Chemical Constituents from *Elytropappus rhinocerotis* and *Rhoicissus tridentata*: Structural and Activity Studies

By

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Submitted in the fulfilment of the academic requirements for the degree

DOCTOR OF PHILOSOPHY

In the School of Chemistry and Physics

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

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October 2015

Declaration

I hereby certify that this research is a result of my own investigation, which has not already been accepted in substance for any degree and is not being submitted in candidature for any other degree.

Signed……………..

Bongiwe Pridesworth Mshengu

I hereby certify that this statement is correct

Singed……………...

Professor Fanie R. van Heerden (**Supervisor**)

Acknowledgements

As I reach this stage in my life, my heart is filled with joy and great love for my Almighty God who has always given me the strength to carry on even when I was walking in the darkest valley. *Glory be to the Father, the Son, and the Holy Spirit*.

I express my sincere gratitude to my supervisor, Professor Fanie R. van Heerden for her supervision, guidance, and encouragement throughout my studies.

I have to thank Professor Siegfried E. Drewes, who although not my supervisor has always been open to discussion and has offered me a lot of guidance throughout my studies.

My sincere thanks also go to the following people:

Mr. C. Grimmer for his assistance with NMR experiments

Mrs. C. Janse Van Rensburg for helping with mass spectrometry

Dr. C. Southway and Ms P. Lubanyana for assisting with HPLC

Mr. F. Shaik, B. Dlamini, and S. Ball for technical assistance

My special thanks are due to my fellow PhD and MSc colleagues in the Warren Laboratory, who better understood my everyday struggles in the lab and were always willing to share constructive ideas with me.

My greatest thanks go to my mother (Mrs. Z. Madlala) and father (Mr. B. Madlala) for their support and prayers throughout all the years I have been at the university.

I am deeply grateful to my husband (Bonginkosi Mshengu) for his unconditional love and support that he has given me. My husband had to be the father and mother to our son (Nkanyiso) while I was preoccupied with my studies and he made sure we had healthy home-cooked meals now and then. I am deeply thankful to you *Donga*.

I thank the University of KwaZulu-Natal and National Research Foundation (NRF) of South Africa for the financial support.

Abstract

Traditional medicines are used by approximately 80% of South African population for their primary health care needs, but the chemistry and biological activity of many medicinal plants have not yet been investigated. This study focused on the isolation and structural elucidation of natural products, as well as developing a high-performance liquid chromatography (HPLC) method to fingerprint the crude extract of *Elytropappus rhinocerotis* (L.f.) Less. *E. rhinocerotis* is well known in traditional medicine for the treatment of colic, wind, diarrhoea, indigestion, dyspepsia, gastric ulcers and stomach cancer. This study was also conducted to isolate, elucidate structures and evaluate the uterotonic activity of natural products from *Rhoicissus tridentata* (L.f.) Wild & Drumm. subsp. *cuneifolia*, a medicinal plant used by many South African women to induce labour and to tone the uterus during pregnancy.

From the ethyl acetate extract of the aerial parts of *E. rhinocerotis*, 6,7-dimethoxycoumarin, 5,6,4'-trihydroxyflavone, 5,7-dihydroxy-4'-methoxyflavone, 5,7-dihydroxy-6,4'-dimethoxyflavone, kaempferol-3-methyl ether, (+)-13-*epi*-labdanolic acid, (+)-labdanolic acid, (+)-labdanolic acid methyl ester, and (+)-labdanediol were isolated. These compounds are reported for the first time from *E. rhinocerotis*. The isolated flavonoids may justify the traditional use of this plant in the treatment of cancer, while the labdane diterpenes have shown anti-inflammatory activities in other studies. A HPLC method to fingerprint the crude extract from the aerial parts of *E. rhinocerotis* was successfully developed and minor variations were observed in the chemical compositions of *E. rhinocerotis* plants collected from different geographic locations.

From the acetone fraction of the methanol extracts of the root of *R. tridentata*, catechin, quercetrin, morin 3-*O*-α-L-rhamnopyranoside, *trans*-resveratrol glucoside, an inseparable mixture of asiatic acid and arjunolic acid, β-sitosterol, and linoleic acid were isolated and characterised. Except for catechin and β-sitosterol, these compounds are reported for the first time from *Rhoicissus* and the occurrence of morin 3-*O*-α-L-rhamnopyranoside is reported for the first time from the family Vitaceae.

The uterotonic activity of the crude methanol extract of the root of *R. tridentata* as well as the activity of the isolated pure compounds was evaluated using the isolated uterine

smooth muscle strips obtained from stilboestrol-primed Sprague-Dawley rats. The mixture of asiatic acid and arjunolic acid showed a response of approximately 13% in the force of uterine muscle contractility at 1.23 µg/mL while β-sitosterol demonstrated a change of 40% in the force of uterine muscle contractility at a concentration of 57.1 µg/mL. Hence it was concluded that the mixture of asiatic acid and arjunolic acid is the most uteroactive component in the extract of *R. tridentata*. Morin 3-*O*-α-L-rhamnopyranoside and *trans*-resveratrol glucoside caused a relaxation in the contractions of the uterine smooth muscle. Both compounds showed a higher inhibition in the force of contractions when compared to the rate of contractions. These findings confirmed that *R. tridentata* possesses both oxytocic and tocolytic activities at different dosages. Catechin and quercetrin were cytotoxic to the uterine smooth muscle tissue.

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List of Abbreviations

AcOH :   acetic acid

AIDS :   acquired immune deficiency syndrome

Å   :   angstrom

ALT   :   aspartate transaminase

APCI :   atmospheric pressure chemical ionization

BCE   :   Before the Common Era

CCl_4 :   carbon tetrachloride

$CDCl_3$:   deuterated chloroform

DM   :   diabetes mellitus

DCM   :   dichloromethane

DMF   :   dimethylformamide

DMSO: dimethyl sulfoxide

ESI   :   electrospray ionization

EtOAc :   ethyl acetate

FDA   :   Food and Drug Administration

GC-MS   gas chromatography-mass spectrometry

Glc   :   glucose

GPCR :   G-protein-coupled receptors

EC_{50} :   half maximal effective concentration

IC_{50} :   half maximal inhibitory concentration

Hex   :   hexane

HPLC :   high-performance liquid chromatography

HRMS :   high-resolution mass spectrometry

HIV   :   human immunodeficiency virus

LPO   :   lipid peroxidase

LRMS : low-resolution mass spectrometry

MeOH : methanol

MIC : minimum inhibitory concentration

NP : natural product

NNRTIs: non-nucleoside reverse-transcriptase inhibitors

NMR : nuclear magnetic resonance

NOE : nuclear overhauser effect

NRTIs : nucleoside reverse-transcriptase inhibitors

PDA : photodiode-array detector

PPH : post-partum hemorrhage

PGE2 : prostaglandin E2

PIs : protease inhibitors

R_F : retention value

ASP : serum alanine transaminase

TI : therapeutic index

TLC : thin-layer chromatography

TGI : total growth inhibition

TCM : traditional Chinese medicine

UV : ultraviolet

VCD : vibrational circular dichroism

WHO : World Health Organization

CHAPTER 1: Introduction and aims of study

1.1   General introduction

Since ancient times, humans have relied on medicinal plants to treat a wide spectrum of diseases and these plants have formed the basis of various sophisticated traditional medicine systems (Cragg and Newman, 2013). The Chinese Materia Medica was amongst the earliest documented systems, with the first record dating to 1100 BCE (Huang, 2010). The first records on the Indian Ayurvedic system, namely Charaka (341 drugs), Sushruta and Samhita (561 drugs), dates before 1000 BCE (Dev, 2001; Kapoor, 1989). A famous record, known as "Ebers Papyrus", listing over 700 drugs used in Egypt, was documented in 1500 BCE (Borchardt, 2002).

Approximately 1000 plant-derived products used in Mesopotamia were documented as early as 2600 BCE and these products are still used today to treat several diseases. Examples of these products are the oils from *Cedrus* species (cedar), *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice) (Cragg and Newman, 2013). While plants have been a source of human medicines for thousands of years, the isolation of bioactive components from plants only started about 200 years ago (Cragg and Newman, 2013).

Some of the early plant-derived drugs discovered were morphine (**1.1**), which was isolated from *Papaver somniferum* L. (Papaveraceae), aspirin (**1.3**), which is a synthetic analogue of salicylic acid (**1.2**) present in willow bark (Salicaceae), quinine (**1.4**), isolated from *Cinchona* species e.g. *C. officinalis* (Rubiaceae) and digoxin (**1.5**), obtained from *Digitalis lanata* Ehrh. (Plantaginaceae). These drugs, which are still used today, show activity against pain, rheumatism and headache, malaria, arrhythmia and congestive heart failure respectively (Buss et al., 2003; Butler, 2004; Rishton, 2008; Schuster and Wolber, 2010).

1.1 **1.2** **1.3** **1.4**

1.5
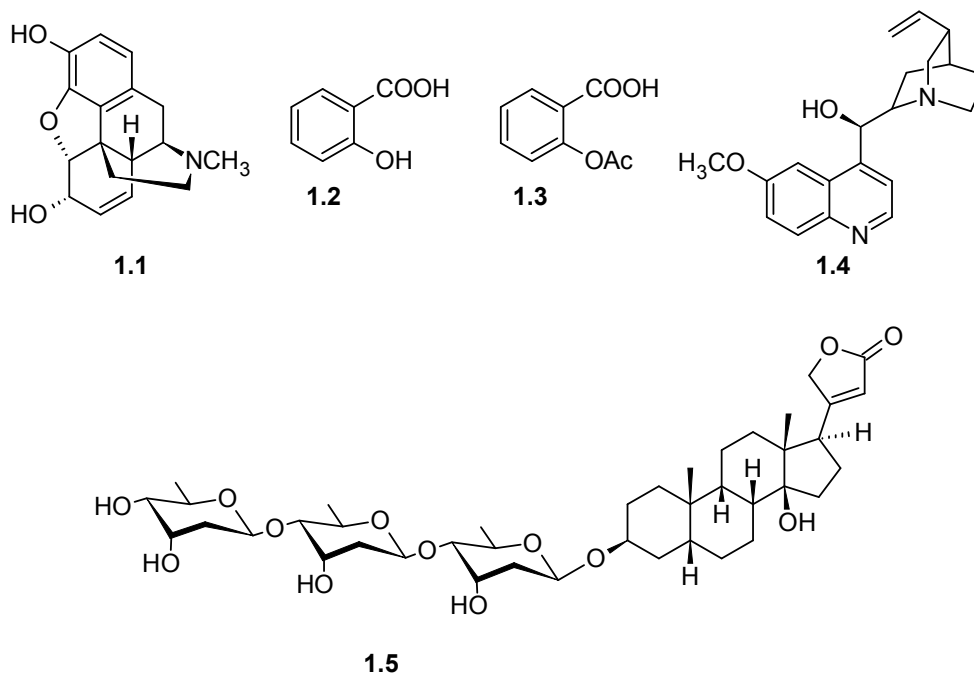
The diverse biological activities exhibited by compounds such as **1.1**-**1.5** prompted many laboratories and pharmaceutical companies to embark on natural product (NP) research. During the 1990's, 80% of drugs were NP or derived from NP. From the year 2000 to 2002, NP or NP-derived drugs were amongst the 35 top-selling drugs worldwide (Butler, 2004; Kingston, 2010). Regardless of this great contribution of NP in drug discovery, most pharmaceutical companies lost interest in NP research during the period of 2001-2008. Approximately 25% and 50% of marketed drugs at that time were from natural sources. The loss of interest in NP was attributed to difficulties experienced in the isolation and identification of hit compounds from crude extracts (Butler, 2004; Kingston, 2010; Li and Vederas, 2009).

At that stage, combinatorial chemistry was identified as the solution to drug discovery as it allowed for the fast production of large numbers of compounds as potential drugs. However, the libraries created through this method often did not produce novel structural types of compounds. This can be attributed to the difficulty in synthesizing complex structures to produce novel compounds. As a result, many scientists regained interest in NP research for drug discovery and development since the complexity of NP structures makes them good lead compounds. In addition, advances in modern technology such as molecular modeling, virtual screening, high-throughput cell-based

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screenings and advanced spectroscopic methods for structural elucidation ensured quicker identification of hit compounds from the crude plant extracts (Kingston, 2010; Rishton, 2008; Schuster and Wolber, 2010).

In particular, natural products have contributed to the treatment of infectious, neurological, cardiovascular and metabolic, immunological and inflammation, and oncological diseases (Fennell et al., 2004; Kingston, 2010; McGaw et al., 2008; Mishra and Tiwari, 2011; Mukhtar et al., 2008; Newman and Cragg, 2012). Example of plant-derived drugs and lead compounds in clinical use for the treatment of infectious diseases are artemisinin (**1.6**) and betulinic acid (**1.8**).

Artemisinin (**1.6**) is an antimalarial drug isolated from *Artemisia annua* (sweet wormwood, *qinghao*), a plant with long historical use in Traditional Chinese Medicine (TCM) for the treatment of fevers. This compound was also isolated from several other *Artemisia* species (for example, *A. vulgaris*, *A. japonica*, *A. vulgaris* L. (mugwort) syn, and *A. nilagirica*), and it is used in many countries for its antimalarial activity (Efferth, 2009; Rashmi et al., 2014). Besides the antimalarial properties, **1.6** and its derivatives have shown *in vitro* anti-cancer activity against radiation-resistant breast cancer cells (Singh and Lai, 2001), drug-resistant small cell lung carcinoma cells (Sadava et al., 2002), human leukemia cell lines (Lai and Singh, 1995), and colon cancer and active melanomas (Efferth et al., 2001).

In addition, **1.6** demonstrated antifungal activity against some plant pathogens (for instance, *Gaeumannomyces graminis* var. *tritici, Rhizoctonia cerealis, Gerlachia nivalis* and *Verticillium dahlia)* (Tang et al., 2000). A synthetic analogue of artemisinin, arterolane (**1.7**) in combination with piperaquine phosphate, is in Phase III clinical trials for the treatment of malaria in India, Bangladesh, and Thailand (Mishra and Tiwari, 2011).

1.6   **1.7**
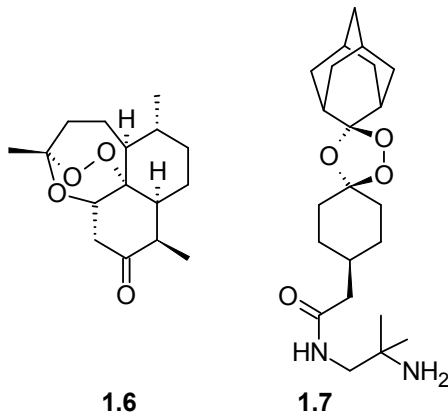
Betulinic acid (**1.8**) was isolated as an anti-HIV principle from the leaves of *Syzigium claviflorum*. This compound showed inhibition of HIV-1 replication in H9 lymphocytes with an EC_{50} of 1.4 μM and a therapeutic index (TI) of 9.3(Lee, 2010; Mishra and Tiwari, 2011; Schuster and Wolber, 2010; Sun et al., 1998). Compound **1.8** is present in many plant species and considerable quantities of this compound can be obtained from the bark of the birch tree (*Betula* spp., Betulaceae) (Moghaddam et al., 2012; Pisha et al., 1995). Betulinic acid was reported to exhibit a variety of other biological properties, for example, anti-bacterial (Chandramu et al., 2003), anti-malarial (Bringmann et al., 1997), anti-inflammatory (Alakurtti et al., 2006; Mukherjee et al., 1997) anthelmintic (Enwerem et al., 2001), antinociceptive (Kinoshita et al., 1998), and anticancer activities (Fulda and Debatin, 2000; Zuco et al., 2002).

Betulinic acid (**1.8**) has served as a valuable anti-HIV lead compound and amongst its derivatives, 3-*O*-(3',3'-dimethylsuccinyl)betulinic acid (Bevirimat[®]) (**1.9**) was extremely potent. Compound **1.9** inhibited HIV replication with an EC_{50} < 0.35 nM and TI of 20 000 (Mishra and Tiwari, 2011). Interestingly, **1.9** retained activity even against virus isolates resistant to NRTIs, NNRTIs, and PIs. Bevirimat[®] also exhibited synergistic effects with other AIDS drugs (Lee, 2010).

1.8 R = H

1.9 R =
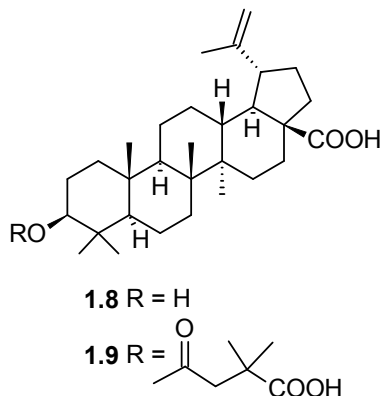
The two vinca alkaloids, vinblastine (**1.10**) and vincristine (**1.11**) were isolated from *Catharanthus roseus* (L.) G. Don (Apocynaceae) or *Vinca rosea* L. (known as "Chang Chung Hua" in Chinese medicine) (Barnett et al., 1978). Compounds **1.10** and **1.11** are some of the well-known plant-derived drugs used to treat Hodgkin's lymphoma and acute childhood leukemia. Local people of Jamaica and India use *C. roseus* as a diuretic, anti-dysenteric, hemorrhagic and antiseptic as well as for the treatment of diabetes. The anticancer activity of this plant was discovered by serendipity when the extracts were investigated as a source for potential oral hypoglycemic agents (Cragg and Newman, 2005). Numerous synthetic analogues of **1.10**, e.g. vinorelbine (Nabelbine[®]) (**1.12**) were designed to target other types of tumor or to minimize side effects shown by compound **1.10**. Vinorelbine (**1.12**) showed activity against non-small cell lung and advanced breast cancer (Cragg and Newman, 2005; Johnson et al., 1996; Potier, 1989).

1.10 R = CH$_3$

1.11 R = CHO

1.12
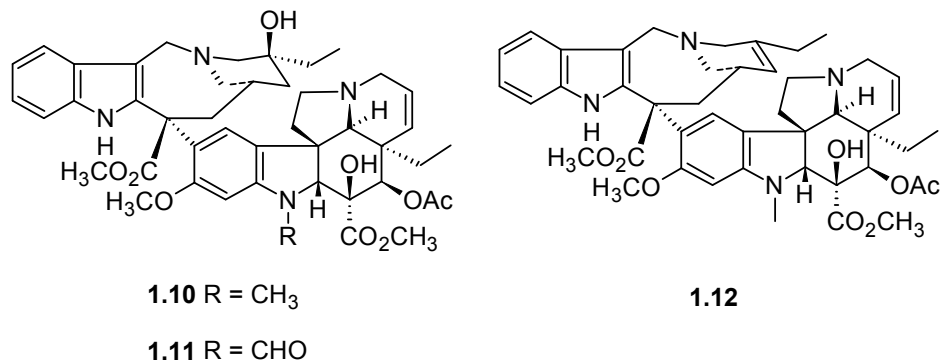
Another important plant-derived anticancer drug approved for clinical use is paclitaxel (Taxol[®]) (**1.13**). In 1992 paclitaxel was approved for the treatment of ovarian cancer,

and later (1994) was approved to treat breast cancer. Paclitaxel (**1.13**) was first isolated from the leaves of *Taxus brevifolia* Nutt. (Taxaceae) (Cragg and Newman, 2005; Wani et al., 1971). Unfortunately, the supply of paclitaxel (**1.13**) was limited since only trace amounts of this compound (0.01% of dry weight of the bark) could be isolated (Cragg and Newman, 2005). Furthermore, the natural source was nonrenewable as the tree grows very slowly. As a result, many studies were pursued to synthesize analogues of **1.13** (Denis et al., 1988; Funk and Yost, 1996; Holton et al., 1994). Docetaxel (**1.14**) is a clinically used anti-cancer drug which was synthesized from 10-deacetyl-baccatin III (**1.15**). Baccatins such as compound **1.15**, are key precursors of paclitaxel and are readily available in various *Taxus* species (e.g. **1.15** was obtained from *T. baccata* L.) (Guenard et al., 1993).

1.13 R^1 = COCH$_3$, R^2 = HNCOC$_6$H$_5$
1.14 R^1 = H, R^2 = HNCOOC(CH$_3$)$_3$

1.15
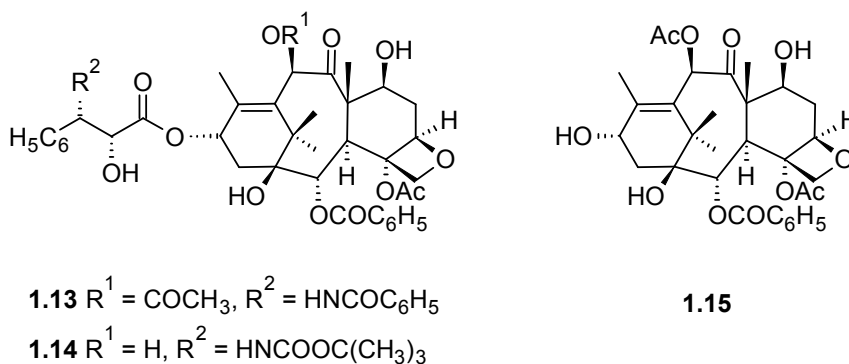
In spite of the great success of the pharmaceutical industries in the search for drug lead compounds from plants, most of the plant biodiversity still remain unexplored (Aremu et al., 2010; Okem et al., 2012). It is estimated that South Africa has more than 24 000 indigenous plant species and about 3000 of these species are used as medicinal plants. A large number of these plant species have never been studied. Moreover, 80% of the South African Black population still relies on traditional medicines for their primary health care needs. This is because traditional medicine is an important part of the culture of African people and traditional herbs are generally more accessible and affordable than the western medicines (Aremu et al., 2010; Okem et al., 2012; Street et al., 2008).

Traditional medicines are normally assumed to be safe for human consumption as plants have a long history of usage in the treatment of diseases. However, recent

research has shown an increase in the number of deaths due to plant poisoning. Some factors linked to this acute poisoning include misidentification of plant species and incorrect preparation or dosage of plant extracts (Fennell et al., 2004; Street et al., 2008). Hence, the identification, isolation, and evaluation of natural products for their biological activities and toxicity before they are applied as therapeutic agents are of great importance.

This project is aimed at the isolation, identification and biological evaluation of natural products from two Zulu medicinal plants, *Elytropappus rhinocerotis* (L.f.) Less. (Asteraceae) and *Rhoicissus tridentata* (L.f.) Wild & Drumm. subsp. *cuneifolia* (Eckl. & Zehr.) N.R. Urton (Vitaceae). The specific aims of the project are listed in Section 1.2. In Section 1.3, a brief description of the organization of this thesis is given.

1.2   Aims of the study

The aims of this study were:

- To isolate secondary metabolites from *Elytropappus rhinocerotis* and to determine the structures of the isolated compounds.

- To develop a method of determining the chemical markers present in *E. rhinocerotis* using HPLC-PDA/LCMS and to compare the chemical profiles of different *E. rhinocerotis* plants collected from different locations.

- To test and compare the uterotonic activity of the methanol (MeOH) extracts of two Zulu medicinal plants (*Rhoicissus tridentata* and *Gunnera perpensa*). These plants are amongst the most cited plants used in the preparation of *Isihlambezo*, a traditional herbal drink taken by pregnant women in their last trimester of pregnancy to induce labor and improve health of the baby and the mother.

- To investigate the phytochemistry of the most uteroactive fraction from *R. tridentata* and to evaluate the uterotonic activity of the isolated pure compounds.

1.3   Organization of the thesis

Following this chapter (Chapter 1), this thesis contains three more chapters. In Chapter 2, a literature review of the family Asteraceae is presented and the isolation, structural characterization, and HPLC-PDA analysis of the compounds from *E. rhinocerotis* are discussed. Chapter 2 also includes a discussion on the variation of chemical profiles of different *E. rhinocerotis* plants collected in different locations. Chapter 3 focuses on the literature review of oxytocic plants and the isolation and structural characterization of compounds from *R. tridentata*. The oxytocic activity of the crude *R. tridentata* extracts, *G. perpensa*, and the compounds isolated from *R. tridentata* will also be discussed in Chapter 3. General conclusions about the findings of this research and future recommendations will be discussed in Chapter 4.

CHAPTER 2: The phytochemistry of *Elytropappus rhinocerotis*

2.1 Introduction

The family Asteraceae includes mainly herbaceous plants, with a rare occurrence of trees, shrubs, and climbers. To date, Asteraceae is documented as the largest flowering plant family, with about 25 000 species grouped into 1700 genera and 12 subfamilies (Funk et al., 2009; Zavada and de Villiers, 2000). These species are widely distributed throughout the world, but a majority are found in the tropical and subtropical regions, such as central America, eastern Brazil, the Andes, the Mediterranean, Levant parts of Middle East, central Asia, South Africa and southwestern China (Bohm and Stuessy, 2001; Jansen and Palmer, 1987; Stuessy, 2010). The species are characterized by the following features: (i) a group of closely packed flowers into heads (known as an inflorescence), (ii) small leaf like structures surrounding the flowers (phyllaries), (iii) the existence of a modified calyx attached to apex of the ovary (pappus) (Barreda et al., 2012; Jansen and Palmer, 1987; Stuessy, 2010).

The Aims of this Chapter are:

- To present an overview of the documented economic and medicinal uses of some Asteraceae species, with a particular focus on the South African indigenous plants.

- To summarise the reported traditional uses, biological activities, and to provide an overview of the phytochemical studies undertaken for the genus Elytropappus (an endemic South African Asteraceae genus).

- To present the findings from the phytochemical investigation of *Elytropappus rhinocerotis* undertaken in the current study.

2.2 Economic and medicinal uses of some Asteraceae plants

Members of the genus *Heliathus* (sunflower) and *Carthamus* (safflower) are cultivated worldwide for their oil and nuts production, as well as the feeding of birds and small animals (Milner et al., 1945; Torres et al., 2014; Weiss, 2000). Other genera, such as, *Lactuva* (lettuce) and *Cynara* (artichokes) are popular in food production. Several genera in the Asteraceae family are important in horticulture, for instance, *Tagetes* and *Chrysanthemums* (ornaments) (Jansen and Palmer, 1987), *Dahlia* (cultigen), *Zinnia* and *Helenium* (garden flower) (Funk et al., 2009).

The orange-yellow carotenoid lutein (**2.1**) extracted from *Tagetes erecta*, is well-known in Europe for providing colour to foods, such as pasta, vegetable oil, margarine, mayonnaise, confectionery, dairy products, citrus juice and mustard (Hadden et al., 1999; Piccaglia et al., 1998; Vasudevan et al., 1997). Lutein is approved as a food colourant in the European Union, Australia and New Zealand, but it is only used in poultry feed in United States (Otterstätter, 1999; Wrolstad and Culver, 2012).

2.1
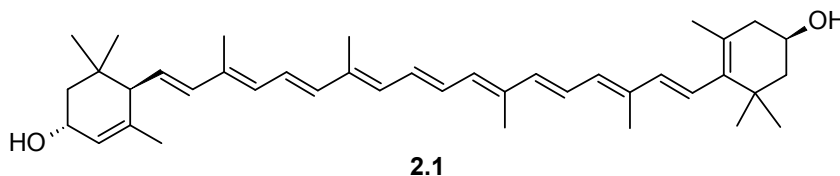
The large number of Asteraceae species are found worldwide and their wide array of natural products make them useful in the treatment of a wide variety of ailments. The largest genus in this family is the genus *Baccharis*, which consists of about 500 species. *Baccharis* plants are mainly found in the warm temperate and tropical regions of Brazil, Argentina, Colombia, Chile and Mexico. The genus *Baccharis*, commonly known as *carqueja*, is popularly used in traditional medicine in southern Brazil, Uruguay and Argentina for the treatment of stomachache, backache, headache and bellyache. The essential oil composition of several *Baccharis* species have been studied and slight variations in the composition is observed. Other types of compounds isolated from this genus are kaurane, labdane and neo-clerodane diterpenes.

Two species from this genus (*B. articulate* and *B. crispa)* have been recently cited amongst the six most popularly used plants for pain relief in Rio Grande do Sul, Southern Brazil (Florão et al., 2012; Stolz et al., 2014). *B. articulate* is also taken as a tonic, febrifuge, diuretic, for digestion, for control of the anemia and weakness, anthelmintic and weight loss. GC-MS analysis of the volatile oil from *B. articulate* from Southern Brazil revealed the major chemical constituents to be β-pinene (39.0%) (**2.2**), *cis*-β-guaiene (9.8%) (**2.3**), γ-muurolene (5.8%) (**2.4**), limonene (4.8%) (**2.5**), α-pinene (4.5%) (**2.6**), α-gurjunene (**2.7**) (4.4%) and spathulenol (4.2%) (**2.8**) (Simionatto et al., 2008).

| 2.2 | 2.3 | 2.4 | 2.5 |

| 2.6 | 2.7 | 2.8 |
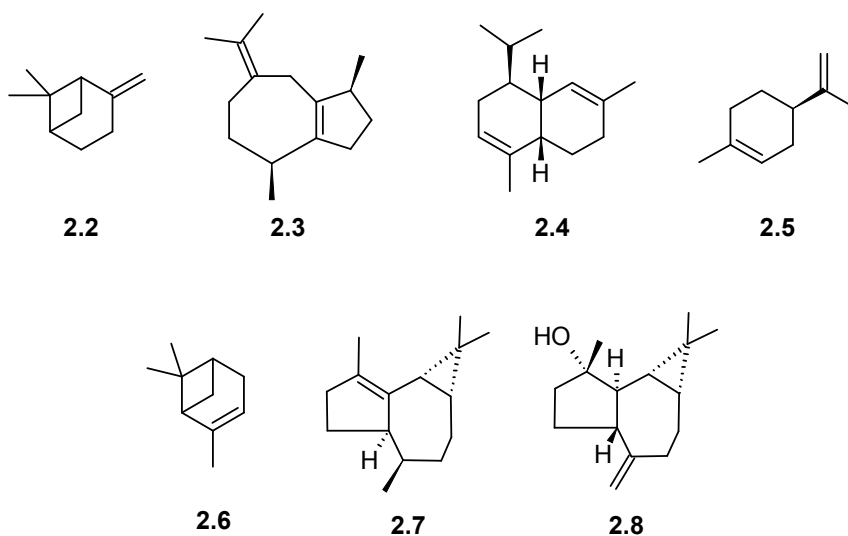
B. crispa is popularly used in Brazil for the treatment of gastrointestinal, liver and kidney diseases as well as inflammation. From pre-clinical studies performed on the crude aerial aqueous extract and butanolic fraction, this plant was shown to possess antinociceptive and anti-inflammatory properties (Gené et al., 1996; Nogueira et al., 2011; Paul et al., 2009; Stolz et al., 2014). These activities have been associated with the presence of a saponin (echinocystic acid, **2.9**), rutin (**2.10**) and other phenolic compounds in the extracts (de Oliveira et al., 2012; Gené et al., 1996; Stolz et al., 2014).

2.9   **2.10**
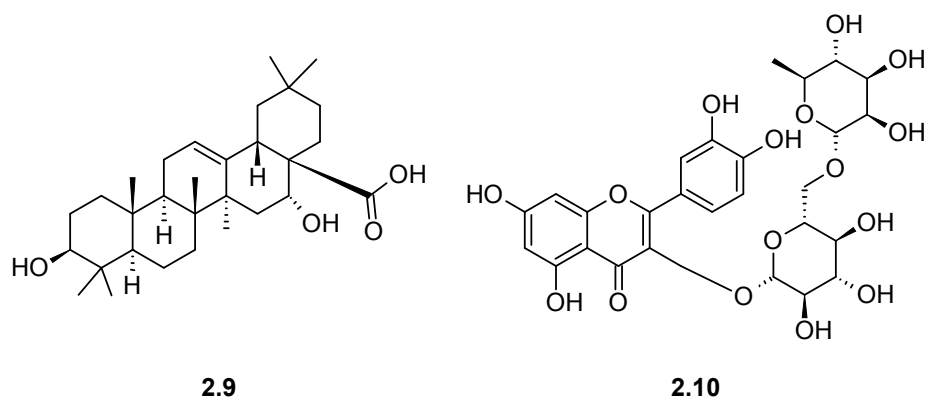
Species from the genus *Brickella* (known as brickellbushes) are native to Mexico and southwestern United States. In traditional medicine, the herbal tea is prepared from these species to cure ulcers, migraines, heart diseases and diabetes. The chemical composition of some *Brickella* species have been studied, and some isolated compounds have hypoglycemic and antioxidant properties (Andrade-Cetto and Heinrich, 2005; Marles and Farnsworth, 1995; Rivero-Cruz et al., 2006). Rivero-Cruz *et al.* (2006) studied the phytochemistry of *B. veronicaefolia* and reported that 86% of its essential oil consists of benzoates and sesquiterpenoids. A hypoglycemic flavone (5,7,3'-trihydroxy-3,6,4'-trimethoxyflavone, **2.11**) was isolated from the chloroform extract of the leaves of *B. veronicaefolia* (Perez G et al., 2000).

