INVESTIGATION OF THE NATURAL PRODUCTS COMPOSITION FROM THE SEAWEED *ULVA CAPENSIS*

By

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Thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in the Faculty of Natural Sciences University of the Western Cape

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ABSTRACT

In modern society, diversity of marine macroalgae has become an inspiration for pharmaceutical companies and researchers because of their numerous health benefits, and a great deal of interest has developed towards the isolation of bioactive compounds to identify novel marine natural products that could eventually be developed into therapeutics or pharmaceutical products. Furthermore, marine macroalgae are valuable source of structurally diverse metabolites with scientifically proven reports.

The search continues as there are many natural bioactive compounds that are in the womb of the ocean which are still a mystery. Thus, the present study investigates the natural products from green seaweed *Ulva capensis*.

Six known compounds namely Isofucosterol (**C1**), 24*R*-Saringosterol (**C2**), 24*S*-Saringosterol (**C3**), Methyl stearidonate (**C4**), Methyl linoleate (**C5**) and Methyl Linolenate (**C6**) were isolated for the first time from *Ulva capensis* using chromatographic techniques. Structural elucidation of the compounds was achieved by spectroscopic analysis: NMR and GC-MS spectroscopy and by comparing with literature.

To our best knowledge and with extensive search from literatures, this dissertation is the first phytochemical report to be carried out from *Ulva capensis*.

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DECLARATION

I, Sazi Selby Thwala, hereby declare that **"Investigation of the natural products composition from the seaweed *Ulva capensis*"** is my original dissertation and to my knowledge, it has not been previously submitted for any degree in any other University, and all the sources I have used have been acknowledged by complete reference.

Date: 28 November 2019 **Signature: ……………………**

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DEDICATION

I would like to dedicate this dissertation to my family, my twin sister **Nonsikelelo Thwala**, my older sister **Thulisile Dimba**, my younger siblings **Ncamiso** and **Lunga Zikalala**. My uncle **Nkosinathi Sibande** who has played a role of a father figure in my life, I will always be grateful for the love, encouragements and the outmost support.

Finally, I would like to pay tribute to my late mother **Nelisiwe Cynthia Sibande** for her enormous support and love throughout my academic journey to the point of her departure, May Her Beautiful Soul Rest In Peace.

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LIST OF ABBREVIATIONS

1D	One dimensional
2D	Two dimensional
^{1}H	Proton
^{13}C	Carbon 13
BC	Before Christ
br.	Broad signal
BuOH	Butanol
$CDCl_3$	Deuterated chloroform
COSY	Correlation spectroscopy
d	Doublet
dd	Doublet of doublet
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
et al.,	et altera
EtOAc	Ethyl Acetate
FAME	Fatty Acids Methyl Esters
g	Gram
GC – MS	Gas Chromatography Mas Spectroscopy
HMBC	Heteronuclear Multiple Bond Connectivity
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
J	Coupling Constant
L	Liter
m	Multiplet

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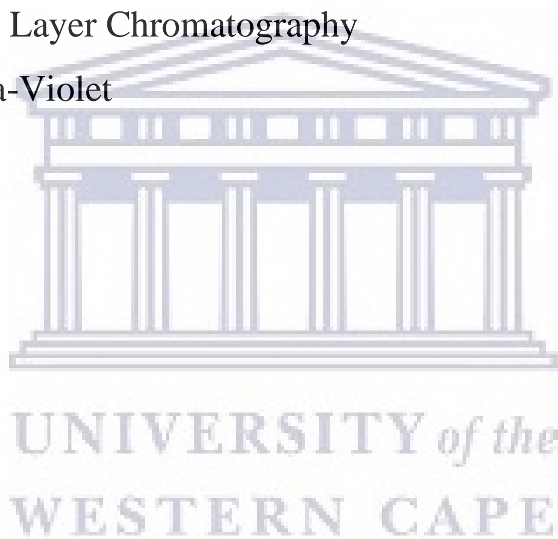
MeOH	Methanol
mg	Milligram
m/z	Mass per Charge
NMR	Nuclear Magnetic Resonance
ppm	Parts Per Million
q	Quartet
s	Singlet
sept	Septet
t	Triplet
TLC	Thin Layer Chromatography
UV	Ultra-Violet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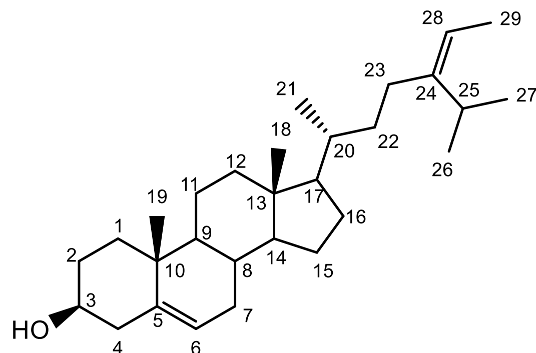
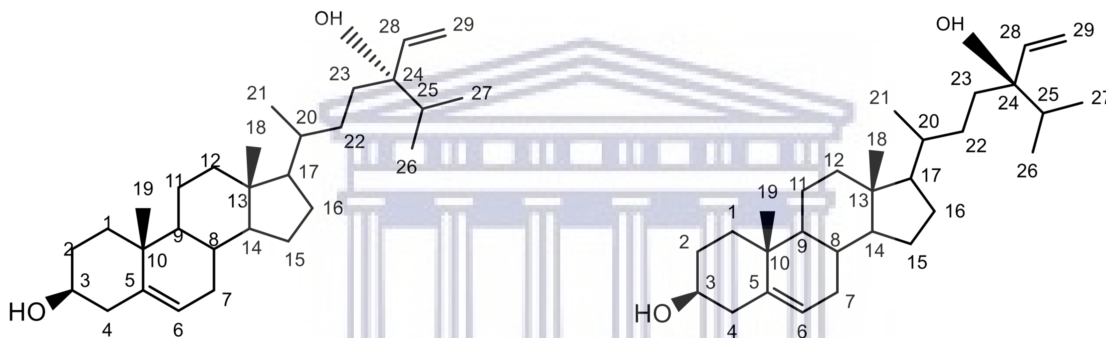
LIST OF ISOLATED COMPOUNDS



Compound 1



Compound 2

Compound 3

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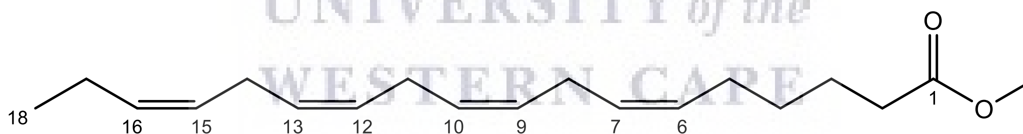

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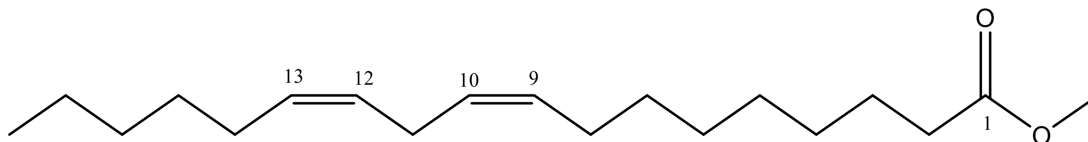

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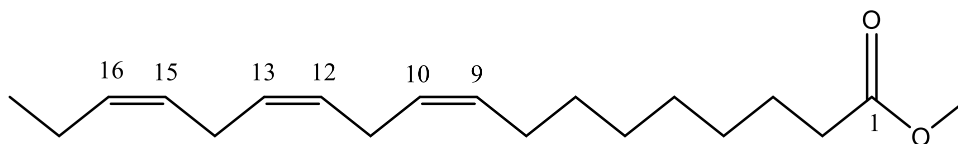

Compound 6

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Chapter One

Introduction and Literature review

1.1 Background

Algae are primitive non-flowering plants without true root, stem and leaves (Leelavathi *et al.,* 2015)**.** They are abundantly found in solid substrates and commonly presenting onto depths of 30 – 40 meters (Makkar *et al.,* 2007). They can be split into two major categories: The macroalgae (seaweeds) and the microscopic sized microalgae (Bangert *et al.,* 2013). Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their morphology (Kuda *et al.,* 2002). Like other plants, seaweeds contain various organic and inorganic substances, which can benefit human health (Cox *et al.,* 2010). Marine seaweeds draw an extraordinary wealth of mineral elements from the sea, which may account for up to 36% of its dry mass (Ito *et al.,* 1989). The mineral nutrients present in seaweeds are diverse, and the main elements are iodine, phosphorus, calcium and potassium (Leelavathi *et al.,* 2015). Seaweeds are rich in many nutrients such as vitamins (A, B1, B12, C, D and E), riboflavin, folic acid, and pantothenic acid. Their amino acids content are well balanced and contain all or most of the essential amino acids required for the maintenance of health (Chia *et al.,* 2015). Unlike vegetables and other land plants, seaweeds have more than 54 trace elements required for human body's physiological functions and these are in great quantities (Dhargalkar and Periera *et al.,* 2005). therefore, making seaweeds one of the important marine living resources that could be termed as the futuristically promising plant.

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Furthermore, seaweeds are rich sources of structurally novel and biologically active metabolites and are classified as the largest remaining reservoir of natural molecules (Rachel *et al.,* 2015). Extensive research has been carried out on seaweeds with greater interest focused on bioactive compounds with antimicrobial qualities (Rachel *et al.,* 2015). The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions (Leelavathi *et al.,* 2015). Seaweeds live in very exigent, competitive, and aggressive surroundings, which are very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules. They have long become valuable sources in many commercial applications such as foodstuffs, animal feed, cosmetics, pollution abatement, and therapeutics (Bangert *et al.,* 2013).

Among the three classes of seaweeds, Brown algae (Phaeophyceae) is one of the most well studied algal families in respect of secondary metabolites (Van Trana *et al.,* 2017). Even though it is vastly studied, it has been classified as the largest remaining reservoir of natural molecules yet to be evaluated for drug activity (Giri and Ohshima *et al.,* 2012). Moreover, the search for novel compounds from natural sources to combat the negative effects of synthetic compounds and their resistance towards multidrug resistant pathogens continue to be a major priority (Rachel *et al.,* 2015). Studies combining both antioxidant and antibacterial activities of macroalgae are rare in comparison with well-known extracts such as grape seeds, grapefruit seeds and green tea. Nevertheless, a report by Cox (2010) has described such a study conducted on a limited selection of seaweed species.

Seaweed extracts are also a very important factor in the traditional herbal medicine found in the market these days and best known for their richness in polysaccharide, minerals and certain

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vitamins (Holdt and Kraan *et al.,* 2011). There are numerous benefits of seaweed extracts which have been known from thousands of years, but it is only during modern time that their bioactive ingredients are beginning to be recognized. They contain bioactive substances like polysaccharide, lipids and polyphenols, with anti-bacterial, anti-fungal and anti-viral properties (Pal *et al.,* 2014). They are also known to contain reactive antioxidant molecules such as ascorbate and glutathione when fresh, as well as secondary metabolites, including carotenoid astaxanthin (Figure.**1.1**), mycosporinelike aminoacids (*e.g.* mycosporine palythine), catechins (*e.g.* catechin, epigallocatechin) phlorotannins (*e.g.* phloroglucinol) and tocopherols (Mendes *et al.,* 2012). Even though seaweeds have been studied extensively in search for biologically active compounds, there remains a multitude of seaweed species with no phytochemical and biological studies that have been reported and hence they are still an attractive source for chemical investigation (Makkar *et al.,* 2007).

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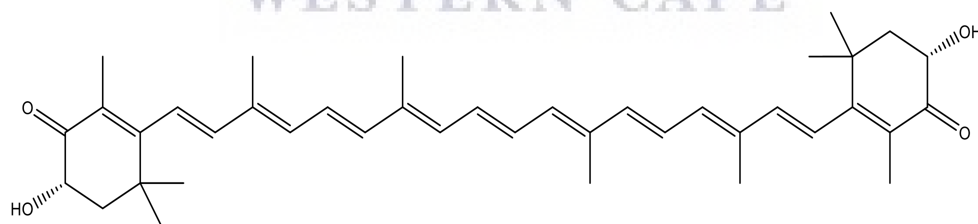

Figure 1.1: Astaxanthin

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1.2 Literature review

Marine algae have been the focus of interest as promising sources of novel and potentially bioactive primary and secondary metabolites. Over the past decade's seaweed utilisation expanded from consumption as food to animal feed, cosmetics, pollution abatement, and therapeutics. Previously, industrialization of seaweeds began with the production of soda and potash from brown seaweeds for the manufacture of soap, glass and iodine (Jensen *et al.,* 1979). Alginates, agar and carrageenan are three commercially important phycocolloids obtained from seaweeds (McLachlan *et al.,* 1985). Alginates have strong antibacterial properties and stimulate reparative process of wounds (Chojnacka *et al.,* 2012). Seaweed genera used for extraction of alginates are *Ascophyllum, Durvillaea, Lessonia, Laminaria, Macrocystis, Sargassum* and *Turbinaria* (Dhargalkar and Verlecar *et al.,* 2009).

The multipurpose uses of seaweed phycocolloids; such as emulsifiers in dairy products, leather, textile and pharmaceutical industries, for the treatment of arthritis and metal poisoning, bone grafting, immobilization of biological catalysts in industrial processes, therapeutic health promoters and beauty enhancer have immense value to human kind (Dhargalkar and Periera *et al.,* 2005). Seaweeds are also used as fertilizer in agriculture and horticulture, food supplement for animals and feed for aquaculture. Presently, seaweed-based food additives are commonly used in the preparation of fast foods. In fact, virtually everyone consumes some processed seaweeds every day (Dhargalkar and Verlecar *et al.,* 2009).

Around the world there are 221 algae species belonging to 32 Chlorophyta, 64 Phaeophyta and 125 Rhodophyta are being utilized for a variety of purposes**.** Of these, about 145 species (66%) are used for food (White and Ohno *et al.,* 1999). The importance of seaweeds for human

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consumption is well known since 300 BC in China and Japan (Dhargalkar and Periera *et al.,* 2005). These two countries are the major seaweed cultivators, producers and consumers in the world. In the Indian Ocean region countries like Malaysia, Indonesia, Singapore, Thailand, and Korea, edible seaweeds are used in salads, jelly, soup (Dhargalkar and Periera *et al.,* 2005).

Edible seaweeds are low calorie foods, with a high concentration of minerals, vitamins and proteins and a low content in lipid (Keyimu and Abuduli *et al.,* 2019). They are excellent sources of vitamins A, B1, B12, C, D and E, riboflavin, niacin, pantothenic acid and folic acid (Nisizawa et al 1988) as well as minerals such as Ca, P, Na, and K. They are also easy to digest. The consumption of seaweed is standard because it has lipid and protein quality compared to other vegetables, mainly because of its better relatively high levels of unsaturated fatty acids and high content in essential amino acids (Murata *et al.,* 2001).

Agar is a typical and traditional food material in Japan, and it is used as a material for cooking and Japanese-style confectionery (Murata and Nakazoe *et al.,* 2001). The important edible seaweed genera are *Porphyra, Chondrus, Rhodymenia, Hypnea, Gigartina, Gracilaria, Laurencia, Iridaea, Phyllophora (red) Undaria, Durvillaea, Ecklonia, Sargassum, Turbinaria* (brown) and *Ulva, Enteromorpha, Monostroma,* Caulerpa (green). In addition to China and Japan, other countries such as Scotland, Chile, Philippines, Malaysia, Bali, Korea, Singapore, and Sri Lanka also consume seaweeds in a variety of forms (Dhargalkar and Verlecar *et al.,* 2009). Furthermore, Murata (2001) reported that the consumption of marine algae was large in Okinawa Prefecture where people are known to relatively live longer than average.

In addition, agar is used for the manufacture of capsules for medical applications and as a medium for cell culture. It was reported that intake of agar leads to a decrease in the concentration of blood

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glucose, exerts anti-aggregation effect of red blood cells and reduce the absorption of ultraviolet rays (Kraan *et al.,* 2012). It has also been indicated that fucoidan exerts anti-blood coagulation and anticancerous effects (Zhuang *et al.,* 1995). Kombu from Hokkaido (Japan) was used to treat diseases of the thyroid gland about 800 BC in China. Recently, it has been suggested that the increase in the incident of adult diseases in Japan, such as diabetes, hypertension and hyperlipidemia was caused by the decrease in the dietary intake of fish, shellfish and marine algae (Murata and Nakazoe *et al.,* 2001). Marine algae contribute to the treatment and prevention of various diseases (Murata and Nakazoe *et al.,* 2001), they are an essential source of n-3 polyunsaturated fatty acids for the maintenance of health. (Galli *et al.,* 1994). Furthermore, Moghadasian and Frohlich (1999) reported that alginic acid leads to a decrease in the concentration of cholesterol, exerts an anti-hypertension effect, prevents effect of absorption of toxic chemical substances, and plays a major role as dietary fibre for the maintenance of human health. Studies on the bioactivities of marine algae have revealed numerous health-promoting effects, including anti-oxidative, anti-inflammatory, antimicrobial, and anti-cancer effects (Lee *et al.,* 2013). Fucoxanthin, a type of xanthophyll and an accessory pigment in the chloroplast of algae, has also shown beneficial effects (Kim and Pangestuti *et al.,* 2011).

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Various literatures have addressed a wide range of issues regarding conventional biofuel production ranging from the use of crops for production to ethical use (Amosu *et al.,* 2006). On the other hand, algae production is promising as the next generation feedstock, capable of significantly higher yields with lower resources inputs that other feedstocks currently used to produce biofuel (Amosu *et al.,* 2006). The lists of fuels that can be derived from algae include biodiesel, butanol, gasoline, methane, ethanol, and jet fuel.

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1.3 Seaweeds from polar regions and their uses

As a highly productive ecosystem, the Southern Ocean supports various potentially high commercial value components. Recently, after the decline in world fisheries, there has been a renewed interest in the "Living Resources of the Southern Ocean" (Dhargalkar and Verlecar *et al.,* 2009). The resulting efforts are aimed at critical stock assessment, harvestable fish quantities, and the region's environmental health for the sustainable management of this invaluable resource. The marine macroalgae or seaweed, however, is another crucial renewable resource that supports the Southern Ocean. All species from the polar regions are adapted to low light and their phenology is finely tuned to the strong seasonal changes of the light conditions (Wiencke *et al.,* 2007). The vast algae beds near the sub-Antarctic islands and the coastal Antarctica hold potential algae for future exploitation. Antarctic red algae have been identified for their chemo diversity, containing compounds possessing antibacterial and other inhibitive properties to marine animals (Gallardo *et al.,* 1999). Table **1.1** below outlines the Antarctic seaweeds and their uses.

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Table 1.1. Antarctic seaweeds and their potential uses.

Species	**Examples**	**Potential uses**
Chlorophyta	*1. Acrosiphonia pacifica*	Pharmaceutical
	2. Monostroma hariotii	Edible
	3. Ulva lactuca	Edible, animal feed.

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Phaeophyta	*4. Ascoseira mirabilis*	Alginate, Pharmaceutical.
	5. Desmarestia anceps	Alginate, Pharmaceutical
	6. D. menzeisii	Alginate, Pharmaceutical
	7. D. Antarctica	Alginate, Pharmaceutical
	8. Himantothallus grandifolius	Alginate
	9. Durvillaea antarcticaand D. willana	Alginate, edible.
	10. Cystosphaera jacquinotii	Alginate
	11. Lessonia flavicans	Alginate, edible.
	12. Macrocystis pyrifera	Alginate
Rhodophyta	*13. Porphyra endiviifolium*	Edible
	14. Ptilonia megellanica	Pharmaceutical
	15. Cladonta ilyalii	Pharmaceutical
	16. Gigartina skottsbergii	Carrageenan
	17. Iridaea cordata	Carrageenan
	18. Palmaria decipiens	Carrageenan
	19. Phyllophora antarcitca	Carrageenan
	20. Plocamium cartilagineum	Pharmaceutical
	21. Callophyllis variegate	Pharmaceutical
	22. Gracilaria spp.	Agar

(Dhargalkar and Verlecar *et al.,* 2009)

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1.4 Natural Products

In medicinal chemistry, the term 'natural product' is used to describe a low molecular weight chemical compound, usually excluding proteins and nucleic acids, produced or extracted from living organisms (Njuguna *et al.,* 2014).

Natural products are metabolic products of plants, animals, insects, marine organisms and microbes. The metabolic products include alkaloids, flavonoids, terpenoids, glycosides, amino acids, proteins and carbohydrates (Amsler *et al.,* 2008). Natural products are commonly known as "secondary metabolites" and are molecules that are not essential to the growth and development of the producing organism (Amsler *et al.,* 2008). They have a broad range of functions, for example, pheromones that act as social signalling molecules with other individuals of the same species, communication molecules that promote symbiosis.

Furthermore, alkaloids, terpenes, ascorbic acid, tocopherols and carotenoids (metabolites) isolated from seaweeds have also been proven to exhibit antioxidant activity (O'Sullivan *et al*., 2011). Over the year's plants have been used for different applications ranging from medicines, to sweeteners, pigment and many more. The latter applications are estimated to be over 40% and have their origins in natural products (Hanson *et al.,* 2003). The beginning of modern natural products chemistry may be looked back to Friedrich Wilhelm Sertürner (1783- 1841), the first person to isolate a pure compound which is now known as morphine the poppy-plant. The discovery paved a new way for natural products chemistry; development of the field opened a channel of collaborations between chemists and biologists, each bringing their own knowledge base to address chemical ecological questions (Harborne *et al.,* 1989).

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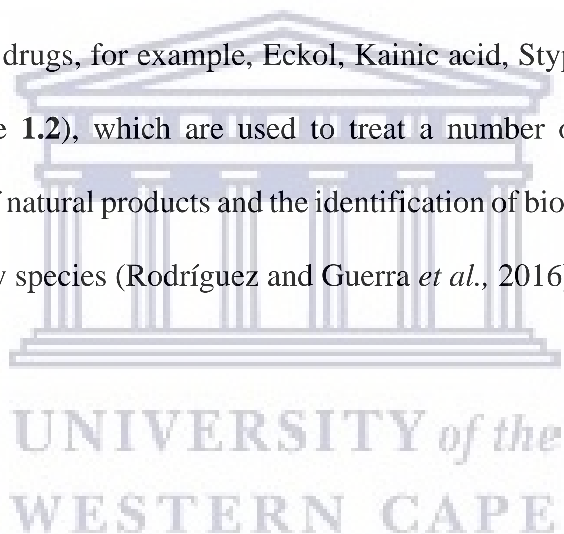
Moreover, Research of natural products inspired improvement of the separation techniques, spectroscopic tactics to form elucidation, and artificial methodologies that now represent the foundation of current organic chemistry. Enthusiasm for normal items was not simply scholarly yet rather was incited by their extraordinary utility as colours, polymers, strands, pastes, oils, waxes, seasoning specialists, fragrances, and medications. Recognition of the biological properties of myriad natural products has fuelled the current focus of this field, namely, the search for new drugs, antibiotics, insecticides, and herbicides (Croteau *et al.,* 2000).

Furthermore, several phytochemical screenings for bioactive compounds were performed which led to the discovery of new drugs, for example, Eckol, Kainic acid, Stypoldione and Trabectedin (Hanson *et al.,* 2003; figure **1.2**), which are used to treat a number of diseases. The detailed chemical characterization of natural products and the identification of bioactive compounds remain largely unexplored for many species (Rodríguez and Guerra *et al.,* 2016).



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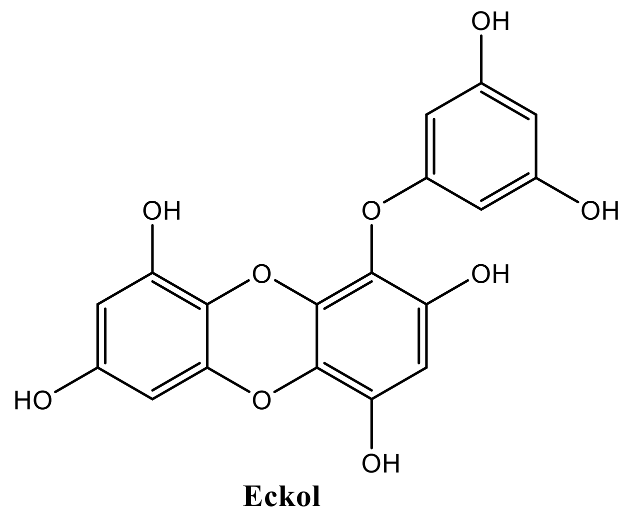
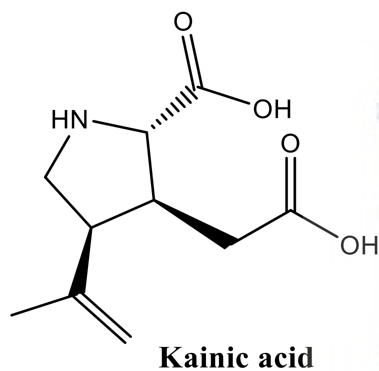
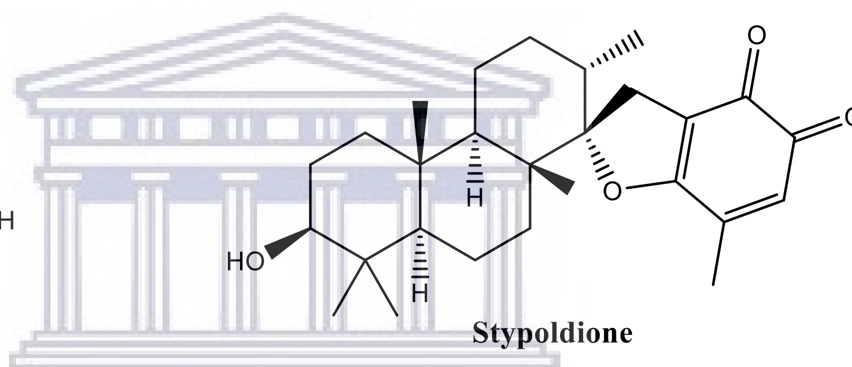


Eckol



Kainic acid



Stypoldione

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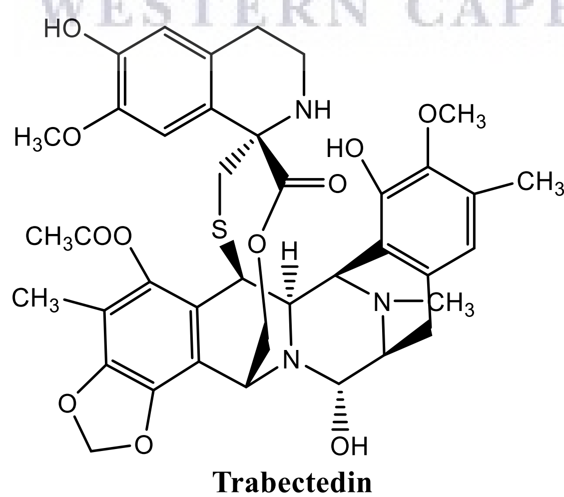

Trabectedin

Figure 1.2: Examples of natural products isolated from marine plants.

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1.4.1 Class of Natural Product

1.4.1.1 Phenolics

Phenolic compounds are classes of chemicals consisting of hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group (Mbaveng *et al.,* 2014), if a compound contains more than two or several hydroxylic groups is called polyphenol. Phenolics represent a diverse class of compounds including flavonoids, phenolic acids, coumarins, tannins, lignans, tocopherols, quinones and xanthones (Murphy *et al.,* 2014). Flavonoids are the biggest group of plant phenolics, representing more than half of the 8,000 phenolic compounds that are evident (Mbaveng *et al.,* 2014), but are also found in marine algae. Nearly 100 flavonoids have been isolated since the discovery of the first marine flavonoids in 1996 **(**Martins *et al.,* 2018**)** and the number is increasing as there is a growing interest concerning their biological activities (Cox *et al.,* 2010)**.**

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Chemically, flavonoids are based upon a 15-carbon skeleton consisting of two benzene rings, ring A and B linked via a heterocyclic pyran ring (C). They have a variety and structural diversity of classes such as flavonols, flavones, flavanones, flavan-3-ols, isoflavones and dihydrochalcones (Kumar and Pandey *et al.,* 2013; figure **1.3**). The different classes of flavonoids differ in the oxidation level and pattern of substitution of the C ring, whereas individual compounds within a class differ in the pattern of A and B rings (Middleton *et al.,* 1998). Seaweeds extracts contain appreciable amounts of phenolics (Chojnacka *et al.,* 2012), compared to brown seaweed species with high phenols concentration, green and red seaweeds have low phenol concentration (Holdt and Kraan *et al.,* 2011).

