# Chemical Constituents from the Bulb of *Eleutherine Americana* (Aubl.) Merr

**Chulida Hemtasin**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the**
**Degree of Master of Science in Chemical Studies**
**Prince of Songkla University**
**2008**

| | |
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| **Thesis Title** | Chemical Constituents from the Bulb of *Eleutherine americana* (Aubl.) Merr |
| **Author** | Miss Chulida Hemtasin |
| **Major Program** | Chemical Studies |

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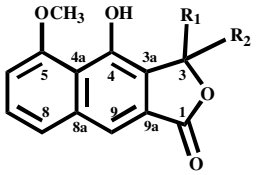
(Assoc. Prof. Dr. Krerkchai Thongnoo)
Dean of Graduate School

| | |
|---|---|
| **ชื่อวิทยานิพนธ์** | องค์ประกอบทางเคมีจากหัวของว่านหอมแดง (*Eleutherine americana* (Aubl.) Merr) |
| **ผู้เขียน** | นางสาวชุลิดา เหมตะศิลป์ |
| **สาขาวิชา** | เคมีศึกษา |
| **ปีการศึกษา** | 2550 |

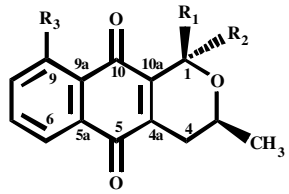
## บทคัดย่อ


การศึกษาองค์ประกอบทางเคมีของหัวว่านหอมแดง (*Eleutherine americana* (Aubl.) Merr) แยกได้สารประกอบที่ยังไม่มีรายงานการวิจัย ประเภทแอนทราควิโนน 2 สาร คือ 2-acetyl-3-hydroxy-8-methoxy-1-methylanthraquinone (**SA12**) และ 3,6,8-trihydroxy-4-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA13**) ประเภทแนปโทควิโนน 2 สาร คือ [8-methoxy-1,4-dioxo-3-(2-oxo-propyl)-1,4-dihydronaphthalen-2-yl]acetic acid methyl ester (**SA9**) และ 2-[2′-(acetyloxy)propyl]-3-hydroxy-5-methoxy-1,4-naphthoquinone (**SA10**) และประเภทอนุพันธ์แนปทาลีน 1 สาร คือ 3,4-dihydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one (**SA14**) นอกจากนี้ยังได้สารที่มีรายงานวิจัยแล้ว 10 สาร ได้แก่ 4-hydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one (**SA1**) (1*R*,3*S*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA2**) (1*R*,3*R*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho [2,3-*c*]pyran-5,10-dione (**SA3**) (1*R*,3*R*)5,10-dihydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho [2,3-*c*]pyran-4(3*H*)-one (**SA4**) (1*R*,3*S*)3,4-dihydro-9-hydroxy-1,3-dimethyl-1*H*-naphtho [2,3-*c*]pyran-5,10-dione (**SA5**) 4,8-dihydroxy-3-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA6**) 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA7**) 1,2,4,4a-tetrahydro-6-methoxy-2-methyl-10*H*-naphtho[2',3':2,3]cyclobuta [1,2-*b*]furan-5,10(3a*H*)-dione (**SA8**) 1,3,6-trihydroxy-8-methylanthraquinone (**SA11**) 1,2-dihydroxy-8-methoxy-3-methylanthraquinone (**SA15**) โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี UV IR NMR และ MS เปรียบเทียบกับสารที่มีรายงานการวิจัยแล้ว



**SA1**: $R_1 = CH_3$; $R_2 = H$

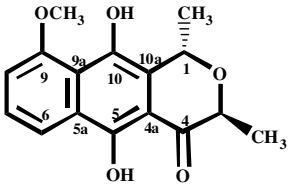
**SA14**: $R_1 = CH_3$; $R_2 = OH$



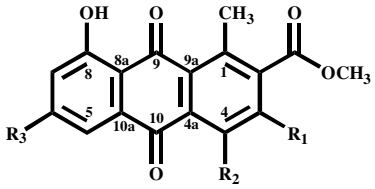
**SA2**: $R_1 = CH_3$; $R_2 = H$; $R_3 = OCH_3$

**SA3**: $R_1 = H$; $R_2 = CH_3$; $R_3 = OCH_3$

**SA5**: $R_1 = CH_3$; $R_2 = H$; $R_3 = OH$



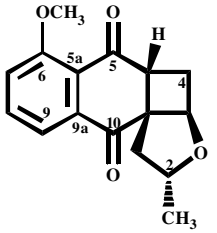
**SA4**



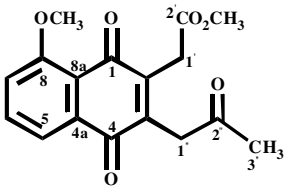
**SA6:** $R_1 = OCH_3$; $R_2 = OH$; $R_3 = H$

**SA7:** $R_1 = R_2 = OCH_3$; $R_3 = H$

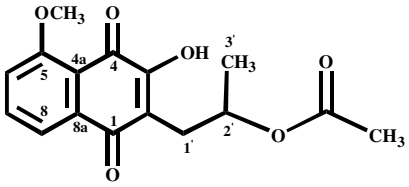
**SA13:** $R_1 = R_3 = OH$; $R_2 = OCH_3$



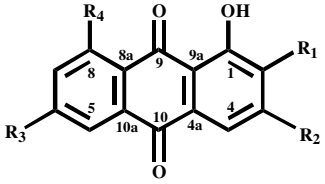
**SA8**



**SA9**

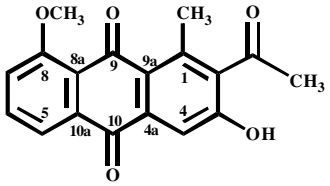


**SA10**



**SA11**: $R_1 = H$; $R_2 = R_3 = OH$; $R_4 = CH_3$

**SA15**: $R_1 = OH$; $R_2 = CH_3$; $R_3 = H$; $R_4 = OCH_3$



**SA12**: $R_1 = CH_3$; $R_2 = R_3 = H$; $R_4 = OCH_3$

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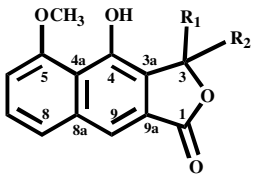
| **Author** | Miss Chulida Hemtasin |
| **Major Program** | Chemical Studies |
| **Academic Year** | 2007 |

# ABSTRACT

Investigation of the chemical constituents of the bulb of *Eleutherine americana* (Aubl.) Merr yielded two new anthraquinones: 2-acetyl-3-hydroxy-8-methoxy-1-methylanthraquinone (**SA12**) and 3,6,8-trihydroxy-4-methoxy-1-methyl anthrquinone-2-carboxylic acid methyl ester (**SA13**), two new naphthoquinones: [8-methoxy-1,4-dioxo-3-(2-oxo-propyl)-1,4-dihydronaphthalen-2-yl]acetic acid methyl ester (**SA9**) and 2-[2′-(acetyloxy)propyl]-3-hydroxy-5-methoxy-1,4-naphthoquinone (**SA10**), and one naphthalene derivative: 3,4-dihydroxy-5-methoxy-3-methyl naphtho[2,3-*c*]furan-1(3*H*)-one (**SA14**). Ten known compounds were also obtained: 4-hydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one (**SA1**), (1*R*,3*S*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA2**), (1*R*,3*R*) 3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA3**), (1*R*,3*R*)5,10-dihydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-4(3*H*)-one (**SA4**), (1*R*,3*S*)3,4-dihydro-9-hydroxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA5**), 4,8-dihydroxy-3-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA6**), 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA7**), 1,2,4,4a-tetrahydro-6-methoxy-2-methyl-10*H*-naphtho [2',3':2,3]cyclobuta[1,2-*b*]furan-5,10(3a*H*)-dione (**SA8**) 1,3,6-trihydroxy-8-methyl anthraquinone (**SA11**) and 1,2-dihydroxy-8-methoxy-3-methylanthraquinone (**SA15**). Their structures were determined on the basis of UV, IR, NMR and MS by comparison their spectroscopic data including the optical rotations, with those reported.



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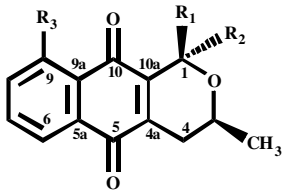
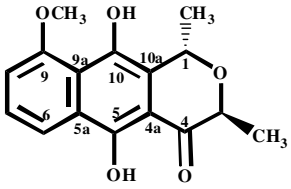


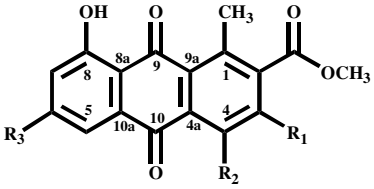
**SA2**: $R_1 = CH_3$; $R_2$ =H; $R_3 = OCH_3$

**SA3**: $R_1 = H$; $R_2 = CH_3$; $R_3 = OCH_3$

**SA5**: $R_1 = CH_3$; $R_2$ =H; $R_3 = OH$



**SA4**



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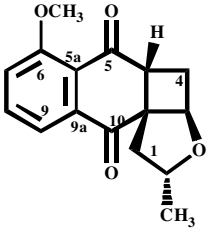
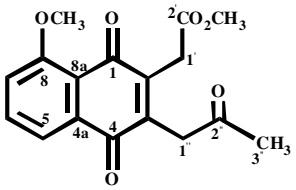
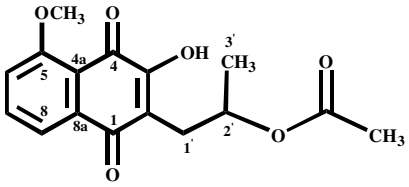


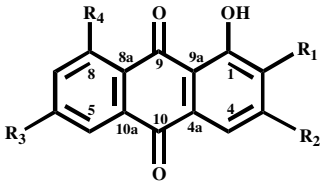
**SA8**



**SA9**

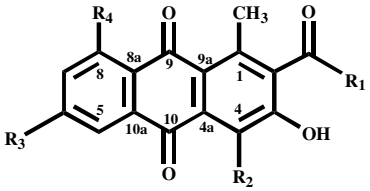


**SA10**



**SA11**: $R_1 = H$; $R_2 = R_3 = OH$; $R_4 = CH_3$

**SA15** $R_1 = OH$; $R_2 = CH_3$; $R_3 = H$; $R_4 = OCH_3$



**SA12**: $R_1 = CH_3$; $R_2 = R_3 = H$; $R_4 = OCH_3$

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Chulida Hemtasin

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# LIST OF ILLUSTRATIONS (Continued)

# LIST OF ABBREVIATIONS AND SYMBOLS

| | | |
|---|---|---|
| *s* | = | singlet |
| *d* | = | doublet |
| *t* | = | triplet |
| *m* | = | multiplet |
| *dd* | = | doublet of doublet |
| *dt* | = | doublet of triplet |
| *td* | = | triplet of doublet |
| *ddd* | = | doublet of doublet of doublet |
| *br s* | = | broad singlet |
| g | = | gram |
| kg | = | kilogram |
| mg | = | milligram |
| % | = | percent |
| nm | = | nanometer |
| m.p. | = | melting point |
| $cm^{-1}$ | = | reciprocal centimeter (wave number) |
| $\delta$ | = | chemical shift relative to TMS |
| *J* | = | coupling constant |
| $\lambda_{max}$ | = | maximum wavelength |
| $\nu$ | = | absorption frequencies |
| $\varepsilon$ | = | molar extinction coefficient |
| $^{\circ}C$ | = | degree celcius |
| MHz | = | Megahertz |
| ppm | = | part per million |
| IR | = | Infrared |
| UV | = | Ultraviolet-Visible |
| NMR | = | Nuclear Magnetic Resonance |
| 2D NMR | = | Two Dimentional Nuclear Magnetic Resonance |
| COSY | = | Correlated Spectroscopy |

## LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

| | | |
|---|---|---|
| DEPT | = | Distortionless Enhancement by Polarization Transfer |
| HMBC | = | Heteronuclear Multiple Bond Correlation |
| HMQC | = | Heteronuclear Multiple Quantum Coherence |
| CC | = | column chromatography |
| TMS | = | tetramethylsilane |
| Acetone-$d_6$ | = | deuteroacetone |
| DMSO-$d_6$ | = | deuterodimethylsulphoxide |
| $CDCl_3$ | = | deuterochloroform |
| MeOH | = | Methanol |
| $CH_2Cl_2$ | = | Dichloromethane |
| TLC | = | Thin-Layer Chromatography |
| MIC | = | Minimum Inhibition Concentration |

# CHAPTER 1
# INTRODUCTION

## 1.1 Introduction

Nowadays, people around the world pay more attention to health care, especially “natural therapy”. Herb medicines play important roles in many countries. Various kinds of cosmetics have herbs or some kinds of plants as parts of their ingredients. People gained knowledge from ancestors, in term of folkloric medicines. Accordingly, various parts of the plants, bulb, stems, leaves and roots etc. can be used to treat illness. *Curcuma longa* Linn. (ขมิ้นชัน) protects and heals ulcer, *Andrographis paniculata* Wall.ex Nees (ฟ้าทะลายโจร) reliefs the symptom of cold, whereas *Rhinacanthus nasutus* Linn. kurz (ทองพันชั่ง) and *Streblus aspera* Lour. (ข่อย) are used to treat skin diseases. Medicinal plant are used worldwide, it is estimated by the World Health Organization that approximately 75-80% of the world's population uses plant medicines either in part or entirely (Taylor *et al.,* 2000).

*Eleutherine americana* (Iridaceae), known as Wann-Hom-Daeng in Thailand, is a herbal plant cultivated in China, Indonesia and Thailand as an ornamental and medicinal plant. The red bulb is used for treatment of cardiac diseases, especially coronary disorders (Komura *et al*., 1983) and as an ingredient for treating illness such as cold or people who have to suffer from nasal congestion (Saralamp, *et al*., 1996). Anthraquinones, naphthalenes and naphthoquinones have been isolated and identified from the bulb of *E. americana* (Xu *et al*., 2005). Some of the compounds in this species display important biological activities such as eleutherin and isoeleutherin had the effect of increasing coronary flow in an isolated guinea pig heart (Chen *et al*., 1986) and showed antifungal activity (Alves *et al*., 2003), However, there are only a few reports on the chemical constituents. We were therefore motivated to investigate its constituents in detail.

## 1.2 Review of Literatures

*Eleutherine* is in the family of Iridaceae. They are distributed throughout South America and Asian (Goldblatt, *et al*., 1991). The chemical constituents and biological activity of five species: *E. americana* (Aubl.) Merr, *E. bulbosa, E. subaphylla* Gagnep, *E. plicata and E. palmifolia* were reported for. *E. americana* (Aubl.) Merr is only one species of *Eleutherine* found in Thailand (สมิตินันทน์, 2523).

### 1.2.1 The Chemical Constituents and Biological Activity of *Eleutherine* genus

Naphthoquinones, naphthalenes and anthraquinones were the major components isolated from the rhizome of *Eleutherine* genus. Eleutherin, isoelutherin and eleutherol were common in this genus (Chen, *et al*., 1981). Glycosides were also reported (Shibuya, *et al*., 1997). The chemical constituents isolated from the *Eleutherine* genus were summarized in **Table 1**. (The literature survey from SciFinder Scholar databases).

*Eleutherine* genus is a herbal plant (Schultes & Raffaut, 1990). *E. bulbosa* is used for painful and irregular menstruation (Hodge & Taylor, 1956) and as an abortive and antifertility agent (Weniger, *et al*., 1982). The rhizome of *E. subaphylla* Gagnep has been used as a folk medicine for antibacterial agents and as haemostatic (Dan & Mai, 1990). *E. americana* has been used for treatment of coronary disorders in some countries such as China (Ding & Huang, 1983 and Chen, *et al*., 1997). In Thailand, it was locally used for treatment of fever and skin disease.

Napthoquinone and naphthalene derivatives from the bulb of *E. bulbosa*, eleutherin and eleutherol displayed antibacterial activities against *Bacillus subtilis, Micrococcus pyogenes var. aureus* and *Streptococcus hemolyticus* (Schmid, *et al*., 1951 and Bianchi, *et al*., 1975). Eleutherin isolated from *E. americana* was reported to show inhibitory activities against human topoisomerase II with the MIC 50 μg/mL, whereas isoeleutherin and isoeluetherol demonstrated inhibitory activity against HIV replication in H9 lymphocytes with $IC_{50}$ 8.55 μg/mL and 1.41μg/mL, respectively (Hara, *et al*., 1997). Furthermore, eleutherin, isoeleutherin and

4-hydroeleutherin could inhibit human erythroleukemia as well as their activities against the growth of *pyricularia oryzae* (Xu, *et al*., 2005).

### 1.2.2 *Eleutherine americana* (Aubl.) Merr

*E. americana* (Aubl.) Merr is in the family of Iridaceae. Its local names in Thailand is "Wann-Hom-Daeng" (ว่านหอมแดง; ภาคกลาง), Wann-Kai-Daeng" (ว่านไก่แดง; เชียงใหม่), "Bore-Jer (บ่อเจอ; แม่ฮ่องสอน) (สมิตินันทน์, 2523). *E. americana* is an annual, perennial herb, subterranean shallot-like with purplish red, scale-leaf. Leaf simple, basal, linear, blade-like, apex acute, base narrow, smooth margin, 2-4 cm wide, 20-40 cm long, palm-like. Inflorescence in fascicle, protruding from bulb. Flowers are spray, its white, peduncles 2.5-4 cm, calyx is green. Fruit is oblong capsule (Saralamp, *et al*., 1996).

**Figure 1** *Eleutherine americana* (Aubl.) Merr

**Table 1** Compounds from the *Eleutherine* genus

| Compounds | structures | Bibliography |
|---|---|---|
| ***E. americana*** (Rhizome) | | |
| eleutherol | **2** | Chen, *et al*., |
| eleutherin | **18** | 1981 |
| isoeleutherin | **19** | |
| ***E. americana*** (Bulb) | | |
| 4,8-dihydroxy-3-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester | **28** | Komura, *et al*., 1983 |
| 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester | **29** | |
| 9,10-dihydro-6,8-dihydroxy-3,4-dimethoxy-1-methyl-9,10-dioxo-anthracene-2-carboxylic acid methyl ester | **31** | |
| 3,4,8-trimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester | **32** | |
| elecanacin | **21** | Hara*, et al*., 1997 |
| dihydroeleutherinol | **3** | Xu, *et al*., |
| hongconin | **1** | 2005 |
| 2-acetyl-3,6,8-trihydroxy-1-methyl-9,10-anthracenedione | **33** | |
| 1,3,6-trihydroxy-8-methylanthraquinone | **30** | |
| 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester | **29** | |
| 3,4-dihydro-4-hydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione | **25** | |
| 9,9′-dihydroxy-8,8′-dimethoxy-1,1′-dimethyl-[4,4′-binaphtho[2,3-*c*]furan]-3,3′(1*H*,1′*H*)-dione | **13** | |

**Table 1** (continued)

| Compounds | structures | Bibliography |
|---|---|---|
| 2-(2-hydroxypropyl)-5-methoxy-1,4-naphthalenedione | **26** | Nielson, |
| 5-methoxy-(1-methoxymethoxy)-α-methyl-2-naphthaleneethanol | **5** | *et al*., 2006 |
| 2-[(2*R*)-2-(ethenyloxy)propyl]-5-methoxy-1,4-naphthalenedione | **27** | |
| ***E. bulbosa*** (Bulb) | | |
| 4-ethyl-2,3-dihydro-6-methoxy-2-methylnaphtho[1,2-*b*]furan-5-ol | **7** | Schmid, |
| 3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione | **17** | *et al*., 1950 |
| 3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-diol | **9** | |
| 5-ethoxy-3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-10-ol | **10** | |
| 5-ethoxy-3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-10-ol | **16** | |
| 3,4,6,7,8,9-hexahydro-5,10-dimethoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran | **8** | |
| 4-ethyl-2,3-dimethyl-6-methoxy-2-methylnaphtho [1,2-*b*]furan-5-ol acetate | **6** | |
| 2,3,6,7,8,9-hexahydro-2-methylnaphtho[1,2-*b*]furan-5-ol | **4** | |
| 3-ethyl-2-(2-hydroxypropyl)-5-methoxy-1,4-naphthoquinone acetate | **22** | |
| 3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-diol | **12** | |
| 3,4,6,7,8,9-hexahydro-5-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-10-ol | **11** | |

**Table 1** (continued)

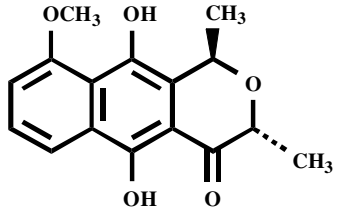
| Compounds | structures | Bibliography |
|---|---|---|
| ***E. bulbosa*** (Bulb) | | |
| 3-ethyl-2-(2-hydroxypropyl)-5-methoxy-1,4-naphthoquinone | **23** | Schmid, *et al*., 1950 |
| 3,4-dihydro-5,9,10-trimethoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran | **14** | |
| 3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*] pyran-5,10-diol | **15** | |
| 2-acetonyl-3-ethyl-5-methoxy-1,4-naphthoquinone | **24** | |
| eleutherol | **2** | Alves, *et al*., 2003 |
| eleutherin | **18** | |
| isoeleutherin | **19** | |
| eleutherinone | **20** | |
| ***E. palmifolia*** (Bulb) | | |
| eleutheside A | **35** | Shibuya, *et al*., 1997 |
| eleutheside B | **36** | |
| eleutheside C | **37** | |
| ***E. plicata*** (Bulb) | | |
| eleutherol | **2** | Yogogawa, *et al*., 1978 |
| eleutherin | **18** | |
| isoeleutherin | **19** | |
| hongconin | **1** | |
| dihydroeleutherinol | **3** | |
| 16-hydroxy-androst-4-ene-3,17-dione | **34** | Pinto, *et al*., 1961 |

**Table 1** (continued)

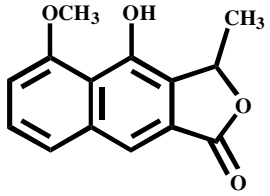
| Compounds | structures | Bibliography |
|---|---|---|
| ***E. subapylla*** (Bulb) | | |
| eleutherol | **2** | Nguyen, |
| eleutherin | **18** | *et al*., 1978 |
| isoeleutherin | **19** | |

## Structures of compounds from the *Eleutherine* genus

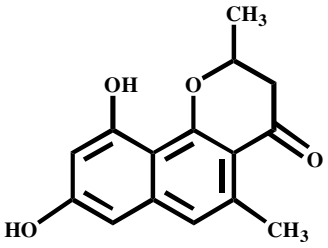
### Naphthalenes



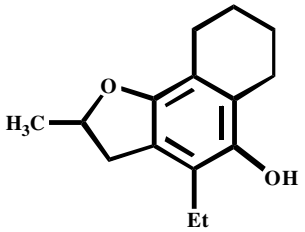

**1 :** hongconin



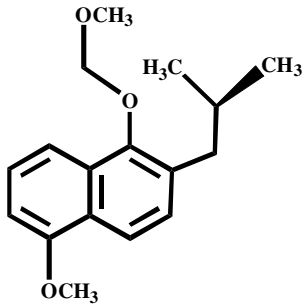

**2 :** eleutherol



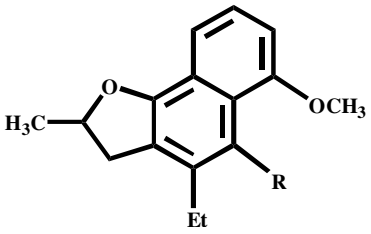

**3 :** dihydroeleutherinol




**4 :** 2,3,6,7,8,9-hexahydro-2-methyl naphtho[1,2-*b*]furan-5-ol

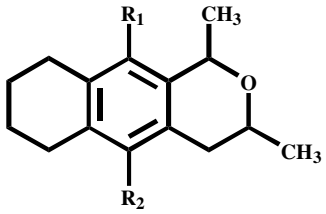



**5 :** 5-methoxy-(1-methoxymethoxy)-α-methyl-2-naphthaleneethanol




**6 :** R = OAc **:** 4-ethyl-2,3-dimethyl-6-methoxy-2-methylnaphtho[1,2-*b*]furan-5-ol acetate

**7 :** R = OH **:** 4-ethyl-2,3-dihydro-6-methoxy-2-methylnaphtho[1,2-*b*]furan-5-ol




**8 :** $R_1 = R_2 = OCH_3$ **:**

3,4,6,7,8,9-hexahydro-5,10-dimethoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran

**9 :** $R_1 = OH$, $R_2 = OAc$ **:**

3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-diol

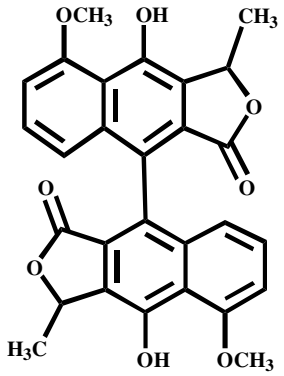
**10 :** $R_1 = OH$, $R_2 = OEt$ **:**

5-ethoxy-3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-10-ol

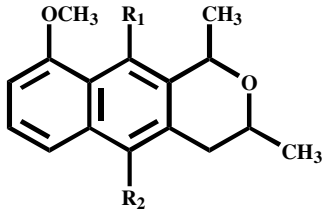
**11 :** $R_1 = OH$, $R_2 = OCH_3$ **:**

3,4,6,7,8,9-hexahydro-5-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-10-ol

**12 :** $R_1 = R_2 = OH$ **:** 3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-diol




**13 :** 9,9′-dihydroxy-8,8′-dimethoxy-1,1′-dimethyl-[4,4′-binaphtho[2,3-*c*]furan]-3,3′(1*H*,1′*H*)-dione




**14 :** $R_1 = R_2 = OCH_3$ **:**

3,4-dihydro-5,9,10-trimethoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran

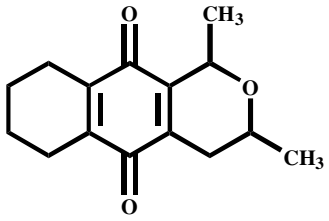
**15 :** $R_1 = OH$, $R_2 = OAc$ **:**

3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-diol

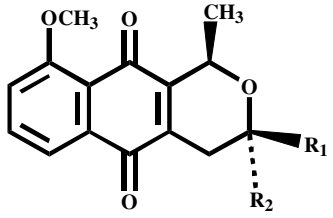
**16 :** $R_1 = R_2 = OCH_3$ **:**

5-ethoxy-3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-10-ol

## Naphthoquinones

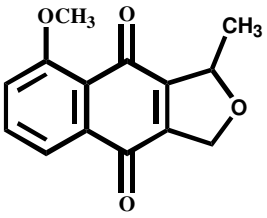



**17 :** 3,4,6,7,8,9-hexahydro-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione

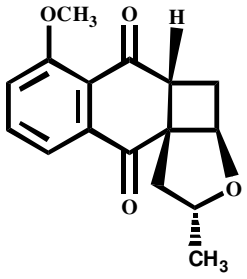



**18 :** $R_1 = CH_3$, $R_2 = H$ **:** eleutherin

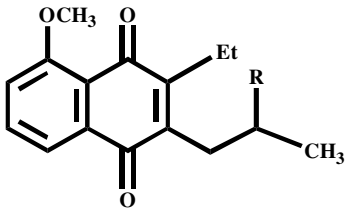
**19 :** $R_1 = H$, $R_2 = CH_3$ **:** isoeleutherin




**20 :** eleutherinone

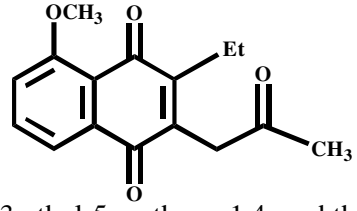



**21 :** elecanacin

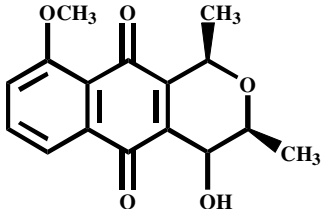



**22 :** R = OAc **:** 3-ethyl-2-(2-hydroxypropyl)-5-methoxy-1,4-naphthoquinone acetate

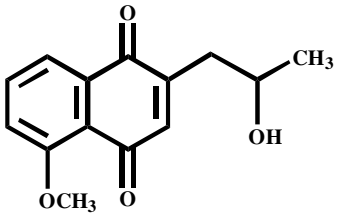
**23 :** R = OH **:** 3-ethyl-2-(2-hydroxylpropyl)-5-methoxy-1,4-naphthoquinone



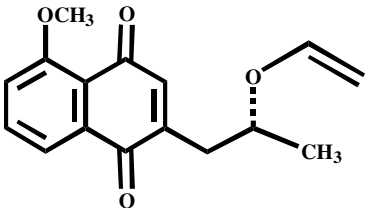

**24 :** 2-acetonyl-3-ethyl-5-methoxy-1,4-naphthoquinone




**25 :** 3,4-dihydro-4-hydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione

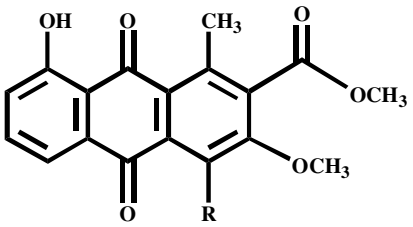



**26 :** 2-(2-hydroxypropyl)-5-methoxy-1,4-naphthalenedione




**27 :** 2-[(2*R*)-2-(ethenyloxy)propyl]-5-methoxy-1,4-naphthalenedione

## Anthraquinones

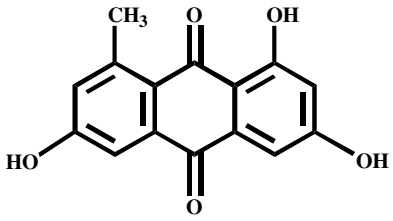



**28 :** R = $OCH_3$ **:**

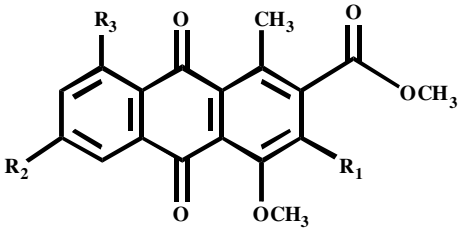
4,8-dihydroxy-3-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester

**29 :** R = OH **:**

8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester




**30:** 1,3,6-trihydroxy-8-methylanthraquinone

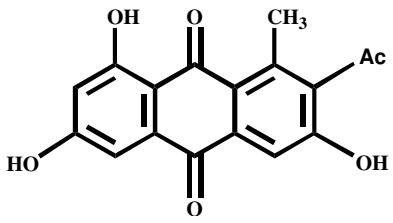



**31 :** $R_1$ = $OCH_3$, $R_2$ = $R_3$ = OH **:**

9,10-dihydro-6,8-dihydroxy-3,4-dimethoxy-1-methyl-9,10-dioxo-anthracene-2-carboxylic acid methyl ester

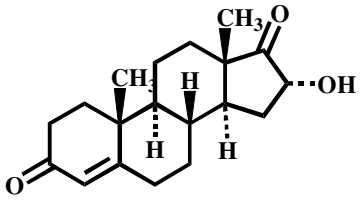
**32 :** $R_1$ = $OCH_3$, $R_2$ = H, $R_3$ = $OCH_3$ **:**

3,4,8-trimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester



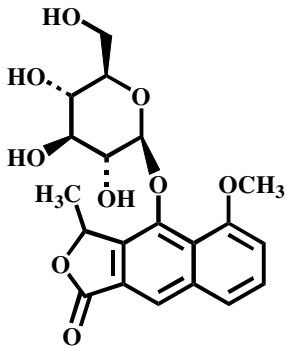

**33 :** 2-acetyl-3,6,8-trihydroxy-1-methyl-9,10-anthracenedione

## Steroid

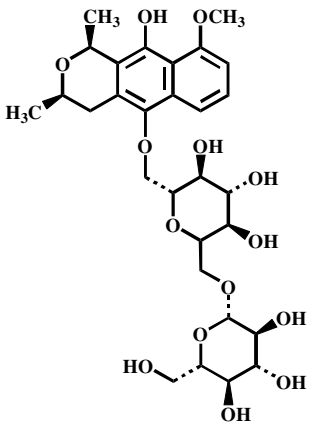


**34:** 16-hydroxy-androst-4-ene-3,17-dione

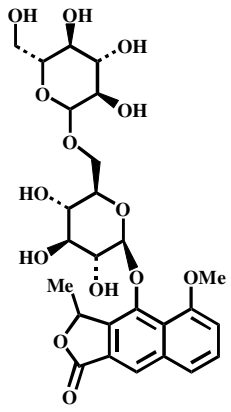
## Glycosides



**35:** eleutheside A



**36:** eleutheside B



**37:** eleutheside C

## 1.3 The Objective

The objective of this work is to investigate the chemical constituents from the bulb of *E. americana* (Aubl.) Merr.

# CHAPTER 2
# EXPERIMENTAL

## 2.1 General Method

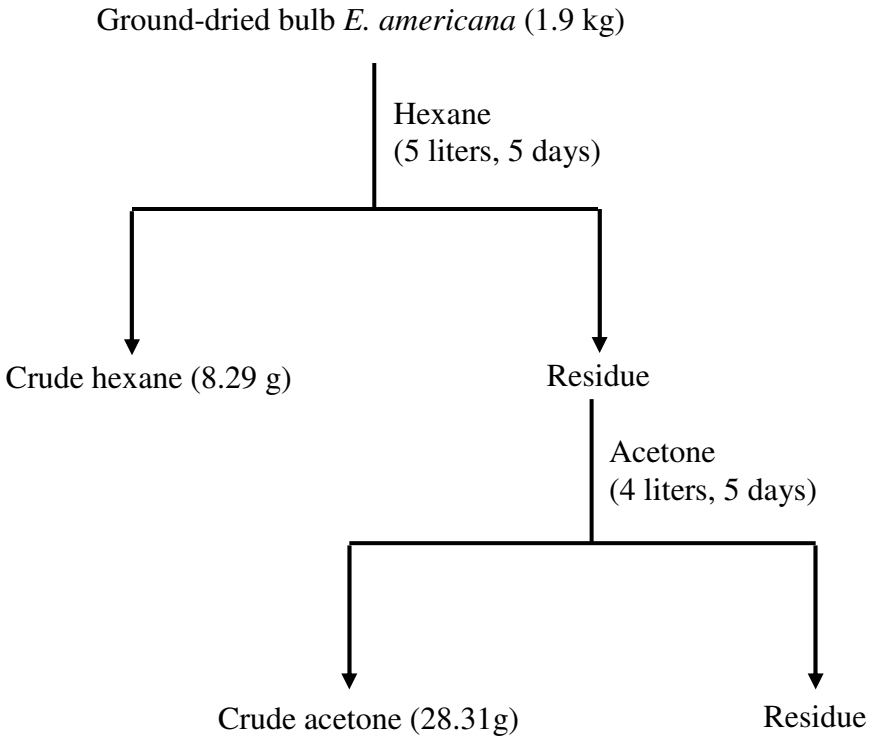
Melting point was recorded in $^{\circ}$C on a digital Electrothermal 9100 Melting Point Apparatus. Ultraviolet spectra were measured with UV-160A spectrophotometer (SHIMADZU). Principle bands ($\lambda_{max}$) were recorded as wavelengths (nm) and log ε in methanol solution. Infrared spectra were obtained on a FTS165 FT-IR spectrophotometer and were recorded in wave number ($cm^{-1}$). $^{1}H$ and $^{13}C$ Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield$^{TM}$ 300 and 500 MHz at Department of Chemistry, Faculty of Science, Prince of Songkla University. Spectrum were recorded in deuterochloroform, deuteroacetone, hexadeutero-dimethylsulphoxide and were recorded as $\delta$ value in ppm downfield from TMS (internal standard $\delta$ 0.00). Solvents for extraction and chromatography were distilled at their boiling ranges prior to use. For thin layer chromatography, aluminum sheets of silica gel 60 GF254 (20×20 cm, layer thickness 0.2 mm) were used for analytical purposes and the compounds were visualized under ultraviolet light. Column chromatography was performed using silica gel 100 (70-230 Mesh ASTM, Merck).

## 2.2 Plant material

The bulbs of *E. americana* were collected from Phang-nga province in the southern part of Thailand, in April 2007. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University. The specimen (C. Hemtasin 1 Phang-nga:Kuraburi 2/4/07) have been deposited in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

## 2.3 Extraction and Isolation

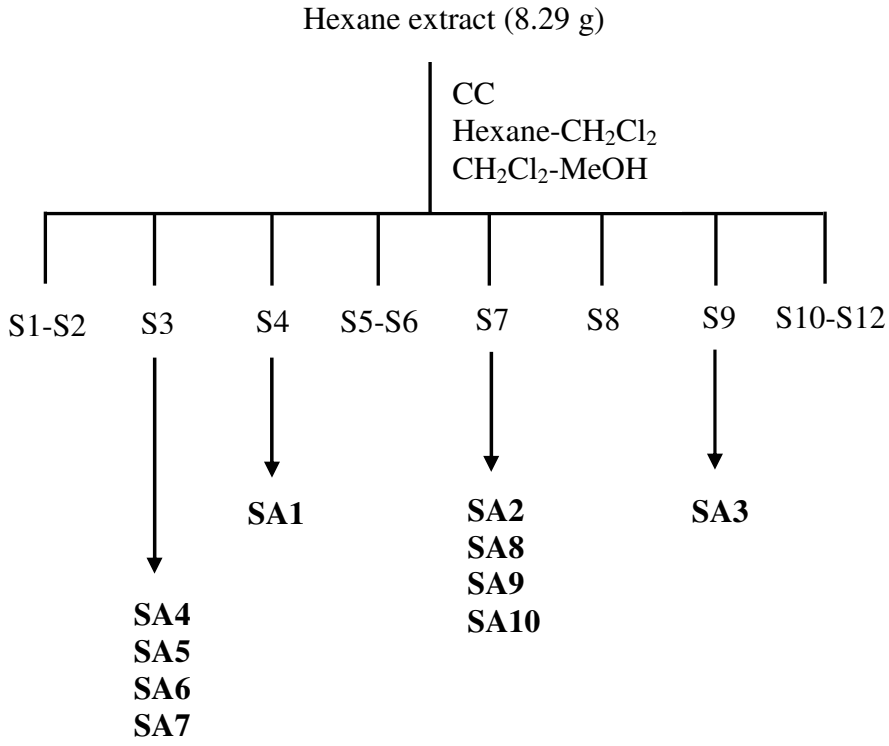
Ground-dried bulb of *E. americana* (1.9 kg) were immersed at room temperature in hexane and acetone for 5 days successively. After evaporation, the yellow-brown solid of hexane extract (8.29 g) and the viscous liquid of acetone extract (28.31 g) were obtained. The process of extraction was shown in **Scheme 1**.




**Scheme 1** Extraction of crude extracts from the bulb of *E. americana*

### 2.3.1 Purification of hexane extract

Hexane extract (8.29 g) was subjected to column chromatography using silica gel as stationary phase and eluted with hexane-dichloromethane, dichloromethane, dichloromethane-methanol and methanol as eluents. On the basis of their TLC characteristic, the fractions which contained the same major compounds were combined to give fractions S1-S12. Ten pure compounds were obtained as shown in **Scheme 2**.




**Scheme 2** Isolation of compounds **SA1-SA10** from hexane extract

**Table 2** Physical characteristic and weight of the fractions from hexane extract

| Fraction | Weight (g) | Physical characteristic |
|---|---|---|
| S1 | 1.6781 | brown wax |
| S2 | 0.9653 | yellow-brown viscous |
| S3 | 1.4159 | yellow solid mixed with orange solid |
| S4 | 0.8732 | Yellow solid |
| S5 | 0.6362 | yellow solid mixed with brown solid |
| S6 | 0.5091 | yellow solid mixed with brown solid |
| S7 | 1.4272 | brown solid mixed with yellow solid |
| S8 | 0.4253 | brown solid mixed with yellow solid |
| S9 | 0.4265 | brown solid |
| S10 | 2.3754 | brown solid |
| S11 | 0.1810 | brown viscous liquid |
| S12 | 0.3930 | brown viscous liquid |

**Isolation of SA1, SA2 and SA3**

Fraction S4 (0.873 g), S7 (1.427 g) and S9 (0.426 g), which contained the major components, were each dissolved in dichloromethane to form solid of **SA1** (193.7 mg), **SA2** (387.0 mg) and **SA3** (129.2 mg), respectively.

**SA1**

Melting point : 190-192 $^{\circ}$C

$[\alpha]^{29}_{D}$ +87° (*c* 0.045, $CHCl_3$)

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 245 (3.01), 301 (1.63), 314 (1.87), 348 (1.95), 364 (2.07)

IR (Neat) ν ($cm^{-1}$) : 3354 (O-H stretching), 1752 (C=O stretching), 1287 (C-O stretching)

$^{1}$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 9.64 (1H, *s*, 4-OH), 7.82 (1H, *s*, H-9), 7.53 (1H, *d*, *J* = 8.1 Hz, H-8), 7.39 (1H, *t*, *J* = 8.1 Hz, H-7), 6.92 (1H, *d*, *J* = 8.1 Hz, H-6), 5.71 (1H, *q*, *J* = 6.6 Hz, H-3), 4.12 (3H, *s*, 5-$OCH_3$), 1.73 (3H, *d*, *J* = 6.6 Hz, 3-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 170.5 (C=O), 156.5 (C), 149.1 (C), 137.1 (C), 127.8 (C), 126.6 (CH), 125.7 (C), 123.5 (CH), 117.4 (C), 116.4 (CH), 106.2 (CH), 77.3 (CH), 56.3 ($OCH_3$), 19.1 ($CH_3$)

**SA2**

Melting point : 174-176 °C

$[\alpha]^{29}_{D}$ +255° (*c* 15.3, $CHCl_3$)

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 235 (2.68), 267 (1.54), 273 (1.40), 348 (1.43), 396 (1.51)

IR (Neat) ν ($cm^{-1}$) : 1653 (C=O stretching), 1290 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 7.72 (1H, *dd*, *J* = 1.2, 8.4 Hz, H-6), 7.64 (1H, *t*, *J*= 8.4 Hz, H-7), 7.28 (1H, *dd*, *J* = 1.2, 8.4 Hz, H-8), 4.86 (1H, *ddq*, *J* = 2.7, 3.6, 6.6 Hz, H-1), 3.99 (3H, *s*, 9-$OCH_3$), 3.59 (1H, *ddq*, *J* = 2.7, 5.7, 10.5 Hz, H-3), 2.75 (1H, *td*, *J* = 2.7, 18.3 Hz, H-4), 2.19 (1H, *ddd*, *J* = 3.6, 10.5, 18.3 Hz, H-4), 1.54 (3H, *d*, *J* = 6.6 Hz, 1-$CH_3$), 1.34 (3H, *d*, *J* = 5.7 Hz, 3-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 184.0 (C=O), 183.7 (C=O), 159.3 (C), 148.7 (C), 139.9 (C), 134.5 (CH), 133.9 (C), 120.2 (C), 118.9 (CH), 117.7 (CH), 70.2 (CH), 68.7 (CH), 56.4 ($OCH_3$), 29.9 ($CH_2$), 21.2 ($CH_3$), 20.7 ($CH_3$)

**SA3**

Melting point : 173-175 °C

$[\alpha]^{29}_{D}$ -24° (*c* 8.3, $CHCl_3$)

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 222 (3.20), 244 (3.55), 266 (3.46), 271 (3.46), 395 (3.04)

IR (Neat) ν ($cm^{-1}$) : 1653 (C=O stretching), 1290 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 7.74 (1H, *dd*, *J* = 1.2, 8.4 Hz, H-6), 7.65 (1H, *t*, *J* = 8.4 Hz, H-7), 7.29 (1H, *dd*, *J* = 1.2, 8.4 Hz, H-8), 5.01 (1H, *dq*, *J* = 2.7, 6.9 Hz, H-1), 4.01 (3H, *s*, 9-$OCH_3$), 4.00 (1H, *m*, H-3), 2.70 (1H, *dd*, *J* = 3.3, 18.9 Hz, H-4), 2.23 (1H, *ddd*, *J* = 2.7, 10.2, 18.9 Hz, H-4), 1.54 (3H, *d*, *J* = 6.9 Hz, 1-$CH_3$), 1.35 (3H, *d*, *J* = 6.9 Hz, 3-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 184.2 (C=O), 182.7 (C=O), 159.7 (C), 148.0 (C), 139.3 (C), 134.7 (CH), 134.0 (C), 119.7 (C), 119.0 (CH), 117.8 (CH), 67.4 (CH), 62.4 (CH), 56.4 ($OCH_3$), 29.5 ($CH_2$), 21.5 ($CH_3$), 19.7 ($CH_3$)

**Isolation of SA4, SA5, SA6 and SA7**

Fraction S3 (1.415 g) was further purified by column chromatography over silica gel and eluted with mixed solvent of hexane-dichloromethane to give fractions S3.1-S3.6. Fraction S3.3 was rechromatographed on column chromatography and eluted with hexane-dichloromethane (7:3) to afford **SA4** (2.4 mg). Fraction S3.4 was further purified by column chromatography eluted with hexane-dichloromethane (1:4) to give a yellow-brown viscous of **SA5** (3.0 mg) and **SA6** (5.0 mg) as an orange solid, whereas fraction S3.6 was purified by crystallization in hexane-dichloromethane (1:9) to afford **SA7** (7.5 mg) as a yellow needles.