2.11
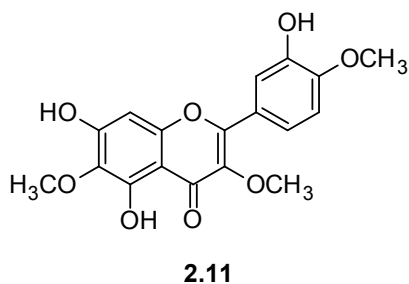
Brickella cavanillesii (prodigiosa or hamula) is a bitter-tasting herb widely commercialized in Mexico (alone or in combination with other plants) for treating ulcers, dyspepsia, and diabetes. This plant is amongst the 306 most frequently used species for the treatment of type-II diabetes mellitus (DM), and is sold as a cheaper alternative to insulin (Andrade-Cetto and Heinrich, 2005; Escandón-Rivera et al., 2012; Eshiet et al., 2014). Phytochemical studies on the aerial parts led to the isolation of 6-acetyl-5-hydroxy-2,2-dimethyl-*2H*-chromene (**2.12**) (Rodríguez-López et al., 2006),

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pendulin (**2.13**), and atanasin (**2.14**) (Flores and Herrán, 1960; Flores and Herrán, 1958; Mata et al., 2013). An *O*-methylated flavonol (brickellin, **2.15**) (Iinuma et al., 1985) was reported as a major constituent of *B. cavanillesii* and was associated with the antidiabetic properties shown by this plant (Eshiet et al., 2014). Bioassay-guided fractionation led to the isolation of several natural products, including, 6-hydroxyacetyl-5-hydroxy-2,2-dimethyl-*2H*-chromene (**2.16**), sesquiterpene lactones (caleins C, **2.17**) and several flavonoids [isorhamnetin (**2.18**) and quercetin (**2.19**)]. Compound **2.16-2.19** showed a significant inhibitory activity against the enzyme α-glucosidase.
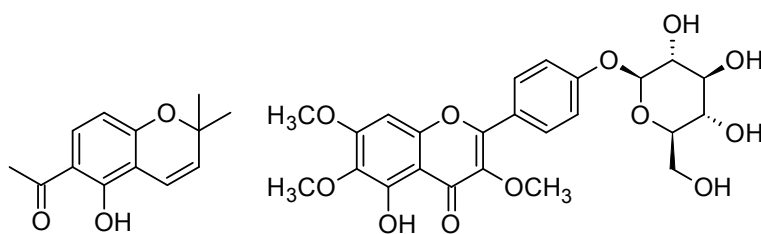
2.12   **2.13**
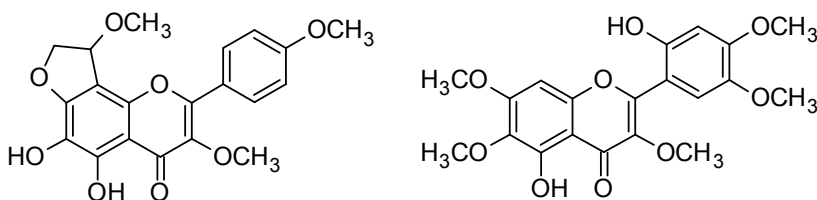
2.14   **2.15**
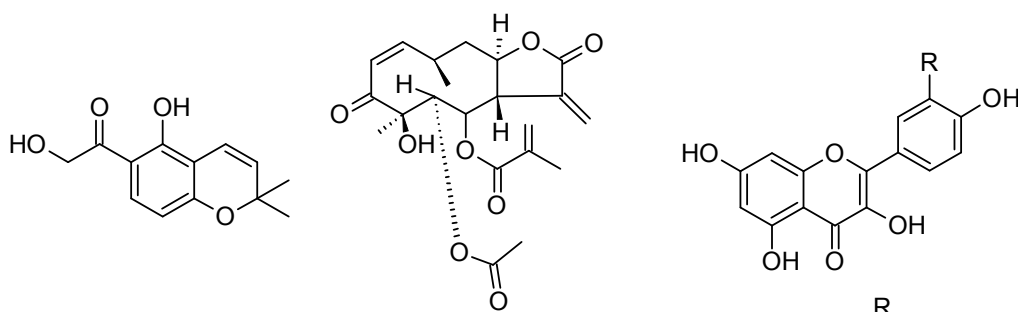
2.16   **2.17**   **2.18** OCH$_3$
                                2.19 OH

Members of the genus *Echinacea* are well known in North America and Europe due to their ability to stimulate the immune system, which is important in the treatment and prevention of upper respiratory tract infections (Barrett, 2003; Toselli et al., 2009). In

traditional medicine, native American Indians use *Echinacea* species for the treatment of wounds, burns, insect and snake bites. The roots of these plants are chewed to cure toothache, throat infection, pain, cough and stomach cramps (Percival, 2000; Shah et al., 2007). *Echinacea* extracts and whole plant extracts are commercially prepared as direct pressed juices, freeze-dried ethanolic or hydrophilic extracts, and powdered dried leaves and flowers. These products are available in groceries, pharmacies, and health food stores throughout the world (Barrett, 2003). In the United States alone, *Echinacea* products annual sales are estimated to be worth $300 million (Barrett, 2003; Brevoort, 1998).

Artemisia L. is one of the widely used Asteraceae genera across different traditional medicine systems worldwide. Local communities of India, Myanmar, Pakistan, Nepal, Bhutan, Afghanistan and Japan uses *Artemisia* species for fever and eczema, treatment of wounds and skin diseases, febrifuge, depurative properties, digestive disorders, epilepsy, psychoneurosis, depression, irritability, insomnia, anxiety, stress, treatment of amenorrhea and dysmenorrhea. These plants are also used for their anthelminthic, antiseptic, antispasmodic properties and in ethnoveterinary medicine (Govindaraj et al., 2008; Rajeshkumar and Hosagoudar, 2012).

The significance of this genus in medicinal chemistry was increased due to the isolation of an antimalarial drug, artemisinin (**1.5**) from *Artemisia annua* (De Vries and Dien, 1996; Rashmi et al., 2014). As discussed in Chapter 1, artemisinin (**1.5**) was first isolated from Asian *Artemisia* specie, *A. annua*. *A. annua* is cultivated in Africa and its tea is well-known for the treatment of malaria (Abad et al., 2012). The only indigenous member of *Artemisia* in South Africa is *Artemisia afra* Jacq. ex Willd (*wilde als*). *A. afra* is amongst the oldest indigenous plants used in the traditional medicine in South Africa (Van Wyk, 2008a). The local uses of this plant, as well as uses of several South African Asteraceae plants are discussed below.

2.3 Medicinal uses of some South African Asteraceae plants

As mentioned earlier, Asteraceae is the largest family of flowering plants worldwide. In South Africa these plants often occur in the Cape fynbos biome. Cape fynbos is the flora of the Western Cape which forms part of the Cape Floral Kingdom and consists of about 8550 species. Since this fynbos is dominated by Asteraceae, many traditional medicines used by indigenous people here are derived from plants belonging to this family (Salie et al., 1996). Several Asteraceae species discussed below are indigenous to South Africa, with a high occurrence in the Cape fynbos.

Artemisia afra Jacq. ex Willd (*wilde als* in Afrikaans, *lengana* in Sotho, and *umhlonyane* in Xhosa and Zulu) is a South African indigenous species popularly used in traditional medicine as a bitter tonic and a stimulant for Cape herbal medicine (Thring and Weitz, 2006; Van Wyk, 2008b). Amongst other ailments treated by this plant are respiratory disorders, colic, flatulence, constipation, gastritis, poor appetite, heartburn, measles, headache, earache, gout, diabetes, malaria, diarrhea and wounds (Hutchings et al., 1996; Neuwinger, 2000; Van Wyk, 2008a; Watt and Breyer-Brandwijk, 1962).

Biological studies on *A. afra* extracts showed that this plant has antimicrobial, antioxidant, anti-nematodal, antimalarial, cardiovascular, cytotoxic and sedative properties. *A. afra* is one of the commercially important medicinal plants in South Africa, with its first commercial product based on low-thujone material developed as a tincture (1996) and tablets (2002) under the brand names Healer's Choice and Phyto Nova, respectively (Van Wyk, 2011).

Flavonoids found in *A. afra* extracts include apigenin, chrysoeriol, tamarixetin, acacetin, genkwanin and kaempferol (Avula et al., 2009; Kraft et al., 2003; Waithaka, 2004). Significant amounts of luteolin and quercetin were isolated from the aqueous extract (Muganga, 2007; Mukinda et al., 2010; Waithaka, 2004). Luteolin (**2.20**) and quercetin (**2.19**) are reported to be easily extractable, stable under various processing conditions and selectively quantifiable using HPLC. These compounds were therefore

assigned as ideal markers when evaluating *A. afra* extracts (Markham, 1982; Mjiqiza, 2006; Mjiqiza et al., 2013; Mukinda et al., 2010; Waithaka, 2004).

Several other types of compounds have been isolated from *A. afra*. These include, sesquiterpene lactones (for instance, guaianolides and glaucolides) (Jakupovic et al., 1988), triterpenes (such as, α-amyrin, β-amyrin, and friedelin), as well as alkanes (e.g. ceryl cerotinate and *N*-nonacosane) (Silbernagel et al., 1990). Volatile oil from this plant is very useful and has been used as a substitute for armois oil. Armois oil is produced by *A. vulgarisa* L. and is used in perfumes and as a flavoring agent. Although the composition of this oil varies with geographic origin, some common constituents have been identified such as 1,8-cineole (**2.21**), α-thujone (**2.22**), β-thujone (**2.23**), camphor (**2.24**) and borneol (**2.25**) (Van Wyk, 2008a, 2011).

2.20   **2.21**   **2.22**

2.23   **2.24**   **2.25**
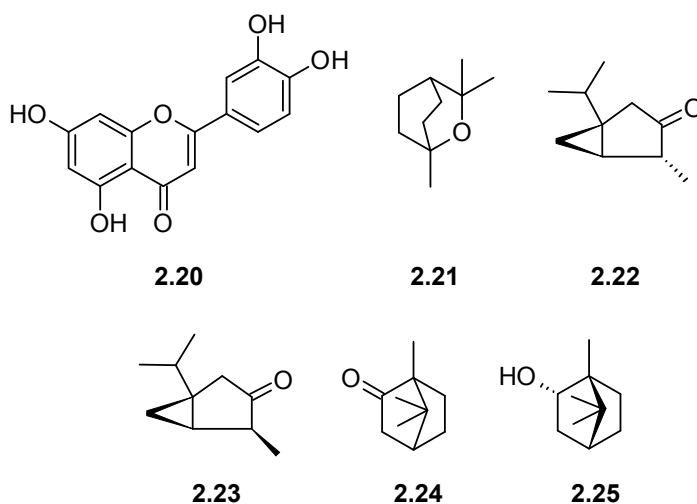
Species of the genus *Eriocephalus* L. are used by South African local communities for treatment of coughs and colds, flatulence and colic, digestive disorders, as well as stomach pain. *Eriocephalus punctulatus* DC. (Cape chamomile) and *E. africanus* L. (wild rosemary) were used as diuretic and diaphoretic (Mierendorff et al., 2003). *E. africanus* is also used for treating gastro-intestinal, gynaecological complaints, inflammatory and other dermal complications. The oil extracted from wild rosemary and Cape chamomile is commercially important in the preparation of perfume, skin care and beauty products (Amabeoku et al., 2000; Njenga and Viljoen, 2006). The sesquiterpene, 8-*O*-isobutanoylcumambrin B (**2.26**) with antiplasmodial properties was

isolated from *E. tenuifolius* DC. The compound had a low IC_{50} of 6.25 μg/mL against *Plasmodium falciparum* D10, but was unfortunately also cytotoxic (Nthambeleni, 2008).
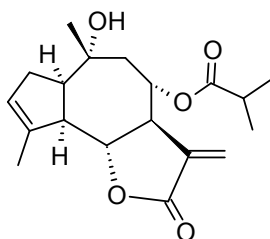
2.26

Other Asteraceae species commonly cited in South African traditional medicines are *Tarchonanthus camphoratus* L. (camphor bush) and *Athrixia phylicoides* DC. (bush tea or Zulu tea). The green leaves of the camphor bush are used for a range of ailments depending on how they are prepared. While the burnt leaves are inhaled to cure blocked sinuses, asthma and headache (Pretorius, 2008), the infusion of boiled leaves is taken orally to treat coughs, toothache, abdominal pain and bronchitis. Camphor bush leaves are also used for massaging the body and as a perfume (Aiyegoro and Van Dyk, 2013; Amabeoku et al., 2000; Hutchings et al., 1996; Watt and Breyer-Brandwijk, 1962).

The herbal tea prepared from leaves of *Athrixia phylicoides* is used as a "blood purifier" for sores and boils. The decoction of leaves and stems is used as a lotion for sore feet, boils, acne and infected wounds. Leaf infusions are also used as a stimulant, aphrodisiac drink and gargle for infected throat. In addition, leaf infusions have been reported to treat hypertension, heart diseases, diabetes, diarrhea and vomiting. Roots are used as a purgative and to treat coughs (Hutchings et al., 1996; Joubert et al., 2008; Rampedi and Olivier, 2005; Van Wyk and Gericke, 2000; Watt and Breyer-Brandwijk, 1962).

Phytochemical investigation on the aerial parts of *A. phylicoides* led to the isolation of germacren D, linoleic acid and *p*-hydroxyphenylpropan-3-ol coumarate (Bohlmann and Zdero, 1977; Joubert et al., 2008). Mashimbye et al. (2006) reported on the isolation of a new flavonoid, 5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavon-3-ol (**2.27**) from the leaves of *A. phylicoides* (Joubert et al., 2008; Mashimbye et al., 2006). Several diterpenes related to kaurane, triterpene and thymol derivatives were isolated from the

root extracts of some *Athrixia* species (e.g. *Athrixia spp.* and *A. pinifolia*) (Joubert et al., 2008).
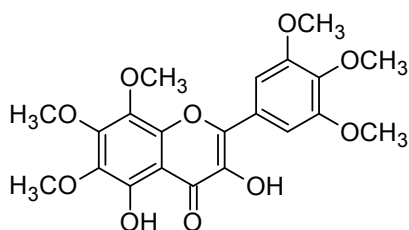
2.27

Fouche et al. (2008) conducted a study which was aimed at evaluating the *in vitro* anticancer activity of South African plants against breast MCF7, renal TK10 and melanoma UACC62 human cell lines. For Asteraceae, the hit rate for cytotoxicity against cancer cell lines was substantially larger than for any of the other plant families. *Schkuhria pinnata* (Lam) Kuntze demonstrated anticancer activity and the active principle was identified as eucannabinolide (**2.28**). Compound **2.28** showed a total growth inhibition (TGI) of < 6.25 µg/mL for melanoma UACC62, 7.75 µg/mL for breast MCF7 and 12.00 µg/mL for renal TK10 cancer cell lines. Eucannabinolide (**2.28**) was also isolated from other members of the genus *Schkuhria* (e.g. *S. virgata*), where it exhibited *in vivo* antileukemic activity (Herz and Govindan, 1980).
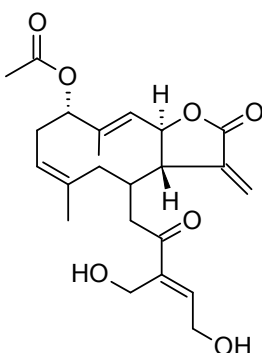
2.28

Another Asteraceae representative in the study mentioned above was *Oncosiphon piluliferum*. Two active constituents, namely, tetradin A (**2.29**) and deacetyl-β-cyclopyrethrosin (**2.30**) were isolated from the dichloromethane extract of *O. piluliferum*. Tetradin A (**2.29**) exhibited a TGI of 4.50 µg/mL for TK10, 86.10 µg/mL

for MCF7 and 18.72 µg/mL for UACC62. Compound **2.30** TGI for TK10 was 5.89 µg/mL, 86.15 µg/mL for MCF7 and 16.92 µg/mL for UACC62 (Fouche et al., 2008).

2.29　　　　**2.30**
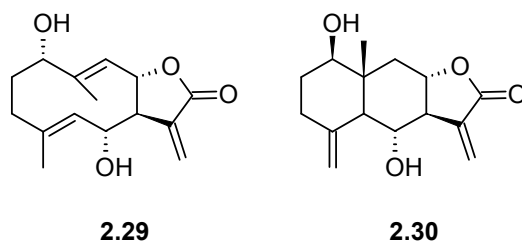
Salie et al. (1996) evaluated the *in vitro* antimicrobial activity of four indigenous Asteraceae species (*Arctotis auriculata* Jacq., *Eriocephalus africanus* L., *Felicia erigeroides* DC. and *Helichrysum crispum* (L.) D. Don). The organic extract of *A. auriculata* and *E. africanus* showed antimycobacterial activity against *Mycobacterium smegmatis*. These findings were very interesting as they showed that these plants also could inhibit the growth of *M. tuberculosis* (Salie et al., 1996).

These extracts, as well as extract from *F. erigeroides* also inhibited growth of *Pseudomonas aeruginosa*, a microorganism causing one of the most difficult infections to treat with normal antibiotics (Levinson and Jawetz, 2002). Organic extracts of *E. africanus* and *H. crispum* and aqueous extract of *F. ergeroides* exhibited antifungal activity against *Candida albicans*. Activity against *Staphyllococcus aureus* was shown by organic extracts of *A. auriculata* and *E. africanus* (Salie et al., 1996). The antimicrobial activity of the genus *Helichrysum* has been extensively studied and other examples of active species in this genus are mentioned below.

Members of the genus *Helichrysum* are widely used in Southern African traditional medicine. *Helichrysum* is one of the largest genus in the family Asteraceae, consisting of approximately 500-600 species. About 244-250 of these species occur in South Africa. Plants from this genus are very popular in traditional medicine for their use in invoking the goodwill of the ancestors, to induce trances and the aerial parts of several species that are used for these purposes are commercially available. Examples of these plants are *H. griseum* Sond, *H. herbaceum* (Adrews) Sweet, *H. epapposum* Bolus and *H. natalitium* DC. *Helichrysum* plants are also used to treat respiratory and gastro-intestinal disorders, eye conditions, pain and inflammation, menstrual pains,

rheumatism and headache. Leaves are often applied as a wound dressing and these plants are also used to fumigate huts and as bedding to repel insects (Afolayan and Meyer, 1997; Arnold et al., 2002; Hutchings et al., 1996; Lourens et al., 2008; Mathekga et al., 2000; Watt and Breyer-Brandwijk, 1962).

Several research groups have extensively studied the antimicrobial activity of *Helichrysum* species and most crude extracts usually exhibited higher activity against Gram-positive organisms than Gram-negative species. Antibacterial compounds, which are commonly flavonoids, have been isolated from some species. The active compound isolated from *H. aureonitens* was galangin (3,5,7-trihydroxyflavone) (**2.31**).

In a study conducted by Afolayan and Meyer (1997), galangin showed antibacterial activity against four Gram-positive bacteria (three *Bacillus* species and *Micrococcus kristinae*) and one Gram-negative species (*Enterobacter cloaceae*). Galangin (**2.31**) also exhibited activity against several bacteria and fungi, for instance, six β-lactam-sensitive and resistant strains of *Staphylococcus aureus*, sixteen strains of 4-quinolone-resistant strains of the bacterium, and *Aspergillus tamari*. In another study, galangin demonstrated antiviral activity against Herpes simplex virus type 1 and Coxsackie virus (MIC of 6 µg/mL) (Lourens et al., 2008; Meyer et al., 1997).
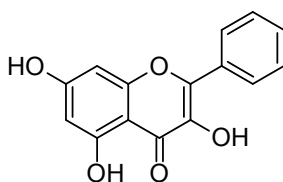
2.31

The active principle from *H. odoratissimum* was identified as 3-*O*-methylquercetin. This compound has antibacterial activity against a wide variety of microorganisms, including *Salmonella typhimurium* (Gram-negative, MIC = 50 µg/mL), *Staphylococcus aureus* (Gram-positive species, MIC = 6.25 µg/mL) and the fungi (e.g. *Candida albicans*, MIC = 12.5 µg/mL) (Van Puyvelde et al., 1989). Pinocembrin chalcone (**2.32**) was isolated from *H. trilineatum*, while pinocembrin (**2.33**) was obtained as an artefact during the isolation process from this plant. Both these compounds exhibited anti-staphylococcal activity (Bremner and Meyer, 1998). Another flavonoid, 5,7-

dibenzyloxyflavanone was isolated from *H. gymnocomum* and it showed activity against a diverse group of Gram-positive and Gram-negative bacteria as well as a yeast (Drewes and van Vuuren, 2008; Lourens et al., 2008).
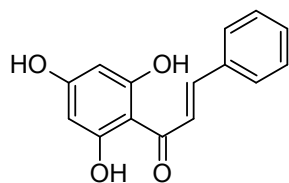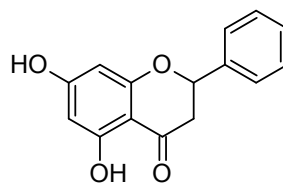
2.32   **2.33**

Interesting antibacterial activity was observed for two chalcones (**2.34** and **2.35**) isolated from *H. melanacme*. 2',4',6'-trihydroxy-3'-prenylchalcone (**2.34)** and 1-(3,4-dihydro-3,5,7-trihydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-yl)-3-phenyl-(*E*)-2-propen-1-one (**2.35)** inhibited growth of a drug-sensitive H37Rv strain of *Mycobacterium tuberculosis* with a MIC of 0.05 mg/mL. The crude extract together with the isolated chalcones were also evaluated for antiviral activity against the influenza A virus. The chalcones **2.34** and **2.35** showed lower activity than the crude extract, but the activity was higher when the chalcones were combined (Lall et al., 2006; Lourens et al., 2008).
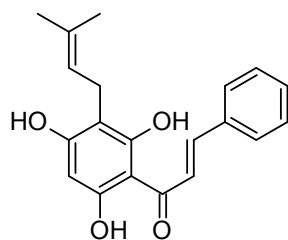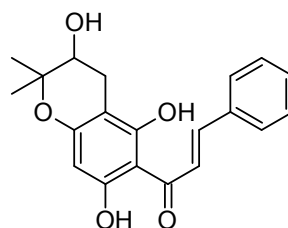
2.34   **2.35**

Compounds other than flavonoids with antimicrobial activity have also been isolated from *Helichrysum* species. Linoleic and oleic acid were obtained from *H. pedunculatum* and they showed antibacterial activity against *S. aureus* and *Micrococcus kristinae* (MIC = 1.0 mg/mL) (Dilika et al., 2000). The active compound (kaurenoic acid) from *H. kraussii* inhibited growth of *E. coli*, *Bacillus cereus*, *B. subtilis*, *S. aureus* and *Serratia marcescens*. A prenylated butyrylphloroglucinol (**2.36**) (MIC = 100 µg/mL) was also isolated from *H. kraussii* and it showed activity against similar bacteria as

kaurenoic acid (**2.37**) as well as *B. pumilis* and *Micrococcus kristinae* (Bremner and Meyer, 2000; Lourens et al., 2008)
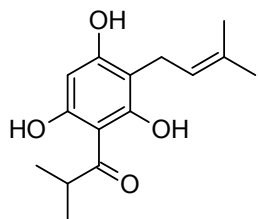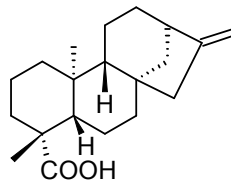
2.36   **2.37**

From the crude extract of *H. caespititium*, two phloroglucinols (caespitin **2.38** and **2.39**) with antimicrobial activity were isolated. Caespitin (**2.38**) inhibited growth of *S. aureus*, *Streptococcus pygenes*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum canis* (Mathekga et al., 2000). 2-Methyl-4-[2′,4′,6′-trihydroxy-3′-(2-methylpropanoyl)-phenyl]but-2-enyl acetate (caespitate) (**2.39**) was active against several microorganisms, including *Bacillus cereus*, *B. pumilis*, *B. substilis*, *Microsporum kristinae* and *S. aureus* (Mathekga et al., 2000). At a concentration of 0.5-1.0 μg/mL, the antifungal activity of caespitate (**2.39**) was observed against *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *C. cucumerinum*, *C. sphaerospermum* and *Phytophtora capsici* (Lourens et al., 2008; Mathekga et al., 2000)
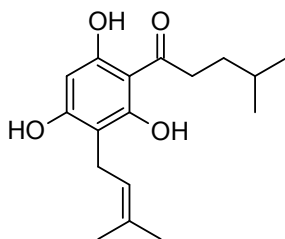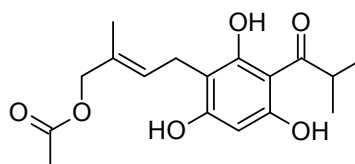
2.38   **2.39**

In summary, Asteraceae plants are used extensively in ethnomedicines worldwide and many of the uses are associated with the treatment of infectious diseases, for instance, many genera are used to treat respiratory disorders and wounds. The chemical compositions of many genera in the family have been studied, and a wide variety of compounds have been isolated. This is not surprising since this family has a large

morphological diversity of genera. The most common groups of compounds identified in this family include flavonoids, sesquiterpenes, diterpenes and acylated phloroglucinols. The biological/ pharmacological activities of the plant extracts and the compounds isolated from Asteraceae species have up to now not received enough attention. In this chapter, we intend to report on the isolation and structural elucidations of the compounds isolated from *Elytropappus rhinocerotis*.

2.4   The genus *Elytropappus*

2.4.1   Introduction

The genus *Elytropappus* Cass. (Asteraceae, tribe Gnaphaliea) derives its name from the Greek words *elytron* (sheath) and *pappos* (fluff) which is appropriate considering the fluffy feathery appearance of the top part of the seeds displayed by several species in this genus. This genus was first described by Cassini in 1816 based on *Gnaphalium hispidum* (recently known as *Elytropappus hispidus*). A comprehensive taxonomical study on *Elytropappus* was later carried out by Levyn in 1935. Her findings led to the grouping of the eight *Elytropappus* species into three groups (Group 1: *E. cyathiformis* and *E. hispidus*; Group 2: *E. longifolius*, *E. gnaphaloids*, *E. scaber* and *E. glandulosus*; Group 3: *E. rhinocerotis* and *E. adpressus*) (Levyns, 1926, 1935).

Koekemoer re-assessed the taxonomy of *Elytropappus* to establish the rank of its formal and informal grouping. In this study Koekemoer divided *Elytropappus* into three genera. The species which were previously in Group 2, together with *Stoebe intricata*, were grouped under a new genus *Mytovernic*, and Group 3 species (*E. rhinocerotis* and *E. adpressus*) were grouped under a new genus *Dicerothamnus* (Koekemoer, 2002). However, the proposed reclassification has not yet been published in a scientific journal; therefore, we will refer to *Elytropappus* in this thesis as a single genus.

All *Elytropappus* species are endemic to South Africa, particularly they are common in Cederberg (part of Cape floristic region). With the exception of *E. rhinocerotis*, *Elytropappus* species are not well known and there is no literature about their medicinal or ecological uses. *E. rhinocerotis* (Fig. 2.1), commonly known as "renosterbos or rhinoceros bush" is a bush-shrub of 1-2 m in height with small grayish-green leaves and tiny flower heads. During the shedding of the seeds, the brown chaffy bracts around each flower head open up, giving the plant a brownish fluffy appearance (Dorchin and Gullan, 2007; Levyns, 1935; Pool et al., 2009).

Figure 2.1: Habitat and aerial parts of *E. rhinocerotis*

(Photo: http://botany.cz/cs/dicerothamnus-rhinocerotis/)

"Renosterbos" is a dominant plant in Renosterveld. This vegetation, which is believed to have derived its name from "Renosterbos", was first described by Sparrman (a distinguished South African traveler) in 1775. "Renosterbos" is also found as far north as the Namaqualand and Richtersveld, the great escarpment around Molteno, and it also extends to the southern parts of Eastern Cape to East London. This plant tolerates both snow and fire and is abundant in heavily grazed areas as it is unpalatable to livestock (Proksch et al., 1982). As a result, farmers consider "renosterbos" as a major weed and a lot of research has been done on the eradication strategies and its biocontrol (Koekemoer, 2002).

2.4.2   Traditional uses

In traditional medicine, the powdered young tips and branches of *E. rhinocerotis* are used to treat colic, wind and diarrhoea in children. Adults take the infusions of the twigs (in brandy or wine) to treat indigestion, dyspepsia, gastric ulcers and stomach cancer. These infusions are also used as a tonic drink to improve appetite. *E. rhinocerotis* became popular for its use in the treatment of influenza and fever in the flu epidemic of 1918 (Hutchings et al., 1996; Thring and Weitz, 2006; Watt and Breyer-Brandwijk, 1962).

2.4.3   Phytochemistry and biological activities

Dekker et al. (1988) isolated a new labdane diterpene, rhinocerotinoic acid (**2.40**) from the aerial parts of *E. rhinocerotis*. This compound exhibited anti-inflammatory activity in both non-adrenalectomised and adrenalectomised rats. Gray et al. (2003) tried to isolate rhinocerotinoic acid from *E. rhinocerotis* but could not obtain this compound. Studies on the chemical composition of the leaf resin indicated that the methoxylated flavones, cirsimaritin (**2.41**), hispidulin (**2.42**), eupafolin (**2.43**) and quercetin (**2.44**) were the major products (Proksch et al., 1982).

Benzoic acid (**2.45**), its derivatives [hydroxybenzoic acid (**2.46**), protocatechuic acid (**2.47**) and veratric acid (**2.48**)], as well as cinnamic acid derivatives [p-coumaric acid (**2.49**), ferulic acid (**2.50**) and sinapic acid (**2.51**)] were reported as minor products from the leaf resin of *E. rhinocerotis* (Proksch et al., 1982). However, it is unclear whether compounds **2.41**-**2.48** are produced by *E. rhinocerotis* or by the insects that use this plant as a habitat. To date, three species of gall-inducing Diptera (*Spathulina peringueyi Bezzi* and two species belonging to the Cecidomyiidae family) are reported to reside within *E. rhinocerotis* (Dorchin and Gullan, 2007). Cardiac glycosides, saponins and tannins were detected from the crude sample of *E. rhinocerotis* (Scott et al., 2004).

However, these observations were based on colour reactions and the results should be treated with care.

2.40

2.41

2.42

2.43

2.44

2.45 R^1 = H, R^2 = H

2.46 R^1 = OH, R^2 = H

2.47 R^1 = OH, R^2 = OH

2.48 R^1 = OCH$_3$, R^2 = OCH$_3$

2.49 R^1 = H, R^2 = H

2.50 R^1 = OCH$_3$, R^2 = H

2.51 R^1 = OCH$_3$, R^2 = OCH$_3$
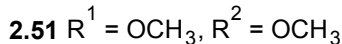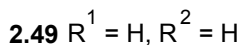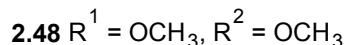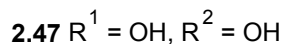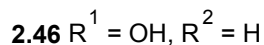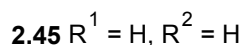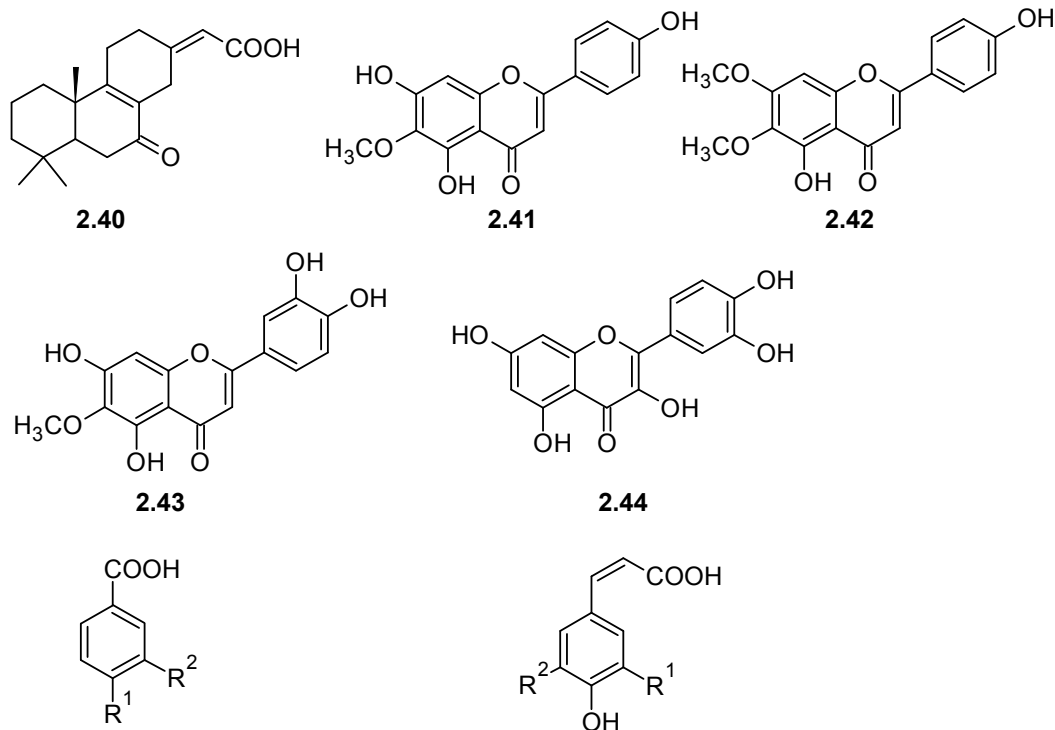
Knowles (2005) reported that the extracts of *E. rhinocerotis* showed antifungal properties against *Botrytis cinerea*, a fungal pathogen causing grey mould rot on a large number of economically and horticulturally important crops. This activity was more effective when the extracts were combined with synthetic fungicides. The methanol extract of the aerial parts showed moderate antimicrobial activity against *S. aureus*. A zone inhibition of 13 mm was observed compared to 9 mm (no activity) and 27 mm for ciprofloxacin (control) (Knowles, 2005).

The above discussion demonstrates that the phytochemistry of *E. rhinocerotis* is not well understood. Even though some compounds were reported to occur in this plant, further studies are required to confirm their occurrence and to isolate new compounds.

