The synthesis and electrochemical studies of Chalcones and Flavanones: An Investigation of their antioxidant activity

By

Carlo Baugaard

M.Sc. thesis submitted in fulfilment of the requirements for the degree of Magister Scientiae,

In the Department of Chemistry

University of Western Cape

Principal Supervisor: Prof. IR Green

Department of Chemistry

Co. Supervisor: Dr Jahed

Department of Chemistry

DECLARATION

I declare that the "The synthesis and electrochemical studies of Chalcones and Flavanones: An Investigation of their antioxidant activity", is my own work, that it has not been submitted for any degree or examination in any other University and that all sources I have used or quoted have been indicated and acknowledged by complete references.

Full name …**Carlo Secondo Baugaard**……....Date ……

Signed……

ABSTRACT

Flavonoids, one of the biggest classes of secondary metabolites, are found abundantly in nature in a broad range of fruits, vegetables and beverages such as tea, coffee, beer, wine and fruit drinks. Flavonoids have been reported to exert multiple biological functions as well as tremendous pharmacological activity, including anticancer activity, protection, antioxidant activity, cardiovascular protection, antibacterial, antifungal and antiviral activity. The antioxidant activity of flavones is reported to be associated with those bearing hydroxyl functions.

In the present study, several reaction steps have been carried out to synthesize three sub classes of flavonoids namely; chalcones, dihydrochalcones and flavanones with various substituents attached. The first step involved protection of hydroxyl groups of acetophenone and benaldehyde as starting materials. Thereafter the Clasien Schmidt condensation reaction, under basic conditions, was performed to afford chalcone intermediates. Treatment of these chalcones with sodium acetate, under reflux, afforded flavanones as a single product in high yields. Thereafter all protecting groups where removed to yield the final products. All products and intermediates where purified by column chromatography and were characterized by Nuclear Magnetic Resonance Spectroscopy (NMR) (^{1}H NMR and ^{13}C NMR).

An electrochemical analysis on all flavonoid compounds was performed by Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV) to give information on the accessible redox couples identified by their oxidation potentials. Oxidation potentials, which gave valuable information about reducing ability and hence the antioxidant activity, where used to compare all compounds. The antioxidant activity was observed to increase with the addition of hydroxyl groups on the B-ring. Compounds with a combination of hydroxyl groups on the A-ring and methoxy groups on the B-ring showed increased antioxidant activity when compared to those with only hydroxyl groups on the base structure. 2, 5, 4'-trihydroxy dihydrochalcone showed moderate antioxidant ability. However the 2, 5, 4'-trihydroxychalcone, containing the α, β unsaturated double bond, proved to have the greatest antioxidant ability.

ACKNOWLEDGMENTS

This paper could not be written to its fullest without Professor Ivan Green, who served as my supervisor despite his other academic commitments. His wisdom, knowledge and commitment to the highest standards inspired and motivated me. He has constantly challenged and encouraged me throughout the time I have spent studying under him. He would have never accepted anything less than my best efforts, and for that, I thank him.

To my co-supervisor Dr Jahed and professor Iwuoha, your thoughtful criticism, time and attention was immensely appreciated. Thank you for providing me with the advice and financial support needed to complete my study.

I would like to express my deepest gratitude to Dr Titinchi, who over the past year has become my mentor and my friend, thank you for your assistance, guidance and support. I look forward to working with you in the near future.

Many thanks to my colleagues at the University of the Western Cape for sharing your enthusiasm, and the best if times providing a much needed escape from my studies.

To the department of Chemistry at the University of the Western Cape I extend my gratitude for providing me with the platform necessary for completing my MSc. program.

A special thanks to my employer, Fine Chemicals Corporation, for being accommodating towards the completion of my studies.

Most importantly, none of this would have been possible without the love and patience of my family, whom have always supported, encouraged and believed in me throughout all my endeavours.

Special thanks to my uncle and aunt, Spencer and Tracey Williams, who were always behind me supporting and encouraging me with their best wishes.

To my invaluable network of friends “the Ryt-offs”, you know who you are, thank you for the laughter, fun times and for always stoking my buzz.

To my light, Kaeli O’Ryan, my friend, cheerleader, drill sergeant, voice of reason, counsellor and life raft. Thank you for your unconditional love, support and inspiration. The positive energy you continue to transfer has enlightened and enriched my spirit. Your love is my source of strength, your belief in me is my source of motivation. You remain my only constant through the enormous pressures that life endures.

To my mother, Wendy Anne Baugaard, without whom, none of this would have been possible. There are no words that can fully describe my appreciation. Your love, support and constant patience throughout my long years of studying has taught me so much about sacrifice, discipline and compromise. Throughout my life, you have actively supported me in my determination to find and realise my potential, and to make this contribution to our world. Your motivation has helped me persevere through great pressures and encouraged me to strive for excellence.

Dedicated to my mother Wendy Anne Baugaard

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ABBREVIATIONS

Ag/AgCl	Silver/Silver chloride
$CDCl_3$	Deuterated chloroform
CV	Cyclic voltammetry
d	Doublet
DCM	Dichloromethane
EtOH	Ethanol
FT-IR	Fourier transform infrared spectroscopy
g	Gram
h	Hours
Hz	Hertz
M	Molar
m	Multiplet
MeOH	Methanol
ml	Millilitres
nm	Nanometre
NMR	Nuclear magnetic resonance
ppm	Parts per million
s	Singlet
SWV	Square wave voltammetry
TLC	Thin layer chromatography
t	Triplet
UV/Vis	Ultraviolet-visible spectroscopy
°C	Degrees Celsius

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1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

The term "flavonoid", derived from the Latin word "*flavus*" meaning Yellow, is used to describe a broad collection of naturally occurring polyphenolic compounds having a C_6-C_3-C_6 skeleton structure consisting of two aromatic rings (A and B) and a heterocyclic ring (C) **(Figure 1.1)**. These compounds, which are one of the biggest classes of secondary metabolites [1], are found abundantly in nature in a broad range of fruits, vegetables and beverages such as tea, coffee, beer, wine and fruit drinks.

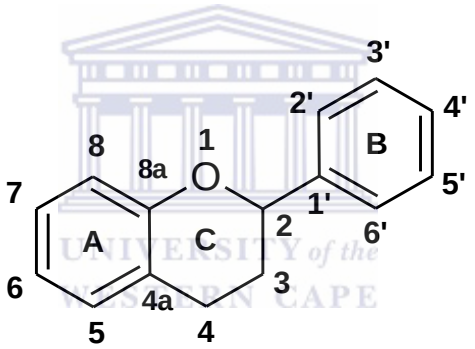
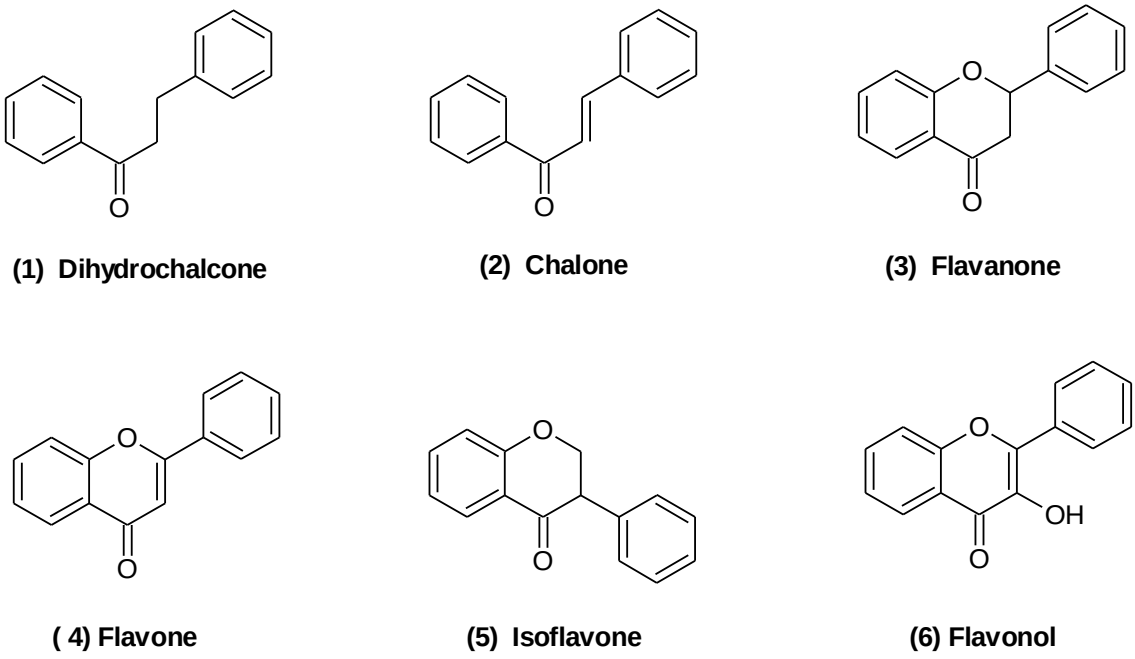


Figure 1.1: Basic skeleton of flavonoids

Over 8000 different naturally occurring flavonoids have been discovered [2] and the list is still growing. Most of these structurally different flavonoids can be subdivided into several classes of compounds, based on the degree of saturation present on the heterocyclic C-ring; they include the dihydrochalcone **(1)**, chalcone **(2)**, flavanone **(3)**, flavone **(4)**, isoflavone **(5)** and Flavanol **(6) (Figure 1.2)** [3].



(1) Dihydrochalcone **(2) Chalone** **(3) Flavanone**

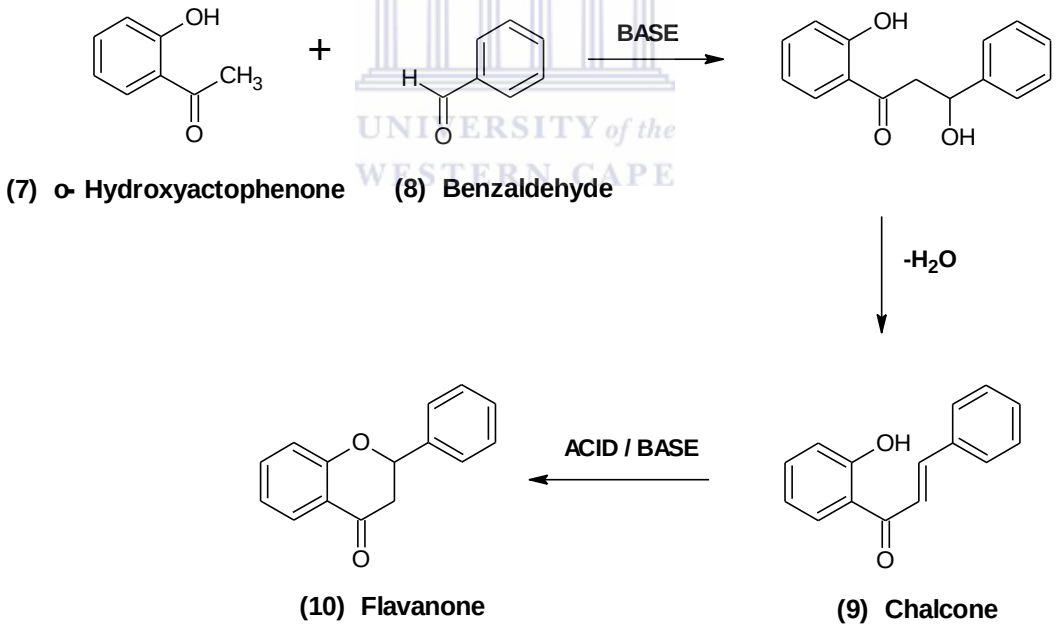
(4) Flavone **(5) Isoflavone** **(6) Flavonol**

Figure 1.2: Flavonoid groups

Flavonoids are considered to be one of the most important natural products around the world and have aroused considerable interest in recent years due to their therapeutic effects on human health. Flavonoids and its derivatives, have reported to exert multiple biological and pharmacological activity including antimicrobial, anti-inflammatory, analgesic, antimalarial, antioxidant, antibacterial, antibiotic, immunomodulatory as well as antitumor activity [4 -13]. It has also been reported that flavonoids and its derivatives have proven to be important intermediates in the synthesis of antiviral HIV-1 drugs [14-15]. The expected antioxidant ability of these compounds is attributed to the conjugated double bonds and completely delocalized π-electron system on both benzene rings, which is why they are increasingly thought to be responsible for the longer life expectancy of populations with well-balanced, healthy diets [16 – 17]. Such a diet consists of a high amount of fruits and vegetables as well as beverages from vegetable origin. Among compounds of known structure, flavanoids and flavonoids deserve special attention because they are present in large amounts in practically all-dietary plants, fruits and roots, and are consumed daily in substantial amounts.

1.1.1 Synthesis of Chalcones and Flavanones

The tremendous biological properties and antioxidant activity of flavonoids has resulted in intense synthetic efforts towards their synthesis. Chalcones **(2)**, also found abundantly in edible plants, are well known intermediates in the metabolic pathway as well as the chemical synthesis of Flavanoids [18]. They have a general chemical structure of two phenyl groups, both containing hydroxyl groups, connected by a three carbon α,β unsaturated carbonyl system [(-C=C-(CO)-]. Chalcones **(9)** are readily accessible via two well-established synthetic routes comprising of a base-catalyzed aldol/Claisen Schmidt condensation or acid-mediated aldolization of equimolar o-hydroxyacetophenones **(7)** and benzaldehydes **(8)** [19].Cyclization of the o-hydroxychalcones, a fundamental step in biosynthesis of flavonoids [20], is achieved through an intermolecular reaction, under basic or acidic conditions, to produce the corresponding flavanone **(10) Scheme (1.1)**. This reaction produces the chromane core that is characteristic of many flavonoids.



Scheme 1.1: Claisen Schmidt condensation of 2'-hydroxyacetophenone and benzaldehyde, followed by aldol condensation.

1.1.2 Radicals and Antioxidants

1.1.2.1 Free radicals

Radicals, often referred to as 'free radicals', are described as atoms, molecules or ions with unpaired electrons in an open shell configuration and are formed by single electron oxidation or reduction. Their formation is associated with the natural metabolism of aerobic cells whereby the oxygen consumption, inherent to cell growth, leads to the generation of free radicals. Examples of these reactive oxygen species (ROS) include superoxide, peroxyl, alkoxyl, hydroxyl and nitric oxide. Free radicals play an important role in cellular energy producing systems and the synthesis of biological compounds, however if they are produced in excess, they could attack lipid in the cell membranes, proteins, enzymes and DNA. These oxidative damages can lead to aging and several degenerative diseases such as cancer, stroke, myocardial infarction and diabetes [21-23]. Many forms of cancer are believed to be the result of reactions between free radicals and DNA, resulting in mutations that can affect the cell cycle and potentially lead to malignancy. Termination of these free radicals occurs through enzymatic means or by free radical scavenging by antioxidants; therefore antioxidants play a key role in these defence mechanisms [24].

1.1.2.2 Antioxidants

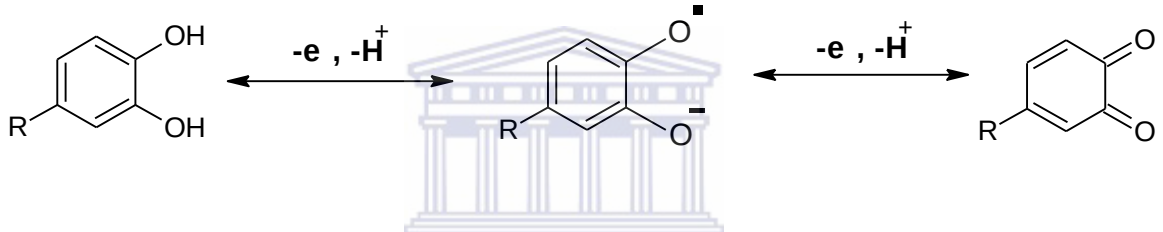
Oxidation reactions are crucial for life; however they can also damage or kill cells. An antioxidant is a molecule that inhibits oxidation reactions, therefore preventing the formation of free radical intermediates, and diminishing its undesired effect. The radical scavenging is achieved by being oxidized themselves and therefore antioxidants are often found to be reducing agents such as thiols, ascorbic acid, or polyphenols. Due to their cellular protective abilities, antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and many others linked to oxidative stress [25].

1.1.3 Antioxidant activity and Electrochemistry of Flavonoids

1.1.3.1 Antioxidant Activity of Flavonoids

Polyphenolic compounds such as chalcones, flavonoids and prenylated flavanoids have shown to have tremendous radical scavenging ability and are therefore regarded as

excellent antioxidants [26, 27]. The radical scavenging within the flavonoid system is said to occur via an electron transfer process, in which the phenol is transformed into a phenoxyl radical resulting in the formation of the semiquinone **(Scheme 1.2)** [28]. Therefore flavonoids can be described as hydrogen donating antioxidants due to the reducing properties of the multiple hydroxyl groups attached to aromatic ring systems, along with their ability to delocalize the resulting phenoxyl radical within the structure [29].Various structural parameters such as aromatic hydroxyl groups, the catechol group, the α, β-double bond in conjugation with a 4-carbonyl group as well as additional resonance effective substituents are said to play a role in the antioxidant activity, however there is much contradiction around this particular aspect.



Scheme 2.1: Mechanism leading to o-semiquinone radical

1.1.3.2 Electrochemistry of Flavonoids

Most Flavonoids are electroactive due to the presence of phenolic groups. The oxidation of flavonoids, or more importantly the ease to which flavonoids are oxidized is of great interest as it can be directly related to its activity as an antioxidant in the human body [29, 30]. Therefore electrochemical methods, such as differential pulse, cyclic and square wave voltammetry can be useful as a mode of detection as well as provide us with information regarding its reactivity as a hydrogen- or electron-donating. Using electrochemistry, the reducing capacity of the flavonoid substrate can be determined by measuring its oxidation potential and therefore providing a useful mechanism to further understand its structure-activity relationship.

1.2 Research objectives

i. To determine a suitable method for the synthesis of chalcones, dihydrochalcones, flavanones, and prenylated flavanones.
ii. To synthesis the above mentioned compounds with varying hydroxy, methoxy and prenyl group substituents.
iii. To determine a suitable electrochemical method for the detection and characterization of the above mentioned compounds.
iv. To elucidate structures of all compounds using proton and carbon nuclear magnetic resonance and Infrared Spectroscopy.
v. To provide further understanding about the structure-activity relationship of using oxidation potentials obtained from electrochemical studies.

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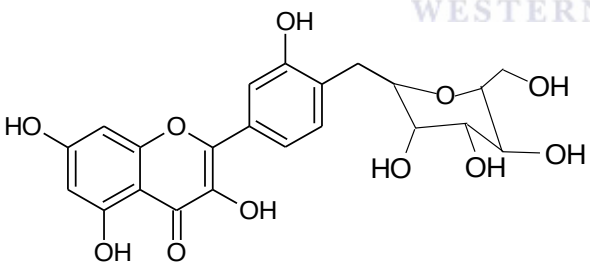
CHAPTER 2

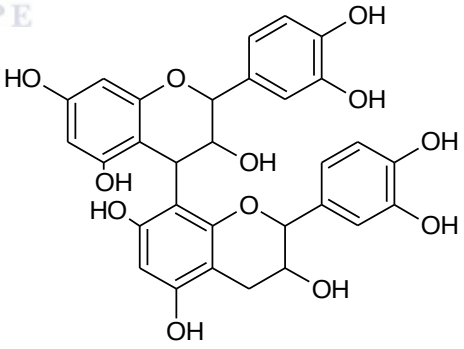
2. LITERATURE REVIEW

2.1 Flavonoids in foods

The definition of a "healthy" diet is debatable as there are beneficial and detrimental properties about many of the foods we eat. There is however, considerable evidence that suggests the high consumption of fruits and vegetables is extremely beneficial for ones health. Of the compounds with known structure present in fruits and vegetables, flavonoids deserve special attention as they are present in large amounts. Besides the flavonoids mentioned earlier, foods also contain flavonoid conjugates in the form of glycosides, flavonoid dimers, oligomers and polymers [31]. Two examples of these conjugates can be seen below, namely Quercetin-4-O-B-glucoside **(11)** and Procyanidin B_1 dimer **(12)**. Concentrations of flavonoids in foods do vary and are influenced by a number of factors such as species, variety, climate, degree of ripeness and storage.



(11) Quercetin - 4' - O - B - glucoside



(12) Procyanidin B_1 Dimer

Figure 2.1: Examples of Flavonoid glucoside and Dimer [31]

2.1.1 Fruits

Almost all fruits are good sources of flavonoids such as catechins, flavonols and proanthocyanidins. Fruits that are red or purple such as blueberries, cranberries, raspberries, and strawberries are said to contain high amounts of flavonoids [31]. Catechins are the most abundant flavonoids found in fruits, usually situated in the flesh of fruits, with the richest sources found in black grapes and apples. However in fruits such as apples, flavonols are predominantly found in the skin and therefore peeling fruits would significantly reduce the levels of flavonoid intake. Flavanones and flavones, a unique class of flavonoid, are found in citrus fruits and juices and include flavanones hesperetin, naringenin, quercetin and rutin [31, 32].

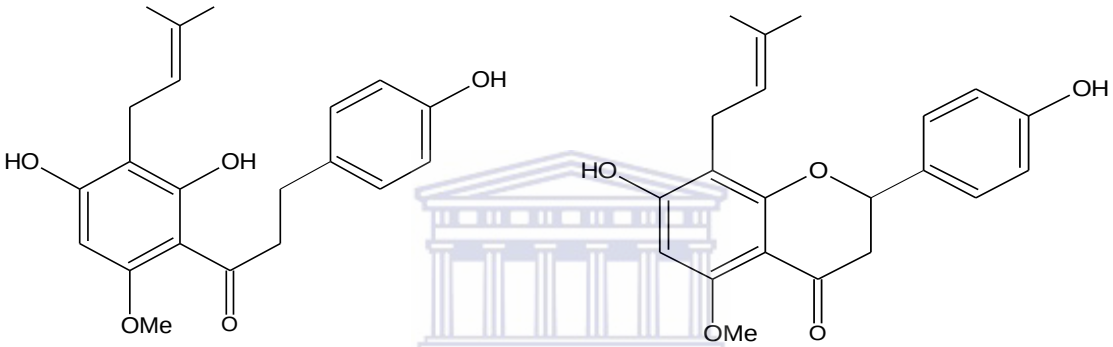
2.1.2 Vegetables

As with fruits, almost all vegetables contain flavonoids although their variety is less. Quercetin is the key flavonoid found in garlic, leeks and onions and is best known for its ability to spark the immune system into action and is rapidly taking its place at the top of the antioxidant pyramid [32]. Onions are considered as one of the most important sources of the flavonoid quercetin and form a common staple in most diets around the world. Flavones have been found in celery, sweet peppers and lettuce. On the other hand, catechins and type B procyanidins have been found in legumes. Green chilly pepper is one of the few vegetables to contain flavonols and flavones as well as having detectable levels of quercetin and luteolin respectively [32]. Soy is a good source of isoflavones. Soy flavonoids have been linked to greatly reduced blood cholesterol levels and the prevention of osteoporosis and can also play a role in managing the effects of menopause [33].

2.1.3 Beverages

Up to 30% of white, green, and black tea leaves consist of flavonoids. However due to the processing of black tea and to a lesser extent green tea, the percentages are lower than in white tea. All members of the tea family are packed with flavonols, catechin, epicatechin, epigallocatechin and epicatechin gallate [34].Perhaps surprisingly; wine and more surprisingly beer are great sources of the flavonoids. Red grapes and the red grape skins which are used to make red wine contain high amounts of flavonols and flavan-3-ols such as catechin, epicatechin and procyanidins [31]. It has been suggested that the low

occurrence of coronary heart disease in the French, despite their cholesterol-rich diet, could be directly linked to their relatively high intake of red wine. In recent times isoprenyl flavanoids namely, Xanthohumol **(13)**, Isoxanthohumol (**14) (Figure 2.2)** and 8-prenylnaringenin have been found in certain beers [35]. They occur due to the inflorescence (hop cones or hops) which is widely used in the brewing industry to give beer its characteristic fragrance, aroma, and taste. These flavonoids may be responsible for the antioxidant activity of lager beer, which is said to be higher than that of green tea, red wine, or grape juice [36].



(13) Xanthohumol **(14) Isoxanthohumol**

Figure 2.2: Structures of isoprenyl flavanoids; Xanthohumol and Isoxanthohumol

2.1.4 Other

Flavonoids have also been found in many foods besides fruits and vegetables; Table 2.1 provides more examples of these foods [37-38].

Structure	Flavonoid	Fruit and vegetables	Beverages	Other Foods
Flavonol	quercetin,	onions, hot peppers, kale, broccoli, rutabagas and spinach	Black and green tea	honey
	Kaempferol	cabbage, kale, bens, endive, leek, tomato, strawberries, grapes	Teas	Olive oil
	Myricetin	grapes, berries, fruits, vegetables, herbs	Red wine	Walnuts
Flavanol / Flavan-3-ol	Catechins	Legumes	Oolong tea	Dark chocolate
	Epicatechins	Peaches	All teas and red wines	Chocolate, argan oil, vinegar
Flavones	Apigenin	Celery, onions, lettuce, berries	Berry juice	Parsley, red pepper
	Luteolin	beets, Brussels sprouts, cabbage and cauliflower		Thyme
	Tangeritin	Tangerine , other citrus peels	Fruit juice	
Flavanones	Eriodictyol	Lemons, grape	Red and white	peppermint

		fruits	wines	
	Naringenin	grapefruits, oranges and tomatoes (skin)	Red and white wines	cooked tomato paste
	Hesperetin	Citrus fruits	Red and white wines	
Prenylated Flavanoids	Isoxanthohumol	Hops	Beer	

Table 2.1: Examples of foods containing flavonoids

2.2 Flavonoids in nature

2.2.1 Function in Plants

Flavonoids, and their conjugates, first appearing in algae 500 million years ago, form a very large and remarkably diverse group of natural products. They are found in plant tissues, where they are present inside the cells or on the surfaces of different plant organs and are best known for the characteristic red, blue and purple anthocyanin pigments of plant tissues [39]. However their role in the beautiful display of colour is not the only biological function of flavonoids which is why there has been a great resurgence in research activity on the function of flavonoids in plants.

2.2.2. Flavonoids and UV protection

UV radiation reaching the earth's surface is divided into lower energy UV-A (320-400 nm), higher energy UV-B (280-320 nm) and UV-C (254-280 nm) regions [40]. The most severe damage caused by ultraviolet (UV) light particularly UV-B band, which causes most severe damage, is on the increase due to ozone reduction, which is the primary screen of solar radiation. Over exposure to UV-B radiation can cause cellular damage and damage to the chloroplast which leads to a reduction in photosynthesis [40, 41]. Flavonoids, situated in the epidermis of the plant, generally absorb light in the280 – 315 nm regions and thus act as UV filters, preventing UV-B radiation from reaching the mesophyll and

therefore protecting the tissue from damage and ensuring optimum plant photosynthesis [40].

2.2.3 Stress protection

2.2.3.1 Temperature stress

Flavonoids have been shown to play a role in the responses to chilling and heat stress due to extreme low and high temperatures. In a study of two species of hawthorn, *Crataegus laevigata* and *C. monogyna*, an increase in levels of colourless flavonoids (-)-epicatechin and hyperoside (quercetin 3-galactoside) was observed when plants were subjected to both cold treatment and drought stress [42]. Under these treatments it was found the antioxidant capacity of the shoot extracts were enhanced due to the increase in flavonoid content [42].

2.2.3.2 Heavy metal tolerance

It is evident that flavonoids have a role in the resistance of high levels of metals in the soil. Aluminium toxicity in soil, said to be the most extensive problem of toxicity stress in plants, is a major factor constraining crop performance in tropical climates [42]. High levels of aluminium is said to cause oxidative stress within the plant. However the metal binding activity of many flavonoids results in stable complexes with aluminium, therefore combating its undesirable effects [42, 43]. More evidence suggesting that flavonoids play an important role in metal resistance is shown by the high levels of these phenolic compounds that are exuded when roots of plants are exposed to aluminium. This together with evidence of a high stability constant for the Al complexes with pentahydroxy-flavones and flavan-pentols strongly supports a role for the flavonoid-type phenolics in Al resistance [42, 43].

2.2.3.3 Oxidative stress

The antioxidant activity of flavonoids in humans has been well documented in the literature; however these biologically active compounds have been proven to serve a similar function in plants. Plants undergoing severe stress conditions build up dihydroxy B-ring-substituted flavonoids, which are effective scavengers of reactive oxygen species (ROS) [44]. Colourless flavonoids have been found in the vacuoles and toad lesser extent in the cytoplasm, with the latter better sited to interact with reactive oxygen species [43].

There is much evidence that flavonoid oxidation has a protective function during plant development and growth; for example the browning of onion scales during aging is caused by the oxidation of quercetin glucosides, which in turn leads to the formation of the antifungal agent3,4-dihydroxybenzoic acid [45]. Quinones, formed by Flavonoid oxidation has been shown to protect plants against pathogens as well as protecting germinating embryos [43].

2.3 Salutary effects in human health

In many cultures traditional healing is sill a very popular alternative to conventional medicine. For centuries, preparations that contain flavonoids and there conjugates have been used by physicians and healers in attempts to treat disease, for example, propolis was prescribed by Hippocrates (460-377 BC) in Ancient Greece for the treatment of sores and ulcers [46]. We now understand the antimicrobial properties of propolis are attributed to its high flavonoid content. Other examples include the plant Tagetes miuta, containing the flavonoid conjugate quecetagetin-7-arabinosyl-galactoside, which was used extensively in Argentine traditional medicine to treat various diseases [10]. Huangchin is another example, used by the Chinese in which this herbal medicine has been used for the treatment of oral wounds. In this case the flavone baicalein is reported to be largely responsible for this plant's antimicrobial effects [46]

2.3.1 Antioxidant activity

2.3.1.1 Scavenging of free radicals by flavonoids

One of the more prominent therapeutic properties of the flavonoids is their excellent antioxidant capacity, which is due to their radical scavenging ability. Radicals or reactive oxygen species (ROS) are highly reactive and are formed *in vivo* during normal aerobic metabolism, generally during oxidation reactions catalyzed by oxygenases [46, 47]. These reactions are very common since molecular oxygen is a very good electron acceptor. The transfer of electrons to dioxygen generates molecules of water, a safe product. However partial reduction leads to the formation of highly toxic radicals which when produced in excess can cause damage to DNA, proteins, and lipids, all of which contribute to the onset of cardiovascular disease [46, 47]. Flavonoids possess ideal structural chemistry for the efficient scavenging of free radicals such as super oxide anions, singlet oxygen, nitric

oxide, and peroxynitrite. Flavonoids can be oxidized by radicals, resulting in more stable and less reactive radicals. This is due to the high reactivity of the hydroxyl group of the flavonoids with the reactive compound of the radicals. Therefore, flavonoids can stabilize the ROS and prevent oxidative injury [48].It is also a valuable aspect for therapeutic and prophylactic applications of flavonoids, e.g., after infection, inflammation, burns, or radiation injury [49].

2.3.2 Anti-inflammatory

Inflammation is a defence system of the body to an invasion of a foreign body of any kind, for example bacterial cells, viruses and even wooden splinters. Inflammation involves, among others, the action of the complement system, blood coagulation, humeral and cellular immunity, cytokines, tissue hormones, angiogenesis, and repair processes [48]. The immune system has two branches to defend itself, the humeral and the cellular. Both of these branches are stimulated by flavonoids [48]. The treatment of a sore throat and fever with an ethanol extract of propolis is a classical example of a quick cure brought about by flavonoids. The pain and fever caused are a result of chemical signals from the point of invasion to the brain. Eicosanoids are, the compounds which release the alarm, and are formed from arachidonic acid by a series of enzymes. Many of these enzymes are inhibited by flavonoids, therefore by down-regulating them, these phenolic compounds can reduce thrombosis and chronic inflammatory reactions [48]

2.3.3 Anti-bacterial, anti-fungal and antimicrobial activity

Many flavonoids have been found to have excellent anti-bacterial, anti-fungal and antimicrobial activity [48, 50-54]. Flavonoids have been used to kill the bacterial or fungal cells and to counteract the spread and the effects of the bacterial toxin. Many, but not all, of the bacterial strains commonly encountered by humans are killed by flavonoids; however the mechanism by which this is accomplished is not known yet. Metabolic perturbation has been suggested to play a role, fungi which often accompany bacterial infections, may be killed by flavonoids due to this particular mechanism [48].

The antimicrobial and antifungal activity of prenylated flavonoids from five different medicinal plants were evaluated against the fungi; Candida albicans and Saccaromyces

cerevisiae, gram negative bacteria; E. coli and Salmonella typhimurium, and gram positive bacteria; Staphylococcus epidermis and S. aureus. All but one of the compounds tested showed antibacterial and/or antifungal activity with varying degrees of potency, which suggests that the antimicrobial activity of prenylated flavonoids is common. Furthermore kurarinone, a 5-methoxyflavanonecompletely inhibited the growth of all tested bacteria. Although further research is necessary to establish the pharmacological mechanism of the antimicrobial and antifungal activity it was suggested that since prenylated flavonoids are more hydrophobic than the conventional flavonoids, they may easily penetrate through the cell membrane [55].

