SYNTHESIS OF 1,3,7-TRIHYDROXYXANTHONE AND ITS PRENYLATED

DERIVATIVES AND THEIR ANTITUMOUR ACTIVITIES

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By

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ABSTRACT

In this study, a xanthone building block and three prenylated xanthones were synthesized and isolated. They were 1,3,7-trihydroxyxanthone **(1)**, 1,7-dihyroxy-3-(3-methylbut-2-enyloxy)xanthone **(2),** 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** and 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone **(4)**. The prenylated xanthones **(2)**, **(3),** and **(4)** were obtained from the reaction of 1,3,7-trihydroxyxanthone with prenyl bromide in alkaline medium. The structures of the compounds were established by means of IR, UV, MS, and NMR (^{1}H, ^{13}C, HMQC, and HMBC) techniques.

The cytotoxic activities of 1,3,7-trihydroxyxanthone **(1),** 1,7-dihyroxy-3-(3-methylbut-2-enyloxy)xanthone **(2),** 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** on the *in vitro* growth of two human tumor cell lines, HeLa and MDA-MB-231 were evaluated by using MTT method. The result indicated that 1,3,7-trihydroxyxanthone **(1)** showed a moderate inhibitory activity towards HeLa cancer cell line with IC$_{50}$ value of 21 μg/ml, however the compound showed insignificant inhibitory activity towards MDA-MB-231 cancer cell line. The prenylated xanthones, 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone **(2)** and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** gave insignificant inhibitory activities against both HeLa and MDA-MB-231 cancer cell lines with IC$_{50}$ value of more than 50μg/ml

suggesting that o-prenylation to carbon position C-3 was found to reduce the cytotoxic activities of the prenylated xanthone **(2)** and **(3)** against the two cell lines.

ABSTRAK

Dalam kajian ini, satu blok xanthone dan tiga sebatian prenilasi telah disisntesiskan dan diasingkan. Mereka ialah 1,3,7-trihidroksixanthone**(1)**, 1,7-dihidroksi-3-(3-metilbut-2-enilaksi)xanthone**(2)**, 1,7-dihidroksi-2-(3-metilbut-2-enil)-3-(3-metilbut-2- enilaksi)xanthone**(3)**, dan 1-hidroksi-4-(3-metil-but-2-enil)-3,7-di(3-methilbut-2- enilaksi)xanthone**(4)**. Xanthone prenilasi **(2)**, **(3)** dan **(4)** didapati dari reaksi 1,3,7-trihidroksixanthone**(1)** dengan bromida prenil di dalam media alkali. Struktur sebatian kemudian dikenalpasti dengan menggunakan teknik IR, UV, MS and NMR (^{1}H, ^{13}C, HMQC, and HMBC).

Aktiviti sitotoksik tiga sebatian xanthone **(1)**, **(2)** dan **(3)** terhadap pertumbuhan *in vitro* sel kanser HeLa dan MDA-MB-231 dengan menggunakan kaedah MTT juga dijelaskan. Keputusan kajian menunjukkan bahawa 1,3,7-trihidroksixanthone**(1)** menunjukkan aktiviti halangan sederhana dengan nilai IC_{50} 21 µg/ml terhadap sel kanser HeLa tetapi tiada aktiviti terhadap sel kanser MDA-MB-231. Xanthone prenilasi 1,7-dihidroksi-3-(3-metilbut-2-enilaksi)xanthone**(2)** dan 1,7-dihidroksi-2-(3-metilbut-2-enil)-3-(3-metilbut-2- enilaksi)xanthone**(3)** tidak menunjukkan aktiviti halangan terhadap sel kanser HeLa dan MDA-MB-231 dengan memberikan nilai IC_{50} lebih daripada 50µg/ml mencadangkan o-prenilasi di karbon C-3 mengurangkan aktiviti sitotoksik xanthone prenilasi **(2)** dan **(3)** terhadap dua sel kanser tersebut.

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Last but most importantly, my gratitude goes to my parents and sister for their continual support and patience throughout the course of this work, without their support this work would not be successful.

APPROVAL SHEET

I certify that, this project report entitled "**SYNTHESIS OF 1,3,7-TRIHYDROXYXANTHONE AND ITS PRENYLATED DERIVATIVES AND THIER ANTITUMOR ACTIVITIES**" was prepared by **GOH YI FAN** and submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (Hons.) in Chemistry at Universiti Tunku Abdul Rahman.

Approved by

Supervisor

_____   Date: _____

(Asst. Prof. Dr. Lim Chan Kiang)

FACULTY OF SCIENCE

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Date: _____

PERMISSION SHEET

It is hereby certified that **GOH YI FAN** (ID No: **08ADB02667**) has completed this report entitled **"SYNTHESIS OF 1,3,7-TRIHYDROXYXANTHONE AND ITS PRENYLATED DERIVATIVES AND THIER ANTITUMOR ACTIVITIES"** under the supervision of **ASST. PROF. DR. LIM CHAN KIANG** from the Department of Chemistry, Faculty of Science.

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

DECLARATION

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

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(GOH YI FAN)

Date:

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LIST OF ABBREVIATIONS

δ	Chemical shift ppm
^{1}H	Proton
^{13}C	Carbon-13
d	Doublet
dd	Doublet of doublet
h	Hour
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
IR	Infrared
J	Coupling constant in Hz
Lit.	Literature
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
TLC	Thin Layer Chromatography
UV-Vis	Ultra Violet-Visible

CHAPTER 1

INTRODUCTION

1.1 General Introduction

According to the WHO, cancer is a leading cause of death worldwide: it accounted for 7.4 million deaths (around 13% of all deaths) in 2004 and there is an urgent need to find better cures for this disease. Although novel drugs have been developed for cancer treatment, cancer remains a dangerous disease. Many methods, including chemical and biological methods, have been used in order to develop more potent anticancer drugs (Younghwa, 2009).

Using chemical methods, many structural scaffolds have been created for cancer treatments. Among these scaffolds, xanthones or xanthen-9-ones (**Figure 1**) have attracted many scientists to isolate from natural products or synthesize because of its interesting pharmacological importance, such as antioxidant (Yoshikawa *et al*., 1994), antitumoral (Ho *et al*., 2002), antiallergic (Chairungsrilerd *et al.,* 1996), anti-inflammatory (Shankaranarayan *et al.*, 1979) antibacterial (Sundaram *et al.,* 1983), and antiviral (Chen *et al.,* 1996) activities. The major two sources of xanthone deriavatives are synthesis and isolation from natural resources.
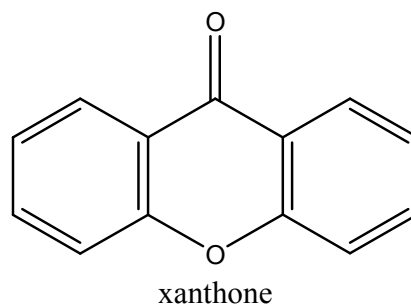
xanthone

Figure 1.1: Molecular structure of xanthone

The xanthone nucleus is known as 9-xanthenone or dibenzo-γ-pyrone and it is symmetric (Vieira and Kijjoa, 2005; Pinto *et al.,* 2005; Souza and Pinto, 2005; Gales and Damas, 2005). Xanthones have been classified into five groups: (a) simple oxygenated xanthones, (b) xanthone glycosides, (c) prenylated xanthones, (d) xanthonolignoids and (e) miscellaneous xanthones (Sultanbawa, 1980; Jiang *et al.*, 2004).

According to Pedraza-Chaverri *et al.* (2008), numerous studies have been designed to examine the anticancer activities of xanthones isolated from mangosteen-fruit pericarp. A study carried out by Ho *et al.* (2002), it was found that garcinone E has a potent cytotoxic effect on hepatocellular carcinoma cell lines. Jung *et al.* (2006) isolated two new xanthones (8-hydroxycudraxanthone G and mangostinone) as well as 12 known xanthones from mangosteen-fruit pericarp. They determined their antitumoral properties in preneolplastic lesions

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induced by 7,12-dimethylbenpa[anthracene (DMBA) in a mouse mammary organ culture. α-Mangostin inhibited DMBA-induced preneoplastic lesions with an IC_{50} of 1.0 µg/mL (2.44 µM). These results suggest that xanthones isolated from natural products would be candidates for preventive and therapeutic application for cancer treatment.

Since xanthone derivatives from natural products are relatively limited in type and position of the substituents imposed by the biosynthetic pathways, the chemical syntheses of new compounds can enlarge the possibilities of having different nature and positions of the substituents on the xanthonic nucleus. This allows scientists to rationalize and characterize the structure features that are important to their bioactivity (Pedro *et al*., 2002). Based on these considerations, Madalena Pedro *et al* (2002) have synthesized and assessed twenty-seven oxygenated xanthones for their capacity to inhibit *in vitro* growth of three human cancer cell lines, MCF-7 (breast cancer), TK-10 (renal cancer) and UACC-62 (melanoma). In another study, oxime- and methyloxime-coupled xanthones were prepared and tested for cytotoxic activity. The results showed that these compounds were efficient cancer cell growth inhibitors (Wang *et al.,* 2005).

Recently, prenylated xanthones have aroused great interest in the search for novel bioactive compounds (Pinto *et al*., 2009) due to their interesting pharmacological properties. Despite the importance of prenylated xanthones, a

few works on their synthesis (Rao *et al.*, 2001; Helesbeux *et al*., 2004) have been reported. Hereby, we report the synthesis of 4 xanthones, 1,3,7-trihydroxyxanthone **(1)**, 1,7-dihyroxy-3-(3-methylbut-2-enyloxy)xanthone **(2)**, 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)**, 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone **(4)** and the study of the *in vitro* cytotoxic effect of 1,3,7-trihydroxyxanthone **(1)**, 1,7-dihyroxy-3-(3-methylbut-2-enyloxy)xanthone **(2)** and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** towards HeLa and MDA-MB-231 cancer cell lines.

1.2 Objectives

i. To synthesize 1,3,7-trihydroxyxanthone and its prenylated compounds and isolate them by column chromatography

ii. To identify and characterize the structures of 1,3,7-trihydroxyxanthone and its derivatives by using 1D- and 2D- NMR, FTIR and UV-Vis spectroscopies

iii. To investigate the pharmacological activities of 1,3,7-trihydroxyxanthone and its prenylated compounds against HeLa (cervical) and MDA-MB-231 (breast) cancer cell lines

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of Xanthones

According to Noungoue Tchamo Diderot *et al.* (2006), there are generally six general methods to synthesize xanthones, namely Michael-Kostanecki method, Friedel-Crafts method, Robinson-Nishikawa method, Asahina-Tanase method, Tanase method and Ullman method.

Kostanecki (1892) carried out the first xanthone synthesis by heating an equimolar mixture of a polyphenol and an O-hydroxybenzoic acid in the presence of acetic anhydride or zinc chloride which act as dehydrating agent. Similar to Kostanecki (1892), Grover *et al.* (1955) prepared xanthone using a polyphenol and an O-hydroxybenzoic acid but with a mixture of phosphorus oxychloride and zinc chloride as condensing agent. Noungoue Tchamo Diderot *et al.* (2006) stated that the Grover method requires lower temperature and have good results but if the reacting polyphenol are hydroquinone, resorcinol or pyrogallol, the yields are not satisfactory. Madalena Pedro *et al.*(2002) works showed that the synthesis of

1-hydroxyxanthone gives 48% yield only, in contrary to synthesis of 1,3-dihydroxy-2-methylxanthone's 86% yield, probably due to the use of resorcinol as reactants. As shown in the **Figure 2.1**, Sangwook Woo *et al.* (2006) prepared xanthone core using phloroglucinol and salicylic acid.
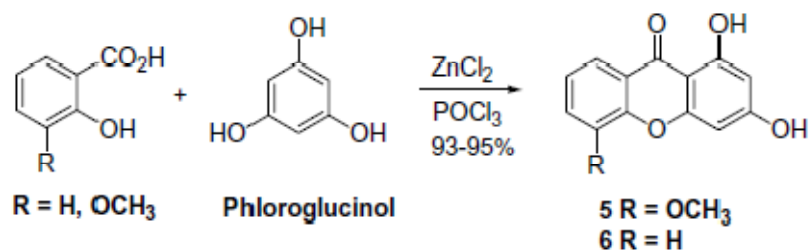
Figure 2.1: Preparation of xanthone using phloroglucinol and salicylic acid

In the work by Yan Liu *et al* (2006), it was reported that microwave irradiation can efficiently accelerate the condensation of salicylic acid with polyphenols in slightly improved yields. Moreover, Martine Varacher-Lembege *et al* (2008) reported that the use of Eaton's reagent (phosphorus pentoxide and methanesulfonic acid: P_2O_5/ CH_3SO_3H) as the coupling agent afforded the xanthones in high yield (90%).

Reported by Lin *et al.* (1993), another standard method for the synthesis of xanthones involves the benzophenone intermediates. An appropriately substituted benzoyl chlorides and phenolic derivatives undergo Friedel-Crafts acylation to

give benzophenone under certain conditions. Cyclization of the resulting 2-2'-dioxygenated benzophenones through a dehydrative or oxidative process completes the formation of xanthones. Various hydroxyl, dihydroxy and methoxy xanthones were synthesized by Pedro *et al* (2002) using benzophenone method. Xanthone was formed involving the formation of benzophenones followed by the quantitative elimination of methanol in the presence of alkali as presented by Barton *et al*., as shown in the **Figure 2.2** below.
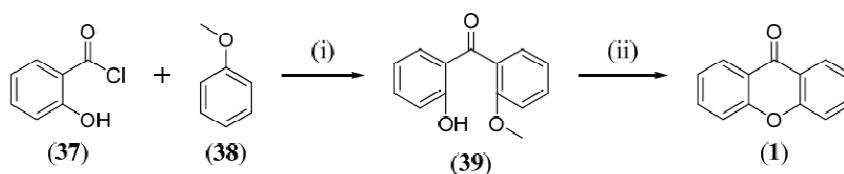
Figure 2.2: Synthetic route for xanthone. Reagents and conditions:

(i) AlCl₃, dry ether, rt, 1h; (ii) NaOH, methanol, reflux, 6h.

As shown in the **Figure 2.3** below, Nishikawa *et al.* synthesized 1,3-dihydroxyxanthone via a ketimine intermediate. Starting materials were reacted in the presence of zinc chloride to afford the ketimine derivative in moderate yield. The ketimine was then subjected to aqueous basic conditions to form the 1,3-dihydroxyxanthone. However, the yield is low.
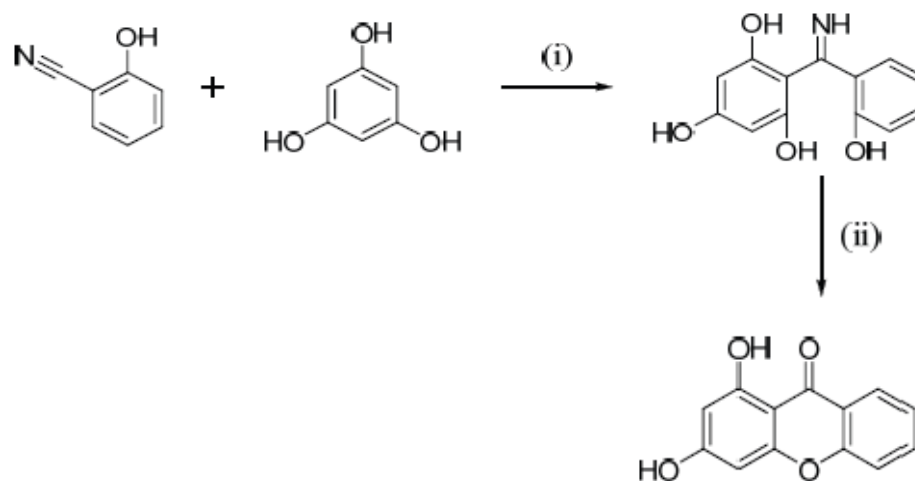
Figure 2.3: Synthesis route for 1,3-dihydroxyxanthone. Reagents and conditions: (i) Dry ether, ZnCl$_2$, HCl, 2h; (ii) NaOH $_{(aq),}$ reflux.

This method has been modified by Atkinson and Heilbron (1926) to synthesize 2,6-dihydroxyxanthone as shown in the **Figure 2.4**.
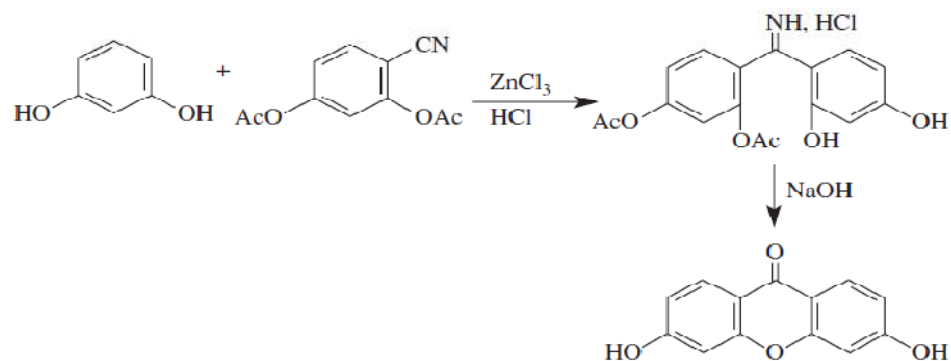
Figure 2.4: Synthesis of 2,6-dihydroxyxanthone using method modified by Atkinson and Heilbron (1926)

Asahina-Tanase method is a useful method for synthesizing methyoxylated xanthones or xanthones with aid sensitive substituents (Granoth and Pownall, 1975). Vitale *et al.* (1994) modified the procedure as shown in the **Figure 2.5**.

2-Methoxyxanthone (52%)
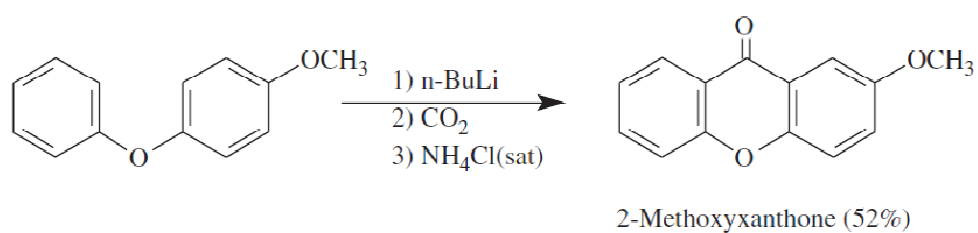
Figure 2.5: Synthesis of 2-methoxyxanthone by Vitale *et al* (1994)

Polyhydroxyxanthones can be synthesized through this method. With pre-established orientation of substituents, partially methylated polyhydroxyxanthones has been prepared. The synthesis of 1,3-dihydroxyxanthone is carried out by Pillai *et al* (1986) as shown in the **Figure 2.6.**
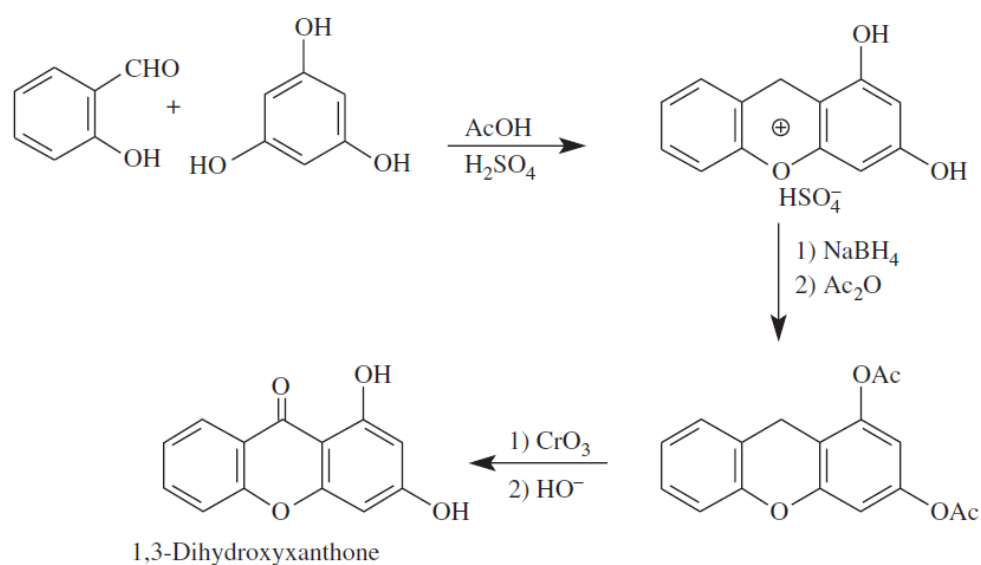
Figure 2.6: Synthesis of 1,3-dihydroxyxanthone by Pillai *et al.* (1986)

Ullman method involves biphenyl ether intermediate with the ring formation by one-step conversion by lithium diisoprophylamide or by acethyl chloride. For example, Ulmann and Pauchaud (1906) synthesized euxanthone using this mehod as shown in the **Figure 2.7**.
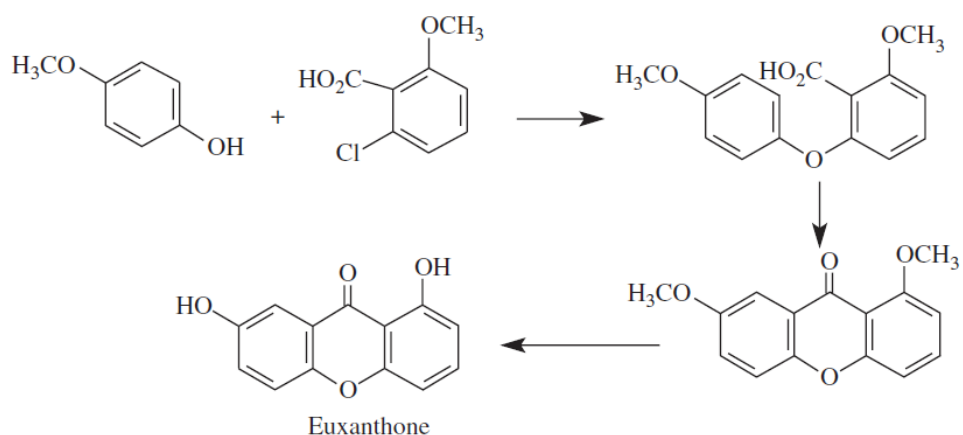
Figure 2.7 The Ullman method for the preparation of euxanthone.

For example, Martina Pickert and August Wilhelm Frahm (1998) synthesized xanthone using 2-chlorobenzoic acid and a cresol or hydroxybenzonitrile (R=CH$_3$, CN) under Ullmann conditions using a copper catalyst at temperatures of 180-200°C leading to substituted 2-phenoxybenzoic acids. After reacting with phosphoric acid and phosphorus oxychloride, they cyclise to the desired xanthone under Friedel-Crafts conditions in high yields. Rajan Giri *et al.* (2010) found the use of a non-nucleophilic base, such as 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) to be essential for the coupling reaction to form the diaryl ethers **(Figure 2.8)**.
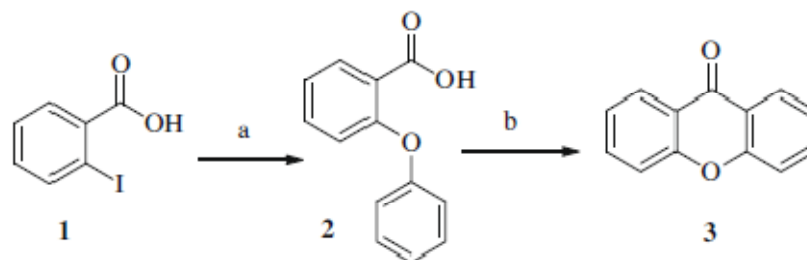
Figure 2.8: (a) phenol, Cu, CuI, pyridine, DBU, DMF, reflux; (b) H$_2$SO$_4$.
100°C.

2.2 Prenylation of Xanthones

There has been little work done on the O-prenylation of xanthones. Recently, Raquel A.P. Castanheiro carried out the synthesis of oxyprenylation xanthones by prenylation of 3 xanthone blocks with prenyl bromide and K$_2$CO$_3$ in acetone. These syntheses gave products yield percentage from 2% ~ 72%(**Figure 2.9)**
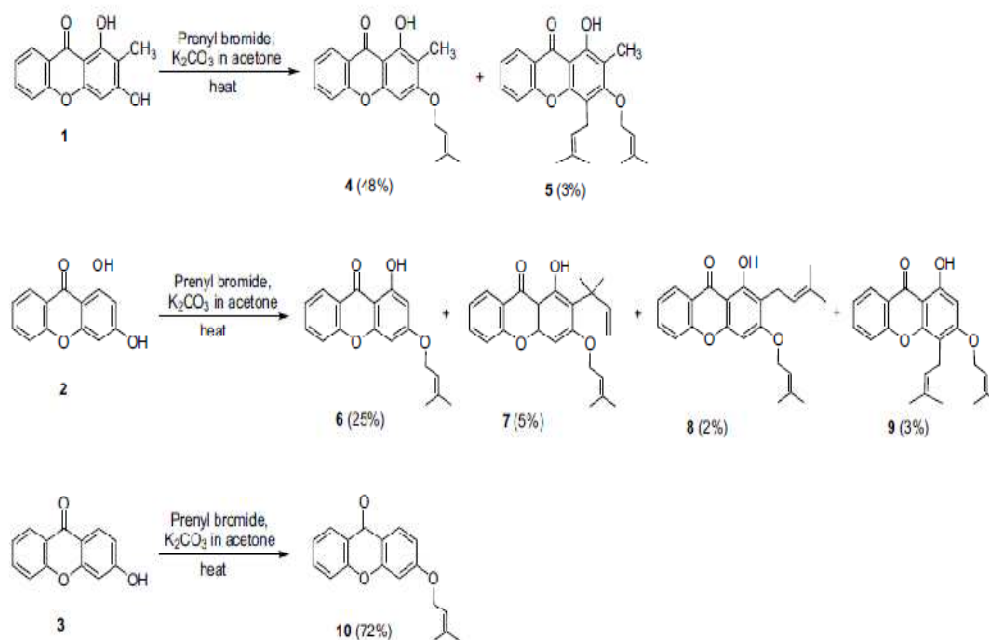
Figure 2.9: O-prenylation of xanthones

Further reaction of O-prenylated xanthones with catalytic amount of zinc chloride gives cyclic derivatives of xanthones according to Raquel A.P. Castanheiro *et al* (2007).
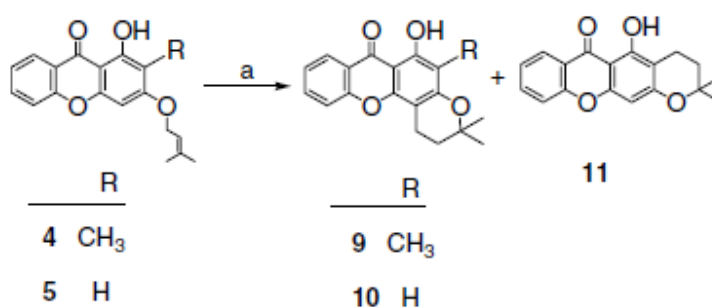
Figure 2.10: Synthesis route for pyranoxanthones. Reagents and conditions: (a) ZnCl₂, *o*-xylene, 200°C, 21 h.

