



Chemical Constituents from the Roots of *Clausena excavata* Burm. f.

Nitima Bindulem

**A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemical Studies**

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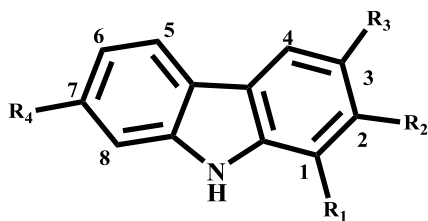
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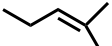
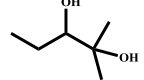
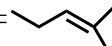
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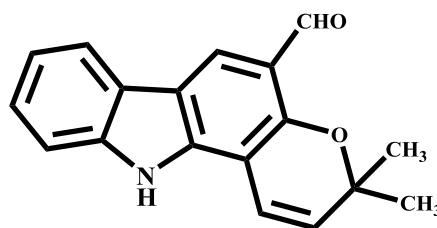
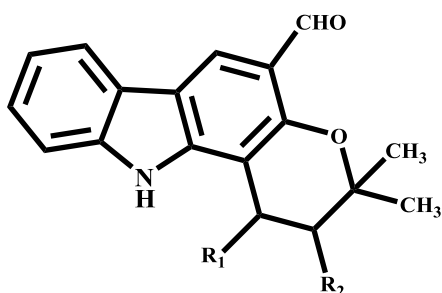
ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากรากหมูย (<i>Clausena excavata</i> Burm. f.)
ผู้เขียน	นางสาวนิติมา บินดุเหล็ก
สาขาวิชา	เคมีศึกษา
ปีการศึกษา	2553

บทคัดย่อ

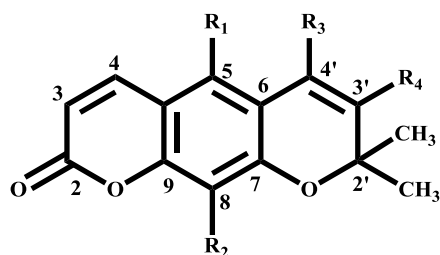
การศึกษาองค์ประกอบทางเคมีของส่วนสกัดเมทิลีนคลอไรด์จากรากหมูยสามารถแยกสารใหม่ได้ 4 สาร เป็นสารประกอบประเภท alkaloids 3 สาร คือ clausebazole A (RM7), clausebazole B (RM8) และ clausebazole C (RM26) สารประเภท furanocoumarin 1 สาร คือ clausemarin (RM5) นอกจากนี้ยังได้พบสารที่มีการรายงานมาแล้ว 23 สาร ประกอบด้วยสารประเภท carbazole alkaloids 14 สาร คือ heptaphylline (RM1), mukonal (RM6), mukonidine (RM9), mukonine (RM11), murrayacine (RM12), murrayanine (RM13), 7-methoxymukonal (RM14), *O*-methylnukonal (RM17), 3-formyl-2,7-dimethoxycarbazole (RM19), clausine L (RM20), 7-hydroxyheptaphylline (RM21), clausine K (RM22), clausine H (RM24) และ isomukonidine (RM27) สารประเภท pyranocoumarins 6 สาร คือ clausinidin (RM2), dentatin (RM3), xanthoxylatin (RM4), nordentatin (RM15), kinocoumarin (RM16) และ 7-hydroxy-8-(1,1-dimethylallyl)citrusarin (RM18) สารประเภท limonoids 2 สาร คือ *O*-methylclausenolide (RM23) และ clausenarin (RM25) และ สารประเภทอนุพันธ์ benzoic acid 1 สาร คือ 4-hydroxy-2-methoxybenzoic acid (RM10) โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี UV IR NMR MS และเปรียบเทียบกับสารที่มีรายงานการวิจัยแล้ว



- RM1:** R₁ = , R₂ = OH, R₃ = CHO, R₄ = H; heptaphylline
- RM6:** R₁ = H, R₂ = OH, R₃ = CHO, R₄ = H; mukonal
- RM7:** R₁ = , R₂ = OH, R₃ = CHO, R₄ = H; clausebazole A
- RM9:** R₁ = H, R₂ = OH, R₃ = COOCH₃, R₄ = H; mukonidine
- RM11:** R₁ = OCH₃, R₂ = H, R₃ = COOCH₃, R₄ = H; mukonine
- RM13:** R₁ = OCH₃, R₂ = H, R₃ = CHO, R₄ = H; murrayanine
- RM14:** R₁ = H, R₂ = OH, R₃ = CHO, R₄ = OCH₃; 7-methoxymukonal
- RM17:** R₁ = H, R₂ = OCH₃, R₃ = CHO, R₄ = H; *O*-methyilmukonal
- RM19:** R₁ = H, R₂ = OCH₃, R₃ = CHO, R₄ = OCH₃; 3-formyl-2,7-dimethoxycarbazole
- RM20:** R₁ = H, R₂ = OCH₃, R₃ = COOCH₃, R₄ = H; clausine L
- RM21:** R₁ = , R₂ = OH, R₃ = CHO, R₄ = OH; 7-hydroxyheptaphylline
- RM22:** R₁ = H, R₂ = OCH₃, R₃ = COOH, R₄ = OCH₃; clausine K
- RM24:** R₁ = H, R₂ = OCH₃, R₃ = COOCH₃, R₄ = OCH₃; clausine H
- RM27:** R₁ = H, R₂ = OCH₃, R₃ = COOH, R₄ = H; isomukonidine



- RM8:** R₁ = H, R₂ = OH; clausebazole B **RM12:** murrayacine
- RM26:** R₁ = OH, R₂ = OH; clausebazole C

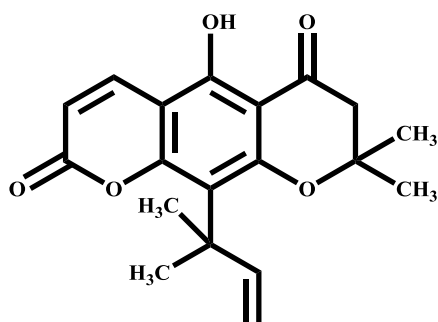


RM3: $R_1 = \text{OCH}_3$, $R_2 = \text{CH}_2\text{CH}=\text{CH}_2$, $R_3 = \text{H}$, $R_4 = \text{H}$; dentatin

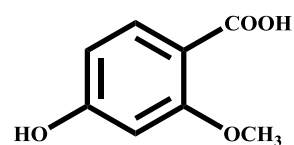
RM4: $R_1 = \text{OCH}_3$, $R_2 = \text{H}$, $R_3 = \text{H}$, $R_4 = \text{H}$; xanthoxylatin

RM15: $R_1 = \text{OH}$, $R_2 = \text{CH}_2\text{CH}=\text{CH}_2$, $R_3 = \text{H}$, $R_4 = \text{H}$; nordentatin

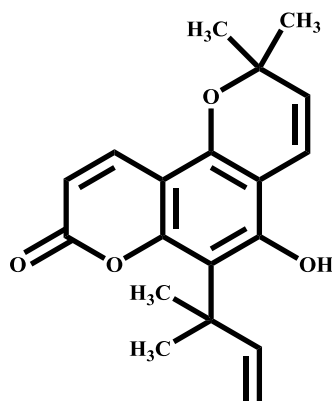
RM16: $R_1 = \text{OH}$, $R_2 = \text{CH}_2\text{CH}=\text{CH}_2$, $R_3 = \text{H}$, $R_4 = \text{CH}_2\text{CH}=\text{CH}_2$; kinocoumarin



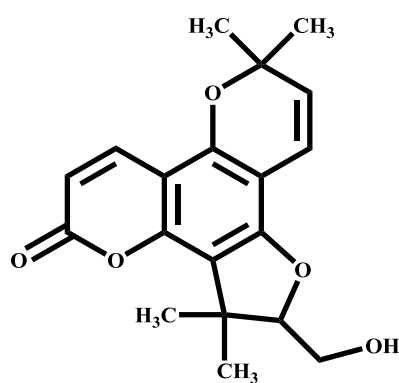
RM2: clausinidin



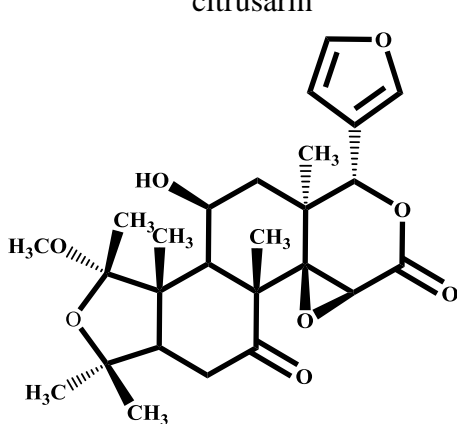
RM10: 4-hydroxy-2-methoxybenzoic acid



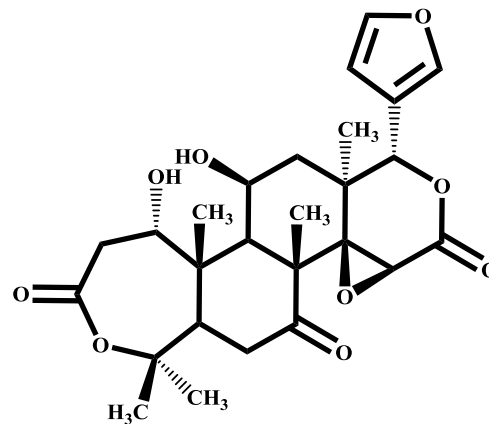
RM18: 7-hydroxy-8-(1,1-dimethylallyl)
citrusarin



RM5: clausemarin



RM23: *O*-methylclausenolide

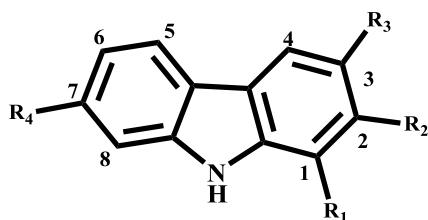


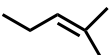
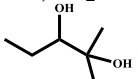
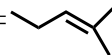
RM25: clausenarin

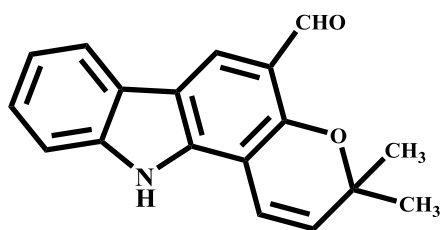
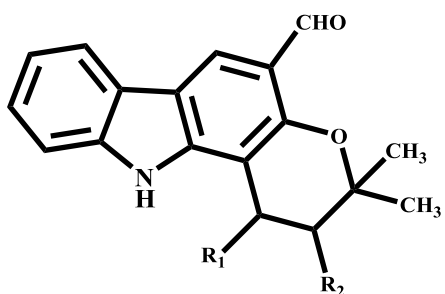
Thesis Title	Chemical Constituents from the Roots of <i>Clausena excavata</i> Burm. f.
Author	Miss Nitima Bindulem
Major Program	Chemical Studies
Academic Year	2010

ABSTRACT

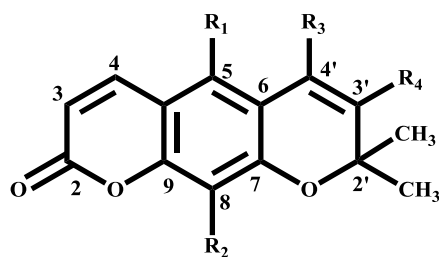
Investigation of the crude methylene chloride extract of the roots of *Clausena excavata* Burm. f. yielded four new compounds; three carbazole alkaloids: clausebazole A (**RM7**), clausebazole B (**RM8**) and clausebazole C (**RM26**), a new coumarin: clausemarin (**RM5**), together with twenty-three known compounds: fourteen carbazole alkaloids; heptaphylline (**RM1**), mukonal (**RM6**), mukonidine (**RM9**), mukonine (**RM11**), murrayacine (**RM12**), murrayanine (**RM13**), 7-methoxymukonal (**RM14**), *O*-methylumukonal (**RM17**), 3-formyl-2,7-dimethoxy carbazole (**RM19**), clausine L (**RM20**), 7-hydroxyheptaphylline (**RM21**), clausine K (**RM22**), clausine H (**RM24**) and isomukonidine (**RM27**), six pyranocoumarins: clausinidin (**RM2**), dentatin (**RM3**), xanthoxylatin (**RM4**), nordentatin (**RM15**), kinocoumarin (**RM16**) and 7-hydroxy-8-(1,1-dimethylallyl)citrusarin (**RM18**), two limonoids: *O*-methylclausenolide (**RM23**) and clausenarin (**RM25**) and one benzoic acid derivative: 4-hydroxy-2-methoxybenzoic acid (**RM10**). Their structures were determined on the basis of UV, IR, NMR, MS and by comparison of their spectroscopic data with those reported.



- RM1:** R₁ = , R₂ = OH, R₃ = CHO, R₄ = H; heptaphylline
- RM6:** R₁ = H, R₂ = OH, R₃ = CHO, R₄ = H; mukonal
- RM7:** R₁ = , R₂ = OH, R₃ = CHO, R₄ = H; clausebazole A
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- RM13:** R₁ = OCH₃, R₂ = H, R₃ = CHO, R₄ = H; murrayanine
- RM14:** R₁ = H, R₂ = OH, R₃ = CHO, R₄ = OCH₃; 7-methoxymukonal
- RM17:** R₁ = H, R₂ = OCH₃, R₃ = CHO, R₄ = H; *O*-methylmukonal
- RM19:** R₁ = H, R₂ = OCH₃, R₃ = CHO, R₄ = OCH₃; 3-formyl-2,7-dimethoxycarbazole
- RM20:** R₁ = H, R₂ = OCH₃, R₃ = COOCH₃, R₄ = H; clausine L
- RM21:** R₁ = , R₂ = OH, R₃ = CHO, R₄ = OH; 7-hydroxyheptaphylline
- RM22:** R₁ = H, R₂ = OCH₃, R₃ = COOH, R₄ = OCH₃; clausine K
- RM24:** R₁ = H, R₂ = OCH₃, R₃ = COOCH₃, R₄ = OCH₃; clausine H
- RM27:** R₁ = H, R₂ = OCH₃, R₃ = COOH, R₄ = H; isomukonidine



- RM8:** R₁ = H, R₂ = OH; clausebazole B **RM12:** murrayanine
- RM26:** R₁ = OH, R₂ = OH; clausebazole C

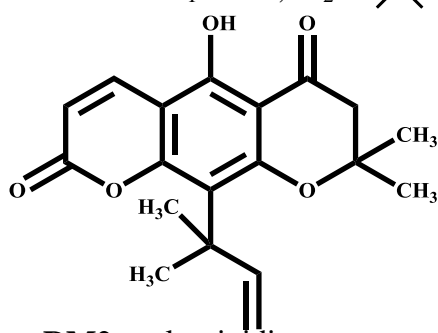


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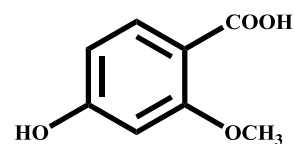
RM4: $R_1 = \text{OCH}_3$, $R_2 = \text{H}$, $R_3 = \text{H}$, $R_4 = \text{H}$; xanthoxylatin

RM15: $R_1 = \text{OH}$, $R_2 = \text{CH}_2\text{CH}(\text{CH}_3)_2$, $R_3 = \text{H}$, $R_4 = \text{H}$; nordentatin

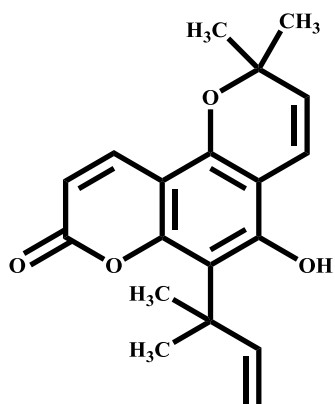
RM16: $R_1 = \text{OH}$, $R_2 = \text{CH}_2\text{CH}(\text{CH}_3)_2$, $R_3 = \text{H}$, $R_4 = \text{CH}_2\text{CH}(\text{CH}_3)_2$; kinocoumarin



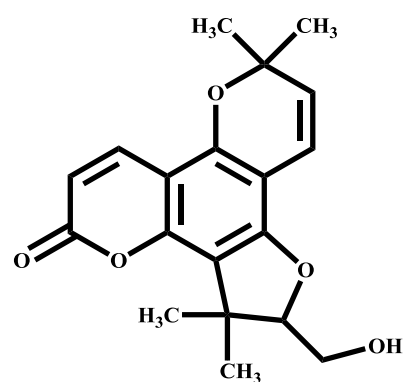
RM2: clausinidin



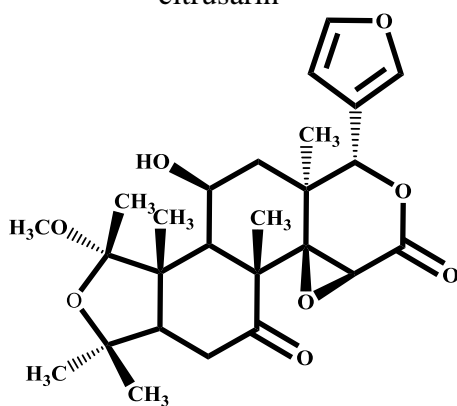
RM10: 4-hydroxy-2-methoxybenzoic acid



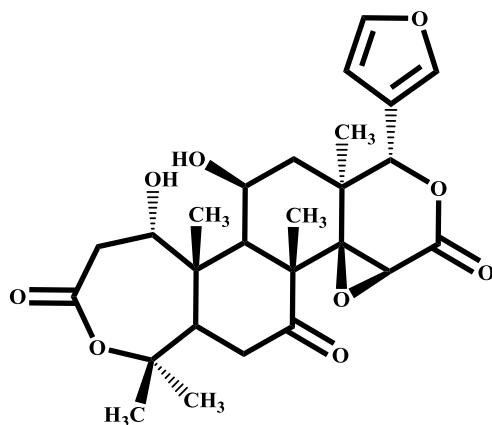
RM18: 7-hydroxy-8-(1,1-dimethylallyl) citrusarin



RM5: clausemarin



RM23: *O*-methylclausenolide



RM25: clausenarin

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Nitima Bindulem

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents from the roots of *Clausena excavata* Burm.f. They are a part of the basic research on the Thai medicinal plants. A derivative of benzoic acid, two limonoids, one furanocoumarin, six pyranocoumarins, and seventeen carbazole alkaloids were isolated from the roots of *Clausena excavata*.

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LIST OF ABBREVIATIONS AND SYMBOLS

<i>s</i>	=	singlet
<i>d</i>	=	doublet
<i>t</i>	=	triplet
<i>q</i>	=	quartet
<i>m</i>	=	multiplet
<i>dd</i>	=	doublet of doublet
<i>dt</i>	=	doublet of triplet
<i>br s</i>	=	broad singlet
<i>br d</i>	=	broad doublet
<i>g</i>	=	gram
nm	=	nanometer
mp	=	melting point
cm ⁻¹	=	reciprocal centimeter (wave number)
δ	=	chemical shift relative to TMS
<i>J</i>	=	coupling constant
[α] _D	=	specific rotation
λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
ϵ	=	molar extinction coefficient
<i>m/z</i>	=	a value of mass divided by charge
°C	=	degree celcius
MHz	=	Megahertz
ppm	=	part per million

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

<i>c</i>	=	concentration
IR	=	Infrared
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 Spectroscopy
CC	=	Column Chromatography
QCC	=	Quick Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
TLC	=	Thin Layer Chromatography
TMS	=	tetramethylsilane
CDCl ₃	=	deuteriochloroform
CD ₃ OD	=	deuteromethanol
CD ₃ COCD ₃	=	deuteroacetone
DMSO- <i>d</i> ₆	=	hexadeuterodimethyl sulfoxide

CHAPTER 1

INTRODUCTION

1.1 Introduction

Clausena excavata Burm. f. (Rutaceae) known locally in Thailand as " Mui " is a tall shrub or small to medium-sized tree, which can grow up to 1-3 meter high. Their branchlets are pubescent. Bark gray and smooth. The leaves was pinate, alternate, ovate to lanceolate, leaflets 5-8 pairs, 3-5 cm long, upper surface with numerous oil dots. The flower is white, arranged in a many-flowered and pedicles up to 3 mm long. The fruits was green when unripe and berry-like pink-orange when ripe, up to 1 cm in diameter, fleshy. This plant commonly found in the forests and limestone areas in Southeast Asia. Traditionally, this plant was used as herbal medicine by local people, *Clausena excavata* is used as a folk medicine for the treatment of snakebite, abdominal pain and as a detoxification agent [Wu *et al.*, 1982], and remedy to treat paralysis, ulcerated nose, colic, stomach trouble, fever and head-ache. It is insecticide, tonic and vermifuge [Trinh, 1999]. The leaves of this plant are used as traditional medicine to cure cold, abdominal pain, malaria and dysentery [Wu *et al.*, 1993].

According to Smitinand (2001), there are six species of Genus *Clausena* found in Thailand as follows.

1. *Clausena wallichii* Oliv. Var
2. *Clausena lansium* (Lour.) Skeels
3. *Clausena wallichii* Oliv.Var.guillauminii (Tanaka) J.P.Molino
4. *Clausena harmandiana* (Pierre) Pierre ex Guillaumin
5. *Clausena lenis* Drake
6. *Clausena excavata* Burm. f.

All parts of this tree, stem, leaves, rhizome and root are employed medicinally for variety indications. The crude ethanolic extract from the root and stem bark showed antibacterial effect [Wu *et al.*, 1982], and the crude methanol extract from the stem barks, partitioned layers and chromatographic fractions revealed the

presence of promotive and inhibitive constituents, simultaneously [Wu *et al.*, 1996]. The ethanolic extract of leaves, given orally at dose of 125.25 and 500 mg/kg body weight, showed significant antinociceptive activity on acetic acid induced writhing in mice [Rahman *et al.*, 2002].



Trees



Stem



Flowers



Leaves



Flowers



Fruits

Figure 1 Different parts of *Clausena excavata*

1.2 Review of Literatures

The chemical constituents isolated from the *Clausena excavata* were summarized in **Table 1**. Information obtained from SciFinder Scholar copyright in 2009 will be presented and classified into groups: Carbazole alkaloids, Coumarins, Flavonoids and Limonoids.

1.2.1 The Biological Activity of *C. excavata*

The Plants of *Clausena* species are known to be rich sources of carbazole alkaloids and coumarins [Ito *et al.*, 1996].

The compounds isolated from *C. excavata* have been investigated for biological activity. For example, The IC₅₀ value of clausine D on arachidonic acid and collagen-induced platelet aggregation were calculated to be 9.0 +/-1.1 and 58.9+/-0.9 µM, respectively [Wu *et al.*, 1994]. Dentatin, nordentatin, clausinidin, 3-formyl carbazole, mukonal, 3-methoxycabonylcarbazole, 2-hydroxy-3-formyl-7-methoxy carbonylcarbazole and clauszoline J showed antimycobacterial active at a minimum inhibitory concentration MIC₅₀ 50, 100, 200, 100, 200, 50, 100 and 100 µg/ml, respectively and 3-formylcarbazole, mukonal, 3-methoxycabonylcarbazole and 2-hydroxy-3-formyl-7-methoxycabonylcarbazole showed antifungal activity with IC₅₀ values of 13.6, 29.3, 9.5 and 2.8 µg/ml, respectively [Sunthitikawinsakul *et al.*, 2003]. Clausenidin and nordentatin suppressed hepatitis B virus surface antigen in HepA2 cells, and in addition, clausenidin, nordentatin and clausarin showed cytotoxic activity against four human cancer cell lines (A549, MCF7, KB, and KB-VIN). The most interesting result in the cytotoxicity assay was the significant activity of clausenidin against the multi-drug resistant cell line, KB-VIN, without activity against the KB cell line [Su *et al.*, 2009]. Clausenaguinone-A shows potent inhibitory activity of the rabbit platelet aggregation as well as cytotoxicity in HCT-8, RPMI-7951, and TE671 tumor cells [Wu *et al.*, 1994]. Clausenamine-A and *O*-demethylmurrayafoline A showed potent cytotoxic activities against a variety of human cancer cell lines in vitro [Zhang *et al.*, 2000]. Clauslactones A-D were found to exhibit inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus

early antigen activation in Raji cells [Ito *et al.*, 2000]. A limonoid, clausenolide-1-Et ether exhibited HIV-1 inhibitory activity and two coumarins, dentatin and nor-dentatin related to an anti-HIV-1 substance, (+)-calanolide A, were obtained from the crude chloroform extract of the rhizomes, induced toxicity to cells used in a syncytium assay for anti-HIV-1 activity. These compounds did not show any cytotoxic effect against KB and BC-1 cell lines (IC₅₀ value > 20 µg/mL) [Sunthitikawinsakul *et al.*, 2003]. Clausine Z exhibits inhibitory activity against cyclin-dependent kinase 5 (CDK5) and shows protective effects on cerebellar granule neurons *in vitro* [Potterat *et al.*, 2005]. *O*-methylmukonal, 3-formyl-2,7-dimethoxycarbazole, clauszoline J and clausenidin displayed anti-HIV-1 activity in a syncytial assay with EC₅₀ values of 12, 29.1, 34.2 and 5.3 µM, respectively, and thus exhibited potential therapeutic index (PTI) values of 56.7, 8.0, 1.6 and 7.0, respectively. These compounds demonstrated a lack of cytotoxicity against the KB and BC-1 cancer cell lines [Kongkathip *et al.*, 2005]. 3-Carbomethoxy-2-hydroxy-7-methoxycarbazole shows significant cytotoxicity against CEM-SS cell line [Taufiq-Yap *et al.*, 2007]. Clauslactones R, S and T were found to show moderate topoisomerase II inhibitory effects at 50 µM [Xin *et al.*, 2008].

Table 1 Compounds from *Clausena excavata*

a. Carbazole alkaloids

c. Flavonoids

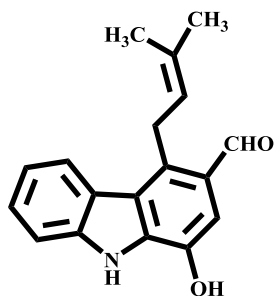
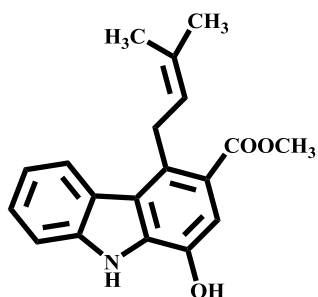
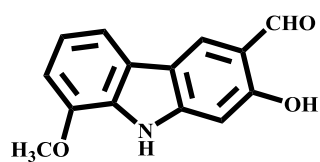
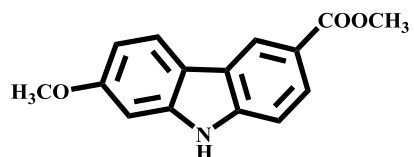
b. Coumarins

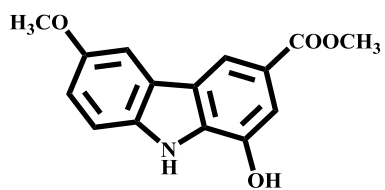
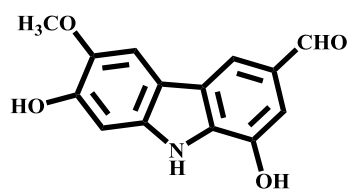
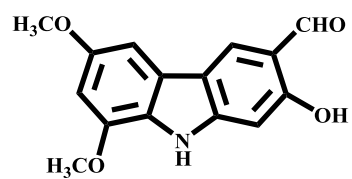
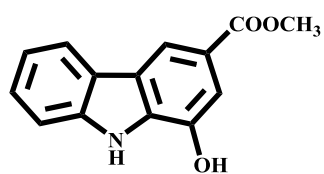
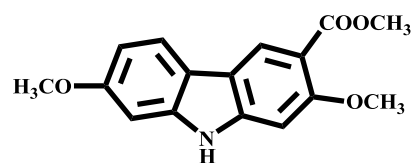
d. Limonoids

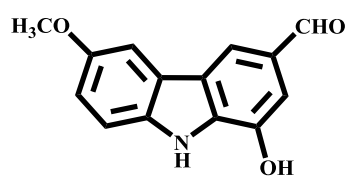
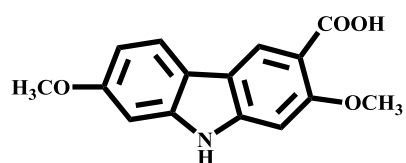
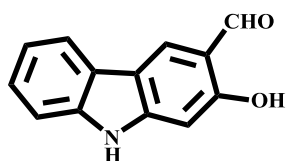
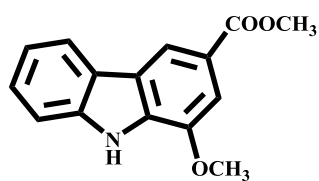
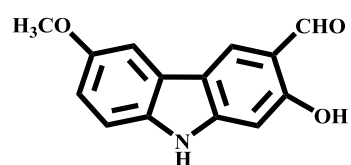
Scientific name	Part	Compounds	Bibliography
<i>Clausena excavata</i>	Stem Barks	Clausine D, a1 Clausine F, a2 <i>O</i> -Methylclausinolide, d1 Zapoterin, d2 Clausine A, a3 Clausine C, a4 Clausine G, a5 Clausine J, a6 Clausine B, a7 Clausine E, a8 Clausine H, a9 Clausine I, a10 Clausine K, a11 Mukonal, a12 Mukonine, a13 Lasine, a14 Glycozolidal, a15 3-Methylcarbazole, a16 Heptasoline, a17 Heptaphylline, a18	Wu <i>et al.</i> , 1992 Wu <i>et al.</i> , 1993 Wu <i>et al.</i> , 1996 Wu <i>et al.</i> , 1996

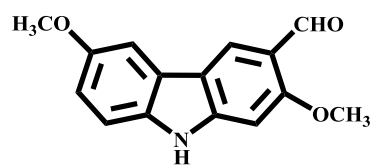
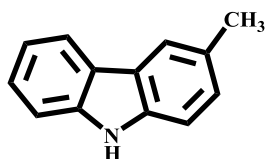
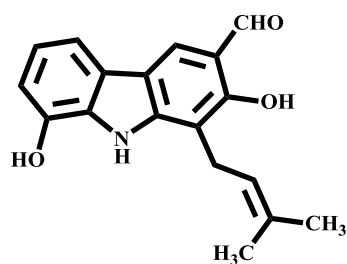
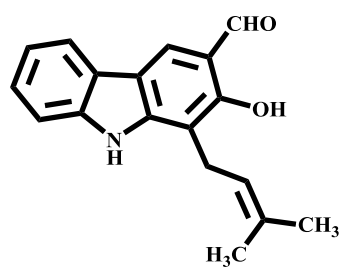
Scientific name	Part	Compounds	Bibliography
<i>Clausena excavata</i>	Stem Barks	Murrayanine, a19 2-Hydroxy-3-methylcarbazole, a20 Clauszoline A, a21 Clauszoline B, a22 Clauszoline D, a23 Clauszoline E, a24 Clauszoline F, a25 Clauszoline G, a26 Clauszoline K, a27 Clauszoline L, a28 Clausine TY, a29	Wu <i>et al.</i> , 1996 Ito <i>et al.</i> , 1996 Ito <i>et al.</i> , 1997 Taufiq-Yap <i>et al.</i> , 2007
	Roots and Stem Barks	Clausenin, b1 Clausenidin, b2	Bose <i>et al.</i> , 1973
	Root Barks	Clausenidinaric acid, b3 Nordentatin, b4 Xanthoxylatin, b5 Claucavatin A, b6 Claucavatin B, b7 Xanthyletin, b8 Kinocoumarin, b9 Osthol, b10 Liquiritigenin, c1 Clausine M, a30 Clausine W, a31 Clausine T, a32 Furoclausine A, a33	Wu <i>et al.</i> , 1982 Huang <i>et al.</i> , 1996 Wu <i>et al.</i> , 1999 Wu <i>et al.</i> , 1997

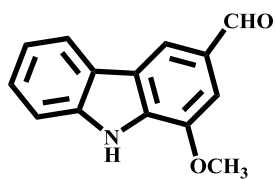
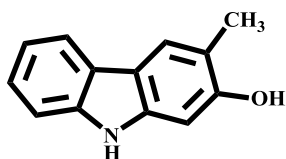
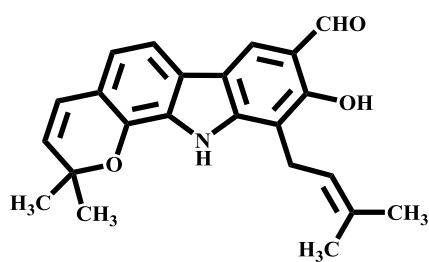
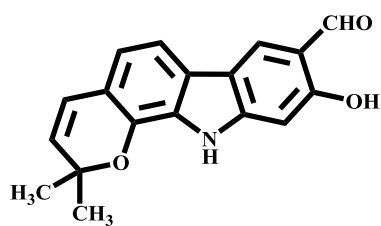
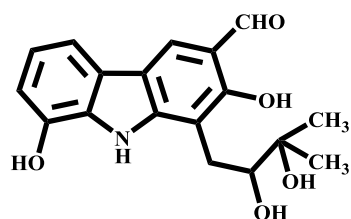
Scientific name	Part	Compounds	Bibliography
<i>Clausena excavata</i>	Root Barks	Furoclausine B, a34	Wu <i>et al.</i> , 1997
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		Clausevatin E, a36	
		Clausevatin F, a37	
		Clausevatin G, a38	
	Roots	Clauszoline H, a39	Ito <i>et al.</i> , 1997
	Leaves	Clausine L, a40	Wu <i>et al.</i> , 1993
		Rutin, c2	
		Clauszoline M, a41	Ito <i>et al.</i> , 1997
	Rhizome	Clausenolide-1-Et ether, d3	Sunthitikawinsakul <i>et al.</i> , 2003
	Leaves and Stems	Clausine Z, a42	Potterat <i>et al.</i> , 2005
		Clauslactone R, b11	Xin <i>et al.</i> , 2008
		Clauslactone S, b12	
	Clauslactone T, b13		
Fruits and Stems	Seselin, b14	Laphookhieo <i>et al.</i> , 2009	

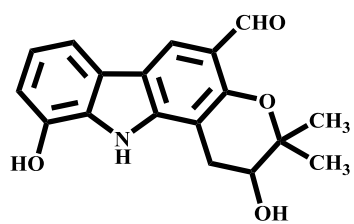
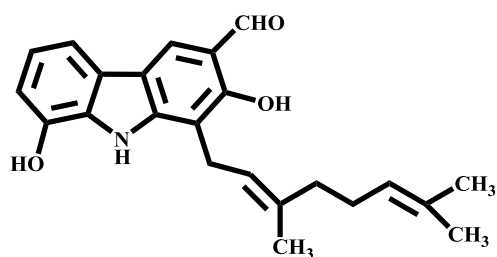
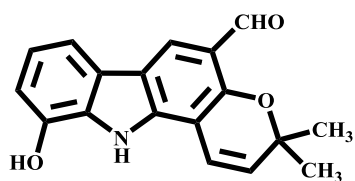
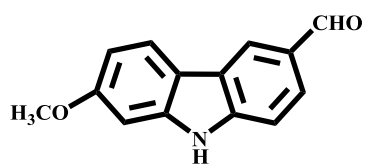
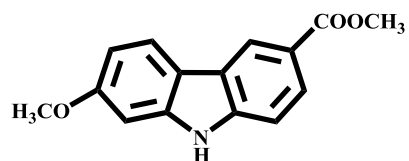
a. Carbazole alkaloidsClausine D, **a1**Clausine F, **a2**Clausine A, **a3**Clausine C, **a4**

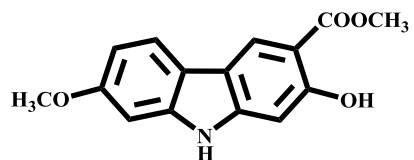
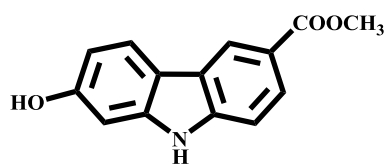
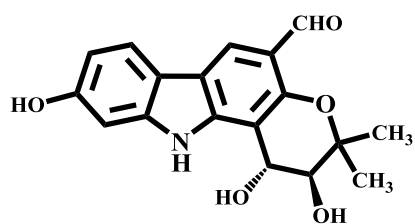
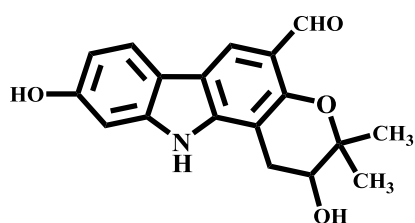
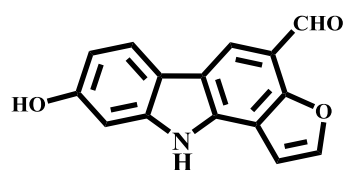
Clausine G, **a5**Clausine J, **a6**Clausine B, **a7**Clausine E, **a8**Clausine H, **a9**

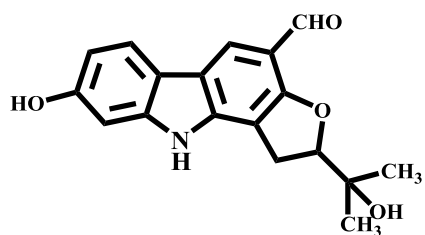
Clausine I, **a10**Clausine K, **a11**Mukonal, **a12**Mukonine, **a13**Lasine, **a14**

Glycozolidal, **a15**3-Methylcarbazole, **a16**Heptasoline, **a17**Heptaphylline, **a18**

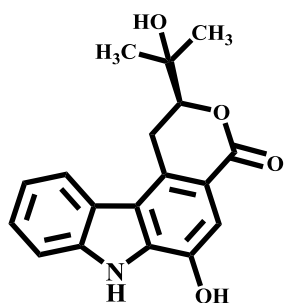
Murrayanine, **a19**2-Hydroxy-3-methylcarbazole, **a20**Clauszoline A, **a21**Clauszoline B, **a22**Clauszoline D, **a23**

Clauszoline E, **a24**Clauszoline F, **a25**Clauszoline G, **a26**Clauszoline K, **a27**Clauszoline L, **a28**

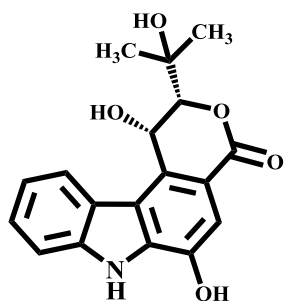
Clausine TY, **a29**Clausine M, **a30**Clausine W, **a31**Clausine T, **a32**Furoclausine A, **a33**



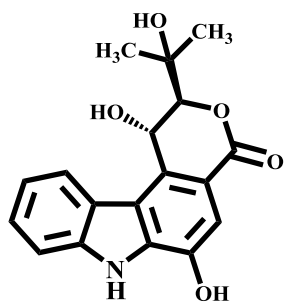
Furoclausine B, a34



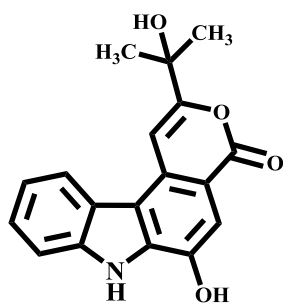
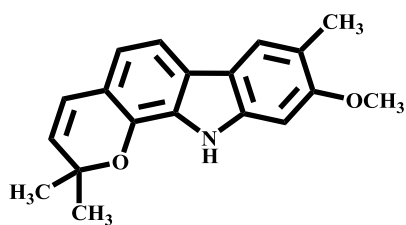
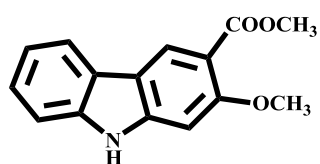
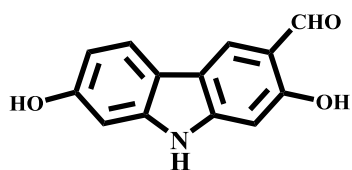
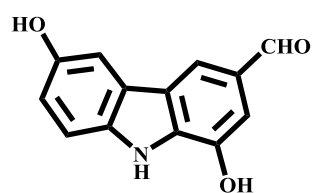
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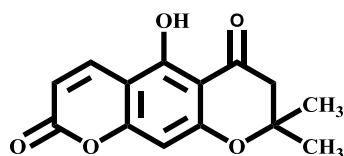
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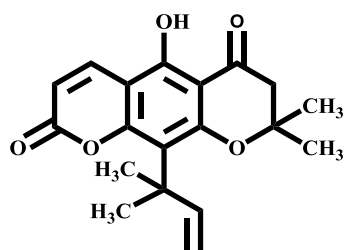
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Clausevatin G, **a38**Clauszoline H, **a39**Clausine L, **a40**Clauszoline M, **a41**Clausine Z, **a42**

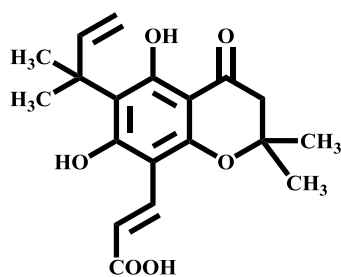
b. Coumarins



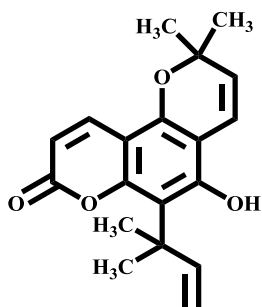
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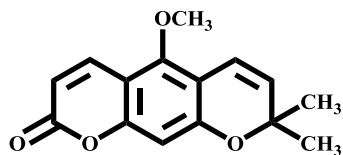
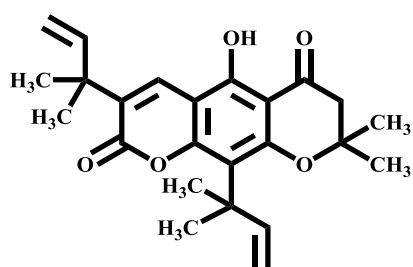
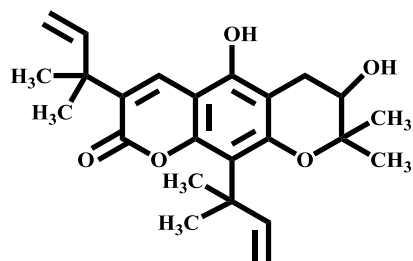
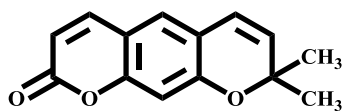
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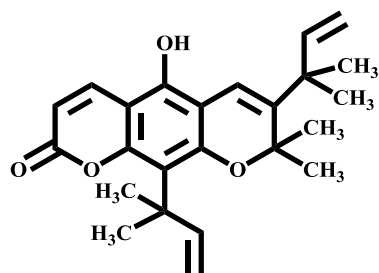
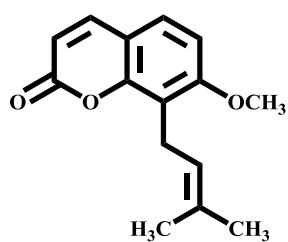
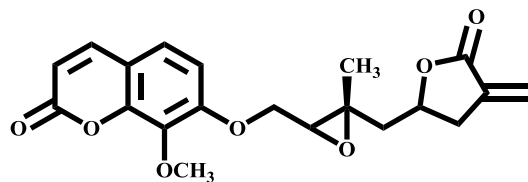
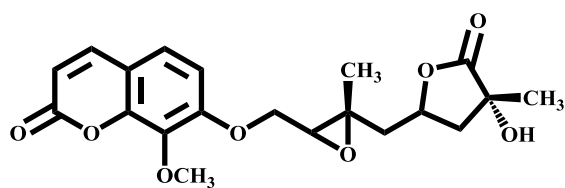


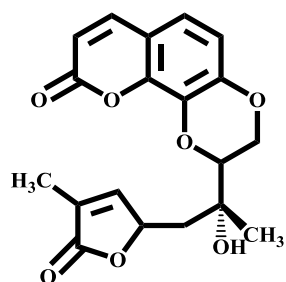
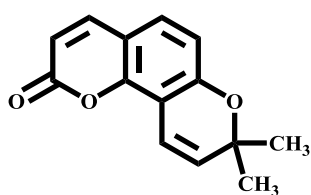
Clausenidinic acid, **b3**



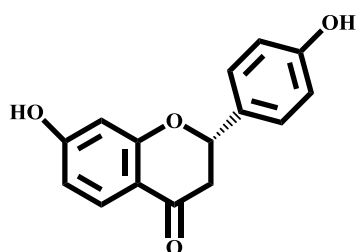
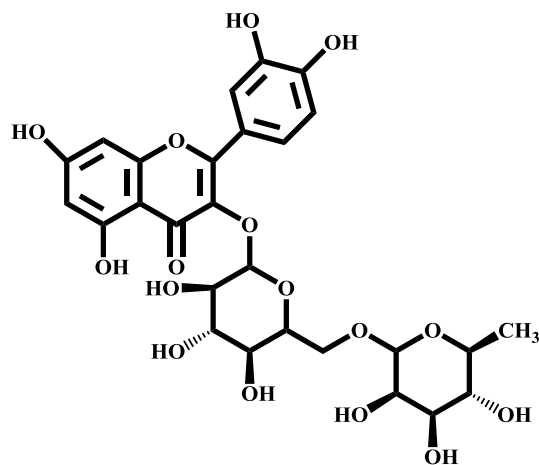
Nordentatin, **b4**

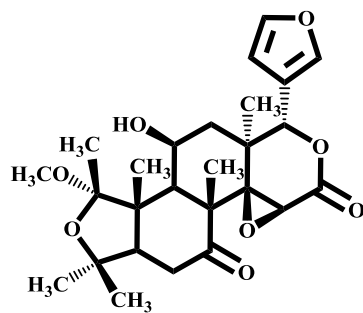
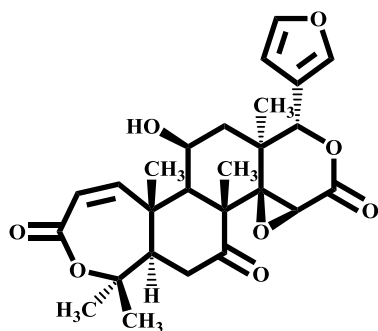
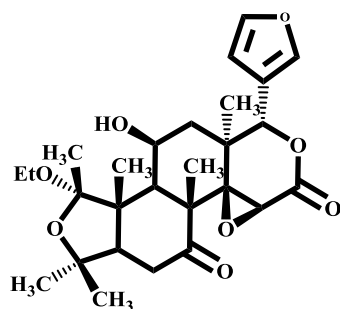
Xanthoxylatin, **b5**Claucavatin A, **b6**Claucavatin B, **b7**Xanthyletin, **b8**

Kinocoumarin, **b9**Osthol, **b10**Clauslactone R, **b11**Clauslactone S, **b12**

Clauslactone T, **b13**Seselin, **b14**

C. Flavonoids

Liquiritigenin, **c1**Rutin, **c2**

d. Limonoids*O*-Methylclausinolide, **d1**Zapoterin, **d2**Clausenolide-1-Et ether, **d3**

1.3 Objective

This research work involved isolation, purification and structural elucidation of chemical constituents from the roots of *Clausena excavata*.

CHAPTER 2

EXPERIMENTAL

2.1 Instruments and Chemicals

Melting point was recorded in °C on a digital Electrothermal 9100 Melting Point Apparatus. Ultraviolet spectra were measured with a UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and $\log \epsilon$ in methanol solution. The optical rotation $[\alpha]_D$ was measured in acetone and methanol 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. ^1H and ^{13}C – Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield™ 300 and 500 MHz spectrometer at Department of Chemistry, Faculty of Science, Prince of Songkla University. Spectra were recorded in deuteriochloroform and deuterioacetone as δ value in ppm down field from TMS (internal standard δ 0.00) and coupling constant (J) are expressed in hertz. Quick column chromatography (QCC) and column chromatography 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 F₂₅₄ (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.

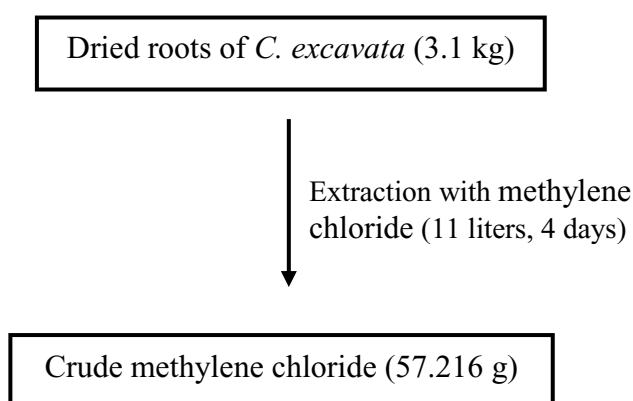
2.2 Plant material

The dried roots of *C. excavata* were collected from Songkhla province in the Southern part of Thailand, in September, 2009. Identification was made by Assoc.Prof. Dr. Kitichate Sridith, Department of Biology, Faculty of Science, Prince of Songkla University. The specimen (N. Bindulem 1) with Herbarium number

(0013589) has been deposited in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

2.3 Extraction and Isolation

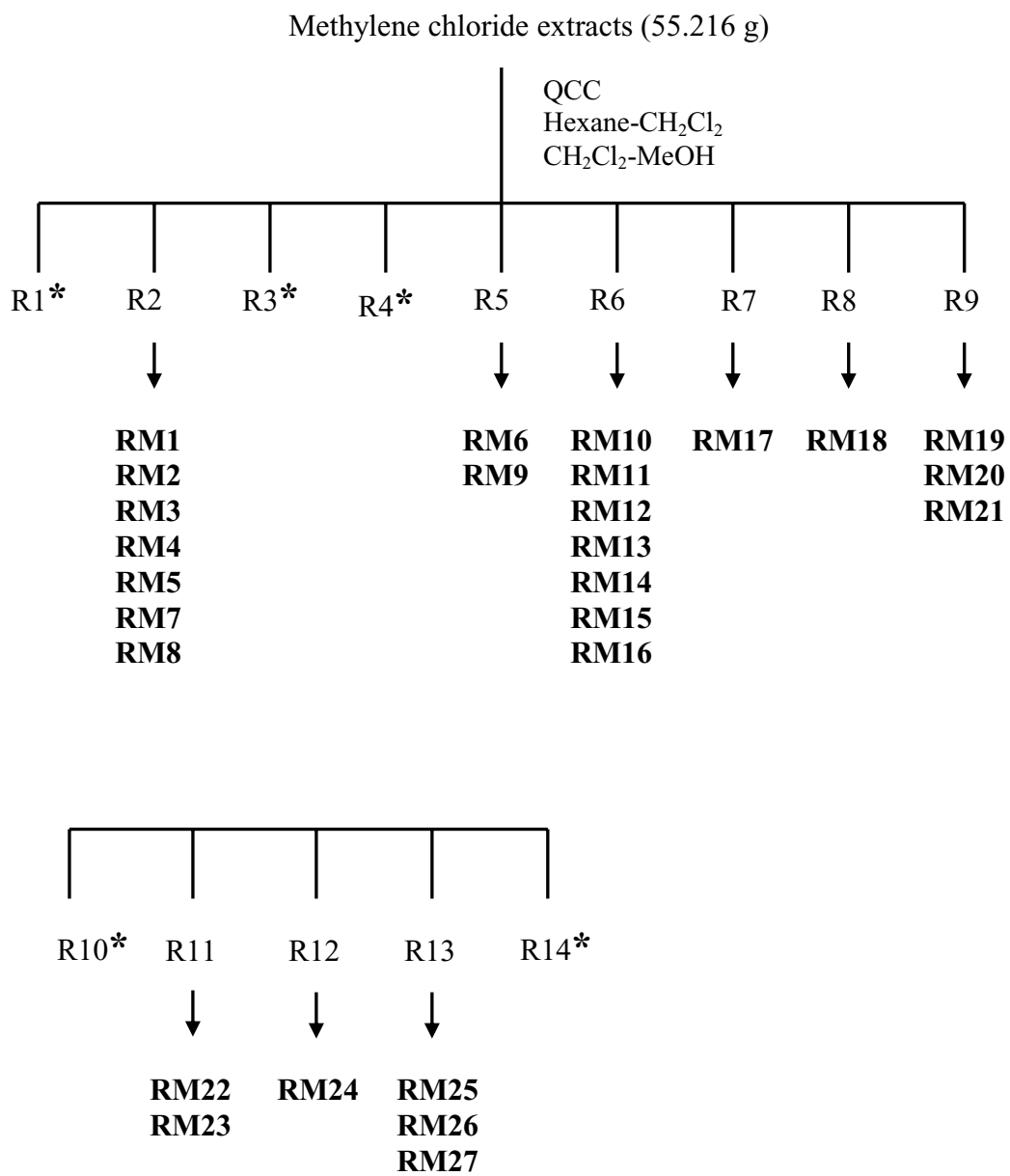
Dried roots of *C. excavata* (3.1 kg) were extracted three times (each 4 days) with methylene chloride at room temperature. Filtration and evaporation of the solvent in *vacuo* afforded a dark brown residue (57.216 g). The process of extraction was shown in **Scheme 1**.



Scheme 1 Isolation of crude extract from the roots of *C. excavata*

2.4 Isolation and Chemical Investigation

Methylene chloride extract (55.216 g) was subjected to quick column chromatography using silica gel as stationary phase and eluted with a gradient of hexane, hexane-methylene chloride, methylene chloride, methylene chloride-methanol and methanol as eluents. On the basis of their TLC characteristics, the fractions which contained the same major components were combined to give fractions R1-R14. Twenty-seven pure compounds were obtained as shown in **Scheme 2**.



* No further investigation

Scheme 2 Isolation of compounds **RM1-RM27** from methylene chloride extract

Table 2 Physical characteristics and weights of the fractions from the methylene chloride extract

Fraction	Weight (g)	Physical characteristic
R1	0.0320	yellow viscous liquid
R2	29.3762	yellow viscous liquid
R3	1.9873	yellow viscous liquid
R4	0.3700	brown viscous liquid
R5	0.9000	brown viscous liquid
R6	1.7295	brown viscous liquid
R7	2.4016	brown viscous liquid
R8	4.5235	brown viscous liquid
R9	1.0415	brown viscous liquid
R10	1.8973	brown viscous liquid
R11	3.7952	brown viscous liquid
R12	2.4877	brown viscous liquid
R13	1.1938	black viscous solid
R14	3.3425	black solid
total	55.078	

Fraction R2 (29.3762 g) was separated by column chromatography over silica gel and eluted with ethyl acetate-hexane (2.0:8.0) to afford 7 fractions (2A-2G).

Subfraction 2C (0.0285 g) was filtered and washed with hexane to give a yellow powder of **RM1**: heptaphylline (0.0236 g).

Subfraction 2D (0.0460 g) was filtered and washed with hexane to give a yellow prismatic rods of **RM2**: clausinidin (0.0404 g).

Subfraction 2F (9.0045 g) was filtered and washed with hexane to give a colorless prism of **RM3**: dentatin (0.0455 g). The Filtrate of 2F (8.3303 g) was further purified by column chromatography over silica gel and eluted with ethyl acetate-hexane (2.0:8.0) to afford 13 fractions (2F1-2F13).

Subfraction 2F5 (2.8594 g) was purified by column chromatography over silica gel and eluted with ethyl acetate-hexane (2.0:8.0) to afford 6 fractions (2F5A-2F5E).

Subfraction 2F5C (1.8440 g) was further purified by column chromatography over silica gel and eluted with ethyl acetate-hexane (2.0:8.0) to afford 6 fractions (2F5C1-2F5C6).

Subfraction 2F5C3 (1.3940 g) was purified by column chromatography over silica gel and eluted with ethyl acetate-hexane (2.0:8.0) to afford 6 fractions (2F5C3A-2F5C3H).

Subfraction 2F5C3D (0.0631 g) was separated by column chromatography with Sephadex LH-20 and eluted with methanol-methylene chloride (1.0:1.0) to give a colorless prisms of **RM4**: xanthoxylatin (0.0085 g).

Subfraction 2F9 (0.0268 g) was further purified on preparative TLC and eluted with ethyl acetate-methylene chloride (1.0:9.0) to give yellow gum of **RM5**: clausemarin (0.0069 g).

Subfraction 2F11 (0.0168 g) was separated by column chromatography with Sephadex LH-20 and eluted with ethyl acetate-methylene chloride (0.3:9.7) to give a colorless crystalline solid of **RM7**: clausebazole A (0.0075 g).

Subfraction 2F12 (0.0366 g) was filtered and washed with a mixture of hexane and methylene chloride (9.5:0.5) to give a colorless solid of **RM8**: clausebazole B (0.0087 g).

Fraction R5 (0.9000 g) was filtered and washed with a mixture of hexane and methylene chloride (9.5:0.5) to afford 2 fractions (5A-5B).

Subfraction 5A (0.0370 g.) was further purified on preparative TLC and eluted with ethyl acetate-hexane (3.0:7.0) to give an orange solid of **RM6**: mukonal (0.0133 g) and a pale yellow crystalline solid of **RM9**: mukonidine (0.0023 g).

Fraction R6 (1.7295 g) was further purified by column chromatography over silica gel and eluted with methylene chloride-hexane (7.0:3.0) to afford 16 fractions (6A-6P).

Subfraction 6G (0.0075 g) was filtered and washed with a mixture of hexane and methylene chloride (8.5:1.5) to give a colorless crystalline solid of **RM10**: 4-hydroxy-2-methoxybenzoic acid (0.0058 g).

Subfraction 6E (0.0080 g) was separated by preparative TLC and eluted with ethyl acetate-hexane (3.5:6.5) to afford 2 fractions (6E1-6E2).

Subfraction 6E2 (0.0017 g) was further purified by preparative TLC eluted with ethyl acetate-hexane (4.0:6.0) to give an orange crystalline solid of **RM11**: mukonine (0.0014 g).

Subfraction 6H (0.0277 g) was separated by preparative TLC and eluted with methylene chloride-ethyl acetate-hexane (3.0:2.0:5.0) to afford 2 fractions (6H1-6H2).

Subfraction 6H2 (0.0050 g) was purified by preparative TLC and eluted with methylene chloride-ethyl acetate-hexane (2.0:2.0:6.0) to give a colorless crystalline solid of **RM12**: murrayacine (0.0021 g).

Subfraction 6J (0.0159 g) was separated by preparative TLC and eluted with methylene chloride-hexane (6.5:3.5) to give an orange crystalline solid of **RM13**: murrayanine (0.0114 g).

Subfraction 6N (0.0087 g) was filtered and washed with a mixture of hexane and methylene chloride (8.5:1.5) to give a yellow crystalline solid of **RM14**: 7-methoxymukonal (0.0067 g).

Subfraction 6O (0.1612 g) was further purified by Sephadex LH-20 and eluted with methylene chloride-methanol (1.0:1.0) to afford 11 fractions (6O1-6O11).

Subfraction 6O6 (0.0185 g) was further purified by Sephadex LH-20 and eluted with methylene chloride-methanol (1.0:1.0) to afford 6 fractions (6O6A-6O6F).

Subfraction 6O6C (0.0154 g) was separated by preparative TLC and eluted with methylene chloride to afford 3 fractions (6O6C1-6O6C3). Subfraction 6O6C1 gave a colorless viscous liquid of **RM16**: kinocoumarin (0.0040 g).

Fraction R7 (2.4016 g) was filtered and washed with a mixture of hexane and methylene chloride (8.5:1.5) to give a pale yellow prism of **RM15**: nordentatin (0.8299 g).

Fraction R8 (4.5235 g) was further purified by Sephadex LH-20 and eluted with methylene chloride–methanol (1.0:1.0) to afford 7 fractions (8A-8G). Subfraction 8D give a brownish crystalline solid of **RM17**: *O*-methylnukonal (0.0515 g).

Subfraction 8E (0.0253 g) was further purified by column chromatography over silica gel and eluted with ethyl acetate-hexane (2.5:7.5) to give an orange crystalline solid of **RM18** : 7-hydroxy-8-(1,1-dimethylallyl)citrusarin (0.0019 g).

Subfraction R9 (1.0415 g) was further purified by column chromatography over silica gel and eluted with methylene chloride to afford 12 fractions (9A-9L). Subfraction 9F give a brownish crystalline solid of **RM19**: 3-formyl-2,7-dimethoxycarbazole (0.0120 g).

Subfraction 9H (0.0287 g) was further purified by preparative TLC and eluted with methanol-methylene chloride (0.2:9.8) to afford 2 fractions (9H1-9H2). Subfraction 9H2 gives a brownish crystalline solid of **RM20**: clusine L (0.0216 g).

Subfraction 9H1 (0.0287 g) was further purified by preparative TLC and eluted with methylene chloride to give yellow viscous liquid of **RM21**: 7-hydroxyheptaphylline (0.0025 g).

Fraction R11 (3.7952 g) was further purified by Sephadex LH-20 and eluted with methylene chloride–methanol (1.0:1.0) to afford 5 fractions (11A-11E). Subfraction 11C give a brownish powder of **RM22**: clusine K (0.0305 g).

Subfraction 11B (1.1214 g) was filtered and washed with methylene chloride to give a colorless crystalline solid of **RM23**: *O*-methylclausenolide (0.2955 g).

Subfraction R12 (2.4877 g) was further purified by column chromatography over silica gel and eluted with methanol-methylene chloride (0.2:9.8) to afford 12 fractions (12A-12L).

Subfraction 12E (0.0058 g) was further purified by preparative TLC and eluted with methanol-methylene chloride (0.2:9.8) to give a yellow crystalline solid of **RM24**: clusine H (0.0030 g).

Fraction R13 (1.1938 g) was filtered and washed with hexane and methylene chloride (1.5:8.5) to give a colorless crystalline solid of **RM25**: clausenarin

(0.0085 g). The filtrate (1.1850 g) was further purified by Sephadex LH-20 and eluted with methylene chloride–methanol (1.0:1.0) to afford 5 fractions (13A-13E).

Subfraction 13E (0.0258 g) was further purified by preparative TLC and eluted with methanol-methylene chloride (0.4:9.6) to afford 6 fractions (13E1-13E6). Subfraction 13E3 gave yellow viscous liquid of **RM26**: clausebazole C (0.0065 g).

Subfraction 13E5 (0.0035 g) was further purified by preparative TLC and eluted with methylene chloride to give a colorless crystalline solid of **RM27**: isomukonidine (0.0025 g).

Compound RM1: heptaphylline, as a yellow powder, m.p. 169-170°C; UV λ_{\max} (MeOH) (log ϵ : 239 (3.98), 303 (4.22) and 351 (3.37) nm; IR (neat) (cm^{-1}): 3335 (O-H stretching), 1645 (aldehyde) and 1618, 1590 (cm^{-1} (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 3.

Compound RM2: clausinidin, yellow prismatic rods, m.p. 133-134°C; UV λ_{\max} (MeOH) (log ϵ : 223 (3.37), 285 (3.63) and 327 (3.42) nm; IR (neat) (cm^{-1}): 1731 (ester carbonyl), 1628 (cm^{-1} (chelated carbonyl), 1603, 1563 (cm^{-1} (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 5.

Compound RM3: dentatin, colorless prisms, m.p. 93-94 °C; UV λ_{\max} (MeOH) (log ϵ : 227 (3.28), 272 (3.37) and 328 (4.00) nm; IR (neat) (cm^{-1}): 1728 (ester carbonyl), 1612, 1590 (cm^{-1} (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 7.

Compound RM4: xanthoxylatin, colorless prisms, m.p. 130-131 °C; UV λ_{\max} (MeOH) (log ϵ : 226 (4.29), 268 (4.32) and 346 (4.01) nm; IR (neat) (cm^{-1}): 1733 (ester carbonyl), 1618, 1565 (cm^{-1} (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 9.

Compound RM5: clausemarin, yellow gum; UV λ_{\max} (MeOH) (log ϵ : 217 (4.33), 253 (4.41) and 299 (4.50) nm; IR (neat) (cm^{-1}): 3440 (O-H

stretching), 1728 (C=O stretching), 1603, 1464 cm^{-1} (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 11.

Compound RM6: mukonal, orange solid, m.p. 239-240°C; UV_{max} (MeOH) ($\log \epsilon$): 224 (4.35), 238 (4.39) and 330 (4.25) nm; IR (neat) (cm^{-1}): 3439 (O-H stretching), 1637 (aldehyde), 1618, 1520 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 13.

Compound RM7: clausebazole A, colorless crystalline solid, m.p. 180-181°C; UV_{max} (MeOH) ($\log \epsilon$): 202 (4.53), 234 (4.10), 276 (4.22), 299 (4.28) and 343 (3.79) nm; IR (neat) (cm^{-1}): 3414 (O-H stretching), 1629 (aldehyde), 1608, 1585 cm^{-1} (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 15.

Compound RM8: clausebazole B, crystalline solid, m.p. 170-171°C; UV_{max} (MeOH) ($\log \epsilon$): 237 (3.84), 277 (3.81), 297 (3.93) and 319 (3.49) nm; IR (neat) (cm^{-1}): 3416 (O-H stretching), 1663 (aldehyde), 1606, 1488 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 16.

Compound RM9: mukonidine, pale yellow crystalline solid, m.p. 158-159 °C; UV_{max} (MeOH) ($\log \epsilon$): 243 (4.10), 284 (4.28) and 338 (3.72) nm; IR (neat) (cm^{-1}): 3357 (O-H stretching), 1650 (ester carbonyl), 1633, 1464 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 17.

Compound RM10: 4-hydroxy-2-methoxybenzoic acid, colorless crystalline solid, m.p. 163-164°C; UV_{max} (MeOH) ($\log \epsilon$): 235 (3.37), 293 (2.61) and 308 (2.82) nm; IR (neat) (cm^{-1}): 3384 (O-H stretching), 1648 (carboxy carbonyl), 1621, 1615, 1562 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 19.

Compound RM11: mukonine, orange crystalline solid, m.p. 170-171°C; UV_{max} (MeOH) ($\log \epsilon$): 236 (4.38), 276 (4.42) and 310 (3.14) nm; IR (neat) (cm^{-1}): 3415 (O-H stretching), 1700 (ester carbonyl), 1606, 1578 (aromatic system);

For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 20.

Compound RM12: murayacine, colorless crystalline solid, m.p. 239-241 °C; UV λ_{max} (MeOH) (log ϵ : 225 (4.10), 281 (4.10), 299 (4.07) and 359 (3.49) nm; IR (neat) (cm^{-1}): 3335 (O-H stretching), 1636 (aldehyde carbonyl), 1602, 1575 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 22.

Compound RM13: murrayanine, orange crystalline solid, m.p. 162-163 °C; UV λ_{max} (MeOH) (log ϵ : 205 (4.18), 297 (3.58) and 330 (3.70) nm; IR (neat) (cm^{-1}): 3356 (O-H stretching), 1636 (aldehyde), 1606, 1578 (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 23.

Compound RM14: 7-methoxymukonal, yellow crystalline solid, m.p. 205-207 °C; UV λ_{max} (MeOH) (log ϵ : 202 (3.93), 240 (3.52), 300 (3.81) and 339 (3.14) nm; IR (neat) (cm^{-1}): 3415 (O-H stretching), 1627 (aldehyde), 1598, 1467 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 25.

Compound RM15: nordentatin, pale yellow prism, m.p. 178-180 °C; UV λ_{max} (MeOH) (log ϵ : 206 (4.82), 226 (4.69) and 336 (4.46) nm; IR (neat) (cm^{-1}): 3412 (O-H stretching), 1712 (ester carbonyl), 1600, 1563 (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 27.

Compound RM16: kinocoumarin, colorless viscous liquid; UV λ_{max} (MeOH) (log ϵ : 204 (3.42), 225 (3.18), 283 (3.39) and 336 (3.06) nm; IR (neat) (cm^{-1}): 3285 (O-H stretching), 1712 (ester carbonyl), 1592, 1566 (aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 29.

Compound RM17: *O*-methylumkonal, brownish crystalline solid, m.p. 195-197 °C; UV λ_{max} (MeOH) (log ϵ : 235 (3.24), 277 (3.20), 296 (3.27) and 351 (2.79) nm; IR (neat) (cm^{-1}): 3446 (O-H stretching), 1666 (aldehyde), 1622, 1602

(aromatic system); For ^1H NMR (CDCl_3 , 300 MHz) and ^{13}C NMR (CDCl_3 , 75 MHz) spectral data, see Table 31.

Compound RM18: 7-hydroxy-8-(1,1-dimethylallyl)citrusarin, pale yellow crystalline solid, m.p. 185-187°C; UV $_{\text{max}}$ (MeOH) (log ϵ : 205 (4.03), 227 (3.89), 280 (3.98) and 336 (3.76) nm; IR (neat) (cm^{-1}): 3335 (O-H stretching), 1717 (ester carbonyl), 1617, 1570, 1457 (aromatic system); For ^1H NMR (CDCl_3 , 500 MHz) and ^{13}C NMR (CDCl_3 , 125 MHz) spectral data, see Table 33.

Compound RM19: 3-formyl-2,7-dimethoxycarbazole, brownish crystalline solid, m.p. 221-223°C; UV $_{\text{max}}$ (MeOH) (log ϵ : 241 (4.20), 299 (4.31) and 347 (3.71) nm; IR (neat) (cm^{-1}): 3237 (O-H stretching), 1661 (aldehyde), 1602, 1508 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 35.

Compound RM20: clausine L, brownish crystalline solid, m.p. 131-132°C; UV $_{\text{max}}$ (MeOH) (log ϵ : 241 (3.36), 267 (3.37), 320 (2.60) and 333 (2.48) nm; IR (neat) (cm^{-1}): 3414 (O-H stretching), 1699 (ester carbonyl), 1637, 1461 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 37.

Compound RM21: 7-hydroxyheptaphylline, yellow viscous liquid; UV $_{\text{max}}$ (MeOH) (log ϵ : 239 (3.98), 303 (4.22) and 351 (3.37) nm; IR (neat) (cm^{-1}): 3410 (O-H stretching), 1620 (aldehyde), 1578, 1456 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) spectral data, see Table 39.

Compound RM22: clausine K, brownish powder, m.p. 254-256°C; UV $_{\text{max}}$ (MeOH) (log ϵ : 237 (4.02), 277 (3.95) and 351 (3.37) nm; IR (neat) (cm^{-1}): 3410 (O-H stretching), 1620 (carboxy carbonyl), 1603, 1548 (aromatic system); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 40.

Compound RM23: *O*-methylclausenolide, colorless crystalline solid, m.p. 189-190°C, $[\alpha]_{\text{D}}^{26} = -39.5^\circ$ ($c = 0.05$, MeOH); UV $_{\text{max}}$ (MeOH) (log ϵ : 207 (4.44) and 331 (4.32) nm; IR (neat) (cm^{-1}): 3493 (O-H stretching), 1710, 1630 (ester and ketone carbonyl), 840 (β -substituted furan); For ^1H NMR (CD_3COCD_3 , 300 MHz) and ^{13}C NMR (CD_3COCD_3 , 75 MHz) spectral data, see Table 42.

Compound RM24: clausine H, yellow crystalline solid, m.p. 187-189°C; UV_{max} (MeOH) (log ϵ : 246 (4.43), 282 (4.41) and 309 (3.96) nm; IR (neat) (cm⁻¹): 3404 (O-H stretching), 1702 (ester carbonyl), 1618, 1581 (aromatic system); For ¹H NMR (CD₃COCD₃, 300 MHz) and ¹³C NMR (CD₃COCD₃, 75 MHz) spectral data, see Table 44.

Compound RM25 clausenarin, colorless crystalline solid, m.p. 190-191°C; [α]_D²⁵ = -98.7° (*c* 1.04, Me₂CO) UV_{max} (MeOH) (log ϵ : 205 (4.42), 256 (3.49) and 331 (3.93) nm; IR (neat) (cm⁻¹): 3422 (O-H stretching), 1704, 1630 cm⁻¹ (ester and ketone carbonyl), 850 (*β*-substituted furan); For ¹H NMR (CDCl₃+CD₃OD (1 drop), 300 MHz) and ¹³C NMR (CDCl₃+CD₃OD (1 drop), 75 MHz) spectral data, see Table 46.

Compound RM26: clausebazole C, yellow viscous liquid; UV_{max} (MeOH) (log ϵ : 237 (4.27), 278 (4.23), 296 (4.35) and 352 (3.72) nm; IR (neat) (cm⁻¹): 3330 (O-H stretching), 1665 (aldehyde), 1607, 1580 (aromatic system); For ¹H NMR (CD₃COCD₃, 300 MHz) and ¹³C NMR (CD₃COCD₃), 75 MHz) spectral data, see Table 48.

Compound RM27: isomukonidine, colorless crystalline solid, m.p. 224-225°C °C; UV_{max} (MeOH) (log ϵ : 204 (4.67), 224 (4.62), 248, (4.56) and 331 (4.47) nm; IR (neat) (cm⁻¹): 3410 (O-H stretching), 1620 (carboxy carbonyl), 1603, 1548 (aromatic system); For ¹H NMR (CD₃COCD₃, 300 MHz) and ¹³C NMR (CD₃COCD₃, 75 MHz) spectral data, see Table 49.

Cyclization of nordentatin (RM15): RM15 (10 mg) was treated with conc.HCl (1 mL) in CH₂Cl₂ at room temperature for overnight. The water was poured into reaction mixture and then extracted with CH₂Cl₂. The organic layer was dried with anhydrous Na₂SO₄ and the solvent was evaporated. The residue was subjected to preparative TLC (100% CH₂Cl₂) to give the cyclized product (**III**) (3.4 mg) as colorless viscous liquid, together with the starting material (1.9 mg); UV_{max} (MeOH) (log ϵ : 204 (3.42), 225 (3.35), 283 (3.33) and 338 (3.15) nm; IR (Neat) (cm⁻¹): 1723 (C=O stretching), 1593, 1451 (aromatic system); For ¹H NMR (CDCl₃, 300 MHz) spectral data, see Table 34. This product was found to have ¹H NMR spectrum identical with citrusarin-A.

Cyclization of 7-hydroxy-8-(1,1-dimethylallyl)citrusarin: RM18 (5.0 mg) was treated with conc.HCl (0.5 mL) in CH₂Cl₂ at room temperature for overnight. The water was poured into reaction mixture and then extracted with CH₂Cl₂. The organic layer was dried with anhydrous Na₂SO₄ and the solvent was evaporated. The residue was subjected to preparative TLC (100% CH₂Cl₂) to give the cyclized product (**III**) (2.4 mg) together with the starting material (1.1 mg).

CHAPTER 3

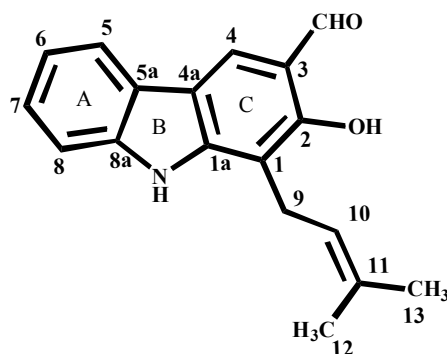
RESULTS AND DISCUSSION

3.1 Structure elucidation of compounds from the roots of *C. excavata*

The crude methylene chloride extract from the roots of *C. excavata* was subjected to quick column chromatography and repeated column chromatography over silica gel to furnish twenty-seven compounds: heptaphylline (**RM1**), clausinidin (**RM2**), dentatin (**RM3**), xanthoxylatin (**RM4**), clausemarin (**RM5**), mukonal (**RM6**), clausebazole A (**RM7**), clausebazole B (**RM8**), mukonidine (**RM9**), 4-hydroxy-2-methoxybenzoic acid (**RM10**), mukonine (**RM11**), murrayacine (**RM12**), murrayanine (**RM13**), 7-methoxymukonal (**RM14**), nordentatin (**RM15**), kinocoumarin (**RM16**), *O*-methyilmukonal (**RM17**), 7-hydroxy-8-(1,1-dimethylallyl) citrusarin (**RM18**), 3-formyl-2,7-dimethoxycarbazole (**RM19**), clausine L (**RM20**), 7-hydroxyheptaphylline (**RM21**), clausine K (**RM22**), *O*-methylclausenolide (**RM23**), clausine H (**RM24**), clausenarin (**RM25**), clausebazole C (**RM26**) and isomukonidine (**RM27**).

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

3.1.1 Compound RM1



RM1 was obtained as a yellow powder, m.p. 169-170°C [lit. 170-171°C]. The IR spectrum showed absorption bands at 3335 cm^{-1} (OH and NH), 1645 cm^{-1} (aldehyde) and 1618, 1590 cm^{-1} (aromatic system).

In the ^1H NMR spectrum of **RM1**, two singlets at δ 9.79 (CHO) and 7.90 (H-4) and a broad singlet at δ 8.15 (NH) were observed. In the aromatic region, a set of four adjacent proton signals at δ 7.18 (*m*, 1H), 7.30 (*m*, 1H), 7.31 (*m*, 1H) and 7.86 (*d*, $J = 7.7$ Hz, 1H) were assigned for H-6, H-7, H-8 and H-5, respectively, suggesting no substituent on the A-ring. Furthermore, the presence of a prenyl moiety in the molecule was indicated by ^1H NMR signals at δ 1.68 (*s*, H₃-13), 1.80 (*s*, H₃-12), 3.53 (*d*, $J = 6.8$ Hz, H₂-9) and 5.22 (*br t*, $J = 6.8$ Hz, H-10). The intramolecular hydrogen bonding between 3-CHO and a downfield hydroxyl at δ 11.57 inferred that a phenolic hydroxyl was located at C-2, thus, a prenyl moiety should be located at C-1. The HMBC correlations between δ 157.8 (C-2) and H-9 (δ 3.53), H-4 (δ 7.90) and aldehydic proton (δ 9.79) confirmed the placement of the hydroxyl group at C-2, while correlations between C-1 (δ 109.0) and H-10 (δ 5.22) and a hydroxyl proton (δ 11.57) sited the prenyl group at C-1. On the basis of these results, the structure of **RM1** was proposed for heptaphylline [Ruangrunsi *et al.*, 1990].

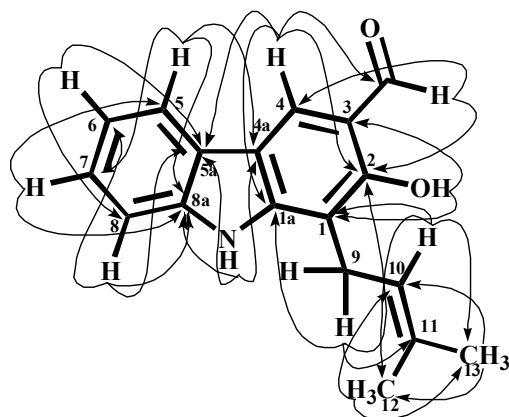


Figure 2 Selected HMBC correlations of **RM1**

Table 3 ^1H , ^{13}C NMR and HMBC spectral data of **RM1** (CDCl_3)

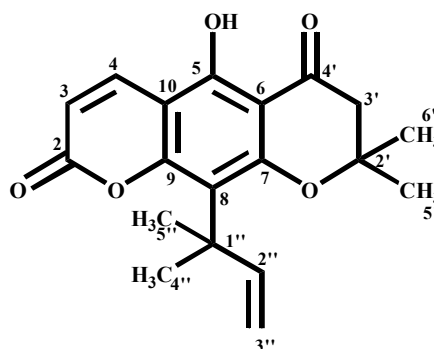
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	145.0 (C)	-
1	-	109.0 (C)	-
2	-	157.8 (C)	-
3	-	115.4 (C)	-
4	7.90 (1H, <i>s</i>)	125.3 (CH)	C-1, C-1a, C-5a, C-2, CHO
4a	-	117.3 (C)	-
5a	-	123.6 (C)	-
5	7.86 (1H, <i>d</i> , $J = 7.7$ Hz)	119.8 (CH)	C-4a, C-7, C-8a
6	7.18 (1H, <i>m</i>)	120.8 (CH)	C-5a, C-8, C-8a
7	7.30 (1H, <i>m</i>)	125.8 (CH)	C-5, C-8a
8	7.31 (1H, <i>m</i>)	110.9 (CH)	C-5a, C-6, C-8a
8a	-	140.1 (C)	-
9	3.53 (2H, <i>d</i> , $J = 6.8$ Hz)	22.8 (CH_2)	C-1, C-1a, C-2, C-10, C-11
10	5.22 (1H, <i>br t</i> , $J = 6.8$ Hz)	121.2 (CH)	C-1, C-12, C-13
11	-	134.1 (C)	-
12	1.80 (3H, <i>s</i>)	18.1 (CH_3)	C-10, C-11, C-13
13	1.68 (3H, <i>s</i>)	25.7 (CH_3)	C-10, C-11, C-12
2-OH	11.57 (1H, <i>s</i>)	-	C-1, C-2, C-3
3-CHO	9.79 (1H, <i>s</i>)	195.4 (CHO)	C-2, C-4
NH	8.15 (1H, <i>br s</i>)	-	C-4a, C-5a, C-8a

Table 4 ^1H and ^{13}C NMR spectral data of **RM1** and **Heptaphylline (R)** (CDCl_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM1 ^a	R ^b	RM1 ^c	R ^d
1a	-	-	145.0	145.3
1	-	-	109.1	109.7
2	-	-	157.8	158.0
3	-	-	115.4	115.7
4	7.90 (<i>s</i>)	8.04 (<i>s</i>)	125.3	126.0
4a	-	-	117.3	117.7
5a	-	-	123.6	124.1
5	7.86 (<i>d</i> , $J = 7.7$ Hz)	7.97 (<i>d</i> , $J = 7.6$ Hz)	119.8	120.7
6	7.18 (<i>m</i>)	7.27 (<i>t</i> , $J = 7.6$ Hz)	120.8	120.0
7	7.30 (<i>m</i>)	7.40 (<i>t</i> , $J = 7.6$ Hz)	125.8	125.9
8	7.31 (<i>m</i>)	7.40 (<i>d</i> , $J = 7.6$ Hz)	110.9	111.6
8a	-	-	140.1	141.5
9	3.53 (<i>d</i> , $J = 6.8$ Hz)	3.64 (<i>d</i> , $J = 6.8$ Hz)	22.8	23.0
10	5.22 (<i>br t</i> , $J = 6.8$ Hz)	5.32 (<i>br t</i> , $J = 6.8$ Hz)	121.2	121.7
11	-	-	134.1	132.7
12	1.80 (<i>s</i>)	1.77 (<i>s</i>)	18.1	17.6
13	1.68 (<i>s</i>)	1.90 (<i>s</i>)	25.7	25.3
2-OH	11.57 (<i>s</i>)	11.57 (<i>s</i>)	-	-
3-CHO	9.79 (<i>s</i>)	9.91 (<i>s</i>)	195.4	196.3
NH	8.15 (<i>br s</i>)	8.20 (<i>s</i>)	-	-

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

3.1.2 Compound RM2



RM2 was obtained as yellow prismatic rods, m.p. 133-134°C [lit. 135-136°C]. The IR spectrum showed absorption bands at 1731 cm^{-1} (ester carbonyl), 1628 cm^{-1} (chelated carbonyl) and 1603, 1563 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM2** showed AB system of α,β -unsaturated lactone at δ 6.14 and δ 8.02 (1H each, d , $J = 9.5$ Hz) which were characteristic of H-3 and H-4, respectively, of the coumarin skeleton. The presence of a downfield singlet signal at δ 12.96 (5-OH), suggested that a hydroxyl proton was chelated by an adjacent carbonyl oxygen. A sharp two-proton singlet at δ 2.73 (H₂-3') and a six-proton singlet at δ 1.47 (H₃-5' and H₃-6') suggested a methylene adjacent to a carbonyl group and a *gem*-dimethyl group which was confirmed by HMBC correlations between δ 198.2 (C-4') and H-3' (δ 2.73) and δ 47.6 (C-3') and H₃-5'/H₃-6'. The above features indicated that **RM2** contained a 2',2'-dimethyl-4-pyranone ring linearly attached to the coumarin ring. The remaining peak disclosed a 1,1-dimethylallyl group at δ 1.61 (s , $2 \times \text{CH}_3$), 4.85 (dd , $J = 7.8, 1.2$ Hz, 1H), 4.90 (dd , $J = 15.9, 1.2$ Hz, 1H) and 6.21 (dd , $J = 15.9, 7.8$ Hz, 1H). The HMBC spectrum showed correlations between a chelated hydroxyl proton (5-OH) and C-5 (δ 159.0), C-6 (δ 104.0) and C-10 (δ 103.2) as well as correlations between H-4 (δ 8.02) and C-5 (δ 159.0) and H-3 (δ 6.14) and C-10 (δ 103.2), confirming the presence of a 5-hydroxypyranocoumarin moiety. The 1,1-dimethylallyl group was placed at C-8 based on the result of HMBC correlations between δ 114.4 (C-8) and H-2'' (δ 6.21) and H₃-4''/H₃-5'' (δ 1.61). It was therefore suggested that compound **RM2** was clausinidin [Huang *et al.*, 1996].

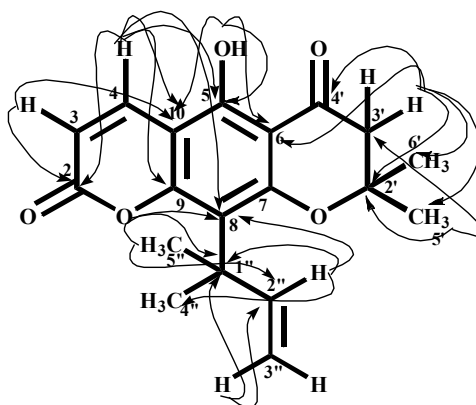


Figure 3 Selected HMBC correlations of **RM2**

Table 5 ^1H , ^{13}C NMR and HMBC spectral data of **RM2** (CDCl_3)

Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	-	-	-
2	-	160.0 (C)	-
3	6.14 (1H, <i>d</i> , $J = 9.5$ Hz)	110.7 (CH)	C-2, C-10
4	8.02 (1H, <i>d</i> , $J = 9.5$ Hz)	138.6 (CH)	C-2, C-5, C-8, C-9, C-10
5	-	159.0 (C)	-
6	-	104.0 (C)	-
7	-	160.6 (C)	-
8	-	114.4 (C)	-
9	-	159.0 (C)	-
10	-	103.2 (C)	-
1'	-	-	-
2'	-	80.0 (C)	-
3'	2.73 (2H, <i>s</i>)	47.6 (CH_2)	C-6, C-2', C-4', C-5', C-6'
4'	-	198.2 (C)	-
5'	1.47 (3H, <i>s</i>)	26.5 (CH_3)	C-2', C-3', C-6'
6'	1.47 (3H, <i>s</i>)	26.5 (CH_3)	C-2', C-3', C-5'
1''	-	40.9 (C)	-
2''	6.21 (1H, <i>dd</i> , $J = 15.9, 7.8$ Hz)	149.5 (CH)	C-8, C-1'', C-4'', C-5''
3''	4.85 (1H, <i>dd</i> , $J = 7.8, 1.2$ Hz)	108.4 (CH_2)	C-1'', C-2''
4''	4.90 (1H, <i>dd</i> , $J = 15.9, 1.2$ Hz)	29.4 (CH_3)	C-8, C-1'', C-2'', C-5''

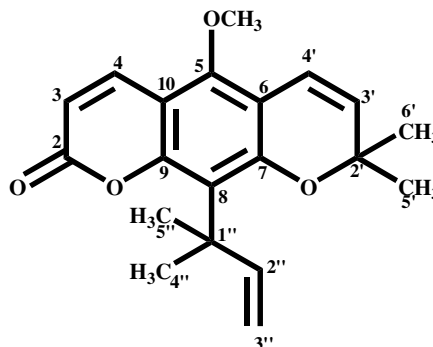
5''	1.61 (3H, <i>s</i>)	29.4 (CH ₃)	C-8, C-1'', C-2'', C-4''
5-OH	12.96 (1H, <i>s</i>)	-	C-5, C-6, C-10

Table 6 ¹H NMR spectral data of **RM2** and **Clausinidin (R)** (CDCl₃)

Position	δ_{H} (multiplicity)	
	RM2 ^a	R
1	-	-
2	-	-
3	6.14 (<i>d</i> , <i>J</i> = 9.5 Hz)	6.17 (<i>d</i> , <i>J</i> = 9.8 Hz)
4	8.02 (<i>d</i> , <i>J</i> = 9.5 Hz)	8.05 (<i>d</i> , <i>J</i> = 9.8 Hz)
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
1'	-	-
2'	-	-
3'	2.73 (<i>s</i>)	2.76 (<i>s</i>)
4'	-	-
5'	1.47 (<i>s</i>)	1.64 (<i>s</i>)
6'	1.47 (<i>s</i>)	1.64 (<i>s</i>)
1''	-	-
2''	6.21 (<i>dd</i> , <i>J</i> = 15.9, 7.8 Hz)	6.23 (<i>dd</i> , <i>J</i> = 17.5, 10.4 Hz)
3''	4.85 (<i>dd</i> , <i>J</i> = 7.8, 1.2 Hz)	4.89 (<i>dd</i> , <i>J</i> = 10.4, 1.0 Hz)
	4.90 (<i>dd</i> , <i>J</i> = 15.9, 1.2 Hz)	4.92 (<i>dd</i> , <i>J</i> = 17.5, 10.4 Hz)
4''	1.61 (<i>s</i>)	1.49 (<i>s</i>)
5''	1.61 (<i>s</i>)	1.49 (<i>s</i>)
5-OH	12.96 (<i>s</i>)	12.99 (<i>s</i>)

^a300 MHz

3.1.3 Compound RM3



RM3 was obtained as a colorless prisms, m.p. 93-94°C [lit. 95°C]. The IR spectrum showed absorption bands at 1728 cm^{-1} (ester carbonyl) and 1612, 1590 cm^{-1} (aromatic system).

The spectral data of **RM3** showed a similar pattern to that of **RM2**. The major differences were that the additional two olefinic doublets at δ 5.68 (H-3') and 6.55 (H-4') (1H each, $J = 9.9$ Hz) in **RM3** replaced a singlet methylene signal δ 2.73 in **RM2** and the disappearance of a carbonyl carbon signal at δ_{C} 198.2 in **RM2**, suggesting that a dimethylpyran ring in **RM3** replaced a dimethylpyranone ring in **RM2**. In addition a methoxyl singlet signal was observed at δ_{H} 3.81. The C-5 (δ 151.2) showed a HMBC correlation to H-4 (δ 7.86), H-4' (δ 6.55) and methoxyl protons (δ 3.81), suggesting that **RM3** was a linear pyranocoumarin derivative having the methoxyl group at C-5. Therefore, compound **RM3** was identified as dentatin [Govindachari *et al.*, 1968].