2.3.4 Antiviral activity of Quercetin

Quercetin has been reported to have the ability to bind to viral coat protein and polymerases as well as interfere with viral nucleic acid synthesis. For example, methyl quercetin was found to block polio virus replication by interfering with the single-stranded RNA. Quercetin has been further shown to have antiviral activity against enveloped viruses such as herpes simplex type I, respiratory syncytial, pseudorabies, parainfluenza type 3, and Sindbis. Quercetin has also been found to protect against macrophage-dependent murine cardio virus (mengovirus) infection and also shown to enhance the antiviral activity of agents such as interferon. This ability to enhance interferon makes quercetin a key flavanoid for further investigation against superior viral infections such as HIV [56]

2.3.4.1 HIV Aids

Acquired immunodeficiency syndrome (AIDS), which is caused by the human immunodeficiency virus (HIV), is a devastating life-threatening pandemic. In recent times flavonoids have been investigated for their antiviral activity. Theoretically, flavonoids should have an excellent chance of preventing the proliferation of HIV since they inhibit reverse transcriptase, induce IFNs, and inactivate the enzyme that prepares the precursor of the protein building blocks of the viral capsid [48]. In an approach to discover novel products as new lead compounds for potential anti-HIV agents, flavonoids derived from organic extracts of four Desmos Lour. Species were screened against HIV-1 in infected H9 lymphocytic cells. Three flavanones were found to exhibit antiviral activity, which

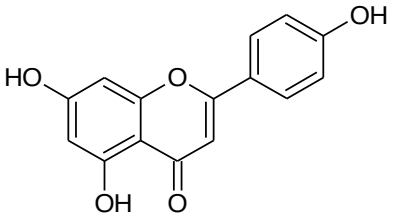
suggests that the flavanone C2–C3 double bond may not be necessary for antiviral activity. The most potent compound was found to be a chalcone [57]. The toxicity of flavonoids is much lower than that of current HIV drugs. The flavonoid quercetin glucuronide extracted from the leaves of *N. nucifera*, was found to have moderate anti-HIV activity and low cytotoxicity, which suggested that the glycosyl moiety bound to the C-3 hydroxyl group might also play an important role in the anti-HIV activity [58].

2.3.5 Anticancer Activity of Flavonoids

Cancer, known medically as malignant neoplasm, embraces a group of diseases that are all caused by an unregulated disturbance in growth metabolism. Cancerous diseases, after cardiovascular disorders and accidents, kill more people before their normal life span has been reached. Although cancer is curable when discovered early, radical cancer treatment coincides with considerable life threatening dangers and discomfort and therefore there has been a rising interest in developing anticancer drugs from relatively non-toxic sources that offer some promise of a moderate, long-term life-saving cure. Flavonoids are some of the most promising anticancer natural products that have been tried. Related synthetic substances, e.g., flavone acetic acid, have been subjected to Phase I clinical trials already, and they may soon become adopted into the general repertoire of treatment [48]. Most flavonoids have proved to inhibit proliferation in many types of cultured human cancer cell lines, whereas they have little or no toxicity to normal human cells [47]

2.3.5.1 Apigenin as a chemopreventitive agent

Apigenin, found in many herbal teas, is one of the most bioactive plant flavones. Various studies have demonstrated the anti-proliferate effects of apigenin on various cancer cell lines including; breast, colon, cervical, lung, prostate and skin cancer cell lines. In many cases the cancer cell line showed complete retardation after exposure to Apigenin. These laboratory studies have proven that Apigenin (15) affects several critical pathways and/ or targets which are associated with cancer as well as help in improving cardiovascular conditions to stimulate the immune system, and provide some protection against cancer [59]. The structure of Apigenin can be seen in **Figure 2.3**.



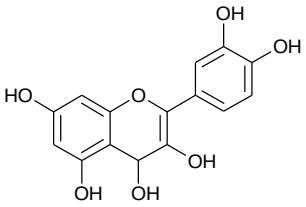
(15) Apigenin

Figure 2.3: Structure of Apigenin

2.3.5.2 Quercetin as an anticancer agent

Several studies have investigated the anti-tumorogenic effect of quercetin in cancer therapy. Quercetin, in a dose-dependent study, has been shown to suppress the prostate (PC-3) tumour growth both *in vitro* and *in vivo*. Importantly, no side effects were observed when serums containing concentrations of up to 12 μ M were administrated to humans. In a phase I clinical study achieved by Ferry *et al*., quercetin was safely administered by intravenous bolus at a dose injection [60]. Results of the plasma levels obtained showed inhibition of lymphocyte tyrosine kinase activity and evidence of antitumor activity [61].

In vitro studies of quercetin has also shown a wide range of inhibitory effects, for example, studies carried out by Debes *et al* that quercetin potentially may be useful in cancer therapy as a thermosensitizer by increasing the cell killing effect of hyperthermia and chemotherapy due to its ability to suppress heat shock protein expression [62]. The structure of Quercetin can be seen in **Figure 2.4.**



(16) Quercetin

Figure 2.4: Structure of Quercetin

2.4 The synthesis of flavonoids

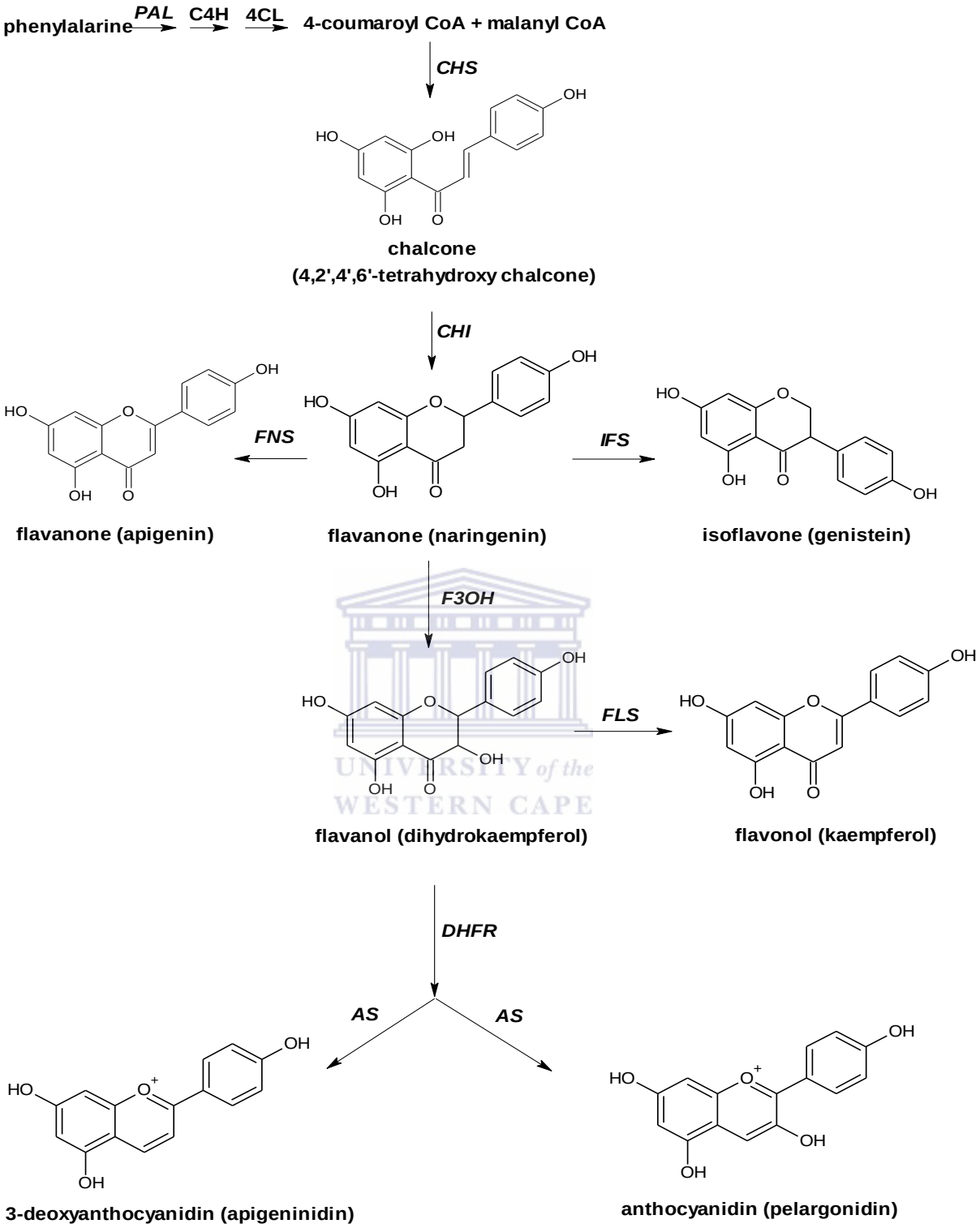
2.4.1 Biosynthesis

Flavonoid biosynthesis **(Scheme 2.1)** has been well characterized in the petunia and snap dragon flowers and in the kernels of maize, and is therefore one of the most well studied pathways among plant secondary metabolisms [63]. The first key step in the biosynthesis of all flavonoids is the condensation of4-coumaroyl-CoA with three molecules of malonyl-CoA catalysed by chalcone synthase (CHS); the chalcone is stereo specifically cyclized by the action of chalcone isomerase (CHI) [63].

The cyclization of 2´-hydroxychalcone is a fundamental step in the biosynthesis of all simple flavanoids. The reaction produces the chromane core, characteristic of many flavonoids, and which is responsible for the colourful appearance of many flowers and the bitter taste of grapefruit [64]. The flavonoid Naringenin is a precursor for almost every other flavonoid. Unsaturation of the C-2/C-3 bond forms flavones, which have a more planar skeletal ring structure. This change is able to cause a significant change the recognition properties of the flavonoid. When the B-ring is transferred from C-2 to C-3 the corresponding isoflavonoids are formed. Flavonols are flavones with a hydroxyl group at C-3 and in flavan-3-ols the C-4 carbonyl group has been eliminated [65].

2.4.2 Enzymes

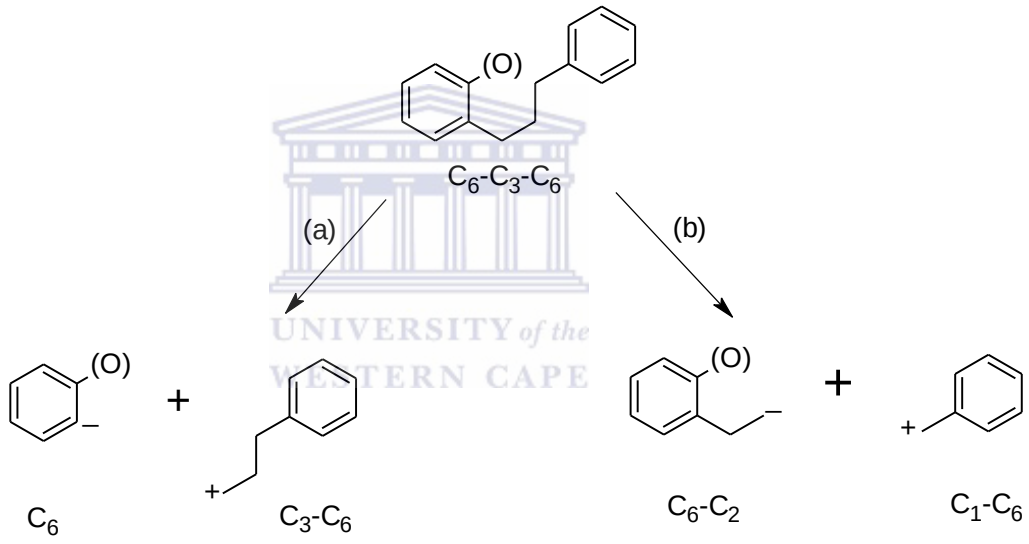
The flavonoid structures are derived from central and key metabolites; phosphenol pyruvate and erythrose-4-4phosphate via the shikimate and phenyl propanoid pathways and acetyl-CoA via the acetone-malonate pathway. This initial condensation reaction is catalyzed by chalcone synthase (CHS), thereafter the other selected enzymes are; CHI (chalcone isomerase); IFS (isoflavone synthase complex); FNS (flavone synthase); F3OH (flavanone-3-hydroxylase); FLS (flavonol synthase); DHFR (dihydroflavonol reductase) and AS (anthcyanidin synthase) [66] **(Scheme 2.1).**



Scheme 2.1: Biological pathway of the major flavonoid structural classes

2.4.3 Retrosynthetic Strategy

The C_6-C_3-C_6 flavonoid skeleton can be retro synthetically assessed using two of the more obvious analysis. One way would be to unravel the structure into a C_3-C_6 cinnamic acid type synthon that could either be a carboxylic acid, an aldehyde or an alcohol **(a)**. However, these reactions are usually not simple and an alternative strategy is often preferred [67]. In the second strategy the flavonoid skeleton is unravelled into a C_6-C_2 acetophenone and a C_1-C_6 benzaldehyde unit **(b)**. This route is used more frequently in literature and requires easily accessible starting materials; viz., acetophenones and benzaldehydes. Both retro synthetic strategies can be seen in **Scheme 2.2**.



Scheme 2.2: Retrosynthetic analysis of C_6-C_3-C_6 skeleton

2.4.4 Chalcones and Dihydrochalcones

Chalcones **(16)**, found abundantly in edible plants, are well known precursors in the metabolic pathway as well as the chemical synthesis of flavanoids [68]. They have a general chemical structure of two phenyl groups connected by a three carbon α, β unsaturated carbonyl system [(-C=C-(CO)-], see **Figure 2.5**. Compounds having the chalcone backbone have also displayed various biological and therapeutic activities [69] and in some cases have shown an increased antioxidant and photo-antioxidant activity due to the presence of the p-coumeric acid and 2-hydroxylbenzoyl moieties **(Figure 2.6)** [68]. The saturation of the C2-C3 double bond produces the corresponding dihydrochalcone **(17)** **(Figure 2.5)**

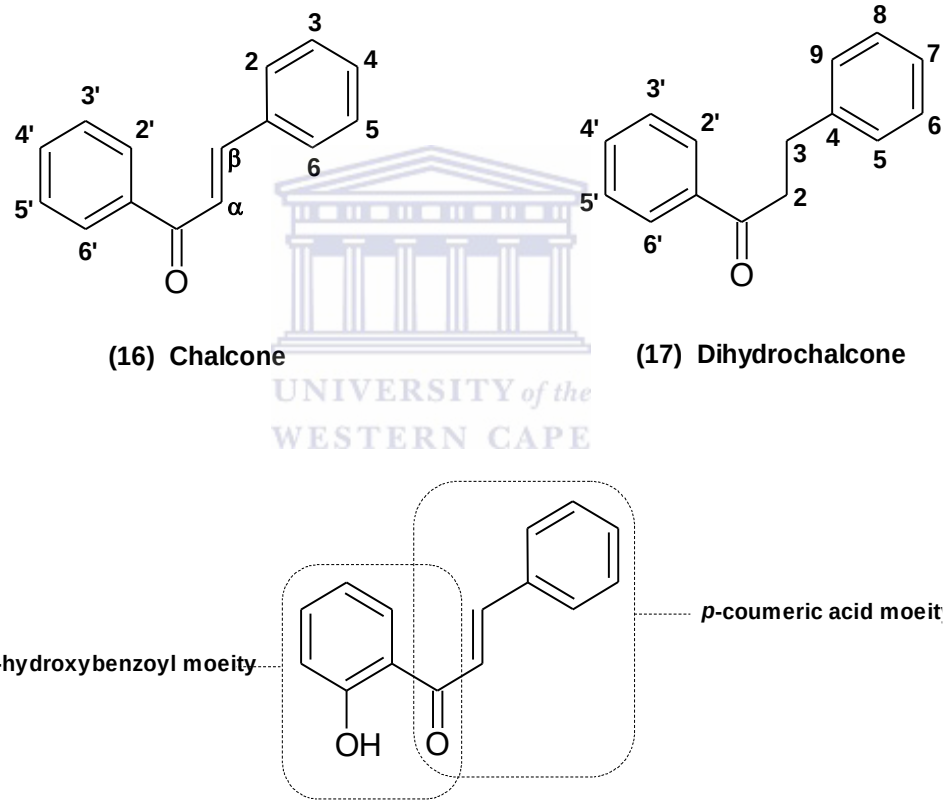
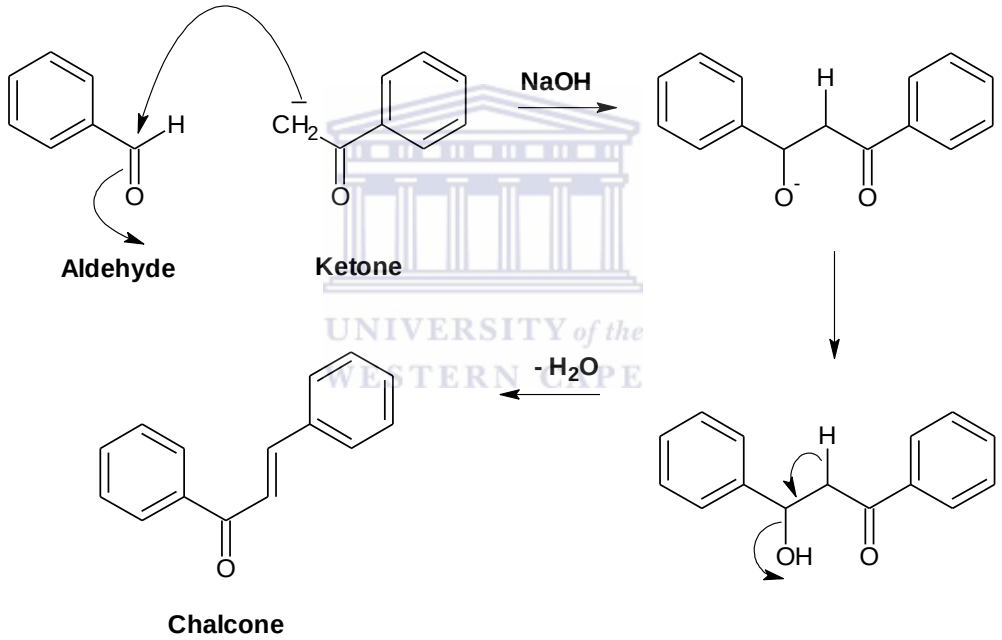


Figure 2.5: Chalcone **(16)** and Dihydrochalcone **(17)** base structures, p-coumeric acid and 2'-hydroxylbenzoyl moieties of the 2'-hydroxychalcone structure.

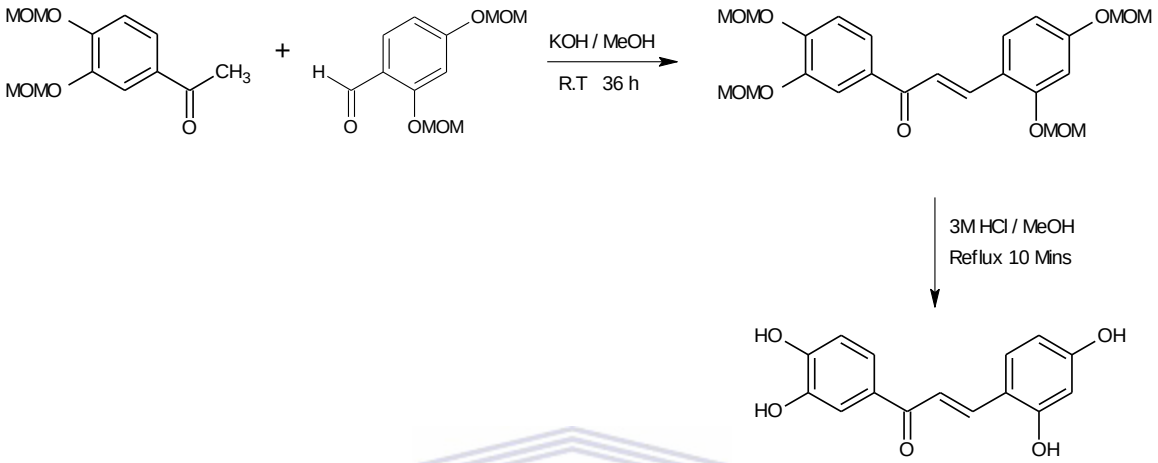
The base-catalyzed aldol condensation of equimolar amounts of substituted acetophenones and aldehydes in the presence of alcoholic alkali has been reported to show good yields [70-73]. This reaction is best known as the Claisen-Schmidt condensation in which a α-proton on the acetophenone moiety is removed by a strong base, resulting in the formation an enolate anion which is made relatively stable by the delocalization of electrons. Next, the carbonyl carbon of the benzaldehyde is nucleophilically attacked by the enolate anion. The alkoxide is reprotonated to form an OH group, then under the basic conditions water is eliminated to form the thermodynamically stable α, β-conjugated enone system viz., chalcone. The mechanism for the Claisen-Schmidt condensation can be seen in **Scheme 2.3.**



Scheme 2.3: Mechanism of the Claisen-Schmidt condensation

In their synthetic protocol towards hydroxychalcones, Jun et al. [74] added, drop wise, a solution of MOM protected acetophenone and MOM protected benzaldehyde in methanol, to a stirred solution of KOH in water, under argon. The reaction mixture was kept at 0°C for 3 hours and then at room temperature for 72 hours. The mixture was then poured in ice water, adjusted to pH 3-4 using 1M HCl, and then extracted with ethyl

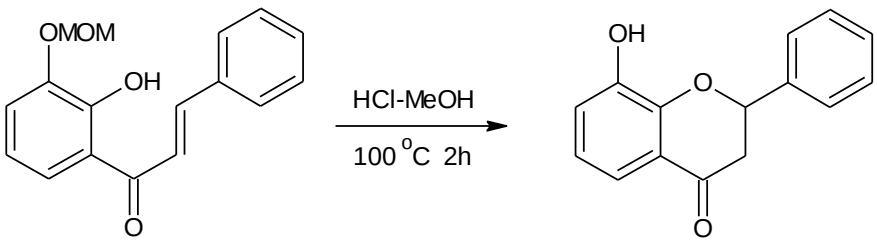
acetate. To remove the protecting groups the corresponding chalcone was dissolved in methanol and to this 3M HCl was added drop wise. The mixture was refluxed for 10 min, diluted with water and extracted with ethyl acetate **(Scheme 2.4)**.



Scheme 2.4: Synthetic protocol towards hydroxychalcones using the Claisen-Schmidt condensation

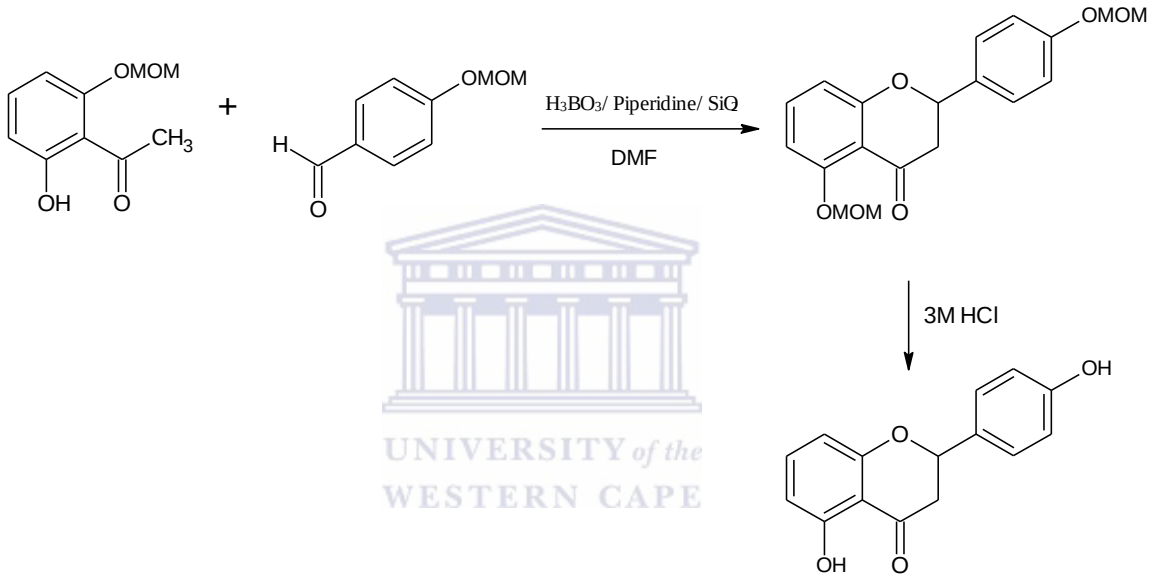
2.4.5 Flavanones

In the synthetic protocol towards monohydroxyflavanones, Kagawa et al [75] cyclized 2´-hydroxychalcones in an acidic solution; to a solution of 2´-hydroxy-3´-methoxy-methoxychalcone in MeOH to which 26% HCl-MeOH was added and the mixture then stirred at 100 °C for 2 hours. Neutralization with a saturated $NaHCO_3$ solution and extraction with ethyl acetate afforded 8-hydroxyflavanone as colourless crystals **(Scheme 2.5)**.



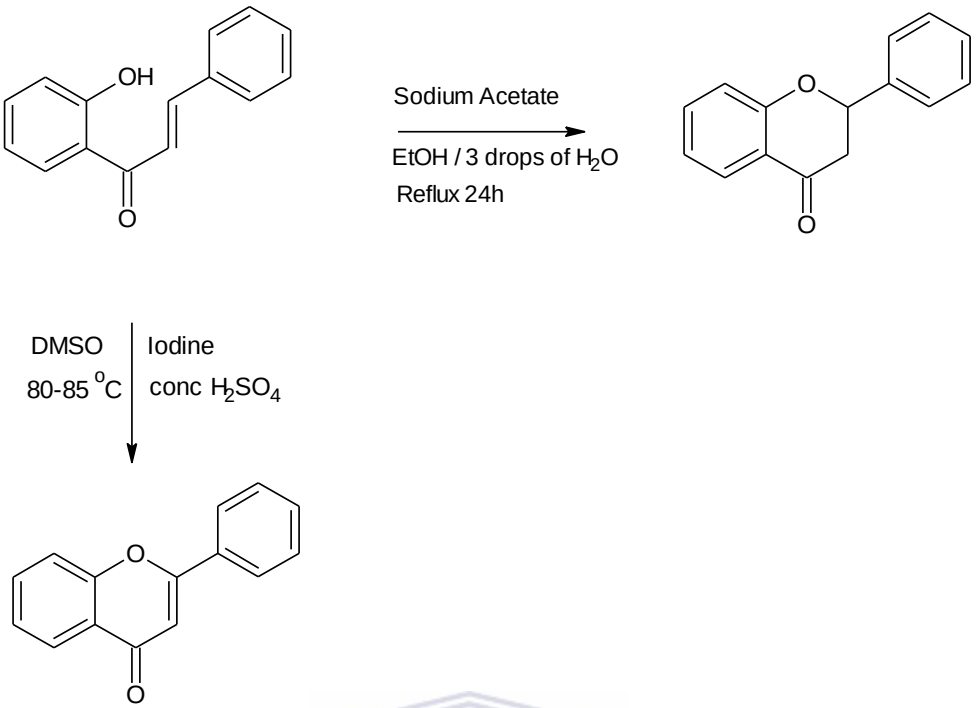
Scheme 2.5: Synthetic protocol towards 8-hydroxyflavanone using acidic solution

Soon Sung Lim et al [76] used a very different approach in their synthetic protocol towards 6, 4´-dihydroxyflavanone **(Scheme 2.6)**. Their synthesis started from 2´-hydroxy-5´-methoxymethoxyacetophenone and 4-methoxymethoxy-benzaldehyde which were added to a mixture of H_3BO_3, piperidine and SiO_2 in DMF. The mixture was stirred and heated to 120 °C under nitrogen purge for 6-12 hours. The cooled mixture was diluted with acetone, filtered and evaporated to afford crude 5, 4´-di (methoxy-methoxy) flavanone. Thereafter the MOM protecting groups were removed by treating the crude flavanone with 3M HCl for 3 hours.



Scheme 2.6: Synthetic protocol towards 5, 4´di-hydroxyflavanone using H_3BO_3, Piperidine and SiO_2

R. Sheng at al [77] prepared flavanone derivatives by refluxing 2´-hydroxychalcones and sodium acetate in a solution of ethyl alcohol and 3 drops of water for 24 hours. On the other hand flavone derivatives were prepared by adding the 2´-hydroxychalcones and iodine to a solution of concentrated H_2SO_4 in DMSO and warming the mixture to 80-85 °C and stirring for 24 hours. These two methods can be seen in **Scheme 2.7.**

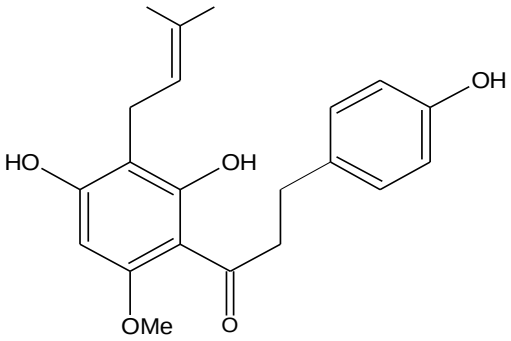


Scheme 2.7: Synthetic protocol towards flavanones using a sodium acetate reflux and a synthetic protocol towards flavones using iodine and conc. H_2SO_4 in DMSO

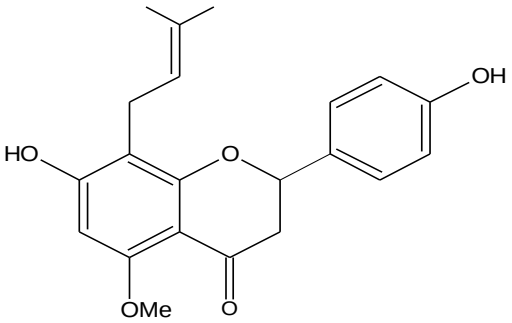
2.4.6 Prenylated Flavanoids

Prenylated flavanoids are a unique class of naturally occurring flavonoids, widely distributed throughout the plant kingdom and characterized by having a prenylated side chain in the flavonoid skeleton. The addition of a prenyl group as an alkyl side chain has been shown to increase the antioxidant activity as well as to increase the degree of bioactivity [78]. This comes to no surprise as the prenyl group is the isoprene unit which is a significant building block in biological compounds with immense activity such as terpenes and monocyclic terpenoids.

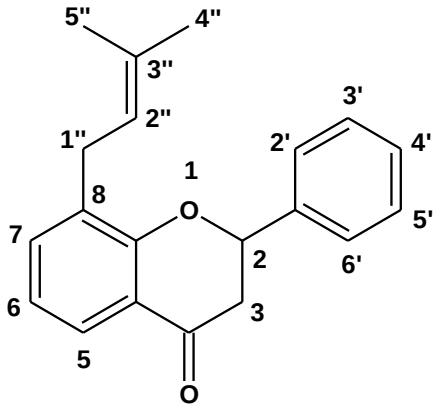
In recent times isoprenyl flavanoids namely, Xanthohumol **(18)**, Isoxanthohumol **(19)** and 8-prenylnaringenin **(20)** have been found present in certain beers [79]. They occur due to the inflorescence (hop cones or hops) which is widely used in the brewing industry to give beer its characteristic fragrance, aroma, and taste. Although the brewing value of hop flavanoids is not well understood, these flavanoids may be responsible for the antioxidant activity of lager beer, which is said to be higher than that of green tea, red wine, or grape juice [80]. A study by C. Gerhauser *et.al* showed that Xanthohumol **(18)**, and Isoxanthohumol **(19) (Figure 2.6)** both displayed excellent cancer preventative properties, while 8-prenylnaringenin **(20)** was found to be the most potent phytoestrogen known to date [79].These biological activities suggest that prenylflavanoids have the potential for application in cancer prevention programs and in prevention or treatment of (post-) menopausal 'hot flashes' and osteoporosis.



