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SYNTHESIS, CHARACTERISATION AND CYTOTOXIC ACTIVITIES OF 1, 3, 6, 8 – TETRAOXYGENATED XANTHONE DERIVATIVES

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ABSTRACT

Interest has been shown in the synthesis of xanthonic species because of their interesting pharmacological activities. In this project, a xanthonic block and two alkenylated xanthone derivatives have been synthesised and studied for their cytotoxic activity. The three synthetic xanthones were 1, 3, 6, 8-tetrahydroxyxanthone, 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one. 1, 3, 6, 8-Tetrahydroxyxanthone was the building block for the synthesis of alkenylated xanthones: 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthene-9-one. Reaction of 5-bromo-1-pentene with the xanthonic block in the presence of potassium carbonate in organic medium was found to promote *o*-alkenylation to the block.

The structures of the compounds synthesised were established by means of IR, UV, MS and NMR (^{1}H, ^{13}C, HMQC and HMBC) techniques. The xanthonic compounds were examined for their cytotoxic activity by using MTT assay and the inhibitory activities of these compounds toward the *in vitro* growth of HeLa (cervical carcinoma) and MDA-MB-231 (human breast adenocarcinoma) cancer cell lines were studied. All compounds tested were found to be weakly active against MDA-MB-231 cell. In HeLa cell line test, 1, 3, 6, 8-tetrahydroxyxanthone gave weak inhibitory activity with IC_{50} value of more than 50.0 μg/mL. On the other hand, 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-

one showed moderate activities against the cell line with IC_{50} values of 7.0 μg/mL and 11.0 μg/mL, respectively.

ABSTRAK

Tumpuan telah diberikan dalam penghasilan spesies xanthone kerana mereka mempunyai banyak kegiatan farmakologi. Dalam projek ini, satu blok xanthone dan dua sebatian alkenilisi telah disintesis dan dikaji activity sitotoksik masing-masing. Sebatian-sebatian yang telah dihasilkan ialah 1, 3, 6, 8-tetrahidroksixanthone, 1, 8-dihidroksi-3, 6-bis(pent-4-eniloksi)-9*H*-xanthen-9-one dan 1, 3, 8-trihidroksi-6-(pent-4-eniloksi)-9*H*-xanthen-9-one. 1, 3, 6, 8-Tetrahidroksixanthone adalah blok dalam proses alkenilisi untuk mendapat sebatian alkenilisi: 1, 8-dihidroksi-3, 6-bis(pent-4-eniloksi)-9*H*-xanthen-9-one dan 1, 3, 8-trihidroksi-6-(pent-4-eniloksi)-9*H*-xanthen-9-one. Alkenilisi dengan menggunakan alkenyl bromide dalam medium organik telah menghasilkan produk *o*-alkenilisi.

Sintesis, pengenalan struktur dan activiti sitotoksik ke atas tiga spesis xanthone yang disintesis telah dilaporkan. Teknik IR, UV, MS and NMR (^{1}H, ^{13}C, HMQC and HMBC) telah pun digunakan untuk pengenalan struktur ke atas ketiga-tiga spesis xanthone tersebut. MTT assay telah digunakan untuk mengkaji aktiviti sitotoksik dan kesan-kesan terhadap pertumbuhan *in vitro* sel kanser HeLa dan MDA-MD-231. Keputusan kajian menunjukkan bahawa semua spesis xanthone yang disintesis tidak mempunyai kesan sitotoksik yang nyata terhadap sel MDA-MD-231. Manakala, hanya 1, 8-dihidroksi-3, 6-bis(pent-4-eniloksi)-9*H*-xanthen-9-one dan 1, 3, 8-trihidroksi-6-(pent-4-eniloksi)-9*H*-xanthen-9-one mengenakan aktiviti sitotoksik terhadap sel kanser

HeLa. Mereka masing-masing menunjukkan nilai IC_{50} 7.0 µg/mL dan 11.0 µg/mL. 1, 3, 6, 8-Tetrahidroksixanthone menunjukkan nilai IC_{50} melebihi 50.0 µg/mL terhadap sel kanser HeLa.

ACKNOWLEDGEMENTS

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A special thanks is extended to all the UTAR's lab assistants for their cooperation and assistance during this project.

Lastly, I would like to thank my beloved family members for their continuous support, concern and encouragement in completing this project.

APPROVAL SHEET

I certify that, this project report entitled "**SYNTHESIS, CHARACTERISATION AND CYTOTOXIC ACTIVITIES OF 1, 3, 6, 8-TETRAOXYGENTED XANTHONE DERIVATIVES**" was prepared by **LIM SHIAN HOI** and submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Hons.) in Chemistry at Universiti Tunku Abdul Rahman (UTAR).

Approved by

Supervisor

_____ Date: _____

(Dr. Lim Chan Kiang)

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date:

PERMISSION SHEET

It is hereby certify that **LIM SHIAN HOI** (ID No: **08 ANB 04599**) has completed this report entitled "**SYNTHESIS, CHARACTERISATION AND CYTOTOXIC ACTIVITIES OF 1, 3, 6, 8-TETRAOXYGENATED XANTHONE DERIVATIVES**" under supervision of **DR. LIM CHAN KIANG** from the Department of Chemistry, Faculty of Science.

I hereby give permission to my supervisor to write and prepare manuscript of these research findings for publishing in any form, if I 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 the 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 it has not been previously or concurrently submitted for any other degree at Universiti Tunku Abdul Rahman (UTAR) or other institutions.

_____

(Lim Shian Hoi)

Date:

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LIST OF ABBREVIATIONS

AcOH	Acetic acid
Ac_2O	Acetic anhydride
α	alpha
$AlCl_3$	Aluminium chloride
NH_4Cl (sat)	Ammonium chlroride (saturated)
HCO_2NH_4	Ammonium formate
AIBN	Azobisisobutyronitrile
Bn	Benzyl
BnBr	Benzyl bromide
β	Beta
BF_3	Boron trifluoride
Br	Bromine atom
CsF	Caesium fluoride
CSA	Camphoresulfonic acid
CO_2	Carbon dioxide
^{13}C-NMR	Carbon-Nuclear Magnetic Resonance
Cl	Chlorine atom
CCl_4	Chloroform
CrO_3	Chromium trioxide
Cu	Copper
CuI	Copper Iodide
DNA	Deoxyribonucleic acid

CH_2Cl_2	Dichloromethane
Et_2O	Diethyl ether
DIBAL-H	Diisobutylaluminium hydride
(-) DIPT	Diisopropyltryptamine
DMAP	4-Dimethylaminopyridine
$(CH_3)_2SO_4$	Dimethyl sulphate
DMF	Dimethylformamide
$(EtO)_2CO$	2-Ethoxy-1,3-dioxolane
EtOAc	Ethyl acetate
$(C_2H_5)_2O$	Ethyl ether
FT-IR	Fourier Transform- Infrared
γ	gamma
Δ	Heat
h	Hour
HCl	Hydrochloric acid
H	Hydrogen atom
HBr	Hydrogen bromide
HI	Hydrogen Iodide
HO^-	Hydroxide ion
IR	Infrared
IBX	2-Iodoxybenzoic acid
Pb	Lead
mCPBA	Meta-chloroperoxybenzoic acid
MsCl	Methanesulfonyl chloride

CH_3SO_3H	Methanesulphonic acid
MeOH	Methanol
MOMCl	Methyl chloromethyl ether
CH_3	Methyl group
MeI	Methyl iodide
MAOS	Microwave-Assisted Organic Synthesis
NBS	N-bromosuccinimide
n-BuLi	n-butyl lithium
N,N-DEA	N-nitrosodiethylamine
1D-NMR	One Dimensional- Nuclear Magnetic Resonance
OsO_4	Osmium tetroxide
O	Oxygen atom
H_3PO_4	Phosphoric acid
$POCl_3$	Phosphorus chloride
P_2O_5	Phosphorus pentoxide
PPA	Polyphosphoric acid
KI	Potassium Iodide
^{1}H- NMR	Proton-Nuclear Magnetic Resonance
Py	Pyrene
Sat	Saturated
NaH	Sodium Hydride
$NaHSO_3$	Sodium hydrogen sulfite
NaI	Sodium Iodide
$NaIO_4$	Sodium metaperiodate
NaOMe	Sodium methoxide
S	Sulphur atom

H_2SO_4	Sulphuric acid
SC-CO_2	Supercritical Fluid- Carbon Dioxide
TBSCl	Tert-butyldimethylsilyl chloride
TBAF	Tetrabutylammonium fluoride
THF	Tetrahydrofuran
t-BuOOH	Tert-butyl hydroperoxide
Ti(*i*-Opr)$_4$	Titanium propoxide
Bu_3SnH	Tributylstannane
$CHCl_3$	Trichloromethane
Et_3N	Triethylamine
PPh_3	Triphenylphosphine
2D-NMR	Two Dimensional- Nuclear Magnetic Resonance
UV-Vis	Ultraviolet- Visible
$ZnCl_3$	Zinc chloride

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Xanthone is a compound that possesses a tricyclic scaffold and can also be called as dibenzo-γ-pyrone or 9-xanthenone. This compound is normally yellow in colour and has a symmetrical structure. Xanthones were reported to have anti-tumor, anti-oxidant, anti-inflammatory, anti-allergy, anti-bacterial, anti-fungal and anti-viral properties [Diderot *et al*., 2006]. All these biological properties possessed by xanthone are depending on the position and the type of substituents that are attached to xanthonic block and this has attracted the researchers to further their study on xanthone. In this project the main focus is on cytotoxic property of alkenylated xanthones and the basic skeleton of xanthone is shown as below:

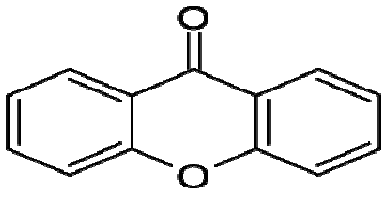


Figure 1.1: Basic skeleton of xanthone

Xanthones can be obtained through synthesis or via extraction from the natural resources such as plants from the families of *Gentianaceae, Guttiferae,*

Polygalaceae, Leguminosae, Lythraceae, Moraceae, Loganiaceae, and *Rhamnaceae*; fungi and lichens [Diderot *et al*., 2006]. Generally, natural xanthones can be classified into five groups, which are simple oxygenated xanthones, glycoside xanthones, prenylated xanthones, xantholiganoids and miscellaneous. Figures 1.2, 1.3, 1.4 and 1.5 showed the examples of xanthone extracted from natural sources.

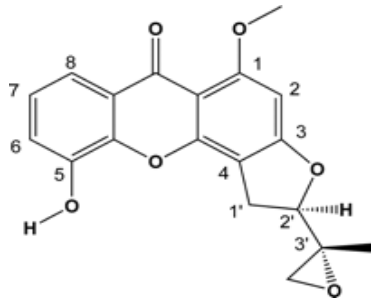


Figure 1.2: Structure of psorospermin from *Psoroepermum febrifugum*

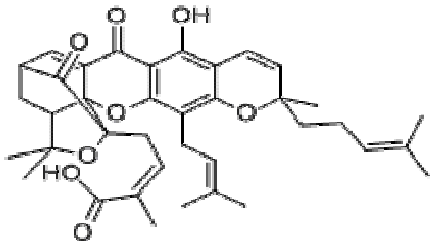


Figure 1.3: Structure of gambogic acid from *Garcinia hamburyi*

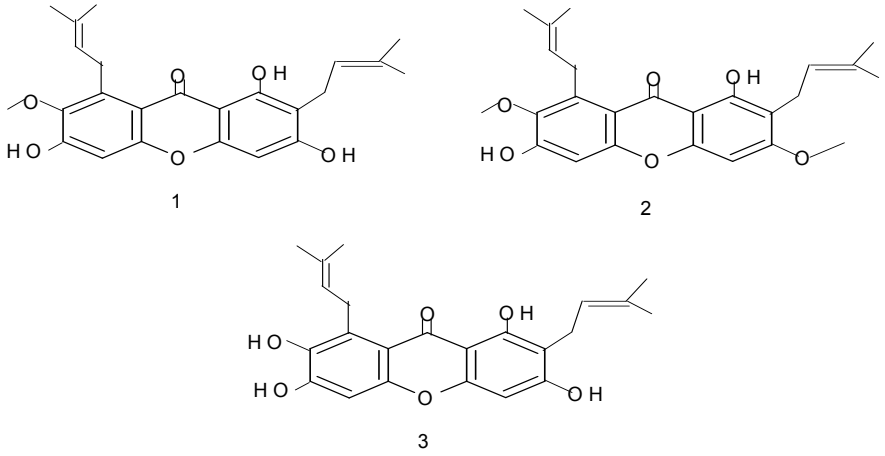


Figure 1.4: Structure of α-mangostin (1), β-mangostin (2) and γ-mangostin (3) from *Garcinia mangostana*

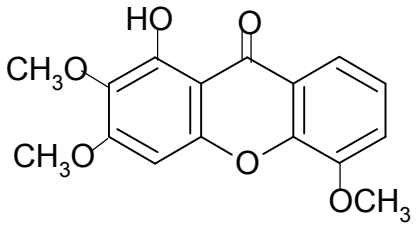


Figure 1.5: Structure of 1-hydroxy-2, 3, 5-trimethoxyxanthone from *Helenia elliptica*

Due to the facts that, xanthone derivatives extracted from the natural sources were found to be limited in types and positions of the substituent, through synthesis, various types of xanthone can be produced to allow comprehensive structural activity relationship study to be carried out for identification of the cytotoxic functional group present in various xanthone derivatives. So, in this project, synthesis of alkenylated xanthones is the main focus.

From the previous studies, there are six methods being practiced in synthesising xanthones. These methods included Michael-Kostanecki method, Friedel-Crafts method, Robinson-Nishikowa method, Ashina-Tanase method, Tanase method and Ullman method [Diderot *et al*., 2006]. Characterisation of the synthetic xanthone were done by means of UV-Vis, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR including HMQC and HMBC, IR and mass analyses. Purification of xanthones were done by using column chromatography.

1.2 Objectives

The objectives of this project are to:

1. Synthesise xanthonic building block and alkenylated xanthone derivatives.

2. Purify the synthetic compounds by using column chromatography.

3. Characterise and identify the structure of synthetic compounds by using FT-IR, 1D- & 2D-NMR, UV-Vis spectroscopic and mass spectrometric methods.

4. To investigate the cytotoxic activity of synthetic xanthones against cancer cell lines: HeLa and MDA-MB-231.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of Xanthone and Its Prenylated Derivatives

Xanthone, having a molecular formula of $C_{13}H_8O_2$, with the structure displayed in Figure 2.1, can have lots of derivatives bearing different substituent and each of these derivatives has different functions. From the past review, different derivatives of xanthone have been reported to show several biological properties as discussed before in the previous chapter. Those biological properties of xanthone derivatives are depend on the position and the types of substituent that attached to the xanthonic block.

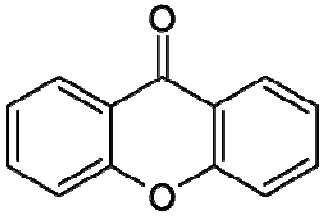


Figure 2.1: Structure of xanthonic block

Due to the interesting biological properties of xanthone, the main focus of this project is to synthesis alkenylated xanthones that have cytotoxic effect. From the past researches, alkenylated xanthone obtained can be classified into *o*-alkenylated xanthone or *c*-alkenylated xanthone. In *o*-alkenylation, alkenyl

bromide is reacted with the –OH group of xanthone through nucleophilic substitution. While, *c*-alkenylation involves electrophilic substitution on the carbon atom of the xanthonic block.

Alkenylated xanthone can be synthesised, either through synthesising the desired xanthonic nucleus (Section 2.1.1) and followed by alkenylation (Section 2.1.2) or by using the alkenylated starting materials to synthesised alkenylated xanthones (Section 2.1.3).

2.1.1 Synthesis of Xanthonic Block

Xanthonic block can be synthesised by using Grover, Shah and Shah's Method, via diaryl ether intermediate or other's methods.

2.1.1.1 Grover, Shah and Shah's Method

Grover, Shah and Shah's method is the conventional method used in synthesising xanthones. This method has been proven to give high yield of xanthonic nucleus about 90% [Varacha-Lembege *et al*., 2008].

This method utilises Eaton's reagent as coupling reagent to promote coupling reaction between phloroglucinol and salicylic acid or substituted salicylic acid. But in other studies, Eaton's reagent was reported to be replaced by polyphosphoric acid (PPA) or $ZnCl_3$/ $POCl_3$ (93-95%) which acts as coupling agent in the reaction.

2.1.1.2 Synthesis via Benzophenone and Diaryl Ether Intermediate

In other research, another method used in synthesising xanthonic nucleus has been established by using benzophenone (Figure 2.2) and diaryl ether as intermediate (Figure 2.3).

Figure 2.2 shows the pathway in synthesising xanthonic nucleus via benzophenone intermediate. There are two pathways: **a** and **b,** that are different in their starting materials. Path **a**, involves the synthesis of benzophenone intermediate through condensation reaction carried out by phosphorus oxychloride and zinc chloride on *ortho*-oxygenated benzoic acid and activated phenol. Whereas in path **b**, benzophenone intermediate is obtained through condensation reaction by Friedel-Crafts acylation of the appropriately substituted benzoyl chlorides with phenolic derivatives. Then, paths **a** and **b** undergo oxidative or dehydrative process to enable cyclisation of 2, 2'-dioxygenatedbenzophenone to form xanthonic block [Demikiran, 2007].

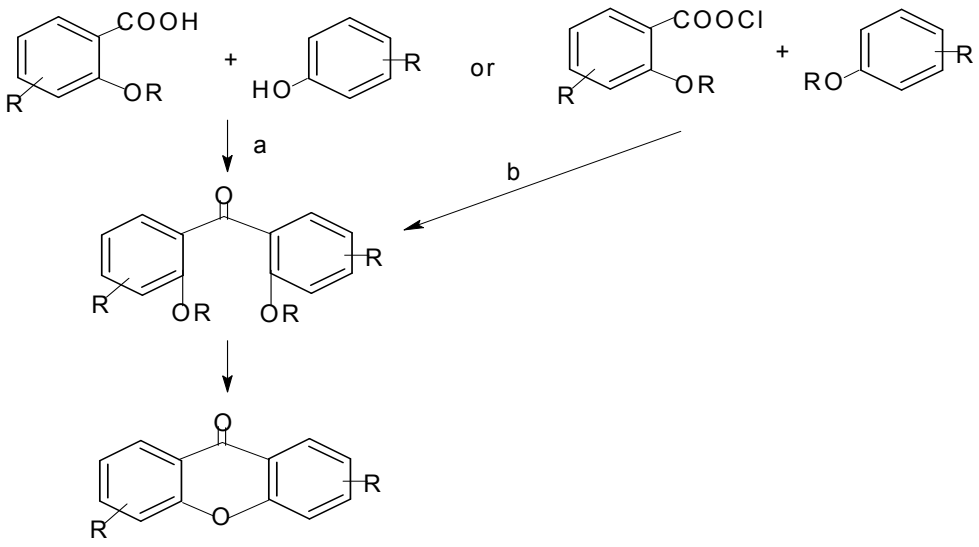


Figure 2.2: Synthesis of xanthonic nucleus via benzophenone intermediate

Synthesis of xanthonic nucleus via diaryl ether intermediate is shown in Figure 2.3. This method utilises the condensation reaction of phenol and *O*-chloro or bromobenzoic acid. Then, one step reaction is carried out by using lithium diisopropylamide or acetyl chloride to convert the biphenyl intermediate to xanthonic block. This method is called Ulmann synthesis [Demikiran, 2007].

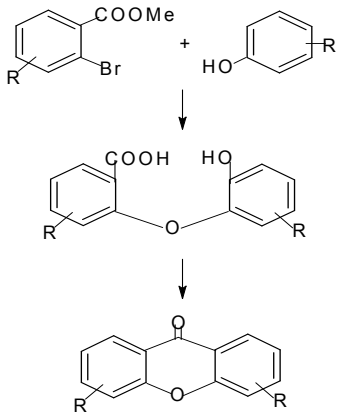


Figure 2.3: Synthesis of xanthonic nucleus through diaryl ether intermediate

2.1.1.3 Other Synthesis Methods

There are still many methods that have been applied to produce the desired xanthonic block such as by using readily accessible salicylates and saliaryl triflate. This method makes use of CsF to afford efficient one-step synthesis of biological interesting xanthone and thioxanthone. This reaction presumably proceeds by a tandem intermolecular nucleophilic coupling of the benzoate and aryne with subsequent intramolecular electrophilic cyclisation [Zhao *et al.*, 2007].

Besides that, the formation of xanthone can also be done by coupling of phenol and aryl halide in the presence of copper to generate aryl ethers. Cyclisation is then completed by using *o*-phenylsalicylic acid derivative with concentrated H_2SO_4 heated at 100 ^oC (Figure 2.4). The drawback of this method is that many steps are involved in the production of xanthone, at which, this will decrease the percentage yield.

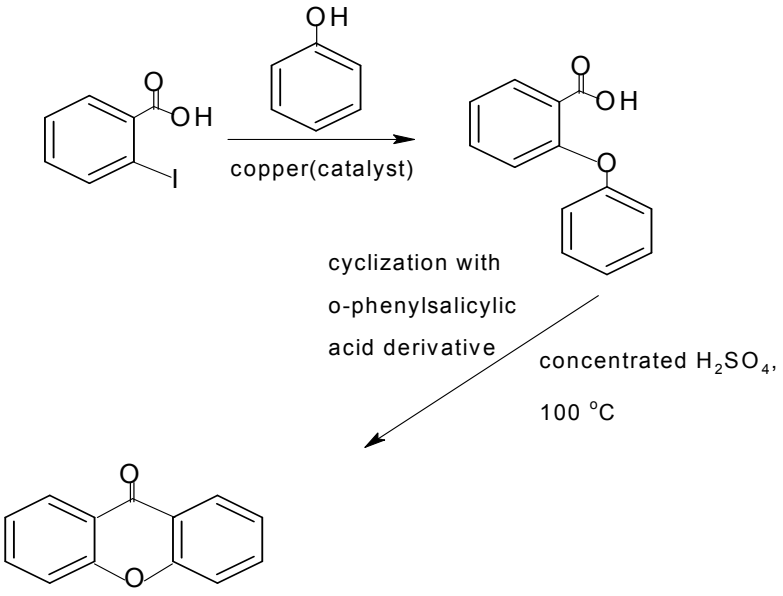


Figure 2.4: Synthesis of xanthone nucleus using copper as catalyst

Other methods that can be used to prepare xanthonic nucleus are shown in Figures 2.5, 2.6, 2.7, 2.8 and 2.9.

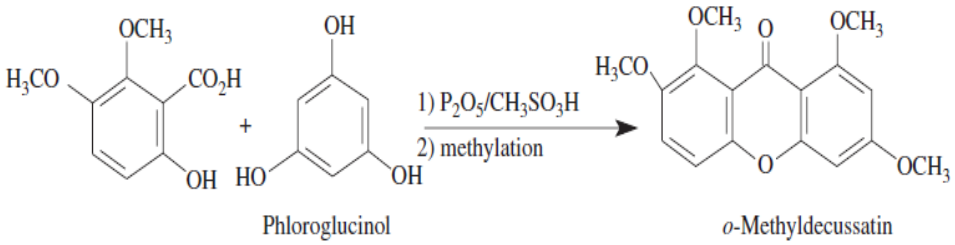


Figure 2.5: Synthesis of *O*-methyldecussatin [Pillai *et al*., 1986]

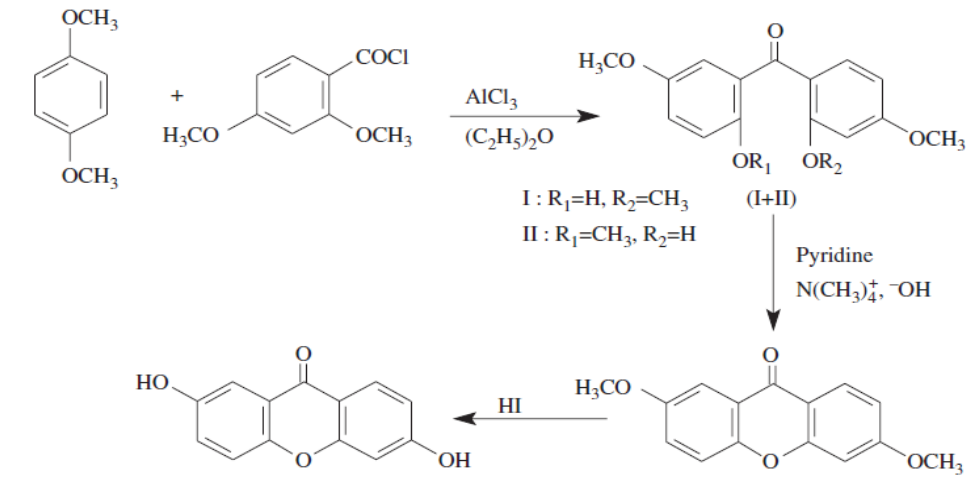


Figure 2.6: Preparation of 3, 7-dihydroxyxanthone [Lin *et al.*, 1993]

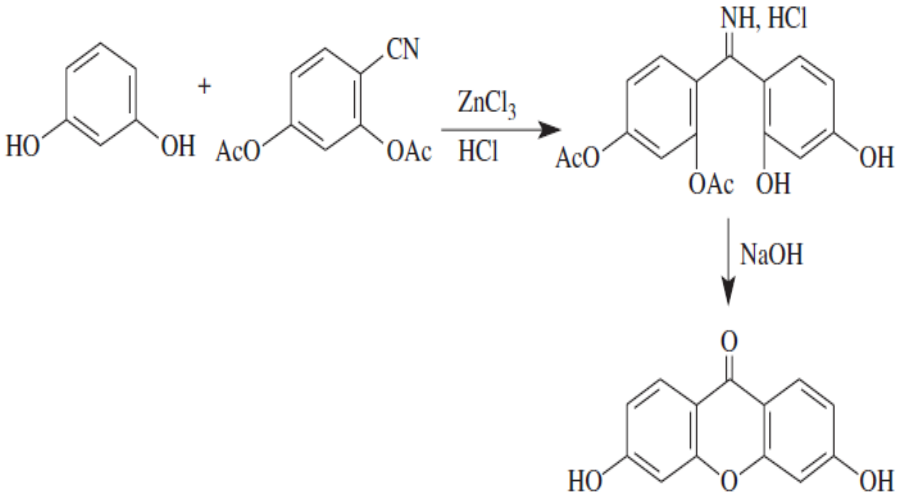


Figure 2.7: Preparation of 3, 7-dihydroxyxanthone via Ketimino intermediate [Atkinson and Heilbron, 1926]

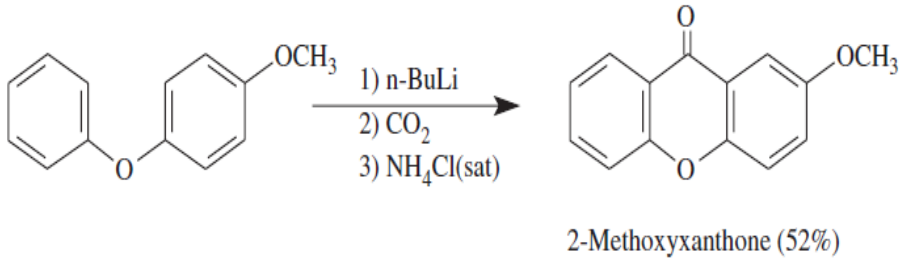


Figure 2.8: Preparation of methoxylated xanthones [Vitale *et al.*, 1994]

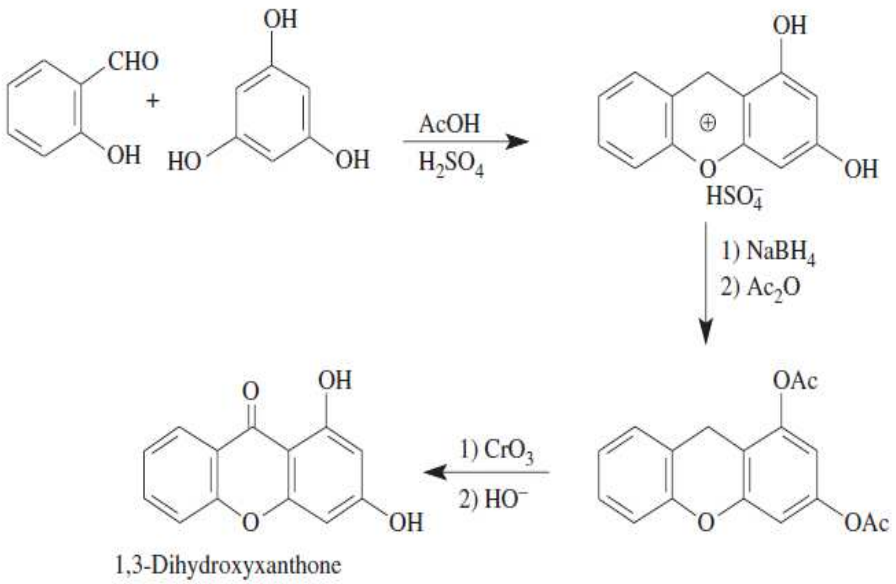
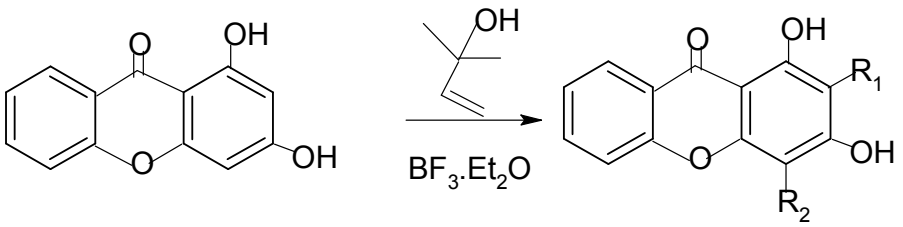


Figure 2.9: Synthesis of 1, 3-dihydroxyxanthone [Pillai *et al.*, 1986]

2.1.2 Prenylation of Xanthonic Block

After synthesising the xanthonic block, prenylation is then carried out through reacting the xanthone block with prenyl bromide in either organic medium or aqueous medium to produce *o*-prenylated or *c*-prenylated xanthones. In organic medium such as in the presence of acetone with prenyl bromide and K_2CO_3, *o*-prenylated xanthone can be produced and to produce *c*-prenylated xanthone, aqueous medium is used. There are several methods shown in Figures 2.10, 2.11, 2.12 and 2.13 to synthesis *c*-prenylated xanthones.



