

**STRUCTURE AND SYNTHESIS OF
PHLOROGLUCINOL DERIVATIVES FROM
*HYPERICUM ROEPERIANUM***

Thesis submitted in fulfilment of the requirements for the degree

Master of Science



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ABSTRACT

The research presented in this study combines natural product chemistry with organic synthesis. The natural product research involved a phytochemical investigation of a South African plant, *Hypericum roeperianum*, which belongs to the Hypericaceae (previously Clusiaceae/Guttiferae) family. A combination of chromatographic techniques resulted in the isolation of two known compounds: [3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone (3-geranyl-2,4,6-trihydroxybenzophenone) and 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4*H*-pyran-4-one (hyperenone-A). A third compound was also isolated and is provisionally assigned as 1-methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**). However, the small amount of compound isolated did not provide sufficient data for a conclusive structural assignment.

To the best of our knowledge 3-geranyl-2,4,6-trihydroxybenzophenone has not been isolated previously from the *Hypericum* genus but has been isolated from *Tovomita krukovii*, *T. longifolia*, *Helichrysum monticola* and *Garcinia vieillardii*. It has been reported to exhibit inhibitory effects against *Candida albicans*, *Candida neoformans*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella gallinarum* and *Mycobacterium smegmatis*. The activity exhibited against *Mycobacterium smegmatis* is of great interest as this bacterium belongs to the same genus as *Mycobacterium tuberculosis*, which is the bacterium that is the cause of tuberculosis (TB), a bacterial infectious disease affecting 1.7 billion people per annum.

Due to the interesting biological activity exhibited by 3-geranyl-2,4,6-trihydroxybenzophenone the synthetic section of our research involved the synthesis of this secondary metabolite as well as seven structural analogues. These analogues, namely, phenyl-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]methanone, 1-[3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]ethanone, 1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone, 1-[3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-trihydroxy phenyl]-2-methylbutan-1-one, 2-methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]butan-1-one, [3-(3,7-dimethylocta

-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylpropan-1-one, 2-methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]propan-1-one, as well as 3-geranyl-2,4,6-trihydroxybenzophenone were successfully synthesized via a Friedel-Crafts acylation reaction of phloroglucinol with an appropriate acyl chloride. Alkylation of the acyl phloroglucinol derivatives, with geranyl and prenyl moieties, was accomplished by Friedel-Crafts-type alkylation reactions. With the synthesis of these compounds we hoped to explore the significance of the structural features of 3-geranyl-2,4,6-trihydroxybenzophenone and determine whether variation in length of the prenyl side chain and the type of acyl group present would affect the level of activity.

PREFACE

The experimental work described in this dissertation was carried out in the School of Chemistry, Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Fanie van Heerden.

I hereby declare that these studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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K. SMITH

I hereby certify that the above statement is correct.

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PROFESSOR F. VAN HEERDEN

SUPERVISOR

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ABBREVIATIONS

COSY	COrrrelation SpectroscopY
DCM	dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer.
DMF	<i>N,N</i> -dimethylformamide
DNA	deoxyribonucleic acid
EtOAc	ethyl acetate
Hex	hexanes (a mixture of hexane isomers)
HIV	human immunodeficiency virus
HMQC	Heteronuclear Multiple Quantum Coherence
HSQC	Heteronuclear Single Quantum Coherence
Hz	hertz
IR	infrared
MeOH	methanol
NMR	nuclear magnetic resonance
RNA	ribonucleic acid
TB	tuberculosis
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMS	tetramethylsilane
UV	ultraviolet

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CHAPTER 1

Introduction and Aim of Study

1.1 Plants as a Source of Medicinal Compounds

Over a period of at least five thousand years, mankind has utilized natural products as its main source of medicinal compounds.¹ In particular, plants on the African continent have a long history of medicinal use for various diseases and ailments. These are of such importance that in some African countries, the majority of the population (up to 90%) still relies solely on plants for their healthcare.²

Africa is known for its diverse range of flora, comprising of thousands of different species, many of which are indigenous to South Africa and are commonly used in traditional medicine to treat a range of diseases and illnesses.^{2,3} An investigation of these plants, selected on the basis of their use in traditional medicine, can lead to the discovery of potential new molecules with biological activity, which may have a significant influence on the advancement of drug discovery.^{2,3}

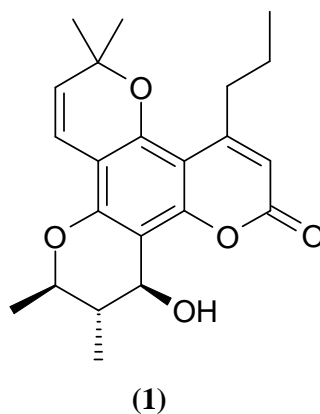
1.2 Tuberculosis

Tuberculosis (TB) is a subject of great concern throughout the globe, but predominantly in poverty stricken Asia and Africa.⁴ This contagious airborne disease is caused by *Mycobacterium tuberculosis*.⁵

It is statistically estimated that one third of the world's population (approximately 2 billion people) are hosts to the TB causing bacilli, resulting in 1.7 million deaths in 2006 (World Health Organization, 2008).^{4,6}

Although effective TB treatments are available, the development of multi-drug resistant strains (MDR-TB) as well as the co-prevalence of the human immunodeficiency virus (HIV) and TB has resulted in an urgent need for the discovery and implementation of

novel anti-tuberculosis drugs, preferentially with alternative mechanisms of action.⁶ The potential use of natural products, or the derivatives thereof, has been previously mentioned and indicates that natural product chemistry may play a vital role in the discovery of innovative treatments for TB. To substantiate this claim, recent research on (+)-calanolide A (**1**), isolated from *Calophyllum lanigerum* (Clusiaceae), has shown that this coumarin derivative inhibits *Mycobacterium tuberculosis*.^{7,8} Furthermore, this compound is not only active against the drug-susceptible strains, but has also been found to inhibit MDR-TB strains and in addition inhibits some strains of HIV.⁸ Calanolide A (**1**) exhibited promising results in phase I clinical trials, making it worthy for further investigation in phase II trials.^{9,10} Phase II trials were initiated, however the development of calanolide A is currently on hold, whilst awaiting a decision by the Sarawak government, who owns the pharmaceutical company to which this compound is licensed to.⁹



The isolation of (+)-calanolide A (**1**), as well as many other biologically active compounds from the *Clusiaceae* family, prompted us to explore the phytochemistry of plants in South Africa belonging to this family.

1.3 Thesis Aims

The aim of the research presented in this study was to combine natural product research with organic synthesis. This involved an investigation of a South African plant, *Hypericum roeperianum*, which belongs to the Hypericaceae (previously Clusiaceae/Guttiferae) family.

The major aims of this study were:

- To carry out a phytochemical investigation on *H. roeperianum* in order to identify some secondary metabolites present in the plant
- To examine the biological activity of the isolated compounds
- To synthesise a biologically active compound isolated from *H. roeperianum*
- To synthesise structural analogues (**3** – **9**) of the bio-active compound (**2**) (**Figure 1.1**)
- In the synthesis of these compounds we hoped to explore the significance of the structural features of the bioactive compound **2** and determine whether variation in length of the prenyl side chain and the type of acyl group present would affect the level of biological activity.

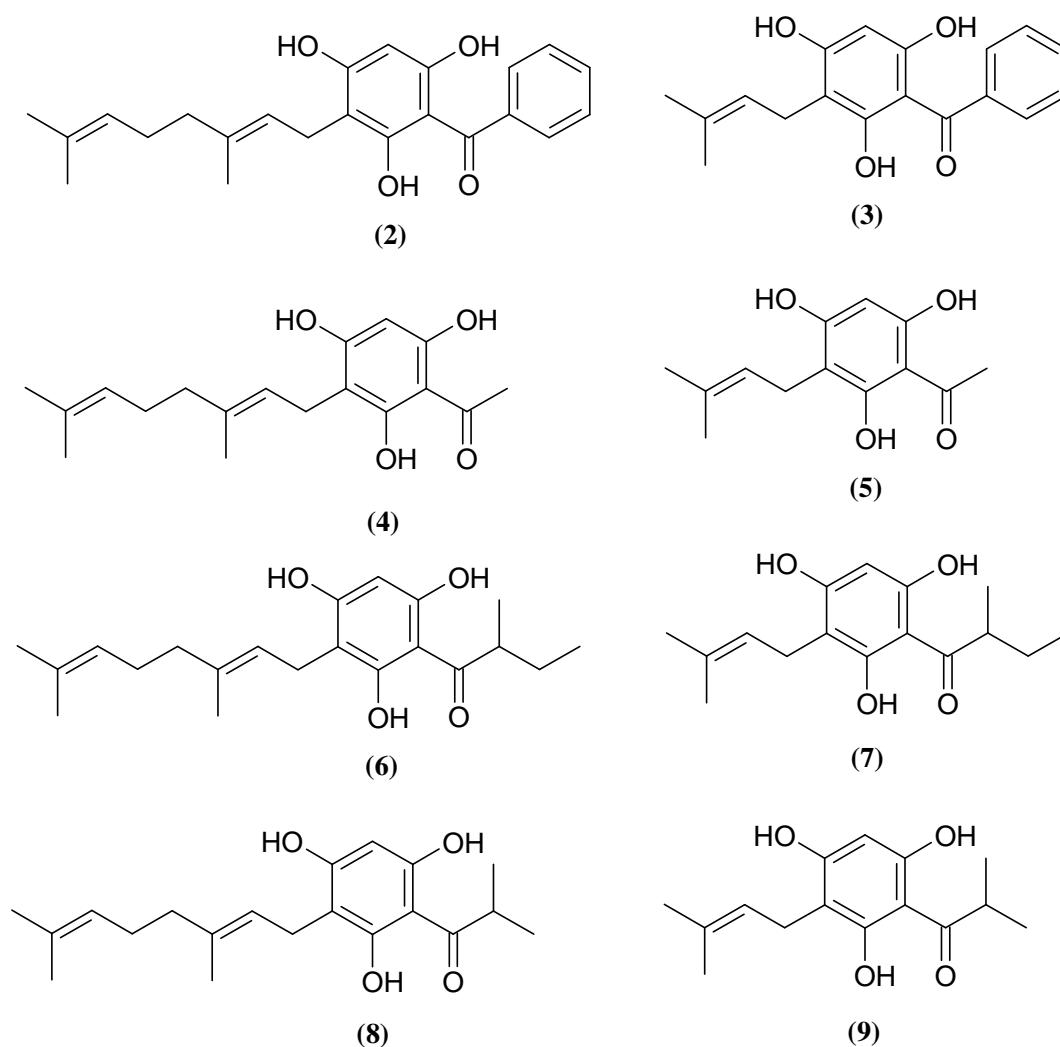


Figure 1.1 The bioactive compound with the structural analogues.

In this chapter we briefly presented the major concerns of TB and revealed how natural product chemistry may lead to the discovery of new potential drugs. The aims of the project were also introduced. In the following chapter we discuss the genus *Hypericum*.

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CHAPTER 2

An Overview of the Genus *Hypericum*

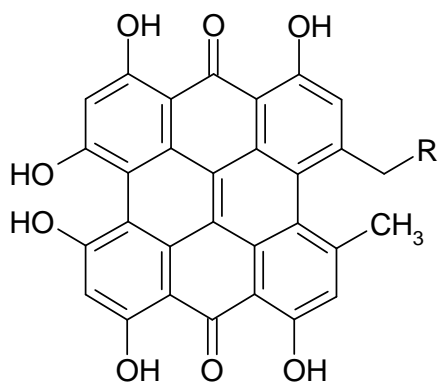
2.1 Description and Distribution

The genus *Hypericum* L. belongs to Hypericaceae, alternatively known as Clusiaceae or Guttiferae.¹ *Hypericum* is a large genus comprised of approximately 400 species, which are widely distributed throughout the temperate regions of the world, though only one species occurs in South America, two in Australia and New Zealand and six (*Hypericum revolutum*, *H. roeperianum*, *H. lalandii*, *H. aethiopicum*, *H. natalense*, *H. wilmsii*) are indigenous to South Africa.^{2,3}

The *Hypericum* species vary from small trees and shrubs (up to 12 m tall) to herbs.^{3,4} Although this genus is widely distributed, it is not present in habitats that are particularly dry, hot or cold and are rarely found in water. The species that occur in the tropics are most commonly located at high altitude. Thus *Hypericum* is seldomly found in deserts, polar regions and tropical lowlands.^{3,4}

Most species of *Hypericum* are recognizable by their characteristic leaves, flowers and fruit.³ The light-green leaves are simple (consisting of a single blade), oval in shape and arranged opposite to each other. The leaves frequently contain glandular secretions which are evident as transparent, red or black spots.³ The translucent spots contain cells that secrete essential oils, whilst the red and black spots contain hypericin (**10**) or pseudohypericin (**11**).³

The flowers (**Figure 2.1**) are comprised of green (occasionally red tinged) sepals and a corolla containing five petals (rarely four), which are usually a shade of yellow varying from lemon-yellow to deep orange yellow.^{3,4} This yellow pigment (due to xanthonenes) is a common occurrence in the tissues of plants belonging to the Clusiaceae family.^{1,3} The inner whorls of the flower are comprised of pollen-producing stamens (3-5 groups) and an ovary with 3-5 styles.³



10: R = H
11: R = OH

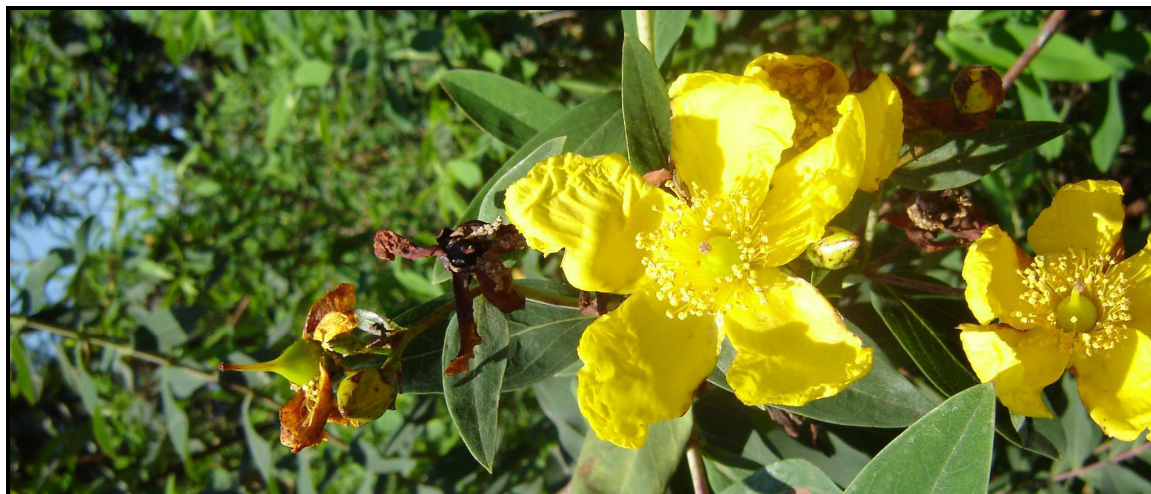


Figure 2.1 The characteristic bright-yellow flowers of the *Hypericum* species: *H. perforatum* (top left),⁵ *H. revolutum* (top right)⁶ and *H. roeperianum* (bottom).

Hypericum species bear capsular fruit (**Figure 2.2**) (most often dry), which splits open to release a copious amount of cylindrically shaped yellow-brown/purple-brown seeds.³ In some *Hypericum* species the capsular fruit is fleshy.⁴



Figure 2.2 Capsular fruit of *H. perforatum*.⁵

2.2 Origins of the Name *Hypericum*

Some attempts were made to derive the meaning of *Hypericum* from hypo or hyper-ericum suggesting that the name means beneath or above the heath. However, the name *Hypericum* is thought to have originated from the ancient Greeks who named the plant $\nu\pi\epsilon\rho\epsilon\iota\kappa\omicron\nu$ ($\nu\pi\epsilon\rho$ meaning ‘above’ and $\epsilon\iota\kappa\omicron\nu$ meaning ‘image’).³ This name arose as the ancient Greeks used *Hypericum* species to hang on their religious images to ward off evil spirits.^{3,7} In other regions the herb was referred to as *fuga daemonum* which translates to “make the demons flee”. Evil presence was believed to be most intense on Midsummers Eve (23rd June – celebration of the pagan feast) and Halloween (31st October). On these days of the year the Greeks emphasized the importance of safeguarding their religious images and homes by decorating them with *Hypericum*.³

The name St. John’s Wort, which refers to the *Hypericum* species in general, but in most cases specifically to *H. perforatum*, came about during the Middle Ages when the pagan feast was Christianized and dedicated to Saint John the Baptist. Traditionally, St. John’s

Wort was used to produce an anointing oil by infusing the herb in olive oil for a number of days. This anointing oil was blood red in colour and allegedly symbolized the blood of the saint.⁸ In Old English the word “wort” meant “plant”.⁸ Today St. John’s Wort (more correctly Common St. John’s Wort) refers to *H. perforatum*, which is the species most commonly found in nearly all of Europe.³ The species name ‘*perforatum*’ is believed to be due to the translucent glandular dots present on the leaves of this plant, which when held toward light appear as though the leaves are perforated.⁷

2.3 Traditional Uses of the *Hypericum* genus

The traditional uses of the *Hypericum* species were mainly related to spiritual significance, such as the protection from evil spirits as was previously mentioned.^{3,7} However, many members of the *Hypericum* genus were also used traditionally by ancient communities for treatment of various ailments, thus signifying the medicinal value of this genus.

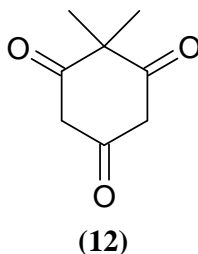
Hypericum has been used therapeutically for thousands of years. A Greek physician and botanist, Dioscorides, described its applications as a diuretic and in the treatment of nerve pain and malaria.⁸ The Japanese used *H. chinense*, indigenous to their country, to treat female abnormalities⁹ and in China, *H. japonicum* was used as a herbal medicine in the treatment of tumours, infectious hepatitis as well as some bacterial diseases.^{10,11} Native Americans used *Hypericum* as an abortifacient, anti-inflammatory, antiseptic, an astringent (substance that decreases the release of blood or mucus by constricting veins and body tissues)¹² as well as in the treatment of kidney problems, nosebleeds, diarrhea, fever and colic.^{8,13,14}

H. perforatum (St. John’s Wort) is considered to have antibacterial, antiviral and anti-inflammatory properties.⁸ In folk medicine this particular *Hypericum* species is recorded to have been used in various parts of the world as a remedy to a broad range of ailments. These include: psychological disorders, insomnia, gastritis, nausea, kidney and respiratory problems, open wounds and burns.^{7,15}

Based on the evidence showing that *Hypericum* has been used medicinally for many centuries and has been recorded in the traditional pharmacopoeia of several nations, its discovery by modern medicine is not unexpected.³

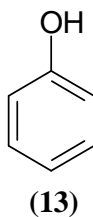
2.4 Biosynthesis of Classes of Compounds within the Genus

Hypericum has been found to be an excellent source of a large range of compounds with the most active being of phenolic nature such as flavonoids, xanthenes, phloroglucinol derivatives and naphthodianthrone structures.⁷ Some compounds have also been found to possess a filicinic acid (**12**) moiety, which is also derived from phloroglucinol.^{16,17}



2.4.1 Phenolic Compounds

Phenolic derivatives are characterized by the possession of one or more hydroxyl (OH) groups attached to an aromatic ring system.¹⁸ Phenol (**13**) is the simplest member of this class and is the substance from which the term “phenolic” is derived.



Most phenolic derivatives originate from one or more of three building blocks, namely, acetyl co-enzyme A (**14**), erythrose-4-phosphate (**15**) and phosphoenol pyruvate (**16**) (**Figure 2.3**).¹⁹ The latter two molecules are both involved in the shikimic acid pathway, whereas the acetate pathway relies on the presence of acetyl co-enzyme A (**14**) as well as malonyl co-enzyme A (activated from of acetyl co-enzyme A).¹⁹

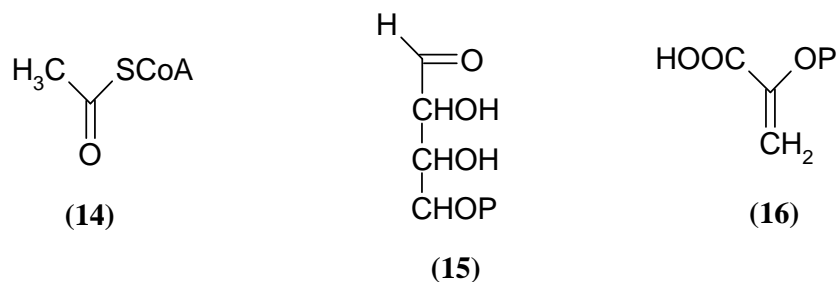
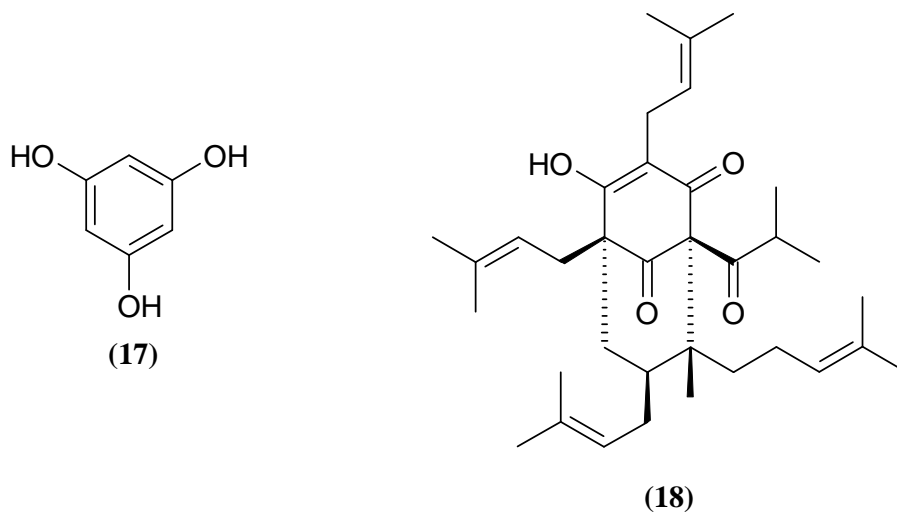


Figure 2.3 Three building blocks of common biosynthetic pathways.

The biosynthesis of phenolic metabolites may be based exclusively on the acetate pathway (e.g. phloroglucinol derivatives and anthrones), solely on the shikimic acid pathway (e.g. coumarins) or may involve a combination of these two pathways (e.g. flavonoids and xanthenes).^{19,20} The biosynthesis of some phenolic metabolites will be reviewed in greater depth within this chapter.

2.4.2 Phloroglucinol Derivatives

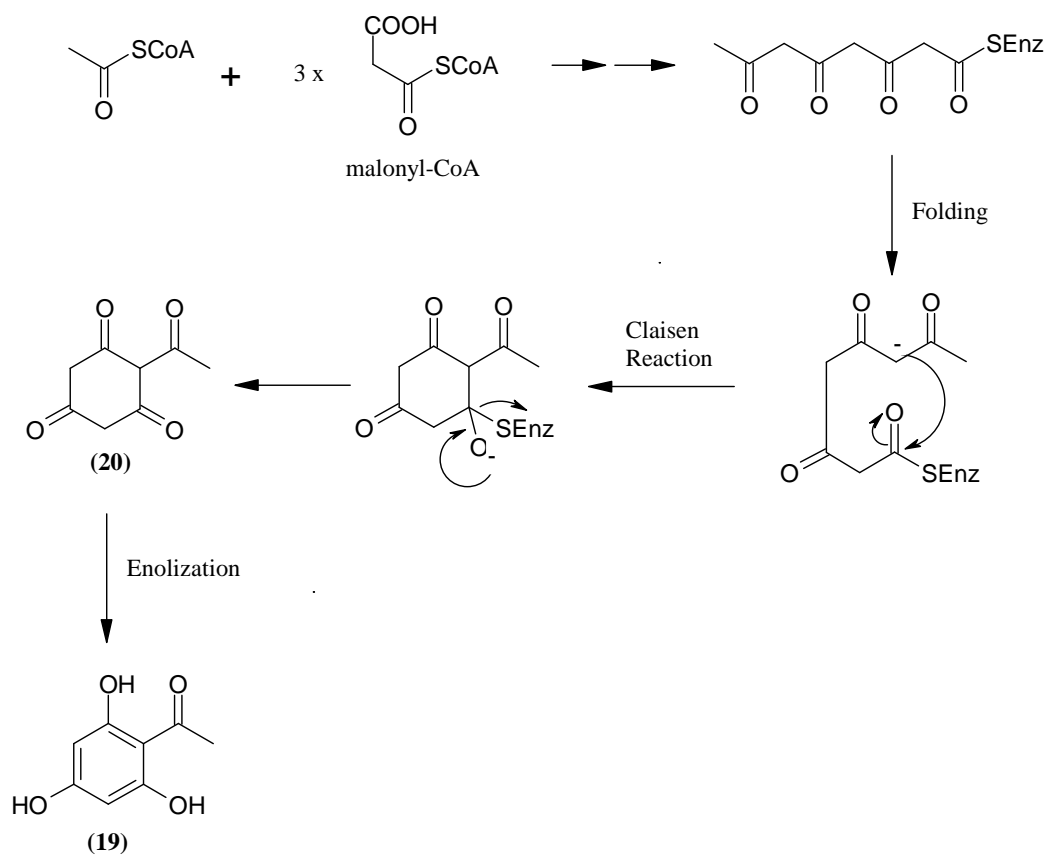
Phloroglucinol derivatives are derived from 1,3,5-trihydroxybenzene (**17**). Hyperforin (**18**)¹⁵, isolated from *H. perforatum*, is a phloroglucinol derived structure that has expanded into a bicycle [3,3,1]nonaendionol, which is substituted with a number of isoprene units.²¹



Biosynthetically this class of compound arises solely from the acetate pathway.¹⁹ The building block, acetyl co-enzyme A (**14**), polymerizes to afford a chain of carbon atoms

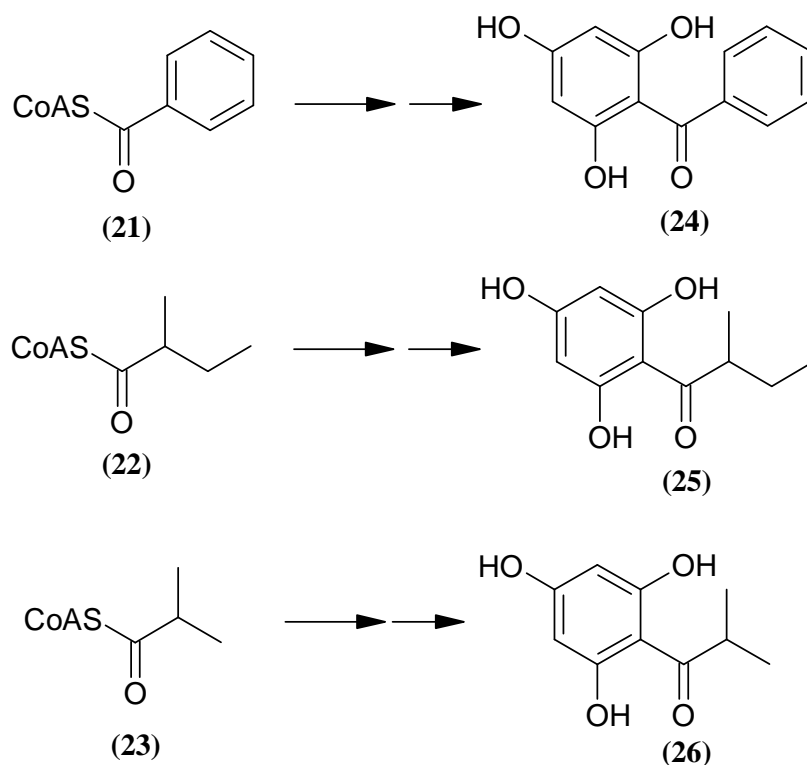
where every alternate carbon has a keto function. For this reason the acetate pathway may often be referred to as the polyketide pathway. In order for polymerization to occur, acetyl co-enzyme A (**14**) is first converted into its more active form, malonyl co-enzyme A. This is achieved through the addition of CO_2 , which is available to the plant through photosynthesis. On condensation of the two molecules, the added CO_2 is released.¹⁹

Scheme 2.1 shows the biosynthetic pathway of phloroacetophenone (**19**), where folding of the polyketide chain allows cyclization to occur *via* a Claisen reaction. This involves the elimination of the thiol-leaving group, which in turn results in the formation of cyclohexatrione (**20**). This intermediate transforms into the more stable aromatic form by keto-enol tautomerism affording the final product, phloroacetophenone (**19**), which possesses oxygen atoms on alternate carbons. This distinctive oxygenation pattern is indicative that the metabolite is acetate-derived.²⁰



Scheme 2.1 Biosynthesis of phloroacetophenone (**19**).²⁰

Other acylphloroglucinols may be formed by changing the starter unit, e.g. the use of benzoyl-CoA (**21**), isovaleryl-CoA (**22**) or isobutyryl-CoA (**23**) in place of acetyl-CoA (**14**) will undergo the same mechanism to form 2,4,6-trihydroxybenzophenone[phenyl-(2,4,6-trihydroxyphenyl)methanone] (**24**), 2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**25**) 2-methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**), respectively (Scheme 2.2).^{22,23}



Scheme 2.2 Biosynthesis of acylphloroglucinols.

2.4.3 Coumarins

Coumarins (**Figure 2.4**) are phenolic compounds exhibiting a C_6C_3 backbone, which is cyclic in nature and referred to as a 2*H*-chromen-2-one system or by its older name 1,2-benzopyrone.^{19,24} These molecules are derived from cinnamic acid *via* the shikimic acid pathway.¹⁹

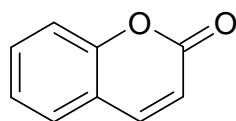
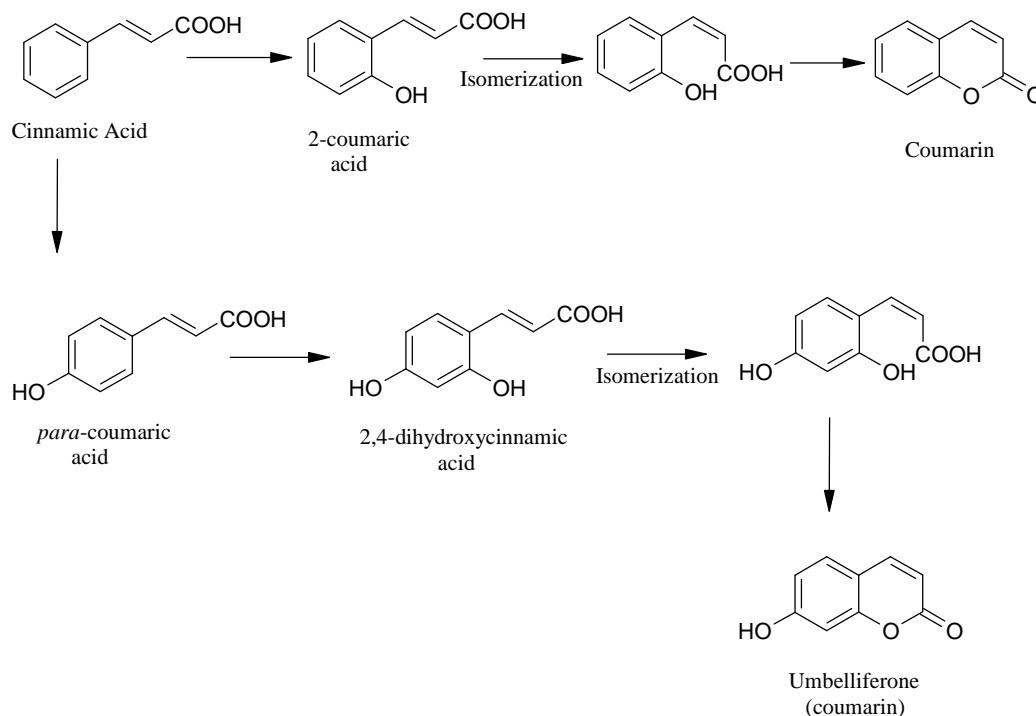


Figure 2.4 Basic structure of coumarins.

Para-coumaric acid is believed to be the significant precursor, which has to undergo two crucial steps in the process of forming coumarins (**Scheme 2.3**).¹⁹ Firstly, the aromatic ring must undergo hydroxylation *ortho* to the side chain to afford 2,4-dihydroxycinnamic acid. The double bond present in the side-chain of this intermediate must then be converted from the *trans*-configuration to the *cis*-configuration.^{19,20} Due to the fact that the *cis*-configuration is less stable, it is thought that this transformation would be unfavourable. Conversely, the conjugated system of cinnamic acid facilitates the isomerisation.²⁰ Once the molecule is in the *cis*-configuration, cyclization can occur between the hydroxyl group and the carboxylic acid resulting in the formation of a lactone and the elimination of water as a by-product.¹⁹



Scheme 2.3 Biosynthesis of coumarins.²⁰

2.4.4 Flavonoids

Flavonoids, found abundantly in the plant kingdom, have a $C_6C_3C_6$ skeleton, where the two aromatic rings (A and B rings) are joined by the C_3 unit, which most often forms the C-ring.^{18,19} Several substructures (**Figure 2.5**) can be formed and are frequently described according to the degree of oxidation in the C-ring. For instance, reduction of the flavones 2,3-double bond results in the formation of a flavanone.^{19,24}

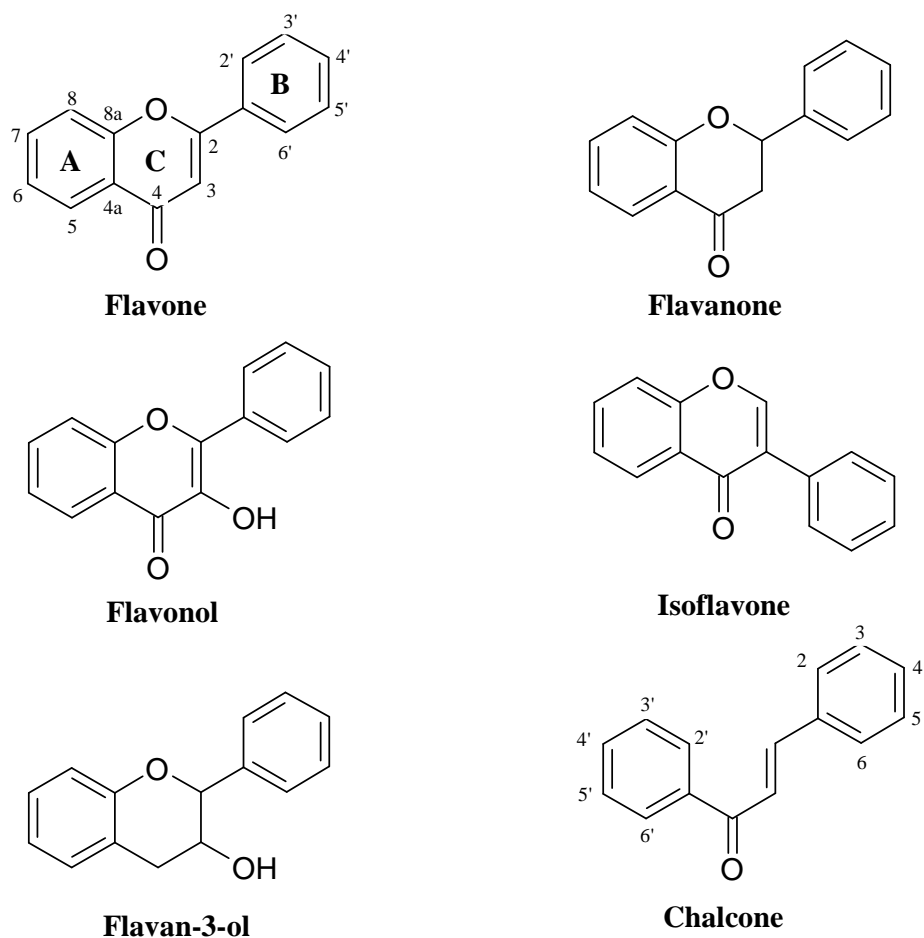
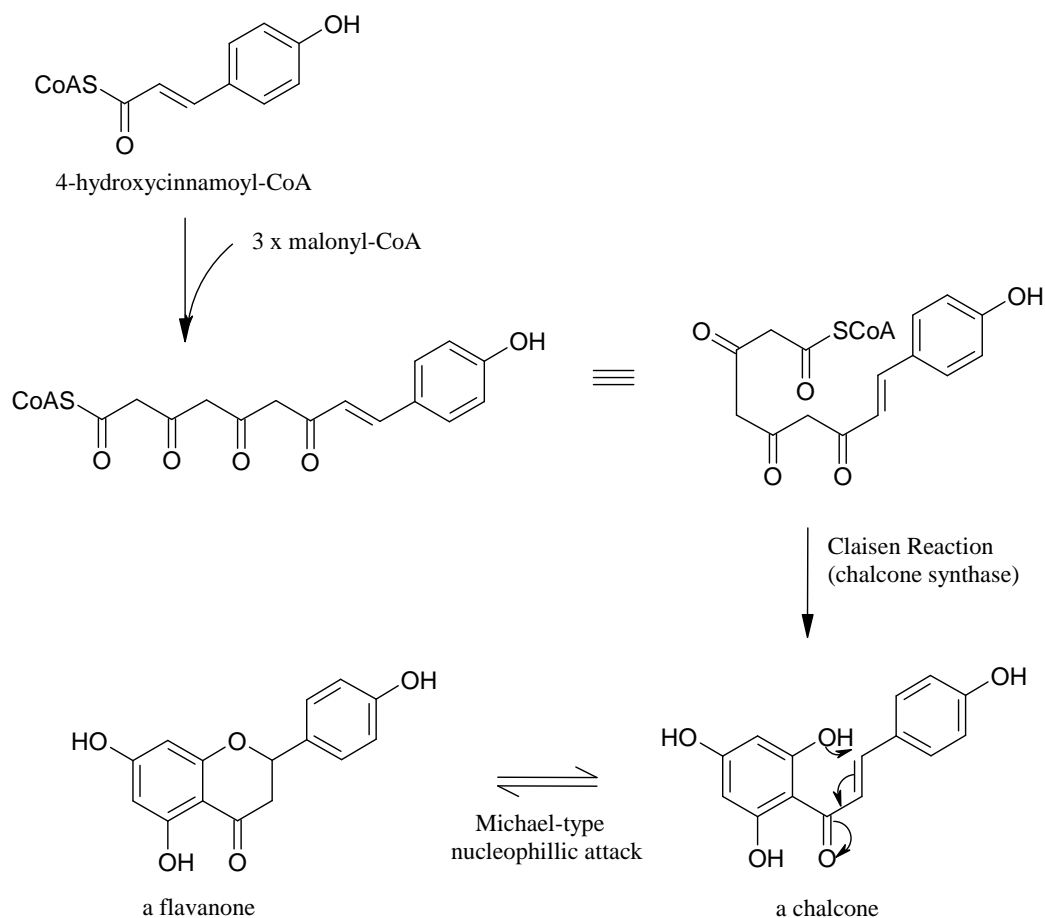


Figure 2.5 Subclasses of flavonoids.²⁵

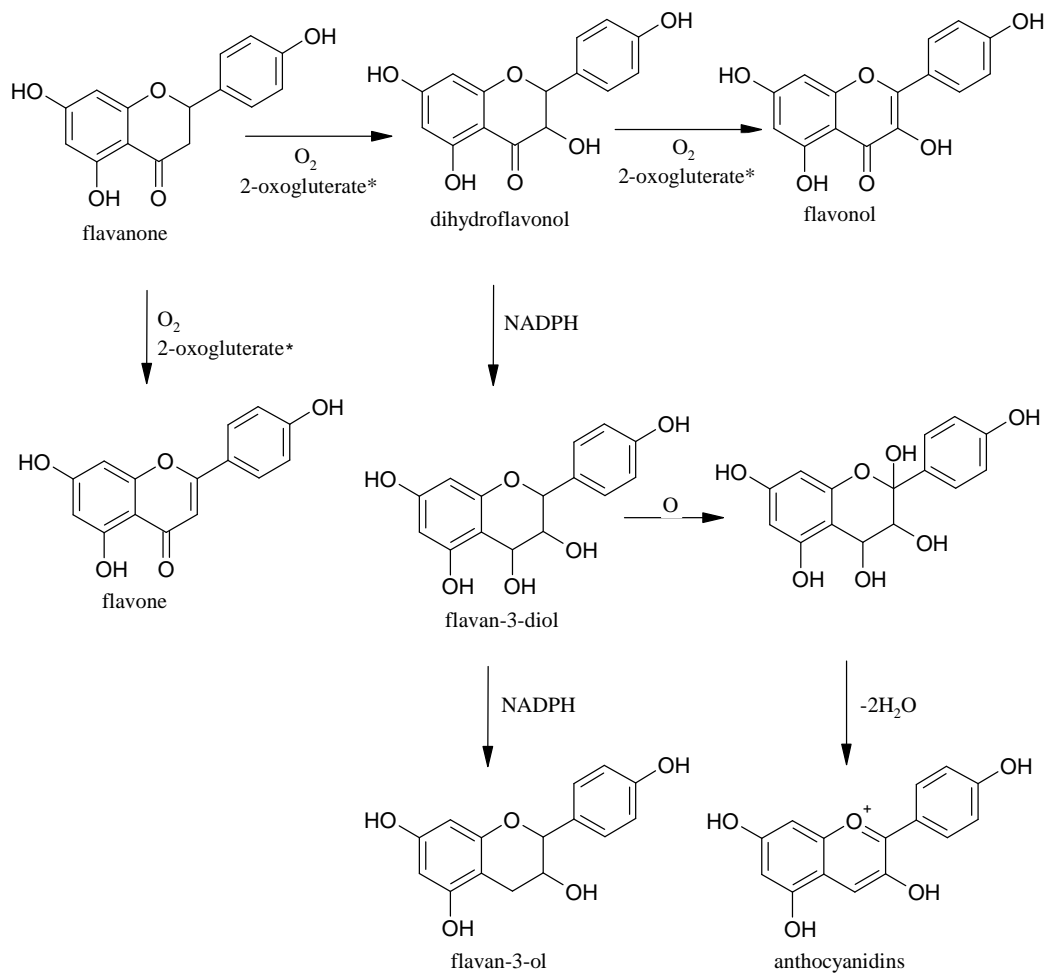
Many phenolic secondary metabolites, of which flavonoids are a good example, arise from the amalgamation of the two previously mentioned pathways (shikimic acid pathway and acetate pathway).²⁰ In the initial biosynthesis of flavonoids, a polyketide is formed from a cinnamoyl-CoA building block (shikimate) where the chain has been extended by the

addition of malonyl-CoA units (acetate) (**Scheme 2.4**). Folding of the polyketide allows a Claisen reaction to occur, which results in the formation of aromatic rings. This step is catalyzed by an enzyme (chalcone synthase) and generates chalcones, which are important precursors for a large number of flavonoid derivatives.²⁰

Flavanones are produced from the chalcone precursor *via* a Michael-type nucleophilic attack of the hydroxyl group on the α,β -unsaturated ketone moiety (**Scheme 2.4**).²⁰ In turn, modifications of the simple flavanone skeleton, generally the pyran-4-one ring, may produce a variety of molecules including flavones, flavonols, anthocyanidins and flavan-3-ols (**Scheme 2.5**).²⁰



Scheme 2.4 Biosynthesis of flavonoid precursors.²⁰

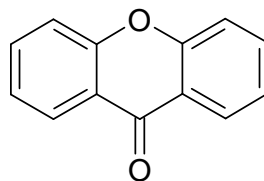


*Various 2-oxoglutarate-dependent oxygenases are required

Scheme 2.5 Modifications of flavanone to form various types of flavonoids.²⁰

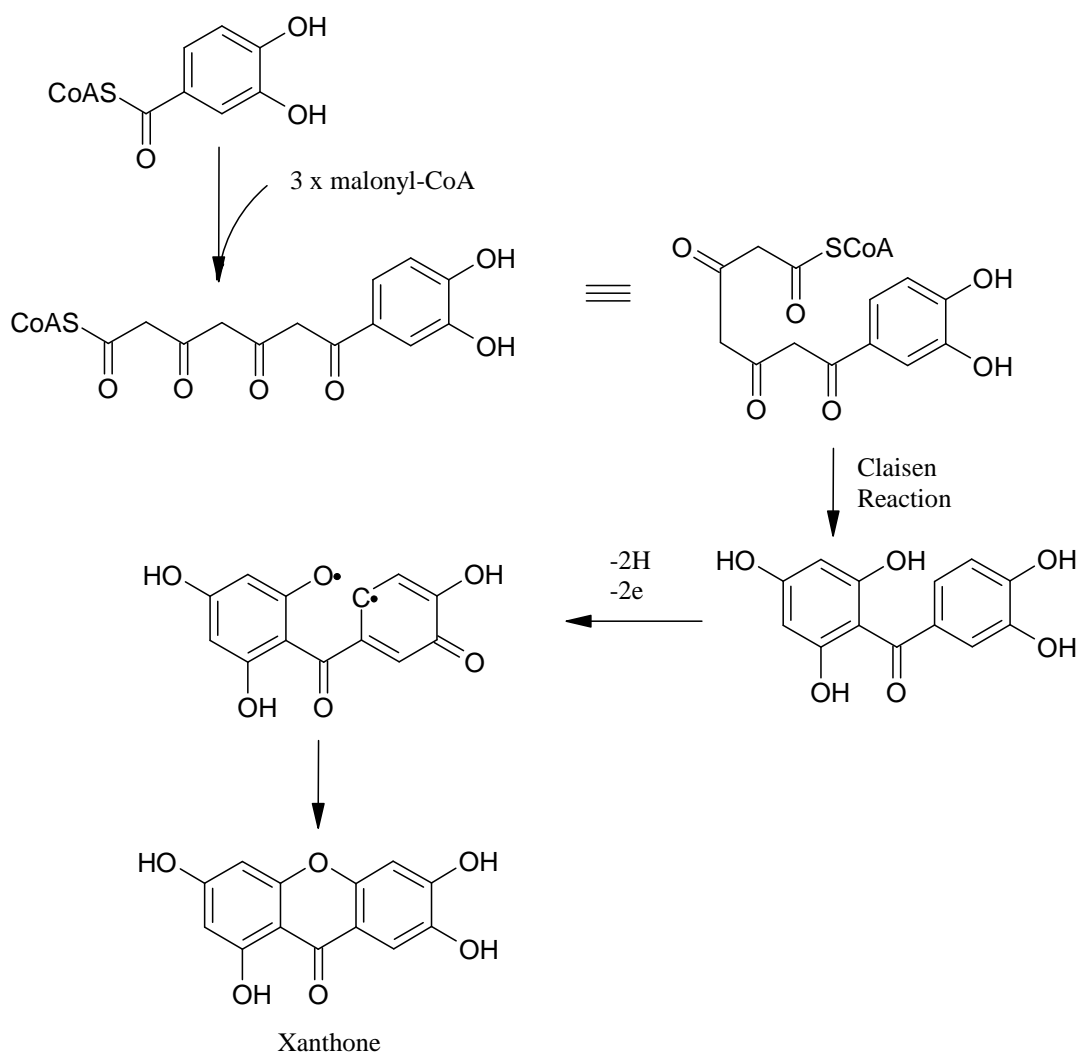
2.4.5 Xanthenes

In chemical terms, the expression xanthone refers to compounds with a $C_6C_1C_6$ carbon skeleton (**27**), where two benzene moieties are linked through a carbonyl group and an oxygen atom. No free rotation of C-C bonds is possible as the rings are joined in a fused formation. Various chemical groups may be attached to this backbone and these substituents define the particular functionalities or properties of the compound.²⁶



(27)

Xanthenes, similarly to flavonoids, are derived from a combination of the shikimate and acetate pathways.¹⁹ A major difference in xanthone biosynthesis (**Scheme 2.6**) is that the shikimate starter unit is of the form C_6C_1 rather than C_6C_3 as evident in the biosynthesis of flavonoids. Besides this difference the biosynthetic mechanism is comparable to that illustrated for flavonoids.¹⁹

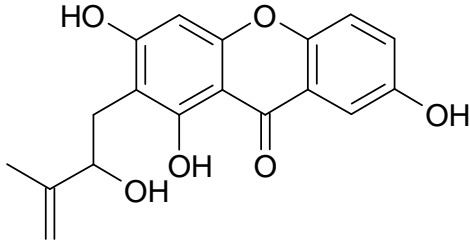
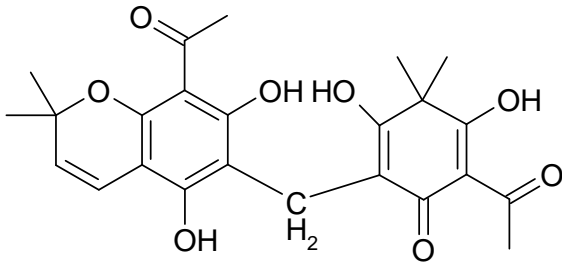
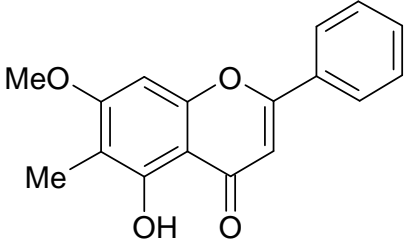


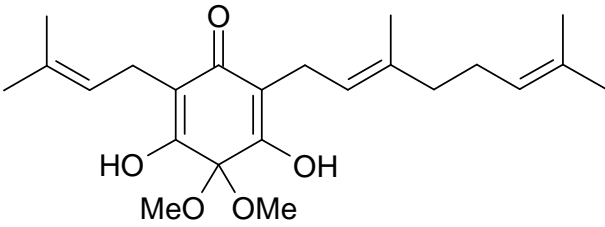
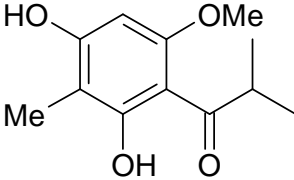
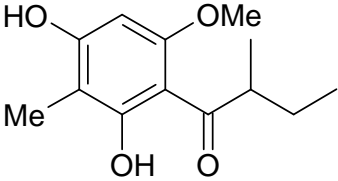
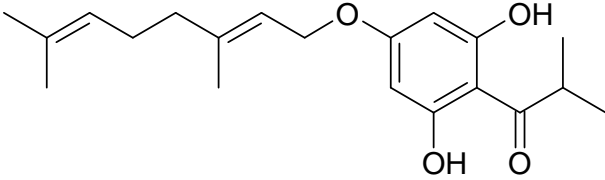
Scheme 2.6 Biosynthesis of xanthenes.¹⁹

2.4.6 Examples of Secondary Metabolites Isolated from *Hypericum* Species

A large range of secondary metabolites have been previously isolated from the *Hypericum* species. Many of the isolated compounds are phenolic in nature and belong to the following classes: flavonoids, xanthenes, phloroglucinol derivatives and acylphloroglucinols. Some secondary metabolites have also been found to possess a filicinic acid moiety.

Table 2.1 Classes of secondary metabolites isolated from *Hypericum* species.

Class of Compound	Compound Structure	<i>Hypericum</i> species isolated from
Xanthone		Leaves of <i>H. chinense</i> ⁹
Filicinic acid derivative		Leaves and stems of <i>H. drummondii</i> ^{17,27}
Flavonoid		Aerial parts of <i>H. ericoides</i> ²⁸

Phloroglucinol derivative		Flowers of <i>H. erectum</i> ²⁹
Acyl-phloroglucinols		Aerial parts of <i>H. beanii</i> ³⁰
		Aerial parts of <i>H. beanii</i> ³⁰
		Roots of <i>H. erectum</i> ²⁹

2.5 Biological Activity Associated with the *Hypericum* Species

The genus *Hypericum* belongs to the Hypericaceae family, which is acknowledged to be a rich source of xanthenes. These xanthenes exhibit various biological activities including: cytotoxic, mutagenic, anti-microbial, anti-tumour and anti-inflammatory effects.^{9,31}

Xanthenes isolated from the roots of *H. roeperianum* exhibited anti-fungal activity against *Candida albicans*.

