LAI CHOOI KUAN

B. Sc. (Hons.) Chemistry

2012

SYNTHESIS AND CHARACTERISATION OF 1,3-DIHYDROXYXANTHONE DERIVATIVES AND THEIR ANTIOXIDANT ACTIVITIES

LAI CHOOI KUAN

BACHELOR OF SCIENCE (HONS.) CHEMISTRY

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

OCTOBER 2012

SYNTHESIS AND CHARACTERISATION OF 1,3-DIHYDROXYXANTHONE DERIVATIVES AND THEIR ANTIOXIDANT ACTIVITIES

By

LAI CHOOI KUAN

A Project Report Submitted to the Department of Chemical Science,

Faculty of Science,

Universiti Tunku Abdul Rahman

in Partial Fulfillment of the Requirement for the

Degree of Bachelor of Science (Hons.) Chemistry

October 2012

ABSTRACT

SYNTHESIS AND CHARACTERISATION OF 1,3-DIHYDROXYXANTHONE DERIVATIVES AND THEIR ANTIOXIDANT ACTIVITIES

Lai Chooi Kuan

In this study, a xanthonic block 1,3-dihydroxyxanthone (**39**) and three new prenylated xanthone derivatives, namely 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**), 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**), and 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**) were successfully synthesized. These pure compounds were isolated by using column chromatography and their structures were elucidated through ^{1}H-NMR, ^{13}C-NMR and UV-Vis analyses, and were further confirmed on the basis of 2D-NMR including HMQC and HMBC analyses. Among these compounds, compound (**42**) was found to bear a novel skeleton of xanthen-3,9-dione.

The xanthonic block was synthesized using salicylic acid and phloroglucinol in the presence of Eaton's reagent and was subsequently used as starting material for

prenylation with two different bases, potassium carbonate and potassium hydroxide in aqueous medium.

The DPPH radical scavenging assay was carried out to study the antioxidant activities of the isolated compounds, and the results indicated compound **43** to exhibit significant antioxidant activity, in which the activity was found to be weaker as compared to the reference compounds, ascorbic acid and kaempferol. On the other hand, the xanthonic block (**39**) and other prenylated derivatives, **41** and **42** showed no significant antioxidant activities.

ABSTRAK

Dalam projek ini, satu blok xanthone iaitu 1,3-dihidroksixanthone (**39**) dan tiga prenilasi xanthone, iaitu 1-hidroksi-2,4-bis(3-metil-but-2-enil)-3-(metil-but-2-eniloksi)-xanthen-9-one (**41**), 1-hidroksi-2,4,4-tris(3-metil-but-2-enil)-4*H*-xanthen-3,9-dione (**42**), dan 1,3-dihidroksi-2,4-bis(3-metil-but-2-enil)- xanthen-9-one (**43**) telah berjaya dihasilkan dan dikenalpastikan. Hasil sintesis ini telah dipisah melalui kromatografi kolom dan identiti sebatian-sebatian tersebut telah dikenalpasti melalui kaedah spektroskopi seperti UV-Vis, IR, 1D- dan 2D-NMR.

Blok xanthone telah disintesis menggunakan asid salisilik dan phloroglucinol. Hasil sintesis tersebut digunakan sebagai bahan permulaan untuk prenilasi dalam larutan akueus, dengan menggunakan dua jenis alkali yang berbeza, iaitu potassium hidroksida dan potassium karbonat.

Semua xanthone yang dihasilkan telah diuji aktiviti antioksidan masing-masing dengan menggunakan kaedah DPPH dan didapati hanya 1,3-dihidroksi-2,4-bis(3-metil-but-2-enil)-xanthen-9-one (**43**) menunjukkan aktiviti yang nyata. Manakala blok xanthone (**39**) dan prenilasi xanthone yang lain, **41** dan **43** tidak memberikan aktiviti antioksidan yang efektif.

ACKNOWLEDGEMENT

First of all, I gratefully express deep appreciation to my supervisor, Dr. Lim Chan Kiang, who has shown his kind and invaluable interaction, support, and advice for my academic success.

Special thanks to my seniors Koo Hooi Tee and Lim Cheng Hoe, for their unconditional guidance and help.

I would like to thank my group mates and lab mates, for their assistance and companionship in the laboratory. It has been a pleasant experience to work with all of them.

Finally, I most deeply acknowledge my families and friends for their unceasing encouragement, support, and patience throughout the course of this project.

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 it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

_____

(LAI CHOOI KUAN)

APPROVAL SHEET

This project report entitled "**SYNTHESIS AND CHARACTERISATION OF 1,3-DIHYDROXYXANTHONE DERIVATIVES AND THEIR ANTIOXIDANT ACTIVITIES**" was prepared by LAI CHOOI KUAN 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:

_____ Date:_____

(Dr. Lim Chan Kiang)

Supervisor

Department of Chemical Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date:_____

PERMISSION SHEET

It is hereby certified that **LAI CHOOI KUAN** (ID No: **10ADB00867**) has completed this report entitled "**SYNTHESIS AND CHARACTERISATION OF 1,3-DIHYDROXYXANTHONE DERIVATIVES AND THEIR ANTIOXIDANT ACTIVITIES**" supervised by Dr. Lim Chan Kiang from the Department of Chemical Science, Faculty of Science.

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

Yours truly,

_____

(LAI CHOOI KUAN)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
δ	Chemical shift in ppm
^{o}C	Degree Celsius
^{1}H	Proton
^{13}C	Carbon-13
cm	Centimetre
μL	Microlitre
μm	Micromitre
%	Percent sign
λ_{max}	Wavelength maxima in nm
A_0	Absorbance of negative control in DPPH assay
A_1	Absorbance of test compound in DPPH assay
Ace	Acetone
$AlCl_3$	Aluminium chloride
Aq.	Aqueous
C	Carbon
C-prenylated	Carboprenylated
CAN	Ceric ammonium nitrate
CH_3SO_3H	Methanesulfonic acid
CO_2	Carbon dioxide

CoA	Coenzyme A
Cu	Copper
dd	Doublet of doublet
d	Doublet
DCM	Dichloromethane
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EA	Ethyl acetate
et. al.	et alii (and others)
$FeCl_3$	Iron (III) chloride
g	Gram
G. mangostana	*Garcinia mangostana*
GSS	Grover, Shah and Shah
h	Hour
H	Hydrogen
H_2O	Water
HBF_4	Fluoroboric acid
HCl	Hydrochloric acid
HEX	Hexane
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
HSCoA	Coenzyme A

IC_{50}	50% Inhibitory Concentration
IR	Infrared
J	Coupling constant in Hz
KBr	Potassium bromide
K_2CO_3	Potassium carbonate
KOH	Potassium hydroxide
MAOS	Microwave-assisted organic synthesis
Me	Methyl
mg	Milligram
MHz	Megahertz
ml	Millilitre
min	Minute
mmol	Milimole
mol	Mole
MW	Microwave
MS	Mass Spectrometry
n-Buli	n-Butyllithium
NH_4Cl	Ammonium chloride
nm	nanometer
NMR	Nuclear Magnetic Resonance
2D-NMR	Two dimensional Nuclear Magnetic Resonance
NO_2	Nitogen dioxide
o	Ortho

O-prenylated	Oxyprenylated
P_2O_5	Phosphorus pentoxide
PPA	Polyphosphoric acid
ppm	Parts per million
$POCl_3$	Phosphorus oxychloride
r.t.	Room temperature
R_f	Retention factor
ROS	Reactive oxygen species
s	Singlet
SAR	Structure-activity relationship
SiO_2	Silicon dioxide
t	Triplet
td	Triplet of doublet
$TiCl_4$	Titanium tetrachloride
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV-Vis	Ultraviolet-Visible
W	Watt
$ZnCl_2$	Zinc chloride

CHAPTER 1

INTRODUCTION

1.1 Background

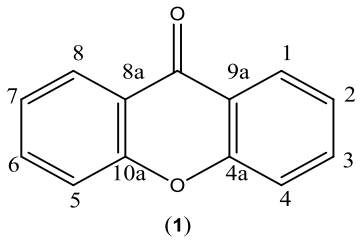
Mangosteen (*Garcinia mangostana* Linn.) is a tropical fruit belonging to the Guttiferae family and because of its popularity mangosteen is considered ‘‘queen of the tropical fruit’’ (Zarena & Sankar, 2011). Mangostin (now designated α-mangostin) from the fruit hulls of *G. mangostana* appears to be the first biologically significant chemical constituent to be isolated from *Garcinia* plant. This pioneering work was published in 1855 by Dr W. Schmid, a German chemist. Because of the bright yellow colour of mangostin, he coined the word xanthone, based on the Greek word xanthos for yellow, to name the new chemical class he had discovered during its structural investigation and reported mangostin as the first naturally occurring xanthone derivative. Since then active investigations into the isolation, characterization, and biological effects of a wide variety of novel chemical constituents from *Garcinia* species have continued to-date unabated.

1.2 Chemistry and Classification of Xanthones

1.2.1 Chemistry

Chemically xanthones (9H-xanthen-9-ones) (**1**) are heterocyclic compound with the dibenzo-γ-pyrone framework and it is symmetric (Bennett & Lee, 1989). The tricyclic aromatic system makes the xanthone molecule very stable and allows it to be extremely versatile (Zarena & Zankar, 2009).

Looking at Figure 1.1, certain features are evident: (a) two benzene rings are fused to a pyran-4-one ring, (b) the benzene rings are identical, thus imparting symmetry elements to the structure, (c) C-1 (or C-8) and C-4 (or C-5) are acidic sites and (d) either or both rings are susceptible to electrophilic substitution, facilitated and directed by the pyran oxygen on the one hand, while hindered by the carbonyl group, on the other hand. Manipulation allows varying substitution patterns to be accessible. The advantage of this approach is to provide simpler, shorter and more efficient (in certain cases) access to derivatives of xanthone (Odrowaz-Sypniewski, Tsoungas, Varvounis, Cordopatis, 2009).



(**1**)

Figure 1.1: The basic skeletal structure of xanthones

1.2.2 Classification of Xanthones

Xanthones have been classified in five froups: (a) simple oxygenated xanthones, (b) xanthone glycosides, (c) prenylated xanthones, (d) xanthonolignoids and (e) miscellaneous xanthones (Marzano, Caffieri, Fossa, & Bordin, 1997).

1.3 Occurrence and Natural Distribution of Xanthones

Xanthones are secondary metabolites found in some higher plant families, fungi and lichens. They occur in eight families, namely Gentianaceae, Guttiferae, Polygalaceae, Leguminosae, Lythraceae, Moraceae, Loganiaceae, and Rhamnaceae. Extensive studies on xanthones have been made in the Gentianaceae and Guttiferae families (Hostettmann & Hostettmann, 1989).

Garcinia L. (Guttiferae family) is a large genus of polygamous trees or shrubs, distributed in tropical Asia, Africa, and Polynesia. It is indigenous to Malaysia and cultivated in the west coast of India and Ceylon. The fruit is the mangosteen, rated one of the most delectable of the tropics and pulp gives the fruit its reputation as one of the finest and most delicious of fruits. The fruit is a rounded berry 5 to 7 centimeters in diameter, smooth, and dark purple. The rind is firm, spongy, thick, and full of yellow, resinous juice.

The pericarp of mangosteen-fruit has been used as a medicinal agent by Southeast Asians for centuries in the treatment of skin infections and wounds (Mahabusarakam, Wiriyachtra, & Taylor, 1987), amoebic dysentery (Garnett & Sturton, 1932; Chopra, Nayar, & Chopra, 1956), etc. In Ayurvedic medicine, the pericarp of mangosteen-fruit has been widely used against inflammation and diarrhea (Balasubramanian & Rajagopalan, 1988), and cholera and dysentery (Sen et al., 1980).

1.4 Synthetic Xanthones

The type and position of the substituents of natural xanthones are limited where the biological activities of this class of compounds are associated with their tricyclic scaffold and depending on the nature and position of the different substituents (Souza & Pinto, 2005; Mandal, Das & Joshi, 1992).

The limitation of natural xanthones can be overcome through chemical synthesis to extend the possibilities of having different nature and positions of the substituents on the xanthone core. This allows scientists to rationalize and characterize the structure features that are important to their bioactivity (Pedro, Cerqueira, Sousa, Nascimento, & Pinto, 2002).

New xanthones and its derivatives are produced through 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 Prenylated Xanthones

Prenylated xanthones are the major group of naturally occurring xanthones. Prenylated xanthones, including furan and pyran derivatives, have been reported to mediate several interesting biological activities, concerning a large variety of targets with therapeutic value (Pinto, Sousa, & Nascimento, 2005; Pinto & Castanheiro, 2008).

Although the oxygenation pattern of these derivatives can play an important role in their biological activity, the presence of the prenyl side chains seems also to be associated with the enhanced interaction with biological membranes and with target proteins when compared with their non-prenylated analogs (Epifano, Genovese, Menghini, & Curini 2007). For this reason, a series of prenylated xanthone derivatives have been synthesized and evaluated for their

antioxidant activities in this study. The synthesis of the prenylated xanthone derivatives is normally carried out by introduction of the prenyl side chain to the hydroxyxanthone nucleus, in a rather vigorous condition.

1.6 Bioactivities of Xanthones

Xanthones have been reported to inhibit lipid peroxidation, antioxidant activity, neuroprotective properties (Mahabusarakam, Proudfoot, Taylor, & Croft, 2000; Yu, Zhao, Yang, Zhao, & Jiang, 2007; Weecharangsan *et. al*., 2006), and also inhibit prostaglandin E2 synthesis (Nakatani *et. al*., 2002), and HIV-1 protease (Chen, Wan, & Loh, 1996). Furthermore, studies had shown that xanthones exhibit a wide range of microbial and other pharmacological activities, e.g., cytotoxic, anti-inflammatory, antimicrobial, antifungal, xanthine oxidase and monoamine oxidase inhibitory activity (Kosem, Han, & Moongkarndia, 2007; Nkengfack, Kounga, Fomum, Meyer, & Bodo, 2002).

1.7 Antioxidant Activities of Xanthones

Reactive oxygen species (ROS) are resulting in oxidation of various cell constituents as DNA, lipid, and proteins and consequently cause oxidative damage to cellular substance leading to cell death (Boonstra and Post, 2004). The oxidative damage of DNA induced by ROS leads to certain cancers, and

ROS may also play a role in cell cycle progression. ROS is implicated in numerous pathological events including metabolic disorders, cellular aging, reperfusion damage of DNA, inflammation, atherosclerosis and carcinogenesis (Robak, Shridi, Wolbis & Krolikowska, 1988).

Antioxidants may prevent these degenerative processes by various mechanisms including scavenging of free radicals. There are numerous reports about the reduction of the incidence of degenerative diseases due to the consumption of fruits and vegetables (Gordon, 1996; Feskanich *et. al*., 2000; Joshipura *et. al*., 2001; Gardner, White, McPhail, & Duthie, 2000). These positive bioactivities are considered mainly to be due to the presence of various antioxidants in fruits and vegetables. Most of the antioxidant activity in foods is thought to be due to vitamins C and E, polyphenols, and carotenoids (Gardner *et. al*., 2000; Klimczak, Maleecka, Szlachta, & Gliszczyńska-Świgło, 2007).

Xanthones have been extensively exploited both because of their wide-ranging pharmacological properties and also because they serve as important units for donating electrons (Boots, Haenen, den Hartog, & Bast, 2002). Their potent antioxidant activity plays a preventive role against disease by removing the reactive oxygen species (ROS) which cause destructive and irreversible damage to the components of a cell (Lopaczyski & Zeisel, 2001).

1.8 Objectives

The objectives of this study are:

- To synthesize xanthonic block and its prenylated derivatives.

- To purify the synthetic compounds through various chromatographic methods.

- To identify and characterize the pure synthetic compounds through 1D- & 2D-NMR and UV-Vis spectroscopic analyses.

- To evaluate antioxidant activities of the synthetic compounds.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis Approaches of Xanthones

Xanthones, a particular class of plant phytochemicals from mangosteen, are highly biological active natural products. In the field of medicinal chemistry, the groups of compounds that can bind to different classes of receptors have attracted much attention (Bringmann, Ochse, Schupp, & Tasler, 2001). The main objectives of xanthone syntheses are not only for the development of more diverse and complex bioactive compounds for biological activity and structure-activity relationship (SAR) studies but also for other applications in medicinal chemistry, such as preparation of fluorescent probes, due to the photochemical properties of xanthones (Sousa & Pinto, 2005).

The traditional extraction methods used to obtain natural products have several drawbacks; they are time consuming, laborious, have low selectivity and/or low extraction yields. Moreover, these traditional techniques employ large amounts of toxic solvents.

On the other hand, the biosynthetic pathways are a limiting factor for the structural variation of natural-occuring xanthones, the synthesis of new derivatives can help rationalize the relation of structural features versus activity (Sousa & Pinto, 2005). Due to important biological applications of xanthones, some synthetic strategies leading to more complex derivatives have been widely explored in the past years (Sousa & Pinto, 2005).

2.2 Biosynthesis

The biosynthetic pathway of xanthones in plants has been abundantly studied by many authors *in vivo* (Fujita & Inoue, 1980) and *in vitro* with markers (Grȍger *et al*., 1968; Gupta & Lewis, 1971). They attempted to inter-relate the observed oxygen patterns of natural xanthones and correlate them with the recognised oxygen patterns. They had proposed two different processes that are involved in their biosynthesis: acetate polymalonic route (Figure 2.1) and mixed shikimate acetate pathway (Figure 2.2).

2.2.1 Acetate Polymalonic Route

It has been shown for some xanthones in lower plants (micro-organisms and lichens) that their synthesis was totally acetate-derived from seven acetate units (McMaster, Scott, Trippett, 1960; Birch, Hlubucek, Simpson &

Westerman, 1976). The biosynthetic mechanism of ravelenin from *Helminthosporium ravenelii* proposed by Birch *et. al*. in 1976 gives an illustration of the acetate polymalonic route. Benzophenone is involved as an intermediate (Figure 2.1).

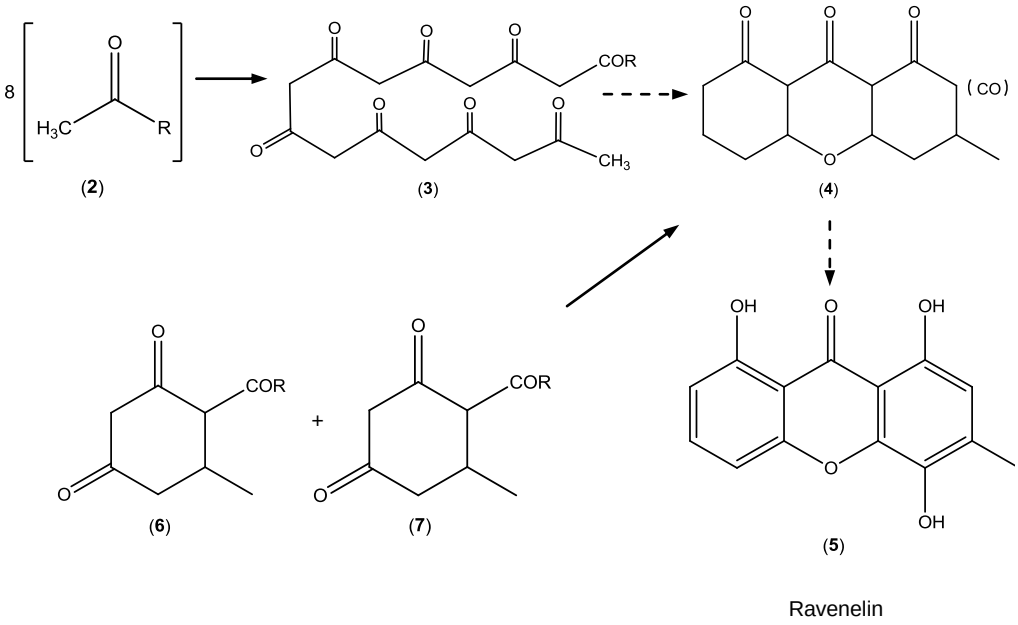


Figure 2.1: Biosynthesis of xanthone through acetate polymalonic route

2.2.2 Mixed Shikimate Acetate Pathway

Biosynthetically, the xanthone backbone is assumed to derive from common benzophenone intermediates. Their oxygenation patterns indicate a mixed shikimate (formation of C ring)-acetate (formation of A ring) pathway (Dewick, 1998, Herrmann & Weaver, 1999, Gottlieb, 1968). The proposed biosynthesis is exemplified with the synthesis of maclurin (**12**) and 1,3,5,6-

tetrahydroxyxanthone (**13**) in Figure 2.2 (Locksley, Moore, & Scheinmann, 1967, Carpenter, Locksley, & Scheinmann, 1969, Bennett & Lee, 1988, Bennett , Lee, & Nagaratnam, 1990). Shikimic acid (**8**), derived from shikimic pathway, can be converted into protocatechuic acid (**9**) after oxidation, dehydration, and enolization. Reaction of protocatechuic acid (**9**) with coenzyme A (HSCoA) can produce an activated ester **10** that can further react with three units of malonyl-coenzyme A to yield intermediate **11**. A Dieckmann condensation gives rise to benzophenones, such as maclurin (**12**). Depending upon the benzophenone produced, this is a branch point in the biogenesis of other benzophenone-type natural products. It is generally accepted that xanthones such as 1,3,5,6-tetrahydroxyxanthone (**13**) are formed by means of phenolic coupling of the benzophenone precursors (Bennet & Lee, 1988, Bennett *et. al*., 1990). Cinnamic acid, benzoic acid, *m*-hydroxybenzoic acid, malonic acid, and 4'-deoxymaclurin as the intermediate benzophenone were found to be efficient precursors.

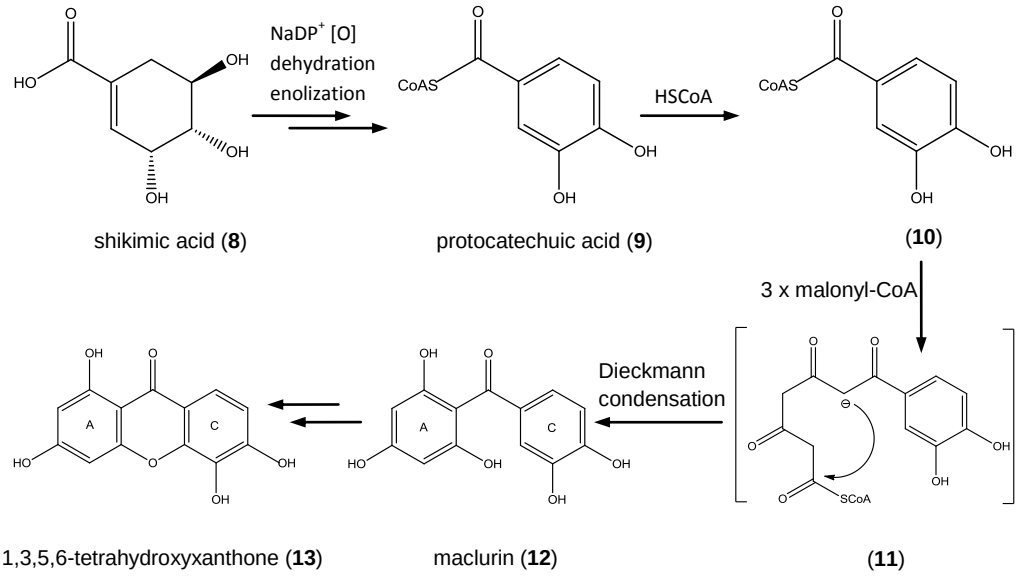


Figure 2.2: Biosynthesis of xanthone through mixed shikimate acetate pathway

2.3 Chemical Synthesis

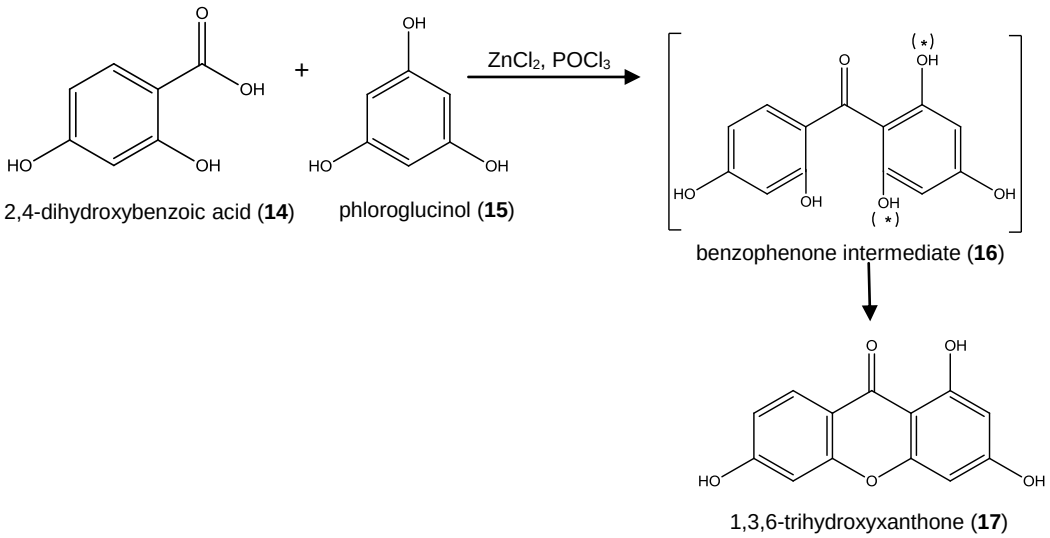
Some xanthones are from natural origins, they have limited type and position of substituents imposed by the biosynthetic pathways. Synthesis of new compounds enables enlargement of the possibilities of having different natures and positions of substituents on the xanthone nucleus. This will allow us to have different structures with a variety of biological activities.

2.3.1 The Grover, Shah and Shah (GSS) Reaction

Distillation of a mixture of a phenol, a phenolic acid, and acetic anhydride is the earliest and simplest method for the synthesis of hydroxyxanthones (Michael, 1883; Kostanecki & Nessler, 1891; Lund, Robertson, & Whalley, 1953), but yields are often poor, experimental conditions are rather drastic, and there is a possibility of decarboxylation, autocondensation, and other side reactions (Lespegnol, Bertrand, & Dupas, 1939)

However in 1955, Grover, Shah and Shah developed a general method for the synthesis of xanthones that still enjoys great popularity today. The Grover, Shah and Shah reaction offers a convenient method for preparing hydroxyxanthones due usually to the accessibility of the starting materials (Sousa & Pinto, 2005). It requires a salicylic acid derivative such as (**14**) and an activated polyphenol for example (**15**) that are heated together with zinc

chloride in phosphoryl chloride as solvent. The Grover, Shah and Shah (GSS) method can afford the xanthone skeleton (**17**) directly only if the benzophenone intermediate (**16**) carries another phenol substituent in an alternate site (*) for cyclization (Figure 2.3).



2,4-dihydroxybenzoic acid (**14**) phloroglucinol (**15**)

benzophenone intermediate (**16**)

1,3,6-trihydroxyxanthone (**17**)

Figure 2.3: Synthesis of xanthone by the Grover, Shah and Shah method

2.3.2 Modifications to the Grover, Shah and Shah Reaction

Recent modifications to the Grover, Shah, and Shah (GSS) reaction have been reported. Better results were obtained using a mixture of phosphorus pentoxide-methanesulfonic acid (Eaton's reagent) instead of phosphorus oxychloride-zinc chloride as catalyst (Pillai *et. al*., 1986; Moreau *et. al*., 2002). The former acylation catalyst was found to be an excellent condensing agent between 3-methylsalicylic acid (**18**) and phloroglucinol (**19**), providing high

yields (90–95%) of the xanthone (**20**) and no detectable amounts of the possible benzophenone (**21**) (Figure 2.4).

