of compound **TA1**

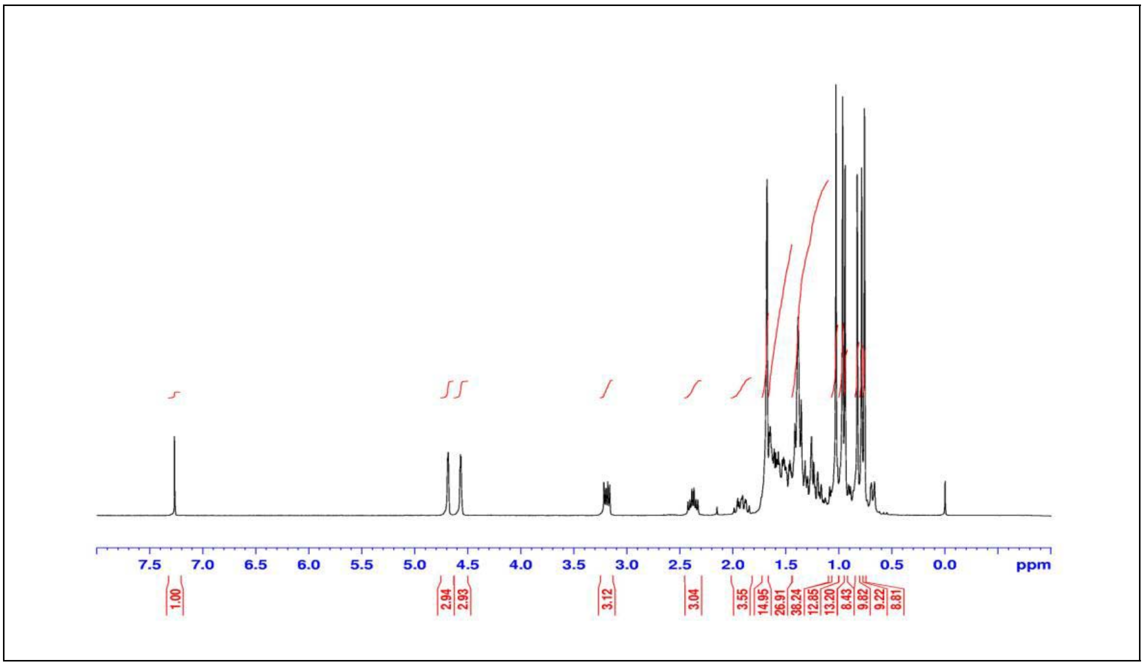


Figure 3 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **TA1**

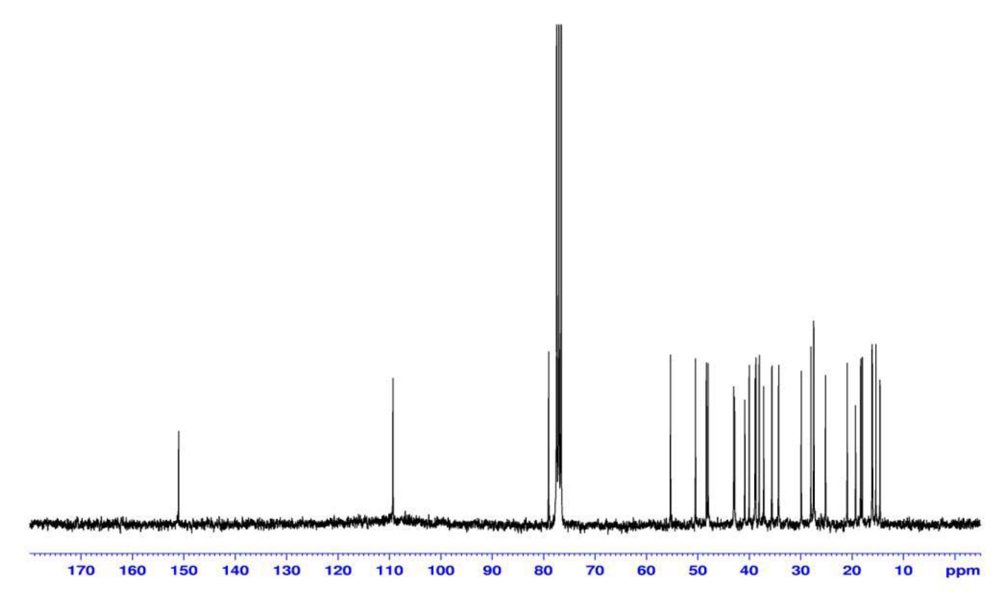


Figure 4 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **TA1**

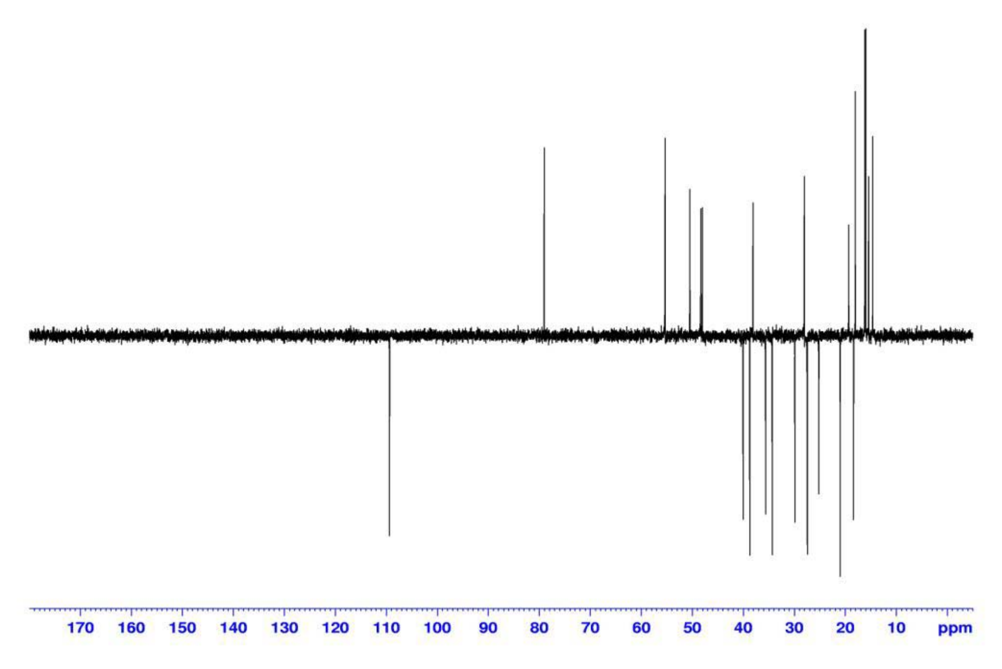


Figure 5 DEPT 135 ($CDCl_3$) of compound **TA1**

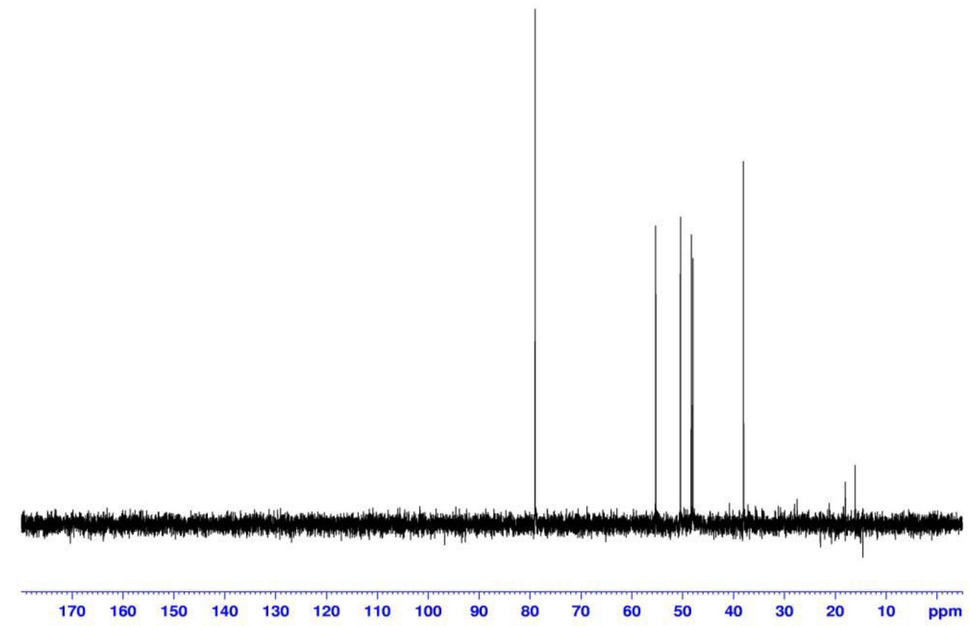


Figure 6 DEPT 90 (CDCl$_3$) of compound **TA1**

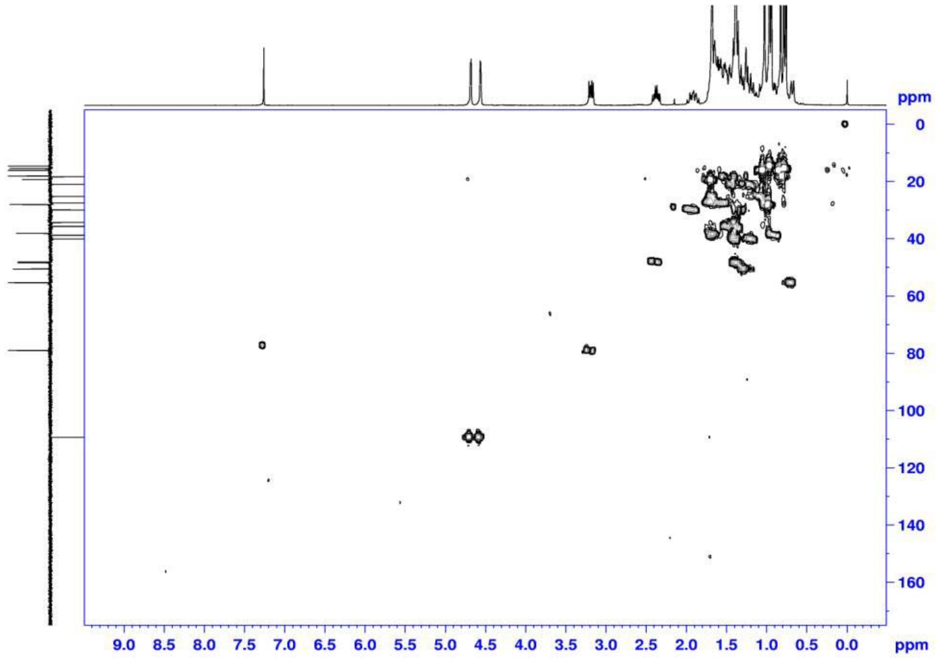


Figure 7 2D HMQC (CDCl$_3$) of compound **TA1**

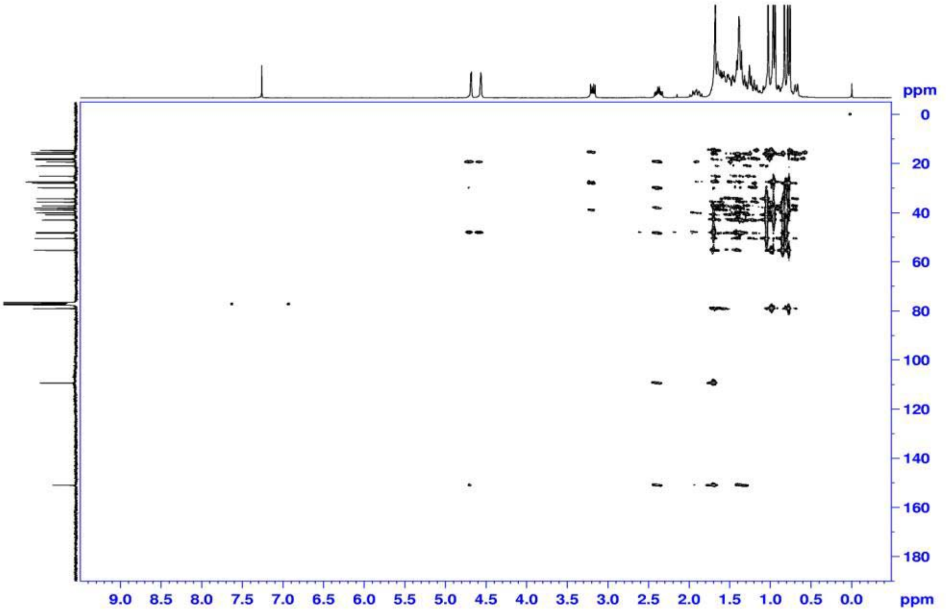


Figure 8 2D HMBC ($CDCl_3$) of compound **TA1**

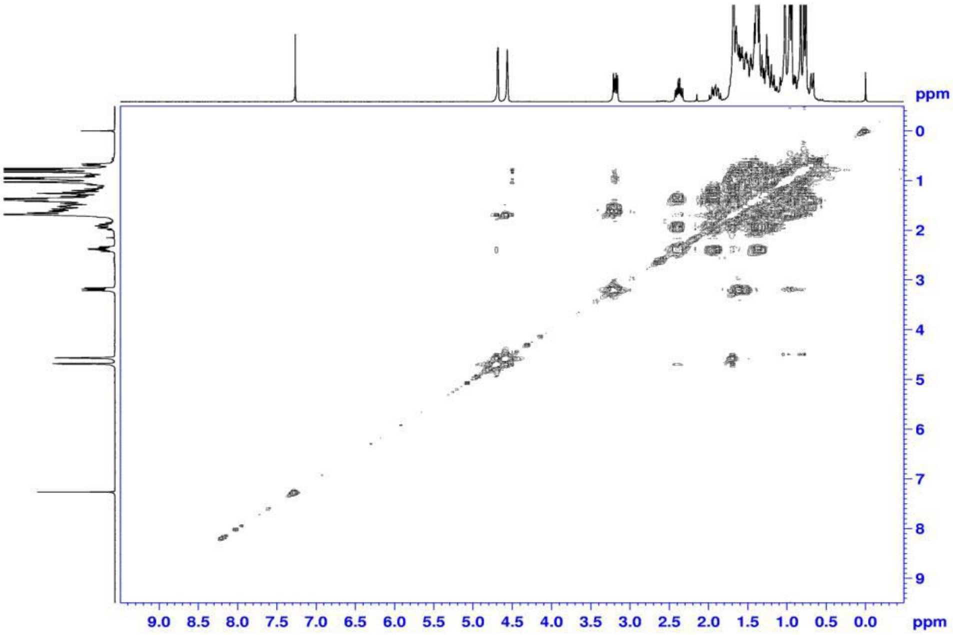


Figure 9 2D COSY ($CDCl_3$) of compound **TA1**

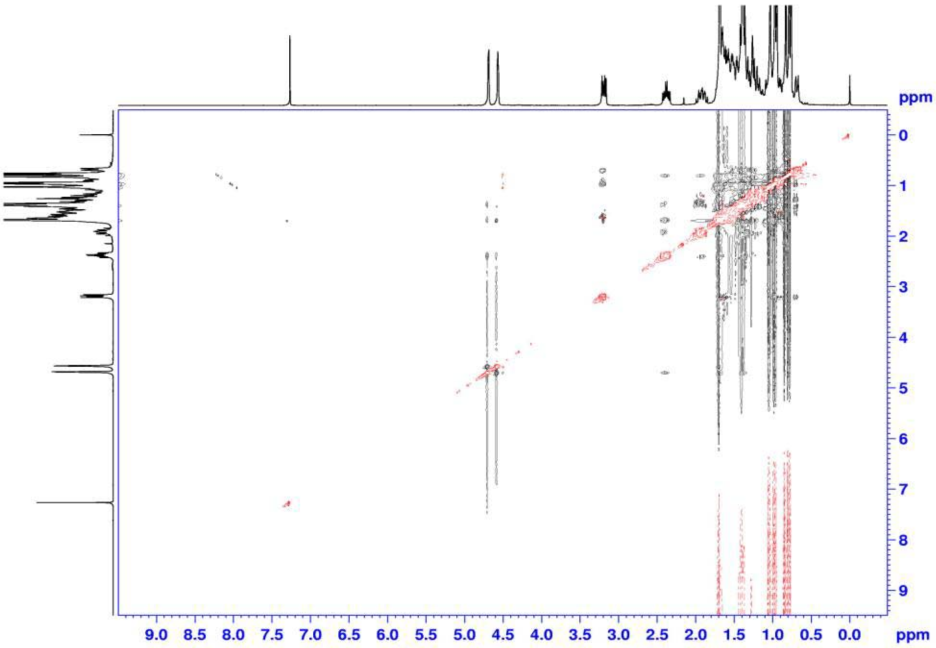


Figure 10 2D NOESY ($CDCl_3$) of compound **TA1**

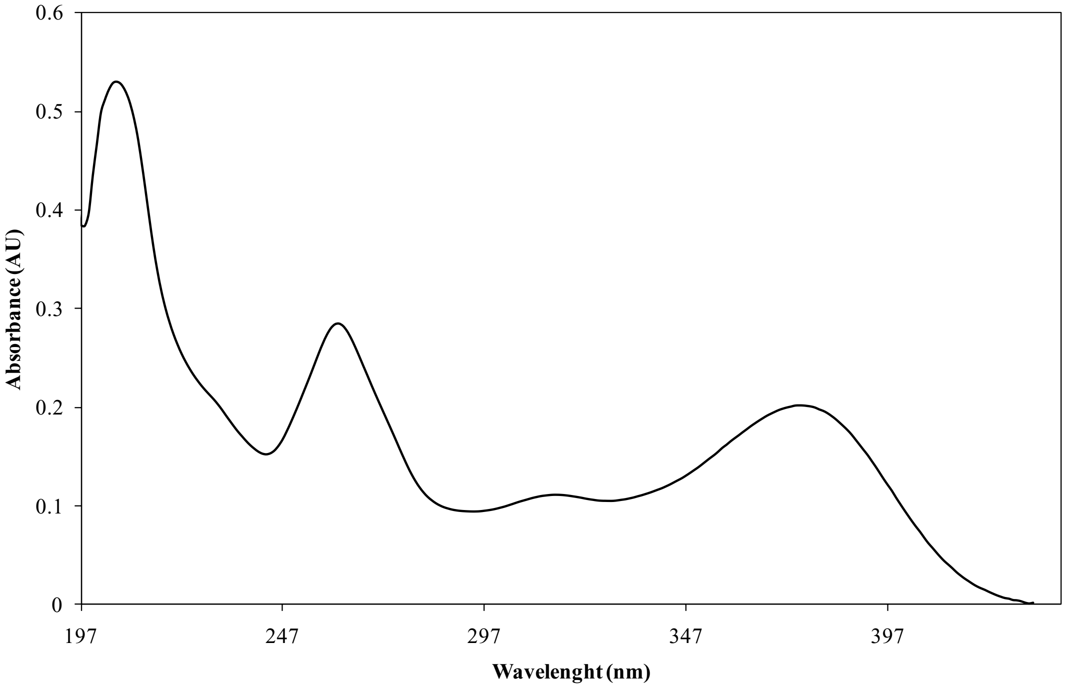


Figure 11 UV (MeOH) spectrum of compound **TA2**

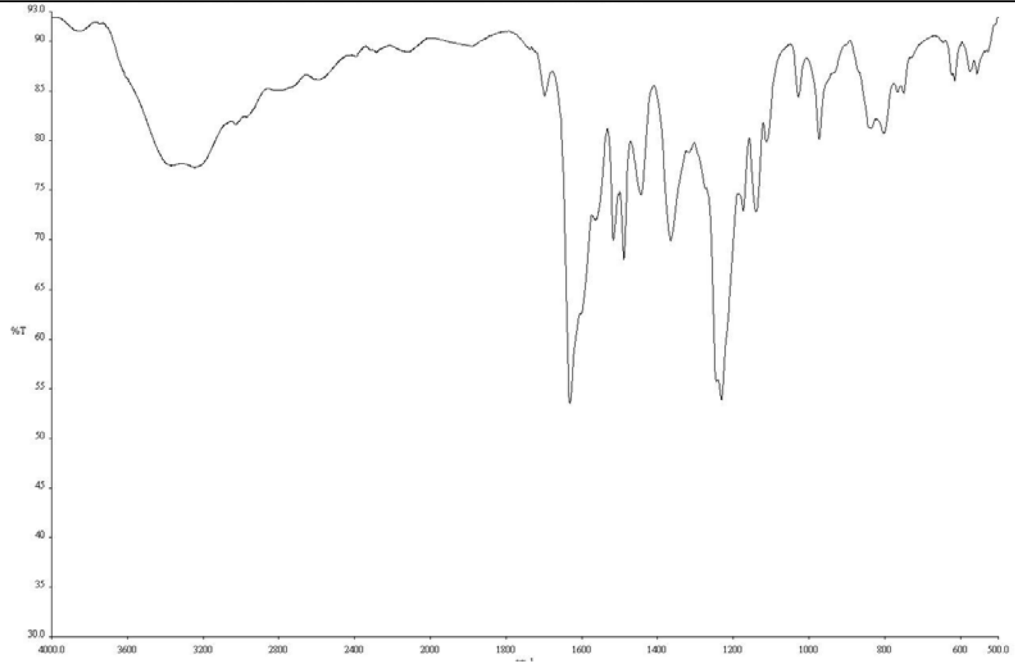


Figure 12 IR (neat) spectrum of compound **TA2**

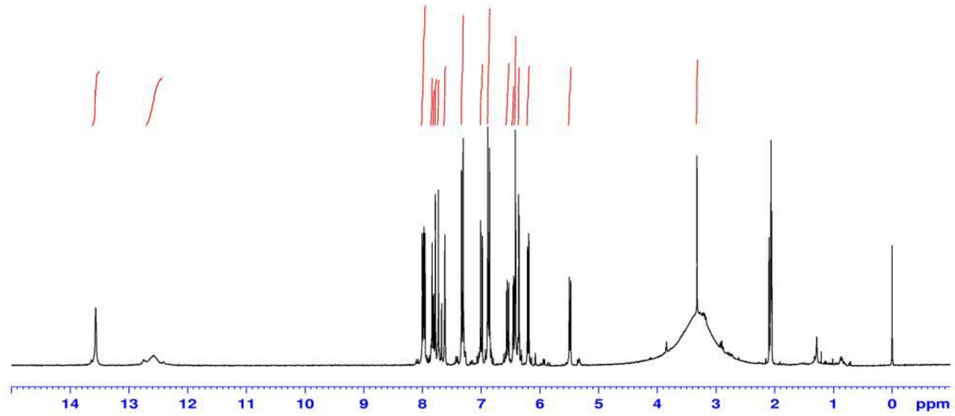


Figure 13 ^{1}H NMR (300 MHz) (Acetone-d_6) of compound **TA2**

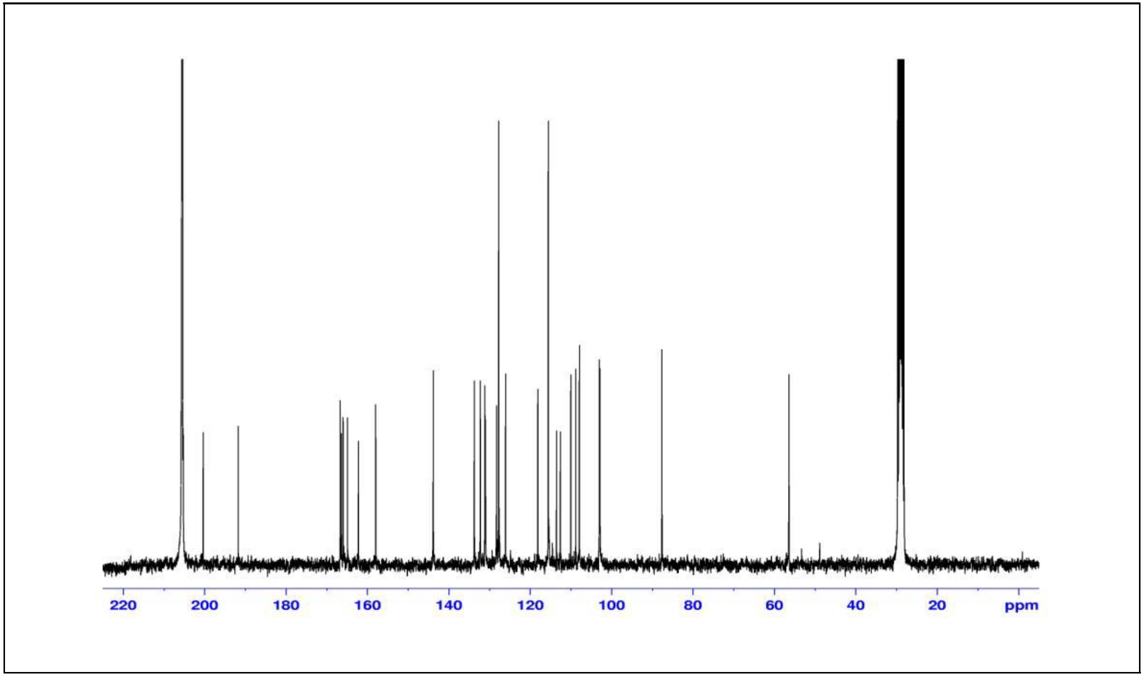


Figure 14 ^{13}C NMR (75 MHz) (Acetone-d_6) of compound **TA2**

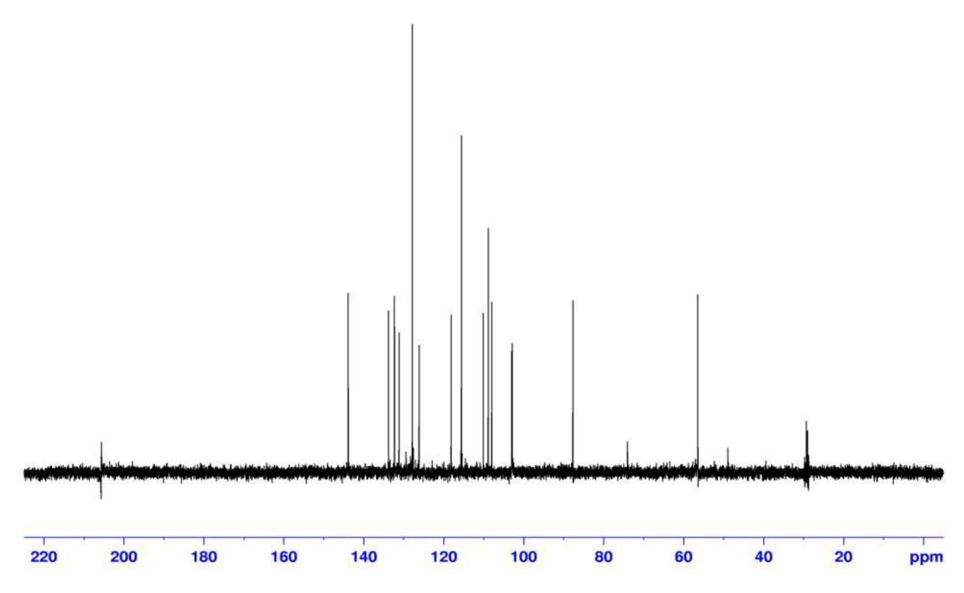


Figure 15 DEPT 135 (Acetone-d_6) of compound **TA2**

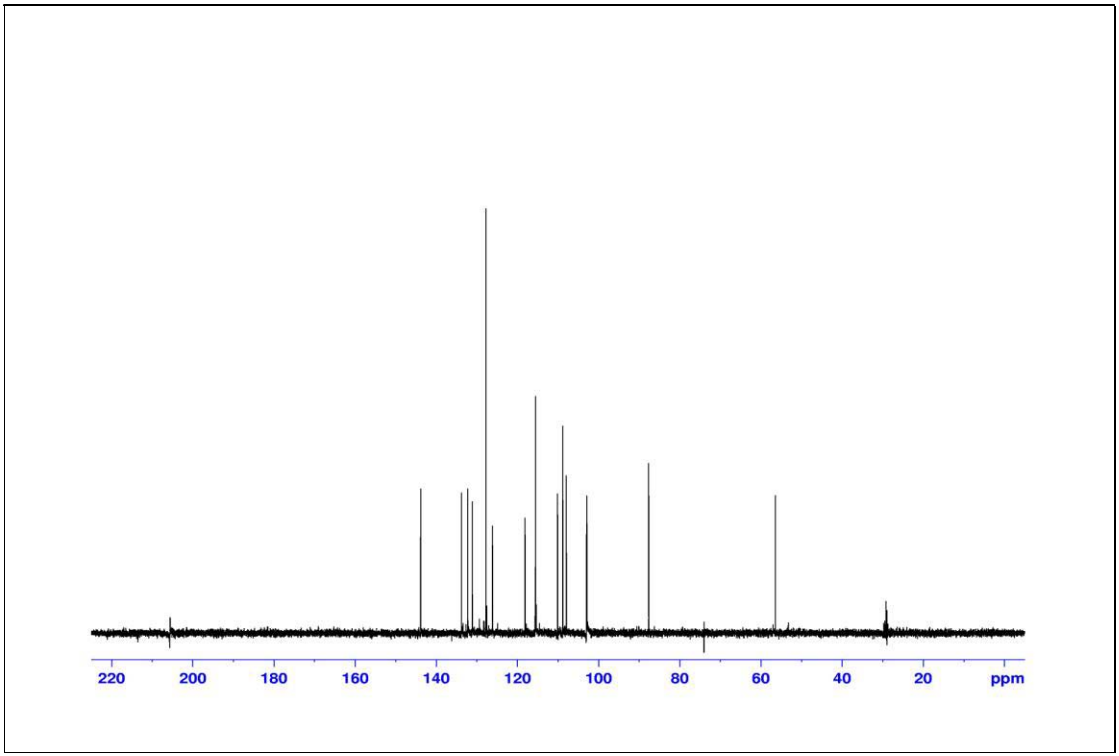


Figure 16 DEPT 90 (Acetone-d_6) of compound **TA2**

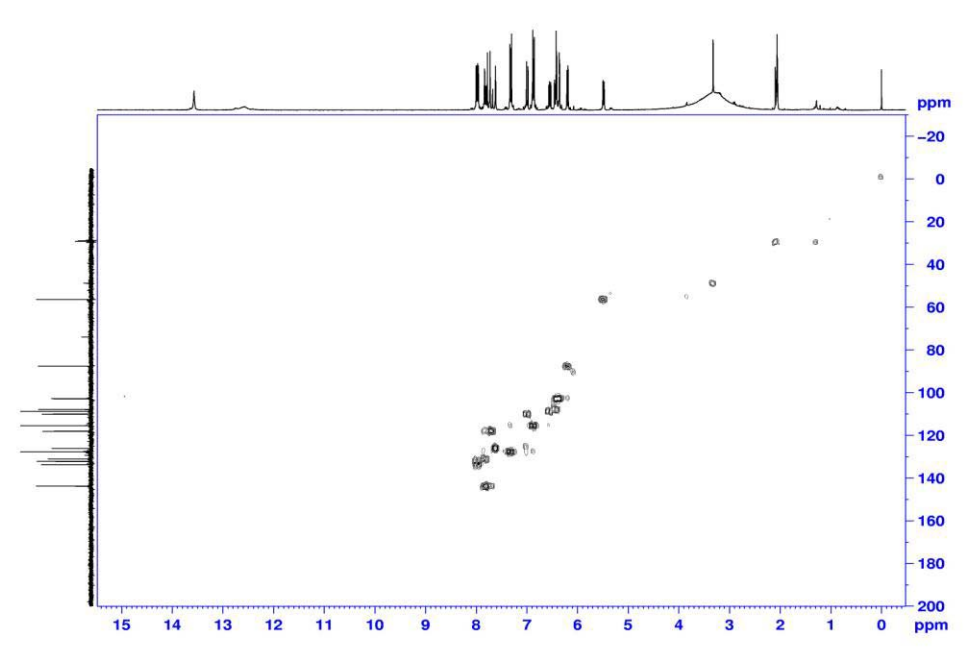


Figure 17 2D HMQC (Acetone-d_6) of compound **TA2**

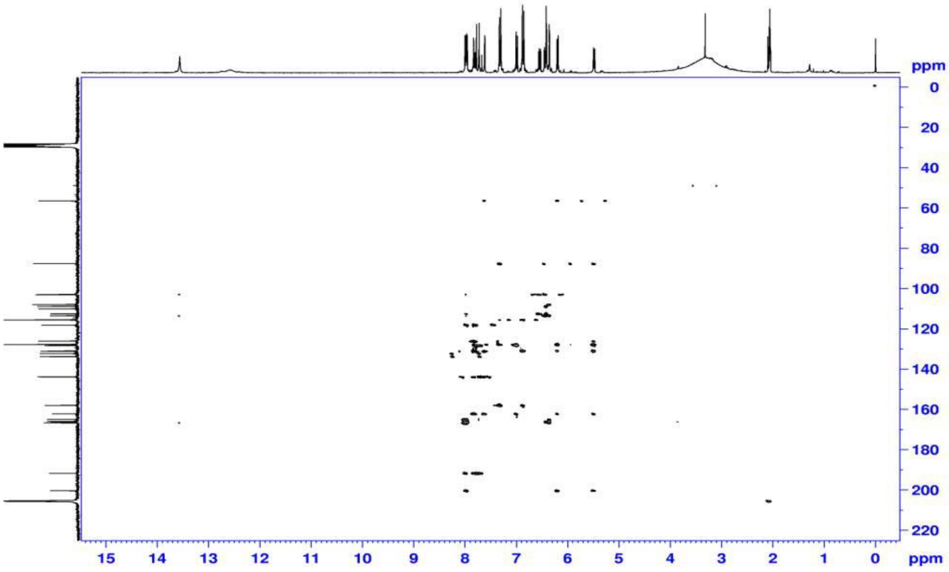


Figure 18 2D HMBC (Acetone-d_6) of compound **TA2**

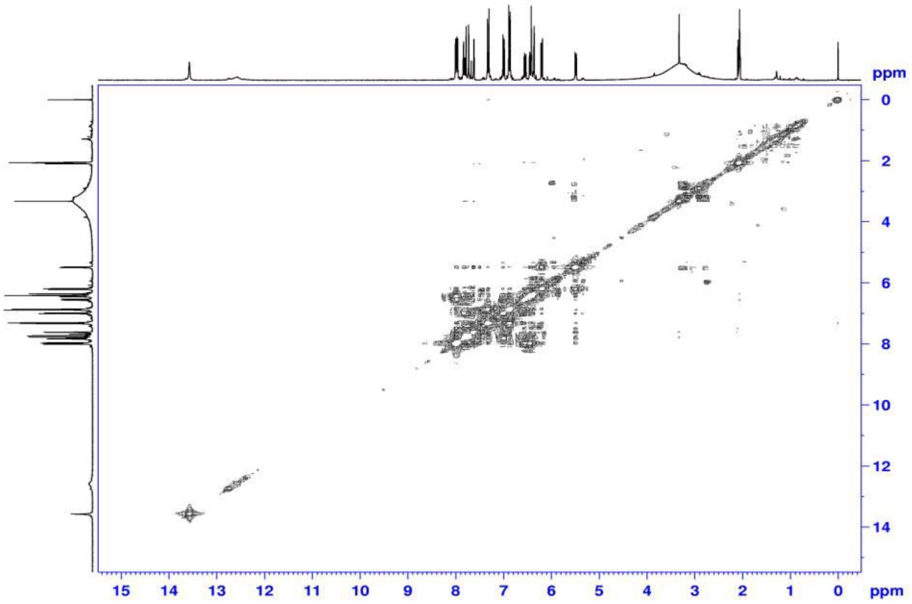


Figure 19 2D COSY (Acetone-d_6) of compound **TA2**

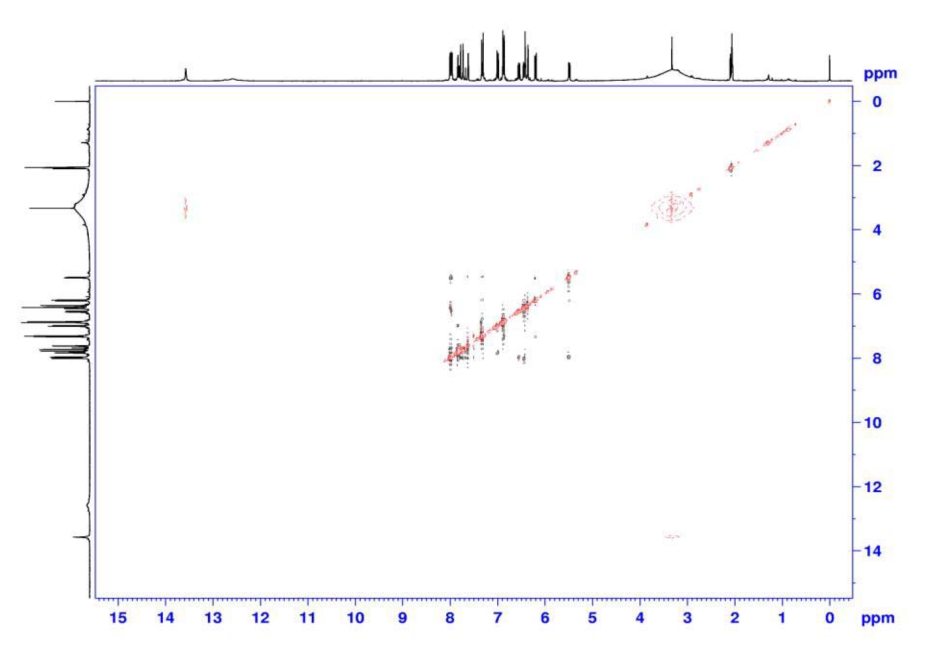


Figure 20 2D NOESY (Acetone-d_6) of compound **TA2**

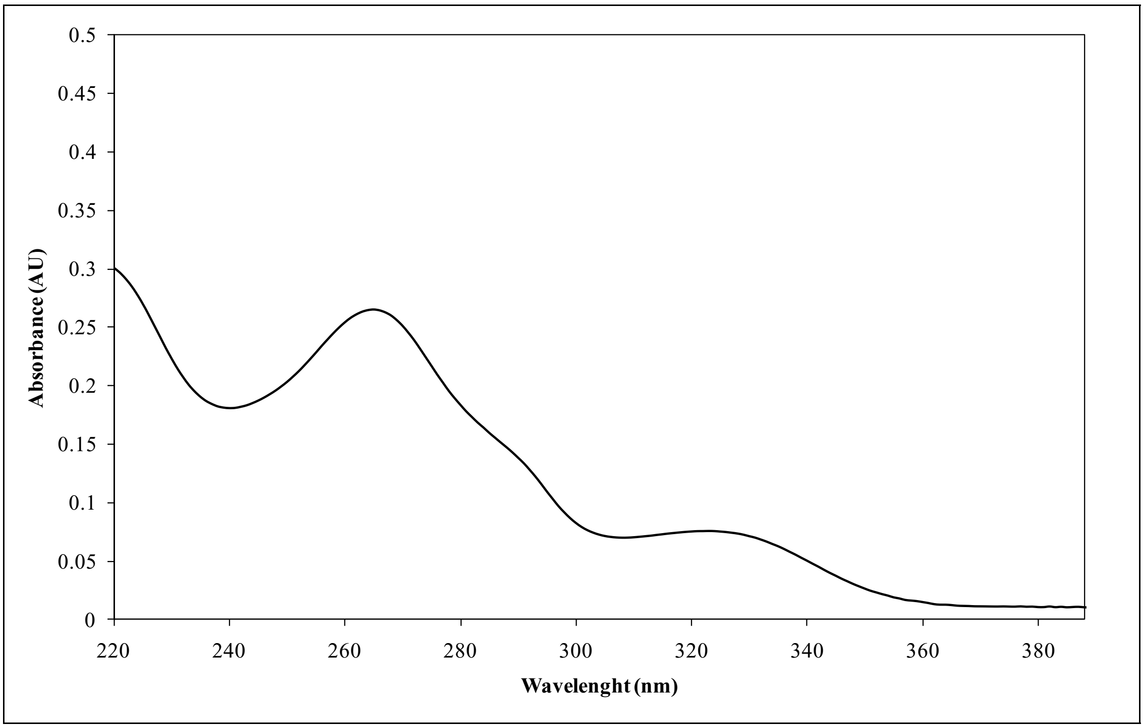


Figure 21 UV (MeOH) spectrum of compound **TA3**

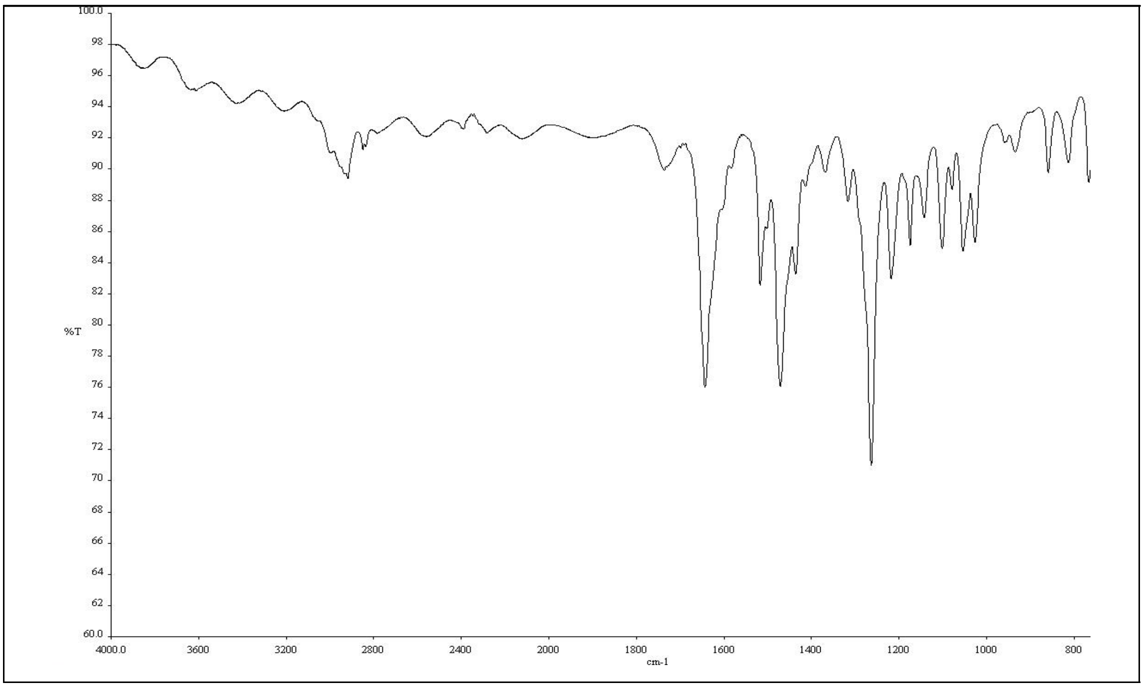


Figure 22 IR (neat) spectrum of compound **TA3**

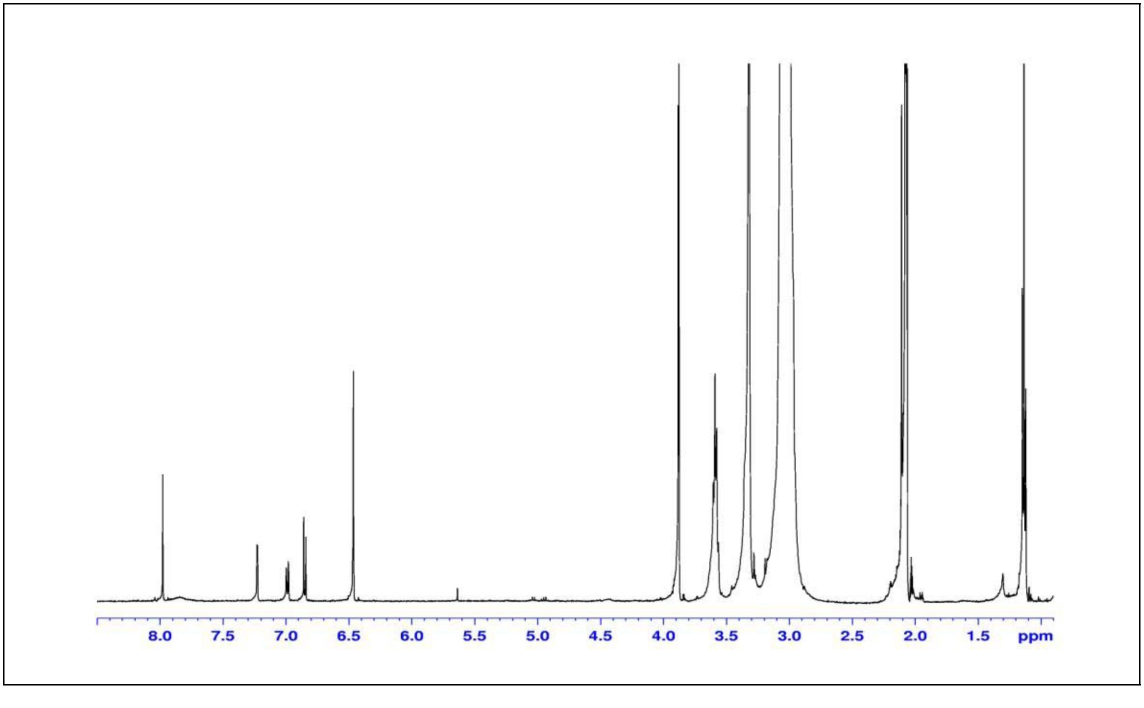


Figure 23 ^{1}H NMR (500 MHz) (Acetone-d_6) of compound **TA3**

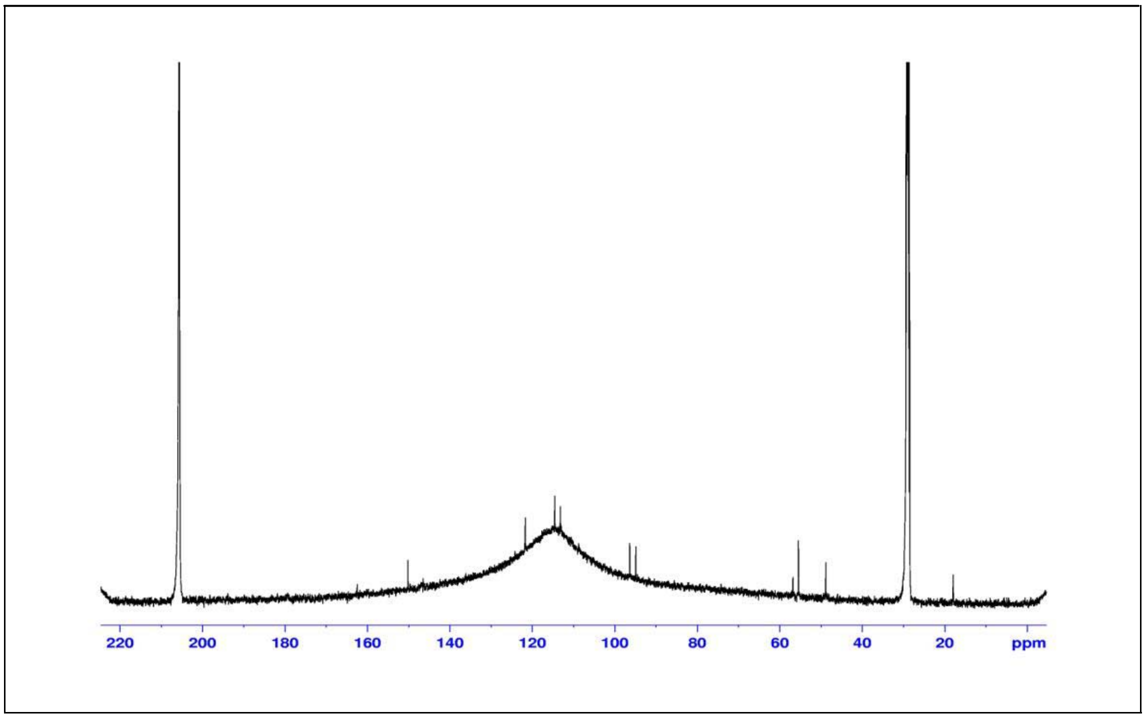