**SA4**

Melting point : 121-123 $^{\circ}$C

$[\alpha]^{29}_{D}$ -8.5° (*c* 0.200, $CHCl_3$)

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 222 (1.79), 259 (2.38), 266 (2.46), 271 (2.37), 430 (1.85)

IR (Neat) ν ($cm^{-1}$) : 3405 (O-H stretching), 1607, 1584 (C=O stretching), 1235, 1101 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 12.82 (1H*, s*, 5-OH), 8.97 (1H*, s*, 10-OH), 8.05 (1H*, d*, *J* = 8.4 Hz, H-6), 7.38 (1H*, t*, *J* = 8.4 Hz, H-7), 7.02 (1H*, d, J* = 8.4 Hz, H-8), 5.48 (1H*, q*, *J* = 6.6 Hz, H-1), 4.69 (1H*, q, J* = 6.6 Hz, H-3), 4.13 (3H*, s*, 9-$OCH_3$), 1.64 (3H*, d*, *J* = 6.6 Hz, 1-$CH_3$), 1.53 (3H*, d, J* = 6.6 Hz, 3-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 202.9 (C=O), 155.7 (C), 154.4 (C), 139.4 (C), 125.9 (C), 125.4 (CH), 120.9 (C), 119.5 (C), 118.1 (CH), 109.2 (CH), 108.0 (C), 71.4 (CH), 69.5 (CH), 56.4 ($OCH_3$), 17.4 ($CH_3$), 16.3 ($CH_3$)

**SA5**

$[\alpha]^{29}_{D}$ +16° (*c* 0.0092, $CHCl_3$)

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 222 (3.15), 245 (3.34), 267 (3.30), 272 (3.35), 420 (2.93)

IR (Neat) ν ($cm^{-1}$) : 3441 (O-H stretching), 1640, 1615 (C=O stretching), 1276 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 12.04 (1H, *s*, 9-OH), 7.63 (1H, *dd*, *J* = 1.8, 7.2 Hz, H-6), 7.58 (1H, *t*, *J* = 7.2 Hz, H-7), 7.24 (1H, *dd*, *J* = 1.8, 7.2 Hz, H-8), 5.01 (1H, *dq*, *J* = 2.7, 6.6 Hz, H-1), 4.00 (1H, *m*, H-3), 2.76 (1H, *dd*, *J* = 3.6, 19.5 Hz, H-4), 2.25 (1H, *ddd, J* = 3.6, 11.4, 19.5 Hz, H-4), 1.55 (3H, *d*, *J* = 6.6 Hz, 1-$CH_3$), 1.36 (3H, *d*, *J* = 6.6 Hz, 3-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 189.0 (C=O), 183.0 (C=O), 162.0 (C), 146.6 (C), 136.2 (CH), 132.1 (C), 124.4 (CH), 119.1 (CH), 115.2 (C), 66.9 (CH), 62.5 (CH), 29.9 ($CH_2$), 21.4 ($CH_3$), 19.7 ($CH_3$)

**SA6**

Melting point : 135-137 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 229 (4.33), 250 (4.05), 265 (3.84), 273 (3.75), 443 (3.75)

IR (Neat) ν ($cm^{-1}$) : 3374 (O-H stretching), 1739, 1617 (C=O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 13.71 (1H, *s,* 8-OH), 12.91 (1H, *s,* 4-OH), 7.83 (1H, *d*, *J* = 6.6 Hz, H-5), 7.66 (1H, *t*, *J* = 7.1 Hz, H-6), 7.33 (1H, *d*, *J* = 8.4 Hz, H-7), 4.10 (3H, *s,* 3-$OCH_3$), 3.99 (3H, *s,* 2-$CO_2CH_3$), 2.64 (3H, *s*, 1-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 189.1 (C=O), 189.0 (C=O), 166.8 (C=O), 162.6 (C), 155.2 (C), 150.2 (C), 137.5 (C), 136.0 (CH), 132.4 (C), 132.3 (C), 125.4 (C), 125.4 (CH), 118.8 (CH), 118.0 (C), 117.0 (C), 61.6 ($OCH_3$), 52.7 ($OCH_3$), 19.6 ($CH_3$)

**SA7**

Melting point : 220-221 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 223 (3.98), 254 (3.89), 264 (3.86), 271 (3.77), 401 (3.46)

IR (Neat) ν ($cm^{-1}$) : 3467 (O-H stretching), 1733, 1674, 1633 (C=O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$ (ppm) : 12.57 (1H, *s*, 8-OH), 7.70 (1H, *d*, *J* = 7.4 Hz, H-5), 7.62 (1H, *t*, *J* = 7.4 Hz, H-6), 7.26 (1H, *d*, *J* = 7.4 Hz, H-7), 4.03 (3H, *s,* 4-$OCH_3$), 3.99 (3H, *s,* 3-$OCH_3$), 3.97 (3H, *s,* 2-$CO_2CH_3$), 2.69 (3H, *s*, 1-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$ (ppm) : 189.8 (C=O), 182.6 (C=O), 166.9 (C=O), 161.9 (C), 155.5 (C), 152.1 (C), 137.0 (C), 136.2 (CH), 136.2 (C), 136.1 (C), 134.3 (C), 129.9 (C), 123.7 (CH), 118.6 (CH), 116.5 (C), 62.0 ($OCH_3$), 61.6 ($OCH_3$), 52.7 ($OCH_3$), 20.1 ($CH_3$)

**Isolation of SA8, SA9 and SA10**

The filtrate of fraction S7 (1.202 g) was further subjected the column chromatography, eluted with hexane-dichloromethane to give fractions S7.1-S7.9. Fraction S7.6 was rechromatographed on column chromatography and eluted with mixed solvent of dichloromethane-methanol to give fraction S7.61 – S7.68. Fraction S7.66, S7.68 and S7.9 were each further purified by column chromatography over silica gel and eluted with dichloromethane-methanol (49:1) solvent system to afford a brown needles of **SA8** (2.0 mg), a yellow-brown solid of **SA9** (3.6 mg) and brown solid of **SA10** (6.4 mg), respectively.

**SA8**

Melting point : 198-199 °C

$[\alpha]^{29}_{D}$ +85° (*c* 0.004, $CHCl_3$)

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 218 (2.72), 231 (3.08), 336 (2.15)

IR (Neat) ν ($cm^{-1}$) : 1679 (C=O stretching), 1276 (C-O stretching)

$^1$H-NMR 500 MHz ($CDCl_3$) $\delta$(ppm) : 7.46 (1H, *dd*, *J* = 1.0, 8.0 Hz, H-9), 7.46 (1H, *t*, *J* = 8.0 Hz, H-8), 7.30 (1H, *dd*, *J* = 1.0, 8.0 Hz, H-7), 4.61 (1H, *dd*, *J* = 6.0, 6.5 Hz, H-3a), 4.56 (1H, *m*, H-2), 3.97 (3H, *s,* 6-$OCH_3$), 3.21 (1H, *dd*, *J* = 2.5, 7.5 Hz, H-4a), 2.58 (2H, *m,* H-4), 2.30 (1H, *dd*, *J* = 11.0, 12.5 Hz, H-1), 2.01 (1H, *dd*, *J* = 4.5, 12.5 Hz, H-1), 1.46 (3H, *d, J* = 5.5 Hz, 2-$CH_3$)

$^{13}$C-NMR 125 MHz ($CDCl_3$) $\delta$(ppm) : 196.0 (C=O), 195.0 (C=O), 159.0 (C), 138.0 (C), 134.8 (CH), 124.0 (C), 119.3 (CH), 117.2 (CH), 80.9 (CH), 75.9 (CH), 61.3 (C), 56.5 ($OCH_3$), 45.0 (CH), 42.0 ($CH_2$), 36.0 ($CH_2$), 19.2 ($CH_3$)

**SA9**

Melting point : 101-103 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 225 (3.52), 240 (3.51), 266 (3.28), 271 (3.26), 405 (2.69)

IR (Neat) ν ($cm^{-1}$) : 1736, 1718, 1656, 1586 (C=O stretching), 1274 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 7.74 (1H, *dd*, *J* = 1.2, 7.8 Hz, H-5), 7.66 (1H, *t*, *J* = 7.8 Hz, H-6), 7.29 (1H, *dd*, *J* = 1.2, 7.8 Hz, H-7), 4.00 (3H, *s,* 8-$OCH_3$), 3.77 (2H, *s,* H-1″), 3.68 (3H, *s,* 2′-$OCH_3$), 3.63 (2H, *s*, $CH_2$-1′), 2.30 (3H, *s*, $CH_3$-3″)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 203.2 (C=O), 184.3 (C=O), 182.7 (C=O), 169.9 (C=O), 159.8 (C), 143.6 (C), 140.4 (C), 134.9 (CH), 134.0 (C), 119.7 (C), 119.4 (CH), 117.9 (CH), 56.5 ($OCH_3$), 52.4 ($OCH_3$), 41.8 ($CH_2$), 32.9 ($CH_2$), 30.1 ($CH_3$)

DEPT 135°($CDCl_3$) $\delta$ (ppm) $CH_3$ : 30.1, 52.4, 56.5; $CH_2$ : 32.9, 41.8; CH : 117.9, 119.4, 134.9.

EIMS m/z (% relative intensity) : 316 [$M^+$] (13), 275 (19), 274 (98), 242 (100), 215 (43), 214 (26), 187 (22), 128 (21), 85 (23), 71 (24)

HR-MS m/z : 316.0943 for $C_{17}H_{16}O_6$ (calcd. 316.0947)

**SA10**

Melting point : 148-150 °C

$[\alpha]^{29}_D$ +4° (*c* 0.890, $CHCl_3$)

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 225 (3.54), 244 (3.49), 278 (3.44), 389 (3.02)

IR (Neat) ν ($cm^{-1}$) : 3245 (O-H stretching), 1736, 1659, 1638 (C=O stretching), 1245 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 7.81 (1H, *dd*, *J* = 1.2, 7.7 Hz, H-8), 7.71 (1H, *t*, *J* = 7.7 Hz, H-7), 7.55 (1H, *dd*, *J* = 1.2, 7.7 Hz, H-6), 5.21 (1H, *sext*, *J* = 6.3 Hz, H-2′), 4.04 (3H, *s,* 5-$OCH_3$), 2.86 (2H, *dd*, *J* = 1.2, 6.3 Hz, H-1′), 1.96 (3H, *s*, CO$\underline{CH_3}$), 1.28 (3H, *d*, *J* = 6.3 Hz, $CH_3$-3′)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 184.1 (C=O), 179.6 (C=O), 170.6 (C=O), 160.1 (C), 154.5 (C), 136.5 (CH), 135.0 (C), 119.8 (CH), 117.6 (C), 116.9 (C), 116.7 (CH), 69.5 (CH), 56.5 ($OCH_3$), 29.7 ($CH_2$), 21.2 ($CH_3$), 20.1 ($CH_3$)

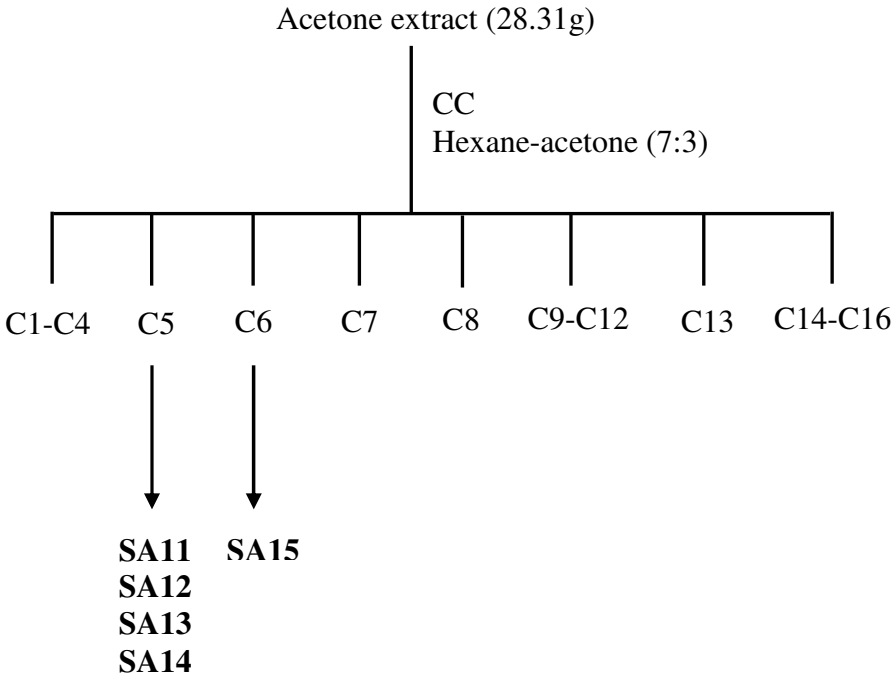
DEPT 135°($CDCl_3$) $\delta$(ppm) $CH_3$ : 20.1, 21.2, 56.5; $CH_2$ : 29.7; CH : 69.5, 116.7, 119.8, 136.5

EIMS m/z (% relative intensity) : 304 [$M^+$] (1), 263 (2), 262 (7), 245 (15), 244 (100), 229 (23), 218 (96), 216 (22), 203 (12), 173 (9)

HR-MS m/z : 304.0943 for $C_{16}H_{16}O_6$ (calcd. 304.0947)

### 2.3.2 Purification of acetone extract

Acetone extract (28.31 g) was separated by column chromatography over silica gel and eluted with hexane-acetone (7:3) solvent system. On the basis of their TLC characteristic, the fractions which contained the same major component were combined to give fractions C1-C16 (Table 3). Five pure compounds were obtained as shown in **Scheme 3**




**Scheme 3** Isolation of compounds **SA11-SA15** from acetone extract

**Table 3** Physical characteristic and weight of the fractions from acetone extract

| Fraction | Weight (g) | Physical characteristic |
|---|---|---|
| C1 | 0.0990 | brown solid mixed with orange solid |
| C2 | 0.3532 | yellow solid mixed with orange solid |
| C3 | 0.8977 | yellow-brown solid |
| C4 | 0.4635 | brown solid |
| C5 | 0.2265 | yellow-brown solid |
| C6 | 0.4543 | brown solid |
| C7 | 0.2636 | brown solid mixed with yellow solid |
| C8 | 0.4165 | brown solid mixed with yellow solid |
| C9 | 0.3069 | brown viscous solid |
| C10 | 0.2696 | brown viscous solid |
| C11 | 0.8652 | brown viscous solid |
| C12 | 0.6370 | brown viscous solid |
| C13 | 8.8576 | gray solid |
| C14 | 0.9402 | brown viscous solid |
| C15 | 0.3172 | brown viscous solid |
| C16 | 0.2554 | brown viscous solid |

**Isolation of SA11-SA14**

Fraction C5 (256.5 g) was rechromatographed on column chromatography and eluted with the mixed solvent of hexane-acetone to give eight fractions (C5.1-C5.8).

Fraction C5.5 was recrystallized from the mixed solvent of dichloromethane-methanol (49:1) to give an orange solid of **SA11** (8.7 mg).

Fraction C5.6 was chromatographed on column chromatography and eluted with dichloromethane, dichloromethane-acetone and acetone to give fraction C5.61-C5.613. Fraction C5.67 and C5.612 were purified by crystallization in dichloromethane-methanol (49:1) to afford a yellow solid of **SA12** (8.4 mg) and **SA13** (7.7 mg), respectively.

Fraction C5.7 was further subjected to column chromatography, eluted with dichloromethane-acetone (19:1) to give seven fractions (C5.71-C5.77). Crystallization of fraction C5.77 in dichloromethane-methanol (49:1) gave a brown needle of **SA14** (4.0 mg).

**SA11**

Melting point : >300 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 222 (3.78), 244 (3.58), 283 (4.21), 344 (3.03), 433 (3.40)

IR (Neat) ν ($cm^{-1}$) : 3416 (O-H stretching), 1648, 1620 (C=O stretching), 1269 (C-O stretching)

$^1$H-NMR 300 MHz (Acetone-$d_6$) $\delta$(ppm) : 13.34 (1H, *s*, 1-OH), 10.25 (1H, *s,* 6-OH), 7.61 (1H, *d*, *J* = 2.4 Hz, H-5), 7.21 (1H, *d*, *J* = 2.4 Hz, H-4), 7.12 (1H, *d*, *J* = 2.4 Hz, H-7), 6.68 (1H, *d*, *J* = 2.4 Hz, H-2), 2.80 (3H, *s*, 8-$CH_3$)

$^{13}$C-NMR 75 MHz (Acetone-$d_6$) $\delta$(ppm) : 189.0 (C=O), 182.4 (C=O), 165.3 (C), 145.4 (C), 137.3 (C), 134.9 (C), 133.2 (C), 127.1 (C), 124.5 (CH), 123.0 (C), 112.2 (CH), 111.0 (C), 108.2 (CH), 107.0 (CH), 23.1 ($CH_3$)

**SA12**

Melting point : 203-204 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 223 (4.36), 246 (4.21), 257 (4.17), 266 (4.29), 273 (4.33), 372 (3.66)

IR (Neat) ν ($cm^{-1}$) : 3328 (O-H stretching), 1702, 1664, 1584 (C=O stretching), 1284 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$+$CD_3OD$) $\delta$(ppm) : 9.98 (1H, *s*, 3-OH), 7.80 (1H, *dd*, *J* = 0.9, 7.8 Hz, H-5), 7.64 (1H, *t, J* = 7.8 Hz, H-6), 7.50 (1H, *s*, H-4), 7.32 (1H, *dd, J* = 0.9, 7.8 Hz, H-7), 4.01 (3H, *s,* 8-$OCH_3$), 2.68 (3H, *s,* 1-$CH_3$), 2.58 (3H, *s,* 2-CO$\underline{CH_3}$)

$^{13}$C-NMR 75 MHz ($CDCl_3$+$CD_3OD$) $\delta$(ppm) : 209.7 (C=O), 188.2 (C=O), 188.1 (C=O), 163.5 (C), 160.8 (C), 143.4 (C), 140.6 (C), 139.4 (C), 138.6 (C), 137.9 (CH), 130.7 (C), 127.6 (C), 122.9 (CH), 122.3 (CH), 115.2 (CH), 60.4 ($OCH_3$), 32.9 ($CH_3$), 22.8 ($CH_3$)

DEPT 135°($CDCl_3$+$CD_3OD$) $\delta$(ppm) $CH_3$ : 22.8, 32.9, 60.4; CH : 115.2, 122.3, 122.9, 137.9

EIMS m/z (% relative intensity) : 309 [$M^+$] (93), 295 (100), 277 (15), 250 (8), 249 (33), 221 (11), 168 (6), 165 (8), 152 (9), 139 (11)

HR-MS m/z : 310.0826 for $C_{18}H_{14}O_5$ (calcd. 310.0841)

**SA13**

Melting point : 209-211 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 216 (3.95), 224 (4.03), 285 (4.26), 351 (3.15), 429 (3.59)

IR (Neat) ν ($cm^{-1}$) : 3390 (O-H stretching), 1713, 1630 (C=O stretching), 1274 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 13.05 (1H, *s*, 8-OH), 9.30 (1H, *br*, 3-OH), 8.50 (1H, *br*, 6-OH), 7.23 (1H, *d*, *J* = 2.4 Hz, H-5), 6.71 (1H, *d*, *J* = 2.4 Hz, H-7), 4.03 (3H, *s*, 2-$CO_2CH_3$), 3.99 (3H, *s*, 4-$OCH_3$), 2.77 (3H, *s*, 1-$CH_3$)

$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 188.3 (C=O), 182.7 (C=O), 167.6 (C=O), 164.9 (C), 163.9 (C), 152.9 (C), 146.7 (C), 137.8 (C), 135.8 (C), 128.1 (C), 127.9 (C), 124.7 (C), 111.7 (C), 108.8 (CH), 107.5 (CH), 62.2 ($OCH_3$), 52.9 ($OCH_3$), 20.7 ($CH_3$)

EIMS m/z (% relative intensity) : 358 [$M^+$] (46), 327 (15), 326 (29), 311 (8), 299 (16), 298 (105), 297 (14), 270 (16), 269 (10), 241 (7)

HR-MS m/z : 358.0692 for $C_{18}H_{14}O_8$ (calcd. 358.0689)

**SA14**

Melting point : 167-169 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log ε) : 226 (4.48), 243 (4.57), 266 (3.79), 313 (3.80), 347 (3.77), 362 (3.81)

IR (Neat) ν ($cm^{-1}$) : 3371 (O-H stretching), 1718 (C=O stretching), 1245 (C-O stretching)

$^1$H-NMR 300 MHz (Acetone-$d_6$) $\delta$(ppm) : 9.99 (1H, *s,* 4-OH), 7.80 (1H, *s*, H-9), 7.70 (1H, *d, J* = 7.8 Hz, H-8), 7.53 (1H, *t, J* = 7.8 Hz, H-7), 7.20 (1H, *d, J* = 7.8 Hz, H-6), 6.60 (1H, *s,* 3-OH), 4.22 (3H, *s*, 5-$OCH_3$), 1.98 (3H, *s*, 3-$CH_3$)

$^{13}$C-NMR 75 MHz (Acetone-$d_6$) $\delta$(ppm) : 167.9 (C=O), 158.0 (C), 151.0 (C), 137.9 (C), 127.5 (CH), 127.0 (C), 125.0 (C), 123.0 (CH), 117.0 (C), 115.1 (CH), 106.8 (CH), 106.0 (C), 56.2 ($OCH_3$), 24.4 ($CH_3$)

DEPT 135°(Acetone-$d_6$) $\delta$(ppm) $CH_3$ : 24.4, 56.2; CH : 106.8, 115.1, 123.0, 127.5

EIMS m/z (% relative intensity) : 260 [$M^+$] (41), 245 (105), 242 (17), 227 (12), 199 (22), 186 (25), 171 (40), 158 (12), 115 (11), 102 (7)

HR-MS m/z : 260.0679 for $C_{14}H_{12}O_5$ (calcd. 260.0685)

**Isolation of SA15**

Fraction C7 (0.2636 g) was chromatographed on column chromatography eluted with dichloromethane-methanol to give ten fractions (C7.1-C7.10). Crystallization of fraction C7.2 in dichloromethane-methanol (49:1) gave an orange solid of **SA15** (6.6 mg).

**SA15**

Melting point : 220-221 °C

UV ($CH_3OH$) $\lambda_{max}$ nm (log $\varepsilon$) : 231 (4.33), 244 (4.30), 267 (4.16), 273 (4.18), 362 (3.62)

IR (Neat) $\nu$ ($cm^{-1}$) : 3372 (O-H stretching), 1653, 1622 (C=O stretching), 1272 (C-O stretching)

$^1$H-NMR 300 MHz ($CDCl_3$) $\delta$(ppm) : 13.18 (1H, *s,* 1-OH), 7.95 (1H, *dd, J* = 0.9, 7.8 Hz, H-5), 7.74 (1H, *t, J* = 7.8 Hz, H-6), 7.63 (1H, *s,* H-4), 7.34 (1H, *dd, J* = 0.9, 7.8 Hz, H-7), 4.07 (3H, *s*, 8-$OCH_3$), 2.36 (3H, *s*, 3-$CH_3$)

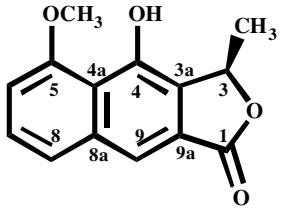
$^{13}$C-NMR 75 MHz ($CDCl_3$) $\delta$(ppm) : 194.2 (C=O), 186.7 (C=O), 165.9 (C), 154.6 (C), 154.0 (C), 141.5 (C), 141.0 (CH), 135.8 (C), 128.7 (C), 127.4 (CH), 125.8 (C), 125.3 (CH), 122.8 (CH), 120.5 (C), 61.7 ($OCH_3$), 21.2 ($CH_3$)

# CHAPTER 3
# RESULTS AND DISCUSSION

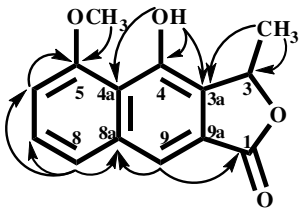
## 3.1 Structural Determination

The bulb of *Eleutherine americana* (Aubl.) Merr was extracted with hexane and acetone, successively. Separation of the hexane extract by column chromatography produced 4-hydroxyl-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1-(3*H*)-one (**SA1**), (1*R*,3*S*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*] pyran-5,10-dione (**SA2**), (1*R*,3*R*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho [2,3-*c*]pyran-5,10-dione (**SA3**), (1*R*,3*R*)5,10-dihydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho [2,3-*c*]pyran-4(3*H*)-one (**SA4**), (1*R*,3*S*)3,4-dihydro-9-hydroxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA5**), 4,8-dihydroxy-3-methoxy-1-methyl anthraquinone-2-carboxylic acid methyl ester (**SA6**), 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA7**), 1,2,4,4a-tetrahydro -6-methoxy-2-methyl-10*H*-naphtho[2′,3′:2,3]cyclobuta[1,2-*b*]furan-5,10-(3*H*)-dione (**SA8**), [8-methoxy-1,4-dioxo-3-(2-oxo-propyl)-1,4-dihydronaphthalen-2-yl]acetic acid methyl ester (**SA9**), 3-hydroxy-2-(2′-acetyloxypropyl)-5-methoxy-1,4-naphthoquinone (**SA10**), whereas purification of the acetone extract gave five compounds: 1,3,6-trihydroxy-8-methylanthraquinone (**SA11**), 2-acetyl-3-hydroxy-8-methoxy-1-methylanthraquinone (**SA12**), 3,6,8-trihydroxy-4-methoxy-1-methyl anthraquinone-2-carboxylic acid methyl ester (**SA13**), 3,4-dihydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one (**SA14**), 1,2-dihydroxy-8-methoxy-3-methyl anthraquinone (**SA15**). Their structures were elucidated by 1D and 2D spectroscopic data.

**SA1: 4-hydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one (eleutherol)**




**SA1** was obtained as white needles, $[\alpha]^{29}_{D}$ +87° (*c* 0.045, $CHCl_3$). The UV spectrum exhibited the absorption bands at 245, 301, 314, 348 and 364 nm. The IR spectrum showed the absorption bands of O-H stretching at 3354 $cm^{-1}$ and of C=O stretching at 1752 $cm^{-1}$. Its $^1$H NMR spectra **(Table 4)** showed resonances at $\delta$ 7.53 (*d*, *J* = 8.1 Hz, H-8), 7.39 (*t*, *J* = 8.1 Hz, H-7), 6.92 (*d*, *J* = 8.1 Hz, H-6), indicating the presence of an aromatic moiety with an ABM pattern. The substituent group at C-5 was assigned to a methoxyl group ($\delta$ 4.12). This was confirmed by the HMBC correlation of 5-$OCH_3$ and H-6 to C-5. The singlet signals at $\delta$ 7.82 and $\delta$ 9.64 were assigned for H-9 and 4-OH. An aromatic proton H-9 was located at the same side as C=O and H-8 from the HMBC correlation of H-9 to C-1 and C-8a. The establishment of C=O of the ester group was indicated by the carbon signal at $\delta$ 170.5 **(Table 4).** The quartet at $\delta$ 5.71 (*J* = 6.6 Hz) and doublet at $\delta$ 1.73 (*J* = 6.6 Hz) indicated the methine proton H-3 and the methyl group 3-$CH_3$, respectively. The HMBC correlation of H-8 to C-8a and of H-9 to C-8a and C-1 indicated that H-9 was located between C-8 and C=O, whereas the correlations of -$CH_3$ to C-3a and of OH to C-3a and C-4a indicated that OH and -$CH_3$ were close to each other. These data and its optical rotation are compatible with those of 4-hydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one, known as eleutherol (Alves *et al*., 2003).

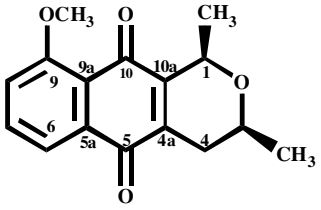



Major HMBC of **SA1**

**Table 4** NMR spectra data of **SA1**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 170.5 (C=O) | - | - |
| 3 | 77.3 (CH) | 5.71 (*q, J* = 6.6 Hz, 1H ) | C-3a, C-4 |
| 3a | 127.8 (C) | - | |
| 4 | 149.1 (C) | - | - |
| 4a | 117.4 (C) | - | - |
| 5 | 156.5 (C) | - | |
| 6 | 106.2 (CH) | 6.92 (*d*, *J* = 8.1 Hz, 1H) | C-4a, C-5, C-8 |
| 7 | 126.6 (CH) | 7.39 (*t*, *J* = 8.1 Hz, 1H) | C-5, C-6, C-8a |
| 8 | 123.5 (CH) | 7.53 (*d*, 8.1 Hz, 1H) | C-6, C-7, C-8a |
| 8a | 137.1 (C) | - | - |
| 9 | 116.4 (CH) | 7.82 (*s,* 1H) | C-1, C-3a, C-4a, C-8a |
| 9a | 125.7 (C) | - | - |
| 3-$CH_3$ | 19.1 ($CH_3$) | 1.73 (*d*, *J* = 6.6 Hz, 3H) | C-3, C-3a |
| 4-OH | - | 9.64 (*s*, OH) | C-3a, C-4, C-4a |
| 5-$OCH_3$ | 56.3 ($OCH_3$) | 4.12 (*s*, 3H ) | C-5, C-6 |

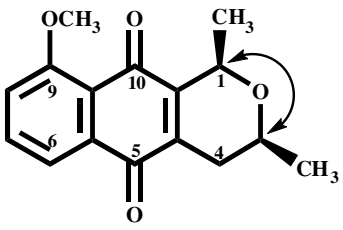
**SA2: (1*R*,3*S*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (eleutherin)**



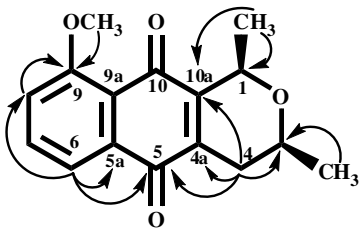

**SA2** was obtained as a yellow solid, $[\alpha]^{29}_{D}$ +255° (*c* 15.30, $CHCl_3$). The UV spectrum exhibited absorption maxima at 235, 267, 273, 348 and 396 nm, indicating a quinone as the basic structure. The IR spectrum showed the absorption band of C=O stretching at 1653 $cm^{-1}$. The $^1H$ NMR spectral data **(Table 5)** showed an ABM pattern of aromatic protons H-6, H-7 and H-8 at $\delta$ 7.72 (*dd*, *J* = 1.2, 8.4 Hz), 7.64 (*t*, *J* = 8.4 Hz) and 7.28 (*dd*, *J* = 1.2, 8.4 Hz), respectively. A singlet of a methoxyl group at $\delta$ 3.99 (3H) was assigned to 9-$OCH_3$. The HMBC correlations of 9-$OCH_3$ and H-8 to C-9 confirmed the location of $OCH_3$ at C-9. Two sets of doublets with an integral of three protons each at $\delta$ 1.54 (*J* = 6.6 Hz) and 1.34 (*J* = 5.7 Hz), together with signals at $\delta$ 4.86 (*ddq, J* = 2.7, 3.6, 6.6 Hz) and 3.59 (*ddq*, *J* = 2.7, 5.7, 10.5 Hz) were assigned for 1-$CH_3$, 3-$CH_3$, and H-1, H-3, respectively. In the COSY experiment, apart from coupling with 3-$CH_3$, H-3 was found to correlate to a non-equivalent -$CH_2$-, that resonated at $\delta$ 2.75 (*td*, *J* = 2.7, 18.3 Hz) and 2.19 (*ddd*, *J* = 3.6, 10.5, 18.3 Hz). The quinone moiety was suggested from the presence of the carbonyl carbon signals at $\delta$ 184.0 (C-5) and 183.7 (C-10). Furthermore, the chemical shifts of the methine carbons C-1 ($\delta$ 70.2) and C-3 ($\delta$ 68.7) indicated that they were connected to an oxygen atom of an ether group. The HMBC correlations of H-4 to C-5 as well as H-6 to C-5 confirmed that H-4 and H-6 were peri C-5 carbonyl groups, consequently 1-$CH_3$ and –$OCH_3$ were then placed at the peri C-10 carbonyl group. In the NOEDIFF experiment, the enhancement of H-1 resonance ($\delta$ 4.86) by irradiation of H-3 resonance ($\delta$ 3.59) indicated their *cis* relative stereochemistry. The optical rotation also confirmed the proposed stereochemistry of **SA2.** Its spectral data and the assignment is in agreement with (1*R*,3*S*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran -5,10-dione, which is known as eleutherin (Komura *et al*., 1983).

**Table 5** NMR spectral data of **SA2**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 70.2 (CH) | 4.86 (*ddq*, *J* = 2.7, 5.7, 6.6 Hz, 1H) | - |
| 3 | 68.7 (CH) | 3.59 (*ddq*, *J* = 2.7, 5.7, 10.5 Hz, 1H) | - |
| 4 | 29.9 ($CH_2$) | 2.75 (*td*, *J* = 2.7, 18.3 Hz, 1H) | C-3, C-4a, C-5, |
| | | 2.19 (*ddd*, *J* = 3.6, 10.5, 18.3 Hz, 1H) | C-10a, |
| 4a | 139.9 (C) | - | - |
| 5 | 184.0 (C=O) | - | - |
| 5a | 133.9 (C) | - | - |
| 6 | 118.9 (CH) | 7.72 (*dd*, *J* = 1.2, 8.4 Hz, 1H) | C-5, C-5a, C-8, |
| | | | C-9a |
| 7 | 134.5 (CH) | 7.64 (*t*, *J* = 8.4 Hz, 1H) | C-6 |
| 8 | 117.7 (CH) | 7.28 (*dd*, *J* = 1.2, 8.4 Hz, 1H) | C-7, C-9, C-9a |
| 9 | 159.3 (C) | - | - |
| 9a | 120.2 (C) | - | - |
| 10 | 183.7 (C=O) | - | - |
| 10a | 148.7 (C) | - | - |
| 1-$CH_3$ | 20.7 ($CH_3$) | 1.54 (*d*, *J* = 6.6 Hz, 3H) | C-1, C-10a |
| 3-$CH_3$ | 21.2 ($CH_3$) | 1.34 (*d*, *J* = 5.7 Hz, 3H) | C-3, C-4 |
| 9-$OCH_3$ | 56.4 ($OCH_3$) | 3.99 (*s*, 3H) | C-9 |

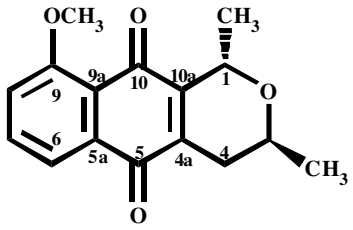



NOE of **SA2**

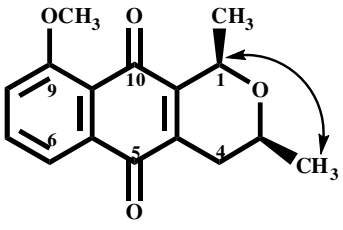



Major HMBC of **SA2**

**SA3: (1*R*,3*R*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (isoeleutherin)**




**SA3** was a yellow solid, $[\alpha]^{29}_{D}$ -24° (*c* 8.30, $CHCl_3$). The UV, IR, $^1$H NMR and $^{13}$C NMR spectral data (**Table 6**) produced a similar pattern to those of **SA2** (eleutherine), with a slight difference in the chemical shifts of H-1 ($\delta$ 5.01, *dq*, *J* = 2.7, 6.9 Hz) and H-3 ($\delta$ 4.00, *m*). It was thus proposed to be a diastereomer of eleutherin. The presence of a characteristic signal of the ABM pattern of aromatic protons was shown at $\delta$ 7.74 (*dd*, *J* = 1.2, 8.4 Hz, H-6), 7.65 (*t*, *J* = 8.4 Hz, H-7), 7.29 (*dd*, *J* = 1.2, 8.4 Hz, H-8). The $^1$H NMR spectrum showed a singlet signal of a methoxy proton 9-$OCH_3$ at $\delta$ 4.01, -$CH_2$- at $\delta$ 2.70 and 2.23, and two methyl group at $\delta$ 1.54 and 1.35. The results from the NOEDIFF experiment indicated that the 1-$CH_3$ and 3-$CH_3$ were *trans*. The optical rotation also confirmed proposed stereochemistry of **SA3**. The HMBC experiment confirmed the assigned structure. Thus **SA3** was proposed to be (1*R*,3*R*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione isoeleutherin (Komura *et al*., 1983).

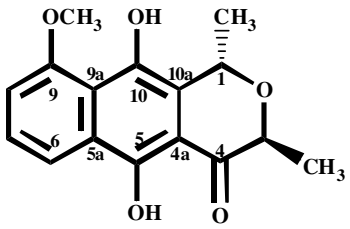



NOE of **SA3**

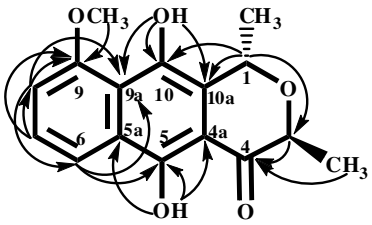
**Table 6** NMR spectral data of **SA3**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 67.4 (CH) | 5.01 (*dq*, *J* = 2.7, 6.9 Hz, 1H) | C-3, C-4a, C-10a, 1-$CH_3$ |
| 3 | 62.4 (CH) | 4.00 (*m*, 1H) | C-1, 3-$CH_3$ |
| 4 | 29.5 ($CH_2$) | 2.70 (*dd, J* = 3.3, 18.9 Hz, 1H) | C-3, C-4a, C-5, |
| | | 2.23 (*ddd*, *J* = 2.7, 10.2, 18.9 Hz, 1H) | C-10a |
| 4a | 139.3 (C) | - | - |
| 5 | 184.2 (C=O) | - | - |
| 5a | 134.0 (C) | - | |
| 6 | 119.0 (CH) | 7.74 (*dd*, *J* = 1.2, 8.4 Hz, 1H) | C-5, C-8, C-9a |
| 7 | 134.7 (CH) | 7.65 (*t*, *J* = 8.4 Hz, 1H) | C-5a, C-8, C-9 |
| 8 | 117.8 (CH) | 7.29 (*dd*, *J* = 1.2, 8.4 Hz, 1H) | C-6, C-7, C-9 |
| 9 | 159.7 (C) | - | - |
| 9a | 119.7 (C) | - | - |
| 10 | 182.7 (C=O) | - | - |
| 10a | 148.0 (C) | - | - |
| 1-$CH_3$ | 19.7 ($CH_3$) | 1.54 (*d*, *J* = 6.9 Hz, 3H) | C-1, C-10a |
| 3-$CH_3$ | 21.5 ($CH_3$) | 1.35 (*d*, *J* = 6.9 Hz, 3H) | C-3, C-4 |
| 9-$OCH_3$ | 56.4 ($OCH_3$) | 4.01 (*s*, 3H) | C-9 |

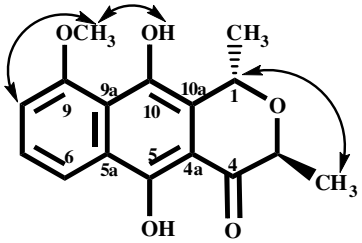
**SA4: (1*R*,3*R*)5,10-dihydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-4(3*H*)-one (hongconin)**




**SA4** was a yellow solid, m.p. 121-123°C, $[\alpha]^{29}_{D}$ -8.5° (*c* 0.200, $CHCl_3$). The UV spectrum showed maximum absorption bands at 222, 266 and 430 nm. The IR spectrum exhibited the stretching bands of hydroxyl group (3405 $cm^{-1}$), aromatic ring (1607 and 1584 $cm^{-1}$) and ether linkage (1235 and 1101 $cm^{-1}$). The $^1$H NMR spectra **(Table 7)** exhibited the signals of H-1 ($\delta$ 5.48, *q*), 1-$CH_3$ ($\delta$ 1.64, *d*), H-3 ($\delta$ 4.69, *q*), 3-$CH_3$ ($\delta$ 1.53, *d*), aromatic protons H-6 ($\delta$ 8.05, *d*), H-7 ($\delta$ 7.38, *t*) and H-8 ($\delta$ 7.02, *d*) with a methoxyl group ($\delta$ 4.13, 9-$OCH_3$), a hydroxyl group ($\delta$ 12.82, 5-OH) and non-chelated hydroxyl group ($\delta$ 8.97, 10-OH). In addition, the $^{13}$C NMR spectrum showed signals of one carbonyl carbon at 202.9 (CO-4), one methoxy carbon ($\delta$ 56.4), two methyl carbons ($\delta$ 17.4 and 16.3), five methine carbons ($\delta$ 125.4, 118.1, 109.2, 71.4 and 69.5) and seven quaternary carbons ($\delta$ 155.7, 154.4, 139.4, 125.9, 120.9, 119.5 and 108.0). The HMBC correlation of OH ($\delta$ 12.82, *s*) to C-4a, C-5 and C-5a suggested its connection at C-5. The assignment of 9-$OCH_3$ was confirmed by the HMBC correlations of this methoxyl group to C-9 and of H-8 to C-9. The correlation of 10-OH to C-9a, C-10 and C-10a confirmed the placement of 10-OH. In addition, the HMBC experiment further showed the correlations of H-8 to C-9a of 10-OH to C-9a, C-10a and of 1-$CH_3$ to C-10a suggesting that the 10-OH was between 8-$OCH_3$ and 1-$CH_3$. The enhancement of H-8 and 10-OH signals in the NOEDIFF experiment by irradiation of 9-$OCH_3$ confirmed the assignment of H-8, 9-$OCH_3$ and 10-OH. Furthermore, irradiation of H-1 enhanced the signal of the methyl protons 3-$CH_3$ indicating that the relative stereochemistry of 1-$CH_3$ and 3-$CH_3$ were *trans*. The optical rotation and assignment agrees with those of hongconin, (1*R*,3*R*)5,10-dihydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-4(3*H*)-one (Chen *et al*., 1986).




Major HMBC of **SA4**




NOE of **SA4**

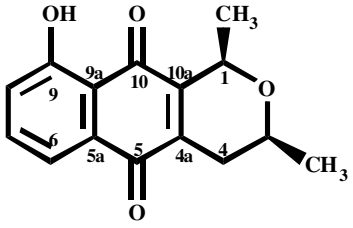
**Table 7** NMR spectral data of **SA4**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 71.4 (CH) | 5.48 (*q*, *J* = 6.6 Hz, 1H) | C-3, C-4a, C-10, C-10a, 1-$CH_3$ |
| 3 | 69.5 (CH) | 4.69 (*q*, *J* = 6.6 Hz, 1H) | C-1, C-4, 3-$CH_3$ |
| 4 | 202.9 (C=O) | - | - |
| 4a | 108.0 (C) | - | - |
| 5 | 154.4 (C) | - | - |
| 5a | 125.9 (C) | - | - |
| 6 | 118.1 (CH) | 8.05 (*d*, *J* = 8.4 Hz, 1H) | C-5, C-9[a] |
| 7 | 125.4 (CH) | 7.38 (*t*, *J* = 8.4 Hz, 1H) | C-8, C-9 |
| 8 | 109.2 (CH) | 7.02 (*d*, *J* = 8.4 Hz, 1H) | C-5, C-9, C-9a |
| 9 | 155.7 (C) | - | - |
| 9a | 119.5 (C) | - | - |
| 10 | 139.4 (C) | - | - |
| 10a | 120.9 (C) | - | - |
| 1-$CH_3$ | 17.4 ($CH_3$) | 1.64 (*d*, *J* = 6.6 Hz, 3H) | C-1,C-10a |
| 3-$CH_3$ | 16.3 ($CH_3$) | 1.53 (*d*, *J* = 6.6 Hz, 3H) | C-3, C-4 |
| 5-OH | - | 12.82 (*s*, 1H) | C-4a, C-5, C-5[a] |
| 10-OH | - | 8.97 (*s*, 1H) | C-9a, C-10, C-10a |
| 9-$OCH_3$ | 56.4 ($OCH_3$) | 4.13 (*s*, 3H) | C-9 |

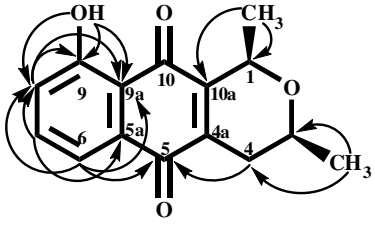
**Table 8** Comparison of the $^{1}$H NMR spectral data of **SA4** and **hongconin**

| Position | **SA4** $\delta_H$ (multiplicity) | **Hongconin** $\delta_H$ (multiplicity) |
|---|---|---|
| 1 | 5.48 (*q*, *J* = 6.6 Hz, 1H) | 5.41 (*q*, *J* = 7.0 Hz, 1H) |
| 3 | 4.69 (*q, J* = 6.6 Hz, 1H) | 4.63 (*q, J* = 7.0 Hz, 1H) |
| 4 | - | - |
| 4a | - | - |
| 5 | - | - |
| 5a | - | - |
| 6 | 8.05 (*d*, *J* = 8.4 Hz, 1H) | 8.00 (*d*, *J* = 7.0 Hz, 1H) |
| 7 | 7.38 (*t*, *J* = 8.4 Hz, 1H) | 7.28 (*t*, *J* = 7.0 Hz, 1H) |
| 8 | 7.02 (*d, J* = 8.4 Hz, 1H) | 6.95 (*d, J* = 7.0 Hz, 1H) |
| 9 | - | - |
| 9a | - | - |
| 10 | - | - |
| 10a | - | - |
| 1-$CH_3$ | 1.64 (*d*, *J* = 6.6 Hz, 3H) | 1.58 (*d*, *J* = 7.0 Hz, 3H) |
| 3-$CH_3$ | 1.53 (*d, J* = 6.6 Hz, 3H) | 1.48 (*d, J* = 7.0 Hz, 3H) |
| 5-OH | 12.82 (*s*, 1H) | 12.72 (*s*, 1H) |
| 10-OH | 8.97 (*s*, 1H) | 8.87 (*s*, 1H) |
| 9-$OCH_3$ | 4.13 (*s*, 3H) | 4.02 (*s*, 3H) |

**SA5: (1*R*,3*S*)3,4-dihydro-9-hydroxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione**




**SA5** was obtained as a yellow-brown viscous oil, $[\alpha]^{29}_{D}$ +16° (*c* 0.092, $CHCl_3$). The UV spectrum showed the maximum absorption bands at 222, 245, 267, 272 and 420 nm. The IR spectrum showed the bands of a hydroxyl group at 3441 $cm^{-1}$ and carbonyl groups at 1640, 1615 $cm^{-1}$. The $^1H$ NMR spectra **(Table 9)** exhibited resonances of a chelated hydroxyl group 9-OH at $\delta$ 12.04 (*s*). The remaining signals were resembled those of **SA2** (eleutherin): the ABM pattern of H-6 ($\delta$ 7.63, *dd*), H-7 ($\delta$ 7.58, *t*) and H-8 ($\delta$ 7.24, *dd*), doublet of 1-$CH_3$ ($\delta$ 1.55), doublet of 3-$CH_3$ ($\delta$ 1.36), doublet of quartet of H-1 ($\delta$ 5.01), multiplet of H-3 ($\delta$ 4.00), *dd* and *ddd* of non-equivalent of -$CH_2$- ($\delta$ 2.76, 1H and $\delta$ 2.25, 1H). The HMBC correlations of 9-OH and H-8 to C-9 confirmed the location of OH at C-9. The quinone moiety was suggested from the presence of the carbonyl carbon signals at $\delta$183.0 (C-5) and 189.0 (C-10). As for **SA2**, the chemical shifts of methine carbons C-1 ($\delta$ 66.9) and C-3 ($\delta$ 62.5) confirmed the ether structure. The HMBC experimental also confirmed the assignments. The optical rotation and the assignment is in agreement with (1*R*,3*S*)3,4-dihydro-9-hydroxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (Gill *et al*., 2004), a demethoxy derivatives of eleutherin.