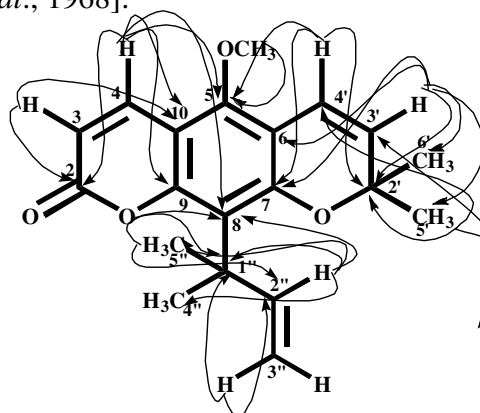


Figure 4 Selected HMBC correlations of **RM3**

Table 7 ^1H , ^{13}C NMR and HMBC spectral data of **RM3** (CDCl_3)

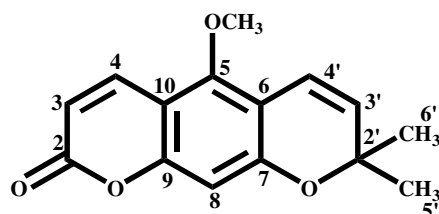
position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	-	-	-
2	-	160.7 (C)	-
3	6.17 (1H, <i>d</i> , <i>J</i> = 9.6 Hz)	111.6 (CH)	C-2, C-10
4	7.86 (1H, <i>d</i> , <i>J</i> = 9.6 Hz)	138.9 (CH)	C-2, C-5, C-8, C-9, C-10
5	-	151.2 (C)	-
6	-	111.7 (C)	-
7	-	156.0 (C)	-
8	-	119.2 (C)	-
9	-	153.9 (C)	-
10	-	107.5 (C)	-
1'	-	-	-
2'	-	77.3 (C)	-
3'	5.68 (1H, <i>d</i> , <i>J</i> = 9.9 Hz)	130.4 (CH)	C-6, C-7, C-2', C-5', C-6'
4'	6.55 (1H, <i>d</i> , <i>J</i> = 9.9 Hz)	116.3 (CH)	C-5, C-7, C-2'
5'	1.43 (3H, <i>s</i>)	27.5 (CH_3)	C-2', C- 3', C-4'
6'	1.43 (3H, <i>s</i>)	27.5 (CH_3)	C-2', C- 3', C-4'
1''	-	41.2 (C)	-
2''	6.28 (1H, <i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	149.8 (CH)	C-8, C- 1'', C-4'', C-5''
3''	4.86 (1H, <i>dd</i> , <i>J</i> = 10.6, 1.1 Hz) 4.92 (1H, <i>dd</i> , <i>J</i> = 17.4, 1.0 Hz)	108.2 (CH_2)	C- 1'', C-2''
4''	1.64 (3H, <i>s</i>)	29.4 (CH_3)	C-8, C- 1'', C-2''
5''	1.64 (3H, <i>s</i>)	29.4 (CH_3)	C-8, C- 1'', C-2''
5-OCH ₃	3.81 (3H, <i>s</i>)	63.4 (CH_3)	C-5

Table 8 ^1H NMR spectral data of **RM3** and **Dentatin (R)** (CDCl_3)

Position	δ_{H} (multiplicity)	
	RM3 ^a	R
1	-	-
2	-	-
3	6.17 (<i>d</i> , <i>J</i> = 9.6 Hz)	6.15 (<i>d</i> , <i>J</i> = 10.0 Hz)
4	7.86 (<i>d</i> , <i>J</i> = 9.6 Hz)	7.90 (<i>d</i> , <i>J</i> = 10.0 Hz)
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
1'	-	-
2'	-	-
3'	5.68 (<i>d</i> , <i>J</i> = 9.9 Hz)	5.75 (<i>d</i> , <i>J</i> = 10.0 Hz)
4'	6.55 (<i>d</i> , <i>J</i> = 9.9 Hz)	6.60 (<i>d</i> , <i>J</i> = 10.0 Hz)
5'	1.43 (<i>s</i>)	1.48 (<i>s</i>)
6'	1.43 (<i>s</i>)	1.48 (<i>s</i>)
1''	-	-
2''	6.28 (<i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	6.15 (<i>dd</i> , <i>J</i> = 17.5, 10.5 Hz)
3''	4.86 (<i>dd</i> , <i>J</i> = 10.6, 1.1 Hz)	4.98 (<i>dd</i> , <i>J</i> = 10.5, 1.3 Hz)
	4.92 (<i>dd</i> , <i>J</i> = 17.4, 1.0 Hz)	4.89 (<i>dd</i> , <i>J</i> = 17.5, 1.3 Hz)
4''	1.64 (<i>s</i>)	1.69 (<i>s</i>)
5''	1.64 (<i>s</i>)	1.69 (<i>s</i>)
5-OCH ₃	3.81 (<i>s</i>)	3.85 (<i>s</i>)

^a300 MHz

3.1.4 Compound RM4



RM4 was obtained as colorless crystalline solids, m.p. 130-131 °C [lit. 132-133 °C]. The IR spectrum showed absorption bands at 1733 cm^{-1} (ester carbonyl) and 1618, 1565 cm^{-1} (aromatic system).

The ^1H and ^{13}C NMR spectra of **RM4** were similar to those of **RM3**, except the absence of the signals of a 1,1-dimethylallyl group and the presence of an aromatic proton singlet signal at δ 6.56 (H-8) which indicated that the difference between **RM3** and **RM4** was the substituent at C-8. The HMBC correlations between aromatic proton H-8 (δ 6.56) and the carbons at δ 155.6 (C-9), 111.3 (C-6), 157.6 (C-7) and 107.4 (C-10) placed the aromatic proton at C-8. It was therefore suggested that compound **RM4** was xanthoxylatin [Cazal *et al.*, 2009].

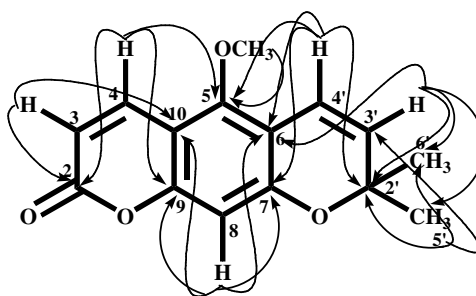


Figure 5 Selected HMBC correlations of **RM4**

Table 9 ^1H , ^{13}C NMR and HMBC spectral data of **RM4** (CDCl_3)

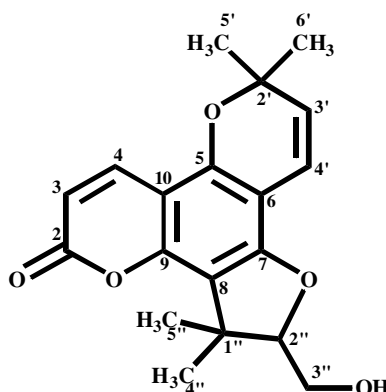
position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	-	-	-
2	-	161.1 (C)	-
3	6.21 (1H, <i>d</i> , $J = 9.6$ Hz)	112.3 (CH)	C-2, C-10
4	7.86 (1H, <i>d</i> , $J = 9.6$ Hz)	138.5 (CH)	C-2, C-5, C-9
5	-	152.8 (C)	-
6	-	111.3 (C)	-
7	-	157.6 (C)	-
8	6.56 (1H, <i>s</i>)	100.8 (CH)	C-9, C-10, C-6, C-7
9	-	155.6 (C)	-
10	-	107.4 (C)	-
1'	-	-	-
2'	-	77.5 (C)	-
3'	5.71 (1H, <i>d</i> , $J = 10.1$ Hz)	130.6 (CH)	C-6, C-2', C-5', C-6'
4'	6.57 (1H, <i>d</i> , $J = 10.1$ Hz)	115.8 (CH)	C-5, C-6, C-7, C-2'
5'	1.47 (3H, <i>s</i>)	28.1 (CH_3)	C-2', C-3', C-6'
6'	1.47 (3H, <i>s</i>)	28.1 (CH_3)	C-2', C-3', C-5'
5-OCH ₃	3.89 (3H, <i>s</i>)	63.6 (CH_3)	C-5

Table 10 ^1H and ^{13}C NMR spectral data of **RM4** and **Xanthoxylatin (R)** (CDCl_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM4 ^a	R ^b	RM4 ^c	R ^d
1	-	-	-	-
2	-	-	161.1	161.1
3	6.21 (<i>d</i> , <i>J</i> = 9.6 Hz)	6.20 (<i>d</i> , <i>J</i> = 9.6 Hz)	112.3	112.4
4	7.86 (<i>d</i> , <i>J</i> = 9.6 Hz)	7.84 (<i>d</i> , <i>J</i> = 9.6 Hz)	138.5	138.5
5	-	-	152.8	152.9
6	-	-	111.3	111.4
7	-	-	157.6	157.6
8	6.56 (<i>s</i>)	6.54 (<i>s</i>)	100.8	100.9
9	-	-	155.6	155.6
10	-	-	107.4	107.4
1'	-	-	-	-
2'	-	-	77.5	77.6
3'	5.71 (<i>d</i> , <i>J</i> = 10.1 Hz)	5.96 (<i>d</i> , <i>J</i> = 10 Hz)	130.6	130.6
4'	6.57 (<i>d</i> , <i>J</i> = 10.1 Hz)	6.57 (<i>d</i> , <i>J</i> = 10 Hz)	115.8	115.8
5'	1.47 (<i>s</i>)	1.45 (<i>s</i>)	28.1	28.2
6'	1.47 (<i>s</i>)	1.45 (<i>s</i>)	28.1	28.2
5-OCH ₃	3.89 (<i>s</i>)	3.85 (<i>s</i>)	63.6	63.7

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

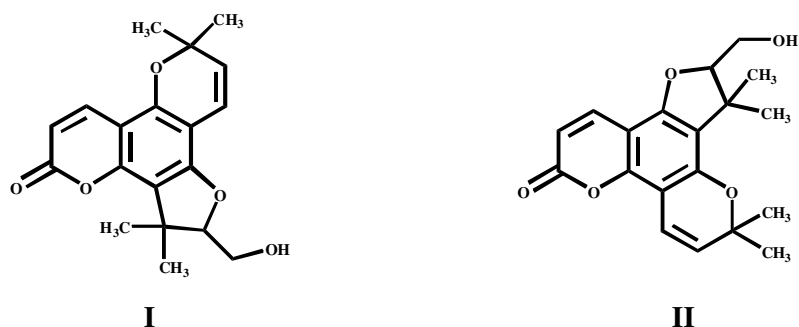
3.1.5 Compound RM5



RM5 was obtained as yellow gum. The IR spectrum showed absorption bands at 3440 cm^{-1} (OH), 1728 cm^{-1} (ester carbonyl) and $1603, 1464\text{ cm}^{-1}$ (aromatic system).

Its EIMS spectrum showed a molecular peak at $m/z\ 330\ [M]^+$ corresponding to the molecular formula $C_{19}H_{20}O_5$. The ^1H NMR spectrum of **RM5** showed the two pair of AB type doublets at $\delta\ 6.03$ and 7.87 (1H each, $J = 9.7\text{ Hz}$) and at $\delta\ 6.41$ and 5.50 (1H each, $J = 9.9\text{ Hz}$), accompanied by signals of two methyl groups attached to oxygenated carbon at $\delta\ 1.40$ (6H, *s*) which were assignable to the proton on an α,β -unsaturated carbonyl system and on the dimethylbenzopyran ring system, respectively. The downfield shift of H-4 at $\delta\ 7.87$ and the absence of the other proton signals in the aromatic region in the ^1H NMR spectrum, suggested the presence of 5,7-dioxygenated-6,8-disubstituted coumarin skeleton having a dimethylpyran ring system in the molecule. The remaining proton signals were assigned to the geminal methyls attached to benzylic carbon at $\delta\ 1.29$ and 1.52 (3H each, *s*), an oxymethine proton at $\delta\ 4.43$ (*dd*, $J = 7.9, 3.7\text{ Hz}$, 1H) and two doublets of hydroxymethylene protons at $\delta\ 3.81$ (*dd*, $J = 12.1, 7.9\text{ Hz}$, 1H), 3.88 (*dd*, $J = 12.1, 3.7\text{ Hz}$, 1H). The HMBC spectrum showed the correlations between the oxymethine proton at $\delta\ 4.43$ (H-2'') and two methyl carbons at $\delta\ 20.9$ (C-4'') and 26.9 (C-5''), which further correlated to an aromatic carbon at $\delta\ 113.7$ (C-8) and an oxygenated aromatic carbon at $\delta\ 157.4$ (C-7), indicating the presence of 2-hydroxymethyl-1,1-dimethyldihydrobenzofuran system in the molecules. Furthermore the HMBC

spectrum suggested the location of a hydroxy methylene group at C-2'' due to the correlations of δ 3.88 and 3.81 (H_2-3'') to the carbons at δ 43.4 (C-1'') and 94.5 (C-2'').



The structure of **RM5** could be depicted by either structure **I** or **II**. Comparison of the NMR data of **RM5**, citrusarin-A and citrusarin-B (Chan *et al.*, 2010) in **Table 12** showed the similarity of the chemical shift of signal at δ 6.41 due to H-4' on the pyran ring of **RM5** to that of citrusarin-A. It was concluded that **RM5** should therefore be constituted as structure **I**. It was therefore suggested that compound **RM5** was a new compound and named as clausemarin.

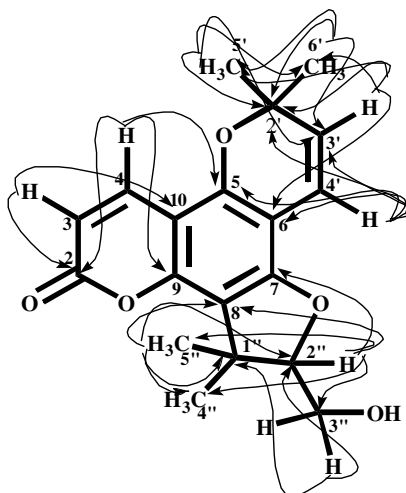
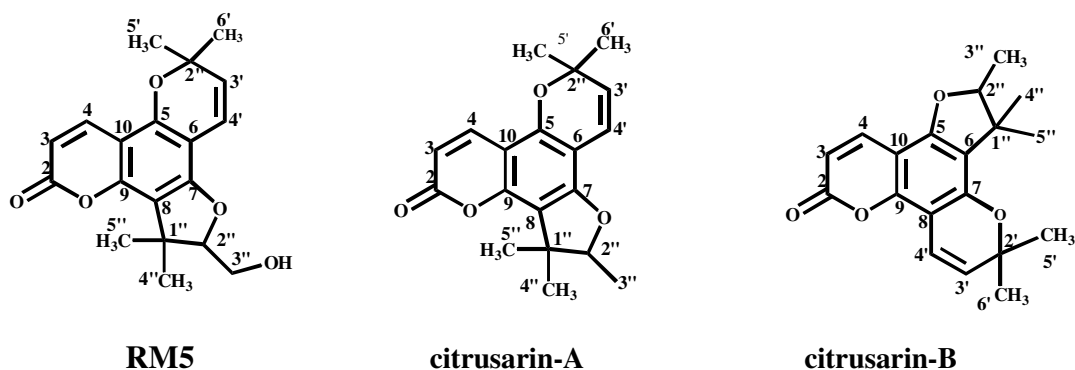


Figure 6 Selected HMBC correlations of **RM5**

Table 11 ^1H , ^{13}C NMR and HMBC spectral data of **RM5** (CDCl_3)

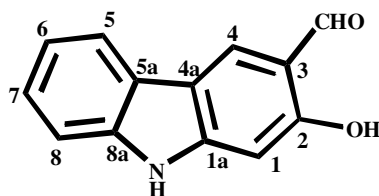
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	-	-	-
2	-	161.0 (C)	-
3	6.03 (1H, <i>d</i> , <i>J</i> = 9.7 Hz)	110.2 (CH)	C-2, C-10
4	7.87 (1H, <i>d</i> , <i>J</i> = 9.7 Hz)	139.2 (CH)	C-2, C-5, C-9
5	-	150.5 (C)	-
6	-	102.0 (C)	-
7	-	157.4 (C)	-
8	-	113.7 (C)	-
9	-	150.9 (C)	-
10	-	103.9 (C)	-
1'	-	-	-
2'	-	78.0 (C)	-
3'	5.50 (1H, <i>d</i> , <i>J</i> = 9.9 Hz)	128.1 (CH)	C-6, C-2', C-5', C-6'
4'	6.41 (1H, <i>d</i> , <i>J</i> = 9.9 Hz)	115.7 (CH)	C-5, C-6, C-7, C-2', C-3'
5'	1.40 (3H, <i>s</i>)	28.0 (CH_3)	C-2', C-3', C-6'
6'	1.40 (3H, <i>s</i>)	28.0 (CH_3)	C-2', C-3', C-5'
1''	-	43.4 (C)	-
2''	4.43 (1H, <i>dd</i> , <i>J</i> = 7.9, 3.7 Hz)	94.6 (CH)	C-7, C-8, C-1'', C-3'', C-4'', C-5''
3''	3.81 (1H, <i>dd</i> , <i>J</i> = 12.1, 7.9 Hz) 3.88 (1H, <i>dd</i> , <i>J</i> = 12.1, 3.7 Hz)	61.7 (CH_2)	C-1'', C-2''
4''	1.29 (3H, <i>s</i>)	20.9 (CH_3)	C-8, C-1'', C-2'', C-5''
5''	1.52 (3H, <i>s</i>)	26.9 (CH_3)	C-8, C-1'', C-2'', C-4''

Table 12 ^1H NMR spectral data of **RM5**, **Citrusarin-A** and **Citrusarin-B** (CDCl_3)

position	RM5 ^a	Citrusarin-A ^b	Citrusarin-B ^b
1	-	-	-
2	-	-	-
3	6.03 (<i>d</i> , <i>J</i> = 9.7 Hz)	6.07 (<i>d</i> , <i>J</i> = 9.6 Hz)	6.11 (<i>d</i> , <i>J</i> = 9.5 Hz)
4	7.87 (<i>d</i> , <i>J</i> = 9.7 Hz)	7.93 (<i>d</i> , <i>J</i> = 9.6 Hz)	7.75 (<i>d</i> , <i>J</i> = 9.5 Hz)
5	-	-	-
6	-	-	-
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-
1'	-	-	-
2'	-	-	-
3'	5.50 (<i>d</i> , <i>J</i> = 9.9 Hz)	5.54 (<i>d</i> , <i>J</i> = 9.9 Hz)	5.56 (<i>d</i> , <i>J</i> = 9.9 Hz)
4'	6.41 (<i>d</i> , <i>J</i> = 9.9 Hz)	6.45 (<i>d</i> , <i>J</i> = 9.9 Hz)	6.80 (<i>d</i> , <i>J</i> = 9.9 Hz)
5'	1.40 (<i>s</i>)	1.46 (<i>s</i>)	1.46 (<i>s</i>)
6'	1.40 (<i>s</i>)	1.46 (<i>s</i>)	1.47 (<i>s</i>)
1''	-	-	-
2''	4.43 (<i>dd</i> , <i>J</i> = 7.9, 3.7 Hz)	4.48 (<i>d</i> , <i>J</i> = 6.6 Hz)	4.49 (<i>d</i> , <i>J</i> = 6.6 Hz)
3''	3.81 (<i>dd</i> , <i>J</i> = 12.1, 7.9 Hz) 3.88 (<i>dd</i> , <i>J</i> = 12.1, 3.7 Hz)	1.39 (<i>d</i> , <i>J</i> = 6.6 Hz)	1.40 (<i>d</i> , <i>J</i> = 6.6 Hz)
4''	1.29 (<i>s</i>)	1.25 (<i>s</i>)	1.18 (<i>s</i>)
5''	1.52 (<i>s</i>)	1.52 (<i>s</i>)	1.42 (<i>s</i>)

^a300 MHz, ^b400 MHz

3.1.6 Compound RM6



RM6 was obtained as an orange solid, m.p. 239-240°C [lit. 238°C]. The IR spectrum showed absorption bands at 3439 cm^{-1} (OH and NH), 1637 cm^{-1} (aldehyde) and 1618, 1520 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM6** showed a signal pattern similar to those of **RM1**, except for the appearance of an additional singlet signal at δ_{H} 6.92 (δ_{C} 96.5) assignable to an aromatic proton, instead of the signal of the prenyl side chain, indicating no side chain at C-1 of a carbazole moiety. The upfield shift of the aromatic proton (H-1) at δ 6.92 indicated shielding by resonance effects from two adjacent heteroatoms. The HMBC correlations between δ 6.92 (H-1) and δ 115.4 (C-3), 161.0 (C-2) and 117.6 (C-4a) as well as correlations from a chelated hydroxyl proton (δ 11.48) to δ 96.5 (C-1) confirmed the position of the additional aromatic proton at C-1. Therefore, compound **RM6** was identified as mukonal [Ruangrungsi *et al.*, 1990].

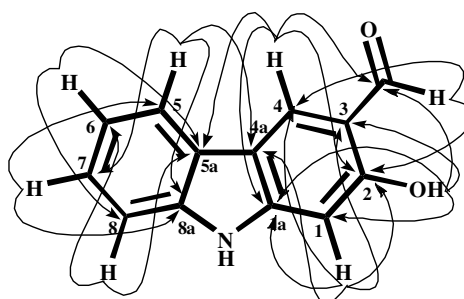


Figure 7 Selected HMBC correlations of **RM6**

Table 13 ^1H and ^{13}C NMR and HMBC spectral data of **RM6** (CD_3COCD_3)

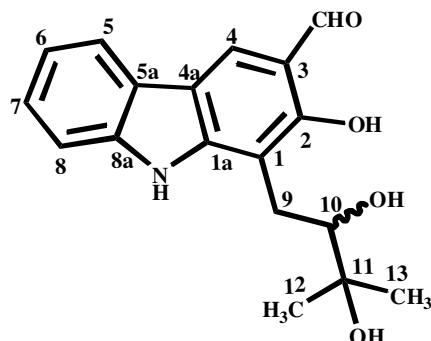
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	146.2 (C)	-
1	6.92 (1H, <i>s</i>)	96.5 (CH)	C-1a, C-2, C-3, C-4, C-4a, CHO
2	-	161.0 (C)	-
3	-	115.4 (C)	-
4	8.46 (1H, <i>s</i>)	127.6 (CH)	C-1a, C-2, C-5a, CHO
4a	-	117.6 (C)	-
5a	-	123.3 (C)	-
5	8.09 (1H, <i>d</i> , $J = 7.7$ Hz)	119.7 (CH)	C-4a, C-7, C-8a
6	7.24 (1H, <i>td</i> , $J = 7.7, 1.0$ Hz)	120.4 (CH)	C-5a, C-8
7	7.40 (1H, <i>td</i> , $J = 8.0, 1.0$ Hz)	125.8 (CH)	C-5, C-8a
8	7.51 (1H, <i>d</i> , $J = 8.0$ Hz)	111.1 (CH)	C-5a, C-6
8a	-	141.1 (C)	-
2-OH	11.48 (1H, <i>s</i>)	-	C-1a, C-1, C-2, C-3, CHO
3-CHO	10.01 (1H, <i>s</i>)	195.7 (CHO)	C-1, C-2, C-4
NH	10.82 (1H, <i>br s</i>)	-	-

Table 14 ^1H and ^{13}C NMR spectral data of **RM6** and **Mukonal (R)** (CD_3COCD_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM6 ^a	R ^b	RM8 ^c	R ^d
1a	-	-	145.0	145.3
1	6.92 (<i>s</i>)	6.89 (<i>s</i>)	109.1	109.7
2	-	-	157.8	158.0
3	-	-	115.4	115.7
4	8.46 (<i>s</i>)	8.43 (<i>s</i>)	125.3	126.0
4a	-	-	117.3	117.7
5a	-	-	123.6	124.1
5	8.09 (<i>d</i> , $J = 7.7$ Hz)	8.07 (<i>d</i> , $J = 7.6$ Hz)	119.8	120.7
6	7.24 (<i>td</i> , $J = 7.7, 1.0$ Hz)	7.22 (<i>t</i> , $J = 7.6$ Hz)	120.8	120.0
7	7.40 (<i>td</i> , $J = 8.0, 1.0$ Hz)	7.38 (<i>t</i> , $J = 7.6$ Hz)	125.8	125.9
8	7.51 (<i>d</i> , $J = 8.0$ Hz)	7.48 (<i>d</i> , $J = 7.6$ Hz)	110.9	111.6
8a	-	-	140.1	141.5
2-OH	11.48 (<i>s</i>)	11.46 (<i>s</i>)	-	-
3-CHO	10.01 (<i>s</i>)	9.98 (<i>s</i>)	195.4	196.3
NH	10.82 (<i>br s</i>)	10.72 (<i>br s</i>)	-	-

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

3.1.7 Compound RM7



RM7 was obtained as a colorless crystalline solid, m.p. 180-181°C. The IR spectrum showed absorption bands at 3414 cm^{-1} (OH and NH), 1629 cm^{-1} (aldehyde) and 1608, 1585 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM7** showed a comparable pattern to those of **RM6**, except that a high field aromatic proton H-1 at δ 6.92 was replaced by signals of a side chain $-\text{CH}_2\text{CH}(\text{OH})\text{C}(\text{CH}_3)_2\text{OH}$ resonance as a set of ABX type of $-\text{CH}_2\text{CH}-$ at δ 2.88 (*dd*, $J = 14.0, 10.0$ Hz, 1H), 3.37 (*dd*, $J = 14.0, 1.9$ Hz, 1H) and 3.76 (*ddd*, $J = 10.0, 5.1, 1.9$ Hz, 1H), two methyl singlets at δ 1.32 and 1.33 and a hydroxyl singlet δ 4.12 (*d*, $J = 5.1$ Hz, 1H). The HMBC correlations between H₂-9 (δ 2.88 and 3.37) to C-1a (δ 146.6), C-2 (δ 158.1) and C-11 (δ 72.4) confirmed the position of the side chain $[-\text{CH}_2\text{CH}(\text{OH})\text{C}(\text{CH}_3)_2\text{OH}]$ at C-1. On the basis of these results, the structure of **RM7** was a new compound and named as clausebazole A.

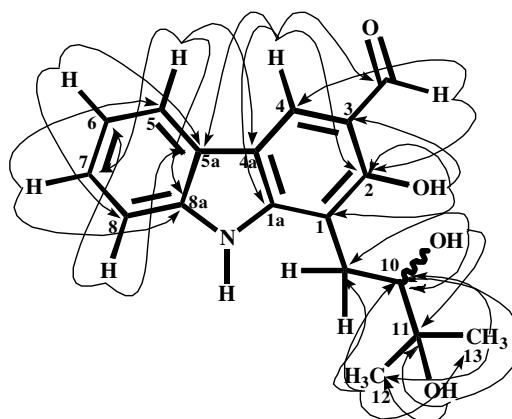


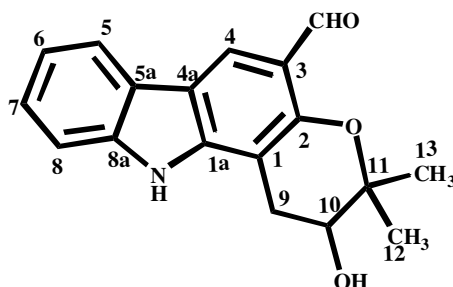
Figure 8 Selected HMBC correlations of **RM7**

Table 15 ^1H and ^{13}C NMR and HMBC spectral data of **RM7** (CD_3COCD_3)

Position	δ_{H} (multiplicity) ^a	δ_{C} (C-type) ^b	HMBC
1a	-	146.6 (C)	-
1	-	108.7 (C)	-
2	-	158.1 (C)	-
3	-	115.2 (C)	-
4	8.36 (1H, <i>s</i>)	125.6 (CH)	C-1a, C-2, C-5a, CHO
4a	-	117.4 (C)	-
5a	-	123.7 (C)	-
5	8.08 (1H, <i>d</i> , $J = 7.7$ Hz)	119.6 (CH)	C-4a, C-7, C-8a
6	7.22 (1H, <i>td</i> , $J = 7.7, 1.0$ Hz)	120.2 (CH)	C-5a, C-8,
7	7.37 (1H, <i>td</i> , $J = 8.0, 1.0$ Hz)	125.6 (CH)	C-5, C-8a
8	7.52 (1H, <i>d</i> , $J = 8.0$ Hz)	111.3 (CH)	C-5a, C-6
8a	-	141.2 (C)	-
9	2.88 (1H, <i>dd</i> , $J = 14.0, 10.0$ Hz) 3.37 (1H, <i>dd</i> , $J = 14.0, 1.9$ Hz)	26.6 (CH_2)	C-1a, C-2, C-11
10	3.76 (1H, <i>ddd</i> , $J = 10.0, 5.1, 1.9$ Hz)	78.4 (CH)	C-9, C-11, C-12
11	-	72.4 (C)	-
12	1.32 (3H, <i>s</i>)	24.5 (CH_3)	C-10, C-11, C-13
13	1.33 (3H, <i>s</i>)	25.1 (CH_3)	C-10, C-11, C-12
2-OH	11.79 (1H, <i>s</i>)	-	C-1, C-2, C-3
3-CHO	9.99 (1H, <i>s</i>)	196.0 (CHO)	C-2, C-4
10-OH	4.12 (1H, <i>d</i> , $J = 5.1$ Hz)	-	C-9, C-10, C-11
11-OH	3.81 (1H, <i>br s</i>)	-	C-10, C-11, C-12
NH	10.77 (1H, <i>br s</i>)	-	-

^a300 MHz, ^b75 MHz

3.1.8 Compound RM8



RM8 was obtained as a colorless solid, m.p. 170-171°C. The IR spectrum showed absorption bands at 3416 cm^{-1} (OH and NH), 1663 cm^{-1} (aldehyde) and 1606, 1488 cm^{-1} (aromatic system).

The spectral data of **RM8** showed a similar signal pattern to those of **RM7**. The major difference from that of **RM7** was only the absence of a chelated hydroxyl proton signal at C-2. Two methyl singlet signals at δ 1.27 and 1.37 and ABX-type signals at δ 2.84 (*dd*, $J = 16.6, 5.6$ Hz, 1H), 3.13 (*dd*, $J = 16.6, 7.2$ Hz, 1H) and 3.90 (*dd*, $J = 7.2, 5.6$ Hz, 1H) in the ^1H NMR spectrum indicated that the side-chain had cyclized to give a 3-hydroxy-2,2-dimethylpyran ring. The aldehydic proton singlet shifted slightly downfield (δ 10.34) while H-4 singlet shifted highfield (δ 8.24) as compared to δ 9.99 and 8.36, respectively, in **RM7**, probably due to stereochemical changes on cyclization. These data led to the identification of compound **RM8** a new compound and named as clausebazole B.

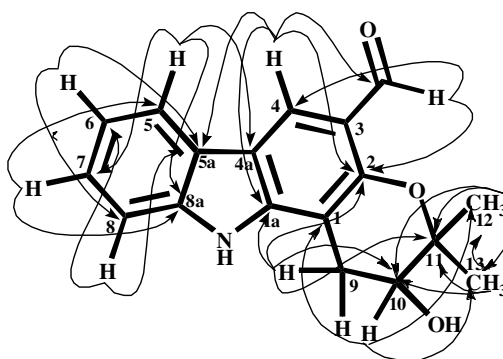


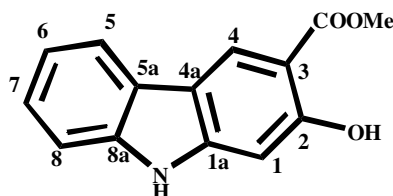
Figure 9 Selected HMBC correlations of **RM8**

Table 16 ^1H and ^{13}C NMR and HMBC spectral data of **RM8** (CD_3COCD_3)

Position	δ_{H} (multiplicity) ^a	δ_{C} (C-type) ^b	HMBC
1a	-	144.1 (C)	-
1	-	102.8 (C)	-
2	-	154.4 (C)	-
3	-	118.5 (C)	-
4	8.24 (1H, <i>s</i>)	118.1 (CH)	C-1a, C-2, C-5a, 3-CHO
4a	-	116.6 (C)	-
5a	-	124.0 (C)	-
5	7.97 (1H, <i>d</i> , $J = 7.7$ Hz)	119.8 (CH)	C-4a, C-7, C-8, C-8a
6	7.08 (1H, <i>td</i> , $J = 7.7, 1.0$ Hz)	120.1 (CH)	C-5a, C-8
7	7.22 (1H, <i>td</i> , $J = 8.1, 1.0$ Hz)	125.3 (CH)	C-5, C-8a
8	7.35 (1H, <i>d</i> , $J = 8.1$ Hz)	111.1 (CH)	C-5a, C-6
8a	-	140.9 (C)	-
9	2.84 (1H, <i>dd</i> , $J = 16.6, 5.6$ Hz) 3.13 (1H, <i>dd</i> , $J = 16.6, 7.2$ Hz)	27.2 (CH_2)	C-1, C-1a, C-2, C-10, C-11
10	3.90 (1H, <i>dd</i> , $J = 7.2, 5.6$ Hz)	68.1 (CH)	C-1, C-12, C-13
11	-	78.2 (C)	-
12	1.27 (3H, <i>s</i>)	19.9 (CH_3)	C-10, C-11, C-13
13	1.37 (3H, <i>s</i>)	24.9 (CH_3)	C-10, C-11, C-12
3-CHO	10.34 (1H, <i>s</i>)	188.2 (CHO)	C-4, C-2
NH	10.52 (1H, <i>br s</i>)	-	-

^a300 MHz, ^b75 MHz

3.1.9 Compound RM9



RM9 was obtained as a pale yellow crystalline solid, m.p. 158-159°C [lit. 162-163°C]. The IR spectrum showed absorption bands at 3357 cm^{-1} (OH and NH), 1650 cm^{-1} (ester carbonyl) and 1633, 1464 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM9** was similar to those of **RM6**, except that in **RM9** a singlet signal of a carbomethoxyl group at δ_{H} 3.96; δ_{C} 51.6 and δ_{C} 171.3 replaced an aldehydic proton at δ_{H} 10.01; δ_{C} 195.7 in **RM6**. By comparison of the ^1H NMR and ^{13}C NMR data of **RM9** and mukonidine in **Table 18**, it was therefore suggested that compound **RM9** was mukonidine [Knolker *et al.*, 2003].

Table 17 ^1H , ^{13}C NMR spectral data of **RM9** (CD_3COCD_3)

Position	δ_{H} (multiplicity)	δ_{C} (C-type)
1a	-	145.7 (C)
1	6.90 (1H, s)	96.8 (CH)
2	-	160.7 (C)
3	-	105.0 (C)
4	8.58 (1H, s)	122.5 (CH)
4a	-	116.9 (C)
5a	-	123.4 (C)
5	8.05 (1H, d, $J = 7.7$ Hz)	119.6 (CH)
6	7.16 (1H, td, $J = 7.7, 0.9$ Hz)	119.9 (CH)
7	7.32 (1H, td, $J = 8.1, 0.9$ Hz)	125.5 (CH)
8	7.43 (1H, d, $J = 8.1$ Hz)	110.9 (CH)

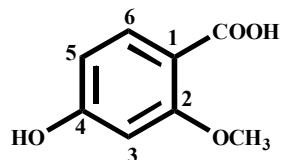
8a	-	141.1 (C)
2-OH	11.02 (1H, <i>br s</i>)	-
3-COOCH ₃	-	171.3 (COOCH ₃)
3-COOCH ₃	3.96 (3H, <i>s</i>)	51.6 (CH ₃)
NH	10.64 (1H, <i>br s</i>)	-

Table 18 ¹H and ¹³C NMR spectral data of **RM9** and **Mukonidine (R)** (CD₃COCD₃)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM9 ^a	R ^b	RM9 ^c	R ^d
1a	-	-	145.7	147.17
1	6.90 (<i>s</i>)	6.93 (<i>s</i>)	96.8	98.3
2	-	-	160.7	162.2
3	-	-	105.0	106.5
4	8.58 (<i>s</i>)	8.43 (<i>s</i>)	122.5	123.8
4a	-	-	116.9	118.4
5a	-	-	123.4	124.9
5	8.05 (<i>d</i> , $J = 7.7$ Hz)	8.06 (<i>d</i> , $J = 7.7$ Hz)	119.6	121.2
6	7.16 (<i>td</i> , $J = 7.7, 0.9$ Hz)	7.19 (<i>t</i> , $J = 7.7$ Hz)	119.9	121.5
7	7.32 (<i>td</i> , $J = 8.1, 0.9$ Hz)	7.36 (<i>t</i> , $J = 8.1$ Hz)	125.5	127.1
8	7.43 (<i>d</i> , $J = 8.1$ Hz)	7.46 (<i>d</i> , $J = 8.1$ Hz)	110.9	112.4
8a	-	-	141.1	142.5
2-OH	11.02 (<i>br s</i>)	11.10 (<i>br s</i>)	-	-
3-COOCH ₃	-	-	171.3	172.8
3-COOCH ₃	3.96 (<i>s</i>)	3.98 (<i>s</i>)	51.6	53.2
NH	10.64 (<i>br s</i>)	10.50 (<i>br s</i>)	-	-

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

3.1.10 Compound RM10



RM10 was obtained as a colorless crystalline solid, m.p. 163-164°C. The IR spectrum showed absorption bands at 3384 cm^{-1} (OH), 1648 cm^{-1} (carboxy carbonyl) and 1615, 1562 cm^{-1} (aromatic system).

The ^1H NMR spectrum displayed the ABX-type signals at δ 7.84 (1H, *d*, $J = 8.4$ Hz, H-6), 6.76 (1H, *dd*, $J = 8.4, 2.2$ Hz, H-5) and 6.99 (1H, *d*, $J = 2.2$ Hz, H-3), a methoxyl signal at δ 3.84, a hydroxyl signal at δ 10.19 (*br s*) and the appearance of a carboxyl carbon signal at δ 172.0 in the ^{13}C NMR spectrum indicating 2,4-dioxygenated benzoic acid. The HMBC correlations of the methoxyl group at δ 3.84 with δ 158.3 (C-2) confirmed the position of the methoxyl group at C-2 leaving the hydroxyl group at C-4. Additionally H-3 at δ 6.99 showed HMBC correlations with δ 117.1 (C-1), 158.3 (C-2), 145.4 (C-4) and 107.5 (C-5). It was therefore suggested that compound **RM10** was 4-hydroxy-2-methoxybenzoic acid.

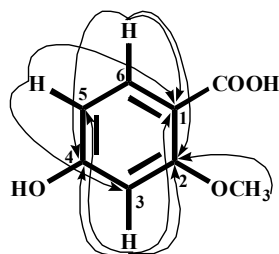


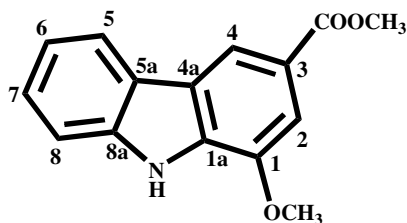
Figure 10 Selected HMBC correlations of **RM10**

Table 19 ^1H , ^{13}C NMR and HMBC spectral data of **RM10** (CD_3COCD_3)

Position	$\delta_{\text{H}}^{\text{a}}$ (multiplicity)	$\delta_{\text{C}}^{\text{b}}$ (C-type)	HMBC
1	-	117.1 (C)	-
2	-	158.3 (C)	-
3	6.99 (1H, <i>d</i> , $J = 2.2$ Hz)	94.8 (CH)	C-1, C-2, C-4, C-5
4	-	141.5 (C)	-
5	6.76 (1H, <i>dd</i> , $J = 8.4, 2.2$ Hz)	107.5 (CH)	C-1, C-3
6	7.84 (1H, <i>d</i> , $J = 8.4$ Hz)	119.7 (CH)	C-1, C-2, C-4
2-OCH ₃	3.84 (3H, <i>s</i>)	54.8 (CH ₃)	C-2
4-OH	10.19 (1H, <i>br s</i>)	-	-
COOH	-	172.0 (COOH)	-

^a300 MHz, ^b75 MHz

3.1.11 Compound RM11



RM11 was obtained as an orange crystalline solid, m.p. 170-171°C [lit. 173-174°C]. The IR spectrum showed absorption bands at 3415 cm^{-1} (NH), 1700 cm^{-1} (ester carbonyl), 1606, 1578 cm^{-1} (aromatic system).

The ^1H NMR spectra were comparable to those of **RM9**. The difference between **RM11** and **RM9** were shown in the splitting patterns in one aromatic ring whose two protons were shown as two singlets at δ 6.90 (H-1) and 8.58 (H-4) in **RM9** but changed to two doublets at δ 7.59 (H-2) and 8.48 (H-4) (1H each, $J = 1.1$ Hz) in **RM11**, thus indicating the change from two *para* protons in **RM9** into two *meta* protons in **RM11**. Additional signal of a methoxyl group was observed at δ 4.08 whose HMBC correlation to δ 145.5 (C-1) suggested its position at C-1. Therefore, compound **RM11** was identified as mukonine [Kuwahara *et al.*, 2005].

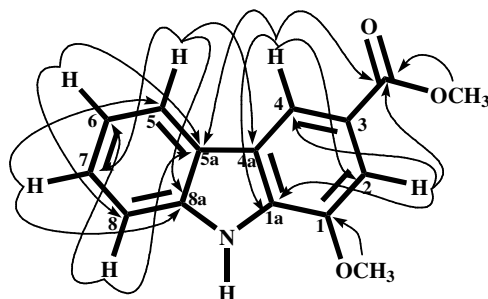


Figure 11 Selected HMBC correlations of **RM11**

Table 20 ^1H , ^{13}C NMR and HMBC spectral data of **RM11** (CD_3COCD_3)

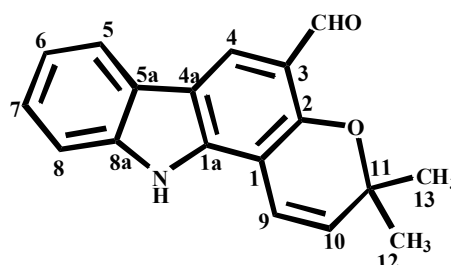
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	133.5 (C)	-
1	-	145.5 (C)	-
2	7.59 (1H, <i>d</i> , $J = 1.1$ Hz)	106.3 (CH)	C-1a, C-4, COOMe
3	-	121.7 (C)	-
4	8.48 (1H, <i>d</i> , $J = 1.1$ Hz)	115.7 (CH)	C-1a, C-2, C-5a, COOMe
4a	-	120.0 (C)	-
5a	-	123.5 (C)	-
5	8.21 (1H, <i>d</i> , $J = 8.8$ Hz)	120.4 (CH)	C-4a, C-7, C-8a
6	7.26 (1H, <i>td</i> , $J = 8.8, 1.0$ Hz)	119.8 (CH)	C-5a, C-8
7	7.46 (1H, <i>td</i> , $J = 8.2, 1.0$ Hz)	126.2 (CH)	C-5, C-8a
8	7.63 (1H, <i>d</i> , $J = 8.2$ Hz)	111.7 (CH)	C-5a, C-6
8a	-	140.5 (C)	-
1-OCH ₃	4.08 (3H, <i>s</i>)	55.2 (CH ₃)	C-1
3-COOCH ₃	-	167.1 (COOMe)	-
3-COOCH ₃	3.92 (3H, <i>s</i>)	51.1 (CH ₃)	COOMe
NH	10.83 (1H, <i>br s</i>)	-	-

Table 21 ^1H and ^{13}C NMR spectral data of **RM11** (CD_3COCD_3) and **Mukonine (R)** (CDCl_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM11 ^a	R	RM11 ^b	R
1a	-	-	133.5	132.9
1	-	-	145.5	145.0
2	7.59 (<i>d</i> , $J = 1.1$ Hz)	7.60 (<i>d</i> , $J = 1.1$ Hz)	106.3	106.7
3	-	-	121.7	121.9
4	8.48 (<i>d</i> , $J = 1.1$ Hz)	8.48 (<i>dd</i> , $J = 1.1, 0.6$ Hz)	115.7	116.2
4a	-	-	120.0	120.7
5a	-	-	123.5	123.6
5	8.21 (<i>d</i> , $J = 8.8$ Hz)	8.10 (<i>m</i>)	120.4	121.9
6	7.26 (<i>td</i> , $J = 8.8, 1.0$ Hz)	7.28 (<i>ddd</i> , $J = 8.1, 1.3, 0.8$ Hz)	119.8	120.3
7	7.46 (<i>td</i> , $J = 8.2, 1.0$ Hz)	7.46 (<i>ddd</i> , $J = 8.1, 6.8, 1.1$ Hz)	126.2	126.3
8	7.63 (<i>d</i> , $J = 8.2$ Hz)	7.49 (<i>ddd</i> , $J = 8.1, 1.3, 0.8$ Hz)	111.7	111.2
8a	-	-	140.5	139.5
1-OCH ₃	4.08 (<i>s</i>)	4.07 (<i>s</i>)	55.2	55.7
3-COOCH ₃	-	-	167.1	168.0
3-COOCH ₃	3.92 (<i>s</i>)	3.98 (<i>s</i>)	51.1	52.0
NH	10.83 (<i>br s</i>)	8.47 (<i>br s</i>)	-	-

^a300 MHz, ^b75 MHz

3.1.12 Compound RM12



RM12 was obtained as a colorless crystalline solid, m.p. 239-241°C [lit. 242-244°C]. The IR spectrum showed absorption bands at 3335 cm^{-1} (NH), 1636 cm^{-1} (aldehyde) and 1602, 1575 cm^{-1} (aromatic system).

The ^1H NMR spectral data of **RM12** showed a similar pattern to those of **RM8**. The differences between **RM8** and **RM12** were that the methylene protons and an oxymethine proton in **RM8** were replaced by two doublets for one proton each at δ 7.07 and 6.03 ($J = 9.9$ Hz) at C-9 and C-10, respectively. By comparison of the ^1H NMR data of **RM12** and murrayacine in **Table 22**, it was therefore suggested that compound **RM12** was murrayacine [Chakraborty *et al.*, 1973].

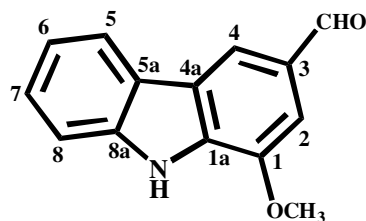
Table 22 ^1H NMR spectral data of **RM12** (CD_3COCD_3) and **Murrayacine (R)** ($\text{DMSO}-d_6$)

Position	δ_{H} (multiplicity)	
	RM12 ^a	R ^b
1a	-	-
1	-	-
2	-	-
3	-	-
4	8.47 (s)	8.40 (s)

4a	-	-
5a	-	-
5	8.23 (<i>d</i> , <i>J</i> = 7.8 Hz)	8.15-8.35 (<i>m</i>)
6	7.33 (<i>td</i> , <i>J</i> = 7.8, 1.2 Hz)	8.15-8.35 (<i>m</i>)
7	7.48 (<i>td</i> , <i>J</i> = 8.1, 1.2 Hz)	8.15-8.35 (<i>m</i>)
8	7.58 (<i>d</i> , <i>J</i> = 8.1 Hz)	8.15-8.35 (<i>m</i>)
8a	-	-
9	7.07 (<i>d</i> , <i>J</i> = 9.9 Hz)	7.00 (<i>d</i> , <i>J</i> = 10.0 Hz)
10	6.03 (<i>d</i> , <i>J</i> = 9.9 Hz)	5.95 (<i>d</i> , <i>J</i> = 10.0 Hz)
11	-	-
12	1.47 (<i>s</i>)	Not reported
13	1.69 (<i>s</i>)	Not reported
3-CHO	10.58 (<i>s</i>)	10.68 (<i>s</i>)
NH	10.97 (<i>br s</i>)	12.00 (<i>s</i>)

^a300 MHz, ^b60 MHz

3.1.13 Compound RM13



RM13 was obtained as an orange crystalline solid and m.p. 162-163°C [lit. 167-168°C]. The IR spectrum showed absorption bands at 3356 cm^{-1} (NH), 1636 cm^{-1} (aldehyde), 1606, 1578 cm^{-1} (aromatic system).

The ^1H NMR spectrum were comparable to those of **RM11**. The differences between **RM13** and **RM11** were that an aldehydic proton (δ_{H} 10.06, δ_{C} 191.9) in **RM13** replaced carbomethoxyl signal of **RM11** at δ_{H} 3.92, δ_{C} 51.1 and δ_{C} 167.1. The HMBC correlations of an aldehydic proton at δ 10.06 with the carbons at δ 146.1 (C-1), δ 103.5 (C-2), 130.2 (C-3) and 120.3 (C-4) placed a formyl group at C-3. Therefore, compound **RM13** was identified as murrayanine [Bernal *et al.*, 2007].

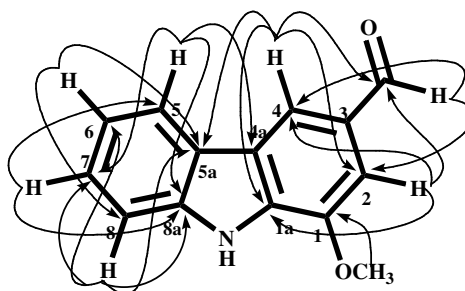


Figure 12 Selected HMBC correlations of **RM13**

Table 23 ^1H , ^{13}C NMR and HMBC spectral data of **RM13** (CDCl_3)

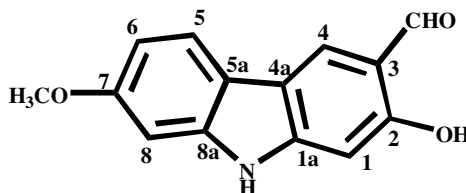
position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	134.1 (C)	-
1	-	146.1 (C)	-
2	7.47 (1H, <i>br s</i>)	103.5 (CH)	C-1a, C-1, C-4, 3-CHO
3	-	130.2 (C)	-
4	8.20 (1H, <i>s</i>)	120.3 (CH)	C-1a, C-1, C-2, C-5a, 3-CHO
4a	-	123.7 (C)	-
5a	-	123.7 (C)	-
5	8.12 (1H, <i>d</i> , $J = 7.8$ Hz)	120.7 (CH)	C-4a, C-7, C-8a
6	7.33 (1H, <i>ddd</i> , $J = 7.6, 7.1, 1.7$ Hz)	120.7 (CH)	C-5a, C-8
7	7.34 (1H, <i>m</i>)	126.6 (CH)	C-5, C-8a
8	7.53 (1H, <i>m</i>)	111.4 (CH)	C-6, C-5a
8a	-	139.4 (C)	-
1-OCH ₃	4.10 (3H, <i>s</i>)	55.8 (CH ₃)	C-1
3-CHO	10.06 (1H, <i>s</i>)	191.9 (CHO)	C-1, C-2, C-3, C-4
NH	8.70 (1H, <i>br s</i>)	-	-

Table 24 ^1H and ^{13}C NMR spectral data of **RM13** and **Murrayanine (R)**
(CDCl_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM13 ^a	R ^a	RM13 ^b	R ^b
1a	-	-	134.1	134.0
1	-	-	146.1	146.0
2	7.47 (<i>br s</i>)	6.89 (<i>s</i>)	103.5	103.4
3	-	-	130.2	130.0
4	8.20 (<i>s</i>)	8.18 (<i>br s</i>)	120.3	120.6
4a	-	-	123.7	123.5
5a	-	-	123.7	124.6
5	8.12 (<i>d</i> , $J = 7.8$ Hz)	8.10 (<i>br d</i> , $J = 7.8$ Hz)	120.7	120.6
6	7.33 (<i>ddd</i> , $J = 7.6, 7.1, 1.7$ Hz)	7.32 (<i>ddd</i> , $J = 7.8, 7.2, 1.6$ Hz)	120.7	120.6
7	7.34 (<i>m</i>)	7.46-7.55 (<i>m</i>)	126.6	126.6
8	7.53 (<i>m</i>)	7.46-7.55 (<i>m</i>)	111.4	111.5
8a	-	-	139.4	139.4
1-OCH ₃	4.10 (<i>s</i>)	4.05 (<i>s</i>)	55.8	55.7
3-CHO	10.06 (<i>s</i>)	10.04 (<i>s</i>)	191.9	192.0
NH	8.70 (<i>br s</i>)	8.72 (<i>br s</i>)	-	-

^a300 MHz, ^b75 MHz

3.1.14 Compound RM14



RM14 was obtained as a yellow crystalline solid, m.p. 205-207°C [lit. 208-209°C]. The IR spectrum showed absorption bands at 3415 cm^{-1} (OH and NH), 1627 cm^{-1} (aldehyde) and 1598, 1467 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM14** showed a similar signal pattern to those of **RM6**, except for the presence of an additional methoxyl signal at δ 3.87 and the downfield ABX system signals at δ 7.95 (*d*, $J = 8.5$ Hz), 6.85 (*dd*, $J = 8.5, 2.2$ Hz) and 7.05 (*d*, $J = 2.2$ Hz) for H-5, H-6 and H-8, respectively, in **RM14** replaced a set of four adjacent proton signals in **RM6**. The HMBC correlations between δ 3.87 (7-OCH₃) and δ 159.3 (C-7) as well as the correlations from δ 159.3 (C-7) to H-5 (δ 7.95) suggested the position of the methoxyl group at C-7. It was therefore suggested that compound **RM14** was 7-methoxymukonal [Ruangrunsi *et al.*, 1990].

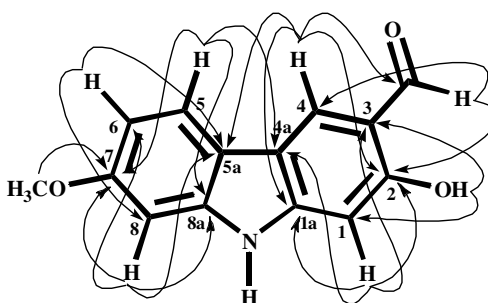


Figure 13 Selected HMBC correlations of **RM14**

Table 25 ^1H , ^{13}C NMR and HMBC spectral data of **RM14** (CD_3COCD_3)

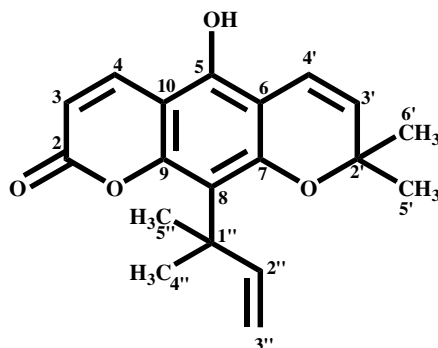
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	146.4 (C)	-
1	6.87 (1H, <i>s</i>)	96.4 (CH)	C-1a, C-2, C-3, C-4a
2	-	160.4 (C)	-
3	-	115.2 (C)	-
4	8.32 (1H, <i>s</i>)	126.2 (CH)	C-1a, C-1, C-2, C-5a, CHO
4a	-	118.0 (C)	-
5a	-	116.7 (C)	-
5	7.95 (1H, <i>d</i> , $J = 8.5$ Hz)	120.4 (CH)	C-4a, C-7, C-8, C-8a
6	6.85 (1H, <i>dd</i> , $J = 8.5, 2.2$ Hz)	108.7 (CH)	C-5a, C-8
7	-	159.3 (C)	-
8	7.05 (1H, <i>d</i> , $J = 2.2$ Hz)	95.6 (CH)	C-5a, C-6, C-8a
8a	-	142.5 (C)	-
2-OH	11.44 (1H, <i>br s</i>)	-	C-1, C-2, C-3
3-CHO	9.98 (1H, <i>s</i>)	195.7 (CHO)	C-2, C-4
7-OCH ₃	3.87 (3H, <i>s</i>)	55.0 (CH ₃)	C-7
NH	10.75 (1H, <i>br s</i>)	-	-

Table 26 ^1H and ^{13}C NMR spectral data of **RM14** and **7-Methoxymukonal (R)**
(CD_3COCD_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM14 ^a	R ^b	RM14 ^c	R ^d
1a	-	-	146.4	147.0
1	6.87 (<i>s</i>)	6.85 (<i>s</i>)	96.4	97.0
2	-	-	160.4	160.9
3	-	-	115.2	115.8
4	8.32 (<i>s</i>)	8.29 (<i>s</i>)	126.2	126.7
4a	-	-	118.0	118.4
5a	-	-	116.7	117.3
5	7.95 (<i>d</i> , $J = 8.5$ Hz)	7.92 (<i>d</i> , $J = 8.8$ Hz)	120.4	121.0
6	6.85 (<i>dd</i> , $J = 8.5, 2.2$ Hz)	6.83 (<i>dd</i> , $J = 8.8, 2.2$ Hz)	108.7	109.2
7	-	-	159.3	159.8
8	7.05 (<i>d</i> , $J = 2.2$ Hz)	7.02 (<i>d</i> , $J = 2.2$ Hz)	95.6	96.1
8a	-	-	142.5	143.0
2-OH	11.44 (<i>br s</i>)	11.42 (<i>s</i>)	-	-
3-CHO	9.98 (<i>s</i>)	9.95 (<i>s</i>)	195.7	196.2
7-OMe	3.87 (<i>s</i>)	3.85 (<i>s</i>)	55.0	55.5
NH	10.75 (<i>br s</i>)	10.72 (<i>br s</i>)	-	-

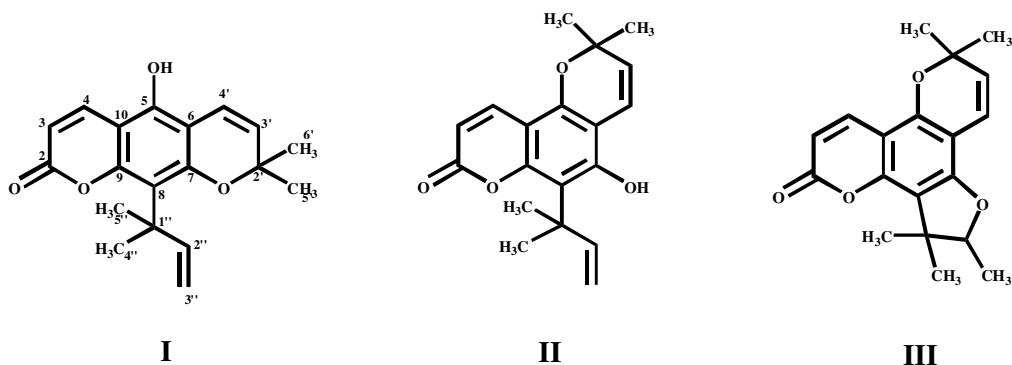
^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

3.1.15 Compound RM15



RM15 was obtained as a pale yellow prism, m.p. 178-180°C [lit. 182°C]. The IR spectrum showed absorption bands at 3412 cm^{-1} (OH), 1712 cm^{-1} (ester carbonyl) and 1600, 1563 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM15** was similar to those of **RM3**, except for the absence of the methoxyl proton signal (δ 3.81) at C-5 (δ 151.2) in **RM3**, and the change of the carbon chemical shift of C-5 to δ 148.0 in **RM15**, suggesting the hydroxyl group at C-8 in **RM15**. On the basis of the above evidence, **RM15** could be constituted as structure **I** or **II**.



However, if **RM15** had the structure **II** it should readily isomerize in acid to give the furan **III**. Upon treatment with conc. H_2SO_4 or conc. HCl , **RM15** did not produce detectable amount of the furan **III**, only a product which showed the same signal pattern as **RM18** (Table 33). It was concluded that **RM15** should therefore be constituted as structure **I**. It was therefore suggested that compound **RM15** was nordentatin [Huang *et al.*, 1996].

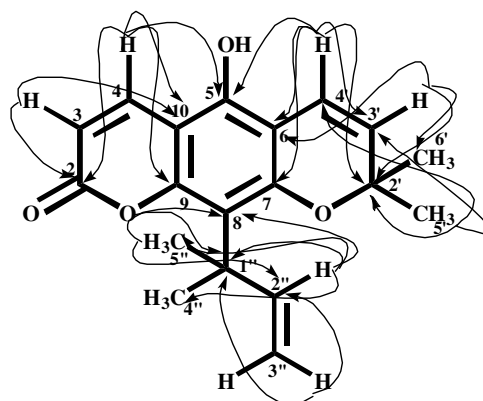


Figure 14 Selected HMBC correlations of **RM15**

Table 27 ^1H , ^{13}C NMR and HMBC spectral data of **RM15** (CDCl_3)

Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	-	-	-
2	-	162.3 (C)	-
3	6.03 (1H, <i>d</i> , $J = 9.6$ Hz)	108.9 (CH)	C-2, C-10
4	8.11 (1H, <i>d</i> , $J = 9.6$ Hz)	140.5 (CH)	C-2, C-5, C-9, C-10
5	-	148.0 (C)	-
6	-	106.9 (C)	-
7	-	156.2 (C)	-
8	-	115.1 (C)	-
9	-	153.9 (C)	-
10	-	104.5 (C)	-
1'	-	-	-
2'	-	76.9 (C)	-
3'	5.56 (1H, <i>d</i> , $J = 10.0$ Hz)	128.7 (CH)	C-6, C-2', C-5', C-6'
4'	6.64 (1H, <i>d</i> , $J = 10.0$ Hz)	116.1 (CH)	C-5, C-6, C-7, C-2', C-3'
5'	1.36 (3H, <i>s</i>)	27.2 (CH_3)	C-7, C-2', C-3', C-4'
6'	1.36 (3H, <i>s</i>)	27.2 (CH_3)	C-7, C-2', C-3', C-4'
1''	-	40.7 (C)	-

2''	6.21 (1H, <i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	150.1 (CH)	C-8, C-1'', C-4'', C-5''
3''	4.78 (1H, <i>dd</i> , <i>J</i> = 10.6, 1.0 Hz) 4.84 (1H, <i>dd</i> , <i>J</i> = 17.4, 1.0 Hz)	107.7 (CH ₂)	C-1'', C-2''
4''	1.57 (3H, <i>s</i>)	29.4 (CH ₃)	C-8, C-1'', C- 2''
5''	1.57 (3H, <i>s</i>)	29.4 (CH ₃)	C-8, C-1'', C- 2''

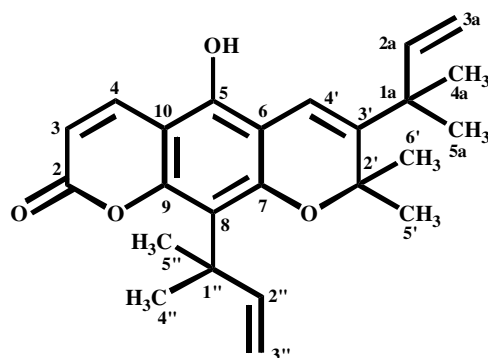
Table 28 ¹H NMR spectral data of **RM15** and **nordentatin (R)** (CDCl₃)

Position	δ_{H} (multiplicity)	
	RM15 ^a	R
1	-	-
2	-	-
3	6.03 (<i>d</i> , <i>J</i> = 9.6 Hz)	6.07 (<i>d</i> , <i>J</i> = 9.7 Hz)
4	8.11 (<i>d</i> , <i>J</i> = 9.6 Hz)	8.11 (<i>d</i> , <i>J</i> = 9.7 Hz)
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
1'	-	-
2'	-	-
3'	5.56 (<i>d</i> , <i>J</i> = 10.0 Hz)	5.73 (<i>d</i> , <i>J</i> = 10.0 Hz)
4'	6.64 (<i>d</i> , <i>J</i> = 10.0 Hz)	6.75 (<i>d</i> , <i>J</i> = 10.0 Hz)
5'	1.36 (<i>s</i>)	1.64 (<i>s</i>)
6'	1.36 (<i>s</i>)	1.64 (<i>s</i>)
1''	-	-
2''	6.21 (<i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	6.30 (<i>dd</i> , <i>J</i> = 17.5, 10.5 Hz)

3"	4.78 (<i>dd</i> , $J = 10.6, 1.0$ Hz) 4.84 (<i>dd</i> , $J = 17.4, 1.0$ Hz)	4.81 (<i>dd</i> , $J = 10.5, 1.3$ Hz) 4.89 (<i>dd</i> , $J = 17.5, 1.3$ Hz)
4"	1.57 (<i>s</i>)	1.49 (<i>s</i>)
5"	1.57 (<i>s</i>)	1.49 (<i>s</i>)

^a300 MHz

3.1.16 Compound RM16



RM16 was obtained as colorless viscous liquid. The IR spectrum showed absorption bands at 3285 cm^{-1} (OH), 1712 cm^{-1} (ester carbonyl) and 1592 , 1566 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM16** was similar to those of **RM15**. The differences were shown as the disappearance of an olefinic proton signal H-3' at δ_{H} 5.56; δ_{C} 128.7 in **RM15** and the carbon chemical shift at C-3' was changed to δ 146.4 in **RM16**. In addition the second 1,1-dimethylallyl side chain was apparent as signals at δ 5.93 (1H, *dd*, $J = 17.5$, 10.6 Hz, H-2a), 4.89 (1H, *dd*, $J = 10.6$, 1.1 Hz, H-3a), 4.95 (1H, *dd*, $J = 17.5$, 1.1 Hz, H-3a), 1.39 (6H, *s*, CH₃-4a, CH₃-5a). The HMBC correlations of CH₃-5a/CH₃-4a at δ 1.39 to the carbons at δ 146.4 (C-3'), 41.7 (C-1a), 146.4 (C-3') and of H-4' at δ 6.46 to the carbons at δ 41.7 (C-1a) suggested the location of the second 1,1-dimethylallyl side chain at C-3'. By comparison of the spectral data, it was therefore suggested that compound **RM16** was kinocoumarin [Huang *et al.*, 1996].

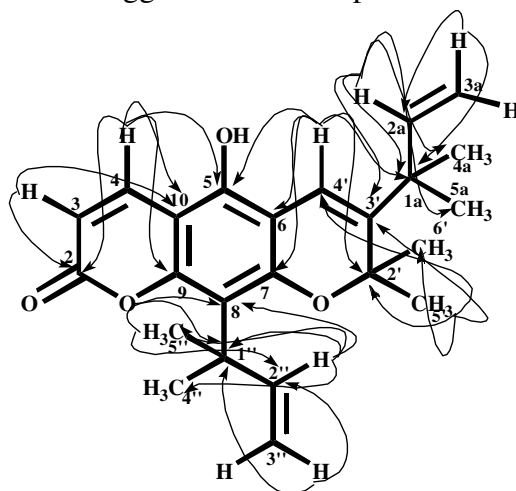


Figure 15 Selected HMBC correlations of **RM16**

Table 29 ^1H , ^{13}C NMR and HMBC spectral data of **RM16** (CDCl_3)

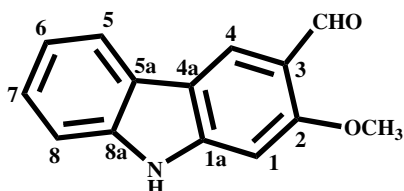
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	-	-	-
2	-	161.4 (C)	-
3	6.15 (1H, <i>d</i> , <i>J</i> = 9.6 Hz)	110.2 (CH)	C-2, C-10
4	8.05 (1H, <i>d</i> , <i>J</i> = 9.6 Hz)	139.2 (CH)	C-2, C-5, C-9, C-10
5	-	153.9 (C)	-
6	-	107.8 (C)	-
7	-	155.1 (C)	-
8	-	115.8 (C)	-
9	-	146.8 (C)	-
10	-	103.9 (C)	-
1'	-	-	-
2'	-	81.2 (C)	-
3'	-	146.4 (C)	-
4'	6.46 (1H, <i>s</i>)	111.4 (CH)	C-5, C-6, C-7, C-2', C-1a
5'	1.49 (3H, <i>s</i>)	27.0 (CH_3)	C-2', C-3', C-4', C-6'
6'	1.49 (3H, <i>s</i>)	27.0 (CH_3)	C-2', C-3', C-4', C-5'
1''	-	41.0 (C)	-
2''	6.29 (1H, <i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	149.9 (CH)	C-8, C-1'', C-4'', C-5''
3''	4.85 (1H, <i>dd</i> , <i>J</i> = 10.6, 1.1 Hz) 4.88 (1H, <i>dd</i> , <i>J</i> = 17.4, 1.1 Hz)	108.2 (CH_2)	C-1'', C-2''
4''	1.65 (3H, <i>s</i>)	29.5 (CH_3)	C-8, C-1'', C-2''
5''	1.65 (3H, <i>s</i>)	29.5 (CH_3)	C-8, C-1'', C-2''
1a	-	41.7 (C)	-
2a	5.93 (1H, <i>dd</i> , <i>J</i> = 17.5, 10.6 Hz)	146.6 (CH)	C-3', C-1a, C-4a, C-5a
3a	4.89 (1H, <i>dd</i> , <i>J</i> = 10.6, 1.1 Hz) 4.95 (1H, <i>dd</i> , <i>J</i> = 17.5, 1.1 Hz)	112.0 (CH_2)	C-1a, C-2a
4a	1.39 (3H, <i>s</i>)	28.0 (CH_3)	C-3', C-1a, C-2a
5a	1.39 (3H, <i>s</i>)	28.0 (CH_3)	C-3', C-1a, C-2a

Table 30 ^1H NMR spectral data of **RM16** and **Kinocoumarin (R)** (CDCl_3)

Position	δ_{H} (multiplicity)	
	RM16 ^a	R
1	-	-
2	-	-
3	6.15 (<i>d</i> , <i>J</i> = 9.6 Hz)	6.15 (<i>d</i> , <i>J</i> = 9.7 Hz)
4	8.05 (<i>d</i> , <i>J</i> = 9.6 Hz)	8.10 (<i>d</i> , <i>J</i> = 9.7 Hz)
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
1'	-	-
2'	-	-
3'	-	-
4'	6.46 (<i>s</i>)	6.47 (<i>s</i>)
5'	1.49 (<i>s</i>)	1.63 (<i>s</i>)
6'	1.49 (<i>s</i>)	1.63 (<i>s</i>)
1''	-	-
2''	6.29 (<i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	5.92 (<i>dd</i> , <i>J</i> = 17.7, 10.0 Hz)
3''	4.85 (<i>dd</i> , <i>J</i> = 10.6, 1.1 Hz)	4.86 (<i>dd</i> , <i>J</i> = 10.0, 1.0 Hz)
	4.88 (<i>dd</i> , <i>J</i> = 17.4, 1.1 Hz)	4.92 (<i>dd</i> , <i>J</i> = 17.7, 1.0 Hz)
4''	1.65 (<i>s</i>)	1.36 (<i>s</i>)
5''	1.65 (<i>s</i>)	1.36 (<i>s</i>)
1a	-	-
2a	5.93 (<i>dd</i> , <i>J</i> = 17.5, 10.6 Hz)	6.29 (<i>dd</i> , <i>J</i> = 17.7, 10.0 Hz)
3a	4.89 (<i>dd</i> , <i>J</i> = 10.6, 1.1 Hz)	5.07 (<i>dd</i> , <i>J</i> = 10.0, 1.0 Hz)
	4.95 (<i>dd</i> , <i>J</i> = 17.5, 1.1 Hz)	5.10 (<i>dd</i> , <i>J</i> = 17.7, 1.0 Hz)
4a	1.39 (<i>s</i>)	1.49 (<i>s</i>)
5a	1.39 (<i>s</i>)	1.49 (<i>s</i>)

^a300 MHz

3.1.17 Compound RM17



RM17 was obtained as a brownish crystalline solid, m.p. 195-197°C [lit. 180-189°C]. The IR spectrum showed absorption bands at 3446 cm^{-1} (NH), 1666 cm^{-1} (aldehyde), 1622, 1602 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM17** showed a similar pattern to those of **RM6**, except that no signal of chelated hydroxyl proton as observed in **RM6** but an additional methoxyl signal was apparent at δ 3.99 in **RM17**. The HMBC correlations between δ 3.99 (2-OCH₃) and δ 161.6 (C-2) as well as correlations from δ 161.6 (C-2) to H-4 (δ 8.56) confirmed the position of a methoxyl group at C-2. It was therefore suggested that compound **RM17** was *O*-methylnukonal [Ruangrunsi *et al.*, 1990].

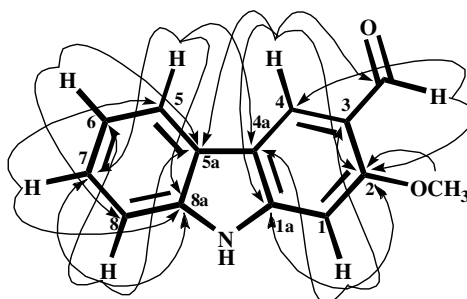


Figure 16 Selected HMBC correlations of **RM17**

Table 31 ^1H , ^{13}C NMR and HMBC spectral data of **RM17** (CDCl_3)

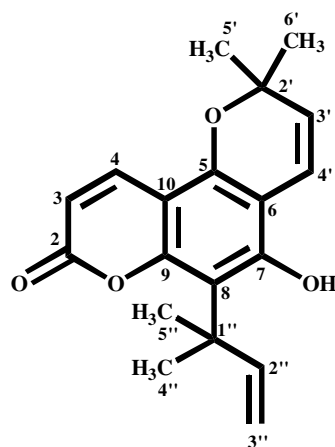
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	145.2 (C)	-
1	6.87 (1H, <i>s</i>)	92.4 (CH)	C-1a, C-2, C-3, C-4a, CHO
2	-	161.6 (C)	-
3	-	118.9 (C)	-
4	8.56 (1H, <i>s</i>)	121.8 (CH)	C-1a, C-2, C-5a, CHO
4a	-	117.4 (C)	-
5a	-	123.7 (C)	-
5	8.01 (1H, <i>d</i> , $J = 7.7$ Hz)	120.1 (CH)	C-4a, C-7, C-8a
6	7.29-7.25 (1H, <i>m</i>)	120.7 (CH)	C-5a, C-8
7	7.39 (1H, <i>m</i>)	125.7 (CH)	C-5, C-6, C-8a
8	7.40 (1H, <i>m</i>)	110.7 (CH)	C-5a, C-6, C-8a
8a	-	140.1 (C)	-
2-OCH ₃	3.99 (3H, <i>s</i>)	55.8 (CH ₃)	C-2
3-CHO	10.48 (1H, <i>s</i>)	189.6 (CHO)	C-2, C-4
NH	8.78 (1H, <i>br s</i>)	-	-

Table 32 ^1H , ^{13}C NMR spectral data of **RM17** (CDCl_3) and ***O*-Methylmukonal (R)** (CD_3COCD_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM21 ^a	R ^b	RM21 ^c	R ^d
1a	-	-	145.2	146.2
1	6.87 (<i>s</i>)	6.88 (<i>s</i>)	92.4	93.5
2	-	-	161.6	162.2
3	-	-	118.9	117.7
4	8.56 (<i>s</i>)	8.56 (<i>s</i>)	121.8	121.2
4a	-	-	117.4	119.3
5a	-	-	123.7	124.2
5	8.01 (<i>d</i> , $J = 7.7$ Hz)	8.00 (<i>d</i> , $J = 8.0$ Hz)	120.1	120.7
6	7.29-7.25 (<i>m</i>)	7.25 (<i>t</i> , $J = 7.7$ Hz)	120.7	120.5
7	7.39 (<i>m</i>)	7.38 (<i>t</i> , $J = 7.7$ Hz)	125.7	126.2
8	7.40 (<i>m</i>)	7.47 (<i>d</i> , $J = 8.0$ Hz)	110.7	111.7
8a	-	-	140.1	141.4
2-OCH ₃	3.99 (<i>s</i>)	4.00 (<i>s</i>)	55.8	56.1
3-CHO	10.48 (<i>s</i>)	10.49 (<i>s</i>)	189.6	188.4
NH	8.78 (<i>br s</i>)	8.88 (<i>br s</i>)	-	-

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

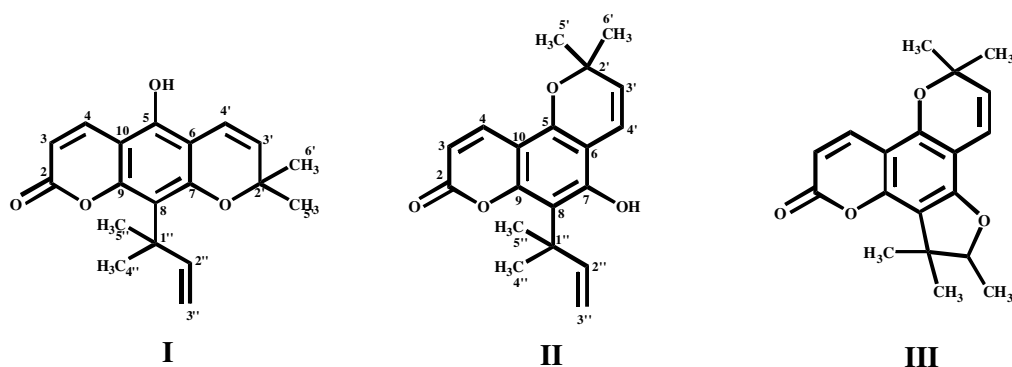
3.1.18 Compound RM18



RM18 was obtained as a pale yellow crystalline solid, m.p. 185-187°C [lit. 182°C]. The IR spectrum showed absorption bands at 3335 cm^{-1} (OH), 1717 cm^{-1} (ester carbonyl) and 1617, 1570, 1457 cm^{-1} (aromatic system).

The ^1H NMR signal pattern of **RM18** resembled to those of **RM15**, except for some difference of chemical shift of the signal due to H-4' (**Table 33**). However, in the HMBC spectrum (**Fig. 17**), observation of the three-bond H-C correlations of H-4' (δ 6.49) to two oxygenated aromatic carbons at δ_{C} 155.9 (C-7) and 154.4 (C-5) suggested the presence of an *O*-substituent at C-7, as in the molecule of **RM15**. These data together with HMBC data shown by arrows in **Fig. 17**, implied that **RM18** should be represented either by structure **I** or **II**. Therefore, **RM15** and **RM18** were found to be regioisomers with regard to the locations of the attachment of a dimethylpyran ring to angular orientation or linear orientation of the coumarin nucleus.

The location of the pyran ring could not be confirmed by H-C long-range in the HMBC spectra of **RM15** and **RM18**, because the problem of the assignment of oxygenated aromatic carbon signals at δ_{C} 148.0 and 156.2 in the spectrum of **RM15** and δ_{C} 155.9 and 154.4 in that of **RM18**, to either C-5 and C-7 or C-7 and C-5, respectively, remained.



To confirm this, **RM15** and **RM18** were treated with conc. HCl or H_2SO_4 which gave only a product with the same signal pattern as **RM18**. It was concluded that **RM15** isomerized to **RM18** in acid condition. When **RM15** was treated with conc. HCl in CH_2Cl_2 at room temperature for overnight, the cyclized product (**III**) was observed. The NMR spectrum showed the disappearance of the 1,1-dimethylallyl group and the appearance of the 2,3,3-trimethyl-dihydrobenzofuran system in the molecule as shown in **Table 34**. On the basis of the above result, the structures of **RM15** and **RM18** were a linear and an angular pyranocoumarin, respectively. Therefore, compound **RM18** was established as 7-hydroxy-8-(1,1-dimethylallyl)citrusarin [Wu *et al.*, 1982].

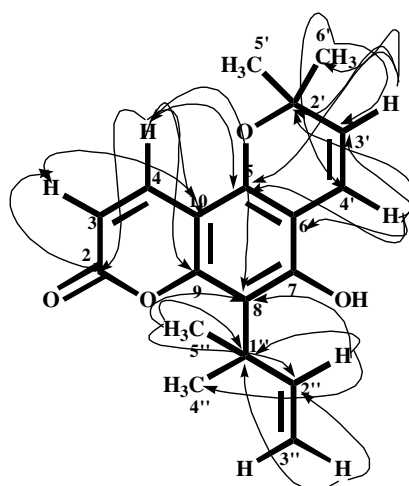


Figure 17 Selected HMBC correlations of **RM18**

Table 33 ^1H and ^{13}C NMR spectral data of **RM18** and **RM15** (CDCl_3)

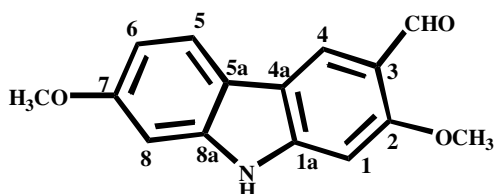
Position	RM18		RM15	
	δ_{H} (multiplicity)	δ_{C} (C-type)	δ_{H} (multiplicity)	δ_{C} (C-type)
1	-	-	-	-
2	-	161.1 (C)	-	162.3 (C)
3	6.15 (<i>d</i> , <i>J</i> = 9.6 Hz)	110.6 (CH)	6.03 (<i>d</i> , <i>J</i> = 9.6 Hz)	108.9 (CH)
4	7.99 (<i>d</i> , <i>J</i> = 9.6 Hz)	138.8 (CH)	8.11 (<i>d</i> , <i>J</i> = 9.6 Hz)	140.5 (CH)
5	-	154.4 (C)	-	148.0 (C)
6	-	105.9 (C)	-	106.9 (C)
7	-	155.9 (C)	-	156.2 (C)
8	-	114.8 (C)	-	115.1 (C)
9	-	146.4 (C)	-	153.9 (C)
10	-	103.8 (C)	-	104.5 (C)
1'	-	-	-	-
2'	-	77.35 (C)	-	76.9 (C)
3'	5.70 (<i>d</i> , <i>J</i> = 9.9 Hz)	130.1 (CH)	5.56 (<i>d</i> , <i>J</i> = 10.0 Hz)	128.7 (CH)
4'	6.49 (<i>d</i> , <i>J</i> = 9.9 Hz)	116.4 (CH)	6.64 (<i>d</i> , <i>J</i> = 10.0 Hz)	116.1 (CH)
5'	1.44 (3H, <i>s</i>)	27.3 (CH ₃)	1.36 (<i>s</i>)	27.2 (CH ₃)
6'	1.44 (3H, <i>s</i>)	27.3 (CH ₃)	1.36 (<i>s</i>)	27.2 (CH ₃)
1''	-	42.0 (C)	-	40.7 (C)
2''	6.28 (<i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	150.1 (CH)	6.21 (<i>dd</i> , <i>J</i> = 17.4, 10.6 Hz)	150.1 (CH)
3''	4.78 (<i>dd</i> , <i>J</i> = 10.6, 0.9 Hz) 4.92 (<i>dd</i> , <i>J</i> = 17.4, 0.9 Hz)	108.1 (CH ₂)	4.78 (<i>dd</i> , <i>J</i> = 10.6, 1.0 Hz) 4.84 (<i>dd</i> , <i>J</i> = 17.4, 1.0 Hz)	107.7 (CH ₂)
4''	1.64 (<i>s</i>)	29.6 (CH ₃)	1.57 (<i>s</i>)	29.4 (CH ₃)
5''	1.64 (<i>s</i>)	29.6 (CH ₃)	1.57 (<i>s</i>)	29.4 (CH ₃)

Table 34 ¹H NMR spectral data of **compound III** and **Citrusarin-A** (CDCl₃)

Position	Compound III	Citrusarin-A ^b
1	-	-
2	-	-
3	6.08 (<i>d</i> , <i>J</i> = 9.7 Hz)	6.07 (<i>d</i> , <i>J</i> = 9.6 Hz)
4	7.94 (<i>d</i> , <i>J</i> = 9.7 Hz)	7.93 (<i>d</i> , <i>J</i> = 9.6 Hz)
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
1'	-	-
2'	-	-
3'	5.55 (<i>d</i> , <i>J</i> = 9.9 Hz)	5.54 (<i>d</i> , <i>J</i> = 9.9 Hz)
4'	6.46 (<i>d</i> , <i>J</i> = 9.9 Hz)	6.45 (<i>d</i> , <i>J</i> = 9.9 Hz)
5'	1.46 (<i>s</i>)	1.46 (<i>s</i>)
6'	1.46 (<i>s</i>)	1.46 (<i>s</i>)
1''	-	-
2''	4.48 (<i>q</i> , <i>J</i> = 6.6 Hz)	4.48 (<i>d</i> , <i>J</i> = 6.6 Hz)
3''	1.40 (<i>d</i> , <i>J</i> = 6.6 Hz)	1.39 (<i>d</i> , <i>J</i> = 6.6 Hz)
4''	1.26 (<i>s</i>)	1.25 (<i>s</i>)
5''	1.53 (<i>s</i>)	1.52 (<i>s</i>)

^a300 MHz, ^b400 MHz

3.1.19 Compound RM19



RM19 was obtained as a brownish crystalline solid, m.p. 221-223°C [lit. 221-223°C]. The IR spectrum showed absorption bands at 3237 cm^{-1} (NH), 1661 cm^{-1} (aldehyde) and 1602, 1508 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM19** was similar to those of **RM14**, except for the presence of an additional methoxyl signal δ 4.01 (2-OCH₃) in **RM19** substituted for the hydroxyl group in **RM14**. The position of a methoxyl group (δ 4.01) was determined by the HMBC correlations between δ 4.01 (2-OCH₃) and δ 160.9 (C-2) as well as correlations from δ 160.9 (C-2) to H-4 (δ 8.39) and CHO (δ 187.8). It was therefore suggested that compound **RM19** was 3-formyl-2,7-dimethoxycarbazole [Ruangrunsi *et al.*, 1990].

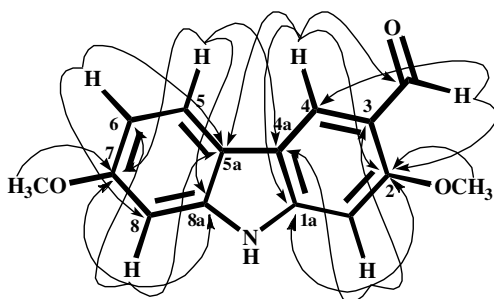


Figure 18 Selected HMBC correlations of **RM19**

Table 35 ^1H , ^{13}C NMR and HMBC spectral data of **RM19** (CD_3COCD_3)

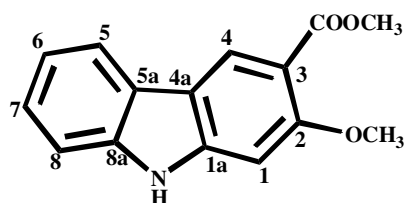
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	145.8 (C)	-
1	7.12 (1H, <i>s</i>)	93.0 (CH)	C-1a, C-2, C-3, C-4a
2	-	160.9 (C)	-
3	-	118.6 (C)	-
4	8.39 (1H, <i>s</i>)	119.3 (CH)	C-1a, C-2, C-5a, CHO
4a	-	117.4 (C)	-
5a	-	117.1 (C)	-
5	8.00 (1H, <i>d</i> , $J = 8.5$ Hz)	120.6 (CH)	C-4a, C-7, C-8a
6	6.85 (1H, <i>dd</i> , $J = 8.5, 2.2$ Hz)	108.7 (CH)	C-5a, C-8
7	-	159.2 (C)	-
8	7.04 (1H, <i>d</i> , $J = 2.2$ Hz)	95.4 (CH)	C-5a, C-6, C-8a
8a	-	142.2 (C)	-
2-OCH ₃	4.01 (3H, <i>s</i>)	55.5 (CH ₃)	C-2
3-CHO	10.45 (1H, <i>s</i>)	187.8 (CHO)	C-2, C-4
7-OCH ₃	3.87 (3H, <i>s</i>)	55.0 (CH ₃)	C-7
NH	10.61 (1H, <i>br s</i>)	-	-

Table 36 ^1H and ^{13}C NMR spectral data of **RM19** and **3-Formyl-2,7-dimethoxy carbazole (R)** (CD_3COCD_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM19 ^a	R ^b	RM19 ^c	R ^d
1a	-	-	145.8	146.6
1	7.12 (<i>s</i>)	7.11 (<i>s</i>)	93.0	93.9
2	-	-	160.9	161.6
3	-	-	118.6	116.0
4	8.39 (<i>s</i>)	8.37 (<i>s</i>)	119.3	121.5
4a	-	-	117.4	119.4
5a	-	-	117.1	118.2
5	8.00 (<i>d</i> , $J = 8.5$ Hz)	7.97 (<i>d</i> , $J = 8.3$ Hz)	120.6	120.2
6	6.85 (<i>dd</i> , $J = 8.5, 2.2$ Hz)	6.83 (<i>dd</i> , $J = 8.3, 2.2$ Hz)	108.7	109.5
7	-	-	159.2	159.7
8	7.04 (<i>d</i> , $J = 2.2$ Hz)	7.02 (<i>d</i> , $J = 2.2$ Hz)	95.4	96.2
8a	-	-	142.2	143.0
2-OCH ₃	4.01 (<i>s</i>)	3.99 (<i>s</i>)	55.5	56.3
3-CHO	10.45 (<i>s</i>)	9.95 (<i>s</i>)	187.8	186.6
7-OCH ₃	3.87 (<i>s</i>)	3.85 (<i>s</i>)	55.0	55.8
NH	10.61 (<i>br s</i>)	10.64 (<i>br s</i>)	-	-

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

3.1.20 Compound RM20



RM20 was obtained as a brownish crystalline solid, m.p. 131-132°C [lit. 133-135°C]. The IR spectrum showed absorption bands at 3414 cm^{-1} (NH), 1699 cm^{-1} (ester carbonyl) and 1637, 1461 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM20** was similar to those of **RM9**. The difference between them was shown as the methoxyl group (δ 3.92) to be the substituent at C-2 in **RM20** instead of the hydroxyl group in **RM9**. The HMBC correlations between δ 3.92 (2-OCH₃) and δ 158.9 (C-2) as well as correlations from δ 158.9 (C-2) to H-4 (δ 8.55) confirmed the position of a methoxyl group at C-2. Thus, the structure of clausine L was deduced as **RM20** [Bhattacharyya *et al.*, 1994].

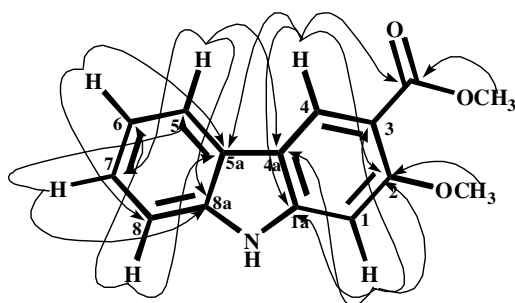


Figure 19 Selected HMBC correlations of **RM20**

Table 37 ^1H , ^{13}C NMR and HMBC spectral data of **RM20** (CD_3COCD_3)

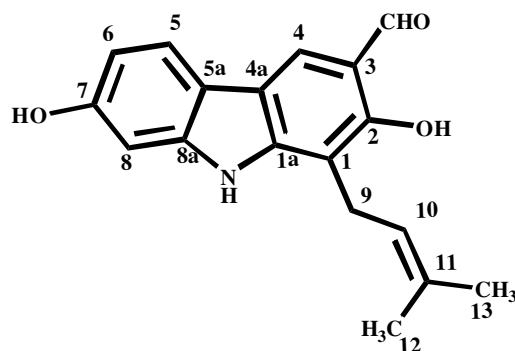
position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	143.8 (C)	-
1	7.15 (1H, <i>s</i>)	93.9 (CH)	C-1a, C-2, C-4, <i>COOMe</i>
2	-	158.9 (C)	-
3	-	112.7 (C)	-
4	8.55 (1H, <i>s</i>)	124.1 (CH)	C-1a, C-2, C-5a, <i>COOMe</i>
4a	-	116.0 (C)	-
5a	-	123.3 (C)	-
5	8.09 (1H, <i>d</i> , $J = 7.7$ Hz)	119.5 (CH)	C-4a, C-7, C-8a
6	7.21 (1H, <i>t</i> , $J = 7.5$ Hz)	119.7 (CH)	C-5a, C-8
7	7.35 (1H, <i>t</i> , $J = 7.7$ Hz)	125.1 (CH)	C-5, C-8a
8	7.50 (1H, <i>d</i> , $J = 8.1$ Hz)	110.9 (CH)	C-5a, C-6
8a	-	140.6 (C)	-
2-OCH ₃	3.92 (3H, <i>s</i>)	55.5 (CH ₃)	C-2
3-COOCH ₃	-	166.6 (COOCH ₃)	-
3-COOCH ₃	3.86 (3H, <i>s</i>)	50.9 (CH ₃)	COOCH ₃
NH	10.62 (1H, <i>br s</i>)	-	-

Table 38 ^1H and ^{13}C NMR spectral data of **RM20** (CD_3COCD_3) and **Clausine L** (**R**) (CDCl_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM20 ^a	R ^b	RM20 ^c	R ^d
1a	-	-	143.8	145.3
1	7.15 (<i>s</i>)	6.98 (<i>s</i>)	93.9	96.3
2	-	-	158.9	144.4
3	-	-	112.7	118.7
4	8.55 (<i>s</i>)	8.10 (<i>s</i>)	124.1	122.4
4a	-	-	116.0	117.7
5a	-	-	123.3	124.1
5	8.09 (<i>d</i> , $J = 7.7$ Hz)	7.80 (<i>d</i> , $J = 8.0$ Hz)	119.5	121.4
6	7.21 (<i>t</i> , $J = 7.5$ Hz)	7.40-7.20 (<i>m</i>)	119.7	120.6
7	7.35 (<i>t</i> , $J = 7.7$ Hz)	7.40-7.20 (<i>m</i>)	125.1	120.5
8	7.50 (<i>d</i> , $J = 8.1$ Hz)	7.40-7.20 (<i>m</i>)	110.9	111.1
8a	-	-	140.6	145.8
2-OCH ₃	3.92 (<i>s</i>)	3.90 (<i>s</i>)	55.5	56.1
3-COOCH ₃	-	-	166.6	167.7
3-COOCH ₃	3.86 (<i>s</i>)	4.10 (<i>br s</i>)	50.9	51.8

^a300 MHz, ^b100 MHz, ^c75 MHz, ^d25 MHz

3.1.11 Compound RM21



RM21 was obtained as yellow viscous liquid. The IR spectrum showed absorption bands at 3394 cm^{-1} (OH and NH), 1615 cm^{-1} (aldehyde) and $1578, 1456\text{ cm}^{-1}$ (aromatic system).

The ^1H NMR spectrum of **RM21** also showed a signal pattern similar to those of **RM1**, except the presence of an additional hydroxyl signal at δ 8.36. The downfield ABX system signals were shown at δ 7.87 (*d*, $J = 8.4\text{ Hz}$), 6.87 (*dd*, $J = 8.4, 2.1\text{ Hz}$) and 6.92 (*d*, $J = 2.1\text{ Hz}$) for H-5, H-6 and H-8, respectively, which were affected by an oxygenated substituent on C-7. Comparison of its ^1H NMR data with those of 7-hydroxyheptaphylline (**Table 39**) indicated that compound **RM21** was 7-hydroxyheptaphylline [Kumar *et al.*, 1995].