Figure 24 ^{13}C NMR (125 MHz) (Acetone-d_6) of compound **TA3**

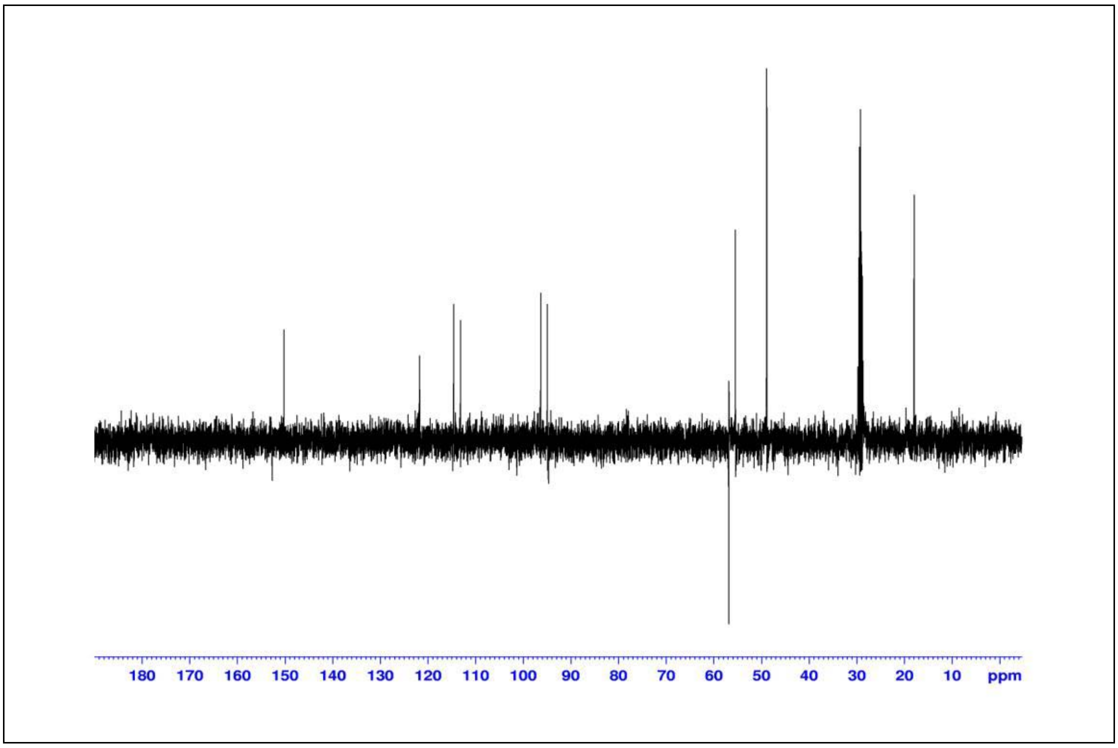


Figure 25 DEPT 135 (Acetone-d_6) of compound **TA3**

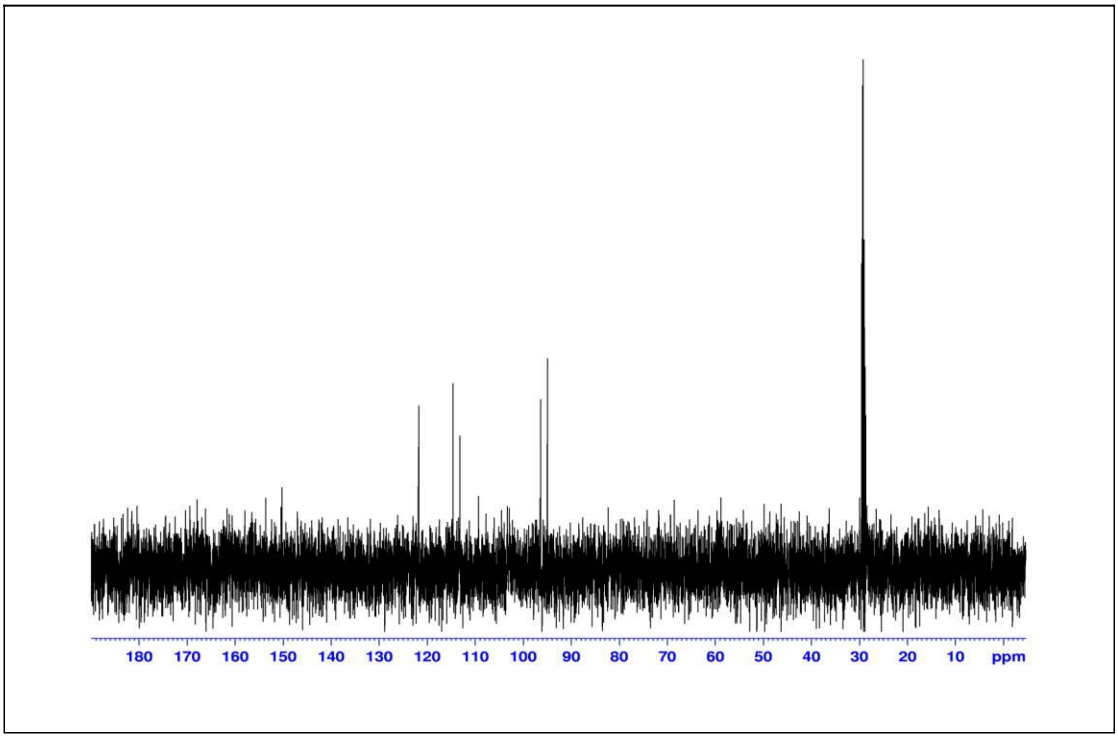


Figure 26 DEPT 90 (Acetone-d_6) of compound **TA3**

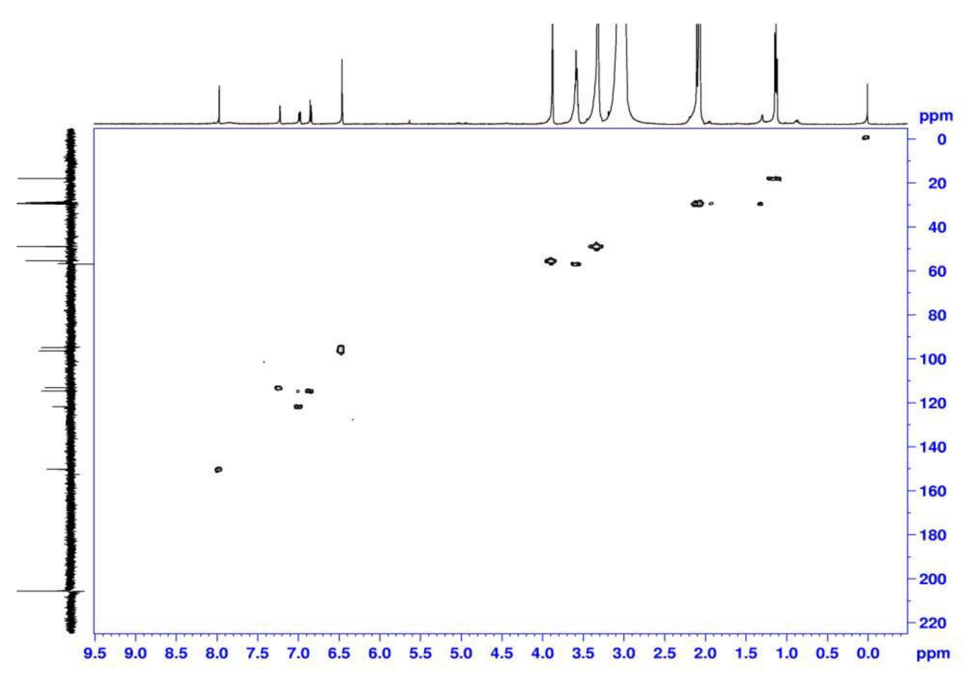


Figure 27 2D HMQC (Acetone-d_6) of compound **TA3**

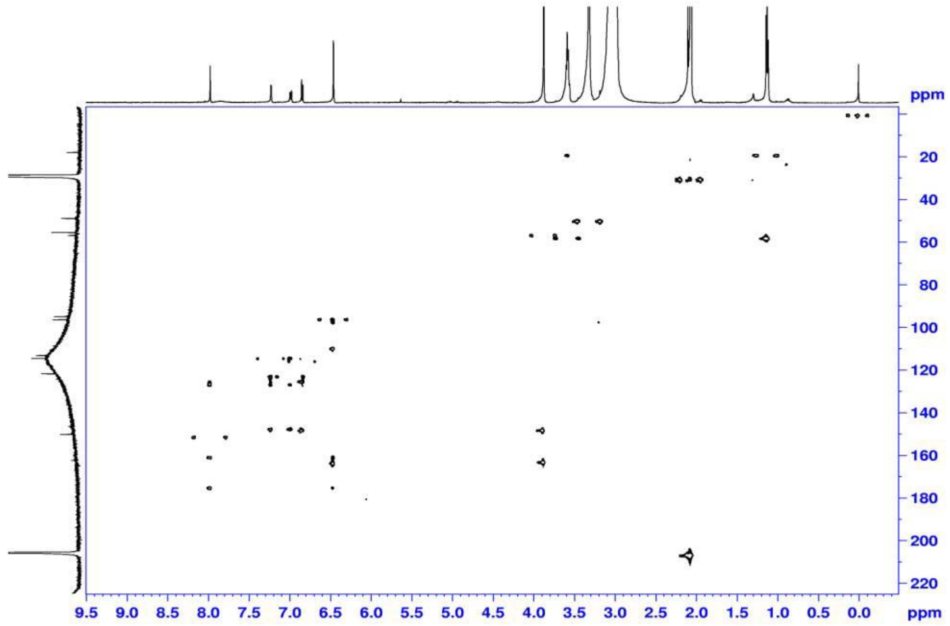


Figure 28 2D HMBC (Acetone-d_6) of compound **TA3**

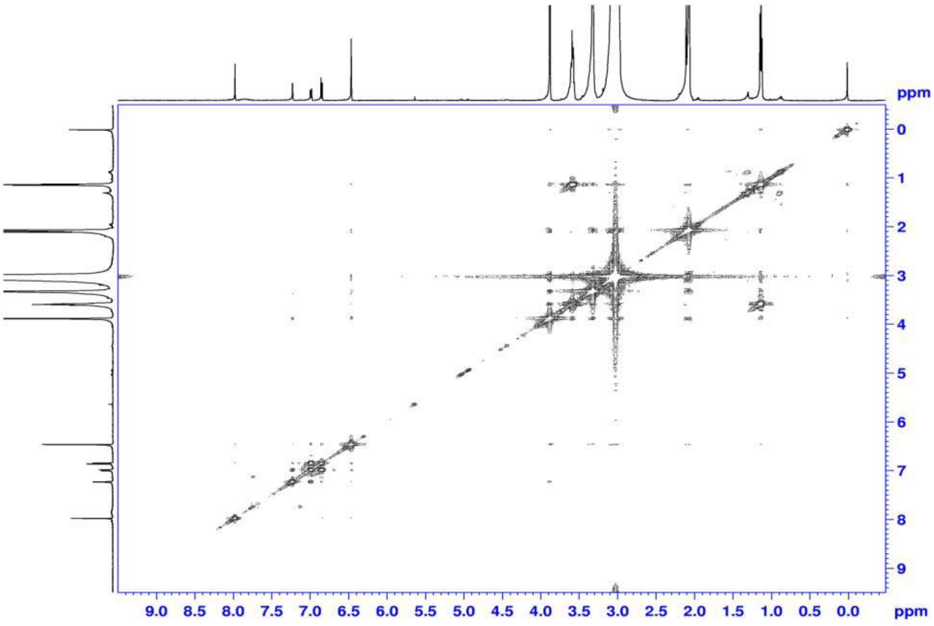


Figure 29 2D COSY (Acetone-d_6) of compound **TA3**

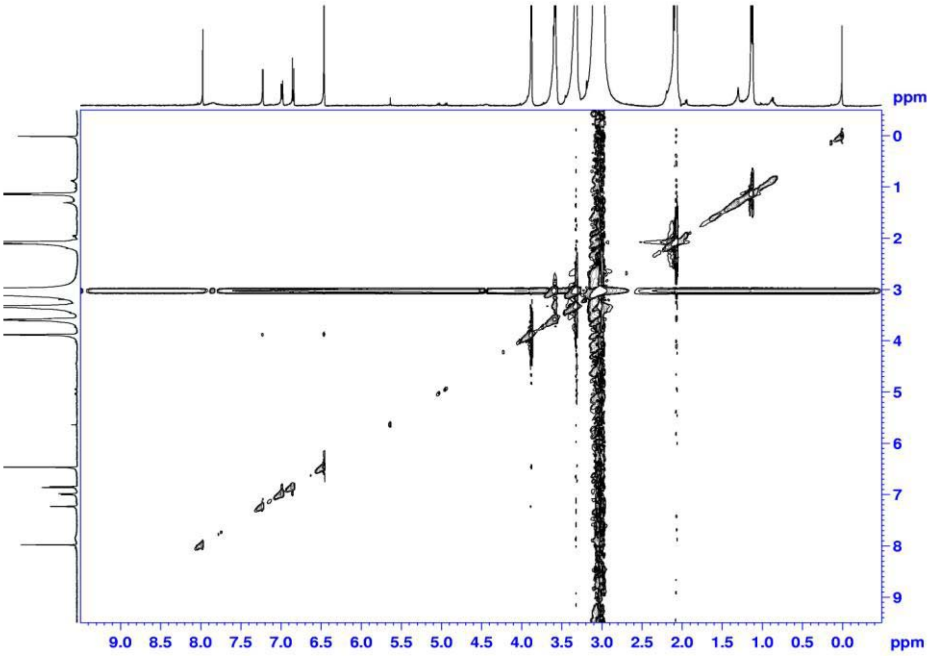


Figure 30 2D NOESY (Acetone-d_6) of compound **TA3**

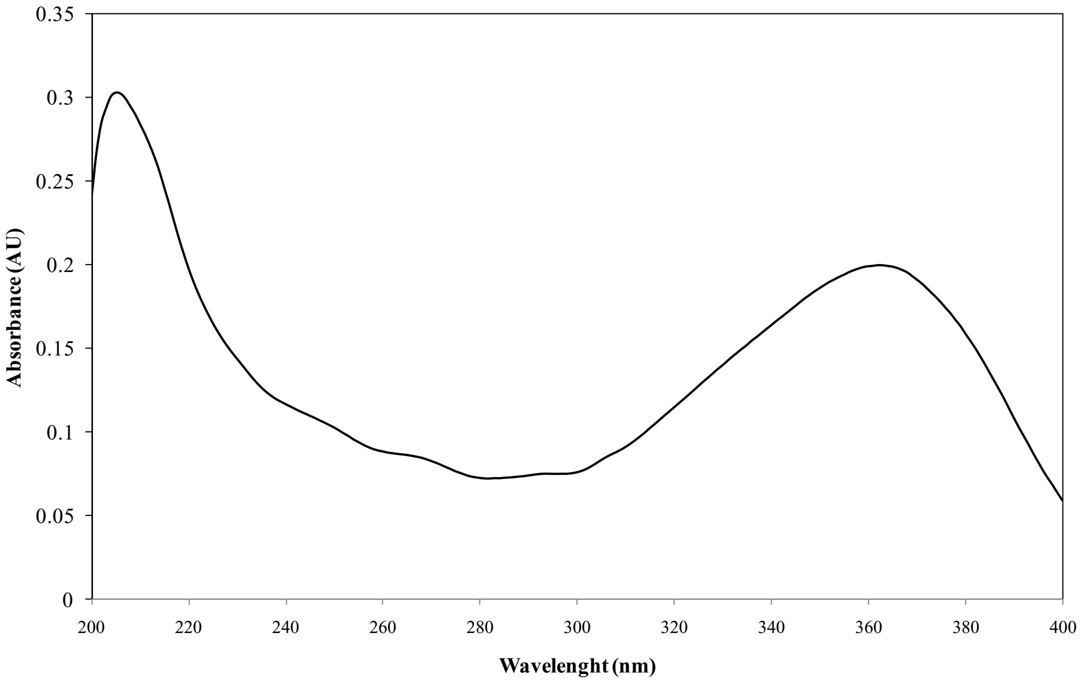


Figure 31 UV (MeOH) spectrum of compound **RD1**

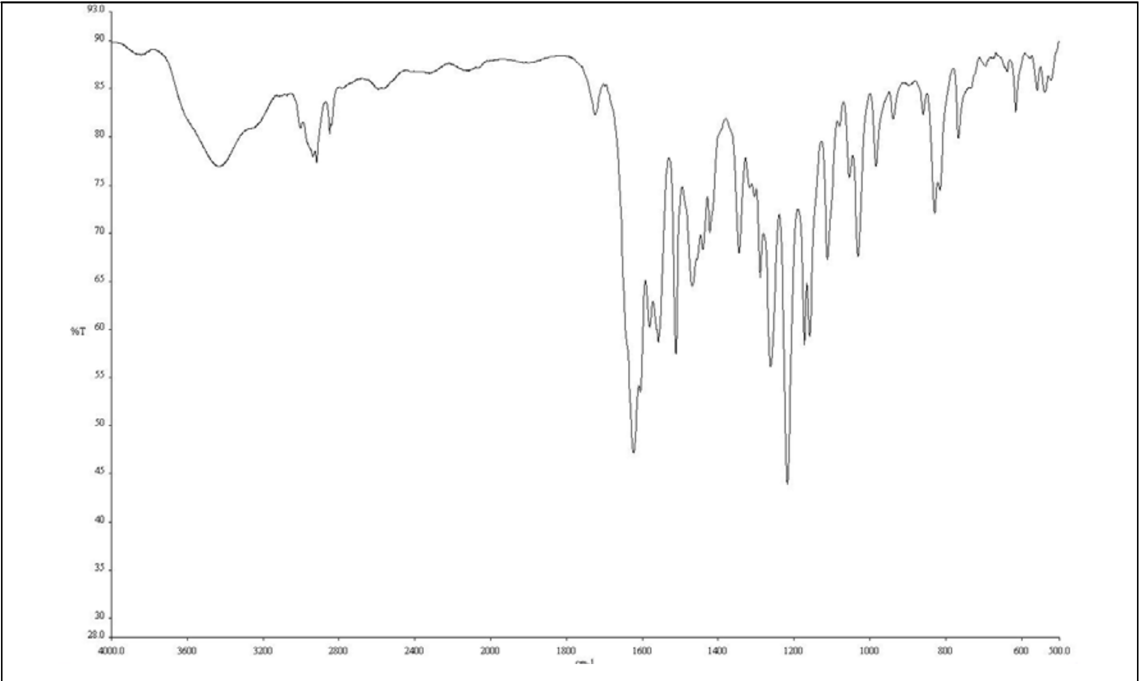


Figure 32 IR (neat) spectrum of compound **RD1**

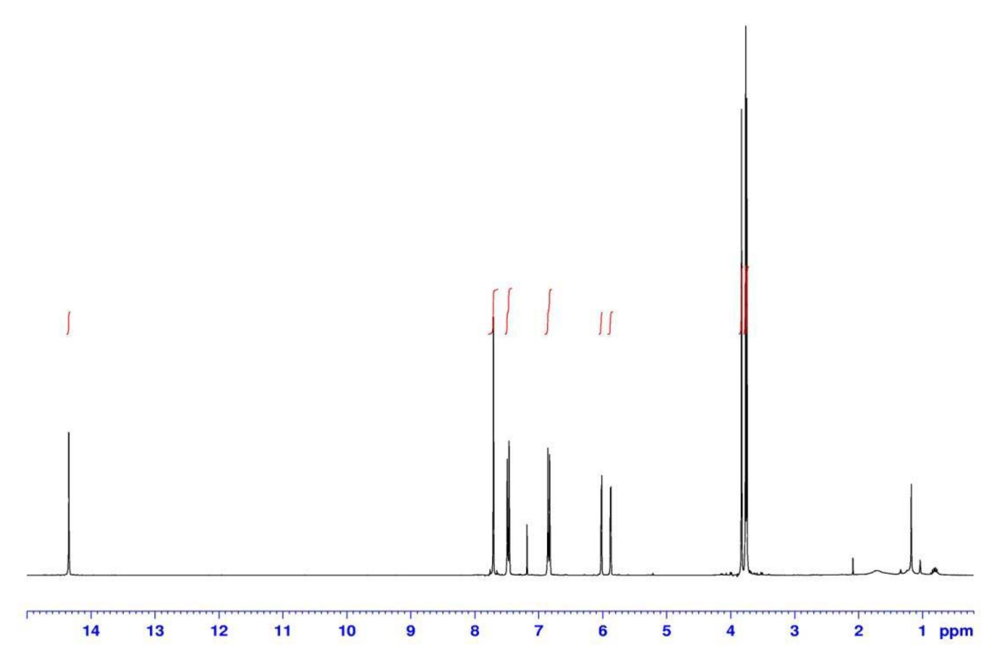


Figure 33 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RD1**

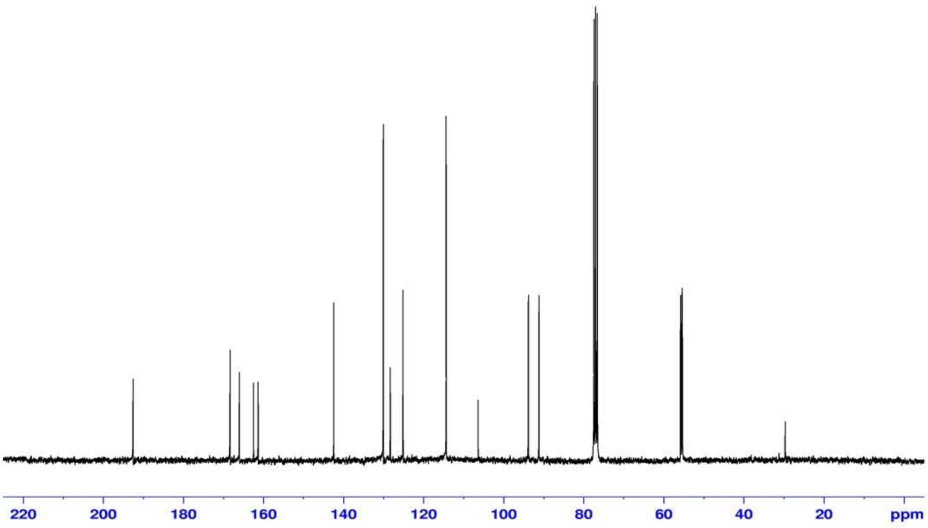


Figure 34 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD1**

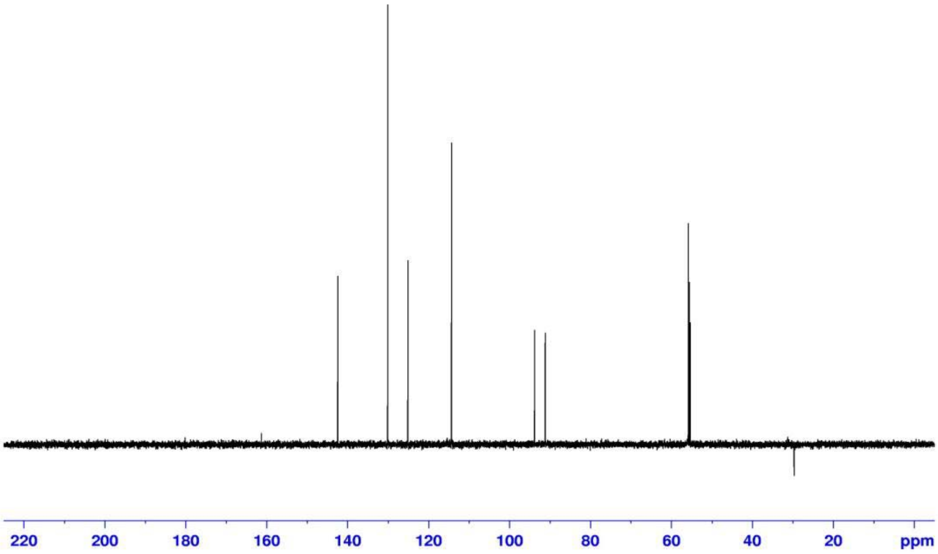


Figure 35 DEPT 135 ($CDCl_3$) of compound **RD1**

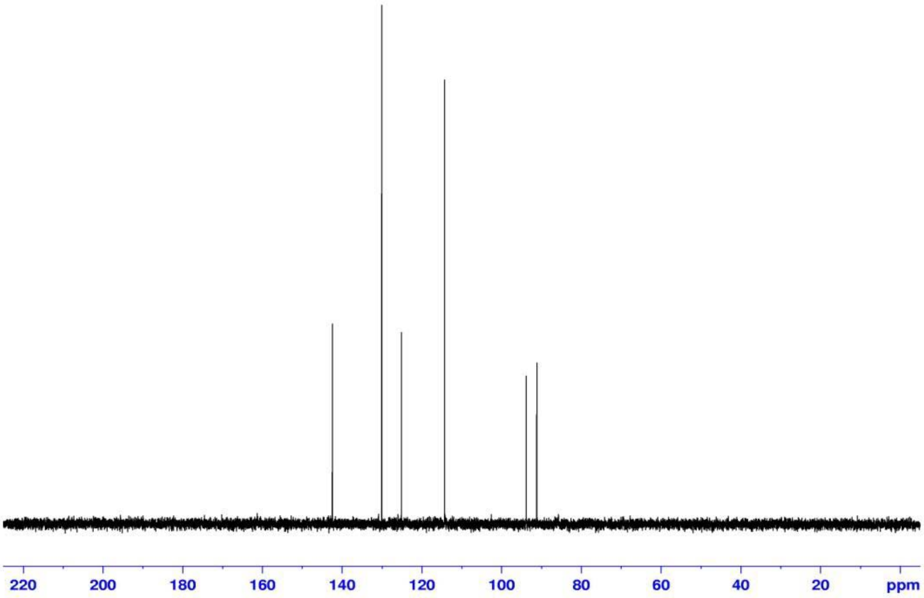


Figure 36 DEPT 90 (CDCl$_3$) of compound **RD1**

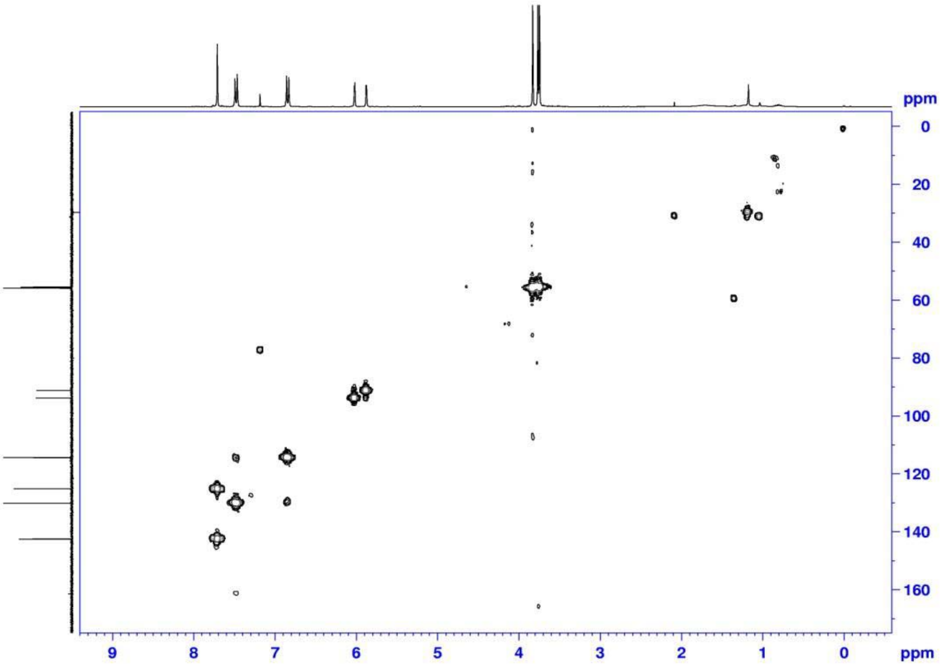


Figure 37 2D HMQC (CDCl$_3$) of compound **RD1**

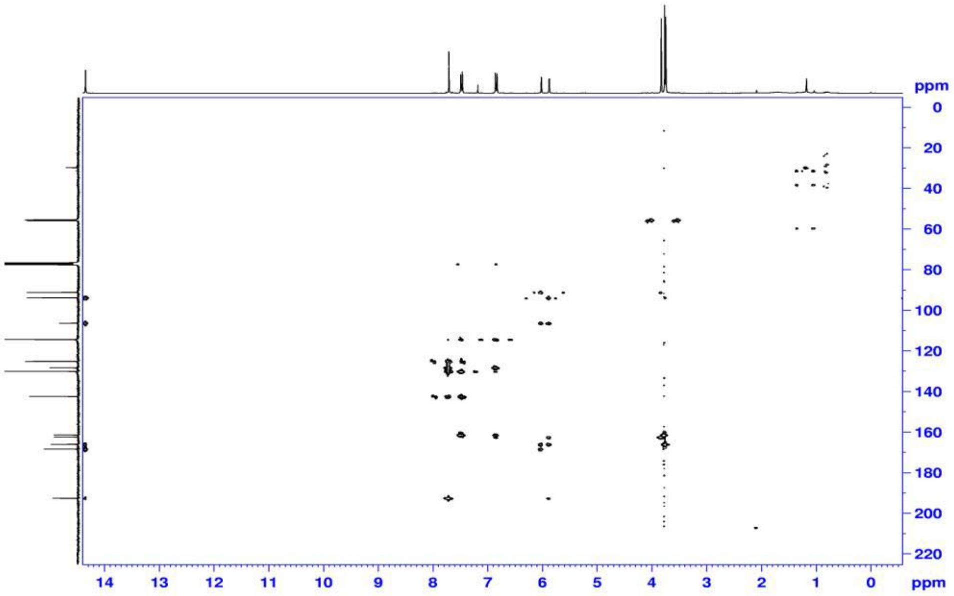


Figure 38 2D HMBC ($CDCl_3$) of compound **RD1**

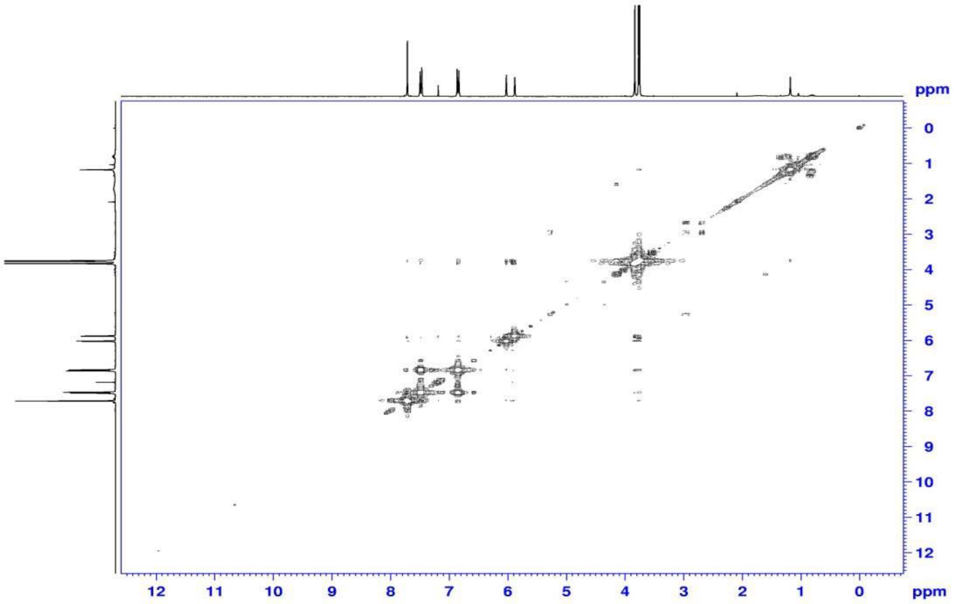


Figure 39 2D COSY ($CDCl_3$) of compound **RD1**

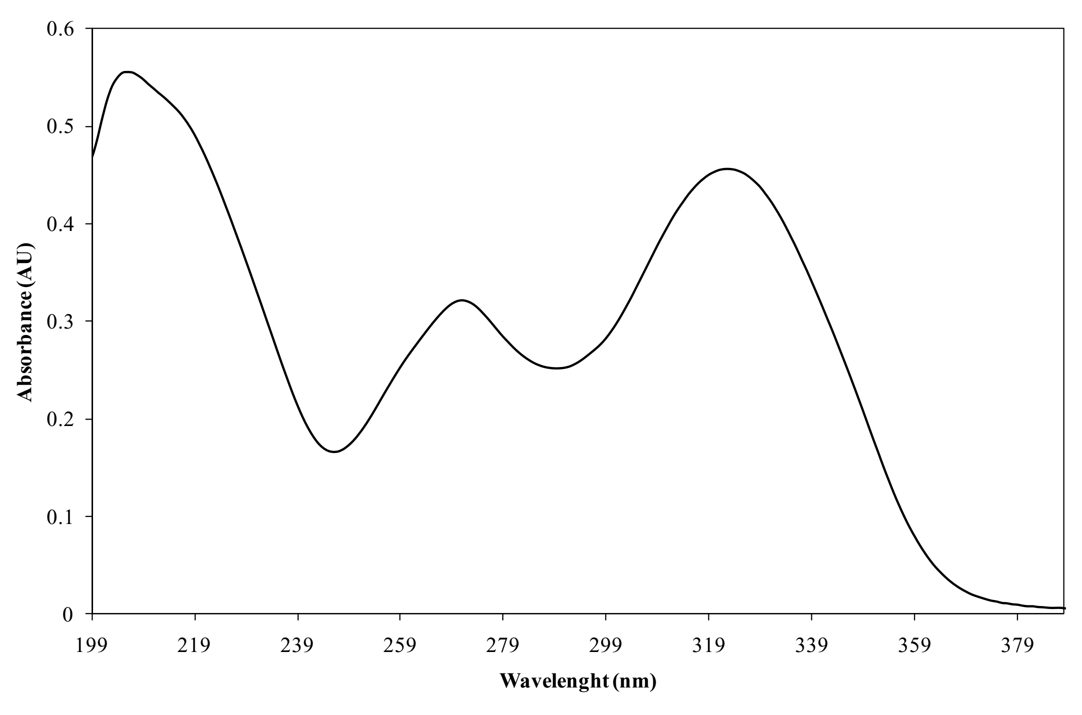


Figure 40 UV (MeOH) spectrum of compound **RD2**

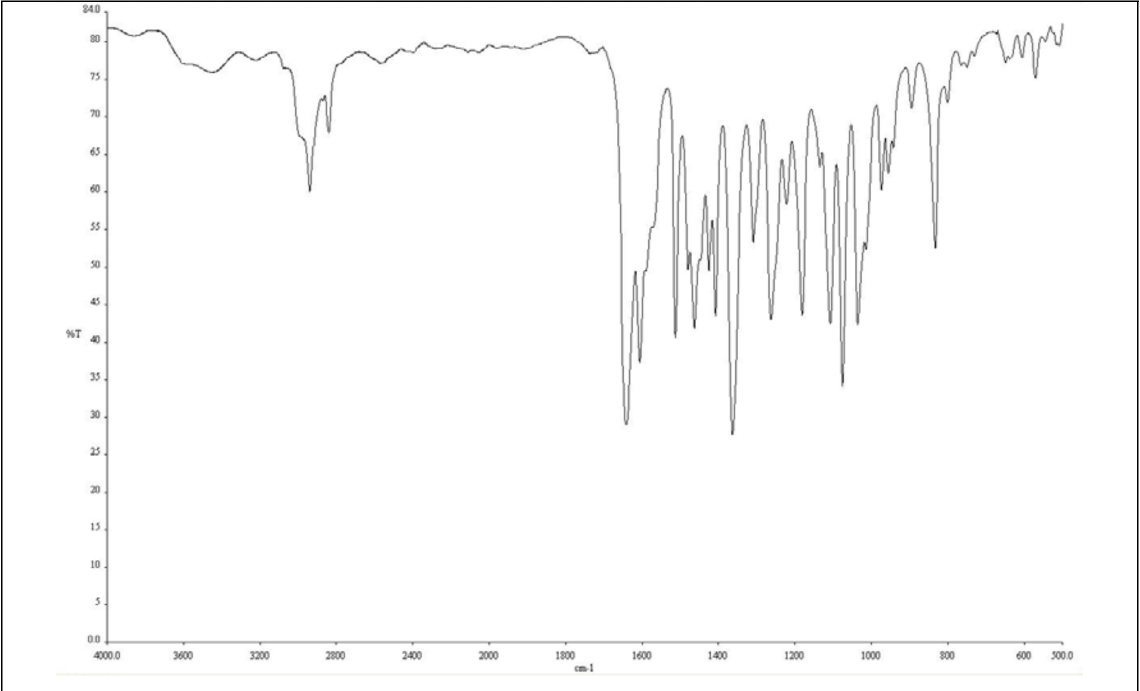


Figure 41 IR (neat) spectrum of compound **RD2**

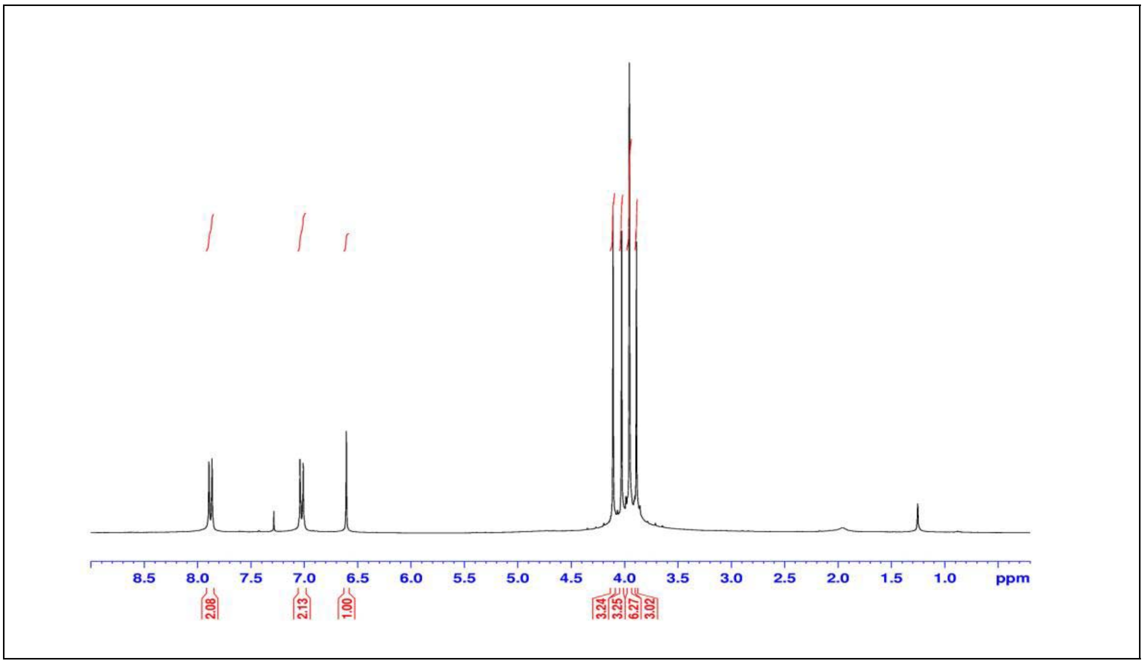


Figure 42 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RD2**

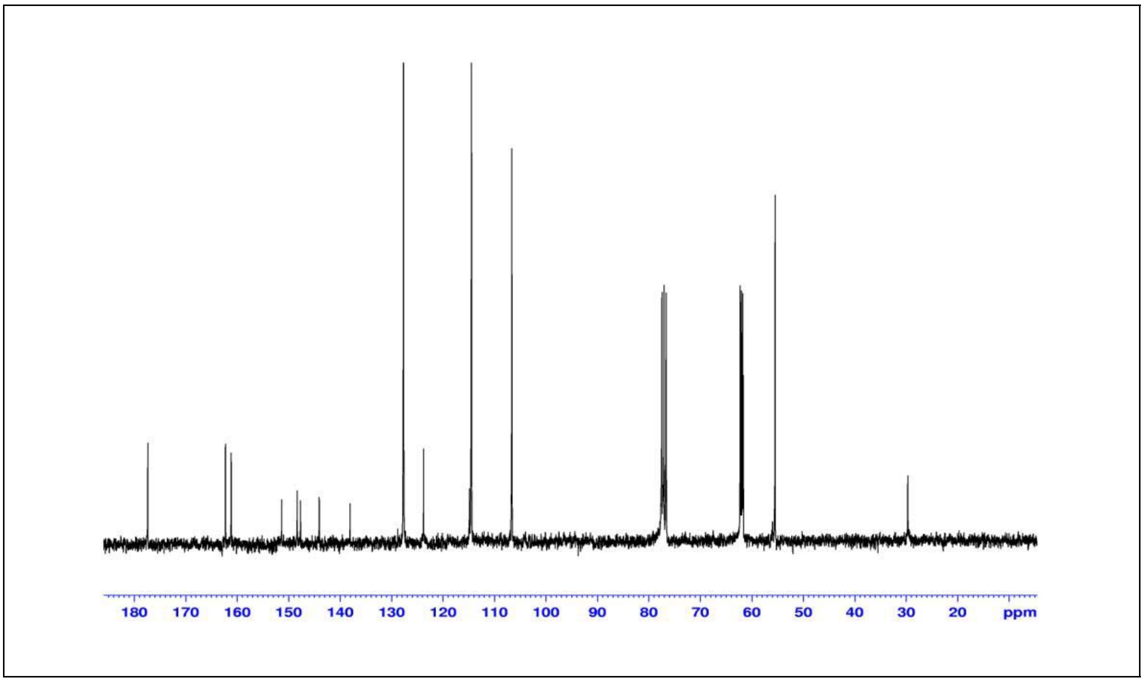


Figure 43 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD2**

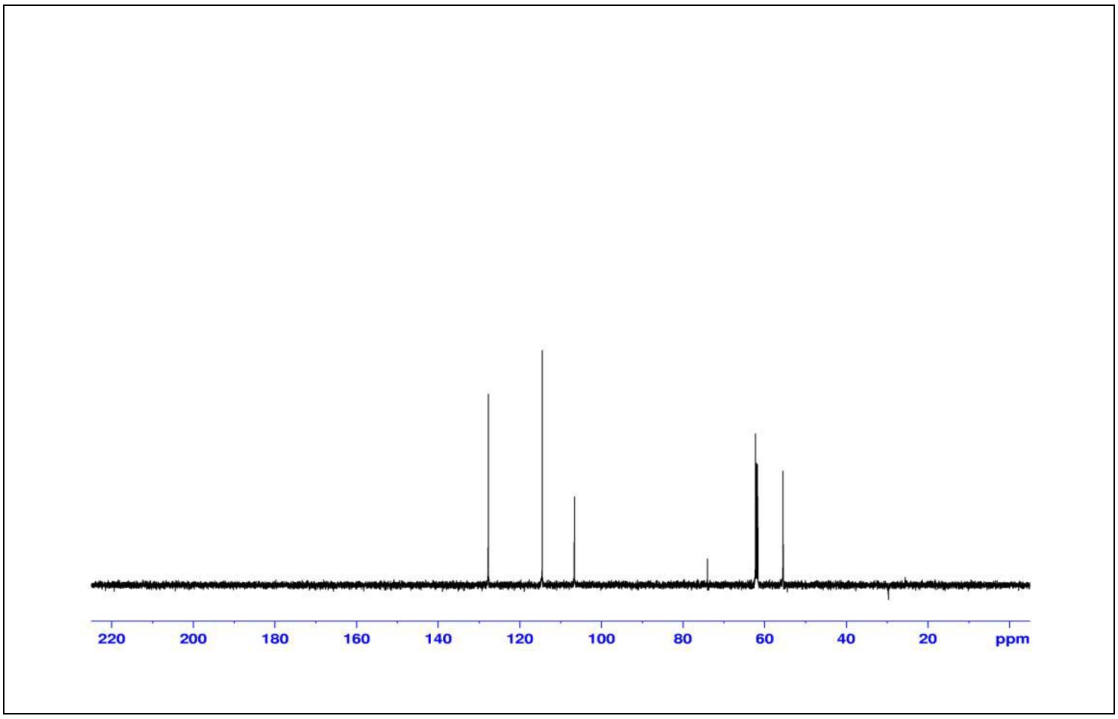


Figure 44 DEPT 135 (CDCl$_3$) of compound **RD2**

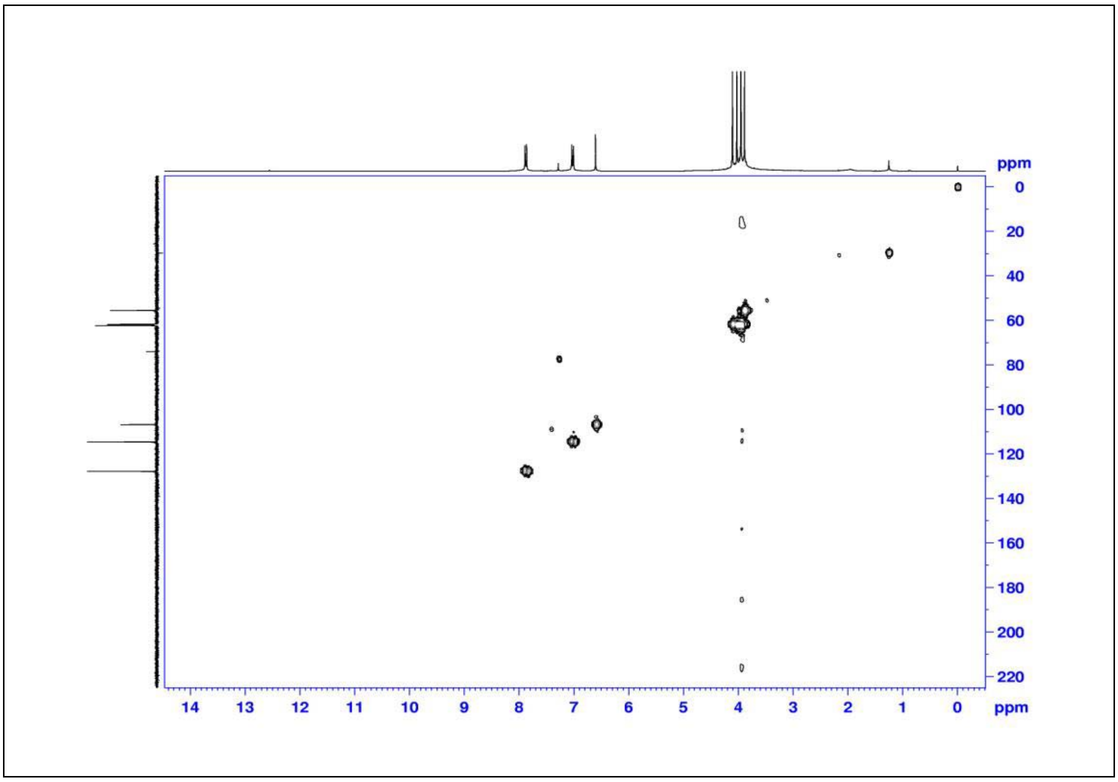


Figure 45 2D HMQC (CDCl$_3$) of compound **RD2**