There are 3 known methods of C-prenylation of xanthones:

1. Boron trifluoride is used as catalyst in C-prenylation of xanthones with 2-methylbut-3-en-2-ol as done by Anand and Jain (1973)(**Figure 2.11**).

(19) R_1=Prenyl, R_2=H 8%

(20) R_2=H, R_2=Prenyl 10%

(21) R_1=R_2=Prenyl 5%
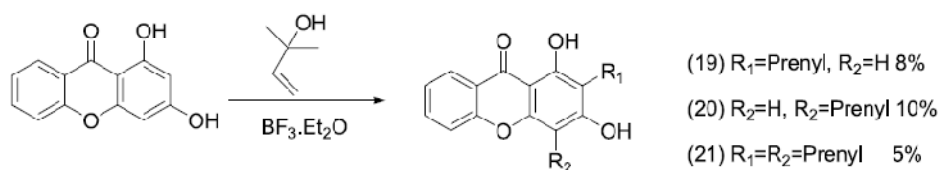
Figure 2.11: C-prenylation of xanthones using boron trifluoride as catalyst

2. Sodium methoxide is used as catalyst in synthesis of O- and C-prenylated xanthones, as in the work by Anand Jain (1974) using 1,3-dihydroxy-5,8-methoxyxanthone as staring material(**Figure 2.12**).

(21) R_1=R_2=R_3=prenyl 4%

(22) R_1=R_2=prenyl R_3=H 4%

(23) R_1=R_3=prenyl R_2=H 5%
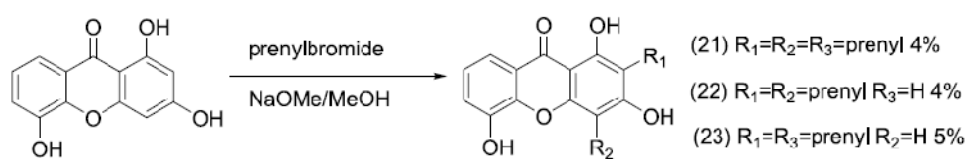
Figure 2.12: O- and C- prenylation of xanthones using sodium methoxide as catalyst

3. C-prenylation takes place through Claisen rearrangement of O-prenylation, but with low yield as done by Patel and Trivedi (1988)(**Figure 2.13**).
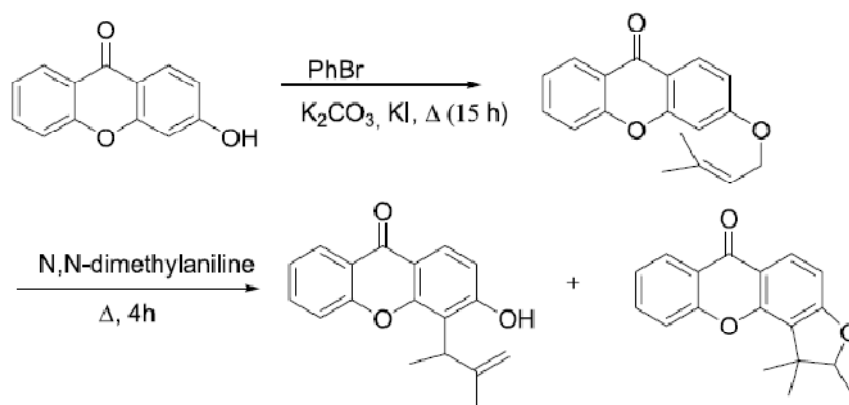
Figure 2.13: C-prenylation of xanthones with Claisen rearrangement

2.3 Cytotoxic Activity of Xanthone Derivatives

Many studies have been designed to examine the anticancer activities of xanthone derivatives from either chemical synthesis or isolation of natural products (Table 1). MCF-7 breast adnocarcinoma, SF-268 CNS cancer, UACC-62 melanoma, NCI-H460 non-small cell lung cancer (R.A.P. Castanheiro *et al*, 2009), MDA-MB-231, CaOV-3, LnCap prostate, HCT 116 colon, Hela cervix, NCl-H460 (Sousa *et al*, 2009), KB oral human epidermoid carcinoma (Suphavanich *et al*, 2009) and TK-10 renal cancer (Pedro *et al*, 2002) cell lines have been used.

Table 2.1: Journals about antitumoral properties of xanthone derivative

from isolation and synthesis

Title	References
Xanthones as Inhibitors of Growth of Human Cancer Cell Lines and Their Effects on the Proliferation of Human Lymphocytes in Vitro	Madalena Pedro *et al* (2002)
CoMFA and CoMSIA studies on a new series of xanthone derivatives against the oral human epidermoid carcinoma (KB) cancer cell line	K. Suphavanich *et al*(2009)
Xanthones from *Hypericum Chinese* and their cytotoxicity evaluation	Naonobu Tanaka *et al* (2009)
Xanthones with growth inhibition against HeLa cells from *Garcinia xipshuanbannaensis*	Quan-Bin Han *et al* (2008)
Bromoalkoxyxanthones as promising antitumor agents: Synthesis, crystal structure and effect on human tumor cell lines	Emilia Sousa *et al* (2009)
Synthesis of new xanthone analogues and their biological activity test- Cytotoxicity, topoisomerase II inhibition, and DNA cross-linking study	Sangwook Woo *et al (*2007)
Bioactive prenylated xanthones and anthraquinones from *Cratoxylum formosum* ssp. Pruniflorum	Nawong Boonnak *et al* (2006)
Cytotoxic caged-polyprenylated xanthonoids and a xanthone from *Garcinia cantleyana*	Khalid A. Shadid *et al* (2007)
Dihydroxyxanthones prenylated derivatives: Synthesis, structure elucidation, and growth inhibitory activity on human tumor cell lines with improvement of selectivity for MCF-7	Raquel A.P. Castanheiro *et al* (2007)
Antioxidant and cytotoxic activities of xanthones from *Cudrania tricuspidata*	Byong Won Lee *et al* (2005)
Antitumor Activity of Some Prenylated Xanthones	Raquel A.P. Castanheiro *et al (*2009)

2.3.1 MTT Assay

The MTT assay is a quantitative colometric assay based on the cleavage of the yellow water-soluble tetrazolim salt, MTT, to form water-insoluble, dark-blue formazan crystals. MTT cleavage occurs only in living cells by the mitochondrial enzyme succinate dehyrogenase. The formazan crystals are solubilised using a suitable organic solvent, usually isopropanol, and the optical density of the resulting solution is measured using a spectrophotometer. The absorbance is directly proportional to the concentration of the blue formazan solution, which is in turn proportional to the number of metabolically active cells (Hughes *et al.*, 2003).

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. In other words, it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50}). It is commonly used as a measure of antagonist drug potency in pharmacological research. Sometimes, it is also converted to the pIC_{50} scale (-log IC_{50}), in which higher values indicate exponentially greater potency. According to

the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition *in vitro*.

2.3.2 Cytotoxic Activity of Synthetic Xanthones Derivatives

The biological activities of xanthone derivatives vary depending on the nature and position of the different substituents (Suphavanich, 2009). K. Suphavanich *et al* (2009) conducted CoMFA and CoMSIA studies on a series of xanthone derivatives against the KB cell line to investigate structure-activity relationship (SAR). They studied the steric and electrostatic properties that are associated with bioactivity of xanthone derivatives.
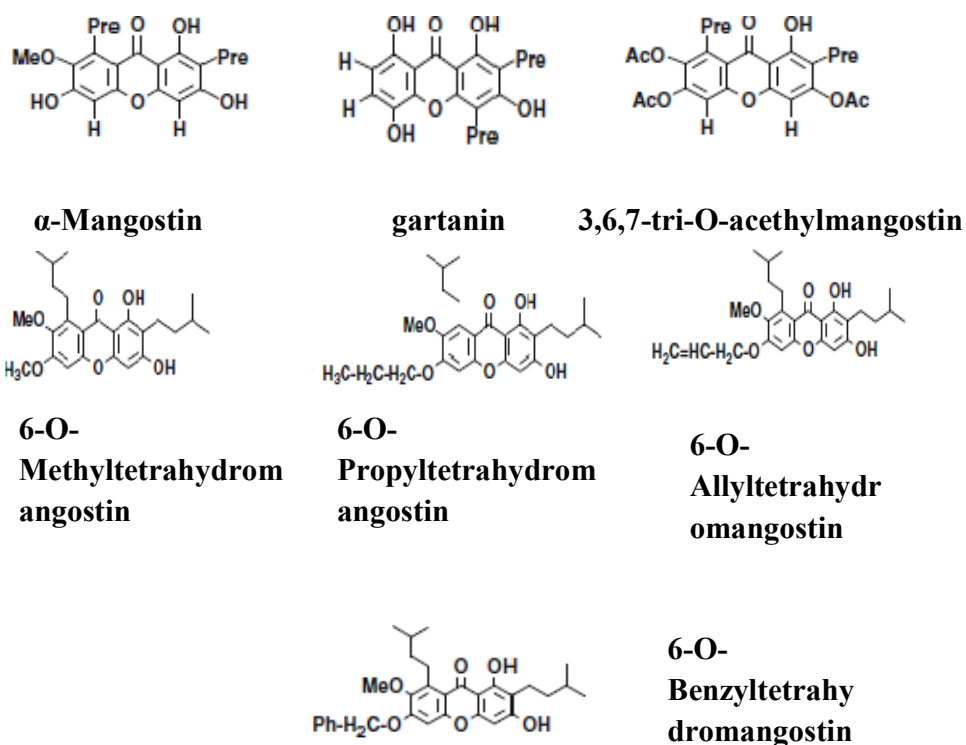
Figure 2.14 Example of xanthone derivatives

They found that α-Mangostin , has the highest bioactivity (IC_{50} = 2.08 μM) as it has bulky substituent (pre) at position R1 and less bulky substituents (OH, H) at the R3 and R5 positions. Gartanin and 3,6,7-tri-O-acethylmangostin have low activities(IC_{50} = 15.63 and 19.65μM respectively) because they have bulky group at position R3. Out of the 33 compounds, compounds that have IC_{50} less than 5 μM, i.e. high bioactivity, are having a bulky group, mostly prenyl group, at the position of R1 and have less bulky group at R2 and R3.   6-O-Methyltetrahydromangostin (IC_{50} = 5.17 μM), 6-O-propyltetrahydromangostin (IC_{50} = 4.63 μM), 6-O- allyltetrahydromangostin (IC_{50} = 5.57 μM) and 6-O-benzyltetrahydromangostin (IC_{50} = 9.15 μM) have same structure except at the R3

position. 6-O-Benzyltetrahydromangostin has poor activity owing to the negative charges in the region where negative charges would decrease the bioactivity.

Emilia Sousa *et al* (2009) reported that no capacity to inhibit the growth of human tumor cell lines was observed for a bisxanthone, 1,1'-[hexane-1,6-diylbis(oxy)]bis[9H-xanthen-9-one] (**a**) whereas 1-(6-bromohexyloxy)-xanthone (**b**) showed some interesting bioactivity against SF-268(GI_{50}=30.2±3.6μM), NCI-H460(GI_{50}=32.7±3.2μM) and MCF-7. Curiously, it shows a growth inhibitory activity against the estrogen receptor (ER+) MCF-7 (GI_{50}=22.7±1.3μM) but not against the ER-MDA-MB-231 (GI_{50}>100 μM). They also carried out the bromoalkylation of 3,4-dihydroxyxanthone , producing 3-(6-bromohexyloxy)-4-hydroxy-9H-xanthen-9-one (**c**) and 3,4-bis(6-bromohexyloxy)-9H-xanthen-9-one(**d**). Xanthone (**c**) showed no growth inhibition whereas (**d**) was found to inhibit only the growth of the breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231). Their results showed that one free hydroxyl group in the bromoalkylxanthone scaffold is unfavorable to the growth inhibitory activity against ER+ MCF-7. (**Figure 2.15**)

a

b

OH

d

c
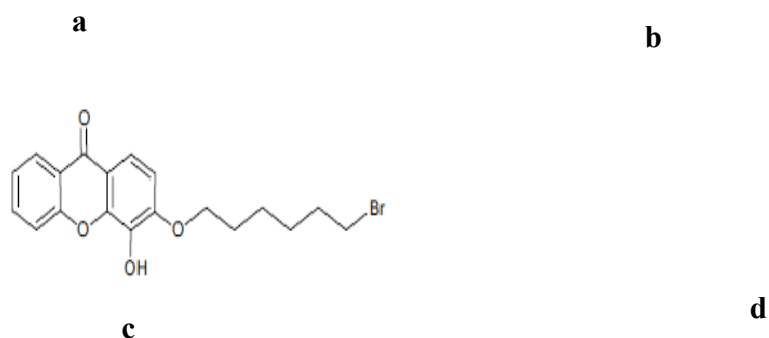
Figure 2.15 Bisxanthone and bromoalkoxyxantones

Sangwook Woo *et al* (2007) prepared some 3-(2', 3'-epoxypropoxy)xanthones and epoxide ring opened halohydrin analogues, and evaluated their cytotoxicity against several human cancer cell lines such as LnCap, MCF-7, HCT 116, MDA-MB231 and Hela. 1,3-Di(2',3'-epoxypropoxy)xanthone (**e**) showed most effective cytotoxic activity against LnCap (IC_{50}= 9.0±0.2µM), MCF-7 (IC_{50}= 3.2±0.8µM), HCT 116(IC_{50}= 10.2±0.7µM), MDA-MB231(IC_{50}= 12.8±0.9µM) and Hela (IC_{50}= 23.3±1.7µM) among the series compounds comparable to the reference, adriamycin. In their studies, they found that two epoxypropoxy group substitutions at 1 and 3 carbon on xanthone core generating

better cytotoxic activity than mono-substituted one. This finding is parallel to the previous report.
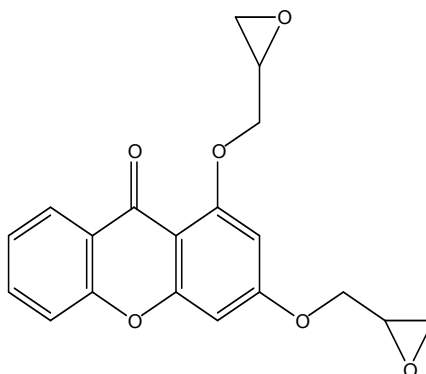
Figure 2.16: 1,3-di(2',3'-epoxypropoxy)xanthone

R.A.P. Castanheiro *et al.* (2007) reported the synthesis, structure, and antitumor activity of 11 xanthones. The scheme is shown below. They found that the existence of an alkyl group at C-2 seems to be important for the antitumor activity against MCF-7 because GI_{50} lowers to $6.0 \pm 0.7\mu M$ and $9.1\pm 1.5\mu M$ for compound **6** and **7**, respectively from their parent block, compound **1** (GI_{50} = 21.9 ± 0.4 μM). Comparing the growth inhibitory effects on MCF-7 cell line of compounds **9-11**, and their precursors, **4** and **5**, they concluded that the extra pyran ring led to the appearance of an effect in compounds **9** and **11**, but not in compound **10**. Interestingly, they pointed out that the higher activity of compound **9** for MCF-7 corresponds to a global angular structure with a methyl group at C-2, while the compound **10**, although with an angular structure but without substituent at C-2, shows no activity. In 2009, they carried out molecular modification of the compounds, 1,3-dihydroxy-2-methylxanthone and 1,3-

dihydroxyxanthone. Three pyranoxanthones were obtained by dehydrogenation of the respective dihydropyranoxanthones with DDQ in dry dioxane whereas two other prenylated xanthones were obtained from the reaction of 1-hydroxyxanthone with prenyl bromide in alkaline medium, or by condensation of xanthone with isoprene in the presence of orthophosphoric acid. The two prenylated xanthones, namely 1-hydroxy-2-(2-methylbut-3-en-2-yl)-9H-xanthen-9-one and 1-hydroxy-2-(3-methylbut-2-enyl)-9H-xanthen-9-one exhibited a moderate growth inhibitory activity against the MCF-7 cell line (**Figure 2.17**, **2.18**).
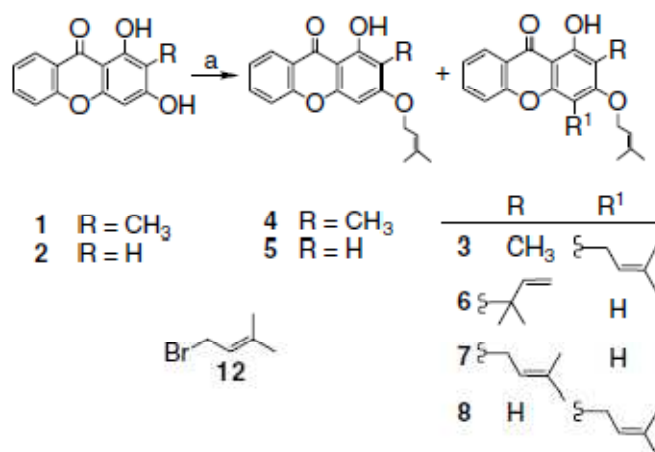
Figure 2.17: Prenylation of xanthone. Reagent and conditions: (a) Prenyl bromide, K$_2$CO$_3$, acetone, reflux, 8h
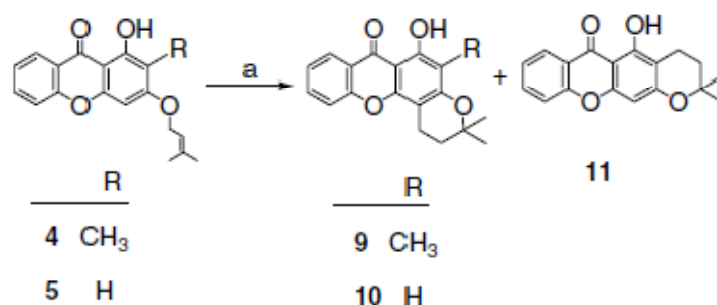
Figure 2.18: Prenylation of xanthone. Reagent and conditions: (a)ZnCl$_2$, *o*-xylene, 200°C, 21h.

In another study, Madalena Pedro *et al* (2002) assessed twenty-seven oxygenated xanthones for their capacity to inhibit *in vitro* growth of three human cancer cell lines, MCF-7, TK-10 and UACC-62. According to Madlanea Pedro *et al* (2002), no capacity to inhibit the growth of the human cancer cell lines was observed with unsubstituted xanthone even when tested at concentrations higher than 200µM, but the introduction of oxygenated groups in the xanthonic nucleus led to the appearance of a dose dependent growth inhibitory effect. Although this effect showed to be not very strong (GI$_{50}$>50 µM) for the majority of the xanthone derivatives, some xanthones derivatives exhibited interesting growth inhibitory effects on the human cancer cell lines tested with GI$_{50}$<20 µM. It was found that 1,2-dihydroxyxanthone, 3,4-dihydroxyxanthone, and 2,3-dihydroxyxanthone-4-methoxyxanthone were significantly much more active to

the melanoma UACC-62 cell line than to MCF-7 and TK-10. The dihydroxylation at 1,2-, 2.3-, 3,4- and 3,5- positions of the xanthonic nucleus was associated with compounds that showed growth inhibitory effects that were significantly stronger to all the three cell lines than those presented by 1,2-, 2.3-, 3,4-, and 3,5- dimethoxyxanthones. Similarly, the hydroxyl and methoxy substituents at 1,2-, 3,4- and 3,5- positions were associated with a significant increase of activity when compared with dimethoxyderivatives in the same positions. They noted that the hydroxyl-methylation at 3,4 position caused a complete loss of activity of the compound and the improvement of the activity from 3-hydroxy-4-methoxyxanthone to 3,4 dihydroxyxanthone is also remarkable especially for UACC cell line.

2.3.3 Cytotoxic Activity of Natural Xanthone Derivatives

According to Nawong Boonnak *et al* (2006), they isolated 31 compounds from the roots and barks of *Cratoxylum formosum* ssp. *Pruniflorum*. There were 24 xanthones reported among these 31 compounds. Cytotoxicity against MCF-7 (breast adenocarcinoma), HeLa (Human cervical cancer), HT-29 (colon cancer) and KB (hunan oral cancer) cell lines was evaluated. Gerontoxanthone I strongly inhibited all cancer cell lines compared to camptothecin whereas β-mangostin, α-mangostin, 3,4-dihydrojacareibun showed less inhibitory activity than

Gerontoxanthone I. Other xanthones were found to be inactive for cytotoxic acitivity.

Eight prenylated xanthones, together with seven other known compounds, were isolated from the acetone extracts of the twigs of *Garcinia xipshuanbannaensis* by Quan-Bin Han *et al* (2008). The cytotoxic activities of these compounds were evaluated using the MTT method. They concluded that xanthones with an unsaturated prenyl group had stronger cytotoxic activity against cancer cells, whereas those with hydroxylated prenyl groups had none. They also found that the number of unsaturated prenyl substitutes can significantly affect the cytotoxic activity of xanthone compounds: the more prenyl groups, the more potent effect, which had been found in their previous study (Quan-Bin Han *et al.*, 2007)

Naonobu Tanaka *et al* (2009) isolated a series of known xanthones and other compounds from stems of *Hypericum chineses*. They evaluated the cytotoxicities of the isolated xanthone derivatives as well as additional 32 xanthones against a panel of human cancer cell lines. They found that although most xanthones were non-cytotoxic, some xanthones were more sensitive against MDR cancer cell lines (KB-C2 and K562/Adr). In addition, some xanthones displayed enhanced cytotoxicities against MDR cancer cells in the presence of colchicines. Since MDR is one of major obstacles to cancer chemotherapy

overcoming MDR is most important to succeed in cancer chemotherapy. These xanthones are considered to be leads for the chemotherapeutic agents against MDR in cancer, and would provide more potent derivative with a suitable modification.

In the investigation by K.A. Shadid *et al* (2007), the isolated compounds shown in the **Figure 2.19** were assayed for their cytotoxic activity towards four cancer cell-lines, namely MDA-MB-231, MCF-7, CaOV-3, HeLa. Compounds **2,4-6** exhibited strong activity against the four cell-lines tested, with IC_{50} values ranging from 0.22 to 7μg/ml, except for compound **6** which was weakly cytotoxic against MDA-MB-231 cell line. Compounds **1** and **3** exhibited moderate to weak activity with IC_{50} varies ranging from 9.67 to 27.50 μg/ml, respectively. They found that the caged nature of ring-B as well as the presence of the peri-hydroxyl group on ring-A are important structure features for bioactivity. These are in agreement to previous findings (Cao *et al*., 1998; Macken *et al*., 2000). Besides, they noted that prenylation on C-2 appears to reduce the cytotoxic acitivity of this class of compounds quite significantly as indicated by the weak cytotoxic activity exihibited by compound **1** (**Figure 2.19**)
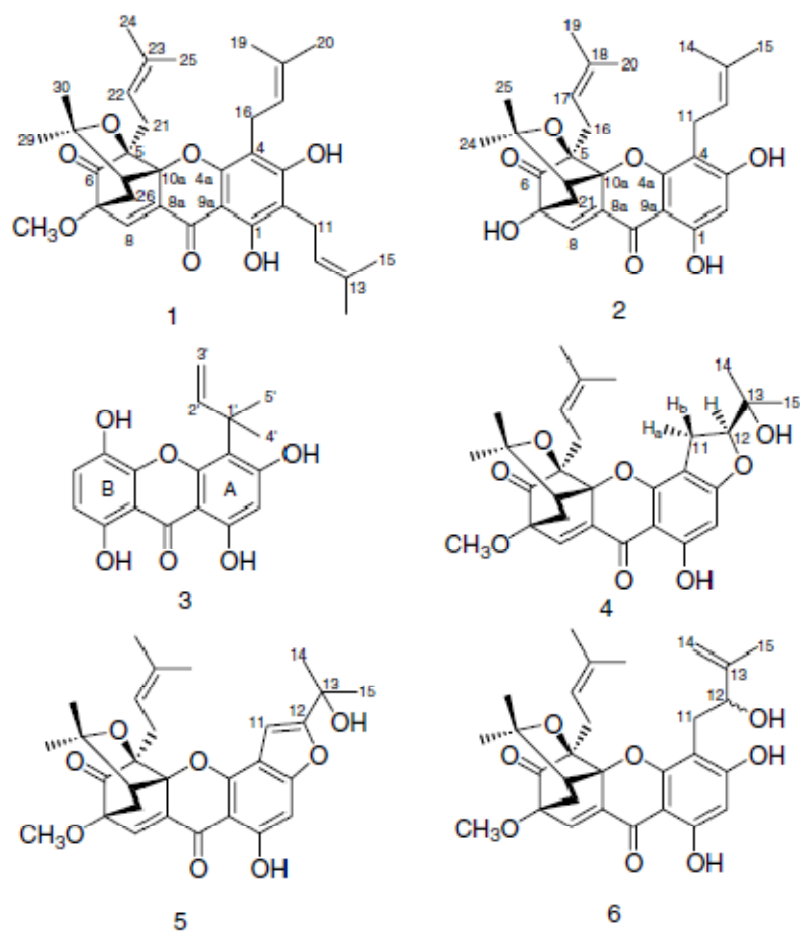
Figure 2.19: Structures of cantleyanone A (1), 7-hydroxyforbesione (2), 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone (3) cantleyanones B-D (4-6)

Chapter 3

MATERIALS AND METHODS

3.1 Chemicals

Chemicals used in the synthesis of 1,3,7-trihydroxyxanthone are listed below:

Table 3.1: Chemicals used in synthesis of 1,3,7-trihydroxyxanthone

Chemical Name	Molecular formula	Source	Molecular weight, (g/mol)
2,5-dihydroxybenzoic acid	$C_7H_6O_4$	Acros Organics	154.12
1,3,5-trihydroxybenzene (phloroglucinol)	$C_6H_6O_3$	Sigma-Aldich	236.11
Eaton's reagent (Phosphorus pentoxide in methanesulfonic acid)	$P_2O_5/MeSO_3H$	Acros Organics	-

Chemicals used in the prenylation of 1,3,7-trihydroxyxanthone are listed below:

Table 3.2: Chemicals used in prenylation of 1,3,7-trihydroxyxanthone

Chemical Name	Molecular formula	Source	Molecular weight, (g/mol)
3,3-dimethylallyl bromide	C_5H_9Br	Sigma-Aldrich	149.03
Hydrochloric acid (37%)	HCl	Fisher Scientific	36.46
Potassium carbonate	K_2CO_3	John Kollin Corporation	138.20
Acetone	CH_3COCH_3	QReC	-

Solvents used in purification of synthesized compounds are listed below:

Table 3.3: Solvents used in purification of synthesized compounds

Chemical Name	Molecular formula	Source	Density (g/ml)
Hexane	$CH_3(CH2)_4CH_3$	Merck	0.659
Dichloromethane	CH_2Cl_2	Fisher Scientific	1.325
Ethyl acetate	$CH_3COOC_2H_5$	LAB-SCAN	0.902
Acetone	CH_3COCH_3	QReC	0.791
Methanol	CH_3OH	Mallinckrodt chemicals	0.791

Table 3.4: Chemical used in determination of cytotoxicity of xanthones

Chemical Name	Source	Country
MTT	Sigma-aldrich	USA
DMSO	Fisher Scientific	UK
RPMI 1640 media	Cellgro	Manassas
Fetal Bovine Serum (FBS)	Hyclone Thermo Scientific	South America
96-cell plate	TPP	Europe
HeLa Cell	ATCC	USA
MDA-MB-231	ATCC	USA

Table 3.5: Deuterated solvents used in NMR

Chemical name	Source	Country
$CDCl_3$	Merck	Germany
Methanol-D4	Merck	Germany
Acetone-D6	Merck	Germany

3.2 Instruments

3.2.1 Infrared Spectroscopy (IR)

An infrared spectrophotometer is an instrument that passes infrared light through an organic molecule and produces a spectrum that contains a plot of the amount of light transmitted on the vertical axis against the wavelength of infrared radiation on the horizontal axis. In infrared spectra the absorption peaks point downward because the vertical axis is the percentage transmittance of the radiation through the sample. Absorption of radiation lowers the percentage transmittance value. Since all bonds in an organic molecule interact with infrared radiation, IR spectra provide data on the identities of functional groups. In this project, Perkin Elmer 2000-FTIR spectrophotometer was used for sample analysis in the range of 4000 cm^{-1} – 400 cm^{-1}. The sample pellet was prepared by grinding sample with KBr powder in ratio of 1:10 and compressed under high pressure.