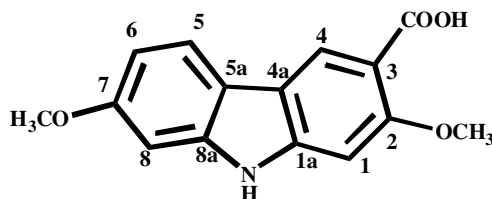
Table 39 ^1H NMR spectral data of **RM21** (CD_3COCD_3) and **7-Hydroxyheptaphylline (R)** ($\text{CDCl}_3 + 2\text{ drops CD}_3\text{OD}$)

Position	δ_{H} (multiplicity)	
	RM21 ^a	R ^b
1a	-	-
1	-	-
2	-	-
3	-	-
4	8.16 (<i>s</i>)	7.86 (<i>s</i>)

4a	-	-
5a	-	-
5	7.87 (<i>d</i> , $J = 8.4$ Hz)	7.72 (<i>d</i> , $J = 8.4$ Hz)
6	6.87 (<i>dd</i> , $J = 8.4, 2.1$ Hz)	6.72 (<i>dd</i> , $J = 8.4, 2.1$ Hz)
7	-	-
8	6.92 (<i>d</i> , $J = 2.1$ Hz)	6.84 (<i>d</i> , $J = 2.1$ Hz)
8a	-	-
9	3.61 (<i>d</i> , $J = 6.6$ Hz)	3.55 (<i>d</i> , $J = 6.8$ Hz)
10	5.34 (<i>br t</i> , $J = 6.6$ Hz)	5.28 (<i>br t</i> , $J = 6.8$ Hz)
11	-	-
12	1.83 (<i>s</i>)	1.83 (<i>s</i>)
13	1.67 (<i>s</i>)	1.68 (<i>s</i>)
2-OH	11.75 (<i>s</i>)	-
3-CHO	9.79 (<i>s</i>)	9.80 (<i>s</i>)
7-OH	8.63 (<i>br s</i>)	-
NH	10.44 (<i>br s</i>)	10.44 (<i>s</i>)

^a300 MHz, ^b500 MHz, ^c125 MHz

3.1.22 Compound RM22



RM22 was obtained as a brownish powder, m.p. 254-256°C [lit. 250-256°C]. The IR spectrum showed absorption bands at 3410 cm^{-1} (NH), 1620 cm^{-1} (carboxy carbonyl) and 1603, 1548 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM22** was similar to those of **RM19**. However, instead of a sharp singlet aldehydic proton as in **RM19**, a carboxylic acid on C-3 was proposed for **RM22**, which was corresponded to the resonance of the carboxyl carbon at δ_{C} 166.2 in the ^{13}C NMR spectrum. The HMBC correlation between δ 166.2 (COOH) to H-4 (δ 8.64) confirmed the position of carboxylic group at C-3. It was therefore suggested that compound **RM22** was clausine K [Wu *et al.*, 1996].

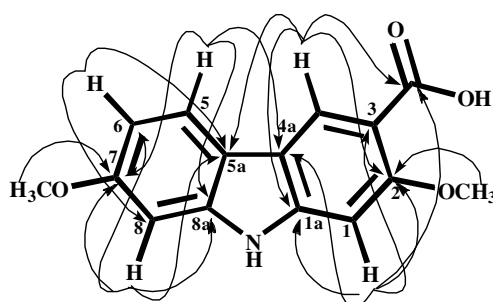


Figure 20 Selected HMBC correlations of **RM22**

Table 40 ^1H , ^{13}C NMR and HMBC spectral data of **RM22** (CD_3COCD_3)

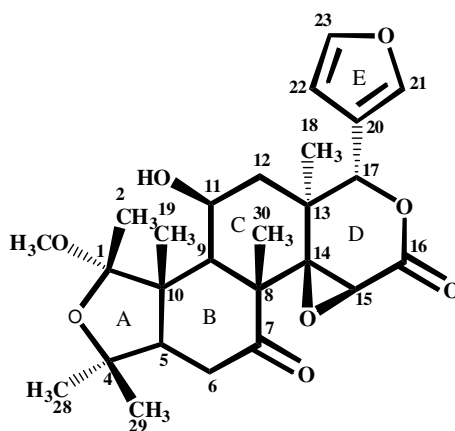
position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	144.0 (C)	-
1	7.24 (1H, <i>s</i>)	93.8 (CH)	C-1a, C-2, C-3, C-4a
2	-	157.2 (C)	-
3	-	110.8 (C)	-
4	8.64 (1H, <i>s</i>)	124.2 (CH)	C-1a, C-2, C-5a, COOH
4a	-	117.8 (C)	-
5a	-	116.5 (C)	-
5	8.02 (1H, <i>d</i> , $J = 8.6$ Hz)	120.6 (CH)	C-4a, C-7, C-8a
6	6.87 (1H, <i>dd</i> , $J = 8.6, 2.2$ Hz)	108.7 (CH)	C-5a, C-8
7	-	159.2 (C)	-
8	7.05 (1H, <i>d</i> , $J = 2.2$ Hz)	95.3 (CH)	C-5a, C-6, C-7, C-8a
8a	-	142.2 (C)	-
2-OCH ₃	4.13 (3H, <i>s</i>)	56.3 (CH ₃)	C-2
3-COOH	-	166.0 (COOH)	-
7-OCH ₃	3.87 (3H, <i>s</i>)	54.9 (CH ₃)	C-7
NH	10.61 (1H, <i>br s</i>)	-	-

Table 41 ^1H and ^{13}C NMR spectral data of **RM22** (CD_3COCD_3) and **Clausine K (R)** ($\text{DMSO}-d_6$)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM22 ^a	R ^b	RM22 ^c	R ^d
1a	-	-	144.0	143.4
1	7.24 (<i>s</i>)	7.30 (<i>s</i>)	93.8	94.0
2	-	-	157.2	157.4
3	-	-	110.8	112.3
4	8.64 (<i>s</i>)	8.39 (<i>s</i>)	124.2	123.1
4a	-	-	117.8	115.8
5a	-	-	116.5	116.3
5	8.02 (<i>d</i> , $J = 8.6$ Hz)	7.94 (<i>d</i> , $J = 8.5$ Hz)	120.6	120.4
6	6.87 (<i>dd</i> , $J = 8.6, 2.2$ Hz)	6.77 (<i>dd</i> , $J = 8.5, 2.0$ Hz)	108.7	108.1
7	-	-	159.2	158.1
8	7.05 (<i>d</i> , $J = 2.2$ Hz)	6.97 (<i>d</i> , $J = 2.0$ Hz)	95.3	95.1
8a	-	-	142.2	141.6
2-OCH ₃	4.13 (<i>s</i>)	3.89 (<i>s</i>)	56.3	55.7
3-COOH	-	-	166.0	167.5
7-OCH ₃	3.87 (<i>s</i>)	3.83 (<i>s</i>)	56.0	55.8
NH	10.61 (<i>br s</i>)	11.27 (<i>br s</i>)	-	-

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

3.1.23 Compound RM23



RM23 was obtained as a colorless crystalline solid, m.p. 193-194°C [lit. 190-191°C]. The IR spectrum showed absorption bands at 3493 cm^{-1} (OH) and 1710 and 1630 cm^{-1} (ester and ketone carbonyl) and 840 (β -substituted furan).

The ^1H NMR spectrum suggested the presence of a β -substituted furan at δ 7.59 (*br s*, 1H), 7.57 (*br d*, $J = 1.5$ Hz, 1H) and 6.49 (*br d*, $J = 1.0$ Hz, 1H). It was further established that compound **RM23** was a limonoid with five tertiary methyl groups resonating as singlets at δ 1.50, 1.45, 1.21, 1.13, 1.10 and a deshielded C-methyl group at δ 1.61 and a methoxyl signal at δ 3.24. The presence of an epoxy lactone moiety of limonoid was revealed by the characteristic H-15 and H-17 singlet signals at δ 3.92 and 5.56 respectively. The presence of a system $\begin{array}{c} | \\ -\text{CH}-\text{CH}_2-\text{C}=\text{O} \end{array}$ in the molecule was inferred from an ABC pattern at δ 2.29 (*dd*, $J = 14.1, 3.5$ Hz, 1H, H-6 α), 3.01 (*dd*, $J = 15.9, 14.1$ Hz, 1H, H-6 β) and 2.67 (*dd*, $J = 15.9, 3.5$ Hz, H-5 α). This result also revealed the presence of two fully substituted carbon atoms alpha to the methine carbon due to the absence of other coupling for these three protons in the ^1H NMR spectrum. Four mutually coupling protons at δ 2.75 (*m*, 1H, H-9), 4.56 (*m*, 1H, H-11) and 1.77-1.75 (*m*, 2H, H-12) were assigned to the moiety $\begin{array}{c} | \\ -\text{CH}-\text{CHOH}-\text{CH}_2- \end{array}$ in which both terminal carbons are quaternary. This result was also supported by a HMBC experiment (**Figure 21**). The optical rotation of this compound is levorotary ($[\alpha]_{\text{D}}^{25} = -39.5^\circ$ (c 0.05, MeOH)), similar to a *O*-methylclausenolide ($[\alpha]_{\text{D}}^{23} = -35.7^\circ$ (c 0.05, MeOH)). Based on these data, the structure of *O*-methylclausenolide was assigned as **RM23** (Wu *et al.*, 1992).

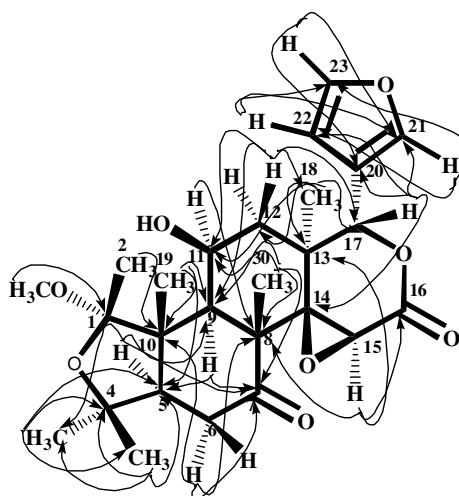


Figure 21 Selected HMBC correlations of **RM23**

Table 42 ^1H , ^{13}C NMR and HMBC spectral data of **RM23** (CD_3COCD_3)

Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	-	108.6 (C)	-
2	1.45 (3H, <i>s</i>)	17.3 (CH_3)	-
3	-	-	-
4	-	79.4 (C)	-
5	2.67 (1H, <i>dd</i> , $J = 15.9, 3.5$ Hz)	55.7 (CH)	C-4, C-6, C-7, C-8, C-9, C-10, C-28, C-29
6	2.29 (1H, <i>dd</i> , $J = 14.1, 3.5$ Hz)	36.8 (CH_2)	C-5, C-7, C-8, C-10
	3.01 (1H, <i>dd</i> , $J = 15.9, 14.1$ Hz)		C-4, C-5, C-7, C-8, C-10
7	-	208.8 (C)	-
8	-	50.6 (C)	-
9	2.75 (1H, <i>m</i>)	44.7 (CH)	C-5, C-6, C-7, C-10, C-11, C-12, C-19, C-30
10	-	51.1 (C)	-
11	4.56 (1H, <i>m</i>)	65.8 (CH)	C-8, C-13
12	1.75-1.77 (2H, <i>m</i>)	43.0 (CH_2)	C-9, C-11, C-13, C-17, C-18
13	-	37.1 (C)	-
14	-	65.8 (C)	-
15	3.92 (1H, <i>s</i>)	54.2 (CH)	C-8, C-11, C-13, C-16
16	-	167.1 (C)	-
17	5.56 (1H, <i>s</i>)	78.1 (CH)	C-11, C-12, C-13, C-19, C-20, C-21, C-22
18	1.10 (3H, <i>s</i>)	18.9 (CH_3)	C-11, C-12, C-13, C-17

19	1.61 (3H, <i>s</i>)	16.6 (CH ₃)	C-5, C-9, C-10
20	-	120.9 (C)	-
21	7.59 (1H, <i>br s</i>)	141.5 (CH)	C-20, C-21, C-23
22	6.49 (1H, <i>d</i> , <i>J</i> = 1.0 Hz)	110.1 (CH)	C-17, C-20, C-21, C-23
23	7.57 (1H, <i>br d</i> , <i>J</i> = 1.5 Hz)	143.2 (CH)	C-20, C-21, C-22
28	1.21 (3H, <i>s</i>)	30.3 (CH ₃)	C-4, C-5, C-29
29	1.13 (3H, <i>s</i>)	22.8 (CH ₃)	C-4, C-5, C-28
30	1.50 (3H, <i>s</i>)	19.7 (CH ₃)	C-7, C-8, C-9, C-11
1-OCH ₃	3.24 (3H, <i>s</i>)	47.8 (CH ₃)	C-1

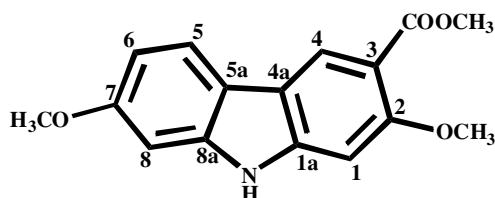
Table 43 ¹H and ¹³C NMR spectral data of **RM23** (CD₃COCD₃) and **O-Methyl-clausenolide (R)** (CDCl₃)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM23 ^a	R	RM23 ^b	R
1	-	-	108.6	108.5
2	1.45 (<i>s</i>)	1.46 (<i>s</i>)	17.3	18.0
3	-	-	-	-
4	-	-	79.4	79.9
5	2.67 (<i>dd</i> , <i>J</i> = 15.9, 3.5 Hz)	2.64 (<i>dd</i> , <i>J</i> = 16.0, 3.3 Hz)	55.7	55.7
6	2.29 (<i>dd</i> , <i>J</i> = 14.1, 3.5 Hz)	2.33 (<i>dd</i> , <i>J</i> = 14.0, 3.3 Hz)	36.8	37.0
	3.01 (<i>dd</i> , <i>J</i> = 15.9, 14.1 Hz)	3.01 (<i>dd</i> , <i>J</i> = 15.9, 14.1 Hz)		
7	-	-	208.8	207.2
8	-	-	50.6	50.7
9	2.75 (<i>m</i>)	2.72 (<i>br s</i>)	44.7	44.7
10	-	-	51.1	51.0
11	4.56 (<i>m</i>)	4.53 (<i>m</i>)	65.8	67.0
12	1.76 (<i>m</i>)	1.89 (<i>dd</i> , <i>J</i> = 15.0, 7.2 Hz)	43.0	43.4
		1.63 (<i>br d</i> , <i>J</i> = 15.0 Hz)		
13	-	-	37.1	37.3
14	-	-	65.8	65.8
15	3.92 (<i>s</i>)	3.99 (<i>s</i>)	54.2	54.4
16	-	-	167.1	167.3

17	5.56 (s)	5.56 (s)	78.1	78.3
18	1.10 (s)	1.11 (s)	18.9	19.3
19	1.61 (s)	1.56 (s)	16.6	17.2
20	-	-	120.9	120.3
21	7.59 (br s)	7.43 (br s)	141.5	141.1
22	6.49 (d, $J = 1.0$ Hz)	6.37 (d, $J = 1.5$ Hz)	110.1	109.9
23	7.57 (br d, $J = 1.5$ Hz)	7.40 (br d, $J = 1.5$ Hz)	143.2	143.0
28	1.21 (s)	1.24 (s)	30.3	30.8
29	1.13 (s)	1.15 (s)	22.8	23.3
30	1.50 (s)	1.45 (s)	19.7	30.8
1-OCH ₃	3.24 (s)	3.23 (s)	47.8	23.3

^a300 MHz, ^b75 MHz

3.1.24 Compound RM24



RM24 was obtained as a yellow crystalline solid, m.p. 187-189°C [lit. 192-194°C]. The IR spectrum showed absorption bands at 3404 cm^{-1} (NH), 1702 cm^{-1} (ester carbonyl) and 1618, 1581 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM24** was similar to those of **RM22**. The difference between **RM24** and **RM22** was that the 3-COOH group in **RM22** was replaced by the carbomethoxyl group in **RM24** observed as signals at δ_{H} 3.80 (COOCH_3); δ_{C} 50.7 and δ_{C} 166.6. The HMBC correlations between δ 3.80 (COOCH_3) and δ 166.6 (COOCH_3) as well as correlation from δ 166.6 (COOCH_3) to H-4 (δ 8.37) confirmed the position of the carbomethoxyl group at C-3. It was therefore suggested that compound **RM24** was clausine H [Wu *et al.*, 1996].

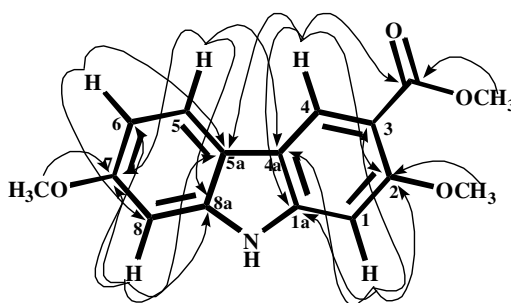


Figure 22 Selected HMBC correlations of **RM24**

Table 44 ^1H , ^{13}C NMR and HMBC spectral data of **RM24** (CD_3COCD_3)

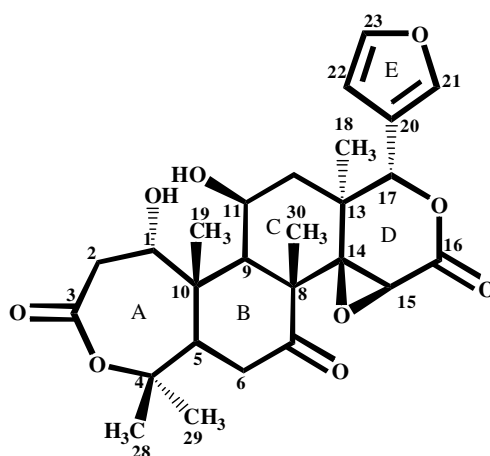
position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	143.8 (C)	-
1	7.08 (1H, <i>s</i>)	94.2 (CH)	C-1a, C-2, C-3, C-4a
2	-	158.1 (C)	-
3	-	112.7 (C)	-
4	8.37 (1H, <i>s</i>)	123.0 (CH)	C-1a, C-2, C-5a, COOCH_3
4a	-	116.4 (C)	-
5a	-	116.9 (C)	-
5	7.91 (1H, <i>d</i> , $J = 8.6$ Hz)	120.2 (CH)	C-7, C-8a, C-4a
6	6.79 (1H, <i>dd</i> , $J = 8.6, 2.2$ Hz)	108.3 (CH)	C-5a, C-8
7	-	158.9 (C)	-
8	6.99 (1H, <i>d</i> , $J = 2.2$ Hz)	95.2 (CH)	C-5a, C-6, C-7, C-8a
8a	-	142.0 (C)	-
2-OCH ₃	3.86 (3H, <i>s</i>)	55.6 (CH ₃)	C-2
3-COOCH ₃	-	166.6 (COOCH ₃)	-
3-COOCH ₃	3.80 (3H, <i>s</i>)	50.7 (CH ₃)	COOCH ₃
7-OCH ₃	3.82 (3H, <i>s</i>)	54.9 (CH ₃)	C-7
NH	10.40 (1H, <i>br s</i>)	-	-

Table 45 ^1H and ^{13}C NMR spectral data of **RM24** and **Clausine H (R)** (CD_3COCD_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM24 ^a	R ^c	RM24 ^b	R ^d
1a	-	-	143.8	144.5
1	7.08 (<i>s</i>)	7.10 (<i>s</i>)	94.2	94.8
2	-	-	158.1	158.7
3	-	-	112.7	113.3
4	8.37 (<i>s</i>)	8.40 (<i>s</i>)	123.0	123.6
4a	-	-	116.4	117.0
5a	-	-	116.9	117.5
5	7.91 (<i>d</i> , $J = 8.6$ Hz)	7.94 (<i>d</i> , $J = 9.0$ Hz)	120.2	120.9
6	6.79 (<i>dd</i> , $J = 8.6, 2.2$ Hz)	6.82 (<i>dd</i> , $J = 9.0, 2.2$ Hz)	108.3	109.0
7	-	-	158.9	159.5
8	6.99 (<i>d</i> , $J = 2.2$ Hz)	7.02 (<i>d</i> , $J = 2.2$ Hz)	95.2	95.8
8a	-	-	142.0	142.6
2-OCH ₃	3.86 (<i>s</i>)	3.90 (<i>s</i>)	55.6	55.6
3-COOCH ₃	-	-	166.6	167.3
3-COOCH ₃	3.80 (<i>s</i>)	3.83 (<i>s</i>)	50.7	51.4
7-OCH ₃	3.82 (<i>s</i>)	3.85 (<i>s</i>)	54.9	56.2
NH	10.40 (<i>br s</i>)	10.34 (<i>br s</i>)	-	-

^a300 MHz, ^b400 MHz, ^c75 MHz, ^d100 MHz

3.1.25 Compound RM25



RM25 was obtained as a colorless crystalline solid, m.p. 289-291°C [lit. 293-294°C]. The IR spectrum showed absorption bands at 3422 cm^{-1} (OH) and 1704, 1630 cm^{-1} (ketone and ester carbonyl) and 850 (β -substituted furan).

The ^1H and ^{13}C NMR spectra of **RM25** were similar to those of **RM23**, except for the absence of a methoxyl and a methyl signals at C-1 and the presence of a system $\text{-CHOH-CH}_2\text{-C=O}$ in the molecule which was inferred from an ABC pattern in ring A at δ 4.04 (*m*, 1H, H-1), 2.78 (*dd*, $J = 15.4, 7.6$ Hz, 1H, H-2 α) and 3.32 (*d*, $J = 15.4$ Hz, H-2 β). This result was also supported by a HMBC experiment (**Figure 46**). The optical rotation of this compound is levorotary ($[\alpha]_{\text{D}}^{25} = -98.7^\circ$ (c 1.04, Me_2CO)), similar to clausenarin ($[\alpha]_{\text{D}}^{23} = -87.5^\circ$ (c 1.04, Me_2CO)). It was therefore suggested that compound **RM25** was clausenarin (Ngadjui *et al.*, 1989).

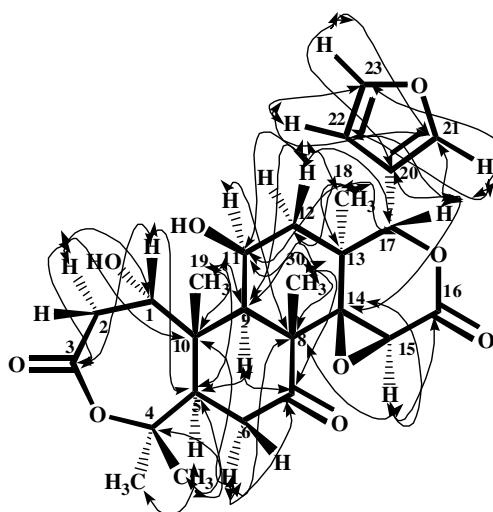


Figure 23 Selected HMBC correlations of **RM25**

Table 46 ^1H , ^{13}C NMR and HMBC spectral data of **RM25** (CD_3COCD_3)

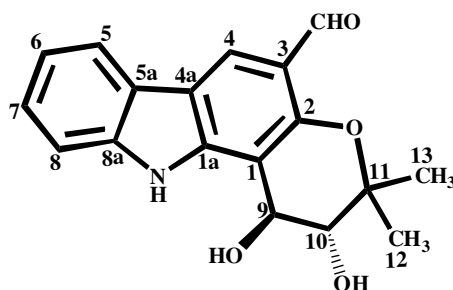
Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1	4.04 (1H, <i>m</i>)	69.6 (CH)	-
2	2.78 (1H, <i>dd</i> , <i>J</i> = 15.4, 7.6 Hz) 3.32 (1H, <i>d</i> , <i>J</i> = 15.4 Hz)	39.3 (CH ₂)	-
3	-	170.0 (C)	-
4	-	83.6 (C)	-
5	2.47 (1H, <i>dd</i> , <i>J</i> = 15.9, 4.1 Hz)	49.8 (CH)	C-4, C-6, C-7, C-8, C-9, C-10, C-28, C-29
6	2.36 (1H, <i>dd</i> , <i>J</i> = 14.0, 4.1 Hz) 2.99 (1H, <i>dd</i> , <i>J</i> = 15.9, 14.0 Hz)	39.1 (CH ₂)	C-5, C-7, C-8, C-10 C-4, C-5, C-7, C-8, C-10
7	-	207.3 (C)	-
8	-	51.5 (C)	-
9	2.52 (1H, <i>m</i>)	46.2 (CH)	C-5, C-6, C-7, C-10, C-11, C-12, C-19, C-30
10	-	45.3 (C)	-
11	4.63 (1H, <i>m</i>)	65.7 (CH)	C-8, C-13
12	1.60 (1H, <i>dd</i> , <i>J</i> = 14.8, 6.3 Hz) 1.74 (1H, <i>d</i> , <i>J</i> = 14.8 Hz)	43.7 (CH ₂)	C-9, C-11, C-13, C-17, C-18
13	-	36.1 (C)	-
14	-	64.6 (C)	-
15	3.61 (1H, <i>s</i>)	53.5 (CH)	C-8, C-11, C-13, C-16
16	-	167.1 (C)	-
17	5.47 (1H, <i>s</i>)	78.1 (CH)	C-11, C-12, C-13, C-19, C-20, C-21, C-22
18	0.94 (3H, <i>s</i>)	19.6 (CH ₃)	C-11, C-12, C-13, C-17
19	1.55 (3H, <i>s</i>)	17.6 (CH ₃)	C-5, C-9, C-10
20	-	120.9 (C)	-
21	7.44 (1H, <i>br s</i>)	141.3 (CH)	C-20, C-21, C-23
22	6.36 (1H, <i>br s</i>)	110.1 (CH)	C-17, C-20, C-21, C-23
23	7.42 (1H, <i>br d</i> , <i>J</i> = 1.8 Hz)	143.2 (CH)	C-20, C-21, C-22
28	1.48 (3H, <i>s</i>)	23.0 (CH ₃)	C-4, C-5, C-29
29	1.29 (3H, <i>s</i>)	32.7 (CH ₃)	C-4, C-5, C-28
30	1.41 (3H, <i>s</i>)	18.7 (CH ₃)	C-7, C-8, C-9, C-11
11-OH	4.59 (1H, <i>d</i> , <i>J</i> = 4.2 Hz)	-	-

Table 47 ^1H and ^{13}C NMR spectral data of **RM25** (CD_3COCD_3) and **Clausenarin (R)** ($\text{DMSO}-d_6$)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM25 ^a	R ^b	RM25 ^c	R ^d
1	4.04 (<i>m</i>)	3.90 (<i>m</i>)	69.6	68.9
2	2.78 (<i>dd</i> , $J = 15.4, 7.6$ Hz) 3.32 (<i>d</i> , $J = 15.4$ Hz)	Not reported	39.3	43.6
3	-	-	170.0	171.1
4	-	-	83.6	84.1
5	2.47 (<i>dd</i> , $J = 15.9, 4.1$ Hz)	Not reported	49.8	49.8
6	2.36 (<i>dd</i> , $J = 14.0, 4.1$ Hz) 2.99 (<i>dd</i> , $J = 15.9, 14.0$ Hz)	Not reported	39.1	41.5
7	-	-	207.3	208.6
8	-	-	51.5	45.1
9	2.52 (<i>m</i>)	Not reported	46.2	46.0
10	-	-	45.3	51.2
11	4.63 (<i>m</i>)	4.45 (<i>m</i>)	65.7	64.6
12	1.60 (<i>dd</i> , $J = 14.8, 6.3$ Hz) 1.74 (<i>d</i> , $J = 14.8$ Hz)	2.35 (<i>m</i>)	43.7	37.1
13	-	-	36.1	35.8
14	-	-	64.6	64.9
15	3.61 (<i>s</i>)	3.75 (<i>s</i>)	53.5	53.2
16	-	-	167.1	167.8
17	5.47 (<i>s</i>)	5.48 (<i>br s</i>)	78.1	78.1
18	0.94 (<i>s</i>)	0.90 (<i>s</i>)	19.6	20.0
19	1.55 (<i>s</i>)	1.45 (<i>s</i>)	17.6	17.7
20	-	-	120.9	120.4
21	7.44 (<i>br s</i>)	7.64 (<i>br s</i>)	141.3	141.8
22	6.36 (<i>br s</i>)	6.46 (<i>br s</i>)	110.1	110.5
23	7.42 (<i>br d</i> , $J = 1.8$ Hz)	Not reported	143.2	143.7
28	1.48 (<i>s</i>)	1.45 (<i>s</i>)	23.0	23.2
29	1.29 (<i>s</i>)	1.25 (<i>s</i>)	32.7	33.4
30	1.41 (<i>s</i>)	1.38 (<i>s</i>)	17.6	17.7
11-OH	4.59 (1H, <i>d</i> , $J = 4.2$ Hz)	5.53 (<i>d</i> , $J = 7.0$ Hz)	-	-

^a300 MHz, ^b90 MHz, ^c75 MHz, ^d22.5 MHz

3.1.26 Compound RM26



RM26 was obtained as yellow viscous liquid. The IR spectrum showed absorption bands at 3330 cm^{-1} (OH and NH), 1665 cm^{-1} (aldehyde) and $1607, 1580\text{ cm}^{-1}$ (aromatic system).

The ^1H and ^{13}C NMR spectra data of **RM26** were similar to those of **RM8**, the major difference were the presence of two oxymethine doublets at δ 4.98 and 3.85 ($J = 7.8\text{ Hz}$) of H-9 and H-10, respectively, indicating that benzylic methylene group in **RM8** was replaced by a hydroxyl methane group in **RM26**. The structure was confirmed by HMBC correlation between δ 4.98 (H-9) and C-1 (δ 106.7), C-2 (δ 153.9) and C-10 (δ 75.8) as well as correlation between δ 3.85 (H-10) and C-9 (δ 68.3), C-11 (δ 80.0) and C-12 (δ 18.6) suggesting the position of two methine doublets at C-9 and C-10, respectively. From NOESY experiment, the oxymethine proton at δ 4.98 (H-9) showed no cross peak with δ 3.85 (H-10) supporting that H-9 and H-10 were *axial-axial*. These results led us to assign structure **RM26** to a new compound and named as clausebazole C.

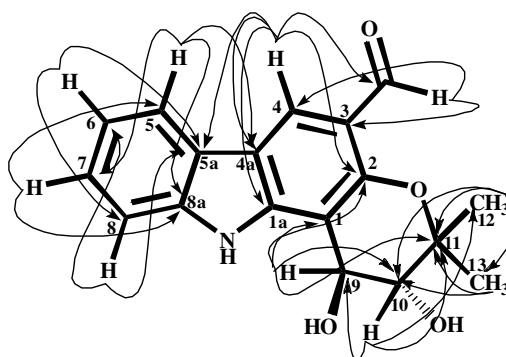


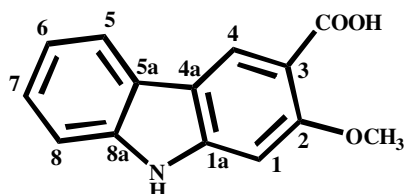
Figure 24 Selected HMBC correlations of **RM26**

Table 48 ^1H , ^{13}C NMR and HMBC spectral data of **RM26** (CD_3COCD_3)

position	$\delta_{\text{H}}^{\text{a}}$ (multiplicity)	$\delta_{\text{C}}^{\text{b}}$ (C-type)	HMBC
1a	-	143.8 (C)	-
1	-	106.7 (C)	-
2	-	153.9 (C)	-
3	-	118.2 (C)	-
4	8.43 (1H, <i>s</i>)	119.6 (CH)	C-1a, C-2, C-4a, C-5a, CHO
4a	-	117.4 (C)	-
5a	-	123.4 (C)	-
5	8.12 (1H, <i>d</i> , $J = 7.5$ Hz)	119.7 (CH)	C-4a, C-7, C-8a
6	7.21 (1H, <i>td</i> , $J = 7.5, 1.0$ Hz)	120.0 (CH)	C-5a, C-8,
7	7.37 (1H, <i>td</i> , $J = 8.1, 1.0$ Hz)	125.5 (CH)	C-5, C-8a
8	7.60 (1H, <i>d</i> , $J = 8.1$ Hz)	111.4 (CH)	C-5a, C-6
8a	-	141.0 (C)	-
9	4.98 (1H, <i>d</i> , $J = 7.8$ Hz)	68.3 (CH)	C-1, C-2, C-10
10	3.85 (1H, <i>d</i> , $J = 7.8$ Hz)	75.8 (CH)	C-9, C-11, C-12
11	-	80.0 (C)	-
12	1.35 (3H, <i>s</i>)	18.6 (CH_3)	C-10, C-11, C-13
13	1.58 (3H, <i>s</i>)	25.9 (CH_3)	C-10, C-11, C-12
3-CHO	10.44 (1H, <i>s</i>)	188.0 (CH)	C-3, C-4
NH	10.48 (1H, <i>br s</i>)	-	-

^a300 MHz, ^b75 MHz

3.1.27 Compound RM27



RM27 was obtained as a colorless crystalline solid, m.p. 224–225°C [lit. 226°C]. The IR spectrum showed absorption bands at 3410 cm^{-1} (NH), 1620 cm^{-1} (carboxy carbonyl) and 1603, 1548 cm^{-1} (aromatic system).

The ^1H NMR spectrum of **RM27** was similar to those of **RM17**. However, instead of a sharp singlet aldehyde proton at δ_{H} 10.48; δ_{C} 189.6 as in **RM17**, a carboxylic acid on C-3 was proposed for **RM27**, which corresponded to the resonance of the carboxyl carbon at δ 165.8 in the ^{13}C NMR spectrum. The HMBC correlations between the carbon at δ 165.8 (COOH) and H-4 (δ 8.76) confirmed the position of carboxylic group at C-3. It was therefore suggested that compound **RM27** was isomukonidine [Fork *et al.*, 2008].

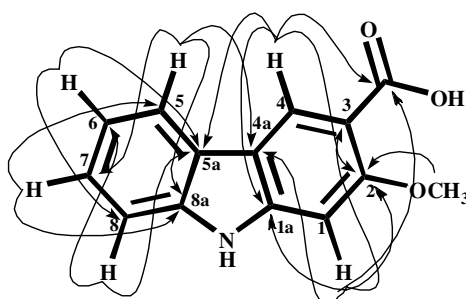


Figure 25 Selected HMBC correlations of **RM27**

Table 49 ^1H and ^{13}C NMR spectral data of **RM27** (CD_3COCD_3)

Position	δ_{H} (multiplicity)	δ_{C} (C-type)	HMBC
1a	-	144.1 (C)	-
1	7.28 (1H, <i>s</i>)	93.8 (CH)	C-1a, C-2, C-3, C-4a, COOH
2	-	158.1 (C)	-
3	-	111.0 (C)	-
4	8.76 (1H, <i>s</i>)	125.3 (CH)	C-1a, C-2, C-5a, COOH
4a	-	117.0 (C)	-
5a	-	123.2 (C)	-
5	8.16 (1H, <i>d</i> , $J = 7.8$ Hz)	119.8 (CH)	C-4a, C-7, C-8a
6	7.24 (1H, <i>td</i> , $J = 7.8, 1.2$ Hz)	119.9 (CH)	C-5a, C-8
7	7.39 (1H, <i>td</i> , $J = 8.1, 1.2$ Hz)	125.5 (CH)	C-5, C-8a
8	7.52 (1H, <i>d</i> , $J = 8.1$ Hz)	111.0 (CH)	C-5a, C-6
8a	-	140.7 (C)	-
2-OCH ₃	4.14 (3H, <i>s</i>)	56.2 (CH ₃)	C-2
3-COOH	-	165.8 (COOH)	-
NH	10.75 (1H, <i>br s</i>)	-	-

Table 50 ^1H and ^{13}C NMR spectral data of **RM27** and **Isomukonidine (R)**
(CD_3COCD_3)

Position	δ_{H} (multiplicity)		δ_{C} (C-type)	
	RM32 ^a	R ^b	RM32 ^c	R ^d
1a	-	-	144.1	145.0
1	7.28 (<i>s</i>)	7.27 (<i>s</i>)	93.8	94.6
2	-	-	158.1	158.9
3	-	-	111.0	111.6
4	8.76 (<i>s</i>)	8.77 (<i>s</i>)	125.3	126.2
4a	-	-	117.0	118.1
5a	-	-	123.2	124.0
5	8.16 (<i>d</i> , $J = 7.8$ Hz)	8.16 (<i>d</i> , $J = 7.8$ Hz)	119.8	120.7
6	7.24 (<i>td</i> , $J = 7.8, 1.2$ Hz)	7.24 (<i>t</i> , $J = 7.8$ Hz)	119.9	120.8
7	7.39 (<i>td</i> , $J = 8.1, 1.2$ Hz)	7.39 (<i>t</i> , $J = 7.8$ Hz)	125.5	126.3
8	7.52 (<i>d</i> , $J = 8.1$ Hz)	7.51 (<i>t</i> , $J = 7.8$ Hz)	111.0	111.9
8a	-	-	140.7	141.6
2-OCH ₃	4.14 (<i>s</i>)	4.13 (<i>s</i>)	56.2	57.1
3-COOH	-	-	165.8	166.6
NH	10.75 (<i>br s</i>)	10.62 (<i>br s</i>)	-	-

^a300 MHz, ^b500 MHz, ^c75 MHz, ^d125 MHz

Conclusion

Investigation of the crude methylene chloride extract from the roots of *M. minutum* led to the isolation of twenty-seven compounds of seventeen carbazole alkaloids: heptaphylline (**RM1**), mukonal (**RM6**), clausebazole A (**RM7**), clausebazole B (**RM8**), mukonidine (**RM9**), mukonine (**RM11**), murrayacine (**RM12**), murrayanine (**RM13**), 7-methoxymukonal (**RM14**), *O*-methylumukonal (**RM17**), 3-formyl-2,7-dimethoxycarbazole (**RM19**), clausine L (**RM20**), 7-hydroxyheptaphylline (**RM21**), clausine K (**RM22**), clausine H (**RM24**), clausebazole C (**RM26**) and isomukonidine (**RM27**), one furanocoumarin: clausemarin (**RM5**), six pyranocoumarins: clausinidin (**RM2**), dentatin (**RM3**), xanthoxylatin (**RM4**), nordentatin (**RM15**), kinocoumarin (**RM16**) and 7-hydroxy-8-(1,1-dimethylallyl)citrusarin (**RM18**), two limonoids: *O*-methylclausenolide (**RM23**) and clausenarin (**RM25**) and one benzoic acid derivative: 4-hydroxy-2-methoxybenzoic acid (**RM10**).

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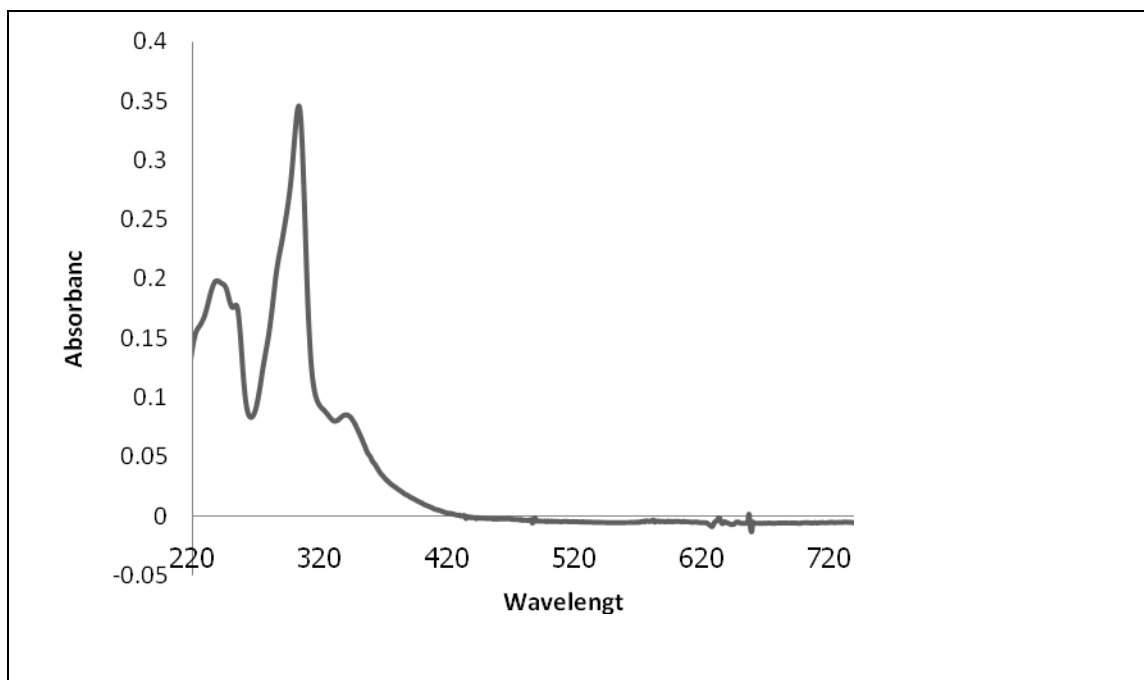