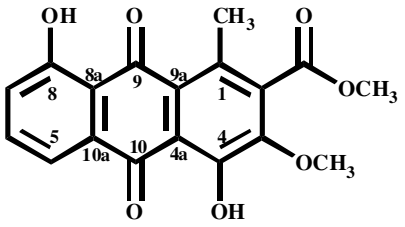

Major HMBC of **SA5**

**Table 9** NMR spectral data of **SA5**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 66.9 (CH) | 5.01 (*dq*, *J* = 2.7, 6.6 Hz, 1H) | C-3, C-10a, 1- $CH_3$ |
| 3 | 62.5 (CH) | 4.00 (*m,* 1H) | - |
| 4 | 29.9 ($CH_2$) | 2.76 (*dd*, *J* = 3.6, 19.5 Hz, 1H) | C-5 |
| | | 2.25 (*ddd*, *J* = 3.6, 11.4, 19.5 Hz, 1H) | |
| 4a | * | - | - |
| 5 | 183.0 (C=O) | - | - |
| 5a | 132.1 (C) | - | - |
| 6 | 119.1 (CH) | 7.63 (*dd*, *J* = 1.8, 7.2 Hz, 1H) | C-5, C-8, C-9a |
| 7 | 136.2 (CH) | 7.58 (*t*, *J* = 7.2 Hz, 1H) | C-5a, C-8 |
| 8 | 124.4 (CH) | 7.24 (*dd*, *J* = 1.8, 7.2 Hz, 1H) | C-9, C-9a |
| 9 | 162.0 (C) | - | - |
| 9a | 115.2 (C) | - | - |
| 10 | 189.0 (C=O) | - | - |
| 10a | 146.6 (C) | - | - |
| 1-$CH_3$ | 19.7 ($CH_3$) | 1.55 (*d*, *J* = 6.6 Hz, 3H) | C-1, C-10a |
| 3-$CH_3$ | 21.4 ($CH_3$) | 1.36 (*d*, *J* = 6.6 Hz, 3H) | C-3, C-4 |
| 9-OH | - | 12.04 (*s*, 1H) | C-8, C-9, C-9a |

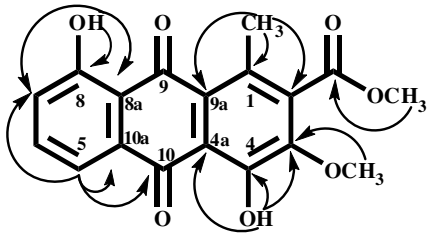
*** Not observed**

**SA6: 4,8-dihydroxy-3-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester**

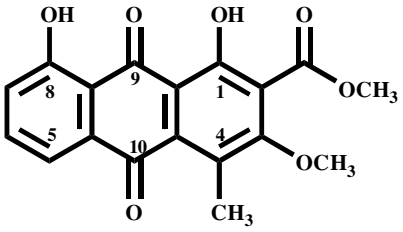



**SA6** was an orange solid. The UV spectrum exhibited absorption maximum at 229, 250, 265, 273 and 443 nm, indicating an anthraquinone as a basic structure. The IR spectrum showed the stretching band of O-H at 3374 $cm^{-1}$ and C=O at 1739 and 1617 $cm^{-1}$. The $^{1}H$ NMR spectra **(Table 10)** showed signals due to chelated hydroxyl protons at $\delta$ 13.71 (8-OH) and $\delta$ 12.91 (4-OH), methyl protons at $\delta$ 2.64 (-$CH_3$) and two methoxyl groups at $\delta$ 4.10 (3-$OCH_3$) and 3.99 (2-CO$\underline{OCH_3}$). The HMBC correlations of 4-OH to C-3, C-4, C-4a and of 8-OH to C-8, C-7, C-8a confirmed the position of OH at C-4 and C-8, respectively. The methyl group was placed at C-1 due to the HMBC correlation of methyl protons to C-1, C-2 and C-9a. The remaining resonances appearing as an ABM system at an aromatic region were assigned for H-5 ($\delta$7.83, *d, J* = 8.4 Hz), H-6 ($\delta$7.66, *t, J* = 8.4 Hz) and H-7 ($\delta$7.33, *d*, *J* = 8.4 Hz). The quinone structure was deduced from the low field carbon resonances at $\delta$ 189.0 (C-10) and 189.1 (C-9), whereas the acyl group was indicated by the chemical shift of a carbonyl carbon at $\delta$166.8 and the proton signal of a methoxyl group at $\delta$3.99. The HMBC correlation of 3-$OCH_3$ to C-3 and of hydroxyl group to C-4 and C-3 indicated that the methoxyl group was placed next to the chelated hydroxyl group (4-OH). The remaining carbon resonances were the resonances of a methyl carbon ($\delta$ 19.6), two methoxy carbons ($\delta$ 61.6 and 52.7), three methine carbons ($\delta$ 118.8, 136.0 and 125.4) and five quaternary carbons ($\delta$ 132.4, 132.3, 125.4, 118.0 and 117.0). According to the biosynthesis of the anthraquinones from a polyketide precursor (Wiley *et al*., 1980), the carboxylic function (2-$COOCH_3$) was placed adjacent to the methyl group (1-$CH_3$). Unfortunately, the HMBC data could not identify the exact location of the chelated hydroxyl group (4-OH), the parallel and the antiparallel chelated hydroxyl group with a quinone moiety, were possible

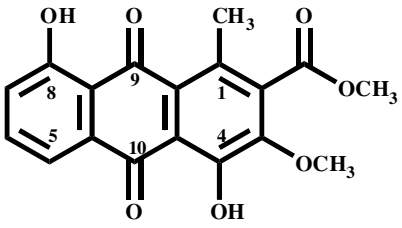
structures. From the IR spectra only one band of the H-bonded carbonyl carbon was obtained, the antiparallel structure was then considered for this compound (Komura *et al*., 1983). **SA6** was therefore proposed to be 4,8-dihydroxy-3-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (Komura *et al*., 1983). The $^{1}H$ and $^{13}C$ NMR spectral data were compatible with those of the known compound.




Major HMBC of **SA6**




Parallel chelated hydroxyl

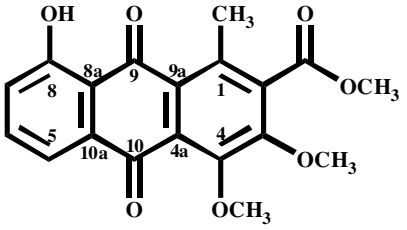



antiparallel chelated hydroxyl

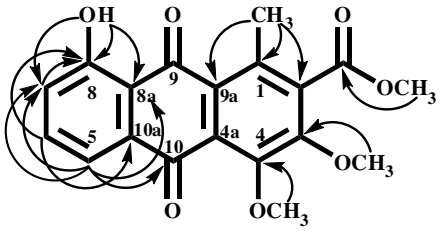
**Table 10** NMR spectral data of **SA6**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 137.5 (C) | - | - |
| 2 | 132.3 (C) | - | - |
| 3 | 150.2 (C) | - | - |
| 4 | 155.2 (C) | - | - |
| 4a | 118.0 (C) | - | - |
| 5 | 118.8 (CH) | 7.83 (*d*, *J* = 8.4 Hz, 1H) | C-7, C-10, C-10[a] |
| 6 | 136.0 (CH) | 7.66 (*t*, *J* = 8.4 Hz, 1H) | C-8, C-10[a] |
| 7 | 125.4 (CH) | 7.33 (*d*, *J* = 8.4 Hz, 1H) | C-5, C-8 |
| 8 | 162.6 (C) | - | - |
| 8a | 117.0 (C) | - | - |
| 9 | 189.1 (C=O) | - | - |
| 9a | 125.4 (C) | - | - |
| 10 | 189.0 (C=O) | - | - |
| 10a | 132.4 (C) | - | - |
| 2-$\underline{CO_2}CH_3$ | 166.8 (C=O) | - | - |
| 1-$CH_3$ | 19.6 ($CH_3$) | 2.64 (*s,* 3H) | C-1, C-2, C-9a |
| 3-$OCH_3$ | 61.6 ($OCH_3$) | 4.10 (*s,* 3H) | C-3 |
| 4-OH | - | 12.91 (*s,* OH) | C-3, C-4, C-4a |
| 8-OH | - | 13.71 (*s*, OH) | C-7, C-8, C-8a |
| 2-$CO_2\underline{CH_3}$ | 52.7 ($OCH_3$) | 3.99 (*s*, 3H) | 2-C=O |

**SA7: 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester**




**SA7** was a yellow solid. The UV spectrum showed the absorption bands at 223, 254, 264, 271 and 401 nm, indicating an anthraquinone skeleton. The IR spectrum indicated the presence of O-H stretching at 3467 $cm^{-1}$ and C=O stretching at 1733, 1674 and 1633 $cm^{-1}$. Its $^{1}H$ and $^{13}C$ NMR spectra (**Table 11**) showed a similar signal pattern to those of **SA6**. The differences were observed as the additional methoxy resonance at $\delta$ 4.03 and the absence of one of a chelated hydroxyl proton. The 4-OH of **SA6** was then replaced by a methoxyl group of **SA7**. The $^{1}H$ NMR spectrum showed the resonances of a chelated hydroxyl group 8-OH at $\delta$ 12.57 (*s*), methyl protons 1-$CH_3$ at $\delta$ 2.69 (*s*), methoxyl group 3-$OCH_3$ at $\delta$ 3.99 (*s*), aromatic protons H-5, H-6 and H-7 at $\delta$ 7.70 (*d*), 7.62 (*t*) and 7.26 (*d*), respectively. The HMBC correlation of OH to C-8, C-7 and C-6 confirmed the position of OH at C-8. The correlations of methyl group to C-1, C-2, C-9 and C-9a indicated that the position of methyl group was nearby the carbonyl group (CO-9). The $^{13}C$ NMR spectrum and DEPT experiments signified the presence of three carbonyl carbons ($\delta$ 189.8, 182.6 and 166.9) three methoxy carbons ($\delta$ 62.0, 61.6 and 52.7), one methyl carbon ($\delta$ 20.1), three methine carbons ($\delta$ 136.2, 123.7 and 118.6) and five quaternary carbons ($\delta$ 136.2, 136.1, 134.3, 129.9 and 116.5). The structure of **SA7** was thus determined as the methoxy derivative of **SA6**, 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (Komura *et al*., 1983).




Major HMBC of **SA7**

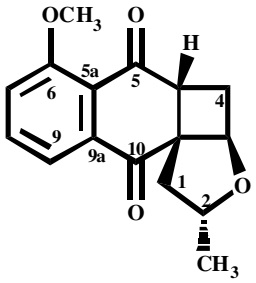
**Table 11** NMR spectral data of **SA7**

| Position | $\delta_C$(C-Type) | $\delta_H$(multiplicity) | HMBC |
|---|---|---|---|
| 1 | 137.0 (C) | - | - |
| 2 | 136.1 (C) | - | - |
| 3 | 152.1 (C) | - | - |
| 4 | 155.5 (C) | - | - |
| 4a | 136.2 (C) | - | - |
| 5 | 118.6 (CH) | 7.70 (*d*, *J* = 7.4 Hz, 1H) | C-7, C-8a, C-10 |
| 6 | 136.2 (CH) | 7.62 (*t,* *J* = 7.4 Hz, 1H) | C-8, C-10[a] |
| 7 | 123.7 (CH) | 7.26 (*d,* *J* = 7.4 Hz, 1H) | C-8, C-8[a] |
| 8 | 161.9 (C) | - | - |
| 8a | 116.5 (C) | - | - |
| 9 | 189.8 (C=O) | - | - |
| 9a | 129.9 (C) | - | - |
| 10 | 182.6 (C=O) | - | - |
| 10a | 134.3 (C) | - | - |
| 2-$\underline{CO_2}CH_3$ | 166.9 (C=O) | - | - |
| 1-$CH_3$ | 20.1 ($CH_3$) | 2.69 (*s*, 3H) | C-1, C-9, C-9[a] |
| 3-$OCH_3$ | 61.6 ($OCH_3$) | 3.99 (*s*, 3H) | C-3 |
| 4-$OCH_3$ | 62.0 ($OCH_3$) | 4.03 (*s*, 3H) | C-4 |
| 8-OH | - | 12.57 (*s,* OH) | C-6, C-7, C-8 |
| 2-$CO_2\underline{CH_3}$ | 52.7 ($OCH_3$) | 3.97 (*s,* 3H) | 2-C=O |

**Table 12** $^{13}C$ NMR spectroscopic data of **SA6** and **SA7**

| Position | **SA6** | **SA7** |
|---|---|---|
| 1 | 137.5 (C) | 137.0 (C) |
| 2 | 132.3 (C) | 136.1 (C) |
| 3 | 150.2 (C) | 152.1 (C) |
| 4 | 155.2 (C) | 155.5 (C) |
| 4a | 118.0 (C) | 136.2 (C) |
| 5 | 118.8 (CH) | 118.6 (CH) |
| 6 | 136.0 (CH) | 136.2 (CH) |
| 7 | 125.4 (CH) | 123.7 (CH) |
| 8 | 162.6 (C) | 161.9 (C) |
| 8a | 117.0 (C) | 116.5 (C) |
| 9 | 189.1 (C=O) | 189.8 (C=O) |
| 9a | 125.4 (C) | 129.9 (C) |
| 10 | 189.0 (C=O) | 182.6 (C=O) |
| 10a | 132.4 (C) | 134.3 (C) |
| 2-$\underline{CO_2}CH_3$ | 166.8 (C=O) | 166.9 (C=O) |
| 1-$CH_3$ | 19.6 ($CH_3$) | 20.1 ($CH_3$) |
| 3-$OCH_3$ | 61.6 ($OCH_3$) | 61.6 ($OCH_3$) |
| 4-$OCH_3$ | - | 62.0 ($OCH_3$) |
| 2-$CO_2\underline{CH_3}$ | 52.7 ($OCH_3$) | 52.7 ($OCH_3$) |

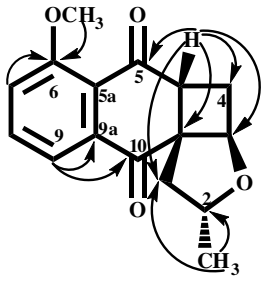
**SA8: 1,2,3a,4a-tetrahydro-6-methoxy-4-methyl-10*H*-naphtho[2′,3′:2,3] cyclobuta [1,2-*b*]furan-5,10(3a*H*)-dione (elecanacin)**




**SA8** was obtained as brown needles, $[\alpha]^{29}_{D}$ +85° (*c* 0.004, $CHCl_3$). The UV spectrum exhibited maximum absorptions at 218, 231, 336 nm. The IR spectrum showed the absorption bands of a carbonyl group at 1679 $cm^{-1}$. The $^1H$ NMR spectra **(Table 13)** showed the signals due to three aromatic protons (H-9, $\delta$ 7.46, *dd, J* = 1.0, 8.0 Hz; H-8, $\delta$ 7.46, *t*, *J* = 8.0 Hz; H-7, $\delta$ 7.30, *dd*, *J* = 1.0, 8.0 Hz), one aromatic methoxyl group (6-$OCH_3$, $\delta$ 3.97 ), three methine protons (H-3a, $\delta$ 4.61, *dd, J* = 6.0, 6.5 Hz; H-2, $\delta$ 4.56, *m*; H-4a, $\delta$ 3.21, *dd, J* = 2.5, 7.5 Hz), non-equivalent methylene protons (H-4, $\delta$ 2.58, *m*), non-equivalent methylene protons (H-$1_a$, $\delta$ 2.30, *dd*, *J* = 11.0, 12.5 Hz; H-$1_b$, $\delta$ 2.01, *dd*, *J* = 4.5, 12.5 Hz), and a methyl group (2-$CH_3$, $\delta$ 1.46, *d*, *J* = 5.5 Hz). The HMBC correlations of a methoxy proton to C-6 as well as of H-7 to C-6 indicated the *ortho* position of H-7 and 6-$OCH_3$. The methyl group was placed at C-2 according to the HMBC correlation of 2-$CH_3$ to C-2 and C-1. Furthermore, the HMBC correlation of H-4a to C-4 and C-5 confirmed that H-4a was adjacent to a carbonyl carbon (CO-5). The $^{13}C$ NMR spectrum showed signals assignable to two carbonyls ($\delta$ 196.0, C-5 and $\delta$ 195.0, C-10) of the quinone moiety and aromatic methoxyl group ($\delta$ 56.5). The chemical shifts of C-3a ($\delta$ 80.9) and C-2 ($\delta$ 75.9) indicated that they are connected to an oxygen atom in an ether group. The correlations of H-9 to C-10 and of H-1 to C-10 confirmed the placements of H-9, C-10, and a furan ring. The COSY and HMBC correlations confirmed all protons and carbon assignments. **SA8** then was identified as 1,2,3a,4a-tetrahydro-6-methoxy-4-methyl-10*H*-naphtho[2′,3′:2,3]cyclobuta[1,2*b*]furan-5,10(3*aH*)-dione. The assignment and its optical rotation are corresponded to the data of elecanacin (Hara *et al*., 1997).

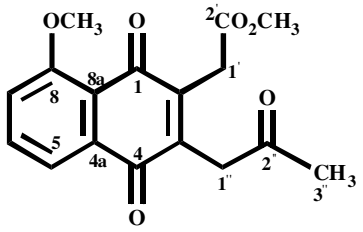
**Table 13** NMR spectral data of **SA8**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 42.0 ($CH_2$) | 2.30 (*dd*, *J* = 11.0, 12.5 Hz, 1H) | C-2, C-4a, C-10a |
| | | 2.01 (*dd*, *J* = 4.5, 12.5 Hz, 1H) | |
| 2 | 75.9 (CH) | 4.56 (*m*, 1H) | - |
| 3a | 80.9 (CH) | 4.61 (*dd*, *J* = 6.0, 6.5 Hz, 1H) | C-2, C-4a, C-10 |
| 4 | 36.0 ($CH_2$) | 2.58 (*m,* 2H) | C-3a, C-4a, C-5, C-10a |
| 4a | 45.0 (CH) | 3.21 (*dd*, *J* = 2.5, 7.5 Hz, 1H ) | C-1, C-3a, C-4, C-5 C-10a |
| 5 | 196.0 (C=O) | - | - |
| 5a | 124.0 (C) | - | - |
| 6 | 159.0 (C) | - | - |
| 7 | 117.2 (CH) | 7.30 (*dd*, *J* = 1.0, 8.0 Hz, 1H) | C-5a, C-6, C-9 |
| 8 | 134.8 (CH) | 7.46 (*t*, *J* = 8.0 Hz, 1H) | C-9 |
| 9 | 119.3 (CH) | 7.46 (*dd*, *J* = 1.0, 8.0 Hz, 1H) | C-9a, C-10 |
| 9a | 138.0 (C) | - | - |
| 10 | 195.0 (C=O) | - | - |
| 10a | 61.3 (C) | - | - |
| 2-$CH_3$ | 19.2 ($CH_3$) | 1.46 (*d*, *J* = 5.5 Hz, 3H) | C-1, C-2 |
| 6-$OCH_3$ | 56.5 ($OCH_3$) | 3.97 (*s*, 3H) | C-6 |

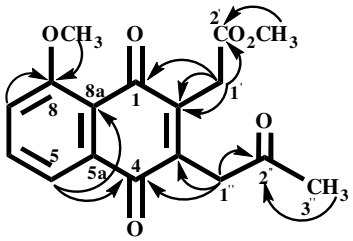



Major HMBC of **SA8**

**SA9: [8-methoxy-1,4-dioxo-3-(2-oxo-propyl)-1,4-dihydronaphthalen-2-yl]acetic acid methyl ester.**




**SA9** was a yellow-brown solid, m.p. 101-103°C. Its molecular formula of $C_{17}H_{16}O_6$ was established on the basis of mass spectrum, EIMS ($[M]^+$ *m/z* 316.0943). The UV spectrum exhibited maximum absorptions at 225, 240, 266, 271 and 405 nm. The IR spectrum showed the absorption bands of C=O stretching at 1736, 1718, 1656 and 1586 $cm^{-1}$. The $^1H$ NMR spectral data **(Table 14)** showed the signals of ABM pattern of aromatic protons H-7 ($\delta$ 7.29, *dd*, *J* = 1.2, 7.8 Hz), H-6 ($\delta$ 7.66, *t*, *J* = 7.8 Hz), H-5 ($\delta$ 7.74, *dd*, *J* = 1.2, 7.8 Hz) and of 8-$OCH_3$ ($\delta$ 4.00, *s*). The HMBC correlations of 8-$OCH_3$ and H-7 to C-8 indicated the *ortho* position of H-7 and 8-$OCH_3$. The quinone moiety was indicated from the presence of the carbonyl carbon signals at $\delta$ 184.3 (C-4) and 182.7 (C-1). The signals of a methoxyl group ($\delta$ 3.68, *s*) and methylene protons (C-1′, $\delta$ 3.63, *s*) whose HMBC correlation to an ester C=O ($\delta$ 169.9) was evidence for the presence of a methyl ethanoyl side chain -$CH_2CO_2CH_3$. The HMBC correlation of H-1′ to C-1 and C-2 indicated the connection of the methyl ethanoyl side chain to the naphthoquinone core at C-2. The proton resonances of $CH_3$-3″ ($\delta$ 2.30, *s*) and $CH_2$-1″ ($\delta$ 3.77, *s*) as well as the HMBC correlations of these protons to C=O (C-2″, $\delta$ 203.2) indicated the presence of an acetonyl side chain -$CH_2C(O)CH_3$. The HMBC correlations of H-5 and H-1″ to C-4 indicated that C=O was in between H-5 and the acetonyl group. **SA9** was therefore identified as a new naphthoquinone derivative, [8-methoxy-1,4-dioxo-3-(2-oxo-propyl)-1,4-dihydronaphthalen-2-yl]acetic acid methyl ester.

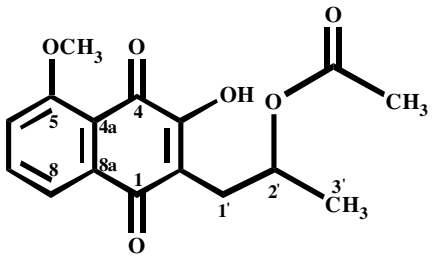



Major HMBC of **SA9**

**Table 14** NMR spectral data of **SA9**

| Position | $\delta_C$(C-Type) | $\delta_H$(multiplicity) | HMBC |
|---|---|---|---|
| 1 | 182.7 (C=O) | - | - |
| 2 | 143.6 (C) | - | - |
| 3 | 140.4 (C) | - | - |
| 4 | 184.3 (C=O) | - | - |
| 4a | 134.0 (C) | - | - |
| 5 | 117.9 (CH) | 7.74 (*dd*, *J* = 1.2, 7.8 Hz, 1H) | C-4, C-8a |
| 6 | 134.9 (CH) | 7.66 (*t*, *J* = 7.8 Hz, 1H) | C-4a, C-5, C-7, C-8 |
| 7 | 119.4 (CH) | 7.29 (*dd*, *J* =1.2, 7.8 Hz, 1H) | C-7, C-8, C-8a |
| 8 | 159.8 (C) | - | - |
| 8a | 119.7 (C) | - | - |
| 8-$OCH_3$ | 56.5 ($OCH_3$) | 4.00 (*s*, 3H) | C-8 |
| 1′ | 32.9 ($CH_2$) | 3.63 (*s*, 2H) | C-1, C-2, C-3, C-2′ |
| 2′ | 169.9 (C=O) | - | - |
| 2′-$OCH_3$ | 52.4 ($OCH_3$) | 3.68 (*s*, 3H) | C-2′ |
| 1″ | 41.8 ($CH_2$) | 3.77 (*s*, 2H) | C-2, C-3, C-4, C-2″ |
| 2″ | 203.2 (C=O) | - | - |
| 3″ | 30.1 ($CH_3$) | 2.30 (*s*, 3H) | C-1″, C-2″ |

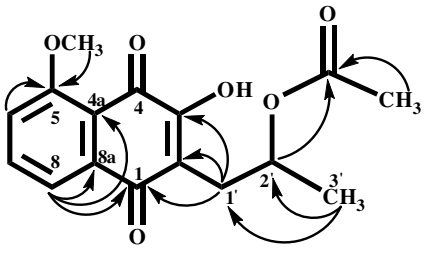
**SA10: 2-[2′-(acetyloxy)propyl]-3-hydroxy-5-methoxy-1,4-naphthoquinone**




**SA10** was obtained as a brown solid, m.p. 148-150 °C, $[\alpha]^{29}_{D}$ +4° (*c* 0.890, $CHCl_3$). Its EIMS exhibited a molecular ion peak at *m/z* 304.0943, consistent with a molecular formula of $C_{15}H_{15}O_6$. The UV spectrum showed the absorption bands at 225, 244, 278 and 389 nm. The IR spectrum indicated the presence of O-H stretching at 3245 $cm^{-1}$ and C=O stretching at 1736, 1659 and 1638 $cm^{-1}$. It was assigned for a naphthoquinone derivative according to the evidence of the resonances of two carbonyl carbons at $\delta$ 184.1 (C-1) and 179.6 (C-4) and of aromatic protons appearing as an ABM pattern at $\delta$ 7.55 (*dd*, *J* = 1.2, 7.7 Hz, H-6), 7.71 (*t*, *J* = 7.7 Hz, H-7) and 7.81 (*dd, J* = 1.2, 7.7 Hz, H-8). The spectrum further showed a methoxyl group at $\delta$ 4.04 (5-$OCH_3$) and it was placed at C-5 due to the HMBC correlations of $OCH_3$ and H-6 to C-5. The presence of an acetyloxypropyl group was indicated from the signals of equivalent methylene protons H-1′ ($\delta$ 2.86, *dd*, *J* = 1.2, 6.3 Hz), a methine proton H-2′ ($\delta$ 5.21, *sext*, *J* = 6.3 Hz), methyl protons H-3′ ($\delta$ 1.28, *d, J* = 6.3 Hz) and an acyl proton ($\delta$ 1.96, *s*) together with the evidence from the resonances of a carbonyl carbon ($\delta$ 170.6) an oxymethine carbon ($\delta$ 69.5) and also HMBC correlation of H-2′ to C=O of an acyl group. The correlation of methylene protons H-1′ and H-8 to C-1 indicated that acetyloxypropyl side chain and H-8 were on the same side as CO-1 **(Table 15). SA10** was therefore identified as 2-[2′-(acetyloxy)propyl]-3-hydroxy-5-methoxy-1,4-naphthoquinone, a new naphthoquinone derivative.

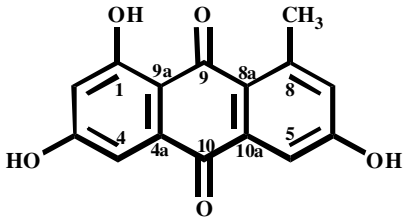
**Table 15** NMR spectral data of **SA10**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 184.1 (C=O) | - | - |
| 2 | 117.6 (C) | - | - |
| 3 | 154.5 (C) | - | - |
| 4 | 179.6 (C=O) | - | - |
| 4a | 116.9 (C) | - | - |
| 5 | 160.1 (C) | - | - |
| 6 | 116.7 (CH) | 7.55 (*dd*, *J* = 1.2, 7.7 Hz, 1H) | C-5, C-8 |
| 7 | 136.5 (CH) | 7.71 (*t*, *J* = 7.7 Hz, 1H) | C-5, C-8a |
| 8 | 119.8 (CH) | 7.81 (*dd*, *J* = 1.2, 7.7 Hz, 1H) | C-1, C-4a, C-7 |
| 8a | 135.0 (C) | - | - |
| 5-$OCH_3$ | 56.5 ($CH_3$) | 4.04 (*s*, 3H) | C-5 |
| 1′ | 29.7 ($CH_2$) | 2.86 (*dd*, *J* = 1.2, 6.3 Hz, 2H) | C-1, C-2, C-3, C-2′, C-3′ |
| 2′ | 69.5 (CH) | 5.21 (*sext*, *J* = 6.3 Hz, 1H) | C-2, C-1′, 2′-O<u>CO</u>$CH_3$ |
| 2′-O<u>CO</u>$CH_3$ | 170.6 (C=O) | - | - |
| 2′-OCO<u>CH</u>$_3$ | 21.2 ($CH_3$) | 1.96 (*s*, 3H) | 2′-O<u>CO</u>$CH_3$ |
| 3′ | 20.1 ($CH_3$) | 1.28 (*d, J* = 6.3 Hz, 3H) | C-1′, C-2′ |

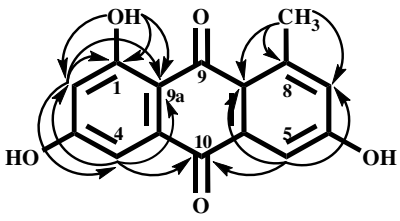



Major HMBC of **SA10**

**SA11: 1,3,6-trihydroxy-8-methylanthraquinone (erythrolaccin)**




**SA11** was an orange solid. The UV spectrum exhibited the absorption bands at 222, 244, 283, 344 and 433 nm, characteristic of an anthraquinone. The IR spectrum showed O-H stretching at 3416 $cm^{-1}$ and C=O stretching at 1648, 1620 $cm^{-1}$. The presence of carbonyl carbons were suggested from the $^{13}C$ NMR signal at $\delta$ 189.0 (C-9) and 182.4 (C-10). The $^{1}H$ NMR spectral data **(Table 16)** showed the signals of a chelated hydroxyl group 1-OH at $\delta$ 13.34, a broad singlet of non-chelated hydroxyl group 6-OH at $\delta$ 10.25 and a downfield shifted methyl group -$CH_3$ at $\delta$ 2.80. The HMBC correlation of 1-OH to C-1, C-2 and C-9a suggested its connection at C-1. The HMBC correlation of $CH_3$ to C-7, C-8 and C-8a indicated that the methyl group was at C-8. The double signals of *meta* proton H-7 and H-5 were observed at $\delta$ 7.12 ($J$ = 2.4 Hz) and $\delta$ 7.61 ($J$ = 2.4 Hz), whereas those of H-4 and H-2 were found at $\delta$ 7.21 ($J$ = 2.4 Hz) and $\delta$ 6.68 ($J$ = 2.4 Hz). The HMBC correlations of H-4 and H-5 to C-10 confirmed the placements of H-4 and H-5 at the peri position to C-10. Its spectral data and the assignments are in agreement with 1,3,6-trihydroxy-8-methylanthraquinone, which is also known as erythrolaccin (Xu *et al*., 2005).

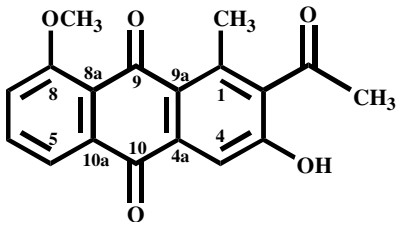



Major HMBC of **SA11**

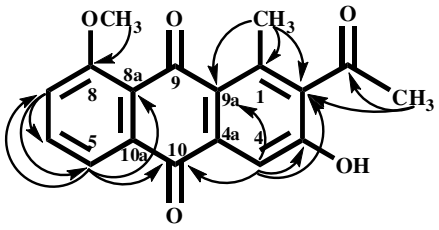
**Table 16** NMR spectral data of **SA11**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 165.3 (C) | - | - |
| 2 | 108.2 (CH) | 6.68 (*d*, *J* = 2.4 Hz, 1H) | C-1, C-4, C-9a |
| 3 | 134.9 (C) | - | - |
| 4 | 107.0 (CH) | 7.21 (*d*, *J* = 2.4 Hz, 1H) | C-2, C-9a, C-10 |
| 4a | 127.1 (C) | - | - |
| 5 | 112.2 (CH) | 7.61 (*d*, *J* = 2.4 Hz, 1H) | C-7, C-8a, C-10 |
| 6 | 145.4 (C) | - | - |
| 7 | 124.5 (CH) | 7.12 (*d*, *J* = 2.4 Hz, 1H) | C-8a |
| 8 | 137.3 (C) | - | - |
| 8a | 123.0 (C) | - | - |
| 9 | 189.0 (C=O) | - | - |
| 9a | 111.0 (C) | - | - |
| 10 | 182.4 (C=O) | - | - |
| 10a | 133.2 (C) | - | - |
| 1-OH | - | 13.34 (*s*, 1H) | C-1, C-2, C-9a |
| 6-OH | - | 10.25 (*s*, 1H) | - |
| 8-$CH_3$ | 23.1 ($CH_3$) | 2.80 (*s*, 3H) | C-7, C-8, C-8a |

**SA12: 2-acetyl-3-hydroxy-8-methoxy-1-methylanthraquinone**




**SA12** was a yellow solid, m.p. 203-204 °C. Its molecular formula of $C_{18}H_{14}O_5$ was established on the basis of mass spectrum, EIMS ($[M]^+$ *m/z* 310.0826). The UV spectrum showed specific absorptions with maxima at 223, 246, 257, 266, 273 and 372 nm. The IR spectrum exhibited absorption bands at 1702, 1664 and 1584 $cm^{-1}$ and the O-H stretching at 3328 $cm^{-1}$. The UV and IR spectral data revealed the anthraquinone derivative. The $^{13}C$ NMR resonances of quinone carbonyl carbons were shown at $\delta$ 188.2 (C-9) and 188.1 (C-10). The $^1H$ NMR spectra (**Table 17**) showed the signals due to a broad singlet of a hydroxyl proton at $\delta$ 9.98 (*br s*, 3-OH), a methoxyl group at $\delta$ 4.01 (*s*, 8-$OCH_3$), methyl protons $\delta$ 2.68 (*s*, 1-$CH_3$), and an isolated aromatic proton at $\delta$ 7.50 (*s*, H-4). The spectrum further showed ABM pattern of aromatic protons H-5 ($\delta$ 7.80, *dd*, *J* = 0.9, 7.8 Hz), H-6 ($\delta$ 7.64, *t*, *J* = 7.8 Hz) and H-7 ($\delta$ 7.32, *dd*, *J* = 0.9, 7.8 Hz). The presence of an acyl group was indicated from the proton resonance of methyl group at $\delta$ 2.58 (*s*) and carbon resonance of C=O at $\delta$ 209.7. The HMBC correlations of H-4 to C-10 and of H-5 to C-10 suggested that aromatic protons H-4 and H-5 were at the peri position to C-10. In addition, H-4 further showed HMBC correlation to C-3 suggesting that the hydroxyl group was *ortho* to H-4. Consequently the acyl group was located at C-2 due to the 3J HMBC correlations of -$CH_3$ and -$COCH_3$ to C-2 confirmed the assignment of 1-$CH_3$ and 2-$COCH_3$. The methoxyl group and methyl group were assigned at C-8 and C-1 from the HMBC correlations of -$OCH_3$ to C-8 and of -$CH_3$ to C-1, C-2 and C-9a. On this finding, the structure of compound **SA12** was determined as 2-acetyl-3-hydroxy-8-methoxy-1-methylanthraquinone. It is a new anthraquinone.

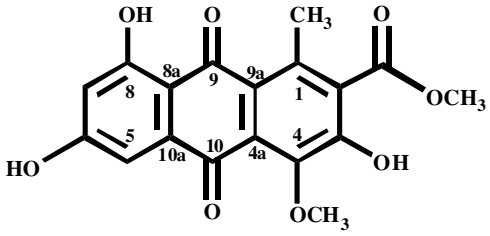



Major HMBC of **SA12**

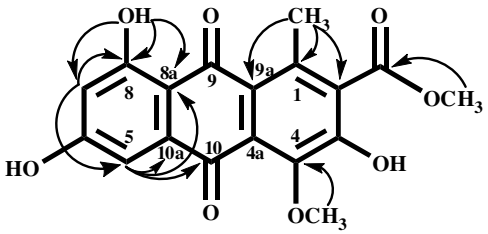
**Table 17** NMR spectral data of **SA12**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 143.4 (C) | - | |
| 2 | 140.6 (C) | - | |
| 3 | 160.8 (C) | - | |
| 4 | 115.2 (CH) | 7.50 (*s*, 1H) | C-2, C-3, C-9a, C-10 |
| 4a | 138.6 (C) | - | - |
| 5 | 122.9 (CH) | 7.80 (*dd*, *J* = 0.9, 7.8 Hz, 1H) | C-7, C-8a, C-10 |
| 6 | 137.9 (CH) | 7.64 (*t*, *J* = 7.8 Hz, 1H) | C-8 |
| 7 | 122.3 (CH) | 7.32 (*dd*, *J* = 0.9, 7.8 Hz, 1H) | C-5, C-6 |
| 8 | 163.5 (C) | - | - |
| 8a | 127.6 (C) | - | - |
| 9 | 188.2 (C=O) | - | - |
| 9a | 130.7 (C) | - | - |
| 10 | 188.1 (C=O) | - | - |
| 10a | 139.4 (C) | - | - |
| 1-$CH_3$ | 22.8 ($CH_3$) | 2.68 (*s*, 3H) | C-1, C-9a |
| 2-$\underline{CO}CH_3$ | 209.7 (C=O) | - | - |
| 2-$CO\underline{CH_3}$ | 32.9 ($CH_3$) | 2.58 (*s*, 3H) | C-2, 2-$\underline{CO}CH_3$ |
| 3-OH | - | 9.98 (*br s*, 1H) | - |
| 8-$OCH_3$ | 60.4 ($OCH_3$) | 4.01 (*s*, 3H) | C-8 |

**SA13: 3,6,8-trihydroxy-4-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester**




**SA13** was an orange solid. m.p. 209-211 °C. It showed a molecular ion peak at *m/z* 358.0692 corresponding to a molecular formula of $C_{18}H_{14}O_8$. The UV spectrum exhibited the absorption bands at 216, 224, 285, 351 and 429 nm, suggesting an anthraquinone skeleton. The IR spectrum showed the stretching bands of O-H at 3390 $cm^{-1}$ and C=O at 1713, 1630 $cm^{-1}$. The $^1H$ and $^{13}C$ NMR spectral data **(Table 18)** were closely related to those of **SA7**, except for the signals of the methoxy protons of **SA7** at $\delta$ 3.99 (3-$OCH_3$) and of aromatic proton H-6 at $\delta$ 7.62 (*t*) were replaced by hydroxyl groups. The $^1H$ NMR spectrum showed the sharp singlet signal of a chelated hydroxyl proton at $\delta$ 13.05 (8-OH), a broad singlet signal of 3-OH ($\delta$ 9.30), 6-OH ($\delta$ 8.50), two doublet signals with *meta* coupling constant of H-5 at $\delta$ 7.23 and H-7 at $\delta$ 6.71. The spectrum further showed signals of methyl protons ($\delta$ 2.77, *s*), a methoxyl group ($\delta$ 3.99, *s*) and a methoxyl ester ($\delta$ 4.03, *s*). The same as **SA6**, the methyl group (1-$CH_3$) and carboxylic function (2-$COOCH_3$) were next to each other according to the biosynthesis of the anthraquinones from polyketide precursor (Wiley *et al*., 1980). The quinone structure was deduced from the low field carbon resonances at $\delta$ 188.3 (C-9) and 182.7 (C-10). The remaining carbon resonances were the resonances of a methyl carbon at $\delta$ 20.7, two methoxy carbons at $\delta$ 62.2 and 52.9, two methine carbons at $\delta$ 108.8 and 107.5, and five quaternary carbons at $\delta$ 135.8, 128.1, 127.9, 124.7 and 111.7. From this finding, the structure of compound **SA13** was determined as a new anthraquinone, 3,6,8-trihydroxy-4-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester.

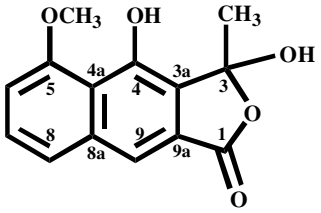



Major HMBC of **SA13**

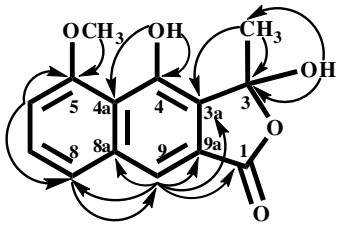
**Table 18** NMR spectral data of **SA13**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 137.8 (C) | - | - |
| 2 | 135.8 (C) | - | - |
| 3 | 152.9 (C) | - | - |
| 4 | 146.7 (C) | - | - |
| 4a | 128.1 (C) | - | - |
| 5 | 107.5 (CH) | 7.23 (*d*, *J* = 2.4 Hz, 1H) | C-7, C-8a, C-10 |
| 6 | 163.9 (C) | - | - |
| 7 | 108.8 (CH) | 6.71 (*d*, *J* = 2.4 Hz, 1H) | C-5, C-8, C-8a |
| 8 | 164.9 (C) | - | - |
| 8a | 111.7 (C) | - | - |
| 9 | 188.3 (C=O) | - | - |
| 9a | 124.7 (C) | - | - |
| 10 | 182.7 (C=O) | - | - |
| 10a | 127.9 (C) | - | - |
| 3-OH | - | 9.30 (*br*, 1H) | - |
| 6-OH | - | 8.50 (*br*, 1H) | - |
| 8-OH | - | 13.05 (*s*, 1H) | C-7, C-8, C-8a |
| 2-$\underline{CO_2}CH_3$ | 167.6 (C=O) | - | - |
| 1-$CH_3$ | 20.7 ($CH_3$) | 2.77 (*s*, 3H) | C-1, C-2, C-9a |
| 4-$OCH_3$ | 62.2 ($OCH_3$) | 3.99 (*s*, 3H) | C-4 |
| 2-$CO_2\underline{CH_3}$ | 52.9 ($OCH_3$) | 4.03 (*s*, 3H) | 2-$\underline{CO_2}CH_3$ |

**SA14: 3,4-dihydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one**




**SA14** was obtained as brown needles, m.p. 167-169 °C. Its molecular formula of $C_{14}H_{12}O_5$ was established on the basis of mass spectrum, EIMS ($[M]^+$ *m/z* 260.0679). The UV spectrum exhibited the maximum absorptions at 226, 243, 266, 313, 347 and 362 nm. The IR spectrum showed the absorption bands of a hydroxyl group at 3371 $cm^{-1}$ and a carbonyl group at 1718 $cm^{-1}$. The $^1H$ NMR spectra **(Table 19)** showed the signals of 4-OH at $\delta$9.99, 3-OH at $\delta$6.60, 3-$CH_3$ at $\delta$1.98 and 5-$OCH_3$ at $\delta$4.22, an aromatic proton H-9 appeared as singlet at $\delta$7.80, whereas H-6, H-7, H-8 resonated as ABM pattern at $\delta$ 7.20, $\delta$ 7.53 and $\delta$ 7.70, respectively. The locations of H-9 and H-8 were assigned from the $^3J$ correlations of H-9 to C-8 and of H-8 to C-9. The correlations of 3-OH to C-3, C-3a and 3-$CH_3$ and of 4-OH to C-3a, C-4 and C-4a confirmed the position of 3-OH and 4-OH. The HMBC correlations of H-8 to C-8a and of H-9 to C-8a and to C-1 suggested that H-9 was in between C-8 and CO-1, whereas the correlations of -$CH_3$ to C-3a and of 4-OH to C-3a and to C-4a indicated that 4-OH and -$CH_3$ were in close proximity. The carbon at $\delta$167.9 was assigned for a carbonyl carbon of ester group of lactone. Therefore **SA14** was assigned to be 3,4-dihydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one, a new naphthalene derivative.

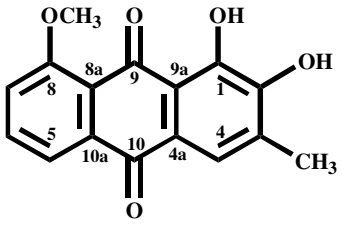



Major HMBC of **SA14**

**Table 19** NMR spectral data of **SA14**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 167.9 (C=O) | - | - |
| 3 | 106.0 (C) | - | - |
| 3a | 127.0 (C) | - | - |
| 4 | 151.0 (C) | - | - |
| 4a | 117.0 (C) | - | - |
| 5 | 158.0 (C) | - | - |
| 6 | 106.8 (CH) | 7.20 (*d, J* = 7.8 Hz, 1H) | C-4a, C-5, C-8 |
| 7 | 127.5 (CH) | 7.53 (*t, J* = 7.8 Hz, 1H) | C-8a |
| 8 | 123.0 (CH) | 7.70 (*d, J* = 7.8 Hz, 1H) | C-4a, C-9 |
| 8a | 137.9 (C) | - | - |
| 9 | 115.1 (CH) | 7.80 (*s,* 1H) | C-1, C-3a, C-4a, C-8, C-8a, C-9[a] |
| 9a | 125.0 (C) | - | - |
| 3-OH | - | 6.60 (*s,* 1H) | C-3, 1-$CH_3$ |
| 3-$CH_3$ | 24.4 ($CH_3$) | 1.98 (*s,* 3H) | C-3, C-3a |
| 4-OH | - | 9.99 (*s,* 1H) | C-3a, C-4, C-4a |
| 5-$OCH_3$ | 56.2 ($OCH_3$) | 4.22 (*s,* 3H) | C-5 |

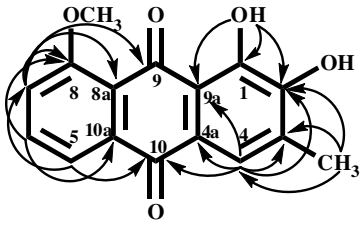
**SA15: 1,2-dihydroxy-8-methoxy-3-methylanthraquinone**




**SA15** was an orange solid. The UV spectrum exhibited the absorption bands at 231, 244, 267, 273 and 362 nm. The IR spectrum showed the O-H stretching at 3372 $cm^{-1}$ and the C=O stretching at 1653 and 1622 $cm^{-1}$. The $^{13}C$ NMR spectra **(Table 20)** exhibited the resonances of two carbonyl carbons of quinone moiety at $\delta$ 194.2 and 186.7 and they were assigned for C-9 and C-10, respectively. The $^{1}H$ NMR spectral data showed the signals of a chelated hydroxyl proton (1-OH) at $\delta$ 13.18, an aromatic proton (H-4) at $\delta$ 7.63 (*s*), the ABM pattern of aromatic protons H-5, H-6 and H-7 at $\delta$ 7.95 (*dd*, *J* = 0.9, 7.8 Hz), 7.74 (*t*, *J* = 7.8 Hz) and 7.34 (*dd*, *J* = 0.9, 7.8 Hz) and singlet signals of the methoxyl group at $\delta$ 4.07 and of methyl group at $\delta$ 2.36. The HMBC correlations of -$OCH_3$ to C-8 and of -$CH_3$ to C-2, C-3 and C-4 confirmed that the position of methoxyl group at C-8 and methyl group at C-3, respectively. The chelated hydroxyl group was placed at C-1 due to the HMBC correlation of OH to C-1, C-2 and C-9a, whereas an aromatic proton H-4 was located at C-4 from the HMBC correlation of H-4 to C-2, C-9a, C-10 and 3-$CH_3$. The HMBC correlations of H-5 to C-10 and of H-4 to C-4a, C-10 indicated that H-4 and H-5 were on the same side of an anthraquinone structure. The $^{13}C$ NMR spectrum further showed four quaternary carbons at $\delta$ 128.7 (C-4a), 125.8 (C-8a), 120.5 (C-9a) and 141.5 (C-10a). From this data, **SA15** was concluded to be 1,2-dihydroxy-8-methoxy-3-methylanthraquinone (Tietze *et al*., 2006).