3.2.2 Melting Point Instrument

Melting point is a physical property that is intrinsic to a compound when an organic compound is pure. Thus, by comparing melting point of a sample to the pure compound, the purity of the sample is tested. In this project, Barnstead Electrothermal 9100 melting point apparatus was used to determine the melting point of the obtained xanthones. The sample was introduced into haematocrit capillaries in solid form and inserted into the apparatus after calibration of apparatus had been carried out.

3.2.3 Nuclear Magnetic Resonance (NMR)

NMR spectroscopy exploits the magnetic properties of certain nuclei. When nucleus is placed in a magnetic field, energy is transferred into the spinning system in the form of radiofrequency to change the spin states. After excitation, relaxation of spinning system occurs and weak signal is released. Since every nucleus feels different magnetic field from their neighbors, different signals can be analyzed based on different environment of the atom. Thus, structure of the molecule can be determined from these signals. In this project, 400MHz JEOL NMR spectrometer was used for sample analysis. ^{1}H- & ^{13}C-NMR, HMBC and

HMQC spectra were obtained by using trimethylsilane (TMS) as internal standard and reference. The samples were prepared by dissolving a small amount of sample in sufficient acetone-d_6 or chloroform-d_6 and filled into a NMR tube to a height of 4 cm and was capped and wrapped with parafilm to prevent solvent evaporation.

3.2.4 Ultraviolet-Visible Spectrophotometer (UV-Vis)

The wavelength at the maximum of the absorption band in the UV-Vis spectrum gives information about the structure of the molecule or ion. In this project, Perkin-Elmer Lambda (25/35/45) UV-Vis spectrophotometer was used for sample analysis. The sample was prepared by dissolving sample with ethanol and measured using quartz cuvette in the range of 100 nm to 800 nm.

3.3 Synthesis Methodology

3.3.1 Synthesis of 1,3,7-Trihydroxyxanthone

7.71 g (50mmol) of 2,5-dihydroxybenzoic acid and 6.3 g (50mmol) of phloroglucinol were mixed in a 25 mL round-bottomed flask. Eaton's reagent was then added into the solution slowly. The mixture was warmed in water bath at 90°C for 30 minutes under stirring. It was followed by cooling the reaction mixture to room temperature. The reaction mixture was then poured into a 1L beaker that was filled with cold ice water. The mixture in the beaker was stirred for 2 hours. The precipitate was filtered using Buchner filtration. The product was then dried in an oven at 60°C.

2,5-dihydroxybenzoic acid   phloroglucinol

1,3,7-trihydroxyxanthone **(1)**
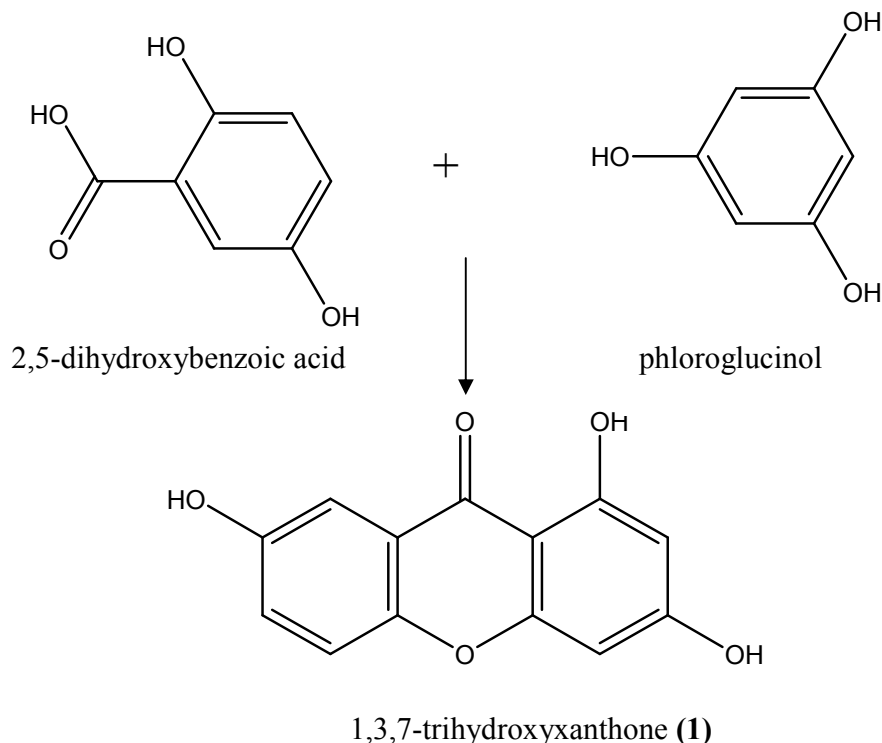
Figure 3.1: Synthesis of 1,3,7-trihydroxyxanthone

3.3.2 Prenylation of 1,3,7-Trihydroxyxanthone in Organic Phase

A mixture of 0.9768 g (4 mmol) of 1,3,7-trihydroxyxanthone and 1.656 g (12 mmol) of K_2CO_3 was stirred for 5 minutes in 100mL of acetone under room temperature. 2.384g (16mmol) of prenylbromide was added into the solution. The solution was then refluxed for 6 hours. After reflux, the solid was filtered and filtrate was dried under reduced pressure.

1,3,7-trihydroxyxanthone

Prenyl bromide

K_2CO_3 in acetone

1,7-diHydroxy-3-(3-methylbut-2-enyloxy)xanthone **(2)**

+

1,7-diHydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)**
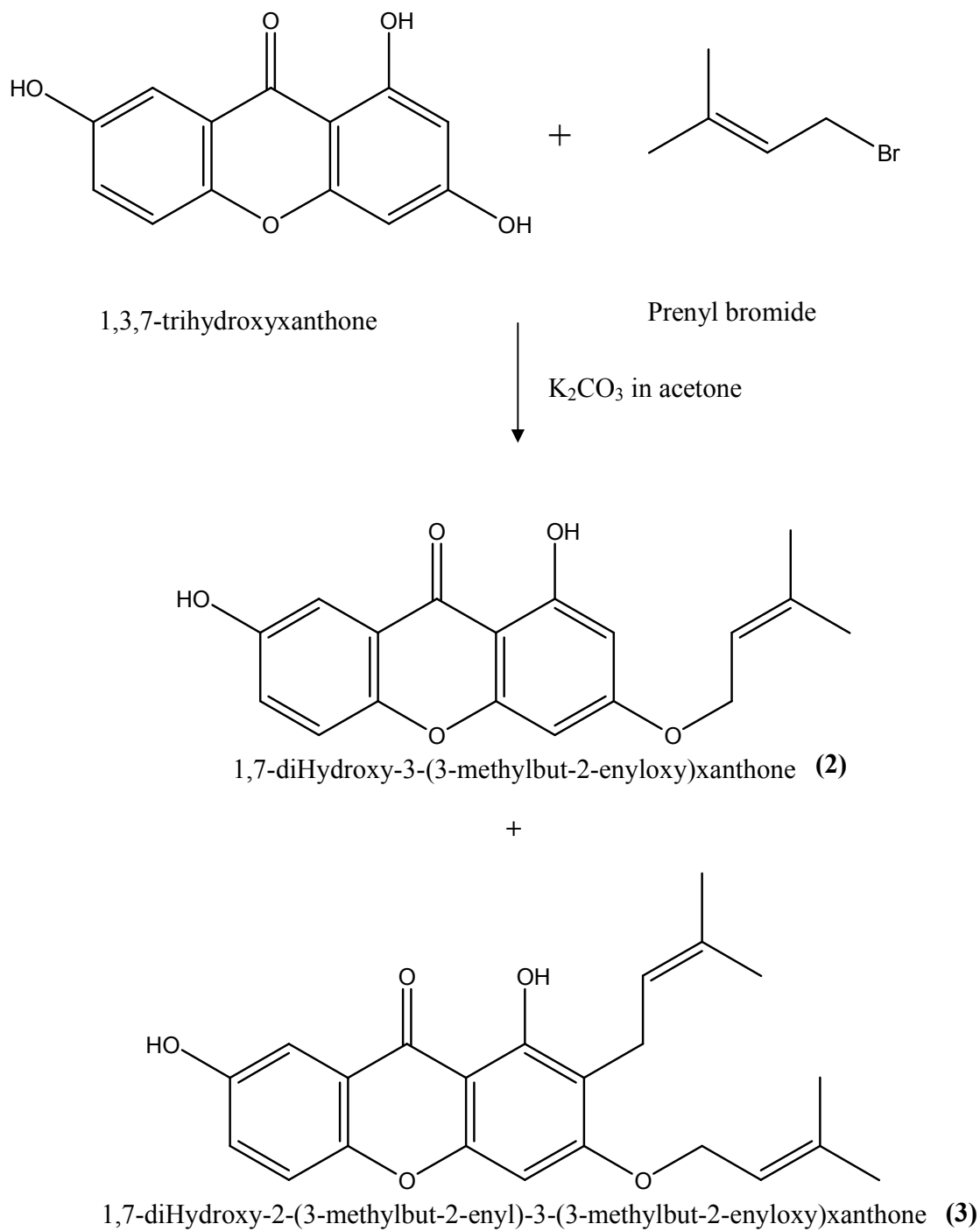
Figure 3.2: Prenylation of 1,3,7-trihydroxyxanthone in organic medium

58

3.3.3 Prenylation of 1,3,7-Trihydroxyxanthone in Aqueous Medium

A mixture of 1.952 g (8mmol) of 1,3,7-trihydroxyxanthone and 35g of K_2CO_3 was stirred for 5 minutes in 100mL of H_2O under room temperature. 5.96 g (40mmol) of prenylbromide was added into the solution. The solution was then refluxed for 6 hours. After reflux, the solid was filtered and filtrate was dried under reduced pressure.

1,3,7-trihydroxyxanthone

prenyl bromide

K_2CO_3 in H_2O

1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone **(4)**
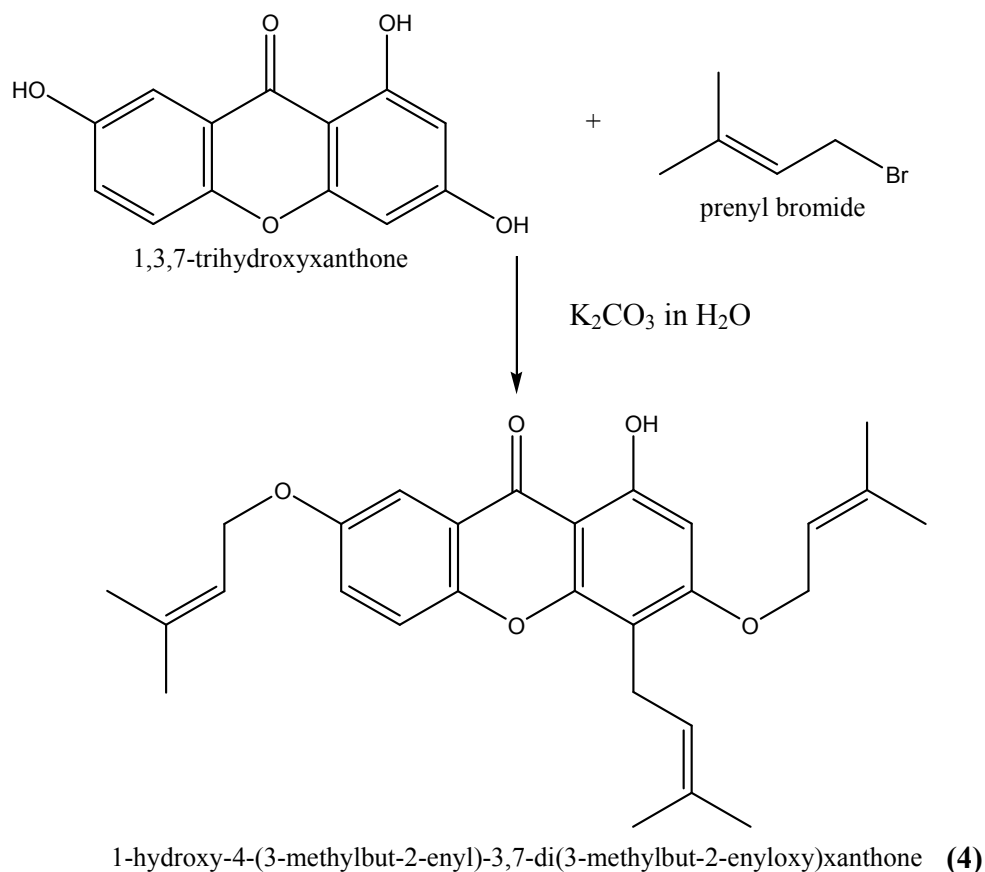
Fig 3.3 Prenylation of 1,3,7-trihydroxyxanthone in aqueous medium

3.4 Chromatographic Methods

3.4.1 Column Chromatography

Sample was dissolved in a small amount of solvent and mixed with silica gel. Saclicycle silica gel of 40-63μm diameter was used as packing materials of column chromatography. Hexane-dissolved silica gel (slurry) was introduced into a column. After eluting the column for at least three times, the sample was then introduced into the column. Separation of the compounds was carried out by eluting the column with gradient elution, starting from less polar solvent. The eluents were collected into beakers. The fractions were monitored by TLC.
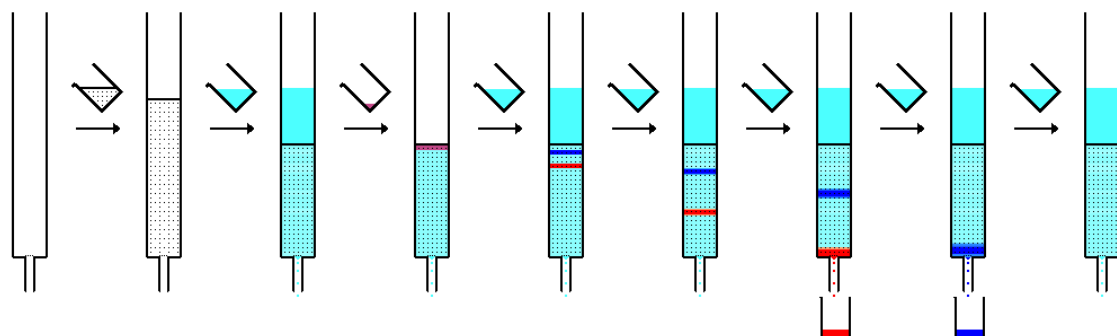
Figure 3.4: Gravity column chromatography sequence

3.4.2 Thin Layer Chromatography (TLC)

Thin layer chromatography was done by using an aluminum plate of dimensions 4.0 cm x 6.7 cm coated with silica gel 60 F_{254} (Merck 1.05554.0001). The dissolved sample was dotted on the baseline at the bottom of the TLC plate by a capillary tube. The plate was then put into and developed in a chromatographic chamber filled with 4 ml of mobile phase with desired composition. The plate was taken out from the chamber when the solvent reached solvent front. The plate was then visualized under UV lamp with both the short (254nm) and long (366nm) wavelengths as shown in **Figure 3.5**. R_f value of each spot was calculated according to the equation below:

$$R_f = \frac{\text{distance of the spot on the TLC-plate}}{\text{distance of the solvent front}}$$
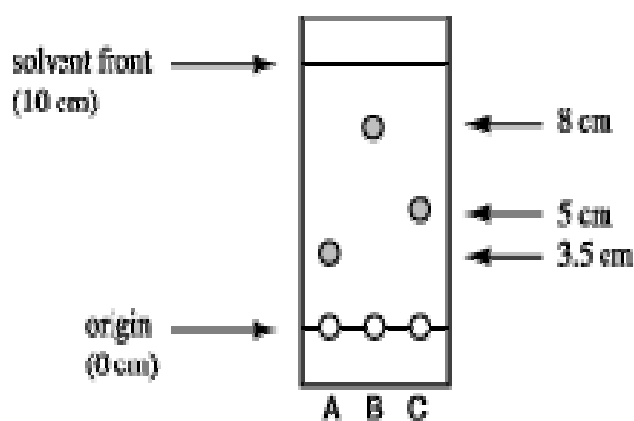
Figure 3.5: Developed TLC plate

3.5 Cytotoxic Assay

The cell viability was evaluated using the colorimetric 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assay. In this MTT assay, HeLa cancer cells and MDA-MB-231 cancer cells were tested. Both of HeLa cells (0.75 x 10^5 cells/mL) and MDA-MB-231 cells (3.5 x 10^5 cells/mL) were cultured in 96-well plates with 0.1 % dimethyl sulfoxide (DMSO) containing drugs at 37 ^{o}C for 72 hours. Blank cell control and blank medium control were also prepared and incubated for 72 hours. After treatment, the cells received 20 μL of 5 mg/mL MTT and were incubated at 37 ^{o}C for another 3 hours. After 3 hours, 70 % of supernatant was removed and 150 μL of DMSO was added into each well. DMSO dissolved the formazan crystal, produced a purple solution. Cell viability was determined by measuring optical density at 570 nm using a Model 550 micro plate reader (Bio-Rad Laboratories, Hercules, CA, USA). Cell viability was calculated as followed:

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

Where x= average absorbance of cell treated with compound

y= average absorbance of blank medium

z= average absorbance of cell control

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis of Xanthone Block

2,5-dihydroxybenzoic acid   +   phloroglucinol

1,3,7-trihydroxyxanthone **(1)**
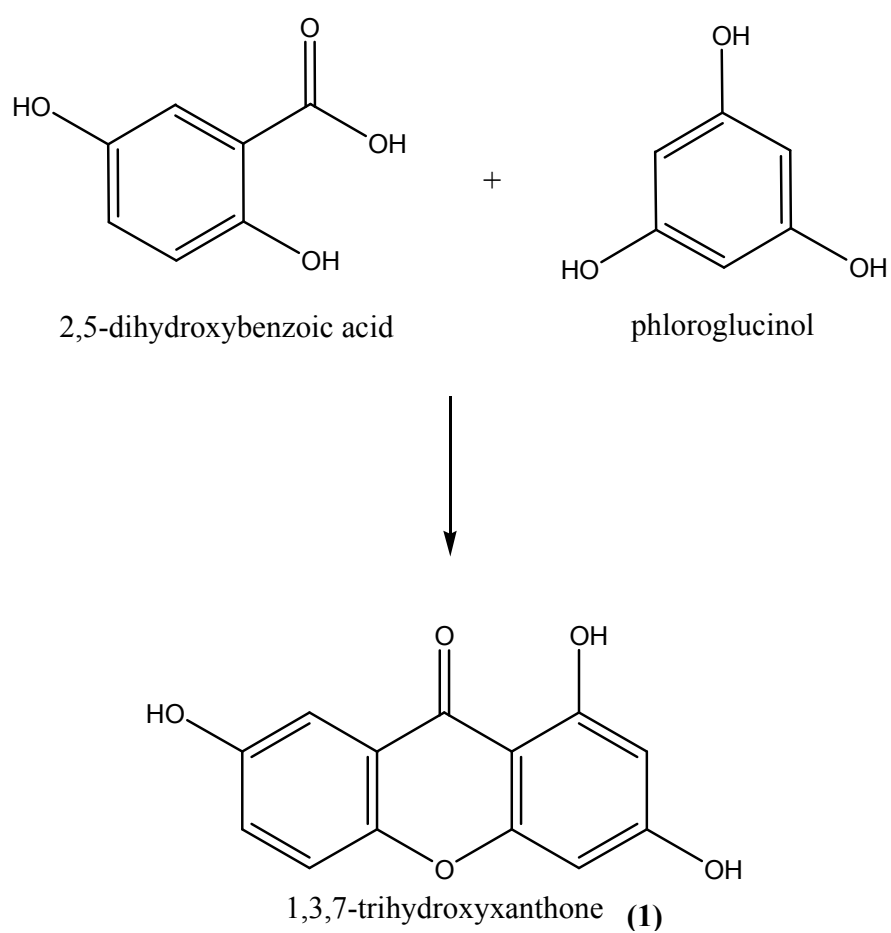
Figure 4.1 Synthesis of xanthone block

4.1.1 Proposed Mechanism of synthesis of Xanthone Block

$$P_4O_{10} + 6H_2O \longrightarrow 4H_3PO_4$$
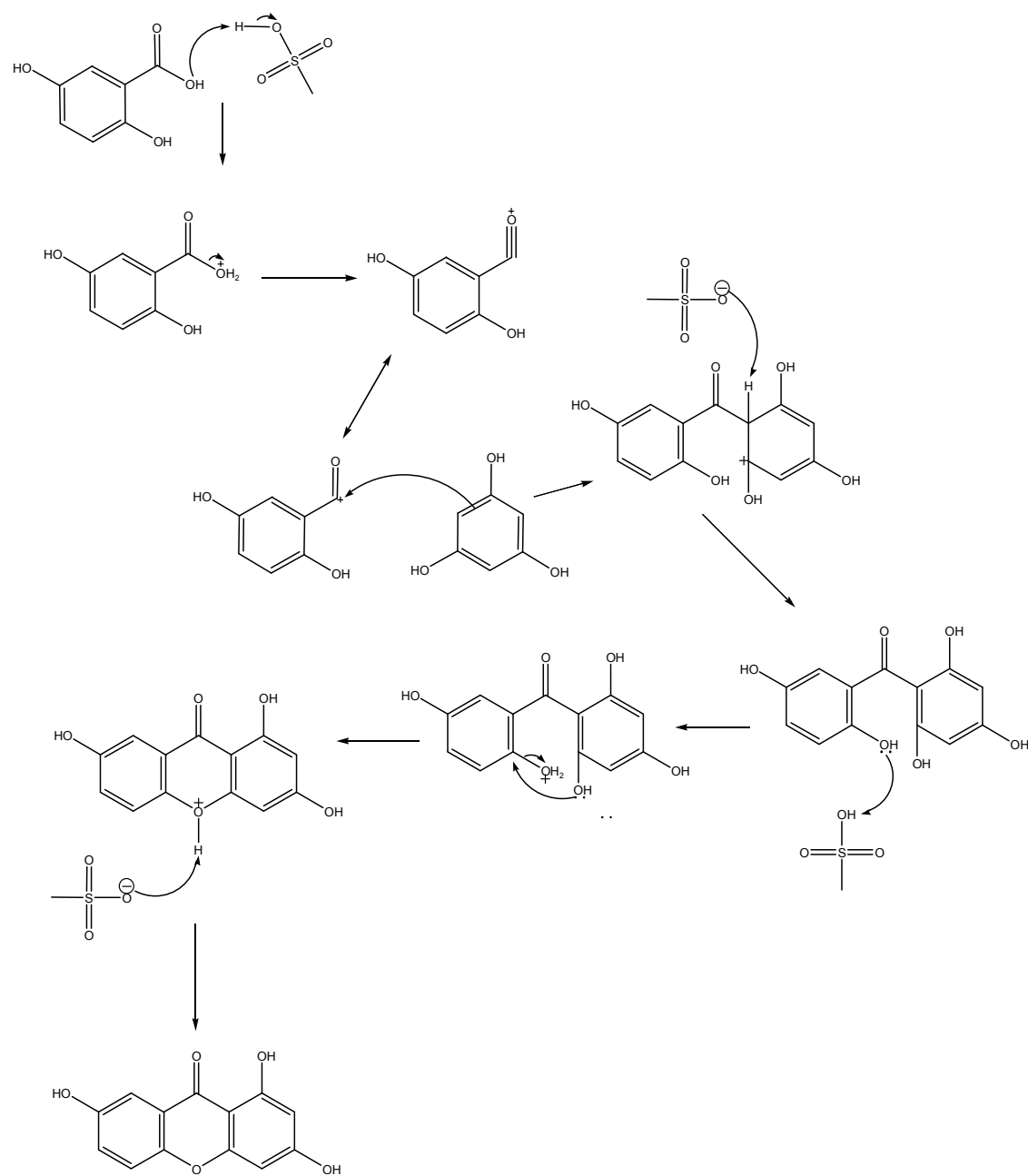
Figure 4.2: Proposed mechanism of xanthone block synthesis

4.1.2 Characterization of 1,3,7-Trihydroxyxanthone (1)

Compound **(1)** was isolated as yellow crystal, and found to have the molecular formula $C_{13}H_8O_5$, having a yield of 37.6% and melting point of 284-287 °C . Its IR spectrum exhibited absorption bands at 3246 (hydroxyl group), 1654 (conjugated carbonyl group) and 1589 cm^{-1} (benzene ring). These data and the UV absorption band at λ_{max} 204, 234, 258, 311, 372 nm for a conjugated carbonyl chromophore suggested that **(1)** is a polyoxygenated xanthone.