Herewith we report further phytochemical investigation of the aerial parts of *E. rhinocerotis*. The biological activities of the isolated compounds will also be evaluated. We will also report on the variation in the chemical composition of *E. rhinocerotis* plants collected in different locations.

2.5   Results and discussion

2.5.1   Introduction

The leaves and branches of *E. rhinocerotis* were collected on farm Weltevreded in Sneeuberg, Murraysburg, Western Cape. After air drying and milling, the aerial plant material was extracted with dichloromethane (DCM) - methanol (MeOH) (1:1). This extract was fractionated by silica gel chromatography to afford 6,7-dimethoxycoumarin (**2.52**), 5,7,4'-trihydroxyflavone (**2.53**), 5,7-dihydroxy-4'-methoxyflavone (**2.54**), 5,7-dihydroxy-6,4'-dimethoxyflavone (**2.55**), kaempferol 3-methyl ether (**2.56**), (+)-13-*epi*-labdanolic acid (**2.57**), (+)-labdanolic acid (**2.62**), (+)-labdanolic acid methyl ester (**2.64**) and (+)-labdanediol (**2.66**). Although other compounds have been isolated from *E. rhinocerotis* (Proksch et al., 1982), the isolation of compounds **2.52**-**2.57**, **2.62**, **2.64** and **2.66** from this species has not yet been reported. In the next section, the structural determination of these compounds is discussed.

2.5.2   6,7-Dimethoxycoumarin (2.52)
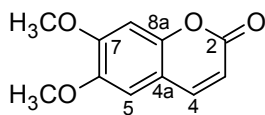
2.52

Compound **2.52** was fluorescing violet on TLC under UV light and showed a strong UV absorption peaks at λ_{max} 228 and 343 nm (Fig. 2.2). These observations suggested the presence of a coumarin chromophore in compound **2.52** (Hammoda et al., 2008; Steck and Bailey, 1969). The structure of **2.52** was further characterised by MS and

NMR data. A pseudo-molecular ion peak at m/z 229.0482 $[M+Na]^+$, which is in agreement with a molecular formula of $C_{11}H_{10}O_4$, was observed in the HRMS spectrum.
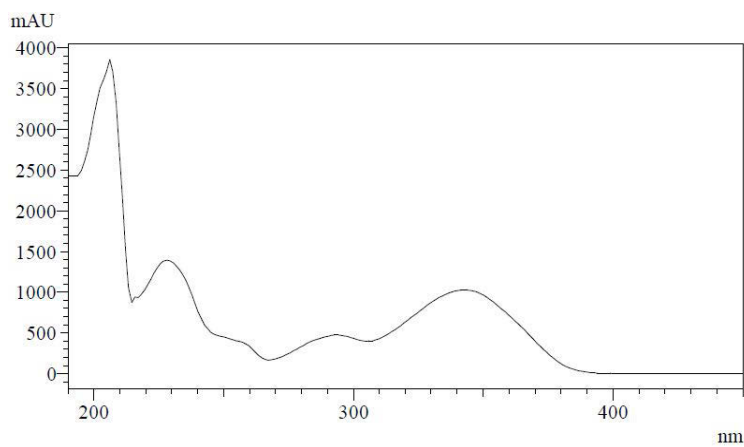
Figure 2.2: UV/Vis absorption spectrum of compound **2.52**

The appearance of two singlets integrating for three protons each at δ_H 3.91 and 3.94 in the ^{1}H NMR spectrum (Plate 1a) indicated the presence of two methoxy groups. The aromatic protons appearing as singlets at δ_H 6.83 (H-5) and 6.85 (H-8) are *para* to each other, and this suggested that the methoxy groups on the aromatic ring are in *ortho* positions (C-6 and C-7). The two doublets observed at δ_H 6.28 and 7.61 in ^{1}H NMR spectrum were assigned to H-3 ($J = 9.5$ Hz) and H-4 ($J = 9.5$ Hz) respectively. In the ^{13}C NMR spectrum (Plate 1b), ten signals corresponding to eleven carbons (the two OCH$_3$ carbon signals have the same chemical shift) were observed.

The DEPT NMR spectrum (Plate 1d) displayed six protonated carbons. The upfield signal at δ_C 56.4 in the ^{13}C and DEPT NMR spectra was assigned to the two methoxy carbons. The remaining four protonated carbon signals in the DEPT NMR spectrum were assigned to the methine carbons (C-3, C-4, C-5, and C-8). Four oxygen-linked non-protonated carbons were observed at δ_C 161.4 (C, C-2), 152.9 (C, C-7), 150.1 (C, C-8a) and 146.4 (C, C-6). The ^{1}H, ^{13}C NMR and the mass spectroscopy data led to the assignment of compound **2.52** as 6,7-dimethoxycoumarin.

Further structural confirmation was achieved by analysing the HMBC spectrum (Plate 1f). Correlations were observed between the aromatic singlets (H-5 and H-8) and the methoxy bearing carbons (C-6 and C-7) (Plate 1f and Fig. 2.3). A correlation between H-4 and the carbonyl carbon (C-2) also confirmed the proposed structural connection (Fig. 2.3). The experimental NMR data of compound **2.52** was in agreement with literature data for 6,7-dimethoxycoumarin, commonly known as scoparone (Céspedes et al., 2006).
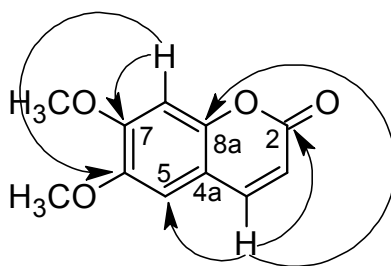
Figure 2.3: Key HMBC correlations in compound **2.52**

Scoparone (**2.52**) was previously isolated from several species in the Asteraceae family, such as *Artemisia tridentate* (Imamura et al., 1977), *Haplopappus foliosus* (Urzua, 2004) and *Artemisia capillaris* (Jang et al., 2006). Coumarins (including 6,7-dimethoxycoumarin) have been reported to exhibit antibacterial and antifungal activity against *S. aureus*, *S. agalactiae*, *S. uberis*, *S. dysgalactiae*, *E. coli* and *Salmonella* (Céspedes et al., 2006). Other activities of scoparone (**2.52**) include vasodilation, immunosuppression, radio-protection and anticoagulation activity (Gakuba, 2010).

2.5.3 5,7,4'-Trihydroxyflavone (2.53)
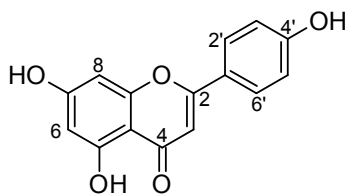
2.53

Compound **2.53** appeared as a brown spot on TLC under UV light and showed up as a bright yellow spot after treating the TLC with *p*-anisaldehyde/ H_2SO_4 stain followed by heating. The UV absorption spectrum (Fig. 2.4) of this compound showed λ_{max} at 267 and 338 nm, which is in agreement with the reported values for flavones (Rijke et al., 2006). A pseudo-molecular ion peak at m/z 269.0454 [M-H]$^-$ observed in the HRMS spectrum is in agreement with a molecular formula of $C_{15}H_{10}O_5$.
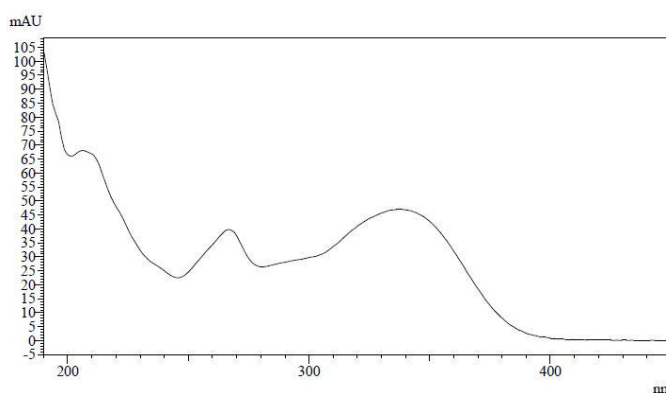
Figure 2.4: UV/Vis absorption spectrum of compound **2.53**

In the ^{1}H NMR spectrum (Plate 2a) of compound **2.53**, two *meta*-coupled doublets were observed at δ_H 6.18 (1H, d, J = 2.0 Hz, H-6) and 6.42 (1H, d, J = 2.0 Hz, H-8). This indicated the presence of a tetra-substituted A-ring of a flavone. A deshielded singlet appearing at δ_H 6.56 was assigned to H-3 of ring C. Two *ortho*-coupled signals, each integrating to two protons was observed at δ_H 6.92 (2H, d, J = 8.8 Hz, H-3', 5') and 7.84 (2H, d, J = 8.8 Hz, H-2', 6'), suggesting that the B-ring of the flavone was di-substituted at *para* positions.

In the ^{13}C NMR spectrum (Plate 2b), 15 signals were observed. The DEPT 135 spectrum (Plate 2d) revealed that the structure contained 7 methine carbons (of which two were overlapping) and 8 non-protonated carbons. A deshielded carbon signal at δ_C 183.7 was assigned to the carbonyl carbon (C-4). The other oxygen-linked carbons appeared at δ_C 166.1 (C, C-5), 165.8 (C, C-2), 162.9 (C, C-4', C-8a) and 159.6 (C, C-7).

In the HMBC spectrum (Plate 2f), correlations were observed between H-3, C-2, C-4, C-1', C-4a and C-8a; H-6, C-5, C-7 and C-4a; H-2'/6', C-2, C-1' and C-4'; as well as between H-3'/5', C-4' and C-2. Some of these correlations are shown in Figure 2.5. Based on the spectral information obtained from NMR, mass, UV/Vis absorption spectra and comparison with literature values (Ersoz et al., 2002), compound **2.53** was assigned as 4',5,7-trihydroxyflavone, also known as apigenin.
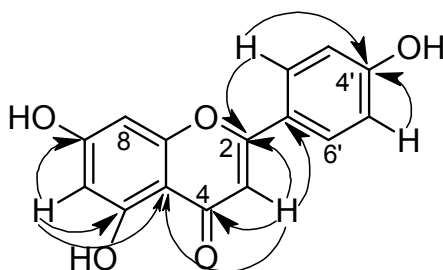
Figure 2.5: Key HMBC correlations in compound **2.53**

Asteraceae plants are well-known for producing a wealth of flavonoids. These plants have been reported to produce nearly all types of known flavonoids. Amongst the flavonoids featuring a six-membered C-ring, flavanones, flavones and flavonols are the most common groups. The most widely occurring substitution pattern displayed by flavones in this family is 5,7,4'-trioxygenation (apigenin type) and 5,7,3',4'-tetraoxygenation (luteolin type). The occurrence of apigenin (**2.53**) and its glycosidic derivatives have been reported in a large number of Asteraceae species and was identified in at least one species in 118 genera out of over 430 genera in this family (Bohm and Stuessy, 2001).

Some genera that are reported to contain of apigenin (**2.53**) belong to the same tribe (Gnaphalieae) as *Elytropappus*. Example of these genera are *Cassina*, *Ozothamnus*, *Odixia*, and *Gnaphalium* (Wollenweber et al., 2005; Wollenweber et al., 1997b; Wollenweber et al., 2008; Zheng et al., 2013). As mentioned earlier, this is the first report on the occurrence of apigenin (**2.53**) in *E. rhinocerotis*. Previously isolated flavones (**2.41**-**2.43**) from this plant displayed 5,6,7,4'-tetraoxygenation substitution pattern (scutellarein type), which is slightly different to the apigenin substitution (5,7,4'-trioxygenation) (Bohm and Stuessy, 2001).

Flavonoids have a potential as chemoprevention and chemotherapeutic agents. The inhibition of malignant human cancer cells was shown to be through mechanisms such as cell cycle arrest, induction of apoptosis, reversal of multi-drug resistance, antiproliferation, antioxidant, inhibition of angiogenesis and inhibition of telomerase activity (Lindenmeyer et al., 2001; Ramos, 2007; Ren et al., 2003). Apigenin was also reported to regulate diabetes mellitus, thyroid dysfunction and lipid peroxidation (Panda and Kar, 2007).

2.5.4   5,7-Dihydroxy-4'-methoxyflavone (2.54)
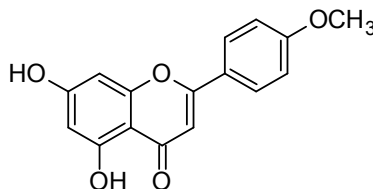
2.54

Similarly to compound **2.53**, compound **2.54** stained bright yellow on TLC treated with *p*-anisaldehyde/ H_2SO_4 stain followed by heating. This was indicative of the presence of a flavonoid moiety in this compound. The UV absorption spectrum (Fig. 2.6) supported the presence of a flavone structure as it showed absorption peaks at λ_{max} 268 and 333 nm (Rijke et al., 2006). In the HRMS spectrum, a pseudo-molecular ion peak at m/z 283.0609 [M-H]⁻, in agreement with the molecular formula of $C_{16}H_{12}O_5$, was observed.
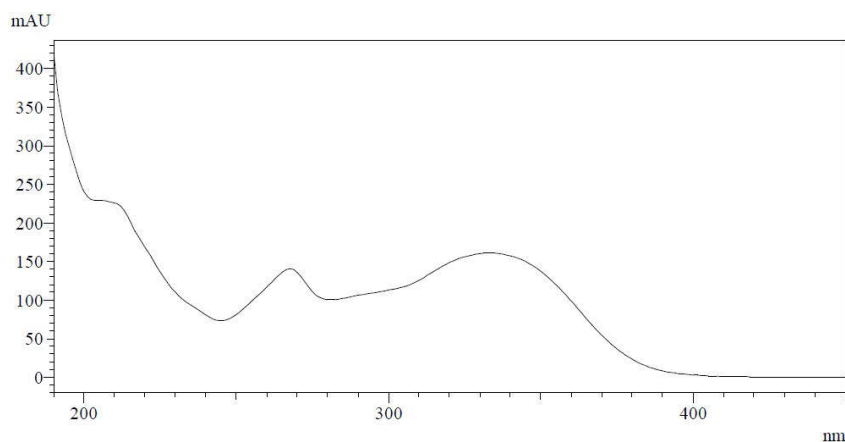
Figure 2.6: UV/Vis absorption spectrum of compound **2.54**

The ^{1}H (Plate 3a) and ^{13}C (Plate 3b) NMR spectra of compound **2.54** were similar to those of compound **2.53**. The only difference between these spectra was the presence of the methoxy singlet at δ_H 3.92 and δ_C 55.1 in the ^{1}H (Plate 3a) and ^{13}C (Plate 3b) NMR spectra of compound **2.54**. The position of this methoxy group was determined by analysing the HMBC NMR spectrum (Plate 3f). A HMBC correlation (Fig. 2.7) was observed between the methoxy proton signal and C-4' (δ_C 162.8), which suggested that the methoxy was attached to C-4'. The structure of compound **2.54** was assigned as 5,7-dihydroxy-4'-methoxyflavone (also known as acacetin or 4-*O*-methyl apigenin) and the experimental NMR data was in agreement with the reported data for this compound (Soon-Ho et al., 2003).
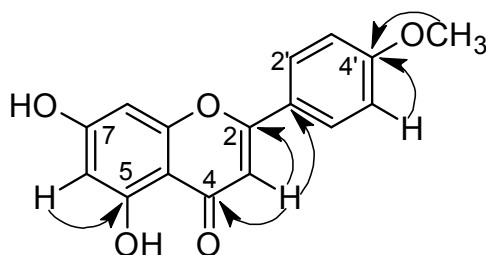
Figure 2.7: Some HMBC correlations in compound **2.54**

33

The isolation of acacetin (**2.54**) is reported for first time from *E. rhinocerotis* but this compound is common in other Asteraceae plants. Acacetin (**2.54**) was isolated from *Blainvillea rhomboidea* (Gomes et al., 2010), *Centaurea furfuracea* (Fakhfakh et al., 2005), *Microglossa pyrifolia* (Kohler et al., 2002), some species in the genus *Arnica* (Merfort, 1984; Schmidt and Willuhn, 2000), several *Artemisia* species (Valant-Vetschera and Wollenweber, 1995) and some *Baccharis* species (Wollenweber et al., 1986a). **2.54** were also isolated from a Korean medicinal plant, *Dendranthema zawadskii* var. *latilobum* Kitamura, where it showed antimicrobial activity against *Candida* species with an inhibition zone of 9-12 mm. This compound also showed moderate anticancer activity against human lung carcinoma (A549), skin melanoma (B16F1) and mouse melanoma (SK-MI-2) with an IC_{50} of > 40 µg/mL (Rahman and Moon, 2007).

2.5.5 5,7-Dihydroxy-6,4'-dimethoxyflavone (2.55)
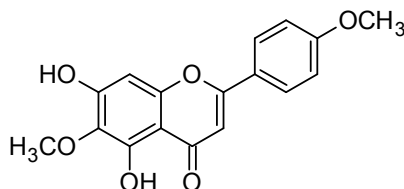
2.55

Compound **2.55** was identified as a flavone based on the colour of its spot on TLC and the UV absorption spectrum pattern. The compound stained yellow on TLC treated with *p*-anisaldehyde stain and the λ_{max} at 274 and 333 nm was obtained from the UV spectrum (Fig. 2.8). A pseudo-molecular ion peak at m/z 313.0714 [M-H]$^-$ observed in the HRMS spectrum is in agreement with the molecular formula ($C_{17}H_{14}O_6$).
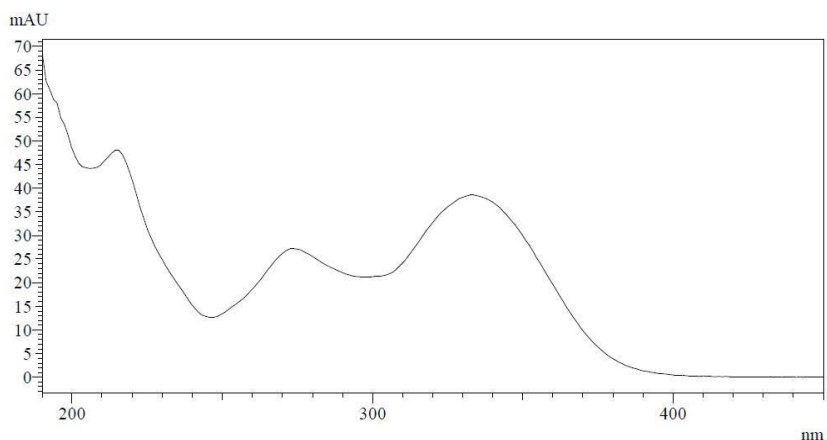
Figure 2.8: UV/Vis absorption spectrum of compound **2.55**

Two methoxy signals were observed in the ^{1}H and ^{13}C NMR spectra (Plate 4a & Plate 4b) at δ_H 3.89/ δ_C 55.5 and 4.04/ δ_C 60.8. The correlation between the methoxy protons at δ_H 3.89 and the C-4' carbon in the HMBC spectrum (Plate 4f) confirmed that the methoxy is attached to C-4'. The HMBC spectrum also showed that the second methoxy was attached to C-6. The 6-OMe experiences steric hindrance from the two *ortho* substituents which affects the aryl-O-bond and causes the methoxy group to adopt an out-of-plane conformation. This conformation results in an inefficient electron conjugation between the lone-pair of the methoxy oxygen and the aromatic ring which decreases electron density at the methoxy group and the ring carbons in the *ortho* and *para* positions to the methoxy group (Agrawal, 1989). This explains the observed downfield shift of the 6-OMe and C-5 signals in the ^{1}H and ^{13}C NMR spectra.

The combination of ^{1}H, ^{13}C NMR, UV/Vis absorption and mass spectral data led to the assignment of compound **2.55** as 5,7-dihydroxy-6,4'-dimethoxyflavone. The proposed structure was further confirmed by HMBC correlations observed between 5-OH signal, the methoxy bearing carbon (C-6) and C-4a; H-3, C-2 and C-1' and between H-8, C-7 and C-6 (Plate 4f, Fig. 2.9).
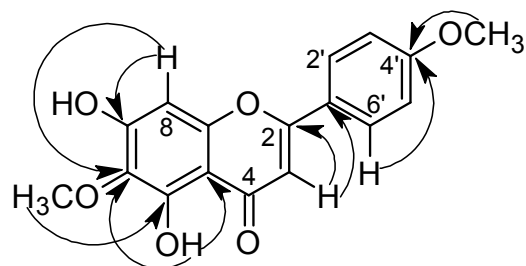
Figure 2.9: Important HMBC correlations in compound **2.55**

5,7-Dihydroxy-6,4'-dimethoxyflavone (**2.55**), commonly known as pectolinarigenin, is isolated for the first time from *E. rhinocerotis*. The substitution pattern (5,6,7,4') shown by this compound is similar to the pattern displayed by flavones **2.41**-**2.43** which were previously isolated from *E. rhinocerotis* (Proksch et al., 1982). Pectolinarigenin (**2.55**) was isolated from several Asteraceae species, including some members of the genus *Artemisia* (Valant-Vetschera and Wollenweber, 1995), *Erigeron breviscapus* (Vant.) Hand.-Mazz. (Zhang et al., 2000), *Heterotheca latifolia* (Rojo et al., 2004), *Arnica* species (Merfort, 1984; Schmidt and Willuhn, 2000), *Tanacetum macrophyllum* Willd (Ivancheva and Stancheva, 1997), *Baccharis* species and *Brickellia californica* (Torrey & Gray) A. (Wollenweber et al., 1997a; Wollenweber et al., 1986b).

Watanabe *et al*. (2014) reported on the isolation and phytotoxicity of pectolinarigenin (**2.55**) from *Onopordum acanthium* L. (Watanabe et al., 2014). **2.55** was also isolated from *Cirsium chanroenicum* and it showed *in vitro* and *in vivo* anti-inflammatory and anti-analgesic activities (Lim et al., 2008). Pectolinarigenin (**2.55)** prevented hepatic injury induced by D-galactosamine (GalN) in rats (Yoo et al., 2008).

2.5.6 Kaempferol 3-methyl ether (2.56)
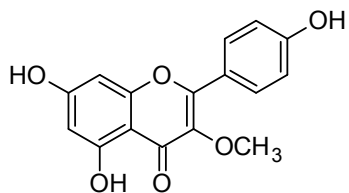
2.56

Compound **2.56** showed a yellow spot on TLC which intensified to a brown colour after spraying the TLC with anisaldehyde in concentrated H_2SO_4. This compound showed strong UV absorption peaks at λ_{max} 266 and 350 nm, which is in agreement with the reported values for flavonols (Fig. 2.10) (Moiseev et al., 2011). A pseudo-molecular ion peak at m/z 299.0721 [M-H]$^-$ was obtained from the LRMS spectrum. Two *ortho*-coupled doublets (integrating to 2H each) were observed in ^{1}H NMR spectrum (Plate 5a) at δ_H 6.95 and 8.00. These indicated the presence of an AA'XX' system of a 1,4 disubstituted aromatic ring. Two more doublets (integrating to 1 proton each) were observed at δ_H 6.23 ($J = 2.0$ Hz) and 6.44 ($J = 2.0$ Hz). These were *meta*-coupled and were assigned to the two protons of tetra-substituted A-ring of a flavone.
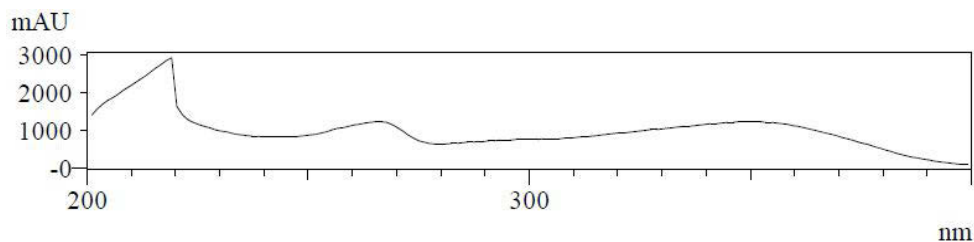
Figure 2.10: UV/Vis absorption spectrum of compound **2.56**

A methoxy signal appeared at δ_H 3.79/ δ_C 60.6 in ^{1}H and ^{13}C NMR spectrum (Plate 5b). This signal is in a deshielded position when compared to the methoxy of 5,7-dihydroxy-4'-methoxyflavone (**2.54**). This deshielding suggested that the methoxy group is in a sterically congested environment, which disturbs conjugation between the methoxy oxygen lone pairs and the C-ring. As a result, the methoxy group is in a less electron dense environment and therefore deshielded. An HMBC correlation (Plate 5f) was observed between the methoxy protons and carbon signal at δ_C 138.1. This carbon was

assigned as C-3 since no proton showed a cross peak to this carbon in the HSQC spectrum (Plate 5e). Also, C-3 is more deshielded in this compound when compared to compound **2.53**-**2.55**. This further indicated that C-3 was a non-protonated carbon in compound **2.56**. Some important HMBC correlations within compound **2.56** are shown in Figure 2.11. The structure of compound **2.56** was assigned as kaempferol 3-methyl ether since the experimental NMR data was in agreement with the reported values (Forgo et al., 2012; Gao et al., 2011; Marie et al., 2006).
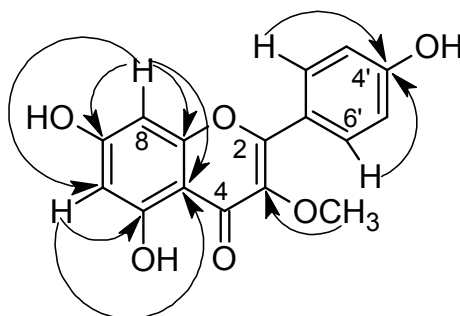
Figure 2.11: HMBC correlation in compound **2.56**

The occurrence of kaempferol 3-methyl ether (isokaempferide) (**2.56**) is reported for the first time from the extracts of *E. rhinocerotis*. A closely related flavonol (quercetin, **2.44**) was previously isolated from *E. rhinocerotis*. Isokaempferide (**2.56**) was previously isolated from several Asteraceae species such as *Cirsium palustre* (L.) Scop. (Nazaruk, 2009), some *Centaurea* species (Flamini et al., 2001; Forgo et al., 2012), *Chrysothamnus nauseosus* (Pallas) Britton ssp. *alhicaulis* (Nutt.) Hall & Clements, *Grindelia* species, *Ericam eria linearifolia* (DC.) Urb. & J. Wussow (Wollenweber et al., 1997a), *Pulicaria dysenterica.* (L.) Bernh, *Senecio viscosa* L. (Wollenweber et al., 1997b) and *Helichrysum italicum* (Wollenweber et al., 2005). Robin, *et al.* (1998) isolated isokaempferide (**2.56**) from *Psiadia dentate* and this compound showed antipoliovirus activity (Robin et al., 1998).

2.5.7 (+)-13-*epi*-Labdanolic acid (2.57)
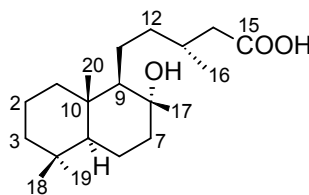
2.57

The ^{13}C NMR spectrum (Plate 6b) of compound **2.57** showed 20 signals. This suggested that compound **2.57** might be a diterpene. Furthermore, a high resolution mass spectrum for this compound showed a pseudo-molecular ion peak at m/z 324.2839 [M-H]$^-$ which is in agreement with the molecular formula of $C_{20}H_{36}O_3$. A non-protonated carbon signal observed at δ_C 177.9 in ^{13}C NMR spectrum is characteristic of a carboxylic acid carbonyl carbon. An HMBC (Plate 6f) correlation is observed between this carboxylic acid signal and the two doublets of doublets appearing at δ_H 2.14 (1H, J = 14.7, 7.9 Hz) and 2.39 (1H, J = 14.7, 6.4 Hz) in the ^{1}H NMR spectrum (Plate 6a). The ^{1}H-^{13}C correlations in the HSQC spectrum (Plate 6e) revealed that these doublets of doublets were due to the protons on the same carbon. This led to the assignment of these doublets of doublets as 14-CH$_2$ protons. An HMBC correlation was observed between 14-CH$_2$ protons and a methyl carbon signal at δ_H 0.98. This methyl carbon was then assigned as 16-CH$_3$. A ^{1}H-^{1}H correlation was observed in the COSY spectrum (Plate 6c) between the 16-CH$_3$ and a multiplet at δ_H 1.98, which led to the assignment of this multiplet as the C-13 methine signal.

Four methyl signals were observed at δ_H 1.15 (3H, s)/ δ_C 23.9, 0.84 (3H, s)/ δ_C 33.4, 0.78 (3H, s)/ δ_C 21.5 and 0.78 (3H, s)/ δ_C 15.4 in the ^{1}H and ^{13}C NMR spectra. This is characteristic of a labdane-type skeleton. Two methine signals at δ_H 0.91 (1H, m, J = 11.9, 1.9 Hz)/ δ_C 56.1 and δ_H 1.02 (1H, m)/ δ_C 61.8 were assigned to H-5 and H-9 respectively. The methylene signals in this compound were overlapping in the range δ_H 0.91 - 1.98 in the ^{1}H NMR spectrum. Long range ^{1}H/^{13}C correlations were observed between 16-Me, C-13 and C-14); Me-17, C-7, C-8, and C-9; Me-20, C-1, C-5 and C-9; H-5, C-4, C-9, C-10, C-18, C-19 and C-20; as well as between H-9, C-1, C-8, C-11 and C-20 (Fig. 2.12).
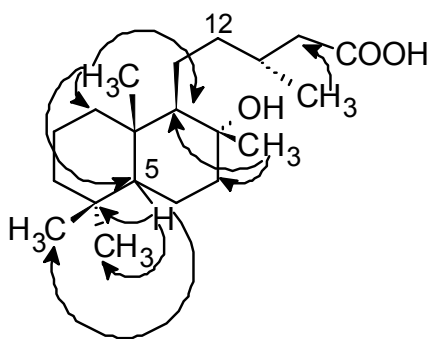
Figure 2.12: Important HMBC correlations in compound **2.57**

Compound **2.57** showed a specific rotation of +7° which was the same as the reported value for (+)-8α-hydroxylabdan-15-oic acid (Ramirez et al., 1998). The relative configuration at C-5, C-8, C-9 and C-10 was determined by analysis of the NOESY spectrum (Plate 6g and Fig. 2.13). Strong NOE correlations between Me-17, H-11 and Me-20 suggested that these groups were in the "same plane" and therefore have an all *cis*-configuration (Waibel et al., 1992). In addition, the Me-20 signal resonated upfield compared to the other methyl signals (Me-18 and Me-19). This also confirmed the *cis*-configuration of the methyl groups and the α-configuration for the OH-8 (Waibel et al., 1992).
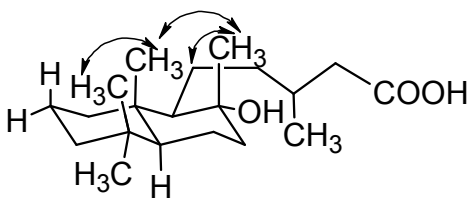
Figure 2.13: NOE correlations observed for compound **2.57**

Based on the NMR data, **2.57**, was assigned as labdanolic acid or its 13-epimer, but it was not possible to assign the relative configuration of C-13 or the absolute configuration of the compound. As will be discussed later, the differences between the NMR data for labdanolic acid and 13-*epi*-labdanolic acid are minimal and it not possible to distinguish between these two compounds using NMR spectroscopy.

40

Therefore, it was envisaged that an X-ray crystal structure analysis of compound **2.57** was necessary to determine the configuration of C-13. The absolute configuration at C-13 was previously determined for (-)-labdanolic acid (**2.58**), a compound which is structurally close to the isolated compound **2.57**. (-)-Labdanolic (**2.58**) was first isolated from *Cistus ladanifer* L. (Cistaceae) in 1956 (Cocker et al., 1956). The structure of **2.58** was determined by chemical degradation and comparison with the specific rotation of sclareol (**2.59**) and manool (**2.60**), but the C-13 configuration was not determined (Cocker and Halsall, 1956). Subsequent studies showed the configuration at C-13 of labdanolic acid to be (*R*) (Bory and Lederer, 1957). This assignment was based on the assumption that no intramolecular hydrogen bonding existed in this molecule, but this was soon challenged as results from other studies showed evidence of intramolecular hydrogen bonding (Bory and Lederer, 1957; Overton and Renfrew, 1967).

2.58   **2.59**   **2.60**
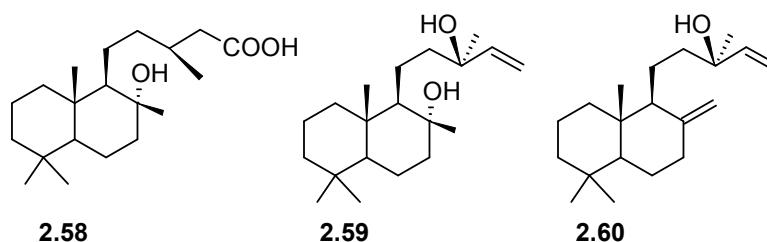
Overton and Renfrew (1967) studied the absolute configuration at C-13 of (-)-labdanolic acid and eperuic acid. In that study, the tricyclic hydroxy-esters of labdanolic acid and eperuic acid were synthesized and the NOE correlations of these esters was analysed. The configuration at C-13 was determined to be (*R*) for both acids (Overton and Renfrew, 1967). Bjamer et al. (1968) investigated the C-13 configuration of **2.58** by X-ray crystallography, where *p*-bromophenacyl esters of (-)-labdanolic acid and eperuic acid were synthesized and analysed. The results obtained in that study confirmed the absolute configuration at C-13 to be (*S*) for both (-)-labdanolic acid and eperuic acid (Bjamer et al., 1968). It is clear from the above discussion that there were controversial arguments used to establish the C-13 absolute configuration of labdanolic acid.