Figure 26 UV (MeOH) spectrum of compound **RM1**

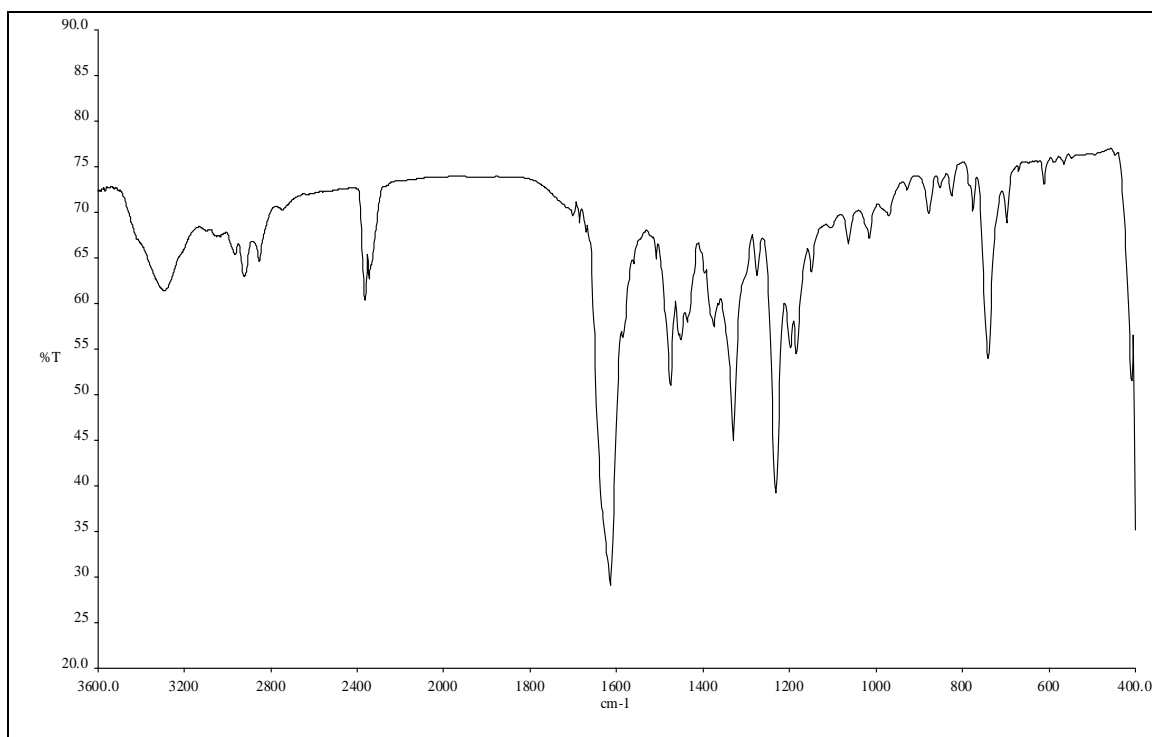


Figure 27 IR (neat) spectrum of compound **RM1**

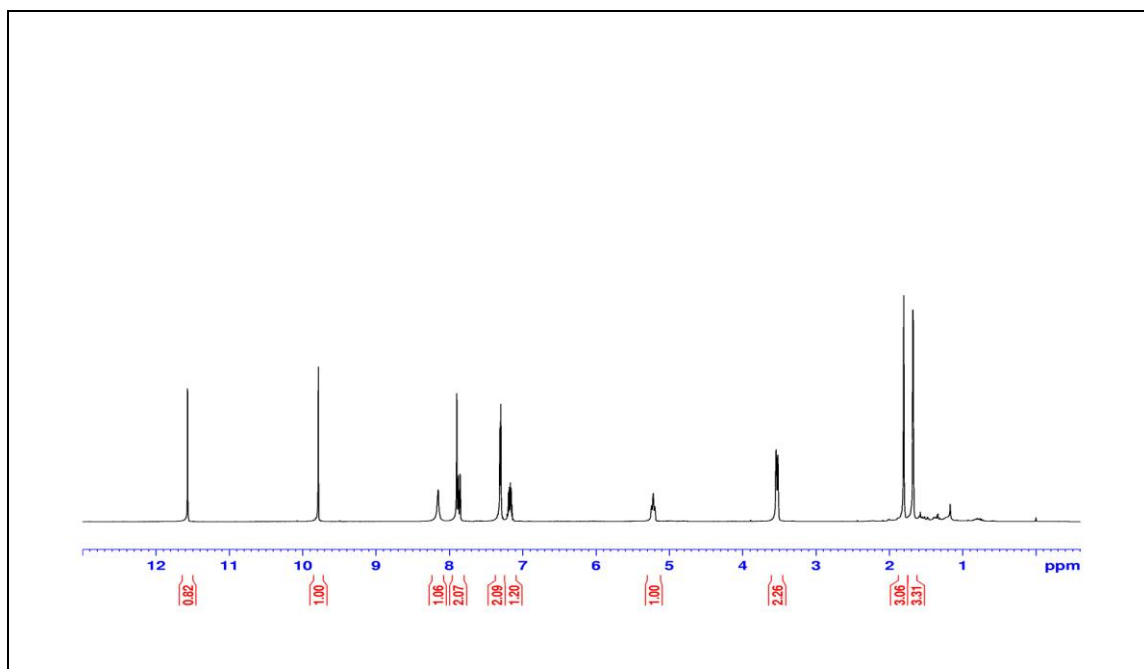


Figure 28 ^1H NMR (300 MHz) (CDCl_3) of compound **RM1**

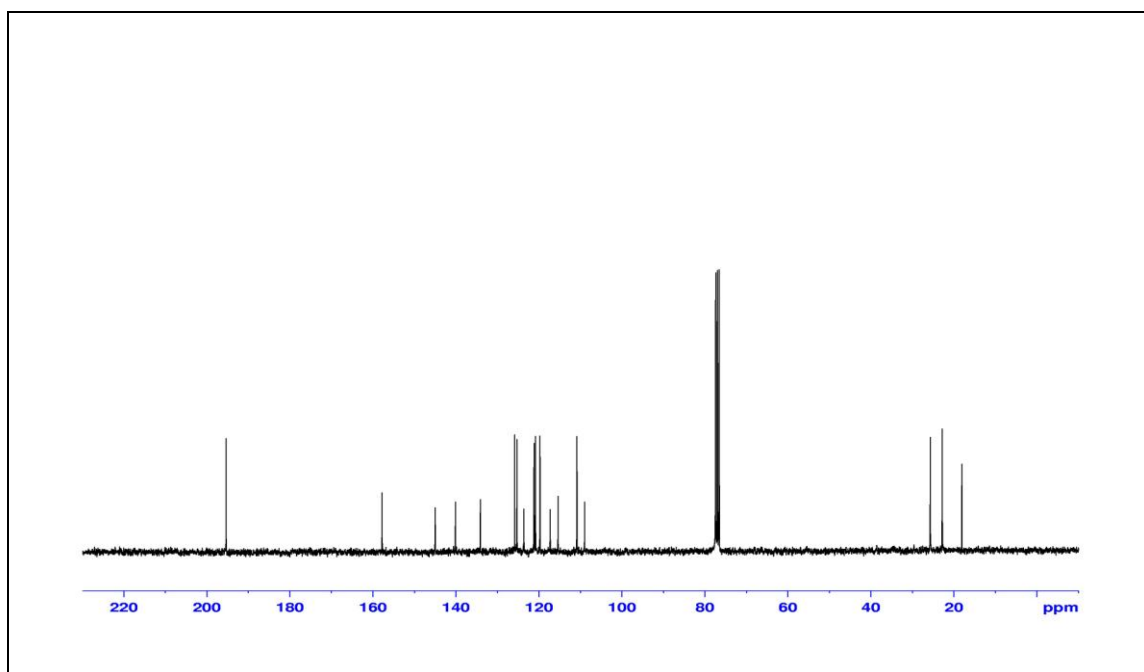


Figure 29 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM1**

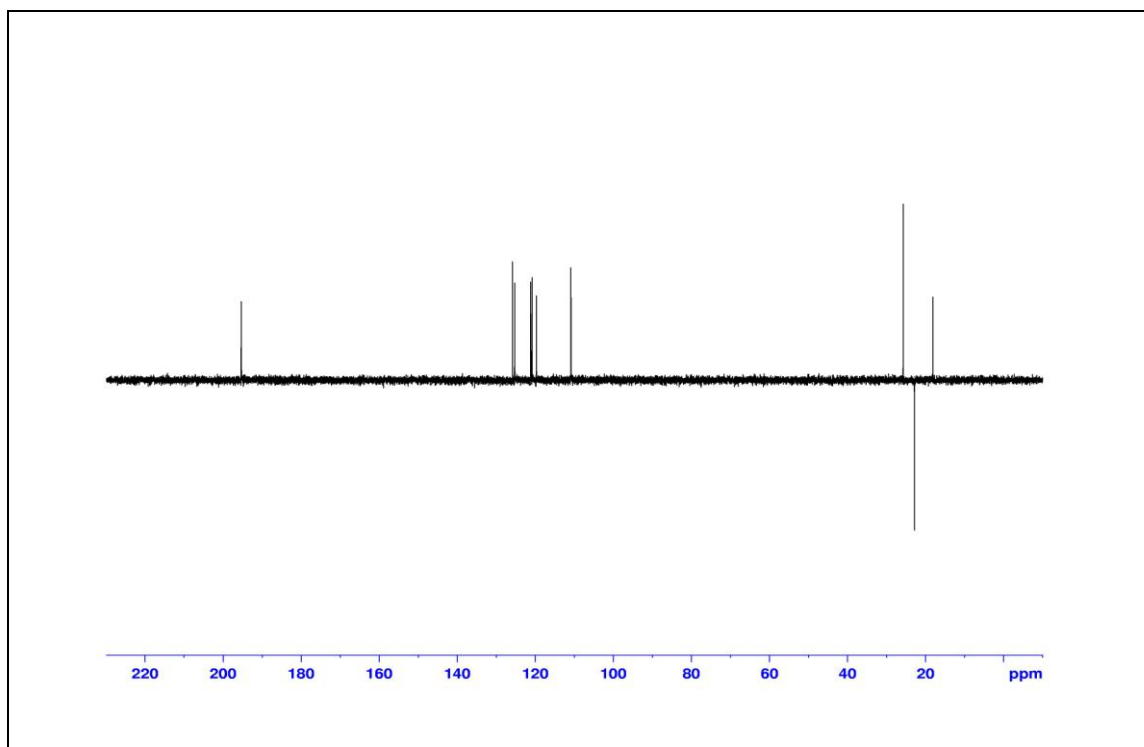


Figure 30 DEPT 135° (CDCl₃) of compound **RM1**

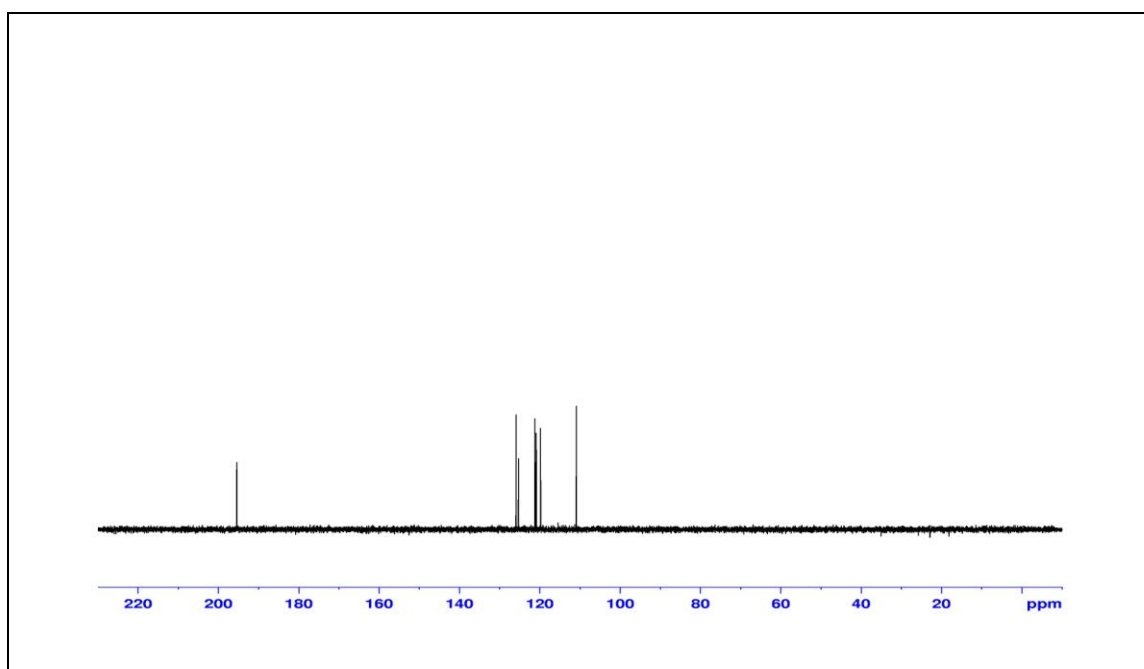


Figure 31 DEPT 90° (CDCl₃) of compound **RM1**

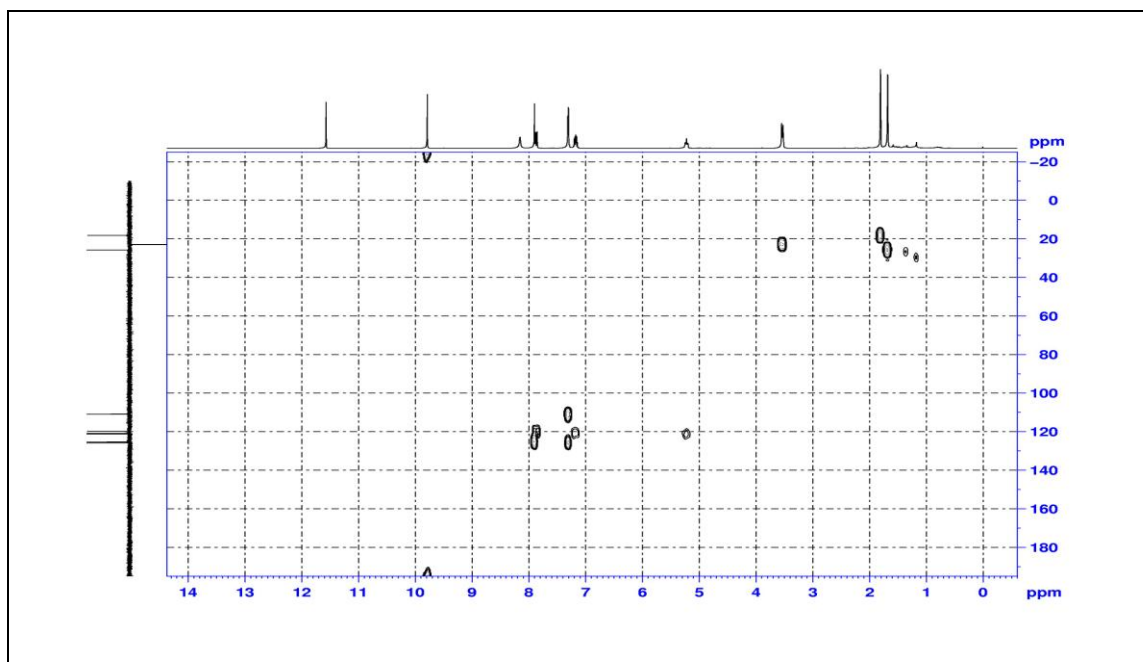


Figure 32 2D HMQC (CDCl₃) of compound **RM1**

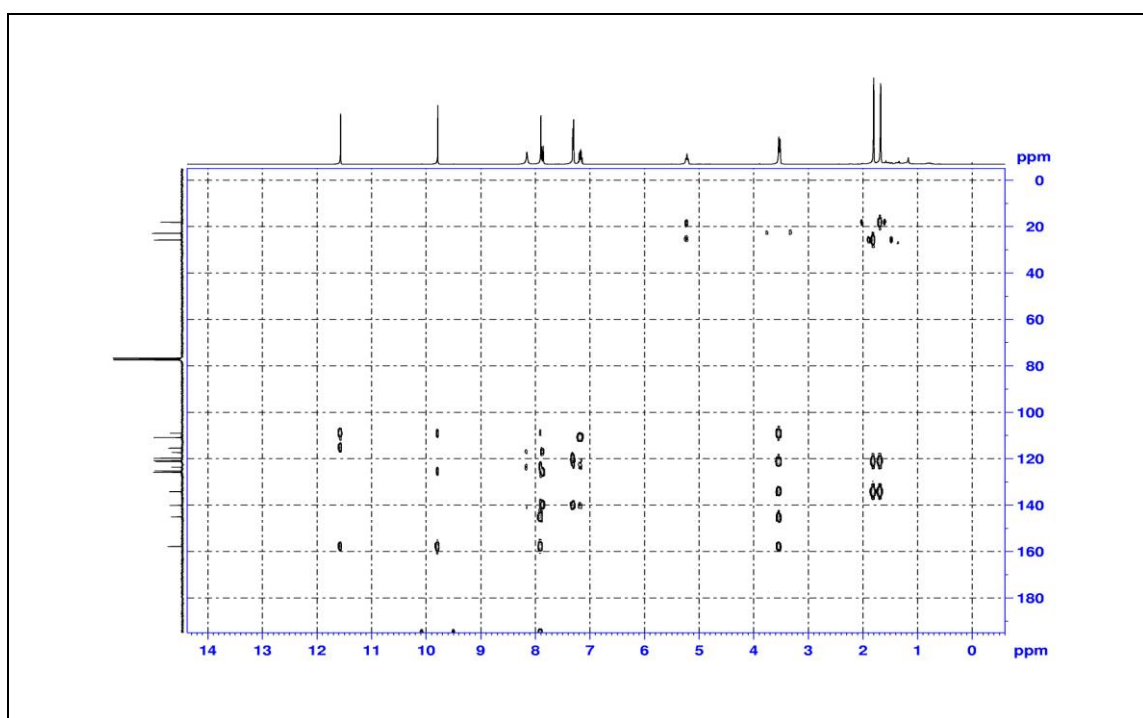


Figure 33 2D HMBC (CDCl₃) of compound **RM1**

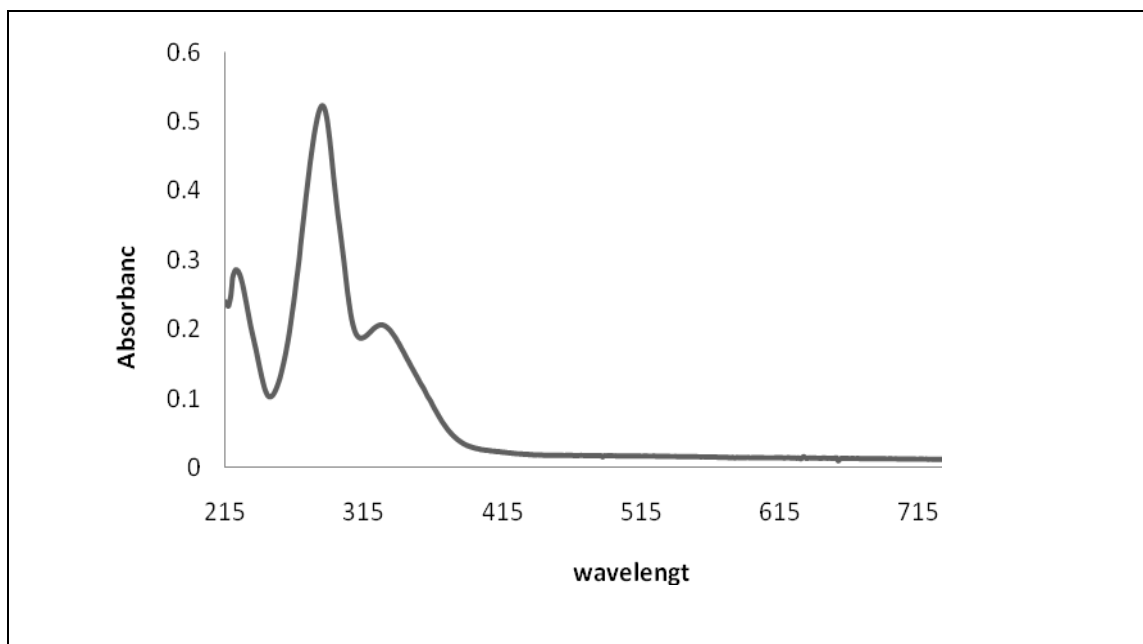


Figure 34 UV (MeOH) spectrum of compound **RM2**

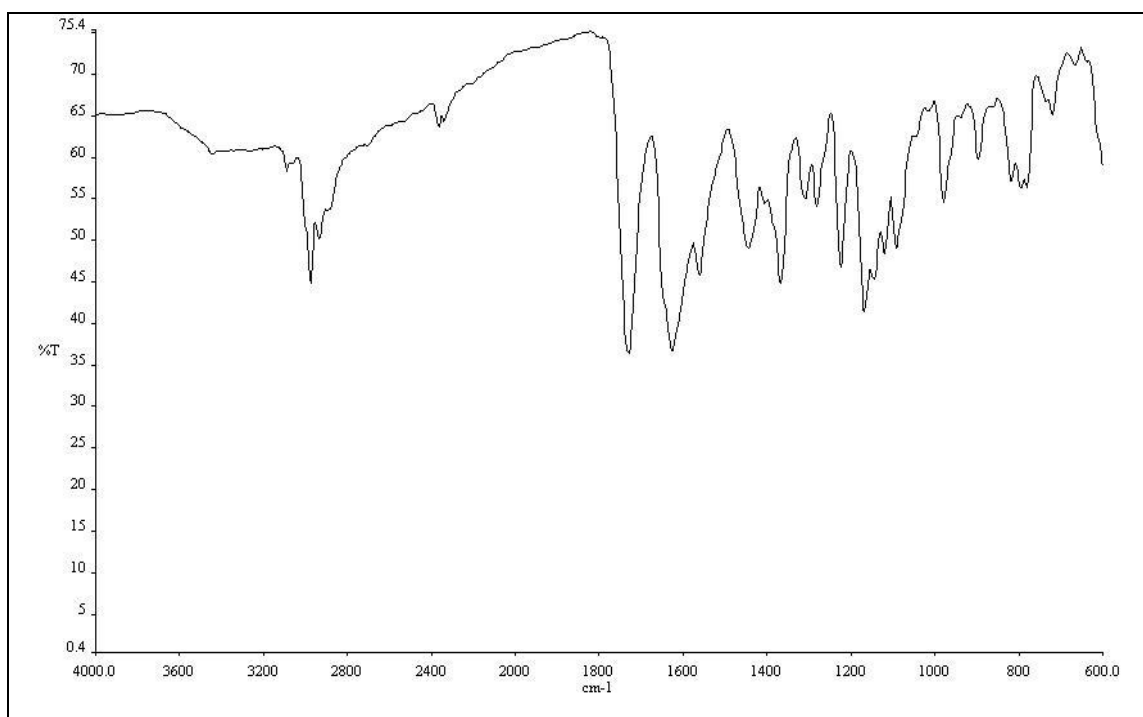


Figure 35 IR (neat) spectrum of compound **RM2**

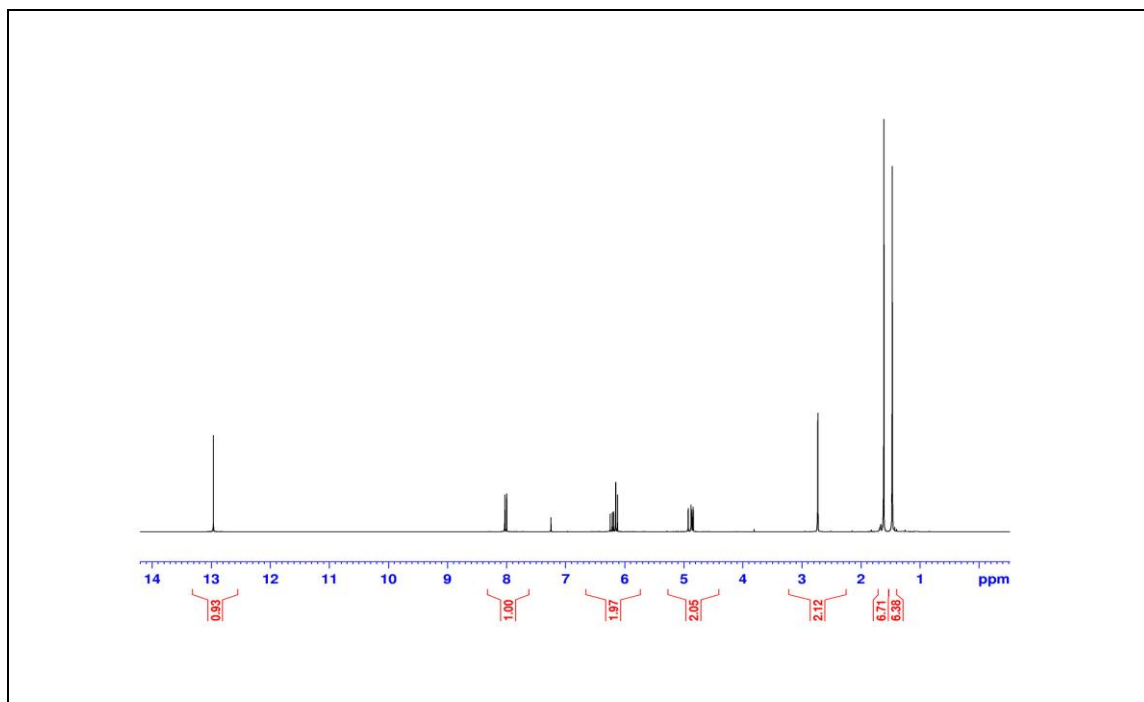


Figure 36 ^1H NMR (300 MHz) (CDCl_3) of compound **RM2**

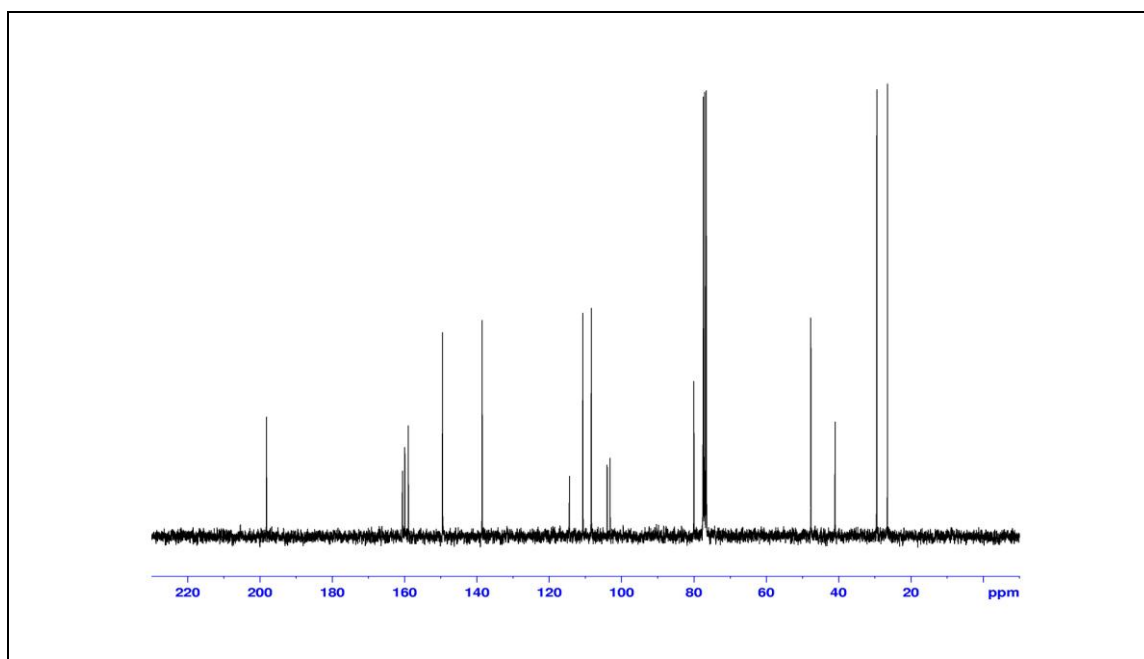


Figure 37 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM2**

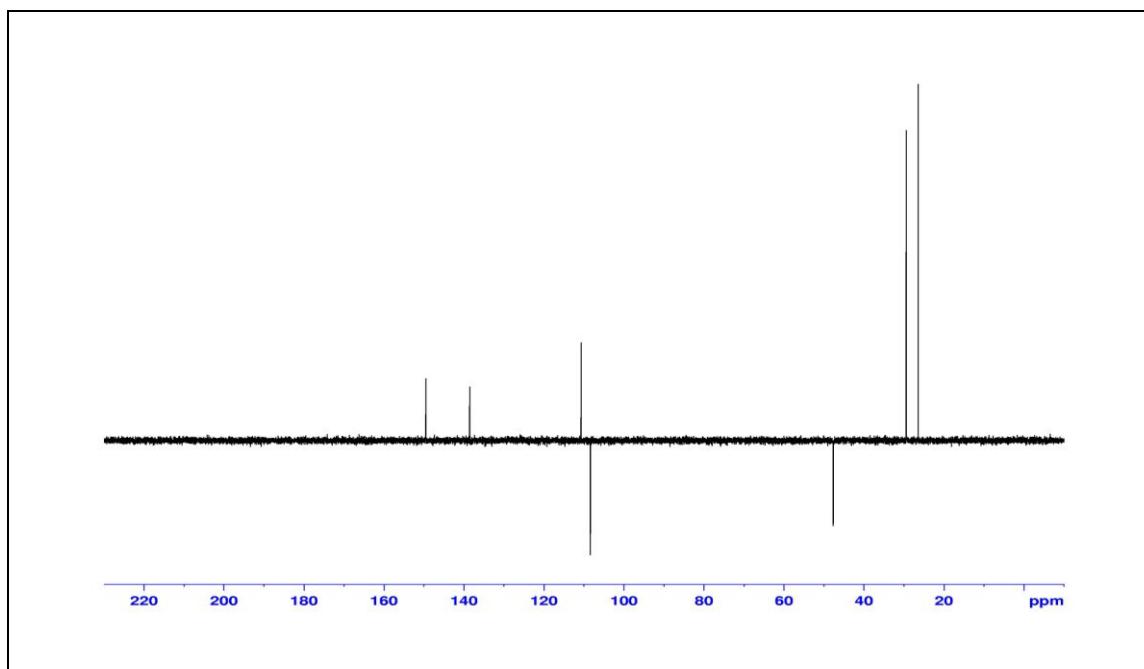


Figure 38 DEPT 135° (CDCl₃) of compound **RM2**

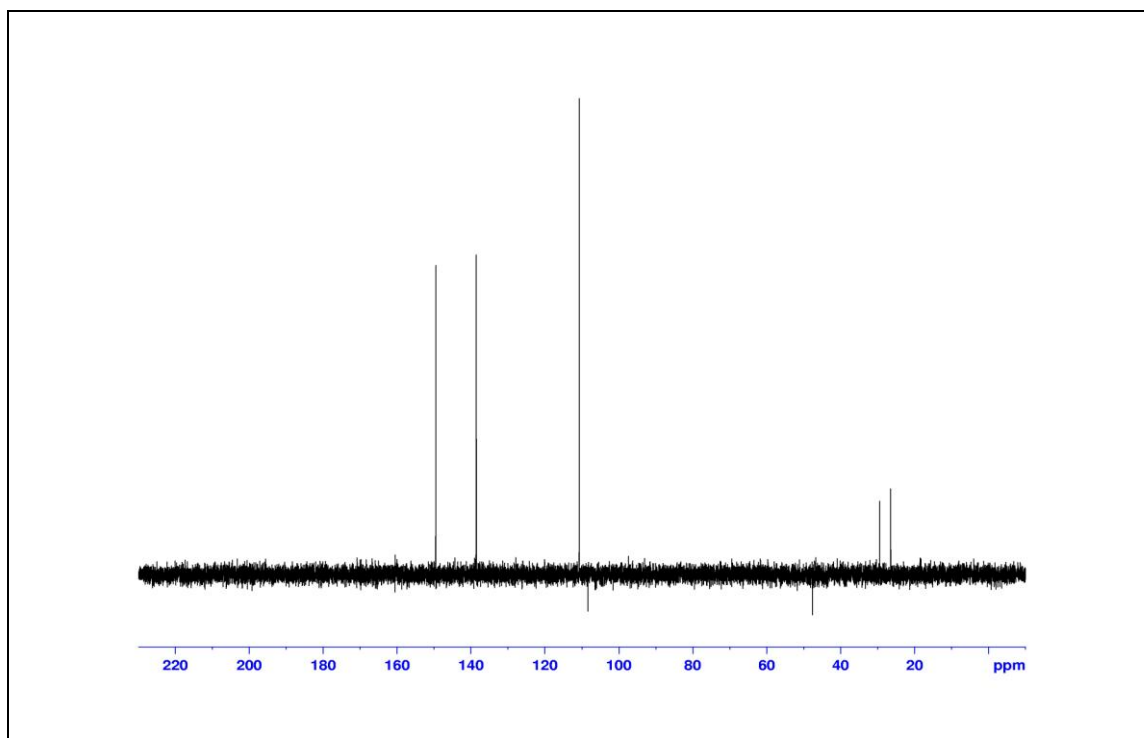


Figure 39 DEPT 90° (CDCl₃) of compound **RM2**

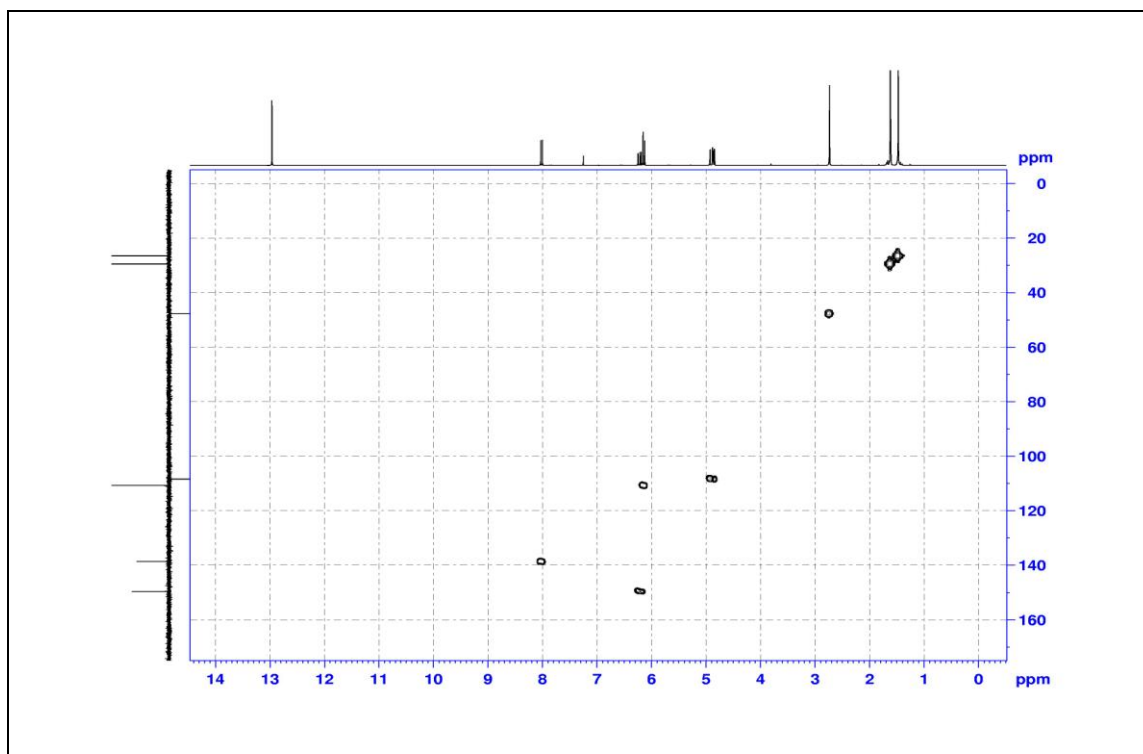


Figure 40 2D HMQC (CDCl₃) of compound **RM2**

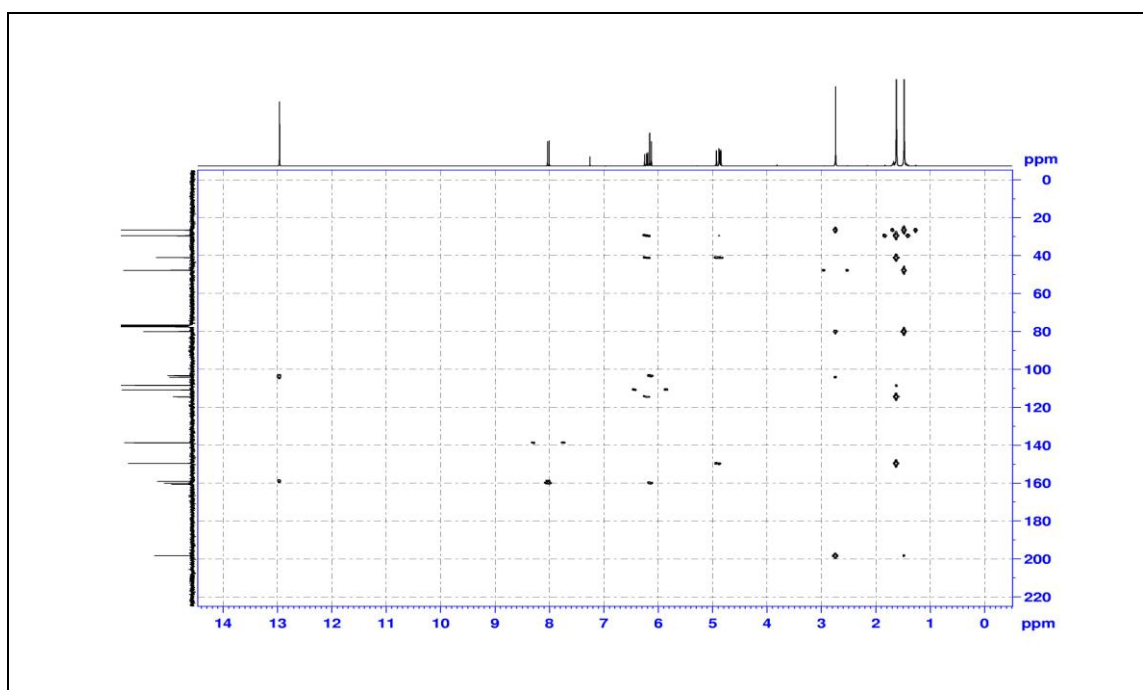


Figure 41 2D HMBC (CDCl₃) of compound **RM2**

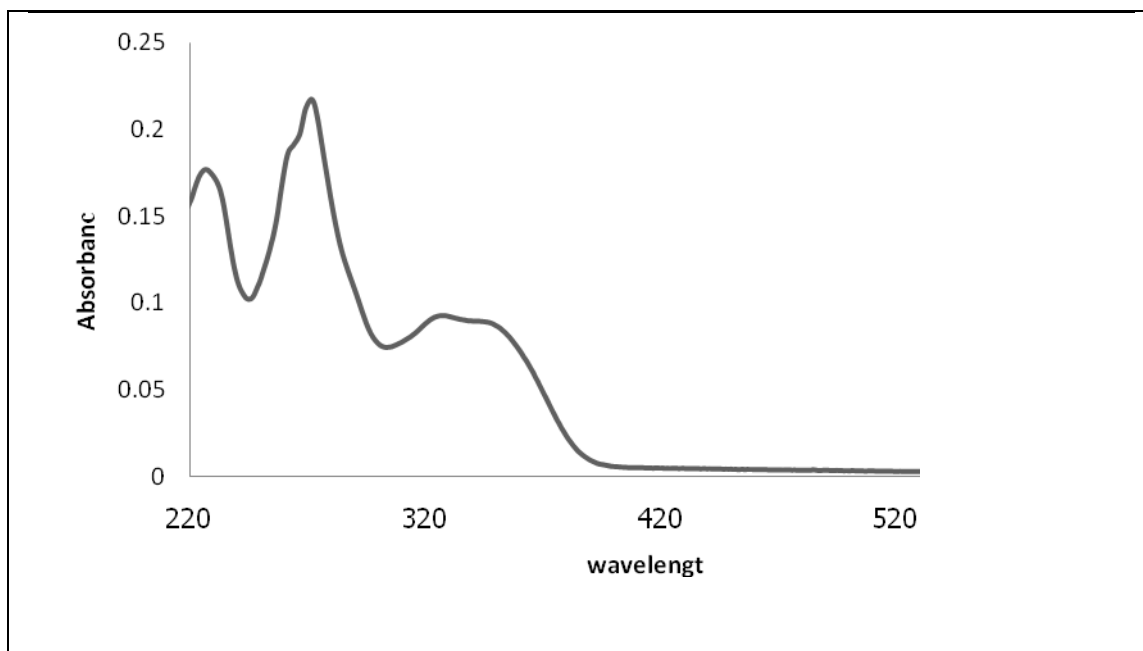


Figure 42 UV (MeOH) spectrum of compound **RM3**

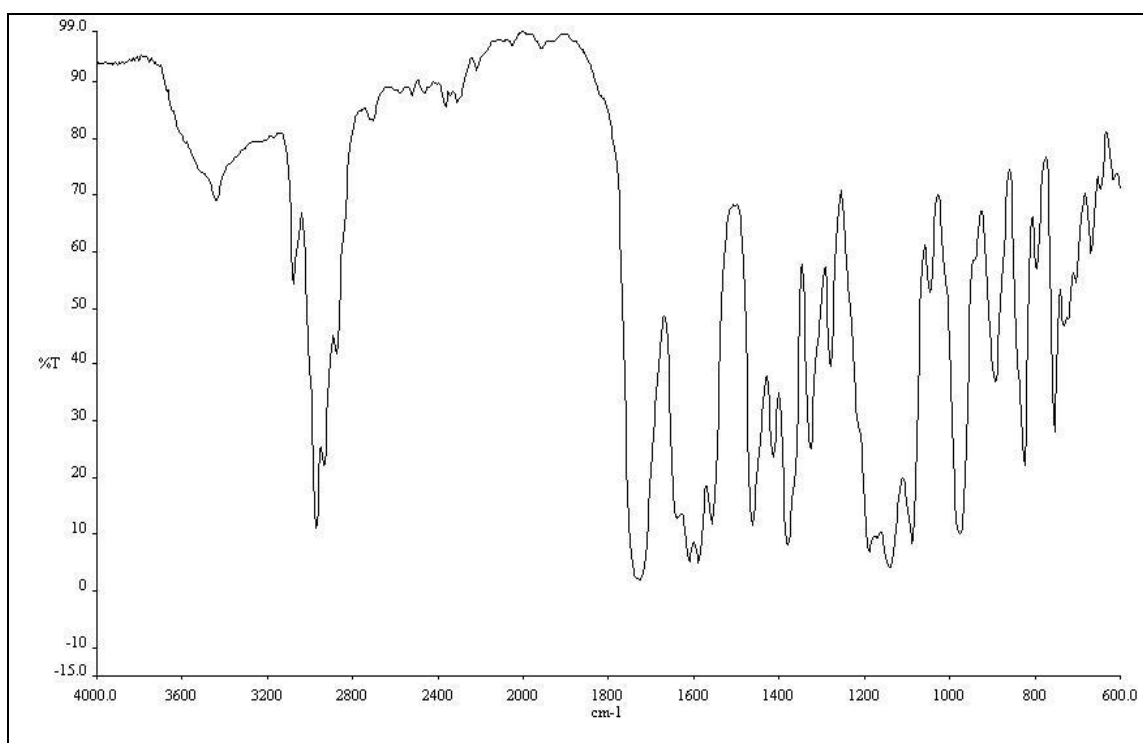


Figure 43 IR (neat) spectrum of compound **RM3**

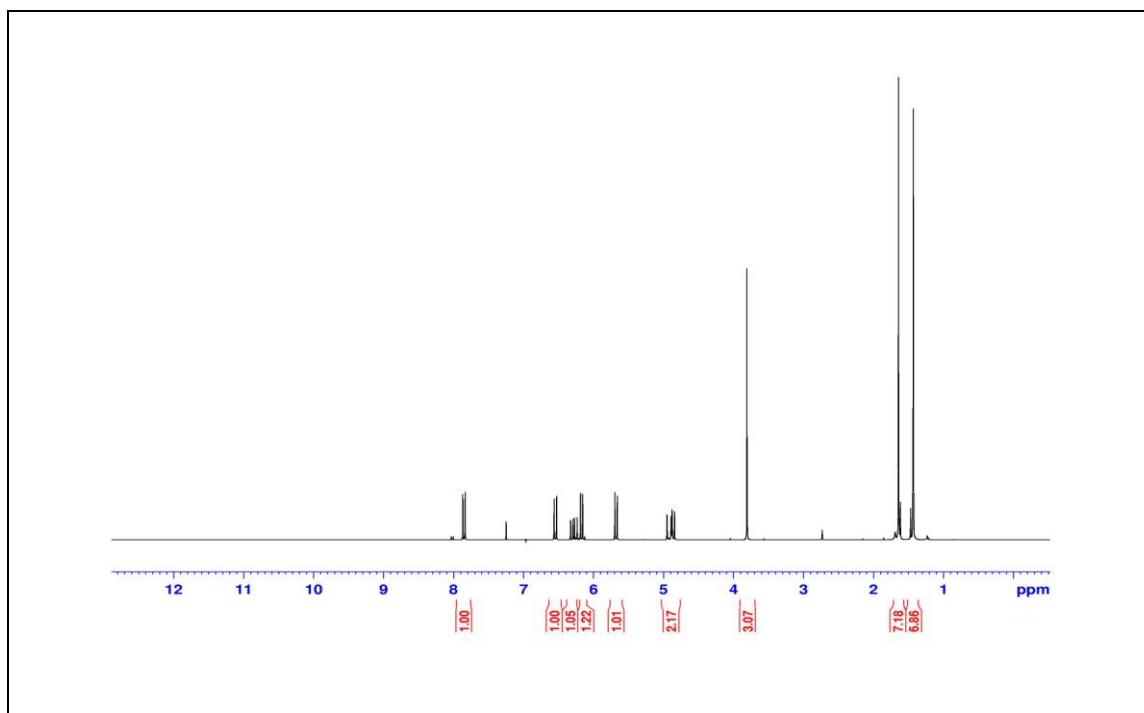


Figure 44 ^1H NMR (300 MHz) (CDCl_3) of compound **RM3**

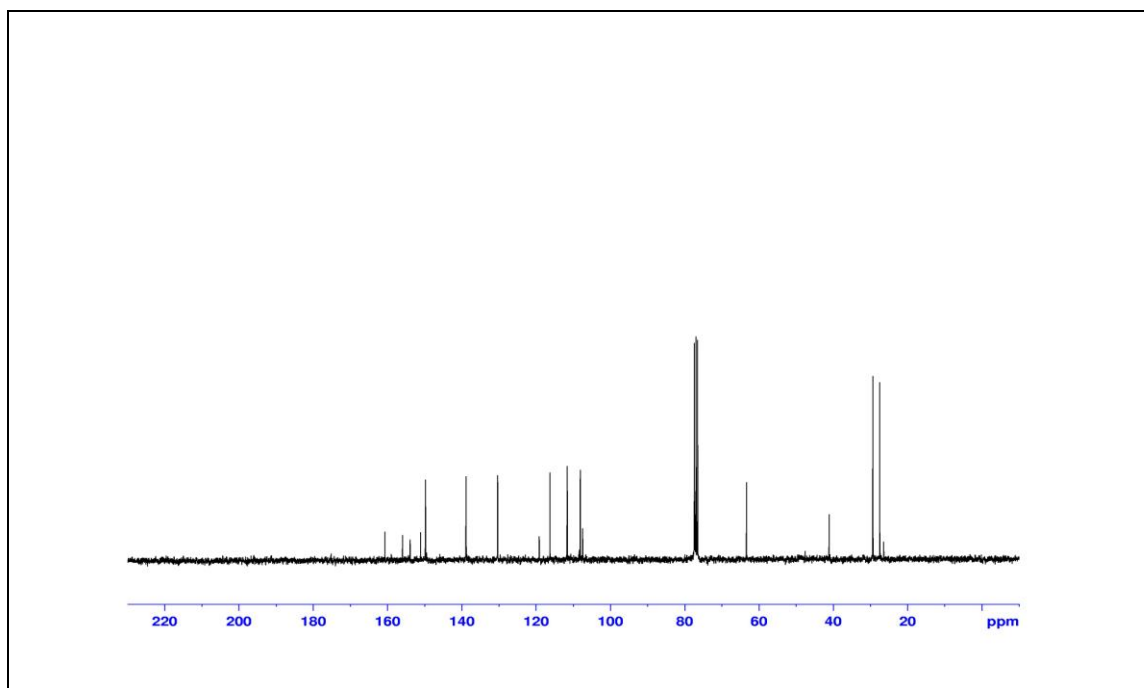


Figure 45 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM3**

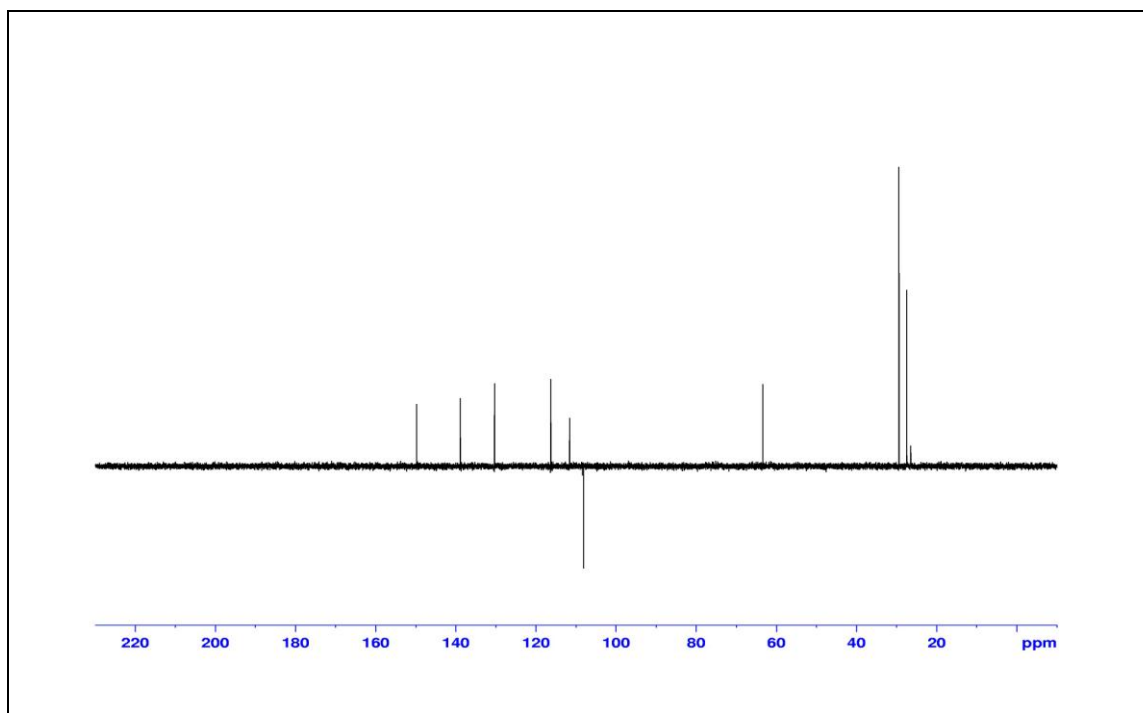


Figure 46 DEPT 135° (CDCl₃) of compound **RM3**

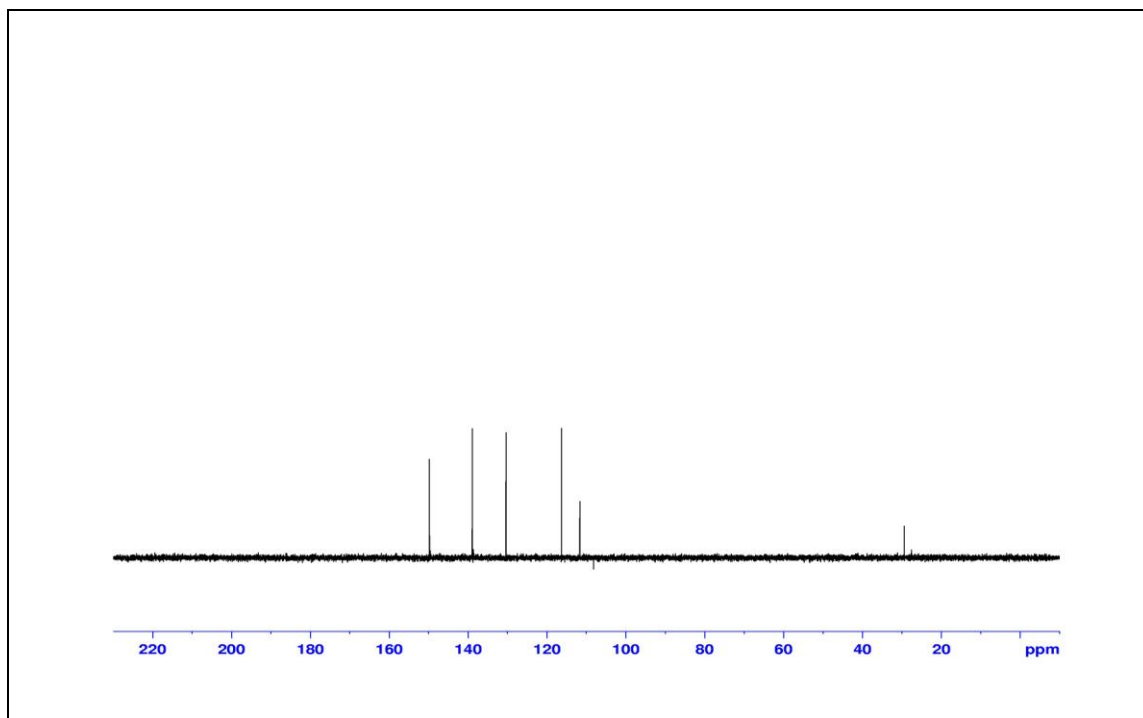


Figure 47 DEPT 90° (CDCl₃) of compound **RM3**

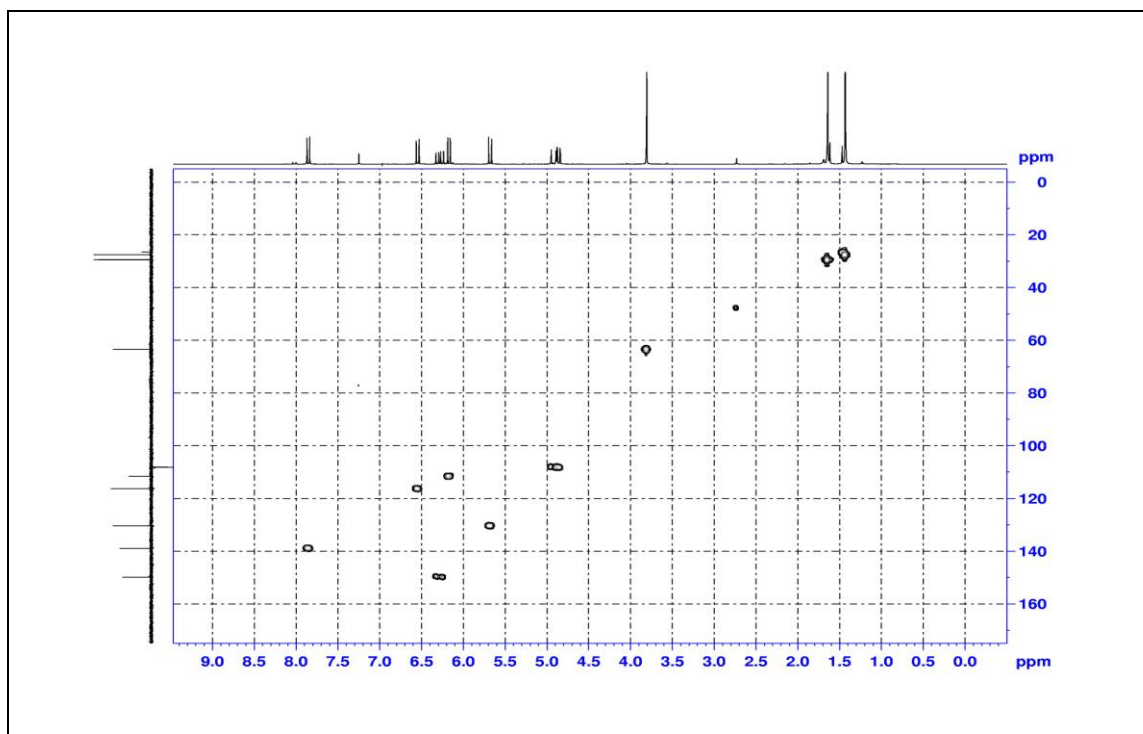


Figure 48 2D HMQC (CDCl_3) of compound **RM3**

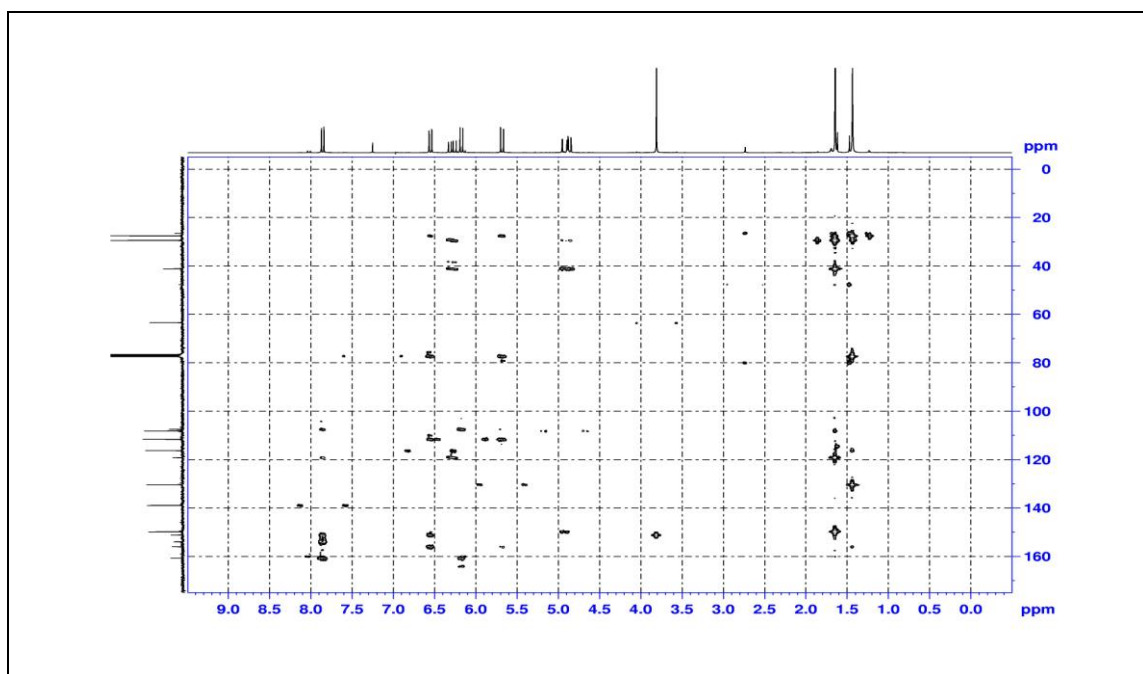


Figure 49 2D HMBC (CDCl_3) of compound **RM3**

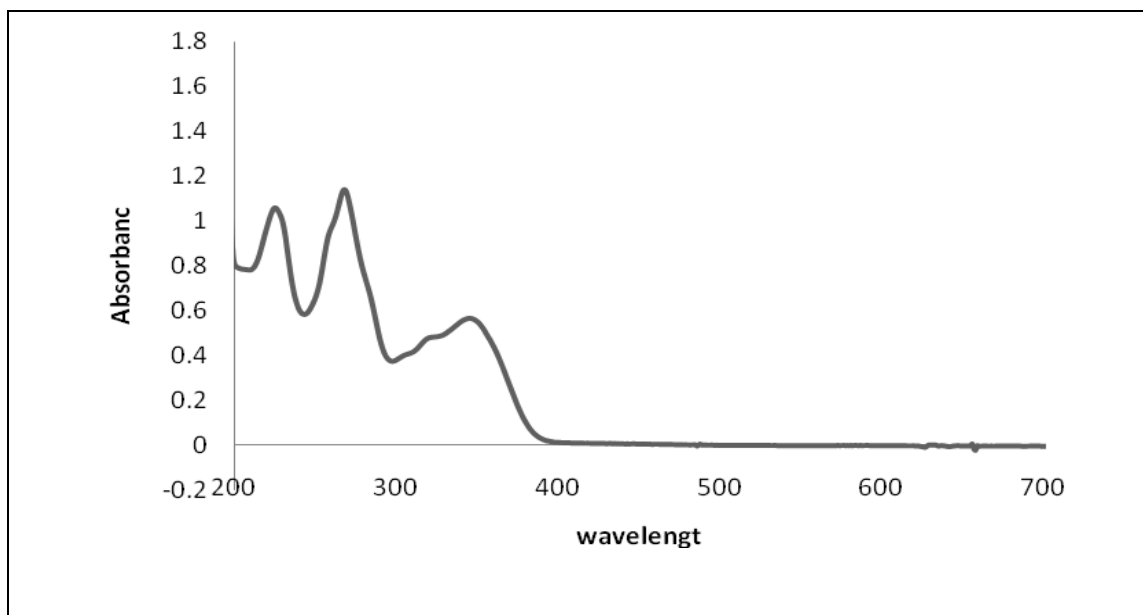


Figure 50 UV (MeOH) spectrum of compound **RM4**

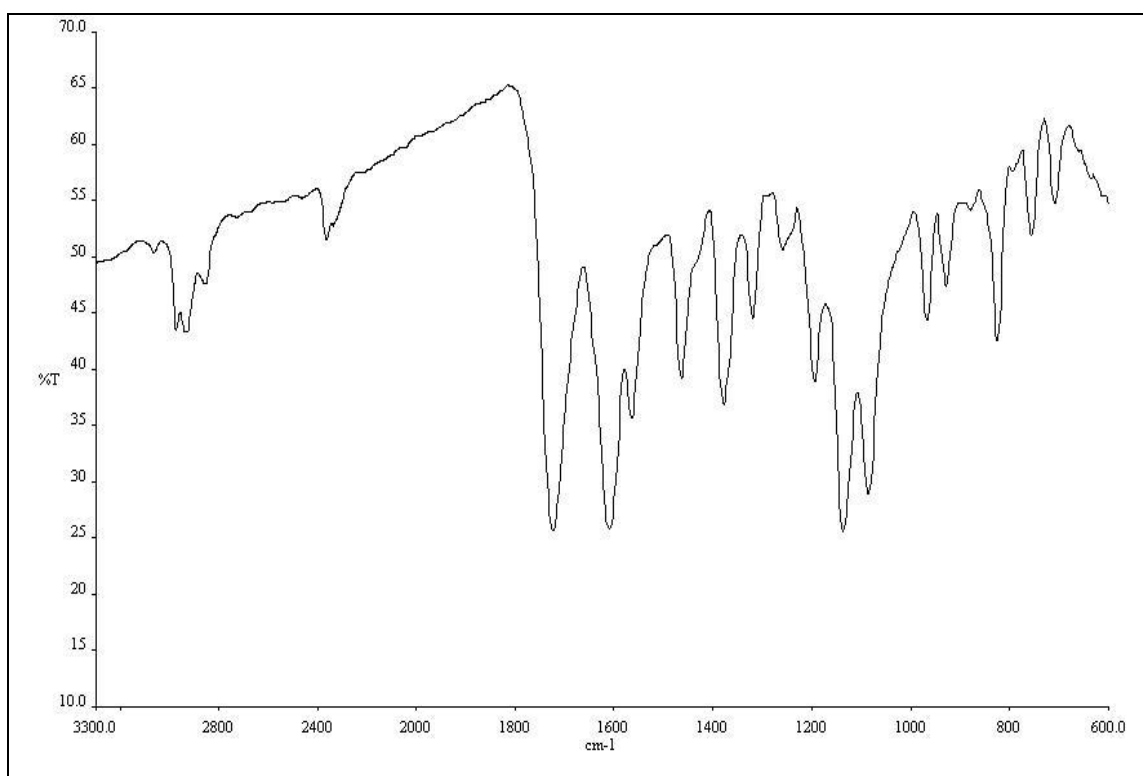


Figure 51 IR (neat) spectrum of compound **RM4**

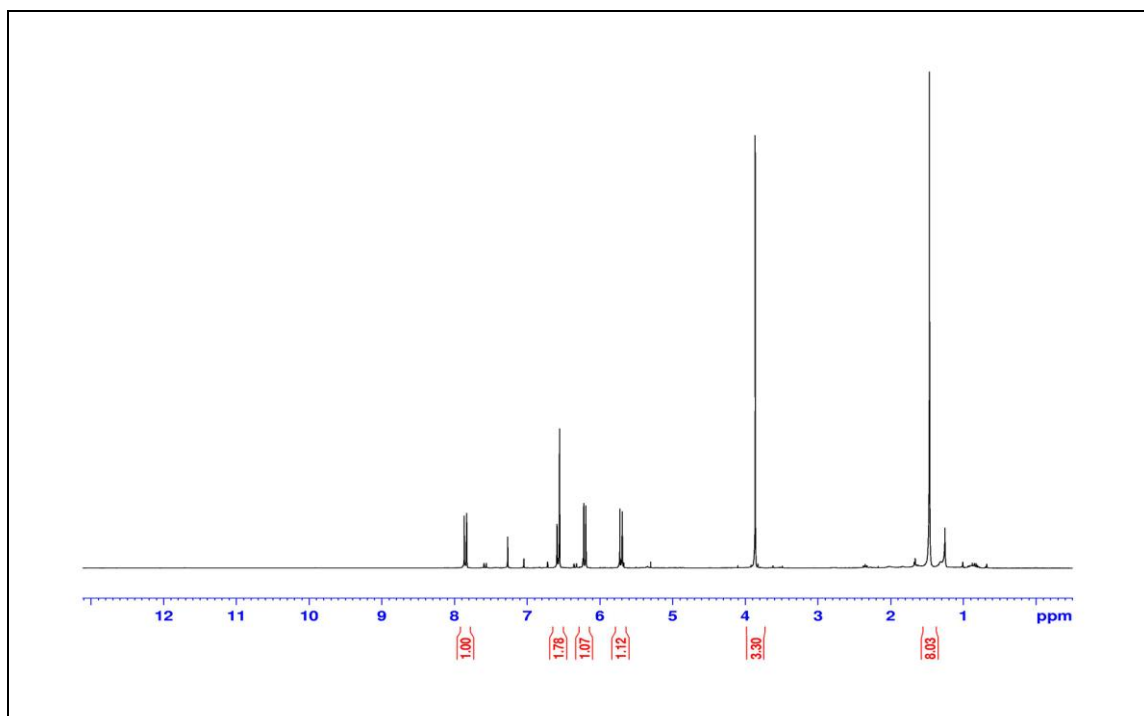


Figure 52 ^1H NMR (300 MHz) (CDCl_3) of compound **RM4**

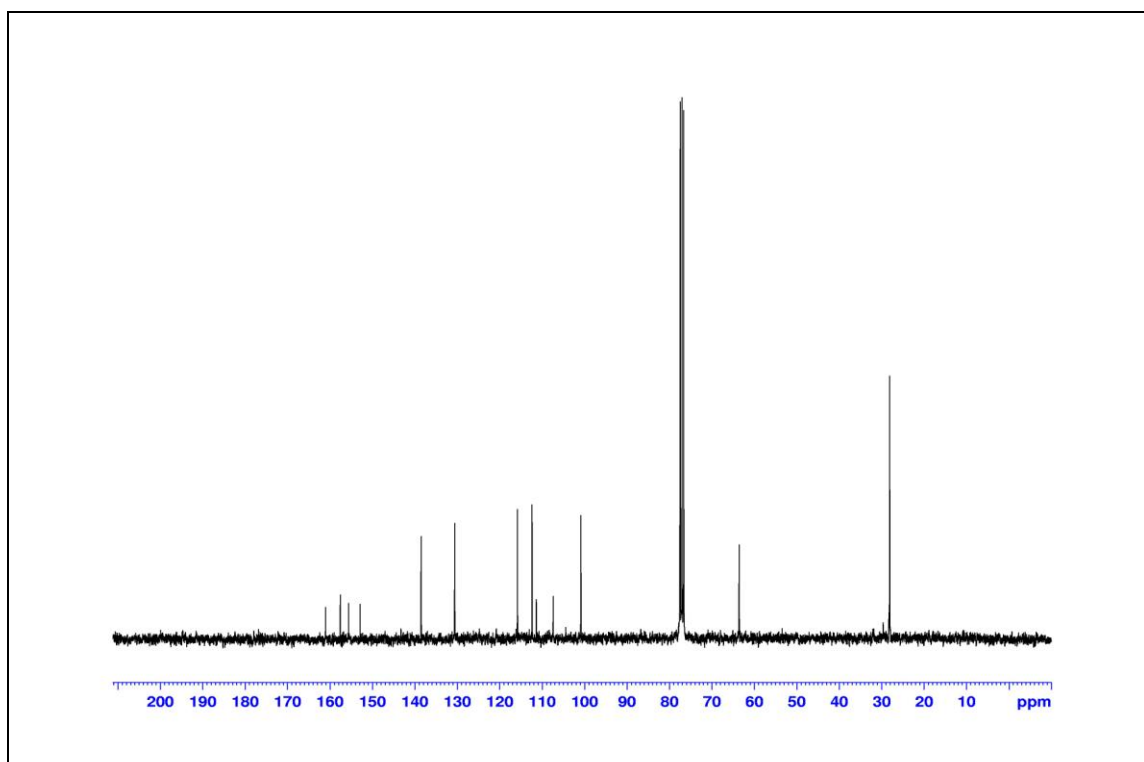


Figure 53 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM4**

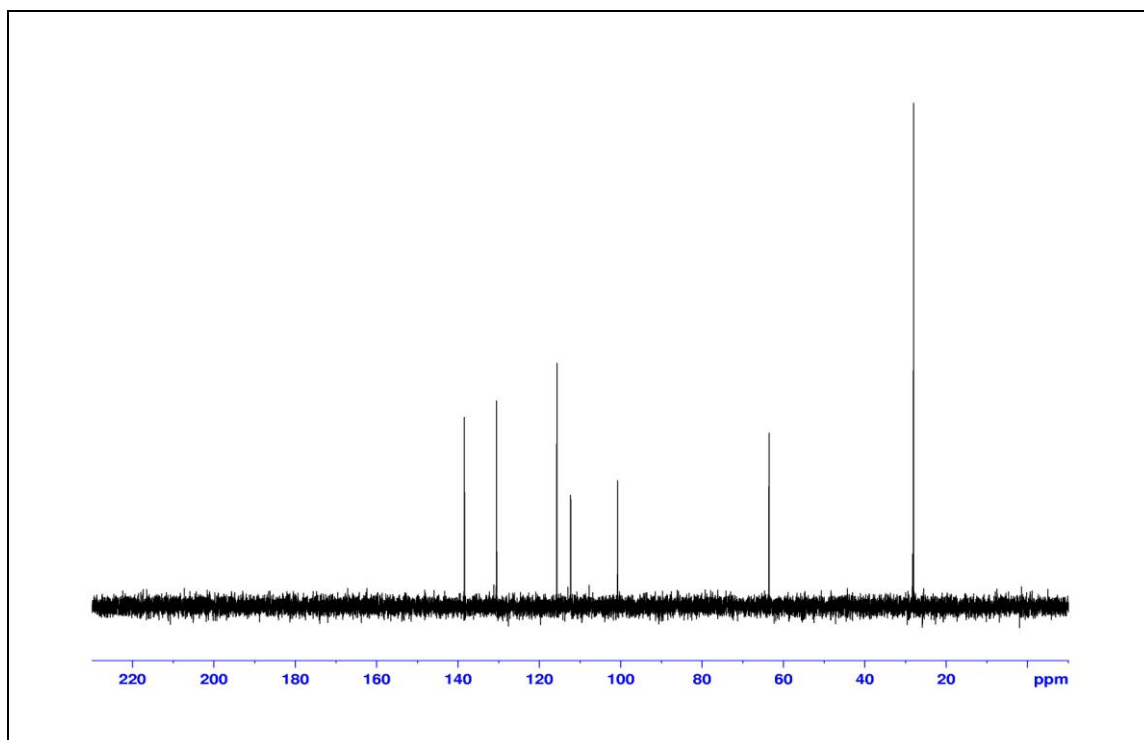


Figure 54 DEPT 135° (CDCl₃) of compound **RM4**

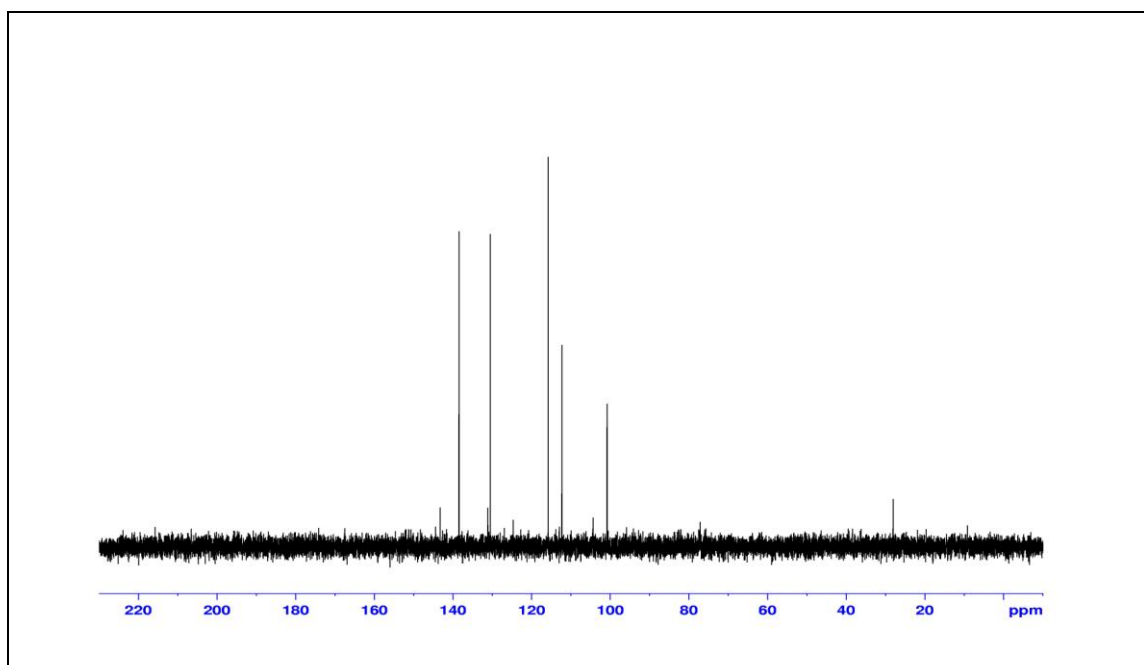


Figure 55 DEPT 90° (CDCl₃) of compound **RM4**

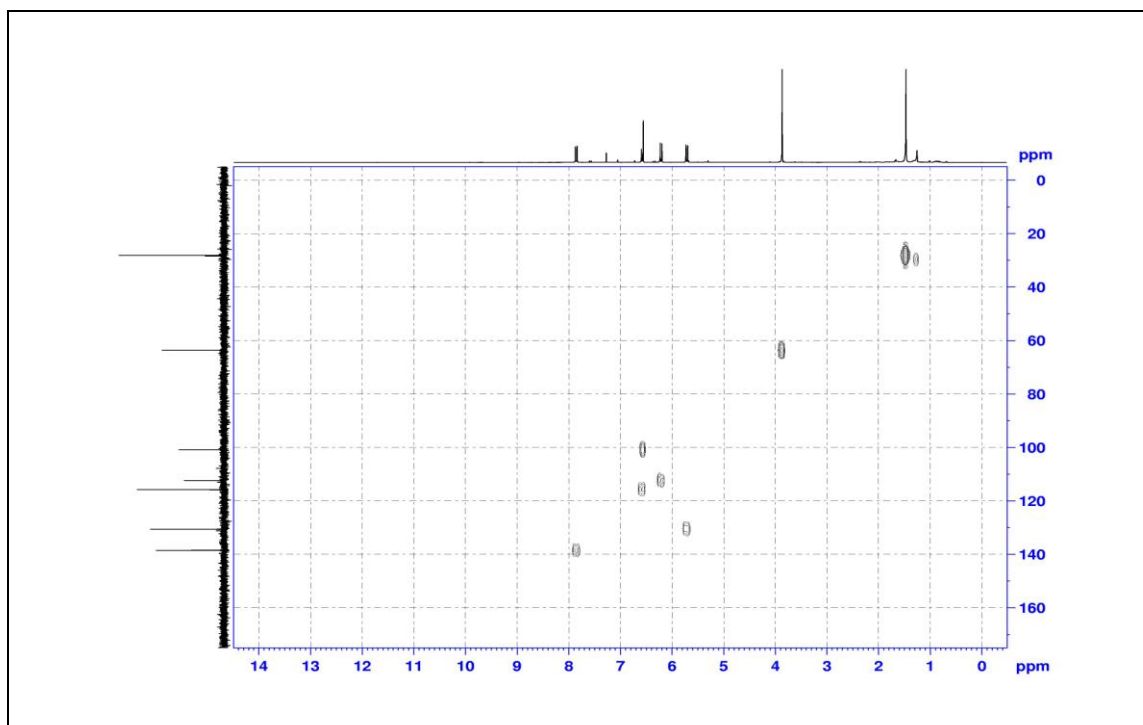


Figure 56 2D HMQC (CDCl_3) of compound **RM4**

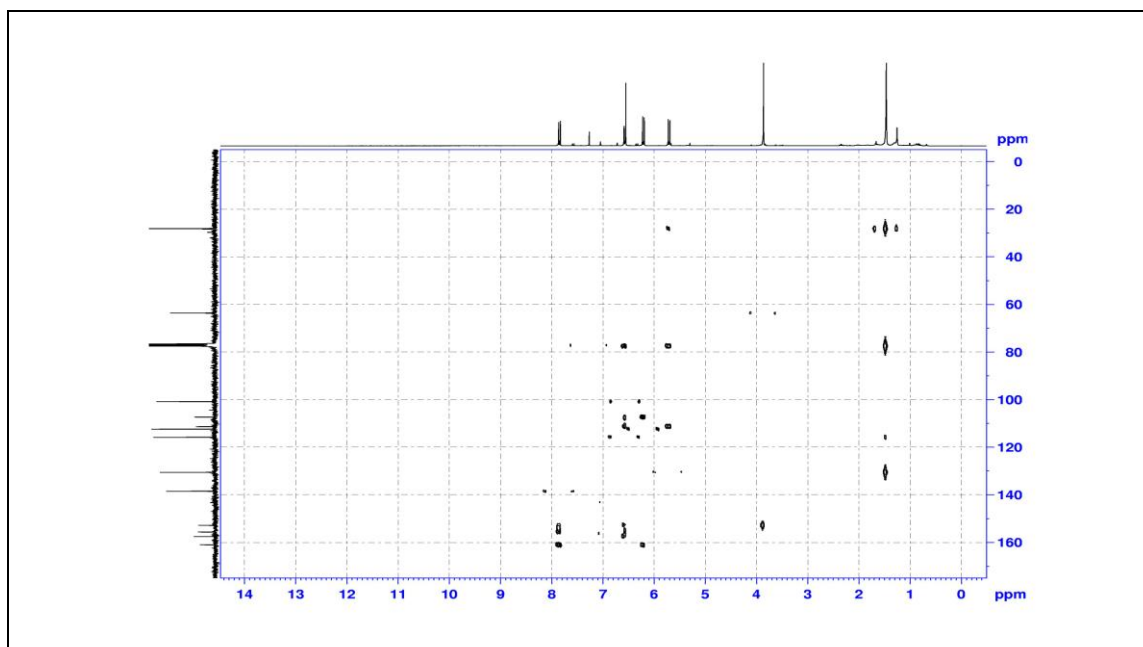


Figure 57 2D HMBC (CDCl_3) of compound **RM4**

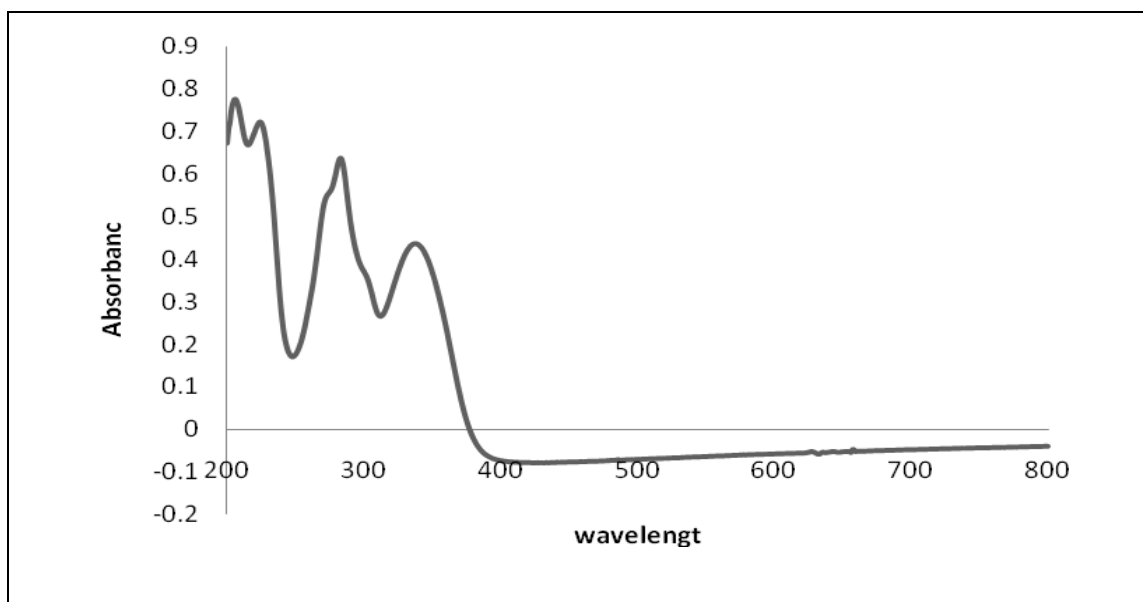


Figure 58 UV (MeOH) spectrum of compound **RM5**

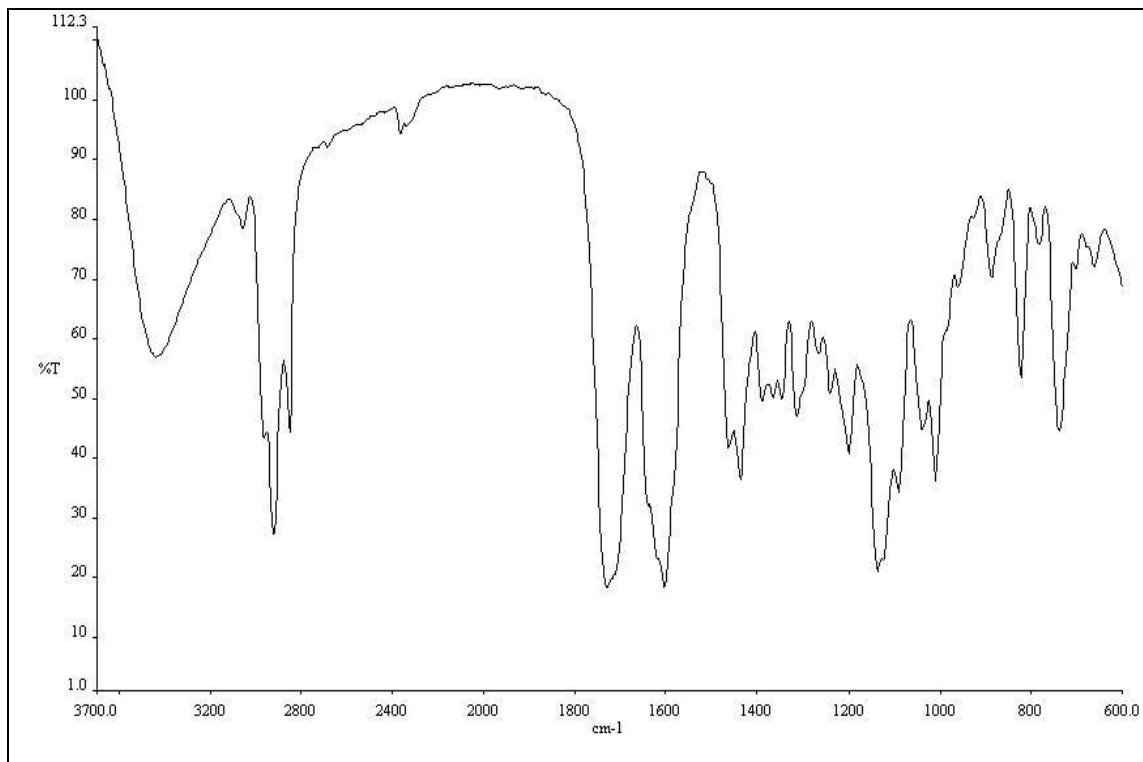


Figure 59 IR (neat) spectrum of compound **RM5**

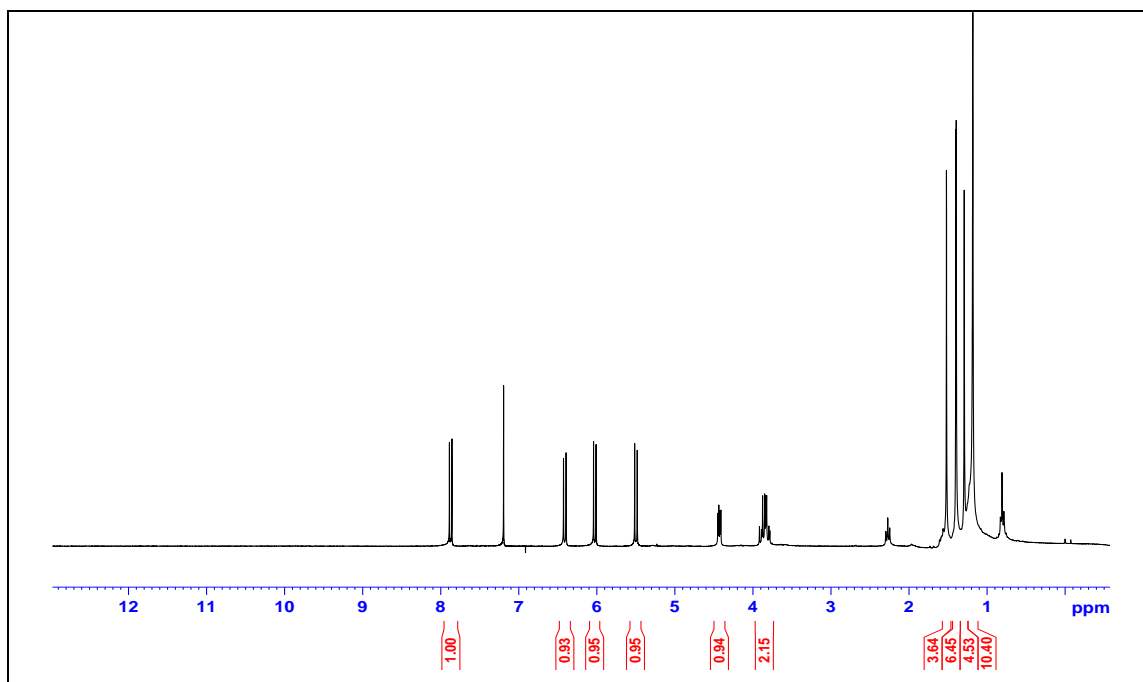


Figure 60 ^1H NMR (300 MHz) (CDCl_3) of compound **RM5**

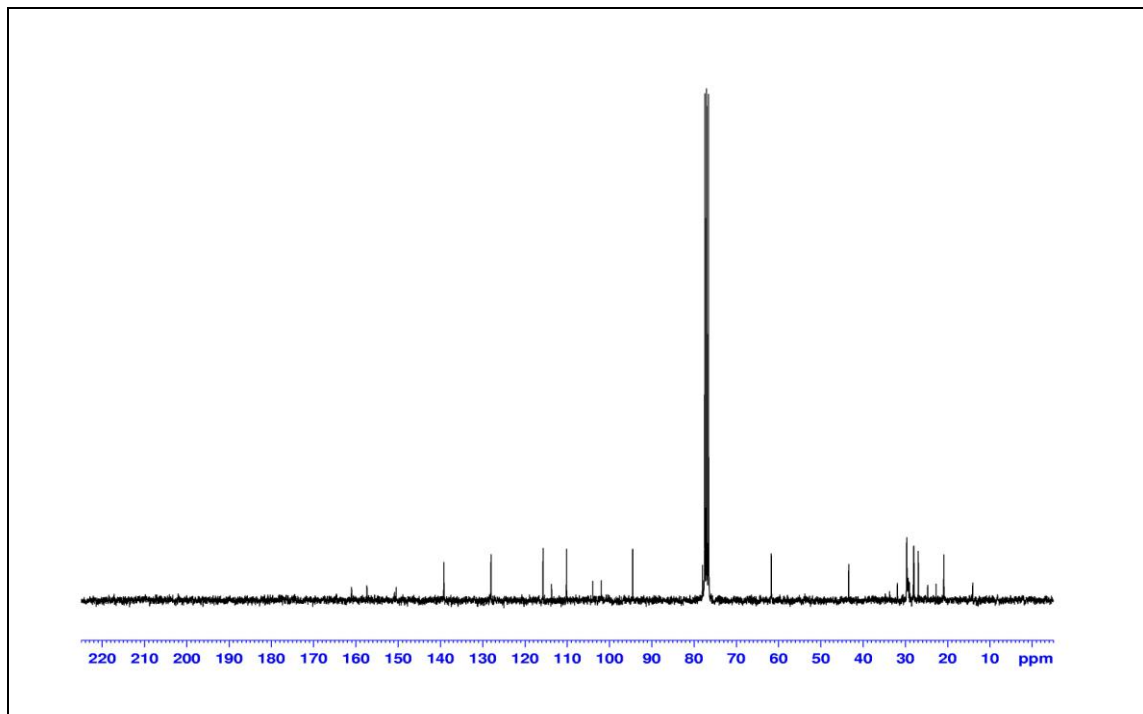


Figure 61 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM5**

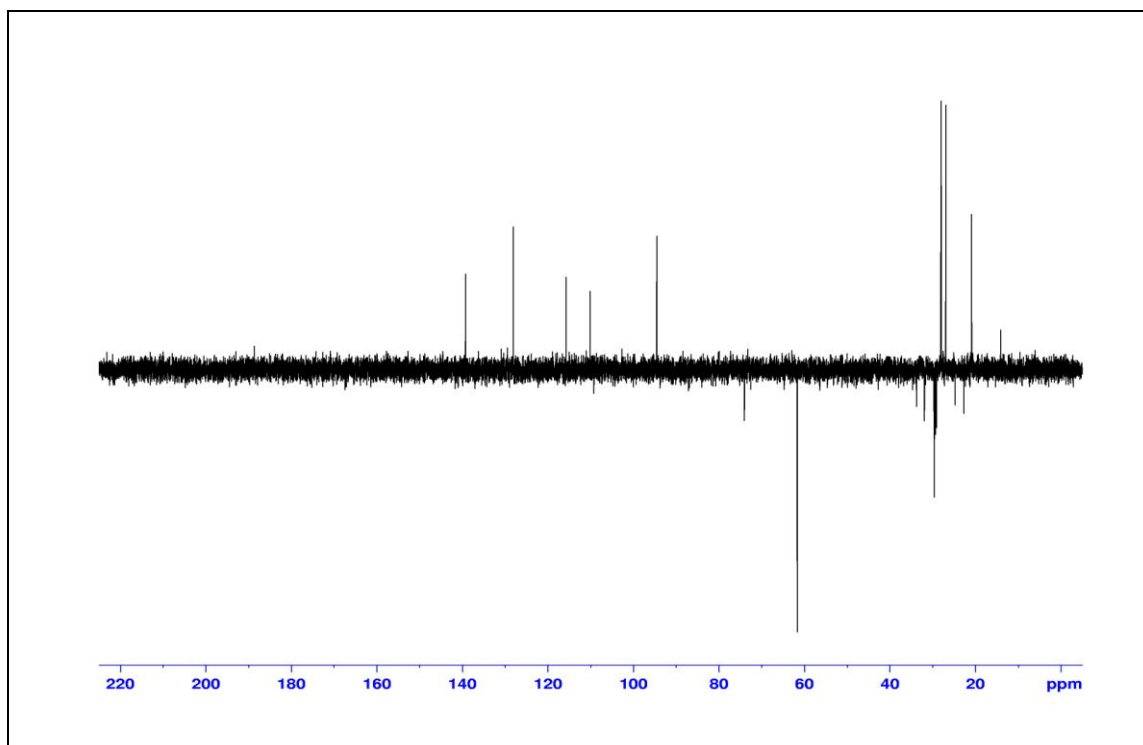


Figure 62 DEPT 135° (CDCl₃) of compound **RM5**

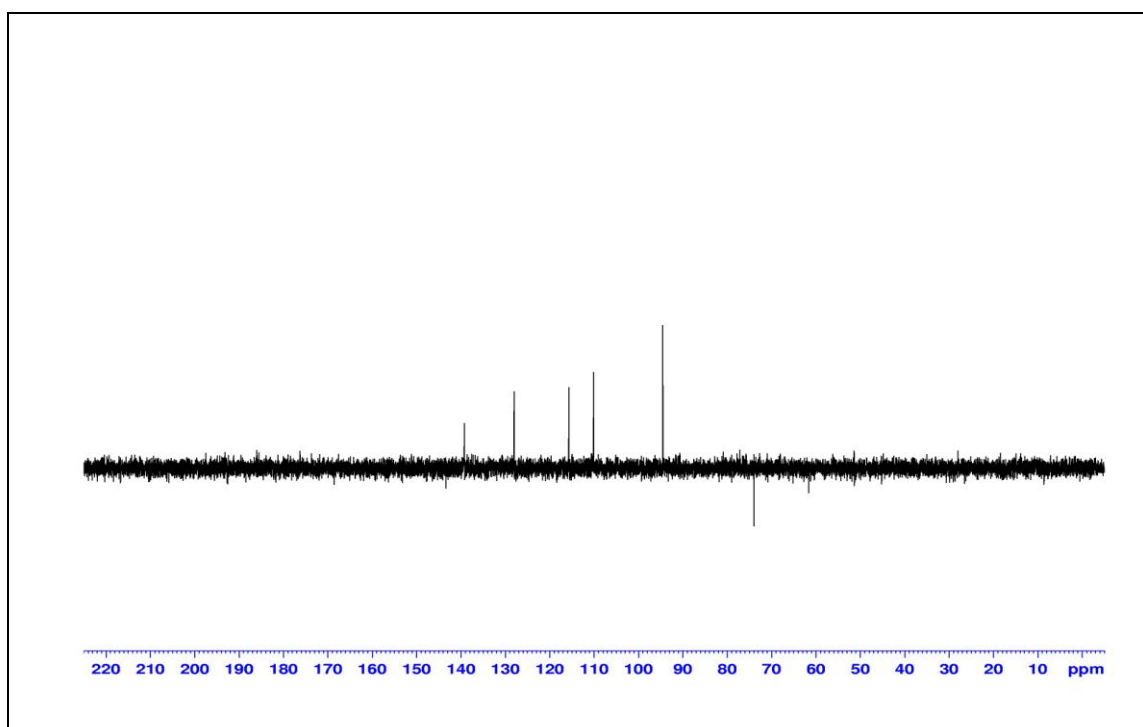


Figure 63 DEPT 90° (CDCl₃) of compound **RM5**

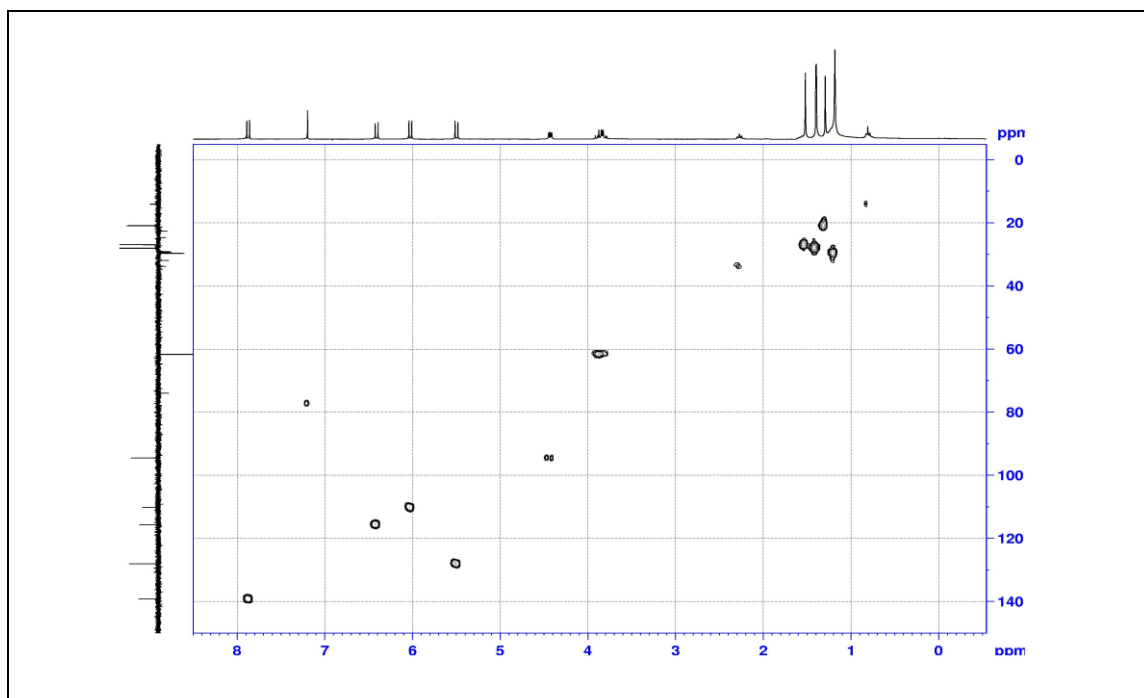


Figure 64 2D HMQC (CDCl_3) of compound **RM5**

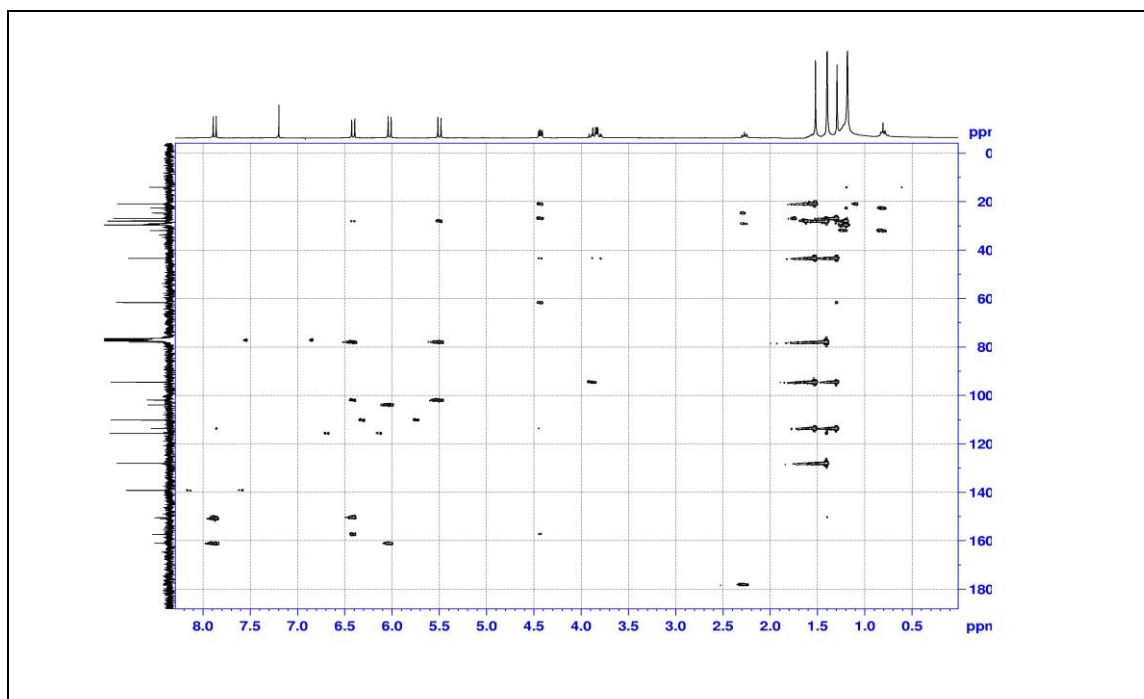


Figure 65 2D HMBC (CDCl_3) of compound **RM5**

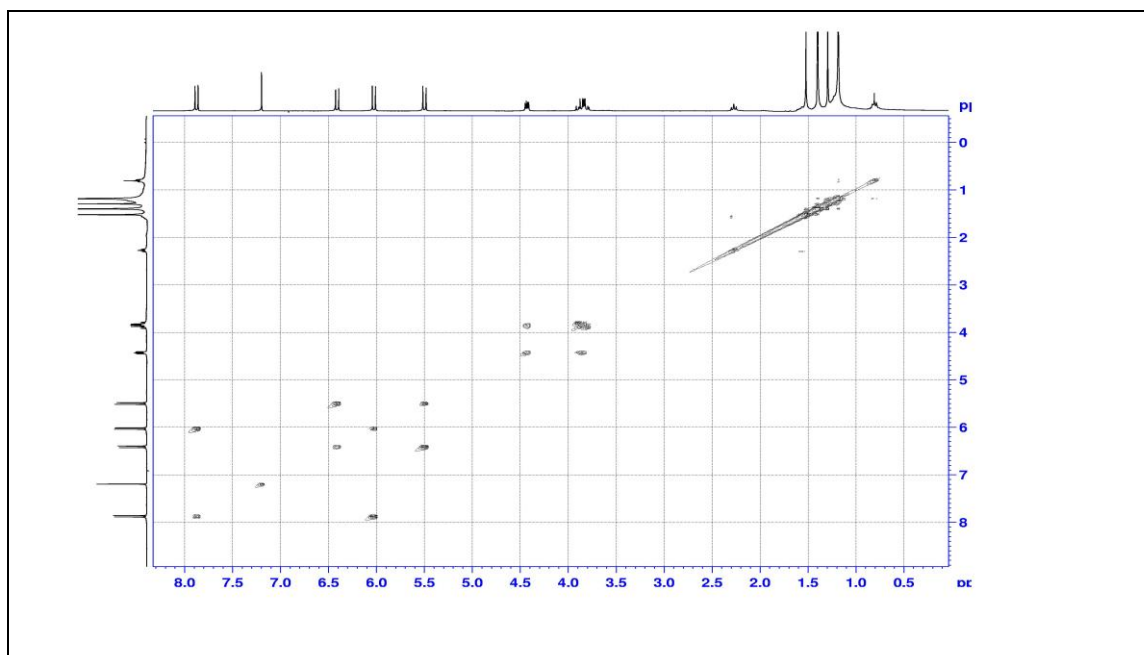


Figure 66 COSY (CDCl₃) of compound **RM5**

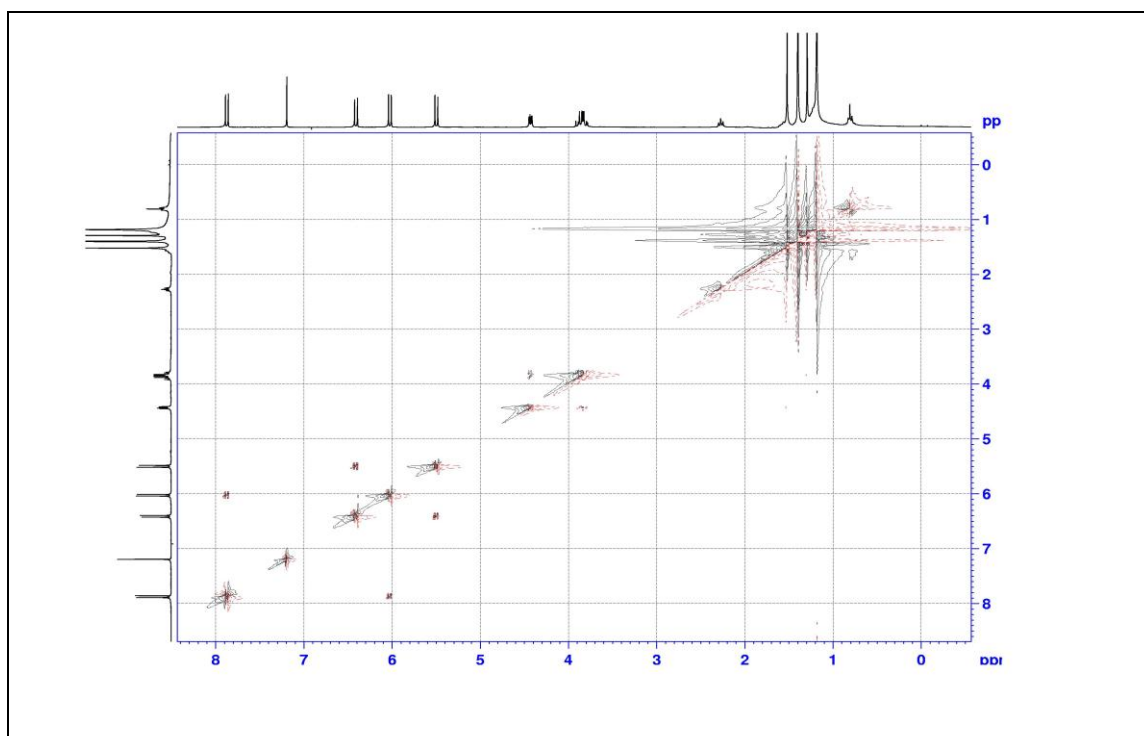


Figure 67 NOESY (CDCl₃) of compound **RM5**

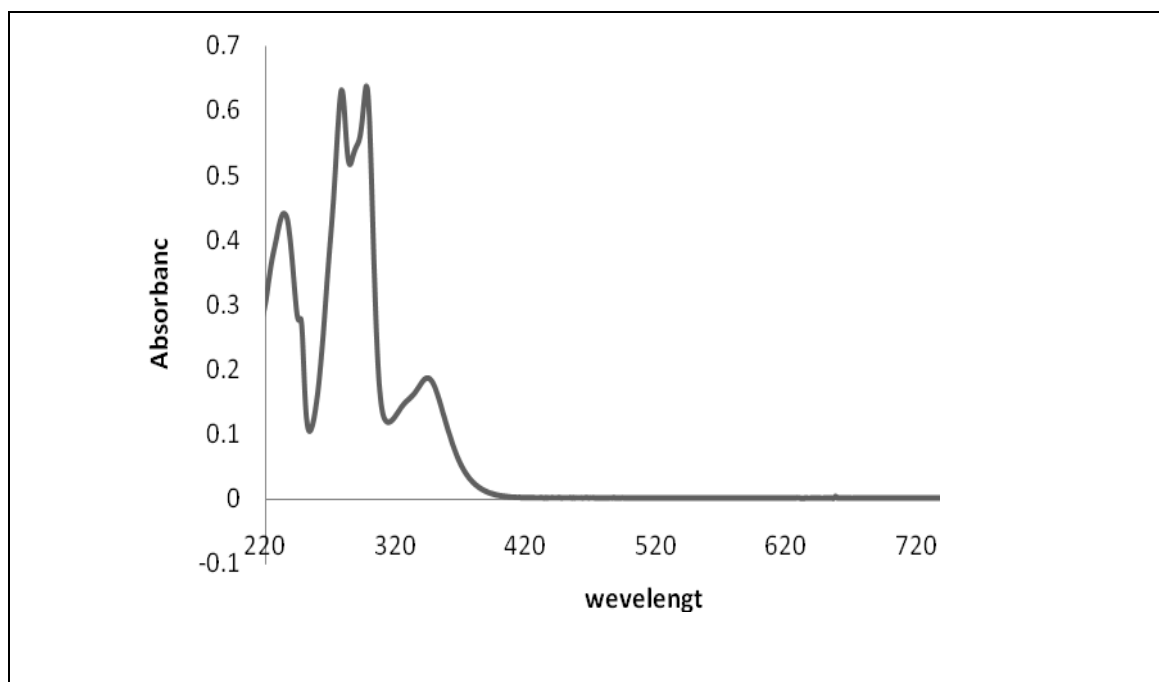


Figure 68 UV (MeOH) spectrum of compound **RM6**

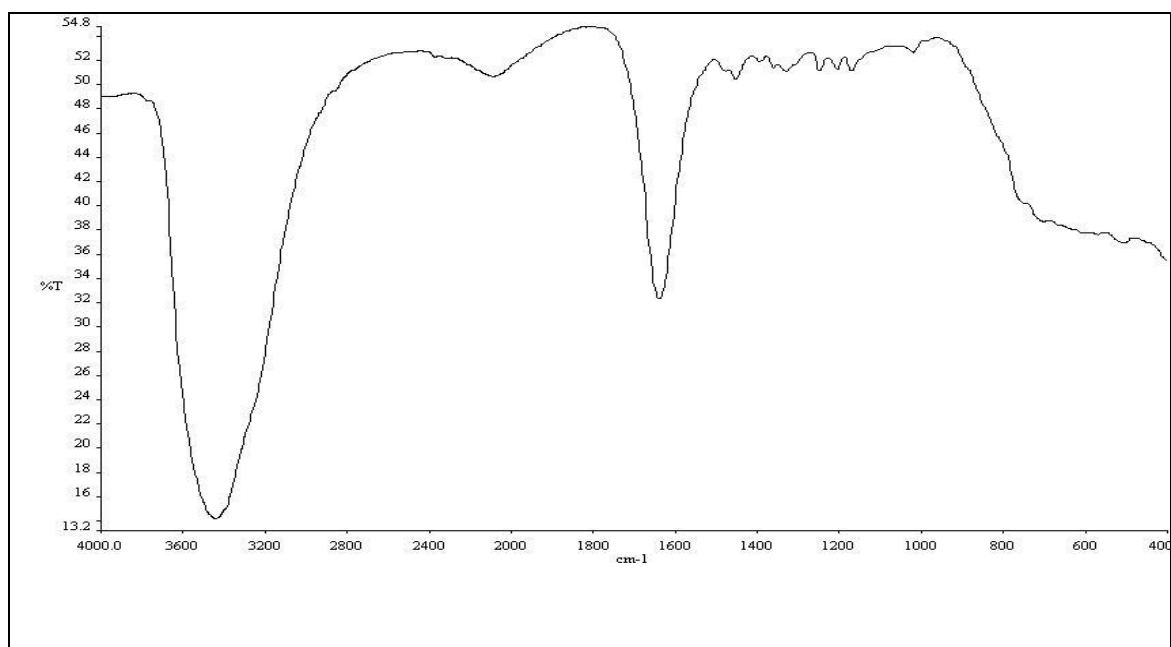