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While phenolic compounds from terrestrial plants are normally derived from gallic and ellagic acids, marine macroalgae phenolics range from simple molecules such as acids to highly complex compounds known as phlorotannins (Mekini´c *et al.,* 2019). Phlorotannins are a group of compounds formed by polymerization of phloroglucinol (1,3,5-trihydroxy benzene), which are unique compounds from brown marine algae (Li *et al.,* 2017). They have a distinctive structure that is not found in terrestrial plants (Freile-Pelegr´ın, and Robledo *et al.,* 2014). Furthermore, the relatively high concentration of phenolic compounds in marine algae species contributes to their antioxidant properties, which help to reduce harmful oxidative reactions to health (Urquiaga and Leighton *et al.,* 2000).

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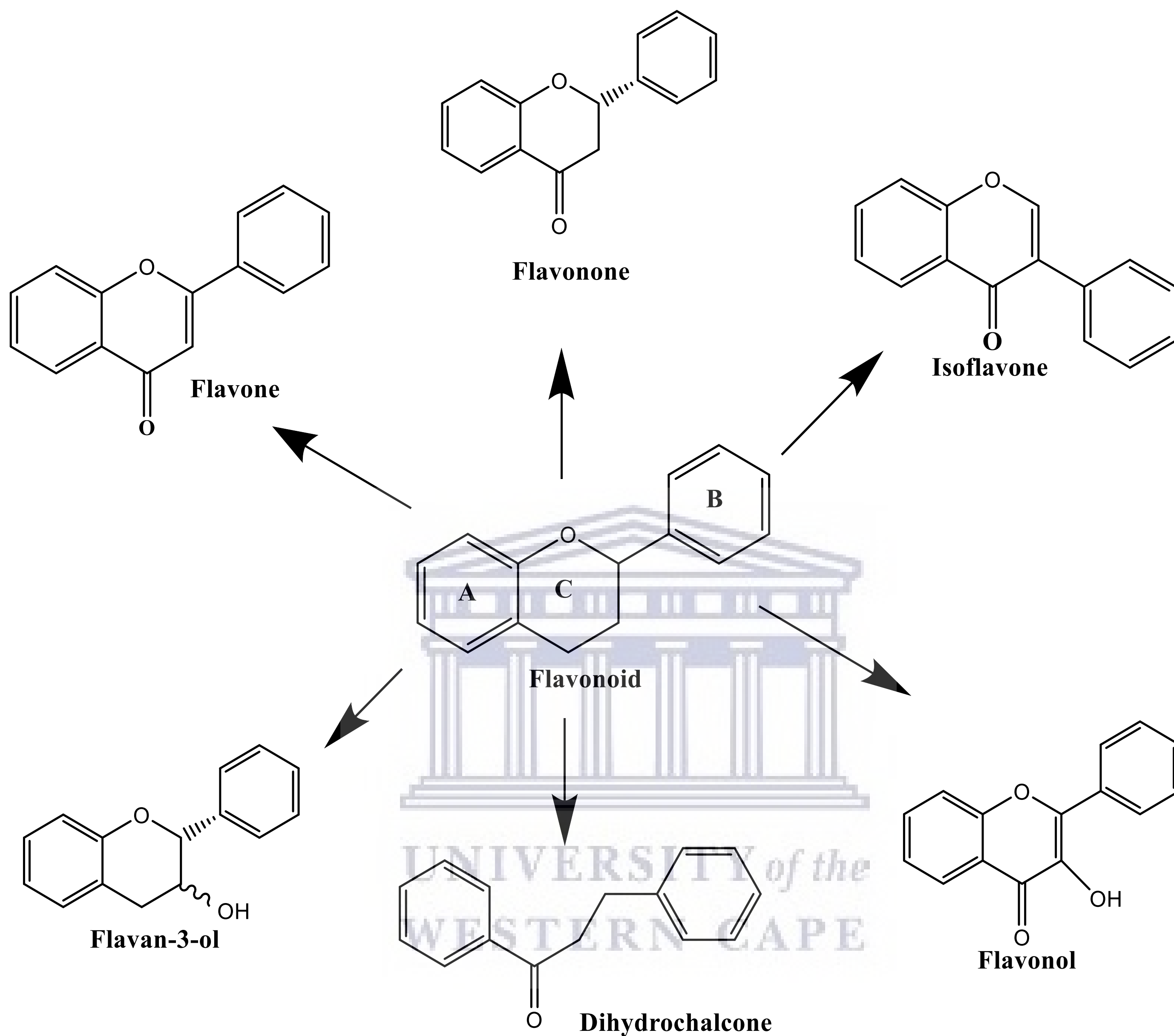

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Figure 1.3: Chemical structure of flavonoid and its derivatives from marine algae.

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1.4.1.2 Alkaloids

Alkaloids are naturally occurring chemical compounds comprising low molecular weight nitrogen and are typically alkaline because of the existence of the heterocyclic ring comprising a nitrogen atom (Matsuura *et al.,* 2015). Alkaloids are found in terrestrial plants, animals, fungi and marine plants, with more than 12, 000 known structures. They represent one of the biggest groups of natural products (Springob and Kutchan *et al.,* 2009). Alkaloid chemistry has been studied extensively in terrestrial plants, and around 30 000 alkaloid compounds have been isolated, but the number of marine algae studies reported is insignificant, with only 44 alkaloids have been isolated (Guven *et al.,* 2013).

Alkaloids found in marine algae are divided into three groups: Phenylethylamine alkaloids, indole and halogenated indole alkaloids and other alkaloids (Alghazeer *et al.,* 2013). The majority of isolated alkaloids from marine algae belong to groups of 2-phenylethlamine and indole. Halogenated alkaloids are not present in terrestrial plants and are specific to algae as well as other marine organisms (Pérez *et al.,* 2016). The majority of the indole alkaloid group are focused in red algae and green algae are rich in alkaloids containing chlorine and bromine (Guven *et al.,* 2013).

Furthermore, Several biological activities have been reported from marine alkaloids such as antifungal, anti-HIV, glucose uptake stimulatory, antileishmanial and anti-inflammatory **(**França *et al.,* 2014). An alkaloid, hordenine (figure **1.4**) which exhibits significant physiological effects (e.g., nicotine- and ephedrine-like effects) was isolated from *Phyllophora nervosa* (Rhodophyta) (Ito and Hori *et al.,* 1989). It was the first alkaloid isolated from a marine alga in 1969 (Güven *et al.,* 2010).

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1.4.1.3 Polysaccharides

Polysaccharides, also known as glycan, are polymers of simple sugars (monosaccharides) linked together by glycosidic bonds (Rajakumar *et al.,* 2018). Marine algae contain large amounts of polysaccharides, which are divided into three groups: Storage polysaccharide, structural polysaccharide and mucopolysaccharides (Holdt *et al.,* 2011). These polysaccharides chemical characteristics vary not only from those found in terrestrial plants, but also among the taxonomic classes of marine algae (Ito and Hori *et al.,* 1989). Polysaccharides are the most significant components of marine algae in terms of chemical content and in commercial value (Ito and Hori *et al.,* 1989). Marine polysaccharides have countless commercial applications such as thickeners, stabilisers, emulsifiers, food and feed (Kraan *et al.,* 2012).

Green algae contain sulphated galactans, sulphuric acid polysaccharide whereas the red algae contain carrageenans, amylopectin like sugar also known as floridean starch, water soluble sulphated galactan, as well as porhyran as mucopolysaccharide that is present in the intracellular spaces (Pal *et al.,* 2014). Brown algae contain alginic acid, β-1, 3 glucan or laminarian and they contain fucoidan as a polysaccharide comprising sulphated esters (Pezoa-Conte *et al.,* 2015).

Fucoidans refer to a group of sulphated polysaccharides mainly found in cell walls of various species of brown seaweed (Ale and Meyer *et al.,* 2013). They do not occur in other division of algae and plants. However, variant fucoidan were found in marine animal species such as sea cucumbers or sea urchins (Voa and Kim *et al.,* 2014), these polysaccharides are referred as sulphated fucans, which are simpler than fucoidans derived from brown algae. Chemically, fucoidans cover several different structural entities that designate a family of fucose containing sulphated polysaccharides (Ale and Meyer *et al.,* 2013). According to Ale *et al.,* (2011), fucoidans

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consist of polysaccharide backbone knows as type I or type II chains. Type I chains contain (1A3)-L-fucopyranose residues and type II is found to contain alternating (1A3) and (1A4)-linked L-fucopyranosyls residues. Fucoidan polysaccharides has numerous biological activities of pharmaceutical interest, including anti-tumor, immunomodulatory, anti-inflammatory, antiallergic, antidiabetes, anticoagulant, anti-hepatopathy and antiviral effects (Voa and Kim *et al.,* 2013).

1.4.1.4 Terpenoids

Terpenes are simple hydrocarbons, while terpenoids are modified class of terpenes with different functional groups and oxidized methyl group moved or removed at various positions (Perveen *et al.,* 2018). Terpenoids are a class of compounds predominantly isolated from marine algae in the 1970–1980s (Dias *et al.,* 2012). They are built up from five carbon isoprene units linked in a head-to-tail manner (Hanson *et al.,* 2003). More than half of the reported secondary metabolites from macroalgae are isoprenoids (Stratmann *et al.,* 1992). Terpenoids are divided into monoterpenes, sesquiterpenes, diterpenes, sesquiterpenes, and triterpenes depending on its carbon units (Perveen *et al.,* 2018).

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Many terpenes have shown potent biological activities against cancer, malaria, inflammation, and a variety of infectious diseases such as viral and bacterial and are used for the treatment of human diseases (Mbaveng *et al.,* 2014).

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1.4.1.5 Steroids

Steroids are terpenoid lipids characterized by the sterane or steroid nucleus that consists of four interconnected rings referred to as A, B, C and D, a hydroxyl group (OH) at C-3, and a side chain (Figure **1.5**) (Lopes *et al.,* 2013). The OH group at C-3 of ring A contributes to hydrogen-bond interactions, while the conformation and length of the side chain and the stereochemistry of the C-24 alkyl group in ring D are key to intermolecular interactions (Milovanovic´ *et al.,* 2009). Steroids differ by the functional groups attached to these rings and the oxidation state of the rings. The specificity of their unique biological actions is because of the various groups attached to a common nucleus. Steroids include a massive group of materials that mediate a completely numerous sets of biological responses (Bhawani *et al.,* 2010). The maximum widespread within the body is cholesterol, a crucial element of cell membranes and the place to begin for the synthesis of different steroids.

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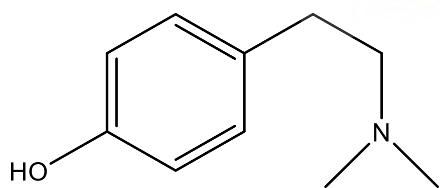

Figure 1.4: Hordenine

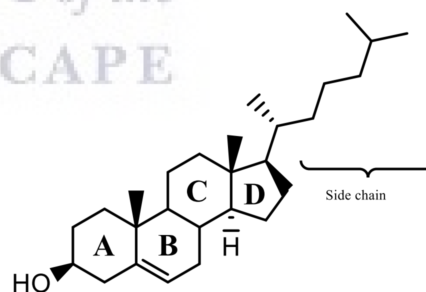

Figure 1.5: Basic structure of steroids

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1.5 Review of *Ulva* genus (Chlorophyta)

Chlorophyta genus *Ulva*, commonly known as “sea lettuce” are one of the well-known algae species in the world. *Ulva genus*, together with Fucus, Conferva and Chara, were some of the original algal general described by Linnaeus in Species Plantarum (1753). *Ulva genus* (Division: Chlorophyta; Class: *Ulvophyceae*; Order: *Ulvales*, Family: *Ulvaceae*), is a green alga which was first identified by Linnaeus in 1753 (Silva *et al.,* 2013). *Ulva genus* is well known for its wide distribution in marine, freshwater and brackish environments throughout the world as shown in Figure **1.6** (Wichard *et al.,* 2015).

Ulva genus is also named *Enteromorpha* as its synonym based on the molecular data (Fanna *et al.,* 2011). *Ulva* and *Enteromorpha* life histories consist of morphologically similar haploid and diploid phases, both of which reproduce by haploid or diploid asexual zoospores formed by mitotic division of vegetative cells (Fakhry *et al.,* 2007). The approved taxa from this genus is currently 196, including 132 species, 6 subspecies, 26 variants, and 32 forms; as per updated status from January 2018 (Rybak *et al.,* 2018).

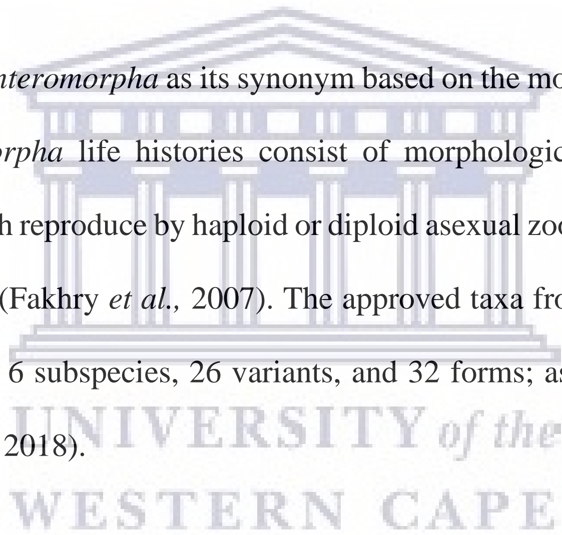

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Figure 1.6: Distribution map of *Ulva* genus (green algae) in the World

1.5.1 Commercial use and value of *Ulva* genus in the world

Moreover, since there is an increase in the *Ulva* biomass in the world, and the fact that green seaweeds are considered to be underexploited or barely exploited natural resources (Rodríguez and Guerra *et al.,* 2016), the scientific world has become more interested in its beneficial biological properties. About 42 countries utilize algal species, where at least 32 Chlorophytes have been recorded along with their uses as summarized in Table **1.2** (White and Ohno *et al.,* 1999).

Ulva genus is an important food source in many south-east Asian countries (Farasat *et al.,* 2013). According to Amosu (2006) the species of *Ulva*, like most vegetables, are among the healthiest foods on the planet, containing essential vitamins, minerals and antioxidants. On average chemical composition of *Ulva* is 9–14% protein, 2–3.6% ether extract (n-3 and n-6 fatty acids 10.4 and 10.9 g/100 g of total fatty acid); 32–36% ash (Rodríguez and Guerra *et al.,* 2016).

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Table 1.2. Algal species utilized world-wide, country and uses.

Species	Use	Country
Chlorophyta		
Acetabularia major	M	Indonesia Philippines
Capspsiphon fulvescens	F	Korea
Caulerpa spp.	F	Malaysia, Thailand
Caulerpa lentillifera	F, M	Philippines
Caulerpa peltata	F, M	Philippines
Caulerpa racemosa	F M	Bangladesh, Japan, Philippines, South Pacific Islands, Vietnam Philippines
Caulerpa sertularioides	F, M	Philippines
Caulerpa taxifolia	F, M	Philippines
Codium spp.	F	Philippines
Codium bartletti	F	Argentina
Codium edule	F	Philippines
Codium fragile	F	Philippines
Codium muelleri	F	Korea, Philippines
Codium taylori	F	Hawaii
Codium tenue	F	Israel
Codium tomentosum	F	Indonesia
Colpomenia sinuosa	F	Indonesia
Dictyosphaeria cavernosa	Ag	Kenya

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	M	Philippines
Enteromorpha spp.	Ag F	Portugal Bangladesh, France, Hawaii Myanmar
Enteromorpha clathrata	F	Korea
Enteromorpha grevillei	F	Korea
Enteromorpha intestinalis	F M	Indonesia, Japan, Korea Indonesia
Enteromorpha linza	F	Korea
Enteromorpha nitidum	F	Korea
Enteromorpha prolifera	F M	Indonesia, Japan, Korea, Philippines Indonesia
Monostroma nitidum	F	Japan
Scytosiphon lomentaria	F	France, Korea
Ulva ***spp.***	Ag F P	Italy. Portugal Argentina, Canada, Chile Hawaii, Japan, Malaysia Italy
Ulva lactuca	F	Vietnam, Indonesia
Ulva pertusa	M	Philippines
Ulva reticulata	F	Vietnam

*****(F = food, A = agar, C = carrageenan, Al = alginate, M = medicine, RoK = Roe on Kelp, Ag = Agricultural, P = paper.)

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1.5.2 Phytochemistry of Green Algae

A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids, have been reported from various marine green algae, the large majority of these are terpenoids compounds (Silva *et al.,* 2013) but fatty acids are also common with nitrogenous compounds (Heo *et al.,* 2005). Green algae are known to produce compounds similar to those from red algae, primarily functionalized sesquiterpenoids and diterpenoids (Melany *et al.,* 2004). However, they are lacking the extensive halogenation of the red algal compounds, for example *Caulerpa brownii* from Australia was reported to yield several bioactive novel diterpenoids and terpenoid esters (Chakraborty *et al.,* 2010).

Furthermore, the main phytosterol found in green algae, results from the alkylation of 24 methylene cholesterol which leads to the production of an isomer of fucosterol, called "Isofucosterol" (Murata and Nakazoe *et al.,* 2001), for example the green alga *Ulva lactuca* Linnaeus contains fucosterol and cholesterol as the most abundant terpenoids. Generally, C29 sterols are the major compounds in brown and green algae (Lopes *et al.,* 2013). However, algae belonging to the Chlorophyceae division present more complex sterols with an asymmetric centre at C-24, like those found in higher plants (Kapetanovic *et al.,* 2005).

The chemistry and biological importance of the isolated compounds and extracts from the green algae with biological activity such as antioxidant, anti-inflammatory, Antifungal, Anti-cancer and antibacterial are highlighted in Table **1.3**.

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Table 1.3: Biological active compounds and extracts from green algae.

Compound	Plant Source	Biological Activity	Reference
Capisterones A & B A = Ac B = H	*Panicillus capitatus*	Antifungal	(Puglisi *et al.,* 2004)
3-O-b-glucopyranosylstigmasta-5,25-diene	*Ulva Lactuca*	Anti-inflammatory	(Awad *et al.,* 2000)
Dimethylsulfoniopropionate		Anti-cancer	(Chakraborty *et al.,* 2010)
Taxifolione	*Caulerpa taxofolia*	Anti-microbial	(Guerriero *et al.,* 2003)
7,7-didehydro-6-hydroxy-6,7-dihydrocaulerpenyne		Anti-microbial	(Lee *et al.,* 2013)
Diterpene Aldehyde	*Halimeda tuna*	Ant-viral	(Koehn *et al.,* 1991)
Beta-caroten	*Dunaliella berdawil*	Anti-oxidant	(Lavy *et al.,* 2003)
Lycopene	*Chlorella marina*	Anti-inflammatory	(Renju *et al.,* 2013)
(1Z,3E)-2-(1-acetoxy-2-(2,6,6-trimethylcyclohex-2-en-1-yl) ethyl buta-1,3-diene-1,4-diyl diacetate.	*C. ashmeati*	Anti-microbial	(Paul *et al.,* 1987)
(E)-2-formyl-4-(2,6,6-trimethylcyclohex-2-en-1-yl) but-2-en-1-yl acetate.	*C. ashmeati*	Anti-microbial	(Paul *et al.,* 1987)

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(E)-2-(2-(2,6,6-trimethylcyclohex-2-en-1-yl) ethylidene) succinaldehyde.	Anti-microbial	(Paul *et al.,* 1987)
(E)-5-oxo-1-(2,6,6-trimethylcyclohex-2-en-1-yl) pent-2-en-3-yl acetate.	Anti-microbial	(Paul *et al.,* 1987)

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Continued….

(3E,3'E)-4,4'-(3,6-dimethyl-1,2-phenylene) bis(2-methylbut-3-en-2-ol)	*Caulerpa Racemosa*	Anti-fungal	(Liu *et al.,* 2013)
(1R,2S,3S)-2-(2-hydroxypropan-2-yl)-4,7-dimethyl-3-(2-methylprop-1-en-1-yl)-2,3-dihydro-1H-inden-1-ol	*Caulerpa Racemosa*	Anti-fungal	(Liu *et al.,* 2013)
Kahalalide F	*Bryopsis. Sp*	cytotoxic, antiviral and antifungal	(*Hamann et al., 1993*)
Methanol Ext.	*Ulva clathrata*(Roth)C.Agardh	Anti-tumorigenic, blood anticoagulant activity	(Tang *et al.,* 2004; Shanmugam *et al.,* 2001)
Methanol Ext.	*Ulva linza* Linnaeus	Antibacterial and anti-inflammatory activity	(Sukatar *et al.,* 2006; Khan *et al.,* 2008)
Methanol Ext.	*Ulva flexuosa* Wulfen	Cytotoxicity against breast ductal carcinoma cell line, high antibacterial activity	(Khanavi *et al.,* 2012)
Methanol Ext.	*Ulva intestinalis* Linnaeus	Antibacterial and antihemolytic activities	(Soltani *et al.,* 2012)

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1.6 Plant Species investigated: *Ulva capensis*

Ulva capensis is a green seaweed which grows well along the coast of the Western Cape peninsula and has been of interest to the South African agriculture and pharmaceutical industries (Stegenga *et al*., 1997). Some members of the *Ulva* genus have been identified as functional foods in some oriental countries and they produce various health benefits, including hepatoprotective, antifungal, anticholinergic, antiadipogenic, blood cholesterol reducing, and blood vessel thrombosis prevention. To our knowledge this species has not been subjected to phytochemical and biological studies, and thus motivated the study which is reported herewith.

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Figure 1.7: Photograph of *Ulva capensis* (source: www.ispotnature.org/ulva-capensis)

Taxonomy:

Kingdom: *Plantae*

Phylum: *Chlorophyta*

Class: *Ulvophyceae*

Order: *Ulvales*

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Family: *Ulvaceae*

Genus: *Ulva*

Species: *Ulva capensis*

1.7 Problem statement

Although green algae (Chlorophyta) are abundant and comparatively easy to gather, their chemistry remains underexplored. They are the least producers of natural compounds compared to brown (Phaeophyta) and red algae (Rhodophyta) (Khanavi *et al.,* 2012). Regardless of their wide utilization in traditional medicine, very little is known about the bioactivity of these seaweeds and up to now, scientific documentation of their bioactivities is minimal (Chia *et al.,* 2015). A comprehensive investigation for the presence of chemical constituents on *Ulva capensis* will be carried out.

1.8 Justification

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Seaweed produces metabolites aiding in the protection against different environmental stresses. These compounds show antiviral, antiprotozoal, antifungal, and antibacterial properties. Macroalgae can be cultured in high volumes and would represent an attractive source of potential compounds useful for unconventional drugs able to control new diseases or multiresistant strains of pathogenic microorganisms. Among all seaweeds, the green seaweeds with less than 300 known compounds are the least producers of natural products when compared to red and brown seaweeds. However, a wide range of compounds, predominantly terpenes and steroids have been reported in various marine green algae. This research should reveal the main constituents which may be used as part of commercial products for the

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cosmeceutical and pharmaceutical industries. The relationships of structure and activity are important for the study.

1.9 Aims of this study

The aim of the project is to investigate the phytochemical composition of *Ulva capensis* by performing solvent extractions techniques and to characterize the isolated compounds.

1.10 Objectives of this study

The main objectives of the study are:

- Extraction of the plant material with methanol.
- To Partition the methanol crude extract by solvent extraction.
- Conduct the preliminary screening on the crude extracts using TLC to identify the various classes of compounds.
- To carry out fractionation of the organic extracts, towards isolation of pure compounds using various chromatographic techniques.
- Characterization of the pure isolates: Pure compounds to be characterized with spectroscopic techniques.

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Chapter Two

Material and reagents

2.1 Experimental Activities

2.1.1 Reagents, Equipment's and Materials

2.1.1.1 Chemicals and Solvents

Solvents that were used in this project were purchased from Kimix Chemical and Lab Supplies and Sigma-Aldrich Chemical Company, which are n-hexane, dichloromethane, ethyl acetate, methanol, deuterated chloroform and deuterated methanol. Sulphuric acid which was used to prepare a reagent called vanillin spray.

2.1.1.2 Equipment's

BÜCHI Rotavapor RE 11 with a water bath at 45°C was used for solvent evaporation, and a freeze-dryer.

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2.1.2 Spectroscopy

2.1.2.1 Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectra were acquired on a 100 MHz frequency Bruker NMR machine (Sigma-Aldrich Norell® Standard Series™ 5 mm NMR tubes) at the University of the Western Cape. Solvent used to prepare samples was deuterated chloroform ($CDCl_3$). The chemical shift of H_1 (δ_H) and C_{13} (δ_C) were measured in δ (ppm) relative to TMS (Tetramethyl silane) as internal standard and coupling constant J in Hz.

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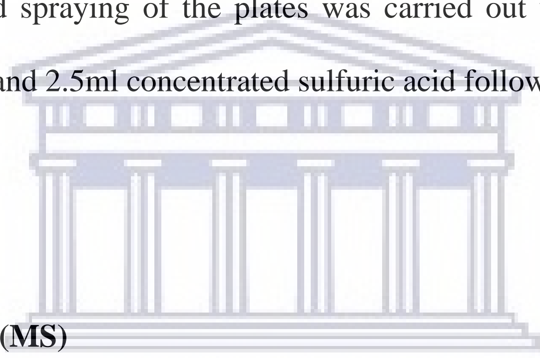
2.1.3 Chromatography Techniques

2.1.3.1 Column Chromatography (CC)

Silica gel 60 (0.040-0.063mm) 230-400 mesh particle size (Merck) was wet packed in a glass column (20-25mm diameter) for column chromatography.

2.1.3.2 Thin Layer Chromatography (TLC)

TLC was performed using Merck Silica gel 60 PF_{254} on a glass (20cm x 20cm) with a thickness of 0.2 mm. Isolated compounds (single spot on the TLC) were visualised under a UV light at (254 mm or 366 mm) and spraying of the plates was carried out using vanillin a mixture containing 15g of vanillin and 2.5ml concentrated sulfuric acid followed by heating until spots were visible.



2.1.4 Mass Spectrometry (MS)

2.1.4.1 Gas Chromatography Mass spectrometry (GC-MS)

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Separation of FAME (fatty acids methyl esters) was achieved on an Agilent technology mass spectroscopy using a TRACE TR-FAME GC column (30 m × 0.25 mm × 0.2 µm). Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. For GC separation, sample injector was maintained at 250°C and sample injection volume was kept at 10 µL. GC-MS analysis was performed at the University of the Western Cape.

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2.2 Seaweed Material

2.2.1 Preparation of plants

A dry alga material (1.8 kg) of *Ulva capensis* (see figure **2.1**) was provided by the CMD Industries, which was already cleaned/washed of salt, epiphytes and other contaminants. About half of the material (0.5 kg) was blended with an overhead stirrer to break down into small pieces and the other half of the material was kept in a cool room until use. The material was macerated with 3L of 20 % H_2O and 80 % MeOH for 62 hours. The MeOH extract was filtered with a cloth and a Whatman filter paper and, the residue was washed with MeOH repeating the process four times. The total extract was concentrated under a reduced pressure on a rotary evaporator to remove MeOH, which was followed by freeze-drying.

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Figure 2.1 Dry *Ulva capensis* material.

2.2.2 Preparation of crude extracts

2.2.2.1 Extraction

The MeOH crude extract form freeze-dryer weighing 182.09 g was grounded to powder and it was then subjected to solvent-solvent partitioning from increasing polarity (600 mL of H_2O and 400 mL of organic solvent mixed in a 5L prep flask) as shown in the Scheme **2.1**.

