

Phytochemical studies of extracts from *Aloe succotrina*

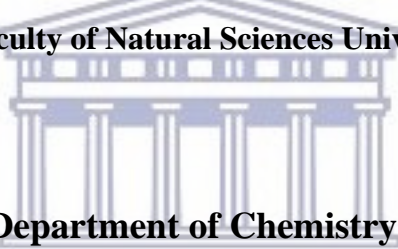


UNIVERSITY of the
WESTERN CAPE

By

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



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ABSTRACT

Global climate change and geographical differences are two major parameters known to have, either, direct or indirect influence on the production of secondary metabolites in plants, which in-turn may affect the quality and/or quantity of the overall metabolites. The primary purpose of this thesis was to evaluate the phytochemistry of the whole leaf of *Aloe succotrina* Lam. spp. – a South African native plant – through a chromatographic spectroscopic approach—against available data accumulated for the cultivated population. Preliminary screening of the crude extracts *i.e.* HEX, DCM and EtOAc on TLC aluminium plates pre-coated with silica gel 60 F₂₅₄ followed by various chromatographic separation, led to the isolation of five known compounds: β -sitosterol (**1**) and two anthrone-C-glycosides (**2** and **5**), including two coumarin derivatives—the aglycone (**3**) and glycoside derivative (**4**). Notably, apart from **5** and **2**, the accumulation of **1**, **3** and **4** in *A. succotrina* Lam. spp. is reported for the first time. Structural elucidation of the individual compounds was achieved by extensive spectroscopic analysis *i.e.* MS, IR, ¹H and ¹³C-NMR spectroscopy and in some cases comparison to the literature. A comparative HPLC chromatogram of the crude MeOH extract of the leaves of *A. succotrina* Lam. was developed for qualitative (and quantitative) identification of the active metabolites, which could be realized by VWD with detection at 290 nm.

Brine shrimp lethality bioassay was used to evaluate *in vivo* cytotoxicity activity of the MeOH, HEX, DCM, and EtOAc crude extracts. The EtOAc extract exhibited the highest toxicity with LC₅₀ values corresponding to 31.13 μ g/mL.

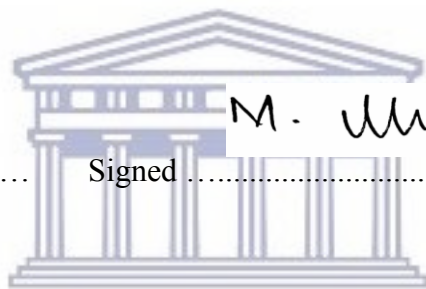
Key word: Phytochemistry, *Aloe succotrina* Lam. spp., HPLC.

DECLARATION

I, **Masixole Makhaba** hereby declare that this work is my original dissertation and to my knowledge, it has not been submitted anywhere else for the award of a degree at any other University. Where other written sources have been quoted, then their words have been re-written but the general information attributed to them has been referenced. I further declare that ethical guidelines were complied with in conducting this study.

Date01/12/2017.....

Signed



M. Makhaba

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I would like to take this opportunity to express my sincere gratitude to my supervisor **Prof W.T. Mabusela**, whom has not just been a mentor but an anchor throughout this project. It has been an honor and a privilege to work with someone who is so dedicated, passionate and humble with his work. He has instilled my passion for research and I've learnt a lot. For that, I will forever be grateful.

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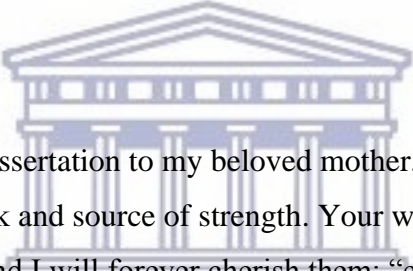
Many thanks to my dear friend, **Mr. Lutho Attwell Klaas**, you know the struggle. Thank you for being a true and a supportive friend. And to the rest of my family, thank you for your uttermost love and support.

I also wish to express my appreciation to the **National Research Foundation** (NRF) for the financial support.

Finally but not the last, my eternal gratitude goes to my Heavenly Father, the Lord Almighty!

DEDICATION

“Let perseverance finish its work so that you may be mature and complete, not lacking anything.” (Declares the Lord) – **James 1:4.**



I would like to dedicate this dissertation to my beloved mother, **Miss Thenjiswa Makhaba.** Thank you for being my rock and source of strength. Your words of encouragement still linger on in my mind and I will forever cherish them: “education is priceless.”

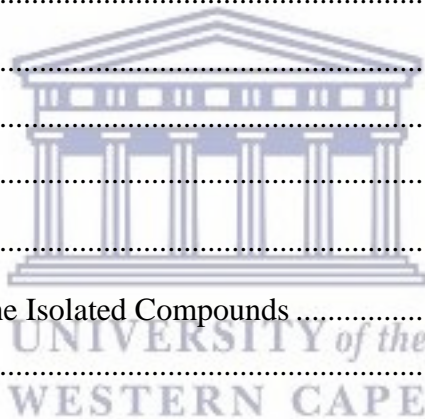
“Ah! Tolo, Zulu, Dlangamandla, Mchenge, Mabhanekazi.”

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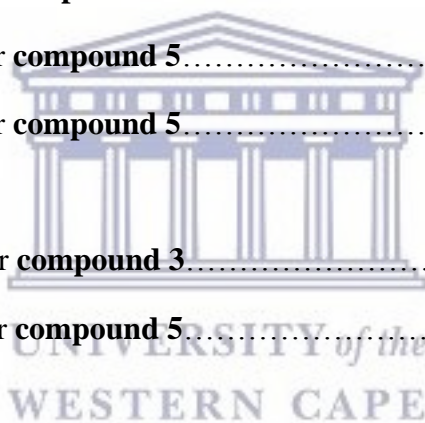
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LIST OF ABBREVIATION

EtOAc	Ethyl Acetate
DCM	Dichloromethane
MeOH	Methanol
EtOH	Ethanol
HEX	Hexane
BuOH	Butanol
ACN	Acetonitrile
dH ₂ O	De-ionised Water
DMSO	Dimethyl sulphoxide
CDCl ₃	Deuterated Chloroform
CD ₃ OD	Deuterated Methanol
1D	One Dimension
2D	Two Dimensions
NMR	Nuclear Magnetic Resonance
¹ H-NMR	Proton Nuclear Magnetic Resonance
¹³ C-NMR	Carbon-13 Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
DPT-135	Distortionless Enhancement by Polarization Transfer
TLC	Thin Layer Chromatography

CC	Column Chromatography
LC-MS	Liquid Chromatography-Mass Spectroscopy
HPLC	High Performance Liquid Chromatography
HPCCC	High Performance Counter -Current Chromatography
DI-ESI-MS	Direct Injection Electrospray Ionization Mass Spectrometry
MS	Mass Spectrometry
FTIR	Fourier Transform Infrared Spectroscopy
IR	Infrared Spectroscopy
UV	Ultra-Violet
DI-ESI-MS	Electrospray Ionization Mass Spectrometry
VWD	Variable Wavelength Detector
°C	Degrees Celsius
MHz	Megahertz
<i>J</i>	Spin-spin Coupling Constant
δ_C	Carbon Chemical Shift
δ_H	Proton Chemical Shift
<i>dd</i>	doublet-of-doublets
<i>d</i>	doublet
<i>t</i>	triplet
<i>m</i>	multiplet
nm	nanometre
appx.	Appendix
K	Potassium

KBr	Potassium Bromide
FA	Formic Acid
H ₂ SO ₄	Sulfuric Acid
Mg	Magnesium
Zn	Zinc
MW	Molecular Weight
ATM	African Traditional Medicine
AFT	African Traditional Medicine
Spp.	Species
LC ₅₀	Lethal Concentration 50



CHAPTER 1

Introduction and Literature Review

1.1 General Introduction

Over the last decades, plant chemistry has emerged as a distinct discipline which focuses on the study of natural products and their chemical composition. The use of these plant derived metabolites in (or as) traditional formulation is quite elaborated among various cultures, particularly, in the African continent. Furthermore, there have been numerous attempts made to isolate these plant metabolites and hence various classes of compounds have since been identified. Nevertheless, due to recent advances in (experimental) technology such as comprehensive isolation and structural determination techniques, subsequent research approaches have been adopted in order to facilitate the implementation of these novel plant derivatives into modern medicine; especially as new lead drugs. As such, there has been an empirical shift – from traditional chemotaxonomic investigation studies – to now more bio-assay guided isolation and identification techniques for the active compounds.

1.2 The Status of Traditional Medicine in the African Context – *an Overview*

Traditional medicine or folklore medicine dates back to the first existence of men. It constitutes knowledge that has been passed on through subsequent generations. This knowledge, which is still popular even today among most cultural countries in the African continent such as South Africa is based on beliefs and practices by indigenous people or traditional healers; whom are entrusted to diagnose and treat illnesses using sacred methods and herbs.

Nevertheless, novel records of these practices (using traditional medicine) can be found documented in various traditional systems of different countries. For instance, the two most ancient records are the Chinese *Material Medica* (11 000 B.C old) and Indian *Ayurveda* (5 000 B.C old) (Zhu, 1998; Gurib-Fakim, 2006; Mukherjee & Wahile, 2006; Dias *et al.*, 2012). By contrast, the African continent is undoubtedly the most diverse continent in as far as flora is concerned. Regrettably, there are, however, no extant records documenting systems of the African Traditional Medicine (ATM) (Gurib-Fakim, 2006).

In Africa, traditional medicine is part of the people's culture and is still considered convenient practise especially in rural areas, where access to 'western-medicine' seems to be hindered. It is therefore rather surprising that there has been no theoretical formulations of medical systems (apart from rock paintings) to allow the understanding of how each 'system' *i.e.* medicinal plant, is conceptualized. Sindiga *et al.* (1995) reports that these misfortunes could, perhaps, be as a result of colonial times which side-lined traditional healers (or "witch doctors" so referred) and ATM use thereof. However, according to Sofowora (1996), various sub-regional centres to coordinate research and innovation in ATM and pharmacopoeia were set up by the Organization of African Unity's' Scientific Technical and Research Commission (AOU/STRC) since 1968 but have not been effective. Nonetheless, the two volumes in 1985 and 86, respectively, of the African Pharmacopoeia (AP) are said to be the first successful records documenting botanical, commercial and phytochemical information of over 100 African native plants (Sofowora, 1996; Elujoba, 2012; Abegaz *et al.*, 2013). In addition to the foretold, the Ghana Herbal Pharmacopoeia (GHP, published in 1992 &2007) and Nigerian Herbal Pharmacopoeia (NHP, published in 2008) have now formed part of the new African Herbal Pharmacopoeia (AHP) spearheaded by the Association African Medicinal Plants Standards (AAMPS), which includes monographs of about 51 plants (Elujoba, 2012; Abegaz *et al.*, 2013). At the same time, various network bodies involved in research and development of medicinal plants within the context of Africa exists in various sub-regions of the African continent. Among these includes the Natural Products Research Network for East and Central Africa (NPRECA), Network for Analytical and Bioassay Services (NABSA), Western Africa Network of Natural Products Research Scientists (WANNPRES) (WHO, 2000). In addition, various other international organizations such the African Biodiversity Network (ABN) and Institute for Natural Resources in Africa (INRI) have also been founded with the view of facilitating novel research efforts (Sofowora, 1996). These strategic attempts which encompass re-evaluation of African medicinal plants as whole, for quality, safety and efficacy are drawn up in accordance with World Health Organization (WHO) guidelines (WHO, 2000).

From a South African perspective, government has encouraged efforts in research and development of medicinal plants and documentation thereof. In its strategic plan, the South African government has adopted the Traditional Health Practitioners Act (Act 35 of 2007) as a framework for all traditional medicine or practices (Republic of South Africa, 2007). In view of this, various research bodies exist; such as the South African Traditional Medicine

Research Group (SATMERG), which is in the process of drawing up pharmaceutical monographs of 60 selected medicinal plants native to South Africa (SATMERG, 2010). The South African Medicinal Research Council (SAMRC) is another novel South African research organization that is devoted in quality and safety assessment of natural products.

1.3 Natural Products

Natural products (also called secondary metabolites) have been rediscovered as strategic tools for drug developments despite recent advancements in combinatorial chemistry (Newman & Cragg, 2007). These novel components from plants (and often animals) have been used in traditional systems of many cultures in the form of herbal concoctions, for instance, to treat flue and cough symptoms.

Unlike primary metabolites which are the fundamental building blocks of all living organisms, secondary metabolites are either present in plants as active components, or as inactive precursors; in which case they may be induced *de novo* after stress via enzyme-catalyzed processes (Osbourn, 1996). For instance, phytoalexins *e.g.* 3-deoxyanthocyanidins and luteolin from *Sorghum bicolor*, capsidiol from *Capsicum annuum*, viniferins from *Vitis vinifera* etc., are synthesized as defense repertoire against microbial and other pathogen infections (Ahuja *et al.*, 2012). It has also been suggested that some (phenolic) phytoalexins have health-promoting effects as anti-oxidants, anti-carcinogenic and cardiac agents (Boue *et al.*, 2009; Jahangir *et al.*, 2009; Pedras *et al.*, 2011).

Nevertheless, for many years research on higher plants has been largely focused on descriptive taxonomic classification *i.e.* based on physical characteristics, and natural product chemistry has been overlooked. However, it was not until the 19th century that scientists began to develop strong interests in phytochemical studies of higher plants. Early publications suggest that among the first secondary metabolite(s) to be reported as active clinical agent(s) were morphine (see **Fig. 1**); which was first isolated in 1806 by a German pharmacist from *Papaver somniferum* opium (Schmitz, 1985). Merck started looking into commercializing of this drug in 1826. This discovery later inspired interests to a lot of scientists and led to the isolation of other secondary metabolites; such as vinblastine and vincristine from *Catharanthus roseus*, quinine and quinidine from *Cinchona spp.*, as well as atropine from *Atropa belladonna* (see **Fig. 1**). Since then, therapeutics has benefited from

numerous novel drug classes that are derived from natural product sources (Ji *et al.*, 2016). Thus, such overwhelming drug preponderance has been accelerated by robust research in the field of natural product chemistry – utilizing highly sophisticated analytical techniques such as HPL chromatography and NMR spectroscopy, which both play a significant role in structural elucidation (Dias *et al.*, 2012).

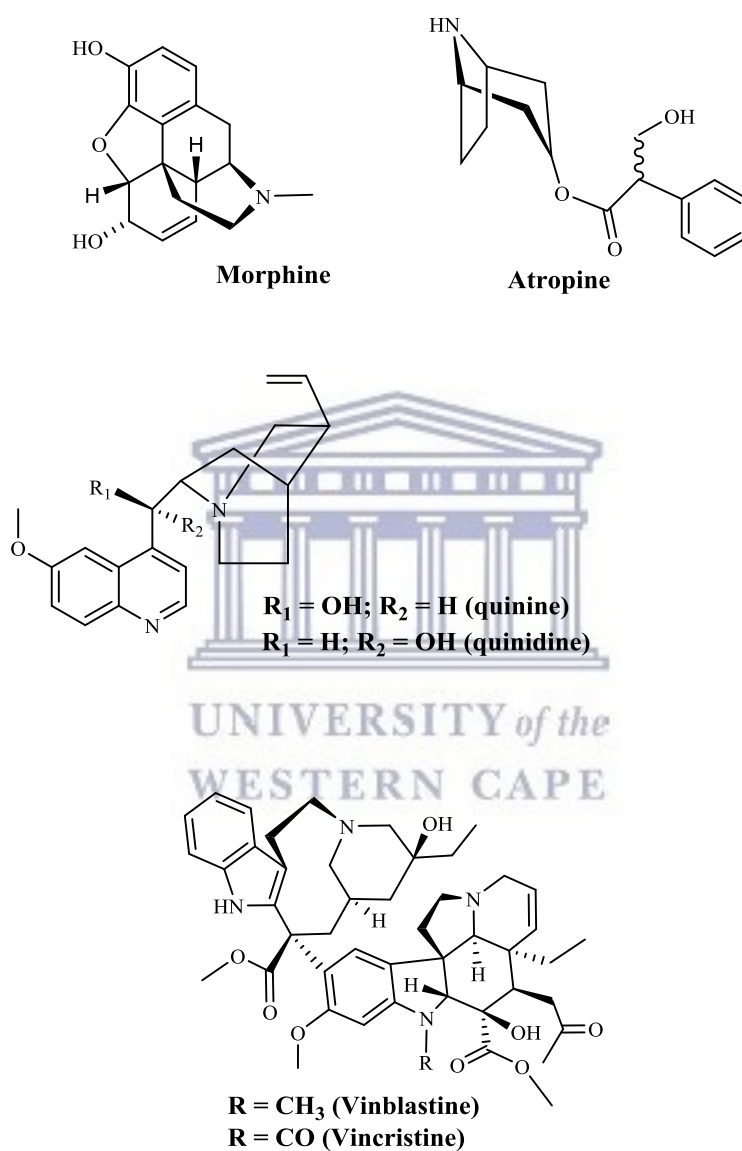


Fig. 1: Showing some of the earlier metabolites to be isolated.

1.4. Classification of Natural Products

The classification of natural products is essentially based on the chemical structure, biological occurrence and taxonomic origin. Classification according to the chemical structure is done based on its molecular skeleton *i.e.* linear, cyclic, aromatic or heterocyclic. These secondary metabolites are very complex and varied from plant to plant; which means each classification is made independent of each other. However, each class of compounds has the same functional group or basic carbon skeleton and to some extent similar biological activity. Alkaloids, glycosides, benzene, naphthalene and furan, chromones, flavonoids, anthraquinoids, pyrans and pyrones, coumarins, terpenoids and other miscellaneous derivatives all represent classes of novel compounds that have been identified from either the roots or the leaf part of the plant. However, only selected classes will be discussed in this thesis.

1.4.1 Glycosides

The term glycoside refers to secondary metabolites that contain a carbohydrate (*e.g.* glucose) linked to a non-carbohydrate moiety *e.g.* steroids via the anomeric position. The process of formation is termed glycosylation. Glycosides can be classified into two categories: O-glycosides and C-glycosides. Some glycosides in plants are typically stored as inactive conjugates. Hence, they become activated by an enzyme called hydrolase via a chemical process known as hydrolysis, which produces two bi-products; glycon (carbohydrate) and aglycon (non-carbohydrate) derivative. The glycone part may be a monosaccharide or a polysaccharide, whereas the aglycone derivative can be any class of natural products such as steroids and terpenoids (Doughari, 2012), or even chromone, anthrone and anthraquinone (Dagne *et al.*, 2000; Reynolds, 2004). In aloes though, most glycosides are elaborated as C-glycosides *e.g.* aloins A/B the major constituents of the leaf organ (Franz & Grun, 1983; Reynolds, 2004), O-glycosides have also been identified in some species. For instance, aloesapanol I-6-O-glucoside, aloesapanol II-6-O-glucoside and aloesapanol III-8-O-glucoside were isolated from *Aloe saponaria* (Yagi *et al.*, 1997), and the O-glycoside 1-O-glucoside of homonataloin B from *Aloe lutescens* (Van Heerden *et al.*, 2002). Furthermore, glycosides have been shown to exhibit potent pharmacological benefits. For example, saponins – specifically steroidal saponins – are known active agents in the treatment of cardiac diseases (Gurib-Fakim, 2006).

1.4.2 Anthraquinoids (Anthraquinones and anthrones)

Anthraquinoids are a class of naturally occurring metabolites found in most Aloe species (and other species). Anthrones and Anthraquinones are the identifiable members of this group. The former is a diketone derivative with two ketone groups at carbon-9 & 10. Anthrones on the other hand are 9-oxoanthracene; anthraquinone derivatives. Their chemical distribution in plants, particularly in Aloes, is varied quite significantly. For example, anthraquinones, *e.g.* aloemodine, are largely distributed in the roots, whereas the leaves constitute mainly of anthrone derivatives which include aloin A/B (Dagne *et al.*, 2000). The distribution of anthraquinoids in aloes is discussed in detail later in this chapter. Nevertheless, the pharmacological roles of Aloe plants have mostly been attributed to Aloemodin and aloin A/B. Aloemodin and aloin A/B exist mainly as glycosides found in the leaf skin or the bitter exudate (Lucini *et al.*, 2013). The glycoside form is typically soluble in water and alcohol; high solubility is necessary for the metabolism of active aglycons. In traditional medicine, reports suggest the use of the bitter exudate as laxatives when ingested orally (Grace *et al.*, 2008). There have also been some potential claims for anti-oxidant activity on the inhibition of peroxidation of linoleic acid (Yen *et al.*, 2000). Other applications of anthraquinoids, especially anthraquinone derivatives, are in the textile industry as dye due to the natural affinity of sulfonic acid groups to bind to the fibre (Malik & Müller, 2016).

1.4.3 Phenols and polyphenols (including flavonoids)

Phenolic compounds obtained from higher plants represent a larger group of naturally occurring metabolites. These secondary metabolites are quite complex structurally and are varied from plant to plant. Hence, they are characterized by having at least one benzene ring with one or more hydroxyl groups attached; in which case they're called phenols. Unlike phenols, polyphenols constitute two benzene rings plus one or more hydroxyl group. These heterocyclic organic molecules occur exclusively on the outer green mesophyll and/or vascular bundles of the aloe leaf (Reynolds, 2004), and therefore have a chemotaxonomic value. Flavonoids are typical examples.

Flavonoids are a major group found within this class of compounds. Despite their elaborated documentation in other genera, flavonoids are neglected chemotaxonomic markers in the genus Aloe (Viljoen *et al.*, 1998). **Fig. 2** below shows structures of major sub-classes of flavonoid compounds found in 31 out of 380 Aloe species screened. It is suggested that in aloes the distribution of these compounds, flavanones and flavones in particular, is mutually exclusive and provide chemotaxonomic evidence (Dagne *et al.*, 2000).

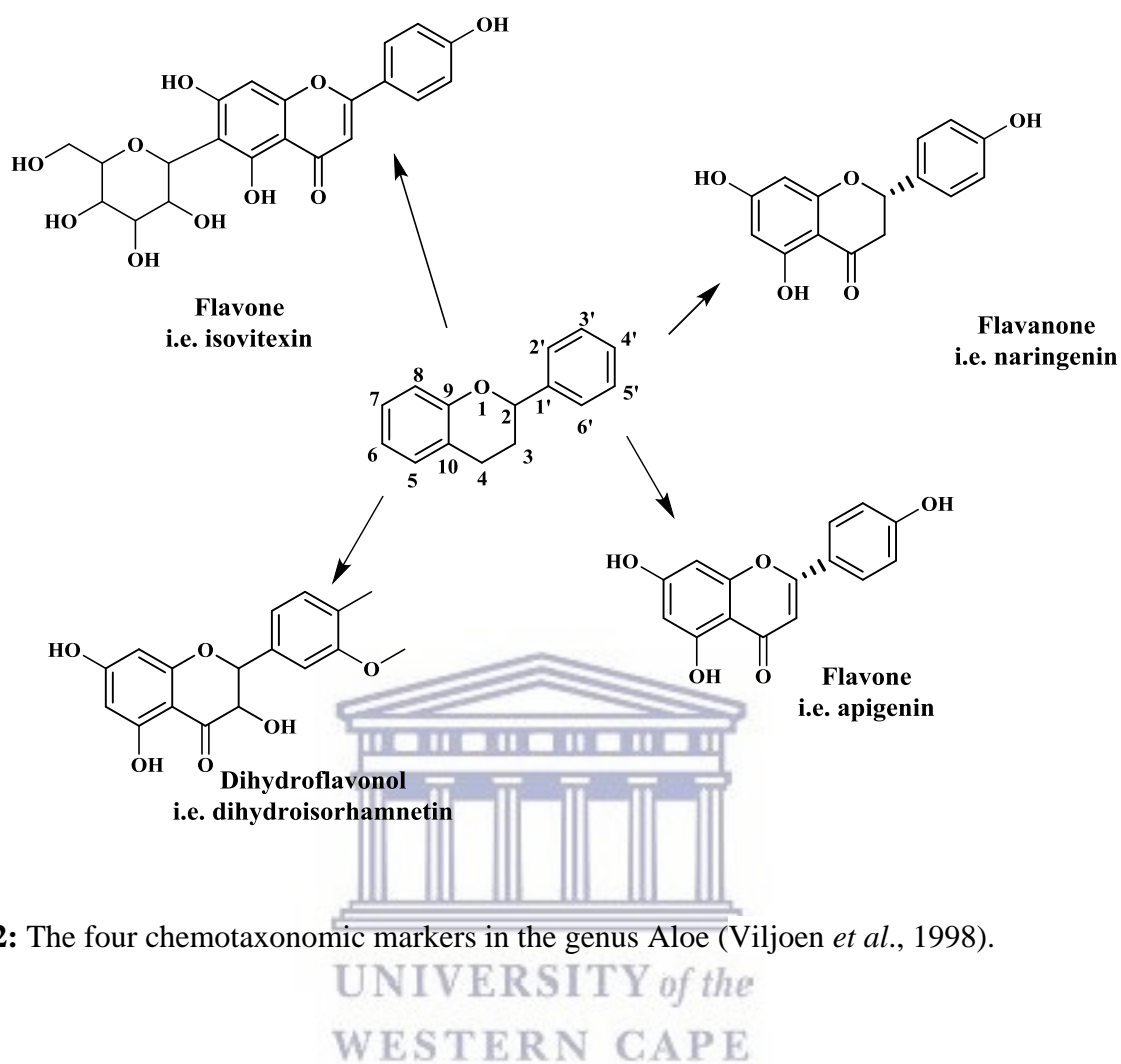


Fig. 2: The four chemotaxonomic markers in the genus *Aloe* (Viljoen *et al.*, 1998).

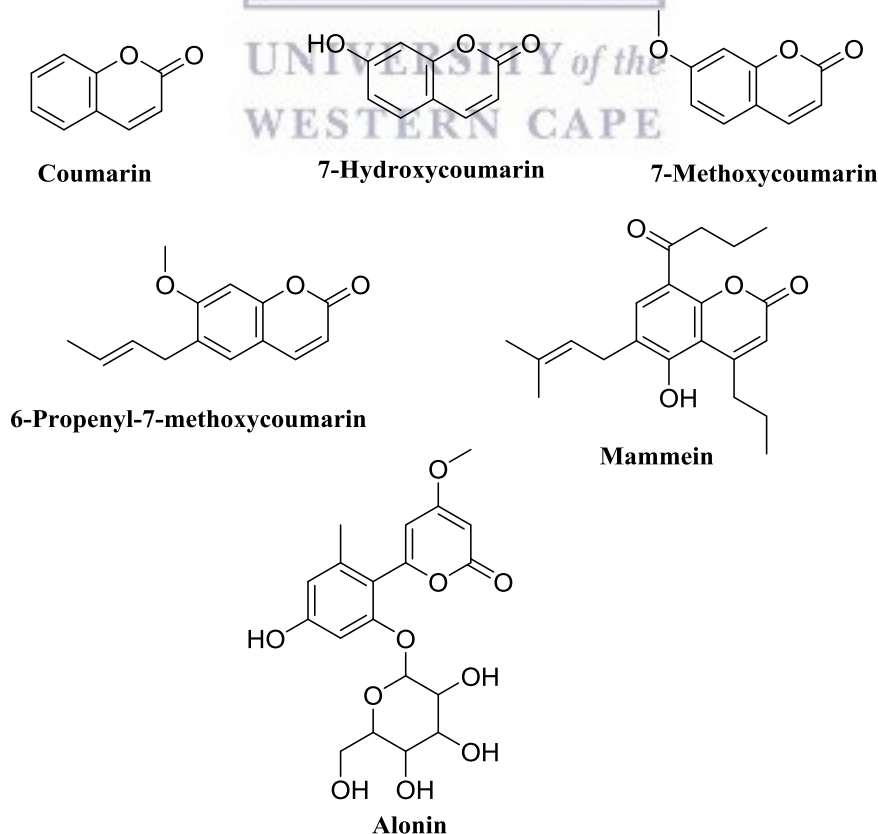
Nevertheless, these heterocyclic metabolites with hydrophilic centres have defined roles in plants. For example, anthocynins are responsible for pigmentation of most fruits as well as protection against excessive radiation from the sun (Koes *et al.*, 1994). In addition, it has been shown that pigmentation (color) also plays a critical role in the plants life cycle, *e.g.* to promote pollination (Treutter, 2006). Some biological roles of flavonoids are seen in the plants' defense mechanism against herbivores and pathogens (Cook & Samman, 1996).

Biological relevance of flavonoids has given them some pharmacological acceptance, especially as dietary supplements present in most foodstuffs due to their regulatory effect in lipid-peroxidation (Gil & Couto, 2013; Zhishen *et al.*, 1999). These phenolic derivatives are also associated with some anti-oxidant activity towards a variety of readily-oxidizable compounds (Cook & Samman, 1996). Free-radicals are major causes of most diseases (or

disorders) among which arthritis and cancer can be mentioned; therefore, flavonoids have been exploited quite extensively as scavenging agents for free-radicals. Other pharmacological roles associated with flavonoids include anti-inflammatory, anti-viral and anti-allergic activities (Cook & Samman, 1996). Tannins, a subgroup of polyphenols, have also been reported to exhibit some chelating effects, which make them potential targets as drug delivery systems to various cells in the body. A particular study reported the relevance of this chelating effect on the basis of total anti-oxidant activity using the ferricthiocyanate method (Lopes *et al.*, 1999).

1.4.4 Coumarins and their derivatives

The nomenclature of coumarins including their derivatives is simple *e.g.* they contain the 5,6-benzo-2-pyrone skeleton and their subsequently classification essentially depends on the substitution of either the benzene and/or pyrone rings (Bhat *et al.*, 2005). These metabolites, in particular coumarin (**Fig. 4**), are widespread throughout higher plants (Kar, 2003). Despite their wide occurrence very few have been identified from aloes *e.g.* dihydroxycoumarin (*Aloe cremnophila*, *Aloe hildebrandtii* and *Aloe jacksonii*) and feralolide (*Aloe ferox*, *Aloe vera* and *Aloe hijazensis*) (Speranza *et al.*, 1993; Vietch *et al.*, 1994; Choi *et al.*, 1996; Abd-Alla *et al.*, 2009).



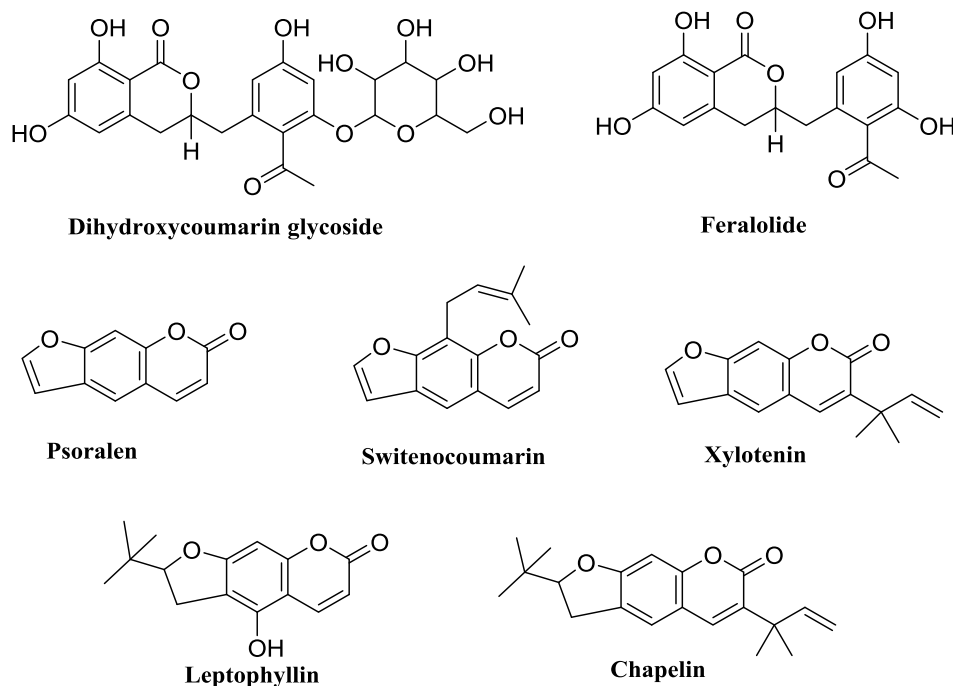


Fig. 3: Some example of naturally occurring coumarins and their derivatives (**Speranza et al., 1993; Vietch et al., 1994; Bhat et al., 2005**).

1.4.5 Terpenoids

Terpenoids are secondary metabolites that are widespread across all higher plants (Wang *et al.*, 2005). These metabolites which are derived from a basic five carbon skeleton (Dubey *et al.*, 2003), are quite potent; in plants they formulate a defence mechanism against viral infection by activating oxidative polymerization and crystallization of resin acids in the infected area (Grassman *et al.*, 2002). Taxol (diterpenoid) and artemisinin (sesquiterpene lactone), are used therapeutically as anti-cancer agent and antimalarial drugs, respectively (Wang *et al.*, 2005). Nevertheless, despite their well-documented chemistry across *all* higher plants (Langenheim, 1994; Umamo *et al.*, 1999; Joseph and Raj, 2010; Mariita *et al.*, 2011; Arowosegbe *et al.*, 2012), however, there is no record documenting their isolation from *Aloe succotrina* Lam. spp.

1.5 Medicinal Plants and Their Role in Modern Medicine

In spite of modern human civilization and recent advances in technology, men still seek inspiration from nature. As stated earlier, the role of medicinal plants as traditional remedies has been known to men for thousands of years (Street & Prinsloo, 2012). Their enhanced use in countries like China, India, including African countries such as South Africa, is well recognized (Newman *et al.*, 2000; Street & Prinsloo, 2012). Medicinal plants are characterized as plants species that have some form of biologically active materials which have the potential/potency to treat illnesses (Rates, 2001). These biologically active materials are referred to as secondary metabolites, and are responsible for a particular function in plants.

Since the discovery of morphine in 1806 and accelerated work in the field of natural product chemistry, natural products, whether as biosynthetic or as direct therapeutic agents of natural origin still continue to inspire new ideas in drug discovery and development. According to Harvey (2008) an estimated amount of over 100 natural-product based active compounds are currently in clinical developments as anti-cancer and anti-infectives. Hence, Newman and co-worker (2016) recently reported that about 25% of newly approved chemical entities in therapeutic use are natural product based; this is excluding formulation of (other) natural product inspired drugs. The relevance of these metabolites in modern medicine is owed to years and years of trial and error by traditional people in their search for treatment of minor conditions like skin burns to severe conditions such as high fever and dry coughs, constipation and diarrhoea (Mukherjee & Wahile, 2006). It has been suggested that the discovery of new drug leads from natural sources if combined with ethnopharmacology and traditional medicine(s) may offer new strategic options in drug-development (Patwardhan & Mashelkar, 2009), which further emphasizes the importance of natural products in drug discovery. Thus, medicinal plant derivatives or secondary metabolites from higher plants are necessary in modern medicine since they provide basis as precursors for the developments of new drugs leads (Newman *et al.*, 2000). **Fig. 4** shows a detailed overview of all newly approved chemical entities according to Newman *et al.* (2000) from medicinal plants and other sources. Surprisingly, pure natural product based drugs, that is, those which have been isolated from medicinal plants (and/or other natural sources) and are currently used as direct drugs in therapeutic preparations account for only 4% of these new chemical entities. The implication thereof may be due to structural complexities associated with natural products as

well as difficulties in obtaining high quantities to meet the economic demand (Cragg & Newman, 2013).

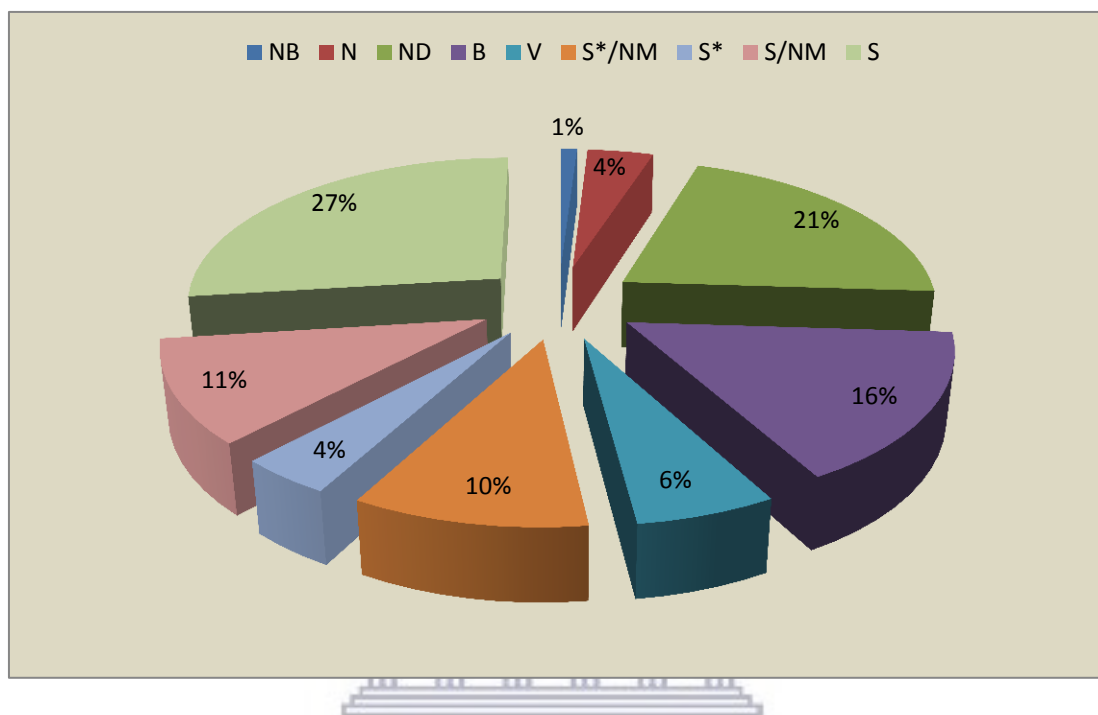


Fig. 4: Showing new approved lead drugs for the period 1981-2016. Where the codes denote: **NB**-natural product botanicals (or mixtures) (1%), **N**-pure natural products (4%), **ND**-natural product derived drugs (21%), **B**-biologics (16%), **V**-vaccine (6%), **S*/NM** – natural product mimic synthetic drugs (10%), **S*** – natural product inspired synthetic drugs (4%), **S/NM** – synthetic natural product mimic drugs (11%), **S**-synthetic drugs (27%).

Furthermore, the commercialization of aloe extracts from two prestige aloe species such as *Aloe vera* and *Aloe ferox*, has also demonstrated a great deal of potential in the development of new lead drugs for medicinal use (discussed later within this chapter). It is worth noting that the role of medicinal plants in modern medicine that is emphasized in this context is on the basis of the development of new lead drugs as premise for treatment of various diseases condition.

1.6 The Terrestrial Biomes of South Africa

Climate change patterns, that is, annual rainfalls and temperatures, are primary factors that predict major changes in biome distribution in South Africa (Low & Rebelo, 1998; Mucina & Rutherford, 2006). According to Rutherford *et al.* (2006), terrestrial biomes are usually classified based on the dominant or similar vegetation exposed to similar climate and geographic locations (see **Fig. 5**). For instance, the Fynbos Biome also called Cape Fynbos is home to approximately 75% of South Africa's rare and threatened plants (SANBI, 2017). These natural shrubs which are found growing in Mediterranean climate conditions in the Western Cape of South Africa represents a unique biodiversity. **Fig. 5** shows the distribution of South Africa's nine biomes.