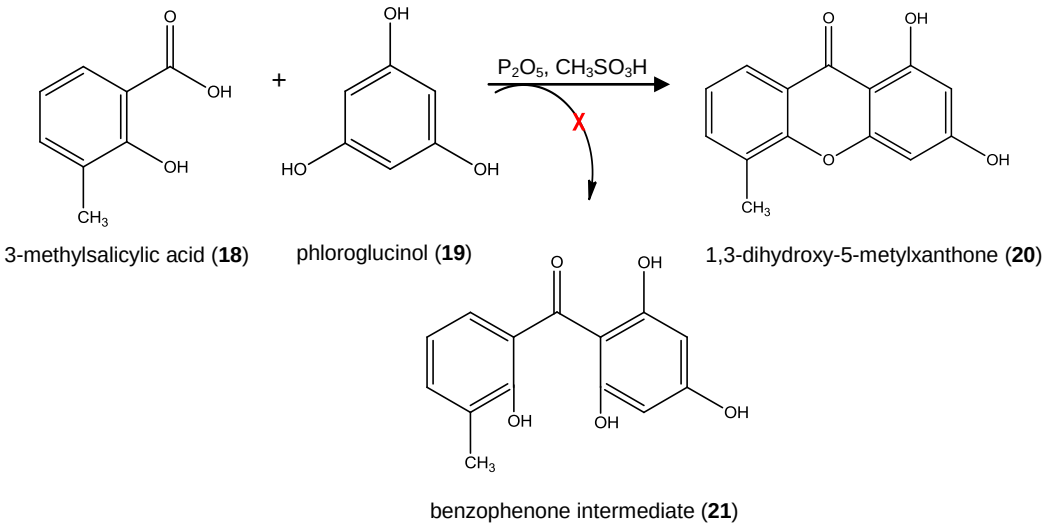
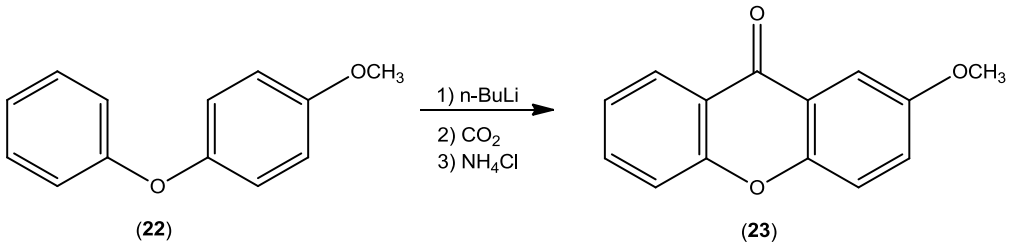


Figure 2.4: Synthesis of xanthone by the modified Grover, Shah and Shah method

2.3.3 Asahina-Tanase Method

This is a useful method for the synthesis of some methoxylated xanthones or xanthones with acid-sensitive substituents (Granoth & Pownall, 1975). Later, Vitale *et al*. (Vitale, Romanelli, Autinio, & Pomilio, 1994) modified the procedure as shown in Figure 2.5.



(**22**) (**23**)

Figure 2.5: Synthesis of xanthone by the modified Asahina-Tanase method

2.3.4 New Approaches to the Synthesis of the Xanthonic Tricyclic System

Different modes to construct the xanthone core have emerged in the last few years. The strategies of these methods were to provide an advantage for building highly polyoxygenated xanthones with regioselectivity (Sousa & Pinto, 2005).

Multi-component reactions give rise to production of complicated molecules in only one process. It is a very fast, efficient, and time-saving manner. Zhang and co-workers have reported that using HBF_4/SiO_2 as an efficient, green, and inexpensive catalytic system has synthesized xanthen-11-one derivatives via a one-pot three-component reaction. The reactions proceeded rapidly at 80^oC under solvent-free conditions (Figure 2.6) (Zhang, Wang, and Ren, 2009).

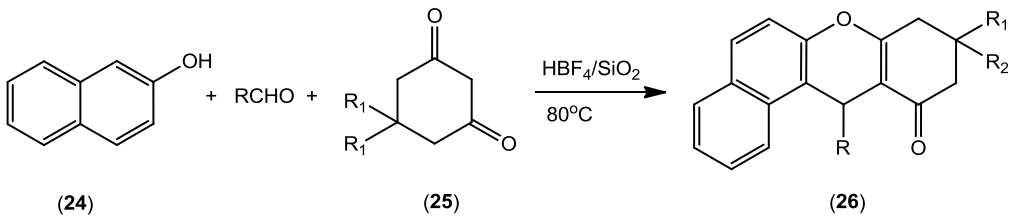


Figure 2.6: Synthesis of xanthone by heterogeneous catalyst reaction

In recent years, the development of sustainable, environmentally friendly, and low-cost C−C bond-forming methods attracted much attention in synthesizing xanthones. Effective and economical catalysts such as ceric ammonium nitrate (CAN), $FeCl_3$, $AlCl_3$, Cu, and $TiCl_4$ were used. In these catalyzing reactions, most of the metal catalysts could be recovered and reutilized (Yang, Ma, Wei, Han, & Gao, 2012). For example, the reaction of (2,4,5-trimethoxyphenyl) (2-hydroxyphenyl) methanone with CAN furnishing the xanthone, 2,3-dimethoxy-9H-xanthen-9-one (Figure 2.7) (Johnson *et al.*, 2010).

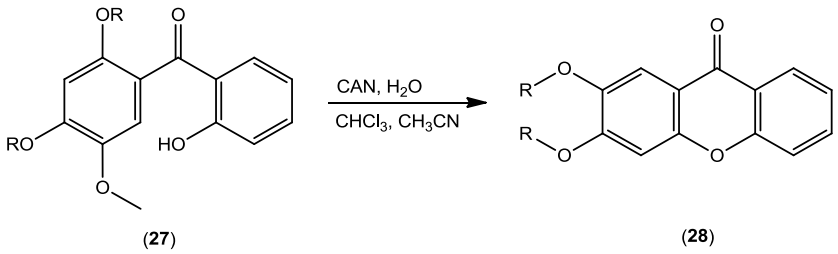


Figure 2.7: Synthesis of xanthone by CAN-mediated oxidation reaction

2.4 Synthesis of Prenylated Xanthones

The synthetic approach used to synthesize prenylated xanthones involved the nucleophilic substitution of xanthonic building blocks (Pinto & Castanheiro, 2009). The two major prenylated xanthones are *O*-prenylated (oxyprenylated) and *C*-prenylated (carboprenylated) xanthones.

2.4.1 O-and C-Prenylated Xanthones

O-alkylation of a hydroxyxanthone with prenyl bromide in alkaline medium, usually K_2CO_3, affords prenyloxy xanthones (Patel & Trivedi, 1988) (Figure 2.8). On the other hand, *C*-prenylation may occur when the reaction is performed in aqueous potassium hydroxide solution (Oger *et. al*., 2003; Helesbeux *et. al*., 2004) (Figure 2.9).

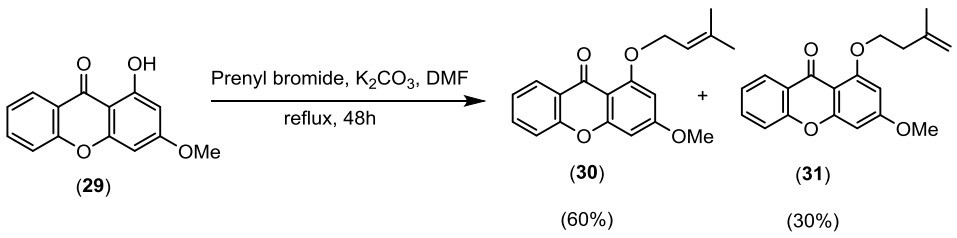


Figure 2.8: *O*-prenylation with prenyl bromide in potassium carbonate

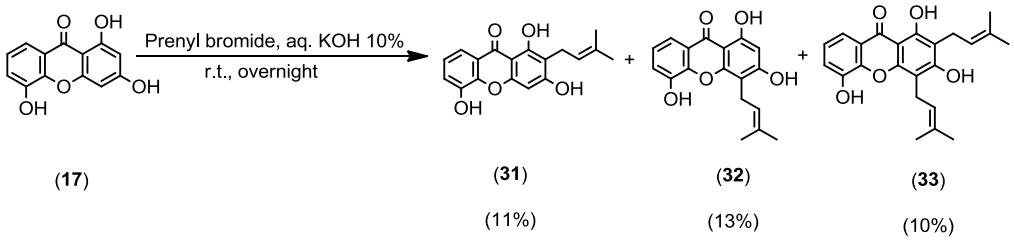


Figure 2.9: *C*-prenylation with prenyl bromide in potassium hydroxide

In order to optimize the synthetic process to obtain biologically active prenylated xanthones, microwave-assisted organic synthesis (MAOS), a methodology not only to dramatically accelerate many organic reactions, but also improve yields and selectivity has been recently used. Castanheiro *et al.* reported that, the usage of microwave (MW) irradiation had led to increased yields and in a remarkably shorter reaction time when compared to the conventional heating in the classical synthesis (Castanheiro *et. al*., 2009) (Figure 2.10).

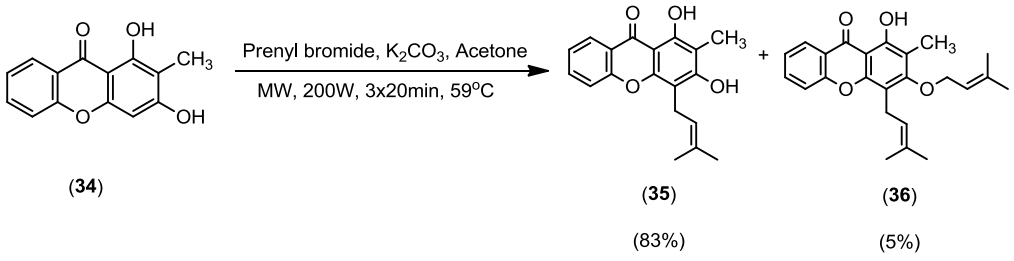


Figure 2.10: Synthesis of prenylated xanthones with MAOS

2.5 DPPH Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is one of the popular method to test the antioxidant activity or hydrogen donating ability of compounds by spectrophotometric method. The scavenging potential is compared with known antioxidants, such as ascorbic acid and kaempferol. The assay is based on one electron reduction of DPPH, in which because of the odd electron, DPPH gives strong absorption maxima at 517 nm by visible spectroscopy. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, i.e., a free radical scavenging antioxidant to form DPPH-H, the absorption intensity is decreased, and the colour changes from violet to yellow. The antioxidants are believed to donate hydrogen from the phenolic hydroxyl groups and break the free radical chain of oxidation forming a stable end product, which does not initiate or propagate further oxidation (Sherwin, 1978). The DPPH radicals get stabilized by accepting the hydrogen donated by the hydroxyl groups present on the phenolic compounds.

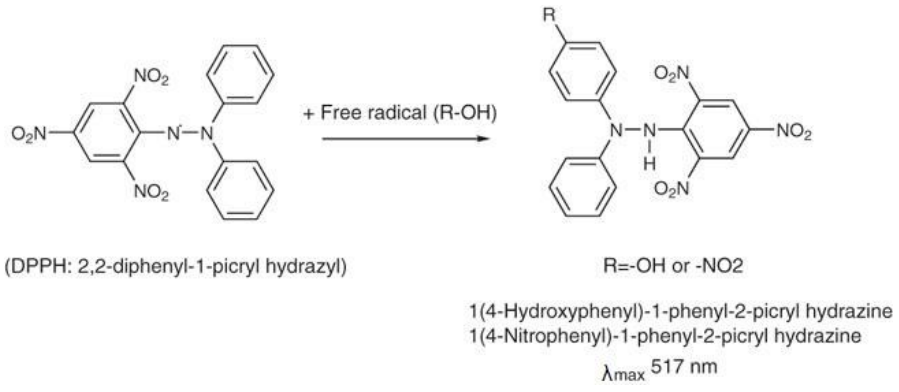


Figure 2.11: Principle of antioxidant (DPPH) assay

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals

The chemicals used in the synthesis of 1,3-dihydroxyxanthone are listed in Table 3.1:

Table 3.1: Chemicals used in synthesis 1,3-dihydroxyxanthone

Chemical reagents	Molecular formula	Molecular weight, ($g\ mol^{-1}$)	Source, Country
Salicylic acid (2-hydroxybenzoic acid)	$C_7H_6O_3$	138.12	Acros Organics, Belgium
Phloroglucinol (benzene-1,3,5-triol)	$C_6H_6O_3$	236.11	Sigma-Aldrich, USA
Eaton's reagent	$P_2O_5/MeSO_3H$	-	Acros Organics, Belgium

The chemicals used for the prenylation of 1,3-dihydroxyxanthone are listed in Table 3.2.

Table 3.2: Chemical used for the prenylation of 1,3-dihydroxyxanthone

Chemical reagents	Molecular formula	Molecular weight, M_w (g mol^{-1})	Source, Country
Acetone	CH_3COCH_3	58.08	QREC, Malaysia
Ethyl Acetate	$CH_3COOC_2H_5$	88.11	LAB-SCAN, Ireland
Hydrochloric acid (37%)	HCl	36.46	Fisher Scientific, UK
Potassium carbonate	K_2CO_3	138.21	John Kollin Corporation, USA
Potassium hydroxide	KOH	56.11	John Kollin Corporation, USA
Prenyl bromide (3,3-dimethylallyl bromide)	C_5H_9Br	149.09	Sigma-Aldrich, USA

The solvents used in purification by using column chromatography are listed in Table 3.3.

Table 3.3: Solvents and materials used in purification of synthesized compounds

Solvents/Materials	Molecular formula	Density, ρ (g ml^{-1})	Source, Country
Acetone	CH_3COCH_3	0.791	QREC, Malaysia
Dichloromethane	CH_2Cl_2	1.325	Fisher Scientific, UK
Ethyl Acetate	$CH_3COOC_2H_5$	0.902	Lab-Scan, Ireland
n-Hexane	$CH_3(CH_2)_4CH_3$	0.659	Merck, Germany
Methanol	CH_3OH	0.791	Mallinckrodt Chemicals, Phillipsburg
Sodium sulphate anhydrous	Na_2SO_4	-	John Kollin Corporation, USA
Silica gel (60 Å)	-	-	a) Silicycle, Canada b) Merck, Germany

Deuterated solvents used in NMR and materials used in chemical analyses are listed in Table 3.4.

Table 3.4: Deuterated solvents and materials used in chemical analyses

Deuterated solvents/ Materials	Source, Country
Acetone-d_6	Acros Organis, Belgium
Deuterated chloroform ($CDCl_3$)	Acros Organis, Belgium
Methanol-d_4	Acros Organis, Belgium
TLC silica gel 60 F_{254}	Merck, Germany

Chemical reagents and materials used in antioxidant assay are listed in Table 3.5.

Table 3.5: List of materials and reagents used in antioxidant assay

Reagents/Materials	Source, Country
96-well plate	Techno Plastic Products AG , Switzerland
Ascorbic acid	Sigma-Aldrich, USA
Kaempferol	Sigma-Aldrich, USA
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Sigma-Aldrich, USA

3.2 Methodology

3.2.1 Synthesis of the Xanthonic Block, 1,3-Dihydroxyxanthone

50 mmol (6.91 g) of salicylic acid and 50 mmol (6.31 g) of phloroglucinol were mixed in a 250 ml flat-bottomed flask. 120 ml of Eaton's reagent was then added slowly into the mixture followed by refluxing the mixture for 30 minutes at 80^{o}C with constant stirring. The reaction was monitored by TLC (thin layered chromatography). After completion of the reaction, the mixture was cooled to room temperature and poured onto crushed ice, filtered using Buchner filtration and extracted using ethyl acetate. After that, the upper organic layer was collected and the solvent was removed under reduced pressure and afforded the crude product that was subsequently purified by column chromatography.

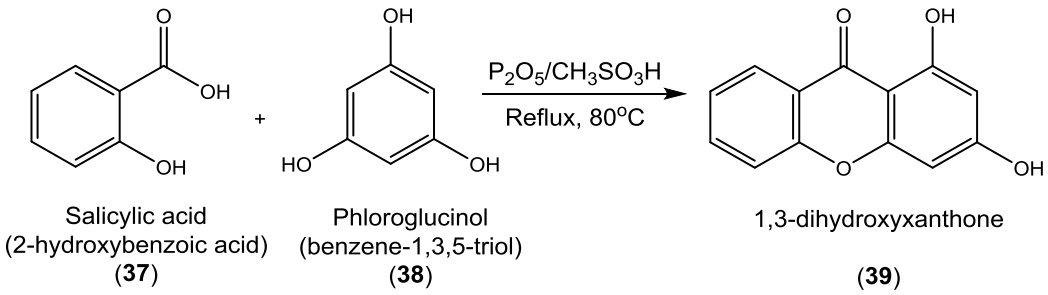


Figure 3.1: Synthesis of 1,3-dihydroxyxanthone

3.2.2 Prenylation of 1,3-Dihydroxyxanthone in Potassium Carbonate Solution

1.83 g (8 mmol) of 1,3-dihydroxyxanthone and 24.15 g (175 mmol) of potassium carbonate in 100 mL of distilled water were mixed and stirred in a 250 ml flat-bottomed flask for 10 minutes at room temperature. After that, 8.94 g (60 mmol) of prenyl bromide in 9 ml of acetone was injected via syringe into the reaction mixture. The mixture was then stirred for 19 hours at room temperature. After that, the reaction mixture was acidified by 100 mL of 10% HCl followed by extracting with 50 mL of ethyl acetate thrice. The upper organic layer was collected, treated with anhydrous sodium sulphate and the solvent was removed using rotary evaporator. The crude product was then subjected to column chromatography with gradient elution.

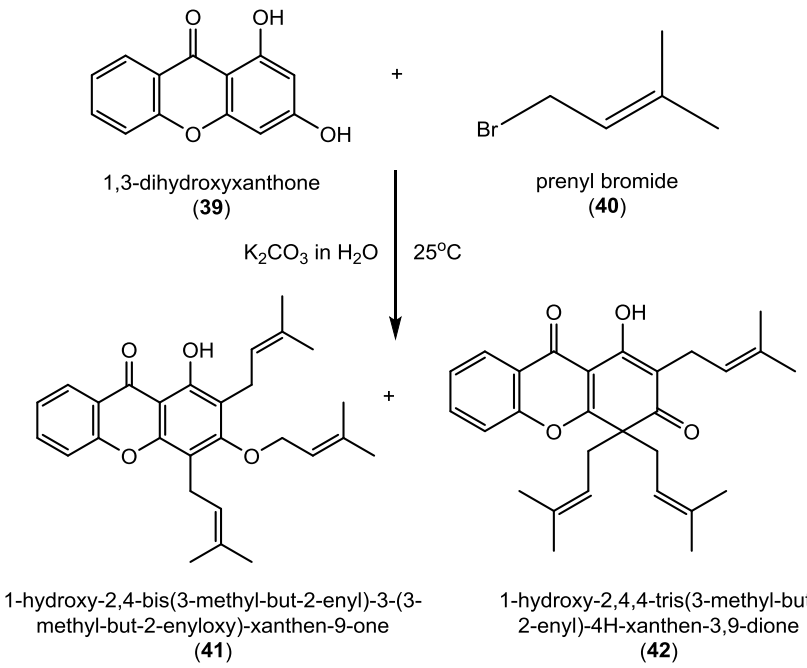


Figure 3.2: Prenylation of 1,3-dihydroxyxanthone in potassium carbonate solution

3.2.3 Prenylation of 1,3-Dihydroxyxanthone in Potassium Hydroxide Solution

0.91 g (4 mmol) of 1,3-dihydroxyxanthone and 1.68 g (30 mmol) of potassium hydroxide in 60 mL of distilled water were mixed and stirred in a 250 ml flat-bottomed flask for 10 minutes at room temperature. After that, 2.98 g (20 mmol) of prenyl bromide in 3 ml of acetone was injected into the reaction mixture via syringe. The mixture was then stirred for 19 hours at room temperature. After that, the reaction mixture was acidified by 100 mL of 10% HCl. The mixture was then extracted with 50 mL of ethyl acetate thrice. The upper organic layer was collected, treated with anhydrous sodium sulphate and the solvent was removed using rotary evaporator. The crude product was then subjected to column chromatography with gradient elution.

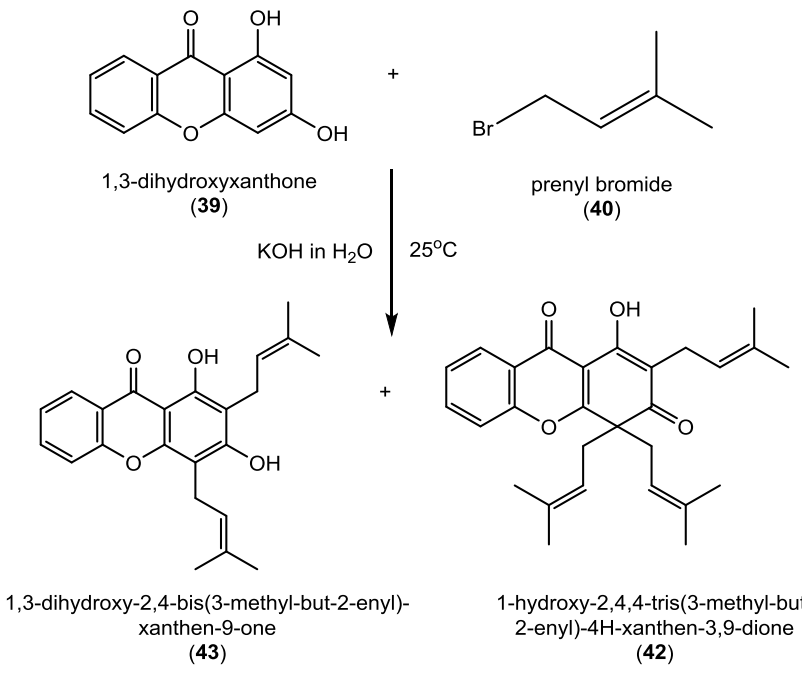


Figure 3.3: Prenylation of 1,3-dihydroxyxanthone in potassium hydroxide solution

3.2.4 Column Chromatography

In column chromatography, the stationary phase, also known as the adsorbent was prepared by mixing Silicycle or Merck silica gel (40-63 μm) and hexane, to form a slurry. The slurry was introduced into a vertical glass column and was allowed to settle down by eluting with the non-polar solvent, hexane.

The packed column was eluted with suitable amount of hexane to compact the silica gel before the sample was introduced into the column. The sample was prepared by using dry packing method in which the sample was dissolved in a least amount of solvent and were mixed evenly with dry silica gel drop by drop. The mixture was left to dry in order to coat onto the silica gel. The sample was then introduced into the packed column followed by gradient elution in increasing polarity to give separation effect to the sample. The fractions collected from column chromatography were analyzed using thin layer chromatography (TLC).

3.2.5 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a simple and inexpensive chromatographic method used to separate compounds based on difference in their polarities. This method was performed on a sheet of aluminium plate coated with Merck brand silica gel 60 F_{254} as adsorbent. A thin capillary tube was dipped into the sample solution and was spotted onto the baseline drawn on the plate. The plate was then put into the developing chamber saturated with the mobile phase. The plate was removed from the chamber when the solvent reached the solvent front. The spots developed were visualized under ultra-violet lamp with both short (254 nm) and long (365 nm) wavelengths. The retention factor, R_f value of each spot was obtained according to the equation below:

$$R_f = \frac{\text{distance travelled by the compound (cm)}}{\text{distance travelled by the solvent (cm)}}$$

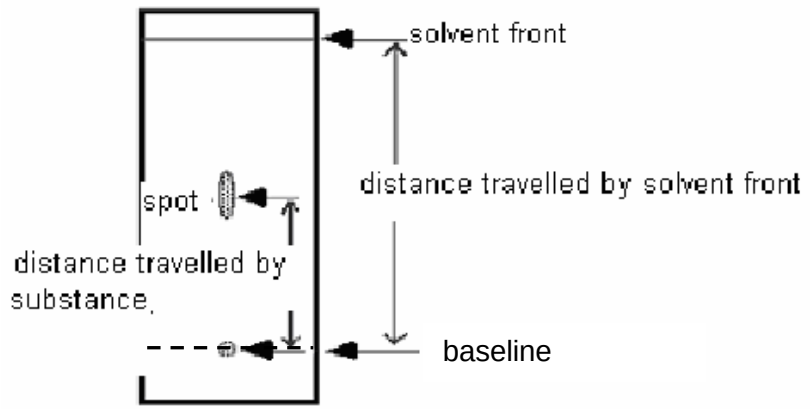


Figure 3.4: Developed thin layer chromatography (TLC) plate

3.3 Instruments

3.3.1 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is applicable to any nucleus possessing spin and is used to determine the number and type of chemical entities in a molecule. JEOL JNM-ECX 400 MHz spectrometer was used in this project. The ^{1}H-NMR and ^{13}C-NMR were devoted to structure elucidation of xanthone derivatives based on chemical shifts, which were estimating from electronic and anisotropic effect. On the other hand, the 2D-NMR, HMQC (Heteronuclear Multiple Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Coherence) were used to solve more complex structures, by observing cross-signals for C, H spin pairs connected by two- or three-bond coupling ($^{2}J_{C\text{-}H}$ or $^{3}J_{C\text{-}H}$) . Tetramethylsilane (TMS) was used as the internal standard and reference.

NMR samples were prepared by dissolving samples in a small amount of deuterated-solvent that filled the NMR tube up to a height of approximately 4 cm. The NMR tube was labelled and the cap was sealed with parafilm to prevent evaporation.

3.3.2 Ultraviolet-Visible (UV-Vis) Spectroscopy

Ultraviolet-visible spectroscopy utilizes the light in the visible and adjacent (near-UV and near-IR) ranges to provide qualitative information for highly conjugated organic compounds. In this project, Perkin-Elmer Lambda (25/35/45) UV-Vis spectrophotometer was used for sample analysis. A small amount of sample was dissolved by absolute ethanol in a quartz cuvette. The absorption maxima were measured in the range of 190 nm to 400 nm.

3.3.3 Melting Point Apparatus

Melting point of a compound is the temperature at which the material changes from solid to a liquid state. Pure crystalline substances have clear and sharp defined melting point, while impurities enlarge the melting range of a substance. In this project, melting point determination of samples was carried out by using Barnstead Electrothermal 9100 melting point apparatus to determine the purity of a sample. The fine solid sample was introduced into a haematocrit capillary before it was inserted into the instrument. The range of temperature at which the solid started to melt and completely melted was recorded.

3.4 Antioxidant Assay

The antioxidant activities of the synthesized compounds were tested using DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay. Firstly, all the samples and standard compounds were dissolved in methanol for preparation of master stocks at a concentration of 1 mg/mL. Then, the master stocks were sonicated for 5 minutes for thorough dissolution. Next, the DPPH powder was dissolved in methanol to get a concentration of 4 mg/mL. The solution was then sonicated and stored in dark at 4^{o}C.

After that, the samples and standard compounds were diluted with methanol into a series of concentrations of 240, 120, 60, 30, 15, 7.5, and 3.75 μg/mL. They were then transferred into a 96-well plate and added with 5 μL of DPPH solution. A DPPH methanolic solution was taken as the negative control. All tests were run in triplicate and averaged.

Immediately after the addition of reagents, the plate was wrapped with aluminium foil to avoid evaporation and stored in dark at room temperature for 30 minutes. After that, the absorbance of the mixture in each well was measured at 520 nm using a microplate reader (Model 680, Bio-Rad Laboratories, Hercules, CA, USA) and results were interpreted by the Microplate Manager®, Version 5.2.1 software. Inhibition rates of the

compounds were calculated, and the resulting data were presented as a plot of inhibition rate against concentration of the samples.

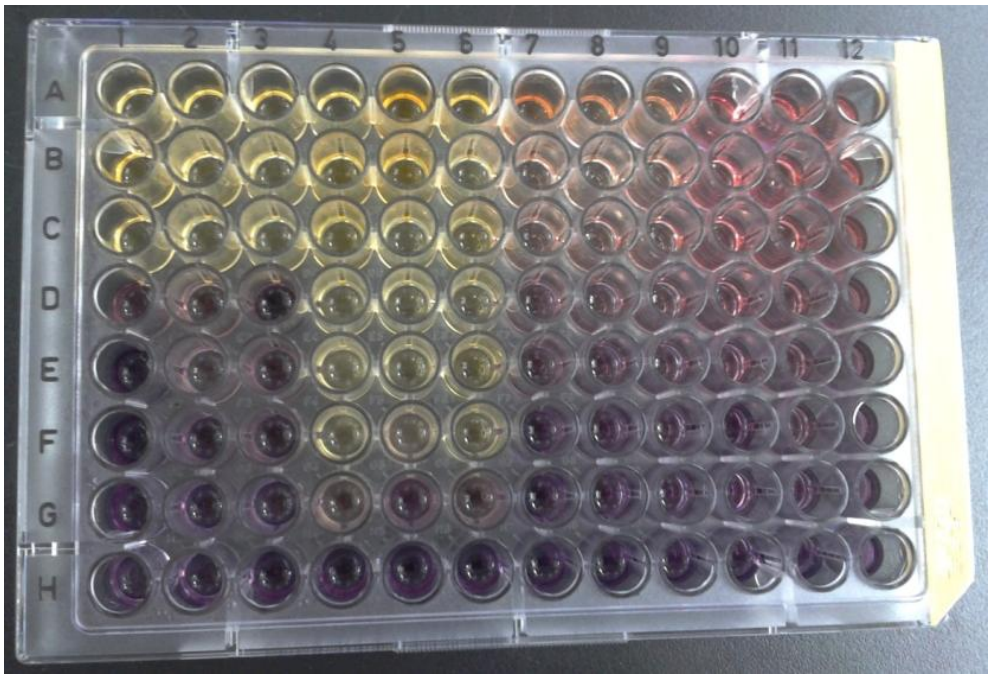


Figure 3.5: DPPH antioxidant assay using 96-well plate

3.5 Calculation

3.5.1 Percentage Yield of Xanthones

The percentage yield of each synthesized xanthone was calculated by using the equation below:

$$\text{Percentage yield} = \frac{\text{experimental yield of xanthone (g)}}{\text{theoretical yield of xanthone (g)}} \text{ x } 100\%$$

3.5.2 Inhibition Rate

Inhibition rates of the test compounds were calculated by using the formula below:

$$\text{Inhibition rate (\%)} = \frac{A_o - A_1}{A_o} \times 100\%$$

Where A_o = Absorbance of the negative control (blank)

A_1 = Absorbance of the test compound

The inhibition rate was plotted against the sample concentration to obtain IC_{50}, defined as the concentration of sample necessary to cause 50% inhibition to the DPPH radical scavenging activity.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis of 1,3-Dihydroxyxanthone

Reaction of 50 mmol of salicylic acid and 50 mmol of phloglucinol with Eaton's reagent as catalyst and coupling agent has afforded the xanthonic block, 1,3-dihydroxyxanthone (**39**) which has a molecular formula of $C_{13}H_8O_4$. A moderate yield of 66% was obtained. The compound appeared as a yellowish-brown solid with melting range of 229-230^oC. The spot of the compound gave R_f value of 0.74 on a TLC plate running with mobile phase of hexane: ethyl acetate: dichloromethane in 1:1:3 ratio.