R_1 =Pre; R_2 = H

or

R_1 = H ; R_2 = Pre

or

R_1 = R_2 = Pre

* Pre = prenyl

Figure 2.10: *c*-Prenylation with 2-methylbut-3-en-2-ol [Anand and Jain, 1973]

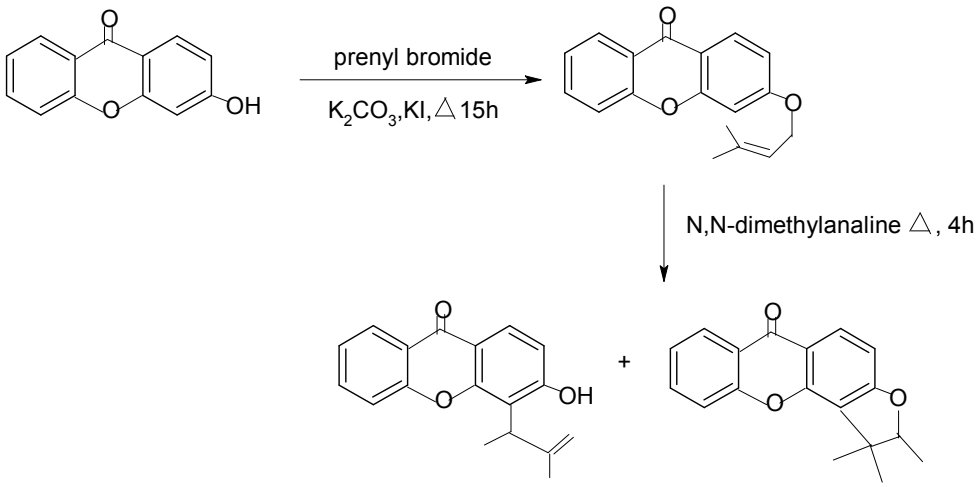


Figure 2.11: *c*-Prenylation with prenyl bromide in the presence of a strong base [Anand and Jain, 1973]

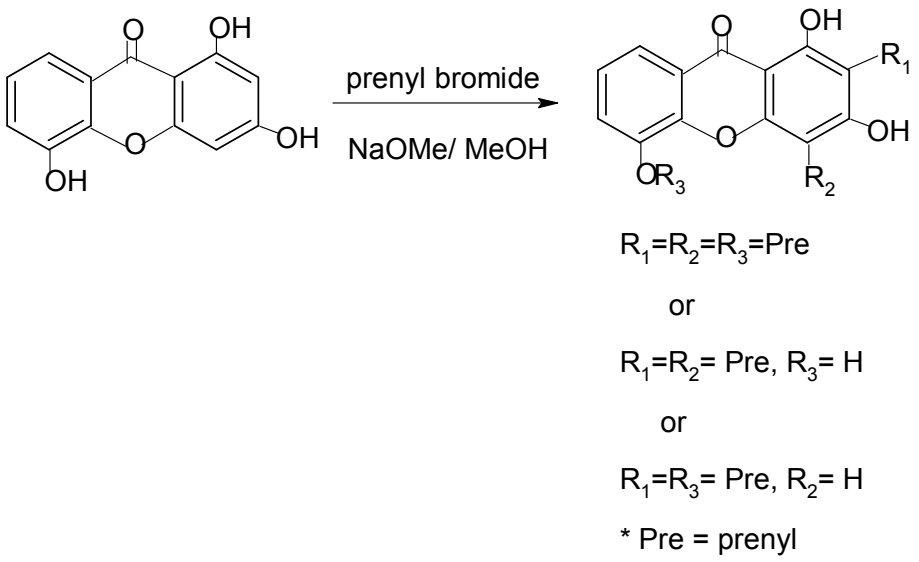


Figure 2.12: *c*-Prenylation through Claisen rearrangement [Patel and Trivedi, 1988]

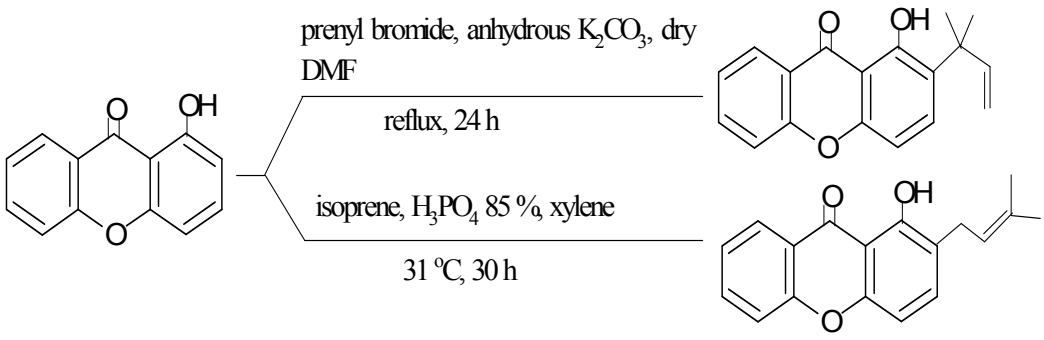


Figure 2.13: Synthesis of *c*-prenylated 1, 1-dimethylally- and 3, 3-dimethylallyl derivatives of xanthone [Castanheiro *et al.*, 2009]

2.1.3 Synthesis via Prenylated Starting Materials

This synthesis method is different from Sections 2.1.1 and 2.1.2; it starts with prenylation of the starting materials before coupling reaction is carried out. This method involves the use of protecting group such as benzyl group in order to improve the high selectivity of the reaction (Figures 2.24, 2.25 and 2.26) [Iikubo *et al.,* 2001].

2.1.4 Improved Synthesis Method

All the synthesis methods mentioned earlier are not environmental friendly. To make the reaction more environmental friendly, a new approach by using microwave has been introduced to synthesise prenylated xanthone. This method is called as microwave-assisted organic synthesis (MAOS) [Castanheiro *et al.*, 2009].

There are pros and cons in the use of this method. At which, it can have high yield of prenylated xanthone but can only be applied to certain types of synthesis. Enhancement has been made by using Mantmorillonite clay as catalyst. This clay enables the reaction to take place in mild condition, increasing the reaction selectivity, yield and decreasing the reaction time. Furthermore, this clay can be easily separated from the reaction mixture,

regenerate and purify. This clay also enables the reaction to be carried out in either solvent free or with solvent condition.

2.1.5 Further Synthesis on Prenylated Xanthone

The reaction does not stop, even though, prenylated xanthone has been synthesised. Prenylated xanthones can further undergo cyclisation to form pyranoxanthone (Figure 2.14, 2.15 and 2.16). Normally, this reaction can be accomplished through refluxing of prenylated xanthone with a catalytic amount of zinc chloride in dry xylene.

The disadvantage of this reaction is that, the yield from the synthesis was low and sometimes the cytotoxic activity was found to be weaker than the prenylated xanthone precursor that was used for cyclisation [Castanheiro *et al.*, 2009].

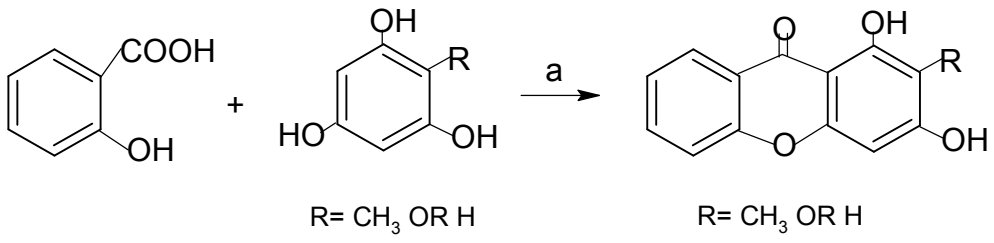


Figure 2.14: Synthesis of xanthone block. Reagents and conditions: (a) $ZnCl_2$, $POCl_3$, 70 ^{0}C, 3 h

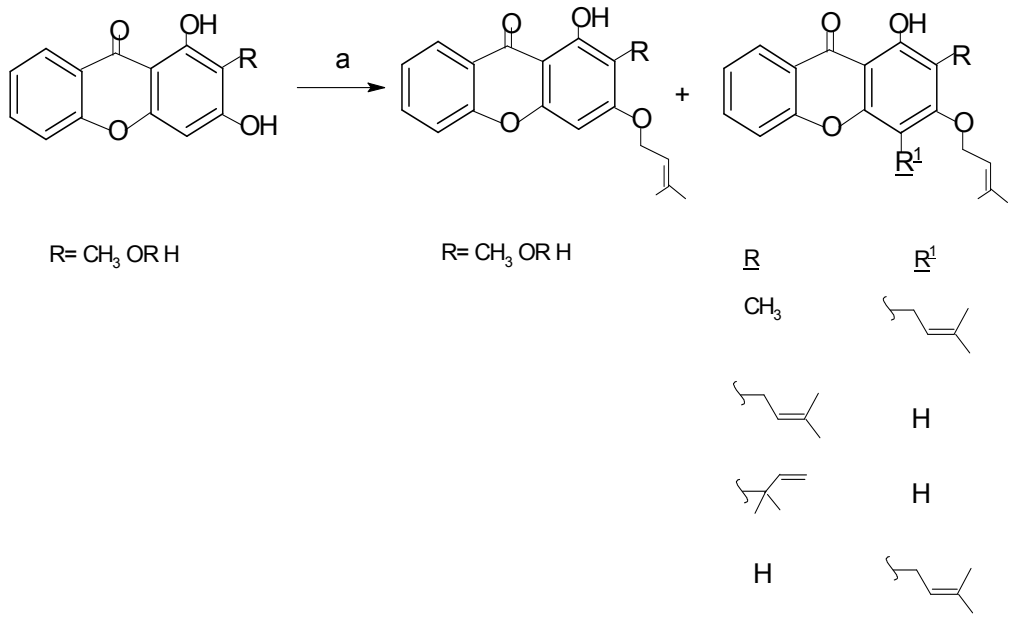


Figure 2.15: Synthesis of prenylated xanthone. Reagents and conditions: (a) prenyl bromide, K_2CO_3, acetone, reflux, 8 h

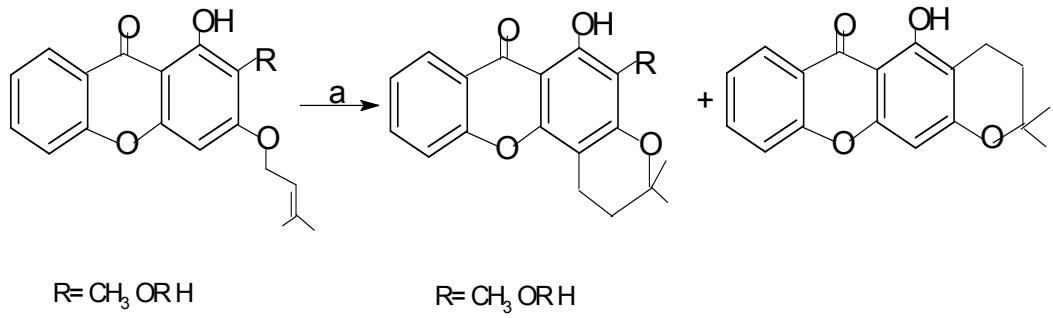


Figure 2.16: Cyclisation of prenylated xanthone. Reagents and conditions: (a) $ZnCl_2$, *o* –xylene, 200 ^oC, 21 h

Noted that, if there is prenyl group attached to the xanthonic block, the size of the prenyl group is important and prenyl group is associated with more selective compound [Castanheiro *et al*., 2009].

2.2 Cytotoxic Activity of Synthetic Xanthone Derivatives

Cyto in greek means cell and *toxic* is poisonous. Thus, combining the two words; cytotoxic means toxic to cell or cell killing. Nowadays, cancer has become a notorious killer around the world and many methods have been carried out to produce anticancer drug. Cancer is a class of disease or disorder characterised by the uncontrolled division of cells and the ability of these cells to invade other tissue or by implantation into distant sites by metastasis [Suphavenich *et al.,* 2009]. Metastasis is defined as spreading of the cancer cell to the neighbouring cell. The example of cancer cell was collected and studied in 1951, called as HeLa cell because this cell was derived from the tumor removed from a woman named Henrietta Laeks.

Normally, cytotoxic activity of the drugs synthesised is tested by using human tumor cell lines KB 3.1(oral squamas cell carcinoma), HeLa cell, breast cancer cell tumor cell line NCI-H460 (non- small lung), MCF-7 (breast adenocarcinoma), UACC-62 (melanoma) and SF-268 (central nervous system). To kill the cell, some drugs are used to interfere with the cell cycle permanently such as to all the cell progress at G_1, S or G_2/M phase [Ding *et al*., 2009]. G_1, S and G2/M phases are shown in Figure 2.17.

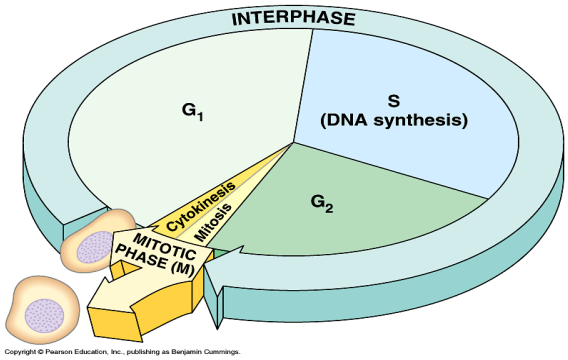


Figure 2.17: Cell cycle

In interphase, including G_1 phase, S phase and G_2 phase, the cell grows and copies chromosomes in preparation for cell division. But chromosomes are only duplicated in S phase. Part of the cell cycle is mitotic phase which can be divided into mitosis and cytokinesis. Mitosis can be divided into 5 stages: prophase, prometaphase, metaphase, anaphase and telophase. Cytokinesis is the last stage in mitotic phase which involved in cleaving the cell's membrane and producing individual cell that completed the whole mitotic phase [Cambell, R. 2005].

As conclusion, cell cycle works through cell growth in G_1 phase, then continue to grow, as the cell copies own chromosomes in S phase and then undergo cell division in G_2 phase. Finally, the cell is divided in mitotic phase. This process repeats to generate new cells. So, by interfering the phases in the cell cycle, this will prevent the cancer cell from replicating and finally die.

In order to interfere the cell cycle of cancer cell, high energy radiation can be used to destruct the DNA in cancer cell. But there is other alternative by using chemotherapy that involves the use of chemotherapeutic drugs to interfere the specific stages in the cell cycle. One of the cytotoxic drugs is xanthone. Xanthone is cytotoxic because the planar structure of xanthone and xanthone derivatives has the ability to intercalate to DNA. Xanthone and xanthone derivatives are then called as DNA intercalator. When DNA alkylating or binding groups are incorporated in the xanthone structure, the resulting compounds might show enhanced DNA interacting capacity by synergic effect from two combined properties; DNA intercalation and DNA alkylation or groove binding [Na, 2009]. Thus, intercalation with the DNA inhibits the cell to replicate and finally the cancer cell stop growing. Based on previous research, the greater the number of prenyl substituents, the higher was the cytotoxic potency [Han *et al*., 2008].

Based on the fact that DNA is the target of prenylated xanthones, xanthones extraction and synthesis have now become a popular study in cytotoxic research.

The cytotoxic activities of xanthone can be evaluated by several methods. These include cytotoxic assay, MTT (Method of Transcription and Translation) assay, SRB (Sulphorhodamine B) assay, WST (Water Soluble Tetrazolium) assay and clonogenic assay. To qualitatively compare the potency of the tested compounds, three parameters including GI_{50}, TGI, and LC_{50} can be used [Han

et al., 2008]. GI_{50} is the concentration of a compound inhibiting 50% of the cell growth [Han *et al*., 2008] whereas TGI is the concentration of a compound completely inhibits the cell growth at 48h and LC_{50} is the concentration at which the tested compound kills 50% of the cell at 48h [Han *et al*., 2008]. The smaller the GI_{50}, TGI, and LC_{50} values, the more cytotoxic the xanthones. Synthesis of xanthone derivatives that have cytotoxic activity are shown in Section 2.3.

2.3 Synthesis of Cytotoxic Xanthone Derivative

As mentioned in Section 2.2, some of the xanthone derivatives have cytotoxic activity. While, in this section, the methods to synthesise various type of cytotoxic xanthones are discussed.

2.3.1 Bromoalkoxyxanthone

In recent research, bromoalkoxyxanthone has been reported to show cytotoxic activity towards SF-268 (CNS-cancer cell, GI_{50}= 30.2 ± 3.6 μM), NCl-H460 (non-small cell lung cancer, GI_{50} = 30.2 ± 3.6 μM) and growth inhibitory activity against MCF-7 (breast adenocarcinoma, estrogen dependent ER(+), GI_{50} = 22.7 ± 1.3 μM). All these effects were due the presence of bromine atom

that could serve as an anchor for the intercalation with DNA [Sousa *et al.,* 2009]. The synthesis of bormoalkoxyxanthone is shown as below:

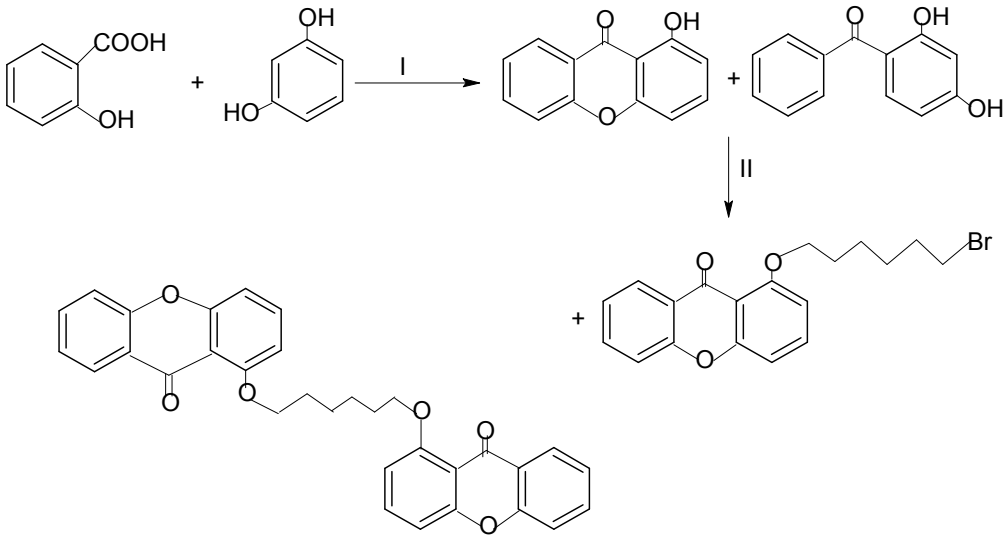


Figure 2.18: Reagents and conditions: (I) zinc chlroride, 200 ^{o}C/ 5 min – 180 ^{o}C/ 4 h; (II) potassium carbonate, dry DMF, 1, 6-dibromobutane, room temperature, 24 h [Sousa *et al.,* 2009]

2.3.2 Epoxyxanthone

Epoxyxanthone compounds and the open ring halohydrin xanthones have been tested for their cytotoxic activity. From the experiment carried out, 1, 3-bisepoxyxanthone was found to be the most active in cell growth inhibition capacity and 1, 3-bisepoxyxanthone also showed concentration dependent DNA cross-linking activity. This observation showed that, two-epoxy group substituted xanthone generated better cytotoxic activity than single epoxy

substitution. Even though, the locations of the two epoxypropoxy groups were different. Two epoxy groups that tethered to 3, 5-position of xanthone can increase the cytotoxic activity dramatically [Na, 2009].

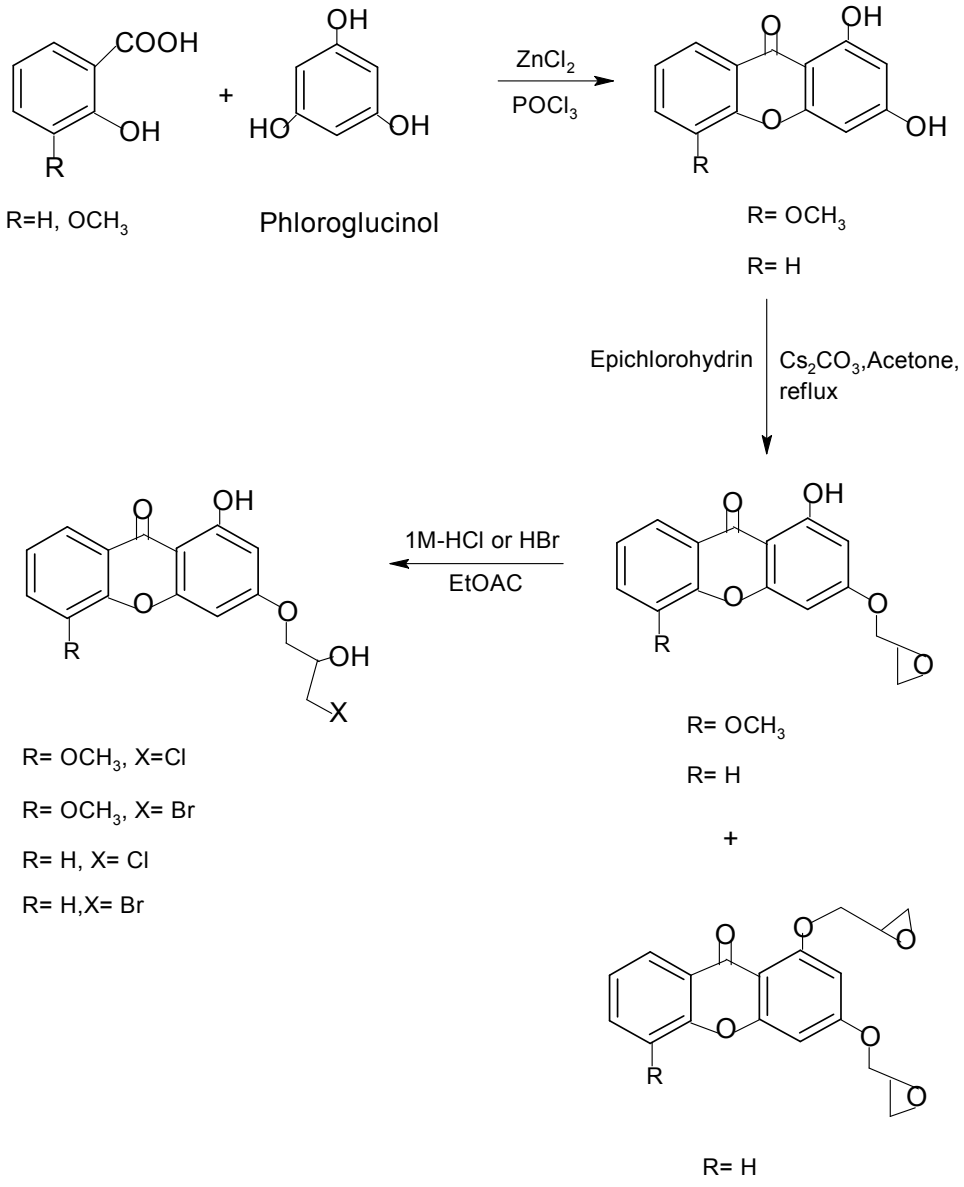


Figure 2.19: Synthesis pathways for epoxyxanthone derivatives [Woo *et al.*, 2006]

Besides that, from the research carried out, it was shown that thioxanthone with the presence of epoxy group was found to possess great cytotoxic activity [Na, 2009].