(18) Xanthohumol



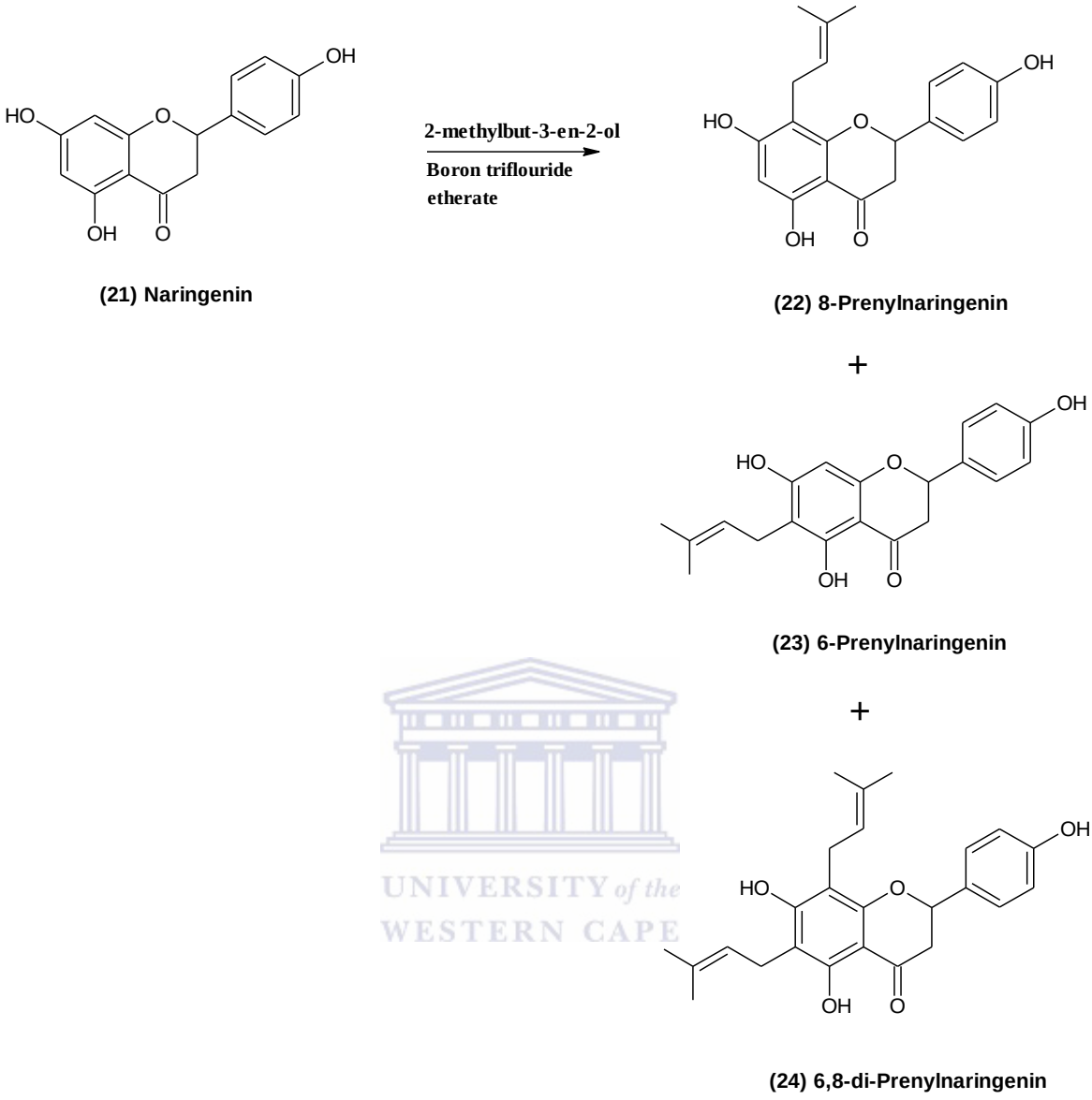
(19) Isoxanthohumol



(20) 8-Prenylnaringenin

Figure 2.6: Structures of the 'beer flavonoids', Xanthohumol, Isoxanthohumol and 8-prenylnaringenin

A. C. Jain *et.al* synthesized prenylated flavanoids using the method of nuclear prenylation of naringenin **(21) (Scheme 2.8)**; with 2-methylbut-3-en-2-ol in the presence of boron triflouride etherate [81]. This reaction produced three products; 8-Prenylnaringenin **(22)**, 6-Prenylnaringenin **(23)** and finally 6, 8-di-Prenylnaringenin **(24)**.



(21) Naringenin

(22) 8-Prenylnaringenin

(23) 6-Prenylnaringenin

(24) 6,8-di-Prenylnaringenin

Scheme 2.8: Synthetic protocol towards prenylated flavanoids, using method of nuclear prenylation of naringenin

2.5 Electrochemistry of Flavonoids

2.5.1 Introduction

Electrochemical methods have been widely used for investigating flavonoids mainly on oxidation mechanism, metal-chelating properties, evaluation of antioxidant capacity and electrochemical detection [82]. The simplicity of electrochemical methods, ease of miniaturization, high sensitivity and relatively low cost are making it an attractive alternative for flavonoid detection. Electrochemical methods such as differential pulse, cyclic and square wave voltammetry have been intensively utilizing for analysis of certain flavonoids [83-87].

2.5.2 Cyclic Voltammetry

Cyclic voltammetry has numerous advantages over the other mentioned methods; it is rapid, inexpensive and sensitive enough for determination of very low concentrations of polyphenols. A study achieved by Hotta et al, on the scavenging activities of polyphenolic antioxidants using cyclic voltammetry, showed an improved correlation between the oxidation potential and the radical scavenging abilities by introducing an anodic peak potential as an additional variable [88].

Medvidović-Kosanović et al, performed an electrochemical study on the properties of three structurally different flavonoids, catechin, quercetin and rutin [89]. All measurements were performed on a glassy carbon electrode and results showed formation of an *o*-semiquinone radical (a good antioxidant) during of the B-ring of a flavonoid molecule. They found that different antioxidant capacities can be explained by the presence of a double bond between C-2 and C-3, conjugated with the 4-oxo group in the ring C, as well as with the presence of 3-OH group in ring C ring.

The oxidation potential of polyphenols has been regarded as an important factor for their antioxidant activities therefore; it has been often used for the evaluation of the antioxidant activity [82, 90-93]. Cyclic voltammetry is one of the most useful methods of evaluating the oxidation potential [94-95].

2.5.3 Square Wave Voltammetry

Square wave voltammetry has advantages when compared to differential pulse and cyclic voltammetry. The analysis speed is greater and there is a lower consumption of electro active species which causes reduced problems with blocking of the electrode surface [89].

This particular method has also been extensively used to detect polyphenolic compounds. In a study performed by Vojtech et al, square wave analysis was successfully used to detect flavonoids: quercetin, quercitin, rutin, chrysin and diosmin in human urine [96].

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CHAPTER 3

3. SYNTHESIS OF CHALCONES, DIHYDROCHALCONES AND FLAVANONES

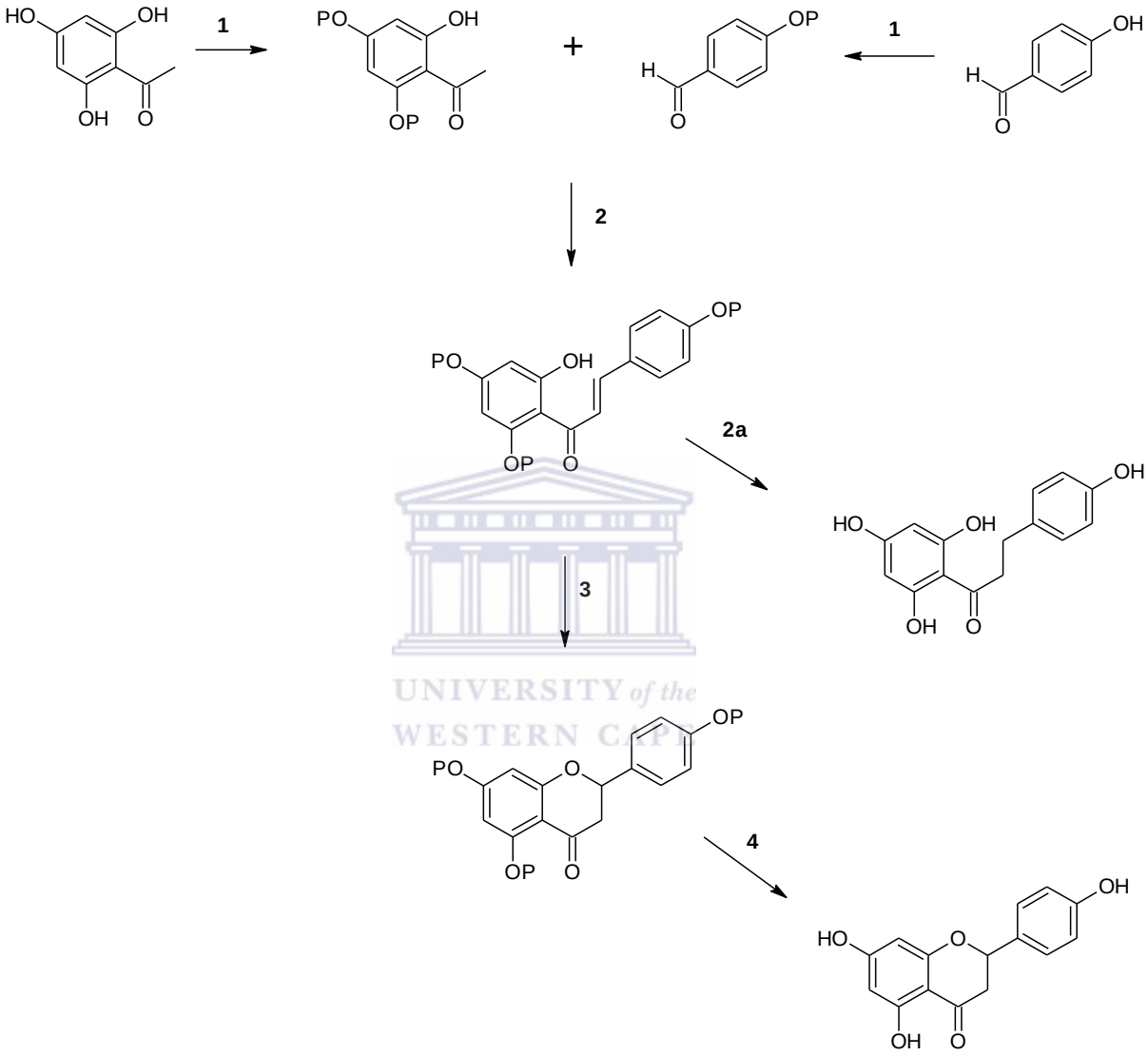
3.1 Introduction

The synthetic strategy used for the synthesis of all compounds is represented in **Scheme 3.1**. Our initial strategy was to prepare starting the materials as alkoxyprotected acetophenones and benzaldehydes. Hydroxyl groups are present in a number of compounds of biological and synthetic interest, including chalcones and flavonoids. Therefore to evaluate how best to maximize yields and selectivity, the OHgroups in the selected acetophenones and benzldehydes were protected using three different masking groups, namely; tetrahydropyranyl ether (THP), methoxy methyl (MOM) and benzyl ether (Bn). All Acetophenone products having the 2′-OH unprotected were preferred as intermediates since this free OH group was required for the further cyclization of the 2-Hydroxychalcones in their corresponding flavanones.

The synthetic approach towards all chalcones and dihydrochalcones was achieved in this work by using the base-catalyzed Claisen-Schmidt condensation between equimolar amounts of substituted acetophenones and substituted aldehydes in the presence of base in ethanol. The mechanism for the reaction is as follows; the α-proton on the acetophenone is removed by a strong base, resulting in the formation an enolate anion, which is made relatively stable by the delocalization of the electrons in the enolate. Next, the electrophilic carbonyl carbon of the benzaldehyde is nucleophilically attacked by the enolate anion. The alkoxy anion group is then protonated to form the corresponding alcohol. Base attack at the proton of the carbonyl results in the illumination of water to form the, β-enone chalcone as the first resonance stabilized product.

Ring closure of the chalcone system occurs through isomerisation and gives rise to the corresponding alkoxyprotected flavanone. This was achieved by reacting

2- Hydroxychalcone intermediates with the basic salt sodium acetate, in ethanol under reflux. Finally, removal of the protective groups produced the hydroxyl flavanones.



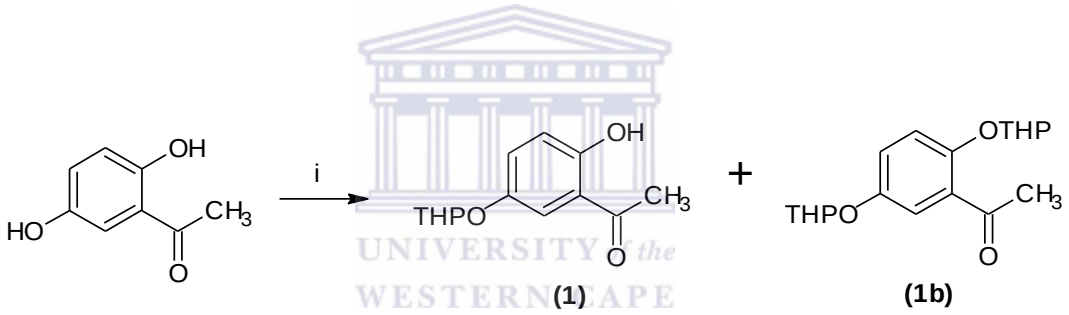
Scheme 3.1: The strategy used for the synthesis of Chalcones, dihydrochalcones and flavanones; step 1: protection of starting materials; step 2: chalcone synthesis; step 3: cyclisation to form flavanones; step 4: removal of protecting groups

3.2 Results and discussion

3.2.1 Synthesis of acetophenones 1-4

3.2.1.1 The use of dihydropyran protection groups

2-Hydroxy- 5-tetrahydropyranylacetophenone **(1)** was formed in good yield by treating 2, 5-dihydroxyacetophenone with 3, 4-dihydro-2-H-pyran (DHP) and pyridinium *p*-toluene sulphonate (PPTS) in the solvent CH_2Cl_2 (DCM) at room temperature for 5 hours **(Scheme 3.2)**. TLC of the reaction mixture after 4 hours revealed that all starting material was consumed. After chromatography of the crude product, the isolated yield of the preferred compound **(1)** was 80 % along with 10 % of the unwanted 2, 5-ditetrahydropyranyl acetophenone **(1b)**. The steric and mainly electronic effects produced due to the hydrogen bonding between the OH group at position 2 and the carbonyl group mitigates against the formation of the dihydropyranyl acetophenone.

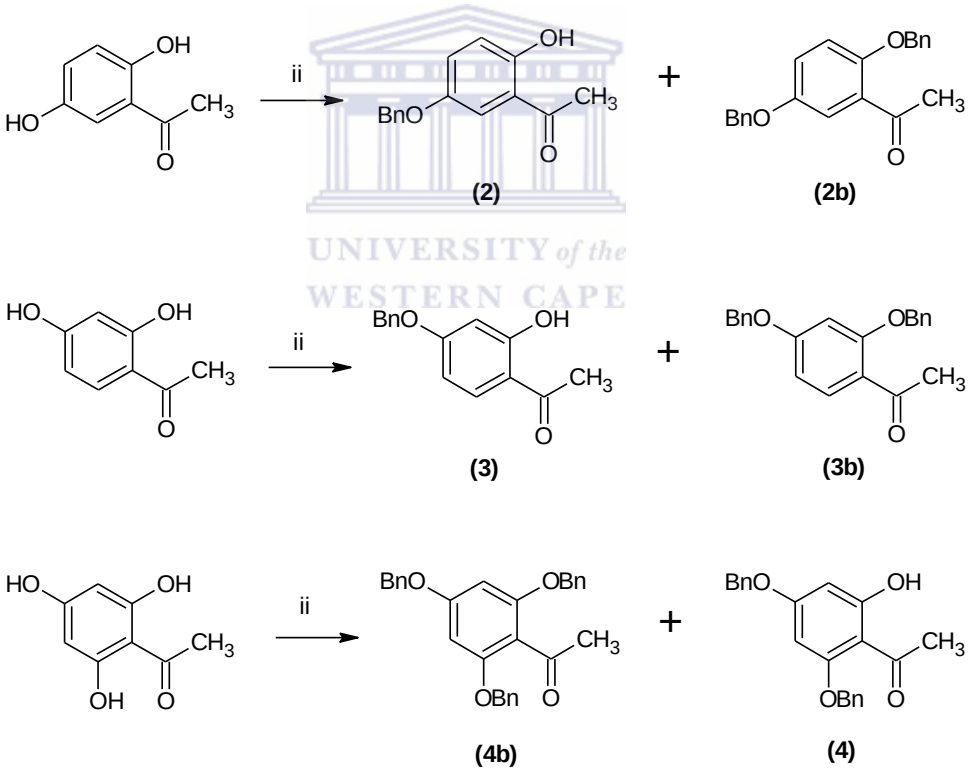


Scheme 3.2: Protection of acetophenones using dihydropyran groups. Reagents and conditions: i) 3, 4-dihydro-2-H-pyran, PPTS (30mg), CH_2Cl_2 (DCM), R.T, 5h.

The use of dihydropyran ethers as protecting groups produced reasonable yields of products. However they were found to decompose over time if not protected from exposure to acidic fumes and environments. This observation was confirmed by comparing the ^{1}H NMR spectra of a single compound over time. It was however found that their stability was maintained during the Claisen-Schmidt formation of the chalcones and subsequent transformation into the corresponding flavanoids.

3.2.1.2 The use of benzyl protection groups

Benzyl ether protected acetophenones **(2-4)** were synthesized by reacting appropriate hydroxy acetopheones with benzyl bromide in the presence of anhydrous potassium carbonate (K_2CO_3) and solvent dimethylformamide **(Scheme 3.3)**. The reaction mixture was stirred for 3 hours at 80 ^oC. Again we observed the formation of unwanted products 2, 5-dibenzyloxyacetophenone **(2b)** and 2, 4-dibenzyloxyacetophenone **(3b)**, produced in low yields, 10 % and 5 % respectively. Conversely, for the protection of 2, 4, 6-trihydroxyacetophenone, the major product was found to be the tribenzyloxy acetophenone **(4b)** with a yield of 80 % and 2-hydroxy-4, 6-dibenzyloxyacetophenone **(4)** was the minor, yield 20 %. Optimization of the yield for (**6**) was not conducted at this stage although this should be possible with some fine tuning of the stoichiometry.

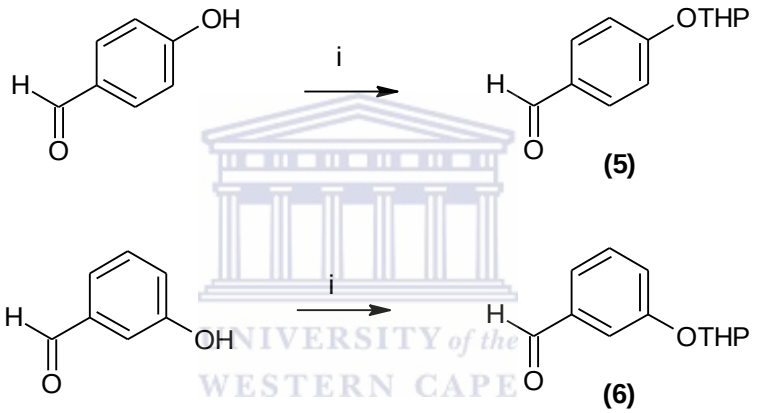


Scheme 3.3: Protection of acetophenones using benzyl groups. Reagents and conditions, ii) Benzyl bromide, K_2CO_3, DMF, 80 ^oC, 3h.

3.2.2 Synthesis of benzaldehydes 5-9

3.2.2.1 The use of dihydropyran protection groups

Pyran ether protected benzaldehydes, 4-tetrahydropyranylbenzaldehyde **(5)** and 3-tetrahydropyranylbenzaldehyde **(6)** were both synthesized in good yields 80 % and 75 % respectively, by stirring the appropriate hydroxyl benzaldehydes with3,4-dihydro-2-H-pyranin the presence of pyridinium *p*-toluene sulphonate (PPTS) and solvent CH_2Cl_2(DCM) for 5 hours at room temperature **(Scheme 3.4)**. Again the pyran protected benzaldehydes proved to be somewhat unstable unless rigorously purified and protected against any acid media. However the stability did increase when compounds **(5)** and **(6)** were converted to their corresponding chalcone analogues.

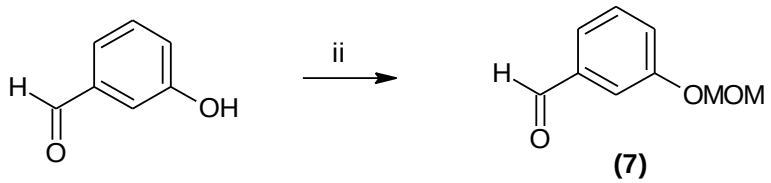


Scheme 3.4: Protection of benzaldehydes using pyran groups. Reagents and conditions: i) 3, 4-dihydro-2-H-pyran, PPTS (30mg), CH_2Cl_2 (DCM), R.T, 5h

3.2.2.2 The use of Methoxy methoxy (MOM) protection groups

5-methoxymethyleneoxy-benzaldehyde **(7)** was synthesized in low yield (40%) by treating 5-hydroxybenzaldehyde with methoxymethyl bromide (MOM-Br) in the presence of sodium hydride (NaH) and solvent DCM, at room temperature for 24 hours **(Scheme 3.5)**. The same reaction was repeated using other solvents viz., acetone and acetonitrile to only produce lower yields in both cases. The methoxy-methylene-oxy masking group is attached via a typical SN_2reaction. The base used removes a proton from the hydroxyl group present on the hydroxyl benzaldehyde forming the corresponding phenoxide anion causing a nucleophilic attack on the MOM-Br with displacement of the bromine. These

protecting groups are usually formed using the reagent MOM-Cl in good yields. However due to the highly cancerous causing nature of the latter reagent it was not used. We ascribed our low yields obtained due to the decomposition of the MOM-Br since upon opening the container, HBr fumes were noticeable and thus we concluded that the MOM-Br reagent was not suitable for the synthesis of the desired MOM derivatives.

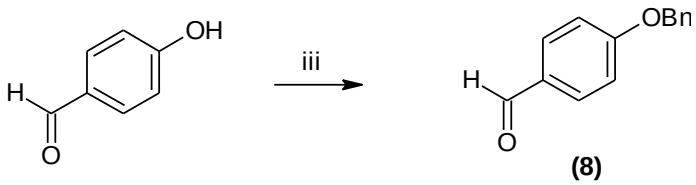


(7)

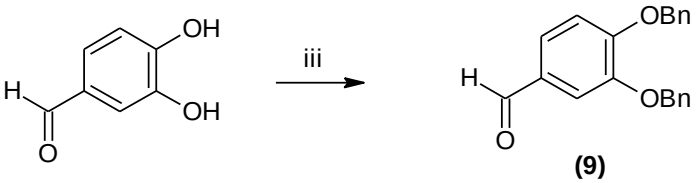
Scheme 3.5: Protection of benzaldehydes using MOM groups. Reagents and conditions: ii) Methoxymethyl bromide (MOM-Br) (1.1eq), NaH, Acetone, R.T, 24h

3.2.2.3 The use of Benzyl protection groups

Benzyl ether protected benzaldehydes, 4-benzyloxybenzaldehyde **(8)** and 3,4,--dibenzyloxybenzaldehyde **(9)** were synthesized in good yield 88 % and 72 % respectively by treating the appropriate hydroxy benzaldehydes with benzyl bromide in the presence of anhydrous potassium carbonate (K_2CO_3) and solvent DMF for 3 hours at 80^{o}C **(Scheme 3.6)**.



(8)



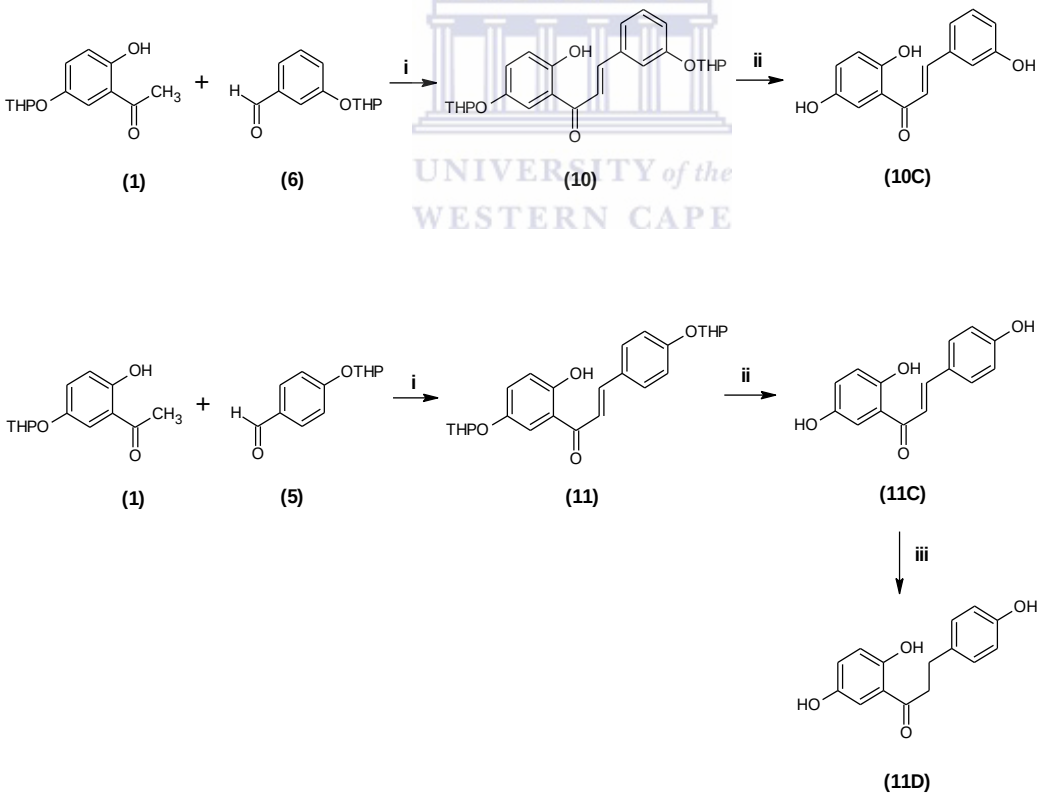
(9)

Scheme 3.6: Protection of benzaldehydes using benzyl groups. Reagents and conditions: iii) Benzyl bromide, K_2CO_3, DMF, 80 ^{o}C, 3h

3.2.3 Synthesis of Chalcones and Dihydrochalones

The tetrahydropyranyl-chalcones **(10)** and **(11)** were prepared in good yields of 82 % and 85 % respectively by the Claisen-Schmidt condensation between acetophenone **(1)** and benzaldehydes **(5)** and **(6)** respectively. An equimolar mixture of the relevant aldehyde and acetophenone in ethanol (95 %) was treated with KOH at 40 °C for 24 hours. Crude products were purified by column chromatography and thereafter the demasking of the pyran protecting groups in **(10)** and **(11)** was achieved by acidic hydrolysis in which the THP protecting groups were removed to produce 2, 5, 4- trihydroxychalcone **(10C)** and 2, 5, 3-trihydroxychalcone **(11C)** respectively b **(Scheme 3.7)**.

Saturation of the α - β double bond was achieved using catalytic hydrogenation, whereby chalcone **11C** was dissolved in ethyl acetate and treated with Pd/C (10% $^{w}/_{w}$) as the heterogeneous catalyst under hydrogen pressure for 5 hours at room temperature to produce the hydroxy-dihydrochalcone **11D (Scheme 3.7)**.



Scheme 3.7: Synthesis of hydroxychalcones 10C and 11C and dihydrochalcone 11D. Reagents and conditions: i) KOH at 40 °C for 24 hours. ii) Acid hydrolysis at room temperature. iii) Catalytic hydrogenation, Pd/C, ethyl acetate, 5 hours at room temperature

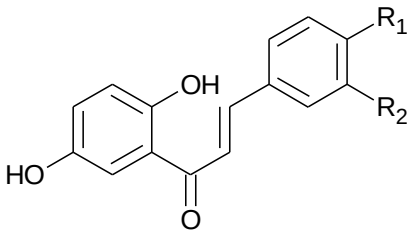
3.2.3.1 Discussion

According to literature the Claisen Schmidt condensation is performed at room temperature for 12-24 hours to achieve good yields (60 – 70 %) [97-100], however through a process of reaction optimization, we found that increasing the reaction temperature to 40 ^{o}C improved yields to 70 – 85 %, as well as reduced the reaction time to 3 - 4 hours.

While pyran protected chalcones **10** and **11**, were prepared were prepared in good yields of 82 and 85 % respectively, their deprotection was achieved through acid hydrolysis and even higher yields of 98 % and 100% for **10C** and **11C** respectively. The IR spectra show characteristic bands for C=O at 1635 - 1659, C=C Ar at 1590 and 1470, while the vinyl CH=CH appeared at 1285-1290 cm^{-1}. The ^{1}H NMR data of hydroxychalcones **10C** and **11C** are understandably similar. Two pairs of *trans*-coupled doublets were observed in regions 7.50-7.90 ppm due to H-α and H-β, this anticipated spectral result was confirmed when compared to the known structures referenced in Sci Finder [101]. The chemical shifts of the H-α protons are at higher field than those of H-β protons in both chalcones. This observation is due to polarization of the C=C double bond which is caused by the electron withdrawing carbonyl group. Thus the β-C has a lower electron density and thus exhibits a weaker shielding effect on the β-H which is consequently less shielded and hence has a δ-H at lower field strength than the α-H. The coupling constant *J* between H-α and H-βin both chalcones is in the region of between 15–16 Hz which indicates an anticipated *trans* configuration [102]. The resonance signals for the hydroxyl groups appear downfield; however the OH group at position 2', in both chalcones (**10C** and **11C**), appear significantly downfield at 11.85-12.87 ppm, with respect to OH signals at positions 5', 4 and 3 (6.50 – 10.34 ppm) and is ascribed to hydrogen bonding with the C4 carbonyl group. The signals for the aromatic protons for both chalcones appear in the range of 6.50-7.50. In the ^{13}C NMR spectra, the C4 for chalcones **10C** and **11C** appears in the region of δ189 and 204 ppm. . The α- and β-C atoms with respect to the carbonyl group gave rise to signals in the range of 121 -137 and again the α-C appears at a higher field than the β-C for the same reasons given *vide infra*. This data is indicative of hydroxylated chalcones and is summarized in Table 2.

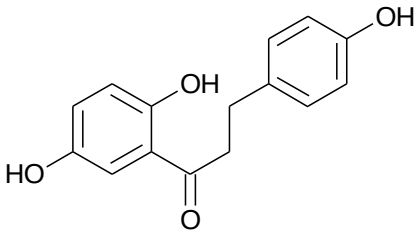
The ^{1}H NMR spectra for hydroxyl-dihydrochalcone **11D,** show two triplets, in the range of 3.00-3.30 ppm, assigned to the hydrogen H-α (J = 7.2 Hz) and H-β (J = 7.2 Hz) of the

ethano chain between to two aryl rings. The H-β signal occurs at a higher field, 3.03 ppm, in comparison to the H-α triplet, 3.24 ppm, this observation is due to the deshielding effects of the electron withdrawing carbonyl group on the H-α causing the chemical shift to appear further downfield. The aromatic protons for dihydrochalcone **11D** appear in the range of 5.50 - 7.50 ppm. In the ^{13}C NMR spectra, C4 displays a signal downfield, in the region of δ 201-205.6, indicative of the carbonyl group functionality [103]. Table 2 represents a summary of the various diagnostic signals for the chalcones discussed *vide infra*. Compound 11D is known and was confirmed by comparing its spectral analysis to the identified compound previously synthesized by Lim *et al.* [104].



(10C) R1 = H, R2 = OH

(11C) R1 = OH, R2 = H



(11D)

	^{1}H			^{13}C			
Compound	**H-α (ppm)**	**H-β (ppm)**	**OH (ppm)**	**C-α (ppm)**	**C- β (ppm)**	**C=O (ppm)**	**C-OH (ppm)**
10C	7.54 (d, J=15Hz)	7.90 (d, J=15Hz)	2' = 12.61, 5' = 10.57, 4 = 9.24	121.4	145.2	190.9	2' = 154.4 5' = 151.6 3 = 157.7
11C	7.56 (d, J=15Hz)	7.90 (d, J=15Hz)	2' = 12.01, 5' = 10.27, 3 = 9.14	121.3	145.2	203.3	2' = 154.4 5' = 151.6 4 = 157.7
11D	3.24 t, J=7.2 Hz	3.03 t, J=7.2 Hz	2' = 11.93 5' = 9.87 4 = 9.54	45.3	26.97	201.4	2' =159.00 5' =146.40 4' =153.20

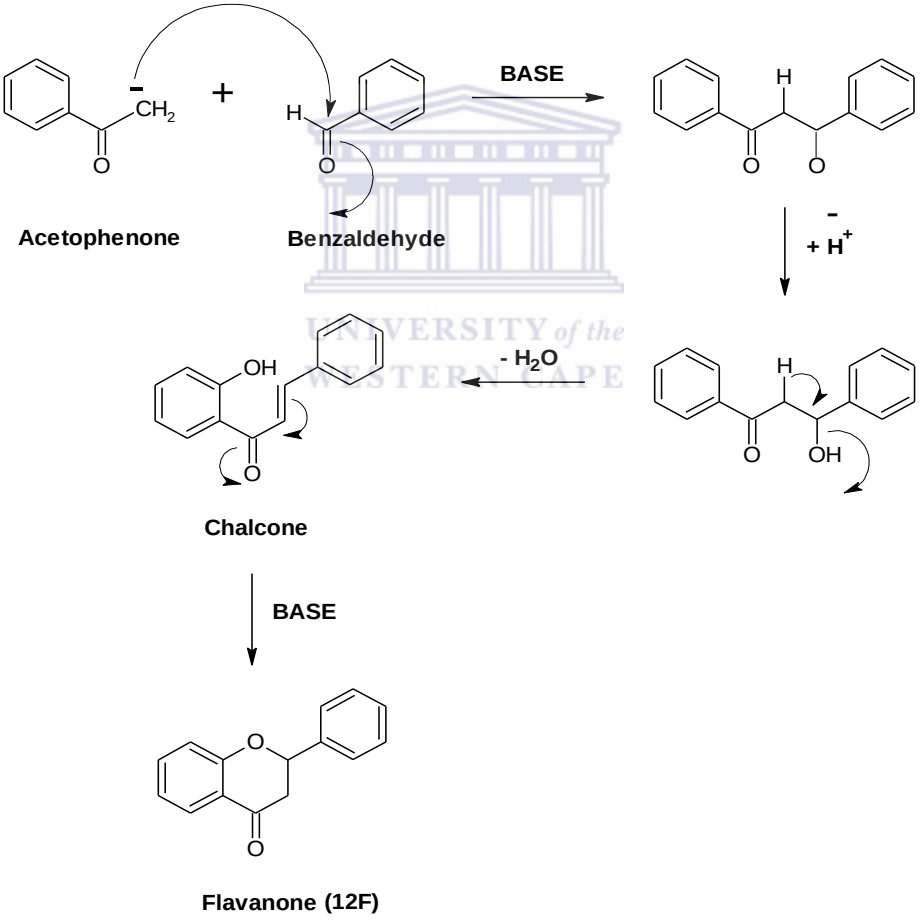
Table 3.1: Characteristic spectroscopic data of hydroxychalcones **10C**, **11C** and dihydrochalcone **11D**

Chalcones **10C** and **11C**, and Dihydrochalcone **11D** are known and were confirmed by spectroscopy with comparisons referenced in Sci Finder [101, 102 and 104].