The ^{1}H NMR spectrum (**Figure 4.4**) revealed the presence of a chelated hydroxyl group at δ 12.94 (s, 1-OH), five aromatic protons at δ 6.21 (1H, d, J= 2.1 Hz), δ 6.35 (1H, d, J= 2.1 Hz), δ 7.38 (1H, d, J= 9.0 Hz), δ 7.30 (1H, dd, J= 9.0, 3.0 Hz), and δ 7.52 (1H, d, J= 3.0Hz). Both doublets observed at δ 6.21 and δ 6.35 were assigned to H-2 and H-4, respectively which are *meta*-coupling to each other with same coupling constant of 2.1 Hz. Doublet of doublets observed at δ 7.30 was assigned to H-6 because it shows couplings with H-5 (J=9.0Hz) and H-8 (J=3.0Hz). Doublet at δ 7.52 was assigned to H-8 because its smaller coupling constant of 3.0Hz compared to that of doublets at δ7.38 which is 9.0 Hz indicates that it is *meta*-coupling with H-6, whereas the latter is *ortho*-coupling with H-6.

The ^{13}C NMR spectrum **(Figure 4.5)** revealed 13 carbon signals, including one carbonyl carbon at δ 180.3, five oxgenated carbons at δ 163.7, δ 165.5, δ

158.1, δ 154.0, and δ 149.8, seven quaternary carbon at δ 97.8, δ 93.7, δ 118.9, δ 124.2, δ 108.4, δ 121.0 and δ 102.6.

The ¹H and ¹³C NMR data were comparable to those of 1,3,7-trihydroxyxanthone (Chang *et al.*, 2007), suggesting **(1)** is 1,3,7-trihydroxyxanthone. Thus, compound **(1)** was elucidated as the following structure in **Figure 4.3**.
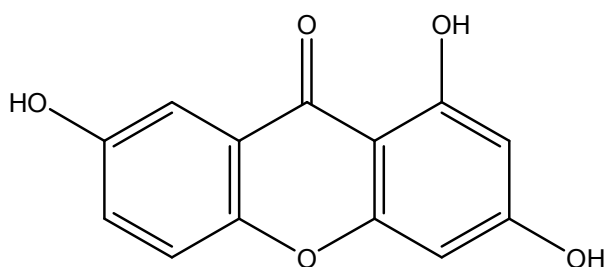
Figure 4.3: 1,3,7-trihydroxyxanthone(1)

Table 4.1: ^{1}H NMR (400MHz, Acetone-D$_6$) and ^{13}C NMR (100MHz, Acetone D$_6$) assignments of 1,3,7-trihydroxyxanthone (1)

Position	δ_H	δ_H (lit.)	δ_c	δ_c (lit.)
1	-	-	163.7	164.7
2	6.21 (1H, d, J= 2.1 Hz)	6.24 (1H, d, J= 2.1 Hz)	97.8	98.5
3	-	-	165.5	166.4
4	6.35 (1H, d, J= 2.1 Hz)	6.39 (1H, d, J= 2.1 Hz)	93.7	94.5
4a	-	-	158.1	159.1
5	7.38 (1H, d, J= 9.0 Hz)	7.47 (1H, d, J= 9.0 Hz)	118.9	119.7
6	7.30 (1H, dd, J= 9.0, 3.0 Hz)	7.30 (1H, dd, J= 9.0, 3.0 Hz)	124.2	125.0
7	-	-	154.0	154.7
8	7.52 (1H, d, J= 3.0Hz)	7.56 (1H, d, J= 3.0Hz)	108.4	109.2
8a	-	-	121.0	121.8
9	-	-	180.3	180.9
9a	-	-	102.6	103.5
10a	-	-	149.8	150.8
1-OH	12.94 (1H, s)	12.47 (1H, s)	-	-

(lit.: Chang *et al*., 2007)
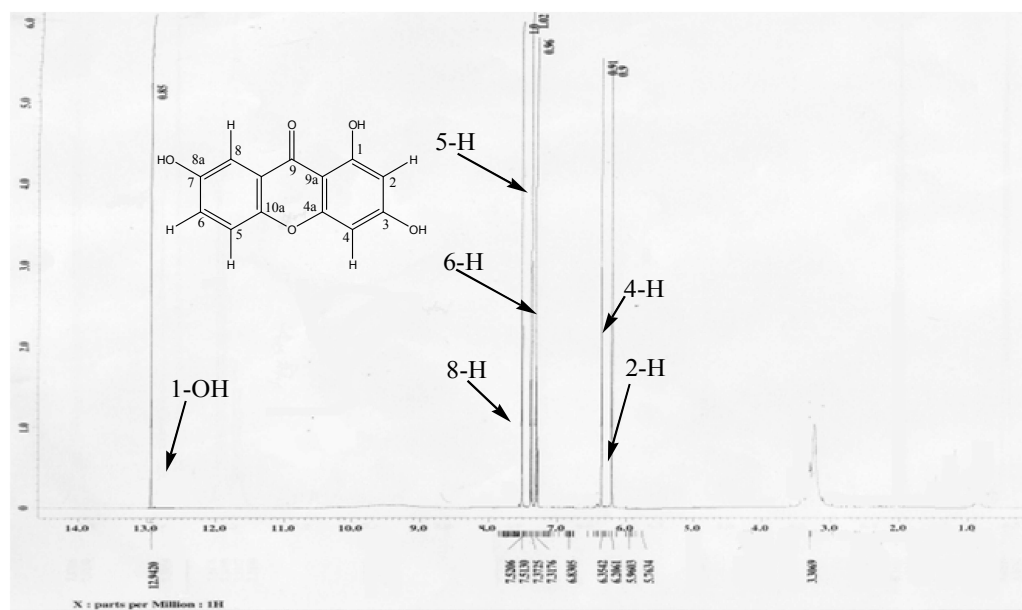
Figure 4.4: ^{1}H-NMR spectrum of 1,3,7-trihydroxyxanthone(1)
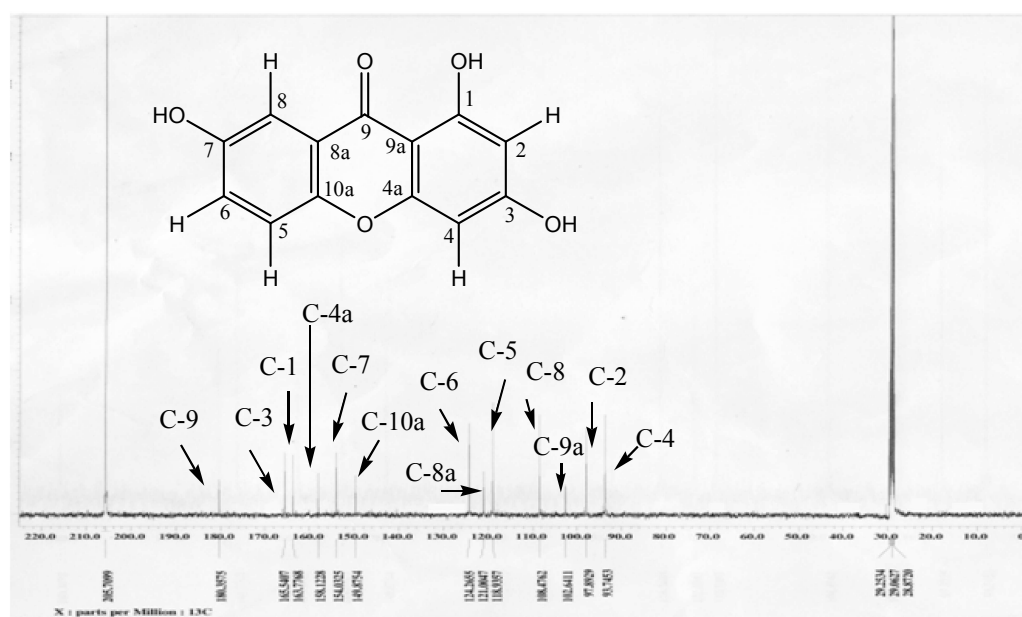
Figure 4.5: ^{13}C NMR spectrum of 1,3,7-trihydroxyxanthone(1)
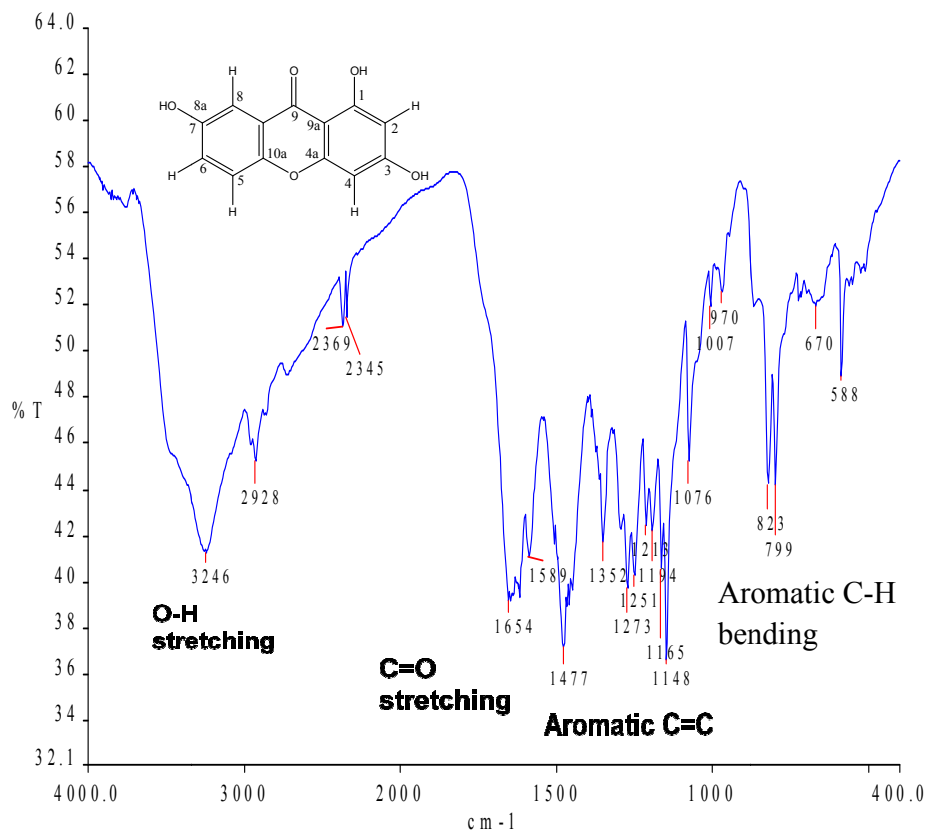
Figure 4.6: IR spectrum of 1,3,7-trihydroxyxanthone (1)
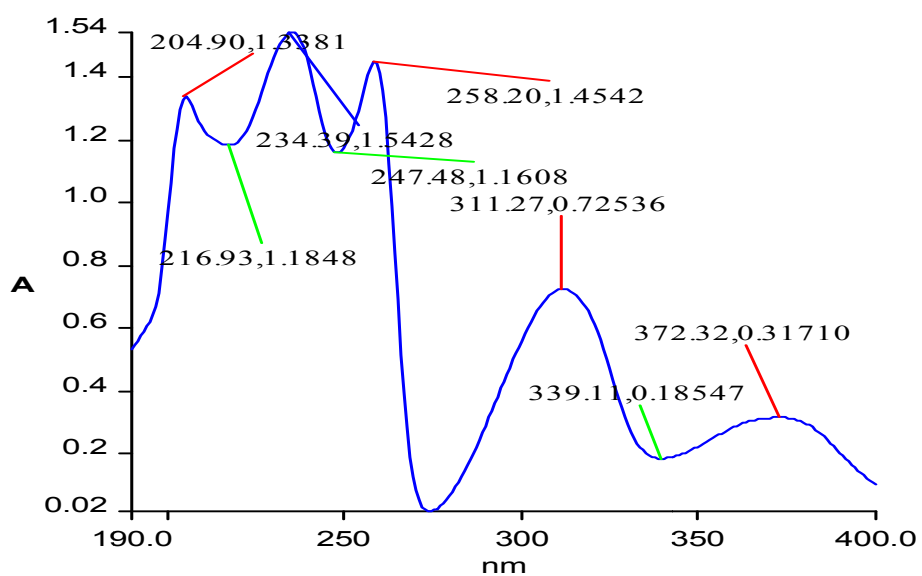
Figure 4.7: UV spectrum of 1,3,7-trihydroxyxanthone (1)

4.2 Prenylation of 1,3,7-Trihydroxyxanthone in Organic Medium

1,3,7-trihydroxyxanthone

prenyl bromide

1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone **(2)**

+

1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)**
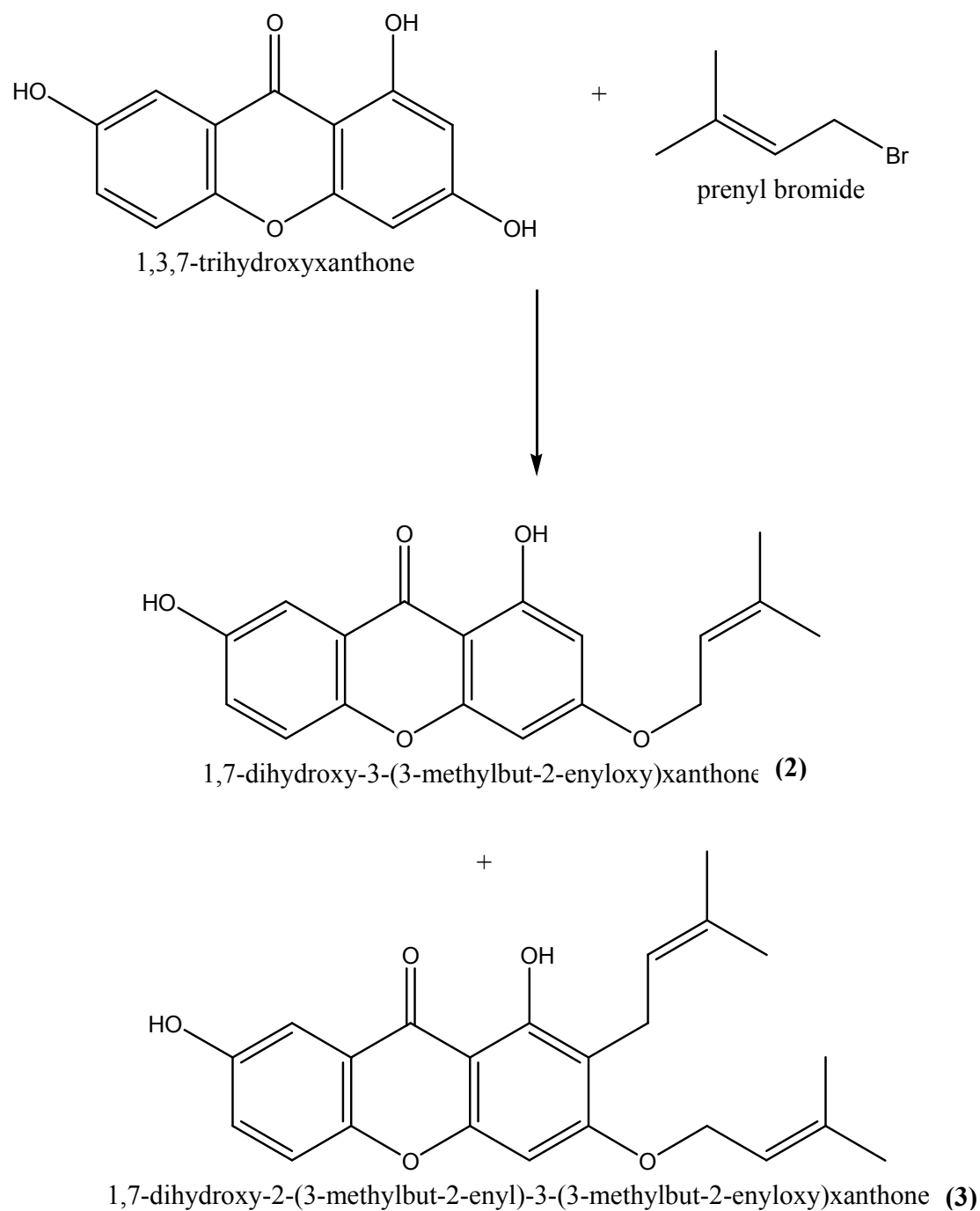
Figure 4.8: Prenylation of 1,3,7-trihydroxyxanthone in organic medium

4.2.1 Proposed Mechanism of Prenylation of 1,3,7-Trihydroxyxanthone in Organic Medium

(2)
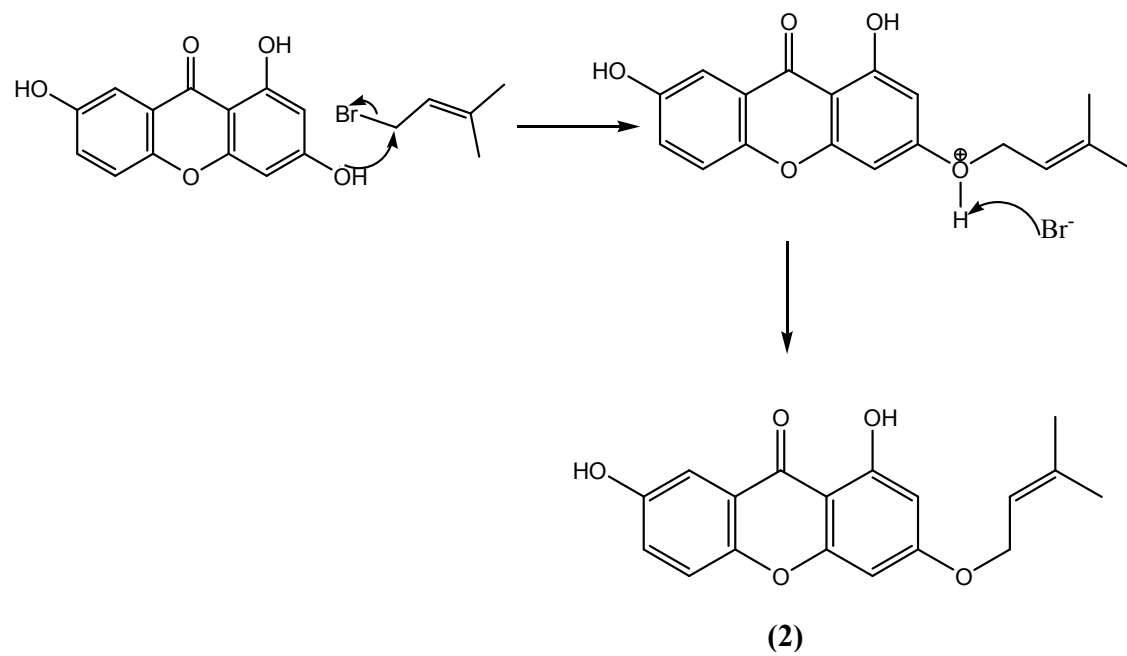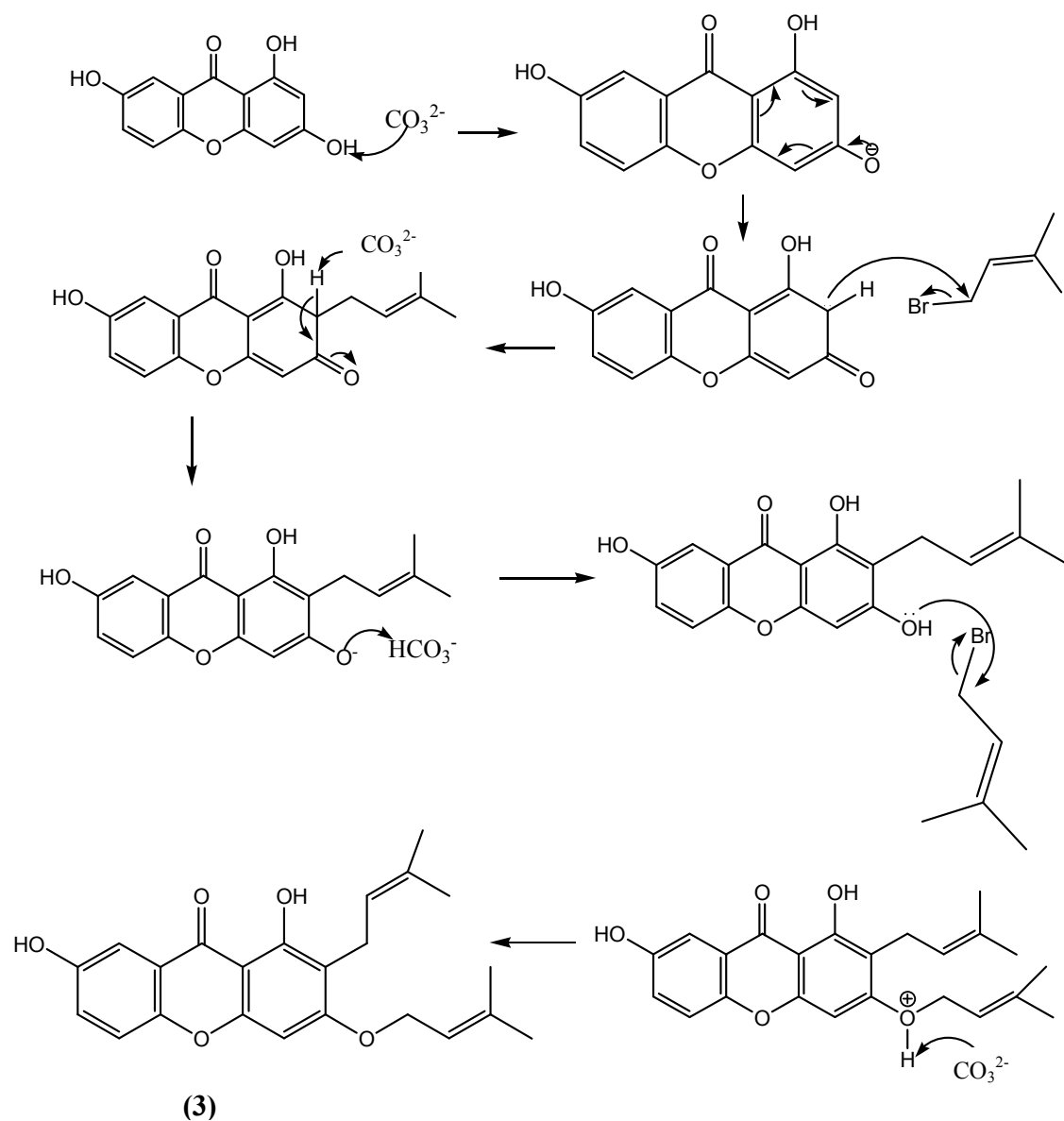
Figure 4.9: Proposed mechanism for formation of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone (2) and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)

4.2.2 Characterization of 1,7-Dihydroxy-3-(3-methylbut-2-enyloxy)xanthone (2)

Compound **(2)** was isolated as yellow crystal, and found to have the molecular formula $C_{18}H_{16}O_5$, having a yield of 2.02%, melting point of 159-164 $^{\circ}$C. Its IR spectrum exhibited absorption bands at 3434 (hydroxyl group), 2923, 2852 (C-H stretching), 1628 (conjugated carbonyl group). These data and the UV absorption band at λ_{max} 213 ,236, 311nm for a conjugated carbonyl chromophore indicated the compound to be polyhydroxyxanthone which was almost similar to the reported 1-hydoxy-3-(3-methylbut-2-enyloxy)xanthone **(Table 4.2)** (Castanheiro *et al.*,2007), suggesting that **(2)** has a close similarity with the structure.

The ^{1}H NMR spectrum (**Figure 4.12**) revealed the presence of a chelated hydroxyl group at δ 12.57 (s, 1-OH), two coupled protons at δ 6.36 (d, 1H, J = 2.1 Hz) and δ 6.43 (d, 1H, J = 2.1 Hz) which were assigned to two aromatic protons in the right ring of xanthone core, three aromatic protons at δ 7.48 (1H, d, J= 9.0 Hz), δ 7.64 (1H, dd, J= 9.0, 3.0 Hz), δ 8.07 (1H, d, J= 3.0Hz) in the left ring, one methylene proton at δ 4.59 (2H, d, J=6.7Hz), one olefinic proton at δ 5.48 (1H, t, J=6.7Hz), two methyl proton at δ 1.81(3H, s) and δ 1.76 (3H, s). The spectrum showed signals that evidence the presence of one 3,3-dimethylallyl (prenyl) group in the proposed structure.

The ^{13}C NMR spectrum (**Figure 4.13**) revealed signals for one carbonyl group at δ 179.6, seven quaternary aromatic carbon at δ 129.6, 121.5, 119.8, 118.5, 103.5, 98.1, 93.8, five oxygenated aromatic carbons at δ 166.6, 163.5, 157.5, 154.4, 144.9, five carbons in the prenyl group at δ 139.7, 118.4, 65.7, 25.9, 18.4.

The ^{13}C NMR data of compound **(2)** was compared to 1-hydoxy-3-(3-methylbut-2-enyloxy)xanthone reported in the literature (Castanheiro *et al.*, 2007) showing characteristic signals for the presence of one prenyl group. However, a closer examination of the ^{1}H spectrum showed that **(2)** is different from 1-hydroxy-3-(3-methylbut-2-enyloxy)xanthone in its left ring moiety. There was an absence of the H-7 signal seen in the ^{1}H spectrum of **(2)**, and the peak splittings shown at H-6 and H-8 are different from their counterparts in 1-hydoxy-3-(3-methylbut-2-enyloxy)xanthone, respectively. H-6 in **(2)** is a doublet of doublets which has *meta*-coupling (J= 3.0Hz) and *ortho*-coupling (J=9.0 Hz) whereas H-6 in 1-hydroxy-3-(3-methylbut-2-enyloxy)xanthone is a triplet of doublets which has two *ortho*-coupling (J=8.3, 7.2Hz) and one *meta*-coupling (J=1.6 Hz). H-8 in **(2)** gave a doublet which is *meta*-coupling (J= 3.0 Hz) whereas H-8 in 1-hydroxy-3-(3-methylbut-2-enyloxy)xanthone is a doublets of doublets which has both *meta*-coupling (J=1.6Hz) and *ortho*-coupling (J=7.9Hz). The only explanation is that 7-position is replaced with other substituent, in this case, a hydroxyl group. The ^{13}C spectrum of **(2)** showed a peak at δ154.4 which was assigned to

oxygenated aromatic carbon, further confirming the presence of a hydroxyl group at carbon position C-7.