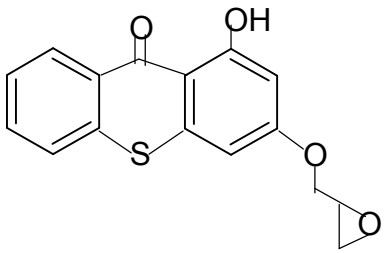


Figure 2.20: Thioxanthone bearing epoxy group [Na, 2009]

2.3.3 Pyranothioxanthone

In another study, some pyranothioxanthones have been proven to have cytotoxic activity. The synthesis method is shown in the Figure 2.21.

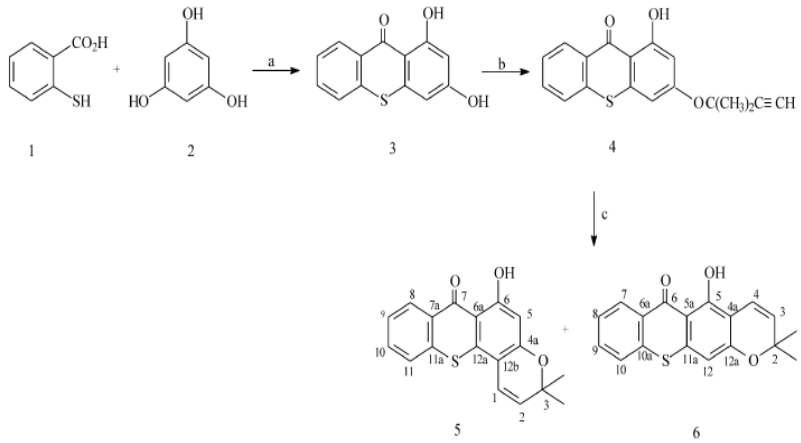


Figure 2.21: (a)Methanesulfonic acid, P_2O_5, Δ; (b)3-chloro-3-methyl-1-butyne, CuI, K_2CO_3, NaI, DMF; (c)N,N-DEA, Δ [Kostakis *et al*., 2001]

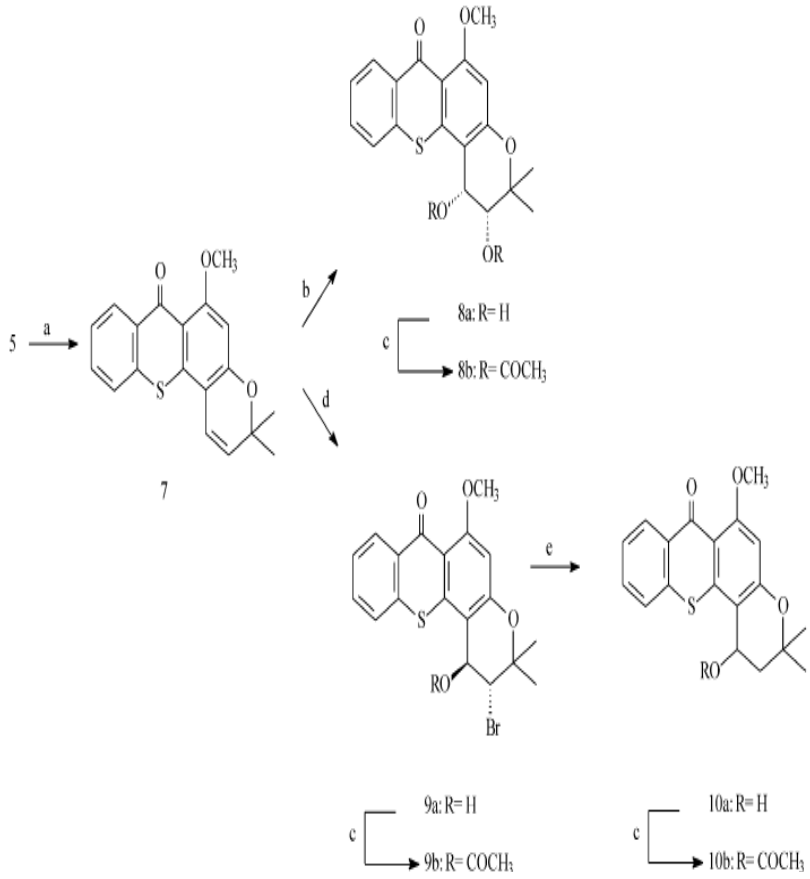


Figure 2.22: (a) (1) NaH, THF; (2) $(CH_3)_2SO_4$, Δ; (b) (1) OsO_4,N-methylmorpholine N-oxide ;(2)$NaHSO_3$; (c) Ac_2O,Py; (d) NBS, H_2O-THF; (e) AIBN, Bu_3SnH, toluene, Δ [Kostakis *et al.*, 2001]

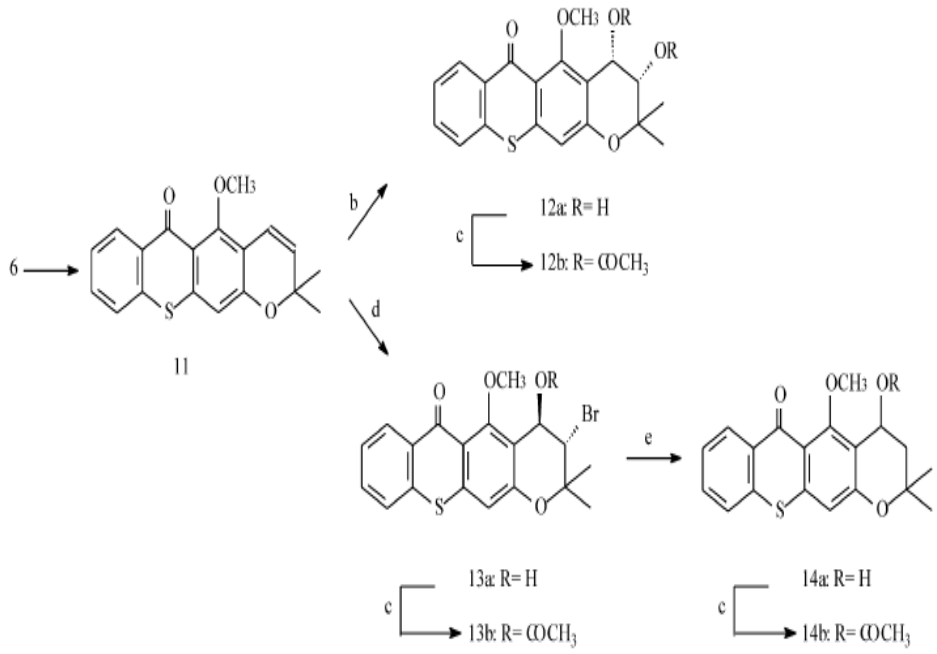


Figure 2.23: (a) (1) NaH,THF; (2) $(CH_3)_2SO_4$, Δ; (b) (1) OsO_4, N-methylmorpholine N-oxide; (2) $NaHSO_3$; (c) Ac_2O, Py; (d) NBS, H_2O-THF; (e) AIBN, Bu_3SnH, toluene, Δ [Kostakis *et al.*, 2001]

From the research carried out by Ioannis Kostakis *et al.,* pyranothioxanthone **7, 8b, 9a, 10a** and **12b** were found to possess good cytotoxic property. The cytotoxic activities of these compounds were evaluated *in vitro* on the L1210 leukemia cell line, with acronycine as reference compound. Compound **9a** (GI_{50} =12.1 ±1.3 μM) exhibited interesting cytotoxic activity, being twice more potent than acronycine (GI_{50} = 25.0 ± 4.1 μM), while compound **7** (GI_{50} = 36.9 ± 3.6 μM), **8b** (GI_{50} = 36.6 ± 2.9 μM), **10a** (GI_{50} = 27.7 ± 2.8 μM) and **12b** (GI_{50} = 33.0 ± 3.2 μM), were as potent as acronycine. In general, in terms of angular isomers, the structure of the compounds that are similar with

acronycine will be more potent than the linear analogues. This suggested that angular orientation of D ring is important for biological activity [Kostakis *et al.,* 2001].

2.3.4 α-Mangostin

α-Mangostin has been proven to have cytotoxic activity too. α-Mangostin can inhibit cell growth and human leukemia cell line by introducing cascape-3 dependent apotosis. Further studies revealed that cellular target of α-mangostin were mitochondria. Recently, research has been carried out in synthesising α-mangostin. In Figure 2.24, fragment **5** was synthesised by protection of 2, 4-dihydroxybenzaldehyde **1** with benzyl (Bn) groups, followed by Baeyer-Villiger oxidation and acid hydrolysis to provide phenol **2**. Phenol **2** was subsequently subjected to bromination and allylation, leading to compound **3** in high overall yield. Upon heating of compound **3** at 160 ^{o}C, Claisen rearrangement occurred and produced allylbenzene. The resulted phenol was protected as methyl ether, leading to compound **4**. The Lemieux-Johnson oxidation of compound **4**, followed by Wittig reaction provided bromobenzene **4** [Iikubo *et al.*, 2001].

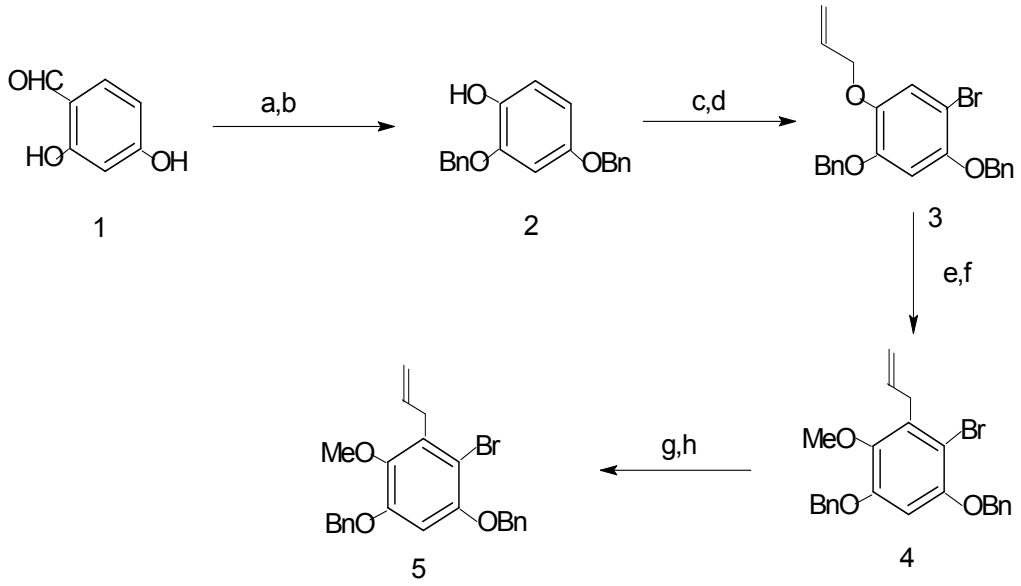


Figure2.24: Synthesis of fragment 5; (a) BnBr, K_2CO_3, DMF, room temperature, 96 % ; (b) mCPBA, CH_2Cl_2, room temperature; 6 M HCl, MeOH, room temperature, 95 % in two steps; (c) Br_2, $CHCl_3$, room temperature, 84 %; (d) allyl bromide K_2CO_3,DMF, room temperature, 80 %; (e) 160 ^oC, 73 %; (f) MeI, K_2CO_3, DMF, room temperature, 87 %; (g) OsO_4, $NaIO_4$, Et_2O)/ H_2O (1/1), room temperature, 95 %; (h) *i*$PrPh_3P^+I^-$, nBuLi, THF, 0 ^oC, 72 % [Iikubo *et al.,* 2001]

Figure 2.25 shows the synthesis of fragment **12** through the reaction of 1, 3, 5-trihydroxybenzene (phloroglucinol) **6**. Phloroglucinol was protected with MOM group (methoxymethyl ether) and followed by prenylation to produce compound **7** in high overall yield. Introduction of ethoxycarbonyl group (EtOOC-) to compound **7** gave compound **8** which was then subjected to acid-catalysed methonolysis, followed by TBS-protection (tetrabutyl silyl), and finally with DIBAL-reduction (diisobutylaluminium hydride) to give benzyl

alcohol **9**. Then, IBS (2-iodobenzoic acid) converted compound **9** to aldehyde compound **10**, with the removal of TBS group adjacent to the prenyl group. Compound **10** was protected as MOM ether for utilisation in regioselective cyclization. The reaction then followed by exchanging of TBS group with benzyl group to produce compound **12** [Iikubo *et al.*, 2001].

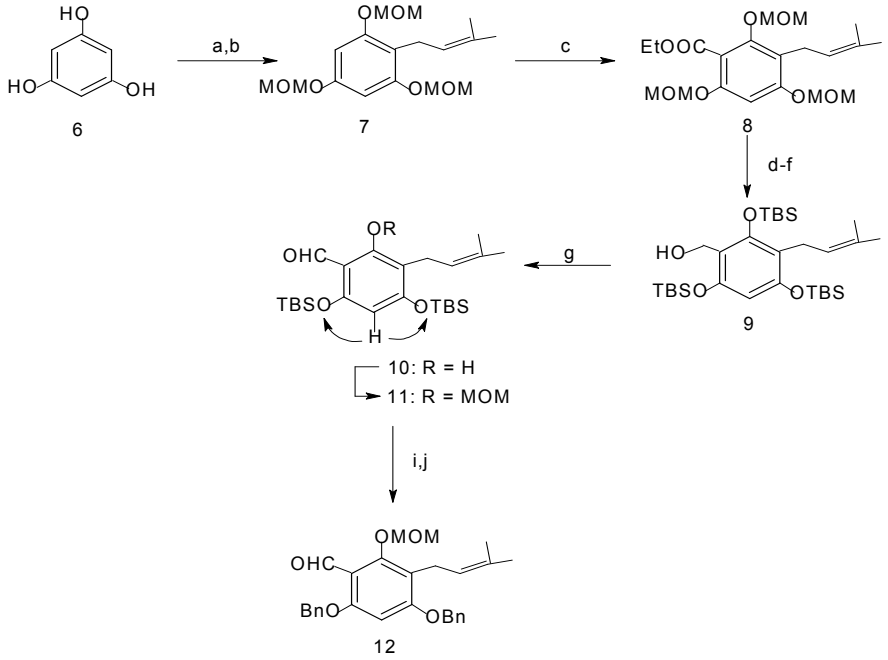


Figure 2.25: Synthesis of fragment 12; (a) NaH, MOMCl, DMF, room temperature, 96 %; (b) nBuLi; prenyl bromide, THF, 0 ^{o}C, 89 %; (c) nBuLi; $(EtO)_2CO$, THF, 0 ^{o}C, 95 %; (d) CSA, MeOH, 60 ^{o}C, 100 %; (e) TBSCl,DMAP, Et_3N, DMF, room temperature, 100 %; (f) DIBAL-H, toluene, 78^{o}C, 78 %; (g) IBX, toluene/DMSO (1/1), room temperature, 76 %; (h) NaH, MOMCl, CH_2Cl_2, room temperature, 65 %; (i) TBAF (tetrabutylammonium floride, THF, 0 ^{o}C, 100 % (j) BnBr, K_2CO_3, DMF, room temperature, 98 % [Iikubo *et al.,* 2001].

Figure 2.26 shows the synthesis of α-mangostin with the coupling of the previously synthesised compounds **5** and **12.**

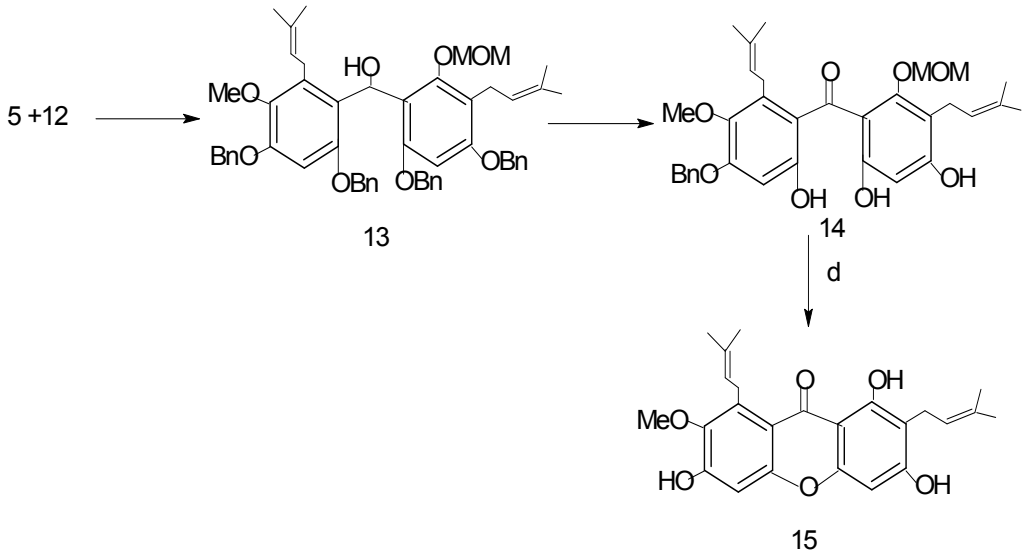


Figure 2.26: Synthesis of α-mangostin; (a) *s*BuLi, THF, 78 ^{o}C, 49 %; (b) IBX, toluene/DMSO (dimethyl sulfoxide) (1/1), room temperature, 76 %; (c) 10 % Pb/C, HCO_2NH_4, acetone, room temperature, 63 %; (d) PPh_3 (triphenylphosphine), CCl_4 (chloroform), THF, room temperature, then silica gel, 43 %; (e) CSA (camphoresulfonic acid), MeOH, room temperature, 76 % [Iikubo *et al.*, 2001]

2.3.5 Psorospermin

Psorospermin was also found to possess cytotoxic activity. It possessed excellent anticancer activity against human cell and marine cell. This is because of intercalation of xanthone group with DNA base pair and alkylation of epoxide by N7-guanine in the presence of topoisomerase II which is the crucial enzyme for DNA cycle. From the past, psorospermin was obtained through extraction from the natural products. But nowadays, as an alternative, psorospermin can be produced via synthetic approach. The synthesis of psorospermin, began with the production of xanthonic block using Grover method. But different from other literature, zinc chloride in $POCl_3$ was heated to 60 ^{o}C for 30 minutes prior to the addition of dimethoxy benzoic acid **5**. This is because zinc chloride was found to be the culprit in decreasing the percentage yield, due to the insolubility of the glass like fused zinc chloride. Compound **5** was heated for another 30 minutes before phloroglucinol **6** was added [Schwaebe *et al*., 2004]. Then, the processes were continued as shown in Figure 2.27.

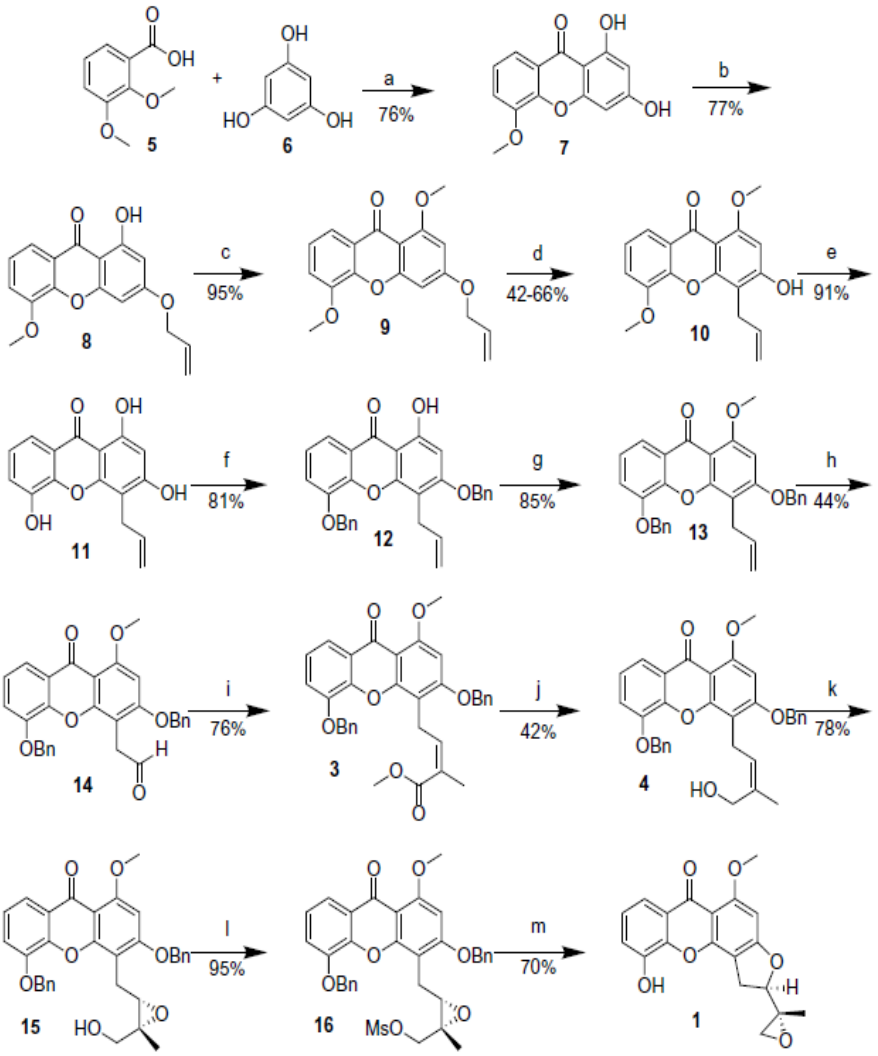


Figure 2.27: Synthesis of psorospermin. Reagents: (a) $ZnCl_3$, $POCl_3$; (b) allyl bromide, K_2CO_3; (C) CH_3I, K_2CO_3; (d) mesitylene, 180°C (e) BBr_3; (F) benzyl bromide, NaH, DMF; (g) CH_3I, DMF; (h) OSO_4, $NaIO_4$; (i) $(CF_3CH_2O)_2POCHCH_3CO_2Me$, KHMDS, 18-CROWN-6; (j) DIBALH/CH_2Cl_2; (k) t-BUOOH, (-) DIPT, Ti(*i*-Opr)$_4$; (i) MsCl, Et_3N; (m) raney nickel/ K_2CO_3, ethanol [Schwaebe *et al.*, 2004]

2.3.6 Xanthonecarboxylic Acid

Xanthonecarboxylic acid can be obtained by oxidation of the methyl group of **3a-f** to **3i-q**, with potassium permanganate in alkaline solution followed by intramolecular Friedel-Craft acylation with polyphosphoric acid [Pickert *et al.,* 1998].

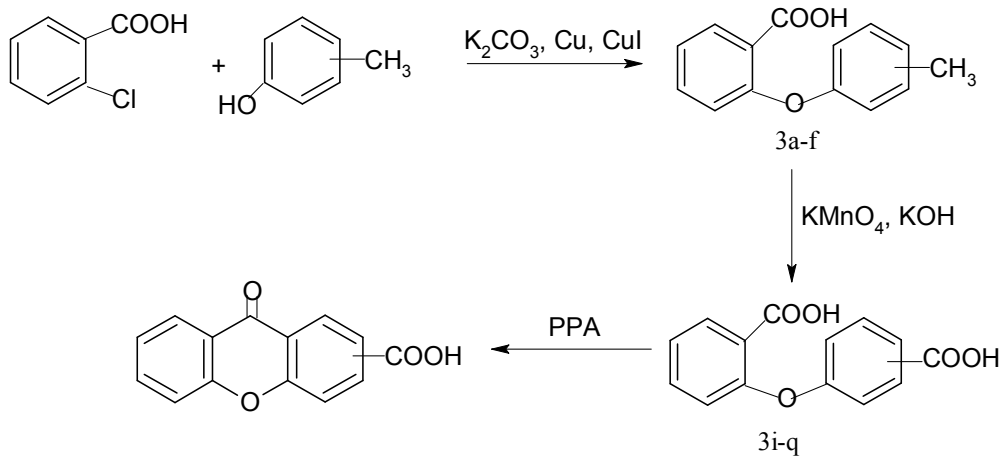


Figure 2.28: Synthesis of xanthonecarboxylic acid [Pickert *et al*., 1998]

In this project, the xanthone block, was synthesised by using two units of substituted benzoic acid via coupling reaction in the presence of Eaton's reagent. Alkenylation was then carried out to produce alkenylated xanthones. Under the same reaction conditions as described in the literature which involved the prenylation of xanthone [Castanheiro *et al*., 2009]. The difference was on the substituent that has been used. Substituent used for prenylation was prenyl bromide while the substituent used for this alkenylation was 5-bromo-1-pentene.

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals

The chemicals used in this project are as below:

Table 3.1: Chemicals used in synthesising xanthone block

Chemical name	Molecular formula	Source	Molecular Weight, M_w (g/mol)	Country
2,4,6-trihydroxybenzoic acid monohydrate	$C_7H_6O_5$	Acros Organics	170.12	Belgium
Eaton's reagent	P_2O_5/ $MeSO_3H$	Acros Organics	-	Belgium
Acetone	C_3H_6O	QRec	58.08	Malaysia
Ethyl acetate	$CH_3COOC_2H_5$	LAB– SCAN	88.10	Ireland

Table 3.2: Chemicals used in synthesising the alkenylated xanthone

Chemical name	Molecular formula	Source	Molecular weight, M_w (g/mol)	Country
Potassium Carbonate	K_2CO_3	John Kollin Corporation	138.20	-
Acetone	CH_3COCH_3	QReC	58.08	Malaysia
Ethyl acetate	$CH_3COOC_2H_5$	LAB – SCAN	88.10	Ireland
Hydrochloric acid (37%)	HCl	Fisher Scientific	36.46	United Kingdom
5-bromo-1-pentene	$Br(CH_2)_3CH=CH_2$	Merck	149.03	German

Table 3.3: Chemical used in NMR analysis

Chemical name	Molecular formula	Source	Molecular Weight,M_w (g/mol)	Country
Acetone-d_6	CD_3COCD_3	Acros Organics	64.12	Belgium

Table 3.4: Chemical and cancer cell lines used in bioassay

Chemical, apparatus and cell	Source	Country
MTT	Sigma - Aldrich	United State of America
DMSO	Fisher Scientific	United Kingdom
RPMI 1640 Media	Cellgro	Manassas
Fetal Bovine Serum (FBS)	Hyclone Thermo Scientific	South America
HeLa Cell	ATCC (American Type Culture Collection)	United State of America (USA)
MDA-MB-231	ATCC	USA

3.2 Instruments

3.2.1 Nuclear Magnetic Resonance Spectrometer (NMR)

NMR provides spectrum that has the information on the number of magnetically distinct atom present in the compound studied. In this project, ^{1}H- & ^{13}C-NMR, HMBC and HMQC spectra were obtained by using trimethylsilane (TMS) as internal standard and reference. The frequency used for ^{1}H-NMR analysis was 400 MHz; while ^{13}C-NMR was performed at 100 MHz. The samples were prepared by dissolving a small amount of sample in

sufficient acetone-d_6 in order to fill up the NMR tube to a height of 4-5 cm and the cap was affixed to the tube and wrapped with parafilm to avoid solvent evaporation

3.2.2 Infrared Spectrophotometer (IR)

Through the IR analysis, IR spectrum was obtained. IR spectrum was used to indicate the presence of certain functional group in the compound analysed. In this project, Perkin Elmer 2000-FTIR spectrophotometer was used for sample analysis and the range used was 4000 cm^{-1} – 400 cm^{-1}. The sample was grinded with KBr powder in the ratio of 1:10. Then, the mixture of the sample with KBr powder was compressed under a high pressure to form KBr sample pellet.