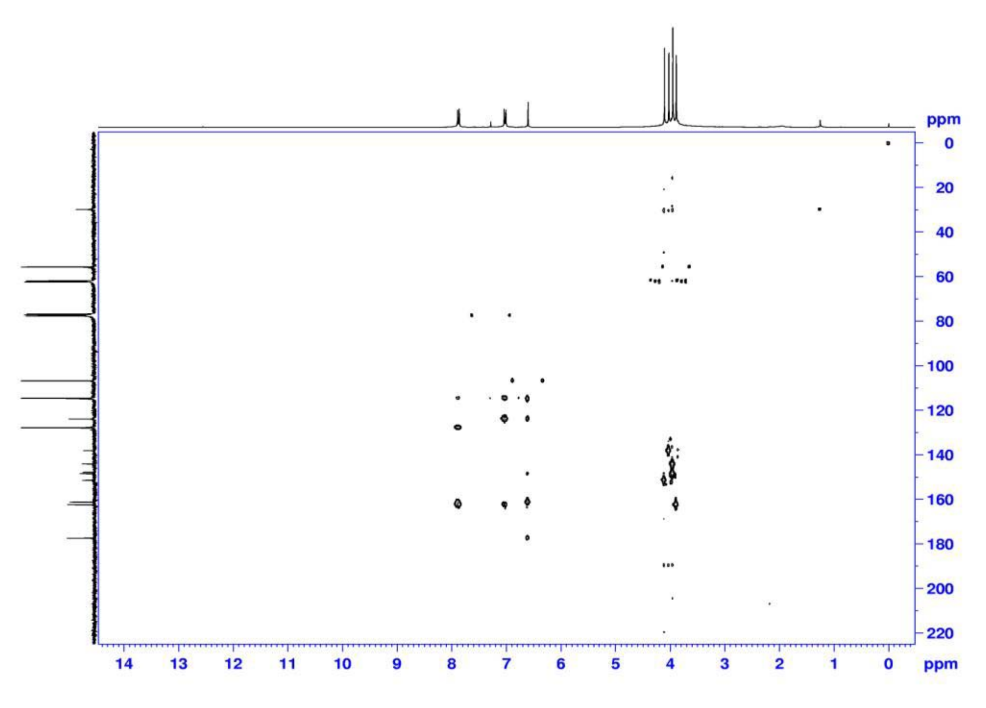


Figure 46 2D HMBC ($CDCl_3$) of compound **RD2**

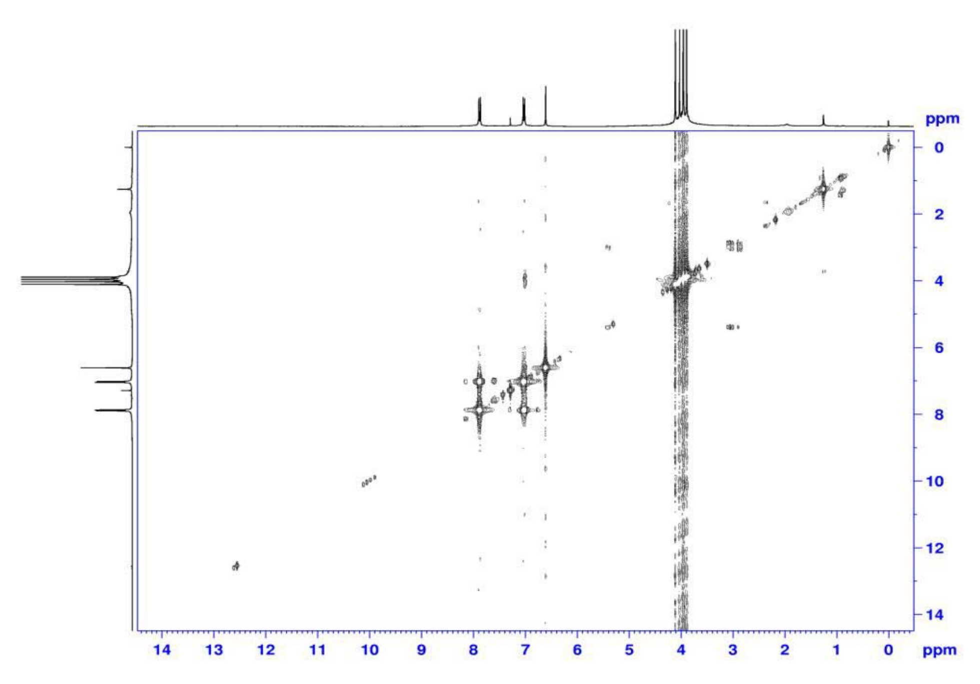


Figure 47 2D COSY ($CDCl_3$) of compound **RD2**

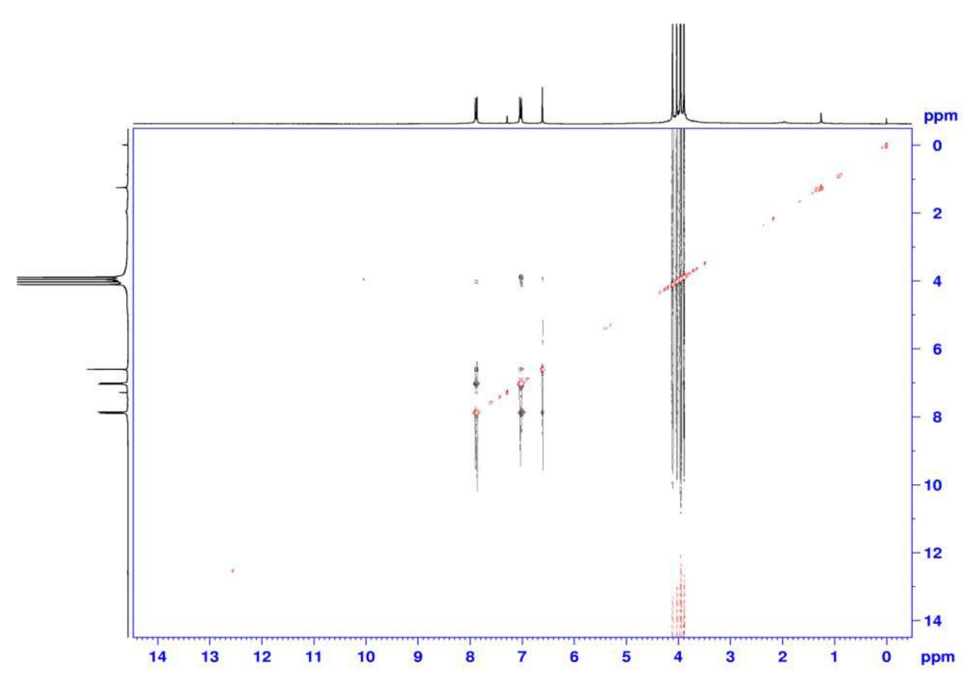


Figure 48 2D NOESY ($CDCl_3$) of compound **RD2**

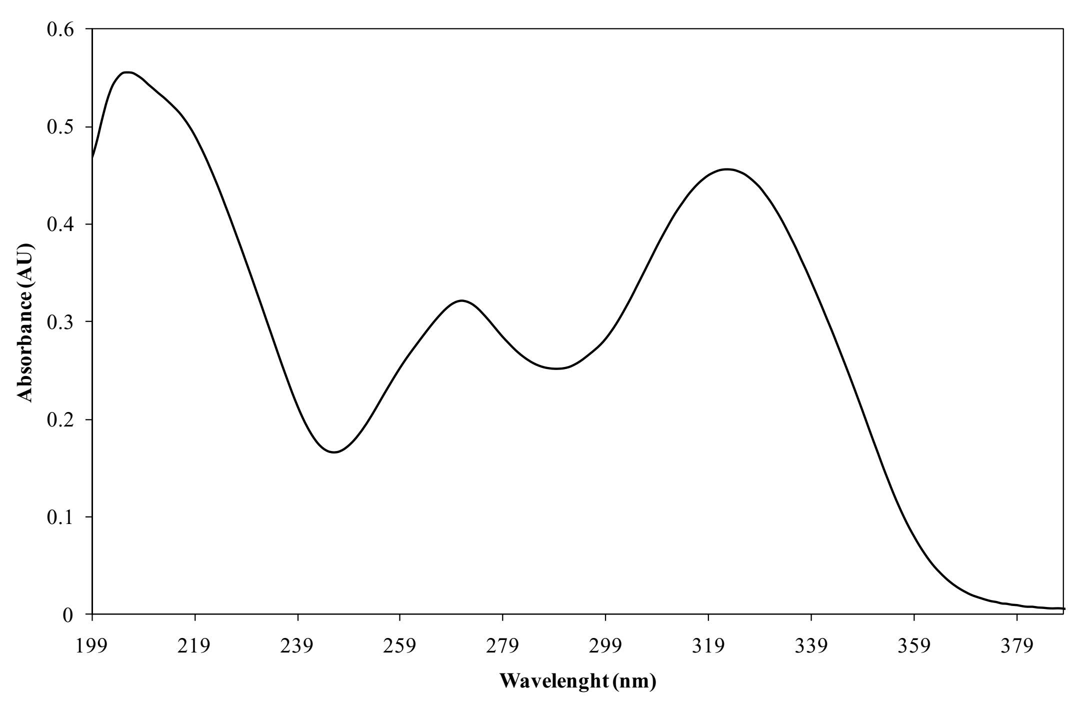


Figure 49 UV (MeOH) spectrum of compound **RD3**

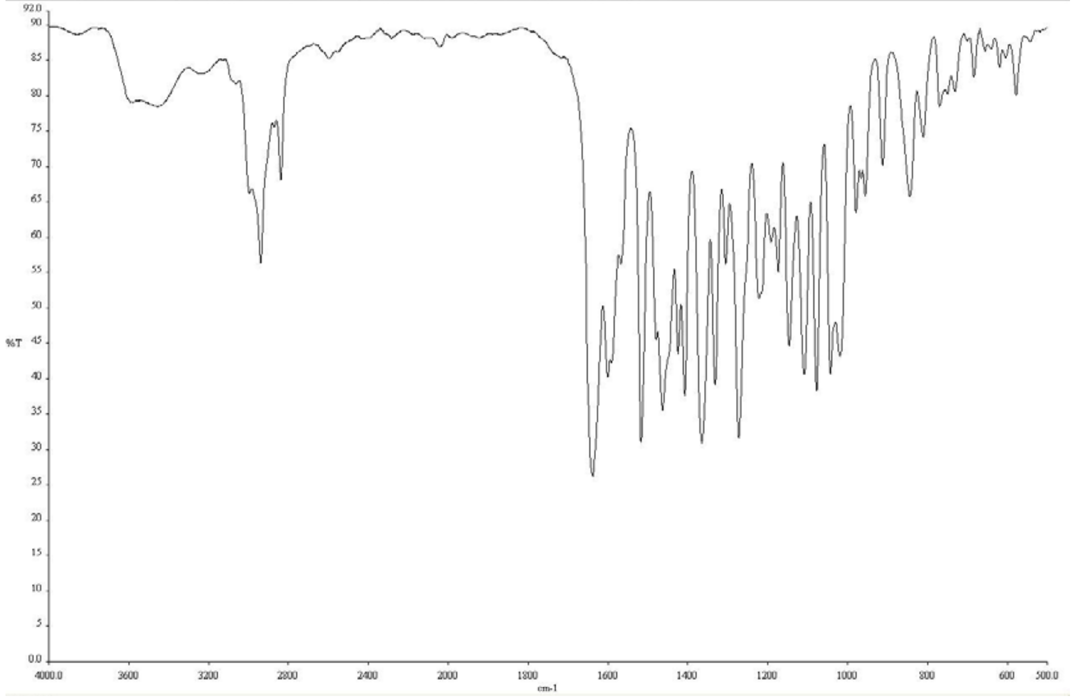


Figure 50 IR (neat) spectrum of compound **RD3**

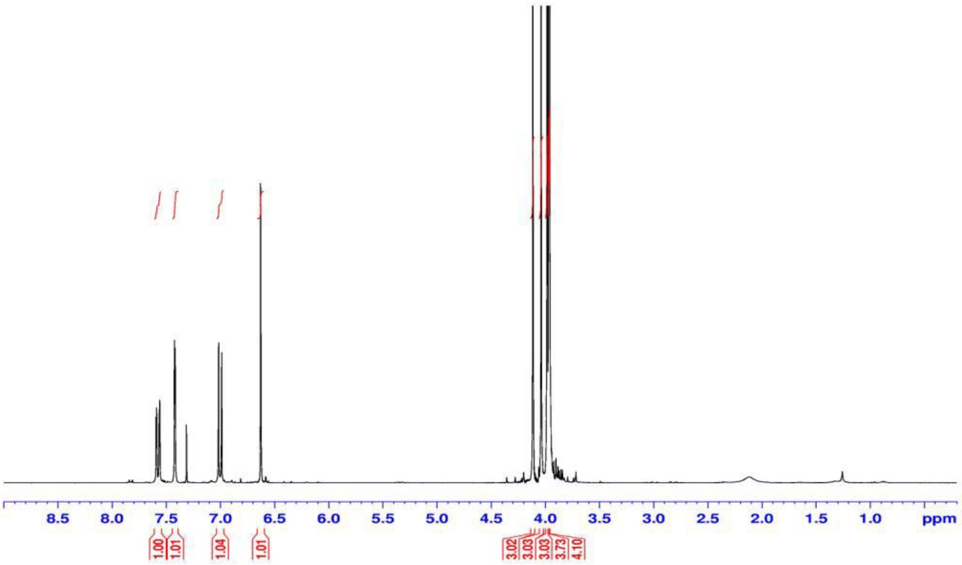


Figure 51 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **RD3**

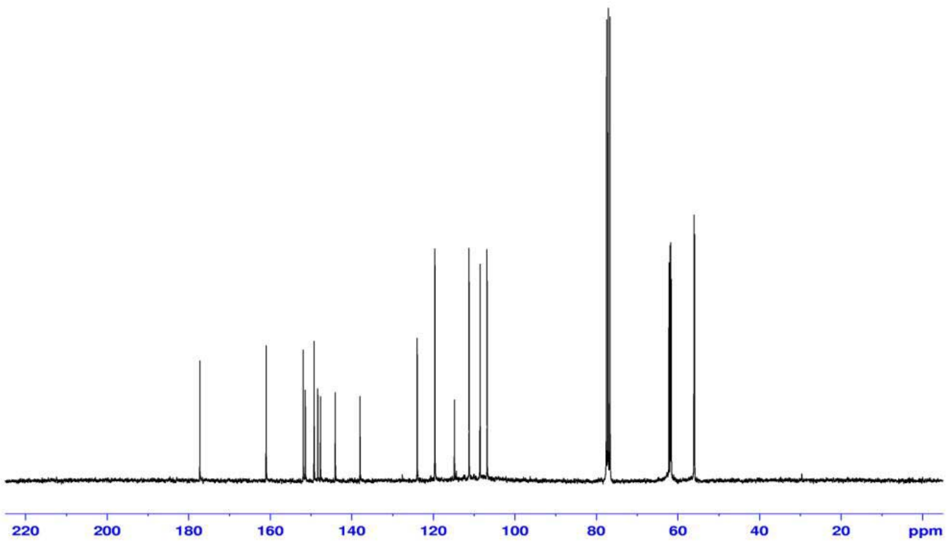


Figure 52 ^{13}C NMR (75 MHz) (CDCl$_3$) of compound **RD3**

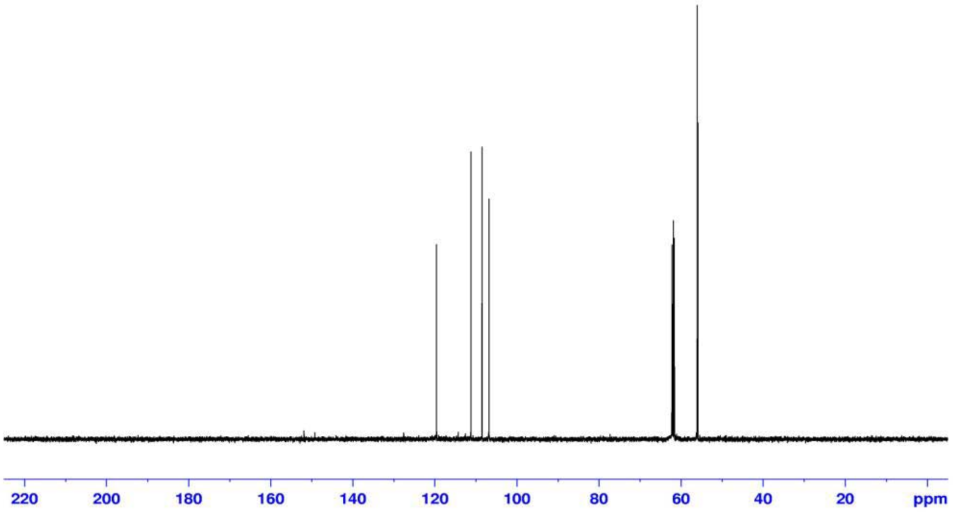


Figure 53 DEPT 135 (CDCl$_3$) of compound **RD3**

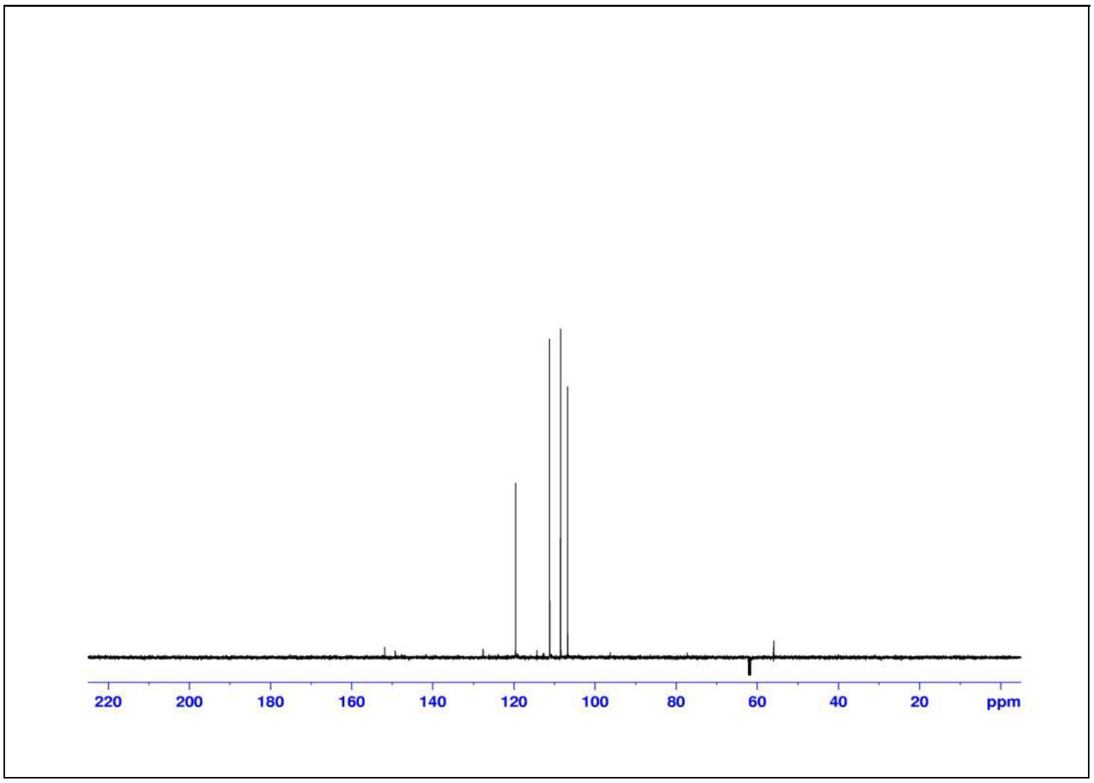


Figure 54 DEPT 90 (CDCl$_3$) of compound **RD3**

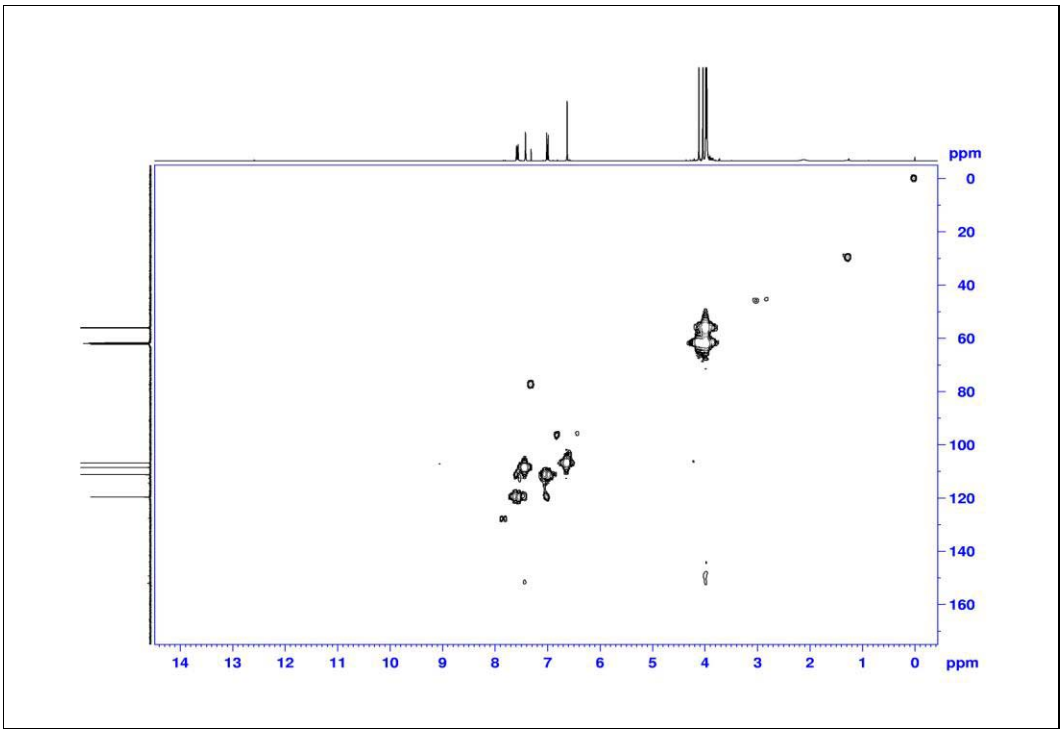


Figure 55 2D HMQC (CDCl$_3$) of compound **RD3**

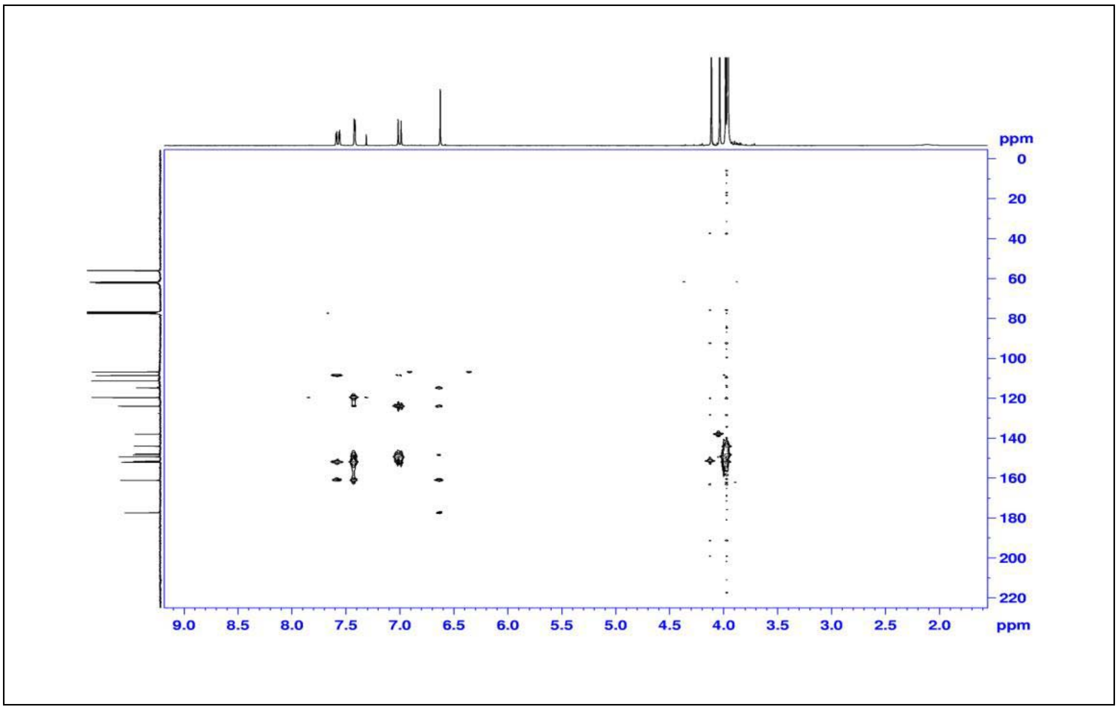


Figure 56 2D HMBC ($CDCl_3$) of compound **RD3**

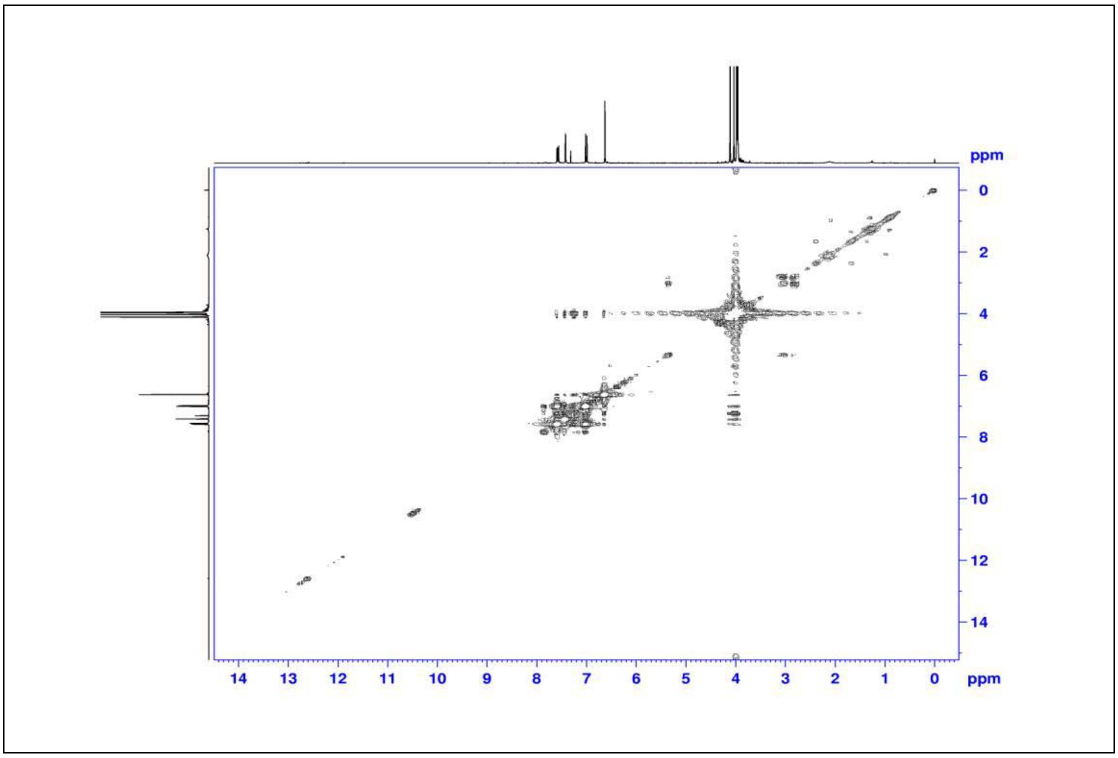


Figure 57 2D COSY ($CDCl_3$) of compound **RD3**

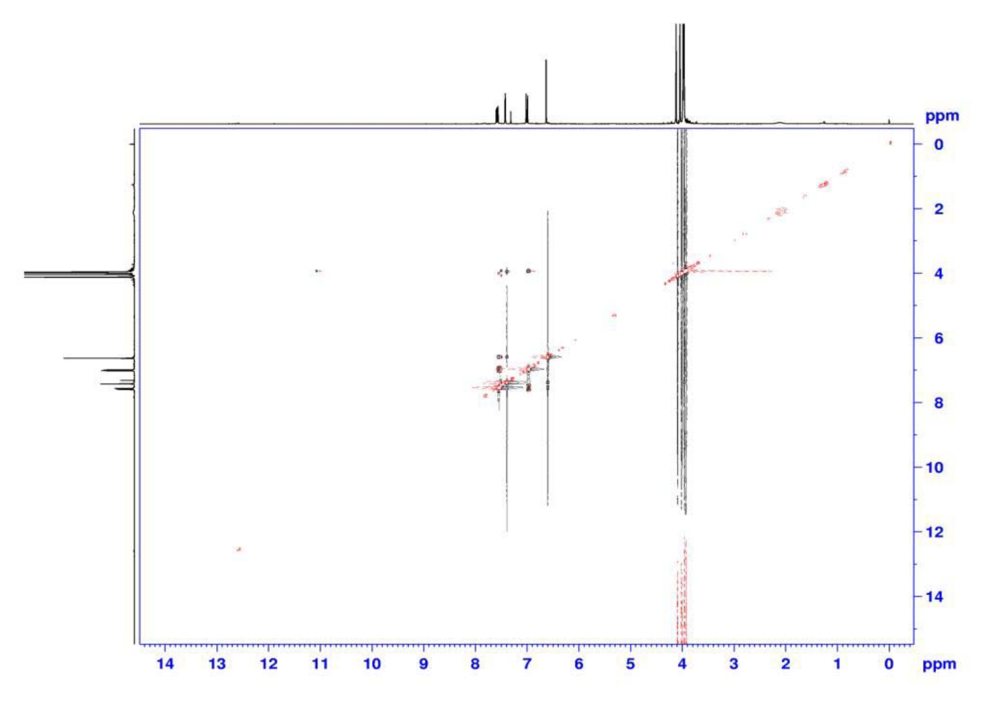


Figure 58 2D NOESY ($CDCl_3$) of compound **RD3**

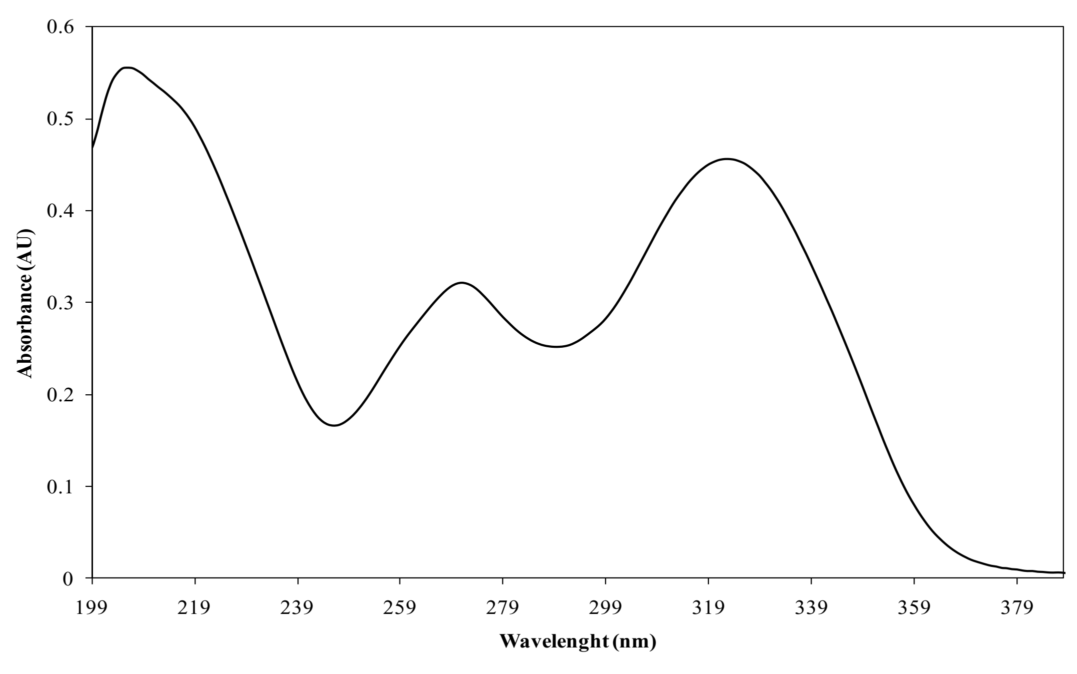


Figure 59 UV (MeOH) spectrum of compound **RD4**

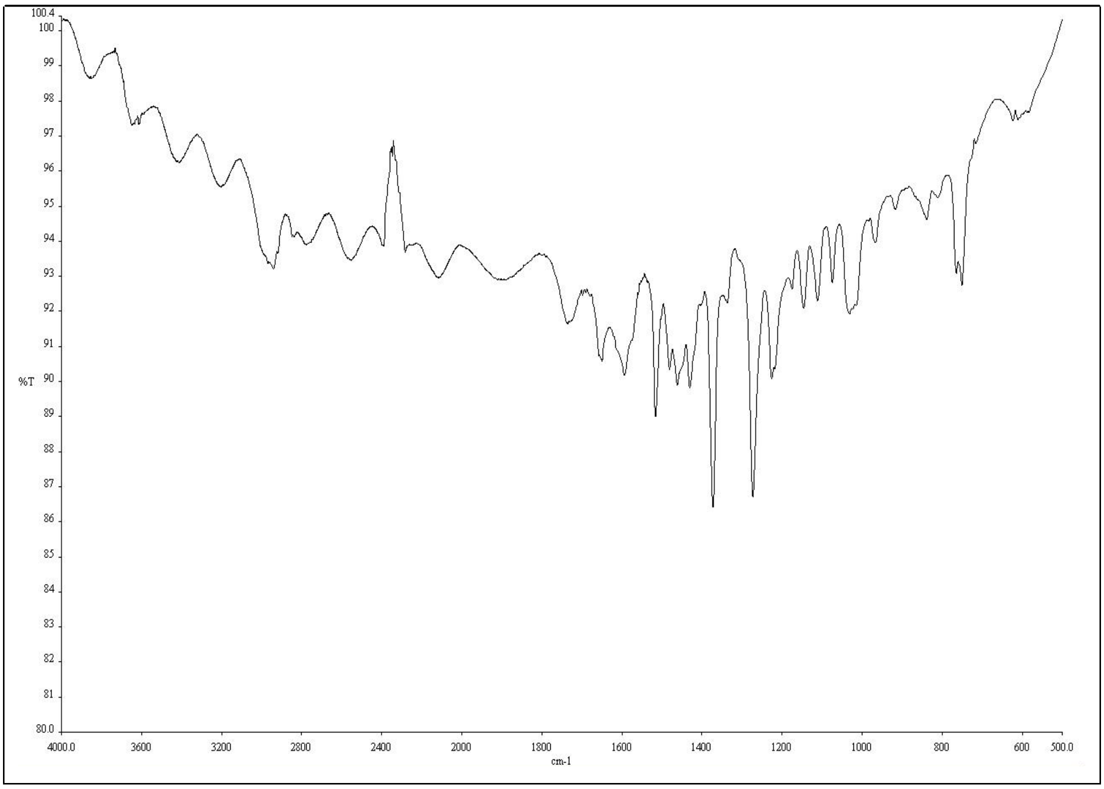


Figure 60 IR (neat) spectrum of compound **RD4**

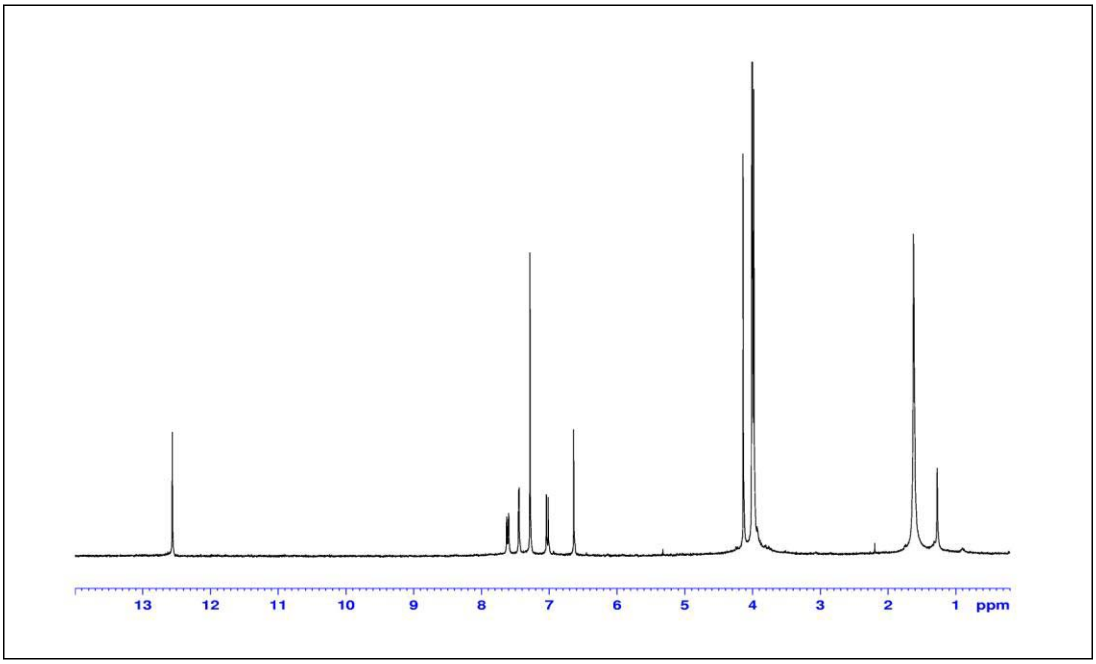


Figure 61 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RD4**

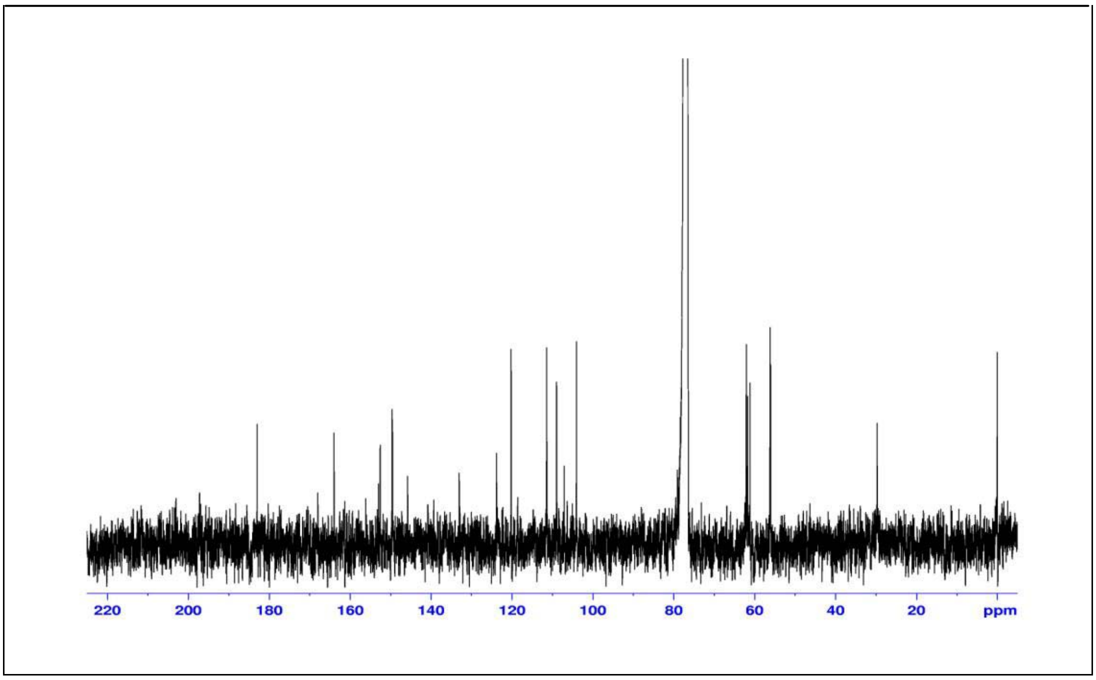


Figure 62 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD4**

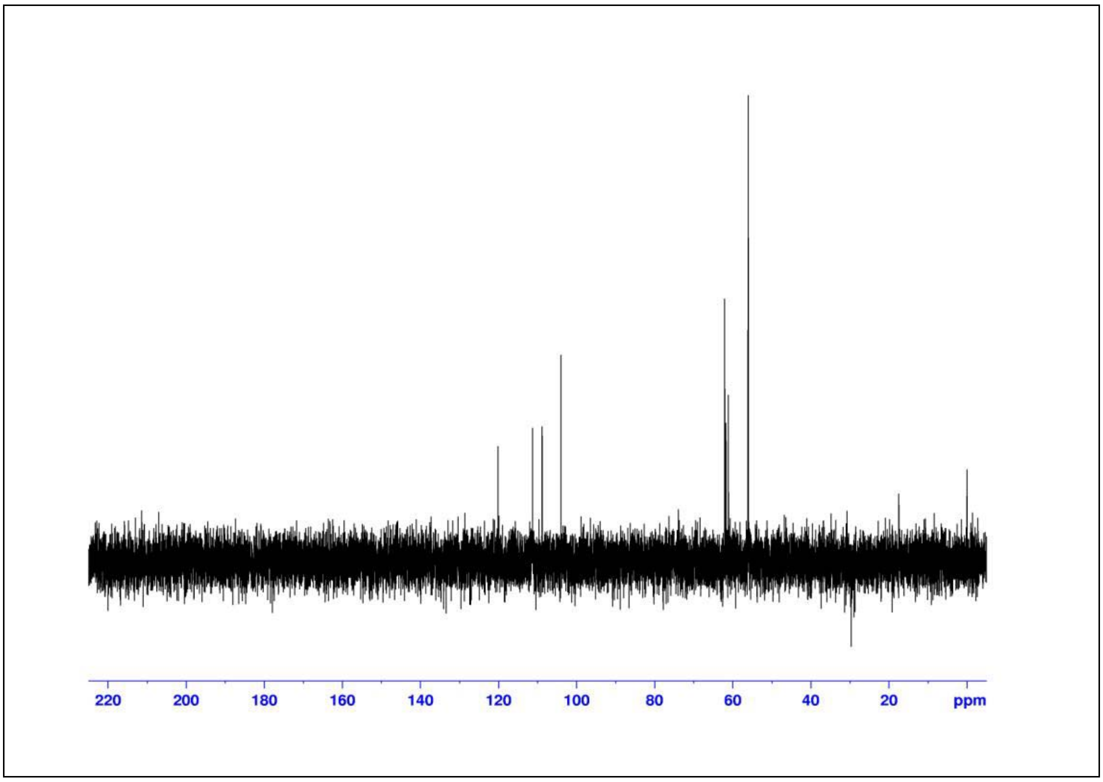


Figure 63 DEPT 135 ($CDCl_3$) of compound **RD4**

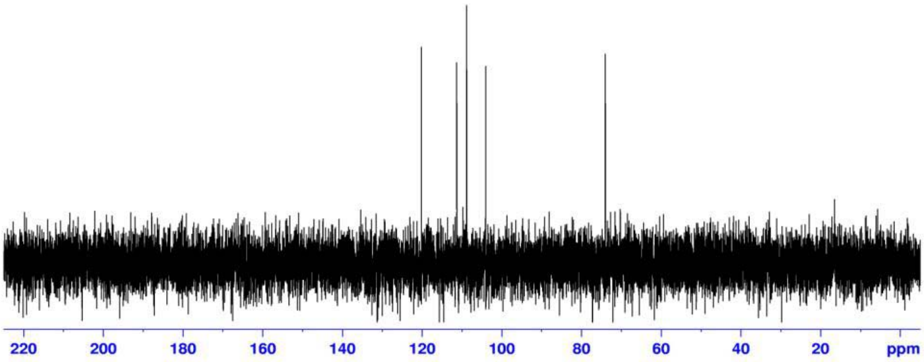


Figure 64 DEPT 90 (CDCl$_3$) of compound **RD4**

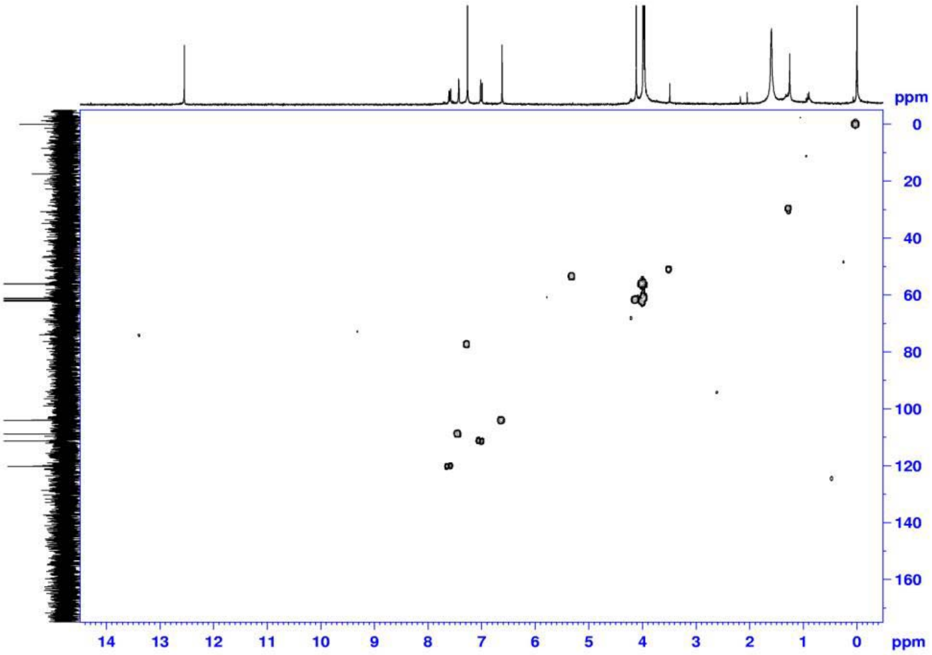


Figure 65 2D HMQC (CDCl$_3$) of compound **RD4**

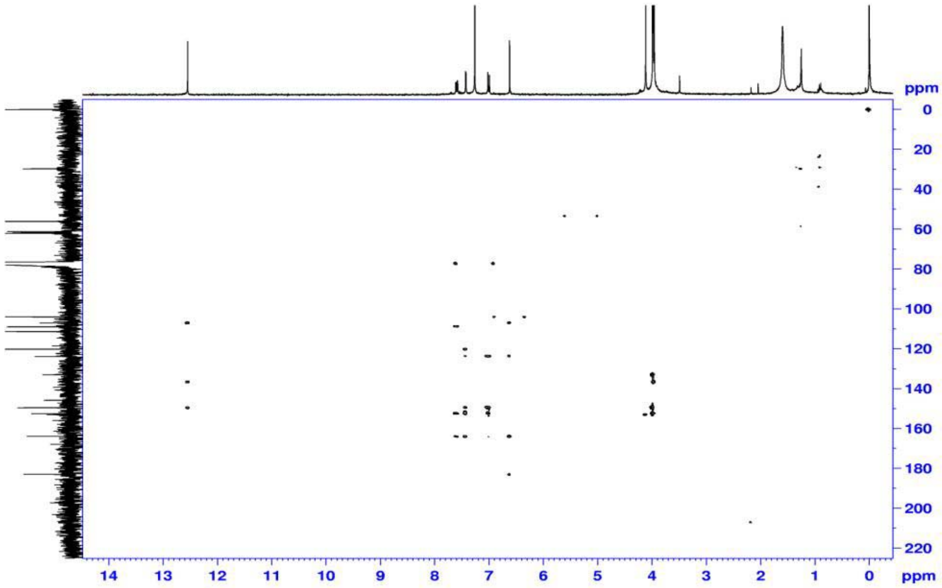


Figure 66 2D HMBC (CDCl$_3$) of compound **RD4**

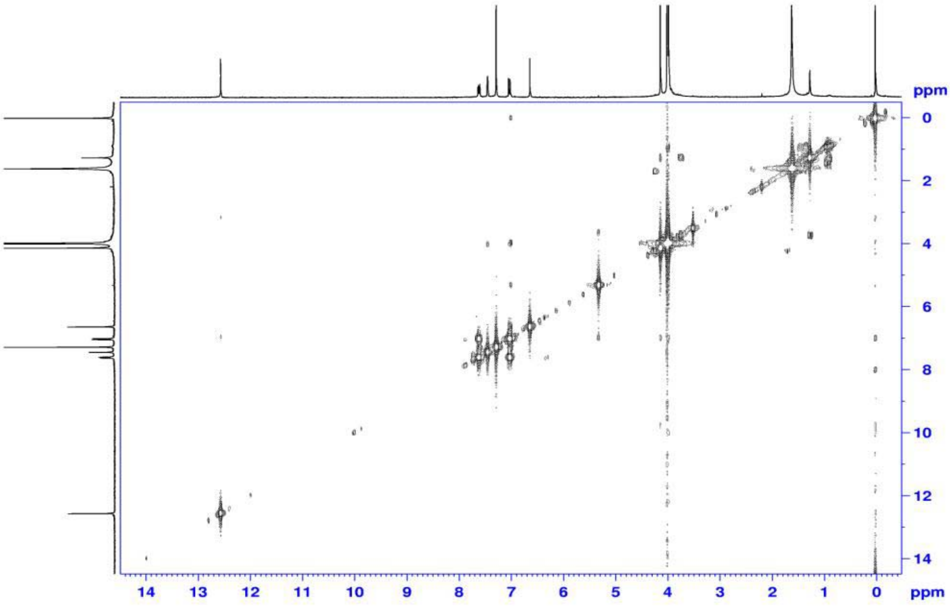


Figure 67 2D COSY (CDCl$_3$) of compound **RD4**