Table 4.1: Summary of physical data of 1,3-dihydroxyxanthone

Molecular formula	$C_{13}H_8O_4$
Molecular weight, g mol^{-1}	228.20
Physical appearance	Yellowish-brown solid
Mass obtained, g	7.5273
Melting point, ^oC	229-230
Percentage yield, %	66.0
R_f value	0.74 (TLC solvent system of Hex: EA: DCM = 1:1:3)

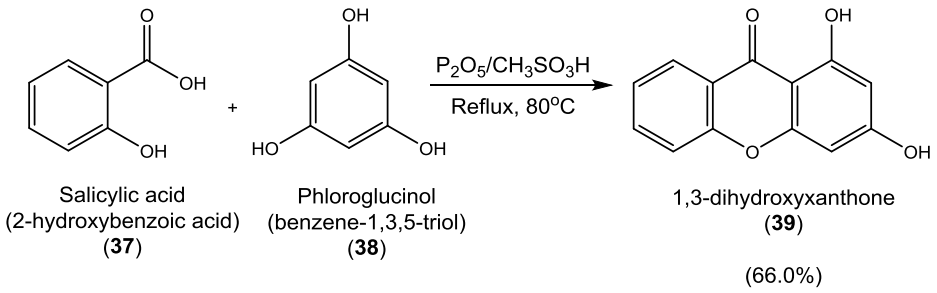


Figure 4.1: Synthesis of 1,3-dihydroxyxanthone

4.1.1 Proposed Mechanism for Synthesis of 1,3-Dihydroxyxanthone

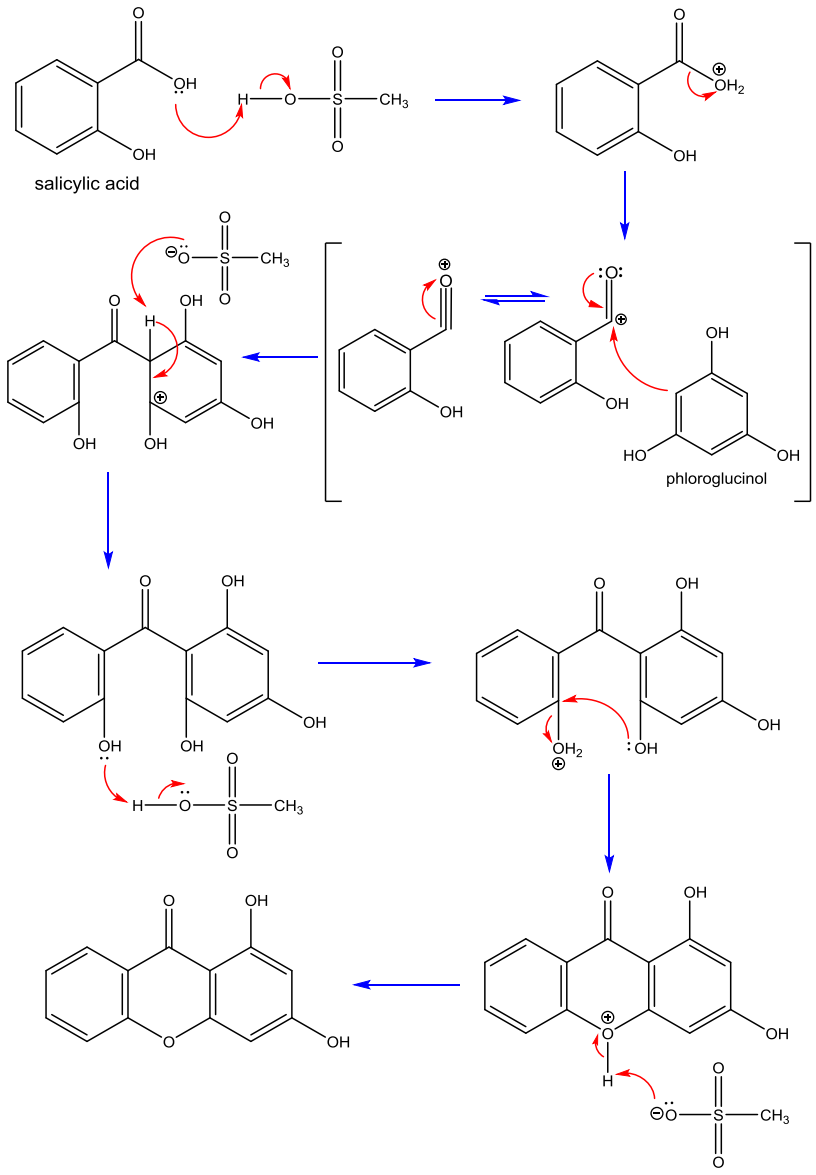


Figure 4.2 Proposed mechanism for synthesis of 1,3-dihydroxyxanthone

4.1.2 Structural Elucidation of 1,3-Dihydroxyxanthone

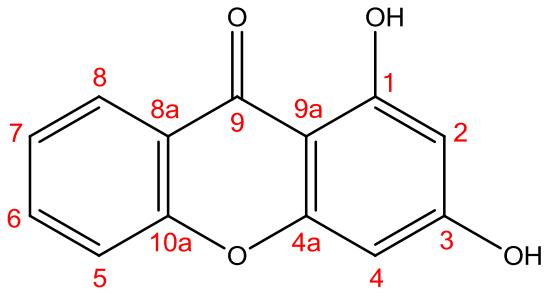


Figure 4.3 Structure of 1,3-dihydroxyxanthone (39)

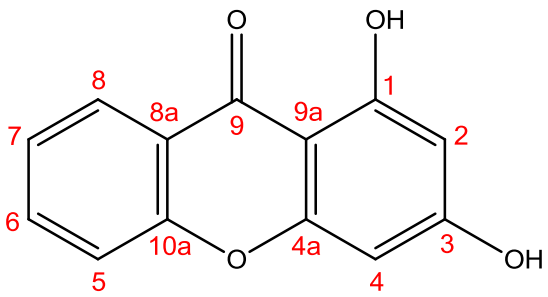
The structure of 1,3-dihydroxyxanthone (**39**) was established by 1D-NMR, 2D-NMR and UV techniques. A total of six proton signals between δ 6-9 were observed in ^{1}H NMR spectrum (Figure 4.4). They were assigned to the six aromatic protons in the structure. The *peri*-hydroxyl group showed intramolecular hydrogen bonding with the carbonyl group and gave a downfield signal at δ 12.88 and this confirmed the presence of hydroxyl substitution at position 1.

The ^{13}C NMR spectrum (Figure 4.5) revealed a total of thirteen carbon signals indicating the presence of thirteen aromatic carbons in the structure. The highly deshielded carbonyl carbon gave a downfield signal at δ 180.5. The four carbon signals having relatively higher shift at δ 165.8, 163.9, 158.0 and 156.0 were assigned to the oxygenated aromatic carbons C3, C1, C4a and

C10a, respectively. On the other hand, signals at δ 135.4, 125.5, 124.2, 117.1, 98.2 and 94.1 were assigned to the six methine carbons, C6, C8, C7, C5, C2 and C4, respectively.

The structure of the compound was further deduced from the 2D-NMR spectra in which the correlations between carbons and hydrogens were shown in Table 4.2. In the HMBC spectrum (Figure 4.7), long range correlations from 1-OH, H2 and H4 to C9a, H5 and H7 to C8a, and from H6 and H8 to C10a were observed. The correlation data was found to be in correspondence to the structure assigned.

The UV spectrum (Figure 4.8) of **39** gave λ_{max} at 213, 235 and 306 nm resembling that the compound was highly conjugated.



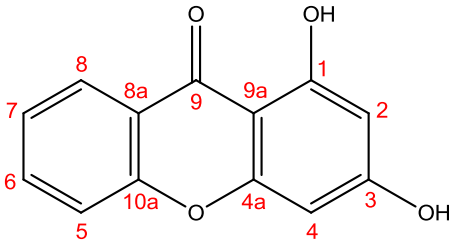
1,3-Dihydroxyxanthone (**39**)

Molecular formula: $C_{13}H_8O_4$

Molecular mass: 228.20 $gmol^{-1}$

Table 4.2: Summary of NMR data of 1,3-dihydroxyxanthone

Position	**δH (ppm)**	**δC (ppm)**	**HMBC**	
			^2J	^3J
1	-	163.9	-	-
2	6.24 (1H, d, *J* = 1.8 Hz)	98.2	C1	C9a, C4
3	-	165.8	-	-
4	6.40 (1H, d, *J* = 1.8 Hz)	94.1	C3, C4a	C2, C9a
4a	-	158.0	-	-
5	7.49 (1H, d, *J* = 8.0 Hz)	117.1	C10a	C7, C8a
6	7.80 (1H, td, *J* = 8.0, 1.2 Hz)	135.4	-	C8, C10a
7	7.42 (1H, t, *J* = 8.0 Hz)	124.2	-	C5, C8a
8	8.15 (1H, dd, *J* = 8.0, 1.2 Hz)	125.5	-	C6, C9, C10a
8a	-	120.4	-	-
9	-	180.5	-	-
9a	-	103.0	-	-
10a	-	156.0	-	-
1-OH	12.88 (OH, s)	-	C1	C2, C9a
3-OH	9.88 (OH, s)	-	-	-



1,3-Dihydroxyxanthone (**39**)

Molecular formula: $C_{13}H_8O_4$

Molecular mass: 228.20 $gmol^{-1}$

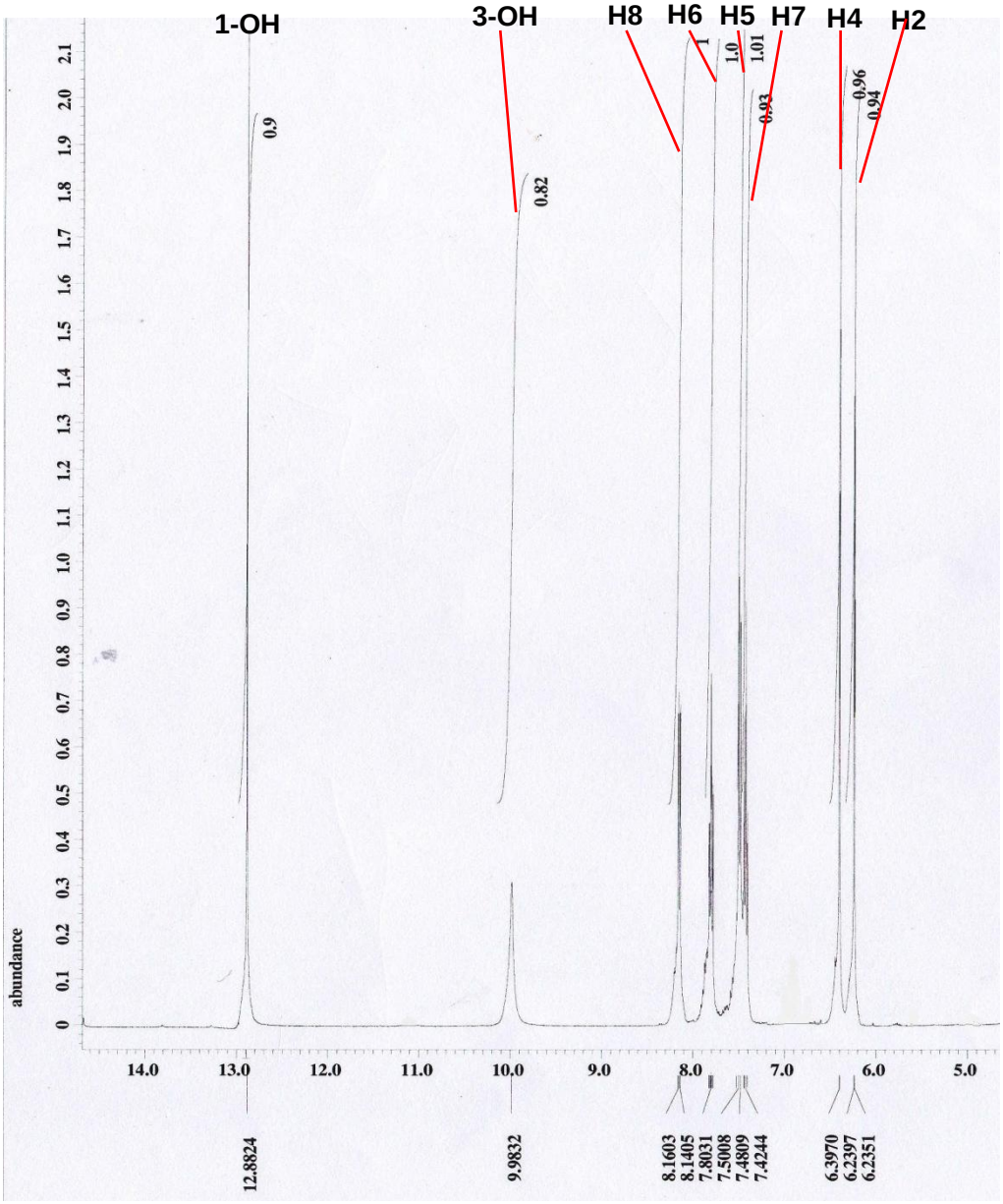
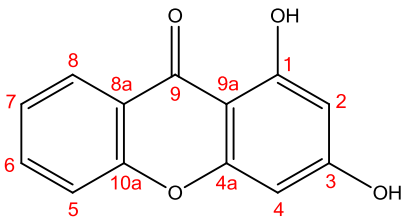


Figure 4.4: ^{1}H NMR spectrum of 1,3-dihydroxyxanthone (400 MHz, acetone-d_6)



1,3-Dihydroxyxanthone (**39**)

Molecular formula: $C_{13}H_8O_4$

Molecular mass: 228.20 $gmol^{-1}$

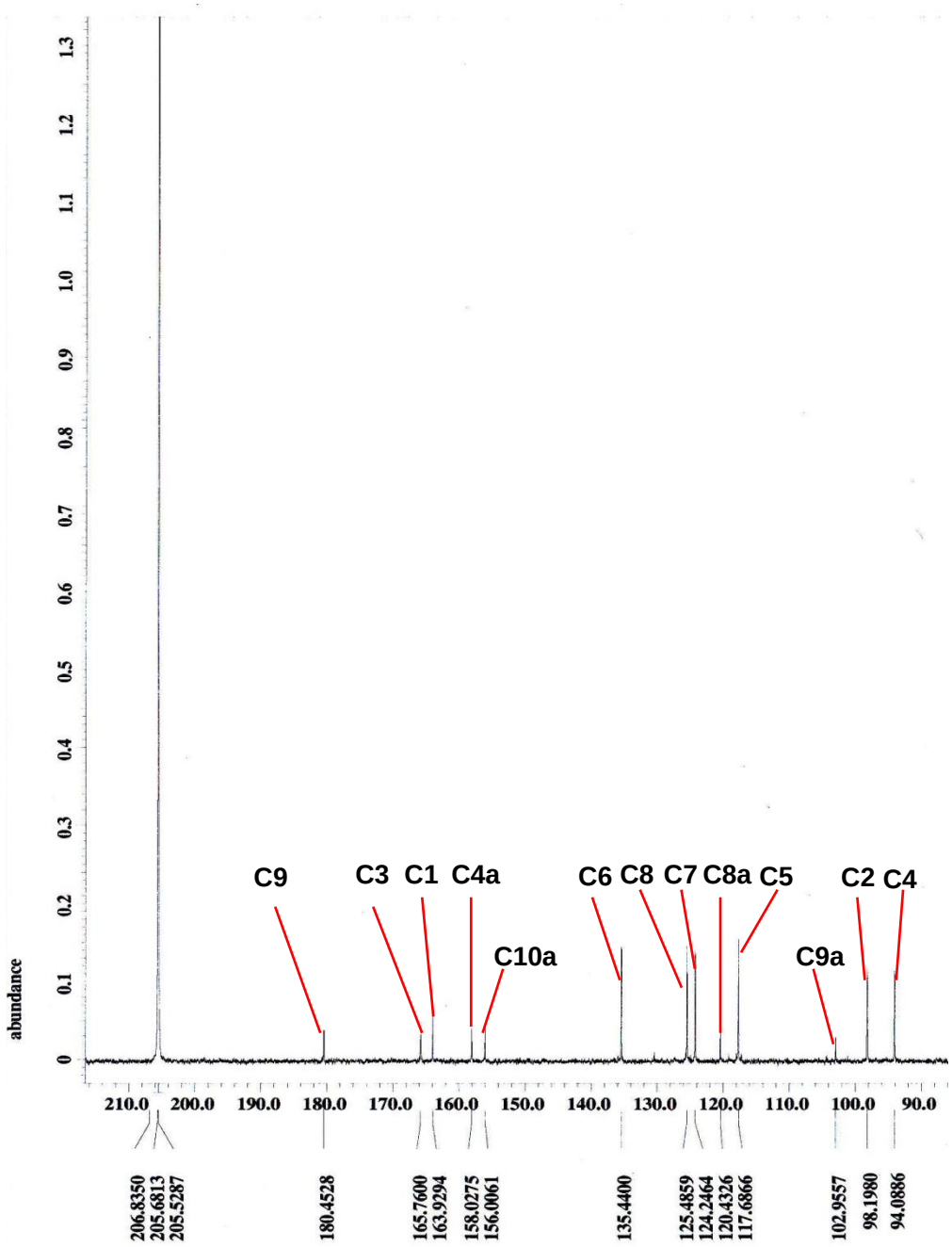
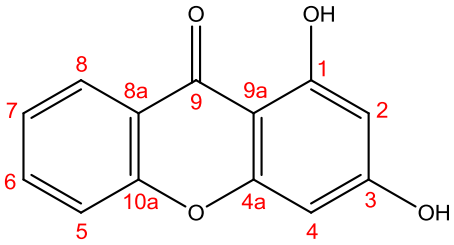


Figure 4.5: ^{13}C NMR spectrum of 1,3-dihydroxyxanthone (400 MHz, acetone-d_6)



1,3-Dihydroxyxanthone (**39**)

Molecular formula: $C_{13}H_8O_4$

Molecular mass: 228.20 $gmol^{-1}$

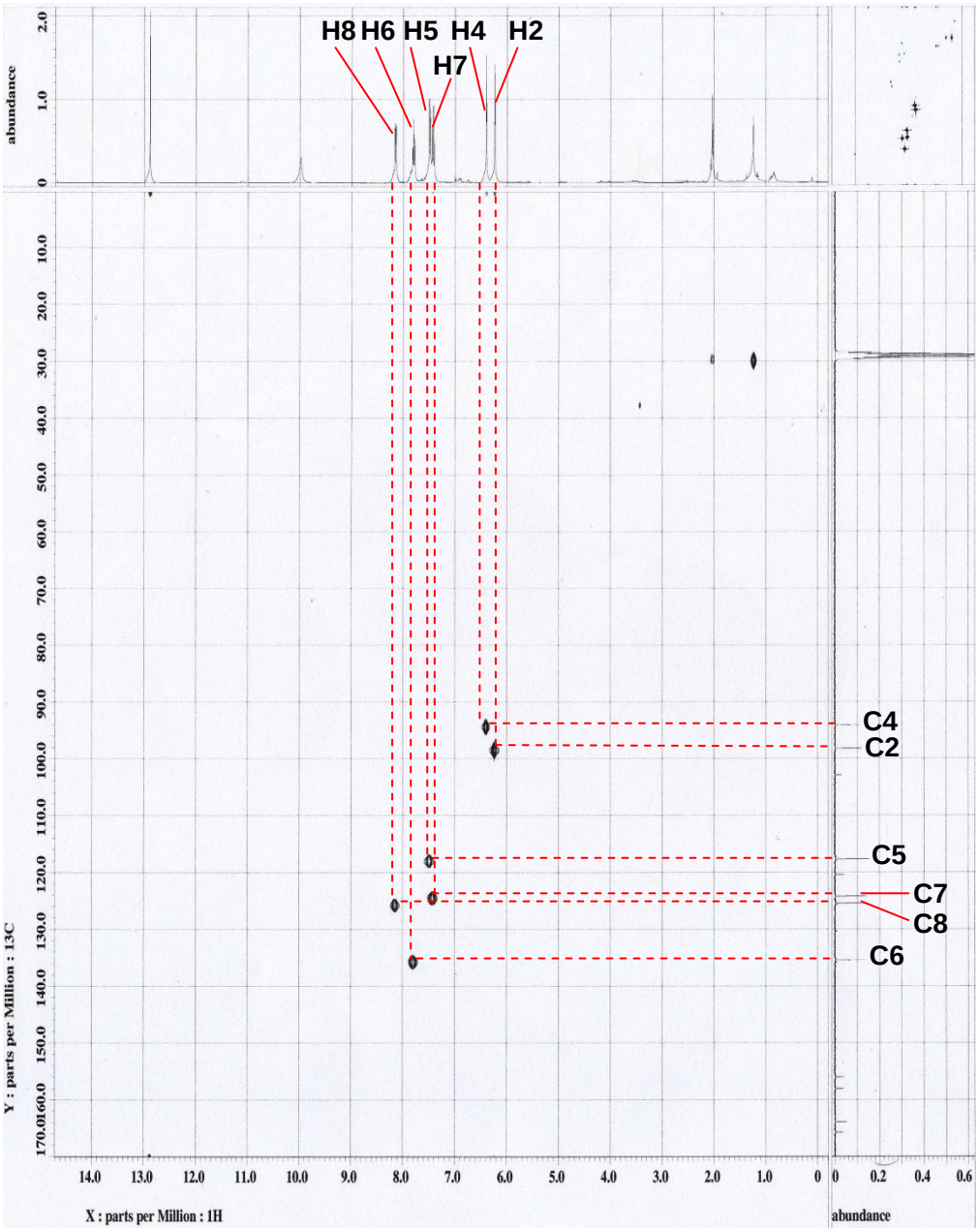
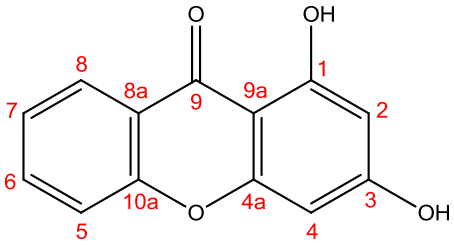


Figure 4.6: HMQC spectrum of 1,3-dihydroxyxanthone (400 MHz, acetone-d_6)



1,3-Dihydroxyxanthone (**39**)

Molecular formula: $C_{13}H_8O_4$

Molecular mass: 228.20 $gmol^{-1}$

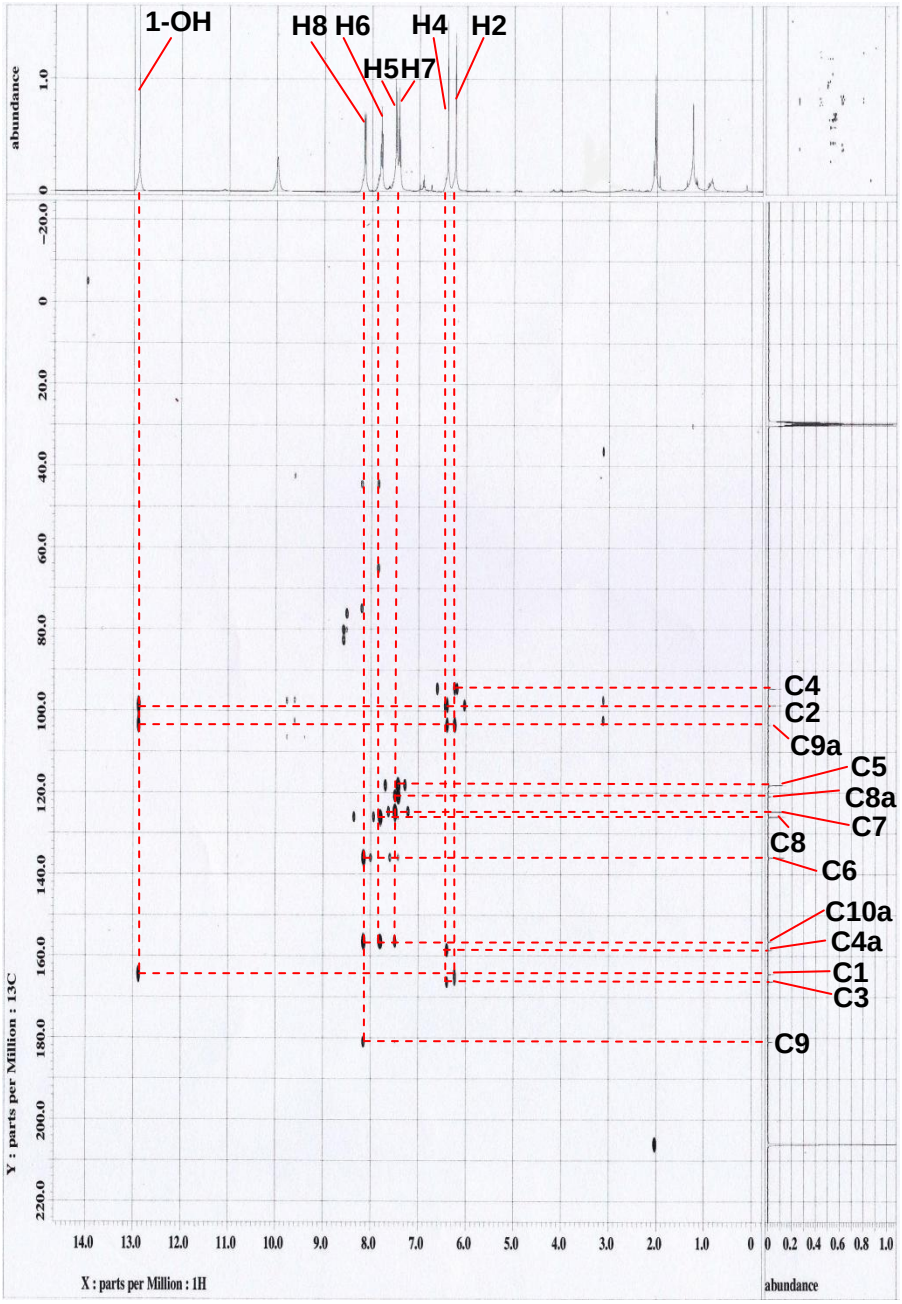
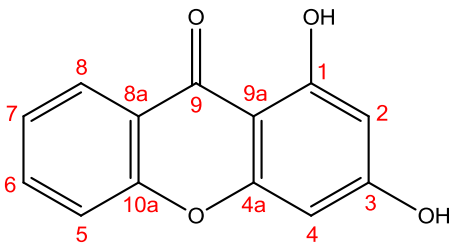


Figure 4.7: HMBC spectrum of 1,3-dihydroxyxanthone (400 MHz, acetone-d_6)



1,3-Dihydroxyxanthone (**39**)

Molecular formula: $C_{13}H_8O_4$

Molecular mass: 228.20 $gmol^{-1}$

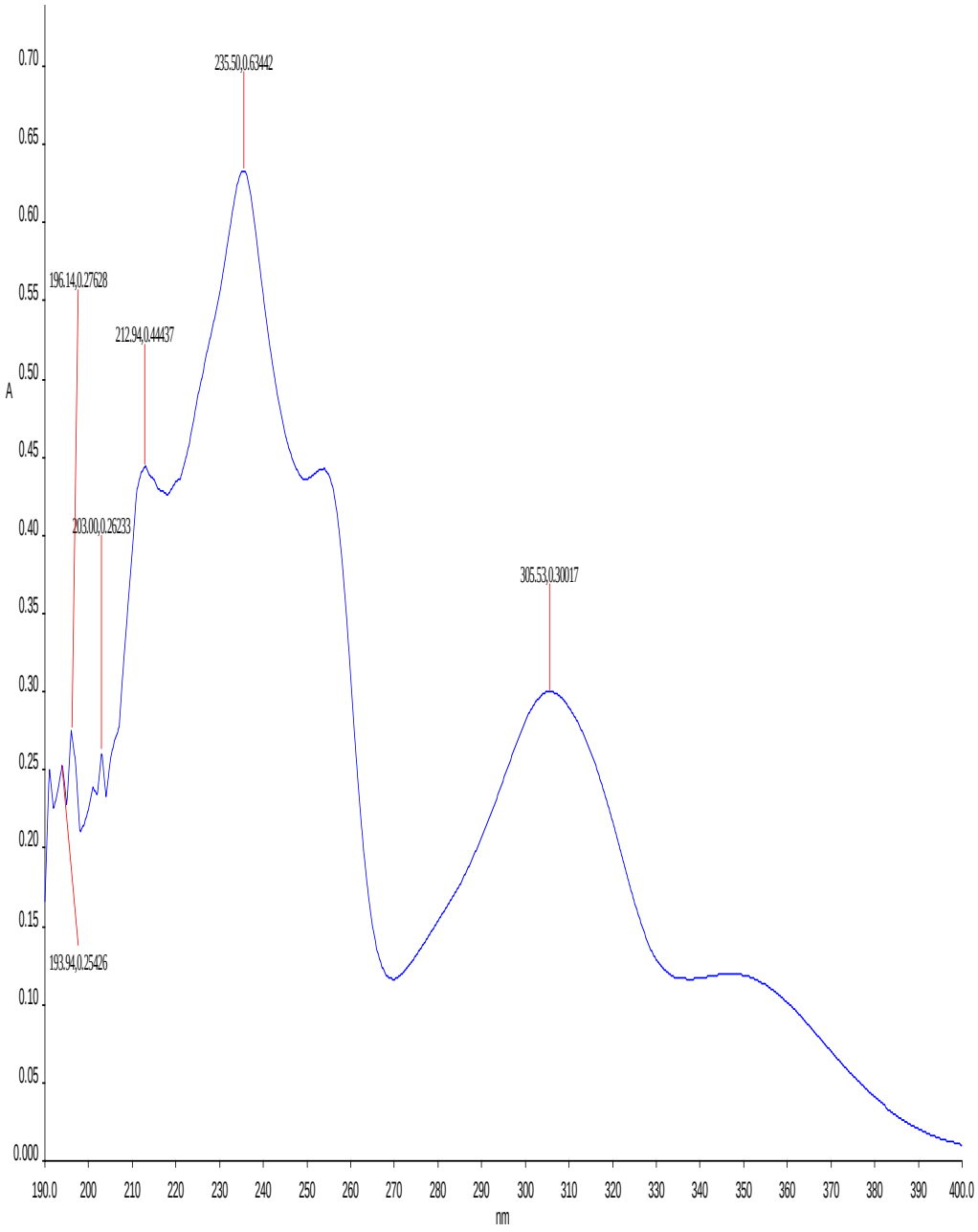


Figure 4.8: UV-Vis spectrum of 1,3-dihydroxyxanthone

4.2 Prenylation of 1,3-Dihydroxyxanthone

The synthetic methods employed for the prenylated compounds are depicted in figures 4.9 and 4.10. Introduction of prenyl group was conducted in two different basic media, potassium carbonate and potassium hydroxide. Consequently, the triprenylated xanthone, **42** appeared as a product for both syntheses. Using potassium carbonate for prenylation gave the carbo-prenylated xanthone **42** as the major product in 8.5% yield and a minor carbo- and oxy-prenylated product **41** (1.2%). On the contrary, synthesis using potassium hydroxide afforded selectively carbo-prenylated xanthones **42** and **43**. With this method, the yield of the prenylated xanthone **42** was increased, to 9.3%. On the other hand, the biprenylated product **43** has a higher yield of 18.3%.