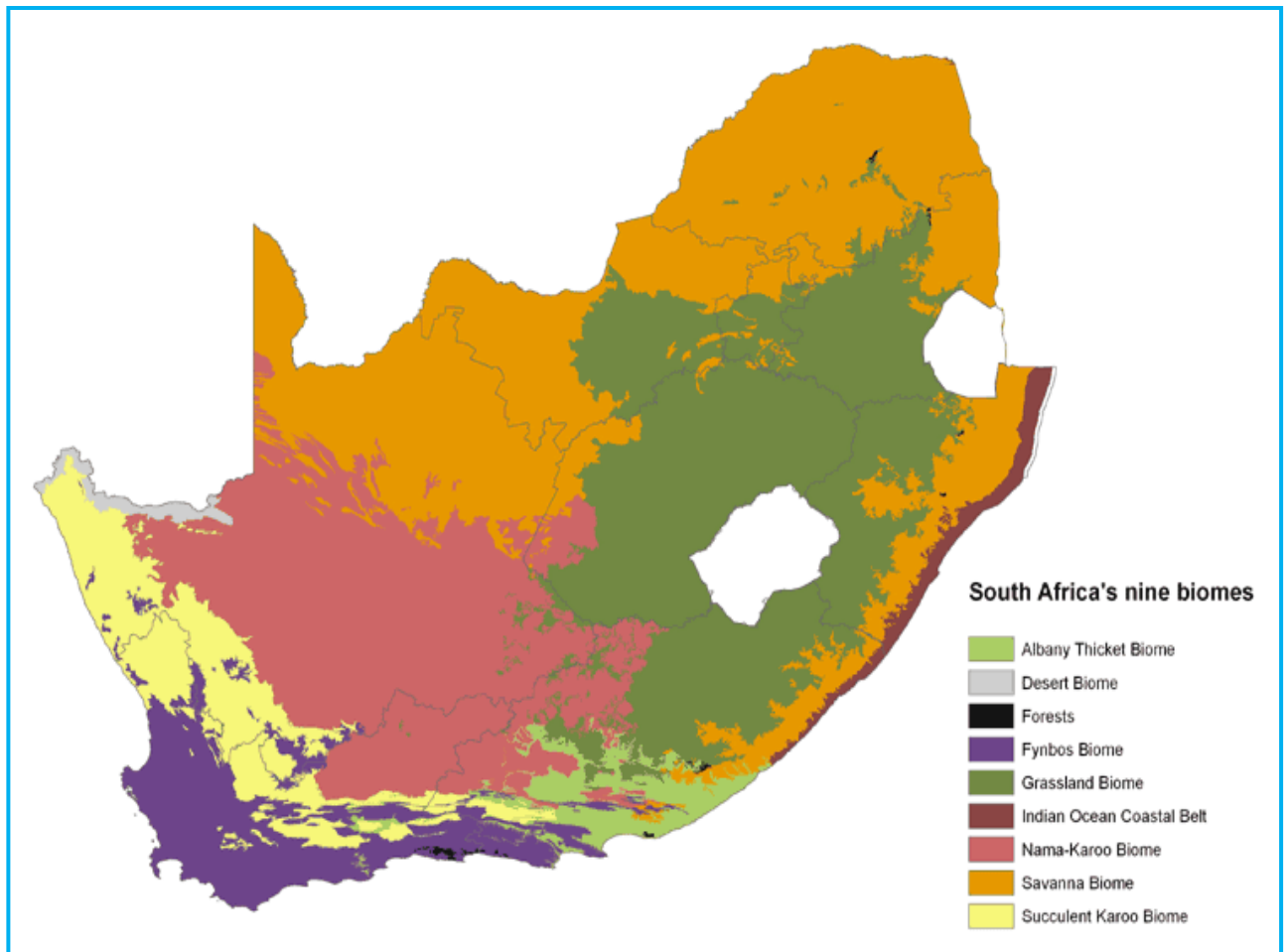


Fig. 5: Terrestrial Biomes of South Africa (SANBI, 2017).

1.7 The genus *Aloe*

Aloes provide an enchanting topic for research from a taxonomic, chemical and biochemical, pharmaceutical as well as economic point of view (Reynolds, 2004). The subsequent use thereof as medicinal plants in traditional medicine is quite elaborated.

The genus *Aloe* is a group of plants containing over 500 species (on the last count) of flowering succulent plants, which belong to the family Asphodelaceae (Mpala *et al.*, 2010). These highly succulent plants are native to Africa; particularly South Africa's Cape Province and extends all the way up to the mountains of tropical Africa including Madagascar (Reynolds, 2004; **Fig. 6**). Aloes are represented quite comprehensively across all South Africa's nine biomes (see **Fig. 5**).

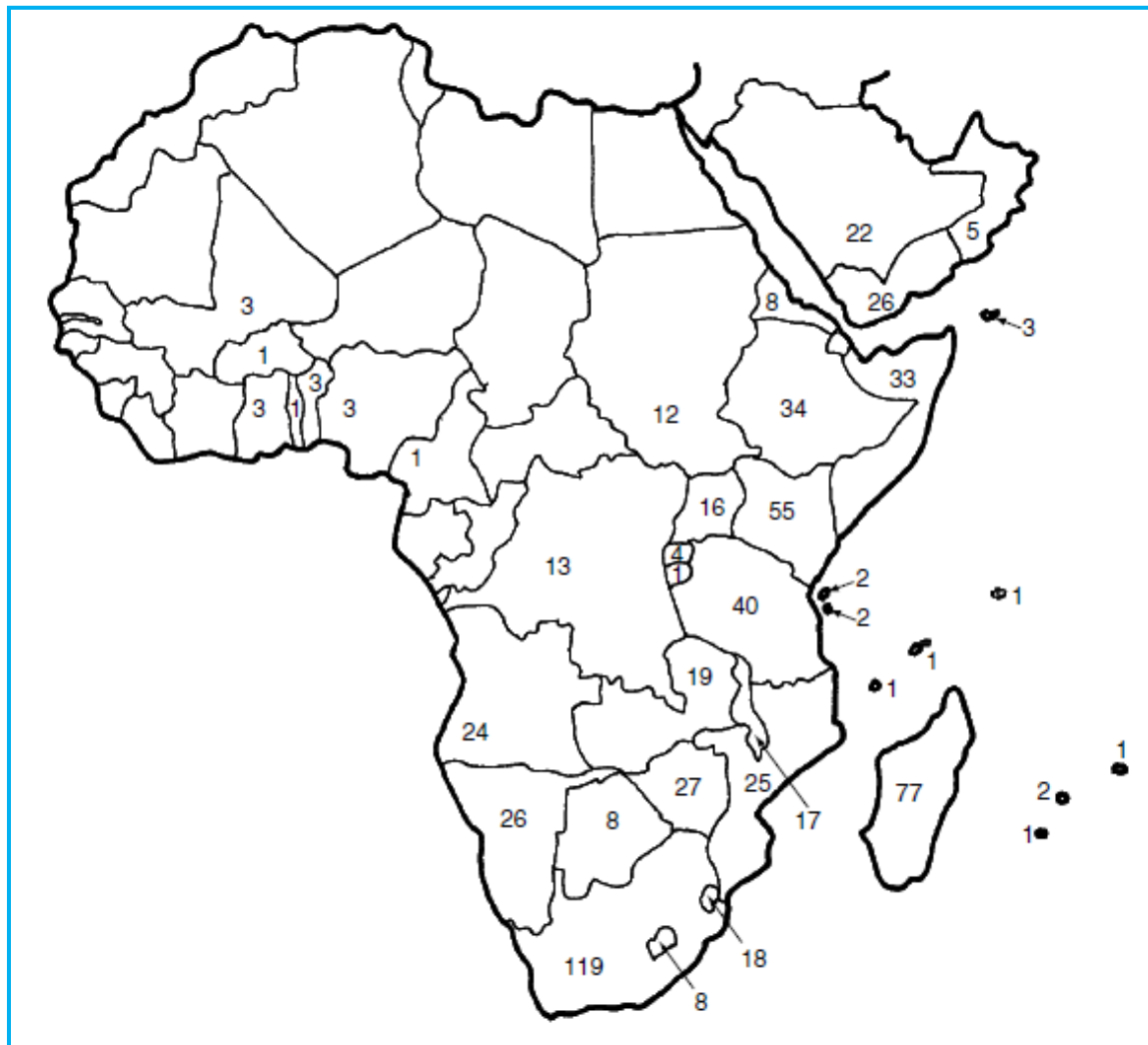


Fig. 6: Geographical distribution of Aloes by country (Reynolds, 2004).

Species of this genus are widely varied in terms of their physiology and perhaps biology, which includes evolution, distribution and growth; some have the potential to grow as tall as a tree *i.e. Aloe ferox*, whereas others are shrubs and stem-less *i.e. Aloe succotrina*. This morphological variation and geographical distribution is demonstrated by their thick-somewhat fleshy-succulent leaves to accommodate high water contents (Holland, 1978). Thus, their habitat inhibition is often areas of poor rainfall – ranging from forests to exposed rock surfaces and cliff faces (Reynolds, 2004). Today, however, some species from this genus are “widely grown in warmer climates globally and indoors” (Bayne, 2002). In view of the fact that aloes grow in varied geographic regions (Reynolds, 2004), their success in cultivation, however, suggests their adaptive capabilities. The wildy cultivated species include *Aloe vera*, formerly known as *Aloe vera* (L.) Brum. f. This species, which is distributed almost everywhere in the world, its true origins are said to be uncertain but likely to be the Arabian Peninsula (Reynolds, 2004). Nevertheless, from a medicinal point of view *Aloe vera* is arguably the *Aloe* spp. that has been thoroughly studied. Phytochemical studies of this species reveal two useable parts, aloe-gel and the bitter exudate. The aloe-gel is used as active ingredient in a range of healthcare and cosmetic products (Reynolds & Dweck, 1999). On the other hand, the bitter exudate is elaborated in *Aloe ferox* due to its purgative properties (Reynolds, 2004).

As illustrated in **Fig. 6**, aloes occur mostly in the southern African region, accounting for approximately 290 species in total (Cock, 2015); the majority being from South Africa, that is, 119 species all together. According to Reynolds (2004), only about 71 of the total South African population are endemic species (Reynolds, 2004), the rest may have been introduced in some form or the other. *Aloe ferox* (Mill.) is one of the oldest South African aloes. The Cape Aloe (as commonly known) has been used quite a lot in traditional medicine due to its purgative and/or laxative properties. Evidence suggests that the laxatives properties derive from the bitter exudate (Reynolds, 2004). The implication of this is that the bitter exudate is reputed to contain phenolic compounds mainly anthranoids and chromones, which are said to be responsible for these purgative properties in one form or the other. Some claims of the bitter exudate include its use in the treatment of arthritis as well as aczema (Mwale & Masika, 2010).

Furthermore, within the South African context, there is still an enormous interest in the use of aloes as medicinal plants especially in traditional medicine despite the lack of comprehensive documentation. Some of the documented South African aloes are shown in **Table 1.1** below.

Table 1.1: Other documented South African aloes.

Name	Aloe Type	Distribution by province	Uses	References
<i>Aloe arborescens</i> Mill.	Multi-stemmed	Eastern Cape, Western Cape, Limpopo, Mpumalanga and KwaZulu-Natal	Traditional Medicine (<i>i.e.</i> skin remedy and decoctions to treat sick-calves), phytotherapy	(Van Wyk & Smith, 1996)
<i>Aloe marlothii</i> A.Berger	Single-stemmed	Northwest, Gauteng, Limpopo, Mpumalanga, KwaZulu-Natal	Traditional Medicine <i>i.e.</i> stomach problems	(Van Wyk & Smith, 1996)
<i>Aloe broomii</i>	Stem-less	Northern Cape, Eastern Cape and Free state	Traditional Medicine <i>i.e.</i> ear remedy for sheep & cattle's	(Van Wyk & Smith, 1996)
<i>Aloe africana</i> Mill.	Single-stemmed	Eastern Cape and Western Cape	Horticulture	(Van Wyk & Smith, 1996)
<i>Aloe brevifolia</i> Mill. var. <i>brevifolia</i>	Dwarf	Western Cape	Horticulture	(Van Wyk & Smith, 1996)
<i>Aloe succotrina</i> Lam.	Multi-stemmed	Western Cape	Horticulture, cosmetic and dye industry	(Marloth, 1905), (Van Wyk & Smith, 1996)

*NOTE. *Aloe succotrina* Lam. is discussed in greater detail later-on in this chapter.

1.7.1 An over view on the phytochemistry of selected Aloe species:

1.7.1.1 *Aloe vera* (L.) Brum. f.

Aloe vera (L.) Brum. f. or *Aloe vera* (referred to hereon-wards) is without any doubt one of the highly documented medicinal plants within the genus *Aloe*. This species which has been widely cultivated almost everywhere in the world is now considered a valuable commercial plant. Evidence suggests that *A. vera* is the first *Aloe* spp. that went to global commercialization for its potent gel-properties (Cock, 2015). The aloe-gel is said to contain 98% water (Bozzi *et al.*, 2007), and the total solid content accounting for the rest (2%).

On dry basis, the aloe-gel contains high levels of polysaccharides (Luta & McAnalley, 2005), which are considered to be the main sources of therapeutic properties (Cock, 2015). In particular, acemannan, a long chain of acetylated mannose residue, is reputed as the active constituent (Femenia *et al.*, 1999). Other gel components include vitamins *i.e.* B₁, B₂, B₆, beta-carotene etc., which have reputed anti-oxidant activities, as well as proteins *e.g.* catalase and minerals such as Zn (Dagne *et al.*, 2000; Van Wyk, 2008; Cock, 2015). Luta and co-worker (2005) reported trace amounts of the phenolic compounds (1%), which have been used throughout the ages as purgatives. However, it is suggested that the concentration of these compounds in the aloe-gel varies according to geographical origin (Choi *et al.*, 1996).

Fig. 7 shows the chemical compositions (%) of the aloe-gel, on dry weight basis.

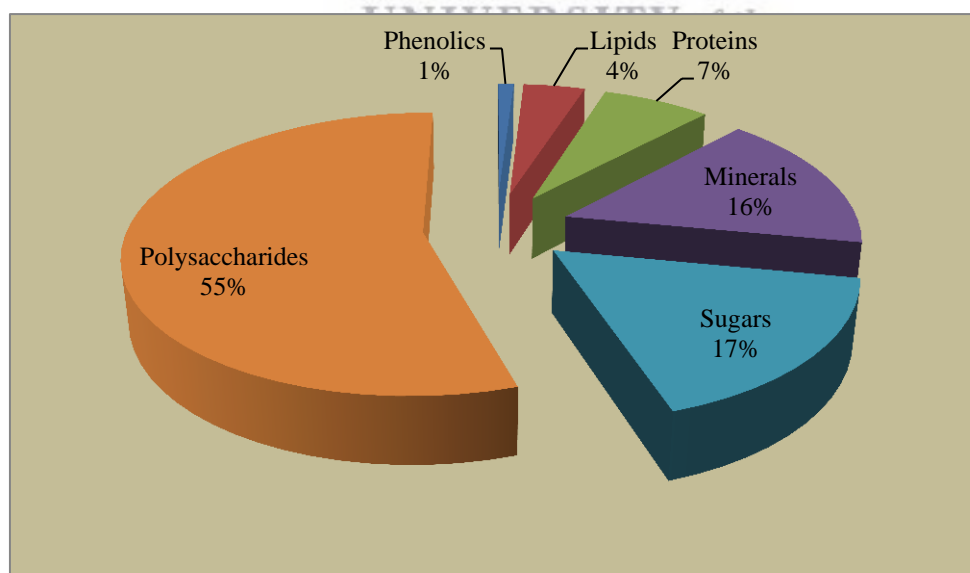


Fig.7: Chemical composition of the *Aloe vera* gel (on dry weight basis) (Luta & McAnalley, 2005).

Interestingly, in spite of modern human civilization, interests in the use of (this) aloe-gel are still eminent. In a report by the International Aloe Science Council in the year 2012, it was reported that of the average 24 000 ha of *Aloe vera* cultivated worldwide, they generate an economic gross of the value US\$300 billion (Zapat *et al.*, 2013). There have been numerous reports suggesting the use of the aloe-gel for wound healing, anti-inflammatory, anti-tumor, antimicrobial, anti-oxidant, antifungal, antiseptic and tyrosinase inhibition activity. The wound healing property of aloe vera gel has been attributed to mannose-6-phosphate (Davis *et al.*, 1994), which may be acts in synergy with acemannan (Femenia *et al.*, 1999). Furthermore, Tanaka *et al.* (2006) evaluated the anti-hyperglycemic effects of *Aloe vera* gel *in vivo* using type 2 diabetic BKS.Cg-m^{+/+}Lepr^{db/J} (*db/db*) mice. In a separate study, the antihyperlipidaemic efficacy of the ethanolic extract from *Aloe vera* leaf gel in streptozotocin (STZ)-induced diabetic rats was evaluated (Rajasekaran *et al.*, 2006). Both studies showed positive results for the use of *Aloe vera* gel as potential antidiabetic agent, owing to the high phytochemical content present in the aloe leaf. A similar phenomenon was reported for *Aloe succotrina*, which is a medicinal plant that is investigated in this thesis, in an *in vivo* study for antihyperlipidemic activity (Dhingra *et al.*, 2014). In addition, the popularity of aloe-gel as active ingredient in a range of healthcare and cosmetic products further emphasize its importance from both the medicinal and economic point of view (Reynolds & Dweck, 1999).

As mentioned earlier, *Aloe vera* is undoubtedly one of the species that has been thoroughly investigated for its phytochemistry as well as therapeutic application(s). Therefore, it warrants no further discussion in this thesis. **Table 1.3** below shows a comprehensive list of the constituents of *A. vera* based on their reputed biological activities.

Table 1.2: Major components isolated from *Aloe vera*, and their associated therapeutic activity.

Structure	Class	Activity	References
Mannose-6-phosphate	Monosaccharides	Wound healing, anti-inflammatory	(Choi & Chung, 2003); (Davis <i>et al.</i> , 1994)
Acemanan	Polysaccharides	Anti-oxidant, wound healing, immunostimulant, antitumor	(Ramamoorthy <i>et al.</i> , 1993); (Choi & Chung, 2003); (Peng <i>et al.</i> , 1991) (Davis <i>et al.</i> , 1994)
Aloin A/B	Anthrone-C-glycoside	Laxative, anti-oxidant, antiprotozoa, antibacterial, cell proliferation, antitumor	(Baruah <i>et al.</i> , (2016); (Choi <i>et al.</i> , 1996); (Choi & Chung, 2003); (El-Shemy <i>et al.</i> , 2010)

(Continued...)

Structure	Class	Activity	References
Emodine	Anthraquinone	Laxative, anti-oxidant, anti-cancer, antimicrobial anti-inflammatory,	(Baruah <i>et al.</i> , 2016); (Choi <i>et al.</i> , 1996); (Das <i>et al.</i> , 2011); (Huang <i>et al.</i> , 2013);
Aloeemodine	Anthraquinone	Laxative, anti-oxidant, anti-cancer, antimicrobial anti-inflammatory,	(Baruah <i>et al.</i> , 2016); (Choi <i>et al.</i> , 1996); (Das <i>et al.</i> , 2011); (Huang <i>et al.</i> , 2013);
Cinnamic acid	Benzyl-esters	Anti-bacterial	(Lawrence <i>et al.</i> , 2009); (Das <i>et al.</i> , 2011)
Elgonica dimer	Anthraquinones	Purgative	(Choi <i>et al.</i> , 1996)

(Continued...)

Structure	Class	Activity	References
Aloesin	Chromone	Anti-hyperpigmentation, cytotoxic, tyrosinase inhibition , anti-inflammatory	(Choi <i>et al.</i> , 2002); (Das <i>et al.</i> , 2011); (Jones <i>et al.</i> , 2002)
10-hydroxyaloin A/B	Anthrone-C-glycosides	Purgative	(Okamura <i>et al.</i> , 1997)
8-O-methylhydroxyaloin A/B	Anthrone-C-glycosides	Purgative	(Okamura <i>et al.</i> , 1997)
<i>p</i> -coumaric acid	Coumarin	Anti-microbial	(Das <i>et al.</i> , 2011)
Aloeresin E	Chromone	Anti-inflammatory, antityrosinase	(Das <i>et al.</i> , 2011); (Baruah <i>et al.</i> , 2016); (Hutter <i>et al.</i> , 1996);

(Continued...)

Structure	Class	Activity	References
Isoaloesin D	Chromones	Anti-oxidant, immunomodulatory	(Zhang <i>et al.</i> , 2006)
Lophenol	Sterols	Anti-diabetic	(Tanaka <i>et al.</i> , 2006)
Lupeol	Sterols	Anti-inflammatory	(Sahu <i>et al.</i> , 2013)
Aloeveroside A	Naphthalene	β -secretase inhibitory	(Yang <i>et al.</i> , 2010)

1.7.1.2 *Aloe ferox* Mill.

Aloe ferox Mill. allude to the accepted taxonomic classification but will from this point be referred as *Aloe ferox* for simplicity. This variable, shallow rooted, succulent plant with persistent dry leaves (about 2 metres long) is native to South Africa (Chen *et al.*, 2012). The *A. ferox* plant is characterized by a single stem which has a potential to grow as tall as a tree. Its flowers are usually varied, ranging from bright red, orange-red to yellow and white forms. Its yellow sap from the leaves dries red (Van Wyk *et al.*, 1997). Furthermore, geographical distribution of this fickle species is limited to the dry areas of Western and Eastern Cape all the way through the southern parts of Kwazulu-Natal province (Van Wyk & Smith, 1996) (see **Fig. 8**). This species is said to flower between May-October (Reynolds, 1982).



Fig. 8: Geographical distribution of *Aloe ferox* in South Africa. *Note. The numbering corresponds to the areas/towns from which the species is found (Kanama *et al.*, 2015).

It would appear that *Aloe ferox* is one of the oldest South African aloes which, according to Reynolds (1982), may have likely been used (then) for “hunting and sticking things on the ground” by early settlers *i.e.* the Bushmans and Khoi-Sans. In fact, Reynolds (1982) also documents records of rock paintings to further support this fact (see **Fig. 9**).

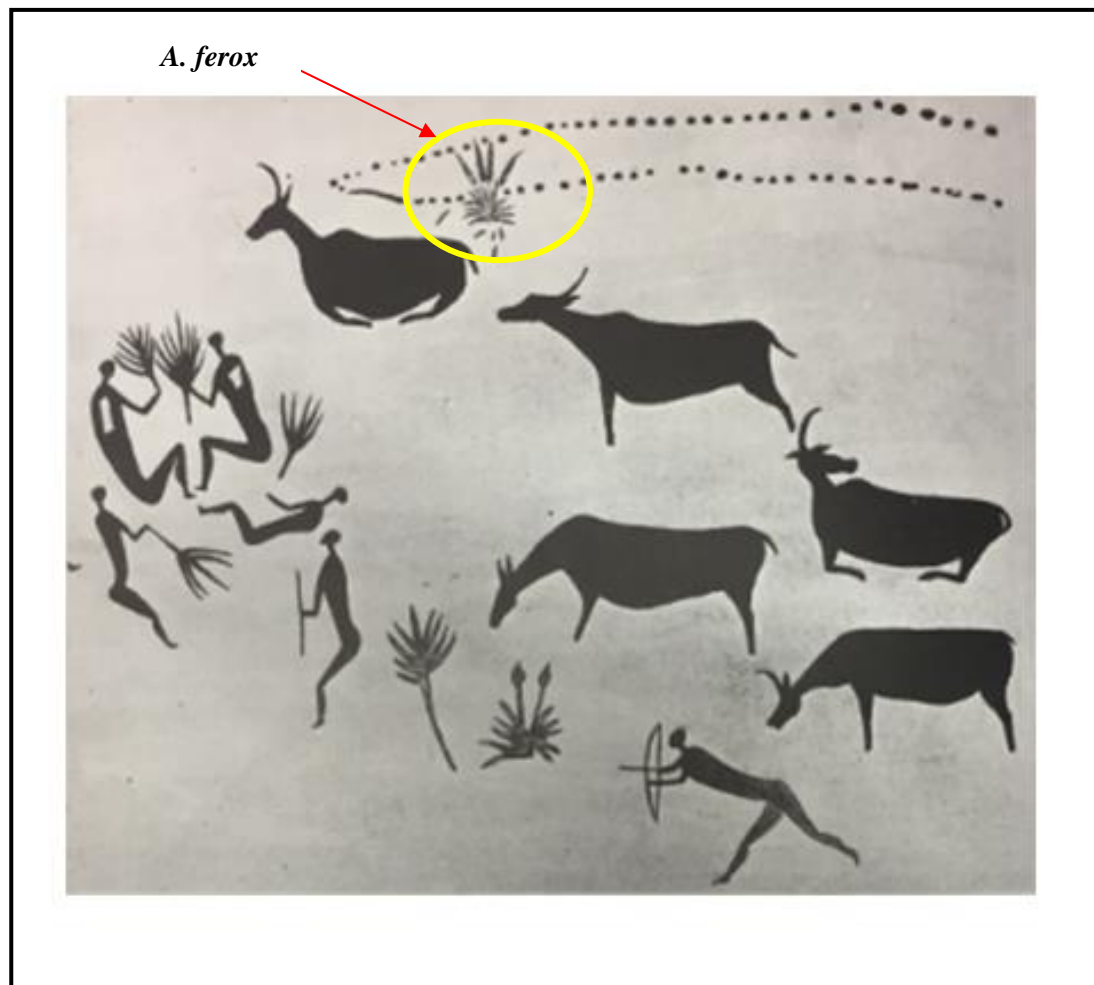


Fig. 9: *Aloe ferox* included in Bushman rock painting (Van Wyk & Smith, 1996).

Today, however, interests in the use of the bitter exudate from this greatly admired species still persist. In terms of the economic scale, the 'Cape aloe' (referred to for this purpose) is reputed to be the most commercially utilized indigenous plants in South Africa (Reynolds, 2004). Grace (2011) states that, in South Africa the tapping industry, which constitutes wild-harvested *Aloe ferox* bitter exudate, was estimated to be worth R 4 million per annum in the late 90s but has seen a significant increase since, from R 12 to R 15 million per annum a decade later. At the same time, Chen *et al.* (2012) reports that in-terms of volumes, South Africa is the largest exporter of the wild harvested bitter exudate. The implication, thereof, is quite significant in terms of socio-economic benefits. Components of this bitter exudate are believed to have been used as laxatives in traditional medicine, and the purgative properties for bowel motions (Reynolds, 2004; Chen *et al.*, 2012). Furthermore, research further suggests that *A. ferox* bitter exudate is common ingredient in a wide variety of healthcare products and cosmetics worldwide (Cock, 2015). Some claims of the bitter exudate include its use in the treatment of arthritis as well as aczema (Mwale & Masika, 2010). Other authors report anti-analgesic, anti-oxidant, anti-microbial and anti-inflammatory activity (Van Wyk *et al.*, 1997; Glen *et al.*, 2000; Kambiz & Afolayan, 2008; Chen *et al.*, 2012). At present, *Aloe ferox* has provoked more research interests due to its ability to supplement the transport of poorly permeable drugs across the intestinal membranes (Chen *et al.*, 2012).

However, adequate progress has been made with regards to the phytochemistry of *Aloe ferox*. The anthraquinone aloe-emodine is suggested as the main component of the leaf exudate (Chen *et al.*, 2012), but chromones and anthrones have also been characterized. Essentially, the distribution of natural products (in aloes) is said to depend on a number of factors, such as concentration ratios between the active (or non-active) constituents as well as their redox states that are in turn affected by seasonal changes (Reynolds, 2004; Cock, 2015). This implies that the accumulation of each constituent is subject to these factors. In a particular study to evaluate the methanol crude extracts of the whole leaf from 183 *Aloe* spp. (Rauwald & Beil, 1993) the presence of 5-hydroxyaloin A, an anthrone-C-glycosyl derivative, was identified in 20 species; yet, 13 of the 20 species showed further variation with regards to the accumulation of this constituent. It was found that the accumulation of this anthrone-C-glycosyl derivative was present at moderate concentrations (0.4%) for *Aloe ferox*. At the same time, aloin A and B were found at higher concentrations than any of the identified constituents, which is not surprising since it (aloin) is considered to be the main component of the bitter exudate (Reynolds, 2004). Hence, the aloin B diastereomer is said to

be predominant. A more recent study by Adhami and co-worker (2015) reported eight biomarkers from the leaf exudate of *Aloe ferox* using high performance counter-current chromatography (HPLC). In another study Speranza *et al.* (1993) reported the accumulation of a rare coumarin derivative *i.e.* feralolide, which has so far been identified in limited species within the genus. This class of compounds is reputed to have a bitter taste (Cock, 2015), which might point towards the laxative properties that is associated with this bitter exudate. On the other hand, although much work on the gel constituents tends to focus on *Aloe vera*, the gel components from *Aloe ferox* have also been considered. Mabusela *et al.* (1990) reported the presence of 14 distinct polysaccharides, which were characterized as arabinogalactans and rhamnogalacturonans. Nonetheless, **Table 1.4** below shows the list of different classes of compounds that have been isolated from *Aloe ferox*, including their associated therapeutic applications.



Table 1.3: Major components isolated from *Aloe ferox*, and their associated therapeutic activity.

Structure	Class	Activity	References
Aloin A/B	Anthrone-C-glycoside	Laxative, anti-microbial, anti-oxidant, antitumor	(Beil & Rauwald, 1993); (Kambiz <i>et al.</i> , 2008); (Adhami & Viljoen, 2015)
Aloinoside A/B	Anthrone-C-glycoside	–	(Rauwald, 1990); (Reynolds, 2004); (Chen <i>et al.</i> , 2012)
Aloesin	Chromone	Anti-inflammatory, anti-oxidant, anti-hyperpigmentation,	(Park <i>et al.</i> , 2011); (Loots <i>et al.</i> , 2011); (Chen <i>et al.</i> , 2012); (Adhami & Viljoen, 2015)
Deoxyreythrolaccin	Anthraquinone	–	(Koyama <i>et al.</i> , 1994)

(Continued...)

Structure	Class	Activity	References
5-hydroxyaloin A	Anthrone-C-glycoside	–	(Beil & Rauwald, 1993); (Chen <i>et al.</i> , 2012); (Adhami & Viljoen, 2015)
Aloeemodine	Anthraquinone	laxative, anti-microbial, anti-cancer	(Koyama <i>et al.</i> , 2001); (Kambiz <i>et al.</i> , 2008); (Van Wyk <i>et al.</i> , 2009)
Aloeresin A	5-methylchromone	Anti-inflammatory, α -glucosidase inhibition	(Lindsey, 2002); (Koyama <i>et al.</i> , 1994); (Speranza <i>et al.</i> 2005); (Adhami & Viljoen, 2015); (Jong-Aurakkun <i>et al.</i> , 2008)

(Continued...)

Structure	Class	Activity	References
Aloeresin C	Chromone	–	(Speranza <i>et al.</i> , 1997); (Adhami & Viljoen, 2015)
Aloeresin H	C,C-glucoside chromone	–	(Manitto <i>et al.</i> , 2003); (Chen <i>et al.</i> , 2012)
Isoaloeresin A	Chromone	–	(Speranza <i>et al.</i> , 1988); (Chen <i>et al.</i> , 2012)
Methyl- <i>p</i> -coumarate	Coumaric acid ester	–	(Reynolds, 2004)
7-hydroxy-2,5-dimethylchromone	Chromone	Anti-cancer	(Kametani <i>et al.</i> , 2007a); (Kametani <i>et al.</i> , 2007b); (Speranza <i>et al.</i> , 1993a)
Furoaloesone	Chromone-furan	–	(Speranza <i>et al.</i> , 1993b)

(Continued...)

Structure	Class	Activity	References
Cape aloes compound 1	Naptha-furans	–	(Koyama <i>et al.</i> , 1994); (Chen <i>et al.</i> , 2012)
Cape aloes compound 2	Naptha-furans	–	(Koyama <i>et al.</i> , 1994); (Chen <i>et al.</i> , 2012)
Cape aloes compound 3	Naptha-furans	–	(Koyama <i>et al.</i> , 1994); (Chen <i>et al.</i> , 2012)
Feroxidin	Tetralins	–	(Speranza <i>et al.</i> , 1992)
Feroxin A	O-glucosylated tetralins	–	(Speranza <i>et al.</i> , 1991)
Feroxin B	O-glucosylated tetralins	–	(Speranza <i>et al.</i> , 1991)

1.7.2 Plant Species Studied In This Thesis: *Aloe succotrina* Lam. spp.



Plate A: *A. succotrina* Lam. growing in the Kirstenbosch National Botanical Gardens. (Notice the purple leaves). Photograph by M. Makhaba.



Plate B: *A. succotrina* Lam. growing in the wild just below Table Mountain (north Kirstenbosch National Botanical Gardens). Photograph by M. Makhaba.

1.7.3 Taxonomy

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Liliopsida
Subclass	: Liliidae
Order	: Liliales
Family	: Asphodelaceae
Genus	: <i>Aloe</i> L.
Species	: <i>succotrina</i> (Raimondo <i>et al.</i> , 2009)



1.7.4 Historical aspects – an Overview

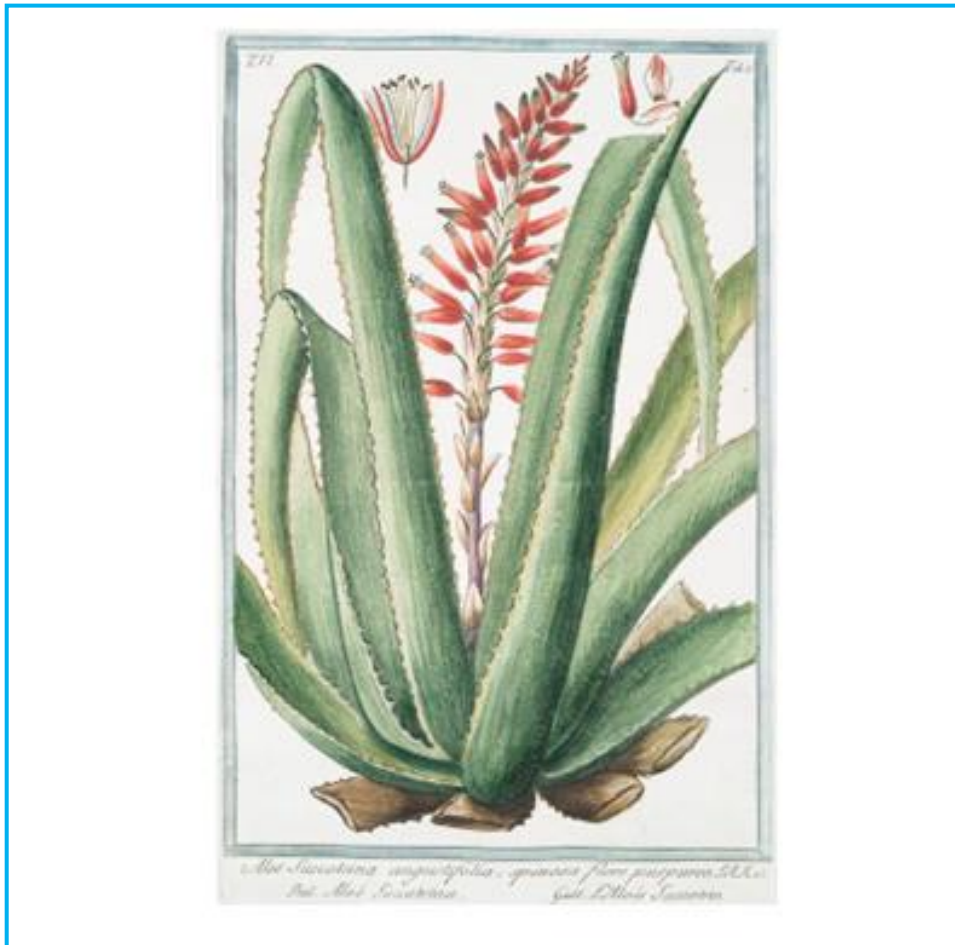


Plate C: First botanic description of *A. succotrina* **Lam.** sourced from (<http://digitalcollections.nypl.org/items/510d47dd-d6d5-a3d9-e040-e00a18064a99>)

The taxonomic history of *Aloe succotrina* **Lam.** followed a long and complicated path. Although this spp. is listed as “Least Concern” in the Red List of South African plants (Raimondo *et al.*, 2009), it is just as much important as any other aloe plant. *A. succotrina* **Lam.** has often been mistaken with *Aloe socotra*, which is an aloe species that grows along the Indian Ocean in the Island Socotra (Gugliemone, *et al.*, 2009; Walker *et al.*, 2015). Thus, naming of this plant has been quite challenging; at first it was thought that the name was derived from the Island Socotra which was highly popular then in the trade industry for the

“sucotrine drug.” It is believed, however, that the name was in fact derived from “succus” which refers to the yellowish sap that is expressed from the plant and “citroen/citron” a mixture of grey/green yellow (Reynolds, 1950; Walker *et al.*, 2015). Other authors though have now placed it under the Section Purpurascens (Raimondo *et al.*, 2009) which accounts for the purple color hence the naming. Reports in the literature further suggest that this prestigious and iconic spp. – naturally occurring in South Africa – was the first South African aloe to be cultivated in Europe for over hundred years, under various names including: *Aloe vera minor*, *Aloe succotrina*, *Aloe americana ananifolia floribus suave-rubentibus*, *Aloe succotrina angustifolia spinosa, flore purpureo* etc., (Gugliemone, *et al.*, 2009; Walker *et al.*, 2015). **Plate C** alongside shows an illustration of the early botanical-description of the species by early authors.

Nevertheless, it was not until early 19th century that the true origins of this phenomenal spp. were rediscovered by Marloth (1905). This distinguished species is found on rocky cliffs of Table Mountain, just above Kisternbosch Botanical Gardens, Western Cape, South Africa. Reynolds (1950), on following work by various scientists gave a conclusive description of the bitter sap in which he said it has a “better and more pleasant smell than that of the usual true aloes.” In addition, Reynolds (1990) in his comparative study of shrubby aloes assigned a TLC zone number P20 for all leaf exudates containing purple staining compounds, which he suggested to be phenolic types. Later, work by Van Wyk and co-worker (1996) described the diagnostic purple features in *Aloe succotrina* **Lam.** Despite this, very little work has since been done to understand the biochemistry that is associated with this purple staining. In fact, only work by Veitch *et al.* (1994) from *Aloe hildebrandtii* suggested dihydroisocoumarin glucoside as one of the compounds responsible for this colour. However, up to now the true chemical nature of ‘those’ compounds responsible for the purple staining remains unknown. At this point it is, perhaps, worth pointing out that there is little published information concerning the phytochemistry of *Aloe succotrina* **Lam.**, and that all the available documentation (reported so far) have been mainly from populations cultivated in National Botanical Gardens and greenhouses elsewhere in the world. It quite is surprising, therefore, that although Africa – South Africa in particular – harbours most of the Aloe species known to men this species has been neglected.

1.7.5 Geographical and Ecological Background

Aloe succotrina Lam. is a South African native medicinal plant. Its geographical distribution appears to be restricted to the better parts of Western Cape and in particular the Cape Fold region (see Fig. 10). This highly succulent shrub that can grow up to 1.5 m tall is classical fynbos vegetation found growing in solitary, but more remotely in dense clumps at elevated rocky cliffs of Table Mountain just above Kirstenbosch National Botanical Gardens (Marloth 1905; Gugliemone, *et al.*, 2009; Walker *et al.*, 2015). Some subpopulation sites have also been described along the coast in rocky cliffs near Hermanus.

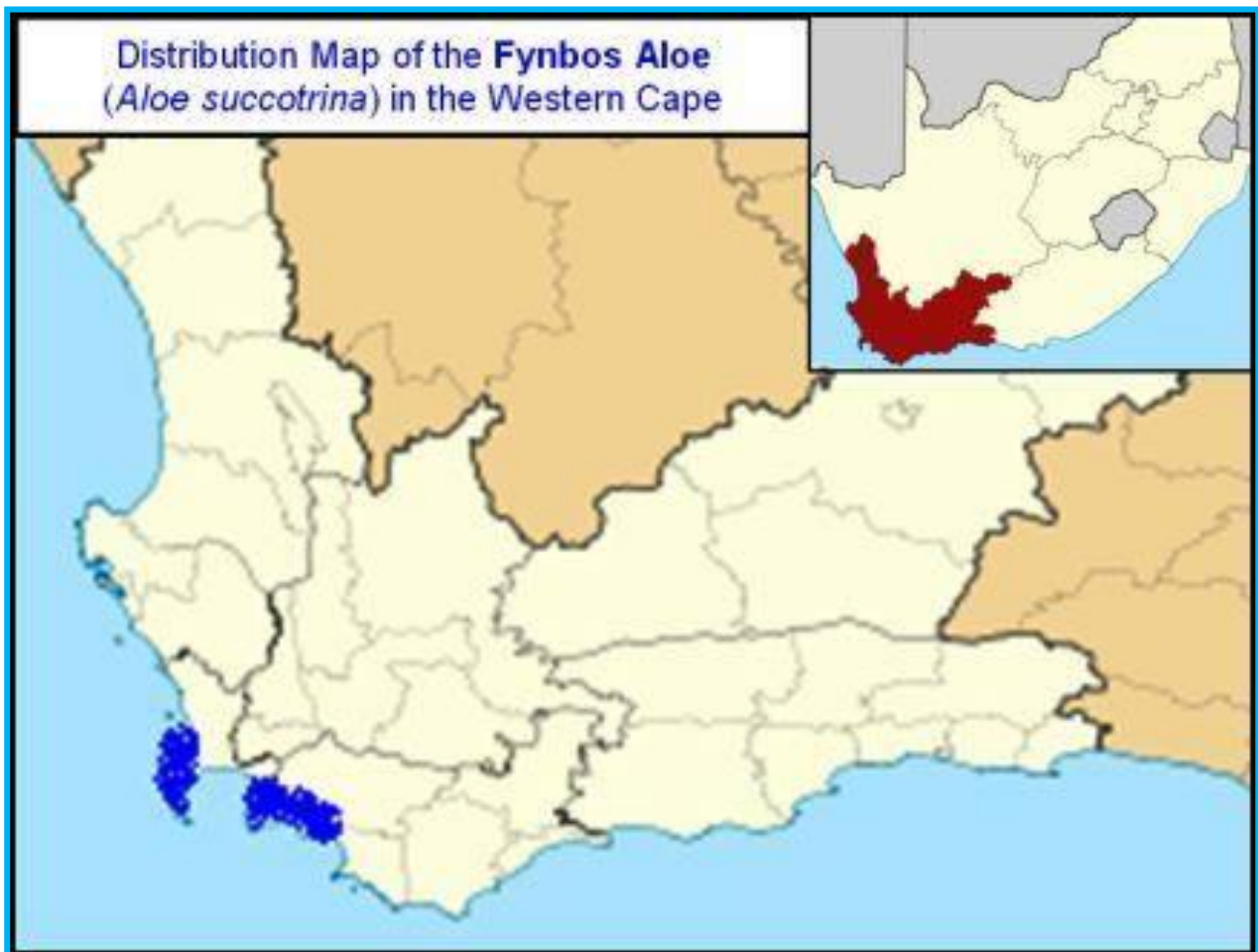


Fig. 10: Geographical distribution of *Aloe succotrina* Lam. **Note:** TM refers to the population that is growing in Table Mountain and HERM. the Hermanus population.