The position of the substituents on xanthone skeleton was determined on the basis of HMQC and HMBC (**Figure 4.14 and 4.15**). In the HMBC spectrum, cross-peaks for the chelated hydroxyl proton{δ_H 12.57(1-OH)/ δ_C 163.5 (C-1), δ_C 103.5 (C-9a), δ_C 98.1 (C-2)} , and the *meta*-coupled pair of aromatic protons { δ_H 6.36 (H-2)/ δ_C 163.5 (C-1), δ_C 166.6 (C-3), δ_C 93.8 (C-4), δ_C 103.5 (C-9a) and δ_H 6.43(H-4)/ δ_C 98.1 (C-2), δ_C 166.6 (C-3), δ_C 144.9 (C-4a), δ_C 103.5 (9a)}were observed, confirming a disubstituted ring feature for **(2),** with hydroxyl groups located at C-1. Similarly, HMBC cross-peaks observed for the two doublet aromatic protons { δ_H 7.48(H-5)/ δ_C 154.4 (C-7), δ_C 121.5 (C-8a), δ_C 144.9 (C-10a) and δ_H 8.07(H-8)/ δ_C 129.6(C-6), δ_C 154.4(C-7), δ_C 144.9(10a)} and one doublet of doublets aromatic proton { δ_H 7.64(H-6)/ δ_C 154.4 (C-7), δ_C 144.9(C-10a) δ_C 118.5(C-8)} established a monosubstituted ring feature to the second ring of the xanthone , with hydroxyl groups located at C-7. The set of signals at δ_H 4.59 (2H, d, H-1'), δ_H 5.48 (1H, t, H-2'), δ_H 1.81 (3H, s, H-4'), δ_H 1.76 (3H, s, H-5') in the ^{1}H spectrum was established to be a 3,3-diallylmethyl (prenyl) group attached to oxygen on C-3 based on the long range coupling between H-1' and C-3 as displayed by correlations { δ_H 4.59/δ_C 166.6(C-3), δ_C 139.7(C-3')} in the HMBC spectrum (**Figure 4.15**). Thus, **(2)** was elucidated to have the following structure in **Figure 4.11**, and was given name as 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone. This structure was further confirmed by the HMQC spectrum

which shows cross-peaks for five aromatic protons {δ_H 8.07/ δ_c 118.5, δ_H 7.64/ δ_c 129.6, δ_H 7.48/ δ_c 119.8, δ_H 6.43/ δ_c 93.8, δ_H 6.36/ δ_c 98.1}, one olefinic proton {δ_H 5.48/ δ_c 118.4}, one methylene proton {δ_H 4.59/65.7}, two methyl protons {δ_H 1.81 δ_c 25.9/, δ_H 1.76/ δ_c 18.4}. The ^{1}H and ^{13}C NMR data of **(2)** were assigned based on the HMQC, and HMBC correlation data.
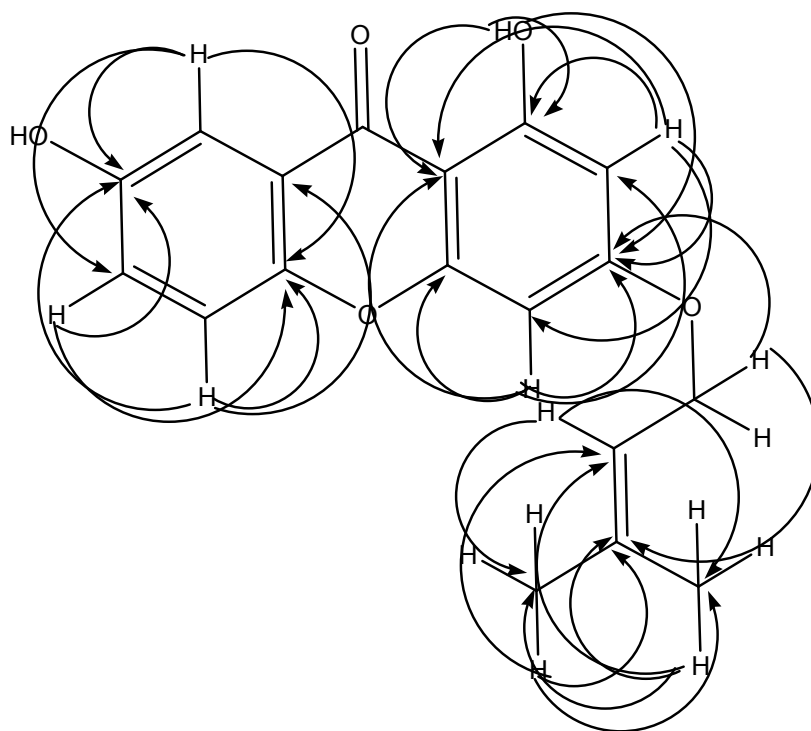
Figure 4.10: Main connectivities found in the HMBC of 1,7-dihydroxy-3-(3-methylbut-2- enyloxy)xanthone (2)
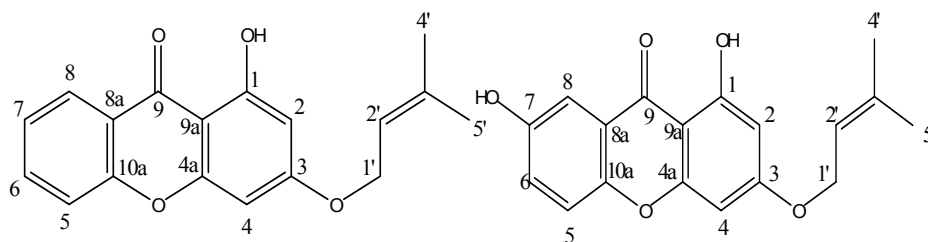
Table 4.2: [^1]H chemical shifts of **1-hydroxy-3-(3-methylbut-2-enyloxy)xanthone** (R.A.P. Castanheiro *et al.*,2007) **and 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone (2)**

	δ_H, 1-hydroxy-3-(3-methylbut-2-enyloxy)xanthone	δ_H, 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone (2)
H-1	12.85 (OH, s)	12.57 (OH, s)
H-2	6.34 (d, J=2.2)	6.36(1H, d, J= 2.1 Hz)
H-4	6.42 (d, J=2.2)	6.43(1H, d, J= 2.1 Hz)
H-5	7.41 (d, J=8.3)	7.48(1H, d, J= 9.0 Hz)
H-6	7.69 (ddd, J=8.3,7.2,1.6)	7.64(1H, dd, J= 9.0, 3.0 Hz)
H-7	7.36 (t,J=7.9,7.2)	-
H-8	8.23 (dd, J=7.9,1.6)	8.07(1H, d, J= 3.0Hz)
H-1'	4.59 (d, J=6.7)	4.59 (2H, d, J=6.7Hz)
H-2'	5.50 (t, J=6.7)	5.48 (1H, t, J=6.7Hz)
H-4', H-5'	1.82,1.77 (2s)	1.81, 1.76 (2s)
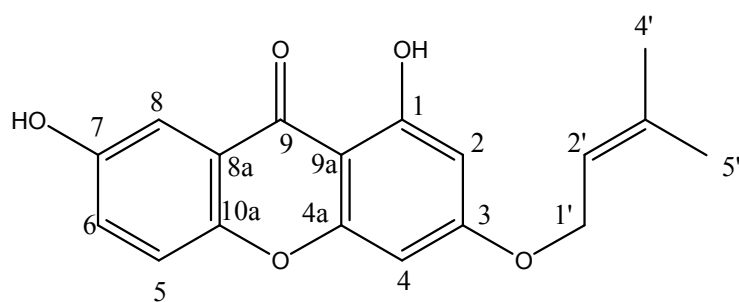
Figure 4.11: 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone (2)

Table 4.3: ^{1}H NMR (400MHz, CDCl$_3$) and ^{13}C NMR (100MHz, CDCl$_3$) assignments of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone (2)

Position	δ_H	δ_c	HMBC
1	-	163.5	-
2	6.36(1H, d, J= 2.1 Hz)	98.1	C- 1(^{2}J), 3(^{2}J), 4(^{3}J),9a(^{3}J)
3	-	166.6	-
4	6.43(1H, d, J= 2.1 Hz)	93.8	C- 2(^{3}J),3(^{2}J),4a(^{2}J),9a(^{3}J)
4a	-	157.5	-
5	7.48(1H, d, J= 9.0 Hz)	119.8	C- 7(^{3}J),8a(^{3}J),10a(^{2}J)
6	7.64(1H, dd, J= 9.0, 3.0 Hz)	129.6	C- 7(^{2}J),10a(^{3}J)
7	-	154.4	-
8	8.07(1H, d, J= 3.0Hz)	118.5	C- 6(^{3}J),7(^{2}J),10a(^{3}J)
8a	-	121.5	-
9	-	179.6	-
9a	-	103.5	-

10a	-	144.9	-
1'	4.59 (2H, d, J=6.7Hz)	65.7	C-3(^{3}J),3'(^{3}J)
2'	5.48 (1H, t, J=6.7Hz)	118.4	C- 4'(^{3}J),5'(^{3}J)
3'	-	139.7	
4'	1.81(3H, s)	25.9	C-2'(^{3}J),3'(^{2}J),5'(^{3}J)
5'	1.76 (3H, s)	18.4	C-2'(^{3}J),3'(^{2}J),4'(^{3}J)
1-OH	12.57(1H, s)	-	C- 1(^{2}J),2(^{3}J),9a(^{3}J)
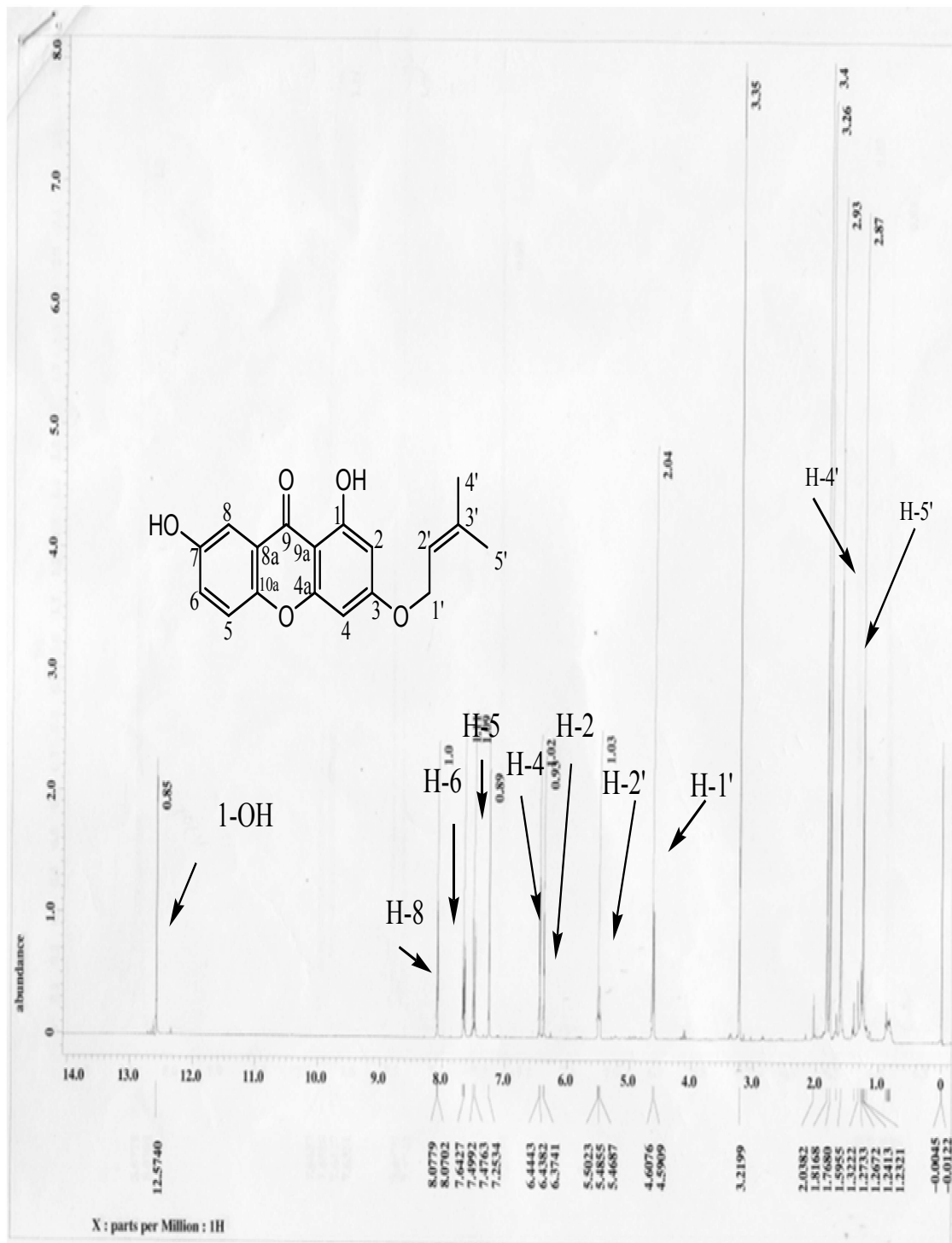
Figure 4.12: ^{1}H NMR spectrum of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone(2)
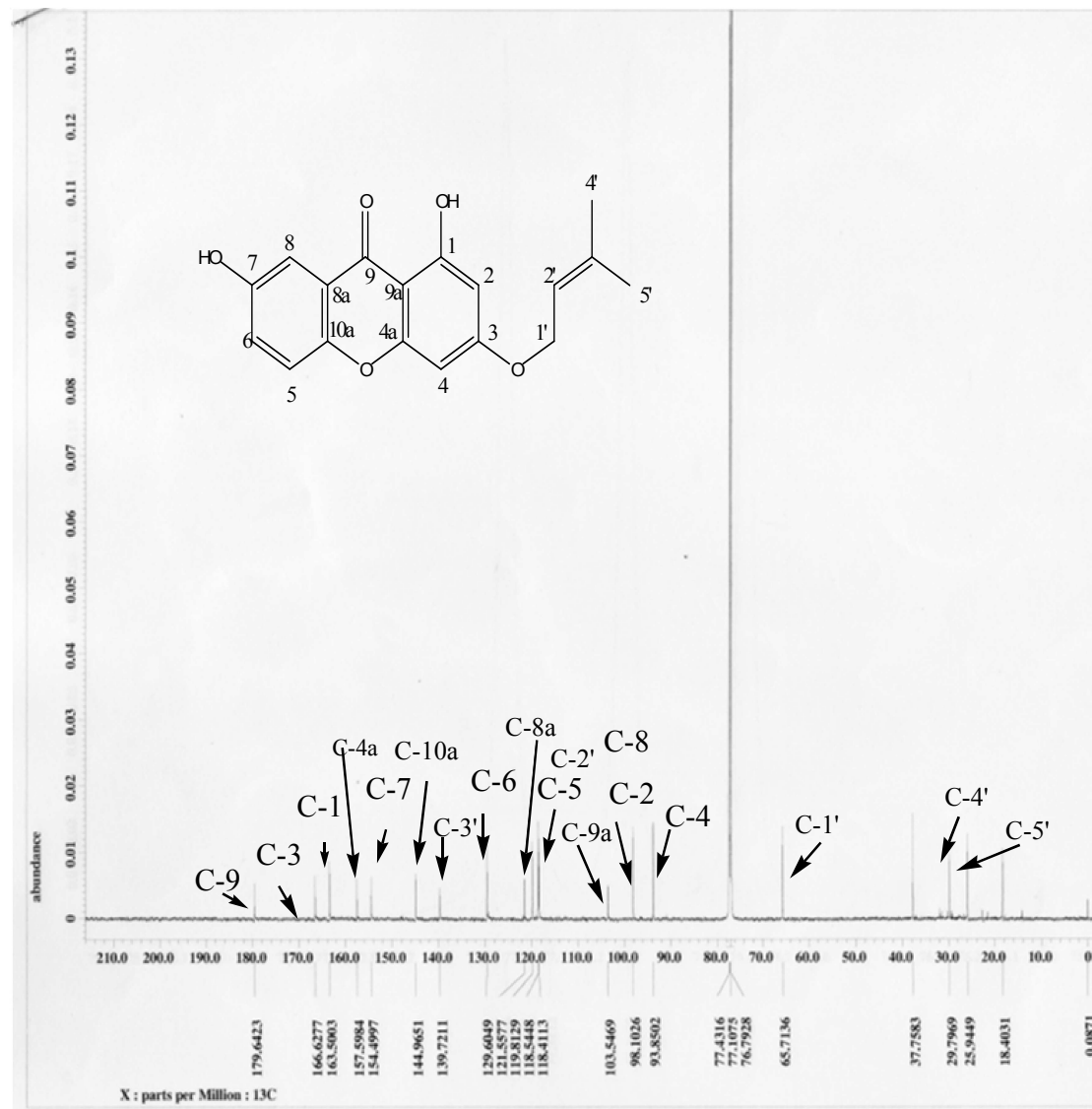
Figure 4.13: ^{13}C NMR spectrum of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone(2)
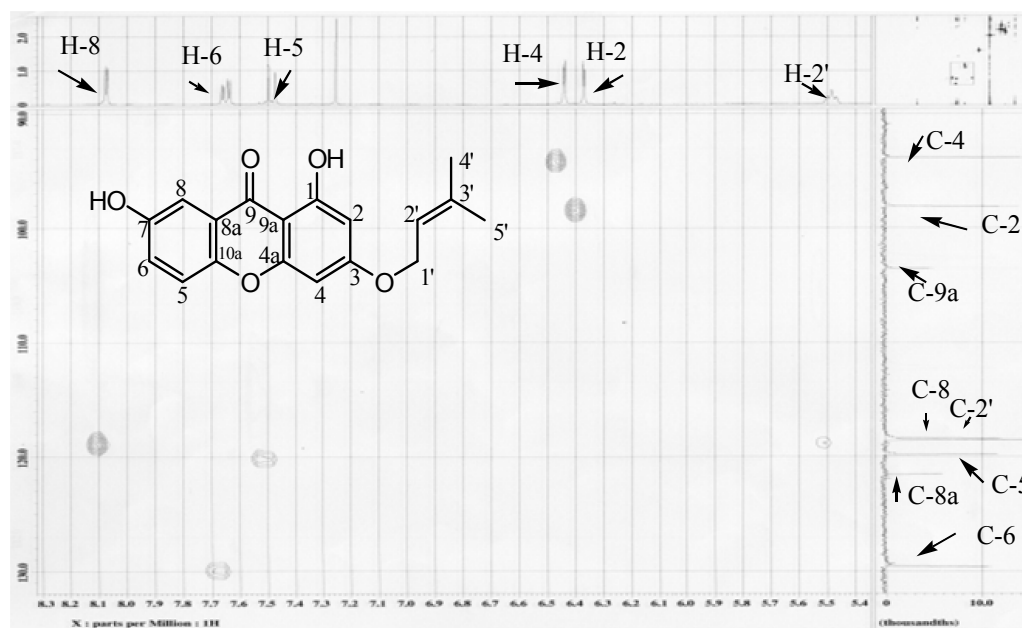
Figure 4.14: HMQC spectrum of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone(2)
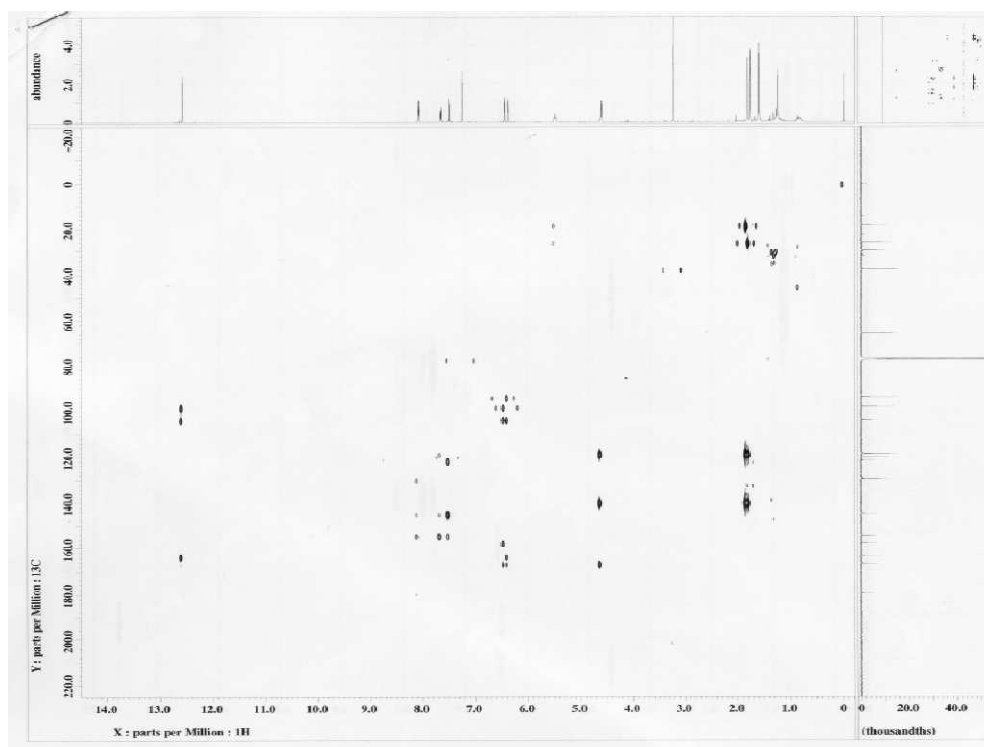
Figure 4.15: HMBC spectrum of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone(2)
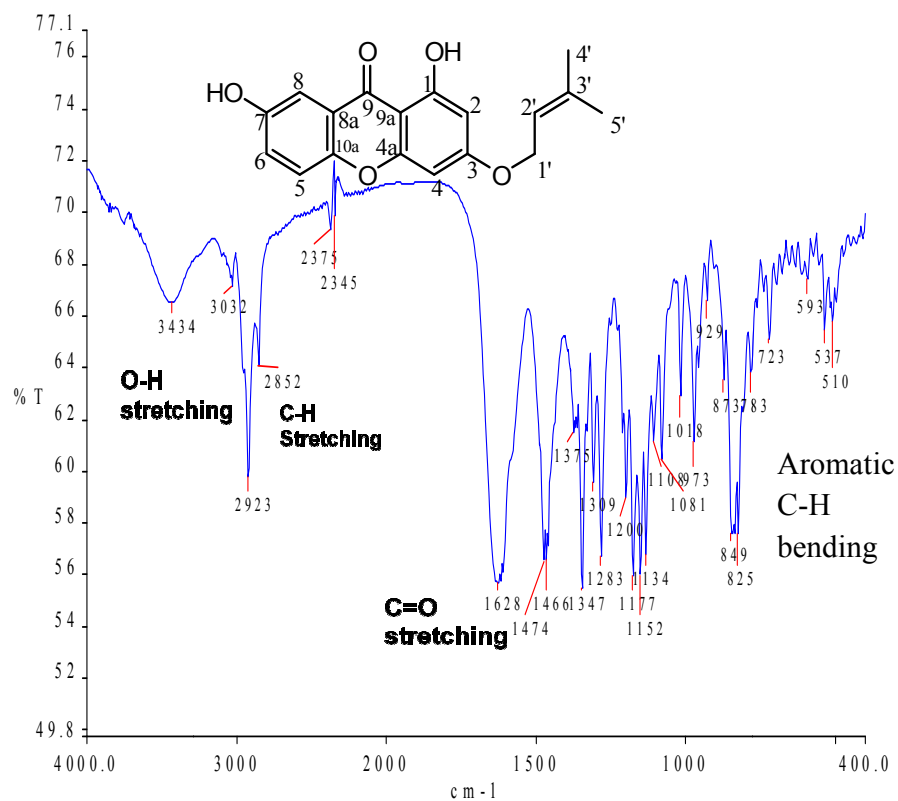
Figure 4.16: IR spectrum of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone(2)
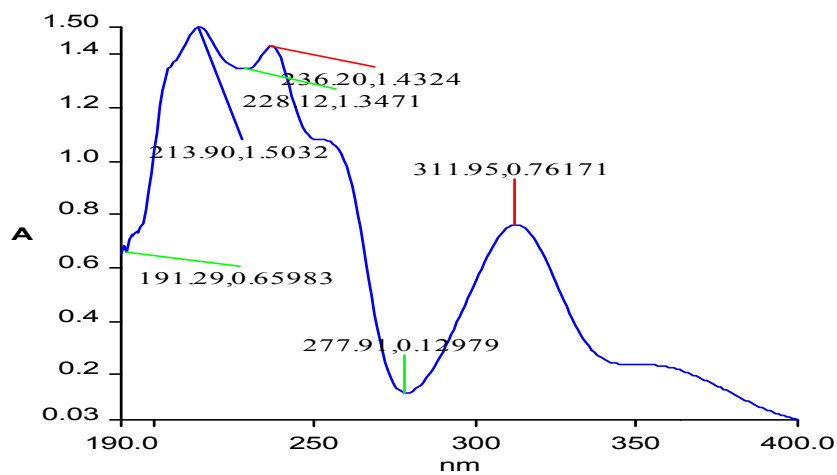
Figure 4.17: UV spectrum of 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone(2)

4.2.3   Characterization   of   1,7-Dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)

Compound **(3)** was isolated as yellow crystal, and was found to have the molecular formula $C_{23}H_{24}O_5$, having a yield of 0.93%, melting point of 130-134°C. Its IR spectrum exhibited absorption bands at 3433 (hydroxyl group), 2959, 2923, 2852 (C-H stretching), 1647 (conjugated carbonyl group). These data and the UV absorption band at λ_{max} 316, 239, 219, 208nm for a conjugated carbonyl chromophore revealed the compound to be polyhydroxyxanthone with the structure almost similar to 1-hydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(Table 4.4)** (Castanheiro *et al.*,2007), suggesting that **(3)**.

The ^{1}H NMR spectrum (**Figure 4.20**) revealed the presence of a chelated hydroxyl group at δ 12.62 (s, 1-OH), one singlet proton at δ 6.43 (s, 1H), three aromatic protons at δ 7.48(1H, d, J= 9.0 Hz), δ 7.64 (1H, dd, J= 9.0, 3.0 Hz), δ 8.07 (1H, d, J= 3.0Hz), two methylene protons at δ 4.62 (2H, d, J=6.7Hz) and δ 3.35 (2H, d, J=7.3Hz), two olefinic protons at δ 5.49 (1H, t, J=6.7Hz)and δ 5.22 (1H, t, J=7.3Hz), four methyl protons at δ 1.81 (3H, s), δ 1.78 (3H, s), δ 1.76 (3H, s), δ 1.67 (3H, s). The spectrum showed signals that evidence the presence of two 3,3-dimethylallyl (prenyl) group.