Phloroglucinol derivatives with biological activity have been isolated from *H. uliginosum* and *H. brasiliense*.¹⁶ The former exhibited anti-fungal activity against *Trichophyton mentagrophytes*, whilst the latter was found to have anti-bacterial activity against *Bacillus subtilis*.¹⁶ Furthermore, the extracts of *H. brasiliense* also showed inhibition of monoamine oxidases (MAO's). The regulation of some physiological amines depends on the MAO enzymes and thus the inhibition of these enzymes is considered to aid the control of depression.¹⁶

Significant activity (values comparable or greater than streptomycin) against *Staphylococcus aureus*, *B. subtilis* and *Mycobacterium smegmatis* was exhibited by the hexane extracts of *H. drummondii*. Isolation of the active compounds showed that the Gram-positive bacterial inhibition was due to filicinic acid derivatives.^{16,17,27}

2.5.1 *Hypericum perforatum*

H. perforatum (St. John's Wort) is by far the most studied species of the *Hypericum* genus. The aerial parts of this plant have been reported to contain: Naphthodianthrones [hypericin (**10**) and pseudohypericin (**11**)], flavonoids, phloroglucinol derivatives [hyperforin (**18**)], coumarins, mono- and sesquiterpenes and phenolic carboxylic compounds. The roots of this species have also been found to contain xanthenes.^{7,32}

2.5.1.1 Hypericin

Hypericin (4,5,7,4',5',7'-hexahydroxy-2,2'-dimethylnaphthodianthrone) (**10**) is a red pigmented anthraquinone derivative, which is one of the major active compounds isolated from *H. perforatum*.⁷ It has also been isolated from other *Hypericum* species, including *H. hirsutum*, *H. maculatum*, *H. nummularium* and *H. triquetrifolium*.⁷ This photochemical compound is found in tiny glands located on the stems, leaves and flowers of the above *Hypericum* species, but is also present in some protozoa and insects.⁷

Hypericin (**10**) is a unique molecule containing both hydrophilic and hydrophobic regions.³² The large conjugated system is responsible for the photochemical properties of the compound. The absorption of UV and visible rays by the chromophore structure may result in photosensitivity if hypericin (**10**) is ingested in large enough amounts.⁸

Originally, hypericin (**10**) was considered to be the active constituent responsible for the antidepressant properties exhibited by St. John's Wort, however, upon further research hyperforin (**18**) was concluded to be the anti-depressant compound.²⁶ Hypericin (**10**) is now often used as a detection standard in *Hypericum* extracts and as a marker compound specifically in *H. perforatum*.^{8,25}

Hypericin (**10**) and chemically similar, pseudohypericin (**11**) have been demonstrated to inhibit numerous retroviruses. This antiviral activity has been tested by *in vitro* and *in vivo* studies, resulting in hypericin (**10**) exhibiting significant inhibition of the replication of viruses such as HIV, influenza A, Epstein-Barr virus, cytomegalo virus, human papilloma virus and herpes.^{8,32}

Mechanistically, the antiviral activity of hypericin (**10**) is unconventional in comparison to other known antiviral agents, as this naphthodianthrone compound inhibits the removal of the lipid sheath surrounding DNA and RNA viruses.³² By doing so, infected cells cannot release replicates of the virus. This mechanism however, limits inhibition by hypericin (**10**) to viruses that contain membranes. Future research of hypericin (**10**) could thus potentially result in the discovery of a new class of anti-HIV drugs.³²

2.5.1.2 Hyperforin

Hyperforin (**18**) is another major constituent of *H. perforatum* and is identified as being the compound associated with the plant's antidepressant effects.¹⁴ Its phloroglucinol-derived structure is chemically incomparable to any other known antidepressant.¹⁴

Biochemical studies indicate that this acylphloroglucinol derivative acts as an antidepressant by hindering the uptake of serotonin (5-HT), dopamine, norepinephrine and gamma-aminobutyric acid (GABA). These neurotransmitters are responsible for conveying messages to and from the brain. The latter neurotransmitter, GABA, is involved

with the decrease of anxiety and the enhancement of relaxation. Inhibition of these neurotransmitters results in accumulation of neurotransmitter levels in the brain, which in turn improves moods and re-establishes emotional stability.³³

Other than its effectiveness as an antidepressant, hyperforin (**18**) also possesses antibacterial activity against penicillin- and methicillin-resistant *Staphylococcus aureus* (MRSA) strains with outstanding MIC values of 0.1-1 $\mu\text{g ml}^{-1}$.¹⁵

This chapter gave an overview of the genus *Hypericum*, discussing various aspects of the genus. The subsequent chapter covers the natural product section of our research, where we introduce *H. roeperianum* and present the findings of our phytochemical investigation of this plant.

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CHAPTER 3

A Phytochemical Investigation of *Hypericum roeperianum*

3.1 Introduction

3.1.1 Description and Distribution

H. roeperianum Schimp. ex. A. Rich., (referred to as 'Isivumelwane' in Zulu) is one of the six *Hypericum* species indigenous to South Africa and occurs as a shrub or small tree ranging from 0.6 to 5 m in height (**Figure 3.1**).¹ As the stems age they become woody and flatten near the inflorescence. This plant gives rise to bright-yellow flowers, which usually possess a number of dark marginal dots and consist of five petals.^{2,3}

This plant grows in central, eastern and south tropical Africa, located in evergreen forests and bushland^{4,5}. It is commonly found growing near rivers or streams, usually at an altitude ranging between 1500 m and 2900 m above sea level.²

Medicinally a root decoction of this particular species is drunk, either alone or in combination with various other plants, to cure female sterility. However, nothing is known about the active constituents of the plant.^{4,5}

3.1.2 Previous Research on *Hypericum roeperianum*

Research performed previously on *H. roeperianum* involved the isolation and characterization of ten xanthenes (**Figure 3.2**) from the DCM extract of the roots of the plant.⁴ Spectroscopic techniques and chemical methods enabled the structures to be identified as 2-hydroxyxanthone (**28**), 5-hydroxy-2-methoxyxanthone (**29**), 1,5-dihydroxy-2-methoxyxanthone (**30**), 2-deprenylrheediexanthone B (**31**), isojacareubin (**32**), 1,6-dihydroxy-5-methoxy-4',5'-dihydro-4',4',5'-trimethylfurano[2',3':3,4]xanthone (5-*O*-methyl-2-deprenylrheediexanthone B) (**33**), 1,6-dihydroxy-5-methoxy-3',3'-dimethyl

pyrano[2',3':3,4]xanthone (5-*O*-methylisojacareubin) (**34**), calycinoxanthone D (**35a**), 1,3,5,6-tetrahydroxy-4-*trans*-sesquilavandulylxanthone (**35b**) and 5-*O*-demethyl paxanthonin (**36**).



Photo: K. Smith

Figure 3.1 *H. roeperianum* tree/shrub (top left) with a close-up view of the woody stem (top right) and opposite, simple leaves (bottom left). The characteristic bright-yellow flowers are shown on the bottom right.

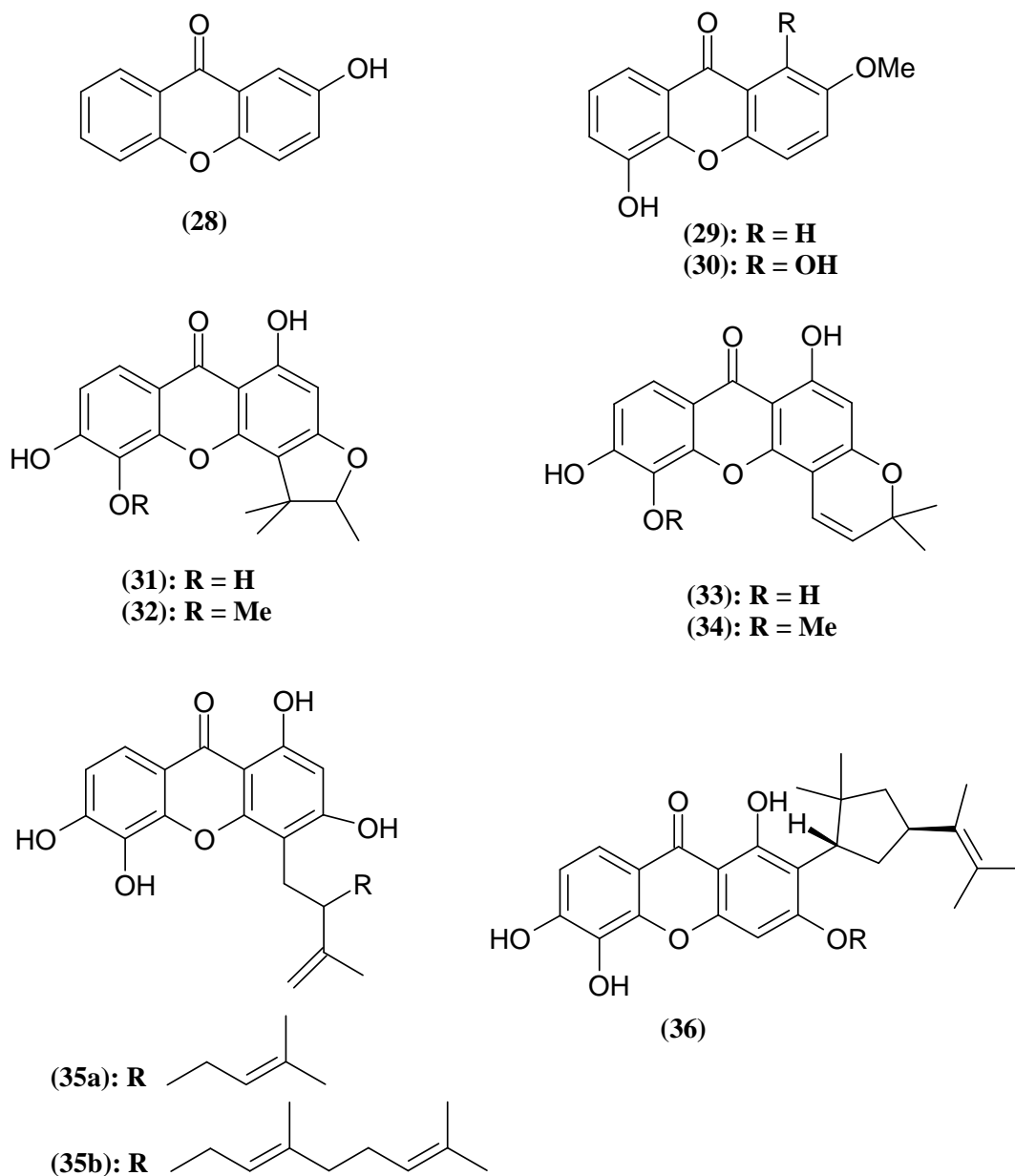
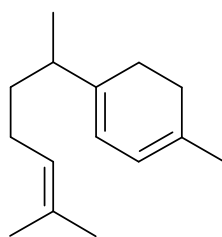


Figure 3.2 Xanthenes isolated from *Hypericum roeperianum*.⁴

Xanthenes **31** - **37** all possess a 1,3,5,6-oxygenation pattern, which is commonly found within the *Clusiaceae* family and compounds **31**, **32**, **34**, **35a**, **35b** and **36** were found to exhibit antifungal activity against *Candida albicans*.⁴

Crockett *et al.* analyzed the volatile constituents of the aerial parts of five *Hypericum* species.⁵ This resulted in the discovery that the major volatile constituent of

H. roeperianum is γ -curcumene (**37**).^{5,6} To the best of our knowledge no other previous work has been reported on the isolation of compounds from the aerial parts of *H. roeperianum*. However, a number of compounds have been isolated from the leaves and stems of other *Hypericum* species. *H. chinense* afforded xanthenes from the MeOH extracts; flavonoids, naphthodianthrones and phloroglucinol derivatives were isolated from the EtOAc extracts of *H. perforatum* and prenylated phloroglucinol derivatives were isolated from the aerial parts of *H. sampsonii*.^{7,8}



(37)

As indicated, the *Hypericum* genus has been found to be an excellent source of a large range of compounds, many of which exhibit useful biological activities.⁴ Hence, interest in this particular genus has recently increased and future research may result in the discovery of potential drugs for various diseases and conditions. For this reason, *H. roeperianum* was considered to be a suitable choice of plant to undergo phytochemical investigation. This selection was further validated by the fact that minimal research has previously been carried out on the aerial parts of this particular *Hypericum* species.

3.2 Results and Discussion

H. roeperianum was chosen for investigation on the basis that if a natural product is found to exhibit interesting therapeutic properties, it is often possible that another species belonging to the same genus (in the case of *Hypericum*) or family (Clusiaceae/Guttiferae/Hypericaceae) may contain similar compounds of equivalent or increased activity.

The current phytochemical investigation* resulted in the isolation of two known molecules (**Figure 3.3**): 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4*H*-pyran-4-one (**38**), and 3-geranyl-2,4,6-trihydroxybenzophenone {[3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone} (**2**). A third compound was also isolated and is provisionally assigned as 1-methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**). However, the small amount of compound isolated did not provide sufficient data for a conclusive structural assignment.

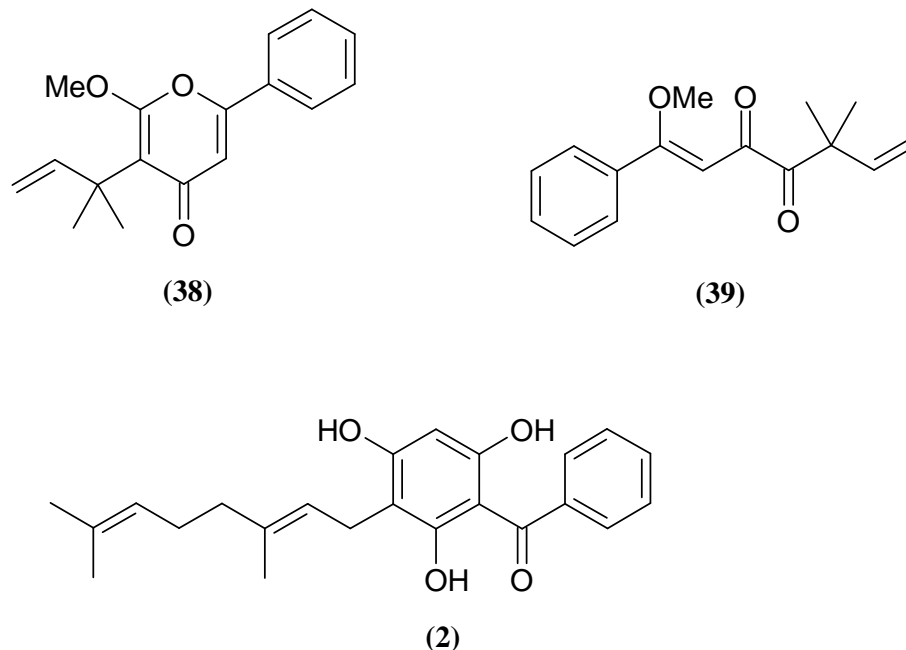
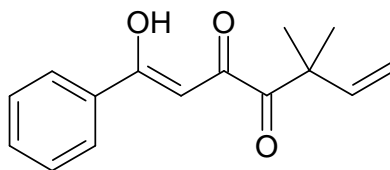


Figure 3.3 Isolated compounds.

Compound **38** was previously isolated from the aerial parts of *Hypericum mysorense* and was named hyperanone A.⁹ To the best of our knowledge **39** has not been previously isolated, however, a compound with a similar structure has been isolated from *Hypericum mysorense* HEYNE.¹⁰ The compound isolated by Kikuchi *et al.*¹⁰ was named mysorenone-C (**40**) and only differs from **39** by the presence of a hydroxyl group where **39** contains a methoxy moiety.

* The phytochemical investigation of *H. roeperianum* was started by S. Mamode as an honours project in the School of Chemistry. Although she has isolated some of the compounds, she has not determined the structures of any of the compounds.



(40)

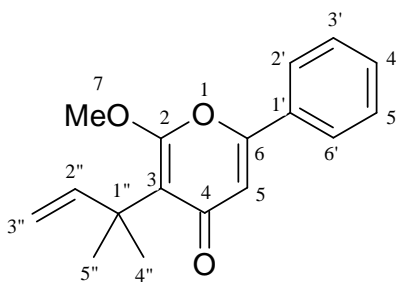
Compounds **38** and **39** exhibit very similar ^1H NMR spectra. In fact, the ^1H NMR spectra show such a close resemblance that at first glance the spectra seem to be of the same compound. When taking a closer look, it becomes evident that there are slight differences in the chemical shifts of certain signals (these will be described in greater depth in the structural elucidation of the two isolated metabolites (**Section 3.2.1.1**)).

3.2.1 Structural Elucidation of Isolated Compounds

The structures of the isolated metabolites were deduced from NMR spectroscopy (^1H , ^{13}C , DEPT90, DEPT135, COSY, HSQC and HMBC experiments), UV-visible spectroscopy, IR-spectroscopy and electrospray mass spectrometry (ESIMS).

3.2.1.1 Structural Elucidation of compounds 38

Refer to the NMR data for **38** (**Plates 1.1 – 1.7 and Table 3.1**).



(38)

The ^1H NMR spectrum of **38** revealed two multiplets at δ_{H} 7.70 and δ_{H} 7.48, two doublets at δ_{H} 4.92 and δ_{H} 4.89, a doublet of doublets at δ_{H} 6.23 and three singlet at δ_{H} 6.61, δ_{H} 4.06 and δ_{H} 1.50.

The multiplets appearing downfield δ_{H} 7.48 (3H, m) and δ_{H} 7.70 (2H) are characteristic of protons in an aromatic moiety. The integration of these signals gives evidence that compound **38** contains a mono-substituted aromatic ring. The multiplet integrating for 3H corresponds to the protons in the *meta* and *para* positions, whilst the signal at δ_{H} 7.70 integrates for 2H and is assigned to the protons in the *ortho* positions of the aromatic ring. The signals observed at δ_{C} 125.5, δ_{C} 129.1 and δ_{C} 130.9 in the ^{13}C NMR spectrum were assigned as the *ortho*, *meta* and *para* methine groups, respectively.

The UV spectra of compound **38** showed a maximum absorption at 277 nm ($\log \epsilon$ 3.63), which confirms that a conjugated aromatic chromophore is present within the structure.

The two doublets and the doublet of doublets observed in the ^1H NMR spectrum suggest the presence of an alkene. The correlation of these signals to a methylene and methine carbon in the ^{13}C NMR spectrum as well as the integration of the signals in the ^1H NMR spectrum implies that the alkene is terminal (i.e. a vinyl moiety).

The ^1H NMR spectrum shows the doublet of doublet appearing at δ_{H} 6.23 (1H, $J = 10.7$ and 17.6 Hz, correlating to δ_{C} 148.3). The coupling constant of 10.7 Hz is characteristic of coupling between two *cis*-olefinic protons. The doublets resonating at δ_{H} 4.89 and δ_{H} 4.92 have coupling constants of 10.8 Hz and 17.5 Hz, respectively and correlate to δ_{C} 108.3 in the ^{13}C NMR spectrum. As already mentioned, the former coupling constant is characteristic of coupling between two *cis*-olefinic protons. The latter coupling constant (17.5 Hz) is commonly observed in the coupling of two *trans*-olefinic protons. This information leads us to conclude that the alkene present within the structure is most likely of the following type (**Figure 3.4**):

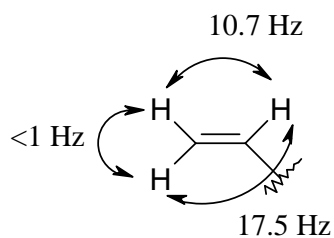


Figure 3.4 Type of alkene present in the structure of **38**.

The upfield singlet observed in the ^1H NMR spectrum at δ_{H} 1.50 correlated to the signal at δ_{C} 27.5 in the ^{13}C NMR spectrum. The DEPT90 and DEPT135 data enabled the singlet to be assigned as a methyl group. The signal in the ^1H NMR spectrum integrates for 6H suggesting that there are two overlapping methyl moieties present within the structure. The sharp singlet at the δ_{H} 4.06 (3H, correlating to δ_{C} 56.0 in the HSQC) occurs in the region characteristic of methoxy groups.

The signals of the ^{13}C NMR spectrum for **38** appearing downfield at δ_{C} 181.2 and δ_{C} 162.7 signify the inclusion of a carbonyl functional group, specifically a ketone, within the structure as well as the presence of an olefinic carbon attached to a methoxy group.

The interpretation of the acquired data, reveals that the following fragments appear in the structures of **38** (Figure 3.5):

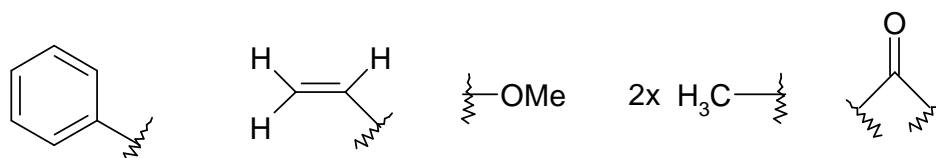


Figure 3.5 Fragments of compounds **38**.

Conclusions were drawn based on the connection of the fragments, which was established by the long range C-H couplings exhibited by the HMBC spectroscopy experiments. The spectral data therefore illustrated that **38** was 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4*H*-pyran-4-one (hyperanone A).

The ESIMS $[\text{M}+\text{H}]^+$ data obtained for compound **38** showed a molecular ion peak at m/z 271.331 $[\text{M}+\text{H}]^+$, which was in agreement with a calculated value of 271.1334 for $\text{C}_{17}\text{H}_{19}\text{O}_3$. This evidence as well as the relatively good correlation of **38** in comparison to the experimental data reported by Kikuchi *et al.*⁹ further substantiated our concluded structural elucidation.

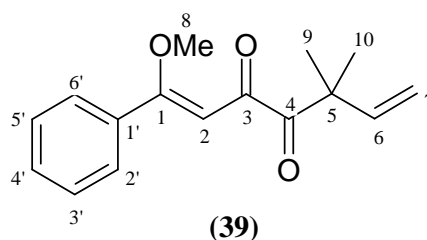
Table 3.1 NMR data of compound **38**.

Compound 38			
Carbon	C	H (Int., Mult., J/Hz)	Assignment
2	162.7		Carbon in pyrone ring on which the methoxy group is attached
3	111.6		Carbon in pyrone ring on which the carbon side chain is attached
4	181.2		Carbonyl C
5	111.3	6.61 (1H, s)	CH in pyrone ring
6	156.8		Carbon in pyrone ring on which the aromatic ring is attached
7	56.0	4.06 (3H, s)	Methoxy group
1'	130.9		Aromatic carbon attached to pyrone ring
2', 6'	125.2	7.70(2H, m)	2 x CH in <i>ortho</i> positions of aromatic ring
3', 5'	129.1	7.48 (3H, m)	CH's in <i>meta</i> positions of aromatic ring
4'	130.9	7.48 (3H, m)	CH in <i>para</i> position of aromatic ring
1''	38.8		Quaternary carbon in alkyl chain
2''	148.3	6.23 (1H, dd, $J = 10.7, 17.5$)	CH of alkene
3''	108.3	4.89 (1H, d, $J = 10.8$) 4.92 (1H, d, $J = 17.5$)	CH ₂ of the alkene
4'', 5''	27.5	1.50 (6H, s)	2 x methyl groups on alkyl chain

3.2.1.2 Provisional Structural Elucidation of Compound 39

Refer to the NMR data for **39** (Plates 2.1 – 2.7 and Table 3.2).

Due to the small amount (2 mg) of compound **39** isolated, the NMR data was obtained from a very dilute solution resulting in poor quality spectra, thus making the signals difficult to assign and hence only a provisional structural assignment was possible.



As in compound **38**, the ^1H NMR spectrum for compound **39** exhibited two multiplets (δ_{H} 7.60 and δ_{H} 7.41), two doublets (δ_{H} 5.27 and δ_{H} 5.24), a doublet of doublets (δ_{H} 6.17) and three singlets (δ_{H} 6.31, δ_{H} 3.75 and δ_{H} 1.48).

The multiplets at δ_{H} 7.60 (2H) and δ_{H} 7.41 (3H) were respectively assigned to be the methine groups in the *ortho* positions and *meta* and *para* positions of a mono-substituted aromatic ring. The HSQC showed these signals correlating to δ_{C} 126.0 (*ortho* positions), 129.0 (*meta* positions) and δ_{C} 131.3 (*para* position) in the ^{13}C NMR spectrum. The presence of the aromatic ring was confirmed by the UV data which showed a maximum absorption band at 276 nm ($\log \epsilon$ 3.46). The band occurring at this wavelength is characteristic of a conjugated aromatic chromophore.

The two doublets and the doublet of doublets observed in the ^1H NMR spectrum suggest the presence of an alkene. The correlation of these signals to a methylene (δ_{C} 116.2) and methine (δ_{C} 140.1) carbon in the ^{13}C NMR spectrum as well as the integration of the signals in the ^1H NMR spectrum implies that the alkene is terminal (i.e. a vinyl moiety).

The ^1H NMR spectrum shows the doublet of doublets appearing at δ_{H} 6.17 (1H, $J = 10.6$ and 17.8 Hz). The doublets resonating at δ_{H} 4.89 and δ_{H} 4.92 have coupling constants of

10.8 Hz and 17.5 Hz. As previously mentioned, these coupling constants are characteristic of coupling between two *cis*-olefinic or *trans*-olefinic protons. This information leads us to conclude that a terminal alkene is present within the structure of the compound **39**.

The singlet appearing at δ_{H} 1.48 in the ^1H NMR spectrum integrated for 6H and was assigned to two methyl moieties (DEPT90 and DEPT135 showed that this signal was a methyl group). The HSQC showed a correlation between the singlet at δ_{H} 1.48 in the ^1H NMR spectrum and δ_{C} 23.5 in the ^{13}C NMR spectrum, whilst the HMQC spectrum showed long-range C-H coupling between the singlet at δ_{H} 1.48 and the following signals in the ^{13}C NMR spectrum: δ_{C} 140.1, δ_{C} 48.6 and δ_{C} 195.5. The latter two signals are not observed in the ^{13}C NMR spectrum as the sample was too dilute, but δ_{C} 48.6 is assigned as the quaternary carbon to which the two methyl groups are attached and the signal at δ_{C} 195.5 is assigned as a carbonyl functional group. The fact that the methyl moieties link to δ_{C} 140.1 suggests that the methyl groups are in close proximity to the methine group of the terminal alkene.

The sharp singlet at the δ_{H} 3.75 (3H, correlating to δ_{C} 51.7 in the HSQC occurs in the region characteristic of methoxy groups. The methoxy moiety correlates to δ_{C} 163.8 in the HMBC. This signal is assigned as the quaternary carbon to which the methoxy group is attached.

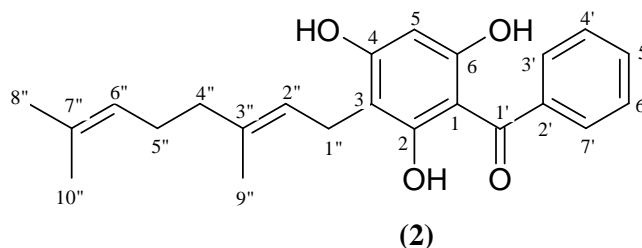
The spectral data allowed **39** to be provisionally assigned as 1-methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione. In order to achieve a conclusive elucidation of **39**, the compound would need to be re-isolated to obtain a greater amount and hence attain better spectral data with which to work with.

Table 3.2 NMR data of compound 39.

Compound 39			
Carbon	C	H (Int., Mult., J/Hz)	Assignment
1	163.8		Carbonyl on which the methoxy group is attached
2	106.8	6.31 (1H, s)	CH of alkene adjacent to a carbonyl C
4	195.5		Carbonyl groups
5	48.6		Quaternary carbon in alkyl chain, to which two methyl groups are attached
6	140.1	6.17 (1H, dd, $J = 10.4, 17.4$)	CH of terminal alkene
7	116.2	5.24 (1H, d, $J = 10.8$) 5.27 (1H, d, $J = 17.5$)	CH ₂ of the alkene
8	51.7	3.75 (3H, s)	Methoxy group
9, 10	23.5	1.48 (6H, s)	2 x methyl groups on alkyl chain
2', 6'	126.0	7.60 (2H, m)	2 x CH in <i>ortho</i> positions of aromatic ring
3', 5'	129.0	7.41 (3H, m)	CH's in <i>meta</i> and <i>para</i> positions of aromatic ring
4'	131.3	7.41 (3H, m)	CH's in <i>meta</i> and <i>para</i> positions of aromatic ring

3.2.1.3 Structural Elucidation of Compound 2

Refer to the NMR data for **2** (Plates 3.1 – 3.6 and Table 3.3).



The ^1H NMR spectrum revealed a multiplet at δ_{H} 7.58 (5H) indicating that an aromatic group is present in the structure. This is further substantiated by the appearance of the signals in the ^{13}C NMR spectrum resonating in the δ_{C} 125 – 135 region. The integration observed in the ^1H NMR spectrum suggests that the aromatic substituent is mono-substituted. The existence of alkenes was established by the presence of signals resonating at δ_{H} 5.27 (1H, t, $J = 7.0$ Hz) and δ_{H} 5.05 (1H, t, $J = 7.0$ Hz) in the ^1H NMR spectrum, which correlated to the signals at δ_{C} 123.7 and δ_{C} 121.5 in the ^{13}C NMR spectrum, respectively.

The signal in the ^{13}C NMR spectrum appearing downfield at δ_{C} 197.7 is characteristic of a ketone (C-7), whilst the quaternary carbon observed at δ_{C} 170.9 is commonly assigned to carbon atoms attached to hydroxy groups within a phenolic ring (C-2, C-4, C-6). A singlet was also observed at δ_{H} 10.31, which was assigned to the hydroxyl hydrogens. Hydroxyl groups that are hydrogen bonded characteristically appear in this region as they are highly deshielded by the close proximity of the electronegative atom. In the structure of compound **2** the hydrogen bonding occurs between the hydrogen atoms of the phenol groups and the oxygen atom of the carbonyl moiety. The three singlet three-proton peaks (δ_{H} 1.80, δ_{H} 1.67 and δ_{H} 1.59) resonating furthest upfield in the ^1H NMR spectrum, are due to the presence of three methyl groups.

From the above information we can conclude that each of the following fragments (**Figure 3.6**) form part of the overall structure of compound **2**.

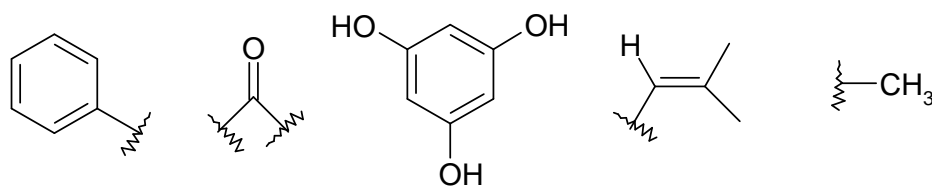


Figure 3.6 Fragments of compound **2**.

The assignments of the chemical shifts of the protonated carbons were allocated on the basis of HSQC and COSY NMR spectroscopy experiments.

The COSY experimental data shows that the triplet at δ_{H} 5.27 couples to the doublet at δ_{H} 3.38 (2H, $J = 7.0$ Hz), as well as to the broadened singlet at δ_{H} 1.80, which has been assigned as a methyl group. This indicates that the proton atoms responsible for the appearance of these three signals are in close proximity to one another.

The NMR experiments therefore indicate that compound **2**, isolated from *H. roeperianum*, is 3-geranyl-2,4,6-trihydroxybenzophenone.

The UV-visible spectrum of **2** exhibited a maximum absorption band at 309 nm ($\log \epsilon$ 3.68). This is indicative that a conjugated system is incorporated in the structure of the compound. The IR spectrum of **2** showed a prominent, sharp peak at 1710 cm^{-1} , which is characteristic of absorption by a ketone moiety. The existence of phenol groups is evident by the appearance of a broad band at 3316 cm^{-1} . The peak at 1605 cm^{-1} suggests that compound **2** contains a carbon-carbon multiple bond, more specifically an alkene. The peak at 700 cm^{-1} substantiates the presence of an alkene as peaks observed in this region are characteristic of the absorption arising from carbon-hydrogen bending vibrations of an alkene. Thus, the information retrieved from the UV-visible data and IR spectra validates that the compound isolated from *Hypericum roeperianum* is 3-geranyl-2,4,6-trihydroxybenzophenone {[3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl] phenylmethanone} (**2**) as was elucidated from the NMR data. The electrospray mass spectrometry data further confirms this structure, as a molecular ion peak was observed at m/z 365.1758 [M-H]⁻, which was in agreement with the calculated value for $\text{C}_{23}\text{H}_{25}\text{O}_4$.

Table 3.3 NMR data of compound 2.

Carbon	C	H (Int., Mult., J/Hz)	Assignment
1	104.6		Carbon on phenolic ring to which the acyl group is attached
2, 4, 6	170.9		Carbons attached to phenol groups
3	108.5		Carbon on phenolic ring to which the alkyl chain is attached
5	96.3	5.94 (1H, s)	CH in phenolic ring
1'	197.7		Carbonyl C
2'	140.1		Carbon of aromatic ring
3', 4', 5', 6', 7'	127.9- 129.1	7.62 (5H, m)	Aromatic protons
1''	21.5	3.38 (2H, d, $J = 7.0$)	CH ₂ in alkyl chain, adjacent to phenolic ring
2''	121.5	5.27 (1H, t, $J = 7.0$)	CH of alkene
3''	135.1		Quaternary C in alkyl chain on which methyl group is attached
4''	39.7	2.05 (2H, m)	CH ₂ in centre of chain
5''	26.4	2.08 (2H, m)	CH ₂ adjacent to terminal alkene of chain
6''	123.7	5.05 (1H, t, $J = 6.9$)	CH of terminal alkene
7''	132.2		Quaternary C in alkyl chain on which terminal methyl groups are attached
8''*	25.6	1.67 (3H, s)	Terminal methyl group
9''	16.2	1.80 (3H, s)	Methyl group on alkene closest to phenolic ring
10''*	17.7	1.59 (3H, s)	Terminal methyl group

* Assignment may be interchanged

3-Geranyl-2,4,6-trihydroxybenzophenone (**2**) was previously isolated from *Tovomita krukovii*, *T. longifolia*, *Helichrysum monticola*, *H. spathulatus*, *H. squarrosus* and *Garcinia vieillardii*.^{11,12,13,14,15} It has been found to exhibit inhibitory effects against *Candida albicans*, *C. neoformans*, *Staphylococcus aureus*, *S. aureus* methicillin resistant strain, *Klebsiella pneumoniae*, *Salmonella gallinarum* and *Mycobacterium smegmatis*.^{11,12} It has also been revealed that **2** is biologically active as an antileishmanial agent against *Leishmania mexicana* and *L. infantum*.¹⁵ Hay *et al.* have deduced that the geranyl constituent of the molecule is likely to be associated with the antileishmanial activity.¹⁵

3.3 Conclusion

Two known compounds were isolated from *H. roeperianum*: 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**) and {[3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone} (**2**). A third metabolite was isolated and was provisionally assigned as 1-methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**). However the elucidation of the structure of this compound is not certain due to insufficient material. Compounds **38** (hyperanone A) and **2** have been isolated previously from other plant sources.

In this study, 3-geranyl-2,4,6-trihydroxybenzophenone (**2**) was isolated for the first time from the *Hypericum* genus, although it has been isolated previously from *Garcinia vieillardii*, *Tovomita krukovii* and *T. longifolia*, all of which originate from the Clusiaceae family.^{11,12,15} Inhibitory effects against various microbes are reported for compound **2**, of which the antimicrobial activity against *Mycobacterium smegmatus* is of great interest, as this bacterium belongs to the same genus as *Mycobacterium tuberculosis*, which is the bacterium that is the cause of tuberculosis (TB).^{11,12,15} Further investigation of this compound or compounds with related structures may therefore lead to the discovery of a potential drug for the treatment of tuberculosis. Thus, the synthetic section of our research incorporated the synthesis of **2** as well as the synthesis of seven structural analogues of this compound.

As **2** was isolated from the DCM leaf extract and was found to have interesting activity, as indicated above, we decided to discontinue the phytochemical investigation of the

remaining plant extracts and focus on the synthesis of this bioactive compound and derivatives thereof. Further investigation of the plant extracts, especially of the more polar extracts, may result in the isolation of interesting compounds.

3.4 Experimental

3.4.1 Standard Experimental Techniques

3.4.1.1 Thin-layer Chromatography (TLC)

Qualitative thin-layer chromatography (TLC) was carried out on Macherey-Nagel aluminium sheets or glass plates, coated with a 0.20 mm layer of silica gel 60 with a fluorescent indicator UV₂₅₄. After development, the plates were viewed under UV light before being sprayed with anisaldehyde. The hexane solvent refers to a mixture of hexane isomers.

3.4.1.2 Flash Column Chromatography

Flash column chromatography was performed on glass columns (of various diameters) containing Merck silica gel 60 (230 – 400 mesh, particle size: 0.040 – 0.063) to a height ranging from 8 cm to 15 cm. The silica gel was loaded into the column as a slurry, using an appropriate solvent system, which was to be the eluting system for that particular column. The plant extract or fraction was dissolved in the appropriate solvent and carefully loaded on the surface of the silica gel. In some cases the fraction was absorbed onto silica gel before being loaded onto the column. Further solvent was applied and the column was allowed to elute as fractions were collected. The elution of the column was performed under pressure (compressed air).

3.4.1.3 The Chromatotron

The chromatotron is a type of thin-layer chromatograph, which is centrifugally accelerated. Solutions of compounds to be separated were applied to chromatotron plates of either 2 mm or 4 mm depending on the mass of the sample. The chromatotron plates were

prepared from Merck silica gel 60 PF₂₅₄ containing gypsum. The separation was conducted on a Harrison Research chromatotron (model 7924T), where elution with a suitable solvent (found using TLC) by gravity flow forms concentric bands of separated compounds. A UV lamp is used to detect the UV active bands, making the concentric bands more visible and easier to collect.

3.4.1.4 Anisaldehyde Stain Reagent

Thin-layer chromatograms were stained with an anisaldehyde solution (13 mL) mixed with concentrated sulfuric acid (17 mL), glacial acetic acid (5 mL) and absolute ethanol (465 mL). The plates were subsequently heated with a heat gun to an approximate temperature of 100 °C in order to ensure the optimum development of colour.

3.4.1.5 Instrumentation

NMR spectra were recorded on a Varian Unity Inova 500 or a Bruker 400 spectrometer. ¹H NMR and ¹³C NMR spectra were referenced to residual protonated solvent signals and deuterated solvent signals, respectively. Coupling constants were calculated in Hertz (Hz). The following abbreviations were used when describing the multiplicities of the peaks observed in the ¹H NMR spectra:

<u>Abbreviation</u>	<u>Peak Multiplicity</u>
s	singlet
d	doublet
dd	doublet of doublets
t	triplet
m	multiplet

Infrared spectra were recorded on a Bruker Alpha FT – IR spectrometer. Mass spectra were obtained either on a Thermofinnigan Trace gas chromatograph coupled to a Polaris Q ion trap electron impact mass spectrometer (GC-EIMS) or on a Waters Acquity liquid chromatograph linked to a Waters LCT Premier time-of-flight mass spectrometer (electrospray ionization in either positive or negative mode). UV-visible absorption spectra were obtained using a Varian Cary 50-Probe UV-visible spectrometer. Melting

points of crystalline compounds were determined by use of a Kofler hot-stage melting point apparatus.

3.4.2 Plant Preparation and Extraction of *Hypericum roeperianum*

3.4.2.1 Collection and Preparation of Plant Material

The *H. roeperianum* Schimp. ex. A. Rich plant material was collected from the Pietermaritzburg National Botanical Gardens, KwaZulu-Natal, South Africa on the 5 February 2007. The plant was identified by Isabel Johnson and voucher specimen [K. Smith 1 (NU)] deposited at the Bews herbarium (Nu), UKZN, Pietermaritzburg). The plant material was separated into leaves and stems and left to dry at room temperature for a period of two weeks. The stems were cut into smaller pieces in order to facilitate the milling of the material. The leaves and stems were ground separately by use of a hammer mill.

3.4.2.2 Extraction of Plant Material

The ground leaves of the plant (556 g dry material) were extracted using DCM (3.0 L) at room temperature for 44 h. The plant material was removed by vacuum filtration and the solvent evaporated by use of a rotary evaporator, to yield 22.9 g of extract. The same leaves were then extracted sequentially with DCM and MeOH in a 1:1 ratio giving a total volume of solvent of 2.2 L. This extraction commenced for twenty-four hours and yielded 50.0 g of extract. A further sequential extraction was carried out using MeOH (2.2 L) as the solvent. The twenty-four hour extraction yielded 44.6 g of extract.

The dried stems (520 g) were extracted initially with DCM (2.0 L) for a period of 24 hours. The solvent-containing extract was separated from the remaining stems by gravity filtration and thereafter the solvent was removed by use of a rotary evaporator. This process yielded 3.88 g of extract. A sequential extraction was carried out with DCM:MeOH (1:1) (1.8 L). The plant material was extracted for 24 h and gave rise to 7.08 g of extract. The next extraction was performed over a 24 h time period with MeOH and yielded a sticky brown extract (3.25 g).

Thin-layer chromatography (TLC) was used to analyse compounds present within the extracts. A number of solvent systems were used to elute the TLC plates until a suitable system was found, and hence could be used to further separate the compounds either by use of a chromatotron or by column chromatography.

3.4.3 Isolation of Compounds from the DCM Crude Leaf Extract

3.4.3.1 Fractionation of Extract

The DCM extract was eluted with the solvent system Hex:EtOAc (4:1) and was viewed under UV light and thereafter with the anisaldehyde stain. Nine spots were observed and appeared to be relatively well separated (**Table 3.4**).

Table 3.4 TLC results of the Crude DCM Leaf Extract eluted with Hex:EtOAc (4:1).

	R _f value	Description		
		No Agent	UV Active	Anisaldehyde Stain
1	0.02	-	-	Orange
2	0.09	Yellow	-	Green
3	0.14	-	Yes	-
4	0.21	Green	-	Sea-green
5	0.26	-	Yes	-
6	0.32	-	-	Purple
7	0.40	-	-	Purple
8	0.56	-	-	Blue-Purple
9	0.74	Yellow	-	Purple

The DCM Extract (4.97 g) was dissolved in DCM and loaded onto a column and eluted a solvent system of Hex:EtOAc in a ratio of 4:1. The polarity of the solvent system was gradually increased during the progression of the column as follows:

Hex:EtOAc (2:1)

Hex:EtOAc (1:1)

EtOAc (100%)

The column was finally washed with 100% MeOH. Eighty-seven fractions (*ca.* 10 mL) were collected. These were spotted on a TLC plate which was eluted with Hex:EtOAc (1:1) solvent system and viewed under UV light followed by anisaldehyde stain reagent. This showed that a relatively good separation of compounds had been obtained. The fractions were appropriately combined into nine fractions, namely A to I (**Table 3.5**).

Table 3.5 Fractions obtained from the column separation of the Crude DCM Leaf Extract of *H. roeperianum*.

	Fractions Combined	Mass (mg)	Description
A	1 - 14	1170	Yellow-green, sticky
B	15 - 29	149	Army green
C	30 - 40	167	Army green
D	41 - 60	604	Dark green, almost black, sticky
E	61 - 70	393	Dark green, almost black, sticky
F	71 - 74	448	Dark green, almost black, sticky
G	75 - 78	195	Dark green
H	79 - 85	167	Dark brown, sticky
I	86 - 87	57	Dark green, almost black

3.4.3.2 Isolation of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (39)

Purification of fraction **D** (**Table 3.5**) (0.60 g) by column chromatography using a solvent system comprised of Hex:EtOAc (1:1) gave rise to 45 fractions (*ca.* 10 mL), which were combined to give a total of 8 fractions (39A – 39G). The column (3 cm in diameter) was packed as a slurry (height of silica gel = 12 cm) and on progression of the column the

polarity of the solvent system was increased by using 100% EtOAc and finally washing with MeOH.

Table 3.6 Masses of combined fractions obtained on Purification of Fraction **D**.

	Fractions Combined	Mass (mg)
39A	1 - 7	4
39B	8 - 12	2
39C	13 - 15	3
39D	16 - 18	3
39E	19 - 21	2
39F	22 -33	7
39G	34 - 39	7
39H	40 - 45	345

Proton NMR spectra were run on the 8 combined fractions. This process yielded a relatively pure fraction, **39B** (**Table 3.6**), which was further analysed by NMR.

1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**) was obtained as a colourless oil (2 mg). UV (DCM) λ_{\max} (log ϵ): 276 (3.46) nm; IR ν_{\max} : 3396, 2920, 2852, 1719, 1645 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ_{H} 1.48 (3H x 2, s, H-9, H-10), 3.75 (3H, s, H-8), 5.24 (1H, d $J = 10.8$ Hz, H-7), 5.27 (1H, d, $J = 17.5$ Hz, H-7), 6.17 (1H, dd, $J = 10.6$ and 17.8 Hz, H-6), 6.31 (1H, s, H-2), 7.41 (3H, m, aromatic protons, H-3', H-4', H-5'), 7.60 (2H, d, aromatic protons, H-2', H-6'); ^{13}C NMR (400 MHz, CDCl_3): δ_{C} 23.5 (C-9, 10), 48.6 (C-5), 51.7 (C-8), 106.8 (C-2), 116.2 (C-7), 126.0 (C-2', 6'), 129.0 (C-3', 5'), 131.3 (C-4'), 140.1 (C-6), 163.8 (C-1).

3.4.3.3 Isolation of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (38)

Fraction **E** (Table 3.5) (390 mg) was dissolved in DCM (5 ml) and loaded onto a column (3 cm in diameter) which had been previously packed as a slurry (height of silica gel: 12 cm) and initially eluted with Hex:EtOAc (1:1). The polarity of the solvent system was gradually increased during the progression of the column, making use of 100% EtOAc and thereafter washing with MeOH. This resulted in the collection of 40 fractions, which were further analysed by TLC and hence similar fractions were combined.

Table 3.7 Masses of combined fractions obtained on Purification of Fraction **E**.

	Fractions Combined	Mass (mg)
41A	1 - 9	5
41B	10 - 13	6
41C	14 - 16	2
41D	17 - 18	124
41E	19 - 24	3
41F	25 - 26	1
41G	27 - 31	1
41H	32 - 40	5

It was evident by proton NMR spectroscopy that fraction **41E** (Table 3.7) was pure. This fraction was thus subjected to further NMR analysis.

3-(1,1-Dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**) was obtained as a colourless oil (3 mg). UV (DCM) λ_{\max} (log ϵ): 277 (3.63) nm; IR ν_{\max} : 1709, 1420, 1358, 1220, 1093, 902, 528, 454, 391 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz): δ_{H} 1.50 (3H x 2, s, H-4'', H-5''), 4.06 (3H, s, H-7), 4.89 (1H, d, $J = 10.8$ Hz, H-3''), 4.92 (1H, d, $J = 17.5$ Hz, H-3''), 6.23 (1H, dd, $J = 10.7$ and 17.6 Hz, H-2''), 6.61 (1H, s, H-5), 7.48 (1H x 3, t, aromatic protons, H-3', H-4', H-5'), 7.70 (1H x 2, d, aromatic protons, H-2', H-6'). ^{13}C NMR; (CDCl_3 , 500 MHz): δ_{C} 27.5 (C-4'', 5''), 38.8 (C-1'') 56.0 (C-7), 108.3 (C-3''),

111.3 (C-5), 111.6 (C-3), 125.2 (C-2', 6'), 129.1 (C-3', 5'), 130.9 (C-1', 4'), 148.3 (C-2''), 156.8 (C-6), 162.7 (C-2), 181.2 (C-4); ESIMS: m/z 271.1331 $[M + H]^+$ (calculated for $C_{17}H_{19}O_3$, 271.1334)

3.4.3.4 Isolation of 3-geranyl-2,4,6-trihydroxybenzophenone (2)

Combined Fraction **G** (Table 3.6) was spotted on TLC plates and eluted with various solvent systems. The solvent system Hex:EtOAc (1:1) showed optimal separation of the evident spots and hence a column was run on sample **G** using this solvent system. Sample **G** (0.132 g) was loaded onto a column (3 cm in diameter, height of silica gel was 11 cm), which had been packed as a slurry. On progression of the column the polarity of the solvent was increased, using 100% EtOAc and finally washing with MeOH. A total of 41 fractions (*ca.* 8 mL) were collected, analysed by TLC (eluted with Hex:EtOAc (1:1), viewed under UV and with anisaldehyde stain) and appropriately combined (10 fractions were obtained).

Table 3.8 Masses of combined fractions obtained on Purification of Fraction **G**.

	Fractions Combined	Mass (mg)
24A	1 - 3	8
24B	4 - 5	4
24C	6 - 9	51
24D	10 - 12	25
24E	13 - 14	6
24F	15 - 19	6
25A	20 - 26	15
25B	27 - 32	9
25C	33 - 37	6
25D	38 - 41	43

Proton NMR spectra on the combined fractions showed that Sample **24C** (Table 3.8) was relatively pure and hence this fraction was further analysed by NMR.

3-Geranyl-2,4,6-trihydroxybenzophenone (**2**) was obtained as a green oil (51 mg). UV (DCM) λ_{\max} . (log ϵ): 309 (3.68) nm; IR ν_{\max} : 3317, 2927, 1710, 1605, 1431, 1327, 1292, 701 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 1.59 (3H, s, H-10''), 1.67 (3H, s, H-8''), 1.80 (3H, s, H-9''), 2.05 (2H, m, H-4''), 2.08 (2H, m, H-5''), 3.38 (2H, d, $J = 7.0$ Hz, H-1''), 5.05 (1H, t, $J = 6.9$ Hz, H-6''), 5.27 (1H, t, $J = 7.0$ Hz, H-2''), 5.94 (1H, s, H-5), 7.62 (5H, m, H-Ar); ^{13}C NMR (CDCl_3 , 400 MHz): δ_{C} 16.2 (C-9''), 17.7 (C-10''), 21.5 (C-1''), 25.6 (C-8''), 26.4 (C-5''), 39.7 (C-4''), 96.2 (C-5), 104.6 (C-1), 121.5 (C-2''), 123.7 (C-6''), 127.9-129.1 (C-3' – C-7'), 132.2 (C-7''), 140.0 (C-2'), 170.9 (C-2, C-4, C-6), 197.9 (C-1); ESIMS: m/z 365.1758 $[\text{M} - \text{H}]^-$ (calculated for $\text{C}_{23}\text{H}_{25}\text{O}_4$, 365.1753)

3.5 References

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CHAPTER 4

Synthesis of Phloroglucinol Derivatives

4.1 Introduction

The aim of this research was to synthesize the isolated phloroglucinol derivative, 3-geranyl-2,4,6-trihydroxybenzophenone ([3-(3,7-dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone) (**2**), as well as 7 structural analogues of this compound (**Figure 4.1**). In synthesizing these molecules, we hoped to explore the significance of the structural features of **2** and determine whether variation in the length of the prenyl side chain and the type of acyl group present, would affect the activity against *Mycobacterium smegmatis*.

Prior to the commencement of synthetic procedures, a thorough literature survey was performed. The aim of this was, not only to establish whether the target compounds had been previously synthesized or isolated from natural product sources, but also to gain insight to methods and synthetic reactions reported in literature that may be useful in our approach to the synthesis of the desired analogues.

To the best of our knowledge, analogues **3** and **5** have not been isolated from natural sources, however, the remaining analogues (**4, 6 – 9**), as well as the parent compound (**2**), have all been isolated previously (**Table 4.1**). Compounds **2, 6, 8** and **9** are all known to exhibit biological activity.

The activity exhibited by **2** has been discussed in Chapter 3 (**Section 3.2.1.2**). Both compounds **6** and **8** are reported to inhibit COX-1 (cyclooxygenase-1), exhibiting IC₅₀ values of 26.2 and 6.0 μM, respectively.¹ In addition, compound **8** also inhibits COX-2 (cyclooxygenase-2) with an IC₅₀ value of 29.9 μM. COX-1 and COX-2 are enzymes that are involved in the prostaglandin pathway.² More specifically, the COX enzymes metabolize arachidonic acid to prostaglandin H₂, which in turn is the substrate that leads to the production of prostaglandins and thromboxane. Prostaglandins play a significant role

in inflammation and pain. Thus, inhibition of COX-1 and/or COX-2 results in an anti-inflammatory effect.²

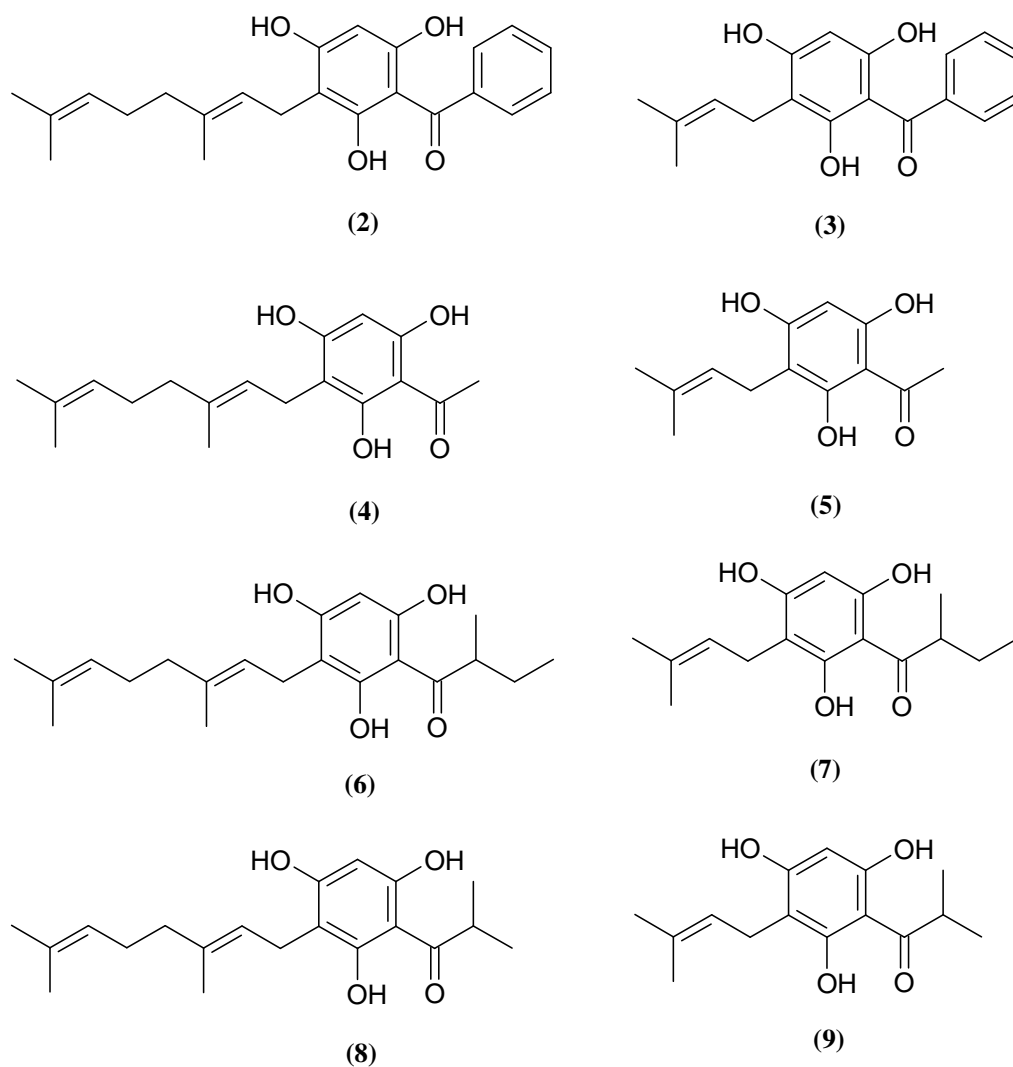


Figure 4.1 The bioactive compound with the structural analogues.