Compound **41** with the molecular formula of $C_{28}H_{32}O_4$ was found to be yellow powder and has melted between 95-96^{o}C. It has a R_f value of 0.87 when developed on a TLC plate eluted with a mobile phase of 85% hexane: 15% ethyl acetate. On the other hand, compound **42**, obtained as orange crystals was established to have a molecular formula $C_{28}H_{32}O_4$ with a melting range of 110-112^{o}C. The spot of **42** gave R_f value of 0.25 on a TLC plate eluted with a solvent mixture of 90% hexane: 10% ethyl acetate. Compound **43** was obtained as yellow powder having the molecular formula $C_{23}H_{24}O_4$ and melted between 131-133^{o}C. It gave R_f value of 0.53 when developed on a TLC plate eluted with a solvent mixture of 50% dichloromethane: 50% hexane. The

physical data of these three prenylated xanthones were summarized in table 4.3.

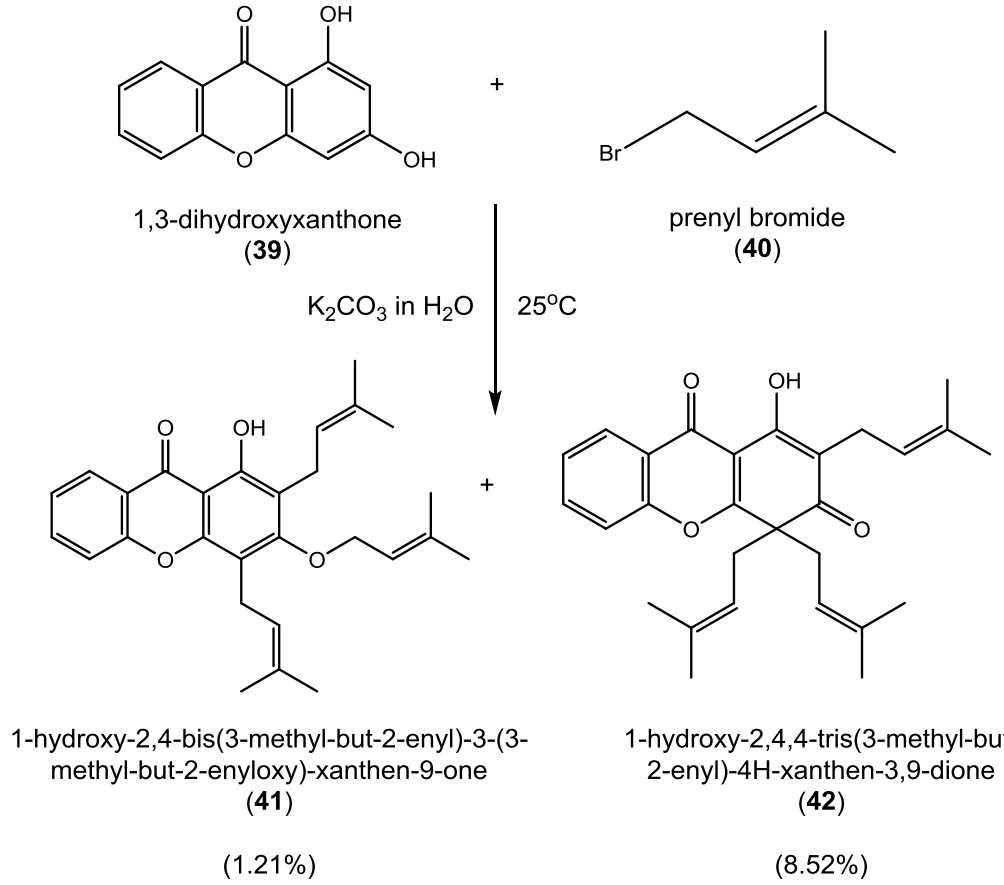


Figure 4.9: Prenylation of 1,3-dihydroxyxanthone in potassium carbonate solution

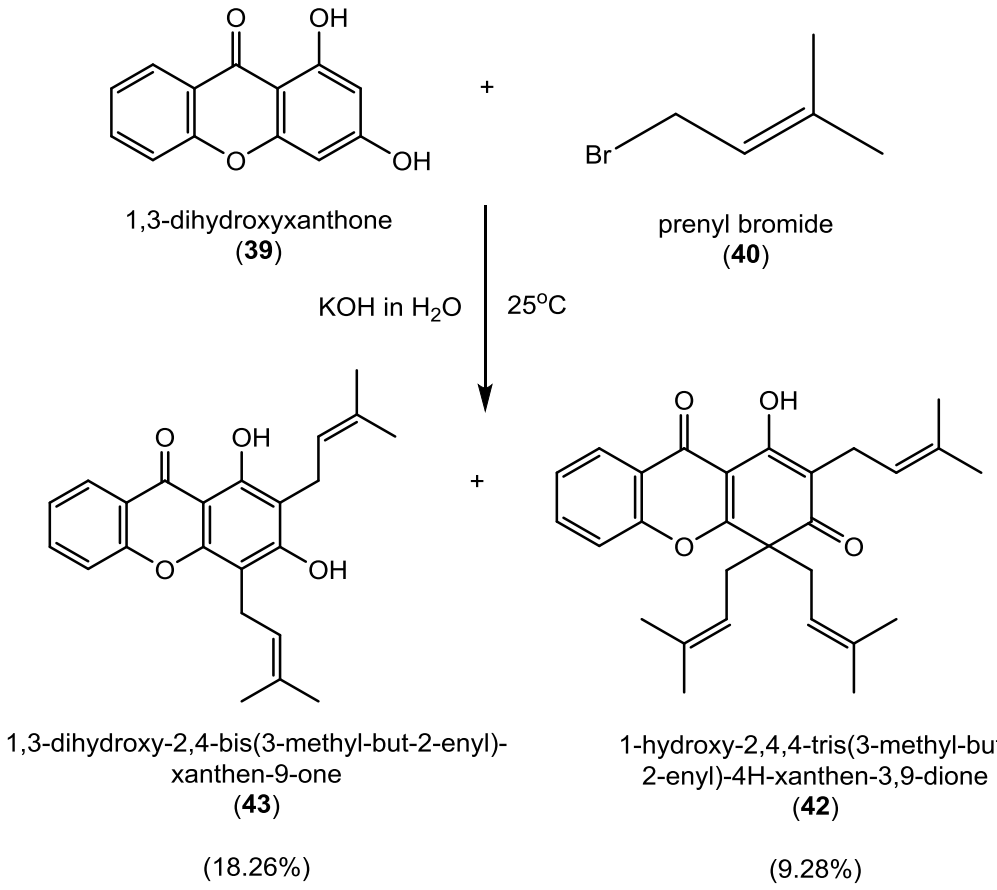


Figure 4.10: Prenylation of 1,3-dihydroxyxanthone in potassium hydroxide solution

Table 4.3: Summary of physical data of prenylated xanthones

Compound	**41**	**42**	**43**
Molecular formula	$C_{28}H_{32}O_4$	$C_{28}H_{32}O_4$	$C_{23}H_{24}O_4$
Molecular weight, g mol^{-1}	432.56	432.56	364.44
Physical appearance	Yellow powder	Orange crystals	Yellow powder
Mass obtained, g	0.0417	0.2947 (synthesis with K_2CO_3 as base) 0.1605 (synthesis with KOH as base)	0.2662
Melting point, ^{o}C	95-96	110-112	131-133
Percentage yield, %	1.21	8.52 (synthesis with K_2CO_3 as base) 9.28 (synthesis with KOH as base)	18.26
R_f value	0.87 (TLC solvent system = 85% Hex: 15% EA)	0.25 (TLC solvent system = 90% Hex: 10% EA)	0.53 (TLC solvent system = 50% DCM: 50% Hex)

4.2.1 Proposed Mechanism for Synthesis of 1-Hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

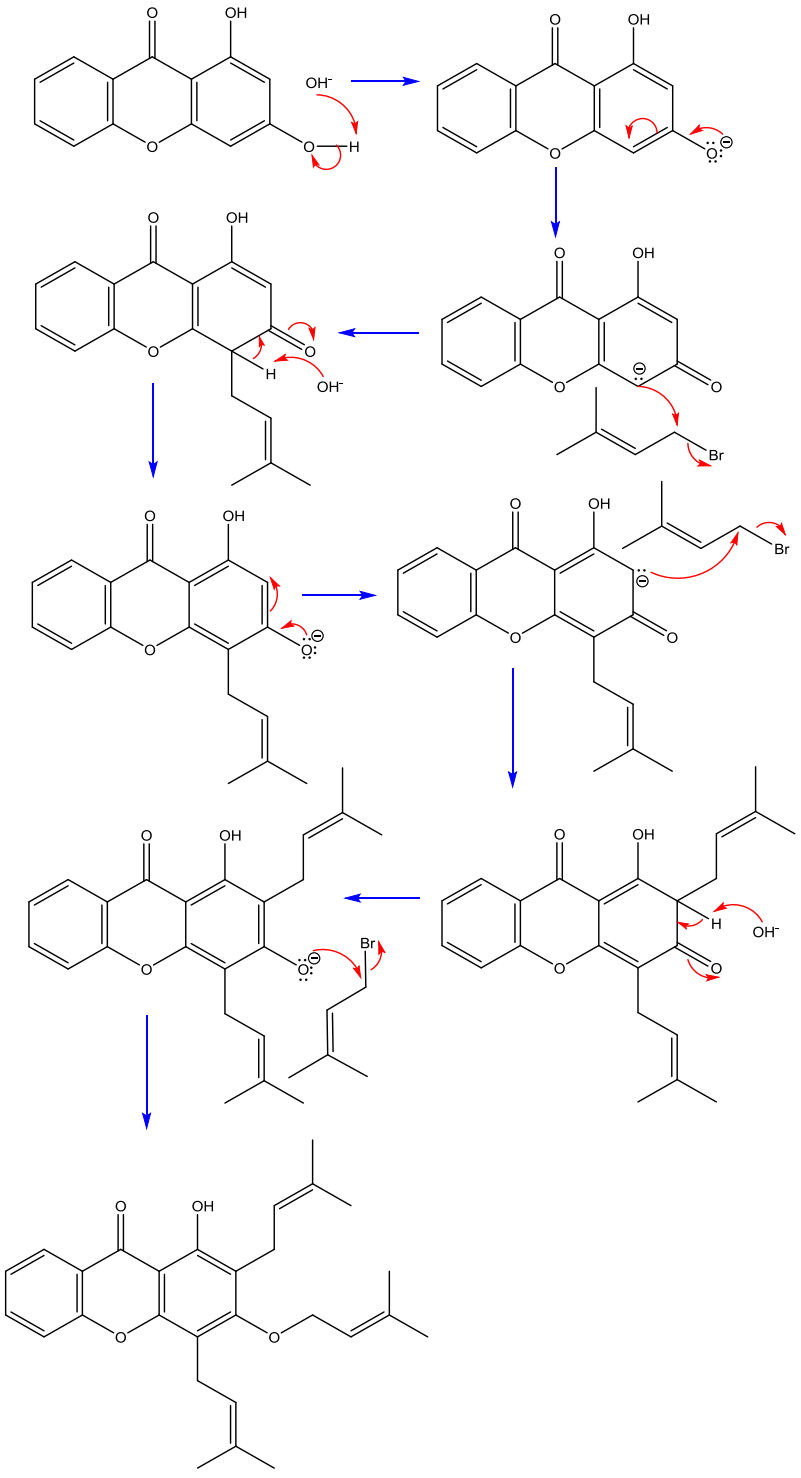


Figure 4.11: Proposed mechanism for synthesis of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

4.2.2 Structural Elucidation of 1-Hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

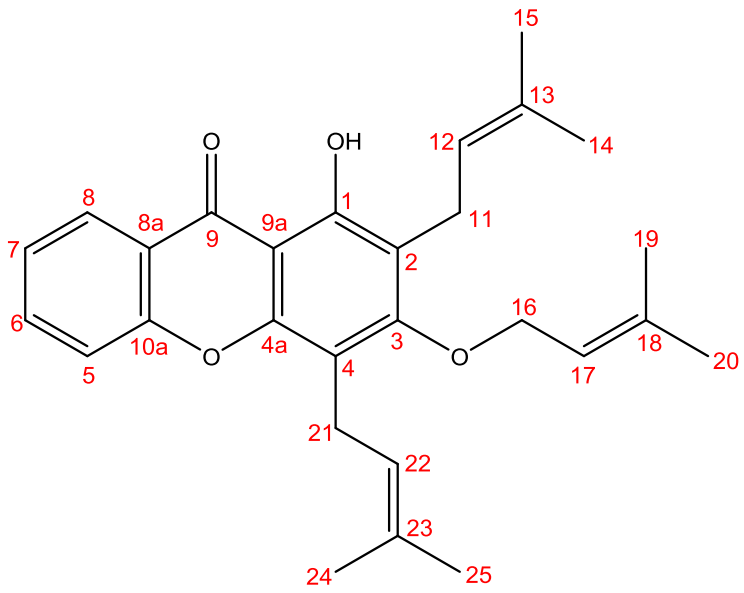


Figure 4.12: Structure of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (41)

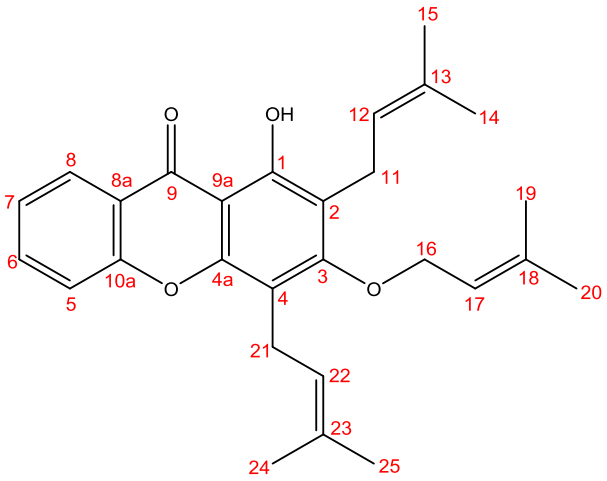
The presence of the C_5-prenyl substituents on compound **41** can be inferred from the ^{1}H and ^{13}C NMR chemical shifts. The ^{1}H NMR spectrum (Figure 4.13) revealed a downfield singlet at δ 12.95, suggesting the presence of chelated hydroxyl group at position 1. In comparison with ^{1}H-NMR spectrum (Figure 4.4) of compound **39**, the absence of proton signals at positions 2 and 4 and hydroxyl signal at position 3 indicating the substitution of three prenyl groups onto the xanthonic block. On top of that, the spectrum exhibited three doublets at δ 4.41, 3.55 and 3.43, each integrated for two protons, indicating the presence of additional three methylene groups. Furthermore, the methyl protons H14, H15, H19, H20, H24, H25 have contributed to six singlets in the

region of δ 1.68 to 1.88. Based on the evidence above, compound **41** was derived to be a xanthone with three prenyl groups.

The ^{13}C NMR spectrum (Figure 4.14) showed a total of 28 resonance signals, in which the signals lying above δ 150 were assigned to the oxygenated sp^2 hybridized carbons, C1, C3, C4a and C10a in the structure. The carbonyl carbon C9 was observed at δ 181.2 in the downfield region.

2D-NMR data collected is essentially needed to prove the structure and the data is found to be in agreement with the proposed structure. In the HMBC spectrum (Figure 4.16), correlations were observed from H11 to C1, C2 and C3, and from H21 to C3, C4, C4a. These correlations were used to assign the positions of two carbo-prenylated units and with that compound **41** was determined to be 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one.

The UV-Vis spectrum (Figure 4.17) has shown that the compound was highly conjugated with absorption maxima detected at 213.05, 236.04, 261.35 and 304.21 nm.



1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**)

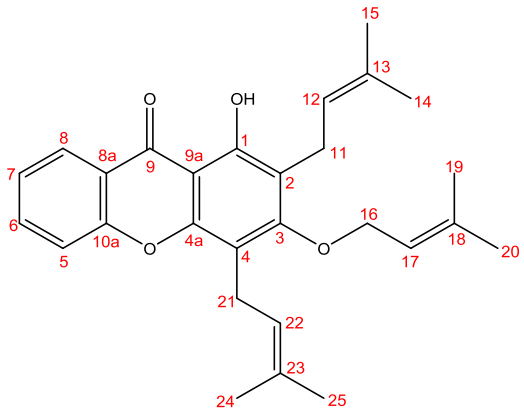
Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

Table 4.4: Summary of NMR data of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

Position	δH (ppm)	δC (ppm)	HMBC	
			^2J	^3J
1	-	159.1	-	-
2	-	117.6	-	-
3	-	162.9	-	-
4	-	113.5	-	-
4a	-	153.2	-	-
5	7.45 (1H, d, *J* = 8.0 Hz)	117.8	C10a	C7, C8a
6	7.70 (1H, td, *J* = 8.0, 1.2 Hz)	135.1	-	C8, C10a
7	7.35 (1H, t, *J* = 8.0 Hz)	123.9	-	C5, C8a
8	8.25 (1H, dd, *J* = 8.0, 1.2 Hz)	126.0	-	C6, C10a
8a	-	120.5	-	-
9	-	181.9	-	-
9a	-	106.2	-	-
10a	-	156.2	-	-

11	3.43 (2H, d, *J* = 6.7 Hz)	22.9	C2, C12	C1, C3, C13
12	5.26 (1H, t, *J* = 6.7 Hz)	122.8	-	C14, C15
13	-	132.2	-	-
14	1.79 (3H, s)	18.1	C13	C12, C15
15	1.69 (3H, s)	25.8	C13	C12, C14
16	4.41 (2H, d, *J* = 6.7 Hz)	71.9	-	C17, C18
17	5.59 (1H, t, *J* = 6.7 Hz)	120.2	-	C19, C20
18	-	138.1	-	-
19	1.68 (3H, s)	18.2	C18	C17, C20
20	1.81 (3H, s)	26.0	C18	C17, C19
21	3.55 (2H, d, *J* = 6.7 Hz)	23.1	C4, C22	C3, C4a, C23
22	5.23 (1H, t, *J* = 6.7 Hz)	123.0	-	C24, C25
23	-	131.8	-	-
24	1.88 (3H, s)	18.2	C23	C22, C25
25	1.69 (3H, s)	25.8	C23	C22, C24
1-OH	12.95 (OH, s)	-	C1	C2, C9a



1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

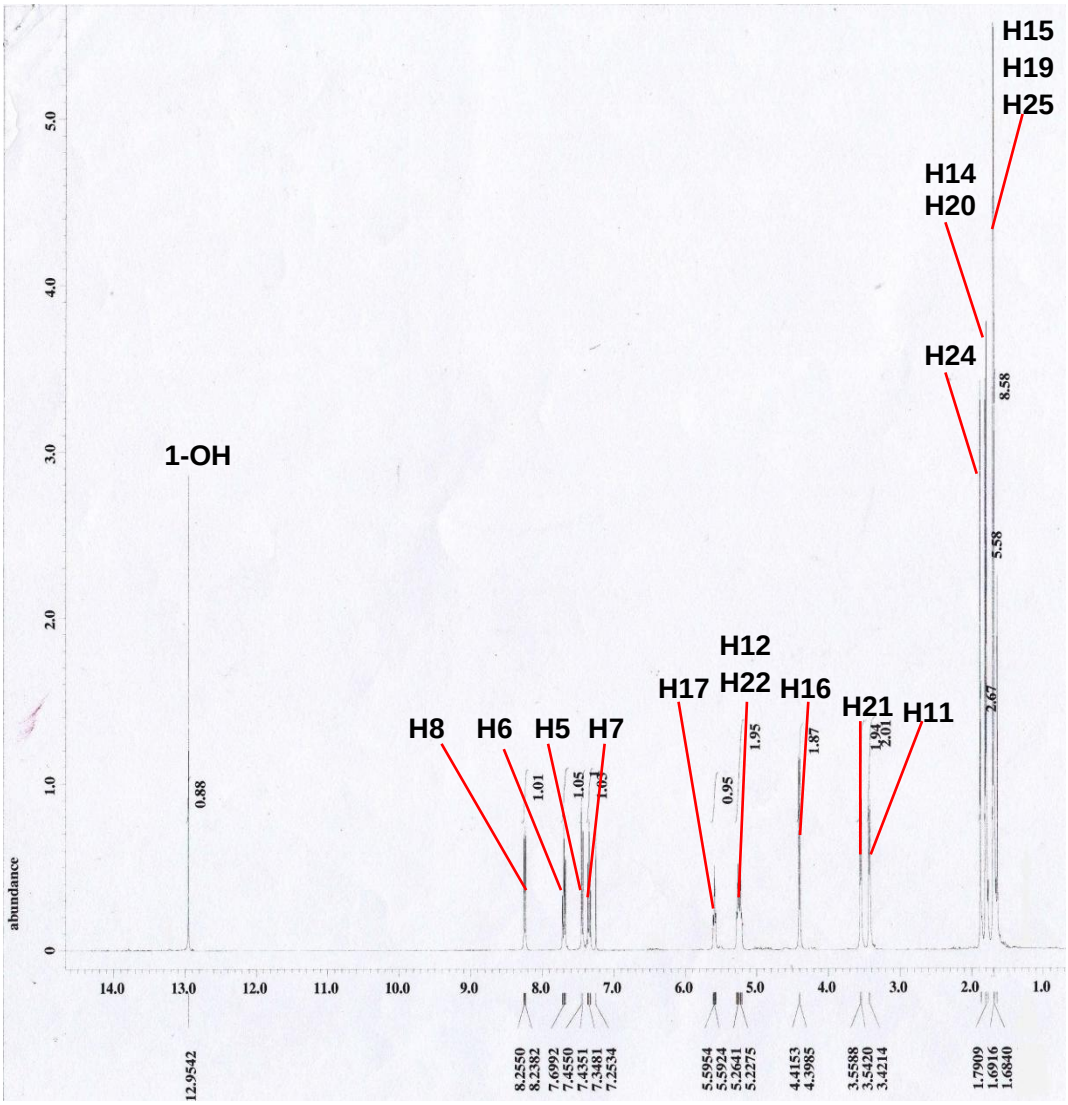
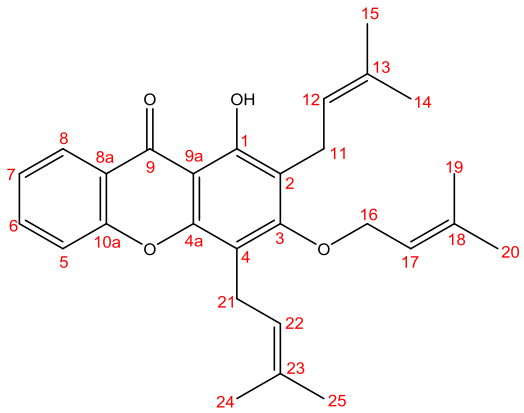


Figure 4.13: ^{1}H NMR spectrum of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, $CDCl_3$)



1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

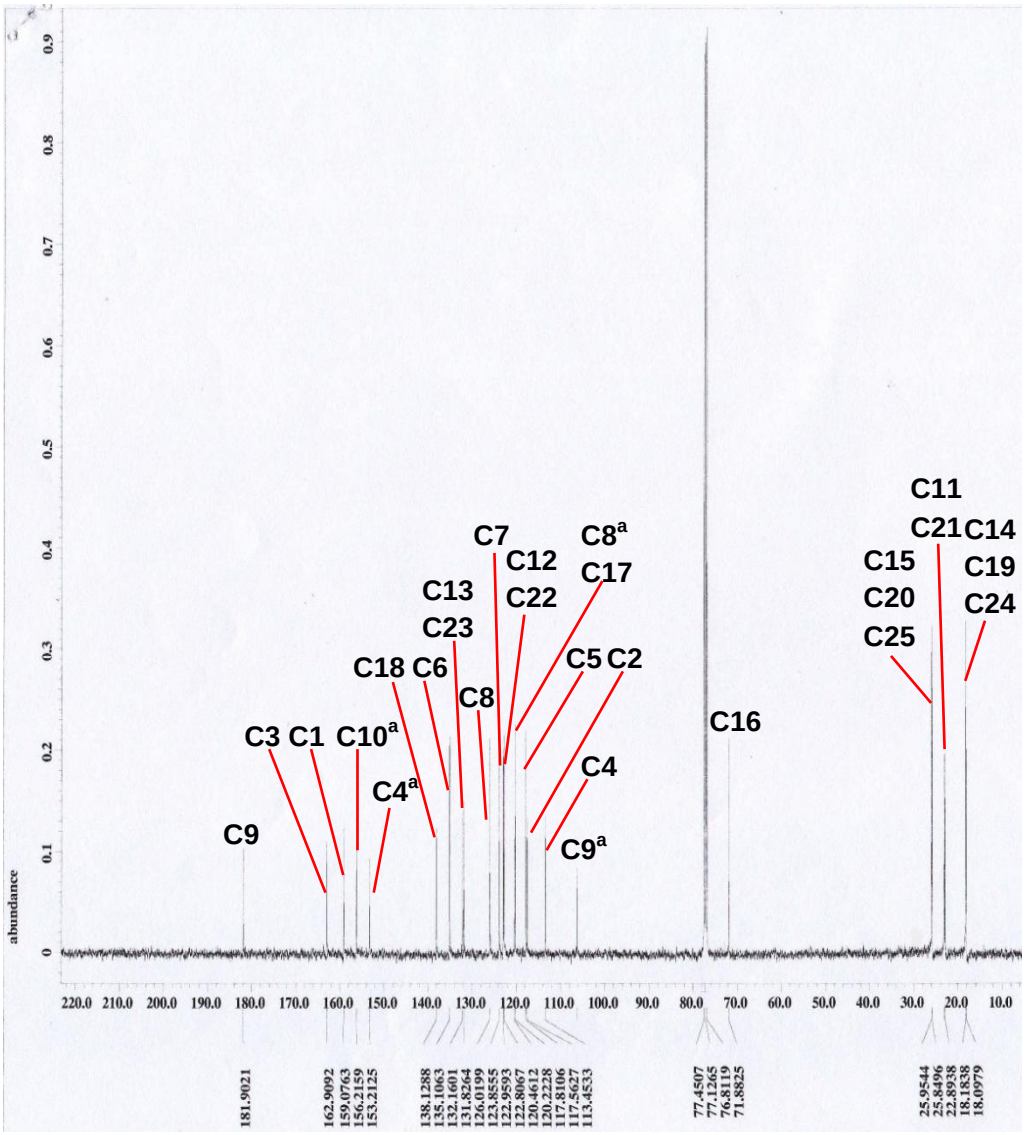
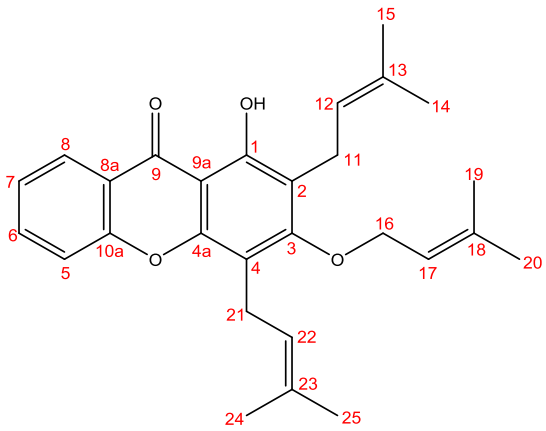