*Data sourced from http://www.eol.org/data_objects/32371460

Their stems are at first simple but die as the plant grows, which seem to allow for further vegetation. Thus they can either be found in these areas protected from direct sunlight by forest trees or on sunny cliff faces where they are highly insulated (by their stalks) (see **Plate B**). The diagnostic patterns of this attractive plant include upright and unbranched inflorescence (about 1 m long) with bright-red flowers (about 25 – 40 mm long); greyish-green leaves (about 70 – 100 mm wide) with scattered white spots and triangular spines on the edges (about 10 mm apart) (Gugliemone, *et al.*, 2009; Walker *et al.*, 2015). Another interesting feature, which perhaps is a necessary chemotaxonomic trait, is the prominent purple color that occurs (Van Wyk & Smith, 1996), especially in dry leaves and when they (leaves) have been exposed to air oxidation (see **Plate A**). It is believed that pollination of this, clearly unique aloe species, occurs during the mid-winter season (July – August) by birds that feed on its nectar (Walker *et al.*, 2015).

Interestingly, it can be noticed that the morphological characters between the two *Aloe succotrina* **Lam.** spp. in **Plate A** (from Kirstenbosch National Botanical Garden) and **Plate B** (from Table Mountain) are quite lucid. The leaves shown in **Plate A** have a very strong purple shade (previously noted especially for the cultivated population), whereas the wild population (**Plate B**) appears to have very little to none. At the same time, it can be observed from **Plate B** that ‘new’ species of the plant appear to be growing from the dichotomous stalks. It is quite welcoming to speculate, therefore, that perhaps climate as well as infection by parasite plays a very big role between these two subpopulation sites.

1.7.6 Phytochemical and biological studies on *Aloe succotrina* Lam. spp.

The phytochemistry of *Aloe succotrina* Lam. is far from being complete, due to various factors. The first account, of course, can be attributed to poor documentation. The other reason may be the long and complex history that the species has suffered (as mentioned earlier). In fact, although this species is known to be native to South Africa, the first analytical study using thin layer chromatography (TLC) and direct column-column chromatography (DCCC) to isolate the secondary metabolites (from the leaves) was reported for the cultivated species (Rauwald & Diemer, 1986). Secondly, re-evaluation – *in this case* – using high performance liquid chromatography (HPLC) for qualitative and quantitative assessment of the whole leaf methanol crude extracts from the cultivated population was also documented (Beil & Rauwald, 1993; Sigler & Rauwald, 1994). In their studies, all workers identified the accumulation of predominantly aloeresin B, and characteristic 7-hydroxyaloin A/B including the acetylated derivatives. It was also reported that the diacetylated form is predominant over the monoacetylated unit (Beil & Rauwald, 1993). While these two analytical studies were mainly focused on the leaf contents, Van Wyk and co-worker (1994) in their chemotaxonomic survey using TLC and HPLC reported the accumulation of anthraquinone and pre-anthraquinones from the roots. To the best of our knowledge, this is the only analytical study that included the native species. Taking this into account, the narrative work that is presented in this thesis (refer to chapter 3) represent the second account of this (native) population. **Table 1.5** shows the different classes of compounds that have so far been identified from *Aloe succotrina* Lam. spp.

On the other hand, biological evaluation of the constituents attainable from this species has also not been examined to any significant extent: anti-inflammatory (Lindsey *et al.*, 2002) and antimalarial activities (Van Zyl, *et al.*, 2002) are the only documented biological studies that have been demonstrated *in vivo*. However, in this thesis it is intended that cytotoxicity and enzyme-inhibition studies (refer chapter 3) be investigated in an attempt to broaden the potential therapeutic applications of *Aloe succotrina* Lam.

Table 1.4: Chemical compounds isolated from *Aloe succotrina* Lam.

Chemical Structure and name of isolated compound	Part of the Plant	References
7-hydroxyaloin A/B	Leaves	(Rauwald & Diemer, 1986);
	Leaves and flowers	(Sigler &Rauwald, 1994)
7-hydroxyaloin-6'-O-monoacetate A/B	Leaves	(Rauwald & Diemer, 1986);
	Leaves and flowers	(Sigler &Rauwald, 1994)
7-hydroxyaloin-3',6'-O-diacetate A/B	Leaves	(Rauwald & Diemer, 1986);
	Leaves and flowers	(Sigler &Rauwald, 1994)
Nataloin A/B	Leaves	(Lindsey <i>et al.</i> , 2002)
Chrysophanol	Roots	(Van Wyk <i>et al.</i> , 1995);
	Leaves	(Sigler &Rauwald, 1994)

(Continued...)

Aloe-emodin	Leaves	(Sigler &Rauwald, 1994)
7-hydroxyaloinaloeemodin	Leaves	(Sigler &Rauwald, 1994)
Chrysophanolanthrone	Leaves	(Sigler &Rauwald, 1994)
Aloeemodinanthrone	Leaves	(Sigler &Rauwald, 1994)
Aloinoside A/B	Leaves	(Van Zyl, <i>et al.</i> , 2002)
Aloesaponol I	Leaves Roots	(Sigler &Rauwald, 1994); (Van Wayk <i>et al.</i> , 1995)
Aloesaponol II	Roots	(Van Wyk <i>et al.</i> , 1995)

(Continued...)

Aloesaponarin I/II	Roots	(Van Wyk <i>et al.</i> , 1995)
Asphodelin	Roots	(Van Wyk <i>et al.</i> , 1995)
Aloesin (or Aloeresin B)	Leaves	(Sigler & Rauwald, 1994); (Beil and Rauwald, 1993); (Lindsey <i>et al.</i> , 2002); (Van Zyl, <i>et al.</i> , 2002)

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1.7.6.1 Anti-inflammatory activity

Inflammation is a normal body-response that is associated with healing. Evidence suggests that unregulated inflammation prompts free-radical formation, which is implicated in cellular and/or tissue damage (Kunchandy & Rao, 1990; Shen *et al.*, 2002). Cyclooxygenase, also called COX-1 or COX-2, are isoenzyme that are responsible for the biosynthesis of prostaglandins (including thromboxane and levuloglandins), which are biological factors that affect almost all known physiological and pathological processes via a reversible interaction with the seven-transmembrane domain receptors (Oshima *et al.*, 1996; Dubois *et al.*, 1998). The mechanism of action is that, COX-1 is continuously stimulated in the body, while COX-2 is induced by inflammatory cytokines (Miyamoto *et al.*, 2000). As a result, these enzymes are highly essential from a medical perspective since they are inhibited by antiinflammatory drugs. Hence, in a separate study, the anti-inflammatory activity of selected African aloes using cyclooxygenase-1 assay was evaluated. Two compounds, aloesin and nataloin A & B (Table 1.5) from the dried leaf MeOH crude extract of *Aloe succotrina*, were found to show some potency against COX-2 inhibition (Lindsey *et al.*, 2002). The content accumulation of each natural product was found to be about 46 percent. It can be concluded, therefore, that a potential molecule that can inhibit COX-2 activity will be of great value pharmacologically. Aloesin is a C-glycosylated chromone (8- β -D-Glucopyranosyl-7-hydroxy-5-methyl-2-(2-oxopropyl)-4H-1-benzopyran-4-one). Chromones are naturally occurring metabolites that are virtually distributed throughout all higher plants, especially in the genus aloe (Reynold, 2004). These secondary metabolites have been associated with holistic biological activities in plants – including antiinflammatory – owing to their inhibitive properties against free-radical formation (Steenkamp & Stewart, 2007; Machado & Marques, 2010). On the other hand, nataloin A & B have so far been defined as chemotaxonomic makers for *Aloe nyrensis* (Dagne *et al.*, 2000), which were previously noted to give a distinct blue TLC staining zone (Conner *et al.*, 1987). However, prior to the above mentioned study their biological importance has not been identified.

1.7.6.2 Antimalarial/antiplasmodial activity

Plasmodium falciparum is a protozoan parasite – a member of the Plasmodium genus, and one of the major causes of malaria (Mariath *et al.*, 2009). According to the world health organization (WHO), malaria is one of the most fatal diseases with the highest rates of complications and mortality. In sub-Saharan Africa, it is believed that malaria accounts for at least 10% of child deaths (Maitland, 2016). Nevertheless, in light of the fact that nature is believed to hold answers to most human challenges, various *in vitro* studies based on natural product-extracts have been adapted as strategic efforts to combat this condition. In a single study to assess the inhibition of chloro-quinine resistant *Plasmodium falciparum* using tritiated hypoxanthine incorporation bioassay, the crude MeOH extract of the leaves from *Aloe succotrina Lam.* tested positive (Van Zyl, *et al.*, 2002). Aloesin and aloinoside A/B (Table 1.5) were subsequently identified as the main active metabolites responsible for the inhibition of 79% of the parasite at 50 µg/mL concentrations (Van Zyl *et al.*, 2002).

1.7.6.3 Anti-hyperlipidemic activity

Hydroxymethylglutarate coenzyme A (HMG-CoA) reductase inhibitors are drug candidates of choice that are currently used for the treatment of hyperlipidemia (Dhingra *et al.*, 2014). According to Kolovou *et al.* (2005) hyperlipidemia is a medical condition that is characterized by high cholesterol and hence is the major cause of cardiac related conditions. *In vivo* study conducted in Wistar albino rats by Dhingra *et al.* (2014) using dried pulp of *Aloe succotrina Lam.* leaves showed positive results in HMG-CoA reductase activity; an enzyme that catalyzes the breakdown of HMG to mevalonate via the cholesterol biosynthesis pathway (Tobert, 1987). This therefore suggests the potential use of *Aloe succotrina Lam.* in the prevention and treatment of hyperlipidemia.

1.8 Problem Statement

As much as the chemical composition of *Aloe succotrina* Lam. spp. is somewhat understood and is, to some extent documented, particularly for species obtained from the National Botanical Gardens and those grown in greenhouses elsewhere in the world – unfortunately, nothing about the endemic population has been reported. It is quite unclear, therefore, just how much variation there is (if any) as far as the chemical composition of this species is concerned, either quantitatively or qualitatively. Furthermore, geographical locations as well as climatic conditions are two parameters known to influence the distribution of the chemical composition in plants. Consequently, these climatic factors can in turn affect the growth patterns of plants that grow naturally in the wild or plants that are cultivated in National Botanical Gardens elsewhere in the world.

1.9 Research Aims and Objectives

The main aim of this current project is to investigate and compare the phytochemicals of *Aloe succotrina* Lam. spp. from the endemic population with those reported in literature, as well as investigate the chemical composition of the characteristic blue dye produced by the leaves.

Thus, specific objectives include:

- a) The extraction of the plant material with methanol (MeOH),
- b) Fractionation of the crude MeOH extract by solvent-partitioning,
- c) Isolation and purification of the phytoconstituents with chromatographic techniques (TLC and column chromatography on silica gel),
- d) Structural studies and characterization of the pure phytoconstituents using NMR, HPLC, FTIR,
- e) To determine the cytotoxicity of the crude extracts and pure compounds where possible,

1.10 The Significance of the Study

Plants that grow in their natural habitat(s) are believed to have the advantage over those grown in botanical gardens and in green houses, which is that they have adapted to their environmental conditions over centuries, through the accumulation of specific metabolites for survival purposes. The significance of this study, therefore, will be to investigate this phenomenon by comparing the results that will be obtained from the endemic population with those reported in literature and show the variations that may exist; either qualitatively or quantitatively. Should the findings of this study show any of these variations (qualitatively or quantitatively); it will therefore serve as an indication about the importance of the natural conditions in the plants' biochemistry. Thus, this study could provide new scientific breakthrough which could change the dynamics of modern medicine entirely. Furthermore, leaves of *A. succotrina* Lam. spp. have been shown to produce a blue dye of as yet unknown chemical structure. Thus, the chemical composition of this dye could well be uncovered.



CHAPTER 2

Materials and Methods

2.1 Reagents and Solvents

Solvents: *n*-hexane, dichloromethane, ethyl acetate, butanol, methanol, ethanol, HPLC grade methanol and acetonitrile, deuterated methanol and deuterated chloroform were all purchased from Sigma-Aldrich, South Africa. Both sulphuric acid (H₂SO₄) and formic acid were purchased from Merck, South Africa. **Reagent:** vanillin was purchased from Merck, South Africa.

2.2. General Experimental Conditions

2.2.1 Solvent Evaporation

Solvent evaporation was performed on a Buchi Rotavapor RE 111, with the temperature of the water bath maintained at 45 °C and 14 mbar.

2.2.2 Spectroscopy

2.2.2.1 FTIR spectroscopy

Attenuated total internal reflectance FTIR measurements were carried out using Spectrum 100 (Perkin Elmer Corp.). Spectra recording were accomplished using the interface “Spectrum”. All analyte-samples were measured against potassium bromide (KBr).

2.2.2.2 NMR spectroscopy

NMR spectroscopic analysis (1D and 2D) were recorded at 25 °C, on a 400MHz Bruker Advanced IIIHD Nanobay spectrometer, using a 5mm BBO probe. The solvents used were deuterated chloroform (CDCl₃) and methanol (CD₃OD). Standard pulse sequences were used to acquire both 1D and 2D NMR spectra, and chemical shifts (δ) were quoted relative to the relevant solvent signal (*e.g.* CD₃OD: ¹H, 3.31-4.72 ppm, ¹³C, 48.1 ppm; CDCl₃: ¹H, 7.24 ppm, ¹³C, 77.0 ppm).

2.2.2.3 Mass spectroscopy (MS)

LC-MS analysis were carried out on Waters Synapt G2 instrument, ESI probe injected into a stream of acetonitrile (ACN), and detection ESI negative mode using TOF-MS detector. The Cone Voltage kept constant at 15 V. The quantity of all identified components was investigated using a per cent relative peak area.

GC-MS analysis was recorded on single-quadrupole GC/MS system (5977B High Efficiency Source (HES) GC/MSD) using ultra-efficient electron ionization (EI).

2.2.3 Chromatography

2.2.3.1 TLC

Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F₂₅₄ plates (Merck) with a 0.2 mm layer thickness. Visualisation of the TLC spots was carried out under UV light at 254nm and/or 366nm, and further detection of compounds was achieved by spraying with vanillin spray reagent (prepared by dissolving 15g of vanillin in 250ml ethanol followed by the addition of 2.5ml concentrated sulphuric acid). After spraying, the TLC plates were heated on a hot plate until spots became visible. On the other hand, reverse – phase TLC aluminium plates coated with modified C₁₈ silica gel with inflorescent indicator F254s, which come with quantity of 20 sizes 20x20 cm (Merck) were used for analysis of polar molecules. TLC plates were developed vertically ascending in a glass-chamber saturated with appropriate solvent mixture(s).

2.2.3.2 Column chromatography (CC)

Silica gel 60 (0.040-0.063mm) with about 230-400 mesh particle size (Merck) – packed in glass columns (20-25mm diameter) as dry or as slurry, was used for column chromatography. Size-exclusion chromatography was performed using Sephadex® LH-20 (Pharmacia).

2.2.3.3 HPLC instrumentation and conditions

Sample purification was carried out using Agilent Technologies 1200 series, equipped with Variable Wavelength Detector (VWD) G1314D /G1314E (SL Plus), manual injector, quaternary pump (G1311A), vacuum degasser (G1322A), column compartment (G1316A) and reversed phase C₁₈ analytical column Zorbax SB (4.6 cm x 150 mm, 3.5 µm). Sample was prepared by dissolving 10 mg of the freeze-dried crude MeOH in 5 mL HPLC grade MeOH (2mg/mL) from which an aliquot of this solution was filtered through ACRODISC PSF (25 mm) GxF/0.45 µm GHP membrane for analysis.

Note: For **gradient elution**, the mobile phase consisted of acetonitrile (A), deionised-water (B) and formic acid (C) (as laid out by Sigler & Rauwald, 1994). The elution system was as follows (A:B:C); 0-40 linear gradient (10:89.5:0.5) to (27.5:72:0.5); 40-55 linear gradient (27.5:72:0.5) to (80:19.5:0.5); 55-70 isocratic elution (80:19.5:0.5). The total run was 70 minutes, flow rate and injection volumes were 1mL/min and 20 μ L, respectively. **Peak identification** was carried out using a VWD to monitor the separation/absorbance of the compounds (290 nm) as well as by comparing the chromatogram with that of Sigler and Rauwald (1994). All experiments were carried out at ambient temperature (~ 25 °C).

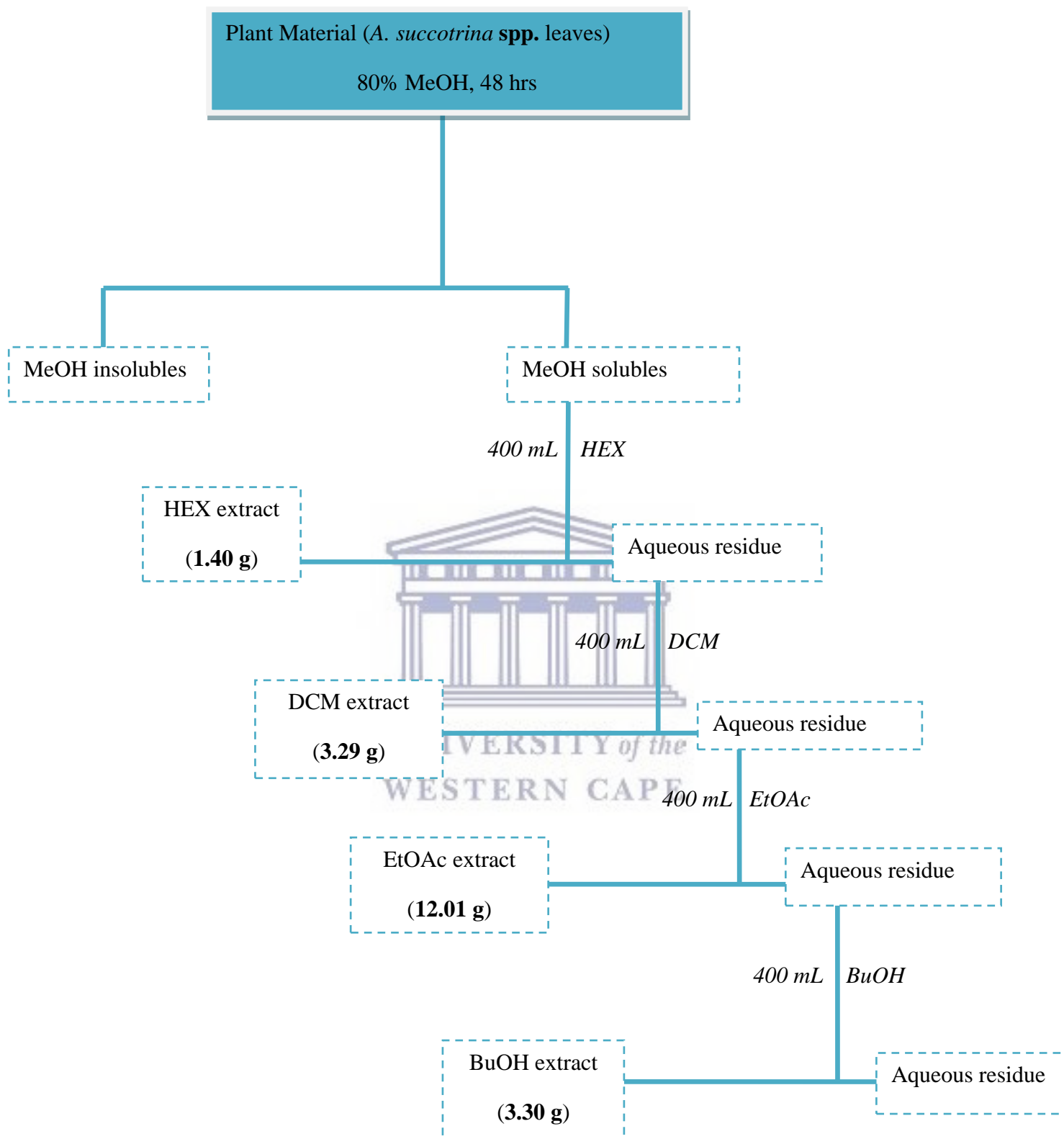
2.3. Plant Material

For phytochemical investigation, fresh leaves of *Aloe succotrina* **Lam.** spp. were obtained from Kirstenbosch National Botanical Gardens, a South African National Botanical Garden that is administered and regulated by the South African National Biodiversity Institute (SANBI). Plant specimen was identified by Mr. E. van Jaarsveld. The material was kept in MeOH at -22 °C until use.

2.3.1. Preparation of Plant Extracts

2.3.1.1 Extraction

The crude MeOH extract of the leaves of *A. succotrina* **Lam.** spp. was evaporated on a rotary evaporator (45 °C), under vacuum and freeze-dried for comparative HPLC analysis as specified by Sigler and Rauwald (1994). Parallel to this, fresh sliced-bodies of the collected species were blended with MeOH and filtered using Whatman N^o1 filter paper. The residue was evaporated in order to remove excess organic solvent (MeOH), which was followed by freeze-drying. This yielded a total extract of 20 g. The freeze-dried sample (20 g) was extracted with 80% MeOH for 48 hours and then subjected to solvent-solvent partitioning (400 mL each) as shown in **scheme 1**. All extracts were evaporated as described above. Purification and isolation of secondary metabolites was achieved through one or a combination of the chromatographic techniques mentioned above.



Scheme 2.1: A schematic diagram representing preparation of extracts.

2.4. Bio-Evaluation of the Crude Extracts

2.4.1 The Brine Shrimp (*Artemia salina*) Lethality Assay: *in vivo*

Cytotoxicity assessment of the crude extracts *i.e.* MeOH, HEX, DCM and EtOAc was carried out as per the design used by Meyer *et al.* (1982) with some modification(s). Artificial seawater was prepared using sea-salt (102 g) dissolved in de-ionized water (3 L). The chamber was made up of a shallow rectangular dish, which was separated with a plastic divider having a number of holes. One side of the chamber was covered with aluminium foil. The Brine shrimp eggs (0.5 g) were obtained and subsequently added to the artificial seawater. Incubation of the eggs was kept for 24 hours at room temperature under continuous illumination (of the uncovered side) and an aquarium aerator pump for agitation and aeration of the eggs for oxygen (both sides).

Following 24 hours incubation, the larvae (nauplii) were observed to be attracted to one side of the vessel that was illuminated with light. Sample preparation was achieved by dissolving 10 mg of each crude extract (MeOH, HEX, DCM, and EtOAc) in 1 mL DMSO to make a stock solution of 10000 µg/mL concentration. From this stock solution, three concentration levels were prepared *i.e.* 10, 100 and 1000 µg/mL, into a series of test tubes and made up to 5 mL by adding sea-water such that the final concentration did not exceed 0.05% of DMSO. Furthermore, DMSO not exceeding 0.05% was used as a negative control for the organic extracts and salt-water for the aqueous extract. Each dosage level was tested in triplicates. The suspensions of the nauplii were incubated for 24 hours at room temperature and the number thereof surviving (nauplii) was determined in order to generate lethality data at each dose (and control) level using Abbotts formula. The mortality values were used to calculate LC₅₀ values *i.e.* the concentration at which 50% of the nauplii died, and data analysed using Microsoft Excel 2010 (see **Table 4.1**).

$$\% \text{ deaths} = \frac{(\text{Control} - \text{Tested})}{(\text{control})} \times 100 \quad \dots(1)$$

2.5. Isolation of Natural Products

2.5.1 Fractionation of Hexane Extract

The crude HEX extract (1.40 g) was chromatographed, isocratically, on a silica-gel column, eluting with HEX/EtOAc (7:3). A total of 20 fractions was obtained and analysed by means of TLC using HEX/EtOAc (7:3) as a mixture. After development, the plate(s) was air dried for complete removal of mobile phase and spots were visualized as described above. Fractions with similar profiles (**F_{V-XX}**) were pooled together, concentrated on a rotary evaporator and purified on Sephadex LH20 column using 100% EtOH as a solvent to yield **compound 1**.

2.5.2 Fractionation of DCM Extract

A total yield of 3.29 g was obtained from the DCM extract. Preliminary screening of the crude extract on TLC for phytochemical constituents was carried out using 10% MeOH/DCM as a mixture. After successful identification of the target compounds, 2 g of the original sample was pre-adsorbed on silica gel and chromatographed on a silica-gel column using gradient elution consisting of the following solvent system; 200 mL DCM/MeOH at ratios (100: 0), (95:5), (90:10), (50:50) and finally (0:100). The resultant fractions were collected in 5 mL portions and a total of 40 fractions were obtained. Profiling of the fractions was achieved by means of TLC, using 10% MeOH/DCM, from which fractions of the target compounds were pooled together (**F_{IV-VII}** and **F_{IX-XVII}**). The sub-fraction **F_{IV-VII}** was purified further on HPLC (C_{18} column) eluting isocratically with 80% MeOH/dH₂O (see **Fig. 2.1**). The peak at 4.449 minutes was collected and later identified as **compound 2**.

On the other hand, sub-fraction **F_{IX-XVII}** crystallized out forming dark-brown crystals. Consequently, an attempt to purify **F_{IX-XVII}** was achieved by means of recrystallization using DCM/MeOH (1:1, v/v), and the resultant crystals were washed successively with DCM and MeOH. TLC profiling, eluting with 10% MeOH/EtOAc, revealed a single spot which was later identified as **compound 3**. (**Note: compound 3 was also identified from the EtOAc extract*).

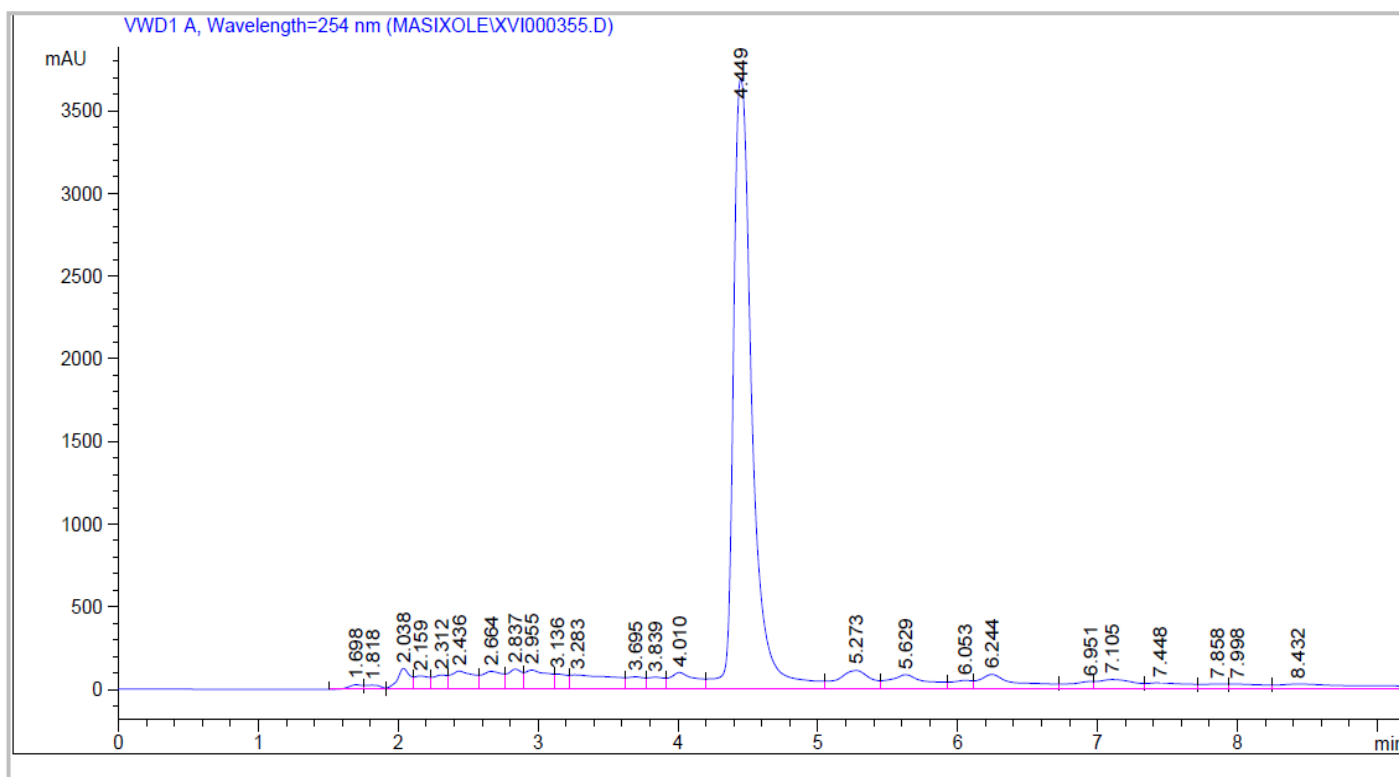


Fig. 2.1: HPLC chromatography (at 254 nm) of **compound 2**. Mobile phase: 80% MeOH/dH₂O, isocratic elution. Flow rate 0.8 mL/min. Peak identification: the peak at 4.449 minutes (retention time) was the compound of interest. *Note.* For column specification see *HPLC conditions (above)*.

2.5.3 Fractionation of EtOAc Extract

Preliminary screening of the crude EtOAc extract (12.01 g) for phytochemical constituents was carried out by TLC using a DCM/MeOH (8.5:1.5) mixture as eluent. After successful identification of the target compounds, 1 g of the crude extract was dissolved and chromatographed isocratically on a silica-gel column using the same solvent system as above. A final volume of 200 mL (DCM/MeOH) was used. Total of 30 fractions were collected in 5 mL portions and were analysed by TLC using DCM/MeOH (9:1). After development, the plates were air dried for complete removal of mobile phase and fractions of the target compounds were pooled together (**F_{XV-XXIV}** and **F_{XXV-XXVIII}**) for further separation. Purification of **F_{XV-XXIV}** was achieved by means of reverse phase silica-gel column eluting isocratically with 90% MeOH/dH₂O (50 mL, v/v), to obtain **compound 4**. On the other hand, **compound 5** was obtained by subjecting **F_{XXV-XXVIII}** on prep-TLC using 15% MeOH/DCM (100 mL, v/v) as a mixture.



2.7 Spectroscopic Data of the Isolated Compounds

HEX, DCM and EtOAc crude extracts were subject to repeated chromatographic separation to achieve pure compounds, the structures of which were unambiguously elucidated by means of spectrometry as well as by comparison with the literature data. All spectroscopy experiments (IR, NMR and MS) were recorded at the department of Chemistry, University of the Western Cape (see method section). MS values are reported in m/z , IR in cm^{-1} and NMR all in ppm:

Compound 1: Isolated from the crude HEX extract as white needle-shaped-crystals (**8 mg**); see **Table 3.1** for ^1H -NMR (CDCl_3 , 400MHz) and ^{13}C -NMR (CDCl_3 , 100MHz) spectra. GC-MSD m/z 414.0 $[\text{M}+\text{H}]^+$ (calculated for $\text{C}_{29}\text{H}_{50}\text{O}$, 414.6). IR_{vamx} (KBr); 3464.6(br.), 2935.7, 2867.9, 1641.6, 1457.3, 1382.5 and 1063.6 cm^{-1} . Compound was identified as **β -sitosterol**. R_f 0.4 (20% EtOAc/HEX).

Compound 2: Isolated from the DCM extract as a white-pink amorphous powder (**8mg**); see **Table 3.2** for ^1H -NMR (CDCl_3 , 400MHz) and ^{13}C -NMR (CDCl_3 , 100MHz) spectra. DI-ESI-MS m/z 343.3 $[\text{M}-\text{H}]^-$ calculated for $\text{C}_{18}\text{H}_{16}\text{O}_7$ (exact calculated mass 343.3154, $[\text{M}-\text{H}]^-$). Compound was identified as **feralolide**. R_f 0.3 (10% MeOH/DCM).

Compound 3: Isolated from the crude DCM extract as pale yellow crystals (**37 mg**); see **Table 3.3** for ^1H -NMR (CDCl_3 , 400MHz) and ^{13}C -NMR (CDCl_3 , 100MHz) spectra. DI-ESI-MS m/z 517.1348 $[\text{M}-\text{H}]^-$ calculated for $\text{C}_{25}\text{H}_{26}\text{O}_{12}$ (exact calculated mass 517.4667, $[\text{M}-\text{H}]^-$). Compound was identified as a **7-hydroxyaloin-4',6'-O-diacetate**. R_f 0.5 (10% MeOH/EtOAc).

Compound 4: Isolated from the crude EtOAc extract as a light purple-red-amorphous powder (**4 mg**); see **Table 3.4** for ^1H -NMR (CDCl_3 , 400MHz) and ^{13}C -NMR (CDCl_3 , 100MHz) spectra. DI-ESI-MS m/z 505.1300 $[\text{M}-\text{H}]^-$ calculated for $\text{C}_{24}\text{H}_{26}\text{O}_{12}$ (exact calculated mass 505.4560, $[\text{M}-\text{H}]^-$). Compound was identified as a **feralolide glycoside**. R_f 0.5 (7:3:1, $\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O}$).

Compound 5: Isolated from the crude EtOAc extract as a purple-amorphous powder (19 mg); see **Table 3.5** for $^1\text{H-NMR}$ (CDCl_3 , 400MHz) and $^{13}\text{C-NMR}$ (CDCl_3 , 100MHz) spectra. DI-ESI-MS m/z 476.1238 $[\text{M-H}]^-$ calculated for $\text{C}_{23}\text{H}_{25}\text{O}_{11}$ (exact calculated mass 476.4380, $[\text{M-H}]^-$). Compound was identified as **7-hydroxyaloïn-6'-O-monoacetate**. R_f 0.2 (15% MeOH/DCM).



CHAPTER 3

Results and Discussion

3.1 Structural Elucidation of Compound 1

One-dimensional (**1D**) and two-dimensional (**2D**) nuclear magnetic resonance (NMR) spectra are useful techniques for structural elucidation. These techniques offer remarkable information about the resonance frequencies of each nucleus in either ^1H or ^{13}C atom, which is essential for peak assignment. Hence, characterization and structural elucidation of **compound 1** (Fig. 3.1) was successfully done on the basis of extensive spectroscopic evidence provided by ^1H -NMR (**1D**), ^{13}C -NMR (**1D**), DEPT-135 (**1D**), gCOSY (**2D**), gHMBC (**2D**) and gHSQC (**2D**).

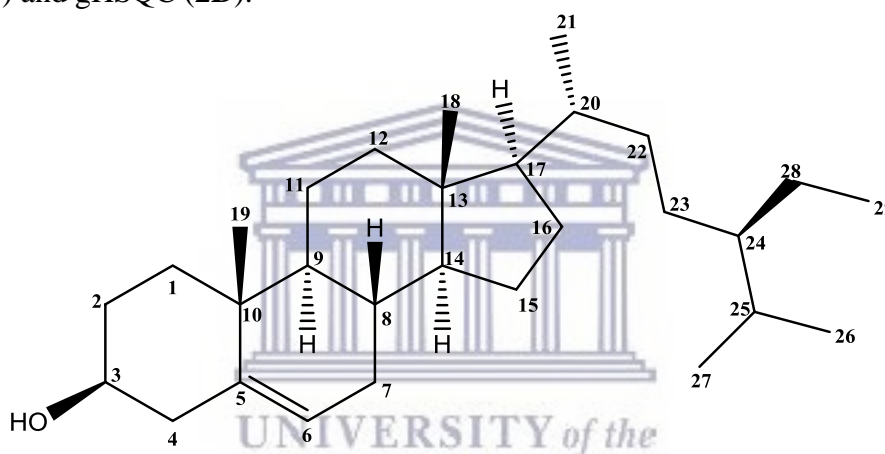


Fig. 3.1: Chemical structure of β -sitosterol.

Compound 1 was isolated as white needle-shaped-crystals (R_f 0.4, 20% EtOAc/HEX). The IR spectrum (Fig. 3.1.4) exhibited characteristic vibration bands at 3464.6 cm^{-1} (ν_{OH} broad stretch), 2935.7 cm^{-1} (ν_{CH_2} cyclic stretch), 2867.9 cm^{-1} (ν_{CH} aliphatic stretch), including a weak vibration for C=C stretch at 1641.6 cm^{-1} . Other absorption bands included the bending frequencies at 1457.3 cm^{-1} (ν_{CH_2} stretch) and 1382.5 cm^{-1} (ν_{CH_3} stretch), as well as 1063.6 cm^{-1} ($\nu_{\text{C-O}}$ stretch) (Pretsch *et al.*, 2000). The spectroscopic evidence that was generated for **1** was found to be consistent with reference data (Ahmed *et al.*, 2010; Chaturvedula & Prakash, 2012; Saeidnia *et al.*, 2014). Evidently, the NMR evidence of this compound showed twenty nine carbon signals, assignable to six methyls, nine methine, eleven methylene as well as, three quaternary carbons, which was further confirmed by the DEPT 135 data (Appx. 1B).

The $^1\text{H-NMR}$ (**Fig. 3.1.2, Table 3.1**) revealed the presence of two signals with δ_{H} at 0.74 and 0.61 ppm, respectively, which were due to singlets characteristic of the methyls' attached to the quaternary carbons *i.e.* C-10 (δ_{C} at 36.5 ppm) and C-13 (δ_{C} at 42.3 ppm). The three other shielded doublets were observed at δ_{H} 0.85 [3H, *d*, $J \sim 6.6$ Hz, Me-21], 0.75 [3H, *d*, $J \sim 1.8$ Hz, Me-26] and 0.75 [3H, *d*, $J \sim 1.8$ Hz, Me-27], respectively. The spectrum further revealed a poorly coupled triplet with δ_{H} at 0.79 ppm ($J \sim 2.2$ Hz), assignable to another methyl attached to C-28 (δ_{C} at 23.1 ppm). In addition, $^1\text{H-NMR}$ also showed the presence of an olefin character with a chemical shift (δ_{H}) at 5.28 ppm, which was confirmed by HMBC studies and by comparison with the literature (Ahmed *et al.*, 2010; Chaturvedula & Prakash, 2012; Saeidnia *et al.*, 2014). A broad multiplet due to splitting by protons at H-2 and H-4 was observed for the proton attached to H-3 (δ_{H} at 3.46 ppm). Indeed, $^{13}\text{C-NMR}$ confirmed the presence of a hydroxylated-carbon with δ_{C} at 71.8 ppm, assignable to C-3. These δ_{H} , with the exception of δ_{H} at 0.79 ppm ($J \sim 2.2$ Hz), are characteristic for the steroidal backbone (Saeidnia *et al.*, 2014).

The HMBC spectrum (**Appx. 1E**) was used to confirm the structure of **1** and hence the selected HMBC correlations are shown in **Fig. 3.1.1** and in **Table 3.1**. Therefore, it was concluded that compound **1** was in-fact β -sitosterol with a molecular formula of $\text{C}_{29}\text{H}_{50}\text{O}$ and its molecular mass was confirmed using GC-MS ($m/z = 414.0$). At this point it is worth pointing out that **1** is reported for the first time in *Aloe succotrina* Lam. spp. (**Note:** for COSY and HSQC refer to **Appx. 1C** and **1D**, respectively).

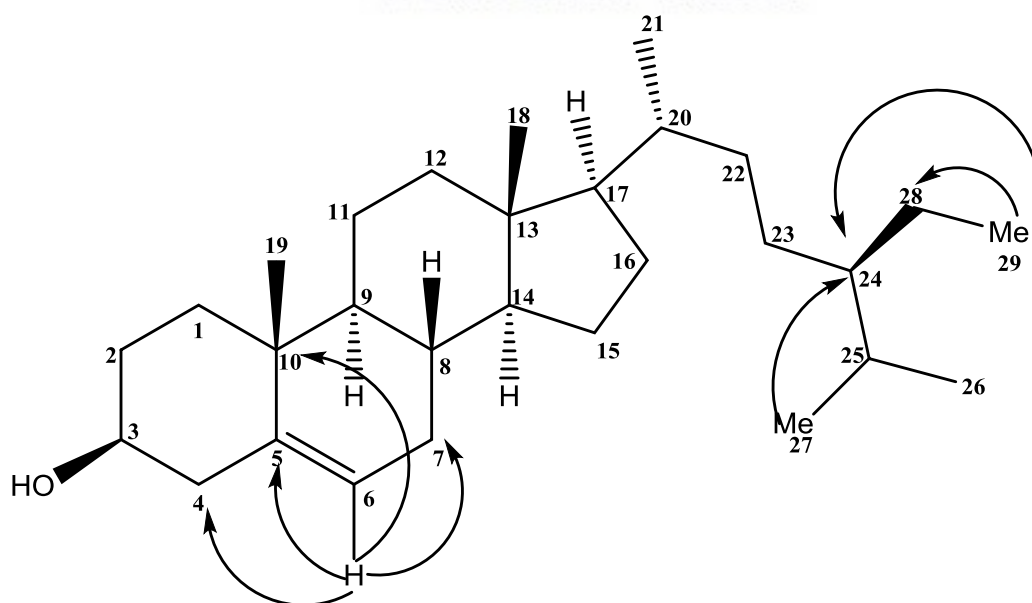


Fig. 3.1.1: Selected HMBC correlations of compound **1** (in CDCl_3 using 400 MHz).