Both **6** and **8** also exhibited activity against the enzyme, which catalyzes the first step of leukotriene synthesis, 5-lipoxygenase (5-LOX).¹ Compound **6** was reported to have an IC_{50} value of 5.8 μM , whilst the value reported for **8** was slightly lower (IC_{50} 2.2 μM). Crockett *et al.*¹ thus concluded that lengthening of the side chain *meta* to the geranyl group significantly reduces the effect on the bioactivity against COX-1, but only minimally reduced the activity against 5-LOX.

Analogue **9** is reported to exhibit significant antimicrobial activity against the following microbes: *Enterococcus faecalis* (IC₅₀ 7.8 μM), *Staphylococcus epidermis* (IC₅₀ 9.8 μM), *S. aureus* (IC₅₀ 6.3 μM), *S. aureus* (methicillin and gentamycin resistant) (IC₅₀ 7.8 μM) and *Bacillus cereus* (IC₅₀ 7.8 μM).³ Activity was also observed against *Pseudomonas aeruginosa* (IC₅₀ 45.0 μM), *Cryptococcus neoformans* (IC₅₀ 26.0 μM) and *Candida albicans* (IC₅₀ 23.8 μM), however, the IC₅₀ values are notably higher than those reported for the previous list of microbes.³

The literature thus illustrates that prenylated acylphloroglucinol derivatives possess potent biological activity against various microbes as well as against enzymes within the prostaglandin and leukotriene pathways.

An in depth investigation of literature revealed that the bioactive compound **2** as well as analogues **3** – **5** and **8** – **9** have been synthesised previously. No synthetic routes to compounds **6** and **7** were described; we therefore believe that this is the first report where compounds **6** and **7** have been achieved by synthetic procedures.

Brajeul *et al.*⁴ reported a synthesis of 2,4,6-trihydroxy-3-prenylbenzophenone (phenyl-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]methanone) (**3**) and a compound similar to **2** (differing only in the presence of a methoxy moiety *para* to the geranyl side chain instead of a hydroxyl group). Their synthetic approach involved a nucleophilic substitution reaction which resulted in the addition of prenyl or geranyl groups to 2,4,6-trihydroxybenzophenone and its trimethyl ether by the use of previously prepared tetrafluoroborate sulfonium salts. This method resulted in the formation of the prenylated (**3**) and geranylated (**2**) products in 42% and 15% yields, respectively.⁴

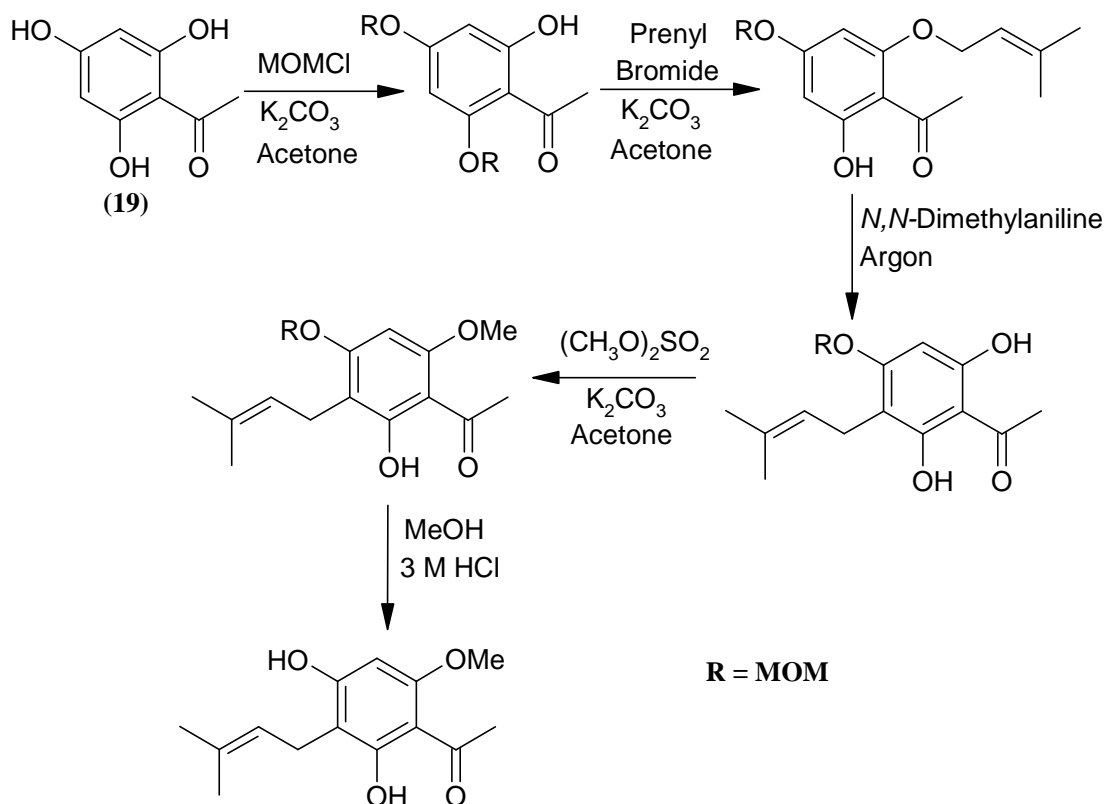
Table 4.1. Plants from which compounds **2**, **4** and **6 – 9** have been isolated.

Compd	Species	Family	Activity
2	<i>Garcinia vieillardii</i> ⁵	Clusiaceae	Active against: <i>Leishmania mexicana</i> and <i>L. infantum</i> ⁵ ; <i>Mycobacterium smegmatus</i> ⁹ ; <i>Candida albicans</i> , <i>C. neoformans</i> , <i>Staphylococcus aureus</i> and <i>S. aureus</i> methicillin resistant strain. ⁸
	<i>Helichrysum monticola</i> ⁶	Asteraceae	
	<i>Helichrysum spathulatus</i> ⁷		
	<i>Helichrysum squarrosus</i> ⁷		
	<i>Tovomita krukovii</i> ⁸	Clusiaceae	
	<i>Tovomita longifolia</i> ⁹		
4	<i>Melicope ptelefolia</i> ¹⁰	Rutaceae	
6	<i>Achyrocline alata</i> ¹¹	Asteraceae	COX-1 and 5-LOX inhibition. ¹
	<i>Esenbeckia nesiotica</i> ¹²	Rutaceae	
	<i>Helichrysum stenopterum</i> ¹³	Asteraceae	
	<i>Hypericum empetrifolium</i> ¹	Clusiaceae	
7	<i>Helichrysum gymnocomum</i> ¹⁴	Asteraceae	
	<i>Helichrysum infaustum</i> ¹⁵		
8	<i>Achyrocline alata</i> ¹¹	Asteraceae	COX-1, COX-2 and 5-LOX inhibition. ¹
	<i>Esenbeckia nesiotica</i> ¹²	Rutaceae	
	<i>Helichrysum infaustum</i> ¹⁵	Asteraceae	
	<i>Helichrysum platypterum</i> ¹⁶		
	<i>Helichrysum stenopterum</i> ¹³		
	<i>Hypericum empetrifolium</i> ¹	Clusiaceae	
	<i>Hypericum jovis</i> ¹⁷		
<i>Hypericum styphelioides</i> ¹⁸			
9	<i>Helichrysum gymnocomum</i> ³	Asteraceae	Active against: <i>Enterococcus faecalis</i> , <i>S. epidermis</i> , <i>S. aureus</i> , <i>S. aureus</i> methicillin and gentamicin resistant strain, <i>Bacillus cereus</i> , <i>Pseudomonas aeruginos</i> , <i>Cryptococcus neoformans</i> and <i>C. albicans</i> . ³
	<i>Helichrysum infaustum</i> ¹⁵		
	<i>Helichrysum platypterum</i> ¹⁶		

Prior research has established that C-prenylation and C-geranylation can be achieved via a nucleophilic substitution reaction in which acylated phloroglucinol derivatives are reacted

with either prenyl or geranyl bromide in the presence of a base such as potassium carbonate or sodium hydride.^{16,19,20} This method has afforded analogues **4**, **5**, **8** and **9** with yields ranging from 28% to 40%. These relatively low yields are considered to be due to the formation of multiple products formed by polyalkylation. Furthermore, *O*-alkylation may also occur instead of the desired *C*-alkylation.

Interestingly, Tan *et al.*²¹ synthesised a compound similar to 2,4,6-trihydroxy-3-prenylacetophenone (1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone) (**5**) (differing only in the presence of a methoxy group *para* to that of the prenyl side chain) where they utilized the more easily achieved *O*-alkylation reaction followed by a Claisen rearrangement followed by a Cope rearrangement to afford the *C*-alkylated product (**Scheme 4.1**).²¹ This five-step synthetic approach attained good yields per individual step (63%, 91%, 80%, 86% and 75% respectively, for bis-protection of phloracetophenone (**19**), *O*-prenylation, Claisen/Cope rearrangement, methylation and deprotection).²¹



Scheme 4.1 Synthesis of *C*-alkylated product via *O*-alkylation²¹

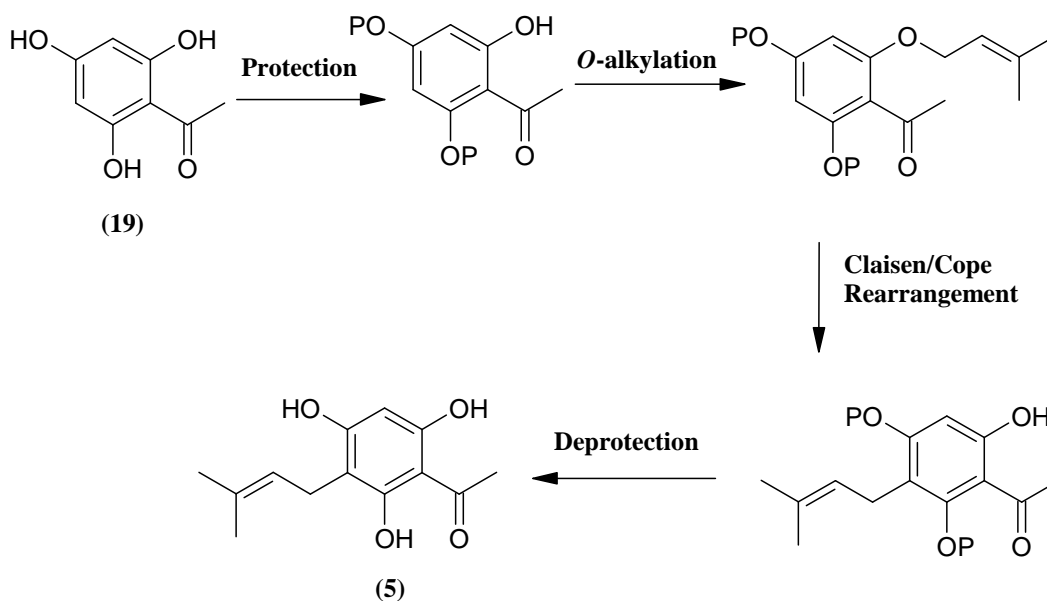
The high yields obtained at each step, especially those of the *O*-prenylation and Claisen rearrangement, suggest that this synthetic strategy may be preferable in comparison to the direct *C*-alkylation, although the latter approach is advantageous as the number of steps involved is reduced from four (excluding methylation) to one.

4.2 Results and Discussion

4.2.1 *C*-Prenylation via *O*-alkylation followed by Claisen/Cope Rearrangement

This synthetic approach was adapted from the method reported by Tan *et al.*²¹ and is a four-step reaction sequence involving protection, *O*-prenylation, consecutive Claisen and Cope rearrangements and lastly deprotection (**Scheme 4.2**).

The initial step of the synthetic strategy involves selective protection of two of the three hydroxyl groups of phloroacetophenone (**19**). This step ensures that the subsequent *O*-alkylation is restricted to a specific free hydroxyl group. Protection with MOMCl, as reported by Tan *et al.*²¹ was not considered owing to the fact that this reagent is highly carcinogenic and is not commercially available. Benzyl ether protection and methylation were selected as alternatives.



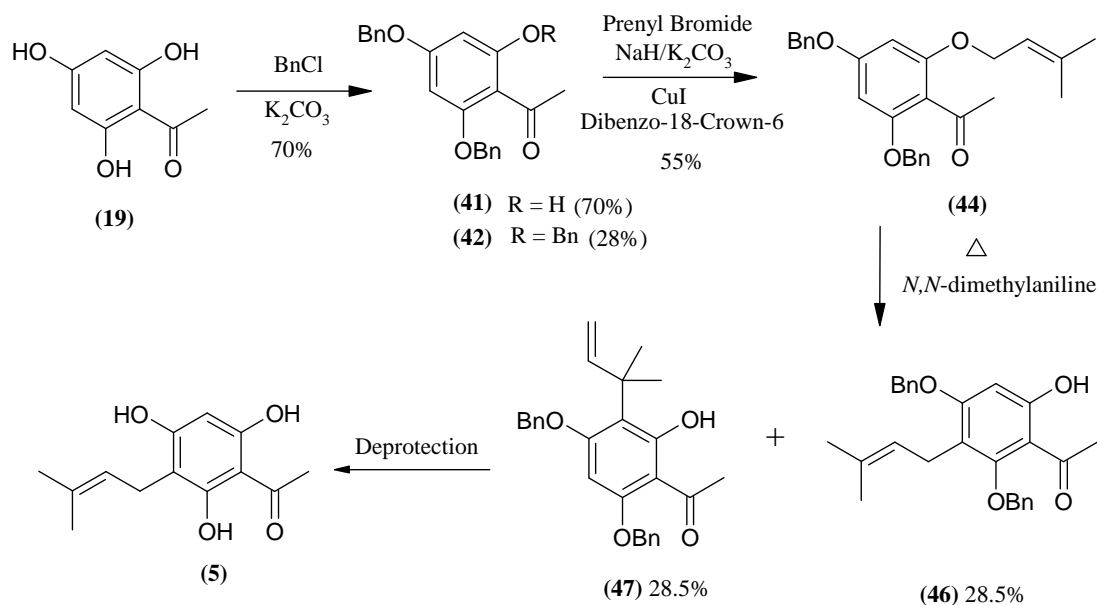
Scheme 4.2 Proposed four-step synthesis of 2,4,6-trihydroxy-3-prenylacetophenone (**5**).

Bisbenzylated phloroacetophenone (1-(2,4-Bis-benzyloxy-6-hydroxyphenyl)ethanone) (**41**) was successfully obtained in 70% yield (**Scheme 4.3**). This was achieved by dissolving three equivalents of K_2CO_3 and one equivalent of phloroacetophenone (**19**) in dry DMF. Two equivalents of benzyl chloride (BnCl) was added and the reaction mixture was heated to 80 °C. The reaction was quenched, by adding 1 M HCl, after a time period of 2.5 h. The 1H NMR spectrum for this compound revealed a multiplet at δ_H 7.40 (10H) in the region characteristic to that of aromatic protons. This multiplet correlated to the peaks in the region δ_C 127.6 – 128.7 in the ^{13}C NMR spectrum. These signals were assigned to the two mono-substituted aromatic rings of the benzyl ether protecting groups. The methylene groups of the protecting group were observed as a singlet (δ_H 5.06, 4H) in the 1H NMR spectrum and appearing at δ_C 70.3 and δ_C 71.1 in the ^{13}C NMR spectrum. The methyl moiety of phloroacetophenone (**19**) was observed as a singlet in the 1H NMR spectrum resonating at δ_H 2.55 and integrating for three protons.

The 1H NMR also revealed a singlet at δ_H 13.99 (1H) as well as two doublets at δ_H 6.10 (1H, $J = 2.3$ Hz) and δ_H 6.16 (1H, $J = 2.3$ Hz). The downfield singlet (δ_H 13.99) arises due to the proton of the unprotected hydroxyl group, which forms a hydrogen bond with the oxygen of the ketone. This ketone is observed in the ^{13}C NMR spectrum as a quaternary carbon at δ_C 203.1. The doublets in the 1H NMR spectrum are characteristic of the two *meta*-aromatic protons of phloroacetophenone derivatives. Quaternary carbons were also observed in the ^{13}C NMR spectrum at δ_C 162.0, 165.1 and 167.6, which were assigned to the three oxygenated aromatic carbons.

The NMR data thus gave evidence that our compound obtained was the desired bisbenzylated phloroacetophenone (**41**). This conclusion was confirmed by the EIMS data, which revealed a molecular ion peak at m/z 371.1262 $[M+Na]^+$, which was in agreement with the calculated value for the sodium adduct of the molecular formula $C_{22}H_{20}O_4Na$.

The tribenzylated phloroacetophenone (**42**) was obtained as a by-product in a yield of 28%. This compound was recognized by the aromatic signal in the 1H NMR spectrum (δ_H 7.38), which integrated for 15 protons, as well as the lack of the singlet downfield at δ_H 13.99.



Scheme 4.3 Synthesis of 2,4,6-trihydroxy-3-prenylacetophenone (**5**).

As was expected, the reaction favoured the bisbenzylated product **41** over the tribenzylated product **42**, which was only obtained in a low yield. This is attributed to the presence of the acyl group and the *O*-hydroxy groups in phloroacetophenone (**19**), which can form an intramolecular hydrogen bond (H-bond) (**Figure 4.2**).

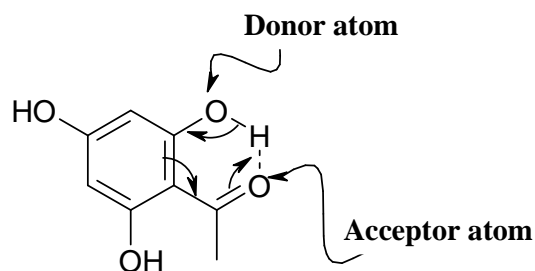
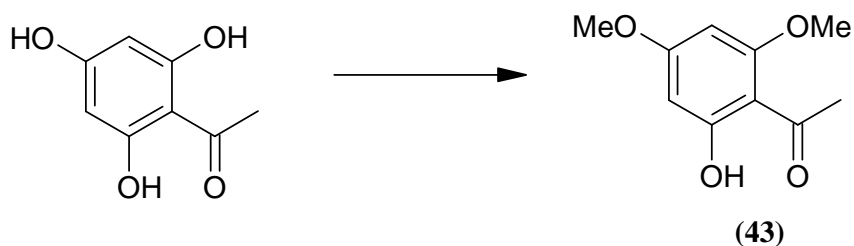


Figure 4.2 Intramolecular bonding of phloroacetophenone (**19**).

This intramolecular H-bond is stabilized due to the conjugated double bonds occurring between the donor (oxygen atom of hydroxy group) and acceptor (oxygen atom of the carbonyl group) atoms (**Figure 4.2**).²² This effect is referred to as “resonance-assisted hydrogen bonding” by Bertolasi *et al.*²³ The delocalisation of the π -electrons results in the C=O and C=C weakening as their electrons are shared amongst the C-O and O-H bonds,

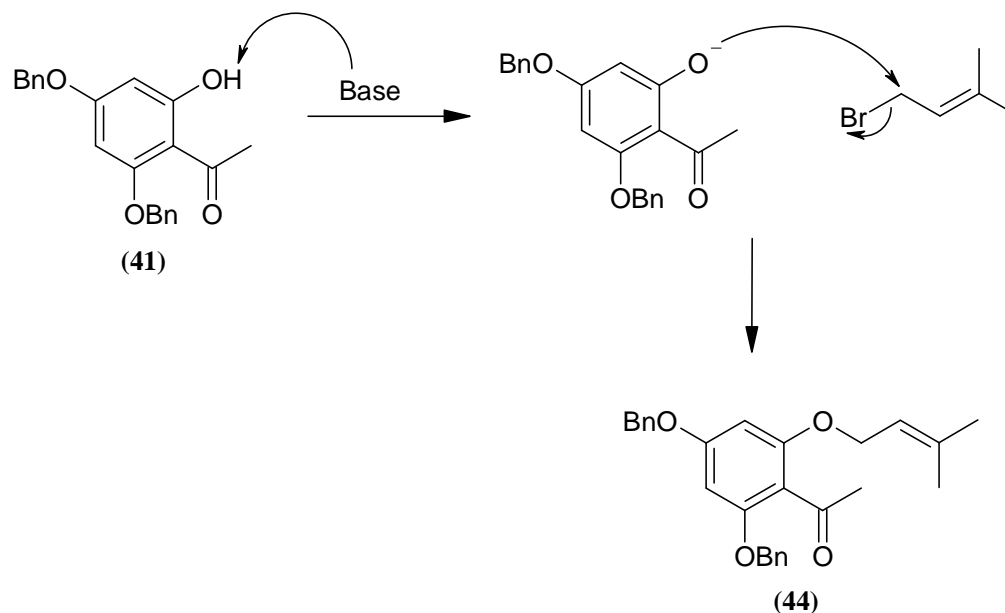
which in turn shorten as they gain partial double bond character.²⁴ Furthermore, the conformation of the acyl group and the position of intramolecular bond is also sterically favoured as the closing of the H-bond forms a six-membered ring.²² Thus, due to the strength and stability of this H-bond, it is difficult for the proton of the hydroxy group to be abstracted by a base (e.g. K_2CO_3 or NaH) and replaced by a protecting group. Therefore, as anticipated, the tribenzylated product **42** is not favoured and is consequently obtained in poor yields.

Protection by methylation to form bismethylated phloroacetophenone (**43**) was also attempted but very low yields (1 – 4%) were achieved and therefore this route (**Scheme 4.4**) was deemed unsuccessful and was not pursued further.



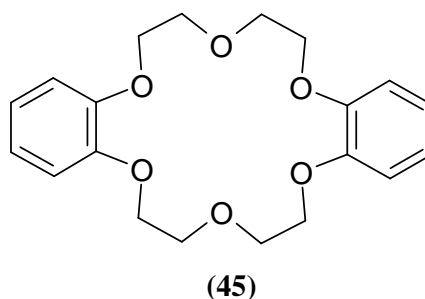
Scheme 4.4 Methylation of phloroacetophenone (**19**).

The second step involved *O*-alkylation of the bisbenzylated phloroacetophenone (**41**) with prenyl bromide (3,3-dimethylallyl bromide). In this step a base was used to deprotonate the remaining unprotected hydroxy group, forming a phenoxide ion. The phenoxide ion then undergoes a nucleophilic substitution reaction on addition of prenyl bromide (**Scheme 4.5**).



Scheme 4.5 *O*-Alkylation of 1-(2,4-bis-benzyloxy-6-hydroxyphenyl)ethanone (**41**).

This reaction was carried out using either NaH or K₂CO₃ as the base and various solvents, namely acetone, DMF and THF. In the reactions where NaH was used, yields of 45% and 54% were obtained in THF and DMF, respectively. When deprotonating with K₂CO₃, the DMF reaction was unsuccessful and the reaction in acetone only yielded 34% of *O*-alkylated product (**44**). In an attempt to increase this yield, dibenzo-18-crown-6 (**45**) and copper iodide (CuI) were added. This resulted in a higher yield of 55% being accomplished.



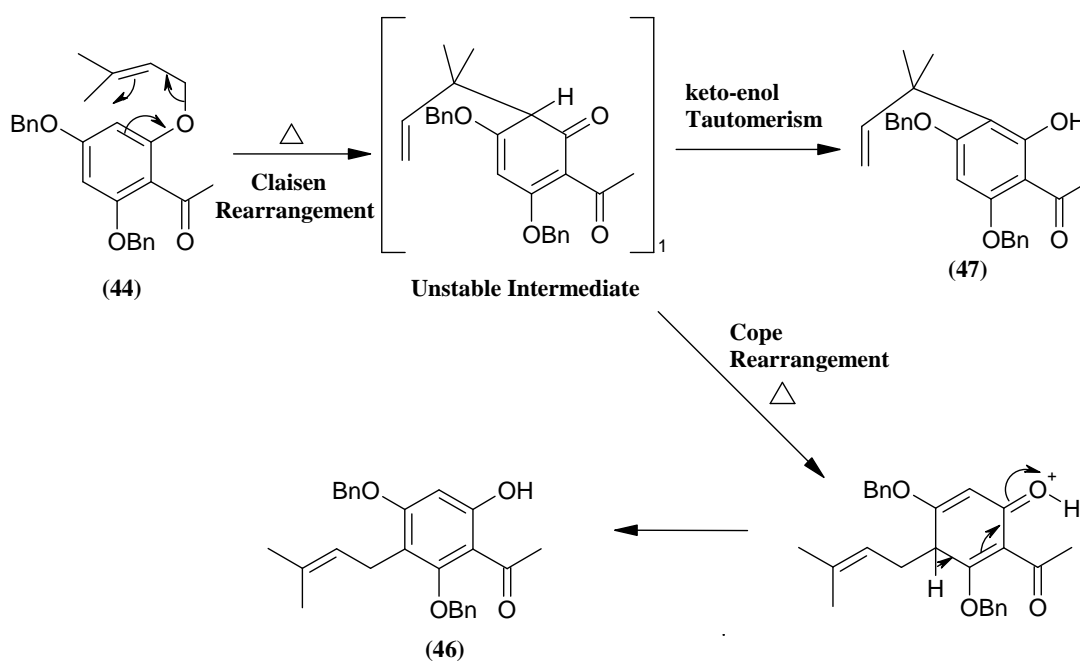
Dibenzo-18-crown-6 (**45**) is a crown ether whose central cavity coordinates particularly well with potassium cations. Thus, the addition of this compound assists in the dissolution

of K_2CO_3 into organic solvents and hence enables the base to react more efficiently in deprotonating the free hydroxy group.

CuI was added to act as a catalyst in converting the prenyl bromide to the more reactive prenyl iodide *via* halogen exchange.

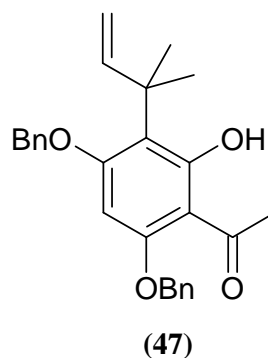
The *O*-prenylated adduct, 1-[2,4-bis-benzyloxy-6-(3-methylbut-2-enyloxy)phenyl] ethanone (**44**), was converted to the *para C*-prenylated derivative *via* a thermal Claisen rearrangement, also known as the [3,3]-sigmatropic rearrangement (**Scheme 4.6**), followed by a Cope rearrangement. This was achieved by refluxing **44** in *N,N*-dimethylaniline.

This type of rearrangement is concerted in nature, meaning that the one bond forms and the other breaks at the same time. This results in the formation of the unstable keto tautomer as an intermediate.²⁵ The keto tautomer rapidly undergoes a proton shift, transforming into its enol conformation and hence regaining aromaticity as well as stability.²⁵



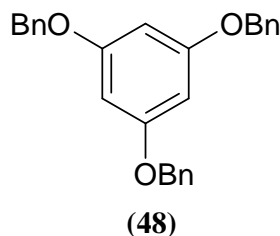
Scheme 4.6 Mechanism of Claisen/Cope rearrangement.²⁵

The Claisen/Cope rearrangement reaction was successful in achieving the desired *para* C-prenylated product (**46**) (Plates 4.1 and 4.2), although a by-product was also present. This by-product, 1-[4,6-bis-benzyloxy-3-(1,1-dimethylallyl)-2-hydroxyphenyl]ethanone (**47**) (Plates 4.1 and 4.2), was obtained by the Claisen rearrangement, which was not followed by the Cope rearrangement. These two compounds were obtained in a 50:50 mixture, each with a yield of 28.5%. An attempt to purify and separate these compounds (which appeared at the same R_f value) by TLC and column chromatography was unsuccessful.



Due to the poor yields achieved in the Claisen/Cope rearrangement, it was decided to attempt the one-step *C*-alkylation reaction of phloracetophenone (**19**) with prenyl bromide. In spite of the low yields reported in literature, this synthetic strategy is advantageous as the number of steps involved, is reduced from four to one.

Another synthetic route investigated, was the introduction of the alkyl group *via* a directed-*ortho* metallation (DOM) type reaction. This strategy would require all the hydroxyl groups present to be protected. Starting with the acetophenone precursors may make the protection of all three hydroxyl moieties difficult to achieve in good yield due to the intramolecular H-bonding that occurs within these types of molecules as discussed previously. For this reason, we proposed phloroglucinol (**17**) as the starting material and protected the hydroxyl groups with the benzyl ether protecting group. The reaction was successful as tribenzylated phloroglucinol (**48**) was obtained in a yield of 46%.



The ^1H NMR data for **48** showed a multiplet in the aromatic region (δ_{H} 7.40), which integrated for 15 protons, thus indicating the presence of the three mono-substituted rings of the benzyl ether protecting groups. Furthermore, the ^1H NMR spectra also revealed singlets at δ_{H} 5.03 and δ_{H} 6.31, integrating for six and three protons, respectively. The former was assigned to the three CH_2 groups of the benzyl ether protecting groups, whilst the latter was assigned to the three methine protons present on the unsubstituted carbons of the phloroglucinol ring.

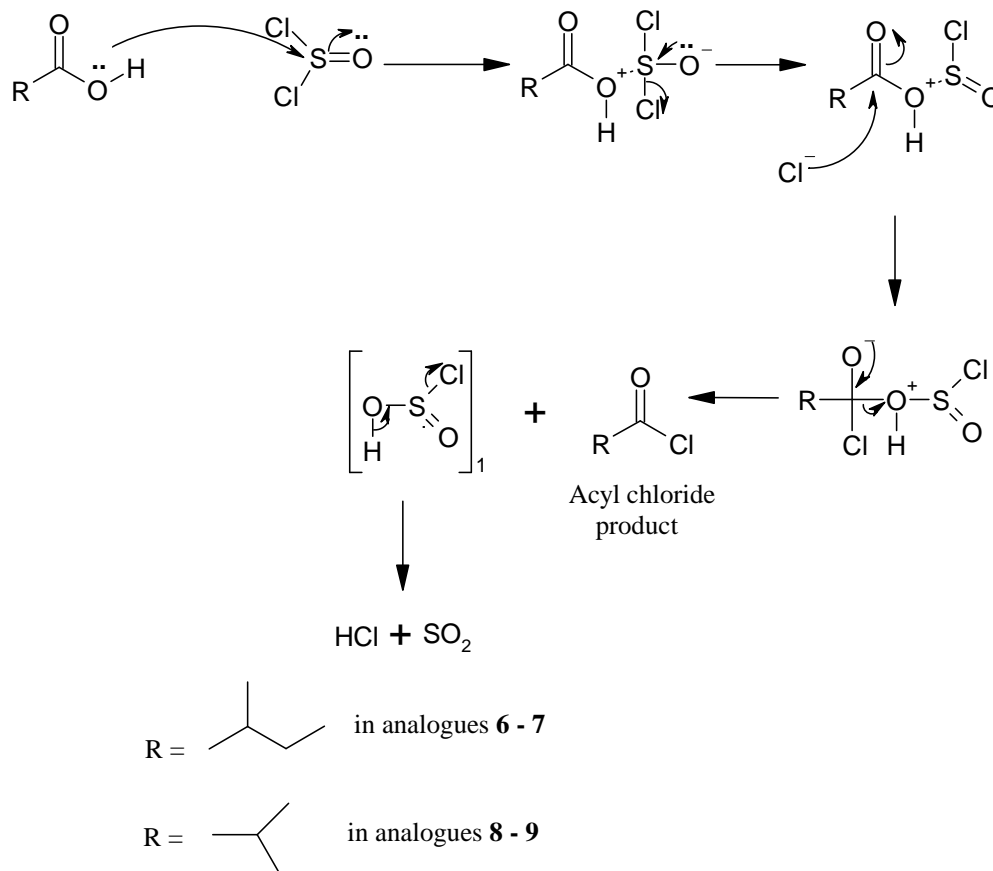
The yield of 46% obtained for the tribenzylated phloroglucinol (**48**) was not very promising for the first step of this synthetic route, thus we decided to attempt the direct C-alkylation method before continuing with this synthetic strategy.

4.2.2 Acylation of Phloroglucinol in the Synthesis of Target Compounds

In order to synthesize the isolated phloroglucinol derivative **2** and the structural analogues (**3**, **6 - 9**), an acylation reaction is required using the appropriate acyl chloride in each case. For analogues **2** and **3**, commercial benzoyl chloride was used. The acyl chlorides, 2-methylbutyryl chloride and isobutyryl chloride, required for the synthesis of analogues **6 - 7** and **8 - 9**, are also commercially available, however, their respective carboxylic acids (2-methylbutyric acid and isobutyric acid) are more stable and therefore have a longer shelf life and are also much less expensive. For these reasons 2-methylbutyryl chloride and isobutyryl chloride were prepared by a nucleophilic addition-elimination reaction between their respective carboxylic acids and an inorganic acid chloride. Phosphorous trichloride (PCl_3), phosphorous pentachloride (PCl_5) and thionyl chloride (SOCl_2) are common inorganic acid chlorides known to give high yields of acyl chlorides when reacted with carboxylic acids.²⁵ We used SOCl_2 , which reacted with the respective carboxylic acids to form a protonated acyl chlorosulfite intermediate (**Scheme 4.7**). This intermediate

is highly reactive as its acyl constituent is a better leaving group compared to that of the acyl chloride.²⁵

The gases released as by-products of the reaction were bubbled through H₂O and hence entrapped. The acyl chloride was then obtained by distillation. Isobutyryl chloride distilled at 92 °C, which agreed with the literature value (91-93 °C) quoted for the boiling point of this compound.²⁶ 2-Methylbutyryl chloride was collected at a temperature of 112 °C. This temperature is slightly lower than the value of 117 – 121 °C as stated in literature.²⁶ As 2-methylbutyric acid and SOCl₂ have boiling points of 176-177 °C²⁶ and 79 °C²⁶ respectively, which differ substantially from that expected for the acyl chloride, the compound collected at 112 °C was believed to be the intended 2-methylbutyryl chloride.



Scheme 4.7 Mechanism for the preparation of acyl chlorides.²⁵

The two prepared acyl chlorides and the commercial benzoyl chloride were reacted with phloroglucinol (**17**) to form three aryl ketones (**24** - **26**) (**Figure 4.3**) via a Friedel-Crafts acylation reaction (**Scheme 4.9**). The acylation reactions were performed using various methods in order to achieve the highest possible yields. The methods differed in the reaction temperature as well as in the solvent (DCM/nitrobenzene) and lewis acid ($\text{AlCl}_3/\text{ZnCl}_2$) that was used. The method where phloroglucinol and aluminium trichloride were dissolved in nitrobenzene and refluxed with the appropriate acyl chloride at 80 °C was observed to achieve the highest yields. This method resulted in compounds **24**, **25** and **26** being obtained in 49%, 57% and 66% yields, respectively.

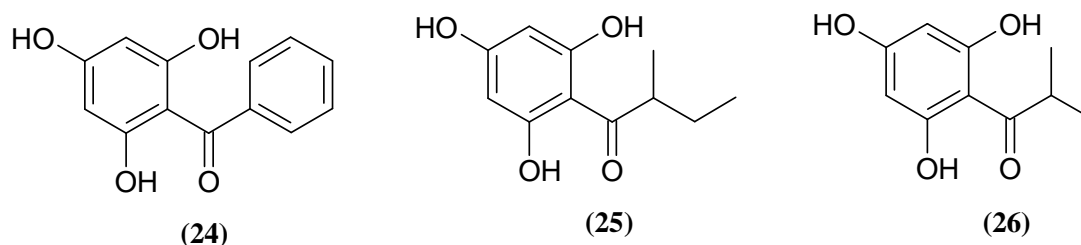
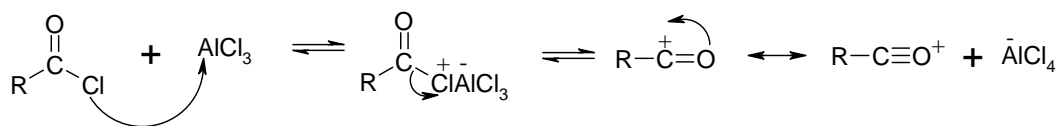
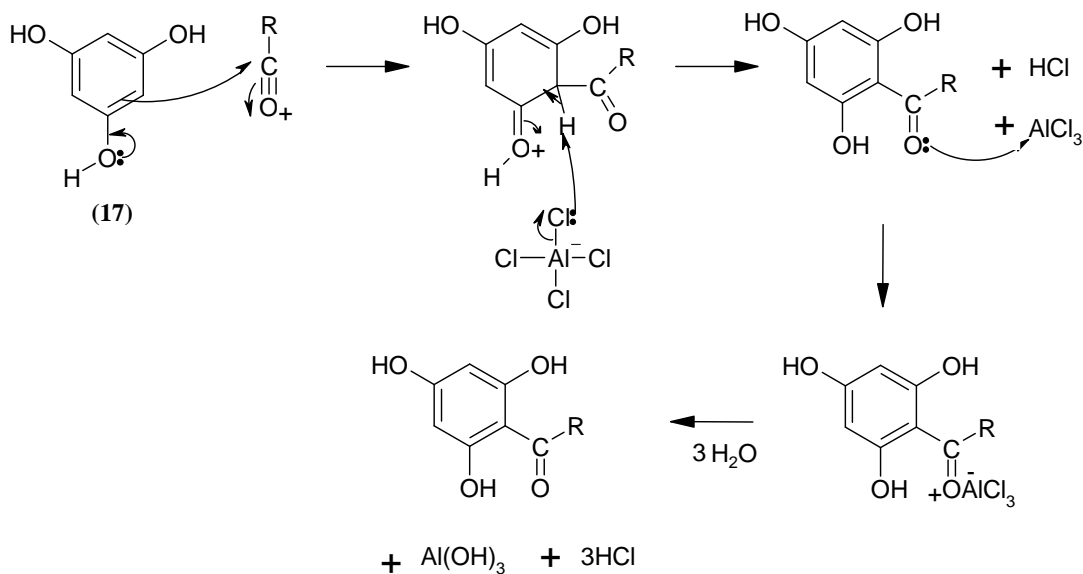


Figure 4.3 The three prepared aryl ketones.

The Friedel-Crafts acylation reaction is an electrophilic aromatic substitution reaction where most often the electrophile is an acylium ion (acyl cation). This acylium ion is created when the Lewis acid catalyst (commonly AlCl_3) forms a complex with the acyl halide and abstracts the halide atom, resulting in the formation of a resonance stabilized acylium ion (**Scheme 4.8**).²⁵



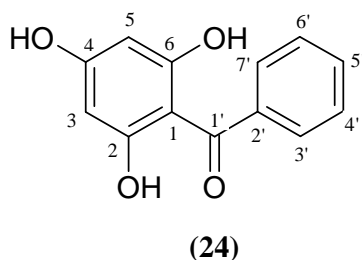
Scheme 4.8 Formation of the acylium ion.²⁵



Scheme 4.9 Mechanism of the acylation reaction.²⁵

4.2.2.1 Structural Elucidation of Compounds 24 - 26

The prepared acyl phloroglucinol derivatives **24** (Plates 5.1 and 5.2), **25** (Plates 6.1 and 6.2) and **26** (Plates 7.1 and 7.2) were fully characterized by NMR (¹H, ¹³C, DEPT90, DEPT135, COSY, HSQC and HMBC) spectroscopy, ultraviolet spectroscopy (UV), infrared spectroscopy (IR) and electrospray mass spectrometry (ESIMS).



The molecular mass of 2,4,6-trihydroxybenzophenone [phenyl-(2,4,6-trihydroxyphenyl)methanone] (**24**) was confirmed by ESIMS where a peak was observed at m/z 253.0473 [$M+Na$]⁺, which agrees the calculated value for C₁₃H₁₀O₄Na, the sodium adduct of the molecular formula. The UV spectrum of **24** exhibited a maximum absorption band at 306 nm (log ϵ 4.36). This indicated that a conjugated system is incorporated in the structure of the compound.

The ^{13}C NMR spectrum (**Table 4.2**) (**Plate 5.2**) showed only 9 of the 13 expected signals and therefore it was concluded that 4 pairs of signals were overlapping. The appearance of the methine signals resonating in the δ_{C} 125-140 region suggested the presence of an aromatic group. This was confirmed by the signals (δ_{H} 7.36, 7.45, 7.58) corresponding to the *para*, *meta* and *ortho* protons in the ^1H NMR spectrum, thus signifying that the aromatic ring was unsubstituted.

The signals occurring at δ_{C} 143.1, 164.0, 166.1 and 200.8 in the ^{13}C NMR spectrum were assigned to quarternary carbons, as there was no evidence of these signals in the DEPT experiments. The latter signal is characteristic of a carbonyl moiety, whilst those resonating at 164.0 and 166.1 are characteristic of carbons attached to hydroxyl groups within a phenolic ring. This implied that our structure contained a phloroglucinol moiety. As only two signals were observed in this region, it is expected that two of the three C-OH groups of the phloroglucinol ring will be in a similar environment and thus these signals will overlap to show only one peak. Further confirmation of the presence of a phloroglucinol moiety was the appearance of the broad band observed in the region 3000-3500 cm^{-1} in the IR spectrum, which is characteristic of alcohol groups, specifically hydrogen-bonded phenols. The IR spectrum also shows a sharp peak in the region where carbonyl functional groups are observed (1635 cm^{-1}).

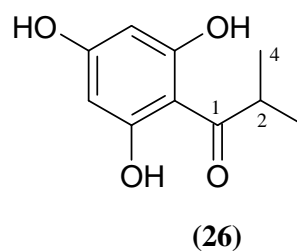
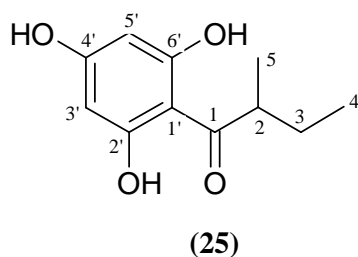
Other than the aromatic protons, the ^1H NMR spectrum (**Plate 5.1**) also revealed a sharp singlet (2H, δ_{H} 5.81). The HSQC experiment showed that this signal correlated to δ_{C} 96.0 in the ^{13}C spectrum, and were assigned to be the protons on the two unsubstituted carbons of the phloroglucinol ring.

The long-range ^{13}C - ^1H couplings observed in the HMBC spectrum enabled the correct connection of the fragments to be established. The experimental data therefore indicates that the synthesis of **24** was successful.

Table 4.2 NMR data for **24**

Carbon	C	H (Int., Mult., J/Hz)	HMBC Correlation
1	106.1		
2,6	164.0		
3,5	96.0	5.81 (2H, s)	C-1, C-1', C-4, C-2, C-6
4	166.1		
1'	200.8		
2'	143.1		
3',7'	129.4	7.58 (2H, d, $J = 7.4$)	C-1'
4',6'	128.8	7.36 (2H, t, $J = 7.4$)	C-2'
5'	132.2	7.45 (1H, t, $J = 7.4$)	C-4', C-6'

The ^{13}C NMR data acquired for **25** and **26** (Table 4.3) (Plates 6.2 and 7.2, respectively), was similar to that of **24** in regards to the carbonyl moiety (δ_{C} 211.6 and 211.9, respectively) as well as the carbons in the phenolic ring on which the hydroxyl groups are attached which were observed at δ_{C} 166.0 and 165.8, respectively. The HSQC experiment shows that the signal resonating at δ_{C} 96.1 in each spectrum correlates to a singlet at δ_{H} 5.82 (2H) in the ^1H NMR spectrum. As with **24**, the signals mentioned denote the presence of a carbonyl group, a phloroglucinol ring and the latter are assigned as the protons on the unsubstituted carbons of the phloroglucinol ring.



The ^1H NMR spectrum for **26** (Plate 7.1) shows a doublet at δ_{H} 1.12 (6H), which is recognized to be the two methyl groups of the isopropyl fragment of the acyl phloroglucinol derivative. This signal was found to correlate to δ_{C} 19.8 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **26** also revealed a heptet at δ_{H} 3.97 (1H, $J = 6.7$ Hz).

The HMBC experiment showed long range C-H coupling of this methine signal to the two methyl groups, as well as coupling between the methyl groups and the carbonyl moiety. This resulted in the full characterization of our isopropyl constituent which is attached to our carbonyl group, which in turn is the substituent on our monosubstituted phloroglucinol ring.

The elucidation of the structure of **26** was substantiated by the experimental data obtained from ESIMS, UV-vis and IR spectroscopy. A molecular ion peak of m/z 219.0633 $[M+Na]^+$ was observed on the ESIMS spectrum, which agrees the calculated value for $C_{10}H_{12}O_4Na$. The UV-vis data confirmed the presence of conjugated bonds in the structure by the exhibition of absorption bands at 229 nm ($\log \epsilon$ 4.60) and 287 nm ($\log \epsilon$ 4.73). The IR spectrum of **26** showed a prominent peak at 1570 cm^{-1} and a broad band in the region of $3000 - 3500\text{ cm}^{-1}$, the former is characteristic of a ketone moiety whilst the broad band gives evidence of phenolic groups.

Compound **25** exhibited a triplet (δ_H 0.89, 3H, $J = 7.4\text{ Hz}$) and a doublet (δ_H 1.10, 3H, $J = 6.7\text{ Hz}$) which were assigned to be methyl groups attached to a methylene and methine carbon, respectively. In the 1H NMR spectrum (**Plate 6.1**), the two methylene protons were not equivalent and appeared as two multiplets resonating at δ_H 1.36 and δ_H 1.80 with an integration of one proton each. This observation is attributed to the diastereotopic relationship of the methylene protons due to the adjacent stereocentre. The HSQC experiment showed that these protons correlated with the carbon resonating at δ_C 28.3 in the ^{13}C NMR spectrum and the HMBC showed long range C-H coupling to the two methyl groups, the carbonyl function and the methine group. The latter proton appears at δ_H 3.85 (1H, $J = 6.7\text{ Hz}$) in the 1H NMR spectrum and correlates to the carbon resonating at δ_C 46.8 in the ^{13}C NMR spectrum. Further investigation of the HMBC experiment aided in establishing the connection pattern of all the fragments present, resulting in the conclusion that compound **25** was the desired 2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one.

The UV-vis data acquired for **25** displayed two absorption bands at 228 ($\log \epsilon$ 4.52) and 288 ($\log \epsilon$ 4.68) which confirmed the presence of a conjugated system within the structure of the compound. This was further substantiated by the broad band observed at 3206 cm^{-1} in the IR spectrum denoting the presence of a phenolic substituent. The IR data also gave

evidence of a ketone group (1567 cm^{-1}). ESIMS revealed a molecular ion peak m/z 211.0968 $[\text{M}+\text{H}]^+$, which is in agreement with the calculated value for the formula $\text{C}_{11}\text{H}_{15}\text{O}_4$.

Table 4.3 NMR data of compounds **25** and **26**

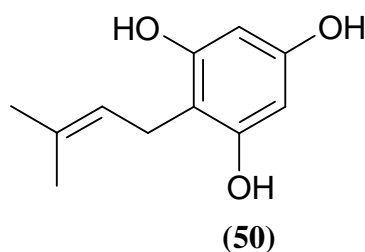
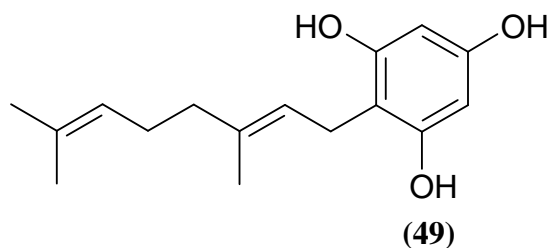
Carbon	Compound 25			Compound 26		
	δ_C	δ_H (Int., Mult., J/Hz)	HMBC Correlation	δ_C	δ_H (Int., Mult., J/Hz)	HMBC Correlation
1	211.6			211.9		
2	46.8	3.85 (1H, sextet, $J = 6.7$)	C-1, C-3, C-4, C-5	40.0	3.97 (1H, heptet, $J = 6.7$)	C-3, C-4
3	28.3	1.36 (1H, multiplet)	C-1, C-2, C-4, C-5	19.8	1.12 (6H, d, $J = 6.7$)	C-1, C-2
	28.3	1.80 (1H, multiplet)	C-1, C-2, C-4, C-5			
4	12.5	0.89 (3H, t, $J = 7.4$)	C-2, C-3	19.8	1.12 (6H, d, $J = 6.7$)	C-1, C-2
5	17.3	1.10 (3H, d, $J = 6.7$)	C-1, C-2, C-3	-		
1'	105.5			104.8		
2',4',6'	166.0			165.8		
3',5'	96.1	5.81 (2H, s)	C-1', C-2', C-4', C-6'	96.1	5.82 (2H, s)	C-1', C-2', C-4', C-6'

4.2.3 Direct C-alkylation of Acylated Phloroglucinol Derivatives

The fact that we were unable to obtain a pure product from the method adapted from Tan *et al.*,²¹ as well as the low yields obtained (Section 4.2.1), prompted us to investigate alternative routes to the desired product and its analogues.

The literature review revealed that the direct introduction of the prenyl side chain may be problematic. This may be attributed to the lack of control over regioselectivity, chemoselectivity and the occurrence of polyalkylation.^{27,28} A further concern is that prenyl moieties have the ability to cyclize and hence often form closed ring structures.²⁹

We attempted to alkylate phloroglucinol (**17**) with geranyl bromide and prenyl bromide to form 1-geranylphloroglucinol [2-(3,7-dimethylocta-2,6-dienyl)benzene-1,3,5-triol] (**49**) and 1-prenylphloroglucinol [2-(3-methylbut-2-enyl)benzene-1,3,5-triol] (**50**), respectively. This was performed by two methods, the first being a Friedel-Craft-type alkylation where ZnCl₂ was used as the Lewis acid. The second method involved a nucleophilic substitution reaction where the base, K₂CO₃, abstracts a proton from one of the phloroglucinol hydroxyl groups resulting in the formation of a phenoxide ion. The phenoxide ion can then be prenylated in the *ortho* position by alkylating with prenyl or geranyl bromide. This is achieved as the phenoxide anion acts as a nucleophile and attacks the alkyl halide at the electrophilic carbon. The bromide ion, being a good leaving group, is simultaneously released and the nucleophilic substitution reaction is complete. The major disadvantage associated with this type of C-alkylation is the possibility that a number of products can form. These products arise from the various resonance structures available for the phenoxide ion, which allows alkylation to occur at the *ortho* and *para* positions as well as on the phenoxide oxygen. Unfortunately both of these methods were unsuccessful.



On reviewing the literature, an article was found that reported that prenylation of acetophenone precursors was more successful than prenylation of the phloroglucinols.²⁹ For this reason we changed our approach, and first prepared the acylated phloroglucinol derivatives before attempting the direct *C*-alkylation.

A Friedel-Craft type alkylation was attempted where **24** (1 eq.) was reacted with geranyl bromide (1.5 eq.) in the presence of a Lewis acid, AlCl₃. A second reaction was also carried out where prenyl bromide was used as the alkylating agent instead of geranyl bromide. Both these reactions were unsuccessful.

Qi and Porco³⁰ reported a prenylation of **24** *via* a nucleophilic substitution reaction. In this reaction a base, KOH, abstracts a proton from one of the phloroglucinol hydroxyl groups. A phenoxide ion is formed which acts as a nucleophile and attacks the alkyl halide. As previously mentioned, the major disadvantage associated with this type of *C*-alkylation is the possibility that a number of products can form. This was evident in the research by Qi and Porco, who obtained a bisprenylated product in 45% yield.³⁰ This approach, utilizing an aqueous KOH solution, was attempted for the alkylation step in the synthesis of analogues **4** and **5** using geranyl bromide and prenyl bromide, respectively. Unfortunately, analysis by NMR spectroscopy showed that the desired mono-alkylated products were not obtained.

An alternative method of direct *C*-alkylation by nucleophilic substitution resulted in promising yields of 74% and 78% by Lee *et al.*²⁰ and Huang *et al.*¹⁹, respectively. In an attempt to reproduce these results, commercially available 2,4,6-trihydroxy acetophenone (**19**) was refluxed with geranyl bromide in the presence of anhydrous potassium carbonate in dry acetone. Although analogue **4** was obtained, our yield of only 23% was much lower than those previously obtained by Lee *et al.*²⁰ and Huang *et al.*¹⁹ The same reaction conditions were used to alkylate **24**, which resulted in a poor yield of 6% for the bioactive compound **2**. The TLC gave evidence that many other products were produced during the reaction, one of which was isolated and found to be the *O*-alkylated product. This product was obtained in a greater yield (12%) than the desired compound and thus indicated that the reaction conditions favour the *O*-alkylation over the *C*-alkylation.

An attempt to alkylate **24** and 2,4,6-trihydroxyacetophenone (**19**) with prenyl bromide (instead of geranyl bromide) *via* this method was unsuccessful in the production of **3**, however, **5** was obtained in a yield of 32%.