Figure 69 IR (neat) spectrum of compound **RM6**

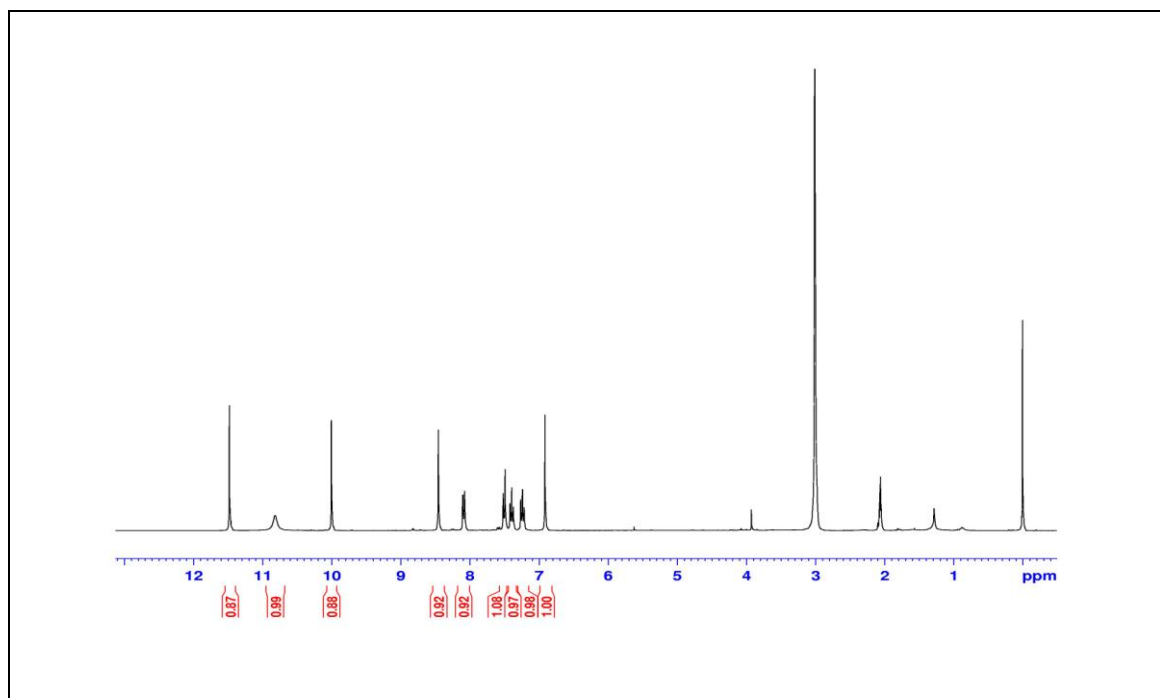


Figure 70 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM6**

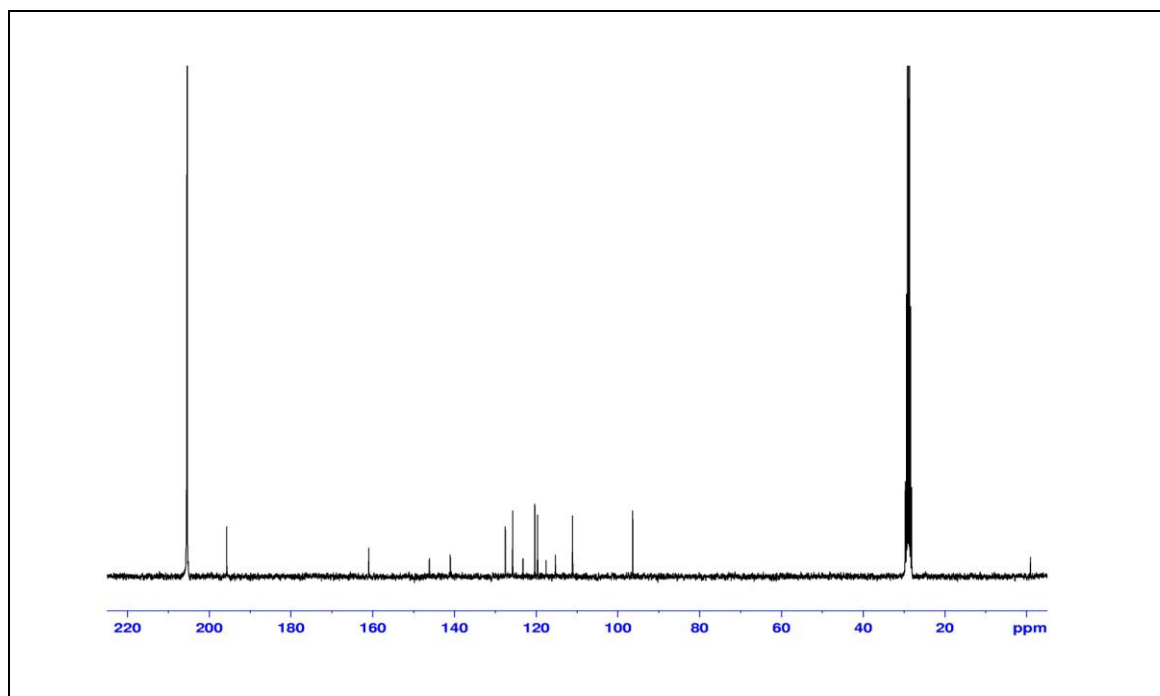


Figure 71 ^{13}C NMR (300 MHz) (CD_3COCD_3) of compound **RM6**

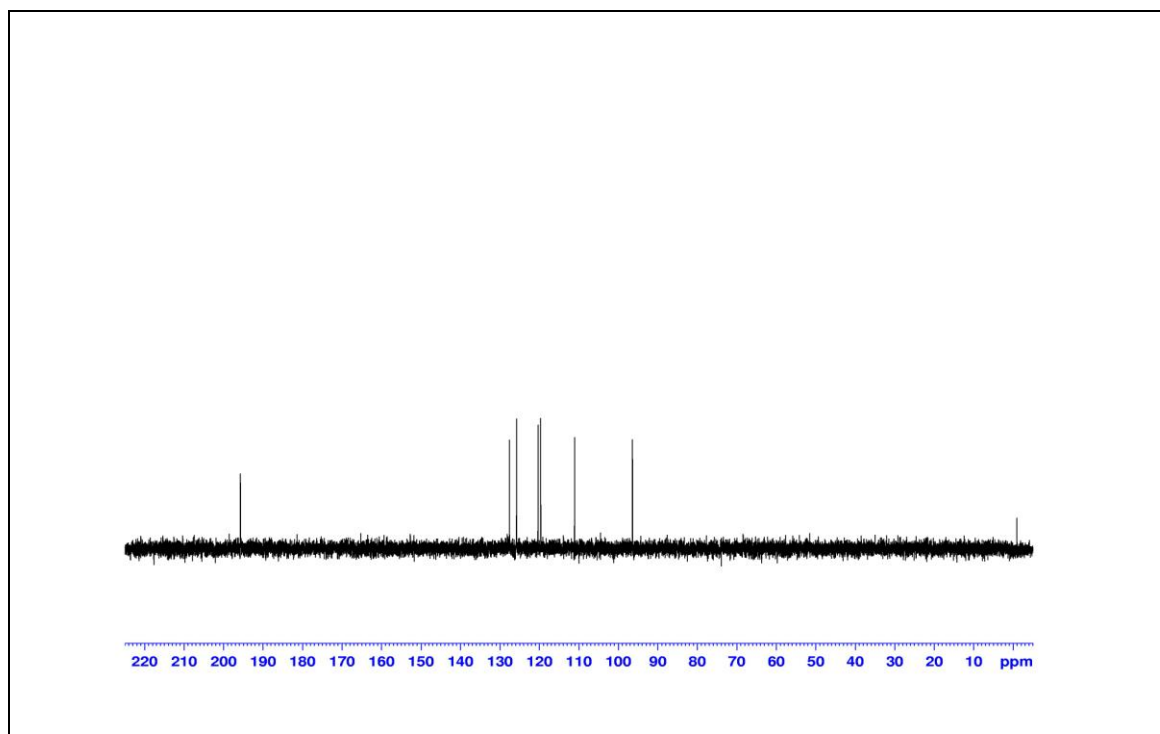


Figure 72 DEPT 135° (CD₃COCD₃) of compound **RM6**

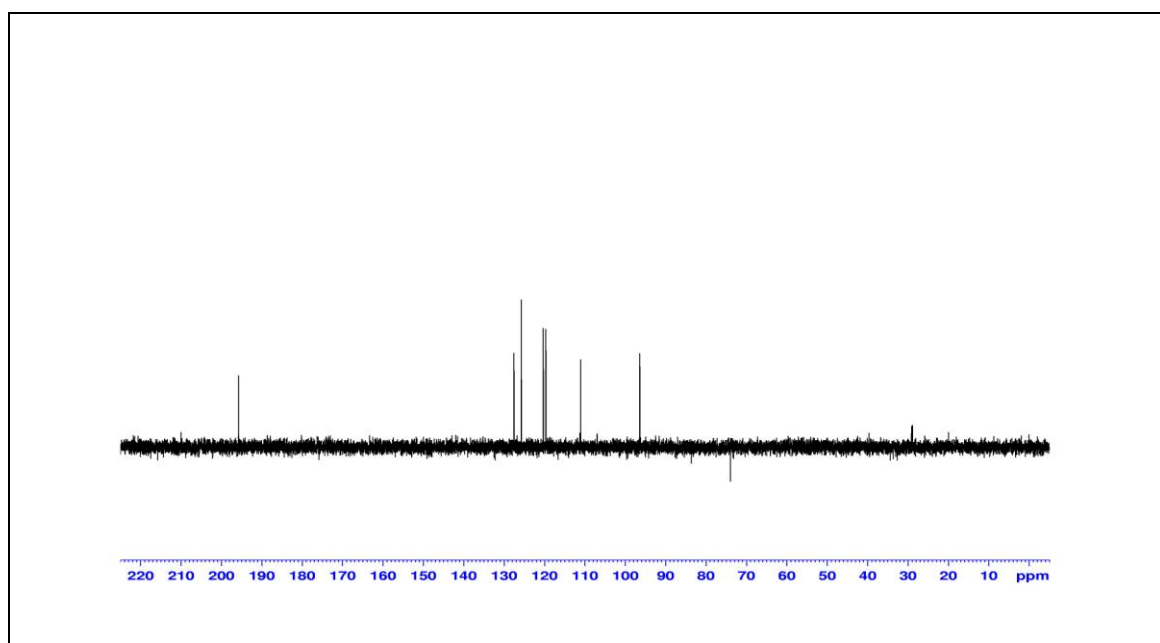


Figure 73 DEPT 90° (CD₃COCD₃) of compound **RM6**

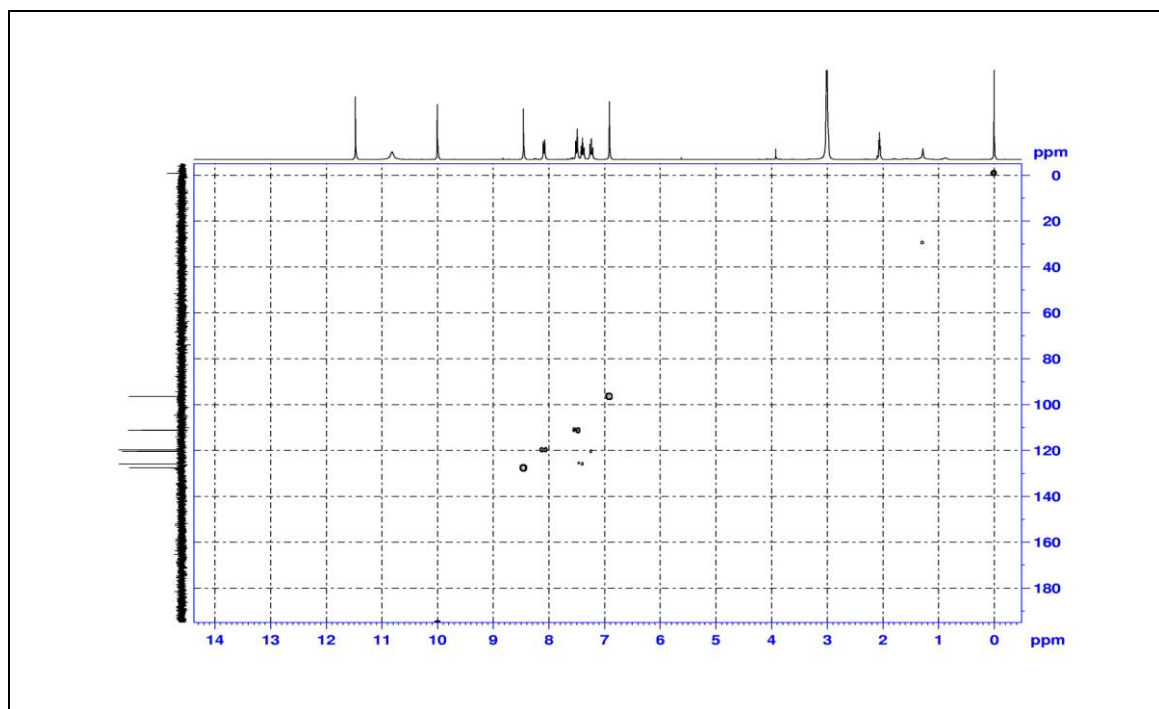


Figure 74 2D HMQC (CD_3COCD_3) of compound **RM6**

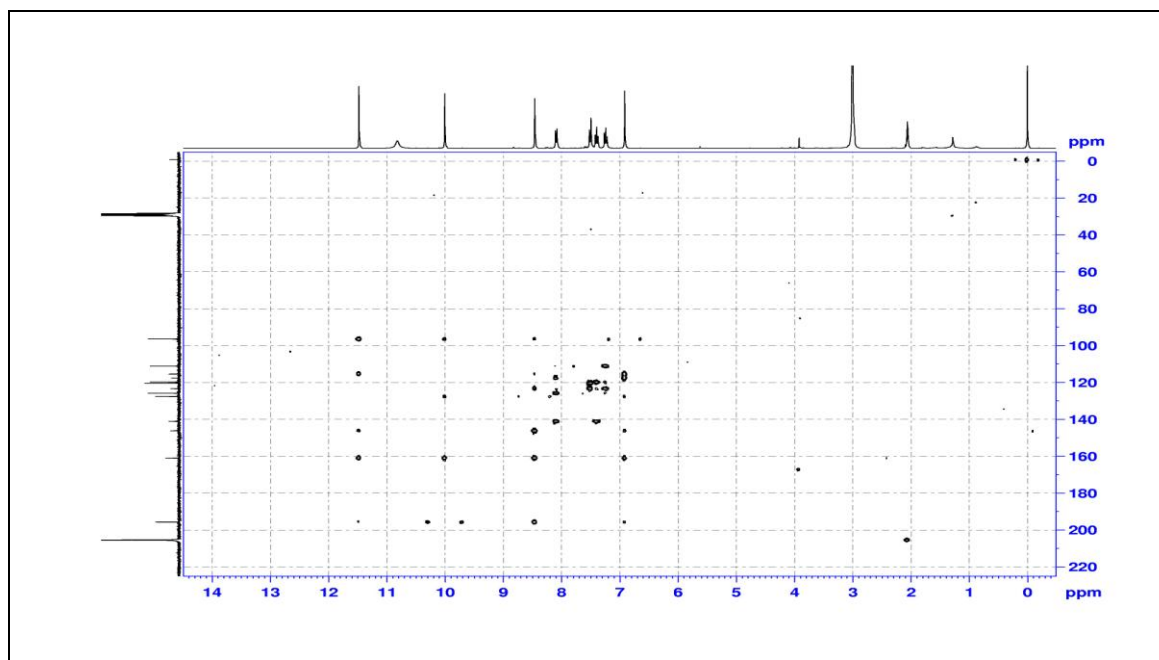


Figure 75 2D HMBC (CD_3COCD_3) of compound **RM6**

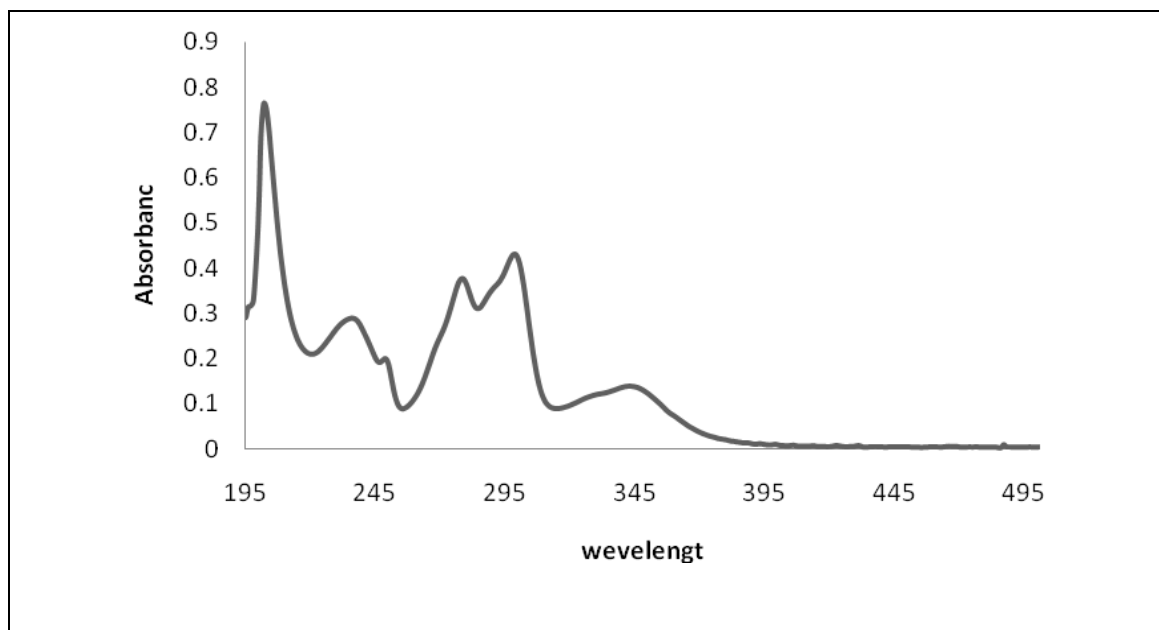


Figure 76 UV (MeOH) spectrum of compound **RM7**

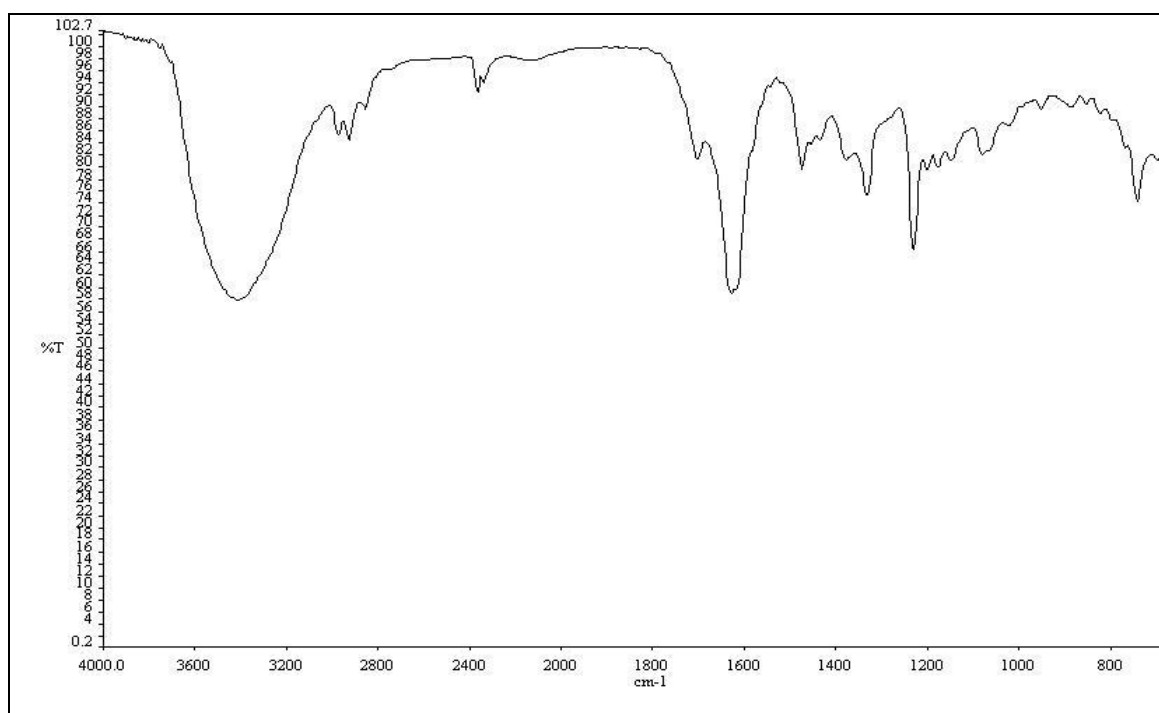


Figure 77 IR (neat) spectrum of compound **RM7**

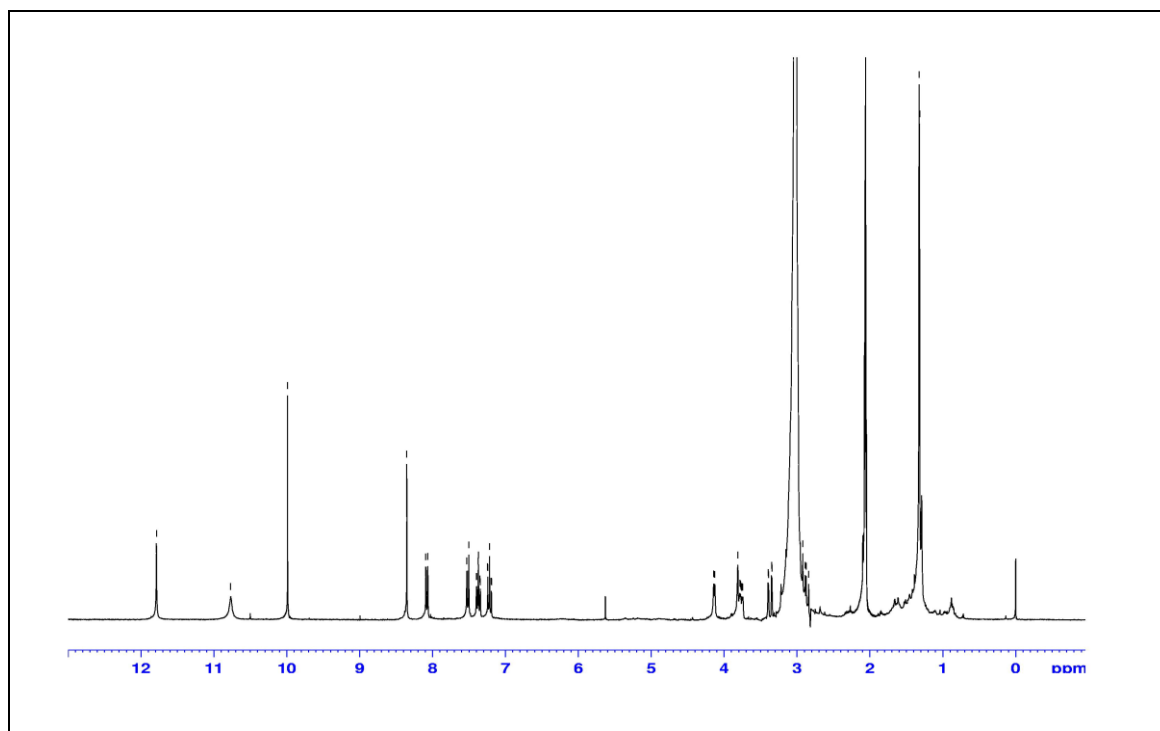


Figure 78 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM7**

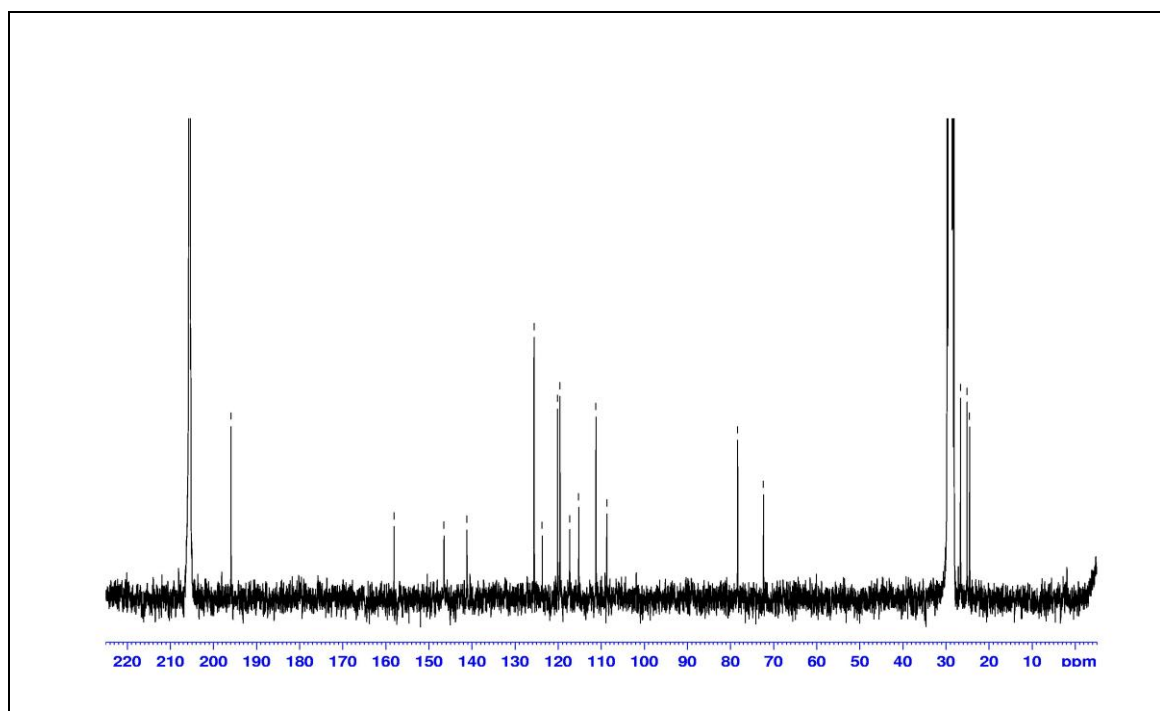


Figure 79 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM7**

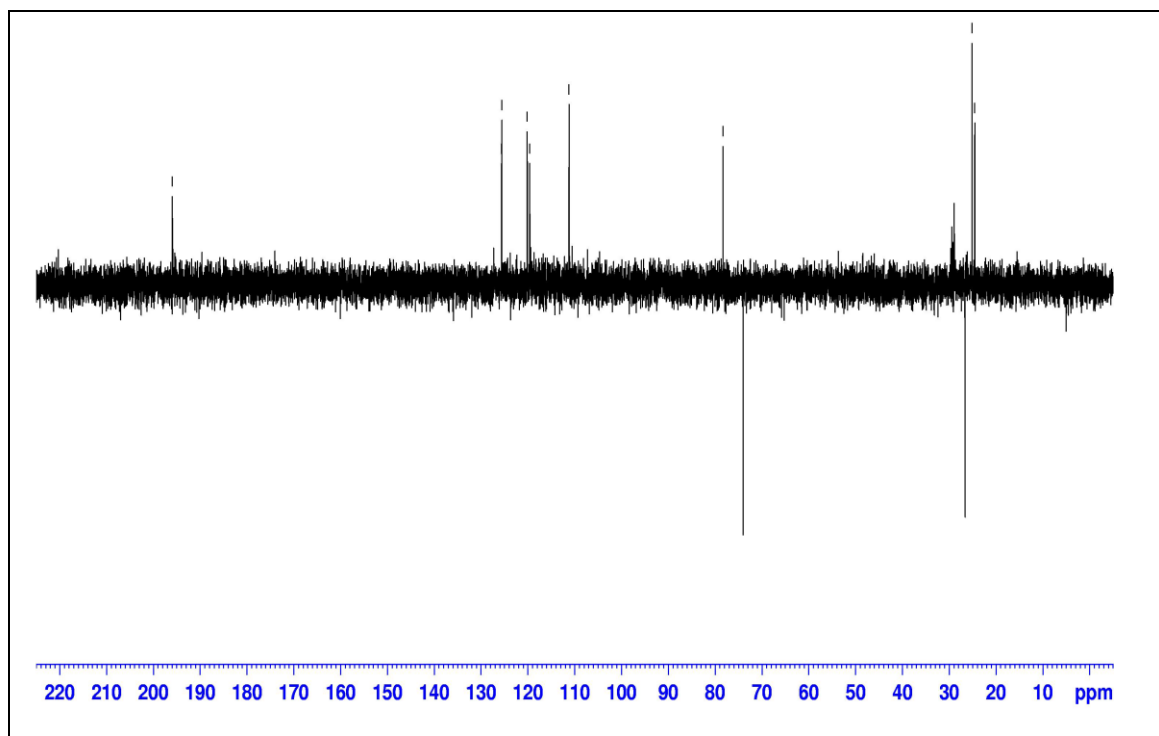


Figure 80 DEPT 135° (CD_3COCD_3) of compound **RM7**

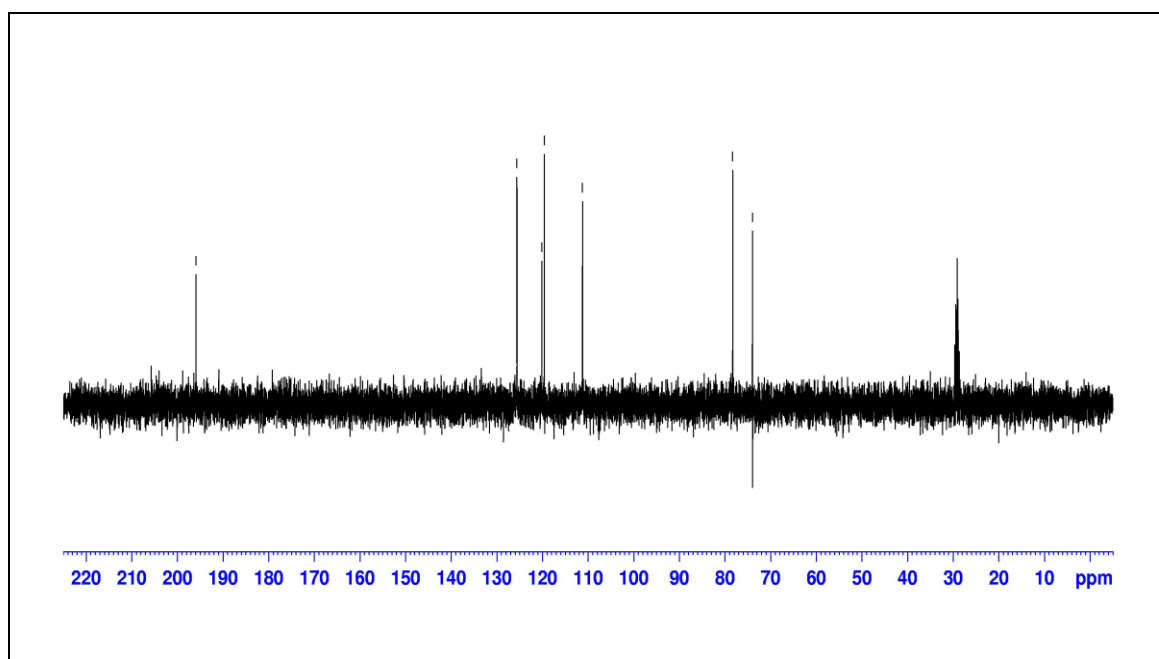


Figure 81 DEPT 90° (CD_3COCD_3) of compound **RM7**

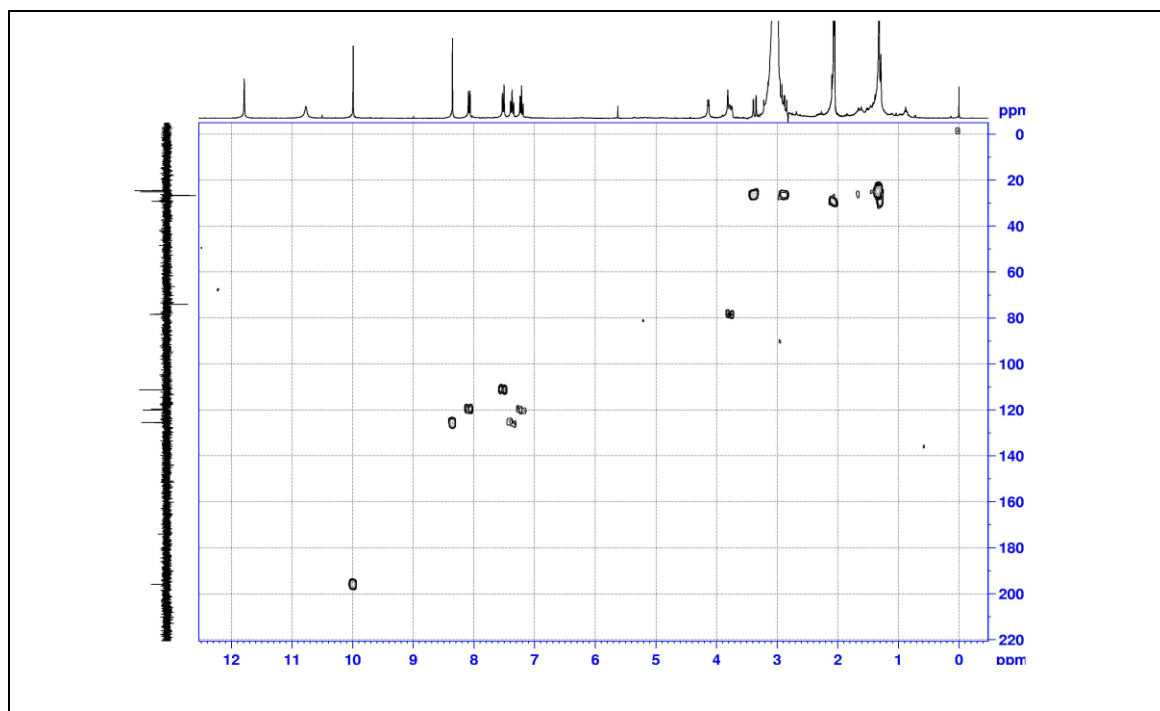


Figure 82 2D HMQC (CD₃COCD₃) of compound **RM7**

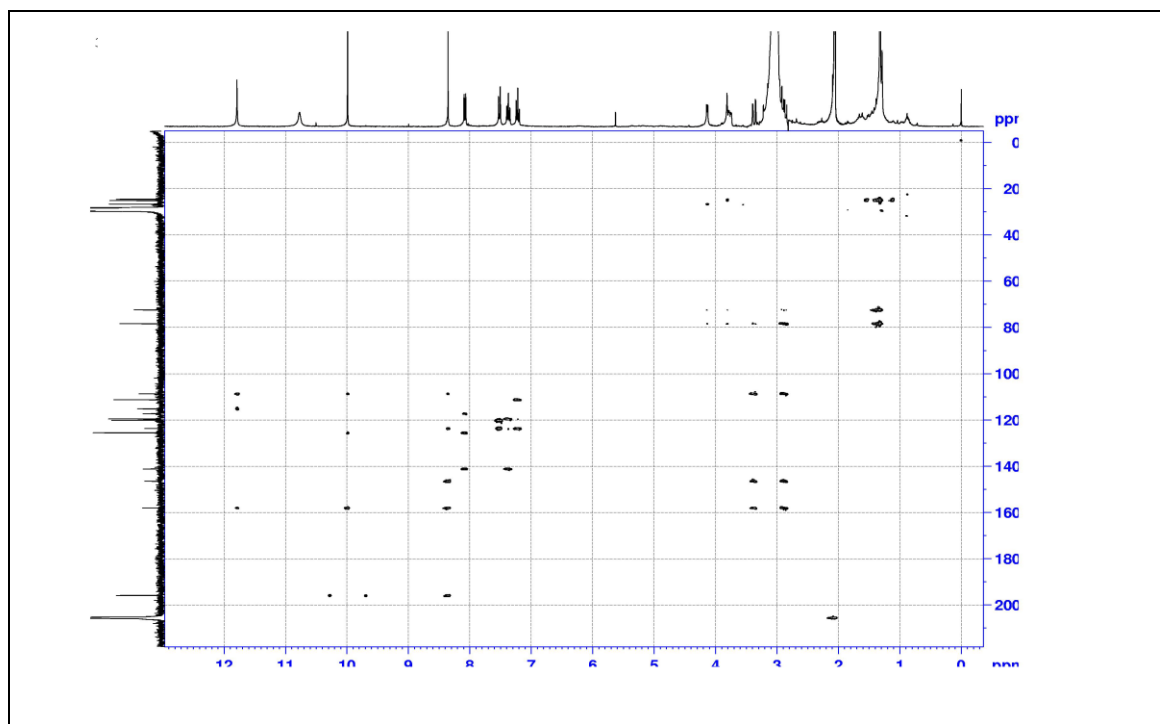


Figure 83 2D HMBC (CD₃COCD₃) of compound **RM7**

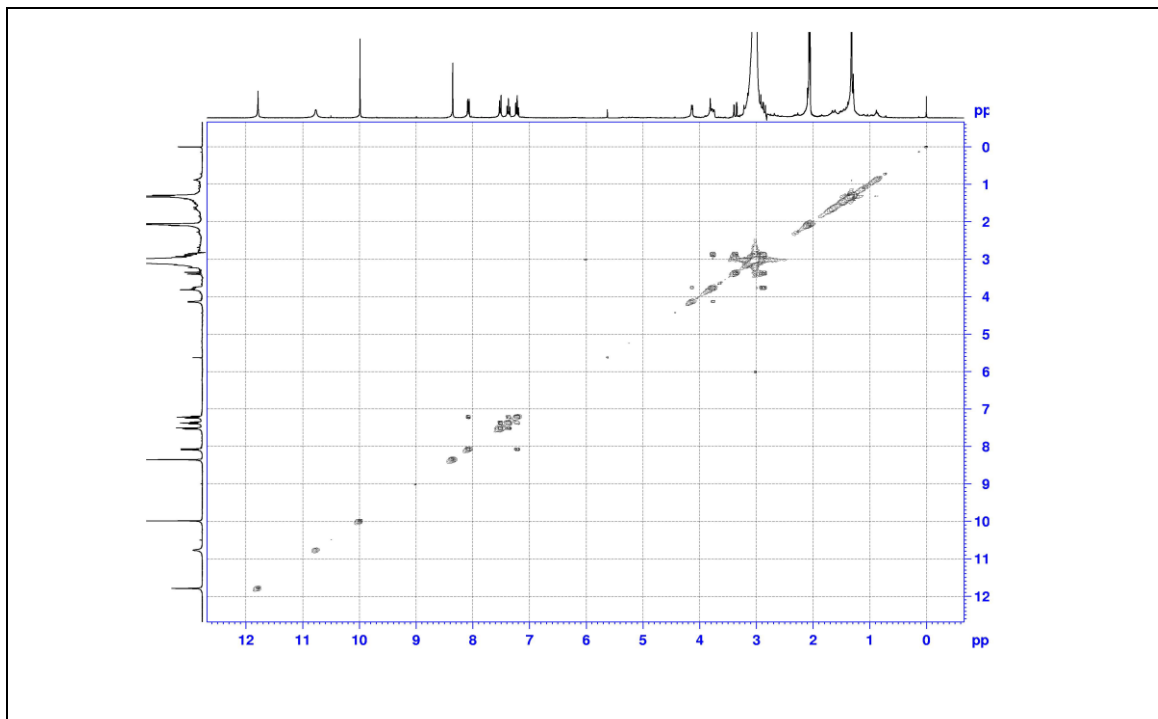


Figure 84 COSY (CD_3COCD_3) of compound **RM7**

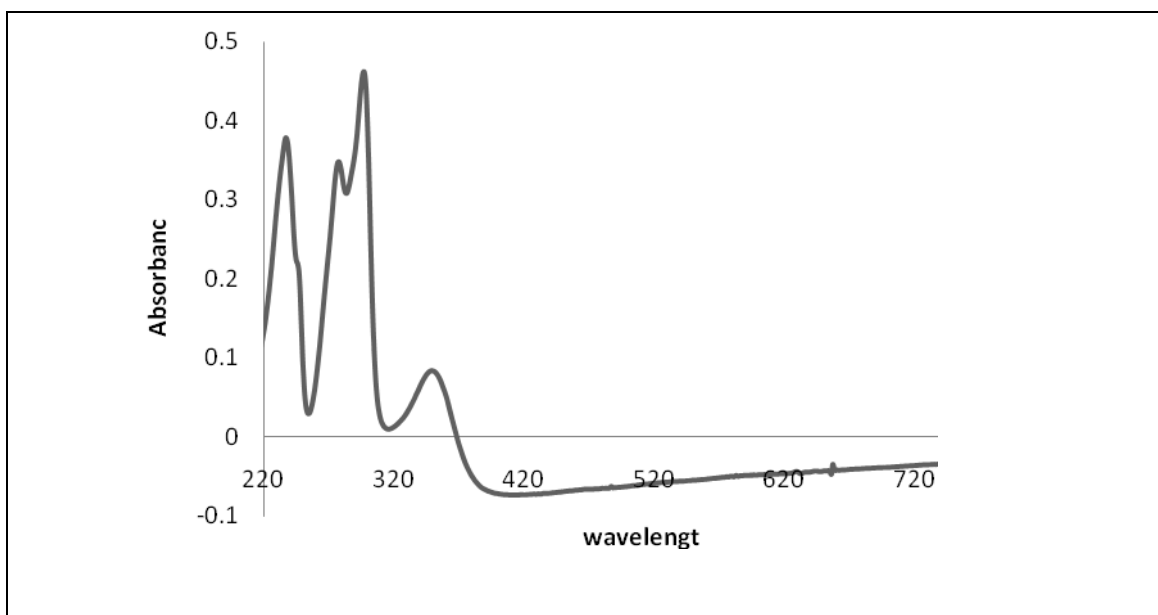


Figure 85 UV (MeOH) spectrum of compound **RM8**

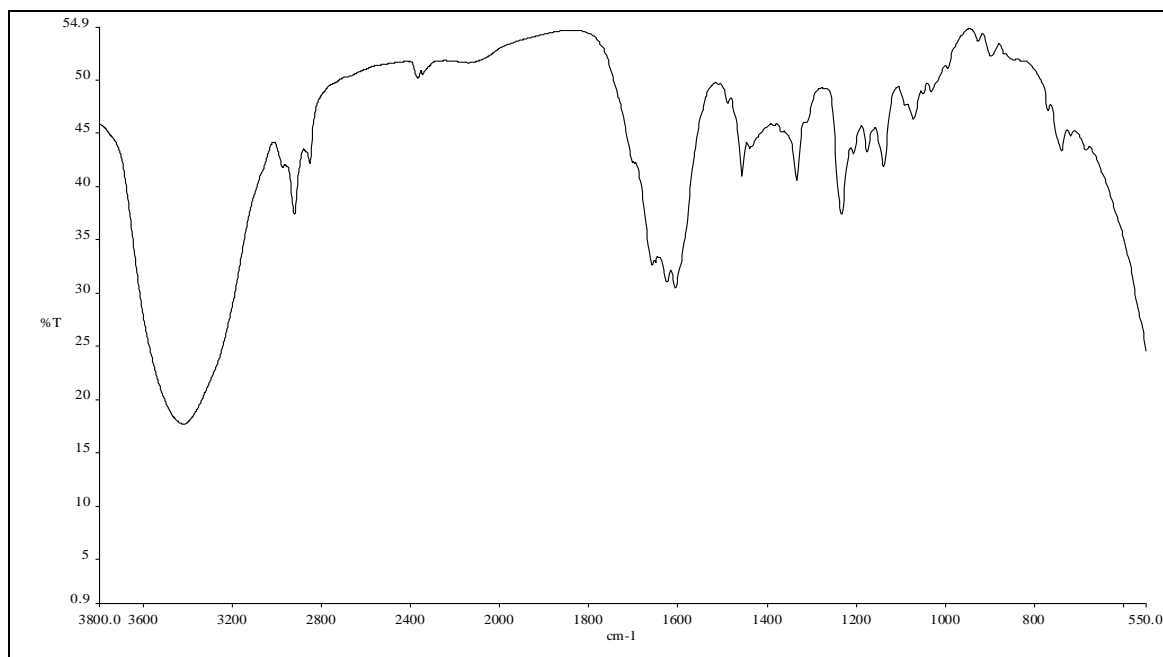


Figure 86 IR (neat) spectrum of compound **RM8**

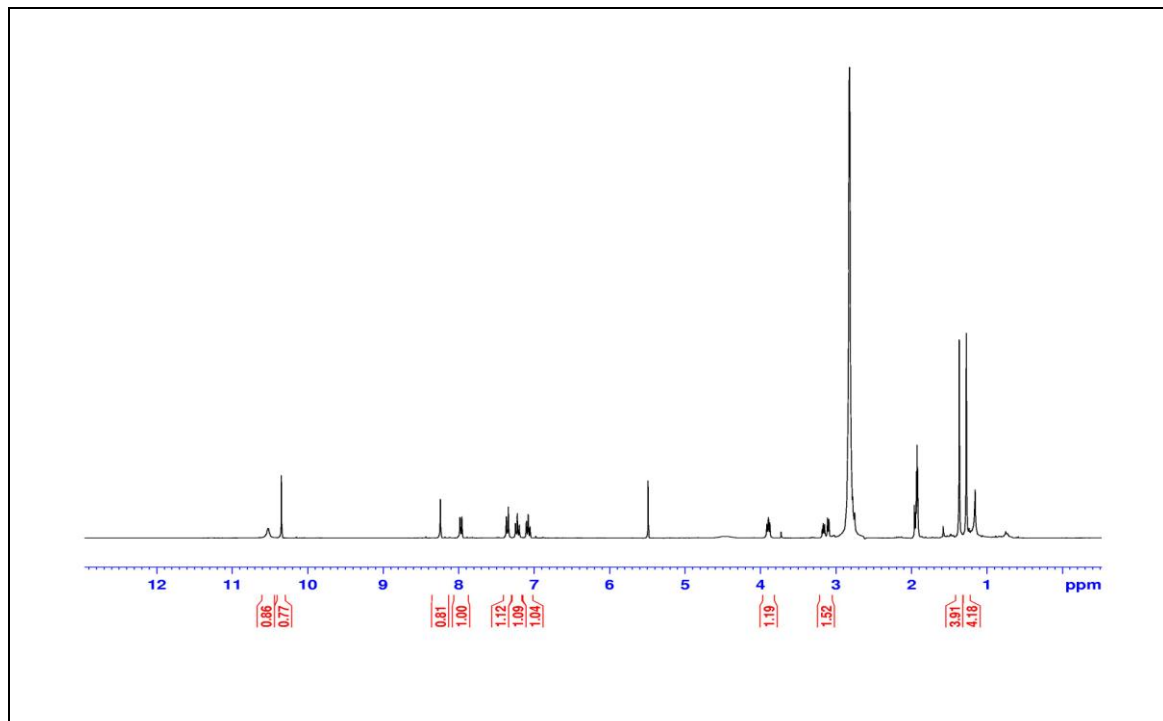


Figure 87 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM8**

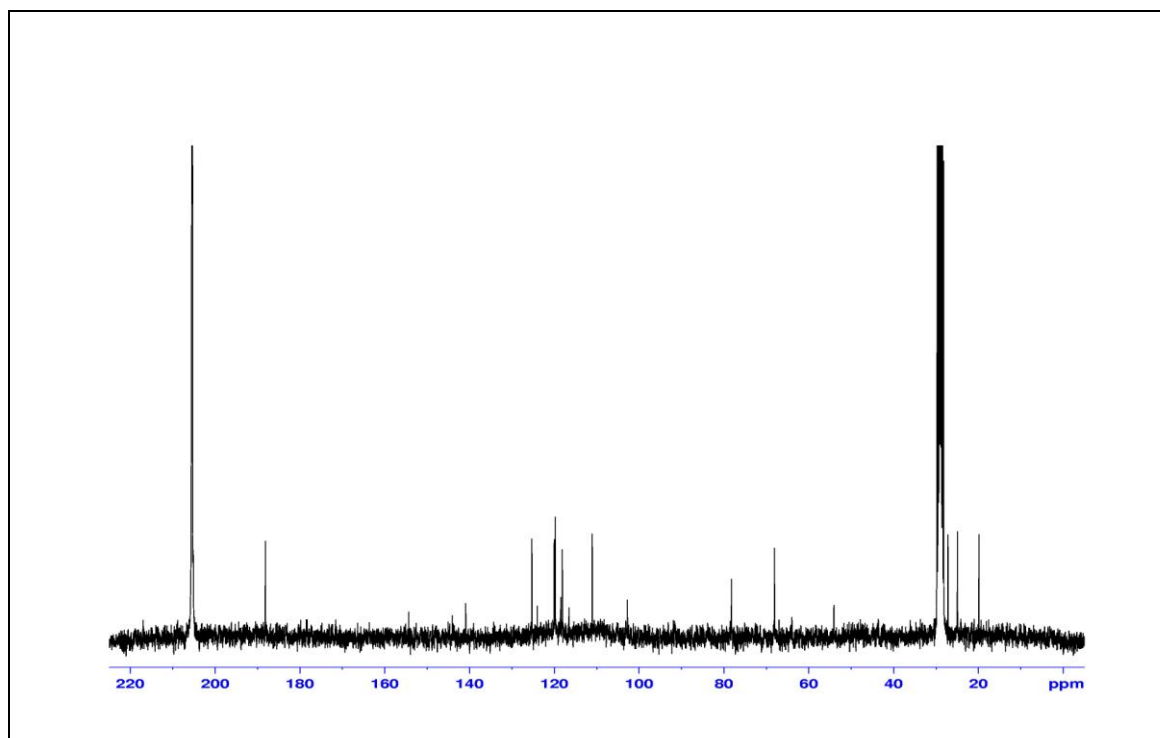


Figure 88 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM8**

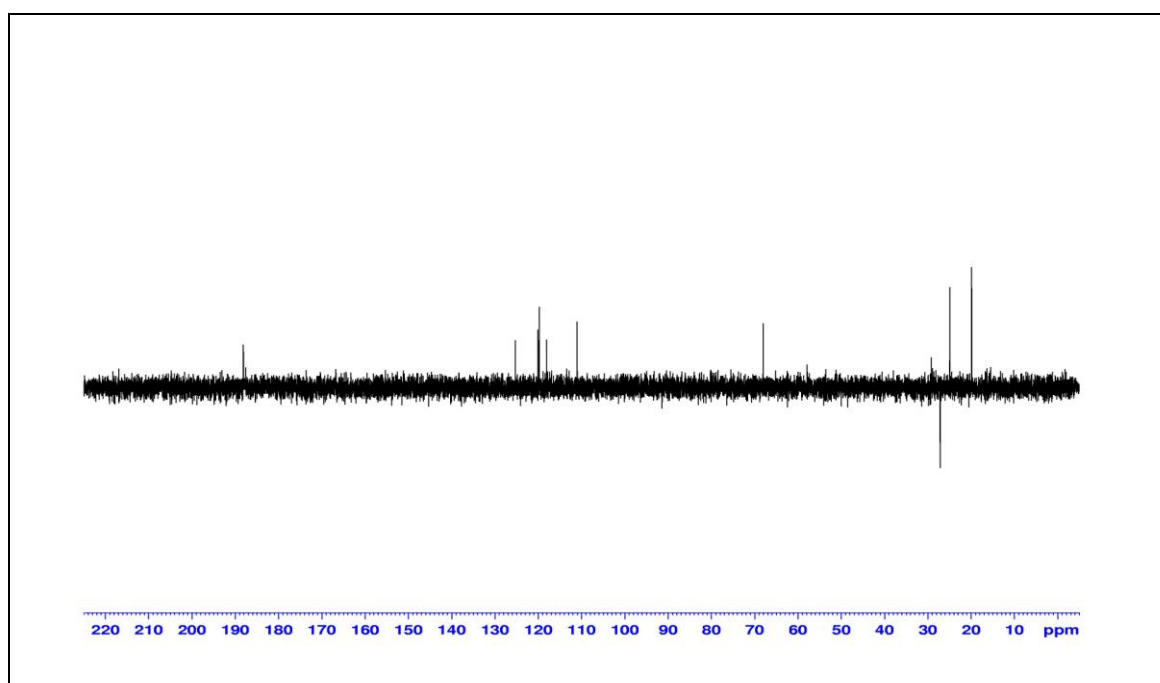


Figure 89 DEPT 135° (CD_3COCD_3) of compound **RM8**

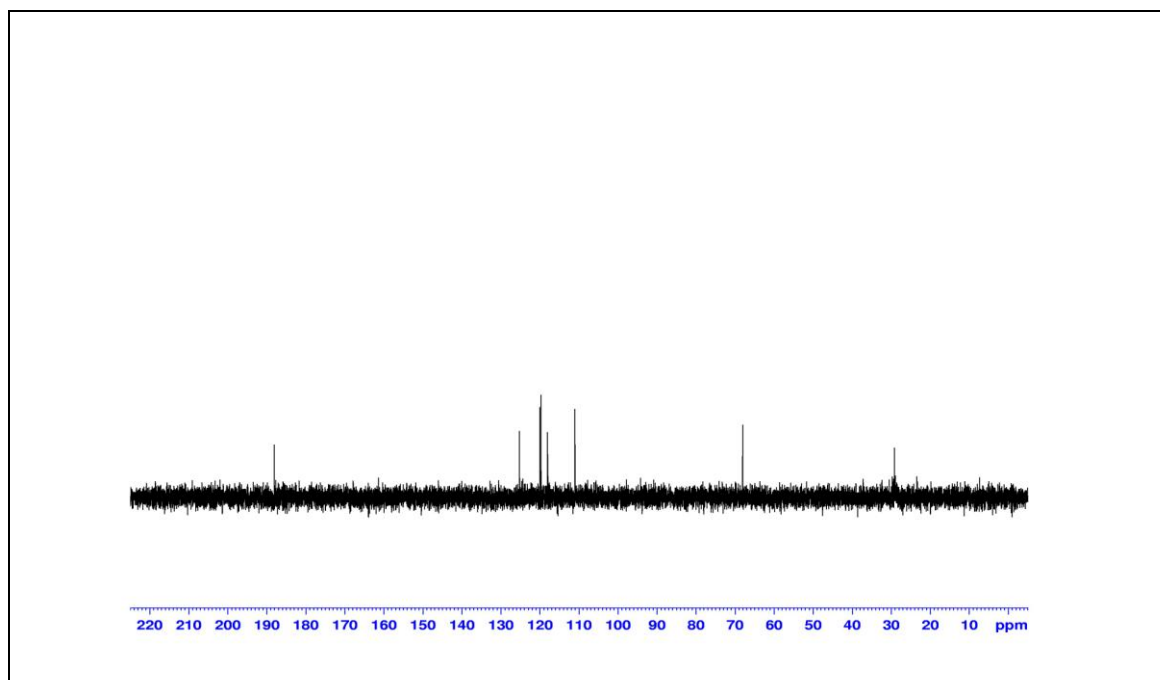


Figure 90 DEPT 90° (CD_3COCD_3) of compound **RM8**

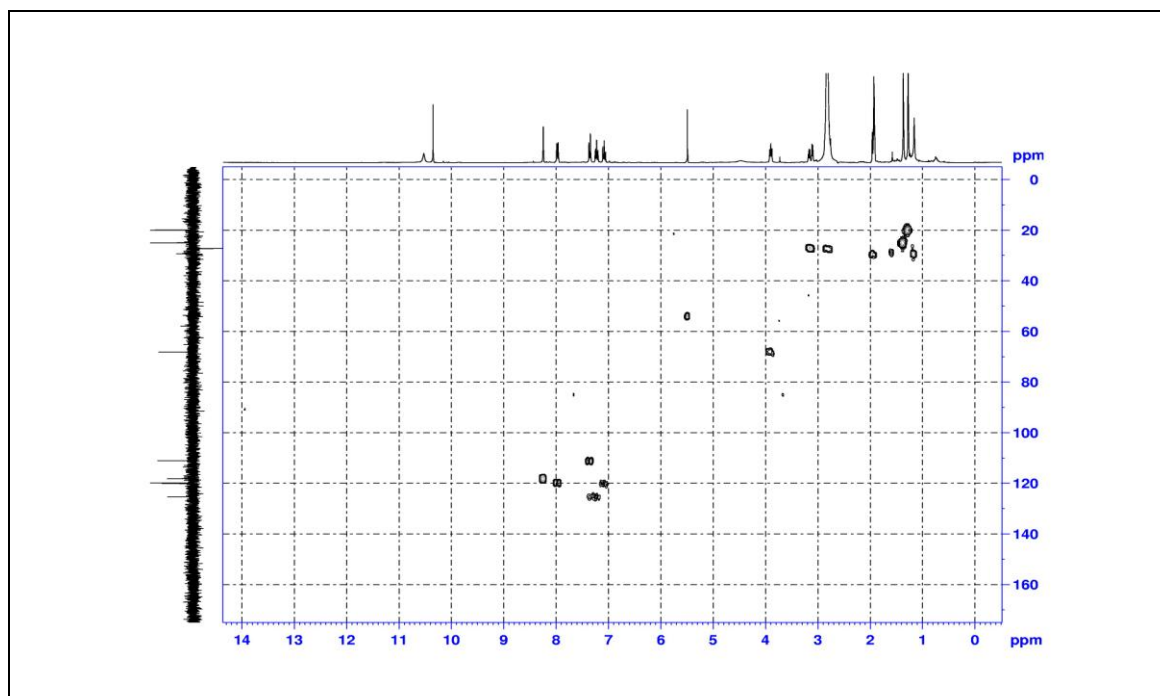


Figure 91 2D HMQC (CD_3COCD_3) of compound **RM8**

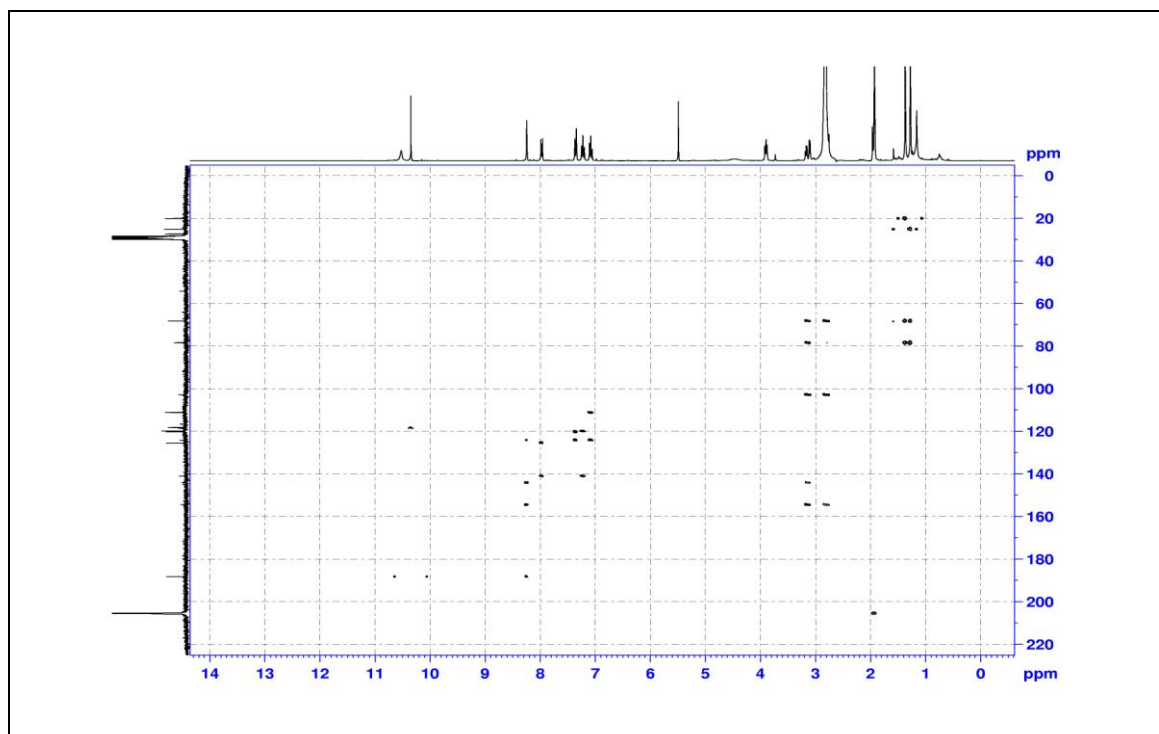


Figure 92 2D HMBC (CD_3COCD_3) of compound **RM8**

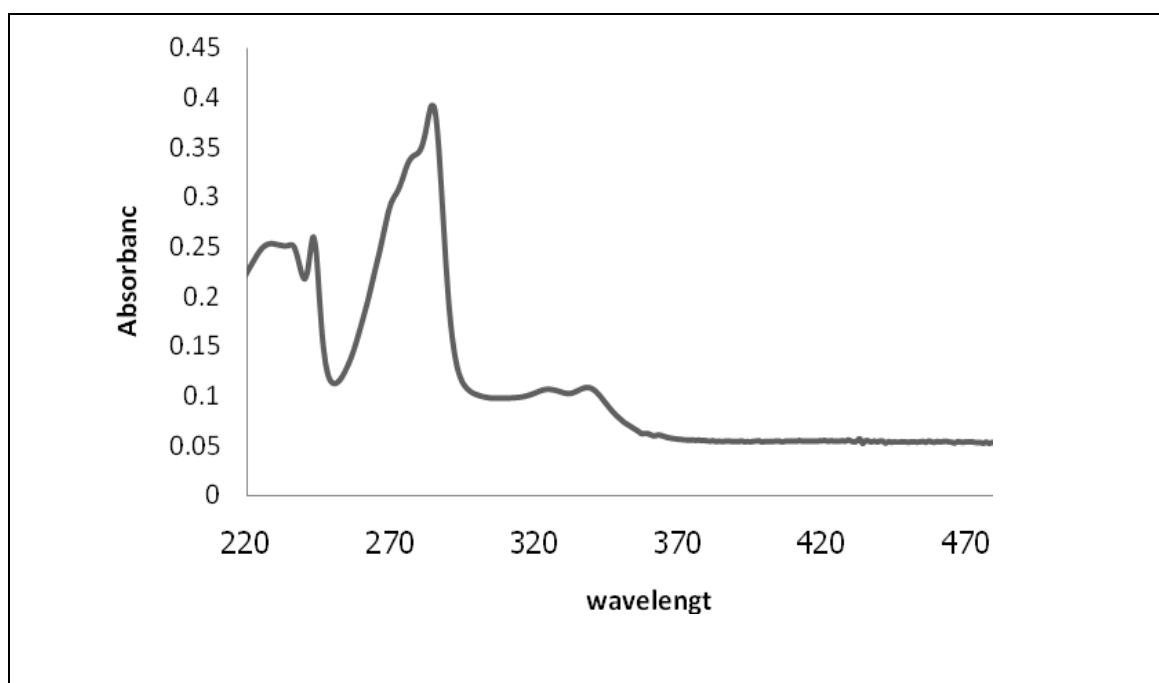


Figure 93 UV (MeOH) spectrum of compound **RM9**

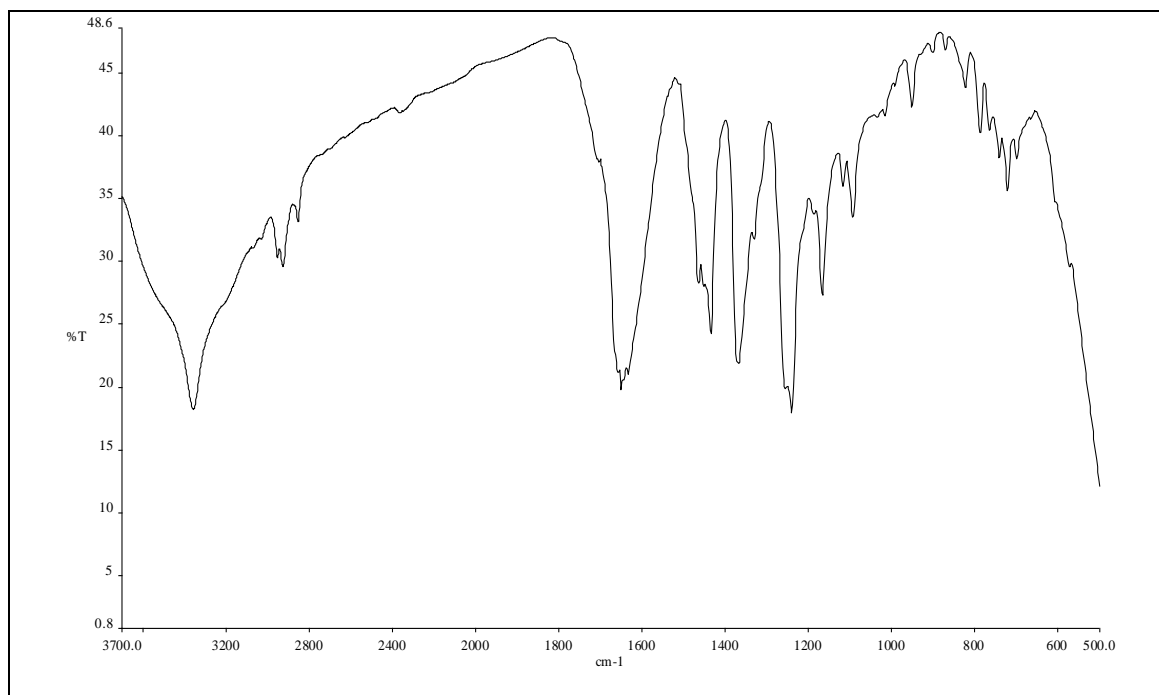


Figure 94 IR (neat) spectrum of compound **RM9**

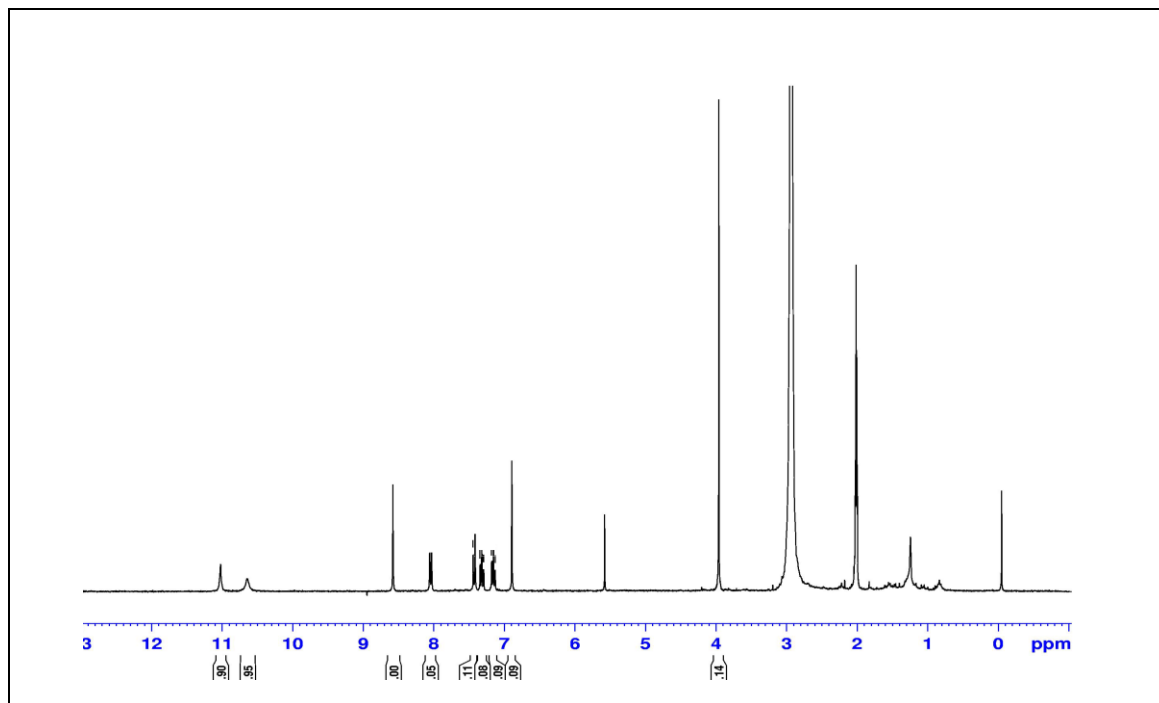


Figure 95 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM9**

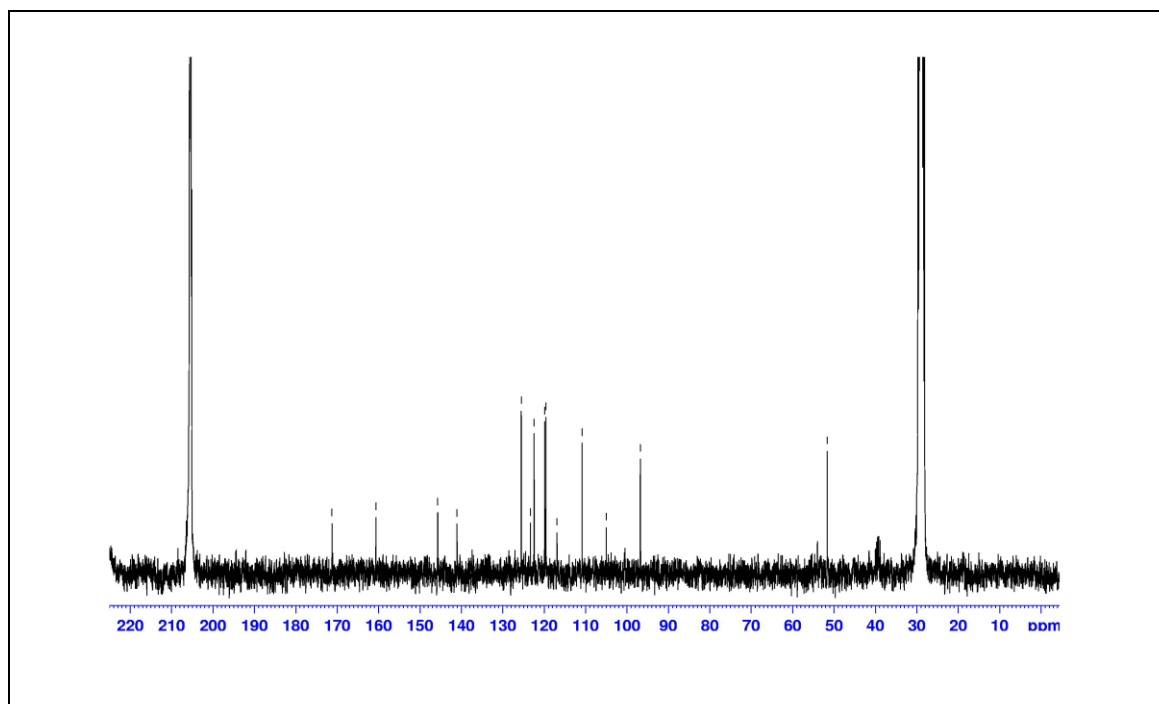


Figure 96 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM9**

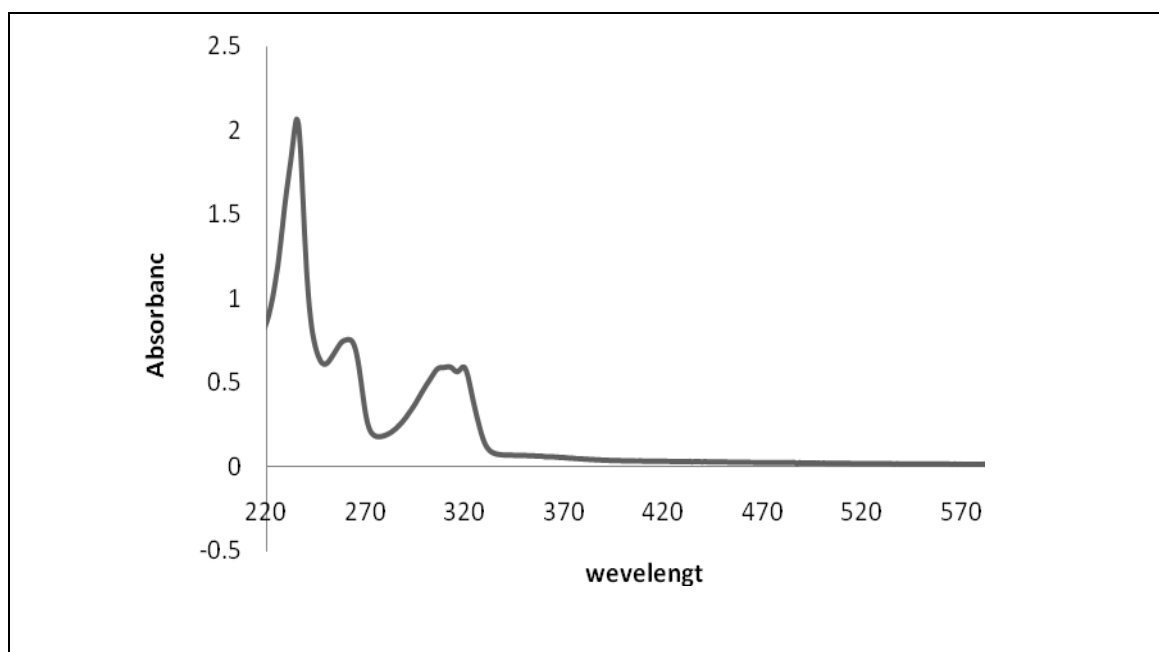


Figure 97 UV (MeOH) spectrum of compound **RM10**

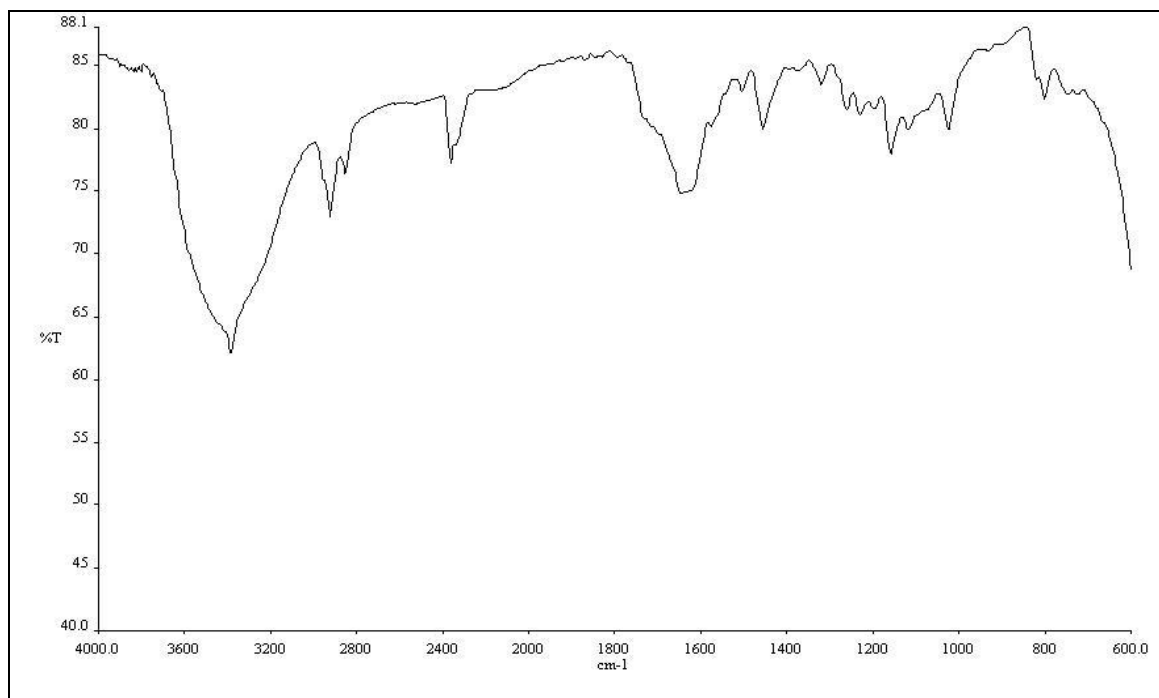


Figure 98 IR (neat) spectrum of compound **RM10**

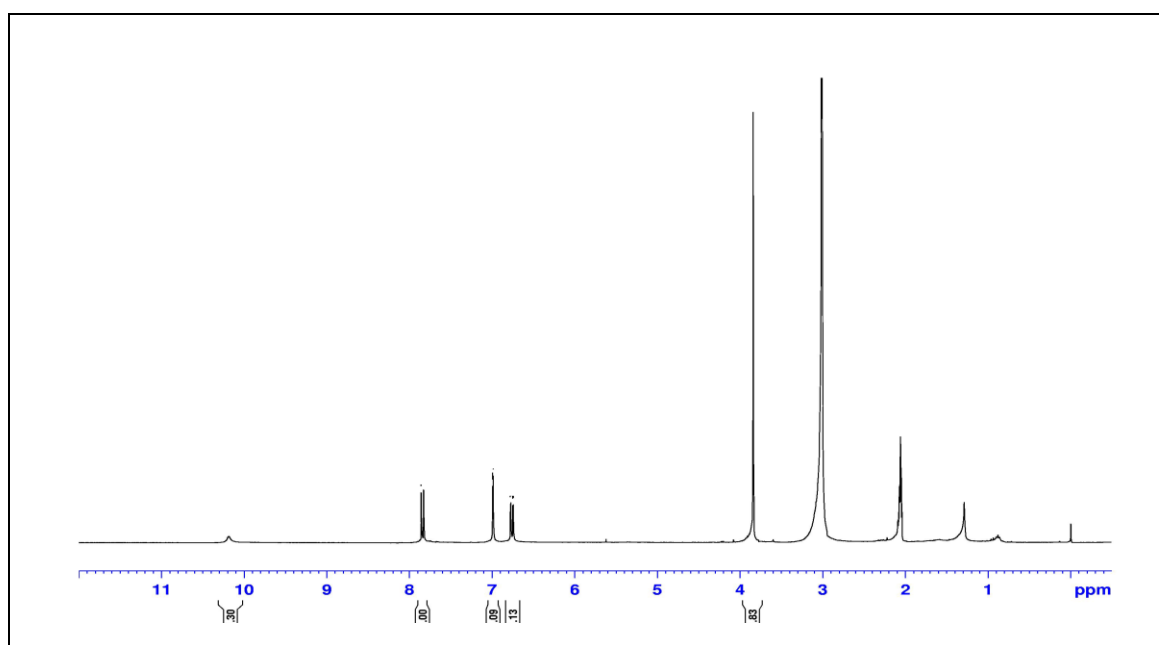


Figure 99 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM10**

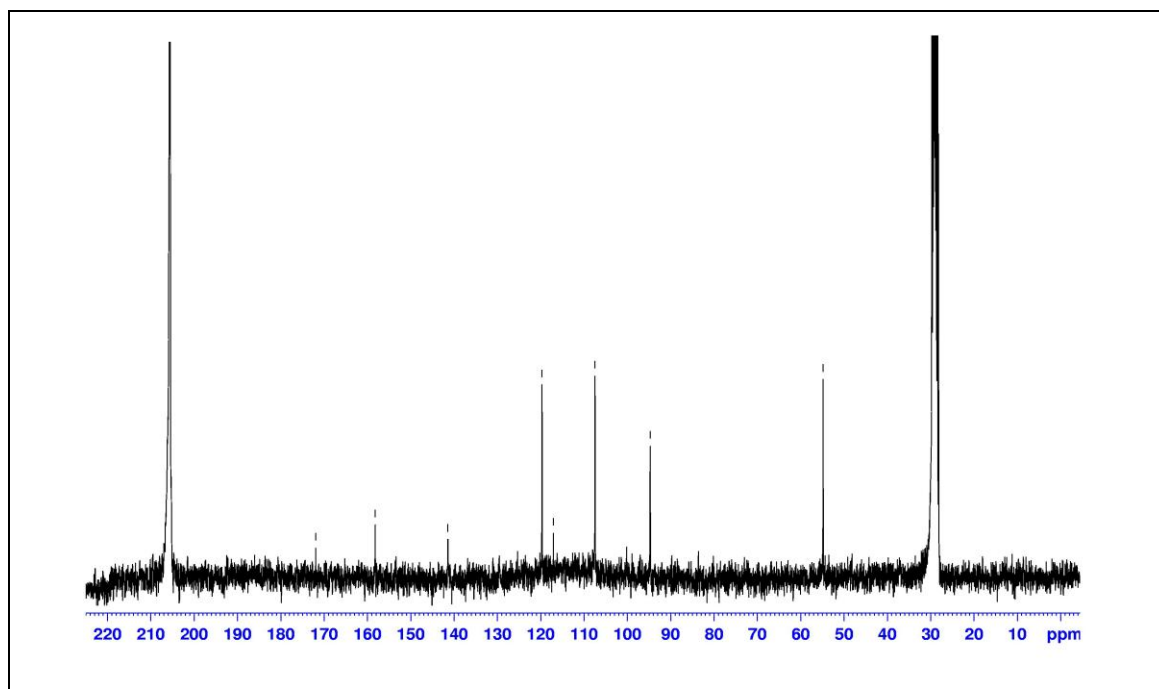


Figure 100 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM10**

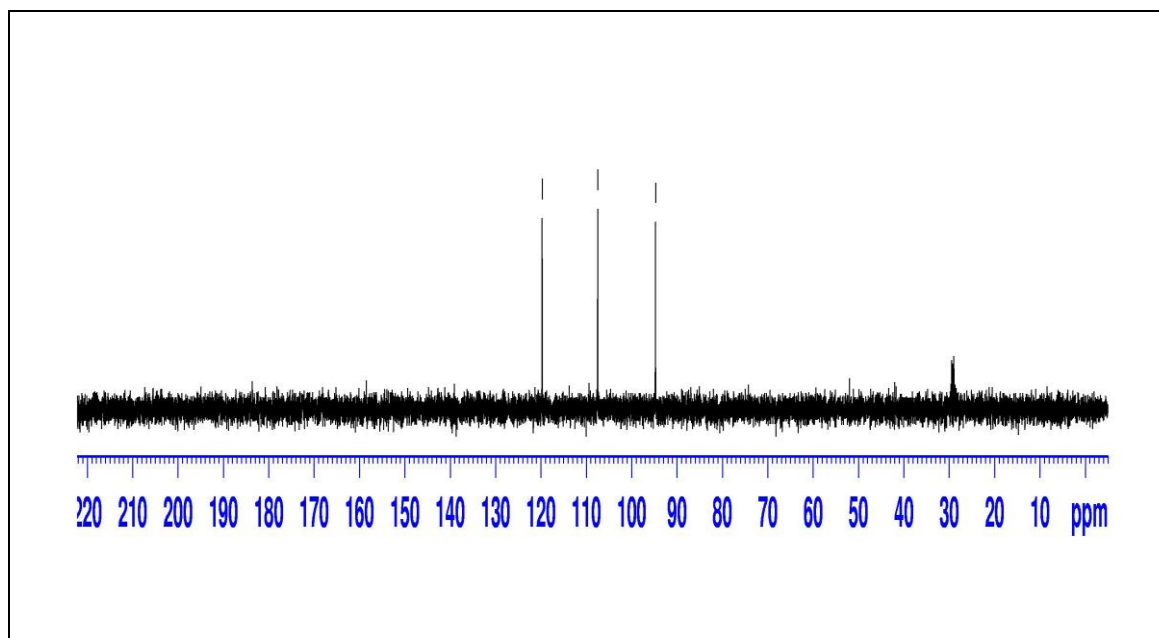


Figure 101 DEPT 90° (CD_3COCD_3) of compound **RM10**

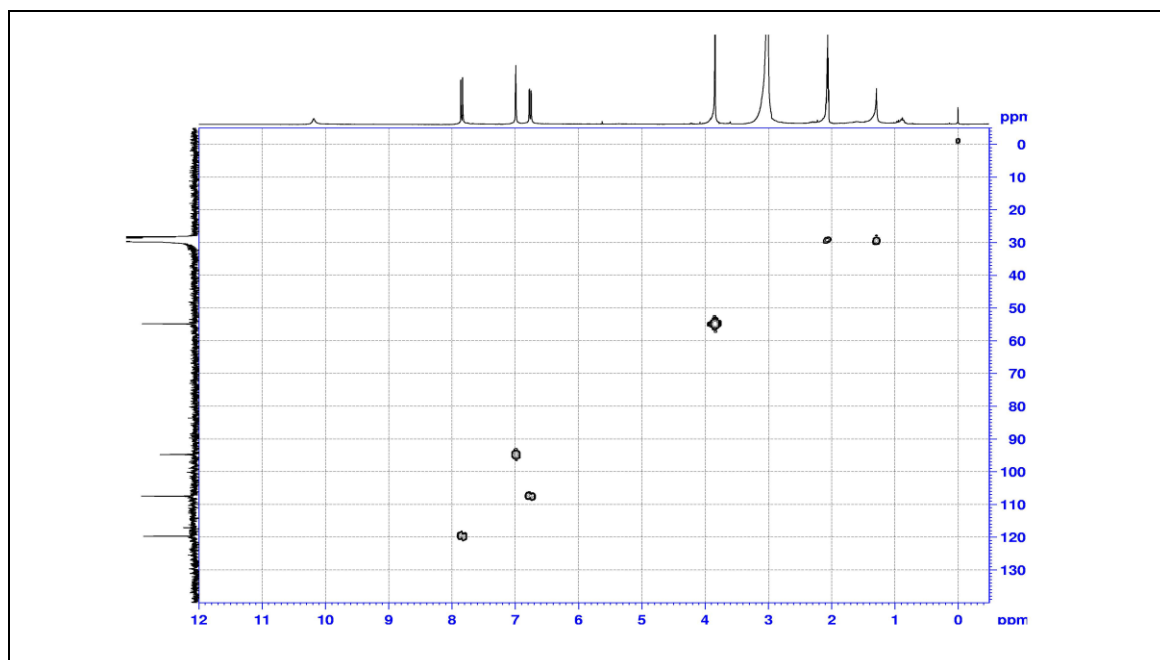


Figure 102 2D HMQC (CD_3COCD_3) of compound **RM10**

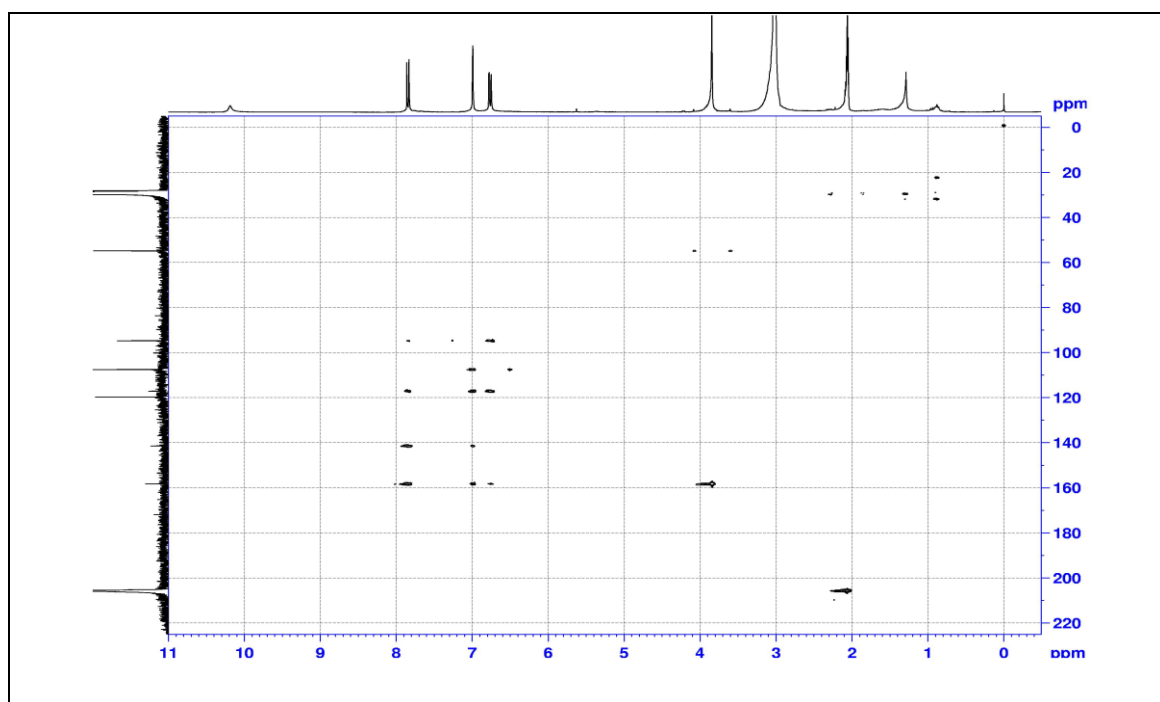


Figure 103 2D HMBC (CD_3COCD_3) of compound **RM10**

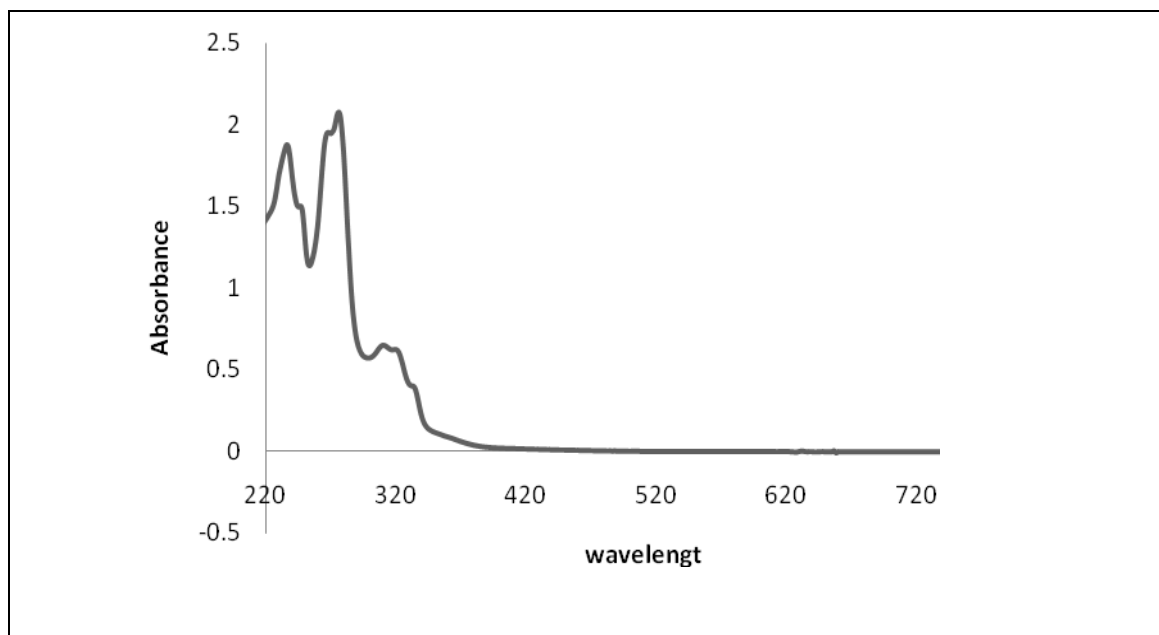


Figure 104 UV (MeOH) spectrum of compound **RM11**

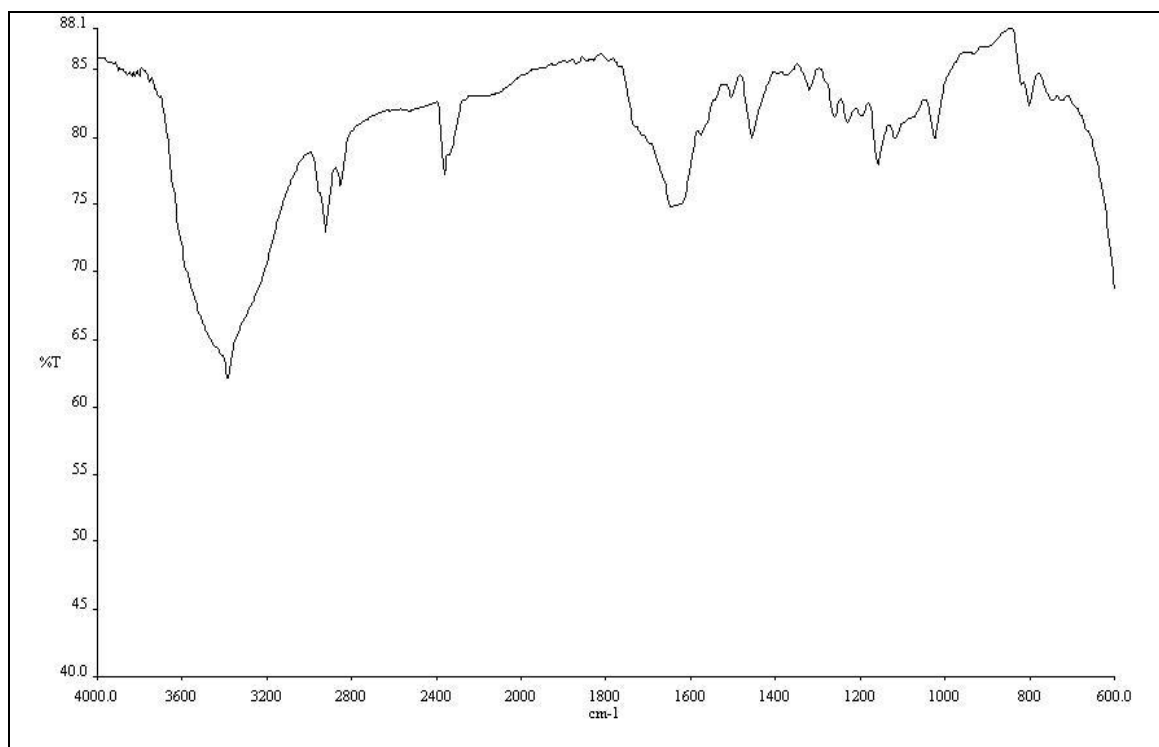


Figure 105 IR (neat) spectrum of compound **RM11**

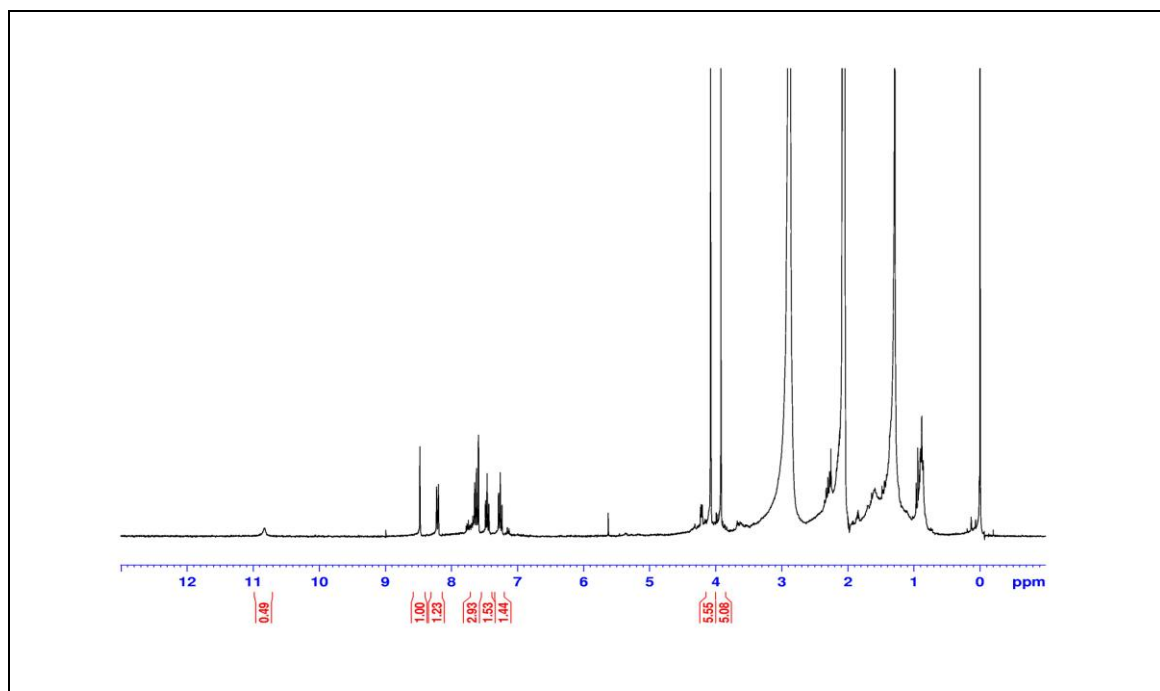


Figure 106 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM11**

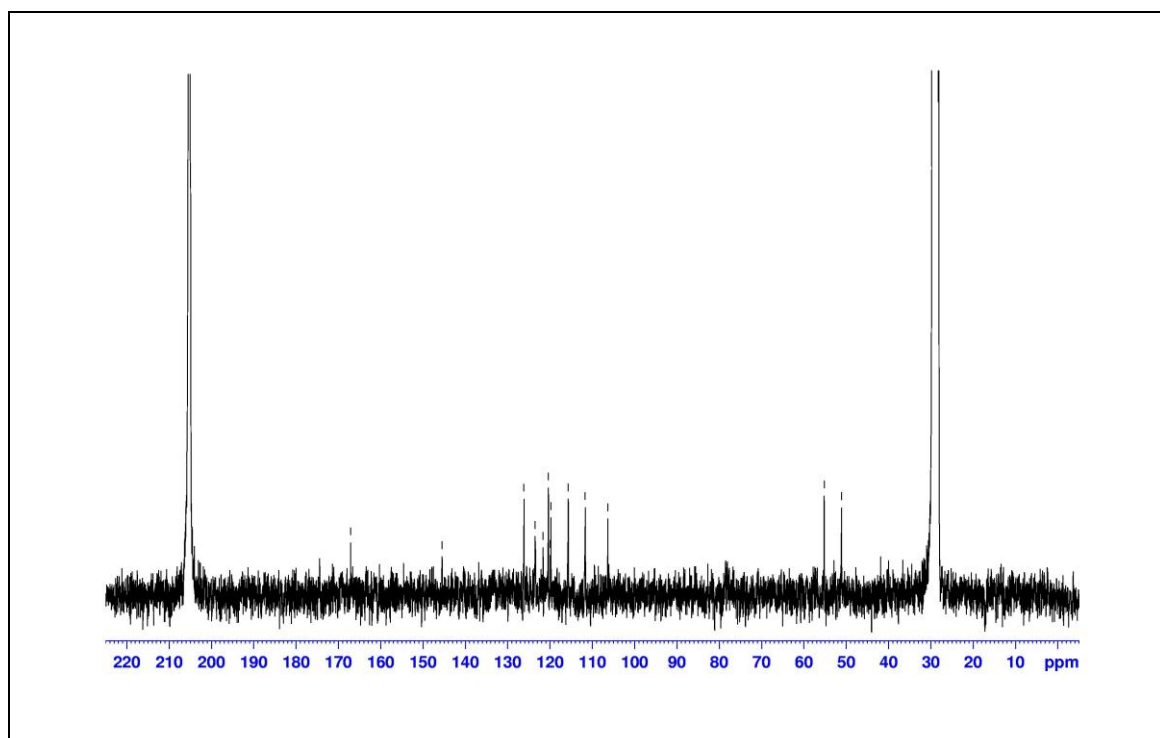


Figure 107 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM11**

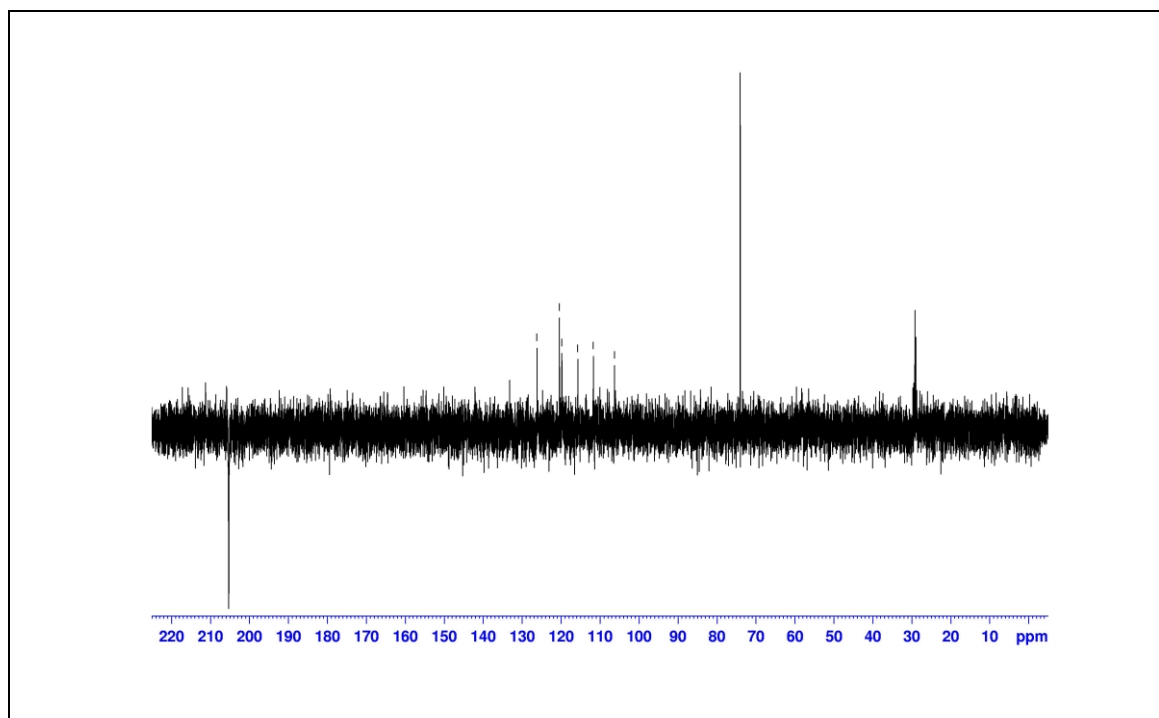


Figure 108 DEPT 90° (CD_3COCD_3) of compound **RM11**

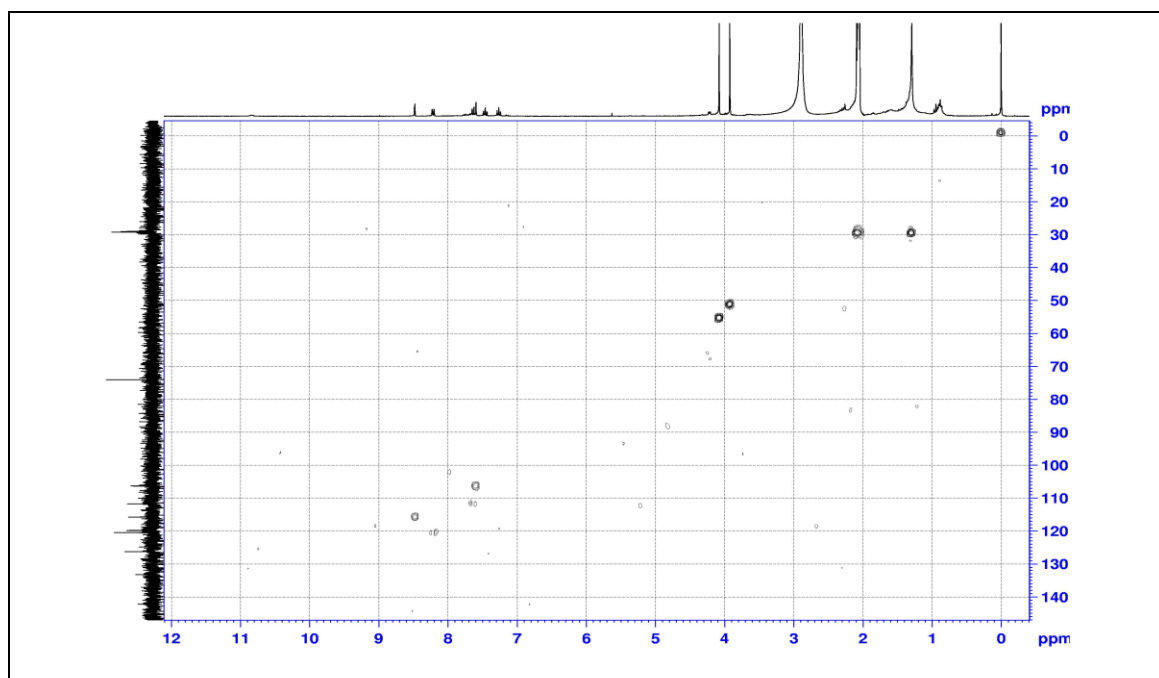


Figure 109 2D HMQC (CD_3COCD_3) of compound **RM11**