**Table 20** NMR spectral data of **SA15**

| Position | $\delta_C$ (C-Type) | $\delta_H$ (multiplicity) | HMBC |
|---|---|---|---|
| 1 | 154.0 (C) | - | - |
| 2 | 154.6 (C) | - | - |
| 3 | 135.8 (C) | - | - |
| 4 | 127.4 (CH) | 7.63 (*s*, 1H) | C-2, C-9a, C-10, 3-$CH_3$ |
| 4a | 128.7 (C) | - | - |
| 5 | 125.3 (CH) | 7.95 (*dd*, *J* = 0.9, 7.8 Hz, 1H) | C-7, C-10 |
| 6 | 141.0 (CH) | 7.74 (*t*, *J* = 7.8 Hz, 1H) | C-8, C-10a |
| 7 | 122.8 (CH) | 7.34 (*dd*, *J* = 0.9, 7.8 Hz, 1H) | C-5, C-8, C-8a, C-9 |
| 8 | 165.9 (C) | - | - |
| 8a | 125.8 (C) | - | - |
| 9 | 194.2 (C=O) | - | - |
| 9a | 120.5 (C) | - | - |
| 10 | 186.7 (C=O) | - | - |
| 10a | 141.5 (C) | - | - |
| 1-OH | - | 13.18 (*s*, 1H) | C-1, C-2, C-9a |
| 3-$CH_3$ | 61.7 ($OCH_3$) | 2.36 (*s*, 3H) | C-2, C-3, C-4 |
| 8-$OCH_3$ | 21.2 ($CH_3$) | 4.07 (*s*, 3H) | C-8 |




Major HMBC of **SA15**

## Conclusion

Investigation of the constituents from the bulb of *E. americana* led to the isolation of five new compounds : two anthraquinones; 2-acetyl-3-hydroxy-8-methoxy-1-methylanthraquinone (**SA12**) and 3,6,8-trihydroxy-4-methoxy-1-methyl anthraquinone-2-carboxylic acid methyl ester (**SA13**), two naphthoquinones; [8-methoxy-1,4-dioxo-3-(2-oxo-propyl)-1,4-dihydronaphthalen-2-yl]acetic acid methyl ester (**SA9**) and 2-[2′-(acetyloxy)propyl]-3-hydroxy-5-methoxy-1,4-naphthoquinone (**SA10**) and one naphthalene derivative; 3,4-dihydroxy-5-methoxy-3-methylnaphtho [2,3-*c*]furan-1(3*H*)-one (**SA14**) together with ten known compounds; 4-hydroxy-5-methoxy-3-methylnaphtho[2,3-*c*]furan-1(3*H*)-one (**SA1**), (1*R*,3*S*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA2**), (1*R*,3*R*)3,4-dihydro-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA3**), (1*R*,3*R*)5,10-dihydroxy-9-methoxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-4(3*H*)-one (**SA4**), (1*R*,3*S*) 3,4-dihydro-9-hydroxy-1,3-dimethyl-1*H*-naphtho[2,3-*c*]pyran-5,10-dione (**SA5**), 4,8-dihydroxy-3-methoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA6**), 8-hydroxy-3,4-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**SA7**), 1,2,4,4a-tetrahydro-6-methoxy-2-methyl-10*H*-naphtho[2',3':2,3]cyclobuta[1,2-*b*] furan-5,10(3a*H*)-dione (**SA8**) 1,3,6-trihydroxy-8-methylanthraquinone (**SA11**) and 1,2-dihydroxy-8-methoxy-3-methylanthraquinone (**SA15**).

