# CHEMICAL SYNTHESIS OF 1,3,8-TRIOXYGENATED XANTHONE DERIVATIVES AND THEIR CYTOTOXIC ACTIVITIES

By

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## ABSTRACT

Nowadays there is more and more interest in the synthesis of xanthones and its derivatives due to their broad usage and pharmaceuticals activities. In this study, four xanthonic compounds were successfully synthesized and identified, which were 1,3,8-trihydroxyxanthone, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one, 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione and 1,8-hydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one. They were labeled as SCD 5, SCE 22, SCR 5 and SCT 2, respectively. 1,3,8-Trihydroxyxanthone was a xanthone building block, and it was synthesized through Grover, Shah and Shah method. This xanthone building block was then subjected to prenylation reactions in both organic and aqueous media. Prenylation in organic medium gave 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one, while 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione and 1,8-hydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one were obtained from the prenylation carried out in aqueous medium. The structures of these compounds were elucidated by means of IR, UV-Vis, MS and NMR (1D-NMR and 2D-NMR) techniques.

Both 1,3,8-trihydroxyxanthone and 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one were subjected to cytotoxic assay toward HeLa and MDA-MB-231 cancer cell lines via MTT colorimetric method. Cytotoxic assay results indicated that HeLa cancer cells were strongly susceptible to 1,3,8-trihydroxyxanthone with an $IC_{50}$ value of 5.5 μg/mL. However, the compound gave no significant activity against MDA-MB-231 cancer cell line with $IC_{50}$ value more than 50 μg/mL. On the other hand, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one gave insignificant inhibitory activity with $IC_{50}$ value more than 50 μg/mL toward the two cell lines tested.

# ABSTRAK

Baru-baru ini, ramai penyelidik telah menunjukkan perhatian mereka ke atas xanthone disebabkan kepelbagaian kegunaan dan kegiatan farmakologinya. Dalam kajian ini, empat sebatian xanthone telah berjaya dihasilkan dan dikenalpastikan, iaitu 1,3,8-trihidroksixanthone, 1,8-dihidroksi-3-(3-metil-but-2-enylosi)-xanthen-9-one, 8-hidroksi-2,2,4,4-tetrakis-(3-metil-but-2-enil)-4H-xanthene-1,3,9-trione dan 1,8-hidroksi-2-(3-metil-but-2-enil)-3-(3-metil-but-2-enylosi)-xanthen-9-one. Keempat-empat sebatian ini telah dilabelkan sebagai SCD 5, SCE 22, SCR 5 dan SCT 2 masing-masing. 1,3,8-Trihidroksixanthone telah disintesis melalui kaedah Grover, Shah dan Shah dan ia seterusnya digunakan sebagai bahan permulaan untuk prenilasi dalam pelarut organic dan juga air. Melalui sintesis dalam pelarut organik, 1,8-dihidroksi-3-(3-metil-but-2-enylosi)-xanthen-9-one telah dihasilkan, sebaliknya, 8-hidroksi-2,2,4,4-tetrakis-(3-metil-but-2-enil)-4H-xanthene-1,3,9-trione dan 1,8-hidroksi-2-(3-metil-but-2-enil)-3-(3-metil-but-2-enyloxy)-xanthen-9-one telah berjaya diperolehi dari prenilasi dalam keadaan air sebagai pelarut. Struktur ketiga-tiga sebatian ini disahkan melalui teknikal IR, UV-Vis, MS dan also NMR (1D-NMR dan 2D-NMR).

Selain itu, kedua-dua jenis xanthone, 1,3,8-trihidroksixanthone dan 1,8-dihidroksi-3-(3-metil-but-2-enylosi)-xanthen-9-one telah diuji terhadap sel

kanser HeLa dan sel kanser MDA-MB-231 dengan menggunakan kaedah MTT. Di antara sebatian-sebatian di atas, didapati 1,3,8-trihidroksixanthone menunjukkan aktiviti yang mampu menghalang pertumbuhan *in vitro* sel kanser HeLa dengan nilai $IC_{50}$ 5.5 μg/mL. Walaupun sebation ini menunjuk activity ke atas sel kanser HeLa, tetapi ia tidak memberi kesan terhadap sel kanser MDA-MB-231 dengan nilai $IC_{50}$ lebih daripada 50.0 μg/mL. O-prenylasi xanthone, 1,8-dihidroksi-3-(3-metil-but-2-enylosi)-xanthen-9-one tidak memberi kesan yang nyata terhadap kedua-dua jenis sel kanser dengan nilai $IC_{50}$ lebih daripada 50.0 μg/mL.

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solving and scientific discussion. It has been a pleasant time working with them. Without their support and help, this research work would not been successful.

## APPROVAL SHEET

I certify that, this project report entitled "**CHEMICAL SYNTHESIS OF 1,3,8-TRIOXYGENATED XANTHONE DERIVATIVES AND THEIR CYTOTOXIC ACTIVITIES"** was prepared by **TAN SU CHIN** and submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (Hons.) in Chemistry at Universiti Tunku Abdul Rahman.

**Approved by**

Supervisor

_____ Date: _____

(Dr. Lim Chan Kiang)

**FACULTY OF SCIENCE**

**UNIVERSITI TUNKU ABDUL RAHMAN**

Date: _____

**PERMISSION SHEET**

It is hereby certified that **TAN SU CHIN** (ID No: **08ADB05467**) has completed this report entitled **"CHEMICAL SYNTHESIS OF 1,3,8-TRIOXYGENATED XANTHONE DERIVATIVES AND THEIR CYTOTOXIC ACTIVITIES"** under the supervision of **DR. LIM CHAN KIANG** from the Department Science, Faculty of Science.

I hereby give the permission to my supervisor to write and prepare manuscript of these research findings for publishing in any form, if I did not prepare it within six (6) months time from this date provided that my name is included as one of the authors for this article. Arrangement of my name depends on my supervisor.

## DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that is has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

_____

( TAN SU CHIN )

Date:_____

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| | |
|---|---|
| δ | Chemical shift in ppm |
| $^{o}$C | Celsius |
| % | Percent |
| $^{1}$H | Proton |
| $^{13}$C | Carbon-13 |
| cm | Centimeter |
| d | Doublet |
| dd | Doublet of doublet |
| ds | Doublet of singlet |
| g | gram |
| h | Hour |
| HMBC | Heteronuclear Multiple Bond Coherence |
| HMQC | Heteronuclear Multiple Quantum Coherence |
| Hz | Hertz |
| IR | Infrared |
| *J* | Coupling constant in Hz |
| *m* | *meta* |
| min | Minute |
| mg | Milligram |
| ml | Milliliter |
| mmol | Milimole |
| mol | mole |
| MHz | Megahertz |
| MS | Mass Spectrometry |
| nm | Nanometer |
| NMR | Nuclear Magnetic Resonance |
| *o* | *ortho* |
| ppm | Parts per million |

| | |
|---|---|
| rt | Room temperature |
| s | Singlet |
| t | Triplet |
| TLC | Thin Layer Chromatography |
| μL | Microliter |
| μm | Micrometer |
| UV-Vis | Ultraviolet-Visible |

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction to Xanthone

Xanthones are a class of yellow coloration heterocyclic organic compounds containing oxygen, with a molecular formula of $C_{13}H_8O_2$. Chemically, xanthones have dibenzo-γ-pyrone as the basic skeleton (Figure 1.1). The xanthone basic skeleton is numbered with carbons 1-4 being assigned as acetate-derived ring A and carbon 5-8 assigned as shikimate-derived ring B. The other carbon atoms are then being assigned as 4a, 5a, 8a, 9 and 9a for structure elucidation purposes (Pedro *et al.*, 2002).

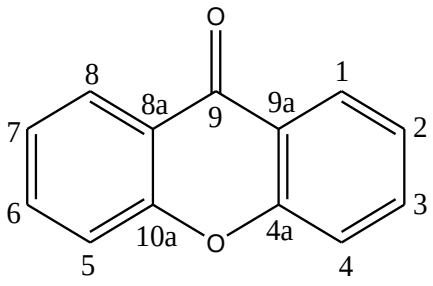



**Figure 1.1: Dibenzo-γ-pyrone**

The general skeleton of xanthone comprises of a planar three-membered ring structure as a backbone which consists of two benzene rings fused together through a carbonyl group and an oxygen atom. Both of these benzene rings are identical to each other, hence it imparts symmetry system in the structure. Due to the multiple double bonds on xanthone structure, there is limitation to free rotation and hence an increase in the rigidity of structure. This rigid framework imparts a tough stability which capable to resist extreme high temperature while remains its integrity.

## 1.2 History of Xanthone

Long time ago, the important to get the right amount of antioxidants or nutrients in order to keep our bodies healthy and ward of disease were more and more mentioned by people. To date, there's a new classification of antioxidants called xanthones**,** first discovered in the mangosteen fruit, which are poised to be one of the biggest discoveries of modern science in terms of health benefits.

In the late $19^{th}$ century, an English botanist, David Fairchild who commented on the gastronomical value of the mangosteen: "It outranks in delicacy, if not all the fruits in the world, certainly all others of the tropical

zone, and it is a joy to the eye as well as to palate to feast on mangosteens" (Fairchild, 1915). Besides Fairchild, there also found that the traditional medical value of mangosteens was confirmed in Ming Dynasty by Benemerito at year 1936. In addition, these mangosteen's medicinal properties also further confirmed by Aryuvedic sages of India (Pedraza-Chaverri *et al.*, 2008).

As a matter of fact, xanthones were first discovered when scientists began studying the health benefits and antioxidants of mangosteen based on its traditional indigenous medical uses. Mangosteens contained major type of polyphenolic xanthone derivatives (Bennett and Lee, 1989) which have a great deal interest in the medicine. In 1855, the first xanthone (*mangostin*) was isolated by Schmid W., who was a German chemist. More recently, many researchers around the globe became curious about the traditional uses of xanthones and began doing experiments and scientific studies on xanthones and mangosteens.

**Figure 1.2: The fruit of *Garcinia mangostana***

## 1.3 Occurrence and Natural Distribution of Xanthone

In 1961, chemist Roberts noticed that polyphenolic xanthones had been isolated from lower fungi, lichens and from only three families which were Gentianaceae, Guttiferae and Anacardiaceae. In 1992, Mandal *et al.* found that many plants belonging to other 20 families also produced xanthones. They noticed that Gentianaceae and Guttiferae families were, in this order, the principal sources of xanthone derivatives.

Besides Gentianaceae, Guttiferae and Anacardiaceae, the family Clusiaceae also one of the families which produced secondary metabolites, xanthones (Peres and Nagem, 1996; Peres *et al*., 2000). *Garcinia mangostana* L. (Clusiaceae) commonly known as mangosteen can be found in the region of Southeast Asia. The fruit and pericarp of *G. mangostana* have been used as a traditional medicine in Southeast Asia for treatment of diarrhea, dysentery, inflammation and ulcers.

The xanthones isolated from natural product can be classified into six main groups, which are simple xanthones, xanthone glycosides, prenylated xanthones, xanthonolignoids, bis-xanthones, and miscellaneous xanthones (Sultanbawa, 1980; Jiang *et al*., 2004).

Xanthones and its derivatives have a variety of biological and cytotoxic activities, such as antioxidant, anticancer and antifatigue due to their unique backbone along with type and position of the attached chemical groups define specific functionalities of xanthones. The interesting xanthone scaffold and its biological efficacy enforced many researchers try to isolate and obtain new xanthones through synthetic method.

## 1.4 Synthetic Xanthone

Due to the xanthones that obtained from extraction of natural products are relatively limited in the type and substituent's position, synthesis of new xanthones become increasingly important. Through synthetic methods, possibilities of new xanthones with different nature and position of substituent on the xanthone building block can be obtained.

There are two synthetic ways used to synthesize new xanthones and its derivatives, which are biosynthesis and chemical synthesis. Biosynthesis involves enzymatic reactions in living organisms to produce various xanthone's derivatives from precursor units, while chemical synthesis involves catalytic reactions carried out in laboratory. In chemical synthesis, reaction of benzoic acid derivative with polyhydroxybenzene happens

with cyclization to enable both the benzene rings to fuse together forming a tricyclic structure of xanthones.

## 1.5 Biological Activities of Xanthone

Xanthones are tricyclic dibenzopyrans with diversed physicochemical and pharmacological activities. Commonly, different xanthones perform a general property which is helping to fight cancer. For instance, one study performed in Japan showed that the xanthone alpha-mangostin had apparent properties that suppressed the development of tumors. Another study in China found the xanthone garcinone E to show significant anticancer effects in terms of liver, lung, and gastric cancer cells (Kosem *et al.,* 2007).

In addition, some xanthones have shown significant antimicrobial effects in fighting MRSA (methicillin-resistant *staphylococcus aureus*), which is a bacterial strain known for its tenacious resistance to pharmacologic antibiotics. One scientific study also showed that alpha-mangostin, beta-mangostin, and garcinone B to have pronounced inhibition effects on a strain of tuberculosis (Pedraza- Chaverri *et al.,* 2008).

As a conclusion, xanthones are active against a variety of pathogens and have exhibited interesting pharmaceutical properties such as antimicrobial (Malet-Cascon *et al.*, 2003), anti-inflammatory (Lin *et al*., 1996), anticancer (Ho *et al*., 2002), antioxidant (Minami *et al.*, 1994), antifungal (Rocha *et al.*, 1994), and antiviral (Groweiss *et al*., 2000).

## 1.6 Objectives

The main purposes of this study are:

- To synthesize xanthone building block and its derivatives.
- To purify the crude xanthone building block and its derivatives by using different types of chromatography methods.
- To characterize the structure of compounds by means of FT-IR, 1D- and 2D-NMR, UV-Vis spectroscopic and mass spectrometric methods.
- To determine the cytotoxic activity of xanthone building block and its derivatives against HeLa and MDA-MB-231 cancer cell lines.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Sources of Xanthones

### 2.1.1 Natural Product

Xanthones are natural products that have been found a wide range of pharmacological properties (Peres *et al*., 2000). These biological properties include anticancer, antibacterial, antiplatelet aggregration, and inhibition of HIV-1 protease.

In natural, mangosteen is the richest known source of natural xanthones compounds, which has tremendous medical values (Radha and Mathew, 2007). Mangosteen or *Garcinia mangostana* is a member from the tree family of Clusiaceae or alternatively, Guttiferae and the genus- *Garcinia* is named after the French botanist, Laurent Garcinia (Ploetz, 2003). The pericarp of *Garcinia mangostana* is a source of xanthones and other bioactive substances. Hence, xanthones have been isolated from pericarp, as well as from whole fruit, heartwood, and leaves.

*Hypericum ascyron* (Pinarosa, 2005) is a species that widely distributed throughout temperate regions and it has been used as traditional medicine in the treatment of wounds, swelling, headache, nausea and abscesses. According to JiangSu New Medical College, there was found that flavonoids and xanthones have been confirmed as components of the *Hypericum ascyron*. Those flavonoids and xanthones were found to show a various bioactivities, including tumor-promoting inhibition (Ito *et al.*, 2003) and inhibitory effects on platelet-activating factor (Oku *et al.*, 2005).

Besides *Garcinia mangostana* and *Hypericum ascyron*, *Swertia chirayita* (Buch.-Ham.) which grows in the temperate regions also contains a number of xanthones. *Swertia chirayita* is used in Tibetan folk medicine as a traditional remedy for chronic fever, anaemia, asthma, liver disorder and hepatitis. These extracted xanthones possess hypoglycaemic activity (Rakesh *et al*., 1991).

Natural products, including xanthones are extracted sequentially using different solvent which are increase in polarity. The extracted xanthones are then separated by using chromatography and their structures have been elucidated based on UV-Vis, IR, MS and NMR data.

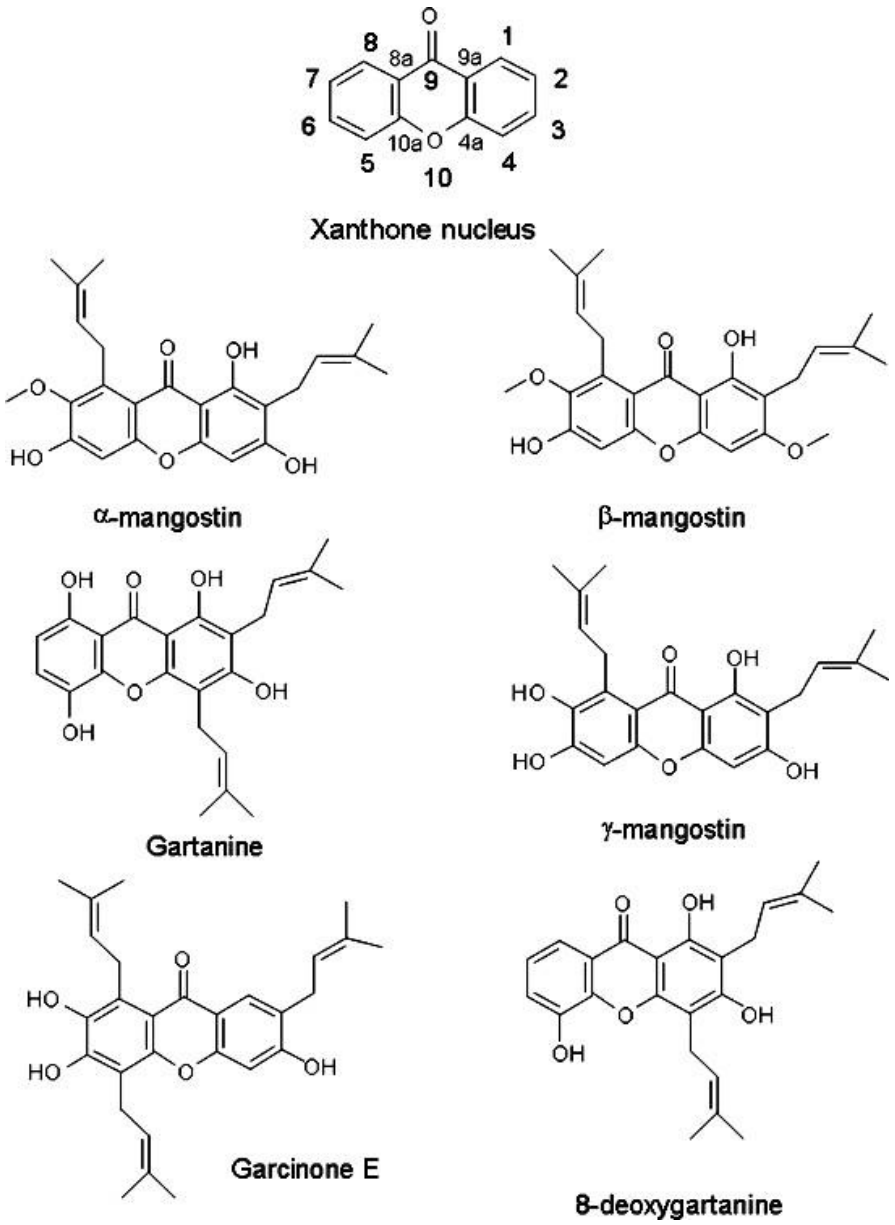



**Figure 2.1: Common xanthone structures extracted from natural products**

### 2.1.2 Biosynthesis of Xanthones

The biosynthetic pathways to xanthones have been discussed for 40 years. Biosynthesis is known as an enzyme-catalyzed process in cells of living organisms by which substrates are converted to more complex products, such as the conversion of extracted constituents from natural products to xanthones. Typically the biosynthesis process consists of several enzymatic steps in which the product of one step is used as starting material in the following step.

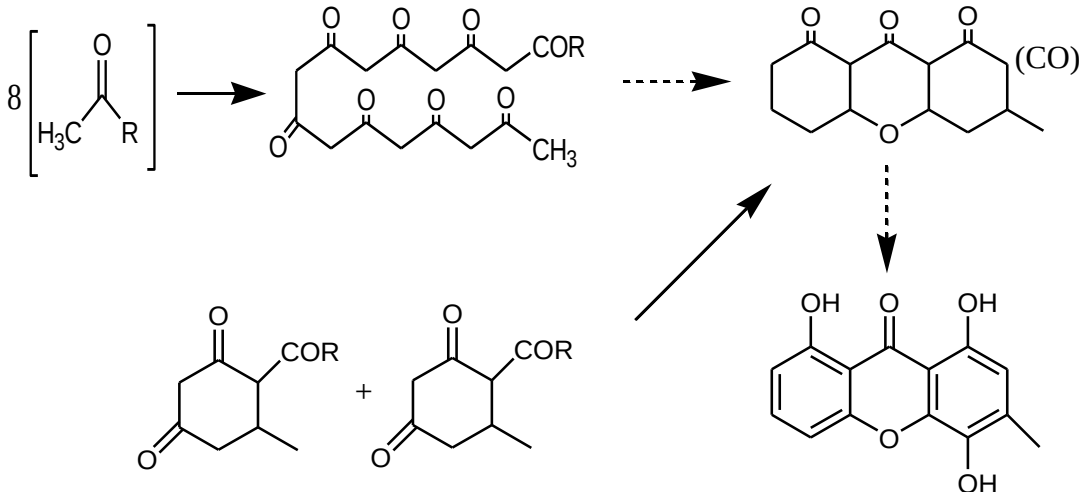
Nowadays, many researchers have studied on the biosynthetic pathway to xanthones in plants *in vivoly* and *in vitroly* with markers (Diderot *et al*., 2006). Through biosynthesis, researchers can inter-relate the observed oxygen patterns of natural xanthones and correlate natural xanthones with recognized oxygenation patterns. There are two processes which involve in biosynthesis.

(i) Acetate polymalonic route

(ii) Mixed shikimate acetate pathway

### 2.1.2.1 Acetate Polymalonic Route

Acetate polymalonic route is the biosynthesis method that used for some xanthones in lower plants such as micro-organisms and lichens. In this biosynthesis method, the end product, xanthone is totally derived from a total of seven acetate units (Diderot *et al*., 2006). Below is one of the illustrations of biosynthesis mechanisms of lower plant involved ravelnelin from *Helminthosporium ravenelii* proposed by Birch *et al*. (1976).




Ravenelin

**Figure 2.2: Biosynthesis of the xanthone ravenelin in lower plants**

### 2.1.2.2 Mixed Shikimate Acetate Pathway

Different from acetate polymalonic route, the mixed shikimate acetate pathway is the biosynthesis method applied to xanthones produced in higher plants. In the biosynthesis mechanism of this mixed shikimate acetate pathway, a phenylalanine, which is formed from shikimate by losing two carbon atoms from the side chain, is oxidized to form an *m*-hydroxybenzoic acid. This *m*-hydroxybenzoic acid is then combined with three units of acetate (via malonate) to produce the shikimate-acetate intermediate. Suitable folding and ring closure of this intermediate will give a substituted benzophenone, which generates a central ring of xanthone moiety by an oxidative phenol coupling reaction. The other mechanisms for the intramolecular reaction of benzophenone involve quinine addition and dehydration between hydroxyl groups on acetate- and shikimate-derived rings and subsequent rearrangement to form the xanthone (Diderot *et al*., 2006).

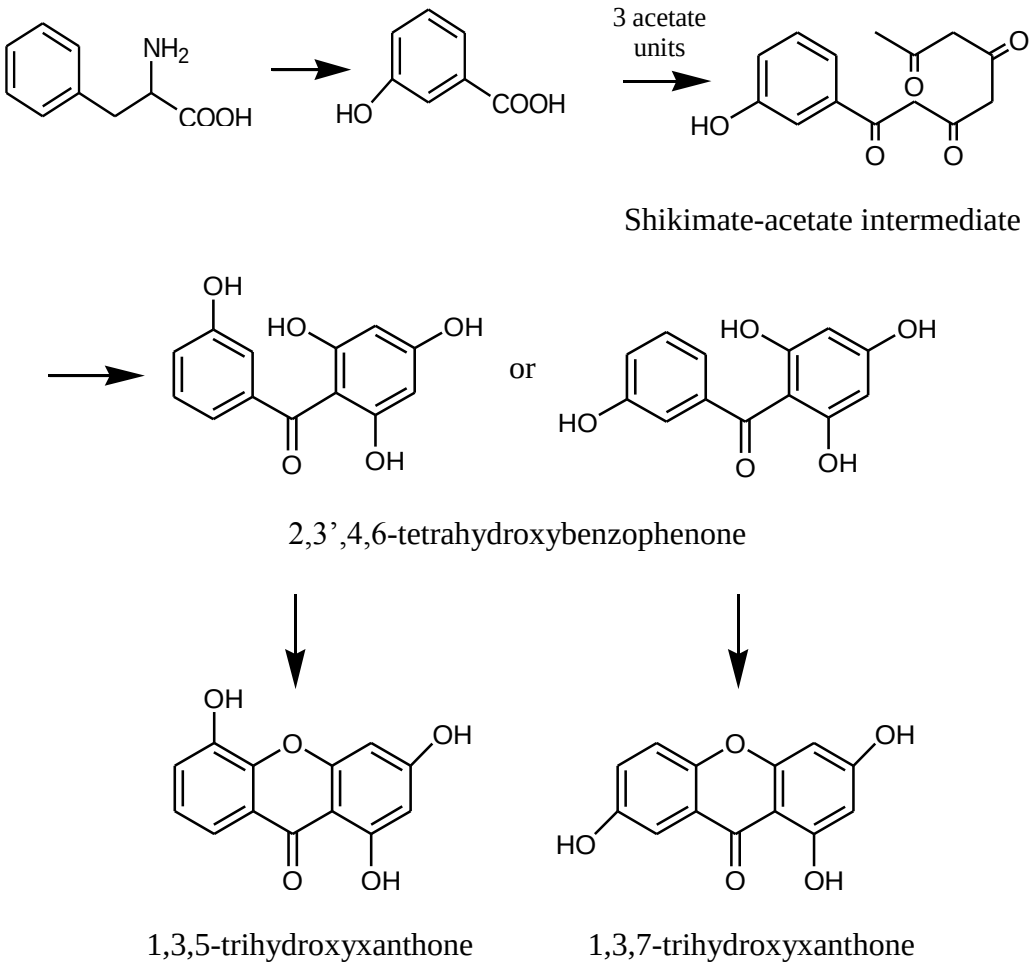



**Figure 2.3: Mixed shikimate acetate pathway**

### 2.1.3 Chemical Synthesis of Xanthones

Recently, xanthones are found to be interesting due to their diverse pharmacological activities. Although xanthones can obtained and isolated from natural products and through biosynthesis of natural products, but the studies of synthesized xanthones by laboratory skills are more focused since years back. The main reason why there is an increase interest in chemical synthesis is that synthetic approach can afford to prepare different type of xanthones with diverse structure. Moreover, through chemical synthesis, the percent yield of synthesized xanthones can be increased.

Nowadays, a lot of synthetic methods to synthesize xanthones have been designed. One of the methods for preparing xanthones is Nencki reaction (Sousa *et al.*, 2009) (Figure 2.4). By using zinc chloride as catalyst and dehydrating agent, xanthones and benzophenone are synthesized. However, this method only gives a low yield. Latter, in order to improve the yield percent, a few standard methods of synthesis of xanthones were introduced, which are:

(i) Grover, Shah and Shah reaction

(ii) Synthesis via benzophenone intermediate

(iii) Synthesis via diaryl ether intermediate

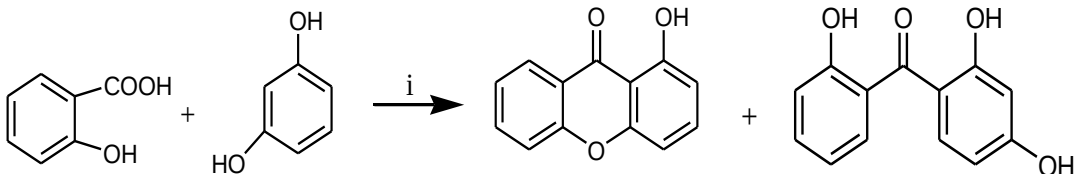



**Figure 2.4: Nencki reaction: (i) zinc chloride, 200°C/ 5 min – 180°C/ 4 h**

### 2.1.3.1 Grover, Shah and Shah Reaction

Through the Grover, Shah and Shah reaction, hydroxyxanthones can be easily prepared due to the ease of accessibility starting. In this synthetic method, it requires a polyphenol and a salicylic acid derivative as starting materials. Both of these starting materials are heated together with a mixture of phosphorus oxychloride and zinc chloride (Grover *et al.,* 1955). The using of a mixture of phosphorus oxychloride and zinc chloride as condensing agent obtained good results in yield percent (Figure 2.5).

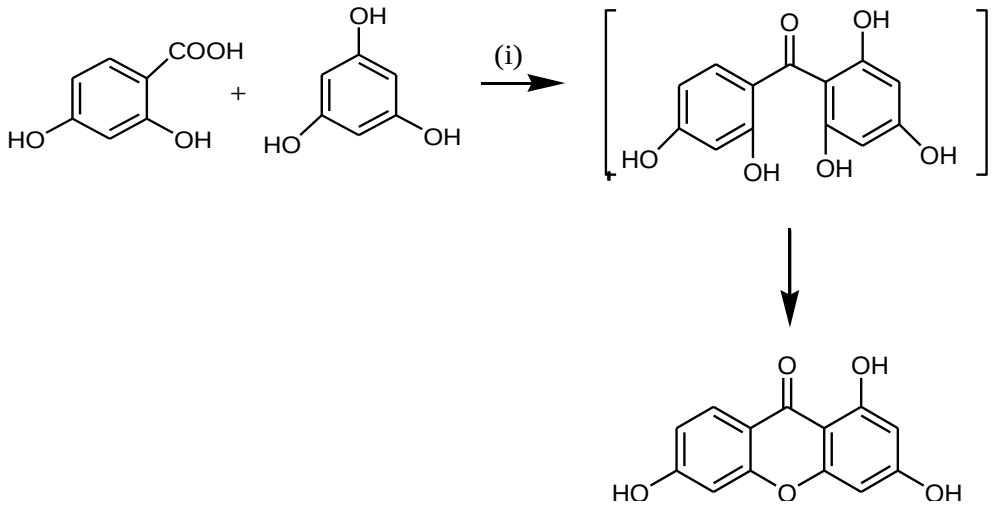



**Figure 2.5: Synthesis route for polyhydroxyxanthone. Reagents and conditions: (i) $ZnCl_2$, $POCl_3$, 70 $^oC$, 2h**

However, in this case, the benzophenone intermediate is being required. Besides, the Grover, Shah and Shah reaction consists of a number of limitations. For example, there is necessity of phenolic group to promote direct cyclization instead of formation of benzophenone intermediate. This may lower the overall yield of synthesized xanthones.

An alternative to overcome these limitations, there has been a modification to Grover, Shah and Shah reaction which can provide a high yield of xanthones without producing any benzophenone. This synthetic method involves the condensation between phoroglucinol and polyphenol. The use of Eaton's reagent (phosphorus pentoxide and methanesulfonic

acid: $P_2O_5$ / $CH_3SO_3H$) as the coupling agent and catalyst instead of mixture of a phosphorus oxychloride and zinc chloride (Sousa and Pinto, 2005) (Figure 2.6).