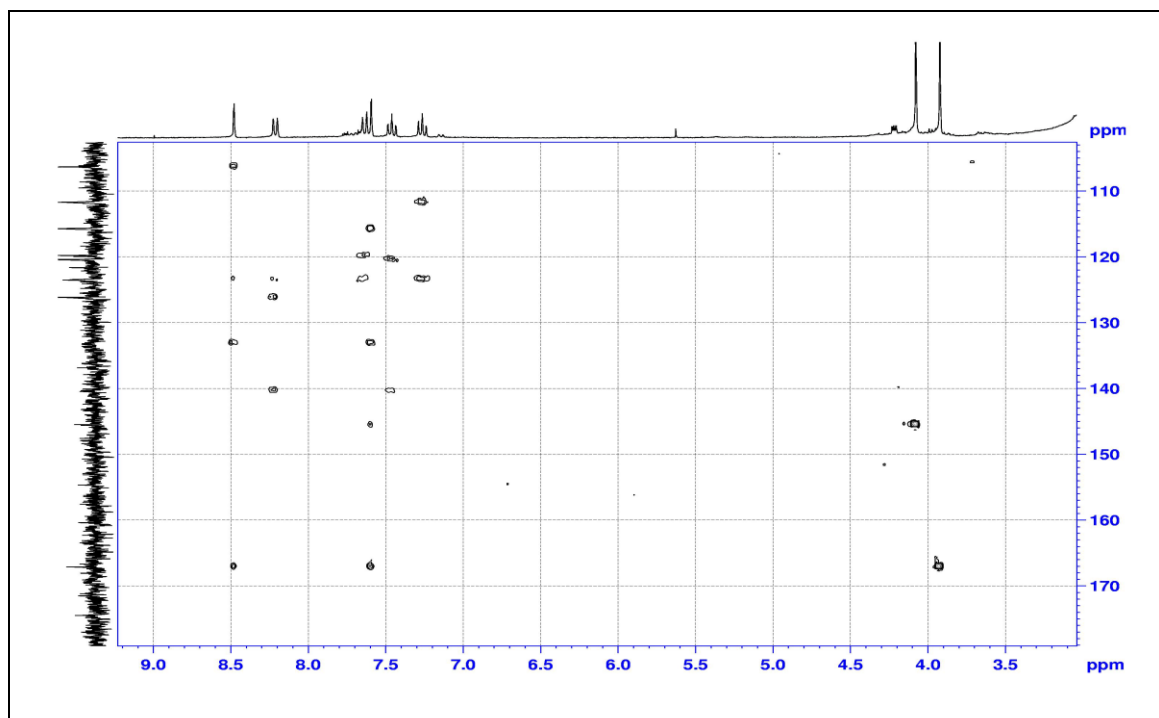


Figure 110 2D HMBC (CD_3COCD_3) of compound **RM11**

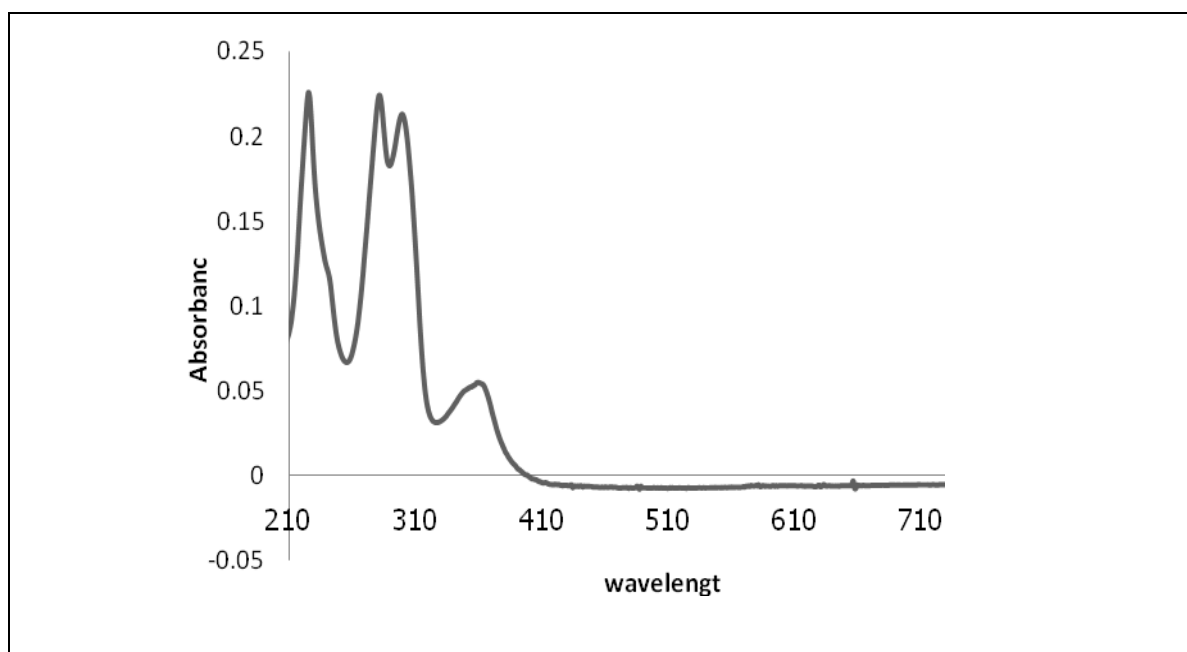


Figure 111 UV (MeOH) spectrum of compound **RM12**

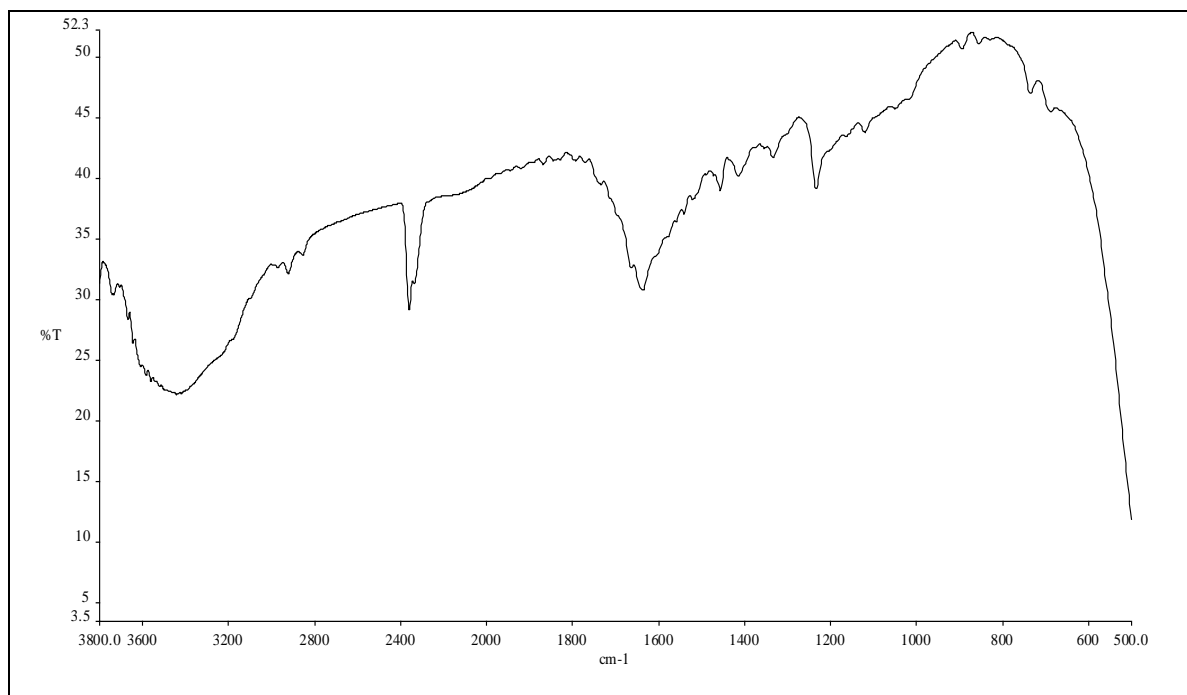


Figure 112 IR (neat) spectrum of compound **RM12**

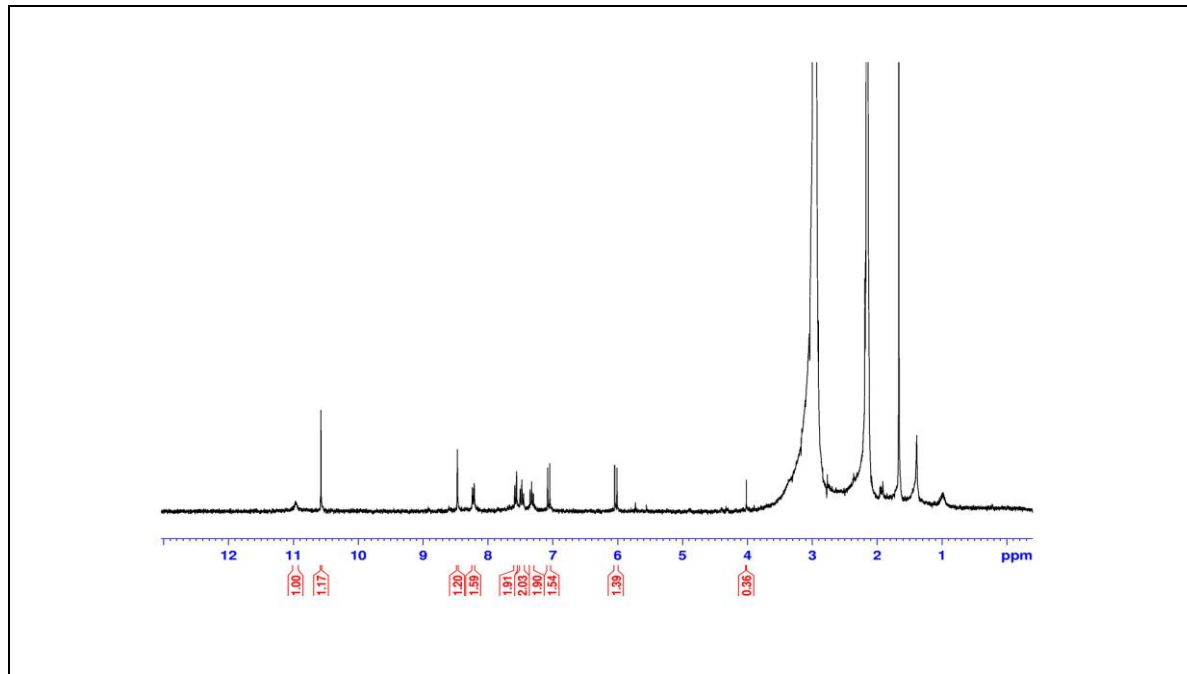


Figure 113 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM12**

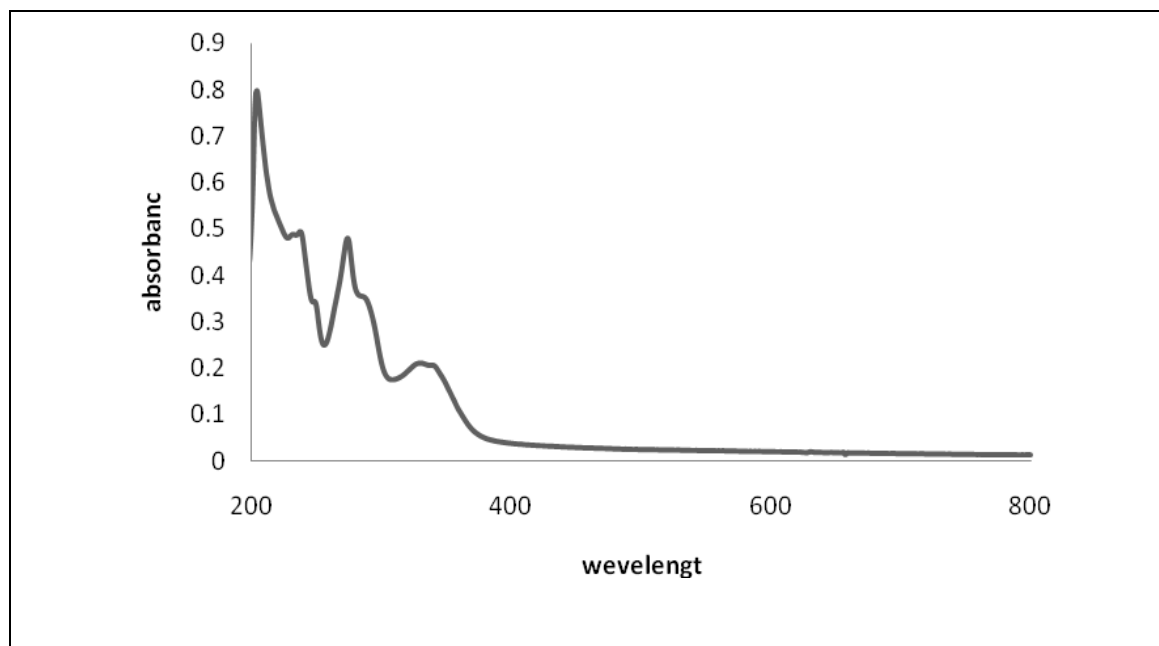


Figure 114 UV (MeOH) spectrum of compound **RM13**

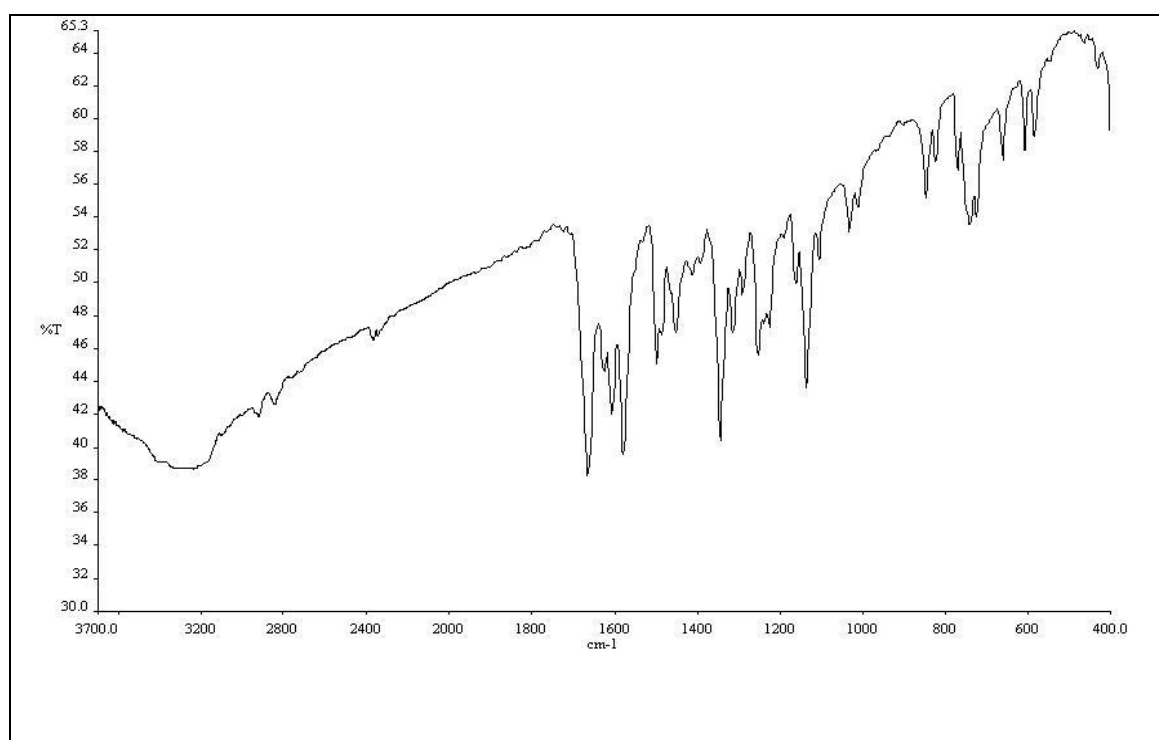


Figure 115 IR (neat) spectrum of compound **RM13**

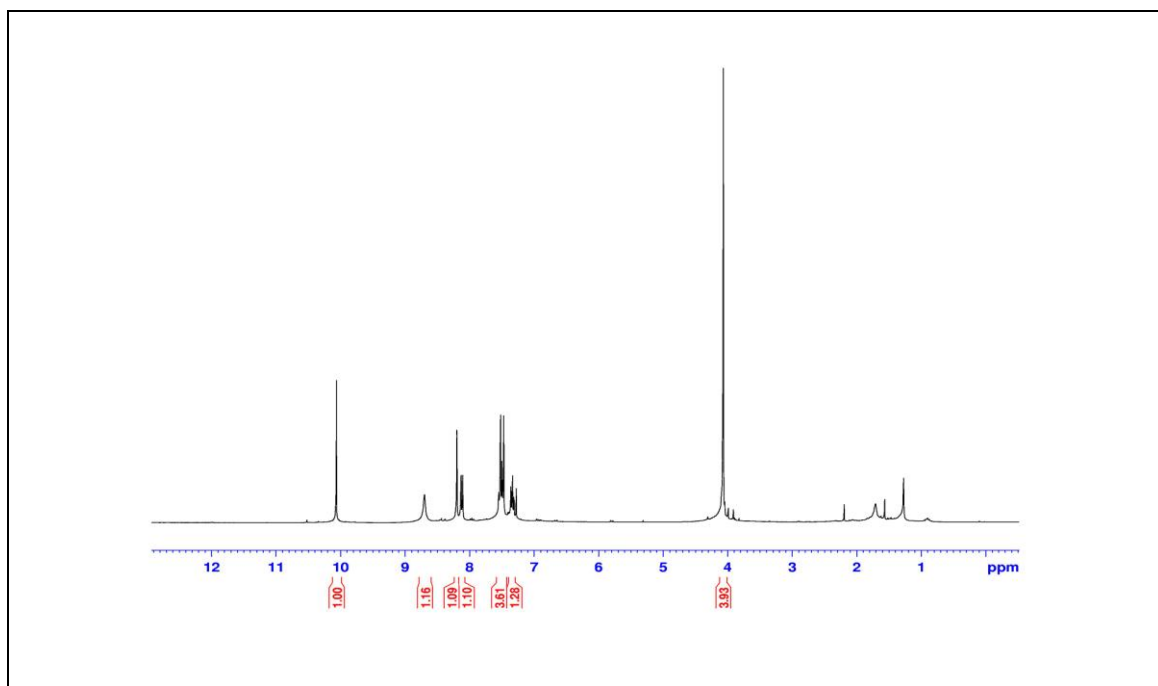


Figure 116 ^1H NMR (300 MHz) (CDCl_3) of compound **RM13**

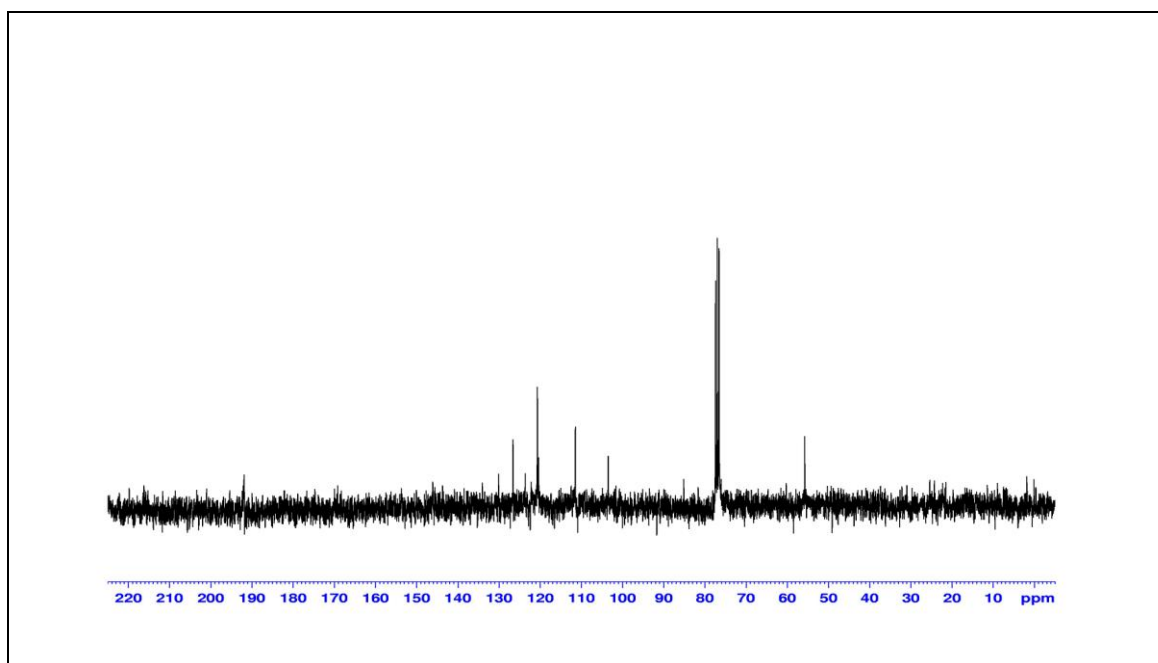


Figure 117 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM13**

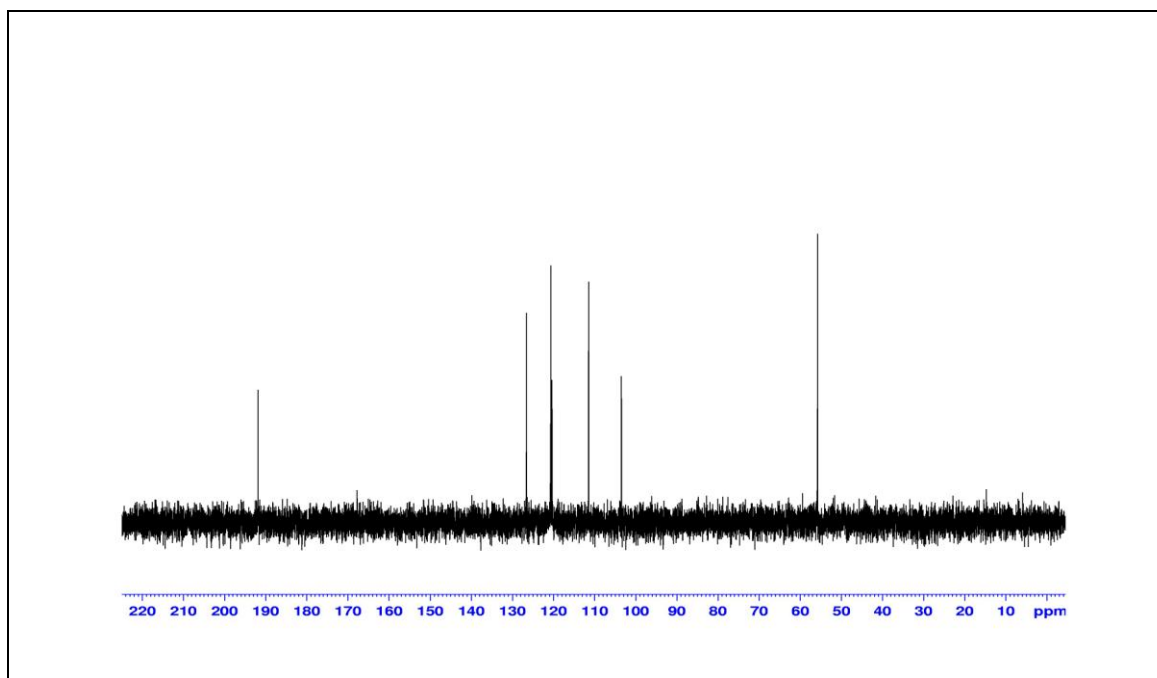


Figure 118 DEPT 135° (CDCl₃) of compound **RM13**

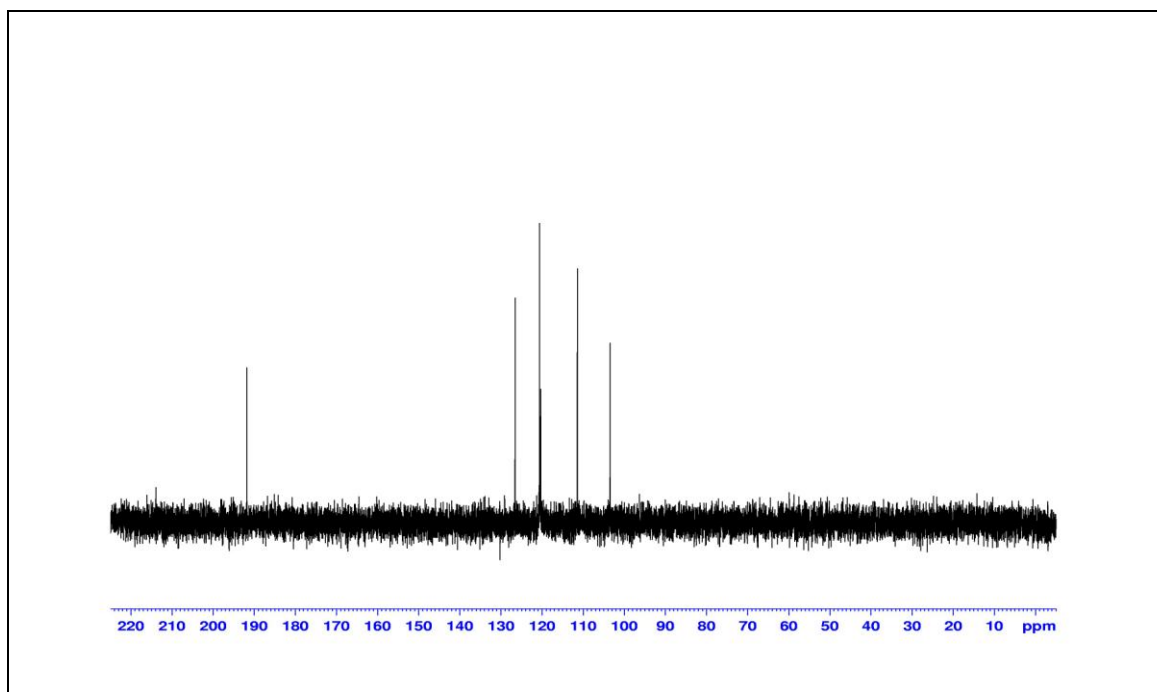


Figure 119 DEPT 90° (CDCl₃) of compound **RM13**

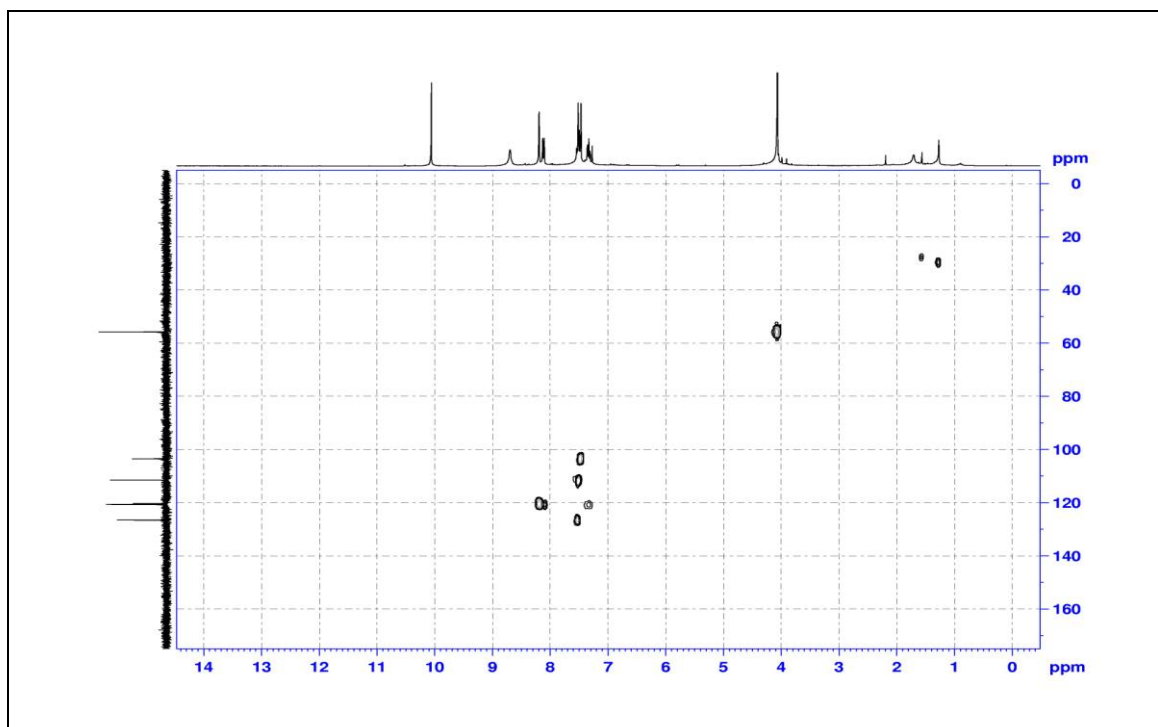


Figure 120 2D HMQC (CDCl₃) of compound **RM13**

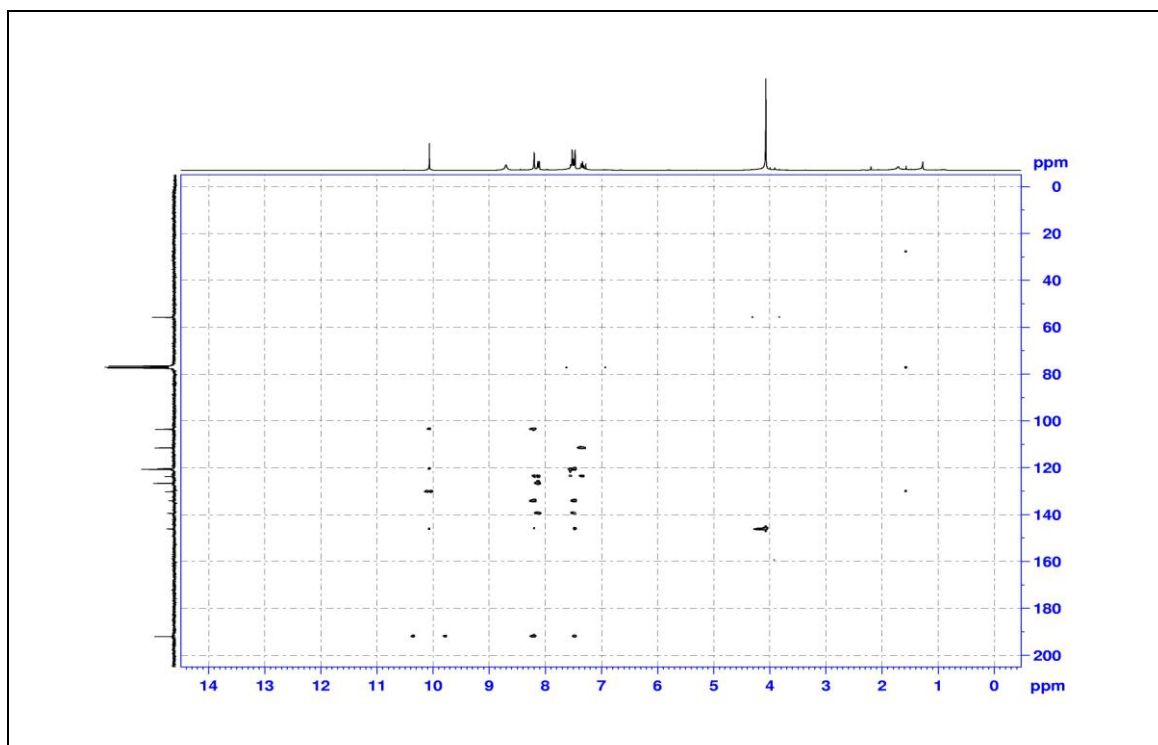


Figure 121 2D HMBC (CD₃COCD₃) of compound **RM13**

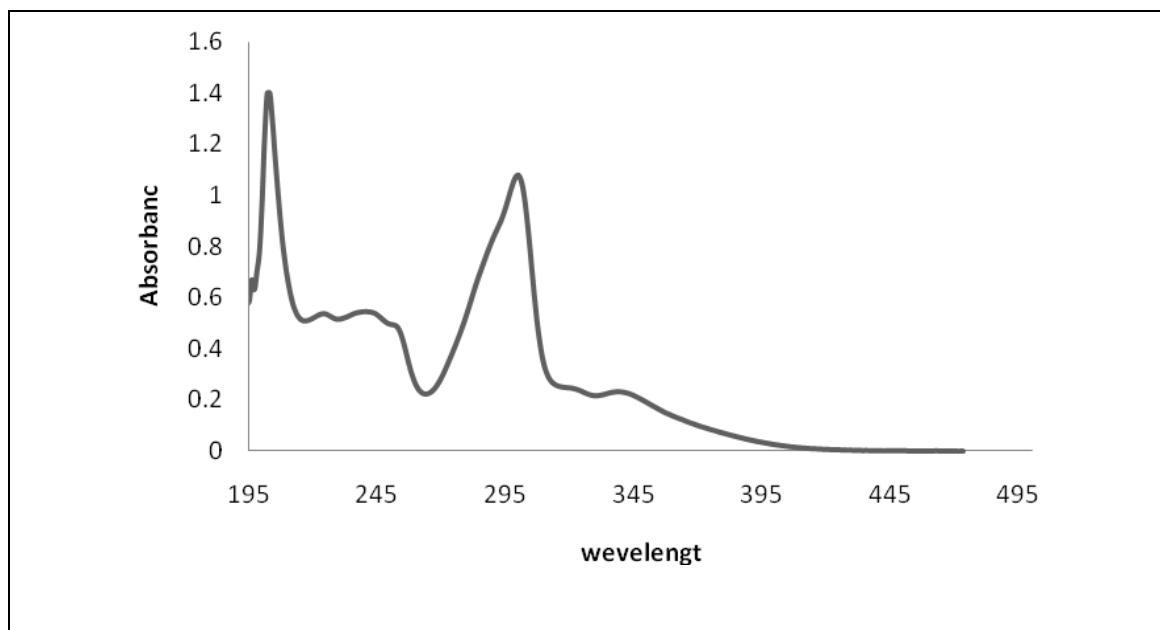


Figure 122 UV (MeOH) spectrum of compound **RM14**

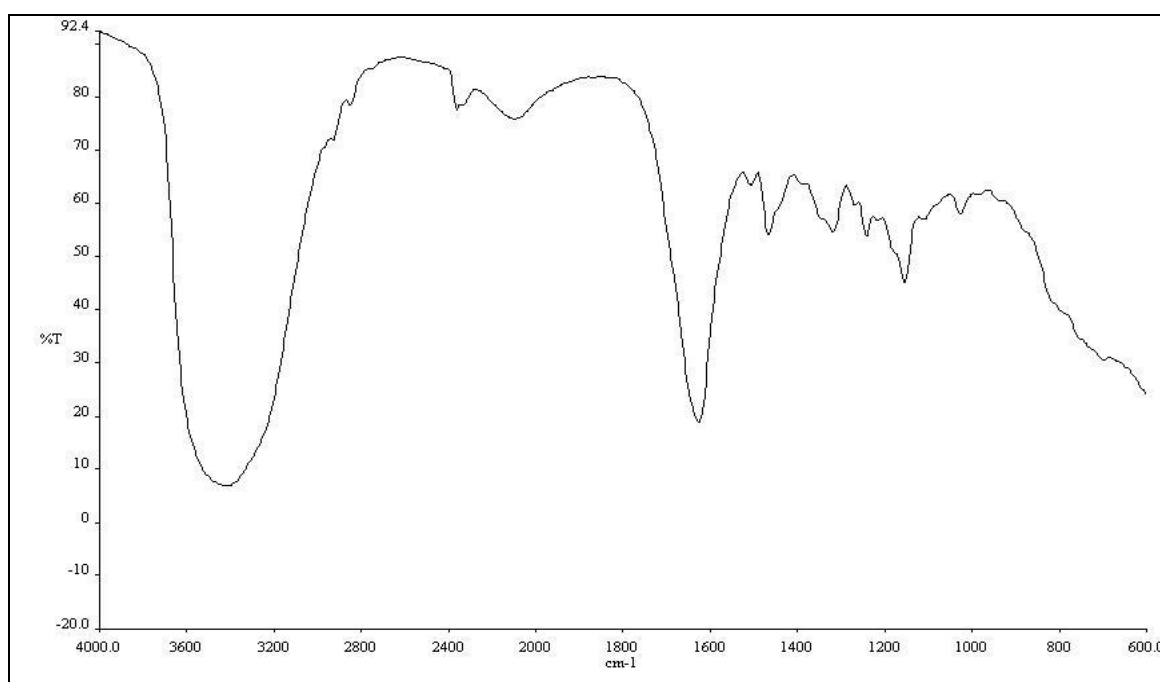


Figure 123 IR (neat) spectrum of compound **RM14**

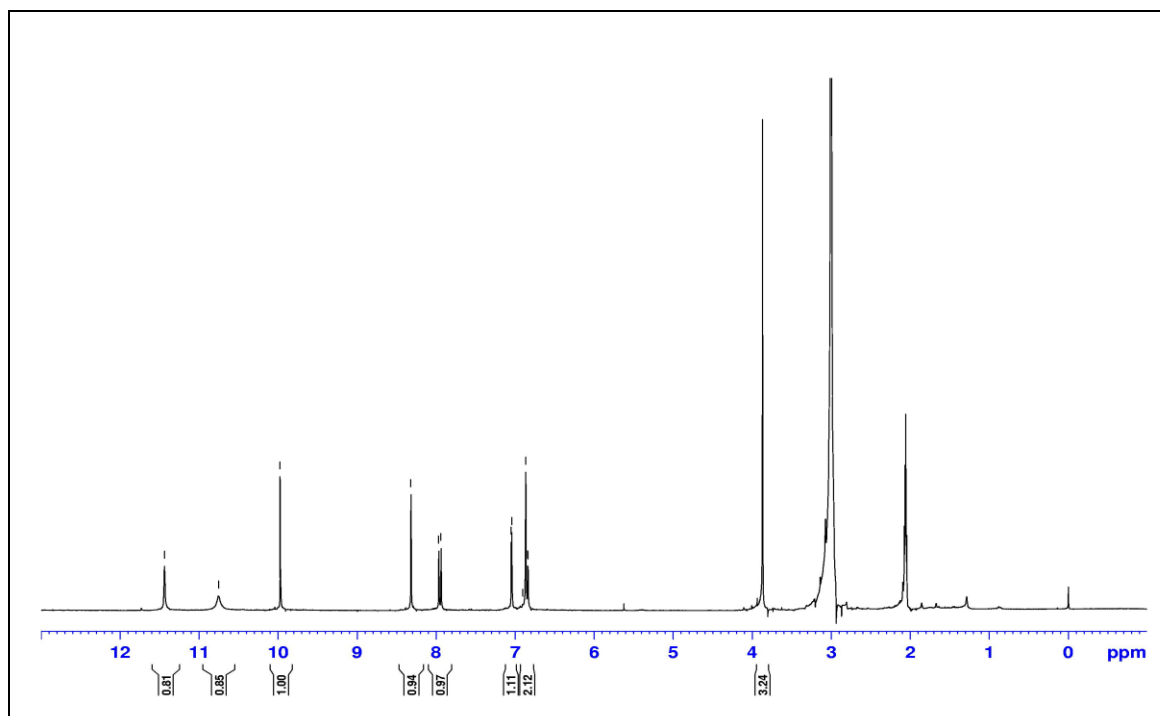


Figure 124 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM14**

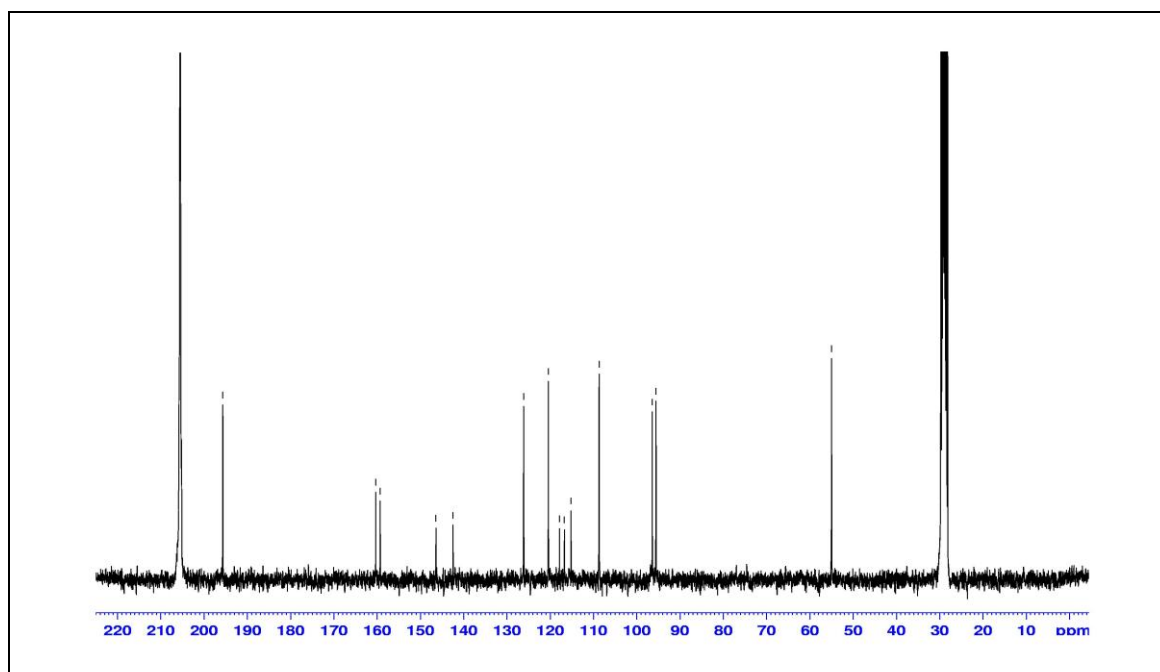


Figure 125 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM14**

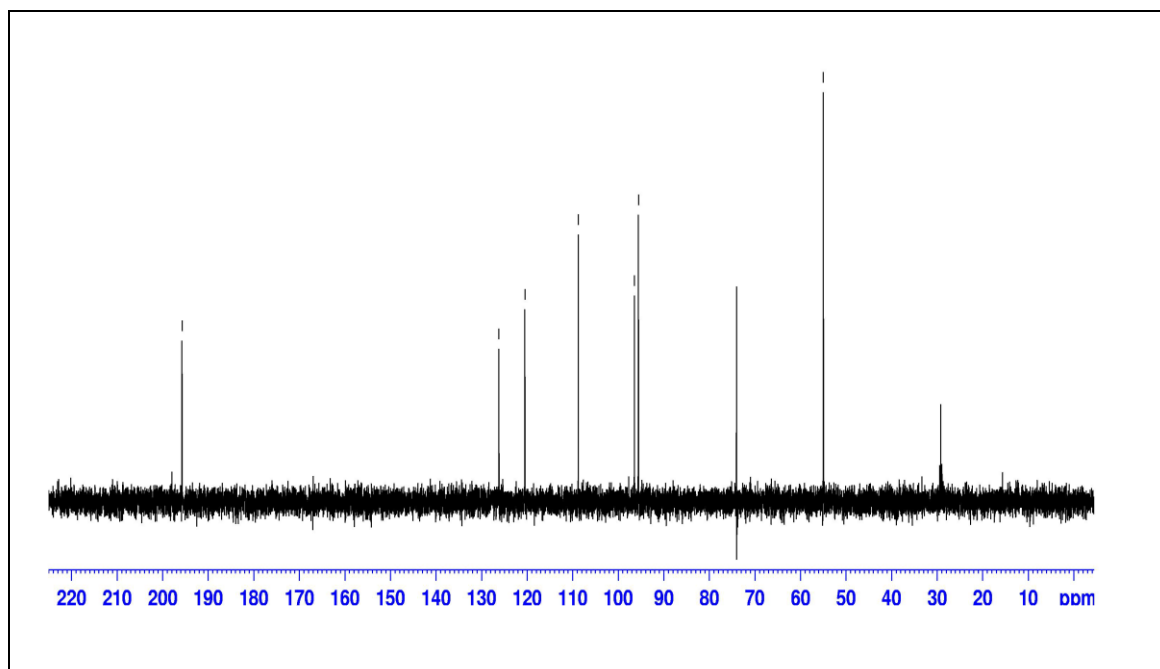


Figure 126 DEPT 135° (CD_3COCD_3) of compound **RM14**

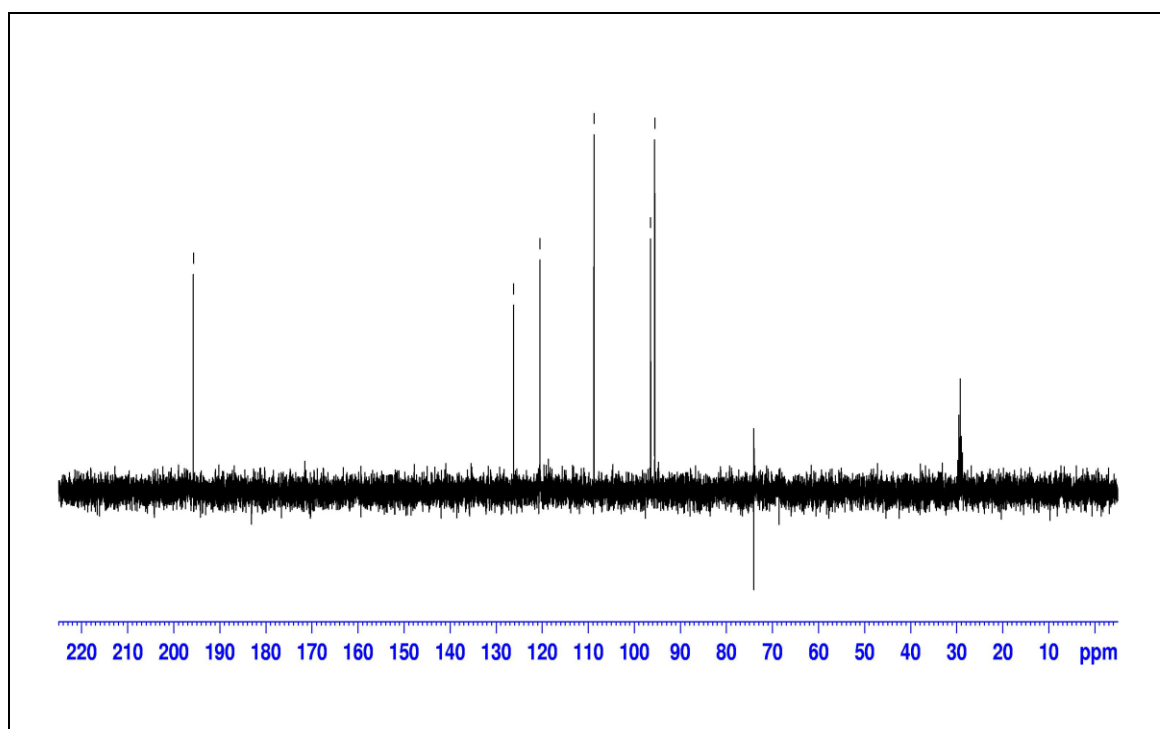


Figure 127 DEPT 90° (CD_3COCD_3) of compound **RM14**

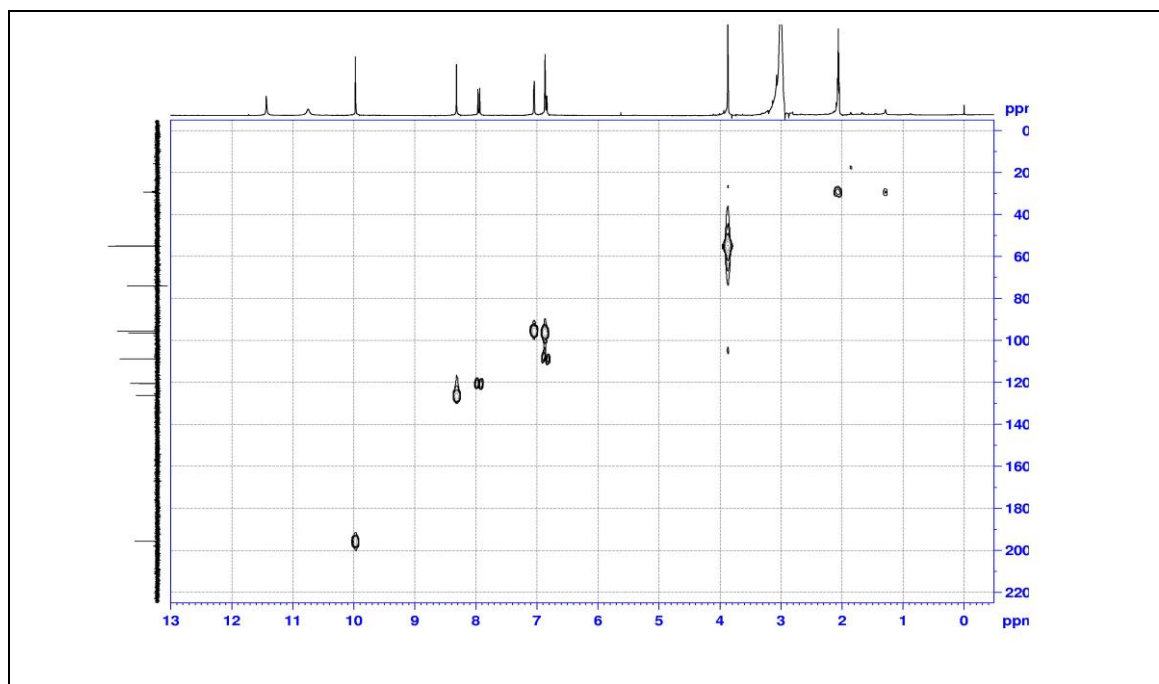


Figure 128 2D HMQC (CD₃COCD₃) of compound RM14

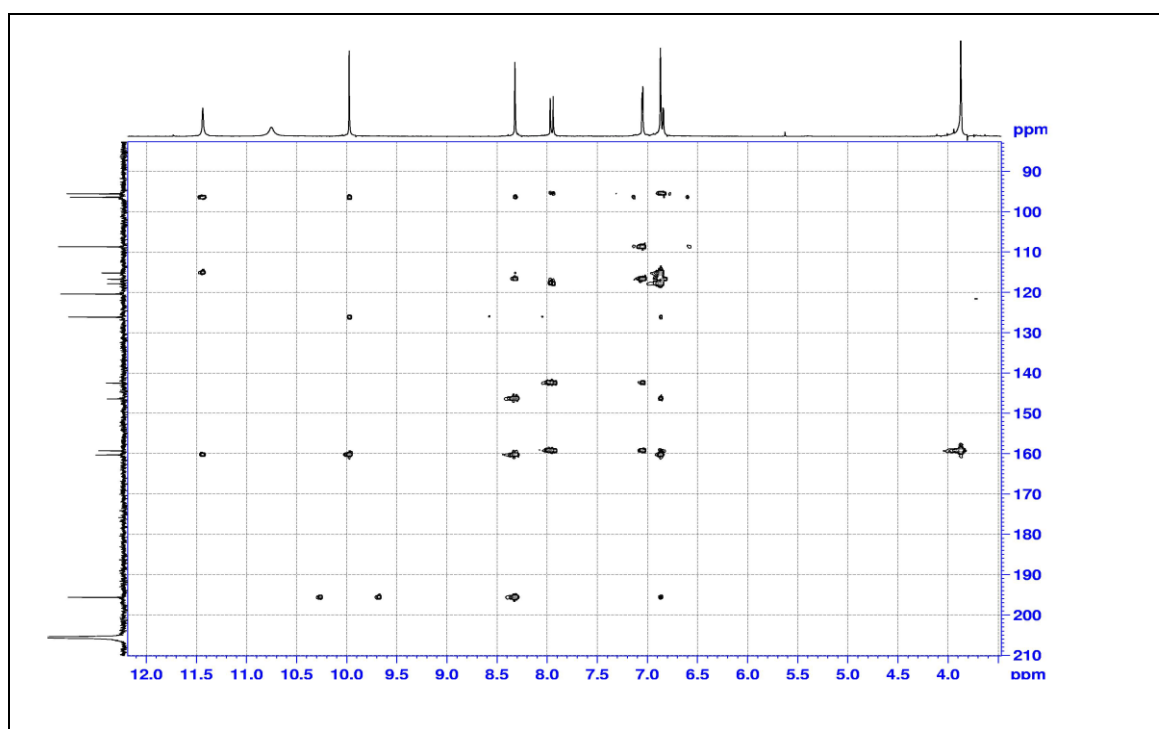


Figure 129 2D HMBC (CD₃COCD₃) of compound RM14

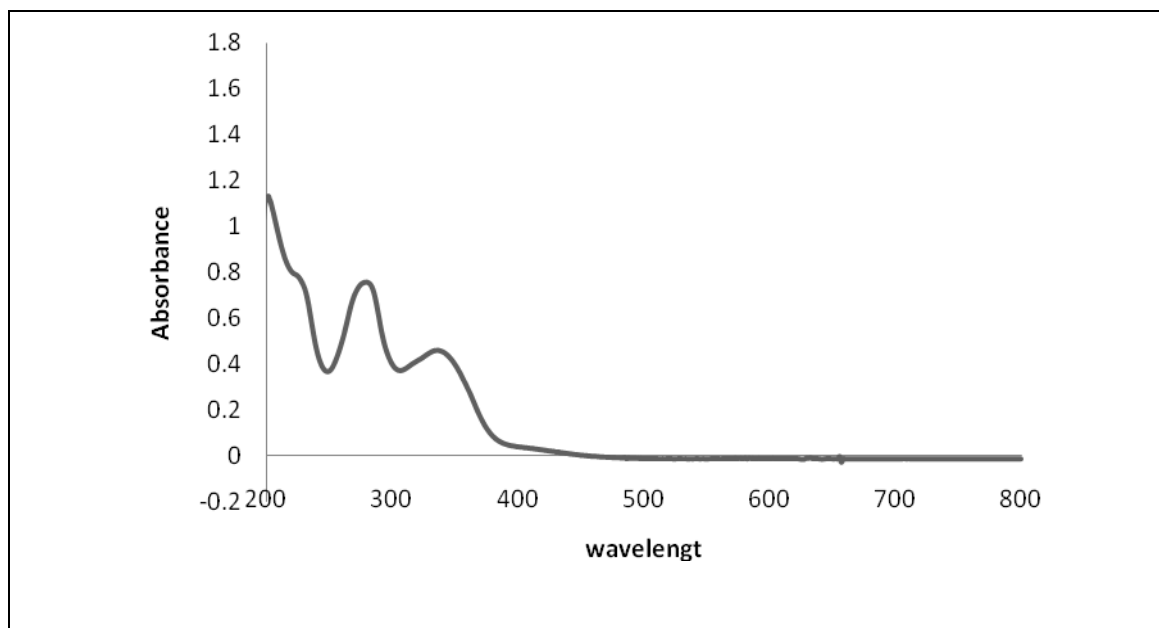


Figure 130 UV (MeOH) spectrum of compound **RM15**

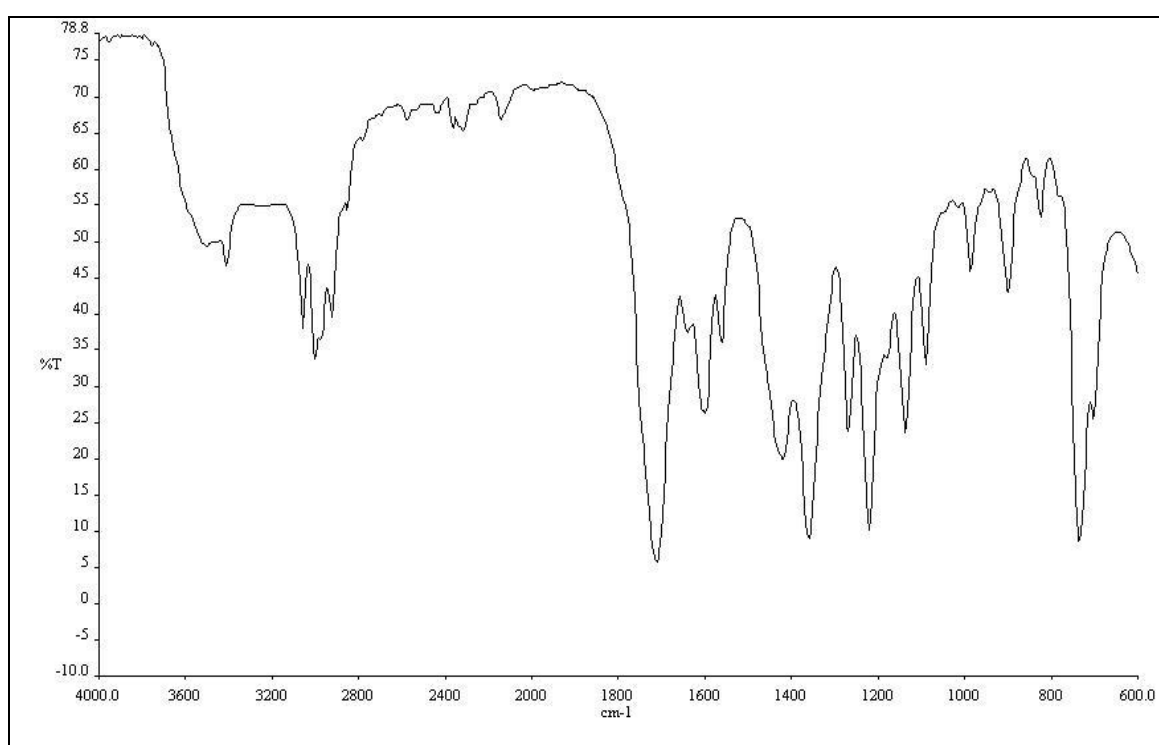


Figure 131 IR (neat) spectrum of compound **RM15**

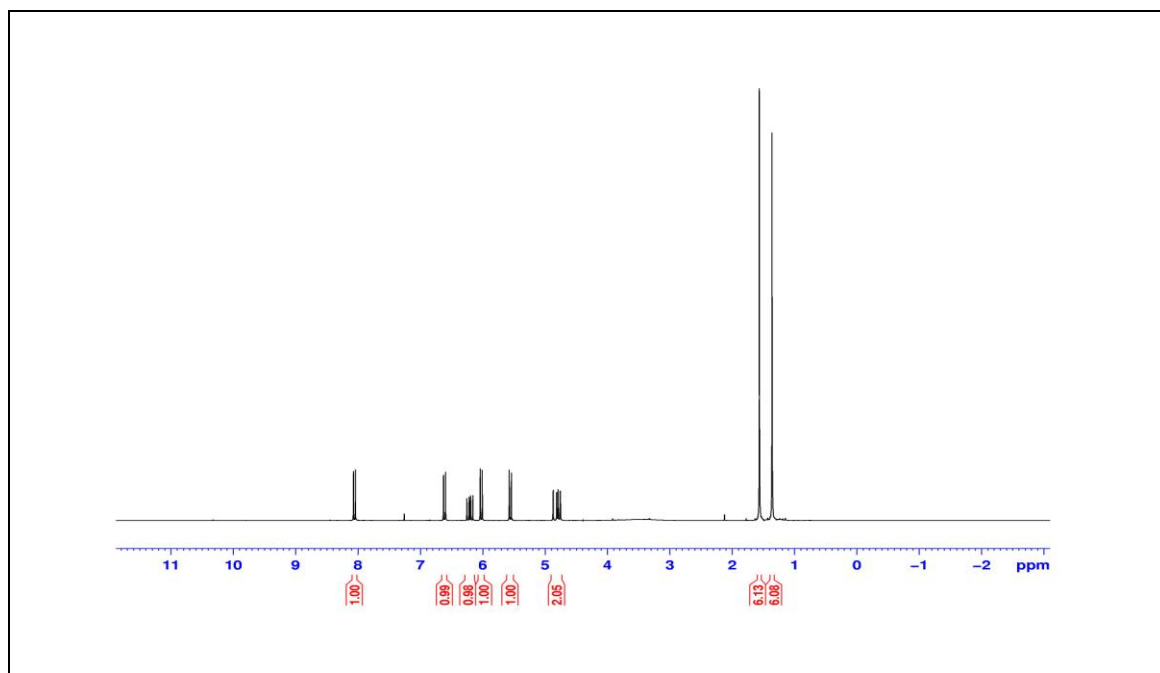


Figure 132 ^1H NMR (300 MHz) (CDCl_3) of compound **RM15**

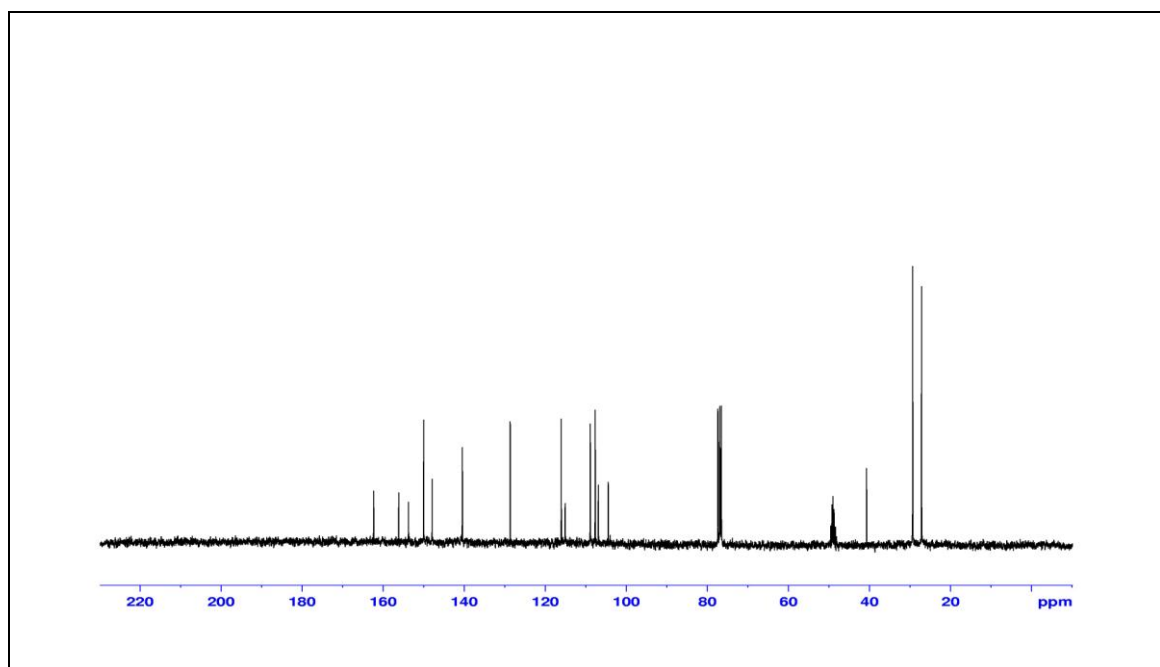


Figure 133 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM15**

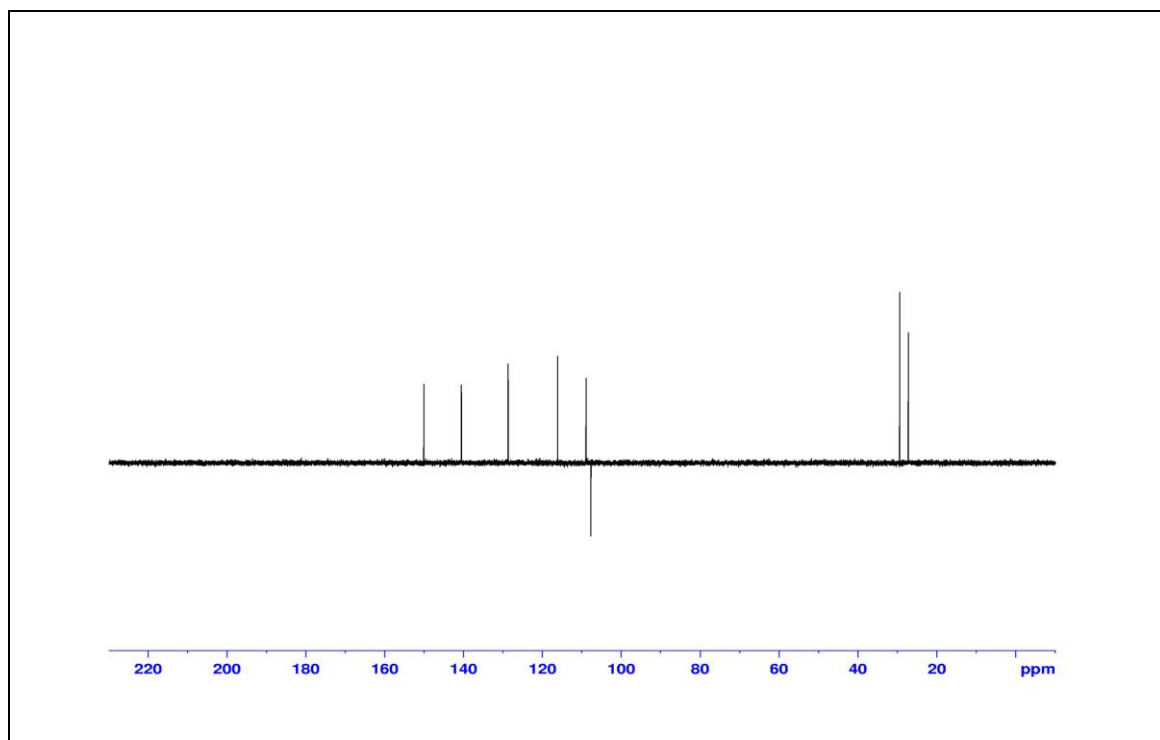


Figure 134 DEPT 135° (CDCl₃) of compound **RM15**

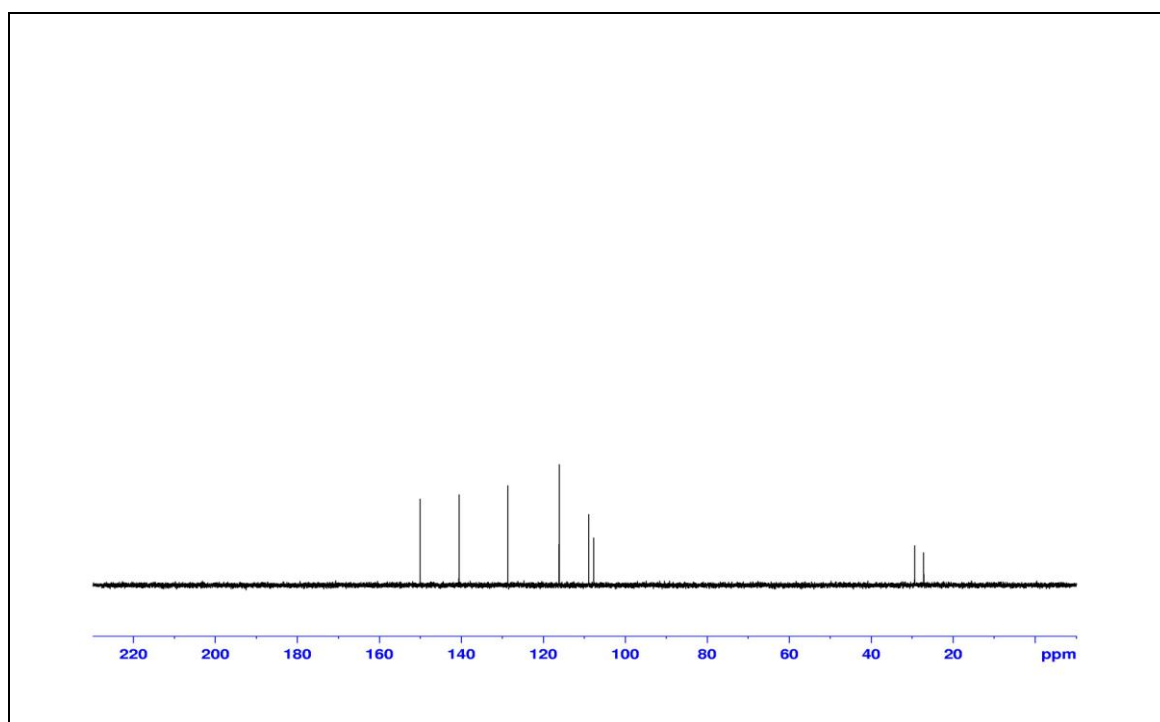


Figure 135 DEPT 90° (CDCl₃) of compound **RM15**

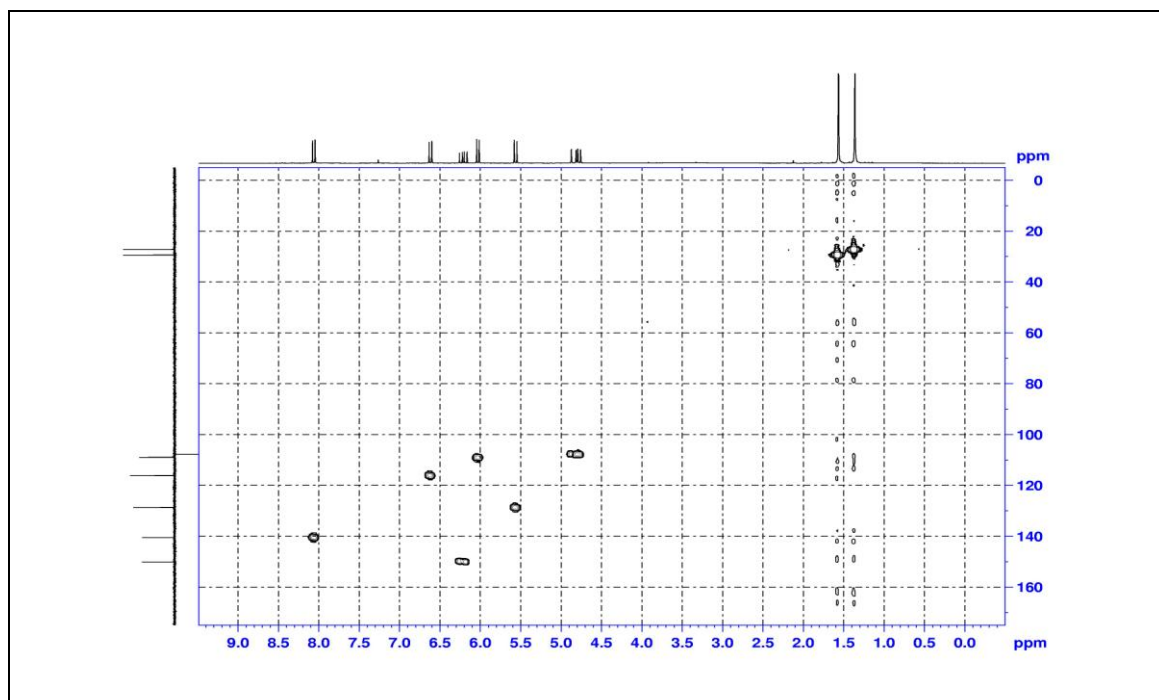


Figure 136 2D HMQC (CDCl_3) of compound **RM15**

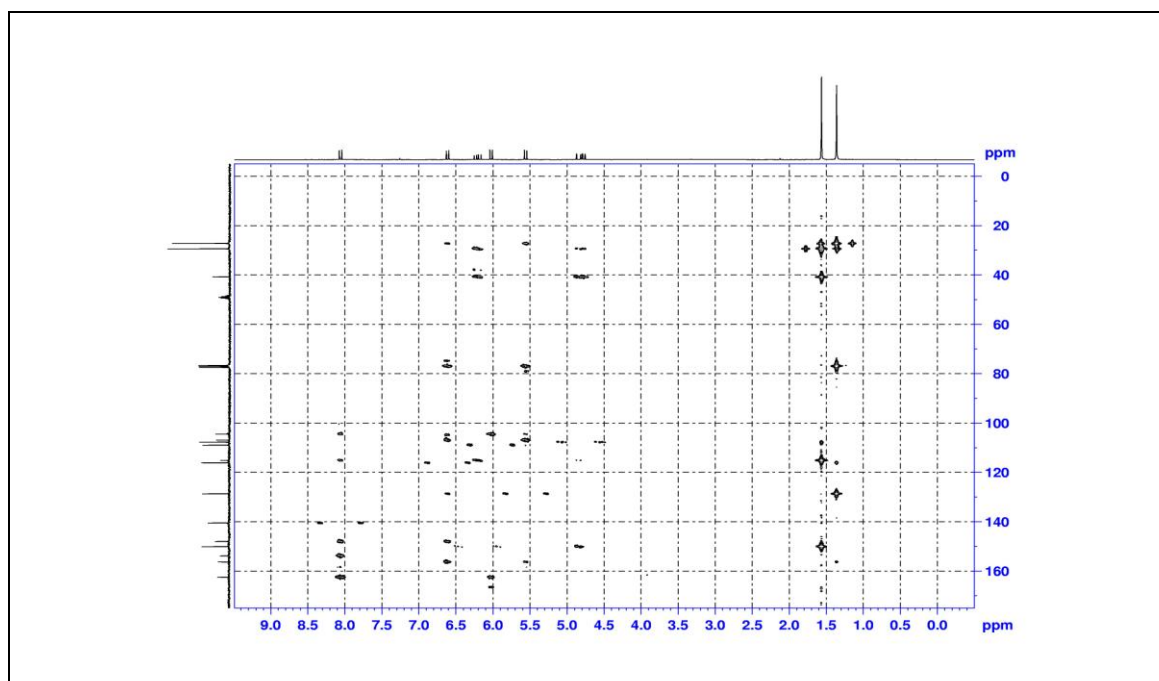


Figure 137 2D HMBC (CDCl_3) of compound **RM15**

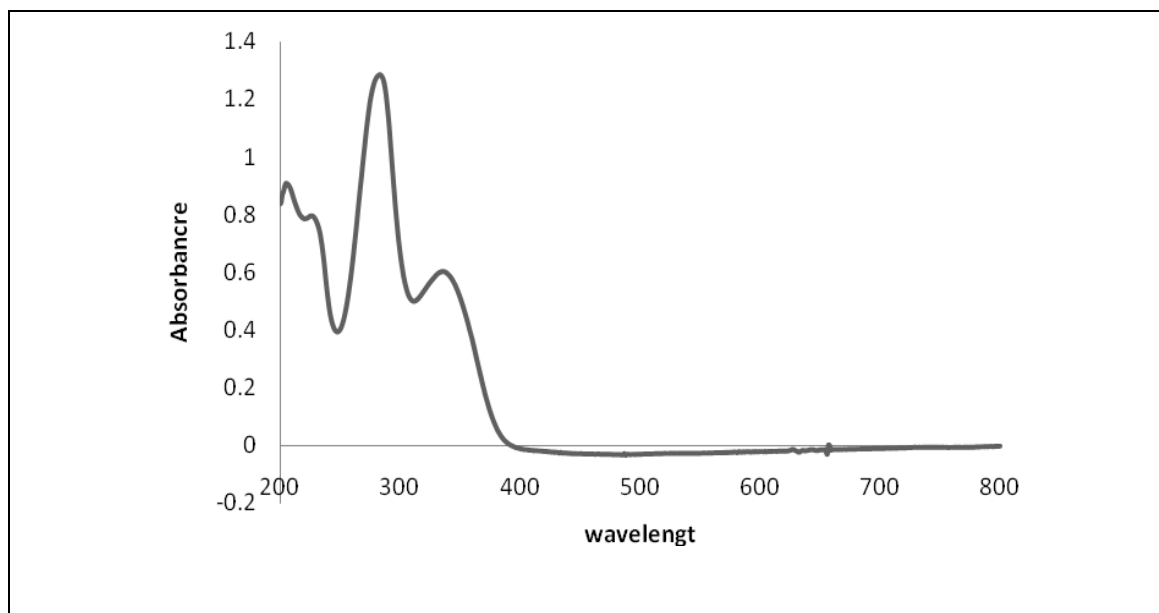


Figure 138 UV (MeOH) spectrum of compound **RM16**

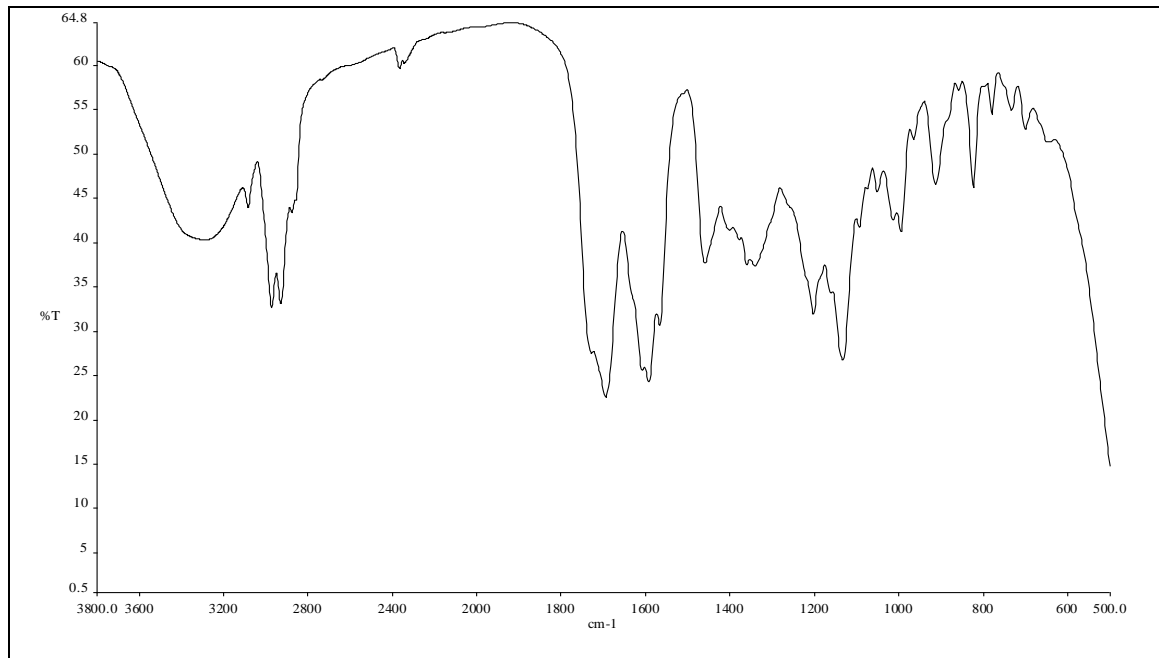


Figure 139 IR (neat) spectrum of compound **RM16**

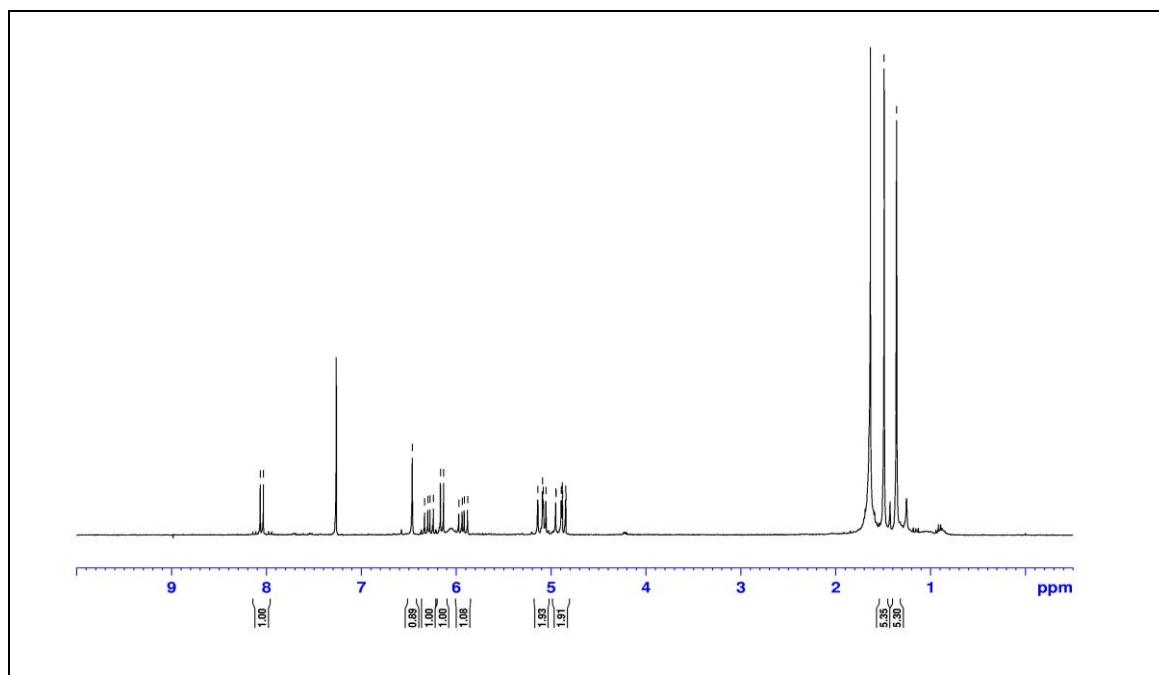


Figure 140 ^1H NMR (300 MHz) (CDCl_3) of compound **RM16**

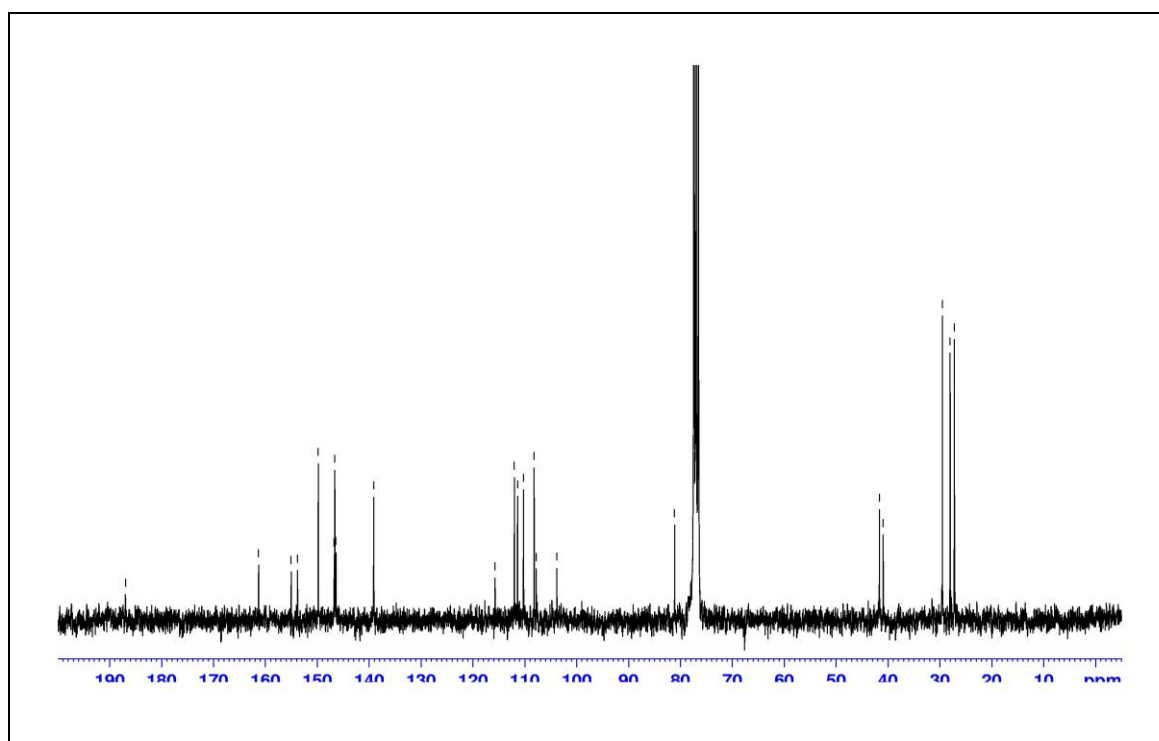


Figure 141 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM16**

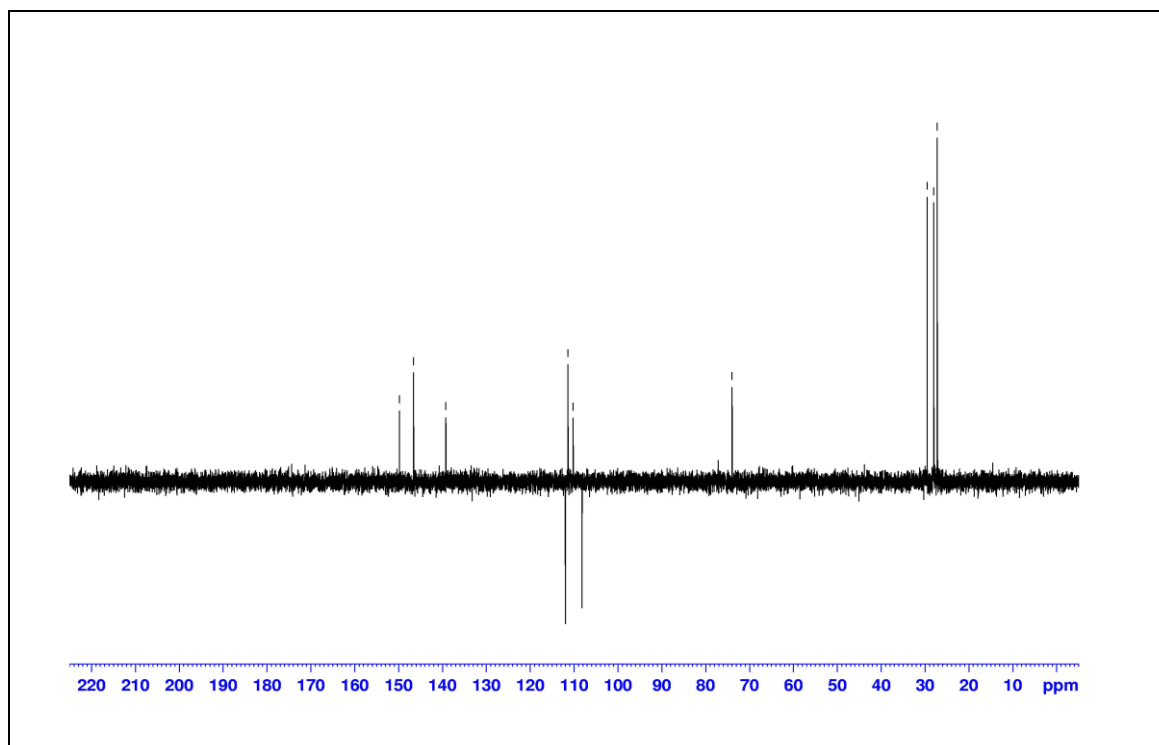


Figure 142 DEPT 135° (CDCl₃) of compound **RM16**

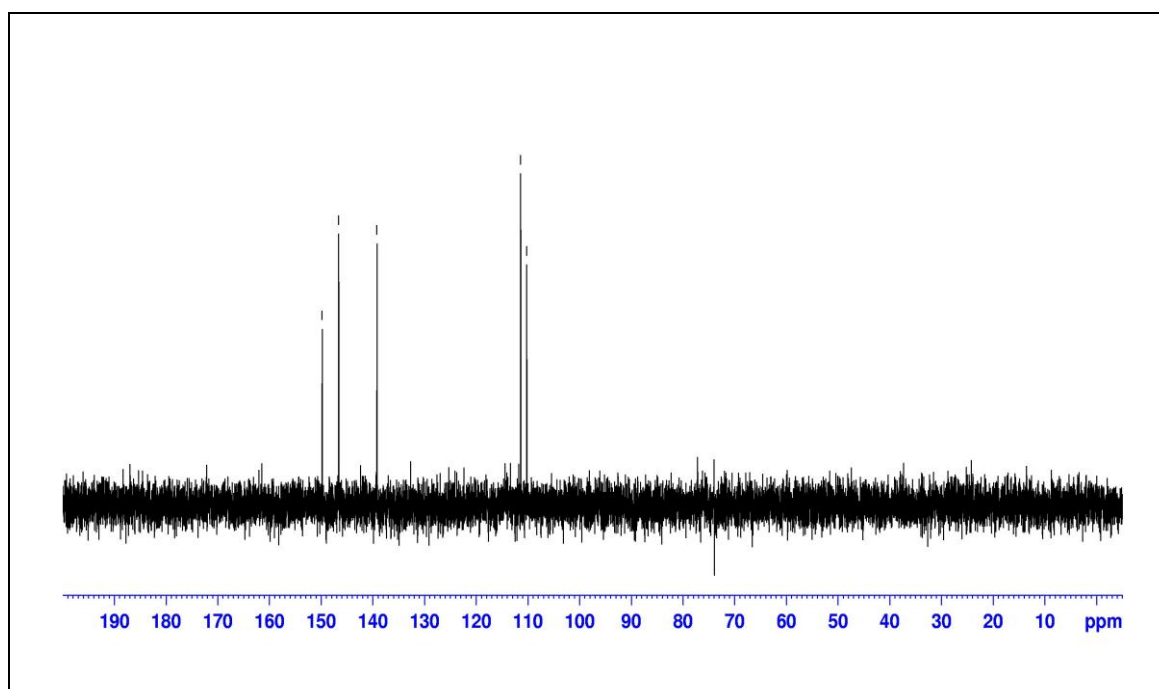


Figure 143 DEPT 90° (CDCl₃) of compound **RM16**

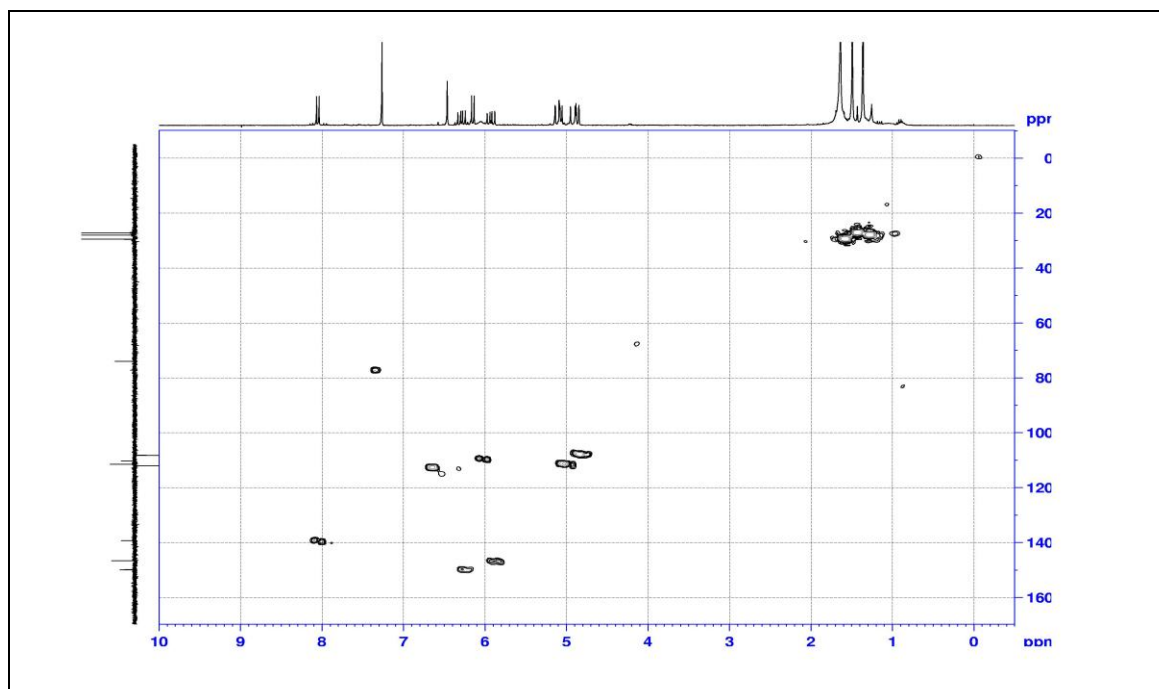


Figure 144 2D HMQC (CDCl_3) of compound **RM16**

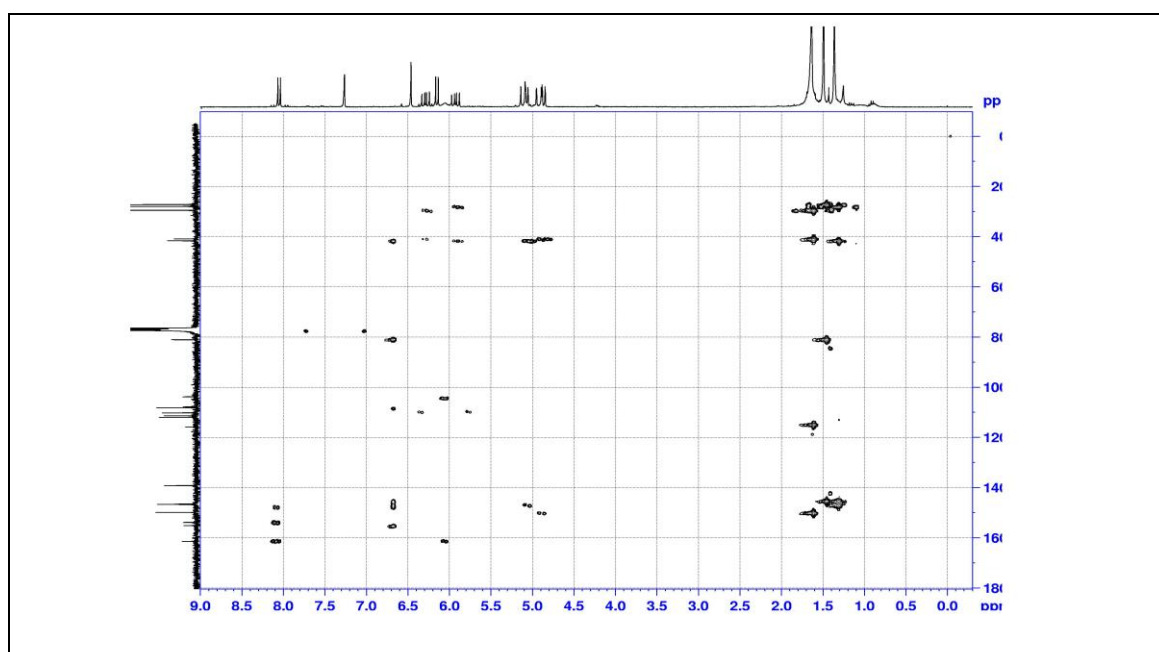


Figure 145 2D HMBC (CDCl_3) of compound **RM16**

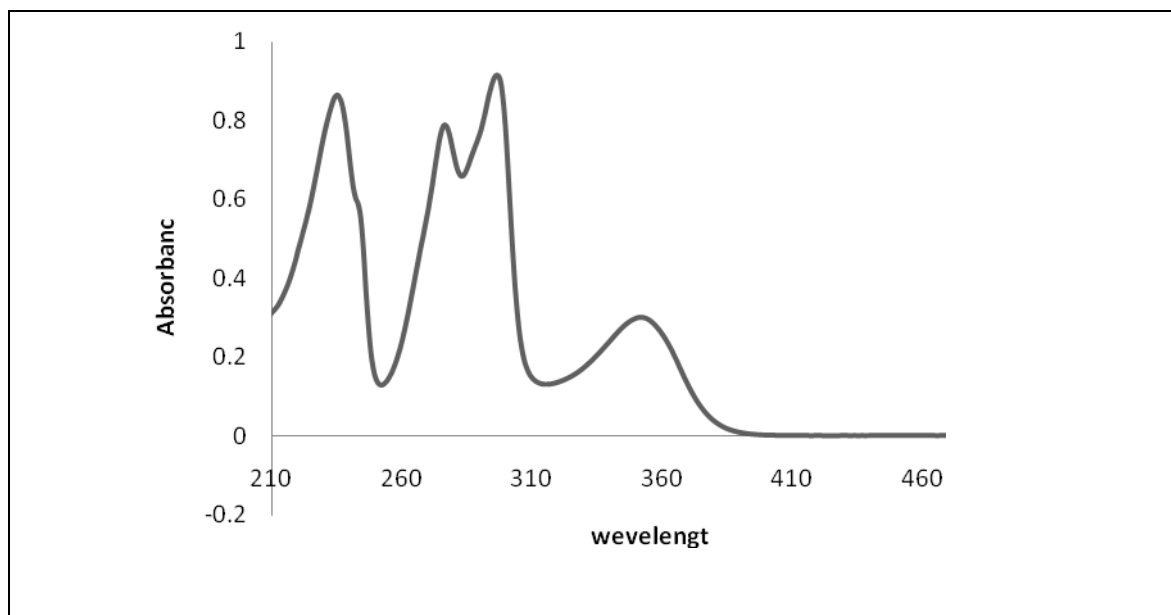


Figure 146 UV (MeOH) spectrum of compound **RM17**

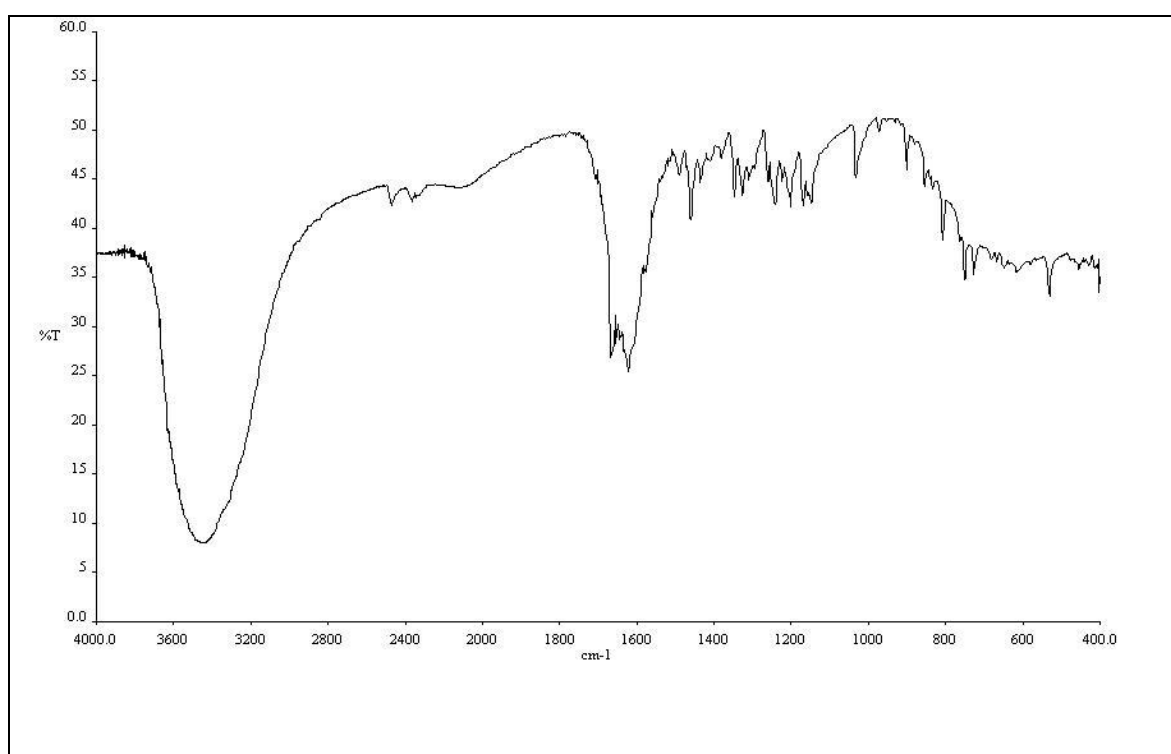


Figure 147 IR (neat) spectrum of compound **RM17**

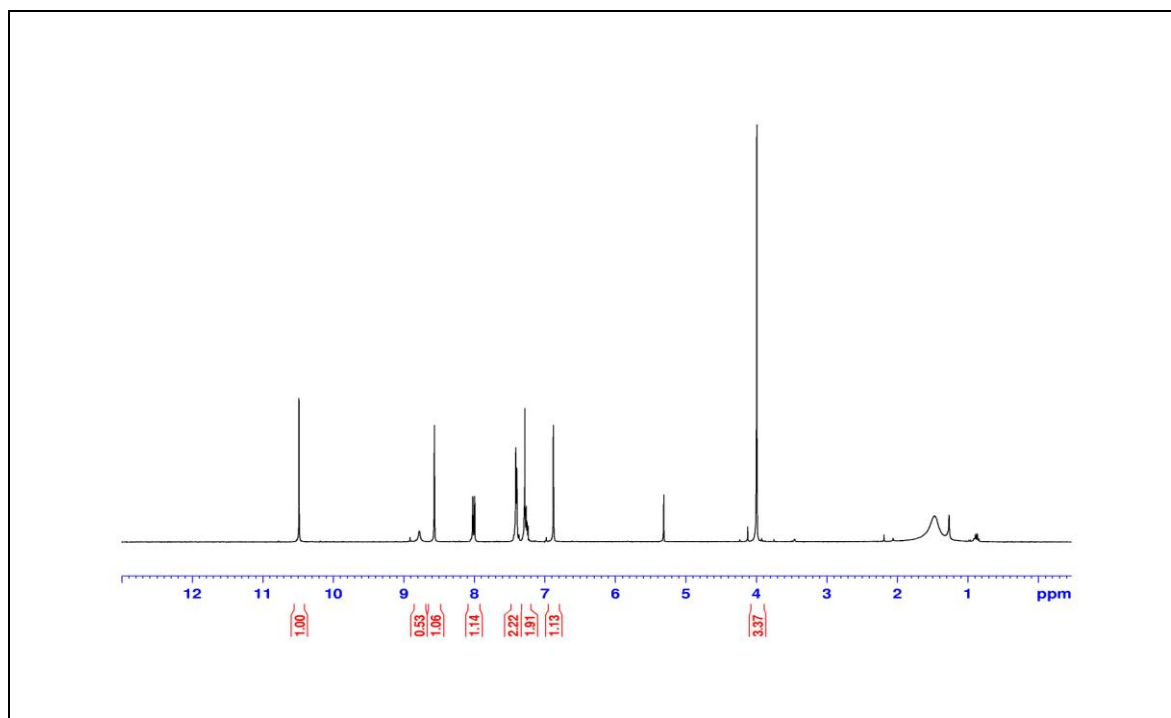


Figure 148 ^1H NMR (300 MHz) (CDCl_3) of compound **RM17**

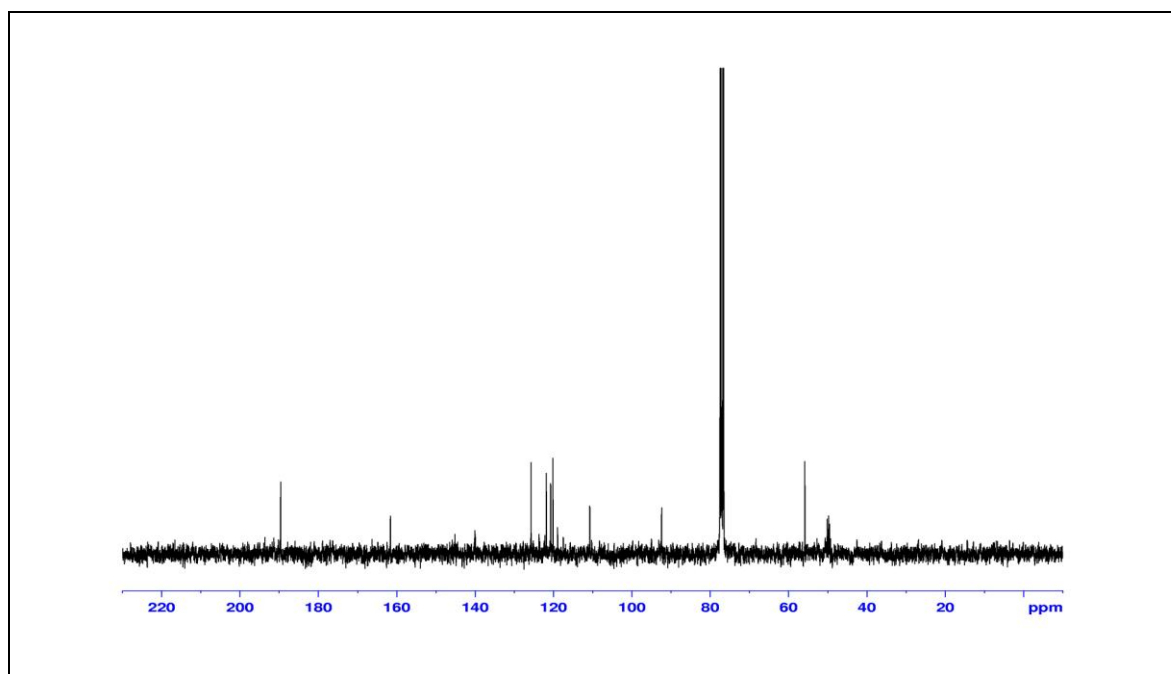


Figure 149 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM17**

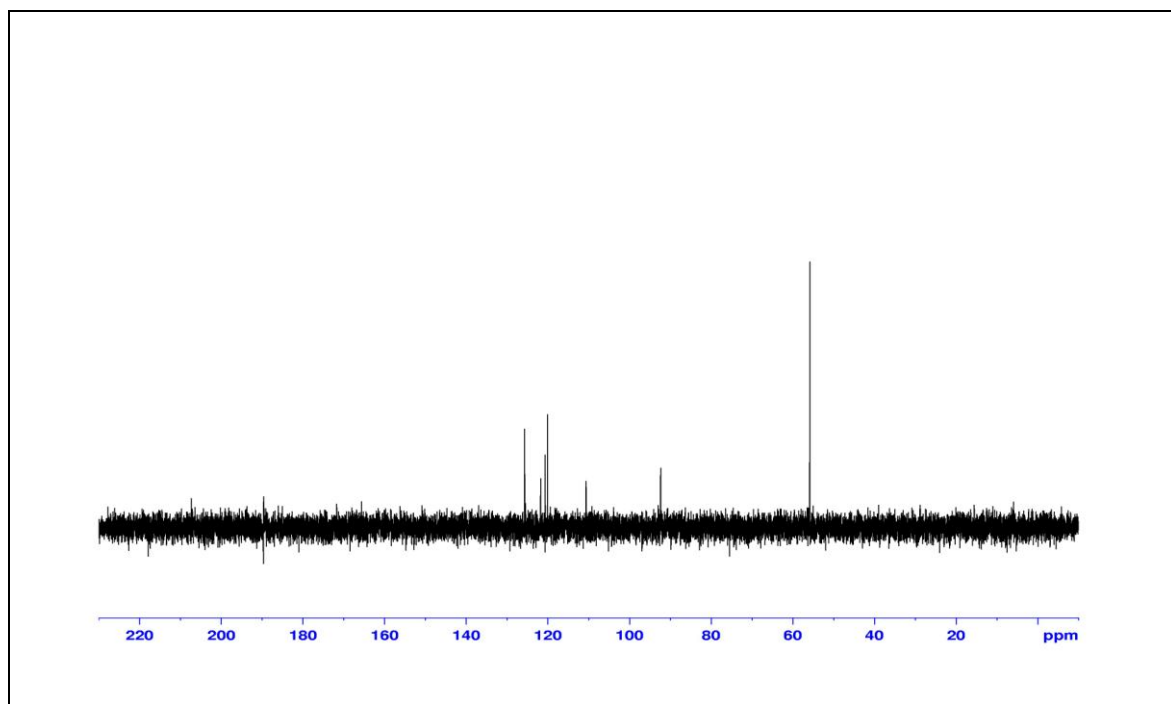


Figure 150 DEPT 135° (CDCl₃) of compound **RM17**

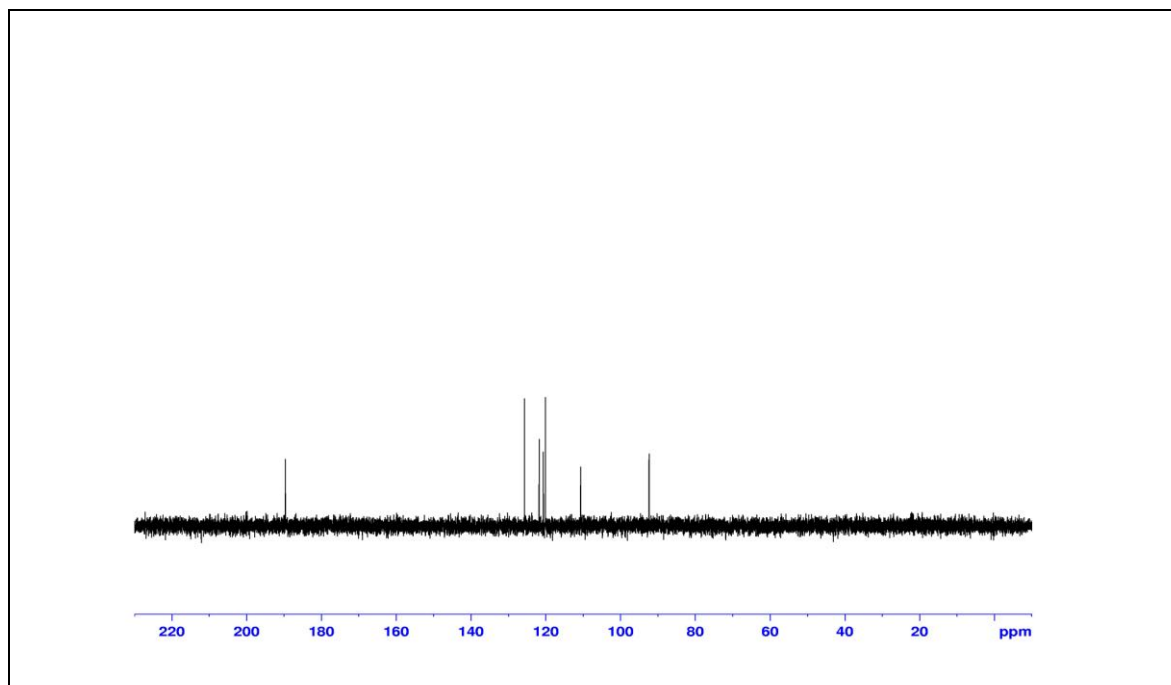