Recently, García-Sánchez et al. (2014) investigated the stereochemical assignment of labdane diterpenes isolated from *Ageratina jacotepecana* (Asteraceae) using vibrational circular dichroism (VCD) measurements. In this study, (+)-13-*epi*-labdanolic acid was

amongst the isolated compounds and its C-13 absolute configuration was confirmed as (*R*). This was based on the results reported by Bjamer et al (1968) for (-)-labdanolic acid (**2.58**) (García-Sánchez et al., 2014).

In our study, the X-ray crystal data will be compared with the literature data for (-)-labdanolic acid (**2.58**) and (+)-13-*epi*-labdanolic acid. In an effort to obtain crystals suitable for X-ray crystal structure analysis, the *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid was synthesised and the results obtained are discussed in the following section.

2.5.8 Synthesis of the *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61)

2.61
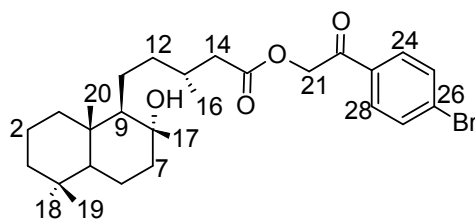
(+)-13-*epi*-Labdanolic acid (**2.57**) was treated with *p*-bromophenacyl bromide and triethylamine in *N,N*-dimethylformamide at 70 °C. After separation with column chromatography using hexane-ethyl acetate (8:2), the desired *p*-bromophenacyl ester was obtained as a white solid which recrystallized from $CHCl_3$-Hex (1:1) to afford well-formed monoclinic crystals, space group $P2_1$. This space group indicated that there is no centre of inversion in the crystal. While two molecules are observed in the unit cell (Fig. 2.14), these are not mirror images of each other. Therefore, compound **2.61** crystallized as enantiopure crystals. The absolute configuration was determined by studying the anomalous dispersion effect. As the structure contains a relatively heavy bromine atom and a high intensity Cu radiation was used for data collection, the dispersion effects became significant and absolute configuration was determined.
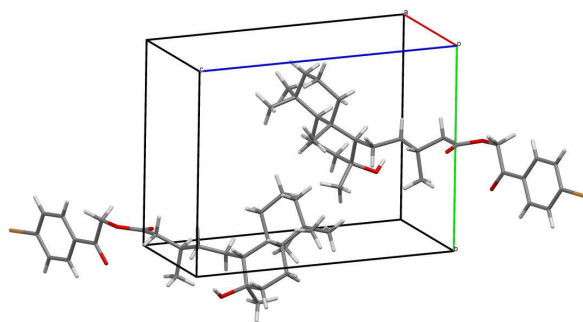
Figure 2.14: The unit cell of compound **2.61**.

A single molecule of the asymmetric unit of compound **2.61** is shown in Figure 2.15. The X-ray structure shows the absolute configurations to be 5*S*, 8*R*, 9R, 10*S* and 13*R*. These carbons are labelled as C-12, C-9, C-25 and C-13 respectively in Figure 2.15. The observed orientation of Me-17 and Me-20 in this X-ray structure of **2.61** is in agreement with the NOE correlation observed between these methyl groups in the isolated compound **2.57** (Plate 6g & Fig. 2.13). The length of the two C=O bonds averages to 1.206 Å while that of C-O bond averages to 1.445 Å. These values are in agreement with the values reported in literature for the average bond lengths of C=O and C-O bonds (Sutton, 1965). In addition, the C-Br bond measured to 1.885 Å, which is in agreement with the literature value of 1.85 ± 0.01 Å (Bjamer et al., 1968; Sutton, 1965).
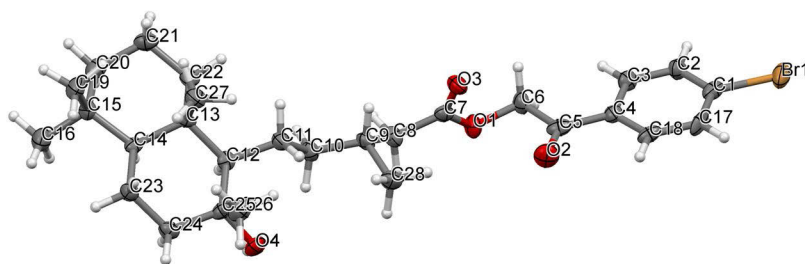
Figure 2.15: A partially labelled thermal ellipsoid plot of compound **2.61** showing 50% probability surfaces. All hydrogen atoms are shown as small spheres of arbitrary radius.

As stated above, the analysis of the X-ray crystal structure led to the assignment of C-13 absolute configuration as (*R*). Noteworthy, the X-ray crystal structure demonstrated the presence of intramolecular hydrogen bonding within compound **2.61** (Fig. 2.16). Therefore, compound **2.61** was assigned as the *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid and the isolated natural product (**2.57**) was assigned as (+)-(5*S*, 8*R*, 9*R*, 10*S*, 13*R*)-8-hydroxylabdan-15-oic acid, also known as (+)-13-*epi*-labdanolic acid. The X-ray crystal structure of an ester of (-)-labdanolic has been reported (Bjamer et al., 1968), but this is the first report of the X-ray crystal structure of *epi*-labdanolic acid or ester derivative of either enantiomers.
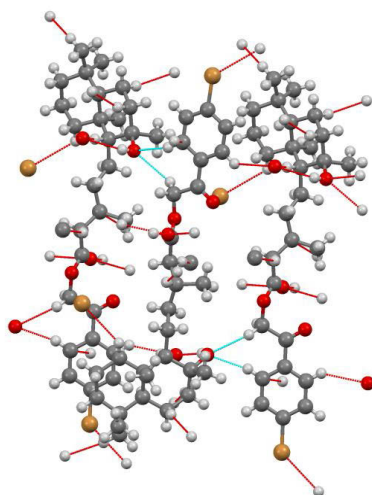
Figure 2.16: Hydrogen-bonded chains adopted by compound **2.61** running parallel to the b-axis. Hydrogen bonds between the molecules are shown in blue lines and red lines represent all bonds to atoms participating in hydrogen bonding.

To the best of our knowledge, this is a first report of the isolation of (+)-13-*epi*-labdanolic acid (**2.57**) from *E. rhinocerotis*. Compound **2.57** was previously isolated from some Asteraceae species such as *Espeletiopsis muiska* Cuatr (Cuatr) (Ramírez et al., 2000; Ramirez et al., 1998), *Stevia lucida* Lagasca (Amaro-Luis and Hung, 1988), and *Ageratina jocotepecana* (discussed earlier) (García-Sánchez et al., 2014). (+)-13-*epi*-Labdanolic acid (**2.57**) was obtained from the oxidation of (-)-13-*epi*-labdan-8α,15-diol which was isolated from *Oxylobus glanduliferus* (Amaro and Adrian, 1982; García-

Sánchez et al., 2014). (+)-13-*epi*-Labdanolic acid (**2.57**) was also isolated from some species in the genus *Cistus* (Cistaceae) (Venditti et al., 2014).

(+)-13-*epi*-Labdanolic acid (**2.57**) showed moderate antibacterial activity against *Bacillus subtilis* (MIC 1.56 mg/mL) and *Staphylococcus aureus* (MIC 2.34 mg/mL) (García-Sánchez et al., 2014). Labdane-type diterpenes (other than compound **2.57**) occur abundantly in nature and in the family Asteraceae and have been shown to exhibit a broad spectrum of biological activities, including, antibacterial, antifungal and cytotoxicity (Chinou, 2005; García-Sánchez et al., 2014).

2.5.9 (+)-Labdanolic acid (2.62)
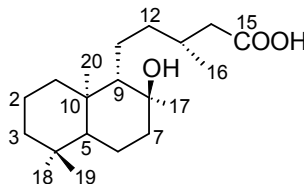
2.62

In the assignment of the structure of compound **2.62** it was observed that the [1]H (Plate 8a), [13]C (Plate 8b) and HRMS spectra were closely related to those of compound **2.57**. Slight differences in the chemical shifts were observed in the ^{1}H NMR spectral data (Plates 6a and 8a, and Table 2.1) at H-11 (1.25 / 1.40 in **2.57** and 1.32 / 1.32 in **2.62**), H-12 (0.91 / 1.61 in **2.57** and 1.32 / 1.48 in **2.62**), H-13 (1.98 in **2.62** and 1.93 in **2.62**), H-14 (2.14 / 2.39 in **2.57** and 2.20 / 2.35 in **2.62**). These differences in chemical shifts were not observed in the ^{13}C NMR spectra (Plate 6b and 8b). Similarly to (+)-13-*epi*-labdanolic acid (**2.57**), NOE correlations (Plate 8f and Fig. 2.12) were observed between the methyl signals in positions 17, 18, and 20, which indicated that these methyl groups were in the same plane. Although compound **2.57** and **2.62** had almost the same NMR spectra, their R_f values were different on TLC.

Compounds **2.57** and **2.62** showed pink spots on a TLC plate when treated with anisaldehyde/ H_2SO_4 stain and heated. Compound **2.57** showed an R_f value of 0.4 while the R_f value of compound **2.62** was 0.2 when using DCM-EtOAc (1:1). This separation on TLC revealed that compound **2.57** and **2.62** were diastereomers. A specific rotation

of (+7°) was obtained for compound **2.62** and this is same as the value obtained for **2.57**.

The NOE data (Fig. 2.12) showed that the relative configuration of C-5, 8, 9 and 10 of **2.57** and **2.62** were the same and thus the difference between the structures of these two compounds had to the configuration of C-13. Therefore, the structure of **2.62** was assigned as labdanolic acid. The specific rotation of **2.62** (+7°) is opposite to that reported for (-)-labdanolic acid (**2.58**) which proved that **2.62** is the enantiomer of **2.58.**

The relative configuration at C-13 in diterpene **2.62** was suggested to be (*R*) based on the observed positive specific rotation (+7°) which is similar to the specific rotation for 13-*epi*-labdanolic acid (**2.57**). There is only one chromophore in both **2.57** and **2.62.** It is clear that the specific rotation of these compounds is influenced by the C-13 stereocentre in close proximity to this chromophore rather than the configuration of the stereocenters in the more remote decahydronaphthaleme ring.
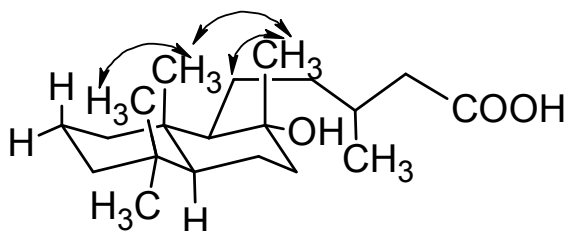
Figure 2.17: NOE correlations observed in compound **2.62**

Table 2.1: ^{1}H and ^{13}C NMR data (400 MHz, CDCl$_3$) of compounds **2.57** and **2.62**.

Position	δ$_C$ (2.57)	δ$_H$ (2.57)	δ$_C$ (2.62)	δ$_H$ (2.62)
1	39.8 (CH$_2$)	1.40 (6H, m)	39.7 (CH$_2$)	0.93 (1H, m,) 1.63 (4H, m)
2	18.4 (CH$_2$)	1.40 (6H, m) 1.61 (4H, m)	18.4 (CH$_2$)	1.42 (2H, m) 1.56 (1H, dt, 13.5, 3.2)
3	41.9 (CH$_2$)	1.15 (4H, s) 1.40 (6H, m)	41.9 (CH$_2$)	1.15 (4H, s) 1.38 (4H, m)
4	33.2 (C)		33.2 (C)	
5	56.1 (CH)	0.91 (2H, m, 11.9, 1.9)	56.1 (CH)	0.89 (1H, m,)
6	20.5 (CH$_2$)	1.25 (3H, m) 1.61 (4H, m)	20.5 (CH$_2$)	1.63 (4H, m) 1.26 (7H, m)
7	44.3 (CH$_2$)	1.40 eq (6H, m) 1.86 ax (1H, dt, 12.3, 3.1)	44.2 (CH$_2$)	1.38 (4H, m) 1.86 (1H, dt, 12.3, 2.8)
8	74.7 (C)		74.6 (C)	
9	61.8 (CH)	1.02 (1H, m)	62.2 (CH)	1.02 (1H, m, 3.85)
10	39.1 (C)		39.1 (C)	
11	21.9 (CH$_2$)	1.25 (3H, m) 1.40 (6H, m)	22.9 (CH$_2$)	1.32 (4H, m)
12	39.6 (CH$_2$)	0.91 (2H, m, 11.9, 1.9) 1.61 (4H, m)	40.6 (CH$_2$)	1.32 (4H, m) 1.48 (2H, m)
13	30.8 (CH)	1.98 (1H, m)	31.2 (CH)	1.93 (1H, m)
14	40.9 (CH$_2$)	2.14 (1H, dd, 14.7, 7.9) 2.39 (1H, 14.7, 6.4)	41.0 (CH$_2$)	2.20 (1H, dd, 14.8, 7.5) 2.35 (1H, 14.8, 5.9)
15	177.9 (C)		177.0 (C)	
16	19.9 (CH$_3$)	0.98 (3H, d, 6.8)	19.9 (CH$_3$)	0.99 (3H, d, 6.7)
17	23.9 (CH$_3$)	1.15 (4H, s)	24.0 (CH$_3$)	1.15 (4H, s)
18	33.4 (CH$_3$)	0.84 (3H, s)	33.4 (CH$_3$)	0.86 (3H, s)
19	21.5 (CH$_3$)	0.78 (6H, s)	21.5 (CH$_3$)	0.79 (6H, s)
20	15.4 (CH$_3$)	0.78 (6H, s)	15.4 (CH$_3$)	0.79 (6H, s)

2.5.10 Synthesis of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63)

2.63
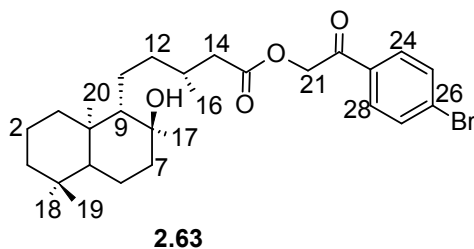
(+)-Labdanolic acid (**2.62**) was treated with *p*-bromophenacyl bromide and triethylamine in DMF at 70 °C. The product was purified by a chromatotron using hex-EtOAc (8:2) to furnish a white solid of *p*-bromophenacyl ester of (+)-labdanolic acid. Recrystallization of this ester from CHCl$_3$-Hex (1:1) afforded white needle-like crystals. Unfortunately, we could not obtain suitable crystals for X-ray crystal structure analysis. The structure was therefore analysed using NMR and HRMS spectral data. Two singlets were observed in the ^{1}H NMR spectrum (Plate 9a) at δ_H 5.26 (1H, s) and 5.29 (1H, s). A ^{1}H/^{13}C short range correlation was observed (Plate 9e) between these singlets and a deshielded methylene carbon signal at δ_C 65.6 and these protons were assigned as H-21 methylene protons.

Two sets of aromatic protons appeared at δ_H 7.63 (2H, d, *J* = 8.5 Hz) and 7.77 (1H, d, *J* = 8.5 Hz) in ^{1}H NMR spectrum (Plate 9a). This was indicative of the presence of a 1,4-disubstituted aromatic ring system. The remaining signals in ^{1}H NMR spectrum were closely similar to those observed for compound **2.63**. 26 Signals were obtained from ^{13}C NMR spectrum (Plate 9b). Two carbonyl carbon signals at δ_C 172.6 and 191.6 were present. The first signal (172.6 ppm) suggested the presence of an ester group in this compound, while the second signal (191.6 ppm) represented a ketone carbonyl carbon. Four aromatic signals existed at δ_C 129.1 (C, C-23), 129.3 (CH, C-25, 27), 132.2 (CH, C-24, 28) and 132.9 (C, C-26) in ^{13}C NMR spectrum, this was in agreement with the proposed structure. Other signals observed in ^{13}C NMR spectrum were similar to those discussed for compound **2.62**.

To assist with the determination of the relative and absolute configuration of the compounds **2.57** and **2.62**, the electronic circular dichroism (ECD) spectra of both the phenacyl ester derivatives of these two compounds were run. However, no absorptions

were observed in the spectra. It is clear that free rotation in the side chain containing the chromophore prevent ECD to be useful in determining the absolute configuration of these compounds.

2.5.11   (+)-Labdanolic acid methyl ester (2.64)
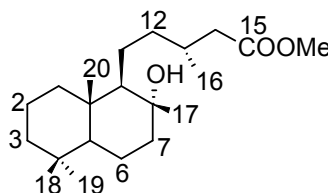
2.64

A compound (**2.64**) with pseudo-molecular ion peak at m/z 361.2633 [M+Na]$^+$, which is in agreement with a molecular formula of $C_{21}H_{38}O_3$, was isolated. Most of the signals in the ^1H and ^{13}C NMR (Plates 10a and 10b) spectra of this compound were similar to those of 13-*epi*-labdanolic acid (**2.57**). But, this compound was different to compound **2.57** as the carboxylic acid signal at 177 ppm in ^{13}C NMR spectrum (Plate 10b) was not observed. Instead, a shielded signal was observed at δ_C 173.9 in ^{13}C NMR spectrum, which is characteristic of an ester carbonyl carbon. In addition, a methoxy signal was observed at δ_H 3.61 and δ_C 51.3 in ^1H and ^{13}C NMR spectra respectively. Further comparison between the experimental and literature data, suggested that compound **2.64** was the methyl ester derivative of (+)-*epi*-labdanolic acid.

The assignment of the relative configuration of compound **2.64** was achieved by analysing NOE correlations and by comparison of signals in ^1H and ^{13}C NMR spectra with those of both (+)-*epi*-labdanolic acid (**2.57**) and (+)-labdanolic acid (**2.62**). NOE correlations were observed between Me-17, Me-18 and Me-20 (Fig. 2.18). ^1H NMR spectra of compound **2.57**, **2.62** and **2.64** were overlapped to observe differences between these compounds. Compound **2.64** was observed to be similar to (+)-13-*epi*-labdanolic acid (**2.57**). Therefore, the relative configuration in compound **2.64** was predicted as 9*R*, 10*S*, 13*R*, 17*R*.

Several steps in our extraction process involved the use of methanol. It is possible that the (+)-labdanolic acid methyl ester (**2.64**) that was isolated was an artefact formed from (+)-epi-labdanolic acid during the extraction process.
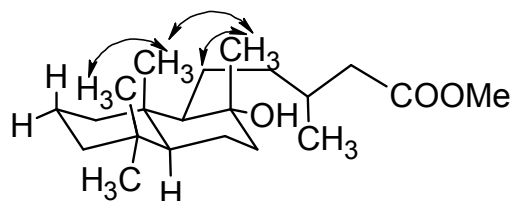
Figure 2.18: NOE correlations observed for compound **2.64**

The C-13 epimer of **2.64** [(-)-labdanolic acid methyl ester (**2.65**)] was prepared by the oxidation of (-)-labdanediol with Jones reagent to give the carboxylic acid derivative which was subsequently methylated with an excess of MeSiCHN$_2$ (Girón et al., 2010). (-)-Labdanolic acid methyl ester (**2.65**) showed *in vitro* anti-inflammatory effects when tested on lipopolysaccharide (LPS)-treated RAW 264 macrophages and it exhibited *in vivo* anti-inflammatory activity when tested in mice. The potency showed by compound **2.65** was similar to that of the commonly used anti-inflammatory drug, indomethacin, which indicated the potential of using this compound for the design of new anti-inflammatory drugs (Cuadrado et al., 2012a; Girón et al., 2010).

2.65
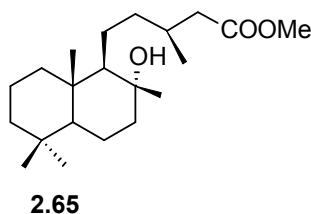
2.5.12   (+)-Labdanediol (2.66)
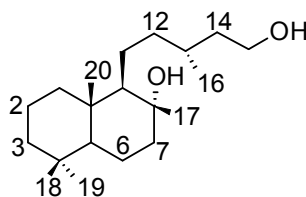
2.66

Another labdanolic acid derivative (**2.66**) was isolated from the aerial parts of *E. rhinocerotis*. A majority of signals in ^{1}H and ^{13}C NMR spectra (Plate 11a and 11b) of this compound (**2.66**) were similar to labdanolic acids **2.57** and **2.62**. A remarkable difference between compound **2.66** and the labdanolic acids **2.57** and **2.62** was the existence of a deshielded methylene signal at δ_H 3.7 in ^{1}H NMR spectrum of compound **2.66**. This downfield shift of this methylene signal suggested that this methylene was oxygen-linked, and thus was assigned as H-15. After comparison of the ^{1}H and ^{13}C NMR data with the literature data, compound **2.66** was confirmed to be (+)-labdanediol. The experimental specific rotation (+12°) of compound **2.66** was also in agreement with the literature value for labdanediol (+10°) (Dawson et al., 1966).

The occurrence of (+)-labdanediol (**2.66**) is reported for the first time from the family Asteraceae. (-)-Labdanediol (**2.67**) was previously isolated from one Asteraceae specie, *Oxylobus glanduliferus* (Amaro and Adrian, 1982). Recently, Girón et al (2010) used (-)-labdanediol as a starting material in the preparation of several derivatives, including (-)-labdanolic acid (**2.58**) and (-)-labdanolic acid methyl ester (**2.65**). The results obtained in this study showed that (-)-labdanediol (**2.67**) together with some derivatives, are potent inhibitors of LPS-induced NO and PGE$_2$ production in macrophage cells and showed anti-inflammatory activity in mice (Cuadrado et al., 2012b; Girón et al., 2010).
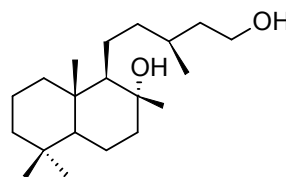
2.67

There are also several reports about the anti-inflammatory properties of labdane diterpenes (De Las Heras et al., 1999; Girón et al., 2008; Matsuda et al., 2002; Shen et al., 2002). (-)-Labdanediol (**2.67**) was also isolated as a major constituent from *Cistus ladaniferus* L. and was converted to 12-nor-ambreinolide which is a precursor of ambrox, a valuable amber-type odorant (Urones et al., 1992).

2.5.13   Conclusion

The occurrence of flavonoids **2.52**-**2.56** are reported for the first time from *E. rhinocerotis*, however, other flavonoids were isolated from this species (Proksch et al., 1982). The labdane diterpenoids **2.57**, **2.62**, **2.64** and **2.66** are not common in the Asteraceae family, but other labdane-type diterpenoids occur abundantly in this family. The biological activities of the isolated flavones and diterpenes were obtained from literature and these activities were compared with the traditional uses of this plant. The reported anticancer activity of *E. rhinocerotis* crude extracts may be attributed to the flavonoids found in this plant. While we isolated four substituted flavones, various flavonoids were present in small quantities in the ethyl acetate extract of this plant (Fig. 2.15).

The previously isolated labdane diterpene (rhinocerotinoic acid), which showed anti-inflammatory properties (Dekker et al., 1988) was not isolated nor detected in the *E. rhinocerotis* extracts used in this study. However, the 13-C epimers of the isolated labdane diterpenes **2.57**, **2.62**, **2.64** and **2.66** showed anti-inflammatory activities (Girón et al., 2010). This suggests that this plant may be a useful source of natural diterpenes with anti-inflammatory properties. *E. rhinocerotis* is one of the plants that have been reported to be used in South Africa during the 1918 influenza pandemic. From the constituents present in this plant it is most likely that the anti-inflammatory effect of the plant extracts might have played a role in its use.

2.6   HPLC studies

2.6.1   Introduction

Several studies have shown variations in the chemical profiles of the same plant collected in different geographic areas. These variations are normally associated with climatic changes, and the differences in the genetic type of the species (Dong et al., 2003). It is therefore important to evaluate the chemical profiles of species collected in different geographic locations before making a general statement on the composition and biological activities of the plants. Reversed-phase high performance liquid chromatography coupled to photodiode array detection/electrospray or atmospheric pressure ionization mass spectrometry (HPLC-PDA/ESI-MS/APCI-MS) has been used as a rapid and powerful tool to define the chemical profiles of complex raw extracts from plants without isolating and cleaning-up their individual constituents (Ferreres et al., 2011). The type of compounds present in the plant extracts are determined by comparing their retention times with those of standard samples run under the same conditions. Additionally, the UV/Vis absorption profile and the data obtained from the mass spectra of the unknown compounds are compared with those reported in literature for various groups of compounds.

Therefore, the aim of this study was to use HPLC-PDA/ESI-MS/APCI-MS in the analysis of the chemical variations in *E. rhinocerotis* collected from different locations (location 1, 2 and 3) in a farm in Weltevrede, Western Cape. The phytochemistry of the sample collected from location 1 was studied in this research and the results were discussed in Section 2.6. The flavonoids **2.52**-**2.56** isolated from this extract were used as reference compounds. The HPLC method which separated these flavonoids was developed and this method was used to analyze the three crude extracts (collected from location 1, 2, and 3).

2.6.2   Analysis of *E. rhinocerotis* collected from location 1 on farm Weltevreded.

The aerial parts of *E. rhinocerotis* were collected from location 1 on farm Weltevreded, Sneeuberg, Murraysburg (Western Cape). After drying, crushing, and extraction with DCM-MeOH (1:1), the crude extract was fractionated using column chromatography to obtain EtOAc extract. The crude EtOAc extract was further purified on silica gel as described in Section 2.6 and the pure compounds were assayed using HPLC-PDA. Compounds **2.52**-**2.56** (0.1 mg each) were redissolved in methanol and were analyzed by reverse phase HPLC using gradient elution method with MeOH/1% AcOH in H_2O. The most polar coumarin **2.52** eluted at 7.445 minutes followed by apigenin (**2.53**) at 16.635 min from HPLC (Fig. 2.19 A & B). As expected, 5,7-dihydroxy-4'-methoxyflavone (**2.54**) and 5,7-dihydroxy-6,4'-dimethoxyflavone (**2.55**) were retained for a longer time in the column and they eluted at 31.338 and 31.290 min, respectively (Fig. 2.19 C & D). This is because the methyl ether substituents (-OMe) in compound **2.54** and **2.55** are more non-polar than the hydroxy (-OH) substituents in apigenin (**2.53**).

A Phenomenex Luna C18 (2) column (250 x 4.60 mm, 5μ) was blocked while we were in the process of analyzing kaempferol 3-methyl ether (**2.56**). Compound **2.56** was then analyzed using Phenomenex kinetex C18 column (100 x 4.60 mm, 2.6μ). Kaempferol 3-methyl ether eluted at 10.298 min (Fig. 2.19 E) and this peak showed the same UV/Vis absorption spectrum to the peak at 17.771 min in the crude *E. rhinocerotis* sample. The structural characterization of compounds **2.52**-**2.56** was confirmed by comparing their UV/Vis absorption spectra (Fig. 2.19) with literature, as well as analyzing their NMR and HRMS spectral data (discussed in Section 2.5).

The second step was to analyse the *E. rhinocerotis* crude extract from location 1 using HPLC-PDA under the same conditions as the pure analytes to determine its chemical composition. Figure 2.20 shows the HPLC chromatogram of the crude extract and the isolated flavonoids **2.52**-**2.56** is labelled. The HPLC method used to analyse this sample separated all the reference analytes, therefore, this method was used to analyse the other *E. rhinocerotis* plants using HPLC-PDA/MS.
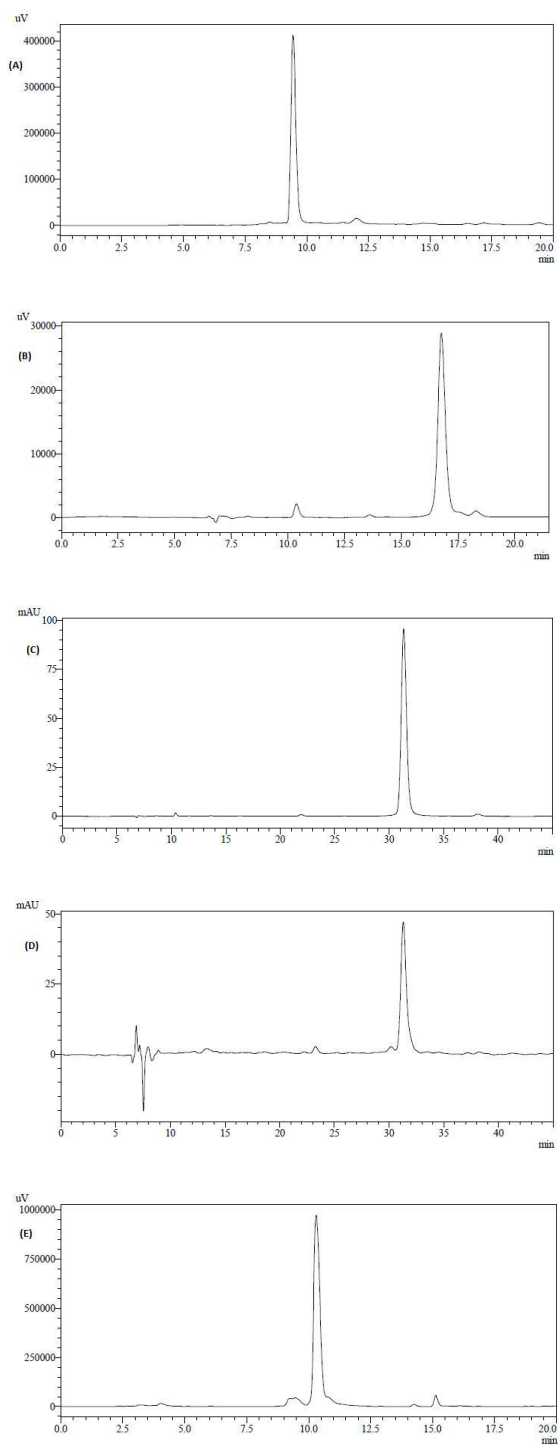
Figure 2.19: HPLC chromatogram and UV/Vis absorption spectra of flavonoids **2.52-2.56** from *E. rhinocerotis* (location 1).
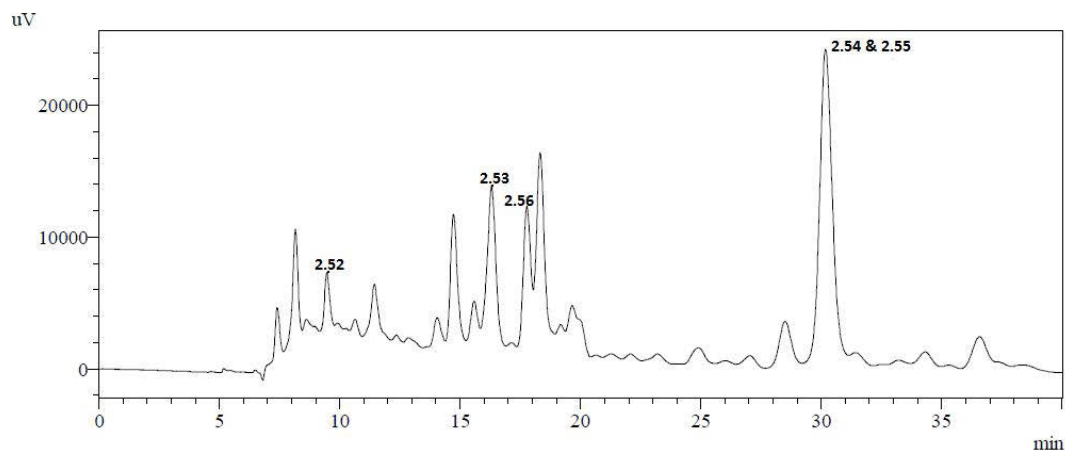
Figure 2.20: HPLC-PDA chromatogram of *E. rhinocerotis* collected from location 1 on farm Weltevreded.

The *E. rhinocerotis* extract from location 1 was further analysed by HPLC-ESI-MS and APCI-MS to identify and characterise the structures of both the UV/Vis absorbing and UV/Vis non-absorbing compounds. Flavonoids **2.52**-**2.56** were identified in the APCI-MS(-) and ESI-MS(+) spectra (Fig. 2.21 B & C). (+)-13-*Epi*-labdanolic acid (**2.57**), (+)-labdanolic acid (**2.62**) were identified from the APCI-MS(-) mode (Fig. 2.21 B), while labdanolic acid methyl ester (**2.64**) was identified from APCI-MS(+) (Fig. 2.21 A). (+)-Labdanediol (**2.66**) was not identified in the crude extract, which may be due to the very small amounts of this compound relative to the other compounds in the crude extract.
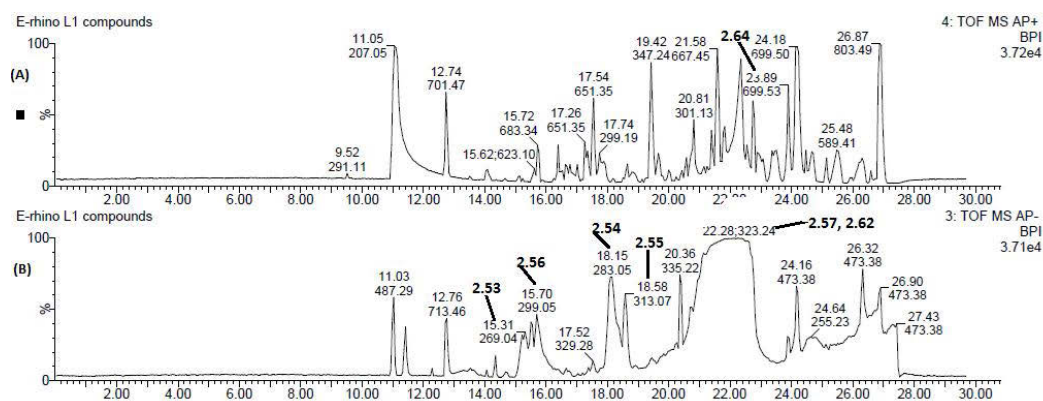
Figure 2.21: HPLC-LCMS chromatogram of the aerial parts of *E. rhinocerotis* collected from location 1 on farm Weltevreded.