3.2.3 Ultraviolet-Visible Spectrophotometer (UV-Vis)

The UV-Vis spectrum was used to determine the qualitative information and the position of the hydroxyl group in xanthone block. From the transition energy of the highly conjugated organic compound, colour produced by the compound can be identified. In this project, Perkin-Elmer Lambda (25/35/45) UV-Vis spectrophotometer was used and the samples were prepared by using absolute ethanol to dissolve the sample. Then, the sample was measured by using quartz cuvette in the range of 190 nm to 400 nm.

3.2.4 Mass Spectrometer (MS)

Mass spectrometry was used to provide the information on the molecular mass, molecular formula and the structure of the molecule. This was done by analysing the molecular ion and fragmentation pattern of the compound. In this project, the sample was analysed by using Agilent 5975 MSD (nominal mass) mass spectrometers.

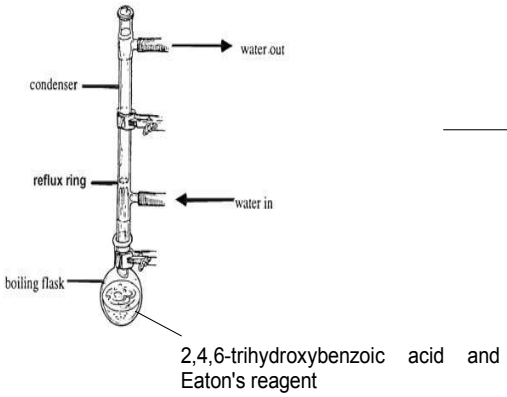
3.2.5 Melting Point Instrument

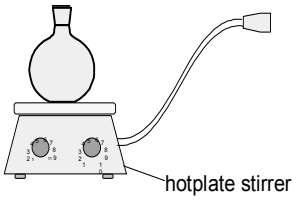
Melting point instrument was used to provide melting point of the tested compounds. By obtaining the melting point, the characteristic and the purity of the tested compound can be determined by comparing the range difference with the pure compound. In this project, Barnstead Electrothermal 9100 melting point instrument was used. Heamatocrit capillaries were used to contain the solid form sample for testing.

3.3 Methodology

3.3.1 Synthesis of 1, 3, 6, 8-Tetrahydroxyxanthone

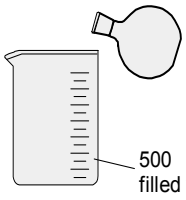
14.1 g (75 mmol) of 2, 4, 6-trihydroxybenzoic acid monohydrate was added with 75 ml of Eaton's reagent and warmed in water bath at 80 ^{o}C for 10 minutes whilst stirring. The mixture was cooled at room temperature and was stirred for 20 minutes. The mixture was poured into 500 mL beaker initially filled with ice and was left to soak in ice bath for 30 minutes. The precipitate was then filtered via Buchner filtration and the precipitate was placed in oven at 50 ^{o}C overnight, while, the filtrate was extracted with ethyl acetate. After drying overnight in the oven, the dried precipitate was then extracted with acetone. Both the extracts were dried under reduced pressure separately.





1. The mixture was warmed in water bath at 80 °C for 10 minutes while stirring.

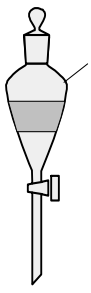
2. The mixture was cooled at room temperature and stirred for 20 minutes.



3. The mixture was poured into the beakers filled with ice and left to soak for 30 minutes.

4. The precipitate formed was filtered with Buchner filtration and dried in the oven overnight at 50 °C.

5. The filtrate was extracted with ethyl acetate.



7. Both extracts were dried under reduce pressure separately.

6. After overnight drying in the oven, the dried precipitate was extracted with acetone.

Figure 3.1: Flow chart on the synthesis of 1, 3, 6, 8-tetrahydroxyxanthone

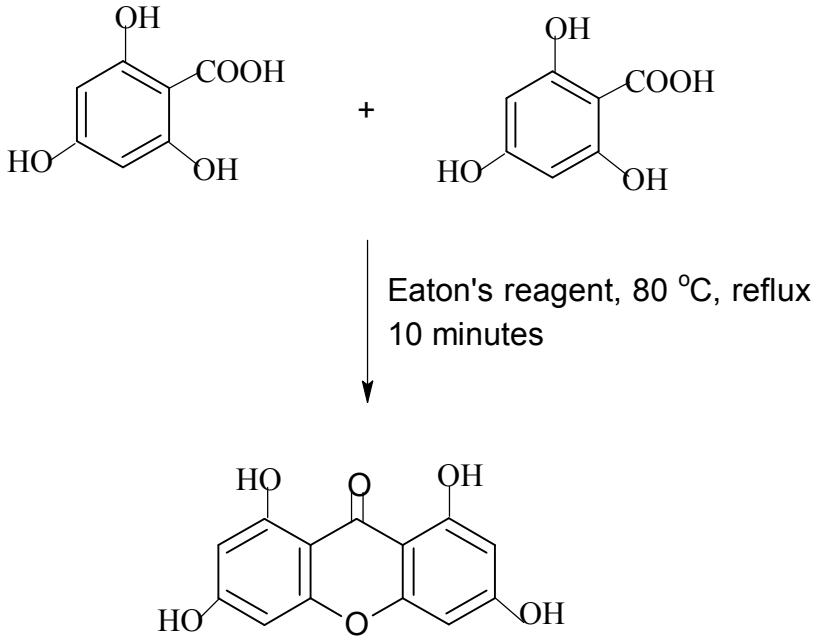
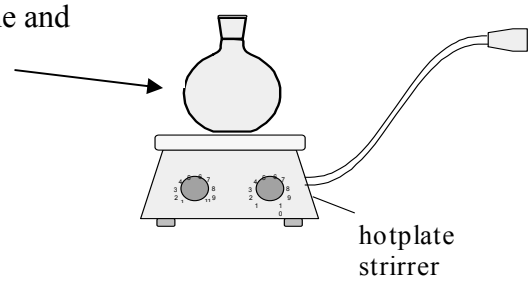


Figure 3.2: Synthesis pathway of 1, 3, 6, 8-tetrahydroxyxanthone

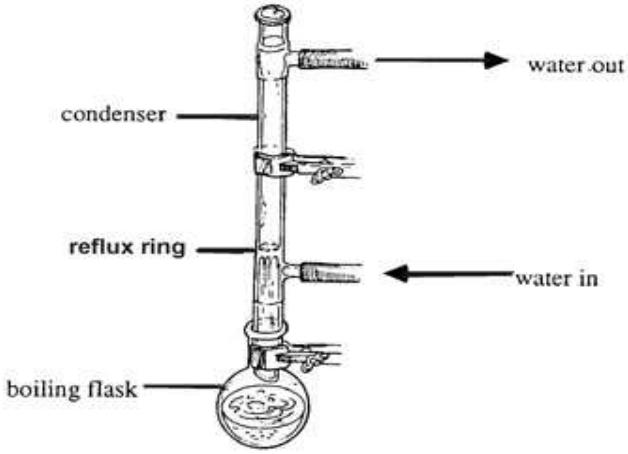
3.3.2 Alkenylation of Xanthonic Block in Organic Solvent

A mixture of 1.04 g (4 mmol) of 1, 3, 6, 8-tetrahydroxyxanthone and 1.656 g (12 mmol) of K_2CO_3 was stirred for 5 minutes in 100 mL acetone under room temperature. 2.384 g (16 mmol) of 5-bromo-1-pentene was added into the solution. The mixture was refluxed for 6 hours at 65 ^{o}C. The solid was filtered and the filtrate was dried under reduced pressure.

Flat bottom flask filled with the mixture of 1, 3, 6, 8-tetrahydroxyxanthone and K_2CO_3 in 100mL acetone



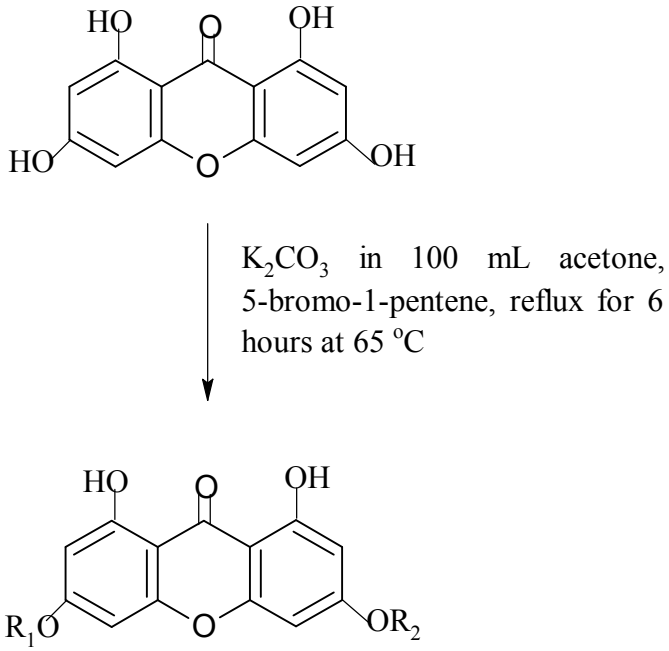
1. The mixture was stirred for 5 minutes at room



2. 5-bromo-1-pentene was added into the mixture and the mixture was refluxed for 6 hours at 65 ^{o}C

3. The solid was filtered and the filtrate was dried under reduced pressure

Figure 3.3: Flowchart on the synthesis of *o*-alkenylated xanthone



(1) $R_1 = R_2$ = pent-4-en-1-yl

(2) R_1= H, R_2 = pent-4-en-1-yl

Figure 3.4: Synthetic route for synthesising *o*-alkenylated 1, 3, 6, 8-tetrahydroxyxanthone

3.4 Chromatography Method

3.4.1 Thin Layer Chromatography (TLC)

Thin layer chromatography was used in this project to identify the pure compound, to test for a suitable solvent system for column chromatography

and to identify the identical compounds by comparing the R_f value of the compounds.

Thin layer chromatography consists of two phases which are mobile phase and stationary phase. Mobile phase used in this project are hexane, dichloromethane, acetone and ethyl acetate. The stationary phase of the TLC plate is made of silica gel 60 F_{254} (Merck 1.05554.0001) coated on aluminium plate of 4.0 cm x 6.7 cm dimension.

Before spotting the compounds on the TLC plate, the base line and the solvent front line were drawn on the TLC plate as shown in Figure 3.5.

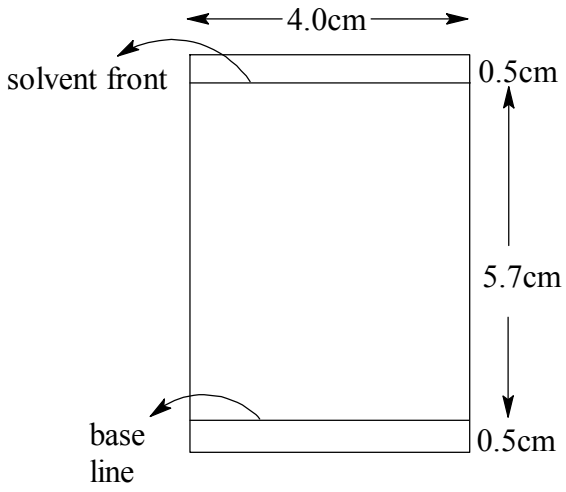


Figure 3.5: Feature of TLC plate used

Identification of the pure compound, test for a suitable solvent system and to identify identical compounds can be done by comparing the retention factor, R_f

value of the compounds. The compounds must first be dissolved and diluted in a solvent. The samples dotted on the TLC plate were checked for its thickness. This was to make sure the compound spotted on the TLC plate was not too thick to prevent tailing and also not too light to avoid difficulty of detecting the spots on the TLC developed.

Once all the compounds were spotted on the TLC plate, the TLC plate was placed into a solvent chamber filled with 4 mL of the desired solvents for separation. The solvent chamber was sealed with aluminium foil to enable chamber to be saturated with the solvent's vapour.

The TLC plate was removed from the solvent chamber when the solvent reached the solvent front and the developed spot was identified and seen under ultraviolet light with both short (254 nm) and long (365 nm) wavelenghts. After that, the spots were circled up by using a pencil.

The retention factor, R_f can be calculated by using the equation as follow:

$$R_f = D / L$$

Where,

D = Distance travelled by the compound from the base line (cm)

L = Distance between solvent front and the base line (cm)

3.4.2 Column Chromatography

Chemical synthesis afforded a mixture of products. Thus, column chromatography was needed for purification of crude products. In this study, normal phase column chromatography with polar stationary phase and relatively non-polar mobile phase was used. The stationary phase used was Silicycle silica gel (40-63μm) and the mobile phase used was a mixture of different solvent such as hexane, acetone, dichloromethane, methanol and ethyl acetate at different ratio increasing polarity. Thus, gradient elution was applied to elute and separate different compounds out from the column. Column chromatography enables the separation of the desired compound out from the side products based on the principle of differences in the strength of interaction between various compounds and the stationary phase.

The sample was prepared via dry packing before subjected into the column. Firstly, the sample solution was introduced drop by drop and mixed with small amount of silica gel in a beaker. Then, the silica gel was swirled lightly to enable the remaining silica gel to cover the droplets that formed. Spatula was used to mix and grind the sample with silica gel until homogenous powdery solid was formed. The process was repeated until all the sample was coated onto the silica gel.

When packing the column, the column was first filled with hexane up to 1/3 of the column's height. A small piece of cotton wool was plugged to the bottom of column. Then, small amount of sea sand was introduced into the column to form a thin layer of sand above the plugged column with hexane continuing running down the column and collected into a conical flask. The conical flask was sealed with aluminium foil to prevent evaporation of the solvent into the air.

A mixture of silica gel and hexane was prepared. The slurry of silica gel was then poured into the column and the stopcock of column was turned on to allow hexane to run down the column into the conical flask. This was to enable even distribution of the silica gel in the column and prevent formation of bubbles. Before introduction of the dry packed sample into the column, the column must be run for a few times to enable the silica gel to pack firmly in the column.

Before loading the sample into the column, the hexane level was run and maintained at 5 cm above the surface of the silica gel. The hexane collected before loading of sample can be reused by pouring back into the column. Gradient elution was performed by using a suitable solvent system that was tested earlier by using TLC. The column was covered with aluminium foil to prevent solvent evaporation. The fraction collected was then examined by using TLC.

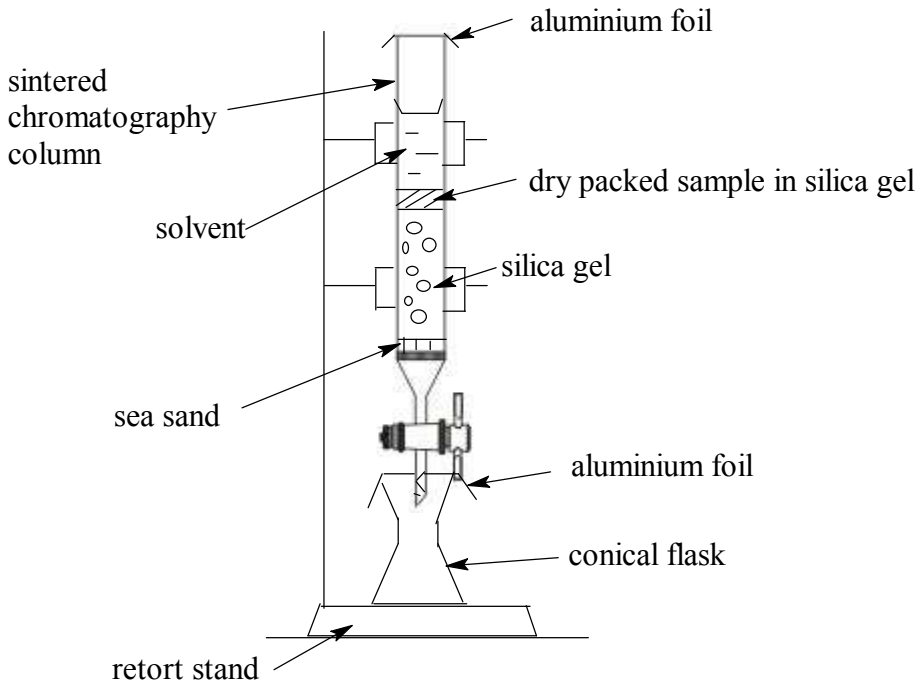


Figure 3.6: Setup of sintered chromatography column

3.5 Bioassay

The cell viability was evaluated by using the colorimetric 3-(4, 5-dimethylthiazo-2-yl)-2, 5-diphenyl- tetrazolium bromide (MTT) assay. In this MTT assay, HeLa and MDA-MB-231 cancer cells were tested. Both of HeLa cells (0.75×10^5 cells/mL) and MDA-MB-231 cells (3.5×10^5 cells/mL) were cultured in 96-well plates with 0.1 % dimethyl sulfoxide (DMSO) containing tested compound 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 to produce 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 follow:

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

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 1, 3, 6, 8-Tetrahydroxyxanthone and Alkenylated Xanthones

The synthesis of 1, 3, 6, 8-tetrahydroxyxanthone involved the reaction of 14.1 g (~75 mmol) of 2, 4, 6-trihydroxybenzoic acid monohydrate with 75 ml of Eaton's reagent. The present of Eaton's reagent enable the coupling reaction of 2, 4, 6-trihydroxybenzoic acid, yielded 4.69 g (43.5 %) of 1, 3, 6, 8-tetrahydroxyxanthone.

Reaction of (1.04 g) of 1, 3, 6, 8-tetahydroxyxanthone with 5-bromo-1-pentene in acetone and in the presence of K_2CO_3 as catalyst, yielded 0.033 g (5.04 %) of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one and 0.21 g (26.5 %) of 1, 8-dihydroxy-3,6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one.

The structures of 1, 3, 6, 8-tetrahydroxyxanthone, 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one were elucidated by using 1D-NMR, 2D-NMR, IR, and UV-Vis analyses.

4.2 Structural Elucidation of Xanthone

4.2.1 Structural Elucidation of 1, 3, 6, 8-Tetrahydroxyxanthone

1, 3, 6, 8-Tetrahydroxyxanthone was synthesised by reacting 2, 4, 6-trihydroxybenzoic acid monohydrate with Eaton's reagent. The function of Eaton's reagent was to enable the coupling of 2, 4, 6-trihydroxybenzoic acid with the loss of water molecules to form 1, 3, 6, 8-tertrahydroxyxanthone-2-carboxylic acid or 1, 3, 6, 8-tertrahydroxyxanthone-4-carboxylic acid. However, subsequent decarboxylation caused the loss of carboxylic group and produced 1, 3, 6, 8-tetrahydroxyxanthone. The overall reaction was shown in Figure 4.1.

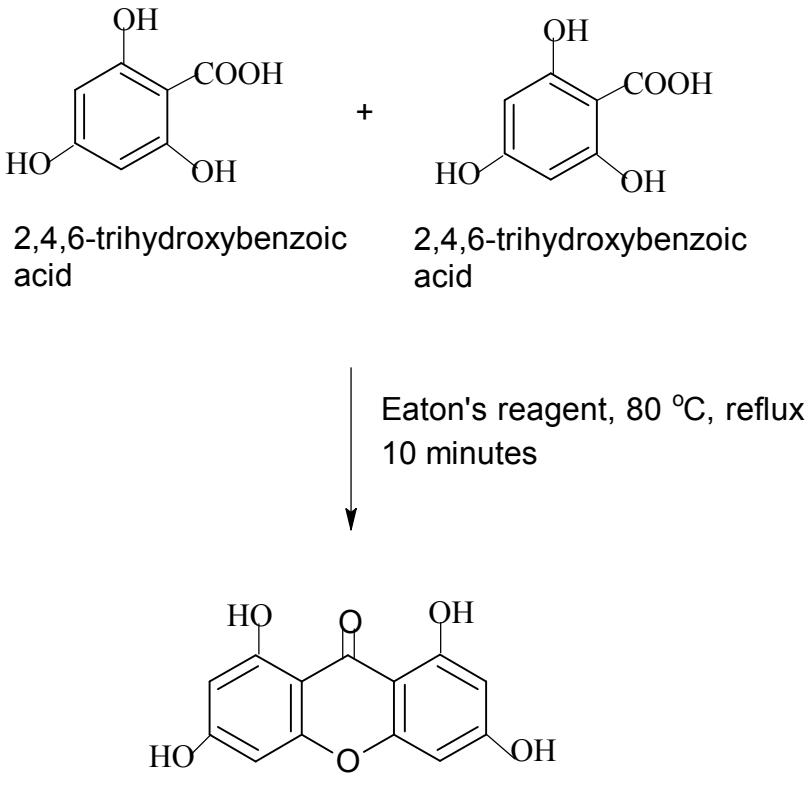


Figure 4.1: Reaction scheme of 1, 3, 6, 8-tetrahydroxyxanthone

1, 3, 6, 8-Tetrahydroxyxanthone was isolated from the crude as yellow solid, m.p. 257 to 259 ^{o}C. From the thin layer chromatography, this compound gave a single spot with R_f value 0.35 by using solvent system 50 % hexane and 50 % ethyl acetate. The UV absorption maxima at 210.0, 234.0, 250.0 and 330.0 nm were characteristic of hydroxyl xanthone derivative. The IR spectrum (Figure 4.7) showed absorption band at 3528 (O-H), 3467 (O-H), 3082 (aromatic C-H), 1637 (C=O), 1522 (aromatic C=C), 1458 (sp^2 C-H bending), and 1183 (C-O) cm^{-1}. The mass spectrum (Figure 4.6) showed a molecular ion, M^+ at m/z 260.0, indicating a molecular formula of $C_{13}H_8O_6$.

Initial analysis of ^{1}H-NMR spectral data indicated that the molecule consisted of a symmetrical xanthone skeleton. The ^{1}H-NMR spectrum (Figure 4.4) exhibited singlet signal for a chelated hydroxyl group at δ 11.93. This chelation caused deshielding effect to the hydroxyl protons thus a tremendous shift of signal to the downfield region. The four hydroxyl group at carbons 1, 3, 6, and 8 gave a symmetrical structure. The proton signal at δ 6.31 (1H, d, J = 2.1 Hz) and δ 6.19 (1H, d, J = 2.1 Hz) were assigned to protons H-4 & -5 and H-2 & -7 respectively. The coupling constant value of 2.1 Hz for the two signals indicated that H-2 was meta-coupling with H-4 and H-5 was meta-coupling with H-7.

The ^{13}C-NMR spectrum (Figure 4.5) showed signals for 7 non-equivalent carbons in the xanthone skeleton. The chelated carbonyl carbon: C-9 (δ 183.1) was deshielded to downfield region because of anisotropy effect. The other 6

non-equivalent carbons on xanthonic skeleton were C-1 & -8 (δ 163.0), C-3 & -6 (δ 165.8), C-4a & -5a (δ 157.8), C-8a & -9a (δ 101.1), C-2 & -7 (δ 98.5) and C-4 & -5 (δ 94.3). The chemical shift of C-1 & -8, C-3 & -6 and C-4a &-5a were higher than C-8a & -9a, C-2 & -7 and C-4 & -5 because the carbon atom was directly attached to an electronegative oxygen atom. The spectral data of ^{1}H-NMR and ^{13}C-NMR were summarised in Table 4.1.