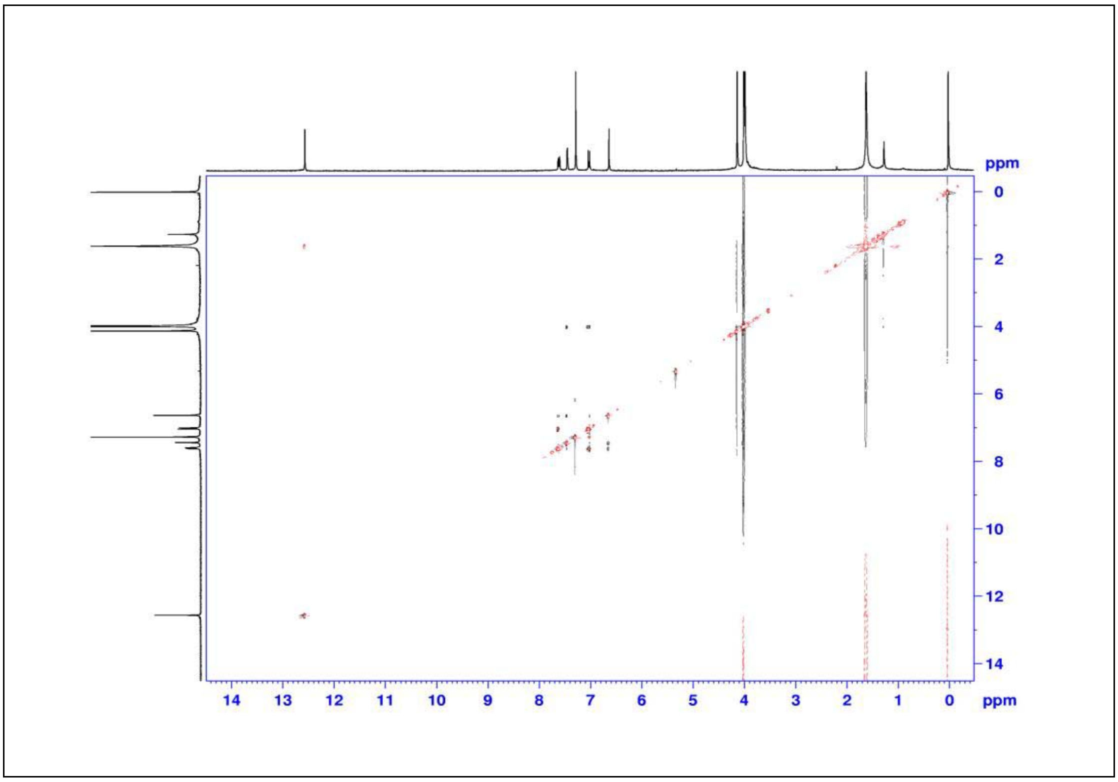


Figure 68 2D NOESY ($CDCl_3$) of compound **RD4**

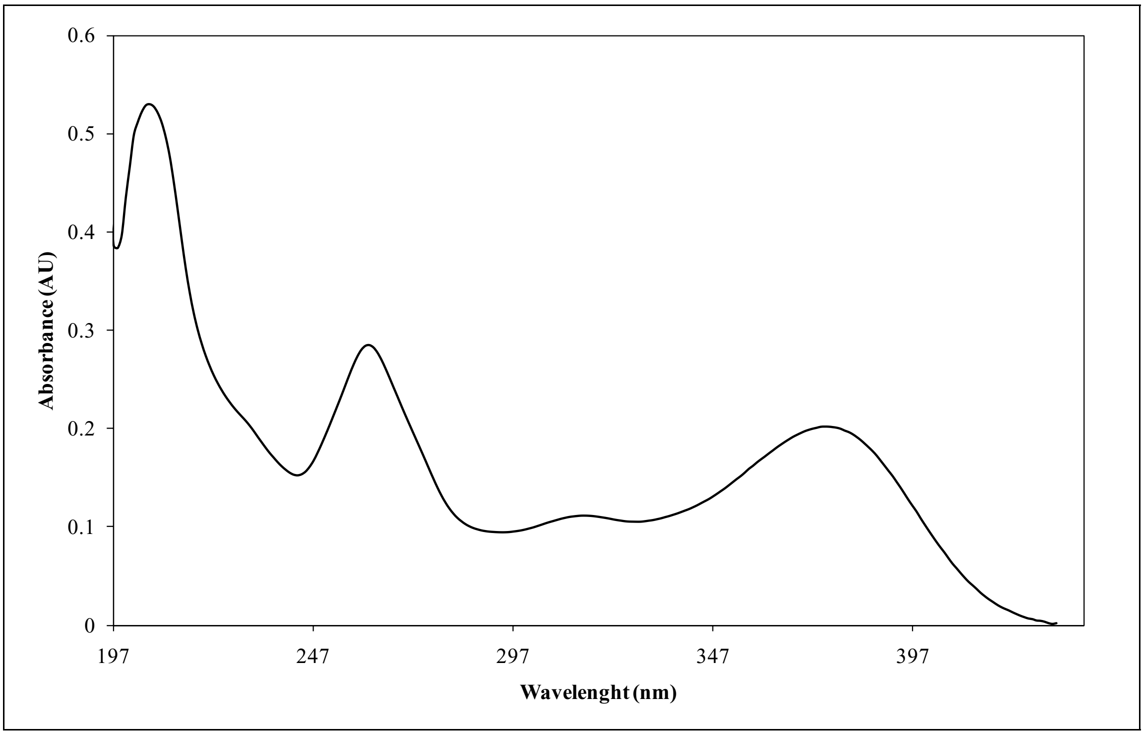


Figure 69 UV (MeOH) spectrum of compound **RD5**

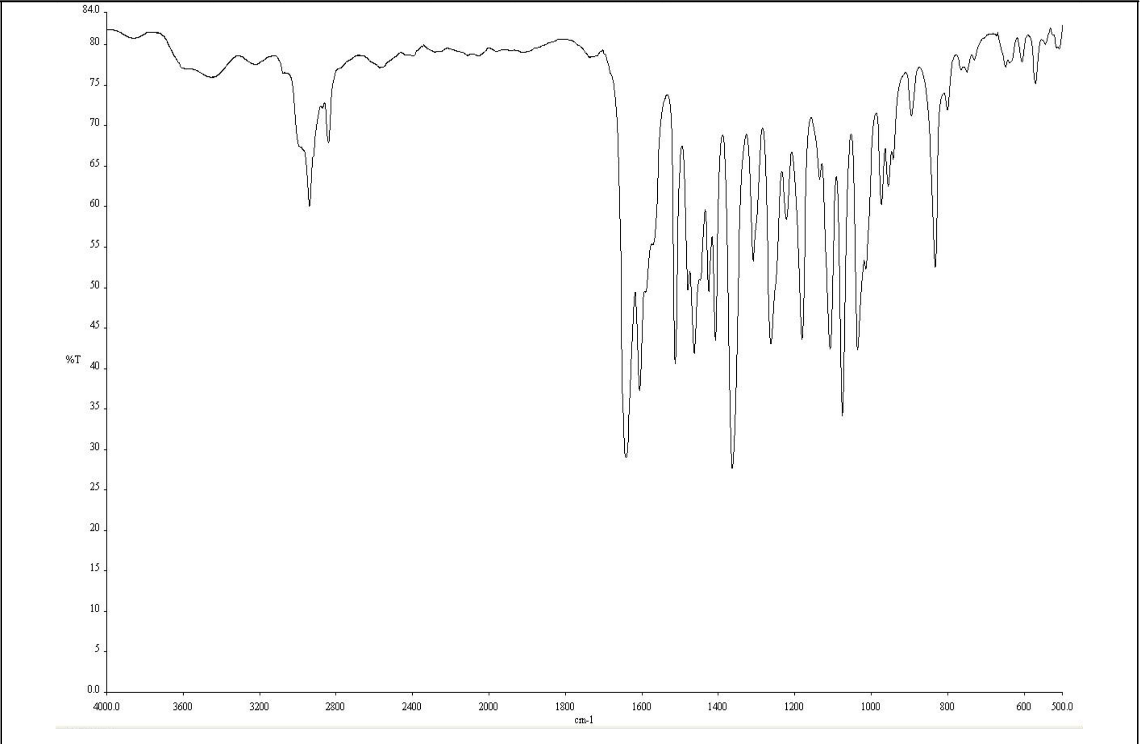


Figure 70 IR (neat) spectrum of compound **RD5**

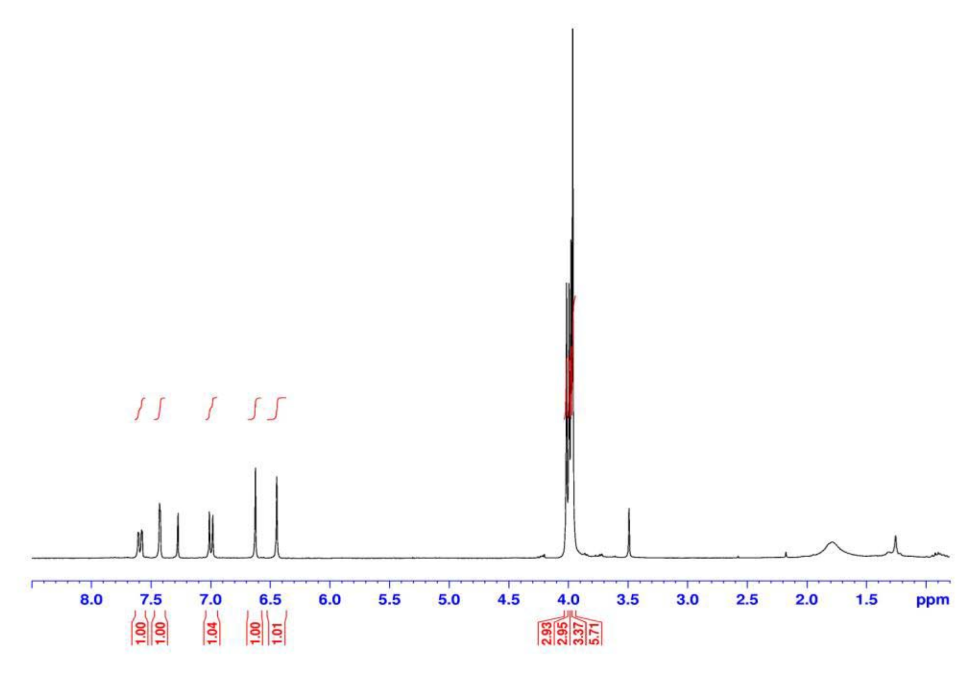


Figure 71 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RD5**

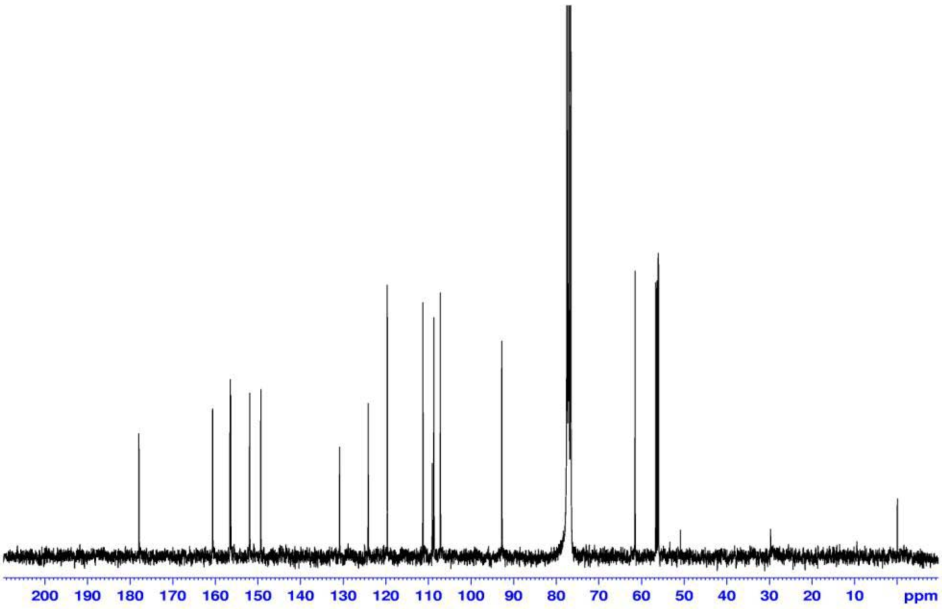


Figure 72 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD5**

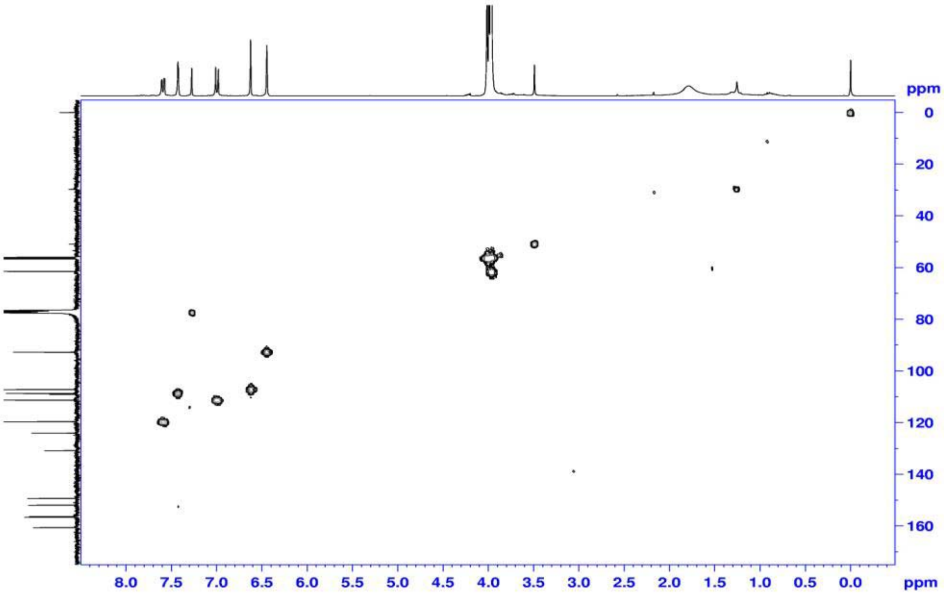


Figure 73 2D HMQC ($CDCl_3$) of compound **RD5**

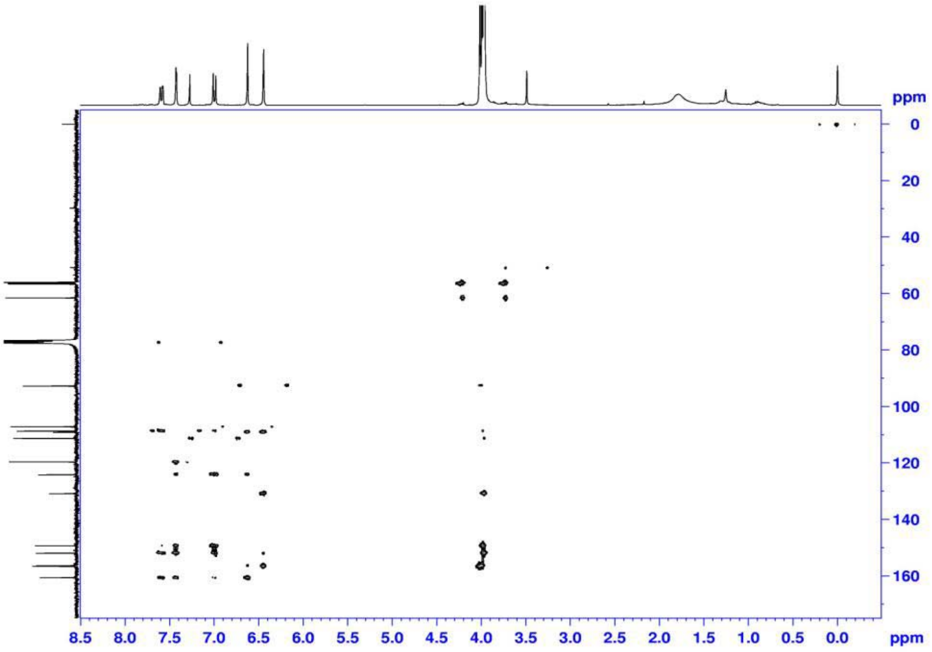


Figure 74 2D HMBC ($CDCl_3$) of compound **RD5**

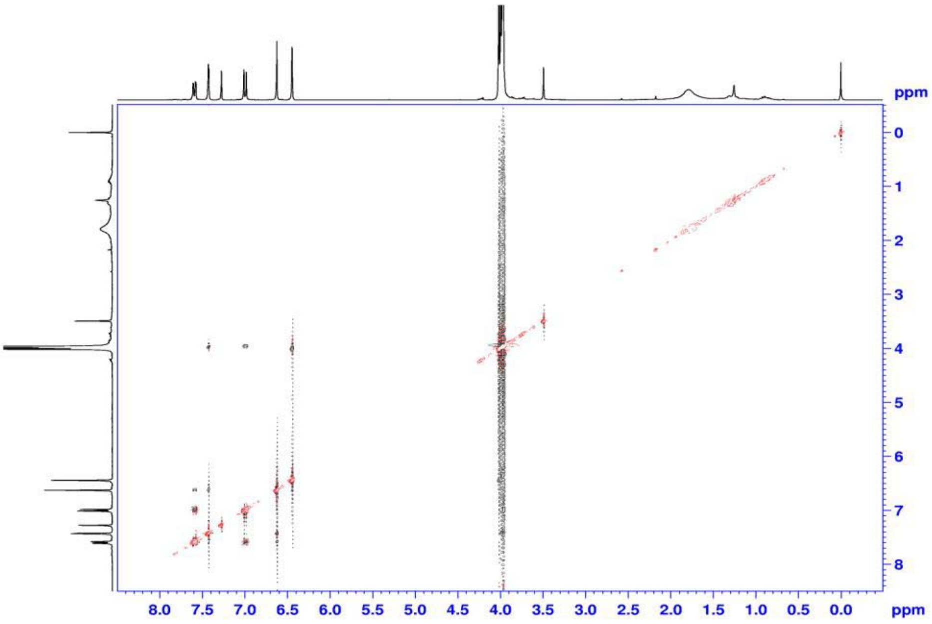


Figure 75 2D NOESY ($CDCl_3$) of compound **RD5**

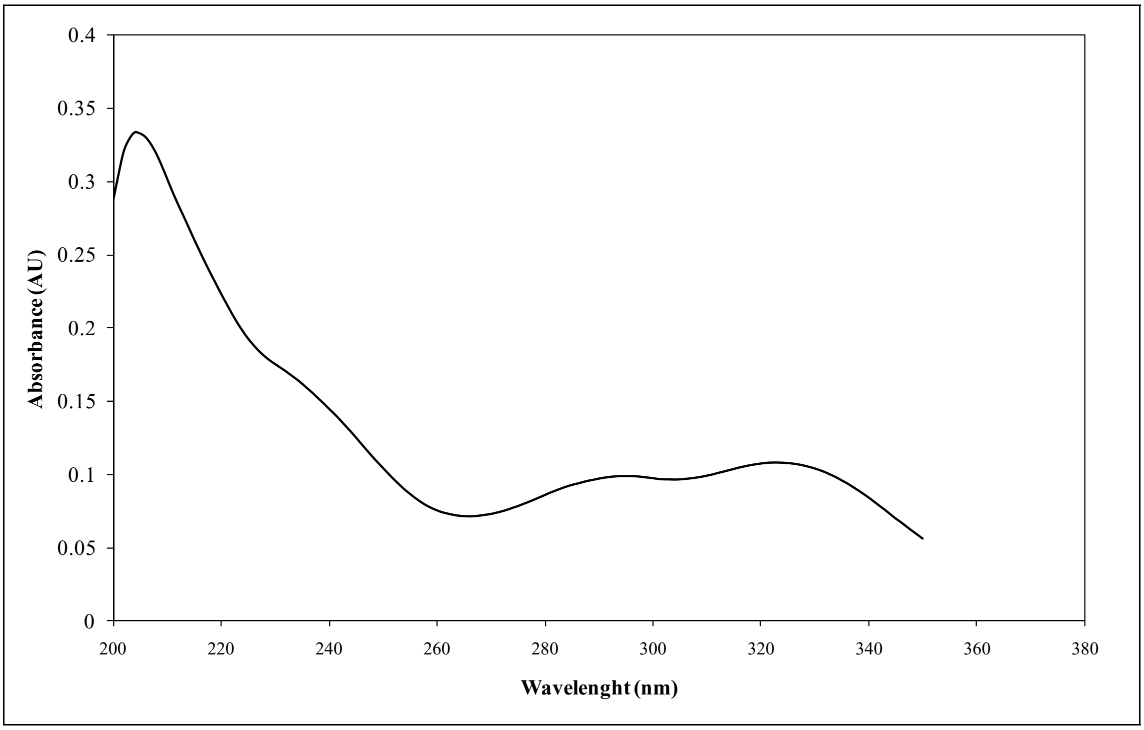


Figure 76 UV (MeOH) spectrum of compound **RD6**

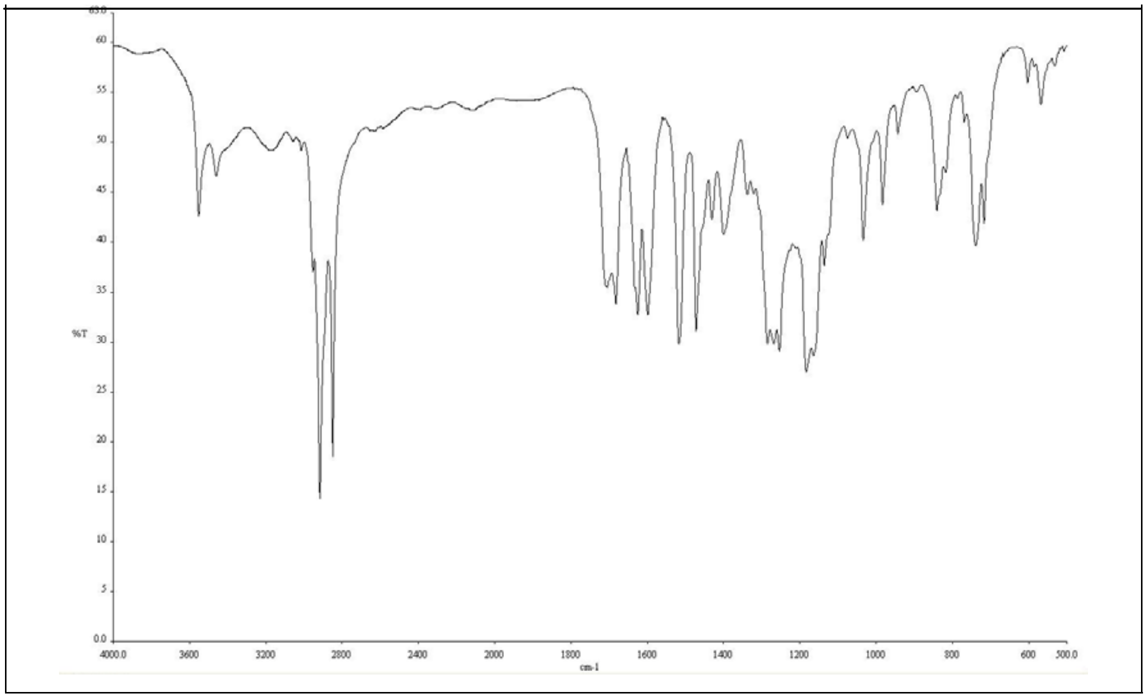


Figure 77 IR (neat) spectrum of compound **RD6**

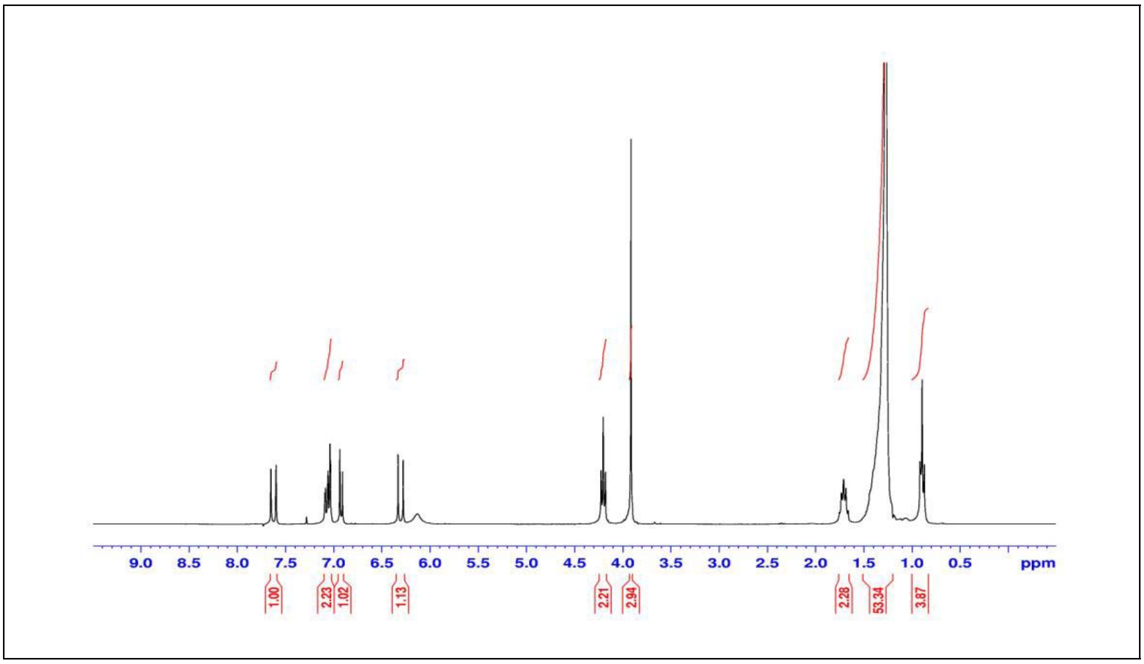


Figure 78 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RD6**

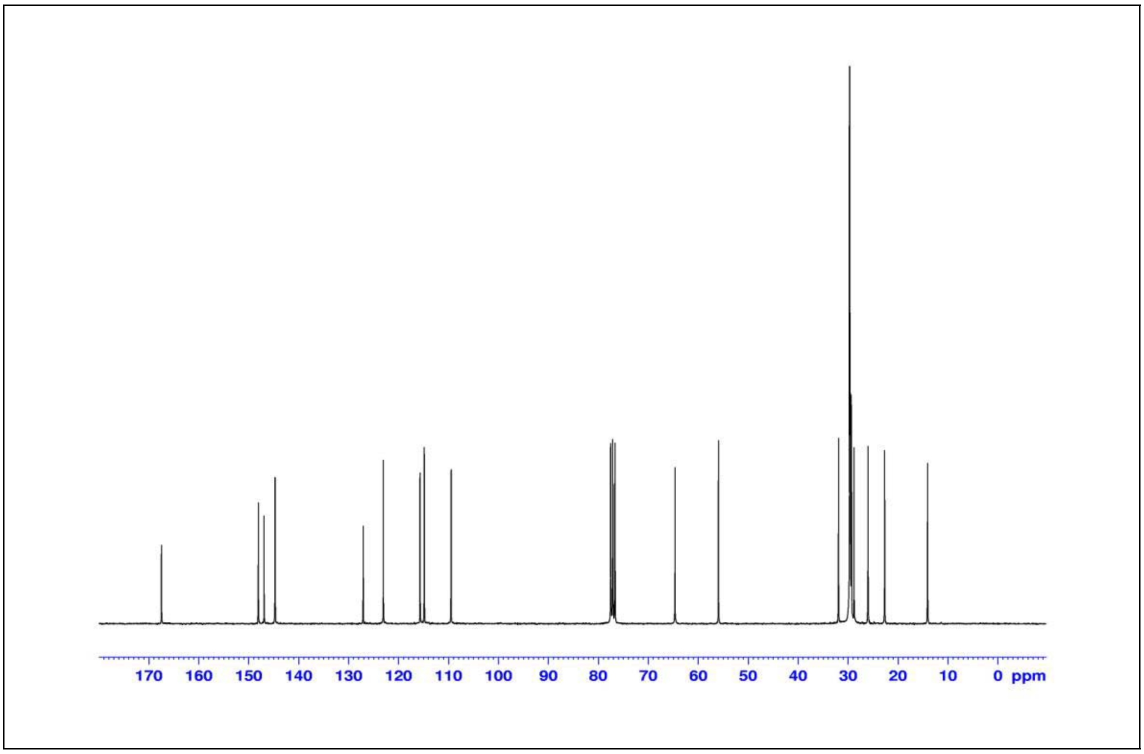


Figure 79 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD6**

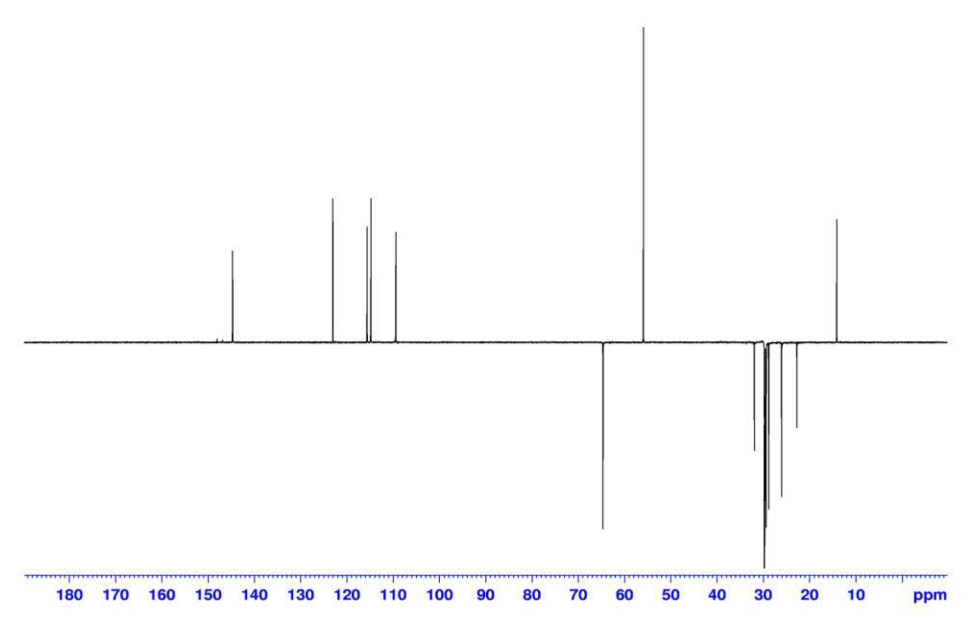


Figure 80 DEPT 135 (CDCl$_3$) of compound **RD6**

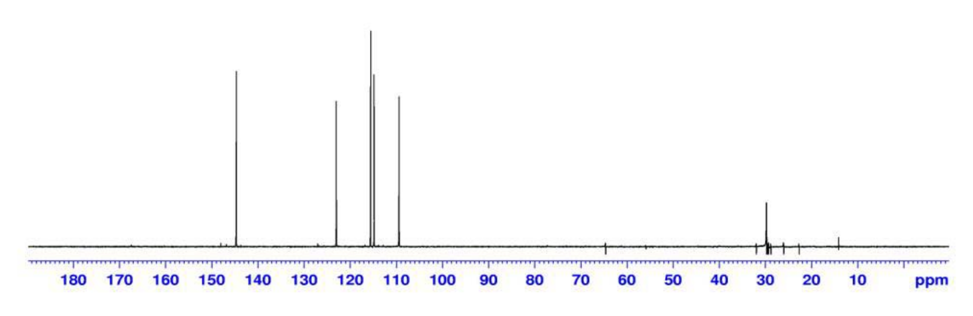


Figure 81 DEPT 90 (CDCl$_3$) of compound **RD6**

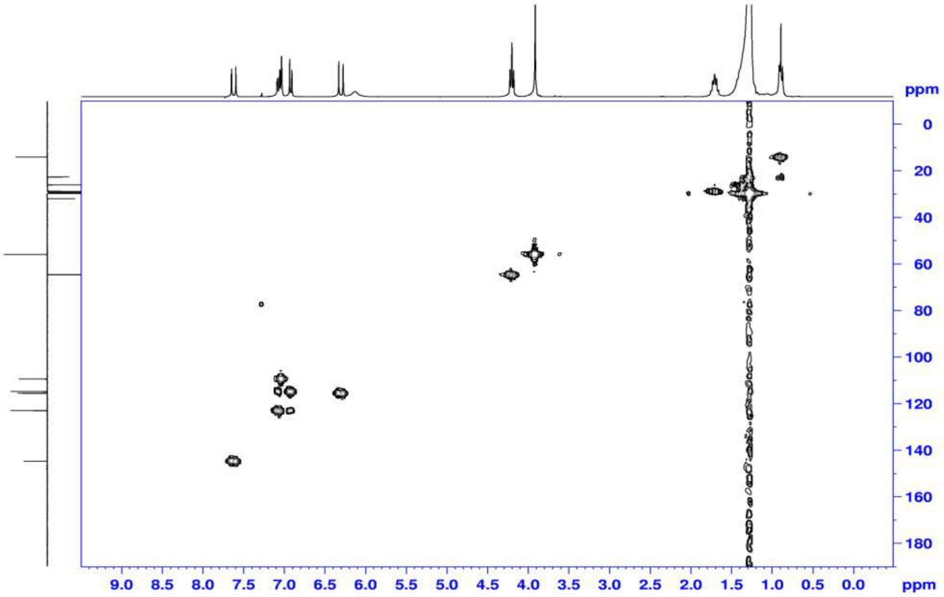


Figure 82 2D HMQC ($CDCl_3$) of compound **RD6**

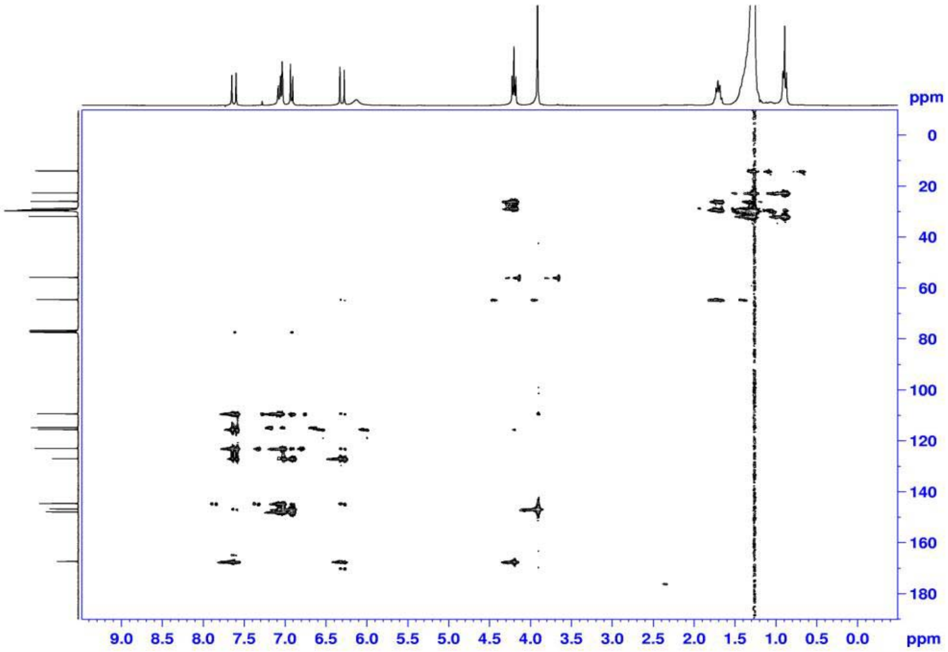


Figure 83 2D HMBC ($CDCl_3$) of compound **RD6**

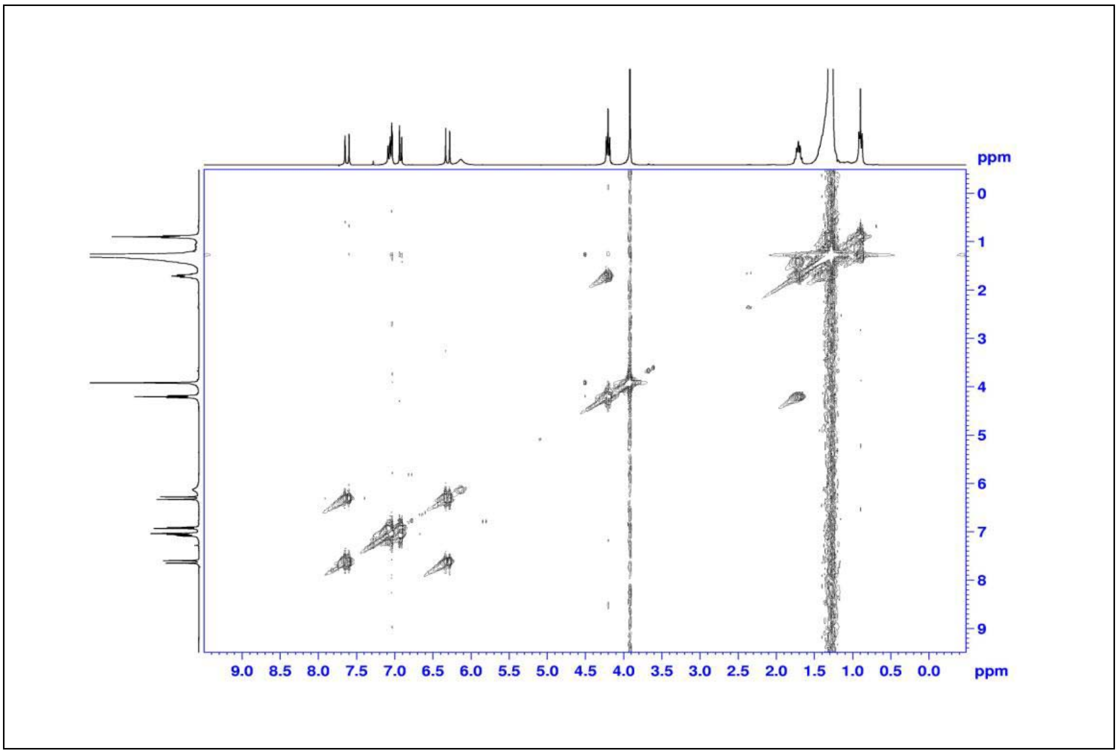


Figure 84 2D COSY ($CDCl_3$) of compound **RD6**

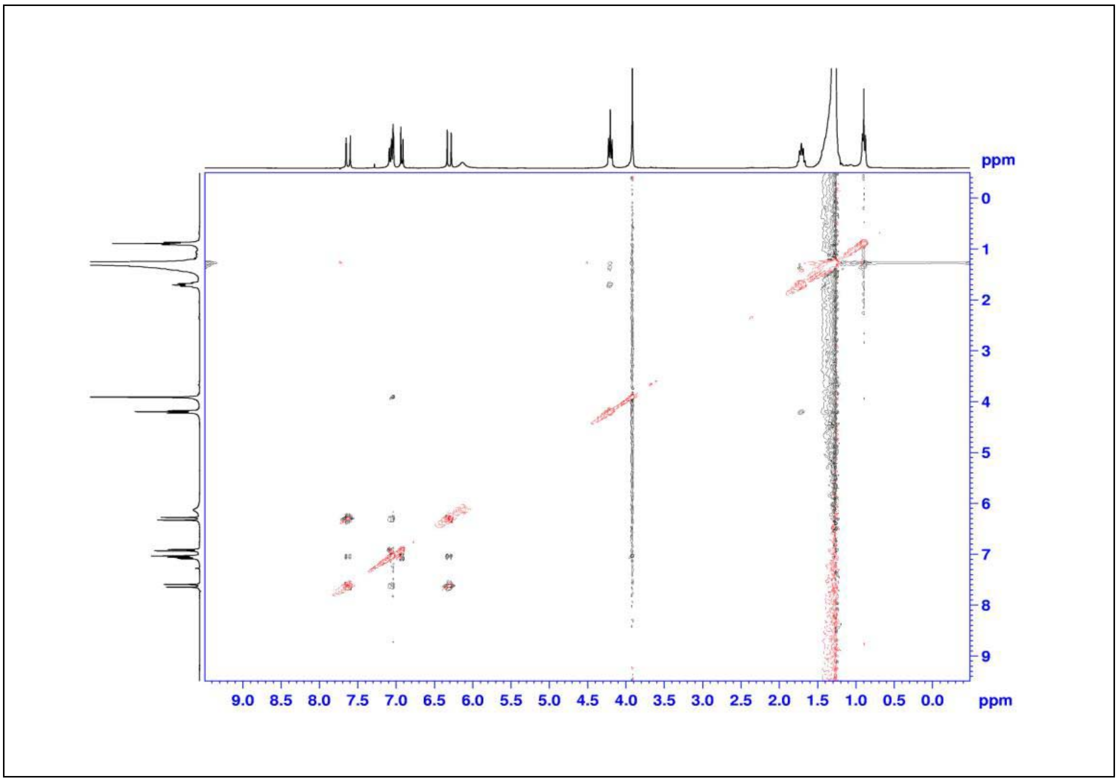


Figure 85 2D NOESY ($CDCl_3$) of compound **RD6**

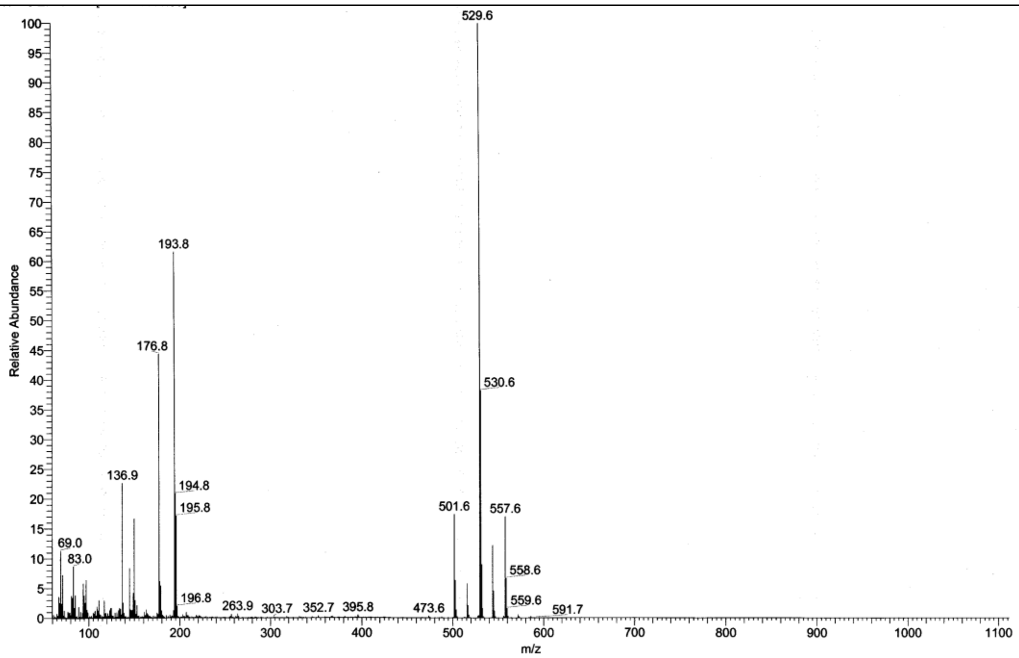


Figure 86 EI-MS of compound **RD6**

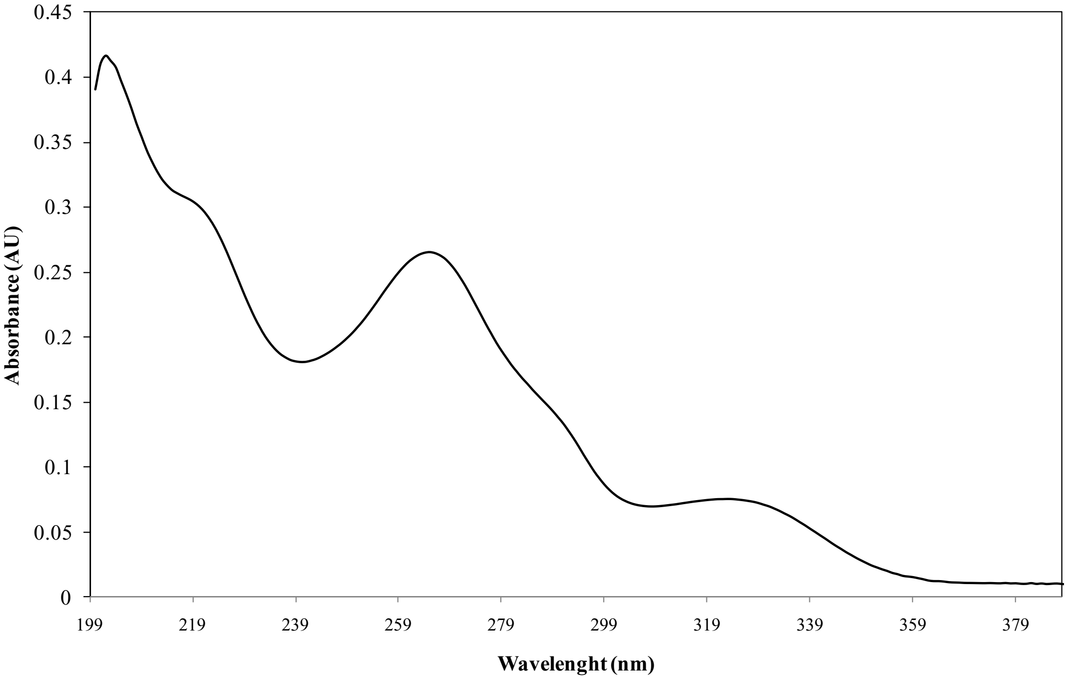


Figure 87 UV (MeOH) spectrum of compound **RD7**

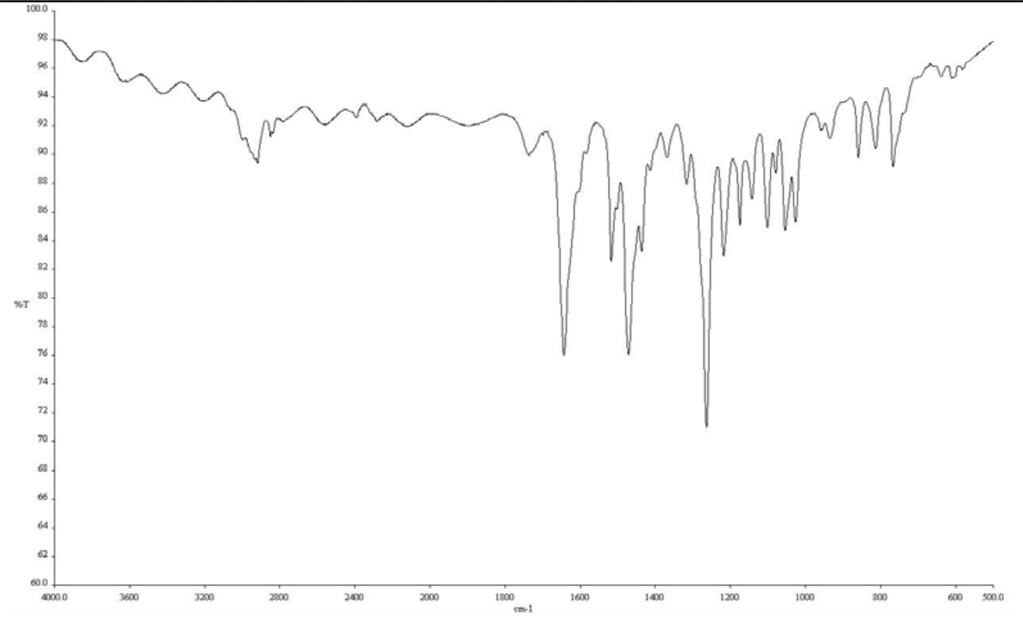


Figure 88 IR (neat) spectrum of compound **RD7**

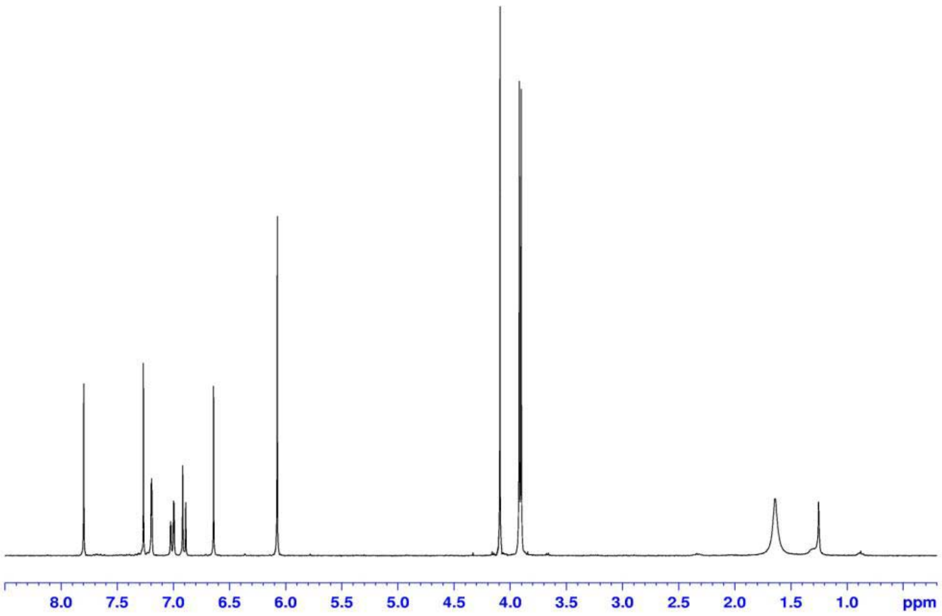


Figure 89 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **RD7**