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# APPENDIX

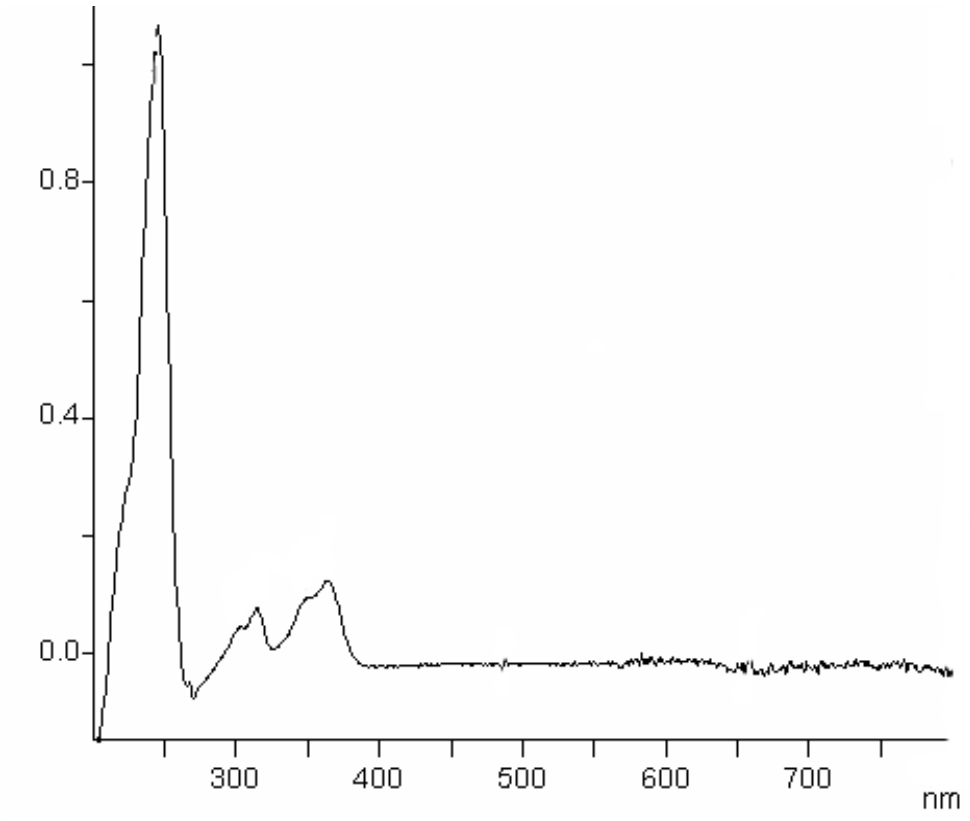


**Figure 2** UV ($CH_3OH$) spectrum of **SA1**

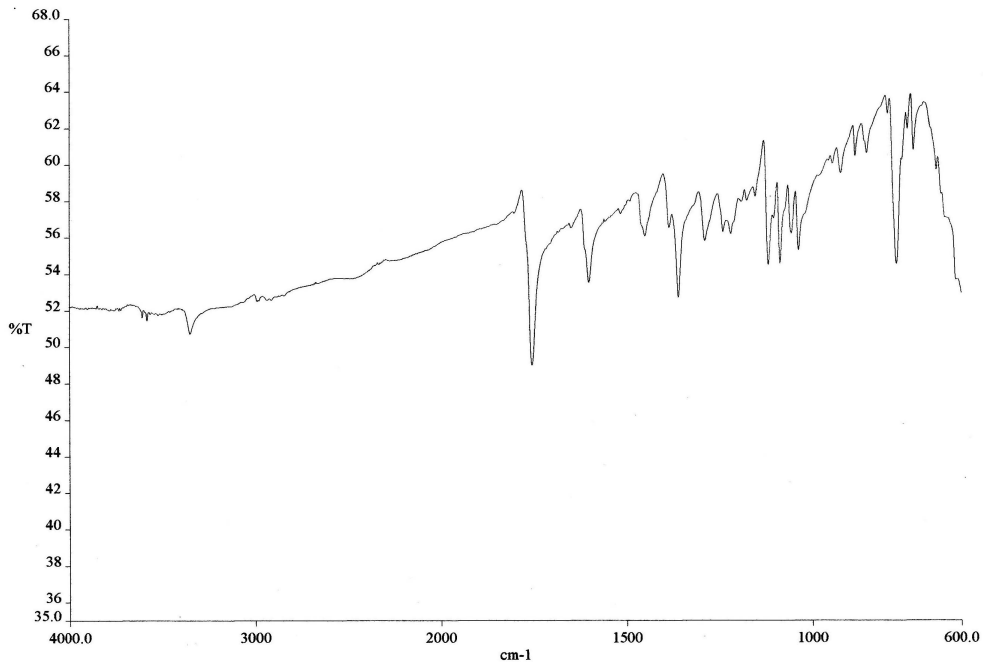


**Figure 3** FT-IR (Neat) spectrum of **SA1**

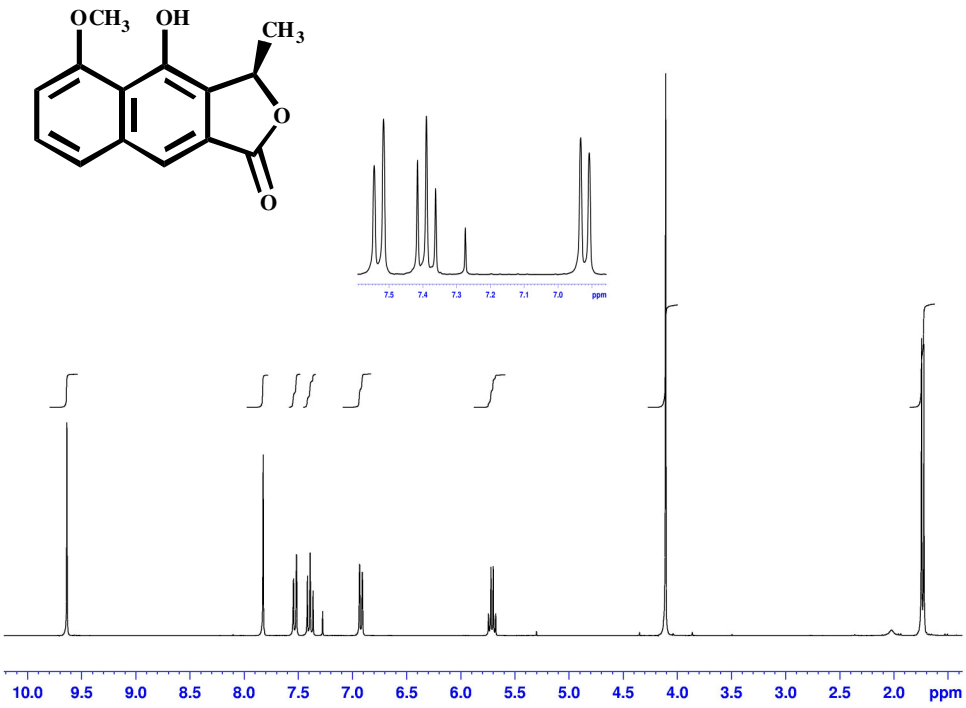


**Figure 4** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA1**

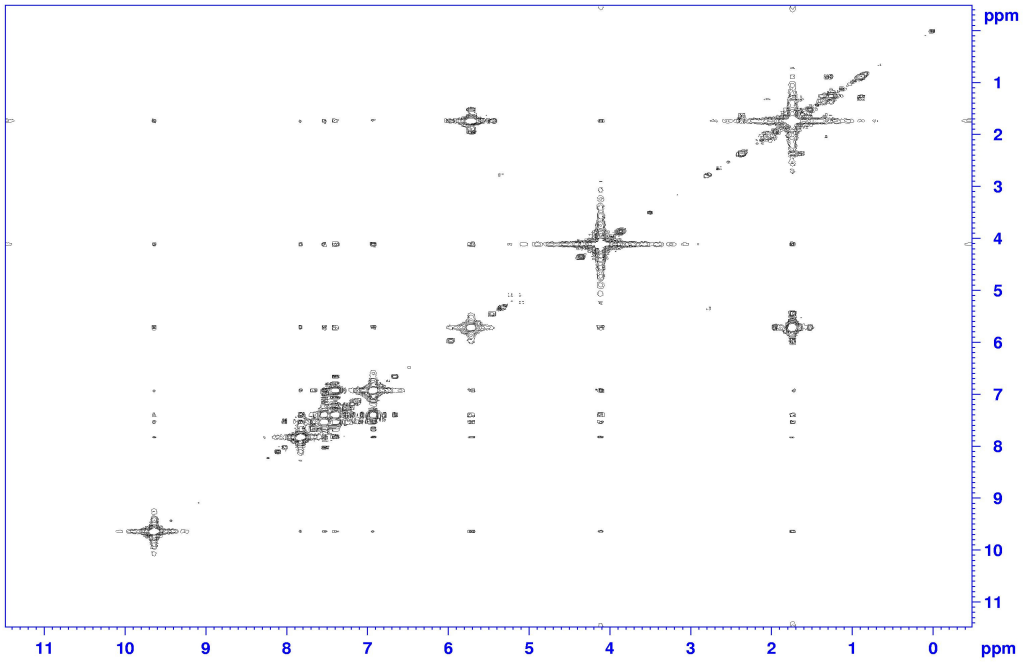


**Figure 5** $^{1}$H-$^{1}$H COSY spectrum of **SA1**

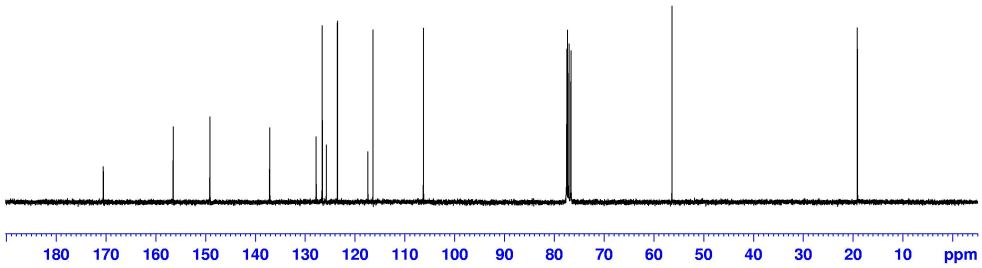


**Figure 6** $^{13}C$ NMR (75 MHz) ($CDCl_3$) spectrum of **SA1**

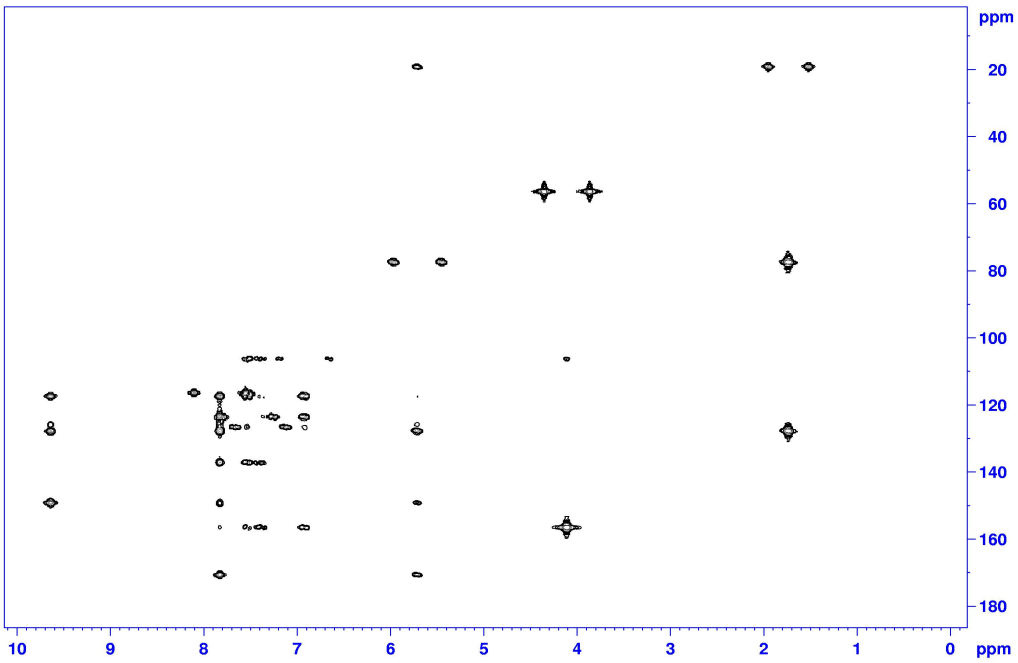


**Figure 7** 2D HMBC spectrum of **SA1**

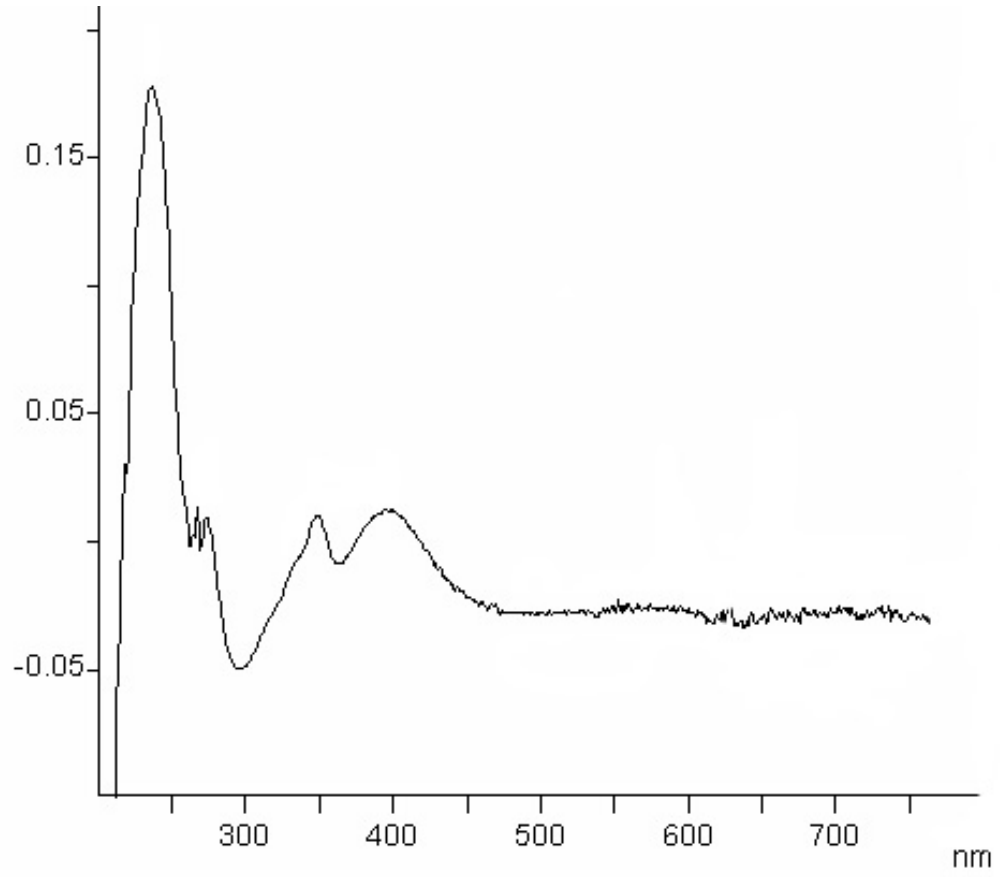


**Figure 8** UV ($CH_3OH$) spectrum of **SA2**

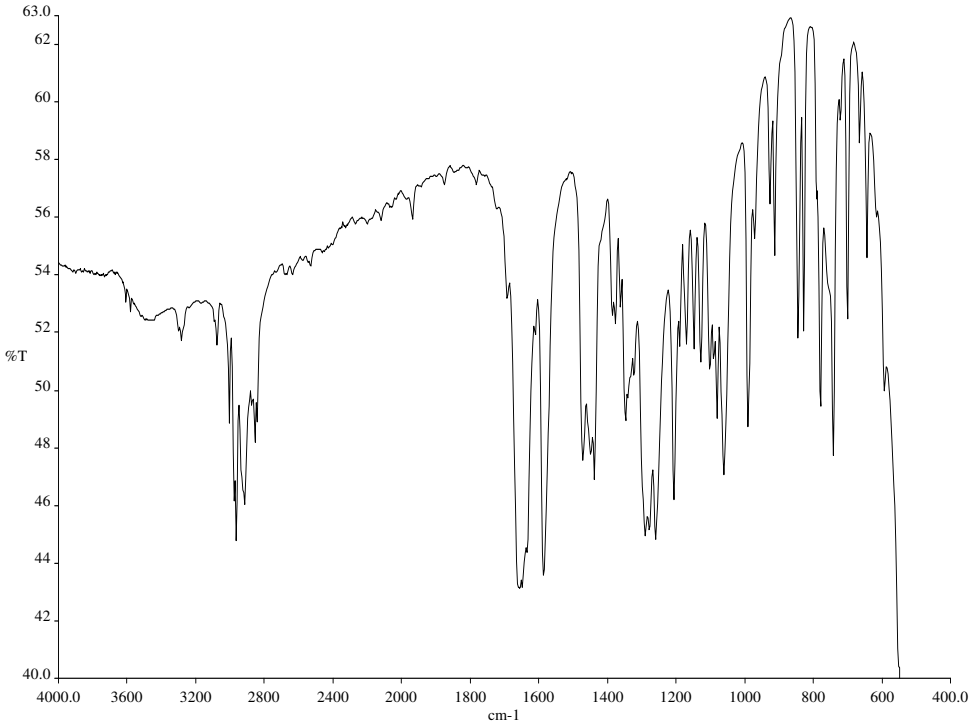


**Figure 9** FT-IR (Neat) spectrum of **SA2**

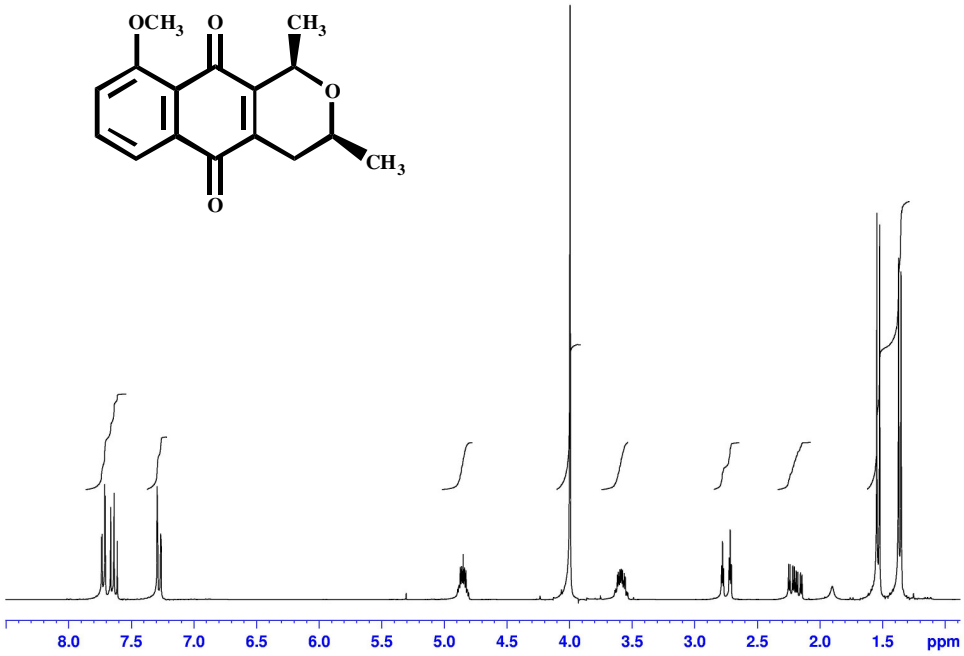


**Figure 10** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA2**

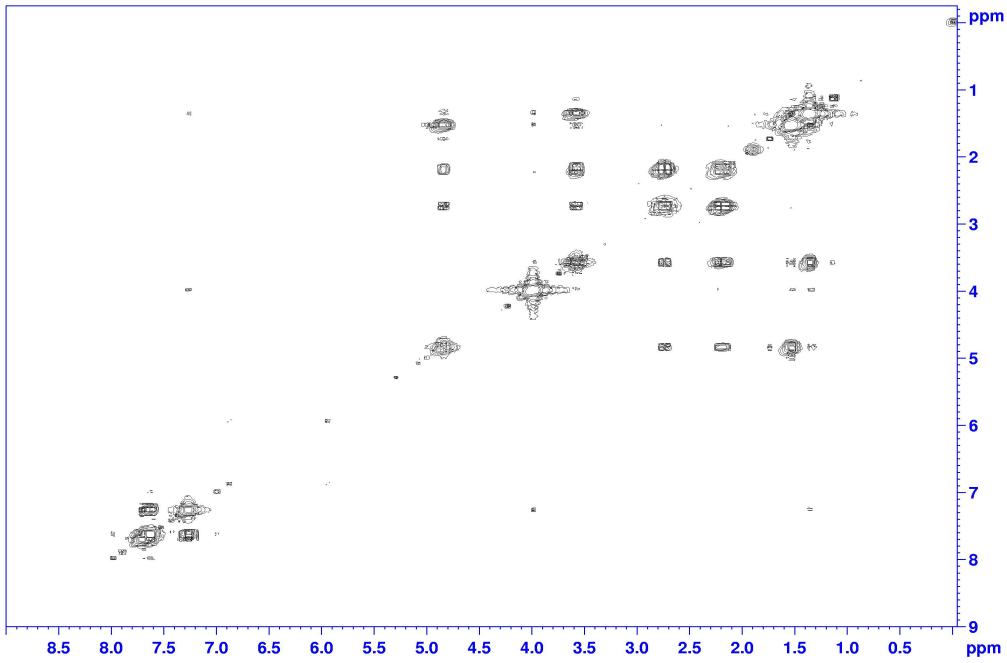


**Figure 11** $^{1}$H-$^{1}$H COSY spectrum of **SA2**

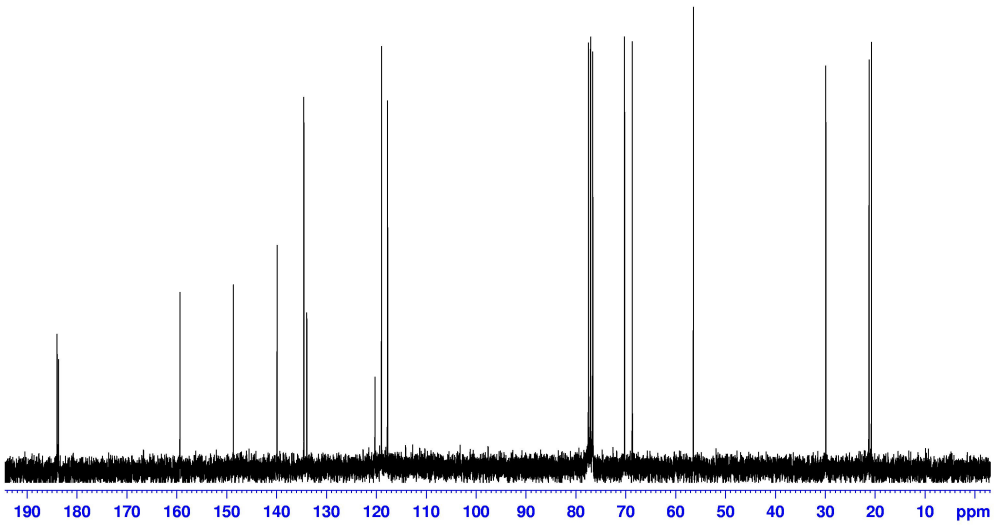


**Figure 12** $^{13}C$ NMR (75 MHz) ($CDCl_3$) spectrum of **SA2**

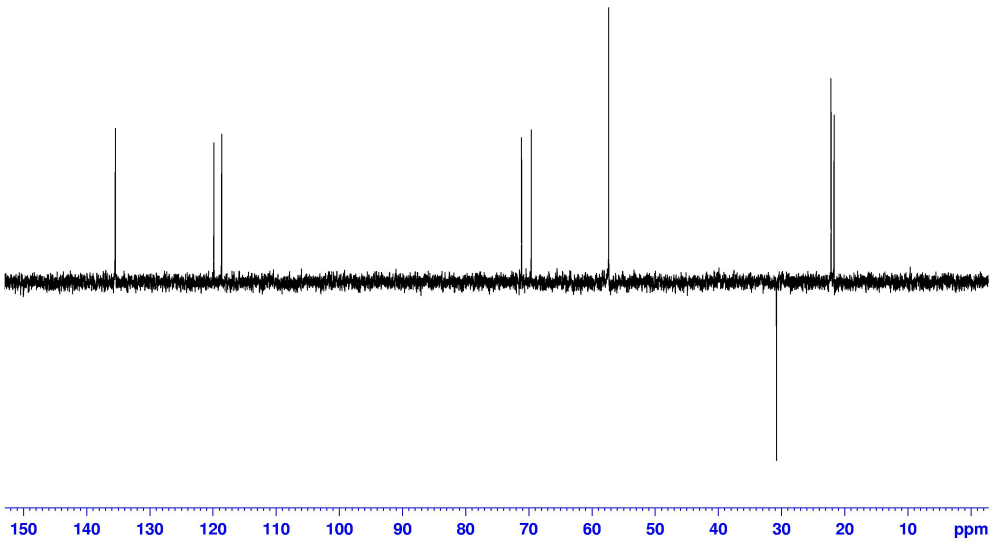


**Figure 13** DEPT 135° ($CDCl_3$) spectrum of **SA2**

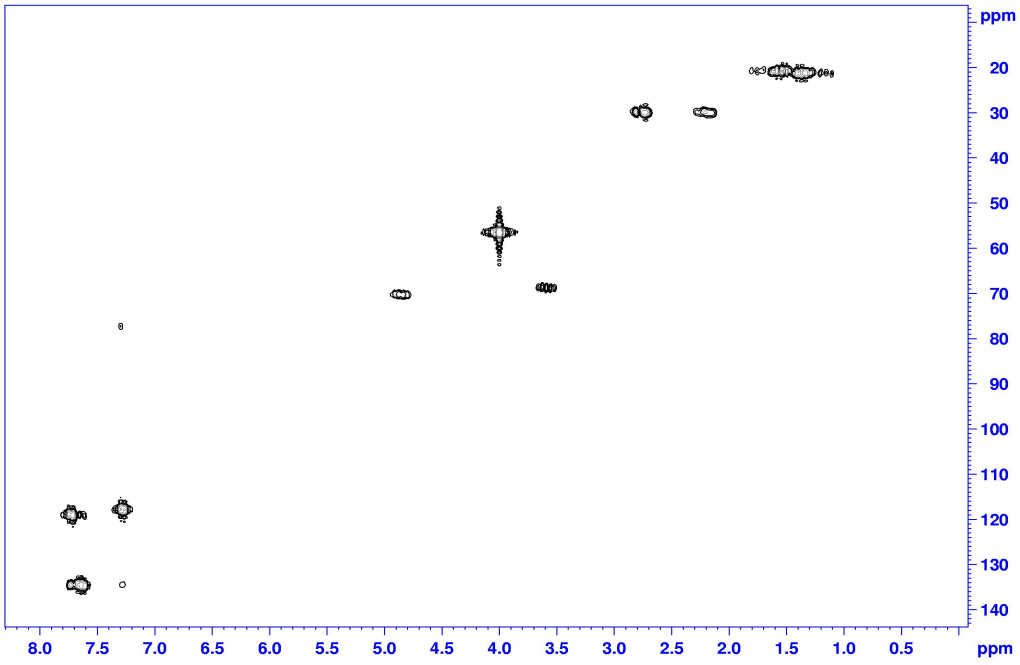


**Figure 14** 2D HMQC spectrum of **SA2**

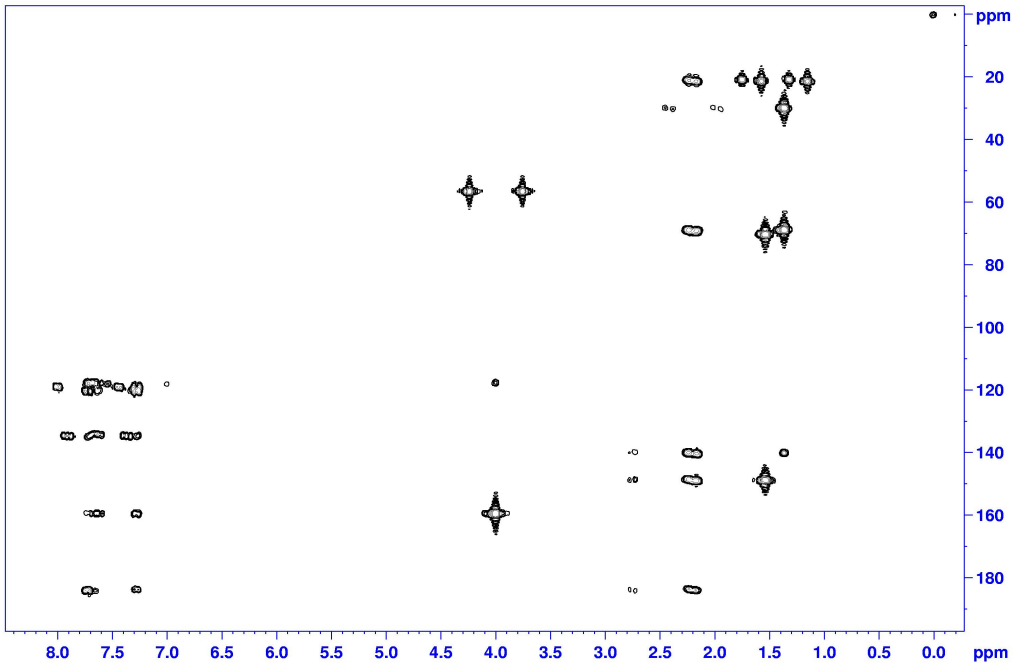


**Figure 15** 2D HMBC spectrum of **SA2**

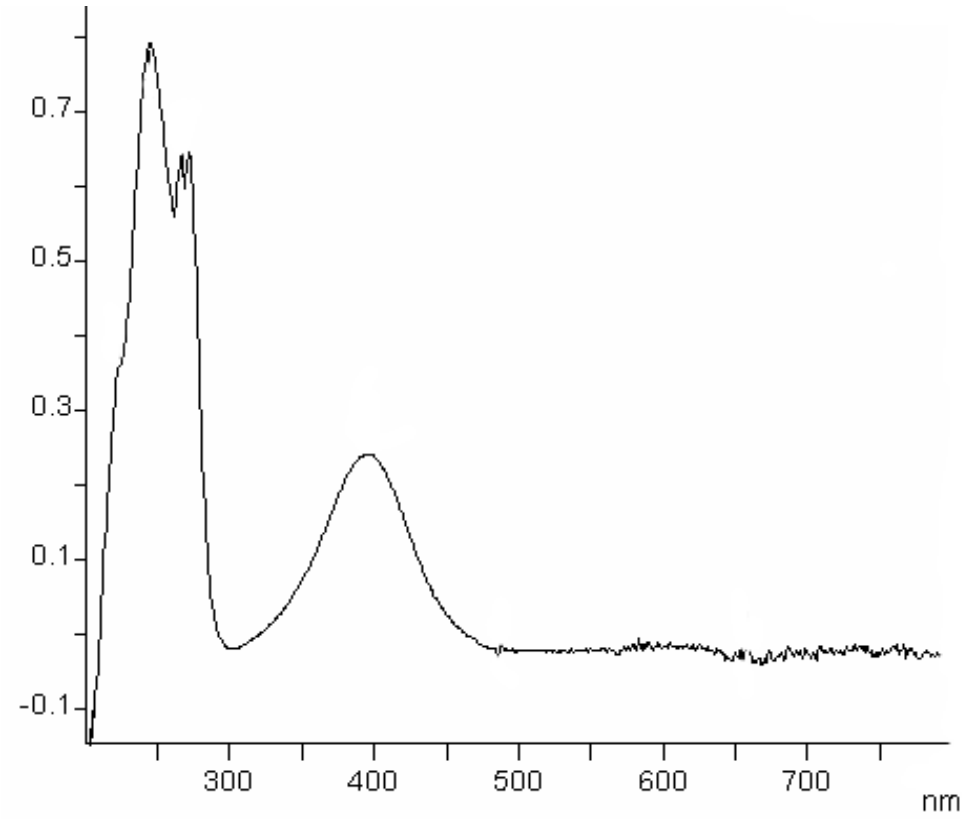


**Figure 16** UV ($CH_3OH$) spectrum of **SA3**

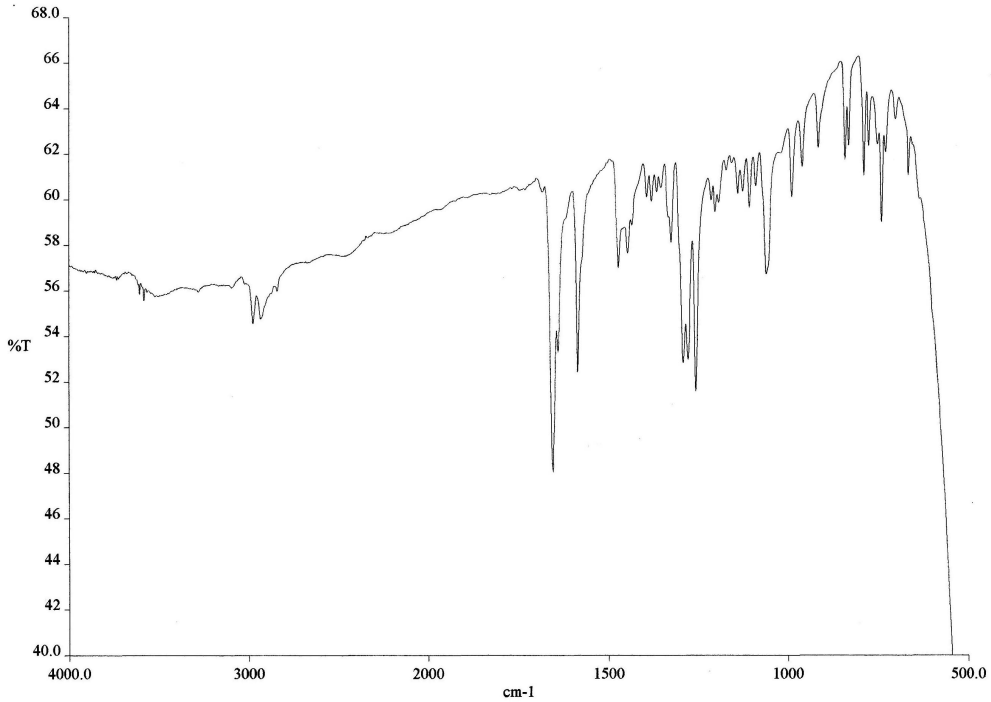


**Figure 17** FT-IR (Neat) spectrum of **SA3**

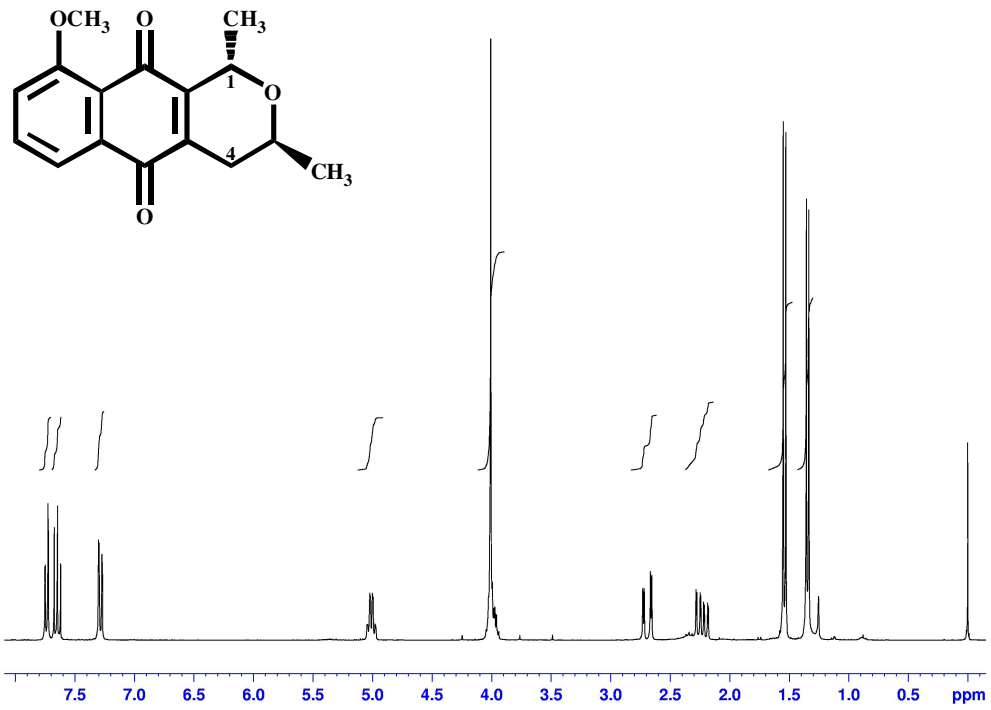


**Figure 18** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA3**

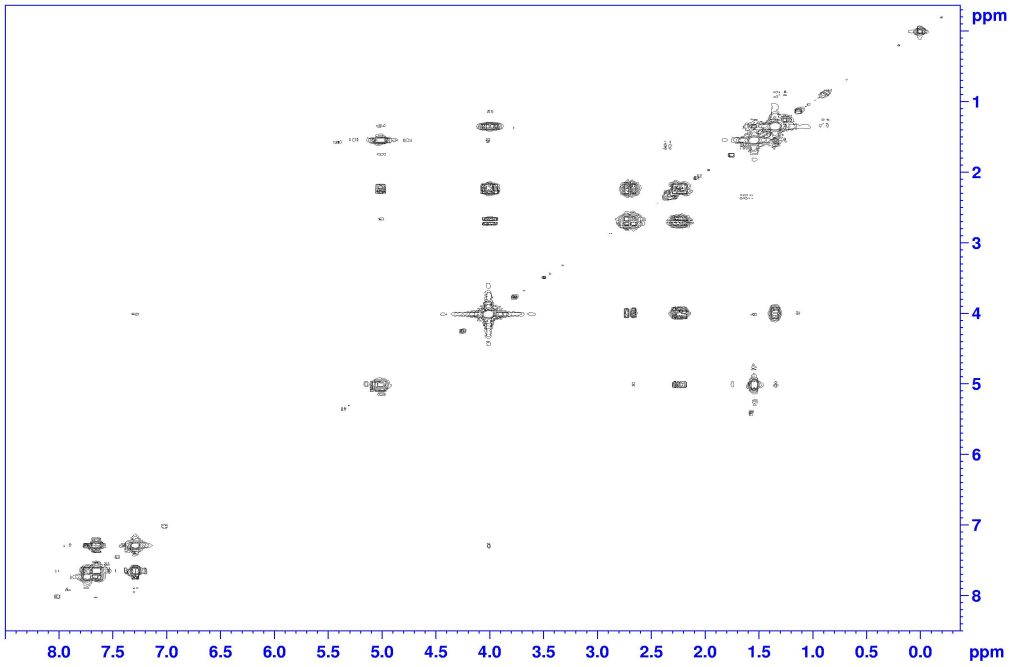


**Figure 19** $^{1}$H-$^{1}$H COSY spectrum of **SA3**

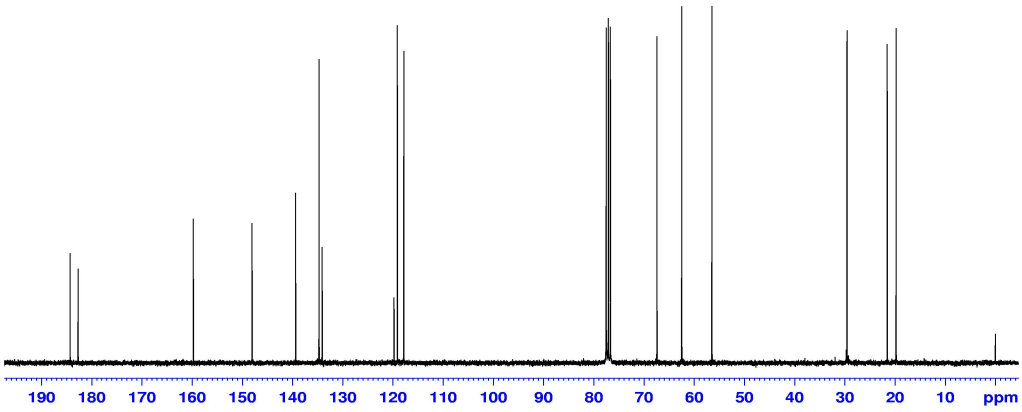


**Figure 20** $^{13}C$ NMR (75 MHz) ($CDCl_3$) spectrum of **SA3**

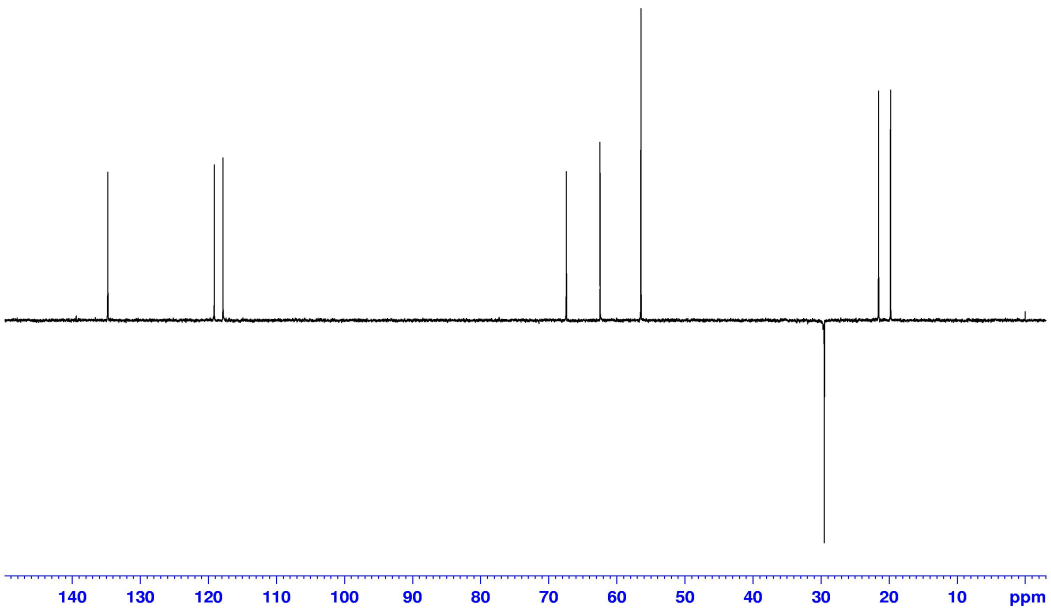


**Figure 21** DEPT 135° ($CDCl_3$) spectrum of **SA3**

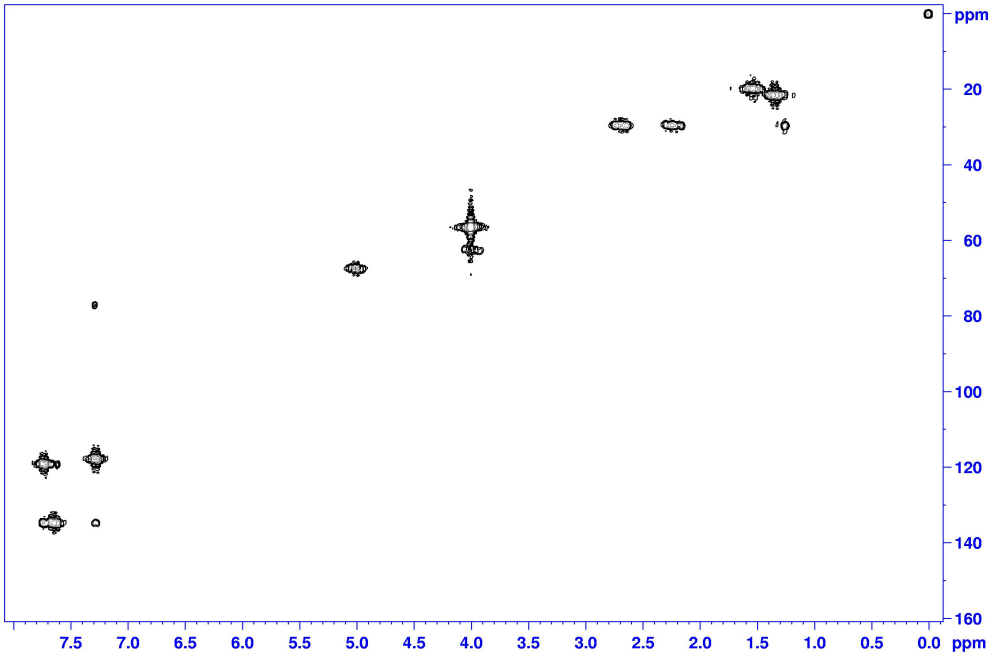


**Figure 22** 2D HMQC spectrum of **SA3**

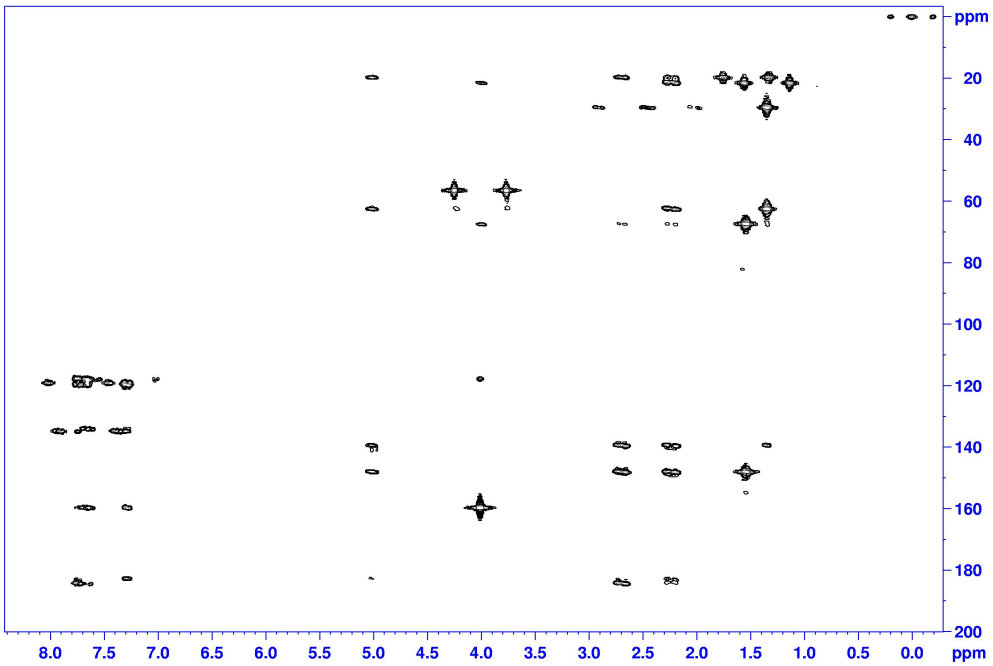


**Figure 23** 2D HMBC spectrum of **SA3**

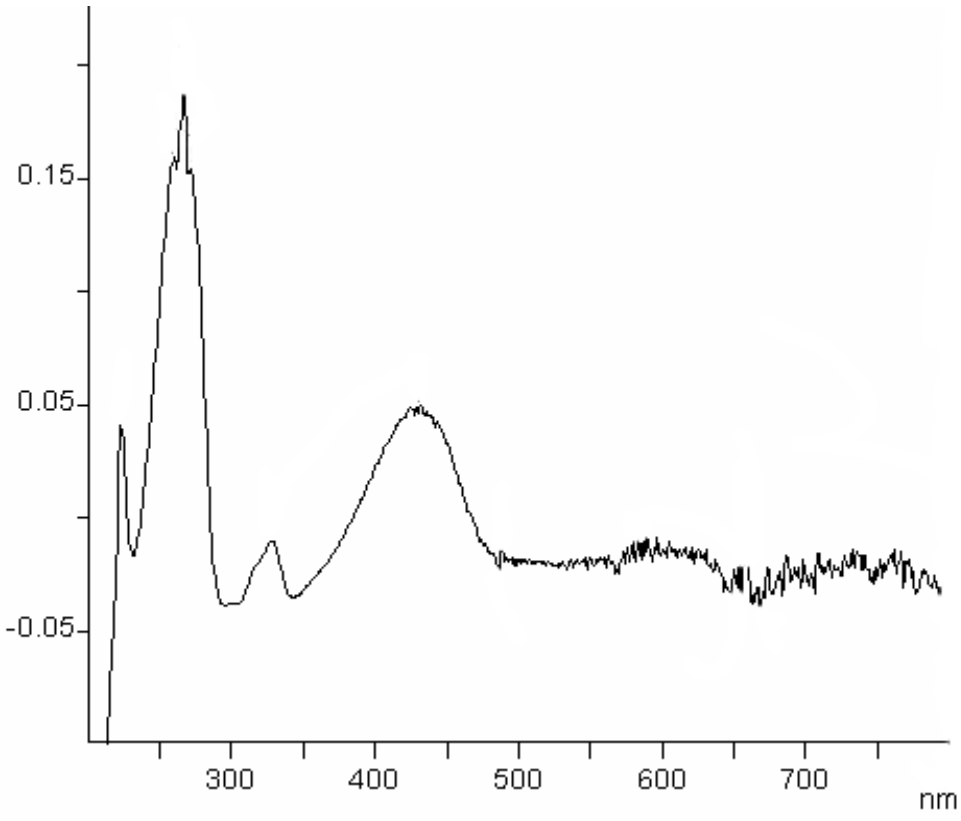


**Figure 24** UV ($CH_3OH$) spectrum of **SA4**

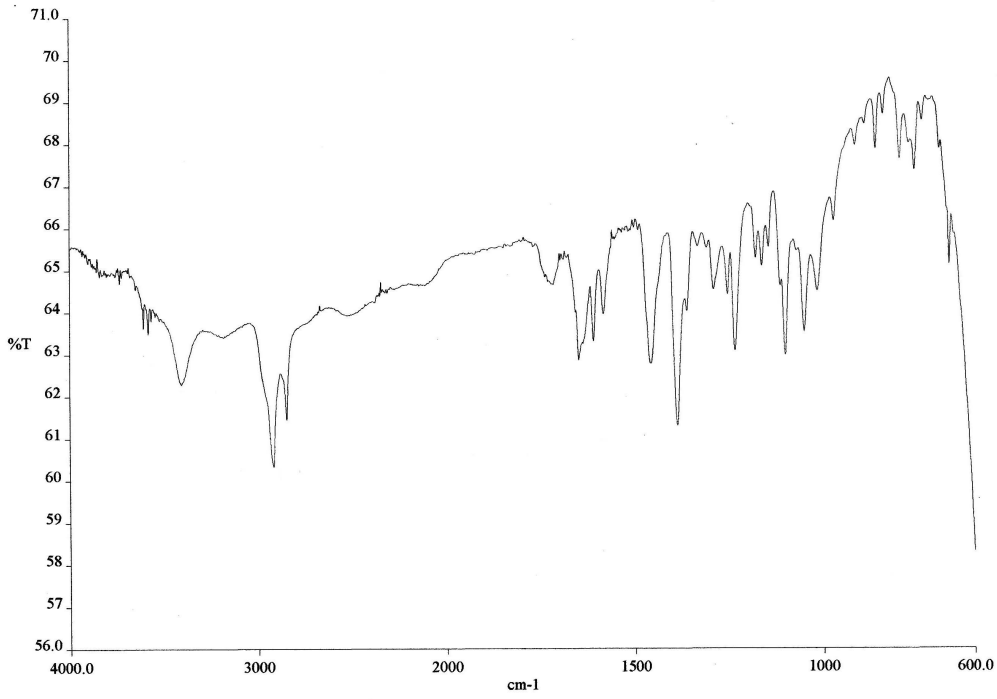


**Figure 25** FT-IR (Neat) spectrum of **SA4**

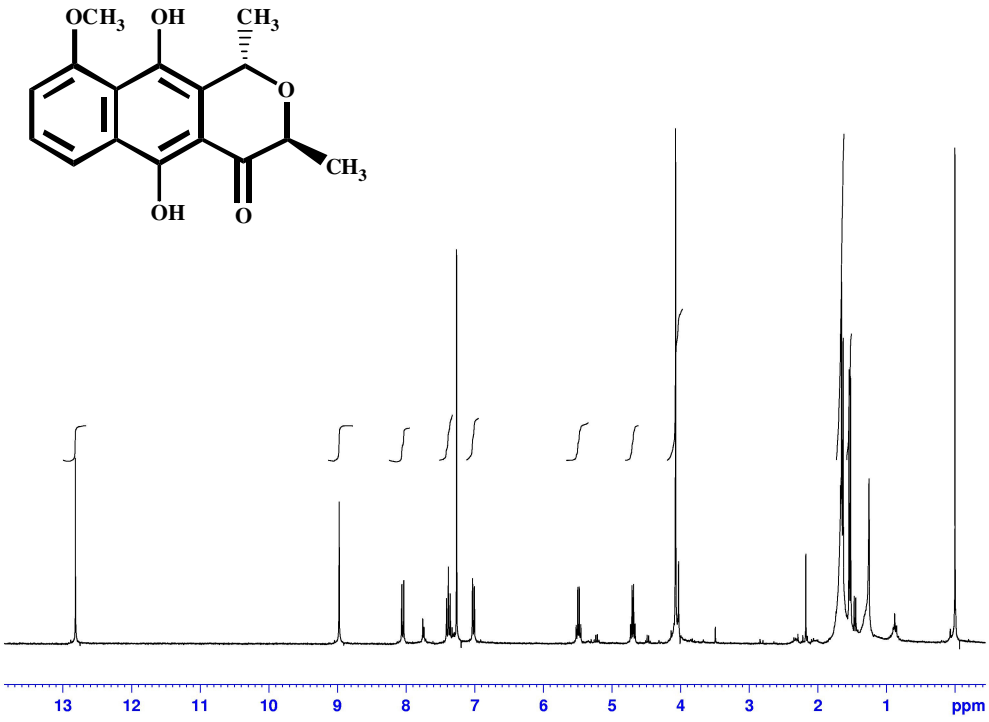


**Figure 26** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA4**

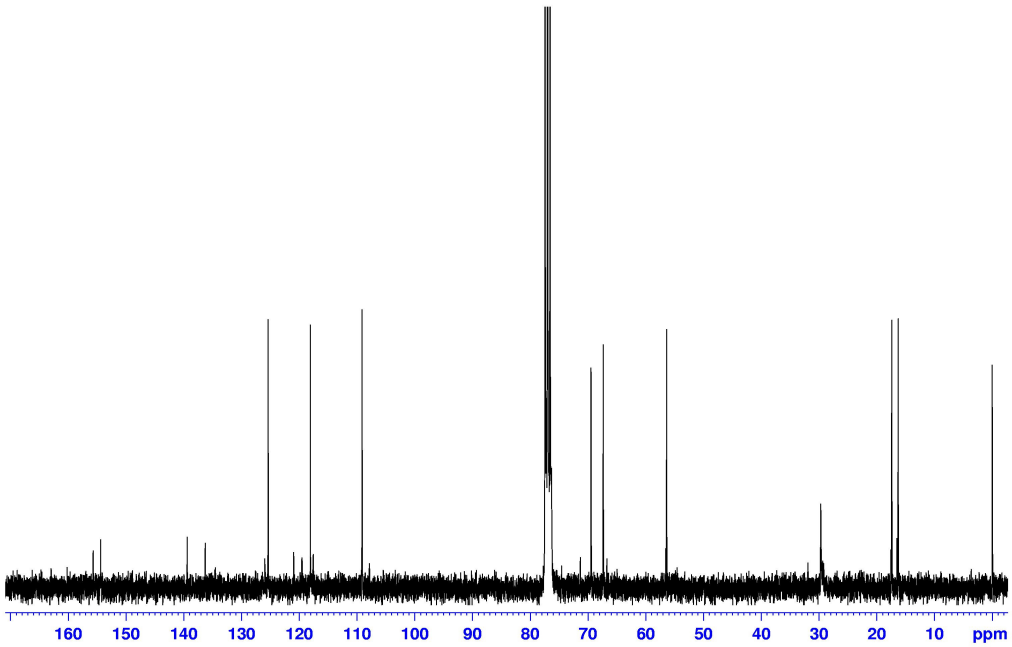


**Figure 27** $^{13}$C NMR (300 MHz) ($CDCl_3$) spectrum of **SA4**

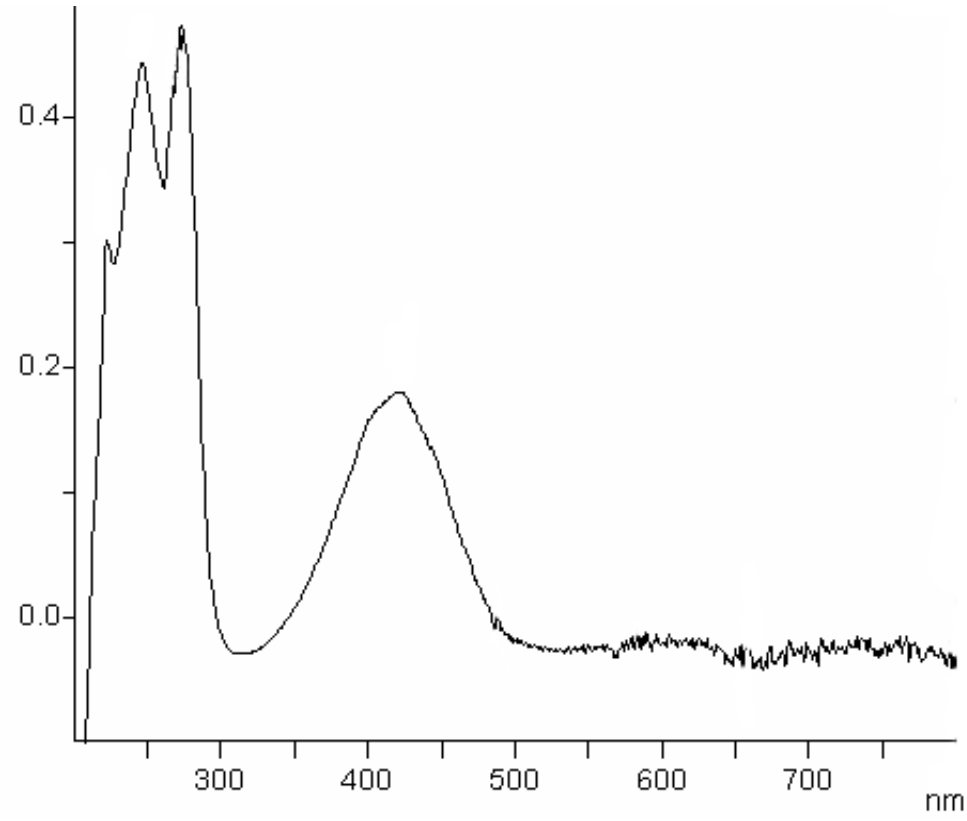


**Figure 28** UV ($CH_3OH$) spectrum of **SA5**

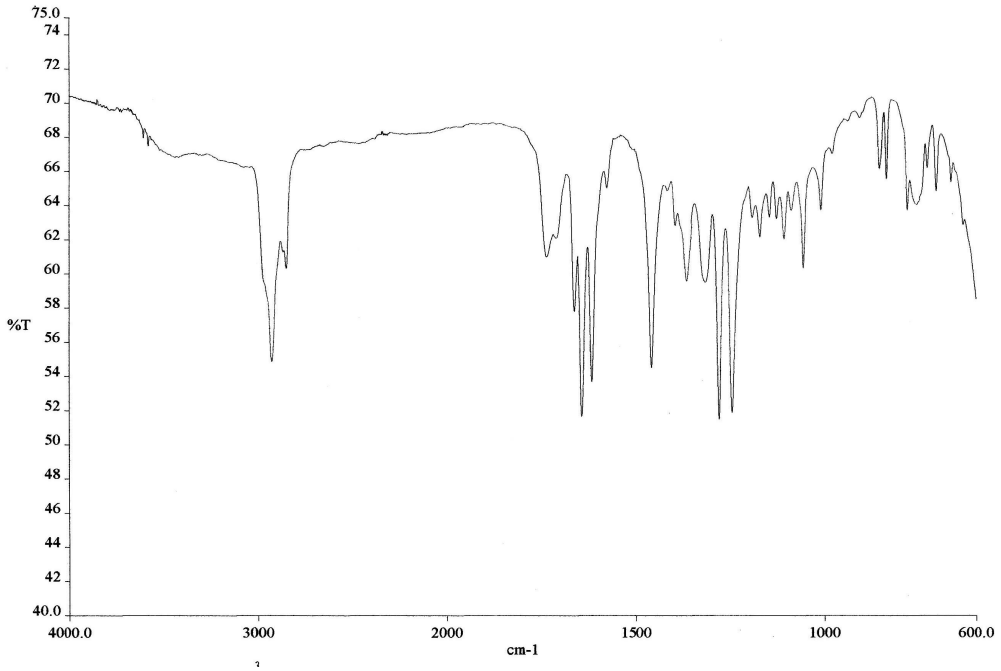