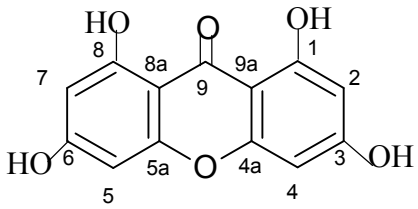
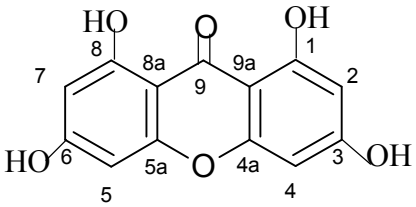


Figure 4.2: The structure of 1, 3, 6, 8-tetrahydroxyxanthone

Table 4.1: Summary of assignment of ^{1}H-NMR and ^{13}C-NMR spectra data of 1, 3, 6, 8-tetrahydroxyxanthone

Position	δ_H (ppm)	δ_C (ppm)
1 & 8	-	163.0
2 & 7	6.19 (1H, d, J = 2.1 Hz)	98.5
3 & 6	-	165.8
4 & 5	6.31 (1H, d, J = 2.1 Hz)	94.3
4a & 5a	-	157.8
8a & 9a	-	101.1
9	-	183.1
1- & 8- OH	11.93 (1H, s)	-



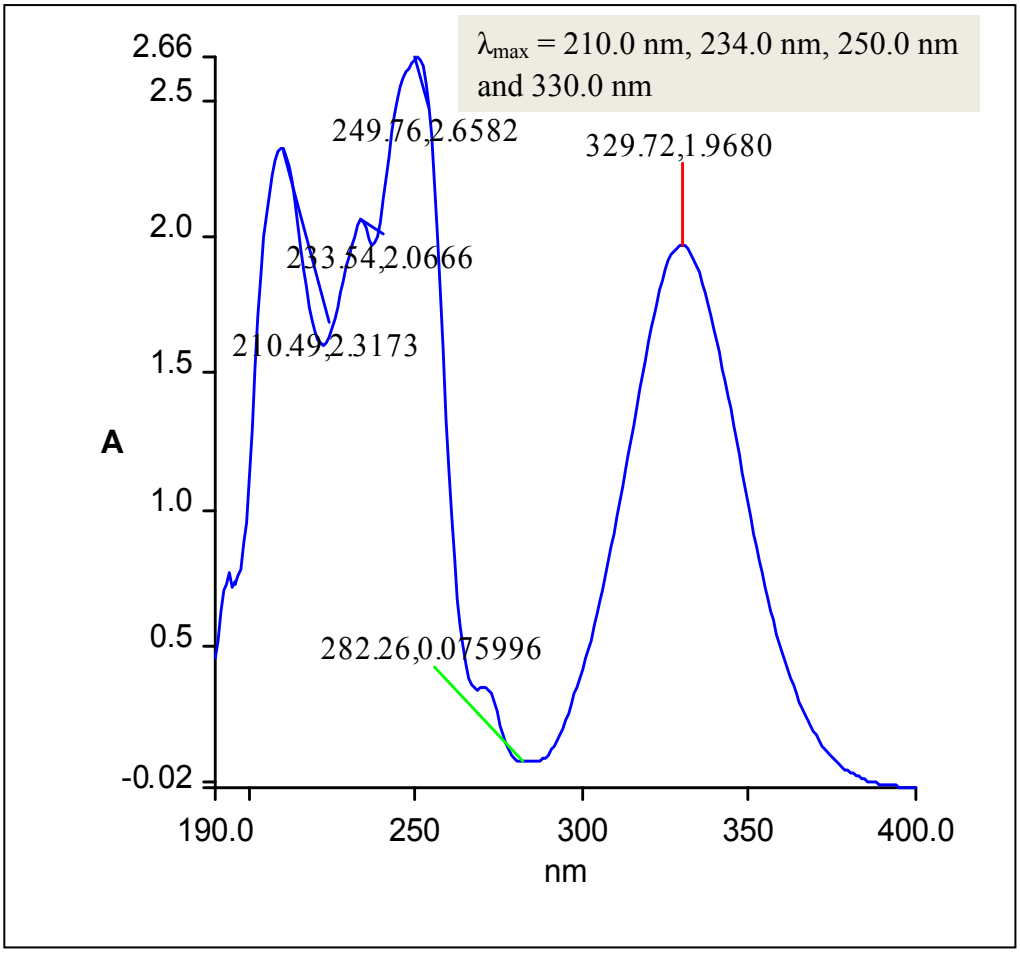
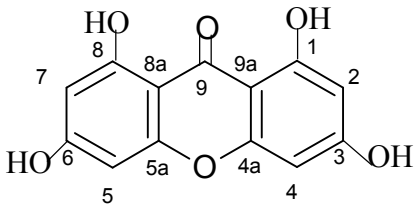


Figure: 4.3: UV-Vis spectrum of 1, 3, 6, 8-tetrahydroxyxanthone



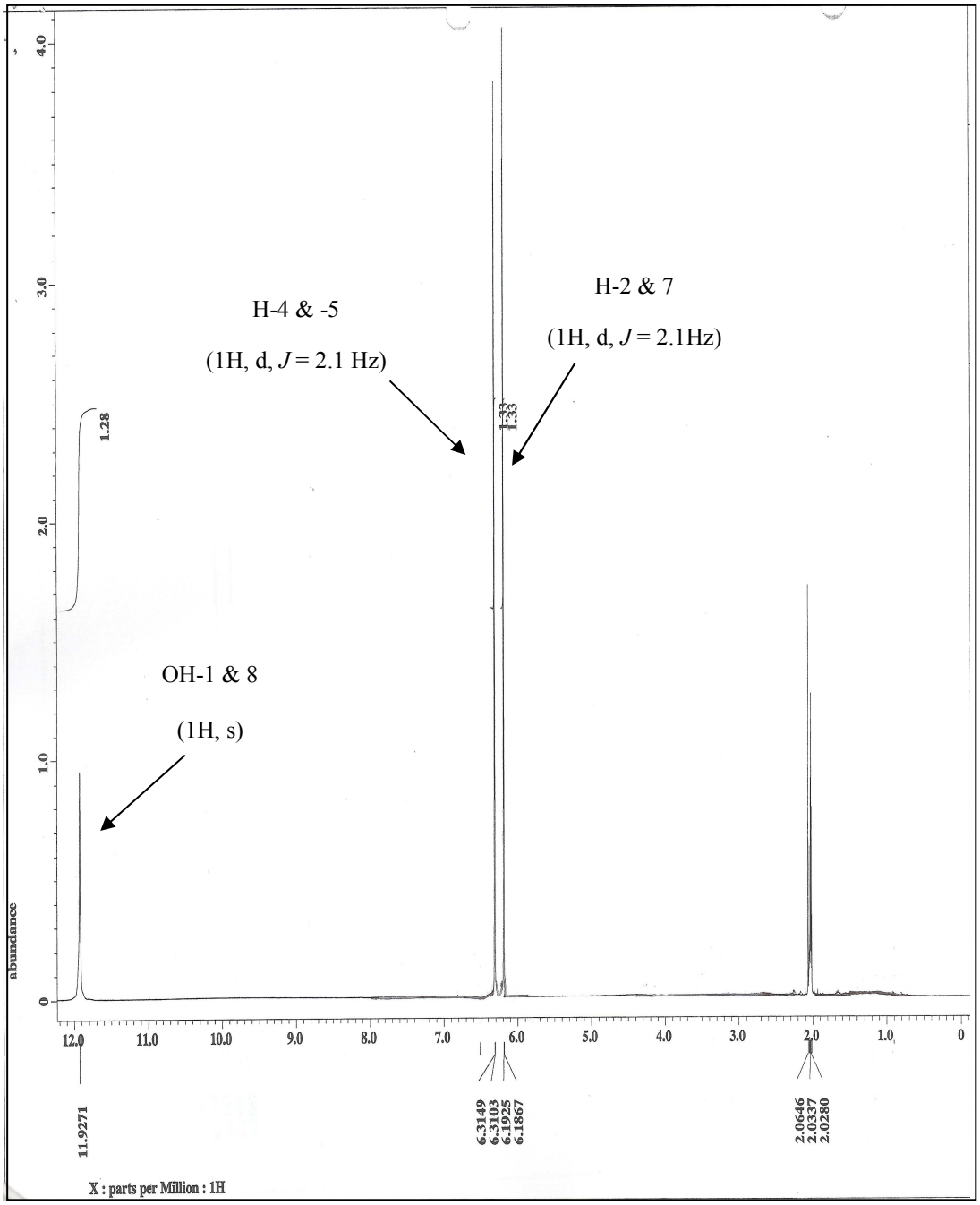
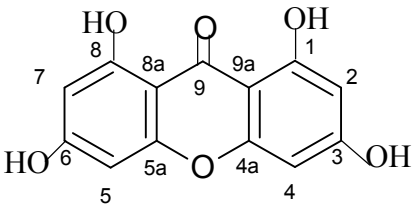


Figure 4.4: ^{1}H-NMR of 1, 3, 6, 8-tetrahydroxyxanthone (400 MHz, acetone -d_6)



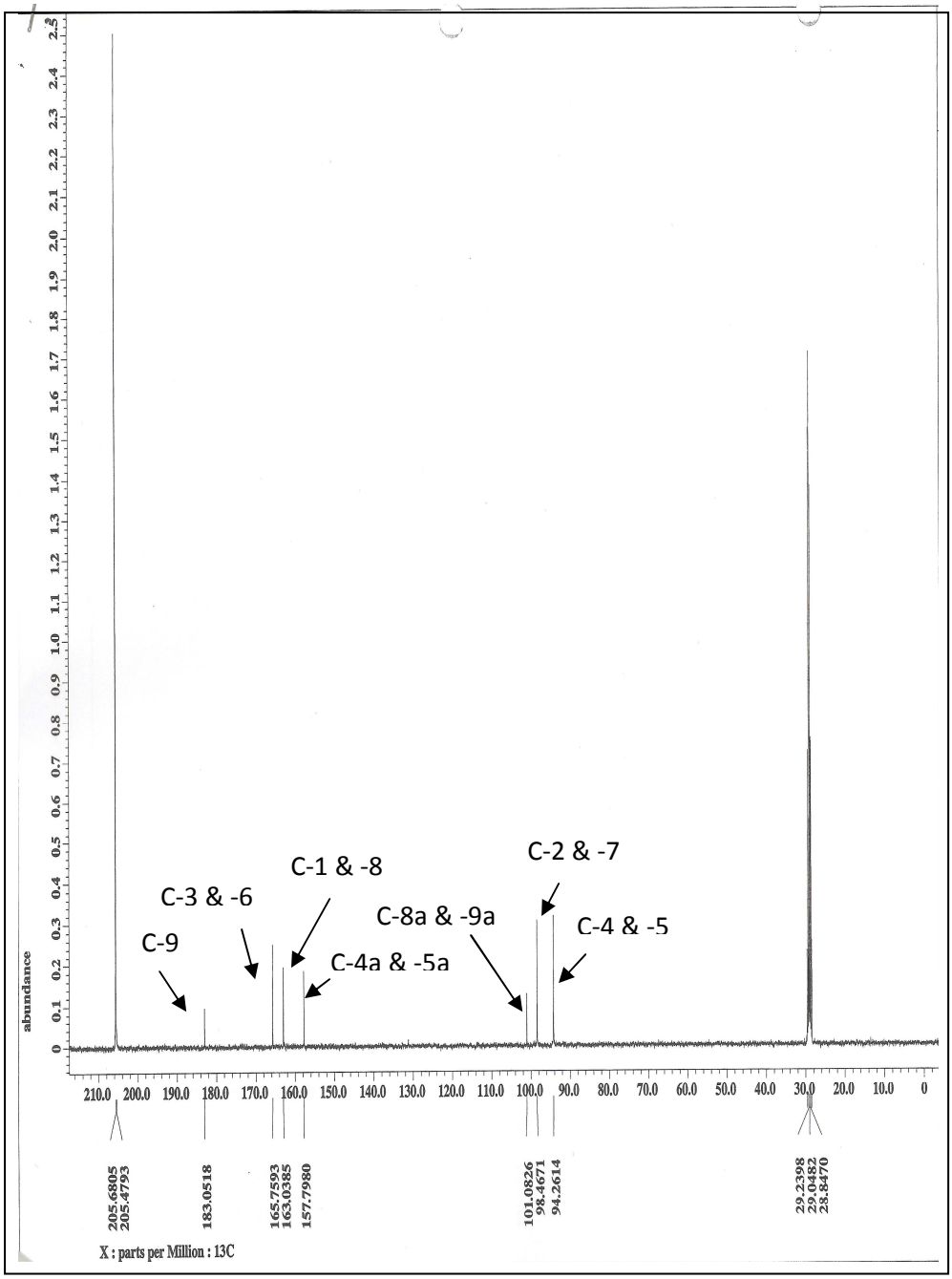
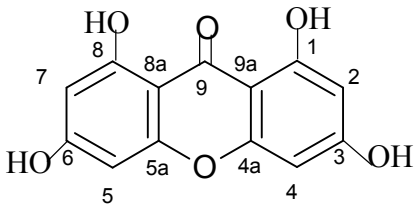


Figure 4.5: ^{13}C-NMR of 1, 3, 6, 8-tetrahydroxyxanthone (100 MHz, acetone- d_6)



(M.W. = 260.0 $gmol^{-1}$)

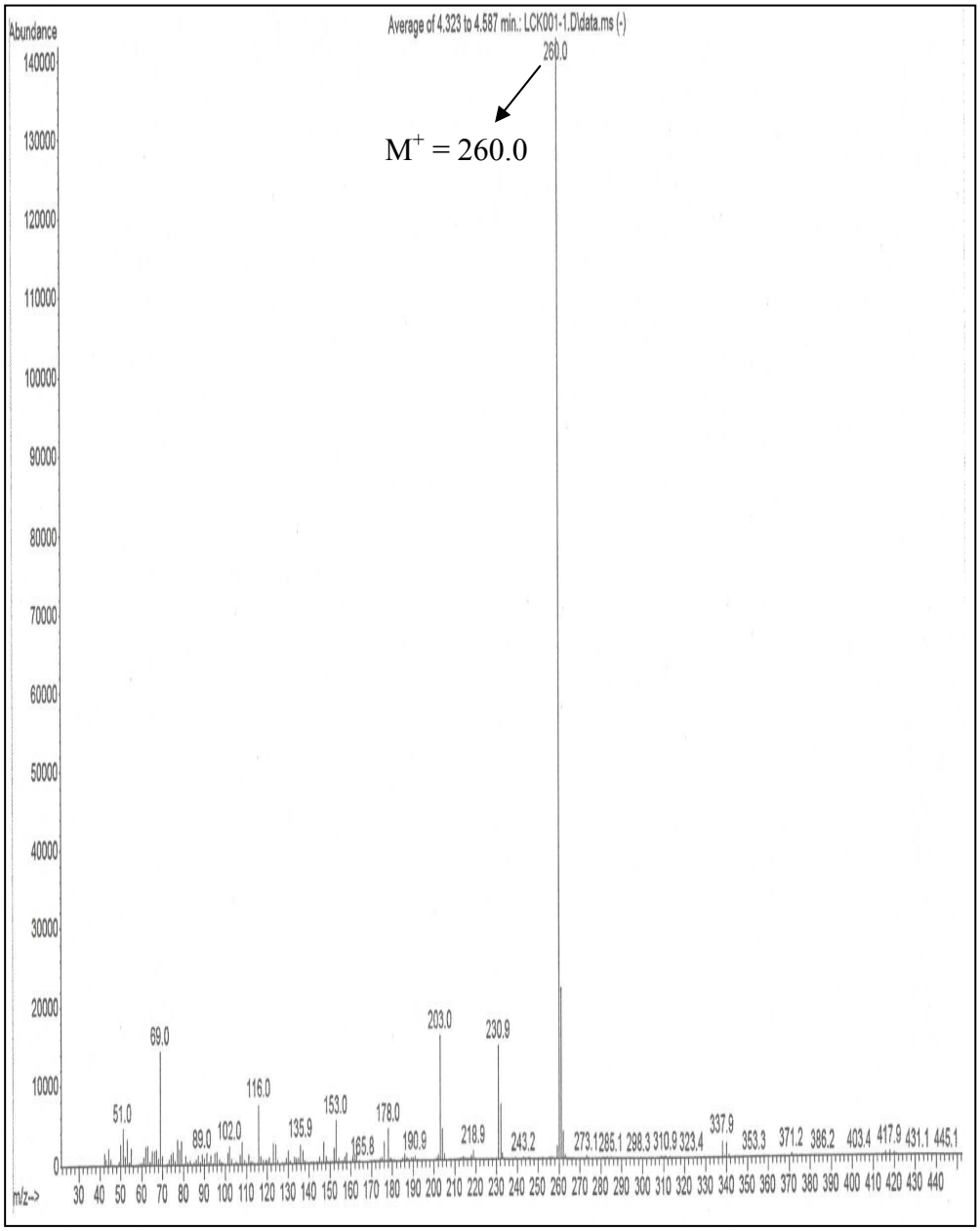


Figure 4.6: Mass spectrum of 1, 3, 6, 8-tetrahydroxyxanthone

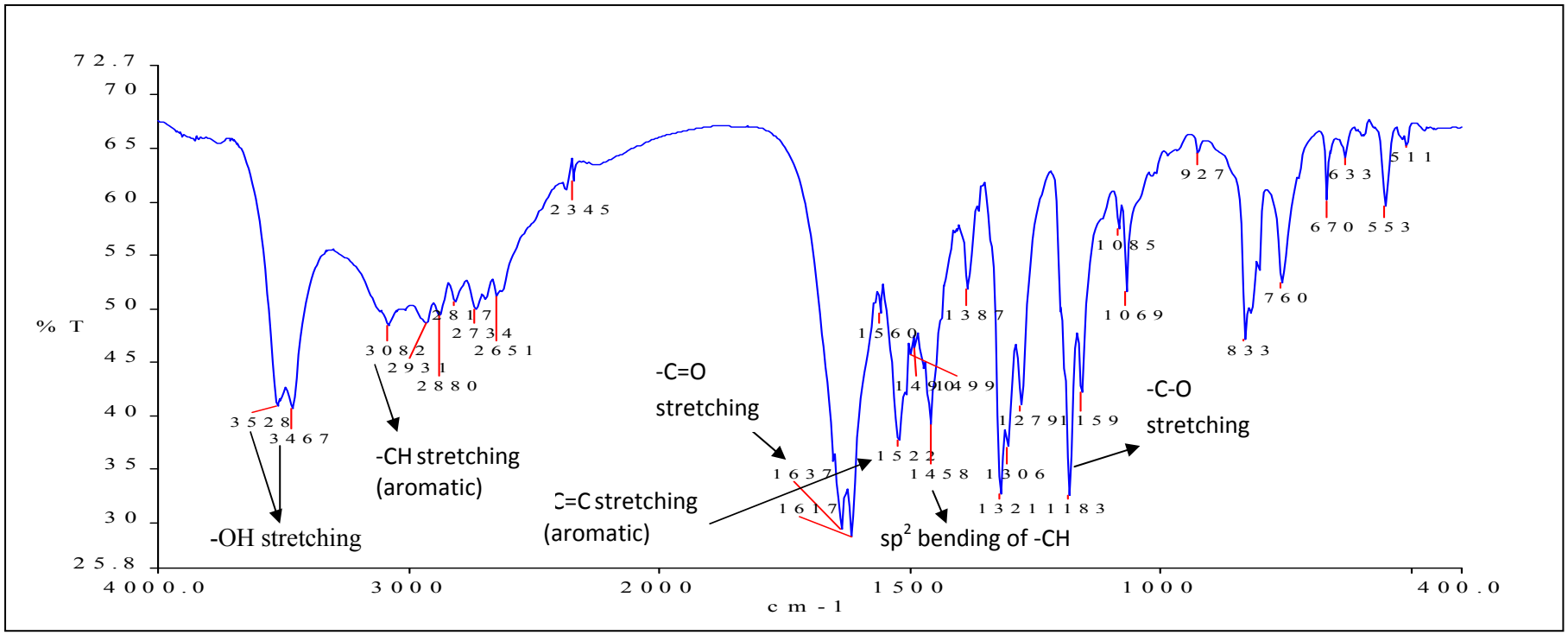


Figure 4.7: IR spectrum of 1, 3, 6, 8-tetrahydroxyxanthone

4.2.2 Alkenylation of 1, 3, 6, 8-Tetrahydroxyxanthone

1, 3, 6, 8-Tetrahydroxyxanthone was subjected to alkenylation by using 5-bromo-1-pentene in acetone as organic medium and potassium carbonate as catalyst. The alkenylation reaction produced two alkenylated xanthones. They are 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one.

1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one appeared as white crystals. Their melting point ranges were 100 to 103 ^{o}C and 138 to 140 ^{o}C, respectively. 1, 8-Dihydroxy-3,6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one gave a single spot of R_f value 0.79 with 50 % hexane and 50 % dichloromethane as the solvent system of the developed TLC. Whereas 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one gave a single spot of R_f value 0.44 with 30 % hexane and 70 % dichloromethane as the solvent system of the developed TLC.

4.2.2.1 Structure Elucidation of 1, 8-Dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one

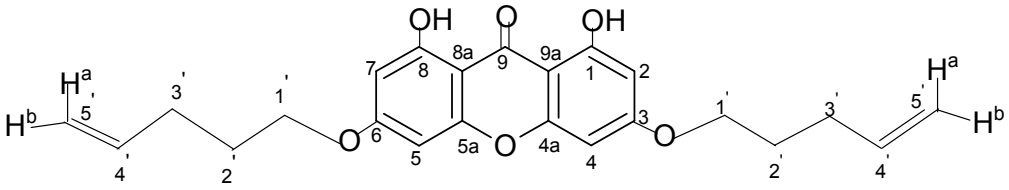


Figure 4.8: Structure of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one

Initial analysis of ^{1}H-NMR spectral data indicated that the molecule consisted of a symmetrical xanthone skeleton. The ^{1}H-NMR spectrum (Figure 4.10) exhibited singlet signal at δ 11.91 for the presence of chelated hydroxyl group. This chelation caused deshielding effect to the hydroxyl protons thus a tremendous shift of signal to the downfield region. The two hydroxyl groups at C-1 & -8 and the two pent-4-en-1-yl groups at C-3 & -6 gave a symmetrical plane to the structure. The proton signals at δ 6.32 (2H, d, J = 2.1 Hz) and δ 6.47 (2H, d, J = 2.1 Hz) were assigned to proton H-2 & -7 and H-4 & -5, respectively. As indicated by the coupling constant 2.1 Hz, of the two signals, H-2 was found to meta-coupled with H-4 and H-5 was found to meta-coupled with H-7. These chemical shifts were almost similar to 1, 3, 6, 8-tetrahydroxyxanthone. The difference was the presence of additional proton signals which were due to the *o*-alkenylation at OH- group on carbons C-3 and C–6 in the xanthonic block.

The ^{13}C-NMR spectrum (Figure 4.11) showed signals for 7 magnetically non-equivalent carbons in the xanthone skeleton. The chelated carbonyl carbon; C-9 (δ 183.1) was deshielded and appeared in downfield region because of anisotropy effect. The other 6 pairs of non-equivalent carbons in the xanthonic skeleton were C-1 & -8 (δ 162.7), C-3 & -6 (δ 166.5), C-4a & -5a (δ 157.5), C-8a & -9a (δ 101.7), C-2 & -7 (δ 97.6) and C-4 & -5 (δ 93.1). The chemical shift of C-1 & -8, C-3 & -6 and C-4a & -5a were higher than C-8a & -9a, C-2 & -7 and C-4 & -5 because the carbon atom was directly attached to an electronegative oxygen atom.

There were a total of 6 characteristic signals appeared in ^{1}H-NMR spectrum (Figure 4.10) which was due to the presence of pent-4-en-1-yl group. The six signals were δ 4.15 (4H, t, J = 7.3 Hz), 1.90 (4H, quin, J = 7.3 Hz), 2.24 (4H, q, J = 7.3 Hz), 5.88 (2H, multiplet), 5.06 (H^a, d, J = 17.2 Hz) and 4.97 (H^b, d, J =12.2 Hz), at which they were assigned to H-1', H-2', H-3', H-4', H^a-5' and H^b-5', respectively. Proton H-1' was deshielded, due to the electron withdrawing effect imposed by the electronegative oxygen atom which attached to carbons C-1'. Whereas, H-4' and H^a-5' & H^b-5' were deshielded due to the anisotropy effect of the C=C in pent-4-en-1-yl group. The two hydrogen atoms attached at C-5' are magnetically non-equivalent because they were restricted from free rotation at C=C bond. Thus, proton H^a-5' and H^b-5' gave two different doublet signals at δ 5.06 and δ 4.97, respectively. Proton H^a-5' was trans-coupling with proton H-4' and proton H-^{b}5' was cis-coupling with proton H-4' as indicated by their coupling constant 17.2 Hz and 12.2 Hz, respectively.

The ^{13}C-NMR spectrum (Figure 4.11) showed signals for 5 magnetically non-equivalent carbons on the pent-4-en-1-yl group. The 5 magnetically non-equivalent carbons were C-1' (δ 68.1), C-2' (δ 28.1), C-3' (δ 29.9), C-4' (δ 137.9) and C-5' (δ 114.8).

The assignment of the proton and carbon signals to the structure was accomplished by referring to HMQC and HMBC spectral data. The direct ^{1}J coupling between carbons and protons were observed in HMQC spectrum (Appendix 2). HMBC spectrum (Appendix 1) was used for the assignment of long range ^{2}J and ^{3}J couplings between protons and carbons in the structure. Based on the long range coupling, it can be used to identify the location where alkenylation has taken place. The HMBC spectrum showed that H-1' was ^{3}J coupling with C-3 and C-3'. The symmetrical structure of this compound also indicated that H-1' was ^{3}J coupling with C-6 and C-3'. Thus, the two pent-4-en-1-yl group, each was attached to the oxygen atoms at C-3 & -6, respectively. All the spectra data were summarised in Table 4.2

Table 4.2 Summary of the assignment of ^{1}H-NMR , ^{13}C-NMR and HMBC spectra data of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one

Position	δ_H (ppm)	δ_C (ppm)	HMBC
1 & 8	-	162.7	-
2 & 7	6.32 (2H, d, *J* = 2.1 Hz)	97.6	C-1 (^{2}J), C-3 (^{2}J), C-4 (^{3}J), C-9a (^{3}J)
3 & 6	-	166.5	-
4 & 5	6.47 (2H, d, *J* = 2.1 Hz)	93.1	C-4a (^{2}J), C-3 (^{2}J), C-2 (^{3}J), C-9a (^{3}J)
4a & 5a	-	157.5	-
8a & 9a	-	101.7	-
9	-	183.1	-
1’	4.15 (4H, t, *J* = 7.3 Hz)	68.1	C-3 (^{3}J), C-3’ (^{3}J), C-6 (^{3}J)
2’	1.90 (4H, quin, *J* = 7.3 Hz)	28.1	C-4’ (^{3}J), C-3’ (^{2}J)
3’	2.24 (4H, q, *J* = 7.3 Hz)	29.9	C-5’ (^{3}J), C-4’ (^{2}J), C-2’ (^{2}J), C-1’ (^{3}J)
4’	5.88 (2H, multiplet)	137.9	C-3’ (^{2}J)
5’	5.06 (2H^{a}, d, *J* = 17.2 Hz)	114.8	C-3’ (^{3}J), C-4’ (^{2}J)
5’	4.97 (2H^{b}, d, *J* =12.2 Hz)	114.8	C-3’ (^{3}J), C-4’ (^{2}J)
1- & 8-OH	11.91 (2H, s)	-	-

The IR spectrum (Figure 4.12), exhibit broad absorption band at 3449 cm^{-1} indicated the presence of O-H in the compound. The other absorption bands observed were at 3082 (aromatic C-H), 2949 (sp^3 C-H), 1641 (C=O), 1594 (C=C), 1168 (C-O), 1068 (C-O) and 745 cm^{-1} (aromatic C-H out-of-plane bending).

The UV (Figure 4.9) absorption maxima at 211, 253 and 328 nm indicated that this compound was conjugated.