Figure 2.22: HPLC-LCMS chromatogram of the aerial parts of *E. rhinocerotis* collected from location 1 on farm Weltevreded.

2.6.3   Analysis of *E. rhinocerotis* collected from location 2 on farm Weltevreded.

E. rhinocerotis was collected in a different location (location 2) on farm Weltevreded. Leaves were separated from branches, washed with water, dried, and each extracted with DCM-MeOH (1:1). The crude extracts were analysed by HPLC-PDA/ESI-MS and APCI-MS. From the leaves, apigenin (**2.53**), 5,7-dihydroxy-4'-methoxyflavone (**2.54**) and kaempferol 3-methyl ether (**2.56**), were identified from the APCI-MS(-) and ESI-MS(-) chromatograms (Fig. 2.22 B & C). In addition, 13-*epi*-labdanolic acid (**2.57**), (+)-labdanolic acid (**2.62**) and (+)-labdanolic acid methyl ester (**2.64**) were also observed from the APCI-MS(-) and ESI-MS(+) spectra (Fig. 2.22 B & D). 5,7-Dihydroxy-6,4'-dimethoxyflavone (**2.55**) and (+)-labdanediol (**2.66**) were not observed in the LCMS spectra. It is possible that this plant does not produce compounds **2.55** and **2.66** or the concentrations of these compounds are too small relative to the other compounds present.

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Figure 2.23: HPLC-PDA/LCMS chromatogram of the leaves of *E. rhinocerotis* collected from location 2 on farm Weltevreded.

From the branches, pseudo-molecular ion peaks in agreement with the molecular masses of flavones 5,7-dihydroxy-4'-methoxyflavone (**2.54**), 5,7-dihydroxy-6,4'-dimethoxyflavone (**2.55**) and kaempferol-3-methyl ether (**2.56**) were identified from the APCI-MS(-) (Fig. 2.23 B). Interestingly, no peaks corresponded to labdanolic acid and its derivatives (**2.57**, **2.62**, **2.64** and **2.66**). These results show that there are a lot of variations between the chemical composition of the leaves and the branches of this plant.

Figure 2.24: HPLC-PDA/LCMS chromatogram of the branches of *E. rhinocerotis* collected from location 2 on farm Weltevreded.

2.6.4 Analysis of *E. rhinocerotis* collected from location 3 on farm Weltevreded.

E. rhinocerotis plant was collected from location 3 on farm Weltevreded. Leaves were separated from branches and the dried plant material was extracted with DCM-MeOH (1:1). The crude extracts were also analysed by HPLC-PDA/ESI-MS/APCI-MS. From the UV/Vis absorption chromatogram, compounds **2.52**-**2.56** were identified (Fig. 2.24 A). The pseudo-molecular ion peaks corresponding to the molecular masses of compounds **2.53**-**2.56** was observed in the APCI-MS(-) (Fig. 2.24 B). Scoparone (**2.52**) co-eluted with an unidentified compound at 11.01 min from the HPLC column. Compound **2.52** showed a pseudo-molecular ion peak at m/z 229.0422, while compound (**x**) showed a peak at m/z 261.0707 in the ESI-MS(+) mode (Fig. 2.24 D). 13-*Epi*-labdanolic acid (**2.57**) and (+)-labdanolic acid (**2.62**) were also observed from the APCI-MS(-) spectrum (Fig. 2.24 B). Labdanolic acid methyl ester (**2.64**) and labdanediol (**2.66**) were not identified in this extract. Five of the seven identified compounds in this extract (**2.53**, **2.54**, **2.55**, **2.57** and **2.62**) were also detected from *E.*

rhinocerotis extract collected from location 2. This showed very close similarities in the chemical composition of the leaves collected from these two locations.
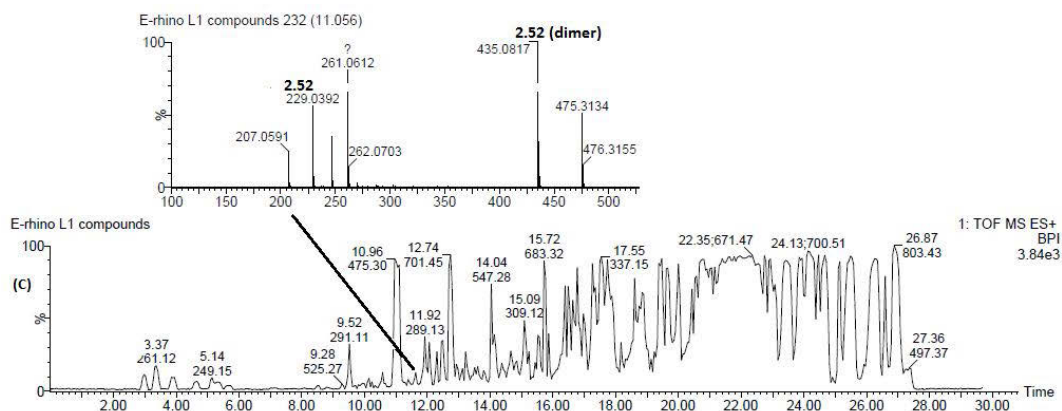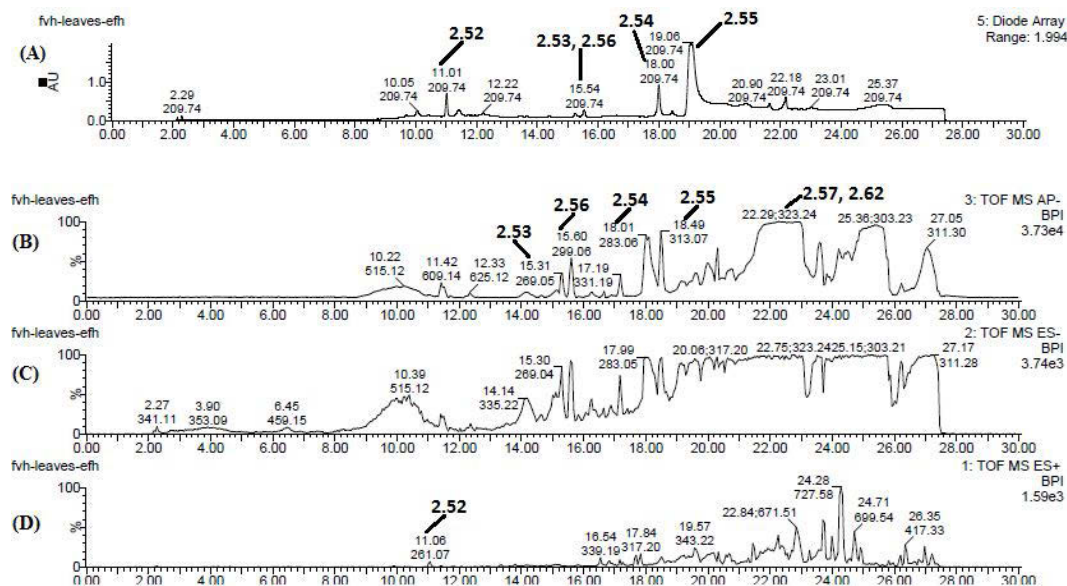

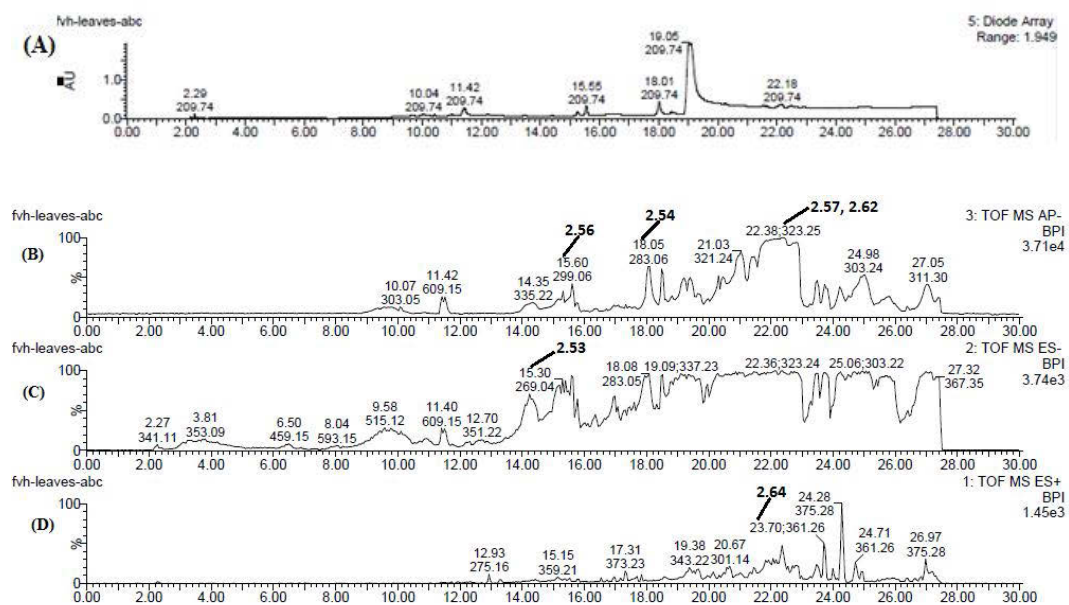**Figure 2.25**: HPLC-PDA/LCMS chromatogram of the leaves of *E. rhinocerotis* collected from location 3 on farm Weltevreded.

The crude extract of the branches from location 3 was assayed by HPCL-LCMS and the molecular masses and retention times in agreement with 5,7-dihydroxy-4'-methoxyflavone (**2.54**), 13-*epi*-labdanolic (**2.57**), (+)-labdanolic acid (**2.62**) and labdanediol (**2.66**) were observed in the LC-MS spectra (Fig. 2.25 B & C). The chemical composition of this sample was very different to that of branches from the *E. rhinocerotis* collected in location 2. The only common compound between these samples was dihydroxy-4'-methoxyflavone (**2.54**). In general, this sample (branches from location 3) was dominated by the labdanolic acids (**2.57**, **2.62** and **2.66**) while only flavonoids could be detected from the location 2 sample.
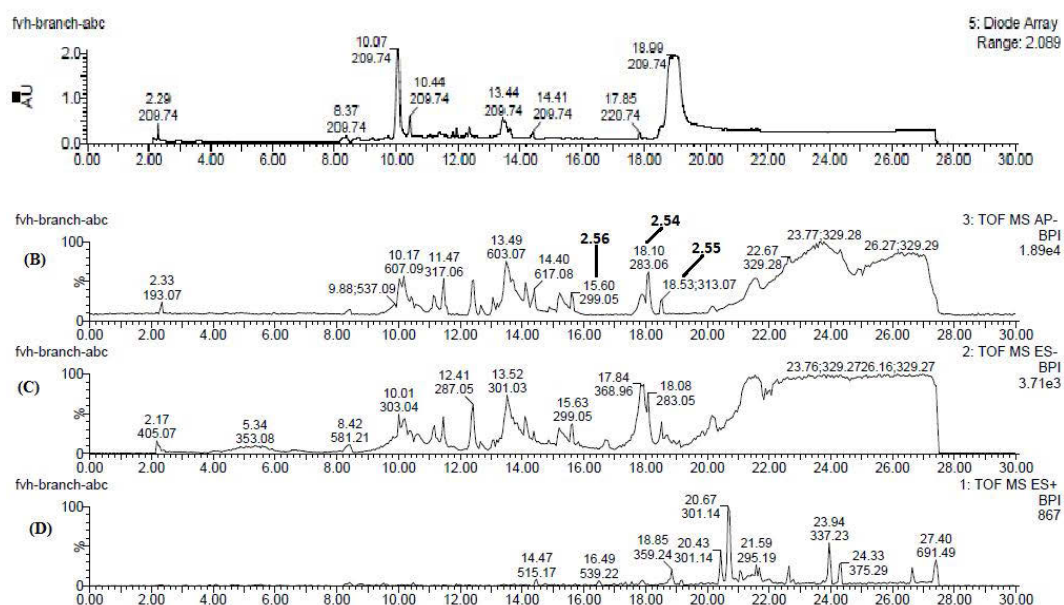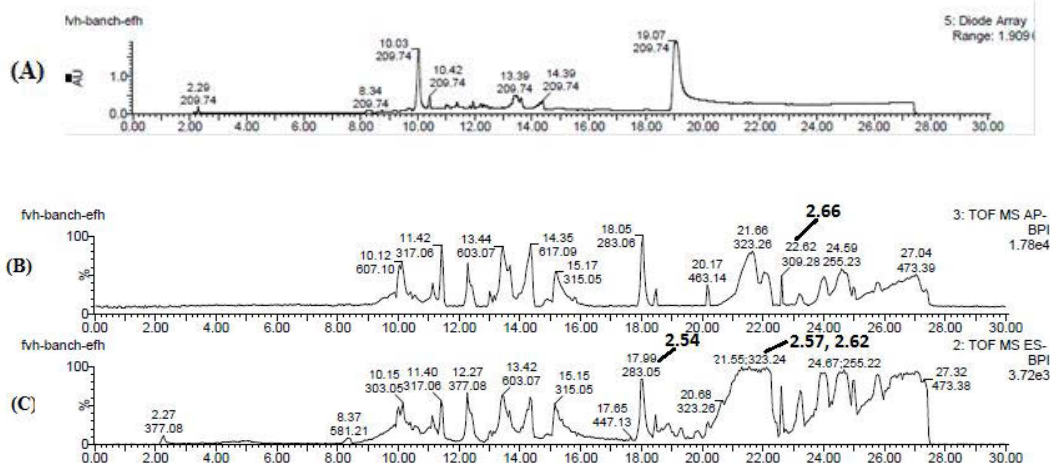
Figure 2.26: HPLC-PDA/LCMS chromatogram of the branches of *E. rhinocerotis* collected from location 3 on farm Weltevreded.

In summary, slight variations in the chemical profiles were observed between the leaves and branches of the same plant. Minor variations were also observed in the constituents of leaves from different plants (collected in different locations). The chemical constituents of the branches collected from different locations were significantly different. Basically, the sample from location 2 was rich in flavonoids [5,7-dihydroxy-4'-methoxyflavone (**2.54**), 5,7-dihydroxy-6,4'-dimethoxyflavone (**2.55**) and kaempferol-3-methyl ether (**2.56**)] while that from location 3 was dominated by the labdanolic acid derivatives [13-*epi*-labdanolic (**2.57**), (+)-labdanolic acid (**2.62**) and labdanediol (**2.66**)]. However, the overall chemical profiles of the aerial parts (leaves and branches) of *E. rhinocerotis* plants collected in locations 1, 2 and 3 were similar. The observed variation in the chemical constituents of the branches collected from different locations was not unexpected. The exposure to different environmental conditions (light, temperature and humidity) and the age of the plant might have influenced the chemical profiles. *E. rhinocerotis* was reported to support a rich fauna of insects, including, three species of gall-inducing Diptera (*Spathulina peringueyi Bezzi* and two species belonging to the Cecidomyiidae family (Dorchin and Gullan, 2007). It is therefore possible that some chemical constituents in this plant are produced by these species. This might cause variations in the chemical profiles if different species (producing different compounds) occur in the plants collected from different locations.

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2.6.5   Conclusion

We developed a HPLC-PDA/MS method for the analysis of *E. rhinocerotis* extracts. The chromatographic results obtained from the leaves collected in different locations farm Weltevreded in the Western Cape indicated minor variation in the chemical profiles of these extracts, while significance variations were observed in the composition of the branches. The overall chemical composition of the aerial parts (leaves and branches) of the *E. rhinocerotis* extracts collected from the three different locations was similar. Since the present study focused on plants collected during the same season, further investigation is required to evaluate the impact of seasonal change on the chemical composition of *E. rhinocerotis*.

2.7   Experimental

2.8.1   Instrumentation and chemicals

Thin-layer chromatography (TLC) plates (Kieselgel 60 F_{254}, 0.25 mm) were used for the inspection of compounds in the crude extracts and the purified fractions. Visualization was achieved by placing the TLC plate under UV radiation (254 or 365 nm) or by staining with *p*-anisaldehyde/H_2SO_4 and heating. The *p*-anisaldehyde stain was prepared by cooling methanol (85 mL) in ice, followed by addition of *p*-anisaldehyde (0.5 mL), sulfuric acid (4 mL) and glacial acetic acid (10 mL). After vigorous shaking, this solution was stored in the refrigerator. Columns packed with silica gel 60 and centrifugal chromatography (Chromatotron™ model 7924, Harrison Research) were used for the purification of the extracts. The circular plates used on the Chromatotron were coated with preparative silica gel to a thickness of 1, 2 or 4 mm.

^{1}H and ^{13}C NMR spectra were recorded on either a Bruker Avance III 500 or Bruker Avance III 400 at frequencies of 500 MHz/ 400 MHz (^{1}H) and 100 MHz/ 125 MHz (^{13}C) using either a 5 mm BBOZ probe or a 5 mm TBIZ probe. All protons and carbons

chemical shifts are quoted relative to the relevant solvent signal (*e.g.* CD$_3$OD: ^{1}H, 3.33 ppm, ^{13}C, 49.0 ppm; CDCl$_3$: ^{1}H, 7.26 ppm, ^{13}C, 77.0 ppm; acetone-d$_6$: ^{1}H, 2.05 ppm, ^{13}C, 29.9 ppm). Proton-proton coupling constants are reported in Hertz. All experiments were conducted at 30 °C unless specified otherwise.

HPLC-PDA analysis was performed on a Shimadzu HPLC consisting of a controller, solvent supply unit, photodiode-array detector and an autosampler, using either a Phenomenex Luna C18 (2) column (250 x 4.60 mm, 5 μ) or a Phenomenex Kinetex C18 column (100 x 4.60 mm, 2.6 μ) operated at 40 °C. The mobile phase consisted of two solvents, 1% acetic acid (A) and methanol (B). Elution was started with 30% of B and using a linear gradient to obtain 90% at 20 min, 100% at 25 min, and returning to 30% at 30 minutes. The injection volume was 4.00 uL and the flow rate was 0.400 μL/min. UV detection for all peaks was from 210-450 nm, and the chromatograms were recorded at 340 nm. UPLC-PDA/LCMS analysis was carried out on the ACQUITY UPLC system consisting of a binary pump, photodiode-array detector, autosampler, and a Waters BEH C18 column (2.1 x 100 mm, 1.7μm) operated at 25 °C. Mass spectra data were collected on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization (ESI) or atmospheric pressure chemical ionisation (APCI) in the positive or negative mode. Optical rotation was determined using an ADP 440$^+$ model polarimeter manufactured by Bellingham and Stanley.

X-ray diffraction data were recorded on a Bruker Apex Duo diffractometer equipped with an Oxford Instruments Cryojet operating at 100(2) K. The data was collected with copper radiation (λ=1.5418Å) at a crystal-to-detector distance of 50 mm using omega and phi scans with exposures taken at 30 W X-ray power and 0.50° frame widths. The data was reduced with the programme SAINT using outlier rejection, scan speed scaling, as well as standard Lorentz and polarisation correction factors. Direct methods (SHELXS-97 and WinGX) were used to solve the structures. All non-hydrogen atoms were located in the E-map and refined anisotropically with SHELXL-97. Hydrogen atoms were included as idealised contributors in the least squares process. All diagrams were generated using Mercury 2.2.

2.8.2   Plant material

For bulk extraction, the aerial parts (leaves and branches) of *Elytropappus rhinocerotis* were collected from farm Weltevreded in Sneeuberg (Murraysburg, Western Cape, GPS coordinates 32.1182 S, 24.0095E) in December 2008. The plant was identified by Mrs. Alison Young from the University of KwaZulu-Natal Botanical Gardens, voucher specimen (van Heerden 3) deposited in the Bews Herbarium, University of KwaZulu-Natal (Pietermaritzburg). For analytical studies, three plants about one kilometre from each other were collected from the same farm (location 1, 2, and 3). The voucher specimens (van Heerden 3) were deposited in the Bews Herbarium, University of KwaZulu-Natal (Pietermaritzburg).

2.8.3   Extraction and isolation

The aerial parts of *E. rhinocerotis* were air dried, ground into a powder (1.9 kg) and then extracted with a mixture of methanol (MeOH)-dichloromethane (DCM) (1:1) at room temperature for 48 hours. This yielded 189 g of crude extract. 19 g of this extract was redissolved in acetone and subjected to a short silica gel column chromatography. Successive elution of this column with hexanes (Hex)-ethyl acetate (EtOAc) (10:0, 9:1, 7:3, 3:7, 5:5 and 0:10) and finally washing with methanol resulted in four fractions (A-D) with the masses of 10.009 g, 6.837 g, 1.9601 g and 3.251g, respectively. Fraction A was purified on a column chromatography using Hex-EtOAc (7:3) to afford five fractions (A1-A5). Further purification of fraction A3 using Hex-EtOAc (7:3) resulted in three fractions (A3.1-A3.3). Fraction A3.1 was further subjected to column chromatography using Hex-EtOAc (8:2) to afford compound **2.56** (0.12 g). Separation of fraction A3.3 on a column with DCM-EtOAc (8:2) yielded six fractions (A3.3.1-A3.3.6). Fraction A3.3.6 (4.02 g) was further purified on a chromatotron using DCM: EtOAc (1:1) to afford labdanolic acid methyl ester (**2.64**) (45 mg), labdanediol (**2.66**) (5 mg), (+)-13-*epi*-labdanolic acid (**2.57**) (250 mg) and (+)-labdanolic (**2.62**) (135 mg).

Fraction B was further separated by column chromatography using Hex-EtOAc (4:6). This resulted into four fractions (B1-B4). Further separation of fraction B2 on a column

using DCM-MeOH (9.5:0.5) yielded seven fractions (B2.1-B2.7). Purification of fraction B2.3 using Hex-EtOAc (4:6) afforded **2.52** (14 mg). Separation of fraction B2.4 by column chromatography eluting with Hex-EtOAc (1:1) resulted in the isolation of **2.53** (2 mg) and **2.55** (5 mg). When fractions B2.5 and B2.6 were re-dissolved in methanol, a light yellow precipitate of compound **2.54** (12 mg) was obtained.

The purified compounds were subjected to HPLC to confirm their purity and to characterise their structures. The structures of isolated compounds were further characterised using NMR and mass spectrometry and the compounds were identified as 6,7-dimethoxycoumarin (**2.52**) (Céspedes et al., 2006), 5,7,4'-trihydroxyflavone (**2.53**) (Ersoz et al., 2002), 5,7-dihydroxy-4'-methoxyflavone (**2.54**), 5,7-dihydroxy-6,4'-dimethoxyflavone (**2.55**) (Soon-Ho et al., 2003), kaempferol-3-methyl ether (**2.56**) (Forgo et al., 2012; Gao et al., 2011), (+)-13-*epi*-labdanolic acid (**2.57**), (+)-labdanolic (**2.62**), (+)-labdanolic acid methyl ester (**2.64**) and (+)-labdanediol (**2.66**) (Cocker and Halsall, 1956; Ramirez et al., 1998).

2.8.4   Physical data of isolated compounds

6,7-Dimethoxycoumarin (2.52) was isolated as off-white crystals.^{1}H NMR (400 MHz, CDCl$_3$): δ_H 3.91 (3H, s, 6-OMe), 3.94 (3H, s, 7-OMe), 6.28 (1H, d, J = 9.5, H-3), 6.83 (1H, s, H-5), 6.85 (1H, s, H-8), 7.61 (1H, d, J = 9.5, H-4) (Plate 1a); ^{13}C NMR (100 MHz, CDCl$_3$): δ_C 56.4 (CH$_3$, 6-OCH$_3$), 56.4 (CH$_3$, 7-OCH$_3$), 108.0 (CH, C-8), 100.1(CH, C-5), 111.4 (C, C-4a), 113.9 (CH, C-3), 143.2 (CH, C-4), 146.4 (C, C-6), 150.1 (C, C-8a), 152.9 (C, C-7), 161.4 (C, C-2) (Plate 1b); HPLC: t$_R$ 9.445, λ_{max} 228 and 343 nm; HRMS(+), m/z 229.0482 [M+Na]$^+$ (calc. for C$_{11}$H$_{10}$O$_4$Na: 229.0477)

5,7,4'-Trihydroxyflavone (2.53) was isolated as a yellow powder. ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 6.18 (1H, d , J = 2.0, H-6), 6.42 (1H, d, J = 2.0, H-8), 6.56 (1H, s, H-3), 6.92 (2H, d, J = 8.8, H-3',5'), 7.84 (2H, d, J = 8.8, H-2',6') (Plate 2a); ^{13}C NMR (125 MHz, CD$_3$OD), Plate 2b: δ_C 95.4 (CH, C-8), 100.7 (CH, C-6), 103.7 (CH, C-3), 104.9 (C, C-4a), 117.1 (CH, C-3',5'), 123.3 (C, C-1'), 129.4 (CH, C-2',6'), 159.6 (C, C-7), 160.8 (C, C-8a), 162.9 (C, C-4'), 165.8 (C, C-2), 166.1 (C, C-5), 183.7 (C, C-4) (Plate

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2b); HPLC: t_R 16.734, λ_{max} 267 and 338 nm; HR-ESIMS(-), m/z 269.0454 [M-H]$^-$ (calc. for $C_{15}H_9O_5$: 269.0450) .

5,7-Dihydroxy-4'-methoxyflavone (2.54) was isolated as a yellow powder. ^{1}H NMR (400 MHz, Acetone-d_6): δ_H 3.92 (3H,s, 4'-OMe), 6.26 (1H, d , $J_=$ 1.7, H-6), 6.54 (1H, d, $J=$ 1.7, H-8), 6.66 (1H, s, H-3), 7.13 (2H, d, $J=$ 8.8, H-3', 5'), 8.02 (2H, d, $J=$ 8.8, H-2', 6'), 12.98 (5-OH) (Plate 3a); ^{13}C NMR (125 MHz, Acetone-d_6): δ_C 55.1 (CH$_3$, 4'-OMe), 93.9 (C, C-8), 99.0 (CH, C-6), 103.7 (CH, C-3), 114.5 (CH, C-3',5'), 123.5 (C, C-1'), 128.2 (CH, C-2',6'), 157.9 (C, C-8a), 162.5 (C, C-5), 162.8 (C, C-4'), 163.8 (C, C-2), 164.7 (C, C-7), 182.1 (C, C-4) (Plate 3b); HPLC: t_R 31.290, λ_{max} 268 and 333 nm; HR-ESIMS(-), m/z 283.0609 [M-H]$^-$ (calc. for $C_{16}H_{11}O_5$: 283.0606).

5,7-Dihydroxy-6,4'-dimethoxyflavone (2.55) was isolated as a green solid. ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 3.89 (3H, s, 4'-OMe), 4.04 (3H,s, 6-OMe), 6.56 (1H, s, H-3), 6.58 (1H, s, H-8), 7.01 (2H, d , $J=$ 9.0, H-3',5'), 7.83 (2H, d, $J=$ 9.0, H-2', 6'), 13.09 (1H, s, 5-OH) (Plate 4a); ^{13}C NMR (125 MHz, CDCl$_3$): δ_C 55.5 (CH$_3$, 4'-OMe), 60.8 (CH$_3$, 6-OMe), 93.3 (CH, C-8), 103.8 (CH, C-3), 105.7 (C, C-4a), 114.5 (CH, C-3', 5'), 123.6 (C, C-1'), 128.0 (CH, C-2', 6'), 130.3 (C, C-6), 152.1 (C, C-8a), 153.1 (C, C-5), 154.9 (C, C-7), 162.6 (C, C-4'), 164.1 (C, C-2), 182.9 (C, C-4) (Plate 4b); HPLC: t_R 31.290, λ_{max} 274 and 333 nm; HR-ESIMS(-), m/z 313.0714 [M-H]$^-$ (calc. for $C_{17}H_{13}O_6$: 313.0712) .

Kaempferol 3-methyl ether (2.56) was isolated as yellow crystals. ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 3.79 (3H, s, 3-OMe), 6.23 (1H, d, $J=$ 2.0, H-6), 6.44 (1H, d, J = 2.0, H-8), 6.95 (2H, d, $J=$ 8.8, H-3', 5'), 8.00 (2H, d, $J=$ 8.8, H-2', 6') (Plate 5a); ^{13}C NMR (125 MHz, CD$_3$OD): δ_C 60.6 (CH$_3$, 3-OMe), 94.2 (CH, C-8), 98.5 (CH, C-6), 104.5 (C, C-4a), 115.2 (CH, C-3', 5'), 121.2 (C, C-1'), 130.0 (CH, C-2', 6'), 138.1 (CH, C-3), 156.7 (C, C-8a), 157.1 (C, C-2), 160.3 (C, C-4'), 161.7 (C, C-5), 164.5 (C, C-7), 178.6 (C, C-4) (Plate 5b); HPLC: t_R 17.771, λ_{max} 266 and 350 nm; LRMS(-), m/z 299.0721 [M-H]$^-$ (calc. for $C_{16}H_{12}O_6$: 300.2629).

13-*Epi*-labdanolic acid (2.57) was isolated as an off-white solid. ^{1}H and ^{13}C NMR data (Table **2.1**, Plate 6a and 6b); mp 66-69 °C (lit. 68 °C); $[\alpha]_D^{30}$ +7 (c 1.0, CHCl$_3$) (lit., +5)

(Ramirez et al., 1998), HR-ESIMS(-), m/z 323.2582 [M-H]$^-$ (calc. for $C_{20}H_{35}O_3$: 323.2586)

(+)-labdanolic acid (2.62) was isolated as an off-white solid; ^1H and ^{13}C NMR data (Table **2.1**, Plate 8a and 8b); mp 67 °C (lit. 68 °C); $[\alpha]_D^{30}$ +7.3 (*c* 2.2, CHCl$_3$) (lit., +5) (Ramirez et al., 1998) , HR-ESIMS(-), m/z 323.2582 [M-H]$^-$ (calc. for $C_{20}H_{35}O_3$: 323.2586).

Compound 2.64, light brown solid, was identified as **(+)-labdanolic acid methyl ester**; ^1H NMR (400 MHz, CDCl$_3$): δ_H 0.76 (6H, s, 19 and 20-OMe), 0.84 (3H, s, 18-OMe), 0.88 (2H, m, H-1, H-5), 0.92 (3H, d, *J* = 6.7, 16-OMe), 0.99 (1H, t, *J* = 3.5, H-9), 1.11 (4H, s, H-3, H-17), 1.38 (6H, m, H-2, H-3, H-7, H-11, H-12), 1.59 (3H, s, H-1, H-2, H-6), 1.83 (1H, dt, *J* = 12.0, 2.9, H-7), 1.92 (1H, m, H-13), 2.09 (1H, dd, *J* = 14.6, 8.0, H-14), 2.33 (1H, dd, *J* = 14.6, 6.1, H-14) (Plate 10a); ^{13}C NMR (125 MHz, CDCl$_3$): δ_C 15.4 (CH$_3$, 20-OMe), 18.5 (CH$_2$, C-2), 19.9 (CH$_3$, 16-OMe), 20.5 (CH$_2$, C-6), 21.4 (CH$_3$, 19-OMe), 22.4 (CH$_2$, C-11), 23.9 (CH$_3$, 17-OMe), 31.1 (CH, C-13), 33.2 (C, C-4), 33.4 (CH$_3$, 18-OMe), 39.1 (C, C-10), 39.7 (CH$_2$, C-1), 40.3 (CH$_2$, C-12), 41.2 (CH$_2$, C-14), 41.9 (CH$_2$, C-3), 44.4 (CH$_2$, C-7), 56.1 (CH, C-5), 61.9 (CH, C-9), 74.1 (C, C-8), 173.9 (C, C-15) (Plate 10b); mp 71-73 °C (lit. 72-74 °C); $[\alpha]_D^{28}$ + 9.5 (*c* 16.4), CHCl$_3$) (lit., $[\alpha]_D^{22}$ +10, *c* 2.03) (Henrick et al., 1964) LRMS(+), m/z 361.2633 [M+Na]$^+$ (calc. for $C_{21}H_{38}O_3Na$: 361.5246).

Compound 2.66 was identified as **(+)-labdanediol**; ^1H NMR (400 MHz, CDCl$_3$): δ_H (6H, s, 19, 20-OMe), 0.86 (3H, s, 18-OMe), 0.92 (5H, d, *J* = 6.8, H-1a, 5, 16-OMe), 1.03 (1H, t, *J* = 3.7, H-9), 1.15 (4H, s, H-3a, 17-OMe), 1.26 (4H, s, H-6a, H-11a), 1.38 (6H, m, H-2a, 3, 7a, 11b, H-14), 1.61 (9H, m, H-1b, 2b, 6b, 12, H-13), 1.85 (1H, dt, *J* = 12.3, 3.0, H-7b), 3.68 (2H, m, H-15) (Plate 11a); ^{13}C NMR (125 MHz, CDCl$_3$): δ_C 15.4 (CH$_3$, 20-OMe), 18.5 (CH$_2$, C-2), 20.0 (CH$_3$, 16-OMe), 20.6 (CH$_2$, C-6), 21.5 (CH$_3$, 19-OMe), 22.3 (CH$_2$, C-11), 24.1 (17-OMe), 30.1 (CH, C-13), 33.2 (C, C-4), 33.4 (CH$_3$, 18-OMe), 39.2 (C, C-10), 39.3 (CH$_2$, C-12), 39.8 (CH$_2$, C-1), 40.5 (CH$_2$, C-14), 42.0 (CH$_2$, C-3), 44.6 (CH$_2$, C-7), 56.2 (CH, C-5), 61.3 (CH$_2$, C-15), 61.9 (CH, C-9), 74.4 (C, C-7) (Plate 11b), $[\alpha]_D^{28}$ +12 (*c* 0.4), CHCl$_3$) (lit., $[\alpha]_D^{22}$ +10, *c* 2.03) (Dawson et al., 1966); HR-ESIMS(-), m/z 307.2798 [M-H]$^-$ (calc. for $C_{20}H_{37}O_2$: 307.2794) .