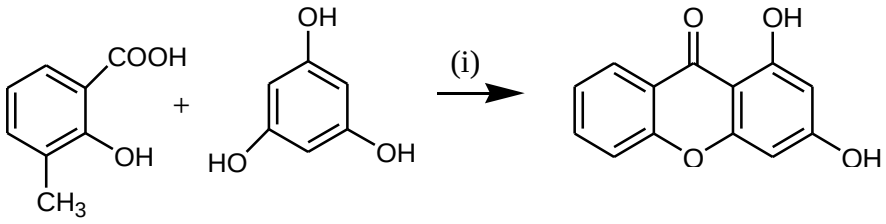



**Figure 2.6: Synthesis route for xanthones. Reagents and conditions: (i) $P_2O_5$ , $CH_3SO_3H$, 80 $^0$C, 20 min**

### 2.1.3.2 Synthesis of Xanthones via Benzophenone Intermediate

Commonly, intermediate benzophenone derivatives can be obtained through condensation by Friedal-Crafts acylation of appropriately substituted benzoyl chlorides with phenolic derivatives involving the formation of benzophenone intermediate, 2-hydroxy-2'-methoxy-benzophenones. Then, the dehydration process causes the cyclization of 2-hydroxy-2'-methoxy-benzophenones to xanthone (Barton and Scott, 1958) (Figure 2.7).

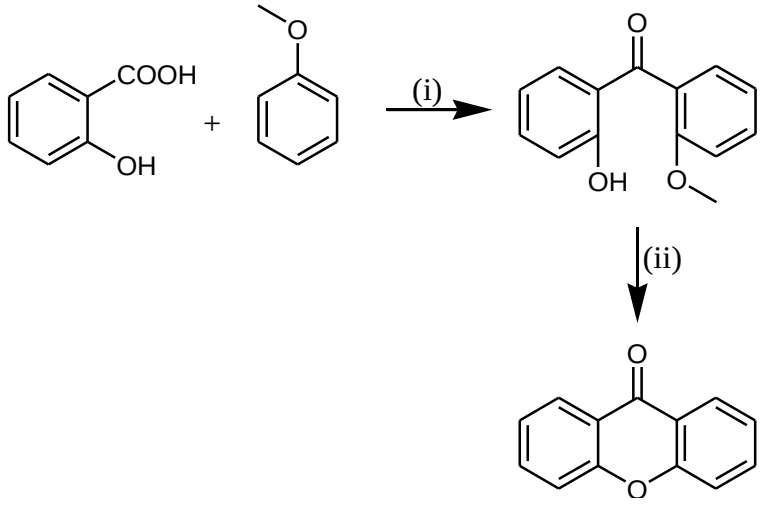



**Figure 2.7: Synthesis route for xanthones nucleus. Reagents and conditions: (i) $AlCl_3$, dry ether, rt, 1h; (ii) NaOH, methanol, reflux, 6 h**

Through Friedel-Crafts acylation, the synthesis of benzophenone intermediate as precursors for cyclization to xanthones can be achieved conveniently. In the case of Friedel-Crafts acylation of methoxybenzene derivatives and substituted benzoyl chloride in ether, the acylation process occurs adjacent to the methoxy group, and a selective demethylation in the presence of aluminium trichloride occurs at the *ortho* position to the carbonyl group. In fact, this *o*-monodemethylation of the substituted benzophenone intermediate through cyclization by elimination of methanol can occur by extending the reaction time to 30 hours (Figure 2.8).

In addition, there is modification of the Friedel-Crafts reaction reported by Elix *et al*., which involves the acylation process in the presence of TFAA, demethylations and subsequent cyclization of benzophenone in aqueous medium under pressurized heating (Elix *et al*., 1993) (Figure 2.9).

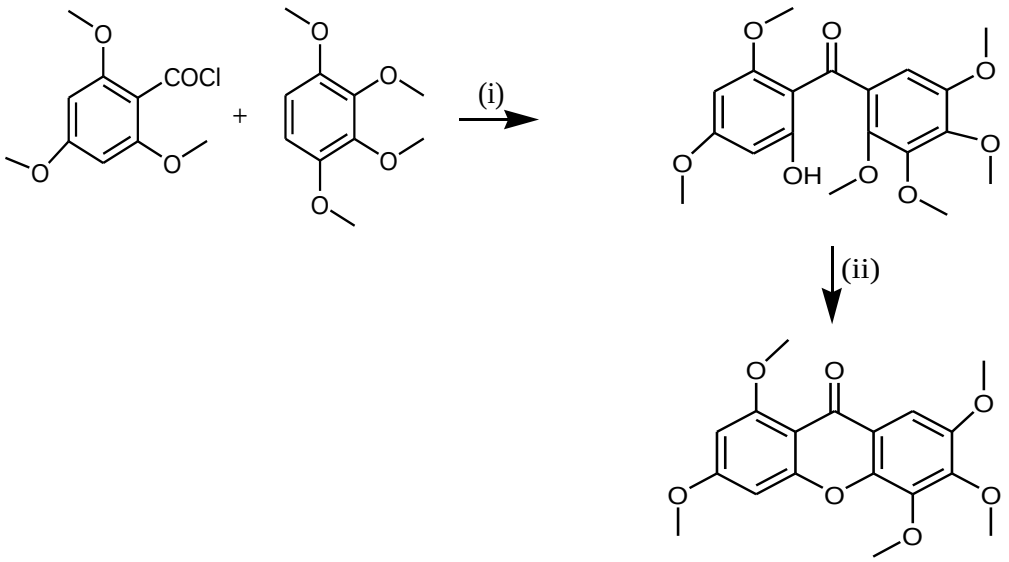



**Figure 2.8: Synthesis route for polymethoxyxanthones. Reagents and conditions: (i) $AlCl_3$, dry ether, rt, 30h;(ii) NaOH, methanol, reflux, 6 h**

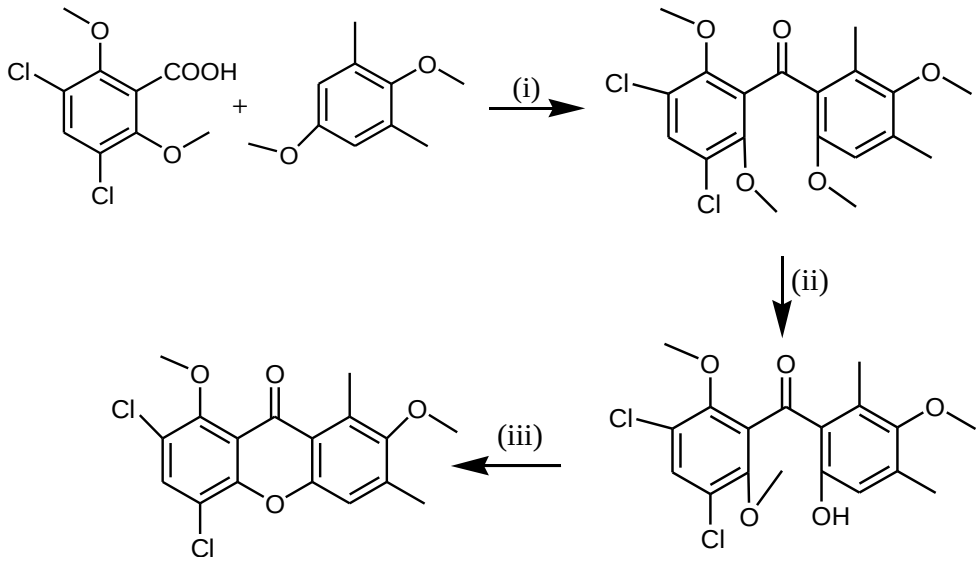



**Figure 2.9: Synthesis route for xanthones. Reagents and conditions: (i) TFAA, dry $CH_2Cl_2$, reflux, 18h; (ii) $BBr_3$, dry $CH_2Cl_2$, -10 $^oC$, 2 h; (iii) water, 120 $^oC$, 16 h**

### 2.1.3.3 Synthesis of Xanthones via Diaryl Ether

Another method to produce synthesized xanthones is through the diaryl ether intermediate. The diaryl ether intermediate can be obtained from the condensation of a phenol with an *o*-chloro or –bromobenzoic acid. Then, the biphenyl intermediate is converted to xanthone by ring formation which can be accomplished through electrophilic cycloacylation (Sousa and Pinto, 2005) (Figure 2.10).

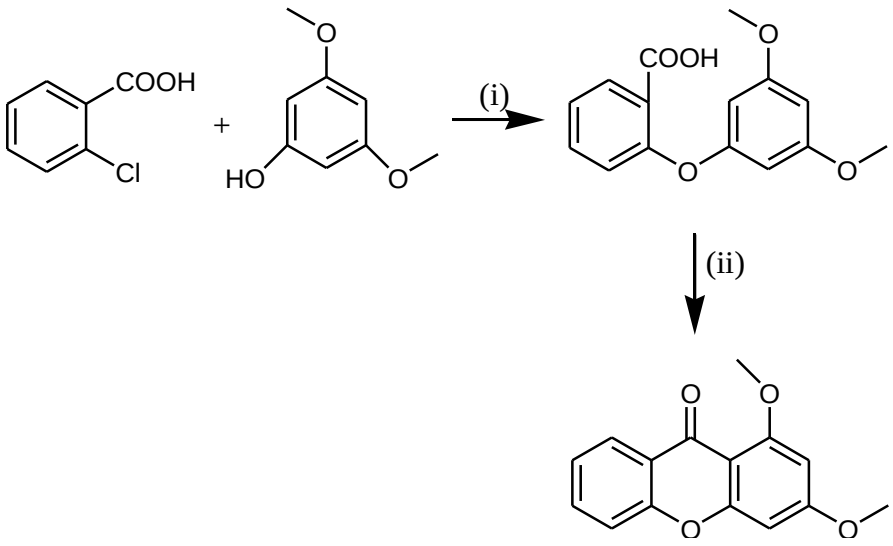



**Figure 2.10: Synthesis route for xanthones via diaryl ether. Reagents and conditions: (i) Cu, $K_2CO_3$, dry DMF, reflux, 3.5 h; (ii) $H^+$, 100 $^oC$, 3 h**

Ullmann coupling reaction is also one of the synthetic methods via diaryl ether. In Ullmann method, polyphosphoric acid (PPA) is used to cyclize the biphenylether into the desire xanthone like structure (Figure 2.11).

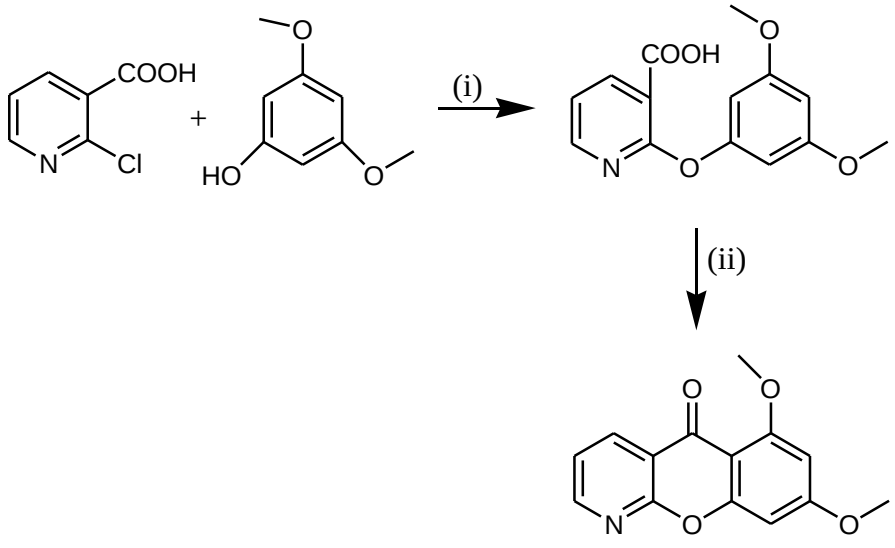



**Figure 2.11: Synthesis route for xanthones. Reagents and conditions: (i) Cu, $K_2CO_3$, dry DMF, reflux, 3.5 h; (ii) PPA, 100 $^oC$, 3 h**

## 2.2 Prenylation of Xanthones

Xanthones show interesting biological activities which is associated with their tricyclic scaffold depending on the nature or the position that different substituents bonded to. Although simple xanthones exhibit cytotoxic activity, but this group of compounds has a lower cytotoxic activity than prenylated xanthones. In order to modulate and improve the cytotoxic activity of xanthones, prenylated xanthones are synthesized by using the simple xanthones as building block.

Castanheiro *et al.* (2007) reported a synthetic method for prenylation of xanthones. The reaction was carried out by nucleophilic substitution on the xanthone building block, with prenyl bromide in alkaline medium (Figure 2.12). In addition, Castanheiro *et al*. also reported a synthetic approach on the cyclization of prenylated xanthones, through refluxing the starting materials by using zinc chloride in dry xylene as catalyst (Figure 2.13) (Castanheiro *et al.*, 2007).

Two years later, Castanheiro *et al*. (2009) reported another two new synthetic approaches on the prenylation xanthones. Both of these new synthetic methods involve different reagent and condition. However, these

synthetic approaches only gave a low yield after long reaction times (Figure 2.14). (Castanheiro *et al*., 2009)

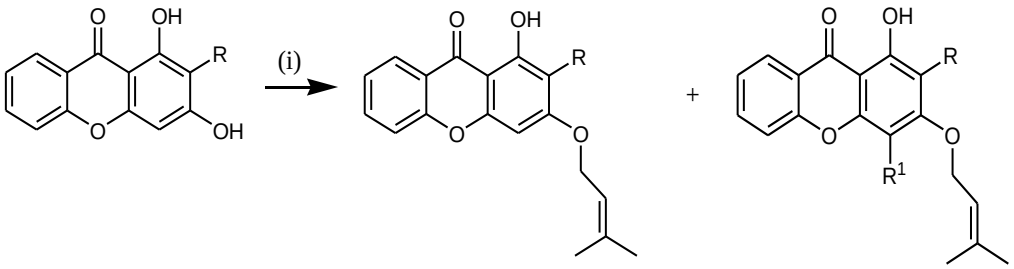



**Figure 2.12: Synthesis route for *o*-prenylated xanthones. Reagents and conditions: (i) Prenyl bromide, $K_2CO_3$, acetone, reflux, 8 h**

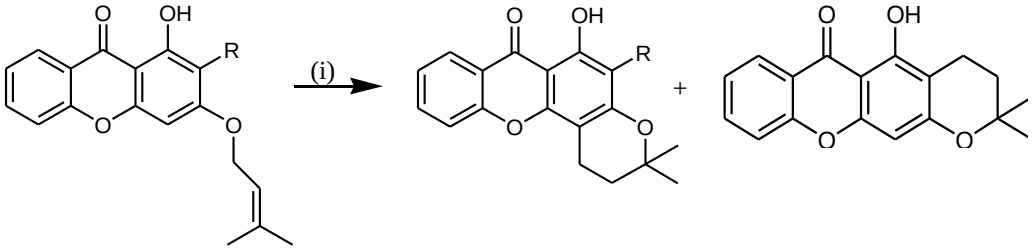



**Figure 2.13: Synthesis route for pyranoxanthones. Reagents and conditions: (i) $ZnCl_2$, *o*-xylene, 200 $^oC$, 21 h**

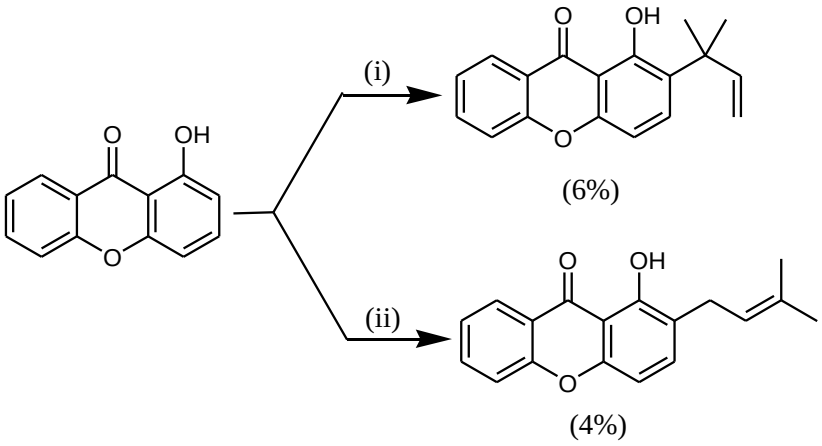



**Figure 2.14: Synthesis route for *c*-prenylated xanthones. Reagents and conditions: (i) Prenyl bromide, anhydrous $K_2CO_3$, dry DMF, reflux, 24 h; (ii) Isoprene, $H_3PO_4$ 85 %, Xylene, 31 $^oC$, 30 h**

Generally, the synthesis of prenylated xanthone is carried out by introduction of the prenyl side chain to hydroxyxanthone building block, in a vigorous condition. This kind of synthesis usually involves toxic reagents and the process is environmentally unfriendly due to the process will consume a lot of solvent.

For this reason, an alternative to obtain the prenylated xanthone is through microwave-assisted organic synthesis (MAOS). This alternative method not only can accelerate organic reactions and reduce the amount of chemical used, but it also can improve yields and selectivity. In this MAOS, there is incorporated microwave (MW) to improve yield of

oxyprenylated xanthone instead of conventional heating (Figure 2.15) (Castanheiro *et al*., 2009).

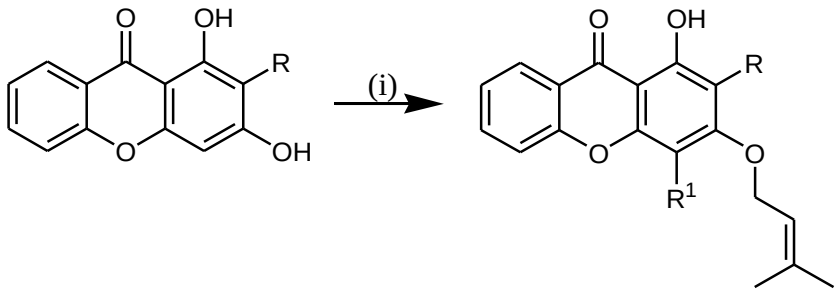



**Figure 2.15: Synthesis route for *o*-prenylated xanthones. Reagents and conditions: (i) Prenyl bromide, $K_2CO_3$, acetone, heat / MW**

On the other hand, Castanheiro *et al*. also mentioned on clays, which can act as solid catalyst for a wide range of organic reactions and a subgroup of clays, Montmorillonite, is particular useful. By using Montmorillonite as catalyst in reaction, the reaction can proceed under mild condition instead of vigorous condition. Besides, it brings lots of benefits such as selectivity, good yields and shorter reaction time. Furthermore, this catalyst can be easily separated from reaction mixture and regenerated for future use.

According to Castanheiro *et al.* report, the cyclization method of prenylated xanthone which is shown in Figure 2.13 can be improved by

using Montmorillonite K10 clay as catalyst and this can lead to a high yield (Figure 2.16).

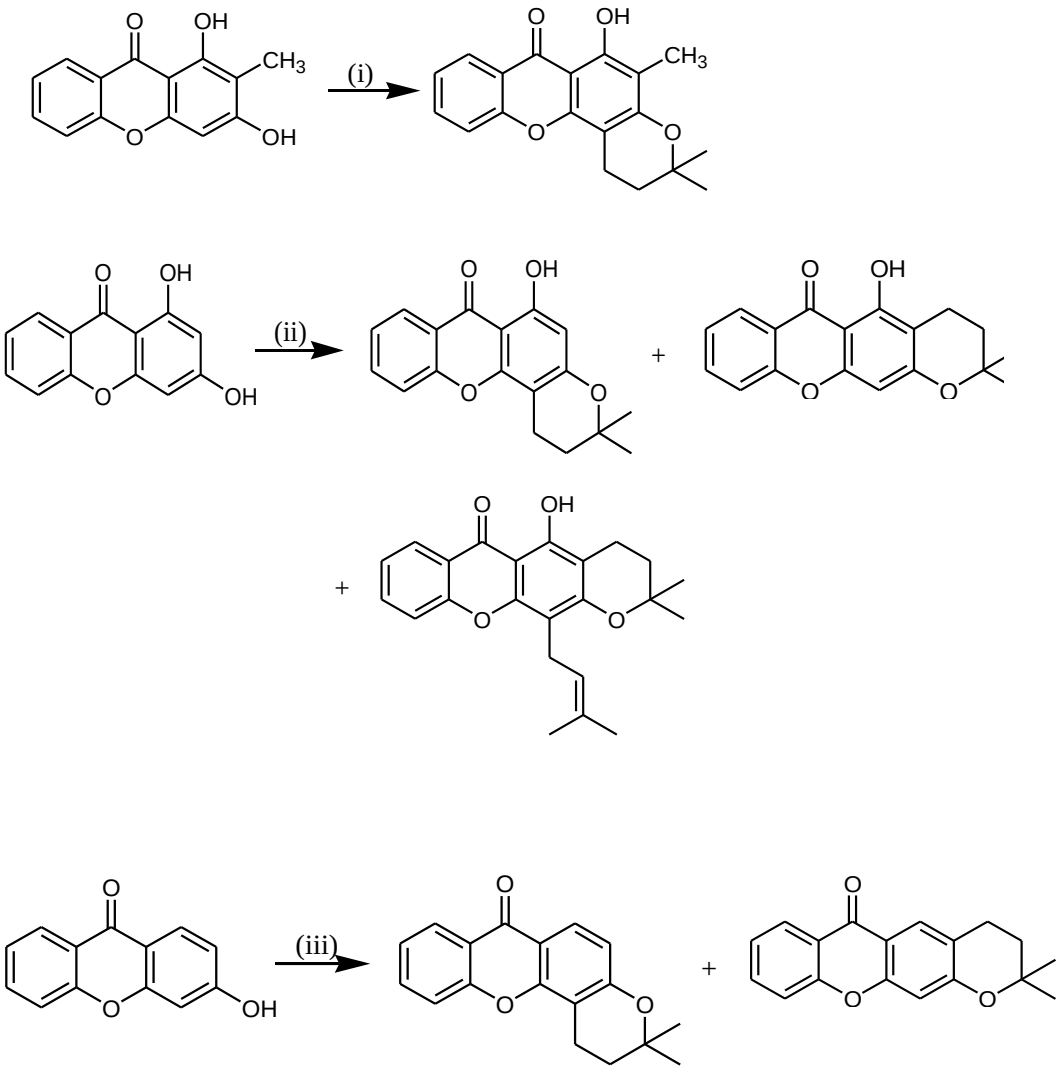



**Figure 2.16: Synthesis route for pyranoxanthones. Reagents and conditions: (i) Montmorillonite K10 clay, Prenyl bromide, rt; (ii) Montmorillonite K10 clay, Prenyl bromide, 100 $^{o}$C, conventional thermal heating; (iii) Montmorillonite K10 clay, Prenyl bromide, MW irradiation**

## 2.3 Cytotoxic Activity of Xanthones

Xanthones are tricyclic dibenzopyrans, either obtained from natural sources or synthesis exhibit significant cytotoxic and antitumor effects. Due to these significant effects, xanthones are much mentioned by many researchers. Through extractions and synthetic approaches, different kinds of xanthones and its derivatives are obtained.

The cytotoxic activities and antitumor properties of xanthones are evaluated using bioassay methods. To quantitatively compare the cytotoxic potency of the tested compounds, four parameters are calculated which include $GI_{50}$, $IC_{50}$, TGI, and $LC_{50}$. $GI_{50}$ is the concentration of the anti-cancer drug that inhibits the growth of cancer cells by 50%. In other words, after giving the drug, there is a 50% reduction in cancer cell proliferation. $IC_{50}$ is the concentration of a drug that inhibits a biological activity by 50%. Besides $GI_{50}$ and $IC_{50}$, TGI also be one of parameters which is the concentration of test drug and it also signifies cytostatic effect. Differ from TGI; $LC_{50}$ is Lethal Concentration 50, which signifies cytotoxic effect. In other words, $LC_{50}$ is the concentration in water having 50% chance of causing death to aquatic life.

Among xanthones, furanoxanthone derivatives have been shown to be the most potent inhibitors of tumor cell growth. Kupchan *et al.* (1980) and

Ioannis *et al.* (2001) found that dihydrofuranoxanthone psorespermine (Diderot *et al*., 2006)(Figure 2.17) exhibited significant cytotoxicity against tumor activity in the cells culture which were derived from a human carcinoma of the nasopharinx (K.B) *in vitro* system and also on leukemia P388 of mice *in vivo* system.

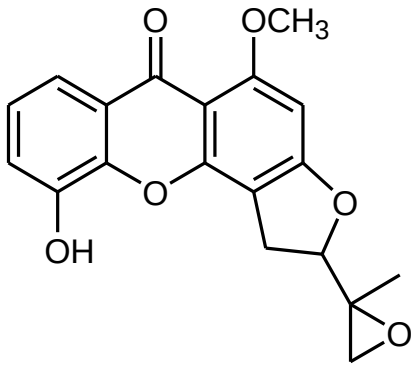



**Figure 2.17: Psorespermine**

Besides furanxanthones, Abou-Shoer *et al*. (1989) reported xantholignoids from *Guttiferae* were responsible for cytotoxic of mammary cancer. Prenylated xanthones which isolated from *Garcinia* or Guttiferae especially 4-(3',7'-dimethylocta-2',6'-dienyl)-1,3,5-trihydroxyxanthone (Diderot *et al*., 2006) (Figure 2.18) have been shown *in vitro* to have a great inhibition activity on colon cancer (Sordat *et al*., 1992).

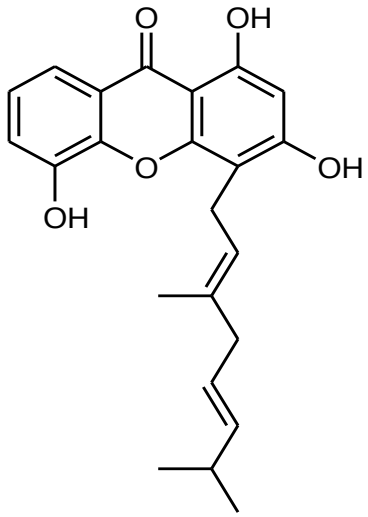



**Figure 2.18: 4-(3',7'-dimethylocta-2',6'-dienyl)-1,3,5-trihydroxyxanthone**

In addition, Ho *et al.* (2002) found that the garcinone E (Figure 2.19), xanthone which extracted from mangosteen fruit pericarp has a potent cytotoxic effect on heptocellular carcinoma cell lines. Garcinone E also had shown cytotoxic effects against other cancer cell lines such as NCI-HUT 125 lung carcinoma cell lines and AGS gastric carcinoma cell lines.

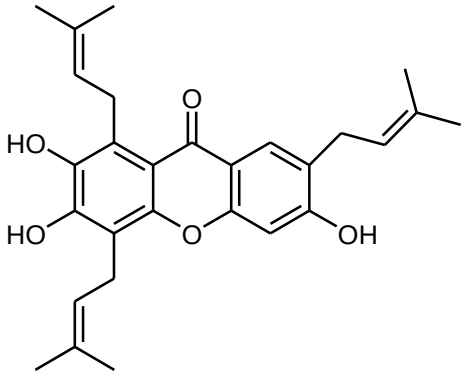



**Figure 2.19: Garcinone E**

In year 2003, Matsumoto *et al.* studied on the effect of xanthones isolated from mangosteen fruit pericarp on the cell growth inhibition of human leukemia cell line HL60. Matsumoto *et al.* reported that α-mangostin (Figure 2.20) has cytotoxicity on leukemia cell lines, for example K562, NB4 and U937. Additionally, *α*-mangostin was also investigated in the short-term chemopreventive effects on putative preneoplastic lesions involved in rat colon carcinogenesis (Nabandith *et al*., 2004).

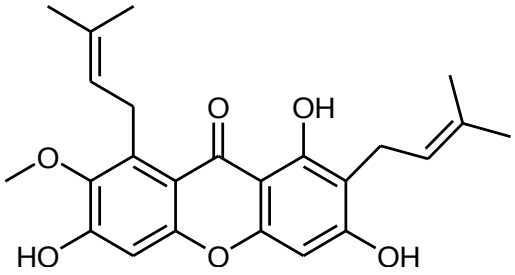



**Figure 2.20: *α*-Mangostin**

Recently, Suksamrarn *et al.* (2006) isolated the mangostenones C, D and E from mangosteen fruit pericarp. These xanthones showed a good cytotoxicity against three different human cancer cell lines: epidermoid carcinoma of mouth (KB), breast cancer (BC-1), and small cell lung cancer (NCI-H187).

Throughout all of these xanthones, Han *et al.* (2007) proved that the number of unsaturated prenyl substituents can significantly affect the cytotoxic activity of xanthone derivatives. The more prenyl groups the compounds have, the more potent effects on the compounds. However, Han *et al.* investigated and found that increasing the polarity of the prenyl group actually reduced the cytotoxic activity of the prenylated xanthones. Furthermore, Castanheiro *et al.* (2009) found that the more rigid of the structure of xanthone compounds or dihydropyran ring, the less the activity of compounds. On the other hand, the introduction of lipophilic prenyl group at C-2 position on xanthonic building block was the reason of appearance of the cytotoxic activity.

## 2.4 Bioassay

One of the most active research areas in xanthones is the study of xanthones cytotoxic activity. Through cell biology, cytotoxic activity of xanthones can be determined through the cytotoxic effect which is accompanied by damage of structural mitochondrial of the cell. By performing colorimetric 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, the cell viability can be determined. The MTT assay which is using, is a simple yet accurate way of measuring the cytotoxic activity of potential medicinal agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth. The assay looks specifically at mitochondrial dehydrogenase activity.

In the MTT assay, a solution containing 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide or MTT is incubated with the cells. If the mitochondrial dehydrogenase is active, the cell is considered viable. Active mitochondrial dehydrogenase cleaves the tetrazolium ring of MTT, yielding purple MTT formazan crystals. Once the crystals have been solubilized (in an HCl / isoproponal solution) the purple solution can be measured using a microplate reader.