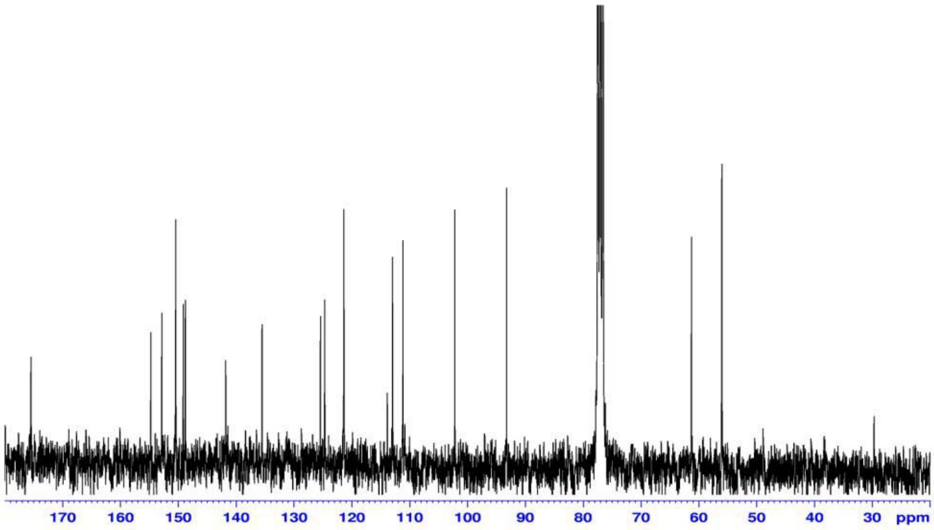


Figure 90 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD7**

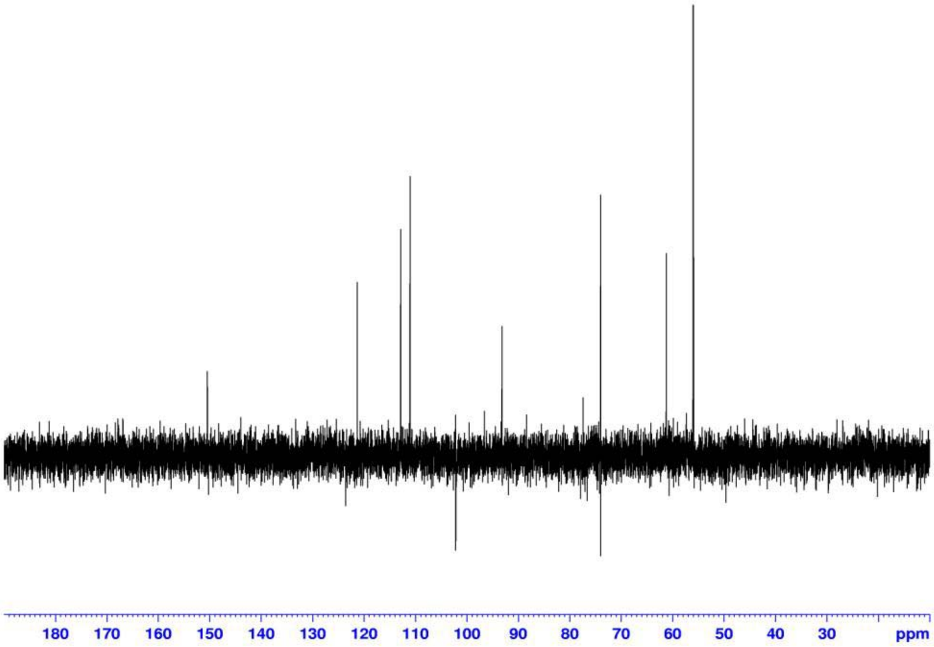


Figure 91 DEPT 135 ($CDCl_3$) of compound **RD7**

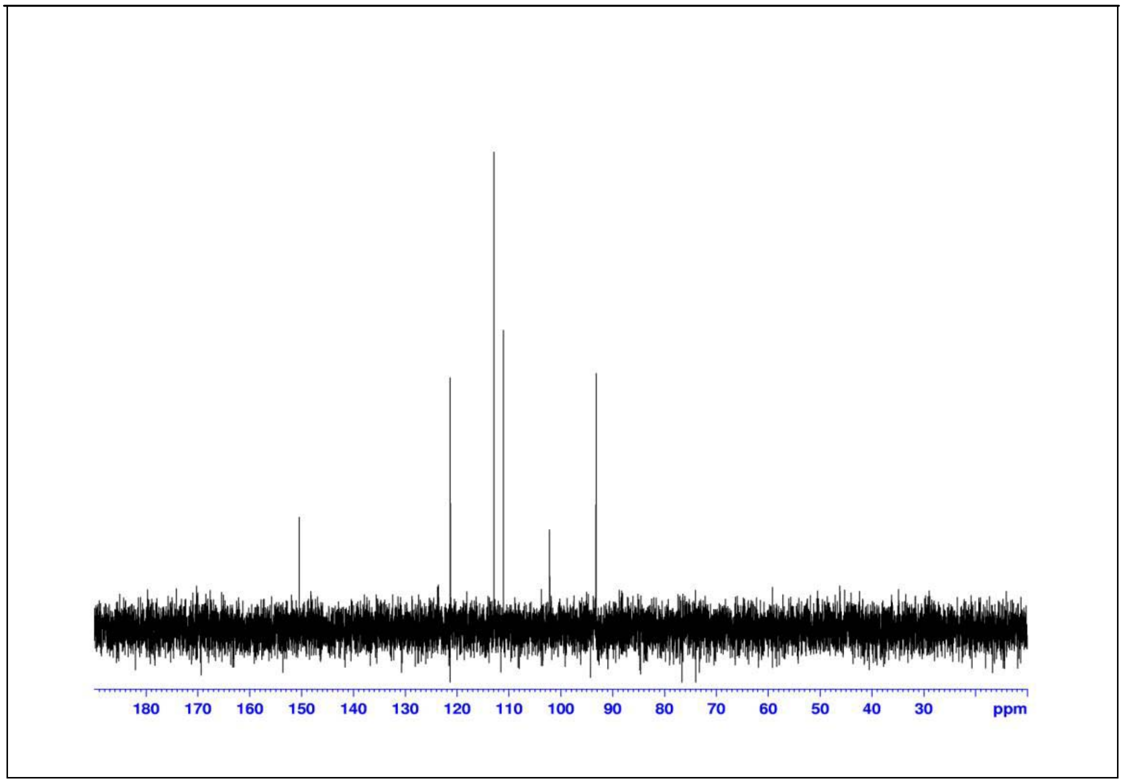


Figure 92 DEPT 90 (CDCl$_3$) of compound **RD7**

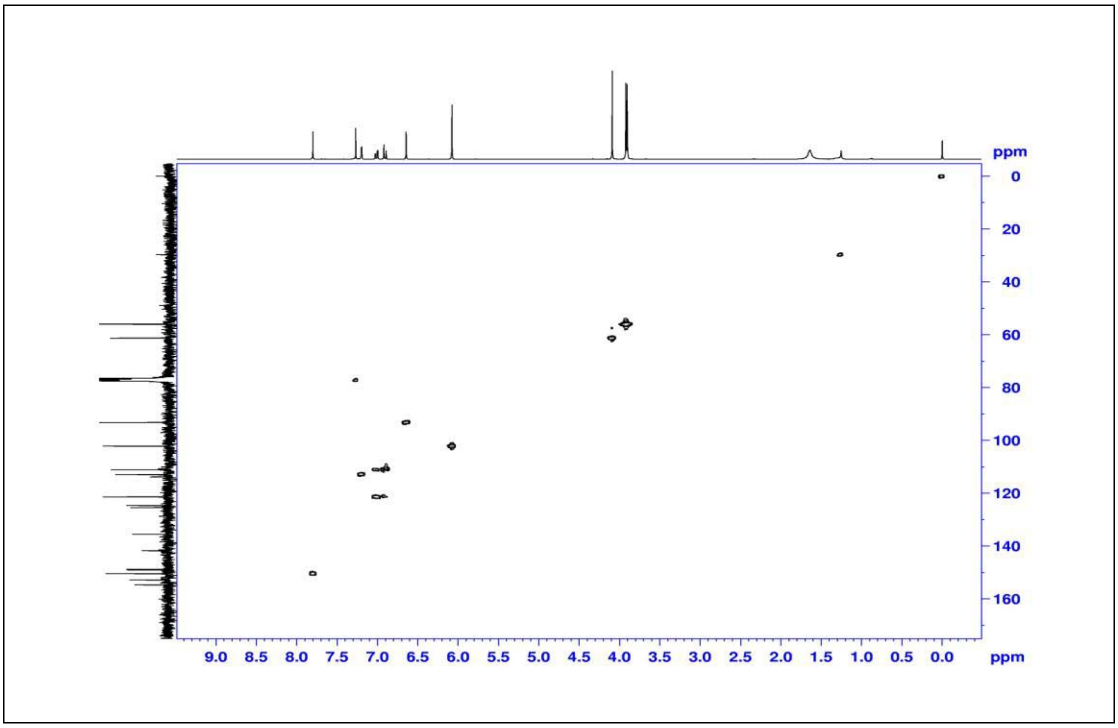


Figure 93 2D HMQC (CDCl$_3$) of compound **RD7**

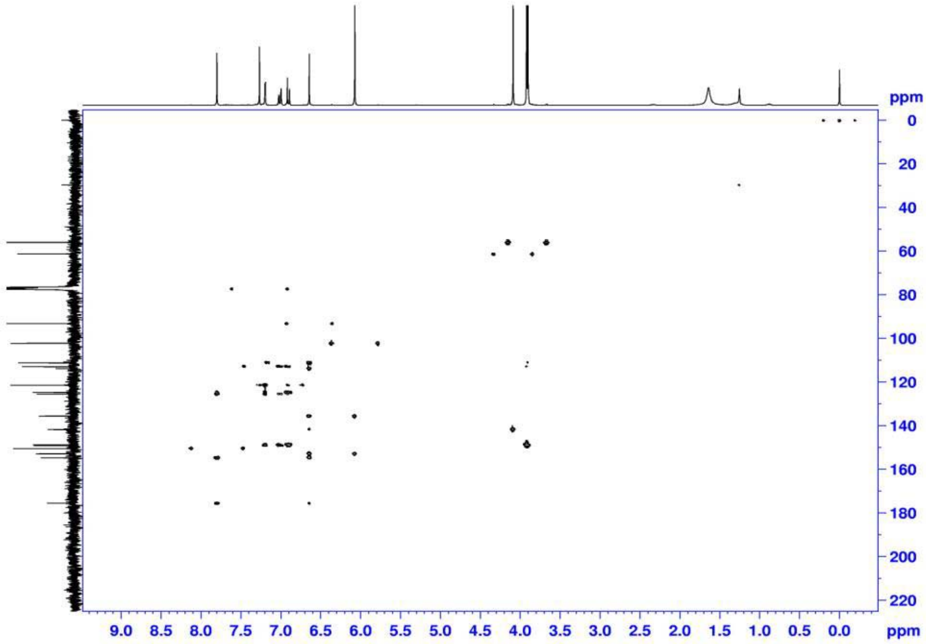


Figure 94 2D HMBC ($CDCl_3$) of compound **RD7**

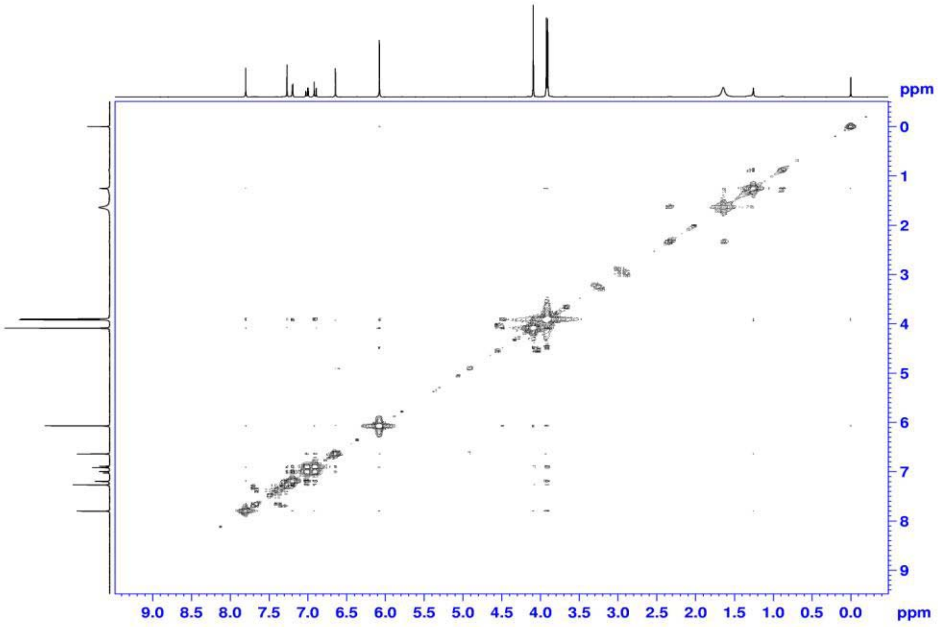


Figure 95 2D COSY ($CDCl_3$) of compound **RD7**

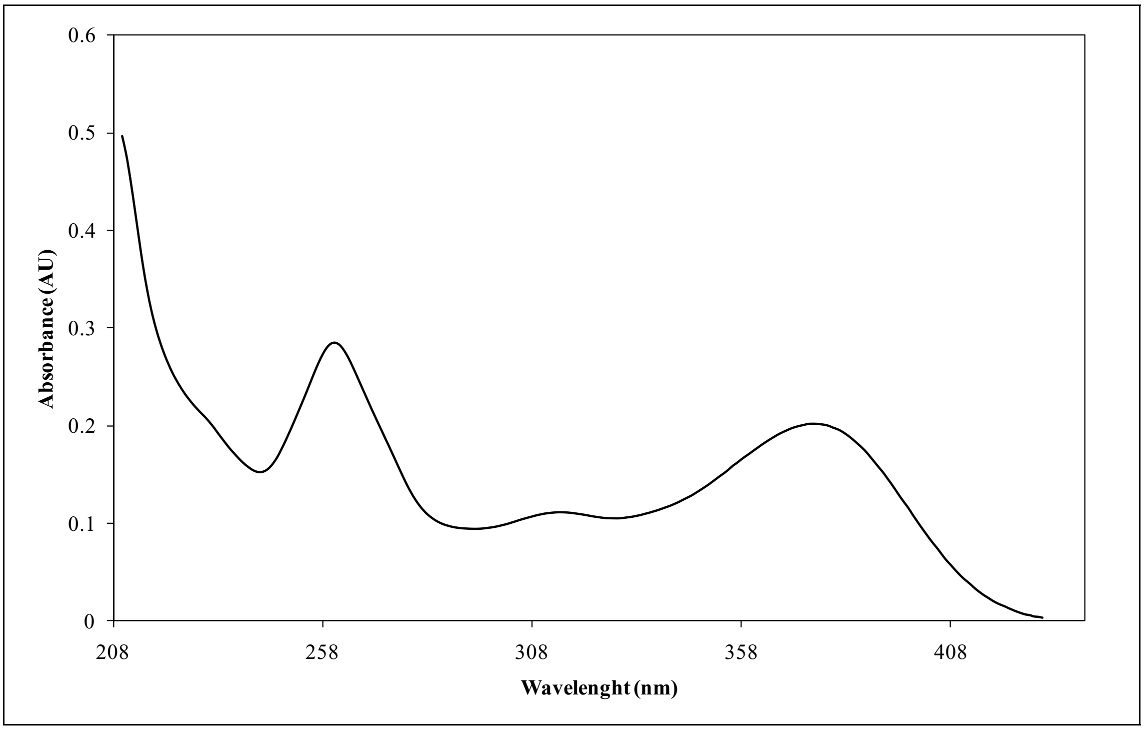


Figure 96 UV (MeOH) spectrum of compound **RD8**

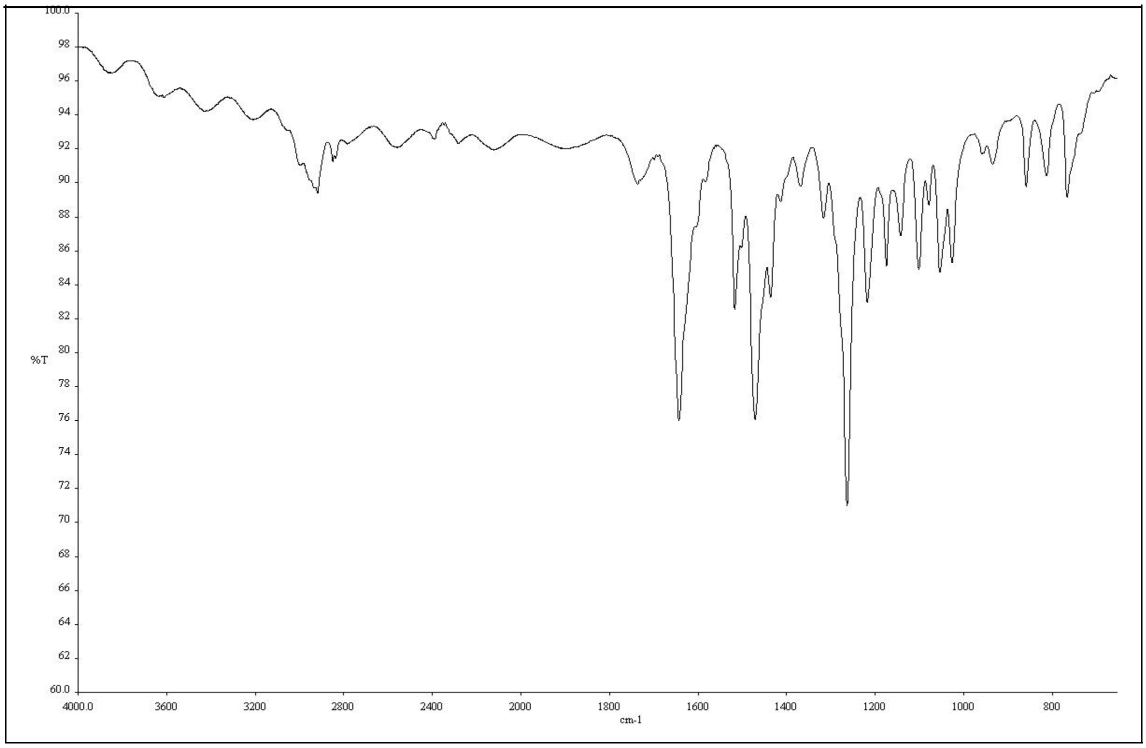


Figure 97 IR (neat) spectrum of compound **RD8**

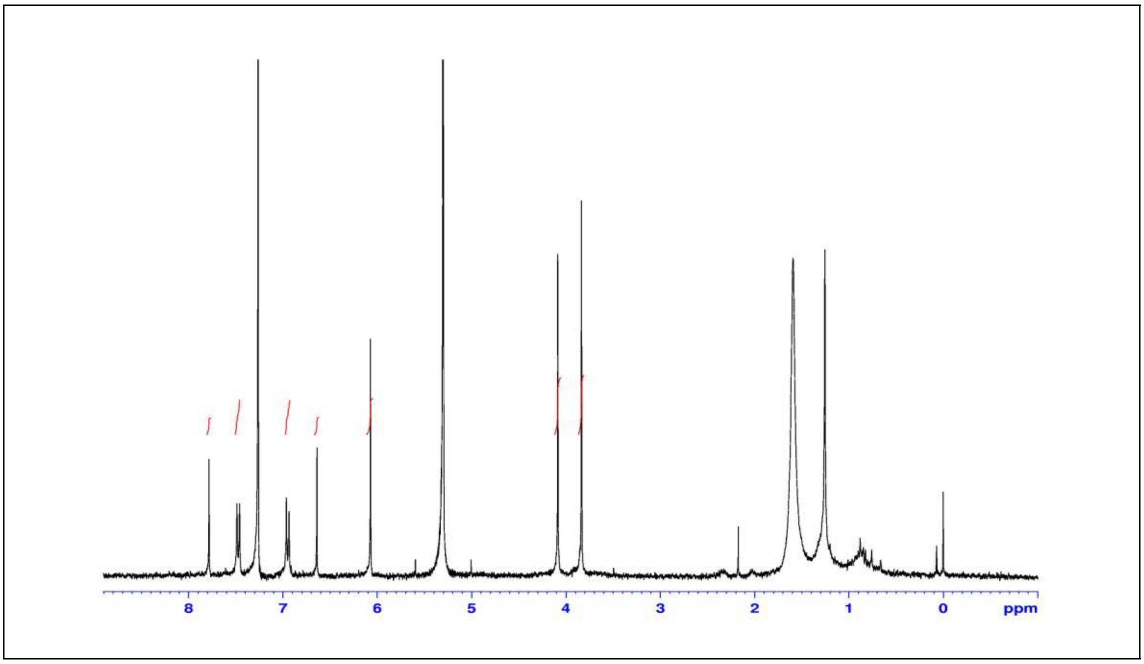


Figure 98 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **RD8**

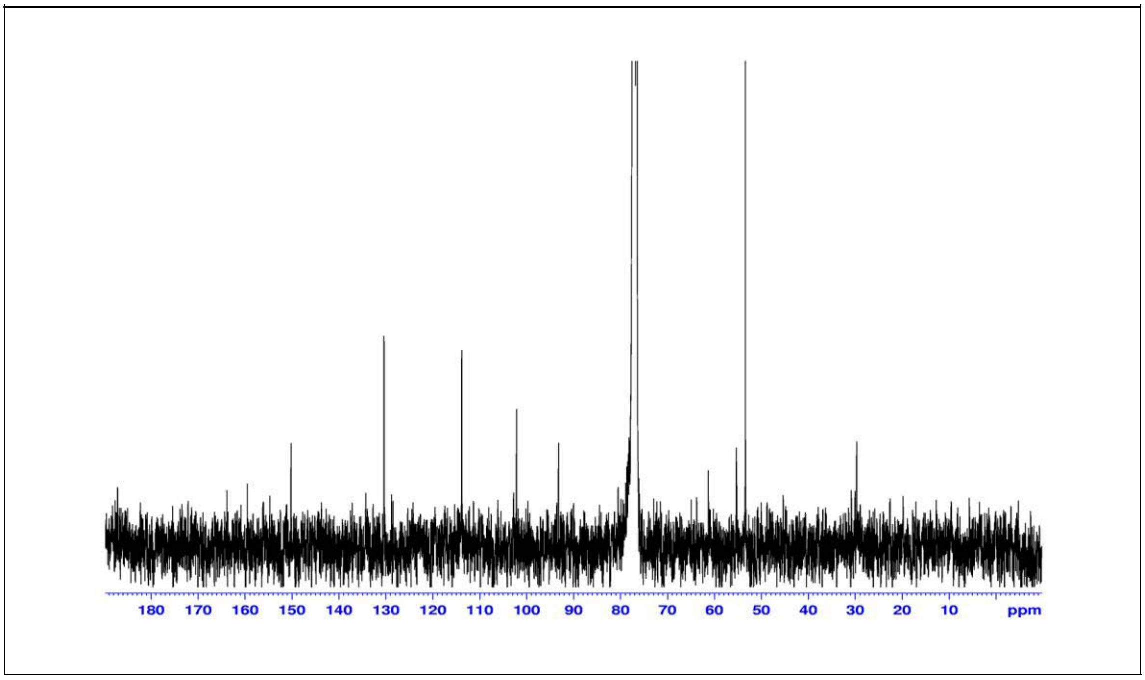


Figure 99 ^{13}C NMR (75 MHz) (CDCl$_3$) of compound **RD8**

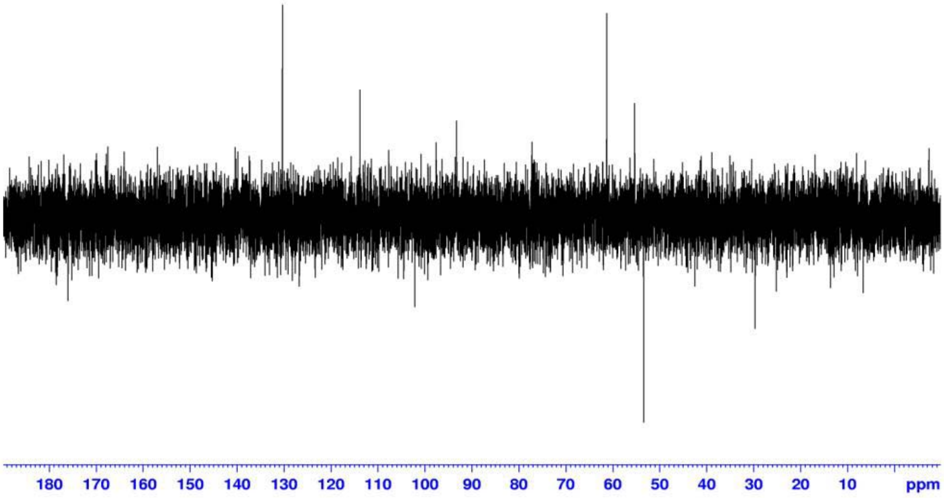


Figure 100 DEPT 135 (CDCl$_3$) of compound **RD8**

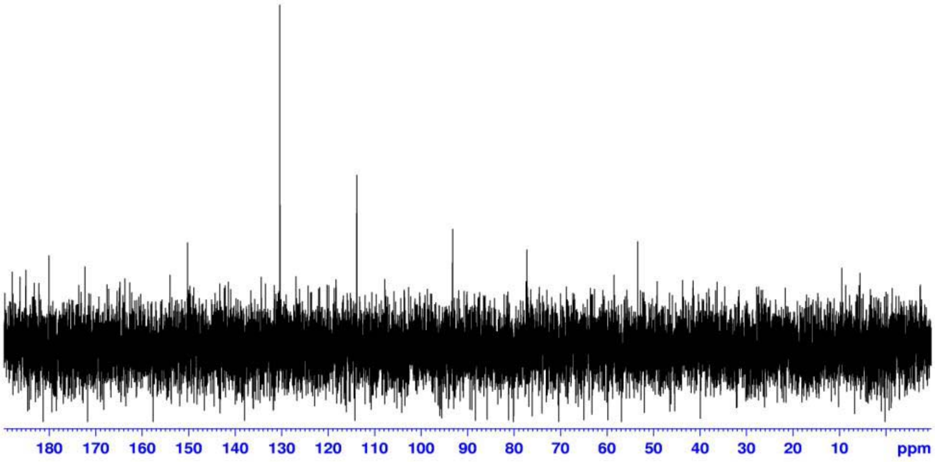


Figure 101 DEPT 90 (CDCl$_3$) of compound **RD8**

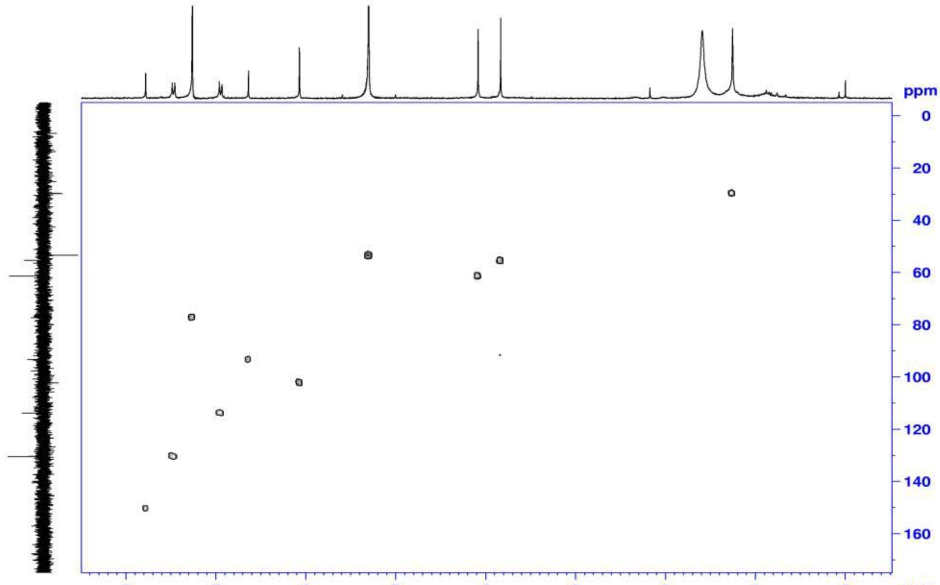


Figure 103 2D HMQC ($CDCl_3$) of compound **RD8**

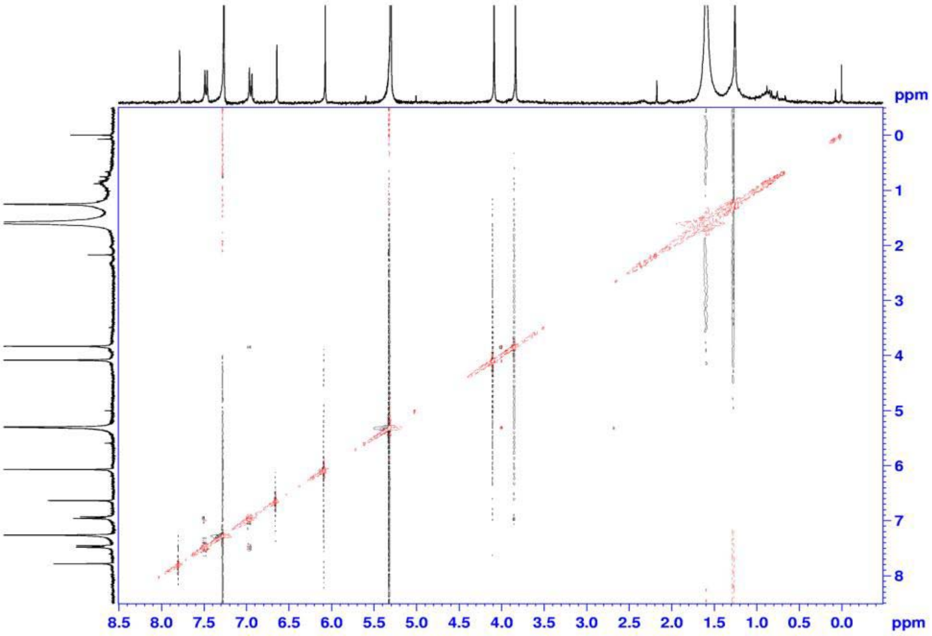


Figure 104 2D NOESY ($CDCl_3$) of compound **RD8**

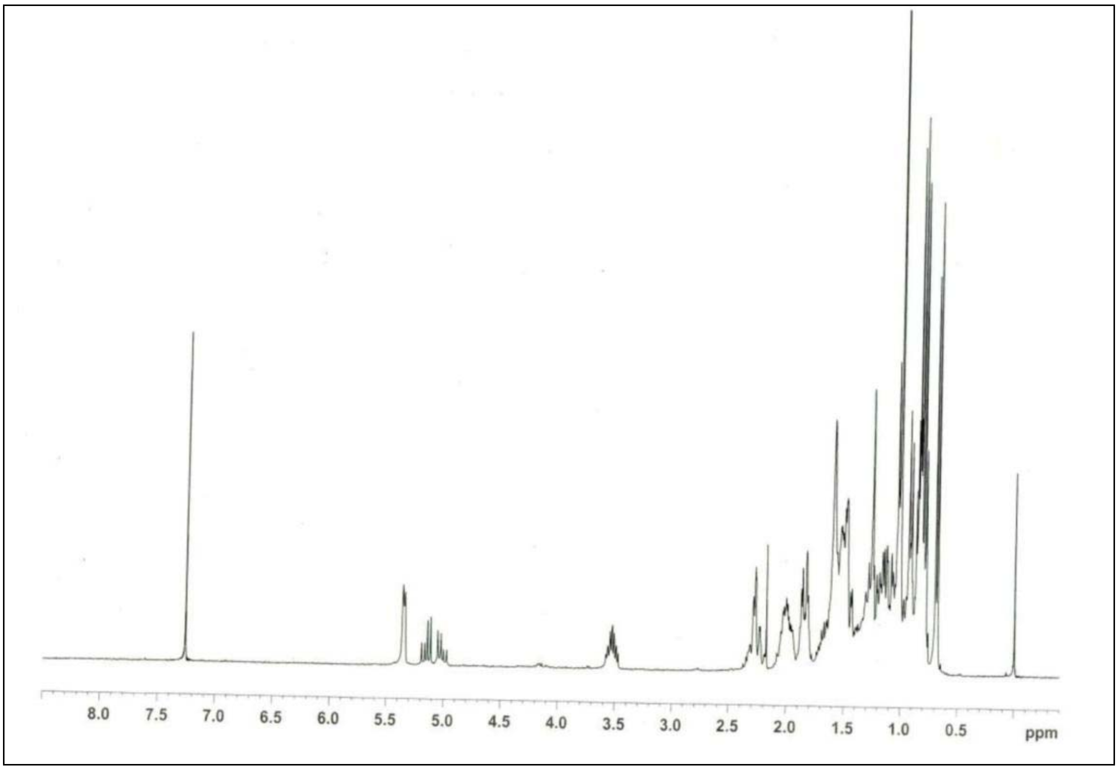


Figure 105 ^{1}H NMR (300 MHz) (CDCl$_3$) of compound **RD9** and **RD10**

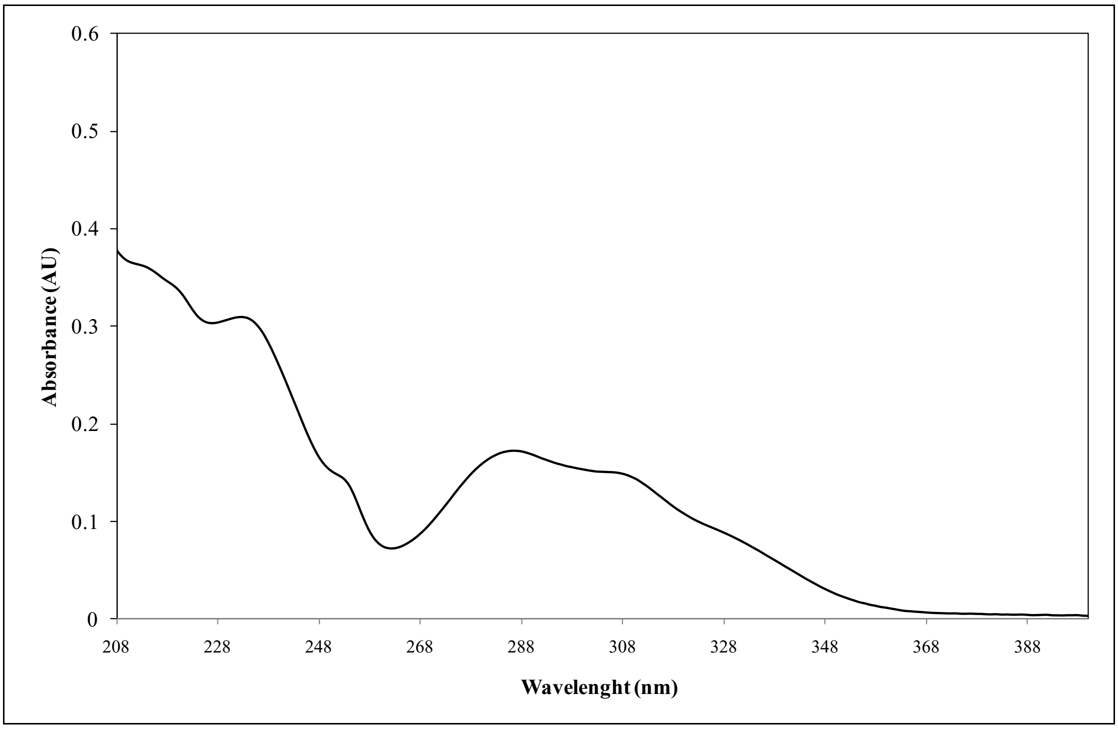


Figure 106 UV (MeOH) spectrum of compound **RD11**

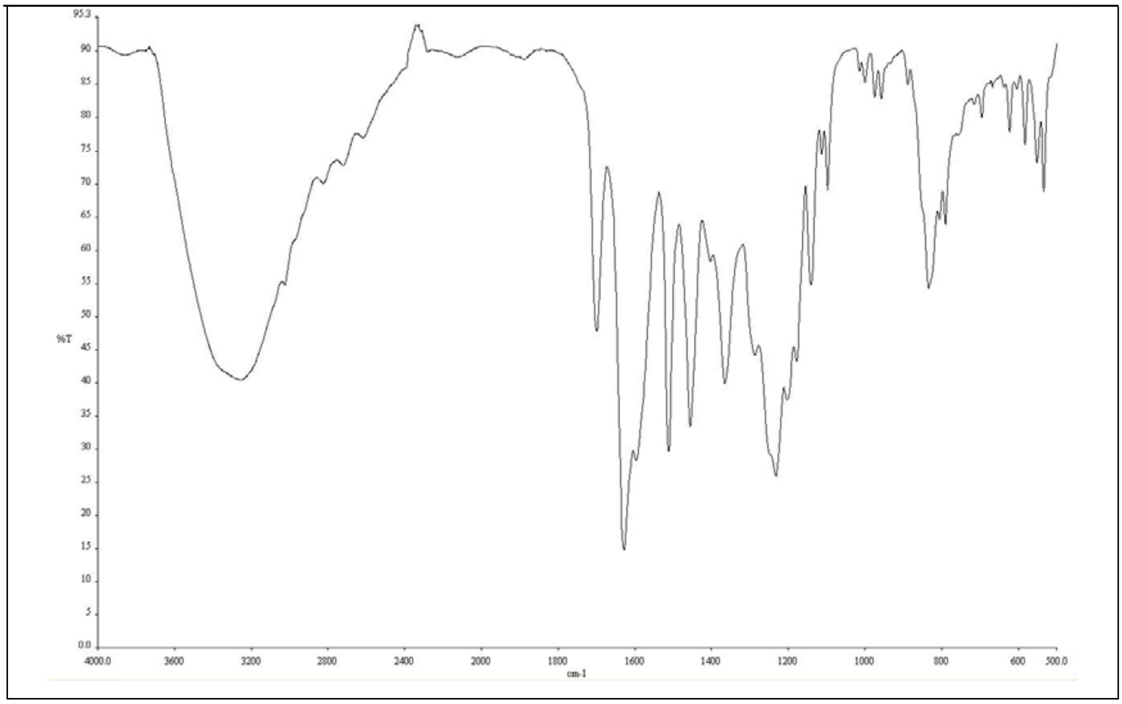


Figure 107 IR (neat) spectrum of compound **RD11**

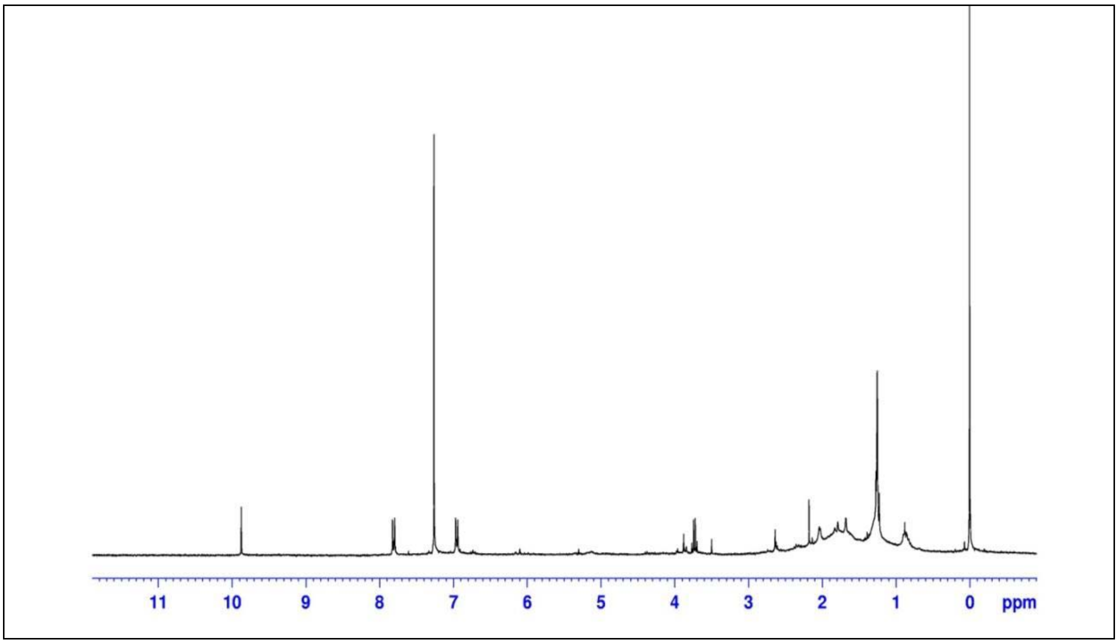


Figure 108 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RD11**

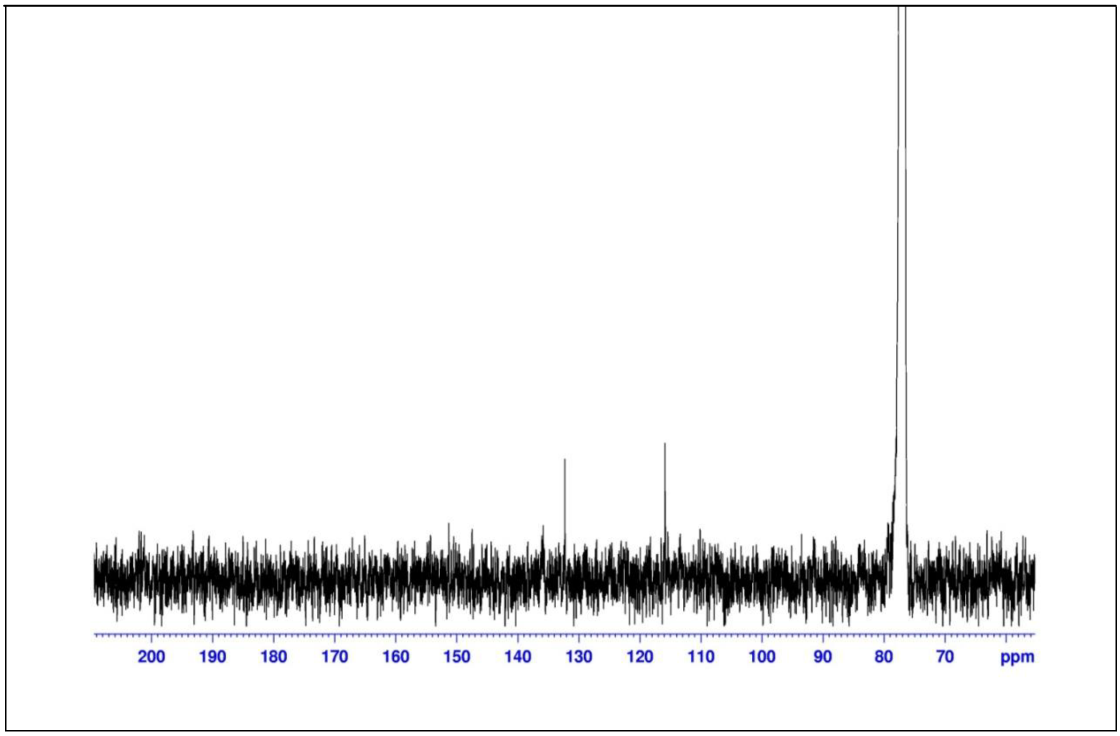


Figure 109 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD11**

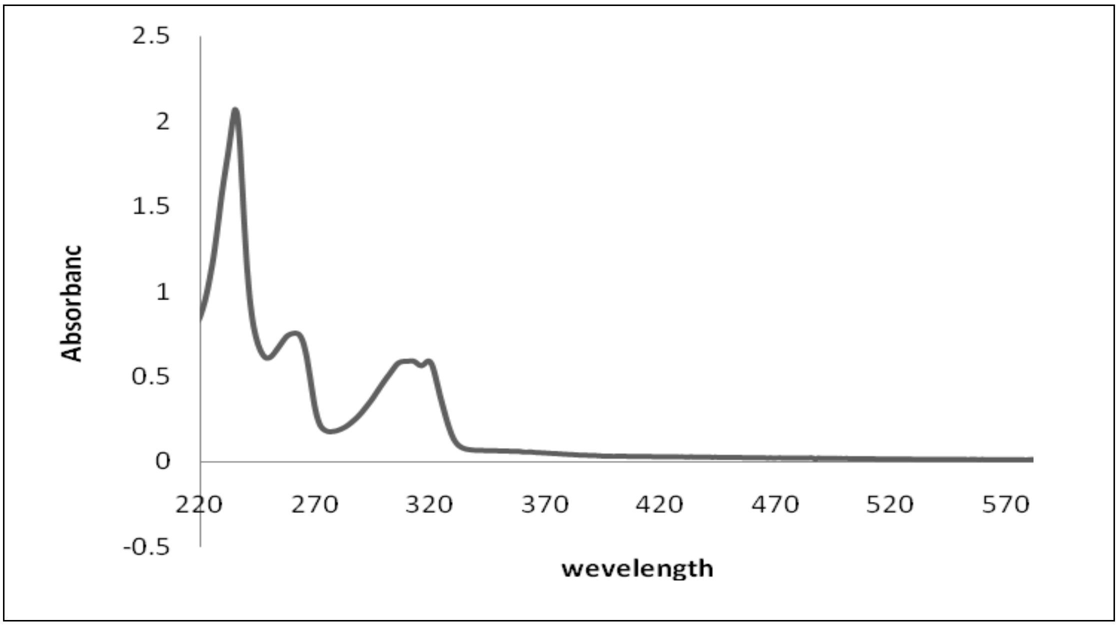


Figure 110 UV (MeOH) spectrum of compound **RD12**

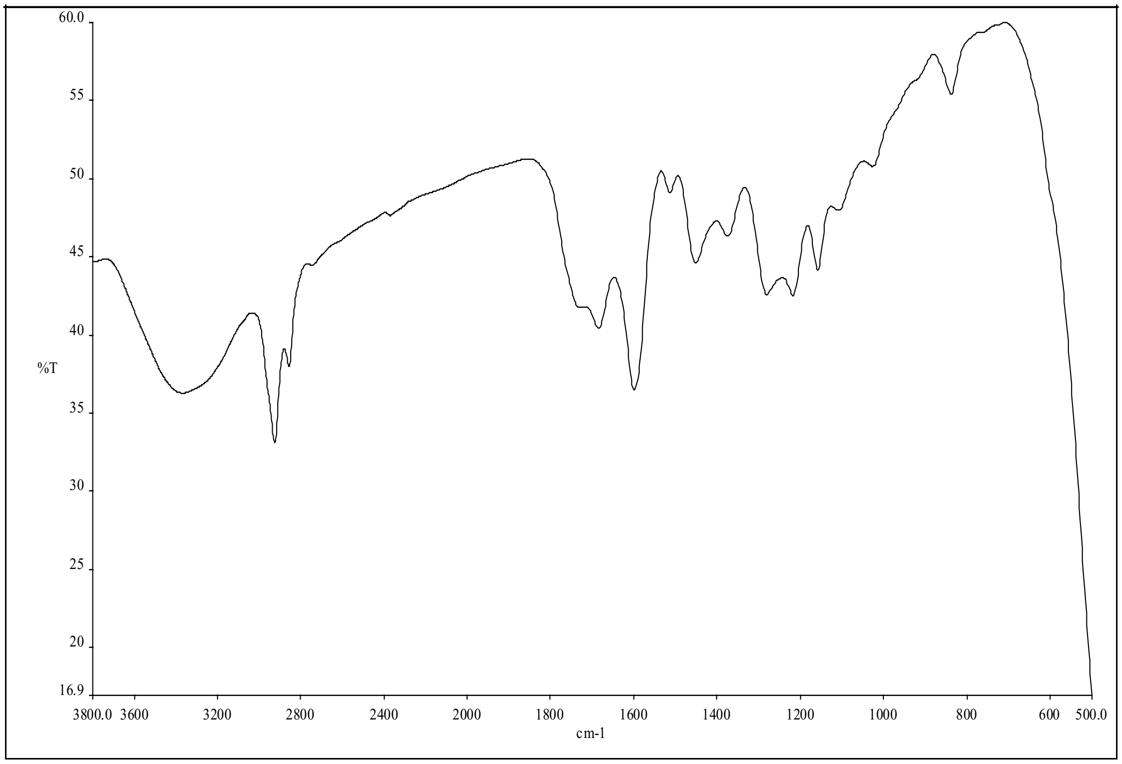


Figure 111 IR (neat) spectrum of compound **RD12**