Table 3.1: ^1H NMR and ^{13}C NMR data of *compound 1* in CDCl_3 at 400MHz

Position	^{13}C -NMR δ (m)	^1H -NMR δ (m, integration, J)	HMBC
1	37.3 [CH_2]	1.01, 1.78 (m, 2H)	C-2, C-10
2	29.2 [CH_2]	1.45, 1.78 (m, 2H)	C-1, C-3
3	71.8 [O-CH]	3.46 (m, 1H)	C-2, C-4
4	42.3 [CH_2]	2.17, 2.21 (m, 2H)	C-3, C-5, C-6
5	140.8 [C]	–	–
6	121.7 [=CH]	5.28 (m, 1H)	C-4, C-7, C-10, C-5
7	31.6 [CH_2]	1.88, 1.9 (m, 2H)	C-5(<i>long range</i>), C-6, C-8
8	31.9 [CH]	0.85 (m, 1H)	C-7, C-9, C-14
9	50.1 [CH]	1.59 (m, 1H)	C-8, C-10, C-11
10	36.5 [C]	–	–
11	21.1 [CH_2]	1.37, 1.4 (m, 2H)	C-9, C-12
12	39.8 [CH_2]	1.09, 1.9 (m, 2H)	C-11, C-13
13	42.3 [C]	–	–
14	56.1 [CH] ^g	1.04 (m, 1H)	C-8, C-13, C-15
15	24.3 [CH_2]	1.02, 1.51 (m, 2H)	C-14, C-16
16	28.3 [CH_2]	1.18, 1.78 (m, 2H)	C-15, C-17
17	56.8 [CH] ^g	0.93 (m, 1H)	C-13, C-20, C-16, C-21
18	11.9 [CH_3]	0.61 (s, 3H)	C-14
19	19.0 [CH_3]	0.74 (s, 3H)	C-5
20	36.2 [CH]	1.29 (m, 1H)	C-17, C-21, C-22
21	18.8 [CH_3]	0.85 (d, 3H, J = 6.6 Hz)	C-17, C-20, C-22
22	37.3 [CH_2]	1.01, 1.76 (m, 2H)	C-24, C-20, C-23
23	26.1 [CH_2]	1.10 (m, 2H)	C-22, C-24
24	45.8 [CH]	0.85 (m, 1H)	C-23, C-22, C-29

25	31.9 [CH] ^g	1.37 (<i>m</i> , 1H)	C-24, C-26, C-27
26	19.8 [CH ₃]	0.75 (<i>d</i> , 3H, <i>J</i> = 1.8 Hz)	C-25, C-24
27	19.0 [CH ₃]	0.75 (<i>d</i> , 3H, <i>J</i> = 1.8 Hz)	C-25, C-24
28	23.1 [CH ₂]	1.16, 1.18 (<i>m</i> , 2H)	C-23, C-24, C-29
29	12.0 [CH ₃]	0.79 (<i>t</i> , 3H, <i>J</i> = 2.2 Hz)	C-24, C-28

NB: δ 3.65 (*s*, OH), based on HSQC and HMBC

^g based on HSQC and HMBC.



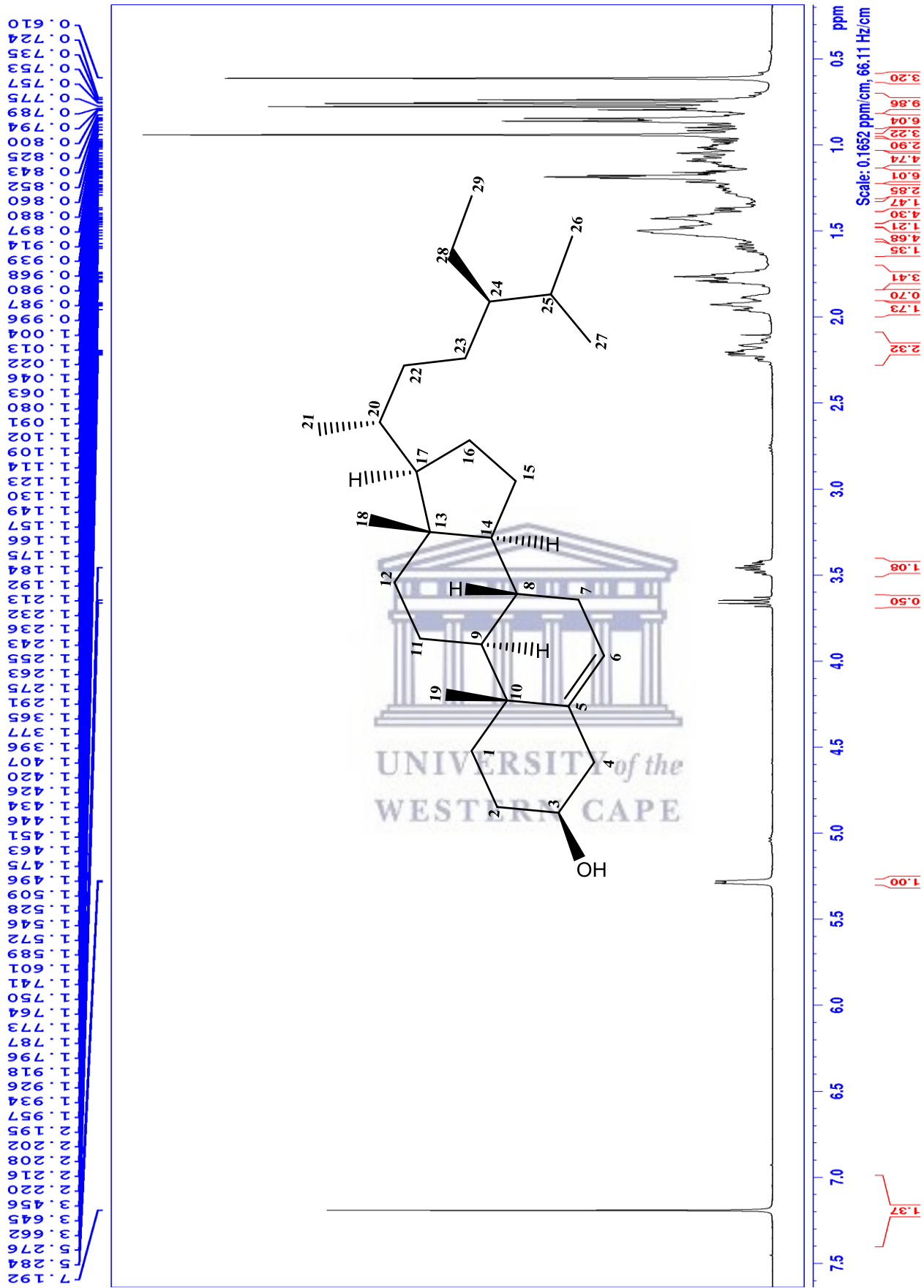


Fig. 3.1.2: ¹H-NMR spectrum of compound 1 (400MHz; CDCl₃; δ_H at 7.19 ppm is due to solvent).

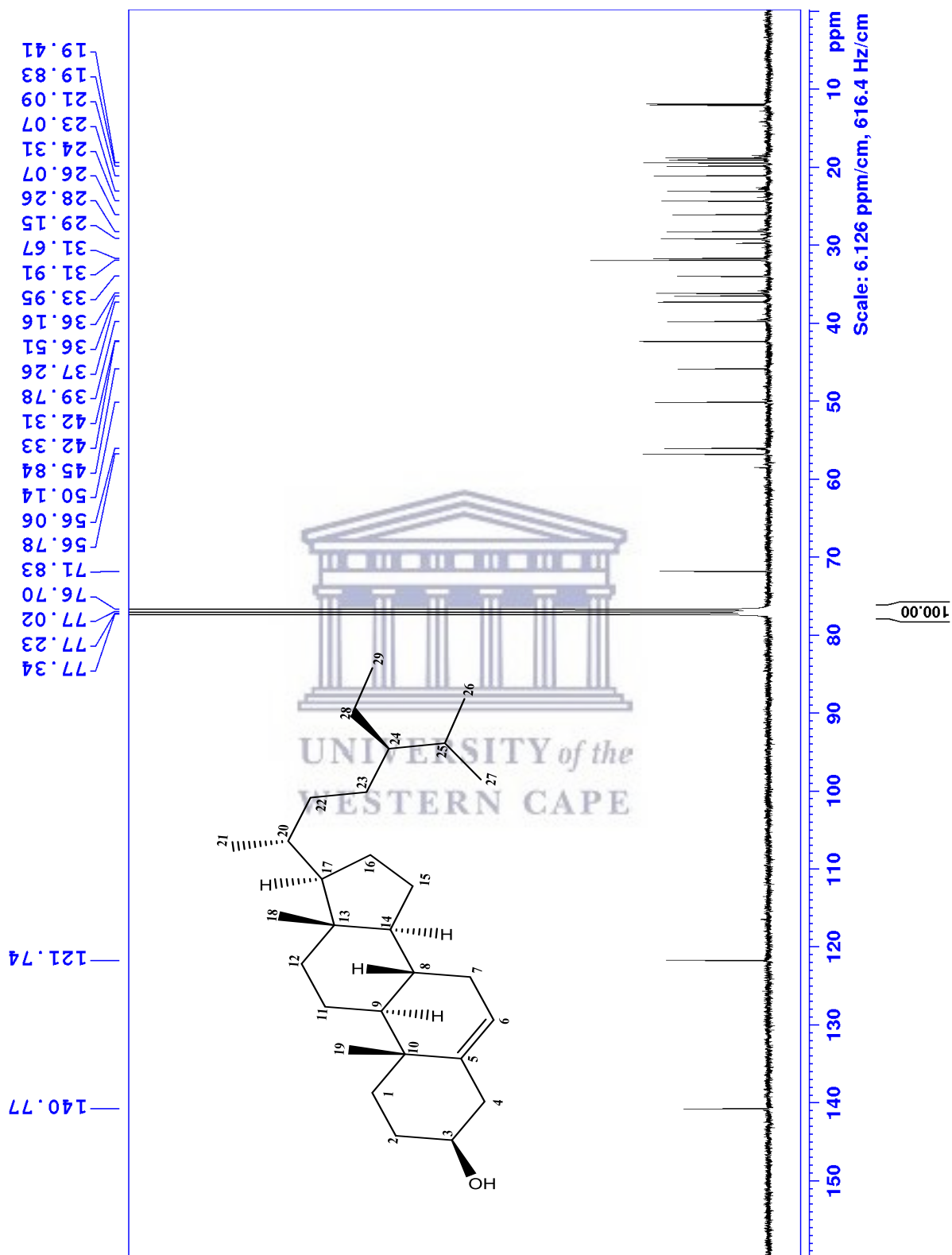


Fig. 3.1.3: ¹³C-NMR spectrum of compound 1 in CDCl₃ (100MHz; CDCl₃; δ_C at 76.7-77.3 ppm are due to solvent).

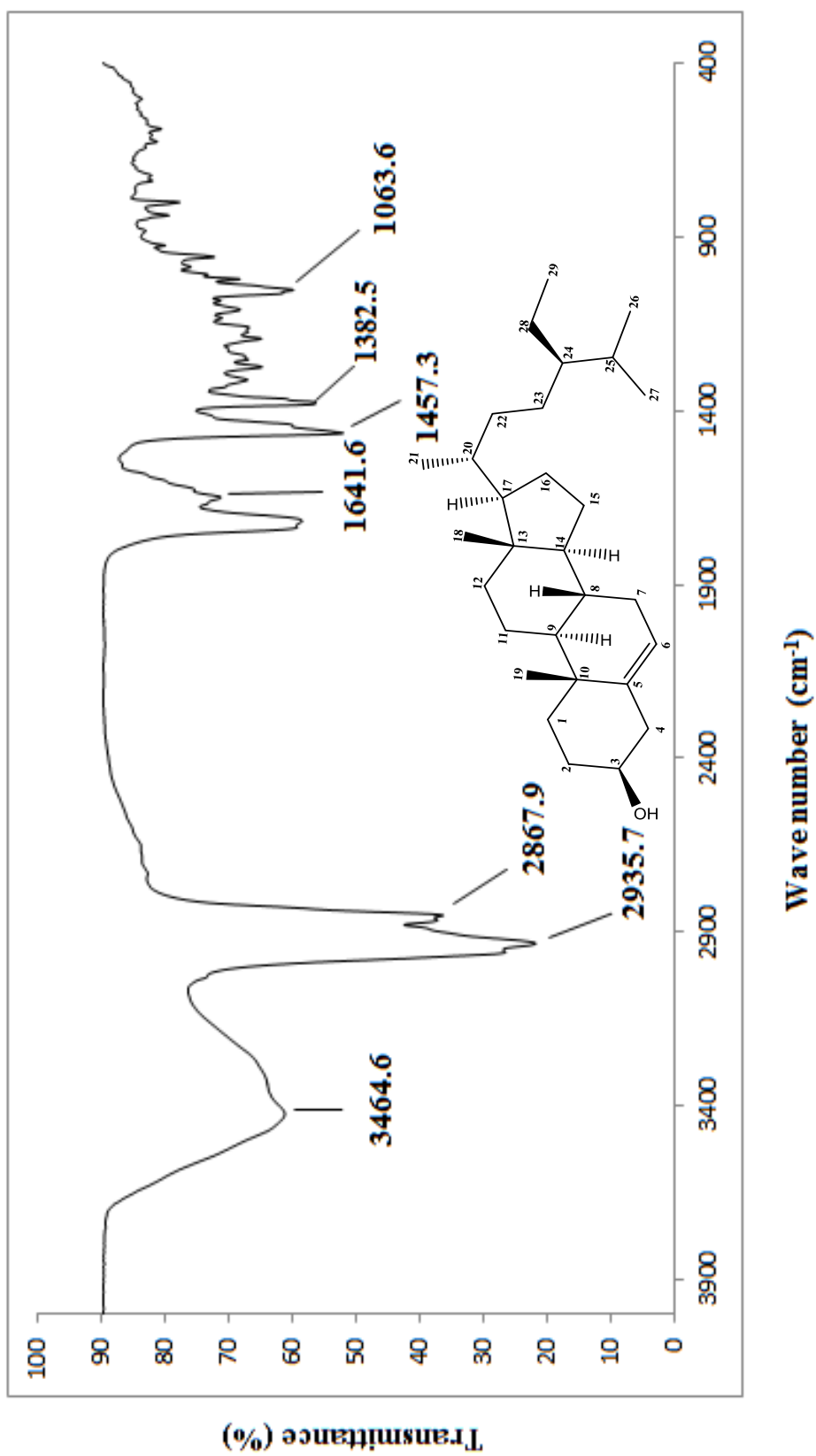


Fig. 3.1.4: IR spectrum for β -sitosterol

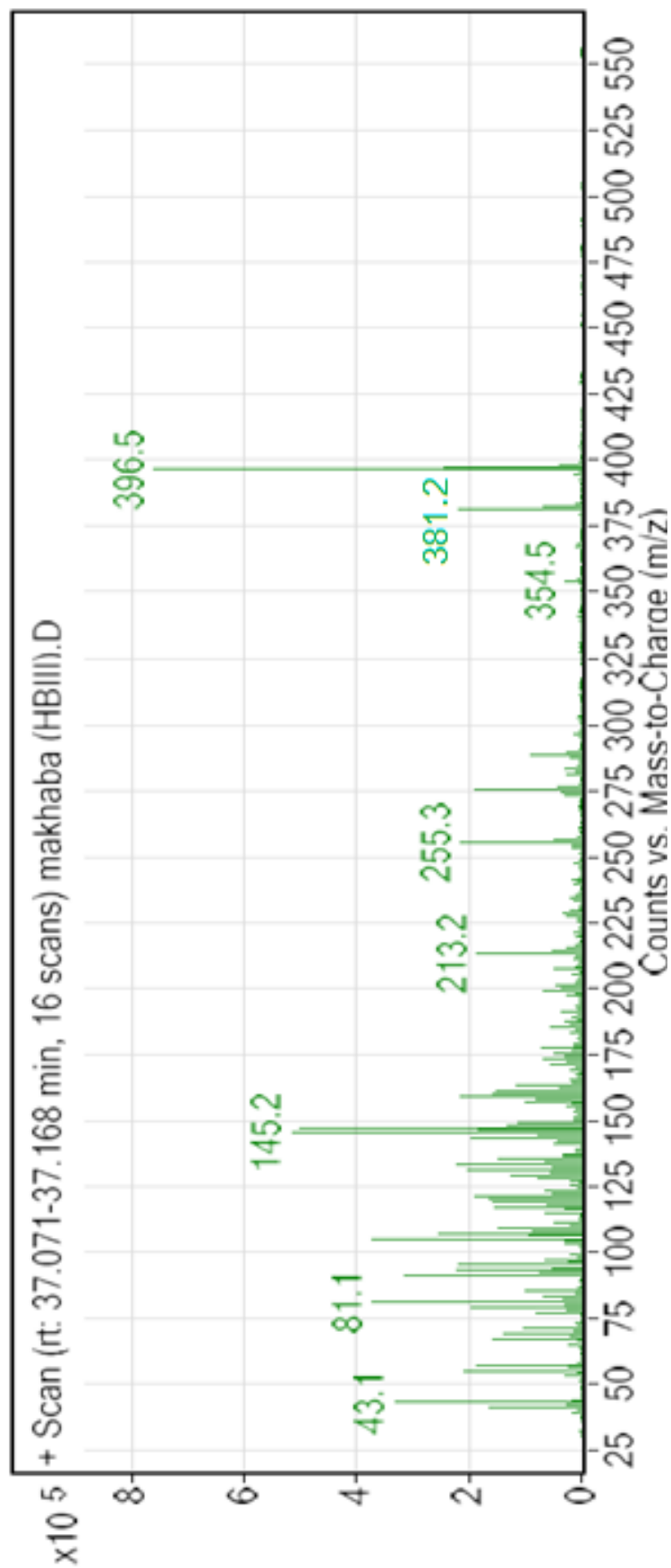


Fig. 3.1.5: GC-MS for compound 1

*

Characteristic fragment ions observed in EI-MS:

Fragment ions (<i>m/z</i>)
396.5
381.2
255.3
213.2

3.2 Structural Elucidation of Compound 2

Compound 2 was readily identified as **feralolide**; a (3R)-isomer of 8-Hydroxy-3-(2''-oxo-propyl)-6-(2',4',6'-trihydroxyphenyl)-isochroman-1-one derivative, according to available literature data previously described for *Aloe ferox*, *Aloe vera* and *Aloe hijazensis* (Speranza *et al.*, 1993; Choi *et al.*, 1996; Abd-Alla *et al.*, 2009). The identity of **2** was, therefore, achieved by means of detailed spectroscopic evidence including direct comparison to reference data (see **Fig. 3.2**). At this point, it is worth pointing out that **2** is reported for the first time for *Aloe succotrina* Lam. spp.

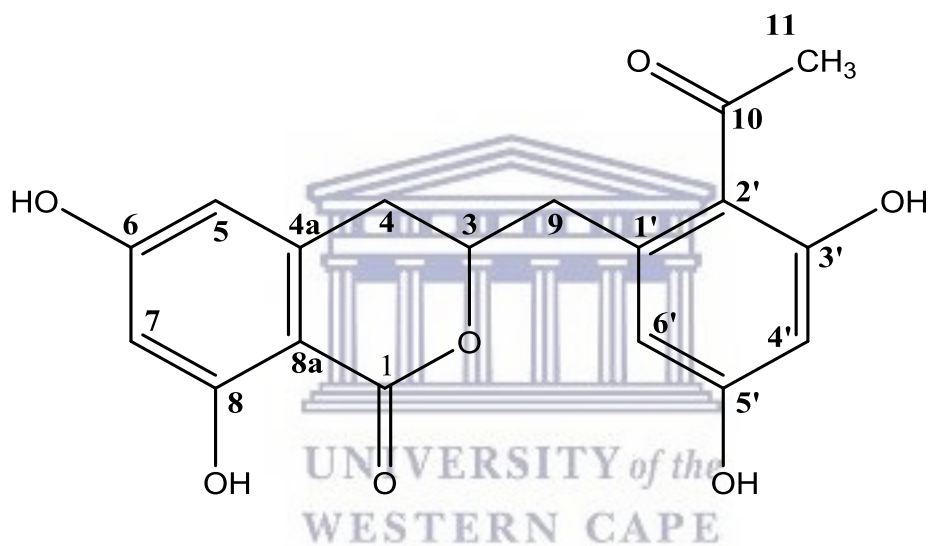


Fig. 3.2: Chemical structure of 8-Hydroxy-3-(2''-oxo-propyl)-6-(2',4',6'-trihydroxyphenyl)-isochroman-1-one.

Compound 2 was isolated as a white-pink amorphous powder (R_f 0.3, 10% MeOH/DCM). Mass spectral data (**Fig. 3.2.4**) using DI-ESI-MS showed a pseudomolecular ion at m/z **343.9000** corresponding to $C_{18}H_{16}O_7$ (exact calculated mass 343.3154, $[M-H]^-$) and another poor signal at m/z **325.90**, formed by the loss of H_2O (Speranza *et al.*, 1993; Choi *et al.*, 1996; Abd-Alla *et al.*, 2009). ^{13}C -NMR spectrum of **2** (**Fig. 3.2.3**) showed an absorption for 18 carbon signals, two of which appeared at δ_C 205.4 and 169.9 ppm, assignable to a carbonyl carbon (C-10) and an ester carbon (C-1). In addition, two up-field signals with δ_C at 32.1 ppm (C-4) and 38.2 ppm (C-9) integrating for two methylene along

with one deshielded signal (δ_C at 80.1 ppm), indicative of the oxymethine at C-3. While eight signals, four of which were signals corresponding to the aromatic carbons (δ_C at 101.1-110.1 ppm) and another four for the meta-hydroxylated-carbons (δ_C at 158.8-165.2 ppm). The position of the acetyl group was identified as C-2' (δ_C at 120.4 ppm) of the phenyl ring (see **Fig. 3.2**). The assignment of this was confirmed by HMBC correlation between the acetate methyl group and C-2' (**Appx. 1J**).

On the other hand, $^1\text{H-NMR}$ spectrum (**Fig. 3.2.2**) revealed the presence of four meta-coupled aromatic protons ($J \sim 2.2\text{Hz}$), assignable to H-5, H-7, H-4' & H-6', including two methylene protons for H-4 & H-9 as well as one oxymethine for H-3 (Speranza *et al.*, 1993; Choi *et al.*, 1996; Abd-Alla *et al.*, 2009). The vicinal proton δ_H at 4.73 ppm (H-3, *m*) was found to correlate with the unresolved peaks assigned to H-4 (δ_H at 2.86-2.89^d ppm) based on COSY correlation (Speranza *et al.*, 1993), as well as, the two methylene δ_H signals at 3.09 [H-9 β , *dd*, J (H-9 β , H-3 β) ~ 7.2 Hz, J (H-9 β , H-9 α) ~ 14.0 Hz]^e; 2.98 [H-9 α , *dd*, J (H-9 α , H-3 β) ~ 5.2 Hz, J (H-9 α , H-9 β) ~ 14.0 Hz]^e for the equatorial and axial protons, through $^1\text{H-}^1\text{H}$ COSY connectivity (**Appx. 1H**). In addition, linkage of the dihydroisocoumaric moiety via C-9 was confirmed by long range HMBC correlation of the oxymethine proton at C-3 with C-9 and C-4 (**Appx. 1J**). Among the important correlations observed in the HMBC spectrum were correlations between H-3 with C-4 (δ_C at 32.1 ppm) and C-1' (δ_C at 137.9 ppm) as well as H-6' with C-9 (δ_C at 38.2 ppm) and C-2' (δ_C at 120.4 ppm) (see **Fig. 3.2.1**; **Table 3.2**). Detailed analysis of DEPT 135 (**Appx. 1G**) and HSQC (**Appx. 1I**) further confirmed the presence of two methylene carbons. Thus, the complete ^1H and $^{13}\text{C-NMR}$ assignment for **2** is shown in **Table 3.2**.

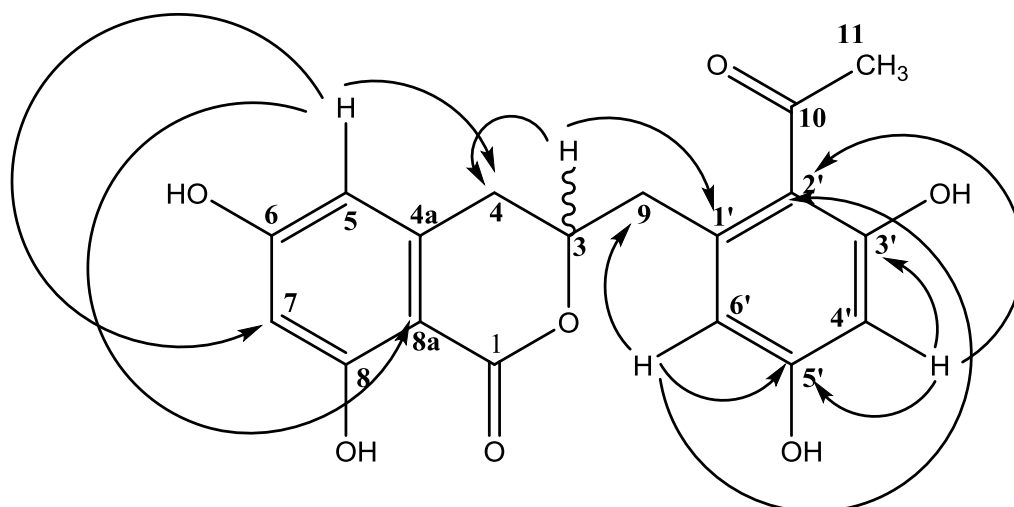


Fig. 3.2.1: Selected HMBC correlations for **compound 2** (CD_3OD using 400 MHz).

Table 3.2: ^1H NMR and ^{13}C NMR data of compound 2 in CD_3OD at 400MHz

Position	^{13}C -NMR δ (m)	^1H -NMR δ (m, integration, J)	HMBC
1	169.9 [O-C=O]	–	–
3	80.1[CH]	4.73 (m, 1H)	C-1', C-4a,
4	32.1 [CH ₂]	2.86-2.89 ^d	C-3, C-5, C-4a
4a	141.8 [C]	–	–
5	106.7 [CH]	6.21 (d, 1H, J = 2.2Hz) ^a	C-4, C-8a, C-7
6	165.2 [=C-OH]	–	–
7	100.8 [CH]	6.20 (d, 1H, J = 2.2Hz) ^a	C-5, C-8a
8	164.2 [=C-OH]	–	–
8a	100.1 [C]	–	–
9	38.2 [CH ₂]	3.09 _b (dd, 1H, J = 7.2, 14.0 Hz) ^c 2.98 _a (dd, 1H, J = 5.2, 14.0 Hz) ^c	C-3, C-6' C-2', C-1', C-3, C-6'
10	205.4 [C=O]	–	–
11	31.5 [CH ₃]	2.51 (s, 3H)	C-10, C-2'
Coum.			
1'	137.9 [C]	–	–
2'	120.4 [C]	–	–
3'	158.8 [=C-OH]	–	–
4'	101.1 [CH]	6.27 (d, 1H, J = 2.2Hz) ^a	C-5', C-6', C-2'

5'	160.1 [=C-OH]	–	–
6'	110.1 [CH]	6.31 (<i>d</i> , 1H, $J = 2.2\text{Hz}$) ^a	C-2', C-4', C-9

^a show poor coupling, which is common for meta-coupling carbons. ^ε correlation based on COSY. ^d Not amenable to first order analysis. **Note.** The *observed* chemical shifts (δ) vary slightly with *reference* chemical shifts (δ) since reported data on literature (Choi *et al.*, 1996; Abd-Alla *et al.*, 2009) was done using 300 MHz for the ¹H-NMR and 125 MHz for ¹³C-NMR (in 10% CDCl₃/CD₃OD). Whereas, another study reported δ in CD₃OD using 300 MHz instrument for ¹H-NMR and 75 MHz for ¹³C-NMR (Speranza *et al.*, 1993).



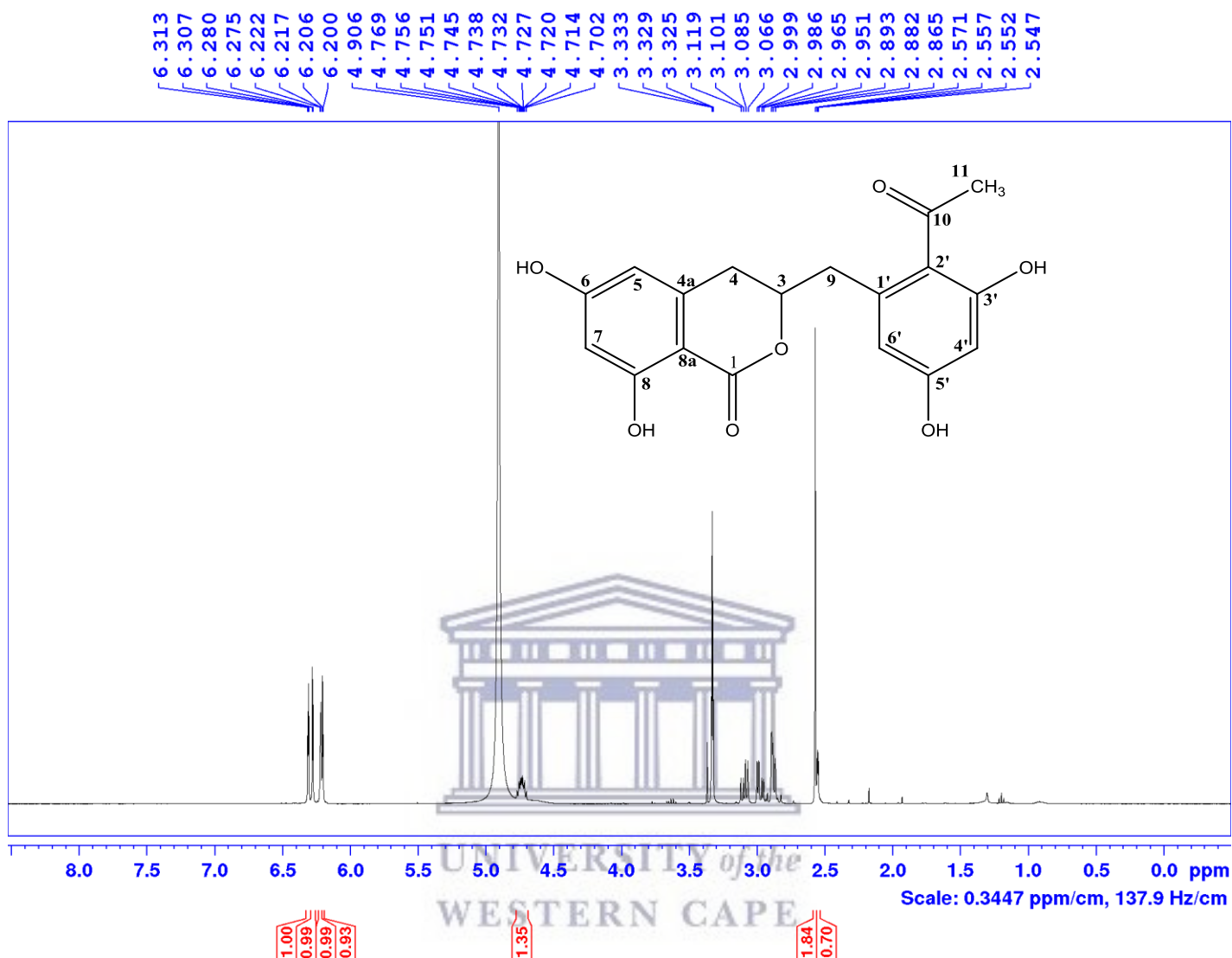


Fig. 3.2.2: ¹H-NMR spectrum of compound 2 (400 MHz; CD₃OD; δ_H at 4.91 ppm and 3.33 ppm are due to solvent).

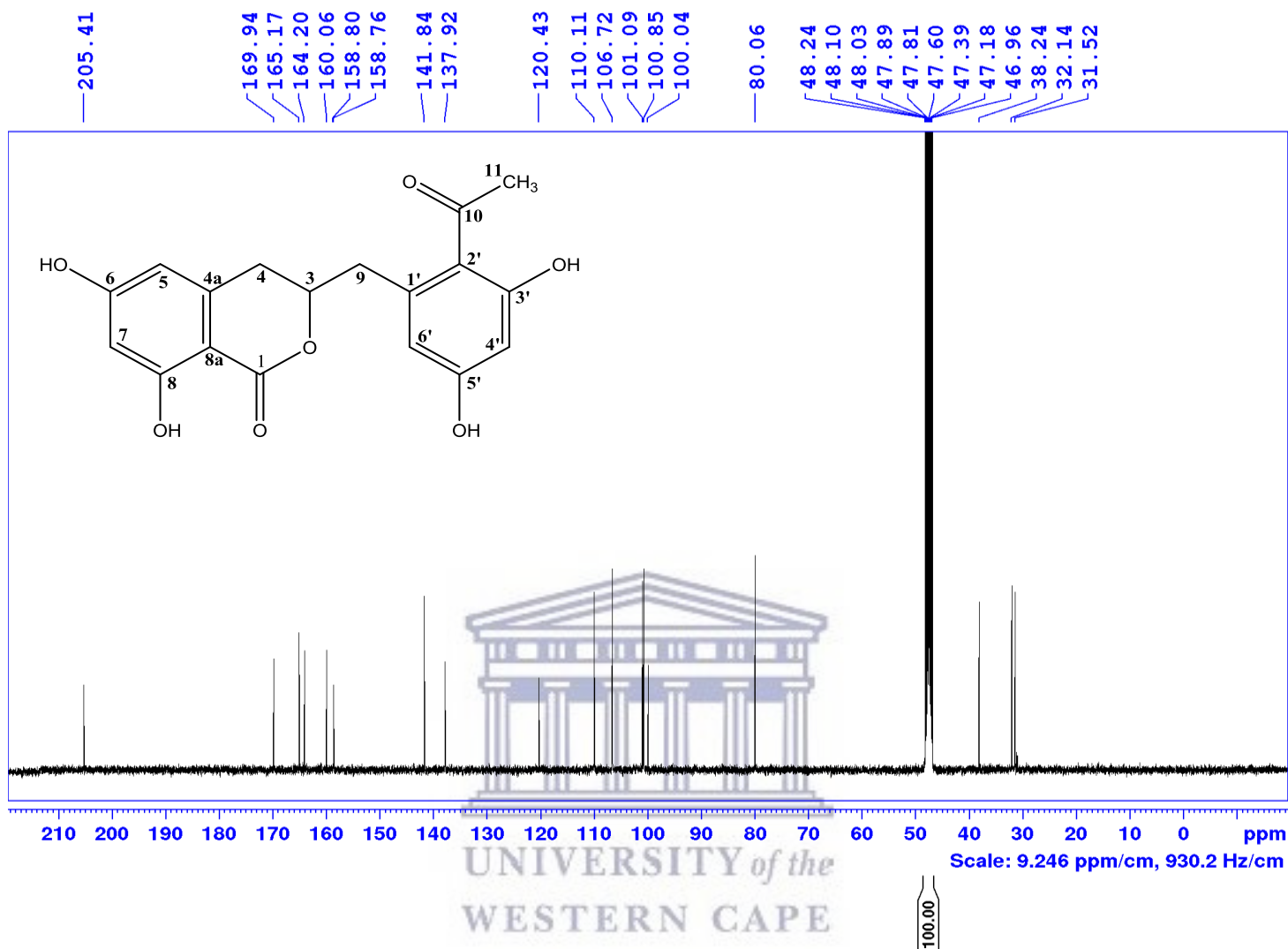


Fig. 3.2.3: ^{13}C -NMR spectrum of compound 2 (100 MHz; CD_3OD ; δ_{C} at 46.9-48.2 ppm are due to solvent).

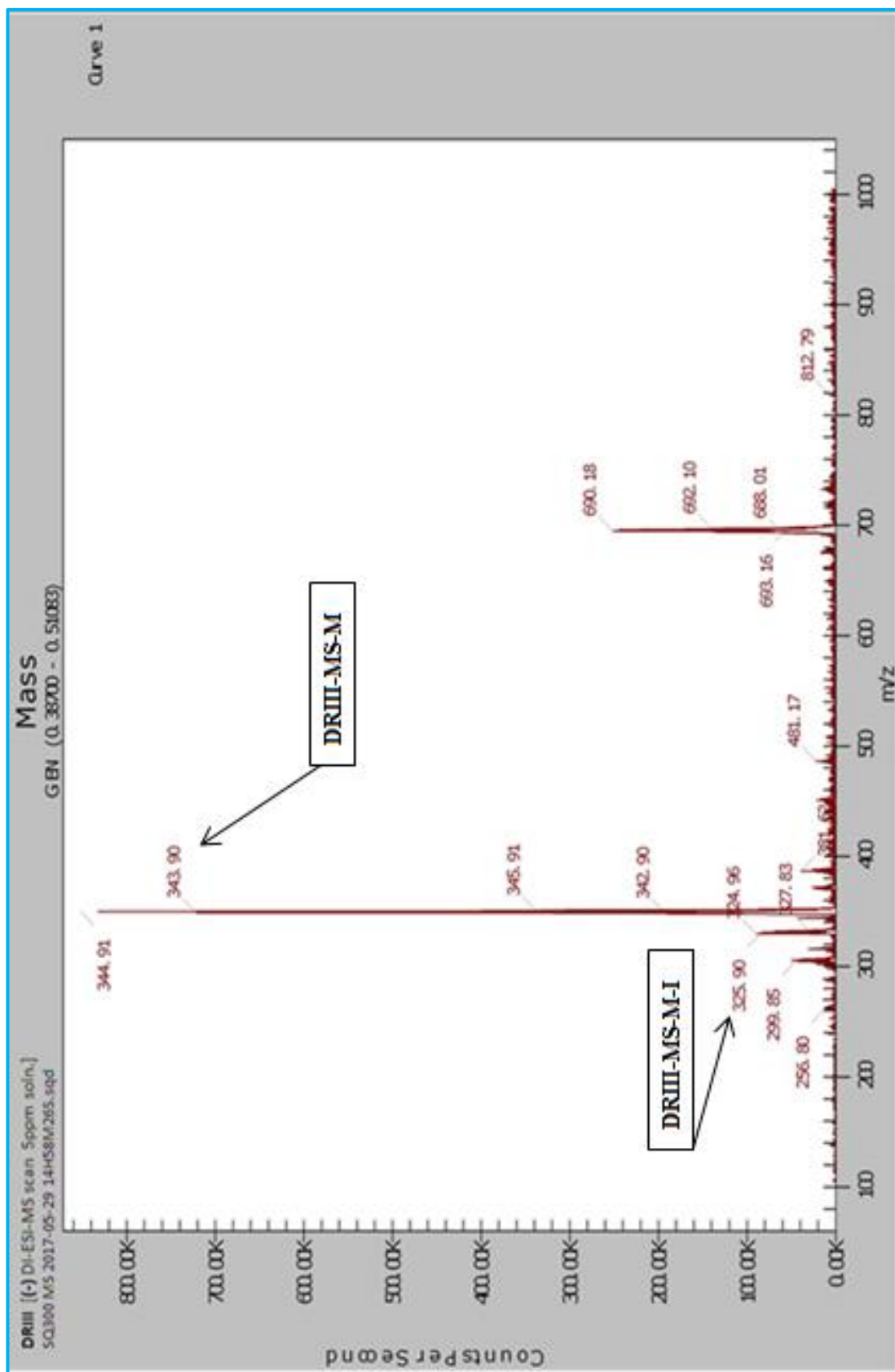


Fig. 3.2.4: LC-MS spectrum for **compound 2**. MS solvent 50 % aqueous deuterated methanol. **DRIII-MS-M** showing the molecular ion $[M-H]^+$ (m/z) 343.90 and **DRIII-MS-M-I** is the characteristic MS-fragment (m/z) 325.90 due to loss of H_2O .

3.3 Structural Elucidation of Compound 3

The accumulation of **3**, a diacetylated anthrone-C-glycoside derivative, in the genus *Aloe* has so far been restricted to *Aloe succotrina* Lam. spp. (Sigler & Rauwald, 1994). Structural characterization of this compound suggests the potential for two dia-stereoisomers which differ in orientation at C-10 of the anthrone-skeleton (Franz & Grün, 1983; Rauwald & Diemer, 1986). Subsequently, structural elucidation of **3** was achieved by means of detailed spectroscopic comparison of the experimental data with reference data as well as conversion to the known 7-hydroxyaloin derivative (Dagne *et al.*, 1998; Teka *et al.*, 2016), which was unequivocally identified as 10-C- β -D-glucopyranosyl-1,7,8-trihydroxy-3-(hydroxymethyl)-10H-anthracen-9-one, commonly known as 7-hydroxyaloin-3',6'-O-diacetate (see **Fig. 3.3**).

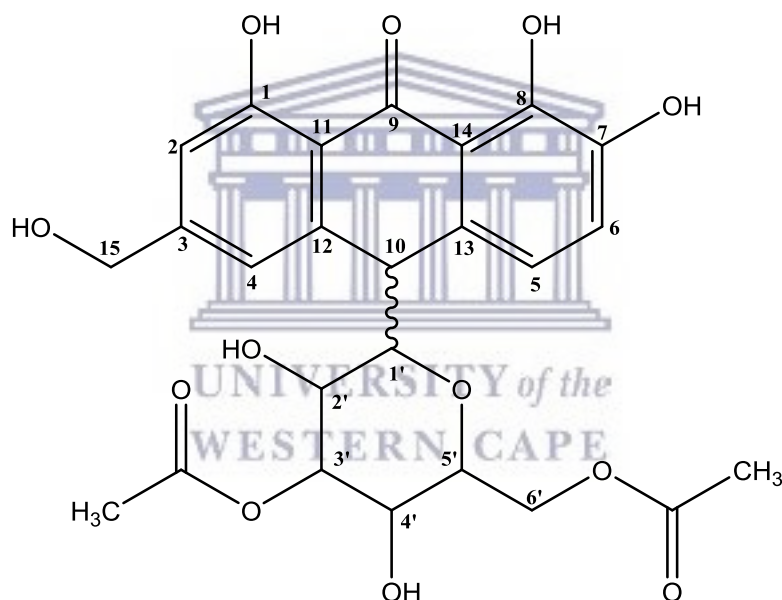


Fig. 3.3: Chemical structure for 10-C- β -D-glucopyranosyl-1,7,8-trihydroxy-3-(hydroxymethyl)-10H-anthracen-9-one.