Figure 151 DEPT 90° (CDCl₃) of compound **RM17**

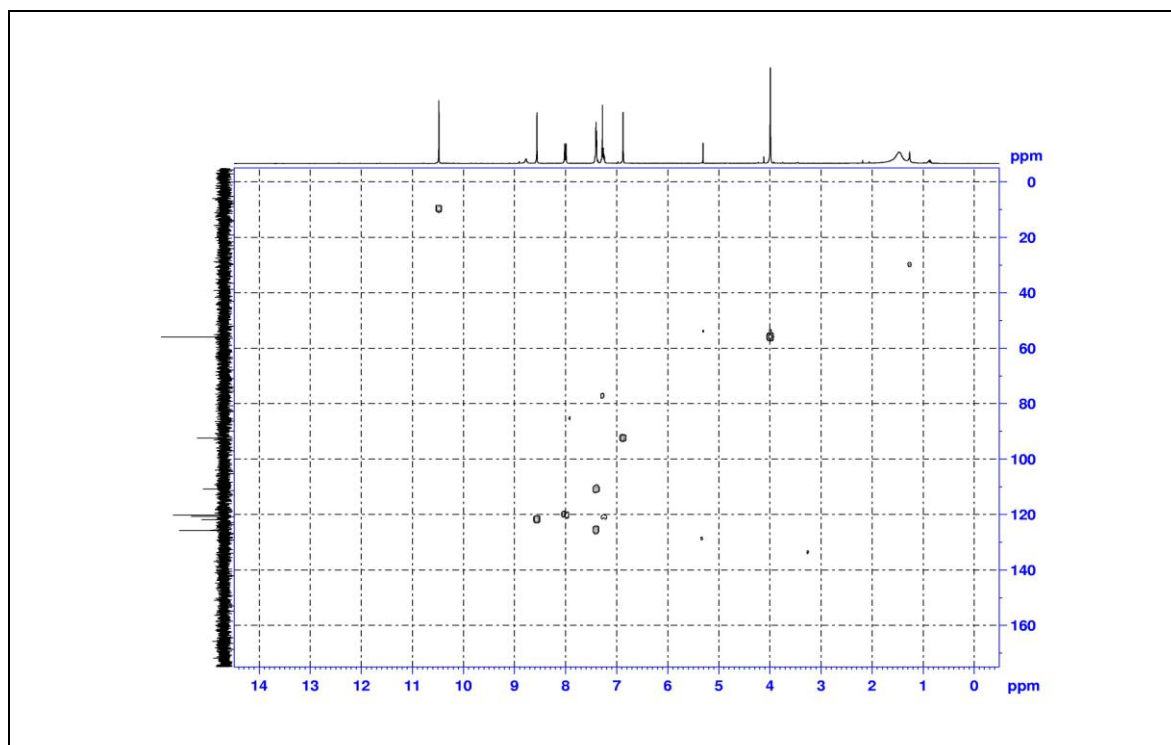


Figure 152 2D HMQC (CDCl_3) of compound **RM17**

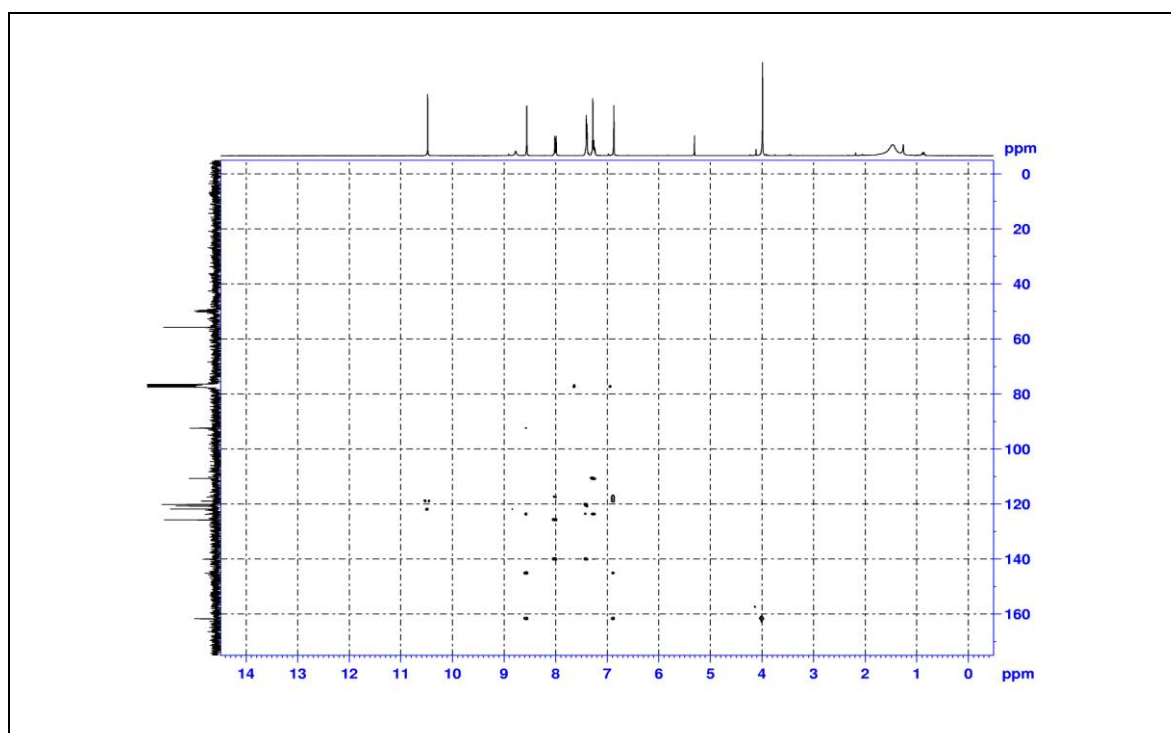


Figure 153 2D HMBC (CDCl_3) of compound **RM17**

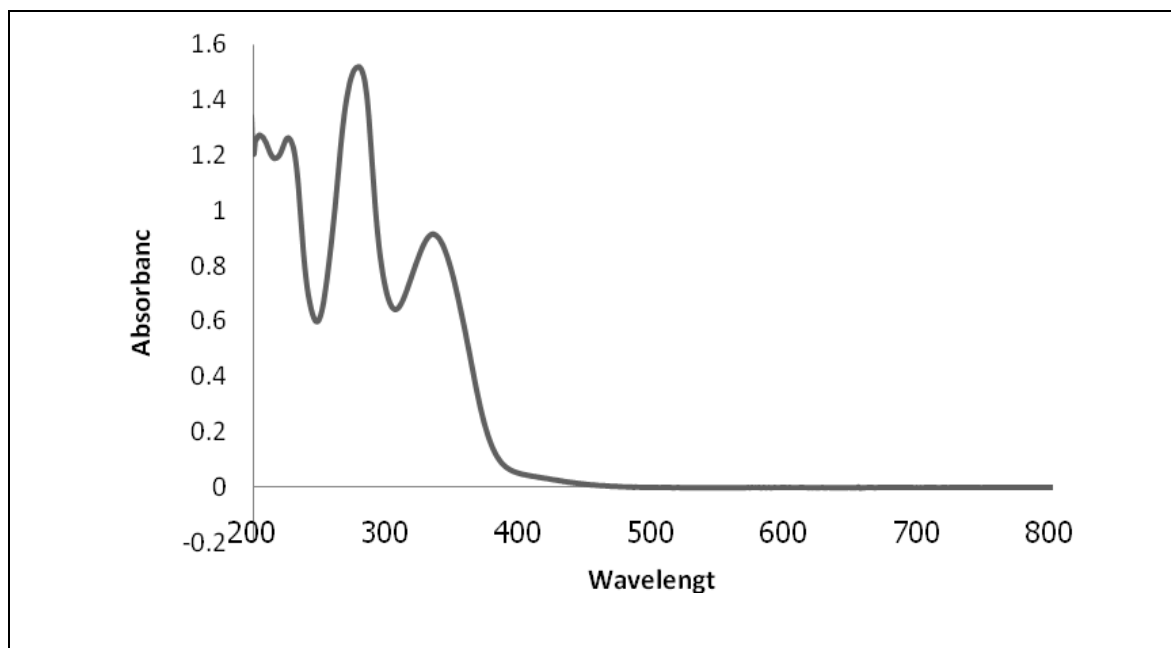


Figure 154 UV (MeOH) spectrum of compound **RM18**

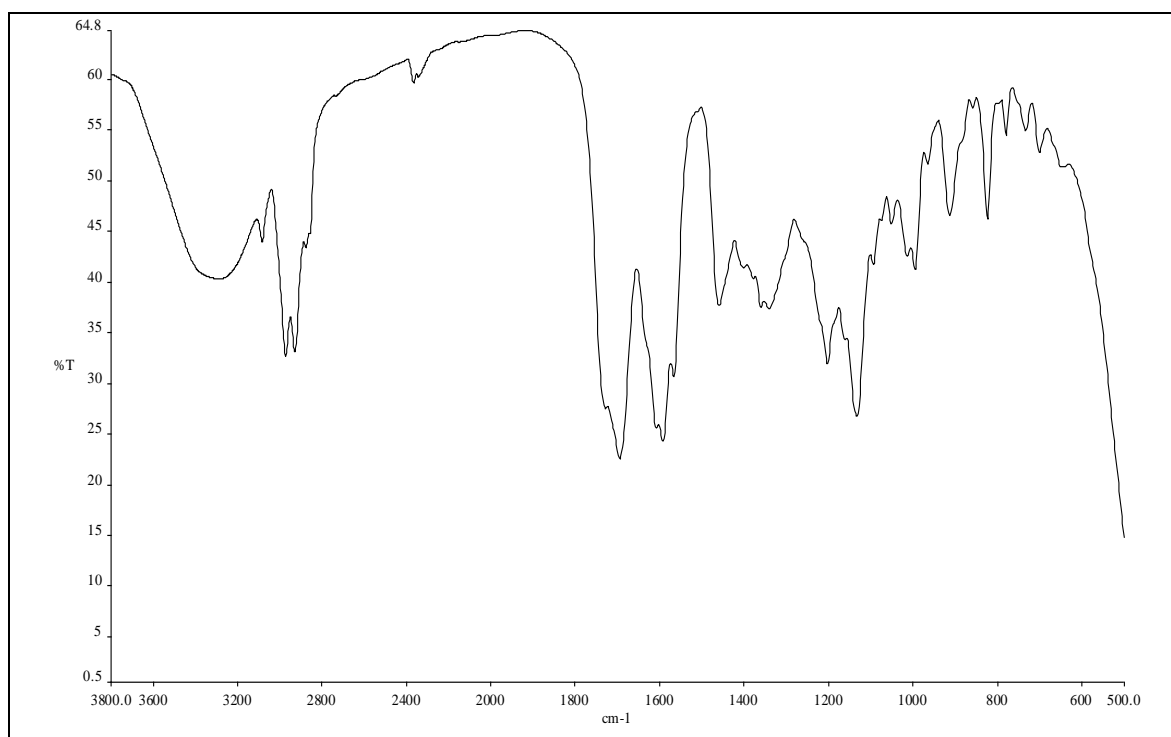


Figure 155 IR (neat) spectrum of compound **RM18**

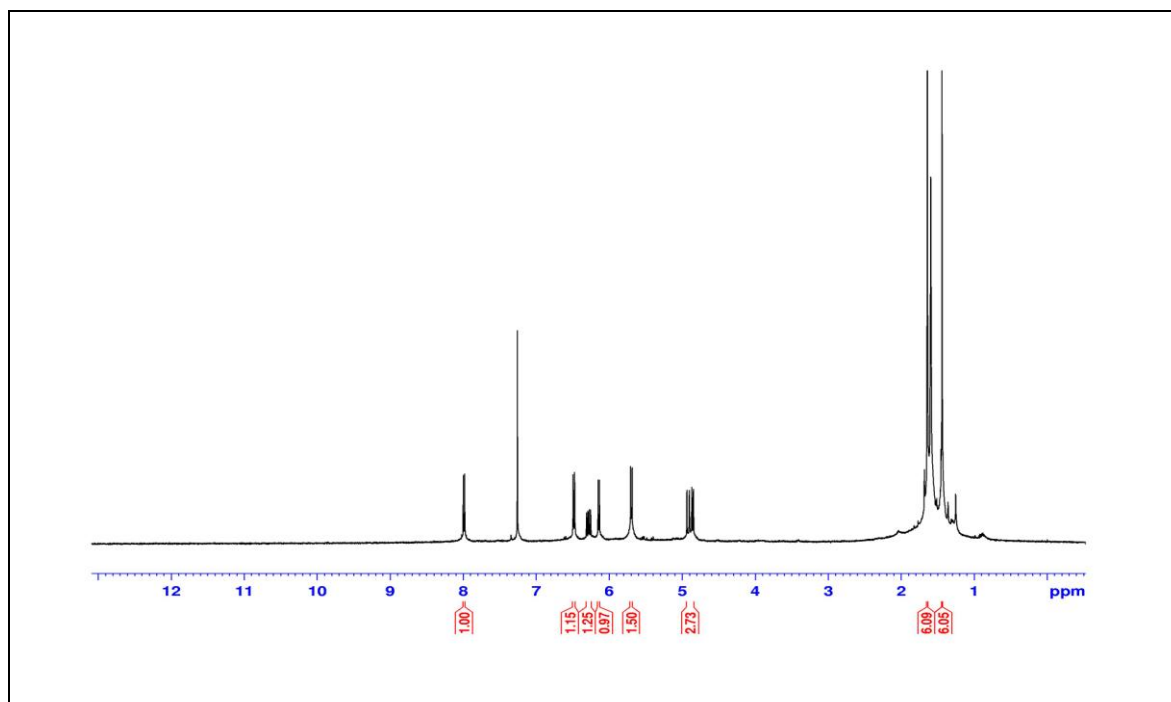


Figure 156 ^1H NMR (300 MHz) (CDCl_3) of compound **RM18**

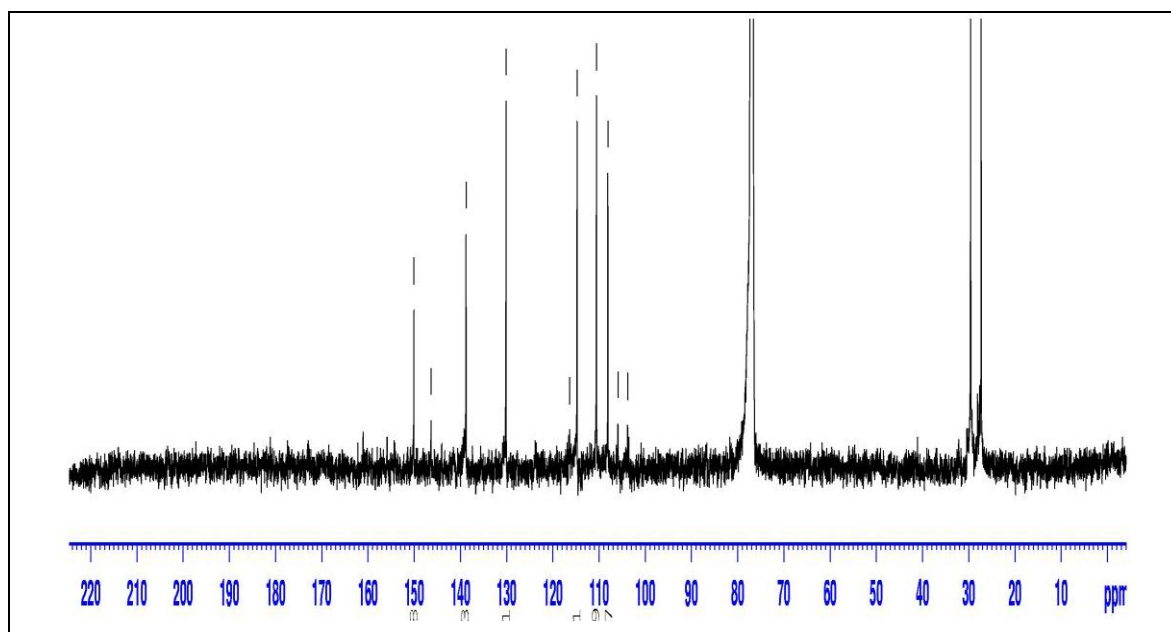


Figure 157 ^{13}C NMR (75 MHz) (CDCl_3) of compound **RM18**

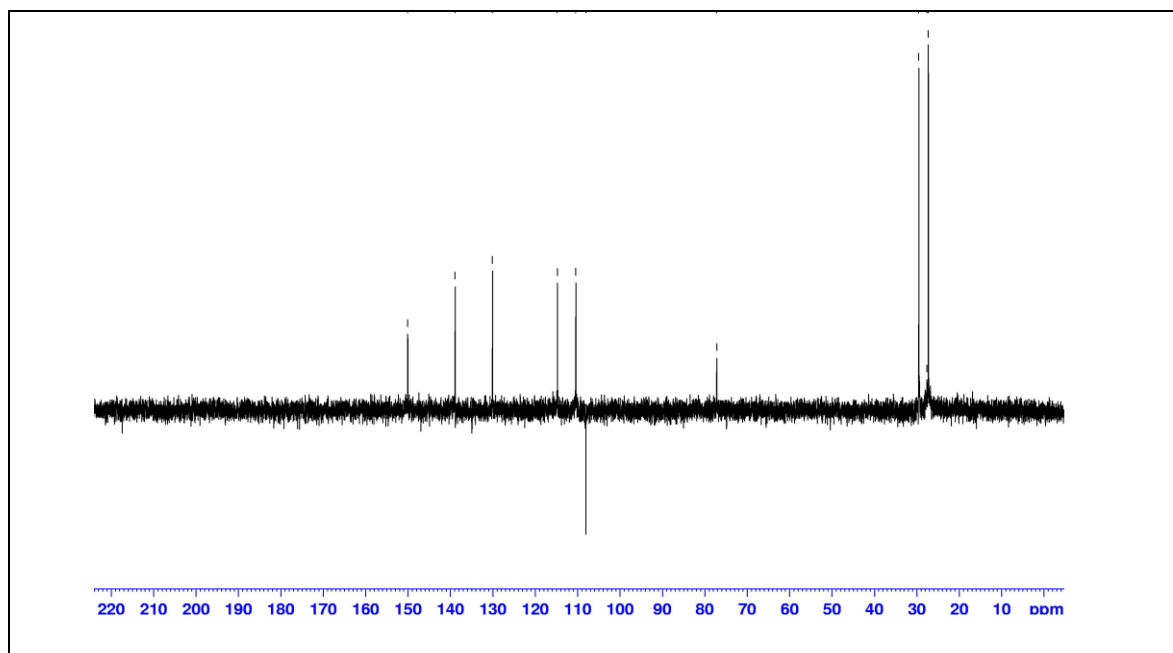


Figure 158 DEPT 135° (CDCl₃) of compound **RM18**

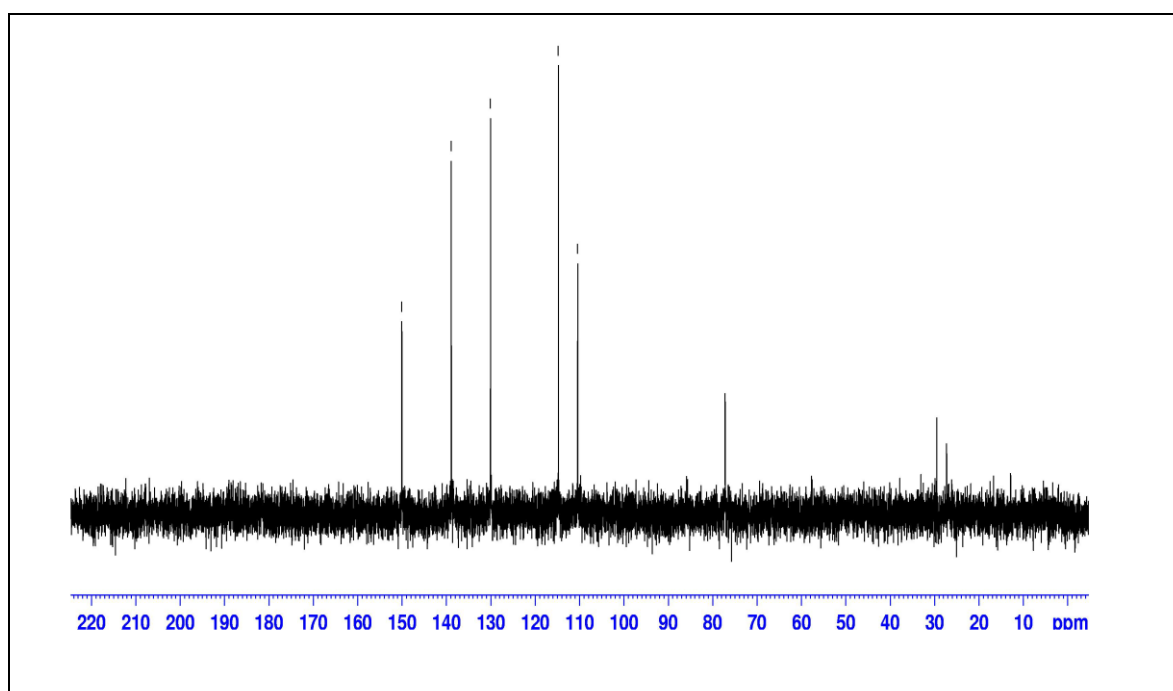


Figure 159 DEPT 90° (CDCl₃) of compound **RM18**

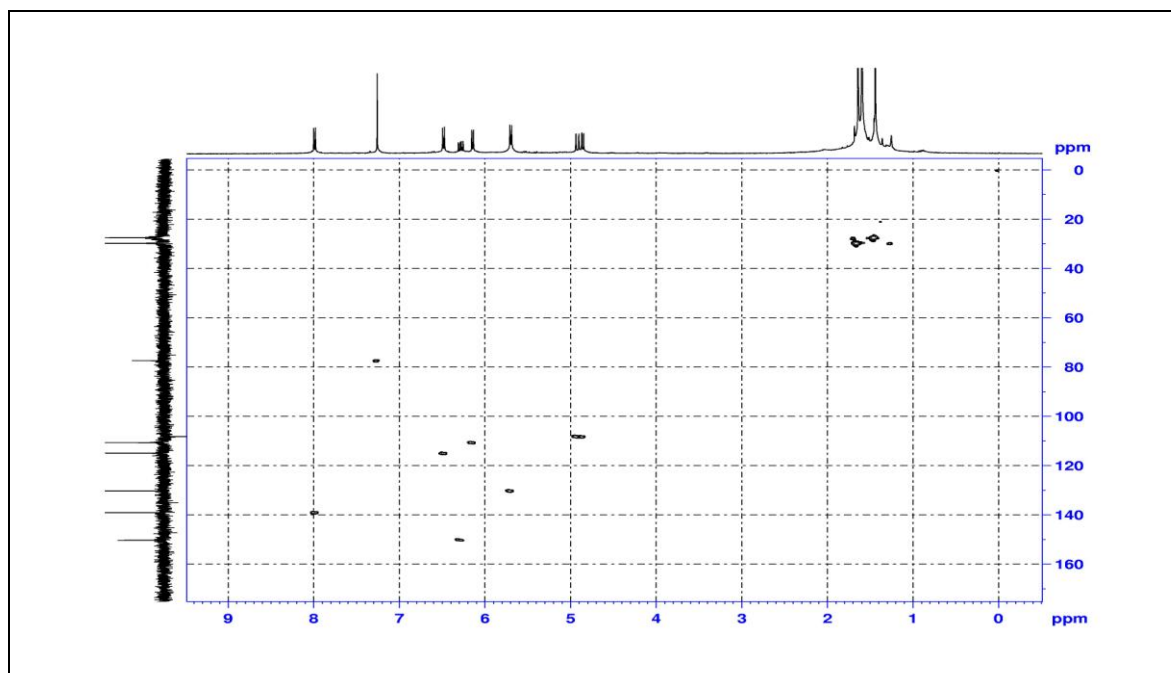


Figure 160 2D HMQC (CDCl₃) of compound **RM18**

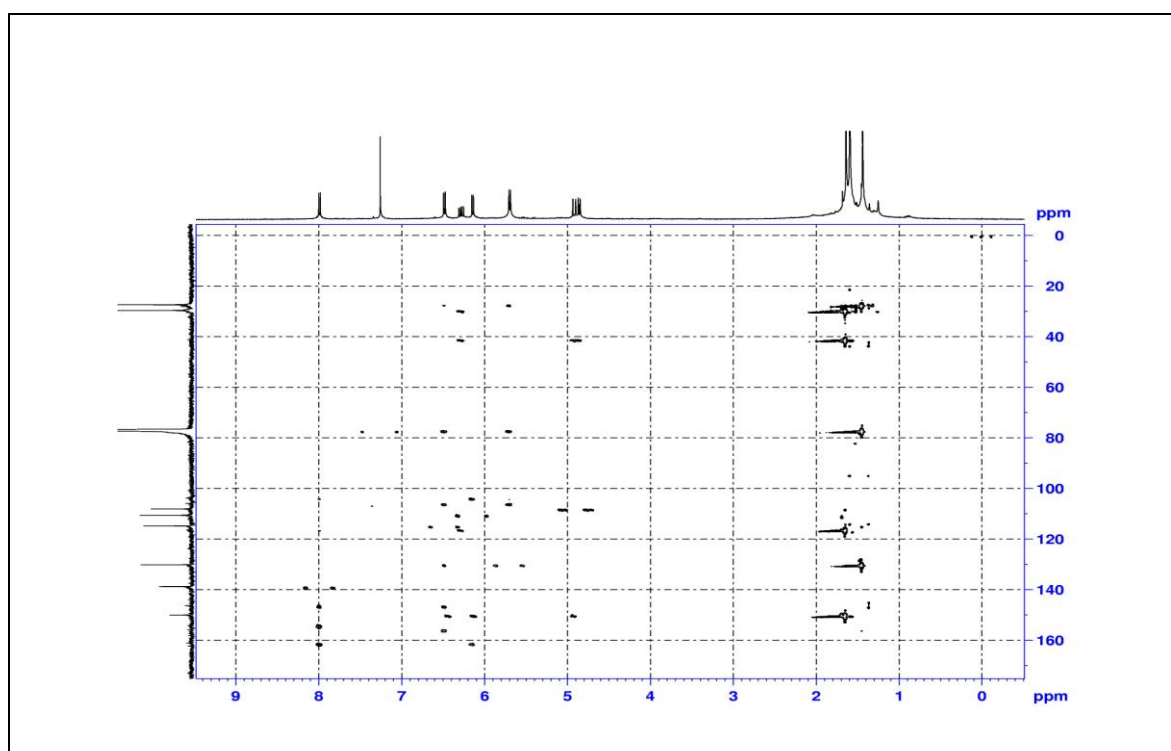


Figure 161 2D HMBC (CDCl₃) of compound **RM18**

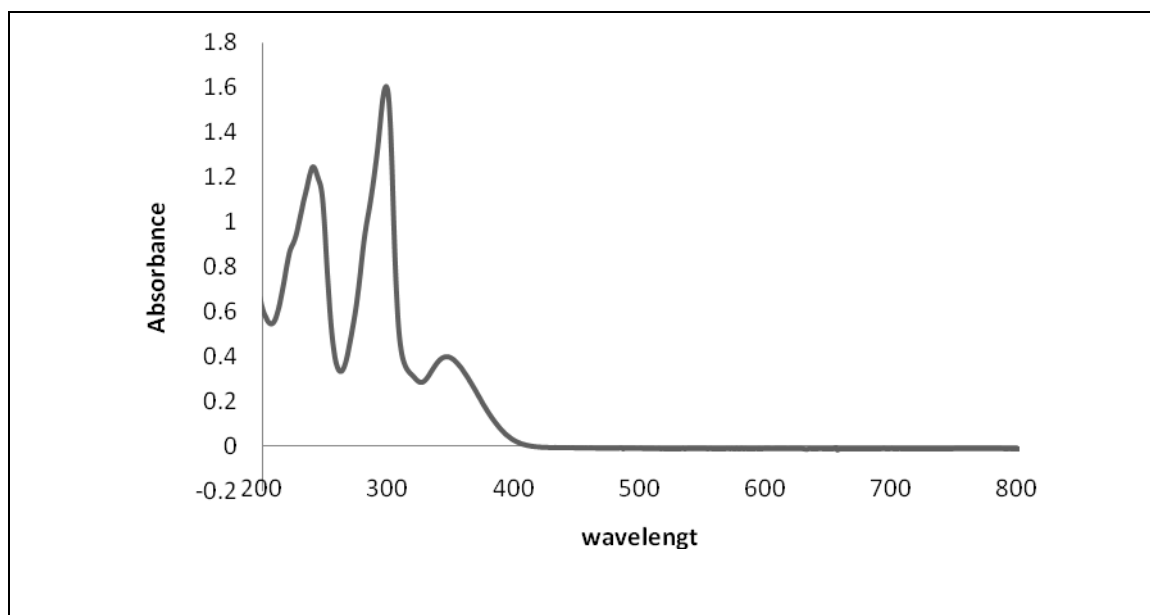


Figure 162 UV (MeOH) spectrum of compound **RM19**

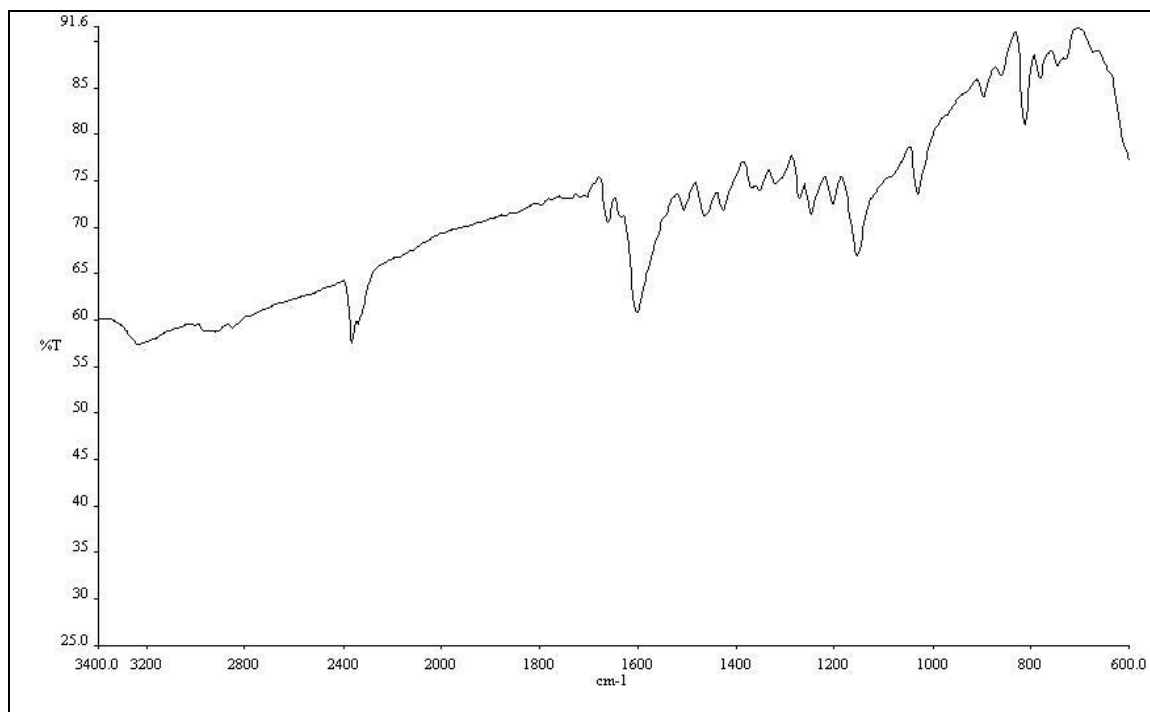


Figure 163 IR (neat) spectrum of compound **RM19**

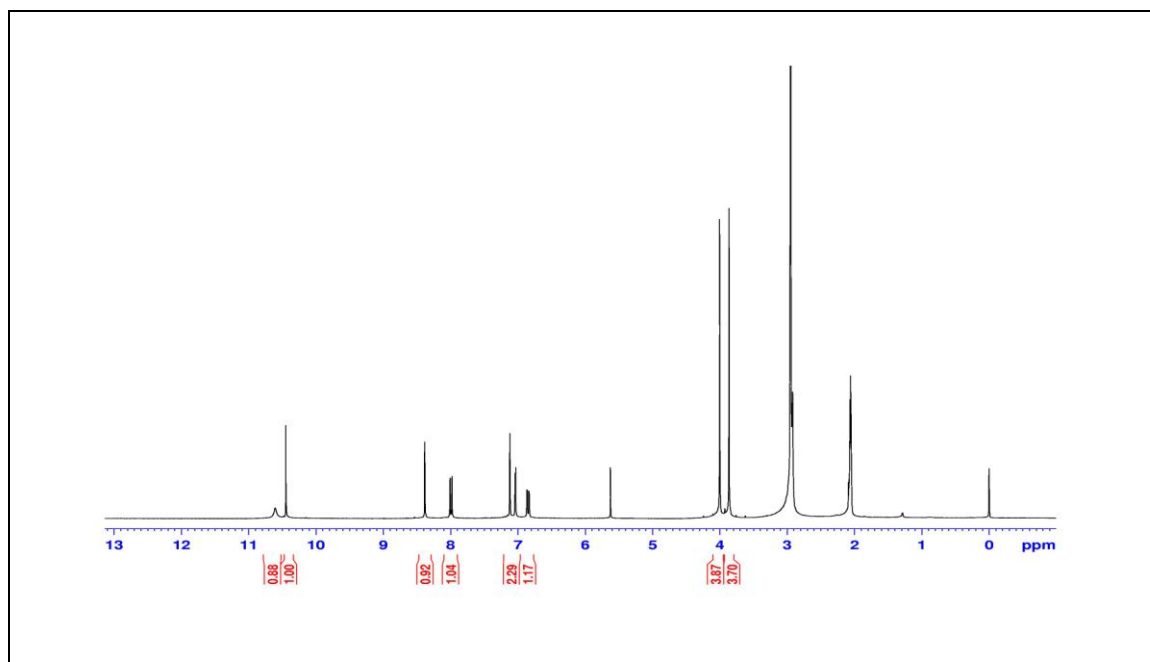


Figure 164 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM19**

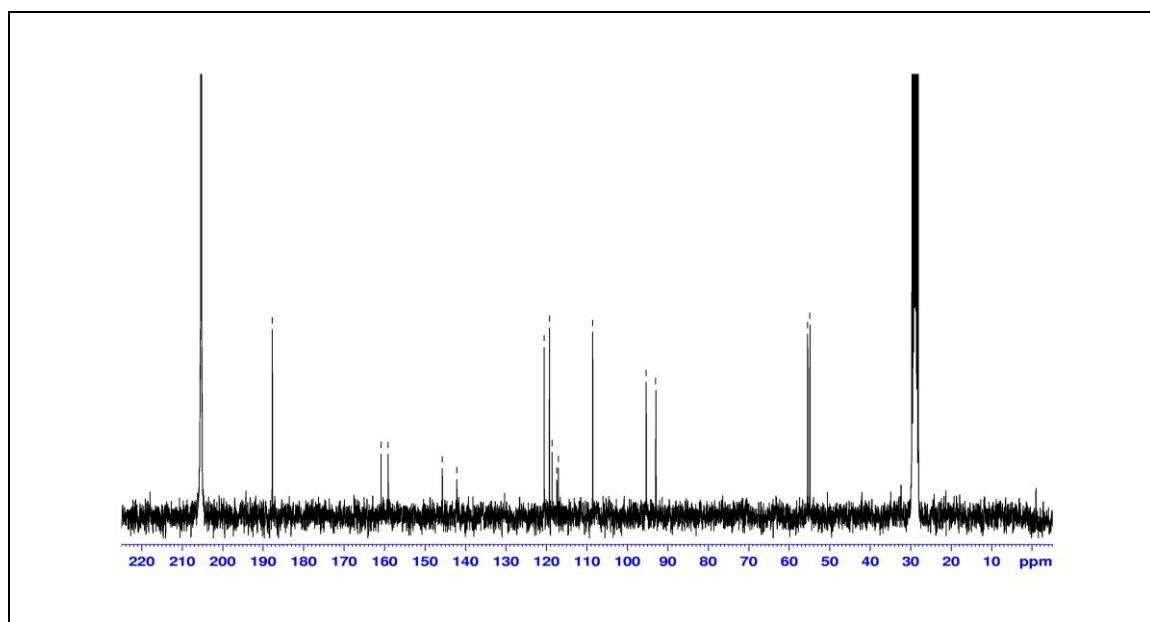


Figure 165 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM19**

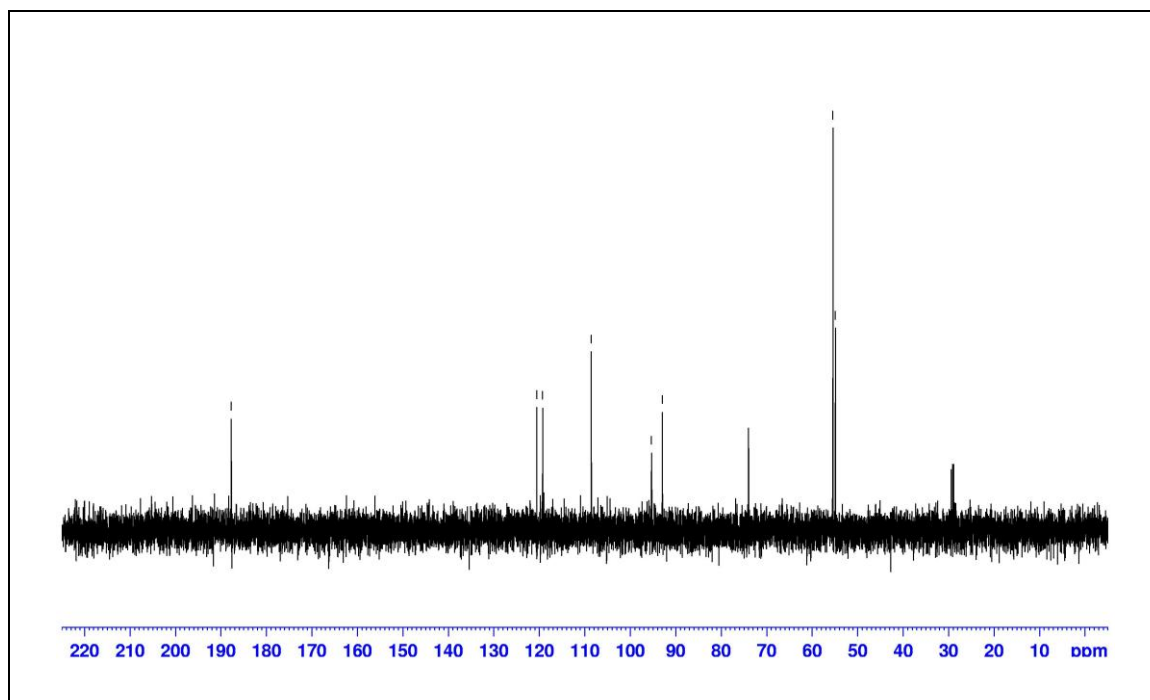


Figure 166 DEPT 135° (CD_3COCD_3) of compound **RM19**

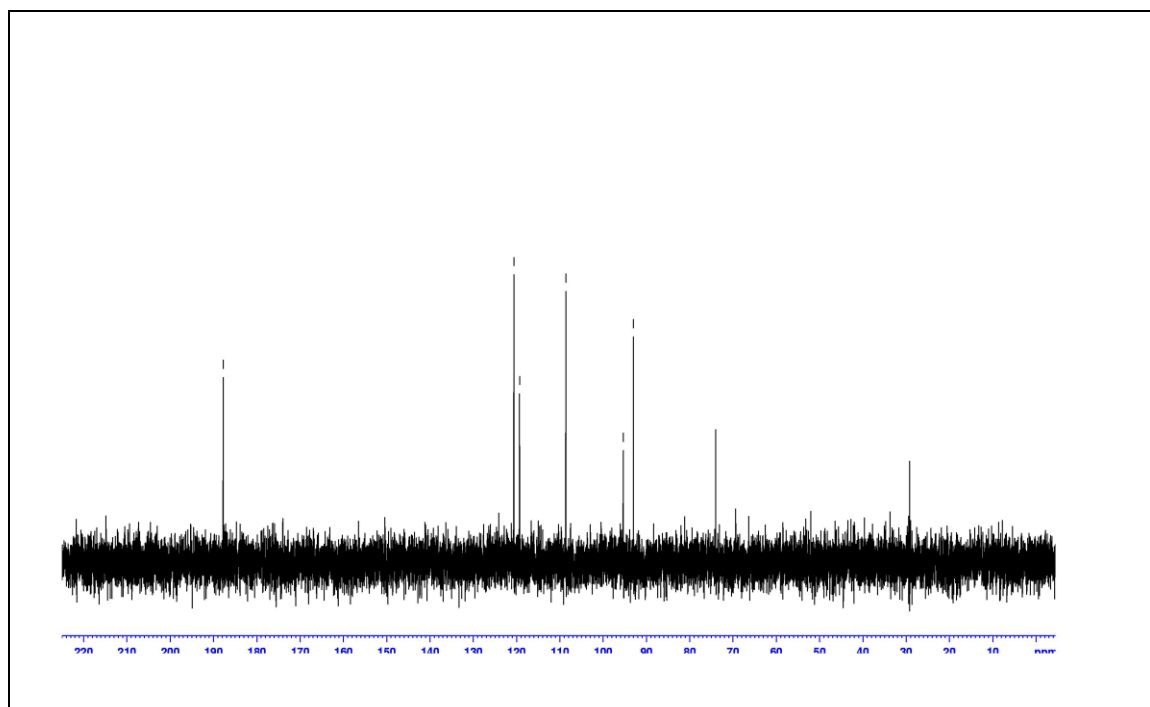


Figure 167 DEPT 90° (CD_3COCD_3) of compound **RM19**

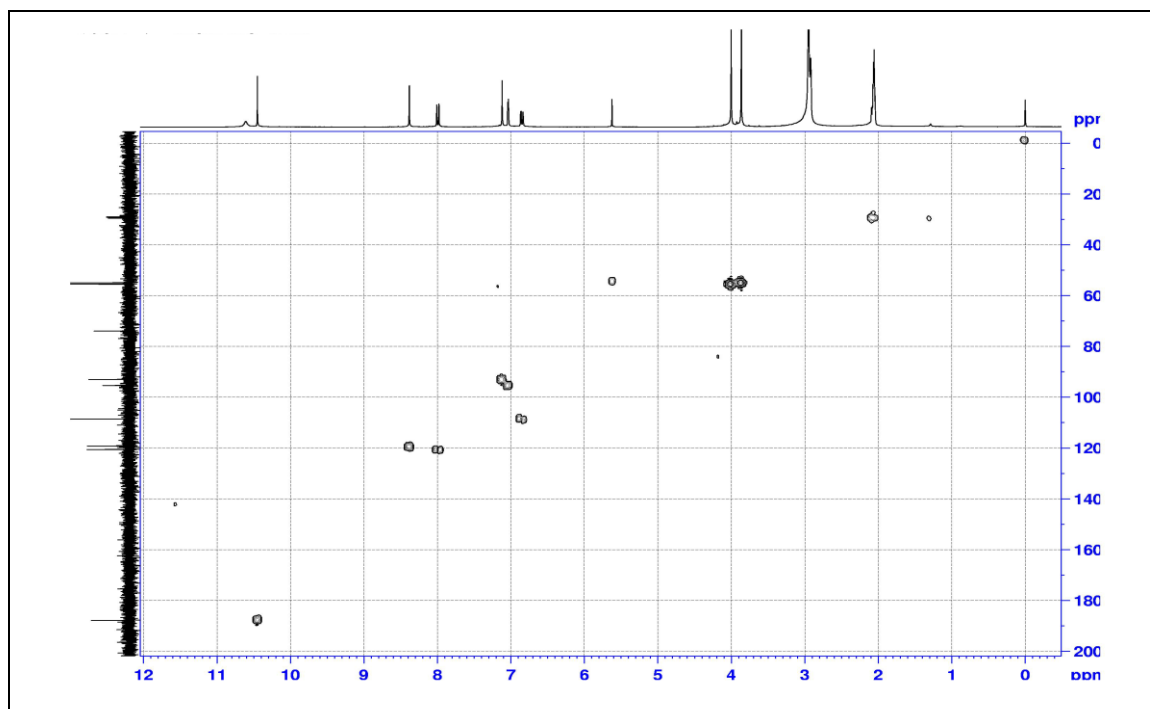


Figure 168 2D HMQC (CD_3COCD_3) of compound **RM19**

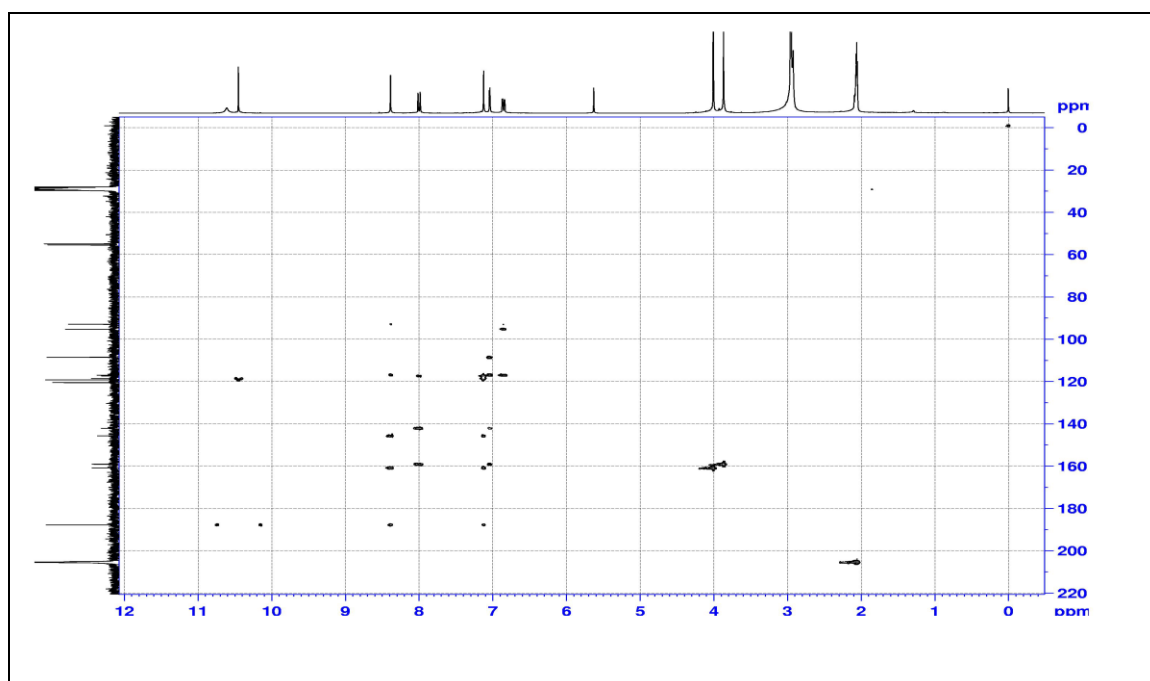


Figure 169 2D HMBC (CD_3COCD_3) of compound **RM19**

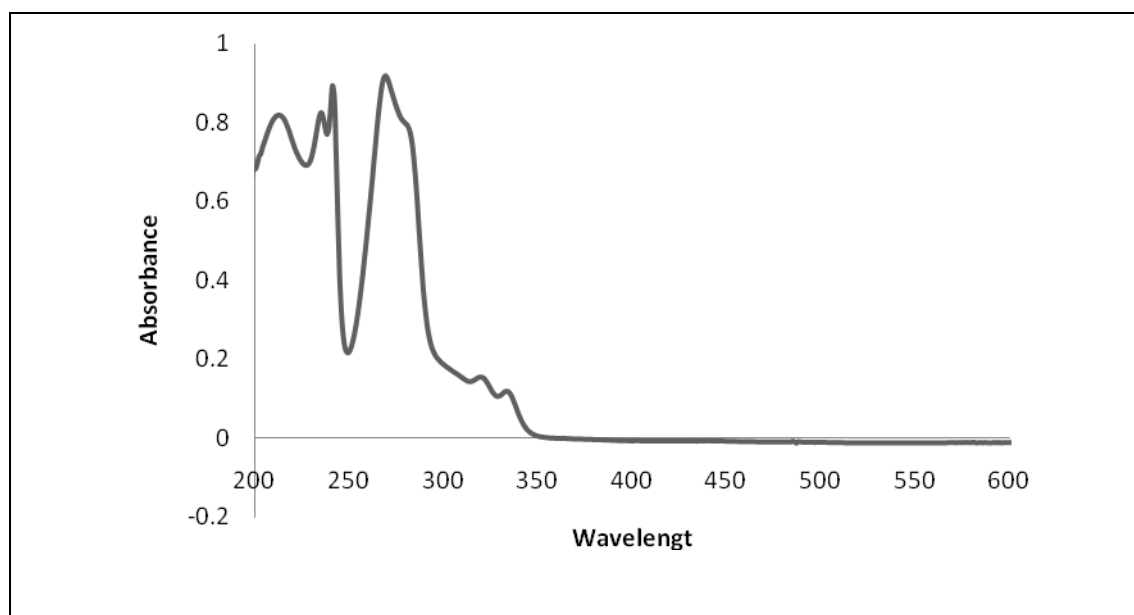


Figure 170 UV (MeOH) spectrum of compound **RM20**

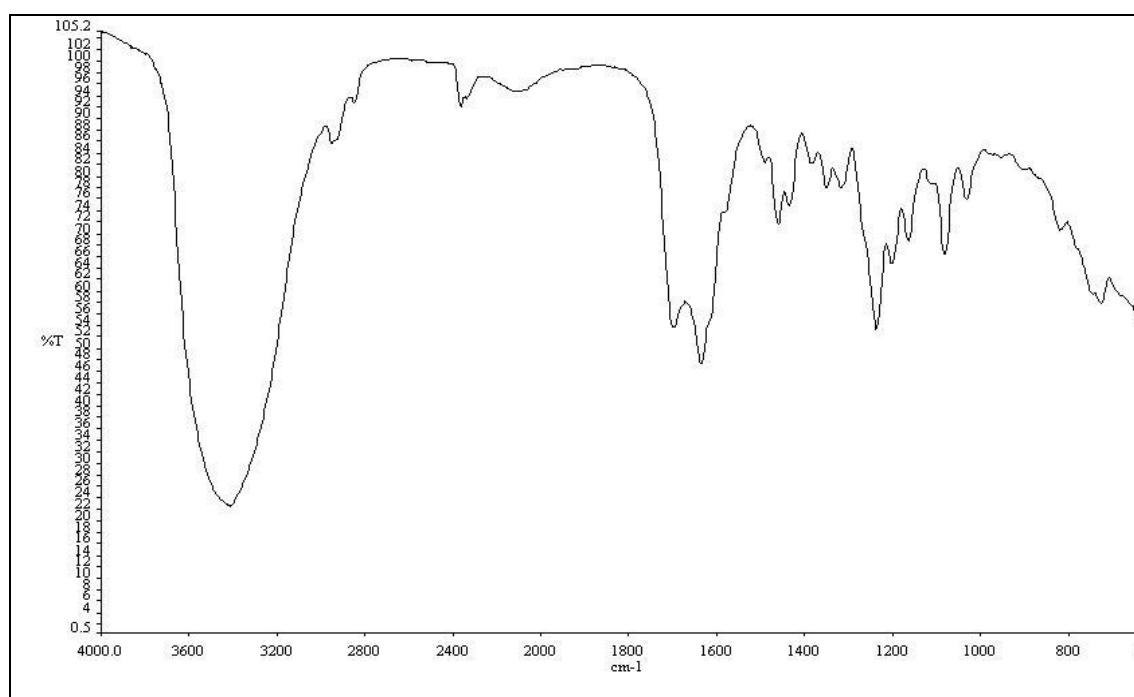


Figure 171 IR (neat) spectrum of compound **RM20**

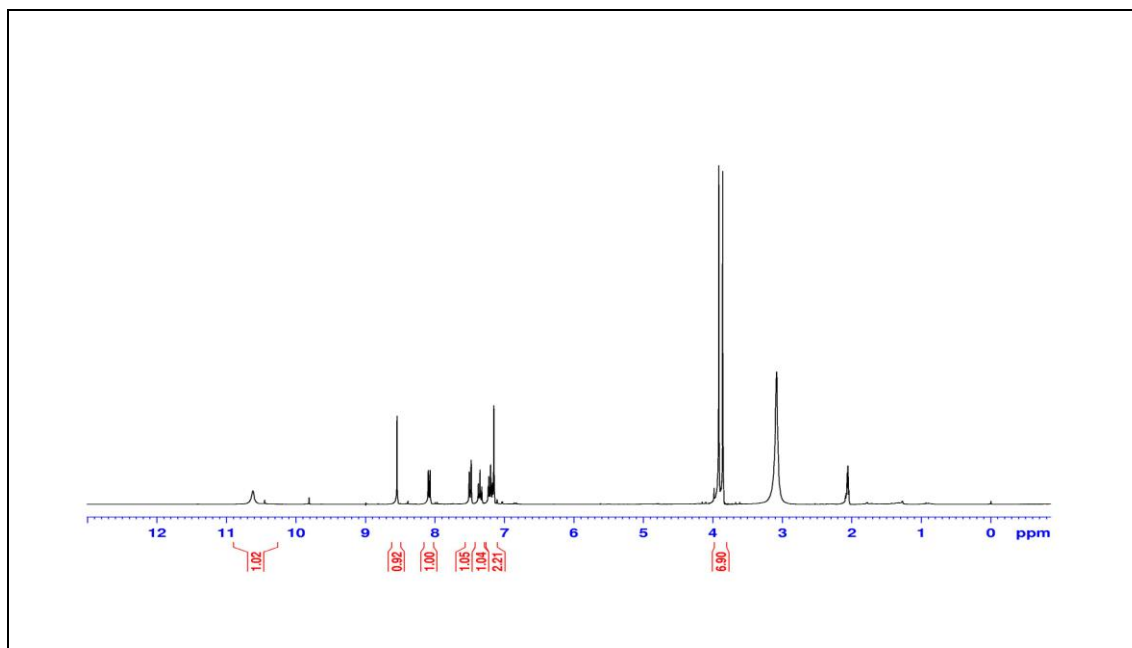


Figure 172 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM20**

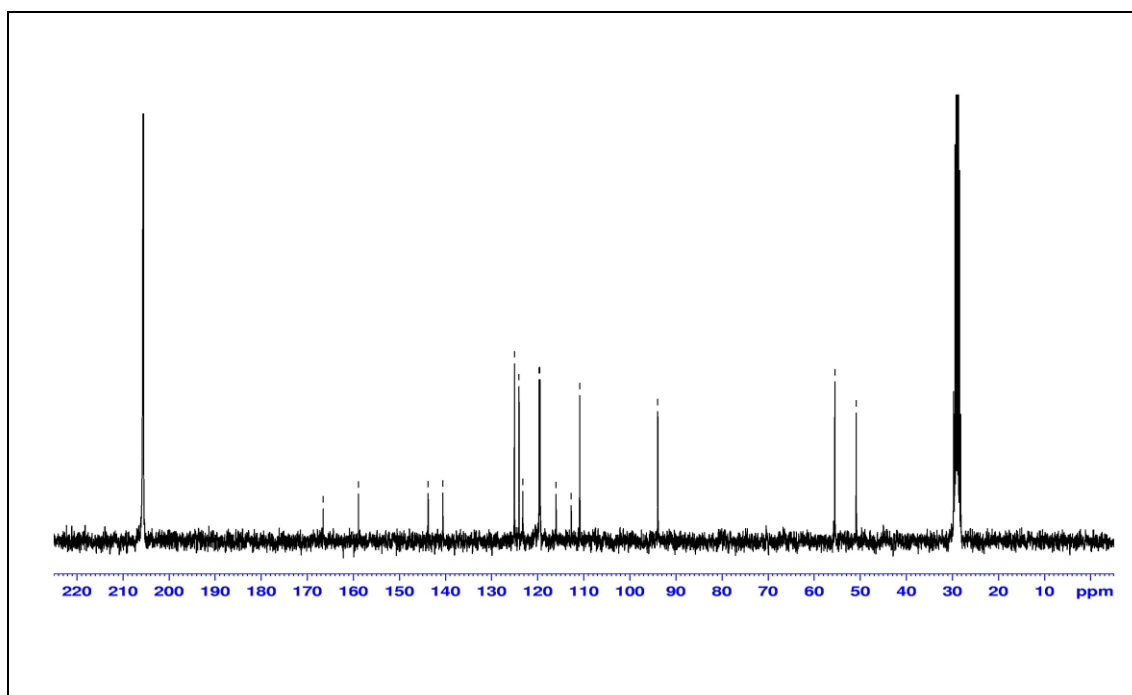


Figure 173 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM20**

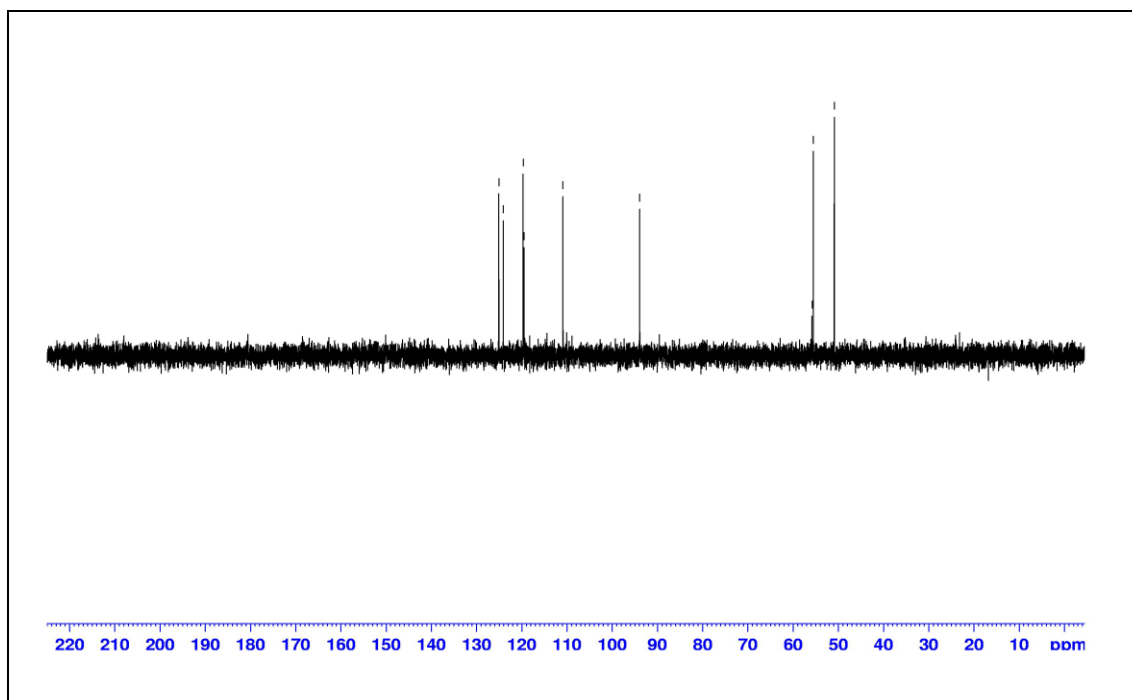


Figure 174 DEPT 135° (CD₃COCD₃) of compound **RM20**

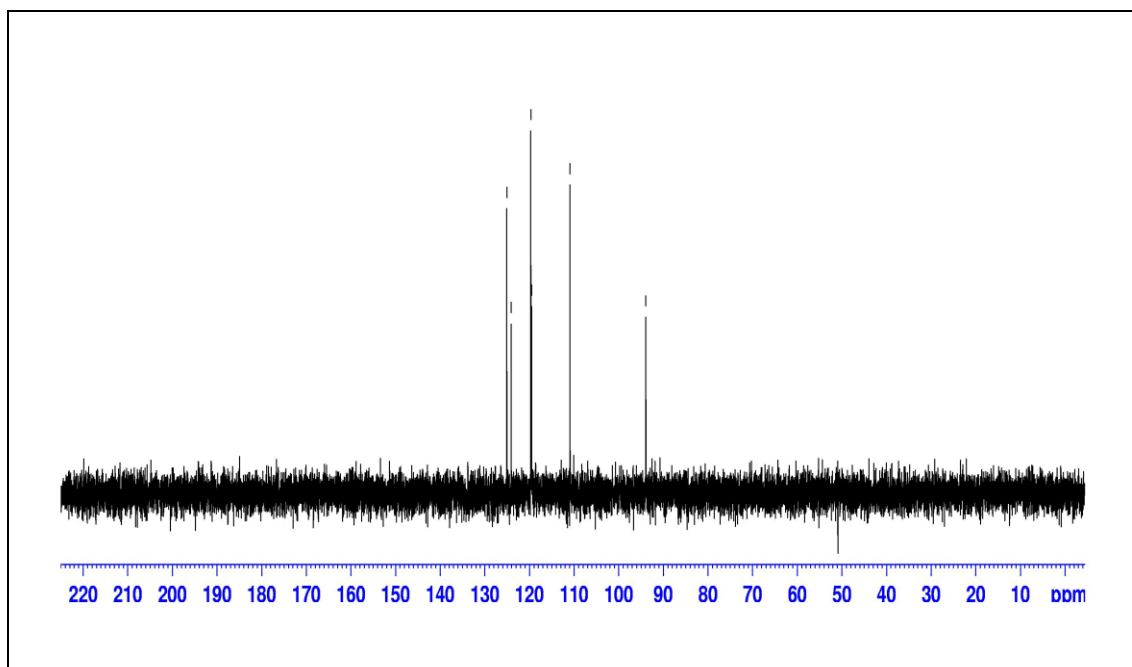


Figure 175 DEPT 90° (CD₃COCD₃) of compound **RM20**

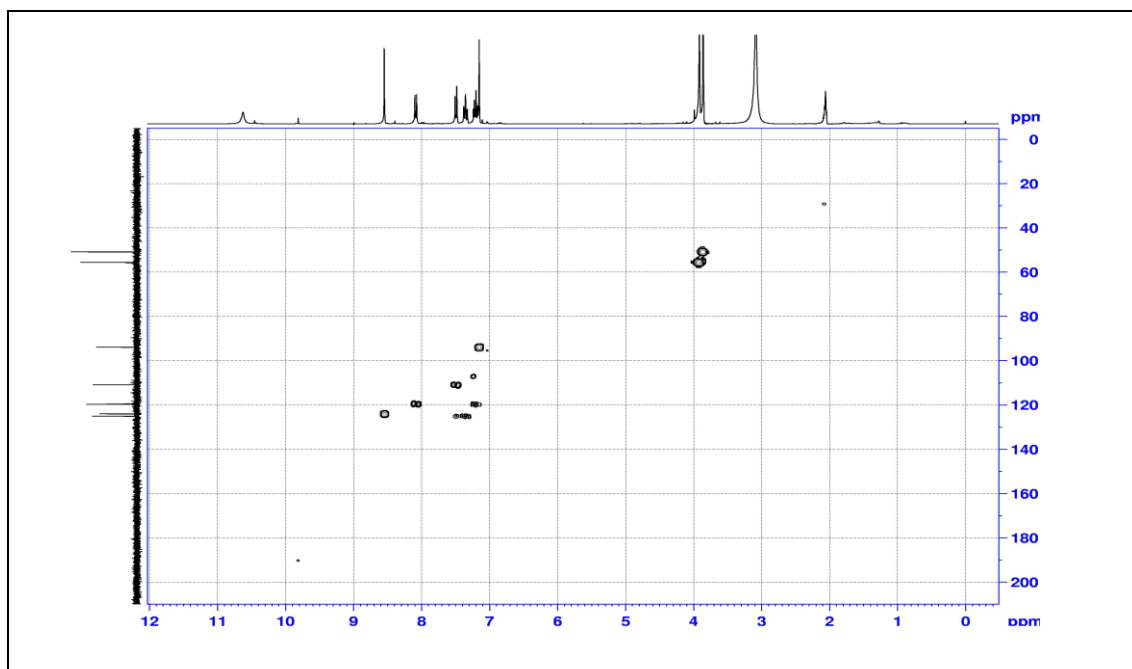


Figure 176 2D HMQC (CD₃COCD₃) of compound **RM20**

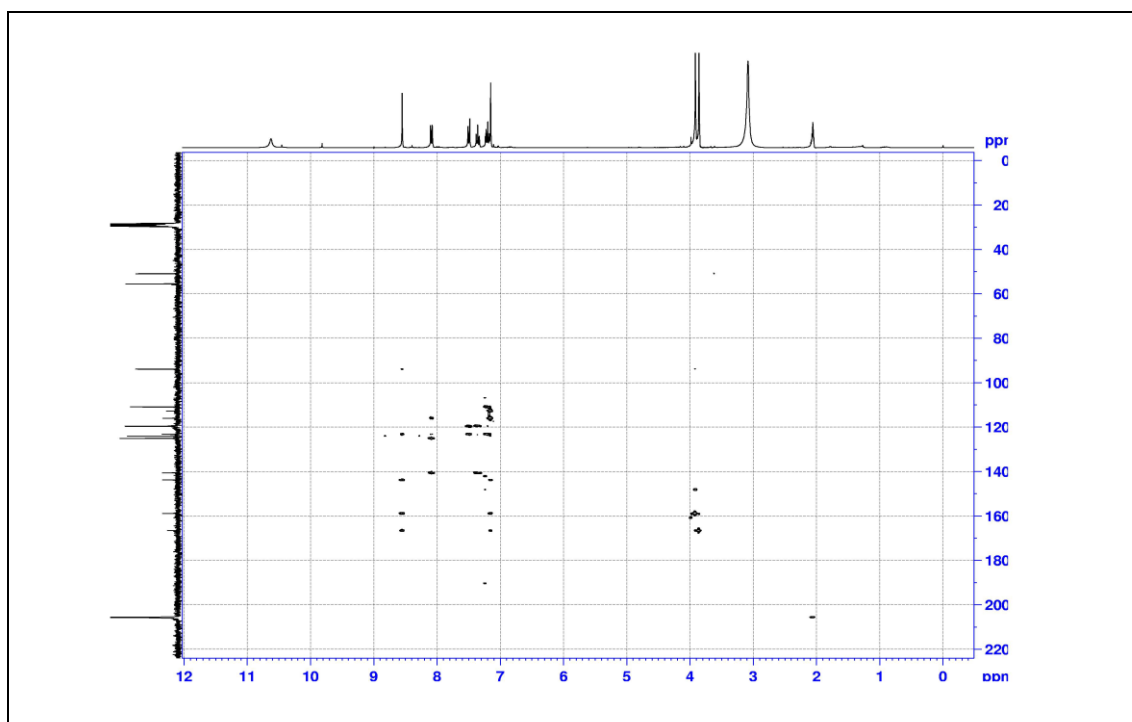


Figure 177 2D HMBC (CD₃COCD₃) of compound **RM20**

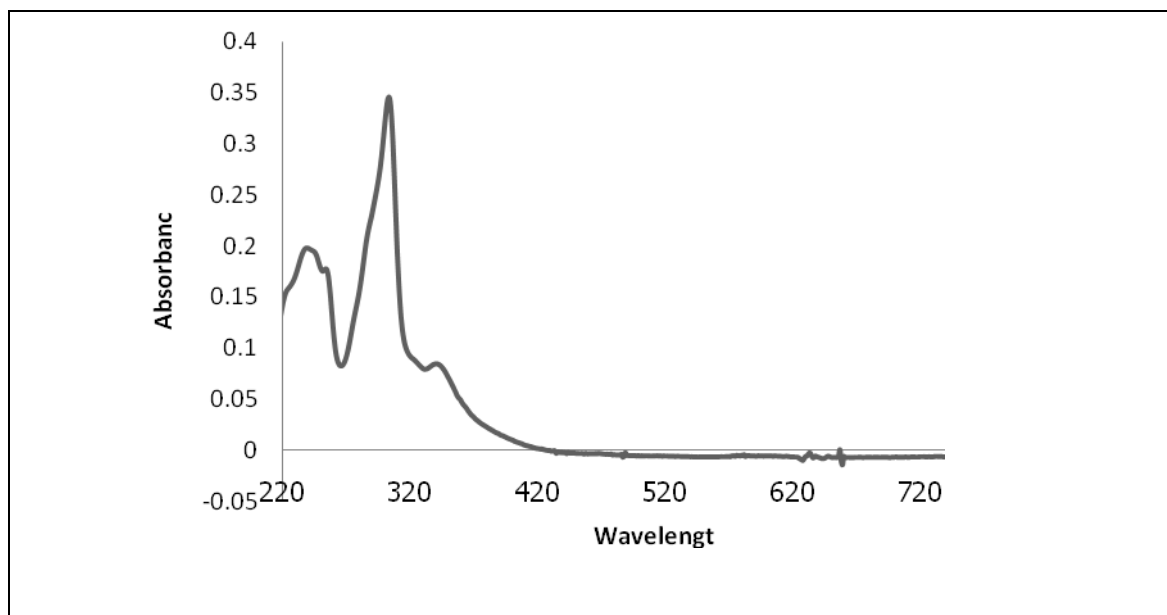


Figure 178 UV (MeOH) spectrum of compound **RM21**

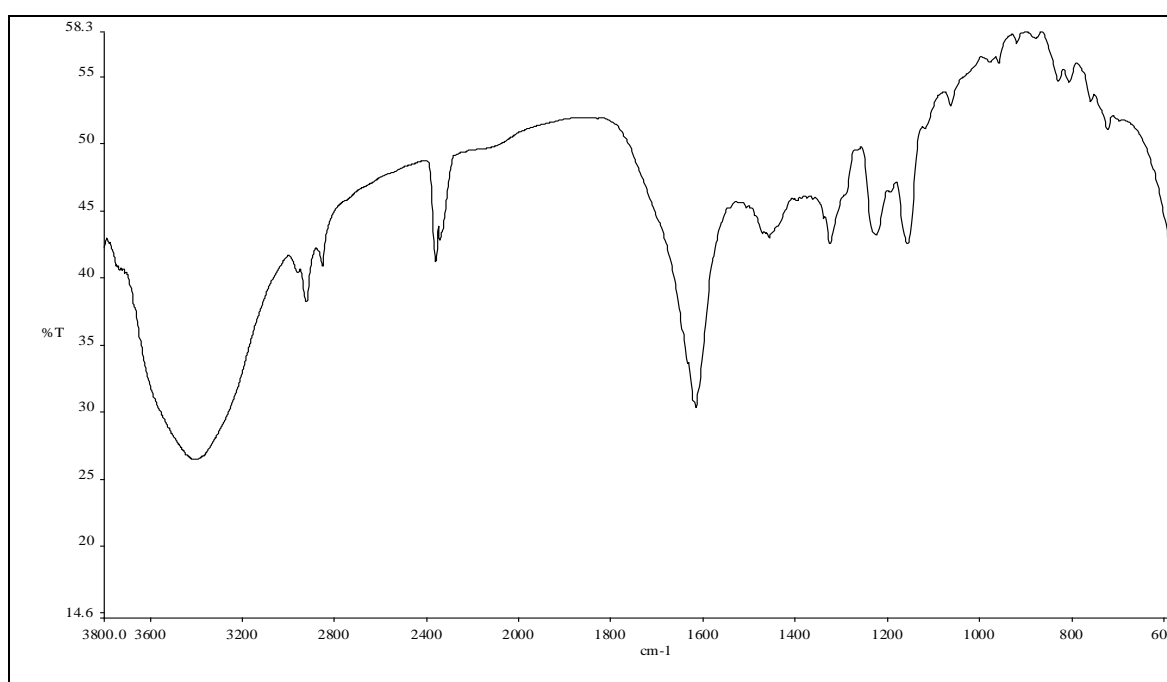


Figure 179 IR (neat) spectrum of compound **RM21**

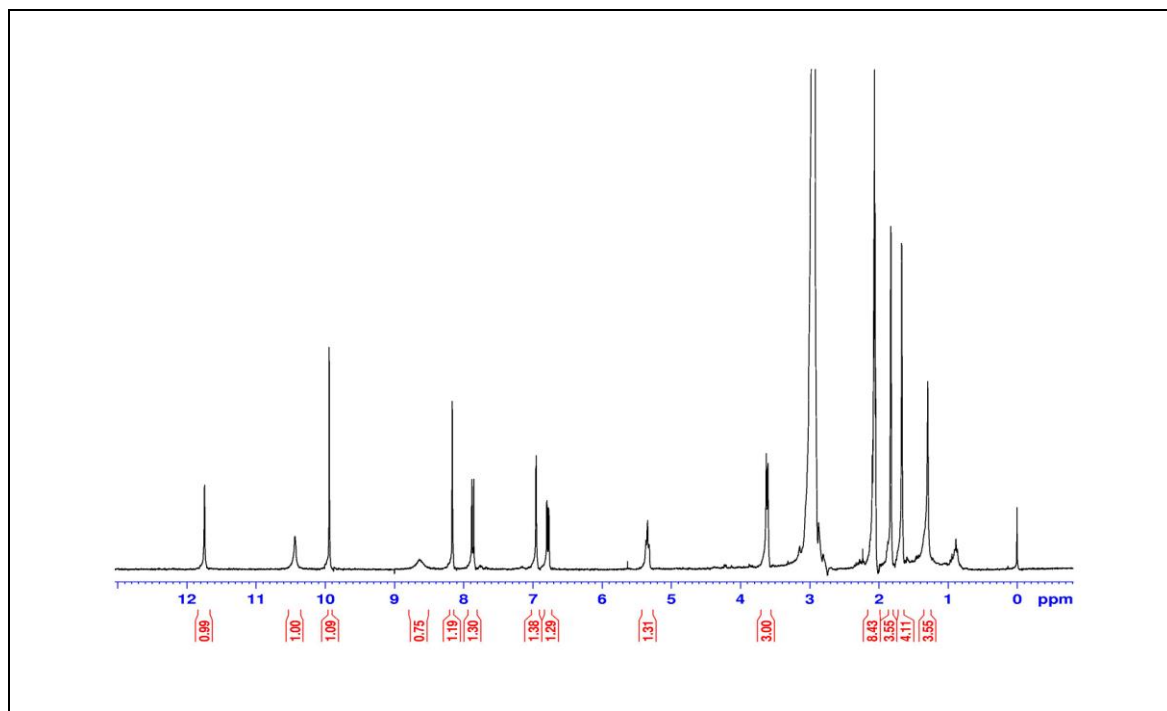


Figure 180 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM21**

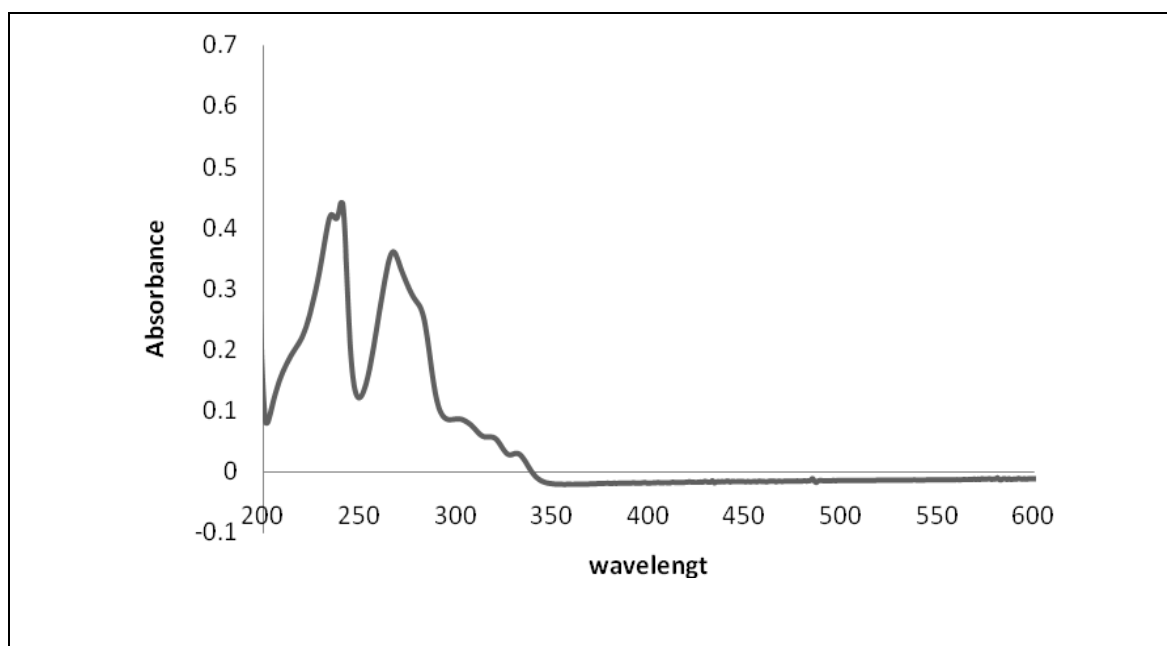


Figure 181 UV (MeOH) spectrum of compound **RM22**

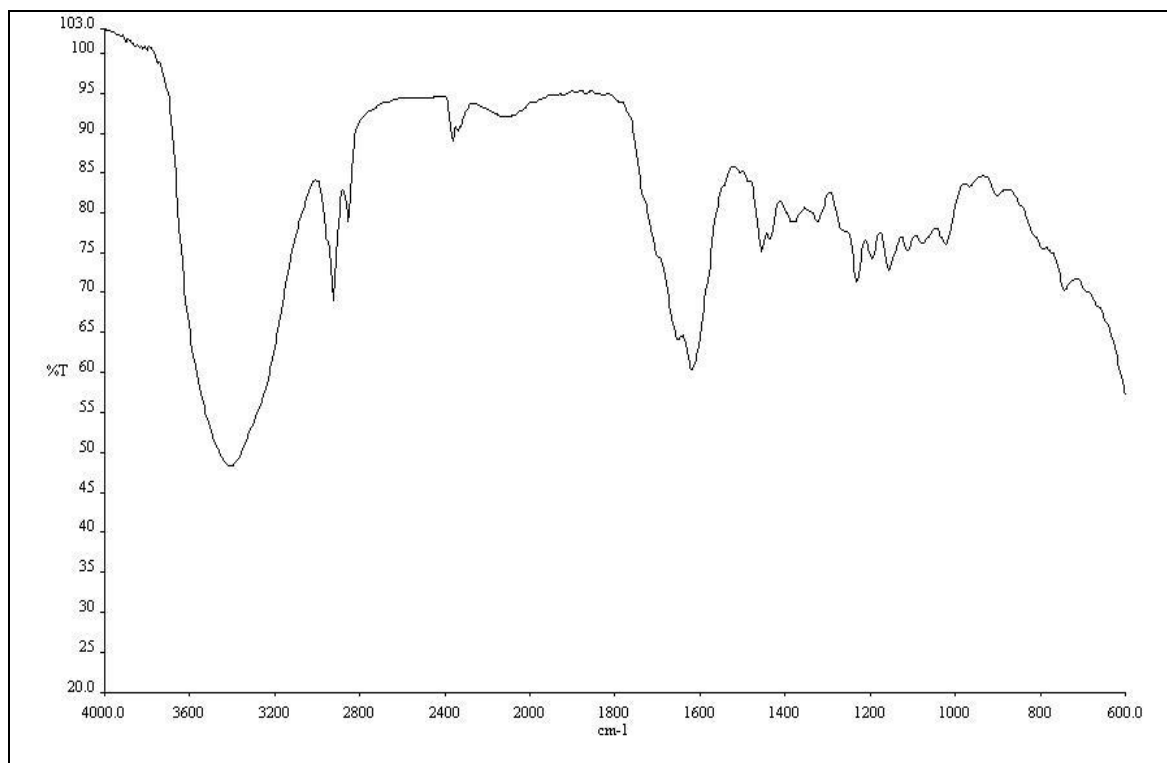


Figure 182 IR (neat) spectrum of compound **RM22**

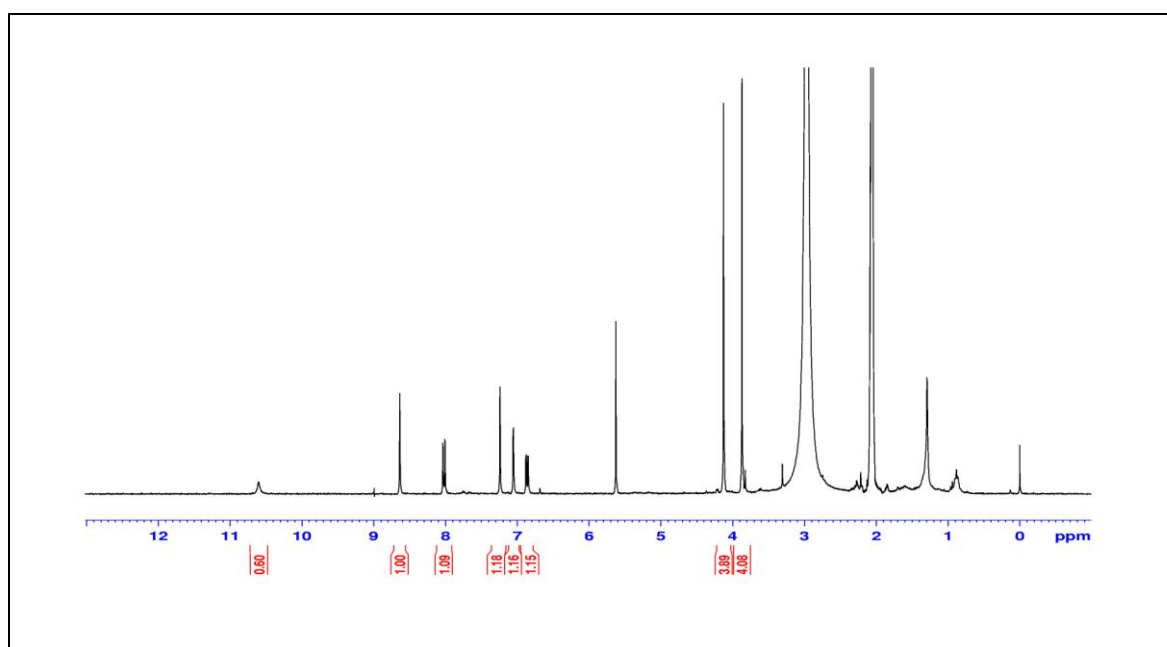


Figure 183 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM22**

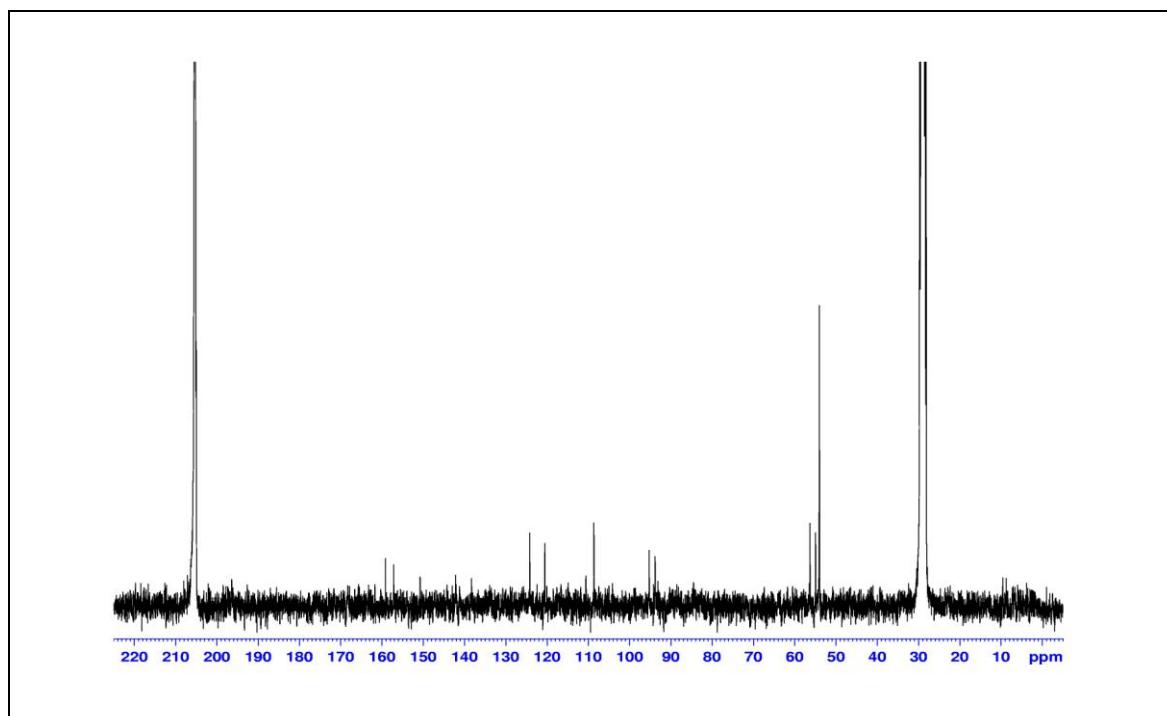


Figure 184 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM22**

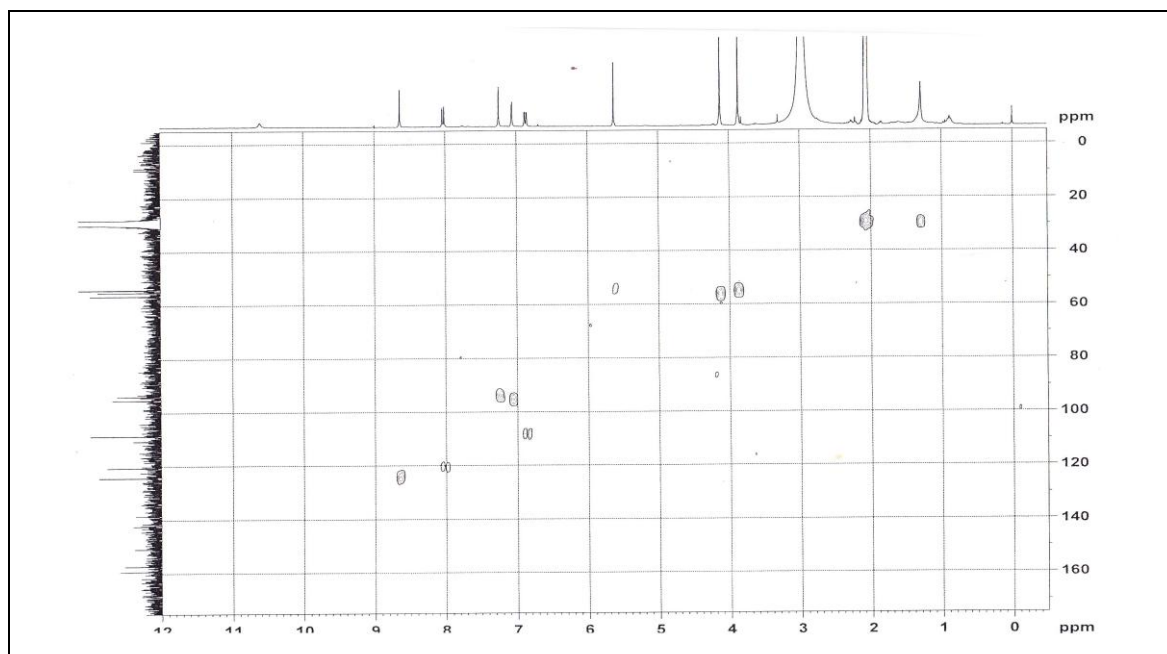


Figure 185 2D HMQC (CD_3COCD_3) of compound **RM22**

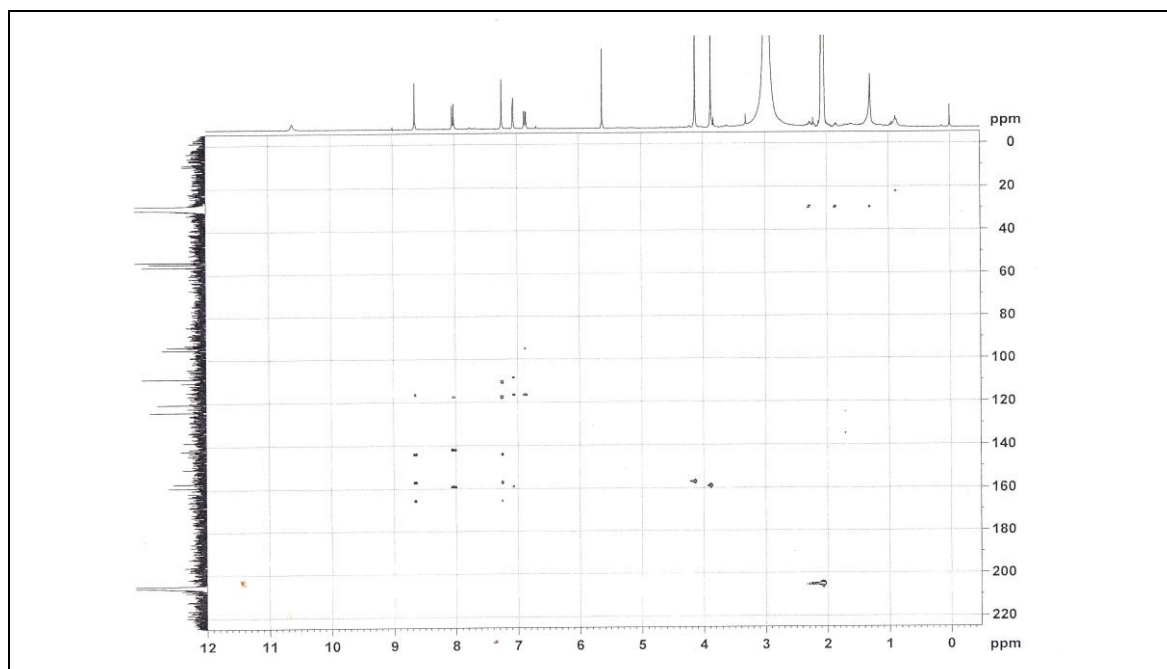


Figure 186 2D HMBC (CD_3COCD_3) of compound **RM22**

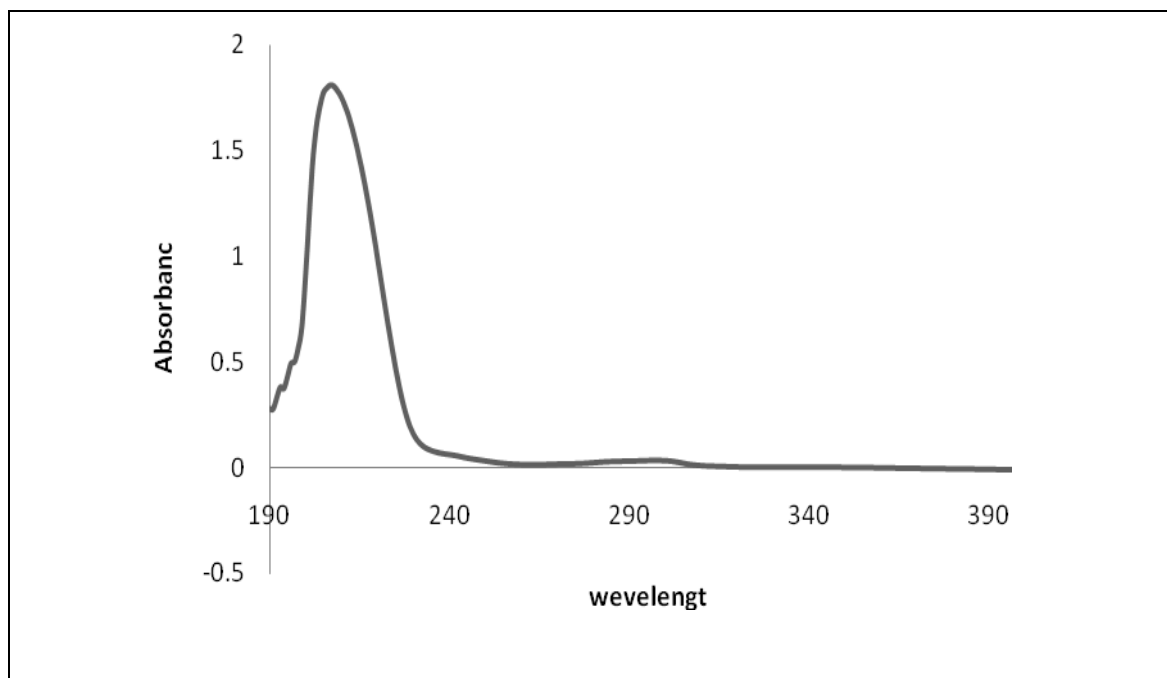


Figure 187 UV (MeOH) spectrum of compound **RM23**

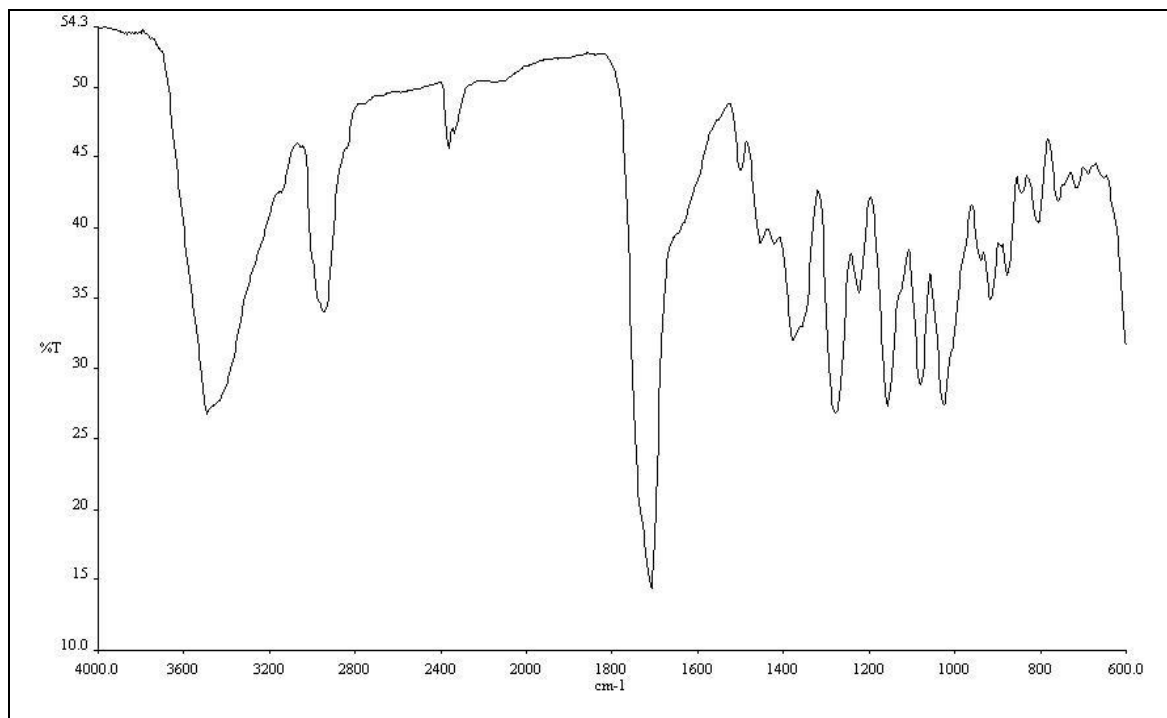


Figure 188 IR (neat) spectrum of compound **RM23**

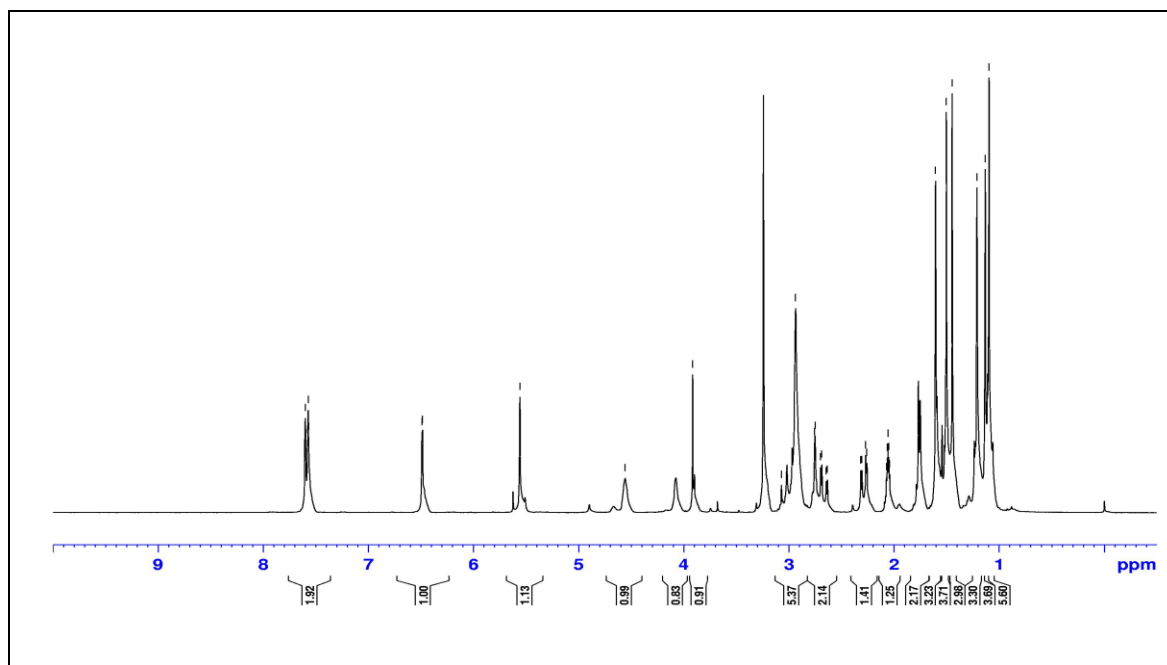


Figure 189 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM23**

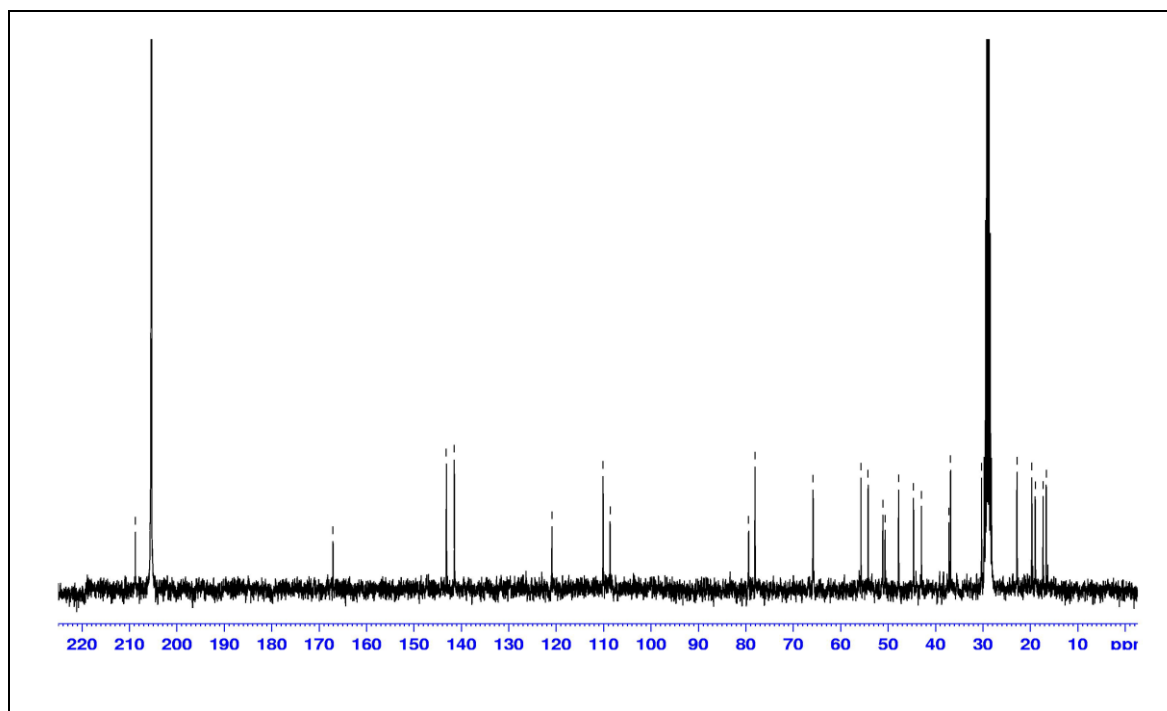


Figure 190 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM23**

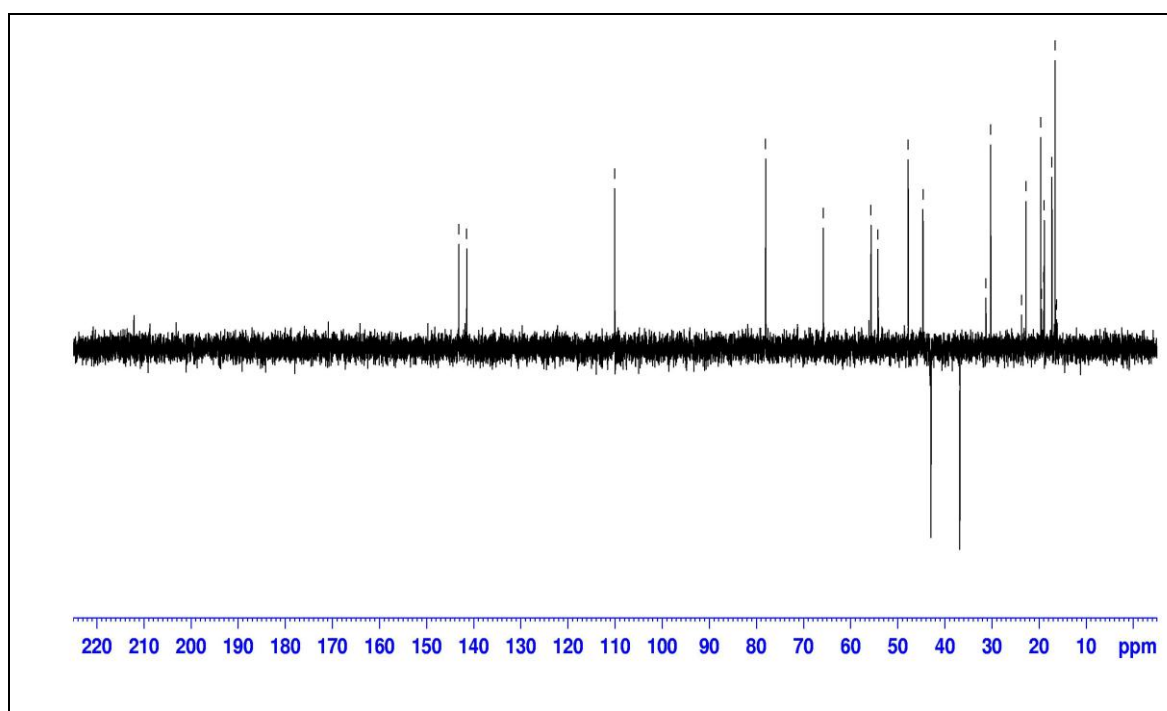


Figure 191 DEPT 135° (CD_3COCD_3) of compound **RM23**

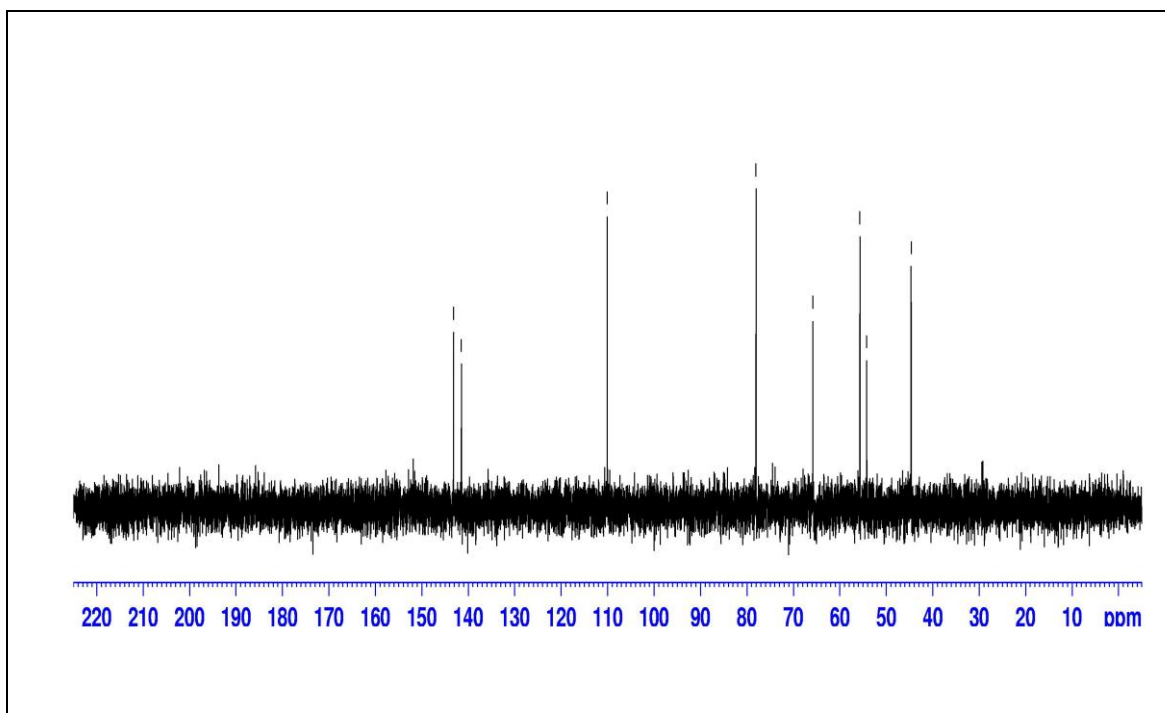