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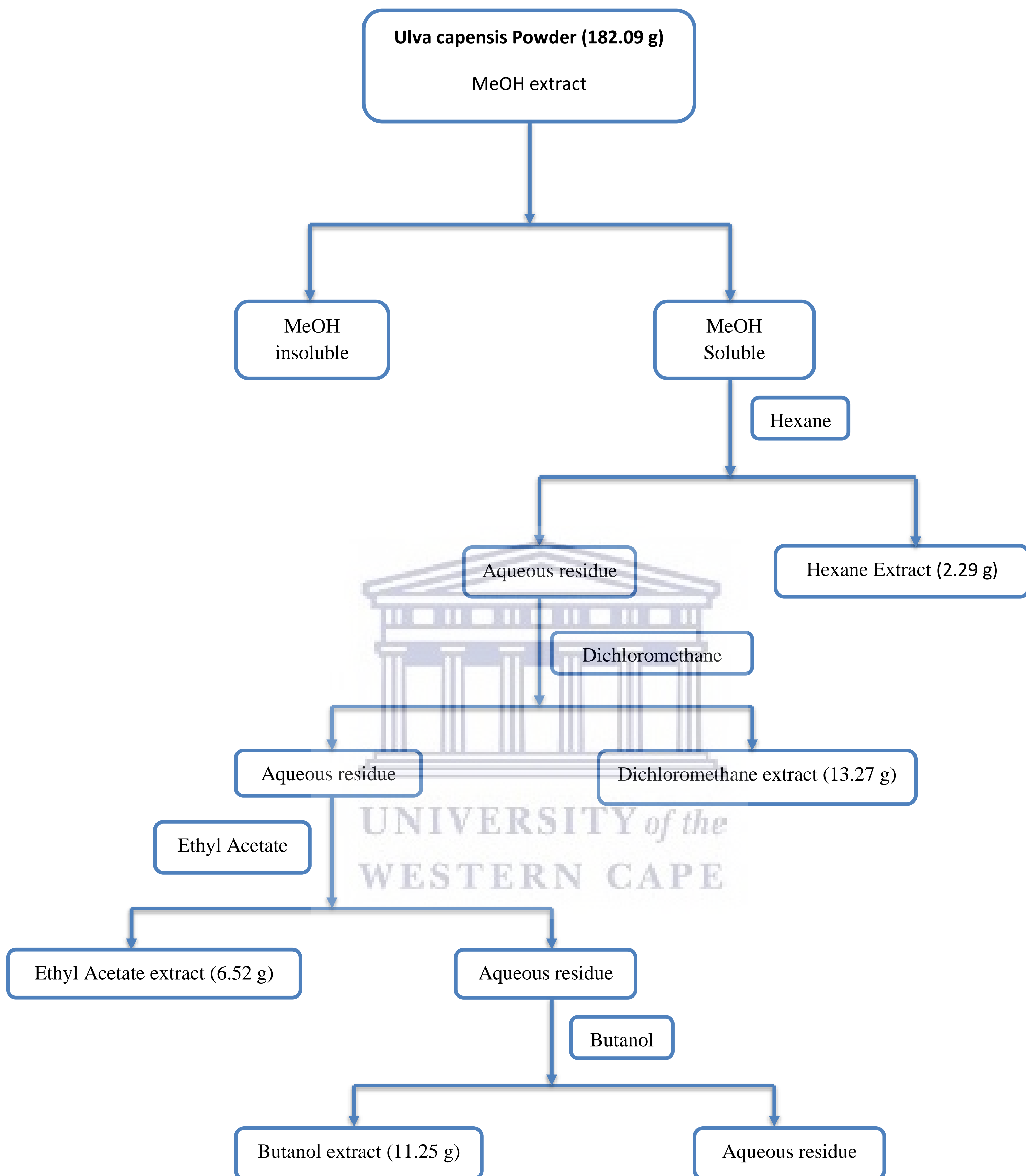

Scheme 2.1: A schematic diagram representing the preparation of the crude extracts.

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2.3 Isolation and purification natural products from *Ulva capensis*

2.3.1 Fractionation of Hexane Extract

The hexane extract (2.29 g) was pre-adsorbed on a silica gel and loaded on a column for fractionation by gravity elution using gradient mixtures of increasing polarity, Hex : EtOAc at the following ratios ; (90:10), (80:20), (70:30), (60:40), (50:50), (40:60), (30:70), (20:80), (10:90) and (100:0) followed by EtOAc : MeOH at the following ratios; (95:5), (90:10), (85:15), (80:20), ending with (70:30). Fractions were collected and analysed using TLC, those with the same Rf were pooled together (SII and SIII) and concentrated on a rotary evaporator. Fraction SIII was eluted with n-Hex : EtOAc (8:2) and showed a single spot on a TLC plate, which was sent for NMR and was identified as compound **1**.

The other fraction SII was further purified by introducing it into a flash column using Hex: EtOAc (9:1) after being dissolved in the same solvent system. This led to isolation of compound **2** and compound **3**.

2.3.2 Fractionation of Ethyl Acetate extract

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The EtOAc extract (6.52 g) was subjected to a column after pre-adsorption of extract on silica gel. Fractionation was achieved through gravity column chromatography with the use of gradient elution. Eluents employed were 500 mL volumes of Hex: EtOAc mixtures at ratios; (100:0), (70:30), (50:50), (30:70), (0:100) finally adding small ratios of MeOH by 500mL volumes of EtOAc: MeOH mixtures at ratios; (95:5), (90:10) and (85:15). Fractions were collected and analysed by TLC using Hex: EtOAc (70:30) fractions with the same profile were pooled together, concentrated on a rotary evaporator, which was then dissolved in a mixture of Hex: DCM (90:10) before being introduced into a flash column. Fractions were collected and pooled together, the fraction was subjected into a prep-TLC using DCM: EtOAc (95:5) to

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obtain a mixture of fatty acid methyl esters (FAME), which lead to the isolation of compound **4, 5** and **6**.

2.4 Spectroscopic Data of the isolated Compounds

2.4.1 Compound 1

Isolated as a white powder (20.7 mg) with a chemical formula of $C_{29}H_{48}O$. ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 1.92 (2H, m, H-1), δ 1.90 (2H, m, H-2), δ 3.51 (1H, m, H-3), δ 2.24 (2H, d, J=1.76 Hz, H-4), δ 5.33 (1H, br. d, J=5.0 Hz, H-6), δ 1.50 (2H, m, H-7), δ 2.04 (1H, m, H-8), δ 0.94 (1H, m, H-9), δ 0.92 (2H, m, H-11*ax*), δ 1.02 (2H, m, H-11*eq*), δ 2.01 (2H, m, H-12), δ 0.97 (1H, m, H-14), δ 1.58 (2H, m, H-15), δ 2.06 (2H, m, H-16), δ 1.11 (1H, m, H-17), δ 0.68 (3H, s, H-18), δ 1.00 (3H, s, H-19), δ 0.95 (1H, q, J=6.6 Hz, H-20), δ 0.93 (3H, d, J=6.6 Hz, H-21), δ 1.47 (2H, m, H-22), δ 1.76 (2H, m, H-23), δ 2.80 (1H, sept, H-25), δ 0.97 (3H, d, J=6.8 Hz, H-26, H-27), δ 5.10 (1H, q, J= 6.6 Hz, H-28), δ 1.57 (3H, d, J=6.6 Hz, H-29). ^{13}C-NMR (100MHz, $CDCl_3$, ppm): δ 37.3 (C-1), δ 31.7 (C-2), δ 71.3 (C-3), δ 42.3 (C-4), δ 140.8 (C-5), δ 121.7 (C-6), δ 31.9 (C-7, C-8), δ 50.1 (C-9), δ 36.5 (C-10), δ 21.1 (C-11,C-26, C-27), δ 39.8 (C-12), δ 42.3 (C-13), δ 56.8 (C-14), δ 24.3 (C-15), δ 28.2 (C-16), δ 56.0 (C-17), δ 11.9 (C-18), δ 19.4 (C-19), δ 36.2 (C-20), δ 18.8 (C-21), δ 36.0 (C-22), δ 27.9 (C-23), δ 145.9 (C-24), δ 28.6 (C-25), δ 116.4 (C-28), δ 12.8 (C-29).

2.4.2 Compound 2 and 3

Both compounds **2** and **3** were isolated as a mixture of epimers, a colourless oil (5.1 mg), having the same chemical formula $C_{29}H_{48}O_2$, (M.W 428).

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2.4.2.1 Compound 2

^{1}H NMR (400MHz, $CDCl_3$): δ 1.82 (2H, m, H-1), δ 1.95 (2H, m, H-2), δ 3.53 (1H, m, H-3), δ 2.28 (2H, s, H-4), δ 5.35 (1H, br. d, J=5.2 Hz, H-6), δ 1.84 (1H, m, H-7), δ 1.40 (1H, m, H-8), δ 0.93 (1H, m, H-9), δ 1.48 (2H, m, H-11), δ 2.01 (2H, m, H-12), δ 0.99 (1H, m, H-14), δ 1.58 (2H, m, H-15), δ 1.85 (2H, m, H-16, H-22), δ 1.13 (1H, m, H-17), δ 0.69 (3H, s, H-18), δ 0.90 (3H, s, H-19), δ 1.41 (1H, m, H-20), δ 0.85 (3H, d, J=6.8 Hz, H-21), δ 1.26 (2H, m, H-23), δ 1.25 (1H, m, H-25), δ 1.02 (3H, d, J=6.8 Hz, H-26), δ 0.95 (3H, d, J=6.6 Hz, H-27), δ 5.72 (1H, dd, J=17.7, 1.4 Hz, H-28), δ 5.14 (1H, dd, J=17.9, 1.4 Hz, H-29a), δ 5.18 (1H, dd, J=12.7, 1.5 Hz, H-29b). ^{13}C-NMR (100MHz, $CDCl_3$): δ 37.2 (C-1), δ 31.9 (C-2), δ 71.8 (C-3), δ 42.3 (C-4, C-13) δ 140.7 (C-5), δ 121.7 (C-6), δ 31.7 (C-7), δ 36.2 (C-8), δ 50.1 (C-9), δ 36.5 (C-10), δ 21.1 (C-11), δ 39.7 (C-12), δ 56.7 (C-14), δ 24.3 (C-15), δ 28.4 (C-16), δ 55.9 (C-17), δ 11.9 (C-18), δ 16.6 (C-19), δ 35.9 (C-20), δ 17.7 (C-21), δ 31.6 (C-22), δ 28.3 (C-23), δ 89.1 (C-24), δ 29.7 (C-25), δ 19.4 (C-26), δ 18.9 (C-27), δ 137.1 (C-28), δ 116.3 (C-29).

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2.4.2.2 Compound 3

^{1}H NMR (400MHz, $CDCl_3$): δ 1.82 (2H, m, H-1), δ 1.95 (2H, m, H-2), δ 3.53 (1H, m, H-3), δ 2.28 (2H, s, H-4), δ 5.35 (1H, br. d, J=5.2 Hz, H-6), δ 1.84 (1H, m, H-7), δ 1.40 (1H, m, H-8), δ 0.93 (1H, m, H-9), δ 1.48 (2H, m, H-11), δ 2.01 (2H, m, H-12), δ 0.99 (1H, m, H-14), δ 1.58 (1H, m, H-15), δ 1.85 (2H, m, H-16, H-22), δ 1.13 (1H, m, H-17), δ 0.69 (3H, s, H-18), δ 0.90 (3H, s, H-19), δ 1.41 (1H, m, H-20), δ 0.85 (3H, d, J=6.8 Hz, H-21), δ 1.26 (2H, m, H-23), δ 1.25 (1H, m, H-25), δ 1.02 (3H, d, J=6.8 Hz, H-26), δ 0.89 (3H, d, J=6.9 Hz, H-27), δ 5.78 (1H, dd, J=17.4, 11.3 Hz, H-28), δ 5.29 (1H, dd, J=17.7, 1.4, H-29a), δ 5.26 (1H, dd, J=11.6, 1.4 Hz, H-29b). ^{13}C-NMR (100MHz, $CDCl_3$): δ 37.2 (C-1), δ 31.9 (C-2), δ 71.8 (C-3), δ 42.3 (C-4, C-13) δ 140.7 (C-5), δ 121.7 (C-6), δ 31.7 (C-7), δ 36.2 (C-8), δ 50.1 (C-9), δ 36.5 (C-10), δ 21.1 (C-11), δ 39.7 (C-12), δ 56.7 (C-14), δ 24.3 (C-15), δ 28.4 (C-16), δ 55.9 (C-17), δ 11.9 (C-

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18), δ 16.6 (C-19), δ 35.9 (C-20), δ 17.7 (C-21), δ 31.6 (C-22), δ 28.3 (C-23), δ 89.2 (C-24), δ 29.7 (C-25), δ 19.4 (C-26), δ 16.7 (C-27), δ 137.2 (C-28), δ 116.4 (C-29).

2.4.3 Compound 4, 5 and 6

Compounds **4**, **5** and **6** (6.5mg) were isolated as a mixture of fatty acids methyl ester (FAME).

2.4.3.1 Compound 4

Isolated as white flakes with a chemical formula of $C_{19}H_{30}O_2$, GC-MS, m/z 290.22 $[M]^+$. ^1H NMR (400MHz, $CDCl_3$): δ 2.27 (2H, t, J=7.4 Hz, H-2), δ 1.61 (2H, m, H-3), δ 1.41 (2H, m, H-4), δ 2.08 (2H, m, H-5, H-17), δ 5.37 (1H, m, H-6, H-7, H-9, H-10, H-12, H-13, H-15, H-16), δ 2.81 (2H, m, H-8, H-11, H-14), δ 0.97 (3H, t, J=7.5 Hz, H-18), δ 3.66 (3H, s, -OC**H**$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): δ 174.1 (C-1), δ 33.9 (C-2), δ 25.5 (C-3), δ 29.4 (C-4), δ 34.3 (C-5), δ 130.3 (C-6), δ 127.9 (C-7), δ 34.1 (C-8), δ 128.2 (C-9), δ 128.4 (C-10), δ 36.3 (C-11), 128.5 (C-12), δ 128.6 (C-13), δ 34.0 (C-14), δ 128.1 (C-15), δ 132.0 (C-16), δ 26.9 (C-17), δ 14.3 (C-18), δ 51.6 (-O**C**H$_3$).

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2.4.3.2 Compound 5

Isolated as white flakes with a chemical formula of $C_{19}H_{34}O_2$, GC-MS, m/z 294.25 $[M]^+$. ^1H NMR (400MHz, $CDCl_3$): δ 2.27 (2H, t, J=7.4 Hz, H-2), δ 1.61 (2H, m, H-3), δ 1.41 (2H, m, H-4, H-5, H-6, H-7, H-15, H-16, H-17), δ 2.08 (2H, m, H-8, H-14), δ 5.37 (1H, m, H-9, H-10, H-12, H-13), δ 2.81 (2H, m, H-11), δ 0.97 (3H, t, J=7.5 Hz, H-18), δ 3.66 (-OC**H**$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): δ 174.3 (C-1), δ 33.9 (C-2), δ 25.5 (C-3), δ 29.1 (C-4), δ 29.4 (C-5), δ 29.7 (C-6), δ 29.2 (C-7), δ 28.0 (C-8), δ 130.3 (C-9), δ 127.0 (C-10), δ 25.6 (C-11), δ 127.1 (C-12), δ 129.7 (C-13), δ 27.2 (C-14), δ 29.6 (C-15), δ 31.9 (C-16), δ 22.8 (C-17), δ 14.3 (C-18), δ 51.6 (-O**C**H$_3$).

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2.4.3.3 Compound 6

Isolated as white flakes with a chemical formula of $C_{19}H_3O_2$, GC-MS, m/z 292.45 $[M]^+$. ^1H NMR (400MHz, $CDCl_3$): δ 2.27 (2H, t, J=7.4 Hz, H-2), δ 1.61 (2H, m, H-3), δ 1.41 (2H, m, H-4, H-5, H-6, H-7), δ 2.08 (2H, m, H-8, H-17), δ 5.37 (1H, m, H-9, H-10, H-12, H-13, H-15, H-16), δ 2.81 (2H, m, H-11, H-14), δ 0.97 (3H, t, J=7.5 Hz, H-18), δ 3.66 (-OC**H**$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): δ 173.6 (C-1), δ 33.9 (C-2), δ 24.9 (C-3), δ 29.1 (C-4), δ 29.4 (C-5), δ 29.7 (C-6), δ 29.2 (C-7), δ 27.2 (C-8), δ 130.3 (C-9), δ 127.0 (C-10), δ 25.6 (C-11), δ 128.5 (C-12), δ 128.6 (C-13), δ 25.5 (C-14) , δ 128.0 (C-15), δ 132.0 (C-16) δ 20.6 (C-17), δ 14.3 (C-18) and δ 51.6 (-O**C**H$_3$).

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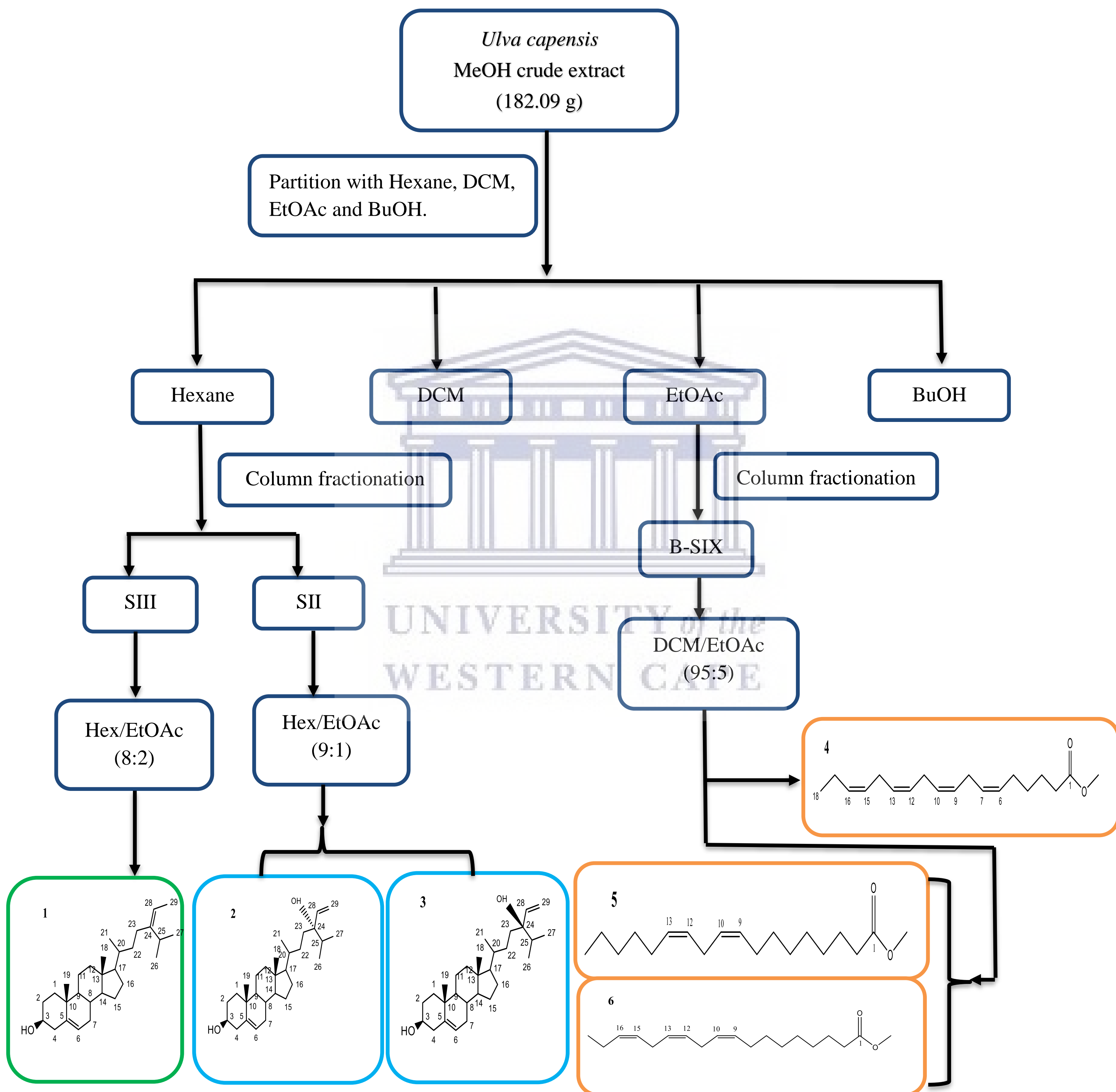
CHAPTER 3

RESULTS AND DISCUSSION



Scheme 2.2: Schematic diagram for the isolation and purification of constituent's form *Ulva capensis*.

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Seaweed material *Ulva capensis* was extracted with MeOH (80%) to yield a crude extract, which was fractionated by solvent-solvent partition with Hex, DCM, EtOAc and BuOH, followed by column fractionation of the four extracts which yielded variety of fractions. Two fractions (SII and SIII) from the hexane extract were subjected to column fractionation using Hex : EtOAc (8:2) and Hex : EtOAc (9:1) as solvent systems which led to the isolation of compound **1**, **2** and **3,** from the EtOAc extract fraction B-SIX was subjected to column fractionation using DCM : EtOAc (95:5) as solvent system which led to the isolation of compound **4**, **5** and **6**, as indicated in scheme **2.2**. No compounds were isolated from the DCM and BuOH extracts as the amounts obtained from column fractionation were too small to further conduct other fractionation techniques.

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3.1 Compound 1

Fraction SIII was further purified by repeated column chromatography which led to the isolation of compound **1** (Figure **3.1**), which gave a dark blue color on TLC thus showing a positive response for a terpenoid upon spraying with the vanillin H_2SO_4 spray (reagent).

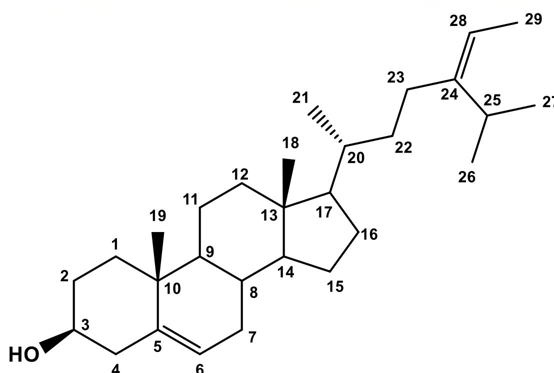

Figure 3.1: Chemical Structure of Compound **1**

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Compound **1** (20.7 mg) was isolated as a white powder and had the molecular formula $C_{24}H_{48}O$. The steroidal nature of compound **1** was deduced from a combination of NMR-experiments 1D (^{1}H, ^{13}C, DEPT 135) and 2D (COSY, HMBC and HSQC), Table **3.1**.

^{1}H NMR spectrum (Appendix 1c) data showed two olefinic protons one which gave a broad doublet at δ 5.33 (1H, br. d, J=5.0 Hz, H-6) and another quartet at δ 5.10 (1H, q, J= 6.6 Hz, H-28). Three doublets at δ 0.93 (3H, J = 6.6 Hz, H-21), δ 0.97 (6H, J = 6.8, H-26, H-27) and δ 1.57 (3H, J = 6.6 Hz, H-29). There are two singlets at δ 0.68 (3H, s, H-18) and δ 1.00 (3H, s, H-19). The chemical shifts and the coupling patterns of proton signals for the six methyl groups at δ 0.68 (3H, s, H-18), δ 1.00 (3H, s, H-19), δ 0.93 (3H, d, J=6.6 Hz, H-21), δ 0.97 (3H, d, J=6.8 Hz, H-26), δ 0.97 (3H, d, J=6.8 Hz, H-27) and δ 1.57 (3H, d, J=6.6 Hz, H-29) indicated that compound **1** has sterol moieties with an allyl methyl (Bang *et al.,* 2011). An oxygenated methine proton signal at δ 3.51 (1H, m) indicated the presence of (-OH). Furthermore, two olefinic proton signals at δ 5.34 (1H, br. d, J=5.0 Hz, H-6) and δ 5.10 (1H, q, J= 6.6 Hz, H-28) indicated that this compound could be a stigmast-dien type sterol (Bang *et al.,* 2011).

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The ^{13}C NMR spectrum (Appendix 1a) revealed the presence of 29 carbons and DEPT 135 (Appendix 1b) showed six methyl carbon signals resonating at δ 11.9 (C-18), δ 19.4 (C-19), δ 18.8 (C-21), δ 21.1 (C-26), δ 21.1 (C-27) and δ 12.8 (C-29), ten methylene carbon signals occurring at δ 37.3 (C-1), δ 31.7 (C-2), δ 42.3 (C-4), δ 31.9 (C-7), δ 21.1 (C-11), δ 39.8 (C-12), δ 24.3 (C-15), δ 28.2 (C-16), δ 36.0 (C-22) and δ 27.9 (C-23), nine methine carbon signals resonating at δ 71.3 (C-3), δ 121.7 (C-6), δ 31.9 (C-8), δ 50.1 (C-9), δ 56.8 (C-14), δ 56.0 (C-17), δ 36.2 (C-20), δ 28.6 (C-25) and δ 116.4 (C-28) and four quaternary carbon signals resonating at δ 140.8 (C-5), δ 36.5 (C-10), δ 42.3 (C-13) and δ 145.9 (C-24). The downfield signal appearing at δ 71.3 ppm was suggestive of a C-atom that is connected to an oxygen atom and attributed to C-3.

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The stereochemistry at the side chain double bond (C-24-C-28) can be explained by the observing the allylic proton H-25 which resonates at δ 2.80 ppm in the case of (Z)-isomer and δ 2.20 ppm for (E)-isomer (Frost *et al.,* 1986). The signal of the C-21 methyl protons appeared at δ 0.95 ppm, rather than at δ 0.99 ppm as in (E)-isomer, further confirming the presence of a cis-double bond at C-24/28 (Alarif *et al.,* 2010). The C-29 methyl group is *cis* to the isopropyl group and hence the resonances for C-25, C-26, C-27 and C-29 are all shifted to higher field. The assignment of C-1 through C-29 was made by direct comparison with spectra data (McInnes *et al.,* 1980) which contains the same hydrocarbons.

^{1}H-^{1}H COSY long range (^{4}J) correlation was observed between the proton signal at δ 2.24 with the olefinic proton resonating at δ 5.33 and another correlation was observed between the proton signal at δ 1.76 with the olefinic proton resonating at δ 5.10, this suggest the linkage of the methylene protons to the double bonds. A correlation was observed between proton signal at δ 3.51 with H-4 at δ 2.24 and a similar interaction was observed between proton signal at δ 3.51 with H-2 at δ 1.90. and therefore, the proton signal δ 3.51 was attributed with H-3. Furthermore, the ^{13}C spectrum distinguished itself by absorbing at δ 71.3 ppm which is oxygenated.

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The HMBC spectrum (Appendix 1d), showed correlations between proton signal at δ 2.01 (H-12) with C-11 and C-18, δ 5.34 (H-6) with C-4, C-3 and C-7, and another correlation was observed between proton signal at δ 1.57 (H-29) with C-24 and C-28 and similarly proton signal δ 2.80 (H-25) correlates with C-26 and C-27. All-important HMBC's are presented in Figure **3.2.** Based on these results and comparing with the literature (McInnes *et al.,* 1980) compound **1** was thus identified as **Isofucosterol** [(24Z)-Stigmasta-5,24(241)-dien-3~-ol].

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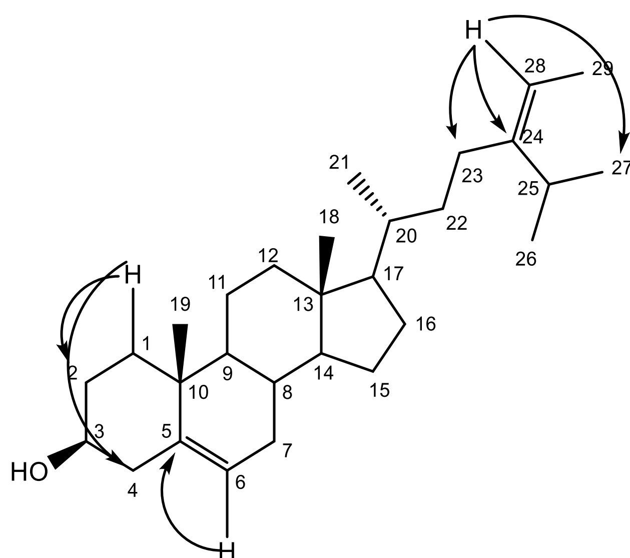

Figure 3.2: HMBC correlation of compound **1**

Table 3.1: 1D and 2D, ^1H and ^{13}C NMR data of compound 1 in $CDCl_3$ at 400 and 100 MHz.