Compound 3 was isolated as pale yellow crystals (**37 mg**). The mass spectral data using DI-ESI-MS showed a pseudomolecular ion at m/z 517.1348 corresponding to $C_{25}H_{26}O_{12}$ (exact calculated mass 517.4667, $[M-H]^-$) (**Appx. 2A**). Both 1H -NMR (**Fig. 3.3.2**) and ^{13}C -NMR (**Fig. 3.3.3**) spectral data were found to be consistent with reference data (Dagne & Alemu, 1991; Dagne *et al.*, 1998; Teka *et al.*, 2016). ^{13}C -NMR including DEPT 135

experiments showed absorption for 25 carbon signals, two of which were carbonyl carbons with δ_C at 170.9^e and 170.5^d ppm for the two acetyl units attached to the sugar moiety and two other δ_C at 19.4^d and 19.0^e ppm for the corresponding methyls. The HMBC spectrum (**Appx. 1O**) was used to differentiate between the acetyl units; the signal corresponding to δ_C at 170.5^d ppm was assigned to the acetyl unit attached at C-3' and the other δ_C at 170.9^e ppm to an acetyl at C-6'. In agreement with this, the HMBC spectrum (see **Fig. 3.3.1**) also showed long range correlation of the OC $\underline{\text{CH}_3}$ proton (δ_H at 1.90 ppm) with δ_C at 170.9^e ppm (for the acetyl unit attached at C-6') as well as correlations of the OC $\underline{\text{CH}_3}$ proton (δ_H 2.01 ppm) with δ_C at 170.5^d ppm (for the acetyl unit attached at C-3'). This was further confirmed by ¹H-¹H COSY connectivity between H-6' and OC $\underline{\text{CH}_3}$ (δ_H 1.90) protons (**Appx. 1M**). Two additional signals with δ_C at 63.1 and 62.4 ppm were assignable to the oxymethylenes at C-15 and C-6', respectively, including one peak corresponding to a tertiary carbon at C-10 (δ_C at 43.6 ppm). Furthermore, additional five δ_C at 71.0 (C-2'), 70.2 (C-4'), 161.6 (C-1), 144.6 (C-7), and 161.6 ppm (C-8) were assignable to two oxymethine carbons of the sugar moiety, as well as three hydroxylated aromatic-carbons of the anthrone skeleton, respectively. The ¹³C-NMR spectrum also showed four aromatic signals with δ_C at 112.6 (C-2), 116.0 (C-4), 119.4 (C-6) and 119.9 ppm (C-5) including five signals δ_C 151.0 (C-3), 117.8 (C-11), 146.3 (C-12), 149.8 (C-13) and 116.4 ppm (C-14), assignable to the quaternary carbons.

The strong de-shielding effects observed at C-12 and C-13 compared to C-11 and C-14 of the anthrone skeleton, may be attributed to the effects of steric hindrance as consequence of the substituents at C-10 (Rao *et al.*, 1951), which disturb the overall planarity of the molecule. Another signal corresponding to a keto-group at C-9 (δ_C 194.6 ppm) was also observed from the ¹³C-NMR spectrum. The anomeric signal was found to resonate at δ_C at 84.6 ppm (C-1') (Dagne *et al.*, 1998; Teka *et al.*, 2016), which was confirmed by ¹H-NMR spectrum that showed a broad singlet with δ_H at 3.36 ppm integrating for one proton assignable to the anomeric proton (H-1'). Further studies *i.e.* molecular modelling and optical rotation would need to be done to confirm the absolute stereochemistry at C-10. Nevertheless, the ¹H-NMR spectrum further showed two strongly intense singlet peaks with δ_H at 2.01 and 1.90 ppm, which is a common region for methyl protons attached to an acetyl unit (Haynes *et al.*, 1970). The assignments of the aromatic protons (H-2, H-4, H-5 and H-6) as well as other ¹H-NMR signals are shown in **Table 3.3**. Furthermore, detailed HSQC (**Appx. 1N**) and DEPT-135 (**Appx. 1L**) experiments which support results presented herein are shown in the appendix section.

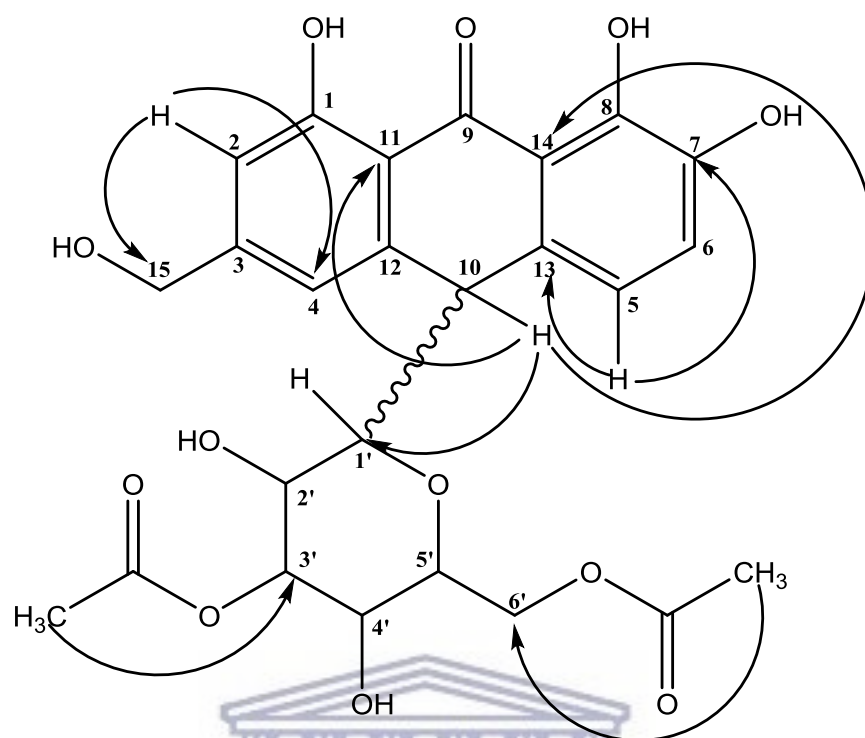


Fig. 3.3.1: Selected HMBC correlations for compound 3 (CD₃OD using 400 MHz).

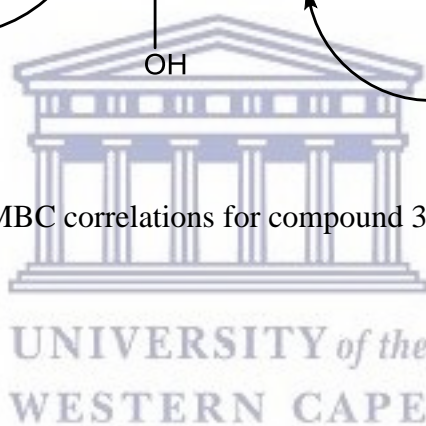


Table 3.3: ¹H NMR and ¹³C NMR data of compound 3 in CD₃OD at 400MHz

Position	¹³ C-NMR δ (m)	¹ H-NMR δ (m, integration, J)	HMBC
1	161.6 [=C-OH]	(s) ^h	–
2	112.6 [=CH]	6.88 (d, 1H, J = 0.6Hz)	C-4, C-15
3	151.0 [C]	–	–
4	116.0 [=CH]	7.02 (d, 1H, J = 0.6Hz)	C-2, C-10(long range)
5	119.9 [=CH]	7.07 (dd, 1H, J = 8.0Hz)	C-7, C-13
6	119.4 [=CH]	6.93 (dd, 1H, J = 8.0Hz)	C-7, C-9(long range)
7	144.6 [=C-OH]	(s) ^h	–
8	161.6 [=C-OH]	(s) ^h	–
9	194.6 [C=O]	–	–
10	43.6 [CH] ^a	4.53 (d, 1H, J = 1.8 Hz)	C-1', C-11, C-14
11	117.8 [C]	–	–
12	146.3 [C]	–	–
13	149.8 [C]	–	–
14	116.4 [C]	–	–
15	63.1 [CH ₂]	4.67(d, 2H, J = 2.4)	C-2, C-3, C-4

C-glc.

1'	84.6 [CH] ^a	3.36 (s, 1H)	C-10, C-2', C-3'
2'	71.0 [CH]	4.35 (t, 1H, J = 9.6, 9.7 Hz)	C-3'
3'	76.0 [CH]	3.48 (t, 1H, J = 9.0, 9.1 Hz)	C-4
4'	70.2 [CH]	3.20 (m, 1H)	–

5'	75.1 [CH]	3.21 (<i>m</i> , 1H)	–
6'	62.4 [CH ₂] ^{<i>b</i>}	3.72 (<i>dd</i> , 1H, <i>J</i> = 6.4, 12.0 Hz)	C-5
		3.81 (<i>dd</i> , 1H, <i>J</i> = 2.4, 12.0 Hz)	C-5
<u>OC</u> CH ₃	170.9 ^{<i>c</i>}	–	–
OC <u>CH</u> ₃	19.0 ^{<i>c</i>}	1.90 (<i>s</i> , 3H)	C-6'
<u>OC</u> CH ₃	170.5 ^{<i>d</i>}	–	–
OC <u>CH</u> ₃	19.4 ^{<i>d</i>}	2.01 (<i>s</i> , 3H)	C-3'

^{*a*} anomeric shift based on COSY, HMBC and HSQC. ^{*b*} based on HMBC and HSQC. ^{*c*} shift due to one OCCH₃ unit at C-6'. ^{*d*} shifts due to one OCCH₃ unit at C-3'. ^{*h*} not amenable to first order analysis.



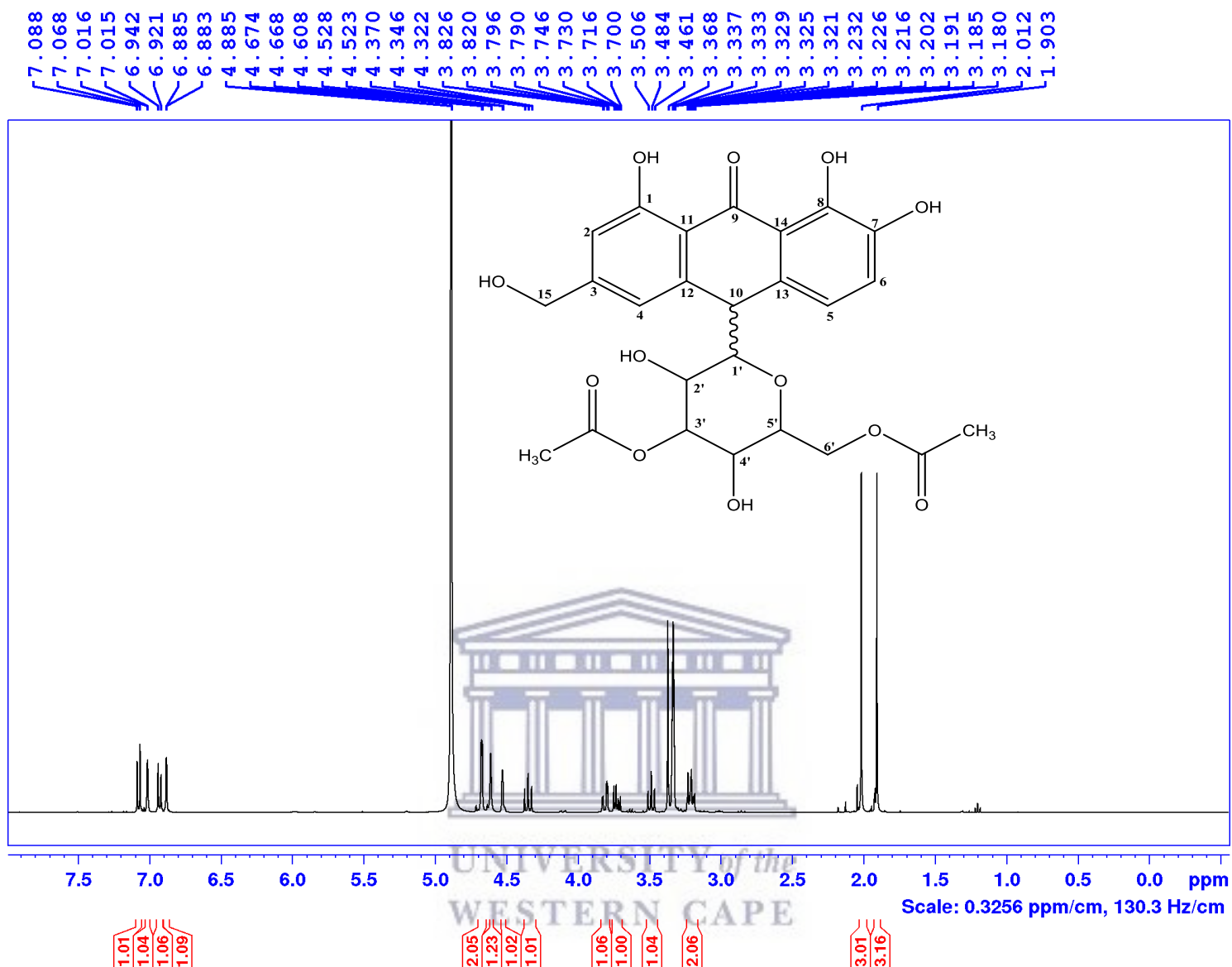


Fig. 3.3.2: ¹H-NMR spectrum of **compound 3** (400 MHz; CD₃OD; δ_H at 4.88 ppm and 3.33 ppm are due to solvent).

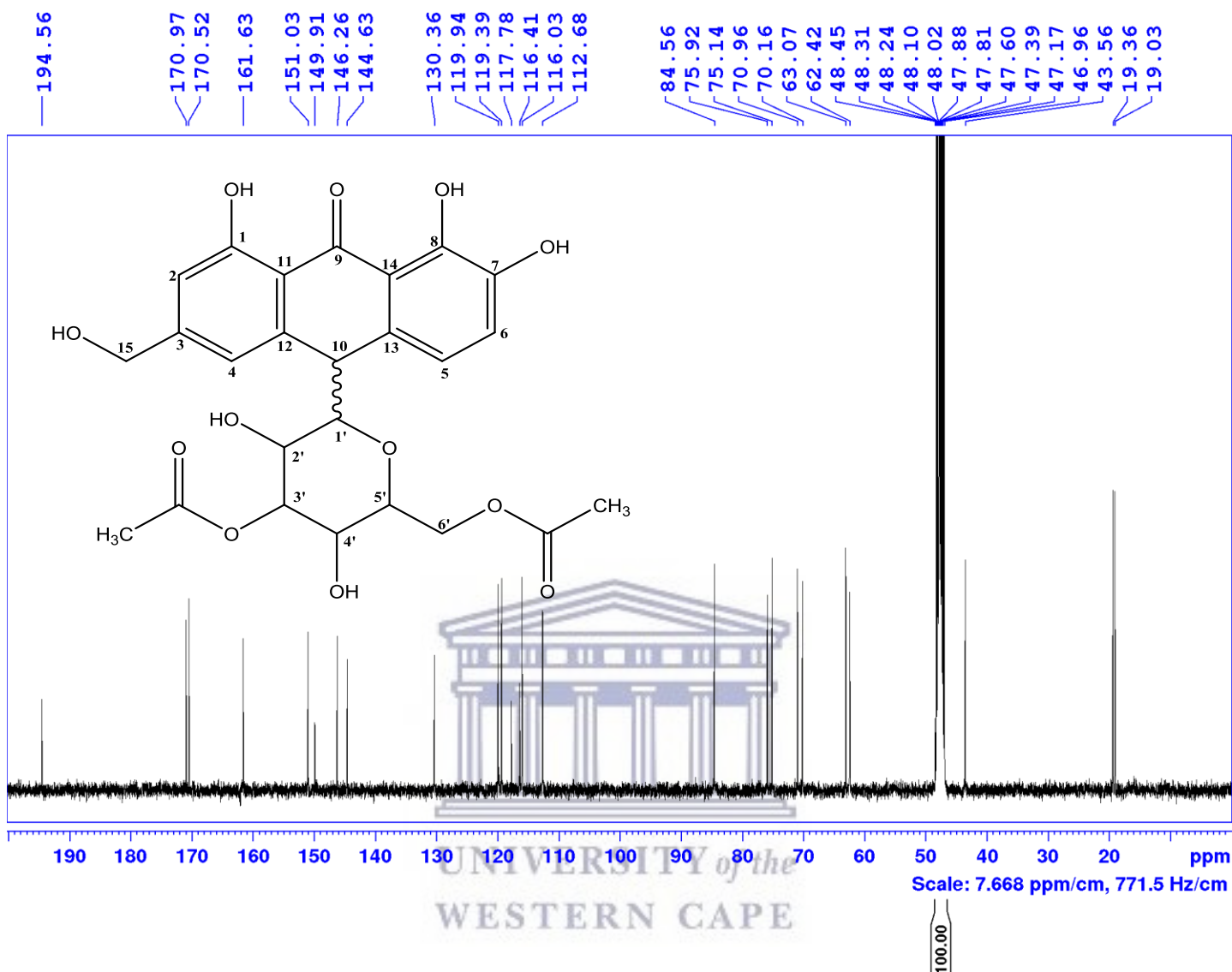


Fig. 3.3.3: ^{13}C -NMR spectrum of **compound 3** (100 MHz; CD_3OD ; δ_{C} at 46.9–48.6 ppm are due to solvent).

3.4 Structural Elucidation of Compound 4

Compound 4, the derivative of **2**, was readily identified on the basis of its coherent NMR and LC-MS spectral evidence as 3,4-dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O-beta-D-glucopyranosyl-5'-hydroxyphenyl)methyl-2(1H)-benzopyran-1-one, which is commonly known as *dihydro-isocoumarin glucoside* (see **Fig. 3.4**). The thin layer chromatography (TLC) profile for this compound gave a strong purple staining zone prior to spraying with vanillin but after vanillin spraying, a single deep-red TLC zone was observed characteristic for the compound (R_f 0.5, with $\text{CHCl}_3\text{-EtOH-H}_2\text{O}$) (**Appx. 1P**). The physico-chemical properties including spectroscopy evidence generated for **4** was found to be consistent with reference data (Vietch *et al.*, 1994). However, it is worth pointing out that **4** is reported for the first time for *Aloe succotrina* Lam. spp.

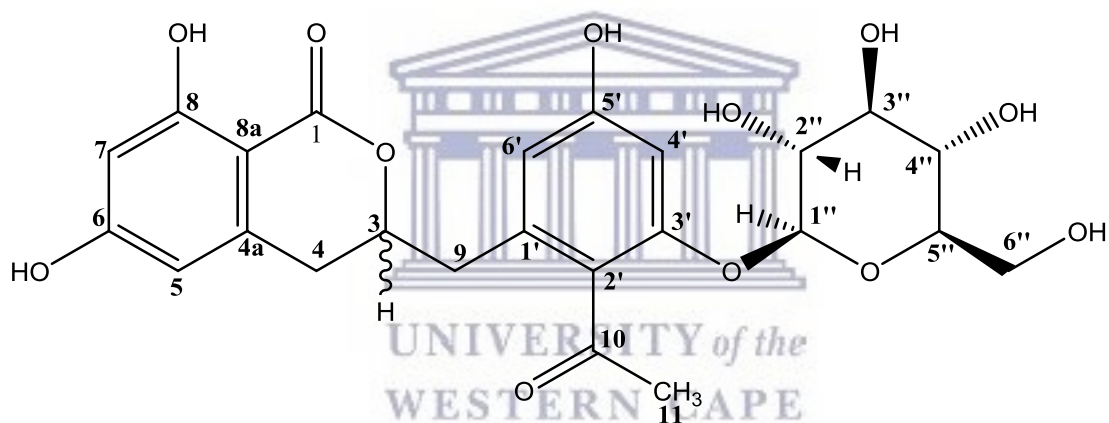


Fig. 3.4: Chemical structure for 3,4-dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl)methyl-2(1H)-benzopyran-1-one.

Compound 4 was isolated as a light purple-red-amorphous powder (R_f 0.5, 7:3:1, $\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O}$). Mass spectral data using DI-ESI-MS showed a pseudomolecular ion at m/z 505.13 corresponding to $\text{C}_{24}\text{H}_{26}\text{O}_{12}$ (exact calculated mass 505.4560, $[\text{M}-\text{H}]^-$) (see **Fig. 3.4.4**). The $^1\text{H-NMR}$ spectrum (see **Table 3. 4; Fig. 3.4.2**) showed peaks at δ_{H} 6.52 [H-6', d , $J \sim 2.2$ Hz]^a; 6.38 [H-4', d , $J \sim 2.2$ Hz]^a; 6.09 [H-5, d , $J \sim 2.2$ Hz]^a; 6.08 [H-7, d , $J \sim 2.2$ Hz]^a; 4.60 [H-3, m]; 2.93 [H-9 β , dd , J (H-9 β , H-3 β) ~ 7.4 Hz, J (H-9 β , H-9 α) ~ 13.9 Hz]^b; 2.82 [H-9 α , dd , J (H-9 α , H-3 β) ~ 5.2 Hz, J (H-9 α , H-9 β) ~ 13.8 Hz]^b; 2.75 [H-4]^c and 2.49 [H-11, s]. Whereas, the peaks at δ_{H} 4.86 [H-1'', d , J (H-1'' α , H-2'' β) ~ 7.5 Hz]; 3.80 [H-6'' β , dd , J (H-6'' β , H-5'' α)

~1.8 Hz, J (H-6'' β , H-6'' α) ~12.0 Hz]; 3.61 [H-6'' α , dd , J (H-6'' α , H-5'' α) ~5.2 Hz, J (H-6'' α , H-6'' β) ~12.0 Hz]; 3.35 [H-2, m]; 3.32-3.34 [H-3'']^d; 3.32-3.34 [H-5'']^d and 3.29 [H-4'', m] were assignable to the sugar moiety as previously described (Vietch *et al.*, 1994). The strong coupling (J ~7.5 Hz) between the ¹H nuclei of H-1'' (anomeric proton) with H-2'' is indicative of a beta-coupling to the sugar moiety. This implies a trans-diaxial relationship between the H-1'' (anomeric proton) and H-2'' (Jacobsen, 2007), suggesting the stereochemistry at H-1'' to be alpha. The assignment of the stereochemistry was validated with comparison to literature data (Vietch *et al.*, 1994). Furthermore, the strong coupling may also be due to an inductive pull of the electrons away from the protons in particular since C-1'' (anomeric carbon) is bonded *additionally* to two oxygens (Jacobsen, 2007), implying a downfield shift especially for the anomeric proton (H-1'', δ_{H} at 4.86).

The ¹³C-NMR (Fig. 3.4.3) including HSQC (Appx. 1R) spectra gave compelling evidence of the δ_{C} typical for O-linked glycosides (Vietch *et al.*, 1994), which allowed the assignment of the sugar moiety (see Table 3.4). Although there were notable differences in the δ_{C} especially for the carbons in close proximity to the glycosylation site, which is to be expected (Vietch *et al.*, 1994), the only two comparable differences were observed at C-3' (δ_{C} 156.9 ppm) as well as C-2' (δ_{C} 123.7 ppm). Needless to say, C-3' (δ_{C} 156.9 ppm) of **4** differed from C-3' (δ_{C} 158.8 ppm) of **2** by a degree of -1.9 ppm, which indicated a more up-field chemical shift (than would be expected) – perhaps brought about by stereo-electronic effects and/or anomeric effects subject to glycosylation (Tvaroska & Bleha, 1989). On the other hand, the examination of long range HMBC connectivity of ¹H–¹³C nuclei atoms was essential to distinguish unambiguously the glycosylation site at C-3', through long range correlation between H-1'' and C-3' (see Fig. 3.4.1). Moreover, the complete assignment of the diagnostic signals shown in Table 3.4 for the δ_{C} of the aglycone skeleton was compared with δ_{C} of **2**, and was also found to be in agreement with reference data (Vietch *et al.*, 1994). The absolute stereochemistry at C-3 (δ_{C} 79.9 ppm) could not be determined due to insufficient amount that was isolated; assignment was made by comparison with compound **2** and literature data (Vietch *et al.*, 1994; Speranza *et al.*, 1993; Choi *et al.*, 1996; Abd-Alla *et al.*, 2009).

Worth noting, the TLC profile of **4** showed a distinct purple staining when the plate was left to stand overnight, after development, without vanillin spraying. According to literature, **4** is suggested as a useful chemotaxonomic maker among Aloe species (Vietch *et al.*, 1994). Hence, *Aloe cremnophila*, *Aloe hildebrandtii* and *Aloe jacksonii* are the only three Aloe species known to elaborate this color (Brandham *et al.*, 1994), which is due to **4**. Thus, taking into account that in the genus, **4** has only been identified in these species, it apparent, therefore, that the identification of **4** in *Aloe succotrina* Lam. spp. 'might' perhaps suggest evidence of its relation to *A. cremnophila*, *A. hildebrandtii* and *A. jacksonii*. Hence, a comprehensive reinvestigation of polyketide accumulation in the aloe plant may be necessary.

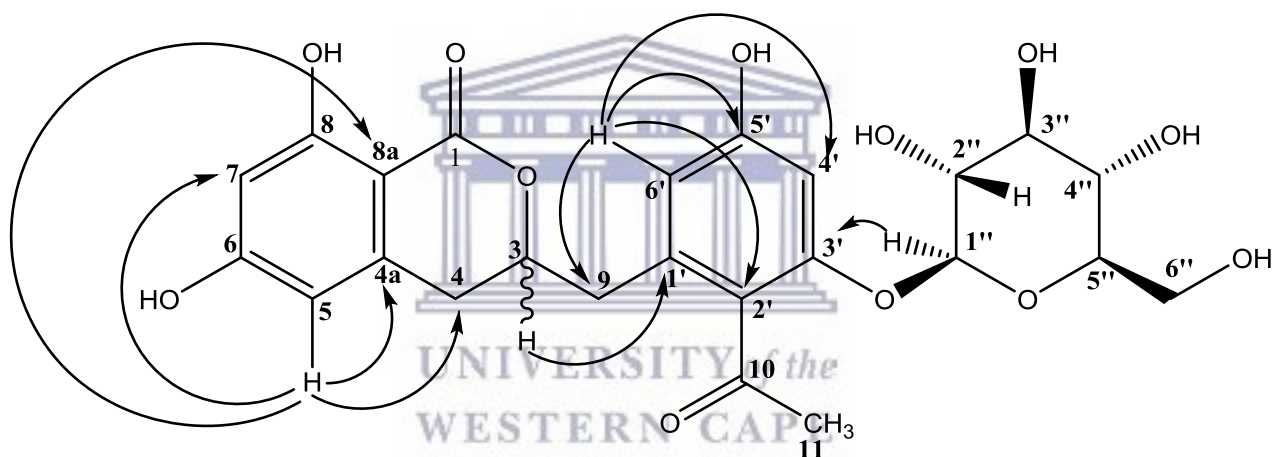


Fig. 3.4.1: Selected HMBC correlations for **compound 4** (CD₃OD using 400 MHz).

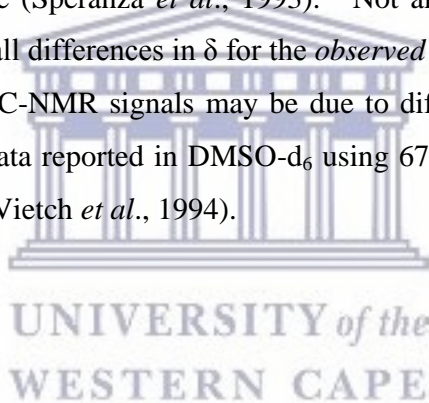
Table 3.4: ^1H NMR and ^{13}C NMR data of compound 4 in CD_3OD at 400MHz

Position	^{13}C -NMR δ (m)	^1H -NMR δ (m, integration, J)	HMBC
1	169.8 [O-C=O]	–	–
3	79.9[CH]	4.60 (m, 1H)	C-1', C-4a
4	32.1 [CH ₂]	2.73 -2.75 ^c	C-5, C-4a, C-8a
4a	141.8 [C]	–	–
5	106.7 [CH]	6.09 (d, 1H, J = 2.2Hz) ^a	C-4, C-8a, C-7, C-6
6	165.1 [=C-OH]	–	–
7	100.9 [CH]	6.08 (d, 1H, J = 2.2Hz) ^a	C-5, C-8a, C-8, C-6
8	164.2 [=C-OH]	–	–
8a	100.0 [C]	–	–
9	37.7 [CH ₂]	2.82 _a (dd, 1H, J = 5.2, 13.8 Hz) ^b 2.93 _b (dd, 1H, J = 7.4, 13.9 Hz) ^b	C-2', C-1', C-3, C-6' C-2', C-1', C-3, C-6'
10	205.7 [C=O]	–	–
11	29.3 [CH ₃]	2.49 (s, 3H)	C-10, C-2'
Coum.			
1'	136.8 [C]	–	–
2'	123.7 [C]	–	–
3'	156.9 [=C-OH]	–	–
4'	101.2 [CH]	6.38 (d, 1H, J = 2.2Hz) ^a	C-6', C-2', C-3', C-5'
5'	159.6 [=C-OH]	–	–
6'	112.0 [CH]	6.52 (d, 1H, J = 2.2Hz) ^a	C-5' C-2', C-4', C-9

O-glc,

1''	100.7 [CH]	4.86 (<i>d</i> , 1H, $J = 7.5$ Hz)	C-3'
2''	73.4 [CH]	3.35 (<i>m</i> , 1H)	–
3''	76.8 [CH]	3.32-3.34 (<i>m</i> , 1H) ^ε	–
4''	69.8 [CH]	3.29 (<i>m</i> , 1H)	–
5''	76.9 [CH]	3.32-3.34 (<i>m</i> , 1H) ^ε	–
6''	61.1 [CH ₂]	3.80 (<i>dd</i> , 1H _a , $J = 1.8, 12.0$ Hz)	–
		3.61 (<i>dd</i> , 1H _b , $J = 5.2, 12.0$ Hz)	–

^a Show poor coupling, which is common for meta-coupling carbons. ^b correlation based on COSY and with reference on literature (Speranza *et al.*, 1993). ^c Not amenable to first order analysis (Vietch *et al.*, 1994). **Note.** Small differences in δ for the *observed* ¹H and ¹³C-NMR signals with respect to *reference* ¹H and ¹³C-NMR signals may be due to different conditions under which data was reported. Reference data reported in DMSO-d₆ using 67.8 MHz for ¹³C-NMR and 500 MHz for ¹H-NMR instrument (Vietch *et al.*, 1994).



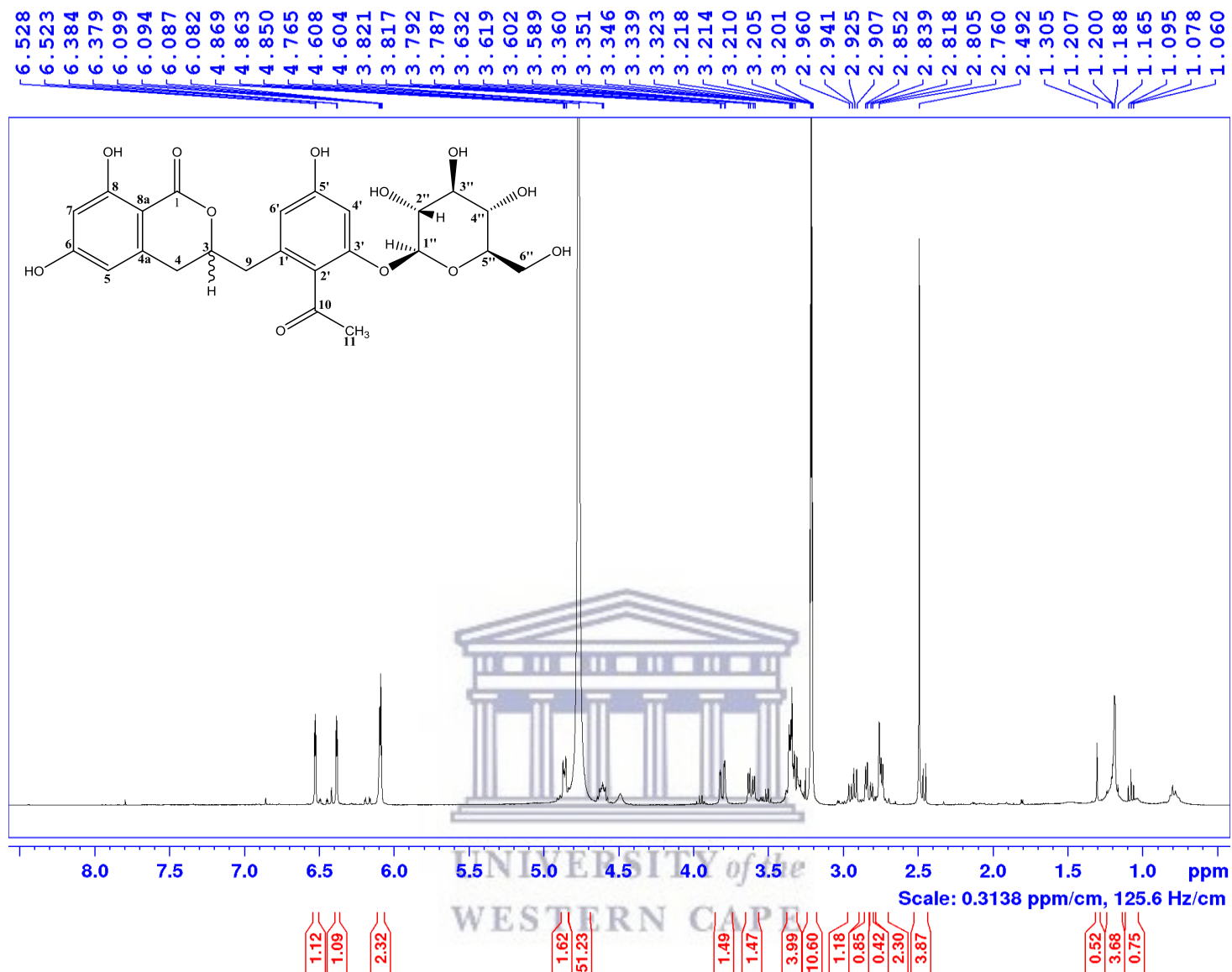


Fig. 3.4.2: ¹H-NMR spectrum of **compound 4** (400 MHz; CD₃OD; δ_H at 4.77 and 3.21 ppm are due to solvent).

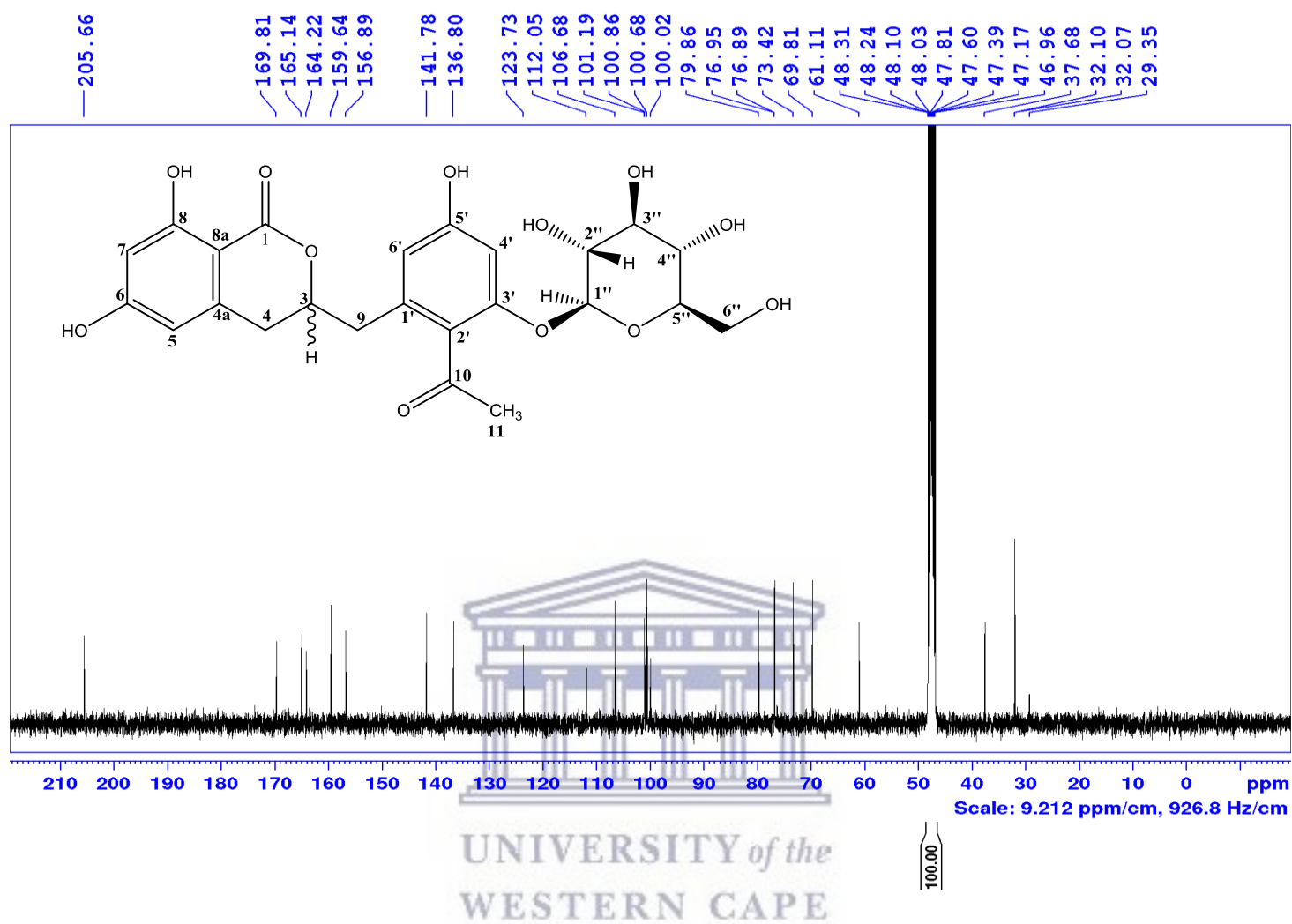


Fig. 3.4.3: ^{13}C -NMR spectrum of **compound 4** (100 MHz; CD_3OD ; δ_{C} at 47.2-48.3 ppm are due to solvent).

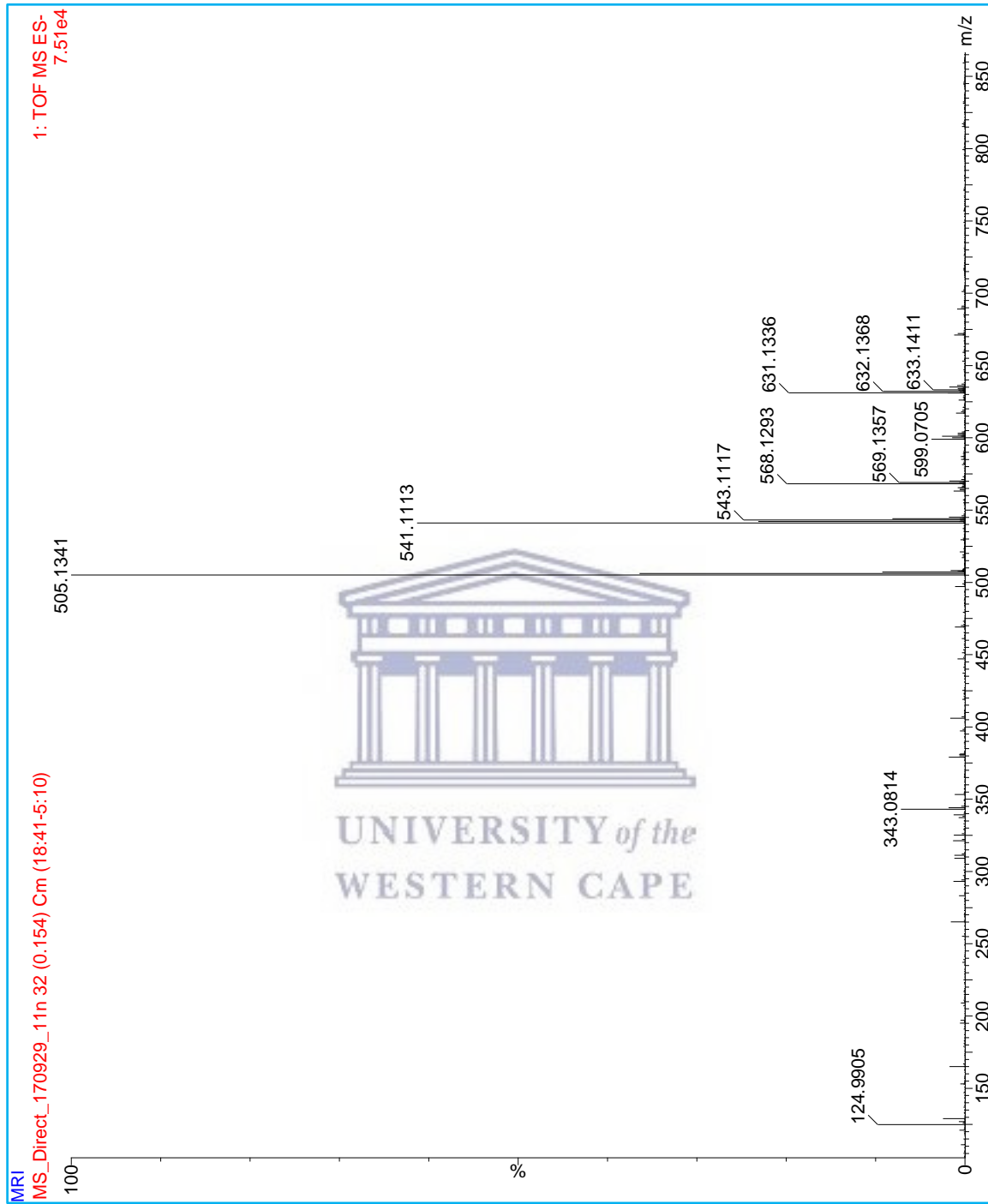


Fig. 3.4.4: LC-MS spectrum for **compound 4**. MS solvent ACN. **MRI-A** shows the molecular ion (m/z) 505.13 $[M-H]^-$. ***Note.** See method section (chapter 2) for conditions.