Figure 4.14: ^{13}C NMR spectrum of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, $CDCl_3$)



1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

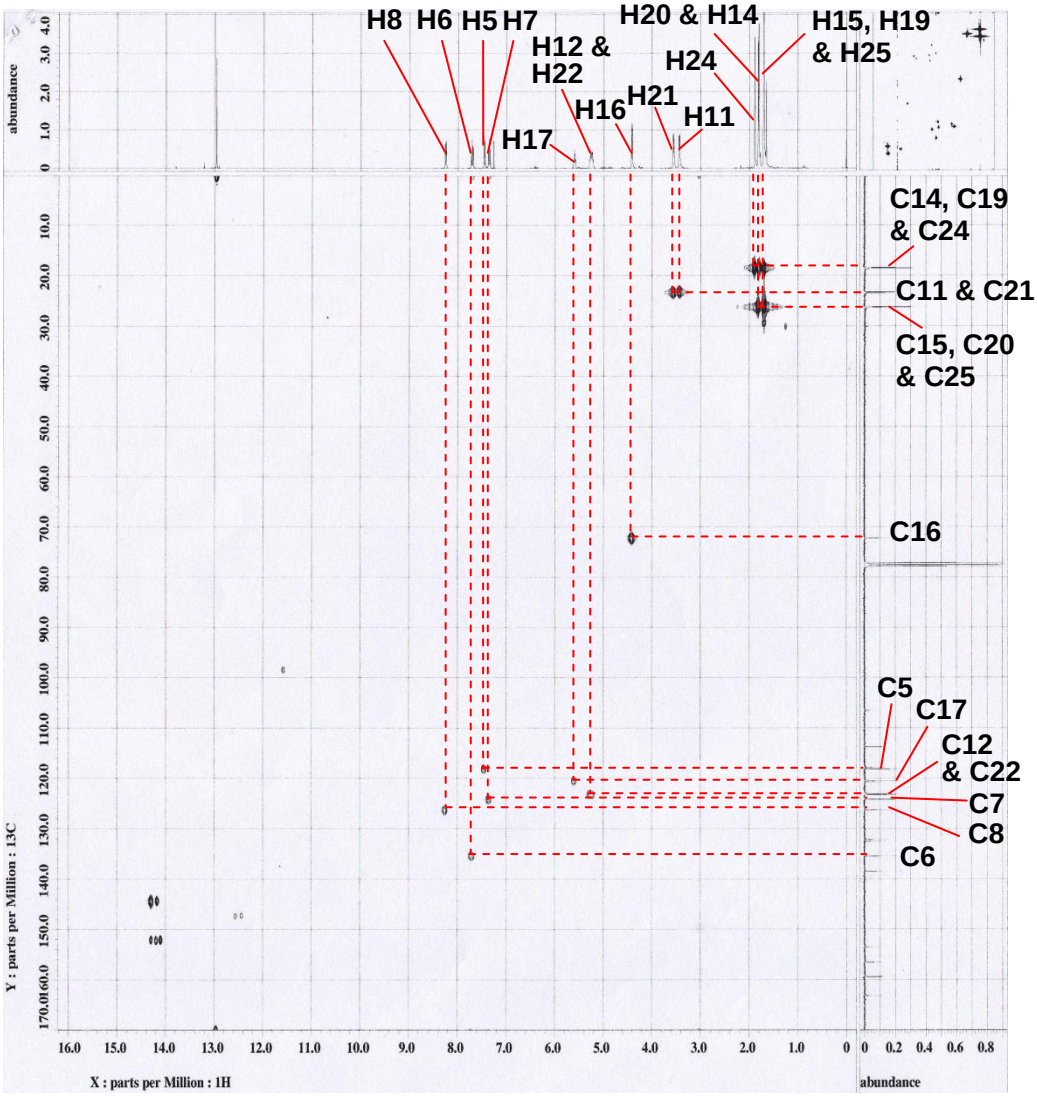
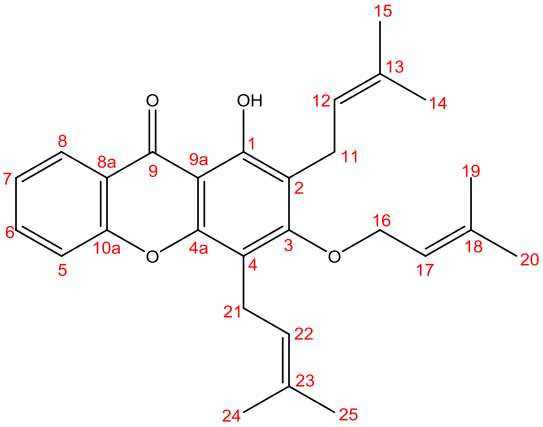


Figure 4.15: HMQC spectrum of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, $CDCl_3$)



1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

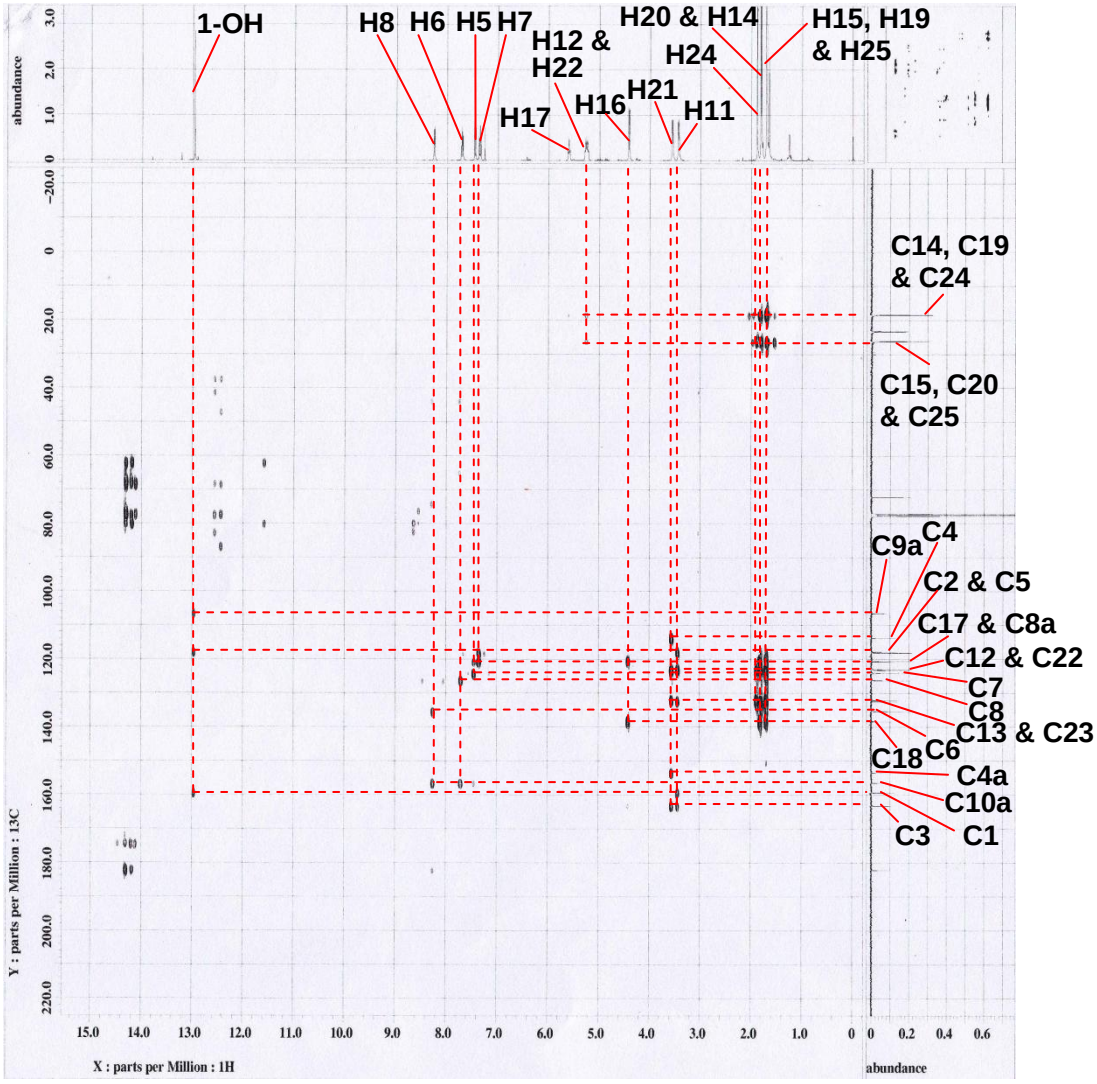
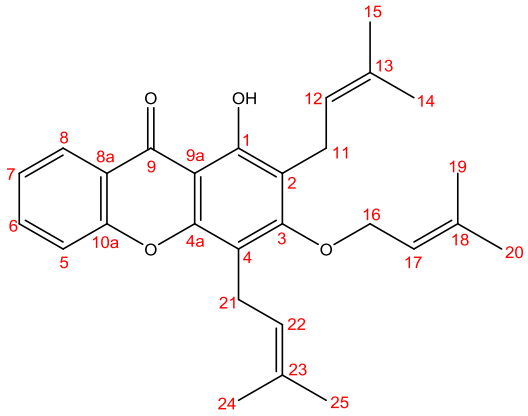


Figure 4.16: HMBC spectrum of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, $CDCl_3$)



1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

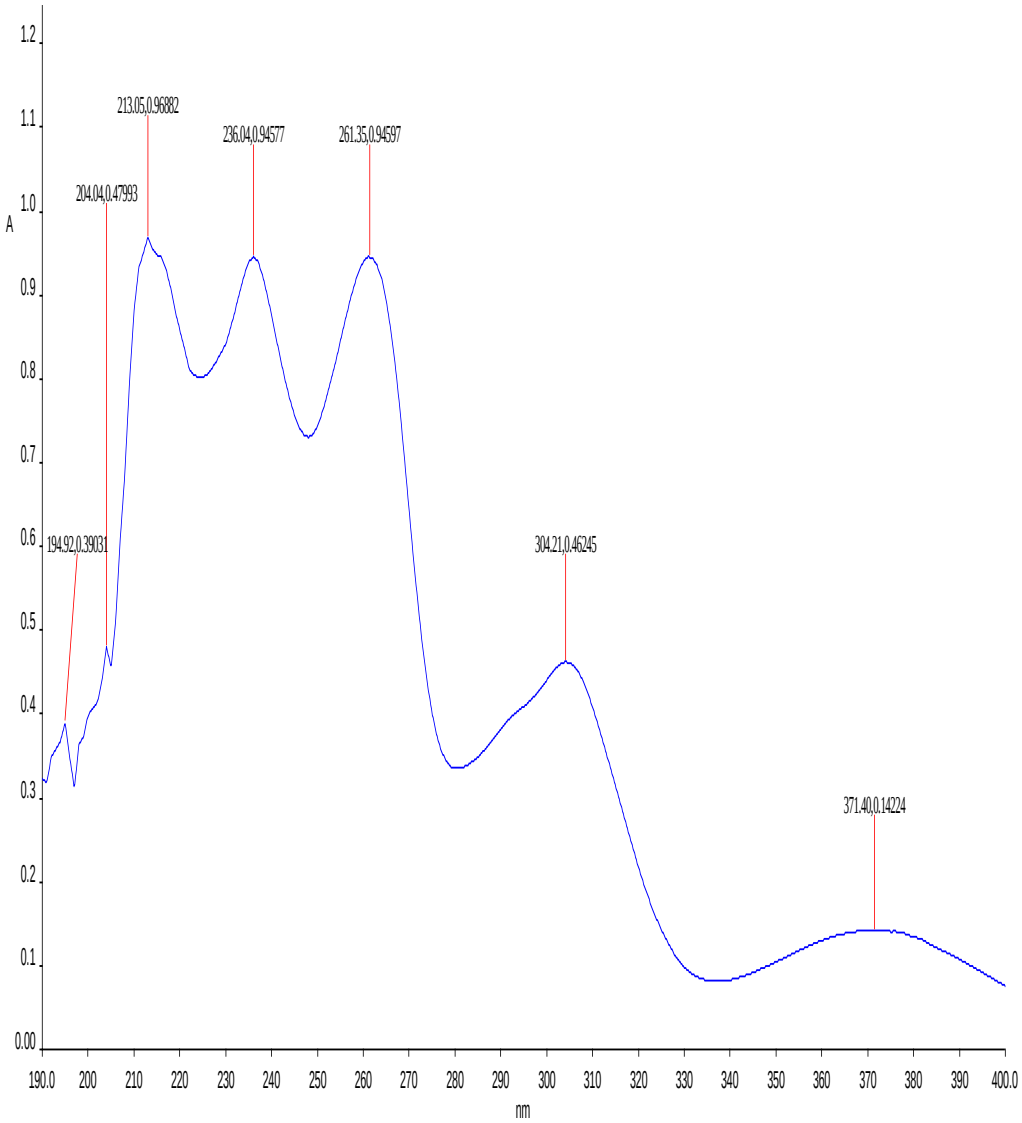


Figure 4.17: UV-Vis spectrum of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

4.2.3 Proposed Mechanism for Synthesis of 1-Hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione

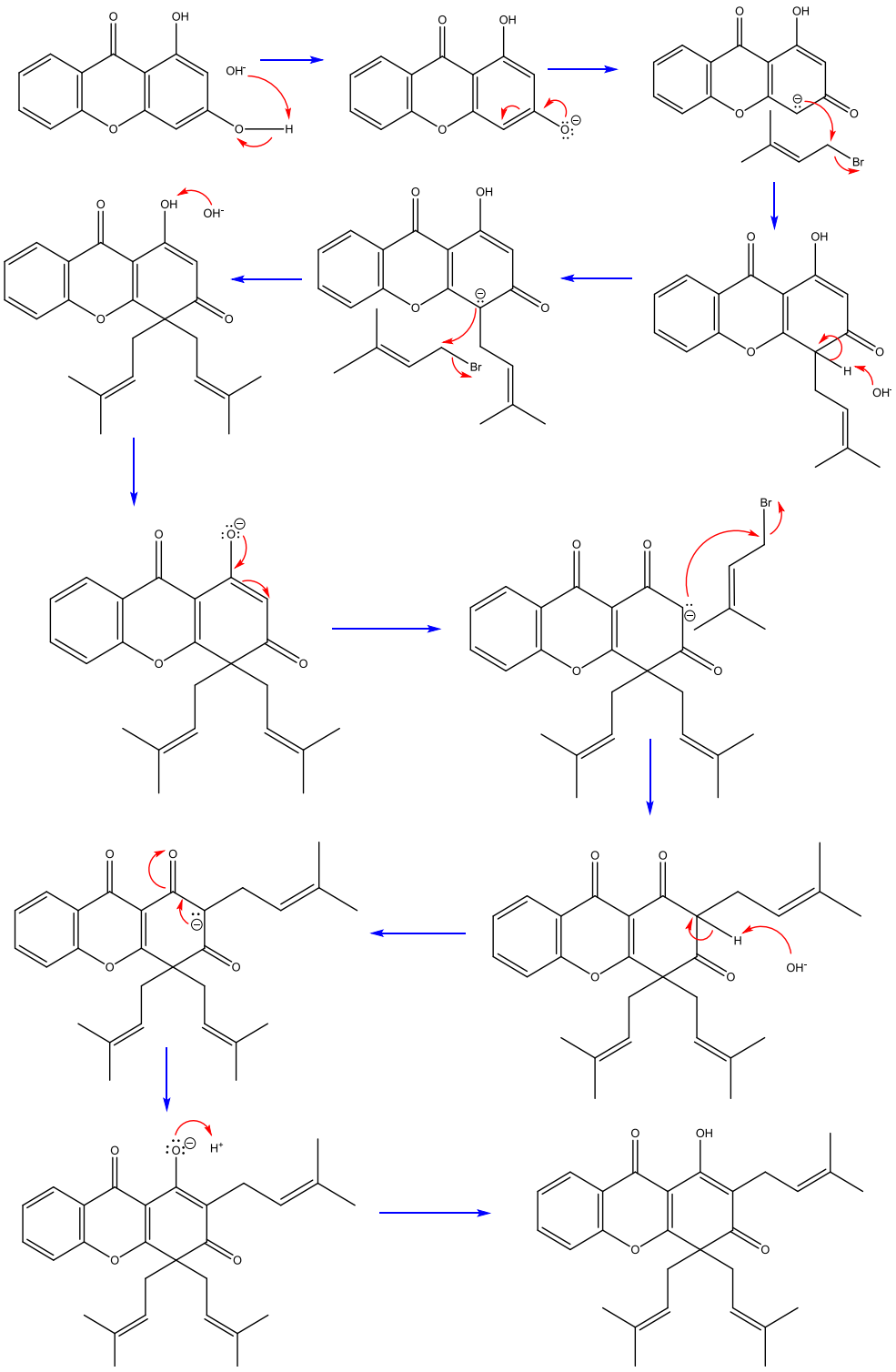


Figure 4.18: Proposed mechanism for synthesis of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione

4.2.4 Structural Elucidation of 1-Hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione

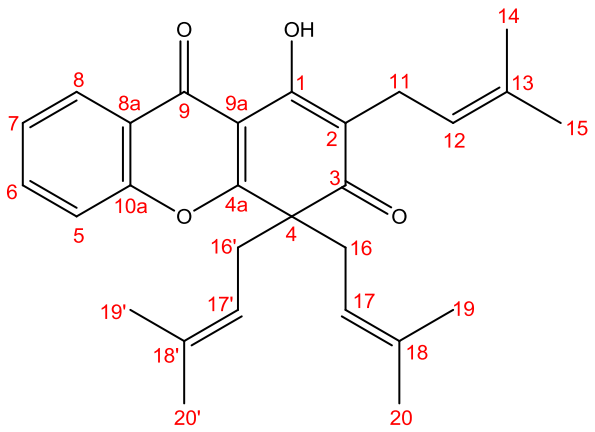


Figure 4.19: Structure of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (42)

By referring to the ^{1}H NMR spectrum (Figure 4.20), the disappearance of proton signals at C2 and C4, in contrast to that of compound **39**, had proven the existence of prenyl groups at these positions. The presence of a pair of doublet of doublets around δ 2.8 suggested the existence of magnetically equivalent methylene protons of the geminal diprenyl chains attached to the same carbon. On the other hand, the presence of doublet at δ 3.10 was due to the methylene protons of the third prenyl chain substituted in xanthone ring A.

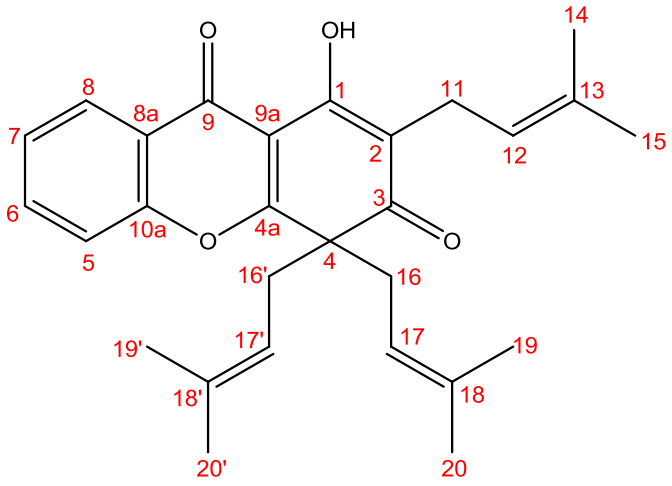
The respective positions of the prenyl groups were determined on the basis of HMBC correlation (Table 4.4). The prenyl methylene proton, H11 showed

linkages with carbons C1, C2 and C3 indicating the location of prenyl group at carbon C2. The ^{3}J correlations of H16 and H16' to both C3 and C4a confirmed the substitution of the diprenyl chains at C4.

The two carbonyl groups in the structure were confirmed by the observation of two downfield signals at δ 195.2 and 179.3 in the ^{13}C NMR spectrum (Figure 4.21). The characteristic signal of the sp^3 hybridised ring carbon C4 was observed at δ 57.8. The methyl carbons of the prenyl groups gave five signals at δ 18.1 (C19), 18.2 (C14), 19.1 (C19'), 26.1 (C20 and C20'), and 26.2 (C15). Meanwhile the protonated olefinic carbons showed signals at δ 122.5 (C12) and 117.6 (C17 and C17').

The structure of compound **42**, therefore can be proposed as 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione. This compound was proven to have a novel skeleton due to the distorted aromatic ring caused by the sp^3 hybridised carbon C4.

The absorption maxima at 261.91, 230.73, 294.35 and 322.88 nm, displayed in the UV-Vis spectrum (Figure 4.24), confirmed that compound **42** is highly conjugated.



1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**)

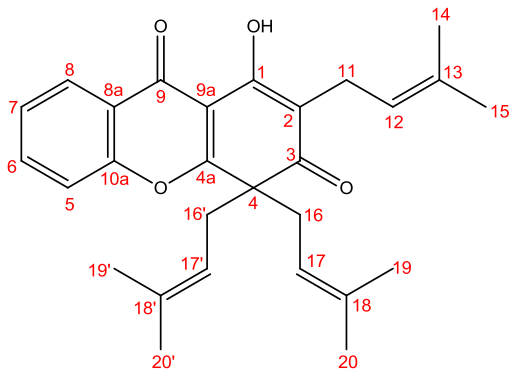
Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

Table 4.5: Summary of NMR data of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione

Position	δH (ppm)	δC (ppm)	HMBC	
			^2J	^3J
1	-	164.9	-	-
2	-	114.8	-	-
3	-	195.2	-	-
4	-	57.8	-	-
4a	-	175.5	-	-
5	7.56 (1H, d, J = 8.0 Hz)	118.3	C10a	C7, C8a
6	7.80 (1H, td, J = 8.0, 1.2 Hz)	135.8	-	C8, C10a
7	7.52 (1H, t, J = 8.0 Hz)	126.7	C8	C5, C8a
8	8.25 (1H, dd, J = 8.0, 1.2 Hz)	126.4	-	C9, C10a

8a	-		-	-
9	-	179.3	-	-
9a	-	110.9	-	-
10a	-	155.5	-	-
11	3.10 (2H, d, J = 8.0 Hz)	21.2	C2, C12	C1, C3, C13
12	5.10 (1H, t, J = 8.0 Hz)	122.5	-	C14, C15
13	-	131.9	-	-
14	1.74 (3H, s)	18.2	-	C15
15	1.65 (3H, s)	26.2	-	C14
16	2.75 (1H, dd, J = 13.4, 6.7 Hz) 2.88 (1H, dd, J = 13.4, 6.7 Hz)	39.3	C4, C17	C3, C4a, C18
17	4.71, (1H, t, J = 6.7 Hz)	117.6	C16	C19, C20
18	-	135.7	-	-
19	1.40 (3H, s)	18.1	-	C20
20	1.44 (3H, s)	26.1	-	C19
16’	2.88 (1H, dd, J = 13.4, 6.7 Hz) 2.75 (1H, dd, J = 13.4, 6.7 Hz)	39.3	C4, C17’	C3, C4a, C18’
17’	4.71, (1H, t, J = 6.7 Hz)	117.6	C16’	C19’, C20’
18’	-	135.7	-	-
19’	1.40 (3H, s)	19.1	-	C20’
20’	1.44 (3H, s)	26.1	-	C19’
1-OH	12.87 (OH, s)	-	C1	C2, C9a



1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

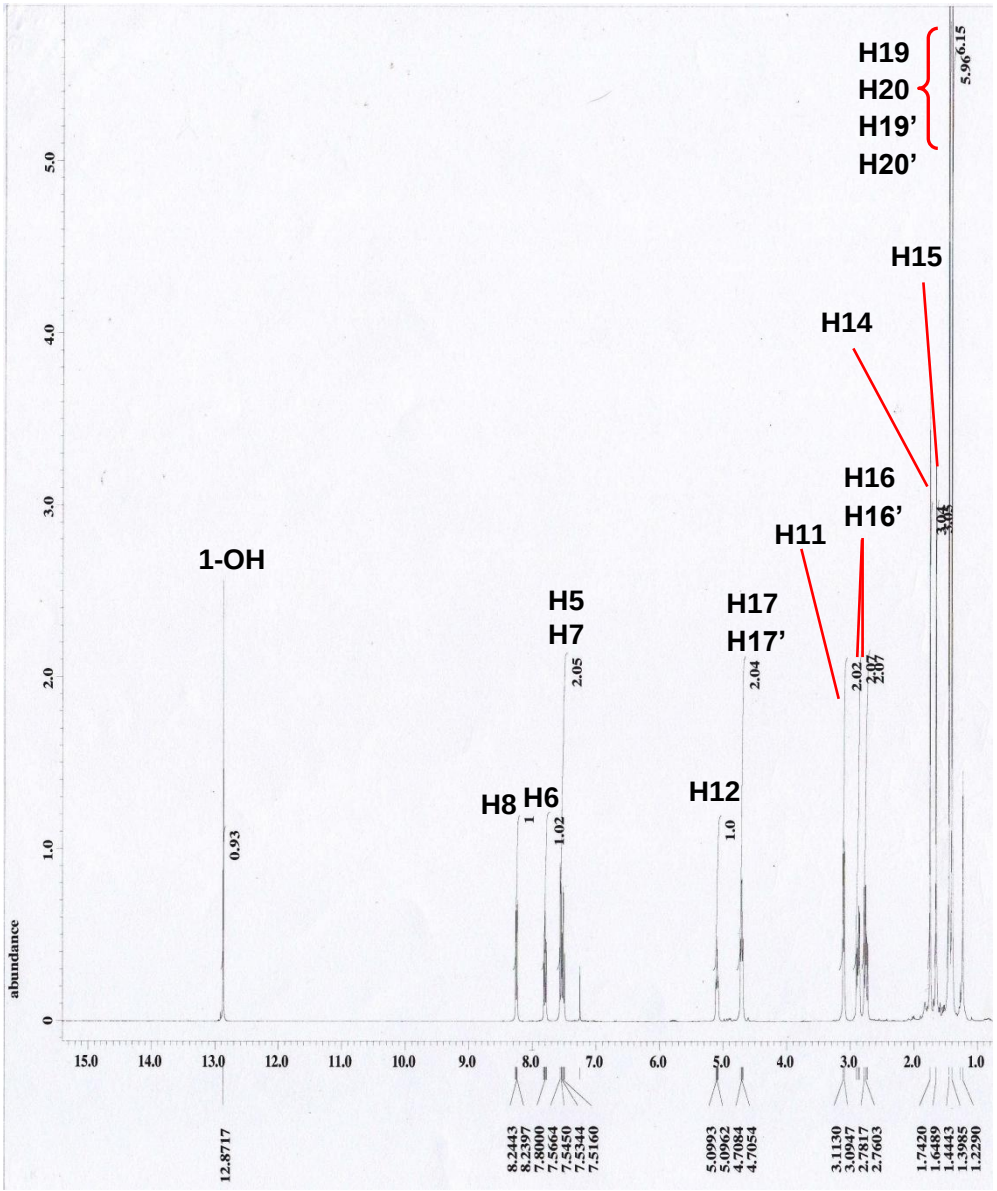
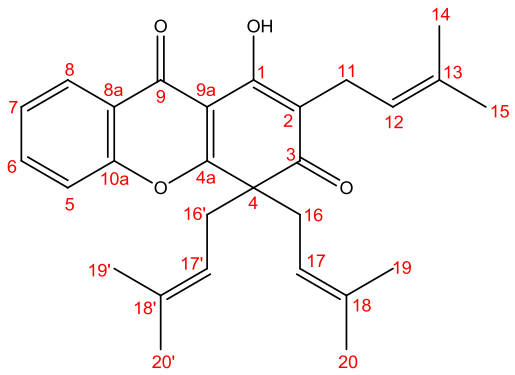


Figure 4.20: ^{1}H NMR spectrum of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (400 MHz, $CDCl_3$)