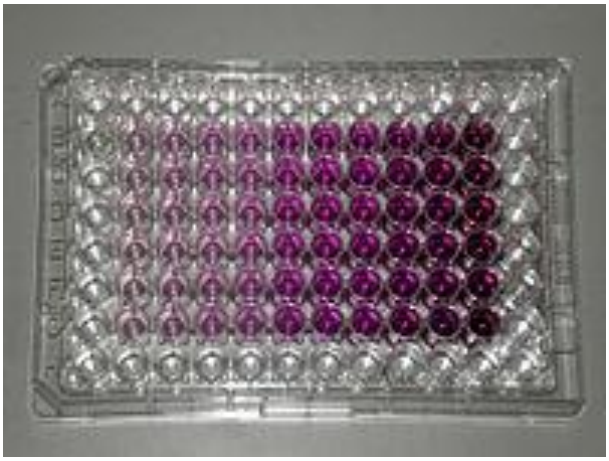
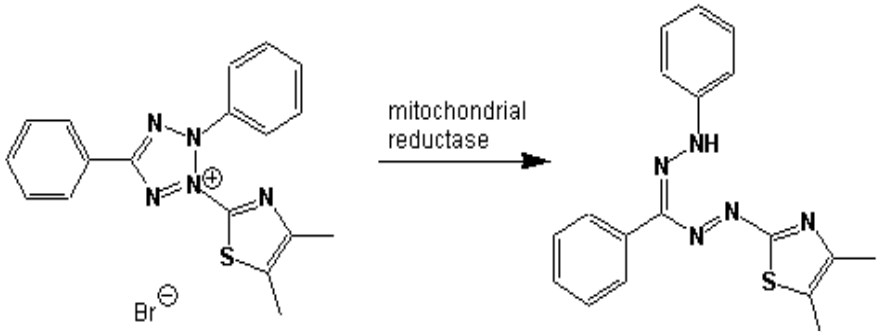


**Figure 2.21: A microtiter plate after an MTT assay. Increasing in the amount of visible cells resulted in increased purple colouring**




MTT

purple MTT formazan

**Figure 2.22: MTT and related tetrazolium salts**

Generally, cells do not require mitochondria as demonstrated by the observation that no differences are observed in the purple formazan production by normal cells in which mitochondria have been poisoned by the nucleic acid toxin, ethidium bromide. Since both serum and plasma, from a variety of species, non-specifically reduce MTT tetrazolium salts to a purple colored formazan product. Hence, fetal bovine serum is particularly used in the MTT assay in order to produce purple MTT formazan crystals.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Materials

All of the chemicals and solvents that were used in this study such as synthesis of xanthone building block, synthesis of prenylated xanthones and isolation of pure xanthone compounds are listed as follow:

**Table 3.1: Chemicals used for synthesis of xanthone building block**

| Chemical | Source | Country |
|---|---|---|
| 2,6-dihydroxybenzoic acid | Acros Organics | Belgium |
| 1,3,5-trihydroxybenzene (Phloroglucinol) | Sigma-Aldrich | USA |
| Eaton's reagent (Phosphorus pentoxide-methanesulfonic acid) | Acros Organics | Belgium |

**Table 3.2: Chemicals used for prenylation of xanthones**

| Chemical | Source | Country |
|---|---|---|
| 3,3-dimethylallyl bromide (Prenyl bromide) | Sigma-Aldrich | USA |
| Potassium carbonate | John Kollin Corporation | - |
| Hydrochloric acid (37 %) | Fisher Scientific | UK |

**Table 3.3: Chemicals and materials used for isolation and chemical analysis**

| Chemical | Source | Country |
|---|---|---|
| Petroleum ether | Fisher Scientific | UK |
| n-Hexane | Merck | Germany |
| Dichloromethane | Fisher Scientific | UK |
| Ethyl acetate | Lab-Scan | Ireland |
| Acetone | QRëC | Malaysia |
| Methanol | Mallinckrodt Chemicals | USA |
| $CDCl_3$ | Merck | Germany |
| Acetone – D6 | Merck | Germany |

| Chemical | Source | Country |
|---|---|---|
| Methanol – D4 | Merck | Germany |
| Silica gel (60Å) | Merck<br>Silicycle | Germany<br>Canada |
| TLC silica gel 60 $F_{254}$ | Merck | Germany |

**Table 3.4: Chemicals and materials used for bioassay**

| Chemical | Source | Country |
|---|---|---|
| 3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyl tetrazolium bromide (MTT) | Sigma-Aldrich | USA |
| Dimethyl sulfoxide (DMSO) | Fisher Scientific | UK |
| RPMI 1640 media | Cellgro | Manassas |
| Fetal Bovine Serum (FBS) | Hyclone Thermo Scientific | South America |
| 96-cell plate | TPP | Europe |
| HeLa cells | American Type Culture Collection (ATCC) | USA |
| MDA-MB-231 cells | American Type Culture Collection (ATCC) | USA |

## 3.2 Methodology

### 3.2.1 Preparation of 1,3,8-Trihydroxyxanthone as Building Block

A mixture of 50 mmol of 2,6-dihydroxybenzoic acid and 50 mmol of phloroglucinol was added slowly with 100 ml Eaton's reagent. The mixture was then warmed at 80 $^{o}$C in water bath for 10 minutes under reflux. After that, the mixture was cooled to room temperature, then poured into ice water and stirred for 1 hour. Precipitate formed was filtered by using Buchner filtration, and then dried in an oven at 50 $^{o}$C, while the filtrate was extracted using ethyl acetate. The crude products were subjected to column chromatography for further purification.

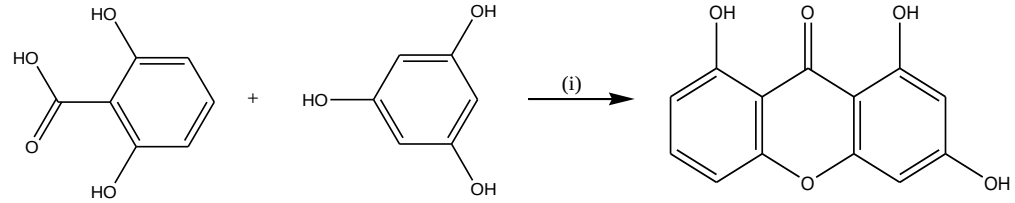
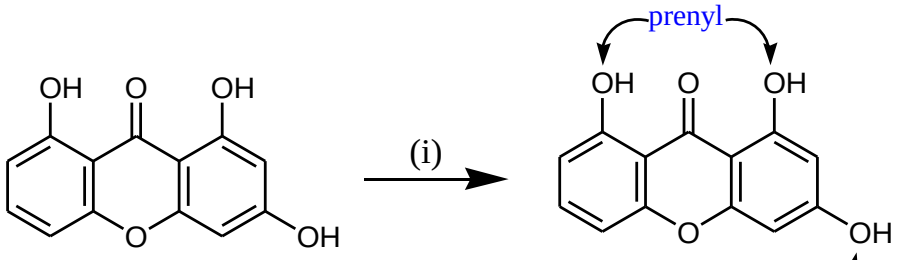



**Figure 3.1: Synthesis route for 1,3,8-trihydroxyxanthone. Reagents and conditions: (i) Eaton's reagent, reflux 10 minutes at 80 $^{o}$C**

### 3.2.2 Prenylation of 1,3,8-Trihydroxyxanthone in Organic Medium

A mixture of 4 mmol of xanthone block and 12 mmol of potassium carbonate ($K_2CO_3$) in 100 mL acetone was stirred for 5 minutes at room temperature. Then, 2.384 g of prenyl bromide was added into the mixture. As prenyl bromide was added, the mixture was then refluxed for 6 hours at 65 $^{o}$C. After 6 hours reflux, the solid formed in the mixture was filtered and the filtrate was then dried under reduced pressure. The crude products were subjected to column chromatography for purification.

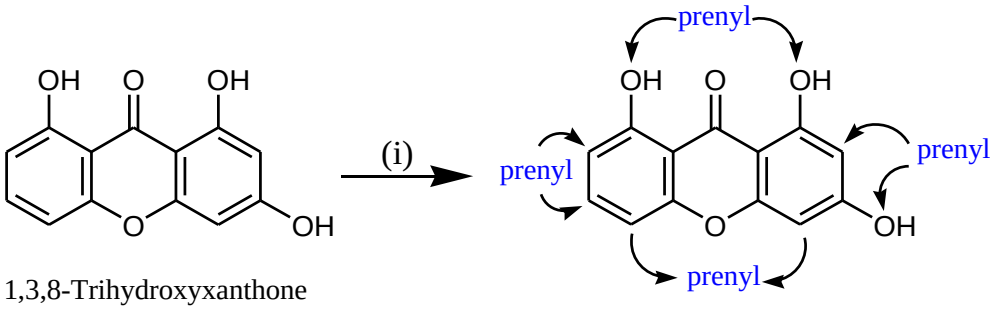



1,3,8-Trihydroxyxanthone

**Figure 3.2: Synthesis route for prenylation of xanthone in organic medium. Reagents and conditions: (i) 1. $K_2CO_3$ in acetone; 2. Prenyl bromide, reflux 6 h**

### 3.2.3 Prenylation of 1,3,8-Trihydroxyxanthone in Aqueous Medium

A mixture of 8 mmol of xanthone block and 35 g of potassium carbonate ($K_2CO_3$) in 100 mL deionized water was introduced into flat bottom flask and stirred for 5 minutes. Meanwhile, 40 mmol of prenyl bromide was prepared in 5 mL of acetone and was then transferred into the mixture in flat bottom flask by using syringe. Next, the reaction mixture was stirred for 20 hours at room temperature, and continually refluxed for 2 hours at 65 $^{o}$C. After 2 hours reflux, the aqueous phase of the mixture was acidified by 10 % hydrochloric acid and then extracted using ethyl acetate. The crude products were subjected to column chromatography for purification.




1,3,8-Trihydroxyxanthone

**Figure 3.3: Synthesis route for prenylation of xanthone in aqueous medium. Reagents and conditions: (i) 1. $K_2CO_3$ in Deionized water; 2. Prenyl bromide in acetone, reflux 2 h at 65 $^{o}$C; 3. 10 % HCl**

### 3.2.4 Bioassay

The cell viability was evaluated using the colorimetric 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assay. In this MTT assay, HeLa cancer cells and MDA-MB-231 cancer cells were tested. Both of HeLa cells (0.75 x $10^5$ cells/mL) and MDA-MB-231 cells (3.5 x $10^5$ cells/mL) were cultured in 96-well plates with 0.1 % dimethyl sulfoxide (DMSO) containing samples at 37 $^oC$ for 72 hours. Blank cell control and blank medium control were also prepared and incubated for 72 hours. After treatment, the cells received 20 μL of 5 mg / mL MTT and were incubated at 37 $^oC$ for another 3 hours. After 3 hours, 70 % of supernatant was removed and 150 μL of DMSO was added into each well. DMSO dissolved the formazan crystal, produced a purple solution. Cell viability was determined by measuring optical density at 550 nm using a Model 550 micro plate reader (Bio-Rad Laboratories, Hercules, CA, USA). Cell viability was calculated as follow:

$$\boldsymbol{cell\ viability} = \frac{\boldsymbol{x - y}}{\boldsymbol{z - y}} \times \mathbf{100}\%$$

Where $x$= average absorbance of cell treated with compound

$y$= average absorbance of blank medium

$z$= average absorbance of cell control

### 3.3 Chromatographic Methods

#### 3.3.1 Thin Layer Chromatogaphy (TLC)

Thin layer chromatography (TLC) is a chromatographic technique used to separate mixture of components. TLC is a simple, quick, and inexpensive procedure that gives a quick answer as how many components are found in a mixture. Thin layer chromatography was performed on a sheet aluminum foil, which was coated with a thin layer of silica gel.

After the sample has been dotted on the marked baseline on the plate, the plate was place into a TLC chamber filled with a solvent or solvent mixture (known as the mobile phase). The mobile phase was drawn up the plate via capillary action. The developed spots on the plate were then visualized under ultraviolet (UV) lamp with both short (254 nm) and long (366 nm) wavelengths.

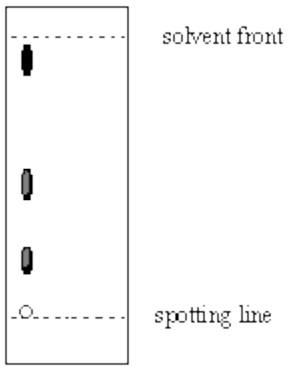



**Figure 3.4: Developed TLC plate**

The components in the mixture can be defined and differentiate by calculating their retention factor ($R_f$). In principle, each of the components will give a different $R_f$ value, hence we can differentiate them as different components can be differentiated. The $R_f$ value can be calculated by using the following equation:

$$R_f = \frac{\text{Distance traveled by component}}{\text{Distance traveled by solvent front}}$$

### 3.3.2 Column Chromatography (CC)

Column chromatography is a common method used to purify individual chemical components from a mixture of compounds. In column chromatography, the stationary phase, a solid adsorbent, Silicycle silica gel (40-63 μm) was prepared in slurry by using hexane and then placed in a vertical glass column. The mobile phase, a chemical solvent, was added through the top and run down the column via gravity force or external pressure.

In this study, dry-packing of sample was prepared and introduced into the packed column as a thin sample layer. As the mobile phase eluted through the column, equilibrium was established between the sample on the silica

gel packed in the column. Due to the different components in the mixture have different strength of interactions with the stationary and mobile phases, they can be separated and eluted out from the column at different retention time along with mobile phase. The individual components were collected as eluent drips from the bottom of the column.

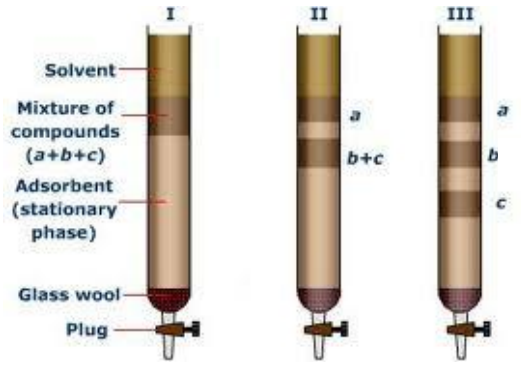



**Figure 3.5: Standard gravity column chromatography setup**

## 3.4 Instruments

### 3.4.1 Nuclear Magnetic Resonance (NMR)

Through NMR spectrum, the structural information of a compound was studied and identified. In this study, JEOL JNM-ECX 400 MHz spectrometer was used to run the proton $^{1}H$, carbon $^{13}C$, and 2D-NMR. In order to run NMR analysis, all of the samples were prepared in appropriate deuterated solvents, such as deuterated-chloroform and deuterated-acetone.

### 3.4.2 Infrared Spectrocopy (IR)

Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum. It covers a range of techniques, mostly based on absorption spectroscopy. By using IR spectroscopy, IR spectrum gives all of the information about the functional groups that present in a compound structure. In this study, Perkin Elmer 2000-Fourier transform infrared (FTIR) spectrometer was used to analyze samples in the range of 4000 $cm^{-1}$ to 400 $cm^{-1}$ and the samples were prepared in form of KBr pellet.

### 3.4.3 Ultraviolet-Visible Spectroscopy (UV-Vis)

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy in the ultraviolet-visible spectral region. The absorption in the visible range directly affects the perceived color of the chemicals involved. Through UV-Vis spectrum, the qualitative information of highly conjugated organic compound can be obtained based on its transition energy give rise to a color. In this project, Perkin Elmer Lambda (25/35/45) UV-Vis spectroscopy was used for sample analysis and the absorption was measured using quartz cuvette in the range of 100 nm to 800 nm.

### 3.4.4 Mass Spectrometry (MS)

Mass spectrometry (MS) is an analytical technique that studies the fragmentations and molecular structure of individual molecules. A mass spectrometer converts them into ions so that they can be moved about and manipulated by external electric and magnetic fields. By using the MS, mass-to-charge ratio of charged particles were measured, which is used for determining actual masses of compounds, determining the elemental composition of a sample and elucidating the chemical structures of molecules. In this study, Agilent 5975C mass spectrometry was used to analyze the samples.

### 3.4.5 Melting Point Instrument

Each pure compound shows a narrow range of melting point. By measuring the melting point of a compound, the purity and identity of the compound can be determined. This mainly due to impurities will cause the melting point to be deviated. In this study, Barnstead Electrothermal 9100 melting point instrument was used to determine the melting point of solid samples in haematocrit capillaries.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Synthesis of 1,3,8-Trihydroxyxanthone (Xanthone Building Block)

This chemical synthesis involved reaction of 50 mmol 2,6-dihydroxybenzoic acid and 50 mmol phloroglucinol in Eaton's reagent to give the xanthone building block. Eaton's reagent served as catalyst and also as a coupling agent in the reaction which afforded the tricyclic 1,3,8-trihydroxyxanthone (Martine *et al*., 2008).

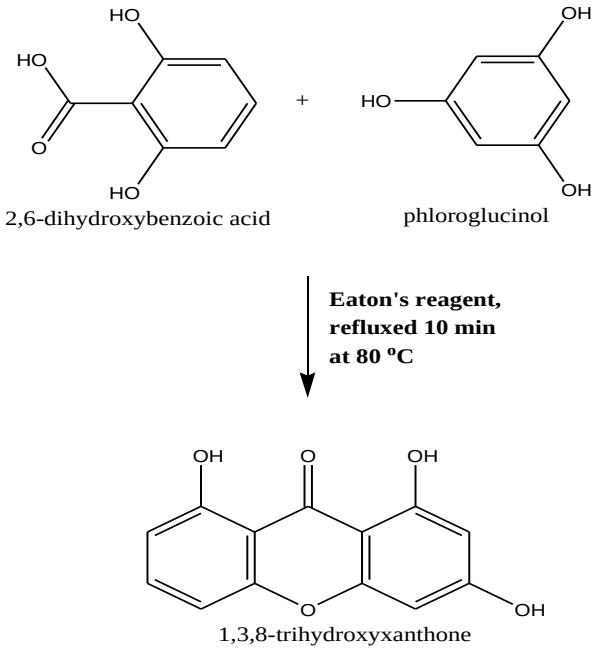
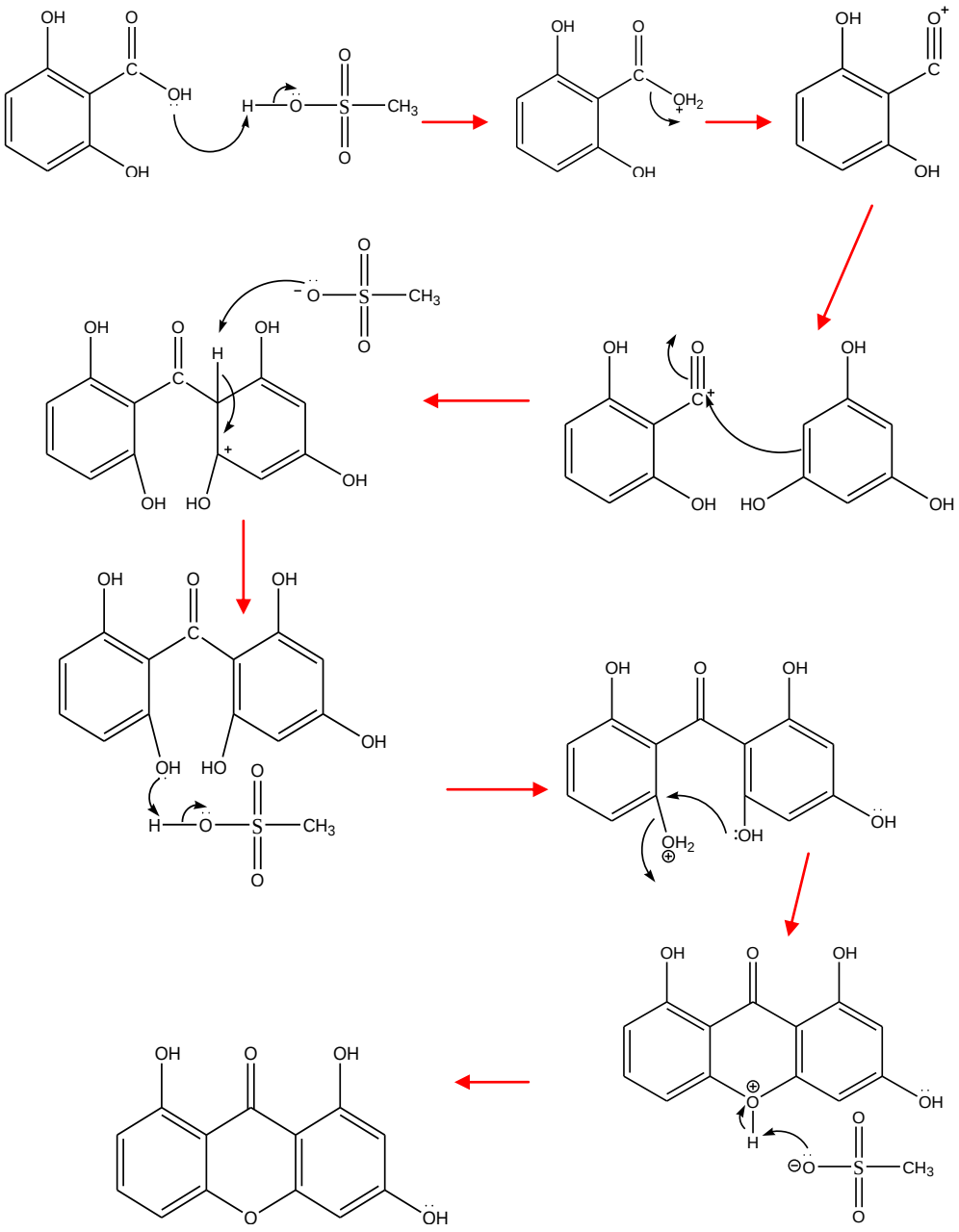



**Figure 4.1: Reaction scheme of 1,3,8-trihydroxyxanthone (xanthone block)**

### 4.1.1 Proposed Mechanism of 1,3,8-Trihydroxyxanthone Synthesized



$$P_4O_{10} + 6\,H_2O \longrightarrow 4\,H_3PO_4$$

**Figure 4.2: Proposed mechanism of simple xanthone, 1,3,8-trihydroxyxanthone synthesized**

### 4.1.2 Characterization of Building Block, 1,3,8-Trihydroxyxanthone

Purification of crude product from the chemical synthesis by using gravity column chromatography packed with silica gel, afforded the isolation of 1,3,8-trihydroxyxanthone. This compound was labeled as SCD 5 and it gave a single spot on the thin layer chromatography (TLC) with retention factor ($R_f$) of 0.33 by using 50% hexane and 50% ethyl acetate as mobile phase. From the synthesis, a total of 4.4355 g of 1,3,8-trihydroxyxanthone was obtained, which contributed to 36.33 % of percentage yield of the compound.

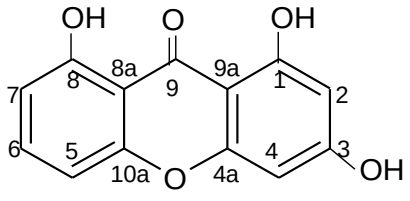



**Figure 4.3: Structure of 1,3,8-trihydroxyxanthone**

SCD 5 was characterized by means of 1D-NMR, 2D-NMR, IR, MS and UV-Vis analyses. Through these analyses, the structure of SCD 5 was confirmed to be 1,3,8-trihydroxyxanthone. It appeared as yellowish solids with a melting point in the range of 262$^{o}$C to 265 $^{o}$C.

The $^{1}$H-NMR spectrum (Figure 4.5) showed two highly deshielded singlets at δ 12.93 (1H, s, 1-OH) and δ 12.86 (1H, s, 8-OH) in the downfield region, which corresponded to the presence of two chelated hydroxyl protons. There were five signals lying in the range of δ 6.00 – 8.00 indicated presence of five aromatic protons, which the signals were δ 7.86 (1H, t, J = 7.7 Hz, H-6), δ 7.53 (1H, d, J = 7.7 Hz, H-7), δ 7.32 (1H, d, J = 7.7 Hz, H-5), δ 6.39 (1H, d, J = 2.1 Hz, H-4), and δ 6.24 (1H, d, J = 2.1 Hz, H-2).

The $^{13}$C-NMR spectrum (Figure 4.6) showed thirteen carbon signals in the region of δ 90.0 – 180.0 which indicated the presence of thirteen nonequivalent $sp^2$ hybridized carbons. Among these carbon signals, the peak with the highest chemical shift at δ 179.6 corresponded to ketone carbonyl carbon, others corresponded to seven quaternary carbons, and five methine's carbons. The assignments of type of carbon signals above were carried out based on HMQC spectral data.

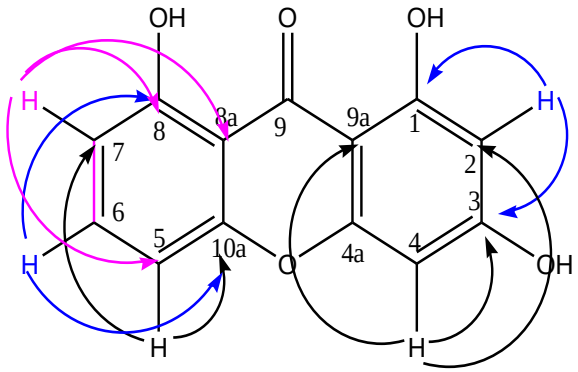
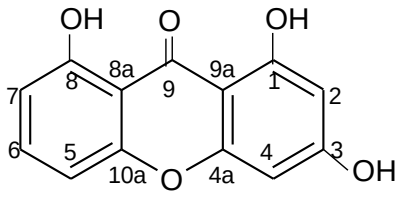



**Figure 4.4: HMBC correlations of 1,3,8-trihydroxyxanthone**

In the HMBC spectrum (Appendix 1b), it was observed that proton H-2 was $^{2}J$ coupling with carbons C-1 and C-3, and $^{3}J$ coupling with carbons C-4 and C-9a. For proton H-4, there was observed that this proton was $^{2}J$ coupling with carbon C-3, and $^{3}J$ coupling with carbons C-2 and C-9a. From these $^{2}J$ and $^{3}J$ couplings, the position of C-9a and C-3 were assigned. In the other ring of 1,3,8-trihydroxyxanthone, H-5 showed $^{2}J$ coupling with carbon C-10a, and $^{3}J$ coupling with carbons C-7 and C-8a, while H-6 was $^{3}J$ coupling with carbons C-8 and C-10a. Another H-7 also showed coupling in HMBC, which showed $^{2}J$ coupling with carbon C-8, and $^{3}J$ coupling with carbons C-5 and C-8a. Through these $^{2}J$ and $^{3}J$ couplings of these three protons, the position of quaternary carbons C-8, C-8a and C-10a were confirmed.

The 1,3,8-trihydroxyxnathone was further characterized through IR, MS and UV analyses. The IR spectrum (Figure 4.7) showed absorption bands at 3408 (O-H), 1654 (chelated C=O) and 1607 (C=C) $cm^{-1}$. The UV absorptions (Figure 4.9) at 204.05, 255.24 and 311.84 nm revealed the compound to be a highly conjugated hydroxyl xanthone derivative. The mass spectrum (Figure 4.8) showed that the compound have a molecular ion, $M^{+}$ at m/z 244.0 indicated the compound to have a molecular formula of $C_{13}H_{8}O_{5}$.



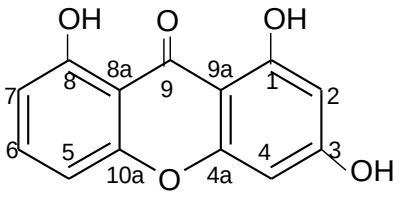

1,3,8-trihydroxyxanthone

244.19 g $mol^{-1}$

$C_{13}H_8O_5$

**Table 4.1: Summary of elucidated spectral data of 1,3,8-trihydroxyxanthone**

| Position | $\delta_H$ (ppm) | $\delta_C$ (ppm) | HMBC |
|---|---|---|---|
| 1 | | 164.1 | - |
| 2 | 6.24 (1H, d, J=2.1Hz) | 98.6 | C-1 ($^2$J), 3 ($^2$J), 4 ($^3$J), 9a ($^3$J) |
| 3 | - | 165.9 | - |
| 4 | 6.39 (1H, d, J=2.1Hz) | 93.8 | C-2 ($^3$J), 3 ($^2$J), 9a ($^3$J) |
| 4a | - | 109.4 | - |
| 5 | 7.32 (1H, d, J=7.7Hz) | 119.8 | C-10a ($^2$J), 8a ($^3$J), 7 ($^3$J) |
| 6 | 7.86 (1H, t, J=7.7Hz) | 135.2 | C-8 ($^3$J), 10a ($^3$J) |
| 7 | 7.53 (1H, d, J=7.7Hz) | 117.2 | C-8 ($^2$J), 5 ($^3$J), 8a ($^3$J) |
| 8 | - | 157.2 | - |
| 8a | - | 114.4 | - |
| 9 | - | 179.6 | - |
| 9a | - | 103.5 | - |
| 10a | - | 147.2 | - |
| 1-OH | 12.93 (1H, s) | - | - |
| 8-OH | 12.86 (1H, s) | - | - |




1,3,8-trihydroxyxanthone

244.19 g $mol^{-1}$

$C_{13}H_8O_5$

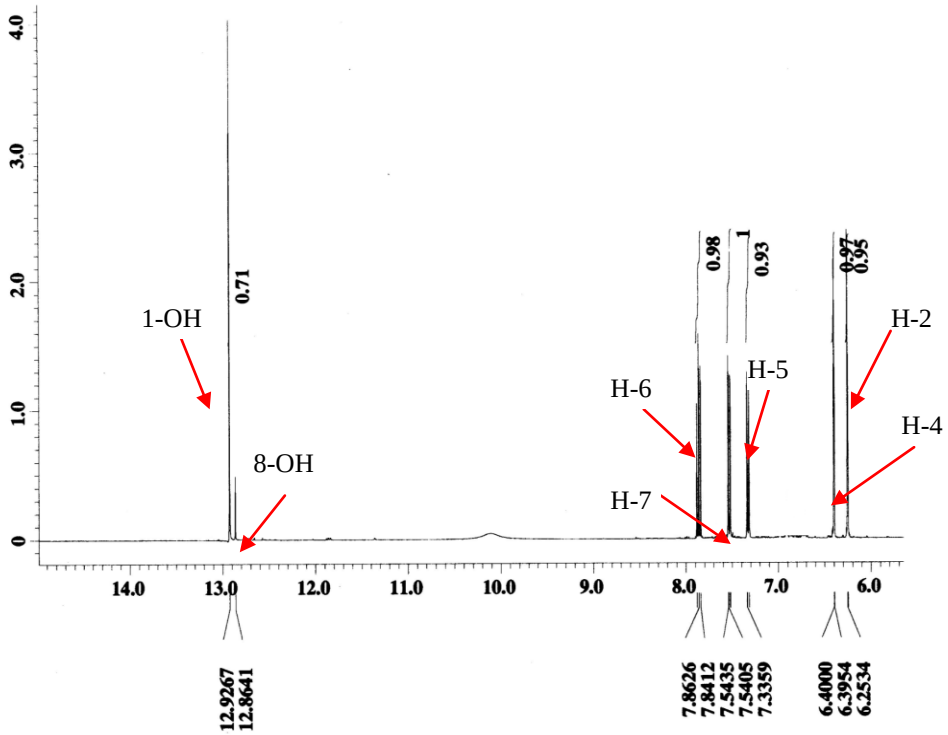
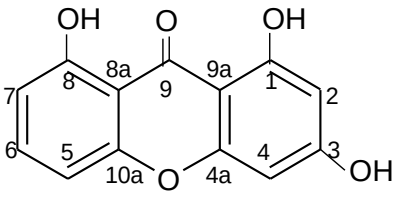