2.8.5 Synthesis of *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61)

A mixture of 13-*epi*-labdanolic acid (176 mg, 0.544 mmol), *p*-bromophenacyl bromide (151 mg, 0.544 mmol) and triethylamine (0.114 mL) was stirred in *N,N*-dimethylformamide (5 mL) at 70 °C for 4 hours. The resulting mixture was extracted with diethyl ether and washed with HCl (0.1 M). The main product was purified by column chromatography using Hex-EtOAc (8:2) to yield an off-white sticky white solid of *p*-bromophenacyl ester of 13-*epi*-labdanolic acid (0.271 mmol, 49%). Recrystallization of this ester (**2.61**) from chloroform-hexane (1:1) afforded white needle-like crystals.

^{1}H NMR (400MHz, CDCl$_3$): δ_H 0.79 (6H, s, 19, 20-OMe), 0.86 (3H, s, 18-OMe), 0.92 (2H, m, H-1a, H-5), 1.02 (4H, m, *J* = 6.8, H-9, 16-OMe), 1.15 (4H, s, H-3a, H-17), 1.26 (3H, m, H-6a, 11a), 1.41 (6H, m, H-2a, H-3b, H-7a, H-11b, H-12), 1.64 (3H, m, H-1b, H-2b, H-6b), 1.86 (1H, dt, *J* = 12.2, 3.1, H-7), 2.05 (1H, m, H-13), 2.27 (1H, dd, *J* = 14.9, 7.9, H-14a), 2.55 (1H, dd, *J* = 14.9, 6.2, H-14), 5.27 (1H, s, H-21a), 5.28 (1H, s, H-21b), 7.63 (2H, d, $J_{25,24}$ = 8.5, H-25, 27), 7.77 (1H, d, *J* = 8.5, H-24, 28) (Plate 7a) ; ^{13}C NMR (100 MHz, CDCl$_3$): δ_C 15.5 (CH$_3$, 20-OMe), 18.5 (CH$_2$, C-2), 19.9 (CH$_3$, 16-OMe), 20.6 (CH$_2$, C-6), 21.5 (CH$_3$, 19-OMe), 22.1 (CH$_2$, C-11), 23.9 (CH$_3$, 17-OMe), 30.9 (CH, C-13), 33.2 (C, C-4), 33.4 (CH$_3$, 18-OMe), 39.1 (C, C-10), 39.7 (CH$_2$, C-1), 40.0 (CH$_2$, C-12), 40.8 (CH$_2$, C-14), 41.9 (CH$_2$, C-3), 44.5 (CH$_2$, C-7), 56.1 (CH, C-5), 62.0 (CH, C-9), 65.6 (CH$_2$, C-21), 74.2 (C, C-8), 129.1 (C, C-23), 129.3 (CH, C-25, 27), 132.2 (CH, C-24, 28), 132.9 (C, C-26), 172.8 (C, C-15), 191.5 (C, C-22) (Plate 7b); HR-ESIMS(+), m/z 543.2076 [M+Na]$^+$ (calc. for C$_{28}$H$_{41}$O$_4$NaBr: 543.2086)

Crystal Data

Formula   C$_{28}$H$_{41}$BrO$_4$

Cell setting   Monoclinic

Space group   P21

M$_r$ (g/mol)   521.18

a/Å   8.0014 (2)

b/Å   10.9459 (3)

c/Å   15.2268 (3)

α/°   90.00

β/°   97.9420 (10)

γ/°   90.00

T/K   100

Z   2

V/Å^3 1320.81

2.8.6   Synthesis of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63)

The mixture of (+)-labdanolic acid (54 mg, 0.166 mmol), *p*-bromophenacyl bromide (46.3 mg, 0.166 mmol) and triethylamine (0.0347 mL) was stirred in *N,N*-dimethylformamide (5 mL) at 70 °C for 4 hours. The mixture was extracted with diethyl ether and washed with HCl (0.1 M). The main product was purified by a chromatotron using Hex-EtOAc (8:2) to obtain an off-white sticky white solid of *p*-bromophenacyl ester of (+)-labdanolic acid (27.4 mg, 32%). Recrystallization of this ester (**2.63**) from CHCl$_3$-Hex (1:1) afforded white needle-like crystals.

^{1}H NMR (400 MHz, CDCl$_3$): δ$_H$ 0.79 (6H, s, 19, 20-OMe), 0.86 (3H, s, 18-OMe), 0.92 (2H, m, H-1a, H-5), 1.02 (4H, m, *J* = 6.8, H-9, 16-OMe), 1.15 (4H, s, H-3a, H-17), 1.26 (3H, m, H-6a, 11a), 1.41 (6H, m, H-2a, H-3b, H-7a, H-11b, H-12), 1.59 (3H, m, H-1b, H-6b), 1.86 (1H, dt, *J* = 12.2, 3.1, H-7), 2.01 (1H, m, H-13), 2.34 (1H, dd, *J* = 14.7, 7.3, H-14a), 2.46 (1H, dd, J = 14.7, 6.2, H-14), 5.26 (1H, s, H-21a), 5.29 (1H, s, H-21b), 7.63 (2H, d, *J* = 8.5, H-25, 27), 7.77 (1H, d, *J* = 8.5, H-24, 28) (Plate 9a); ^{13}C NMR

(100 MHz, CDCl$_3$): δ$_C$ 15.5 (CH$_3$, 20-OMe), 18.5 (CH$_2$, C-2), 19.9 (CH$_3$, 16-OMe), 20.5 (CH$_2$, C-6), 21.5 (CH$_3$, 19-OMe), 22.9 (CH$_2$, C-11), 23.9 (CH$_3$, 17-OMe), 31.5 (CH, C-13), 33.2 (C, C-4), 33.4 (CH$_3$, 18-OMe), 39.7 (C, C-1), 40.6 (CH$_2$, C-10), 41.3 (CH$_2$, C-12), 42.0 (CH$_2$, C-14), 44.3 (CH$_2$, C-3), 53.4 (CH$_2$, C-7), 56.1 (CH, C-5), 62.5 (CH, C-9), 65.6 (CH$_2$, C-21), 74.2 (C, C-8), 129.1 (C, C-23), 129.3 (CH, C-25, 27), 132.2 (CH, C-24, 28), 132.9 (C, C-26), 172.6 (C, C-15), 191.6 (C, C-22) (Plate 9b); HR-ESIMS(+), m/z 543.2073 [M+Na]$^+$ (calc. for C$_{28}$H$_{41}$O$_4$NaBr: 543.2086)

CHAPTER 3: The phytochemistry of *Rhoicissus tridentata*

3.1 Introduction

The onset of labour process during pregnancy has been reported as the main cause of maternal and perinatal deaths in developed countries. It is reported that 28% of the 4 million annual neonatal deaths are due to pre-term births. On the other hand, delayed labour processes are also associated with some neonatal deaths. Therefore, in some cases, induction of labour is required. These are cases such as post-term pregnancy, lack of progress, oligohydramnios and suspected intrauterine growth restriction, where drugs with uterotonic properties are normally used.

Uterotonic drugs are involved in the modulation of uterine muscle contractions at labour, resulting in uterine stimulation. These drugs are divided into three pharmacological groups, namely, ergot alkaloids, oxytocins and prostaglandins (Gruber and O'Brien, 2011). Ergot alkaloids were the first drugs to be used for their uterotonic properties. The first alkaloid with contractile activity was described in 1582 from the fungus *Claviceps purpurea* (commonly known as secale cornutum).

Unfortunately ergot alkaloids were always associated with maternal and fetal deaths. As a result, the use of secale cornutum was halted in 1828. After this, various alkaloids were described, the most important being ergometrine (**3.1**) and methylergometrine (**3.2**) (den Hertog et al., 2001). Ergometrine was isolated from *Claviceps purpurea* by Dudley and Moir in 1932 and was described to be uterine specific and showed less vasospatic properties (Vigneaud et al., 1953). Even though these alkaloids were useful in the prevention of post-partum haemorrhage, their use was limited by severe side effects such as hypertension, nausea, vomiting and maternal death. Ergot alkaloids were unstable when exposed to heat, light and humidity, and this further restricted their use (de Groot et al., 1998; den Hertog et al., 2001; Van Dongen and de Groot, 1995).
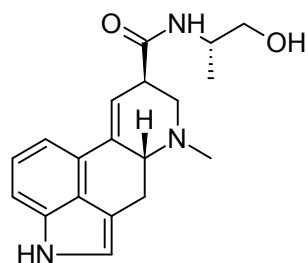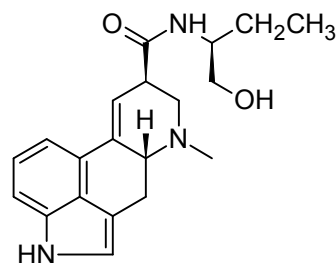
3.1   **3.2**

Oxytocin, an endogenous mammalian cyclopeptide discovered by Sir Henry Dale in 1909, was confirmed to have uterine contractility and milk-ejecting activities. Dale (1909) also concluded that oxytocin was a hormone of the hypothalamus originating from the posterior pituitary gland (Dale, 1909; Wathes and Swann, 1982). Later (1953), oxytocin was described as a polypeptide, and an octapeptide **3.3** with the hormonal activity of oxytocin was synthesised (du Vigneaud et al., 1953; Vigneaud et al., 1953). The octapeptide oxytocin (**3.3**) is used as a WHO standard drug for prevention of post-partum haemorrhage (PPH) (den Hertog et al., 2001). In humans, oxytocin and the closely related vasopressin, act on four cognate receptors (oxytocin and three vasopressin receptors). These all belong to the family of G protein-coupled receptors (GPCR). GPCR are the most promising targets for a drug with about 30% of all marketed drugs acting on them (Gruber and O'Brien, 2011; Koehbach et al., 2013).
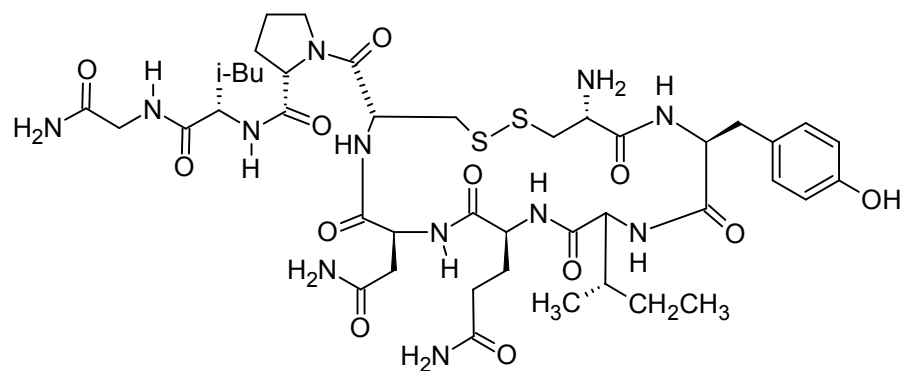

3.3

Prostaglandins have been used to augment labour and cervix ripening since the 1960's. Prostaglandins are members of the eicosanoid family of compounds naturally produced by the uterus, fetal membrane and placenta. The concentration of these compounds increases during the onset of labour and their direct involvement in the parturient process at term and preterm labour has been studied (Keelan et al., 2003; Mitchell et al., 1995). Synthetic prostaglandins play an important role in the ripening of cervix and labour initiation during childbirth. Dinoprostone (**3.4**) and misoprostol (**3.5**) (Cytotec[®]) are the two most commonly used and commercially available prostaglandin analogues. Dinoprostone, which can be obtained as a vaginal insert (Cervidil[®]) and a cervical gel (Prepidil[®]), is approved by the FDA for cervical ripening and induction of labour. Dinoprostone has been less popular in low resource settings as a result of its high costs and special storage conditions (freezing) (Shetty et al., 2001b; Yount and Lassiter, 2013).
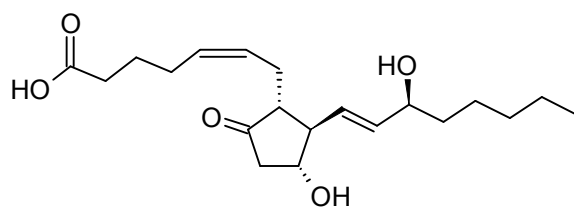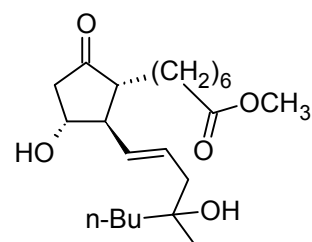
3.4   **3.5**

Misoprostol (**3.5**) is approved by the FDA for the treatment and prevention of gastrointestinal and peptic ulcers; however it is used off-label for the facilitation of cervix ripening and stimulation of labour in obstetric and gynaecological procedures. This drug has gained popularity as an uterotonic agent since it is cheap, heat stable, and orally active. It has been discovered recently that the optimal dosage for misoprostol is 25 mg and that the oral administration route reduces risks of uterine hyperstimulation when compared to the vaginal route. Kundodyiwa et al. (2009) reported that even the 25 mg optimal dosage results into a 6% hyperstimulation rate (Bracken et al., 2014; Hofmeyr et al., 2009; Kundodyiwa et al., 2009; Shetty et al., 2001a).

It is clear from the above discussion that the available uterotonic drugs may lack potency and selectivity, and therefore result to harmful side effects to the mother and the baby

(Attah et al., 2012; Gruber and O'Brien, 2011). Traditional plants have been used for many years by local communities to induce or enhance the labour process during child birth. Their uses are well documented in countries such as Argentina, China, Indonesia, Mexico, Ethiopia, South Africa, northern America and central Europe (Attah et al., 2012; Gruber and O'Brien, 2011). In South Africa, approximately 80% of pregnant women from Zulu and Xhosa populations use traditional medicines (Gruber and O'Brien, 2011; Lindsey et al., 1998; Varga and Veale, 1997). The chemical profiles as well as toxic effects of many oxytocic plants have been reported.

3.2 Non-South African oxytocic plants

Herbal remedies used for pregnancy related ailments are becoming increasingly popular amongst various cultures worldwide. For most populations, this interest in traditional medicine is due to their accessibility and affordability. The majority of African people rely on these remedies because of cultural beliefs, superstitions, circumstantial constraints or a combination of these (Kaido et al., 1997). Some examples of oxytocic plants used in various regions around the world, including Southeast Asia, China, Mexico, North America, Honduras, India, Congo, Nigeria, and Uganda are presented below.

People of low socioeconomic status in the countries in the Southeast Asia region still rely on traditional medicine for their health care needs. Women most frequently use herbal medicines for various health issues, including birth-related ailments, menstrual related disorders, and post-natal issues (post-partum bleeding, lactation increase or decrease). In a recent review, *Leonurus japonicas* and *Ricinus communis* received over 20 species-use reports from the Southeast Asia countries for their induction of labour (de Boer and Cotingting, 2014).

L. japonicas (Lamiaceae) is also traditionally used to treat haemorrhage disorders, and the leaves extracts were found to be analgesic and anti-inflammatory (Wang et al., 2009), reduce PGF_2-α and PGE_2 concentrations and increase levels of progesterone (Jin et al.,

2003). Other species related to *L. japonicas*, for instance *L. artemisia* and *L. heterophyllus* (known as *Yi-Mu-Cao*), have also been used for hundreds of years in Chinese traditional medicine for the initiation and enhancement of labour (Chen and Kwan, 2001). The uterotonic activity of these *Leonurus* herbs was attributed to an alkaloid (leonurine, 4-guanidino-*n*-butyl syringate) (**3.6**) isolated from the fresh and dried leaves extracts (Kong et al., 1976). Together with uterotonic action, *Leonurus* herbs exhibit anti-apoptotic, anti-oxidant, anti-inflammatory, anti-platelet aggregation effects, inhibitory action on pulsating myocardial cells and vasorelaxant activity (Chen and Kwan, 2001; Liu et al., 2012; Sun et al., 2005; Zhu et al., 2004).
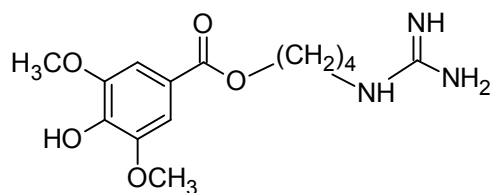

3.6

Ricinus communis (Euphorbiaceae) is used to ease delivery and reduce labour pains, induce labour, expel the placenta, and help the mother recover from the birthing process. Castor oil, derived from the seeds of this plant, increases contractions on isolated human myometrium (O'Sullivan et al., 2010), and induced labour and cervical ripening in pregnant rats (Gao et al., 1999; Gao et al., 1998). It is reported that a castor oil metabolite (ricinoleic acid, **3.7**) may be responsible for the activation of uterine muscle cell (Gao et al., 1999).
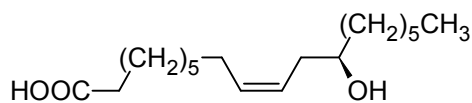
3.7

Women from Mexico use *Montanoa tomentosa* (Asteraceae) for its contraceptive effect and to facilitate labour and child birth. Twenty different compounds have been isolated from *M. tomentosa*, but of particular interest are the three uterotonic tetracyclic diterpenes [kaurenoic acid (**3.8**), grandiflorenic acid (**3.9**) and monoginoic acid (**3.10**)] isolated from the roots (Villa-Ruano et al., 2009). Kaurenoic and grandifloric acids were also isolated from the leaves of *M. tomentosa* (Villa-Ruano et al., 2009) and from various other plants
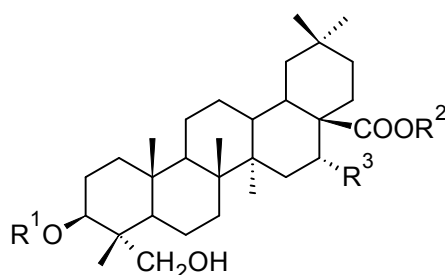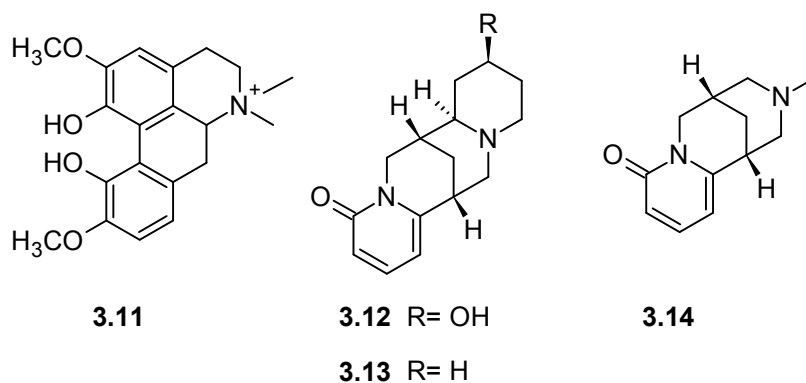
such as *Aspilia mossambicensis* (Asteraceae) (Page et al., 1992). *Aspilia* species in Tanzania are consumed by wild chimpanzees. Interestingly, they are more frequently consumed by female chimpanzees compared to males, which may be explained by the uterotonic activity of this plant (Page et al., 1992).

3.8 R = H
3.9 R = OH

3.10
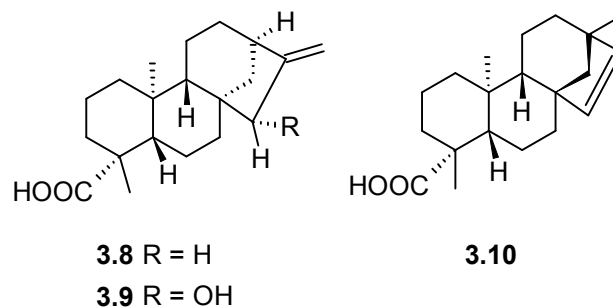
Medicinal herbs are used by pregnant women in North America to tone the uterus, prevent miscarriage and to induce labour. Some common tonic herbs used are *Rubus idaeus* (raspberry leaf) (Rosaceae), *Mitchella repens* (partridge berry) (Rubiaceae) and *Urtica dioica* (stinging nettle) (Urticaceae) (Westfall, 2001). The highly nutritious tea prepared from fresh raspberry leaves is taken for toning the uterus and this tea is also known to prevent miscarriage. The active compound was isolated from the leaves in 1941 and was characterised as an alkaloid (fragarine). Other compounds identified from raspberry (tannins, polypeptides and flavonoids) have been linked to the astringent, stimulant and soothing properties of this plant (Burn and Withell, 1941; Westfall, 2001; Whitehouse, 1941).

Caulophyllum thalictroides (blue cohosh) (Berberidaceae) became popular in North America around 1800 for inducing labour as well as stimulating menstrual flow. This plant is still popular today in western and African American folk medicine and is commercially available as tinctures or tablets. Several alkaloids and saponins have been isolated from *C. thalictroides* (El Tahir, 1991; Flom et al., 1967; Power and Salway, 1913; Rader and Pawar, 2013). In a recent study that focused on the quantification of major alkaloids and saponins in a blue cohosh dietary supplement, magnoflorine (**3.11**), baptifoline (**3.12**), anagyrine (**3.13**), *N*-methylcytisine (**3.14**), leonticin D (**3.15**), cauloside G (**3.16**) and cauloside D (**3.17**) were identified as major constituents (Rader and Pawar, 2013).

Magnoflorine (**3.11**) stimulated contractions on isolated rat uterus and isolated guinea pig ileum (El Tahir, 1991).

| | **3.11** | **3.12** R= OH | **3.14** |
| | | **3.13** R= H | |

	R^1	R^2	R^3
3.15	α-L-Ara	α-L-Rha (1→4)-β-D-Glc (1→4) - β-D-Glc	OH
3.16	β-D-Glc(1→2)- α-L-Ara-	α-L-Rh (1→4)-β-D-Glc(1→4)- β-D-Glc	H
3.17	α-L-Ara	α-L-Rha (1→4)-β-D-Glc (1→4) - β-D-Glc	OH

Ticktin et al. (2005) conducted a survey on the use of medicinal plants for various complications during childbirth by Honduras midwives. *Citrus aurantifolia* (lime tree) (Rutaceae) was by far the most frequently mentioned plant out of the sixteen plants used to accelerate contractions during labour. The root decoction from the lime tree is taken orally (one-half cup) during labour and this plant is also used for its uterotonic activity by the Arunachal Pradesh tribe (India) and Kenyah Leppo'Ke tribe (Indonesia) (Ticktin and Dalle, 2005).

Oldenlandia affinis DC (Rubiaceae) have been used for its oxytocic effects since the 1960's by Lulua women (Kasai in Congo), as well as women from the Central African Republic. Dried leaves from this plant are used to prepare tea, which is sipped and applied to the vagina during labour to facilitate delivery. Gran (1973) isolated two polypeptides (kalata B1 and B2) from the leaves of *O. affinis*. In this study, the major peptide (kalata B1) (Fig. 3.1) was found to induce *in vitro* uterine muscle contractions on isolated rat, rabbit and human uteri strips. Recently, kalata B1, B2 and B7 were re-isolated from *O. affinis* and their uterotonic activities were confirmed (Gran et al., 2008; Gruber and O'Brien, 2011).
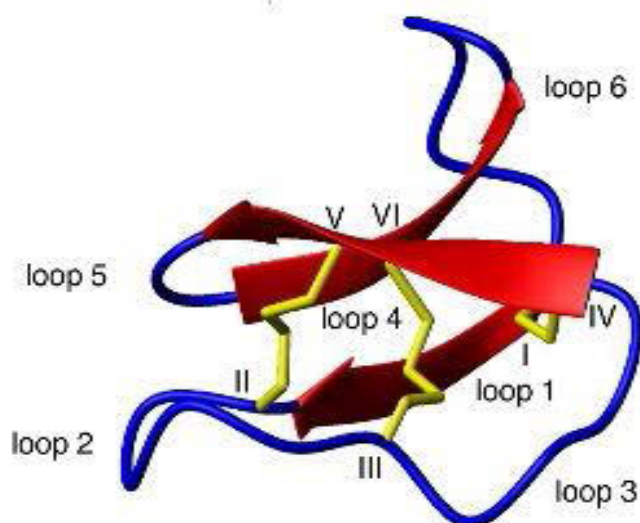

Figure 3.1: Three-dimensional structure of cyclotides kalata B1 (Saether et al., 1995)

Kalata B1 sequencing : Cyclo(L-arginyl-L-asparaginylglycyl-L-leucyl-L-prolyl-L-valyl-L-cysteinylglycyl-L-a-glutamyl-L-threonyl-L-cysteinyl-L-valylglycylglycyl-L-threonyl-L-cysteinyl-L-asparaginyl-L-threonyl-L-prolylglycyl-L-cysteinyl-L-threonyl-L-cysteinyl-L-seryl-L-tryptophyl-L-prolyl-L-valyl-L-cysteinyl-L- threonyl), cyclic(7-21), (11-23), (16-28)-tris(disulfide).

A majority of Nigerians in rural areas still rely on the use of traditional herbs to tone the uterus muscle, induce labour, in the removal of placenta and the management of post-partum haemorrhage. Attah et al. (2012) recently analysed the *in vitro* uterine contractility of nine plant species used in Nigerian traditional medicine. All tested plant extracts elicited

a significant increase in the uterine smooth muscle cell contractility; however, the highest increase was observed for *Commelina africana* (Commelinaceae), *Sida corymbosa* (Malvaceae) and *Vernonia amygdalina* (Asteraceae) (Attah et al., 2012). *C. africana* and *S. corymbosa* are also used in Tanzanian traditional medicine for abortion, and their uterotonic activities were reported by Nikolajsen and co-workers (Nikolajsen et al., 2011).

Bullough and Leary (1982) documented the first report on the use of *V. amygdalina* leaf extract to induce uterine motility and prevent post-partum haemorrhage by Malawian women. Further reports proving the oxytocic property of the aqueous extracts were provided by Kamatenesi-Mugisha (2004), Kamatenesi-Mugisha et al. (2005), and recently by Ijeh et al. (2011). In the former study, the contractility effect of the leaf extract was observed on rabbit jejunum, while there was an increase in the rat uterine motility. The latter study was conducted on guinea pig and an increase in the uterine smooth muscle contractions amplitudes was similar to that of ergometrine. However, uterine contraction amplitudes observed when mammary isolated uterus was used, were lower than ergometrine (Bullough and Leary, 1982; Ijeh and Ejike, 2011; Kamatenesi-Mugisha, 2004; Kamatenesi-Mugisha et al., 2005).

Approximately 90% of the Ugandan population, with the highest portion being women and children, rely on traditional medicine for day-to-day health care needs. Medicinal plants used to increase uterine contractions are commonly used, and about seventy-five uterotonic plants used in the Busheyi and Kasese districts of Western Uganda have recently been documented (Kamatenesi-Mugisha et al., 2007). Some of the plants cited in this survey have the potential to contain potent compounds, since they are used also in other parts of Uganda and other countries to induce labour. For example, *Bidens pilosa* (Asteraceae) (one of the plants cited in this survey) is also used in Rwanda and its methanol extract showed weak stimulating effect on the guinea pig uterine contractions (Chagnon, 1984). Another plant used in both Uganda and Rwanda is *Tetradenia riparia (Iboza)* (Lamiaceae). Goto et al. (1957) reported that the water-methanol extract from *T. riparia* caused a weak stimulation of the guinea pig uterus and ileum smooth muscle contractions (Goto et al.,

1957). The use of *Erlangea cordifolia* (Asteraceae) to augment labour during childbirth is reported in Uganda, as well as in Kenya (Kamatenesi-Mugisha et al., 2007; Mugo, 1977).

3.3   South African oxytocic plants

Veale et al. (1992) conducted a literature survey on the ethnic background, traditional uses and potential toxicity of traditional herbal remedies used by South African (SA) women as antenatal medication or to induce labour. Findings from this study revealed that around 57 plants are used during pregnancy and childbirth in SA (Veale et al., 1992). The Zulu and Xhosa traditional healers prepare an aqueous root extract decoction using a combination of a minimum of four oxytocic plants. This herbal drink, commonly known as *isihlambezo* is reported to be used by ca. 80% of Zulu and Xhosa women and is orally taken in the last trimester of pregnancy. *Isihlambezo* is taken to ensure good health of the mother and the foetus, preventing long and complicated labour, and is believed to protect the baby against evil spirits (Varga and Veale, 1997; Veale et al., 1998).

Some herbal remedies (*imbelekisane, inembe* and *imbiza)* also used in Zulu and Xhosa cultures are closely related to and have been confused with *isihlambezo. Imbelekisane* is orally or administered as an enema in cases of prolonged and difficult labor. *Inembe* is normally taken as an arbortifacient, and its ingredients include *Triumfetta rhomboidea* (Jacq.) (Malvaceae), *Gunnera perpensa* L. (Gunneraceae), *Rhoicissus tridentata* subsp. *cunefolia* (Vitaceae) and *Cyphostemma natalitium* (Szyszyl.) J.v.d. Merwe (Vitaceae). A herbal medicine administered as a drink, emetic, or vaginal douche, with laxative effects is named *Imbiza. Imbiza* is believed to cleanse the uterus thus preparing it to accept the fetus (Varga and Veale, 1997; Veale et al., 1998).

The combination of plants used in the preparation of *isihlambezo* varies from village and from person to person, however, some plants are quoted more than others (Varga and Veale, 1997). Some of the most commonly cited species together with the isolated uterotonic compounds and their mode of actions are shown in Table 3.1. Although the

plants listed here have been thoroughly studied for their biological activities, the activity of other frequently used plants such as *Scadoxus puniceus* (Amaryllidaceae), *Crinum sp.* (Amaryllidaceae), *Typha capensis* (Typhaceae) and *Vernonia neocorymbosa* Hilliard (Asteraceae) are not yet fully understood. The active principles of most of these plants have not been identified. Examples of isolated uterotonic compounds are shown in Figure 3.2.

Table 3.1: Some frequently used herbal ingredients of *isihlambezo* and their active compounds.