1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

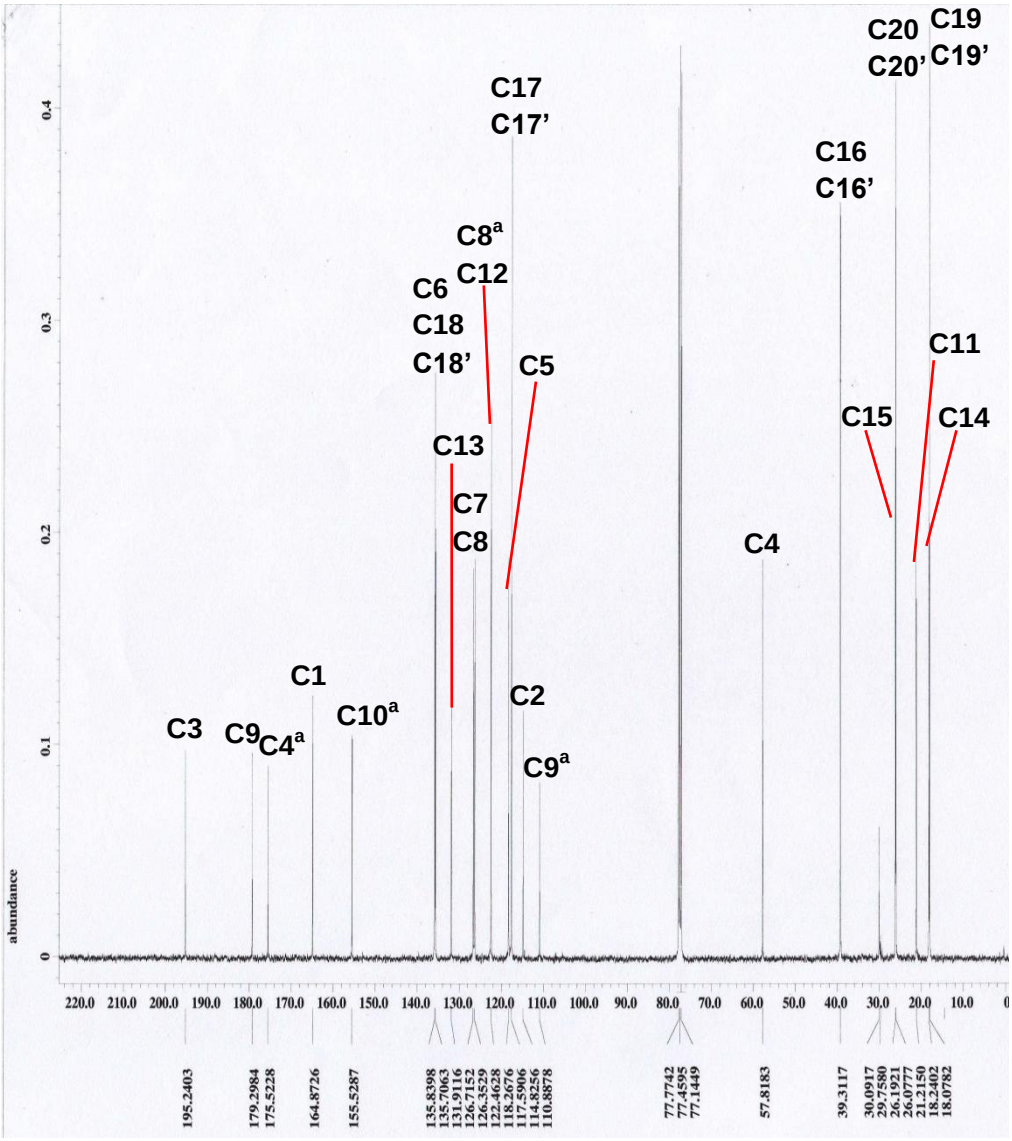
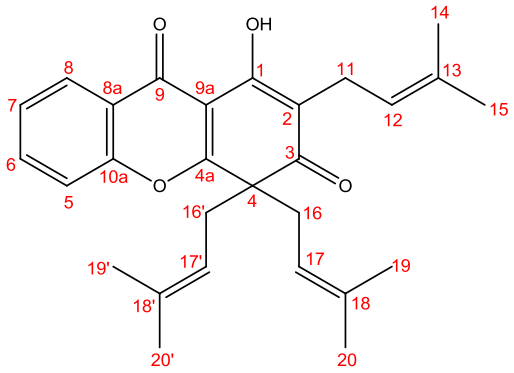


Figure 4.21: ^{13}C NMR spectrum of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (400 MHz, $CDCl_3$)



1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

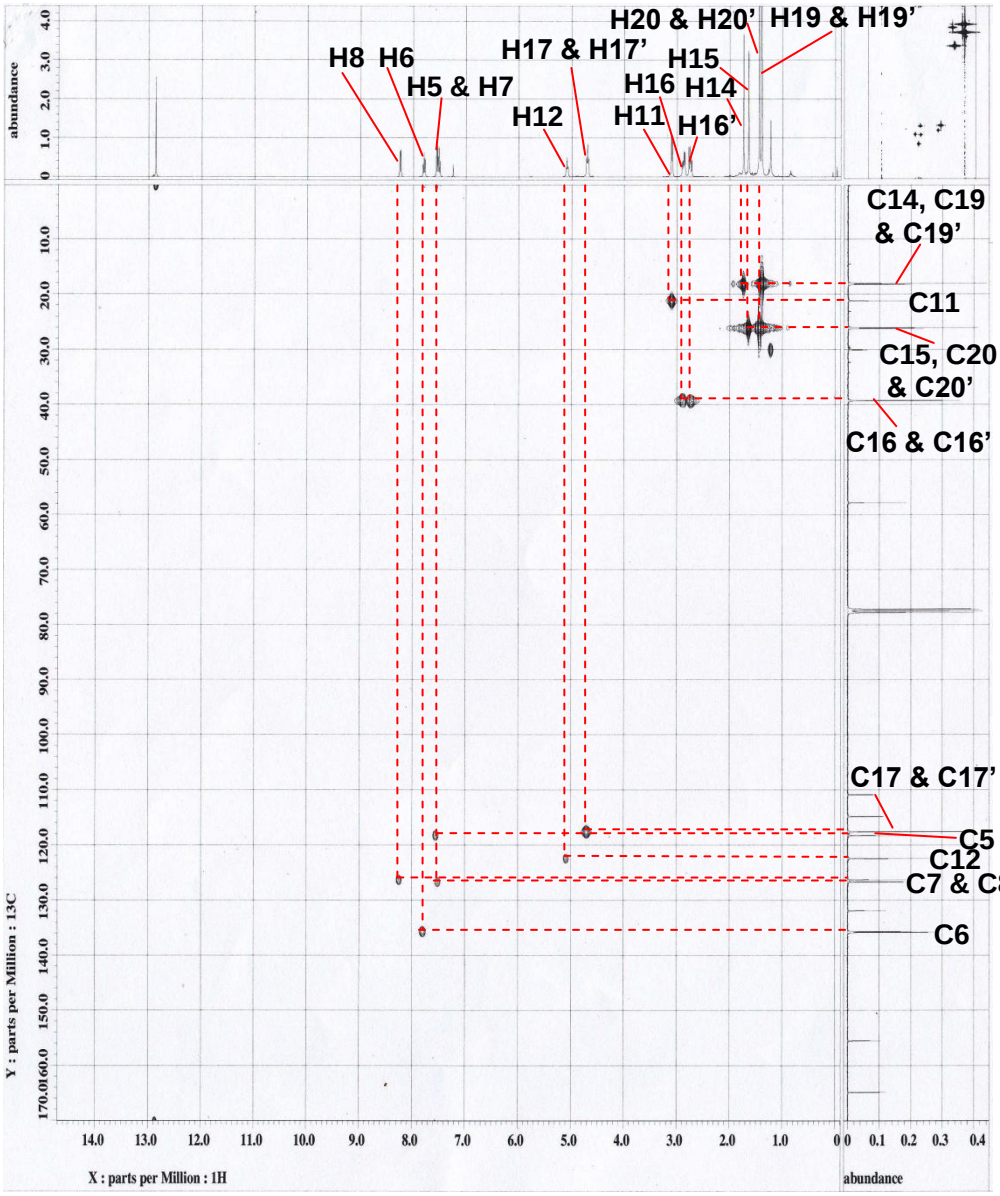
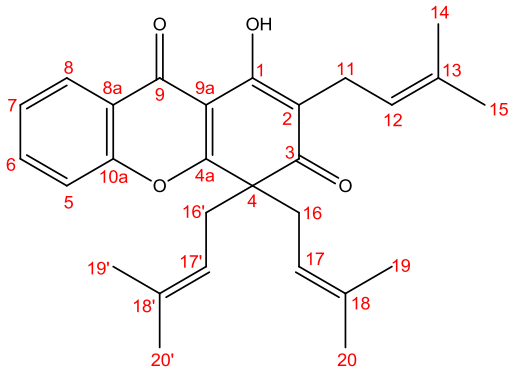


Figure 4.22: HMQC spectrum of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (400 MHz, $CDCl_3$)



1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

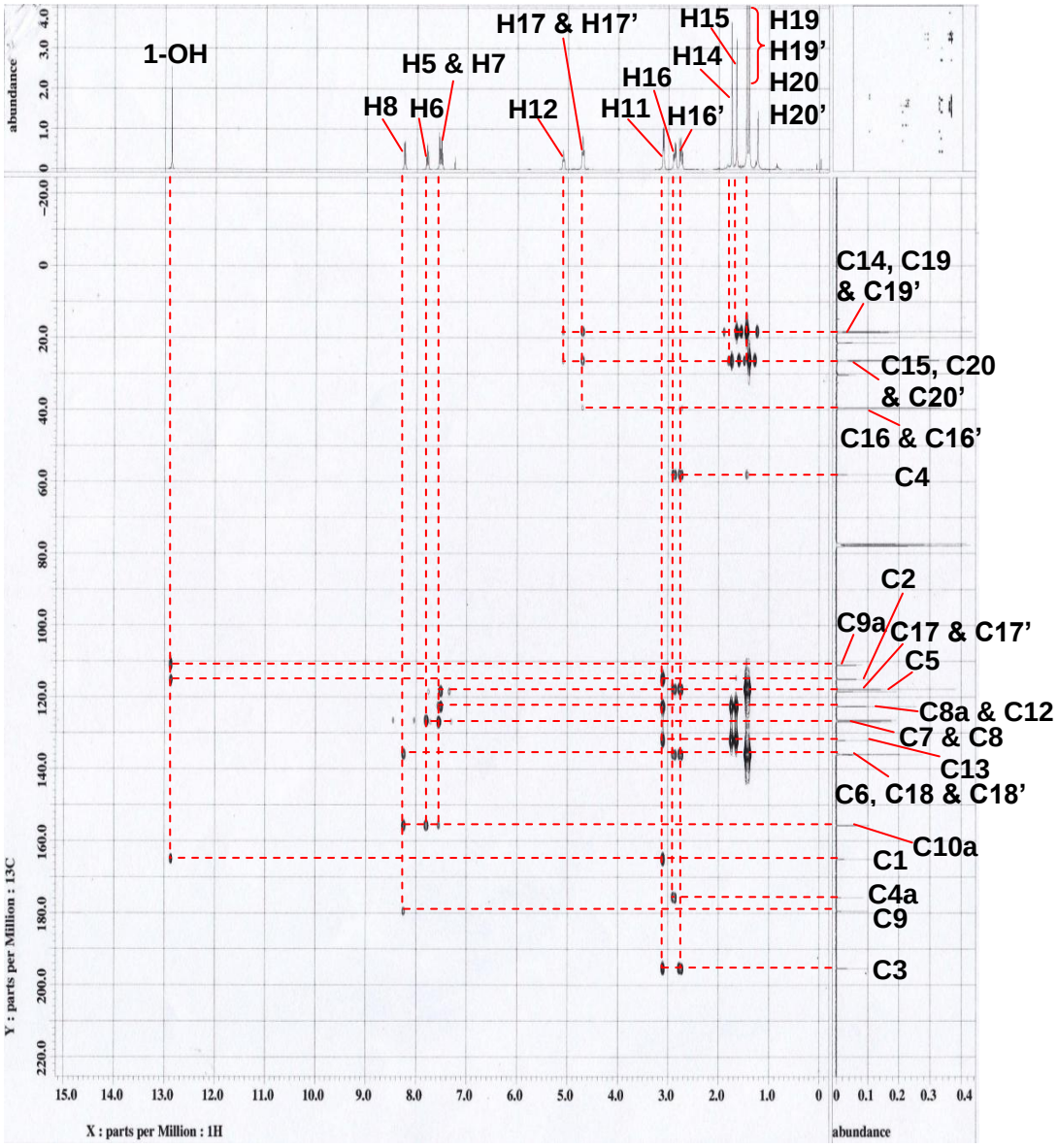
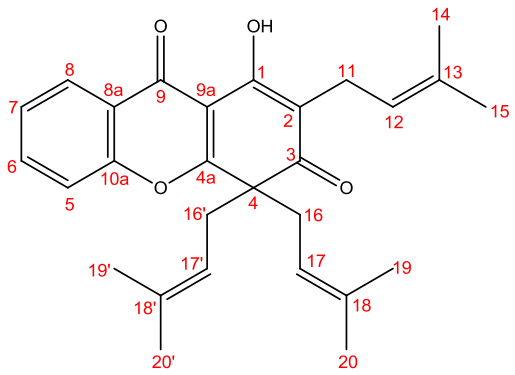


Figure 4.23: HMBC spectrum of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (400 MHz, $CDCl_3$)



1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**)

Molecular formula: $C_{28}H_{32}O_4$

Molecular mass: 432.56 $gmol^{-1}$

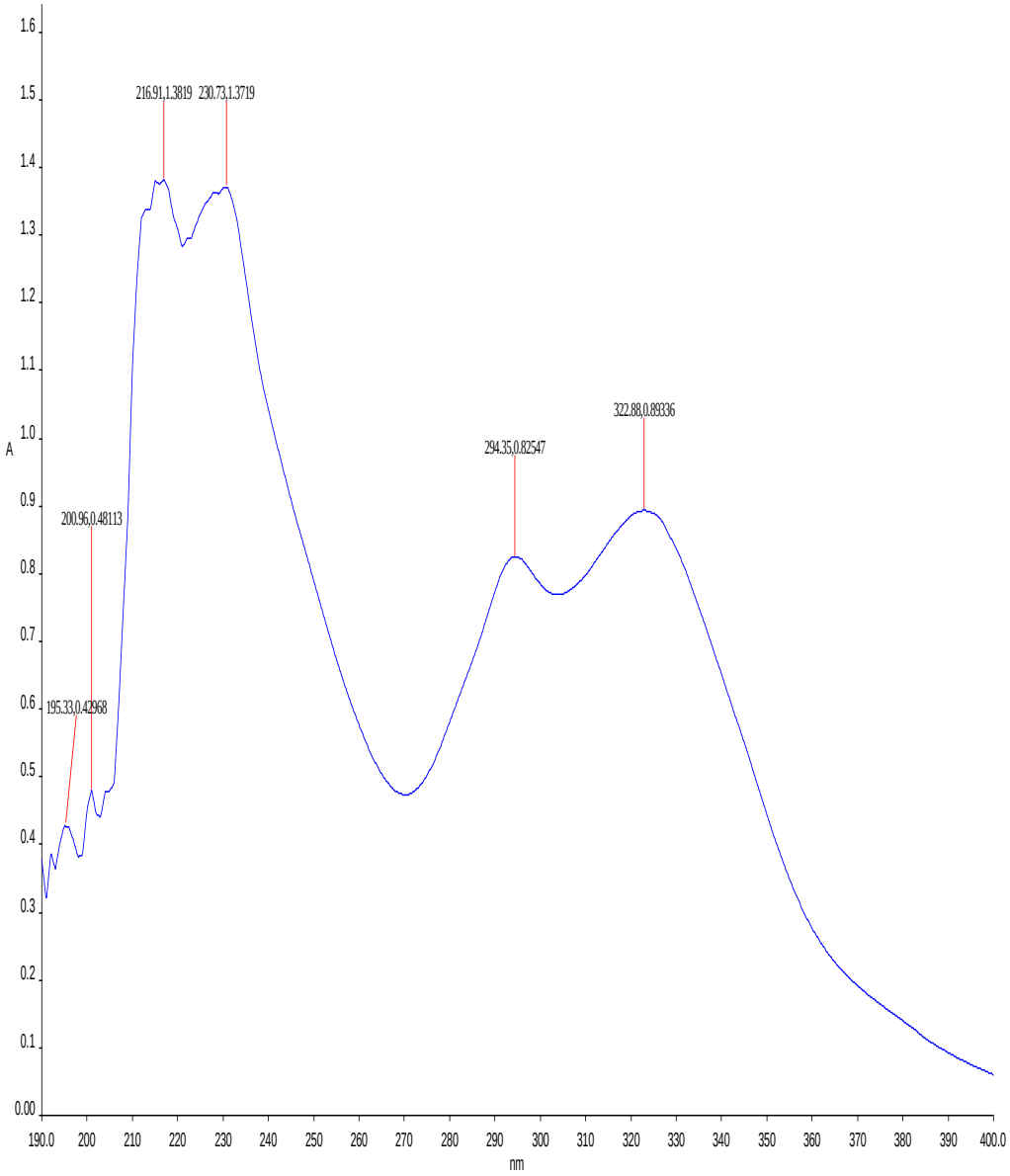


Figure 4.24: UV-Vis spectrum of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione

4.2.5 Proposed Mechanism for Synthesis of 1,3-Dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one

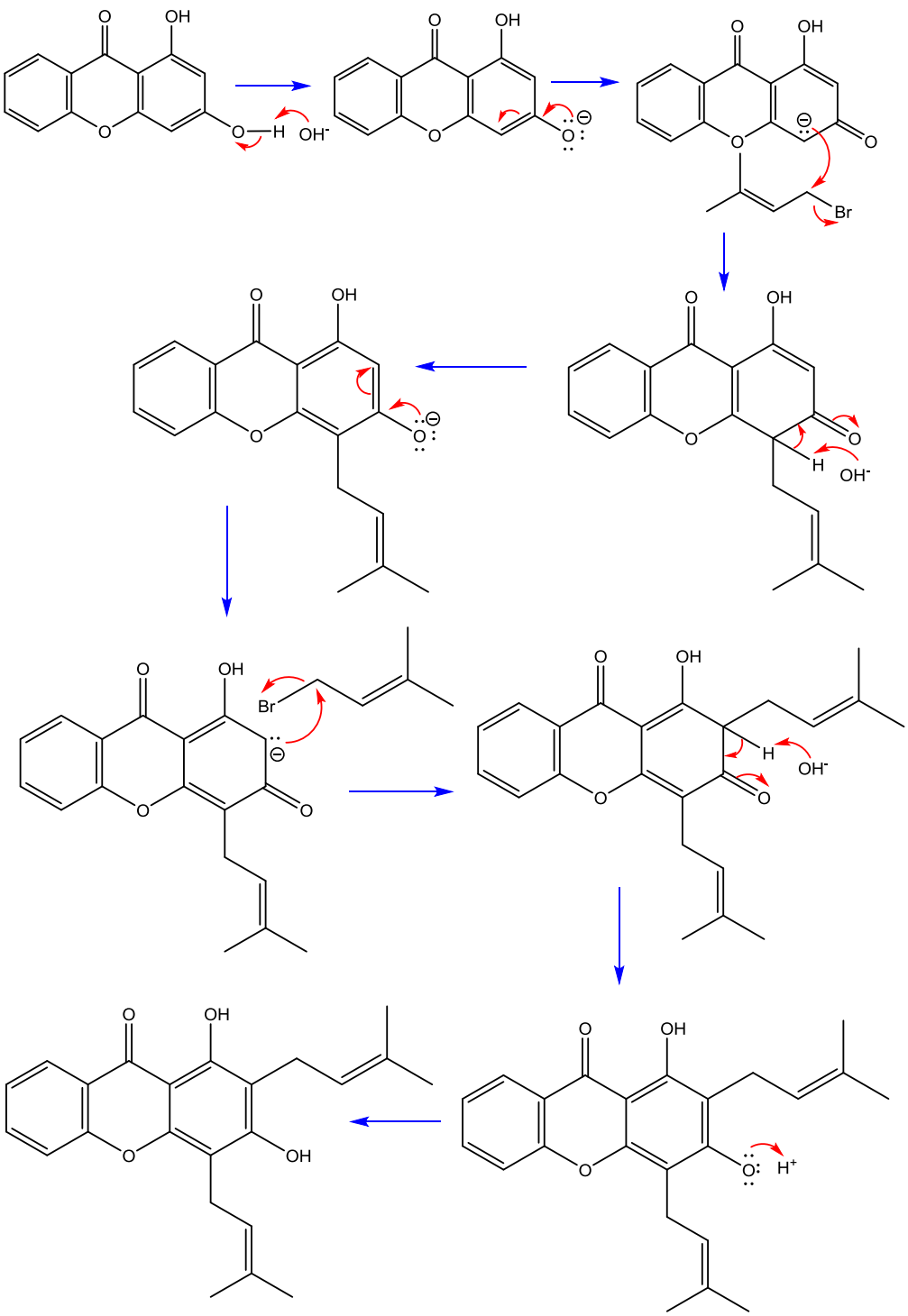


Figure 4.25: Proposed mechanism for the synthesis of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one

4.2.6 Structural Elucidation of 1,3-Dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one

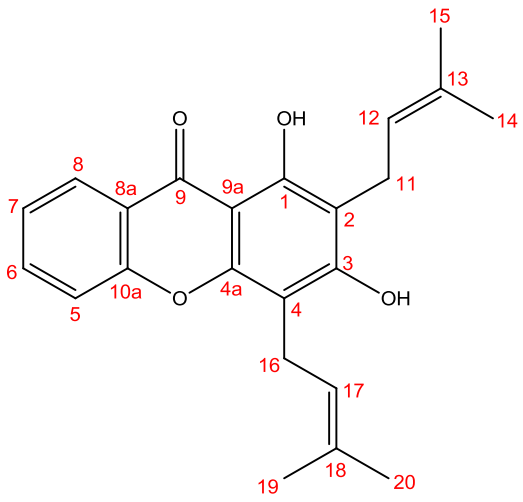


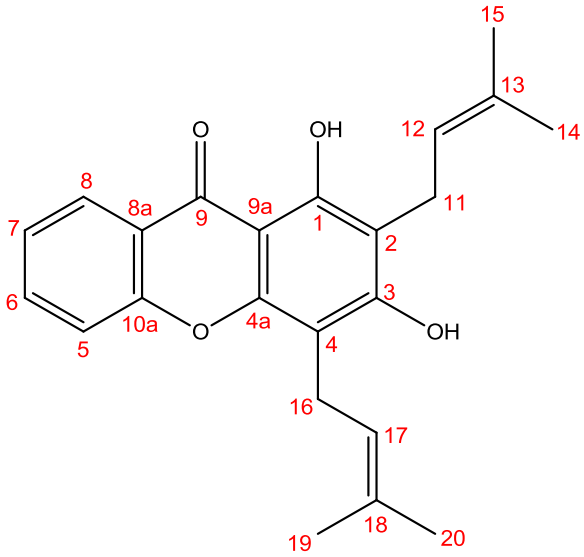
Figure 4.26: Structure of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (43)

In the ^{1}H NMR spectrum of **43** (Figure 4.27), a downfield singlet for chelated hydroxyl group was displayed at δ 13.18, indicating that the *peri*-hydroxyl group was not reacted during the prenylation. Instead, the disappearance of proton signals at C2 and C4, in compared to that of **39**, revealed the existence of carbo-prenylation at these two positions. The two doublets at δ 3.44 and 3.52 assigning to two groups of methylene protons suggested that the compound was diprenylated.

The ^{13}C NMR spectrum (Figure 4.28) showed three methyl carbon signals of prenyl groups at δ 18.1 (C14 and C19), 25.9 (C15) and 26.0 (C20). On the other hand, the methylene carbon signals of the prenyl groups were shown at δ 21.7 (C11) and 21.9 (C16).

The observed key HMBC correlations for the structure assignment are shown in Table 4.5. Linkages of proton H11 with carbons C1 and C3 indicated that the prenyl group was located at C2, whereas the correlation between proton H16 and carbons C3 and C4a established that another prenyl group was attached to carbon C4. Thereby, the complete structure of compound **43** was concluded as 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one.

The UV-Vis spectrum (Figure 4.31) displayed absorption bands at 216.94, 238.06, 259.52 and 316.61 nm. This confirmed that the compound **43** was highly conjugated.



1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**)

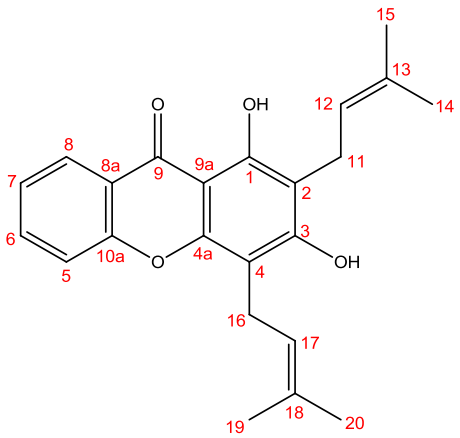
Molecular formula: $C_{23}H_{24}O_4$

Molecular mass: 364.44 $gmol^{-1}$

Table 4.6: Summary of NMR data of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one

Position	δH (ppm)	δC (ppm)	HMBC	
			^2J	^3J
1	-	158.6	-	-
2	-	109.0	-	-
3	-	161.0	-	-
4	-	105.5	-	-
4a	-	152.9	-	-
5	7.39 (1H, d, *J* = 8.0 Hz)	117.6	C10a	C7, C8a
6	7.65 (1H, td, *J* = 8.0, 1.2 Hz)	134.7	C5	C8, C10a
7	7.30 (1H, t, *J* = 8.0 Hz)	123.8	-	C5, C8a
8	8.20 (1H, dd, *J* = 8.0, 1.2 Hz)	125.9	-	C6, C9, C10a

8a	-	120.4	-	-
9	-	181.2	-	-
9a	-	103.5	-	-
10a	-	156.0	-	-
11	3.44 (2H, d, *J* = 6.7 Hz)	21.7	C2, C12	C1, C3, C13
12	5.29 (1H, t, *J* = 6.7 Hz)	121.6	C11	C14, C15
13	-	135.5	-	-
14	1.85 (3H, s)	18.1	C13	C12, C15
15	1.73 (3H, s)	25.9	C13	C12, C14
16	3.52 (2H, d, *J* = 6.7 Hz)	21.9	C4, C17	C3, C4a, C18
17	5.26 (1H, t, *J* = 6.7 Hz)	121.9	C16	C19, C20
18	-	133.8	-	-
19	1.88 (3H, s)	18.1	C18	C17, C20
20	1.77 (3H, s)	26.0	C18	C17, C19
1-OH	13.18 (OH, s)	-	C1	C2, C9a
3-OH	6.49 (OH, s)	-	C3	C2, C4



1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**)

Molecular formula: $C_{23}H_{24}O_4$

Molecular mass: 364.44 $gmol^{-1}$

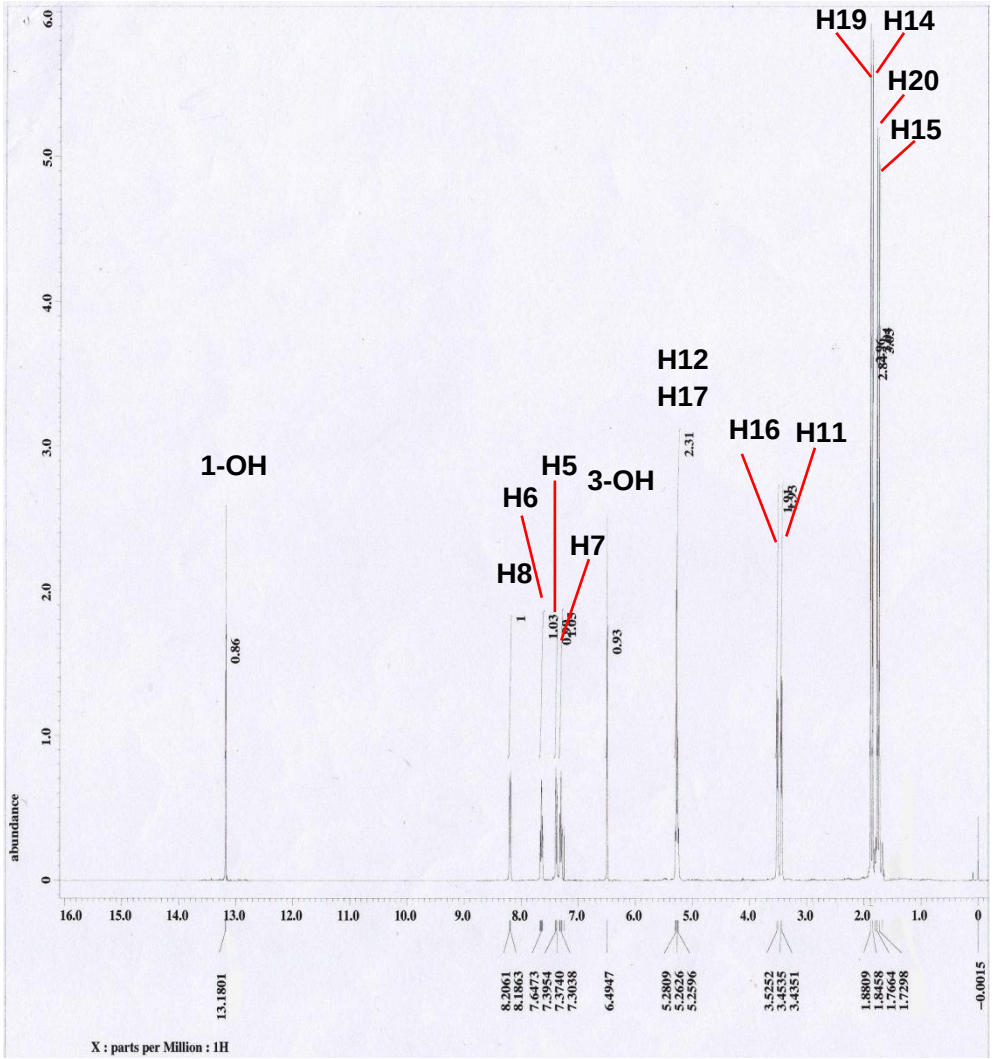
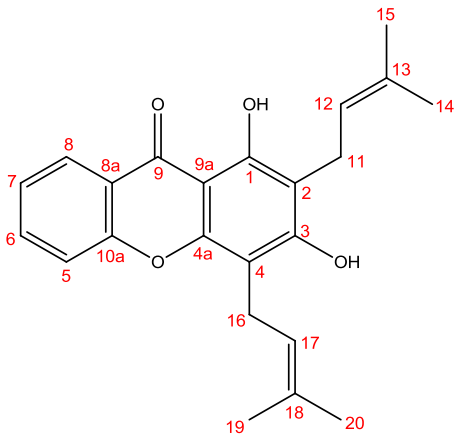