**Figure 29** FT-IR (Neat) spectrum of **SA5**

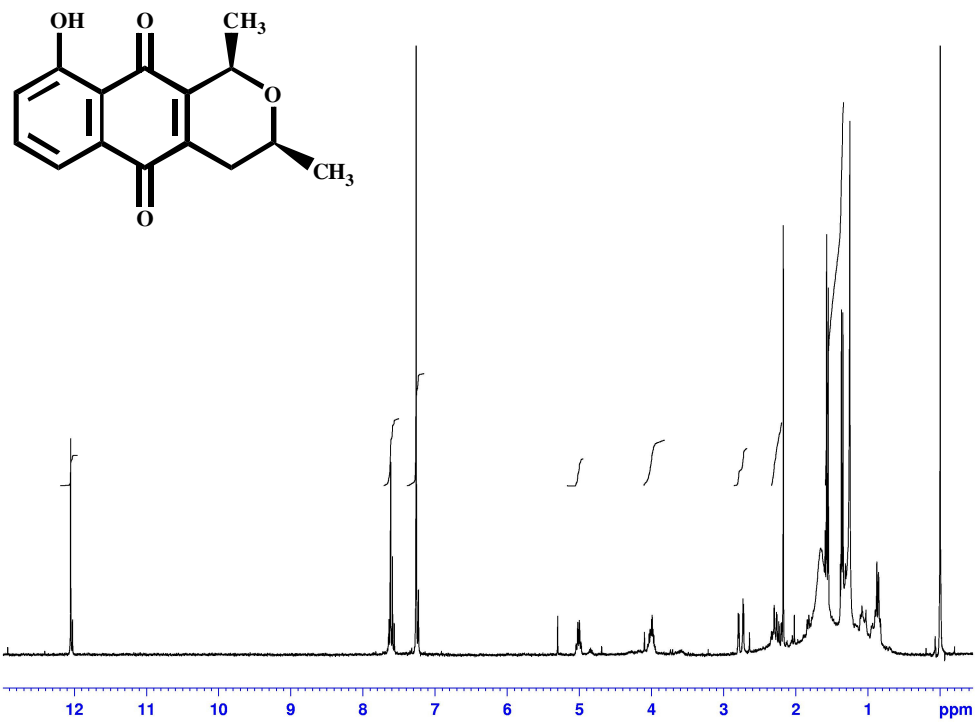


**Figure 30** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA5**

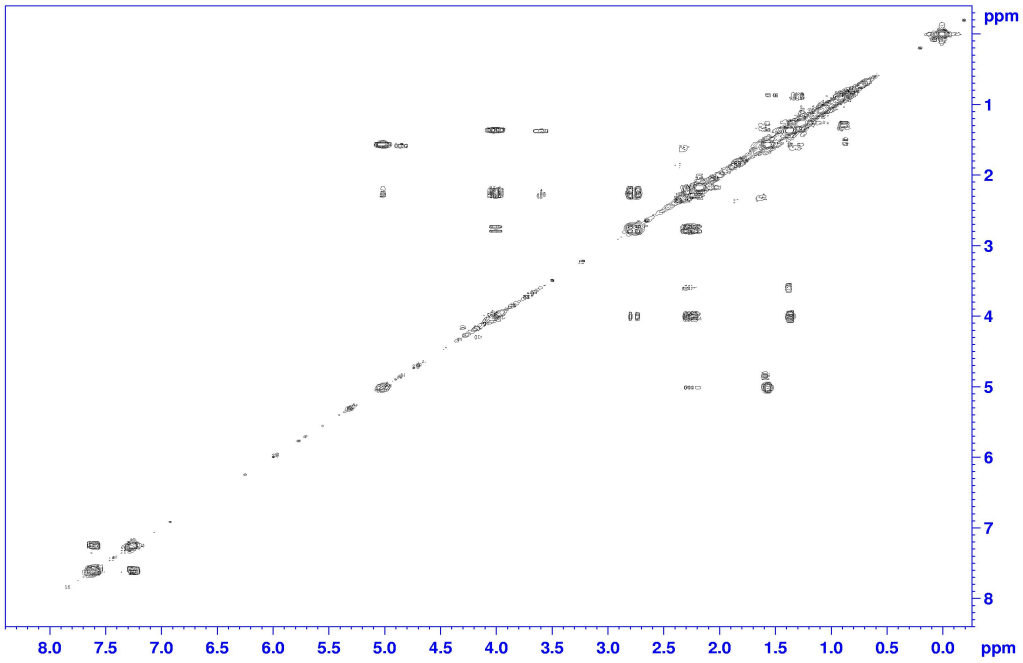


**Figure 31** $^{1}$H-$^{1}$H COSY spectrum of **SA5**

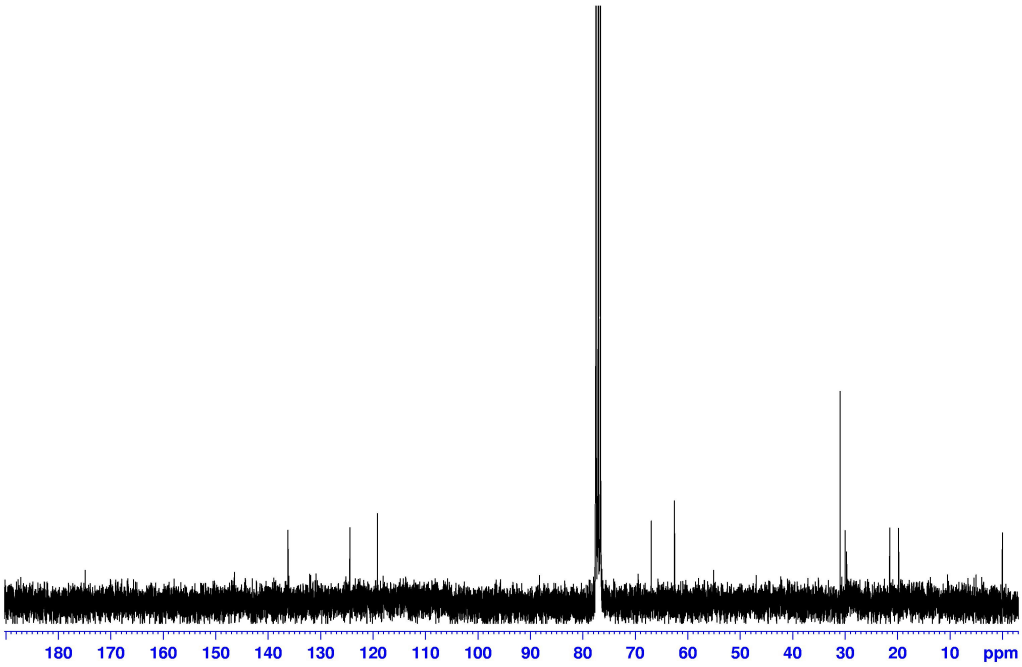


**Figure 32** $^{13}C$ NMR (75 MHz) ($CDCl_3$) spectrum of **SA5**

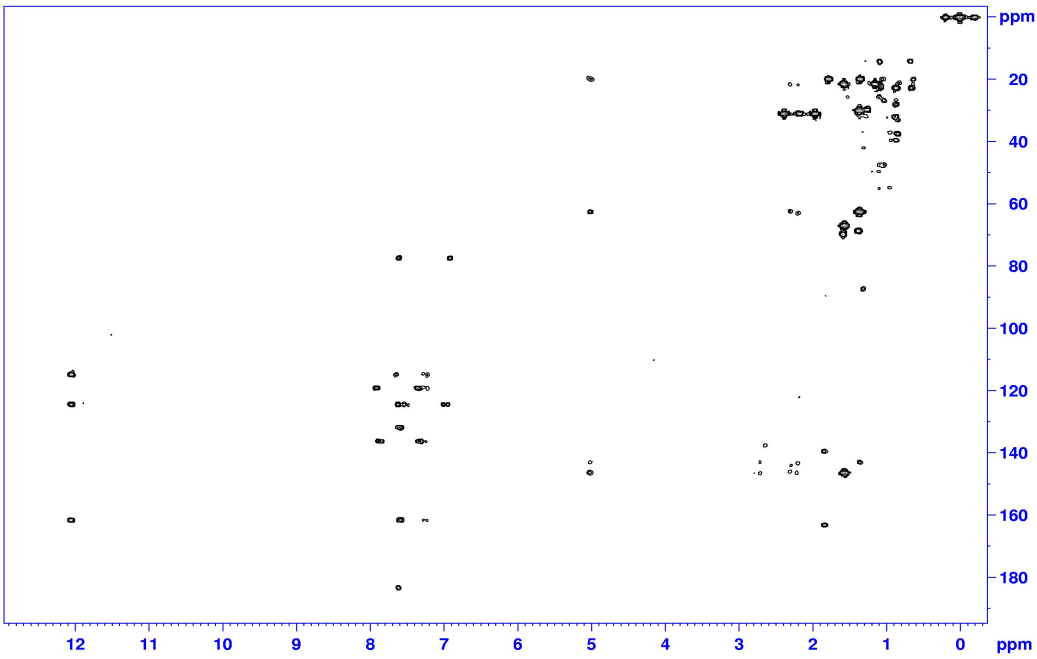


**Figure 33** 2D HMBC spectrum of **SA5**

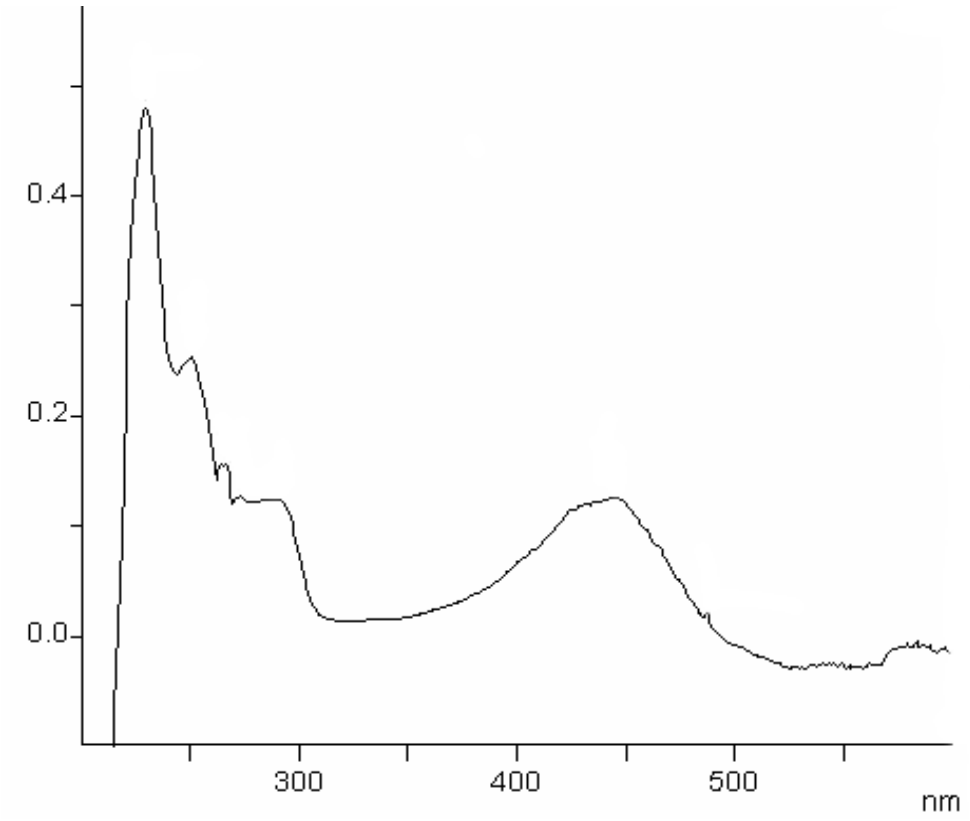


**Figure 34** UV ($CH_3OH$) spectrum of **SA6**

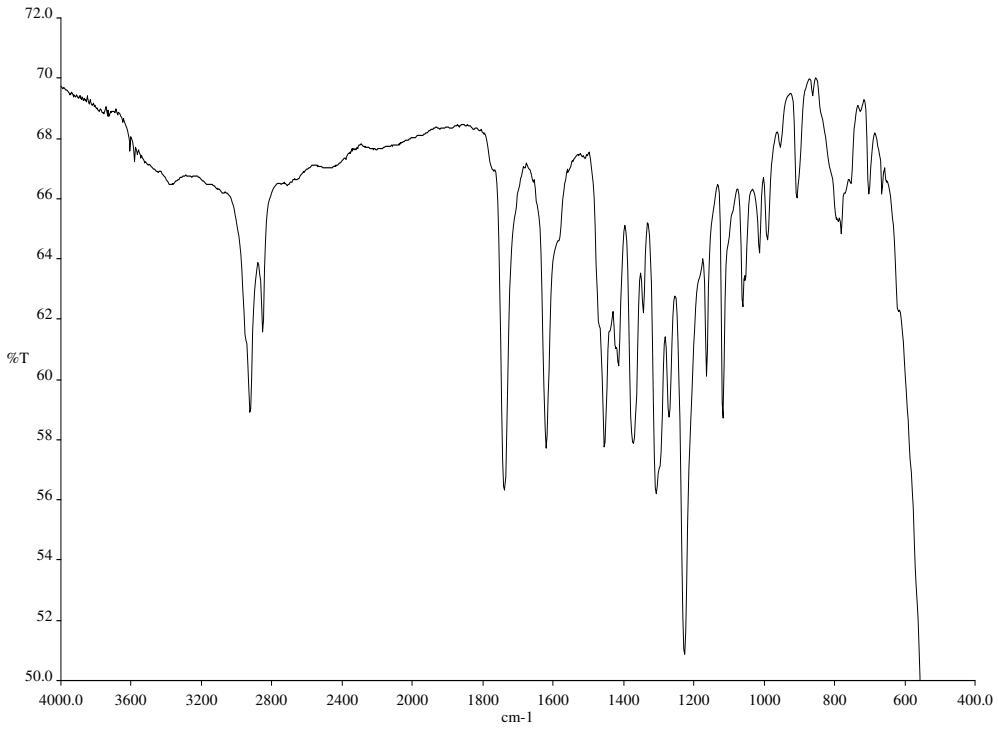


**Figure 35** FT-IR (Neat) spectrum of **SA6**

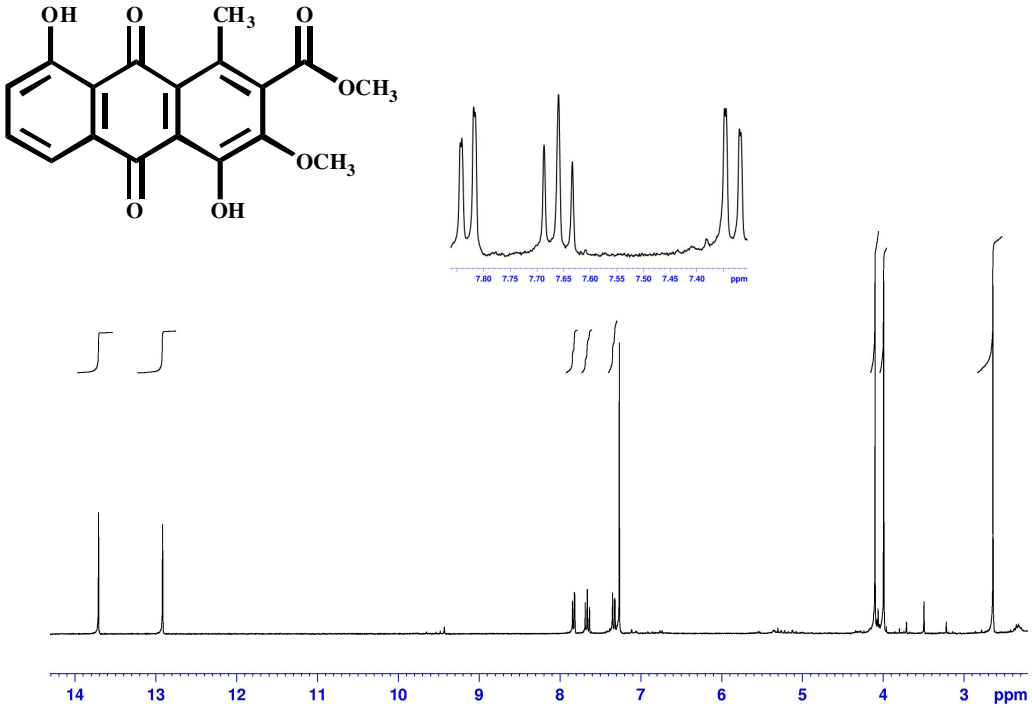


**Figure 36** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA6**

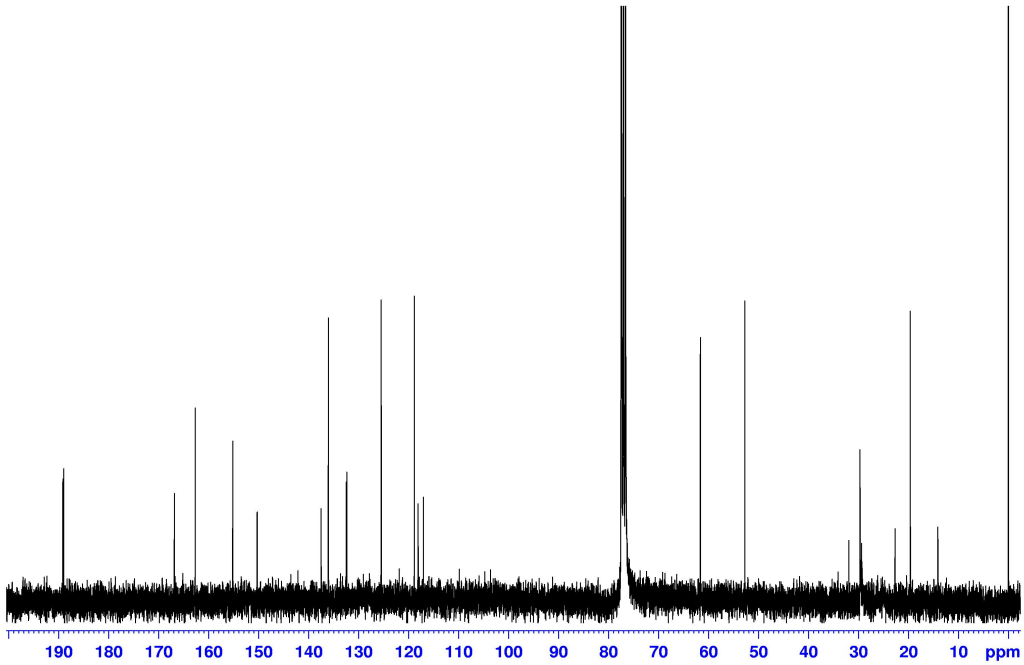


**Figure 37** $^{13}$C NMR (75 MHz) ($CDCl_3$) spectrum of **SA6**

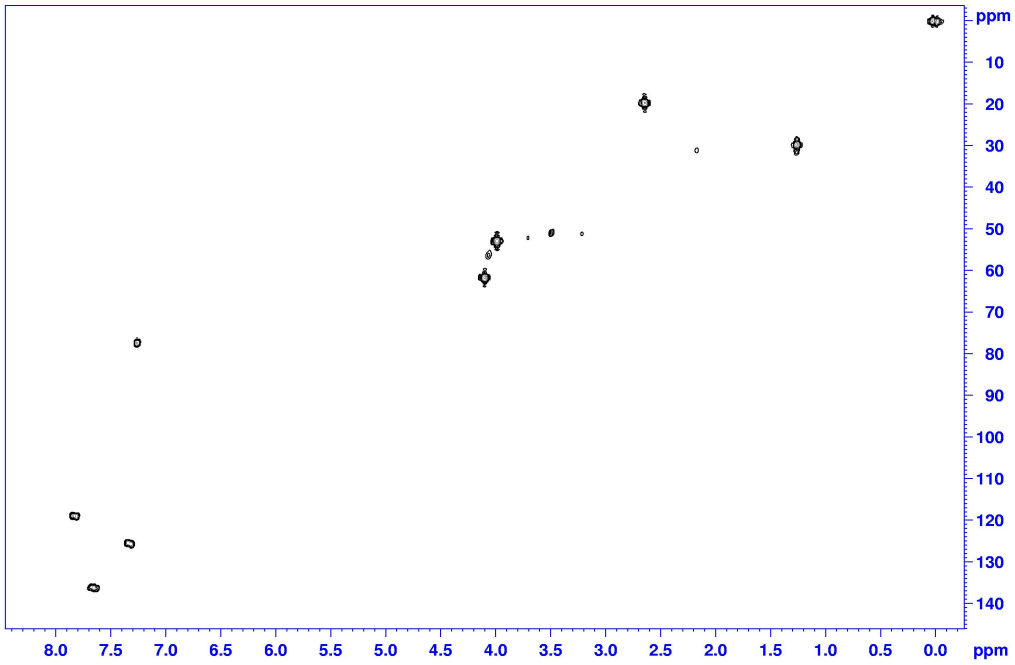


**Figure 38** 2D HMQC spectrum of **SA6**

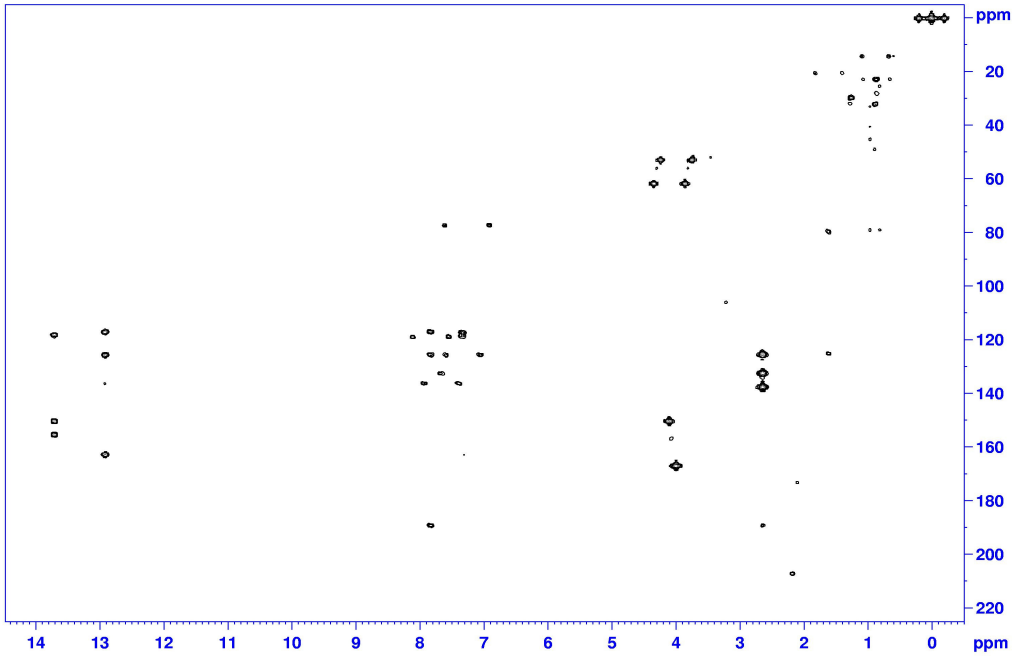


**Figure 39** 2D HMBC spectrum of **SA6**

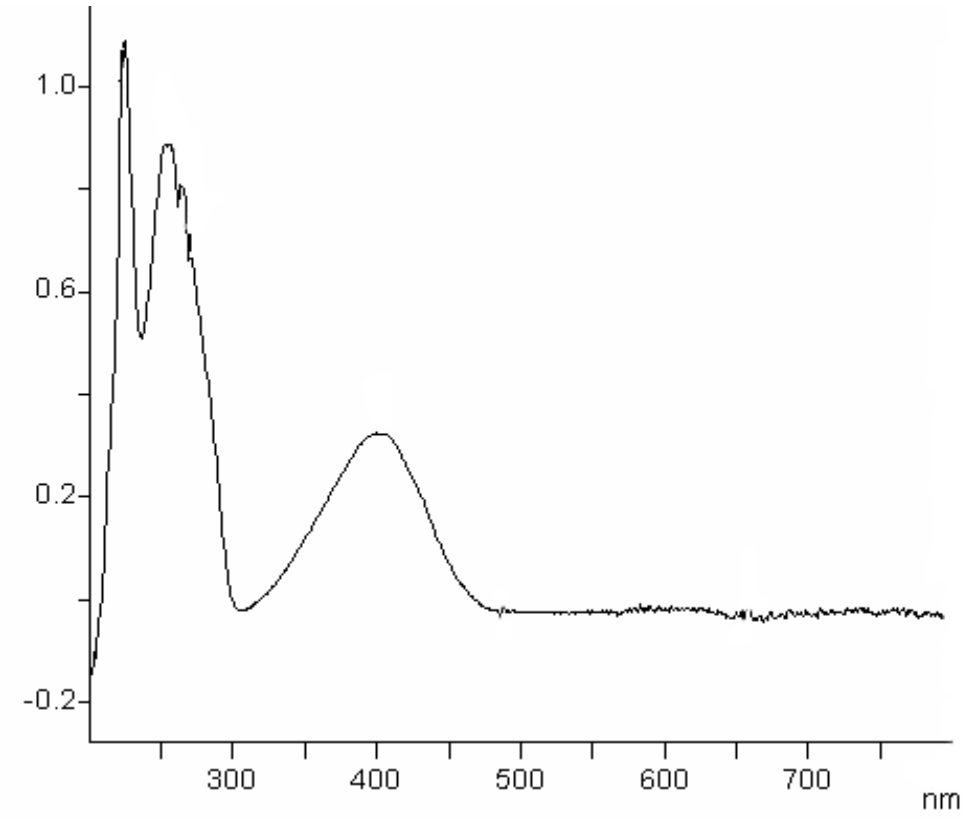


**Figure 40** UV ($CH_3OH$) spectrum of **SA7**

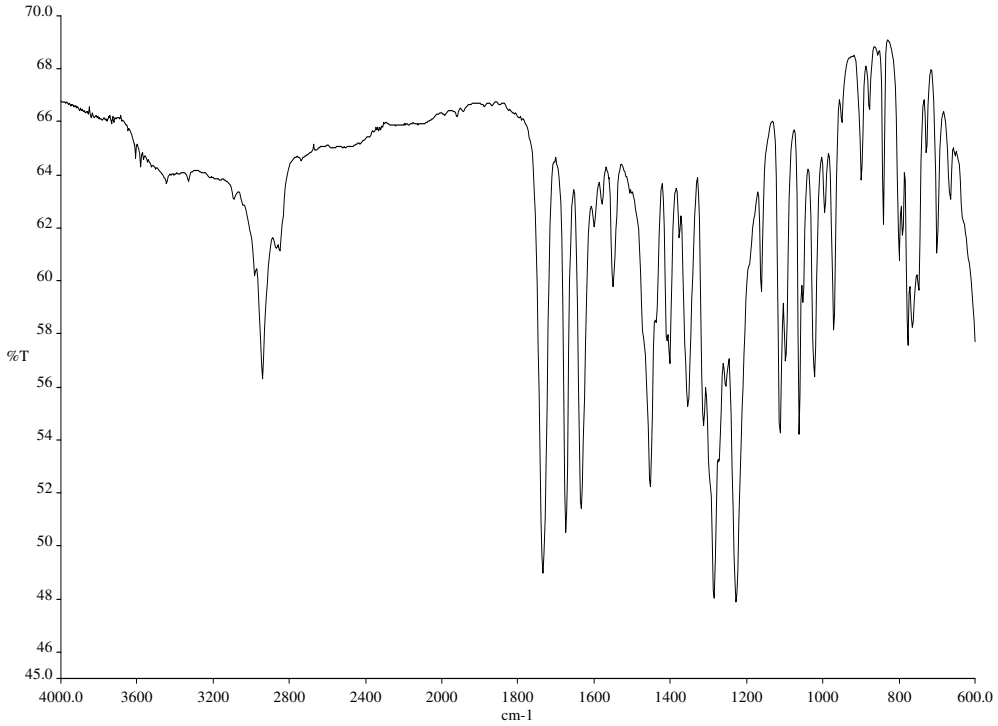


**Figure 41** FT-IR (Neat) spectrum of **SA7**

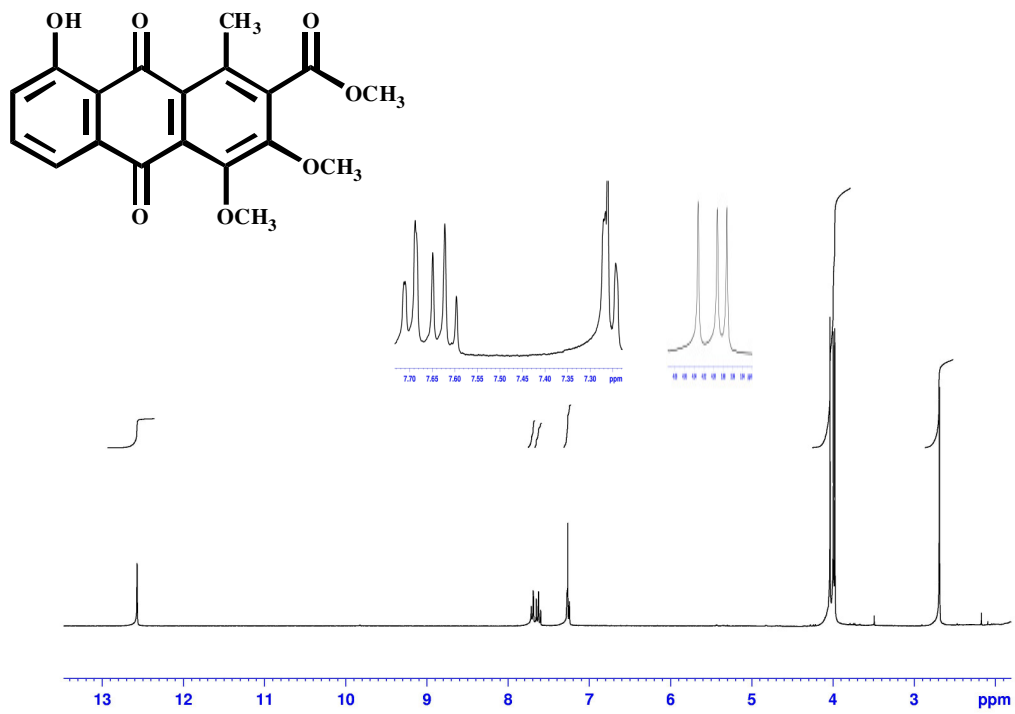


**Figure 42** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA7**

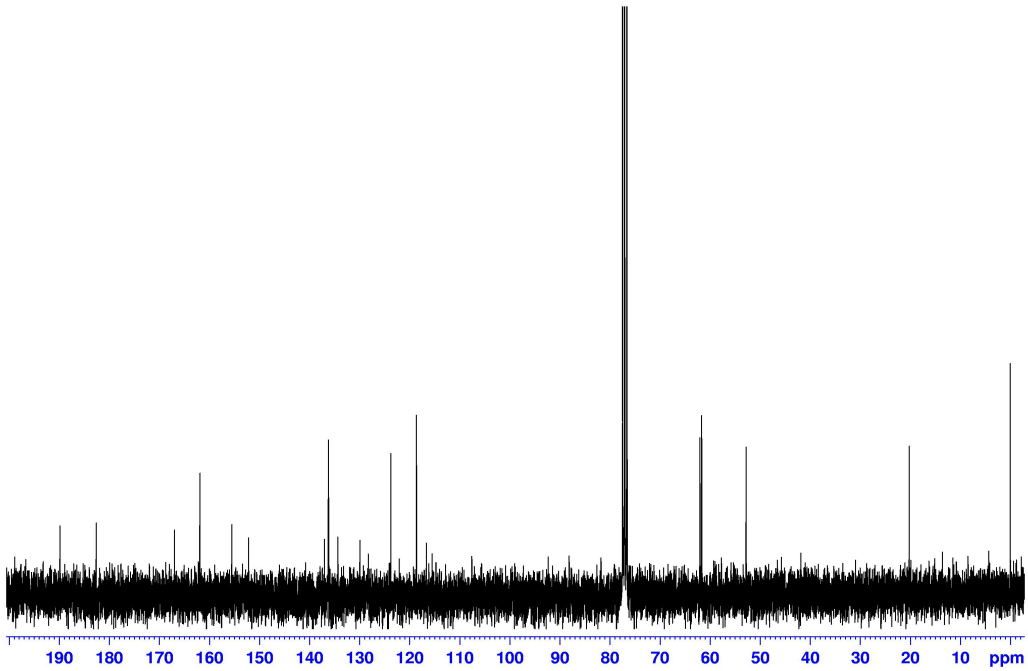


**Figure 43** $^{13}$C NMR (75 MHz) ($CDCl_3$) spectrum of **SA7**

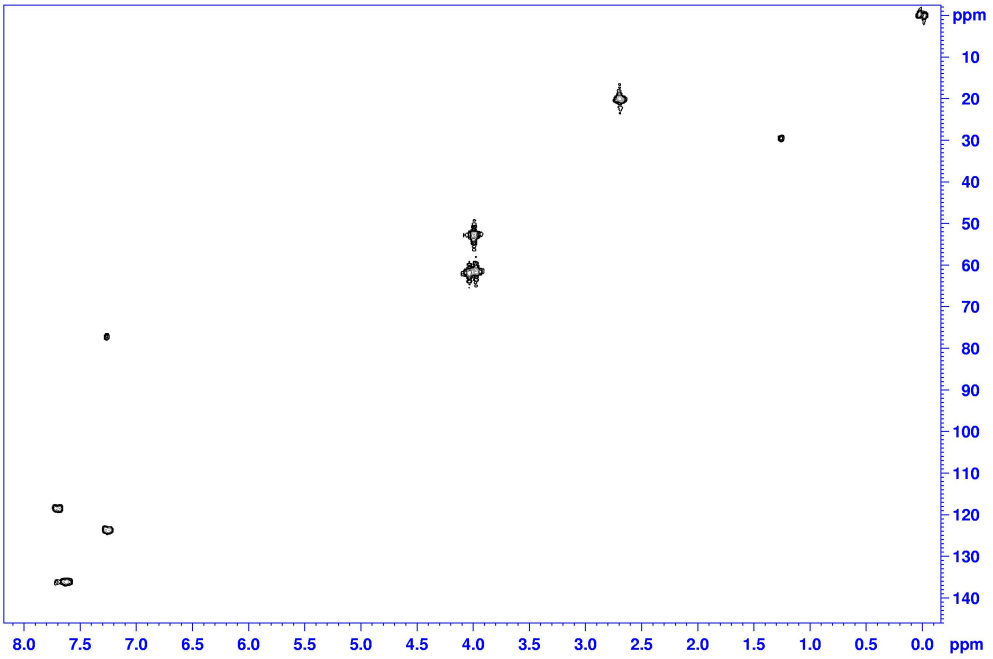


**Figure 44** 2D HMQC spectrum of **SA7**

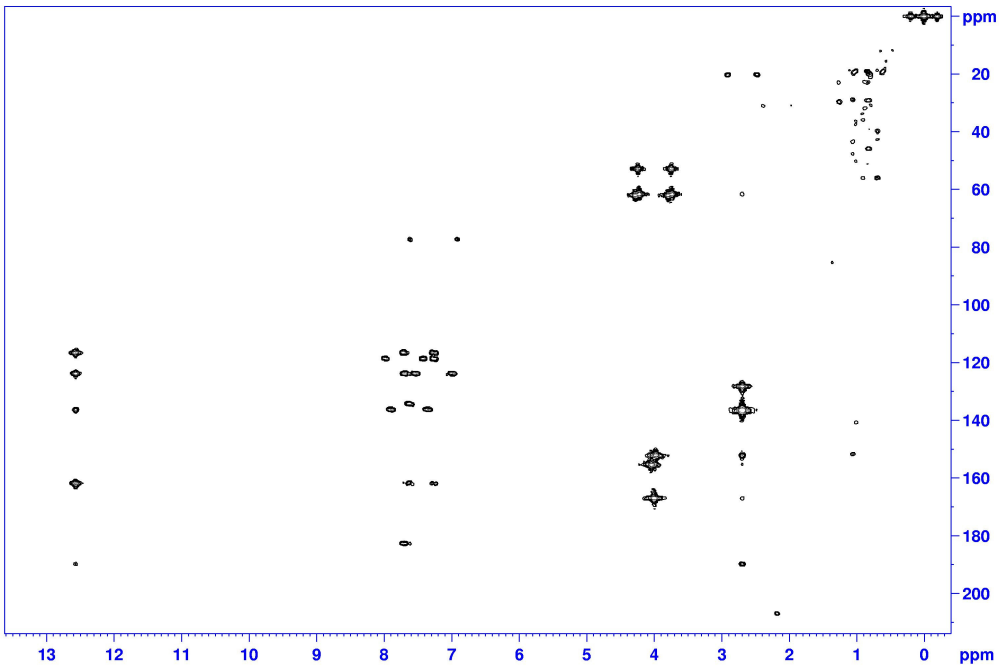


**Figure 45** 2D HMBC spectrum of **SA7**

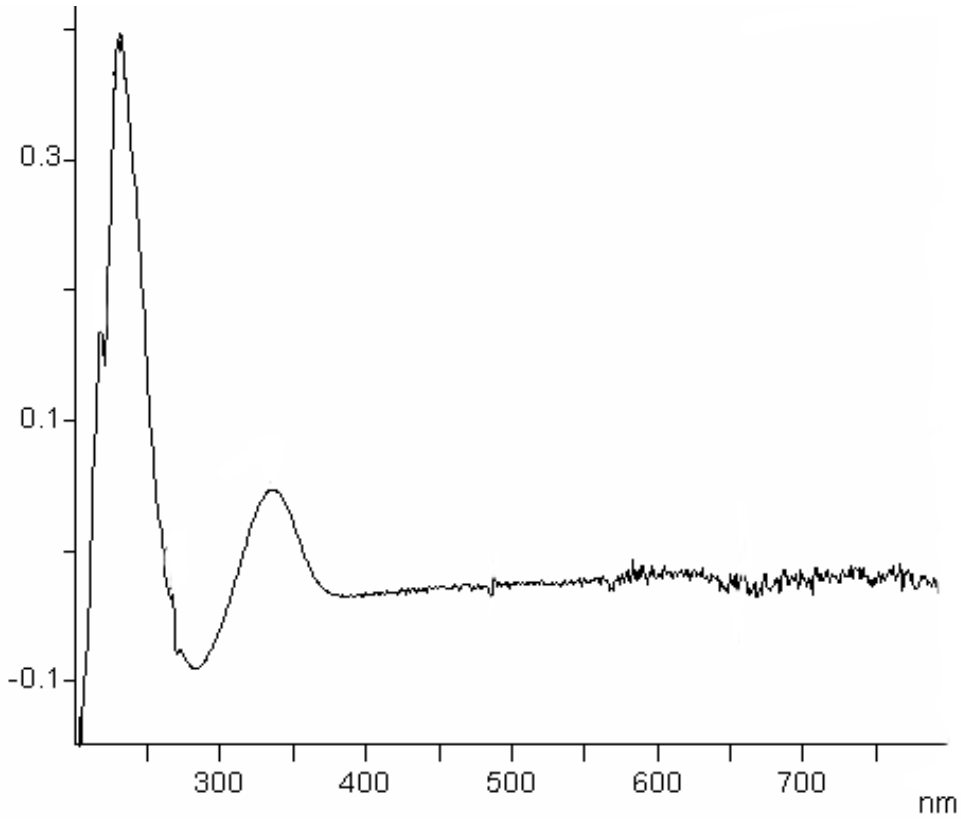


**Figure 46** UV ($CH_3OH$) spectrum of **SA8**

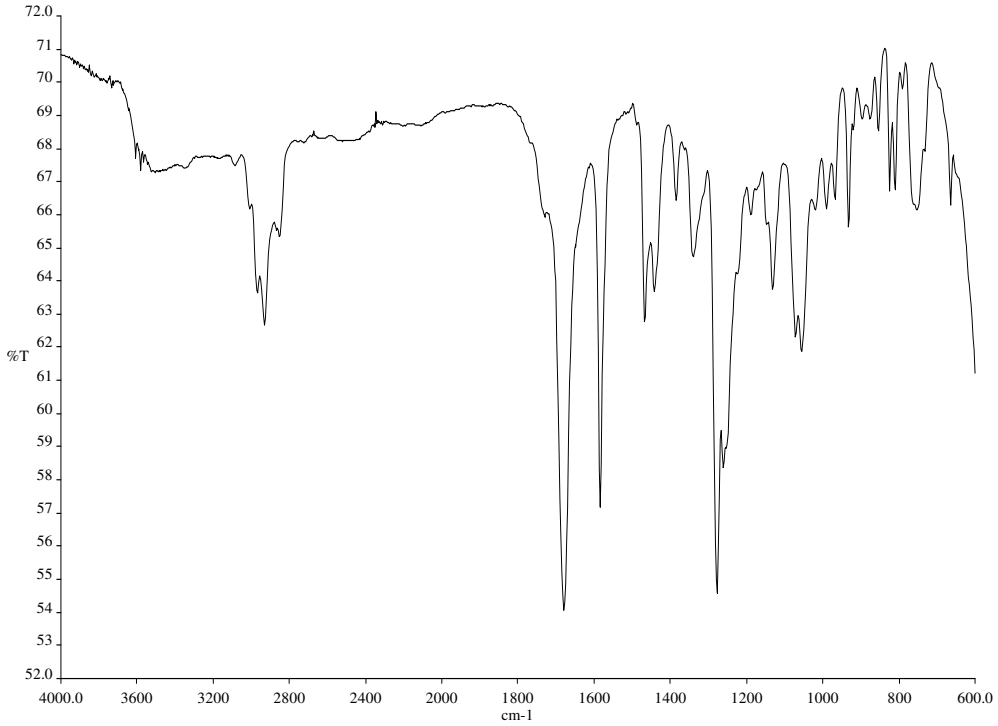


**Figure 47** FT-IR (Neat) spectrum of **SA8**

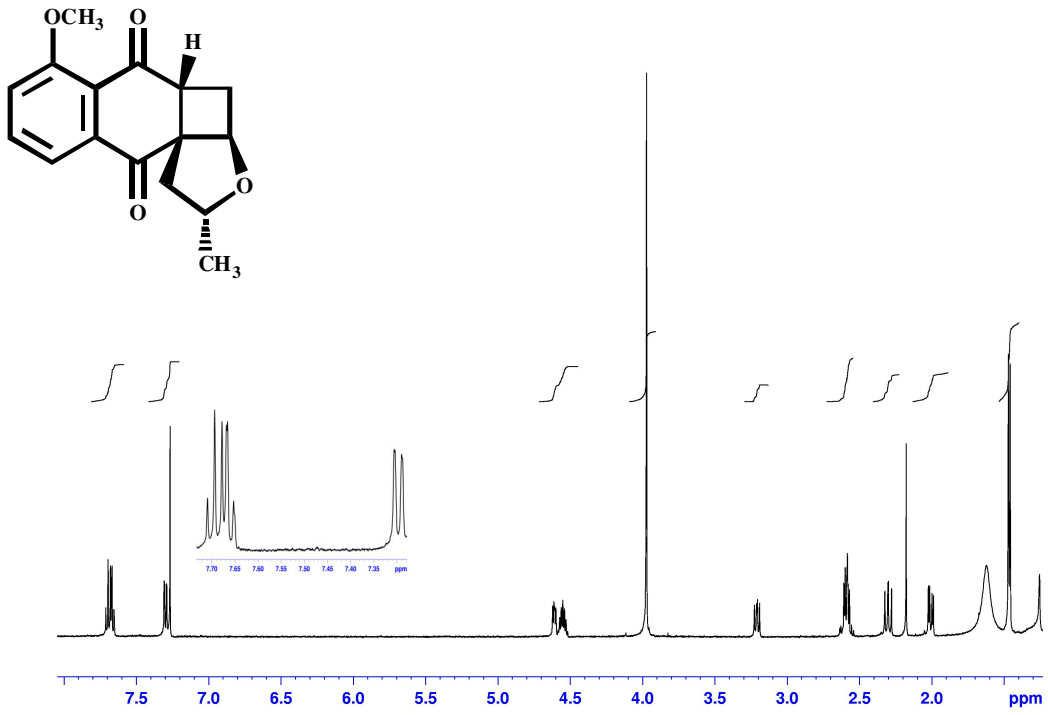


**Figure 48** $^{1}$H NMR (500 MHz) ($CDCl_3$) spectrum of **SA8**

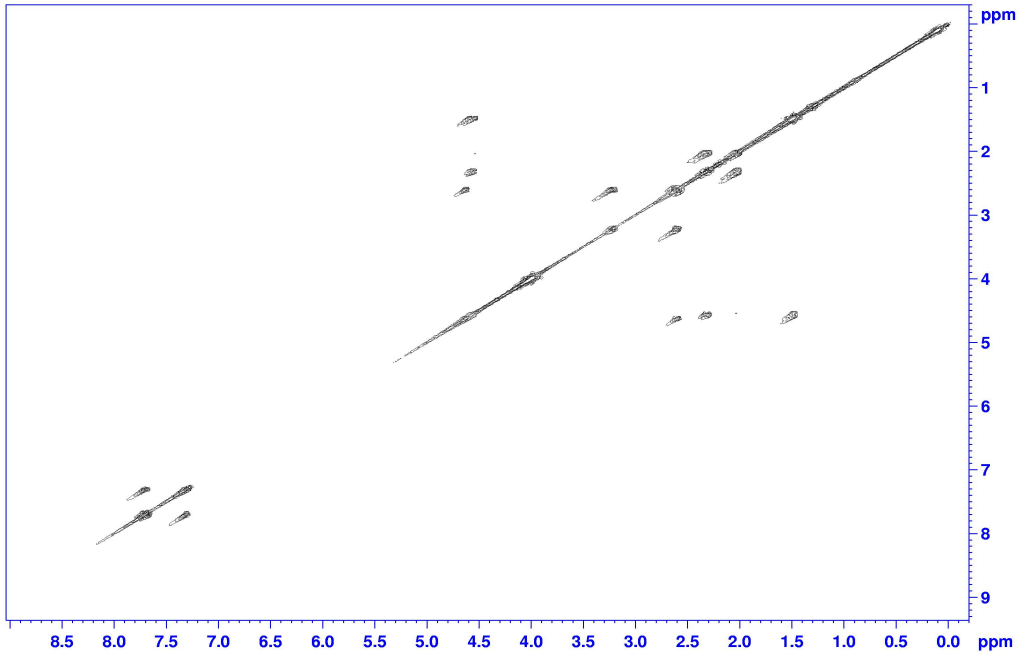


**Figure 49** $^{1}$H-$^{1}$H COSY spectrum of **SA8**

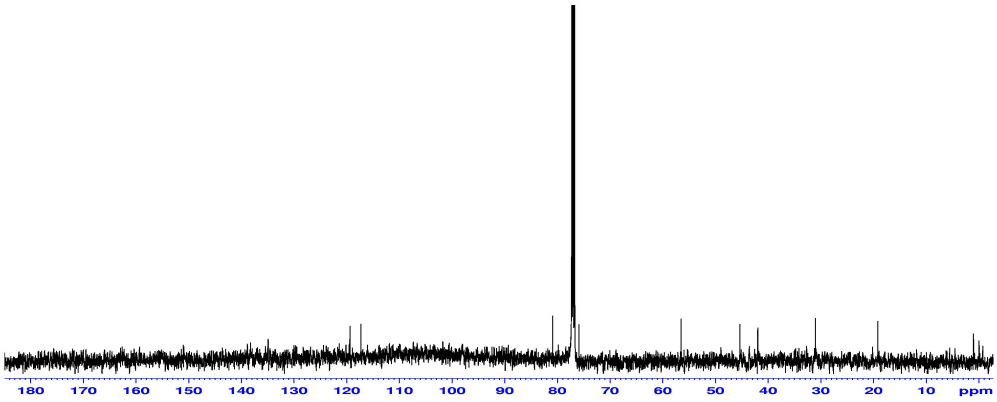


**Figure 50** $^{13}C$ NMR (125 MHz) ($CDCl_3$) spectrum of **SA8**

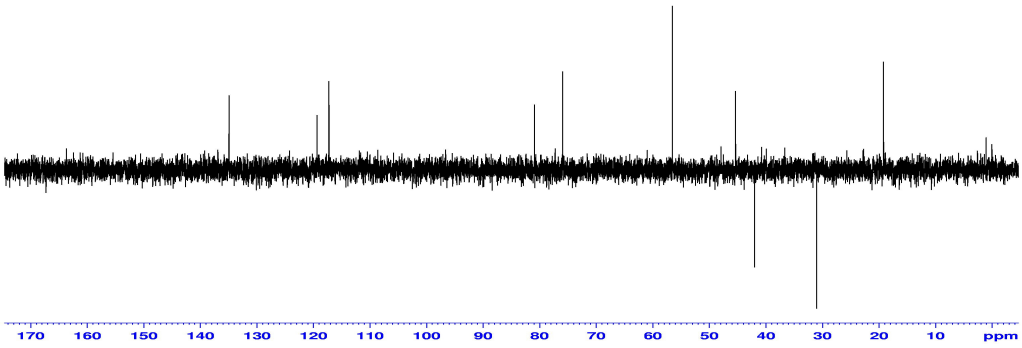


**Figure 51** DEPT 135° ($CDCl_3$) spectrum of **SA8**

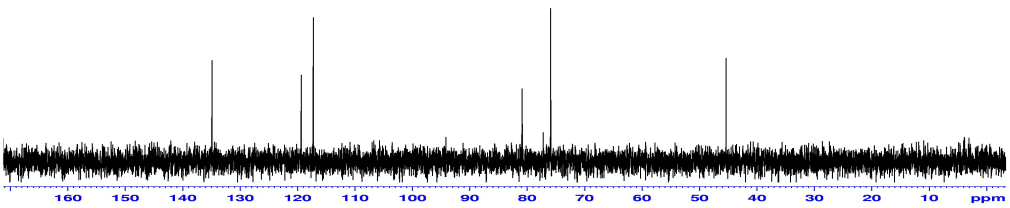


**Figure 52** DEPT 90° ($CDCl_3$) spectrum of **SA8**

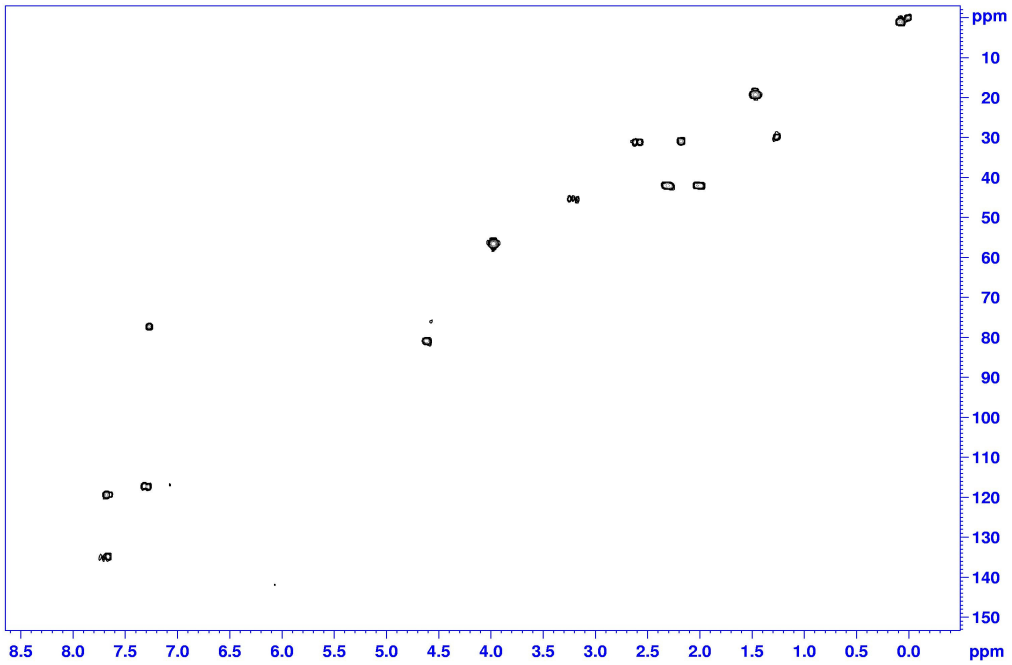


**Figure 53** 2D HMQC spectrum of **SA8**

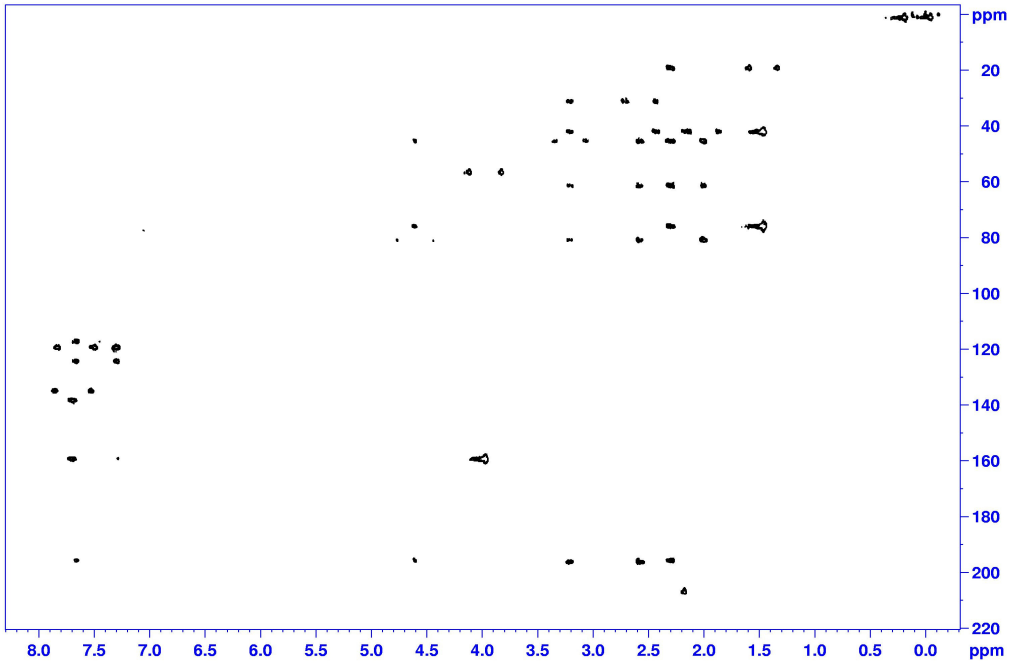


**Figure 54** 2D HMBC spectrum of **SA8**

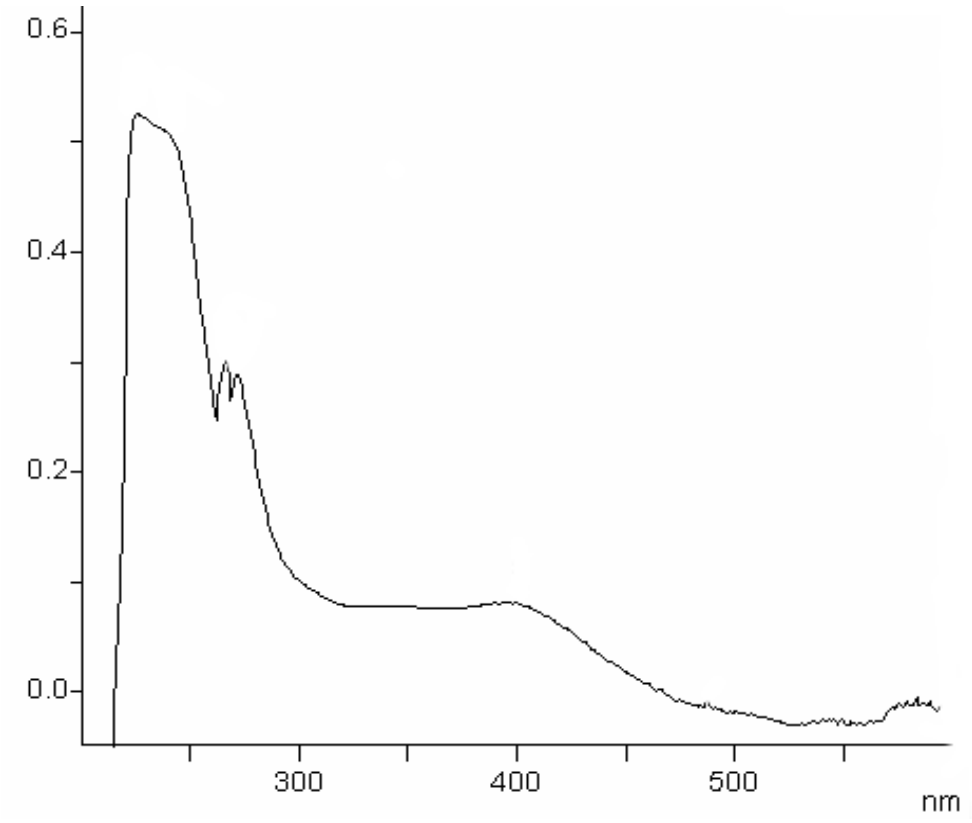


**Figure 55** UV ($CH_3OH$) spectrum of **SA9**

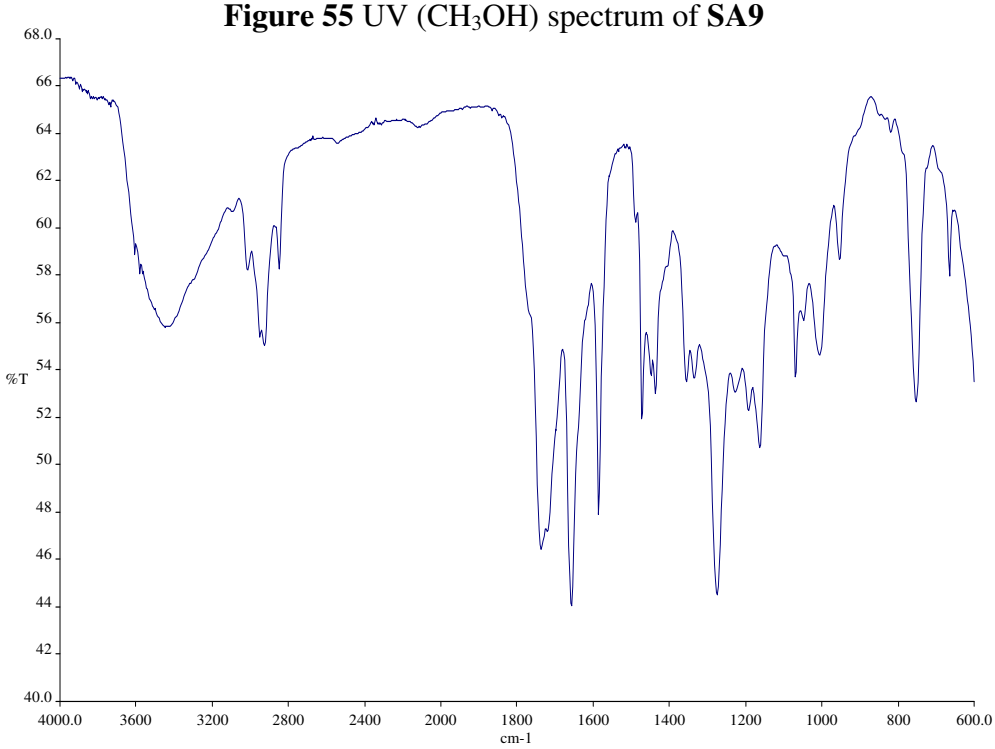


**Figure 56** FT-IR (Neat) spectrum of **SA9**

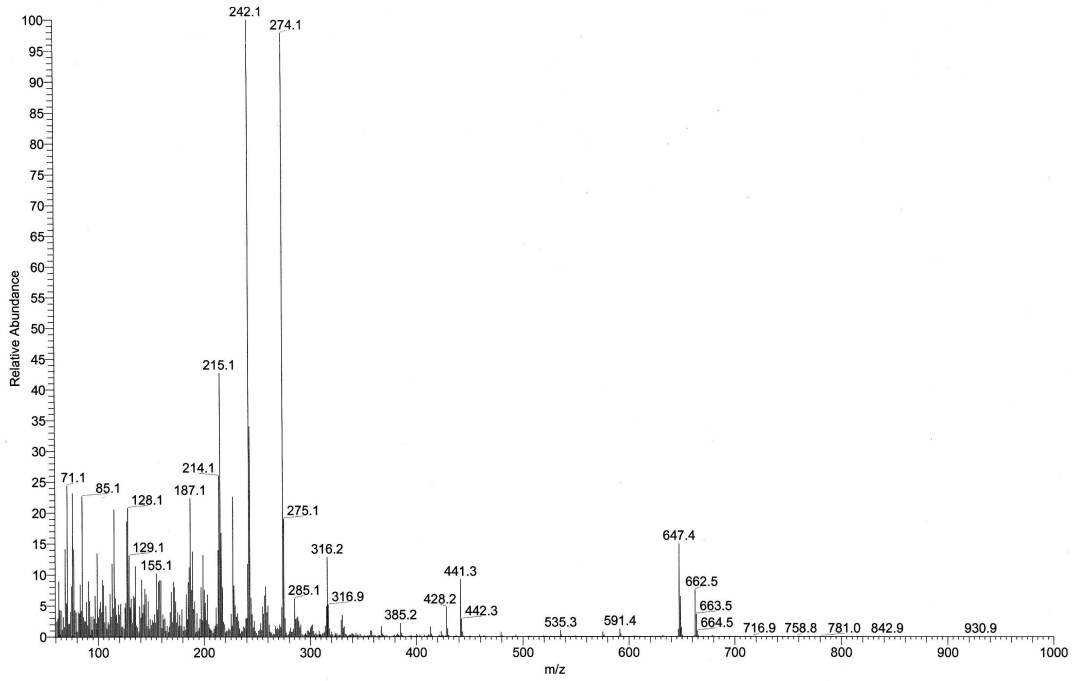


**Figure 57** EI-MS spectrum of **SA9**

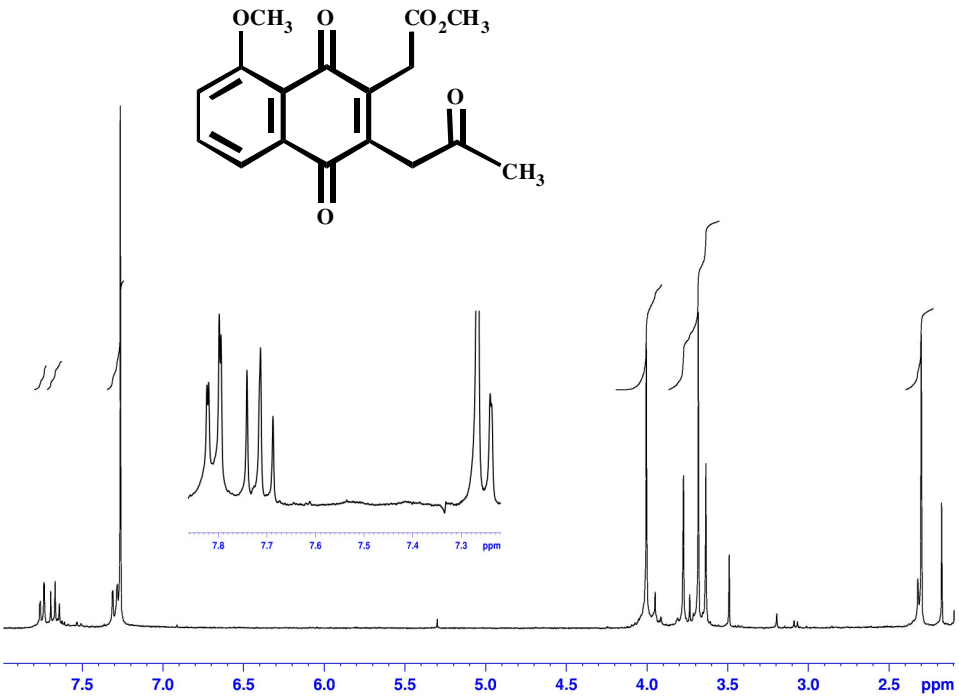