Alkylation of the acylphloroglucinol derivatives was also accomplished by a similar approach where a stronger base, sodium hydride, was used in place of potassium carbonate. The reactions were performed in dry dioxane with either prenyl or geranyl bromide as the alkylating agent. Following the reaction conditions expressed by Kuhnke and Bohlmann¹⁶ and Bharate *et al.*³¹ resulted in the successful production of our bioactive compound **2** (17%) as well as all seven analogues **3 - 9** (19%; 29%; 29%; 17%; 13%; 15% and 10% yields, respectively).

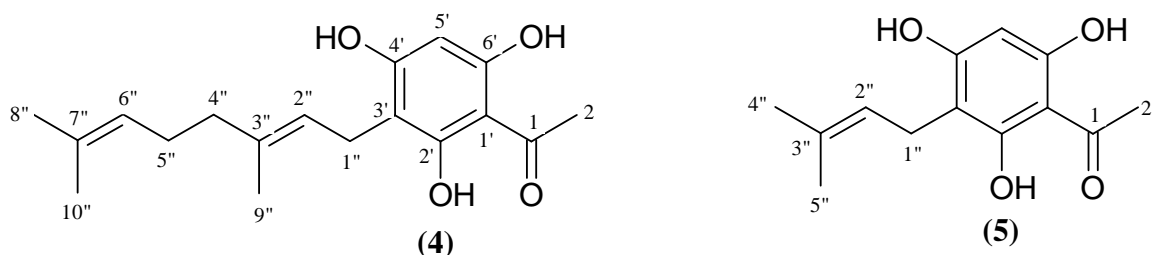
The low yields are most likely attributed to the occurrence of polyalkylation as well as alkylation of the phenoxide oxygen. The fact that our acylphloroglucinol derivatives contain three hydroxyl moieties increases the degree of by-products or competing reactions that may occur. The poor yields obtained suggest that the reaction conditions need to be evaluated and optimized. Unfortunately, due to time constraints, the optimization of the reaction conditions was not carried out.

4.2.3.1 Structural Elucidation of Compounds 2-9

The alkylated acylphloroglucinol derivatives **2 - 9** were fully characterized by NMR (¹H, ¹³C, DEPT90, DEPT135, COSY, HSQC and HMBC) spectroscopy, ultraviolet spectroscopy (UV), infra-red spectroscopy (IR) and electrospray mass spectrometry (ESIMS).

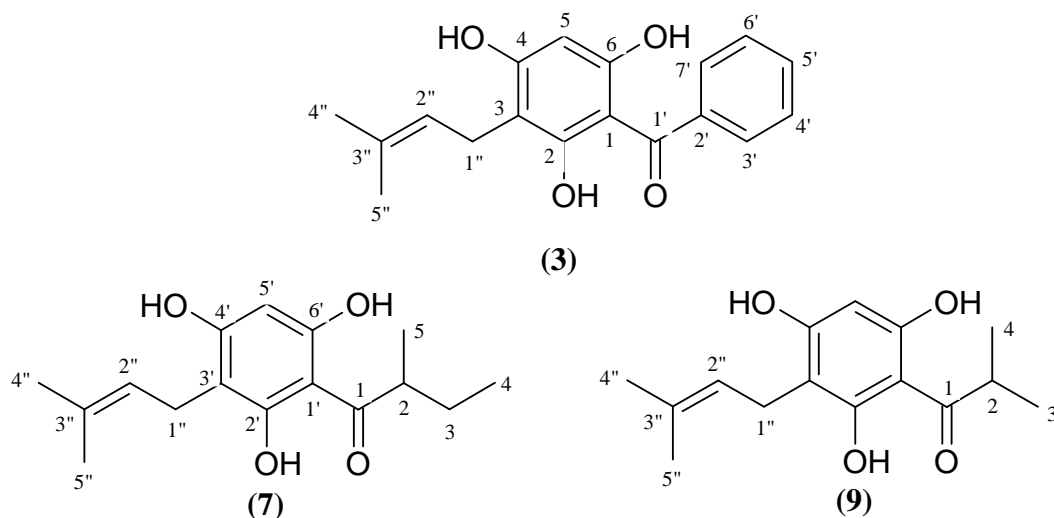
The NMR data for the acylated phloroglucinol fragment (**24**) for **2** (Plates **8.1 - 8.2**) and **3** (Plates **9.1 - 9.2**) was observed to be the same as described in Section 4.2.2.1 (Table 4.2). Similarly, the inclusion of the acylated fragments in **6** and **7**, and **8** and **9** are discussed in greater depth as compounds **25** and **26** (Table 4.3 in section 4.2.2.1), respectively. The major difference is evident in the δ_{H} 5.81 which integrates for two protons in the acylphloroglucinol compounds, but only integrates for one proton in the alkylated compounds. This shows that

the phloroglucinol ring is no longer mono-substituted as observed in **24** – **26**, but is now di-substituted.



The synthesis of analogues **4** and **5** involved the alkylation of commercially available phloroacetophenone (**19**). The phloroacetophenone (**19**) fragment is recognized in the NMR spectra obtained for analogues **4** (**Plates 10.1** and **10.2**) and **5** (**Plates 11.1** and **11.2**). The ^{13}C NMR spectrum shows a set of three quaternary signals in the region characteristic of phenolic carbons thus denoting the presence of a phloroglucinol moiety. Another quaternary carbon resonating further downfield at δ_{C} 204.8 is indicative of a carbonyl functional group.

The ^1H NMR spectrum revealed sharp singlet (δ_{H} 5.90, 1H), which when compared to compounds **24**, **25** and **26** allowed us to assign it as the methine group at the unsubstituted position of the phloroglucinol ring. The fact that this signal, which correlates to the carbon resonating at δ_{C} 95.0, only integrates for one proton indicates that the phloroglucinol core of the alkylated compound is di-substituted. The methyl group of phloroacetophenone appeared at δ_{H} 2.60 (3H, s) and correlated to the carbon resonating at δ_{C} 33.0 in the HSQC. It was also found to correlate to the carbonyl carbon and the quaternary carbon in position C-1' in an HMBC experiment.



Prenylation of **24** – **26** and phloroacetophenone (**19**) resulted in analogues **3**, **7** (Plates **12.1** and **12.2**), **9** (Plates **13.1** and **13.2**) and **5**, respectively (Table **4.4** and Table **4.5**). The ^1H NMR data of **3** (Plate **9.1**) revealed a doublet at δ_{H} 3.24 (2H, $J = 7.2$ Hz), which suggested that *C*-alkylation had occurred. If *O*-alkylation had occurred, we would expect the signal to resonate further downfield at approx. δ_{H} 4. This was further confirmed by the signal at δ_{H} 5.89, which integrated for one proton, indicating that the phloroglucinol ring was disubstituted and no longer monosubstituted as in the acyl phloroglucinol derivatives where the signal with similar chemical shift integrated for two protons. The proton resonating at δ_{H} 3.24 coupled to one of the phenolic carbons (δ_{C} 164.4) of the phloroglucinol ring (HMBC experimental data). This implied that the signal at δ_{H} 3.24 may be assigned to the protons on the carbon of the prenyl side chain that is situated closest to the phenolic ring. The HMBC showed further correlation of this proton signal (δ_{H} 3.24) to two quaternary carbons (δ_{C} 108.5 and δ_{C} 131.8) and a methine carbon (δ_{C} 124.6). The proton of the latter was observed as a triplet (δ_{H} 5.24, 1H, $J = 7.2$ Hz), which correlates to δ_{C} 124.6 in the ^{13}C NMR spectrum (Plate **9.2**). This denotes the presence of an alkene.

Two methyl group protons appearing in the ^1H NMR spectrum at δ_{H} 1.66 and δ_{H} 1.76, (3H, s) correlated to carbons resonating at δ_{C} 26.1 and δ_{C} 18.0. The HMBC experiment revealed long range ^{13}C - ^1H coupling between the two methyl groups and both methyl protons coupled to a quaternary carbon (δ_{C} 131.8) and the assigned methine moiety (C-2''). Combining all the

information described above we concluded that a prenyl side chain had been introduced *via* direct C-alkylation.

A comparison between the data obtained for **3** and the data obtained for **7**, **9** and **5** showed that the prenyl side chain had been successfully introduced to each of our acylphloroglucinol derivatives *via* direct C-alkylation. Our conclusions were further confirmed by comparing our acquired experimental data with that of prenylated compounds reported in literature.^{18, 32}

Table 4.4 NMR data of compound **3**

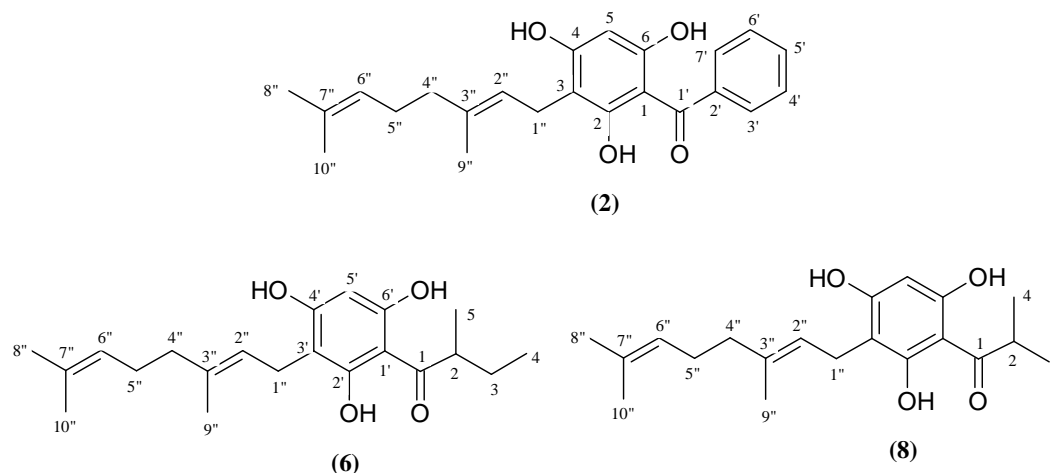
Carbon	C	H (Int., Mult., J/Hz)	HMBC Correlation
1	105.6		
2	164.4		
3	108.5		
4	163.4		
5	95.5	5.89 (1H, s)	C-1, C-3, C-4, C-6, C-1'
6	160.6		
1'	201.2		
2'	143.6		
3', 7'	128.7	7.53 (2H, d, J = 7.0)	C-1', C-4', C-5', C-6'
4', 6'	129.4	7.36 (2H, t, J = 7.3)	C-2', C-3', C-4'
5'	130.8	7.42 (1H, t, J = 7.3)	C-4', C-6'
1''	22.4	3.24 (2H, d, J = 7.2)	C-2, C-3, C-4, C-2'', C-3'',
2''	124.6	5.24 (1H, t, J = 7.2)	
3''	131.8		
4''	18.0	1.76 (3H, s)	C-2'', C-3'', C-5''
5''	26.1	1.66 (3H, s)	C-2'', C-3'', C-4''

Table 4.5. NMR data acquired for analogues **5**, **7** and **9**

Carbon	Compound 5			Compound 7			Compound 9		
	C	H (Int., Mult., J/Hz)	HMBC Correlation	C	H (Int., Mult., J/Hz)	HMBC Correlation	C	H (Int., Mult., J/Hz)	HMBC Correlation
1	204.8			211.7			211.9		
2	33.0	2.60 (3H, s)	C-1, C-1'	46.8	3.88 (1H, sextet, $J = 6.8$)		40.1	4.00 (1H, heptet, $J = 6.7$)	
3				28.4	1.35 (1H, m), 1.80 (1H, m)	C-1, C-2, C-4, C-5	19.9	1.12 (6H,d, $J = 6.9$)	C-1, C-2, C-4
4				12.5	0.89 (3H, t, $J = 7.4$)	C-2, C-3	19.9	1.12 (6H,d, $J = 6.9$)	C-1, C-2, C-3
5				17.4	1.10 (3H, d, $J = 6.8$)	C-1, C-2, C-3			
1'	105.7			105.4			104.7		
2'	164.9			165.4			165.5		
3'	108.1			108.3			108.3		
4'	164.0			163.5			163.6		
5'	95.0	5.90 (1H, s)	C-1', C-3', C-4', C-6'	95.2	5.88 (1H, s)	C-1', C-3', C-4', C-6'	95.1	5.89 (1H, s)	
6'	162.0			161.2			161.2		
1''	22.3	3.17 (2H, d, $J = 7.2$)	C-2', C-3', C-2'', C-3''	22.4	3.17 (2H, d, $J = 7.0$)	C-2', C-3', C-2'', C-3''	22.3	3.17 (2H, d, $J = 7.0$)	C-2''
2''	124.7	5.17 (1H, t, $J = 7.2$)		124.7	5.17 (1H, t, $J = 7.0$)		124.8	5.17 (1H, t, $J = 7.0$)	
3''	131.3			131.3			131.2		
4''*	17.9	1.73 (3H, s)	C-2'', C-3'', C-5''	18.0	1.73 (3H, s)	C-2'', C-3'', C-5''	18.0	1.73 (3H, s)	C-2'', C-3'', C-5''
5''*	26.1	1.63 (3H, s)	C-2'', C-3'', C-4''	26.1	1.64 (3H, s)	C-2'', C-3'', C-4''	26.1	1.64 (3H, s)	C-3'', C-4''

* Assignments of 4'' and 5'' are interchangeable

Alkylation of **24** – **26** and phloroacetophenone (**19**) with geranyl bromide resulted in the bioactive compound **2** and analogues **6** (Plates **14.1** and **14.2**), **8** (Plates **15.1** and **15.2**) and **4**, respectively (Table **4.6** and Table **4.7**). The NMR data acquired showed some similarities when compared to the data obtained for the prenylated compounds. These similarities included the appearance of a singlet at δ_{H} 5.90 (1H), a doublet at δ_{H} 3.18 (2H, $J = 7.2$ Hz) coupling to a triplet at δ_{H} 5.17 (1H, $J = 7.0$ Hz). As mentioned earlier, these signals suggest that C-alkylation has occurred on the phloroglucinol ring with δ_{H} 3.18 being the first carbon (C-1'') in the alkyl side chain, which in turn is attached to the methine moiety (δ_{H} 5.17) of the alkene (C-2'').



The ^1H NMR spectrum also revealed two multiplets at δ_{H} 1.93 and δ_{H} 2.03 (correlating to δ_{C} 41.1 and δ_{C} 27.9, respectively), which integrated for two protons each. The former proton showed long-range ^{13}C - ^1H coupling to C-2'' and to a quaternary carbon (δ_{C} 134.9), a methylene carbon (δ_{C} 27.9) and a methyl group (δ_{C} 16.3), whilst the latter is also coupled to the quaternary carbon at δ_{C} 134.9, as well as to a methylene carbon (δ_{H} 41.1) and a methine carbon (δ_{C} 125.7). The HMBC correlations therefore imply that C-2'' is attached to a quaternary carbon (C-3'') with a methyl substituent (C-9''). C-3'' is also attached to the methylene group (C-4'') that appears at δ_{H} 1.93 in the ^1H NMR spectrum. The fact that signals δ_{H} 1.93 (C-4'') and δ_{H} 2.02 couple to each other indicates that they are adjacent to one another. Thus the signal at δ_{H} 2.02 is assigned to be C-5''. The methine carbon (δ_{C} 125.7) correlates to the triplet resonating at δ_{H} 5.04 (1H) in the ^1H NMR spectrum (C-6'').

The only signals unaccounted for thus far in the ^1H NMR spectrum are the two singlets appearing at δ_{H} 1.61 and δ_{H} 1.55 and integrating for three protons each, thus indicating the presence of two methyl groups. The HMBC data showed long-range ^{13}C - ^1H coupling between the two methyl groups as well as to C-6'' and a quaternary carbon resonating at δ_{C} 132.1. The quaternary carbon was assigned to C-7'', whilst the methyl groups were assigned as C-8'' and C-10'' (these two signals are interchangeable).

The information described above as well as the comparison of our data to that reported in literature¹⁷, led us to conclude that a geranyl substituent had been successfully introduced *via* direct C-alkylation to each of our four acyl phloroglucinol derivatives.

Table 4.6 NMR data of compound **2**.

Carbon	C	H (Int., Mult., <i>J</i> /Hz)	HMBC Correlation
1	105.6		
2	164.4		
3	108.5		
4	163.5		
5	95.5	5.88 (1H, s)	C-1, C-3, C-4, C-6, C-1'
6	160.7		
1'	201.2		
2'	143.7		
3', 7'	129.3	7.50 (2H, d, <i>J</i> = 7.0)	C-1', C-4', C-6'
4', 6'	128.7	7.35 (2H, d, <i>J</i> = 7.3)	C-2', C-3', C-7'
5'	131.8	7.42 (1H, t, <i>J</i> = 7.3)	C-4', C-6'
1''	22.4	3.24 (2H, d, <i>J</i> = 7.0)	C-2, C-3, C-4, C-2'', C-3''
2''	124.6	5.23 (1H, t, <i>J</i> = 7.0)	
3''	135.1		
4''	41.1	1.96 (2H, m)	C-2'', C-3'', C-5'', C-9''
5''	27.9	2.05 (2H, m)	C-4'', C-6'', C-7''
6''	125.7	5.06 (1H, t, <i>J</i> = 7.0)	
7''	132.1		
8''*	26.0	1.61 (3H, s)	C-6'', C-7'', C-10''
9''	16.4	1.76 (3H, s)	C-2'', C-3'', C-4''
10''*	17.9	1.56 (3H, s)	C-6'', C-7'', C-8''

* Assignments are interchangeable

Table 4.7 NMR data acquired for analogues **4**, **6** and **8**

Carbon	Compound 4			Compound 6			Compound 8		
	C	H (Int., Mult., J/Hz)	HMBC Correlation	C	H (Int., Mult., J/Hz)	HMBC Correlation	C	H (Int., Mult., J/Hz)	HMBC Correlation
1	204.7						211.9		
2	33.0	2.60 (3H, s)	C-1, C-1'	46.8	3.88 (1H, sextet, <i>J</i> = 6.7)		40.0	4.00 (1H, heptet, <i>J</i> = 6.6)	C-1, C-3, C-4,
3				28.3	1.36 (1H, m) 1.80 (1H, m)		19.9	1.12 (6H,d, <i>J</i> = 6.9)	C-1, C-2, C-4
4				12.5	0.89 (3H, t, <i>J</i> = 7.4)	C-2, C-3	19.9	1.12 (6H,d, <i>J</i> = 6.9)	C-1, C-2, C-4
5				17.4	1.10 (3H, d, <i>J</i> = 6.7)	C-1, C-2, C-3			
1'	105.7						104.7		
2'	164.9			165.3			165.4		
3'	108.1			106.7			108.4		
4'	164.0			163.4			163.5		
5'	95.0	5.90 (1H, s)	C-1', C-3', C-4', C-6'	95.3	5.90 (1H, s)		95.1	5.89 (1H, s)	C-1', C-3', C-4', C-6'
6'	161.9			163.4			161.1		
1''	22.2	3.18 (2H, d, <i>J</i> = 7.2)	C-3', C-4', C-2'', C-3''	22.2	3.18 (2H, d, <i>J</i> = 7.0)	C-2'', C-3'',	22.3	3.17 (2H, d, <i>J</i> = 7.0)	C-3', C-4', C-2'', C-3''
2''	124.7	5.17 (1H, t, <i>J</i> = 7.0)		124.8	5.17 (1H, t, <i>J</i> = 7.4)		124.8	5.17 (1H, t, <i>J</i> = 7.0)	
3''	134.9			134.9			134.8		
4''	41.1	1.93 (2H, m)		41.0	1.93 (2H, m)		41.0	1.93 (2H, m)	C-3'', C-5'', C-9''
5''	27.9	2.03 (2H, m)		27.8	2.02 (2H, m)		27.8	2.02 (2H, m)	C-4'', C-3'', C-6''

Table 4.7 NMR data acquired for analogues **4**, **6** and **8** continued.

Carbon	Compound 4			Compound 6			Compound 8		
	C	H (Int., Mult., J/Hz)	HMBC Correlation	C	H (Int., Mult., J/Hz)	HMBC Correlation	C	H (Int., Mult., J/Hz)	HMBC Correlation
6"	125.7	5.05 (1H, t, $J = 7.0$)		125.7	5.03 (1H, t, $J = 7.4$)		125.6	5.04 (1H, t, $J = 7.0$)	
7"	132.1			132.1			132.1		
8"*	25.9	1.61 (3H, s)	C-6", C-7", C-10"	26.0	1.60 (3H, s)	C-6", C-7", C-10"	26.0	1.60 (3H, s)	C-6", C-7", C-10"
9"	16.3	1.73 (3H, s)	C-2", C-3", C-4"	16.3	1.73 (3H, s)	C-2", C-3", C-4"	16.3	1.73 (3H, s)	C-2", C-4", C-3"
10"*	17.8	1.55 (3H, s)	C-6", C-7", C-8"	17.9	1.54 (3H, s)	C-6", C-7", C-8"	17.8	1.54 (3H, s)	C-6", C-7", C-8"

*Assignments are interchangeable

4.3 Conclusion

Biological activity and toxicity testing is currently being carried out on our battery of eight synthetic compounds. Therefore future work would involve the evaluation of the results obtained in order to draw some conclusions on the structure-activity relationships of the various groups present in our molecules. If significant biological activity is exhibited by one or more of our synthesized analogues, future work would need to include an attempt to increase the yields of the compounds of interest. This may be achieved by optimizing the reaction conditions of the direct *C*-alkylation method by which the analogues were produced or investigation into alternative routes may be considered. Although the carcinogenic nature of the MOM protecting group needs to be taken into account, as well as the fact that it is no longer commercially available, it may be interesting to synthesize MOMCl and hence attempt the *C*-prenylation *via* *O*-alkylation followed by the Claisen rearrangement as good yields are expressed by Tan *et al.*²¹ The MOM protecting group is also known to be a good *ortho* directing group. Another potential method involves alkylating *via* a direct-*ortho* metallation (DOM) type reaction as discussed in **Section 4.2.1**.

4.4 Experimental

4.4.1 Standard Experimental Techniques

The chromatographical techniques as well as instrumentation utilized in the synthetic section of the research, are the same as that reported in **Chapter 3 (Section 3.4.1)**. IR data is only reported between 1500 and 3500 cm^{-1} .

All anhydrous reactions were performed under an inert nitrogen atmosphere with solvents previously dried using an Innovative Technology Pure Solv MD-7 solvent purification system (THF and DCM) or by drying the solvent over an appropriate drying agent (Acetone and DMF: molecular sieves, dioxane: Na) followed by distillation under nitrogen. The Pure Solv MD-7 solvent purification system is composed of steel canisters under a positive N_2 pressure and uses a drying agent comparable to molecular sieves. This technique does not require any heat and thus no peroxides are formed. Reagents such as

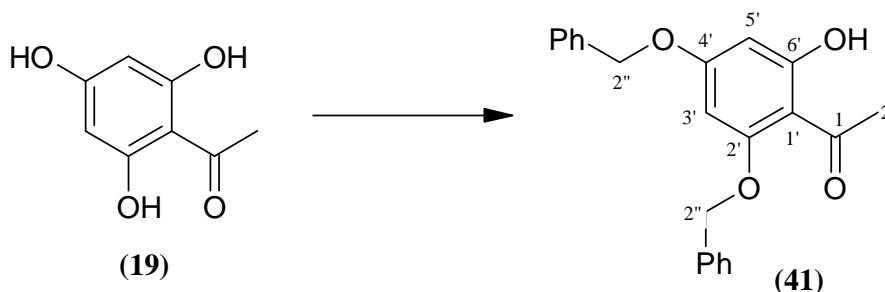
phloroglucinol (**17**) and phloroacetophenone (**19**) were dried in an oven at 120 °C for 24 hours and thereafter stored in a desiccator prior to use. The glassware required for the reactions were also dried in an oven and flushed with nitrogen prior to use. All alkylating agents were added with a syringe *via* a septum.

The reactions were monitored using silica coated aluminium or glass TLC plates. Unless specified to the contrary, the TLC plates were eluted with Hex:EtOAc (2:1) and viewed under UV light before being sprayed with anisaldehyde stain reagent and heated with a heat gun.

Unless stated otherwise, the standard work-up procedure involved: quenching the reaction with 1 M HCl or dist. H₂O, extracting the reaction mixture with DCM (3 x 20 mL) or EtOAc (3 x 30 mL), drying the organic extract over MgSO₄ and concentrating *in vacuo*. Purification was then carried out on either a 2 mm or 4 mm chromatotron plate. Pure compounds were analysed by NMR spectroscopy in deuterated chloroform or methanol.

4.4.2 Synthetic Procedures

4.4.2.1 1-(2,4-Bisbenzyloxy-6-hydroxyphenyl)ethanone (**41**)

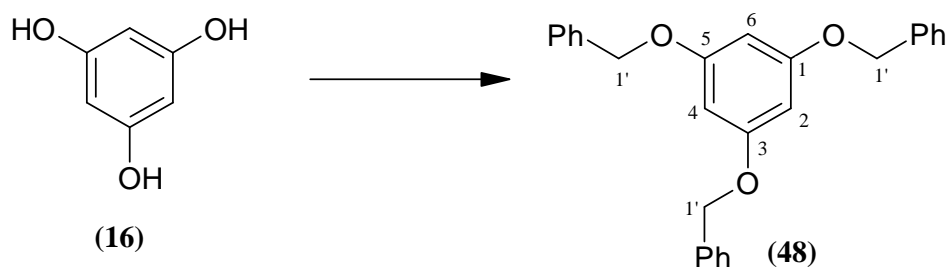


K₂CO₃ (2.47 g, 17.9 mmol) was added to a solution of DMF (20 mL) and phloroacetophenone (**19**) (1.01 g, 6.01 mmol). The reaction mixture was allowed to stir for a time period of 15 minutes before benzyl chloride (1.0 mL, 12.2 mmol) dissolved in DMF (3 mL) was added. The reaction mixture was refluxed for 2 h after which TLC showed that the reaction had gone to completion. The reaction was quenched with 1 M HCl (15 mL) and extracted with DCM. The organic layers were combined, washed with ice

water (6 x 30 mL), dried and concentrated *in vacuo*. Column chromatography (silica height: 14 cm, loaded as a slurry) was used to remove the polar compound observed on the baseline of the TLC plate (Hex:EtOAc 9:1). Further purification of the product was performed on a chromatotron. The organic fraction was loaded onto a 4 mm chromatotron plate and eluted with Hex:EtOAc (9:1). The bisbenzylated product **41** (1.46 g, 70%) was obtained as creamy-yellow needle-like amorphous solid. Melting point: 93-95 °C (MeOH); UV(MeOH) λ_{max} (log ϵ): 287 (4.94) nm; ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 2.55 (3H, s, H-2), 5.06 (4H, s, H-2''), 6.10 (1H, d, $J = 2.3$ Hz, H-5'), 6.16 (1H, d, $J = 2.3$ Hz, H-3'), 7.40 (10H, m, Ar), 13.99 (1H, s, OH); ^{13}C NMR (CDCl_3 , 400 MHz): δ_{C} 33.3 (C-2), 70.3 (C-2''), 71.1 (C-2''), 92.4 (C-3'), 94.8 (C-5'), 106.4 (C-1'), 127.6 – 128.7 (Ar-CH), 135.6 (Ar-C), 135.9 (Ar-C), 162.0 (C-6'), 165.1 (C-4'), 167.6 (C-2'), 203.1 (C-1); ESIMS: m/z 371.1262 $[\text{M}+\text{Na}]^+$ (calculated for $\text{C}_{22}\text{H}_{20}\text{O}_4\text{Na}$, 371.1259).

A by-product was also isolated from the fractions collected in a yield of 28% and was found to be the tribenzylated product (**42**). ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 2.49 (3H, s, H-2), 5.01 (2H, s, H-2''), 5.06 (4H, s, H-1''), 6.26 (1H, s, H-3', H-5'), 7.38 (15H, m, Ar); ^{13}C NMR (CDCl_3 , 400 MHz): δ_{C} 32.6 (C-2), 70.2 (C-2''), 70.6 (C-1''), 93.6 (C-3', C-5'), 115.2 (C-1'), 128.6 – 128.7 (Ar-CH), 136.4 (Ar-C), 157.1 (C-2', C-6'), 161.1 (C-4'), 201.3 (C-1).

4.4.2.2 1,3,5-Trisbenzyloxybenzene (**48**)

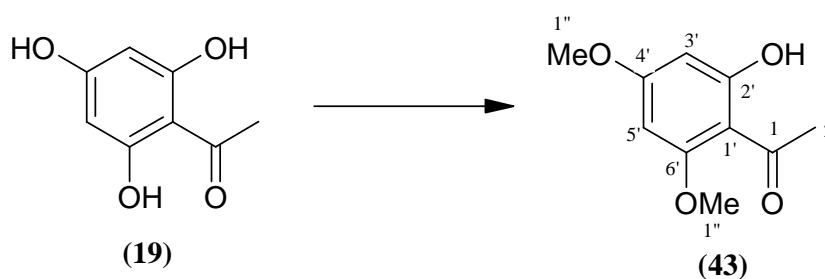


Phloroglucinol (**17**) (602 mg, 4.77 mmol) and K_2CO_3 (3.29 g, 28.83 mmol) were dissolved in DMF (20 mL) and allowed to stir for 20 min before benzyl chloride (1.76 mL, 21.4 mmol) was added. The reaction mixture was heated to 80 °C. The reaction was quenched after 9 h with 1 M HCl (15 mL) and extracted with DCM. The organic fractions were combined and washed with ice water (6 x 30 mL) before being dried and

concentrated *in vacuo*. Column chromatography (diameter: 3 cm, silica height: 15 cm, loaded as a slurry) yielded the trisbenzylated phloroglucinol **48** (869 mg, 46%).

IR (KBr) ν_{\max} : 3028, 2868, 1592 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 5.01 (6H, s, H-1''), 6.27 (3H, s, H-2, H-4, H-6), 7.38 (15H, m, Ar-H); ^{13}C NMR (CDCl_3 , 400 MHz): 70.2 (C-1'), 95.0 (C-2, C-4, C-6), 127.6 – 128.6 (Ar-CH), 136.9 (Ar-C), 160.7 (C-1, C-3, C-5).

4.4.2.3 1-(2-Hydroxy-4,6-dimethoxyphenyl)ethanone (**43**)



Method 1

Phloroacetophenone (**19**) (100 mg, 0.56 mmol) and NaH (90 mg, 3.75 mmol) were dissolved in THF (15 mL) and allowed to stir at 0 °C for 1 h before MeI (0.17 mL, 2.68 mmol) was added. The reaction was quenched with dist. H_2O (5 mL) after 18 h when no more starting material was present. The reaction products were extracted with DCM (3 x 15 mL); the organic fractions were combined and dried before being concentrated *in vacuo*. Purification was performed on a 2 mm chromatotron plate, which yielded dimethylated phloroacetophenone **43** (2 mg, 2%). IR (KBr) ν_{\max} : 2940, 1584 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 2.61 (3H, s H-2), 3.83 (6H, 2 x s, H-1''), 5.92 (1H, d, $J = 2.4$ Hz, H-5'), 6.06 (1H, d, $J = 2.4$ Hz, H-3'), 14.0 (1H, s, OH); ^{13}C NMR (CDCl_3 , 400 MHz): 32.9 (C-2), 55.6 (C-1''), 90.8 (C-5'), 93.6 (C-3'), 106.1 (C-1'), 162.9 (C-6'), 166.1 (C-4'), 167.6 (C-2'), 203.1 (C-1).

Method 2

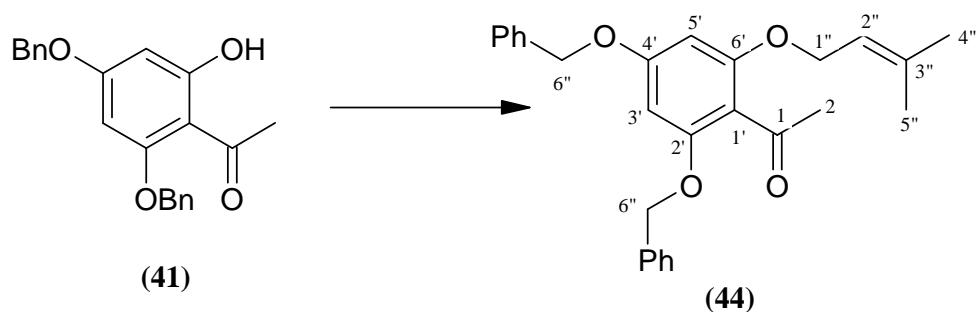
Phloroacetophenone (**19**) (202 mg, 1.20 mmol) and NaH (180 mg, 7.50 mmol) were dissolved in THF (15 mL) and allowed to stir for 1 h before MeI (0.33 ml, 5.35 mmol) was

added. The reaction mixture was heated until gently refluxing (70 °C). The reaction was quenched after 4 h with dist. H₂O (15 mL) and extracted with DCM. The organic fractions were combined, dried and concentrated. The residue was purified on a 2 mm chromatotron plate, eluted with Hex:EtOAc (9:1). Dimethylated phloroacetophenone **43** was not obtained. This reaction was unsuccessful.

Method 3

Phloroacetophenone (**19**) (306 mg, 1.82 mmol) and K₂CO₃ (1.23 g, 5.92 mmol) were dissolved in acetone (20 mL) and allowed to stir under N₂ for 30 min. before MeI was added. The reaction mixture was heated until gently refluxing (60 °C). After refluxing for 6 h TLC showed that no starting material remained and thus the reaction was quenched with dist. H₂O (10 mL) and extracted with EtOAc. The organic layers were combined, dried and concentrated *in vacuo*. Purification was performed on a 2 mm chromatotron plate, which yielded dimethylated phloroacetophenone **43** was obtained in a low yield of 12.6% (45 mg).

4.4.2.4 1-[2,4-Bisbenzyloxy-6-(3-methylbut-2-enyloxy)phenyl]ethanone (**44**)



Method 1

Anhydrous K₂CO₃ (163 mg, 1.18 mmol) was added to bisbenzylated phloroacetophenone **41** (198 mg, 0.568 mmol) dissolved in acetone (8 mL) and the resulting reaction mixture was allowed to stir for 15 minutes before prenyl bromide (87 mg, 0.58 mmol), dissolved in acetone (3 mL), was added. The prenyl bromide addition

was carried out slowly. The reaction mixture was heated until it was gently refluxing (58 °C). The progress of the reaction was monitored by TLC. Although the reaction had not yet gone to completion, it was quenched with water (10 mL) after refluxing for a time period of 100 h. The reaction mixture was then partitioned with DCM and water. The organic layer was dried and the remaining solvent was removed *in vacuo*. The sample underwent purification on a 2 mm chromatotron plate (Hex:EtOAc 9:1) yielding the desired *O*-prenylated product (**44**) (81 mg, 34%).

¹H NMR (CDCl₃, 400 MHz): δ_H 1.73 (3H, s, H-5''), 1.79 (3H, s, H-4''), 2.51 (3H, s, H-2), 4.51 (2H, d, *J* = 7.0 Hz, H-1''), 5.05 (4H, s, H-6''), 5.44 (1H, t, *J* = 7.0 Hz, H-2''), 6.25 (1H, s, H-5') 6.27 (1H, s, H-3'), 7.40 (10H, m, Ar-H); ¹³C NMR (CDCl₃, 400 MHz): 18.0 (C-5''), 25.7 (C-4''), 32.6 (C-2), 65.5 (C-1''), 70.2 (C-6''), 93.0 (C-5'), 93.2 (C-3'), 119.3 (C-2''), 126.9-128.5 (C-Ar), 136.4, 137.5, 160.9, 157.3, 156.9, 201.2 (C-1); ESIMS: *m/z* 417.2067 [M+H]⁺ (calculated for C₂₇H₂₉O₄, 417.2066)

Method 2

Bisbenzylated phloroacetophenone **41** (350 mg, 1.01 mmol) was dissolved in DMF (10 mL) and placed in a 3-neck round bottom flask equipped with a stirrer bar, condenser, N₂ line and a septum. Anhydrous K₂CO₃ (280 mg, 2.03 mmol) was added to the vessel and the resulting reaction mixture was allowed to stir for 15 minutes before prenyl bromide (152 mg, 1.02 mmol) dissolved in DMF (3ml) was added. The prenyl bromide addition was carried out slowly and the resulting reaction mixture was heated to 80 °C. The progress of the reaction was monitored by TLC which showed that after 17 h only starting material was present therefore a further equivalent of K₂CO₃ (143 mg, 1.04 mmol) and 2 equivalents of prenyl bromide (310 mg, 2.08 mmol) was added and the temperature increased to 100 °C. Proton NMR spectra of the reaction mixture after 24 and 40 hours showed starting material but no *O*-alkylated product **44**. A small amount of dibenzo-18-crown-6 (**45**) was added. Although the reaction had not yet gone to completion it was quenched with 1 M HCl (10 mL) after 4.5 days. The reaction mixture was then extracted with DCM and the aqueous layer discarded. The combined DCM layers were washed with ice water (5 x 40 mL), brine (3 x 30 mL) and dried over MgSO₄. The remaining solvent was removed *in vacuo*. The sample underwent purification on a chromatotron (4 mm plate, Hex:EtOAc 9:1). The fractions collected were spotted on TLC (Hex:EtOAc 2:1).

The TLC did not show a good separation, thus the desired product **44** could not be isolated and hence the reaction was believed to be unsuccessful.

Method 3

Bisbenzylated phloroacetophenone **41** (100 mg, 0.29 mmol) dissolved in acetone (6 mL) was placed in a 2-necked reaction vessel equipped with a stirrer bar, thermometer, condenser and N₂ line. Anhydrous K₂CO₃ (119 mg, 0.86 mmol) and dibenzo-18-crown-6 (**45**) (106 mg, 0.29 mmol) was added and the reaction mixture was allowed to stir for 30 minutes before prenyl bromide (90 mg, 0.60 mmol) dissolved in acetone (2 mL) and copper iodide (CuI) (4 mg, 0.023 mmol) was added. The reaction mixture was heated until it was gently refluxing (59 °C). TLC showed that after 5 h the reaction had gone to completion. The reaction was quenched with dist. H₂O (5 mL). The reaction products were extracted with DCM (3 x 15 mL), the organic fractions were combined and the aqueous layer discarded. The organic layer was dried and the remaining solvent was removed. The sample underwent purification on a chromatotron (2 mm plate, Hex:EtOAc 9:1) yielding the desired *O*-prenylated product **44** (66 mg, 55%).

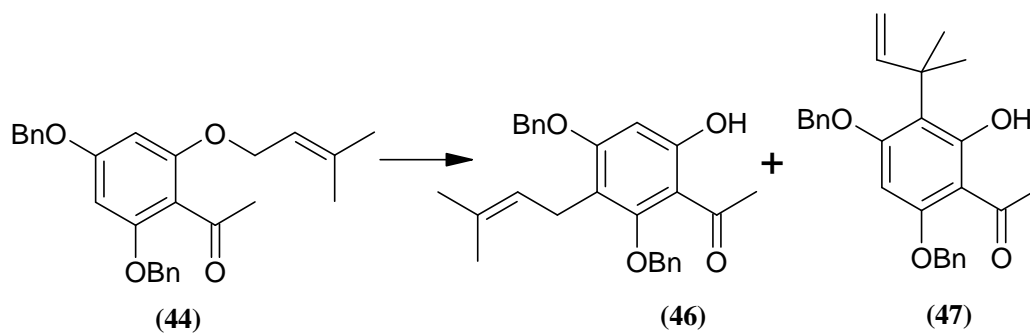
Method 4

Sodium hydride (NaH) (110 mg, 4.58 mmol) was placed into a 3-necked round-bottom flask fitted with a stirrer bar, thermometer, condenser N₂ line and a septum. Bisbenzylated phloroacetophenone **41** (502 mg, 1.44 mmol) was dissolved in THF (20 mL) and allowed to stir for 1.5 hours before adding prenyl bromide (430 mg, 2.89 mmol) dissolved in THF (2 mL). The reaction mixture was heated to 60 °C. After 4.5 h, TLC showed that the desired prenylated product **44** was present. The reaction was quenched with dist. H₂O (10 mL) and extracted with DCM (3 x 15 mL). The organic layer was dried and concentrated. The aqueous layer was discarded. The organic fraction was purified on a chromatotron, where the fraction was dissolved in DCM, loaded onto a 4 mm chromatotron plate and eluted with Hex:EtOAc (9:1). 1-[2,4-bisbenzyloxy-6-(3-methylbut-2-enyloxy)phenyl]ethanone **44** was obtained in 54% yield (322 mg).

Method 5

NaH (23 mg, 0.96 mmol) was placed into a 3-necked round bottom flask and dissolved in DMF (15 mL added to vessel *via* a canula). Bisbenzylated phloroacetophenone **41** (99 mg, 0.28 mmol) was added and the reaction mixture allowed to stir for a time period of 1.5 h before prenyl bromide (88 mg, 0.59 mmol) dissolved in DMF (2 mL) was added. The reaction mixture was heated to 80 °C. TLC showed that alkylation had taken place after 4 h. The reaction was quenched after 4.5 h with 1 M HCl (10 mL) and extracted with DCM. The aqueous layer was discarded and the organic fraction was washed with ice water (6 x 30 mL), dried and concentrated. Column chromatography (10 mL syringe plugged with cotton wool and filled to the 8 mL mark with silica gel, Hex:EtOAc 9:1) was used to remove the polar compound observed on the baseline of the TLC plate. Further purification of the product was performed on a chromatotron (2 mm plate, Hex:EtOAc 9:1). 1-[2,4-Bisbenzyloxy-6-(3-methylbut-2-enyloxy)phenyl]ethanone **44** was obtained in 45% yield (53 mg).

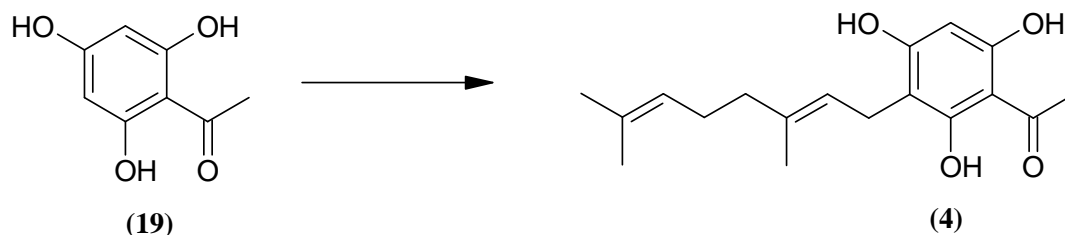
4.4.2.5 1-[2,4-Bisbenzyloxy-6-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**46**)



The *O*-prenylated bisbenzylated phloroacetophenone **44** (146 mg, 0.35 mmol) was dissolved in *N,N*-dimethylaniline (3 mL) and heated to 200 °C. The reaction was monitored by TLC, which showed that the reaction had gone to completion after a time period of 3 h, thus the reaction was quenched with 1 M HCl (10 mL). The reaction products were extracted with EtOAc and the organic fractions combined. The organic fraction was then washed with 1 M HCl (2 x 15 mL), dist. H₂O (2 x 15 mL) and brine (2 x 15 mL) before being dried and concentrated on a rotatory evaporator. The remaining residue was purified on a 2 mm chromatotron plate (Hex:EtOAc 9:1). NMR spectroscopy

showed that 50:50 mixture of the desired product **46** and (1-[4,6-bisbenzyloxy-3-(1,1-dimethylallyl)-2-hydroxyphenyl]ethanone **47** was obtained in 57% yield (83 mg).

4.4.2.6 1-[3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]ethanone (**4**)



Method 1

K_2CO_3 (130 mg, 0.94 mmol) was added to a solution of acetone (6 mL) and phloracetophenone (**19**) (110 mg, 0.654 mmol). The mixture was allowed to stir for 10 minutes before geranyl bromide (0.11 mL, 1.00 mmol) dissolved in acetone (2 mL) was added slowly and refluxed (58 °C) for 4 h. Although the reaction had not gone to completion it was quenched after 4 h by adding 1 M HCl (10 mL). The reaction products were extracted with DCM. The organic layers were combined, dried and concentrated. The extract was dissolved in DCM and purified on a 2 mm chromatotron plate eluted initially with Hex:EtOAc 9:1. As the separation progressed, the polarity of the solvent system was increased as follows: Hex:EtOAc 3:1, Hex:EtOAc 2:1, and finally washed with MeOH. The desired alkylated product **4** was obtained in a low yield of 11% (17 mg). UV(MeOH) λ_{max} (log ϵ): 287 (4.37) nm; IR ν_{max} : 3400, 3306, 2917, 1623 cm^{-1} ; ^1H NMR (CD_3OD , 400 MHz): δ_{H} 1.55 (3H, s, H-10''), 1.61 (3H, s, H-8''), 1.73 (3H, s, H-9''), 1.93 (2H, m, H-4''), 2.03 (2H, m, H-5''), 2.60 (3H, s, H-2), 3.18 (2H, d, $J = 7.3$ Hz, H-1''), 5.05 (1H, t, $J = 7.0$ Hz, H-6''), 5.17 (1H, t, $J = 7.0$ Hz, H-2''), 5.90 (1H, s, H-5'); ^{13}C NMR (CD_3OD , 400 MHz): δ_{C} 16.3 (C-9''), 17.8 (C-10''), 22.2 (C-1''), 25.9 (C-8''), 27.9 (C-5''), 33.0 (C-2), 41.1 (C-4''), 95.0 (C-5'), 108.1 (C-3'), 105.7 (C-1'), 124.8 (C-2''), 125.7 (C-6''), 132.1 (C-7''), 134.9 (C-3''), 161.9 (C-6'), 164.1 (C-4'), 164.9 (C-2'), 204.7 (C-1); ESIMS: m/z 327.1571 [$\text{M}+\text{Na}$] $^+$ (calculated for $\text{C}_{18}\text{H}_{24}\text{O}_4\text{Na}$, 327.1572).

Method 2

K₂CO₃ (333 mg, 2.41 mmol) was added to a solution of acetone (10 mL) and phloroacetophenone (**19**) (198 mg, 1.18 mmol). The mixture was allowed to stir for 10 minutes before geranyl bromide (0.23 mL, 1.19 mmol), dissolved in acetone (2 mL), was added slowly and refluxed for 4 h. The reaction mixture was removed from the heat source after 1 day and the acetone evaporated off, leaving a dark brown oil-like residue. 1 M HCl (20 mL) was added to the residue and extracted with EtOAc (3 x 50 mL). The organic layers were combined, dried and concentrated *in vacuo*. The organic layer was dissolved in DCM and purified on a 4 mm chromatotron plate eluted initially with Hex:EtOAc (7:1). As the separation progressed the polarity of the solvent system was increased as follows: Hex:EtOAc (3:1), Hex:EtOAc (1:1), Hex:EtOAc (1:2), 100% EtOAc and finally washed with MeOH. 3-Geranyl-2,4,6-trihydroxyacetophenone **4** was obtained in 23% yield (83 mg).

Method 3

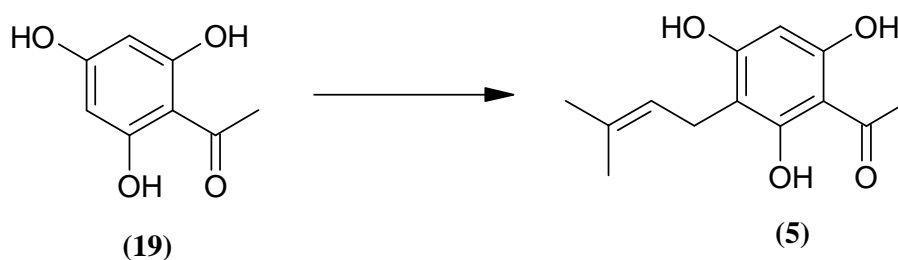
Phloroacetophenone (**19**) (203 mg, 1.21 mmol) was dissolved in an aq. KOH solution [KOH (98 mg, 1.75 mmol) dissolved in 3 mL dist. H₂O] and placed in an ice-bath (0 °C). Geranyl bromide (0.23 mL, 1.19 mmol) was added dropwise over a period of 15 minutes. The reaction mixture was allowed to stir at 0 °C for 2 h. The reaction was quenched after 2 h by pouring the reaction mixture over 1 M HCl (6 mL). The reaction products were extracted with EtOAc; organic fractions were combined, dried and concentrated. The remaining residue was purified on a 4 mm chromatotron plate eluted with: Pet. Ether:EtOAc (7:1), Pet. Ether:EtOAc (3:1), Pet. Ether:EtOAc (1:1), Pet. Ether:EtOAc (2:1), 100% EtOAc. The desired product **4** was not obtained and thus the reaction was deemed to be unsuccessful.

Method 4

Phloroacetophenone (**19**) (200 mg, 1.20 mmol) and NaH (86 mg, 3.59 mmol) was dissolved in dioxane (10 mL). The reaction mixture was allowed to stir for 30 min at room temperature (26 °C). Geranyl bromide (0.34 mL, 1.80 mmol) was added and the resulting

mixture was heated to 60 °C and stirred for a further 4 hr. The reaction was quenched after this time period with dist. H₂O (10 mL). The reaction products were extracted with EtOAc (2 x 30 mL); organic fractions were combined, dried and concentrated. The remaining residue was purified on a 2 mm chromatotron plate (eluted with: Hex:EtOAc 9:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1, Hex:EtOAc 1:1, 100% EtOAc), which yielded the desired product **4** (105 mg, 29 %).

4.4.2.7 1-[2,4,6-Trihydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**5**)



Method 1

Phloroacetophenone (**19**) (210 mg, 1.25 mmol) was dissolved in acetone (20 mL) before K₂CO₃ (338 mg, 2.45 mmol) was added and the reaction mixture was allowed to stir for a time period of 10 minutes. Prenyl bromide (0.14 mL, 1.19 mmol) dissolved in acetone (3 mL) was added to the reaction vessel and refluxed (60 °C) for 1 day. The acetone was removed *in vacuo* leaving a brown residue in the flask. The residue was acidified with 1 M HCl (20 mL) and extracted with EtOAc (4 x 30 mL). The organic layers were combined, dried and concentrated. The organic layer was purified on a 4 mm chromatotron plate (Hex:EtOAc 7:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1, Hex:EtOAc 1:1, Hex:EtOAc 2:1, 100% EtOAc), which yielded 2,4,6-trihydroxy-3-prenylacetophenone (**5**) (89 mg, 34%).

UV (MeOH) λ_{\max} (log ϵ): 289 (360) nm; IR ν_{\max} : 3527, 3287, 2930, 2453, 1598 cm⁻¹; ¹H NMR (CD₃OD, 400 MHz): δ_{H} 1.63 (3H, s, H-5''), 1.73 (3H, s, H-4''), 2.60 (3H, s, H-2), 3.17 (2H, d, $J = 7.2$ Hz, H-1''), 5.17 (1H, t, $J = 7.2$ Hz, H-2''), 5.90 (1H, s, H-5'); ¹³C NMR (CD₃OD, 400 MHz): δ_{C} 17.9 (C-4''), 22.3 (C-1''), 26.1 (C-5''), 33.0 (C-2), 95.0 (C-5'), 105.7 (C-1'), 108.1 (C-3'), 124.7 (C-2''), 131.3 (C-3''), 162.0 (C-6'), 164.0 (C-4'),

164.9 (C-2'), 204.8 (C-1); ESIMS: m/z 259.0948 $[M+Na]^+$ (calculated for $C_{13}H_{16}O_4Na$, 259.0946).

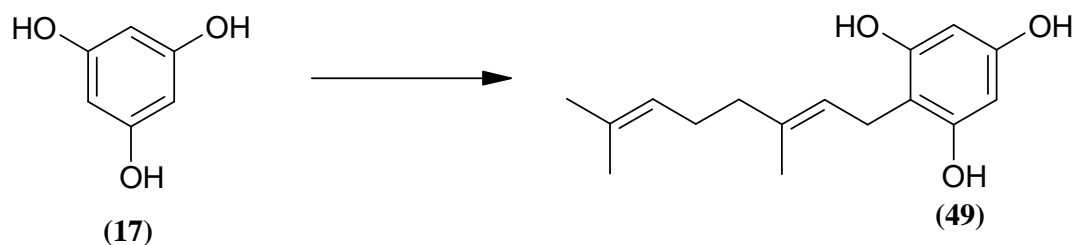
Method 2

Phloroacetophenone (**19**) (215 mg, 1.28 mmol) was dissolved in an Aq. KOH solution and cooled to 0 °C in an ice bath. The aq. KOH solution was prepared by dissolving KOH (92 mg, 1.64 mmol) in dist. H₂O (4 mL). Prenyl bromide (0.14 mL, 1.19 mmol) was added dropwise over a period of 10 minutes. The reaction mixture was allowed to stir at 0 °C for 1 h, after which it was poured over 1 M HCl (6 mL). The reaction products were extracted with EtOAc and the combined organic fractions were dried and solvent removed on a rotatory evaporator. The remaining residue was purified on a 4 mm chromatotron plate (Pet. Ether:EtOAc 7:1, Pet. Ether:EtOAc 4:1, Pet. Ether:EtOAc 2:1, Pet. Ether:EtOAc 1:1, 100% EtOAc). Fractions were analysed by NMR spectroscopy, of which none of the spectra illustrated the presence of the desired prenylated phloroacetophenone **5**. Thus this reaction was deemed to be unsuccessful.