Position	δ ^{13}C	δ ^{1}H (int, mult., J)	COSY	HMBC
1	37.3	1.92 (2H, m)	H-2, H-9	C-4, C-2
2	31.7	1.90 (2H, m)		
3	71.3	3.51 (1H, m)	H-4, H-2	C-2
4	42.3	2.24 (2H, d, J=1.76 Hz)	H-3, H-6, H-2	
5	140.8	-	-	-
6	121.7	5.33 (1H, br. d, J=5.0 Hz)	H-4, H-7	C-3, C-2, C-5
7	31.9	1.50 (2H, m)	H-6	
8	31.9	2.04 (1H, m)		
9	50.1	0.94 (1H, m)		C-1, C-5
10	36.5	-	-	-
11	21.1	*ax* 0.92, *eq* 1.02 (2H, m)		
12	39.8	2.01 (2H, m)		
13	42.3	-	-	-
14	56.8	0.97 (1H, m)		C-13, C-12
15	24.3	1.58 (2H, m)	H-14	
16	28.2	2.06 (2H, m)		
17	56.0	1.11 (1H, m)		C-20
18	11.9	0.68 (3H, s)	H-17	
19	19.4	1.00 (3H, s)		
20	36.2	0.95 (1H, q, J=6.6 Hz)		C-13, C-29
21	18.8	0.93 (3H, d, J= 6.6 Hz)		
22	36.0	1.47 (2H, m)		
23	27.9	1.76 (2H, m)		C-29, C-22
24	145.9	-	-	-
25	28.6	2.80 (1H, sept)	H-21	
26	21.1	0.97 (3H, d, J=6.8 Hz)		
27	21.1	0.97 (3H, d, J=6.8 Hz)		
28	116.4	5.10 (1H, q, J= 6.6 Hz)	H-23	C-24, C-27, C-23
29	12.8	1.57 (3H, d, J= 6.6 Hz)		

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3.2 Compound 2 and 3

Column chromatography of fraction SII from the hexane extract yielded a mixture of the epimers **2** and **3** (5.1 mg) which were inseparable from each other. Compounds **2** and **3** figure **3.3** were isolated as a white crystalline solid, for which GC-MS showed molecular ion peak at m/z: 428 $[M]^+$ (figure **3.8**) corresponding with the molecular formula $C_{29}H_{48}O_2$.

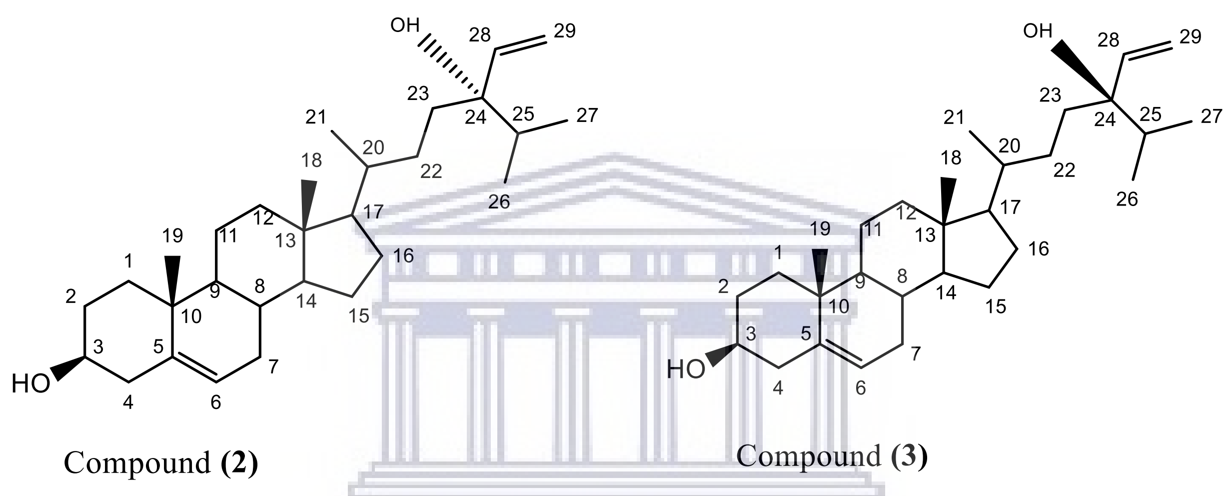

Figure 3.3: Chemical strucrure of compounds **2** and **3**

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The structure was established based on 1D (^{1}H and ^{13}C) and 2D-NMR experiments (HSQC, COSY and HMBC), Table **3.2** and **3.3**.

The ^{1}H NMR spectrum (Appendix 2a) revealed the presence of four olefinic protons for both compounds, δ 5.35 (1H, br. d, J=5.2Hz, H-6), H-28 for compound **2** and **3 :** [δ 5.72 (1H, dd, J=17.7; 11.2 Hz, H-28), δ 5.78 (1H, dd, J=17.4; 11.3 Hz, H-28)] and signal twining of the olefinic protons, for compound **2** : H-29 [δ 5.14 (1H, dd, J= 17.9; 1.4 Hz, H-29a), δ 5.18 (1H, dd, J=12.7; 1.5 Hz, H-29b)] and for compound **3** : H-29 [δ 5.29 (1H, dd, J= 17.4; 11.4 Hz, H-29a), δ 5.26 (1H, dd, J=11.6; 1.4 Hz, H-29b)] details of H-28 and H-29 for both compound **2**

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and **3** can be seen in figure **3.4**. The presence of a hydroxyl group at δ_C 71.8 (C-3) was confirmed by the presence of a multiplet at δ 3.53 ppm, respectively.

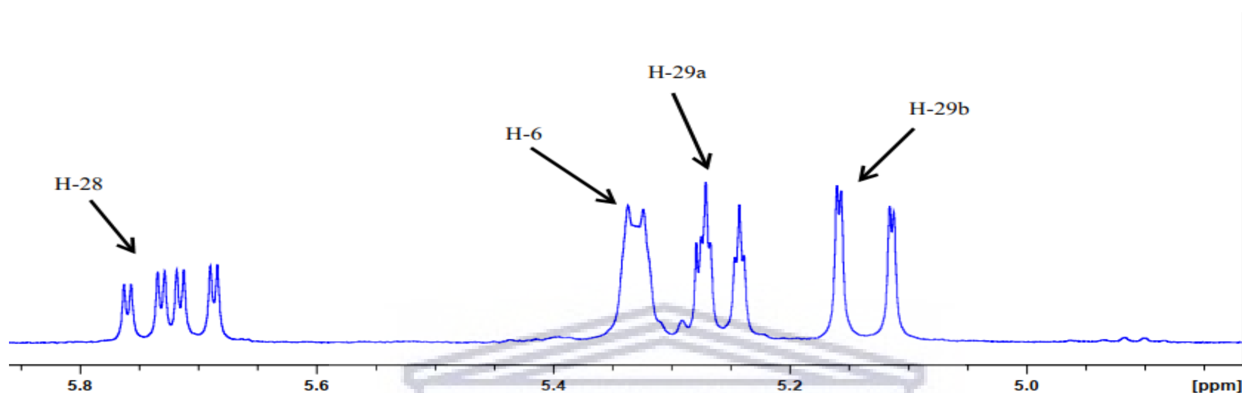

Figure 3.4: Details for ^{1}H NMR spectra of a mixtutre of compound **2** and **3.**

The ^{13}C NMR spectrum (Figure **3.5** / Appendix 2b) exhibited 29 carbon signals and DEPT 135 (Appendix 2c) indicated the presence of five methyl carbon signals at δ 11.9 (C-18), δ 16.6 (C-19), δ 17.7 (C-21), δ 19.4 (C-26), δ 16.7 (C-27) for **3** and δ 18.9 (C-27) for **2**, eleven methylene carbon signals at δ 37.2 (C-1), δ 31.9 (C-2), δ 42.3 (C-4), δ 31.7 (C-7), δ 21.1 (C-11), δ 39.7 (C-12), δ 24.3 (C-15), δ 28.4 (C-16), δ 31.6 (C-22), δ 28.3 (C-23), δ 116.3 (C-29) for **2** and δ 116.4 (C-29) for **3**, nine methine carbon signals at δ 71.8 (C-3), δ 121.7 (C-6), δ 36.2 (C-8), δ 50.1 (C-9), δ 56.7 (C-14), δ 55.9 (C-17), δ 29.7 (C-25), δ 137.1 (C-28) for **2** and δ 137.2 (C-28) for **3**. The remaining ^{13}C signals are four quaternary carbon signals at δ 140.7 (C-5), δ 36.5 (C-10), δ 42.3 (C-13), δ 89.1 (C-24) for **2** and δ 89.2 (C-24) for **3**. The duplicate signals (C-17, C-24, C-25, C-27 to C-29) of **2** and **3** indicated the presence of a 1:1 mixture of 24*R* and 24*S* epimers (Bouzidi *et al.,* 2014). This was further confirmed by observing C-27 which resonates at δ 18.9 ppm in the case of 24*R*-isomer, at δ 16.7 ppm for 24*S*-isomer and

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C-28 which resonates at δ 137.1 ppm in the case of 24*R*-isomer (Huh *et al.,* 2012), at δ 137.2 ppm for 24*S*-isomer (Bouzidi *et al.,* 2014). C-29 which resonates at δ 116.3 ppm in the case of 24*R*-isomer (Huh *et al.,* 2012), at δ 116.4 ppm for 24*S*-isomer.

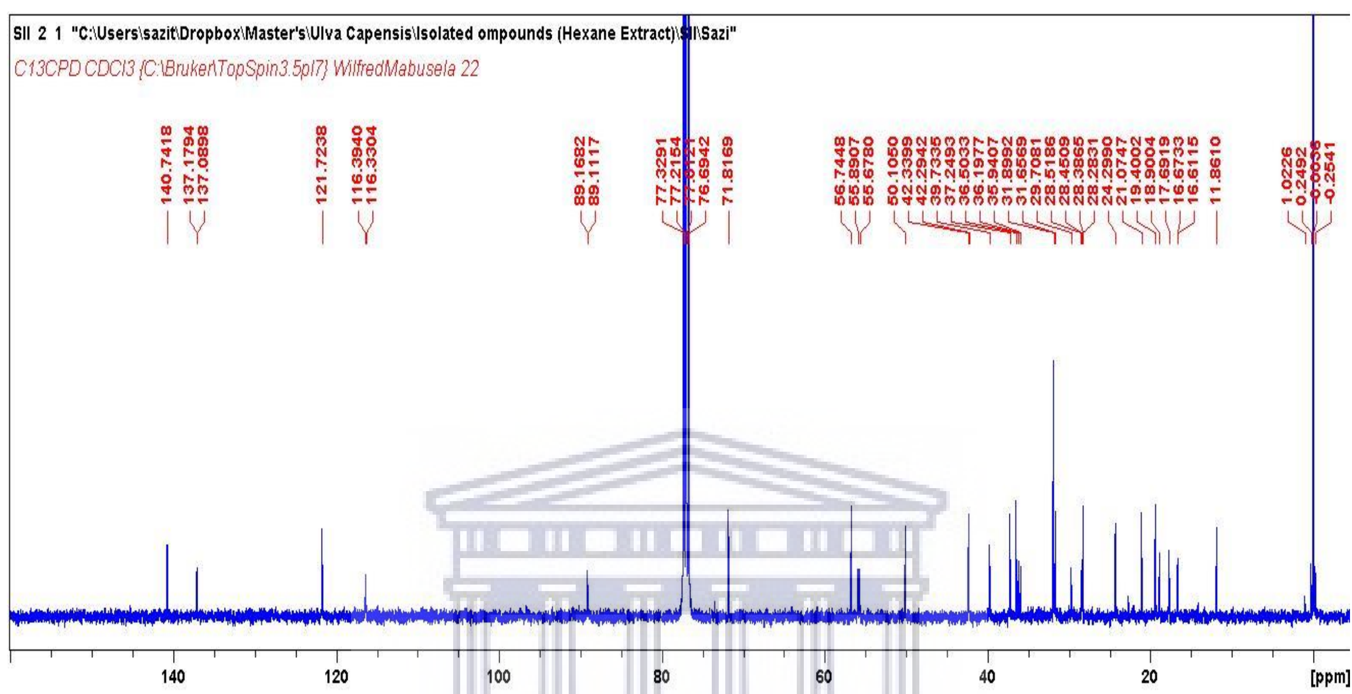

Figure 3.5: ^{13}C NMR Spectrum of compound **2** and **3** in $CDCl_3$

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The NMR spectral data suggested that compounds **2** and **3** have steroidal carbon skeleton. The signal due to C-28 [δ 137.1 (**2**), δ 137.2 (**3**)] and C-29 [δ 116.3 (**2**), δ 116.4 (**3**)] could be associated with the presence of a vinyl group at C-24. The relevant protons each gave rise to three doublet of doublets at δ 5.72 ppm, δ 5.14 ppm and δ 5.18 ppm for compound **2** and at δ 5.78 ppm, δ 5.29 ppm and δ 5.26 ppm for compound **3**. The appearance of a doublet of doublets at δ 5.72 and δ 5.78 ppm indicated the absence of a proton at C-24, thus allowing for the placement of a hydroxyl group at C-24 (Ahmad *et al.,* 1992). This was further confirmed by the presence of downfield quaternary carbon signals at δ 89.1 and δ 89.2 ppm for compounds **2** and **3**, respectively.

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To confirm the position of the partial structure ^1H-^1H COSY and HMBC experiments were carried out. The ^1H-^1H COSY spectra (Appendix 2d) of compounds **2** and **3** showed correlations between H_1 to H_4, H_6-H_9, H_9-H_{11}-H_{12}, H_{14} to H_{17} and H_{21}-H_{22}-H_{23}, as expected (Murtihapsari *et al.,* 2019) supporting the presence of steroidal structure. The HMBC spectrum (Figure **3.6** / appendix 2e), for compound **2** and **3** showed correlation between H-8 with C-9 and C-6. H-9 showed correlation to C-8, C-10 and C-11, confirming the placement of C-8 and C-9. The correlation arising from the tertiary methyl protons at δ 0.69 and δ 1.00 to their neighbouring carbons enabled the assignment of the two-singlet methyl at C-13 and C-10, respectively. The protons of the terminal carbon at H-29 showed correlation with C-24 and C-28. Another correlation at H-28 with C-24 and C-29, HMBC correlation in figure **3.7**.

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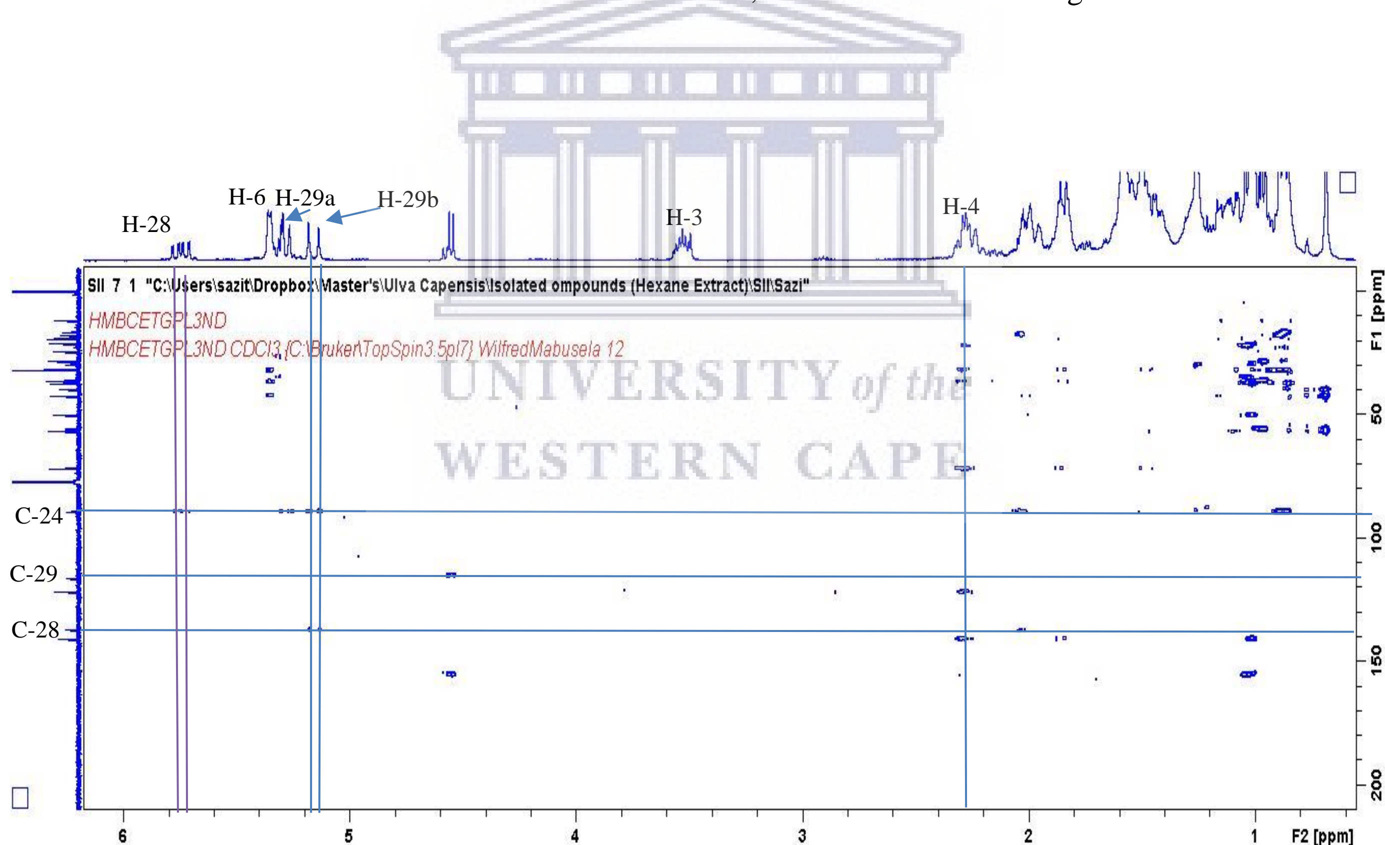

Figure 3.6: HMBC Spectrum of compound **2** and **3** in $CDCl_3$

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Table 3.2: 1D and 2D, ^{1}H and ^{13}C NMR data of compound 2 in $CDCl_3$ at 400 and 100 MHz.

Position	δ ^{13}C	δ ^{1}H (int, mult., J)	COSY	HMBC
1	37.2	1.82 (2H, m)	H-2, H-3	C-2
2	31.9	1.95 (2H, m)	H-2	C-3, C-4
3	71.8	3.53 (1H, m)	H-2, H-4	C-2, C-4, C-5
4	42.3	2.28 (2H, s)	H-3, H-6	C-3, C-6
5	140.7	-		-
6	121.7	5.35 (1H, br. d, J=5.2 Hz)	H-4	C-7
7	31.7	1.84 (1H, m)		-
8	36.2	1.40 (1H, m)	H-9	C-6, C-9
9	50.1	0.93 (1H, m)	H-11	C-5, C-8, C-10, C-11
10	36.5	-		
11	21.1	1.48 (2H, m)	H-9, H-12	C-10
12	39.7	2.01 (2H, m)	H-11	C-13, C-14
13	42.3	-		
14	56.7	0.99 (1H, m)	H-15	
15	24.3	1.58 (2H, m)	H-14	
16	28.4	1.85 (2H, m)	H-15	
17	55.9	1.13 (1H, m)	H-15, H-16	C-20, C-22
18	11.9	0.69 (3H, s)		C-13
19	16.6	0.90 (3H, s)		C-10
20	35.9	1.41 (1H, m)		
21	17.7	0.85 (3H, d, J=6.8 Hz)	H-20	C-24
22	31.6	1.85 (2H, m)	H-17, H-20, H-16	
23	28.3	1.26 (2H, m)	H-22	
24	89.1	-		C-29
25	29.7	1.25 (1H, m)	H-22	
26	19.4	1.02 (3H, d, J=6.8 Hz)	H-21	
27	18.9	0.95 (3H, d, J= 6.6 Hz)		
28	137.1	5.72 (1H, dd, J= 17.7, 11.2 Hz)	H-29	C-24, C-29
29a	116.3	5.14 (1H, dd, J= 17.9, 1.4 Hz)	H-28	C-24, C-28
29b		5.18 (1H, dd, J= 12.7, 1.5 Hz)	H-28	C-24, C-28

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Table 3.3: 1D and 2D, ^{1}H and ^{13}C NMR data of compound 3 in $CDCl_3$ at 400 & 100 MHz.

Position	δ ^{13}C	δ ^{1}H (int, mult, J)	COSY	HMBC
1	37.2	1.82 (2H, m)	H-2, H-3	C-2
2	31.9	1.95 (2H, m)	H-2	C-3, C-4
3	71.8	3.53 (1H, m)	H-2, H-4	C-2, C-4, C-5
4	42.3	2.28 (2H, s)	H-3, H-6	C-3, C-6
5	140.7	-		-
6	121.7	5.35 (1H, br. d, J=5.2 Hz)	H-3, H-4	C-7
7	31.7	1.84 (1H, m)		-
8	36.2	1.40 (1H, m)	H-9	C-6, C-9
9	50.1	0.93 (1H, m)	H-11	C-5, C-8, C-10, C-11
10	36.5	-		-
11	21.1	1.48 (2H, m)	H-9, H-12	C-10
12	39.7	2.01 (2H, m)	H-11	C-13, C-14
13	42.3	-		
14	56.7	0.99 (1H, m)	H-15	
15	24.3	1.58 (1H, m)	H-14	
16	28.4	1.85 (2H, m)	H-15	
17	55.9	1.13 (1H, m)	H-15, H-16	C-20, C-22
18	11.9	0.69 (3H, s)		C-13
19	16.6	0.90 (3H, s)		C-10
20	35.9	1.41 (1H, m)		
21	17.7	0.85 (3H, d, J=6.8 Hz)	H-20	C-24
22	31.6	1.85 (2H, m)	H-17, H-20, H-16	
23	28.3	1.26 (2H, m)	H-22	
24	89.2	-		-
25	29.7	1.25 (1H, m)	H-22	
26	19.4	1.02 (3H, d, J=6.8 Hz)	H-21	
27	16.7	0.89 (3H, d, J= 6.9 Hz)		
28	137.2	5.78 (1H, dd, J= 17.4, 11.3 Hz)	H-29	C-24, C-29
29a	116.4	5.29 (1H, dd, J= 17.7, 1.4 Hz)	H-28	C-24, C-28
29b		5.26 (1H, dd, J= 11.6, 1.4 Hz)	H-28	C-24, C-28

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Hence, based on these findings and comparison with literature data (Bouzidi *et al.,* 2014) was possible to propose that this sample is a mixture of epimers. Compound **2** was identified as **24*R*-Saringosterol** and compound **3** as **24*S*-Saringosterol**. According to a number of literatures, Saringosterol was also isolated as a mixture of epimers (Ayyad *et al.,*2003; Ayyad *et al.,* 2011; Bouzidi *et al.,* 2014; Chen *et al.,* 2014; Rahelivao *et al.,* 2015), only few literatures were able to isolate 24*R*-Saringosterol and 24*S*-Saringosterol as separate compounds (Huh *et al.,* 2012).

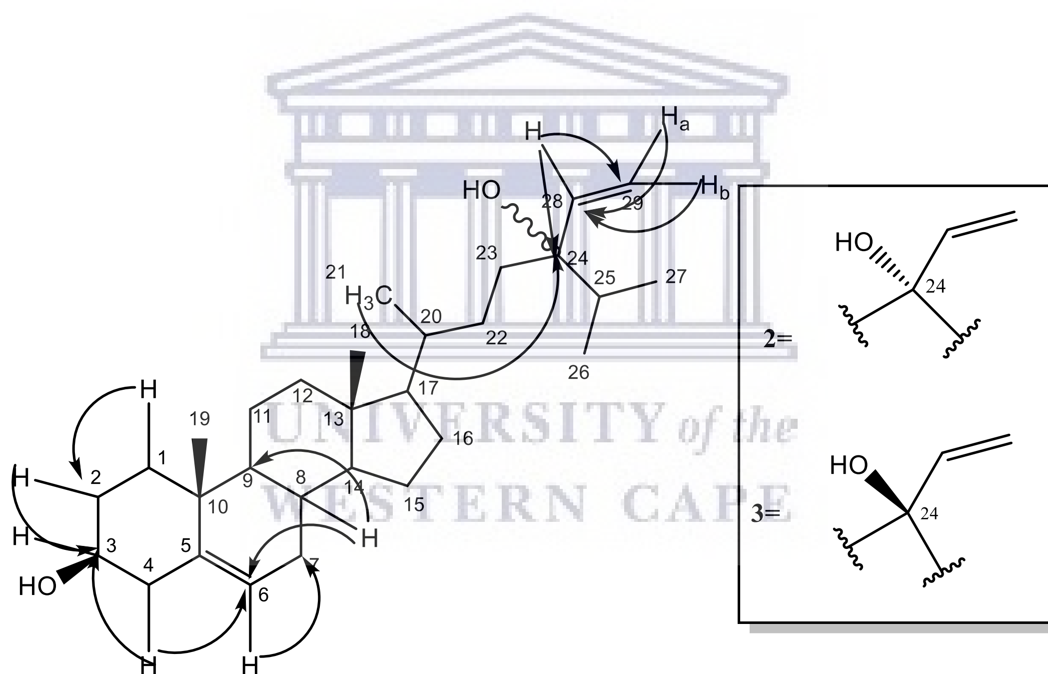

Figure 3.7: HMBC correlation for Compound **2** and **3**

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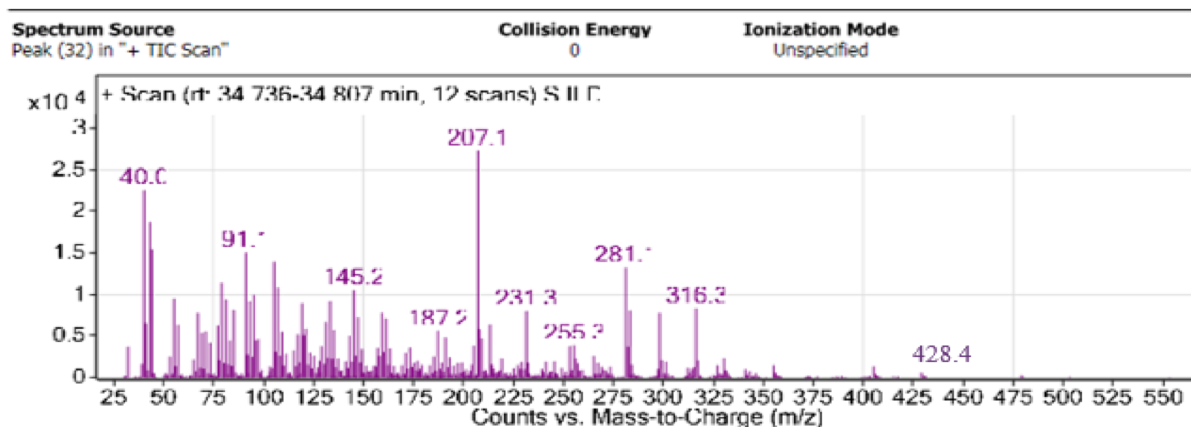

Figure 3.8: GC-MS data of compound **2** and **3**

3.3 Compound 4

Column chromatography of fraction B-SIX of the EtOAc extract yielded compound **4** (figure **3.9**). Compound **4** (6.5mg) was isolated as white flakes and a chemical formula of $C_{19}H_{30}O_2$ as established based on GC-MS, m/z 290.22 $[M]^+$, m/z 261.3 $[M-C_2H_5]^+$ (Figure **3.10**). The structure was established based on 1D and 2D-NMR experiments, Table **3.5**.

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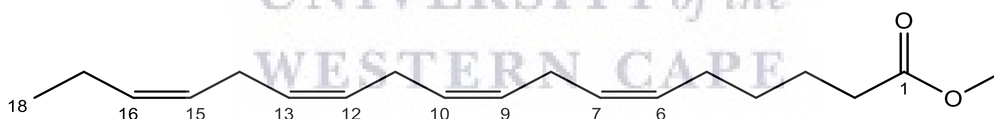

Figure 3.9: Chemical structure of Compound **4**

The ^{1}H NMR spectrum (Appendix 3a) of compound **4,** showed olefinic protons at δ 5.37 ppm which integrated for 8 protons assigned as follows: (8H, m, H-6, H-7, H-9, H-10, H-12, H-13, H-15 and H-16). The signal at δ 2.81 (6H, m), corresponds to the bis-allylic protons (H-8, H-11 and H-14). Two triplets were observed at δ 2.27 (2H, t, J=7.5 Hz, H-2) and at δ 0.97 (3H, t, J=7.5 Hz, H-18). A singlet was observed at δ 3.6 (3H, s) from the hydrogens of methylic

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group. A multiplet resonating at δ 2.08 ppm was attributed to the two methylene protons H-5 and H-17 (4H, m).