Figure 4.27: ^{1}H NMR spectrum of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (400 MHz, $CDCl_3$)



1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**)

Molecular formula: $C_{23}H_{24}O_4$

Molecular mass: 364.44 $gmol^{-1}$

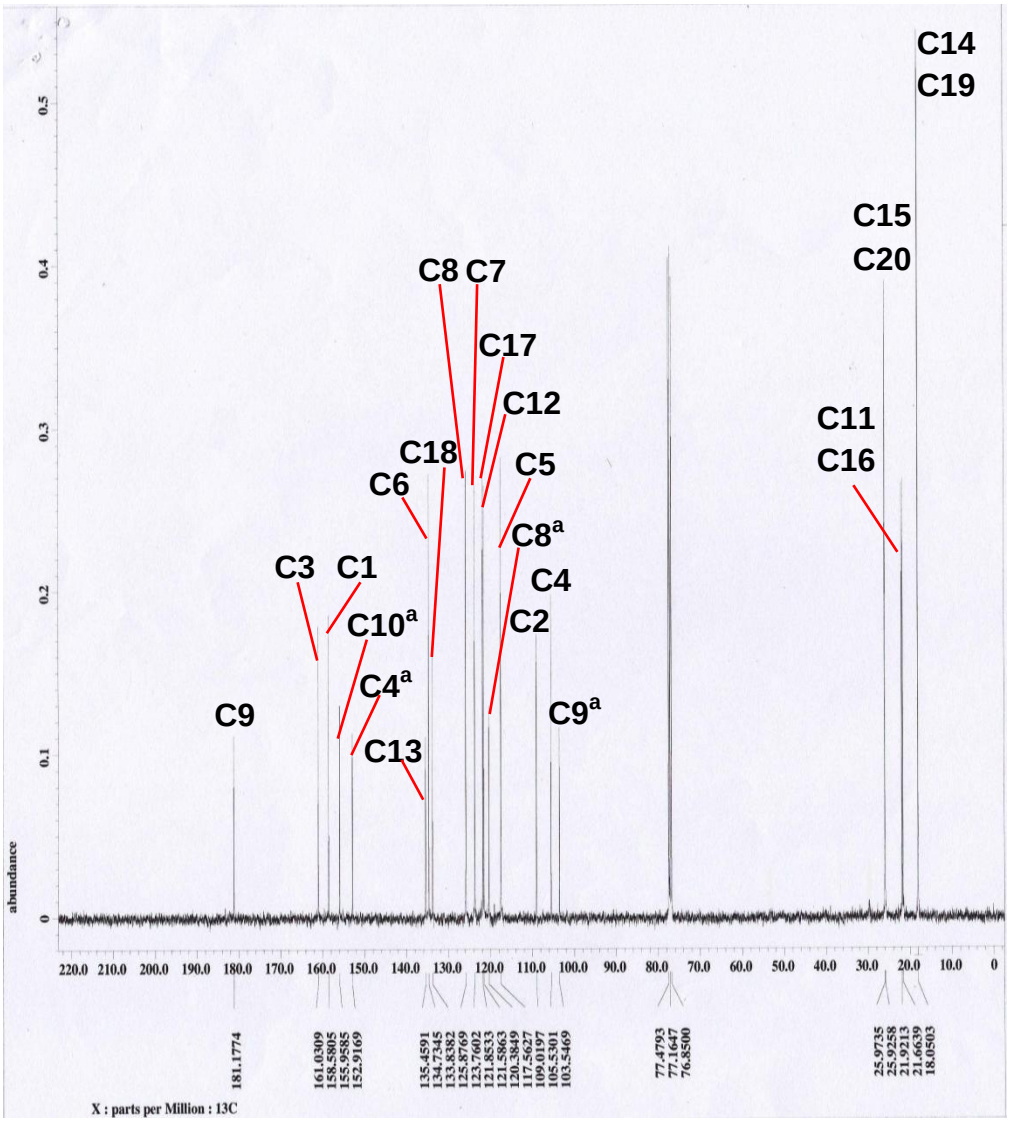
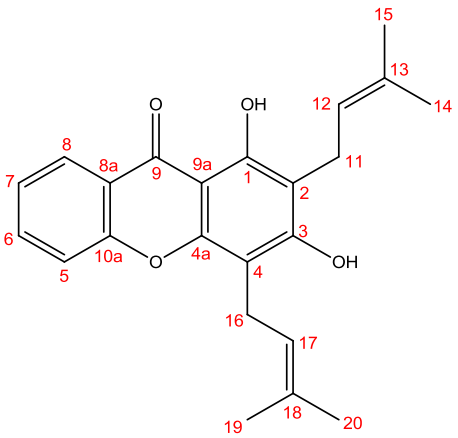


Figure 4.28: ^{13}C NMR spectrum of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (400 MHz, $CDCl_3$)



1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**)

Molecular formula: $C_{23}H_{24}O_4$

Molecular mass: 364.44 $gmol^{-1}$

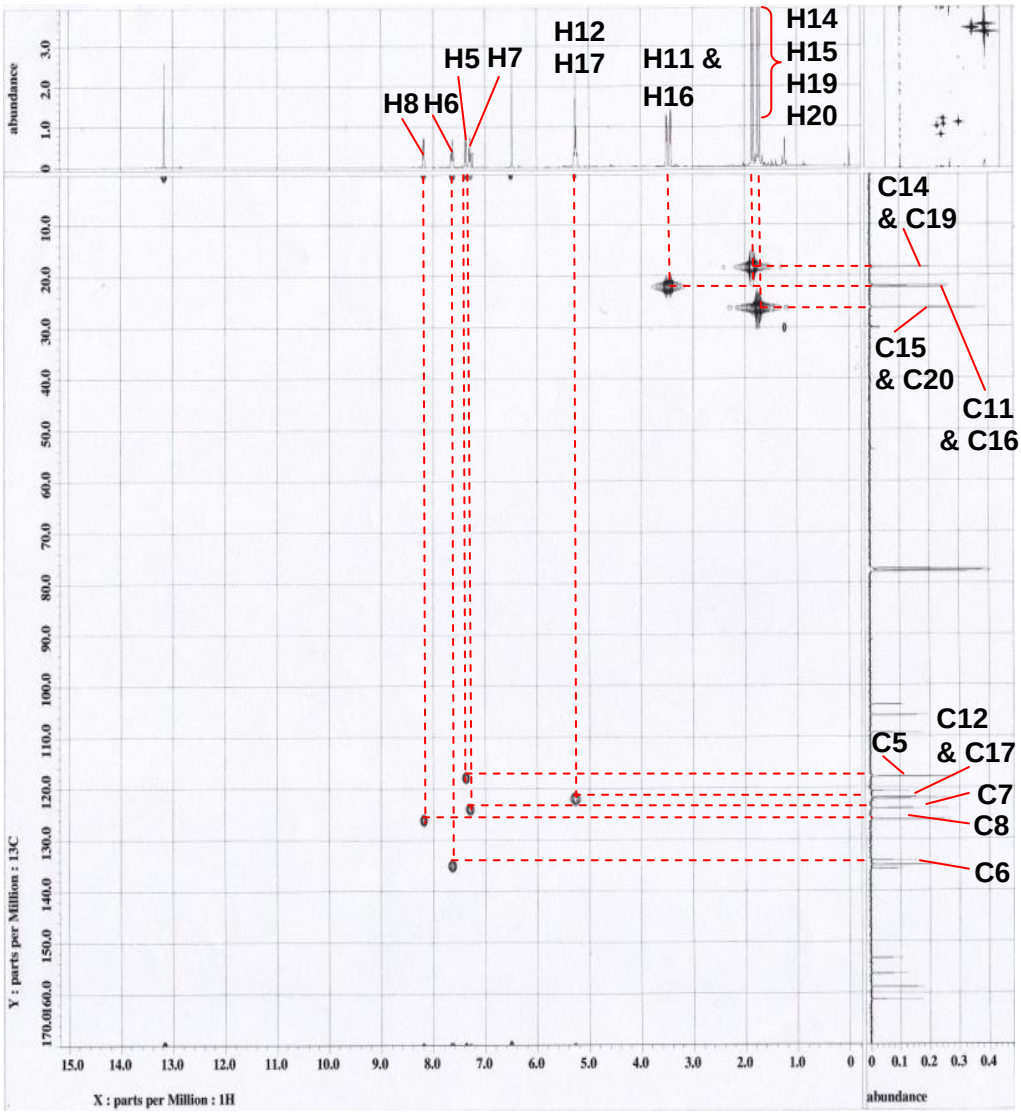
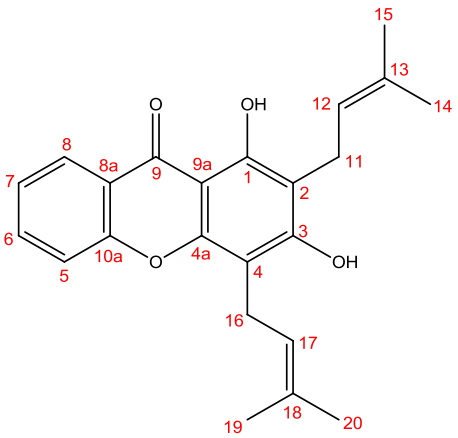


Figure 4.29: HMQC spectrum of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (400 MHz, $CDCl_3$)



1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**)

Molecular formula: $C_{23}H_{24}O_4$

Molecular mass: 364.44 $gmol^{-1}$

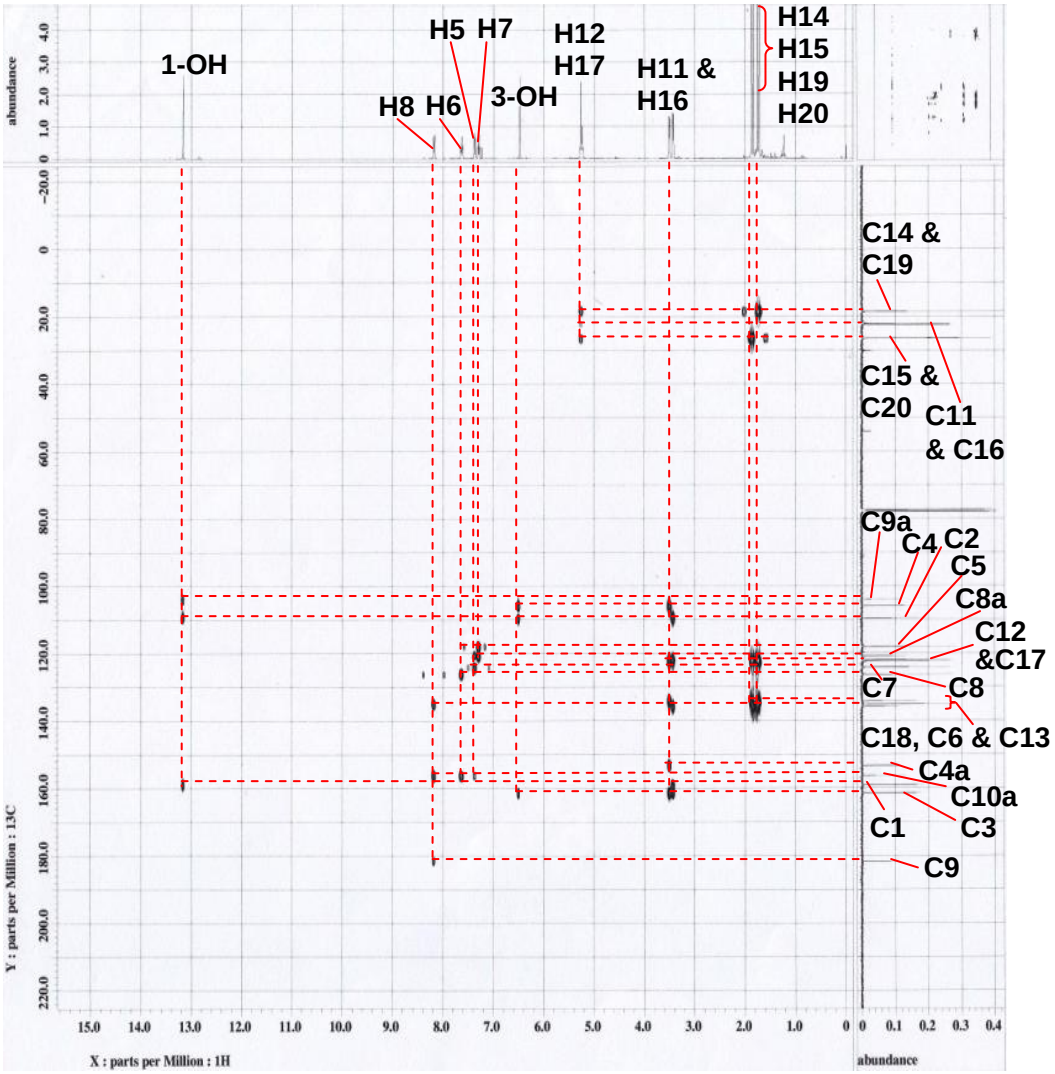
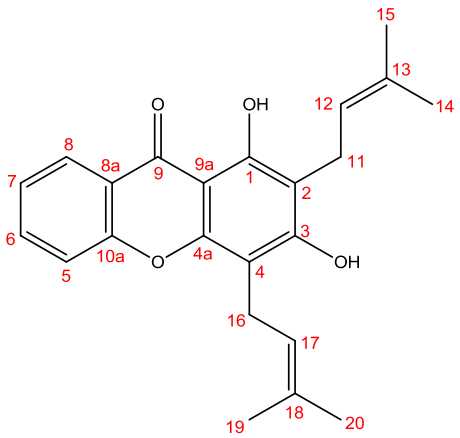


Figure 4.30: HMBC spectrum of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (400 MHz, $CDCl_3$)



1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**)

Molecular formula: $C_{23}H_{24}O_4$

Molecular mass: 364.44 $gmol^{-1}$

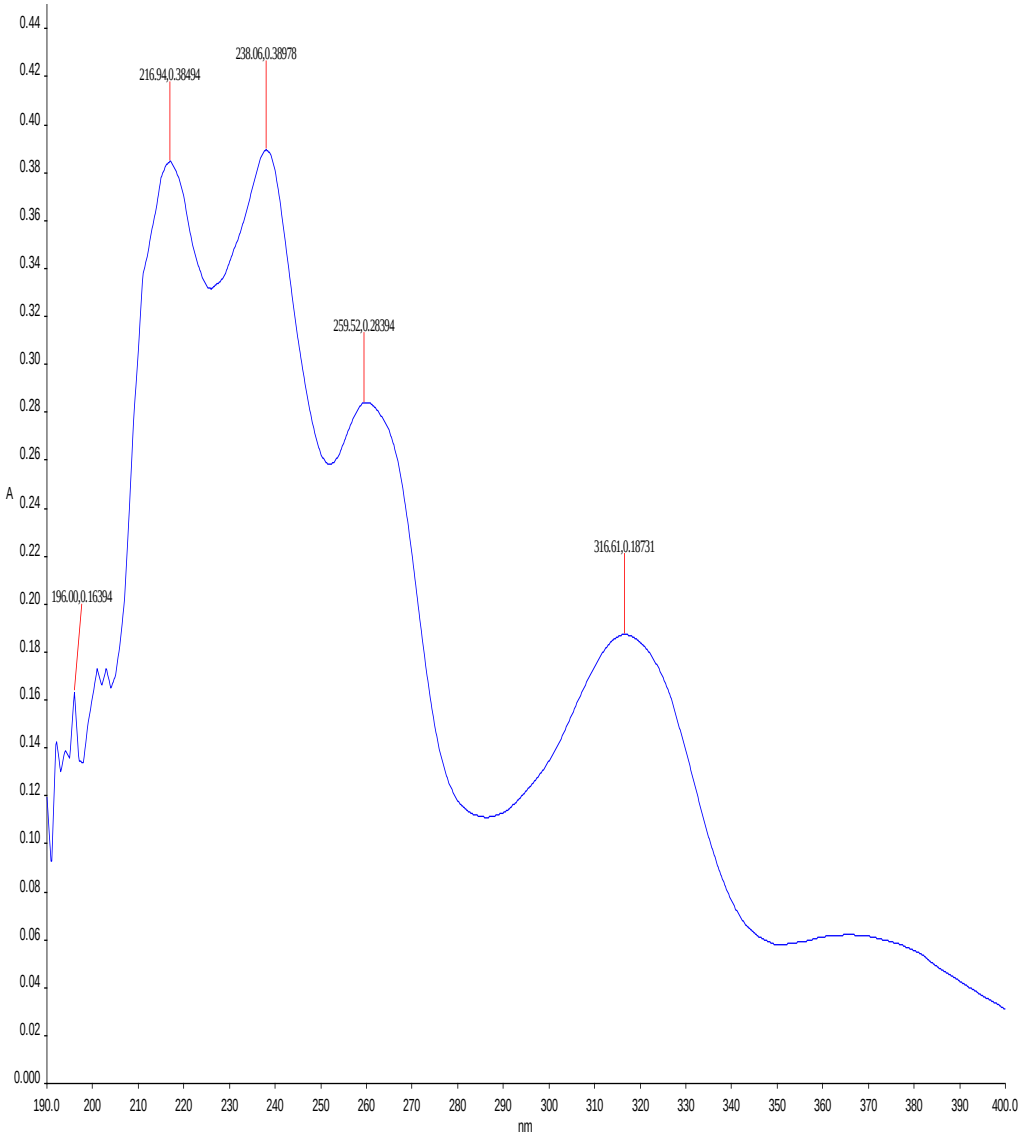


Figure 4.31: UV-Vis spectrum of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one

4.3 Antioxidant Assay

Phenolic compounds, such as benzoic acid derivatives have been extensively exploited because of their multiple biological activities, including antioxidant effects. Since they contain at least one hydroxyl-substituted aromatic ring system, they can form chelate complexes with metal ions and are easily oxidized, as well as serving important units for donating electrons (Rice-Evans, Miller & Paganga, 1997). Research has shown that the majority of phenolic compounds in the fruit hull of *G. mangostana* belong to the xanthone family, may help to offset chronic diseases related with ROS.

The isolated xanthones were screened in view of their antioxidant activities. They were examined with DPPH (1,1-diphenyl-2-dipicrylhydrazyl), of which DPPH is widely used for assessing the ability of polyphenols to transfer labile H-atoms to radicals. The IC_{50} values (effective concentration leading to a 50% loss of DPPH radical activity) were obtained by linear regression analysis of the dose response curves, which were plots of percentage radical scavenging versus concentration depicted in Figures 4.32, 4.33, 4.34, 4.35, 4.36 and 4.37.

The dihydroxyl substitutions at C1 and C3 of compound **43** was consistent with its elicited antioxidant effect (IC_{50}= 85 μg/mL). Although the DPPH radical scavenging ability of **43** was weaker than that of ascorbic acid (IC_{50}= 5

μg/mL) and kaempferol (IC_{50}= 10 μg/mL), it was evident that **43** did show proton-donating ability on DPPH radical to form DPPHH molecules. Unexpectedly, compound **39** with two hydroxyl group as well did not show noticeable antioxidant activity.

Meanwhile compounds **41** and **42** did not show significant antioxidant activities although they both possess a hydroxyl group at C1. From the result it was suggested that the chelating effect between the hydroxyl group and the adjacent carbonyl group has weakened the radical scavenging activities of the compounds. The electron-withdrawing induction effect of the carbonyl group has retarded the homolytic cleavage of the O-H bond.

Table 4.7: Free radical scavenging activities of the test compounds

Compound	**IC_{50} (μg/mL)**
1,3-dihydroxyxanthone (**39**)	> 200
1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**)	> 200
1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**)	>200
1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**)	85
Ascorbic acid (Vitamin C)	5
Kaempferol	10

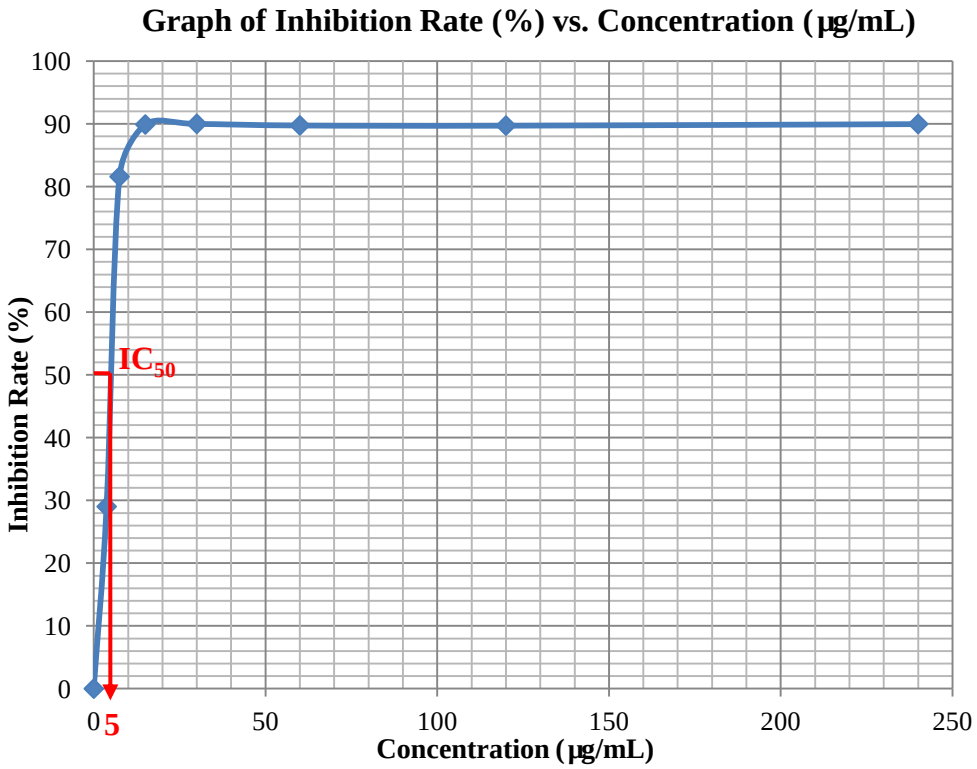


Figure 4.32: Graph of inhibition rate (%) vs. concentration (µg/mL) of ascorbic acid

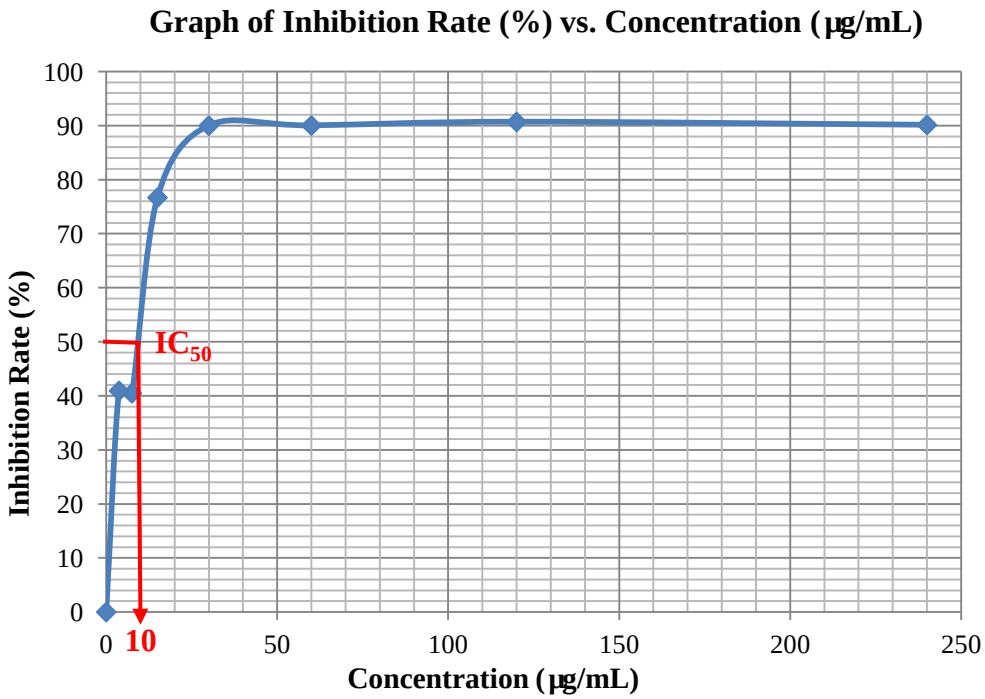


Figure 4.33: Graph of inhibition rate (%) vs. concentration (µg/mL) of kaempferol

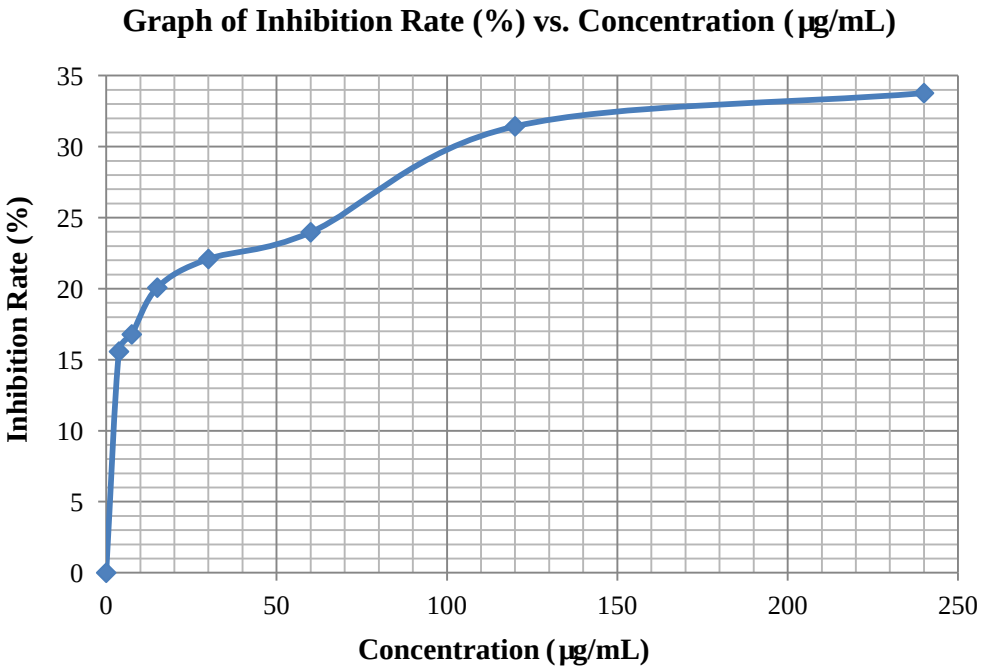


Figure 4.34: Graph of inhibition rate (%) vs. concentration (µg/mL) of 1,3-dihydroxyxanthone (39)