Plant (scientific name, family)	Zulu name/ Xhosa	Plant parts, extract	Active compounds	Uterotonic principle (mode of action)	Reference
Agapanthus africanus (L.) Hoffmg. (Amaryllidaceae)	Ubani, uhlakahla/ Isilakati	Leaf, aqueous extract		Uterotonic activity on isolated rat uterus and ileum, extract possess agonistic activity of uterus muscarinic receptors and promoted prostaglandin synthesis	(Kaido et al., 1997; Veale et al., 1999)
Asclepias fructicosa L. (Apocynaceae)	Ishinga	Wood, aqueous extract		Stimulate uterine muscle contractions	(Sewram et al., 1998)
Clivia miniata (Lindl.) Regel (Amaryllidaceae)	Umayime	Leaf and root, boiled aqueous extract	linoleic acid (**3.18**), 5-hydroxymethyl-2-furancarboxaldehyde (**3.19**)	Extracts increased frequency of spontaneous uterus contractions with no effect on maximal uterus response to oxytocin or acetylcholine, activation of cholinergic receptors	(Sewram et al., 2001; Veale et al., 1989; Veale et al., 2000)
Ekebergia capensis Sparrm. (Meliaceae)	Umnyamathi, Umathunzini, Isimanaye	Wood, aqueous extract	oleanolic acid (**3.20**), 3-*epi*-oleanolic acid (**3.21**)	Extract stimulated uterine muscle contractions and increase their frequency, cholinergic receptors activated	(Sewram et al., 2000; Sewram et al., 1998)
Combretum kraussii Hochst (Combretaceae)	Umdubu	Root, boiled methanol extract	β-sitosterol (**3.22**), combretastatin B-1 (**3.23**), 2'-*O*-β-D-glucopyranoside of combretastatin B-1 (**3.24**), 2'-*O*-β-D-glucopyranoside of combretastatin A-1 (**3.25**)	uterotonic activity at low acetylcholine concentrations, but inhibited the response of isolated uterus to acetylcholine at higher concentrations	(Brookes et al., 1999)

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| Plant (scientific name, family)	Zulu name/ Xhosa	Plant parts, extract	Active compounds	Uterotonic principle (mode of action)	Reference
Gunnera perpensa L. (Gunneraceae)	Ugobo, Uklenya/ Iphuzamlambo	Dry rhizomes, boiled aqueous extract	Z-venusol (**3.26**)	Extract stimulated direct contractile response of the uterus smooth muscle, pure venusol showed no effect but after its removal, the tissue experienced a state of spontaneous contractility	(Kaido et al., 1997; Khan et al., 2004)
Grewia occidentalis L. (Tiliaceae)	Iklolo	Wood, aqueous extract	coniferaldehyde (**3.27**), oleanolic acid	Tonic rhythm of extremely low amplitude and low response observed; regular, rhythmic contractions produced at optimal pressure.	(Sewram et al., 1998)
Pentanisia prunelloides Walp. (Rubiaceae)	Icishamlilo	Roots and leaf, aqueous extract		Uterotonic activity and extract induce uterine and ileum response to low doses of oxytocin and acetylcholine respectively.	(Kaido et al., 1997)
Rhoicissus tridentata (L.f.) subsp. *cuneifolia* (Vitaceae)	Isinwazi	Roots and lignotuber, boiled aqueous extract		Uterus and ileum showed direct contractile response which may be due to the activation of muscarinic receptors and cyclooxygenase metabolites production.	(Katsoulis et al., 2000; Naidoo et al., 2006)
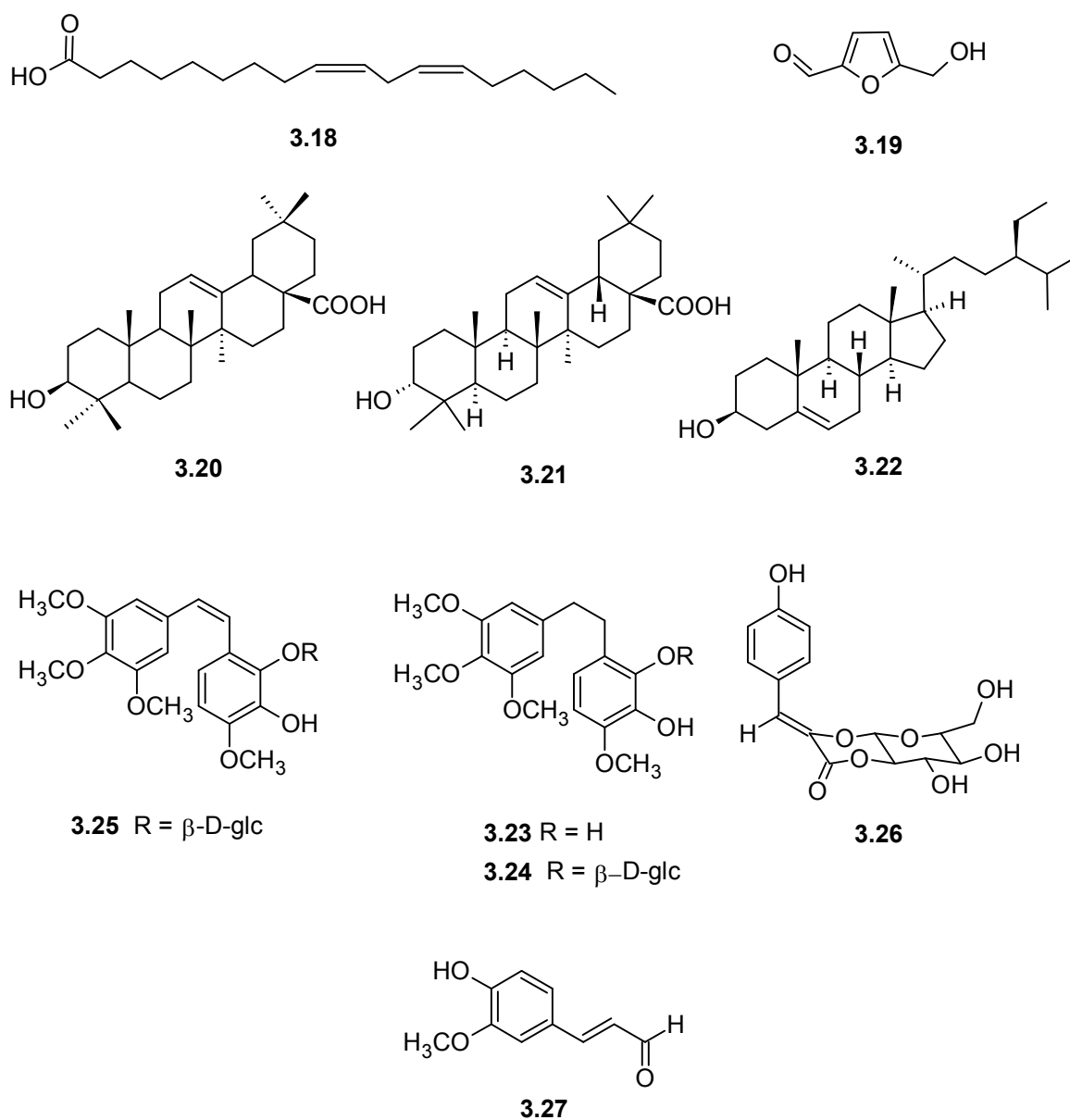
Figure 3.2: Structures of uterotonic compounds isolated from some oxytocic plants.

Structures shown are of linoleic acid (**3.18**), 5-hydroxymethyl-2-furancarboxaldehyde (**3.19**), oleanolic acid (**3.20**), 3-*epi*-oleanolic acid (**3.21**), β-sitosterol (**3.22**), combretastatin B-1 (**3.23**), 2'-*O*-β-D-glucopyranoside of combretastatin B-1 (**3.24**), 2'-*O*-β-D-glucopyranoside of combretastatin A-1 (**3.25**), *Z*-venusol (**3.26**) and coniferaldehyde (**3.27**).

The aqueous extract of the leaves from *Agapanthus africanus* showed uterotonic effects on both isolated rat uterus and ileum, and the mode of action of this extract is well understood (Kaido et al., 1997). Pure active principles have not yet been reported, but some compounds isolated from this plant are yuccagenin (**3.28**), sapogenin agapanthagenin (**3.29**), 7-dehydroagapanthagenin (**3.30**), 8(14)-dehydroagapanthagenin (**3.31**) and 9(1l)-dehydroagapanthagenin (**3.32**) (González et al., 1975; González et al., 1974).
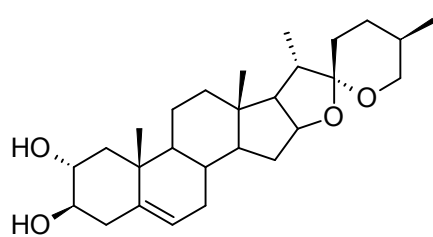

3.28
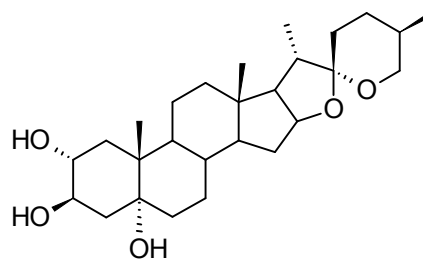
3.29
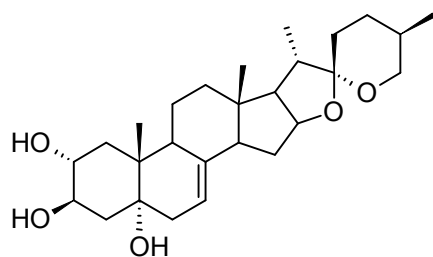
3.30
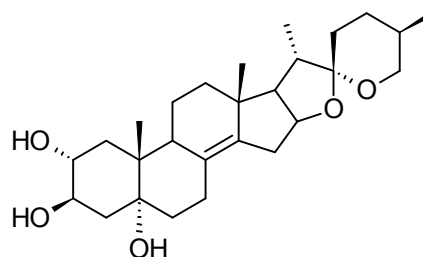
3.31

3.32
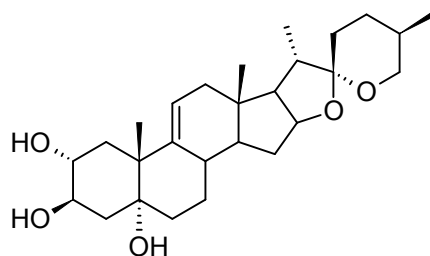
Asclepias fructicosa leaves and stem extracts are very rich in steroidal glycosides. Several of the isolated glycosides are of the pregnane and cardenolide types. Examples of major glycosides from leaves are uscharin (**3.33**), 3'-epigomphoside 3'-acetate (**3.34**), and 3'-epiafroside 3'-acetate (**3.35**). Afroside (**3.36**) was isolated as a major constituent from the

stem of *A. fructicosa* (Cheung et al., 1983). Another type of glycosides isolated from this plant is the megastigmane glycosides. (6*S*,9*R*)-Roseoside (**3.37**) and ascleposide A (**3.38**) represent some of the megastigmane glycosides isolated from *A. fructicosa* (Abe and Yamauchi, 2000).


	R^1	R^2	R^3
3.33	CHO	(thiazoline)	H
3.34	Me	α-OAc, β-H	H
3.35	Me	α-OAc, β-H	OH
3.36	Me	β-OH, α-H	OH

3.37

3.38
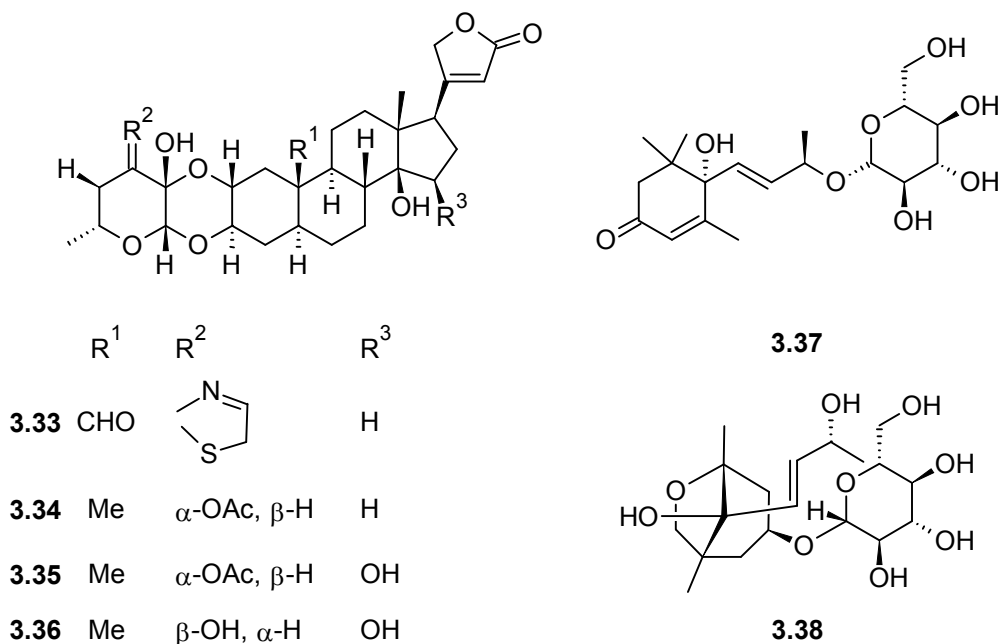
Besides uterotonic activity, *Clivia miniata* was reported as one of the most active antiviral plants in the Amaryllidaceae family. The alkaloid lycorine (**3.39**), isolated as a major compound from the polar fractions of leaves or roots showed antiviral activity against semliki forest, herpes simplex and poliomyelitis viruses (Ieven et al., 1982). Another major alkaloid, clivimine (**3.40**), together with clivonine (**3.41**) and cliviamartine (**3.42**) were also isolated. Alkaloids **3.40-3.42** did not show any antiviral effects, and have not been linked to any biological property of the plant (Ieven et al., 1982).


3.39

3.40
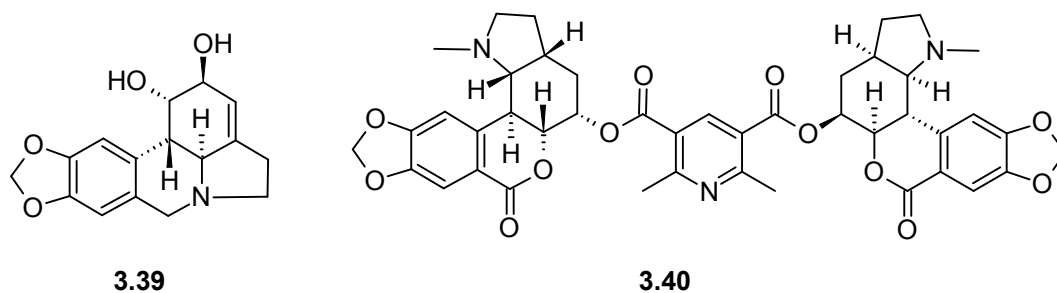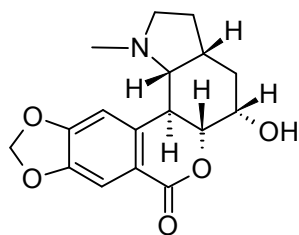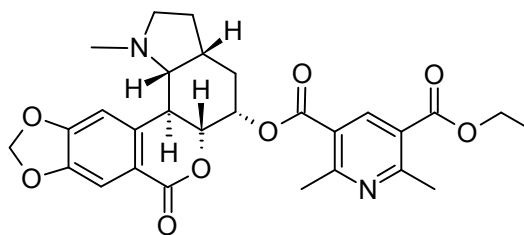
3.41   **3.42**

Apart from *Z*-venusol; small quantities of succinic acid, lactic acid, 1,1'-biphenyl-4,4'-diacetic acid, methyl lespedezate (**3.43**), ellagic acid (**3.44**), the trimethyl ether of ellagic acid glucoside (**3.45**), *p*-hydroxybenzaldehyde and β-sitosterol (**3.22**) were isolated from the dry rhizomes of *Gunnera perpensa* (Brookes and Dutton, 2007; Khan et al., 2004). Brookes and Dutton (2007) also partially characterized two uteroactive phenolic glycosides (named A and B) from *G. perpensa* extracts. These glycosides showed greater uterotonic activity than the water and methanol extracts by increasing the response of the uterus to acetylcholine over a full range of concentrations tested. *G. perpensa* is also used for the treatment of colic during pregnancy by Southern Sotho women (Brookes and Dutton, 2007).
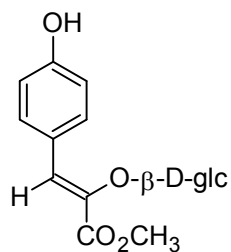
3.43   **3.44** R = H
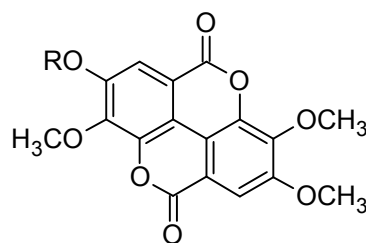
                        3.45 R = β-D-glc

In a phytochemical investigation of *Combretum kraussii*, four pure uteroactive compounds (Table 3.1), as well as combretastatin A-1, ellagic acid (**3.44**) and trimethyl ethers of ellagic acid (**3.45**) were isolated. Two uteroactive phenolic glycosides were also partially characterized from the root extracts. These glycosides exhibited the highest uteroactivity when compared to the other compounds isolated from this plant (Brookes et al., 1999).

The phytochemistry of *Pentanisia prunelloides* has not been extensively studied, and only two compounds have been isolated from this plant. (-)-Epicatechin was obtained from the polar extract, while palmitic acid was identified as a major constituent of the non-polar extract. Recent quantification studies showed that there is 2.29 µg (-)-epicatechin)/g of dry MeOH extract and 0.26 µg/g dry aqueous extract of *P. prunelloides* (Mpofu et al., 2014; Yff et al., 2002).

Brookes et al. (2006) studied the phytochemistry of *Rhoicissus tridentata*. A hydrogel polymer of glucose, which stimulated uterine muscle contractions from low to maximal concentrations of acetylcholine, was partially characterised. The uteroactive oleanolic acid (**3.20**) was purified from the chloroform extract of the roots and branches in this study. Compound **3.20** was also isolated from *Ekebergia capensis* and *Grewia occidentalis* (Table 3.1). β-Sitosterol (**3.22**) and its glucoside, sitosterolin, were also isolated and they showed slight oestrogenic activity causing an increase in the uterine muscle contractility at low acetylcholine concentrations. The uterine response was inhibited at maximum concentrations of acetylcholine (Brookes and Katsoulis, 2006).

An investigation of the hexane extract of the rhizomes of *Typha capensis* led to the isolation of two new compounds, typhaphthalide (**3.46**) and typharine (**3.47**), as well as sitosterol, afzelechin, epiafzelechin, (+)-catechin and (-)-epicatechin (Shode et al., 2002). Some of the compounds (afzelechin and epiafzelechin) isolated from *T. capensis* demonstrated antibacterial activities in previous studies, but the uteroactive principles are not known (Masoko et al., 2008; Xie and Dixon, 2005).
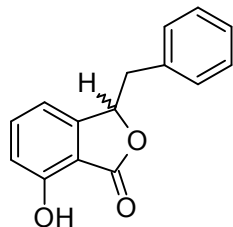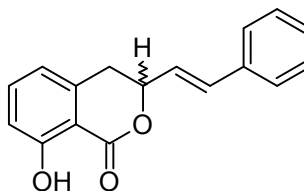
3.46   **3.47**

In the Tswana population, traditional remedies used in pregnancy are known as *kgaba*. Similarly to *isihlambezo*, *kgaba* is taken from the last three months to protect against evil and harm, to hastened prolonged labour, and to augment labour when overdue. In a study carried out by Kooi (2006), several traditional healers from Mogwase district, North West Province revealed that about 18 plants are used in the preparation of *kgaba* and one of the most frequently used is *R. tridentata* (known as *kgaba etona* in this region). Some other items, for instance, ostrich eggshell, baboon urine and mud of wasp, have been reported to stimulate labour amongst Tswana population. Plants with uterotonic activity are sometimes mixed with these items, and these plants are burnt and their ashes used with blessed water in churches (Van der Kooi and Theobald, 2006).

3.3.1   Toxicity of some South African oxytocic plants

Humans have known for a long time that some plants are toxic. They would use these poisonous plants for hunting, war, rituals, murder, suicide, abortions and euthanasia. Some research groups who have focused on identifying the toxic principles in plants, have also shown that most of the toxic chemicals tend to have medicinal properties at lower concentrations. In South Africa, the toxicity of some plants used in *isihlambezo* preparation has been investigated. Yuccagenin and agapanthagenin isolated from the rhizomes of *Agapanthus africanus* caused gastrointestinal track and kidney problems (Ndhlala et al., 2013).

Cardiac glycosides, such as those isolated from *Asclepias fructicosa* (Apocynaceae), are known for their toxic effects on gastro-intestinal, respiratory and nervous system resulting to neuromuscular disorders and death (due to cardiotoxic effects) (Kellerman et al., 1988). A recent review has been published covering the ethnopharmacology and toxicology of Amaryllidaceae alkaloids. It was concluded in this review that although several interesting biological activities are shown by Amaryllidaceae plants, they must be used cautiously because of the toxicity of their alkaloids. Members of this family used during pregnancy and which have shown potential toxicity are *Clivia miniata*, *Scadoxus puniceus*, and *Crinum bulbispermum* (Nair and van Staden, 2013; Ndhlala et al., 2013).

Brookes et al. (2003) studied the cytotoxicity of the aqueous extracts from *R. tridentata*, *G. perpensa* and *C. kraussii* using human fibroblast, monkey vero, and human hepatoma cells. *R. tridentata* extracts had been previously associated with a number of deaths as a result of central nervous system depression and respiratory failure (Brookes and Smith, 2003). Some species from Combretaceae are considered as toxic. In Zimbabwe, 5 women died after the vaginal insertion of powdered root material from *C. erythrophyllum*, while *C. kraussii* butanol extracts showed toxicity to brine shrimps (Rogers and Verotta, 1996).

Brookes (2003) found that the threshold (concentration at which 100% cells survived) for zero monkey vero and human cell death was 250 µg/mL (*G. perpensa*), 67 µg/mL (*C. kraussii*) and 8 µg/mL when *R. tridentata* was used. The blood stream concentration of these extracts were then estimated (taking dilutions into account) to be 4.6 µg/mL for *G. perpensa*, 8.9 µg/mL for *C. kraussii* and 6.1 µg/mL for *R. tridentata*. It was then concluded that the three extracts used in this study were not toxic at cellular level since all the estimated cell concentrations were below the thresholds for zero cell death (Brookes and Smith, 2003). However, traditional healers must be cautions with the dosage of *R. tridentata* as this plant showed the lowest threshold. There are some claims that *R. tridentata* may result into uterine hyperstimulation. Proanthocyanidins, which are found in higher concentrations in summer than winter, have been associated with hyperstimulation. The concentration of these polyphenolic compounds is also reported to vary from one

location to the next (Brookes and Katsoulis, 2006). Therefore, dosage is an important factor to consider when this plant is used.

It is clear from the above discussion that plants are used worldwide for pregnancy-related disorders. But, the phytochemistry of most of these plants has not yet been studied. Moreover, there are several claims concerning the toxicity of these medicinal plants. To minimise the toxicity risks of *isihlambezo* (or any other herbal remedy), correct identification of the species, knowledge of the chemical constituents of the plant, as well as proper dosages must be taken into account. Therefore, more research is needed to study the phytochemistry of oxytocic plants. In this research we focused on a South African indigenous oxytocic plant, *Rhoicissus tridentata*.

3.4   Genus *Rhoicissus*

3.4.1   Introduction

The genus *Rhoicissus* Planch. consists of around 12 to 22 species distributed in the Afrotropical zone of Africa, south of Sahara desert, southern and eastern parts of Arabian Peninsula, Madagascar, south-western Pakistan and the Islands of western Indian Ocean (Chen and Manchester, 2007). The distribution of *Rhoicissus* species is shown in Figure 3.3. *Rhoicissus* belongs to the family Vitaceae. Vitaceae, also known as the grape family, is amongst the smallest flowering plant families with about 14 genera and 900 species. The largest genera in this family are *Cissus* L. and *Cyphostemma* (Planch.) Alston, with ca. half of the species belonging to these genera (Chen and Manchester, 2007; Soejima and Wen, 2006).

Rhoicissus is one of the smallest genera in the family (Nalule et al., 2012; Nie et al., 2010; Soejima and Wen, 2006). As mentioned above, *Rhoicissus* occur naturally in South Africa. Other genera of natural origin in South Africa are *Ampelocissus, Catratia, Cissus* and *Cyphostemma. Ampelopsis*, *Parthenocissus* and *Vitis*, are cultivated in South Africa. In

addition to South Africa, Vitaceae plants are found in the tropical regions of Asia, Australia, neotropics, Pacific Islands and other African countries. *Vitis*, *Parthenocissus* and *Ampelopsis* normally occur in the temperate regions (Chen and Manchester, 2007; Naude, 2012; Nie et al., 2010; Soejima and Wen, 2006).
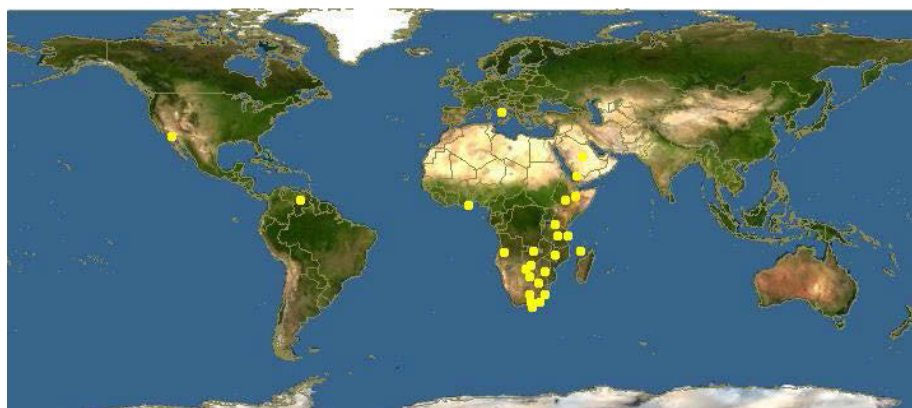

Figure 3.3: Distribution of *Rhoicissus*.

(Map: http://www.discoverlife.org/mp/20q?search=Rhoicissus)

Rhoicissus species, together with other Vitaceae members, are mostly twining vines, but small trees and shrubs also exist. Other characteristic features are (a) the alternating simple or compound leaves, which are often palmately or stipulate lobed, veined or course teeth, (b) tendrils (modified shoots or inflorescences sometimes ending in disk like suckers) opposite the leaves, (c) small unisexual, bisexual or actinomorphic flowers growing as determinate or indeterminate inflorescences and (d) a berry fruit (Chen and Manchester, 2007; Gerrath et al., 2004; Naude, 2012; Nie et al., 2010; Soejima and Wen, 2006).

There are no reports on the economic significance of *Rhoicissus* plants, however members of the sister genera, *Vitis* and *Parthenocissus*, are of high economic value worldwide. Examples of the economically important species are *Vitis vinifera*, *Parthenocissus quinquefolia* and *P. tricuspidata*. Table grapes are obtained from *V. vinifera* (wine grape) and these are used to produce raisins and wine. Wine grape, together with its hybrids, are nowadays cultivated in more than 8 million hectares of land worldwide and annually produce over 200 million litres of wine (Soejima and Wen, 2006; Tröndle et al., 2010). *P.*

quinquefolia (Virginia creeper) and *P. tricuspidata* (Boston ivy) are used as landscape ornaments worldwide (Brickell, 2008; Thornbush, 2012).

Vitaceae plants have been used in various traditional medicine systems for centuries for diseases such as cancer, skin disease, bone fracture, wounds, eye diseases, gonorrhoea, syphilis, menstrual ailments, liver disorders, abdominal pains, stomachaches, headache, diarrhoea, fever, vomiting, typhoid, nausea and malaria (Kanwal, 2011; Khan et al., 2015; Patel et al., 2013; Pathomwichaiwat et al., 2015; Tröndle et al., 2010). This family is also well known for treating gynaecological disorders. Examples of plants used for these disorders are *Ampelocissus latifolia* (used to prevent miscarriages and reduce bleeding during menopause) (Rao, 2010) and several species from the genera *Cyphostemma* and *Rhoicissus* (commonly used to facilitate delivery during pregnancy) (Lin et al., 1999).

In the following sections, we will focus on the genus *Rhoicissus*, its local uses, reported biological activities and phytochemistry. Included in this chapter are also the results from the phytochemical study of *Rhoicissus tridentata*

3.4.2   Local uses

As mentioned earlier, *Rhoicissus* species are popular in traditional medicine for their use in treating gynaecological and obstetrics complaints. *R. tridentata* (L. f.) Wild & Drum subsp. *cuneifolia* (Eckl. & Zeyh.) N.R. Urton, *R. digitata* (L. f) Gilg & Brandt and *R. tomentosa* (Lam.) Wild & Drum are all used to prevent long and complicated labour during child-birth (Brookes and Katsoulis, 2006; Hutchings et al., 1996; Lin et al., 1999). These three plants, as well as *R. rhomboidea* (E. Mey ex Haru.) Planch, are all named *isinwazi/ umthwazi* (isiZulu). The oxytocic properties of *R. tridentata* and *R. digitata* have been confirmed by *in vitro* biological tests (discussed below); however, the use of *R. tomentosa* has not been supported by scientific data. This ambiguity in naming creates confusion during plant identification, and thus uncertainty about the medicinal properties of the collected species (Hutchings et al., 1996; Lin et al., 1999).

Aqueous extract of the root of *R. digitata* have been cited by lay people of Maputaland (KwaZulu-Natal, SA) to be used in combination with other species, for example, *Bridelia cathartica*, *Commiphora neglecta*, *Crotalaria monteiroi*, *Garcinia livingstonei*, *Grewia occidentalis* and *Ochna natalitia* for treating dysmenorrhea, menorrhagia, oligomenorrhoea and infertility. This combination of plants is also used to prevent premature birth, and to cleanse blood after pregnancy (de Wet and Ngubane, 2014). *R. tomentosa* is used as an antihelmintic for calves (Hutchings et al., 1996).

R. revoilli (Planch) root decoctions are used in lactation for both human and cows to increase milk production. Depending on the preparation method, leaves of this plant are used to treat various ailments in African communities. Crushed leaves are externally rubbed to infected skin to hasten wound and ringworm healing (Arwa et al., 2008). Aqueous decoctions prepared from crushed leaves are orally taken to treat intestinal worms such as hookworms (Omino and Kokwaro, 1993). Decoctions are also externally applied to boils to ripen them and thus promote faster healing (Arwa et al., 2008).

3.4.3   Biological activities and phytochemistry

Some Zulu medicinal plants belonging to the Vitaceae family were screened for anti-inflammatory and antimicrobial activities (Lin et al., 1999). A total of twenty-nine crude extracts were evaluated but only five showed significant inhibition of prostaglandin synthesis. With the exception of *Cyphostemma natalitium*, the other four active species (*R. digitata*, *R. rhomboidea*, *R. tomentosa*, and *R. tridentata*) all belong to the genus *Rhoicissus*. *R. digitata* and *R. rhomboidea* extracts showed the highest activity, but these plants are not used as anti-inflammatory agents in traditional medicine. In addition, these extracts exhibited the highest antifungal activity against *Candida albicans* (Lin et al., 1999). Antifungal activity reported for *R. tridentata* against *C. albanicans* and *Saccharomyces cerevisiae* was confirmed in a recent study conducted in Tanzania (Hamza et al., 2006).

Lin et al. (1999) also investigated the antimicrobial activities of the twenty-nine extracts, and it was found that all extracts showed some degree of antimicrobial activity. Gram-positive microorganisms were better inhibited than Gram-negative bacteria. *R. rhomboidea* was one of the plants showing the highest inhibition against different microorganisms (Lin et al., 1999). In another study aimed at screening fourteen medicinal plants used by the Venda community for infectious diseases (such as diarrhoea and fever), antibacterial activities for root/ tubers methanol extracts of *R. tridentata* were confirmed (Samie et al., 2005).

Opoku et al. (2002) investigated the antioxidant activities of four *Rhoicissus* species (*Rhoicissus digitata*, *R. tridentata*, *R. rhomboidea* and *R. tomentosa*). The methanol extracts of the roots, stems and leaves were all tested for: (a) free radical scavenging activity using 1,1'-diphenyl-2-picryhydrazyl free radical, (b) effect on the generation of free radicals by xanthine oxidase, (c) effect on DNA sugar damage and (d) Fe^{2+} chelating activity. *R. tridentata* and *R. rhomboidea* showed more than 50% antioxidant activity, which was comparable to the activities observed for the commercial antioxidants BHA (42%), BHT (50%) and Vitamin E (63%). In Contrast, *R. digitata* and *R. tomentosa* extracts only exhibited pro-oxidative properties at very high concentrations (Atawodi, 2005; Opoku et al., 2002).

Naidoo et al. (2006) further studied the antioxidant properties of the leaves, stem bark and roots acetone extracts of *R. tridentata*. In this study the stem bark acetone extract showed the highest antioxidant activity. Compounds associated with this activity were catechin, epicatechin, gallic acid, epigallocatechin gallate and other unidentified proanthocyanidins (Naidoo et al., 2006). In a recent study the antioxidant property of the root methanol extract of *R. tridentata* has been confirmed (Steenkamp et al., 2013).

Opoku et al. (2000) screened twenty-seven Zulu medicinal plants for antineoplastic activities against HepG2 cell lines and *R. tridentata*, *R. tomentosa*, *R. rhomboidea* and *R. digitata* were amongst the investigated plants. The aqueous root extract of *R. tridentata* exhibited the highest inhibition of proliferation (96%) compared to the other crude extracts. Methanol extract of *R. tridentata* also showed stronger inhibitory activity, with 87%

inhibition of proliferation. Results from this study also showed that all the *Rhoicissus* species, together with other plants tested, have potential antineoplastic activities (Opoku et al., 2000).

The methanol root and leaves extracts of *R. revoilli* were studied to confirm their antimicrobial activity. The root extract was more active than the leaves against three microorganisms (Gram-positive bacteria *Streptococcus pryogenes*, Gram-negative *Salmonella typhi* and a fungal pathogen *Aspergillus niger*). Although the active compounds responsible for antimicrobial activity were not isolated and characterized, the phytochemical screening of the active fractions led to the identification of saponins, flavonoids, ketones, anthraquinones, carbohydrates and alkaloids. In addition to these types of compounds, aldehydes were detected in the leave extract (Arwa et al., 2008).

In a study conducted by Schou et al. (2010), the water extract of *R. digitata* root and the methanol extract of the leaves, increased the acetylcholine-mediated contractions of rat uterine smooth muscle. The MeOH extract of the root inhibited uterine contractility. In additional to providing a rationale for the use of this plant during pregnancy in traditional medicine, results from this study also showed that instead of using the roots, traditional healers can use the leaves (which also showed similar activity to the roots) (Schou et al., 2010).

3.5   *Rhoicissus tridentata*

3.5.1   Introduction

Rhoicissus tridentata (L.F.) Wild & Drum subsp. *cuneifolia* (Eckl. & Zehr.), N.R. Urton, commonly known as wild grape and *Isinwazi* or *Umthwazi* (isiZulu) is a shrubby creeper in the genus *Rhoicissus* (Vitaceae). This plant is characterised by trifoliate leaves with wedge shaped leaflets, and greyish bark with a tubular root (Katsoulis et al., 2000). *R. tridentata*

plants occur throughout the Afrotropical zone (South Africa, south of Sahara desert, southern and eastern parts of Arabian Peninsula, Madagascar, and south-western Pakistan).

Figure 3.4: Leaves and branches of *R. tridentata*

(Photo:https://www.google.co.za/?gfe_rd=cr&ei=DsP1VPExjqnzB4uugIgF#q=rhoicissus+t ridentata+subsp.+cuneifolia)

Local communities in South Africa use *R. tridentata* during pregnancy to enhance the labour process. This plant is also used to prevent miscarriage and diarrhoea, treat abdominal pains and stomach disorders, swelling, menorrhagia, infertility, broken bones, cuts and wounds, epilepsy and to feed children in the absence of their mother (Hutchings et al., 1996; Lin et al., 1999; Veale et al., 1992). The Vhavenda men use *R. tridentata* to treat erectile dysfunction (Rakuambo et al., 2006). Aqueous decoctions and infusions are prepared form crushed dried leaves and roots and are administered orally or as enemas.