3.5 Structural Elucidation of Compound 5

The identity of **compound 5** (Fig. 3.5) was achieved based on the background knowledge that *Aloe succotrina* spp. accumulates 7-hydroxyaloin derivatives (Sigler & Rauwald, 1994), and by making direct comparison of its HMBC (Fig. 3.5.1), HSQC (Appx. 1W) as well as ^1H - ^1H COSY correlation (Appx. 1V) with **3**. Thus, the identity of **3** was found to be 10-C- β -D-glucopyranosyl-1,7,8-trihydroxy-3-(hydroxymethyl)-10H-anthracen-9-one (Dagne *et al.*, 1998; Teka *et al.*, 2016), commonly known as 7-hydroxyaloin-6'-O-monoacetate.

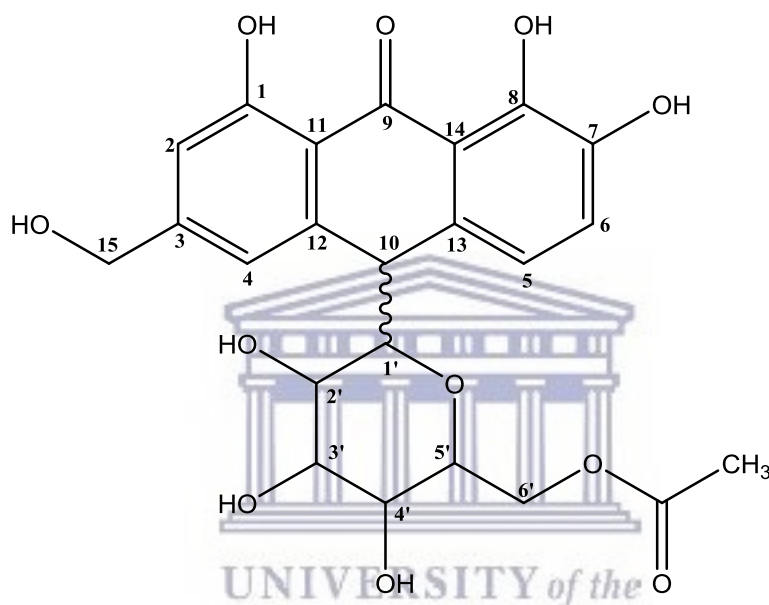


Fig. 3.5: Chemical structure for 10-C- β -D-glucopyranosyl-1,7,8-trihydroxy-3-(hydroxymethyl)-10H-anthracen-9-one.

Compound 5 was isolated as a purple-amorphous powder (R_f 0.2; 15% MeOH/DCM), and its ^1H and ^{13}C -NMR (including COSY, DEPT-135, HMBC & HSQC) spectra presented very close similarities to that of **3**, except **5** had one acetyl unit less. Comparison of the ^{13}C -NMR spectrum of **5** to the existing, reference data for 7-hydroxyaloin (Teka *et al.*, 2016), showed a slightly downfield shift at C-6' (δ_C at 63.4 ppm), which differed by a factor of +1.6 ppm. Hence, the acetylation for **5** was placed at C-6', which was confirmed through long range ^1H - ^{13}C HMBC connectivity between H-6' protons δ_H 3.98 [H-6'' β , *dd*, J (H-6'' β , H-5'' β) \sim 7.1 Hz, J (H-6'' β , H-6'' α) \sim 11.6 Hz] and 3.68 [H-6'' α , *dd*, J (H-6'' α , H-5'' β) \sim 2.0 Hz, J (H-6'' α , H-6'' β) \sim 11.6 Hz] with the ketonyl carbon (δ_C at 171.2 ppm). Another distinction for

the spectra of **5** was a slightly de-shielded δ_C by a factor of +2.4 ppm assignable to the oxymethine at C-3' (δ_C at 78.4 ppm). The HSQC and ^1H - ^1H COSY correlation was necessary to unambiguously assign all the protonated carbons (δ_C) as shown in **Table 3.5**. In addition, direct comparison of the ^1H (**Fig. 3.5.2; Table 3.5**) and ^{13}C -NMR (**Fig. 3.5.3; Table 3.5**) spectral data of **5** with reference data for 10-hydroxyaloin-6'-O-monoacetate as well as 7-hydroxyaloin (Dagne *et al.*, 1998; Teka *et al.*, 2016), allowed further assignment of the chemical shifts (δ). Evidently, the δ_C at 144.5 ppm (C-7) including a broad singlet proton at H-10 (δ_H at 4.38 ppm), served as characteristic features which aid to identify **5** as 10-C- β -D-glucopyranosyl-1,7,8-trihydroxy-3-(hydroxymethyl)-10H-anthracen-9-one, commonly known as 7-hydroxyaloin-6'-O-monoacetate (Dagne *et al.*, 1998; Teka *et al.*, 2016). Unlike in 10-hydroxyaloin-6'-O-monoacetate, the ^1H -NMR of **5** showed two pairs of aromatic signals with δ_H at 6.93 [H-5, *d*, *J* ~8.0 Hz], 6.80 [H-6, *d*, *J* ~8.0 Hz] and 6.89 [H-4, *s*], 6.76 [H-2, *s*], which were assignable to the *ortho* and *meta*-coupled proton-pairs, respectively. The ^1H -NMR also showed a weak signal at δ_H 11.84 ppm, due to the hydroxyl protons at C-1 and C-8 (Dagne *et al.*, 1998). Furthermore, DEPT-135 (**Appx. 1U**) was imperative in confirming the δ_C of the corresponding protonated carbons (see **Table 3.5**). The mass spectral data using DI-ESI-MS was also used to confirmed the molecular formula $\text{C}_{23}\text{H}_{24}\text{O}_{11}$ (exact calculated mass 476.4380, $[\text{M}-\text{H}]^-$) whose pseudomolecular ion at m/z 475.12 was determined explicitly from the chromatogram (**Appx. 2B**). However, further studies such as molecular modelling and optical rotation need to be done to distinguish the absolute stereochemistry at C-10.

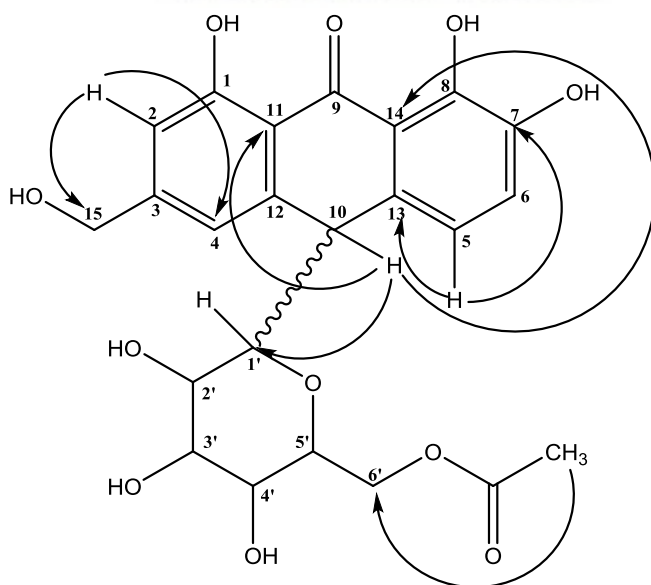


Fig. 3.5.1: Selected HMBC correlations for **compound 5** (CD_3OD using 400 MHz).

Table 3.5: ¹H NMR and ¹³C NMR data of compound 5 in CD₃OD at 400MHz

Position	¹³ C-NMR δ (m)	¹ H-NMR δ (m, integration, J)	HMBC
1	161.6 [=C-OH]	(s) ^d	–
2	112.6 [=CH]	6.76 (s, 1H)	C-4, C-15
3	151.0 [C]	–	–
4	116.0 [=CH]	6.89 (s, 1H)	C-2, C-10(long range)
5	119.8 [=CH]	6.93 (d, 1H, J = 8.0Hz)	C-7, C-13
6	119.4 [=CH]	6.80 (d, 1H, J = 8.0Hz)	C-7, C-9(long range)
7	144.5 [=C-OH]	(s) ^d	–
8	161.6 [=C-OH]	(s) ^d	–
9	194.6 [C=O]	–	–
10	43.6 [CH] ^e	4.39 (brs, 1H)	C-1', C-11, C-14
11	117.8 [C]	–	–
12	146.6 [C]	–	–
13	149.8 [C]	–	–
14	116.5 [C]	–	–
15	63.1 [CH ₂]	4.55 (s, 2H, 15.0, 16.2 Hz)	C-2, C-3, C-4
C-glc.			
1'	84.6 [CH]	3.12 (dd, 1H) ^d	C-10, C-2', C-3'
2'	70.4 [CH]	2.73 (t, 1H, J = 9.2, 9.4 Hz)	C-3'
3'	78.4 [CH]	3.18 (m, 1H)	C-4'
4'	70.2 [CH]	2.99 (t, 1H, J = 9.2, 9.4 Hz)	C-3

5'	77.4 [CH]	2.88 (<i>tdd</i> , 1H, $J = 2.4, 2.0$ Hz)	–
6'	63.4 [CH ₂] ^b	3.68 (<i>dd</i> , 1H, $J = 7.1, 11.6$ Hz)	C-5'
		3.98 (<i>dd</i> , 1H, $J = 2.0, 11.6$ Hz)	C-5'
<hr/>			
1X <u>OC</u>CH₃	171.2	–	–
1X <u>OC</u>CH₃	19.1	1.80 (<i>s</i> , 3H)	C-6'

^acarbon shift based on DEPT, HMBC and HSQC. ^dNot amenable to first order analysis.



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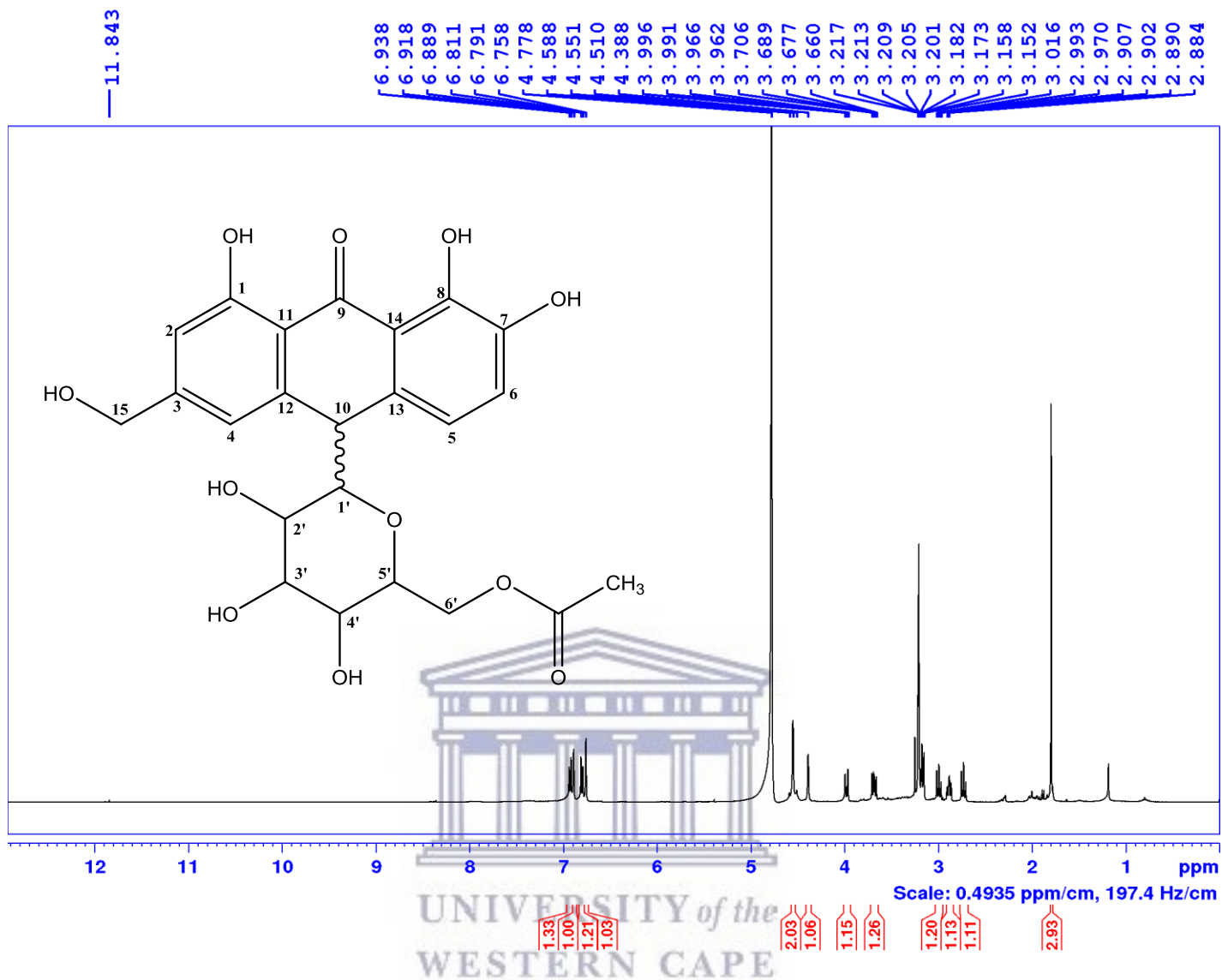


Fig. 3.5.2: ¹H-NMR of compound 5 (100 MHz; CD₃OD; δ_H at 4.78 and 3.21 are due to solvent).

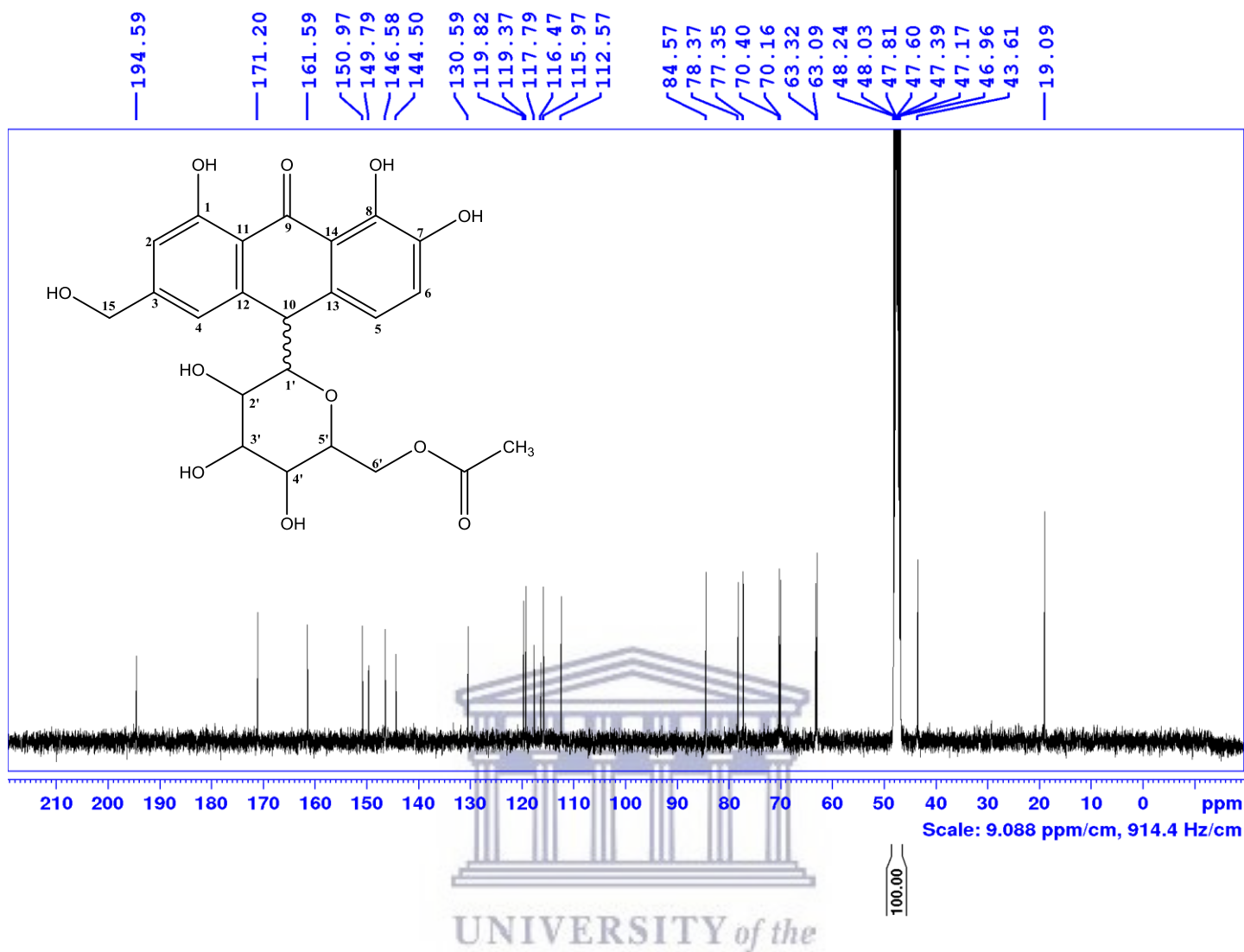
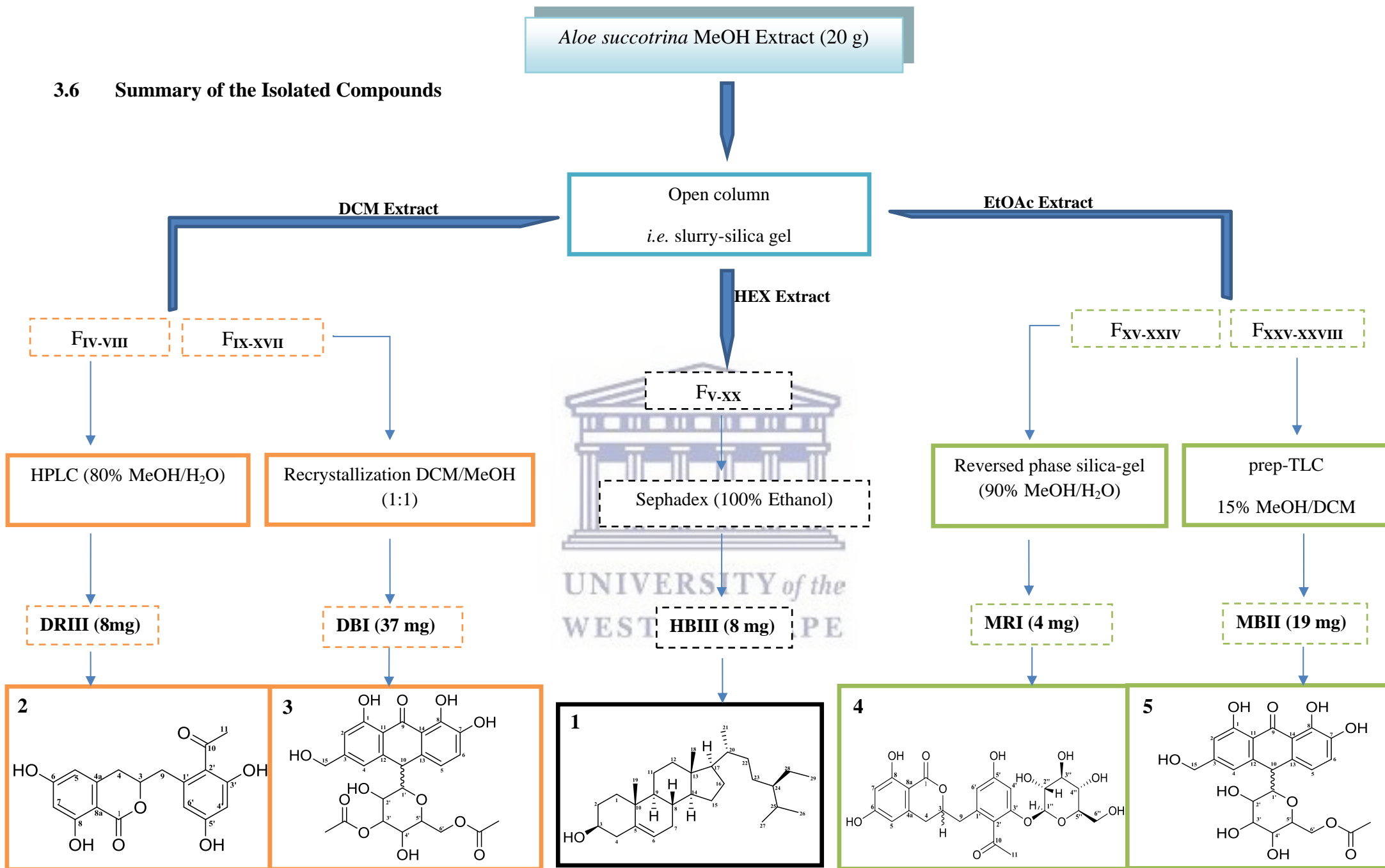


Fig. 3.5.3: ¹³C-NMR of compound 5 (100 MHz; CD₃OD; δ_C at 46.9-48.2 ppm are due to solvent).

3.6 Summary of the Isolated Compounds



Scheme 3.1: Schematic diagram for the isolation and purification of constituents from *Aloe succotrina* Lam.

CHAPTER 4

Biological Evaluation and Comparative HPLC

4.1. Biological Evaluation

In the present study, crude extracts of the leaves of *Aloe succotrina* Lam. spp. were evaluated for their potential cytotoxicity using the **brine shrimp lethality assay** and the percentage mortalities were used to determine the LC₅₀ values at each dosage level (see **Table 4.1**

4.1.1 The Brine shrimp (*Artemia salina*) lethality assay: *in vivo*

Three from the five of the evaluated (crude) extracts of *A. succotrina* Lam. were found to display (some) potent cytotoxicity activity against the brine shrimp larvae *i.e.* EtOAc, HEX as well as the aqueous extract. Interestingly, the aqueous extract, which would be the preferred method of preparing the plant extract(s) when used in traditional medicine (Clarkson *et al.*, 2004), showed moderate cytotoxicity with a corresponding LC₅₀ value at 258.91 µg/mL. By contrast, the MeOH extract, from which most lipophilic compounds that may be associated with toxicity are extractable (Clarkson *et al.*, 2004), had LC₅₀ > 1000 that can be considered as insignificant (Meyer *et al.*, 1982). However, the EtOAc extract had an LC₅₀ value at 31.13 µg/mL, which, according to Meyer *et al.* (1982) represent strong activity. Thus this may, perhaps, suggests selectivity of the potent toxic compounds against the brine shrimp larvae in EtOAc rather than MeOH/DCM or even the aqueous extract. Furthermore, Clarkson *et al.* (2004) also reports that precursors of active compounds may be present in the (crude) extracts but may be modified *in vivo* before (any) activity can be exhibited. Nevertheless, **Table 4.1** shows percentage mortalities of each extract at each dosage level.

Table 4.1: LC₅₀ values of *Aloe succotrina* Lam. screened against Brine shrimp larvae

Parameter	Percentage death after 24 hours			Value	
	10 µg/mL	100 µg/mL	1000 µg/mL	LC ₅₀	Remarks
HEX	14.59	28.76	57.50	580.25	Active
DCM	14.59	28.76	42.92	3160.12	Inactive
EtOAc	42.92	57.37	71.24	31.13	Active
MeOH	14.55	28.32	42.92	3227.52	Inactive
Aqueous	27.25	36.51	63.67	258.91	Active

Note. LC₅₀ values were interpreted as follows: $500 \leq LC_{50} \leq 1000$ weak activity, $100 \leq LC_{50} \leq 500$ moderate activity and $0 \leq LC_{50} \leq 100$ as strong activity (Meyer *et al.*, 1982; Clarkson *et al.*, 2004).

Although the brine shrimp method does not provide any adequate information regarding the mechanism of toxic action, it is a suitable probe for the assessment of the toxic potential of various plants extracts (Naidu *et al.*, 2014). Therefore, acute toxicity evaluation of the active extracts may be required to give conclusive results following this preliminary test.

4.2 Comparative HPLC

Following HPLC profiling of the crude MeOH extract of the leaves of *Aloe succotrina* Lam., as described by Sigler and co-worker (1994), a similar pattern as reported by these results was observed (see Fig. 4.1). Subsequent comparison of the chromatograms *i.e.* for the endemic population (investigated in this thesis) and the botanical gardens population (Sigler & Rauwald, 1994) was done and this allowed us to tentatively assign the peaks as shown. Similarly, **7-hydroxyaloin** type predominated and could be found between the 0-32 minute's retention time intervals. According to Sigler and co-worker (1994), the β - diastereomer(s) concentration is always higher than that of the corresponding α -diastereomer. Evidently, compounds **2**, **4** and **6** corresponding to **7-hydroxyaloin B**, **7-hydroxyaloin-6'-O-**

monoacetate B and **7-hydroxyaloin-4',6'-O-diacetate B**, respectively, showed a strong UV-Vis absorbance, especially **6**. Thus the implication of this is that β -diastereomers are formed *de novo* through biosynthetic pathways while α - diastereomers originate from the isomerization thereof (Franz & Grün, 1983). Furthermore, qualitatively, a direct injection of the MOH crude extract of the leaves of *A. succotrina* **Lam.** that was investigated herein didn't show any significant differences, except, perhaps, for the peaks showing UV-Vis absorption between 56-58 minutes. These are yet to be identified.

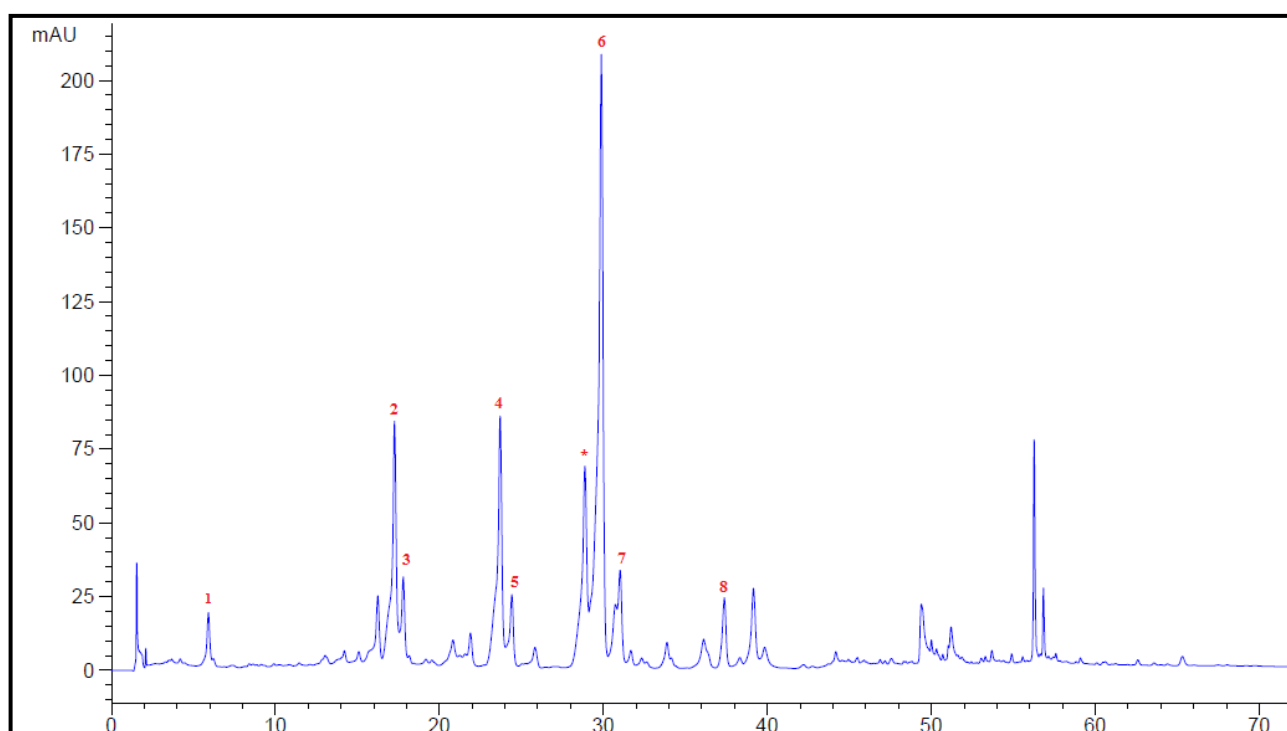


Fig. 4.1: HPL chromatogram of the whole leaf MeOH extract of *Aloe succotrina* **Lam.** (detection at 290 nm). For chromatographic protocol, see experimental section (Chapter 2). **Note.** Peak identification: **(1)** aloeresin B, **(2)** 7-hydroxyaloin B, **(3)** 7-hydroxyaloin A, **(4)** 7-hydroxyaloin-6'-O-monoacetate B, **(5)** 7-hydroxyaloin-6'-O-monoacetate A, **(6)** 7-hydroxyaloin-4',6'-O-diacetate B, **(7)** 7-hydroxyaloin-4',6'-O-diacetate A, **(8)** 7-hydroxyaloeemodine, (*) unknown substance with 7-hydroxyaloin type UV-VIS spectrum (Sigler & Rauwald, 1994), see Chapter 2 for HPLC method.

CHAPTER 5

Conclusion and Future Prospects

5.1 Conclusion

The phytochemistry of the crude extracts of the leaves from *Aloe succotrina* Lam. spp. was successfully studied. Column-column chromatography of the plant extract(s) afforded the isolation of five known compounds which were characterized successfully by means of spectroscopy (1D and 2D NMR) as; 7-hydroxyaloin-6'-O-monoacetate (**5**), 7-hydroxyaloin-3,6'-O-diacetate (**3**), feralolide (**2**) and its glucoside derivative (**4**), as well as β -sitosterol (**1**). Compound **1** was isolated from a fraction of the crude HEX extract, while **2** and **3** were isolated from DCM. The crude EtOAc extract yielded **4** and **5**. Notably, **5** and **3** were found to be consistent with the literature data as previously reported for the botanical gardens population (Rauwald & Sigler, 1994). On the other hand, **1**, **2** and **4** are reported (herein) for the first time from the species. Furthermore, the glycoside **4** and its free aglycone derivative **2** have so far been characterized in a few species within the genus *Aloe* i.e. *Aloe ferox*, *Aloe vera* and *Aloe hijazensis* (Speranza *et al.*, 1993; Choi *et al.*, 1996; Abd-Alla *et al.*, 2009) as well as *Aloe cremnophila*, *Aloe hildebrandtii* and *Aloe jacksonii* (Vietch *et al.*, 1994; Brandham *et al.*, 1994), respectively. Therefore, their identification now in *A. succotrina* (a plant spp. studied in this thesis) may provide evidence which supports the hypothesis that plants that grow in their natural habitats accumulate specific secondary metabolites that aid in their survival.

On the other hand an *in vivo* study on the cytotoxicity of the crude extract using the brine shrimp lethality assay exhibited strong toxicity, especially, for the crude EtOAc extract. The aqueous and HEX extracts showed moderate to weak activity, respectively. The implication of this is that these crude extracts could be subjected for further investigation of the potent compounds, which may be used in drug development for cancer.

The HPLC chromatogram of *A. succotrina* Lam. spp. (for the cultivated population) was previously studied by Sigler and co-worker (1994). In this thesis, a HPLC chromatogram was adopted the same way for comparative determination i.e. qualitative assessment of the crude MeOH extract of the leaves from *A. succotrina* Lam. spp. (studied in this thesis). Despite the two peaks of as yet un-identified structures which showed UV-Vis absorption between 56-58

minutes, qualitatively, the overall chromatogram allowed us to tentatively assign the peaks as shown. Thus this study presents the first evidence of the phytochemistry of *Aloe succotrina* Lam. spp. from the endemic population. Furthermore, it can also be concluded that based on the evidence of this study, climate conditions and geographical differences do play a role in the accumulation of natural products.

5.2 Future Prospects

Since the absolute stereochemistry at C-3' for compound **2** and **4**, including C-10 for **3** and **4** could not be conclusively assigned based NMR spectroscopy *alone* other studies such as circular dichroism (CD), crystallography and molecular modelling will be explored. Furthermore, a LC-MS of the whole leaf of the MeOH extract from *Aloe succotrina* (investigated in this thesis) will be considered to unambiguously assign the diagnostic peaks in the HPLC chromatogram for qualitative comparison with Sigler and Rauwald (1994) (for the cultivated population). In addition, since the HPLC showed evidence of two peaks of as yet unidentified structures, further re-evaluation of the extracts using, either, HPLC or chromatography will be conducted in order to facilitate their structural elucidation. The leaves of *Aloe succotrina* Lam. spp. are known to produce a *blue* dye, which perhaps could be associated with the purple staining that was evident from the crude EtOAc extract, the first attempt therefore, could be to investigate the structural composition that is associated with this dye and/or its biosynthetic pathway. Glycosides are generally known to extract in BuOH. These high molecular weight compounds *e.g.* anthocyanins, which are responsible for color in plants, have reputed pharmacological activity as anti-oxidants, anti-cancer, anti-inflammatory (Kong *et al.*, 2003; Cooke *et al.*, 2005; Yang and Zhai, 2010). Therefore, an initial attempt could be to target these phenolic compounds for their use in food, pharmaceutical and cosmetic industry.

Furthermore, *in vivo* cytotoxicity assessment of the crude extracts using the brine shrimp lethality assay was investigated, from which the crude EtOAc extract was found to be most active. In light of the *inherent* errors that may be associated with this assay, future work could involve subjecting/testing the extracts including the isolated compounds against tumour cell lines in order to give a more comprehensive conclusion; and to possibly identify new leads for anti-cancer chemotherapy.

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APPENDIX 1

TLC and NMR Spectra of the Isolated Compounds



Plate 1A: TLC profile of **compound 1** (HBIII = β -sitosterol) after spraying with vanillin (prepared by dissolving 15g of vanillin in 250ml ethanol followed by the addition of 2.5ml concentrated sulphuric acid). Solvent system: **Hex/EtOAc** (8:2) ($R_f = 0.4$).

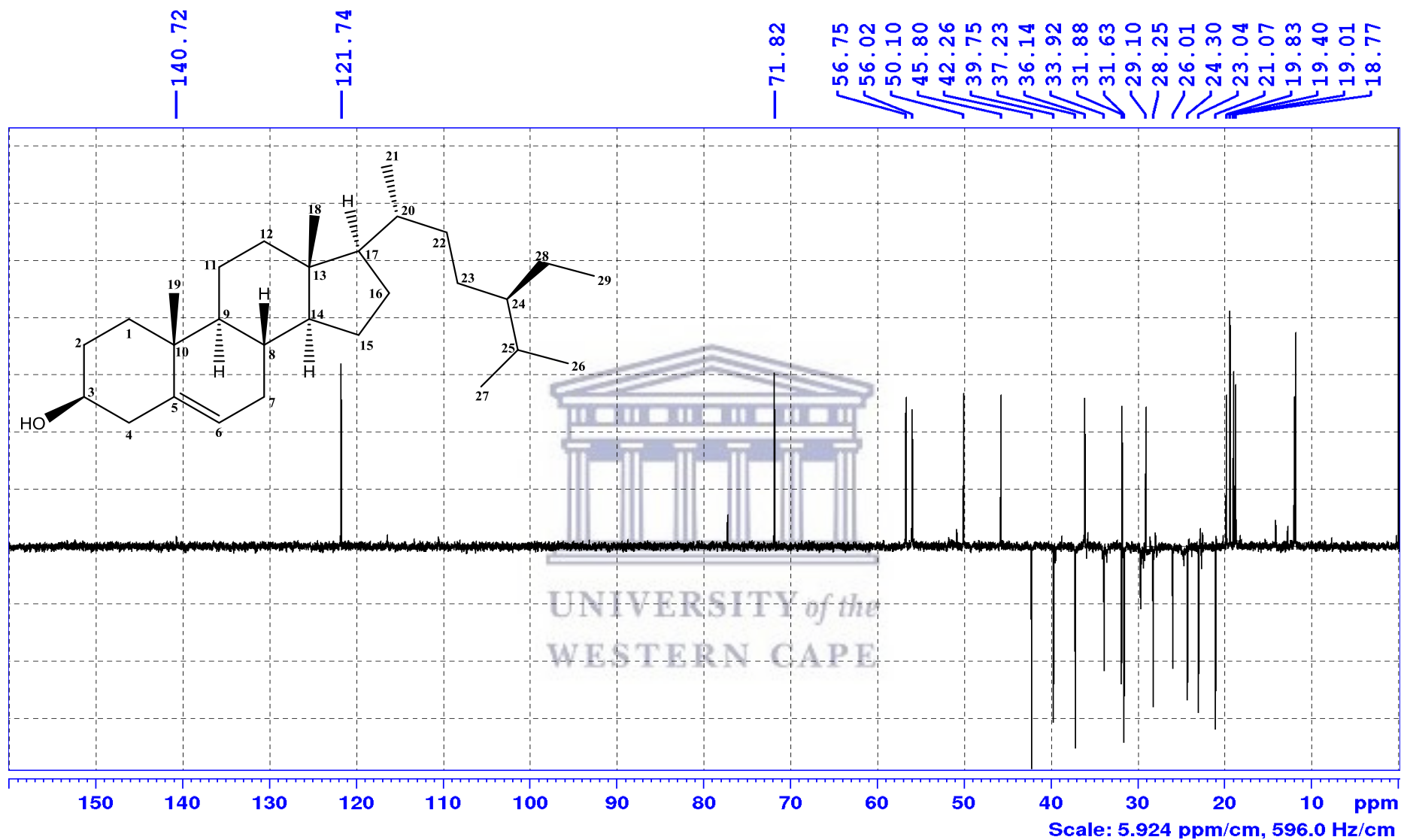


Plate 1B: DEPT-135 spectrum for **compound 1**.

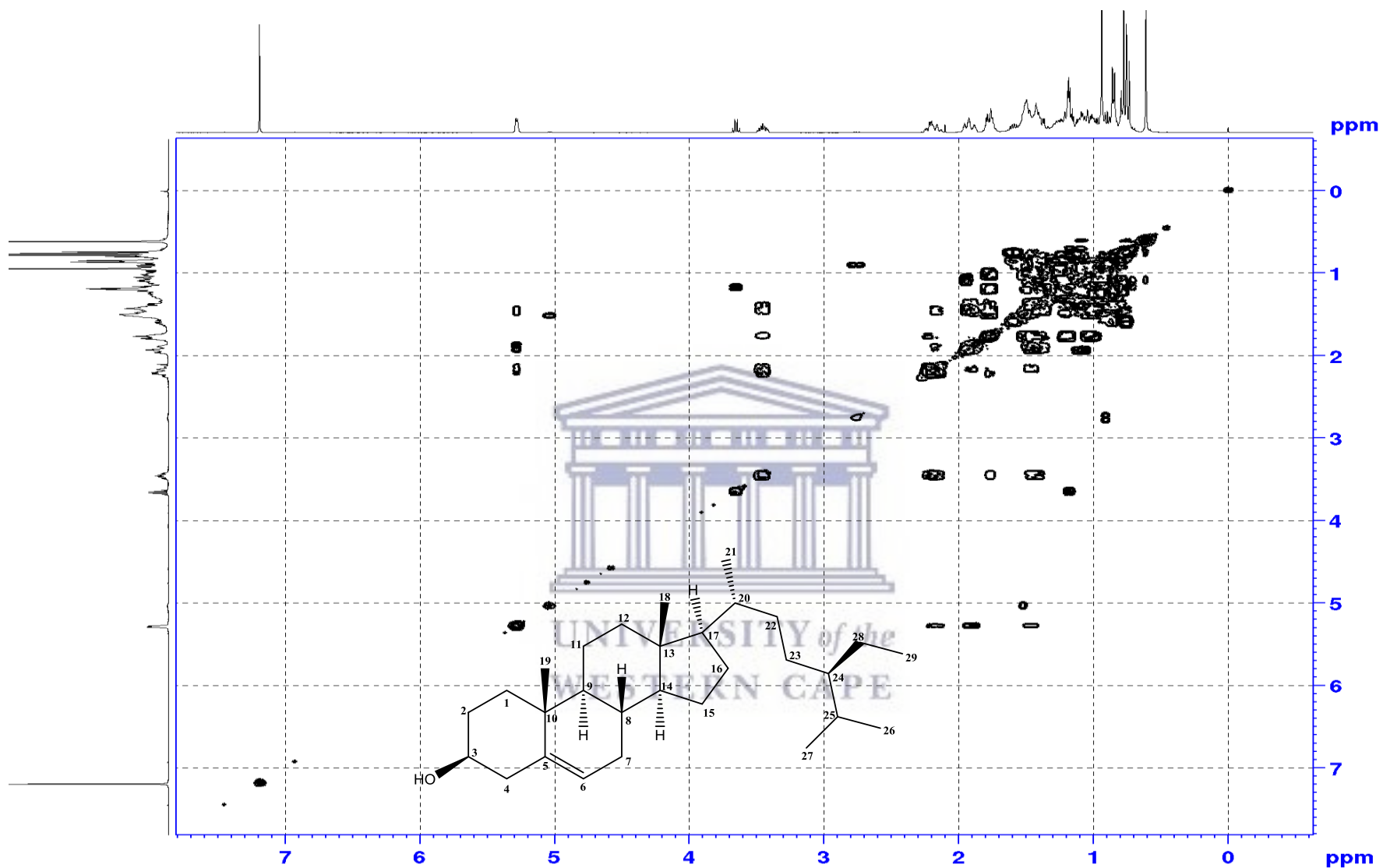


Plate 1C: COSY spectrum for **compound 1**.