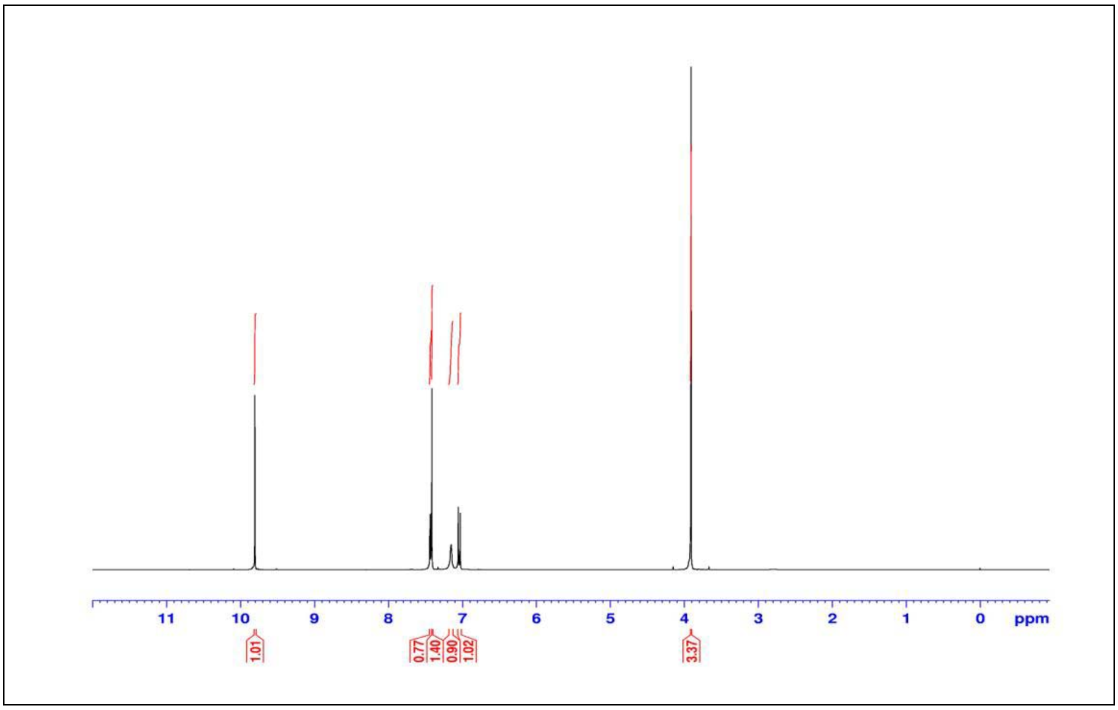


Figure 112 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RD12**

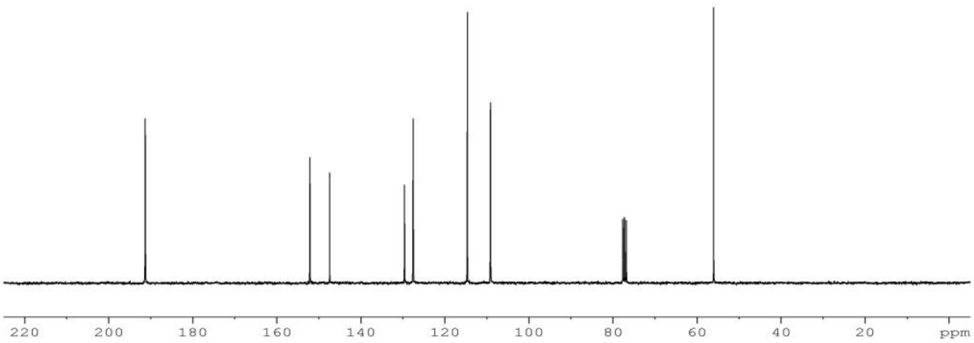


Figure 113 ^{13}C NMR (75 MHz) ($CDCl_3$) of compound **RD12**

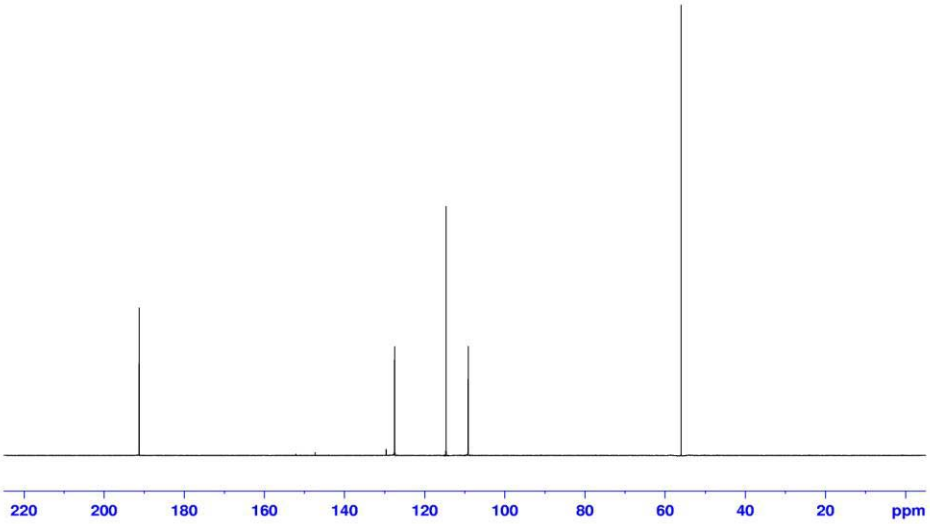


Figure 114 DEPT 135 ($CDCl_3$) of compound **RD12**

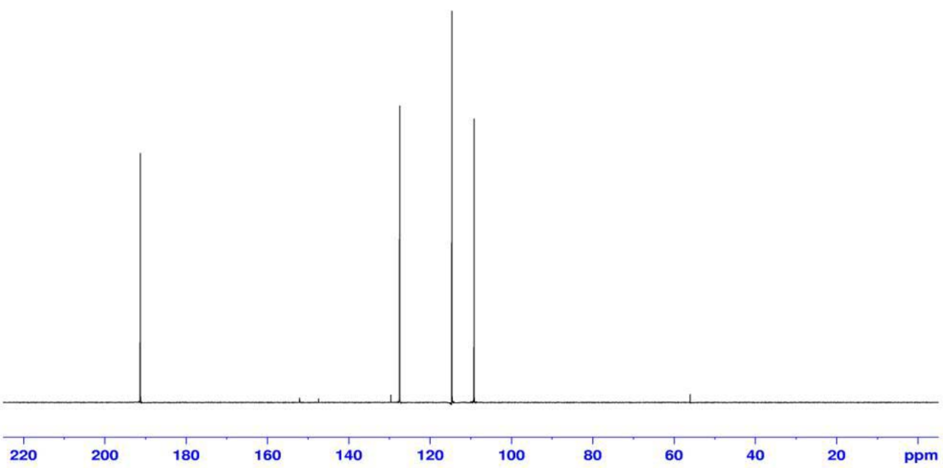


Figure 115 DEPT 90 (CDCl$_3$) of compound **RD12**

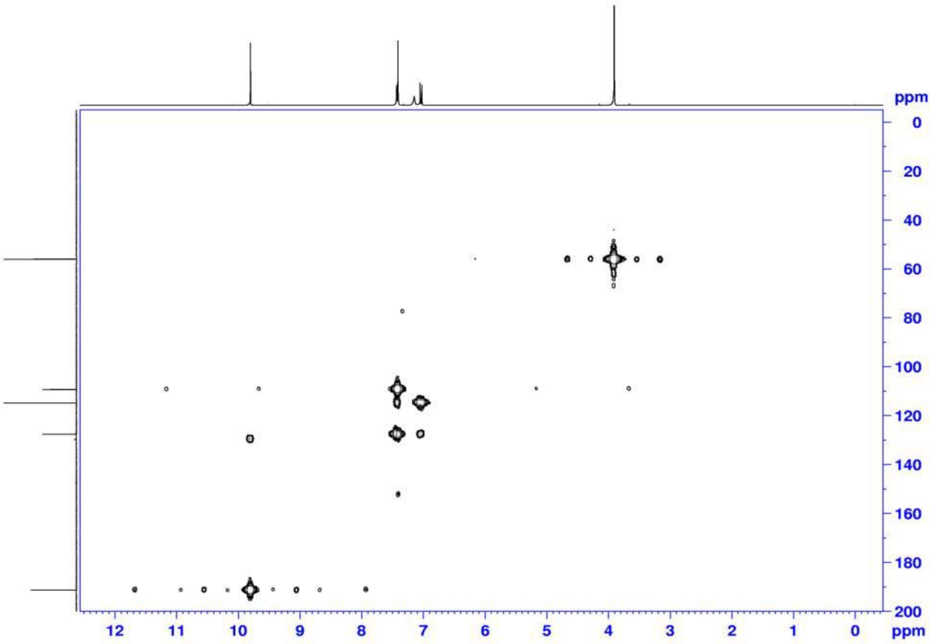


Figure 116 2D HMQC (CDCl$_3$) of compound **RD12**

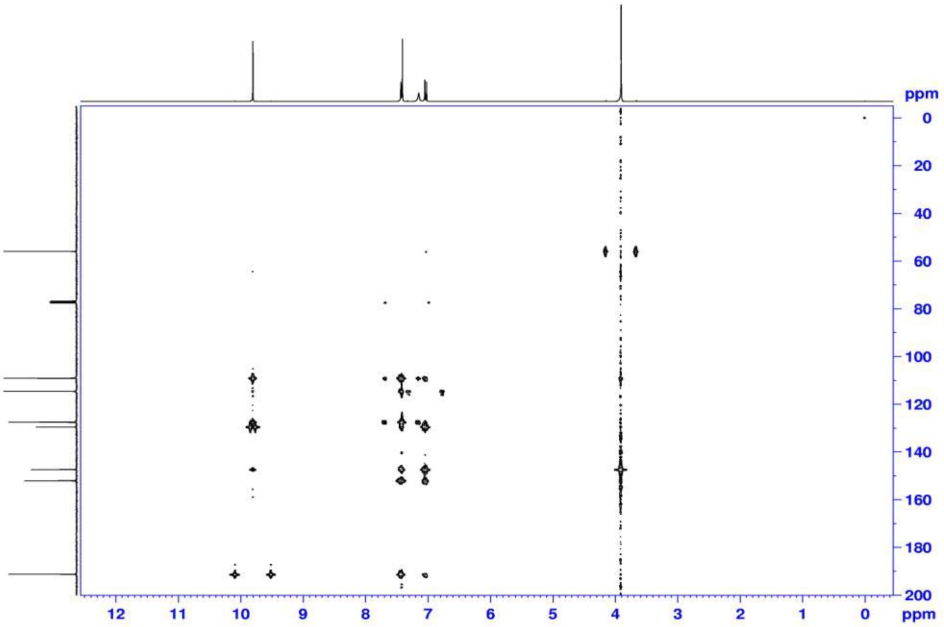


Figure 117 2D HMBC ($CDCl_3$) of compound **RD12**

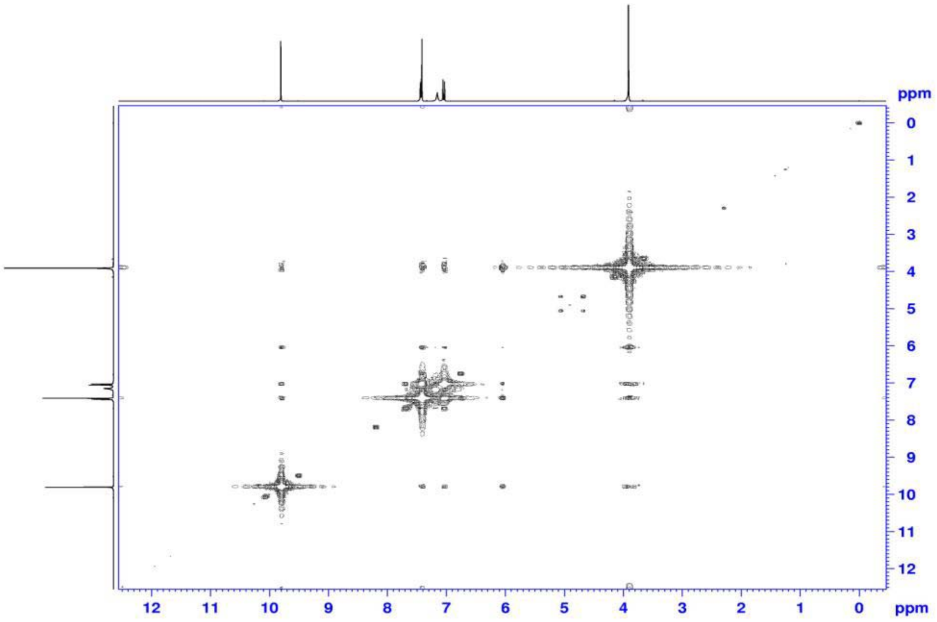


Figure 118 2D COSY ($CDCl_3$) of compound **RD12**

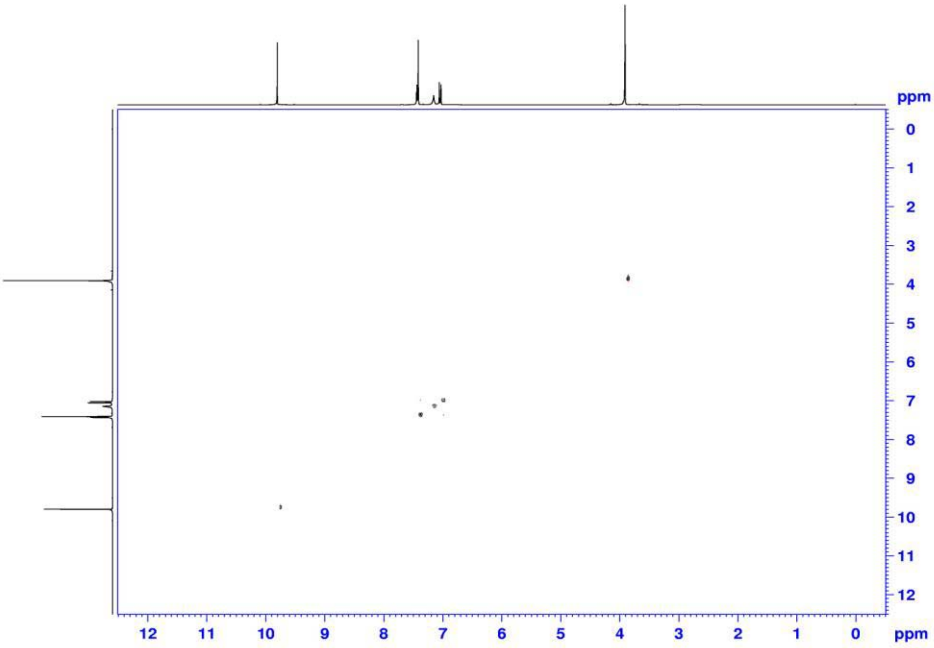


Figure 119 2D NOESY ($CDCl_3$) of compound **RD12**

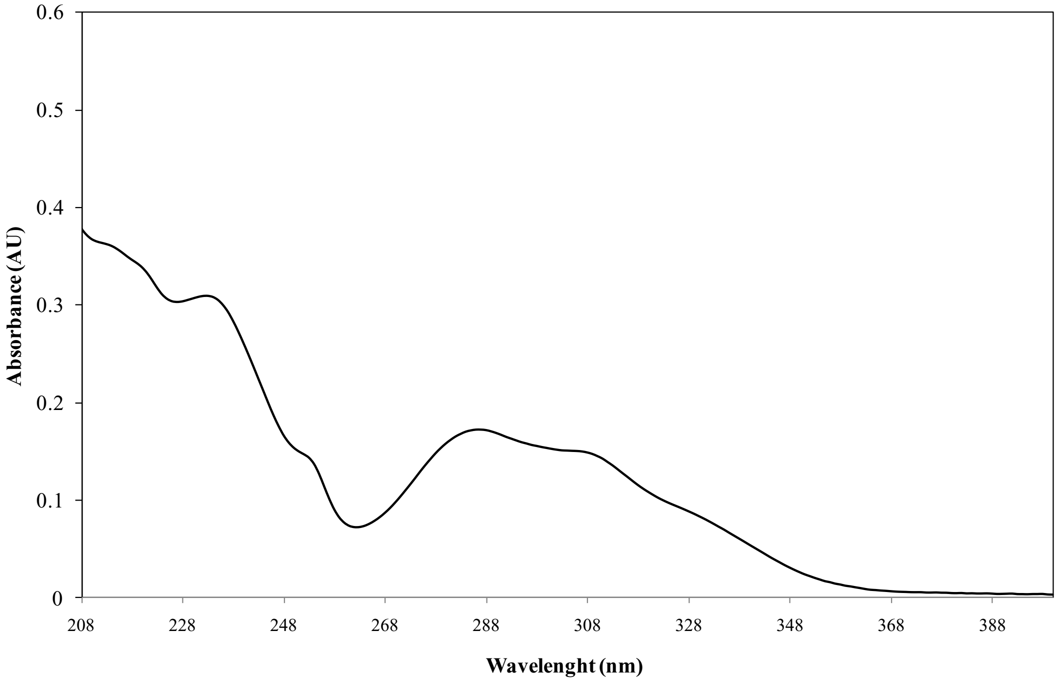


Figure 120 UV (MeOH) spectrum of compound **RA1**

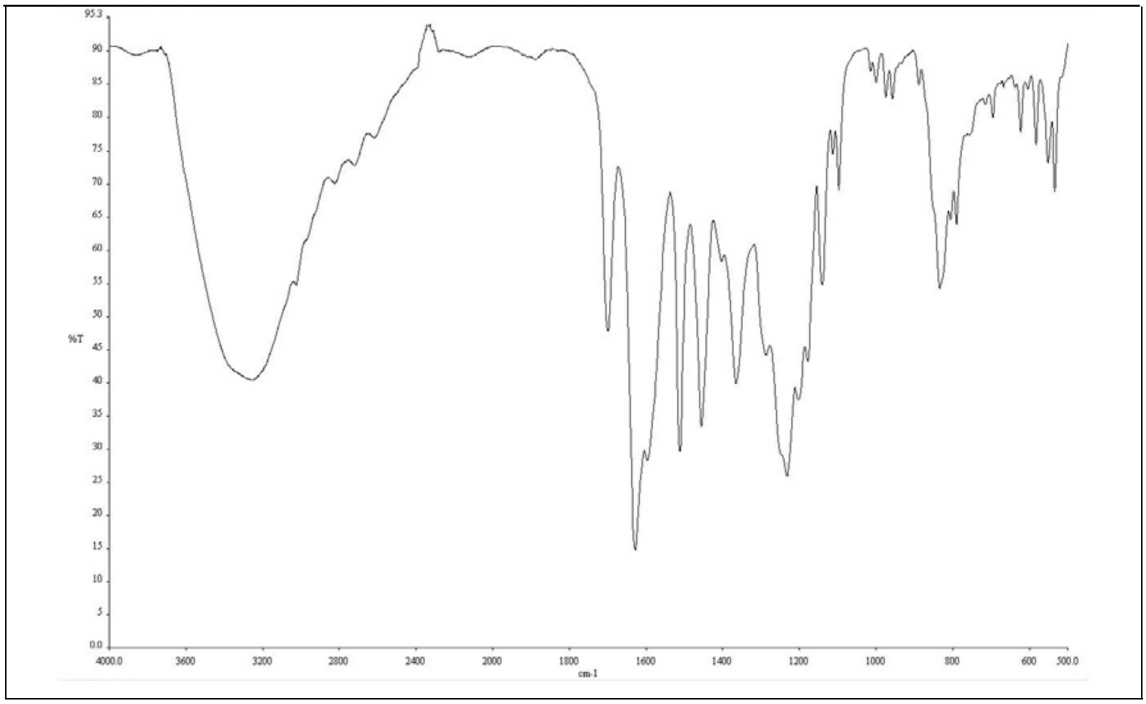


Figure 121 IR (neat) spectrum of compound **RA1**

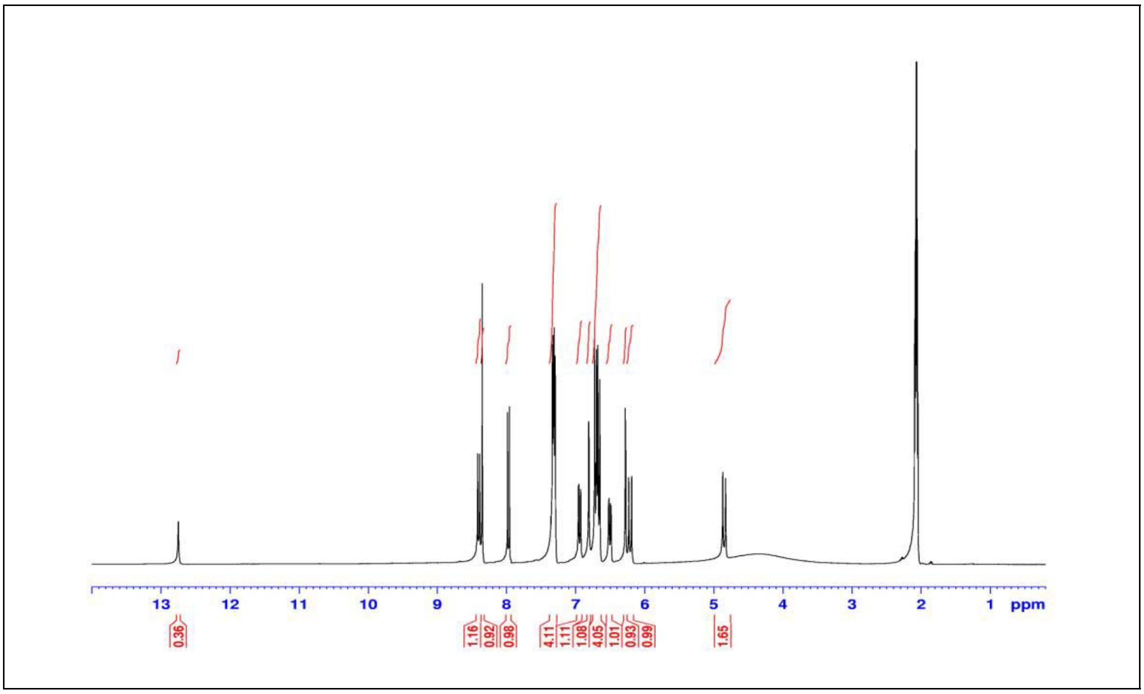


Figure 122 ^{1}H NMR (300 MHz) (Acetone-d_6) of compound **RA1**

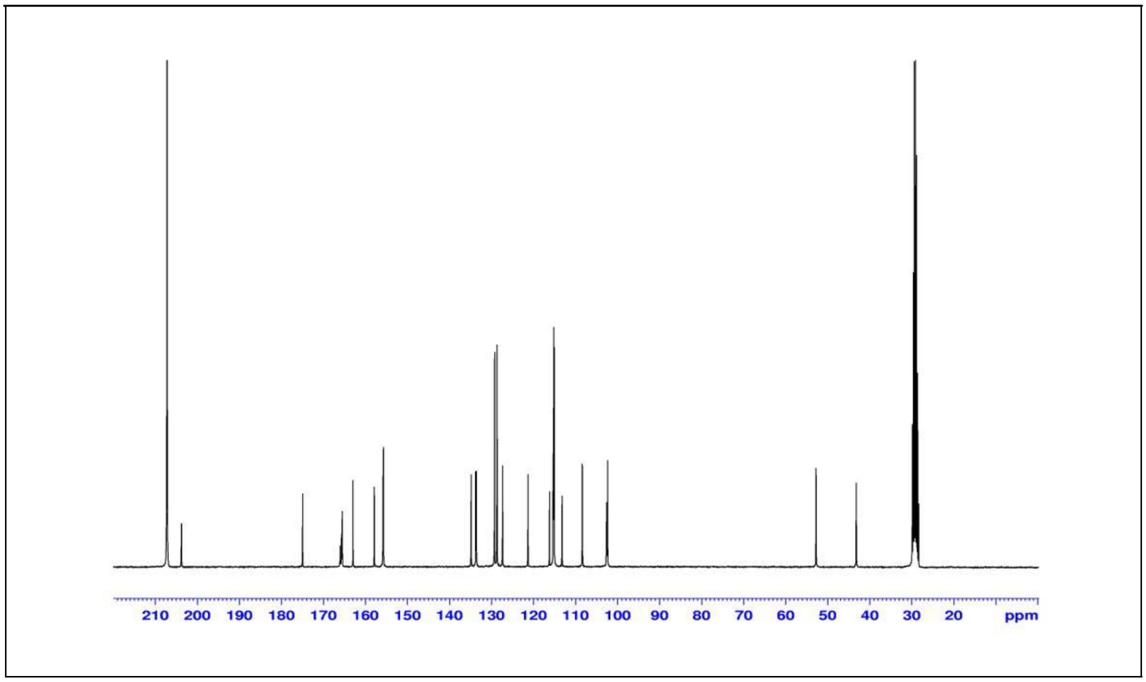


Figure 123 ^{13}C NMR (75 MHz) (Acetone-d_6) of compound **RA1**

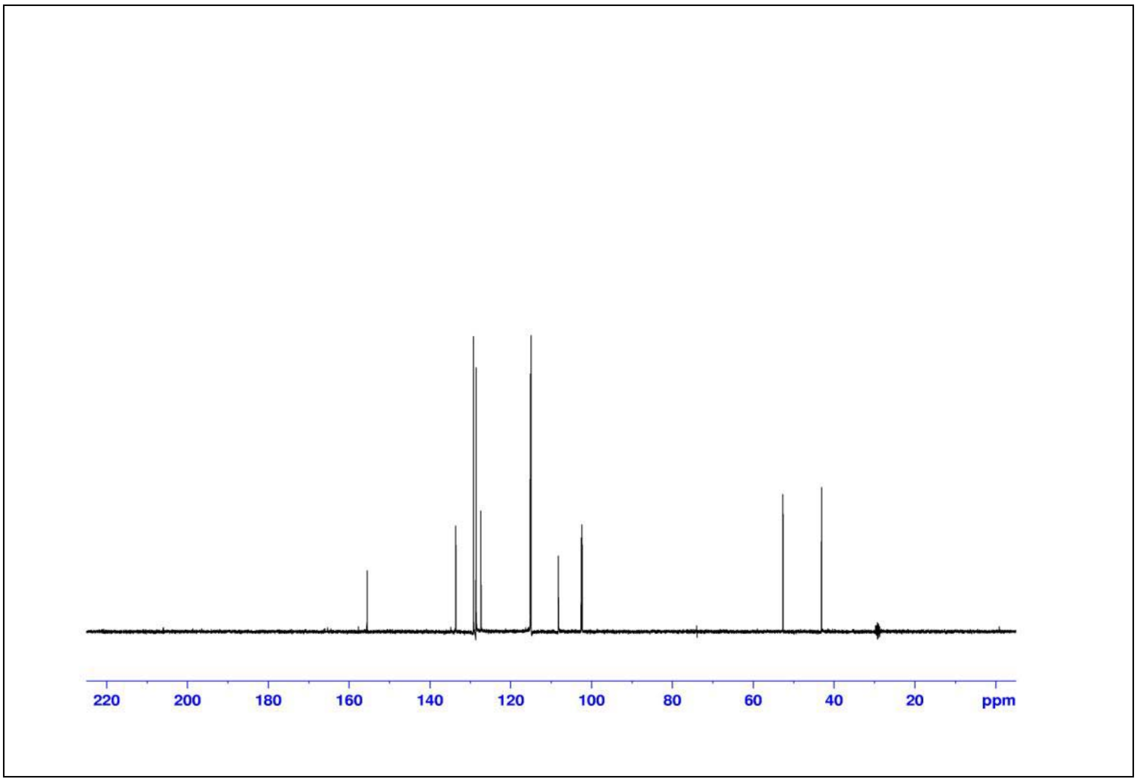


Figure 124 DEPT 135 (Acetone-d_6) of compound **RA1**

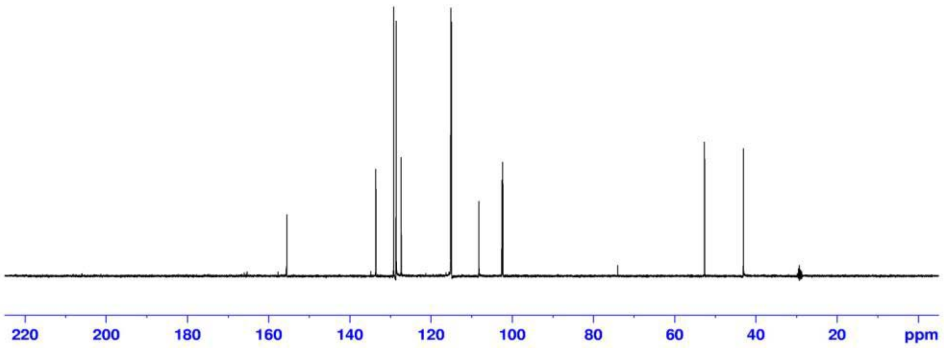


Figure 125 DEPT 90 (Acetone-d_6) of compound **RA1**

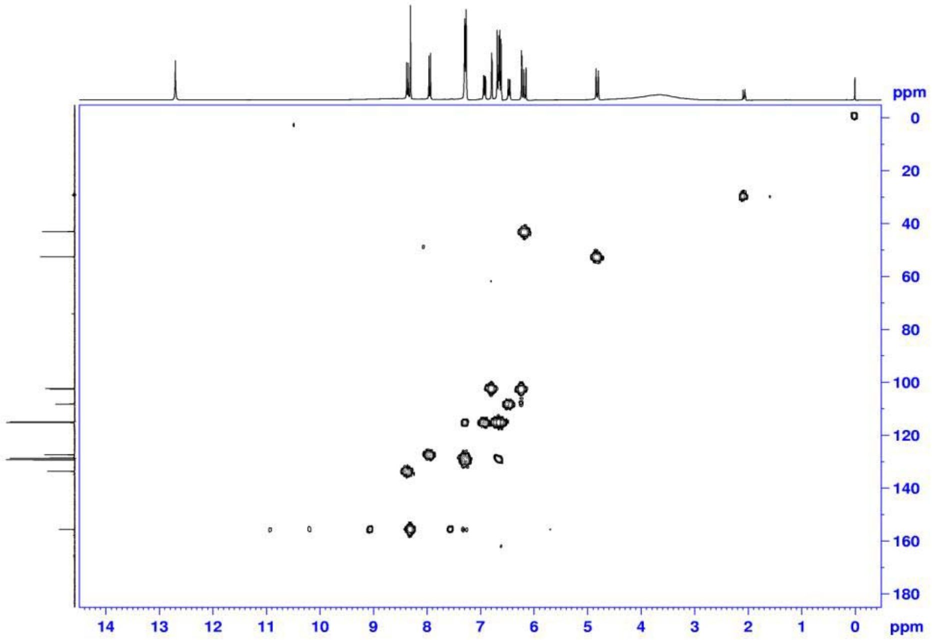


Figure 126 2D HMQC (Acetone-d_6) of compound **RA1**

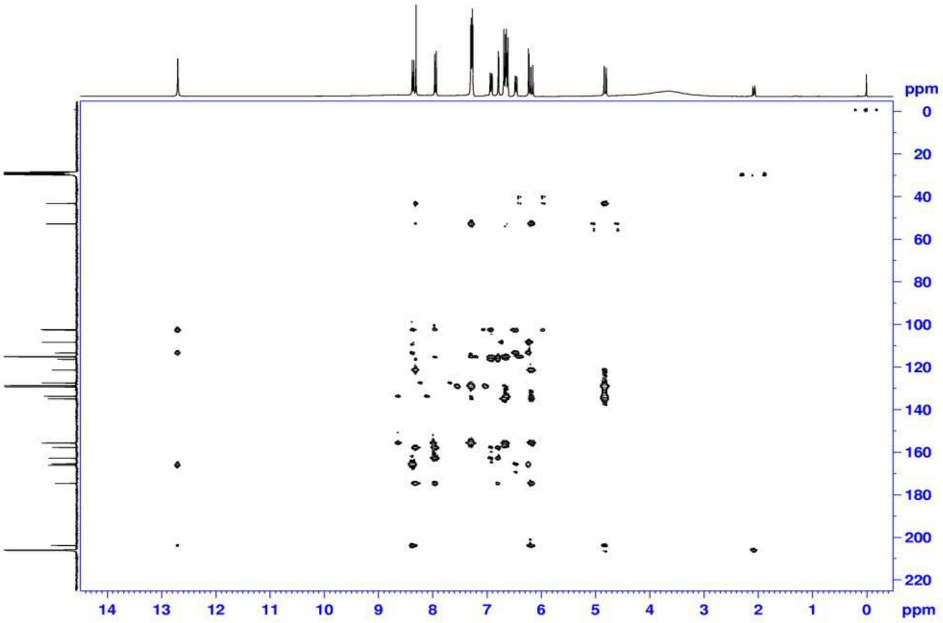


Figure 127 2D HMBC (Acetone-d_6) of compound **RA1**

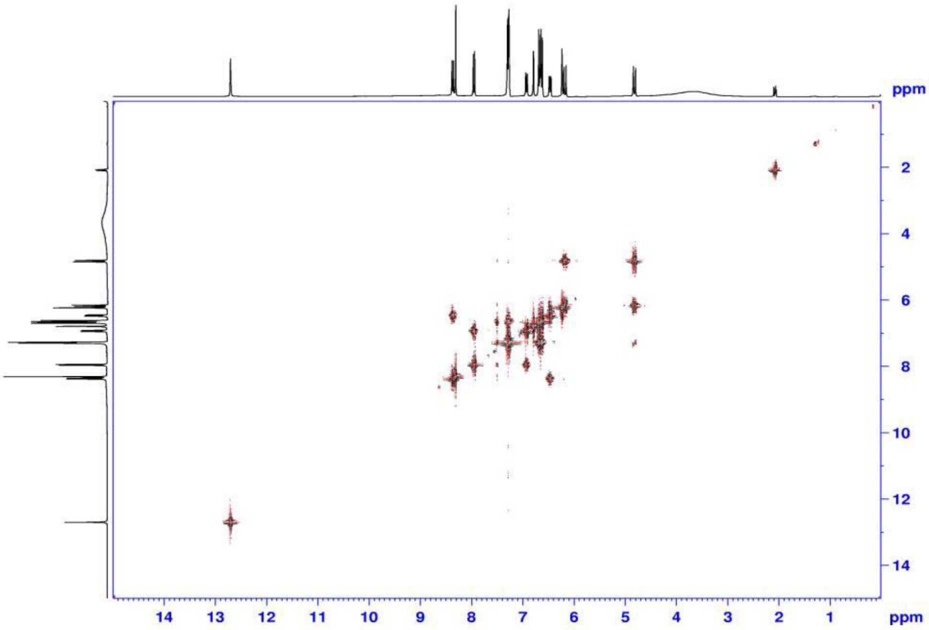


Figure 128 2D COSY (Acetone-d_6) of compound **RA1**

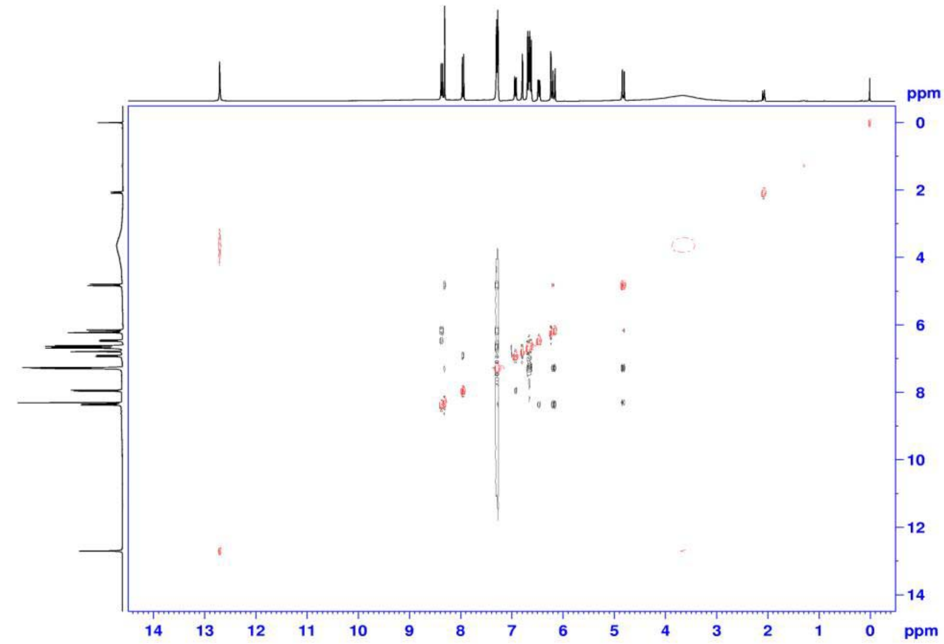


Figure 129 2D NOESY (Acetone-d_6) of compound **RA1**

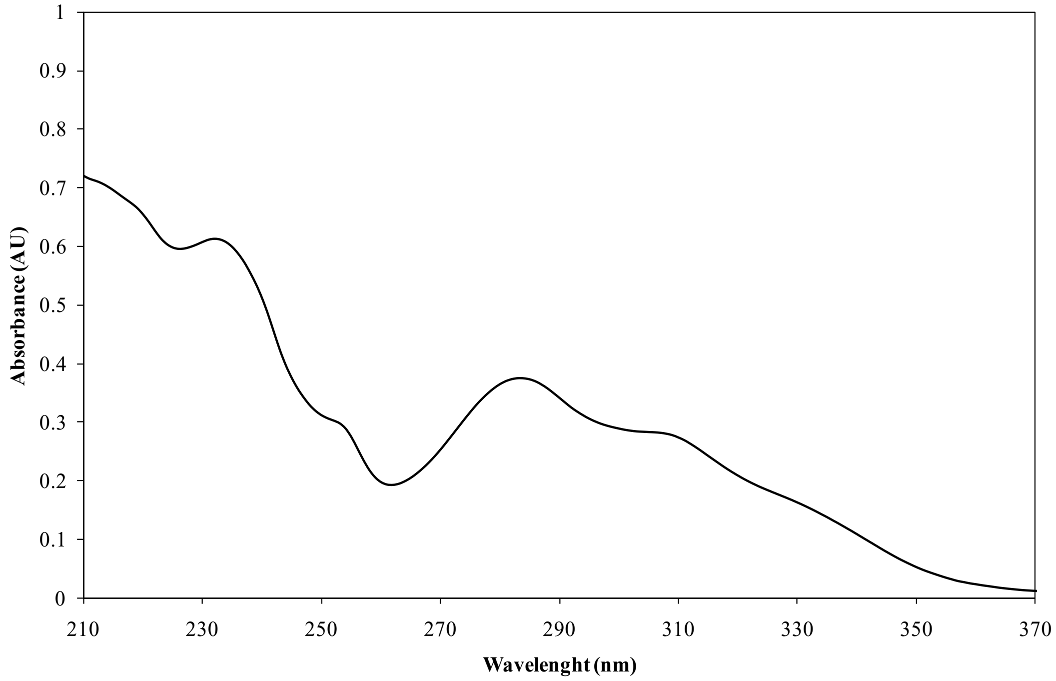


Figure 130 UV (MeOH) spectrum of compound **RA2**

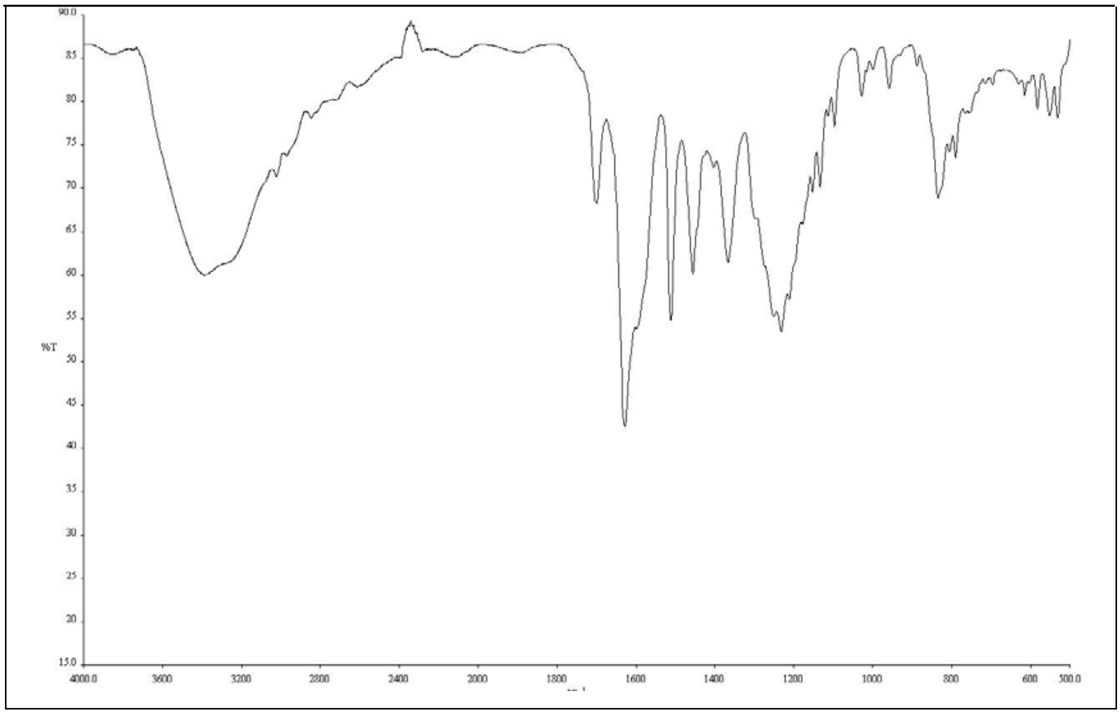


Figure 131 IR (neat) spectrum of compound **RA2**

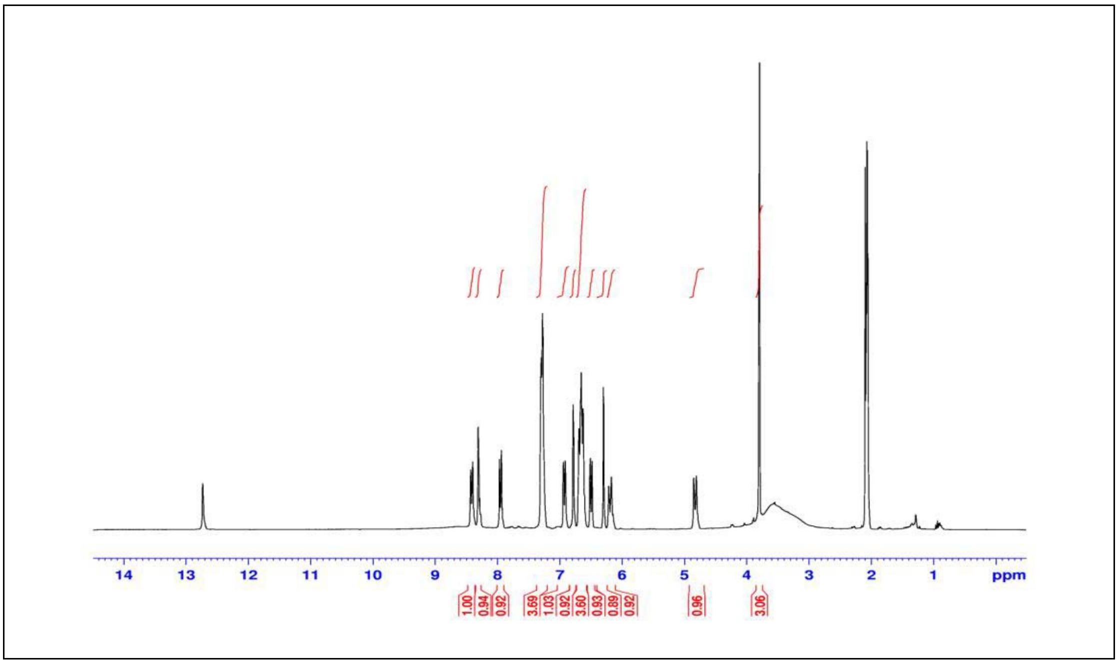


Figure 132 ^{1}H NMR (300 MHz) (Acetone-d_6) of compound **RA2**

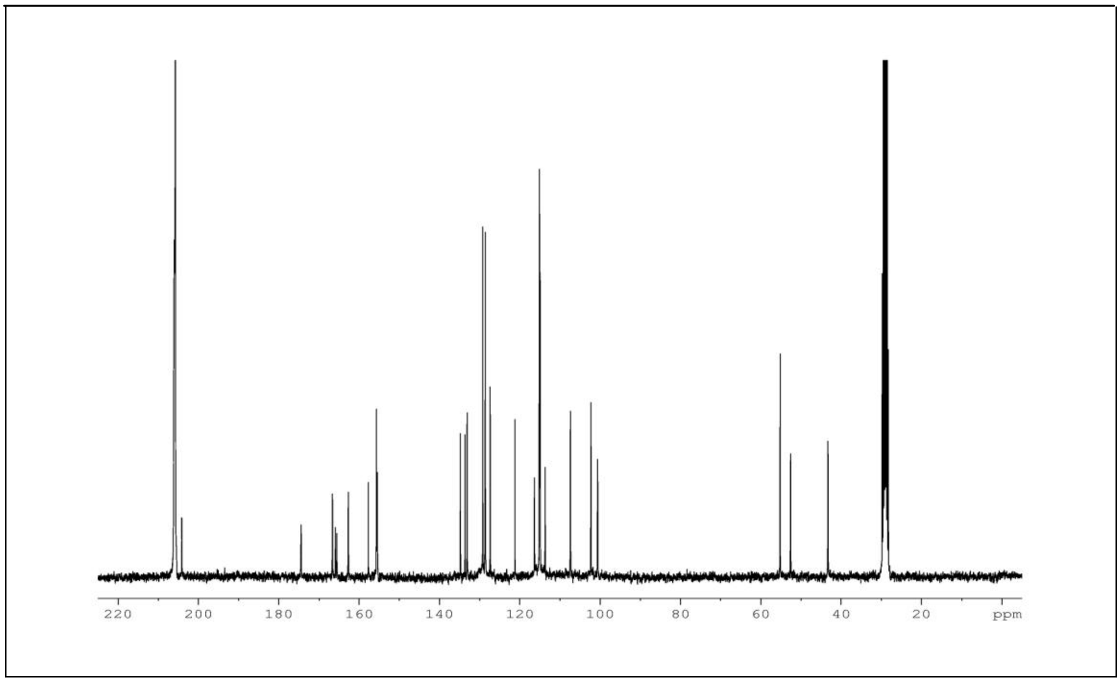