**Figure 58** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA9**

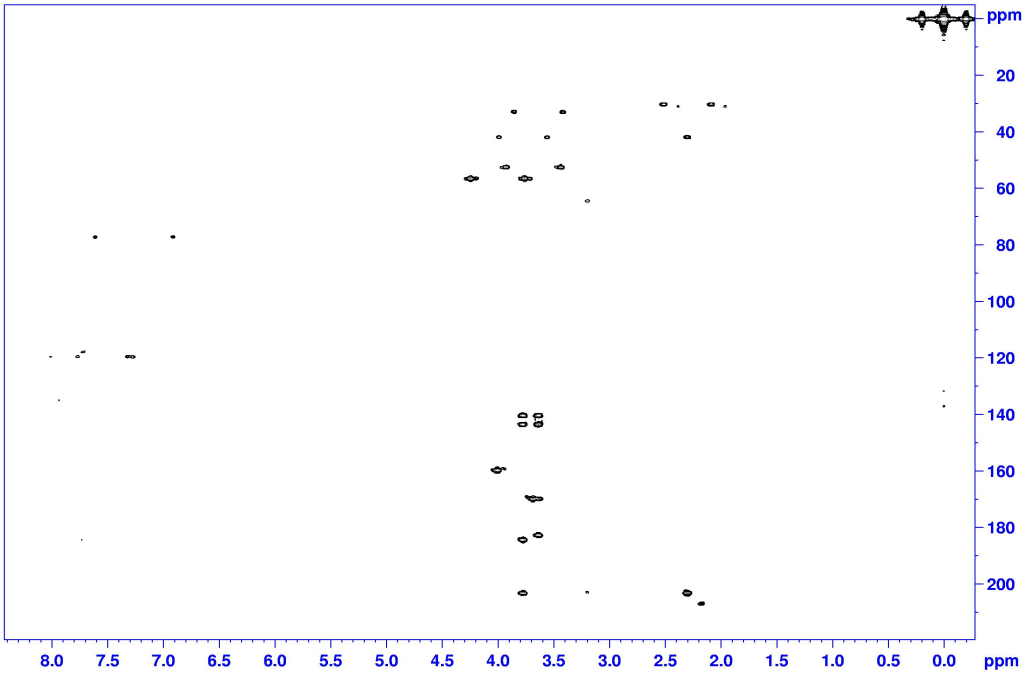


**Figure 59** 2D HMBC spectrum of **SA9**

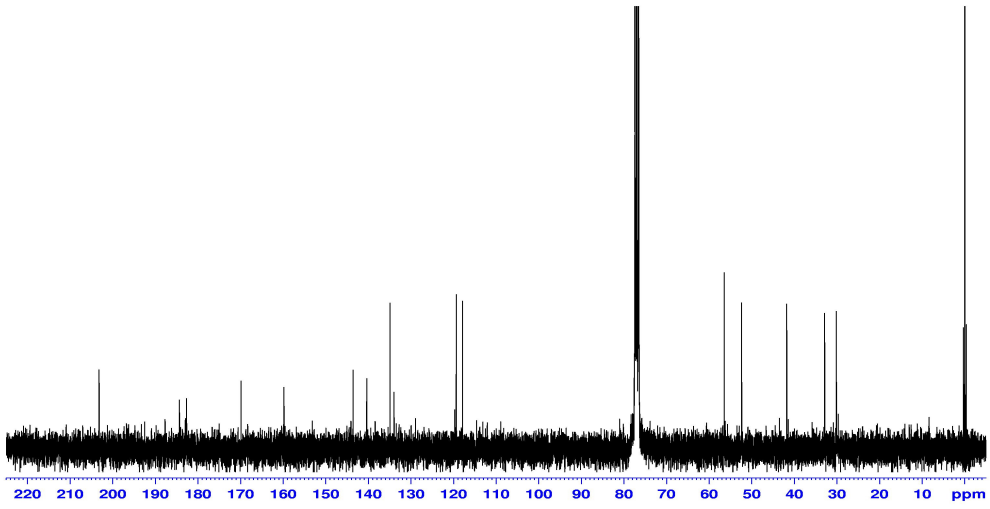


**Figure 60** $^{13}C$ NMR (75 MHz) ($CDCl_3$) spectrum of **SA9**

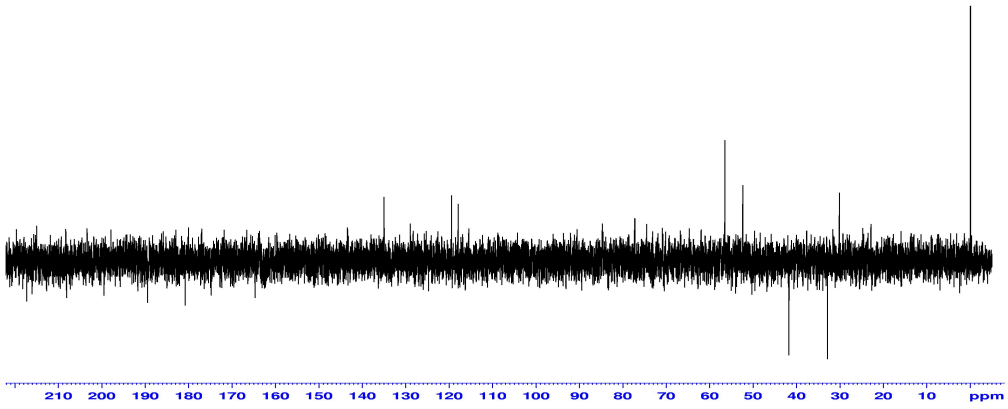


**Figure 61** DEPT 135° ($CDCl_3$) spectrum of **SA9**

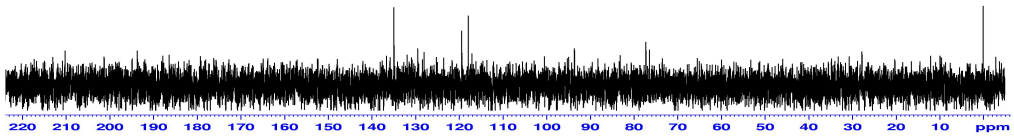


**Figure 62** DEPT 90° ($CDCl_3$) spectrum of **SA9**

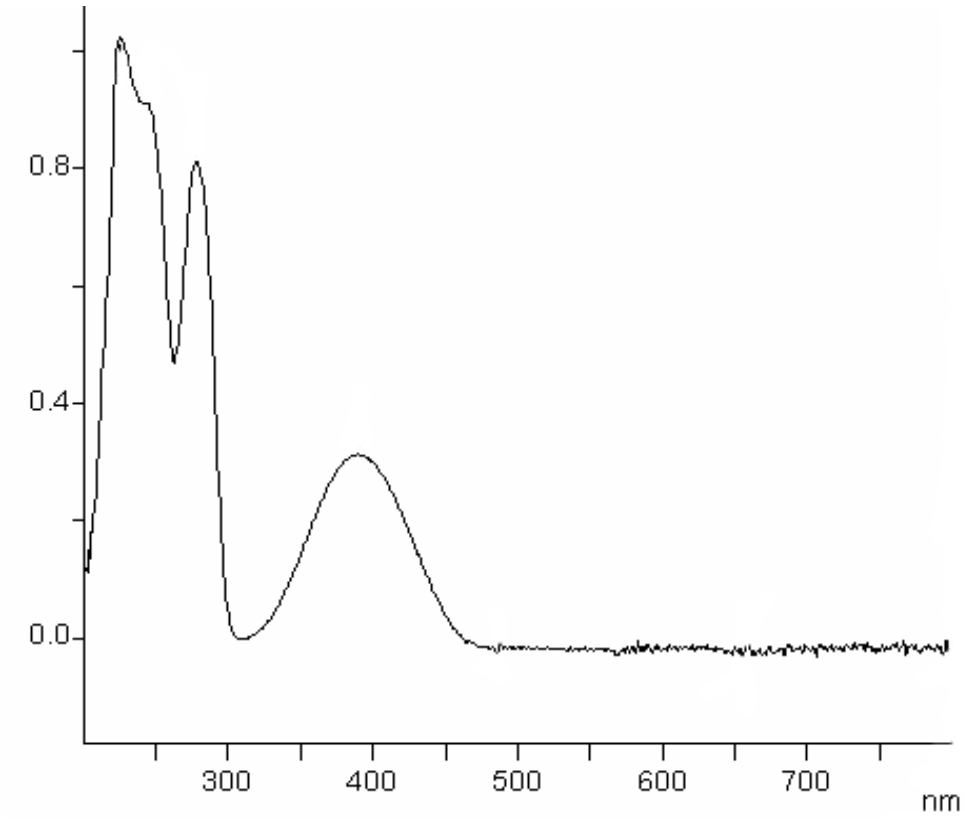


**Figure 63** UV ($CH_3OH$) spectrum of **SA10**

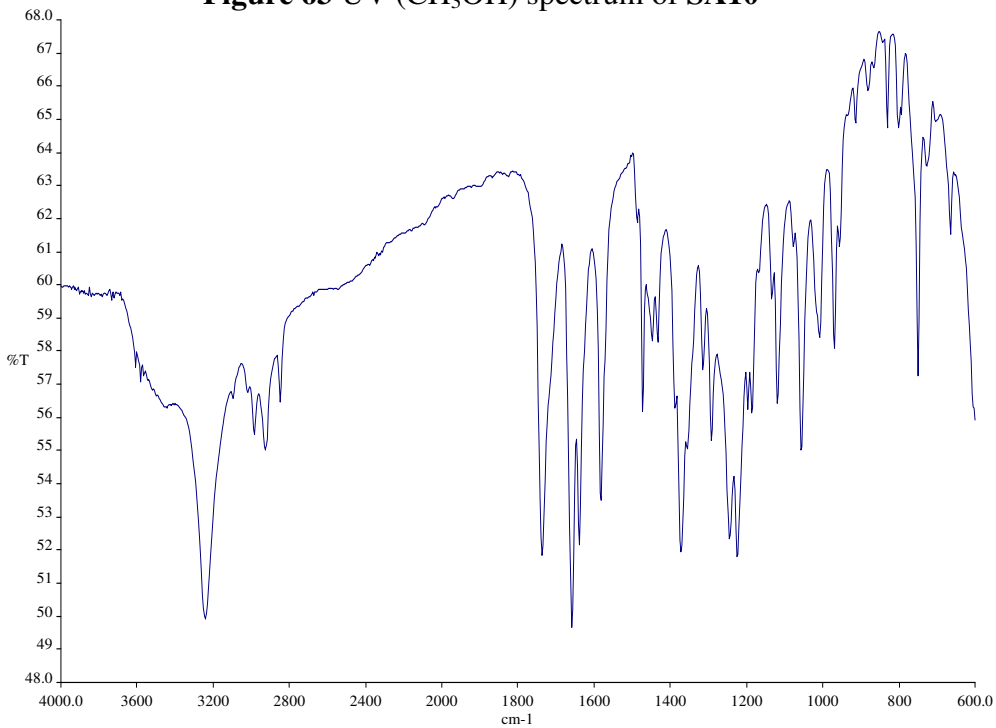


**Figure 64** FT-IR (Neat) spectrum of **SA10**

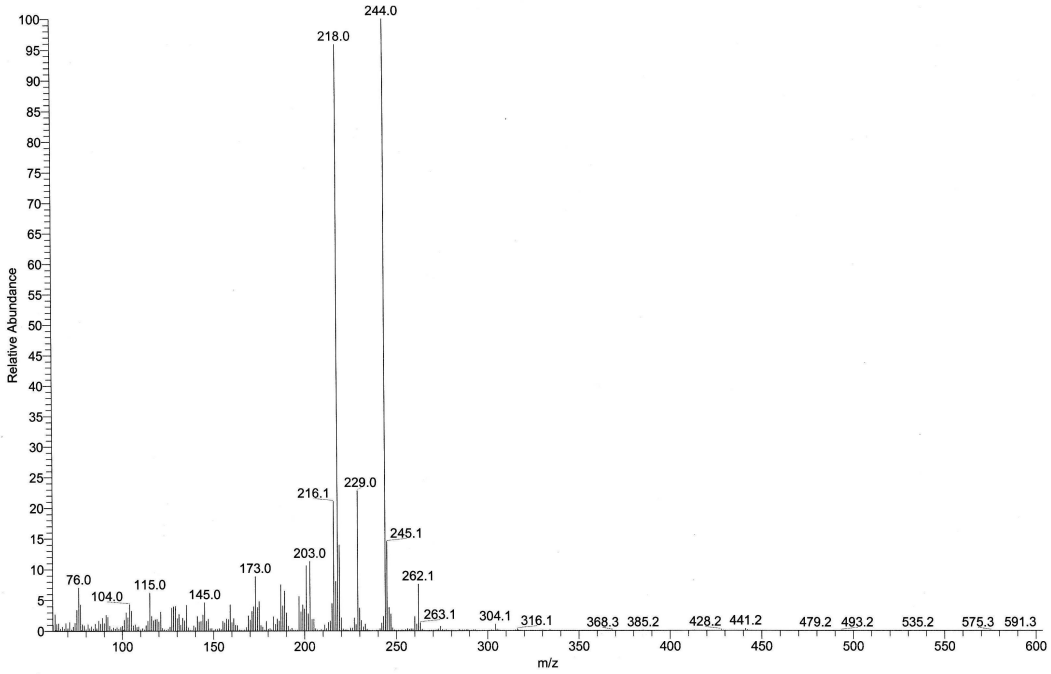


**Figure 65** EI-MS spectrum of **SA10**

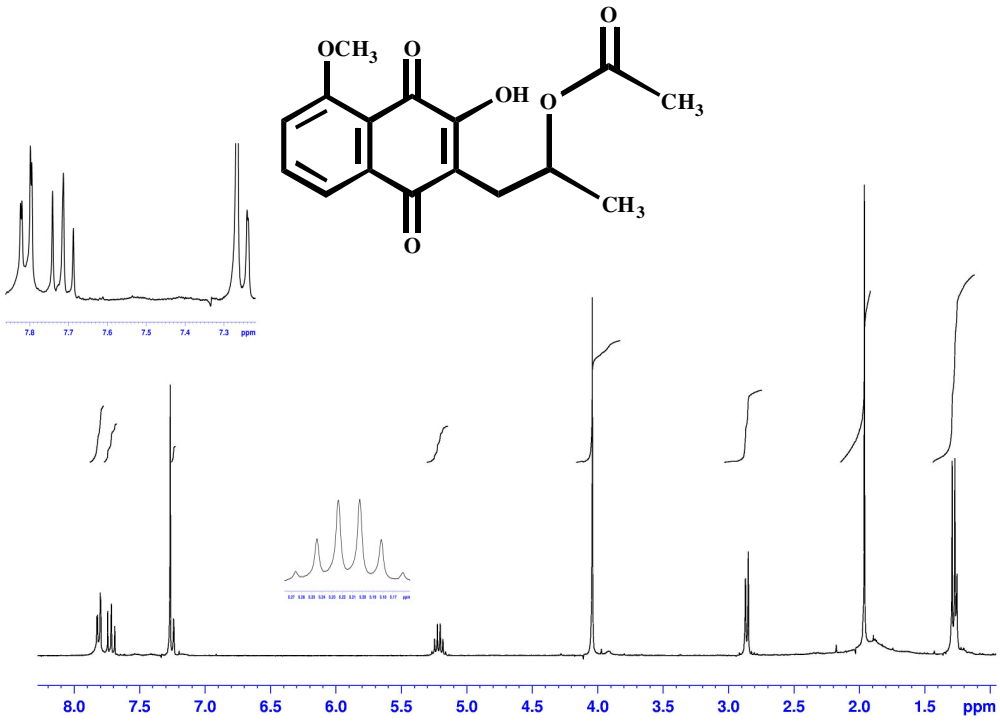


**Figure 66** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA10**

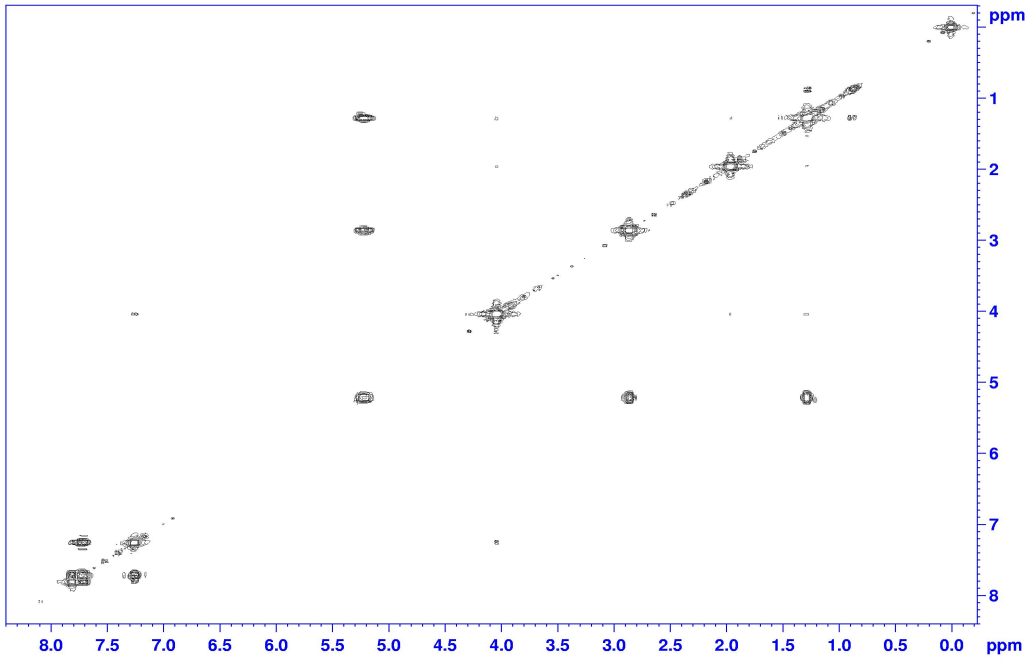


**Figure 67** $^{1}$H-$^{1}$H COSY spectrum of **SA10**

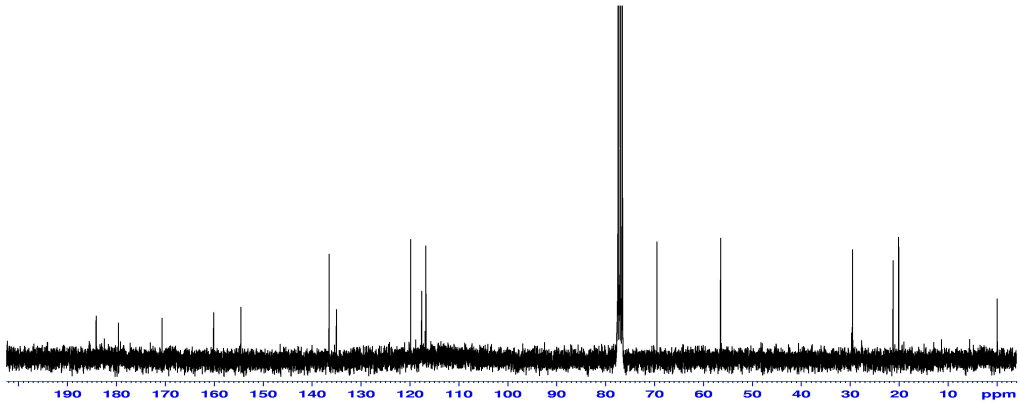


**Figure 68** $^{13}C$ NMR (75 MHz) ($CDCl_3$) spectrum of **SA10**

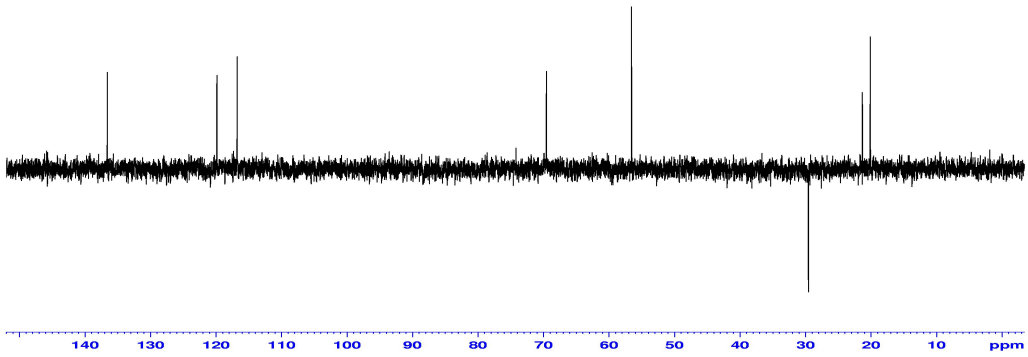


**Figure 69** DEPT 135° ($CDCl_3$) spectrum of **SA10**

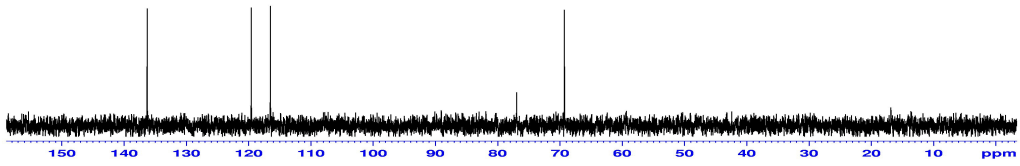


**Figure 70** DEPT 90° ($CDCl_3$) spectrum of **SA10**

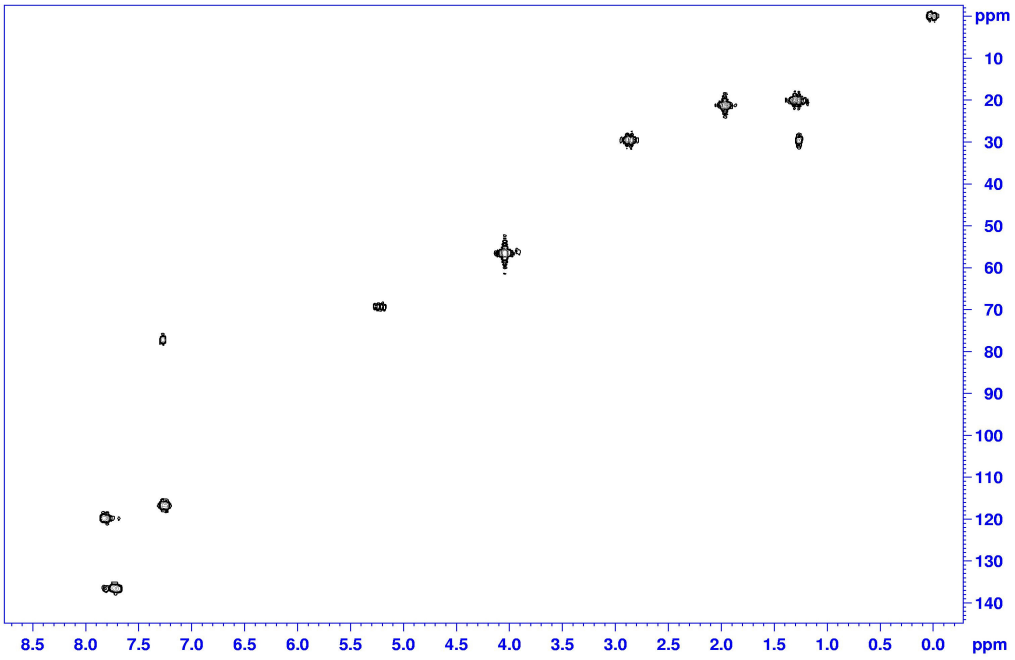


**Figure 71** 2D HMQC spectrum of **SA10**

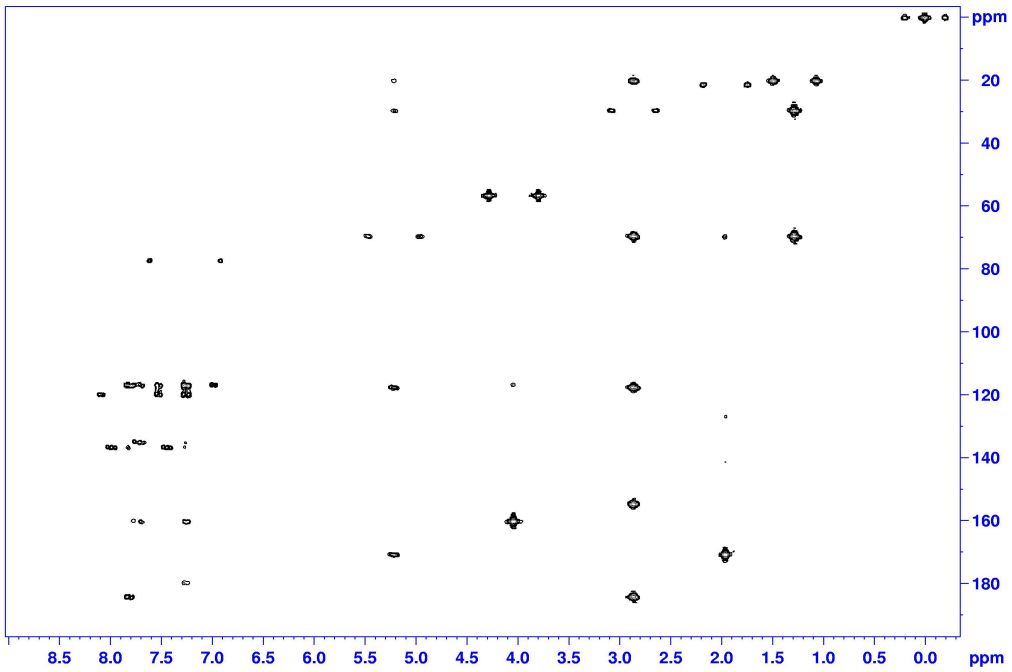


**Figure 72** 2D HMBC spectrum of **SA10**

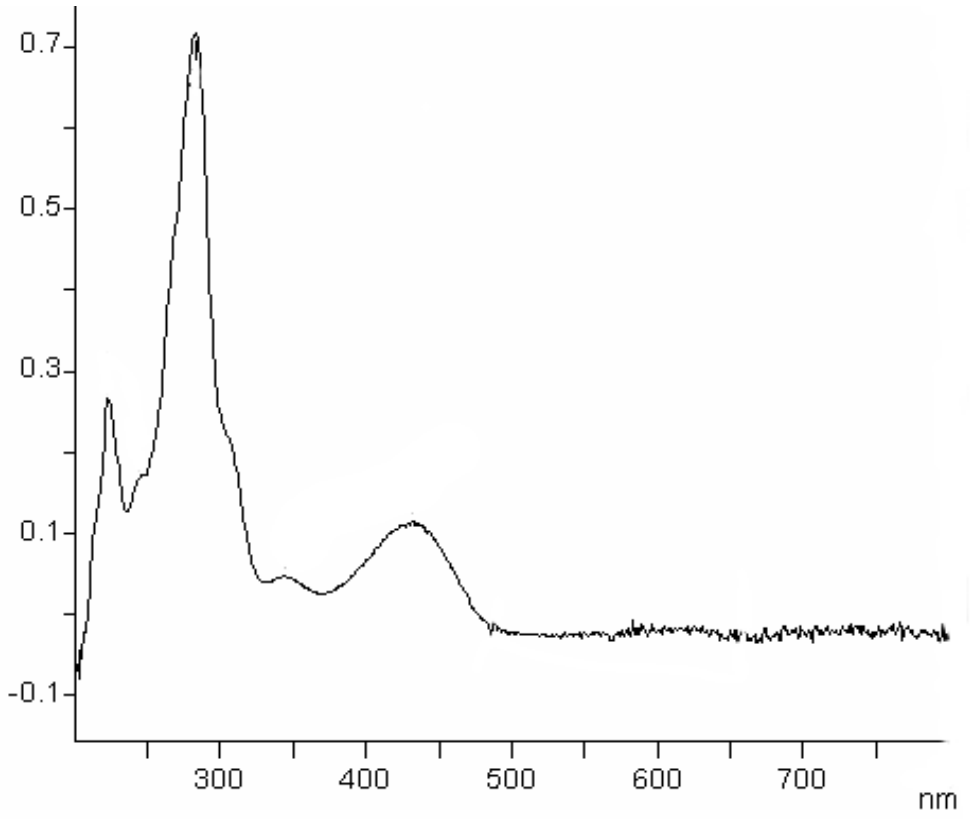


**Figure 73** UV ($CH_3OH$) spectrum of **SA11**

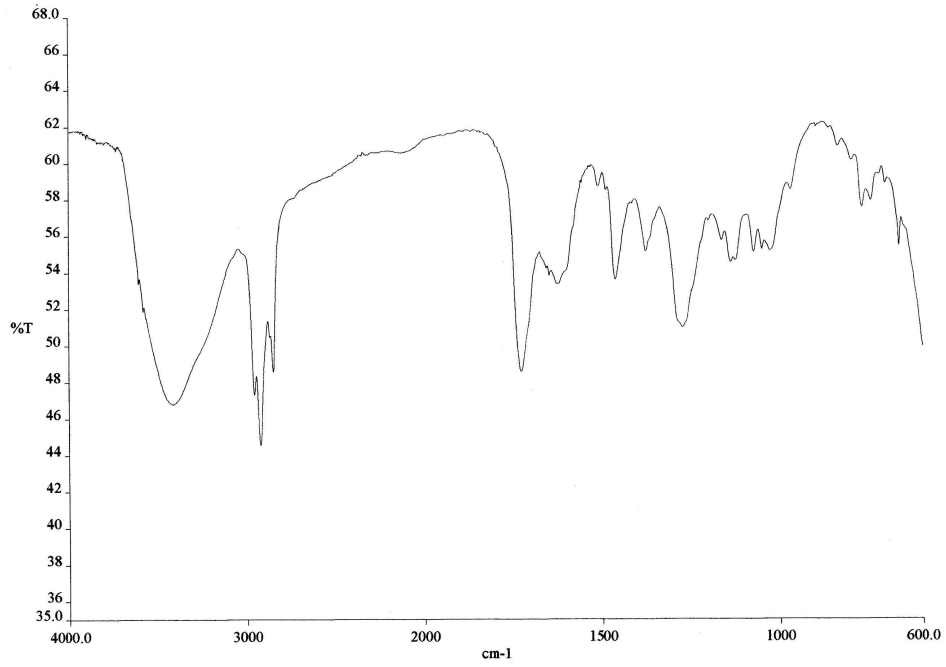


**Figure 74** FT-IR (Neat) spectrum of **SA11**

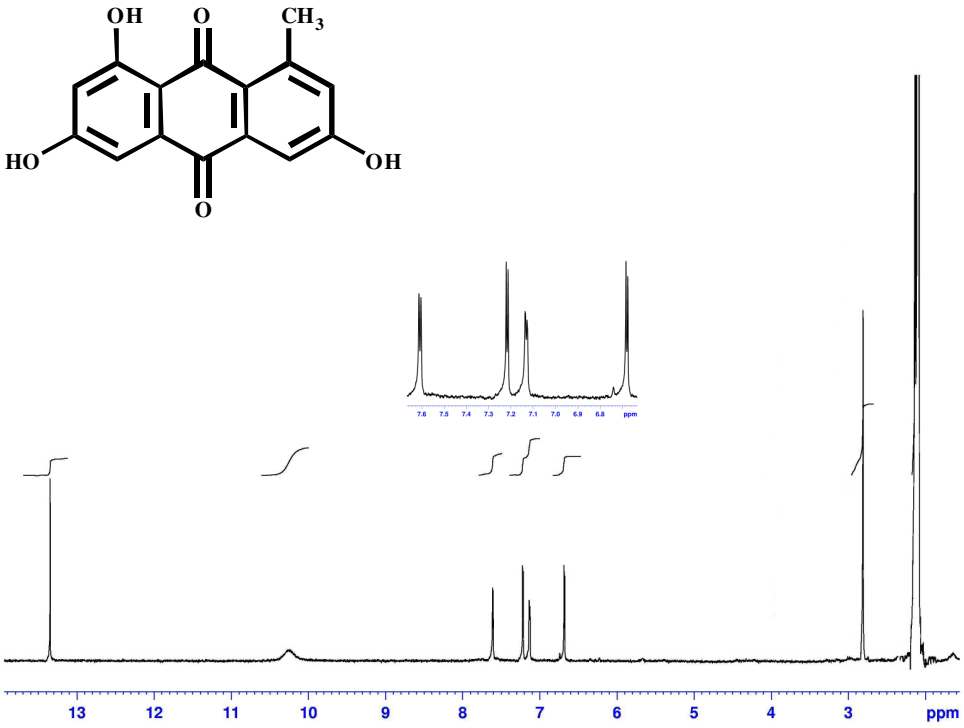


**Figure 75** $^{1}$H NMR (300 MHz) (Acetone-$d_6$) spectrum of **SA11**

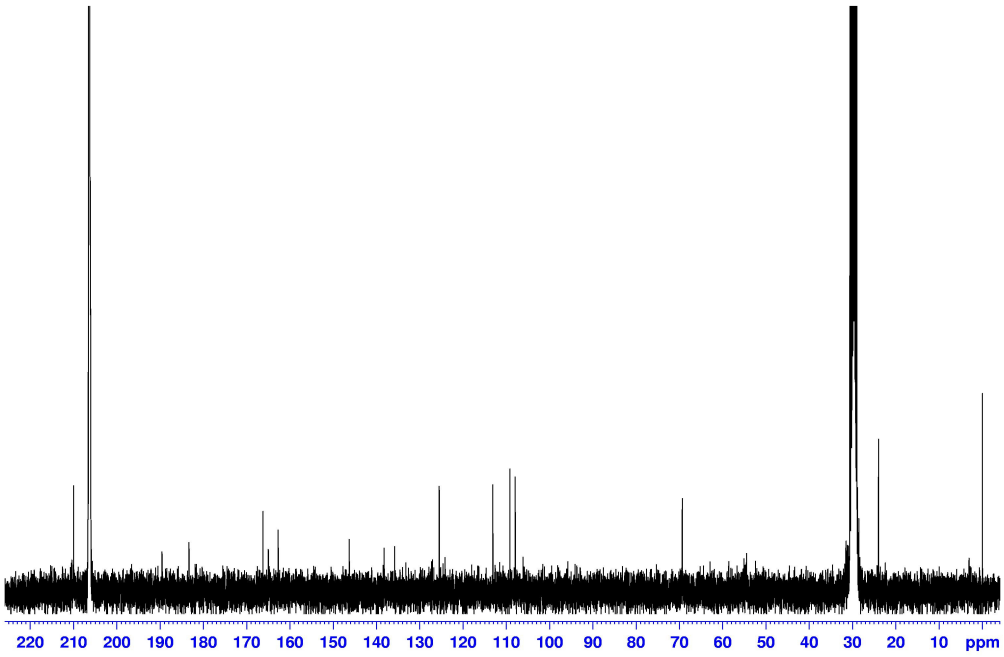


**Figure 76** $^{13}$C NMR (75 MHz) (Acetone-$d_6$) spectrum of **SA11**

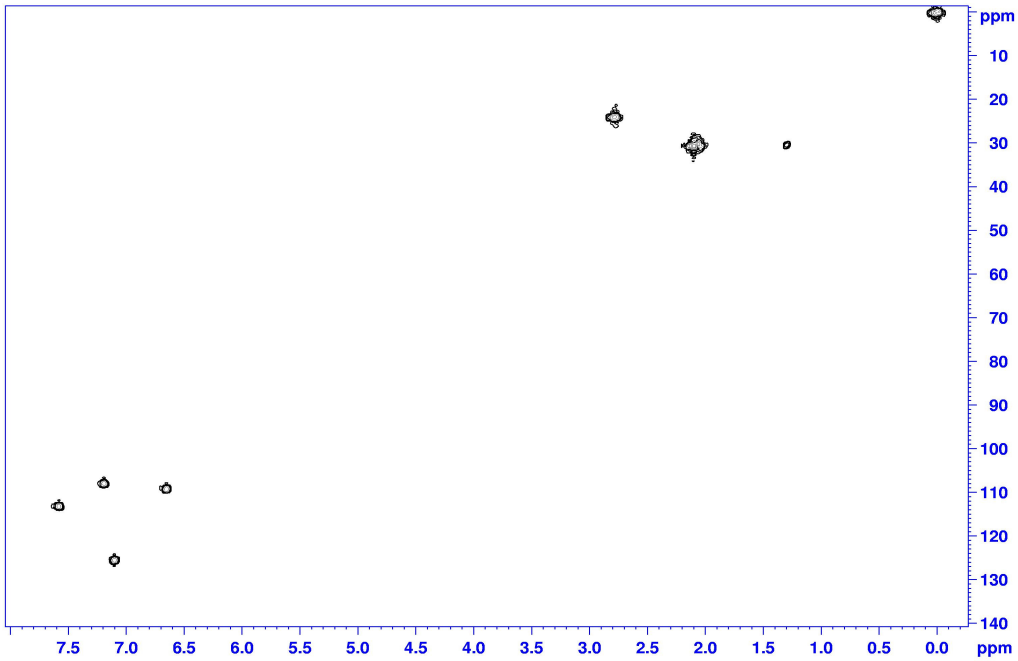


**Figure 77** 2D HMQC spectrum of **SA11**

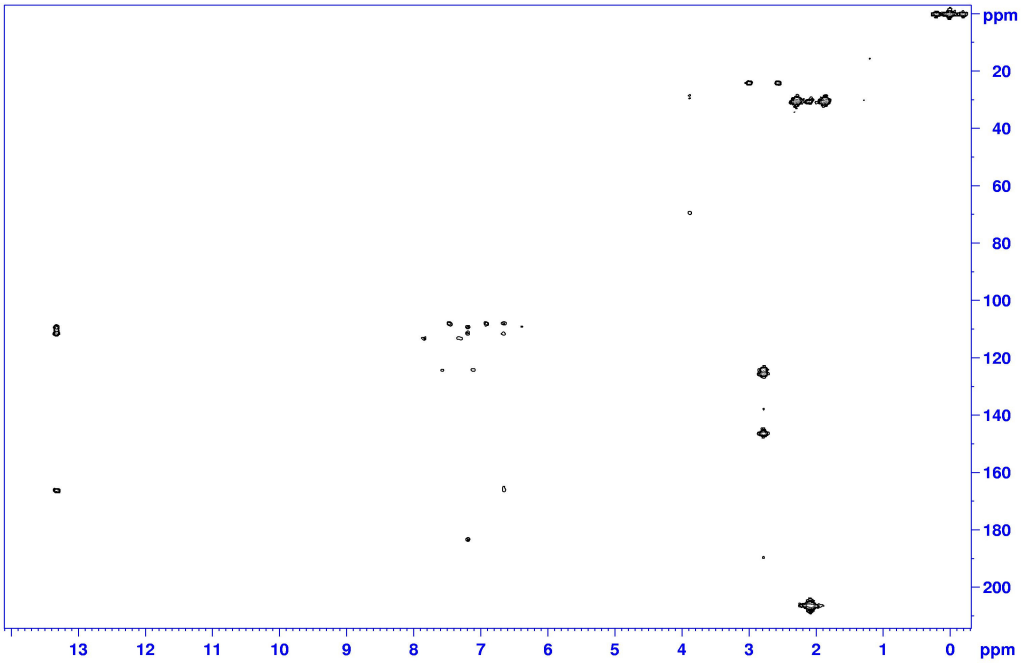


**Figure 78** 2D HMBC spectrum of **SA11**

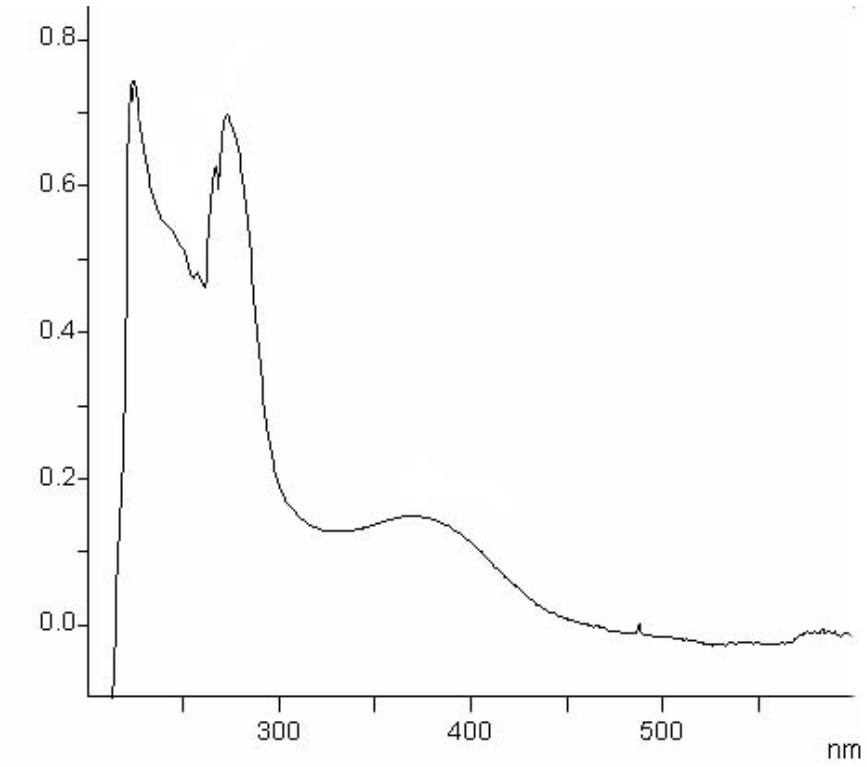


**Figure 79** UV ($CH_3OH$) spectrum of **SA12**

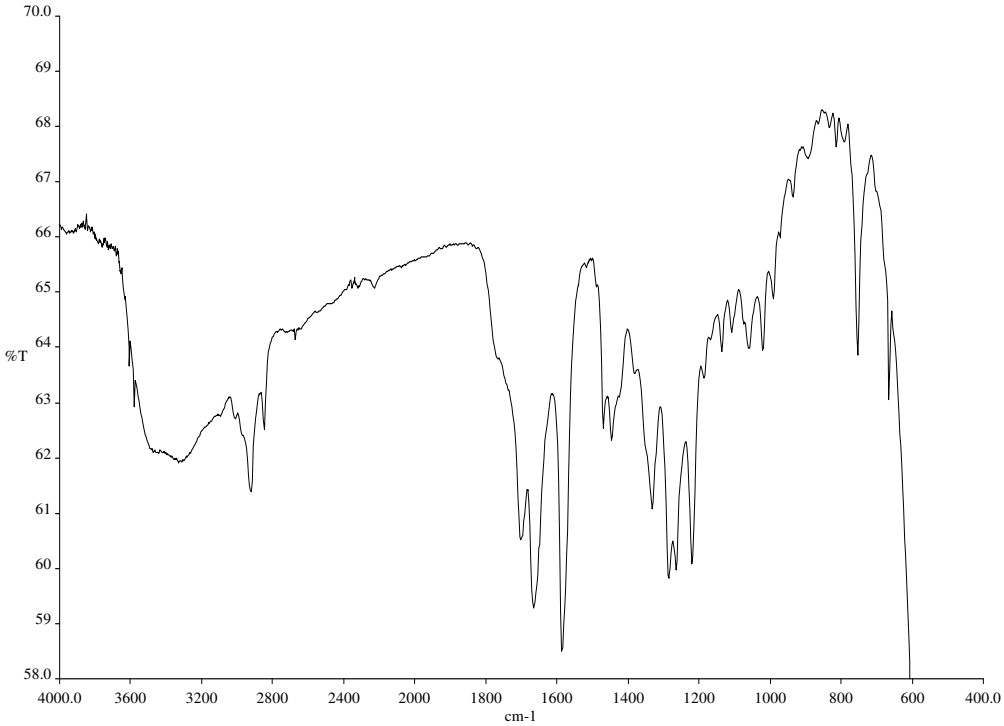


**Figure 80** FT-IR (Neat) spectrum of **SA12**

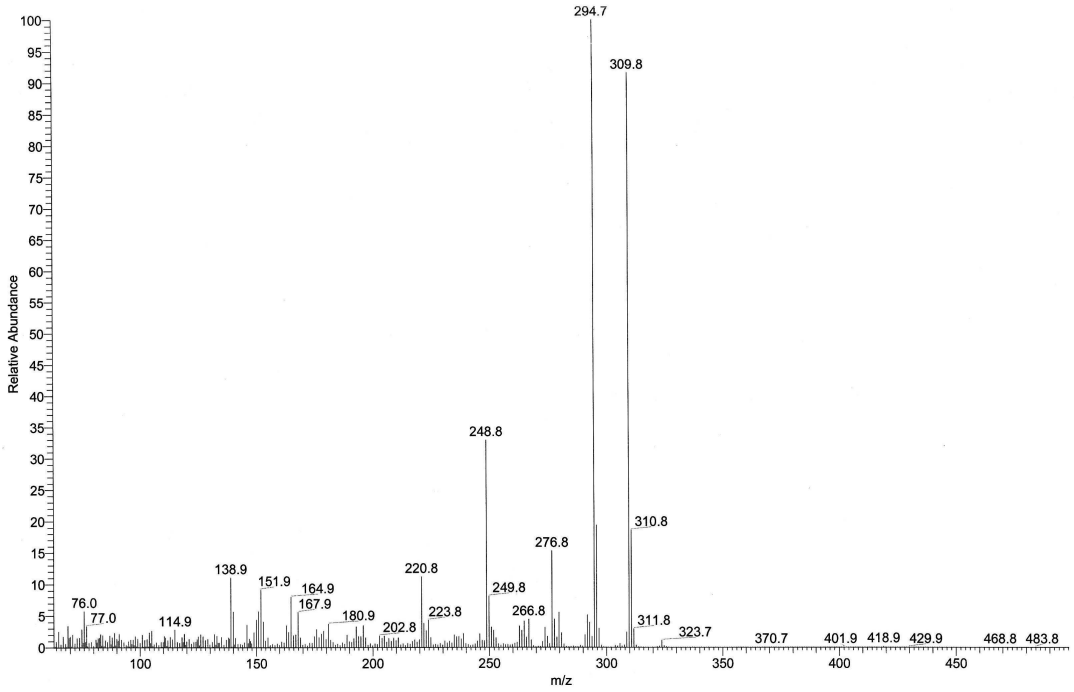


**Figure 81** EI-MS spectrum of **SA12**

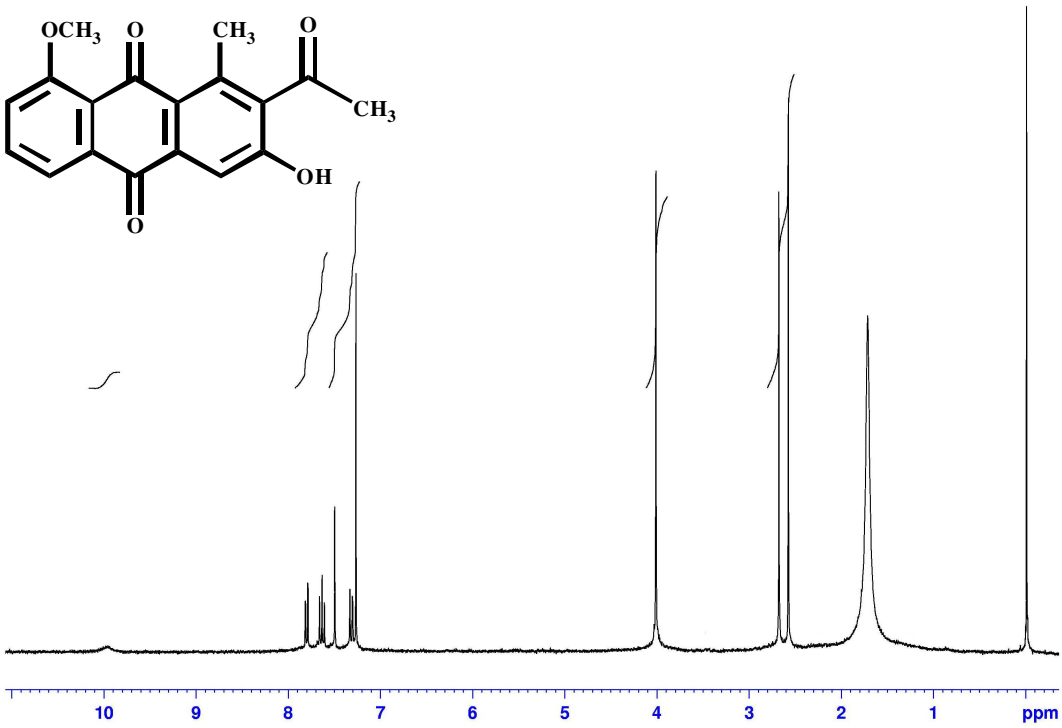


**Figure 82** $^{1}$H NMR (300 MHz) ($CDCl_3$+$CD_3OD$) spectrum of **SA12**

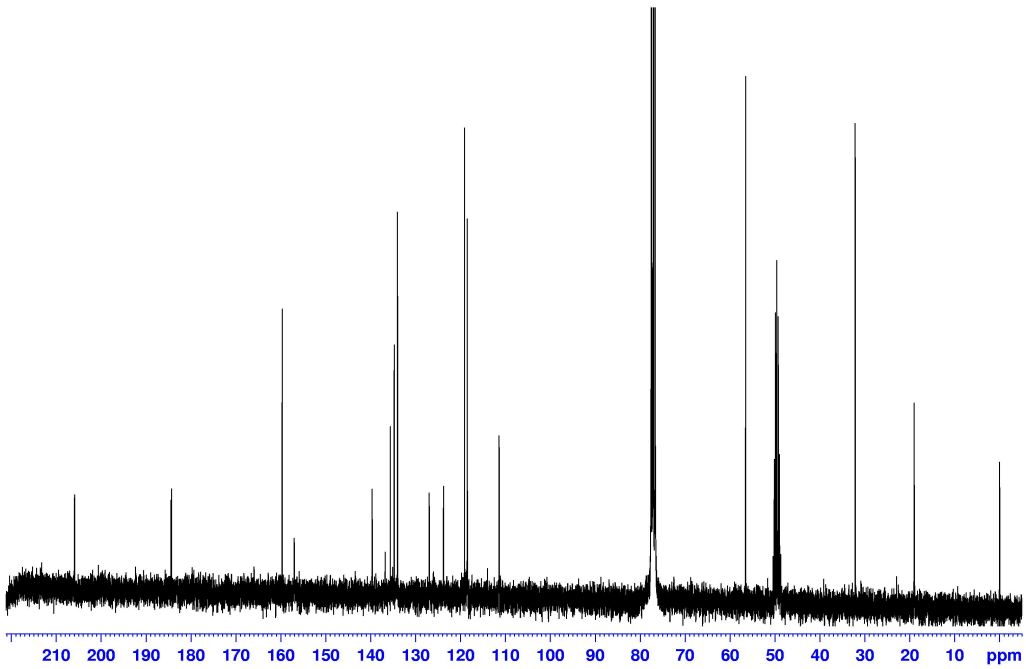


**Figure 83** $^{13}$C NMR (75 MHz) ($CDCl_3$+$CD_3OD$) spectrum of **SA12**

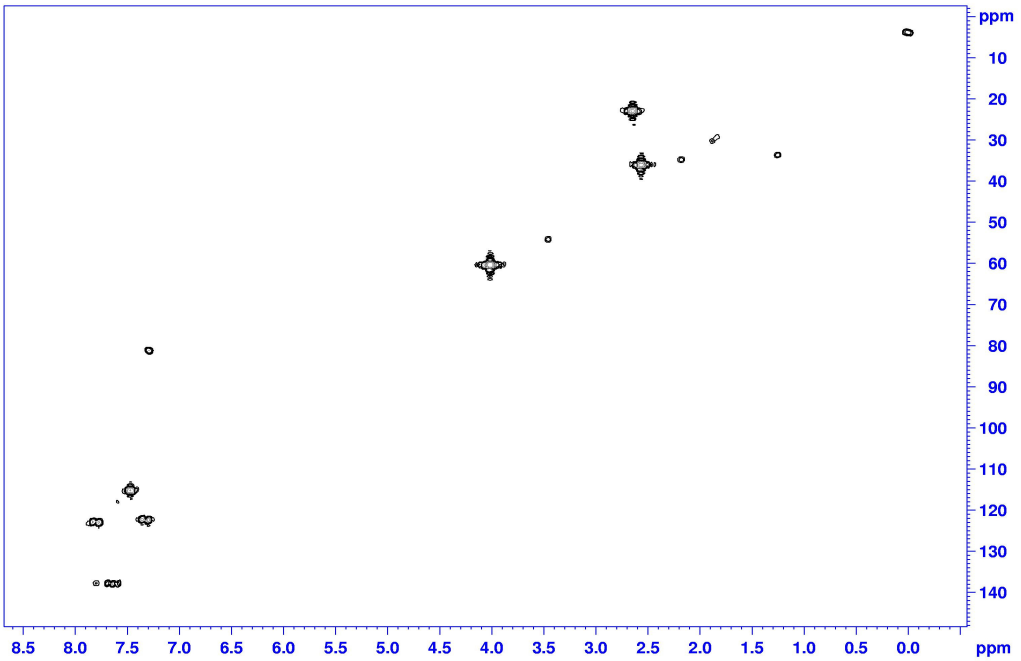


**Figure 84** 2D HMQC spectrum of **SA12**

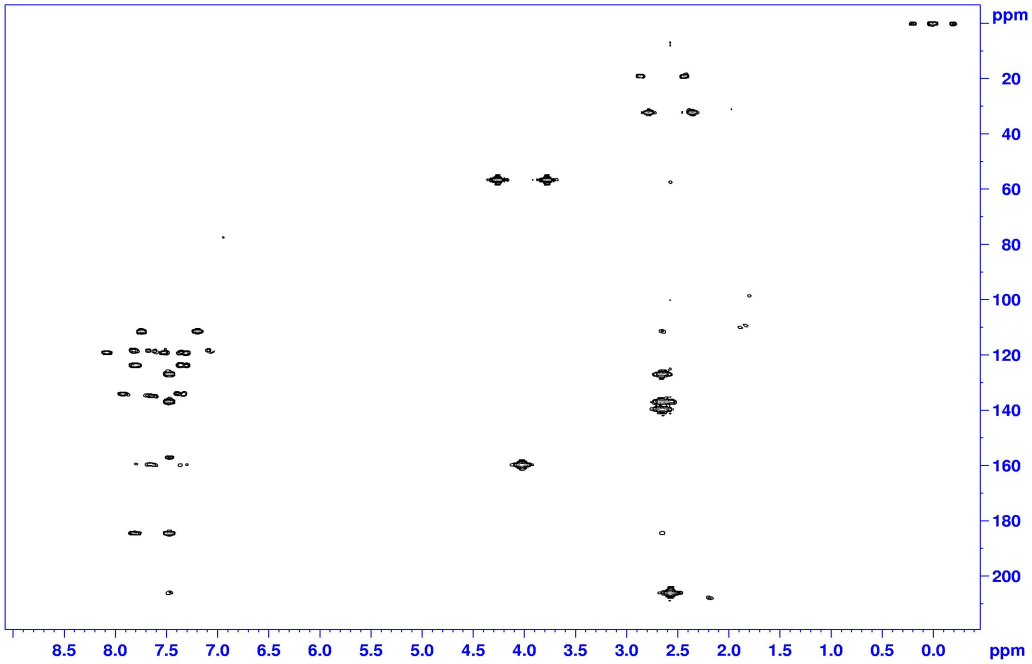


**Figure 85** 2D HMBC spectrum of **SA12**

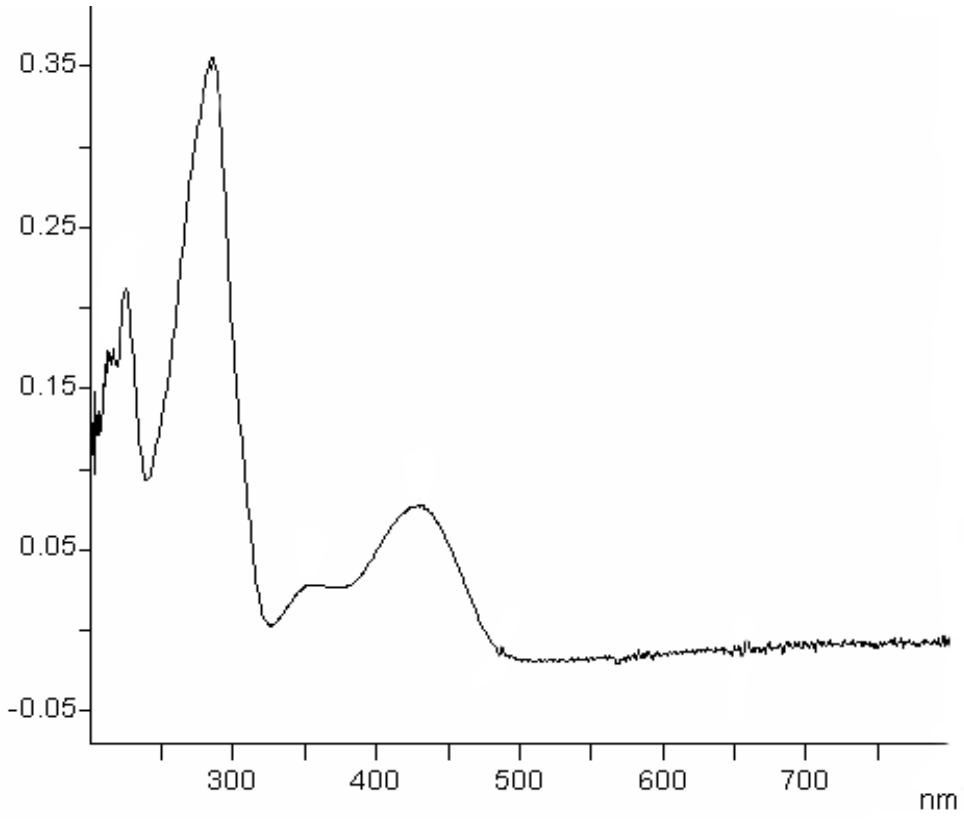