Method 3

Phloroacetophenone (**19**) (200 mg, 1.20 mmol) and NaH (86 mg, 3.59 mmol) was dissolved in dioxane (10 mL). The reaction mixture was allowed to stir for 30 min at room temperature (27 °C). Prenyl bromide (0.21 mL, 1.80 mmol) was added using a syringe and the resulting mixture was heated to 60 °C and stirred for a further 4.5 hr. The reaction was quenched after this time period with dist. H₂O (10 mL). The reaction products were extracted with EtOAc (2 x 30 mL); organic fractions were combined, dried and concentrated. The remaining residue was purified on a 2 mm chromatotron plate (eluted with: Hex:EtOAc 9:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1, Hex:EtOAc 1:1, 100% EtOAc). 2,4,6-Trihydroxy-3-prenylacetophenone (**5**) was obtained in 29% yield (83 mg)

4.4.2.8 Attempted synthesis of 2-(3,7-dimethylocta-2,6-dienyl)benzene-1,3,5-triol (**49**)



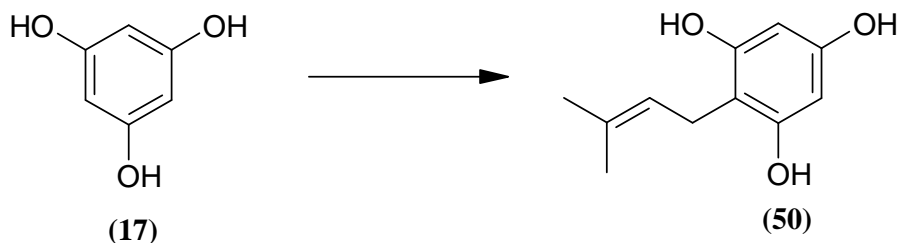
Method 1

Phloroglucinol (**17**) (210 mg, 1.67 mmol) and ZnCl_2 (445 mg, 3.27 mmol) were dissolved in acetone (20 mL). Geranyl bromide (0.45 mL, 2.38 mmol) was added *via* a septum using a syringe. The reaction mixture was refluxed at 60 °C. After a time period of 1 h TLC showed that the reaction had gone to completion, thus the reaction was quenched with dist. H_2O (10 mL) and extracted with DCM. The organic fractions were dried and concentrated *in vacuo*. The organic layer was purified on a 2 mm chromatotron plate (Hex:EtOAc 9:1). The reaction was unsuccessful.

Method 2

Phloroglucinol (**17**) (200 mg, 1.59 mmol) and K_2CO_3 (440 mg, 3.21 mmol) were dissolved in acetone (20 mL) and geranyl bromide (0.30 mL, 1.59 mmol) was added *via* a syringe. The reaction mixture was refluxed (60 °C). The reaction vessel was removed from the heat source after 1 day and the acetone was evaporated off. The remaining residue was acidified with 1 M HCl (20 mL) and extracted with EtOAc (4 x 30 mL). The organic fractions were combined and dried. The concentrated sample was purified on a 4 mm chromatotron plate (Hex:EtOAc 7:1, Hex:EtOAc 4:1, Hex:EtOAc 1:1, Hex:EtOAc 1:2 and 100% EtOAc). The reaction was considered to be unsuccessful as the desired alkylated phloroglucinol **49** was not obtained.

4.4.2.9 Attempted synthesis of 2-(3-methylbut-2-enyl)benzene-1,3,5-triol (**50**)

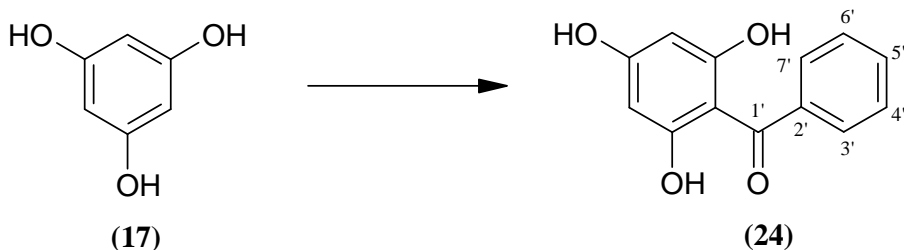


Phloroglucinol (**17**) (205 mg, 1.63 mmol) and ZnCl_2 (425 mg, 3.12 mmol) were dissolved in acetone (20 mL) and prenyl bromide (360 mg, 2.42 mmol) was added *via* a septum using a syringe. The reaction mixture was refluxed at 59 °C. After a time period of 1 h TLC showed that the reaction had gone to completion. Thus the reaction was quenched after 1 h with dist. H_2O (10 mL) and extracted with DCM. The organic fractions were dried, concentrated and further purified on a 2 mm chromatotron plate (Hex:EtOAc 9:1). The desired product **50** was not obtained and therefore the reaction was believed to be unsuccessful.

4.4.2.10 Preparation of acyl chlorides

A 2-necked round bottom flask was set-up with a condenser thermometer and stirring bar. A pipe was attached to the top of the condenser with a funnel on the other end leading into a bath of water. This was done in order to trap the HCl gas that is released as a by-product of the reaction. Isobutyric acid (10 mL, 110 mmol) or 2-methylbutyric acid (10 mL, 91.7 mmol) and 1 equivalent of SOCl_2 (6.7 mL, 91.7 mmol and 8 mL, 110 mmol, respectively) were injected into the vessel and heated until gently refluxing. The reaction mixture was refluxed for 3 h. Distillation was then carried out in order to separate the acyl chloride product from any remaining acid or SOCl_2 . Isobutyryl chloride was collected at a temperature of 92 °C and 2-methylbutyryl chloride was collected 112 °C.

4.4.2.11 Phenyl-(2,4,6-trihydroxyphenyl)methanone (**24**)



Method 1

Phloroglucinol (**17**) (205 mg, 1.63 mmol) and ZnCl_2 (490 mg, 3.60 mmol) were dissolved in DCM (25 mL). Benzoyl chloride (0.28 mL, 2.38 mmol) was added to the vessel through the septum using a syringe. The reaction mixture was gently refluxed (40 °C) for 2.5 h. After this time period the reaction was quenched with dist. H_2O (10 mL) and extracted with DCM. The organic layers were dried and the remaining solvent was removed *in vacuo*. The concentrated sample was purified on a 2 mm chromatotron plate, eluted with Hex:EtOAc (9:1). The reaction was unsuccessful as 2,4,6-trihydroxybenzophenone (**24**) was not obtained.

Method 2

Phloroglucinol (**17**) (200 mg, 1.59 mmol) and AlCl_3 (846 mg, 6.34 mmol) were dissolved in carbon disulfide (2.5 mL). Nitrobenzene (1.5 mL) was added over a time period of 5 min. The reaction mixture was refluxed (50 °C) for 30 min. before benzoyl chloride (0.18 mL, 1.59 mmol) dissolved in nitrobenzene (1 mL) was added to the vessel over 5 min. The reaction mixture was refluxed (50 °C) for a further 30 min. The reaction vessel was then removed from the heat source and allowed to stir whilst cooling to room temperature. Dist. H_2O (10 mL) and 1 M HCl (5 mL) were added. The reaction products were extracted with EtOAc, the organic layers combined, dried and concentrated. The concentrated sample was purified on a 2 mm chromatotron plate, eluted with progressively polar solvent systems: Hex:EtOAc 7:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1, Hex:EtOAc 1:2 and 100% EtOAc. 2,4,6-Trihydroxy-benzophenone (**24**) was obtained in 19% yield (56 mg).

UV(MeOH) λ_{\max} (log ϵ): 306 (4.36) nm; IR ν_{\max} : 3185, 1597 cm^{-1} ; ^1H NMR (CD_3OD , 400 MHz): δ_{H} 5.81 (2H, s, H-3, H-5), 7.36 (2H, t, $J = 7.4$ Hz, H-4', H-6'), 7.45 (1H, t, $J = 7.45$ Hz, H-5'), 7.58 (2H, d, $J = 7.4$ Hz, H-3', H-7'); ^{13}C NMR (CD_3OD , 400 MHz): δ_{C} 96.0 (C-3, C-5), 106.1 (C-1), 128.8 (C-4', C-6'), 129.4 (C-3', C-7'), 132.2 (C-5'), 143.1 (C-2'), 164.0 (C-2, C-6), 166.1 (C-4), 200.8 (C-1'); ESIMS: m/z 253.0473 [$\text{M}+\text{Na}$] $^+$ (calculated for $\text{C}_{13}\text{H}_{10}\text{O}_4\text{Na}$, 253.0477).

Method 3

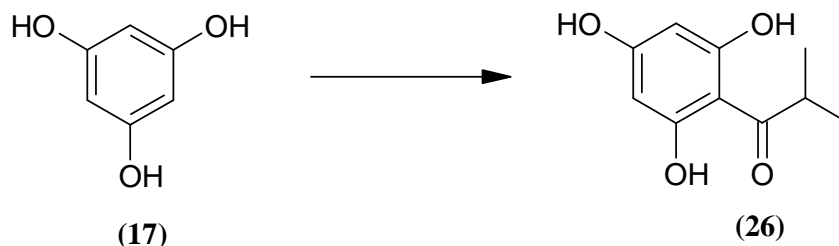
Phloroglucinol (**17**) (500 mg 3.97 mmol) was dissolved in DCM (8 mL) and cooled on ice (0 °C). In a separate flask, AlCl_3 (1.18 g, 8.86 mmol) was dissolved in DCM (10 mL) and cooled on ice (0 °C). Benzoyl chloride (0.46 mL, 3.97 mmol) was added dropwise with a syringe. The resulting mixture was allowed to stir under N_2 for 2.5 h. This mixture was then added slowly to the phloroglucinol solution. The reaction mixture was removed from the ice bath and set aside. Once room temperature was reached the reaction mixture was stirred for a further 22 h before being quenched by the addition of 1 M HCl (15 mL) and dist. H_2O (10 mL). The reaction products were extracted with DCM (2 x 20 mL) followed by EtOAc (3 x 30 mL). All 5 organic fractions were combined, washed with sat. NaHCO_3 (3 x 30 mL), dist. H_2O (3 x 30 mL) and brine (3 x 30 mL), dried over MgSO_4 and concentrated *in vacuo*. Purification was carried out on a 2 mm chromatotron plate, eluting with Hex:EtOAc 6:1 followed by Hex:EtOAc 1:1. The desired product **24** was obtained as a pale yellow solid (415 mg, 46%).

Method 4

Phloroglucinol (**17**) (830 mg, 6.58 mmol) and AlCl_3 (3.41 g, 25.6 mmol) were dissolved in nitrobenzene (12 mL) and allowed to stir at room temperature for 30 min. Benzoyl chloride (0.74 mL, 6.34 mmol) was added with a syringe and the resulting mixture was heated to 80 °C for 1.5 h. During this time the reaction mixture changed colour from pale yellow to black. The reaction mixture was cooled to room temperature before 1 M HCl (20 mL) and dist. H_2O (15 mL) was added. The reaction products were extracted with EtOAc (4 x 30 mL), organic fractions combined and washed with sat. NaHCO_3 (3 x 30 mL), dist. H_2O (3 x 30 mL) and brine (3 x 30 mL). The EtOAc fraction was then dried and concentrated. Purification was carried out on a 4 mm chromatotron plate

(Hex:EtOAc 6:1, Hex:EtOAc 1:1 and 100% EtOAc), which yielded **24** as a pale yellow solid (745 mg, 49%).

4.4.2.12 2-Methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**)



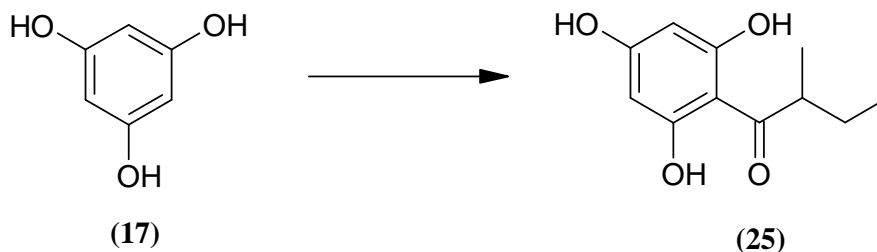
Method 1

Phloroglucinol (**17**) (810 mg, 6.42 mmol) was dissolved in DCM (8 mL) and cooled on ice (0 °C). In a separate flask, AlCl₃ (1.69 g, 12.7 mmol) was dissolved in DCM (10 mL) and cooled on ice (0 °C). Isobutyryl chloride (0.92 mL, 9.52 mmol) was added dropwise with a syringe. The resulting mixture was allowed to stir under N₂ for 1 h. This mixture was then added slowly to the phloroglucinol solution. The reaction mixture was removed from the ice bath and set aside. Once room temperature was reached the reaction mixture was stirred for a further 16 h before being quenched by the addition of 1 M HCl (15 mL) and dist. H₂O (10 mL). The reaction products were extracted with DCM (1 x 20 mL) followed by EtOAc (3 x 30 mL). All organic fractions were combined, washed with sat. NaHCO₃ (3 x 30 mL), dist. H₂O (3 x 30 mL) and brine (3 x 30 mL), dried over MgSO₄ and concentrated *in vacuo*. The concentrated fraction was purified on a 2 mm chromatotron plate, eluting with progressively polar solvent systems: Hex:EtOAc 9:1, Hex:EtOAc 6:1, Hex:EtOAc 1:1 and 100% EtOAc. 2-Methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**) was obtained as a pale pink solid (493 mg, 36%).

UV (MeOH) λ_{\max} (log ϵ): 229 (4.60), 287 (4.73) nm; IR ν_{\max} : 3187, 2975, 1570, 1515 cm⁻¹; ¹H NMR (CD₃OD, 400 MHz): δ_{H} 1.12 (6H, d, $J = 6.7$ Hz, H-3, H-4), 3.97 (1H, heptet, $J = 6.7$ Hz, H-2), 5.82 (2H, s, H-3', H-5'); ¹³C NMR (CD₃OD, 400 MHz): δ_{C} 19.8 (C-3, C-4), 40.0 (C-2), 96.1 (C-3', C-6'), 104.8 (C-1') 165.8 (C-2', C-4', C-6'), 211.9 (C-1); ESIMS: m/z 219.0633 [M+Na]⁺ (calculated for C₁₀H₁₂O₄Na, 219.0633).

Method 2

Phloroglucinol (**17**) (820 mg, 6.50 mmol) and AlCl₃ (3.36 g, 25.2 mmol) were dissolved in nitrobenzene (15 mL) and allowed to stir at room temperature for 30 min. Isobutyryl chloride (0.92 mL, 9.52 mmol) was added with a syringe and the resulting mixture was heated to 80 °C. The reaction mixture was quenched after 1 h by adding 1 M HCl (15 mL) and dist. H₂O (10 mL). The reaction products were extracted with EtOAc (3 x 30 mL), organic fractions combined and washed with sat. NaHCO₃ (3 x 30 mL), dist. H₂O (3 x 30 mL) and brine (3 x 30 mL). The EtOAc fraction was then dried and concentrated. The concentrated fraction was purified on two 4 mm chromatotron plates, eluting with progressively polar solvent systems: Hex:EtOAc (9:1), Hex:EtOAc (6:1), Hex:EtOAc (3:1), Hex:EtOAc (1:1) and 100% EtOAc. The collected fractions were combined appropriately and the desired product **26** was obtained in 66% yield (848 mg).

4.4.2.13 2-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (25)**Method 1**

Phloroglucinol (**17**) (395 mg 3.13 mmol) was dissolved in DCM (8 mL) and cooled on ice (0 °C). In a separate flask, AlCl₃ (850 mg, 6.38 mmol) was dissolved in DCM (10 mL) and cooled on ice (0 °C). 2-Methylbutyryl chloride (0.39 mL, 3.17 mmol) was added dropwise with a syringe. The resulting mixture was allowed to stir under N₂ for 1 h. This mixture was then added slowly (dropwise) to the phloroglucinol solution. The reaction mixture was removed from the ice bath and set aside. Once room temperature was reached the reaction mixture was stirred for a further 15 h before being quenched by the addition of 1 M HCl (10 mL) and dist. H₂O (10 mL). The reaction products were extracted with DCM (1 x 20 ml) followed by EtOAc (3 x 30 mL). All organic fractions were combined, washed with sat. NaHCO₃ (3 x 30 mL), dist. H₂O (3 x 30 mL) and brine (3 x 30 mL), dried and

concentrated *in vacuo*. The concentrated fraction was purified on a 2 mm chromatotron plate (Hex:EtOAc 6:1 followed by 100% EtOAc), yielding **25** (298 mg, 45%).

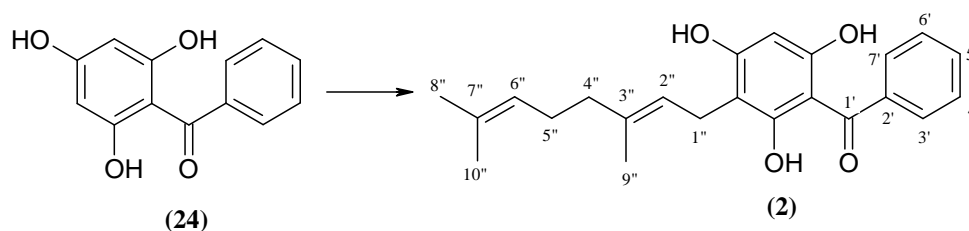
UV (MeOH) λ_{max} (log ϵ): 228 (4.52), 288 (4.68) nm; IR ν_{max} : 3206, 2975, 1567 cm^{-1} ; ^1H NMR (CD_3OD , 400 MHz): δ_{H} 0.89 (3H, t, $J = 7.4$ Hz, H-4), 1.10 (3H, d, $J = 6.7$ Hz, H-5), 1.36 (1H, m, H-3), 1.80 (1H, m, H-3), 3.85 (1H, sextet, $J = 6.7$ Hz, H-2), 5.81 (2H, s, H-3', H-5'); ^{13}C NMR (CD_3OD , 400 MHz): δ_{C} 12.5 (C-4), 17.3 (C-5), 28.3 (C-3), 46.8 (C-2), 96.1 (C-3', C-5'), 105.5 (C-1'), 166.0 (C-2', C-4', C-6'), 211.6 (C-1); ESIMS: m/z 211.0968 $[\text{M}+\text{H}]^+$ (calculated for $\text{C}_{11}\text{H}_{15}\text{O}_4$, 211.0970).

Method 2

Phloroglucinol (**17**) (400 mg, 3.17 mmol) and AlCl_3 (1.73 g, 12.9 mmol) were dissolved in nitrobenzene (8 mL) and allowed to stir at room temperature for 30 min. 2-Methylbutyryl chloride (0.39 mL, 3.17 mmol) was added with a syringe and the resulting mixture was heated to 60 °C. The reaction mixture was quenched after 2 h by adding 1 M HCl (15 mL) and dist. H_2O (10 mL). The reaction products were extracted with EtOAc (3 x 30 mL), organic fractions combined and washed with sat. NaHCO_3 (3 x 30 mL), dist. H_2O (3 x 30 mL) and brine (3 x 30 mL). The EtOAc fraction was then dried over MgSO_4 and concentrated *in vacuo*. The concentrated fraction was purified on a 4 mm chromatotron plate (Hex:EtOAc 9:1, Hex:EtOAc 6:1 and 100% EtOAc). 2-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**25**) was obtained in 57% yield (371 mg).

4.4.2.14 [3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone

(2)



Method 1

2,4,6-Trihydroxybenzophenone (**24**) (134 mg, 0.58 mmol) and K_2CO_3 (166 mg, 1.20 mmol) were dissolved in acetone (10 mL) and allowed to stir at room temperature for 15 min. Geranyl bromide (0.11 mL, 0.58 mmol) was injected into the vessel and the reaction mixture was heated until gently refluxing (60 °C). The reaction was quenched after 46 h by adding 1 M HCl (20 mL). The reaction products were extracted with EtOAc (3 x 30 mL), organic fractions were combined, dried and concentrated. Purification was carried out on a 2 mm chromatotron plate eluting with progressively polar solvent systems: Hex:EtOAc 9:1, Hex:EtOAc 6:1, Hex:EtOAc 3:1, Hex:EtOAc 1:1 and 100% EtOAc. The C-alkylated product, 3-geranyl-2,4,6-trihydroxybenzophenone (**2**) was obtained in a low yield of 6% (12 mg). UV (MeOH) λ_{max} (log ϵ): 310 (4.41) nm; 1H NMR (CD_3OD , 400 MHz): δ_H 1.56 (3H, s, H-10''), 1.61 (3H, s, H-8''), 1.76 (3H, s, H-9''), 1.96 (2H, m, H-4''), 2.05 (2H, m, H-5''), 3.24 (2H, d, $J = 7.0$ Hz, H-1''), 5.06 (1H, t, $J = 7.0$ Hz, H-6''), 5.23 (1H, t, $J = 7.0$ Hz, H-2''), 5.88 (1H, s, H-5), 7.35 (2H, t, $J = 7.4$ Hz, H-4', H-6'), 7.42 (1H, t, $J = 7.4$ Hz, H-5'), 7.50 (2H, d, $J = 7.4$ Hz, H-3', H-7'); ^{13}C NMR (CD_3OD , 400 MHz): δ_C 16.4 (C-9''), 17.9 (C-10''), 22.4 (C-1''), 26.0 (C-8''), 27.9 (C-5''), 41.1 (C-4''), 95.5 (C-5), 105.6 (C-1), 108.5 (C-3), 124.6 (C-2''), 125.7 (C-6''), 128.7 (C-4', C-6'), 129.3 (C-3', C-7'), 131.8 (C-5'), 132.1 (C-7''), 135.1 (C-3''), 143.7 (C-2'), 160.7 (C-6), 163.5 (C-4), 164.4 (C-2), 201.2 (C-1'); ESIMS: m/z 389.1733 $[M+Na]^+$ (calculated for $C_{23}H_{26}O_4Na$, 389.1729).

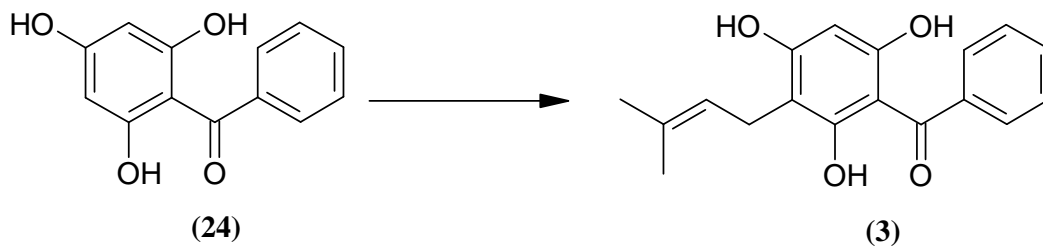
The O-alkylated product was also isolated as the major product in 12% yield (26 mg). 1H NMR (CD_3OD , 400 MHz): δ_H 1.59 (3H, s, H-10''), 1.61 (3H, s, H-8''), 1.66 (3H, s, H-9''), 1.87 (2H, m, H-4''), 1.96 (2H, m, H-5''), 4.26 (2H, d, $J = 6.5$ Hz, H-1''), 4.73 (1H, t, $J = 6.5$ Hz, H-6''), 5.02 (1H, t, $J = 6.8$ Hz, H-2''), 5.94 (1H, d, $J = 2.1$ Hz), 5.99 (1H, d, $J = 2.1$ Hz), 7.46 (5H, m, H-3' - H-7'); ^{13}C NMR (CD_3OD , 400 MHz): δ_C 16.8 (C-9''), 17.9 (C-10''), 26.0 (C-8''), 27.4 (C-5''), 40.5 (C-4''), 66.3 (C-1''), 93.6 (C-5), 96.9 (C-3), 107.2 (C-1), 120.0 (C-6''), 125.1 (C-2''), 128.8 - 129.1 (C-3' - C-7'), 132.2 (C-7''), 132.6 (C-3''), 141.5, 143.4 (C-2'), 162.9 (C-6), 165.0 (C-4), 165.9 (C-2), 200.6 (C-1')

Method 2

2,4,6-Trihydroxybenzophenone (**24**) (196 mg, 0.85 mmol) and AlCl₃ (452 mg, 3.39 mmol) were dissolved in nitrobenzene (15 mL) and allowed to stir at room temperature for 30 min. Geranyl bromide (0.25 mL, 1.30 mmol) was injected into the vessel and the reaction mixture was heated (80 - 100 °C). The reaction was quenched after 42 h by adding 1 M HCl (20 mL). The reaction products were extracted with EtOAc (3 x 30 mL), organic fractions were combined, dried and concentrated. Purification was carried out on a 4 mm chromatotron plate eluting with progressively polar solvent systems: Hex:EtOAc 9:1, Hex:EtOAc 6:1, Hex:EtOAc 3:1, Hex:EtOAc 1:1 and 100% EtOAc. NMR data did not show evidence of the desired product and thus this reaction was deemed to be unsuccessful.

Method 3

2,4,6-Trihydroxybenzophenone (**24**) (146 mg, 0.63 mmol) and NaH (46 mg, 1.92 mmol) were dissolved in dioxane (10 mL). The reaction mixture was heated to 60 °C and allowed to stir for 30 min before geranyl bromide (0.19 ml, 0.98 mmol) was added. The reaction was stirred for a further 3.5 h, after which it was quenched with dist. H₂O (10 mL). The reaction products were extracted with EtOAc, dried and concentrated. Purification on a 2 mm chromatotron plate (Hex:EtOAc 9:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1, Hex:EtOAc 1:1 and 100% EtOAc), which afforded the desired product, 3-geranyl-2,4,6-trihydroxybenzophenone (**2**) (40 mg, 17%).

4.4.2.15 Phenyl-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]methanone (3**)**

Method 1

2,4,6-Trihydroxybenzophenone (**24**) (253 mg, 1.10 mmol) and K_2CO_3 (320 mg, 2.32 mmol) were dissolved in acetone (15 mL) and allowed to stir at room temperature for 30 min. Prenyl bromide (0.13 mL, 0.87 mmol) was injected into the vessel and the reaction mixture was heated until gently refluxing (60 °C). Monitoring by TLC showed no sign of C-alkylation having occurred after a time period of 23 h, thus the temperature was increased to 80 °C. The reaction was quenched after 48 h by adding 1 M HCl (20 mL). The reaction products were extracted with EtOAc (3 x 30 mL), organic fractions were combined, dried and concentrated. Purification was carried out on a 2 mm chromatotron plate eluting with progressively polar solvent systems: Hex:EtOAc 9:1, Hex:EtOAc 7:1, Hex:EtOAc 3:1, Hex:EtOAc 1:1 and 100% EtOAc. This reaction was unsuccessful as the desired product **3** was not obtained.

Method 2

2,4,6-Trihydroxybenzophenone (**24**) (198 mg, 0.86 mmol) and $AlCl_3$ (456 mg, 3.42 mmol) were dissolved in nitrobenzene (15 mL) and allowed to stir at room temperature for 30 min. Prenyl bromide (0.15 mL, 1.30 mmol) was injected into the vessel and the reaction mixture was heated (80 - 100 °C). The reaction was quenched after 42 h by adding 1 M HCl (10 mL). The reaction products were extracted with EtOAc (3 x 30 mL); organic fractions were combined, dried and concentrated *in vacuo*. Column chromatography (diameter: 3 cm, silica height: 15 cm, loaded as a slurry, eluted with progressively polar solvent systems: Hex:EtOAc 9:1, Hex:EtOAc 6:1, Hex:EtOAc 3:1, Hex:EtOAc 1:1 and 100% EtOAc) was used to purify the concentrated organic fraction. The NMR data acquired showed no evidence of **3** and hence the reaction was believed to be unsuccessful.

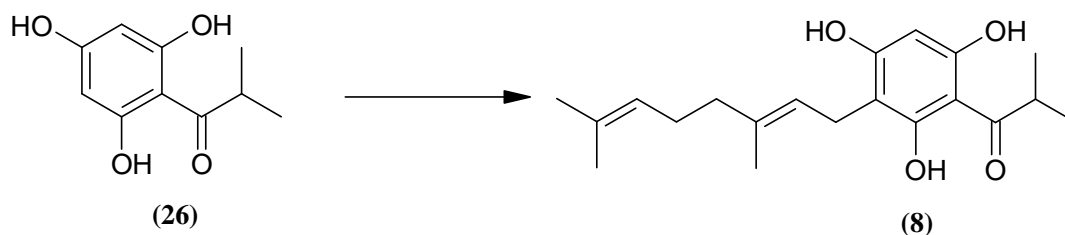
Method 3

2,4,6-Trihydroxybenzophenone (**24**) (199 mg, 0.86 mmol) and NaH (63 mg, 1.96 mmol) were dissolved in dioxane (10 mL). The reaction mixture was heated to 60 °C and allowed to stir for 30 min before prenyl bromide (0.15 mL, 1.30 mmol) was added. The reaction

was stirred for a further 3.5 h, after which it was quenched with dist. H₂O (10 mL). The reaction products were extracted with EtOAc, dried and concentrated. Purification was performed on a 2 mm chromatotron plate, eluting with Hex:EtOAc (9:1), Hex:EtOAc (4:1), Hex:EtOAc (2:1), Hex:EtOAc (1:1) and 100% EtOAc. 2,4,6-Trihydroxy-3-prenylbenzophenone (**3**) was obtained (48 mg, 19%).

UV(MeOH) λ_{\max} (log ϵ): 310 (4.30) nm; IR ν_{\max} : 3179, 2919, 1598 cm⁻¹; ¹H NMR (CD₃OD, 400 MHz): δ_{H} 1.66 (3H, s, H-5''), 1.76 (3H, s, H-4''), 3.24 (2H, d, $J = 7.2$ Hz, H-1''), 5.24 (1H, t, $J = 7.2$ Hz, H-2''), 5.89 (1H, s, H-5), 7.36 (2H, t, $J = 7.4$ Hz, H-4', H-6'), 7.42 (1H, t, $J = 7.4$ Hz, H-5'), 7.53 (2H, d, $J = 7.4$ Hz, H-3', H-7'); ¹³C NMR (CD₃OD, 400 MHz): δ_{C} 18.0 (C-4''), 22.4 (C-1''), 26.1 (C-5''), 95.5 (C-5), 105.6 (C-1), 108.5 (C-3), 124.6 (C-2''), 128.7 (C-4', C-6'), 129.4 (C-3', C-7'), 131.4 (C-5'), 131.8 (C-3''), 143.6 (C-2'), 160.6 (C-6), 163.4 (C-4), 164.4 (C-2), 201.2 (C-1'); ESIMS: m/z 321.1103 [M+Na]⁺ (calculated for C₁₈H₁₈O₄Na, 321.1103).

4.4.2.16 [3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylpropan-1-one (**8**)

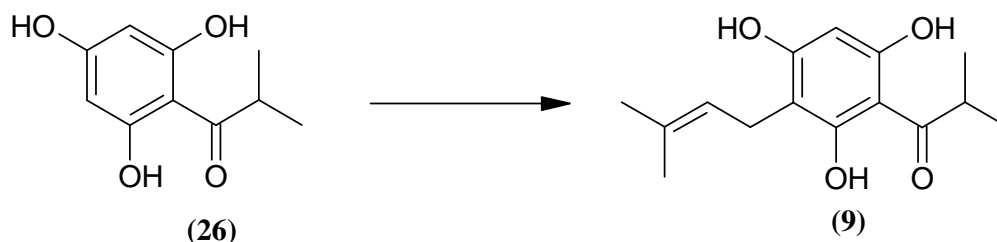


Method 1

2-Methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**) (152 mg, 0.78 mmol) and NaH (55 mg, 2.29 mmol) were dissolved in dioxane (10 mL). The reaction mixture was heated to 60 °C and allowed to stir for 30 min before adding geranyl bromide (0.22 mL, 1.15 mmol). The reaction was stirred for a further 3.5 h before being quenched with dist. H₂O (10 mL). The reaction products were extracted with EtOAc, dried and concentrated. The remaining residue was purified on a 2 mm chromatotron plate (Hex:EtOAc 9:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1 and 100% EtOAc), which yielded the desired product (**8**) (37 mg, 15%).

UV(MeOH) λ_{\max} (log ϵ): 290 (4.22) nm; ^1H NMR (CD_3OD , 400 MHz): δ_{H} 1.12 (6H, d, $J = 6.9$ Hz, H-3, H-4), 1.54 (3H, s, H-10''), 1.60 (3H, s, H-8''), 1.73 (3H, s, H-9''), 1.93 (2H, m, H-4''), 2.02 (2H, m, H-5''), 3.18 (2H, d, $J = 7.0$ Hz, H-1''), 4.00 (1H, heptet, $J = 6.6$ Hz, H-2), 5.04 (1H, t, $J = 7.0$ Hz, H-6''), 5.17 (1H, t, $J = 7.0$ Hz, H-2''), 5.89 (1H, s, H-5'); ^{13}C NMR (CD_3OD , 400 MHz): δ_{C} 16.3 (C-9''), 17.8 (C-10''), 19.9 (C-3, C-4), 22.3 (C-1''), 26.0 (C-8''), 27.8 (C-5''), 40.0 (C-2), 41.0 (C-4''), 95.1 (C-5'), 104.7 (C-1'), 108.4 (C-3'), 124.8 (C-2''), 125.6 (C-6''), 132.1 (C-7''), 134.8 (C-3''), 161.1 (C-6'), 163.5 (C-4'), 165.4 (C-2'), 211.9 (C-1); ESIMS: m/z 355.1886 $[\text{M}+\text{Na}]^+$ (calculated for $\text{C}_{20}\text{H}_{28}\text{O}_4\text{Na}$, 355.1885).

4.4.2.17 2-methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]propan-1-one (**9**)



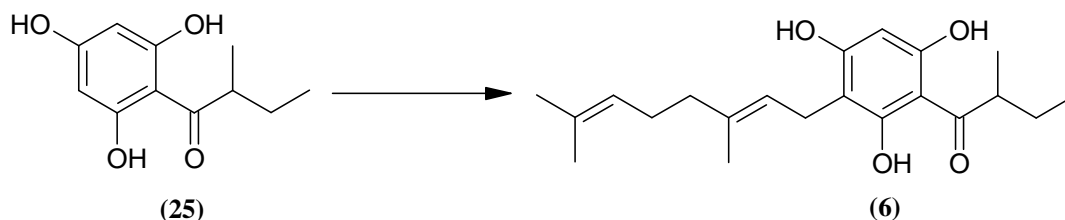
Method 1

2-Methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**) (210 mg, 1.07 mmol) and NaH (75 mg, 3.13 mmol) was dissolved in dioxane (10 mL). The reaction mixture was allowed to stir for 30 min at 60 °C. Prenyl bromide (0.18 mL, 1.53 mmol) was added and the resulting mixture was stirred for a further 4 hr. The reaction was quenched after this time period with dist. H_2O (10 mL). The reaction products were extracted with EtOAc (2 x 30 mL); organic fractions were combined, dried and concentrated. The remaining residue was purified on a 2 mm chromatotron plate eluted with: Hex:EtOAc (9:1), Hex:EtOAc (4:1), Hex:EtOAc (2:1), Hex:EtOAc (1:1), 100% EtOAc. The desired product **9** was obtained in a yield of 10% (29 mg).

UV(MeOH) λ_{\max} (log ϵ): 291 (4.08) nm; IR ν_{\max} : 3426, 3324, 2975, 2916, 1599 cm^{-1} ; ^1H NMR (CD_3OD , 400 MHz): δ_{H} 1.12 (6H, d, $J = 6.9$ Hz, H-3, H-4), 1.64 (3H, s, H-5''), 1.73 (3H, s, H-4''), 3.17 (2H, d, $J = 7.0$ Hz, H-1''), 4.00 (1H, heptet, $J = 6.7$ Hz, H-2), 5.17 (1H, t, $J = 7.0$ Hz, H-2''), 5.89 (1H, s, H-5'); ^{13}C NMR (CD_3OD , 400 MHz): δ_{C} 18.0

(C-4''), 19.9 (C-3, C-4) 22.3 (C-1''), 26.1 (C-5''), 40.1 (C-2), 95.1 (C-5'), 104.7 (C-1'), 108.3 (C-3'), 124.8 (C-2''), 131.2 (C-3''), 161.2 (C-6'), 163.6 (C-4'), 165.5 (C-2'), 211.9 (C-1); ESIMS: m/z 287.1259 $[M+Na]^+$ (calculated for $C_{15}H_{20}O_4Na$, 287.1259).

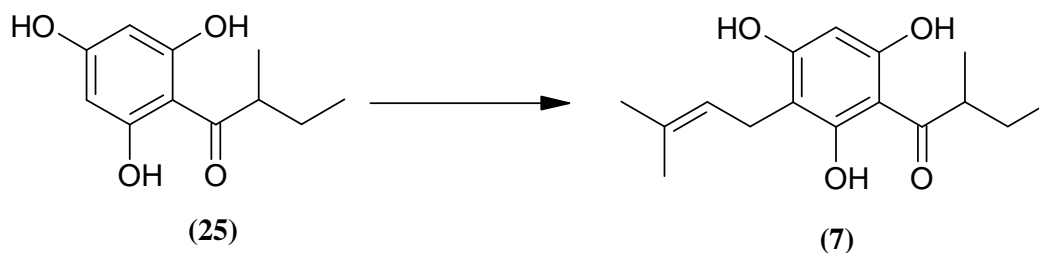
4.4.2.18 1-[3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylbutan-1-one (6)



Method 1

2-Methyl-1-[2,4,6-trihydroxyphenyl]butan-1-one (**25**) (155 mg, 0.74 mmol) and NaH (51 mg, 2.14 mmol) were dissolved in dioxane (10 mL). The reaction mixture was heated to 60 °C and allowed to stir for 30 min before geranyl bromide (0.17 mL, 1.43 mmol) was added. The reaction mixture was stirred for a further 3.5 h before being quenched with dist. H_2O (10 mL). The reaction products were extracted with EtOAc, dried and concentrated. The remaining residue was purified on a 2 mm chromatotron plate, eluting with Hex:EtOAc 9:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1 and 100% EtOAc. The desired product (**6**) was obtained in 17% yield (44 mg). UV (MeOH) λ_{max} (log ϵ): 291 (3.98) nm; 1H NMR (CD_3OD , 400 MHz): δ_H 0.89 (3H, t, $J = 7.4$ Hz, H-4), 1.10 (3H, d, $J = 6.7$ Hz, H-5), 1.36 (1H, m, H-3), 1.54 (3H, s, H-10''), 1.60 (3H, s, H-8''), 1.73 (3H, s, H-9''), 1.80 (1H, m, H-3), 1.93 (2H, m, H-4''), 2.02 (2H, m, H-5''), 3.18 (2H, d, $J = 7.0$ Hz, H-1''), 3.88 (1H, sextet, $J = 6.7$ Hz, H-2), 5.03 (1H, t, $J = 7.4$ Hz, H-6''), 5.17 (1H, t, $J = 7.4$ Hz, H-2''), 5.90 (1H, s, H-5'); ^{13}C NMR (CD_3OD , 400 MHz): δ_C 12.5 (C-4), 16.3 (C-9''), 17.4 (C-5), 17.9 (C-10''), 22.2 (C-1''), 26.0 (C-8''), 27.8 (C-5''), 28.3 (C-3), 41.0 (C-4''), 46.8 (C-2), 95.3 (C-5'), 124.8 (C-2''), 125.7 (C-6''), 132.1 (C-7''), 134.9 (C-3''), 165.3 (C-2'); ESIMS: m/z 369.2044 $[M+Na]^+$ (calculated for $C_{21}H_{30}O_4Na$, 369.2042).

4.4.2.19 2-Methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]butan-1-one (7)



Method 1

2-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**25**) (210 mg, 1.00 mmol) and NaH (69 mg, 2.85 mmol) were dissolved in dioxane (10 mL). The reaction mixture was heated to 60 °C and allowed to stir for 30 min before prenyl bromide (0.17 mL, 1.43 mmol) was added. The reaction was stirred for a further 3.5 h before being quenched with dist. H₂O (10 mL). The reaction products were extracted with EtOAc, dried and concentrated. The remaining residue was purified on a 2 mm chromatotron plate, eluting with Hex:EtOAc 9:1, Hex:EtOAc 4:1, Hex:EtOAc 2:1 and 100% EtOAc. The desired product **7** was obtained in 13% yield (36 mg).

UV(MeOH) λ_{\max} (log ϵ): 292 (4.55) nm; ¹H NMR (CD₃OD, 400 MHz): δ_{H} 0.89 (3H, t, $J = 7.4$ Hz, H-4), 1.10 (3H, d, $J = 6.8$ Hz, H-5), 1.35 (1H, m, H-3), 1.64 (3H, s, H-5''), 1.73 (3H, s, H-4''), 1.80 (1H, m, H-3), 3.17 (2H, d, $J = 7.0$ Hz, H-1''), 3.88 (1H, sextet, $J = 6.8$ Hz, H-2), 5.17 (1H, t, $J = 7.0$ Hz, H-2''), 5.88 (1H, s, H-5'); ¹³C NMR (CD₃OD, 400 MHz): δ_{C} 12.5 (C-4), 17.4 (C-5), 18.0 (C-4''), 22.4 (C-1''), 26.1 (C-5''), 28.4 (C-3), 46.8 (C-2), 95.2 (C-5'), 105.4 (C-1'), 108.3 (C-3'), 124.7 (C-2''), 131.3 (C-3''), 161.2 (C-6'), 163.5 (C-4'), 165.4 (C-2'), 211.7 (C-1); ESIMS: m/z 301.1417 [M+Na]⁺ (calculated for C₁₆H₂₂O₄Na, 301.1416).

4.5 References

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CHAPTER 5

Conclusions

5.1 Conclusions and Future Work

The research presented in this dissertation combined natural product chemistry with organic synthesis. The phytochemical investigation of the aerial plant material of the South African species, *Hypericum roeperianum*, lead to the identification of two secondary metabolites. The isolated compounds, 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4*H*-pyran-4-one (**38**) (hyperanone A) and {[3-(3,7-dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone} (**2**), have been previously isolated from other plant sources.^{1,2,3,4,5,6} A third compound was also isolated and is provisionally assigned as 1-methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**). However, the small amount of compound isolated did not provide sufficient data for a conclusive structural assignment. Future work could therefore include the synthesis of compound **39** in order to prove that the suggested structure of the compound is correct. To the best of our knowledge compound **39** has not been previously isolated.

In this study, 3-geranyl-2,4,6-trihydroxybenzophenone (**2**) was isolated for the first time from the *Hypericum* genus, although it has been isolated previously from other species belonging to the Clusiaceae/Guttiferae family, namely *Garcinia vieillardii*, *Tovomita krukovii* and *T. longifolia*.^{2,5,6} Various inhibitory effects are exhibited by compound **2**, of which the antimicrobial activity against *Mycobacterium smegmatus* is of great interest, as this bacterium belongs to the same genus as *Mycobacterium tuberculosis*, which is the bacterium that is the cause of tuberculosis (TB). Further investigation of this compound or compounds with related structures may therefore lead to the discovery of a potential drug for the treatment of tuberculosis. Hence, the synthetic section of our research incorporated the synthesis of **2** as well as the synthesis of seven structural analogues of the bioactive metabolite.

Synthesis of the bioactive compound **2** and the structural analogues thereof (**3** – **9**) were successfully achieved. If significant biological activity is exhibited by one or more of our synthesized analogues, future work would need to include an attempt to increase the yields as well as an investigation of the cytotoxicity of the compounds of interest. An increase in yields may be achieved by optimizing the reaction conditions of the direct C-alkylation method by which the analogues were produced or investigation into alternative routes may be considered. Although the carcinogenic nature of the MOM protecting group needs to be taken into account, as well as the fact that it is no longer commercially available, it may be interesting to synthesise MOMCl and hence attempt the C-prenylation *via* O-alkylation followed by the Claisen rearrangement as good yields were obtained by Tan *et al.*⁷ following this route. The MOM protecting group is also known to be a good *ortho* directing group. Another potential method involves alkylating *via* a direct *ortho* metallation (DOM) type reaction as discussed in **Section 4.2.1**.

A literature search revealed no synthetic routes to compounds **6** and **7**; we therefore believe that this is the first report where compounds **6** and **7** have been achieved by synthetic procedures. Biological activity and toxicity testing is currently being carried out on our battery of eight synthetic compounds. Future work would thus involve the evaluation of the results obtained in order to draw some conclusions on the structure-activity relationships of the various groups present in our molecules, and hence determine whether further investigation of these molecules as anti-tuberculosis drugs could potentially lead to new TB treatments.

5.2 References

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APPENDIX

NMR Spectra of Isolated Compounds

- Plate 1.1** ^1H NMR spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)
- Plate 1.2** ^{13}C NMR spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)
- Plate 1.3** DEPT90 spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)
- Plate 1.4** DEPT135 spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)
- Plate 1.5** COSY spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)
- Plate 1.6** HSQC spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)
- Plate 1.7** HMBC spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)
-
- Plate 2.1** ^1H NMR spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)
- Plate 2.2** ^{13}C NMR spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)
- Plate 2.3** DEPT90 spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)
- Plate 2.4** DEPT135 spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)
- Plate 2.5** COSY spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)
- Plate 2.6** HSQC spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)
- Plate 2.7** HMBC spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)

- Plate 3.1** ^1H NMR spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)
- Plate 3.2** ^{13}C NMR spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)
- Plate 3.3** DEPT90 spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)
- Plate 3.4** DEPT135 spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)
- Plate 3.5** COSY spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)
- Plate 3.6** HSQC spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)
- Plate 3.7** HMBC spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)

NMR Spectra of Synthesized Compounds

- Plate 4.1** ^1H NMR spectrum (CD_3OD) of 1-[2,4-Bisbenzyloxy-6-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**46**) and 1-[4,6-bis-benzyloxy-3-(1,1-dimethyl allyl)-2-hydroxyphenyl]ethanone (**47**)
- Plate 4.2** ^{13}C NMR spectrum (CD_3OD) of 1-[2,4-Bisbenzyloxy-6-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**46**) and 1-[4,6-bis-benzyloxy-3-(1,1-dimethyl allyl)-2-hydroxyphenyl]ethanone (**47**)
- Plate 5.1** ^1H NMR spectrum (CD_3OD) of Phenyl-(2,4,6-trihydroxyphenyl)methanone (**24**)
- Plate 5.2** ^{13}C NMR spectrum (CD_3OD) of Phenyl-(2,4,6-trihydroxyphenyl)methanone(**24**)
- Plate 6.1** ^1H NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**25**)
- Plate 6.2** ^{13}C NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**25**)
- Plate 7.1** ^1H NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**)
- Plate 7.2** ^{13}C NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**)

- Plate 8.1** ^1H NMR spectrum (CD_3OD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone (**2**)
- Plate 8.2** ^{13}C NMR spectrum (CD_3OD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone (**2**)
- Plate 9.1** ^1H NMR spectrum (CD_3OD) of Phenyl-[2,4,6-trihydroxyphenyl]methanone (**3**)
- Plate 9.2** ^{13}C NMR spectrum (CD_3OD) of Phenyl-[2,4,6-trihydroxyphenyl]methanone (**3**)
- Plate 10.1** ^1H NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]ethanone (**4**)
- Plate 10.2** ^{13}C NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]ethanone (**4**)
- Plate 11.1** ^1H NMR spectrum (CD_3OD) of 1-[2,4,6-Trihydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**5**)
- Plate 11.2** ^{13}C NMR spectrum (CD_3OD) of 1-[2,4,6-Trihydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**5**)
- Plate 12.1** ^1H NMR spectrum (CD_3OD) of 2-Methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]butan-1-one (**7**)
- Plate 12.2** ^{13}C NMR spectrum (CD_3OD) of 2-Methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]butan-1-one (**7**)
- Plate 13.1** ^1H NMR spectrum (CD_3OD) of 2-methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]propan-1-one (**9**)
- Plate 13.2** ^{13}C NMR spectrum (CD_3OD) of 2-methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]propan-1-one (**9**)
- Plate 14.1** ^1H NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylbutan-1-one (**6**)

Plate 14.2 ^{13}C NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylbutan-1-one (**6**)

Plate 15.1 ^1H NMR spectrum (CD_3OD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylpropan-1-one (**8**)

Plate 15.2 ^{13}C NMR spectrum (CD_3OD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylpropan-1-one (**8**)

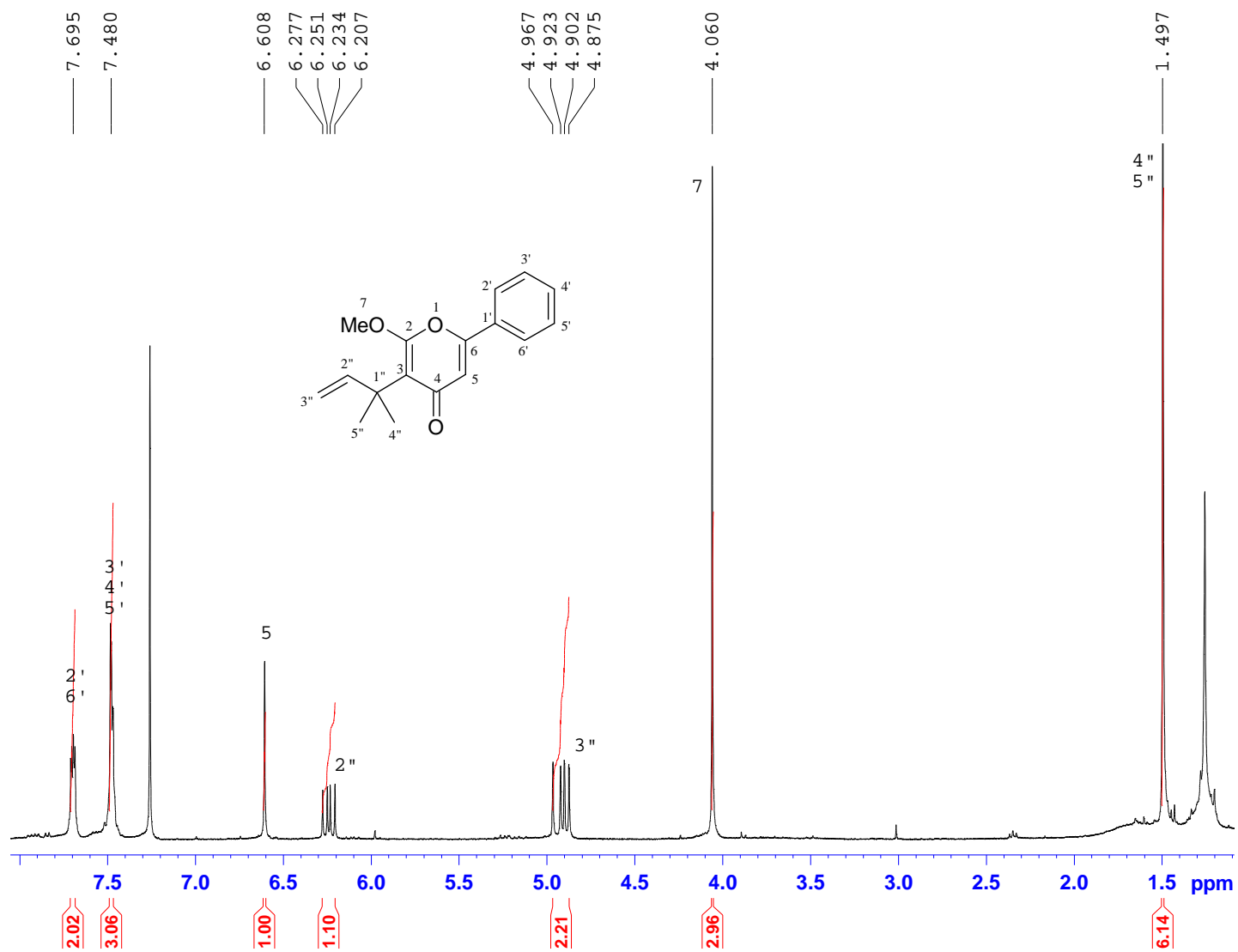


Plate1.1 ^1H NMR spectrum (CDCl₃) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (38)

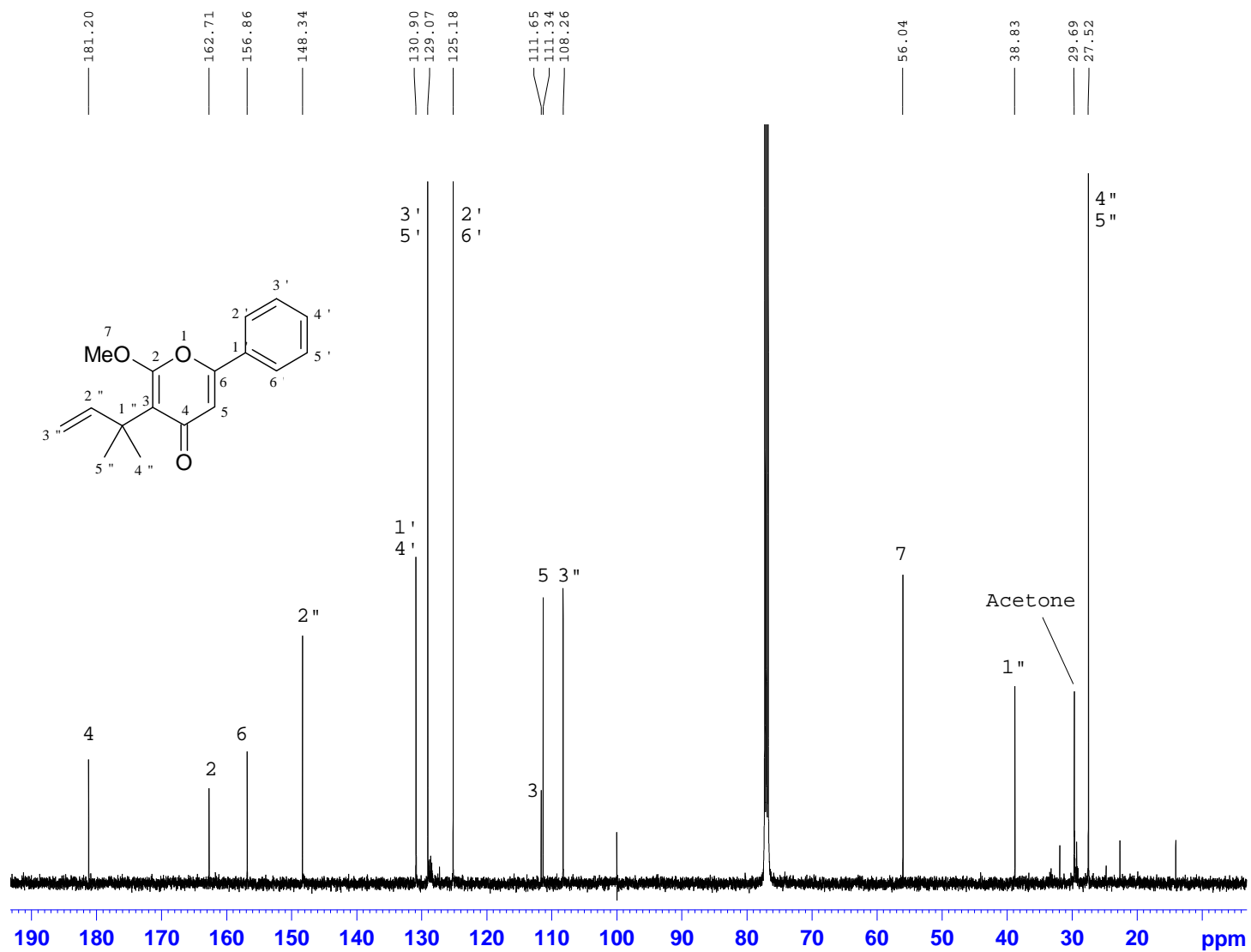


Plate 1.2 ¹³C NMR spectrum (CDCl₃) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (38)