**Figure 4.5: $^1$H-NMR spectrum of 1,3,8-trihydroxyxanthone (400 MHz, acetone-$d_6$)**




1,3,8-trihydroxyxanthone

244.19 g $mol^{-1}$

$C_{13}H_8O_5$

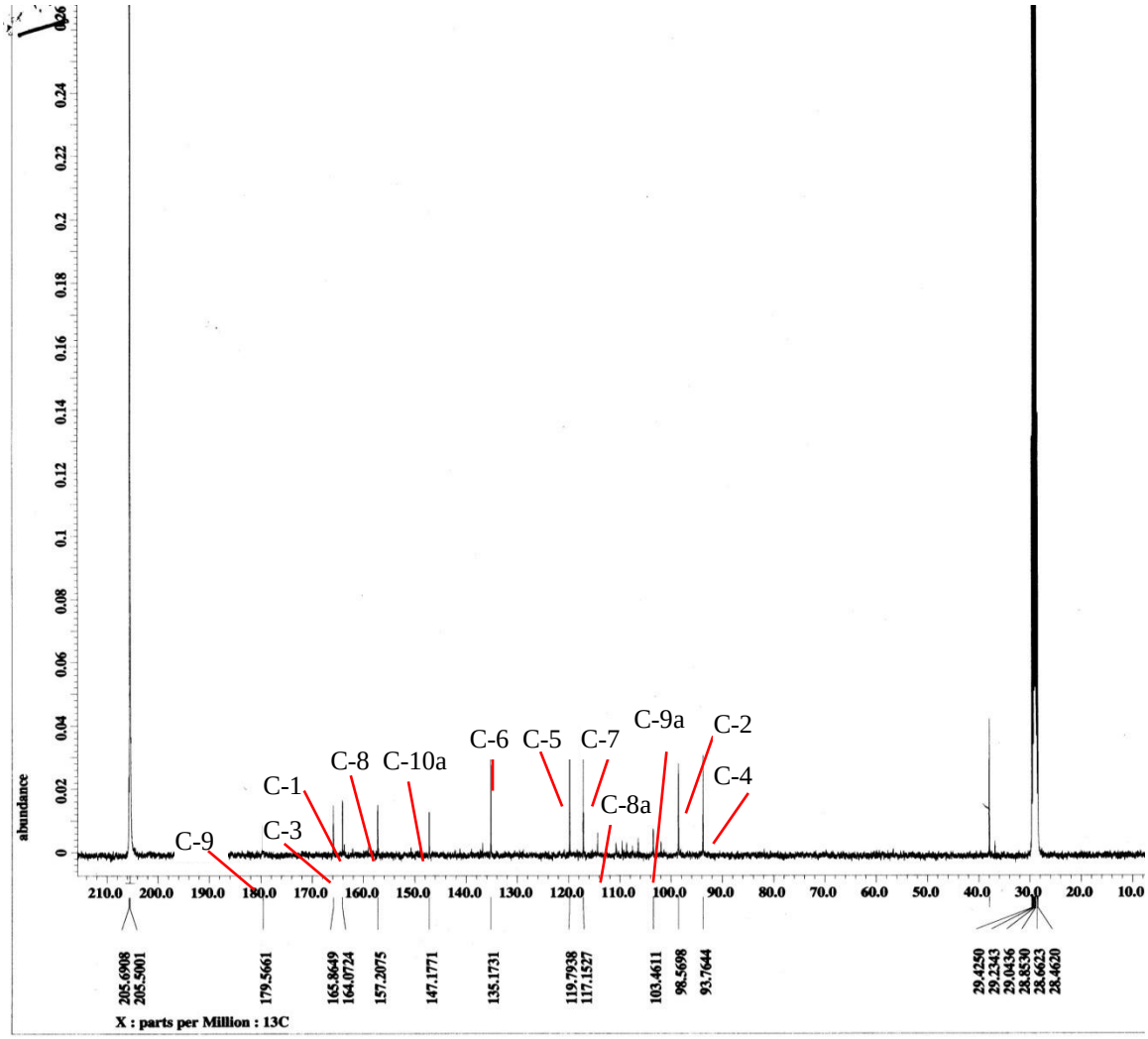
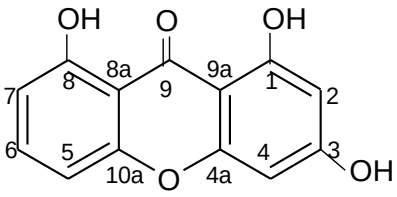



**Figure 4.6: $^{13}$C-NMR spectrum of 1,3,8-trihydroxyxanthone (100 MHz, acetone-$d_6$)**




1,3,8-trihydroxyxanthone

244.19 g $mol^{-1}$

$C_{13}H_8O_5$

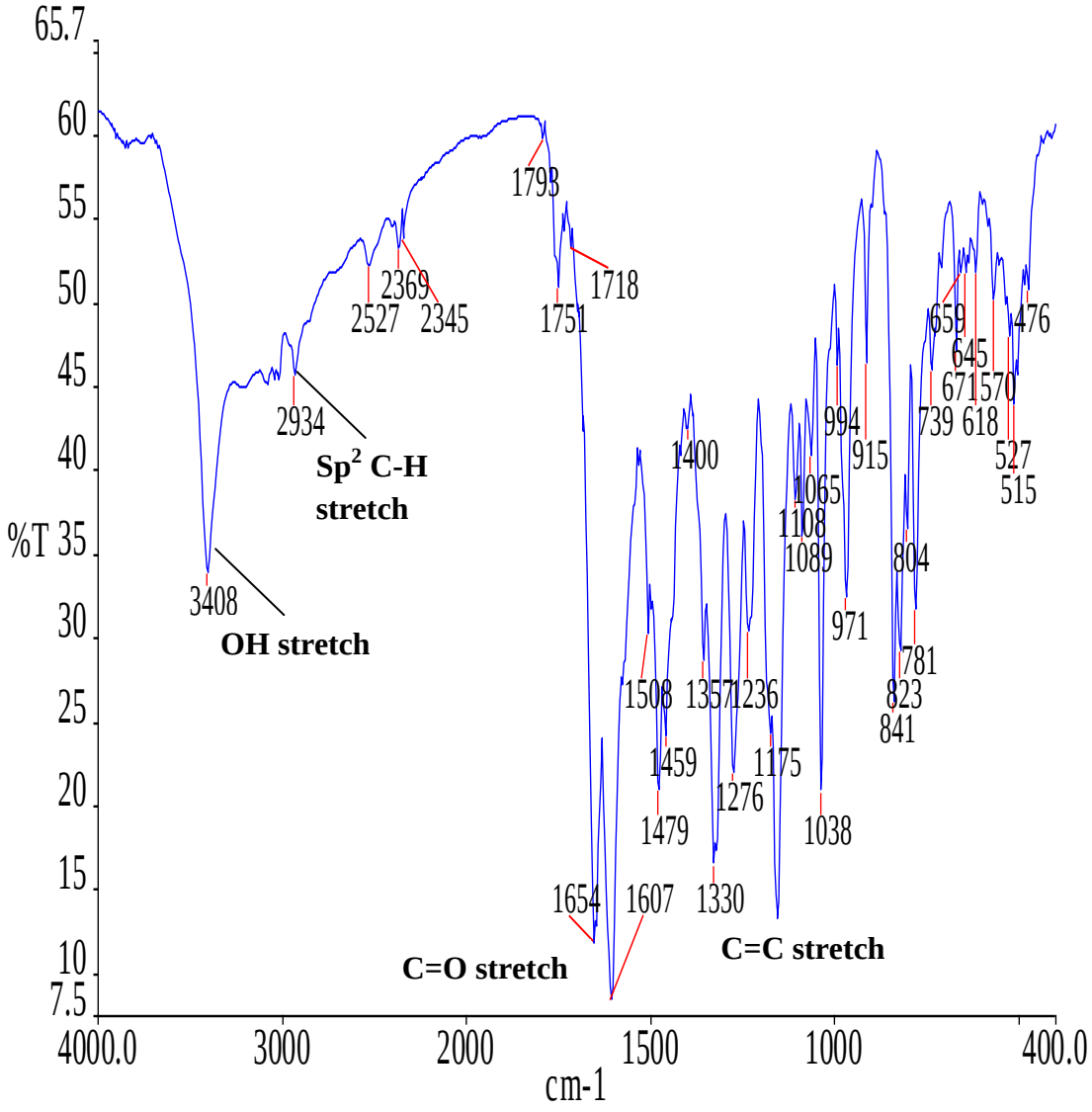
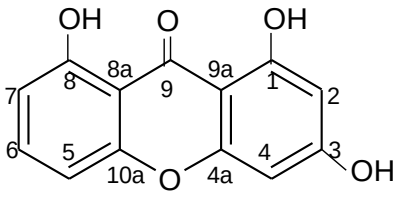



**Figure 4.7: IR spectrum of 1,3,8-trihydroxyxanthone**




1,3,8-trihydroxyxanthone

244.19 g $mol^{-1}$

$C_{13}H_8O_5$

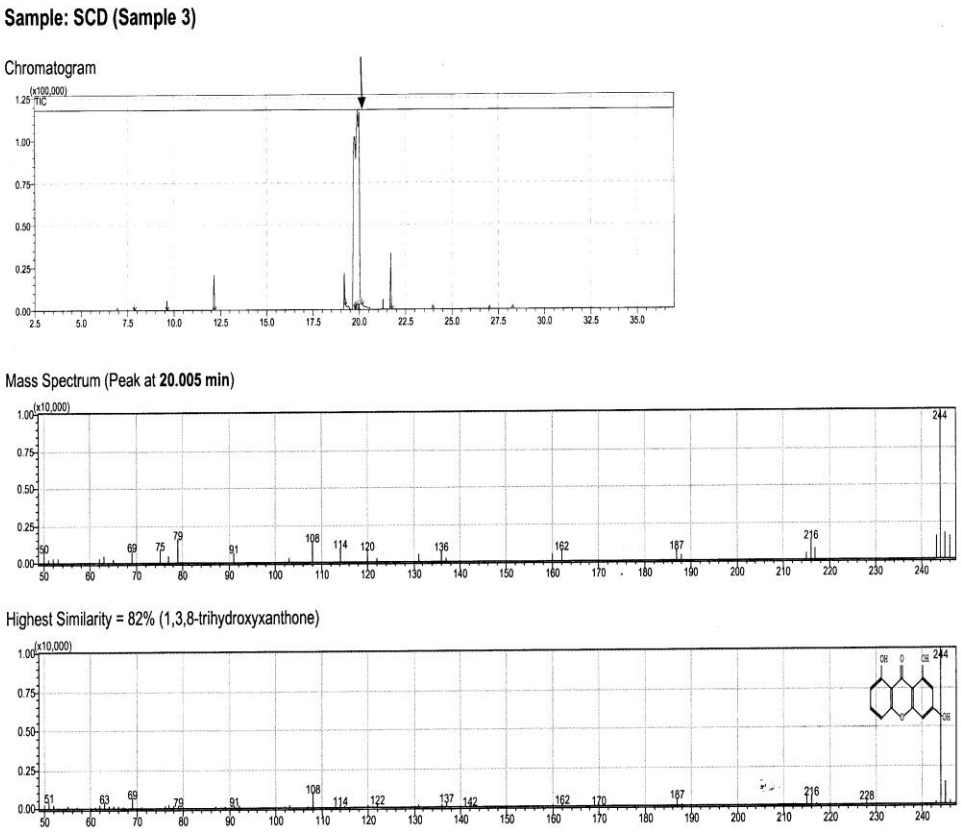
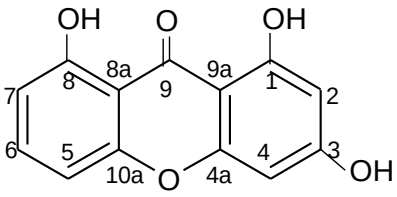



**Figure 4.8: Mass spectrum (MS) of 1,3,8-trihydroxyxanthone**




1,3,8-trihydroxyxanthone

244.19 g $mol^{-1}$

$C_{13}H_8O_5$

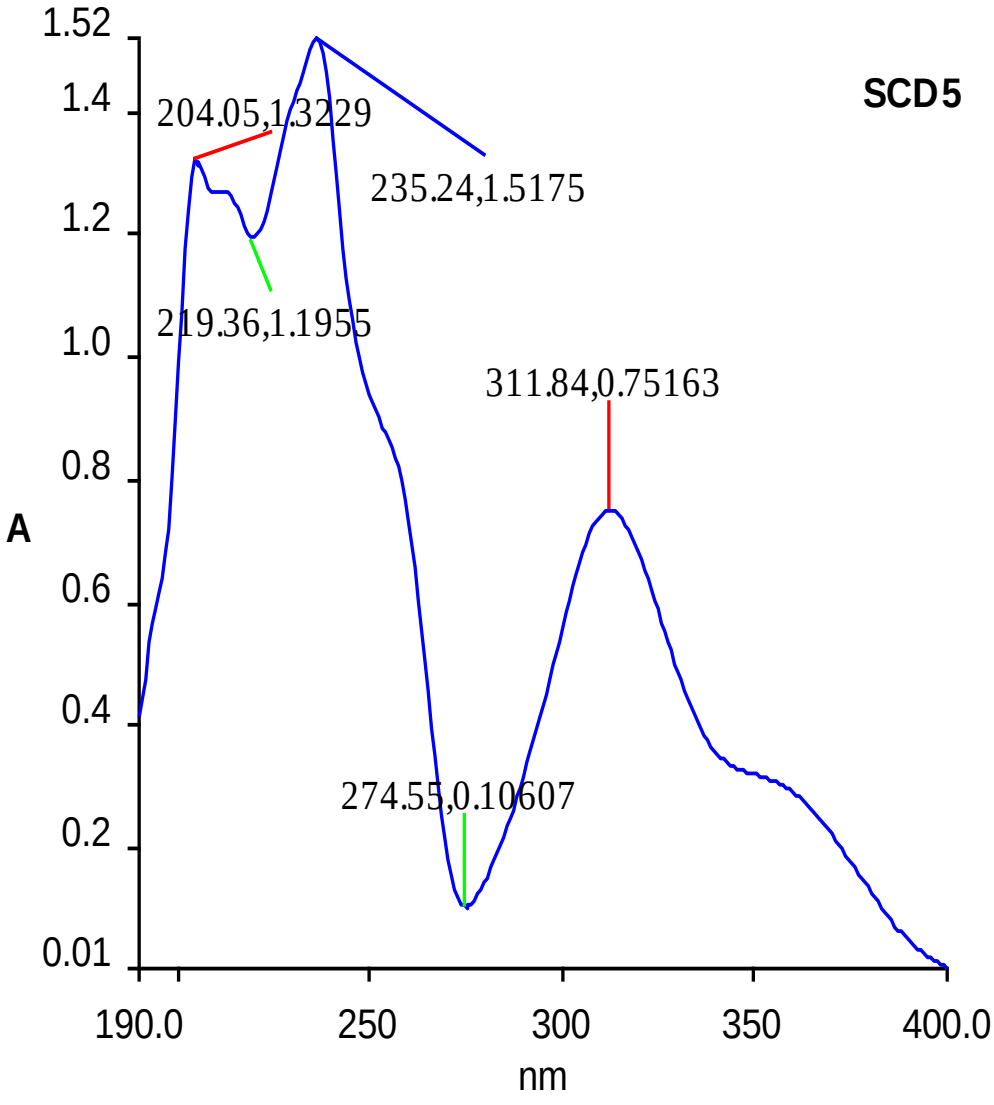



**Figure 4.9: UV-Vis spectrum of 1,3,8-trihydroxyxanthone**

## 4.2 Prenylation of 1,3,8-Trihydroxyxanthone in Organic Medium

This prenylation involved reaction of 4 mmol xanthone block, 1,3,8-trihydroxyxanthone and 2.384 g of prenyl bromide, in the presence of 12 mmol potassium bicarbonate in acetone. In this synthesis, organic medium acetone was used. Due to this organic medium, the reaction mainly occurred through etherification to the hydroxyl groups in xanthone block. From this prenylation, a crude product was obtained and purified through gravity column chromatography. From the purification, a pure prenylated compound, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one was isolated with a total weight of 0.1862 g or 14.91% of percentage yield.

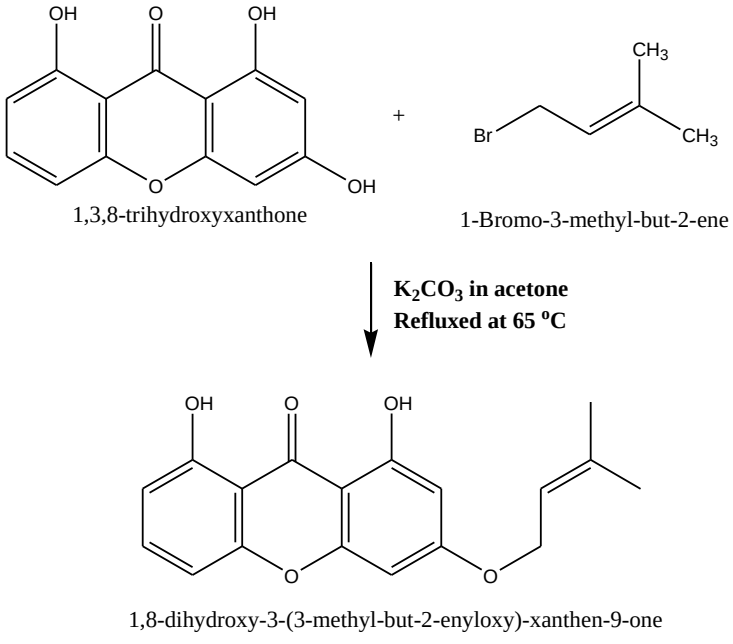
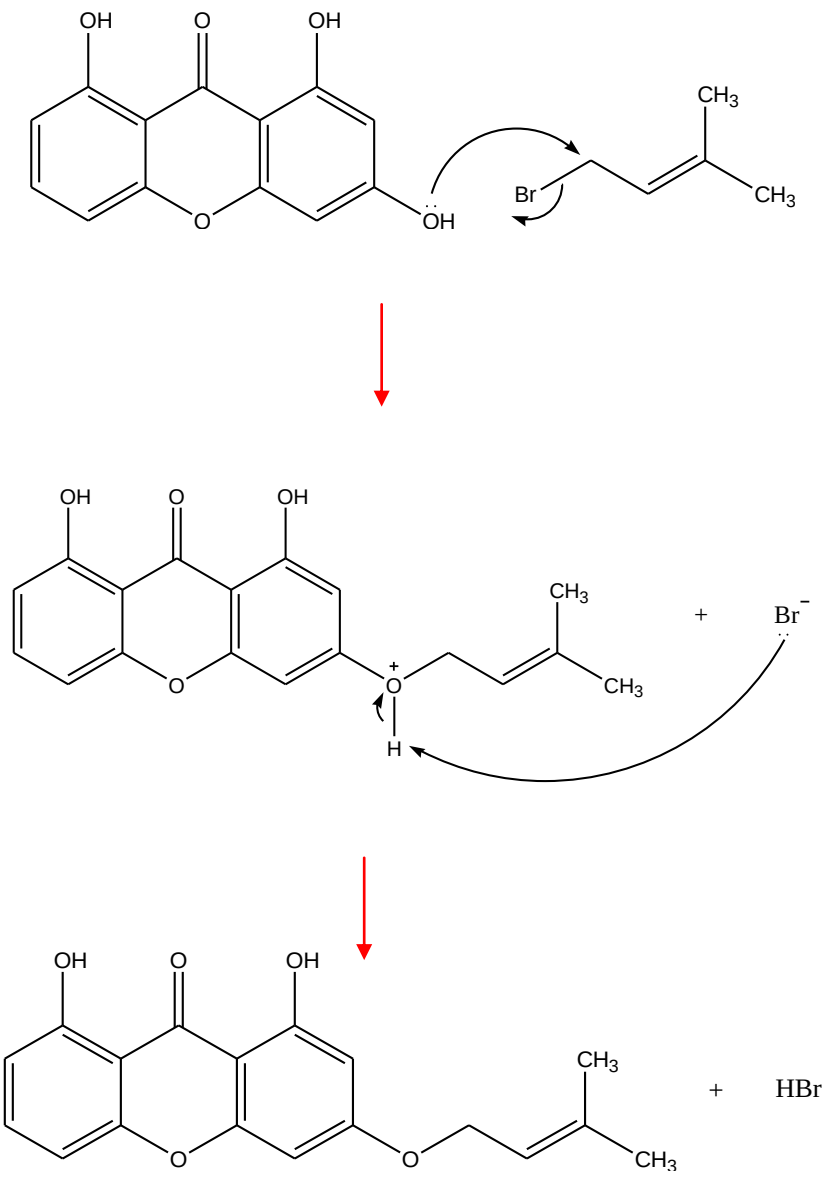



**Figure 4.10: Reaction scheme of O- prenylation synthesis**

### 4.2.1 Proposed Mechanism of 1,8-Dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one Synthesized




[In this synthesis, $K_2CO_3$ only served as catalyst at the surface of the reaction, hence it wound presence in mechanism.]

**Figure 4.11: Proposed mechanism for synthesis of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

### 4.2.2 Characterization of 1,8-Dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

By using gravity column chromatography, a pure compound, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one was obtained. This compound was labeled as SCE 22, which was isolated in the form of yellowish powder-like solid with a melting point range of 148 $^{o}$C to 150 $^{o}$C. This compound gave a single spot on the thin layer chromatography (TLC) with a $R_f$ of 0.43 using solvent system of 90 % hexane and 10 % acetone. The total weight of pure compound isolated was 0.1862 g, which contributed to percentage yield of 14.91 %.

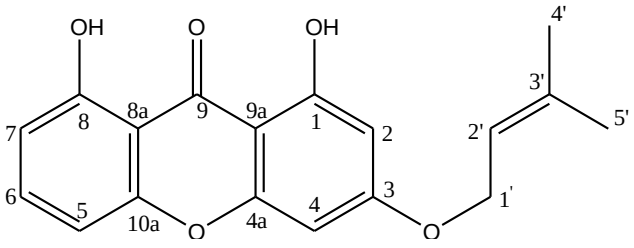



**Figure 4.12: Structure of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

The analysis of $^{1}$H-NMR spectrum (Figure 4.14) indicated that the compound consisted of a xanthone skeleton with one prenyl moiety. The $^{1}$H-NMR spectrum exhibited two singlet signals at δ 11.93 (1H, s, 1-OH) and δ 11.90 (1H, s, 8-OH) indicating the presence of two chelated hydroxyl groups. There was a total five aromatic proton signals in the region δ 6.20 to 7.60. The presence of prenyl group in the structure was evident by the proton signals δ 5.47 (1H, t, J = 6.7 Hz, H-2'), δ 4.57 (2H, d, J = 6.7 Hz, H-1'), δ 1.82 (3H, s, H-4') and δ 1.76 (3H,s, H-5'). Proton H-2' had a higher chemical shift value than protons H-1', H-4' and H-5' because it bonded to $sp^2$ hybridized carbon. A doublet signal observed at H-1' has a higher chemical shift value than protons H-4' and H-5' because it was bonded to electronegative oxygen atom.

The $^{13}$C-NMR spectrum (Figure 4.15) showed eighteen carbon peaks which indicated thirteen carbon signals from xanthone skeleton and the remaining five carbon signals were due to the prenyl group. These five carbon signals were δ 139.7 (C-3'), δ 118.4 (C-2'), δ 65.7 (C-1'), δ 25.9 (C-4') and δ 18.4 (C-5'). Carbons C-3' and C-2' had higher chemical shift because they were the olefinic carbons in the prenyl group.

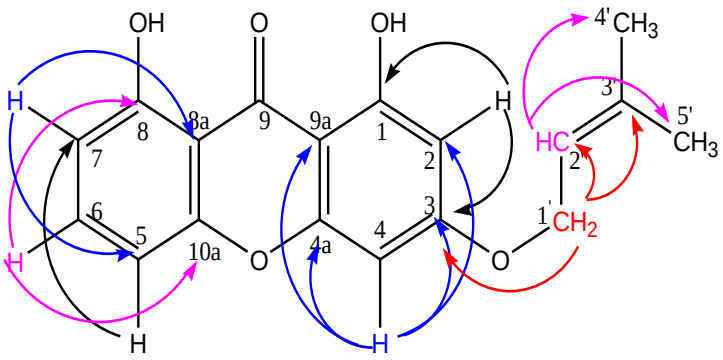
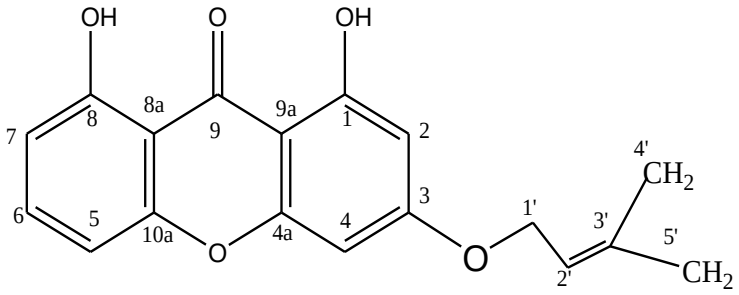



**Figure 4.13: HMBC correlations of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

HMBC analysis revealed long range $^{2}J$ and $^{3}J$ heteronuclear correlations and hence the assignment of quaternary carbon present in the structure can be confirmed and identified. From HMBC spectrum (Appendix 1d), it was observed that H-1' was $^{2}J$ coupling with carbon C-2', and $^{3}J$ coupling with carbons C-3 and C-3'. Due to this $^{3}J$ coupling, the prenyl group was found to be bonded to oxygen at C-3 position.

The prenylated compound, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one was further characterized through IR and UV analyses. The IR spectrum (Figure 4.16) showed absorption bands at 3423 (O-H), 1636 (chelated C=O) and 1608 (C=C) $cm^{-1}$. The UV spectrum (Figure 4.17) showed absorptions at 203.66, 249.92, and 328.86 nm revealed that the compound was highly conjugated and it was in agreement with the structure proposed.




1,8-Dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

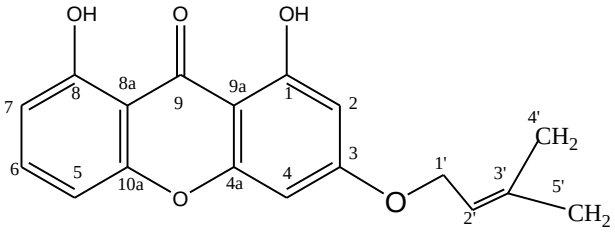
312.308 g $mol^{-1}$

$C_{18}H_{14}O_5$

**Table 4.2: Summary of elucidated spectral data of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

| Position | $\delta_H$ (ppm) | $\delta_C$ (ppm) | HMBC |
|---|---|---|---|
| 1 | - | 162.9 | - |
| 2 | 6.30 (1H, d, J=2.1Hz) | 98.0 | C-1 ($^2$J), 3 ($^2$J), 4 ($^3$J), 9a ($^3$J) |
| 3 | - | 166.7 | |
| 4 | 6.37 (1H, d, J=2.1Hz) | 93.7 | C-2 ($^3$J), 3 ($^2$J), 4a ($^2$J), 9a ($^3$J) |
| 4a | - | 157.7 | - |
| 5 | 6.73 (1H, d, J=8.2Hz) | 110.9 | C-7 ($^3$J), 8a ($^3$J) |
| 6 | 7.52 (1H, t, J=8.2Hz) | 136.8 | C-8 ($^3$J), 10a ($^3$J) |
| 7 | 6.82 (1H, d, J=8.2Hz) | 107.0 | C-5 ($^3$J), 8a ($^3$J) |
| 8 | - | 161.3 | - |
| 8a | - | 107.6 | - |
| 9 | - | 184.5 | - |
| 9a | - | 102.6 | - |
| 10a | - | 156.1 | - |
| 1’ | 4.57 (2H, d, J=6.7Hz) | 65.7 | C-3 ($^3$J), 2’ ($^2$J), 3’ ($^3$J) |
| 2’ | 5.47 (1H, t, J=6.7Hz) | 118.4 | C-4’ ($^3$J), 5’ ($^3$J) |
| 3’ | - | 139.7 | - |

| | | | |
|---|---|---|---|
| 4’ | 1.82 (3H, s) | 25.9 | C-2’ ($^3$J), 3’ ($^2$J), 5’ ($^3$J) |
| 5’ | 1.76 (3H, s) | 18.4 | C-2’ ($^3$J), 3’ ($^2$J), 4’ ($^3$J) |
| 1-OH | 11.93 (1H, s) | - | C-2 ($^3$J), 9a ($^3$J) |
| 8-OH | 11.90 (1H, s) | - | C-7 ($^3$J), 8a ($^3$J) |




1,8-Dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

312.308 g $mol^{-1}$

$C_{18}H_{14}O_5$

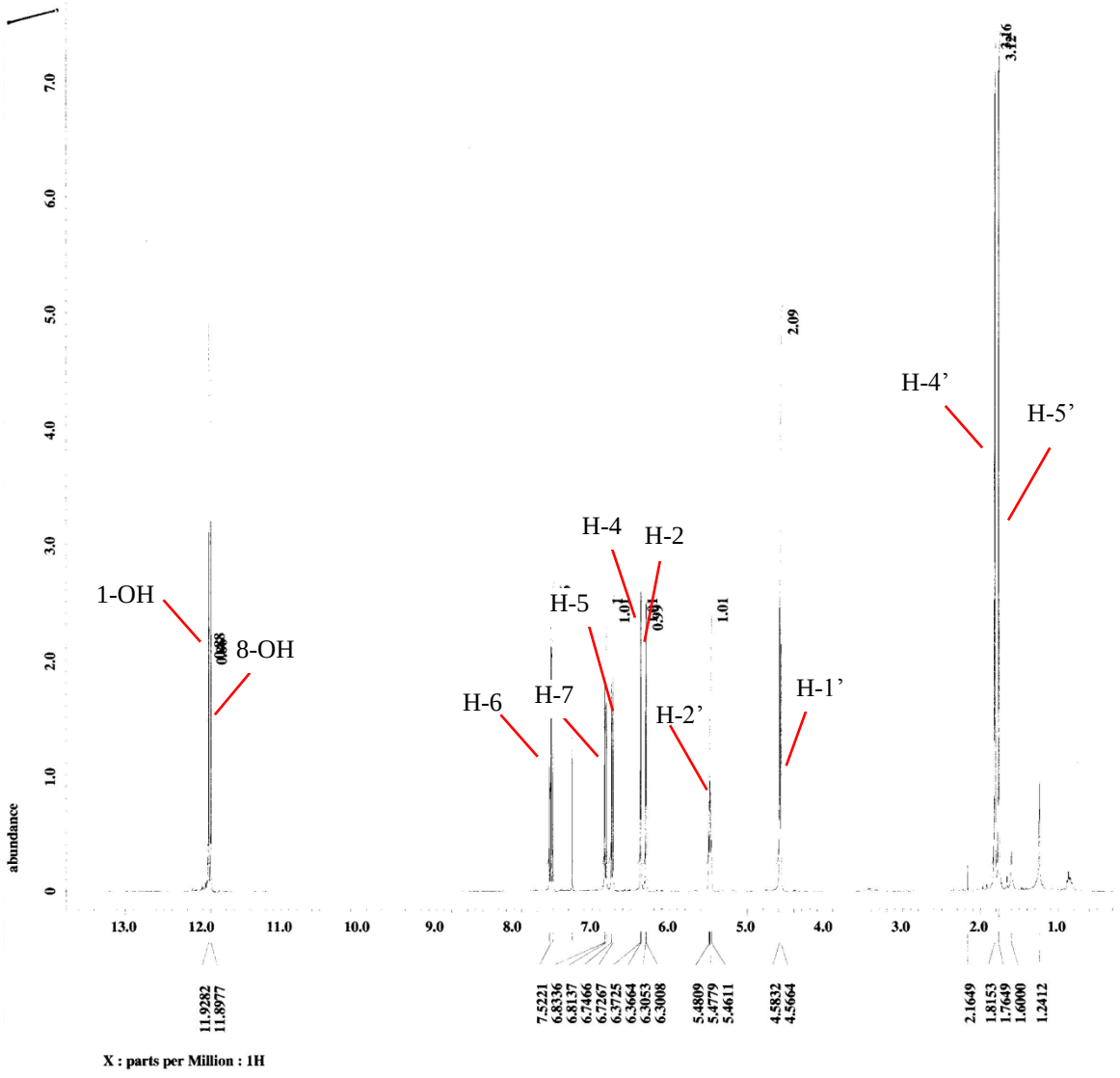
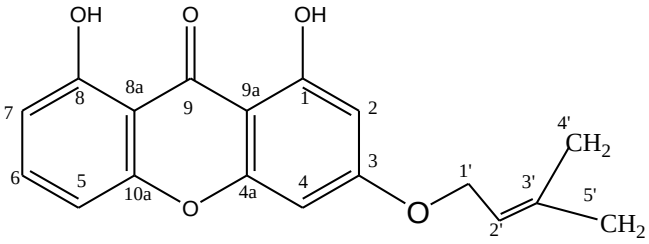