Figure 192 DEPT 90° (CD₃COCD₃) of compound **RM23**

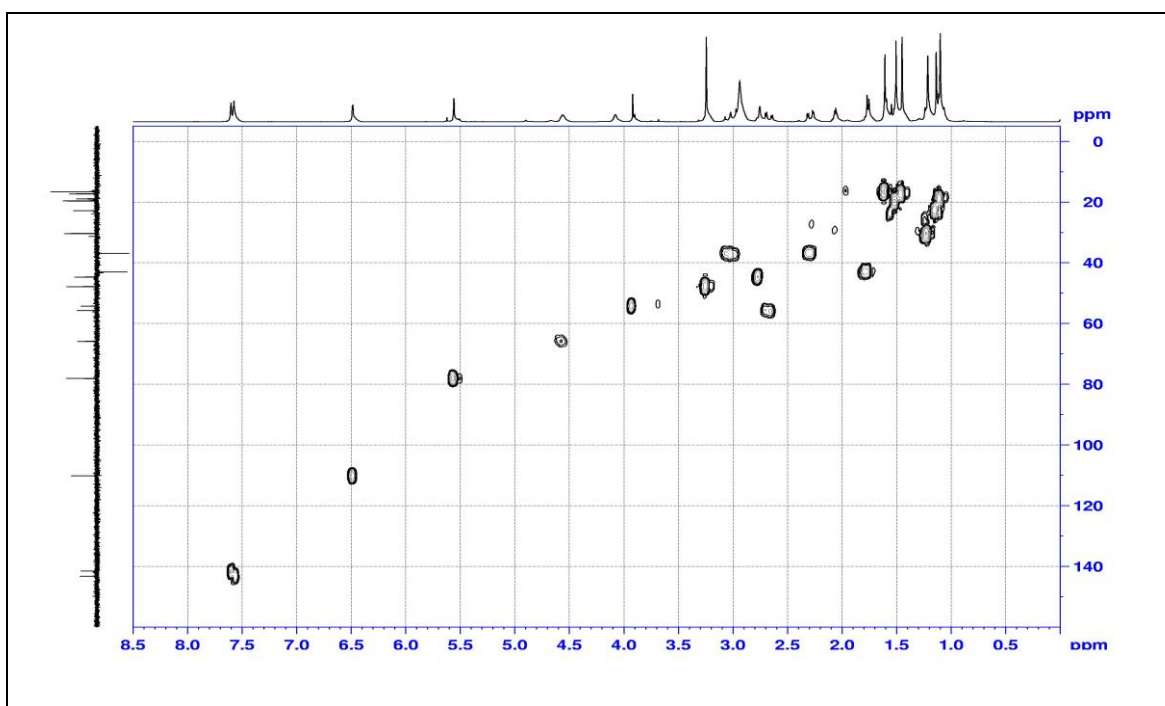


Figure 193 2D HMQC (CD₃COCD₃) of compound **RM23**

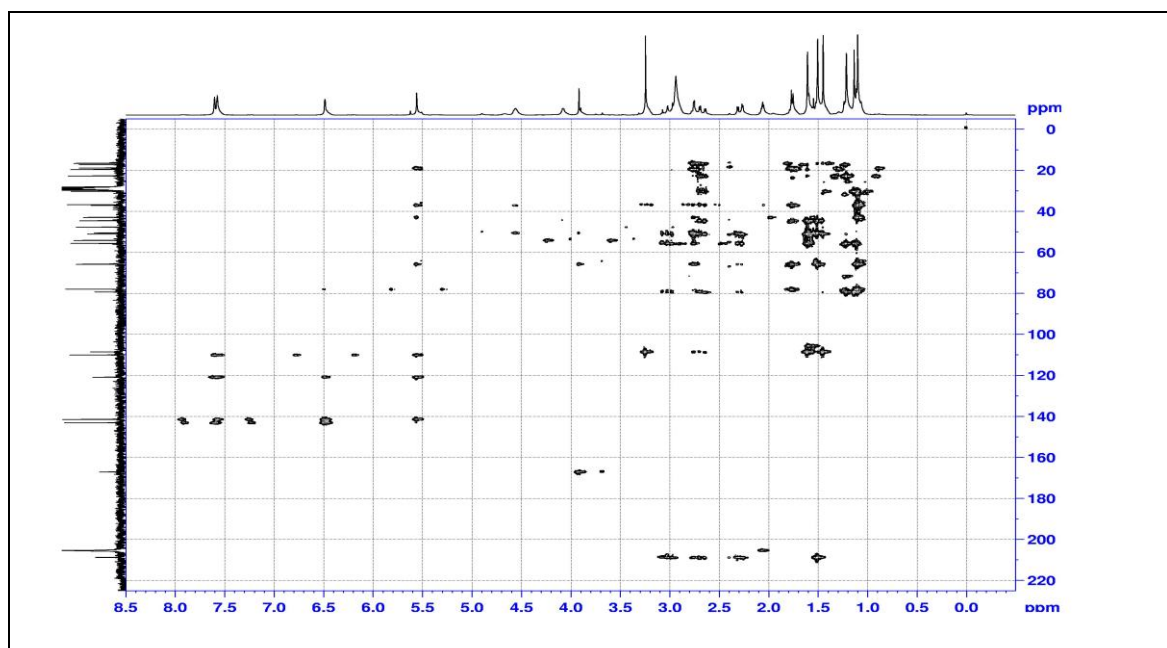


Figure 194 2D HMBC (CD_3COCD_3) of compound **RM23**

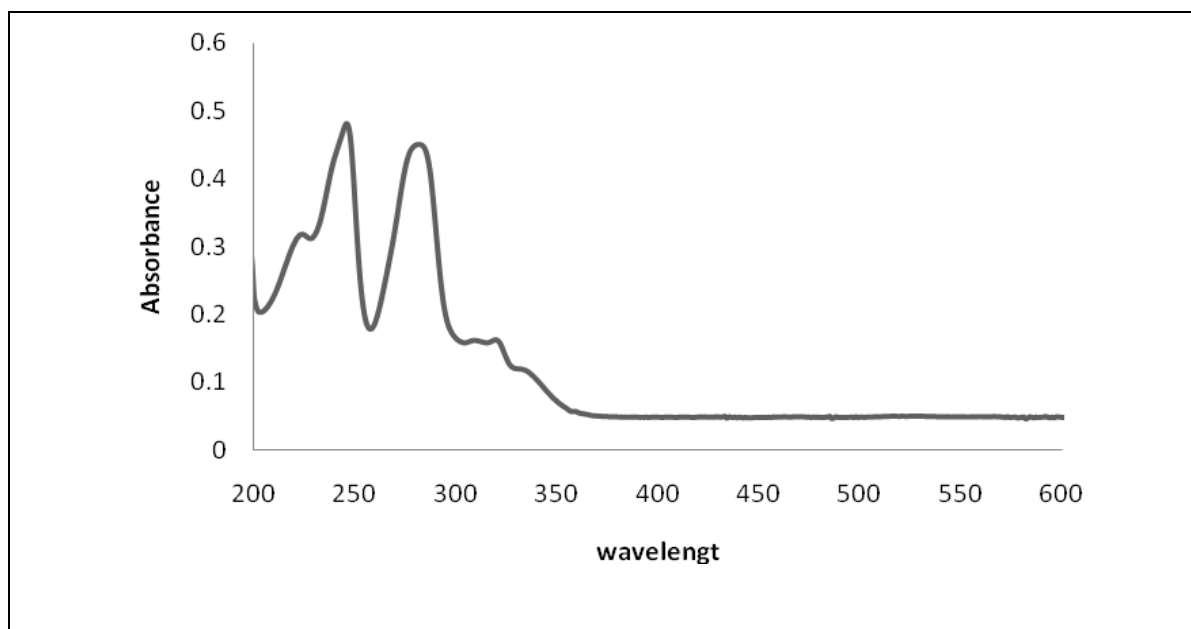


Figure 195 UV (MeOH) spectrum of compound **RM24**

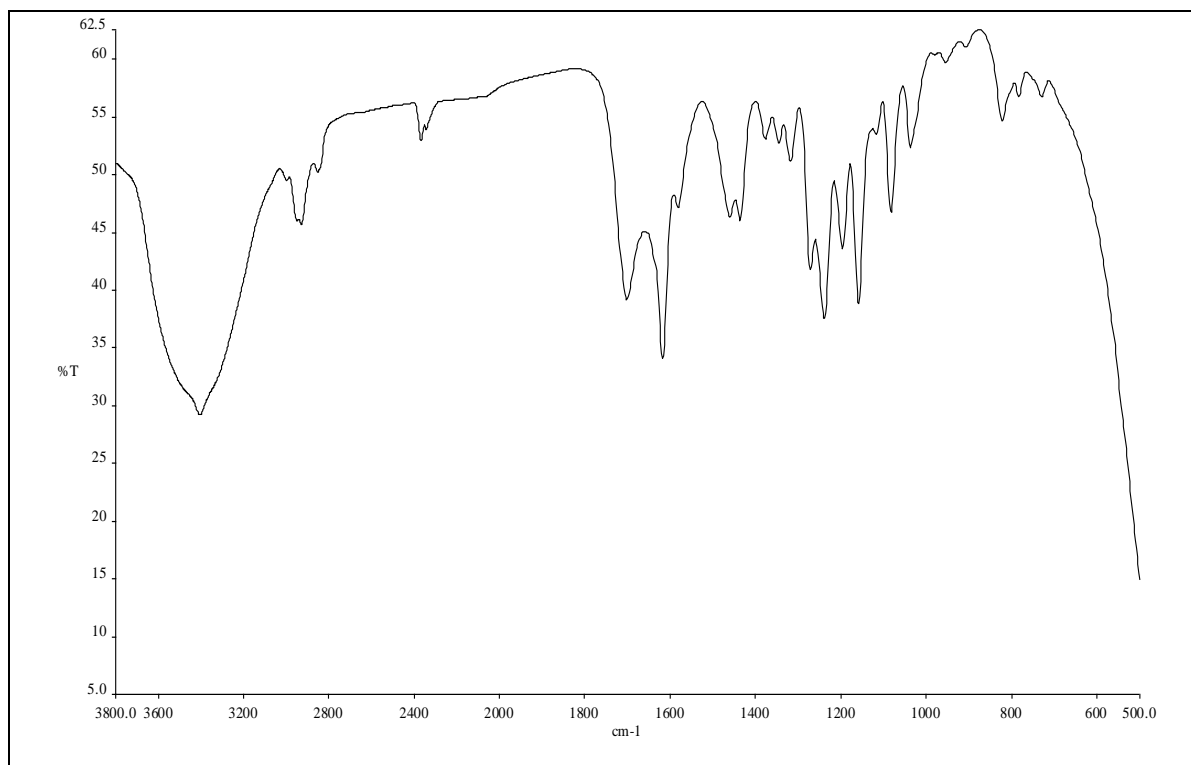


Figure 196 IR (neat) spectrum of compound **RM24**

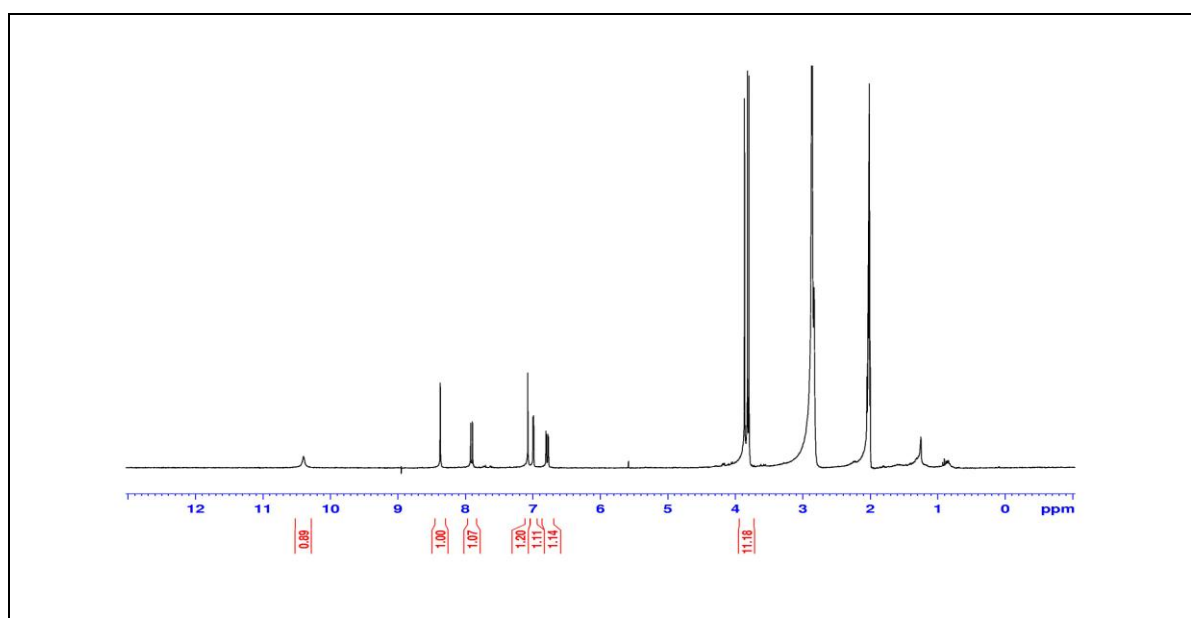


Figure 197 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM24**

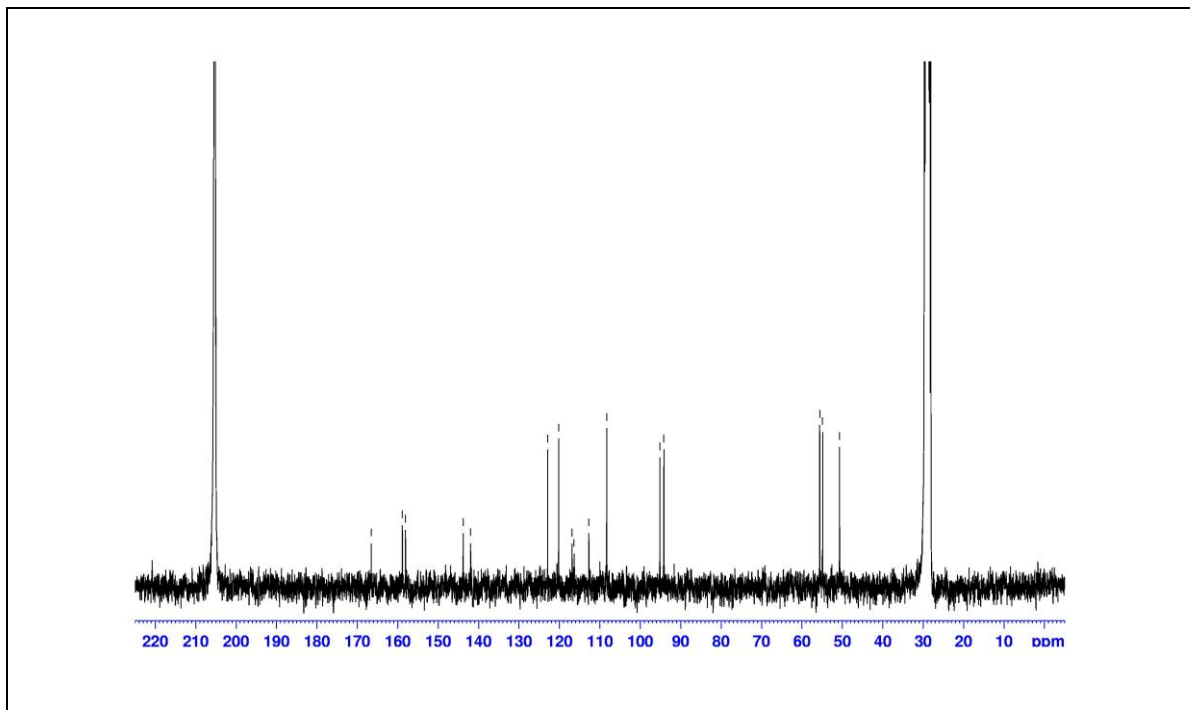


Figure 198 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM24**

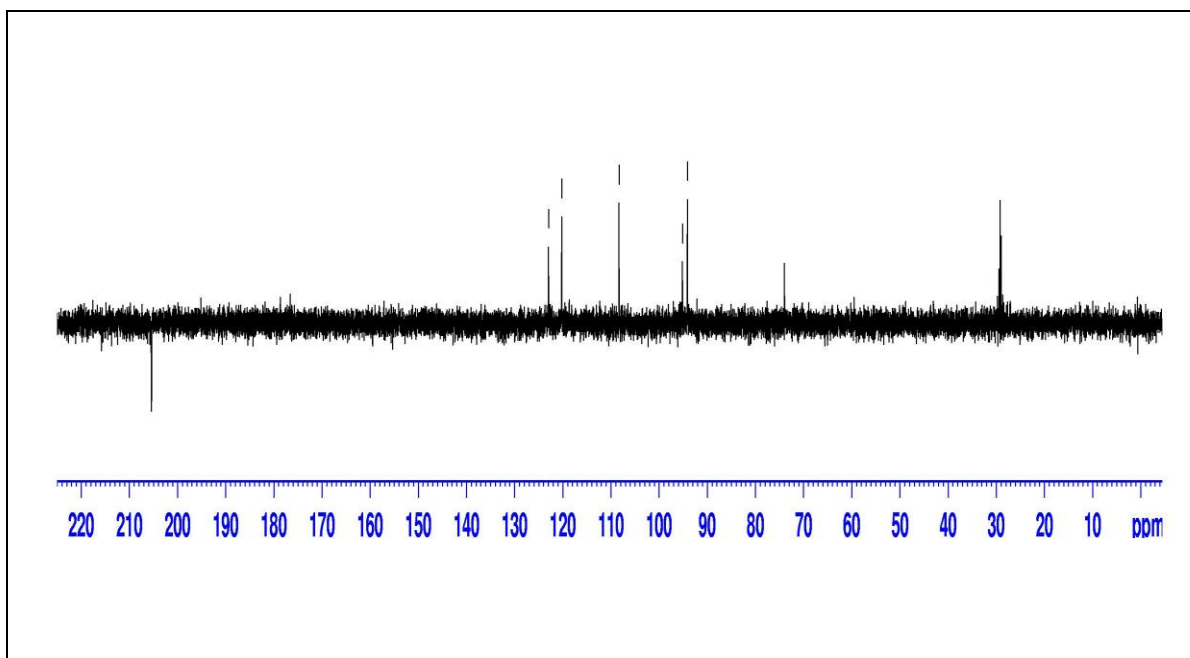


Figure 199 DEPT 90° (CD_3COCD_3) of compound **RM24**

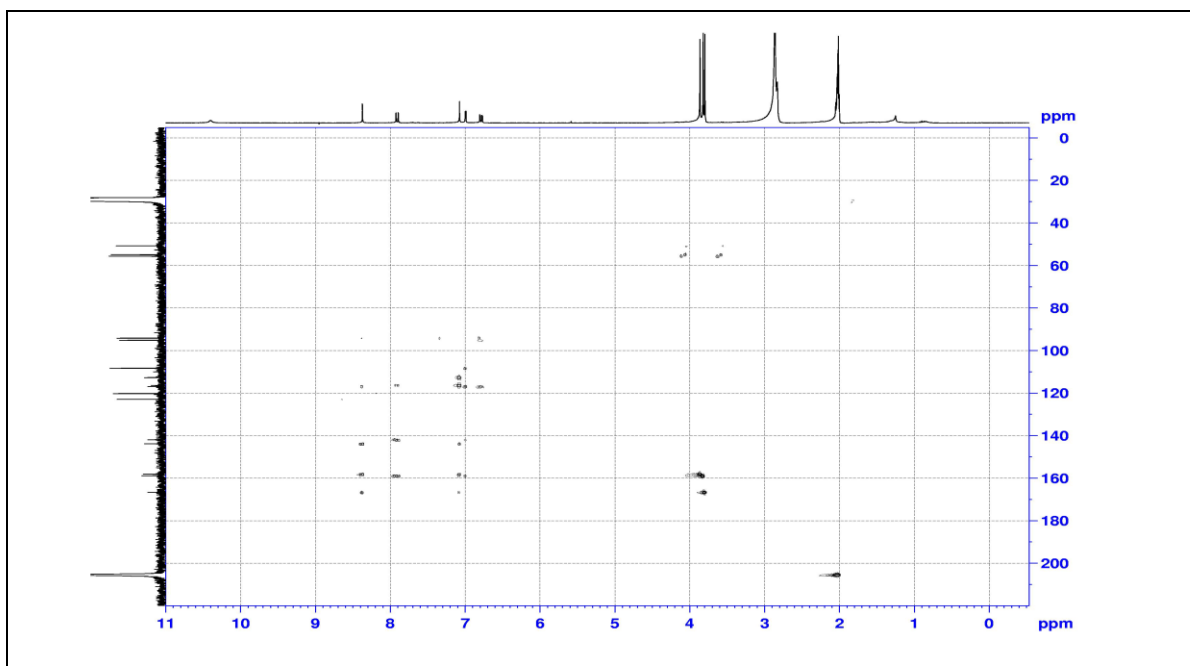


Figure 200 2D HMBC (CD_3COCD_3) of compound **RM24**

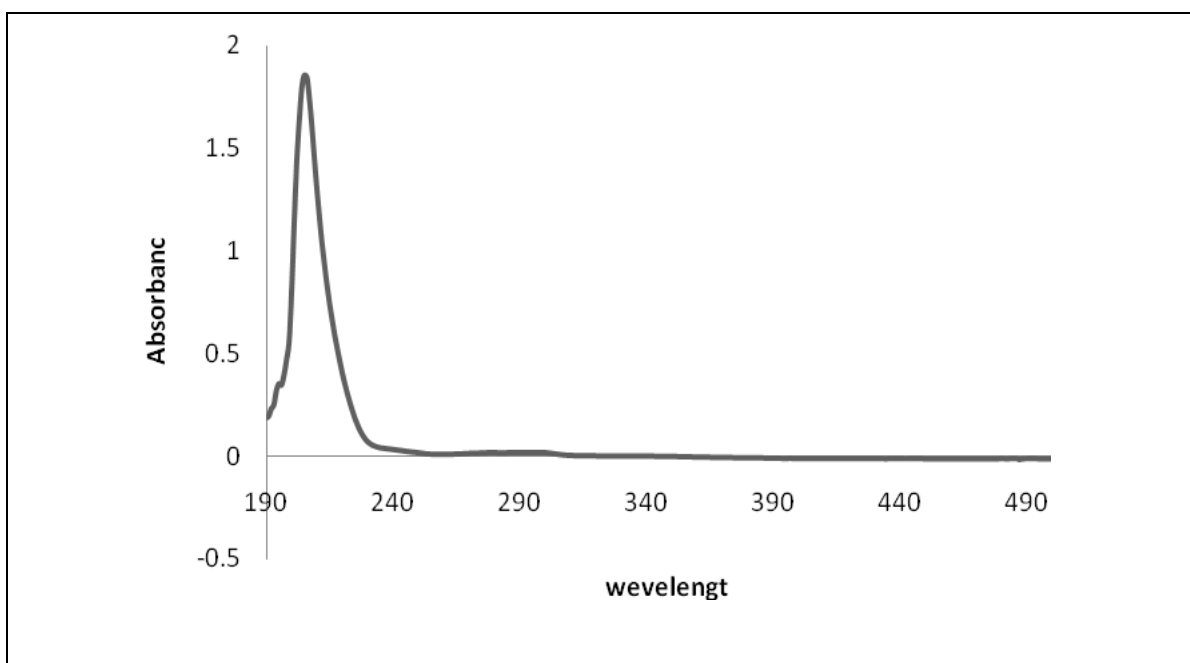


Figure 201 UV (MeOH) spectrum of compound **RM25**

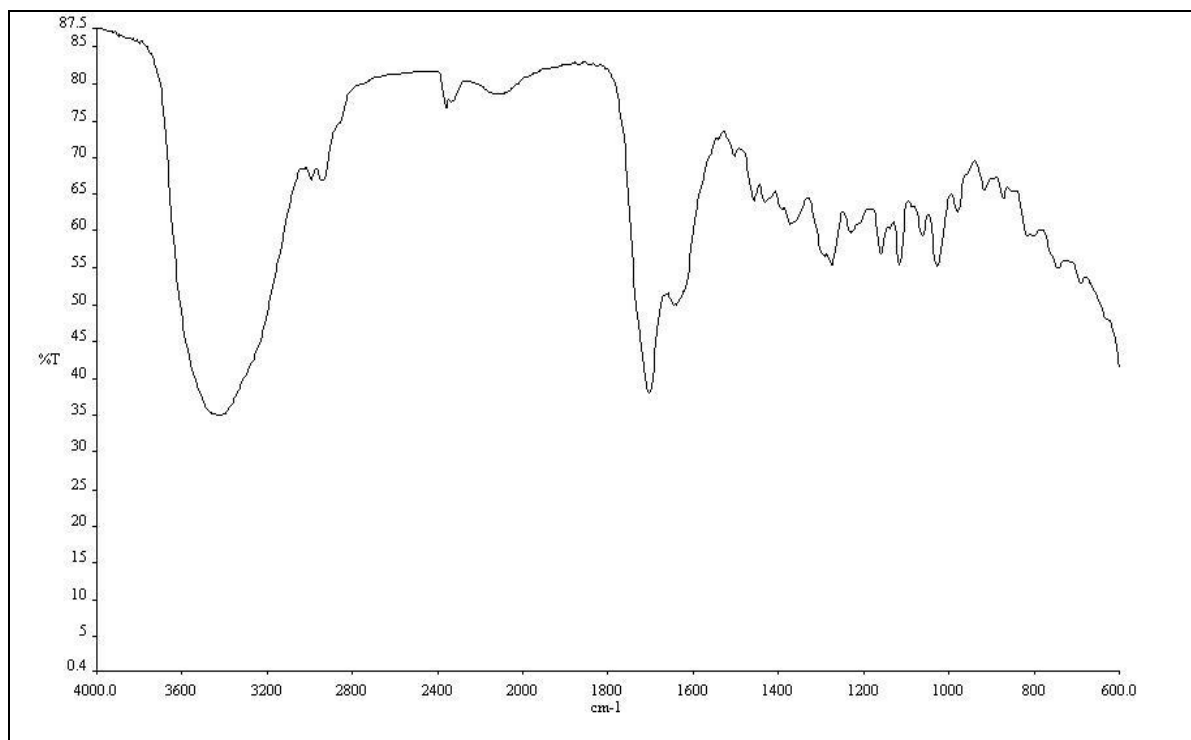


Figure 202 IR (neat) spectrum of compound **RM25**

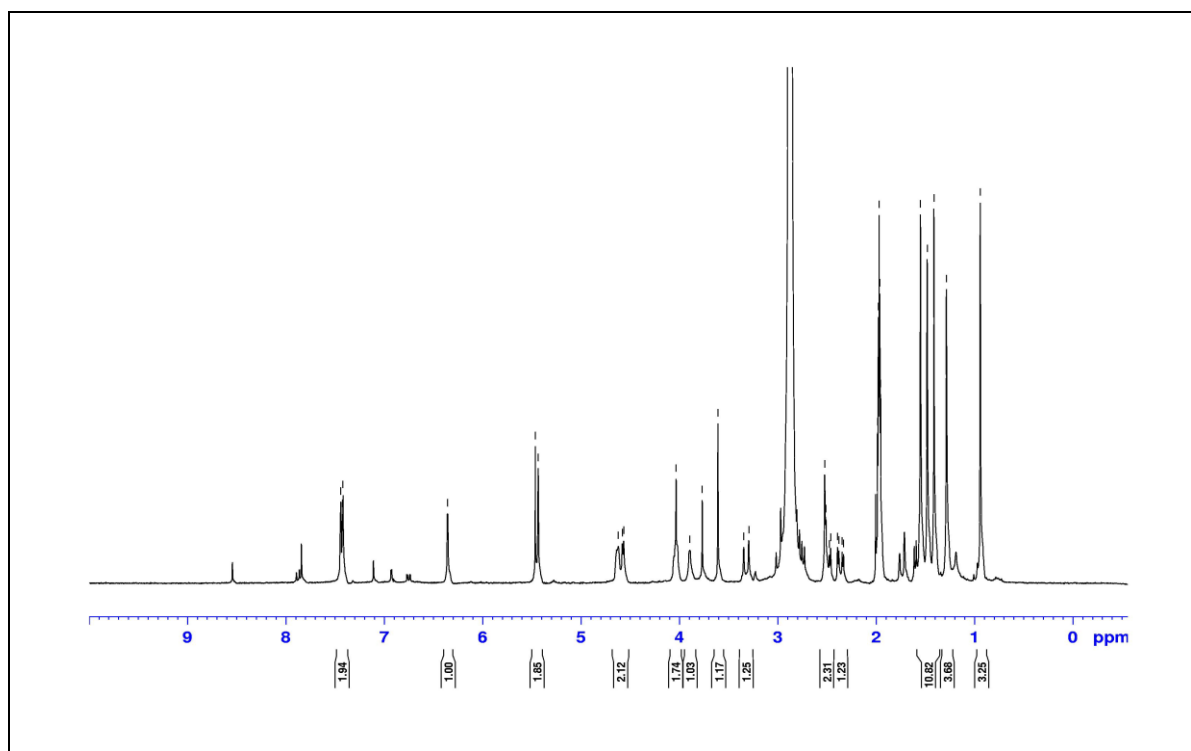


Figure 203 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM25**

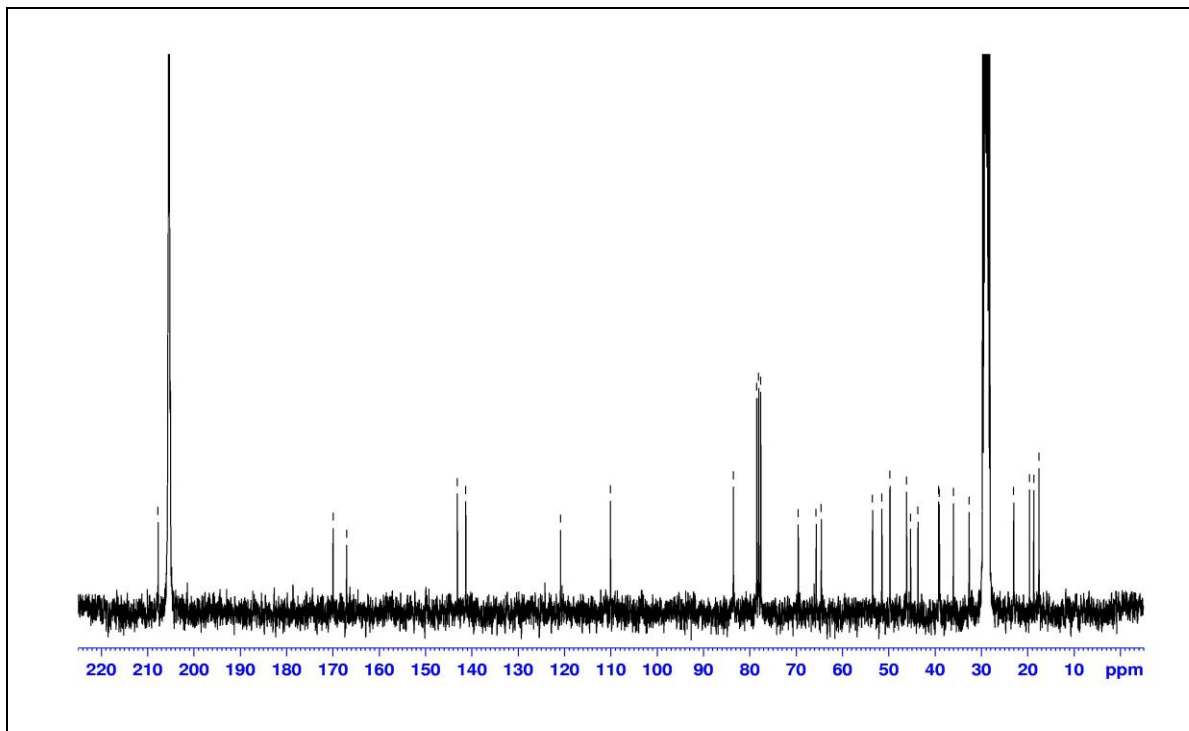


Figure 204 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM25**

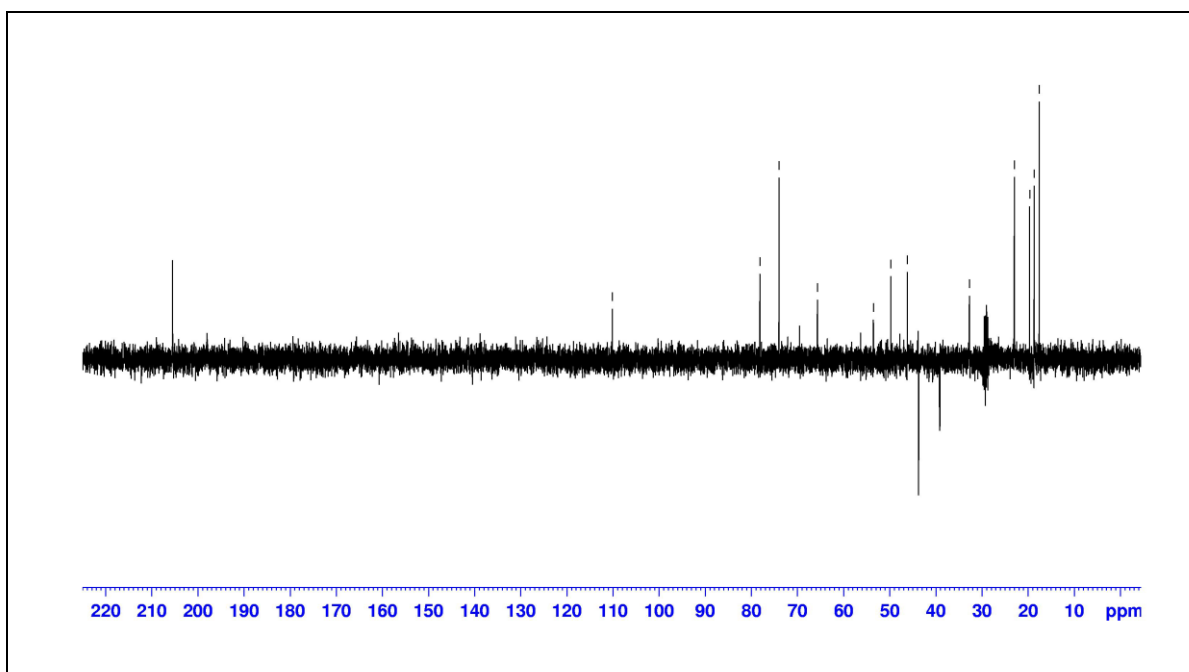


Figure 205 DEPT 135° (CD_3COCD_3) of compound **RM25**

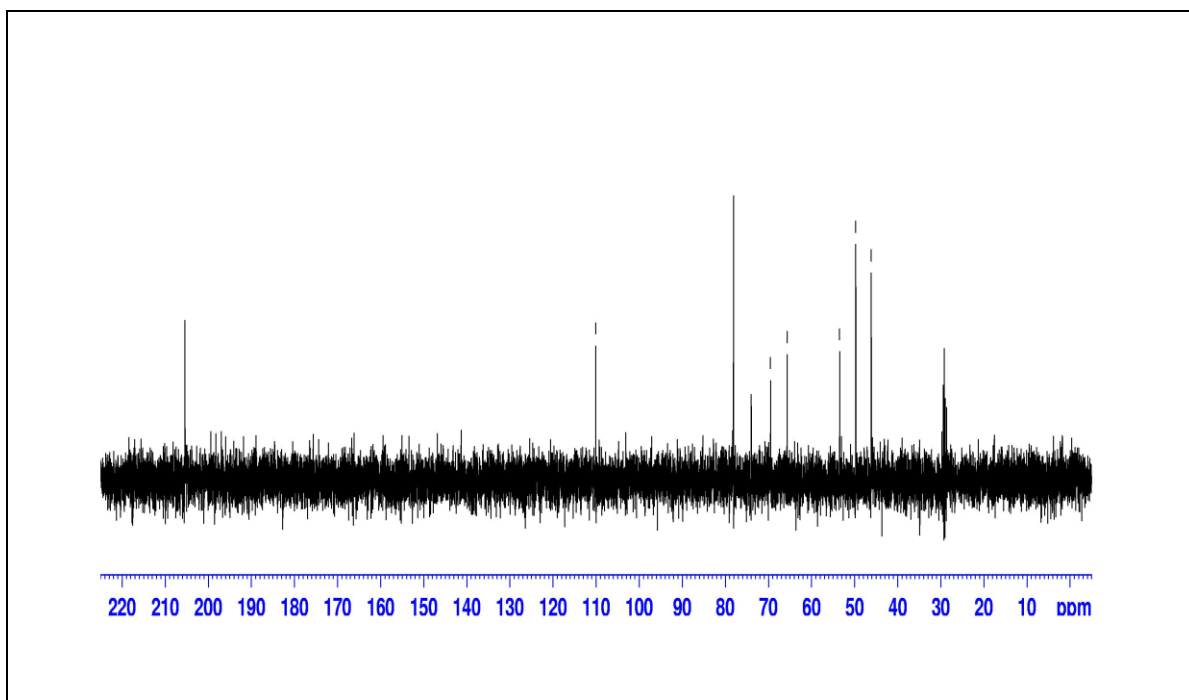


Figure 206 DEPT 90° (CD_3COCD_3) of compound **RM25**

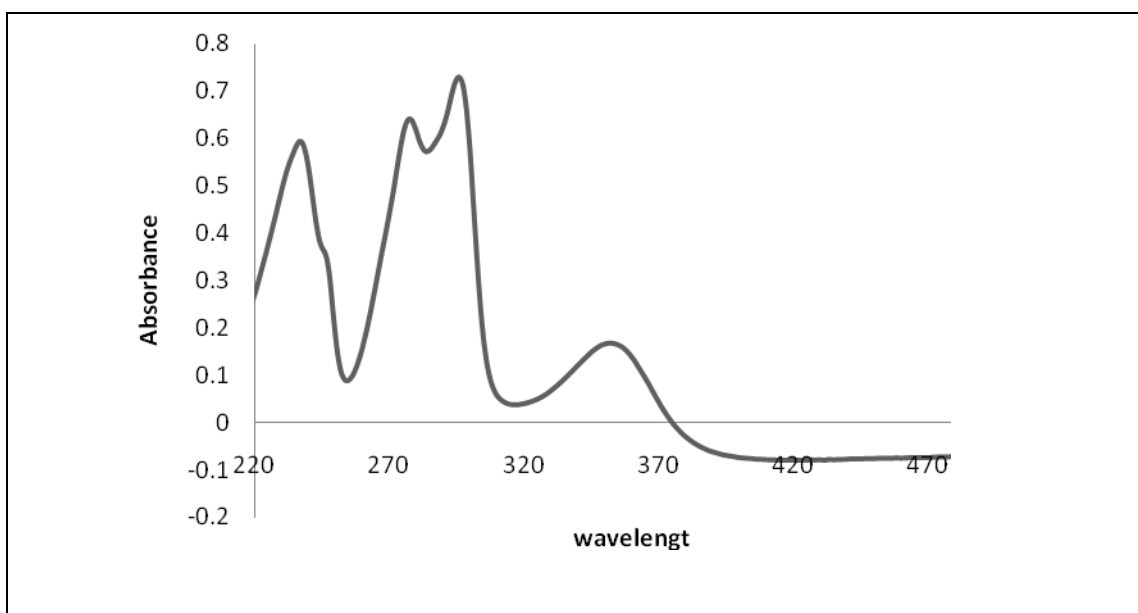


Figure 207 UV (MeOH) spectrum of compound **RM26**

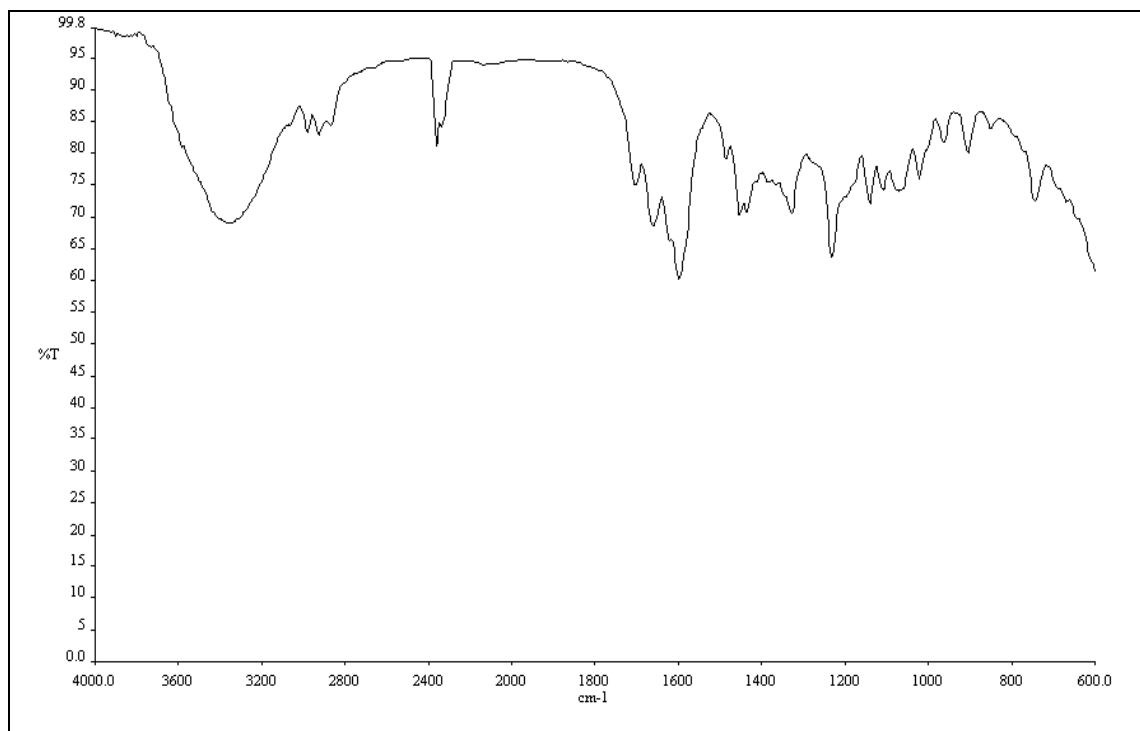


Figure 208 IR (neat) spectrum of compound **RM26**

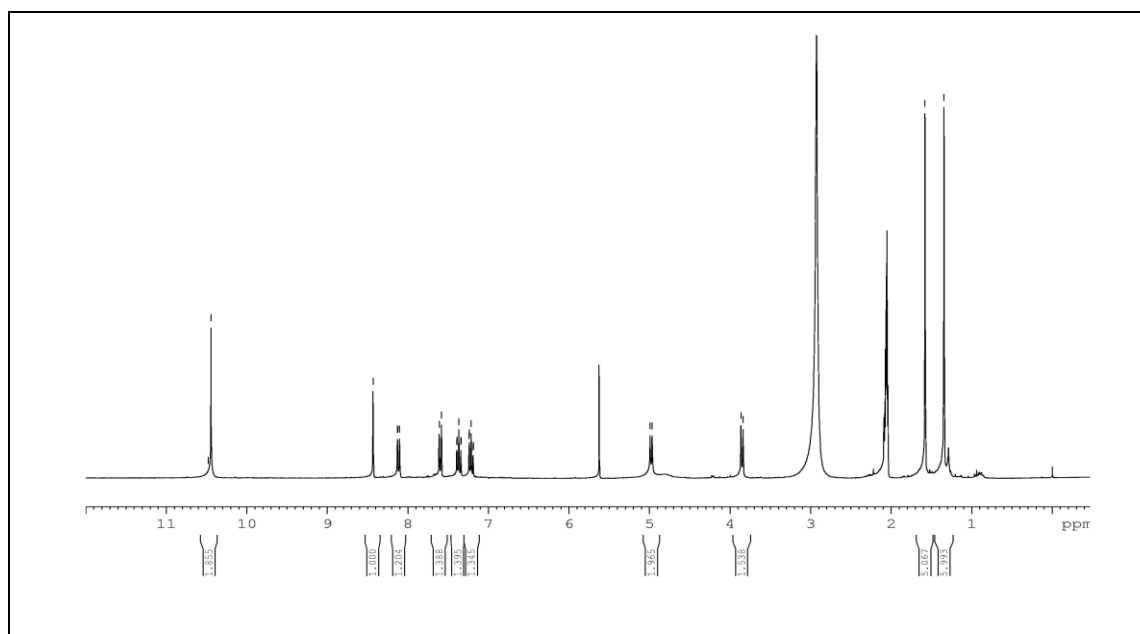


Figure 209 ¹H NMR (300 MHz) (CD₃COCD₃) of compound **RM26**

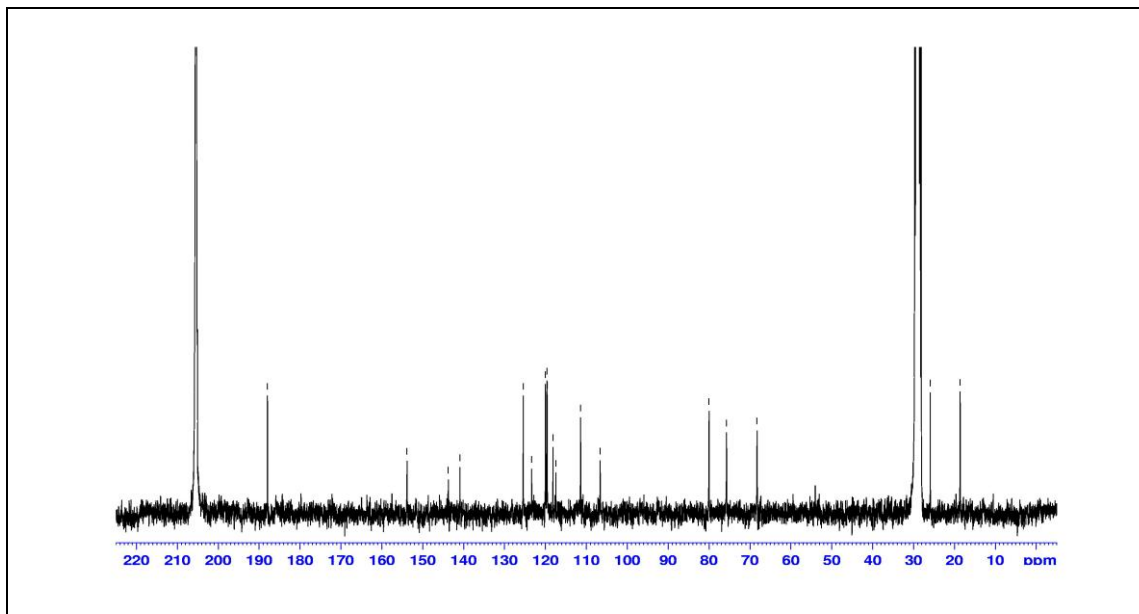


Figure 210 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM26**

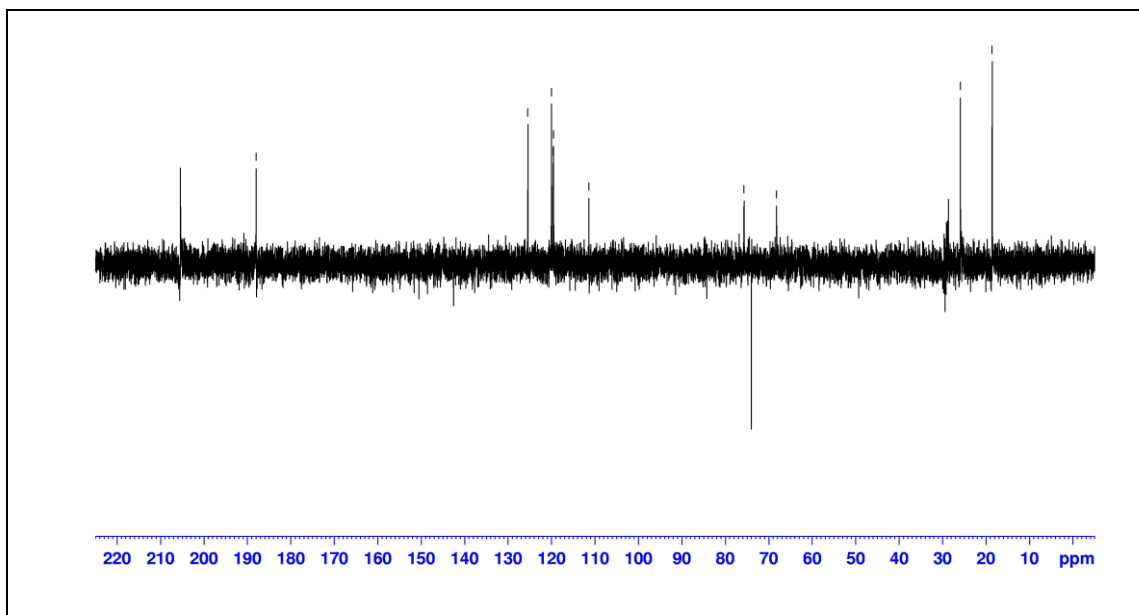


Figure 211 DEPT 135° (CD_3COCD_3) of compound **RM26**

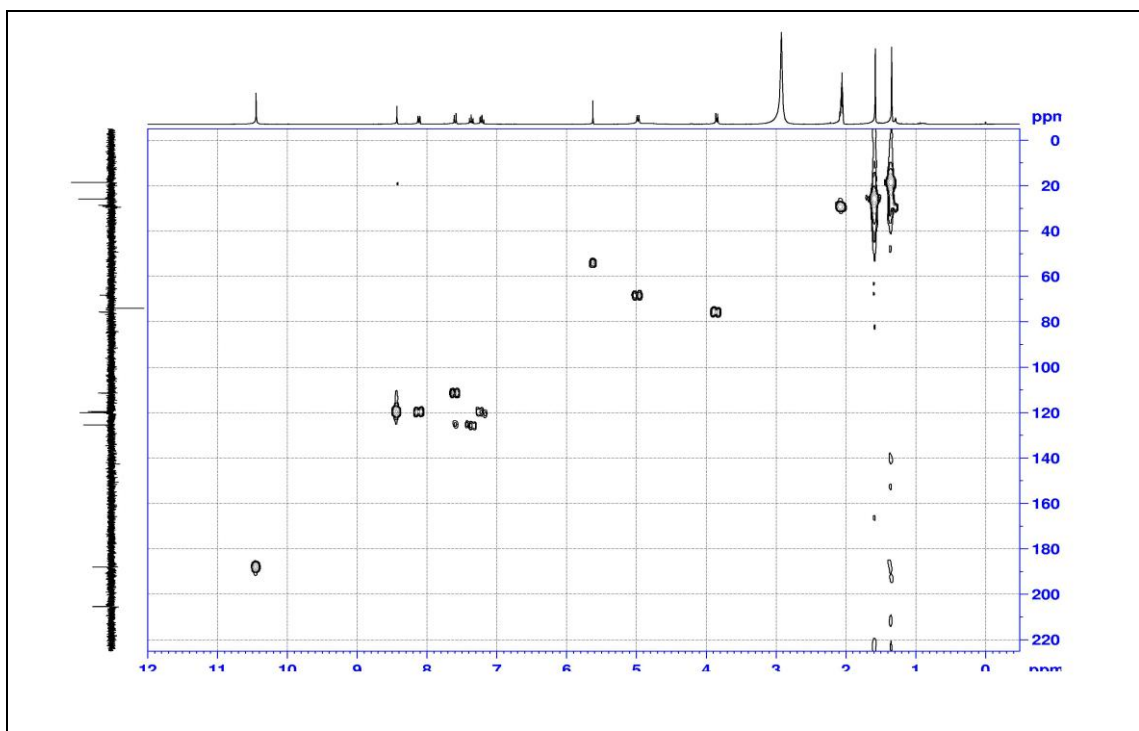


Figure 212 2D HMQC (CD₃COCD₃) of compound RM26

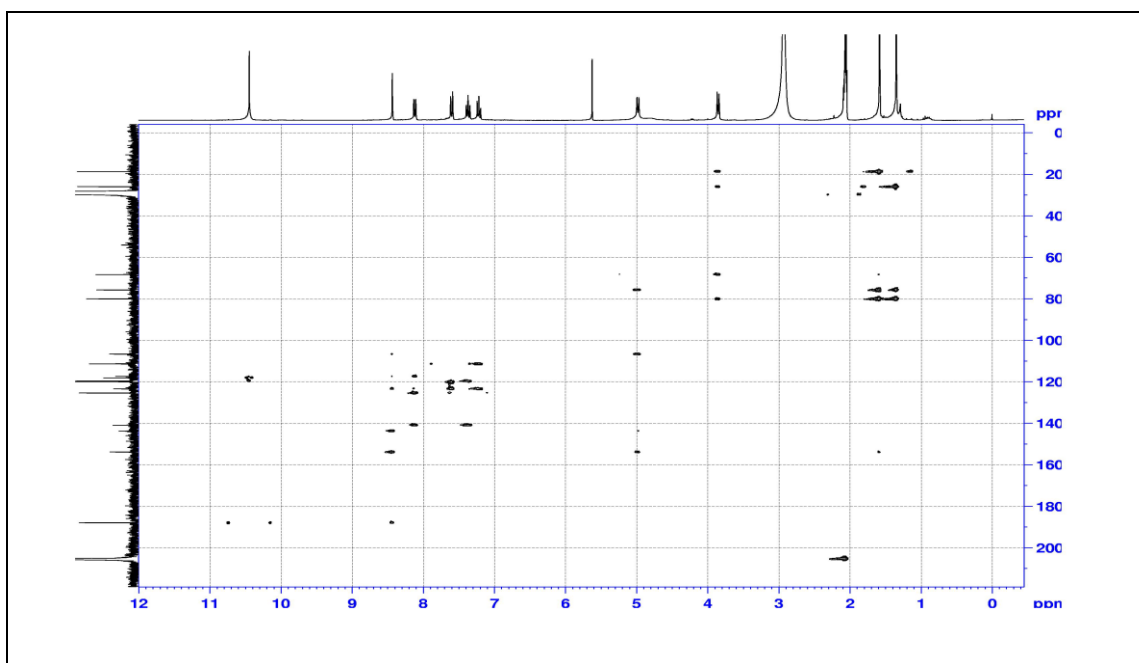


Figure 213 2D HMBC (CD₃COCD₃) of compound RM26

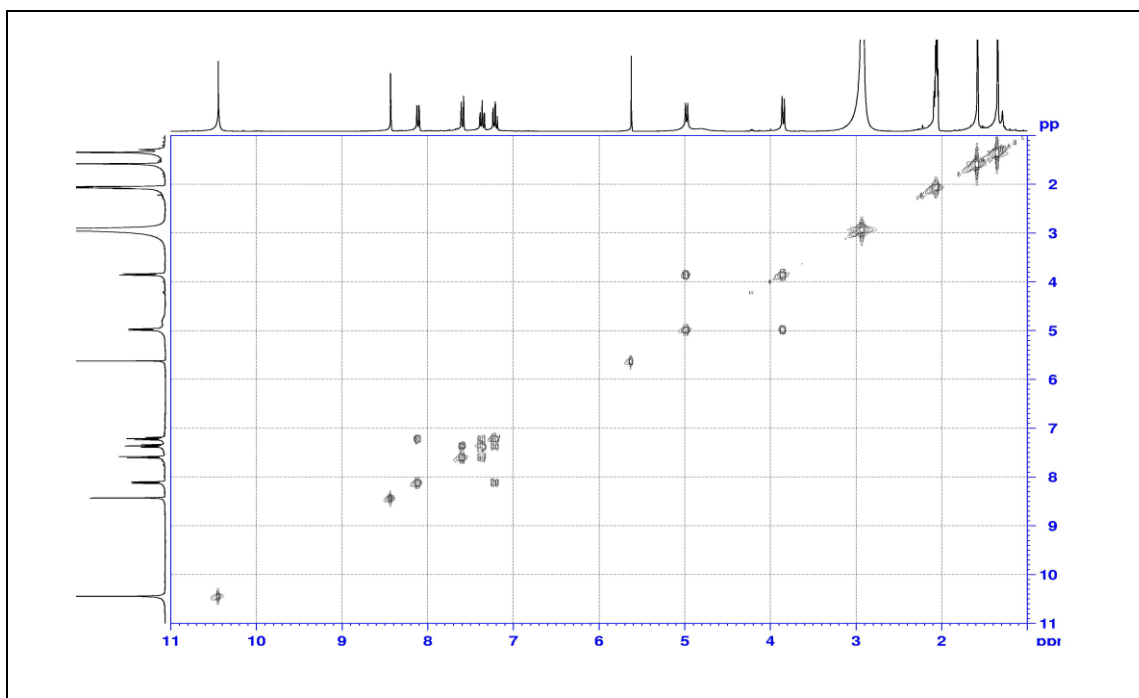


Figure 214 COSY (CD_3COCD_3) of compound **RM26**

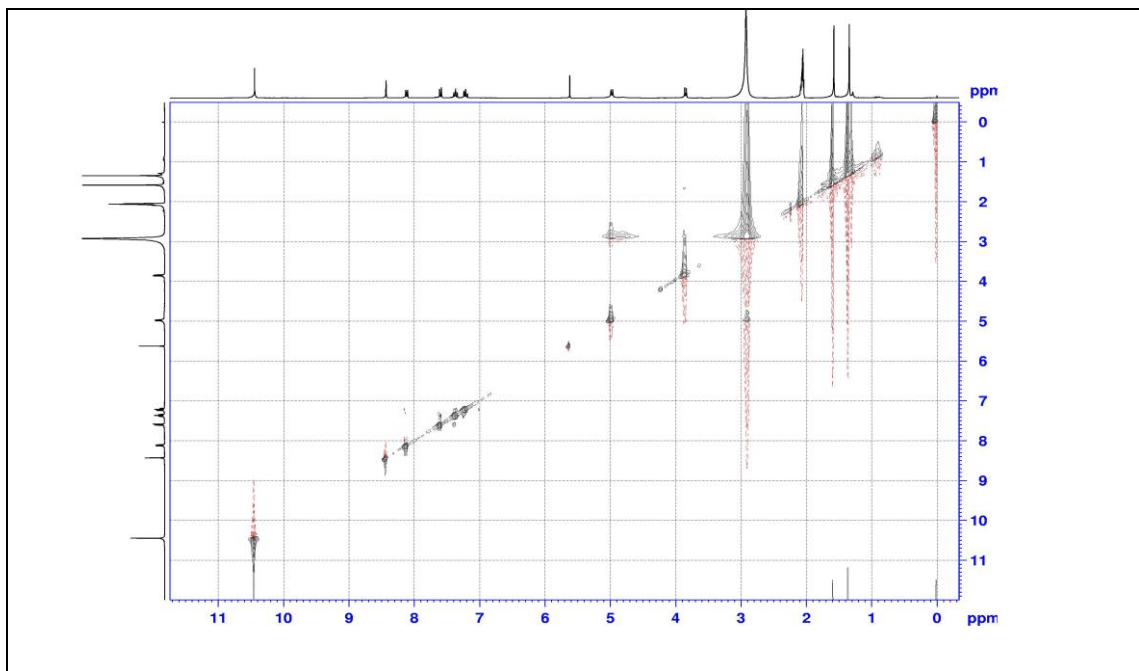


Figure 215 NOESY (CD_3COCD_3) of compound **RM26**

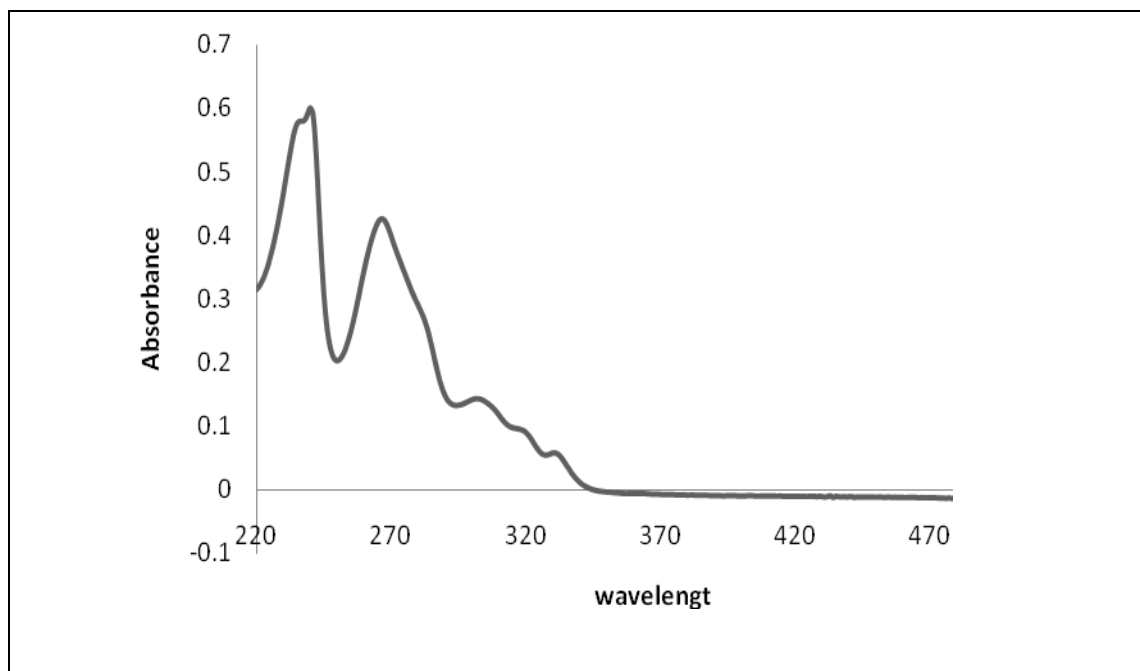


Figure 216 UV (MeOH) spectrum of compound **RM27**

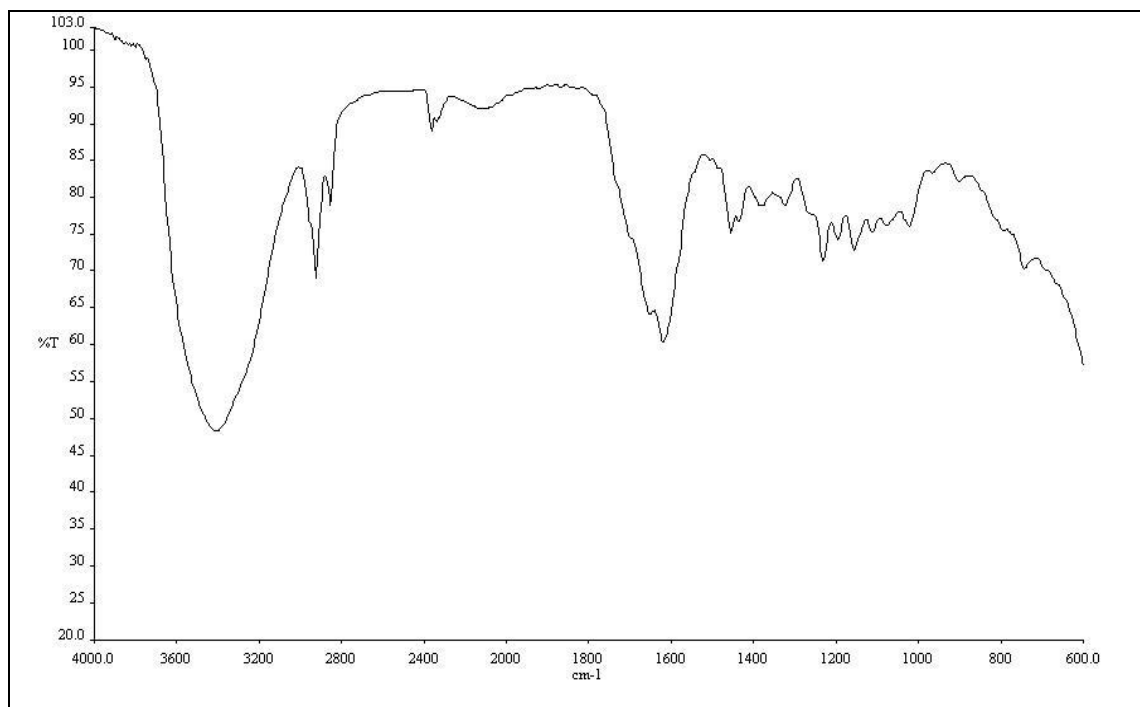


Figure 217 IR (neat) spectrum of compound **RM27**

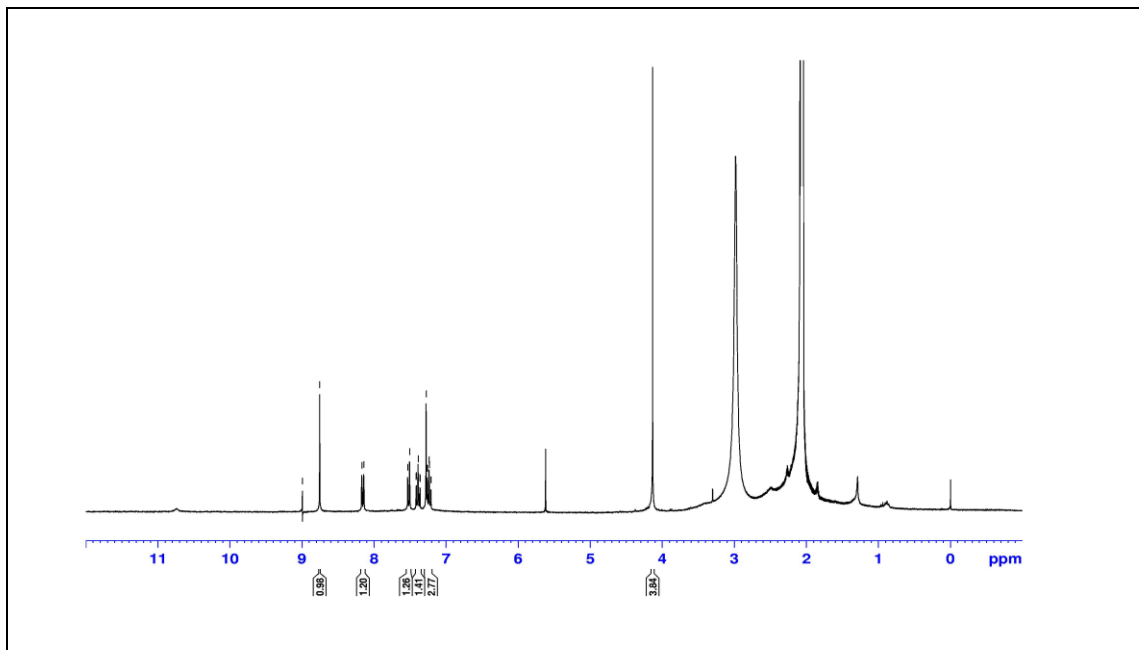


Figure 218 ^1H NMR (300 MHz) (CD_3COCD_3) of compound **RM27**

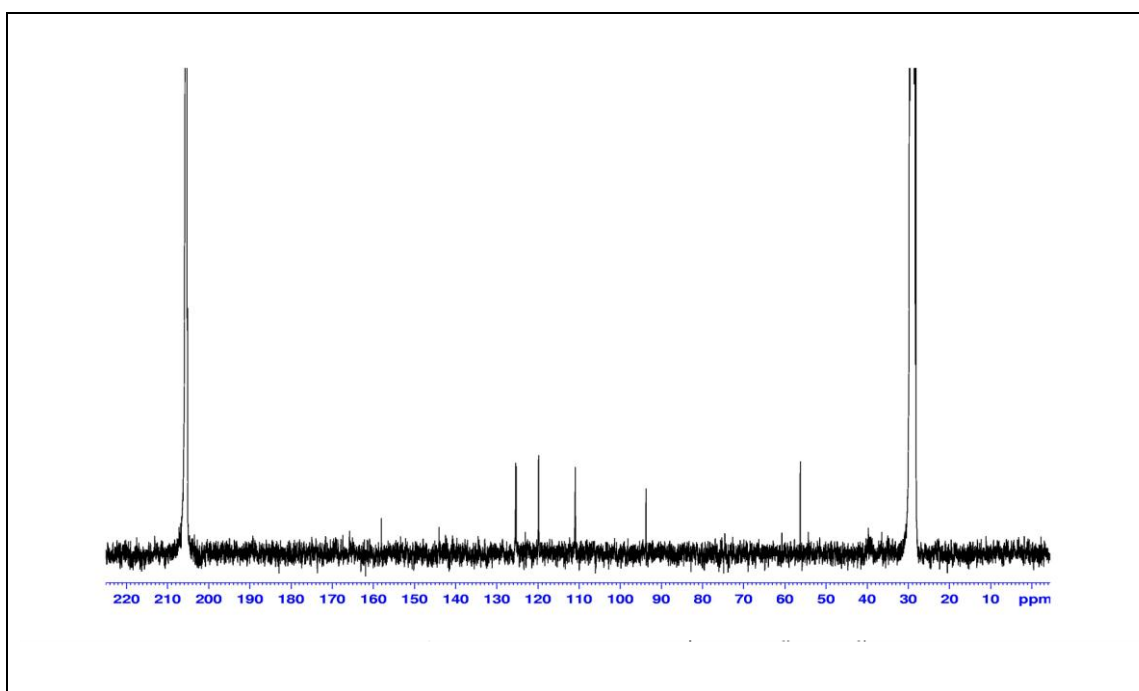


Figure 219 ^{13}C NMR (75 MHz) (CD_3COCD_3) of compound **RM27**

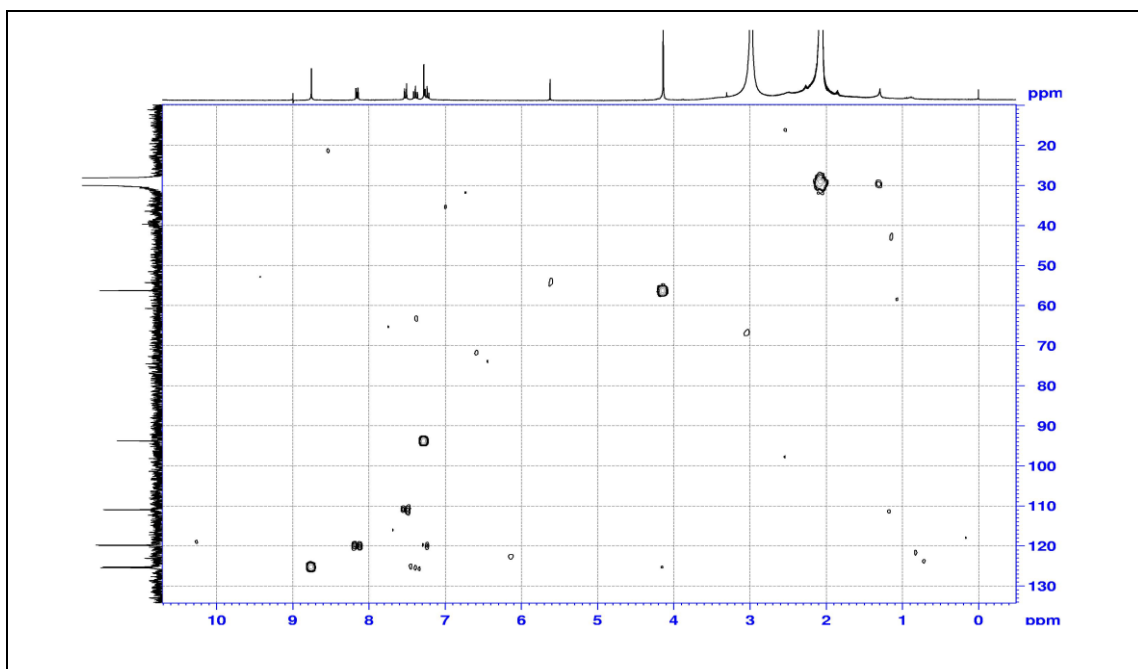


Figure 220 2D HMQC (CD_3COCD_3) of compound **RM27**

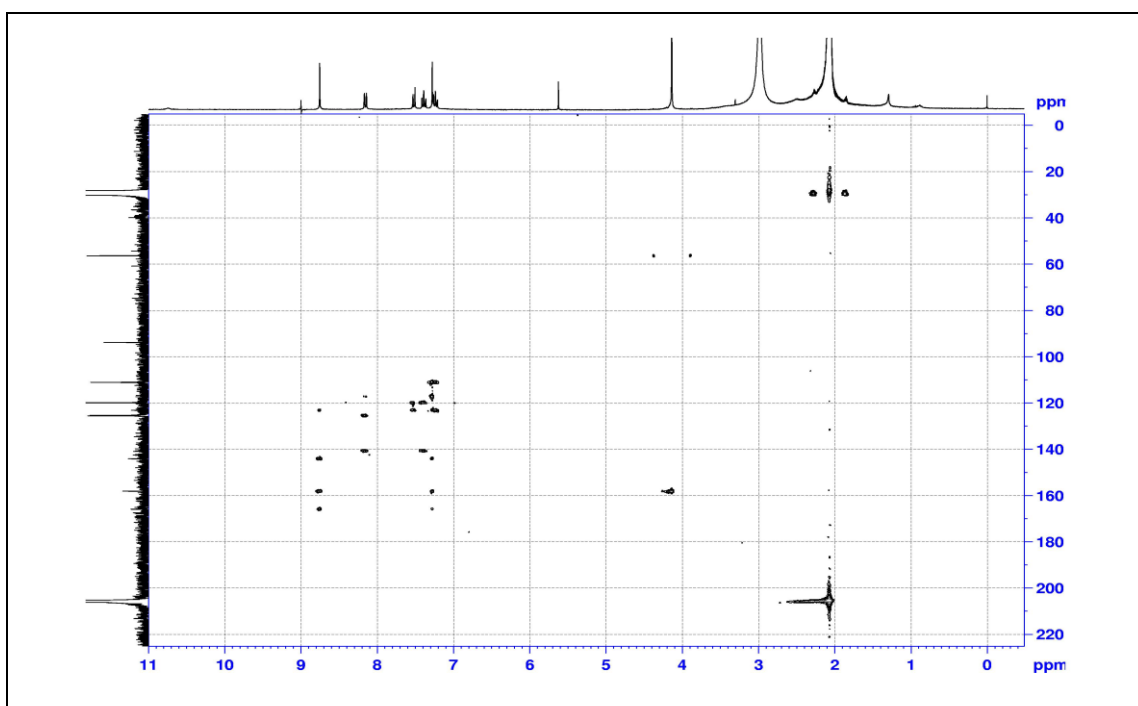


Figure 221 2D HMBC (CD_3COCD_3) of compound **RM27**

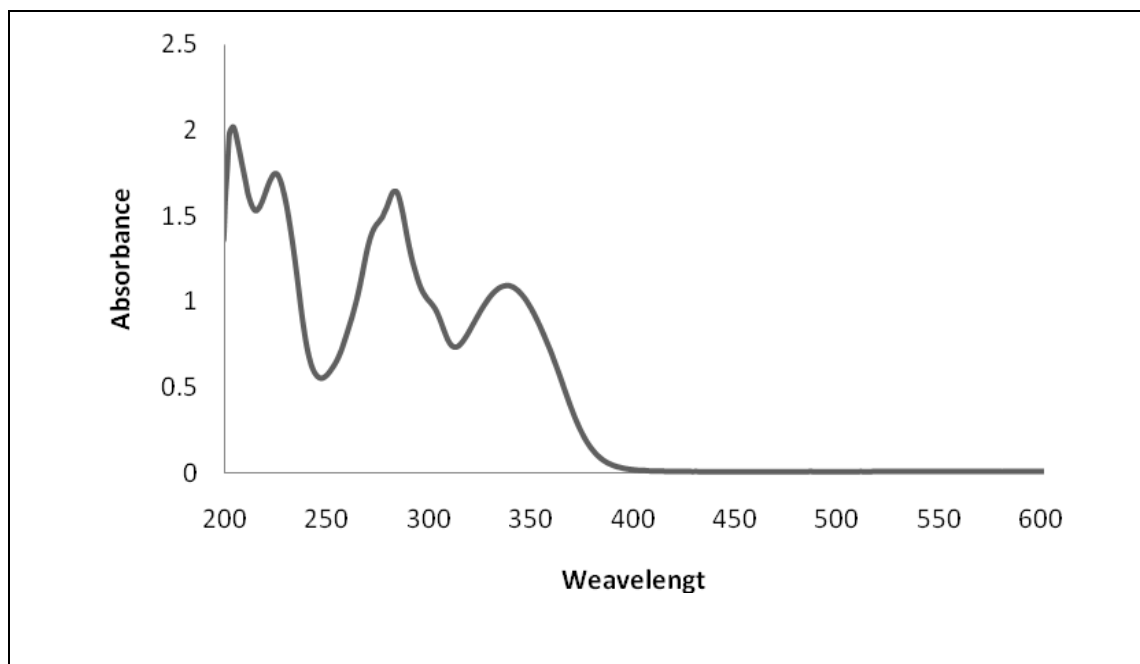


Figure 222 UV (MeOH) spectrum of compound **III** (Citrusarin-A)

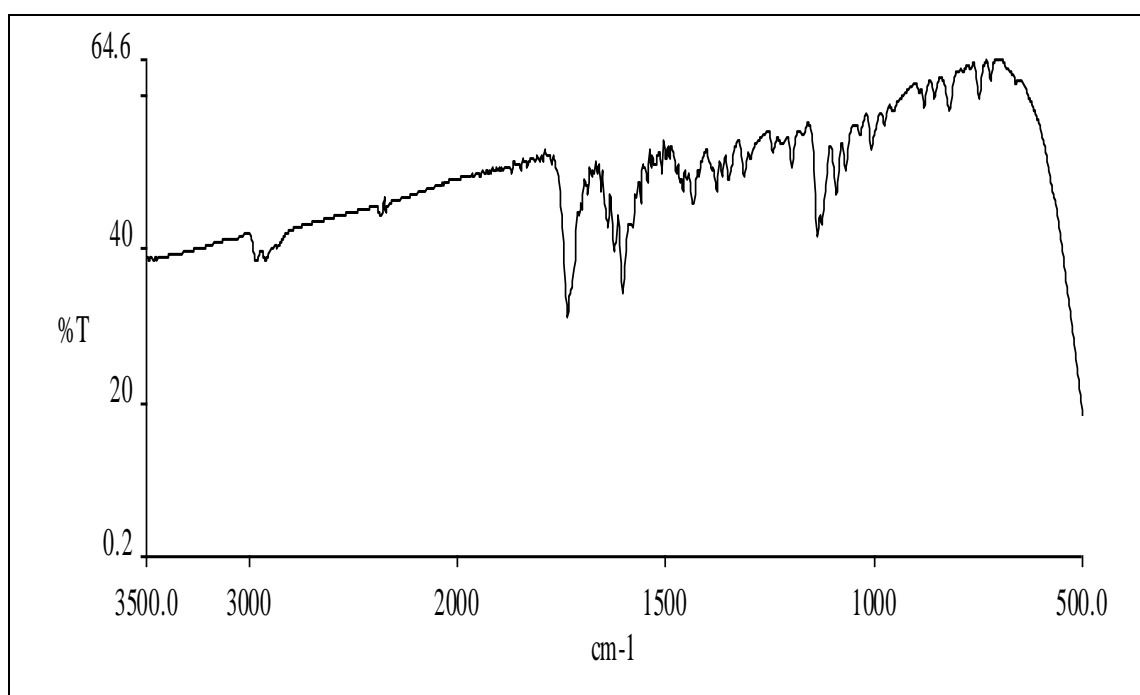


Figure 223 IR (neat) spectrum of compound **III** (Citrusarin-A)

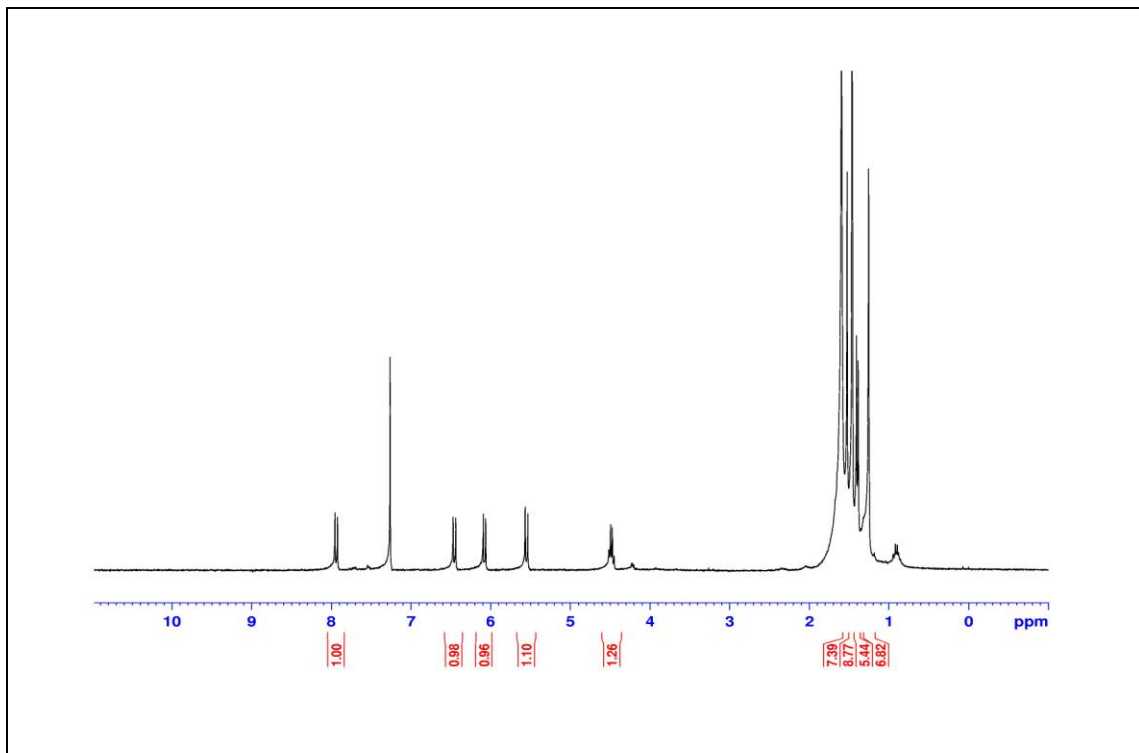


Figure 224 ^1H NMR (300 MHz) (CDCl_3) of compound **III** (Citrusarin-A)

VITAE

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Scholarship Awards during Enrolment

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Ministry of Education

List of Publication and Proceedings

1. Nitima Bindulem and d Carbazole Alkaloids from the Roots of *Micromelum minutum* (G. Forst) Wight & Arn. The 16th National Graduate Research Conference, Maejo University, Chiang Mai, Thailand, 11 March 2010 (Poster presentation)

2. Nitima Bindulem and Suda Chakthong. Carbazole Alkaloids and Pyranocoumarins from the Roots of *Micromelum minutum* (G. Forst) Wight & Arn. The 1st Current Drug Development International Conference, Woraburi Phuket Resort & Spa, Phuket, Thailand, 6-8 May 2010 (Poster presentation, Partial support from Natural Product Research Center)