3.2.4 Synthesis of flavanones

3.2.4.1 The 'simple' flavanone

The simple flavanone **(12F)** was synthesized via the Claisen Schmidt condensation of commercially available 2'-hydroxy-acetophenone and benzaldehyde in base (KOH) to form the chalcone **(12C)** intermediate (yield 78%), this was then cyclized, again under basic conditions, using sodium acetate, to form the corresponding flavanone **12F** (yield 92.8%). **Scheme 3.8** below displays the complete reaction mechanism. Compounds **12C** and **12F** are known, and the characteristic signals for both the chalcone and the corresponding flavanone were compared with the identified structures referenced in Sci-Finder respectively [105, 106].



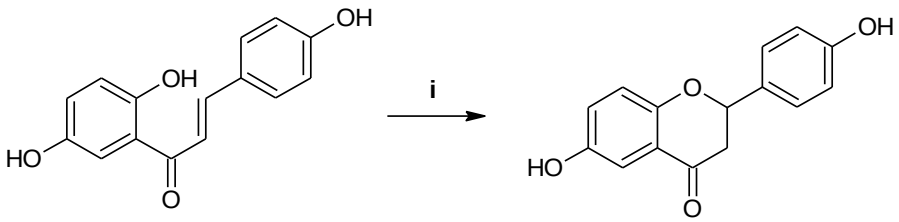
Scheme 3.8: Reaction mechanism for the synthesis of the 'simple flavanone' **(12F)**

3.2.4.2 Hydroxyflavanones and methoxy-hydroxy flavanones

All flavanones **(13F – 18F)** were prepared in good yields (89 – 95 %) via the route synthesis discussed previously. An equimolar mixture of the relevant aldehyde and acetophenone in ethanol (95 %) was treated with KOH at 40 °C for 4 hours to produce the chalcone intermediates **(13C – 18C)**. Crude products were purified by column chromatography and confirmed by NMR spectroscopy. The structural elucidation of compounds **13C – 15C** was achieved by comparing them with references found on Sci-finder, the chalcone moiety can easily be identified by observing two pairs of *trans*-coupled doublets in regions 7.50-7.90 ppm [107-109]. Compounds **16C – 18C** were found to be novel as no references to them were found on Sci-Finder.

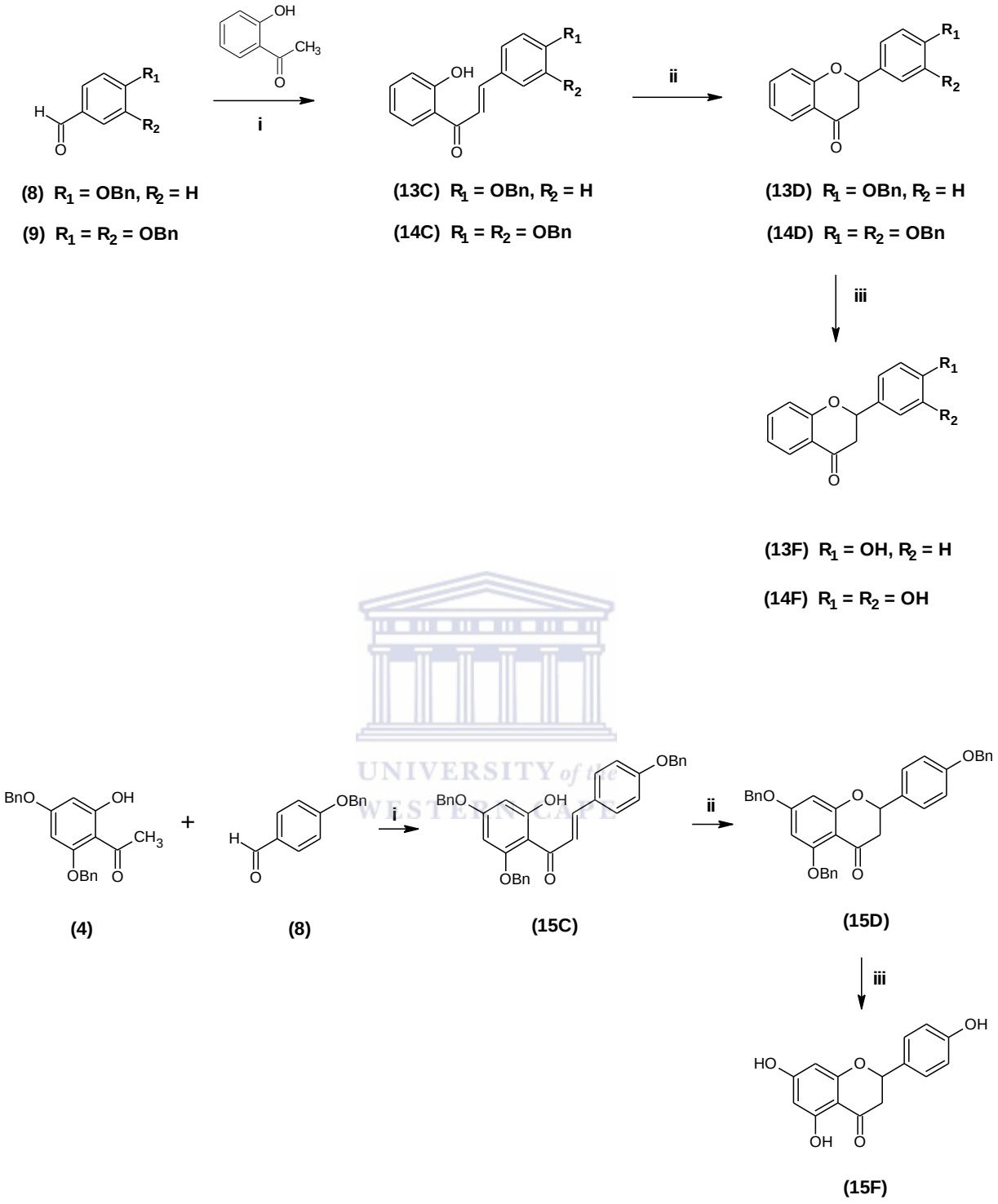
The chalcone intermediate, in ethanol, was treated with sodium acetate and stirred under reflux for 24 hours to produce the corresponding benzyloxy flavanones (**13D – 15D)** and benzyloxy methoxy flavanones (**16D – 18D**). The structure of compound **13D** was compared to 4'-Benzyloxyflavanone prepared by Cabrera *et al*, in their investigation into antitumor agents [107]. Compounds **14D - 18D** were found to be novel as no references to them were found on Sci-finder. The procedure, although seemingly simple needed some fine tuning in order for the reasonable yields of above 85 % to be achieved. Demasking of the benzyl groups for all flavanones was achieved using catalytic hydrogenation. The flavanones were dissolved in ethyl acetate and treated with Pd/C as the heterogeneous catalyst under a hydrogen atmosphere for 5 hours at room temperature to produce hydroxyl-flavanones **13F – 15F (Scheme 3.10)** and hydroxyl methoxyflavanones **16F – 18F (Scheme 3.11)**.

Chalcone **(11C)** was the representative precursor for synthesis of the corresponding hydroxyl flavanone **(11F) (Scheme 3.9)**.

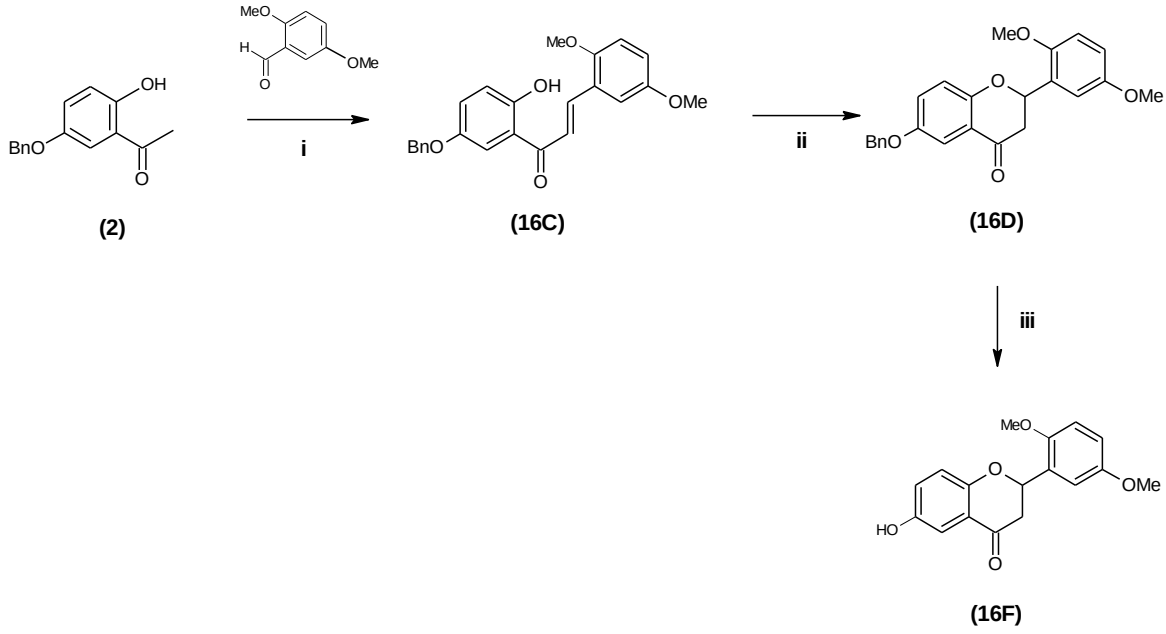


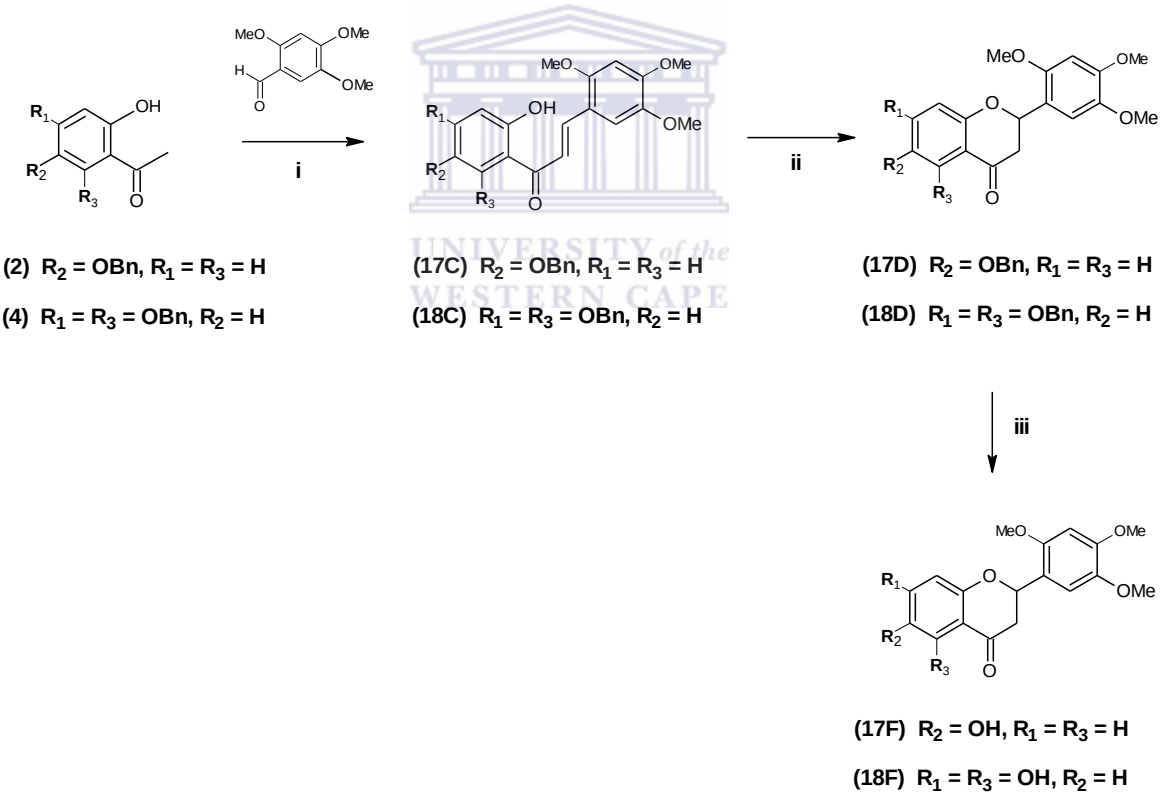
(11C) **(11F)**

Scheme 3.9: Synthesis of hydroxy flavanone **11F**. Reagents and conditions: i) sodium acetate, under reflux for 24 hours.



Scheme 3.10: Synthesis of hydroxyl flavanones **13F – 15F**. Reagents and conditions: i) KOH/EtOH at 40 °C for 4 hours. ii) Sodium acetate, under reflux for 24 hours. iii) Catalytic hydrogenation, Pd/C, ethyl acetate, 5 hours

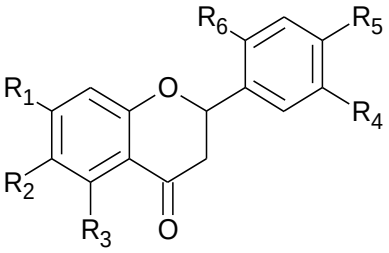




Scheme 3.11: Synthesis of hydroxyl methoxy flavanones **13F – 15F**. Reagents and conditions: i) KOH/EtOH at 40 °C for 4 hours. ii) Sodium acetate, under reflux for 24 hours. iii) Catalytic hydrogenation, Pd/C, ethyl acetate, 5 hours

3.2.4.3 Discussion

Deprotection of benzyloxy flavanones **13D – 18D** by catalytic hydrogenation produced flavanones **13F – 18F** in good yields (90 - 98 %). The ^{1}HNMR spectra of all flavanones **12F – 18F** were all characterized by the presence of two two-proton triplets, in the range of 2.95-3.30 ppm, assigned to the hydrogens of H-3 (J = 7.2 Hz) and H-2 (J = 7.2 Hz) which confirms cyclisation. The H-2 triplets occur at a higher field, 2.95-3.03 ppm, in comparison to the H-3 triplets, 3.17-3.34 ppm, due to the deshielding effects of the electron withdrawing carbonyl group causing the chemical shift ofH-3 to appear further downfield. The resonance signals for the hydroxyl groups appear downfield as expected. However the signals for the OH groups at position 6' appear significantly downfield, 11.85-12.87 ppm, with respect to the other OH signals for OH groups at positions 4', 5', 3 and 4 (6.50 – 10.34 ppm). The strong deshielding of the resonances of OH protons at position 6' is due to intramolecular hydrogen bonding with the *peri*-carbonyl group. In the case of hydroxyl methoxy flavanones **16F – 18F**, the resonance signals for the methoxy groups appear up field as expected, in the region of 3.78 - 3.90 ppm. The aromatic protons for all flavanones **12F – 18F** appear in the range of 5.50 - 7.50 ppm. The ^{13}C NMR spectra, all displayed single signals in the region of δ 201-205.6, which is indicative of a ketone carbonyl group. The 3- and 2-C atoms with respect to the carbonyl group in the pyran ring gave rise to signals in the range of 30 and 75 ppm. Again the resonance signal for the3-C appears at a higher field than the 2-C since the 2-C is directly attached to an Oxygen. Table 3 represents a summary of the various diagnostic signals for the flavanones discussed *vide infra*. The above mentioned flavanones have been structurally compared with their respective compounds found in literature, and their ^{1}HNMR and ^{13}C NMR signals were found to be indicative of the Flavanone structure [110-112].



(12F) R1 = R2 = R3 = R4 = R5 = R6 = H

(13F) R1 = R2 = R3 = R4 = R6 = H, R5 = OH

(14F) R1 = R2 = R3 = R5 = R6 = H, R4 = OH

(15F) R1 = R3 = R5 = OH, R2 = R4 = R6 = H

(16F) R1 = R3 = R5 = H, R2 = OH, R4 = R6 = OMe

(17F) R1 = R3 = H, R2 = OH, R4 = R5 = R6 = OMe

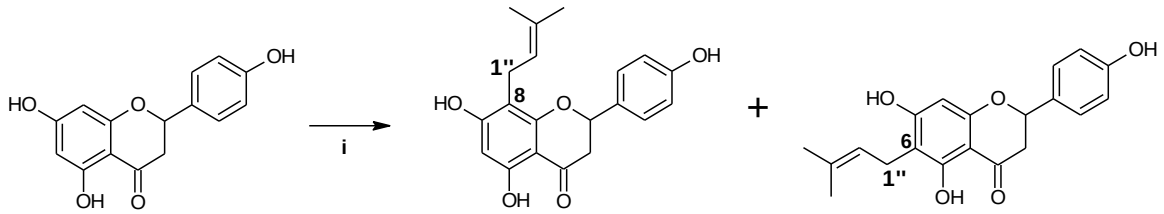
(18F) R1 = R2 = OH, R3 = H, R4 = R5 = R6 = OMe

Compound	H- 3	H- 2	OH	OMe	C- 2	C- 3	C=O	C-OH
12F	3.24 t, J=7.2 Hz	3.03 t, J=7.2 Hz	2’ = 11.93 5’ = 9.4	-	38.79	26.97	205.61	C2’ =156.69 C5’ =153.47
13F	3.17 t, J=7.2 Hz	2.98 t, J=7.2 Hz	2’6’ = 12.87 4’ = 10.34	-	45.3	26.97	201.4	C2’ =159.00 C5’ =126.40 C6’ =143.20
14F	3.17 t, J=7.2 Hz	2.98 t, J=7.2 Hz	2’6’ = 12.87 4’ = 10.34	-	45.3	26.97	201.4	C2’ =159.00 C5’ =126.40 C6’ =143.20
15F	3.17 t, J=7.2 Hz	2.98 t, J=7.2 Hz	2’6’ = 12.87 4’ = 10.34	-	45.3	26.97	201.4	C2’ =159.00 C5’ =126.40 C6’ =143.20
16F	3.32 t, J=7.2 Hz	2.96 t, J=7.2 Hz	2’6’ = 11.85 4’ = 6.50	3.81- 3.90	45.3	26.97	201.4	C2’ =159.00 C4’ =126.40 C6’ =143.20
17F	3.17 t, J=7.2 Hz	2.98 t, J=7.2 Hz	2’ = 12.87 4’ = 10.34	3.83- 3.90	44.3	26.87	201.4	C2’ =159.00 C4’ =155.8
18F	3.21 t, J=7.2 Hz	2.99 t, J=7.2 Hz	2’ = 11.93 5’ = 7.63	3.83- 3.90	45.3	26.97	201.4	C2’ =159.00 C5’ =155.8

Table 3.2: Characteristic spectroscopic data of Flavanones **(12F – 18F)**

3.3. Synthesis of 6- and 8-Prenyl Flavanones

The synthesis of prenylated flavanones **19F** and **20F** was achieved by gradually treating Naringenin **(14F)** with boron trifloride etherate and 2-hydrox-2-methylbutane in dioxin at room temperature for 24 hours. This reaction resulted in the formation of two products, which could be separated chromatographically and identified as 8-C- prenylnaringenin and 6-C- prenylnaringenin in moderate yields of 36 % and 40 % respectively **(Scheme 3.12)**.



(19F) **(20F)**

Scheme 3.12: The synthesis of prenylated flavanones **19F** and **20F**, Reagents and conditions, i) boron trifloride etherate and 2-hydrox-2-methylbutane in dioxin at room temperature for 24 hours

3.3.1 Discussion

The flavanone skeleton of compounds **19F** and **20F** was easily identified using ^{1}H NMR spectroscopy by the characteristic sets of doublets of doublets at δ_H5.25 – 5.39 (J =12.0 and 4.5 Hz) for H-2a, at δ 2.68 – 2 .99 (J = 17 and 4.5 Hz) for H-3e [113]. A third methylene doublet of doublets is observed in the range of δ 3.08 – 3.09 (J = 17.3 and 13.0 Hz) for H – 3a, in which the large coupling is due to geminal coupling between proton H – 3a and H – 3e. The prenyl groups were identified by two singlets corresponding to the two methyl groups, appearing up field in the range of 1.67 – 1.82 ppm, as well as a prominent two proton doublet in the range of 3.35 – 3.37 ppm corresponding to H-1''. Singlets corresponding to H-6 for compound **19F** and H-8 for compound **20F** were observed in the range of δ 5.99 – 6.02 ppm. The ^{1}HNMR spectra for **19F** and **20F** were understandably quite similar. However, according to literature they may be distinguishable

by comparing the δ values of the singlets for H-6and H-8, with the latter appearing slightly downfield in all similar cases published [113]. Based on this premise, compound **19F** was characterized as 8-C- prenylnaringenin (singlet appeared at 5.99 ppm, H-6), and compound **20F** as 6-C- prenylnaringenin (singlet appeared at 6.02 ppm, H-8). Downfield exchangeable singlets at δ 12.00 ppm and 12.39 ppm, characteristic of the chelated OH was observed for **19F** and **20F** respectively. Table 4 provides a summary of the comparative ^{1}H NMR spectra for the two isomers illustrating their similarities and subtle differences as measured on a 200 MHz spectrometer.

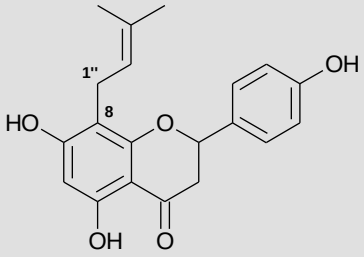
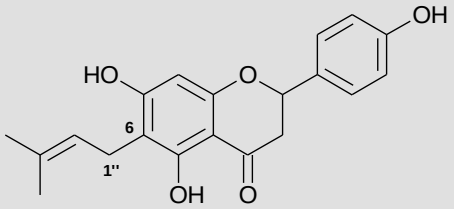
Position	δ H	
	19 F 	**20F** 
2	5.71 dd, *J*=13.0, 3.1Hz	5.39 dd, *J*=13.0, 3.1Hz
3	2.70 dd, *J*=16.3, 3.4Hz, H–3a 2.99 dd, *J*=16.3, 13.0Hz, H–3e	2.68 dd, *J*=16.3, 3.4Hz, H–3[a] 2.89 dd, *J*=16.3, 13.0Hz, H–3e
5 – OH	12.40, s	12.00, s
6	5.99, s	-
7 – OH	11.22, s	11.01, s
8	-	6.02, s
2'	7.33, d, J=8.4Hz	7.32, d, J=8.4Hz
3'	6.88, d, J=8.4Hz	6.88, d, J=8.4Hz
4' – OH	9.67, s	9.60, s
5'	6.88, d, J=8.4Hz	6.88, d, J=8.4Hz
6'	7.33, d, J=8.4Hz	7.32, d, J=8.4Hz
1''	3.35, d, J=8.0Hz,	3.36, d, J=8.0Hz,
2''	5.25-5.37, m	5.20-5.39, m
4''	1.815, s	1.72, s
5''	1.756, s	1.67, s

Table 3.3: [1]H NMR Spectroscopic data of Prenylated flavanoids **19F** and **20F**

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CHAPTER 4

4. EXPERIMENTAL

4.1 Materials

4.1.1 Purification of solvents

All solvents used for reactions and preparative chromatography, were distilled prior to use. Other reagents obtained from commercial sources were used without further purification.

4.1.2 Chromatographic Separations

Preparative column chromatography was carried out on dry-packed columns using Merck silica gel (particle size 0.2 - 0.5 mm) as adsorbent and Merck silica gel 60 (0.063- 0.2 mm) as the stationary phase. Mixtures of ethyl acetate and hexane were used as eluent.

4.1.3 Physical and Spectroscopic Data

All melting points were obtained on a FISCHER-JOHNS melting point apparatus and are uncorrected.

Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectroscopy were used for structural determination of all ligands. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Gemini 2000spectrometer and chemical shifts are indicated in ppm.(^{1}H, 200MHz; ^{13}C, 50MHz).The spectra were run at ambient temperature in deuterated chloroform ($CDCl_3$) solution, with $CHCl_3$ at δ 7.26 for ^{1}H NMR spectra and δ 77.00 for ^{13}C-NMR spectra as internal standards. Sample signals are relative to the resonance of residual protons on carbons in the solvent. Samples were prepared weighing out between 30 – 60 mg of sample and dissolved in deuterated chloroform ($CDCl_3$). In the NMR spectra, assignments of signals with the same superscripts are interchangeable. Splitting patterns are designated as “s”, “d”, “t”, “q”, “m” and “bs”. These symbols indicate “singlet”, “doublet”, “triplet”, “quartet”, “multiplet” and “broad singlet”. FT-IR measurements were used to determine the presence of functional groups in all final stage

unprotected chalcones and flavanones. The ATR-IR measurements were carried out on a Perkin-Elmer Spectrum 100 FTIR spectrometer.

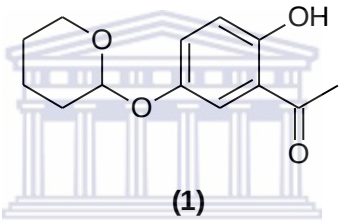
4.1.4 Other General Procedures

The term "residue obtained upon work-up" refers to the residue obtained when the organic layer was separated, dried over magnesium sulphate ($MgSO_4$) followed by filtration and the removal of solvent by evaporation.

4.2 Characterization

4.2.1 Pyran protected acetophenones and benzaldehydes

5-Dihydropyranyl-2-hydroxyacetophenone (1)

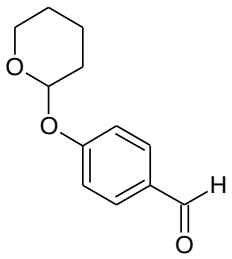


(1)

To a solution of 2, 5-dihydroxyacetophenone (1.38g; 10 mmol) and PPTS (30mg) in CH_2Cl_2 (20ml), 3, 4-dihydro-2-H-pyran (10.5g; 124 mmol) was added. The resulting mixture was stirred for 6 hours at room temperature. The residue obtained upon work-up was chromatographed using EtOAc: hexane (1:4) as eluent to afford the product **(1)** as white thick solid (1.86g; 100% based on 0.21 g recovered starting material).

^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.90 (m, 6H, 3'-, 4'- and 5'-H), 2.61 (s, 3H, C*H*$_3$CO), 3.60 (m, 1H, 6'-Ha), 3.90 (m, 1H, 6'-He), 5.31(t, 1H, *J*=3.2 Hz, 2'-H), 6.91 (d, 1H, *J*=8.8Hz, 3-H), 7.24 (dd, 1H, J=8.8 and 3.0 Hz, 4-H), 7.41 (d, 1H, J=3.0 Hz, 6-H). **^{13}C NMR** δ 18.8, 25.2, 26.6, 30.6, 61.8, 96.4, 113.4, 118.6, 118.7, 132.2, 148.9, 150.9 and 204.0. HRMS: Calc. For $C_{13}H_{16}O_4$ = 236.2637. Found: = 236.2636

4-Dihydropyranylbenzaldehyde (5)



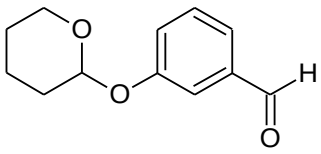
(5)

To a solution of 4-hydroxybenzaldehyde (1.22g; 10 mmol) and PPTS (30mg) in CH_2Cl_2 (20ml), 3, 4-dihydro-2-H-pyran (10.5g; 124 mmol) was added. The resulting mixture was stirred for 6 hours at room temperature. The residue obtained upon work-up was chromatographed using EtOAc: hexane (1:4) as eluent to afford the product **(5)** as a white thick solid (1.14g; 68%).

^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.75 (m, 6H, 3'-, 4'- and 5'-H), 3.65 (m, 1H, 6'-H_a), 3.95 (m, 1H, 6'-H_b), 5.50 (t, 1H, *J*=3.2 Hz, 2'-H), 7.15 (d, *J*=8.8Hz, 2H, H-3 and H-5), 7.24 (d, 2H, *J*=8.8Hz,H-2 and H-6) and 9.86 (s, 1H, ~C*H*O). **^{13}C NMR** δ 18.8, 25.1, 30.3, 61.9, 96.4, 115.2, 129.4, 131.1, 161.3 and 190.7.

HRMS: Calc. For $C_{12}H_{14}O_3$ = 206.2378.Found: = 206.2377

3-Dihydropyranylbenzaldehyde (6)



(6)

To a solution of 3-hydroxybenzaldehyde (1.22g; 10 mmol) and PPTS (30mg) in CH_2Cl_2 (20ml), 3, 4-dihydro-2-H-pyran (10.5g; 124 mmol) was added. The resulting mixture was stirred for 6 hours at room temperature. The residue obtained upon work-up was chromatographed using EtOAc: hexane (1:4) as eluent to afford the product **(6)** (1.09g; yield 53%).

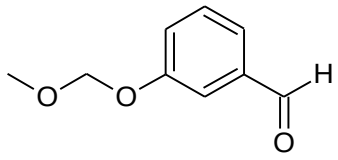
^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.75 (m, 6H, 3'-, 4'- and 5'-H), 3.60 (m, 1H, 6'-H_a), 3.95 (m, 1H, 6'-H_b), 5.31(t, 1H, *J*=3.2 Hz, 2'-H), 7.30 (m, 1H, H-5), 7.36-7.43 (m, 3H, H-2

, H-4 and H-6) and 9.97 (s, 1H, ~C*H*O). **^{13}C NMR** δ 18.8, 25.2, 30.2, 61.8, 96.5, 112.8, 120.4, 123.2, 130.0, 137.8, 158.6 and 192.2.

HRMS: Calc. For $C_{12}H_{14}O_3$ = 206.2378. Found: = 206.2377

4.2.2 MOM protected benzaldehyde

3-Methoxymethyleneoxybenzaldehyde (7)



(7)

To a stirred mixture of 3-hydroxybenzaldehyde (1.22g, 10mmol) and sodium hydride (5.61g, 7mmol) in CH_2Cl_2 (15ml), MOMBr (1.39g, 10.1mmol) was added drop wise. The mixture was stirred at room temperature overnight, diluted with water (20ml), extracted using CH_2Cl_2 , washed with saturated NH_4Cl (3 X 100ml) and evaporated to dryness. The residue obtained upon work-up was chromatographed using EtOAc: hexane (1:4) as eluent to afford 3-methoxymethylene-oxybenzaldehyde **(7)** as a brown solid (0.49g, yield 30%)

^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.49 (s, 3H, OC*H*$_3$), 5.23 (s, 2H, OC*H*$_2$OCH$_3$), 7.29 (m, 1H, 4-H), 7.45 (t, 1H, *J*=7.2 Hz, 5-H), 7.52 (m, 2H, 2- and 6-H). **^{13}C NMR** δ: 56.2, 94.5, 113.3, 120.9, 123.0, 130.9, 137.9, 157.1 and 192.3.

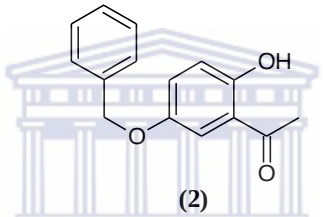
HRMS: Calc. For $C_9H_{10}O_3$ = 166.1739. Found: = 166.1737

4.2.3 Benzyl protection of acetophenones/benzaldehydes

4.2.3.1 General procedure for benzyl protection of acetophenones/bezaldehydes

To a solution of either the acetophenone or benzaldehyde in DMF (30 ml), K_2CO_3 (3.6 mmol equiv) and the same mmol amount of benzyl bromide as the acetophenone or benzaldehyde analogue was added. The reaction mixture was stirred for 3 hours at 80 ^oC. The progress of the reaction was monitored by TLC. The reaction mixture was then poured into water and extracted with ethyl acetate (EtOAc), and the organic portion was washed with brine. The organic fraction was dried over anhydrous Na_2SO_4, and the solvent was removed in *vacuo* to afford a residue which was then chromatographed.