**Figure 4.14: $^1$H-NMR spectrum of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, $CDCl_3$)**




1,8-Dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

312.308 g $mol^{-1}$

$C_{18}H_{14}O_5$

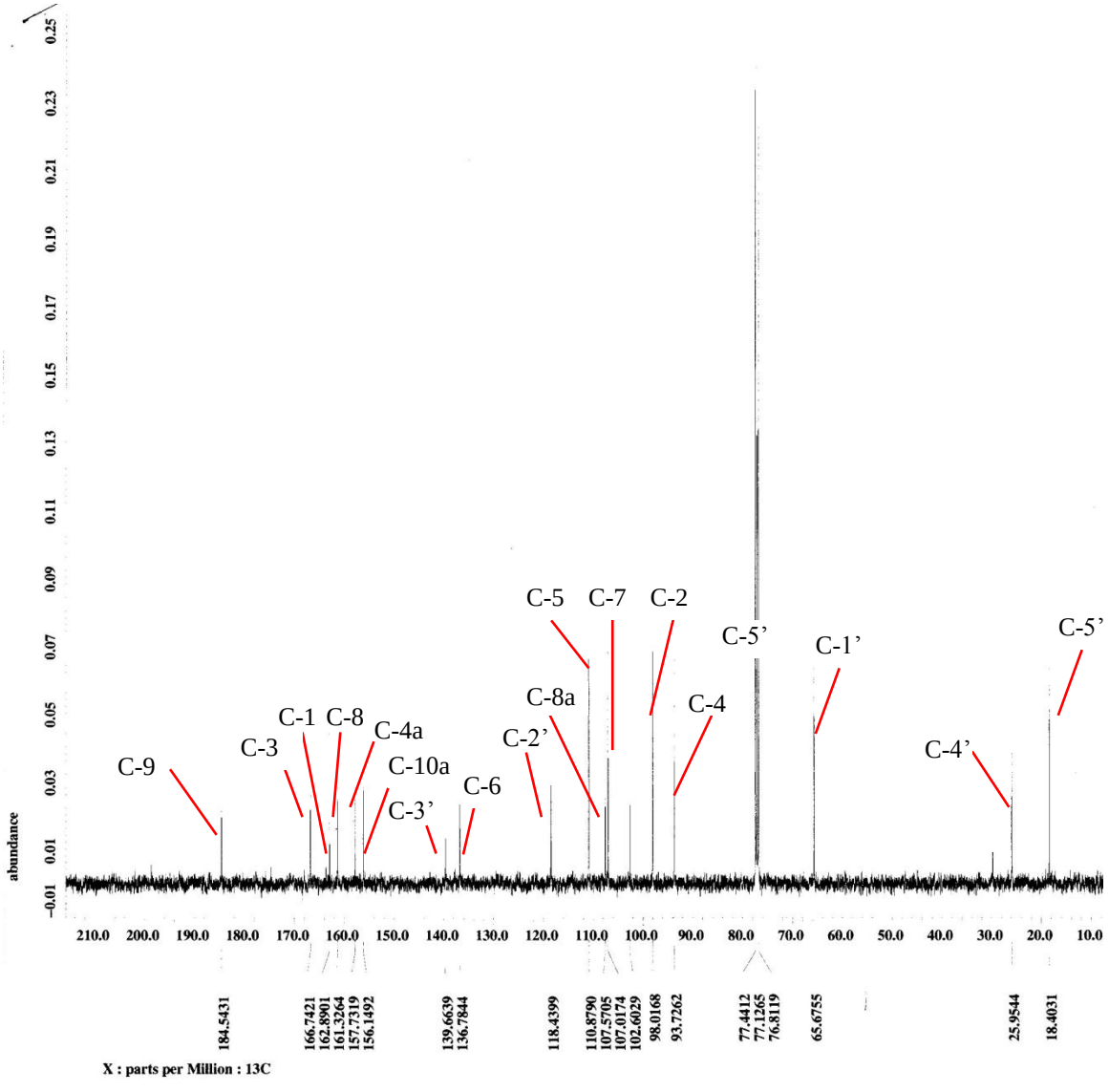
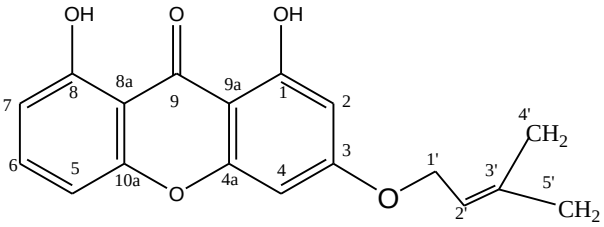



**Figure 4.15: $^{13}$C-NMR spectrum of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (100 MHz, $CDCl_3$)**




1,8-Dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

312.308 g $mol^{-1}$

$C_{18}H_{14}O_5$

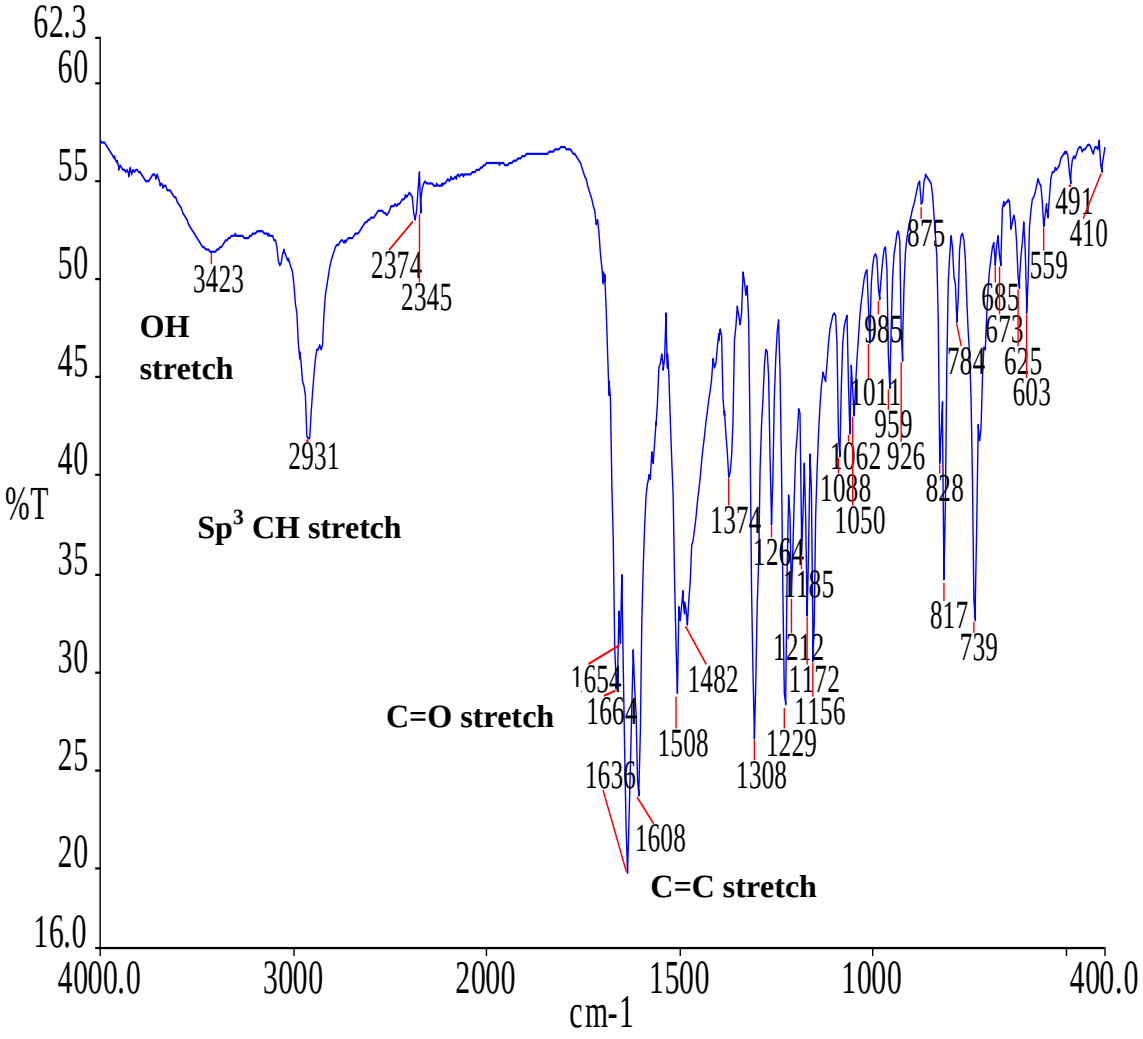
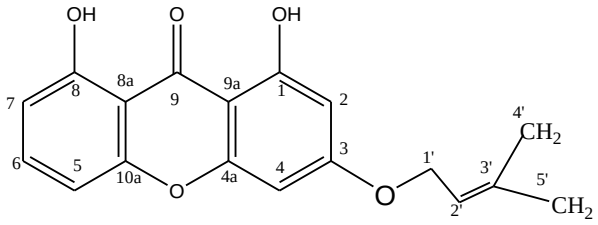



**Figure 4.16: IR spectrum of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**




1,8-Dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

312.308 g $mol^{-1}$

$C_{18}H_{14}O_5$

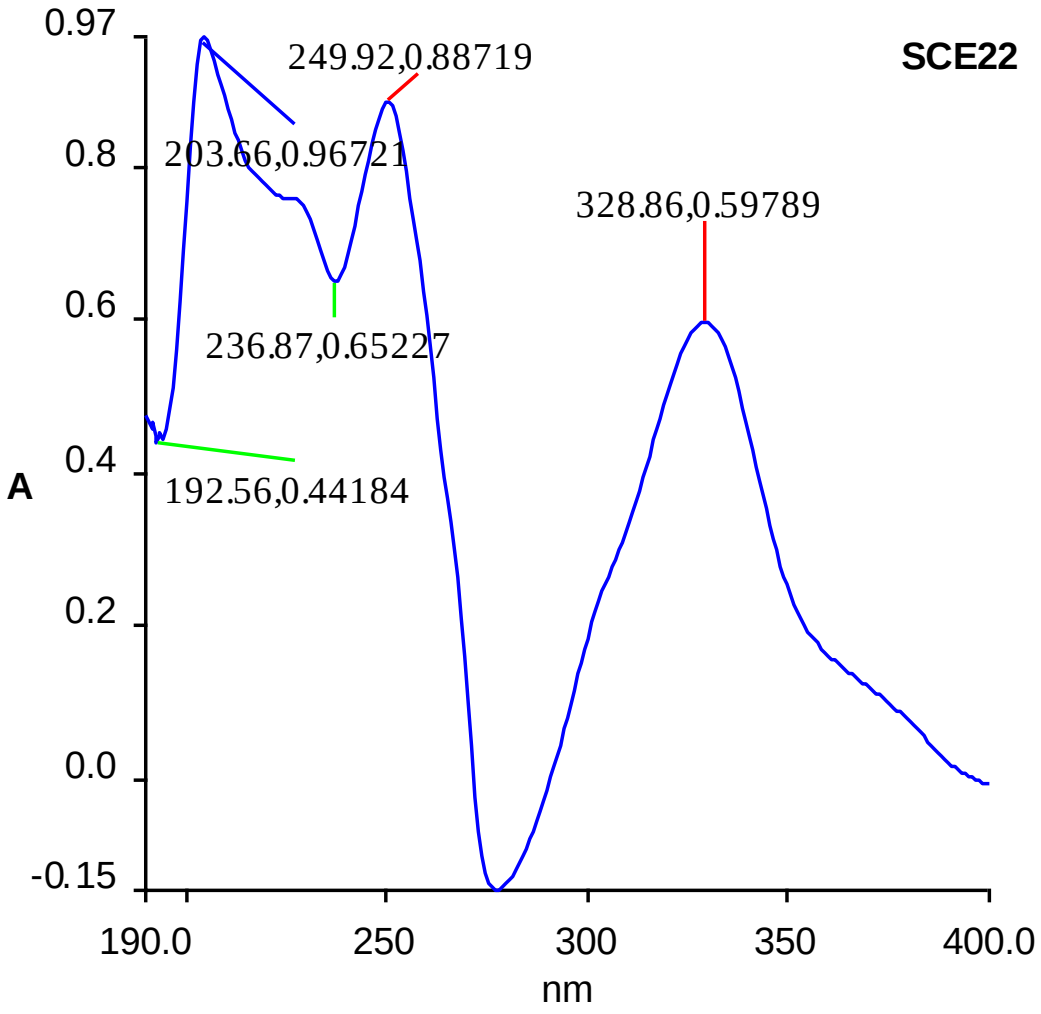



**Figure 4.17: UV-Vis spectrum of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

### 4.3 Prenylation of 1,3,8-Trihydroxyxanthone in Aqueous Medium

This prenylation involved reaction of 8 mmol xanthone block, 1,3,8-trihydroxyxanthone and 40 mmol of prenyl bromide in the presence of 35.0 g potassium bicarbonate in deionized water. In this synthesis, deionized water was used as aqueous medium. The reaction mainly took place at hydroxyl groups and carbons in xanthone block. The crude product that obtained was purify through gravity column chromatography, and two pure compounds were isolated. One of the pure compounds, SCR 5 which was identified as 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione, was found to have four prenyl groups. This compound was isolated with a weight of 0.2365 g or 5.78 % of percentage yield, while the other compound, SCT 2 was found to be 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one, with two prenyl groups attached to it, and gave a yield of 0.1878 g or 6.23 % of percentage yield.

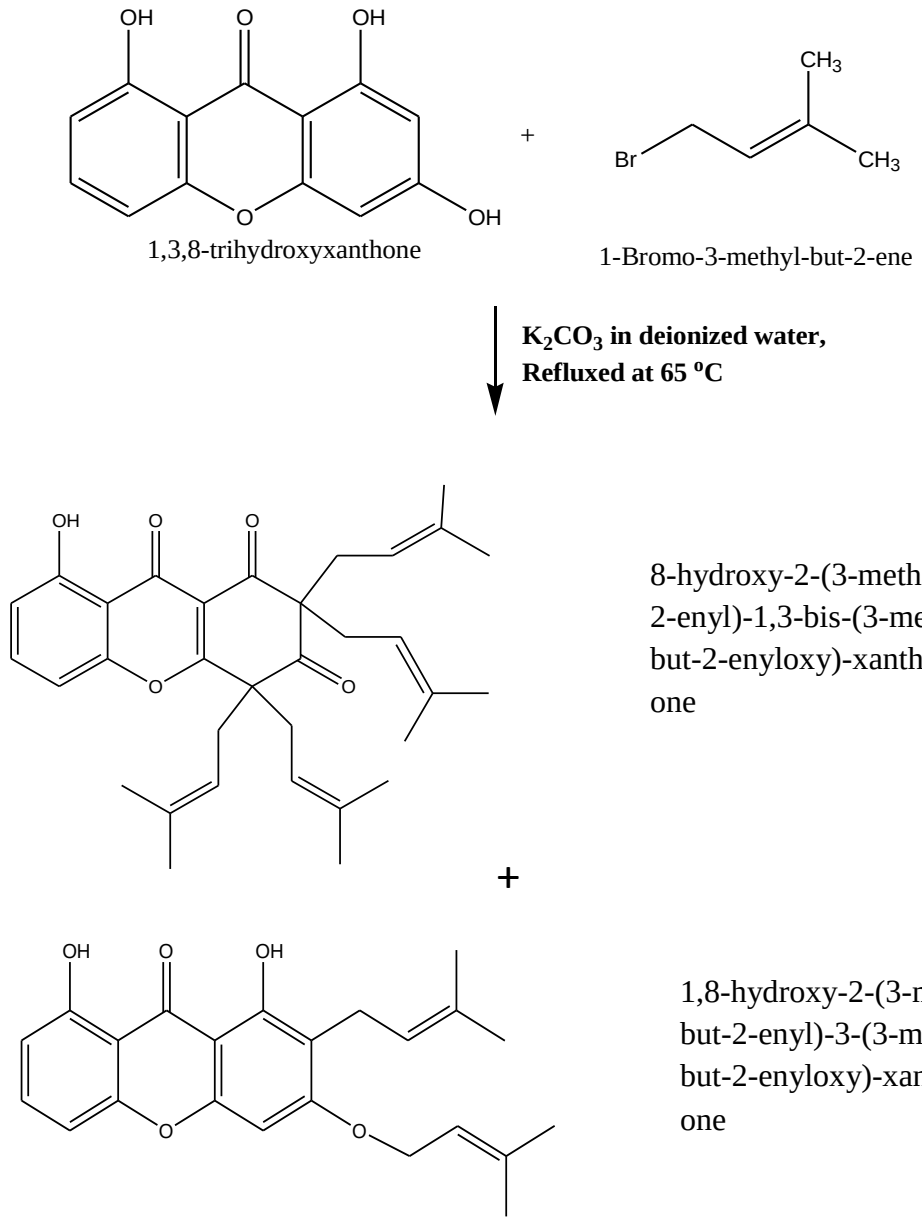



**Figure 4.18: Reaction scheme of O- and C- prenylation synthesis**

### 4.3.1 Compound : 8-Hydroxy-2,2,4,4-tetrakis-(3-methyl- but-2-enyl)-4H-xanthene-1,3,9-trione

#### 4.3.1.1 Proposed Mechanism of 8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione Synthesized

$$K_2CO_3 + H_2O \longrightarrow 2K^+ + CO_3^{2-}$$

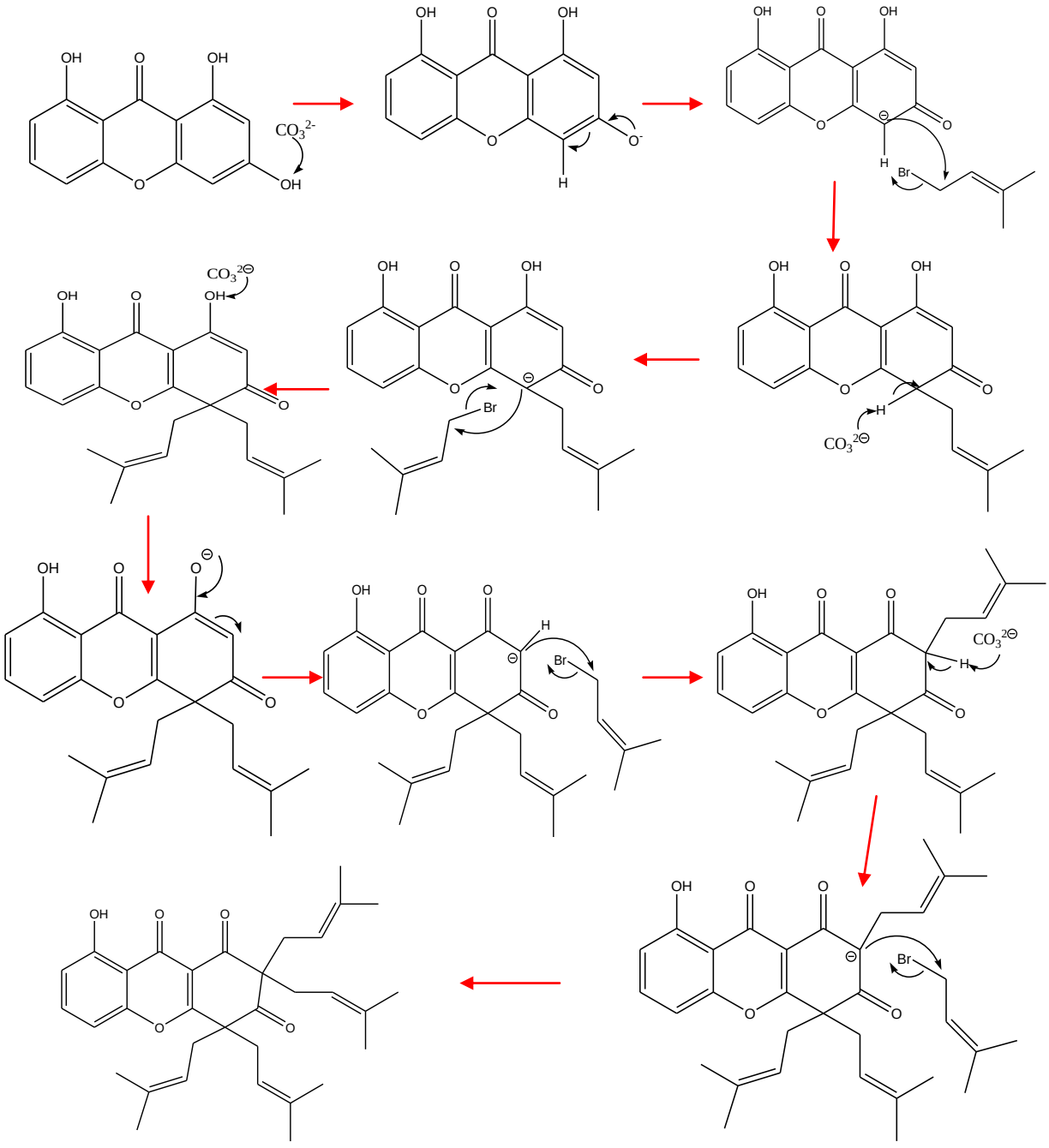


**Figure 4.19: Proposed mechanism for synthesis of 8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione**

### 4.3.1.2 Characterization of Prenylated Xanthone, 8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione

Purification of crude product by using gravity column chromatography afforded a pure prenylated compound, namely 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione. This compound was labeled as SCR 5, which was isolated in the form of yellowish crystal with a melting point of 134 $^{o}$C to 136 $^{o}$C. This compound gave a single spot with $R_f$ value of 0.52 on the thin layer chromatography (TLC) solvent system of 80 % hexane and 20 % ethyl acetate. The total weight of pure compound isolated was 0.2365 g, which contributed to percentage yield of 5.7 %.

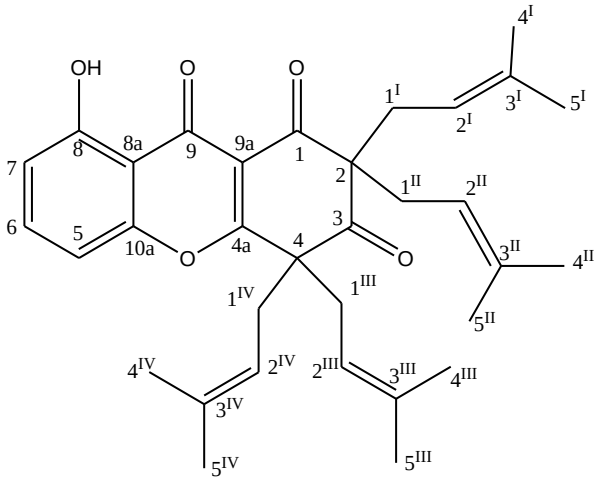



**Figure 4.20: Structure of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione**

The initial analysis of $^{1}$H-NMR spectrum (Figure 4.22) spectral data indicated the molecule to consist of a xanthone skeleton with four prenyl groups. The $^{1}$H-NMR spectrum showed only one chelated hydroxyl proton at 12.49 ppm (1H, s, 8-OH) in the 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione structure. There were presence only three aromatic protons in the structure, which were δ 7.54 (1H, t, J=8.3 Hz, H-6), δ 6.89 (1H, d, J=8.3 Hz, H-7), and δ 6.81 (1H, d, J=8.3 Hz, H-5). The signal peaks which due to the four prenyl groups that presence in the compound were found to be δ 4.91 (2H, t, J=6.7 Hz, H-$2^{I}$ and H-$2^{II}$), δ 4.85 (2H, t, J=7.9 Hz, H-$2^{III}$ and H-$2^{IV}$), δ 2.81 (2H, d, J=7.9 Hz, H-$1^{IV}$), δ 2.64 (2H, d, J= 7.9 Hz, H-$1^{III}$), δ 2.50 (2H, d, J=6.7 Hz, H-$1^{I}$), δ 2.36 (2H, d, J=6.7 Hz, H-$1^{II}$), δ 1.59 (6H, s, H-$4^{I}$ and H-$5^{I}$), δ 1.55 (6H, s, H-$4^{II}$ and H-$5^{II}$), δ 1.50 (6H, s, H-$4^{III}$ and H-$5^{III}$) and δ 1.43 (6H, s, H-$4^{IV}$ and H-$5^{IV}$).

The $^{13}$C-NMR spectrum (Figure 4.23) showed the number and type of nonequivalent carbon present in the compound. For this prenylated compound, the core structure of xanthone nucleus became distorted, which two of carbons C-2 and C-4 were changed from $sp^2$ carbons to $sp^3$ carbons and two of the carbinol groups at C-1 and C-3 were oxidized to carbonyl carbons. A total of three carbonyl carbon signals were found at δ 206.6 (C-3), δ 191.9 (C-1) and δ 179.2 (C-9). Besides that, a total of twenty carbon signals corresponded to four prenyl groups that present in the compound,

which included four methylene carbons, four methine carbons, four quaternary carbons and eight methyl carbons.

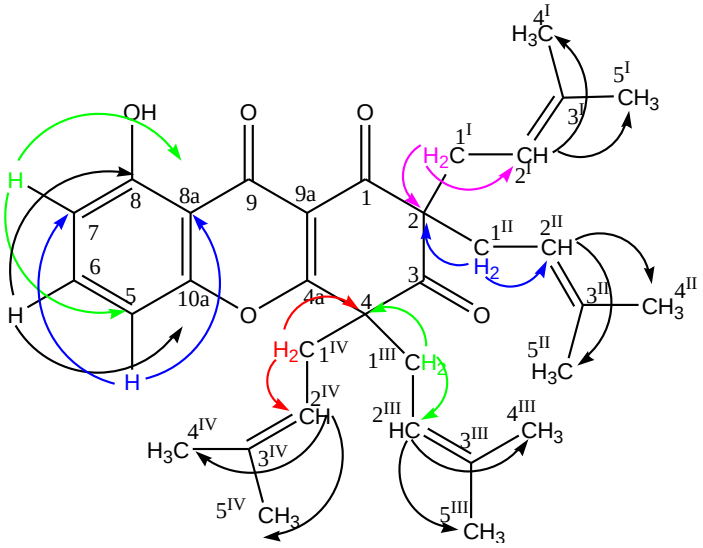
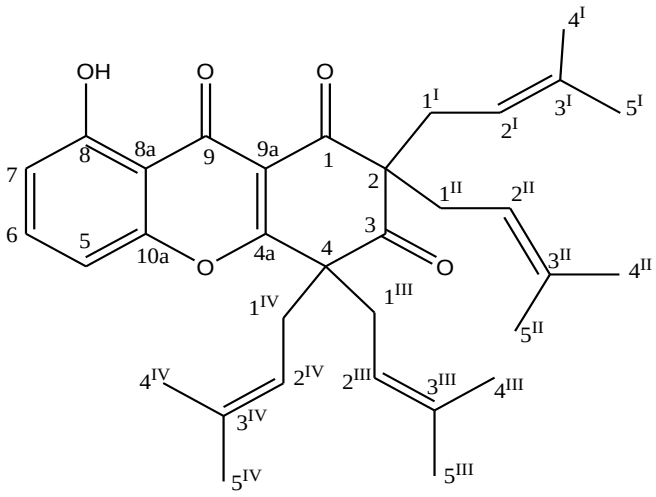



**Figure 4.21: HMBC correlations of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione**

From HMBC spectrum (Appendix 1f), it was observed that methylene proton H-$1^{I}$ and H-$1^{II}$ in the prenyl groups showed $^{2}J$ coupling with carbon C-2, which confirmed that the two prenyl groups were attached at the same carbon position C-2 in the xanthone skeleton. For third and fouth prenyl group, the methylene proton, H-$1^{III}$ and H-$1^{IV}$ showed $^{2}J$ coupling with carbon C-4,and these confirmed that the third and fouth prenyl groups were attached to the xanthone nucleus at the carbon position C-4.

The prenylated compound, 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione was further characterized through IR and UV analyses. The IR spectrum (Figure 4.24) showed absorption bands at 3422 (O-H), 1699 (C=O) and 1422 (C=C) $cm^{-1}$. Meanwhile, the UV spectrum (Figure 4.25) showed absorption maxima at 207.93, 241.22, and 308.66 nm, which confirmed the compound to be a highly conjugated prenylated xanthone.