The ^{13}C NMR spectrum revealed singals for one carbonyl group at δ 179.5, seven quaternary aromatic carbon at δ 129.3, 121.9, 119.7, 118.5, 103.4, 112.8, 90.9, five oxygenated aromatic carbons at δ 164.3, 159.5, 156.0, 154.3, 144.8, two sets of carbon signals for two prenyl groups at δ 138.9, 118.8, 65.7, 25.9, 18.4 and δ 132.0, 121.9, 21.5, 25.9, 17.9.

The ^{13}C NMR data was compared to 1-hydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone reported in the literature (R.A.P. Castanheiro *et al.*,2007) showing characteristic signals for the presence of two prenyl groups. The ^{1}H spectrum showed that **(3)** was different from 1-hydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone in its left ring moiety. There was absence of the H-7 signal seen in the ^{1}H spectrum of **(3)**, and the peak splittings shown at H-6 and H-8 are different from their counterparts in 1-hydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone respectively. H-6 in **(3)** is a doublets of doublets which has *meta*-coupling (J= 3.0Hz) and *ortho*-coupling (J=9.0Hz) whereas H-6 in 1-hydroxy-3-(3-methylbut-2-enyloxy)xanthone is a ddd which has two *ortho*-coupling (J=8.4, 7.0Hz) and one *meta*-coupling (J=1.6 Hz). H-8 in **(3)** gave a doublet which is *meta*-coupling (J= 3.0 Hz) whereas H-8 in 1-hydroxy-3-(3-methylbut-2-enyloxy)xanthone is a doublets of doublets which has both *meta*-coupling (J=1.6Hz) and *ortho*-coupling (J=8.0Hz). Similar to the case of **(2)** mentioned earlier, the only explanation is that 7-position is replaced with other substituent, in this case, a hydroxyl group. The ^{13}C spectrum of **(3)** showed

a peak at δ154.3 which is assigned to 7-position, further confirming the presence of a hydroxyl group at carbon position C-7.

The position of the substituents on xanthone skeleton was determined on the basis of HMQC and HMBC (**Figures 4.22 and 4.23**). In the HMBC spectrum, cross-peaks for the chelated hydroxyl proton{δ_H 12.62 (1-OH)/ δ_C 159.5 (C-1), δ_C 103.4 (C-9a), δ_C 112.8 (C-2)} , and the singlet aromatic proton { δ_H 6.43 (H-4)/ δ_C 112.8 (C-2), δ_C 164.3 (C-3), δ_C 156.0 (C-4a), δ_C 103.4 (9a)}were observed, confirming a trisubstituted ring feature for **(3),** with hydroxyl groups located at C-1. Similarly, HMBC cross-peaks observed for the two doublet aromatic protons { δ_H 7.47 (H-5)/ δ_C 154.3 (C-7), δ_C 121.9 (C-8a), δ_C 144.8 (C-10a) and δ_H 8.08 (H-8)/ δ_C 129.3(C-6), δ_C 154.3(C-7), δ_C 179.5 (C-9), δ_C 144.8(10a)} and one doublet of doublets aromatic proton { δ_H 7.64(H-6)/ δ_C 144.8(C-10a) δ_C 118.5(C-8)} established a monosubstituted ring feature to the second ring of the xanthone, with hydroxyl groups located at C-7. The set of signals at δ_H 4.62 (2H, d, H-1'), δ_H 5.49 (1H, t, H-2'), δ_H 1.81 (3H, s, H-4'), δ_H 1.76 (3H, s, H-5') in the ^{1}H spectrum was established to be a 3,3-diallylmethyl (prenyl) group attached to oxygen on C-3 based on the long range coupling between H-1' and C-3 as displayed by the following correlations, { δ_H 4.62/δ_C 164.3 (C-3), δ_C 138.9 (C-3'), δ_C 118.8 (C-2')} in the HMBC spectrum. Another 3,3-diallylmethyl (prenyl) group was assigned to be attached to carbon C- 2 due to the fact that H-1'' displayed connectivity with C-2 as shown in HMBC spectrum { δ_H 3.35/δ_C 112.8 (C-2), δ_C 132.0 (C-3''), δ_C 121.9 (C-2'')} and that the signal of H-2 disappeared

after prenylation as compared to the ^{1}H spectrum (**Figure 4.4**) of xanthone

building block. Thus, **(3)** was elucidated as the following structure in **Figure 4.19**,

and named as 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-

enyloxy)xanthone. This structure was further confirmed by the HMQC spectrum

which showed cross-peaks between protons and their respective carbons for four

aromatic protons {δ_H 8.08/ δ_c 118.5, δ_H 7.64/ δ_c 129.3, δ_H 7.47/ δ_c 119.7, δ_H 6.43/

δ_c 90.9}, two olefinic protons {δ_H 5.49/ δ_c 118.8 and δ_H 5.22/ δ_c 121.9}, two

methylene protons {δ_H 4.62/65.7 and δ_H 3.35 /21.5}, four methyl protons {δ_H

1.81/ δ_c 25.9, δ_H 1.76/ δ_c 18.4 ,δ_H 1.78/ δ_c 17.9 and δ_H 1.67/ δ_c 25.9}. The ^{1}H and

^{13}C NMR data of **(3)** were assigned based on the HMQC, and HMBC correlations

data (**Table 4.5**). Figure 4.18: Main connectivities found in the HMBC of 1,7-

dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)
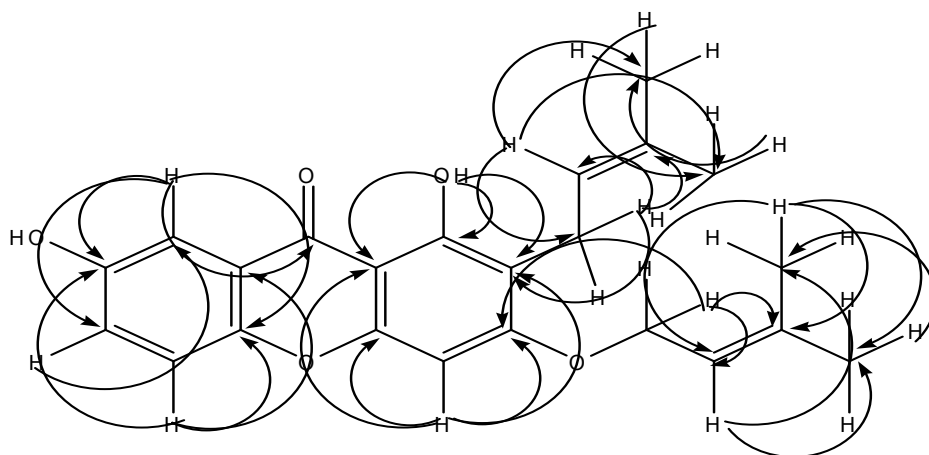
Figure 4.18: Main connectivities found in the HMBC of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)
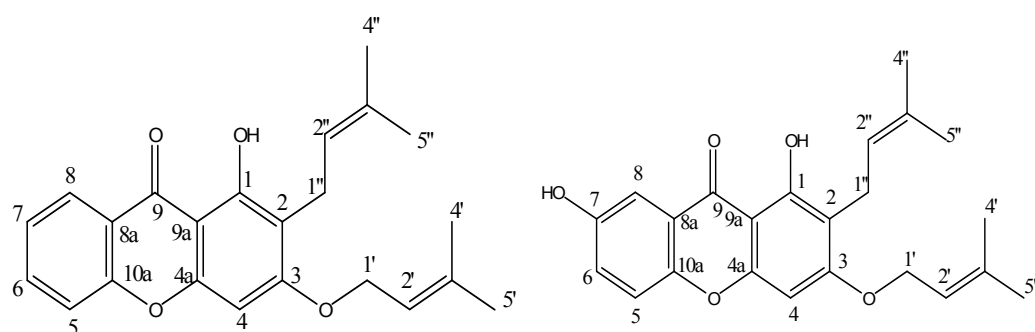
Table 4.4 **¹H chemical shifts of 1-hydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone** (Castanheiro *et al.*,2007) **and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)**

	δ$_H$, 1-Hydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone	δ$_H$, 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)
H-1	12.92 (OH, s)	12.62 (OH, s)
H-2	-	-
H-4	6.44 (s)	6.43(1H, s)
H-5	7.42 (d, J=8.4 Hz)	7.47(1H, d, J= 9.0 Hz)
H-6	7.69 (ddd, J=8.4,7.0,1.6 Hz)	7.64(1H, dd, J= 9.0, 3.0 Hz)
H-7	7.36 (t,J=8.0,7.0 Hz)	-
H-8	8.26 (dd, J=8.0,1.6 Hz)	8.08(1H, d, J= 3.0Hz)
H-1'	4.63 (d, J=6.6 Hz)	4.62(2H, d, J=6.7Hz)
H-2'	5.51 (t, J=6.6 Hz)	5.49(1H, t, J=6.7Hz)
H-4'	1.82,1.77 (2s)	1.81, 1.76 (2s)
H-1''	3.38 (d, J=7.2 Hz)	3.35 (2H, d, J=7.3Hz)
H-2''	5.25 (t, J=7.2 Hz)	5.22(1H, t, J=7.3Hz)
H-4'',	1.79, 1.68 (2s)	1.78, 1.67 (2s)

H-5''		
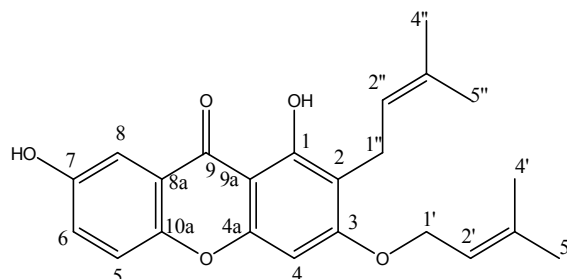
Figure 4.19: 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)

Table 4.5 ^{1}H NMR (400MHz, CDCl$_3$) and ^{13}C NMR (100MHz, CDCl$_3$) assignments of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)

Position	δ$_H$	δ$_c$	HMBC
1	-	159.5	-
2	-	112.8	-
3	-	164.3	-
4	6.43(1H, s)	90.9	C- 2(^{3}J),3(^{2}J),4a(^{2}J),9a(^{3}J)
4a	-	156.0	-
5	7.47(1H, d, J= 9.0 Hz)	119.7	C- 7(^{3}J),8a(^{3}J),10a(^{2}J)
6	7.64(1H, dd, J= 9.0, 3.0 Hz)	129.3	C- 8(^{3}J),10a(^{3}J)
7	-	154.3	-
8	8.08(1H, d, J= 3.0Hz)	118.5	C-6(^{3}J),7(^{2}J),9(^{3}J),10a(^{3}J)
8a	-	121.9	-

9	-	179.5	-
9a	-	103.4	-
10a	-	144.8	-
1'	4.62(2H, d, J=6.7Hz)	65.7	C-2'(^{2}J),3'(^{3}J)
2'	5.49(1H, t, J=6.7Hz)	118.8	C-4'(^{3}J),5'(^{3}J)
3'	-	138.9	-
4'	1.81 (3H, s)	25.9	C-2'(^{3}J),3'(^{2}J),5'(^{3}J)
5'	1.76 (3H, s)	18.4	C-4'(^{3}J)
1''	3.35 (2H, d, J=7.3Hz)	21.5	C-2(^{2}J),2''(^{2}J),3''(^{3}J)
2''	5.22(1H, t, J=7.3Hz)	121.9	C-1''(^{2}J),4''(^{3}J),5''(^{3}J)
3''	-	132.0	-
4''	1.67(3H, s)	25.9	C-5''(^{3}J)
5''	1.78 (3H, s)	17.9	C-4''(^{3}J)
1-OH	12.62 (1H, s)	-	C-1(^{2}J),2(^{3}J),9a(^{3}J)
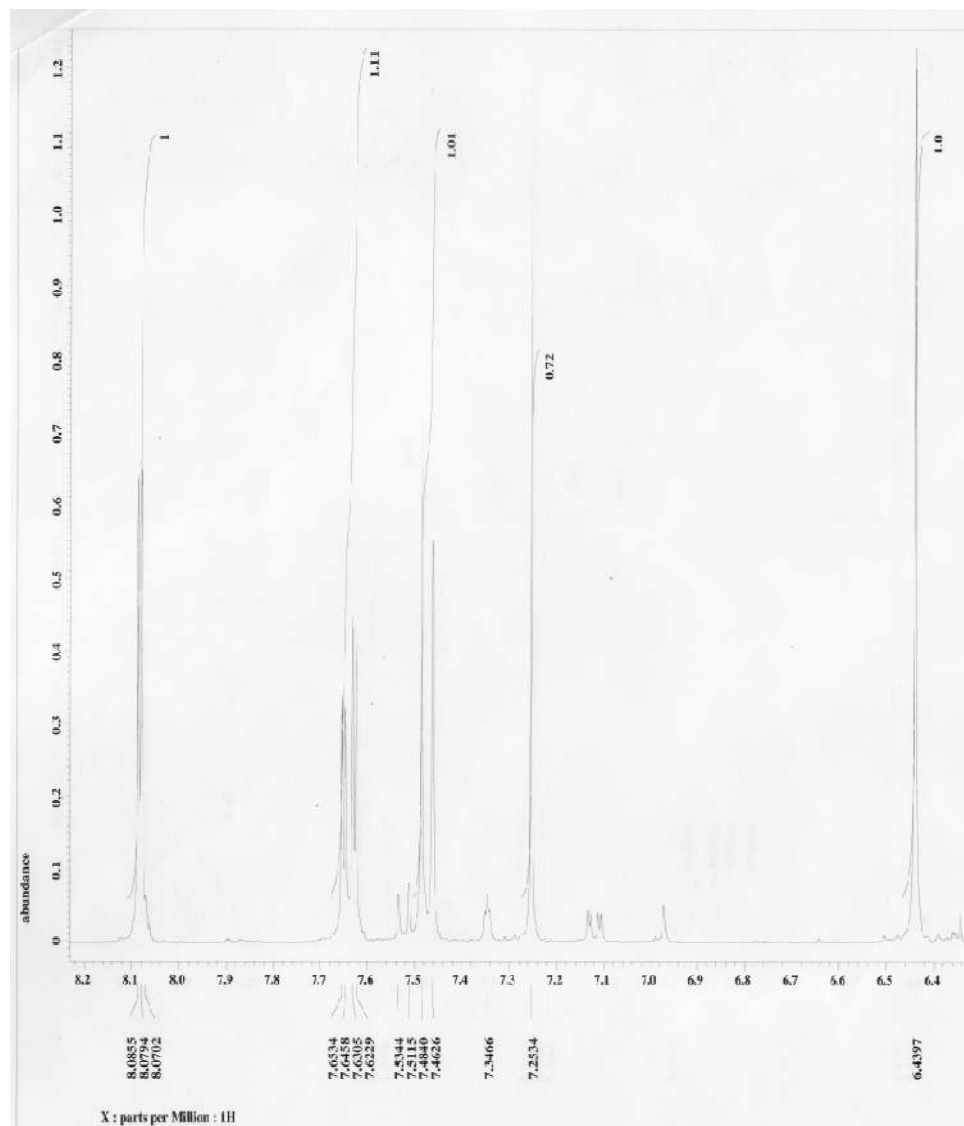
Figure 4.20: ^{1}H NMR spectrum of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)
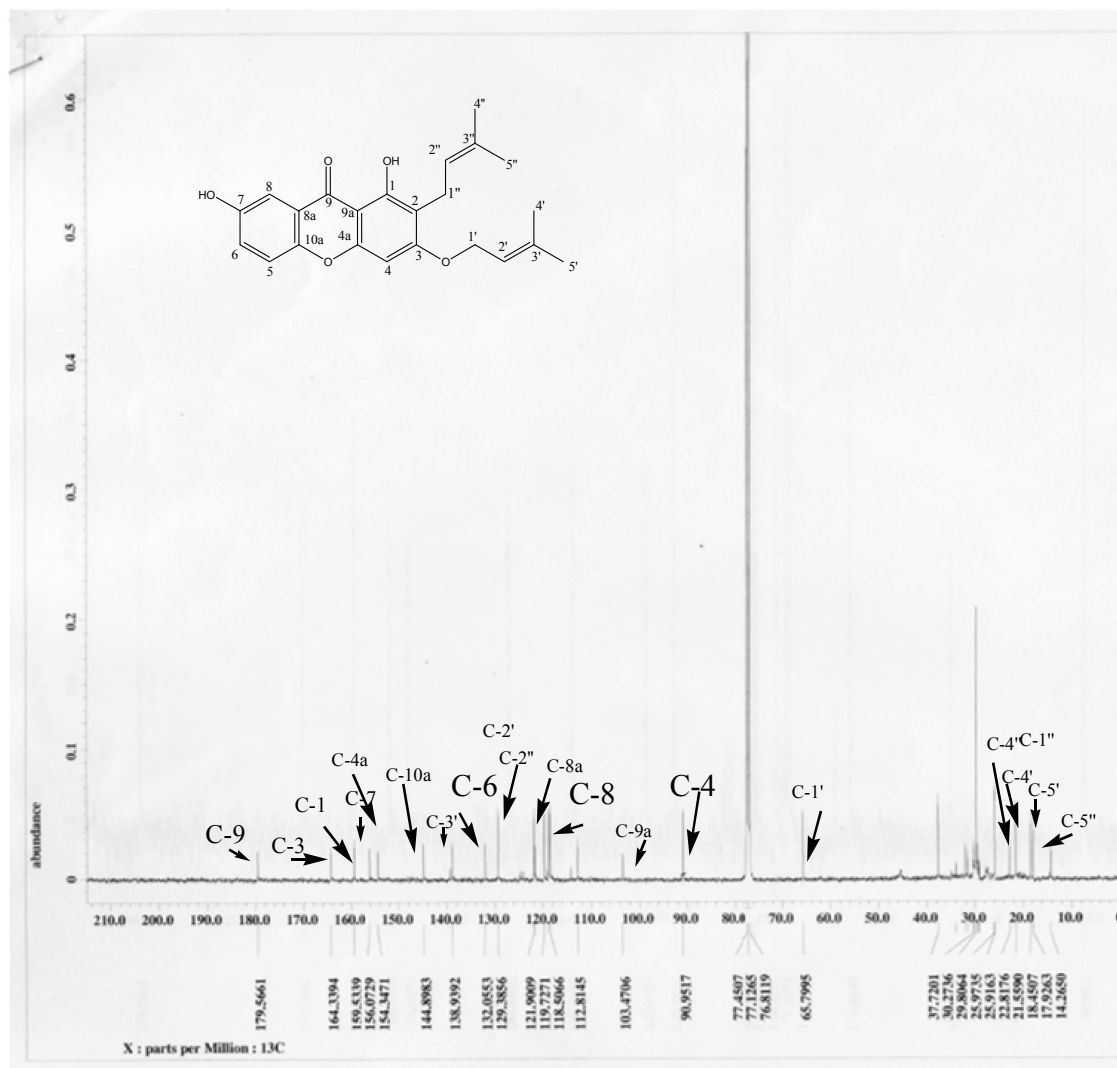
Figure 4.21: ^{13}C NMR spectrum of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)
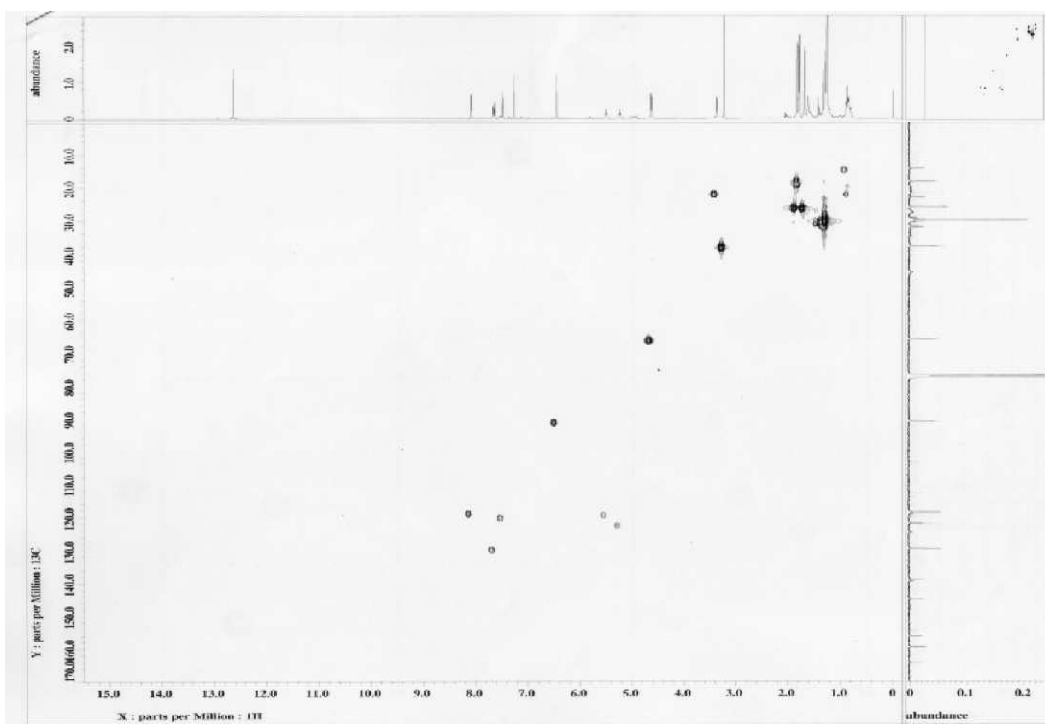
Figure 4.22: HMQC spectrum of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)
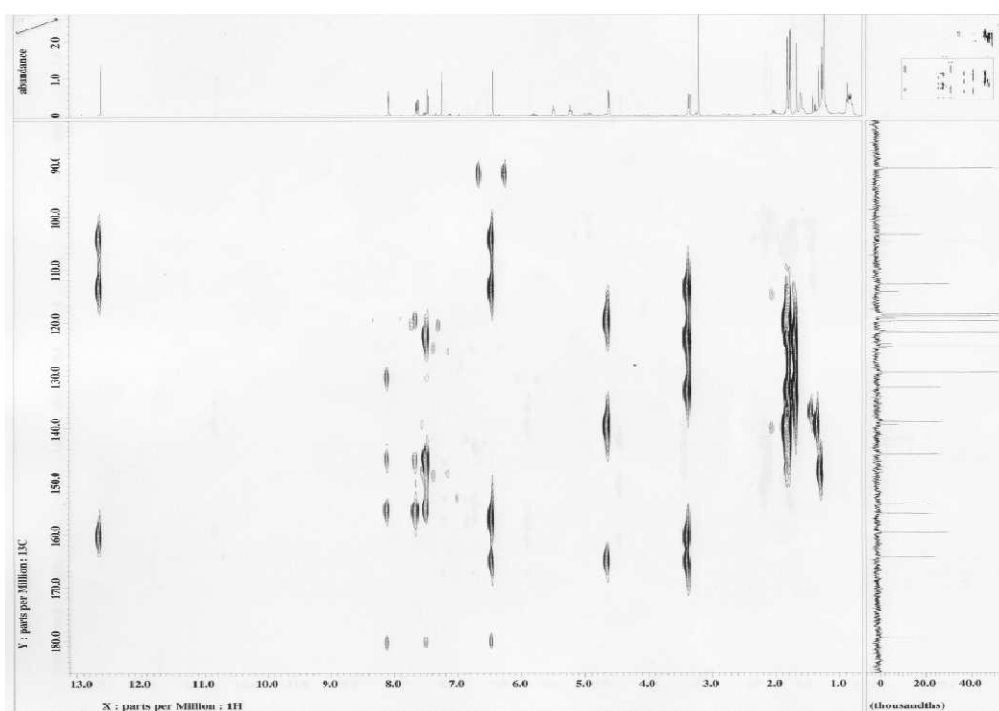
Figure 4.23: HMBC spectrum of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)

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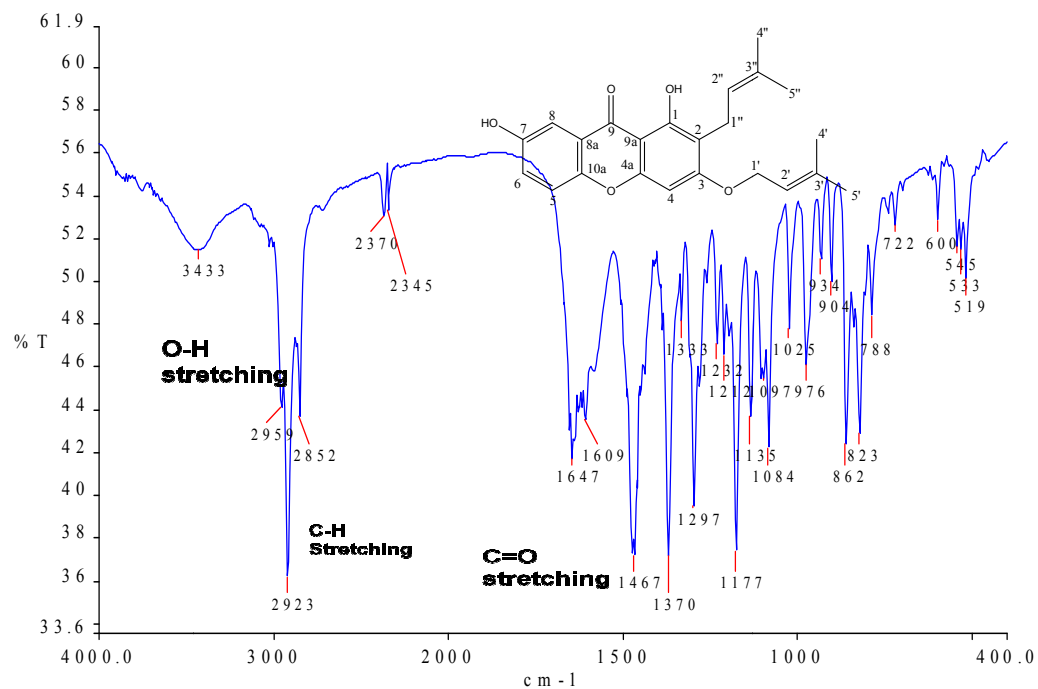
Figure 4.24: IR spectrum of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)
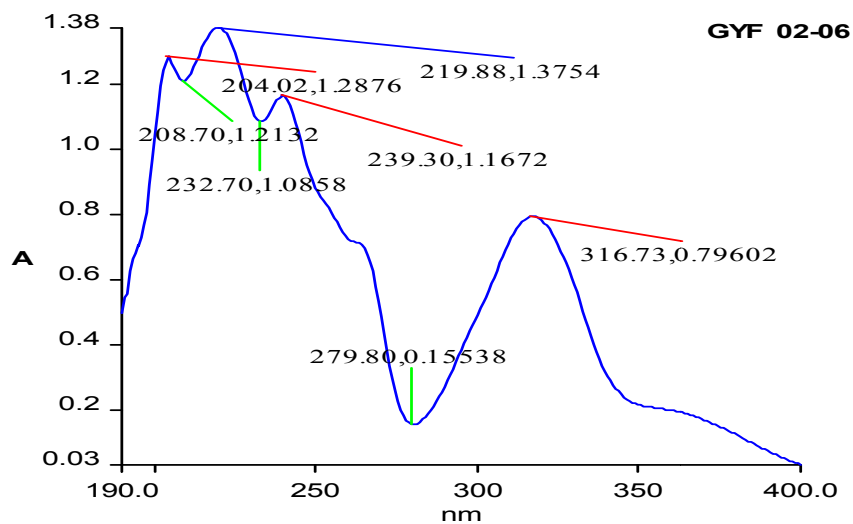
Figure 4.25: UV spectrum of 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3)