The ^{13}C NMR spectrum (Appendix 3b) exhibited 19 carbon signals and DEPT 135 spectrum (Appendix 3c) indicated the presence of two methyl carbon signals resonating at δ 14.3 (C-18) and at δ 51.6 (-O**C**H_3) (Basumatary and Deka *et al.,* 2002). Eight methylene carbon signals were observed at δ 33.9 (C-2), δ 25.5 (C-3), δ 29.4 (C-4), δ 34.3 (C-5), δ 34.1 (C-8), δ 36.3 (C-11), δ 34.0 (C-14) and δ 26.9 (C-17), eight olefinic carbon signals appeared at δ 130.3 (C-6), δ 127.9 (C-7), δ 128.2 (C-9), δ 128.4 (C-10), δ 128.5 (C-12), δ 128.6 (C-13), δ 128.1 (C-15) and δ 132.0 (C-16). The remaining ^{13}C signal at δ 174.1 (C-1) was due to the carbonyl carbon.

The ^{1}H-^{1}H COSY spectrum (Appendix 3d) exhibited a cross peak between the olefinic protons and the signal at δ 2.81 ppm assigned to three methylene protons (H-8, H-11 and H-14) in support of the proposed carbon framework. The terminal methyl proton resonating at δ 0.97 (H-18) showed a correlation with H-17. Based on these findings and comparison with literature data (Cui *et al.,* 2008; Park *et al.,* 2013) the structure was shown to be **Methyl stearidonate** (Methyl 6Z, 9Z, 12Z, 15Z -octadecatetraenoate).

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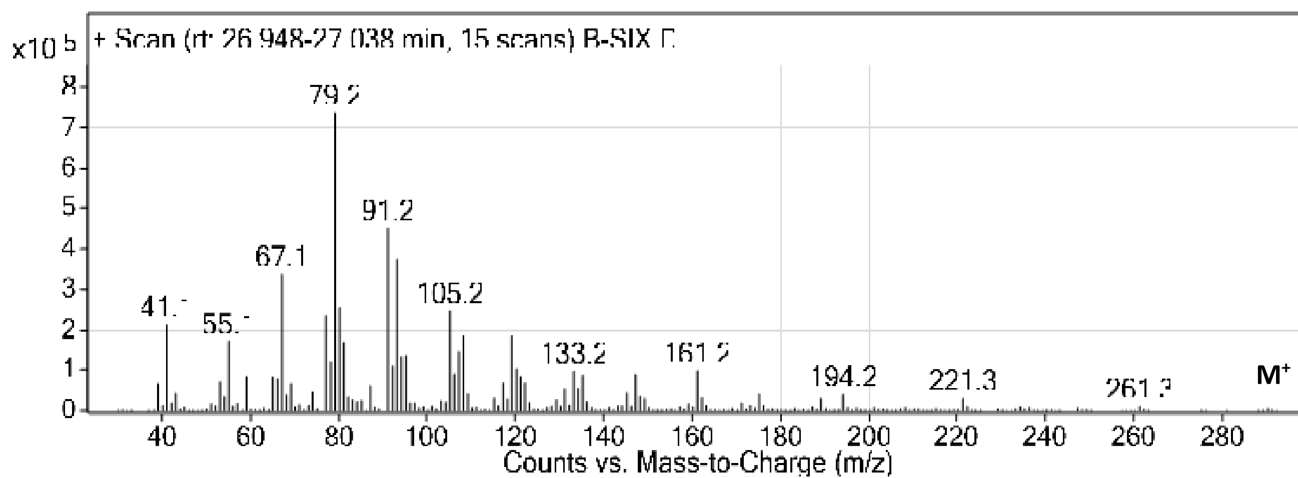

Figure 3.10: GC-MS data of compound **4**

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Table 3.4: 1D and 2D, ^{1}H and ^{13}C NMR data of compound 4 in $CDCl_3$ at 400 and 100 MHz.

Position	δ ^{13}C	δ ^{1}H (int, mult, J)	COSY	HMBC
1	174,1	-		
2	33,9	2.27 (2H, t, J= 7.4 Hz)	H-3	C-1
3	25,5	1.61 (2H, m)	H-2	C-1, C-4
4	29,4	1.41 (2H, m)	H-5	C-3
5	34,3	2.08 (2H, m)	H-4, H-6	
6	130,3	5.37 (1H, m)	H-5	C-7
7	127,9	5.37 (1H, m)	H-8	C-6
8	34,1	2.81 (2H, m)	H-7, H-8	
9	128,2	5.37 (1H, m)	H-8	
10	128,4	5.37 (1H, m)	H-11	
11	36,3	2.81 (2H, m)	H-10, H-12	
12	128.5	5.37 (1H, m)	H-11	
13	128.6	5.37 (1H, m)	H-14	
14	34,0	2.81 (2H, m)	H-13, H-15	
15	128,1	5.37 (1H, m)	H-14	C-16
16	132,0	5.37 (1H, m)	H-17	C-15, C-17, C-18
17	26,9	2.08 (2H, m)	H-16, H-18	C-16
18	14,3	0.97 (3H, t, J=7.5 Hz)	H-17	C-17
- OCH_3	51,6	3.66 (3H, s)	-	

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3.4 Compound 5

Compound **5** (figure **3.11**) was isolated from repeated column chromatography of fraction B-SIX from the EtOAc extract. Compound **5** (6.5mg) was obtained as white flakes with a chemical formula of $C_{19}H_{34}O_2$ as established based on GC-MS, m/z 294.25 $[M]^+$ (Figure **3.12**). The structure was established using 1D and 2D-NMR experiments (Table **3.5**).

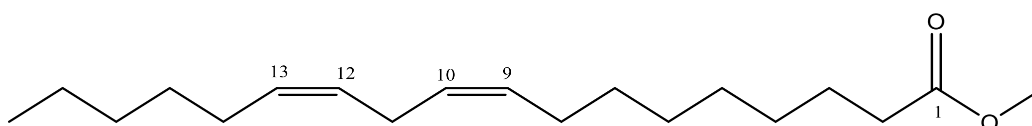

Figure 3.11: Chemical structure of Compound **5**

The ^{1}H NMR spectrum (Appendix 4a) of compound **5**, indicated a multiplet at δ 5.37 ppm which represents the olefinic protons (4H, m, H-9, H-10, H-12 and H-13), a singlet at δ 3.66 ppm representing methoxy protons (-OC**H**$_3$). The bis-allylic proton signal observed at δ 2.81 (2H, m) ppm usually expected for polyunsaturated fatty acids (Basumatary and Deka *et al.,* 2002). A multiplet observed at δ 1.41 ppm which integrated to 14 protons assigned to the following protons (14H, m, H-4, H-5, H-6, H-7, H-15, H-16 and H-17). Two triplets were observed, one appeared at δ 2.27 (2H, t, J=7.4 Hz) and another at δ 0.97 (3H, t, J=7.5 Hz) due to H-2 and H-18 respectively. A multiplet at δ 2.08 (4H, m) was attributed to the allylic protons H-8 and H-14.

The ^{13}C NMR spectrum (Appendix 4b) exhibited 19 carbon signals and DEPT 135 spectrum (Appendix 4c) indicated the presence of two methyl carbon signals resonating at δ 14.3 (C-18) and δ 51.6 (-O**C**H$_3$), twelve methylene carbon signals resonating at δ 33.9 (C-2), δ 25.5 (C-3), δ 29.1 (C-4), δ 29.4 (C-5), δ 29.7 (C-6), δ 29.2 (C-7), δ 28.0 (C-8), δ 25.6 (C-11), δ 27.2 (C-14), δ 29.6 (C-15), δ 31.9 (C-16) and δ 22.8 (C-17), four olefinic carbon signals

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resonating at δ 130.3 (C-9), δ 127.0 (C-10), δ 127.1 (C-12) and δ 129.7 (C-13) and lastly, carbonyl carbon signal at δ 174.3 (C-1).

The ^{1}H-^{1}H COSY spectrum (Appendix 4d), the bis-allylic proton at δ 2.81 ppm showed correlation with H-10, H-12, a long-range correlation with H-9 and H-13. The same correlation was observed between H-10 with H-11, H-12 with H-11 and long-range correlation between H-9 with H-11 and H-13 with H-11.

The HMBC spectrum (Appendix 4e) showed correlation between H-2 with C-1 and C-3, and another between H-3 with C-1 and C-4. Further correlations were observed between H-8 and C-9, H-4 and C-3, and H-9 with C-8 and C-10. The key HMBC correlations are illustrated in figure **3.13**. The assignment of the protons and carbons was also made by direct comparison with (Samuelsson and Johansson *et al.,* 2001) spectra data. Based on these results and comparison with the literature (Knothe *et al*., 2006), the structure was shown to be **Methyl linoleate**.

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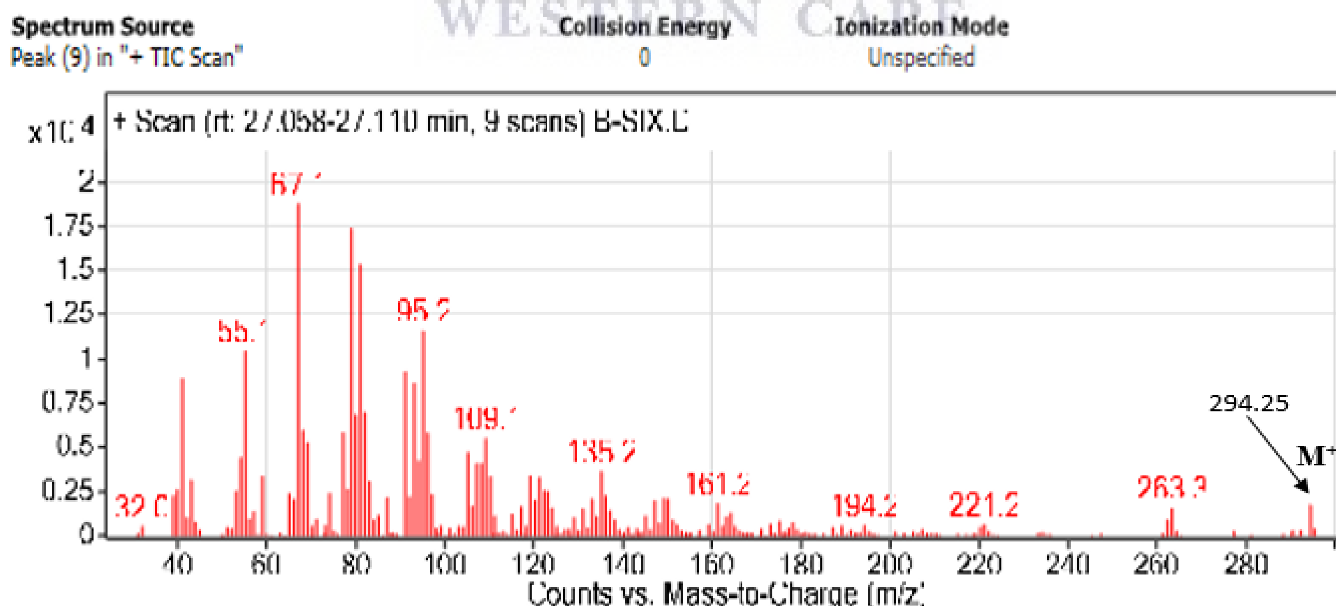

Figure 3.12: GC-MS data of compound **5**

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Table 3.5: 1D and 2D, ^{1}H and ^{13}C NMR data of compound 5 in $CDCl_3$ at 400 and 100 MHz.

Position	δ ^{13}C	δ ^{1}H (int, mult, J)	COSY	HMBC
1	174.3	-		-
2	33.9	2.27 (2H, t, J= 7.4 Hz)	H-3	C-1, C-3
3	25.5	1.61 (2H, m)	H-2, H-4	C-1, C-4
4	29.1	1.41 (2H, m)	H-3	C-3
5	29.4	1.41 (2H, m)		
6	29.7	1.41 (2H, m)		
7	29.2	1.41 (2H, m)		
8	28.0	2.08 (2H, m)	H-9	C-9
9	130.3	5.37 (1H, m)	H-11	C-8, C-10
10	127.0	5.37 (2H, m)	H-11	
11	25.6	2.81 (2H, m)	H-10	
12	127.1	5.37 (1H, m)	H-11	
13	129.7	5.37 (1H, m)	H-11	
14	27.2	2.08 (2H, m)	H-13	
15	29.6	1.41 (2H, m)		
16	31.9	1.41 (2H, m)		
17	22.8	1.41 (2H, m)		
18	14.3	0.97 (3H, t, J=7.5 Hz)	H-17	
- OCH_3	51.6	3.66 (3H, s)		

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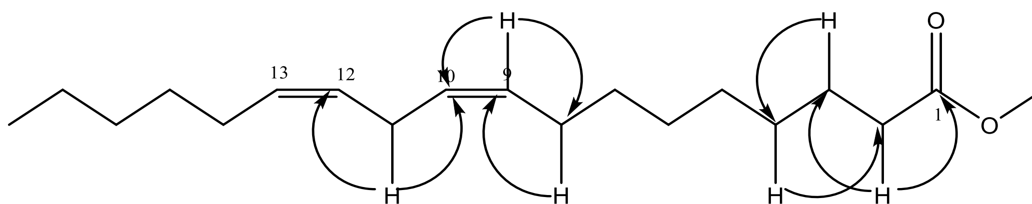

Figure 3.13: HMBC correlation of compound **5**

3.5 Compound 6

Compound **6** (figure **3.14**) was also isolated from repeated column chromatography of fraction B-SIX from the EtOAc extract. Compound **6** was obtained as white flakes with a chemical formula of $C_{19}H_{32}O_2$ as established based on GC-MS, m/z 292.45 $[M]^+$ (figure **3.15**). The structure was established using 1D and 2D-NMR experiments (Table **3.6**).

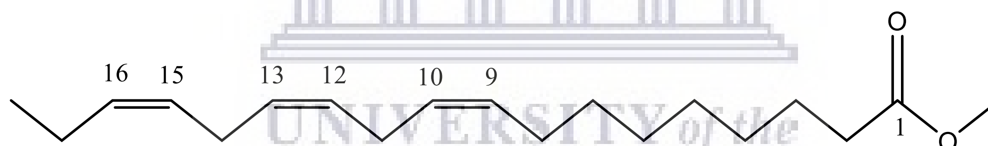

Figure 3.14: Chemical structure of Compound **6**

The ^{1}H NMR spectrum (Appendix 5a) of compound **6**, indicated olefinic protons at δ 5.37 ppm which integrated for 6 protons assigned as follows: (6H, m, H-9, H-10, H-12, H-13, H-15 and H-16), a singlet at δ 3.66 ppm representing methoxy protons (-OC**H**$_3$). The signal at δ 2.81 (4H, m) corresponds to the bis-allylic protons, H-11 and H-14. Two triplets were observed, one appeared at δ 2.27 (2H, t, J=7.4 Hz) and another at δ 0.97 (3H, t, J=7.5 Hz) due to H-2 and H-18, respectively. A multiplet at δ 2.08 (4H, m) was attributed to the allylic protons H-8 and H-17.

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The ^{13}C NMR spectrum (Appendix 5b), exhibited 19 carbon signals and DEPT 135 spectrum (Appendix 5c) indicated the presence of two methyl carbon signals resonating at δ 14.3 (C-18) and δ 51.6 (-OCH_3), ten methylene carbon signals resonating at δ 33.9 (C-2), δ 24.9 (C-3), δ 29.1 (C-4), δ 29.4 (C-5), δ 29.7 (C-6), δ 29.2 (C-7), δ 27.2 (C-8), δ 25.6 (C-11), δ 25.5 (C-14) and δ 20.6 (C-17), six olefinic carbon signals resonating at δ 130.3 (C-9), δ 127.0 (C-10), δ 128.5 (C-12), δ 128.6 (C-13), δ 128.0 (C-15) and δ 132.0 (C-16), and lastly the carbonyl carbon signal at δ 173.6 (C-1).

The ^{1}H-^{1}H COSY spectrum (Appendix 5d), the two bis-allylic protons at δ 2.81 ppm showed correlation one with H-10, H-12 and the other with H-13, H-15 and another between H-10 with H-11. The HMBC spectrum (Appendix 5e) showed correlation between H-2 with C-1 and C-3, and another between H-3 with C-1 and C-4. Further correlations were observed between H-8 and C-9, H-4 and C-3, and H-9 with C-8 and C-10. Based on these results and comparison with the literature (Yang *et al*., 2014; Avula *et al.,* 2017), the structure was shown to be **Methyl linolenate**.

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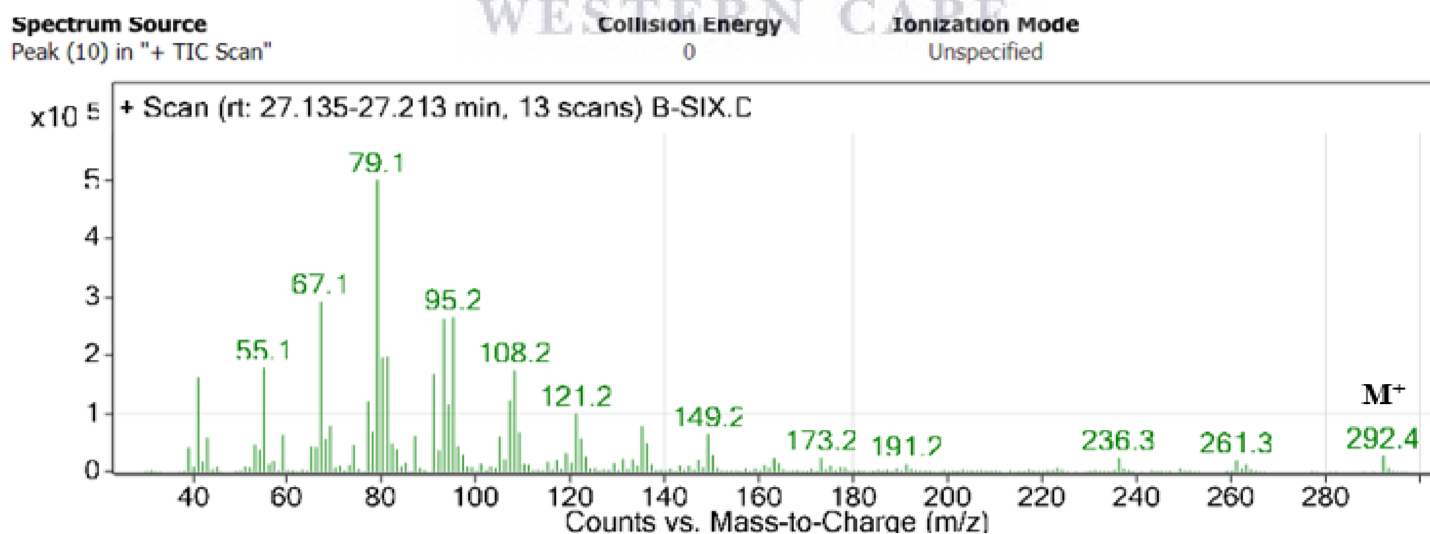

Figure 3.15: GC-MS data of compound **6**

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Table 3.6: 1D and 2D, ^1H and ^{13}C NMR data of compound 6 in $CDCl_3$ at 400 and 100 MHz

Position	δ ^{13}C	δ ^1H (int, mult, J)	COSY	HMBC
1	173.6	-		-
2	33.9	2.27 (2H, t, J= 7.4 Hz)	H-3	C-1, C-3
3	24.9	1.61 (2H, m)	H-2, H-4	C-1, C-4
4	29.1	1.41 (2H, m)	H-3	C-3
5	29.4	1.41 (2H, m)		
6	29.7	1.41 (2H, m)		
7	29.2	1.41 (2H, m)		
8	27.2	2.08 (2H, m)	H-9	C-9
9	130.3	5.37 (1H, m)		C-8, C-10
10	127.0	5.37 (2H, m)	H-11	
11	25.6	2.81 (2H, m)	H-10	
12	128.5	5.37 (1H, m)	H-11	
13	128.6	5.37 (1H, m)		
14	25.5	2.81 (2H, m)	H-13	
15	128.0	5.37 (1H, m)		
16	132.0	5.37 (1H, m)		
17	20.6	2.08 (2H, m)		
18	14.3	0.97 (3H, t, J=7.5 Hz)	H-17	
- OCH_3	51.6	3.66 (3H, s)		

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The isolated compounds have a variety of distribution among the three types of seaweeds, red, green and brown. Isofucosterol (**1**) has been isolated from red seaweed *Gracilaria foliifera* (Alarif *et al.,* 2010), a brown seaweed *Sargassum thunbergii* (Kima *et al.,* 2014) and from two green seaweeds *Enteromorpha intestinalis* and *Ulva lactuca* respectively (Glbbons *et al.,* 1968). Saringosterols (**2** and **3**) occurs predominantly in brown seaweeds, *Turbinaria urnata*, *Sargussum fusiforme*, *Sargussum asperifolium*, *Cystoseira foeniculacea*, *Ascophy zlumnodosw*, *Hizikia fusiformis* (Harvey) Okamur and *Sargassum pallidum* (Turn.) C. Agardh, *Lessonia nigrescens* (Rahelivao *et al.,* 2015; Chen *et al.,* 2014; Ayyad *et al.,* 2003; Bouzidi *et al.,* 2014; Knights *et al.,* 1970; Okano *et al.,* 1985; Liu *et al.,* 2009) and in rare cases, they were identified in both green *Cladophora fascicularis* and red *Acanthophora spicifera* seaweeds (Huang *et al.,* 2007; Dayong *et al.,* 2011). FAME's (**4**, **5** and **6**) are widely occurring in natural fats and dietary oils but has been detected in several species of algae, *Enteromorpha linza* a green seaweed indicated the presence of compounds **4**, **5** and **6**.

Furthermore, There are health benefits associated with the isolated compounds, from the literature isofucosterol was reported to exhibit antioxidant, anticancer activities and acting as anti-obesity agent (Ayyad *et al.,* 2011; Chen *et al.,* 2014), Saringosterol have been shown to exhibit a wide range of biological activities, particularly antitrypanosomal activity, anti-obesity agent and cholesterol-lowering agent (Bouzidi *et al.,* 2014; Hoet *et al.,* 2007; Kima *et al.,* 2014; Chen *et al.,* 2014) and FAMEs are indicated as potential sources for antifungal and antioxidant activity (Pinto *et al.,* 2017). Therefore, the health benefits reported can be related with *Ulva capensis* because of the similar phytochemical composition.

Furthermore, a preliminary study was performed on the ethanol precipitation supernatant to look for possible presence of low molecular weight carbohydrates and unfortunately no carbohydrate were present only long chain fatty acids were noted.

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CHAPTER FOUR

CONCLUSION AND FUTURE RECOMMENDATIONS

4.1 Conclusion

The investigation of the natural products composition from seaweed *Ulva capensis* was successfully performed. Chromatographic fractionation the plant extracts led to the isolation of five known compounds namely: Isofucosterol (**1**), 24*R*-Saringosterol (**2**), 24*S*-Saringosterol (**3**), Methyl stearidonate (**4**), Methyl linoleate (**5**) and Methyl Linolenate (**6**). Compound **1**, **2** and **3** were isolated from the hexane extracts and compounds **4**, **5** and **6** from ethyl acetate (EtOAc) extract. To our knowledge the six compounds are reported for the first time from this plant.

4.2 Future recommendations

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The absolute configuration of the mixtures at C-8, C-9, C-17, C-25 for compound 1 and at C-8, C-9, C-17, C-20, and C-25 for compound 2 and 3, for compound 5 at C-9 and C-12, Could not be conclusively assigned based on NMR spectroscopy and GC-MS alone. Studies such as Nuclear Overhauser Effect Spectroscopy (NOESY) experiment will be explored. Furthermore, LC-MS along with High-Resolution Mass Spectroscopy (HRMS) of compound 4, 5 and 6 will be considered for the confirmation of the assignment of the ambiguous peak signals. Further research is necessary to improve the structural comprehension of the Fatty Acids Methyl Esters (FAME) in addition to what has now been documented.

It is recommended that a reasonable amount of the constituent natural products from the plant be obtained in order to allow for biological studies on the extracts and isolated compounds.

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Further studies to identify additional natural products from the various extracts especially from the polar end of the spectrum, other than the polysaccharides and other bio-macromolecules.