8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione

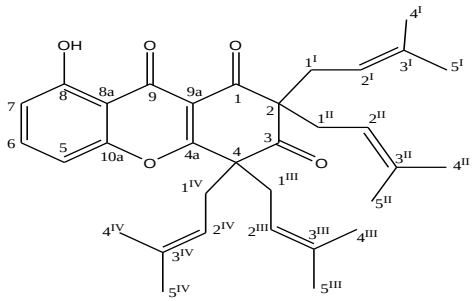
516.65 g $mol^{-1}$

$C_{33}H_{40}O_5$

**Table 4.3: Summary of elucidated spectral data of 8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione**

| Position | $\delta_H$ (ppm) | $\delta_C$ (ppm) | HMBC |
|---|---|---|---|
| 1 | - | 191.9 | - |
| 2 | - | 66.5 | - |
| 3 | - | 206.7 | - |
| 4 | - | 59.0 | - |
| 4a | - | 175.5 | - |
| 5 | 6.85 (1H, d, J=8.3Hz) | 113.0 | C-7 ($^3$J), 8a ($^3$J) |
| 6 | 7.56 (1H, d, J=8.3Hz) | 137.4 | C-8 ($^3$J), 10a ($^3$J) |
| 7 | 6.91 (1H, d, J=8.3Hz) | 106.9 | C-5 ($^3$J), 8a ($^3$J) |
| 8 | - | 161.8 | - |
| 8a | - | 110.7 | - |
| 9 | - | 179.2 | - |
| 9a | - | 117.0 | - |
| 10a | - | 155.1 | - |

| | | | |
|---|---|---|---|
| $1^{I}$ | 2.52 (2H, d, J=6.7Hz) | 33.1 | C-2 ($^{2}$J), $2^{I}$ ($^{2}$J), 1 ($^{3}$J), $3^{I}$ ($^{3}$J) |
| $2^{I}$ | 4.92 (1H, t, J=6.7Hz) | 118.2 | C-$4^{I}$ ($^{3}$J), $5^{I}$ ($^{3}$J) |
| $3^{I}$ | - | 136.2 | - |
| $4^{I}$ | 1.62 (6H, s) | 26.1 | C-$3^{I}$ ($^{2}$J), $2^{I}$, ($^{3}$J) |
| $5^{I}$ | 1.62 (6H, s) | 26.1 | C-$3^{I}$ ($^{2}$J), $2^{I}$ ($^{3}$J) |
| $1^{II}$ | 2.38 (2H, d, J=6.7Hz) | 33.1 | C-2 ($^{2}$J), $2^{II}$ ($^{2}$J), 3 ($^{3}$J), $3^{II}$ ($^{3}$J) |
| $2^{II}$ | 4.92 (1H, t, J=6.7Hz) | 118.2 | C-$4^{II}$ ($^{3}$J),$5^{II}$ ($^{3}$J) |
| $3^{II}$ | - | 136.2 | - |
| $4^{II}$ | 1.58 (6H, s) | 18.0 | C-$3^{II}$ ($^{2}$J), $2^{II}$ ($^{3}$J) |
| $5^{II}$ | 1.58 (6H, s) | 18.0 | C-$3^{II}$ ($^{2}$J), $2^{II}$ ($^{3}$J) |
| $1^{III}$ | 2.83 (2H, d, J=7.9Hz) | 37.6 | C-4 ($^{2}$J), $2^{III}$ ($^{2}$J), 4a ($^{3}$J), $3^{III}$ ($^{3}$J) |
| $2^{III}$ | 4.85 (1H, t, J=7.9Hz) | 117.5 | C-$4^{III}$ ($^{3}$J),$5^{III}$ ($^{3}$J) |
| $3^{III}$ | - | 137.4 | - |
| $4^{III}$ | 1.53 (6H, s) | 25.8 | C-$3^{III}$ ($^{2}$J), $2^{III}$ ($^{3}$J) |
| $5^{III}$ | 1.53 (6H, s) | 25.8 | C-$3^{III}$ ($^{2}$J), $2^{III}$ ($^{3}$J) |
| $1^{IV}$ | 2.66 (2H, d, J=7.9Hz) | 37.6 | C-4 ($^{2}$J), $2^{IV}$ ($^{2}$J), 3 ($^{3}$J), $3^{IV}$ ($^{3}$J) |
| $2^{IV}$ | 4.85 (1H, t, J=7.9Hz) | 117.5 | C-$4^{IV}$ ($^{3}$J), $5^{IV}$ ($^{3}$J), |
| $3^{IV}$ | - | 137.4 | - |
| $4^{IV}$ | 1.46 (6H, s) | 17.9 | C-$3^{IV}$ ($^{2}$J), $2^{IV}$ ($^{3}$J) |
| $5^{IV}$ | 1.46 (6H, s) | 17.9 | C-$3^{IV}$ ($^{2}$J), $2^{IV}$ ($^{3}$J) |
| 8-OH | 12.53 (1H, s) | - | C-8a ($^{3}$J) |



8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione

516.65 g $mol^{-1}$

$C_{33}H_{40}O_5$

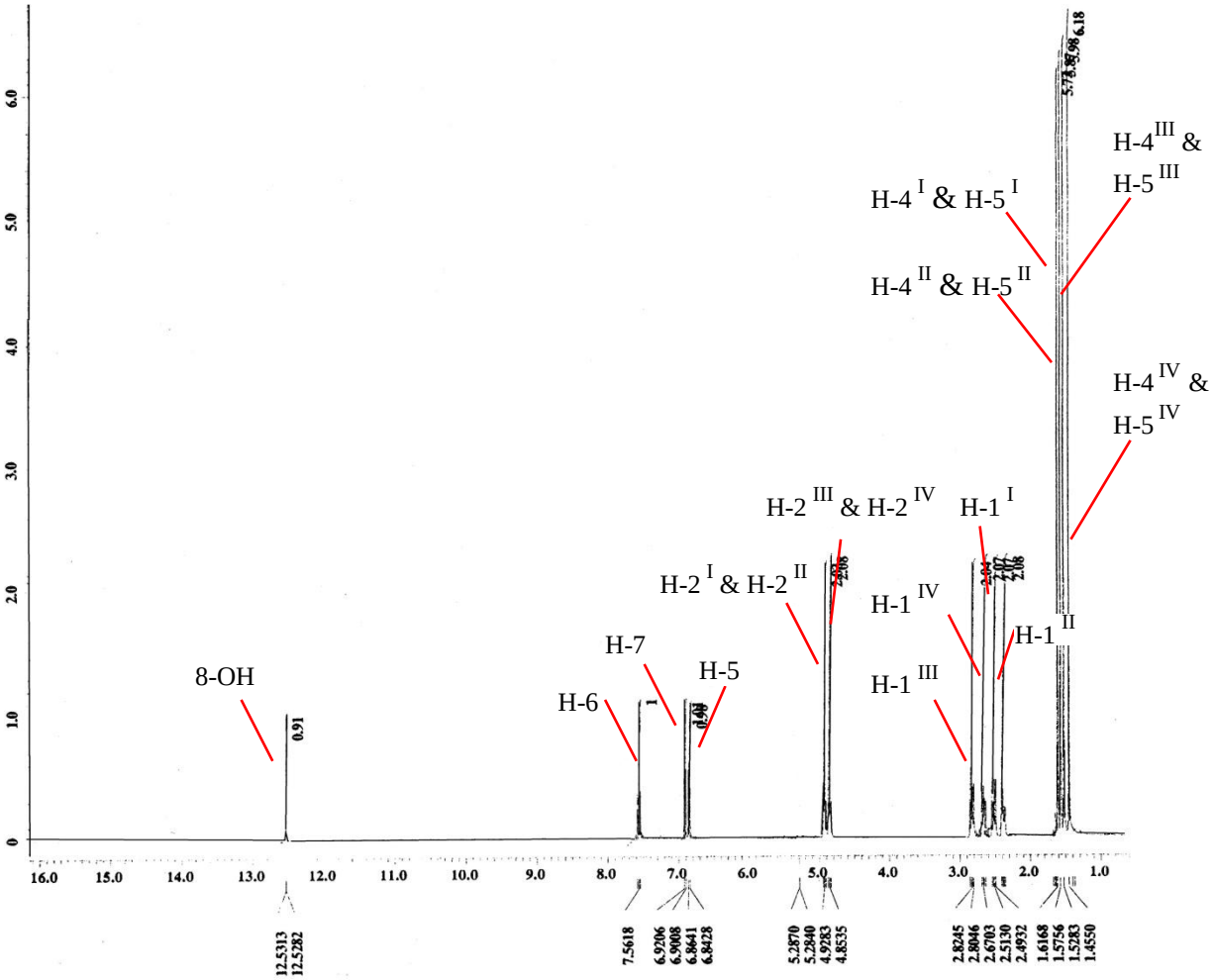
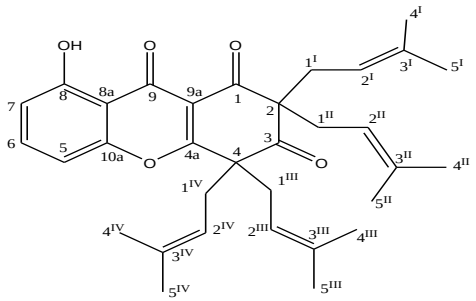



**Figure 4.22: $^1$H-NMR spectrum of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione (400 MHz, $CDCl_3$)**



8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione

516.65 g $mol^{-1}$

$C_{33}H_{40}O_5$

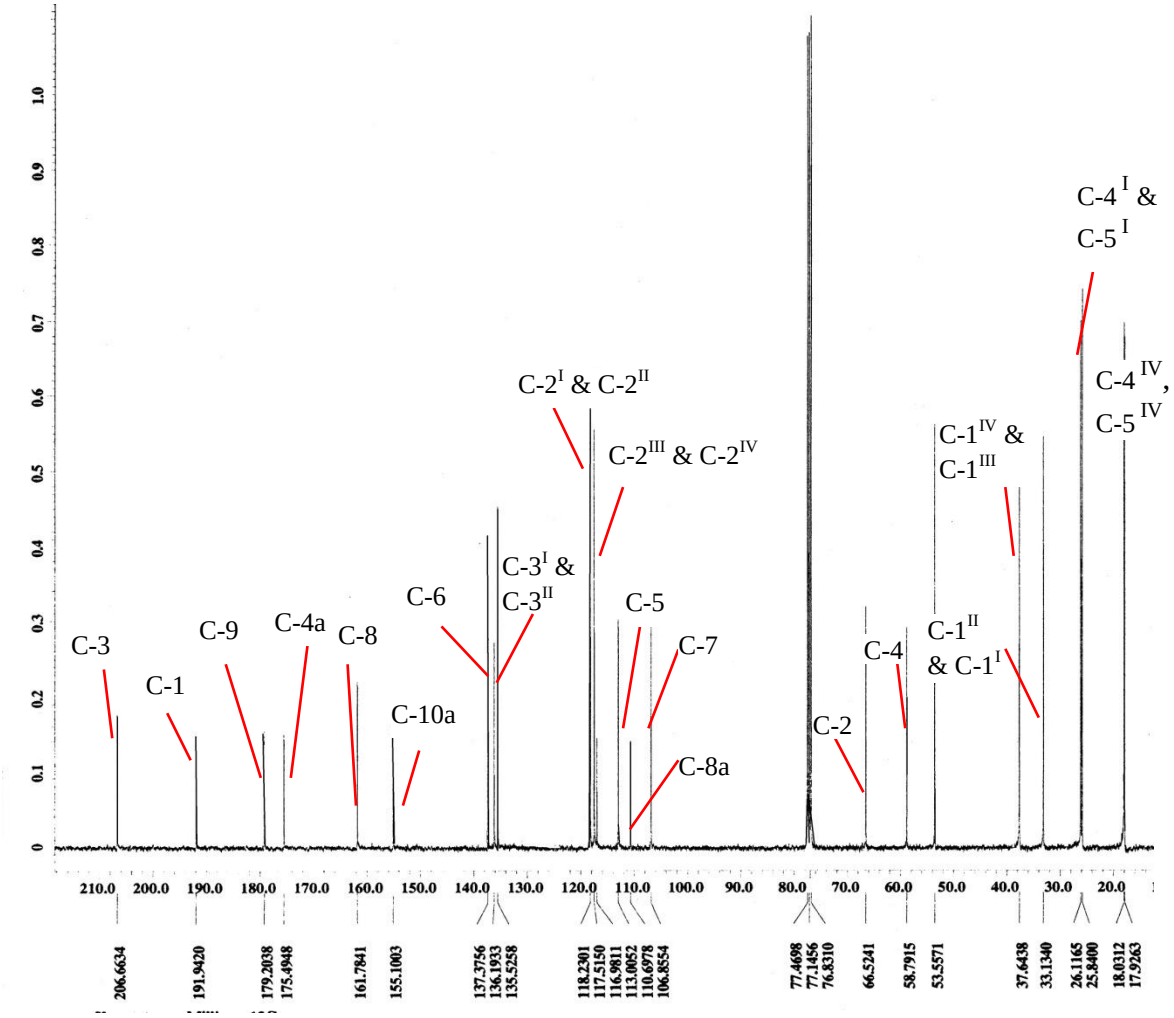
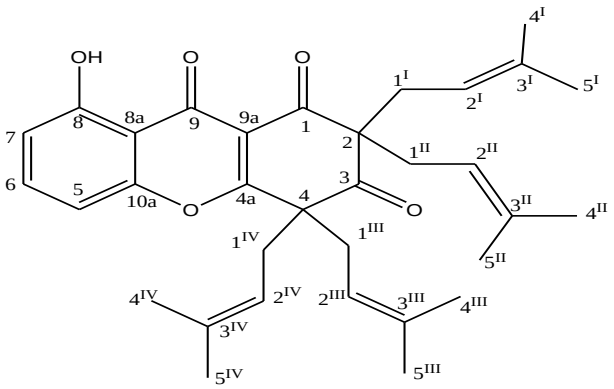



**Figure 4.23: $^{13}$C-NMR spectrum of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione (100 MHz, $CDCl_3$)**




8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione

516.65 g $mol^{-1}$

$C_{33}H_{40}O_5$

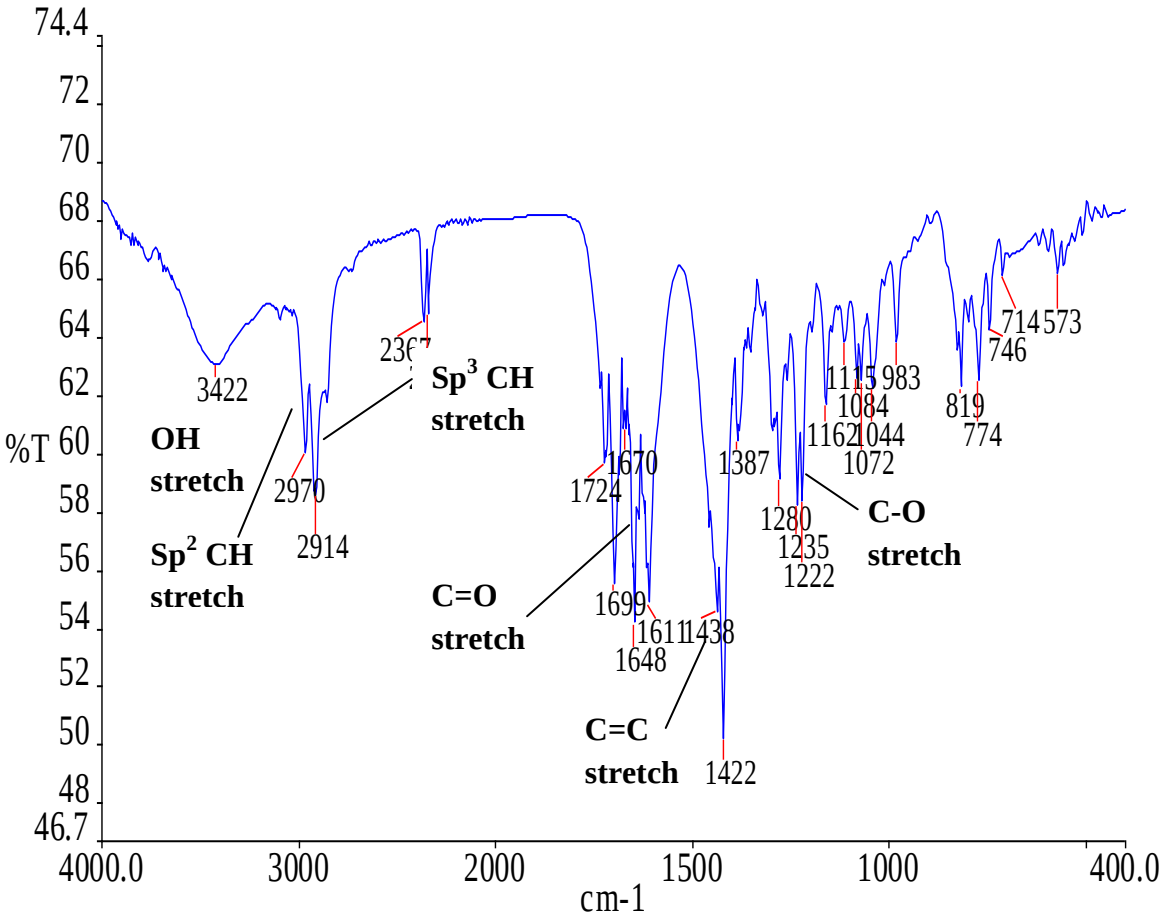
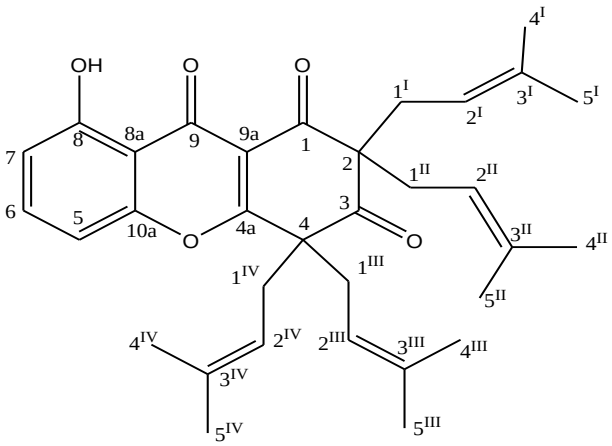



**Figure 4.24: IR spectrum of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione**




8-Hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione

516.65 g $mol^{-1}$

$C_{33}H_{40}O_5$

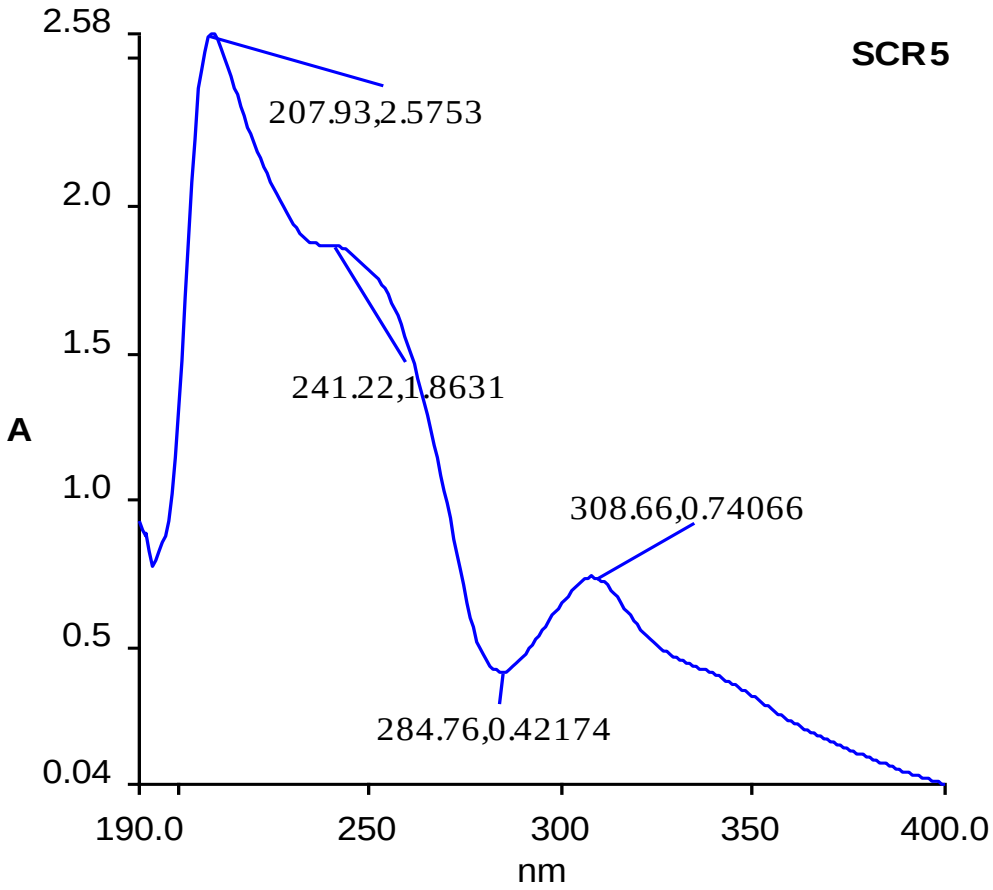



**Figure 4.25: UV-Vis spectrum of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione**

### 4.3.2 Compound : 1,8-Dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

#### 4.3.2.1 Proposed Mechanism of 1,8-Dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one Synthesized

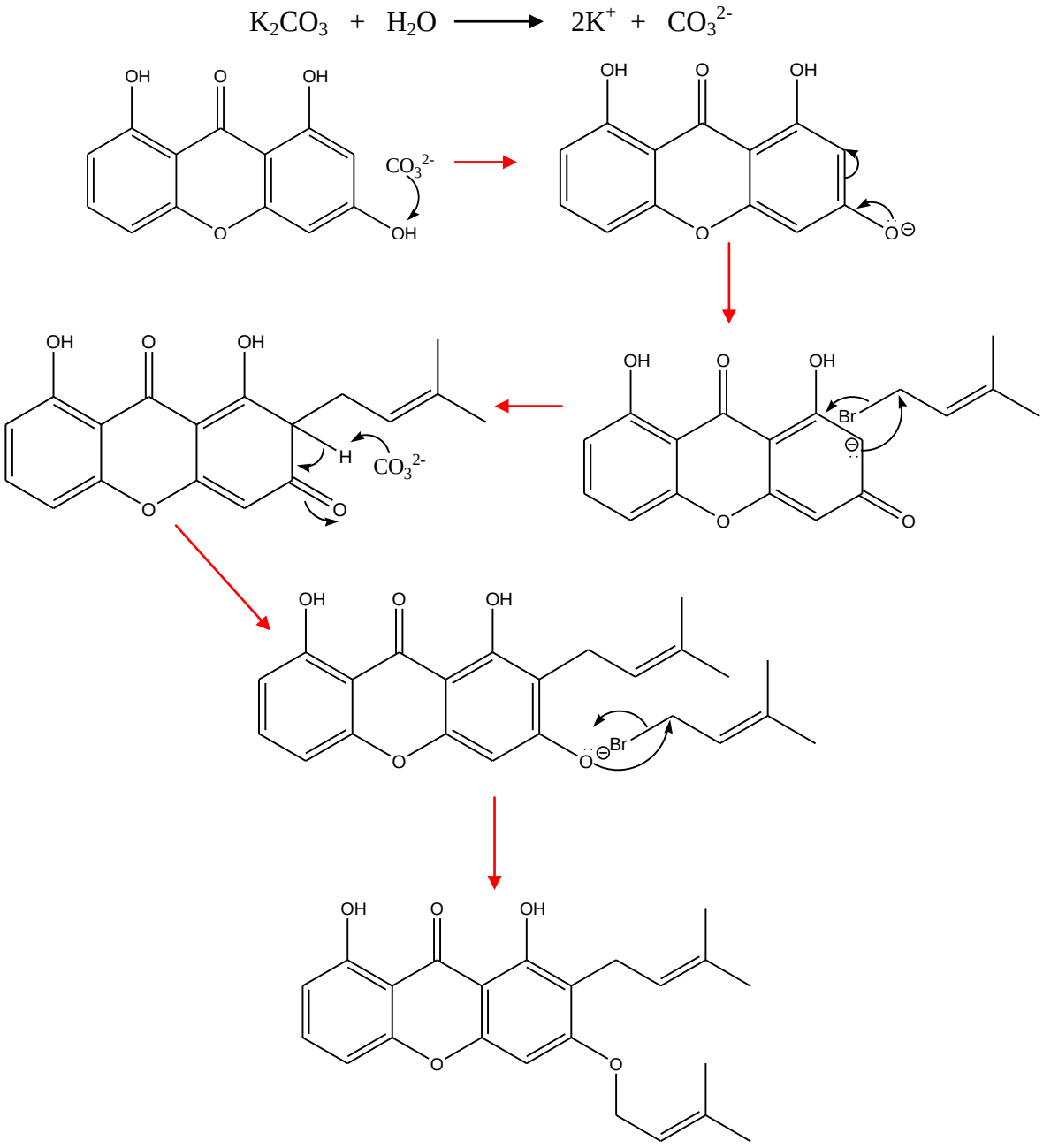



**Figure 4.26: Proposed mechanism for synthesis of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

### 4.3.2.2 Characterization of Prenylated Xanthone, 1,8-Dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

Purification of crude products by using gravity column chromatography yielded a pure prenylated compound, namely 1,8-hydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one. This compound was labeled as SCT 2, which was isolated in the form of yellowish crystal with a melting point range of 144 $^{o}$C to 145 $^{o}$C. This compound gave a single spot with $R_f$ value of 0.41 on the thin layer chromatography (TLC) using a solvent system of 40 % hexane and 60 % dichloromethane.. The total weight of pure compound isolated was 0.1878 g which contributed percentage yield of 6.23 %.

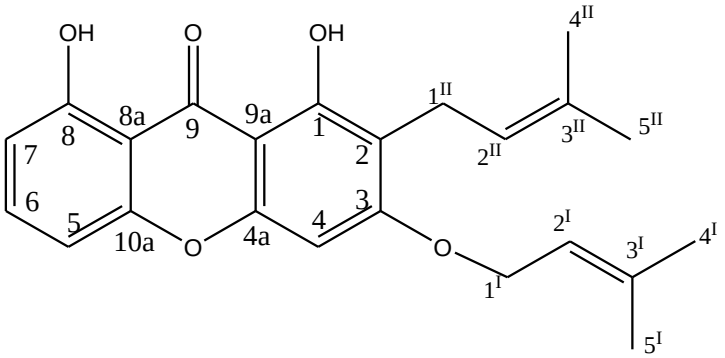



**Figure 4.27: Structure of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)- 3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

The $^{1}$H-NMR spectrum (Figure 4.29) showed chelated hydroxyl proton signal at δ 13.00 (1H, s, 1-OH). The $^{1}$H-NMR spectrum consisted four aromatic proton signals in the range of δ 6.00 – 8.00 and eight proton signals of prenyl groups in the range of δ 1.00 – 5.50. The characteristic signals for the two prenyl groups were observed at δ 5.47 (1H, t, J=6.7 Hz, H-2$^{I}$), δ 5.18 (1H, t, J=7.3 Hz, H-2$^{II}$), δ 4.60 (2H, d, J=6.7 Hz, H-1$^{I}$), δ 3.45 (2H, d, J=7.3 Hz, H-1$^{II}$), δ 1.83(3H, s, H-5$^{II}$), δ 1.80 (3H, s, H-4$^{I}$), δ 1.75 (3H, s, H-5$^{I}$) and δ 1.66 (3H, s, H-4$^{II}$).

The $^{13}$C-NMR spectrum (Figure 4.30) showed the core structure of xanthone block was attached with two prenyl groups, one was *o*-prenylated while another was *c*-prenylated. The $^{13}$C-NMR spectrum showed a total of twenty-three carbon signals, thirteen carbon signals were corresponded to carbons in the xanthone skeleton, while another ten carbon signals were due to the prenyl groups.

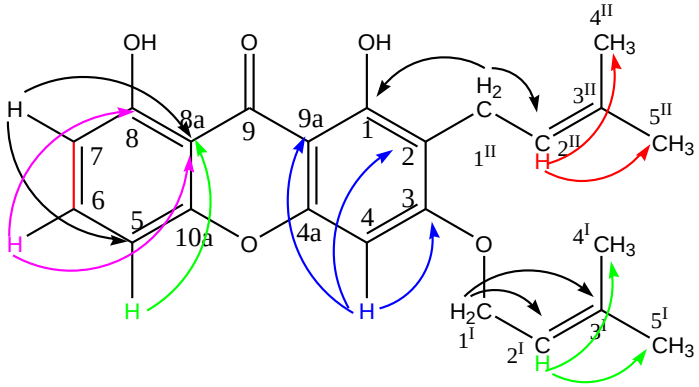
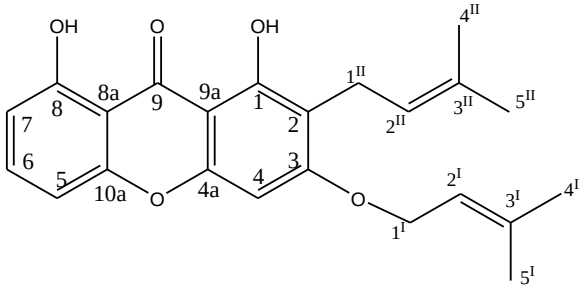



**Figure 4.28: HMBC correlations of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)- 3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

In the HMBC spectrum (Appendix 1h), the methylene proton, H-$1^{I}$ showed $^{3}J$ coupling with carbons C-3, hence, this prenyl groups was found to be attached at the oxygen atom which bonded to C-3 position. For the second prenyl group, H-$1^{II}$ showed $^{2}J$ coupling with carbons C-2 and $^{3}J$ coupling with carbon C-1, therefore, the second prenyl group was confirmed attached at C-2 position in the core structure.

The prenylated compound, 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one was further characterized through IR and UV analyses. The IR spectrum (Figure 4.31) showed absorption bands at 3448 (O-H), 1630 (chelated C=O) and 1466 (C=C) $cm^{-1}$. Meanwhile, the UV spectrum (Figure 4.32) showed absorption maxima at 222.14, 241.22, and 315.24 nm. From these UVsignals, the compound was confirmed to be a highly conjugated structure and was in agreement with the suggested structure, 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one.




1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

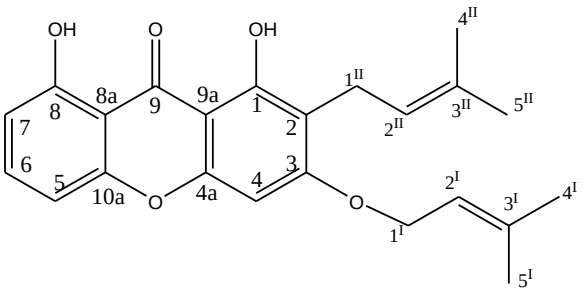
380.42 g $mol^{-1}$

$C_{23}H_{24}O_5$

**Table 4.4: Summary of elucidated spectral data of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

| Position | $\delta_H$ (ppm) | $\delta_C$ (ppm) | HMBC |
|---|---|---|---|
| 1 | - | 162.1 | - |
| 2 | - | 108.0 | - |
| 3 | - | 164.0 | - |
| 4 | 6.36 (1H, s) | 95.7 | C-3 ($^2$J), 9a ($^3$J), 2 ($^3$J) |
| 4a | - | 153.2 | - |
| 5 | 7.29 (1H, d, J=8.6Hz) | 119.6 | C-10a ($^2$J), 8a ($^3$J) |
| 6 | 7.68 (1H, t, J=8.6Hz) | 134.6 | C-8 ($^3$J), 10a ($^3$J) |
| 7 | 7.42 (1H, d, J=8.6Hz) | 117.4 | C-8 ($^2$J), 8a ($^3$J), 5 ($^3$J) |
| 8 | - | 157.7 | - |
| 8a | - | 114.2 | - |
| 9 | - | 180.0 | - |
| 9a | - | 103.9 | - |
| 10a | - | 146.7 | - |
| $1^I$ | 4.60 (2H, d, J=6.7Hz) | 65.9 | C-$2^I$ ($^2$J), $3^I$ ($^3$J), 3 ($^3$J) |
| $2^I$ | 5.47 (1H, t, J=6.7Hz) | 118.9 | C-$5^I$ ($^3$J), $4^I$ ($^3$J) |
| $3^I$ | - | 138.9 | - |
| $4^I$ | 1.80 (3H, s) | 25.9 | C- $3^I$ ($^2$J), $2^I$ ($^3$J) |
| $5^I$ | 1.75 (3H, s) | 18.4 | C- $3^I$ ($^2$J), $2^I$ ($^3$J) |

| | | | |
|---|---|---|---|
| $1^{II}$ | 3.45 (2H, d, J=7.3Hz) | 21.7 | C-1 ($^{3}$J), 2 ($^{2}$J), $3^{II}$ ($^{3}$J), $2^{II}$ ($^{2}$J) |
| $2^{II}$ | 5.18 (1H, t, J=7.3Hz) | 122.0 | C-$4^{II}$ ($^{3}$J), $5^{II}$ ($^{3}$J) |
| $3^{II}$ | - | 131.8 | - |
| $4^{II}$ | 1.66 (3H, s) | 25.9 | C-$2^{II}$ ($^{3}$J), $3^{II}$ ($^{2}$J) |
| $5^{II}$ | 1.83 (3H, s) | 17.9 | C- $2^{II}$ ($^{3}$J), $3^{II}$ ($^{2}$J) |
| 1-OH | 13.00 (1H, s) | - | C-1 ($^{2}$J), 9a ($^{3}$J) |