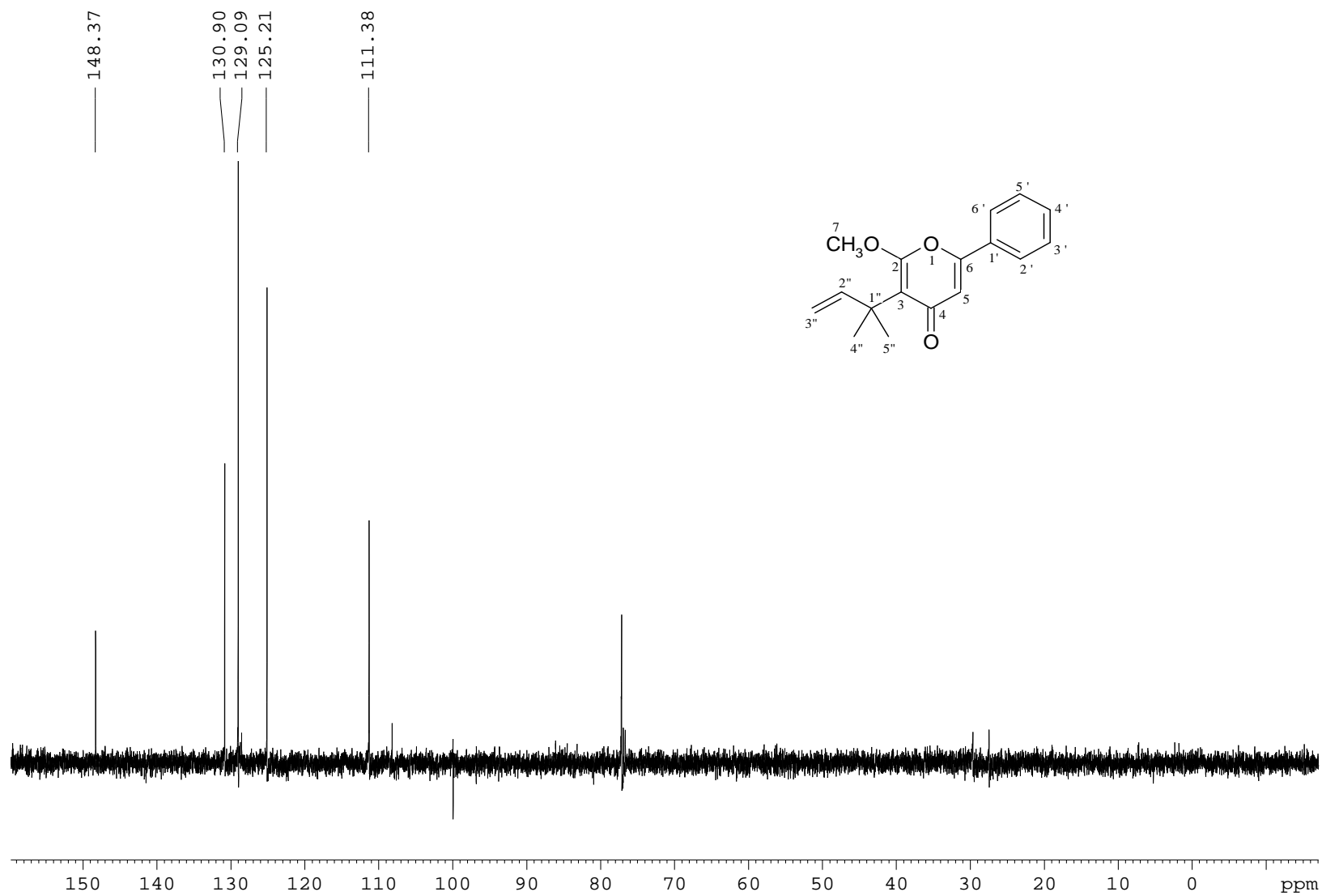


Plate 1.3 DEPT90 spectrum (CDCl₃) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (**38**)

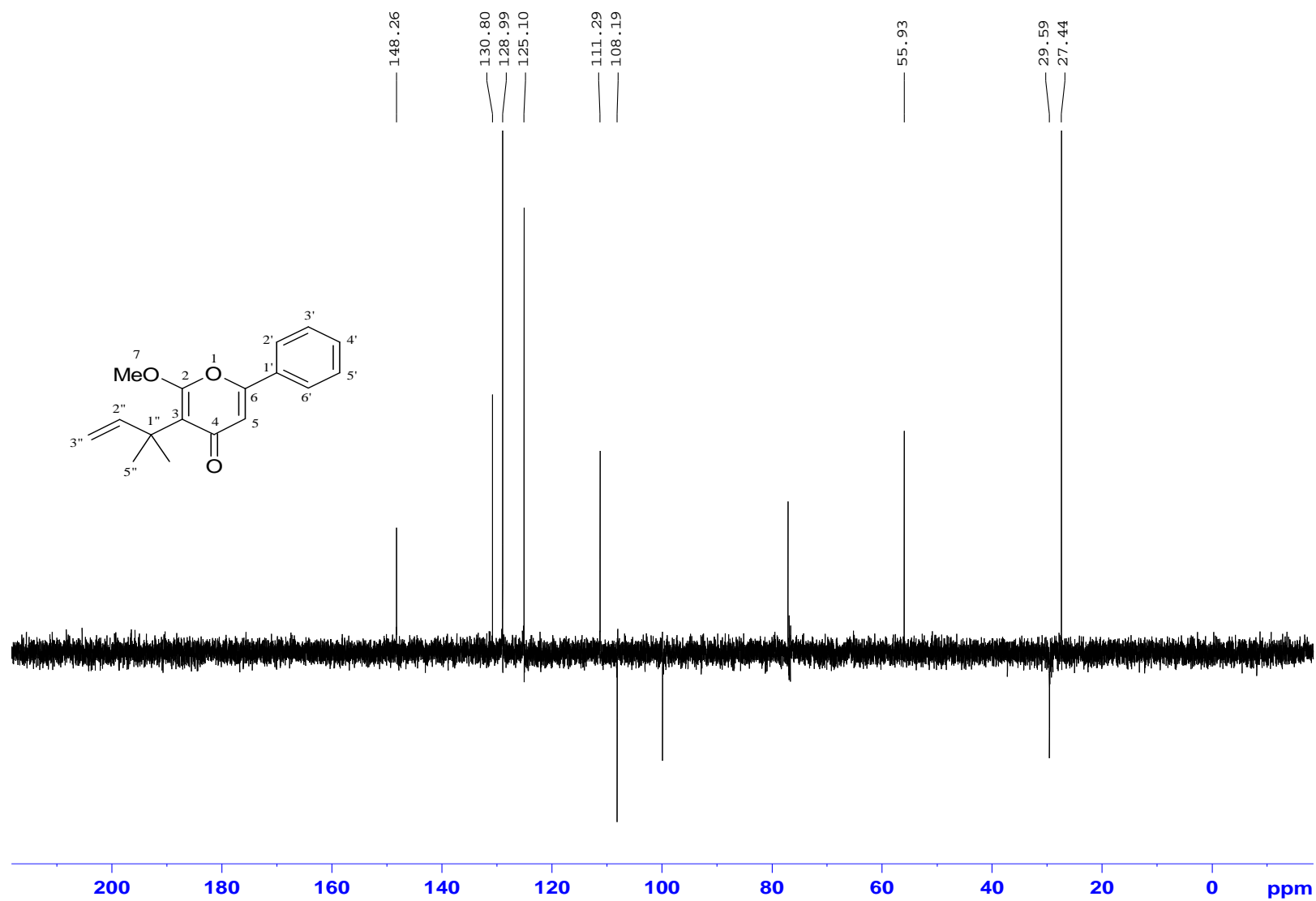


Plate 1.4 DEPT135 spectrum (CDCl₃) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4H-pyran-4-one (38)

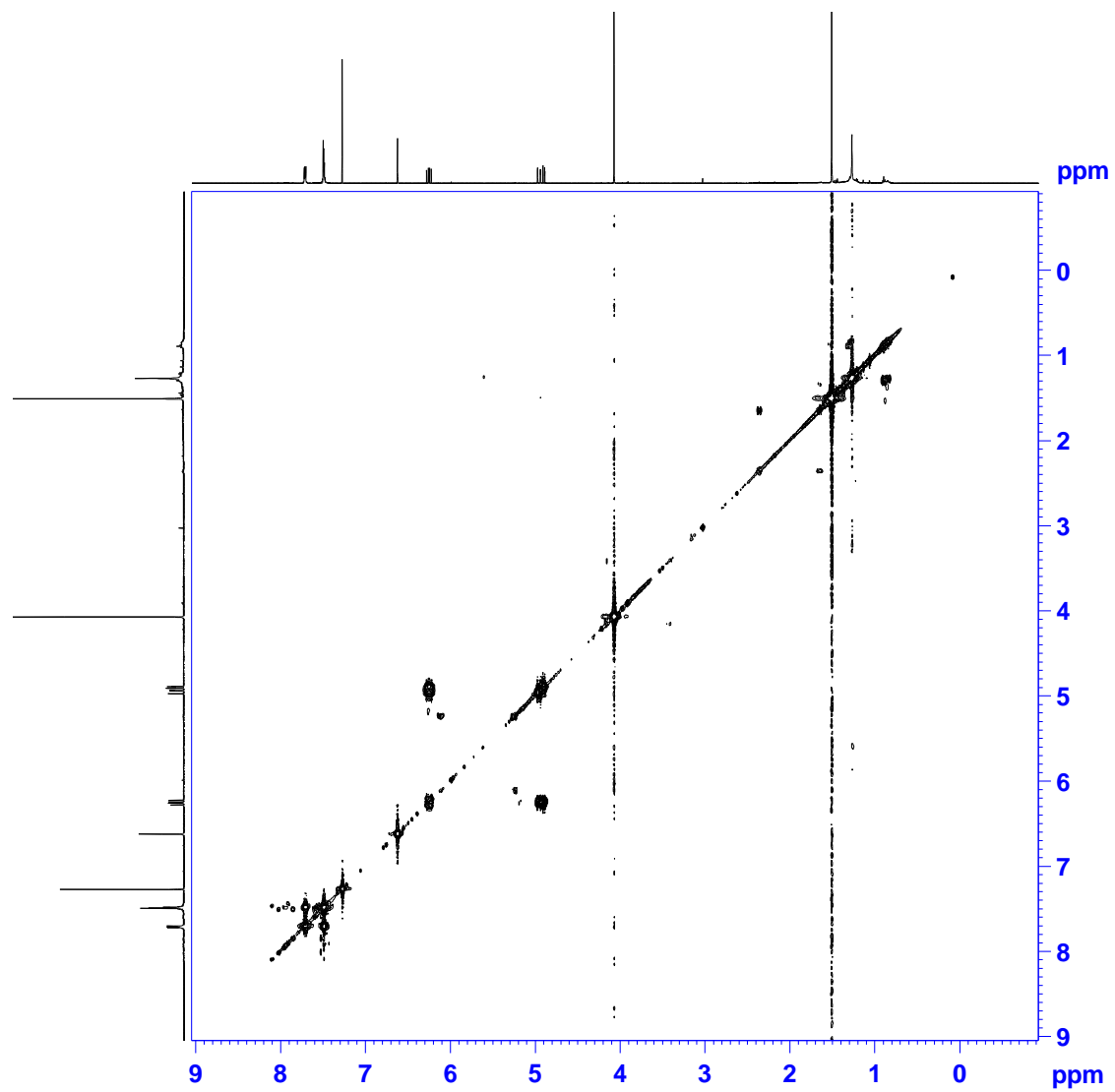


Plate 1.5 COSY spectrum (CDCl₃) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4*H*-pyran-4-one (**38**)

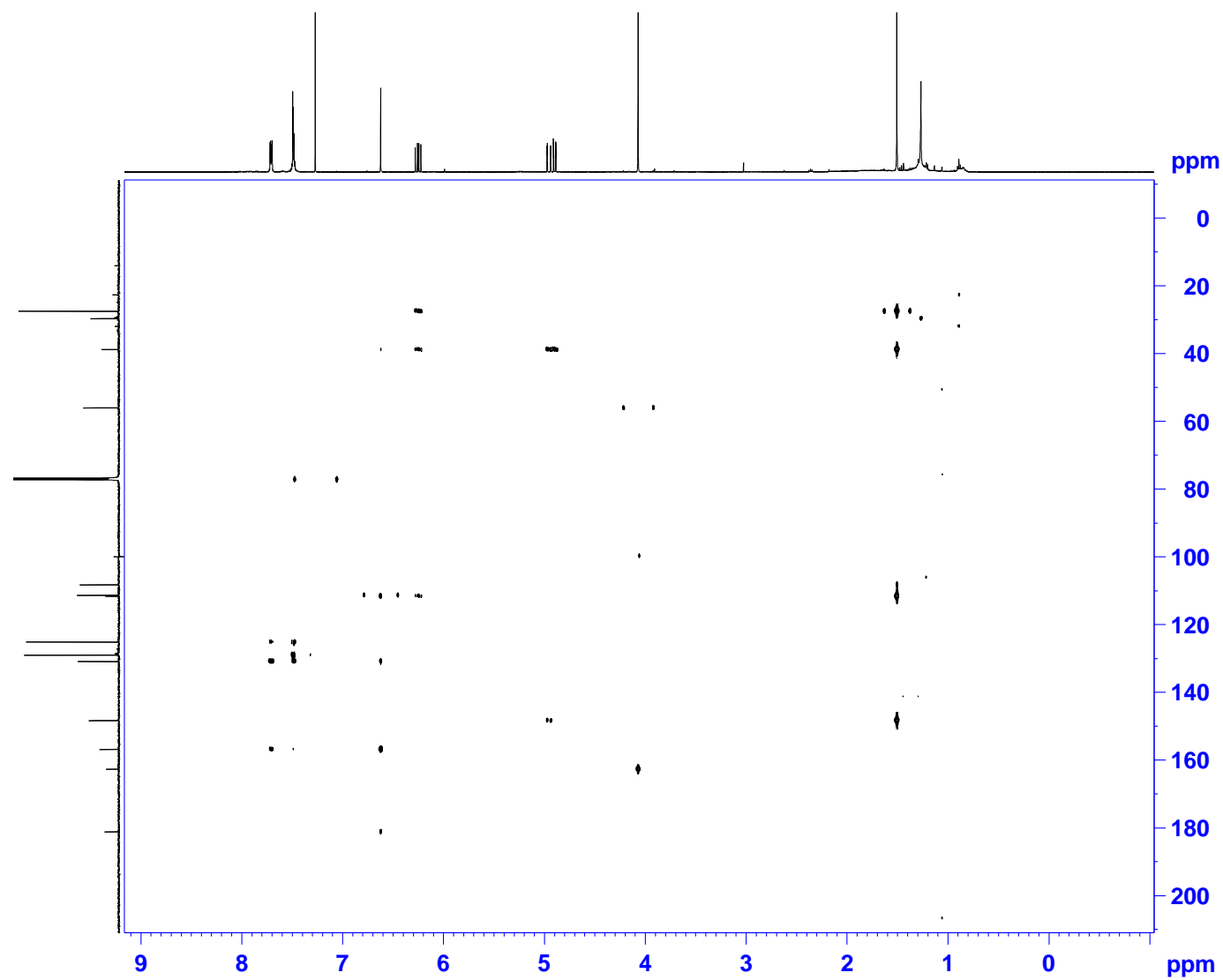


Plate 1.6 HMQC spectrum (CDCl₃) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4*H*-pyran-4-one (**38**)

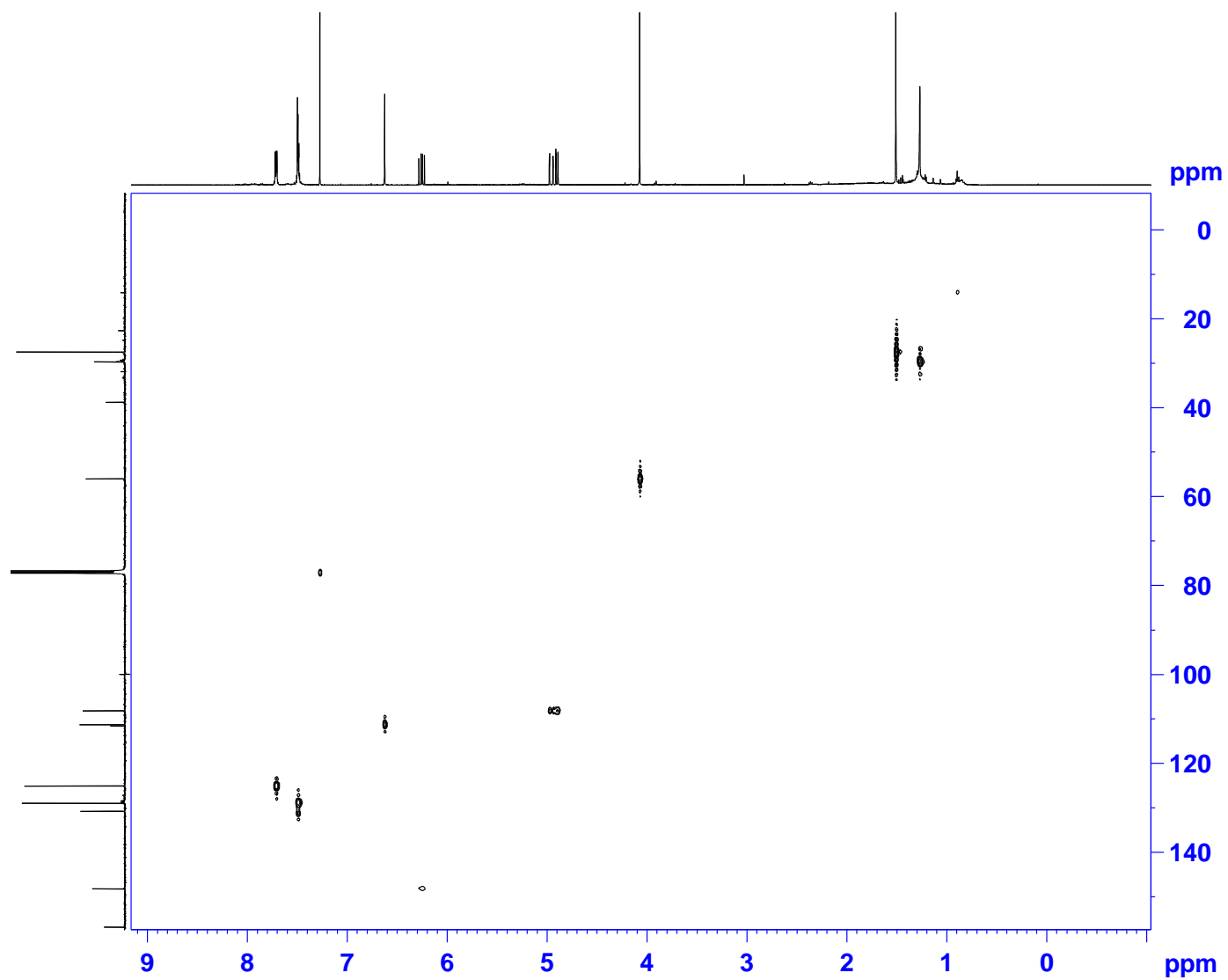


Plate 1.7 HSQC spectrum (CDCl_3) of 3-(1,1-dimethyl-2-propenyl)-2-methoxy-6-phenyl-4*H*-pyran-4-one (**38**)

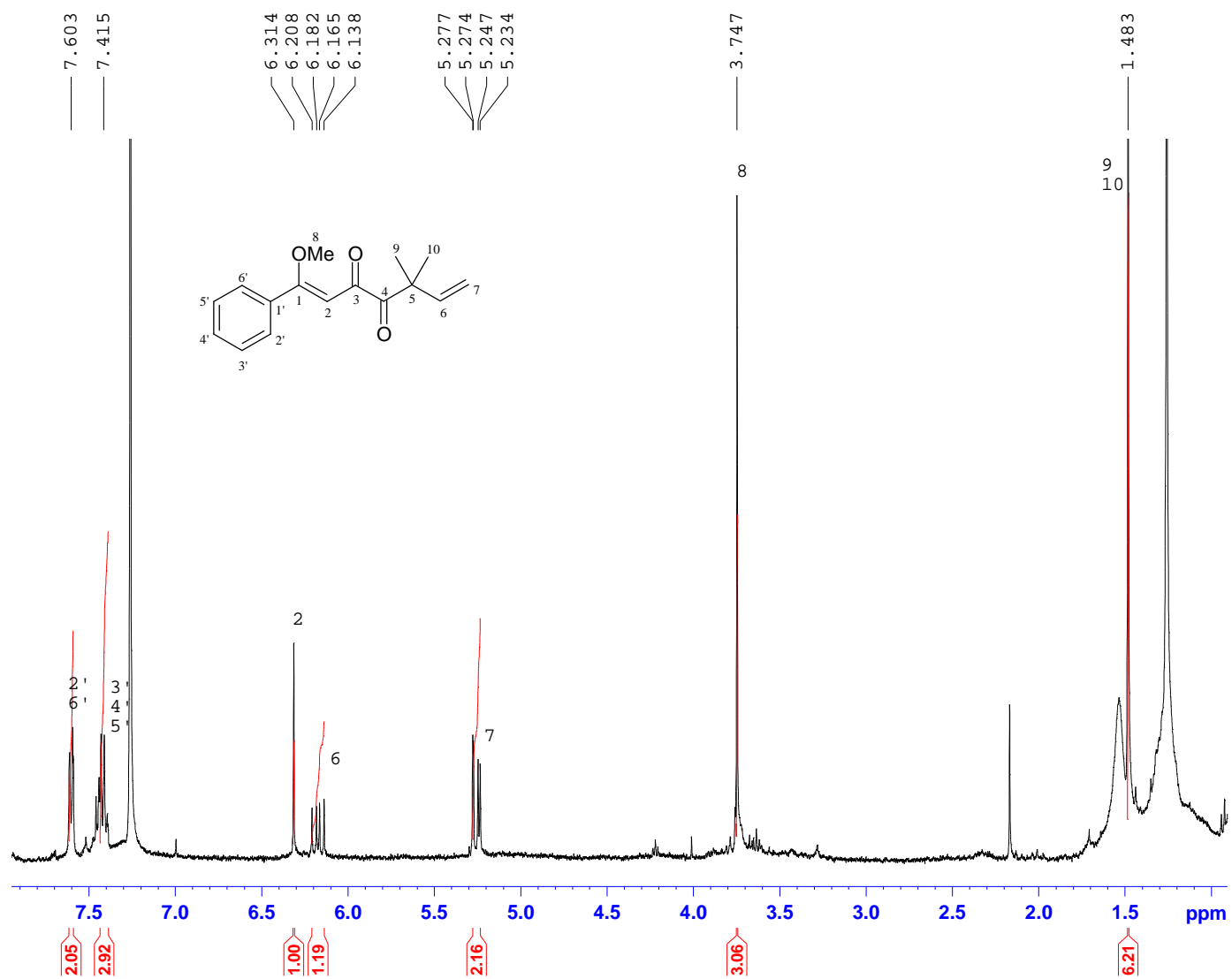


Plate 2.1 ¹H NMR spectrum (CDCl₃) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (39)

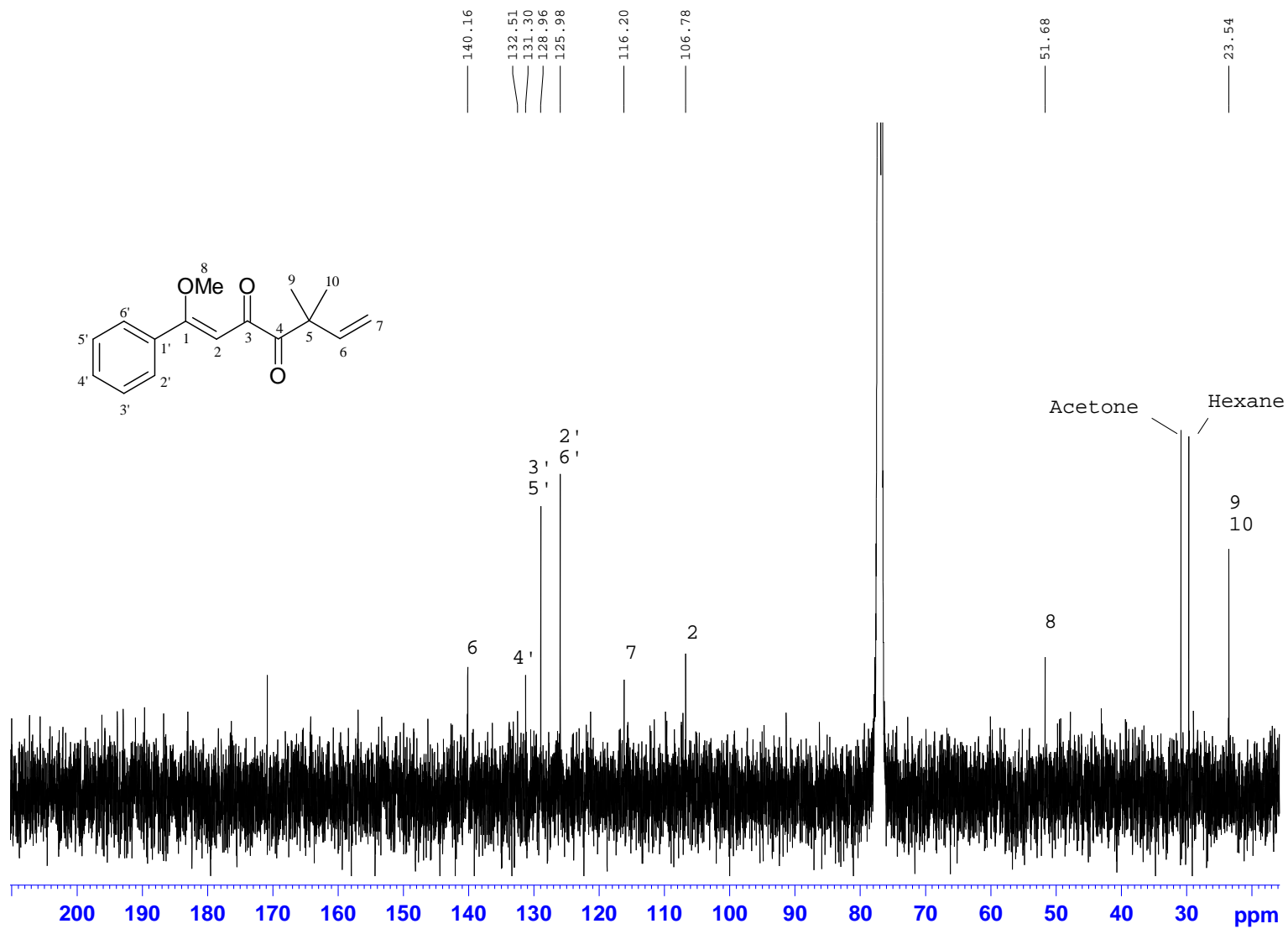


Plate 2.2 ^{13}C NMR spectrum (CDCl₃) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (39)

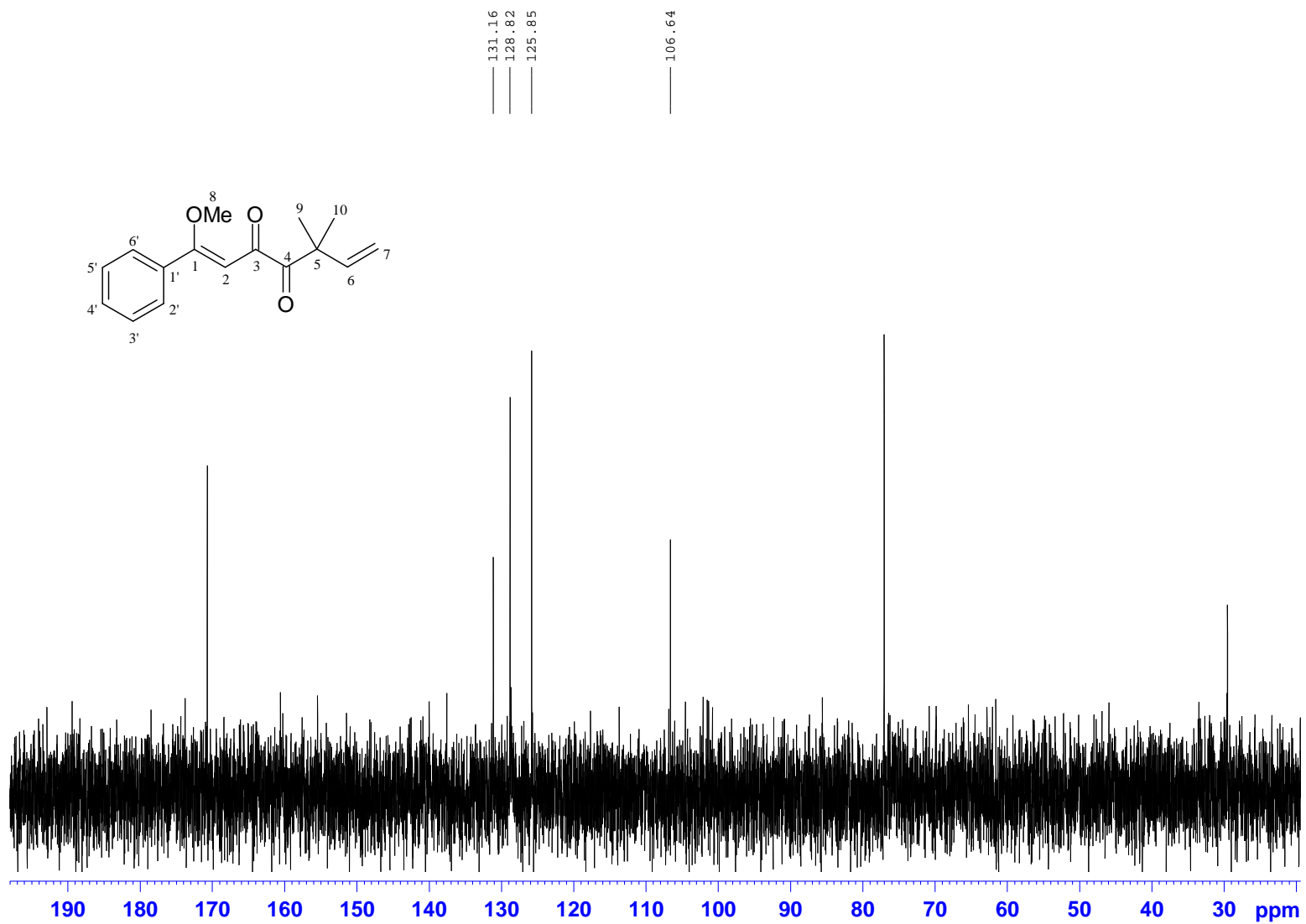


Plate 2.3 DEPT 90 spectrum (CDCl₃) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)

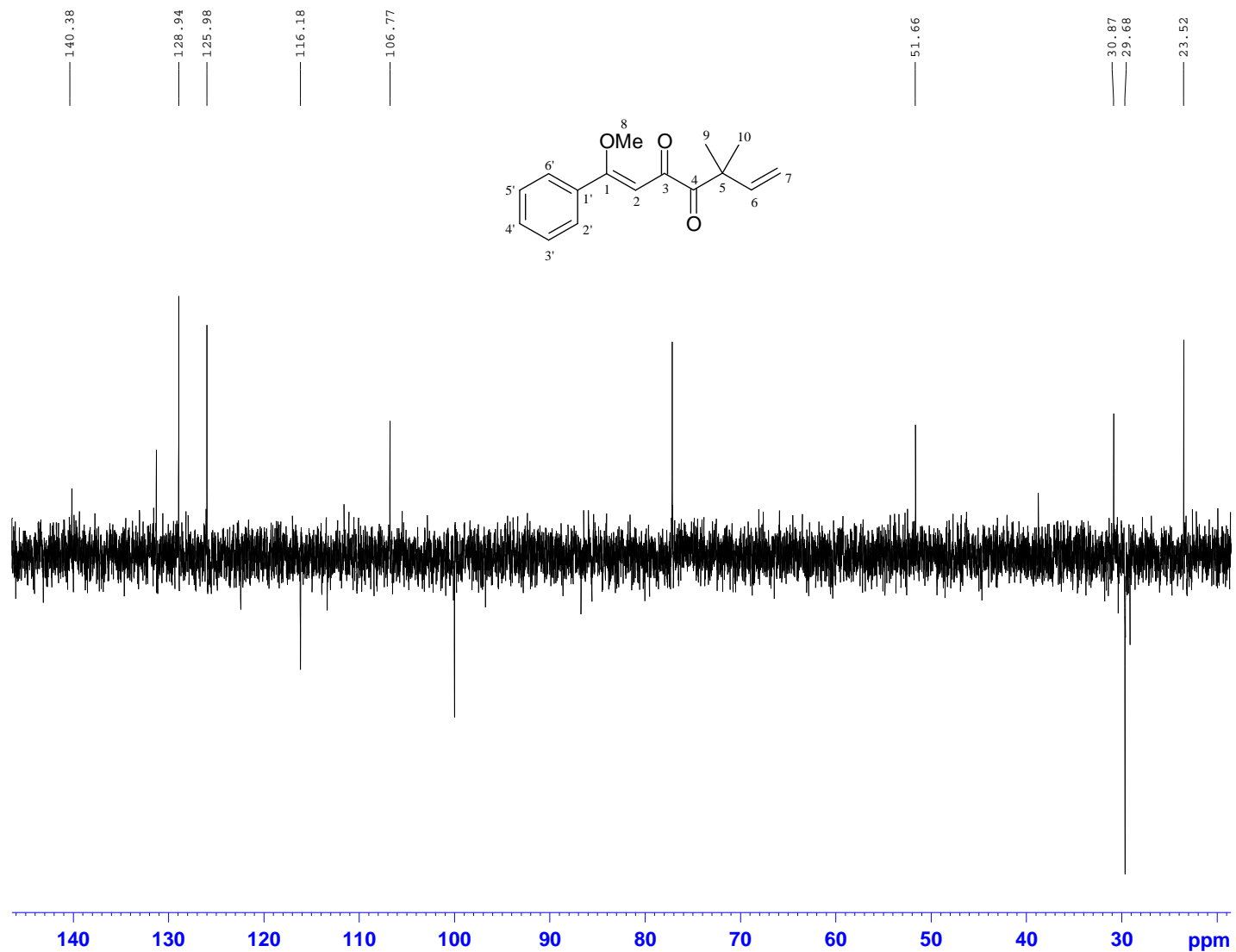


Plate 2.4 DEPT 135 spectrum (CDCl₃) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)

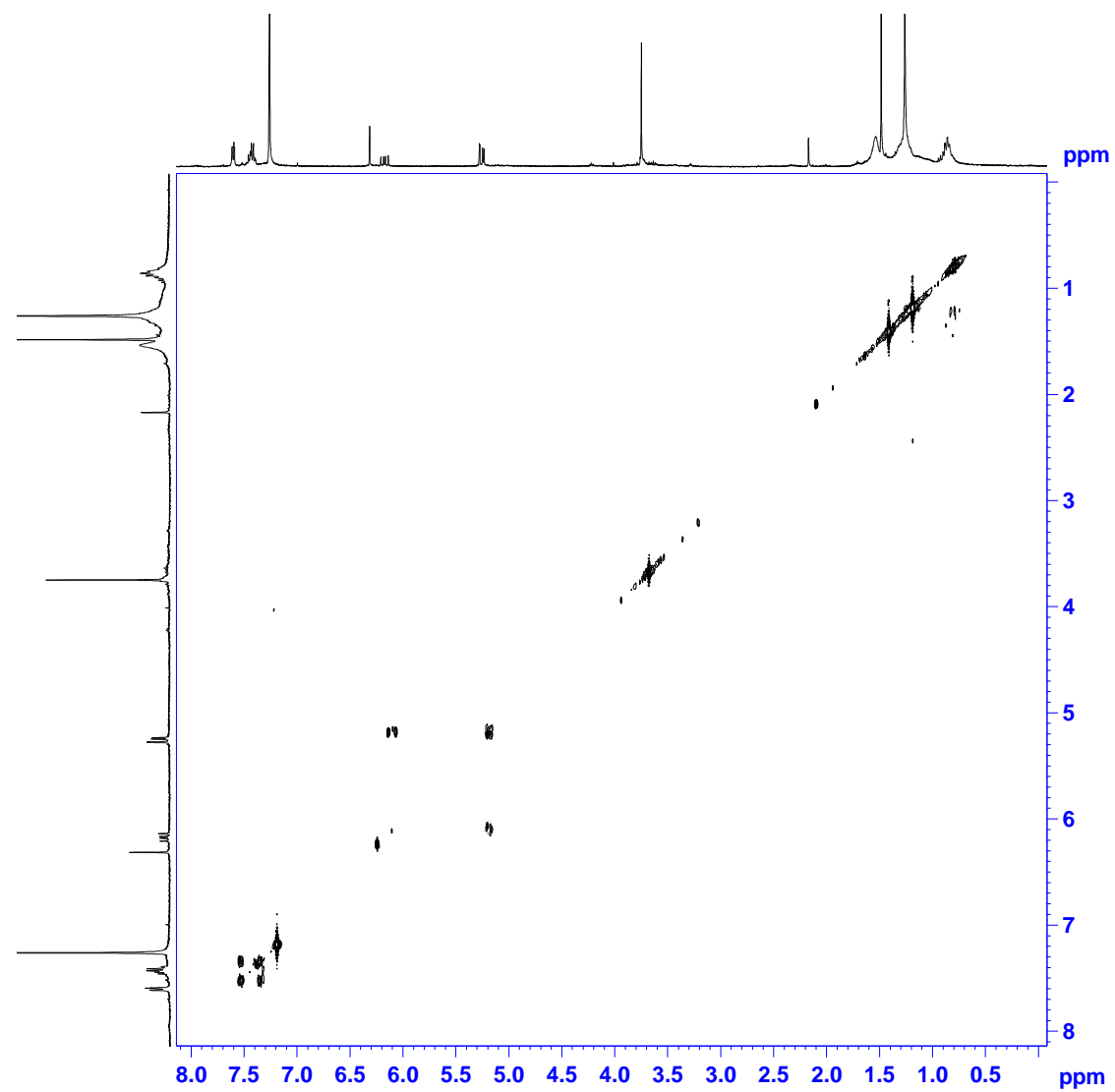


Plate 2.5 COSY spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)

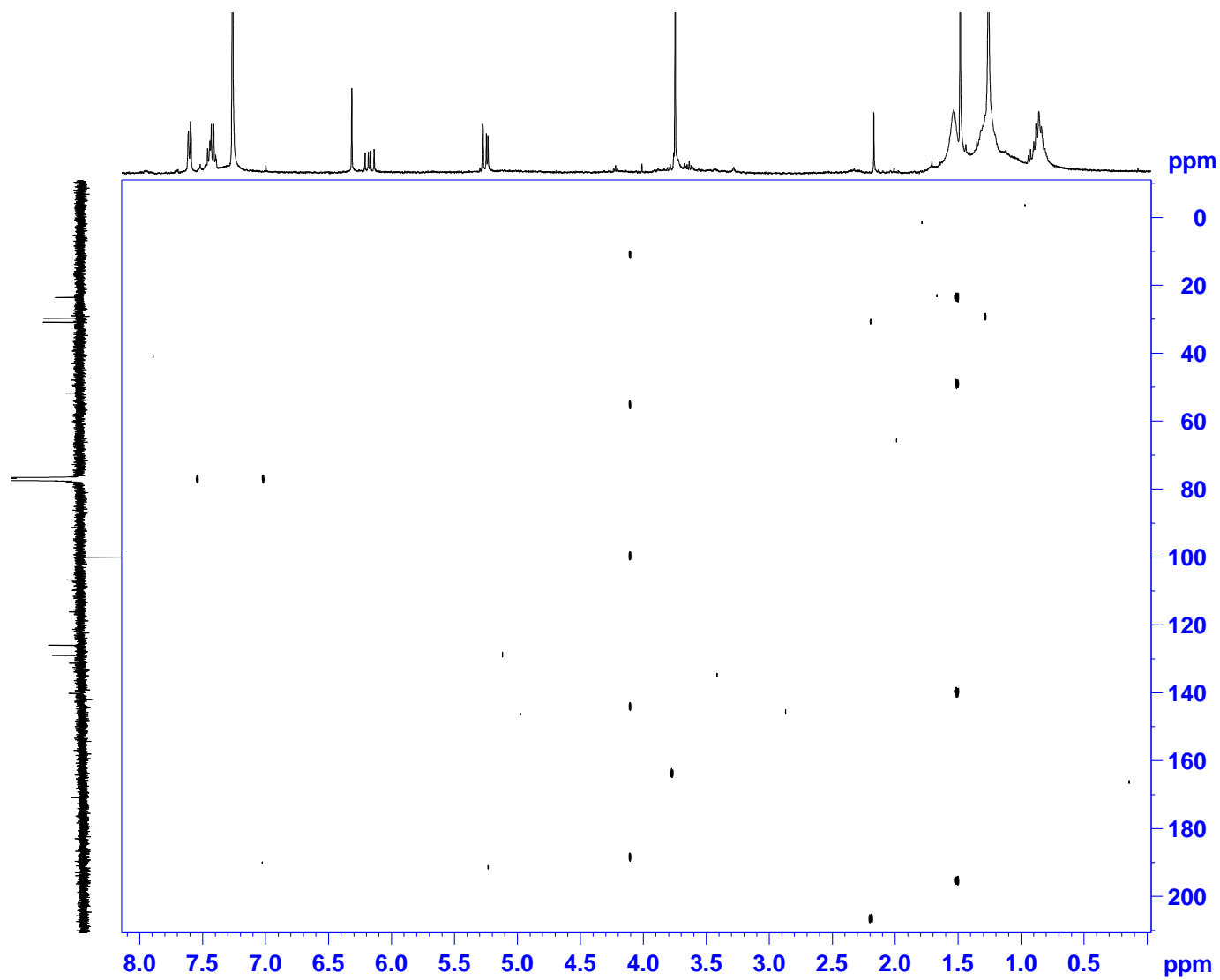


Plate 2.6 HMQC spectrum (CDCl₃) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)

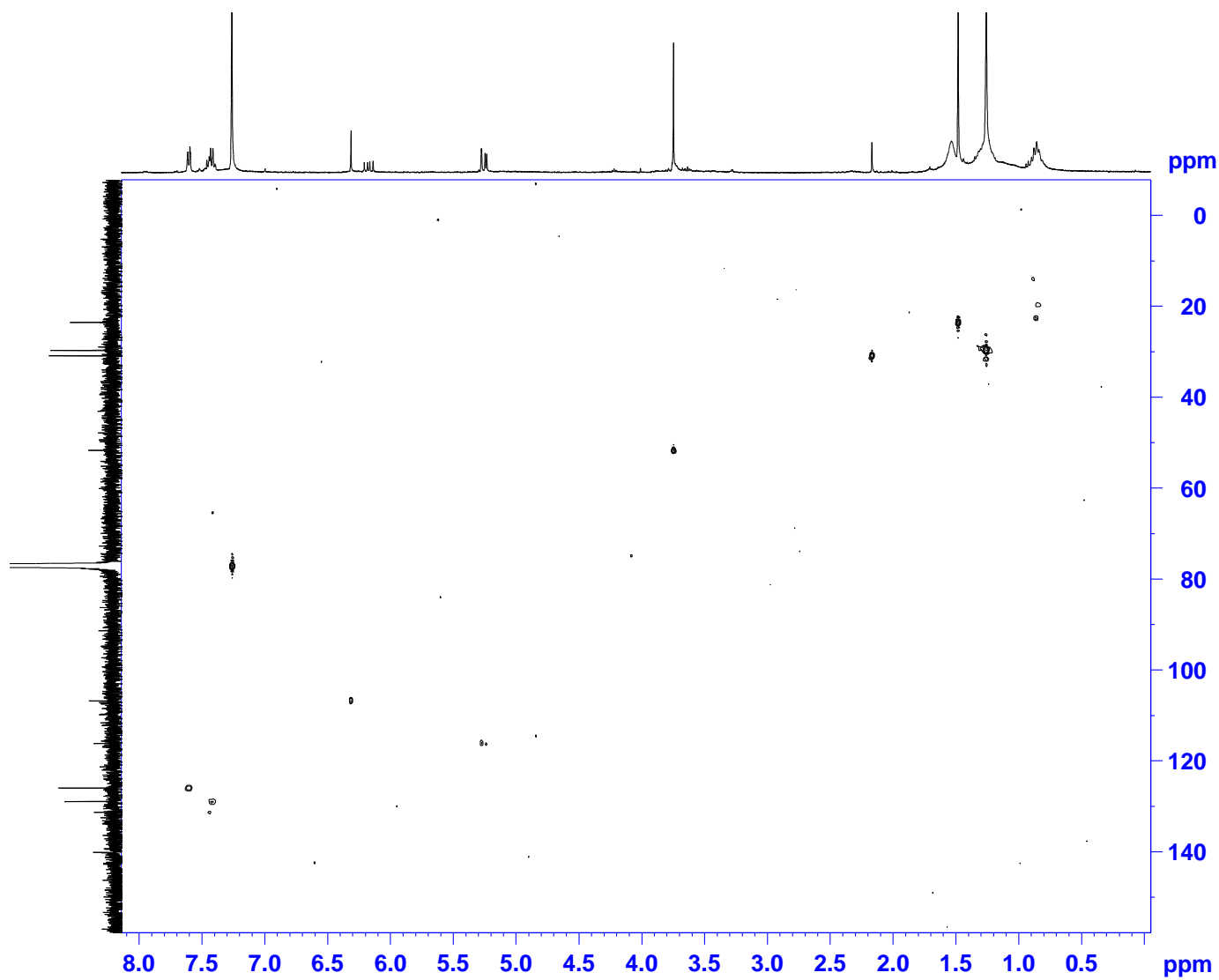


Plate 2.7 HSQC spectrum (CDCl_3) of 1-Methoxy-5,5-dimethyl-1-phenylhepta-1,6-diene-3,4-dione (**39**)

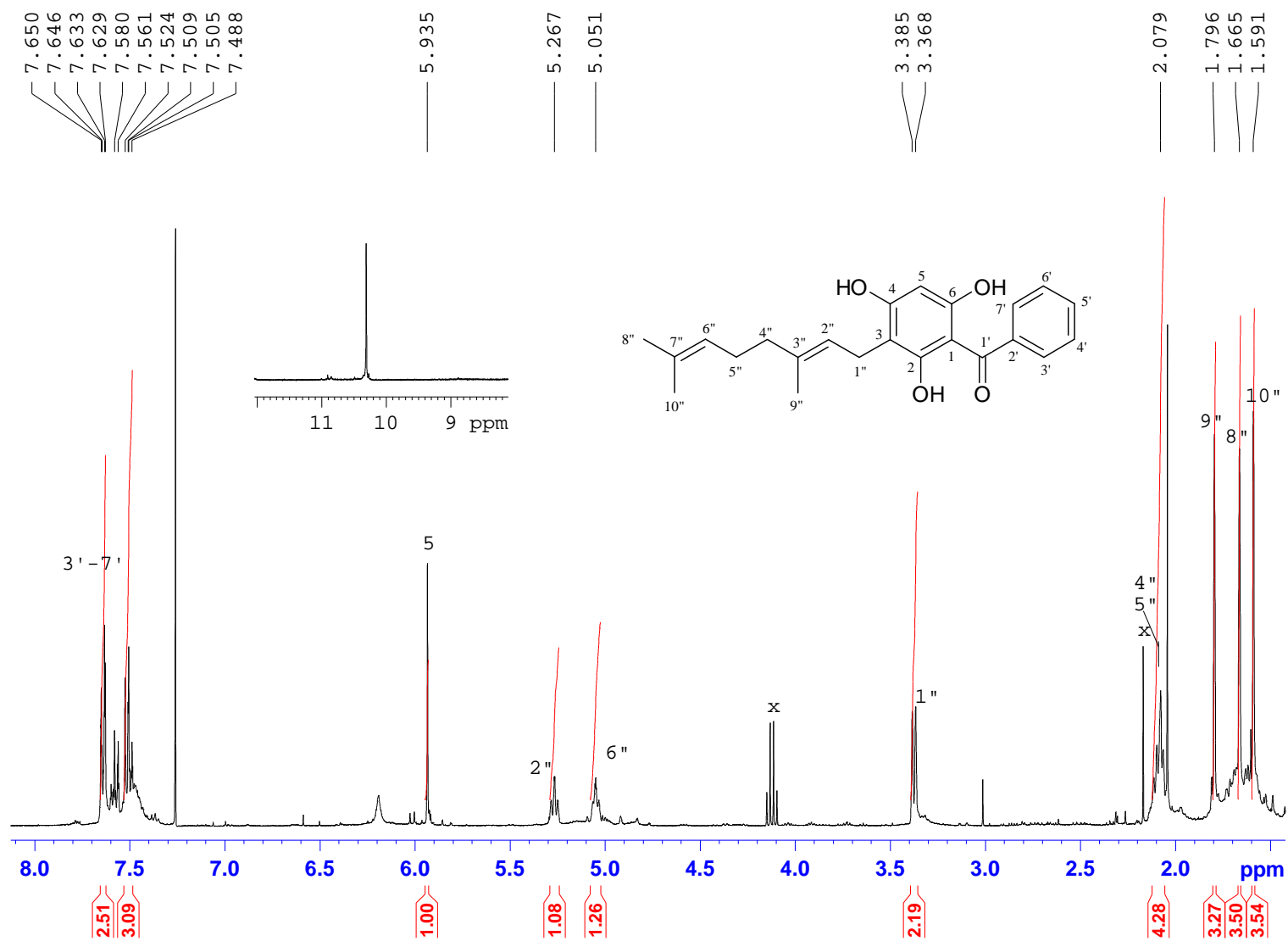


Plate 3.1 ¹H NMR spectrum (CDCl₃) of 3-Geranyl-2,4,6-trihydroxybenzophenone (2)

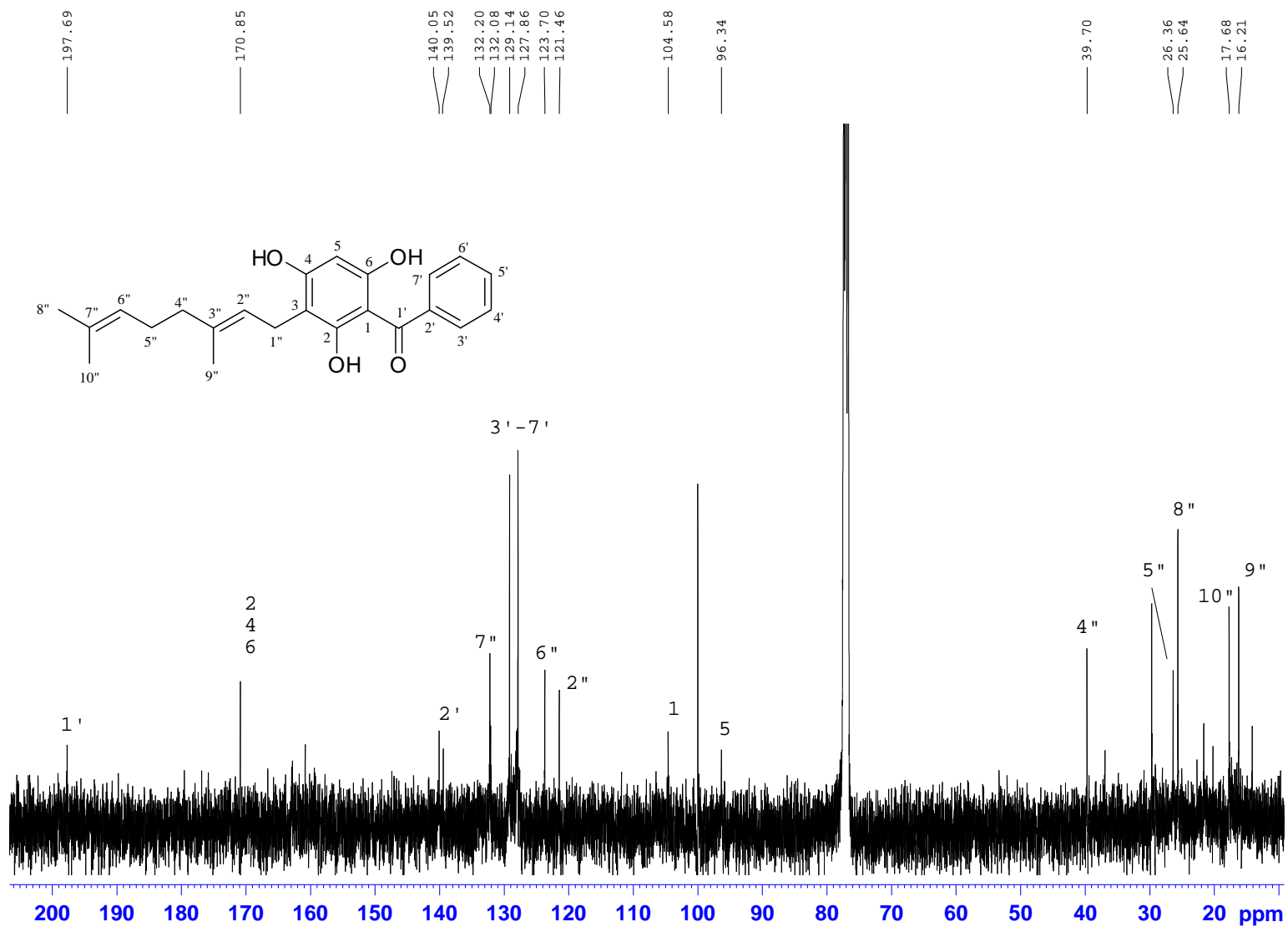


Plate 3.2 ^{13}C NMR spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (2)