4.3 Prenylation of 1,3,7-Trihydroxyxanthone in Aqueous Medium

1,3,7-trihydroxyxanthone

prenyl bromide

aqueous K₂CO₃

1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone **(4)**
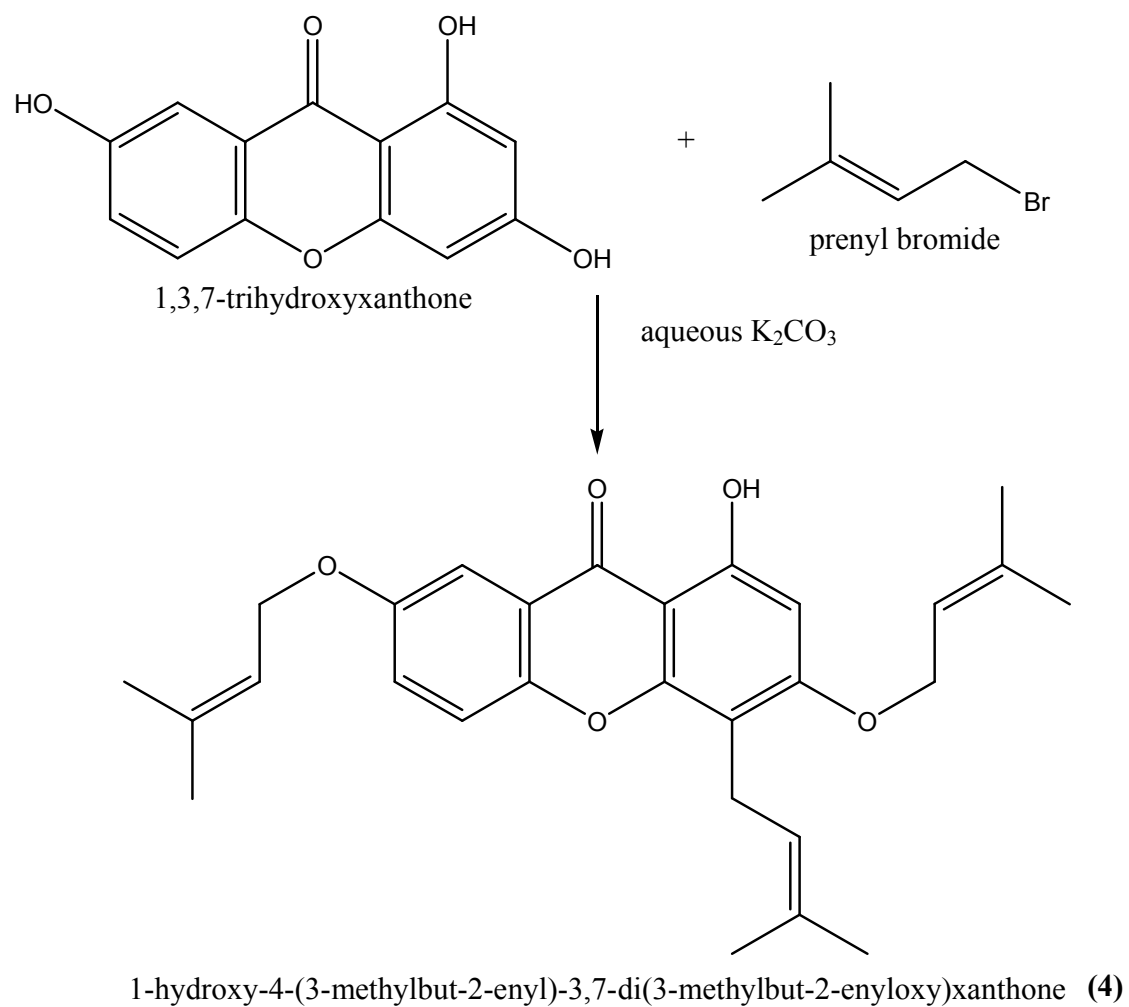
Figure 4.26: Prenylation of 1,3,7-Trihydroxyxanthone in Aquoues Medium

4.3.1 Proposed Mechanism of Prenylation of 1,3,7-Trihydroxyxanthone in Aqueous Medium
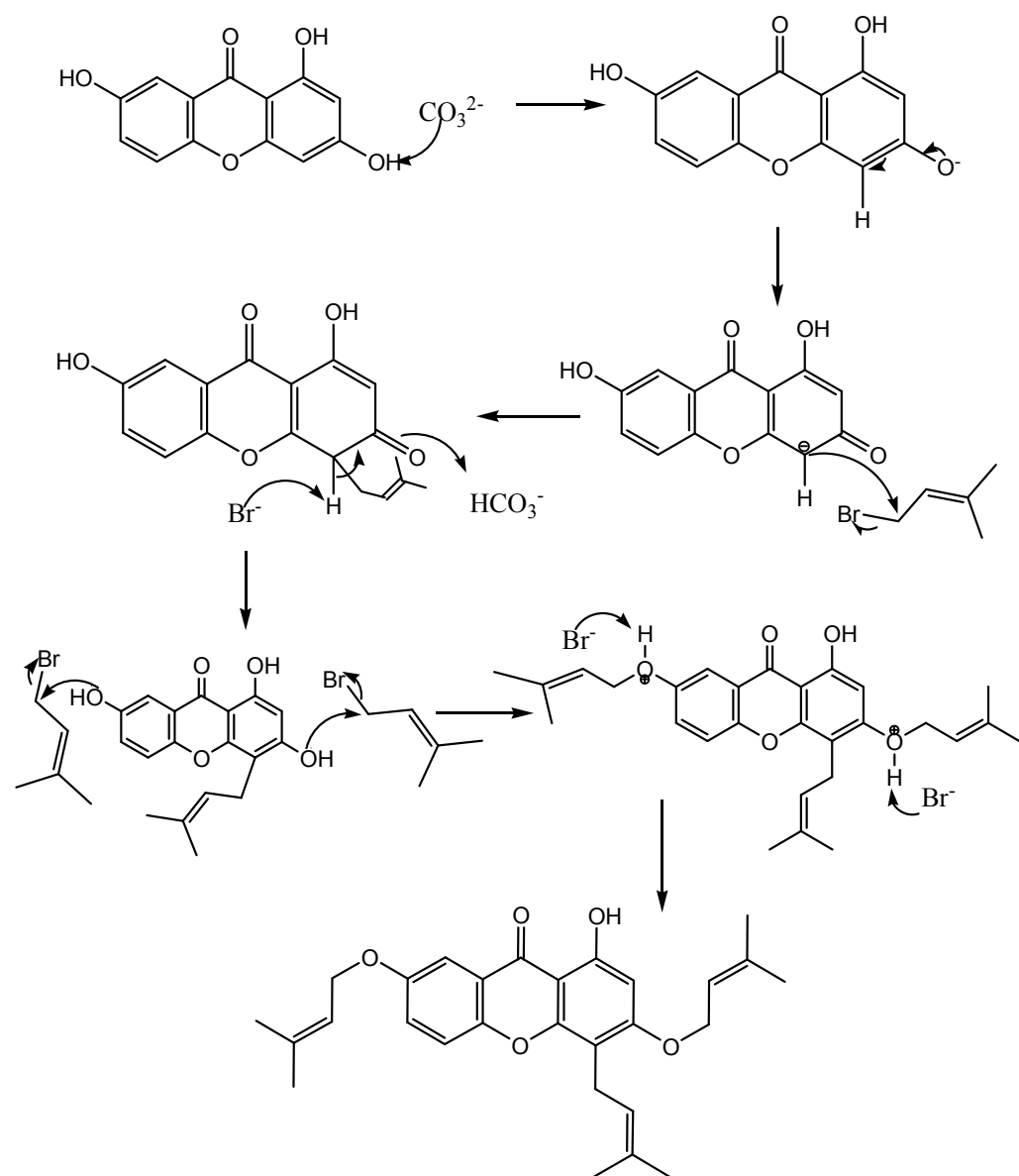
(4)

Figure 4.27: Proposed mechanism for synthesis of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

4.3.2 Characterization of 1-Hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

Compound **(4)** was isolated as yellow crystal, and found to have the molecular formula $C_{28}H_{32}O_5$, having a yield of 4.64%, melting point of 153-156°C. Its IR spectrum exhibited absorption bands at 3423 (hydroxyl group), 2962, 2912, 2856 (C-H stretching), 1654 (conjugated carbonyl group). These data and the UV absorption band at λ_{max} 313, 264, 236, 204nm for a conjugated carbonyl chromophore were almost similar to those reported for 1-Hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(Table 4.6)** (Castanheiro *et al.*,2007), suggesting that **(4)** has close similarity with the structure.

The ^{1}H NMR spectrum (**Figure 4.30**) revealed the presence of a chelated hydroxyl group at δ 12.9 (s, 1-OH), one singlet proton at δ 6.37 (1H, s), three aromatic protons at δ 7.37 (1H, d, J= 9.0 Hz), δ 7.31 (1H, dd, J= 9.0, 3.0 Hz), δ 7.61 (1H, d, J= 3.0Hz), three methylene protons at δ 4.61 (2H, d, J=6.7Hz), δ 4.59 (2H, d, J=6.7Hz), and δ 3.47 (2H, d, J=7.3Hz), three olefinic protons at δ 5.51 (1H, t, J=6.7Hz), δ 5.21 (1H, t, J=7.3Hz) and δ 5.48 (1H, t, J=6.7Hz), six methyl protons at δ 1.81(3H, s), δ 1.79 (3H, s), δ 1.65 (3H, s), δ 1.84 (3H, s), δ 1.77 (3H, s) and δ 1.74 (3H, s) . The spectrum showed signals that evidence the presence of three 3,3-dimethylallyl (prenyl) group in the proposed structure.

The ^{13}C NMR spectrum revealed signals for one carbonyl group at δ181.1, seven quaternary aromatic carbon at δ 129.3, 121.9, 119.7, 118.5, 103.4, 112.8, 90.9, five oxygenated aromatic carbons at δ 163.5, 161.7, 155.1, 154.2, 151.0, three sets of carbon signals for three prenyl groups at δ 139.1, 119.1, 65.7, 25.9, 18.4; δ 132.2, 131.6, 21.7, 25.9, 17.9 and δ 139.1, 119.1, 65.5, 25.9, 18.4.

The ^{13}C NMR data was compared to 1-hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone reported in the liteterature(Castanheiro *et al.*,2007). The difference was only an additional prenyl group in **(4)**. The ^{1}H spectrum showed that **(4)** is different from 1-hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone in its left ring moiety. There was absence of the H-7 signal seen in the ^{1}H spectrum of **(4)**, and the peak splittings shown at H-6 and H-8 are different from their counterparts in 1-hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone, respectively. H-6 in **(4)** is a doublets of doublets which has *meta*-coupling (J= 3.0Hz) and *ortho*-coupling (J=9.0Hz) whereas H-6 in 1-hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone is a ddd which has two *ortho*-coupling (J=8.2, 7.1Hz) and one *meta*-coupling (J=1.6 Hz). H-8 in **(4)** is a doublet which is *meta*-coupling (J= 3.0 Hz) whereas H-8 in 1-hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone is a doublets of doublets which has both *meta*-coupling (J=1.6Hz) and *ortho*-coupling (J=8.0Hz). Similar to the case of **(2)** mentioned

earlier, the only explanation is that 7-position is replaced with C3-methylbut-2-enyloxy group which was confirmed by HMBC spectrum analysis.

The position of the substituents on xanthone skeleton was determined on the basis of HMQC and HMBC analyses. In the HMBC spectrum (**Fig 4.33**), cross-peaks for the chelated hydroxyl proton{δ_H 12.9 (1-OH)/ δ_C 161.7 (C-1), δ_C 103.3 (C-9a), δ_C 95.0 (C-2)} , and the singlet aromatic proton { δ_H 6.37 (H-2)/ δ_C 161.7 (C-1), δ_C 107.9 (C-4), δ_C 103.3 (9a)}were observed, confirming a trisubstituted ring feature in (**4**) on the right side of ring of xanthone nucleus**,** with hydroxyl groups located at C-1. Similarly, HMBC cross-peaks observed for the two doublet aromatic protons { δ_H 7.37 (H-5)/ δ_C 155.1 (C-7), δ_C 120.5 (C-8a), δ_C 151.0 (C-10a) and {δ_H 7.61 (H-8)/ δ_C 125.4(C-6), δ_C 181.1 (C-9), δ_C 151.0(10a)} and one doublet of doublets aromatic proton { δ_H 7.31(H-6)/ δ_C 151.0(C-10a) δ_C 106.0(C-8)} established a monosubstituted ring feature on the left side ring of the compound, with oxygenated group located at C-7. The set of signals at δ_H 4.61 (2H, d, H-1'), δ_H 5.51 (1H, t, H-2'), δ_H 1.81 (3H, s, H-4'), δ_H 1.79 (3H, s, H-5') in the ^{1}H spectrum was established to be a 3,3-diallylmethyl (prenyl) group attached to oxygen on C-3 due to the coupling between H-1' and C-3 as shown by the correlations, { δ_H 4.61/δ_C 163.5 (C-3), δ_C 139.1 (C-3'), δ_C 119.1 (C-2')} in HMBC spectrum. Another 3,3-diallylmethyl (prenyl) group in which the set of signals shown at δ_H 3.47 (2H, d, H-1''), δ_H 5.21 (1H, t, H-2''), δ_H 1.65 (3H, s, H-4''), δ_H 1.84 (3H, s, H-5'') was assigned to be attached to the C- 4 due to the fact that H-1'' has connectivity with C-4 as shown in HMBC spectrum { δ_H 3.47/δ_C

107.9(C-4), δ_C 131.6 (C-3''), δ_C 122.2 (C-2'')} and that the signal of H-4 disappeared after prenylation as compared to **(1)**. The third 3,3-diallylmethyl (prenyl) group in which the set of signals shown at δ_H 4.59 (2H, d, H-1'''), δ_H 5.48 (1H, t, H-2'''), δ_H 1.77 (3H, s, H-4'''), δ_H 1.74 (3H, s, H-5''') was assigned to be attached to the 7-position due to the fact that H-1''' has connectivity with C-7 as shown in HMBC spectrum { δ_H 4.59/δ_C 155.1 (C-7)}. Thus, **(4)** was elucidated as the following structure in **Figure 4.29**, and named as 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone. This structure was further confirmed by the HMQC spectrum (**Figure 4.32**) which showed cross-peaks for protons at four aromatic protons {δ_H 8.08/ δ_c 118.5, δ_H 7.64/ δ_c 129.3, δ_H 7.47/ δ_c 119.7, δ_H 6.43/ δ_c 90.9}, two olefinic protons {δ_H 5.49/ δ_c 118.8 and δ_H 5.22/ δ_c 121.9}, two methylene protons {δ_H 4.62/65.7 and δ_H 3.35 /21.5}, four methyl protons {δ_H 1.81/ δ_c 25.9, δ_H 1.76/ δ_c 18.4 ,δ_H 1.78/ δ_c 17.9 and δ_H 1.67/ δ_c 25.9}. The ^{1}H and ^{13}C NMR data of **(3)** were assigned based on the HMQC, and HMBC correlations (**Table 4.6**).
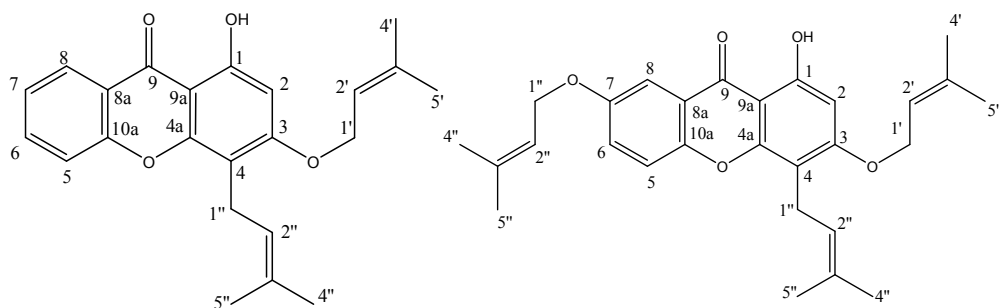
Table 4.6: ^{1}**H chemical shifts of 1-Hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone** (R.A.P. Castanheiro *et al.*,2007) **and 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)**

	δ_H, 1-Hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone	δ_H, 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)
H-1	12.97 (OH, s)	12.9 (OH, s)
H-2	-	6.37(1H,s)
H-4	6.39 (s)	-
H-5	7.46 (d, J=8.2 Hz)	7.37(1H, d, J= 9.0 Hz)
H-6	7.71 (ddd, J=8.2,7.1,1.6 Hz)	7.31(1H, dd, J= 9.0, 3.0 Hz)
H-7	7.36 (t,J=8.0,7.1 Hz)	-
H-8	8.24 (dd, J=8.0,1.6 Hz)	7.61(1H, d, J= 3.0Hz)
H-1'	4.63 (d, J=6.6 Hz)	4.61(2H, d, J=6.7Hz)
H-2'	5.49 (t, J=6.6 Hz)	5.51(1H, t, J=6.7Hz)
H-4', H-5'	1.81,1.76 (2s)	1.81, 1.79 (2s)
H-1''	3.50 (d, J=7.0 Hz)	3.47 (2H, d, J=7.3Hz)
H-2''	5.23 (t, J=7.0 Hz)	5.21(1H, t, J=7.3Hz)

H-4''	1.87, 1.68 (2s)	1.84, 1.65 (2s)
H-1'''		4.59(2H, d, J=6.7Hz)
H-2'''		5.48(1H, t, J=6.7Hz)
H-4''', H-5'''		1.77, 1.74 (2s)
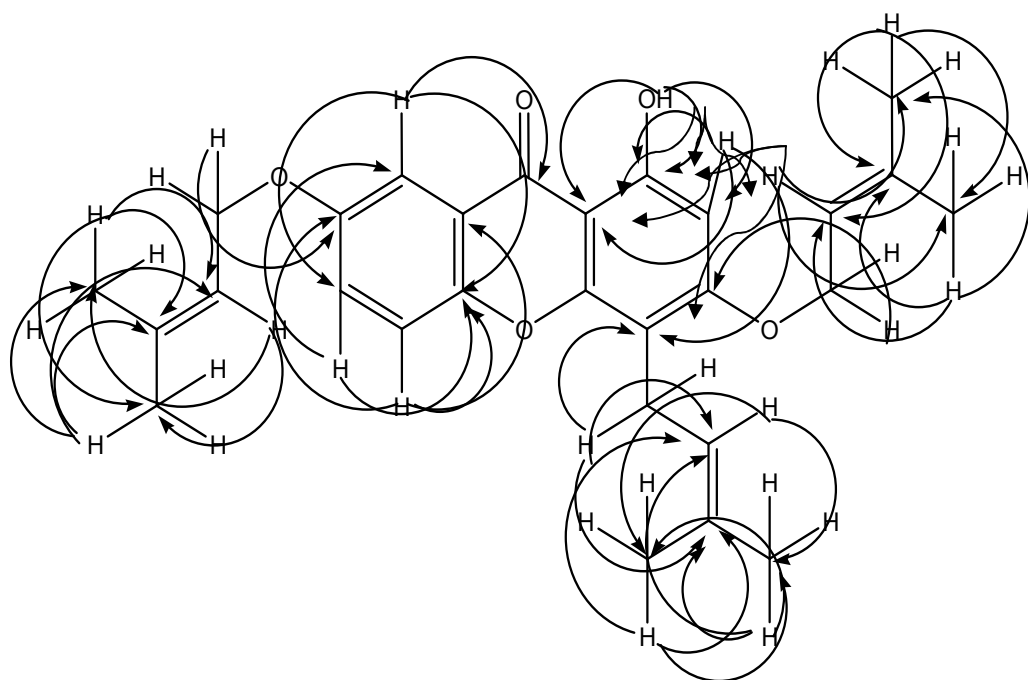
Figure 4.28: Main connectivities found in the HMBC of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)
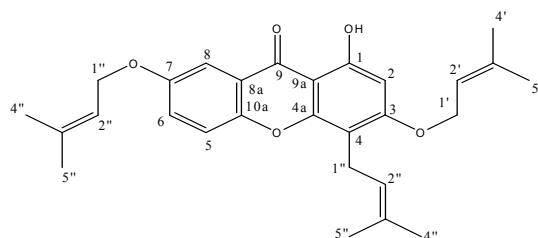
Figure 4.29: 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

Table 4.7: ^{1}H NMR (400MHz, CDCl$_3$) and ^{13}C NMR(100MHz, CDCl$_3$) assignments of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

Position	δ_H	δ_c	HMBC
1	-	161.7	-
2	6.37(1H,s)	95.0	C- 1(^{2}J),4(^{3}J),9a(^{3}J)
3	-	163.5	-
4	-	107.9	-
4a	-	154.2	-
5	7.37(1H, d, J= 9.0 Hz)	119.1	C- 7(^{3}J),8a(^{3}J),10a(^{2}J)
6	7.31(1H, dd, J= 9.0, 3.0 Hz)	125.4	C- 8(^{3}J),10a(^{3}J)
7	-	155.1	-
8	7.61(1H, d, J= 3.0Hz)	106.0	C-6(^{3}J), 9(^{3}J),10a(^{3}J)
8a	-	120.5	-
9	-	181.1	-
9a	-	103.3	-