1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

380.42 g $mol^{-1}$

$C_{23}H_{24}O_5$

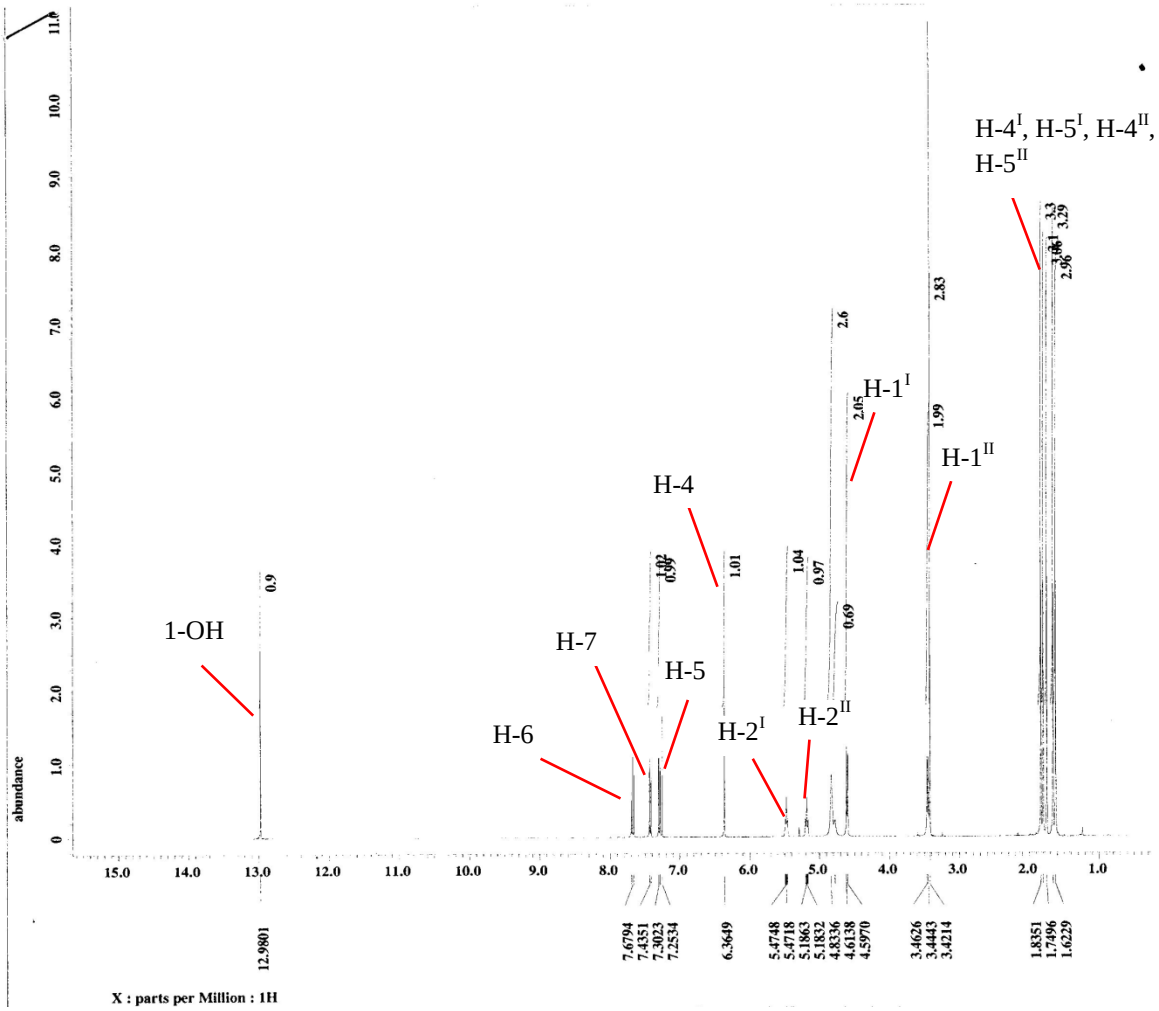
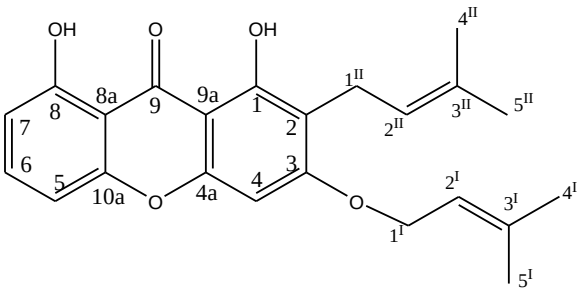



**Figure 4.29: $^1$H-NMR spectrum of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, $CDCl_3$)**




1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

380.42 g $mol^{-1}$

$C_{23}H_{24}O_5$

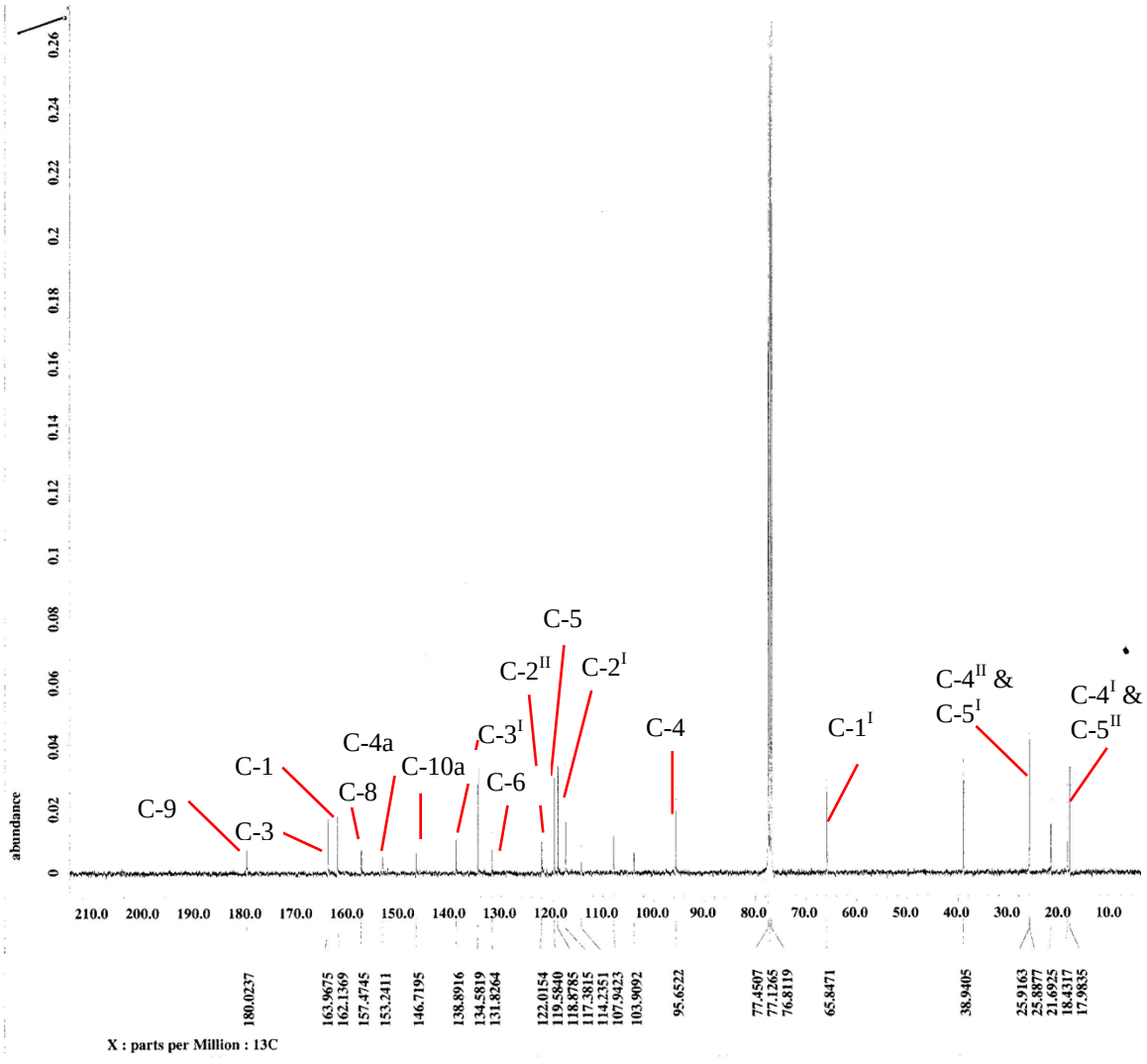
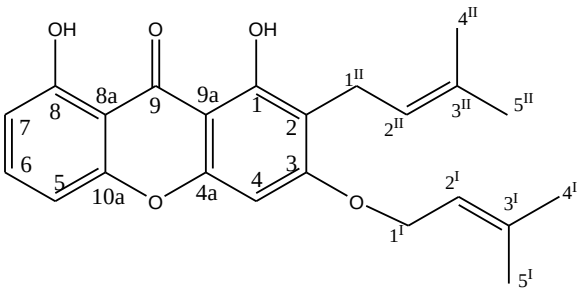



**Figure 4.30: $^{13}$C-NMR spectrum of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (100 MHz, $CDCl_3$)**




1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

380.42 g $mol^{-1}$

$C_{23}H_{24}O_5$

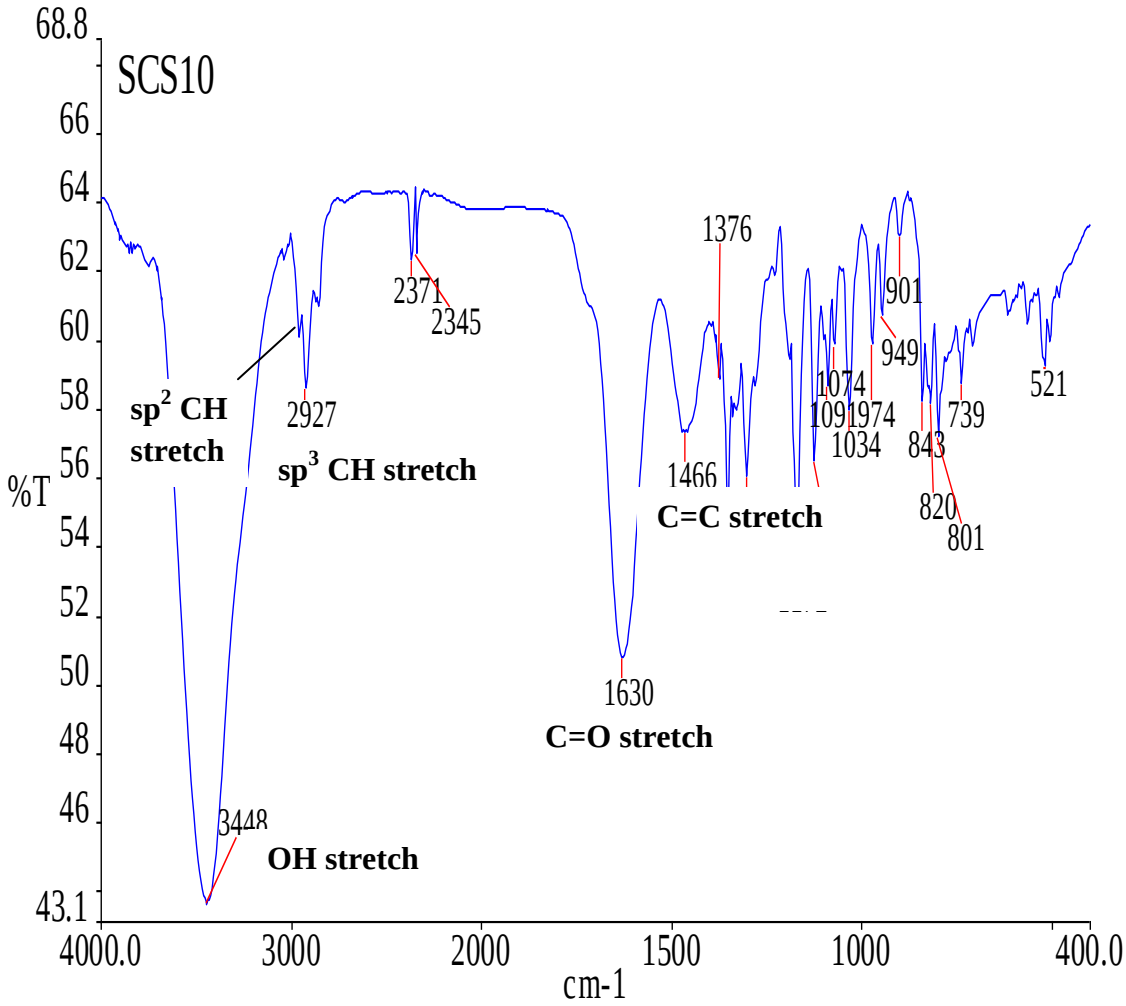
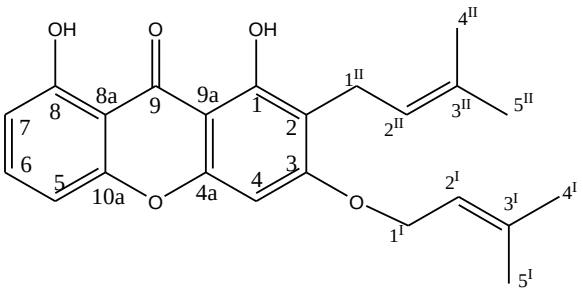



**Figure 4.31: IR spectrum of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**




1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

380.42 g $mol^{-1}$

$C_{23}H_{24}O_5$

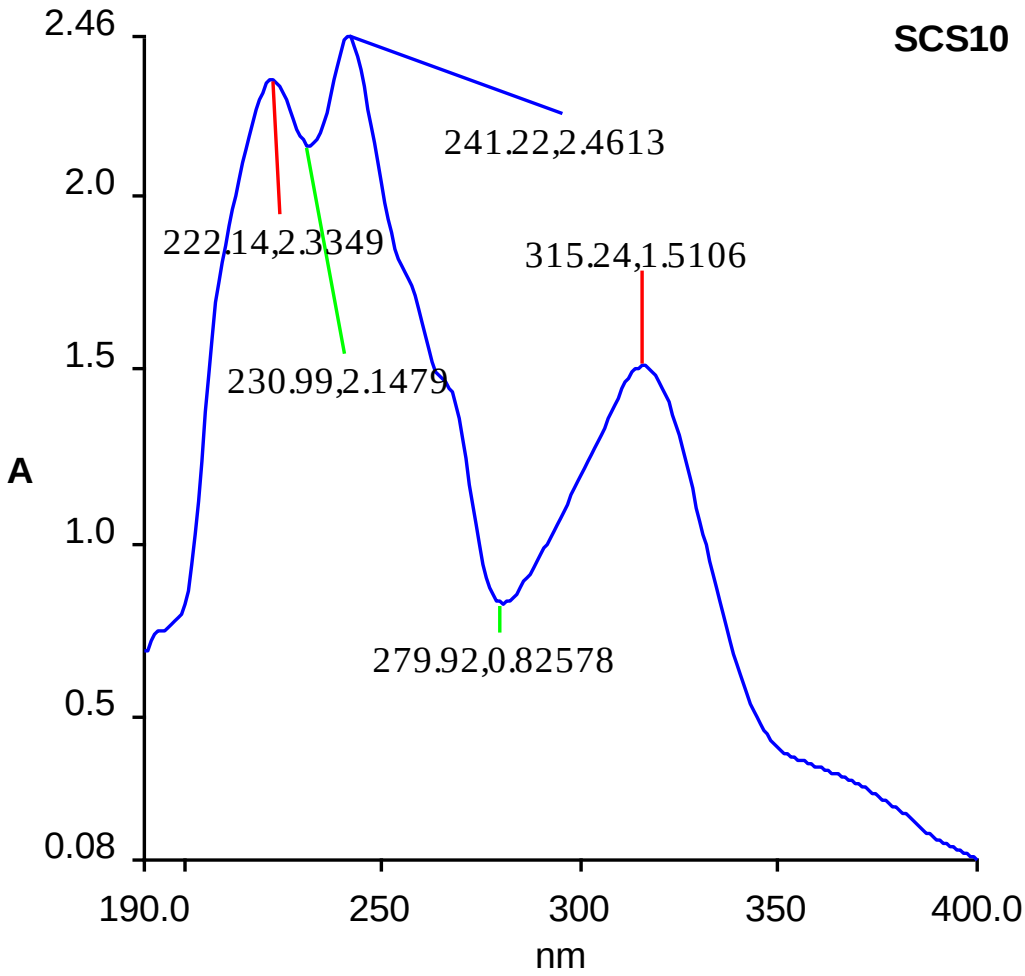



**Figure 4.32: UV-Vis spectrum of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one**

## 4.4 Bioassay

In this study, xanthone block, 1,3,8-trihydroxyxanthone and one of the prenylated xanthones, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one were tested for their cytotoxic activity towards HeLa and MDA-MB-231 cancer cell lines. By performing colorimetric 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, the cytotoxic effect of the compounds toward cancer cells and the cell viability were determined. The cell viability was expressed in $IC_{50,}$ which indicated the concentration of a drug that inhibited a cytotoxic activity by 50%.

For both of the compounds tested, only 1,3,8-trihydroxyxanthone (xanthone block) exerted inhibiting effect on HeLa cell line with an $IC_{50}$ value of 5.5 μg/mL. The prenylated compound, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one did not show any significant cytotoxic activity on HeLa and MDA-MB-231 cancer cell lines, may due to the prenyl groups attached to the O-position. It was suggested that the cytotoxic activity was largely influenced by hydroxyl functional groups that presence in the structure. Once the hydroxyl groups were prenylated, the hydroxyl functional groups disappeared, and this led to the disappearance of cytotoxic activity.

**Table 4.8: Cytotoxicity of xanthone and its derivatives against HeLa and MDA-MB-231 cancer cell lines**

| Compound | Inhibitory concentration, $IC_{50}$ (µg / ml) | |
|---|---|---|
| | HeLa cancer cell line | MDA-MB-231 cancer cell line |
| 1,3,8-trihydroxyxanthone (xanthone block) | 5.5 | > 50.0 |
| 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (o-prenylated xanthone) | > 50.0 | > 50.0 |

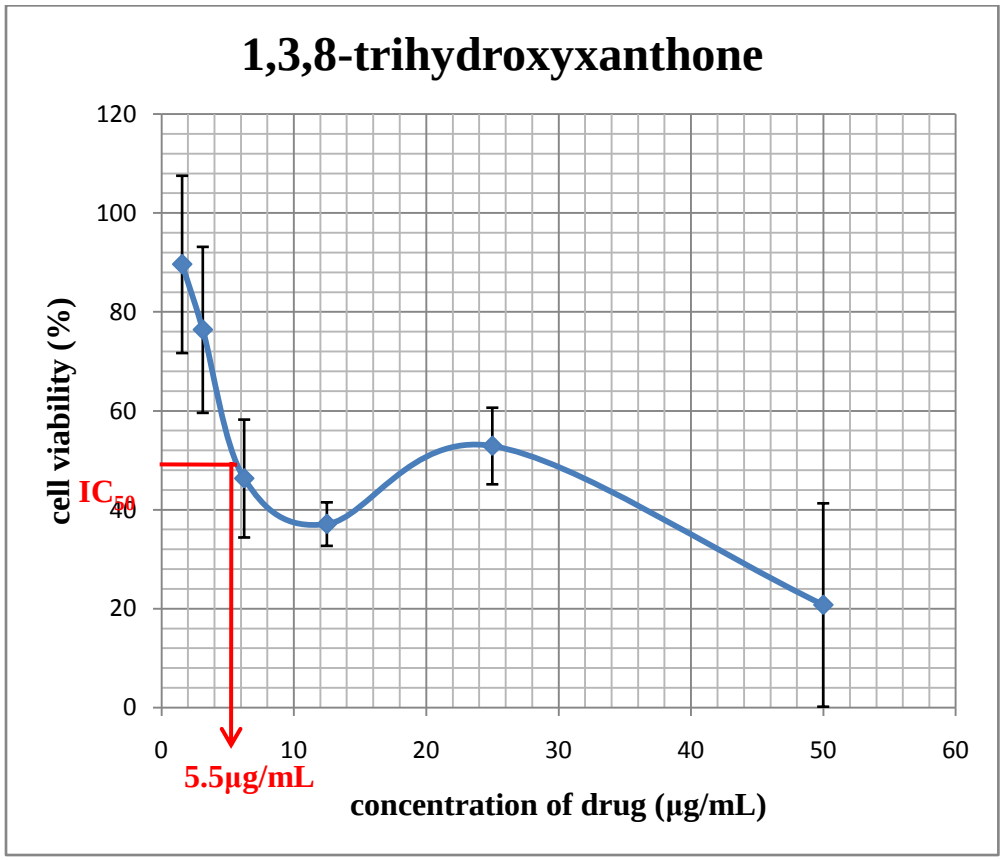



**Figure 4.33: Graph of cell viability against concentration of 1,3,8-trihydroxyxanthone**

# CHAPTER 5

# CONCLUSIONS

## 5.1 Conclusions

In this study, a total of four compounds had been synthesized and identified. They were a xanthone block, 1,3,8-trihydroxyxanthone, and three prenylated xanthones, namely 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one, 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione and 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one. In order to confirm their structures, these compounds were elucidated and characterized through a series of spectroscopy analyses, such as NMR, IR and UV-Vis.

By using colorimetric 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, the cytotoxic activity of 1,3,8-trihydroxyxanthone and 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one were tested against HeLa and MDA-MB-231 cancer cell lines. 1,3,8-Trihydroxyxanthone only exhibited significant cytotoxic effect on HeLa cancer cell line with an $IC_{50}$ value of 5.5 μg/mL and no significant activity was observed against MDA-MB-231 cancer cell line. Meanwhile, 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

was found to give insignificant activity against HeLa and MDA-MB-231 cancer cell lines. This may due to o-prenylation destroyed the hydroxyl functional group attached to C-3 which was found to the essential to elicit cytotoxic activity toward HeLa cancer cell line.

## 5.2 Suggestions for Further Studies

In future, extensive study should be carried out by introduction of other substitution groups to the xanthone block such as methoxy, geranyl and acetyl functional groups instead of prenyl group to provide a more comprehensive insight into the structure-activity relationship study of xanthone compounds.

Moreover, it is suggested the use of more advanced chromatographic methods, such as high performance liquid chromatography, flash column chromatography, centrifugal chromatography and solid-phase extraction instead of gravity column chromatography in the purification of crude product from the synthesis. This mainly because the gravity column chromatography shows low separation efficiency and takes a long time to isolate pure compound.

In addition, other biological activities, such as, antibacterial and antifungal assay can also be done in order to enable wider application of the compounds.

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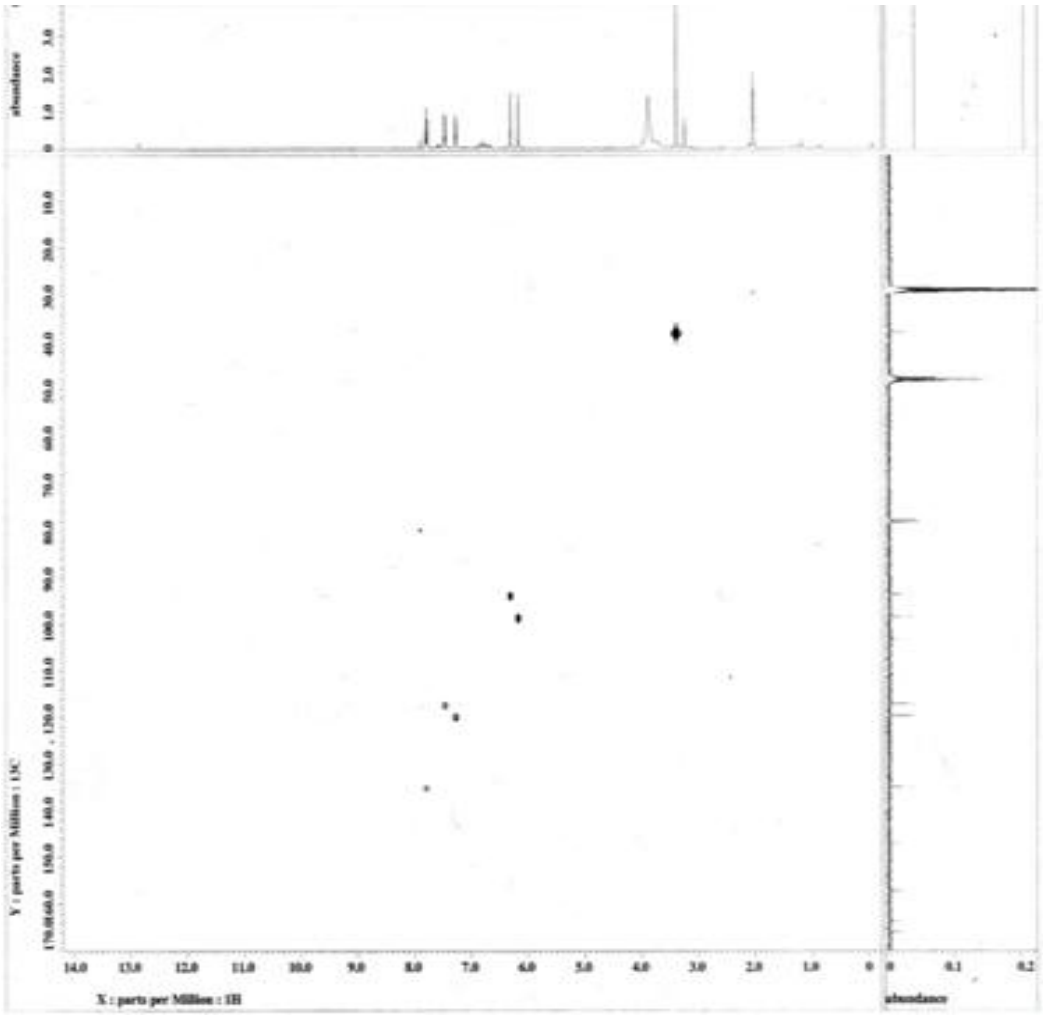
# APPENDICES

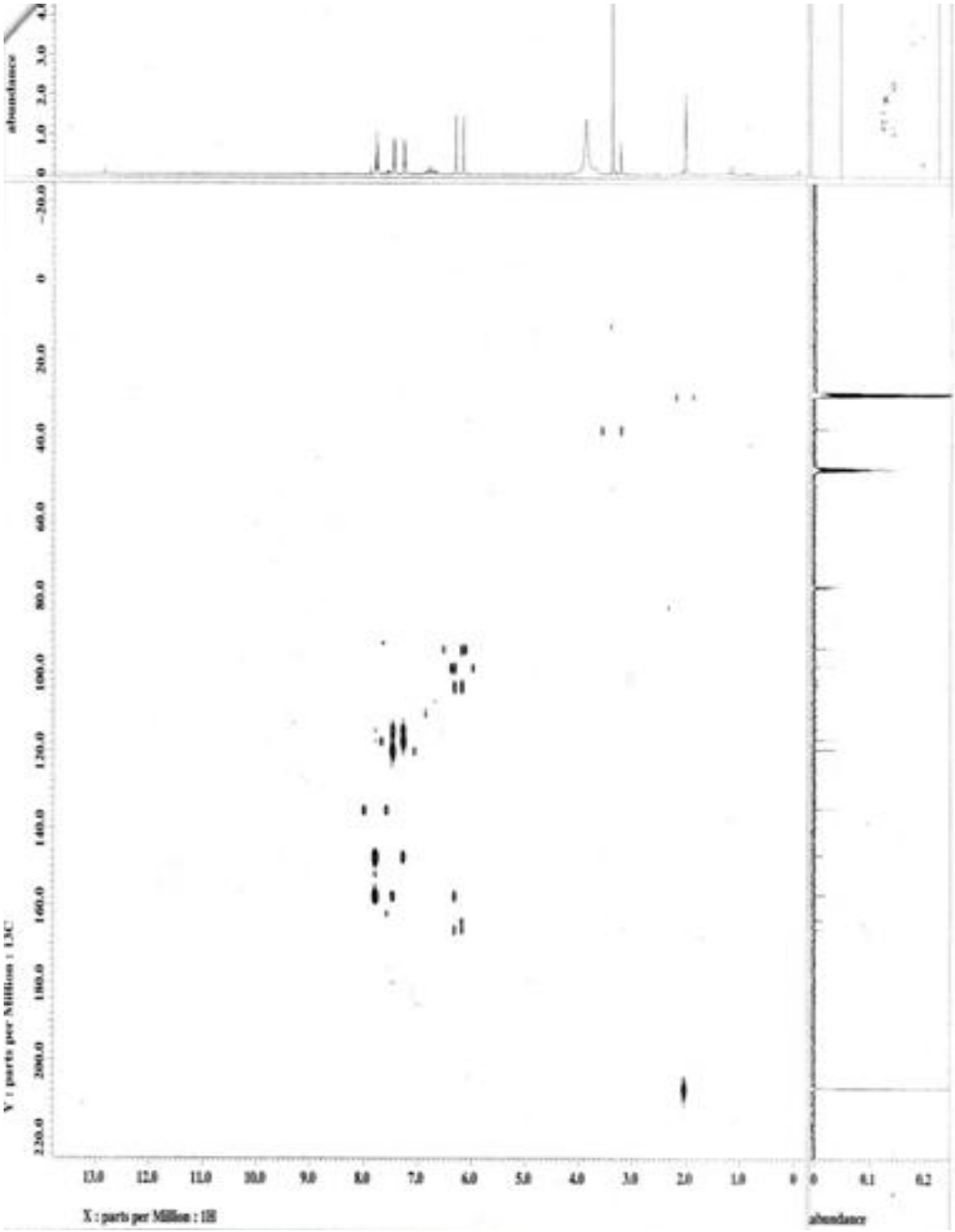
## APPENDIX 1

The following spectrums are 2D-NMR spectra of the prenylated xanthones.