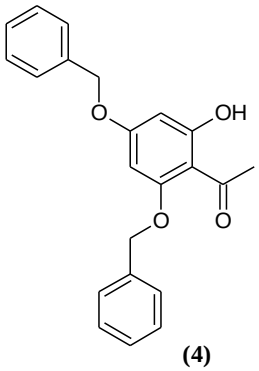
2-Hydroxy-5-benzyloxyacetophenone (2)



(2)

2-Hydroxy-5-benzyloxyacetophenone **(2)** was synthesized from 2, 5-dihydroxyacetophenone (3.043g; 20 mmol), benzyl bromide (1.9 ml; 20 mmol) andK_2CO_3 (10g) according to the general procedure used for benzyl protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **2-Hydroxy-5-benzyloxyacetophenone (2)** as off-white thick solid (3.94g, 16 mmol, 81 % yield). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm, 2.45 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 6.72 (m, 2H, 3,4-H), 7.28 (s, 1H, 6-H), 7.44 (m, 4H, Ar), 11.77 (s, 1H, 2-OH); **^{13}C NMR** (200 MHz, $CDCl_3$) δppm, 30.1 (CH_3), 71.0 (CH_2), 115.1 (C-6), 116.6 (C-3), 119.8 (C-4), 122.1 (C-1), 127.0 – 129.7 (5C-Ar), 141.9 (C-1'), 153.5 (C-5), 154.1 (C-2) and 205.6 (C=O). HRMS: Calc. For $C_{15}H_{14}O_3$ = 242.2699. Found: = 242.2696

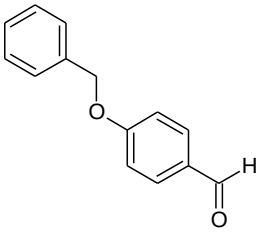
2-Hydroxy-4, 6-dibenzyloxyacetophenone (4)



(4)

2-Hydroxy-4, 6-dibenzyloxyacetophenone **(4)** was synthesized from 2, 4, 6-trihydroxyacetophenone (3.36g; 20 mmol), benzyl bromide (3.8 ml; 40 mmol) andK_2CO_3 (10g) according to the general procedure used for benzyl protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **2-hydroxy-4,6-dibenzyloxyacetophenone (4)** as off-white solid (2.59g, 18 mmol, 37 % yield), **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm, 2.56 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 5.28 (s, 2H, CH_2), 5.88 (s, 1H, 3-H), 6.36 (s, 1H, 5-H), 7.46 (m, 10H, 2xAr), 11.93 (s, 1H, 2-OH); **^{13}C NMR** (200 MHz, $CDCl_3$):δppm, 31.0 (CH_3), 71.1 (CH_2), 71.3 (CH_2), 93.6 (C-5), 94.7 (C-3), 102.3 (C-1), 127.0 – 130.4 (C-Ar), 141.6 (C-1'), 141.9 (C-1''), 162.5 (C-2), 165.6 (C-6), 166.7 (C-4) and 205.6 (C=O). HRMS: Calc. For $C_{22}H_{20}O_4$ = 348.3918. Found: = 38.3915

4-Benzyloxybenzaldehyde (8)

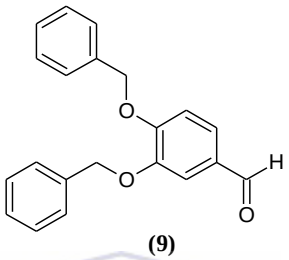


(8)

4-Benzyloxybenzaldehyde **(8)** was synthesized from 4-hydroxybenzaldehyde (2.44g; 20 mmol), benzyl bromide (1.9 ml; 20 mmol) and K_2CO_3 (10g) according to the general procedure used for benzyl protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **4-benzyloxybenzaldehyde (8)** as brown thick solid (3.58g, 16.8 mmol, 84.4 % yield)

^{1}H NMR (200 MHz, $CDCl_3$) δ ppm, 5.18 (s, 2H, CH_2), 6.88 (d, *J* = 7.6 Hz, 2- and 6-H), 7.46 (m, 5H, Ar), 7.96 (d, *J* = 7.6 Hz, 3- and 5-H) and 10.21 (s, 1H, CHO); **^{13}C NMR** (400 MHz, $CDCl_3$) δppm, 71.32 (CH_2), 115.20 (C-3, C-5), 127.0 -129.9 (C-2′, C-3′, C-4′, C-5′ and C-6′), 128.57 (C-1), 131.11 (C-2, C-6), 136.6 (C-1′), 165.78 (C-5), 203.66 (C=O). HRMS: Calc. For $C_{14}H_{12}O_2$ = 212.2439. Found: = 212.2437

3,4-Dibenzyloxybenzaldehyde (9)



(9)

3, 4-Dibenzyloxybenzaldehyde **(9)** was synthesized from 3, 4-dihydroxybenzaldehyde (2.76g; 20 mmol), benzyl bromide (3.8 ml; 20 mmol) and K_2CO_3 (10g) according to the general procedure used for benzyl protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **3,4-dibenzyloxybenzaldehyde (9)** as brown thick solid (4.96g, 15.6 mmol, 77.9 % yield),

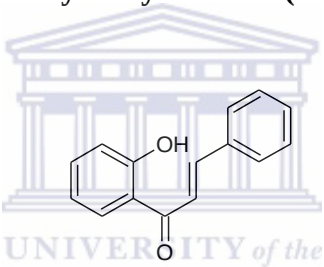
^{1}H NMR (200 MHz, $CDCl_3$): δ ppm, 5.22 (s, 4H, 2xCH_2,), 7.14 (dd, 1H, *J* = 6.4 and 2.0 Hz, 3-H),7.21 (d, 1H, *J* = 2.0 Hz, 6-H), 7.29 (d , 1H, *J* = 6.4 Hz, 2-H), 7.42 (m, 10H, Ar), 10.28 (s, 1H, CH0); **^{13}C NMR** (200 MHz, $CDCl_3$) δppm, 71.3 (CH_2), 71.4 (CH_2), 114.2 (CH_2), 115.1 (CH_2), 123.8 -130.1(C-2′, C-3′, C-4′, C-5′, C-6′, C-2″, C-3″, C-4″, C-5″, C-6″),130.2 (C-1), 136.0 (C-1′), 137.2 (C-1″), 150.8 (C-3), 154.2 (C-4) and 200.2 (C=O). HRMS: Calc. For $C_{21}H_{18}O_3$ = 318.3658. Found: = 318.3655

4.2.4 Synthesis of Chalcones

4.2.4.1 General procedure for the synthesis of Chalcones

To a solution of the substituted acetophenone (10 mmol) in EtOH (10ml-20ml), KOH (40 mmol) and benzaldehyde (10-12 mmol) was added. The resulting mixture was stirred at 40 ^{o}C for 4-5h and the progression of the reaction was monitored by TLC. After all reactants were consumed, the reaction mixture was poured into water and made acidic using 2M HCl solution. The reaction mixture was then extracted with ethyl acetate (EtOAc), and the organic portion washed with brine. The organic fraction was dried over anhydrous Na_2SO_4, filtered and the solvent was removed in *vacuo*. The compound was purified by column chromatography of the residue on silica gel with petroleum ether: ethyl acetate (4:1) as eluent.

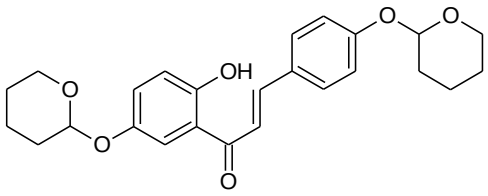
2′-Hydroxychalcone (12C)



(12C)

2′-Hydroxychalcone **(12C)** was synthesized from 2′-hydroxyacetophenone (1.36g; 10 mmol) and benzaldehyde (1.17g; 11 mmol) according to the general procedure for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford 2´- hydroxychalcone **(12C)** as brown crystals (1.75g, 7.8 mmol, 78 % yield) Mp =86 °C (Lit.[114] Mp = 86-88 °C). IR cm^{-1} : 1576 (C=O). **^{1}H NMR** (200 MHz, $CDCl_3$): δ ppm = 6.95(d, 1H, *J*=2.0 Hz, 3'-H), 7.44-7.99 (m, 8H, α-H, Ar-H), 8.01 (d, 1H, *J* = 15.5 Hz, , β-H), 8.12 (d,1H, *J*=2.0 Hz, 6'-H), 9.45 (s, 1 H, 2'-OH). **^{13}C NMR** (200 MHz, $CDCl_3$): δ ppm = 118.0 (3'-H), 120.2 (C-α), 121.4, 126.8, 126.9, 128.6, 129.0, 129.4,131.3, 135.0, 136.8 (C-Ar) 145.9 (C-β), 164.0 (C-2'), 194.1 (C=O). HRMS: Calc. For $C_{15}H_{12}O_2$ = 236.2653. Found: = 236.2651

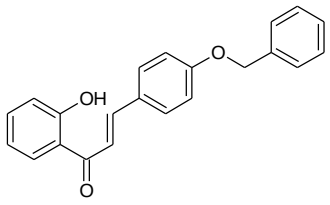
2'-Hydroxy-4, 5'–dihydropyranyl-chalcone (11)



(11)

2'-Hydroxy-4, 5'–dihydropyranyl-chalcone **(11)** was synthesized from 2'- hydroxy-5-dihydropyranyl-acetophenone (1.17g; 5 mmol) and 4-dihydropyranyl-benzaldehyde (1.03g; 5.5 mmol) according to the general procedure for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **2'-Hydroxy-4, 5'–dihydropyranyl-chalcone (11)** as a red thick solid (1.78g, 4.2 mmol, 83.9 % yield) **^{1}H NMR** (200 MHz, $CDCl_3$): δ ppm 1.40-2.16 (m, 12H, 3", 4", 5", 3"', 4"', and 5"'-H), 3.65 (m, 2H, 6"-Ha and 6"'-Ha), 3.90 (m, 2H, 6"-He and 6"'-He), 5.34 (m, 1H, 2"-H), 5.48 (m, 1H, 2"'-H), 6.96 (d, 1H, *J*=9.0 Hz, H-3'), 7.14 -7.39 (m, 5H, 4', 2-,3-,5- and 6-H), 7.57 (d, 1H, *J*=16.0 Hz, β-H), 7.59 (d, 1H, *J*=2.0 Hz, 6'-H), 7.88 (d, 1H, *J*=16.0 Hz, , α-H), 12.44 (s, 1H, 2′-OH). **^{13}C NMR** δ: 18.7(2C), 25.1(2C), 30.2(2C), 61.9 (2C), 96.5 (2C), 112.2, 115.9 (2C), 117.2, 118.0, 119.3, 127.4, 129.8 (2C), 135.3, 144.7, 149.4, 155.3, 156.1 and 192.6 (C=O). HRMS: Calc. For $C_{25}H_{28}O_6$ = 424.4862. Found: = 424.4861

2'-Hydroxy-4-benzyloxychalcone (13C)

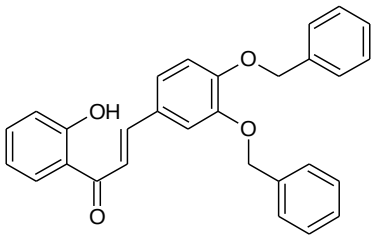


(13C)

2'-Hydroxy-4-benzyloxychalcone **(13C)** was synthesized from 2′-hydroxyacetophenone (1.36; 10 mmol) and 4- benzyloxybenzaldehyde (2.33g; 11 mmol) according to the general procedure used for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **2'- hydroxy-4-benzyloxychalcone (13C)** as a orange thick solid (2.7g, 8.2 mmol, 81.8 % yield). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 5.15 (s, 2H, CH_2), 6.62 (d, 2H, *J*=6.0

Hz, , 3-, 5-H), 6.97 (m, 1H, 3'-H), 7.35-7.46 (m, 7H, Ar-H), 7,55 (d, 1H,*J*=16.0 Hz, α-H), 7.71 (d, 2H, *J*=6.0 Hz, 2-,6-H), 7.81 (d, 1H, *J*=16.0 Hz, β-H), 8.11 (d, 1H, *J*=2.0 Hz, 6'-H), 9.83 (s, 1H, 2′-OH). **^{13}C NMR** (200 MHz, $CDCl_3$) δppm 70.23(CH_2), 113.19 (C-3,5), 116.40 (C-3'), 121.09 (C-α), 121.89 (C-5'), 127.12 (C-2",6"), 127.46 (C-1'), 127.5 - 129.0 (Aryl-Cs) 132.29 (C-β '), 136.56 (C-4'), 159.88 (C-4), 192.22 (C=O). HRMS: Calc. For $C_{22}H_{18}O_3$ = 342.3872. Found: = 342.3870

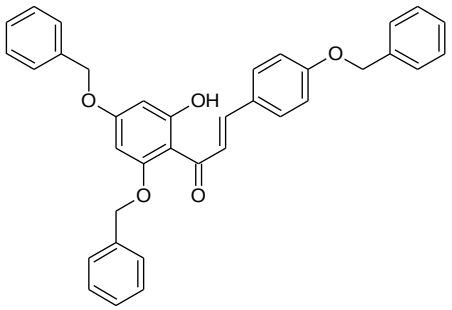
2'-Hydroxy-3,4-dibenzyloxychalcone (14C)



(14C)

2'-Hydroxy-3, 4-dibenzyloxychalcone **(14C)** was synthesized from 2′-hydroxyacetophenone (1.36g; 10 mmol) and 4, 5- dibenzyloxybenzaldehyde (3.5g 11 mmol) according to the general procedure used for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **2'-Hydroxy-3, 4-dibenzyloxychalcone (14C)** as yellowish brown solid (3.67g, 8.4 mmol, 84.2 % yield). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm 5.16 (s, 4H, 2xCH_2), 6.69 (m, 2H, 2- and 5-H), 6.82 (d, 1H, *J*=2.0 Hz, 3'-H), 6.94 (dd, 1H, *J*= 6.0 and 2.0 Hz, 5-H),7.19 (m, 2H, 4′- and 5′-H), 7.33-7.48 (m, 10H, Ar-H), 7.59 (d, 1H, *J*=16.0 Hz, α-H), 7.68 (d, 1H, *J*=2.0 Hz, 6′-H), 7.91 (d, 1H, *J*=16.0 Hz, β-H) and 9.89 (s, 1H, 2′-OH). **^{13}C NMR** (200MHz, $CDCl_3$) δppm 71.3 (2xCH_2), 114.14 (C-2,5), 116.57 (C-1'), 117.98 (C-3'), 121.04 (C- 3′), 121.39 (C-α), 123.40 (C- 6), 127.0 - 129.0 (10Cs of 2xPh rings, 4xAryl Cs), 141.2(C-1′ and C-1″) , 153.9 and 154.0 (C-3 and C-4), 154.3 (C-2'), 189.7 (C=O). HRMS: Calc. For $C_{29}H_{24}O_4$ = 436.4985. Found: = 436.4982

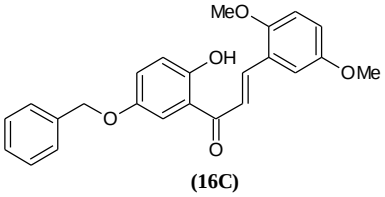
2′-Hydroxy-4′, 4, 6′–benzyloxychalcone (15C)



(15C)

2'-Hydroxy-4', 4, 6'-tribenzyloxychalcone **(15C)** was synthesized from 2′-hydroxy-4′, 6′-dibenzyloxyacetophenone (1.74g; 5 mmol) and 4-benzyloxybenzaldehyde (1.27g; 6 mmol) according to the general procedure used for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **2′-hydroxy-4′, 4, 6′ - tribenzyloxychalcone (15C)** as orange solid (2.26g, 4.2 mmol, 83.7 % yield). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 5.10 (s, 4H, CH_2), 5.19 (s, 2H, CH_2), 6.06 (d, 1H, J=2.0 Hz, 3'-H), 6.29 (d, 1H,*J*=2.0 Hz. 5'-H), 6.82 (d, 2H, *J*= 6.0 Hz, 3,5-H), 7.35-7.49 (m, 15H, Ar-H), 7.56 (d, 1H, *J*=16.0 Hz, α-H), 7.72 (d, 2H, *J*= 6.0 Hz, 2,6-H), 7.91 (d, 1H, *J*=16.0 Hz, β-H), 9.79 (s, 1H, 2-OH). **^{13}C NMR** (200 MHz, $CDCl_3$) δppm 71.2, 71.2, 71.2 (CH_2), 92.8 (C-5´), 93.3 (C-3'), 103.4 (C-1'), 113.1 (C-3,5), 122.1 (C- α), 127.03 (C-2,6), 127.42 (C-β), 127.5 (C-1') 127.72, 127.78 127.80, 127.88, 127.88, 127.9, 129.2, 129.2, 129.3, 129.9, 141.2 , 141.2, 141.3 (C-Ar), 156.6 (C-2'), 163.0 (C-6'), 169.2 (C-4'), 189.9 (C=O). HRMS: Calc. For $C_{36}H_{30}O_5$ = 542.6204. Found: = 542.6203

2′-Hydroxy-5′-benzyloxy-2, 5-dimethoxychalcone (16C)

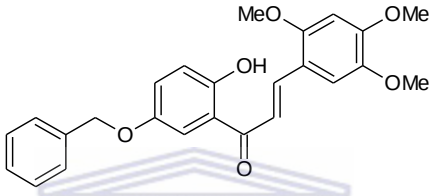


(16C)

2′-Hydroxy-5′-benzyloxy-2, 5-dimethoxychalcone **(16C)** was synthesized from 2′-hydroxy-5′-benzyloxyacetophenone (1.21g, 5 mmol) and 2, 5-dimethoxybenzaldehyde (0.91g, 5.5 mmol) according to the general procedure used for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:5) to afford **2'-Hydroxy-5'-benzyloxy-2,5-methoxychalcone (16C)** as yellowish orange thick

solid (1.64g , 4.2 mmol, 84.0 % yield). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 3.78 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 5.16 (s, 2H, CH_2), 6.85 (m, 4H, 3′,4′,3, 4-H),7.15 (m, 2H, 6′-H and 6-H), 7.35-7.46 (m, 5H, Ar-H), 7.69 (d, 1H, *J*=16.0 Hz, α-H), 8.11 (d, 1H, *J*=16.0 Hz, β-H), 9.42 (s, 1H, 2′-OH). **^{13}C NMR** (400 MHz, $CDCl_3$) δ ppm: 55.8(OCH_3), 55.8 (OCH_3),71.7 (CH_2),111.2 (C-6),113.6 (C-3), 114.6 (C-4),115.8 (C-6'), 116.0 (C-1') 117.9 (C-1), 119.2 (C-3'), 121.6 (C-4'),123.8 (C-α), 127.7, 127.7, 127.8, 129.5, 129.7 (Ar-C), 144.4 (C-β), 148.9 (C-2), 151.3 (C-2′),153.7 (C-5), 154.0 (C-5′),155.0 (C-2′), 187.6 (C=O)**.** HRMS: Calc. For $C_{24}H_{22}O_5$ = 390.4285. Found: = 390.4283

2′-Hydroxy-5′-benzyloxy-2, 4, 5-trimethoxychalcone (17C)

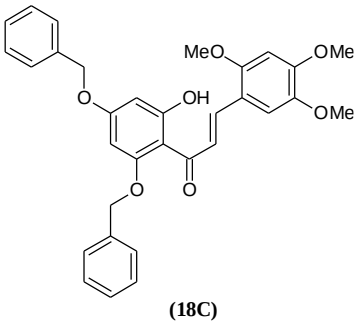


(17C)

2'-Hydroxy-5'-benzyloxy-2, 4, 5-trimethoxychalcone **(17C)** was synthesized from 2'-hydroxy-5'-benzyloxyacetophenone (1.21g, 5 mmol) and 2,4,5-trimethoxybenzaldehyde (0.98g, 5.5 mmol) according to the general procedure used for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:5) to afford **2'-hydroxy-5'-benzyloxy-2, 4, 5-trimethoxychalcone (17C)** as yellowish orange oil (1.78g , 4.2 mmol, 84.4% yield). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 3.74 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3),3.81 (s, 3H, OCH_3), 5.21 (s, 2H, CH_2), 6.18 (s, 1H, 3-H), 6.27 (s, 1H, 6-H),6.78 (d, 1H,*J*=2 Hz, 6'-H), 6.88 (d, 1H, *J*=6.0Hz, 3'-H), 7.15 (d, 1H, *J*=2.0 Hz, 6'-H), 7.24 (dd, 1H, *J* =6.0 and 2.0 Hz, 4'-H), 7.35-7.46 (m, 5H, Ar-H), 7.69 (d, 1H, *J*=16.0 Hz, α-H), 8.11 (d, 1H, *J*=16.0 Hz, β-H), 9.42 (s, 1H, 2'-OH). **^{13}C NMR** (200 MHz, $CDCl_3$)δ ppm: 54.8 (OCH_3), 54.8 (OCH_3),54.3 (OCH_3),71.6 (CH_2), 101.3 (C-3), 109.3 (C-1'), 112,3 (C-6), 113.2 (C-6'), 115.3 (C-3'), 118.9 (C-4'),120.1 (C-1), 123.8 (C-α), 127.7, 127.7, 127.8, 129.5, 129.7(Ar-C), 141.4 (C-1''), 142.4 (C-5), 145.4 (C-β), 149.9 (C-2), 150.3 (C-4), 153.9 (C-5'), 154.9 (C-2'), 197.6 (C=O)

HRMS: Calc. For $C_{24}H_{22}O_5$ = 420.4545. Found: = 420.4542

2'-Hydroxy-4', 6'-dibenzyloxy-2, 4, 5-trimethoxychalcone (18C)



(18C)

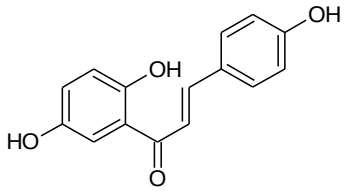
2'-Hydroxy-4',6'-dibenzyloxy-2,4,5-trimethoxychalcone **(18C)** was synthesized from 2'-hydroxy-4',6'-dibenzyloxyacetophenone (1.74g, 5 mmol) and 2,4,5-trimethoxybenzaldehyde (1.08g, 5.5 mmol) according to the general procedure used for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:5) to afford **2'-hydroxy-4',6'-dibenzyloxy-2,4,5-trimethoxychalcone (18C)** as yellowish orange solid (2.25g , 4.3 mmol, 85.0 % yield).

^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.54 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3),3.71 (s, 3H, OCH_3), 5.21 (s, 2H, CH_2), 5.23 (s, 2H, CH_2), 5.98 (d, 1H, *J*=2.0 Hz,3'-H), 6.25 (d, 1H, *J*=2.0 Hz,5'-H),6.45 (s, 1H, 3-H), 7.29-7.56 (m, 12H, 2xAr-H, α-H and 6-H), 8.26 (d, 1H, *J*=16.0 Hz, β-H), 9.62 (s, 1H, 2'-OH). **^{13}C NMR** (200 MHz, $CDCl_3$) 54.2 (OCH_3), 54.8 (OCH_3),54.9 (OCH_3),72.7, 72.7 (CH_2), 92.5 (C-5'), 95.4 (C-3'), 102.2 (C-3), 109.0 (C-1'), 112.1 (C-6), 113.5 (C-6'), 120.3 (C-1), 122.0 (C-α), 127.7, 127.7, 127.7, 127.8, 127.9, 127.9, 129.5, 129.5, 129.6, 129.6 (Ar-C), 141.4 (C-1''), 141.6 (C-1'''),145.8 (C-β), 147.9 (C-2), 151.9 (C-4), 151.9 (C-5), 153.3 (C-4'), 155.0 (C-2'), 189.8 (C=O). HRMS: Calc. For $C_{32}H_{30}O_7$ = 526.5764. Found: = 526.5761

4.2.4.2 General procedure for Pyran deprotection

The pyran, 5mmol, was dissolved in THF (25 mL) and then treated with concentrated HCl (3 drops) and stirred at 25^{o} C. The reaction was monitored by TLC and after all reactants were consumed, the reaction mixture was poured into water (100 mL) and extracted with ethyl acetate (EtOAc). The organic fraction was dried over anhydrous Na_2SO_4, and the solvent was removed in *vacuo*. The compound was purified by column chromatography on silica gel with petroleum ether: ethyl acetate (4:1).

2', 4, 5'-trihydroxychalcone (11C)



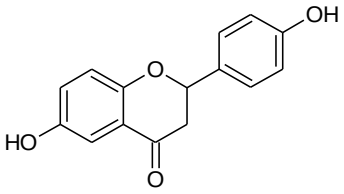
2', 4, 5'-trihydroxychalcone (11C) was synthesized from 2′-2'-Hydroxy-4, 5'–dihydropyran-chalcone (1.17g; 5 mmol) and 4-Benzyloxybenzaldehyde (1.03g; 5.5 mmol) according to the general procedure for chalcone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford 4, 5' –Dihydropyran-chalcone as an orange crystals (1.78g, 4.2 mmol, 83.9 % yield). Mp 215 °C (lit.[118]218 °C); IR cm^{-1} : 3460 (–OH), 1698 (C=O), 1616, 1523 (aromatic). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm = 6.60 (d, 2H, *J*=6.0 Hz, H-3 and H-5), 6.85(dd, 1H, *J* =6.0 and 2.0 Hz, H-4'), 7.12 (d, 1H, *J* = 2.0 Hz, H-3'), 7.27(d,2H, *J* = 6.0 Hz, H-2 and H-6) , 7.32 (s, 1H, H-6), 7.69 (d, 1H, *J*=16.0 Hz, α-H), 8.01 (d, 1H, *J* = 15.5 Hz, , β-H),11.34 (s, 1H, 4-OH and 5'-OH), 11.89 (s, 1H, 2'-OH). HRMS: Calc. For $C_{15}H_{12}O_4$ = 256.2534 Found: = 256.2537

4.2.5 Synthesis of Flavanones

4.2.5.1 General procedure for Flavanone synthesis

The 2′-hydroxychalcones were dissolved in EtOAc (10-20ml). To this solution 500mg of sodium acetate was added and the resulting mixture was stirred under reflux for 5 hours. The progression of the reaction was monitored by TLC. After all reactants were consumed, the reaction mixture was poured into water and extracted with ethyl acetate (EtOAc). The organic medium was washed with brine, dried over anhydrous Na_2SO_4, and the solvent was removed in *vacuo*. The resulting compound was purified by column chromatography on silica gel with petroleum ether: ethyl acetate (4:1) as eluent to afford the final product.

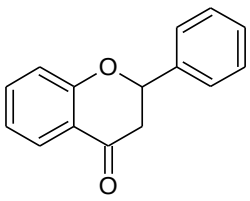
4', 6 –Dihydroxyflavanone (11F)



(11F)

4', 6-Dihydroxyflavanone **(11F)** was synthesized from 2', 4, 5'-trihydroxychalcone **(11C)** (1.0g, 3.9 mmol) according to the general procedure used for flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **4',6-dihydroxyflavanone (11F)** as yellow crystals (0.79g, 3.0 mmol, 79 % yield); Mp = 189 - 192 °C (Lit.[115] Mp = 191 °C); IR cm^{-1} :1679(C=O), 1617, 1540 (aromatic). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 3.17 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.39 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.55 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 6.60 (d, 2H, *J*=6.0 Hz, H-3' and H-5'), 6.85(dd, 1H, *J* =6.0 and 2.0 Hz, H-7), 7.12 (d, 1H, *J* = 2.0 Hz, H-8), 7.27(d,2H, *J* = 6.0 Hz, H-2' and H-6') , 7.32 (s, 1H, H-5), 11.34 (s, 1H, 4'-OH), 11.39 (s, 1H, 6'-OH). **^{13}C NMR** (200 MHz, $CDCl_3$) δ ppm: 42.0 (C-3), 79.0 (C-2), 115.3 (C-5), 115.6 (C-8), 116.9 (C-3', 5'), 120.9 (C-6), 120.4 (C-4a), 129.4 (C-5), 132.9 (C-7), 134.2 (C-1'), 146.7 (C-4'), 146.8 (C-5'), 156.8 (C-8a), 196.9 (C=O).
HRMS: Calc. For $C_{15}H_{12}O_4$ = 256.2534. Found: = 256.25

2-phenyl-2, 3-dihydro-4*H*-chromen-4-one (Flavanone) (12F)

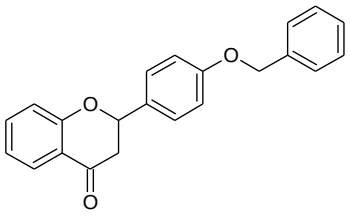


(12F)

2-phenyl-2, 3-dihydro-4*H*-chromen-4-one **(12F)** was synthesized from 2′-hydroxychalcone (1.12g, 5 mmol) according to the general procedure for flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **2-phenyl-2,3-dihydro-4*H*-chromen-4-one (12F)** as brownish white crystals (1.04g, 4.6 mmol, 92.8 % yield); Mp 76 °C (lit.[116] 77–78 °C); IRcm^{-1} : 1715 (C=O); **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 2.99 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.14 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.53 (dd, 1H, *J*=13.0, 3.1Hz, H–2a),

6.67 (d, 1H, *J* = 6.0 Hz, 8-H),6.81 (d, 1H, *J* =6.0 Hz, H-5), 6.95 (t, 1H, *J* = 6.0 Hz, 6-H), 7.23-7.39 (m, 5H, Aryl ring), 7.62 (t, 1H, *J*=6.0 Hz, H-7). **^{13}C NMR** (200 MHz, $CDCl_3$) δppm: 27.0 (C-3), 45.3 (C-2), 116.7, 119.4, 120.4, 120.5, 129.0, 129.4, 130.0, 131.3, 132.5, 135.0, 136.8 (C-Ar), 164.0 (C-8a), 194.4 (C=O).HRMS: Calc. For $C_{15}H_{12}O_2$ = 224.2546. Found: = 224.2545

4′-Benzyloxyflavanone (13D)

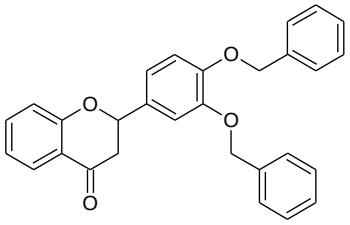


(13D)

4′-Benzyloxyflavanone **(13D)** was synthesized from 2-hydroxy-4′-benzyloxychalcone (1.0g, 3 mmol) according to the general procedure used for flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **4'-benzyloxyflavanone (13D)** [117] as yellow crystals (0.93g, 2.8 mmol, 93 % yield) **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 3.18 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.32 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.15 (s, 2H, CH_2), 5.43 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 6.78 (d, 2H, *J* = 6.0 Hz, 3'-,5'-H), 6.88 (d, 1H, *J* = 6.0 Hz, 8-H), 6.95 (t, 1H, *J* = 6.0 Hz , 6-H), 7.10 (d, 2H, *J* = 6.0 Hz, 2H, 2'-,6'-H), 7.36-7.54 (m, 7H, Aryl-H and H-5 and H-7). **^{13}C NMR** (200 MHz, $CDCl_3$) δ ppm: 42.57 (C-3), 68.31 (CH_2), 78.04 (C-2), 114.2 (C-8), 115.7 (C-3',5'), 120.2 (C-6), 120.5 (C-4a), 127.3 (C-2", C-6"), 127.7 (C-4'), 128.3 (C-2', C-6'), 129.3 (C-3", C5"), 129.7(C-5), 132.5 (C-7), 132.9 (C-1'), 142.0 (C-1"), 156.8 (C-8a), 159.9 (C-4'), 196.5 (C=O)

HRMS: Calc. For $C_{22}H_{18}O_3$ = 332.3906. Found: = 332.3904

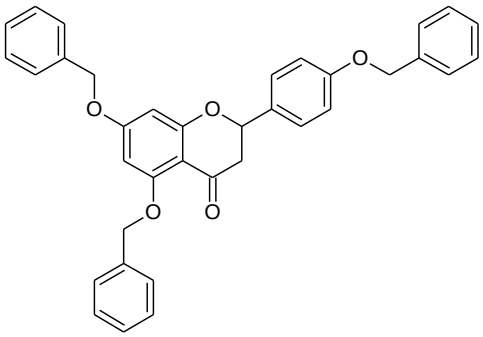
3′, 4′-Dibenzyloxyflavanone (14D)



(14D)

3′, 4′–dibenzyloxyflavanone **(14D)** was synthesized from 2'-Hydroxy-4, 5-dibenzyloxychalcone **(14C)** (2.18g, 5 mmol) according to the general procedure used for flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **3′,4′-dibenzyloxyflavanone (14D)** as yellow thick solid (2.01g, 4.6 mmol, 92 % yield) **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 3.22 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.39 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.15 (s, 4H, 2xCH_2), 5.43 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 6.54 (d, 1H, *J*=2.0 Hz, 2'-H), 6.69 (d, 1H, *J* = 6.0 Hz, 5'-H), 6.90 (dd, 1H, *J* = 6.0 and 2.0 Hz, 6′-H), 6.95 (m, 2H, 6- and8-H), 7.32–7.55 (m, 12-H, Ar). **^{13}C NMR** (200 MHz, $CDCl_3$) δ ppm: 42.07 (C-3), 68.31 (CH_2), 68.3 (CH_2), 78.46 (C-2), 111.78 (C-5'), 114.3 (C-8), 116.7 (C-3'), 120.2 (C-2'), 120.5 (C-4a, C-6), 127.2 (C-2", C-6", C-2'", C-6'"), 127.9 (C-4", C-4'"), 129.3 (C-5", C5'"), 129.9(C-5), 133.8 (C-7), 134.3 (C-1'), 141.0 (C-1", C-1'"), 149.7 (C-4') 150.4 (C-5'), 156.9 (C-8a), 198.29 (C=O). HRMS: Calc. For $C_{29}H_{24}O_4$ = 436.4985. Found: = 436.4984

4′, 5, 7- Tribenzyloxyflavanone (15D)

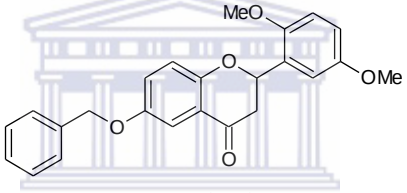


(15D)

4′, 5, 7- Tribenzyloxyflavanone **(15D)** was synthesized from 2'-hydroxy- 4, 4', 6-tribenzyloxychalcone **(15C)** (2.0g, 3.7 mmol) according to the general procedure used for

flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **4′, 5, 7- tribenzyloxyflavanone (15D)** as yellowish brown solid (1.72g, 3.2 mmol, 86 % yield) .**^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm 3.18 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.32 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.12 (s, 4H, 2xCH_2), 5.23 (s, 2H, CH_2),5.43 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 6.14 (s, 2H, 6- and8-H), 6.82 (d, 2H, *J* = 6.0 Hz, 3'-, 5'-H), 7.13 (d, 2H, *J* = 6.0 Hz, 2'-,6'-H), 7.32-7.49 (m, 15-H, Ar). **^{13}C NMR** (200 MHz, $CDCl_3$) δ ppm: 42.6 (C-3), 68.9 (CH_2), 69.2 (CH_2), 69.5 (CH_2), 70.2 (C-2), 92.1 (C-6), 92.8 (C-8),100.9 (C-4a), 114.7 (C-3',5'), 127.1, 127.2 127.2, 127.4, 128.3, 128.38, 128.4, 129.2, 129.2, 129.3, 129.9, 130.1, 130.2, 130.2, 133.0 141.2, 141.3, 141.3 (C-Ar), 157.2 (C-8a), 159.3 (C-4′), 162.3 (C-5), 165.3 (C-7), 197.2 (C=O). HRMS: Calc. For $C_{36}H_{30}O_5$ = 542.6204. Found: = 542.6201

6-Benzyloxy-2′, 5′-dimethoxyflavanone (16D)



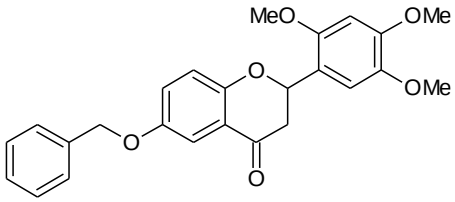
(16D)

6-Benzyloxy-2', 5'-dimethoxyflavanone **(16D)** was synthesized from 2'-hydroxy-6'-benzyloxy-2, 5-methoxychalcone **(16C)** (1.5g, 3.8 mmol) according to the general procedure used for flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **6-benzyloxy-2', 5'-dimethoxyflavanone (16D)** as yellowish brown solid (1.29g, 3.3 mmol, 86 % yield).