10a	-	151.0	-
1'	4.61(2H, d, J=6.7Hz)	65.7	C-3(^{3}J)
2'	5.51(1H, t, J=6.7Hz)	119.1	C- 4'(^{3}J),5'(^{3}J)
3'	-	139.1	-
4'	1.81 (3H, s)	25.9	C-2'(^{3}J),3'(^{2}J),5'(^{3}J)
5'	1.79 (3H, s)	18.4	C-2'(^{3}J),3'(^{2}J),4'(^{3}J)
1''	3.47 (2H, d, J=7.3Hz)	21.7	C-4(^{2}J),2''(^{2}J),3''(^{3}J)
2''	5.21(1H, t, J=7.3Hz)	122.2	C-4''(^{3}J),5''(^{3}J)
3''	-	131.6	-
4''	1.65(3H, s)	25.9	C-2''(^{3}J),3''(^{2}J), 5''(^{3}J)
5''	1.84 (3H, s)	17.9	C-2''(^{3}J),3''(^{2}J), 4''(^{3}J)
1'''	4.59(2H, d, J=6.7Hz)	65.5	C- 7 (^{3}J)
2'''	5.48(1H, t, J=6.7Hz)	119.1	C-4'''(^{3}J),5'''(^{3}J)
3'''	-	139.1	-
4'''	1.77 (3H, s)	25.9	C-2'''(^{3}J),3'''(^{2}J), 5'''(^{3}J)
5'''	1.74 (3H, s)	18.4	C-2'''(^{3}J),3'''(^{2}J), 4'''(^{3}J)
1-OH	12.9 (1H, s)	-	C-1(^{2}J),2(^{3}J),9a(^{3}J)
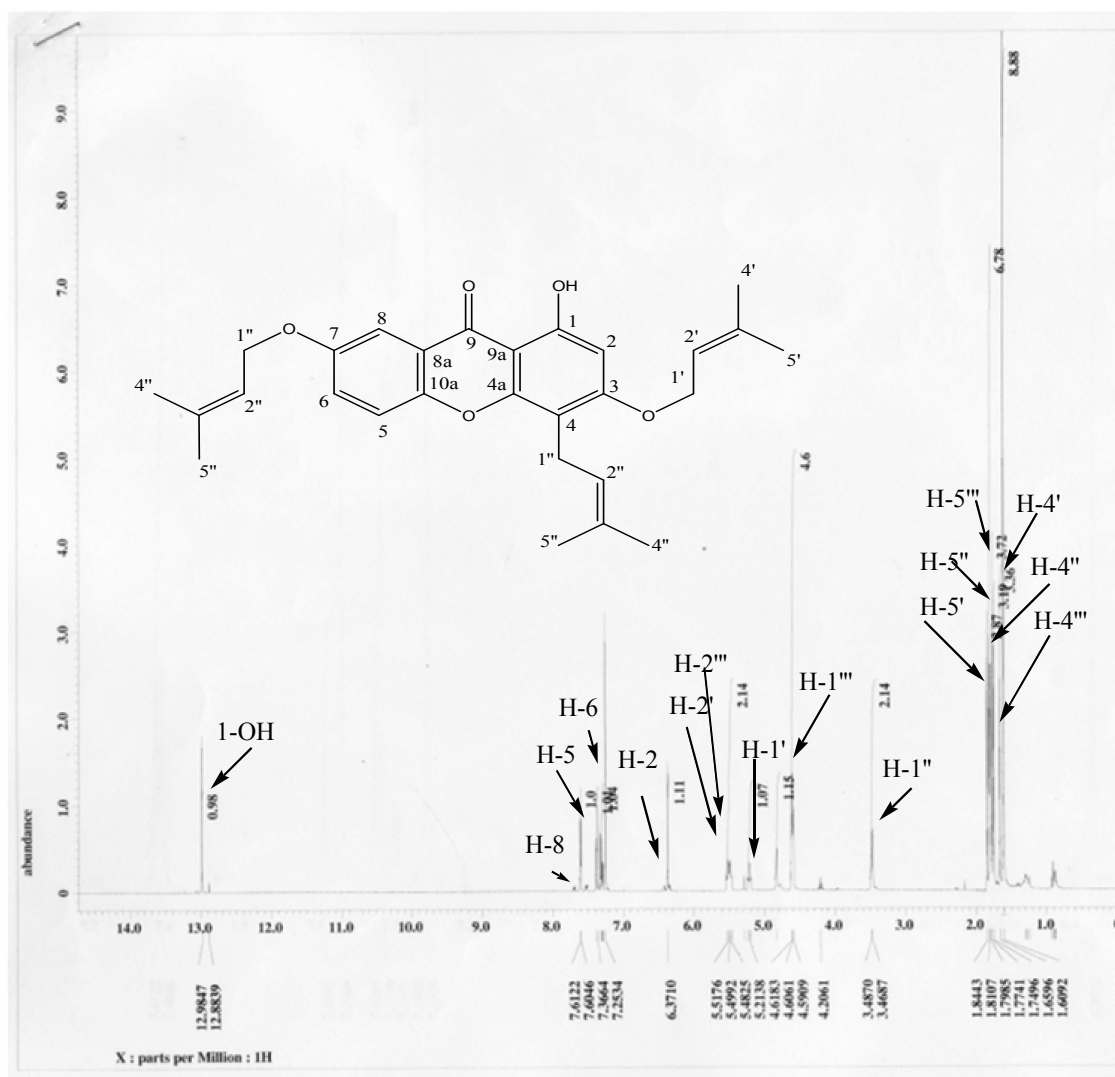
Figure 4.30: ^{1}H-NMR spectrum of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)
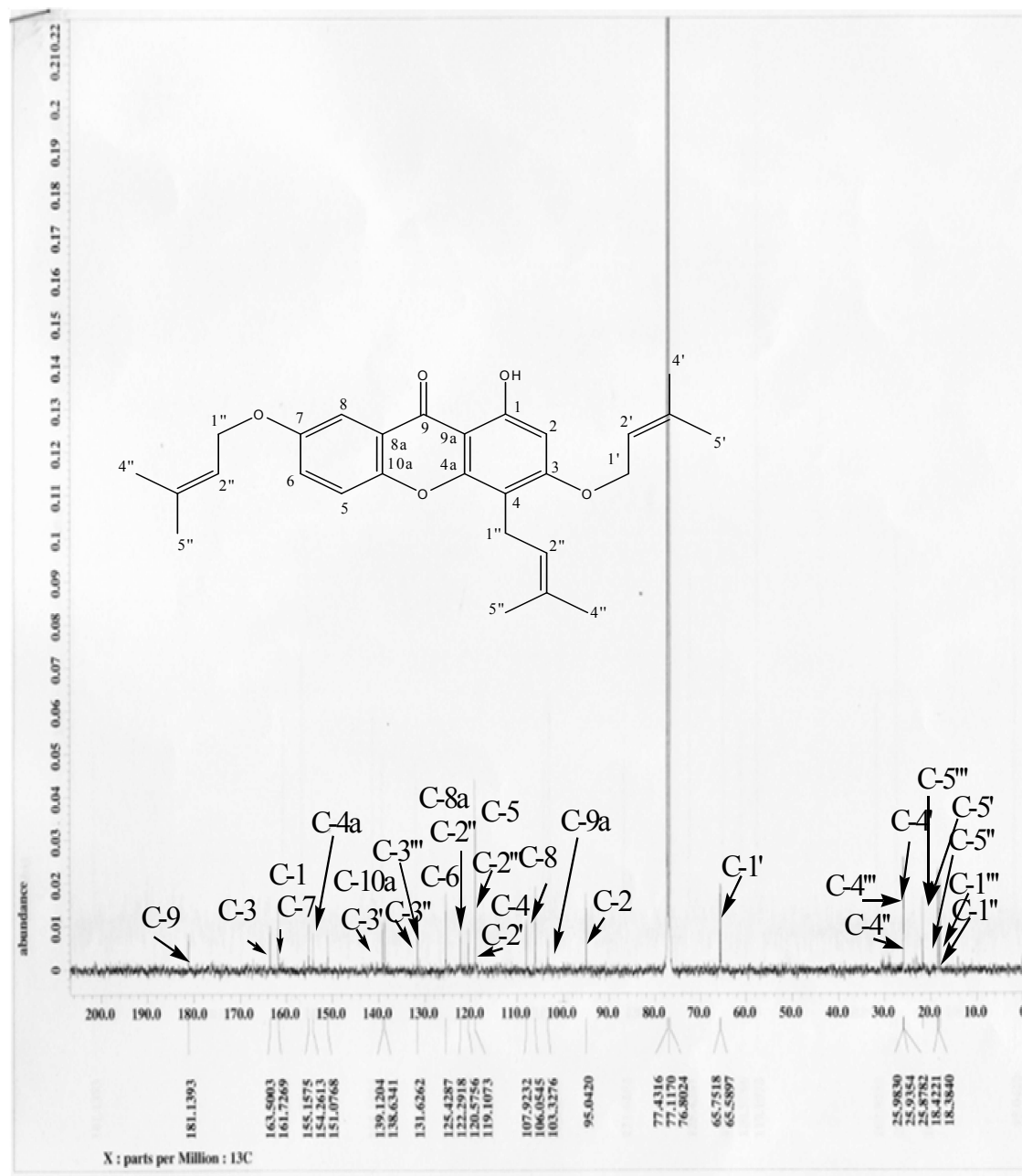
Figure 4.31: ^{13}C-NMR spectrum of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

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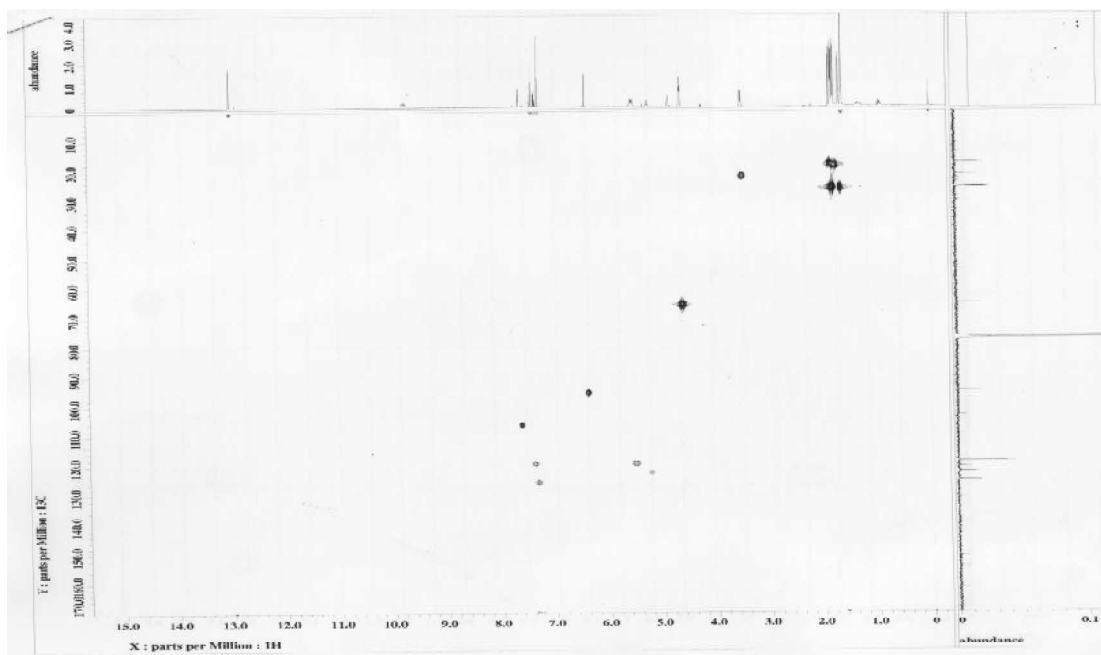
Figure 4.32: HMQC spectrum of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

Figure 4.33: HMBC spectrum of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

Figure 4.34: IR spectrum of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)
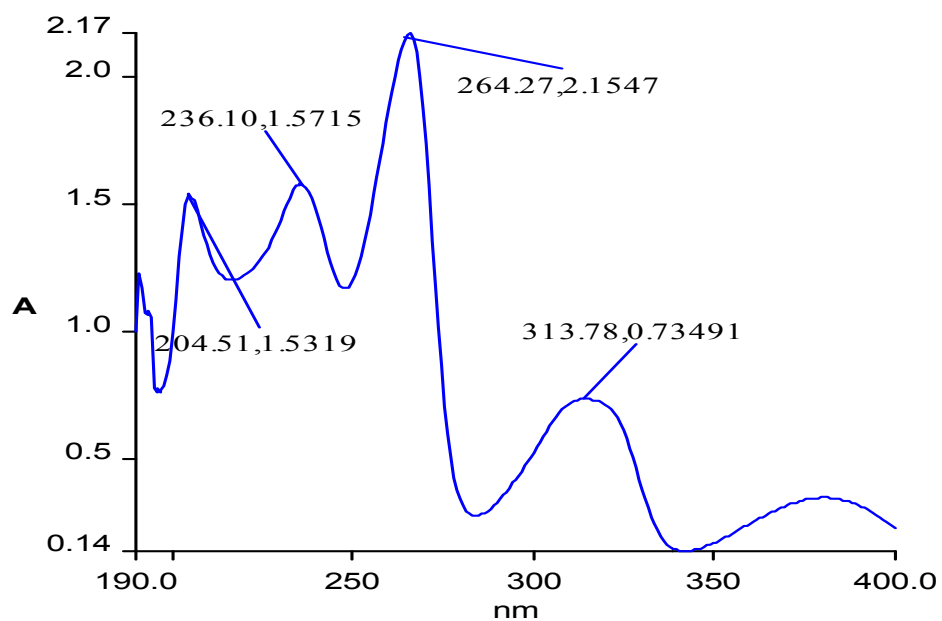
Figure 4.35: UV spectrum of 1-hydroxy-4-(3-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4)

4.4 Biological Studies

1,3,7-Trihydroxyxanthone **(1),** 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone **(2),** 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** were tested for their biological activity towards HeLa and MDA-MB-231 cancer cell lines and the results are shown in **Table 4.7**. The anticancer activity of 1,3,7-trihydroxyxanthone and its derivatives were evaluated based on the cell viability percentage of the tested cancer cell at varying concentrations of the compounds by using MTT method. The inhibitory concentration that kills cell by 50%, IC_{50}, for each compound was then obtained from the graph of cell viability versus concentration of drug **(Figure 4.8)**.

Table 4.7 Cytotoxic Assays Results of Xanthonic Compounds towards HeLa and MDA-MB-231 Cancer Cell Lines

Compound	HeLa Cancer Cell Line		MDA-MB-231 Cancer Cell Line	
	IC_{50} (µg/ml)	IC_{50} (µM/ml)	IC_{50} (µg/ml)	IC_{50} (µM/ml)
1,3,7-trihydroxyxanthone **(1)**	21	86	>50	-
1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone **(2)**	>50	-	>50	-
1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)**	>50	-	>50	-
Doxorubicin	7	12.9	6	11.0
Cisplatin	4	13.3	4	16.7
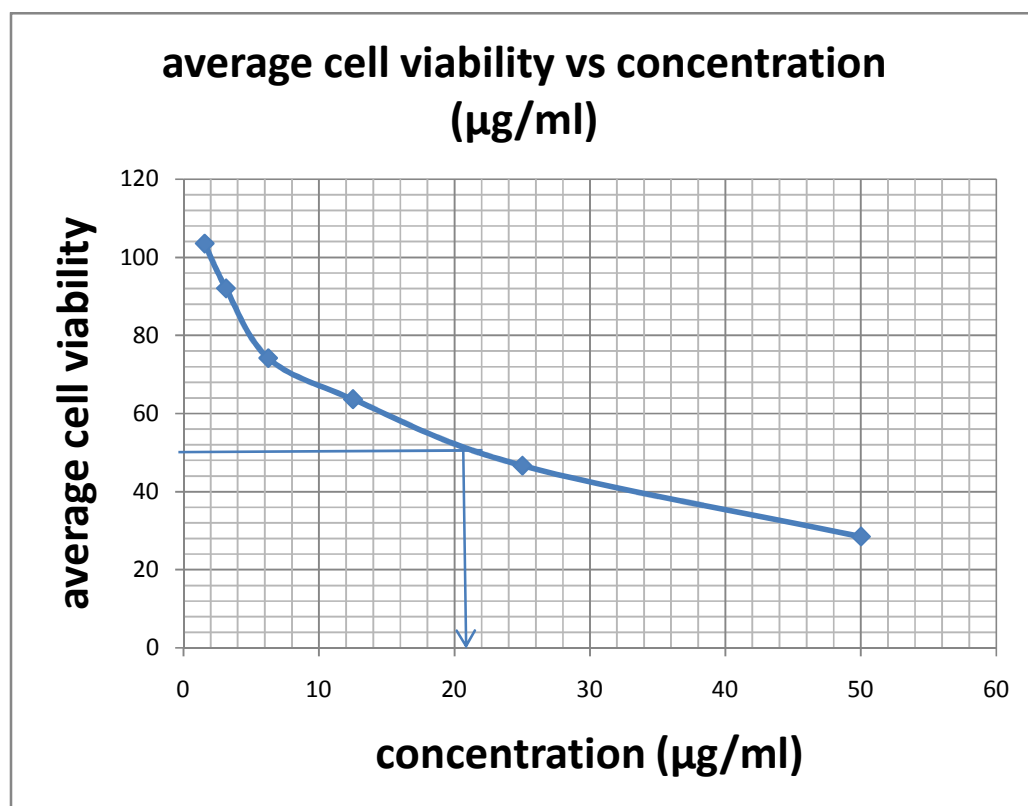
Figure 4.36: Graph of cell viability against concentration of 1,3,7-trihydroxyxanthone (1) for HeLa cancer cell line

The HeLa cancer cell line was found to be moderately susceptible towards 1,3,7-trihydroxyxanthone**(1)** with IC_{50} value of 21μg/ml. However, no significant inhibitory activity was observed for 1,3,7-trihydroxyxanthone**(1)** towards MDA-MB-231cancer cell line. 1,7-Dihydroxy-3-(3-methylbut-2- enyloxy)xanthone **(2)** and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** gave insignificant inhibition towards HeLa and MDA-MB-231 cancer cell lines with IC_{50} value more than 50 μg/ml.

Structurally, it was observed that 1,7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone **(2)** and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** carried a O-prenylated group at their C-3 position compared to 1,3,7-tridhyroxyxanthone **(1)**. The results suggested that the presence of O-prenylated group at C-3 position weakens antitumor activity towards HeLa cancer cell line. Hence, it was suggested that the presence of hydroxyl group at C-3 position is important to elicit significant inhibitory activity towards the HeLa cancer cell line.

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

A total of four compounds have been synthesized and isolated in this study, including a xanthonic block, 1,3,7-tridhyroxyxanthone(1), and three prenylated xanthonic derivatives, 7-dihydroxy-3-(3-methylbut-2- enyloxy)xanthone (2), 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3) and 1-hydroxy-4-(3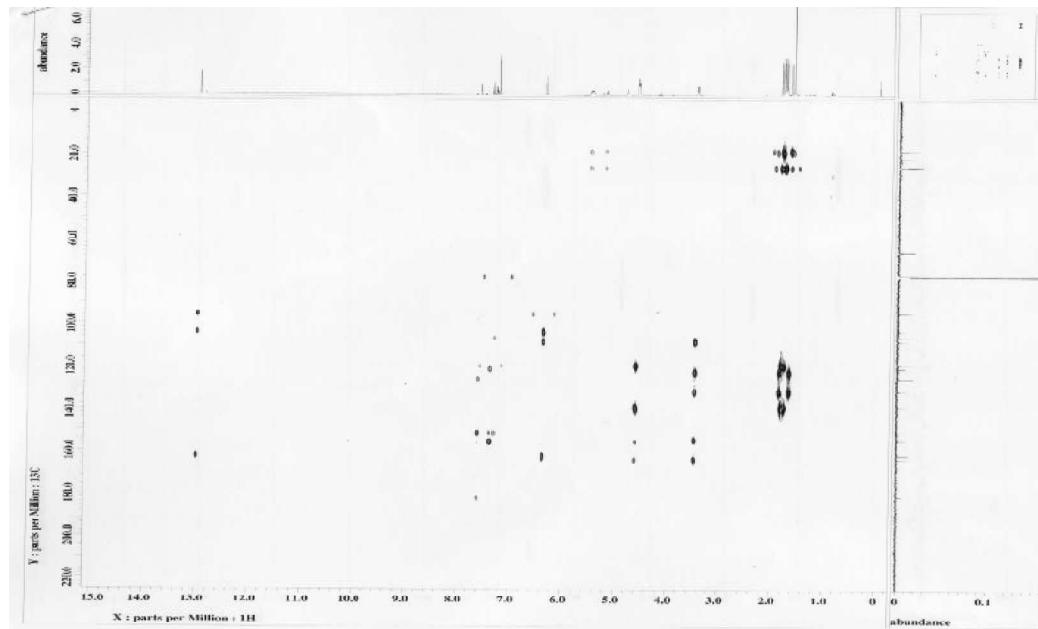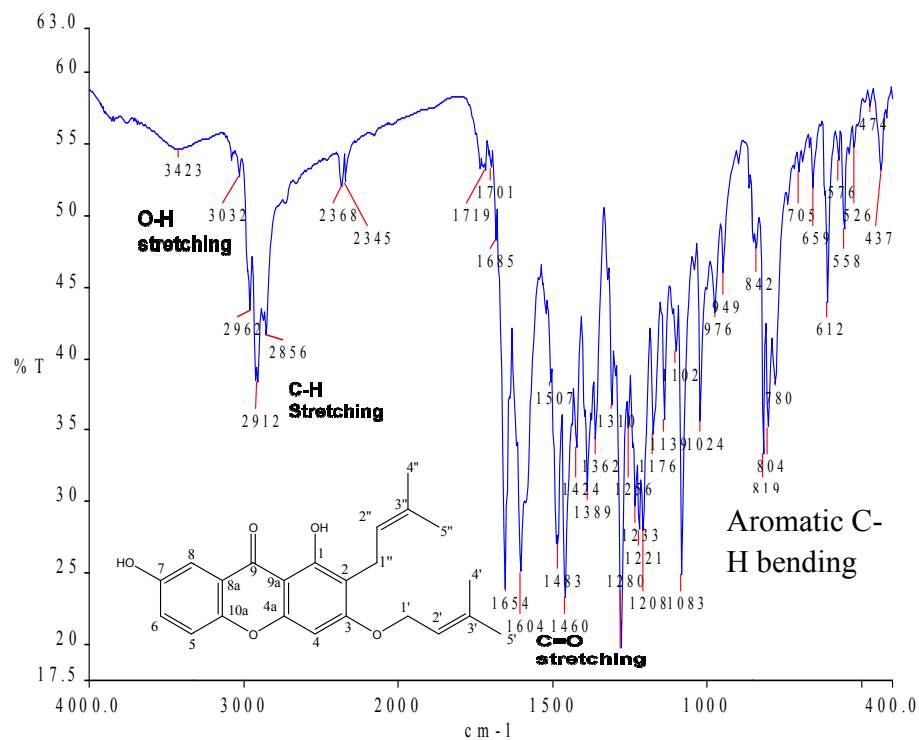-methylbut-2-enyl)-3,7-di(3-methylbut-2-enyloxy)xanthone (4). The structures of these compounds were established through the application of spectroscopic analyses, as well as literature comparison with reported compound for 1,3,7-trihydroxyxanthone.

The bioassay test were conducted for 1,3,7-tridhyroxyxanthone(1), and two prenylated xanthonic derivatives, 7-dihydroxy-3-(3-methylbut-2-enyloxy)xanthone (2), 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3) towards HeLa and MDA-MB-231 cancer cell lines to investigate the inhibitory concentration that kills cells by 50%. 1,3,7-

Tridhyroxyxanthone **(1)** was found to show moderate inhibitory activity against HeLa cancer cell line with IC_{50} value of 21μg/mL but insignificant inhibitory activity against MDA-MB-231 cancer cell line with IC_{50} value > 50 μg/mL. 1,7-Dihydroxy-3-(3-methylbut-2- enyloxy)xanthone **(2)** and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone **(3)** gave insignificant inhibition towards HeLa and MDA-MB-231 cancer cell lines with IC_{50} value more than 50 μg/ml. The results suggested that the presence of O-prenylated group at C-3 position weakens antitumor activity towards HeLa cancer cell line.

5.2 Suggestions for Further Studies

In future, it is suggested that the reaction condition for prenylation of xanthonic block should be modified to enhance the yield. The reaction conditions that can be manipulated are temperature, solvent medium or potential organometallic catalyst which can possibly promote selectivity, thus enhancing the yield.

Second, study can be extended to explore other activities of xanthone derivatives since some previous research had shown that biological activities of xanthone derivatives are versatile. The activities to be explored are antibacterial, anti-inflammatory, anti-oxidant and anti-viral activities and others.

Third, a database for structure-bioactivity was suggested to be created for xanthone derivatives which may help researchers in exploration of new structure and determine the essential functional group and position which may lead to the discovery of potent drug leads.

Finally, different functional group such as geranyl or acetyl group can be introduced into the xanthonic structure to study the effect of these substituents on the biological activities of xanthone derivaties.

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APPENDIX 1

The following table summarise the triplicate results of absorbance analysis for all the compounds for HeLa cancer cell line.

Sample	Conc. (µg/ml)	Replicate 1				Replicate 2				Replicate 3			
		1st	2nd	3rd	Control	1st	2nd	3rd	Control	1st	2nd	3rd	Control
(1)	50	0.649	0.625	0.66	1.596	0.469	0.442	0.446	1.529	0.627	0.472	0.599	1.626
	25	0.847	0.897	0.956	1.533	0.607	0.602	0.724	1.503	0.798	0.877	0.958	1.489
	12.5	1.066	0.978	1.096	1.624	0.889	0.888	1.073	1.505	1.103	1.075	1.227	1.545
	6.25	1.214	1.119	1.158	0.181	1.171	1.058	1.206	0.111	1.211	1.325	1.251	0.18
	3.12	1.434	1.401	1.444	0.183	1.343	1.412	1.487	0.122	1.437	1.415	1.58	0.178
	1.56	1.632	1.659	1.571	0.178	1.59	1.692	1.523	0.106	1.602	1.549	1.573	0.181
(2)	50	1.095	1.045	1.022	1.596	1.142	0.94	1.198	1.529	1.38	1.38	1.382	1.626
	25	1.209	1.252	1.326	1.533	1.208	1.23	1.226	1.503	1.491	1.401	1.591	1.489

	12.5	1.415	1.358	1.375	1.624	1.267	1.362	1.359	1.505	1.473	1.467	1.508	1.545
	6.25	1.452	1.399	1.477	0.181	1.403	1.318	1.599	0.111	1.554	1.476	1.651	0.18
	3.12	1.492	1.41	1.41	0.183	1.416	2.422	1.537	0.122	1.695	1.753	1.641	0.178
	1.56	1.463	1.431	1.424	0.178	1.698	1.717	1.454	0.106	1.855	1.549	1.636	0.181
(3)	50	1.054	1.05	0.988	1.596	1.04	0.974	0.956	1.529	0.975	0.937	0.701	1.626
	25	1.087	1.256	1.182	1.533	1.147	1.241	1.024	1.503	1.034	1.034	1.263	1.489
	12.5	1.392	1.334	1.304	1.624	1.013	1.379	1.399	1.505	1.19	1.182	1.439	1.545
	6.25	1.489	1.412	1.348	0.181	1.352	1.454	1.458	0.111	1.251	1.324	1.354	0.18
	3.12	1.419	1.545	1.533	0.183	1.471	1.608	1.55	0.122	1.359	1.452	1.526	0.178
	1.56	1.789	1.632	1.693	0.178	1.468	1.579	1.569	0.106	1.49	1.512	1.706	0.181

APPENDIX 2

The following table summarise the triplicate results of absorbance analysis for all the compounds for MDA-MB-231 cancer cell line.

Sample	Conc. (µg/ml)	Replicate 1				Replicate 2				Replicate 3			
		1st	2nd	3rd	Control	1st	2nd	3rd	Control	1st	2nd	3rd	Control
(1)	50	2.837	2.737	2.784	2.04	3.328	3.282	3.175	2.566	1.225	1.212	1.189	0.991
	25	3.297	3.283	3.261	1.906	3.455	3.369	3.383	2.294	2.142	1.917	1.653	0.936
	12.5	3.201	3.038	3.146	1.71	3.263	3.199	3.177	2.612	1.552	1.436	1.849	1.032
	6.25	3.054	2.849	2.943	0.143	3.27	3.214	3.341	0.138	1.238	1.121	1.316	0.177
	3.12	2.679	2.824	2.613	0.149	2.99	3.1	3.341	0.139	1.038	0.956	1.365	0.183
	1.56	1.797	1.794	1.894	0.163	2.707	2.535	2.225	0.139	1.014	0.836	0.778	0.173
(2)	50	2.985	3.165	3.303	2.04	3.192	3.254	3.204	2.566	3.185	3.164	3.179	2.54
	25	1.913	1.864	2.032	1.906	3.106	3.236	3.271	2.294	2.976	2.956	2.888	2.479

	12.5	1.244	1.44	1.796	1.71	3.033	3.08	3.108	2.612	2.614	2.805	2.973	2.487
	6.25	1.411	1.482	1.474	0.143	2.77	2.473	2.486	0.138	2.135	2.398	2.445	0.101
	3.12	1.52	1.568	1.536	0.149	2.36	2.481	2.42	0.139	2.035	2.227	2.254	0.118
	1.56	1.482	1.897	1.729	0.163	2.482	2.676	2.714	0.139	2.799	2.664	2.484	0.106
(3)	50	2.051	2.028	2.089	3.237	1.456	1.49	1.447	2.04	2.324	2.317	2.406	2.566
	25	2.568	2.396	2.577	3.223	1.664	1.612	1.651	1.906	2.464	2.433	2.463	2.294
	12.5	2.619	2.678	2.529	3.199	1.665	1.609	1.709	1.71	2.614	2.51	2.475	2.612
	6.25	2.886	2.768	2.832	0.118	1.832	1.826	1.761	0.143	2.713	2.658	2.679	0.138
	3.12	2.989	2.907	2.816	0.115	1.836	1.811	1.852	0.149	2.717	2.729	2.7	0.139
	1.56	3.033	3.202	3.233	0.164	1.884	1.869	2.067	0.163	2.833	2.831	2.836	0.139