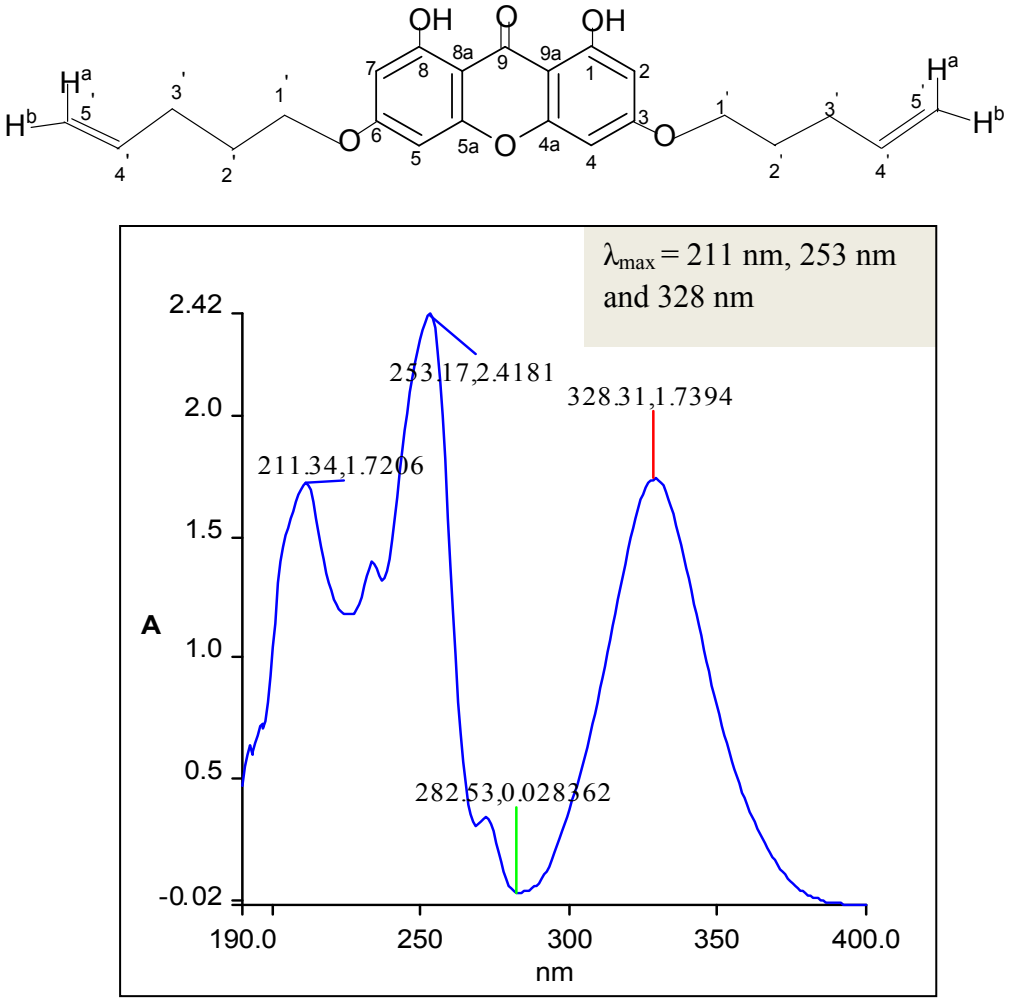


Figure 4.9: UV-Vis spectrum of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one

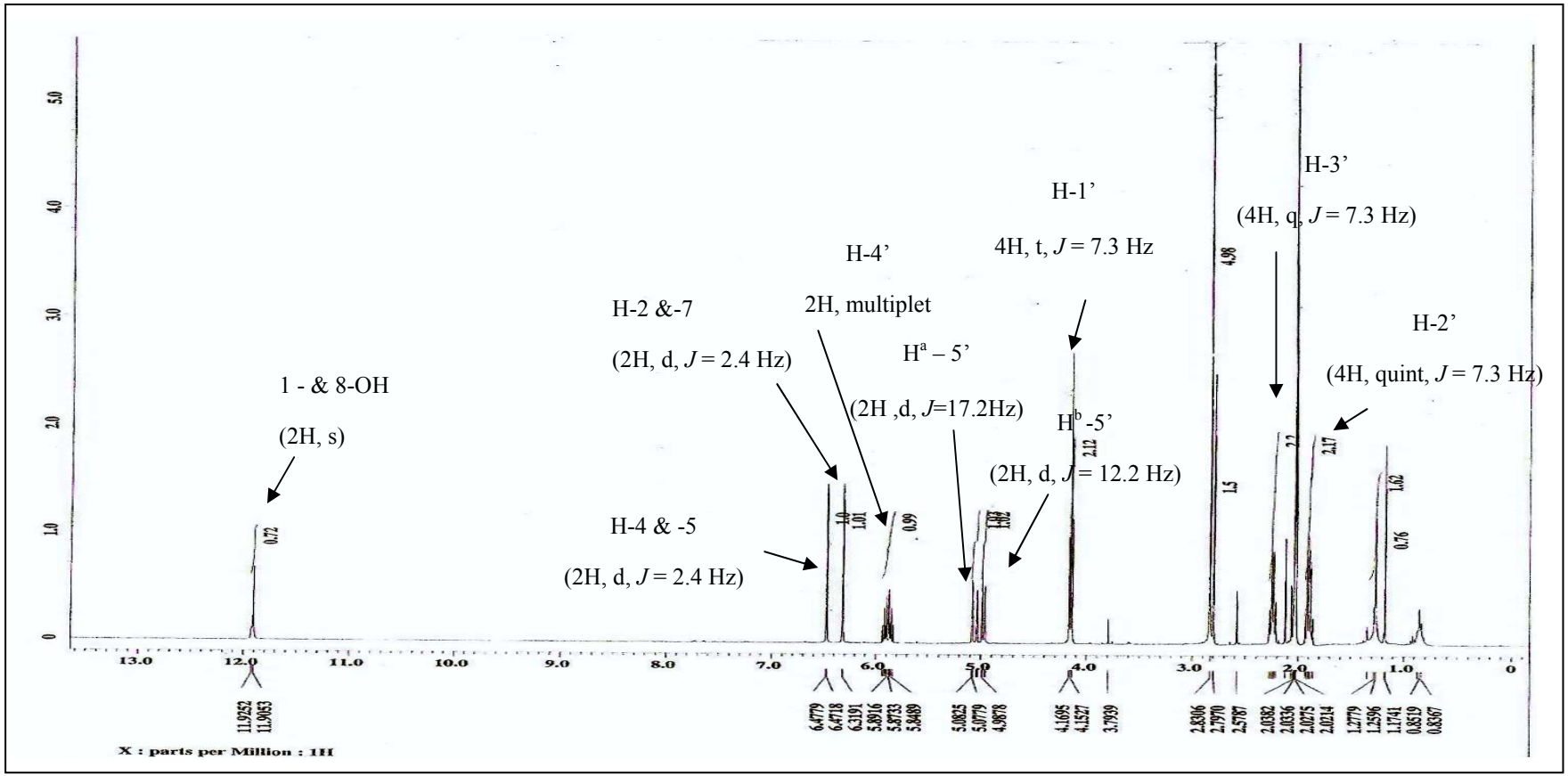
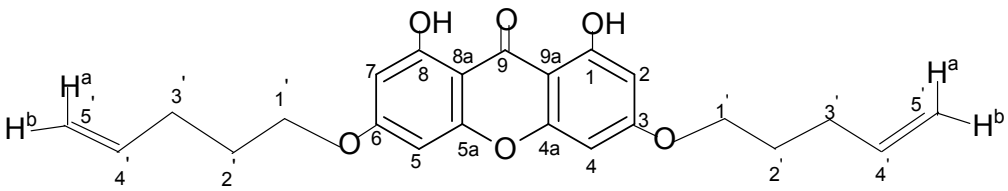


Figure 4.10: ^{1}H-NMR spectrum of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one (400 MHz, acetone-d_6)



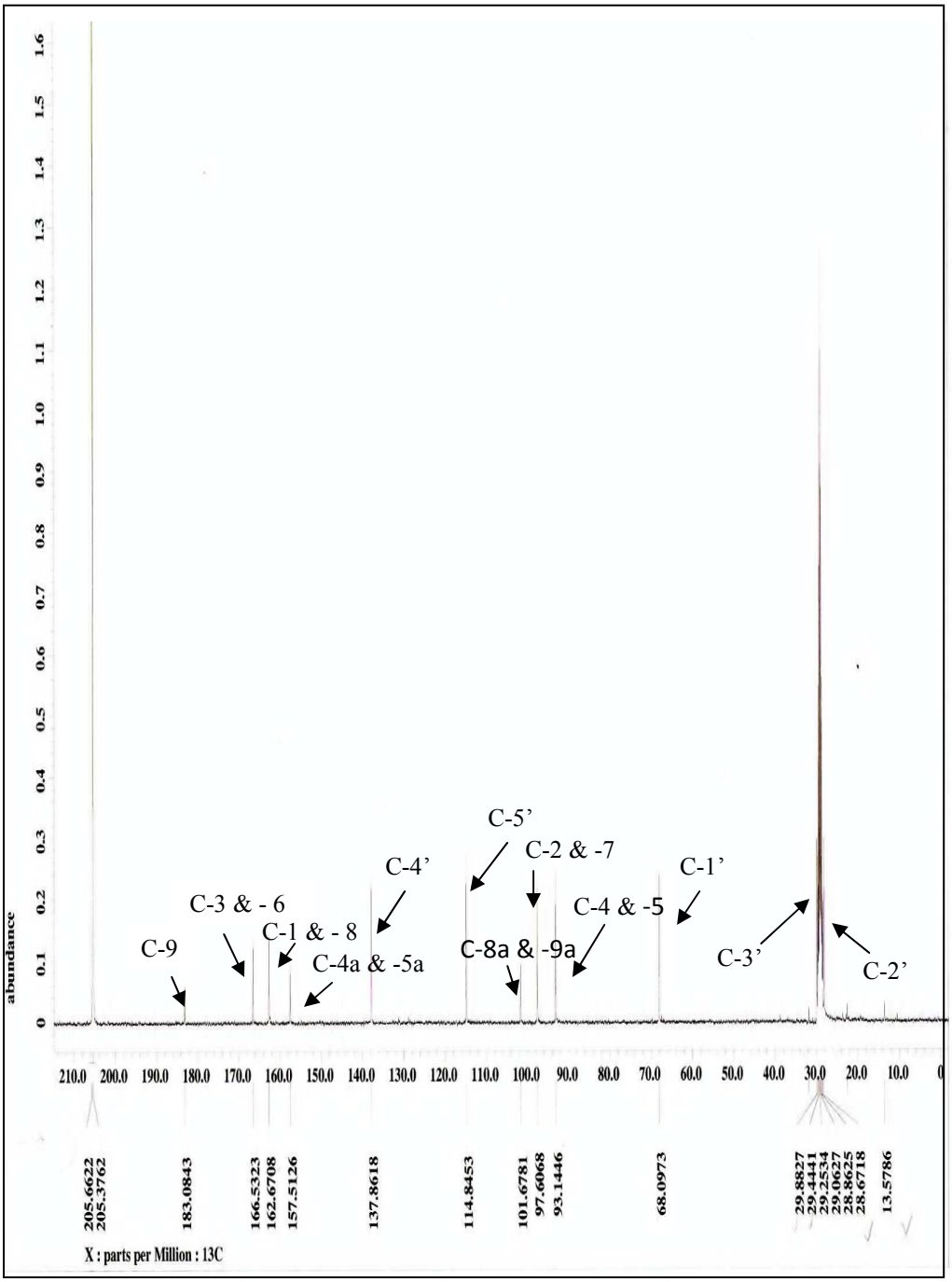
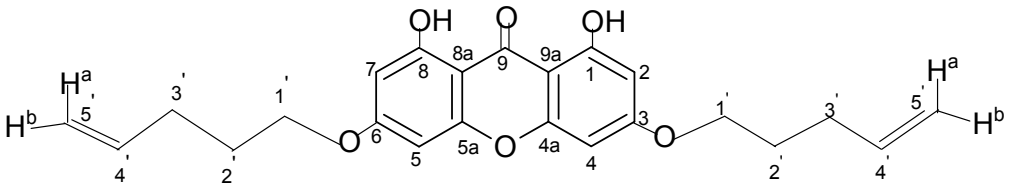


Figure 4.11: ^{13}C –NMR of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one (100MHz, acetone-d_6)



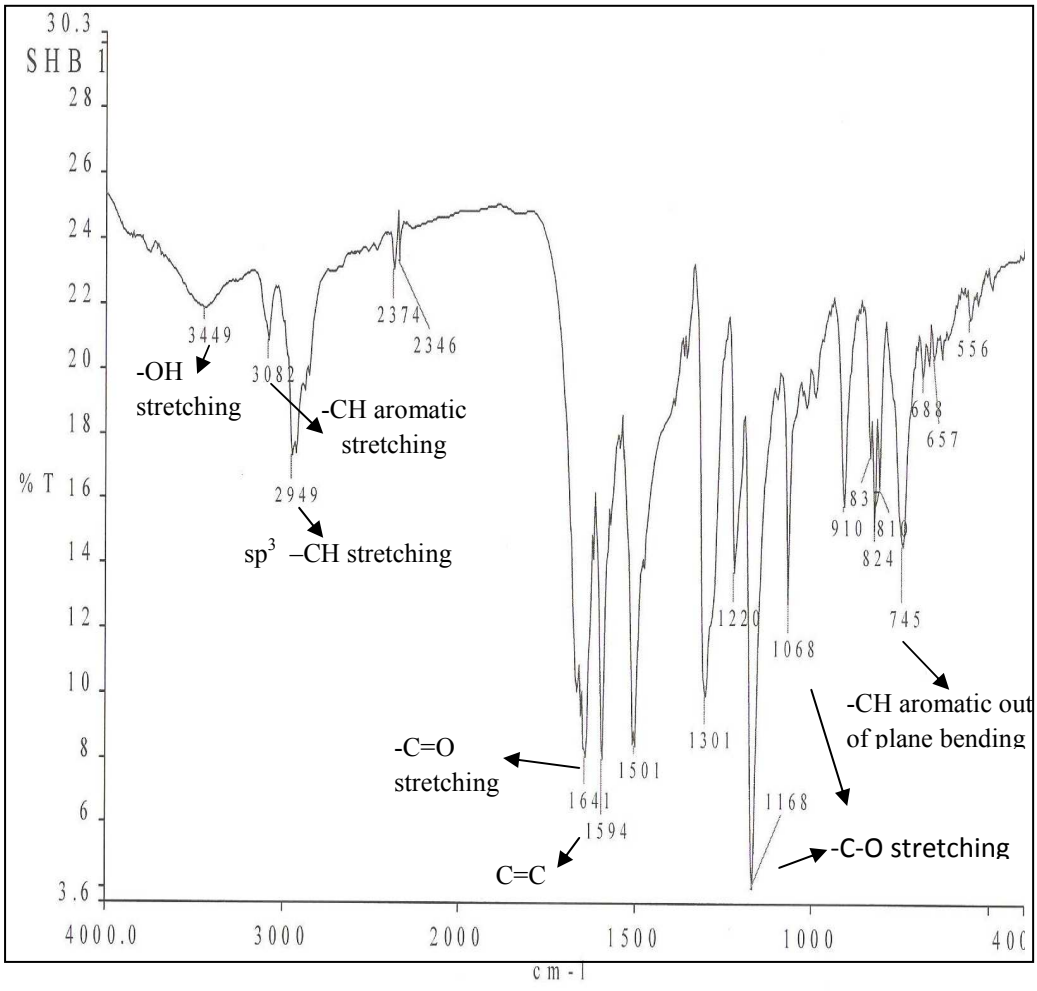


Figure 4.12: IR spectrum of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one

4.2.2.2 Structure Elucidation of 1, 3, 8-Trihydroxy-6-(pent-4-enyloxy)-9*H*- xanthen-9-one

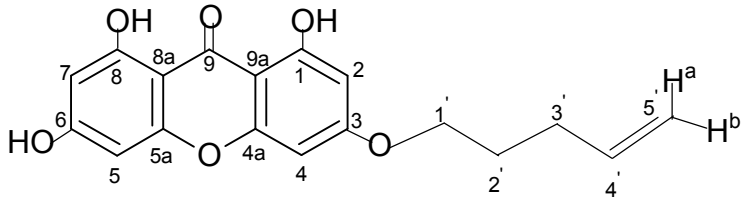


Figure 4.13: Structure of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one

1, 3, 8-Trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one is a mono-substituted *o*-alkenylated xanthone which is different from previous 1, 8-dihydroxy-3, 6-bis(pent-4)-9*H*-enyloxy-xanthen-9-one as described earlier to have two o-alkenylated groups.

Initial analysis of ^{1}H-NMR spectral data indicated that the compound has an asymmetrical xanthone skeleton. The ^{1}H-NMR spectrum (Figure 4.15) exhibited singlet signals at δ 11.69 and δ 12.06 for two chelated hydroxyl groups 1-OH and 8-OH, respectively. These chelations caused deshielding effects to the hydroxyl protons thus a tremendous shift of signals to the downfield region. The presence of three hydroxyl groups at C-1, C-6 & C-8 and one pent-4-en-1-yl group at C-3 gave an asymmetrical structure. The proton signal at δ 6.38 (1H, d, J = 2.3 Hz), δ 6.57 (1H, d, J = 2.3 Hz), δ 6.95 (1H, d, J = 2.3 Hz) and δ 6.75 (1H, d, J = 2.3 Hz) were assigned to proton H-2, H-4, H-5 and H-7 respectively. H-2 was meta-coupling with H-4 and H-5 was

meta-coupling with H-7 as indicated by the coupling constant 2.3 Hz displayed in the four signals.

The ^{13}C-NMR spectrum (Figure 4.16) showed signal for 13 magnetically non-equivalent carbons in the xanthone skeleton. The chelated carbonyl carbon: C-9 (δ 183.9) was deshielded to downfield region because of anisotropy effect. The other 12 non-equivalent carbons in the xanthonic skeleton were C-1 (δ 162.5), C-2 (δ 98.2), C-3 (δ 167.5), C-4 (δ 93.7), C-4a (δ 157.9), C-5a (δ 156.9), C-5 (δ 101.2), C-6 (δ 155.6), C-7 (δ 104.8), C-8 (δ 162.9), C-8a (δ 106.2) and C-9a (δ 102.2).

There were a total of 6 resonances appeared in ^{1}H-NMR spectrum (Figure 4.14) which were due to the presence of pent-4-en-1-yl group. The 6 resonances at δ 4.18 (2H, t, J = 6.7 Hz), 1.91 (2H, quin, J = 1.2 Hz), 2.25 (2H, q, J = 6.7 Hz), 5.88 (1H, multiplet), 5.06 (2H^{a}, d, J = 17.1 Hz) and 4.97 (2H^{b}, d, J =10.4 Hz), at which they were assigned to H-1', H-2', H-3', H-4', H^{a}-5' and H^{b}-5', respectively. Proton H-1' was deshielded, due to the electron withdrawing effect imposed by the electronegative oxygen atoms directly attached to carbons C-1'. Whereas, H-4' and H^{a}-5' & H^{b}-5' were deshielded, due to the anisotropy effect of the C=C in the pent-4-en-1-yl group. The two hydrogen atoms attached at C-5' are magnetically non-equivalent because they were restricted from free rotation at C=C bond. Thus, proton H^{a}-5' and H^{b}-5' gave two different doublet signals at δ 5.06 and δ 4.97, respectively. Proton H^{a}-5' was trans-coupling with proton H-4' and proton H^{b}-5' was cis-coupling with

proton H-4' as indicated by their coupling constant 17.1 Hz and 10.4 Hz, respectively.

The ^{13}C-NMR spectrum (Figure 4.15) showed 5 signals for 5 magnetically non-equivalent carbons present in the pent-4-en-1-yl group. The 5 magnetically non-equivalent carbons were C-1' (δ 68.4), C-2' (δ 28.7), C-3' (δ 30.3), C-4' (δ 137.8) and C-5' (δ 114.9).

The assignment of the proton and carbon signals to the structure was accomplished by referring to HMQC and HMBC spectral data. The direct ^{1}J coupling between carbons and protons were observed in HMQC spectrum (Appendix 4). HMBC spectrum (Appendix 3) was used for the assignment of long range ^{2}J and ^{3}J coupling between protons and carbons in the structure. Based on the long range coupling, it can be used to identify the location where alkenylation has taken place. The HMBC spectrum showed that H-1' was ^{3}J coupling with C-3 and C-3'. Thus, the pent-4-en-1-yl group was attached to the oxygen atoms at C-3. All the spectra data were summarised in Table 4.3

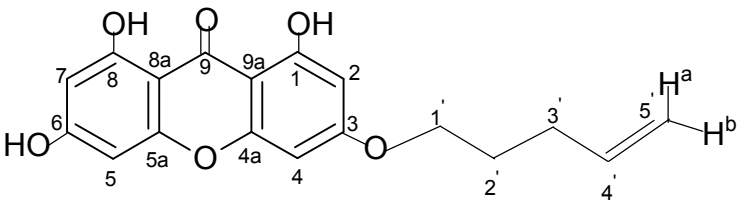
Table 4.3 Summary of assignment of the ^{1}H-NMR, ^{13}C-NMR, HMBC spectra data 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one

Position	δ_H (ppm)	δ_C (ppm)	HMBC
1	-	162.5	-
2	6.38 (1H, d, *J* = 2.3 Hz)	98.2	C-1 (^{2}J), C-3 (^{2}J), C-4 (^{3}J), C-9a (^{3}J)
3	-	167.5	-
4	6.57 (1H, d, *J* = 2.3 Hz)	93.7	C-4a (^{2}J), C-3 (^{2}J), C-2 (^{3}J), C-9a (^{3}J)
4a	-	157.9	-
5a	-	156.9	-
5	6.95 (1H, d, *J* = 2.3 Hz)	101.2	C-7 (^{3}J), C-5a (^{2}J), C-6 (^{2}J)
6	-	155.6	-
7	6.75 (1H, d, *J* = 2.3 Hz)	104.8	C-6 (^{2}J), C-8 (^{2}J), C-5 (^{3}J), C-8a (^{3}J)
8	-	162.9	-
8a	-	106.2	-
9	-	183.9	-
9a	-	102.2	-
1’	4.18 (2H, t, *J* = 1.2 Hz)	68.4	C-3’ (^{3}J), C-3 (^{3}J)
2’	1.91 (2H, quint, *J* = 6.7 Hz)	28.7	C-4’ (^{3}J), C-1’ (^{2}J), C-3’ (^{2}J)
3’	2.25 (2H, q, *J* = 6.7 Hz)	30.3	C-5’ (^{3}J), C-4’ (^{2}J), C-1’ (^{3}J),C-2’ (^{2}J)
4’	5.88 (1H. multiplet)	137.8	-
5’	5.06 (2H^{a}, d, *J* = 17.1 Hz)	114.9	C-3’ (^{3}J)
5’	4.97 (2H^{b}, d, *J* = 10.4 Hz)	114.9	C-3’ (^{3}J)

Position	δ_H (ppm)	δ_C (ppm)	HMBC
1-OH	12.06 (1H, s)	-	-
8-OH	11.69 (1H,s)	-	-

The IR spectrum (Figure 4.17) exhibited broad absorption band at 3431 cm^{-1} indicated the presence of O-H in the compound. The other absorption bands were 3078 (aromatic C-H), 2952 (sp^3 C-H), 2925 (sp^3 C-H), 2855 (sp^3 C-H), 1627 (C=O), 1611 (C=C), 1466 (sp^2 C-H), 1196 (C-O), 1172 (C-O), 1118 (C-O), 1070 (C-O) and 817 (aromatic C-H out-of-plane bending).

The UV (Figure 4.14) absorption maxima at 210, 251, and 329 nm indicated that this compound was conjugated.