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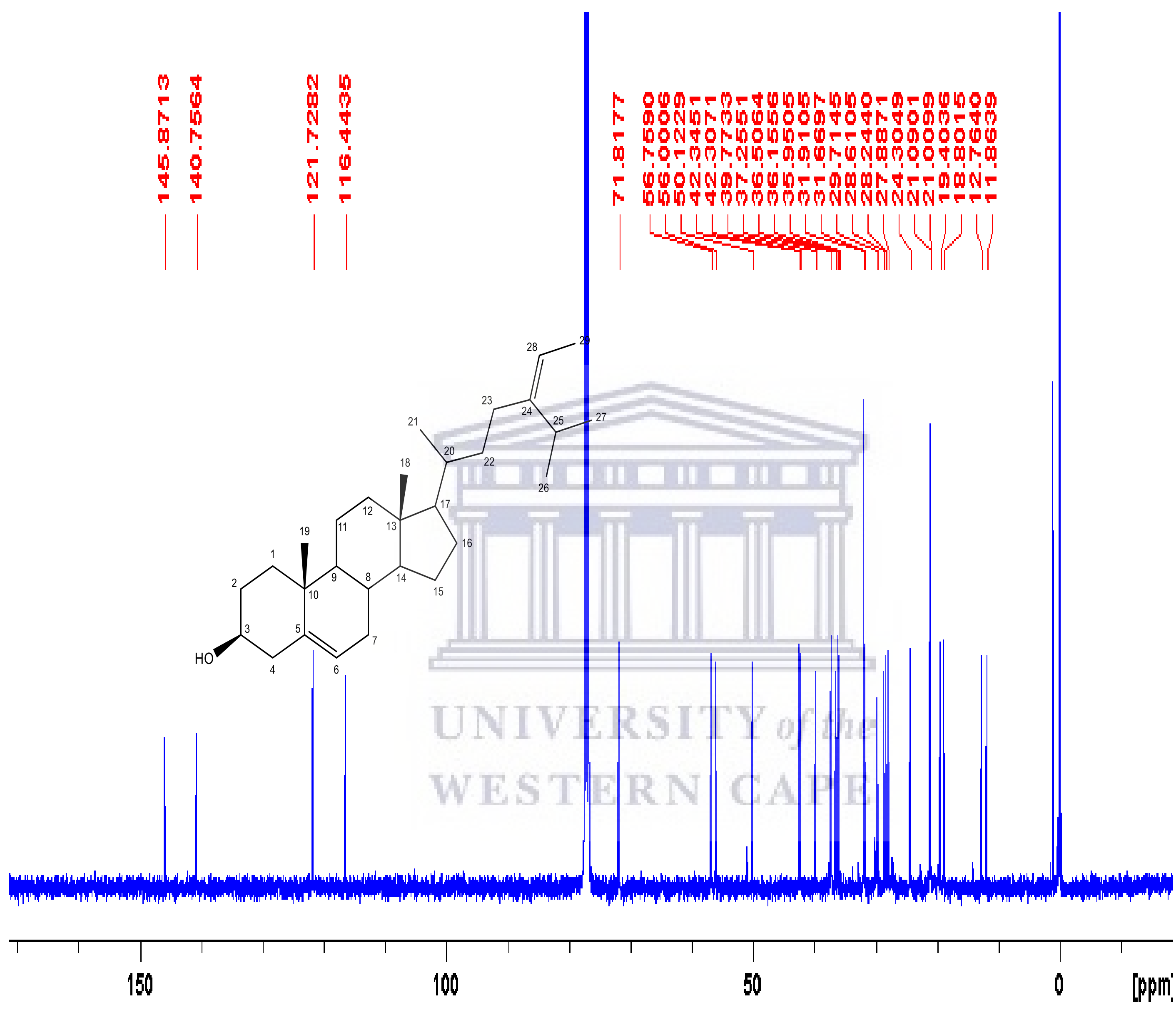
Appendix

Appendix 1a: ^{13}C NMR spectrum of compound 1 (100 MHz, $CDCl_3$)



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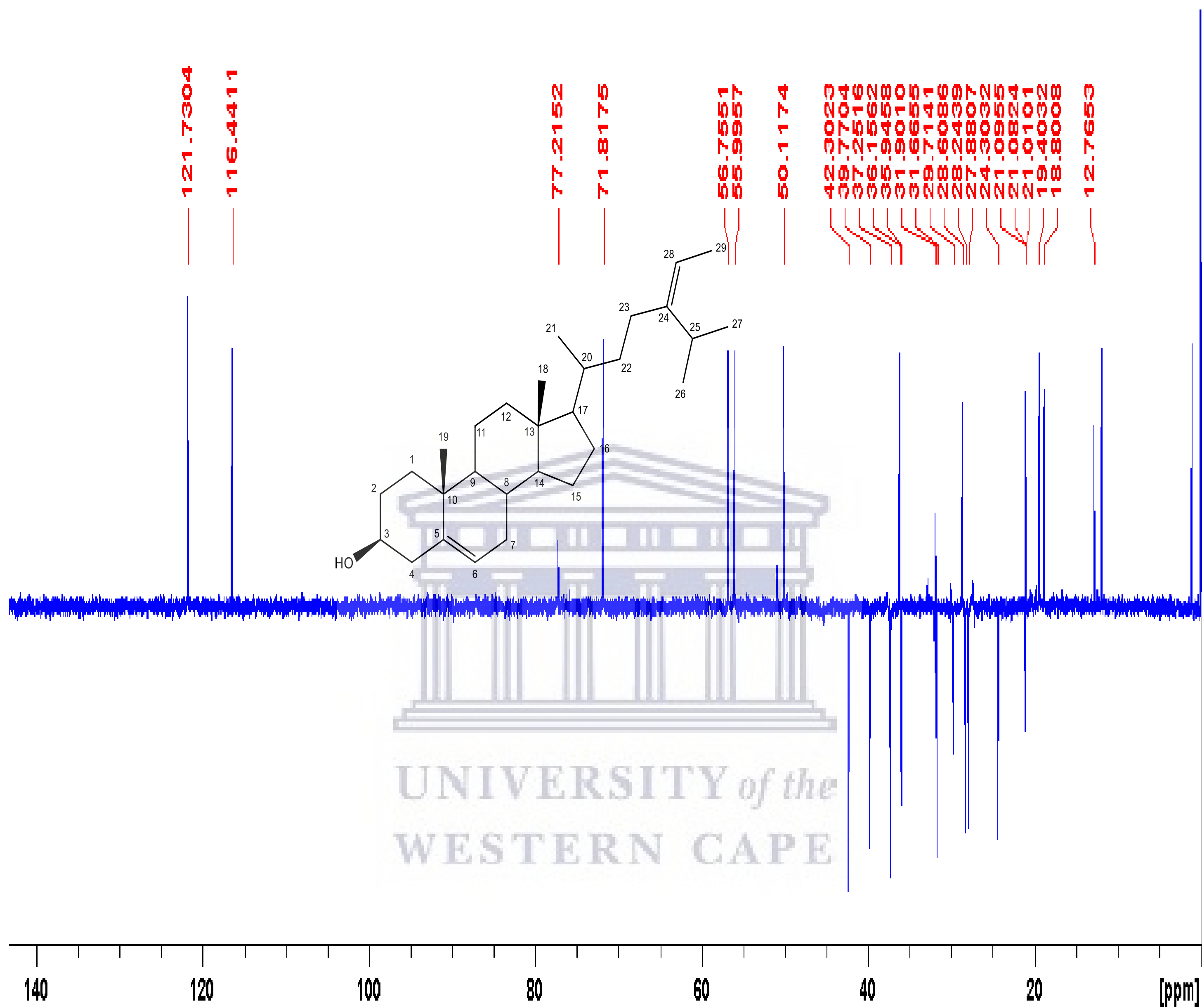
Appendix 1b: **DEPT 135 NMR spectrum of compound 1 (100 MHz, $CDCl_3$).**



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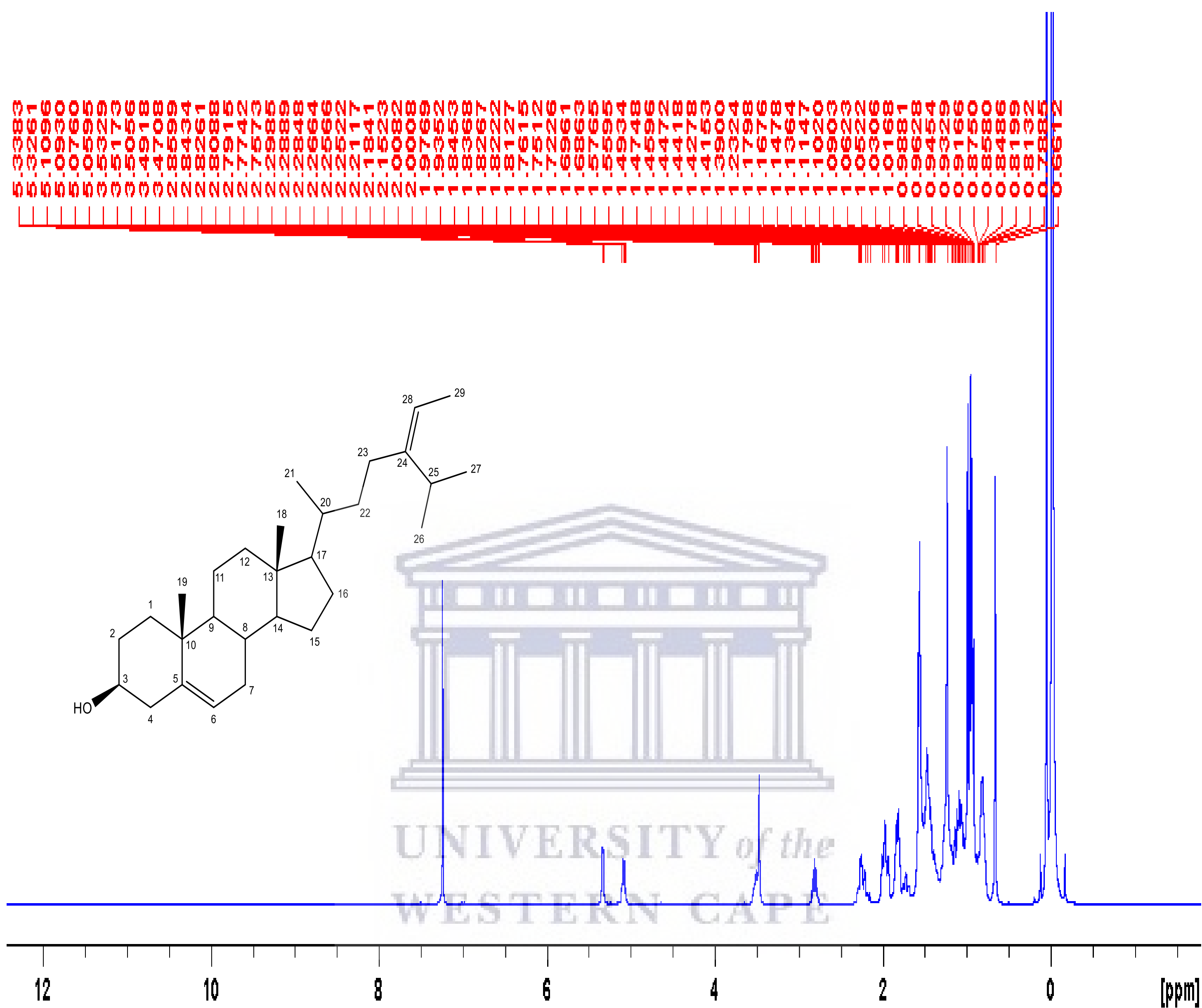
Appendix 1c: **^{1}H NMR spectrum of compound 1 (400 MHz, $CDCl_3$).**



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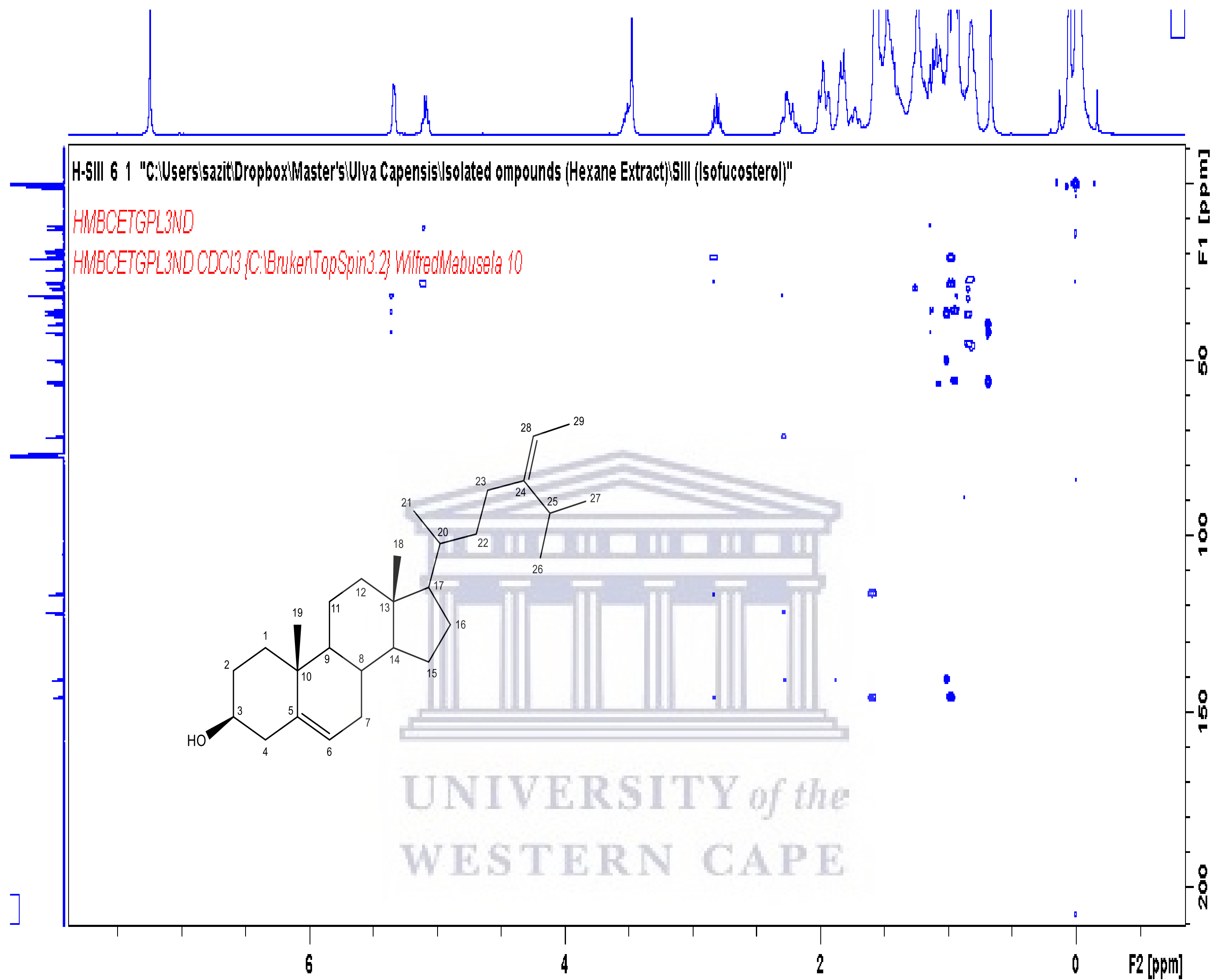
Appendix 1d: **HMBC spectrum of compound 1 (100 & 400 MHz, $CDCl_3$)**



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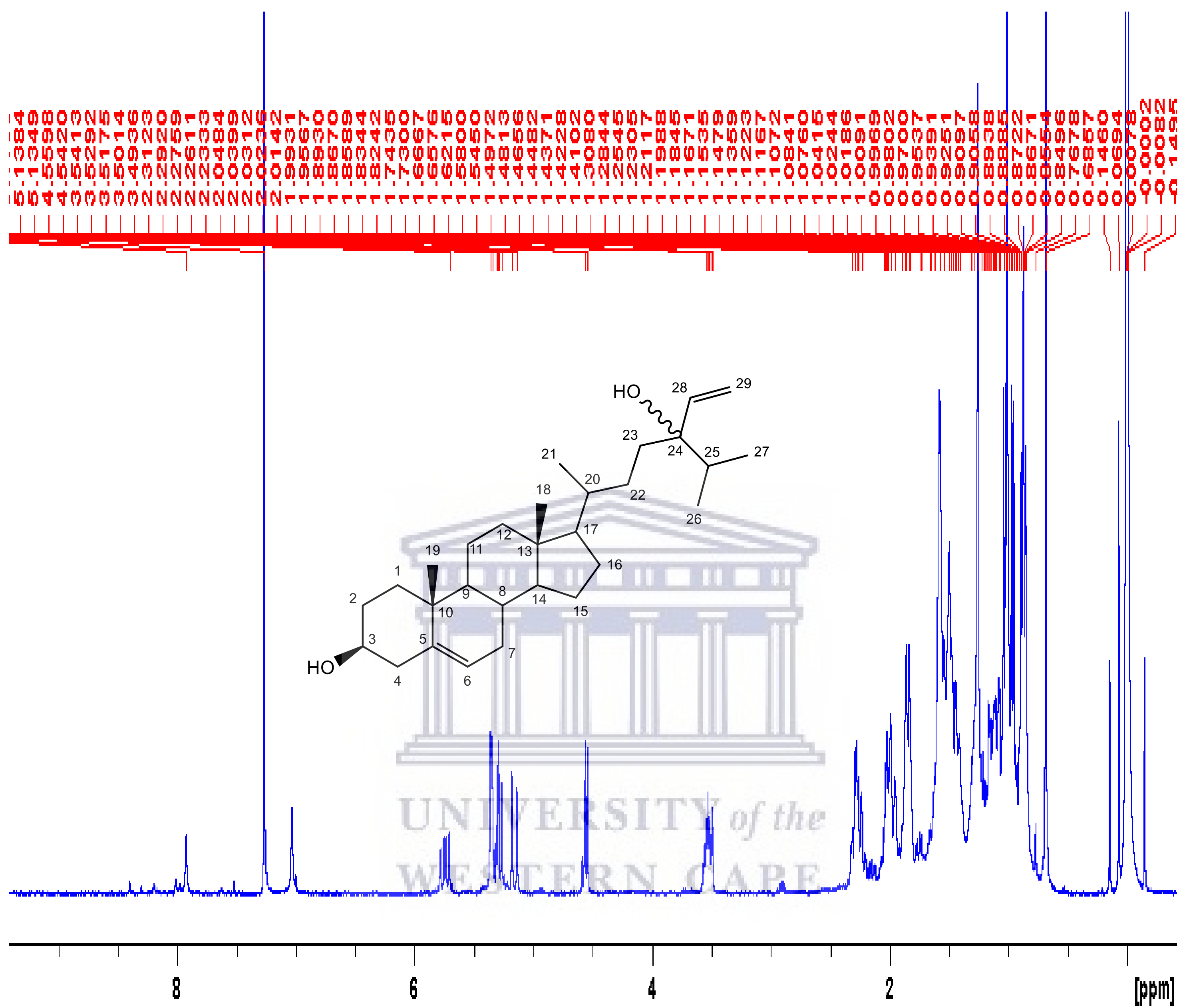
Appendix 2a: **^{1}H NMR spectrum of compound 2 and 3 (400 MHz, $CDCl_3$)**



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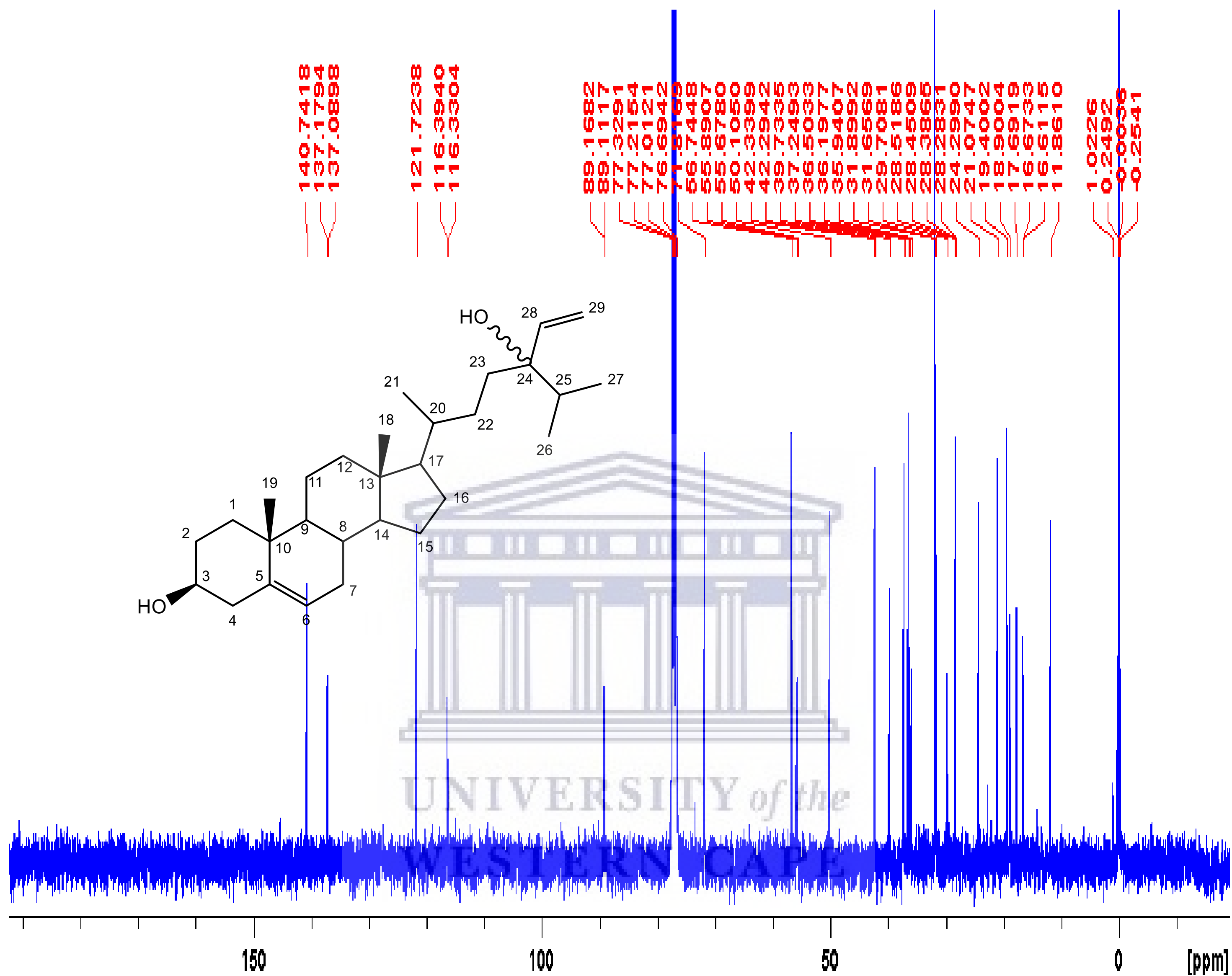
Appendix 2b: **^{13}C NMR spectrum of compound 2 and 3 (100 MHz, $CDCl_3$)**



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Appendix 2c: **DEPT 135 spectrum of compound 2 and 3 (100 MHz, $CDCl_3$)**

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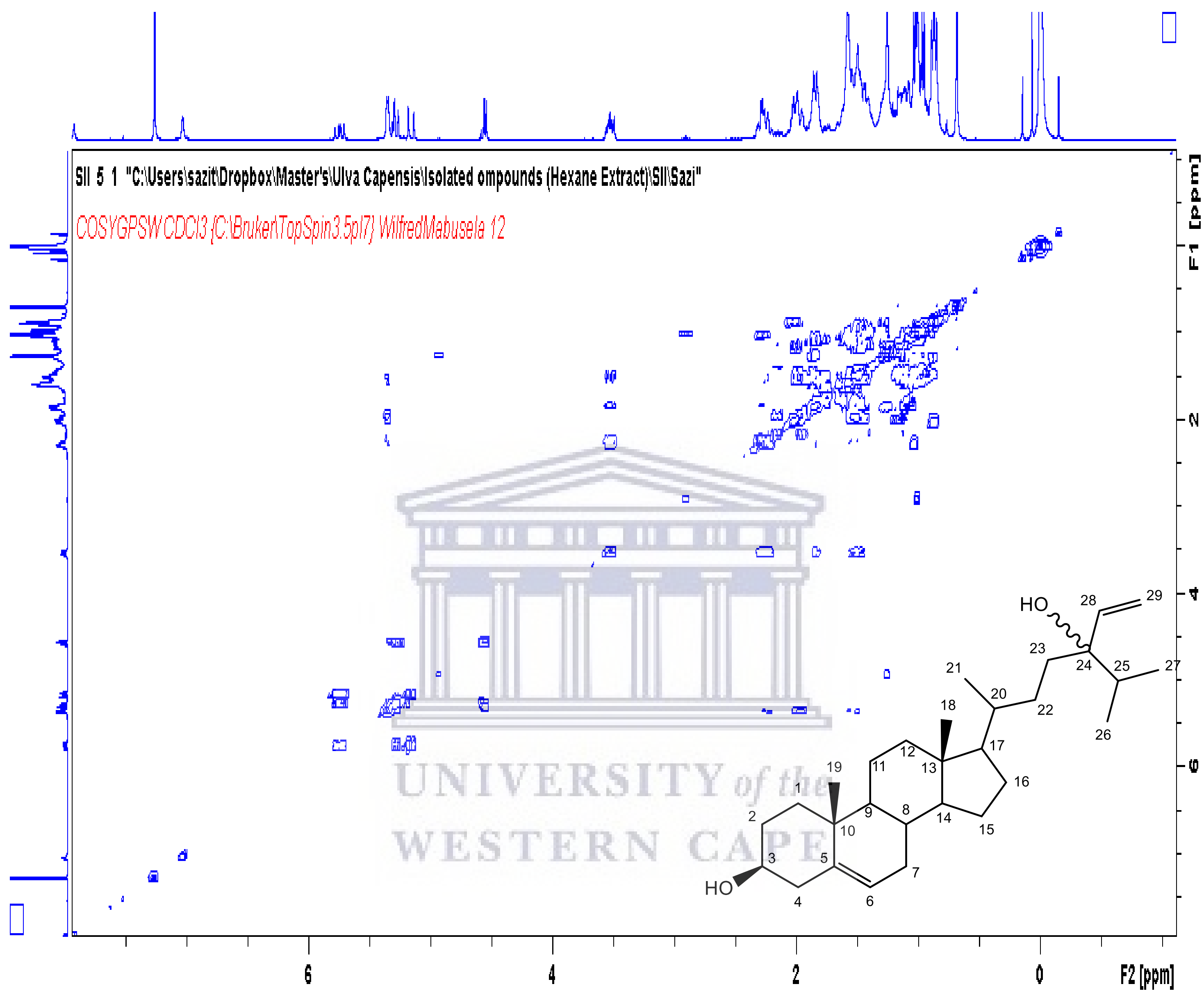
Appendix2d: **COSY NMR spectrum of compound 2 and 3 (400 MHz, $CDCl_3$)**



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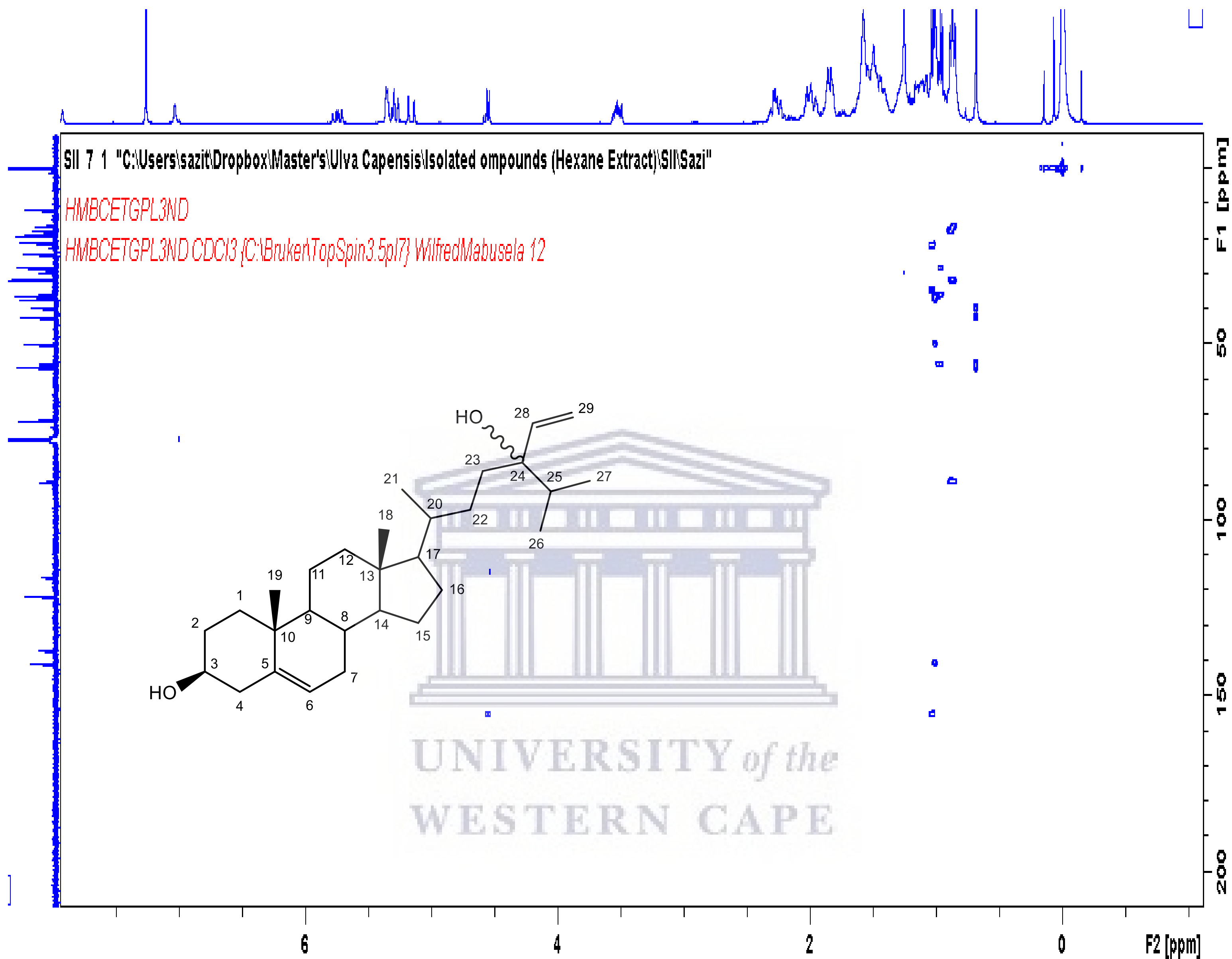
Appendix 2e: **HMBC NMR spectrum of compound 2 and 3 (400 & 100 MHz, $CDCl_3$).**