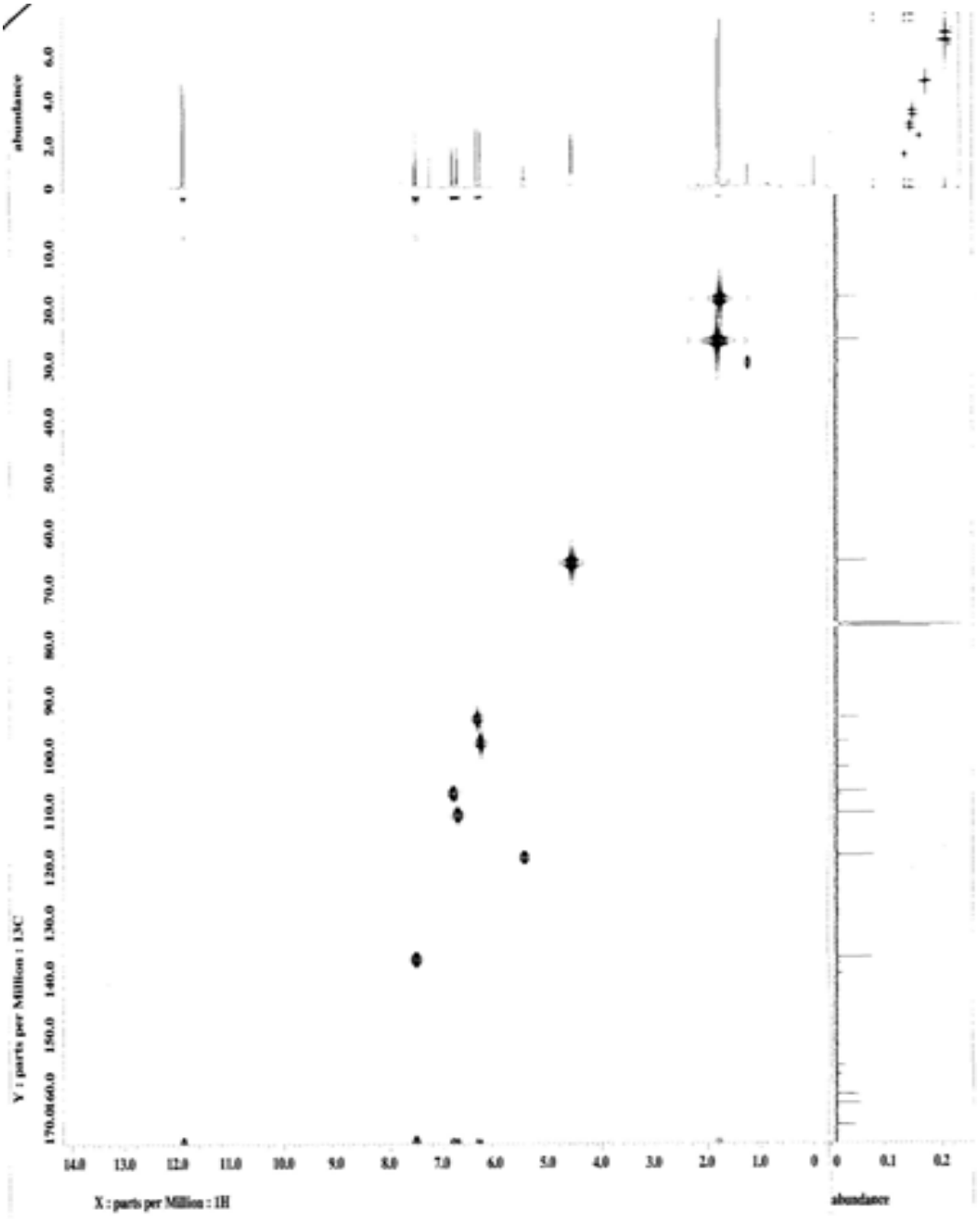
### (a) HMQC spectrum of 1,3,8-trihydroxyxanthone:



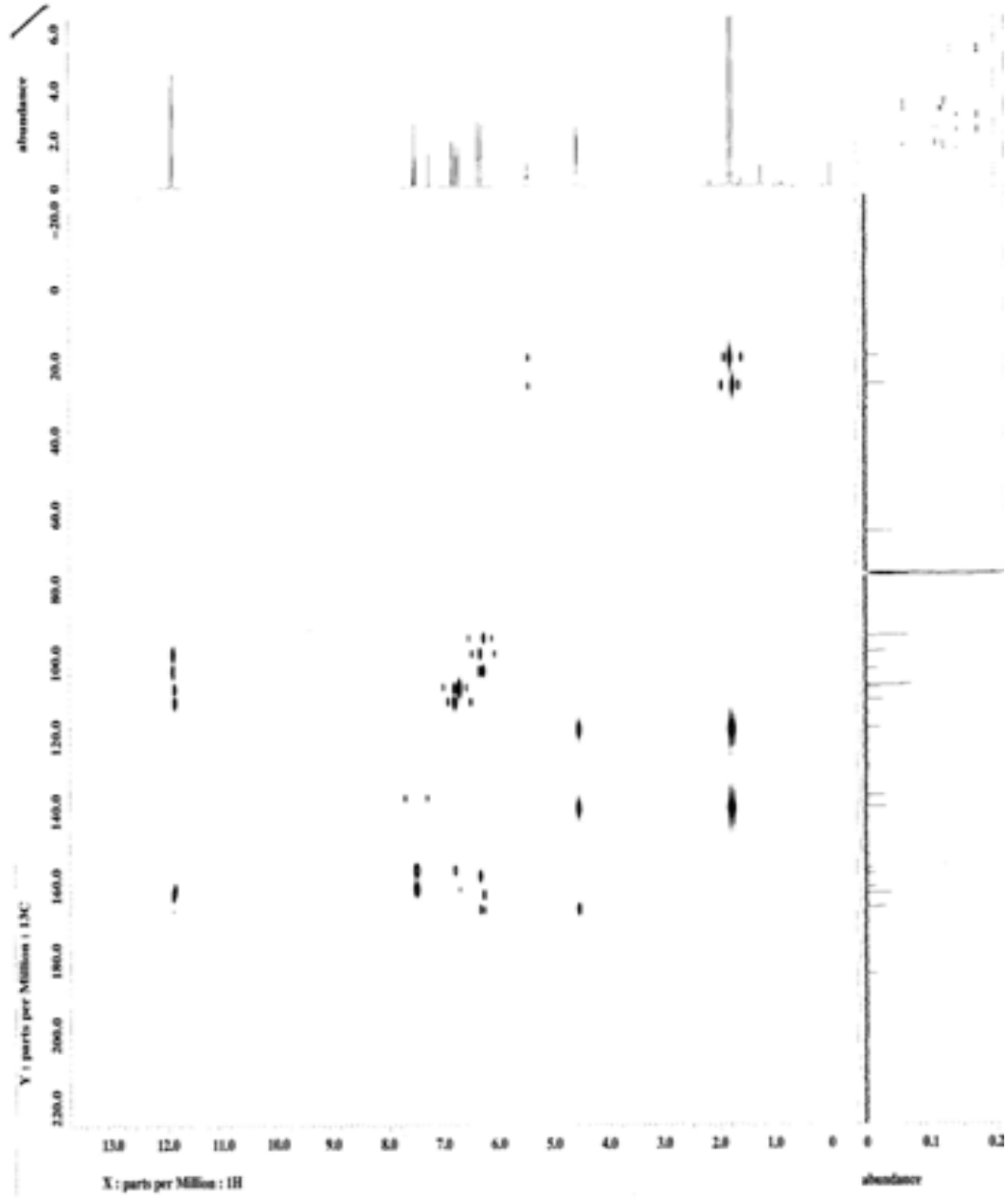
**(b) HMBC spectrum of 1,3,8-trihydroxyxanthone:**



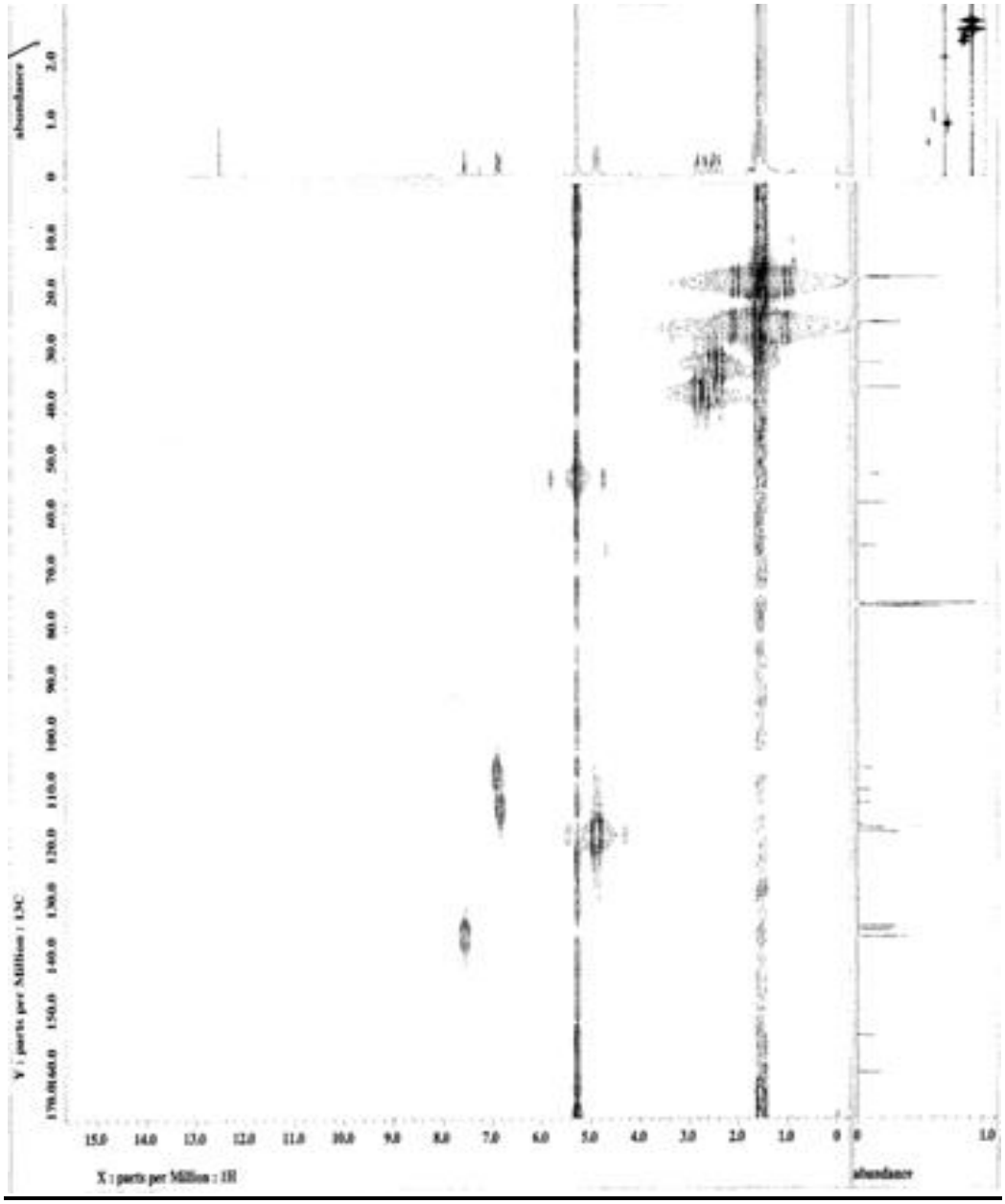
**(c) HMQC spectrum of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one:**



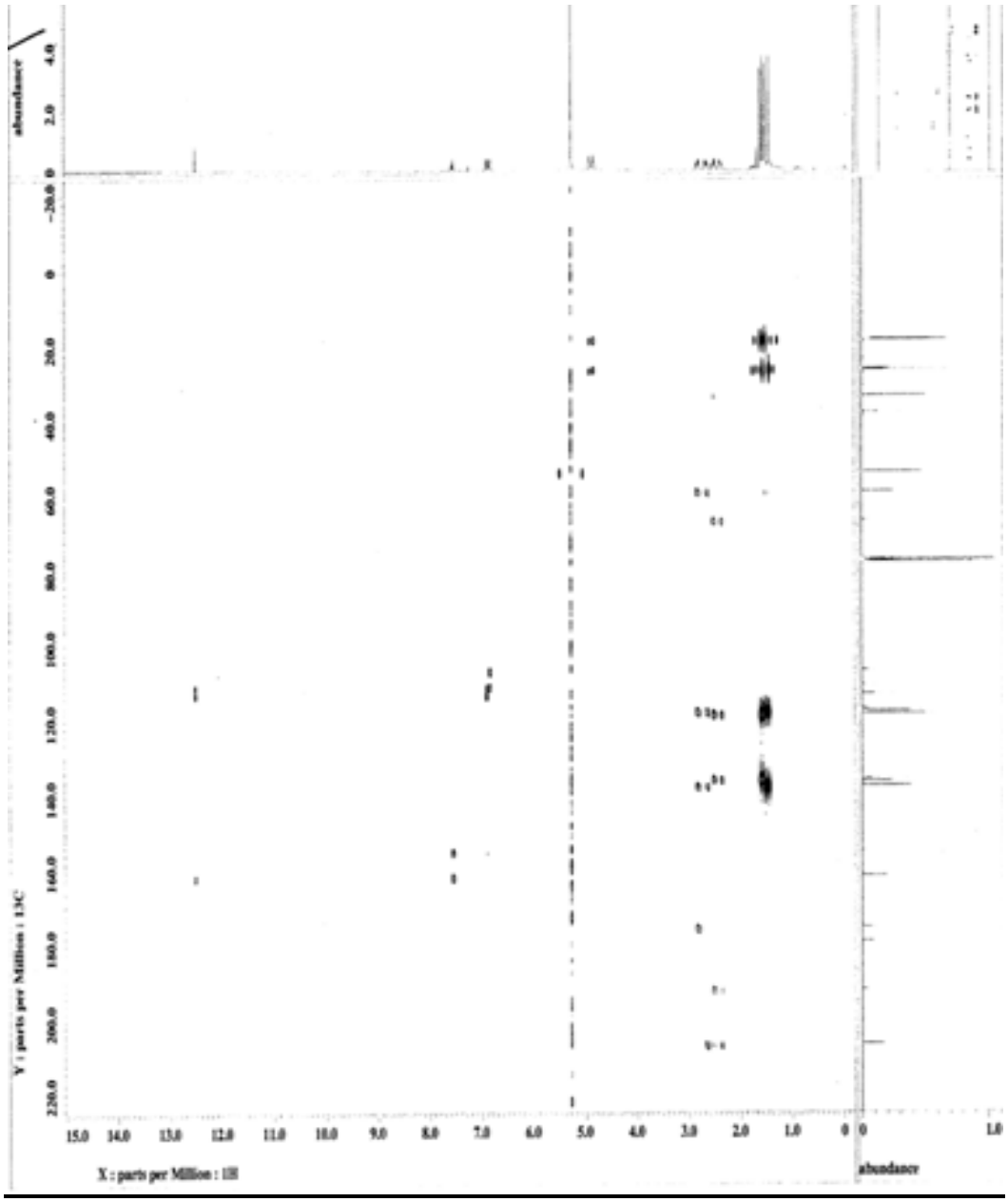
**(d) HMBC spectrum of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one:**



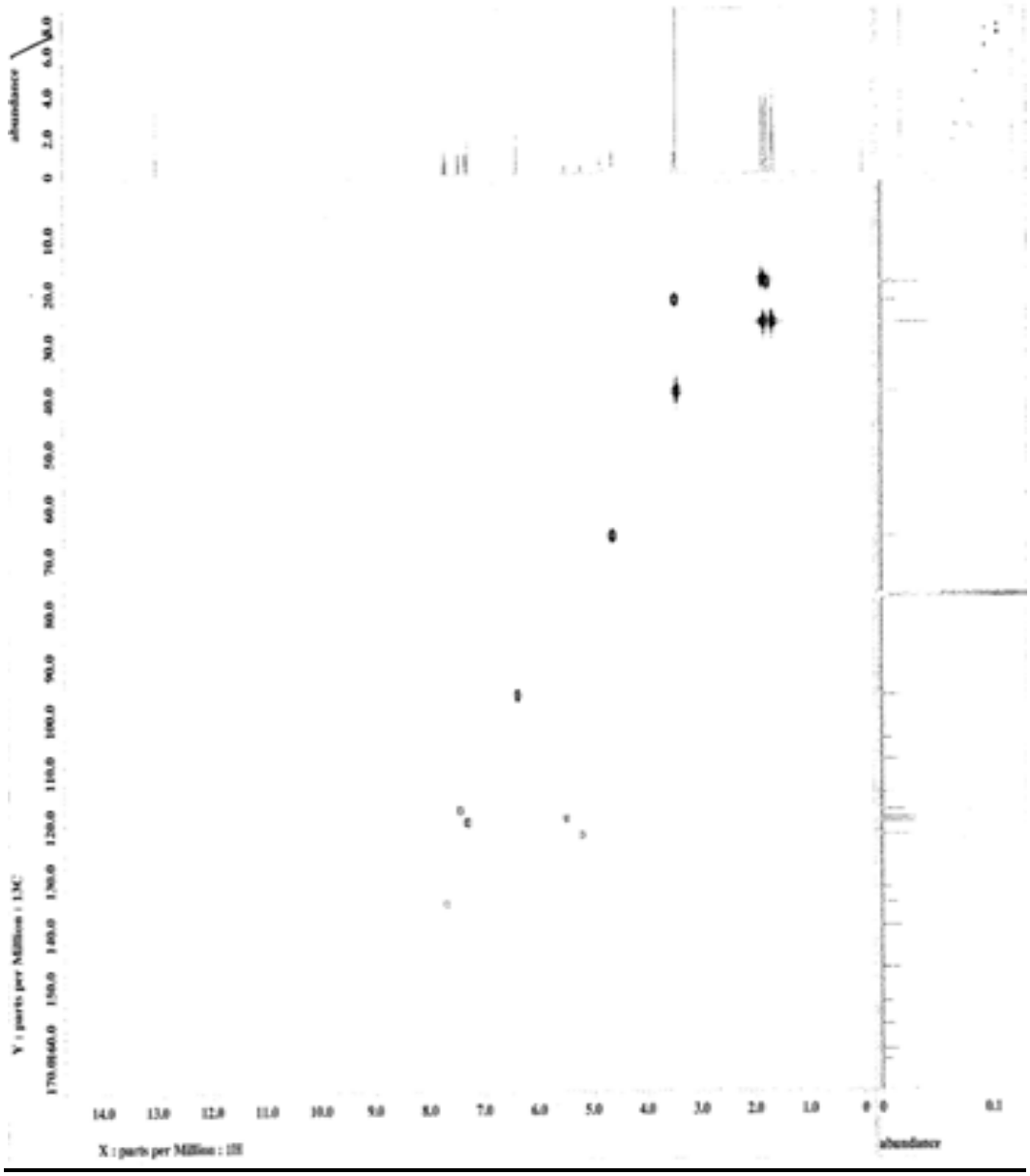
**(e) HMQC spectrum of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione:**



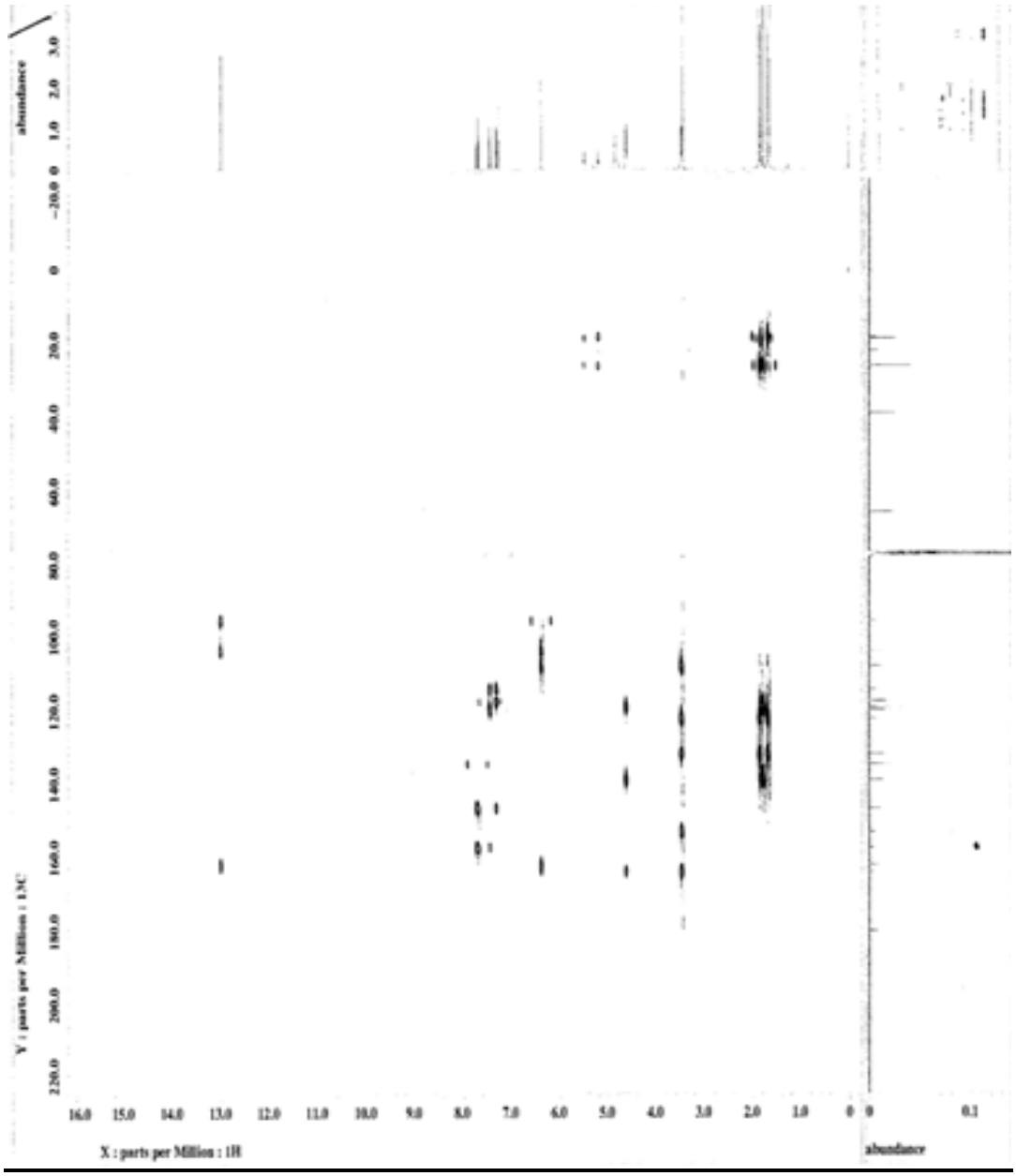
**(f) HMBC spectrum of 8-hydroxy-2,2,4,4-tetrakis-(3-methyl-but-2-enyl)-4H-xanthene-1,3,9-trione:**



**(g) HMQC spectrum of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one :**



**(h) HMBC spectrum of 1,8-dihydroxy-2-(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one:**



**APPENDIX 2**

The following tables summarize the triplicates MTT assay results of absorbance analysis for 1,3,8-trihydroxyxanthone on the HeLa cancer cell line.

**First trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 0.556 | 0.464 | 0.517 | 0.512 | 43.80 |
| **25** | 0.521 | 0.555 | 0.478 | 0.518 | 44.43 |
| **12.5** | 0.498 | 0.479 | 0.511 | 0.496 | 41.99 |
| **6.25** | 0.667 | 0.641 | 0.665 | 0.658 | 59.89 |
| **3.125** | 0.973 | 0.913 | 0.956 | 0.947 | 91.96 |
| **1.563** | 1.089 | 1.144 | 1.054 | 1.095 | 108.38 |
| **Control** | 1.15 | 0.973 | 0.937 | 1.02 | - |
| **Blank** | 0.121 | 0.116 | 0.113 | 0.117 | - |

**Second trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 0.277 | 0.3 | 0.289 | 0.289 | 14.12 |
| **25** | 0.715 | 0.791 | 0.932 | 0.813 | 54.64 |
| **12.5** | 0.541 | 0.492 | 0.583 | 0.539 | 33.45 |
| **6.25** | 0.602 | 0.567 | 0.607 | 0.592 | 37.58 |
| **3.125** | 0.933 | 0.884 | 0.775 | 0.864 | 58.61 |
| **1.563** | 1.113 | 1.107 | 0.917 | 1.046 | 72.65 |
| **Control** | 1.59 | 1.191 | 1.417 | 1.399 | - |
| **Blank** | 0.108 | 0.11 | 0.1 | 0.106 | - |

**Third trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 0.165 | 0.155 | 0.155 | 0.158 | 4.30 |
| **25** | 0.729 | 0.753 | 0.793 | 0.758 | 59.62 |
| **12.5** | 0.534 | 0.413 | 0.555 | 0.501 | 35.86 |
| **6.25** | 0.528 | 0.491 | 0.666 | 0.562 | 41.49 |
| **3.125** | 0.965 | 0.92 | 1.007 | 0.964 | 78.58 |
| **1.563** | 1.096 | 1.079 | 1.018 | 1.064 | 87.83 |
| **Control** | 1.246 | 1.137 | 1.206 | 1.196 | - |
| **Blank** | 0.113 | 0.103 | 0.119 | 0.112 | - |

**APPENDIX 3**

The following tables summarize the triplicates MTT assay results of absorbance analysis for 1,3,8-trihydroxyxanthone on the MDA-MB-231 cancer cell line.

**First trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 1.282 | 1.268 | 1.414 | 1.321 | 77.00 |
| **25** | 2.249 | 2.094 | 2.314 | 2.219 | 135.98 |
| **12.5** | 2.873 | 2.811 | 2.848 | 2.844 | 177.05 |
| **6.25** | 2.688 | 2.888 | 3.077 | 2.884 | 179.70 |
| **3.125** | 2.99 | 2.707 | 3.009 | 2.902 | 180.86 |
| **1.563** | 1.989 | 2.295 | 2.594 | 2.293 | 140.82 |
| **Control** | 1.768 | 1.668 | 1.578 | 1.671 | - |
| **Blank** | 0.145 | 0.126 | 0.177 | 0.149 | - |

**Second trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 1.162 | 1.193 | 1.2 | 1.185 | 74.97 |
| **25** | 1.304 | 1.232 | 1.305 | 1.280 | 81.56 |
| **12.5** | 2.678 | 1.679 | 1.758 | 2.038 | 133.98 |
| **6.25** | 3.068 | 2.974 | 2.978 | 3.007 | 200.95 |
| **3.125** | 2.034 | 1.848 | 2.016 | 1.966 | 128.98 |
| **1.563** | 2.051 | 1.785 | 1.343 | 1.726 | 112.40 |
| **Control** | 1.976 | 1.152 | 1.513 | 1.547 | - |
| **Blank** | 0.105 | 0.097 | 0.101 | 0.101 | - |

**Third trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 1.71 | 1.842 | 1.891 | 1.814 | 76.77 |
| **25** | 2.374 | 2.65 | 2.68 | 2.568 | 111.10 |
| **12.5** | 2.991 | 2.966 | 2.849 | 2.935 | 127.83 |
| **6.25** | 2.536 | 3.16 | 2.705 | 2.800 | 121.68 |
| **3.125** | 2.399 | 2.756 | 2.533 | 2.563 | 110.85 |
| **1.563** | 2.437 | 0.942 | 2.889 | 2.089 | 89.30 |
| **Control** | 2.508 | 2.331 | 2.134 | 2.324 | - |
| **Blank** | 0.126 | 0.126 | 0.134 | 0.129 | - |

**APPENDIX 4**

The following tables summarize the triplicates MTT assay results of absorbance analysis for 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one on the HeLa cancer cell line.

**First trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 1.047 | 1.201 | 1.152 | 1.133 | 77.79 |
| **25** | 1.095 | 1.068 | 1.278 | 1.147 | 78.84 |
| **12.5** | 0.944 | 1.408 | 1.108 | 1.153 | 79.33 |
| **6.25** | 0.993 | 1.009 | 1.172 | 1.058 | 71.99 |
| **3.125** | 1.166 | 1.104 | 1.066 | 1.112 | 76.15 |
| **1.563** | 0.736 | 1.218 | 1.274 | 1.076 | 73.38 |
| **Control** | 1.673 | 1.254 | 1.339 | 1.422 | - |
| **Blank** | 0.11 | 0.125 | 0.132 | 0.122 | - |

**Second trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 0.64 | 0.59 | 0.619 | 0.616 | 62.02 |
| **25** | 0.726 | 0.632 | 0.623 | 0.660 | 67.81 |
| **12.5** | 0.722 | 0.687 | 0.693 | 0.701 | 73.11 |
| **6.25** | 0.769 | 0.76 | 0.752 | 0.760 | 80.95 |
| **3.125** | 0.778 | 0.727 | 0.818 | 0.774 | 82.79 |
| **1.563** | 0.785 | 0.751 | 0.817 | 0.784 | 84.10 |
| **Control** | 0.998 | 0.846 | 0.872 | 0.905 | - |
| **Blank** | 0.14 | 0.139 | 0.154 | 0.144 | - |

**Third trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 0.932 | 0.876 | 0.895 | 0.901 | 86.83 |
| **25** | 1.08 | 0.92 | 0.908 | 0.969 | 94.42 |
| **12.5** | 1.168 | 0.899 | 0.948 | 1.005 | 98.35 |
| **6.25** | 1.065 | 1.22 | 1.014 | 1.0997 | 108.77 |
| **3.125** | 1.048 | 1.451 | 0.969 | 1.156 | 114.97 |
| **1.563** | 1.117 | 1.024 | 0.935 | 1.025 | 100.59 |
| **Control** | 1.15 | 0.973 | 0.937 | 1.02 | - |
| **Blank** | 0.121 | 0.116 | 0.113 | 0.117 | - |

**APPENDIX 5**

The following tables summarize the triplicates MTT assay results of absorbance analysis for 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one on the MDA-MB-231 cancer cell line.

**First trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 3.232 | 3.229 | 2.862 | 3.108 | 134.59 |
| **25** | 2.93 | 2.678 | 3.075 | 2.894 | 124.87 |
| **12.5** | 2.683 | 2.841 | 2.947 | 2.824 | 121.65 |
| **6.25** | 2.695 | 2.796 | 3.198 | 2.896 | 124.96 |
| **3.125** | 2.183 | 2.199 | 3.019 | 2.467 | 105.39 |
| **1.563** | 2.425 | 1.705 | 1.801 | 1.977 | 83.06 |
| **Control** | 2.037 | 2.323 | 2.686 | 2.349 | - |
| **Blank** | 0.225 | 0.116 | 0.123 | 0.155 | - |

**Second trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 1.524 | 1.707 | 1.714 | 1.648 | 50.27 |
| **25** | 1.981 | 2.454 | 2.521 | 2.319 | 72.47 |
| **12.5** | 2.786 | 3.132 | 2.976 | 2.965 | 93.87 |
| **6.25** | 3.317 | 3.216 | 3.165 | 3.233 | 102.75 |
| **3.125** | 3.153 | 3.116 | 3.027 | 3.099 | 98.31 |
| **1.563** | 3.056 | 3.149 | 3.098 | 3.101 | 98.39 |
| **Control** | 3.222 | 3.185 | 3.042 | 3.150 | - |
| **Blank** | 0.123 | 0.125 | 0.145 | 0.131 | - |

**Third trial:**

| Conc. (µg/mL) | Raw data | | | Mean | Cell viability, % |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **50** | 2.108 | 2.126 | 2.37 | 2.201 | 145.25 |
| **25** | 1.648 | 1.634 | 1.96 | 1.747 | 113.85 |
| **12.5** | 1.912 | 1.93 | 1.904 | 1.915 | 125.47 |
| **6.25** | 1.379 | 1.567 | 1.354 | 1.433 | 92.14 |
| **3.125** | 1.504 | 1.572 | 1.362 | 1.479 | 95.32 |
| **1.563** | 1.606 | 1.671 | 1.527 | 1.601 | 103.76 |
| **Control** | 1.976 | 1.152 | 1.513 | 1.547 | - |
| **Blank** | 0.105 | 0.097 | 0.101 | 0.101 | - |

**APPENDIX 6**

The following tables summarize the cell viability of 1,3,8-trihydroxyxanthone on HeLa and MDA-MB-231 cancer cell lines.

**HeLa cancer cell line:**

| Conc. (µg/mL) | Cell viability, % | | | |
|---|---|---|---|---|
| | 1st trial | 2nd trial | 3rd trial | Mean |
| **50** | 43.80 | 14.12 | 4.30 | 20.74 |
| **25** | 44.43 | 54.64 | 59.62 | 52.90 |
| **12.5** | 41.99 | 33.45 | 35.86 | 37.10 |
| **6.25** | 59.89 | 37.58 | 41.49 | 46.32 |
| **3.125** | 91.96 | 58.61 | 78.58 | 76.38 |
| **1.563** | 108.38 | 72.65 | 87.83 | 89.62 |

**MDA-MB-231 cancer cell line:**

| Conc. (µg/mL) | Cell viability, % | | | |
|---|---|---|---|---|
| | 1st trial | 2nd trial | 3rd trial | Mean |
| **50** | 77.00 | 74.97 | 76.77 | 76.25 |
| **25** | 135.98 | 81.56 | 111.10 | 109.55 |
| **12.5** | 177.05 | 133.98 | 127.83 | 146.28 |
| **6.25** | 179.70 | 200.95 | 121.68 | 167.44 |
| **3.125** | 180.86 | 128.98 | 110.85 | 140.23 |
| **1.563** | 140.82 | 112.40 | 89.30 | 114.17 |

**APPENDIX 7**

The following tables summarize the cell viability of 1,8-dihydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one on HeLa and MDA-MB-231 cancer cell lines.

**HeLa cancer cell line:**

| Conc. (µg/mL) | Cell viability, % | | | |
|---|---|---|---|---|
| | 1st trial | 2nd trial | 3rd trial | Mean |
| **50** | 77.78 | 62.02 | 86.83 | 75.55 |
| **25** | 78.84 | 67.81 | 94.42 | 80.36 |
| **12.5** | 79.33 | 73.11 | 98.35 | 83.59 |
| **6.25** | 71.99 | 80.95 | 108.77 | 87.24 |
| **3.125** | 76.15 | 82.79 | 114.97 | 91.30 |
| **1.563** | 73.38 | 84.10 | 100.59 | 86.02 |

**MDA-MB-231 cancer cell line:**

| Conc. (µg/mL) | Cell viability, % | | | |
|---|---|---|---|---|
| | 1st trial | 2nd trial | 3rd trial | Mean |
| **50** | 134.59 | 50.27 | 145.25 | 110.04 |
| **25** | 124.87 | 72.47 | 113.85 | 103.73 |
| **12.5** | 121.65 | 93.87 | 125.47 | 113.66 |
| **6.25** | 124.96 | 102.75 | 92.14 | 106.62 |
| **3.125** | 105.39 | 98.31 | 95.32 | 99.67 |
| **1.563** | 83.06 | 98.39 | 103.76 | 95.07 |