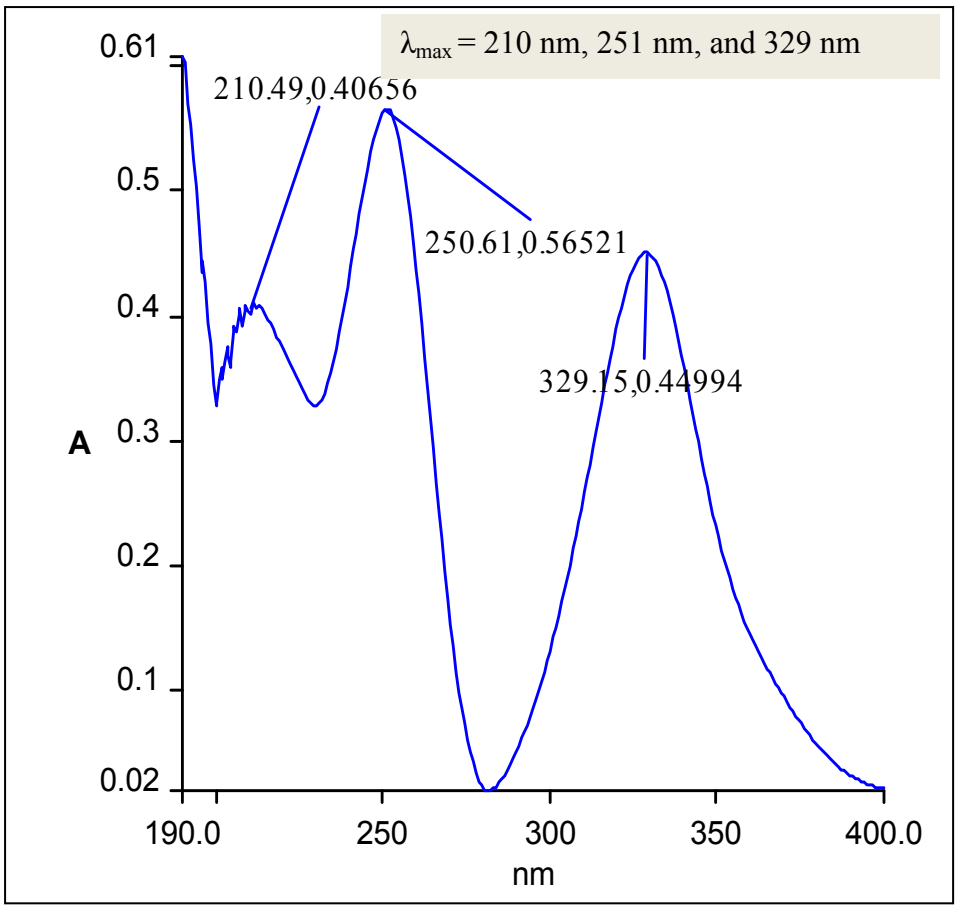


Figure 4.14: UV-Vis spectrum of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one

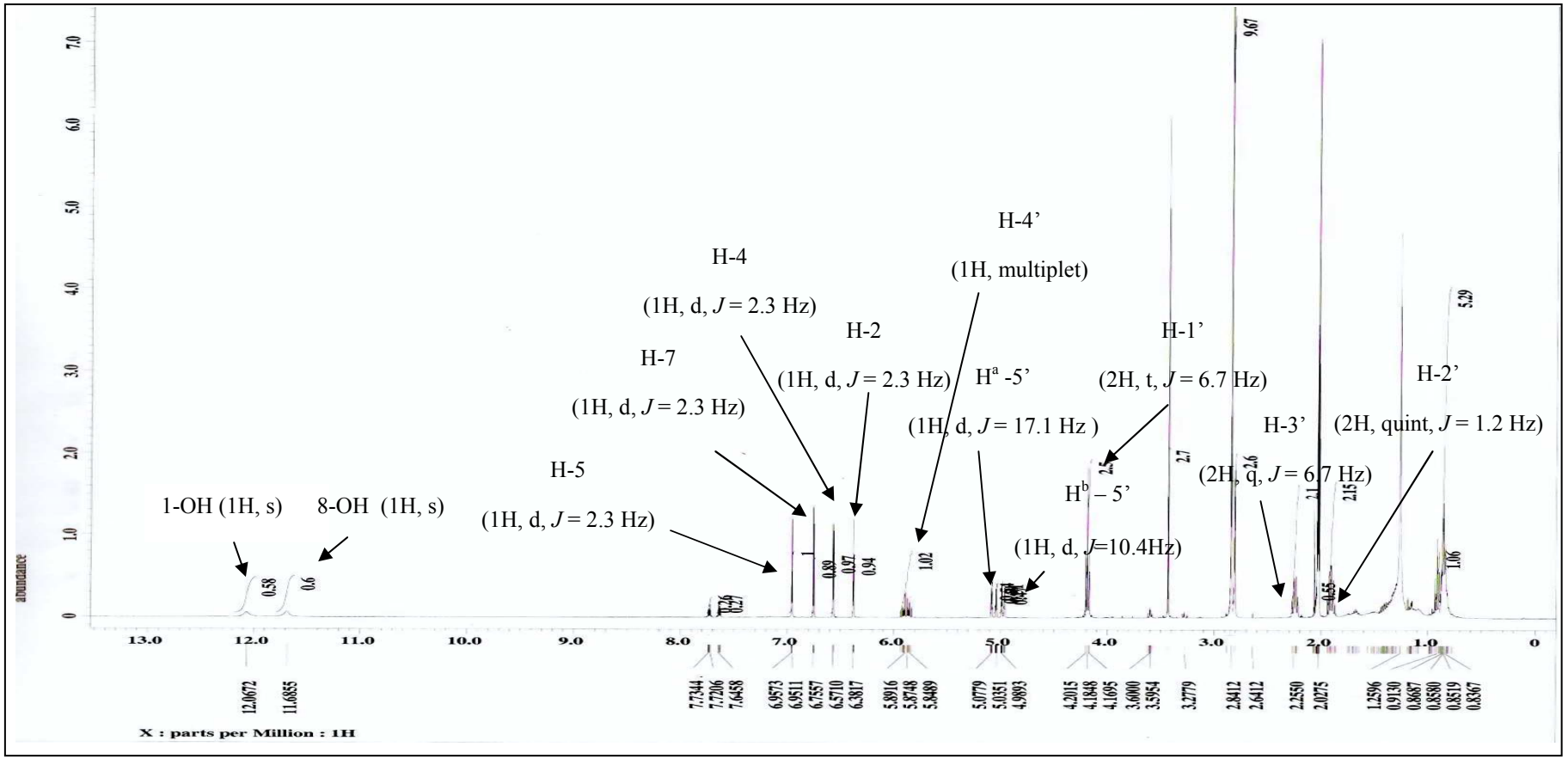


Figure 4.15: ^{1}H-NMR of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one (400 MHz, acetone-d_6)

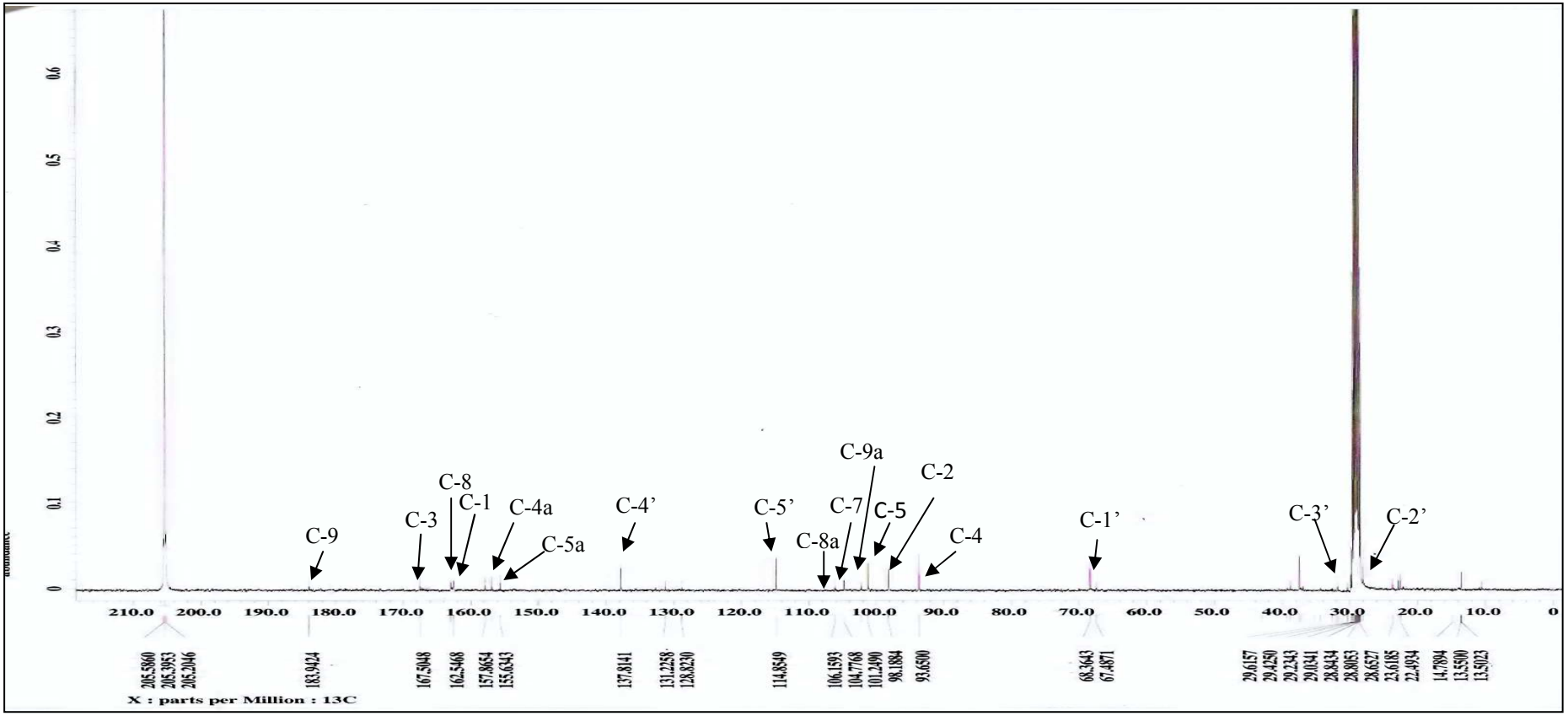


Figure 4.16: ^{13}C-NMR spectrumof 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one (100 MHz, acetone-d_6)

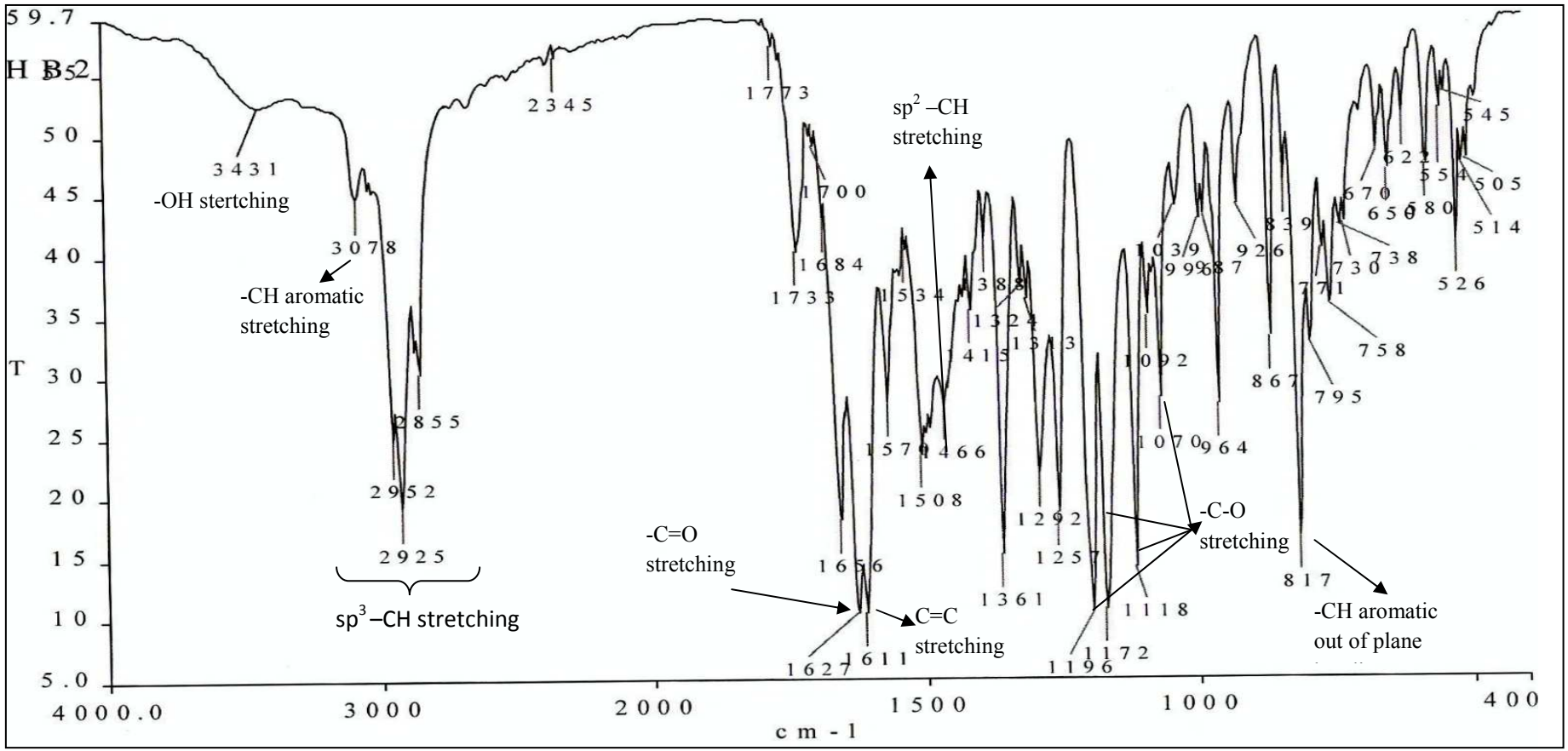


Figure 4.17: IR spectrum of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one

4.3 Mechanism

The mechanisms of formation of 1, 3, 6, 8-tetrahydroxyxanthone and *o*-alkenylated xanthone were shown in Sections 4.3.1 and 4.3.2, respectively.

4.3.1 Mechanism of 1, 3, 6, 8-Tetrahydroxyxanthone

In synthesising 1, 3, 6, 8-tetrahydroxyxanthone, the coupling of 2, 4, 6-trihydroxybenzoic acid was catalysed by Eaton's reagent. Eaton's reagent was made up of phosphorus pentoxide in methanesulfonic acid. The function of phosphorus pentoxide (P_4O_{10}) was as a dehydrating agent, while, methanesulfonic acid (CH_3SO_3H) was a coupling agent. The mechanism was shown in Figure 4.19.

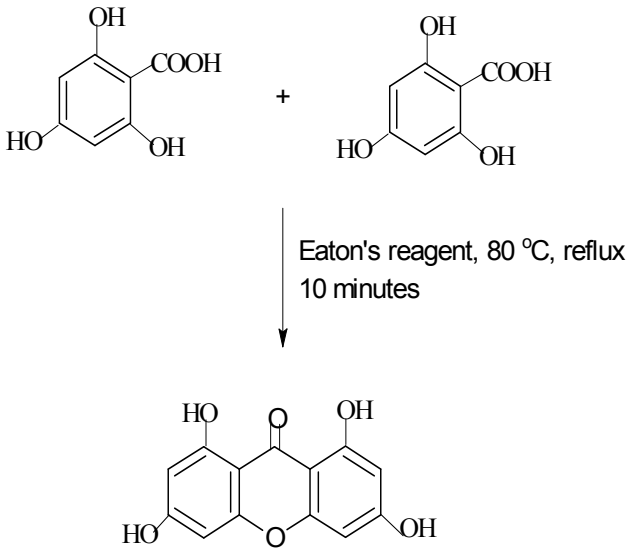


Figure 4.18: Synthesis route of 1, 3, 6, 8- tetrahydroxyxanthone

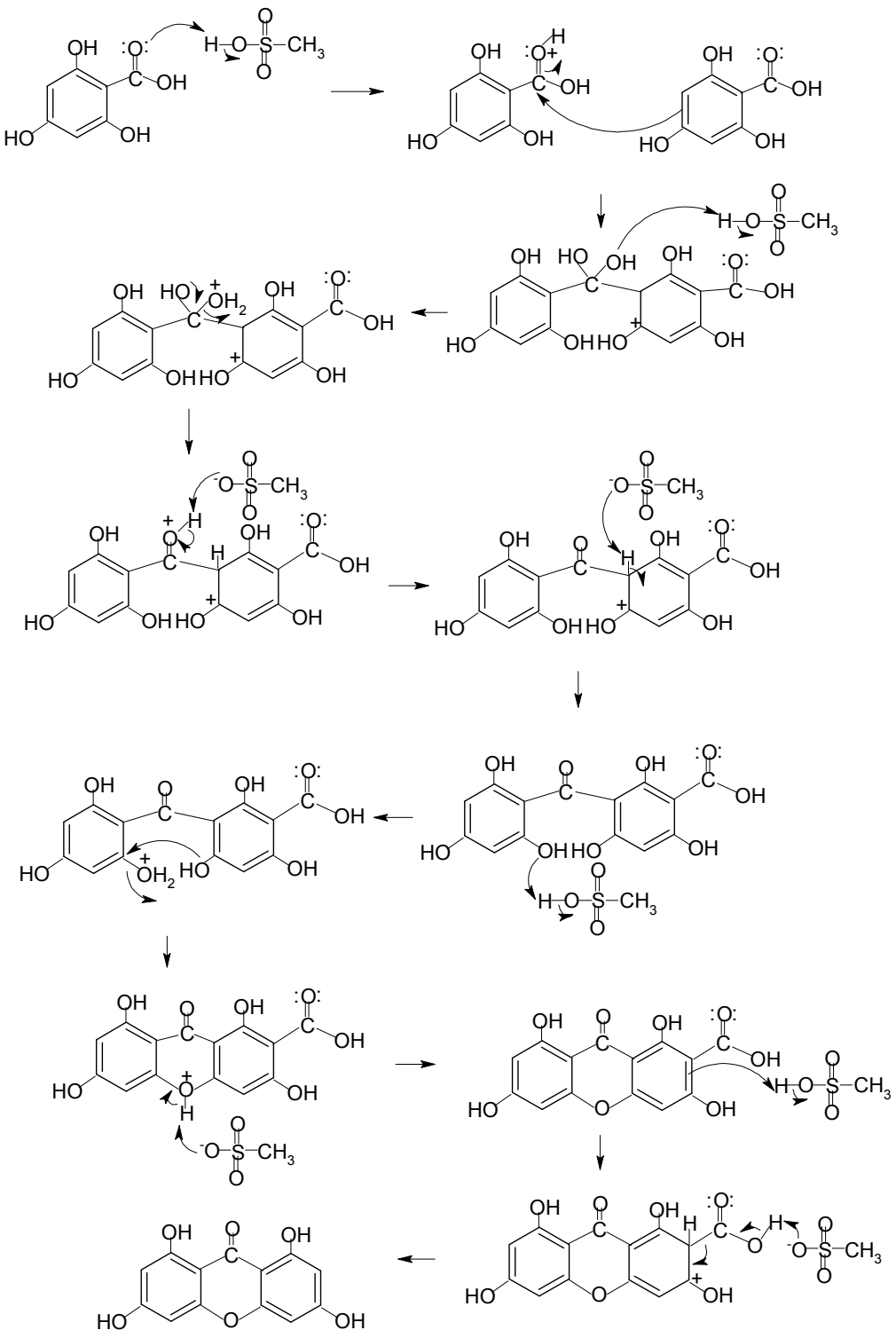


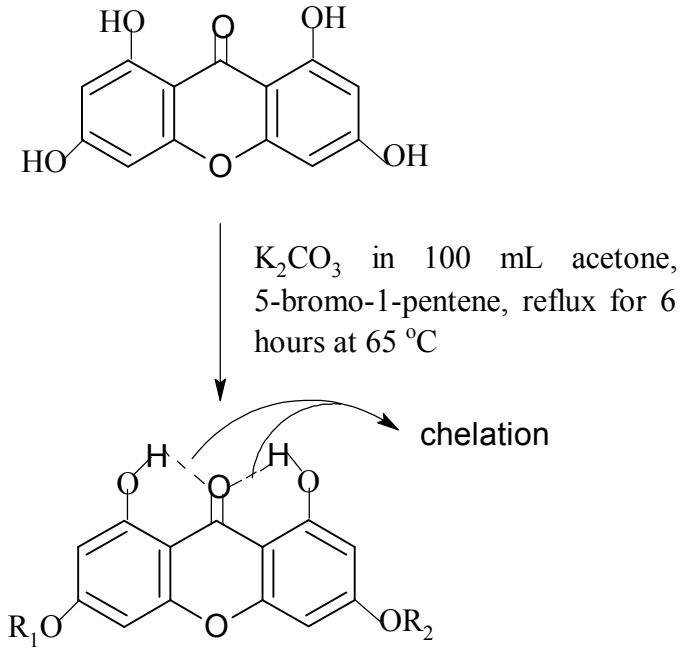
Figure 4.19: Proposed mechanism for synthesis of 1, 3, 6, 8 – tetrahydroxyxanthone

Based on the mechanism in Figure 4.19**,** there were two reactions taken place which were dehydration and decarboxylation. Thus, methanesulfonic acid played two different roles in the reaction. It functioned as coupling agent and also facilitated decarboxylation. This caused the loss of carboxylic group from the expected xanthonic block : 1, 3, 6, 8-tertrahydroxyxanthone-2-carboxylic acid or 1, 3, 6, 8-tertrahydroxyxanthone-4-carboxylic acid to give 1, 3, 6, 8-tetrahydroxyxanthone.

However, this mechanism still needs further identification in terms of its kinetic and thermodynamic properties.

4.3.2 Mechanism of *o*-Alkenylation

From the previous discussion in Section 4.1, *o*-alkenylation of 1, 3, 6, 8-tetrahydroxyxanthone formed two products. They were 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one. The reaction involved was nucleophilic substitution, at which, 1, 3, 6, 8-tetrahydroxyxanthone was a nucleophile and 5-bromo-1-pentene was an electrophile.

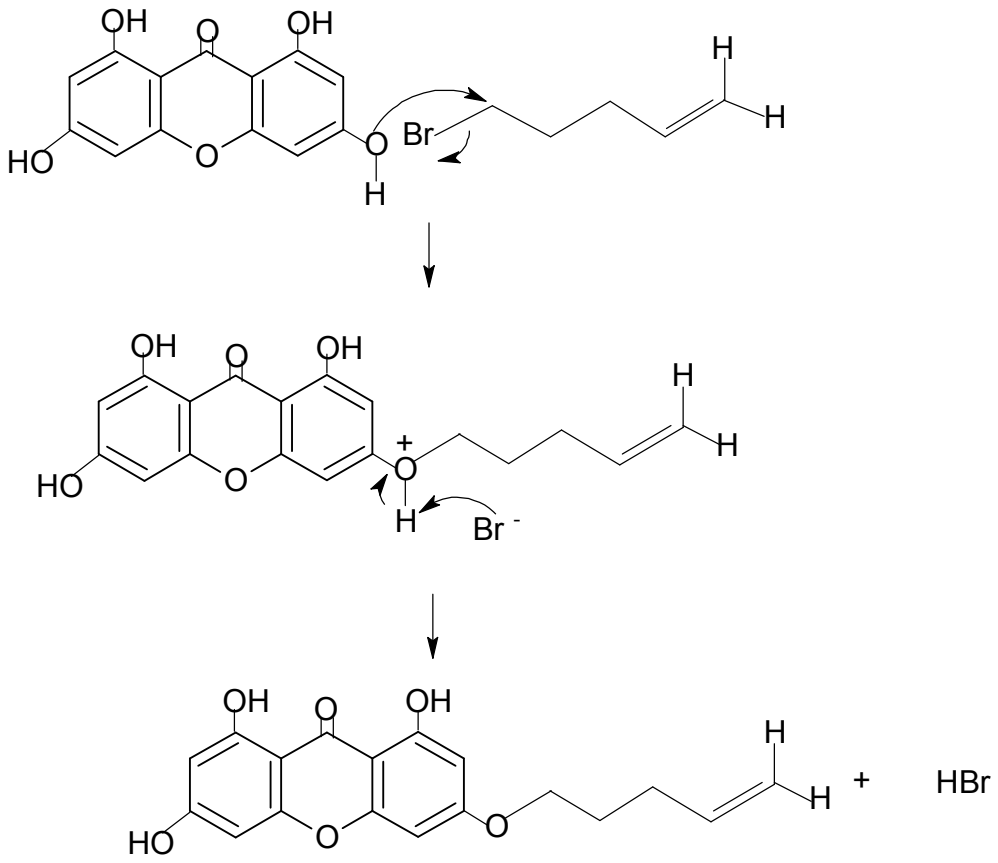


(1) $R_1 = R_2$ = pent-4-en-1-yl

(2) R_1 = H, R_2 = pent-4-en-1-yl

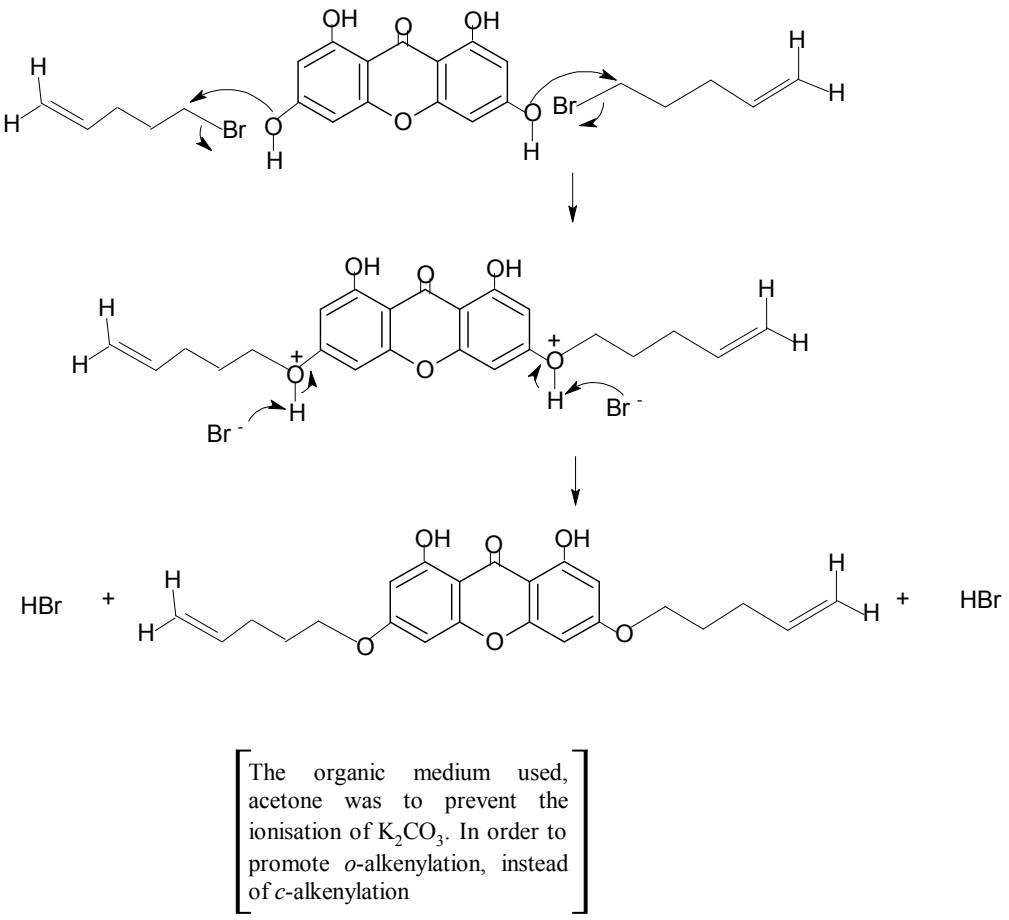
Figure 4.20: Reaction of *o*-alkenylation

Based on Figure 4.20, two free hydroxyl groups in 1, 3, 6, 8-tetrahydroxyxanthone were able to undergo *o*-alkenylation. The other two chelated hydroxyl groups were hardly to undergo *o*-alkenylation. Thus, producing one mono-substituted *o*-alkenylated product, 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one and one di-substituted *o*-alkenylated product, 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one.



The organic medium used, acetone was to prevent the ionisation of K_2CO_3. In order to promote *o*-alkenylation, instead of *c*-alkenylation

Figure 4.21: Proposed mechanism for synthesis of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one



The organic medium used, acetone was to prevent the ionisation of K_2CO_3. In order to promote *o*-alkenylation, instead of *c*-alkenylation

Figure 4.22: Mechanism of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one

The mechanisms involved for 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one were almost similar. The only difference was 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one was mono-substituted and 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one was di-substituted with the same substituent.

Although, potassium carbonate, K_2CO_3 was used as a catalyst in this reaction, but acetone prevented the ionisation of K_2CO_3 and promoted *o*-alkenylation of 1, 3, 6, 8-tetrahydroxyxanthone instead of *c*-alkenylation of 1, 3, 6, 8-tetrahydroxyxanthone.