Figure 133 ^{13}C NMR (75 MHz) (Acetone-d_6) of compound **RA2**

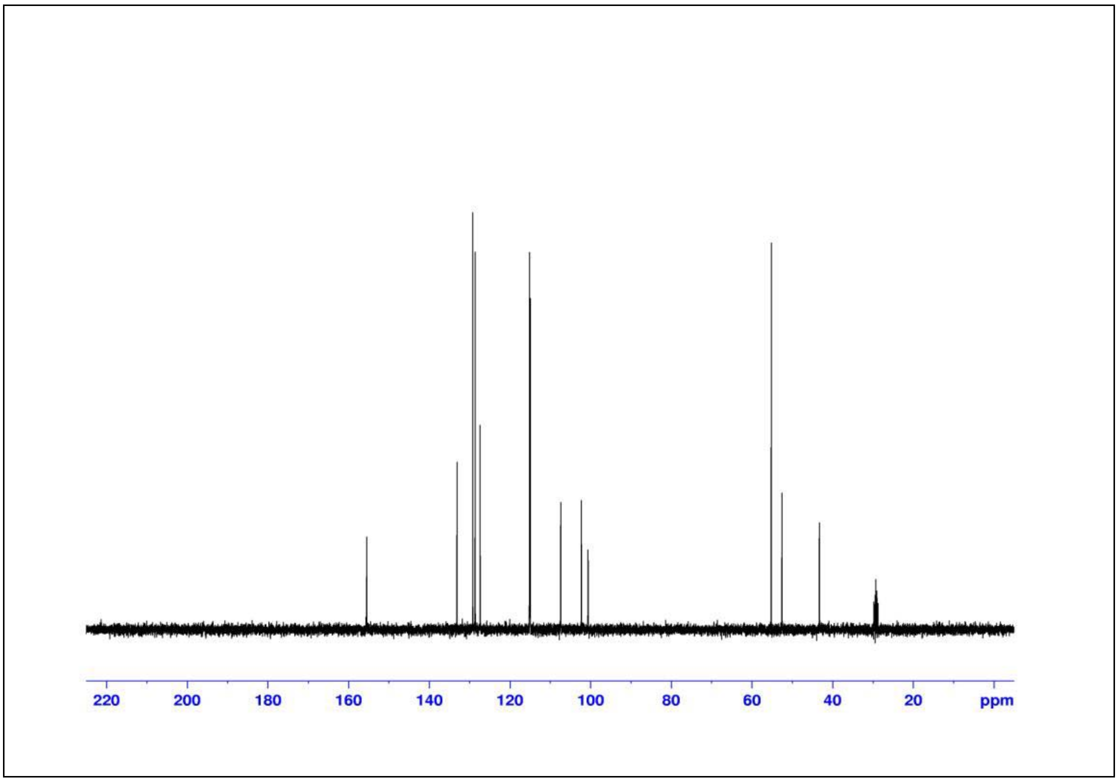


Figure 134 DEPT 135 (Acetone-d_6) of compound **RA2**

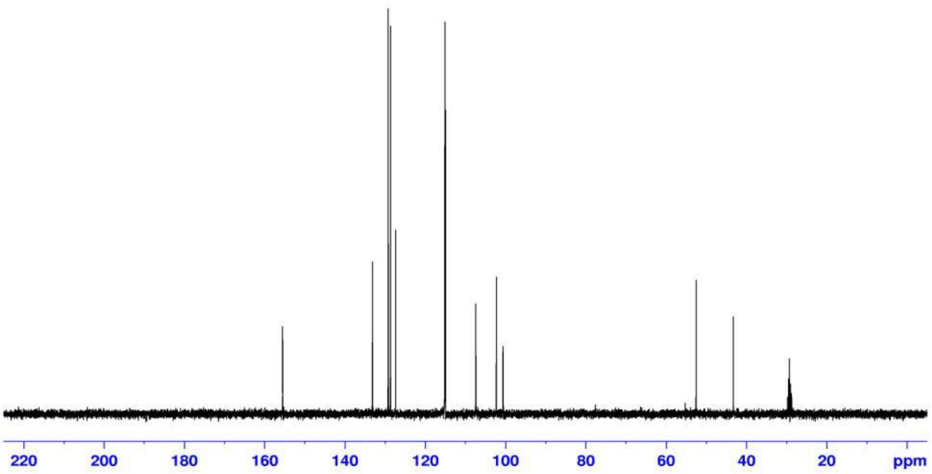


Figure 135 DEPT 90 (Acetone-d_6) of compound **RA2**

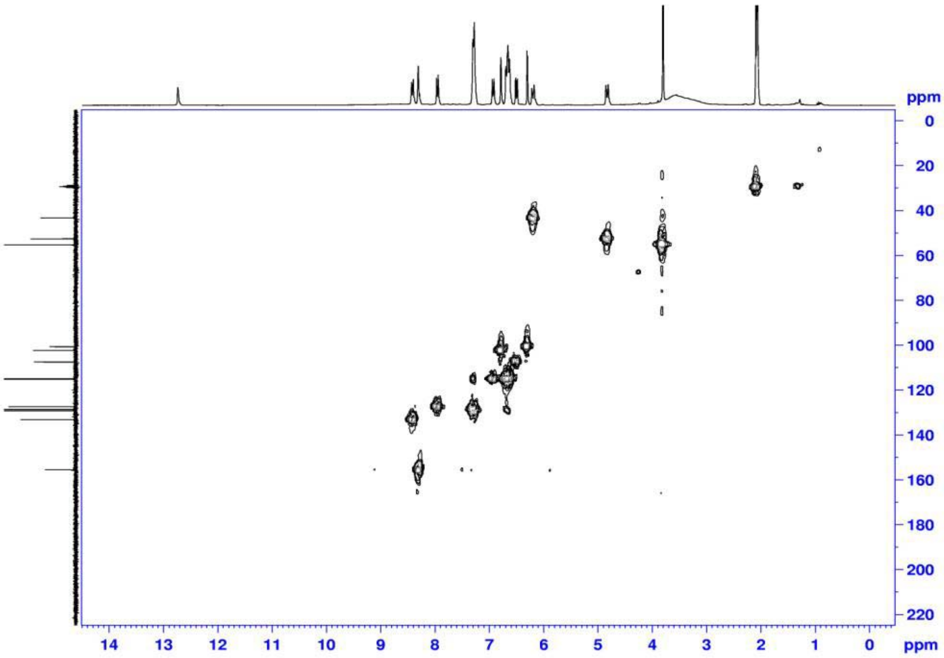


Figure 136 2D HMQC (Acetone-d_6) of compound **RA2**

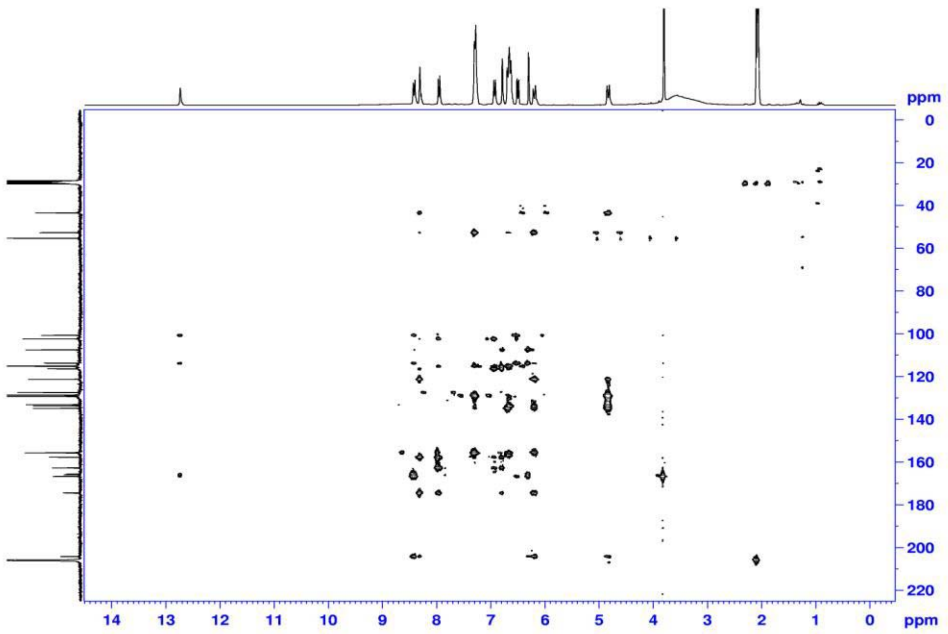


Figure 137 2D HMBC (Acetone-d_6) of compound **RA2**

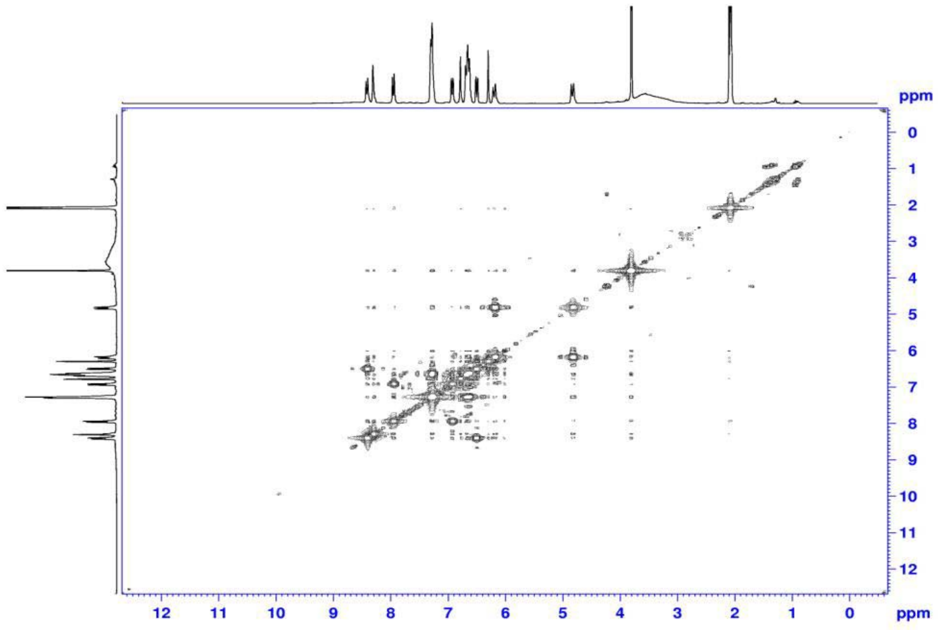


Figure 138 2D COSY (Acetone-d_6) of compound **RA2**

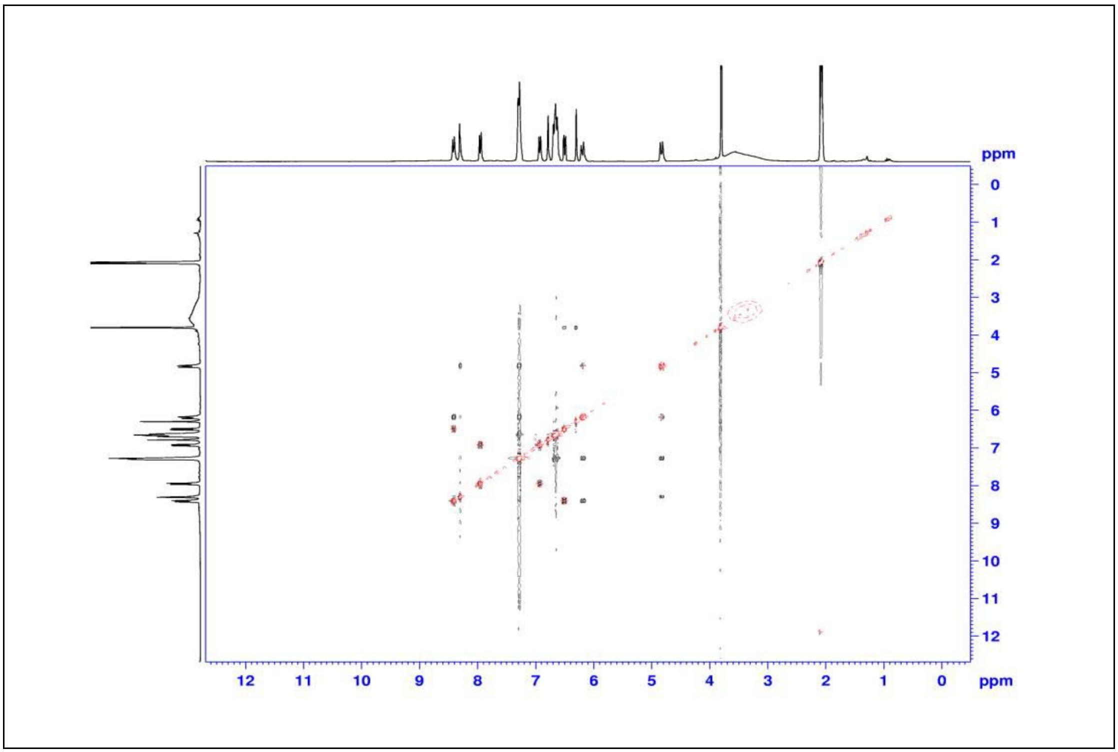


Figure 139 2D NOESY (Acetone-d_6) of compound **RA2**

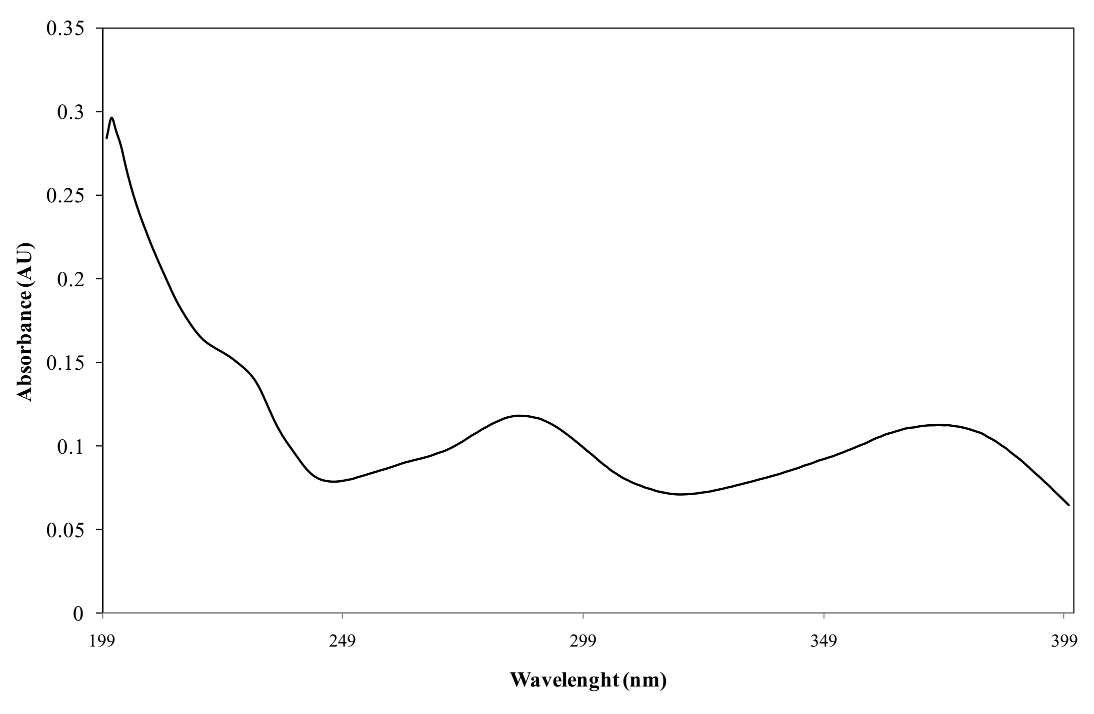


Figure 140 UV (MeOH) spectrum of compound **RA3**

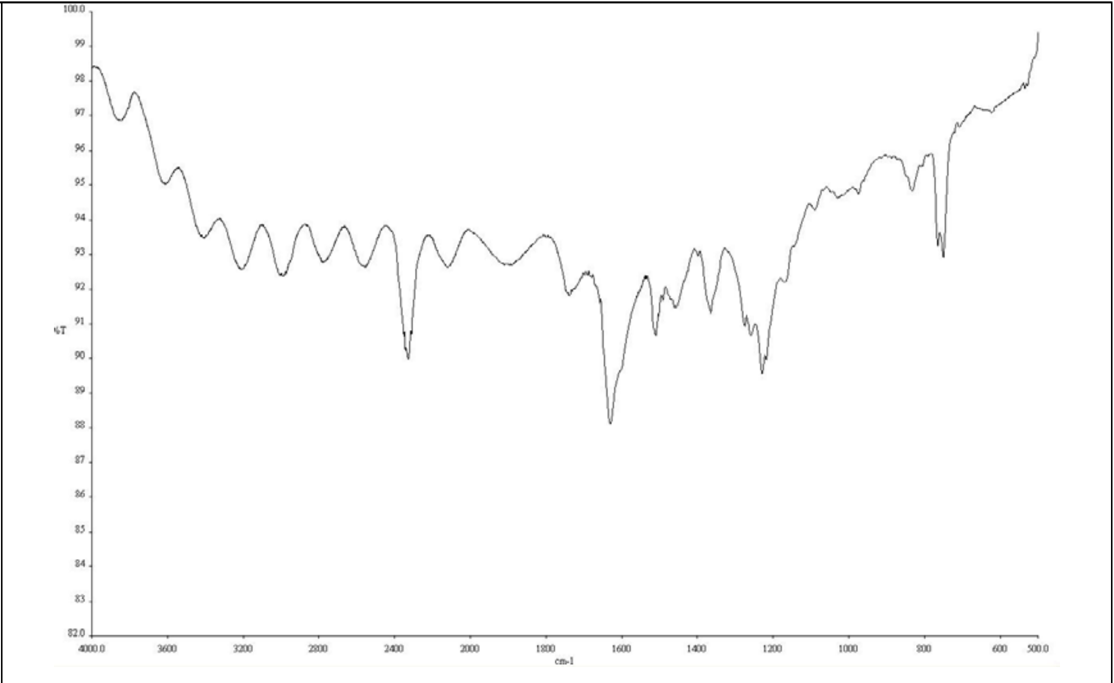


Figure 141 IR (neat) spectrum of compound **RA3**

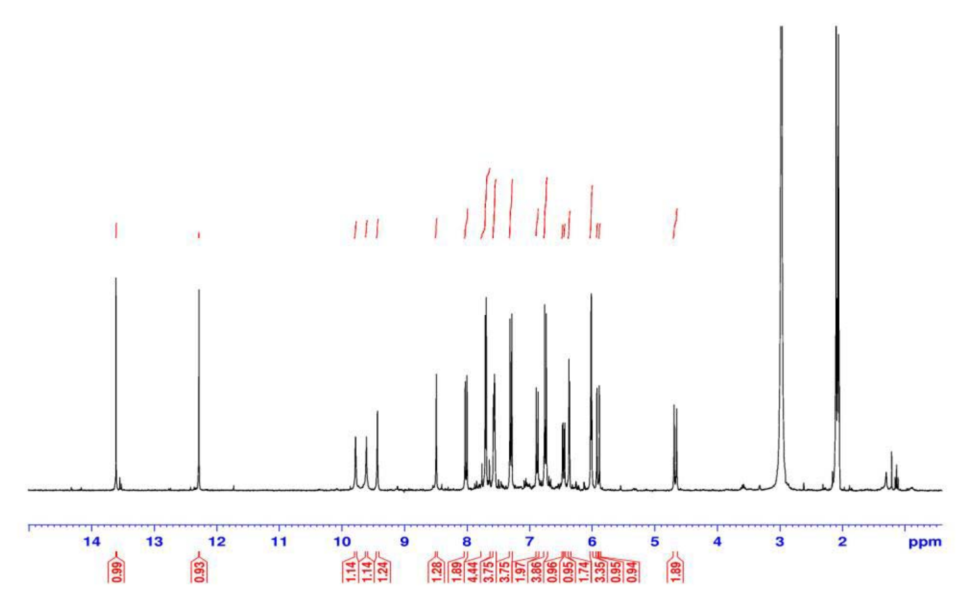


Figure 142 ^{1}H NMR (300 MHz) (Acetone-d_6) of compound **RA3**

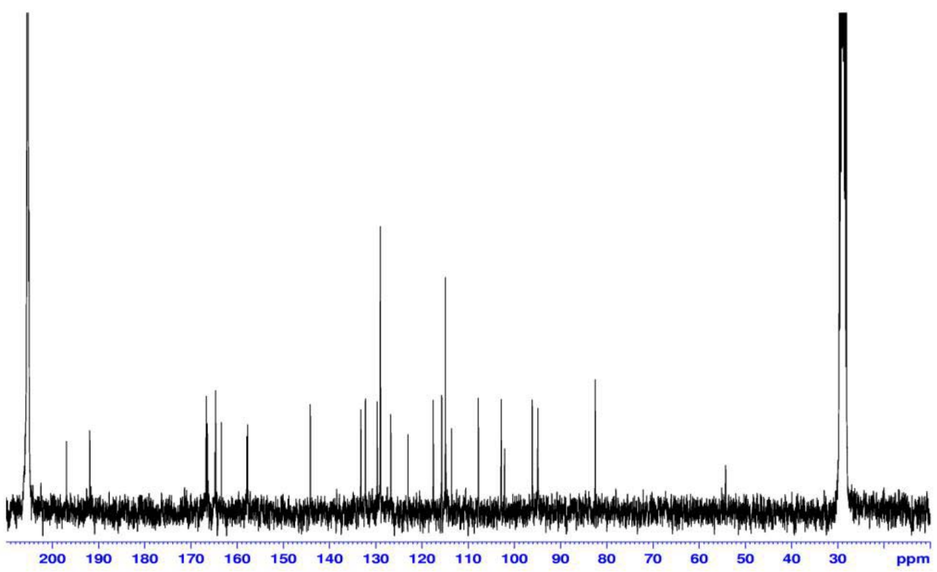


Figure 143 ^{13}C NMR (75 MHz) (Acetone-d_6) of compound **RA3**

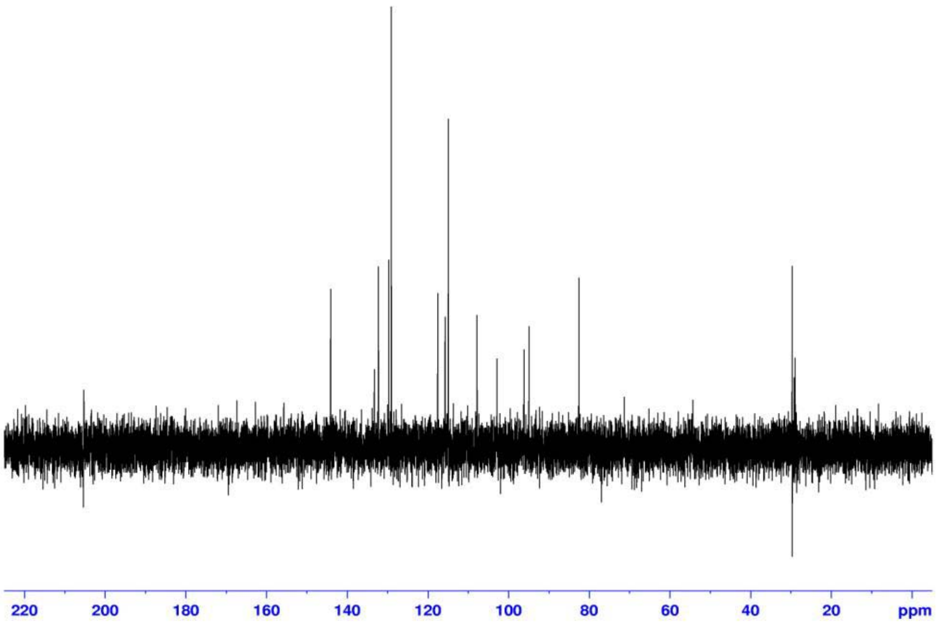


Figure 144 DEPT 135 (Acetone-d_6) of compound **RA3**

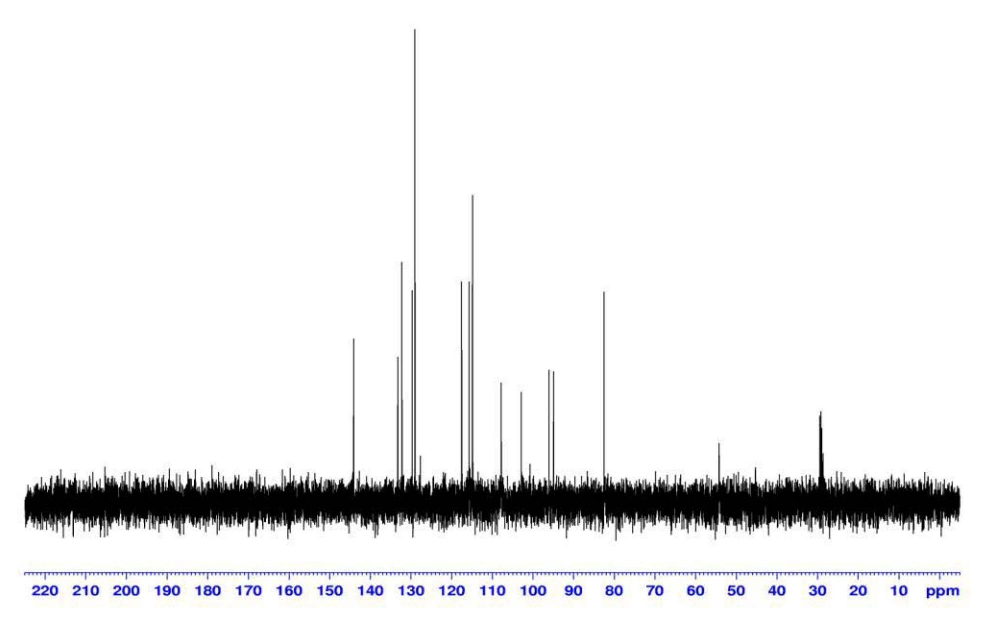


Figure 145 DEPT 90 (Acetone-d_6) of compound **RA3**

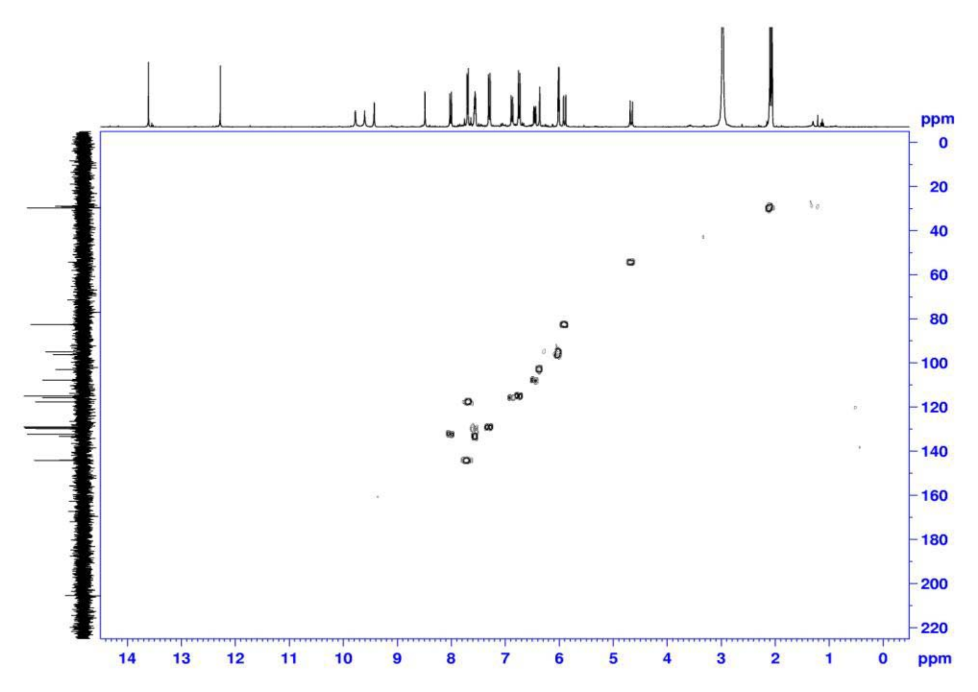


Figure 146 2D HMQC (Acetone-d_6) of compound **RA3**

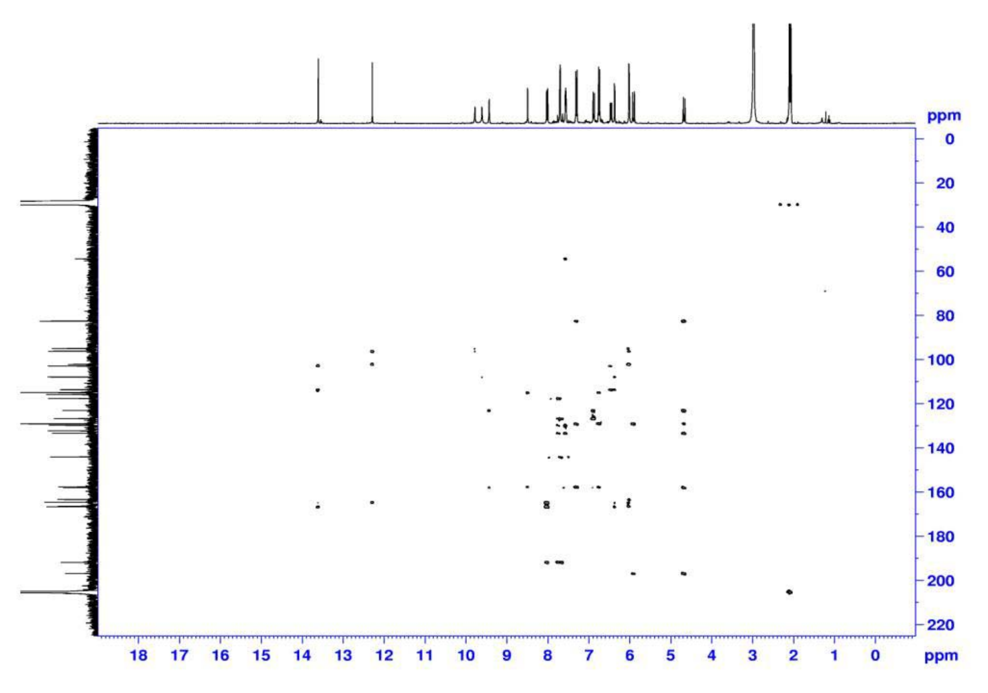


Figure 147 2D HMBC (Acetone-d_6) of compound **RA3**

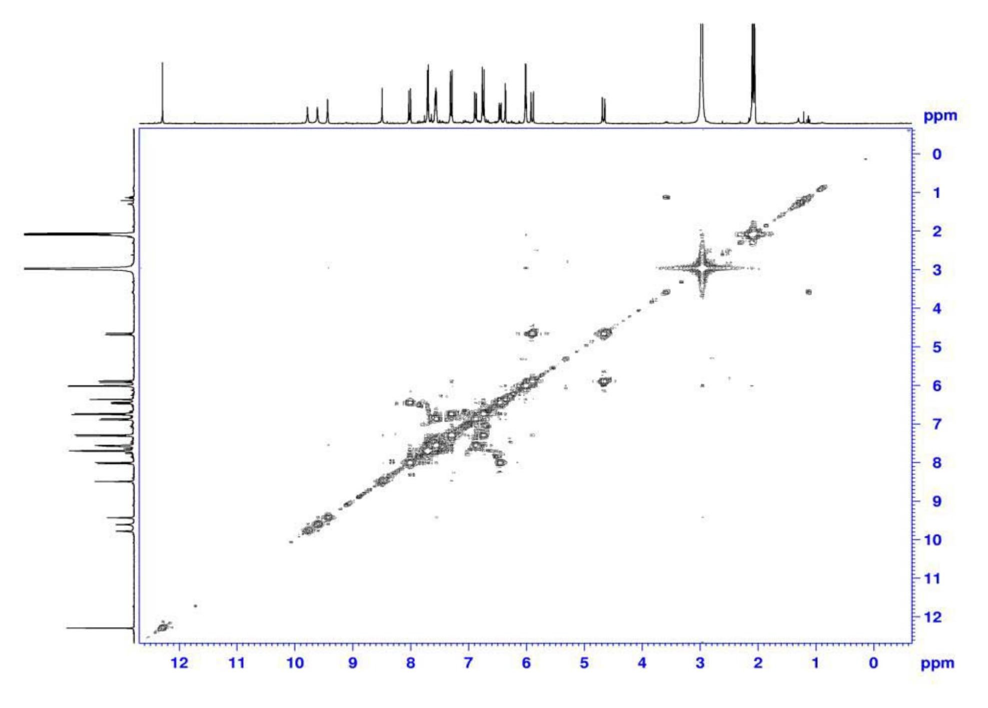


Figure 148 2D COSY (Acetone-d_6) of compound **RA3**

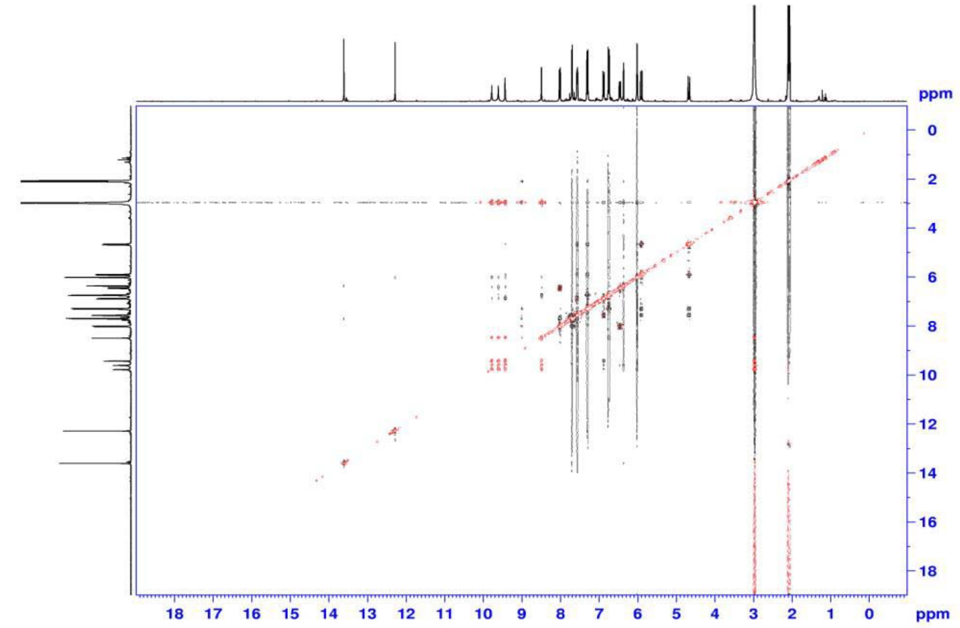


Figure 149 2D NOESY (Acetone-d_6) of compound **RA3**

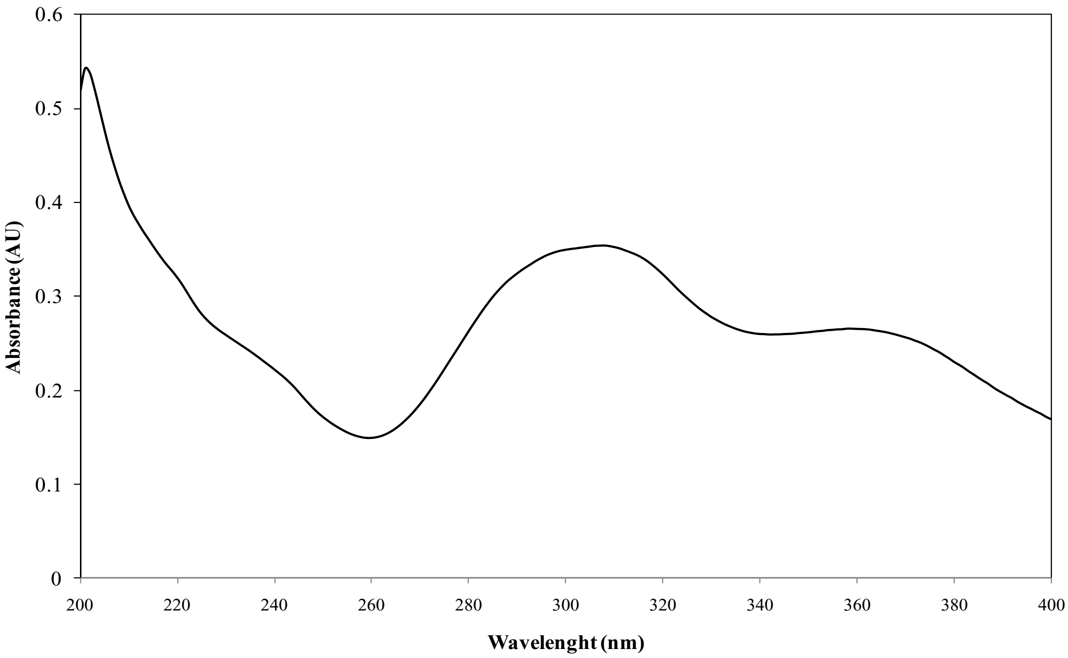


Figure 150 UV (MeOH) spectrum of compound **RA4**

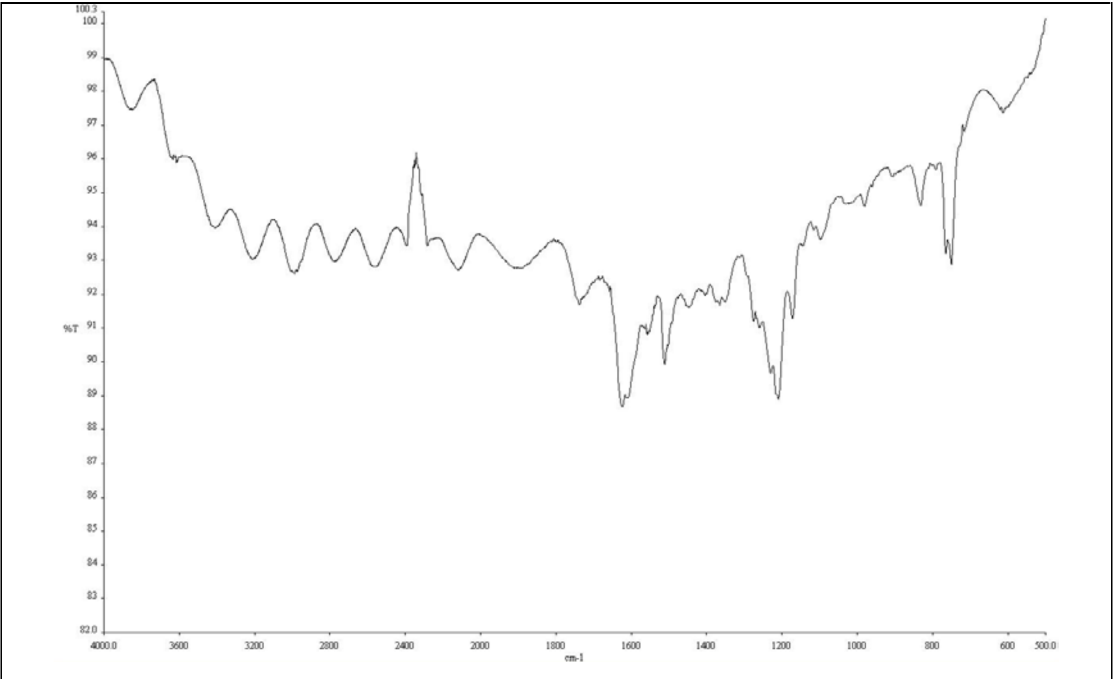


Figure 151 IR (neat) spectrum of compound **RA4**

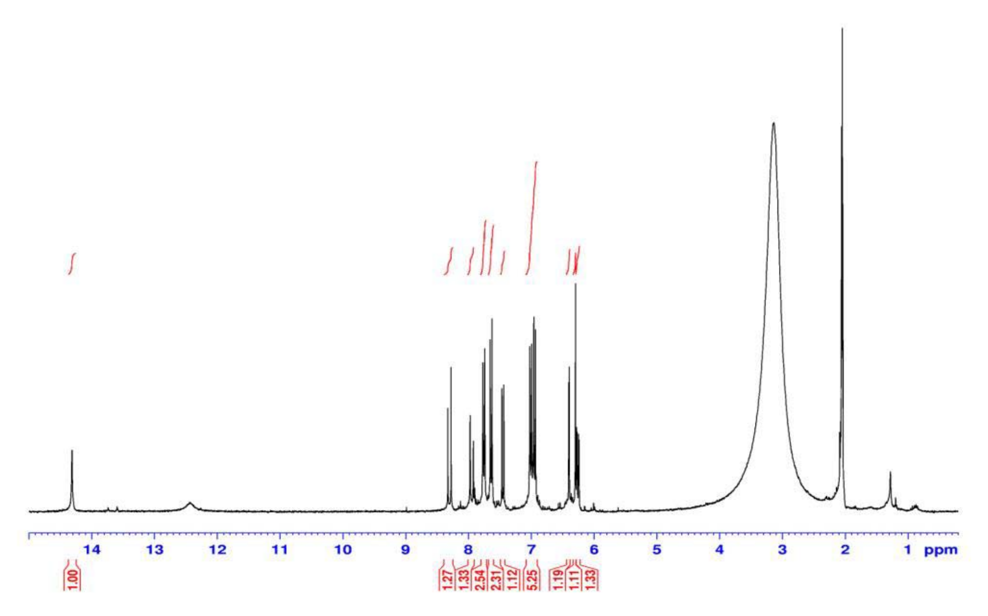


Figure 152 ^{1}H NMR (300 MHz) (Acetone-d_6) of compound **RA4**

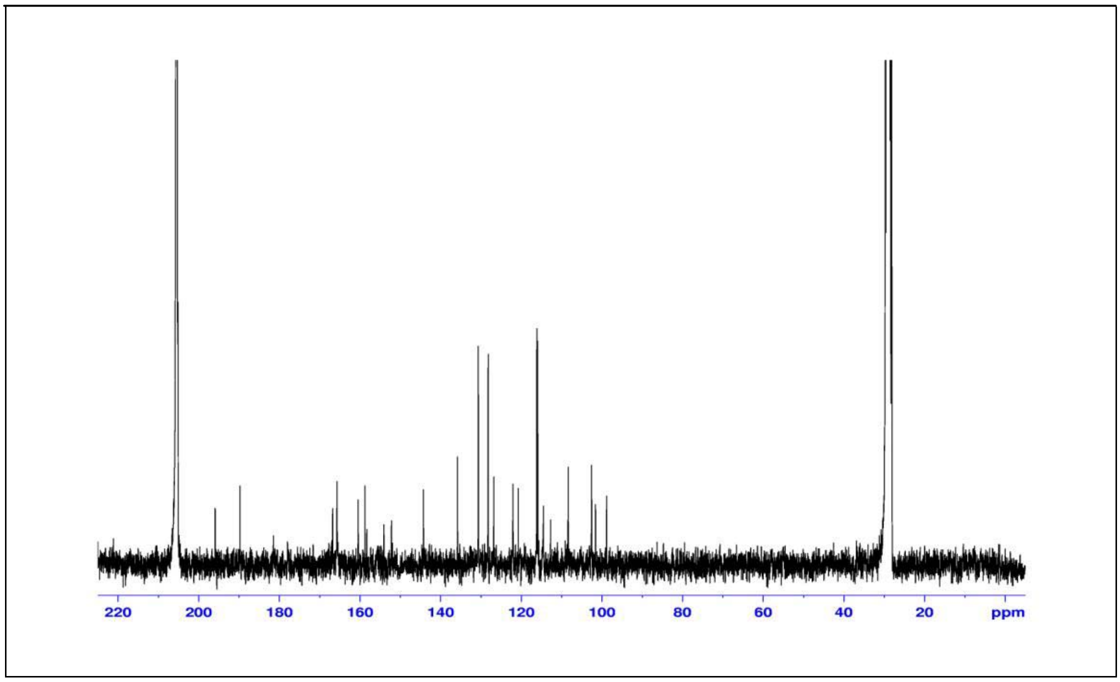


Figure 153 ^{13}C NMR (75 MHz) (Acetone-d_6) of compound **RA4**

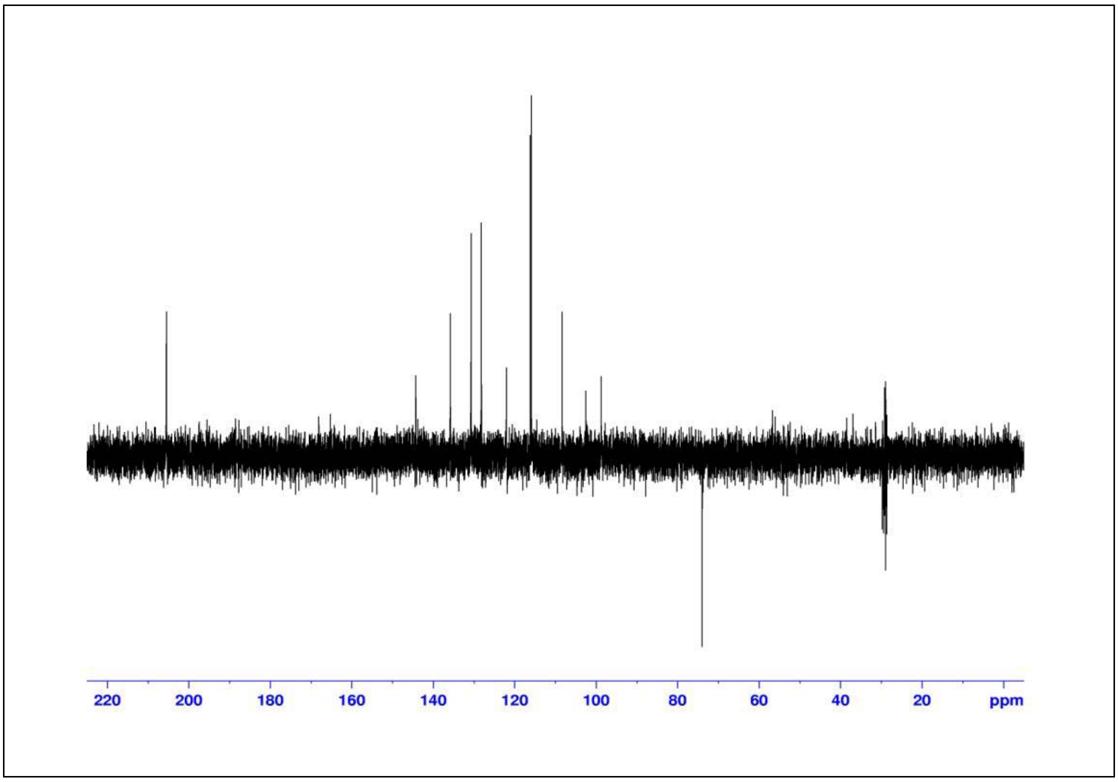