**Figure 86** UV ($CH_3OH$) spectrum of **SA13**

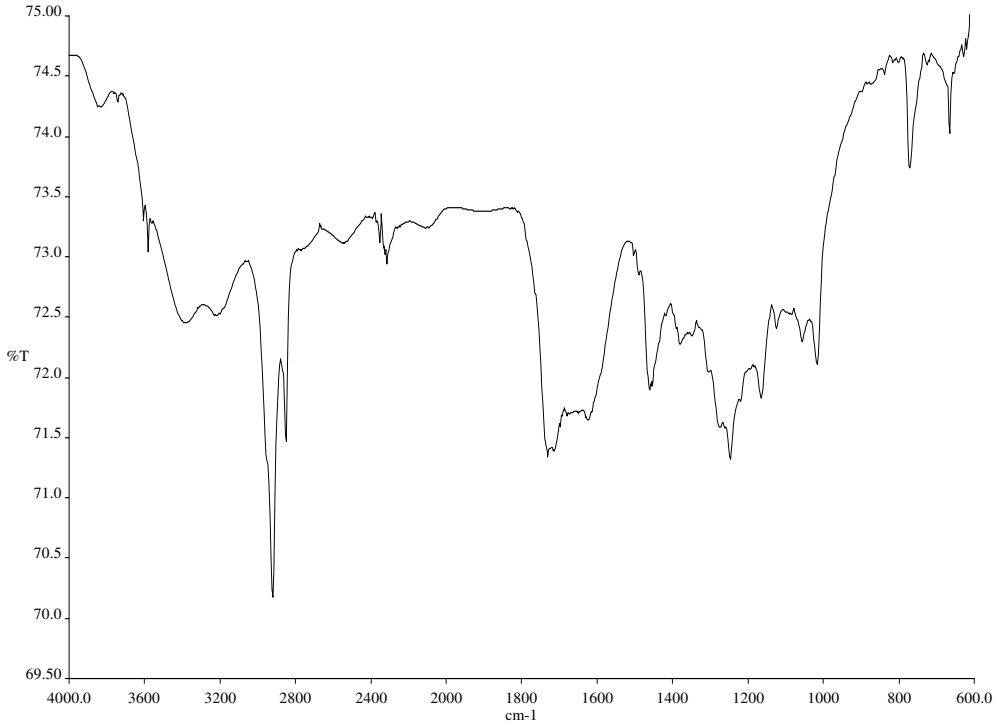


**Figure 87** FT-IR (Neat) spectrum of **SA13**

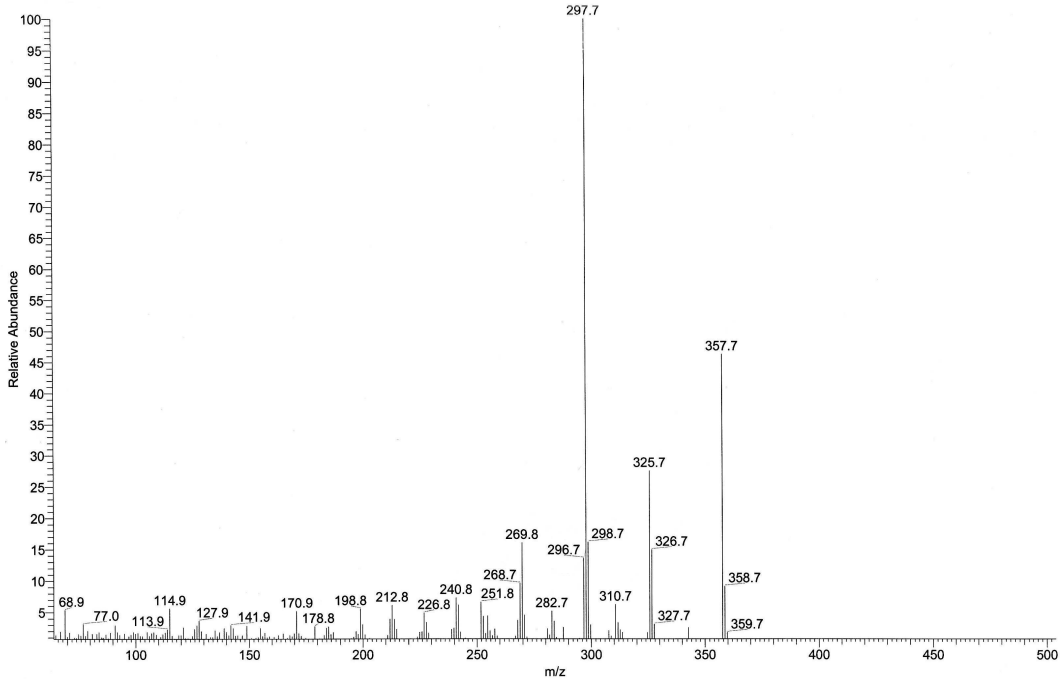


**Figure 88** EI-MS spectrum of **SA13**

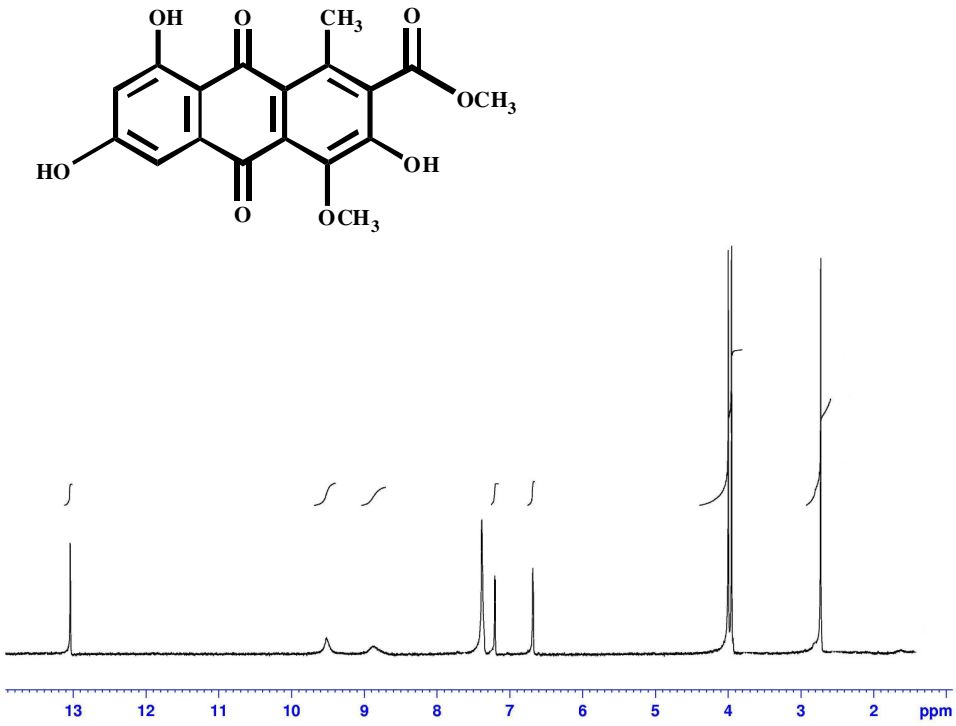


**Figure 89** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA13**

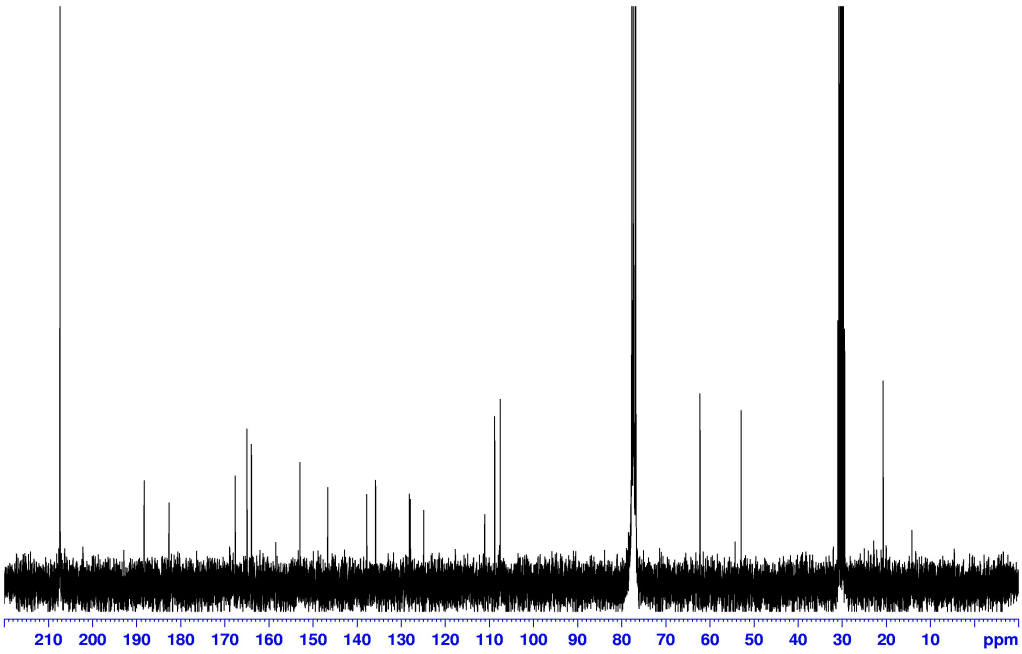


**Figure 90** $^{13}$C NMR (75 MHz) ($CDCl_3$) spectrum of **SA13**

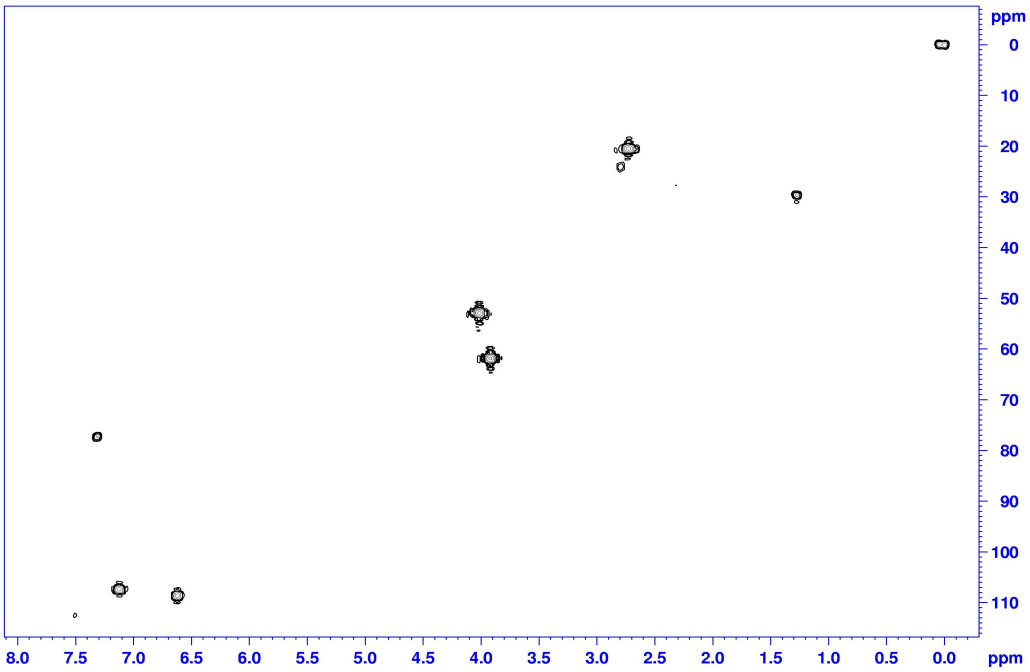


**Figure 91** 2D HMQC spectrum of **SA13**

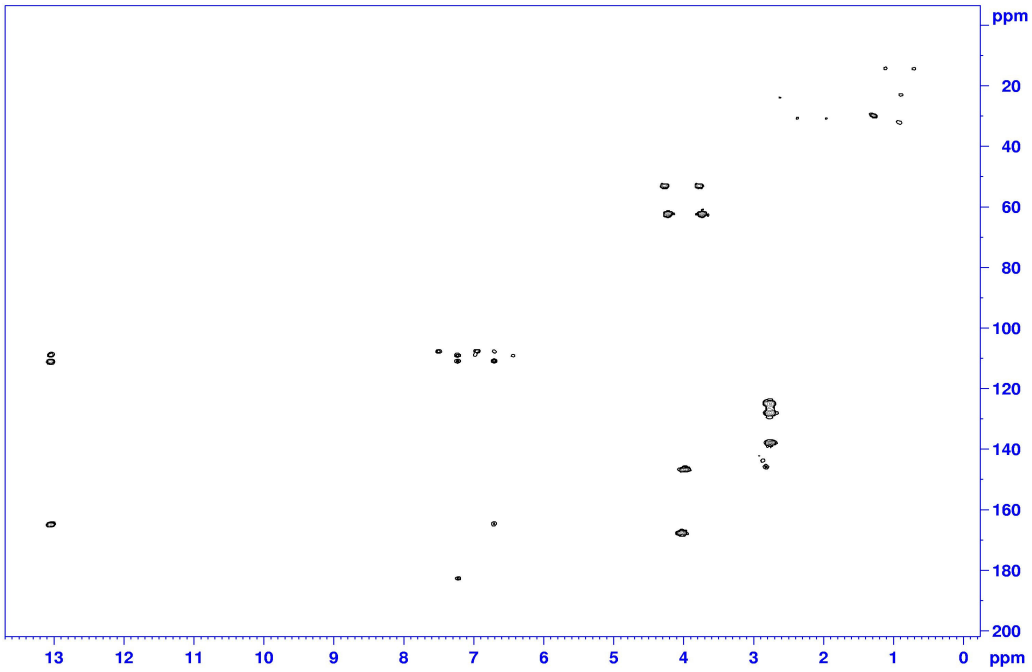


**Figure 92** 2D HMBC spectrum of **SA13**

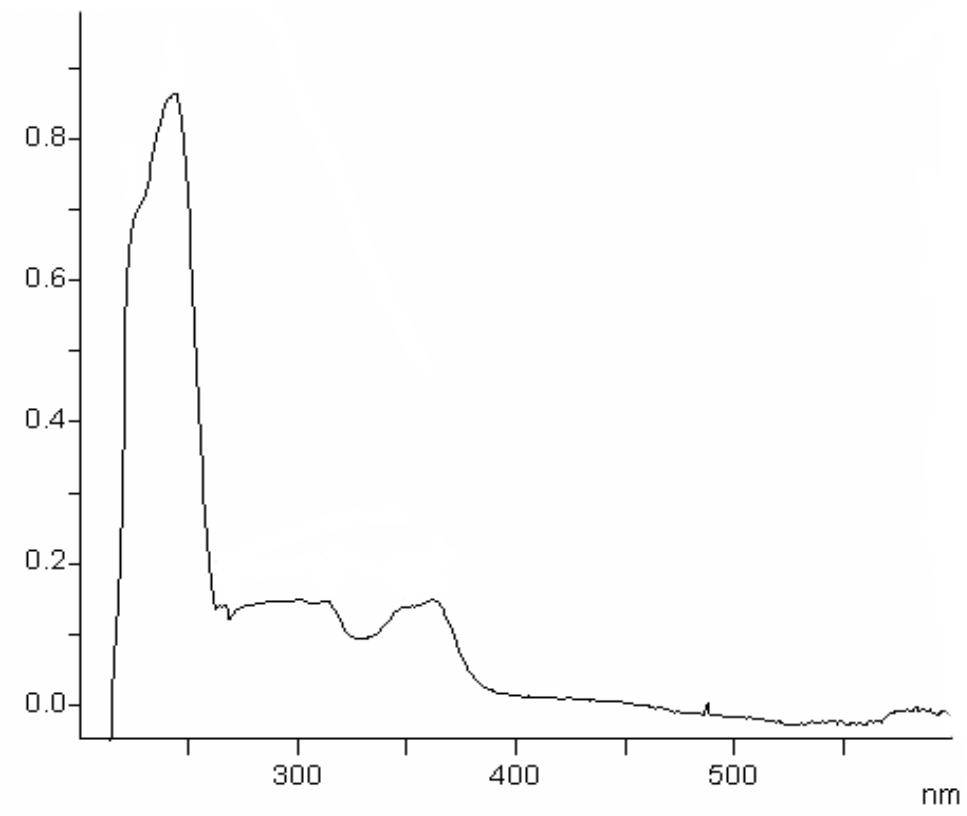


**Figure 93** UV ($CH_3OH$) spectrum of **SA14**

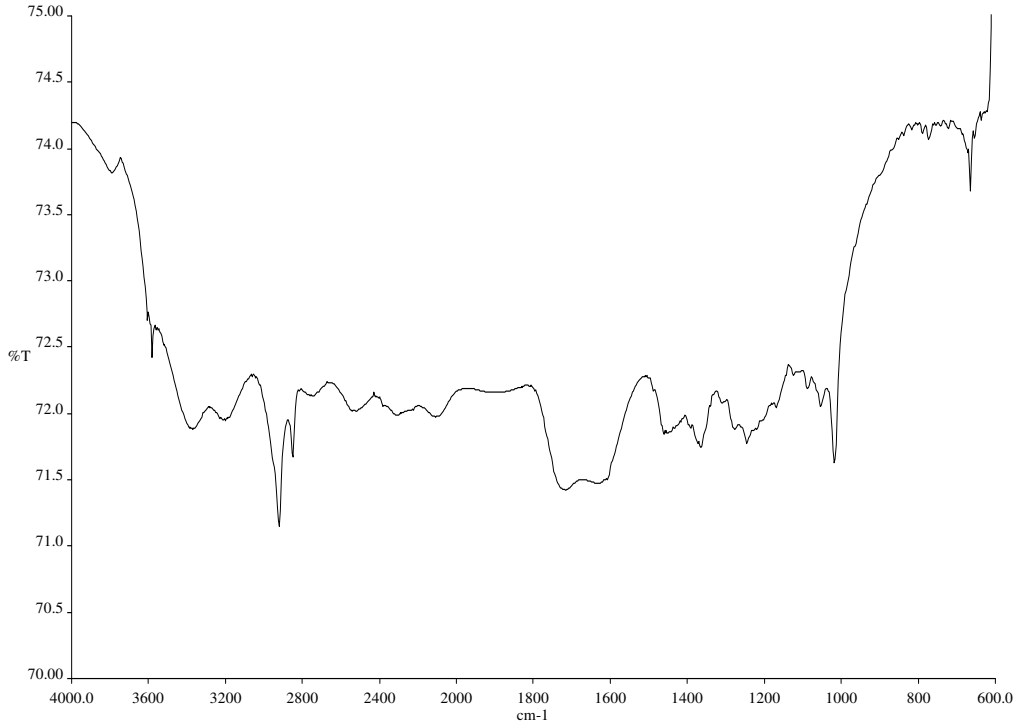


**Figure 94** FT-IR (Neat) spectrum of **SA14**

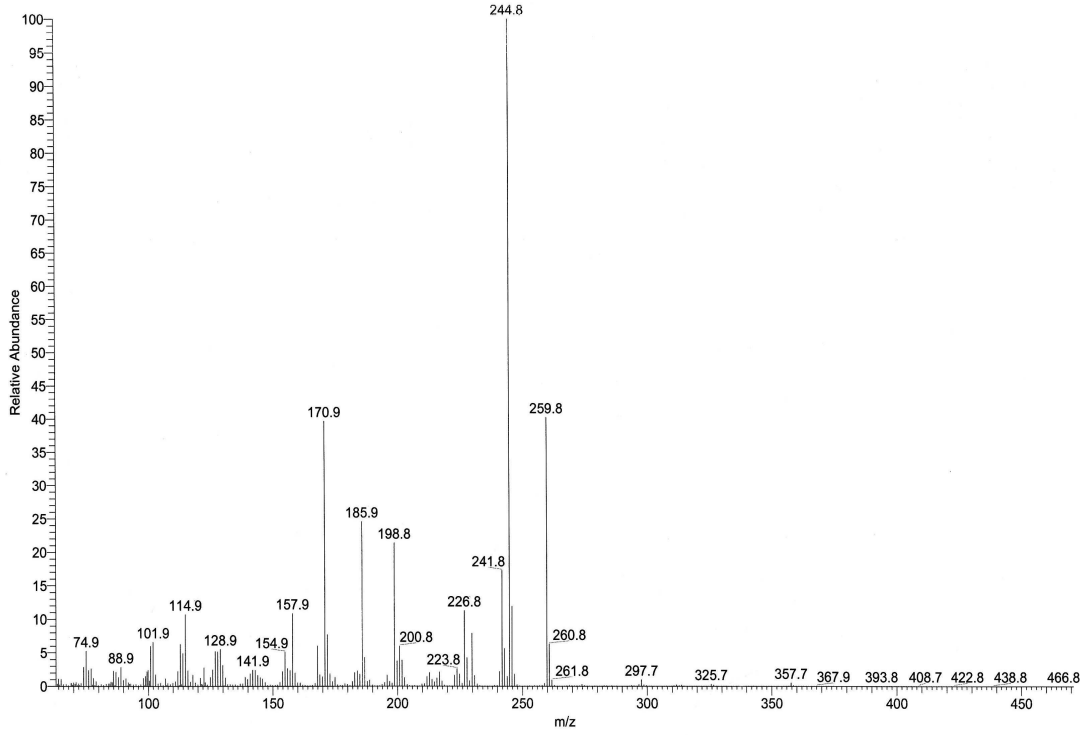


**Figure 95** EI-MS spectrum of **SA14**

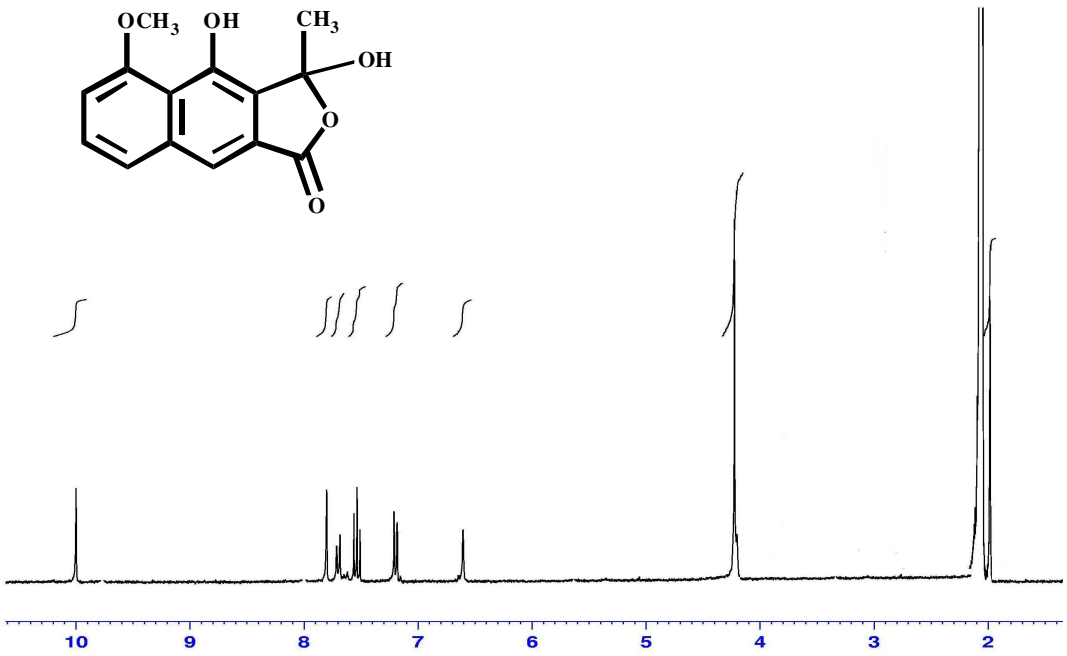


**Figure 96** $^{1}$H NMR (300 MHz) (Acetone-$d_6$) spectrum of **SA14**

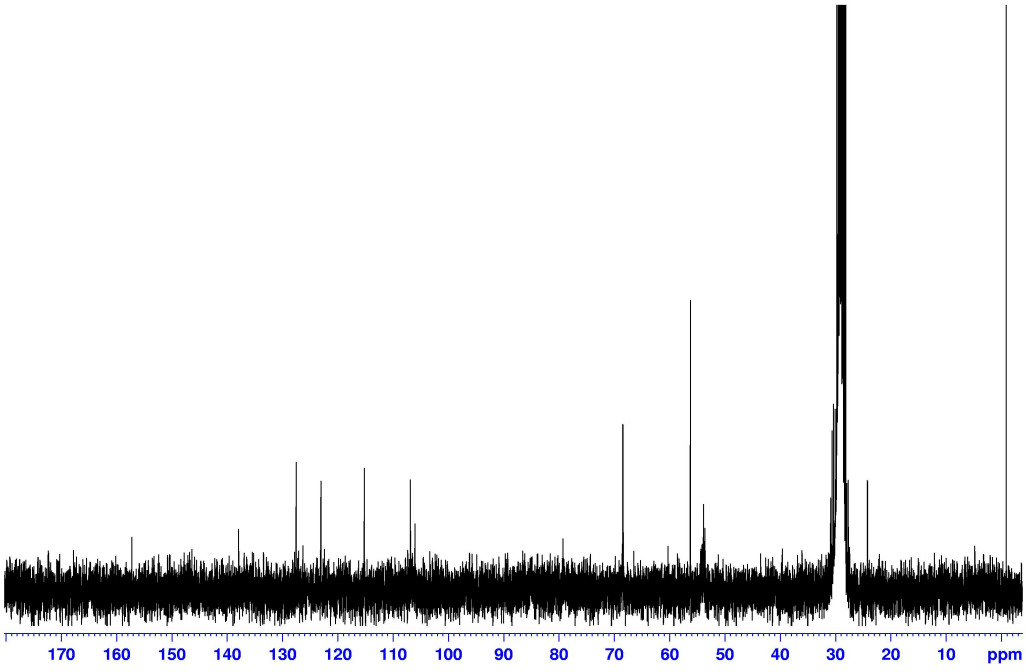


**Figure 97** $^{13}$C NMR (75 MHz) (Acetone-$d_6$) spectrum of **SA14**

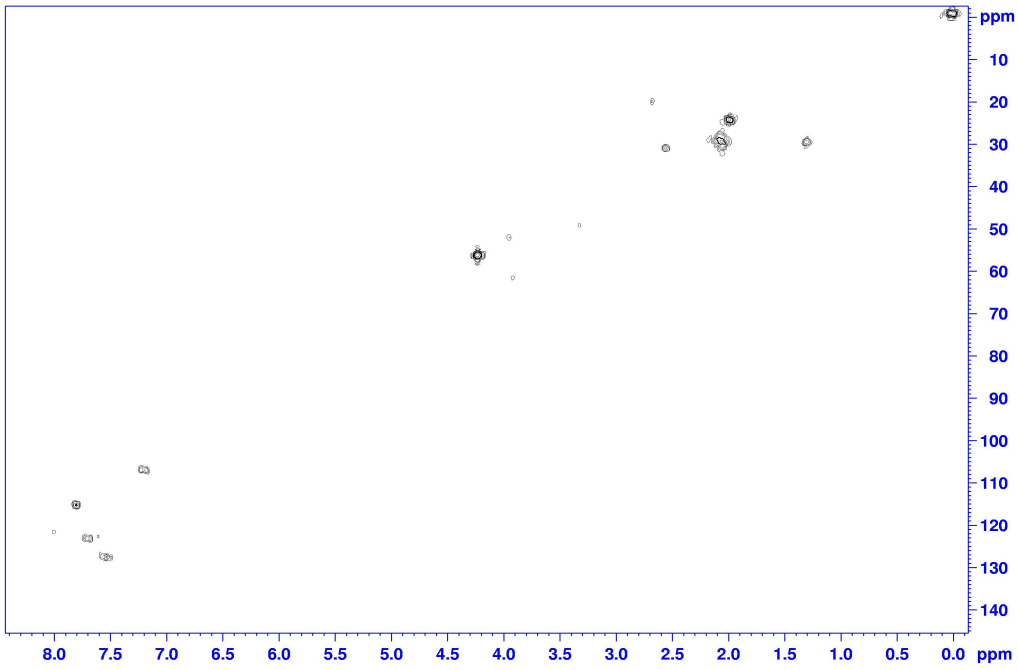


**Figure 98** 2D HMQC spectrum of **SA14**

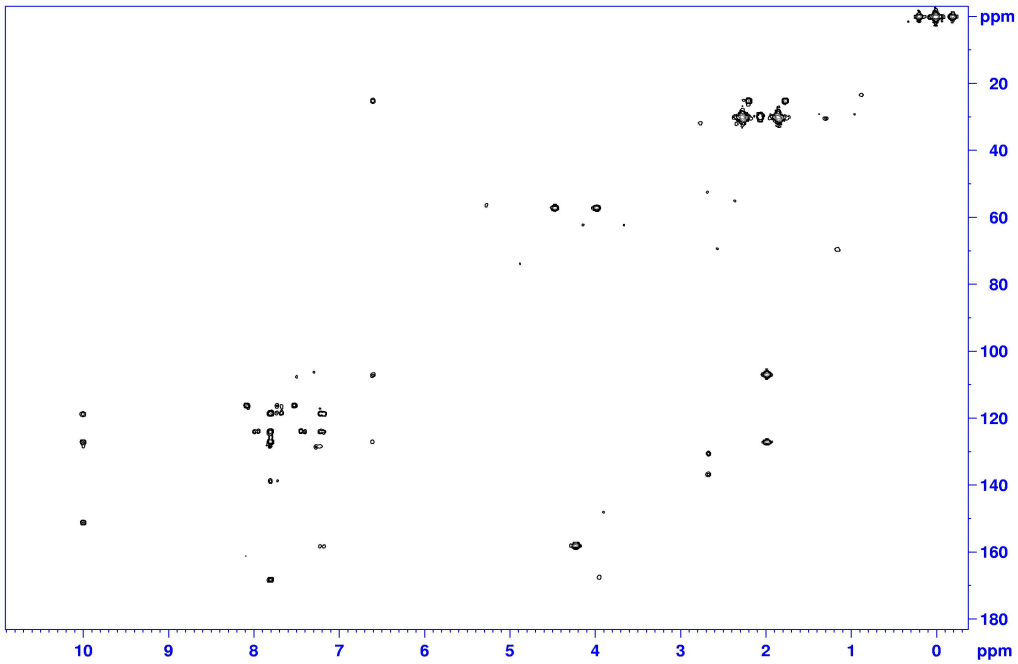


**Figure 99** 2D HMBC spectrum of **SA14**

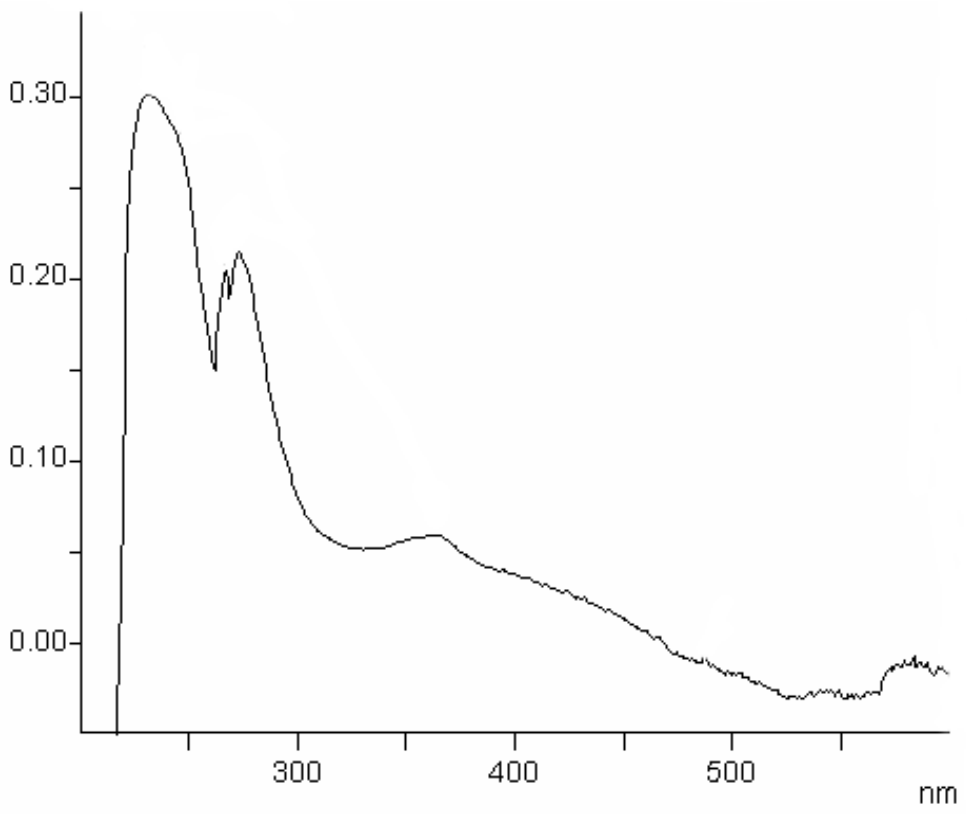


**Figure 100** UV ($CH_3OH$) spectrum of **SA15**

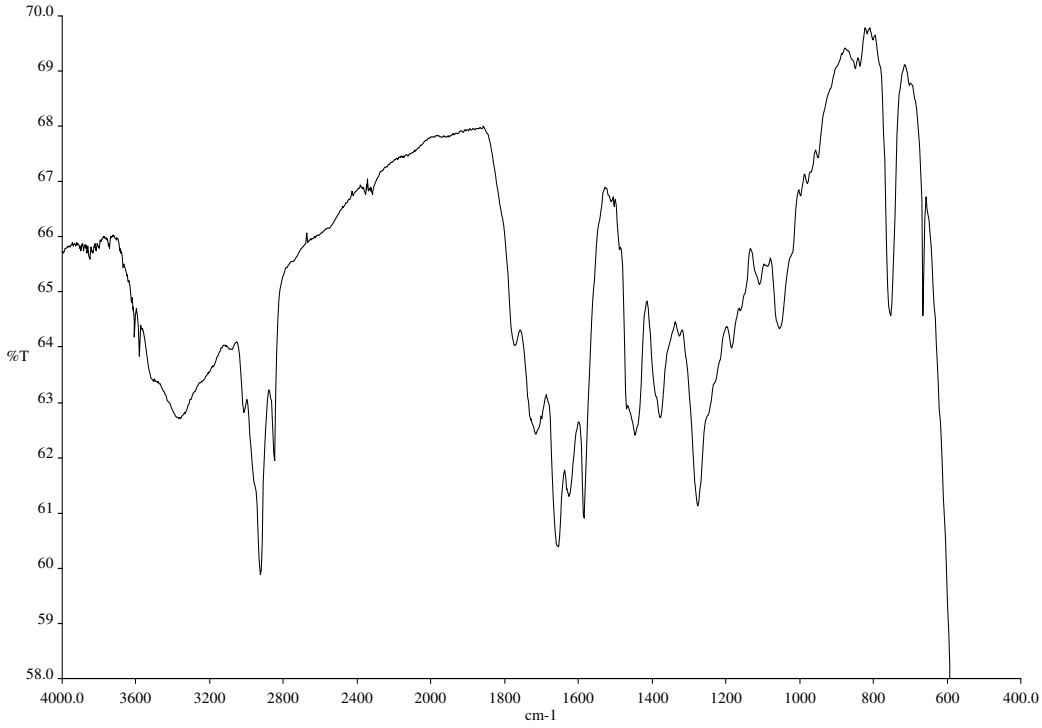


**Figure 101** FT-IR (Neat) spectrum of **SA15**

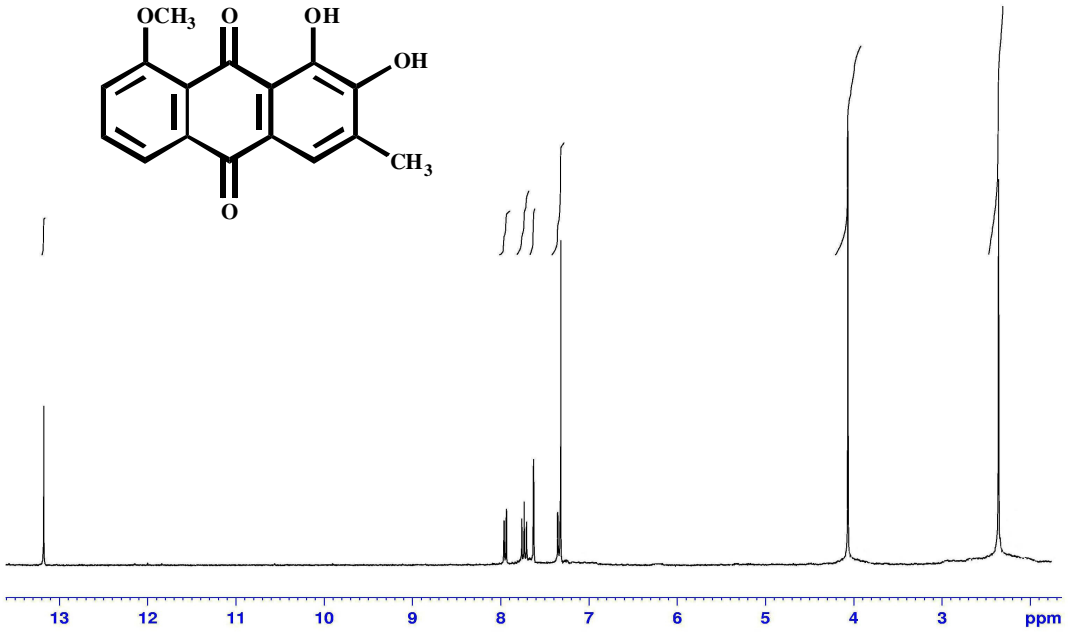


**Figure 102** $^{1}$H NMR (300 MHz) ($CDCl_3$) spectrum of **SA15**

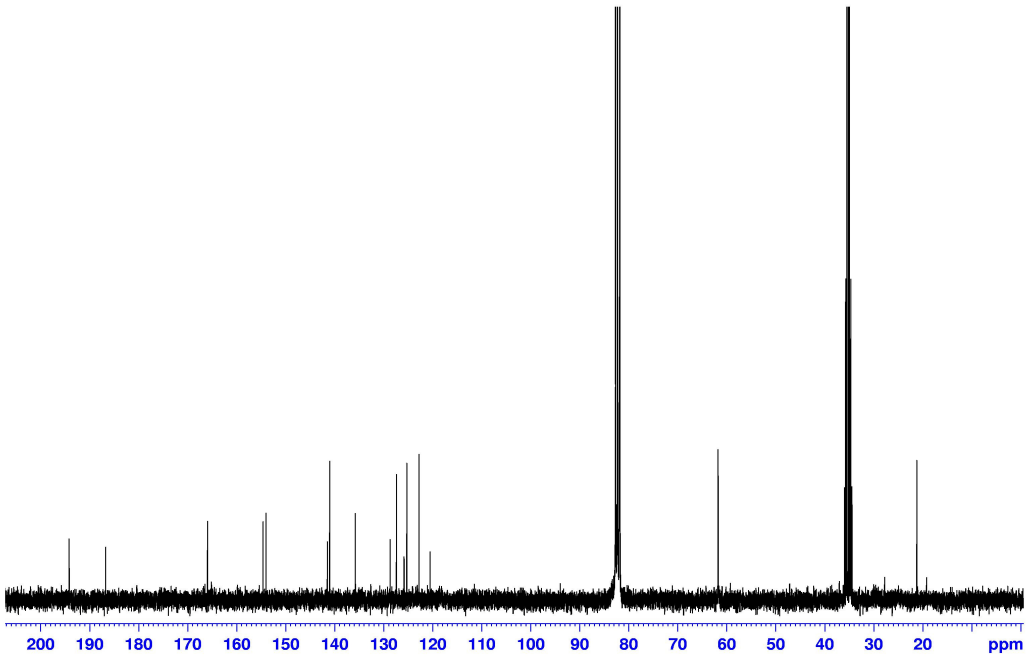


**Figure 103** $^{13}$C NMR (75 MHz) ($CDCl_3$) spectrum of **SA15**

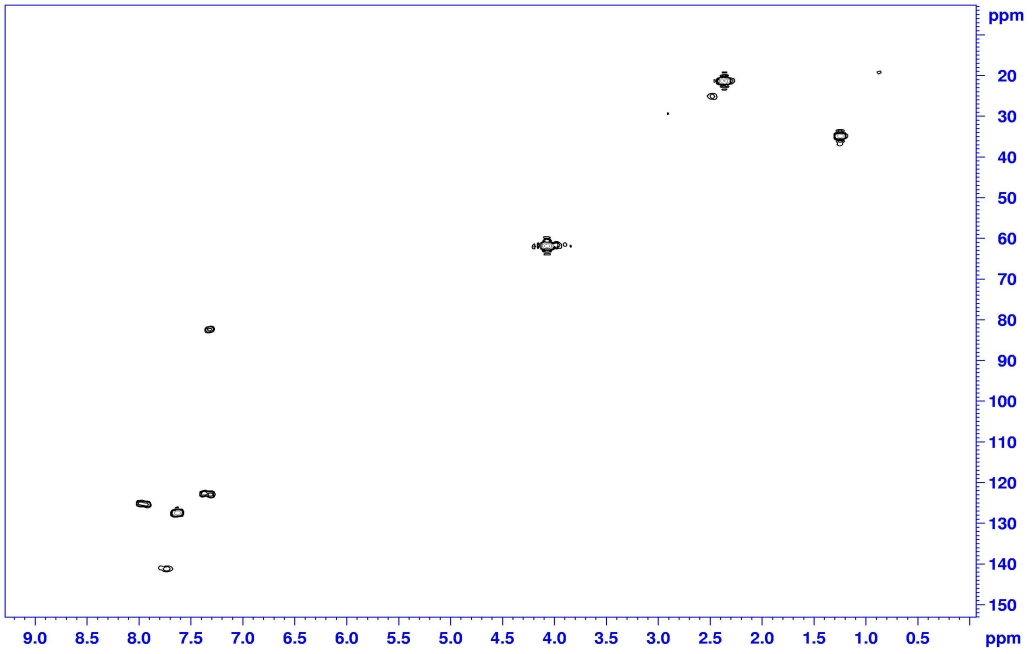


**Figure 104** 2D HMQC spectrum of **SA15**

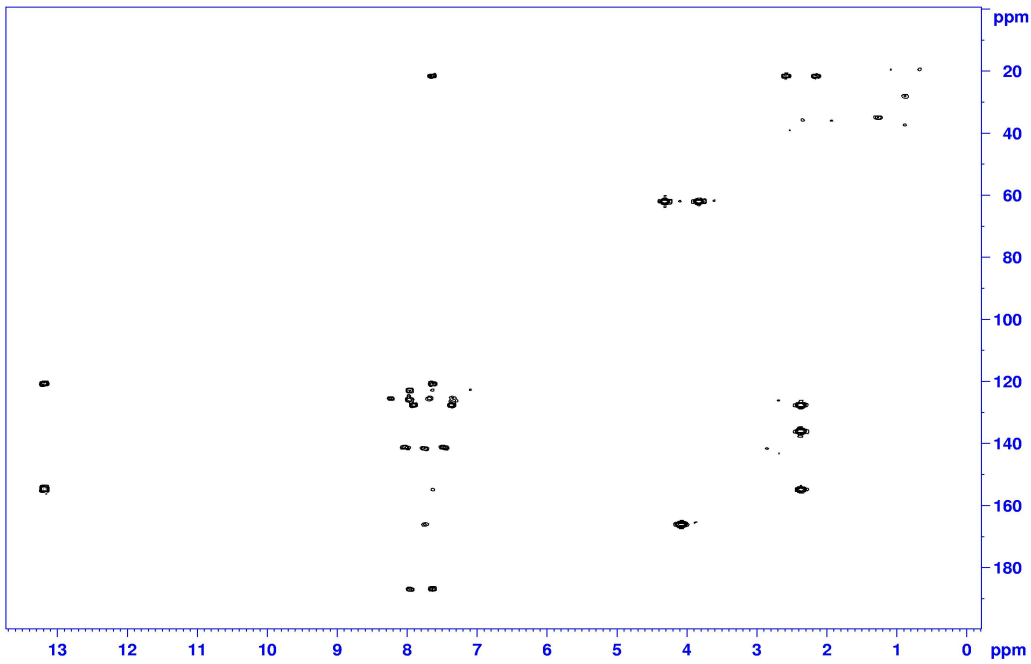


**Figure 105** 2D HMBC spectrum of **SA15**

# VITAE

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**Student ID** 4910220029

**Educational Attainment**

| Degree | Name of Institution | Year of Graduation |
|---|---|---|
| Bachelor of Science (Education) | Prince of Songkla University | 2005 |

**List of Publication and Proceeding**

1. Chulida Hemtasin, Wilawan Mahabusarakam, Supayang Voravuthikunchai and Wumi Ifesan. "A new naphthoquinone from the Bulb of *Eleutherine americana* (Aubl.) Merr" The 6th Princess Chulabhorn International Congress, Shangri-La Hotel, Bangkok, Thailand, 25-29 November 2007. (Poster presentation)
2. Chulida Hemtasin, Wilawan Mahabusarakam and Suda Chakthong. "Chemical constituents of the Bulb of *Eleutherine americana* (Aubl.) Merr" 9th National Grade Research Conference, Burapha University, Chonburi, Thailand, 14-15 March 2008. (Poster presentation)