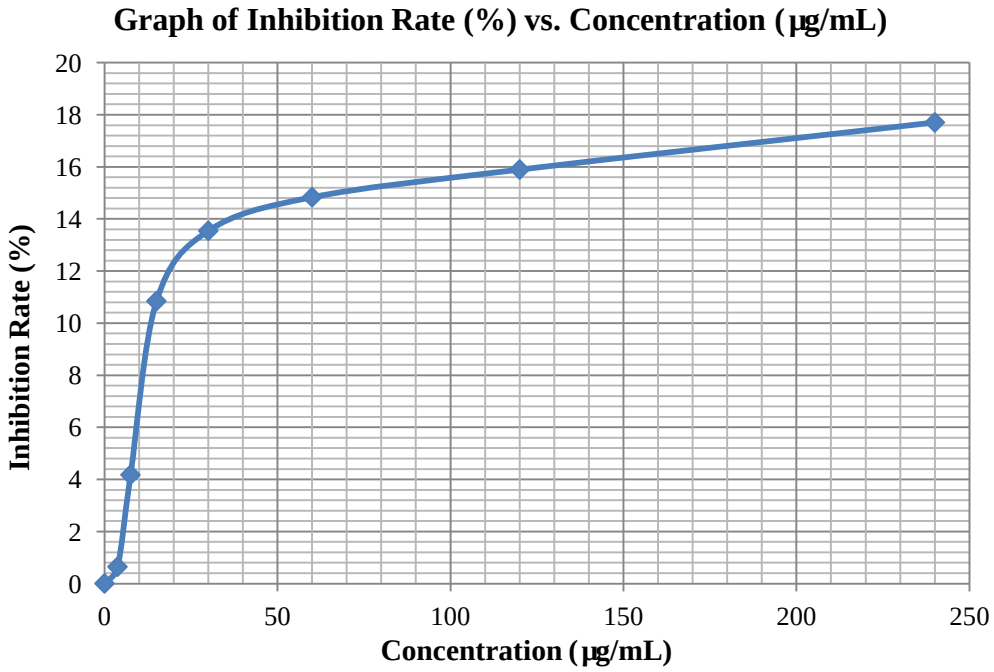


Figure 4.35: Graph of inhibition rate (%) vs. concentration (µg/mL) of 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (41)

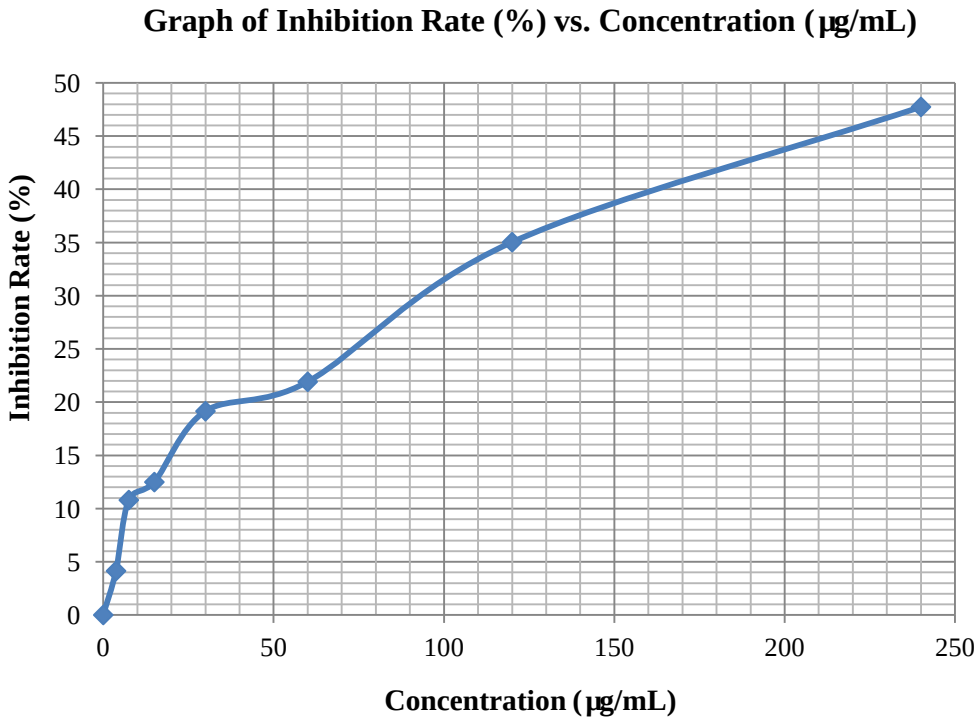


Figure 4.36: Graph of inhibition rate (%) vs. concentration (µg/mL) of 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (42)

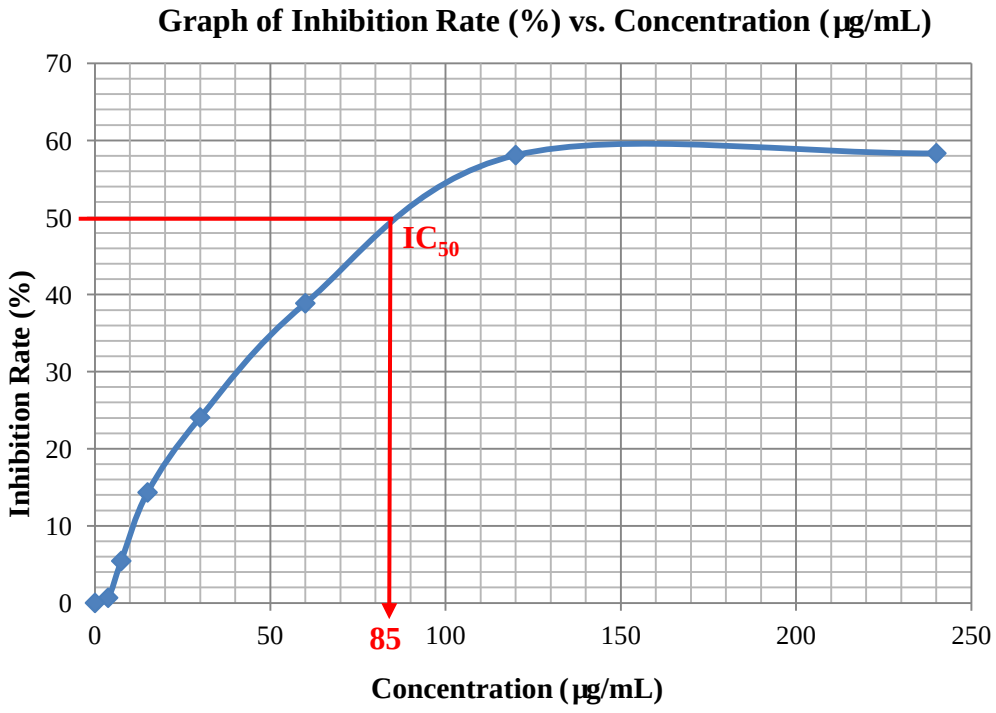


Figure 4.37: Graph of inhibition rate (%) vs. concentration (µg/mL) of 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (43)

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

In this study, a xanthonic block 1,3-dihydroxyxanthone (**39**) and three new prenylated xanthone derivatives, namely 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**), 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**), and 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**) were successfully synthesized. The structures of these pure compounds were elucidated through ^{1}H-NMR, ^{13}C-NMR and UV-Vis analyses, and were further confirmed on the basis of 2D-NMR including HMQC and HMBC analyses. Among these compounds, compound **42** was found to bear a novel skeleton of xanthen-3,9-dione.

The DPPH radical scavenging assay was carried out to study the antioxidant activities of the isolated compounds, and the results indicated compound **43** to exhibit significant antioxidant activity at an IC_{50} value of 85 µg/mL. This activity was found to be weak as compared to the reference compounds, ascorbic acid and kaempferol with their IC_{50} values of 5 µg/mL and 10 µg/mL, respectively.

5.2 Future Studies

The prenylated products suffer a low yield, syntheses which are able to give higher yield can be persued. One example is the application of microwave-assisted organic synthesis (MAOS) which has proven to increase the yield and has remarkable short reaction time. In addition, a combination of a microwave irradiation with the Montmorillonite K10 clay has the possibility to vary the conditions such as the type of clay, the absence and presence of solvent and the temperature. Besides being environmental friendly, this methodology has proven to give a much higher yield when compare to the classical method in which zinc chloride and high temperature are used.

Secondly, the synthesized compounds did not show significant antioxidant activities. It is suggested that a further study on other potential biological activities such as cytotoxic, anti-malarial and anti-bacterial to be carried out.

Besides the gravity column chromatography, other more advanced chromatographic methods such as flash column chromatography, centrifugal chromatography and high performance liquid chromatography are recommended to improve separation to the crude product and to fasten the purification work.

REFERENCES

Balasubramanian, K. & Rajagopalan, K. (1988). Novel xanthones from Garcinia mangostana, structures of BR-xanthone-A and BR-xanthone-B. *Phytochemistry, 27* (5), 1552–1554.

Bennett, G. J. & Lee, H. H. (1988). The biosynthesis of mangostin: the origin of the xanthone skeleton. *Journal of the Chemical Society, Chemical Communications*, pp. 619–620.

Bennett, G. J., Lee, H. H., & Nagaratnam, P. D. (1990). Biosynthesis of Mangostin. Part 1: The origin of the xanthone skeleton. *Journal of Chemistry Society, Perkin Transactions*, *1*(10), 2671-2676.

Bennett, G.J. & Lee, H. H. (1989). Xanthones from Guttiferae. *Phytochemistry*, *28*(4), 967-988.

Birch, A. J., Baldas, J., Hlubucek, J. R., Simpson, T. J., & Westerman, P. W. (1976). Acetate Polymalonic Route of Biosynthesis Xanthones. *Journal of Chemistry Society, Perkin Transactions*, 8, pp. 898-904.

Boonstra, J., & Post, J. A. (2004). Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene*, 337, pp. 1-13.

Boots, A. W., Haenen, G. R., den Hartog, G. J., & Bast, A. (2002). Oxidative damage shifts from lipid peroxidation to thiol arylation by catechol-containing antioxidants. *Biochimica et Biophysica Acta*, *1583*(3), 279-284.

Brigmann, G., Ochse, M.,Schupp, O., & Tasler, S. (2001). *Progress in the Chemistry of Organic Natural Products*. Wien, Germany: Springer.

Cardona, M. L., Fernadez M. I., Pedro J. R., & Serrano A. (1990). Xanthones from Hypericum reflexum. *Pytochemistry, 29*(9), 3003-3006.

Carpenter I., Locksley H. D., & Scheinmann F (1969). Xanthones in higher plants: Biogenetic proposals and a chemotaxonomic survey. *Phytochemistry, 8*(10), 2013-2025.

Castanheiro, R. A. P., Pinto, M. M. M., Cravo, S. M. M., Pinto, D. C. G. A., Silva, A. M. S., & Kijjoa, A. (2009). Improved methodologies for synthesis of prenylated xanthones by microwave irradiation and combination of heterogeneous catalysis (K10 clay) with microwave irradiation. *Tetrahedron, 65*(19), 3848-3857.

Castanheiro, R. A. P., Pinto, M. M. M., Silva, A. M. S., Cravo, S. M. M., Gales, L., Damas, A. M., Pedro, M. M., Nazareth, N., Nascimento, M. S. J., Eaton, G. (2007). Dihydroxyxanthones prenylated derivatives: synthesis, structure elucidation, and growth inhibitory activity on human tumor cell lines with improvement of selectivity for MCF-7. *Bioorganic & Medical Chemistry, 15*(18), 6080-6088.

Chen, S. X., Wan, M., & Loh, B. N. (1996). Active constituents against HIV-1 protease from *Garcinia mangostana*. *Planta Medica, 62*(4), 381–382.

Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1956). *Glossary of indian medicinal plants* (pp. 123). New Delhi, India: The National Institute of Science Communication and Information Resources.

Dewick, P. M. (1998). The biosynthesis of shikimate metabolites. *Natural Product Report, 15*(1), 17-58.

Epifano, F., Genovese, S., Menghini, L., & Curini, M. (2007). Chemistry and pharmacology of oxyprenylated secondary plant metabolites. *Phytochemistry, 68*(7), 939-953.

Feskanich, D., Ziegler, R. G., Michaud, D. S., Giovannucci, E. L., Speizer, F. E., Willett, W. C., & Colditz, G. A. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *Journal of the National Cancer Institute, 92*(22), 1812 1823.

Fujita, M., & Inoue, T. (1980). Biosynthesis of mangiferin in *Anemarrhena asphodeloides* Bunge. I. The origin of the xanthone nucleus. Chemical & *Pharmaceutical Bulletin, 28*(8), 2476–2481.

Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G. (2000). Relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry, 68*(4), 471–474.

Garnett, M., & Sturton, S. D. (1932). Garcinia Mangostana in the treatment of amoebic dysentery. *Chinese Medical Journal*, pp. 969–973.

Gordon, M. H. (1996). Dietary antioxidants in disease prevention. *Natural Product Reports, 13*(4), 265–273.

Gottlieb, O.R. (1968). *Biogeneticproposals regarding aucuparins and xanthones. Phytochemistry, 7*(3), 411-421

Granoth, I., Pownall, H. J. (1975). Synthesis and substituent effects in the nuclear magnetic resonance and mass spectra of dimethyl- and dihaloxanthones. *The Journal of Organic Chemistry, 40*(14), 2088-2091.

Gröger, D., Erge, D., Franck, B., Ohnsorge, U., Flasch, H., & Huper, F. (1968). Mycotoxin from mold fungi. *Chemische Berichte, 101*, pp. 1970-1978.

Grover, P. K., Shah, G. D., & Shah, R. C. (1955). Xanthones. Part IV. A new synthesis of hydroxyxanthones and hydroxybenzophenones. *Journal of the Chemical Society*, pp. 3982-3985.

Gupta, P. & Lewis, J. R. (1971). Xanthones in Cell Suspension Cultures of Two *Centaurium* Species. *Journal of the Chemical Society*, 4, pp. 629-631.

Helesbeux, J. J., Duval, O., Dartiguelongue, C., Séraphin, D., Oger, J. M., & Richomme, P. (2004). Synthesis of 2-hydroxy-3-methylbut-3-enyl substituted coumarins and xanthones as natural products. *Tetrahedron*, *60*(10), 2293-2300.

Herrmann, K. M., & Weaver, L. M. (1999). The Shikimate Pathway. *Annual Review of Plant Physiology and Plant Molecular Biology*, *50*(1), 473-503.

Hostettmann, K., & Hostettmann, M. (1989). Xanthones. In Dey P. M. & Harborne J. B. (Eds.), *Methods in Plant Biochemistry* (Vol. 1). London, UK: Academic Press.

Johnson, M. M., Naidoo, J. M., Fernandes, M. A., Mmutlane, E. M., van Otterlo, W. A., & de Koning, C. B. (2010). CAN-mediated oxidations for the synthesis of xanthones and related products. *The Journal of Organic Chemistry*, *75*(24), 8701-8704.

Joshipura, K. J., Hu, F. B., Manson, J. E., Stampfer, M. J., Rimm, E. B., Speizer, F. E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D., Willett, W. C. (2001). The effect of fruit and vegetable intake on risk for coronary heart disease. *Annals of Internal Medicine*, *134*(12), 1106–1114.

Khan, M. T. H. & Ather, A. (2006). Xanthones as therapeutic agents: chemistry and pharmacology. In *Lead Molecules from Natural Products, Volume 2: Discovery and New Trends* (p. 273). Oxford, UK: Elsevier.

Kinghorn, A. D., Falk, H., & Kobayashi, J. (Eds.). (2011). *Progress in the Chemistry of Organic Natural Products* (Vol. 94). New York: Springer.

Klimczak, I., Maleecka, M., Szlachta, M., & Gliszczyńska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis, 20*(3-4), 313–322.

Kosem, N., Han, Y. H., & Moongkarndia, P. (2007). Antioxidant and cytoprotective activities of methanolic extract from *Garcinia mangostana* hulls. *Science Asia*, 33, pp. 283–292.

Kostanecki, St. V. & Nessler, B. (1891). Synthesen von Oxyxanthonen. *Ber. Dtsch. Chem. Ges., 24*(1), 1894–1897.

Lespegnol, Bertrand, & Dupas (1939). *Bulletin de la Société Chimique de France*, 6, pp. 1925.

Locksley, H. D., Moore, I., & Scheinmann, F. (1967). Extractives from guttiferae—VI : The significance of maclurin in xanthone biosynthesis. *Tetrahedron*, pp. 2229-2234.

Lopaczyski, W., & Zeisel, S. H. (2001). *Nutrition Research, 21*(1-2), 295-307.

Lund, N. A., Robertson, A., & Whalley, W. B. (1953). The chemistry of fungi. Part XXI. Asperxanthone and a preliminary examination of aspergillin. *Journal of the Chemical Society*, pp. 2434-2439.

McMaster, W. J., Scott, A. T., & Trippett, S. (1960). Biosynthetic Pathway of Xanthones: Acetate Polymalonic Route. *Journal of the Chemical Society, 5*(894). 4628-4631.

Mahabusarakam, W., Proudfoot, J., Taylor, W., & Croft, K. (2000). Inhibition of lipoprotein oxidation by prenylated xanthones derived from mangostin. *Free Radical Research, 33*(5), 643–659.

Mahabusarakam, W., Wiriyachtra, P., & Taylor, W. (1987). Chemical constituents of Garcinia mangostana. *Journal of Natural Products, 50*(3), 474–478.

Mandal S., Das P. C., & Joshi P. C. (1992). Xanthones from Natural Resources. *Journal of Indian Chemical Society,* 69, pp. 611-636.

Marzano, C., Caffieri, S., Fossa, P., & Bordin, F. (1997). Activity of 3 carbethoxyangelicin photolysis products. *Journal of Photochemistry and Photobiology B: Biology, 38*(2-3), 189-195.

Michael, A. A. (1883). *Journal* of the *American Chemical* Society, 5, pp. 81.

Moreau, S., Varache-Lembege, M., Larrouture, S., Fall, D., Neveu, A., Deffieux, G., Vercauteren, J., Nuhrich, A. (2002). (2-Arylhydrazonomethyl) substituted xanthones as antimycotics: synthesis and fungistatic activity against Candida species. *European Journal of Medicinal Chemistry*, *37*(3), 237-253.

Nakatani, K., Atsumi, M., Arakawa, T., Oosawa, K., Shimura, S., Nakahata, N., & Ohizumi, Y. (2002). Inhibitions of histamine release and prostaglandin E2 synthesis by mangosteen, a Thai medicinal plant. *Biological & Pharmaceutical Bulletin, 25*(9), 1137–1141.

Nkengfack, E. A., Kounga P. M., Fomum T. Z., Meyer M., & Bodo B. (2002). Globulixanthones A & B, two new cytotoxic xanthones with isoprenoid groups from the root bark of *Symphonia globulifera*. *Journal of Natural Products, 65*(5), 734–736.

Odrowaz-Sypniewski, M. R., Tsoungas, P. G., Varvounis, G., & Cordopatis, P. (2009). Xanthone in synthesis: a reactivity profile via directed lithiation of its dimethyl ketal. *Tetrahedron Letters, 50*(44), 5981–5983.

Oger, J. M., Morel, C., Helesbeux, J. J., Litaudon, M., Séraphin, D., Dartiguelongue, C., Larcher, G., Richomme, P., Duval, O (2003). First 2-hydroxy-3-methylbut-3-enyl substituted xanthones isolated from plants: structure elucidation, synthesis and antifungal activity. *Natural Product Research, 17*(3), 195-199.

Patel, G. N., & Trivedi, K. N. (1988). Abnormal Claisen Rearrangement. *Journal of Indian Chemical Society*, 65, pp. 192-193.

Pedro, M., Cerqueira, F., Sousa, M. E., Nascimento, M. S., & Pinto, M. (2002). Xanthones as inhibitors of growth of human cancer cell lines and their effects on the proliferation of human lymphocytes in vitro. *Bioorganic & Medicinal Chemistry*, *10*(12), 3725-3730.

Pillai, R. K. M., Naiksatam, P., Johnson, F., Rajagopalan, R., Watts, P.C., Cricchio, R., & Borras, S. (1986). Thermorubin II: 1,3-dihydroxy-9H-xanthones and 1,3-dihydroxy-9H-xanthenes. New methods of synthesis. The *Journal of Organic Chemistry, 51*(5), 717-723.

Pinto, M. M. M. & Castanheiro, R. A. P. (2009). Synthesis of Prenylated Xanthones: An Overview. *Current Organic Chemistry, 13*(12), 1215-1240.

Pinto, M. M. M., Sousa, M. E., & Nascimento, M. S. J. (2005). Xanthone Derivatives: New Insights in Biological Activities. *Current Medical Chemistry 12*(21), 2517-2538.

Pinto, M., & Castanheiro, R. (2008). Chapter 11: Natural Prenylated Xanthones: Chemistry and Biological Activities in Natural Products. In Brahmachari, G., (Ed.), *Chemistry, Biochemistry and Pharmacology* (pp 520 - 676). New Delhi, India: Narosa Publishing House.

Poupon, E. & Nay, B. (2011). Polyprenylated Xanthones. In *Biomimetic Organic Synthesis* (p. 54). Weinheim, Germany: Wiley-VCH.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997) Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152- 9.

Richardson, P. M. (1983). The taxonomic significance of C-glycosylxanthones in flowering plants. *Biochemical Systematics and Ecology,11*(4), 371-375.

Robak, J., Shridi, F., Wolbis, M., & Krolikowska, M. (1988). Screening of the influence of flavonoids on lipoxygenase and cycloxygenase activities as well as on nonenzymic lipid peroxidation. *Polish Journal of Pharmacology & Pharmacy, 40*(5), 451- 458.

Robert, J. C. (1961). Naturally occurring xanthones. *Chemical Reviews, 61*(6), 591-605.

Sen, A. K., Uusvuori, R., Hase, T. A., Benerji, N., Sarkar, K. K., & Mazumder, P.C. (1980). A xanthone from Garcinia mangostana. *Phytochemistry 19*(10), 2223–2225.

Sherwin, E. R. (1978). Oxidation and antioxidants in fat and oil processing. *Journal of the American Oil Chemists' Society, 55*(11), 809-814.

Sousa, M. E. & Pinto, M. M. M. (2005). Synthesis of Xanthones: An Overview. *Current Medical Chemistry, 12*(21), 2447-2479.

Vitale, A. A., Romanelli, G. P., Autinio J. C., & Pomilio A. B. (1994). On the substitution-recation of 1-bromo-2-aryloxyethanes with sodium-cyanide. *Journal of Chemical Research, 82*(1), 19-23.

Weecharangsan, W., Opanasopit, P., Sukma, M., Ngawhirunpat, T., Sotanaphun, U., & Siripong, P. (2006). Antioxidative and neuroprotective activities of extracts from the fruit hull of mangosteen (*Garcinia mangostana* Linn.). *Medicanal Principles and Practice, 15*(4), 281–287.

Yang, C. H., Ma, L., Wei, Z. P., Han, F., & Gao J. (2012). Advances in Isolation and Synthesis of Xanthone Derivatives. *Chinese Herbal Medicines, 4*(2), 87-102.

Yu, L., Zhao, M., Yang, B., Zhao, Q., & Jiang, Y. (2007). Phenolics from hull of *Garcinia mangostana* fruit and their antioxidant activities. *Food Chemistry, 104*(1), 176–181.

Zarena, A. S. & Sankar, K. U. (2009). Supercritical carbon dioxide extraction of xanthones with antioxidant activity from *Garcinia mangostana*: Characterization by HPLC/LC–ESI-MS. *The Journal of Supercritical Fluids, 49* (3), 330–337.

Zarena, A. S. & Sankar, K. U. (2011). Xanthones enriched extracts from mangosteen pericarp obtained by supercritical carbon dioxide process. *Separation and Purification Technology, 80* (1), 172–178.

Zhang, Z. H., & Wang, H. J., & Ren X. Q., (2009). A facile and efficient method for synthesis of xanthone derivatives catalyzed by HBF_4/SiO_2 under solvent-free conditions. *Monatshefte für Chemie, 140*(12), 1481-1483.

APPENDICES

APPENDIX A

The following table summarizes the results of inhibition rates vary with concentrations for ascorbic acid in DPPH radical scavenging activity.

Concentration (μg/mL)	Absorbance				Inhibition Rate (%)
	1	2	3	Mean[1]	
240	0.0676	0.0669	0.0680	0.0675 ±0.0006	89.97
120	0.0685	0.0695	0.0695	0.0692 ±0.0006	89.72
60	0.0744	0.0636	0.0690	0.0690 ±0.0054	89.75
30	0.0672	0.0685	0.0662	0.0673 ±0.0012	90.00
15	0.0679	0.0683	0.0677	0.0680 ±0.0003	89.89
7.5	0.0660	0.1646	0.1410	0.1239 ±0.0515	81.59
3.75	0.3523	0.5684	0.5121	0.4776 ±0.1121	29.02
0	0.6803	0.6673	0.6711	0.6729 ±0.0067	0

[1] Each value was obtained by calculating the average of three experiments ± standard deviation.

APPENDIX B

The following table summarizes the results of inhibition rates vary with concentrations for kaempferol in DPPH radical scavenging activity.

Concentration (μg/mL)	Absorbance				Inhibition Rate (%)
	1	2	3	Mean[1]	
240	0.0721	0.0700	0.0698	0.0706 ±0.0013	90.13
120	0.0664	0.0667	0.0666	0.0666 ±0.0002	90.69
60	0.0764	0.0697	0.0680	0.0714 ±0.0044	90.02
30	0.0701	0.0687	0.0759	0.0716 ±0.0038	89.99
15	0.0807	0.2414	0.1781	0.1667 ±0.0810	76.69
7.5	0.3999	0.4737	0.4035	0.4257 ±0.0416	40.48
3.75	0.5513	0.2857	0.4305	0.4225 ±0.1330	40.93
0	0.6297	0.8326	0.6833	0.7152 ±0.1051	0

[1] Each value was obtained by calculating the average of three experiments ± standard deviation.

APPENDIX C

The following table summarizes the results of inhibition rates vary with concentrations for 1,3-dihydroxyxanthone (**39**) in DPPH radical scavenging activity.

Concentration (μg/mL)	Absorbance				Inhibition Rate (%)
	1	2	3	Mean[1]	
240	1.1282	1.1941	0.8737	1.0653 ±0.1692	33.77
120	1.0056	1.2151	1.0877	1.1028 ±0.1056	31.43
60	1.0589	1.7135	0.8964	1.2229 ±0.4325	23.97
30	1.2808	1.6022	0.8763	1.2531 ±0.3637	22.09
15	1.3086	1.2985	1.2496	1.2856 ±0.0316	20.07
7.5	1.1218	1.6164	1.2774	1.3385 ±0.2529	16.78
3.75	1.3498	1.5549	1.1693	1.3580 ±0.1929	15.57
0	1.9581	1.8702	0.9968	1.6084 ±0.5315	0

[1] Each value was obtained by calculating the average of three experiments ± standard deviation.

APPENDIX D

The following table summarizes the results of inhibition rates vary with concentrations for 1-hydroxy-2,4-bis(3-methyl-but-2-enyl)-3-(3-methyl-but-2-enyloxy)-xanthen-9-one (**41**) in DPPH radical scavenging activity.

Concentration (μg/mL)	Absorbance				Inhibition Rate (%)
	1	2	3	Mean[1]	
240	0.8715	0.8061	0.7539	0.8105 ±0.0589	17.70
120	0.7862	0.8897	0.8091	0.8283 ±0.0544	15.89
60	0.7971	0.9006	0.8186	0.8388 ±0.0546	14.83
30	0.8604	0.8303	0.8637	0.8515 ±0.0184	13.54
15	0.8393	0.8547	0.9401	0.8780 ±0.0543	10.84
7.5	0.9046	0.9865	0.9400	0.9437 ±0.0411	4.17
3.75	0.9125	0.9836	1.0393	0.9785 ±0.0636	0.64
0	1.0775	0.9614	0.9154	0.9848 ±0.0835	0

[1] Each value was obtained by calculating the average of three experiments ± standard deviation.

APPENDIX E

The following table summarizes the results of inhibition rates vary with concentrations for 1-hydroxy-2,4,4-tris(3-methyl-but-2-enyl)-4*H*-xanthen-3,9-dione (**42**) in DPPH radical scavenging activity.

Concentration (μg/mL)	Absorbance				Inhibition Rate (%)
	1	2	3	Mean[1]	
240	0.6359	0.5750	0.5216	0.5775 ±0.0572	47.74
120	0.6216	0.7902	0.7422	0.7180 ±0.0869	35.03
60	0.8469	0.9111	0.8790	0.8630 ±0.0321	21.91
30	0.9188	0.8785	0.8833	0.8935 ±0.0220	19.15
15	0.9488	0.9476	1.0049	0.9671 ±0.0327	12.49
7.5	0.9858	0.9990	0.9725	0.9858 ±0.0133	10.80
3.75	1.0753	1.0922	1.0104	1.0593 ±0.0432	4.14
0	1.1190	1.1578	1.0384	1.1051 ±0.0609	0

[1] Each value was obtained by calculating the average of three experiments ± standard deviation.

APPENDIX F

The following table summarizes the results of inhibition rates vary with concentrations for 1,3-dihydroxy-2,4-bis(3-methyl-but-2-enyl)-xanthen-9-one (**43**) in DPPH radical scavenging activity.

Concentration (μg/mL)	Absorbance				Inhibition Rate (%)
	1	2	3	Mean[1]	
240	0.5175	0.4313	0.4422	0.4637±0.0469	58.33
120	0.5035	0.3992	0.4963	0.4663±0.0583	58.09
60	0.6352	0.7138	0.6916	0.6802±0.0405	38.87
30	0.8851	0.8034	0.8465	0.8450±0.0409	24.06
15	0.9447	0.9750	0.9401	0.9533±0.0190	14.33
7.5	1.0787	1.0595	1.0185	1.0522±0.0308	5.44
3.75	1.1549	1.0770	1.0837	1.1052±0.0432	0.67
0	1.0534	1.1847	1.1001	1.1127±0.0666	0

[1] Each value was obtained by calculating the average of three experiments ± standard deviation.