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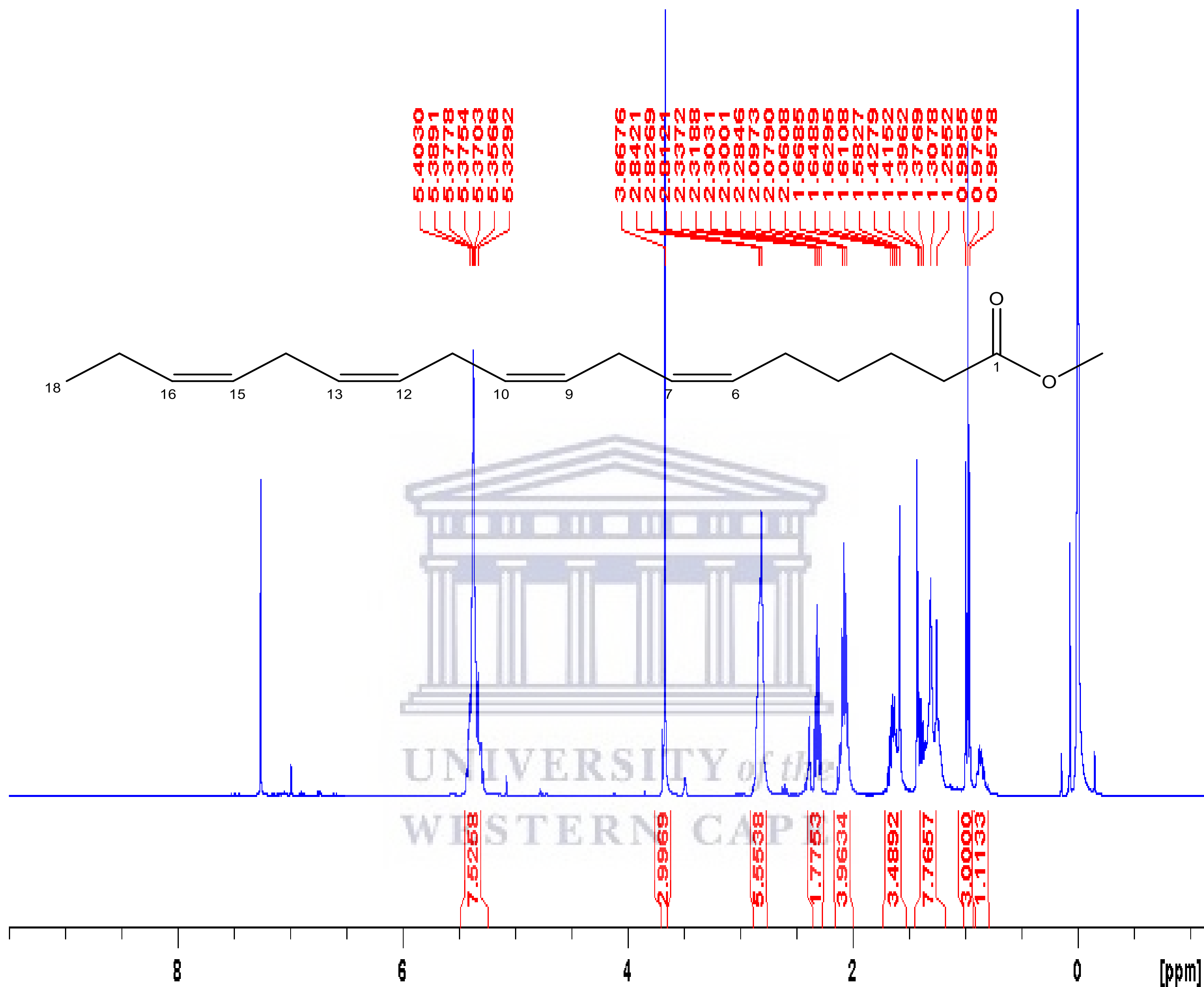
Appendix 3a: **^{1}H NMR spectrum of compound 4 (400 MHz, $CDCl_3$)**



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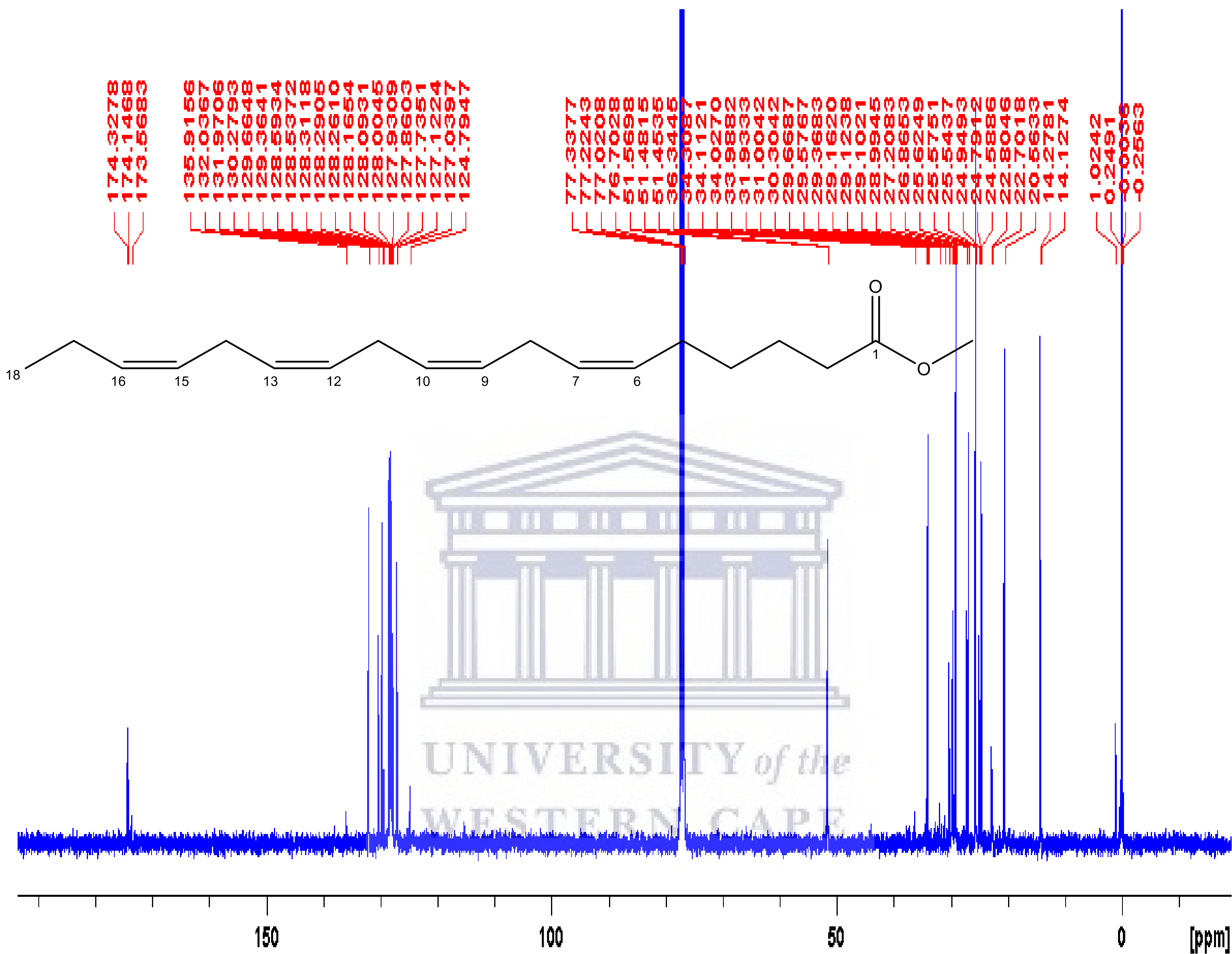
Appendix 3b: **^{13}C NMR spectrum of compound 4 (100 MHz, $CDCl_3$)**



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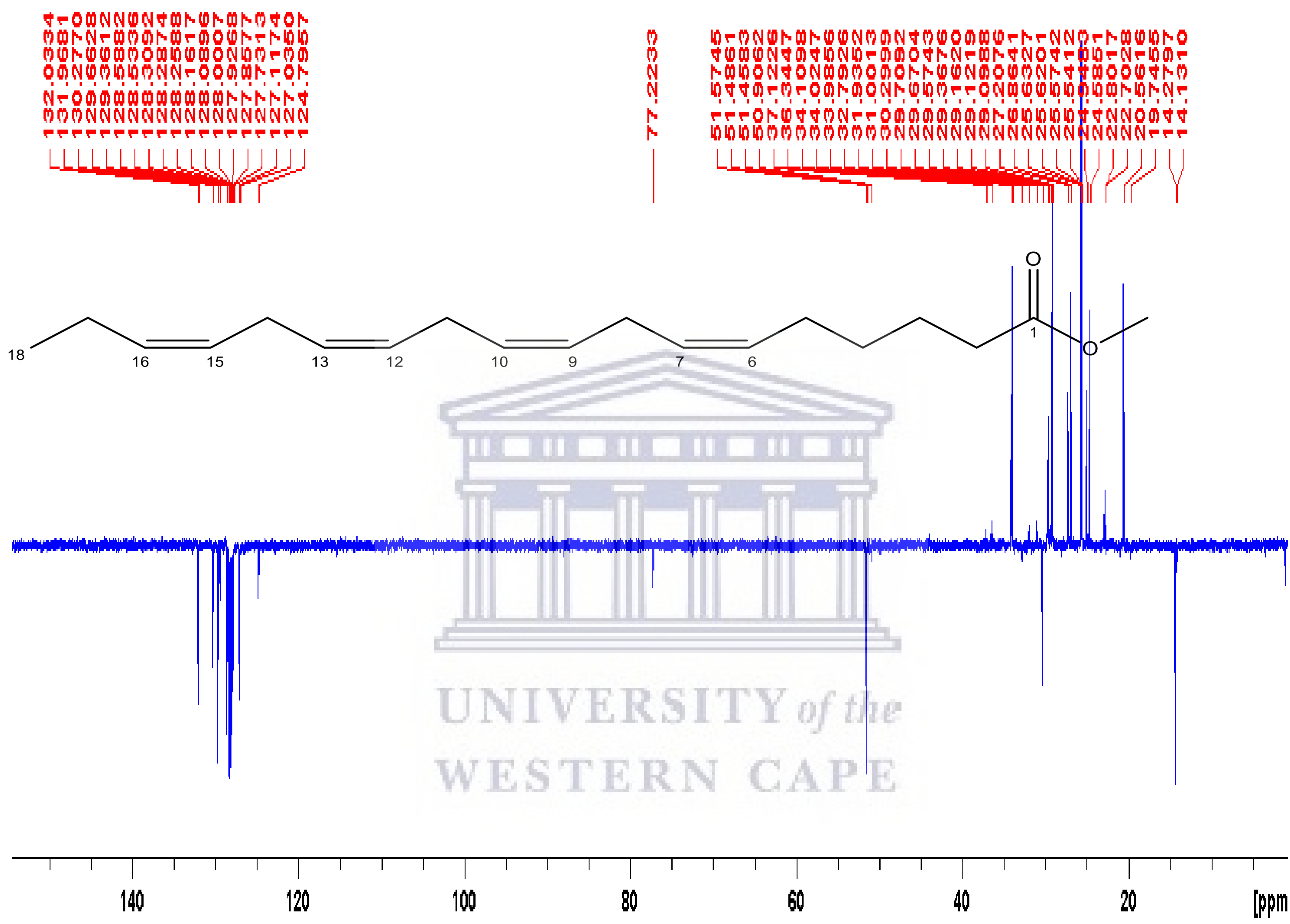
Appendix 3c: **DEPT 135 NMR spectrum of compound 4 (100 MHz, $CDCl_3$)**



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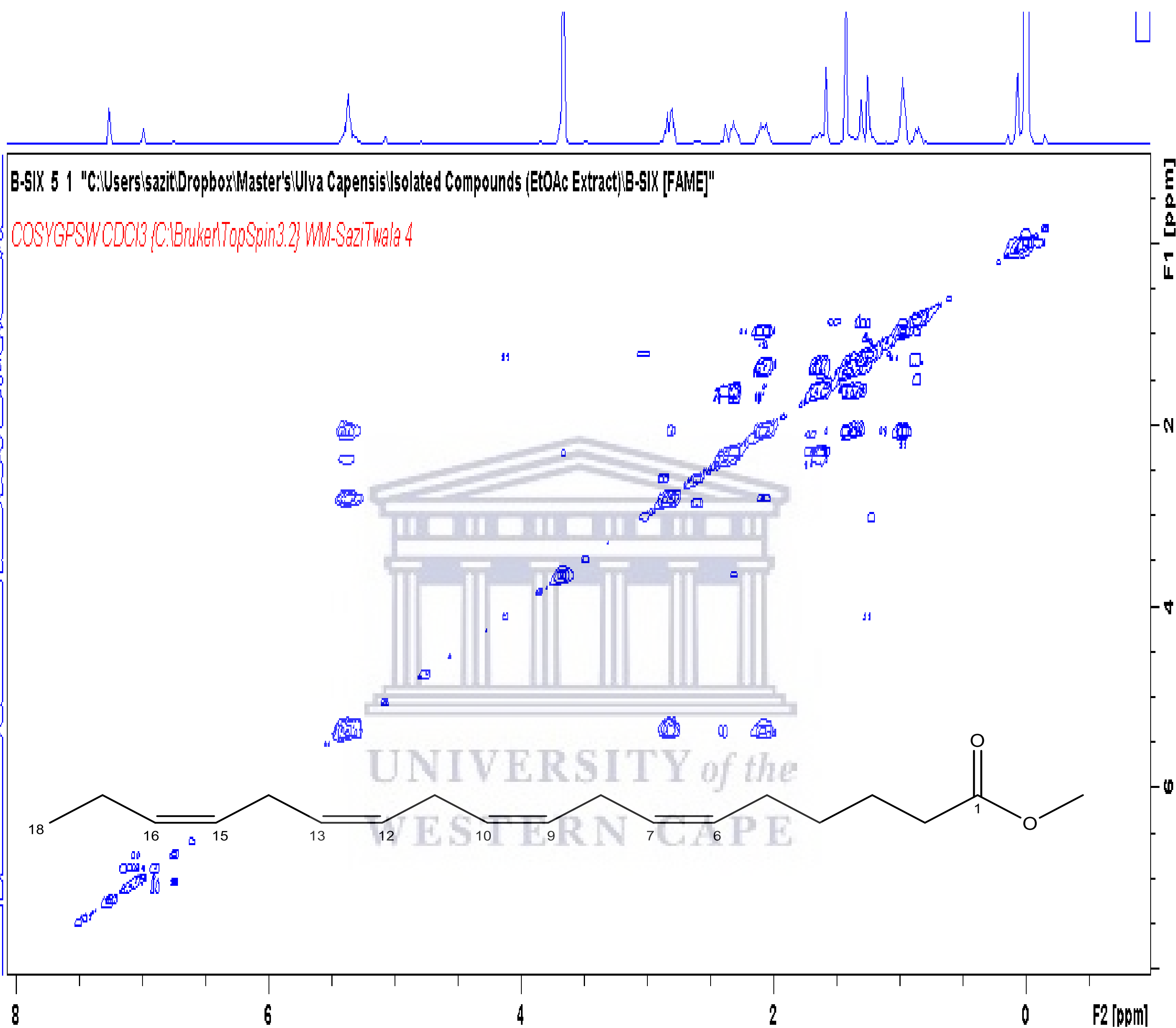
Appendix 3d: **COSY NMR spectrum of compound 4 (400 MHz, $CDCl_3$)**



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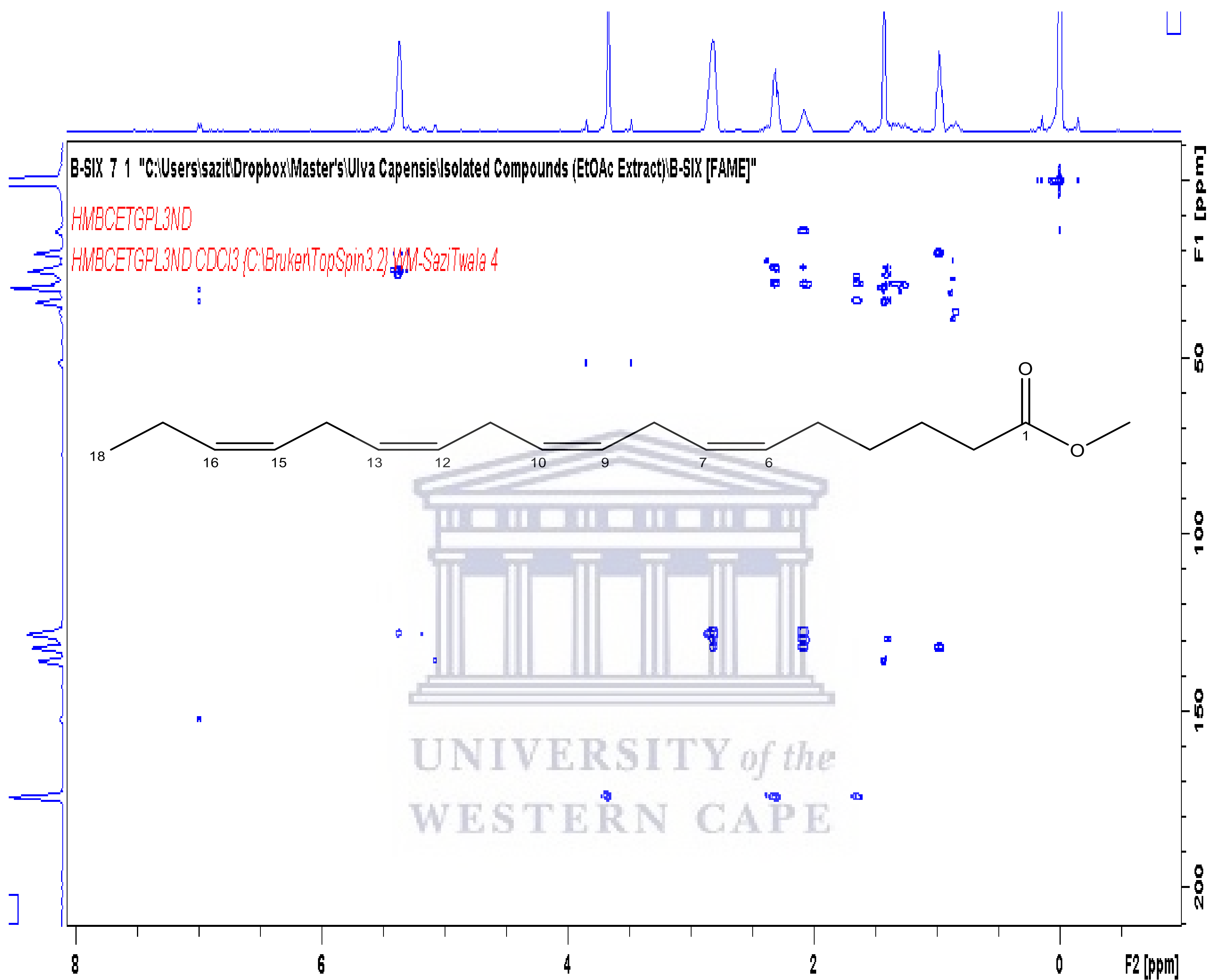
Appendix 3e: **HMBC NMR spectrum of compound 4 (100 & 400 MHz, $CDCl_3$)**



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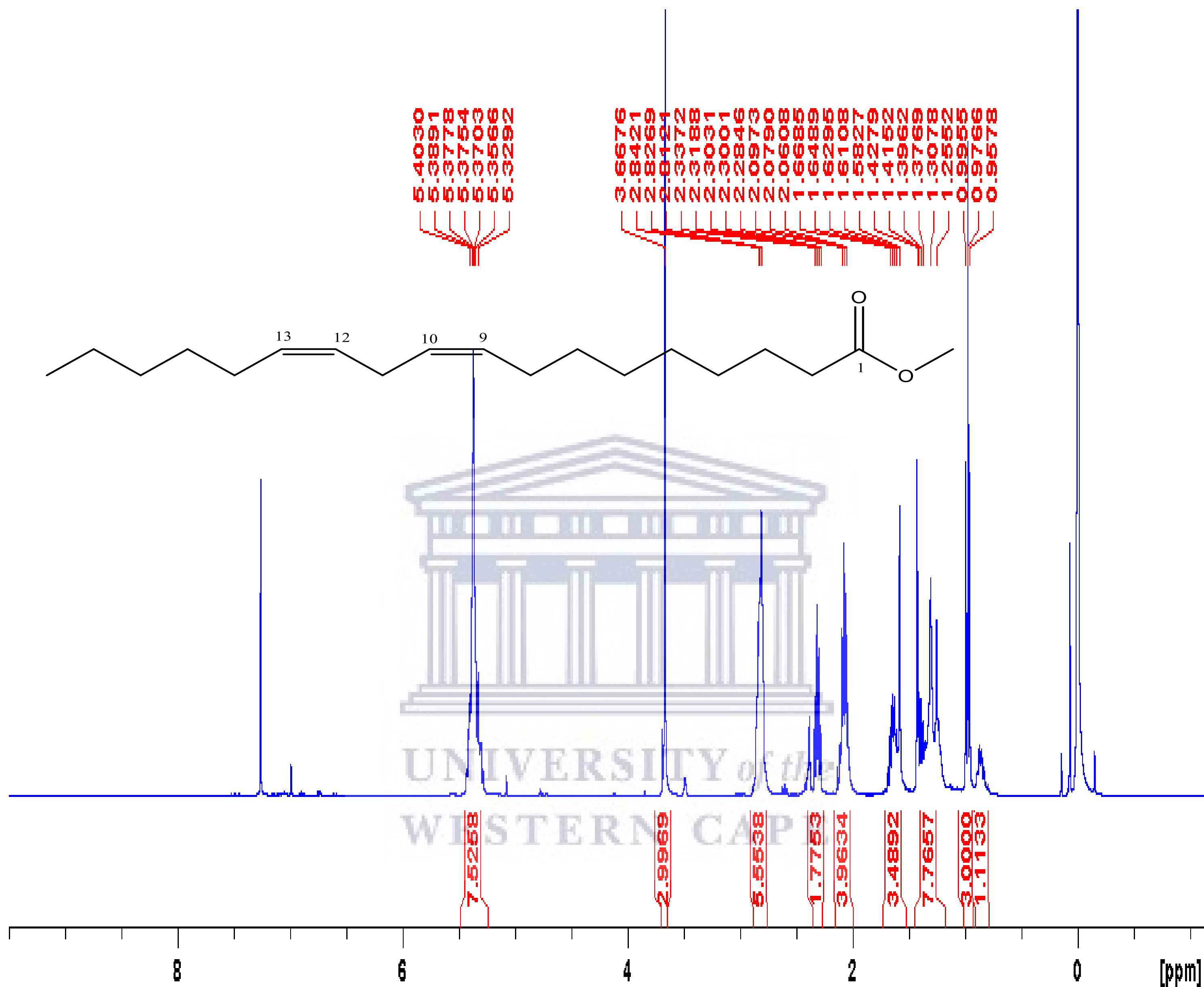
Appendix 4a: **^{1}H NMR spectrum of compound 5 (400 MHz, $CDCl_3$)**



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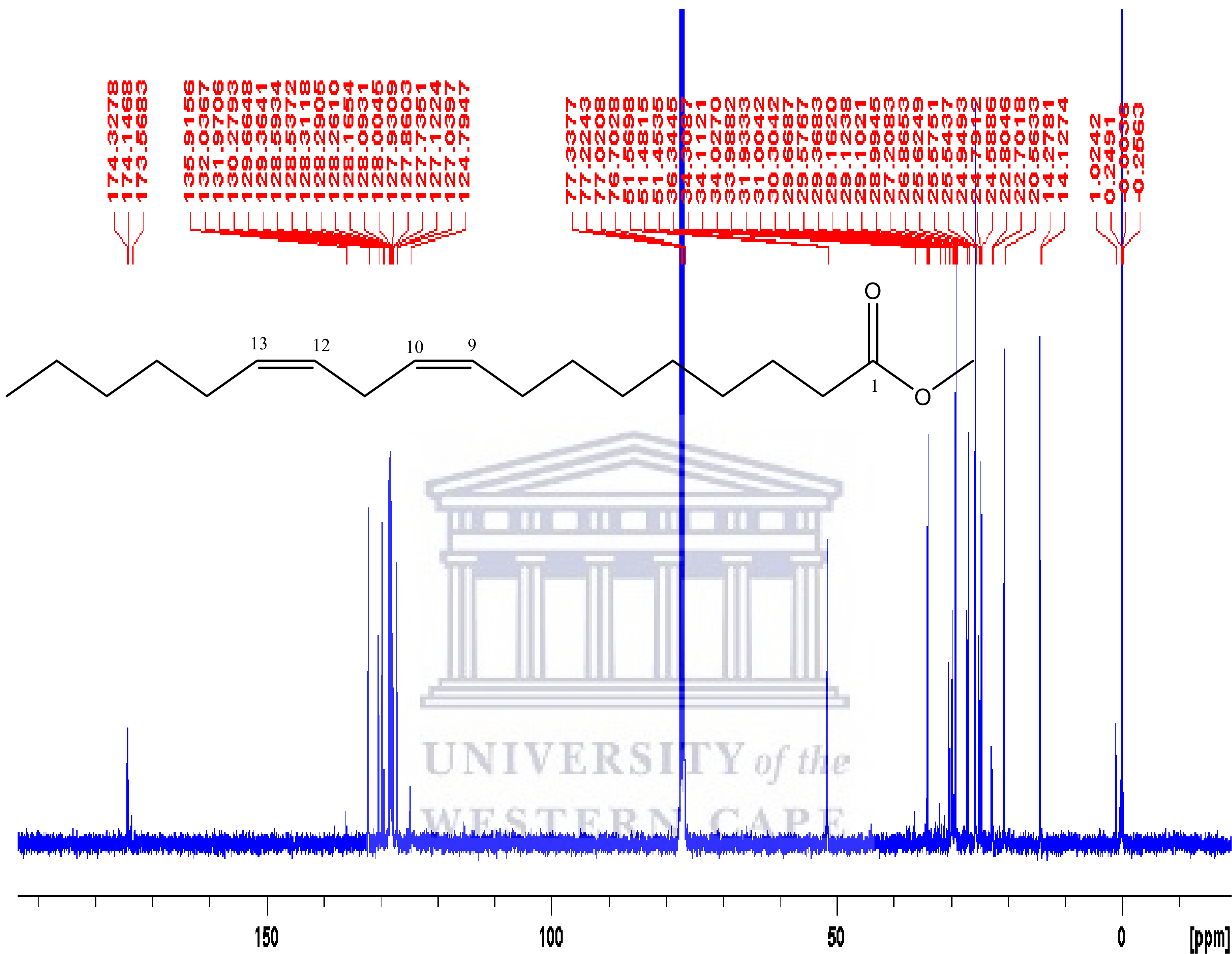
Appendix 4b: **^{13}C NMR spectrum of compound 5 (100 MHz, $CDCl_3$)**



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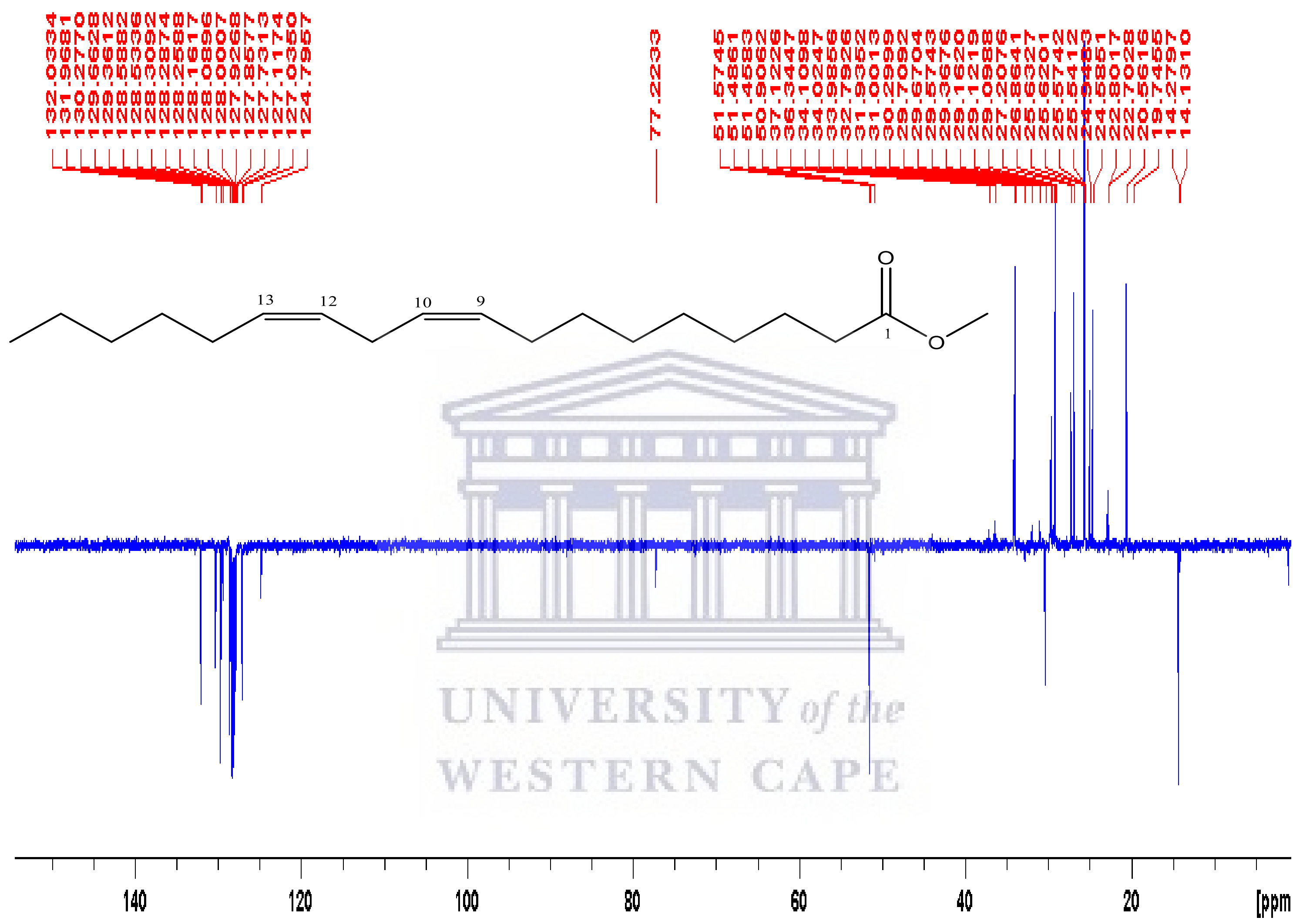
Appendix 4c: **DEPT 135 NMR spectrum of compound 5 (100 MHz, $CDCl_3$)**