^{1}H NMR (400 MHz, $CDCl_3$)δ ppm 3.18 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.33 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 3.69 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2), 5.21 (.dd, *J*=13.0 and 3.4 Hz, H-2a), 6.40 (m, 3H, 3', 4', 6'-H), 6.76 (dd, 1H, *J*=6.0 and 2.0 Hz, H-7), 7.10 (d, 1H, *J*=6.0 Hz H-8), 7.29 (d, 1H, *J*=2.0 Hz, H-5), 7.36 – 7.58 (m, 5H, Ar-H). ^{13}C NMR (200 MHz, $CDCl_3$δ ppm: 43.1 (C-3), 56.2 (CH_3), 57.2 (CH_3), 70.4 (CH_2), 72.3 (C-2), 112.1 (C-6'), 113.6 (C-5), 115.3 (C-8), 116.1 (C-3'), 119.7 (C-7), 122.6 (C-4a), 127.4, 127.6, 127.8, 127.3, 141.8(C-Ar), 141.2 (C-1''), 149.1 (C-8a),149.7 (C-2'), 152.9(C-6),153.2(C-5'),196.9 (C=O).

HRMS: Calc. For $C_{24}H_{12}O_5$ = 390.4285. Found: = 390.4283

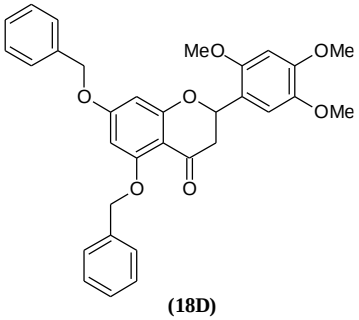
6-Benzyloxy-2′, 4′, 5′-trimethoxyflavanone (17D)



(17D)

6-Benzyloxy-2', 4', 5'-trimethoxyflavanone **(17D)** was synthesized from 2'-hydroxy-6'-benzyloxy-2, 4, 5-methoxychalcone **(17C)** (1.5g, 3.6 mmol) according to the general procedure used for flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **6-benzyloxy-2', 4', 5'-trimethoxyflavanone (17D)** as yellowish-green thick solid (1.42g, 3.4 mmol, 94.6 % yield) **^{1}H NMR** (200 MHz, $CDCl_3$) δppm 3.13 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.38 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 3.69 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 5.13(s, 2H, CH_2), 5.17 (dd, 1H, *J*=13.0 and 3.4 Hz, H-2a)6.10 (s, 1H, 3'-H), 6.52 (s, 1H, 6'-H),6.85 (dd, 1H, *J*=6.1 and 2.0 Hz, H-7), 7.10 (d, 1H, *J*=6.1, 8-H), 7.30 (d, 1H, *J*=2.0 Hz, H-5) and7.36 – 7.58 (m, 5H, Ar-H). **^{13}C NMR** (200 MHz, $CDCl_3$)δ ppm: 43.0 (C-3), 56.1 (CH_3), 57.2 (CH_3), 57.2 (CH_3), 70.9 (CH_2), 73.5 (C-2), 101.8 (C-3'), 112.4 (C-6'), 113.3(C-5), 115.3 (C-8), 119.7 (C-7), 120.1 (C-1'), 122.6 (C-4a), 126.6, 126.7, 126.9, 127.2, 142.7(C-Ar), 141.6 (C-1''), 142.7 (C-5'), 149.1 (C-8a),149.7 (C-4'), 150.3 (C-2'), 153.9(C-6), 198.2 (C=O).HRMS: Calc. For $C_{25}H_{24}O_6$ = 420.4545. Found: = 420.4542

5, 7- Dibenzyloxy-2′, 4′, 5′-trimethoxyflavanone (18D)



(18D)

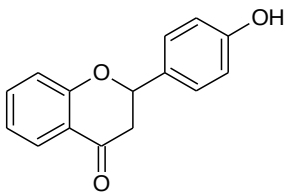
5, 7-dibenzyloxy-2', 4', 5'-trimethoxyflavanone **(18D)** was synthesized from 2'-hydroxy-5', 7'-benzyloxy-2, 4, 5-trimethoxychalcone **(18C)** (1.5g, 2.8 mmol) according to the general

procedure used for flavanone synthesis. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **5, 7-dibenzyloxy-2′, 4′, 5′-trimethoxyflavanone (18D)** as yellowish-green thick solid (1.45g, 2.75 mmol, 96.7 % yield) **^{1}H NMR** (200 MHz, $CDCl_3$) δppm 3.14 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.37 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 3.58 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 5.12 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 5.48 (dd, 1H, *J*=13.0 and 3.4 Hz, H-2a), 6.13 (s, 1H, H-3'), 6.22 (s, 1H, H-6'), 6.35 (d, 1H, *J*=2.0 Hz, H-8) , 6.39 (d, 1H,*J*=2.0 Hz.H-7), , 7.26 – 7.52 (m, 10H, Ar-H). **^{13}C NMR** (200 MHz, $CDCl_3$)δ ppm: 42.4 (C-3), 57.0 (CH_3), 57.2 (CH_3), 57.2 (CH_3), 69.9(CH_2), 67.1 (CH_2), 73.2 (C-2), 91.8 (C-6), 92.8 (C-8), 101.3 (C-4a), 102.1 (C-3'), 113.4 (C-6'), 121.7 (C-1'), 126.6, 126.7, 126.9, 126.9, 128.6, 128.7, 128.7, 129.0, 142.7, 142.8 (C-Ar), 142.9 (C-5'), 148.9 (C-4'), 151.3(C-2'), 159.2 (C-8a), 161.9(C-5), 197.2 (C=O). HRMS: Calc. For $C_{32}H_{30}O_7$ = 526.5764. Found: = 526.5762

4.2.5.2 General procedure for Debenzylation.

Cleavage of the benzyl ethers was performed by catalytic hydrogenation over Pd/C. The protected chalcone/flavanoid (1 gm) was dissolved in ethyl acetate (10ml) and to this 10% Pd/C (20 mg) was added. The apparatus was opened and flushed with hydrogen. Two vacuum/H_2 cycles to replace the air inside with hydrogen was completed. The mixture was vigorously stirred at room temperature under hydrogen pressure for 24 h. The progression of the reaction was monitored by TLC and when all reactants were consumed the reaction mixture was filtered using a membrane filter (Millipore, Millex_-LH, 0.45 mm) and the filtrate was concentrated to provide the crude product.

4′-Hydroxyflavanone (13F)

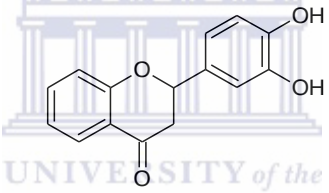


(13F)

4'-Hydroxyflavanone **(13F)** was synthesized from 4´-benzyloxyflavanone **(13D)** (1.0g, 3mmol) according to the general procedure used for benzyl de-protection. The

residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **4′-hydroxyflavanone (13F) [119]**as yellowish white crystals(0.69g, 2.9 mmol, 95.8 % yield). Mp 190 °C (lit.[119] 192-193 °C). IR cm^{-1} : 3459 (–OH), 1699 (C=O), **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm: 3.18 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.32 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.53 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 6.68 (d, 2H, *J* = 6.0 Hz, H-3'and H-5'), 6.85 (d, 1H, *J* = 6.0 Hz, H-8), 6.95 (t, 1H, *J* = 6.2 Hz, H-6), 7.10 (d, 2H, *J* = 6.2 Hz, H-2' and H-6'), 7.41-7.54 (m, 2H, H-5 and H-7). **^{13}C NMR** (200 MHz, $CDCl_3$) δ ppm: 42.55 (C-3), 78.94 (C-2), 115.3 (C-8), 115.72 (C-3',5'), 120.38 (C-6), 120.54 (C-4a), 128.35 (C-2', C-6'), 129.76 (C-5), 132.5 (C-1'), 132.91 (C-7), 156.74 (C-8a), 157.76 (C-4'), 196.89 (C=O). HRMS: Calc. For $C_{15}H_{12}O_3$ = 240.2540. Found: = 240.2543

3', 4’ -Dihydroxyflavanone (14F)

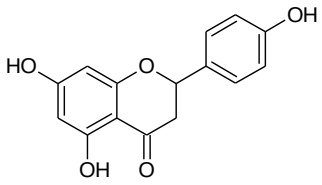


(14F)

3', 4’- dihydroxyflavanone **(14F)** was synthesized from 3', 4’-di-benzyloxyflavanone **(14D)** (1.5g, 3. 4 mmol) according to the general procedure used for benzyl de-protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford 3', **4’-dihydroxyflavanone (14F)** as yellowish-brown crystals (0.80g, 3.1 mmol, 90.9 % yield).Mp 177 °C (lit. [120] 175 - 177 °C). IR cm^{-1} : 3347 (–OH), 1712 (C=O), 1589, 1549 (aromatic). **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm 3.15 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.41 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.59 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 6.44 (d, 1H, *J*=2.0Hz, H-2'), 6.49 (d, 1H,*J* = 6.0 Hz, H-4'), 6.58 (dd, 1H, *J* = 6.0 and 2.0 Hz, H-6'), 6.85 (d, 1H, *J* = 7.0 Hz, H-8), 6.98 (t, 1H, *J* = 7.0 H-6), 7.47 (d, 1H, *J* = 7.0 Hz, 5-H), (t, 1H, *J* = 7.0Hz, H-7), 11.34 (s, 1H, OH), 11.39 (s, 1H, OH).**^{13}C NMR** (200 MHz, $CDCl_3$) δ ppm: 42.8 (C-3), 79.1 (C-2), 114.2 (C-2' and C-6'), 115.7 (C-8), 120.4 (C-6), 120.4 (C-4a), 127.8 (C-3'), 129.4 (C-5), 132.9 (C-7), 134.2 (C-1'), 146.7 (C-4'), 146.8 (C-5'), 156.8 (C-8a), 196.9 (C=O)

HRMS: Calc. For $C_{15}H_{12}O_4$ = 256.2534. Found: = 256.2530

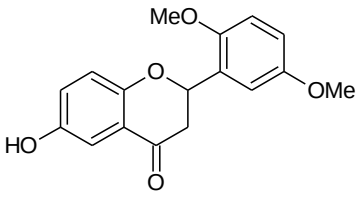
4', 5, 7 Trihydroxyflavanone (15F)



(15F)

4', 5, 7 Trihydroxyflavanone (15F) was synthesized from 4', 5, 7-tribenzyloxyflavanone (1.5 g, 2.8 mmol) according to the general procedure used for benzyl de-protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **4', 5, 7-trihydroxyflavanone (15F)** as yellow crystals (0.69g, 2.5 mmol, 92 % yield). Mp 247 °C (lit. [121] 247 - 249 °C). IR cm^{-1} : 3446 (–OH), 1698 (C=O), 1616, 1540 (aromatic), **^{1}H NMR** (200 MHz, $CDCl_3$) δ ppm 3.13 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.29 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 5.42 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 5.76 (s,1H, 6-H), 5.89 (s,1H, H-8)6.72 (d, 2H, *J* = 6.3 Hz, H-3' and H-5'), 7.13 (d, 2H, *J* = 6.3 Hz, H-2'- and H-6'), 9.79 (s, 1H, 4'-OH),11.34 (s, 1H, 7-OH) and 11.39 (s, 1H, 5-OH). **^{13}C NMR** (200 MHz, $CDCl_3$) δppm 43.7 (C-3), 70.2 (C-2), 94.1 (C-8), 95.3 (C-6), 101.9 (C-4a),116.0 (C-3',5'), 128.1 (C-2',6'), 132.9 (C-1'), 157.2 (C-4'), 159.3 (C-8a), 162.8 (C-5), 164.2 (C-7), 196.8 (C=O).HRMS: Calc. For $C_{15}H_{12}O_5$ = 272.2528. Found: = 272.2525

6-Hydroxy-2′, 5′-dimethoxyflavanone (16F)

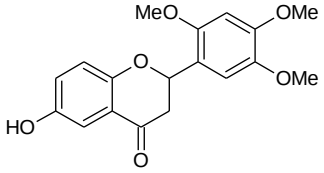


(16F)

6-Hydroxy-2', 5'-dimethoxyflavanone **(16F)** was synthesized from 6-benzyloxy-2', 5'-dimethoxyflavanone (1.0g, 2.56 mmol) according to the general procedure used for benzyl de-protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **6-hydroxy-2', 5'-dimethoxyflavanone (16F)** as red-brown thick solid (0.72g, 2.4 mmol, 94 % yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 2.88 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 3.00 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 5.78 (dd, 1H,

J=13.0, 3.1Hz, H–2a), 6.72 (s, 1H, 6-OH), 6.87 (m, 2H, H-3' and H-4'), 6.98 (d, 1H, *J*=6.8 Hz, H-8), 7.12 (dd, 1H,*J*=6.8, 2.0 Hz, H-7), 7.24(s, 1H, H-2'), 7.42 (d, 1H, *J*=2.0 Hz, H-5). **^{13}C NMR** (400 MHz, $CDCl_3$) δppm 43.7 (C-3), 55.8 (2′-OCH_3), 56.0 (5'-OCH_3), 74.7 (C-2), 111.1 (C-5), 111.6 (C-3'), 112.6 (C-4'), 113.7 (C-8), 119.5 (C-4a), 124.9 (C-7), 128.6 (C-1'), 149.9 (C-5'), 150.1 (C-6'), 150.4 (C-2'), 153.8 (C-6), 156.4 (C-8a), 193.4 (C=O). HRMS: Calc. For $C_{17}H_{16}O_5$ = 300.3059. Found: = 300.3056

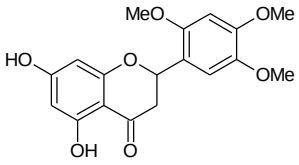
6-Hydroxy-2', 4', 5'-trimethoxyflavanone (17F)



(17F)

6-Hydroxy-2', 4', 5'-trimethoxyflavanone **(17F)** was synthesized from 6-benzyloxy-2', 4', 5'-trimethoxyflavanone (1.0g, 2.4 mmol) according to the general procedure used for benzyl de-protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **6-hydroxy-2', 4', 5'-trimethoxyflavanone (17F)** as yellow crystals (0.70g, 2.1 mmol, 89.1 % yield).Mp 175 °C (lit. [122] 175 - 177 °C). IR cm^{-1} : 3458 (–OH), 1698 (C=O), 1616, 1540 (aromatic). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm:3.00 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.23 (dd, 1H, *J*=16.3, 3.4Hz, H–3a),3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.70 (dd, 1H, *J*=13.0 and 3.4 Hz, H-2a), 6.54 (s, 1H, H-3'), 6.77 (s, 1H, H-6') , 6.88 (d, 1H, *J*=6.0Hz, H-8), 7.26 (dd, 1H, *J*=6.0 and 2.0 Hz, H-7), 7.06 (d, 1H,*J*=2.0 Hz.H-5) 7.63 (s, 1H, 6-OH). **^{13}C NMR** (200 MHz, $CDCl_3$) δppm 26.97 (C-3), 45.3 (C-2), 56.5 (OCH_3), 57.3 (OCH_3), 58.1 (OCH_3), 100.2 (C-3'), 112.5 (C-7, C-6'), 118.2 (C-1'), 119.5 (C-'), 122.3 (C-8), 126.4 (C-4'), 143.2 (C-5), 150.9 (C-4'), 151.8 (C-6), 155.8 (C-5'), 159.0 (C-2'), 201.4 (C=O). HRMS: Calc. For $C_{18}H_{18}O_6$ = 330.3319. Found: = 330.3317

5, 7-Dihydroxy-2', 4', 5'-trimethoxyflavanone (18F)

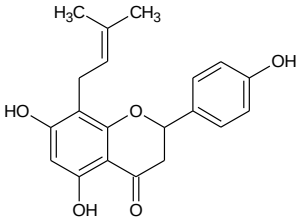


(18F)

5, 7-Dihydroxy-2', 4', 5'-trimethoxyflavanone **(18F)** was synthesized from 5, 7-dibenzyloxy-2′, 4′, 5′-dimethoxyflavonone (1.0g, 1.9 mmol) according to the general procedure used for benzyl de-protection. The residue obtained upon work-up was chromatographed using ethyl acetate/ hexane (1:4) to afford **5, 7-dihydroxy-2', 4', 5'-trimethoxyflavanone (18F)** as reddish-brown thick solid (0.62g, 1.79 mmol, 94 % yield). IR cm^{-1} : 3468 (–OH), 1668 (C=O). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 2.83 (dd, 1H, *J*=16.3, 13.0Hz, H–3e), 3.00 (dd, 1H, *J*=16.3, 3.4Hz, H–3a), 3.84 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 5.76 (dd, 1H, *J*=13.0, 3.1Hz, H–2a), 6.03 (s, 2H, H-6 and H-8), 6.57 (s, 1H, H-3'), 7.10 (s, 1H, H-6'), 9.43 (s, 1H, 7-OH), 12.15 (s, 1H, 5-OH). **^{13}C NMR** (400 MHz, $CDCl_3$) δppm: 42.4 (C-3), 55.9 (OCH_3), 56.09 (OCH_3), 56.4 (OCH_3), 75.4 (C-2), 95.9 (C-8), 96.9 (C-6), 102.6 (C-3'), 103.1 (C-4a), 118.8 (C-6'), 119.6 (C-1'), 144.2 (C-5'), 151.0 (C-4'), 151.8 (C-2'), 165.5 (C-5), 165.8 (C-8a) 167.7 (C-7) 197.8 (C=O). HRMS: Calc. For $C_{18}H_{18}O_7$ = 346.3313. Found: = 346.3312

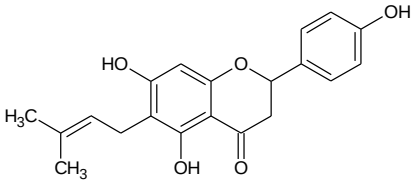
4.2.6 Synthesis of Prenylflavanones

8-C-Prenylnaringenin (19F)



(19F)

6-C Prenylnaringenin (20F)



(20F)

To a stirred solution of naringenin (1.1g, 4mmol) in dioxan (8ml) was added gradually BF_3-etherate (0.6ml, 4.8mmol) at room temperature. To this solution, 2-methyl-but-3-en-2-ol (0.8ml, 7.6mmol) was added and the resulting solution was stirred at room temperature overnight. The residue obtained upon work-up was chromatographed using EtOAc: hexane (1:3) as eluent to afford three fractions.

Fraction A: yellow thick solid (0.38g, 28 % yield) and was characterized as **8-C-prenylnaringenin**. **^{1}H NMR** (200 MHz, $CDCl_3$): 1.76 , 1.82 (2xs, 3H each , Me_2C=), 2.77 (dd, 1H, *J*=16.0 and 4.0Hz, , H-3a), 3.08 (dd, 1H, *J*=16.0 and 12.0Hz, H-3e) , 3.35 (d, 2H, *J*=8.0Hz, H-1"), 5.25-5.37 (m, 2H, H-2 and H-2"), 5.99 (s, 1H, H-6), 6.88 (d, 2H, *J*=8.4Hz, H-3' and H-5'), 7.33 (d, 2H, *J*=8.4Hz, H-2' and H-6') and 12.40 (s, 1H, chelated 5-OH). HRMS: Calc. For $C_{20}H_{20}O_5$ = 340.3698. Found: = 340.36.95

Fraction B: yellow thick solid (0.44g, 32 % yield) and was characterized as **6-C-prenylnaringenin**. **^{1}H NMR** (200 MHz, $CDCl_3$): 1.67, 1.72 (2xs, 3H each , Me_2C=), 2.89 (m, 2H, H-3a and H-3e) , 3.35 (d, 2H, *J*=8.0Hz, H-1"), 5.20-5.39 (m, 2H, H-2 and H-2"), 6.02 (s, 1H, H-8), 6.88 (d, 2H, *J*=8.4Hz, H-3' and H-5'), 7.32 (d, 2H, *J*=8.4Hz, H-2' and H-6') and 12.00 (s, 1H, chelated OH).HRMS: Calc. For $C_{20}H_{20}O_5$ = 340.3698, Found: = 340.36.95

Fraction C upon crystallization afforded the starting material, Naringenin (0.51g, 37 % yield)

4.3 Electrochemistry

4.3.1 Preparation of solutions

Phosphate buffer solution was prepared by mixing appropriate volumes of 0.1Msodium dihydrogen phosphate (NaH_2PO_4) and 0.1M sodium hydrogen phosphate (Na_2HPO_4) with ultra-pure water with a conductivity of 5.5 X 10-6 S.m-1 to produce the desired pH. Ethanol was selected as the most suitable solvent, all chalcones and Flavanones were dissolved in absolute ethanol to produce various concentration ranges between 0.049 and 0.22 M.

4.3.2 Voltammetric measurements

For electrochemical measurements, a three electrode cell consisting of a 3 mm diameter glassy carbon working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (3 M NaCl) reference electrode were used. The surface of the glassy carbon electrode was freshly polished with 1.0 um, 0.05 μm and 0.3 μm alumina powder on a micro cloth pad, rinsed with distilled water and degreased with ethanol in an ultrasonic bath because of possible film formation and memory effects [123]. The GCE was also electrochemically cleaned with 1M H_2SO4 and rinsed with distilled water. After electrochemical cleaning of the GCE, the required volume of sample was added by micro pipette into supporting electrolyte. The potentials were recorded against the silver-silver chloride electrode as a reference electrode used in conjunction with the platinum electrode placed in a 50 ml volume glass cell together with the glassy carbon electrode. Phosphate buffer (5ml) of pH ~ 7 was used as the supporting electrolyte. The scan was taken in a potential range of – 1500 mV and + 1500 mV with the following scan rates 20, 50 and 100 mV/s for cyclic voltammetric measurement and the scan was taken in the same potential window with a frequency of 15 hertz for square wave measurements. Prior to each measurement, the background currents were measured in phosphate buffer alone and subtracted from the currents measured with compound added in phosphate buffer. In order to minimize adsorption onto the electrode surface, measurements were performed immediately after the addition of the compound. The sample in the electrochemical cell was de-aerated by purging with high purity argon during electrochemical measurements. All experiments were carried out at room temperature. For square wave measurements, determination of the flavonoids was carried out at a frequency of 15 Hz, step potential 20 mV/s with phosphate

buffer of pH 7 as supporting electrolyte. For smoothing and baseline correction the software Origin 7 was employed.

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CHAPTER 5

5. ELECTROCHEMISTRY

5.1 Introduction

Cyclic Voltammetry and square wave voltammetry were used to investigate flavonoid characteristics by the peak potential, number and position of peaks at those potentials, the current heights at each peak potential for all flavanoids. Square wave voltammetry, as expected, proved to more sensitive and therefore the peak potentials in its voltammograms were used to determine the oxidation peak potentials. According to literature, the first anodic peak will provide us with the most useful information about the compound's antioxidant activity, it is suggested that the lower the oxidation potential of the first anodic peak the greater the ability of a compound to donate an electron and hence behave as an antioxidant [124, 125]. This was rationalized on the basis that both electrochemical oxidation and hydrogen donating free radical-scavenging involve the breaking of the same phenolic bonds between oxygen and hydrogen, producing the phenoxyl radical and H', which consists of an electron and an H ÷ ion [126]. This will be the main focus, and the antioxidant activity of all flavanoids was determined by comparing the oxidation potential of this first anodic peak.

The antioxidant activity of flavonoids and their metabolites *in vitro* depends upon the arrangement of functional groups about the nuclear structure. In this study we have investigated a number of structural variants namely: a) the number and position of hydroxyl groups attached to the flavanoid backbone; b) the importance of the α, β double bond functionality; c) the effect of ring closure; d) the effects of methoxy groups introduced to the flavanoid backbone; and finally e) the effect of prenyl groups introduced to the flavanoid backbone.

Compound 12F: Flavanone (2-phenyl-2, 3-dihydro-4*H*-chromen-4-one)

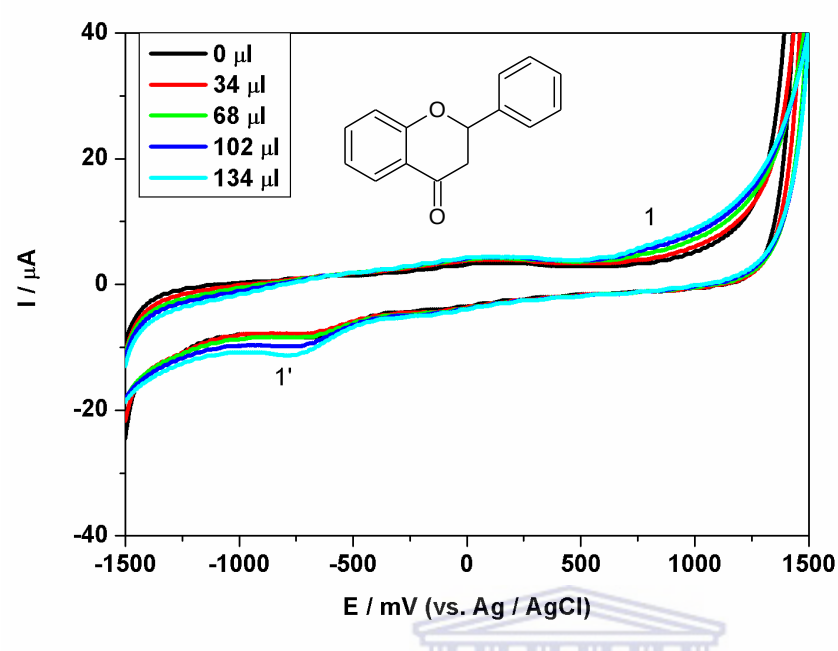


Figure 5.1: Cyclic voltammogram of compound **12F**, scan rate 100 mV/s.

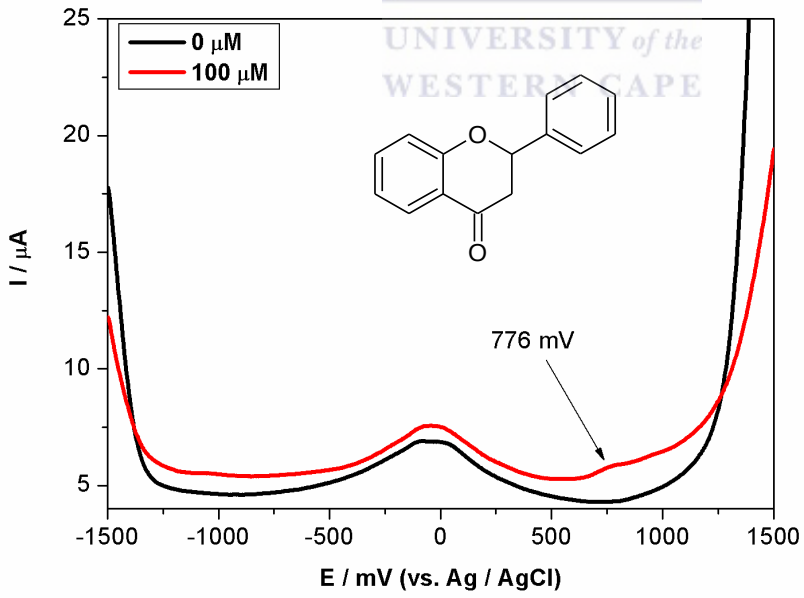


Figure 5.2: Square wave voltammogram of compound **12F**, scan rate 20 mV/s.

Figure 5.1 shows the cyclic voltammogram of compound **12F**, recorded when the potential sweep was started from -1500 mV to +1500 mV and reversed at a scan rate of 100mV/s. The corresponding reduction peak points to reversibility. The square wave voltammogram of compound **12F (Figure 5.2)** shows two sets of peaks; one at 24.4 mV and the other at 776 mV. The first peak at 24.4 mV was observed in the blank run (without addition of sample) as well and was consequently ignored. This initial peak was consistently observed throughout the study, and its peak potential was ignored unless there was clear evidence of oxidation occurring in that specific potential range. The second peak, as shown, is not that clearly defined. However it was validated by performing a number of scans using the specific concentration of 100 µM to observe its repeatability.

Compound 13F: 4'-Hydroxyflavanone

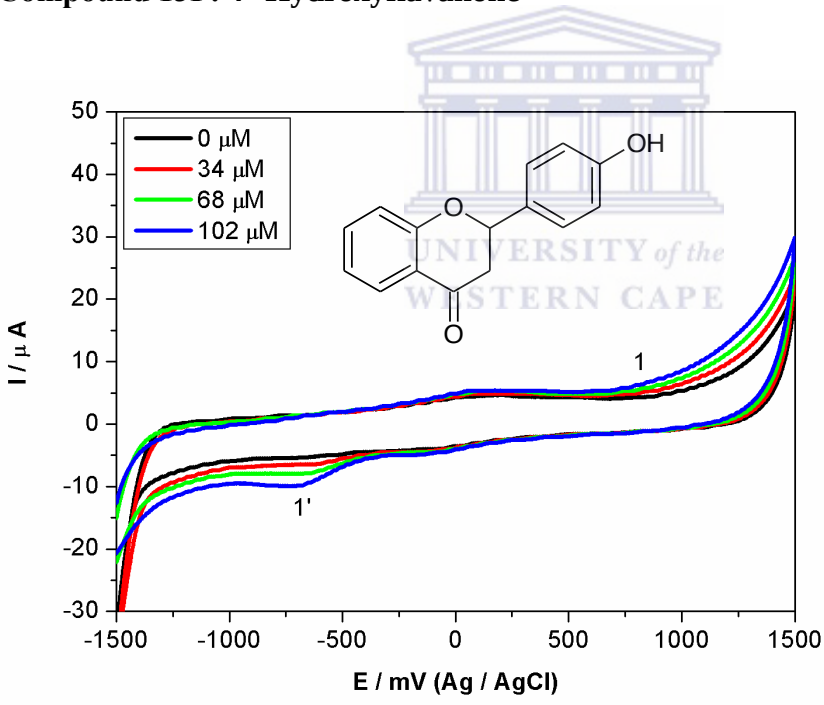


Figure 5.3: Cyclic voltammogram of compound **13F**, scan rate 100 mV/s.