Figure 154 DEPT 135 (Acetone-d_6) of compound **RA4**

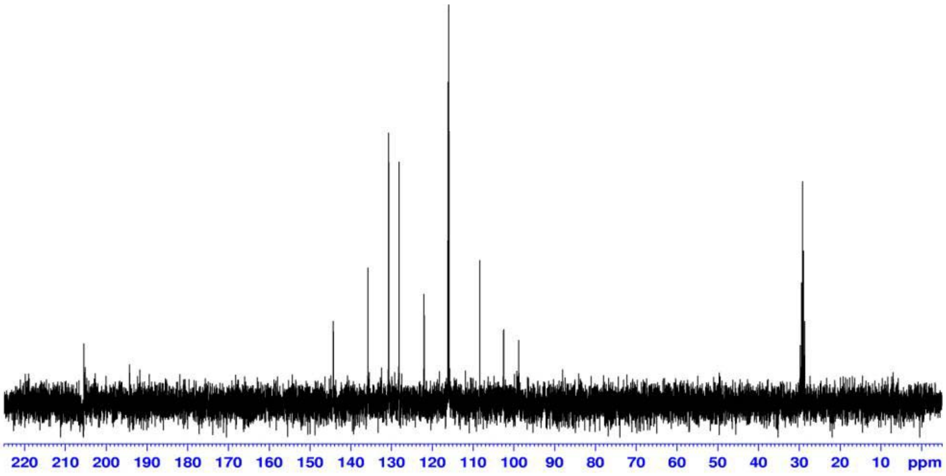


Figure 155 DEPT 90 (Acetone-d_6) of compound **RA4**

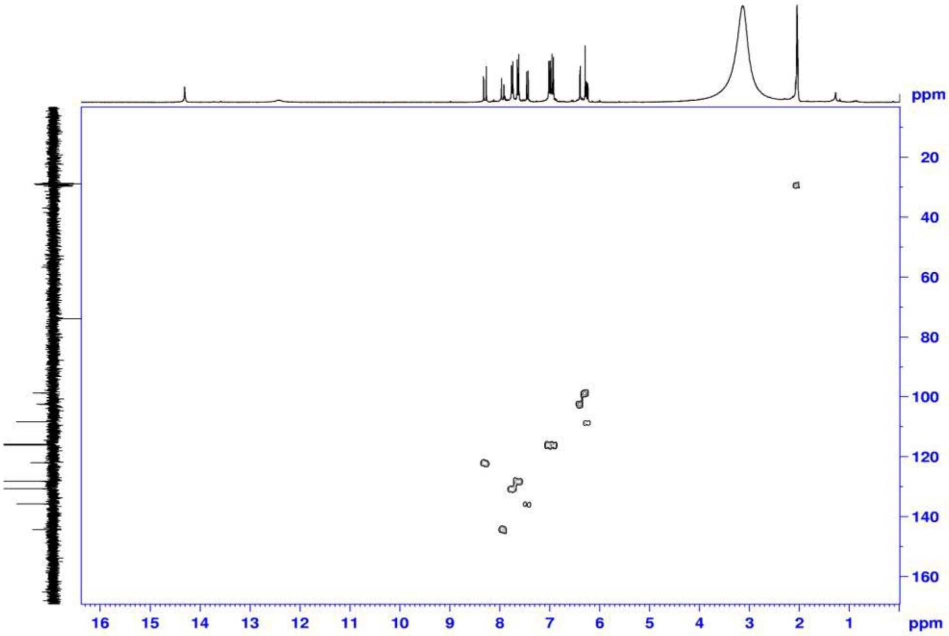


Figure 156 2D HMQC (Acetone-d_6) of compound **RA4**

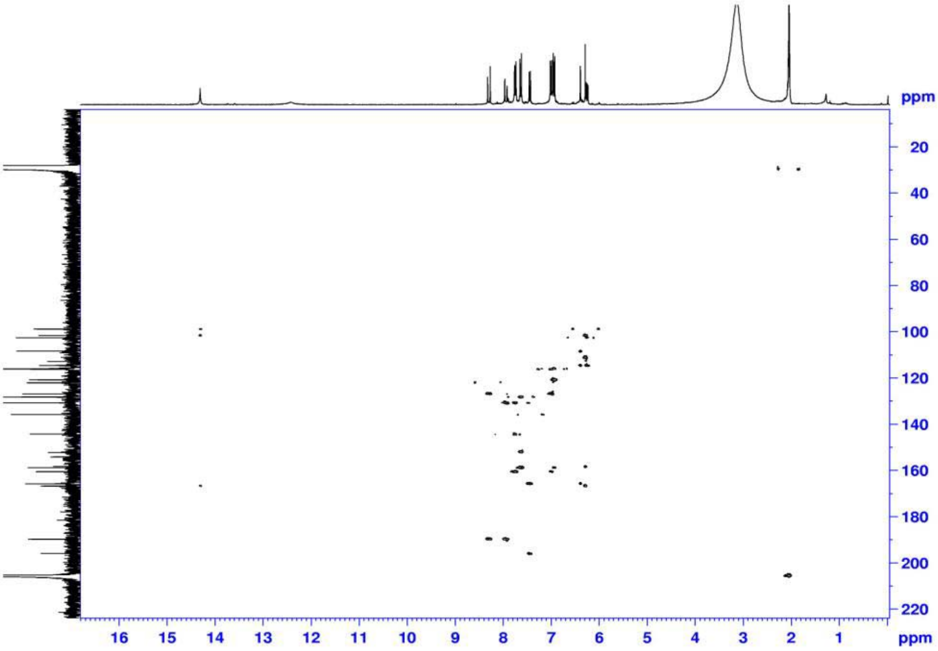


Figure 157 2D HMBC (Acetone-d_6) of compound **RA4**

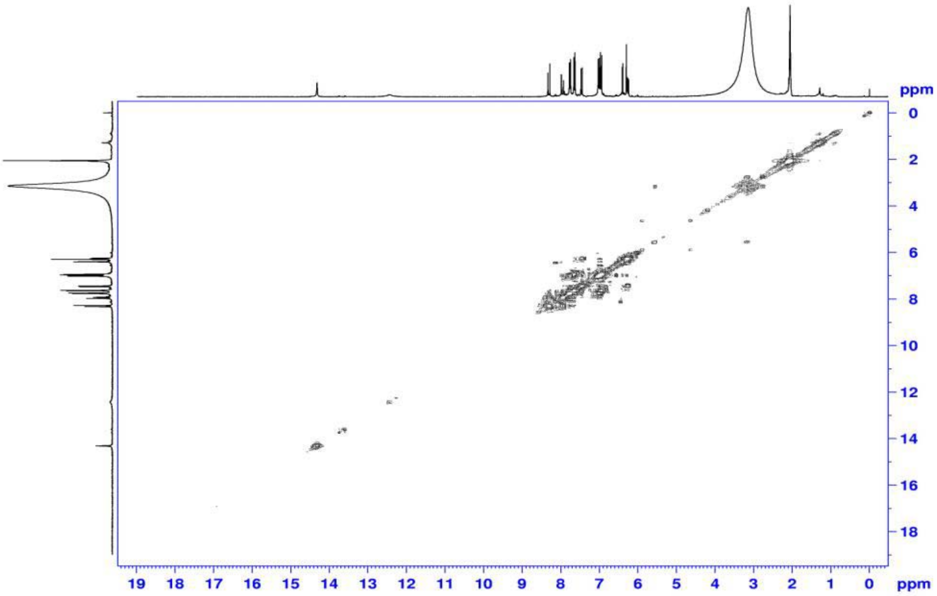


Figure 158 2D COSY (Acetone-d_6) of compound **RA4**

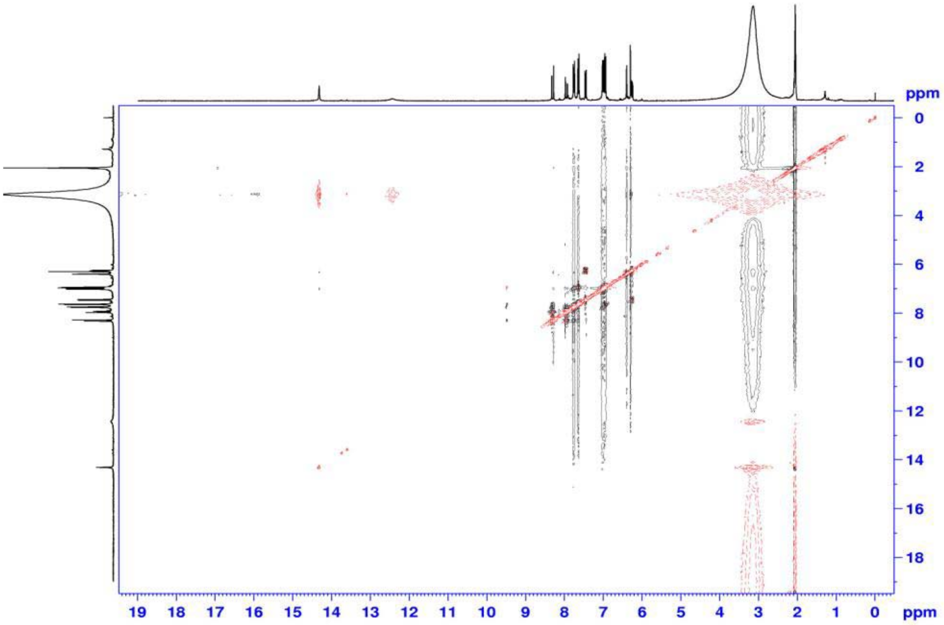


Figure 159 2D NOESY (Acetone-d_6) of compound **RA4**

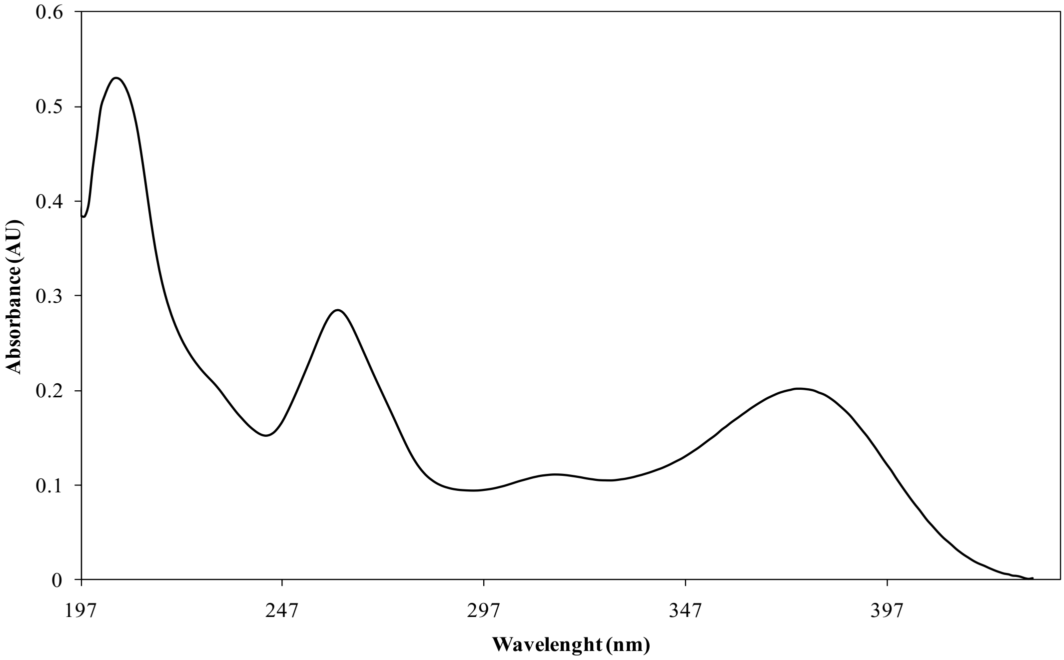


Figure 160 UV (MeOH) spectrum of compound **RA5**

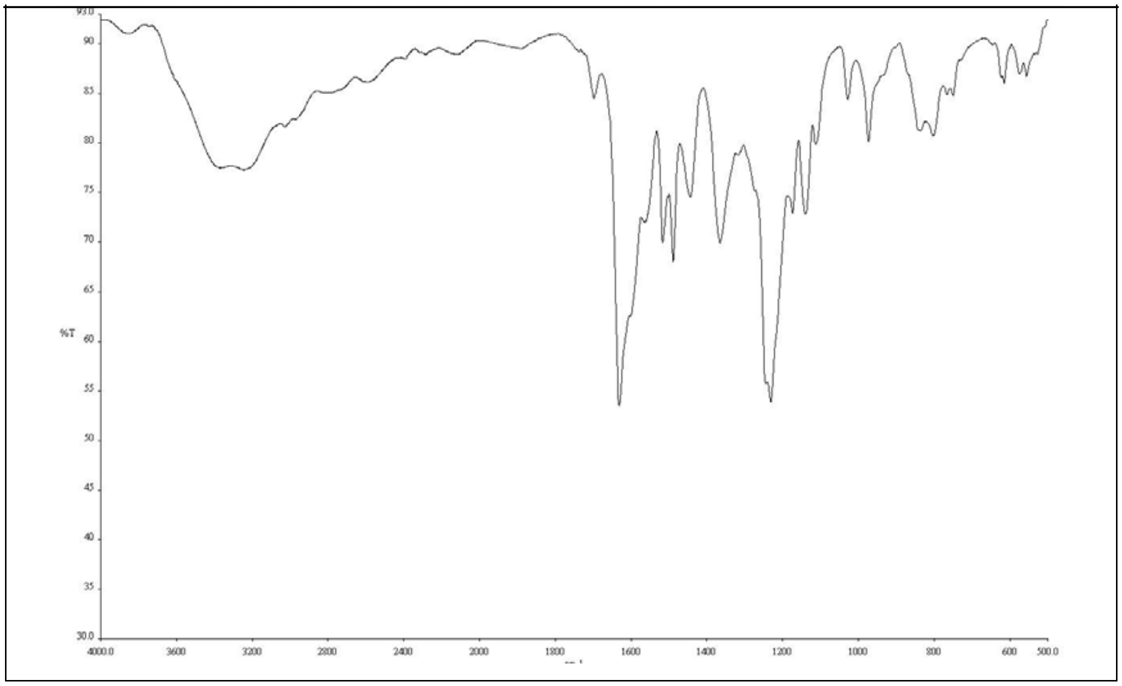


Figure 161 IR (neat) spectrum of compound **RA5**

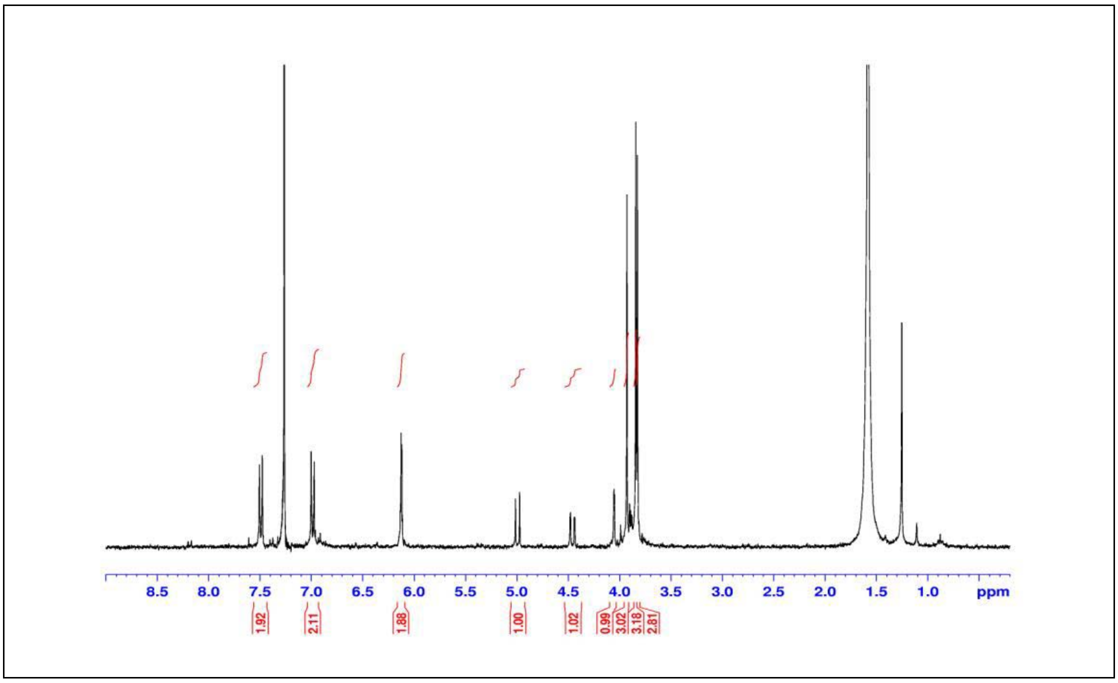


Figure 162 ^{1}H NMR (300 MHz) ($CDCl_3$) of compound **RA5**

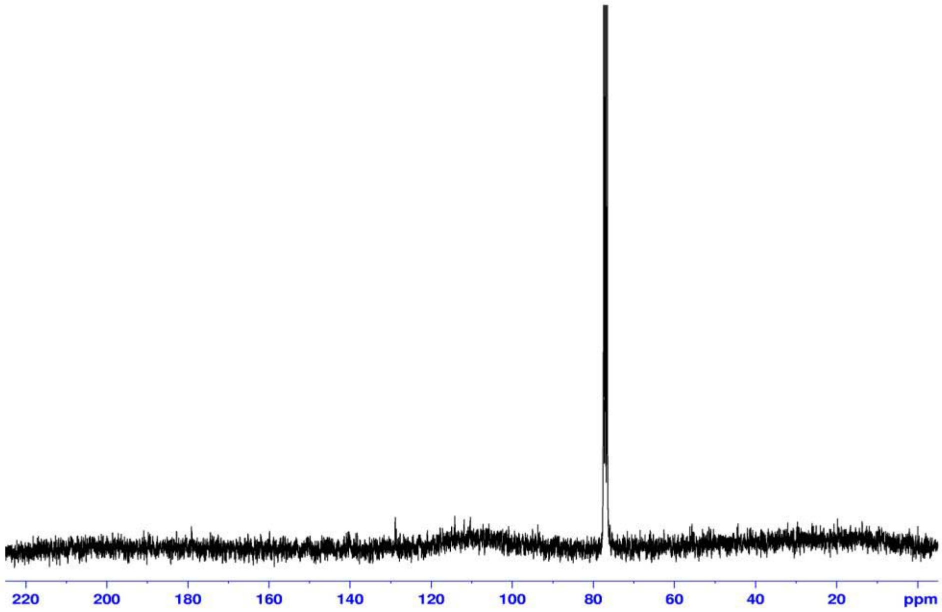


Figure 163 ^{13}C NMR (75 MHz) (CDCl$_3$) of compound **RA5**

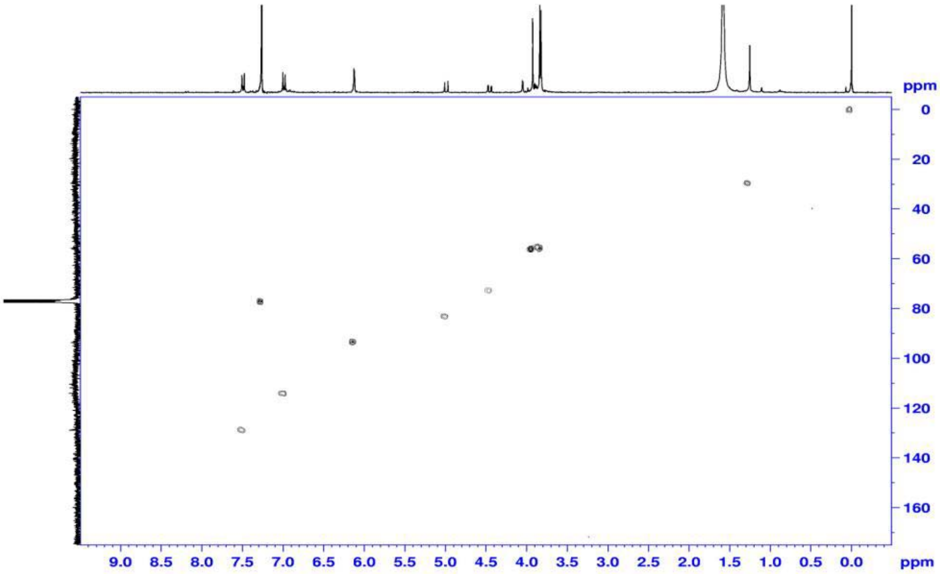


Figure 164 2D HMQC (CDCl$_3$) of compound **RA5**

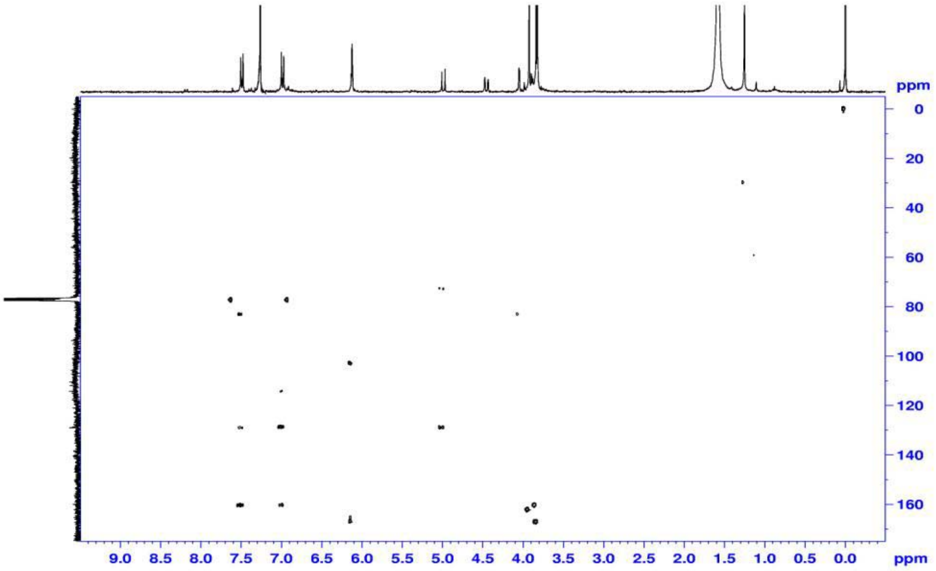


Figure 165 2D HMBC ($CDCl_3$) of compound **RA5**

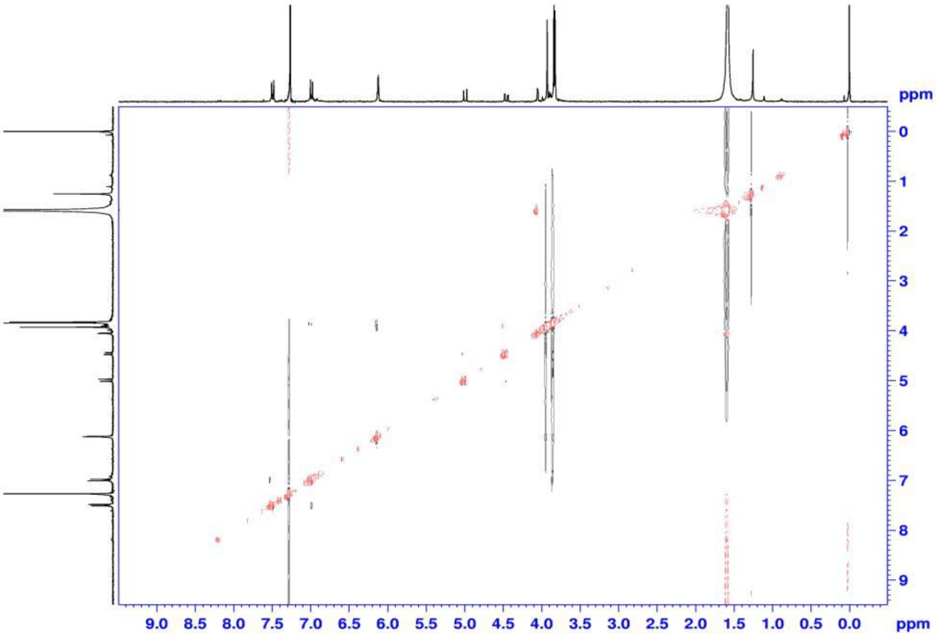


Figure 166 2D NOESY ($CDCl_3$) of compound **RA5**

VITAE

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Student ID 5210220017

Educational Attainment

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Bachelor of Science (Chemistry)	Prince of Songkla University	2009

Scholarship Awards during Enrolment

The Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education

List of Publication and Proceedings

1. Sonprasit, J., Karalai, C. and Ponglimanont, C. Biflavonoid and flavonoid derivatives from the roots of *Ellipanthus tomentosus* Kurz var. *tomentosus*. : PERCH-CIC Congress VII. Jomtein Plam Beach, Pattaya, Chonburi, Thailand. 4-7 May 2011. (Poster presentation)
2. Sonprasit, J., Karalai, C. and Ponglimanont, C. Flavonoids from the roots of *Ellipanthus tomentosus* Kurz var. *tomentosus*. : The 3rd NPRU National Conference 2011, Nakhon Pathom Rajabhat University, Nakhon Pathom, Thailand, 10 August 2011. (Poster presentation and Proceeding)