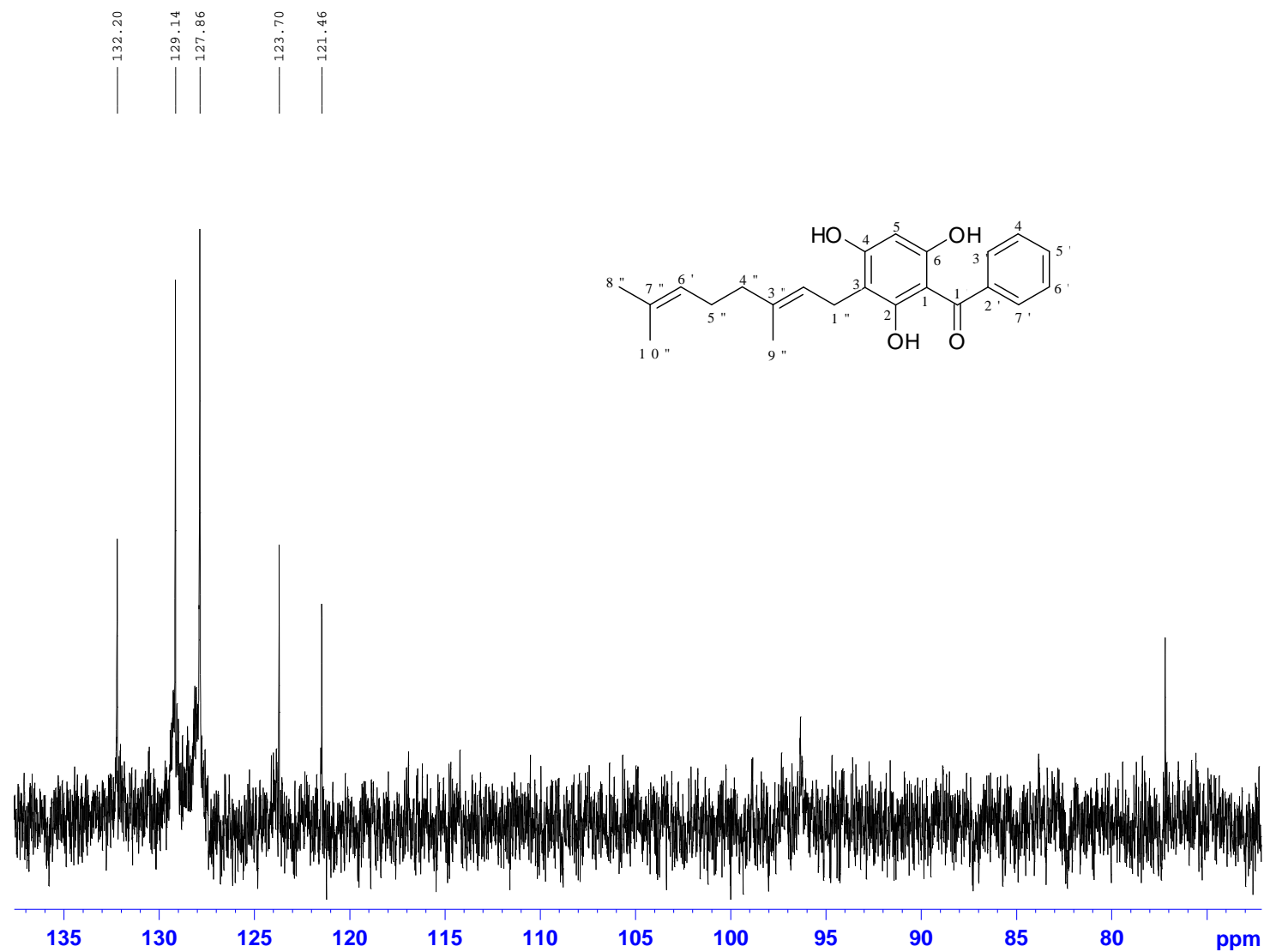


Plate 3.3 DEPT 90 spectrum (CDCl₃) of 3-Geranyl-2,4,6-trihydroxybenzophenone (2)

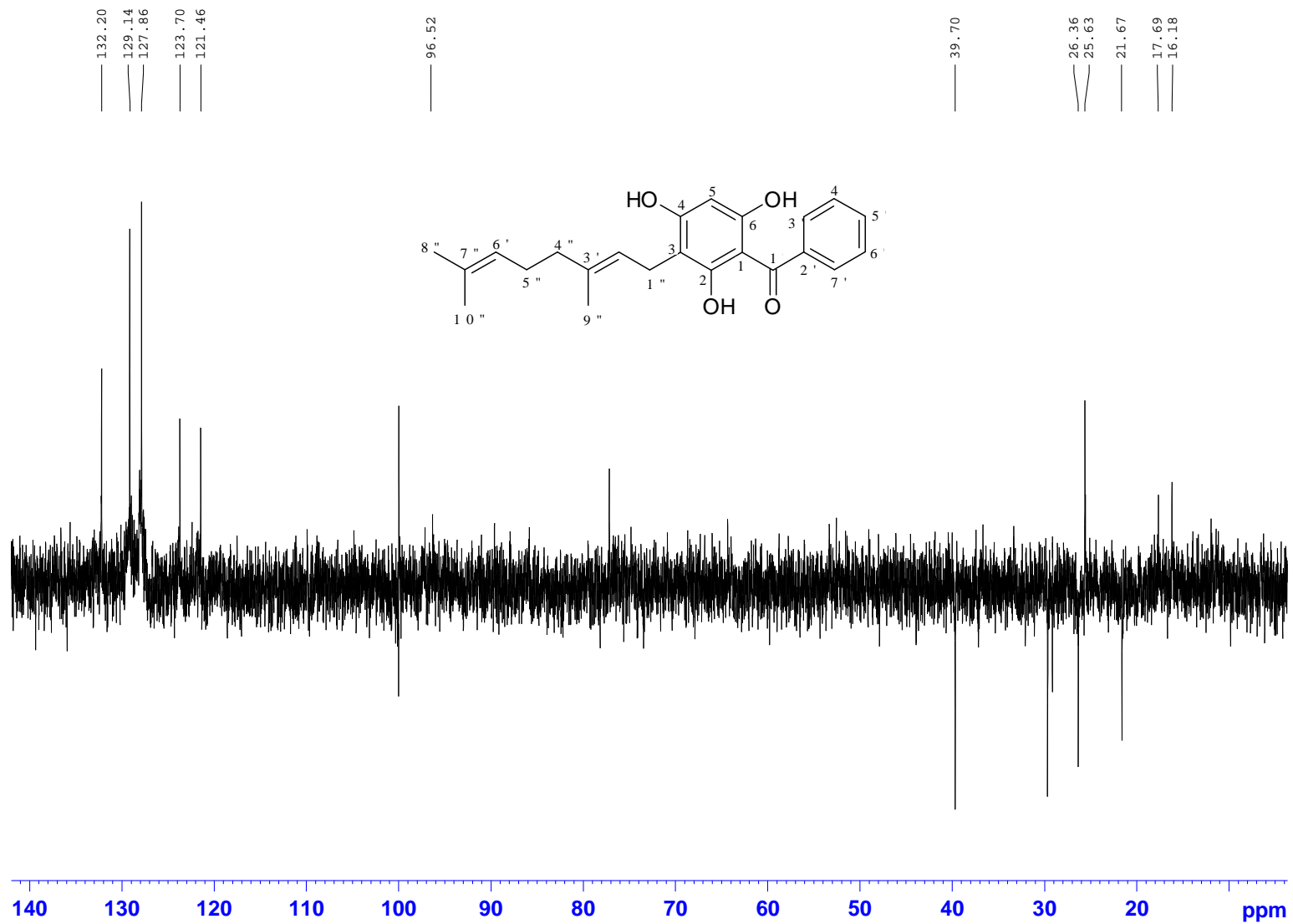


Plate 3.4 DEPT 135 spectrum (CDCl₃) of 3-Geranyl-2,4,6-trihydroxybenzophenone (2)

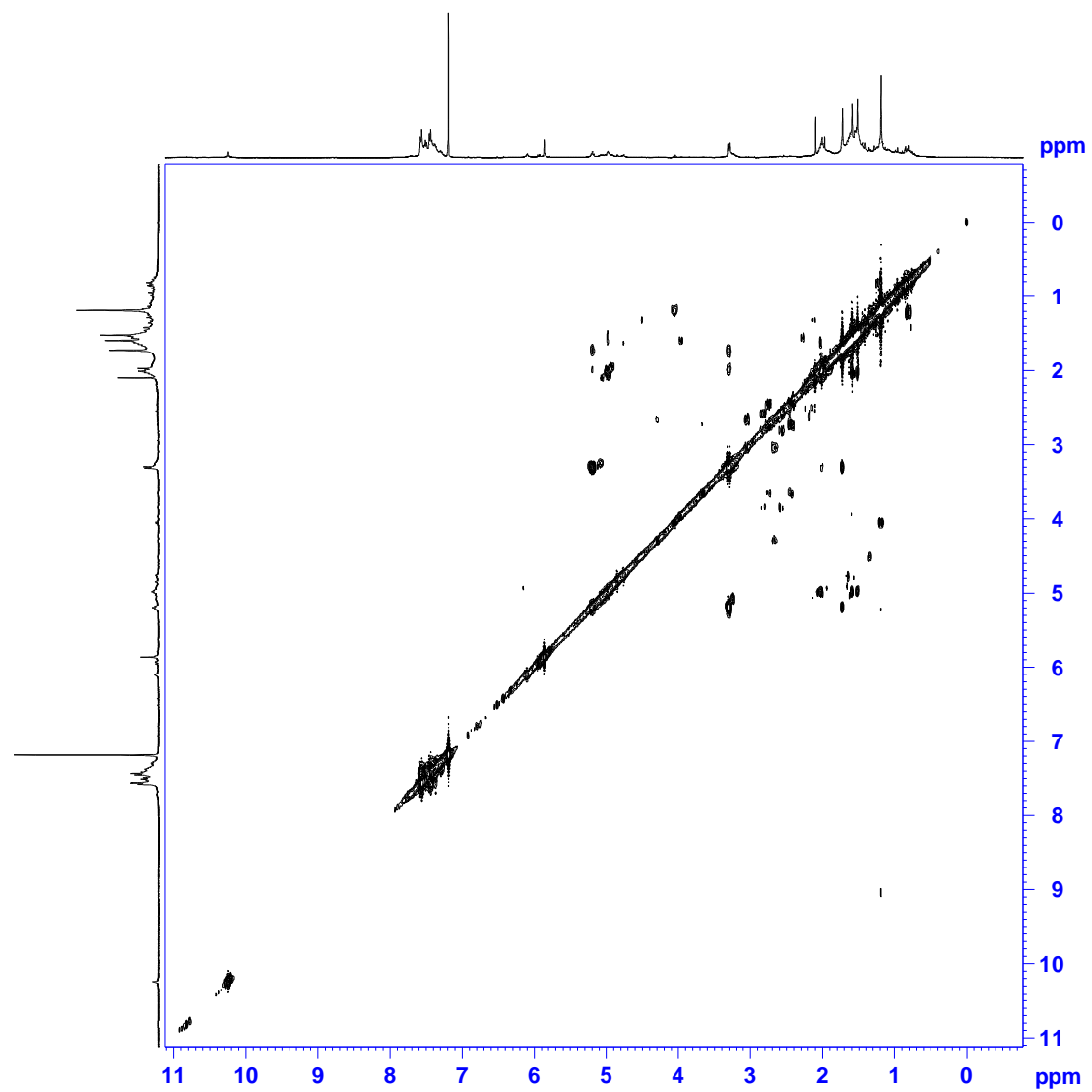


Plate 3.5 COSY spectrum (CDCl_3) of 3-Geranyl-2,4,6-trihydroxybenzophenone (**2**)

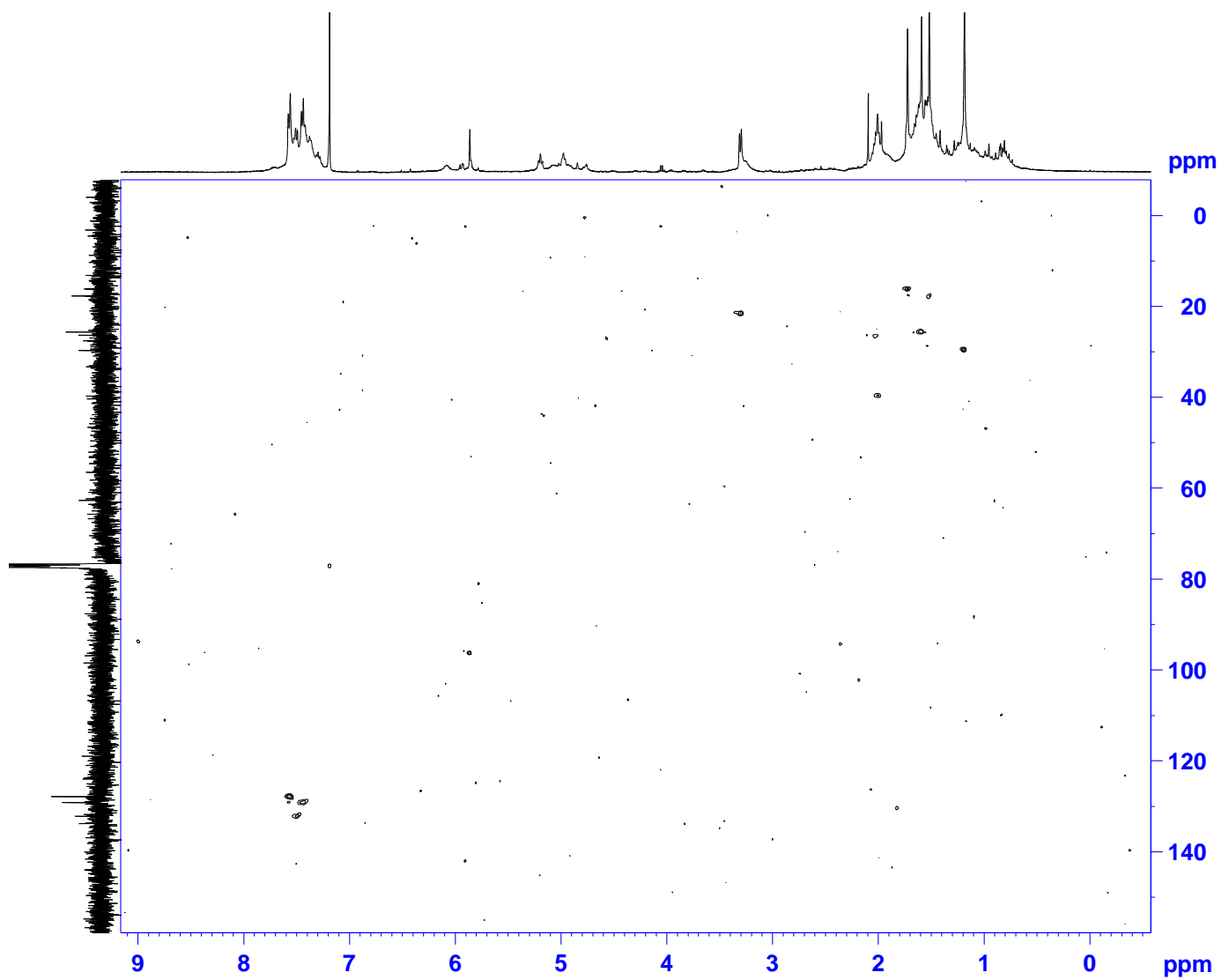


Plate 3.6 HSQC spectrum (CDCl₃) of 3-Geranyl-2,4,6-trihydroxybenzophenone (2)

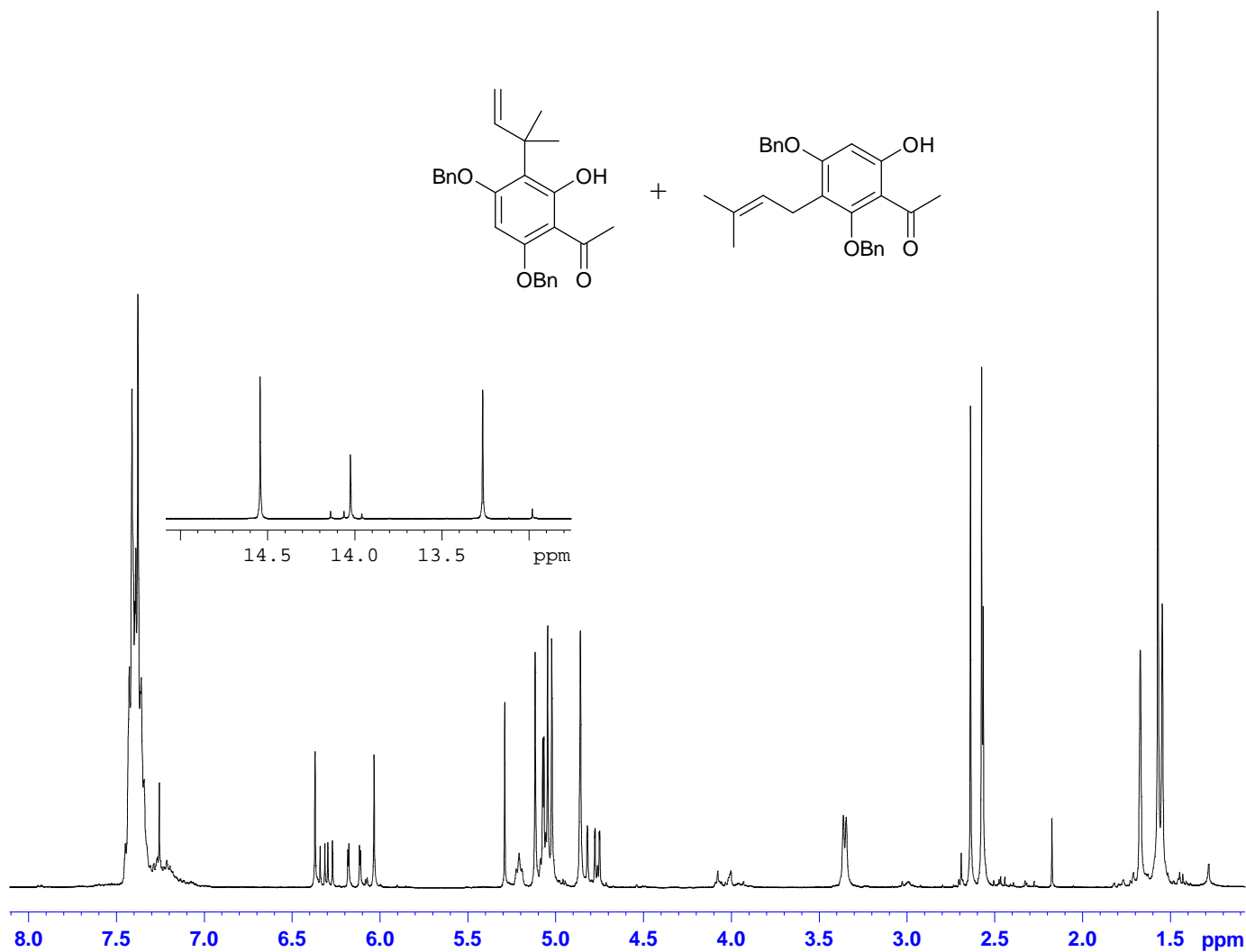


Plate 4.1

¹H NMR spectrum (CD₃OD) of 1-[2,4-Bisbenzyloxy-6-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**46**) and 1-[4,6-bis-benzyloxy-3-(1,1-dimethylallyl)-2-hydroxyphenyl]ethanone (**47**)

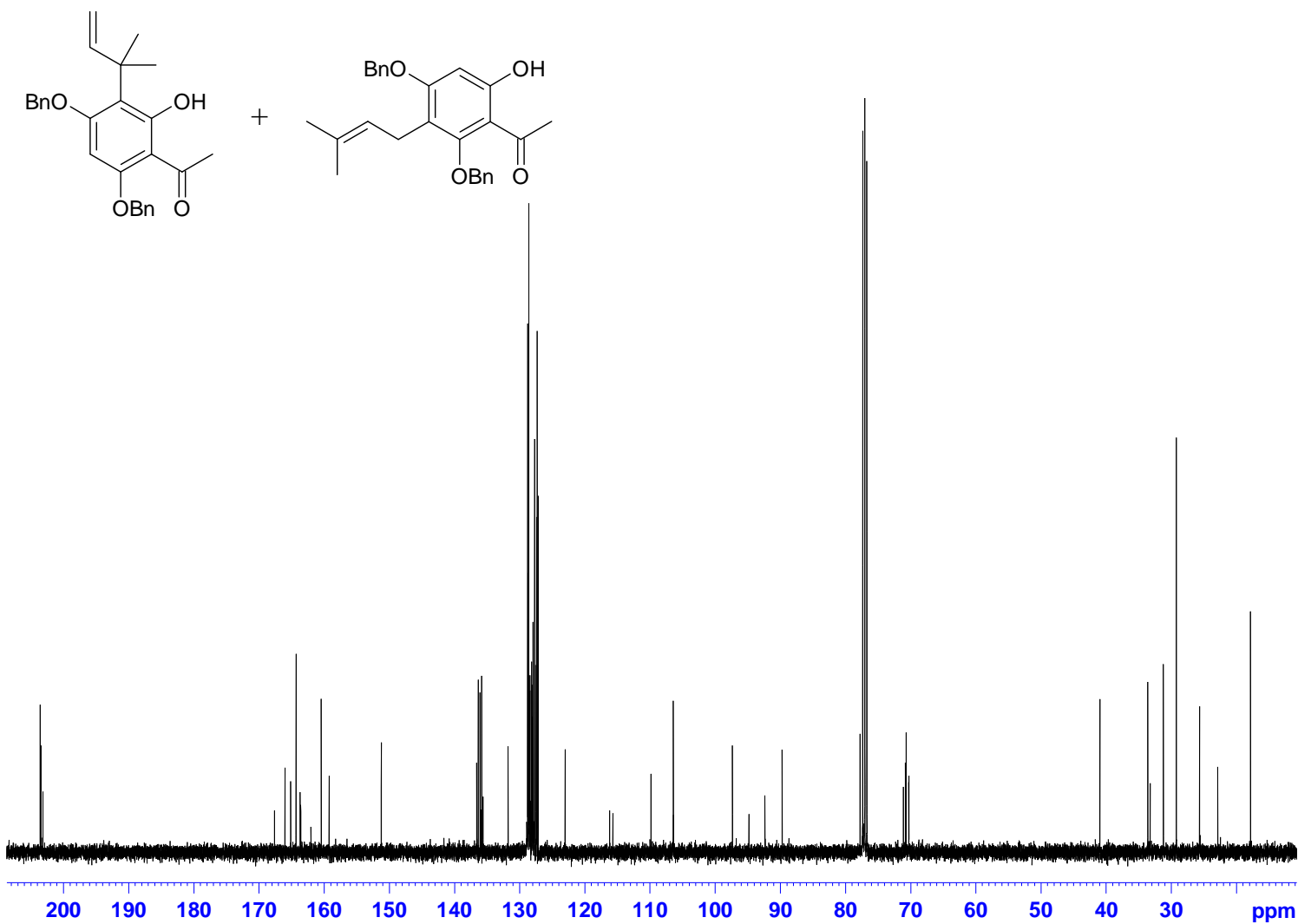


Plate 4.2 ^{13}C NMR spectrum (CD_3OD) of 1-[2,4-Bisbenzyloxy-6-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (**46**) and 1-[4,6-bis-benzyloxy-3-(1,1-dimethylallyl)-2-hydroxyphenyl]ethanone (**47**)

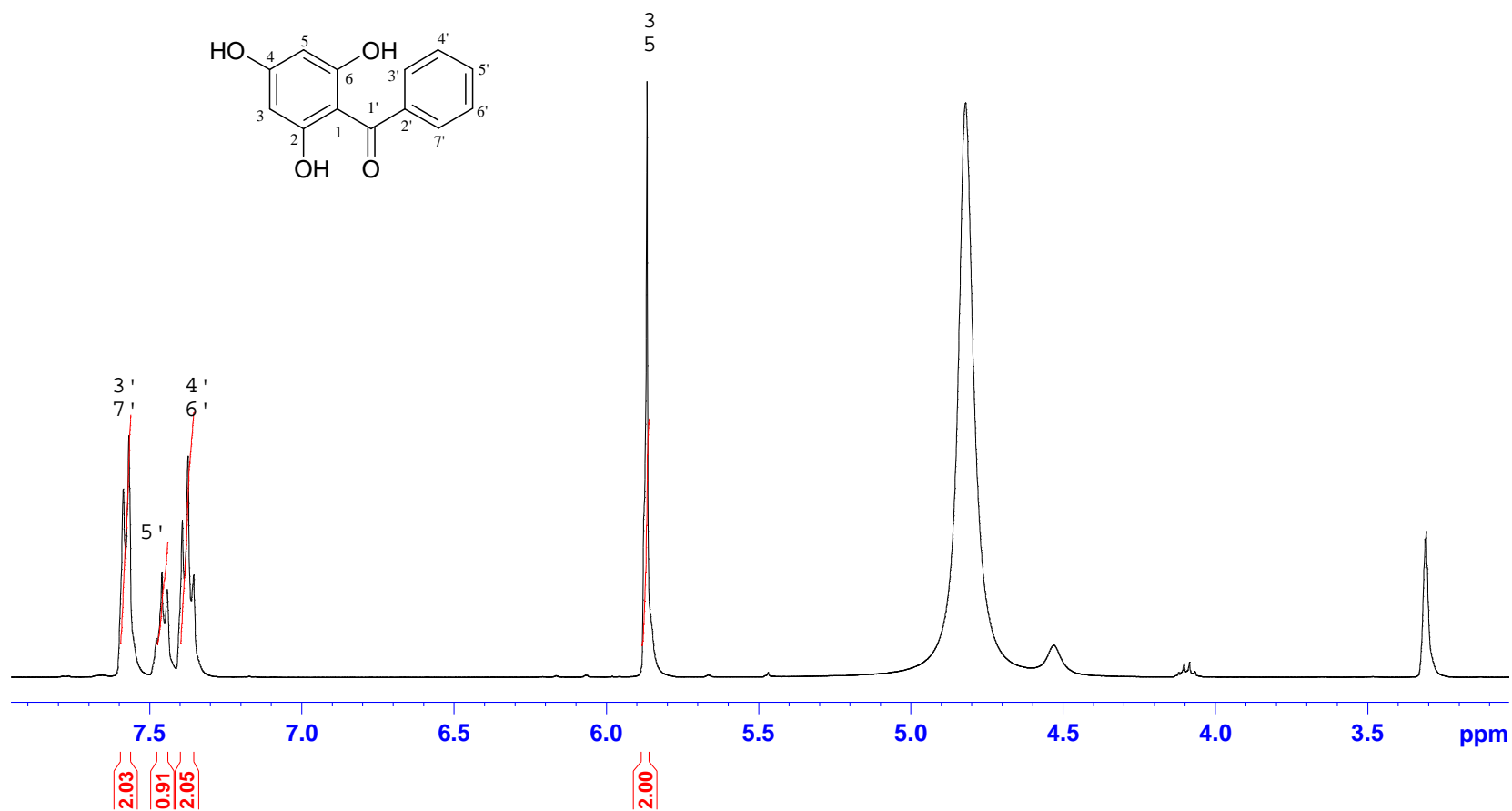


Plate 5.1 ¹H NMR spectrum (CD₃OD) of Phenyl-(2,4,6-trihydroxyphenyl)methanone (**24**)



Plate 5.2 ^{13}C NMR spectrum (CD_3OD) of Phenyl-(2,4,6-trihydroxyphenyl)methanone (24)

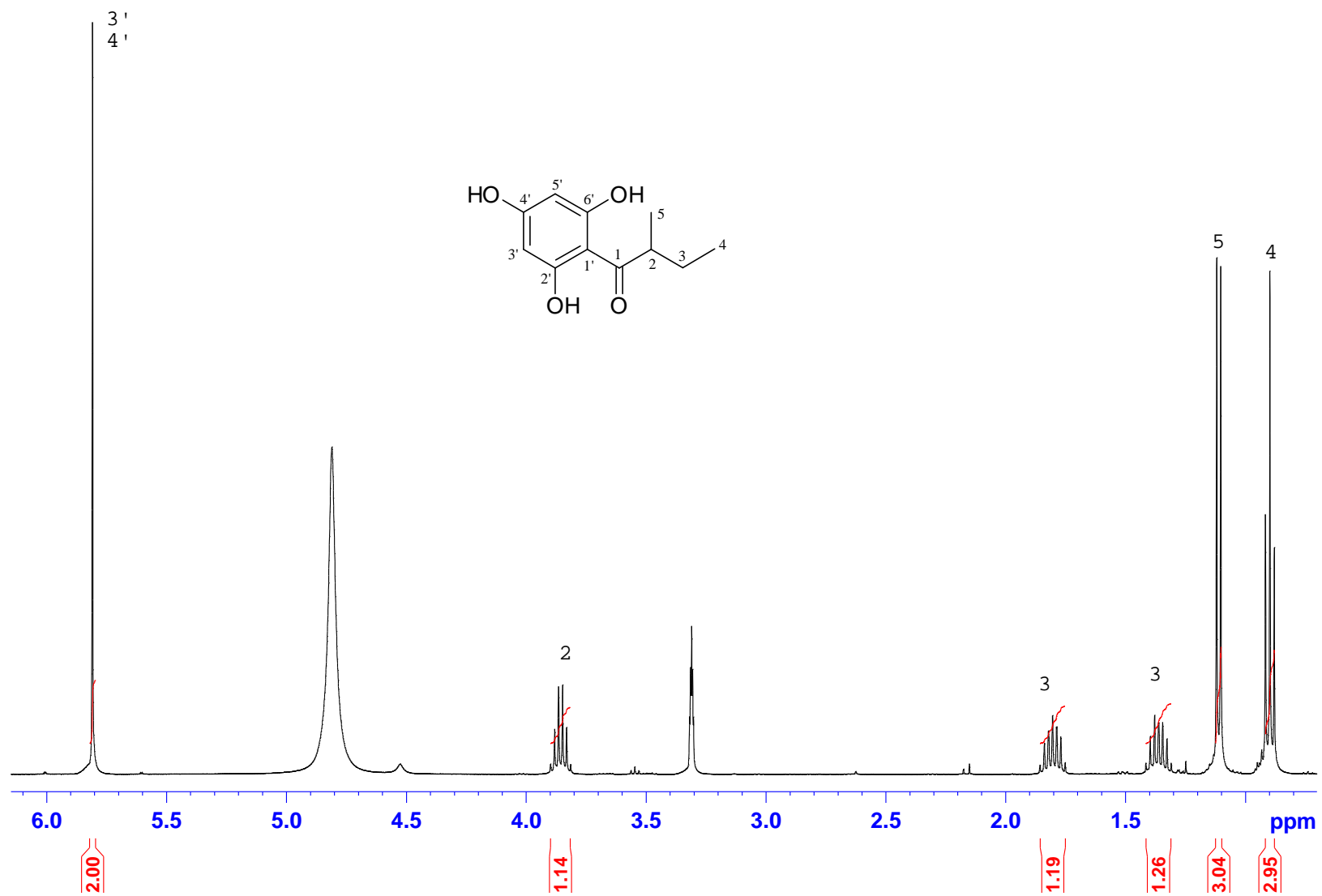


Plate 6.1 ^1H NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (25)

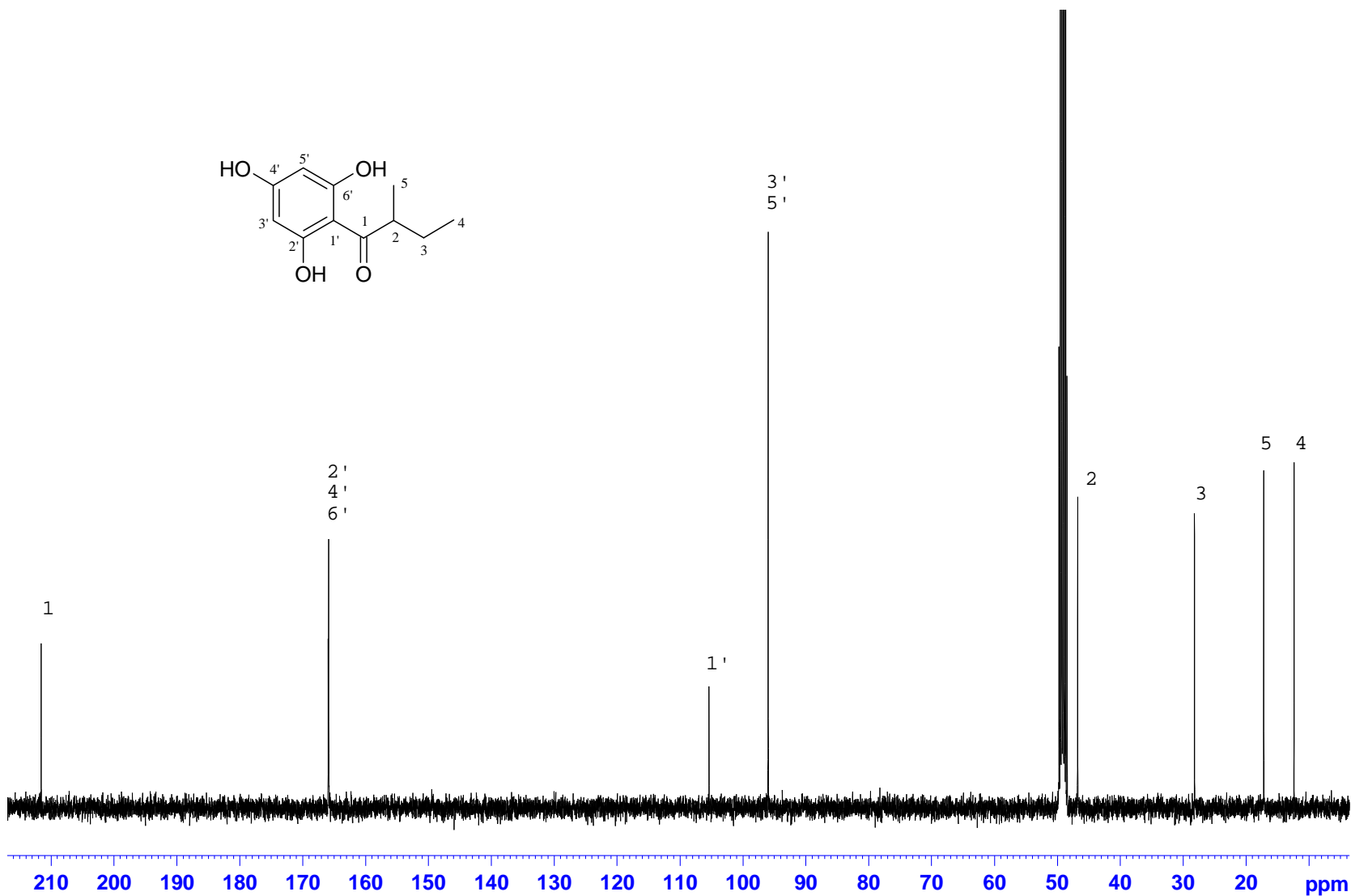


Plate 6.2 ^{13}C NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (25)

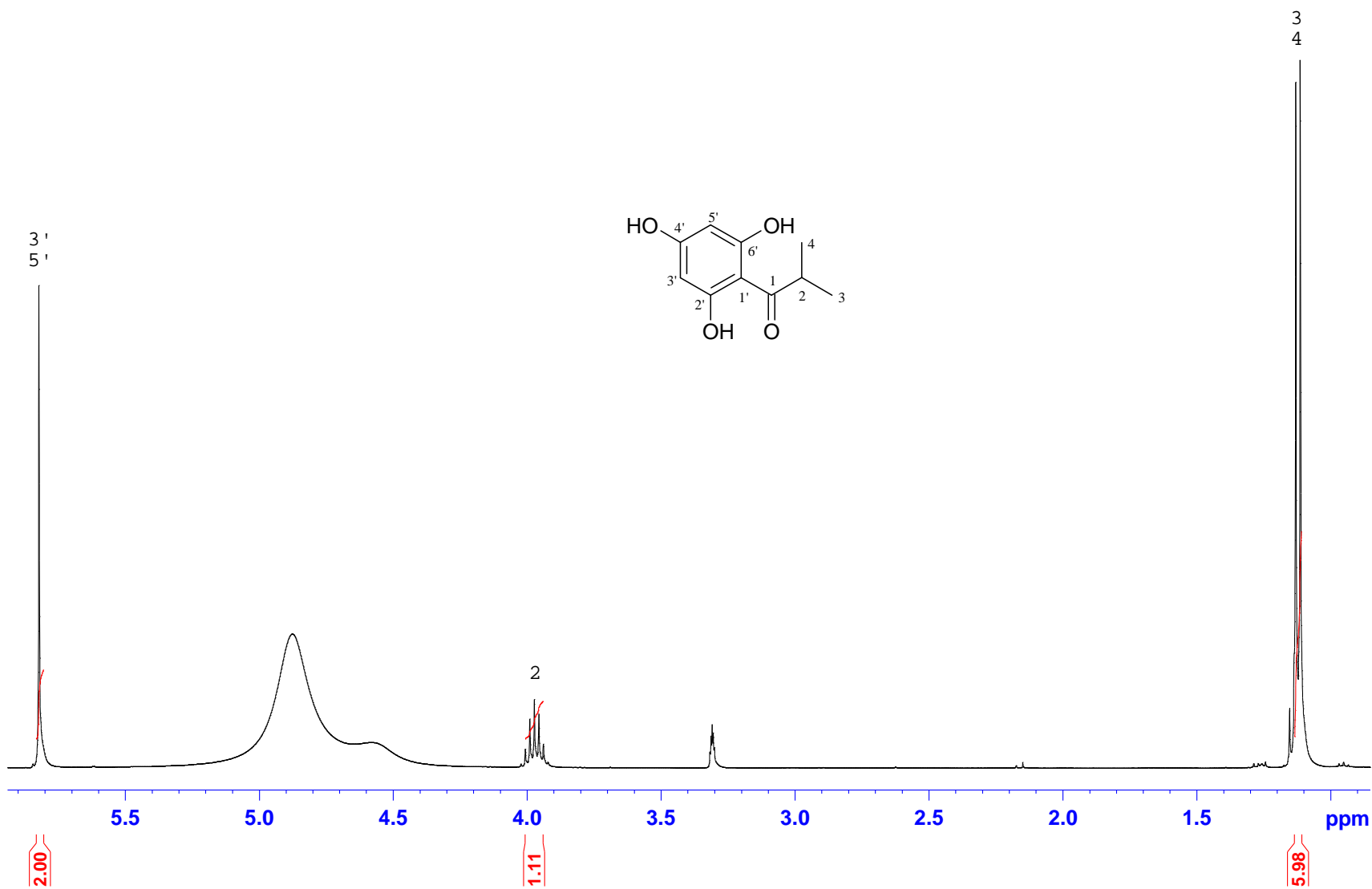


Plate 7.1 ^1H NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**)

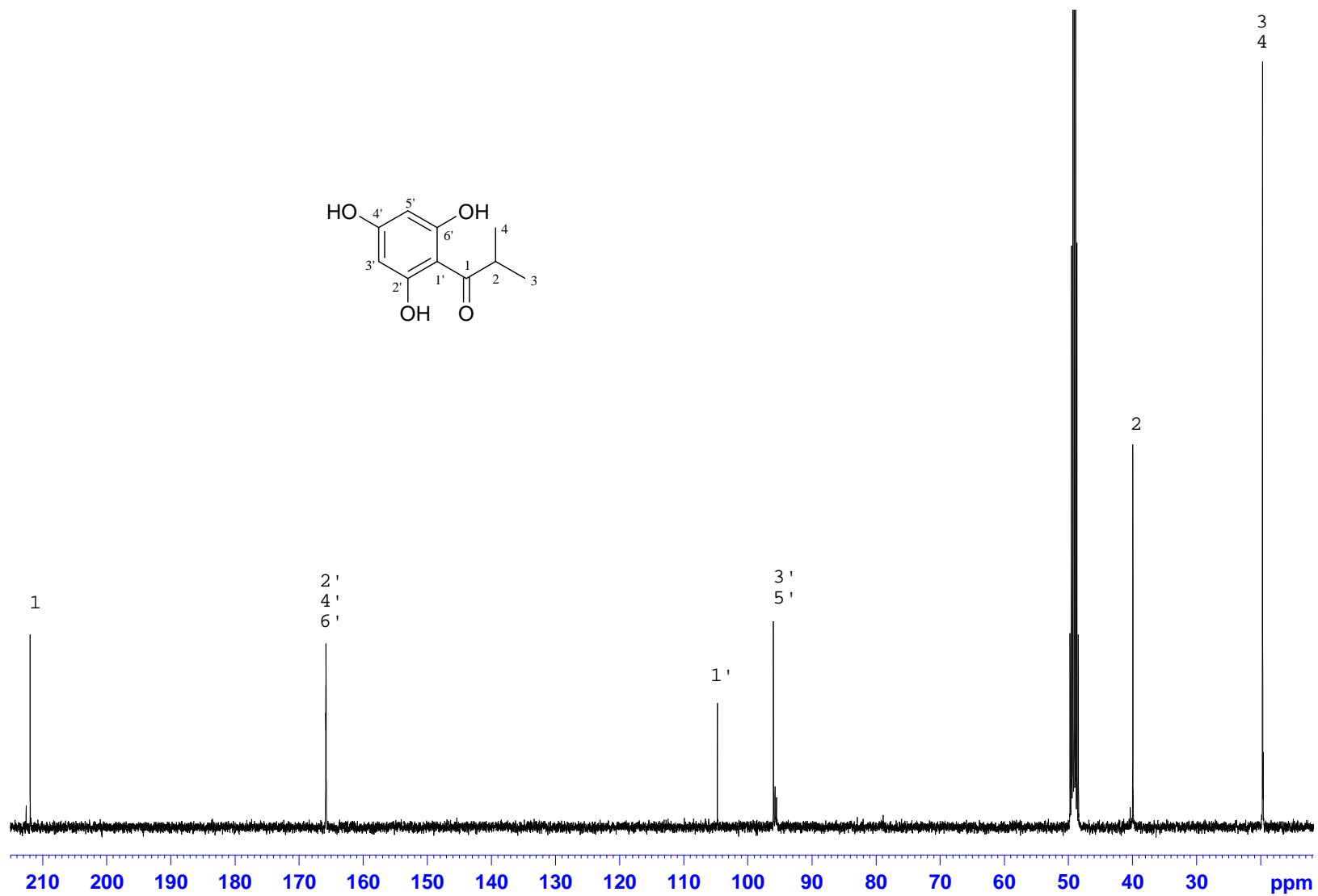


Plate 7.2 ^{13}C NMR spectrum (CD_3OD) of 2-methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**26**)

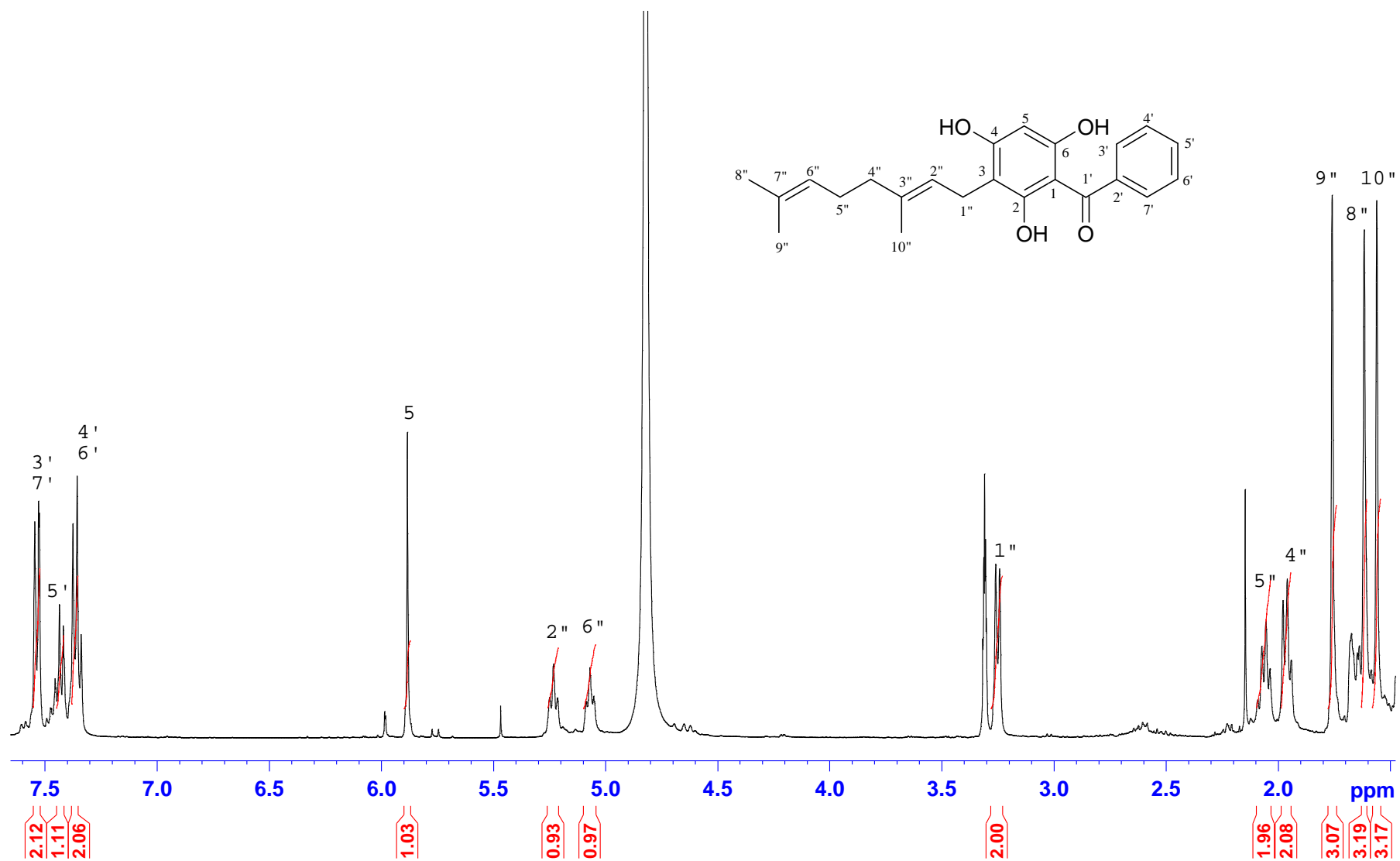


Plate 8.1 ¹H NMR spectrum (CD₃OD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone (2)

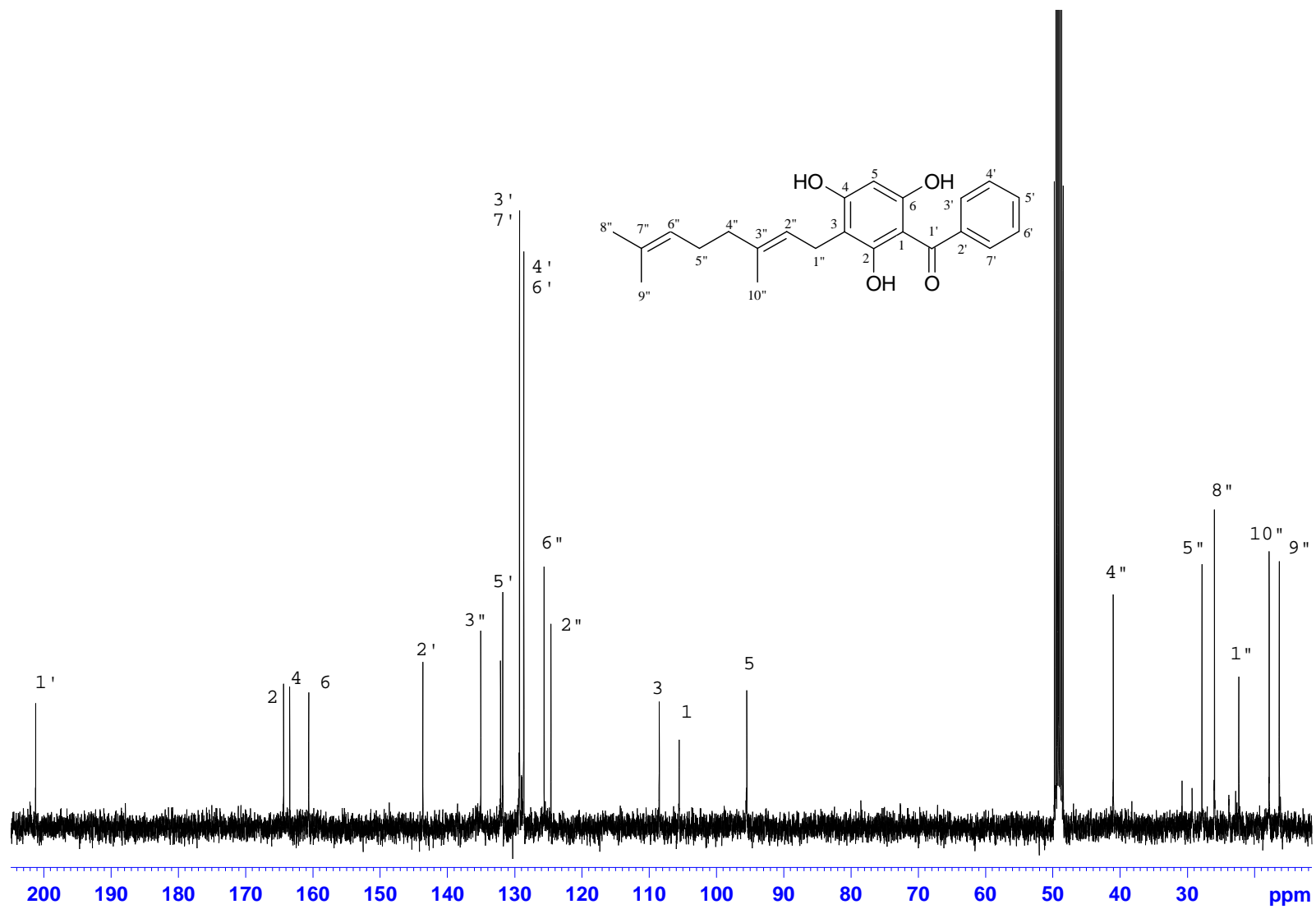


Plate 8.2 ^{13}C NMR spectrum (MeOD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]phenylmethanone (2)