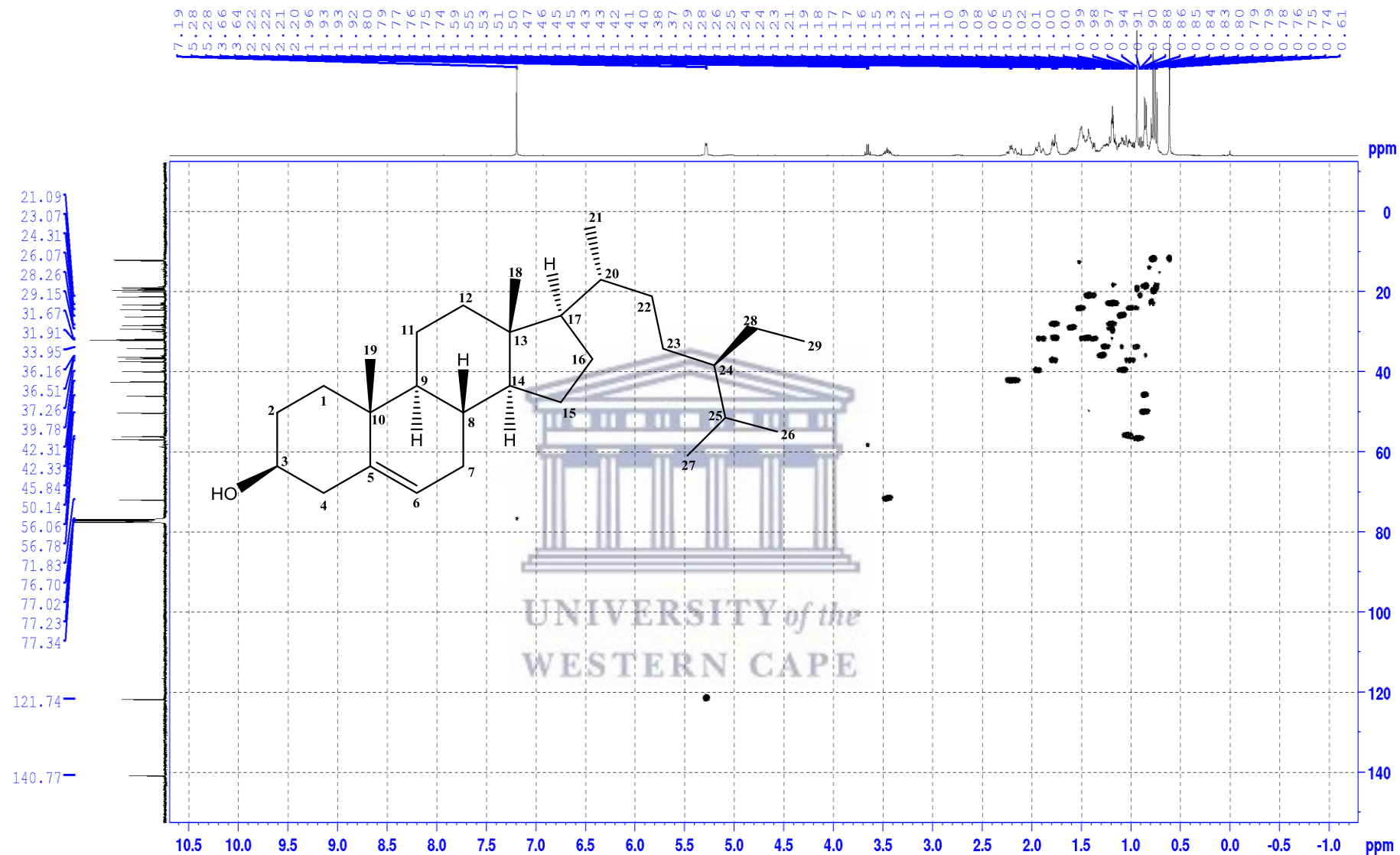


Plate 1D: HSQC spectrum for compound 1.

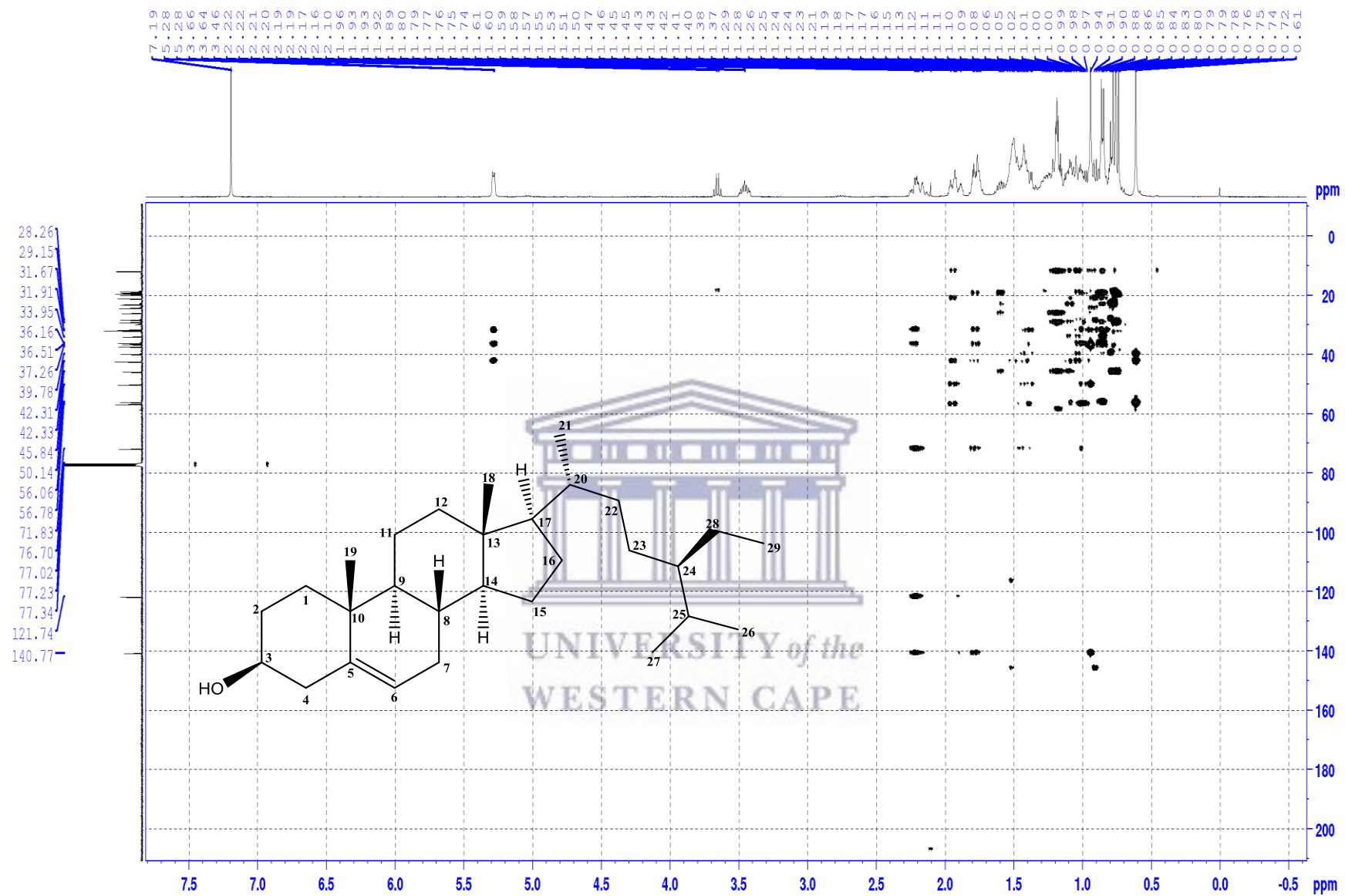


Plate 1E: HMBC spectrum for compound 1.

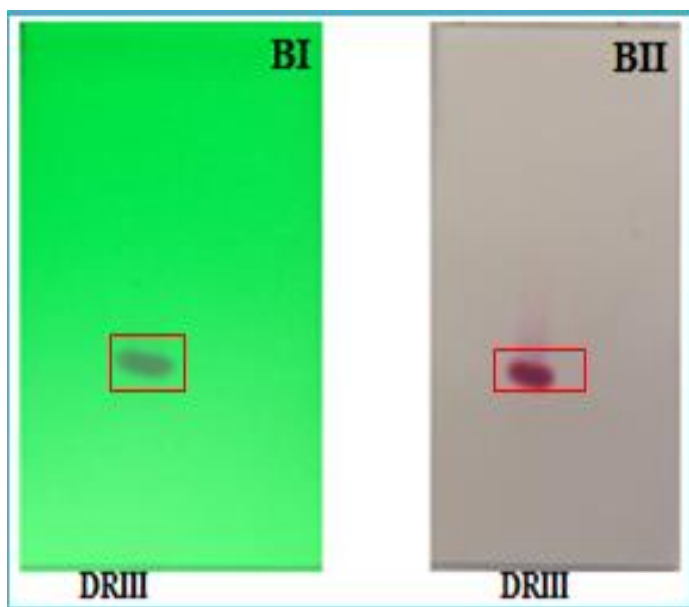
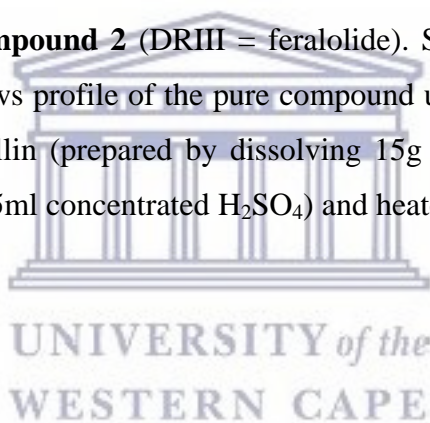


Plate 1F: TLC profile of **compound 2** (DRIII = feralolide). Solvent system: DCM/MeOH (9:1) ($R_f = 0.3$). Plate **BI** shows profile of the pure compound under UV lamp (254 nm) and **BII**, after spraying with vanillin (prepared by dissolving 15g of vanillin in 250ml ethanol followed by the addition of 2.5ml concentrated H_2SO_4) and heated at 110 °C.



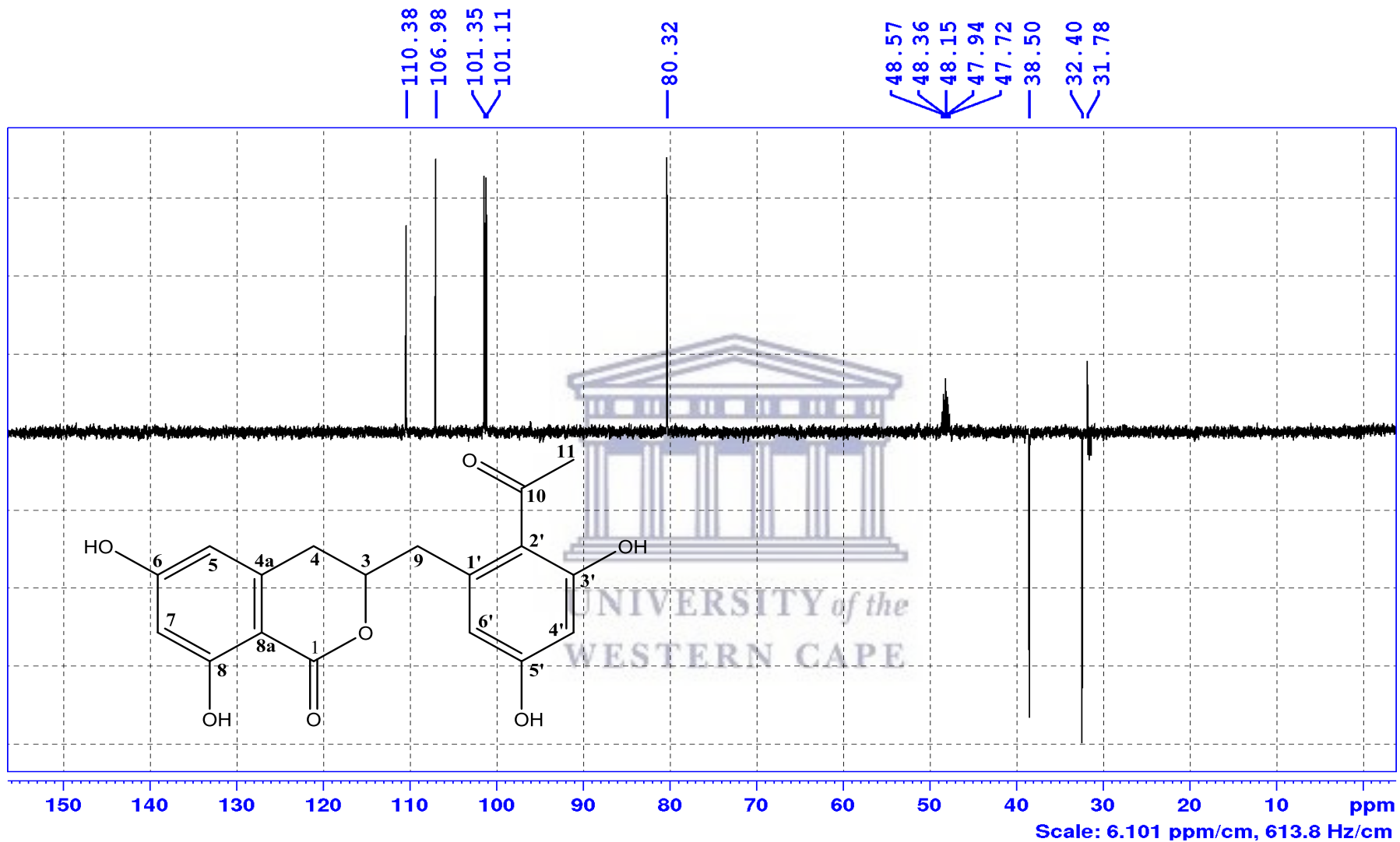


Plate 1G: DEPT-135 spectrum for compound 2.

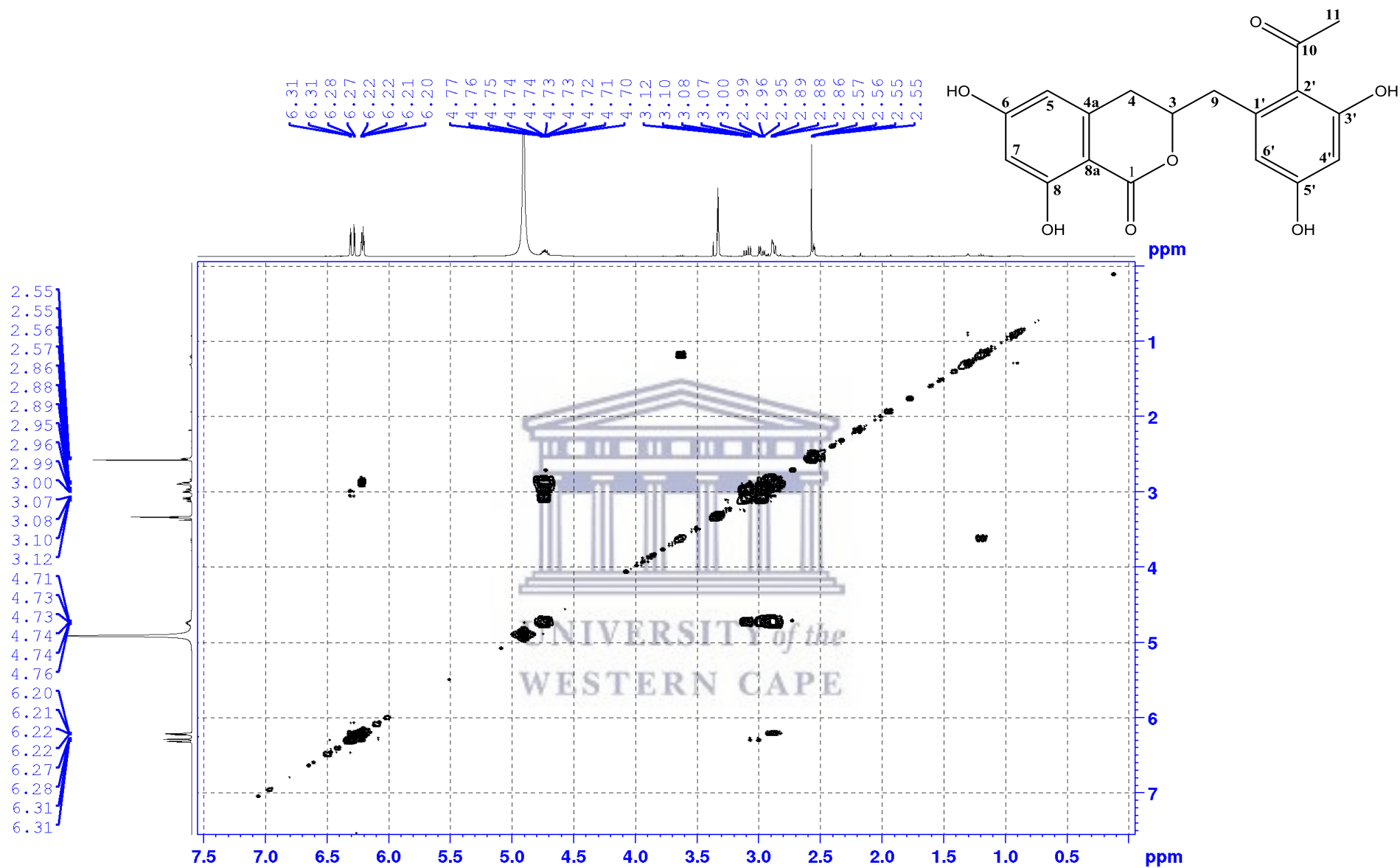


Plate 1H: COSY spectrum for compound 2.

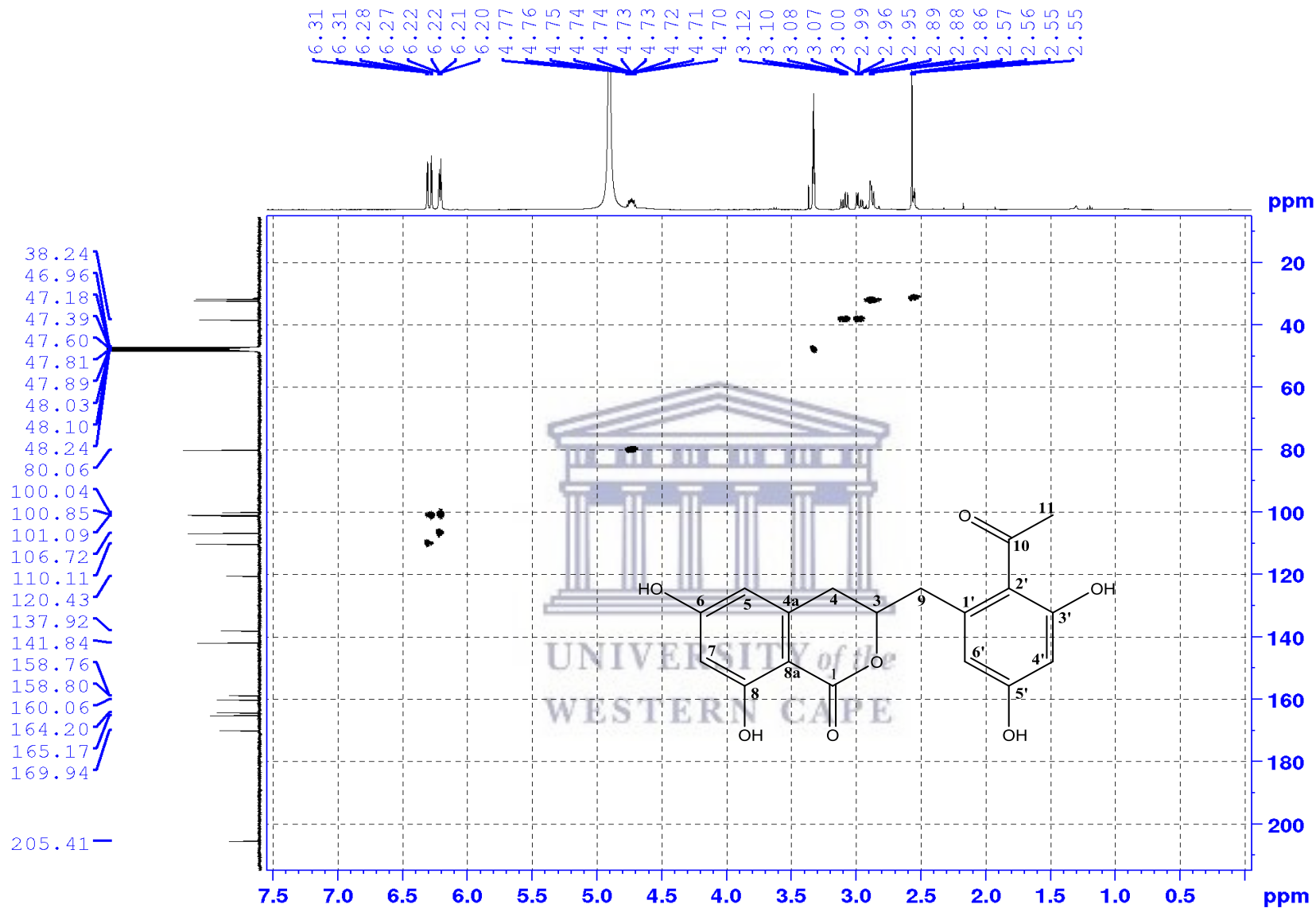


Plate 1I: HSQC spectrum for compound 2.

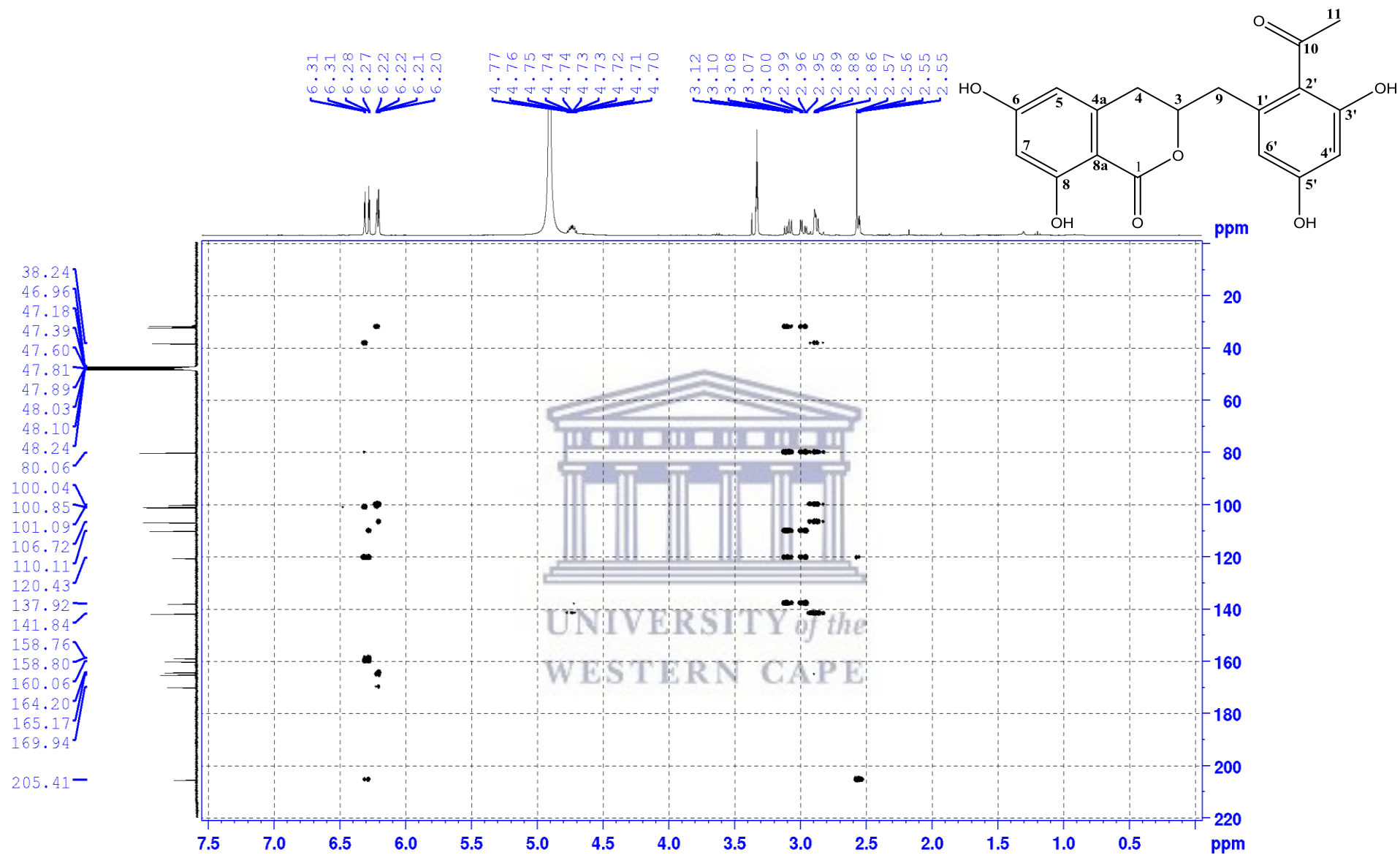


Plate 1J: HMBC spectrum for compound 2.

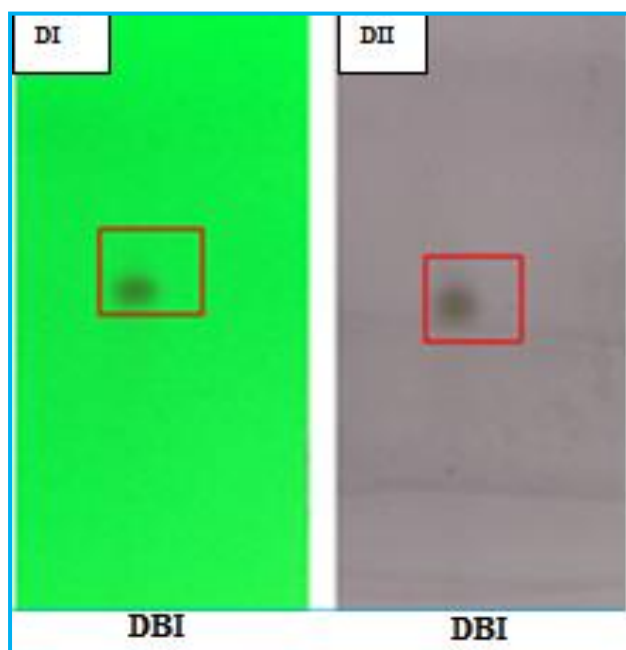
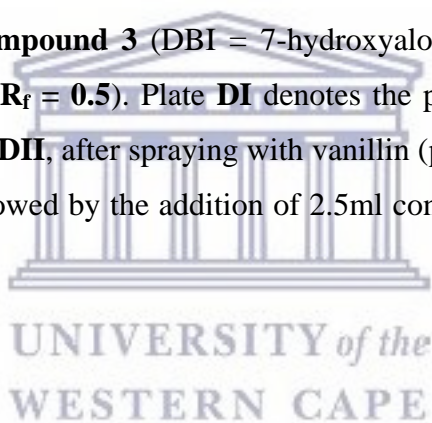


Plate 1K: TLC profile of **compound 3** (DBI = 7-hydroxyaloin-4',6'-O-diacetate), solvent system: EtOAc/MeOH (9:1) ($R_f = 0.5$). Plate **DI** denotes the profile of the pure compound under UV lamp (254 nm) and **DII**, after spraying with vanillin (prepared by dissolving 15g of vanillin in 250ml ethanol followed by the addition of 2.5ml concentrated H_2SO_4) and heated at 110 °C.



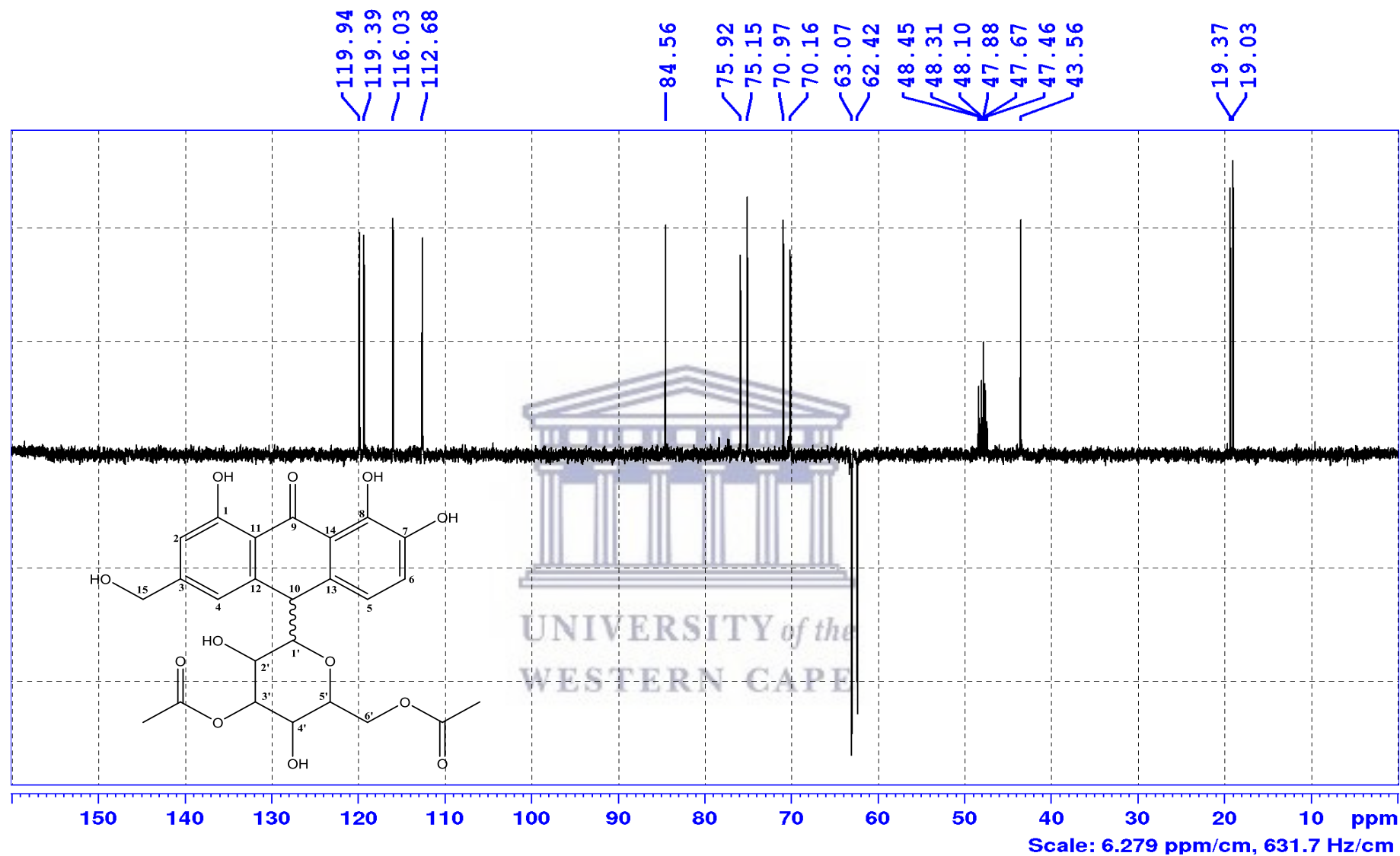


Plate 1L: DEPFT-135 spectrum for compound 3.

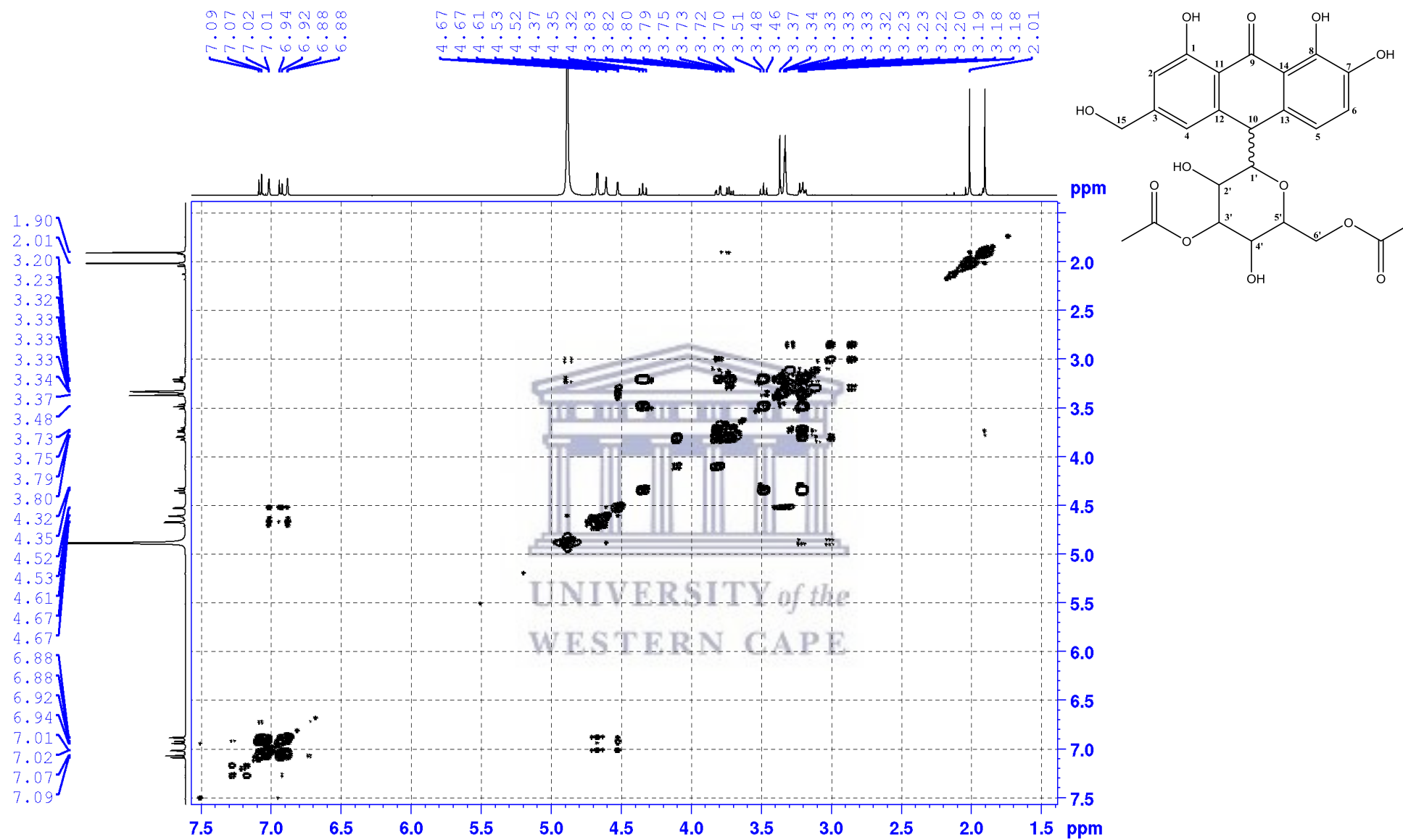


Plate 1M: COSY spectrum for compound 3.

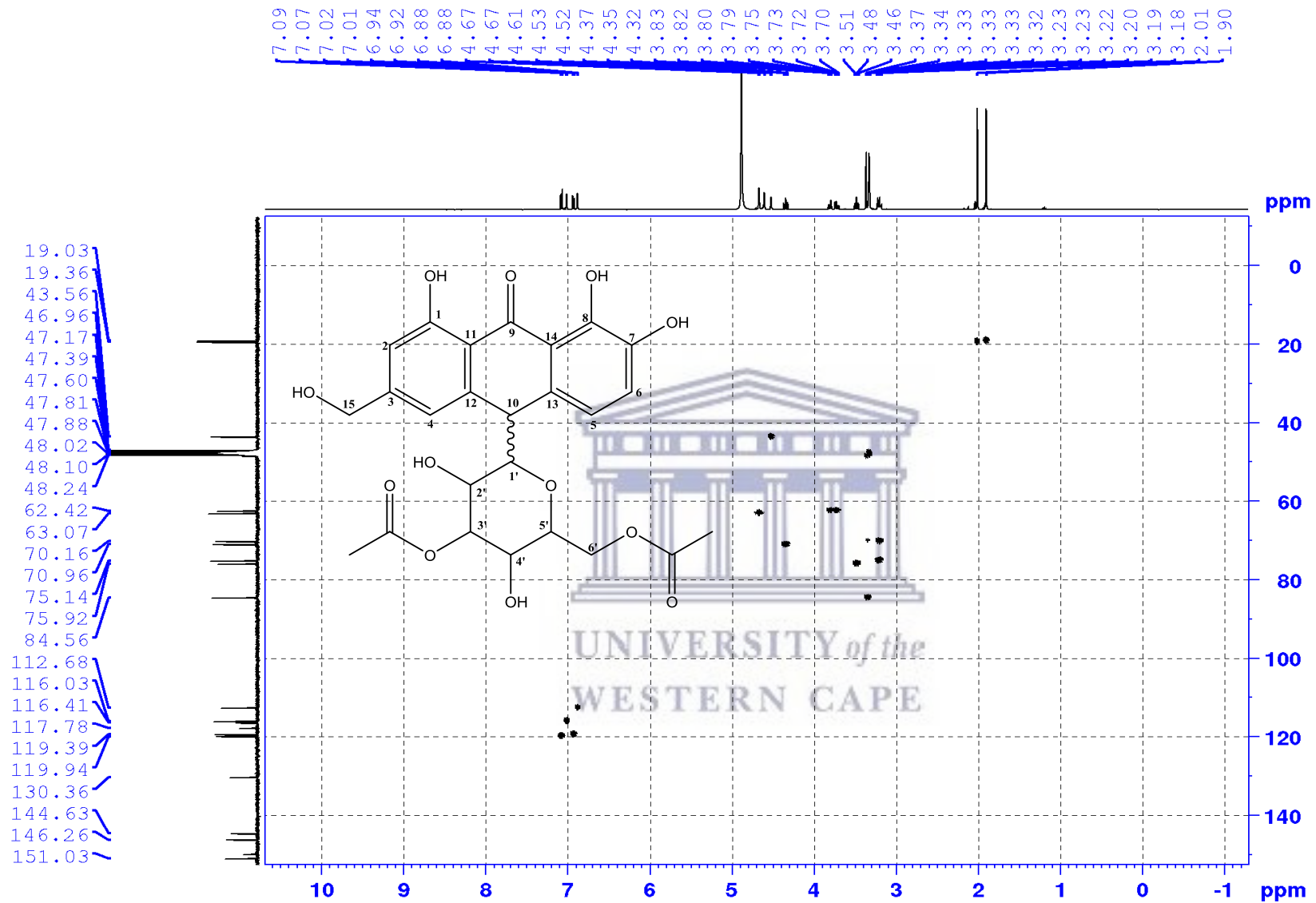


Plate 1N: HSQC for compound 3.