4.4 Bioassay

1, 3, 6, 8-tetrahydroxyxanthone, 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one were subjected to cytotoxic assay. All the compounds were tested for their inhibitory activities toward MDA-MB-231 and HeLa cancer cell lines. The cytotoxic activities of the compounds tested were presented in IC_{50} value, (half maximal inhibitory concentration) or GI_{50} as mention in Section 2.2. IC_{50} value was obtained from the graph of average cell viability versus concentration of each compound tested (Figure 4.23 and Figure 4.24). The cytotoxic activities of those compounds are shown in the Table 4.4

Table 4.4: Cytotoxic activities of compounds against HeLa and MDA-MB-231 cell line

Compound	HeLa cancer cell line,IC_{50} (µg/mL)	MDA-MB-231 cancer cell line,IC_{50} (µg/mL)
1,3,6,8-tetrahydroxyxanthone	>50.0	>50.0
1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one	7.00	>50.0
1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one	11.0	>50.0

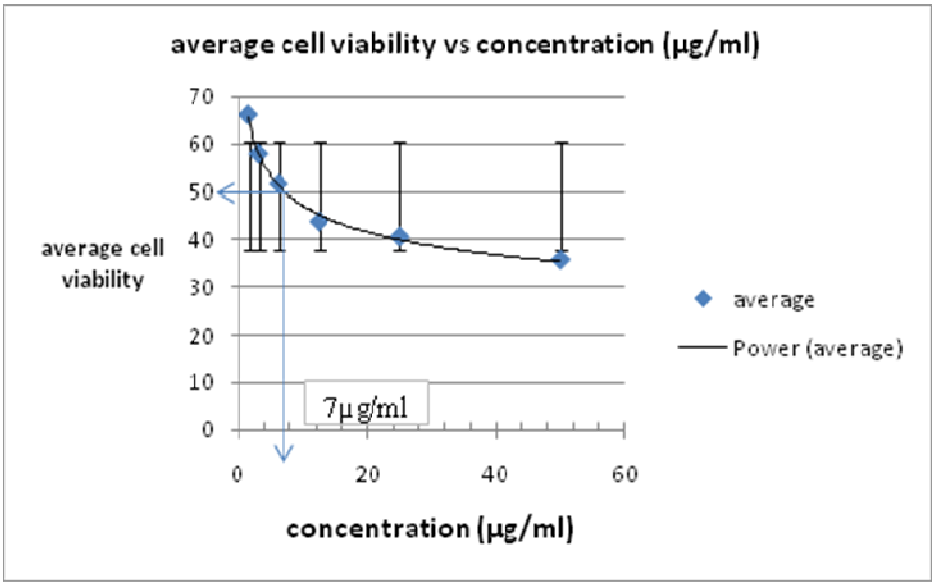


Figure 4.23: Graph of cell viability of Hela cancer cells against concentration of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one

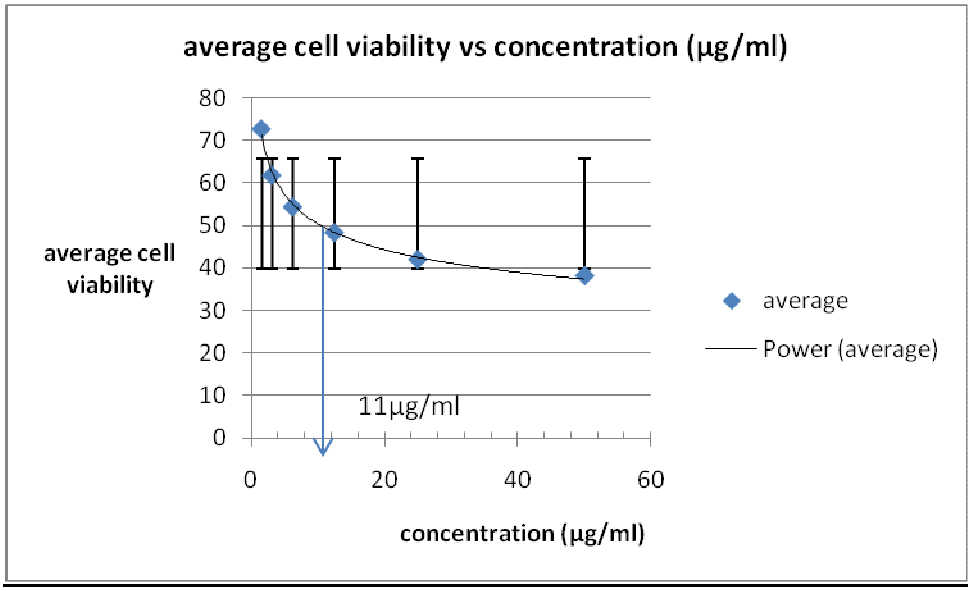


Figure 4.24: Graph of cell viability of HeLa cancer cells against concentration of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen- 9-one

From the results of bioassay, it was found that only 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one showed cytotoxic activity toward HeLa cancer cell line. However, all the compounds tested showed insignificant cytotoxic activity toward MDA-MB-231 cancer cell line.

The smaller the IC_{50}, the more cytotoxic is the compound towards the cancer cell line. The compound with IC_{50} more than 50 µg/mL was considered to be insignificant in their cytotoxic activity. Thus, 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one with IC_{50} value of 7.00 µg/mL was found to be the most cytotoxic toward HeLa cancer cell line as compared to other synthesised

compounds. From the results, it also indicated that the more *o*-substituted xanthonic block exerted more cytotoxic effect toward the HeLa cancer cell line. This can be explained when considering the structure of the compound synthesised.

The xanthonic block, 1, 3, 6, 8-tetrahydroxyxanthone showed insignificant cytotoxic activity towards HeLa cancer cell lines and the cytotoxic effect increases when it is mono-substituted with 5-bromo-1-pentene at the –OH group of C-3. Further increase in the cytotoxic effect when 1, 3, 6, 8-tetrahydroxyxanthone was di-substituted with 5-bromo-1-pentene at –OH groups of C-3 and C-6. Thus, the higher the number of *o*-alkenylated group attached to xanthone, the more cytotoxic the compound towards HeLa cancer cell line.

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

This project was aimed for the synthesis of xanthonecarboxylic acid but due to decarboxylation, the carboxyl group has been detached from the xanthonic ring as carbon dioxide, yielded 1, 3, 6, 8-tetrahydroxyxanthone. There were a total of three xanthonic compounds being synthesised and isolated in this project. This included one xanthonic block and two *o*-alkenylated xanthone. The xanthonic block was 1, 3, 6, 8-tetrahydroxyxanthone (4.69 g). The two *o*-alkenylated xathones were 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one (0.21 g) and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one (0.033 g). All these xanthonic compounds had been fully elucidated, identified and characterised by spectroscopic analyses.

Bioassay had been conducted on HeLa and MDA-MB-231 cancer cell lines by using 1, 3, 6, 8-tetrahydroxyxanthone, 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one as test compounds. All the tested compounds were found to give insignificant cytotoxic activity toward MDA-MB-231 cancer cell line, having IC_{50} of more than 50 μg/mL. In terms of HeLa cancer cell line, only 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one and 1, 3, 8-trihydroxy-

6-(pent-4-enyloxy)-9*H*-xanthen-9-one gave significant cytotoxic activities with IC_{50} values of 7.00 µg/mL and 11.00 µg/mL, respectively. However, the xanthonic block, 1, 3, 6, 8-tetrahydroxyxanthone showed insignificant cytotoxic activity with IC_{50} more than 50 µg/mL toward the same cancer cell line. Thus, it can be concluded that the more *o*-alkenylation on the xanthonic compound, the more cytotoxic the compound towards HeLa cancer cell line.

5.2 Suggestion for Further Studies

For further study, different separation methods should be carried out in order to save the time used for purifying crude products from the synthesis. The other separation methods suggested to be used are flash column chromatography, high performance column chromatography or recrystallisation which are found to give a better separation efficiency.

Besides that, to synthesis xanthonecarboxylic acid, it is suggested a step by step synthesis should be conducted with the use of protecting group (section 2.3.6) to prevent decarboxylation.

On top of that, it is suggested to carry out *c*-alkenylation on 1, 3, 6, 8-tetrahydroxyxanthone to provide insight into the cytotoxic activities of *o*-alkenylated xanthones in comparison with *c*-alkenylated xanthones.

Furthermore, extended pharmacological tests should be carried out in order to find out others pharmacological potential present in the xanthone derivatives. The pharmacological activities to be studied are anti-inflammatory, anti-bacterial, anti-fungal, anti-oxidant, anti-allergy and anti-viral activities.

Lastly, further synthesis is suggested to be done on the xanthonic block in order to produce a wider variety of the xanthonic species for a more comprehensive structure reactivity relationship (SAR) study to look into potential structure of xanthone to be developed into drug leads. The further synthesis might involve cyclisation of the alkenyl group to give additional ring to the structure or introduce other functional groups to the xanthonic nucleus.

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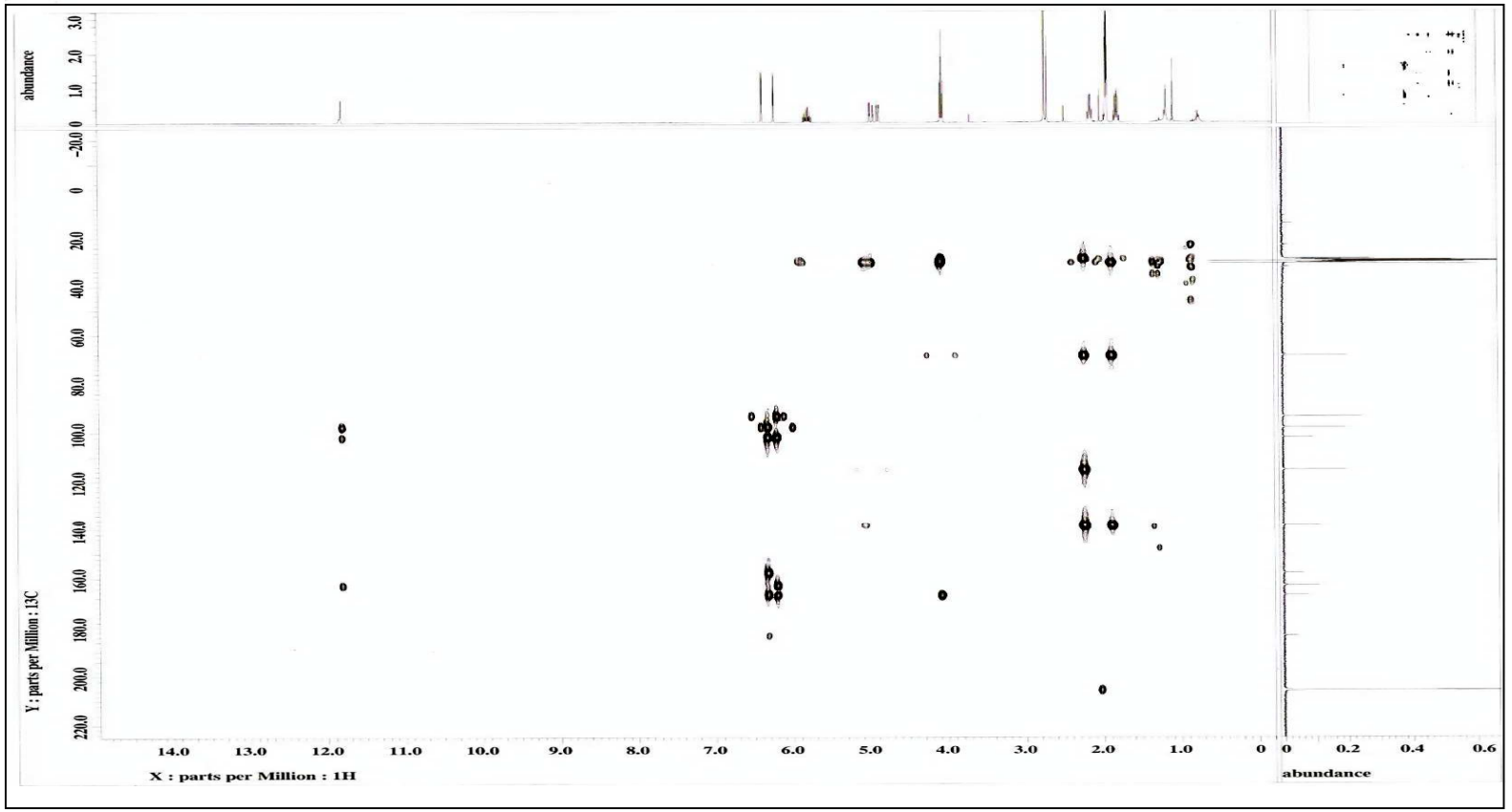
APPENDICES

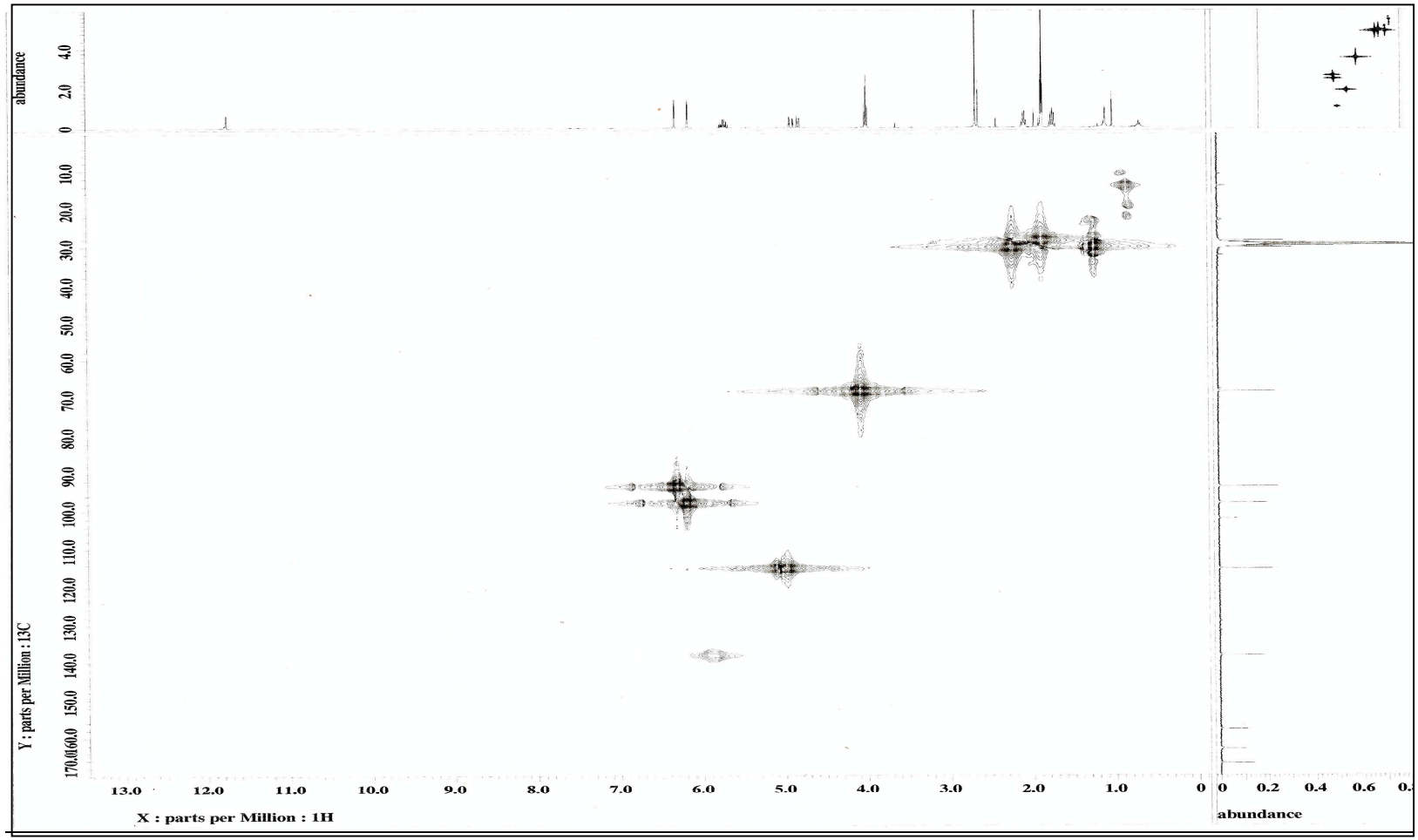
APPENDIX 1

HMBC of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one



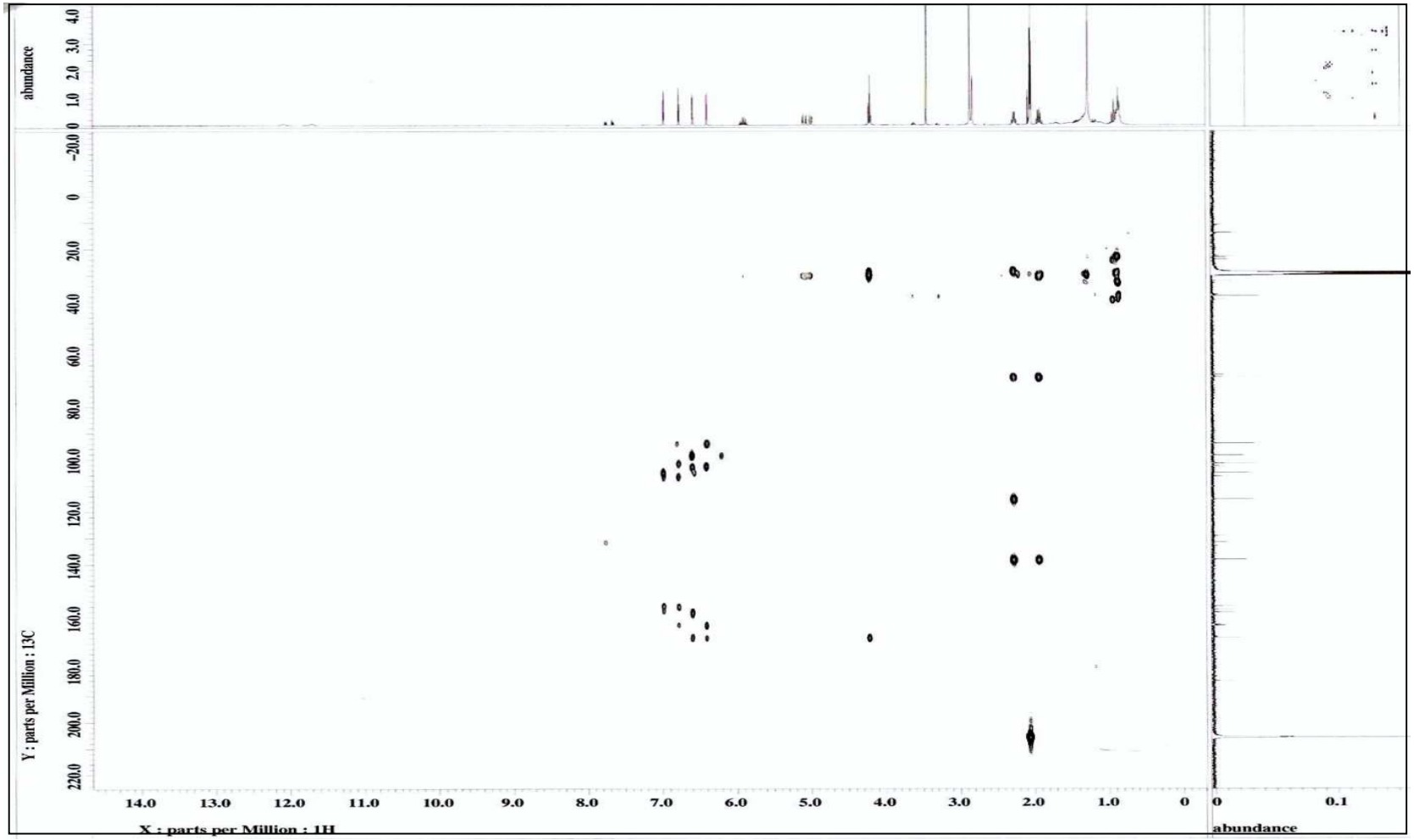
APPENDIX 2

HMQC of 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one



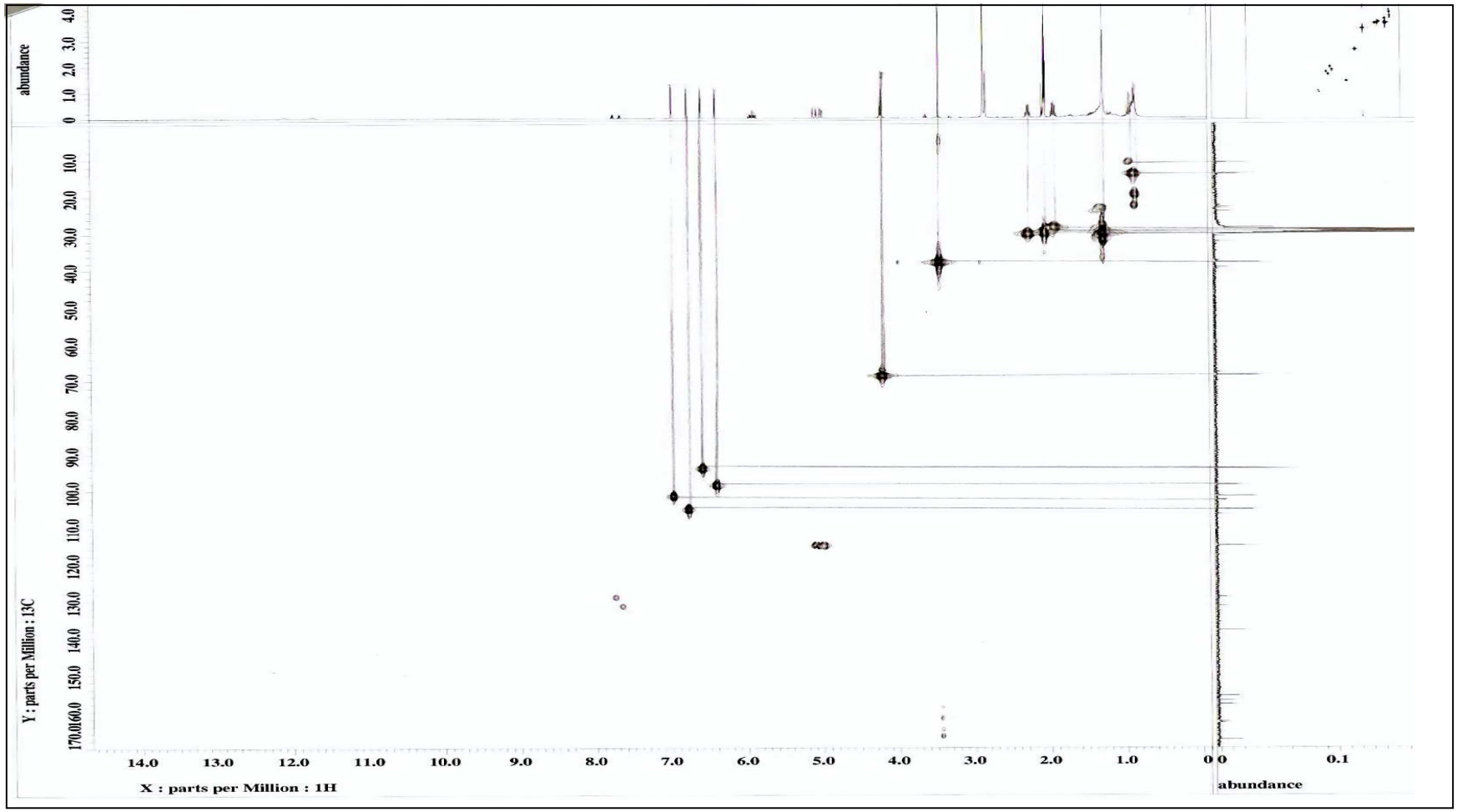
APPENDIX 3

HMBC of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one



APPENDIX 4

HMQC of 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one



APPENDIX 5

Raw Data of MTT assay –HeLa (SHB 1 is 1, 8-dihydroxy-3, 6-bis(pent-4-enyloxy)-9*H*-xanthen-9-one, SHB 2 is 1, 3, 8-trihydroxy-6-(pent-4-enyloxy)-9*H*-xanthen-9-one)

Sample	Conc. (µg/ml)	Replicate 1				Replicate 2				Replicate 3			
		1st	2nd	3rd	Control	1st	2nd	3rd	Control	1st	2nd	3rd	Control
SHB1	50	0.724	0.722	0.726	1.610	0.603	0.609	0.605	1.468	0.454	0.459	0.461	1.201
	25	0.735	0.739	0.731	1.628	0.681	0.678	0.683	1.408	0.542	0.547	0.549	1.153
	12.5	0.756	0.753	0.759	1.580	0.709	0.703	0.701	1.475	0.612	0.619	0.61	1.218
	6.25	0.744	0.879	0.892	0.166	0.806	0.802	0.809	0.103	0.723	0.719	0.721	0.125
	3.12	0.980	1.037	1.043	0.165	0.851	0.849	0.845	0.124	0.751	0.756	0.759	0.130
	1.56	1.201	1.155	1.068	0.174	0.912	0.918	0.915	0.100	0.873	0.879	0.881	0.124
SHB2	50	0.701	0.708	0.809	1.610	0.608	0.610	0.623	1.468	0.432	0.427	0.434	0.979
	25	0.741	0.743	0.743	1.628	0.654	0.649	0.630	1.408	0.501	0.508	0.504	0.923
	12.5	0.767	0.820	0.742	1.580	0.740	0.749	0.739	1.475	0.596	0.589	0.583	0.896
	6.25	0.801	0.940	0.941	0.166	0.801	0.809	0.810	0.103	0.631	0.634	0.638	0.114
	3.12	0.971	1.046	0.861	0.165	0.920	0.912	0.917	0.124	0.721	0.718	0.715	0.115
	1.56	1.045	1.035	1.089	0.174	1.084	1.088	1.078	0.100	0.835	0.811	0.861	0.100

APPENDIX 6

Raw Data of MTT assay- 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
SHB1	50	2.612	2.582	2.890	2.503	2.573	2.09	2.593	2.514	1.024	1.027	1.023	0.953
	25	2.210	2.161	2.635	2.643	1.866	1.963	1.624	2.128	1.002	1.004	1.006	1.029
	12.5	1.688	2.036	2.726	2.117	1.959	1.773	1.872	2.411	0.990	0.989	1.022	0.920
	6.25	1.974	2.549	1.876	0.130	2.060	1.895	1.701	0.106	0.988	0.908	1.037	0.183
	3.12	2.201	2.205	2.209	0.129	2.031	2.038	2.029	0.108	0.935	0.942	0.943	0.185
	1.56	2.201	2.209	2.206	0.136	2.01	2.015	2.012	0.099	0.903	0.905	0.835	0.190
SHB2	50	2.794	2.143	2.827	2.503	3.053	2.381	2.638	2.514	1.001	1.077	1.079	0.953
	25	1.836	1.623	1.751	2.643	2.305	1.939	2.076	2.128	0.998	1.000	0.993	1.029
	12.5	1.570	1.575	1.577	2.117	1.976	1.828	1.782	2.411	1.020	0.986	0.989	0.920
	6.25	1.569	1.567	1.568	0.130	1.724	1.810	1.989	0.106	0.940	0.949	0.954	0.183
	3.12	1.550	1.555	1.557	0.129	1.897	1.895	1.890	0.108	0.939	0.935	0.856	0.185
	1.56	1.669	1.675	1.663	0.136	1.908	1.907	1.902	0.099	0.901	0.900	0.890	0.190