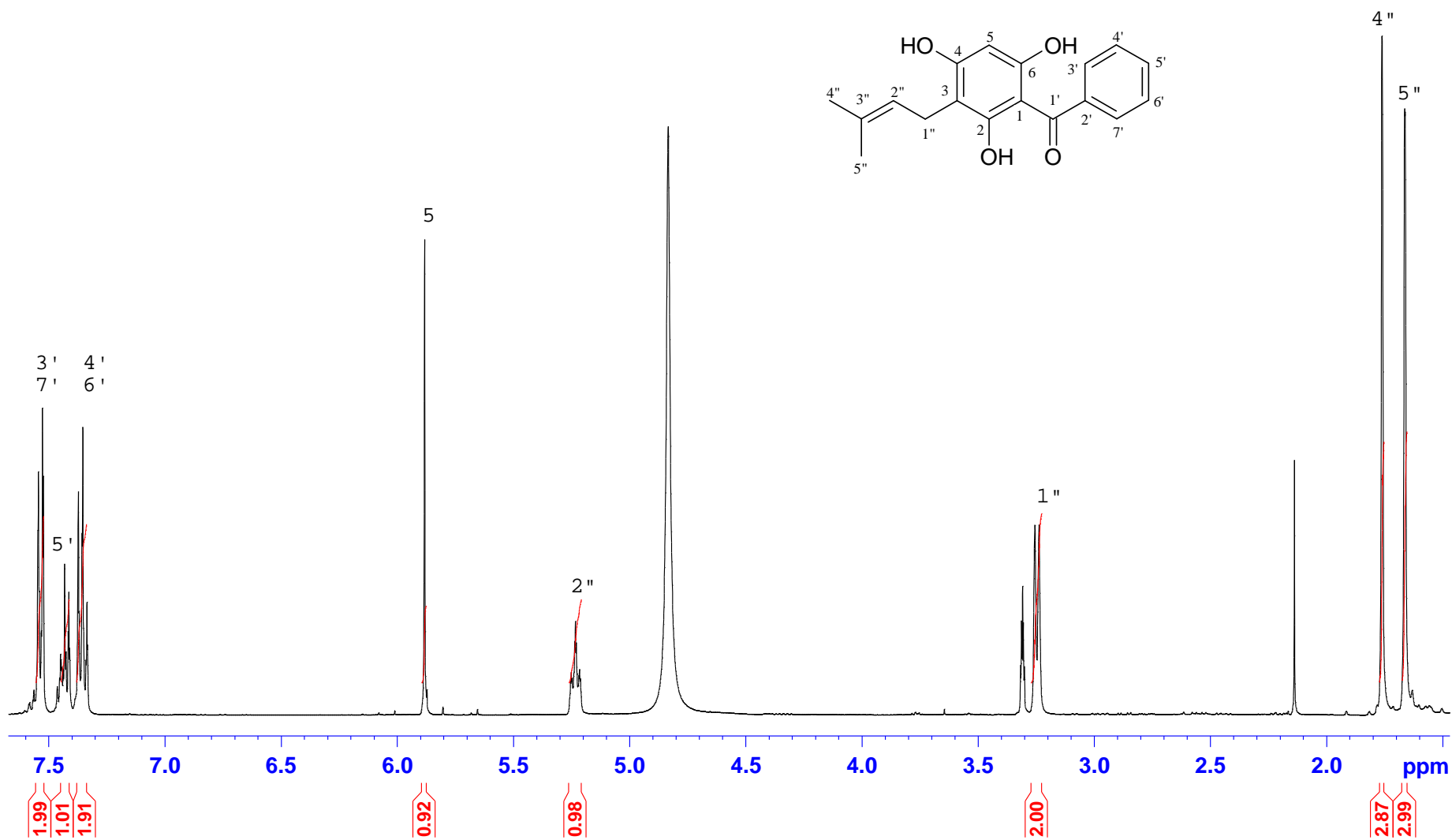


Plate 9.1 ^1H NMR spectrum (CD_3OD) of Phenyl-[2,4,6-trihydroxyphenyl)methanone (**3**)

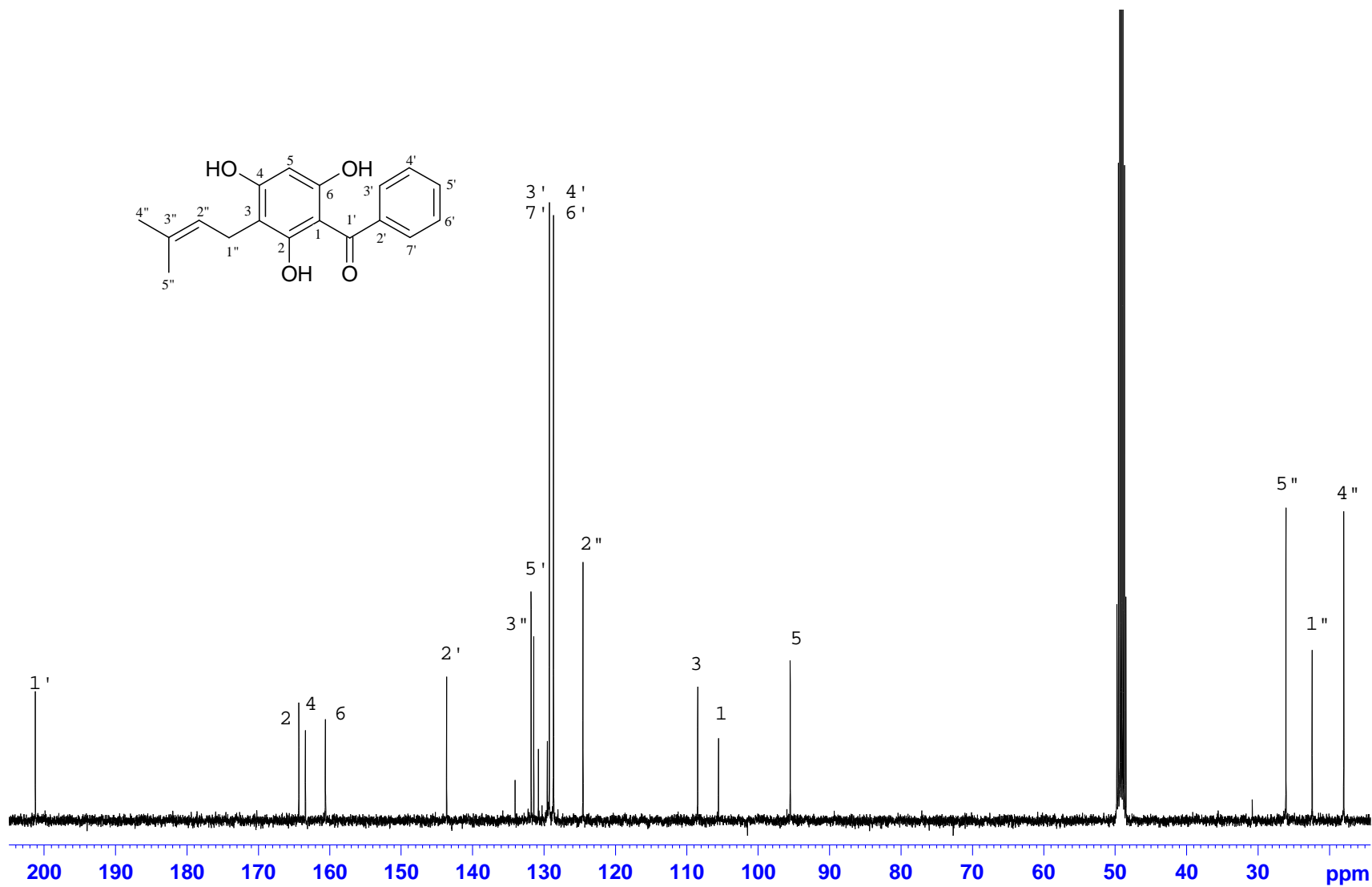


Plate 9.2 ^{13}C NMR spectrum (CD_3OD) of Phenyl-[2,4,6-trihydroxyphenyl)methanone (3)

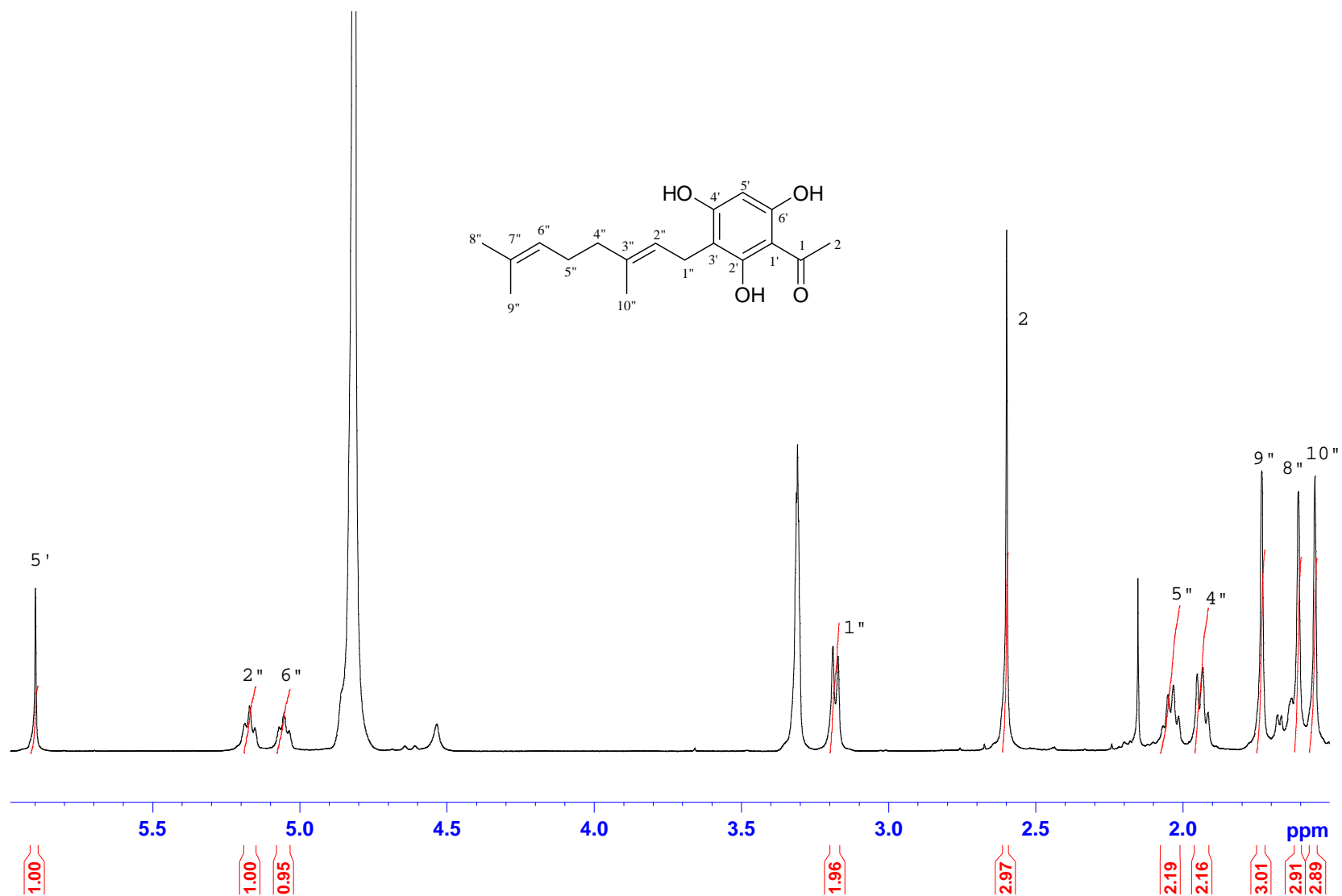


Plate 10.1 ^1H NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]ethanone (**4**)

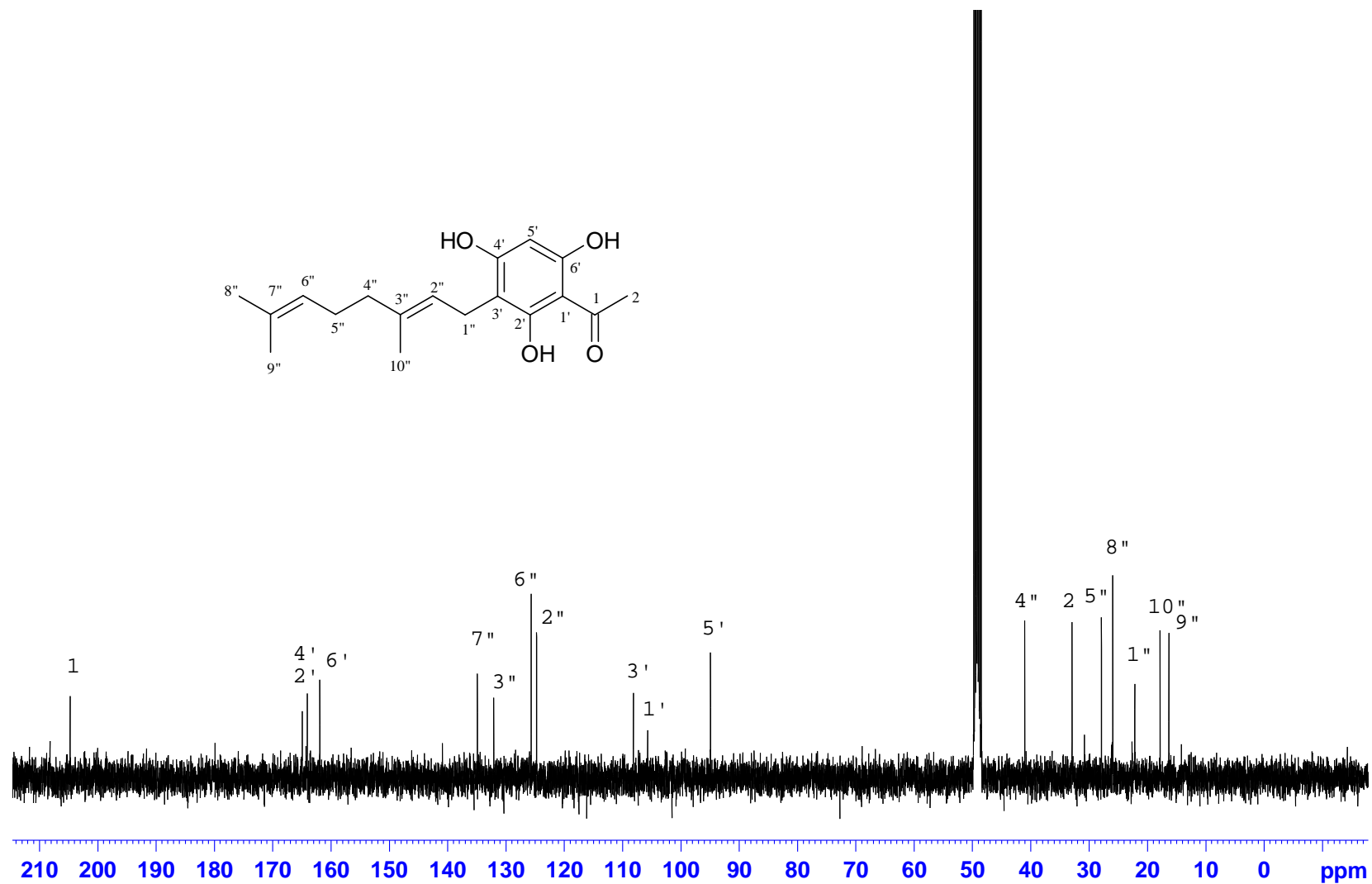


Plate 10.2 ^{13}C NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]ethanone (**4**)

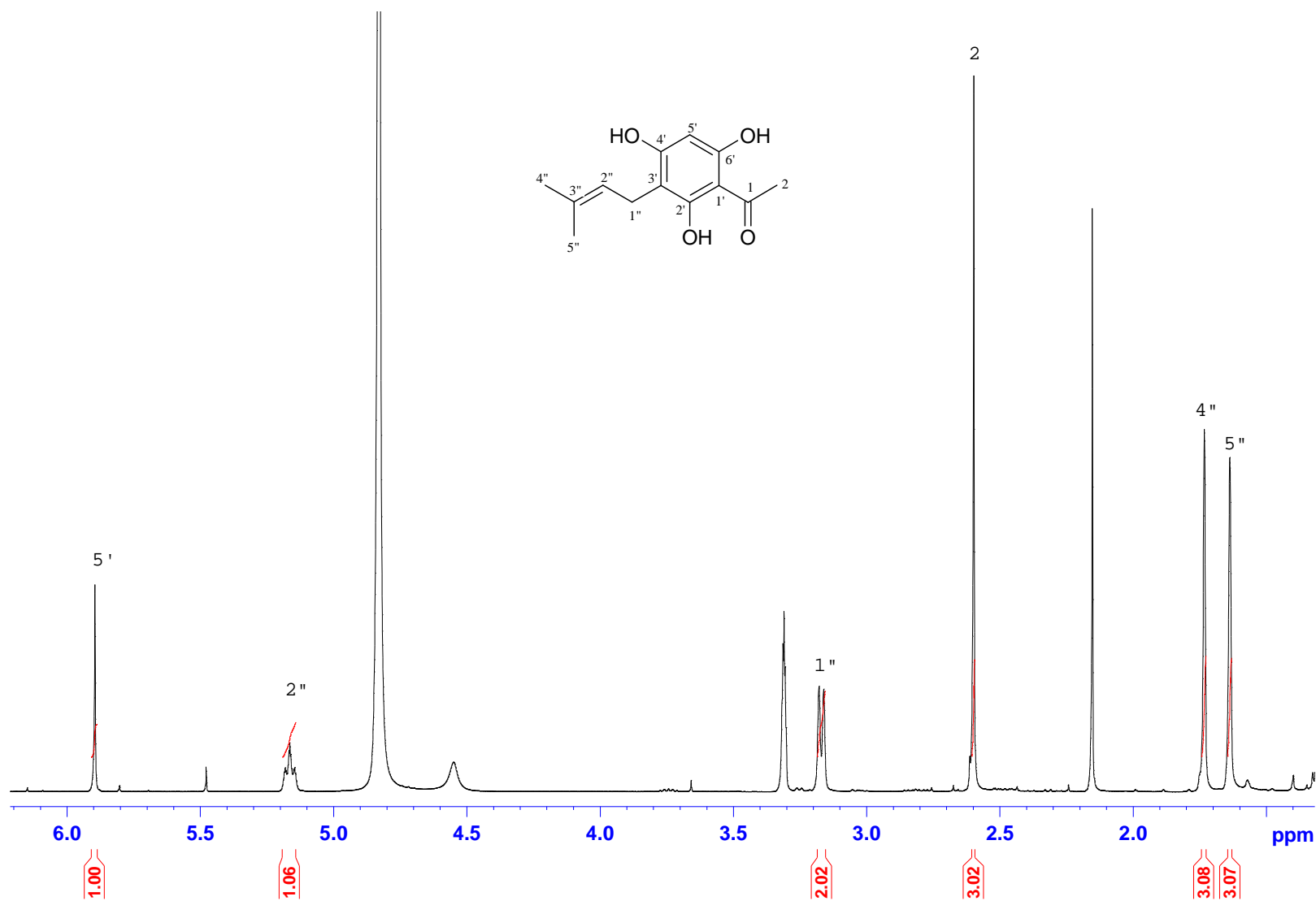


Plate 11.1 ^1H NMR spectrum (CD_3OD) of 1-[2,4,6-Trihydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (5)

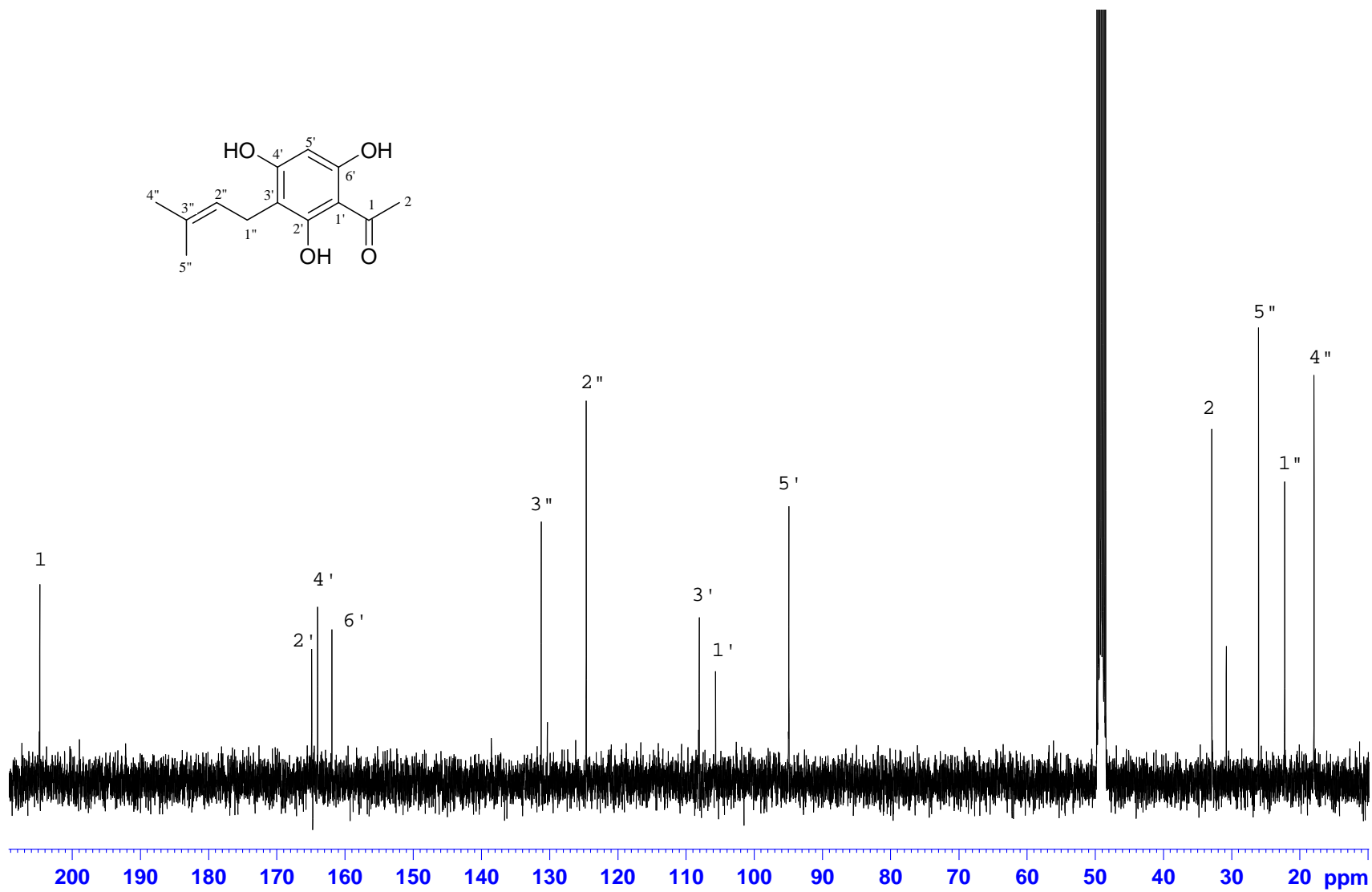
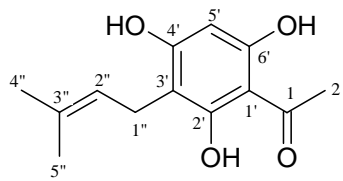


Plate 11.2 ^{13}C NMR spectrum (CD_3OD) of 1-[2,4,6-Trihydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone (5)

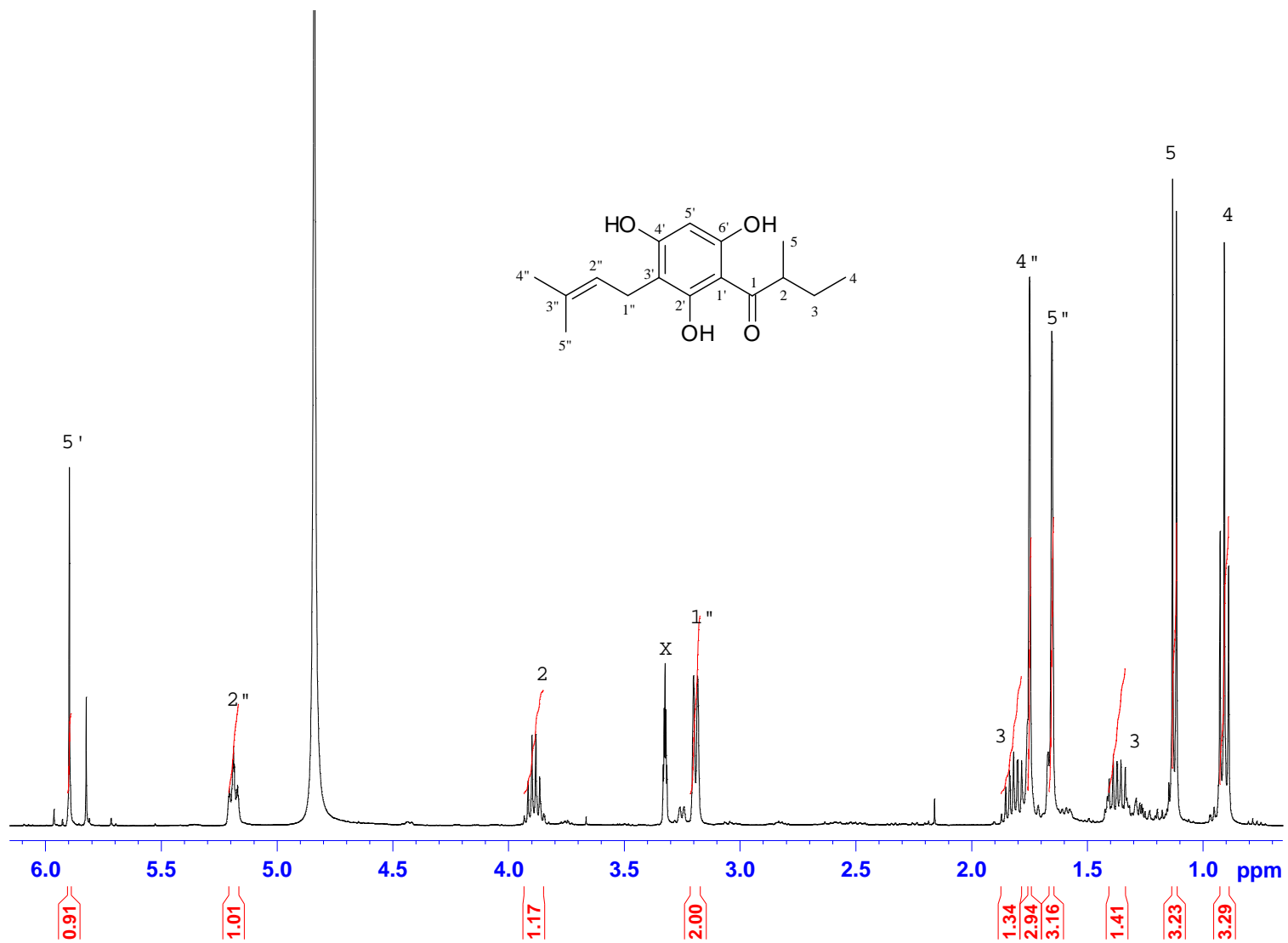


Plate 12.1 ¹H NMR spectrum (CD₃OD) of 2-Methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]butan-1-one (7)

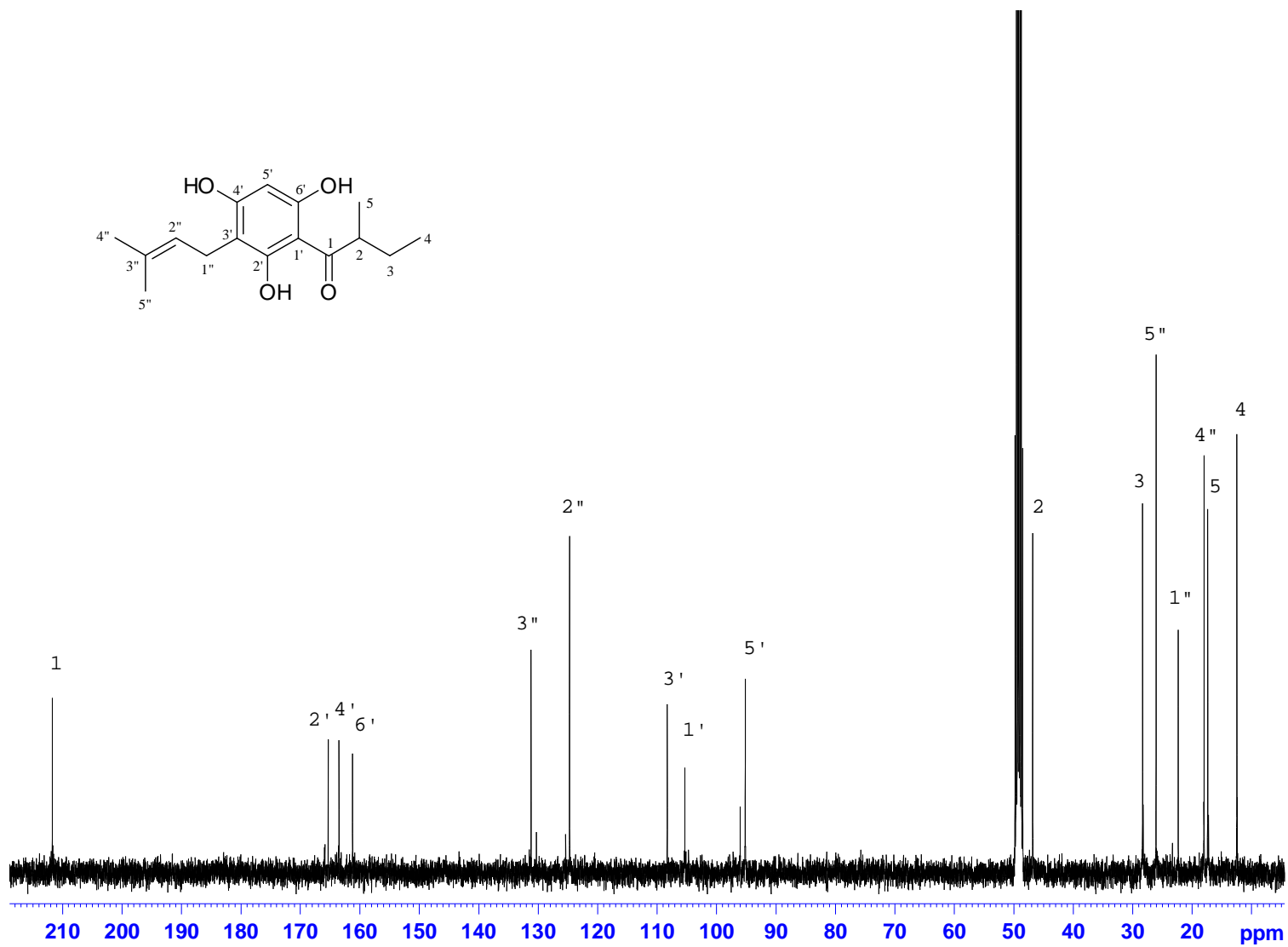
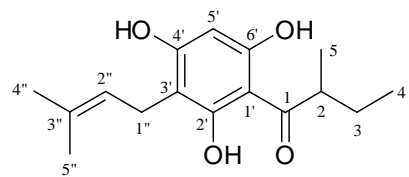


Plate 12.2 ^{13}C NMR spectrum (CD_3OD) of 2-Methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]butan-1-one (7)

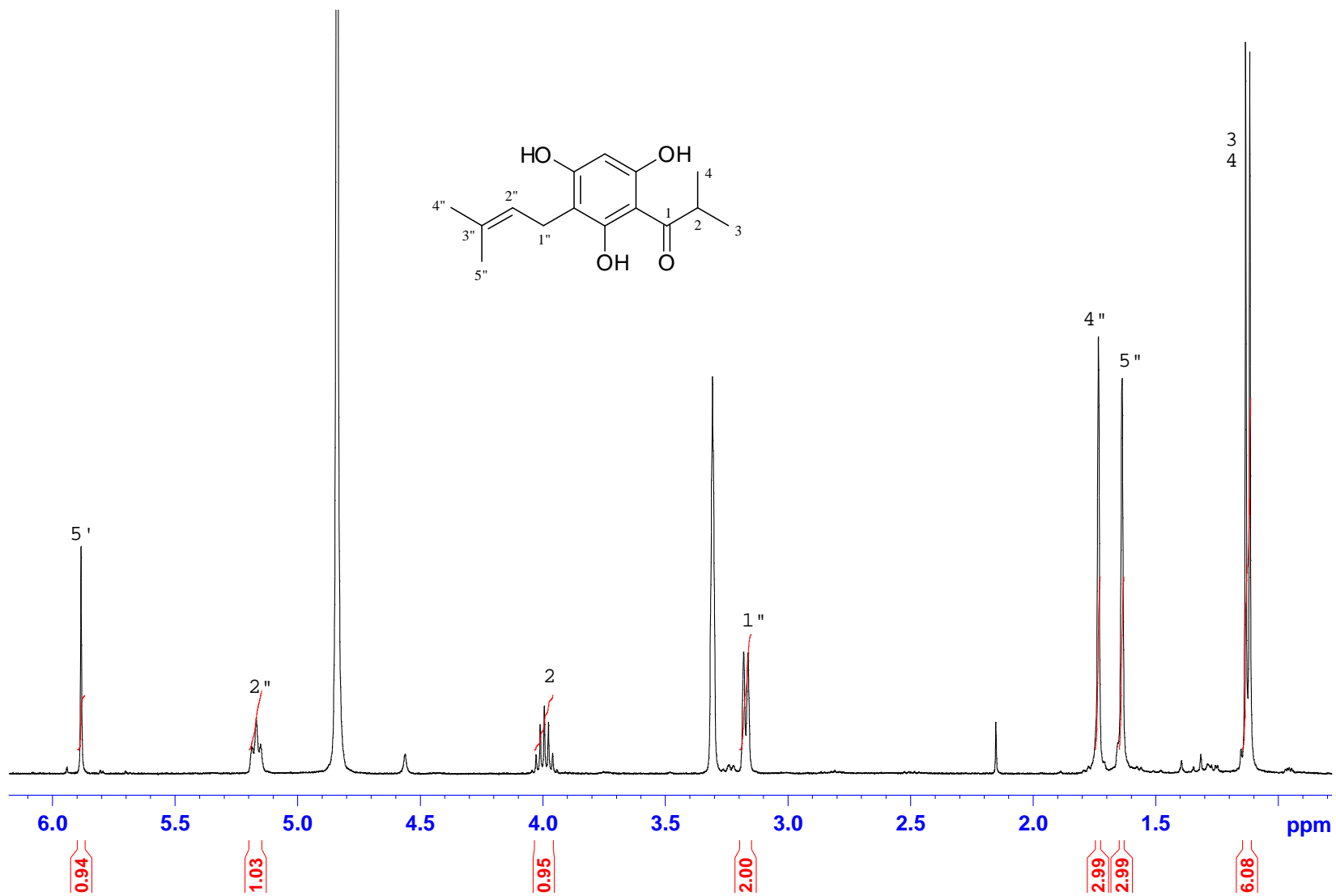


Plate 13.1 ¹H NMR spectrum (CD₃OD) of 2-methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]propan-1-one (**9**)

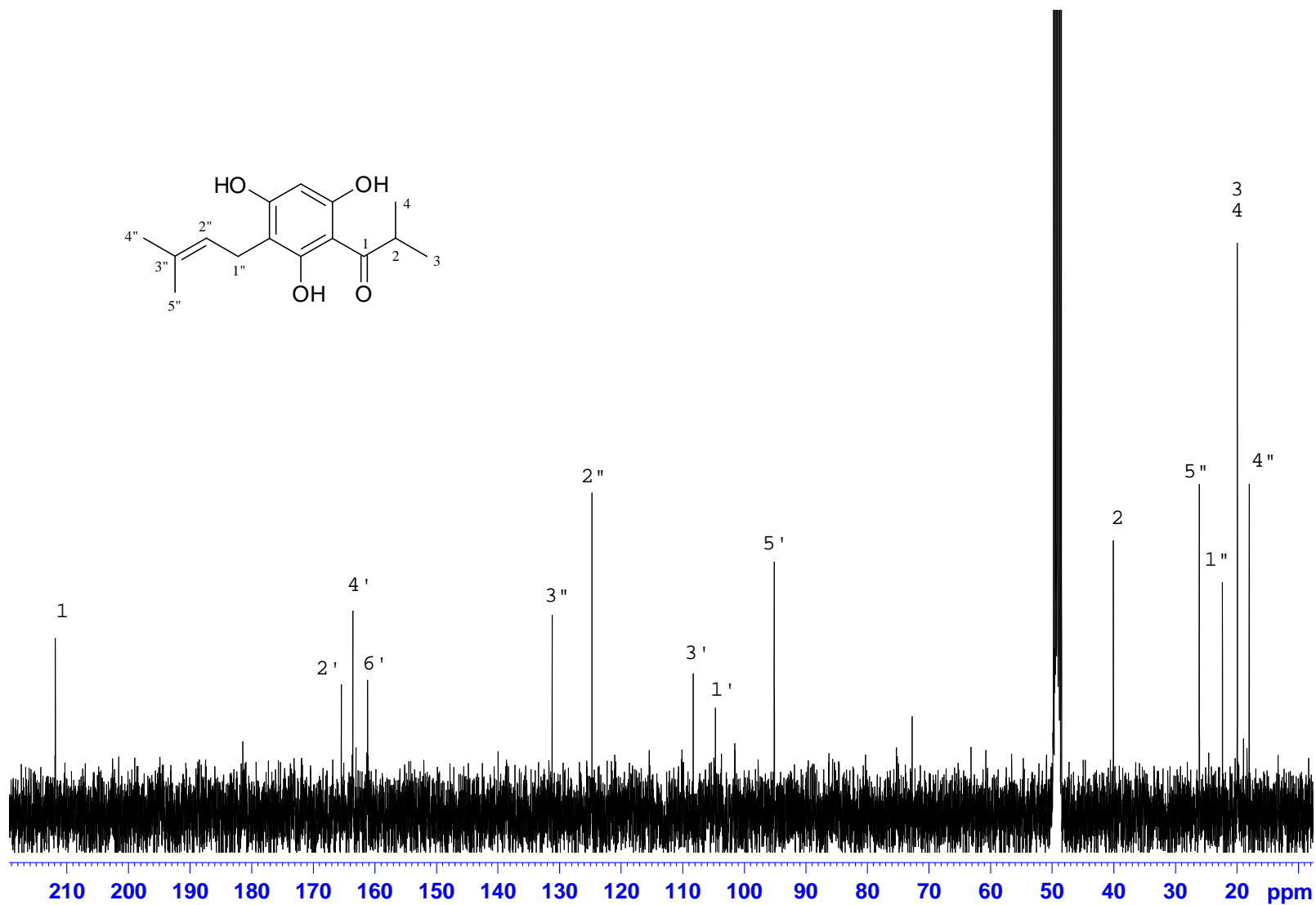
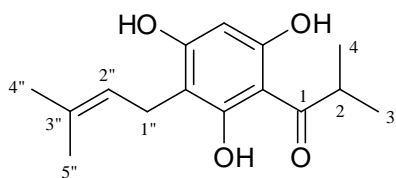


Plate 13.2 ^{13}C NMR spectrum (CD_3OD) of 2-methyl-1-[2,4,6-trihydroxy-3-(3-methylbut-2-enyl)phenyl]propan-1-one (**9**)

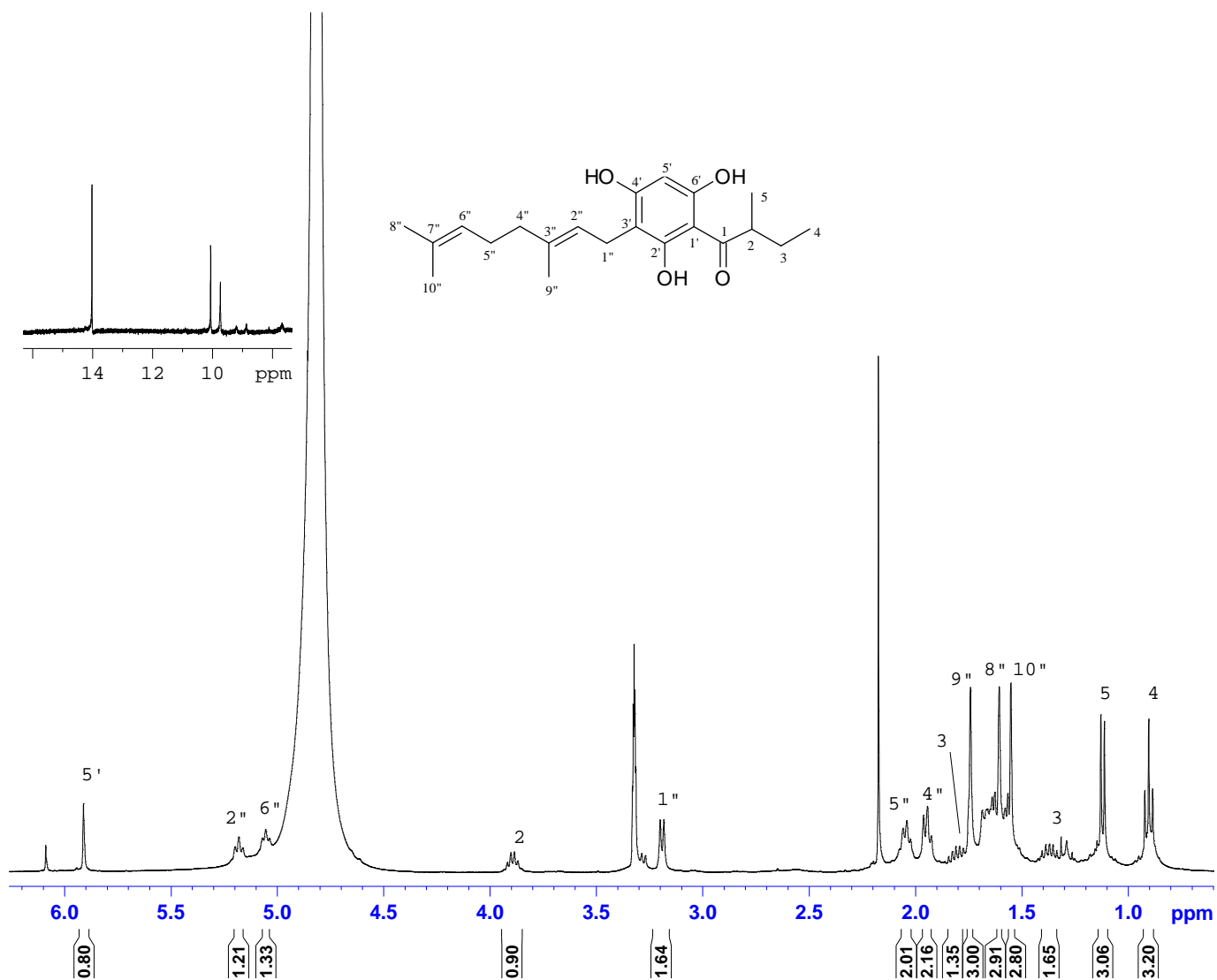


Plate 14.1 ^1H NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylbutan-1-one (**6**)

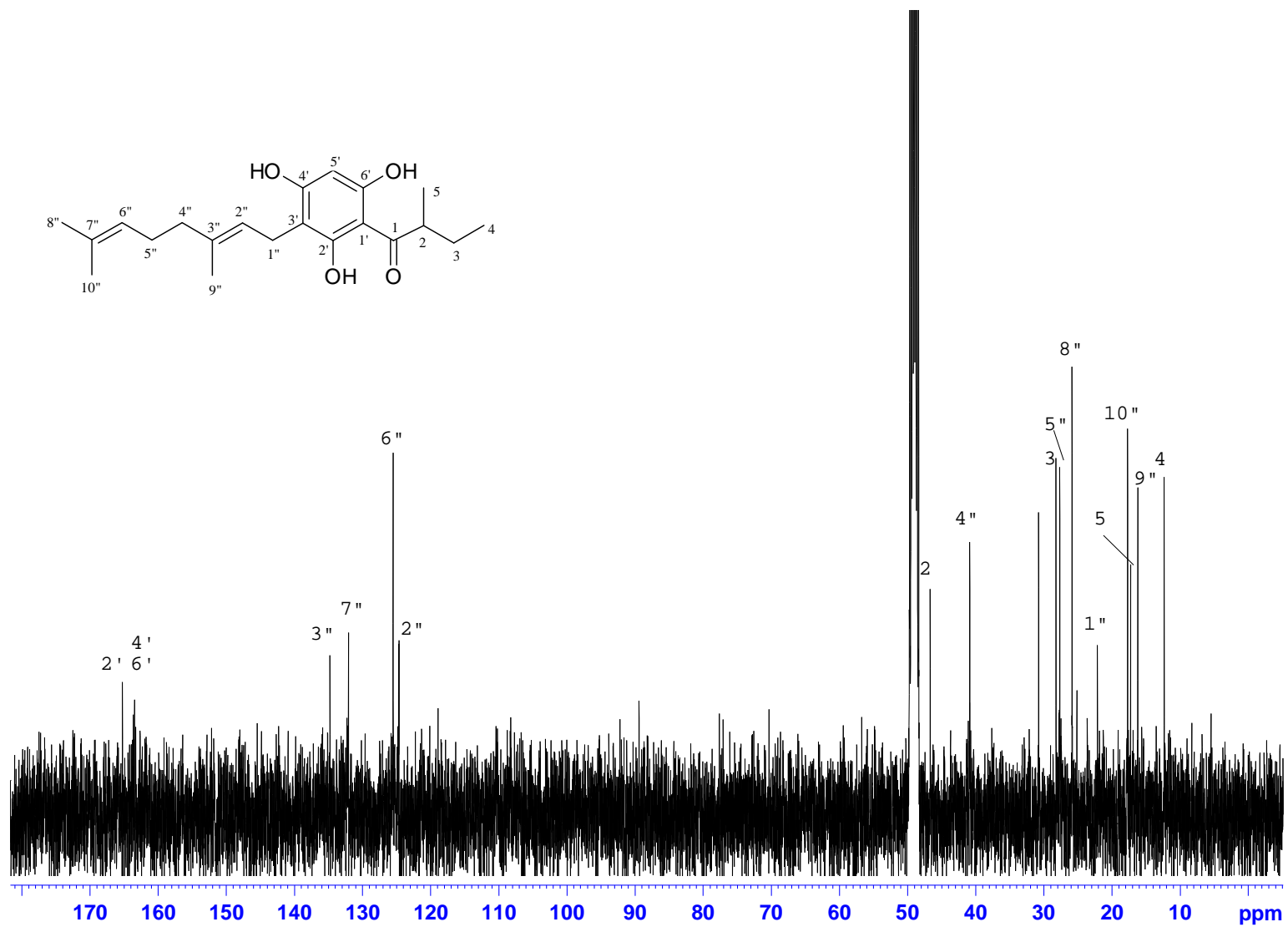


Plate 14.2 ^{13}C NMR spectrum (CD_3OD) of 1-[3-(3,7-Dimethylocta-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylbutan-1-one (6)

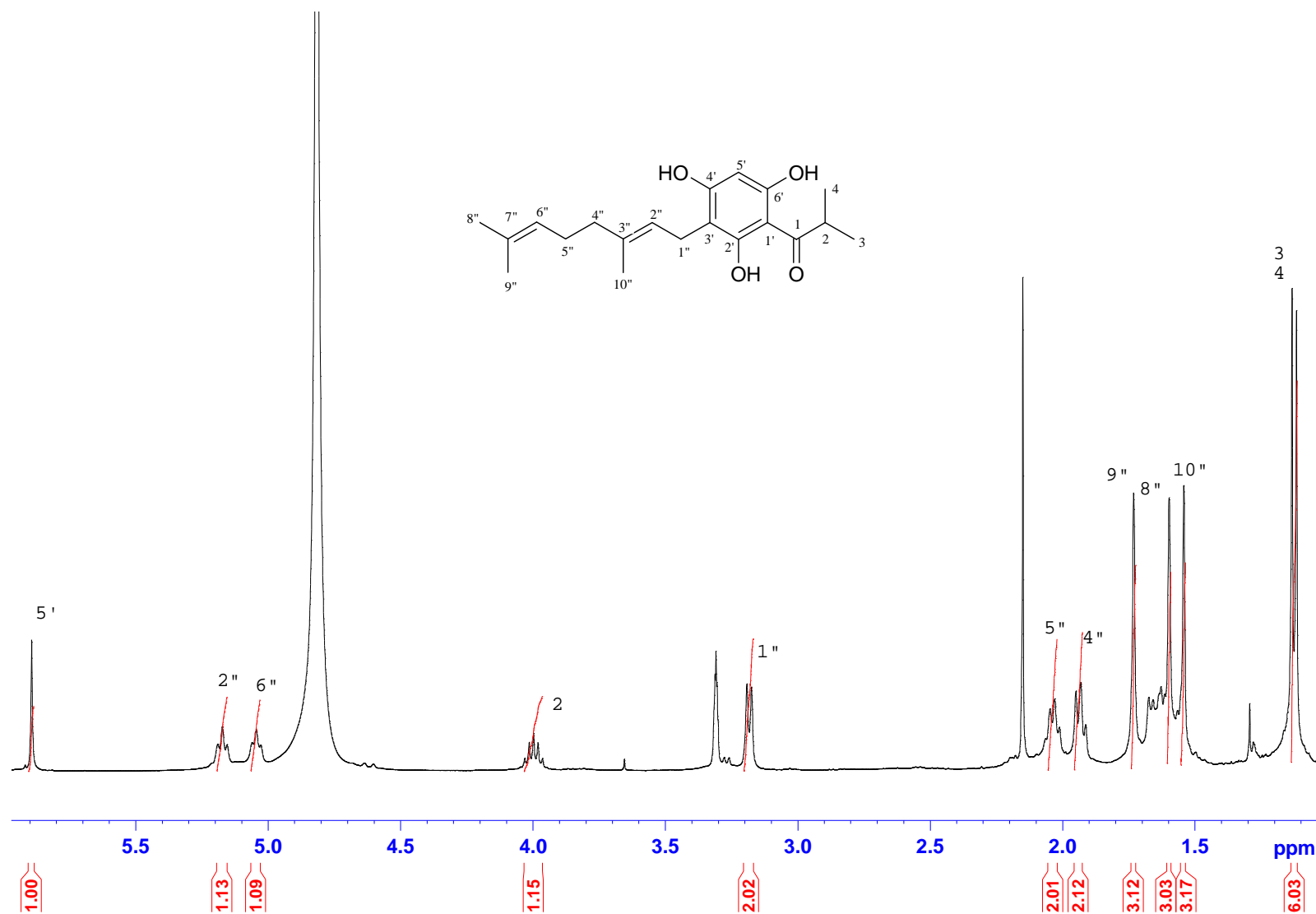


Plate 15.1 ¹H NMR spectrum (CD₃OD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylpropan-1-one (**8**)

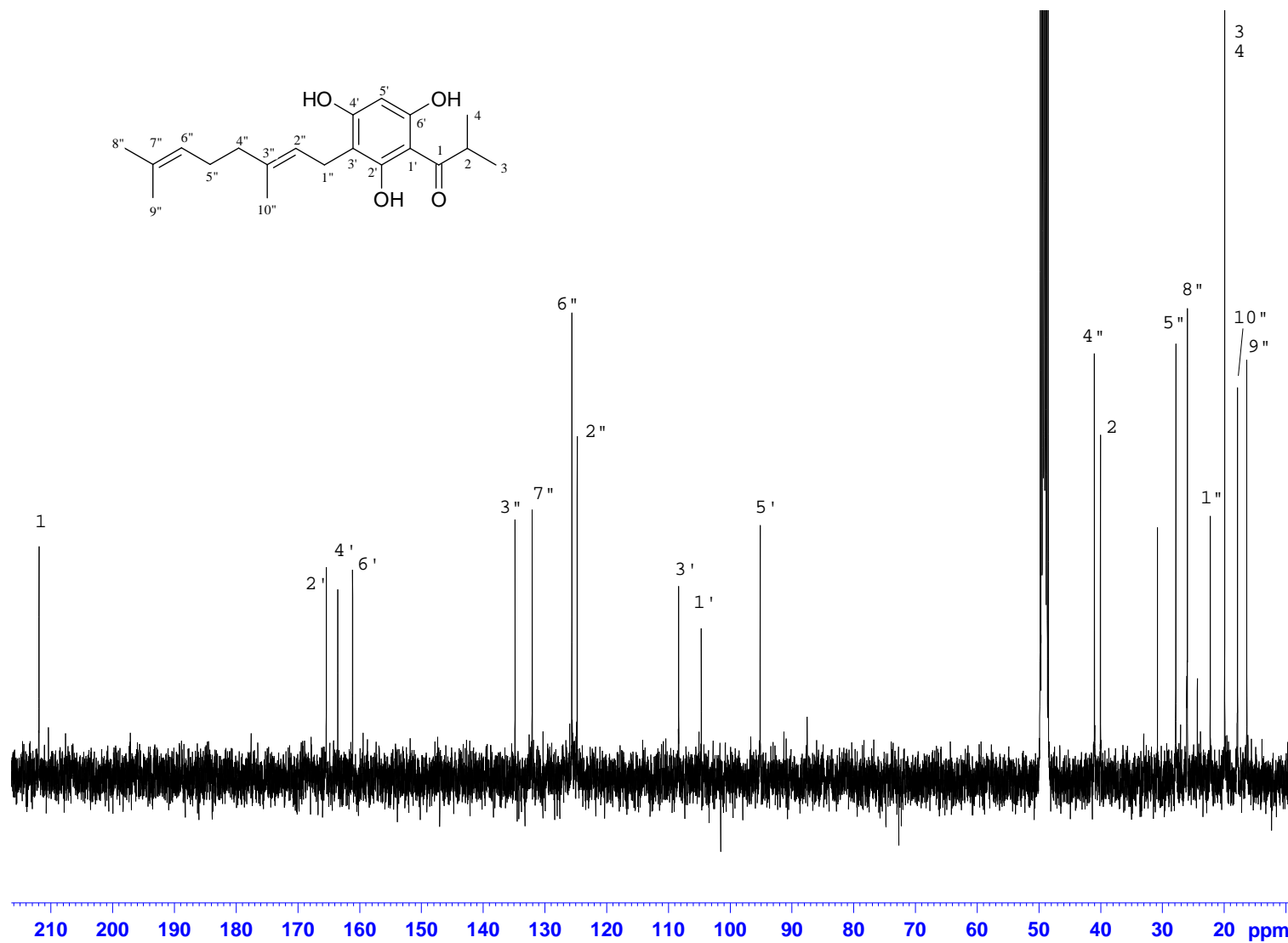


Plate 15.2 ^{13}C NMR spectrum (CD_3OD) of [3-(3,7-Dimethyl-octa-2,6-dienyl)-2,4,6-trihydroxyphenyl]-2-methylpropan-1-one (**8**)