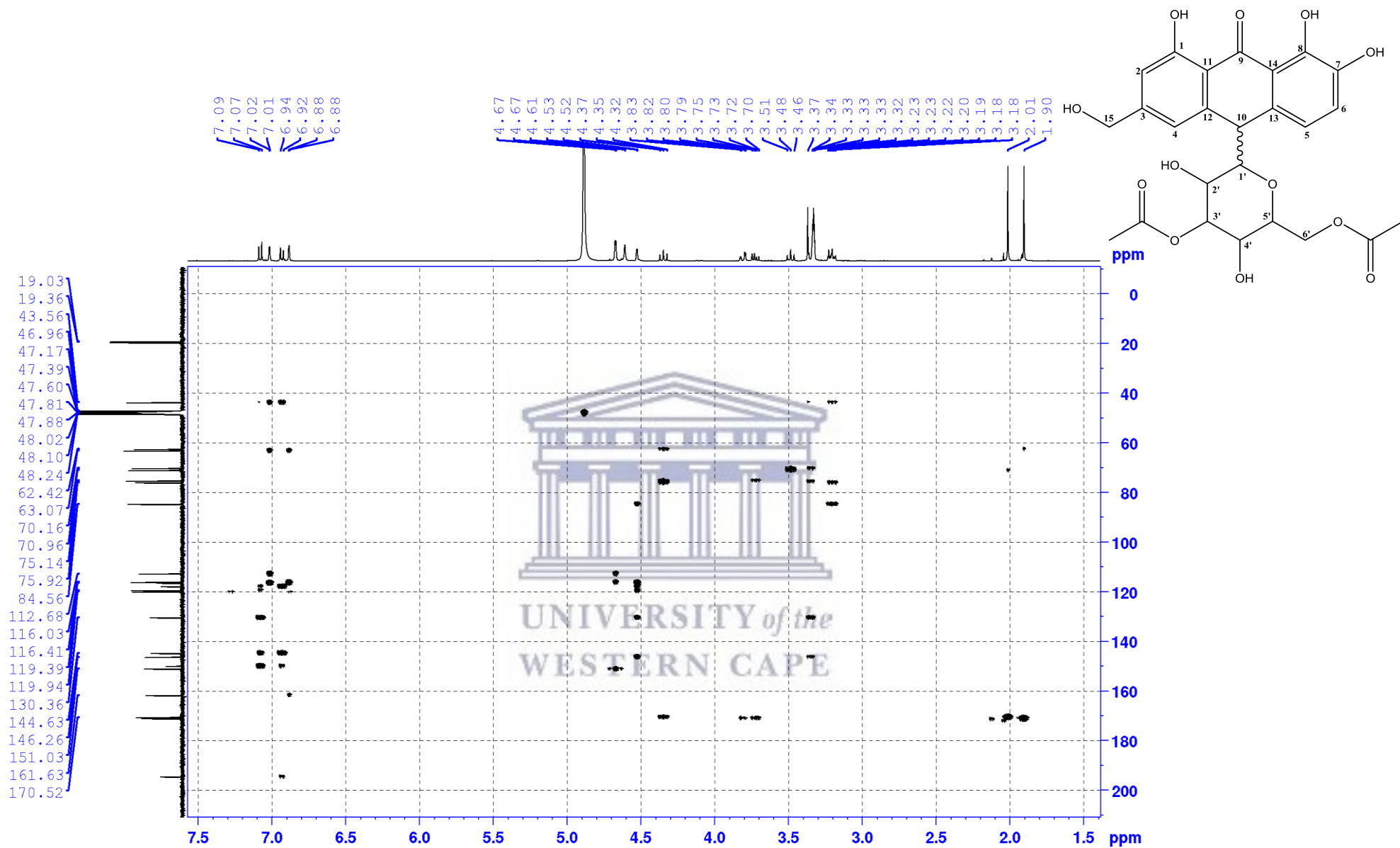


Plate 10: HMBC spectrum for compound 3.

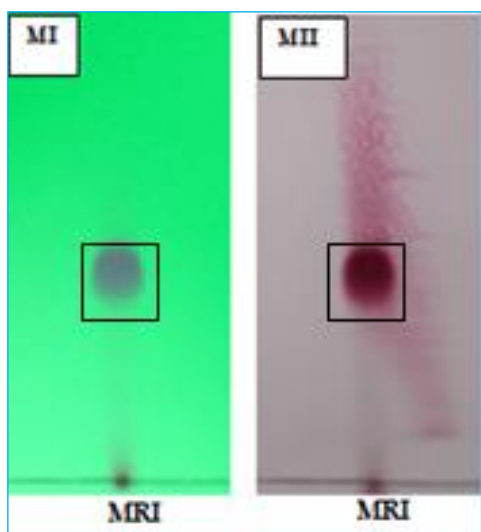


Plate 1P: TLC profile of **compound 4** (MRI = feralolide glycoside). Solvent system: CHCl₃/EtOH/H₂O (7:3:1) (**R_f** = **0.5**). Plate **MI** denotes the profile for the target compound under UV lamp (254 nm) and **MII**, after spraying with vanillin (prepared by dissolving 15g of vanillin in 250ml ethanol followed by the addition of 2.5ml concentrated sulphuric acid).



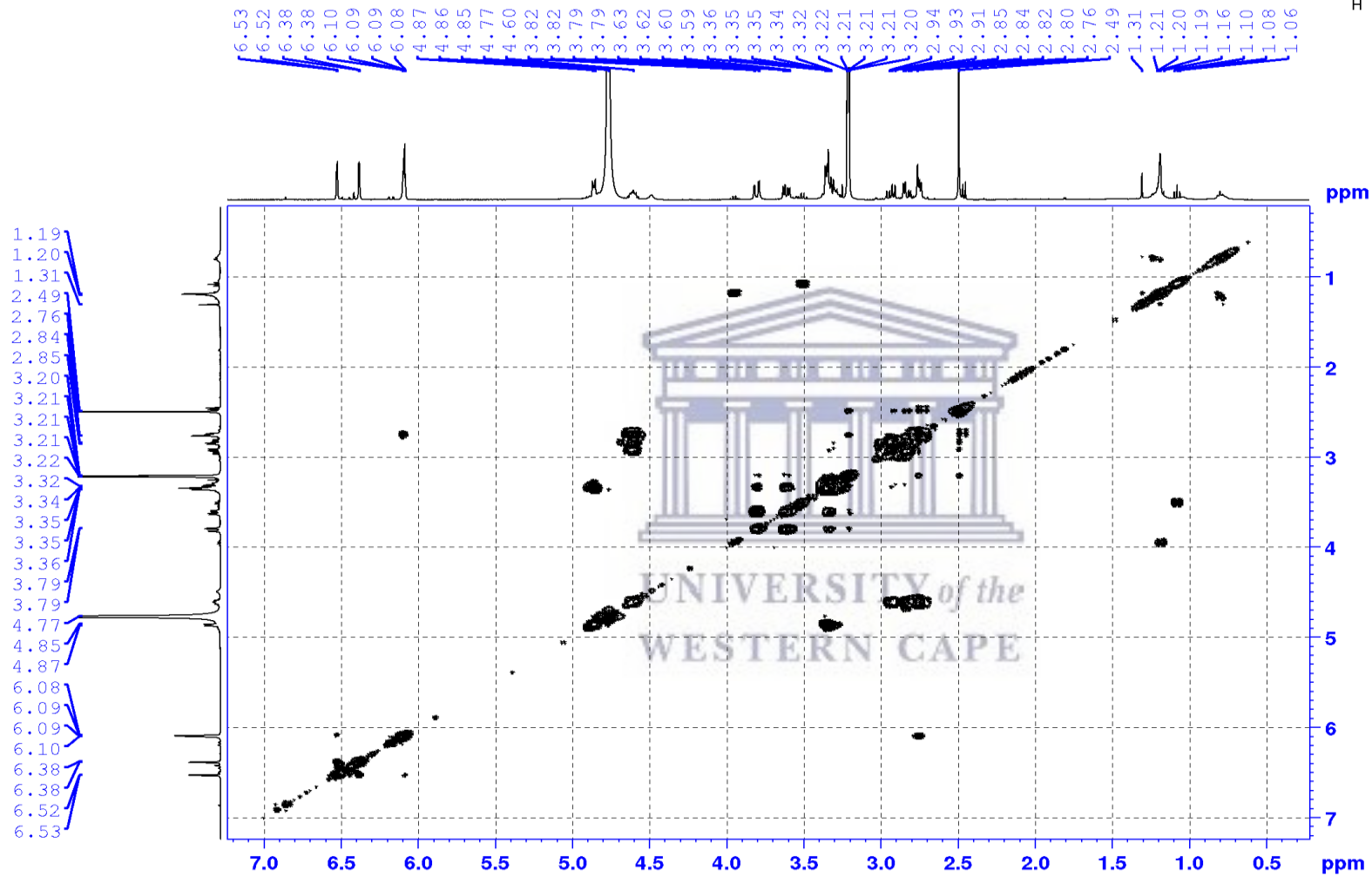
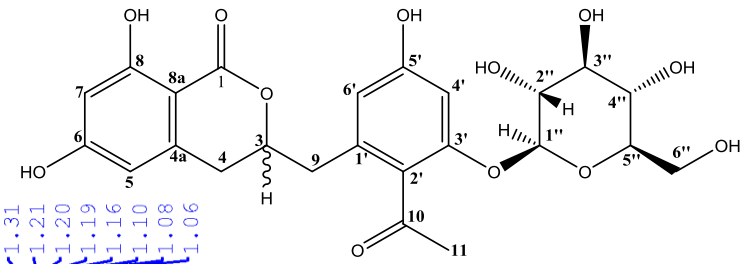


Plate 1Q: COSY spectrum for compound 4.

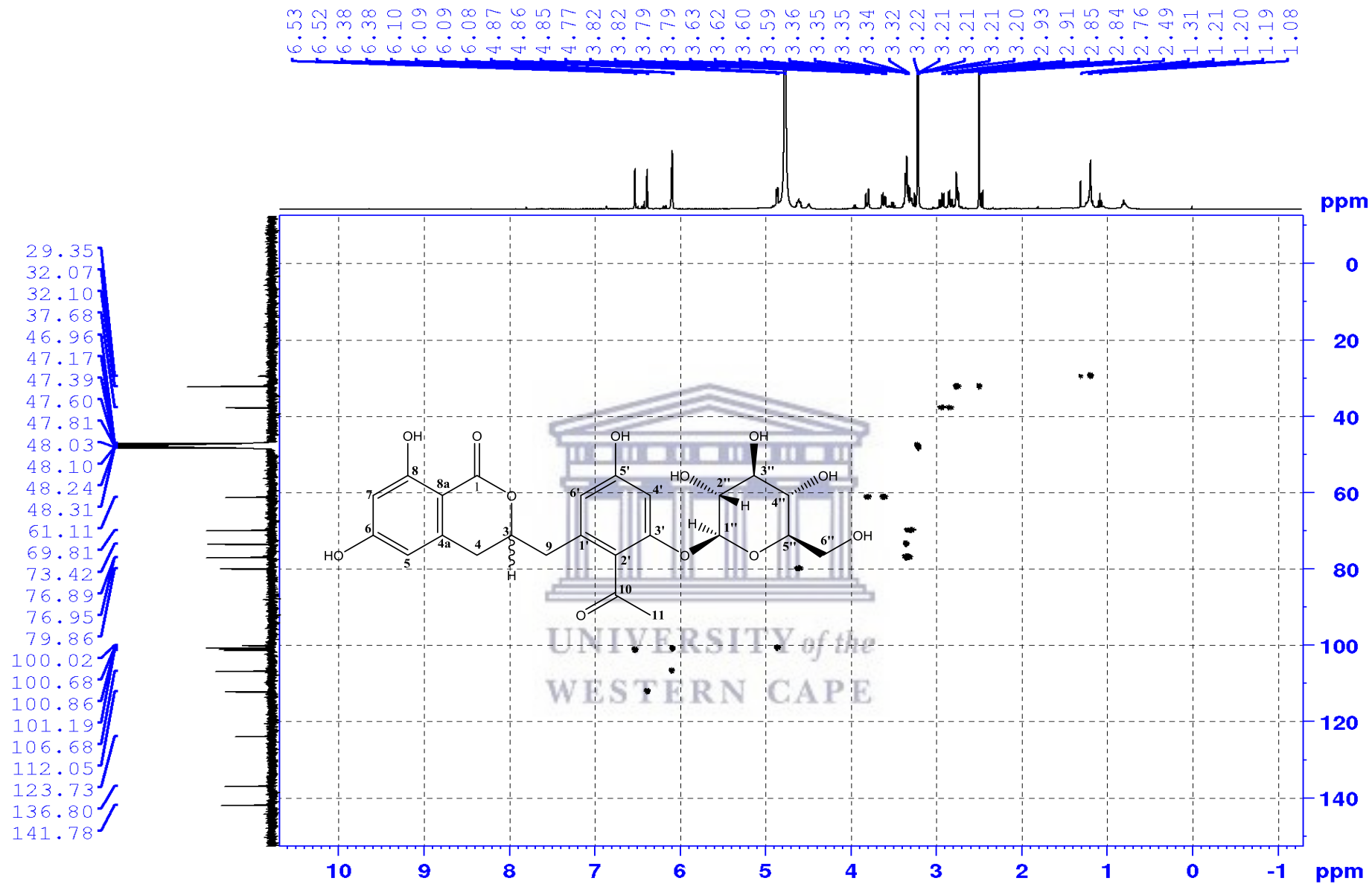


Plate 1R: HSQC spectrum for compound 4.

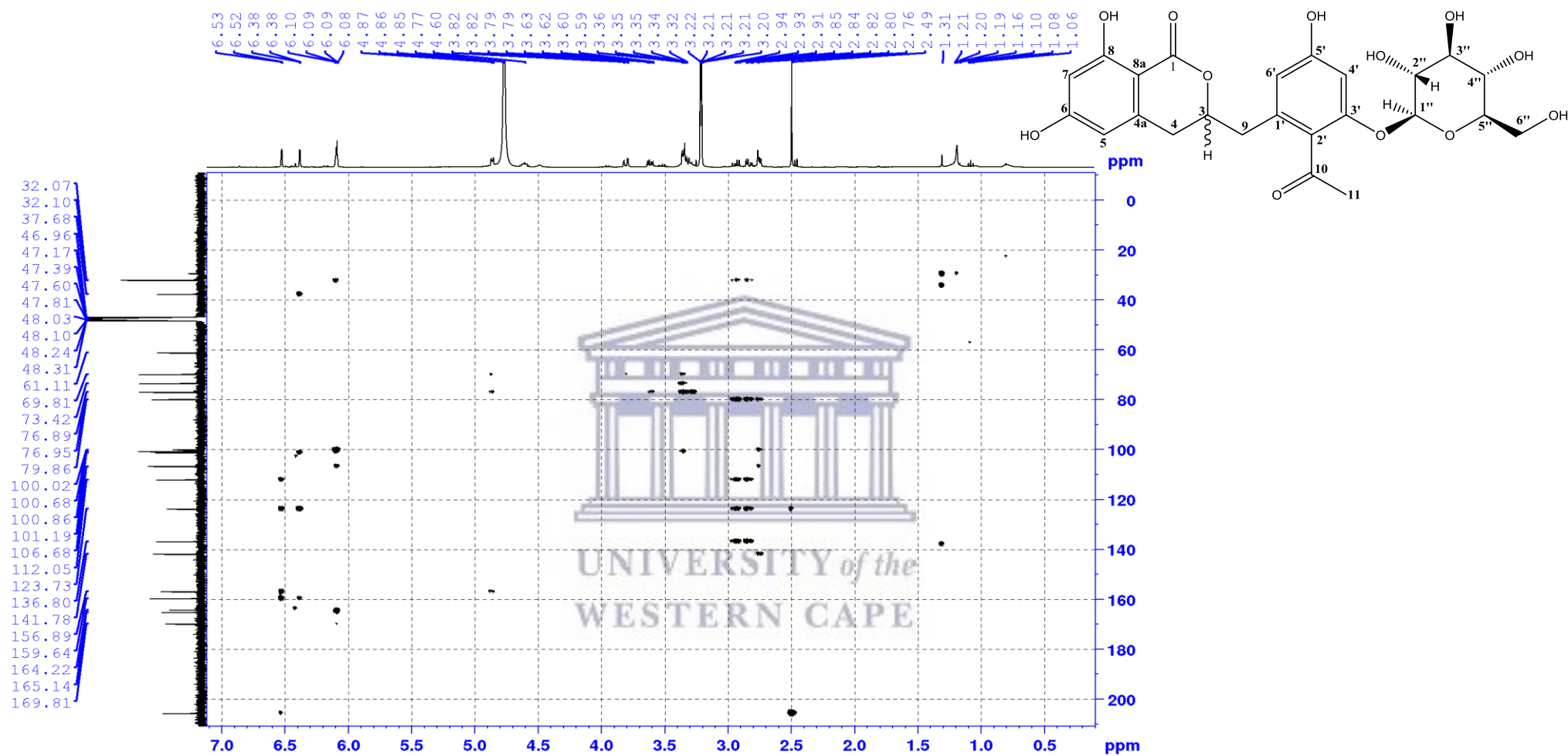


Plate 1S: HMBC spectrum for compound 4.

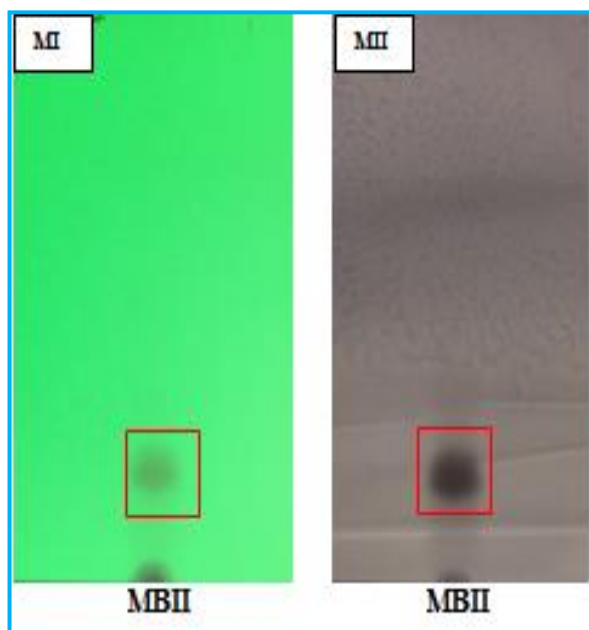
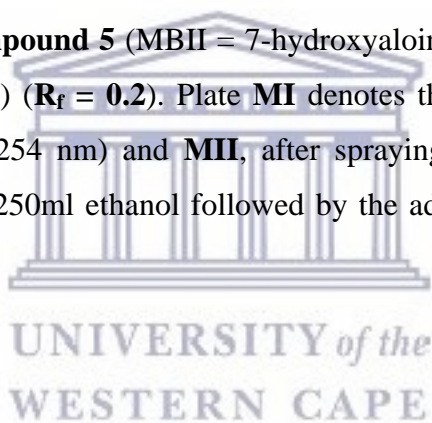


Plate 1T: TLC profile of **compound 5** (MBII = 7-hydroxyaloin-6'-O-monoacetate). Solvent system: DCM/MeOH (8.5:1.5) ($R_f = 0.2$). Plate **MI** denotes the TLC profile for the target compound under UV lamp (254 nm) and **MII**, after spraying with vanillin (prepared by dissolving 15g of vanillin in 250ml ethanol followed by the addition of 2.5ml concentrated sulphuric acid).



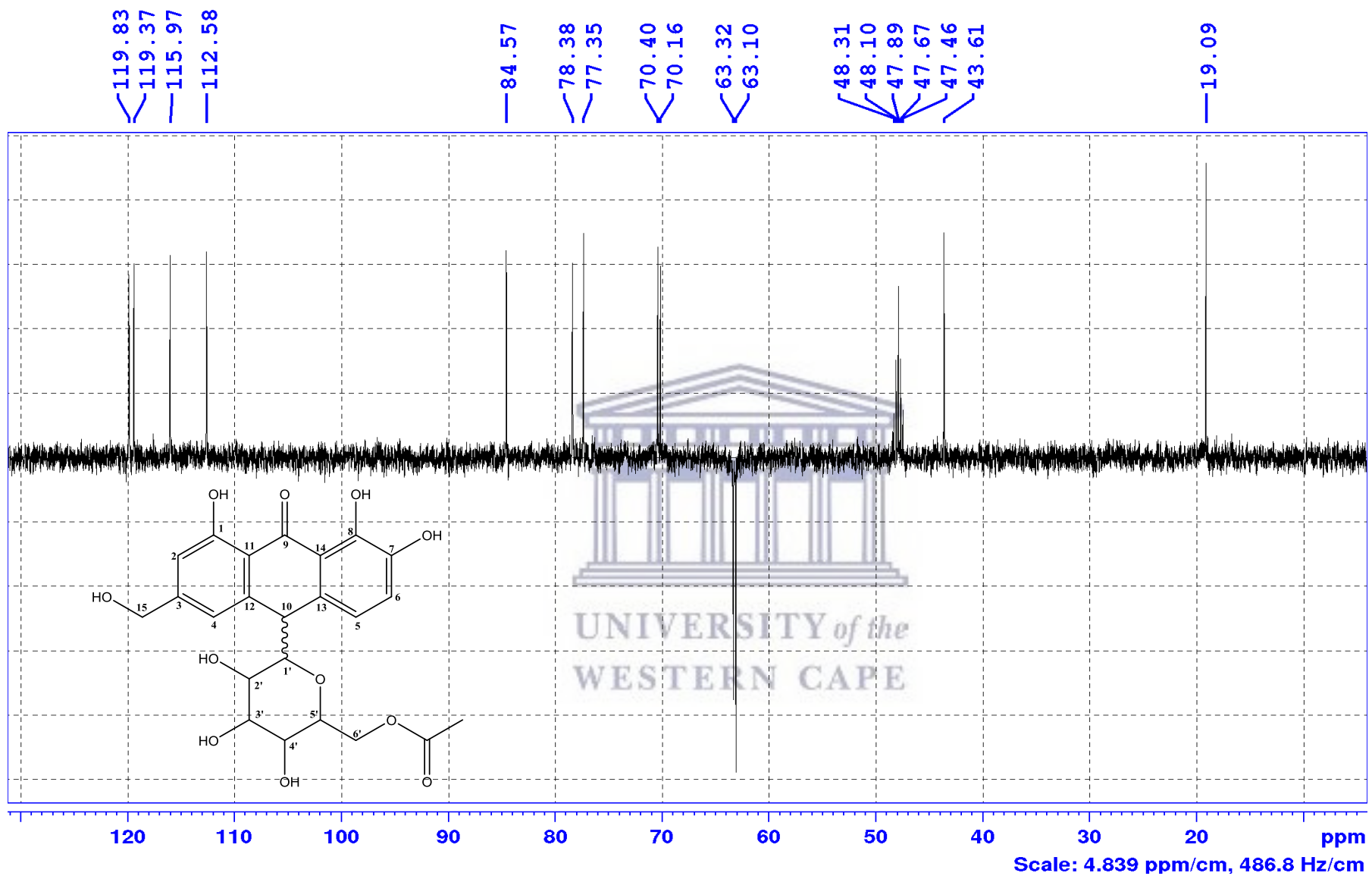


Plate 1U: DEPT-135 spectrum for **compound 5**.

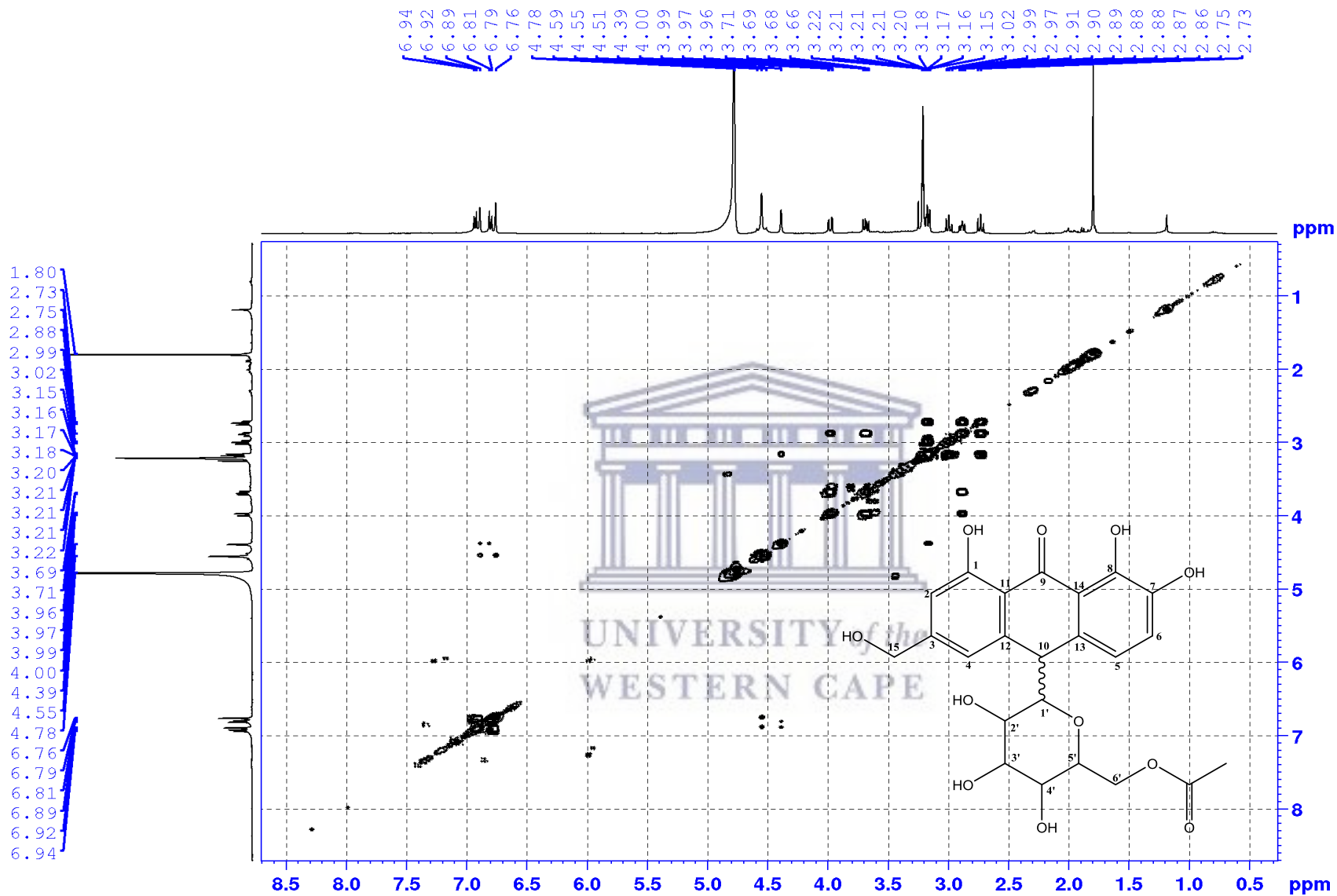


Plate 1V: COSY spectrum for compound 5.

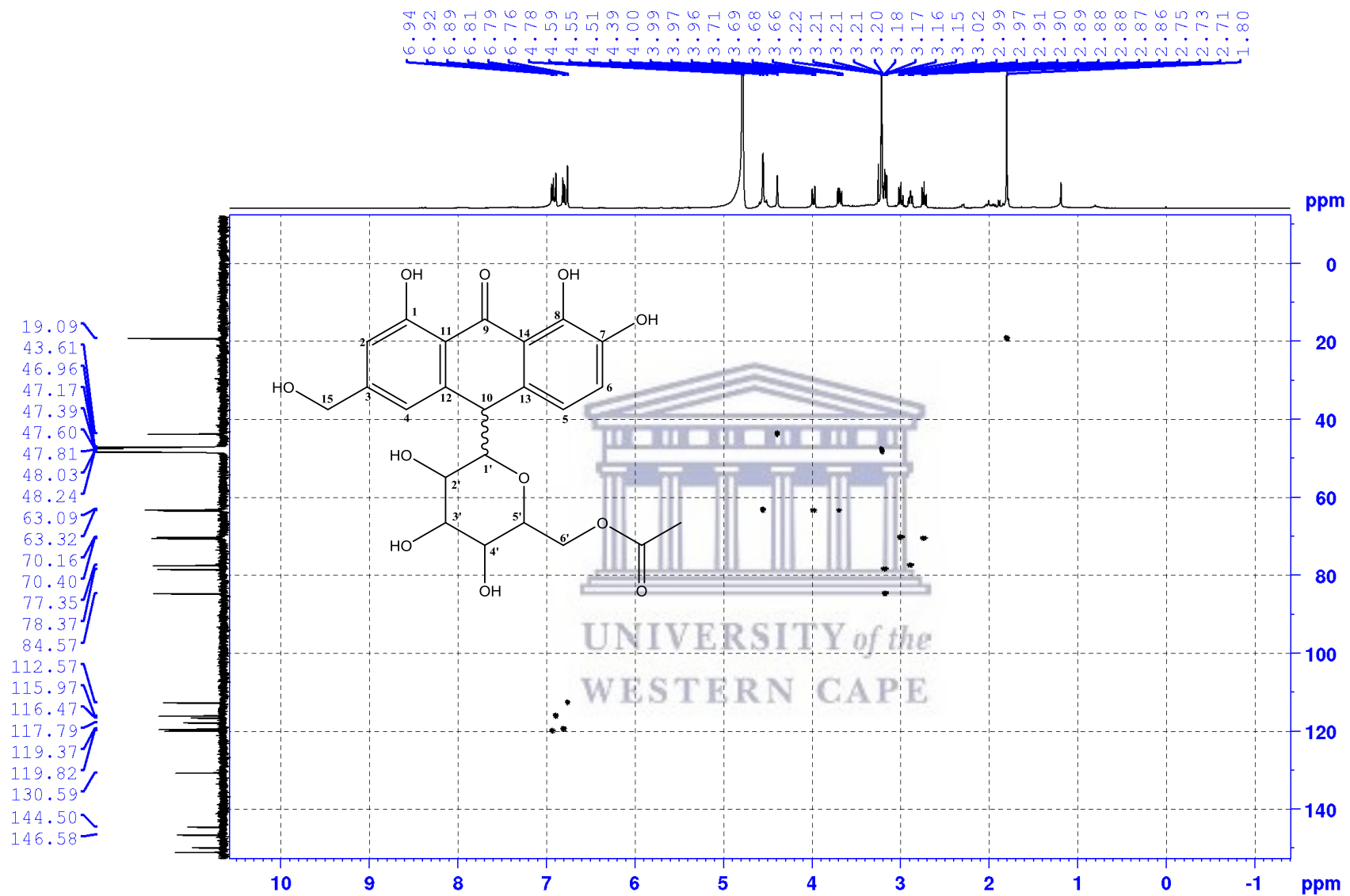


Plate 1W: HSQC spectrum for compound 5.

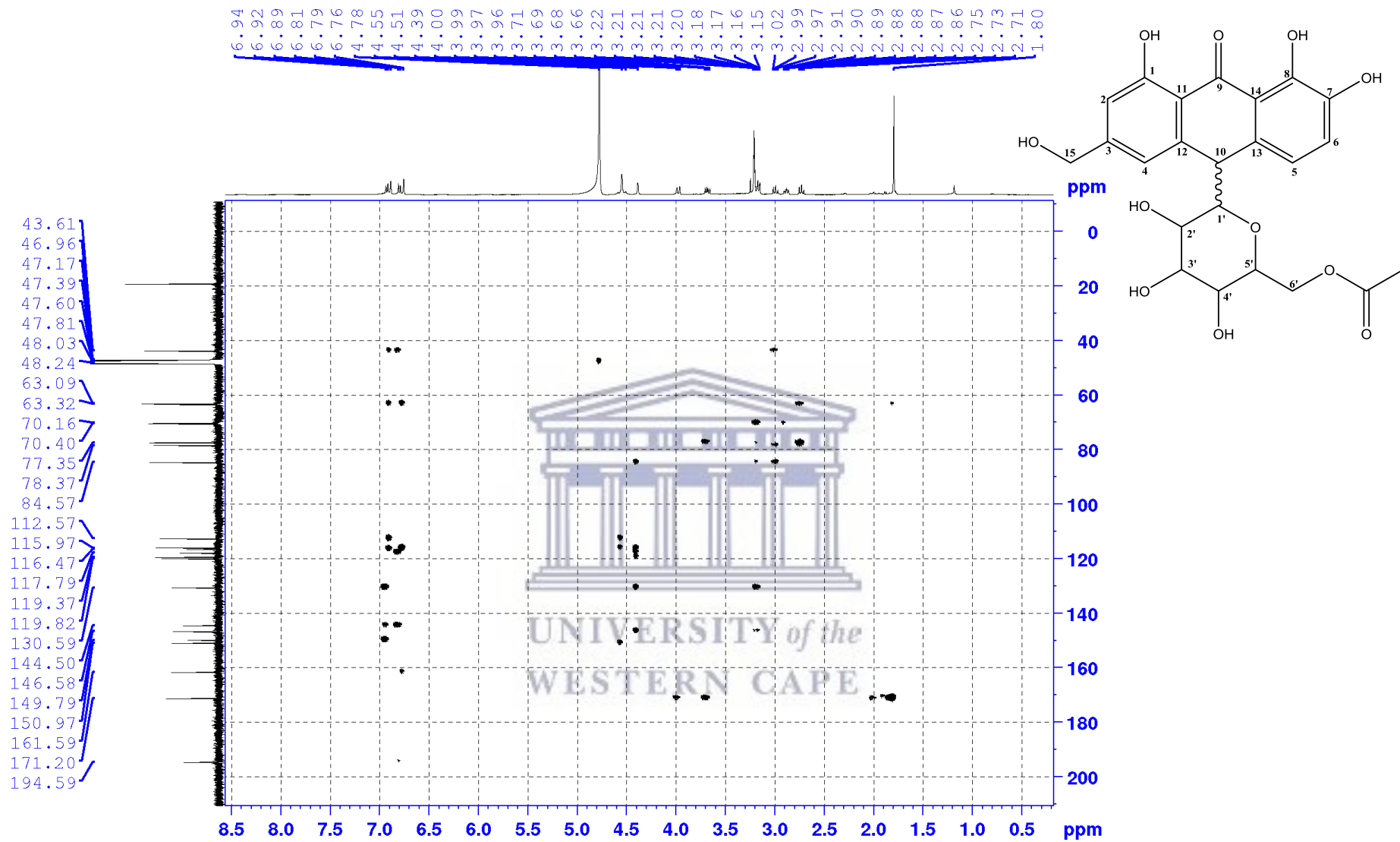


Plate 1X: HMBC spectrum for compound 5.

APPENDIX 2

LC-MS of the Isolated Compounds

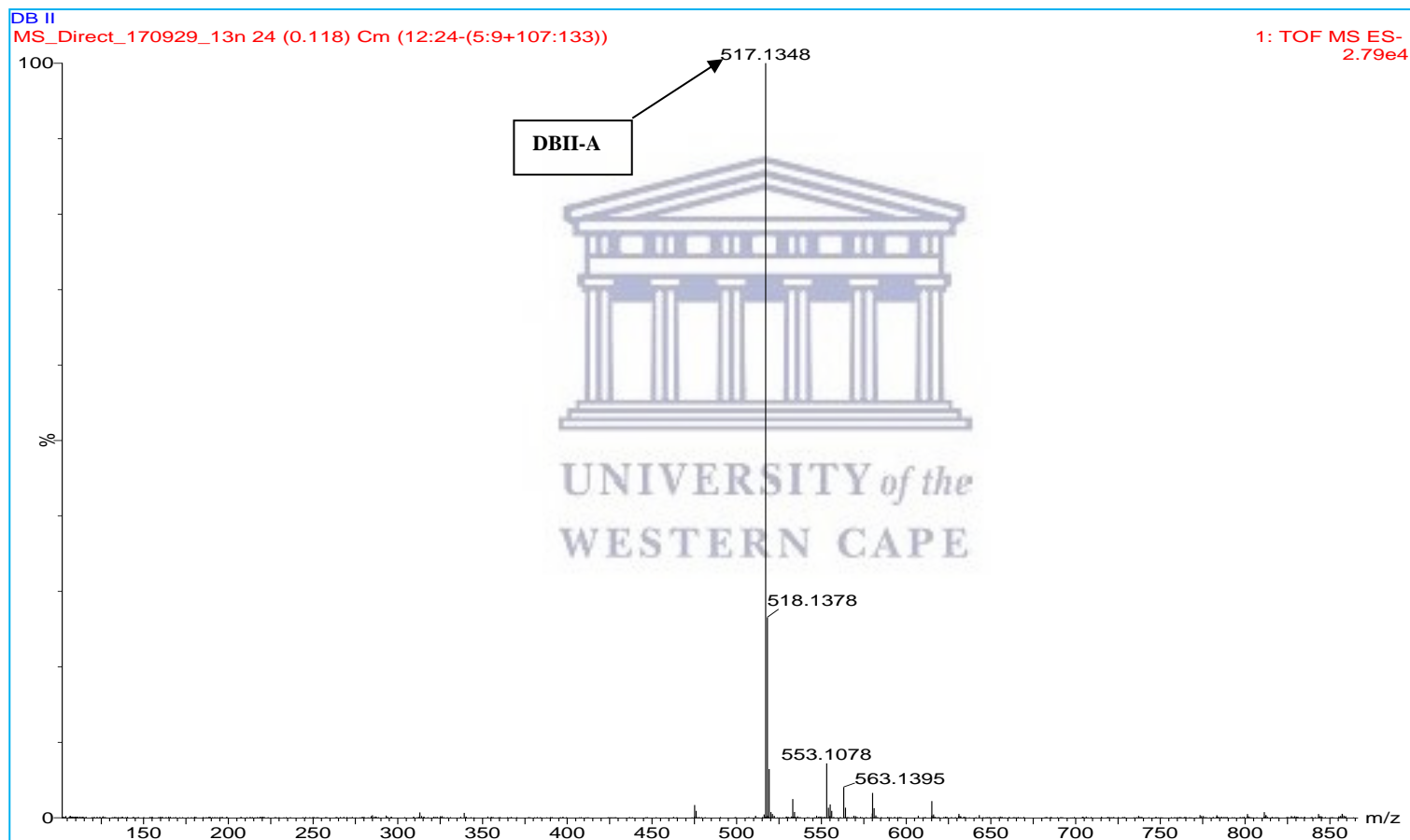


Plate 2A: LC-MS of **compound 3**, MS solvent ACN. **DBII-A** shows the molecular ion (m/z) 517.13 $[M-H]^-$.

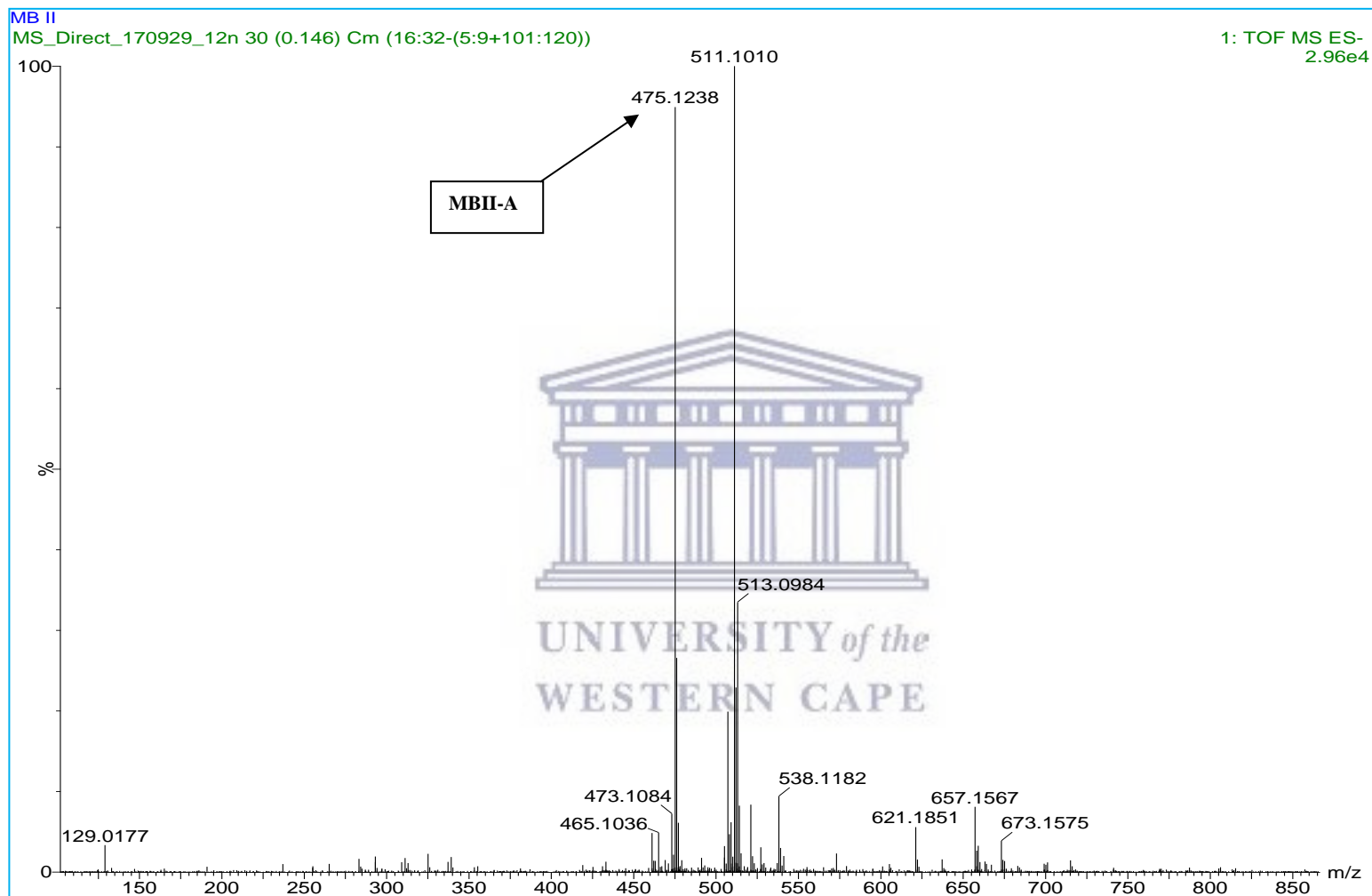


Plate 2B: LC-MS of **compound 5**, MS solvent ACN. **MBII-A** shows the molecular ion (m/z) 476.13 $[M-H]^-$.