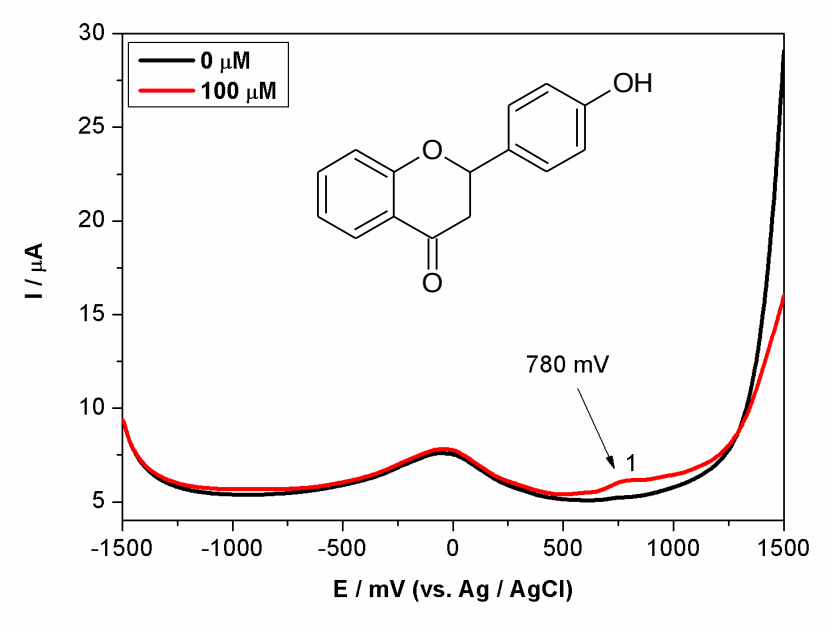


Figure 5.4: Square wave voltammogram of compound 13F, scan rate 20 mV/s.

Figure 5.3 shows the cyclic votammogram of **4'-Hydroxyflavanone,** recorded when the potential sweep began from 1500 mV to + 1500 mV. A distinct peak at 780 mV was observed. The square wave voltammograms between **12F** and **13F** showed a very close similarity and consequently it would appear that the addition of a hydroxyl group at position 4' has had little or no effect to the oxidation potential of the favanoidal system.

Compound 14F: 3', 4'-dihdroxyflavanone

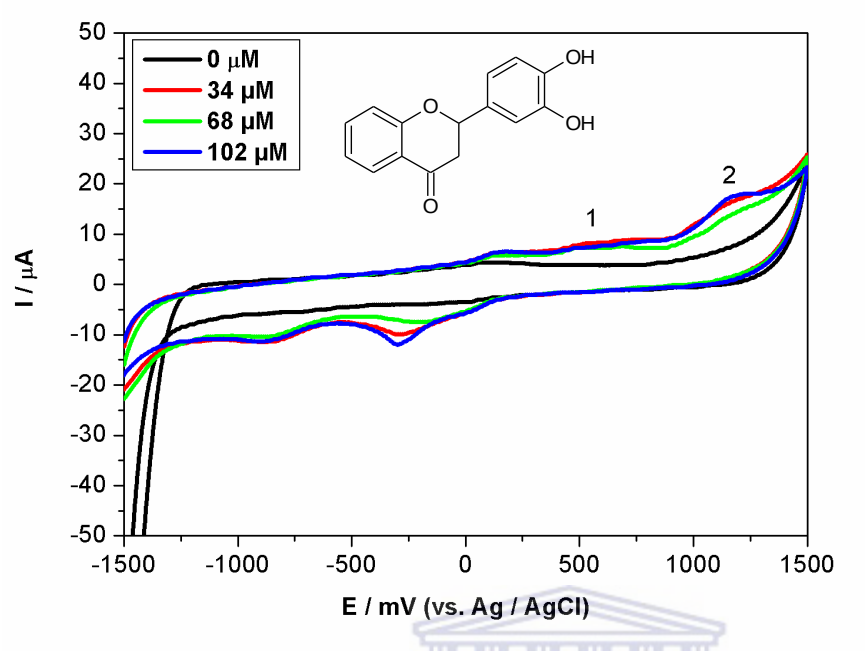


Figure 5.5: Cyclic voltammogram of compound 14F, scan rate 100 mV/s.

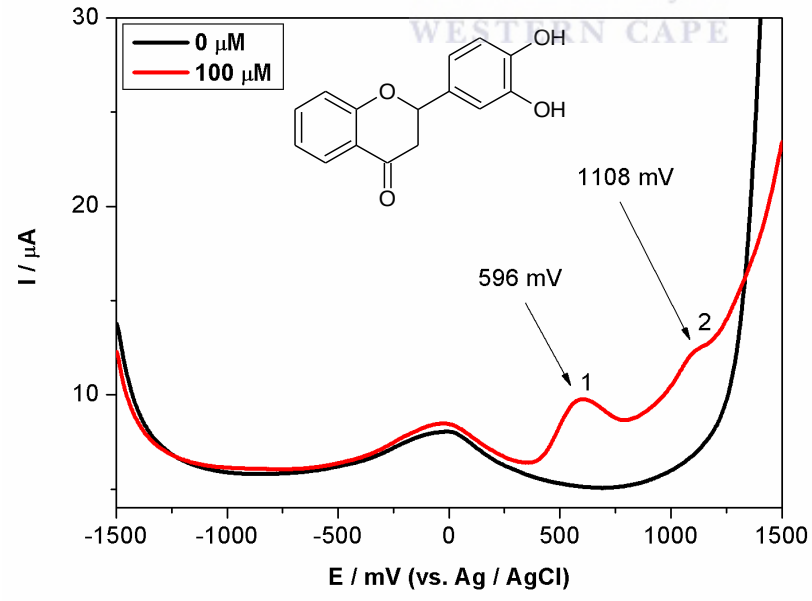
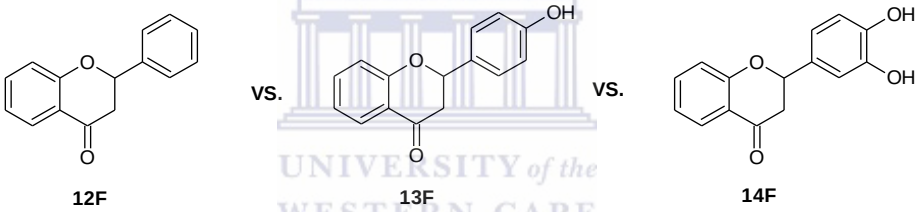


Figure 5.6: Square wave voltammogram of compound 14F, scan rate 20 mV/s.

The cyclic voltammogram **(Figure 5.5)** shows oxidation of the ortho-dihydroxy-phenol groups which results in the formation of a semi-stabile ortho-quinone, which becomes reduced in the reversed scan, appearing as a cathodic peak. The square wave voltammogram of compound **14F (Figure 5.6)** shows a defined peak at 596 mV, and a secondary peak at 1108 mV, which indicates secondary oxidation.

5.2 Comparative Assessment

The B-ring hydroxyl configuration does appear to be a significant factor determining the radical scavenging ability of flavonoids. Hydroxyl groups on the B-ring in general are able to donate an electron to form either hydroxyl, peroxyl or peroxy nitrite radicals, and stabilizing them, giving rise to a relatively stable flavonoid radical system [127]. By comparing the square wave voltammograms of compounds **12F, 13F and 14F** one is able to obtain a greater understanding of the structure-reactivity relationship, namely, what effect the addition of hydroxyl groups on the B-ring has on the oxidation potential.



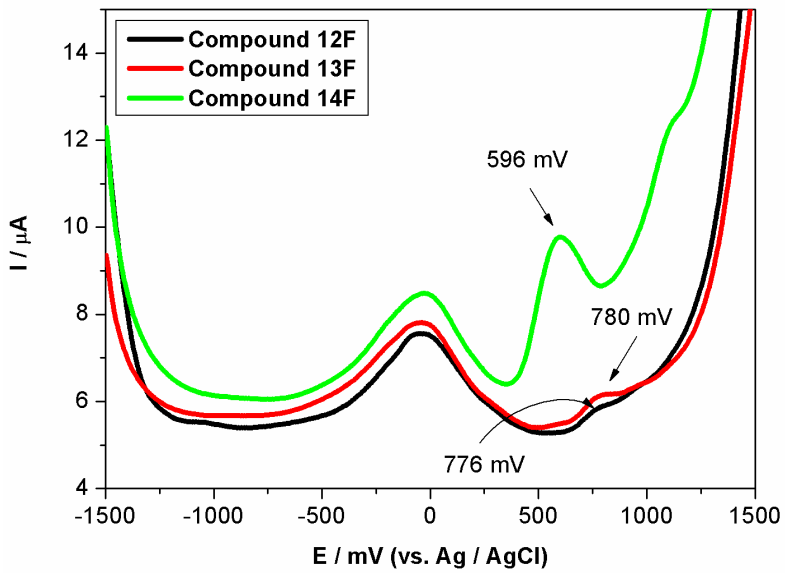
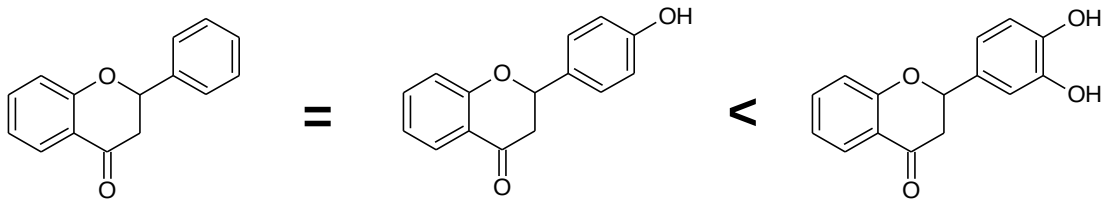


Figure 5.7: Superimposition of the square wave voltammograms of compounds 12F, 13F and 14F

By comparing the oxidation potential of compound **12F** (776 mV) and compound **13F** (780 mV) it is clear that the addition of a hydroxyl group at position 4' on the B-ring had very little effect on the oxidation potential. As the oxidation potential is directly related to antioxidant activity we can assume that the two compounds would perform fairly similarly *in vitro*. In compound **14F** on the other hand, with an additional hydroxyl group at position 5' compared to **13F**, a much lower oxidation potential (596 mV) for the first anodic peak was observed, which again according to literature, would correspond with a higher antioxidant activity [124, 125]. This result was expected as it has been suggested in the literature that the higher the number of hydroxyl groups on the B ring, the more enhanced the flavonoid activity would be [128]. The square wave voltammogram of Compound **14F** also shows a secondary anodic peak at the potential 1108 mV. It is known that oxidation of phenolic groups occur at moderate too high positive potentials forming a phenoxy radical or a phenoxonium ion that can successively undergo different secondary reactions [129]. The secondary peaks can be attributed to secondary oxidations occurring within the compound.

In terms of increasing antioxidant activity, the compounds may thus be arranged as follows:



12F **13F** **14F**

Compound 11D: **2, 5, 4'-trihydroxy dihydrochalcone**

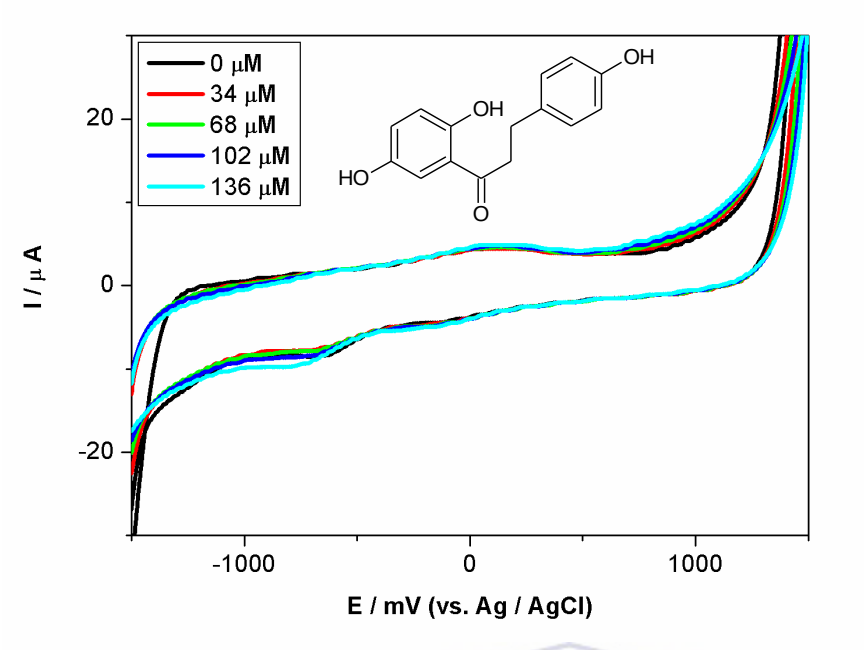


Figure 5.8: Cyclic voltammogram of compound **11D**, scan rate 100 mV/s.

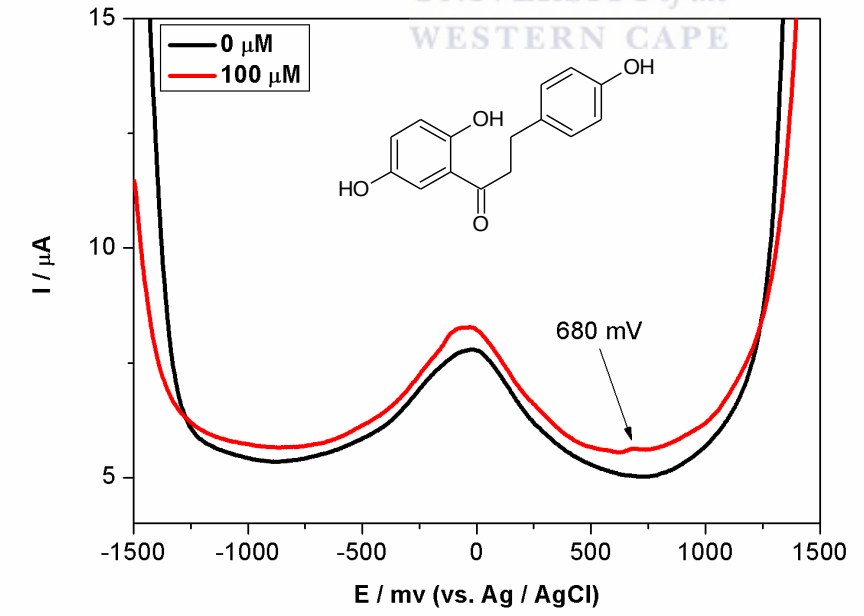


Figure 5.9: Square wave voltammogram of compound **11D**, scan rate 20 mV/s.

Figure 5.8 shows the cyclic voltammogram of compound **11D**, recorded when the potential sweep was begun from -1500 mV to +1500 mV and reversed at a scan rate of 100 mV/s. The square wave voltammogram of compound **11D (Figure 5.9)** shows a very timid peak at 680 mV. Again multiple scans where conducted to test the validity of this peak.

Compound 11C: 2, 5, 4'-trihydroxychalcone

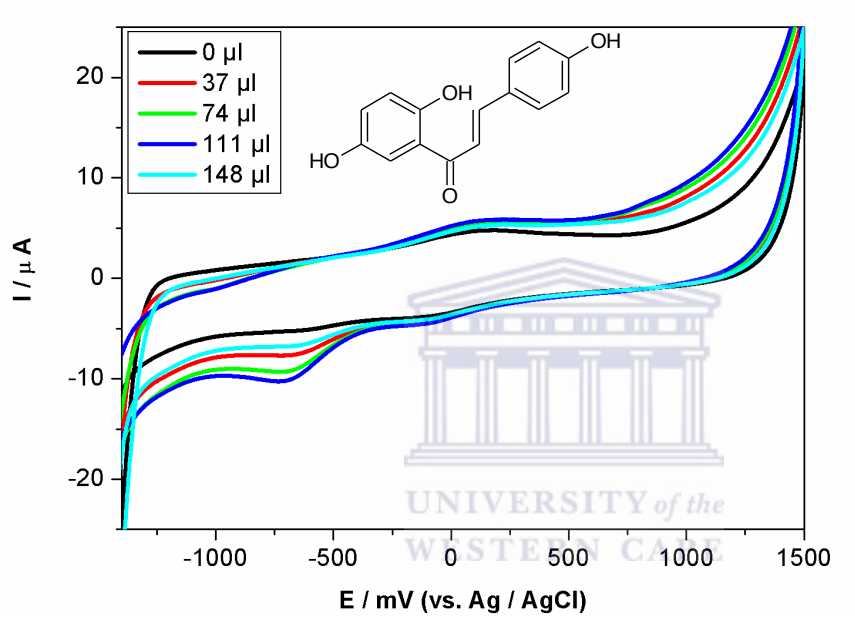


Figure 5.10: Cyclic voltammogram of compound **11C**, scan rate 100 mV/s.

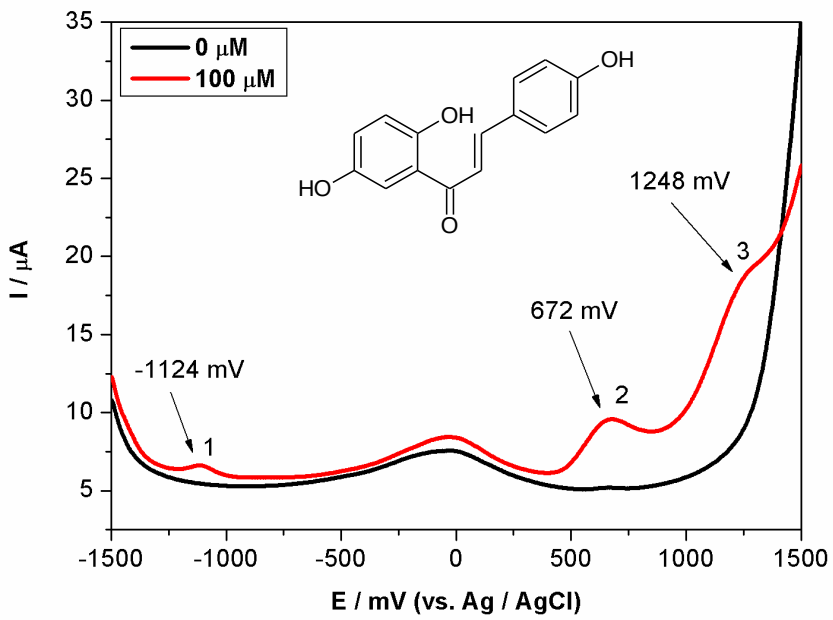


Figure 5.11: Square wave voltammogram of compound **11C**, scan rate 20 mV/s.

The square wave voltammogram of compound **11C** **(Figure 5.10)** shows three sets of defined peaks; one at -1124 mV, the second at 672 mV and the third at 1248 mV. Interestingly the first anodic peak marked as 1 in the voltammogram occurs at a very low oxidation potential which suggests high antioxidant activity [125].

Compound 11F: 4', 6-Dihydroxyflavanone

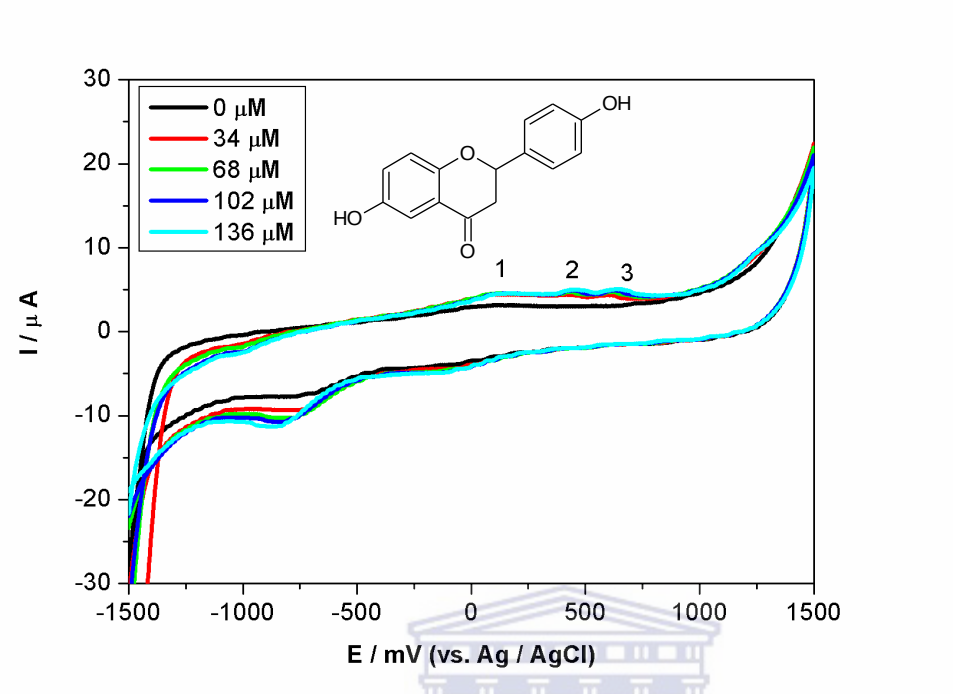


Figure 5.12: Cyclic voltammogram of compound **11F**, scan rate 100 mV/s.

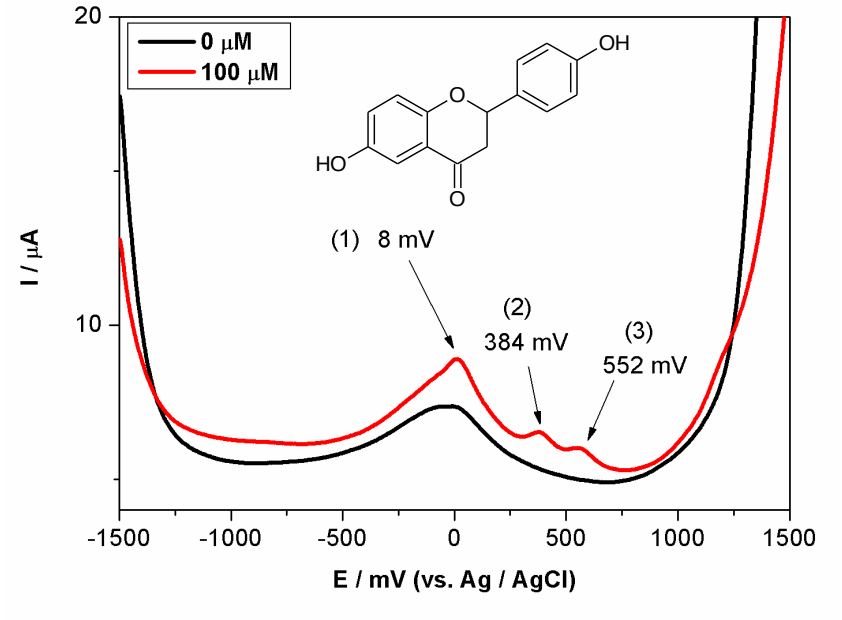


Figure 5.13: Square wave voltammogram of compound **11F**, scan rate 20 mV/s.

The square wave voltammogram of compound **11F (Figure 5.13)** shows three sets of defined peaks; the first at 8 mV marked as 1 in the voltammogram, the second at 348 mV and the third at 552 mV. The first anodic peak occurs in the potential range of the 'ghost peak' found in the blank scan. However there was clear evidence of oxidation occurring and therefore this peak was not ignored. Again this experiment was repeated to ensure the validity of the peak occurring at 8 mV.

5.3 Comparative Assessment

By comparing the square wave voltammograms of compounds **11D, 11C and 11F** one can obtain an improved understanding of the structure-reactivity relationship, namely, the importance of the α, β double bond functionality and the effect of ring closure.

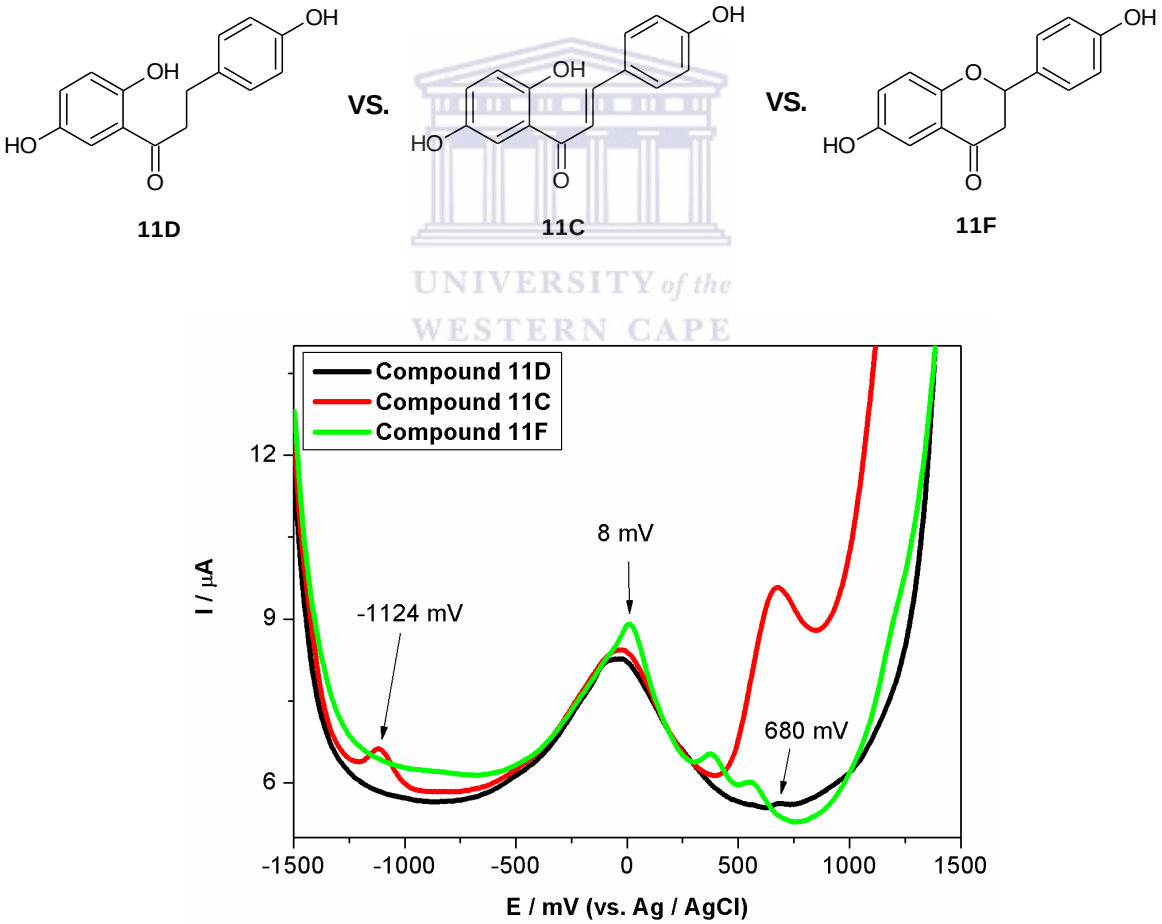
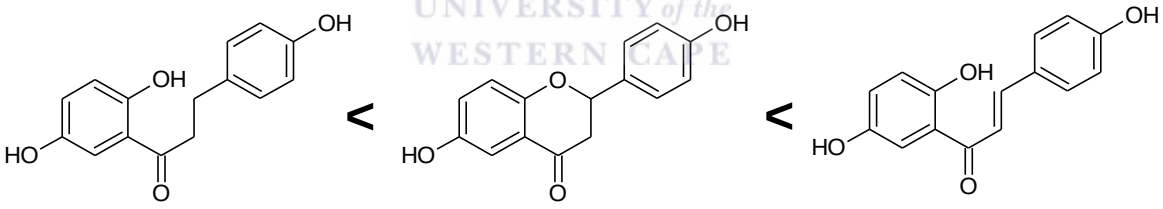


Figure 5.14: Superimposition of the square wave voltammograms of compounds **11D**, **11C** and **11F** at a scan rate of 20mV/s.

This comparison has presented fascinating results. It is clear by comparing the 2, 5, 4'-trihydroxy dihydrochalcone **11D** (680 mV) with the 4', 6-dihydroxyflavanone **11F** (8 mV) that ring closure has significantly lowered the oxidation potential and therefore one can expect the flavanone to have a higher antioxidant activity than its corresponding dihydrochalcone. The first anodic peak for compound **11C**, 2, 5, 4'-trihydroxychalcone, proved to have the lowest oxidation potential (-1124 mV). Such a low oxidation potential renders 2, 5, 4'-trihydroxychalcone a highly potent antioxidant, with a powerful reducing ability. This result proves that the α, β unsaturated double bond has a significant effect on the oxidation potential, and therefore the antioxidant activity is significantly enhanced by its presence. A study by Rice-Evans *et al.* found that conjugation between the A- and B-rings permits a resonance effect of the aromatic nucleus that lends stability to the flavonoid radical and is therefore critical in optimizing the phenoxyl radical-stabilizing effect [130].

In terms of increasing antioxidant activity, the compounds may be arranged as follows:



11D **11F** **11C**

Compound 16F: 6-Hydroxy –2', 5'-dimethoxy flavanone

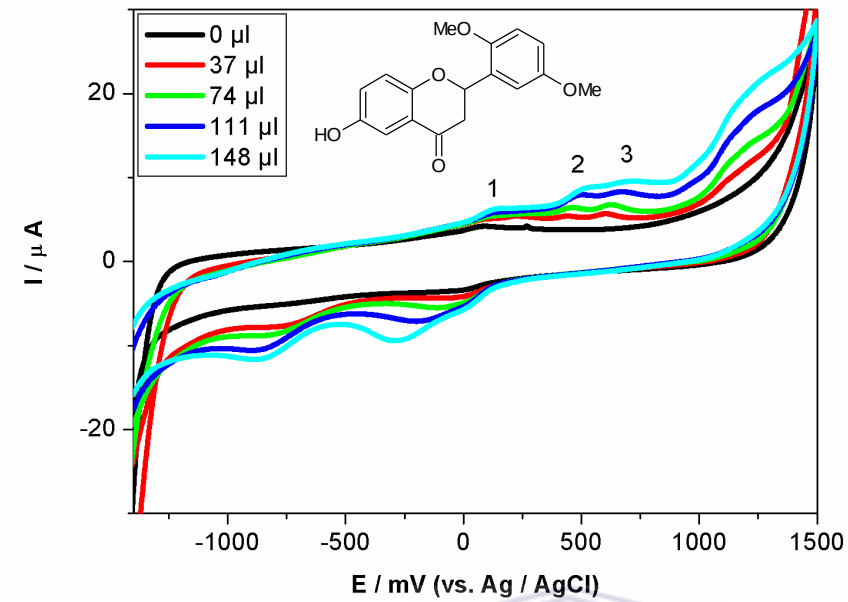


Figure 5.15*:* Cyclic voltammogram of compound **16F**, scan rate 100 mV/s.

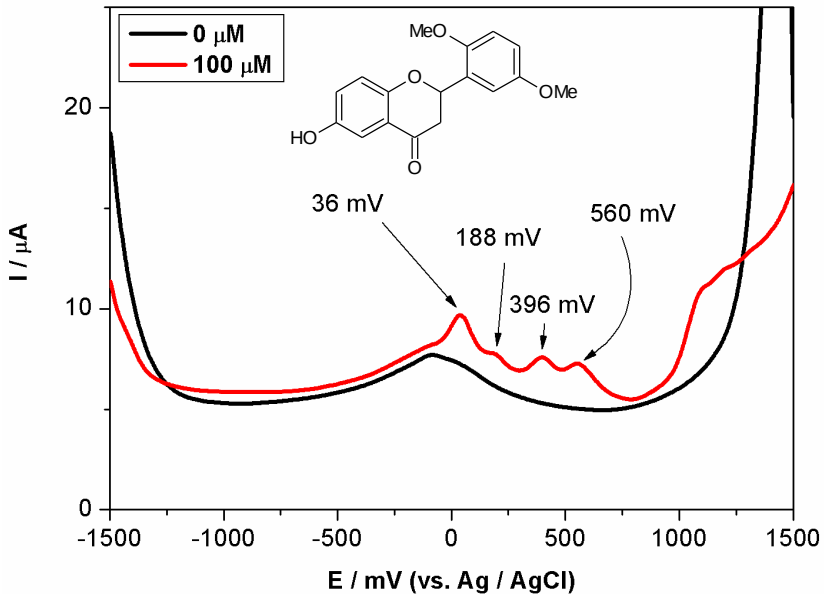


Figure 5.16: Square wave voltammogram of compound **16F**, scan rate 20 mV/s.

Figure 5.16 shows the cyclic voltammogram of compound **16F** at a scan rate of a 100mV/s. The square wave voltammogram of compound **16F (Figure 5.16)** shows five sets of peaks, with four defined peaks in the potential range of 0 – 600 mV. The first anodic peak occurs at 36 mV, the second at 188 mV, the third at 396 mV and the fourth at 560 mV.