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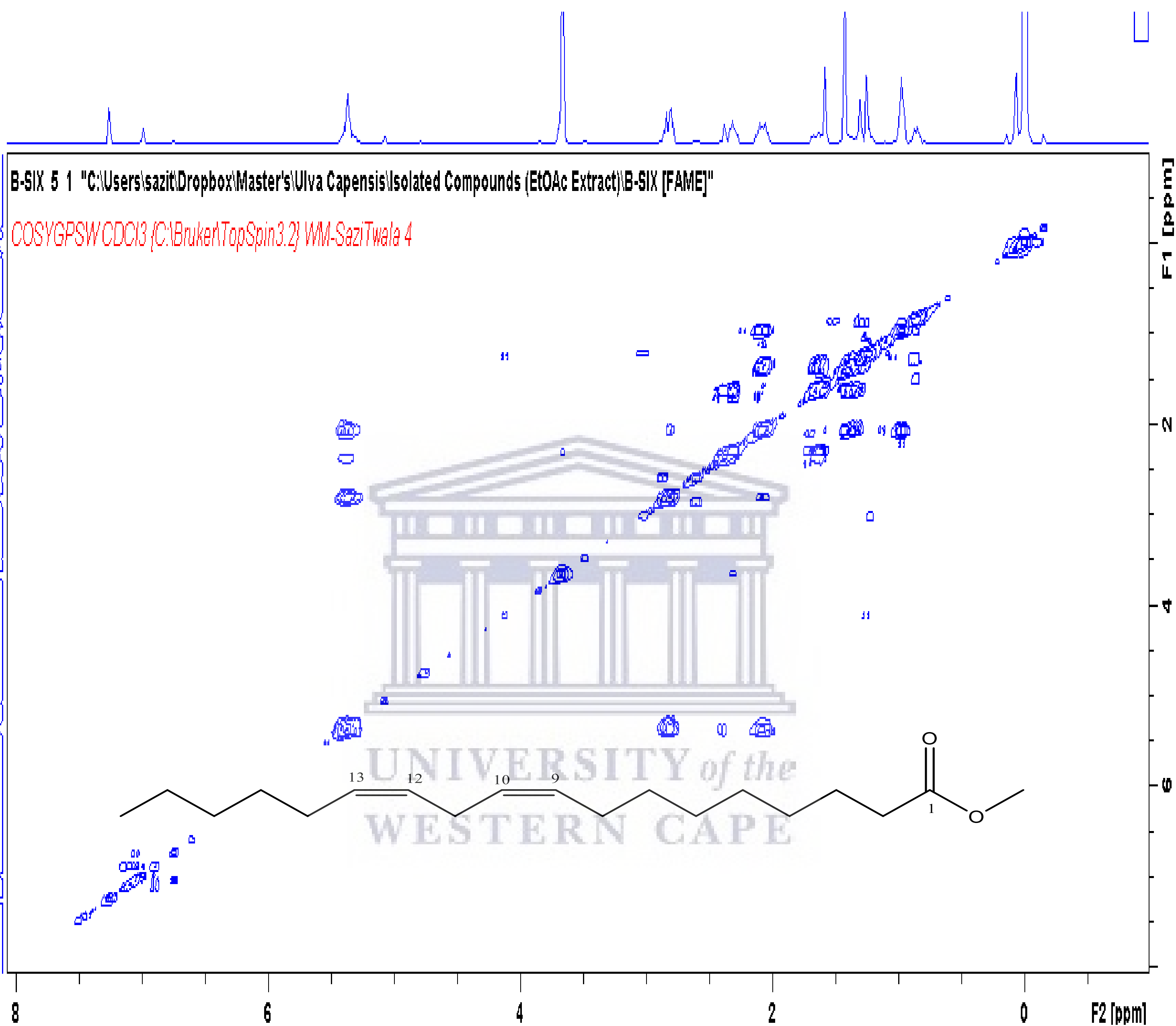
Appendix 4d: **COSY NMR spectrum of compound 5 (400 MHz, $CDCl_3$)**



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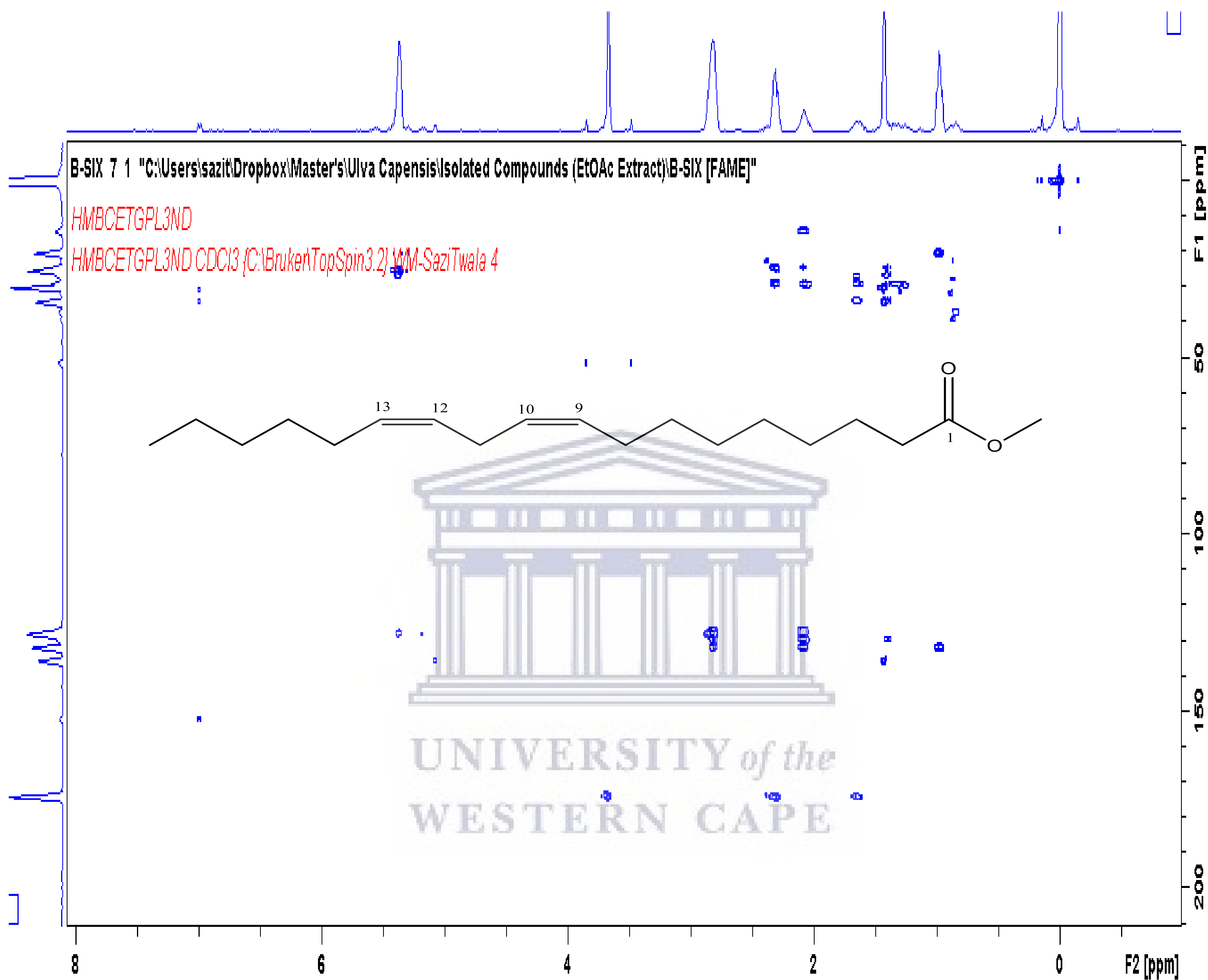
Appendix 4e: **HMBC NMR spectrum of compound 5 (100 & 400 MHz, $CDCl_3$)**



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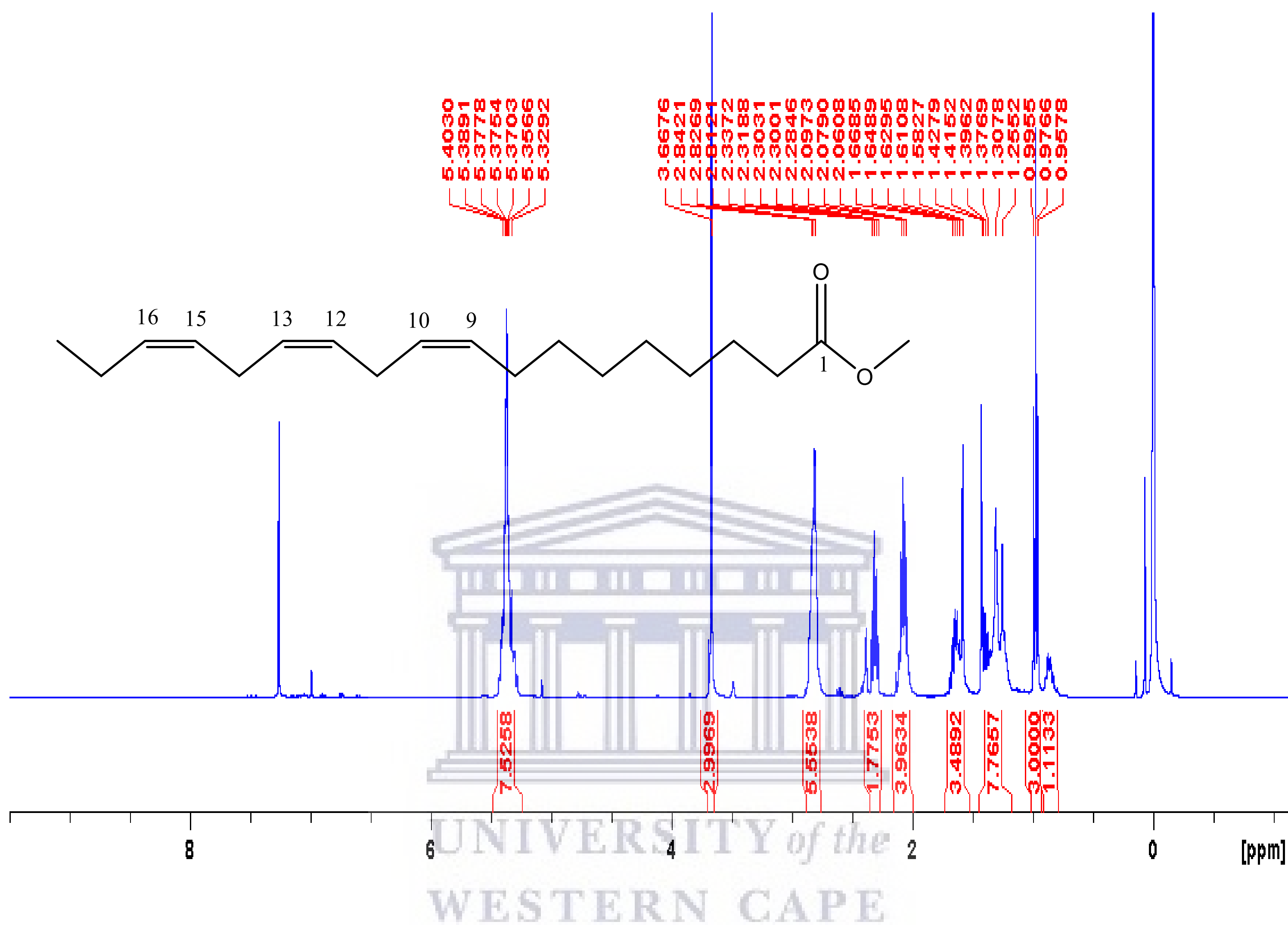
Appendix 5a: **^{1}H NMR spectrum of compound 6 (400 MHz, $CDCl_3$).**



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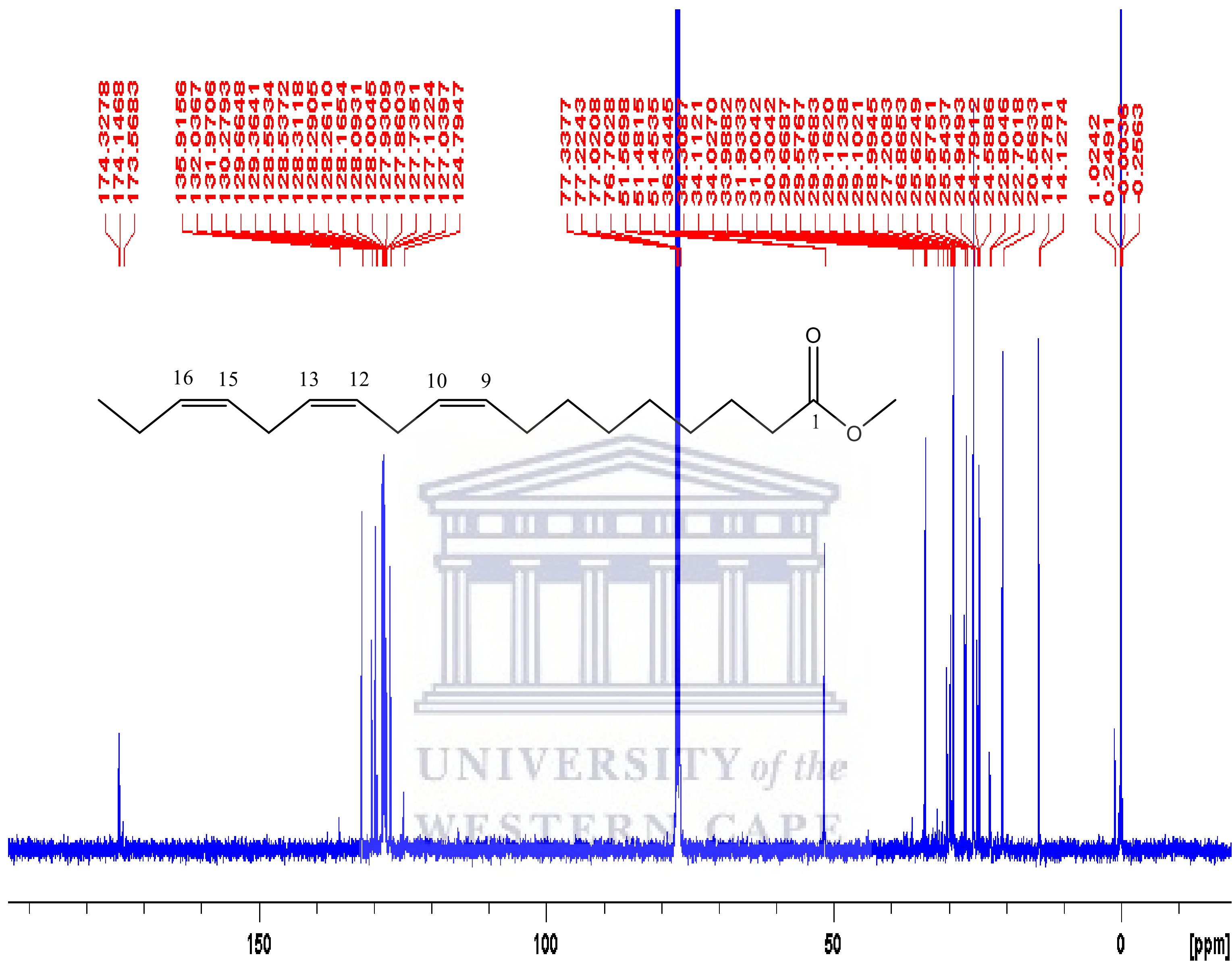
Appendix 5b: **^{13}C NMR spectrum of compound 6 (100 MHz, $CDCl_3$)**



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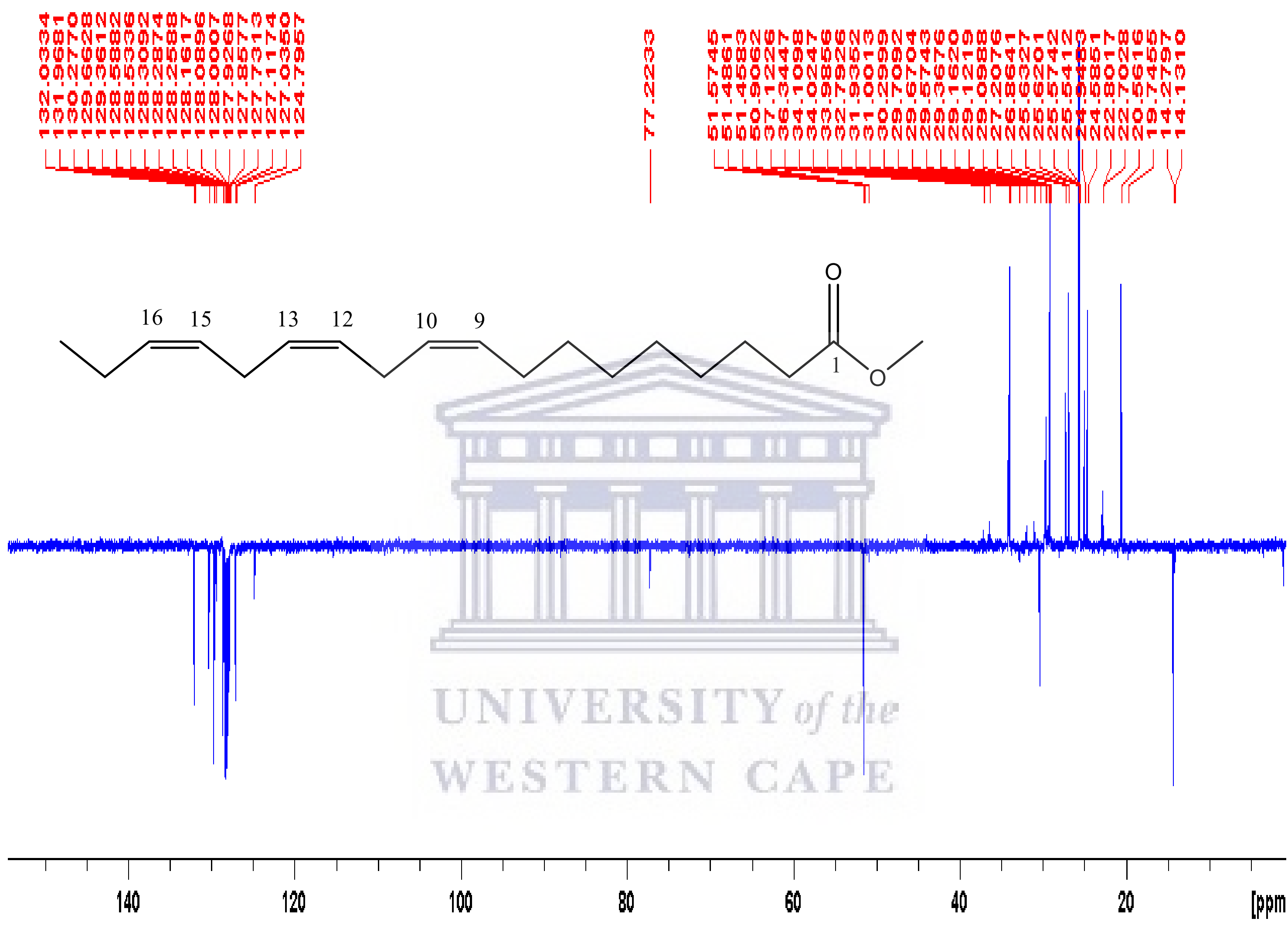
Appendix 5c: **DEPT 135 NMR spectrum of compound 6 (100 MHz, $CDCl_3$)**



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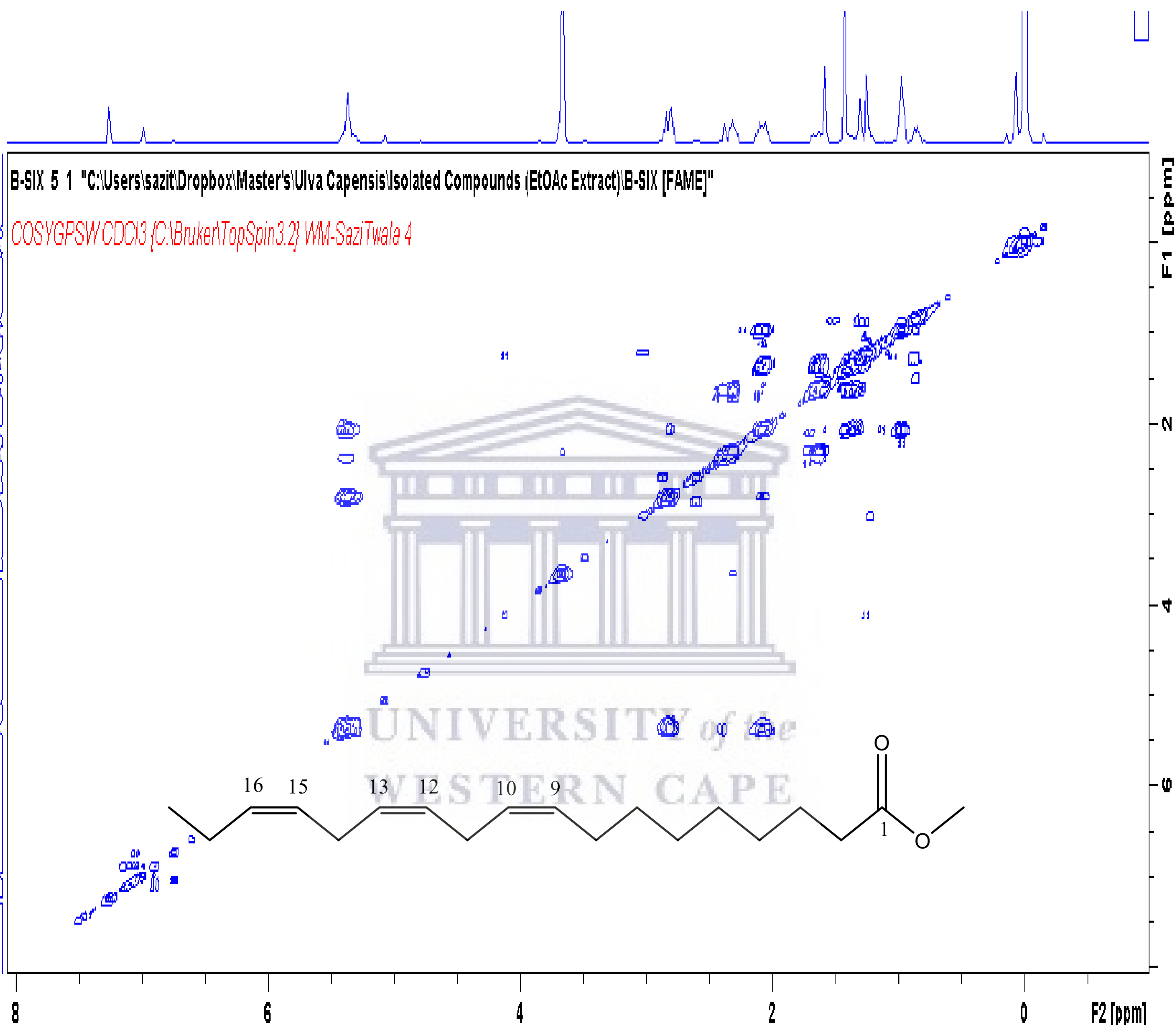
Appendix 5d: **COSY NMR spectrum of compound 6 (400 MHz, $CDCl_3$)**



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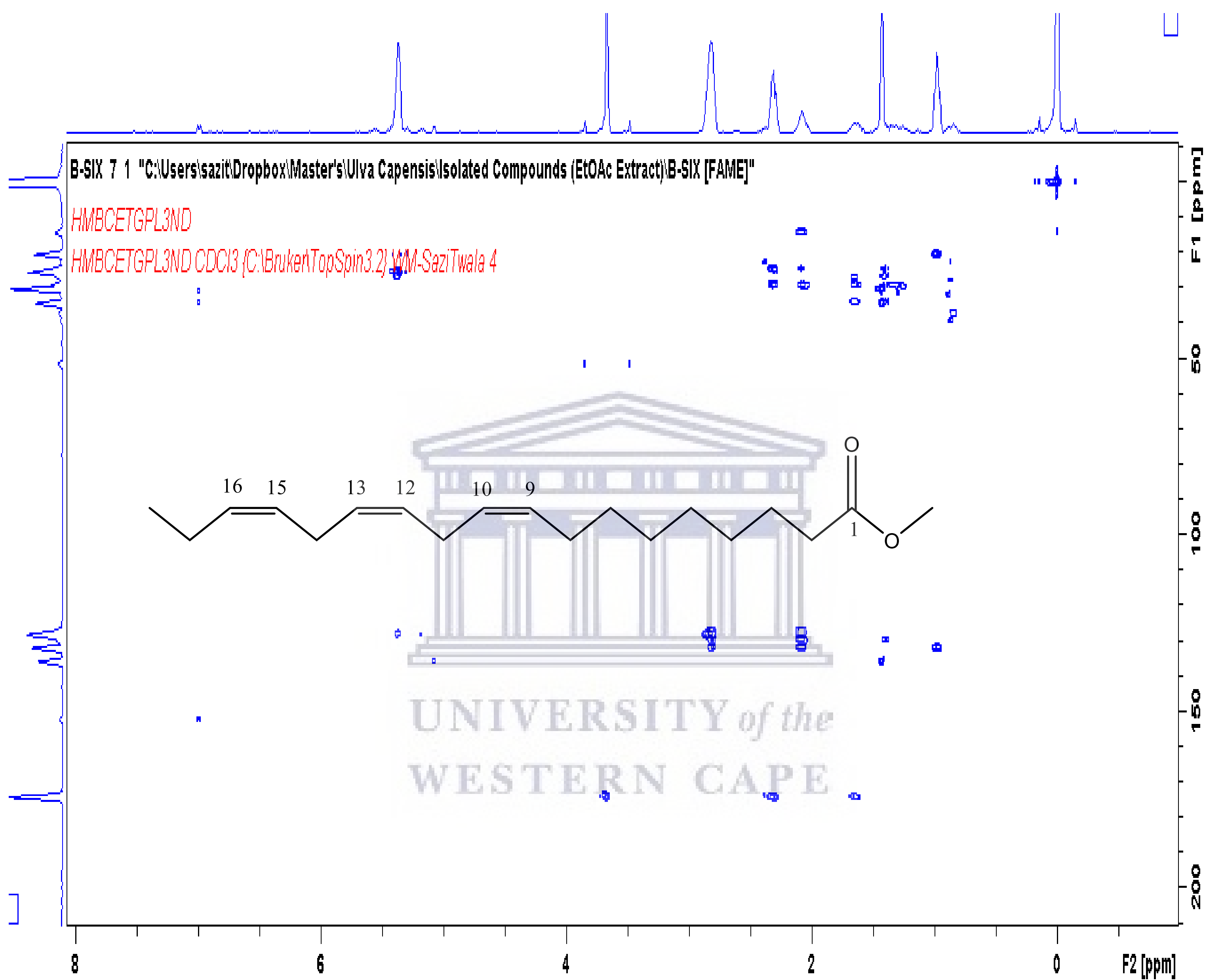
Appendix 5e: **HMBC NMR spectrum of compound 6 (100 & 400 MHz, $CDCl_3$)**



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