Compound 17F: 6-Hydroxy –2', 4', 5'-trimethoxy flavanone

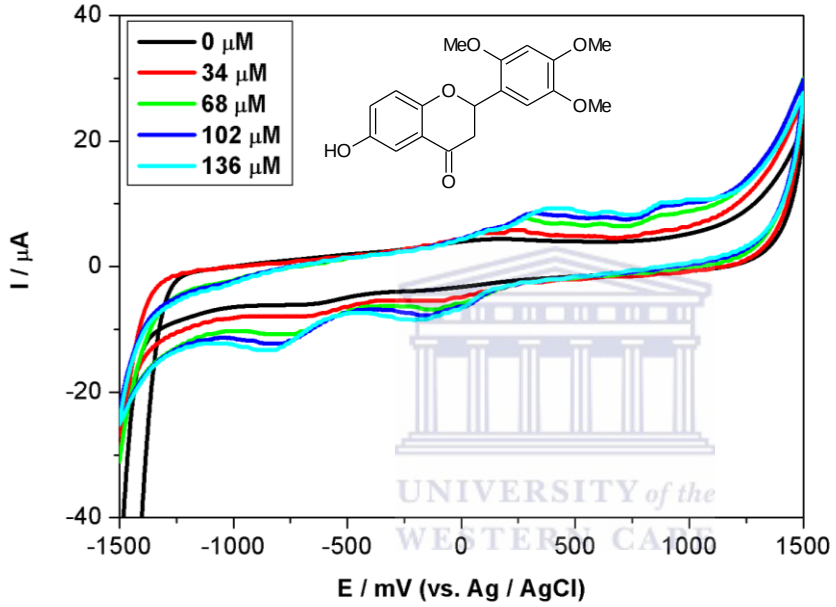


Figure 5.17: Cyclic voltammogram of compound **17F**, scan rate 100 mV/s.

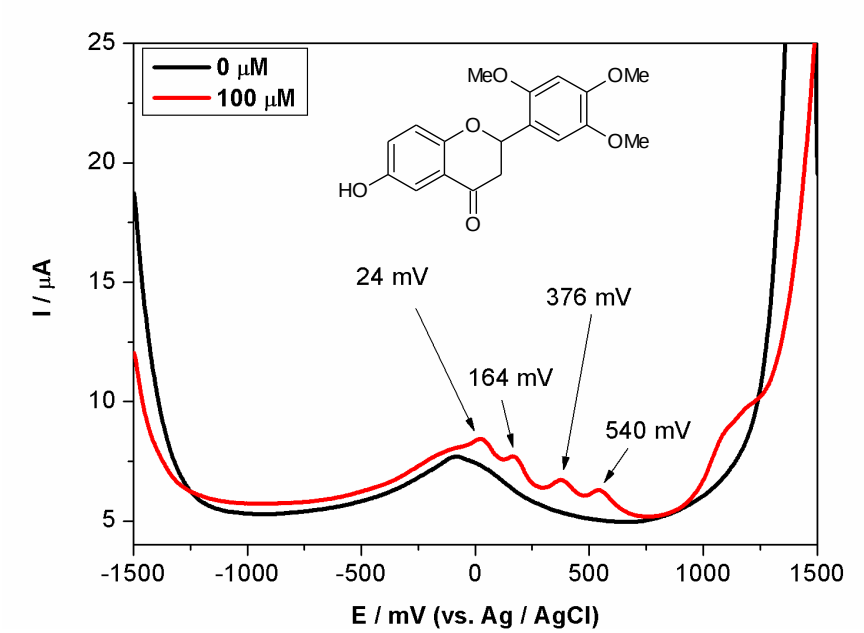


Figure 5.18: Square wave voltammogram of compound **17F**, scan rate 20 mV/s.

The square wave voltammogram of compound **17F (Figure 5.18)** shows five sets of peaks, of which four defined peaks are found in the potential range of 0 – 600 mV. The first anodic peak occurs at 24mV, the second at 164 mV, the third at 376 mV and the fourth at 540 mV.

Compound 18F: **5, 7-dihydroxy-2', 4', 5'-trimethoxy flavanone**

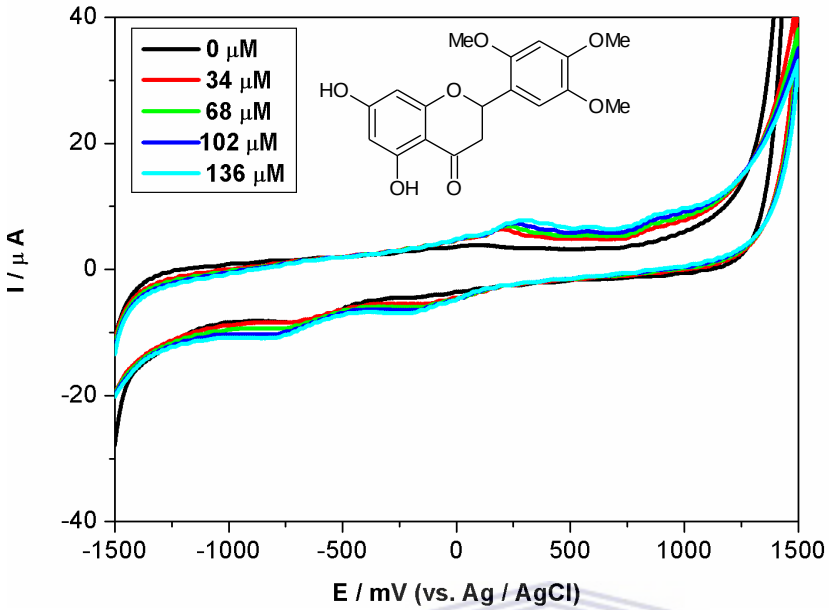


Figure 5.19: Cyclic voltammogram of compound **18F**, scan rate 100 mV/s.

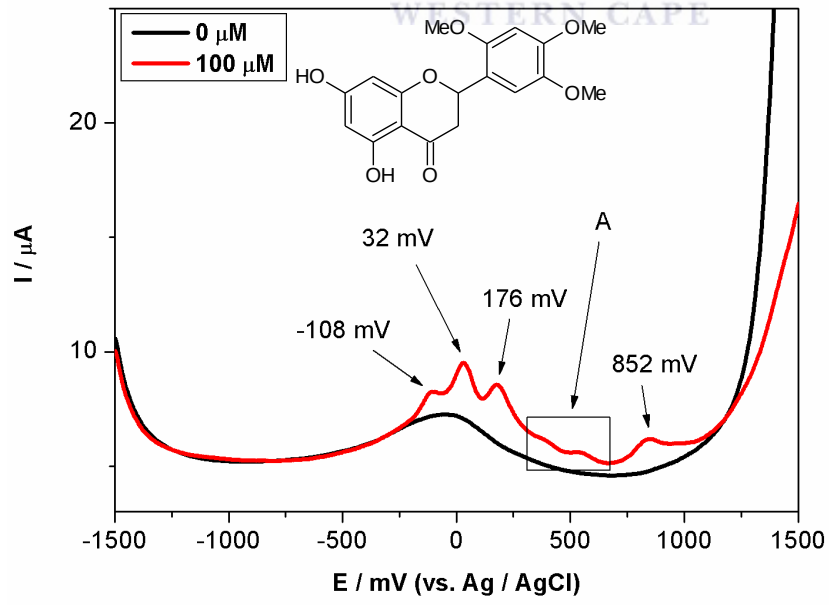


Figure 5.20: Square wave voltammogram of compound **18F**, scan rate 20 mV/s

The square wave voltammogram of compound **18F (Figure 5.20)** shows five sets of peaks, with four defined peaks occurring in the potential range of 0 – 600 mV. The first anodic peak occurs at -108 mV, the second at 32 mV, the third at 176 mV and the fourth at 852 mV.

5.4 Comparative Assessment

By comparing the square wave voltammograms of compounds **16F, 17F and 18F** we can obtain an improved understanding of the structure-reactivity relationship, namely, what effect the addition of methoxy groups on the B-ring have on the oxidation potential.

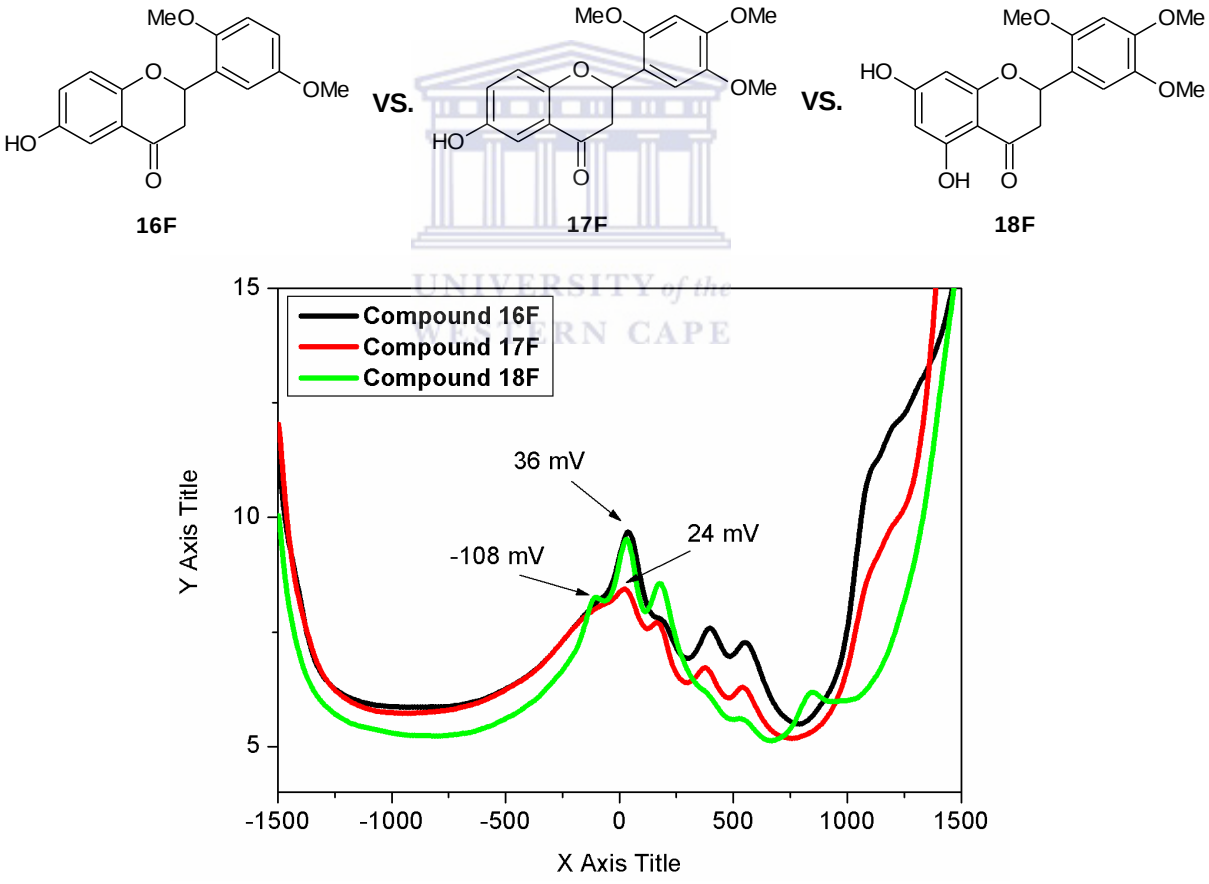
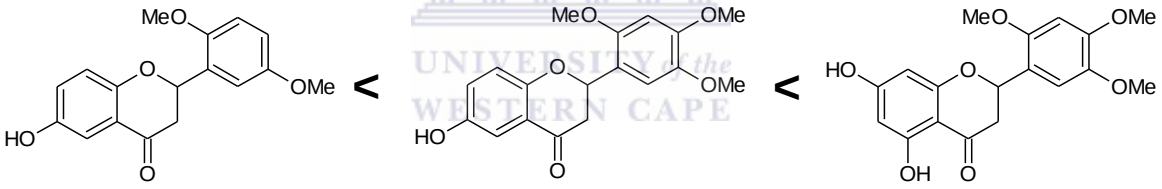


Figure 5.21: Superimposition of square wave voltammogram of compounds **16F, 17F** and **18F**.

There are clear differences between the voltammograms of the polyhydroxylated and polymethoxylated flavonoids. There is an increase in the number of anodic peaks observed as well as a remarkable shift of the first anodic peak to a lower region. Therefore we can conclude that the addition of methoxy groups on the B-ring makes an improvement on the antioxidant ability of such flavanones. The addition of the methoxy group is most likely to affect both hydrophobicity and molecular planarity due to steric affects [131]. These changes could lead to an increase in antioxidant ability. When comparing compounds **16F** and **17F,** we observe that the addition of a methoxy group at position 4' has lowered the potential of the first anodic peak to some extent. Compound **18F,** with two hydroxyl groups on the A-ring at positions 5 and 7, has the lowest peak potential and therefore in the series studied is the best antioxidant. Therefore it seems a specific ratio of hydroxyl groups to methoxy groups might play a role.

In terms of increasing antioxidant activity, the compounds are arranged as follows:



16F **17F** **18F**

Compound 15F: 5, 7, 4'-trihydroxyflavanone (Naringenin)

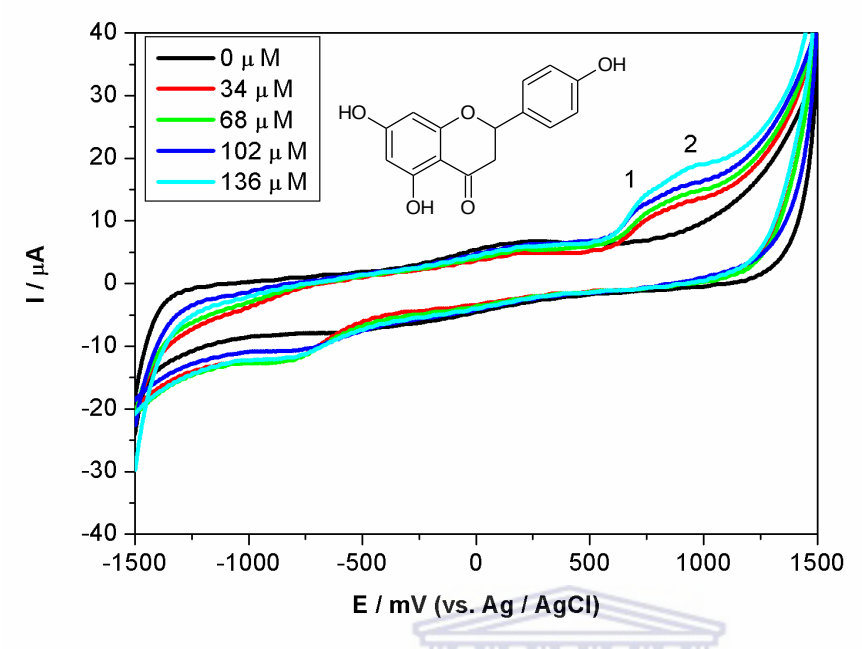


Figure 5.22*:* Cyclic voltammogram of compound **15F**, scan rate 100 mV/s.

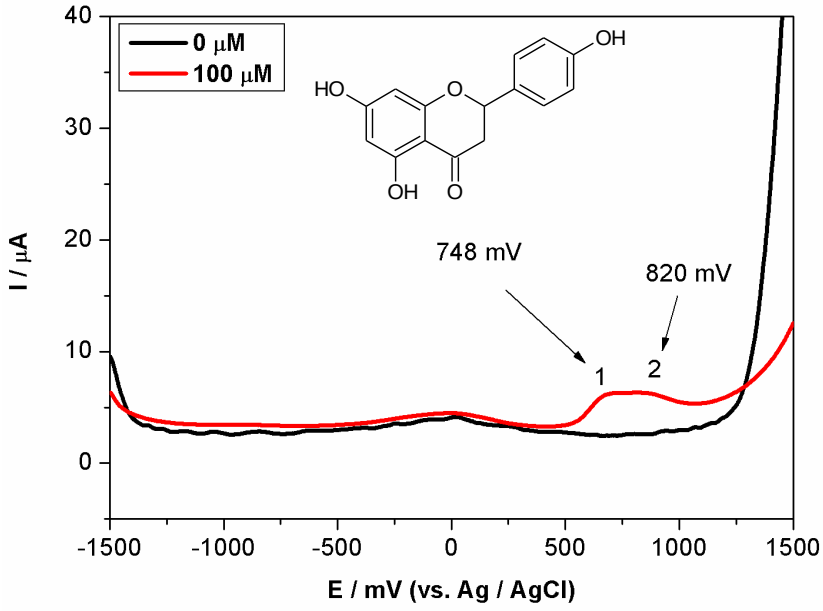


Figure 5.23: Square wave voltammogram of compound **15F**, scan rate 20 mV/s.

The square wave voltammogram of compound **15F (Figure 5.23)** shows two undefined peaks in the range of 500 -1000 mV, with the first peak occurring at 748mV and the second at 820mV.

Compound 20F: 5, 7, 4'-trihydroxy-6-isoprenylflavanone

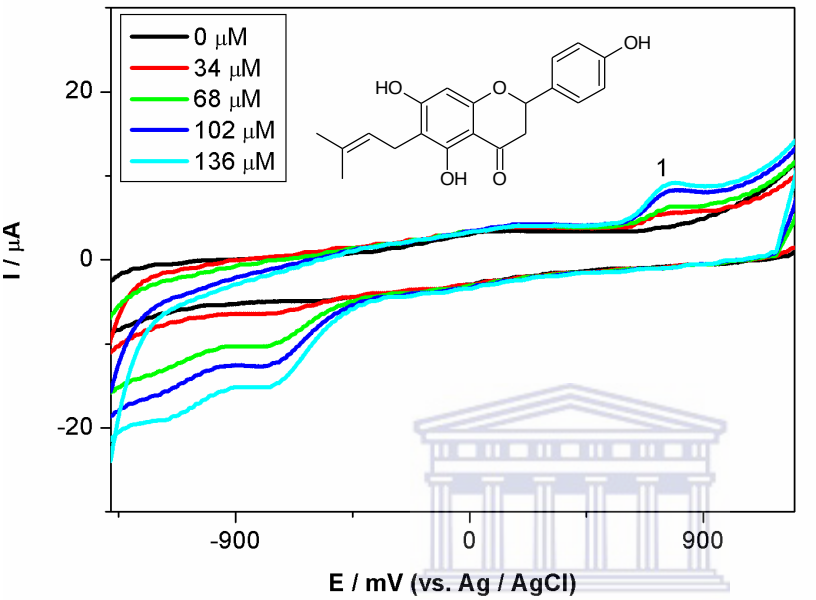


Figure 5.24: Cyclic voltammogram of compound **20F**, scan rate 100 mV/s.

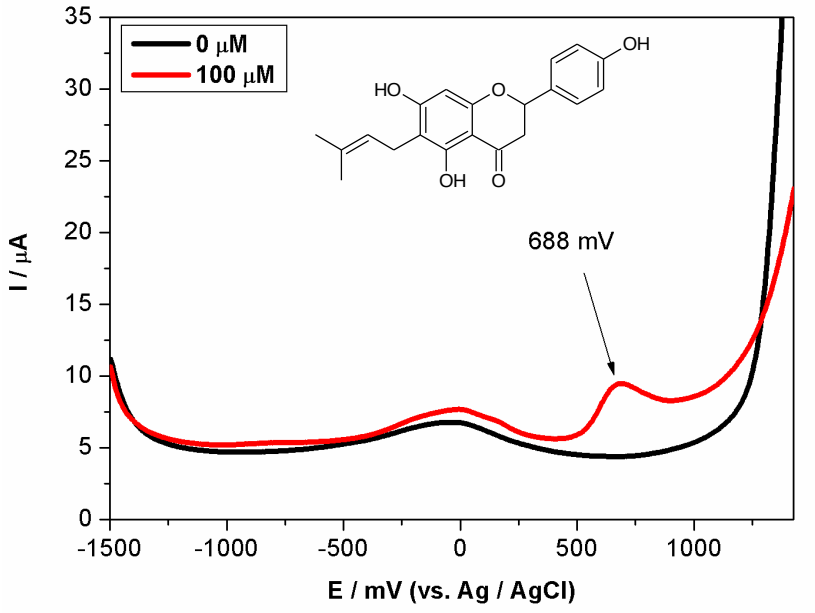


Figure 5.25*:* Square wave voltammogram of compound 20F, scan rate 20 mV/s.

The square wave voltammogram of compound **20F (Figure 5.25)** shows on defined peak at 688mV.

Compound 19F: 5, 7, 4'-trihydroxy-8-isoprenylflavanone

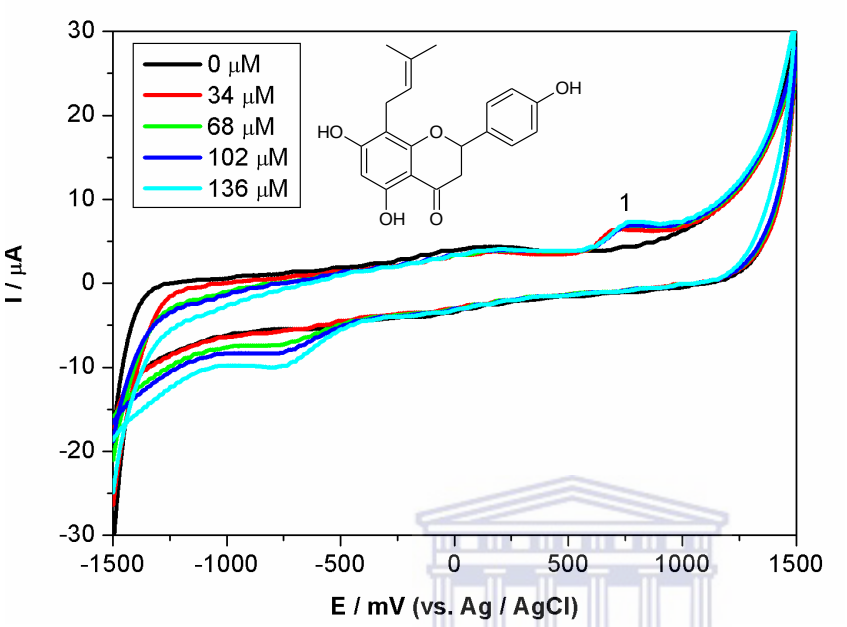


Figure 5.26: Cyclic voltammogram of compound **19F**, scan rate 100 mV/s.

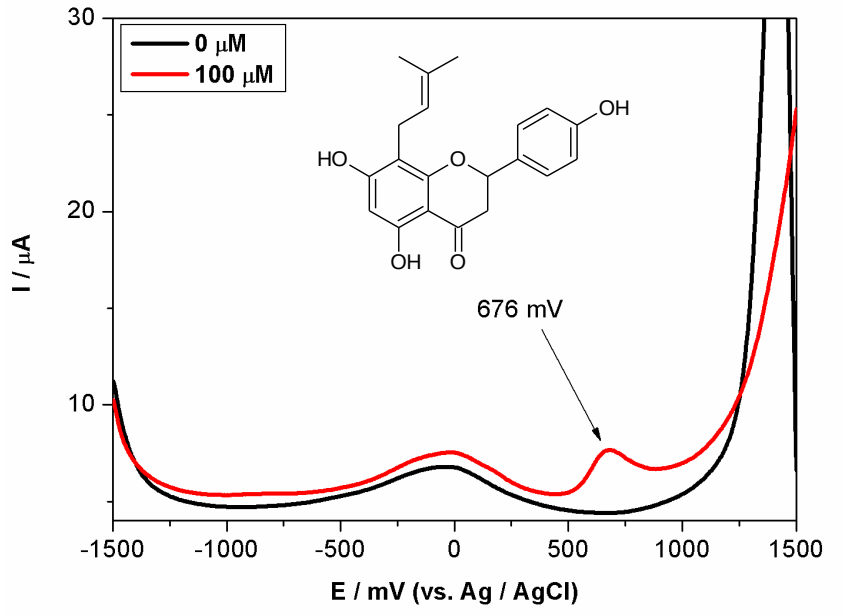


Figure 5.27: Square wave voltammogram of compound **19F**, scan rate 20 mV/s.

Again we have witnessed a single peak at 676mV in the square wave voltammogram of compound **19F (Figure 5.27).**

5.5 Comparative Assessment

By comparing the square wave voltammograms of compounds **15F, 19F and 20F** we can obtain an improved understanding of the structure-reactivity relationship, namely, what effect the addition of a prenyl group, on the A-ring, will have on the oxidation potential.

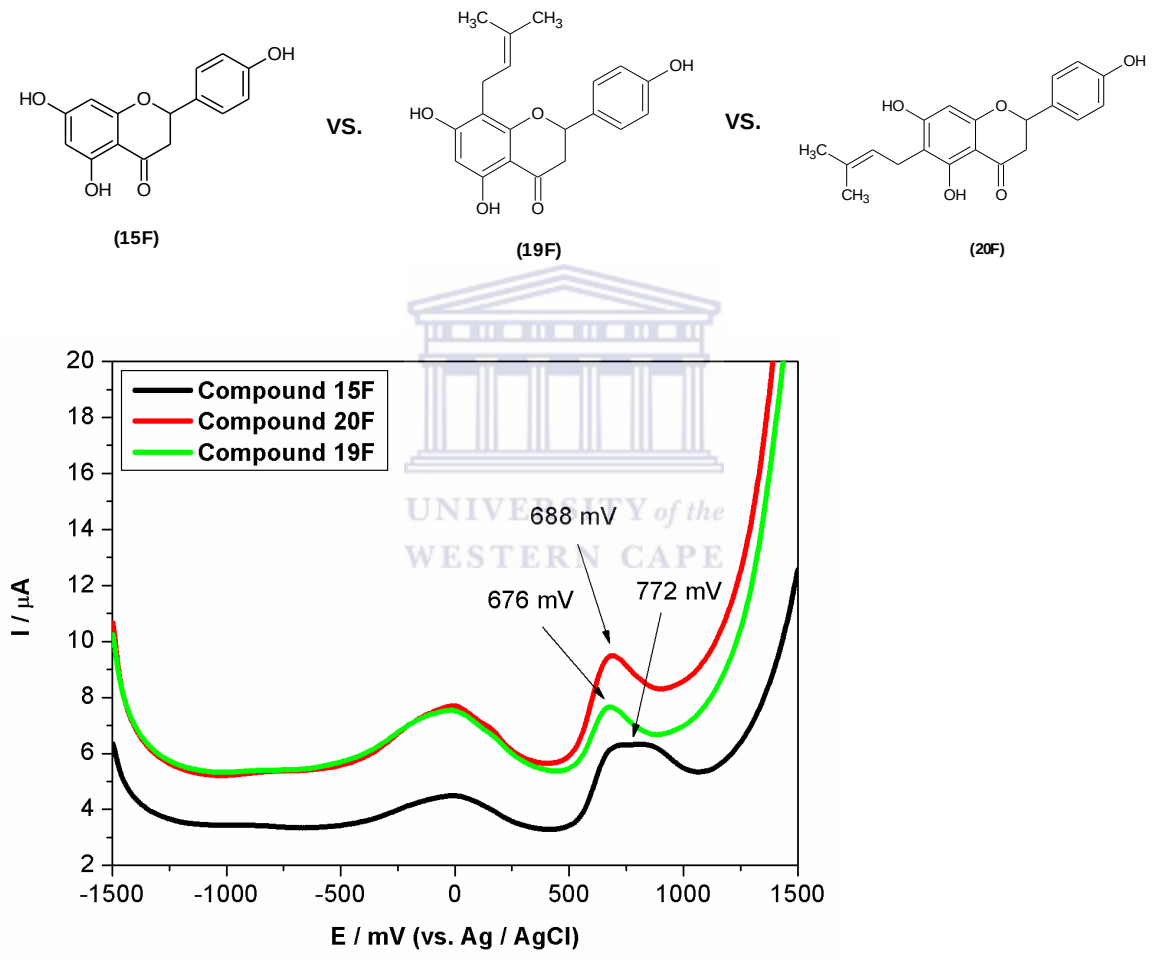


Figure 5.28: Superimposition of square wave voltammogram of compounds **15F**, **19F** and **20F**.

The voltammograms of the three compounds suggests that the addition of the prenyl group, on the A-ring, has had very little effect on the anodic peak potential. There is a very small difference in potentials between compounds, 6- prenyl naringenin and 8 – prenyl naringenin, with the former having the lower peak potential. The anodic peak for compound **14F**, Naringenin, is much broader than compounds **19P** and **20P**; however initial oxidation seems to occur in the 665 – 675 mV region which is concurrent with the both 6- and 8-Prenyl naringenin. What is clear however is the fact that the oxidation peaks for the prenylated naringenins are both more intense and sharper indicating a cleaner oxidation pathway and thus a better antioxidant behaviour.

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chemical reactions following their oxidation. Biochimica et Biophysica Acta (BBA) - General Subjects, 2002. 1572(1): p. 123-132.

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CHAPTER 6

6. CONCLUSION

6.1 Synthesis

12 flavonoid compounds were prepared in order to carry out electrochemical analysis. These compounds consist of one dihydrochalcone **(11D)**, two hydroxy chalcones **(10C and 11C)**, eight flavanones **(11F – 18F)** and finally two prenylated flavanones **(19F and 20F)**. Initial strategies involved finding suitable protecting groups for both the acetophenone and benzaldehyde starting materials. Use of dihydropyran ethers as protecting groups produced reasonable yields. However the products and starting materials were found to decompose over time if not protected from exposure to acidic fumes. The MOM-Br reagent, when opened appeared to be decomposing in the container, and subsequently produced low yields of MOM-protected benzaldehydes. For these reasons the benzyl protecting group was preferred, and subsequent reactions produced good yields and products all appeared to be stable.

Claisen Schmidt condensation between the protected hydroxyacetophenones with protected hydroxybenzaldehydes proved to be ideal for the formation of chalcone intermediates. Chalcone yields improved when the reaction temperature was increased from room temperature to 40 ^{o}C. The Chalcone intermediates later underwent oxidative cyclisation, using sodium acetate in EtOH to form hydroxyl protected flavanones, as a single product in high yields. Acid hydrolysis using HCl was used to demask the pyran protecting groups, whereas catalytic hydrogenation proved to be ideal for removing the benzyl protecting groups for formation of the pure flavanoids.

6.2 Electrochemical analysis

The study continued with the electrochemical analysis of the prepared flavonoids belonging to four flavonoid sub-classes viz., the dihydrochalcones; chalcones; flavanones and prenylated flavanones. The antioxidant activity of flavonoids is related to its ability to donate electrons to stabilize free radicals and therefore its measured oxidation potential renders a useful variable to calculate or postulate its antioxidant activity. The mid-point potentials (E½) of the flavanoids were determined by cyclic and square wave voltammetry. CV and SWV are advantages because they require small amounts of sample, are easy to operate and have short analysis times as compared to other methods. In the case of square wave voltammetry, due to the fast analysis time, a better current response was observed and therefore less interference was expected on the electrode process as compared to cyclic voltammetry. Hence the data from the square wave voltammograms was preferred and used to determine the oxidation potentials. For all flavonoids, there was a single reduction peak at a potential slightly lower than the first peak potential. It is thus logical to conclude that this peak was a result of the reduction of the product of the oxidized functional group corresponding to the first oxidation peak potential.

6.2.1 Relationship between antioxidant activity and structure

6.2.1.1 The effect of substituents

The type of substituent, number and spatial arrangement is perhaps a greater determinant of antioxidant activity than the flavanone backbone alone. Consistent with many other studies we have confirmed that addition of hydroxyl groups, on the B-ring, substantially influences the antioxidant activity of flavanones. There was no observable change with the addition of one hydroxyl group at position 4', however compound **14F,** 4', 5'-dihydroxyflavanone showed a large decrease in oxidation potential when compared to the 4'-hydroxyflananone. We observed that increasing the number of hydroxyl groups on the B-ring caused a lower oxidation potential and therefore an increase in antioxidant activity. Furthermore it is clear that B-ring hydroxyl substitution is possibly a greater determining factor than A-ring hydroxyl substitution. We also noted that a combination of methoxy groups on the B-ring and hydroxyl groups on the A ring had increased the antioxidant activity of the flavanone backbone, even more so than the flavanones with many hydroxyl groups such as Naringenin. The addition of a prenyl group on to Naringenin showed a

modest change to the oxidation potential and therefore has had very little effect on the antioxidant activity. However these additions were on the A-ring only and future work could possibly deal with additions onto the B-ring.

6.2.1.2 The effect the α, β unsaturated double bond and ring closure

When comparing compounds 2, 5, 4'-trihydroxy dihydrochalcone and 4', 6-dihydroxyflavanone, we observe that ring closure has significantly lowered the oxidation potential and therefore one could expect the flavanone to have a higher antioxidant activity than its corresponding dihydrochalcone. However the 2, 5, 4'-trihydroxychalcone, which is in the open ring form, and having the α, β unsaturated double bond, had the lowest oxidation potential and therefore the greatest antioxidant activity. These results suggest that the flavonones, which has a ring C form as well as having the α, β unsaturated double bond between C2 and C3, could be the skeletal framework for future investigations.