3.5.2   Biological activities and phytochemistry

The methanol and aqueous extracts of *R. tridentata* roots have shown antifungal, antibacterial, antioxidant and antineoplastic activities (described above). Further biological studies on the aqueous extract of the roots and lignotubers showed that this extract caused an increase in the contraction of isolated uterine smooth muscle/ileum. This contractility increase was confirmed to be due to the muscarinic receptor system and the production of

cyclooxygenase metabolites (Table 3.1) (Brookes and Katsoulis, 2006; Katsoulis et al., 2000). It is noteworthy that some researchers have confirmed that the uterotonic activity of *R. tridentata* varies seasonally. It is reported that the lignotubers, followed by the stems were the most active plant part; however, this activity was 4-5 times higher for the extracts harvested in autumn and summer compared to those harvested in spring and winter (Katsoulis et al., 2002).

Brookes and Katsoulis (2006) studied the phytochemistry of *R. tridentata* subsp. cuneifolia, and amongst the 20 compounds identified from the methanol and chloroform extracts, the uteroactive oleanolic acid (**3.18**) and slightly oestrogenic β-sitosterol (**3.22**) were isolated. Some proanthocyanidins monomers and dimers from the methanol root extract were identified by HPLC. These included catechin (**3.48**), gallocatechin (**3.49**), fisetinidol (**3.50**), mollisacacidin (**3.51**), epicatechin (**3.52**), epigallocatechin (**3.53**), epicatechin-3-gallate (**3.54**), procyanidin B3 (**3.55**), procyanidin B4 (**3.56**), fisetinidol-(4α→8) catechin (**3.57**), fisetinidol-(4β→8) catechin (**3.58**) and gallic acid.

Another major component of the methanol extract was an unidentified uteroactive sugar fraction (Brookes and Katsoulis, 2006). From the non-polar root extract of *R. tridentata*, the triterpenoids, 20(29)-lupen-3-one (**3.59**), 20-*epi*-ψ-taraxastananol (**3.60**) and γ-sitosterol (which is similar to β-sitosterol (**3.22**), but with a 24*S* configuration), were identified by gas chromatography-mass spectrometry (GC-MS). The young branches afforded triacontanol (Brookes and Katsoulis, 2006).

	R^1	R^2	R^3
3.48	OH	H	H
3.49	OH	H	OH
3.50	H	H	H
3.51	H	OH	H

	R^3	R^4
3.52	H	OH
3.53	OH	OH
3.54	H	O-gallate

	R¹	R⁴
3.55	OH	β-OH
3.56	OH	α-OH
3.57	H	β-OH

3.58
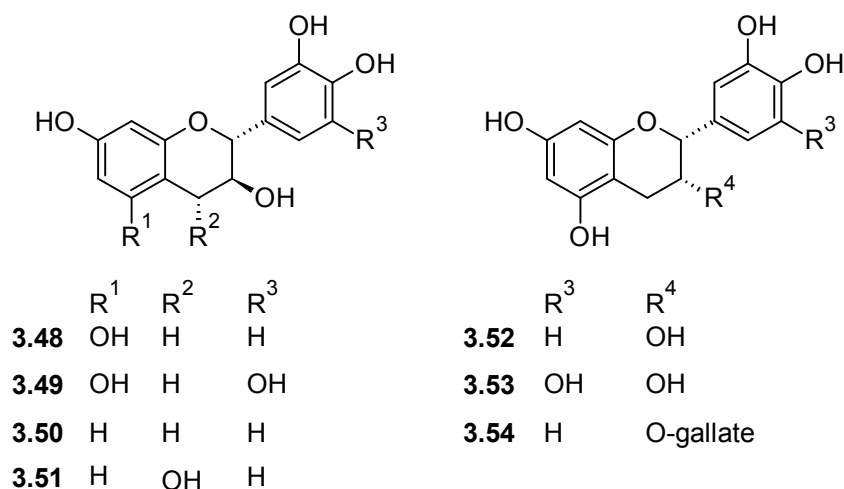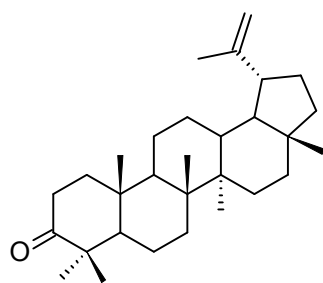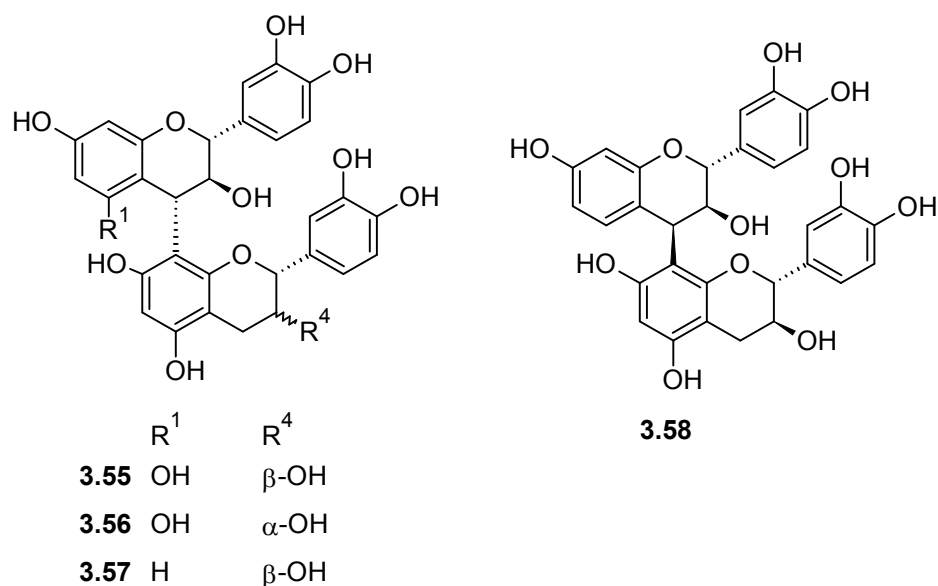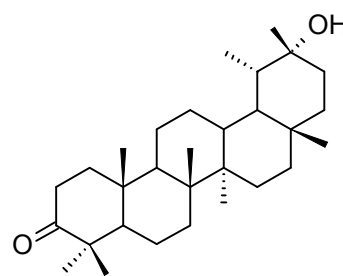
3.59　　　　**3.60**

The *in vitro* anti-oxidative properties of *R. tridentata* motivated Opoku et al. (2007) to evaluate *in vivo* hepatoprotective effects of this plant. The free radical intermediates (malondialdehyde and 4-hydroxy-2-nonenal) produced by carbon tetrachloride (CCl_4) initiated acute liver injury in rats, and the change in the concentrations of serum enzymes [e.g. aspartate transaminase (ALT) and serum alanine transaminase (ASP)] was a useful factor in determining the extent of hepatic damage. Other variables assayed included the effect on the concentration of glucose-6-phosphatase and changes in the levels of lipid peroxidase (LPO). Results obtained in this study demonstrated a decrease in the concentrations of ALT, ASP and LPO, while the glucose-6-phosphatase concentration increased as a result of the administration of *R. tridentata* extract to the CCl_4 intoxicated

rats. These results were an indicator of the hepatoprotective property of *R. tridentata* (Opoku et al., 2007).

R. tridentata was one of the 24 plants mentioned by traditional healers in Limpopo province to be used in the treatment of TB. However, when *R. tridentata* extracts were tested for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Ra$, MTB resistant strain, ripifampicin, ethambutol and streptomycin, no significant activity was observed at concentrations ≤ 100 µg/mL (Green et al., 2010).

Results obtained from the *in vitro* bioassays on the chloroform and ethanol extracts confirmed the erectile dysfunction activity as these extracts caused relaxation of rabbit corpus cavenosal smooth muscle. Significantly higher percentage relaxation was observed at the extract concentration of 13.0 mg/mL instead of 6.5 mg/mL. These results suggest that *R. tridentata* extracts contain promising future candidate for treating erectile dysfunction (Rakuambo et al., 2006).

Nalule et al. (2012) and Tuwangye and Olila (2006) demonstrated the *in vitro* ascaricidal activity of ethanol and water root extract of *R. tridentata* against adult nematodes. The inhibition of adult worm motility increased with increasing extract concentration and incubation. The death of treated worms in this study was attributed to starvation as a result of paralysis. Several active principles previously isolated from *R. tridentata*, including sitosterol, sitosterolin and proanthocyanidins, were associated with the paralysis (Brookes and Katsoulis, 2006; Nalule et al., 2012; Nalule, 2011; Tuwangye and Olila, 2006).

The biological activities of both the polar and non-polar extracts from the roots and lignotubers of *R. tridentata* have been extensively studied. However, the phytochemistry of this plant has received limited attention. A number of compounds have been identified by Brookes, et al. (2006), but their identification relied on the GC-MS and HPLC results and no compounds were isolated from the root polar extract (which is the extract used by traditional healers for the preparation of the uterotonic herbal drink). In the following section (Section 3.6), we will discuss the phytochemistry of the polar extracts from *R.*

tridentata root. In addition, the uterotonic activity of the isolated compounds will be evaluated *in vitro*.

3.6   Results and discussion: Isolation and structural elucidation of compounds from *R. tridentata*

3.6.1   Introduction

Rhoicissus tridentata subsp. *cuneifolia* (leaves, branches and root) were collected from Silverglen nursery in Durban. A second batch of the roots was collected from the vendors in the Warwick Triangle in Durban. After washing under running water, the plant material was separated and dried in an oven at 30 °C. Each plant part was crushed and extracted with DCM (48 hours) and subsequently with MeOH (48 hours). Bioassay guided fractionation of the acetone fraction of the methanol extract of the root led to the isolation of quercetrin (**3.61**), morin 3-*O*-α-L-rhamnopyranoside (**3.62**), catechin (**3.63**), *trans*-resveratrol glucoside (**3.64**) and an inseparable mixture of arjunolic acid (**3.65**) and asiatic acid (**3.66**) were isolated. β-Sitosterol (**3.22**) was the major compound in the DCM fraction from the methanol root extract. Linoleic acid (**3.18**) was also isolated from the DCM fraction.

3.6.2 Quercetrin (3.61)
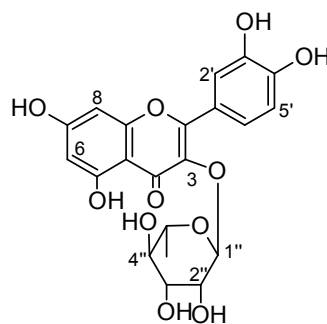
3.61

Compound **3.61** appeared as a yellow band on TLC with an R_f value of 0.62 (7:3 DCM-MeOH), which turned orange after treating the TLC plate with anisaldehyde stain followed by heating. The UV/Vis spectrum (Fig. 3.5) revealed the maximal UV wavelengths (λ_{max}) of 257 and 352 nm, which is in agreement with the UV spectrum of a flavonol (Mabry et al., 1970). In the ESI(+)-HRMS spectrum, a pseudo-molecular ion peak observed at m/z 471.3783 [M+Na]$^+$ is in agreement with a molecular formula of $C_{21}H_{20}O_{11}$.
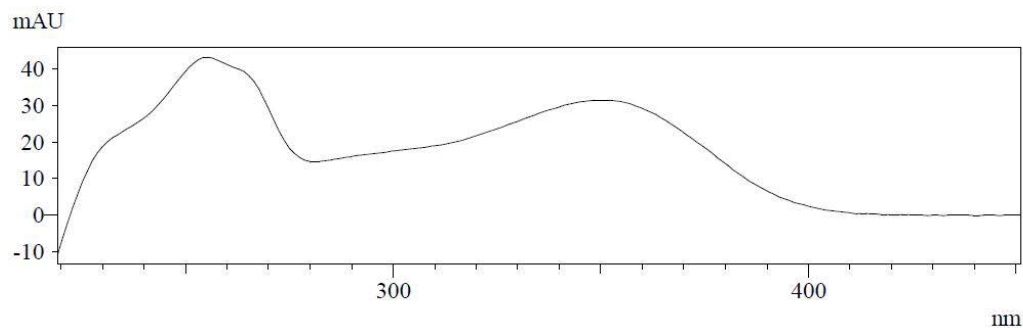
Figure 3.5: UV/Vis absorption spectrum of compound **3.61**

In the ^{1}H NMR spectrum (Plate 12a), two *meta*-coupled aromatic protons were observed at δ_H 6.40 (d, J = 2.3 Hz) and 6.23 (d, J = 2.3 Hz). These are characteristic of flavonoids with oxygen substituents at positions 5 and 7. The ^{1}H NMR spectrum also showed three signals corresponding to an ABX aromatic spin system at δ_H 6.94 (d, J = 8.2 Hz), 7.33 (dd, J = 8.2, 2.3 Hz) and 7.36 (d, J = 2.3 Hz), which represented a 3',4'

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substituted B-ring of a flavonoid derivative. The substitution pattern of ring B was confirmed by the downfield shift of H-2' and H-6' when compared to H-5'.

The ^{1}H and ^{13}C NMR spectra both showed upfield signals at δ_H 0.92 (3H, d, J = 5.9 Hz) and δ_C 17.7, respectively, which is diagnostic of the 6-methyl group of a rhamnose moiety. The anomeric proton signal of this moiety observed at δ_H 5.37 (d, J = 1.7 Hz) correlated in the HSQC NMR spectrum (Plate 12e) with a carbon resonating at δ_C 103.6. The small coupling constant for H-1" (J = 1.7 Hz) confirmed the α-configuration of the anomeric proton (Lu and Foo, 1997). Other rhamnose protons were observed at δ_H 3.44 (1H, t, J = 5.9 Hz, H-5"), 4.24 (1H, m, H-2"), 3.77 (1H, dd, J = 9.4, 3.4 Hz, H-3") and 3.37 (1H, m, H-4").

In the ^{13}C NMR spectrum (Plate 12b) of **3.61**, a total of 21 carbons were present. A carbonyl carbon signal at δ_C 179.6 (C-4) and six oxygen-bonded carbons were observed at δ_C 166.0 (C, C-7), 163.4 (C, C-5), 159.3 (C, C-2), 158.6 (C, C-8a), 149.8 (C, C-3'), 146.4 (C, C-4') and 136.2 (C, C-3). In combination with the ^{1}H NMR data, it was concluded that compound **3.61** was 5,7,3',4'-tetrahydroxy-3-*O*-rhamnopyranoxy flavone, better known as quercetrin.

The proposed structure was further confirmed by correlations observed in the HMBC spectrum (Plate 12f and Fig. 3.6). Correlations between H-8/H-6, C-8a, C-6 and C-4a established the presence of a benzopyran ring system. H-2' showed a long range correlation to C-2, which confirmed the substitution of the B-ring to C-2. HMBC spectrum also revealed a correlation between H-1" and C-3. This proved that the sugar moiety was connected to C-3 of the flavonol.
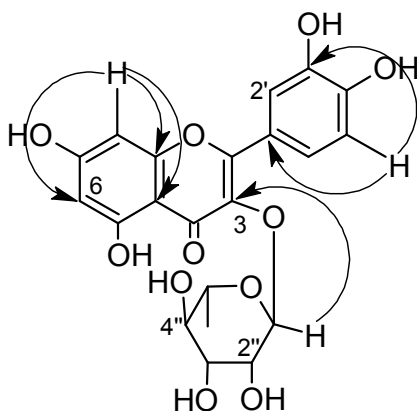
Figure 3.6: Key HMBC correlations in compound **3.61**

The ^{1}H and ^{13}C NMR data of compound **3.61** was in agreement with the reported data for quercetrin (Lu and Foo, 1997). This is the first report of quercetrin (**3.61**) from the genus *Rhoicissus*. However, this compound has been identified in the family Vitaceae. It was isolated from the aqueous extract of the stems and leaves of *Cissus quadrangularis* L. (Sharp et al., 2007) and *Cissus sicyoides* L. (Beltrame et al., 2001). Quercetrin is also present in several species of the genus *Vitis* (Moore and Giannasi, 1994) and has been isolated from *Leea indica* (Joshi et al., 2013).

Flavonoids and their glycosylated derivatives are known for their activity as antioxidants, anti-inflammatory and anticancer activity (Antunes-Ricardo et al., 2014; Veitch and Grayer, 2011). Moreover, quercetrin (**3.61**) have shown *in vitro* antiviral activity against the influenza A/NWS/33 (H1N1) virus and when high concentrations were used, it inhibited neuraminidase activity (Ha et al., 2014).

3.6.3 Morin 3-*O*-α-L-rhamnopyranoside (3.62)
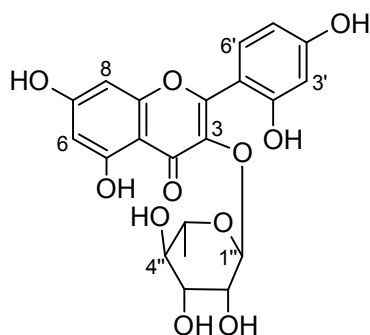
3.62

Compound **3.62** was observed as a yellow spot on TLC, with an R_f value of 0.69 (7:3 DCM-MeOH). Similarly to quercetrin (**3.61**), the UV/Vis absorption spectrum showed λ_{max} at 257 and 352 nm, which is consistent with the UV spectrum of a flavonol (Mabry et al., 1970). A pseudo-molecular ion peak at m/z 471.3851 $[M+Na]^+$ in agreement with the molecular formula, $C_{21}H_{20}O_{11}$, was observed in the ESI(+)-HRMS spectrum. The presence of a tetra-substituted A-ring of a flavonol was confirmed by the two *meta*-coupled aromatic protons observed at δ_H 6.22 (1H, d, *J* = 2.0 Hz) and 6.08 (1H, d, *J* = 2.0) in the ^{1}H NMR spectrum (Plate 13a). An ABX aromatic spin system was observed in the ^{1}H NMR spectrum (Plate 12a) at δ_H 6.73 (d, *J* = 8.2 Hz, H-6'), 7.13 (1H, d, *J* = 2.0 Hz, H-3'), 7.21 (dd, *J* = 8.2 and 2.0 Hz, H-5'), which suggested the presence a 2',4'-substituted B-ring of a flavonol. The signal for H-5' proton was upfield compared to H-3' and therefore confirming the substitution pattern of the B-ring. An upfield signal at δ_H 0.92 (d, *J* = 6.2 Hz) and δ_C 17.7 in the ^{1}H NMR and ^{13}C NMR spectra (Plates 13a and 13b), respectively, was indicative of the 6-methyl group of a rhamnose moiety. A doublet at δ_H 5.29 (1H, *J* = 1.7 Hz) in ^{1}H NMR spectrum represented the anomeric proton (H-1") signal of the rhamnose unit. The α-configuration of this proton was confirmed by small coupling constant for (*J* = 1.7 Hz) (Harborne, 1993; Lu and Foo, 1997).

In the ^{13}C NMR spectrum of compound **3.62**, 21 carbon signals were present. A carbonyl carbon signal was observed at δ_C 178.8 (C, C-4), whereas six oxygen-bonded carbons resonated at δ_C 162.7 (C, C-7), 159.1 (C, C-5), 153.2 (C, C-2), 159.0 (C, C-8a), 156.7 (C,

C-4') and 159.9 (C, C-2'). The rhamnose carbons resonated at δ_C 72.1 (H-5"), 72.0 (H-2"), 72.1 (H-3") and 73.4 (H-4").The ^{1}H and ^{13}C NMR data led to the assignment of the structure of **3.62** as morin 3-*O*-α-L-rhamnopyranoside.

The connectivity of the above-mentioned system was established by ^{1}H/^{13}C correlations observed from the HMBC spectrum (Plate 13f and Fig. 3.7). The presence of the benzopyran ring system in **3.62** was confirmed by the observed long-range ^{1}H/^{13}C correlations between H-6/ H-8, C-4a and C-8a. An HMBC correlation between H-1" and C-3 implied that the sugar unit was connected to C-3 of the flavonol. The trisubstituted aromatic system was established to be linked to C-2 of the benzopyran moiety by the observed long range correlations between H-6'/H-5'/H-3' and C-2.

The experimental NMR data of compound **3.62** was in agreement with the data reported in literature for morin 3-*O*-α-L-rhamnopyranoside (Lu and Foo, 1997; Yen et al., 2009). Morin 3-*O*-α-L-rhamnopyranoside (**3.62**) is isolated for the first time from the genus *Rhoicissus*. Compound **3.62** was reported to exhibit several biological activities, for example, antioxidant, antimicrobial and anti-inflammatory effects (Yen et al., 2009).

3.62
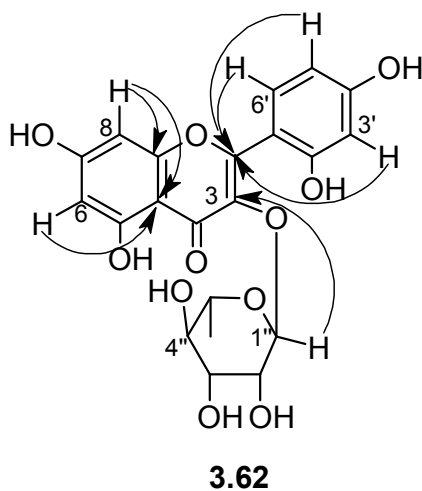
Figure 3.7: HMBC correlations in compound **3.62**

3.6.4 Catechin (3.63)
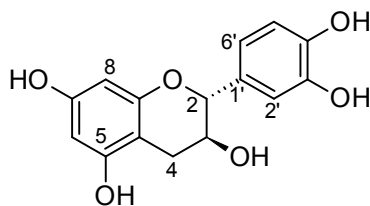
3.63

Compound **3.63** showed a pseudo-molecular ion peak at m/z 313.2611 [M+Na]$^+$ which is in agreement with the molecular formula, $C_{15}H_{14}O_6$. A λ_{max} of 278 nm was observed in the UV/Vis absorption spectrum (Fig. 3.8) of compound **3.63**, which is characteristic of catechin (Zu et al., 2006). The ^{1}H NMR (Plate 14a) and ^{1}H-^{1}H COSY spectra (Plate 14c) of **3.63** showed the presence of an ABX aromatic spin system at δ_H 6.79 (d, *J* = 8.2 Hz), 6.75 (dd, *J* = 8.2, 1.9 Hz) and 6.86 (d, *J* = 1.9 Hz). The tetra-substituted flavonoid derivative was shown by the two *meta*-coupled protons (H-8 and H-6) at δ_H 5.88 and 5.95 respectively. Two methine protons (H-2 and H-3) resonated at δ_H 4.59 (d, *J* = 7.3 Hz) and 3.99 (m). A ^{1}H-^{1}H COSY correlations were observed between H-2 and H-3, as well as between H-3 and the methylene protons (H-4) at δ_H 2.53 (dd, *J* = 8.2 Hz, H-4β) and 2.86 (dd, *J* = 15.9, 5.6 Hz, H-4α).
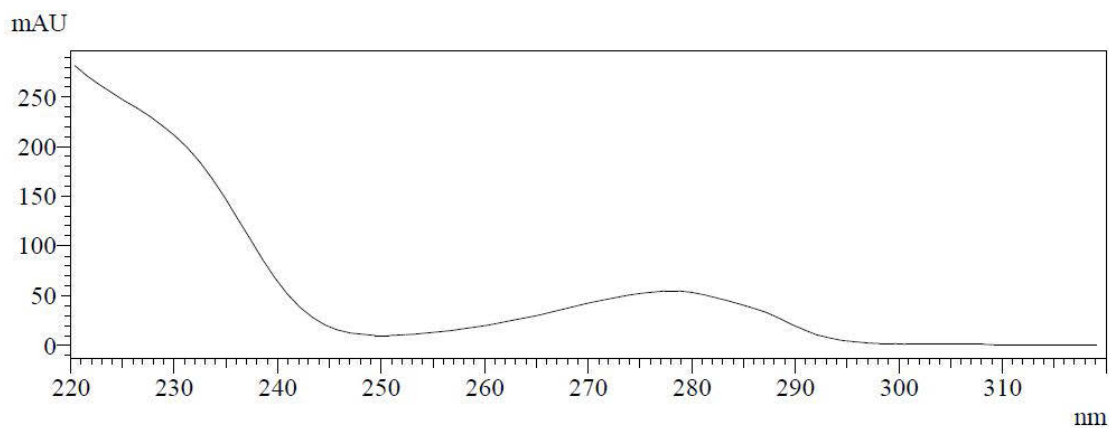
Figure 3.8: UV/Vis absorption spectrum of compound **3.63**

Fifteen signals were observed in the ^{13}C NMR spectrum (Plate 14b) of compound **3.63**, which corresponded to the proposed structure. The presence of 5 aromatic oxygen-

bonded carbons in the proposed structure was confirmed by the signals at δ_C 146.2 (C, C-3'), 146.3 (C, C-4'), 156.9 (C, C-8a), 157.6 (C, C-5) and 157.8 (C, C-7). The signal at δ_C 28.5 in the DEPT-135 experiment (Plate 14d) was assigned to the methylene carbon (C-4). No signal was observed at δ_C 170-190 in ^{13}C NMR spectrum. This confirmed the absence of a carbonyl carbon in this compound.

HMBC correlations (Plate 14f and Fig. 3.9) were used to further confirm the connectivity of the proposed structure. HMBC correlations were observed between H-2' and C-2; H-6' and C-2 which shows that the ABX ring system was connected to the pyran ring through C-2. The presence of the benzopyran ring system was confirmed by long range correlations between H-6, C-4a, C-5, and C-7; as well as between H-8, C-4a, C-8a, C-5, and C-7 (Fig. 3.9).

After comparison of the experimental spectroscopic data with the reported data, compound **3.63** was assigned as catechin (Davis et al., 1996). Catechin (**3.63**) was previously isolated from the roots of *R. tridentata* (Brookes and Katsoulis, 2006). Catechin (**3.63**) was also isolated from several species in the Vitaceae family, including *Ampelopsis megalophylla* Diels et. Gilg and *Leea thorelii* Gagnep. (Lakornwong et al., 2014; Zheng et al., 2009).
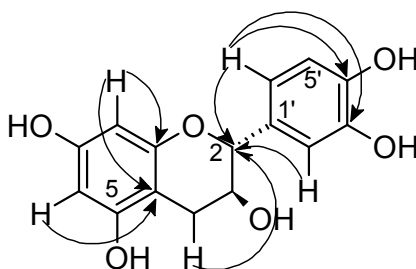
Figure 3.9: HMBC correlations in compound **3.63**

3.6.5   *Trans*-resveratrol glucoside (3.64)
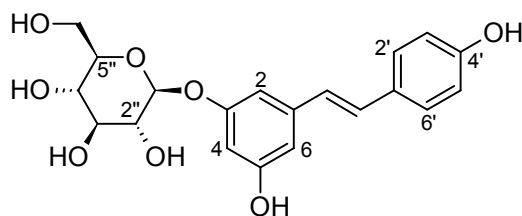
3.64

Compound **3.64** showed a maximal UV absorption at λ_{max} 310 nm in the UV/Vis spectrum (Fig.3.10) and a molecular ion peak at m/z 413.1212 [M+Na]$^+$ was observed in the ESI(+)-HRMS spectrum, which is in agreement with the molecular formula of $C_{20}H_{22}O_8$.
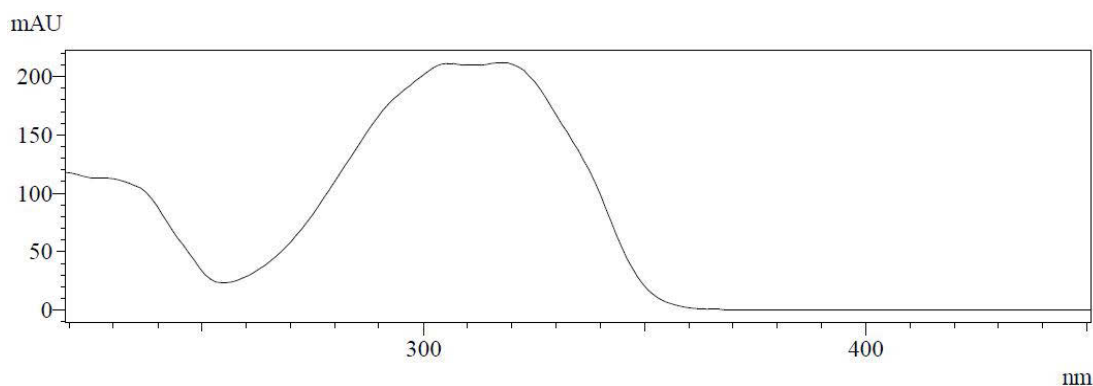
Figure 3.10: UV/Vis absorption spectrum of compound **3.64**

The ^{1}H NMR spectrum (Plate 15a) of compound **3.64** showed two *ortho*-coupled doublets (integrating to two protons each) at δ_H 6.79 (d, J = 8.7 Hz) and 7.39 (d, J = 8.7 Hz). These revealed the presence of an AA'XX' system of a 1,4-disubstituted aromatic ring. Three broad singlets/multiplets were observed at δ_H 6.47 (br.s), 6.64 (m, J = 1.6 Hz) and 6.80 (m, J = 1.6 Hz). These represented the three *meta*-coupled protons of the 1,3,5-trisubstituted aromatic ring. Two doublets with large coupling constants were observed at δ_H 6.87 (d, J = 16.3 Hz) and 7.04 (d, J = 16.3 Hz), suggesting the presence of a *trans*-olefin. A set of signals characteristic of glycosidic protons were observed between δ_H 3 and 5 in the ^{1}H NMR spectrum. An anomeric proton signal appeared at δ_H 4.91 (d, J = 7.5 Hz, H-1") and the other sugar protons

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resonated at δ_H 3.95 (dd, J = 11.9, 2.3 Hz), 3.76 (dd, J = 11.9, 2.3 Hz) and 3.40-3.80 (m). The methylene signals (δ_H 3.95 and 3.76) suggested that this sugar moiety was glucose. The large coupling constant (J = 7.5 Hz) for H-1" was consistent with the β-configuration of the glucosyl bond.

Eighteen carbon signals were observed in the ^{13}C NMR spectrum (Plate 15b) of compound **3.64**. Three of these carbons were oxygen-bonded and they resonated at δ_C 158.5 (C-4'), 159.6 (C-5) and 160.5 (C-3). The three methine carbons of the 1,3,5-trisubstituted aromatic ring resonated at δ_C 104.1 (C-4), 107.1 (C-2) and 108.4 (C-6). These signals were more upfield in comparison to the methine signals of the 1,4-disubstituted aromatic ring. The 1,3,5-trisubstituted aromatic ring is more electron-rich than the 1,4-disubstituted aromatic ring since it is attached to two electron-donating groups, the oxygen of the sugar and the hydroxy group.

The olefinic carbon signals resonated downfield at δ_C 126.7 (C-7) and 130.0 (C-8), which indicated that these carbons were bonded to aromatic rings. The five glucosyl carbon signals were observed at δ_C 71.5 (CH, C-4"), 73.9 (CH, C-2"), 78.1 (CH, C-3"), 78.3 (CH, C-5") and 102.5 (CH, C-1"). The methylene signal (C-6") appeared at δ_C 62.6 in the ^{13}C NMR spectrum. Based on the ^{1}H and ^{13}C NMR data, it was concluded that compound **3.64** was *trans*-resveratrol glucoside.

The long-range ^{1}H-^{13}C correlations observed in the HMBC spectrum (Plate 15f) further confirmed the proposed structural connectivity in compound **3.64**. The HMBC correlation observed between the anomeric proton (H-1") and C-3 confirmed that the sugar unit was connected to the aromatic ring by C-3. Other important HMBC correlations observed in this compound are shown in Figure 3.11.
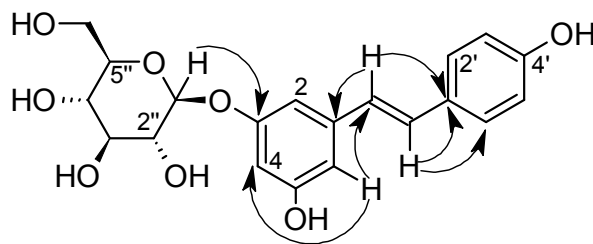
Figure 3.11: Important HMBC correlations in compound **3.64**

Our spectroscopic data for compound **3.64** was in agreement with the reported data for *trans*-resveratrol glucoside, commonly known as piceid (Ha et al., 2009; Teguo et al., 1996). This is the first report on the isolation of *trans*-resveratrol glucoside from the genus *Rhoicissus*. However, the family Vitaceae is dominated by monomeric and oligomeric stilbenoids. The most well-known and studied stilbene in this family is resveratrol, especially *E*-resveratrol found in grapes and red wine from *Vitis vinifera*. Resveratrol and its derivatives have shown a variety of biological activities, namely, anti-inflammatory, anticarcinogenic, antiaging, antiviral, antioxidant, as well as cardioprotective and neuroprotective effects (Burns et al., 2002; Rivière et al., 2012).

Piceid (**3.64**) has been associated with treating atherosclerosis as it was isolated from the two plants used to treat this condition in Asia (leaves of *Eucalyptus* and *Polygonum cuspidatum* roots) (Hillis, 1966; Teguo et al., 1996). Compound **3.64** was also isolated from the leaves of *Veratrum grandifolium* treated with cupric chloride, and it showed antifungal activity (Hanawa et al., 1992).

3.6.6 Asiatic acid (3.65) and arjunolic acid (3.66)

3.65 R^1 = CH$_3$, R^2 = H

3.66 R^1 = H, R^2 = CH$_3$
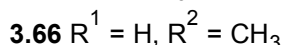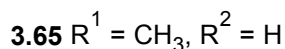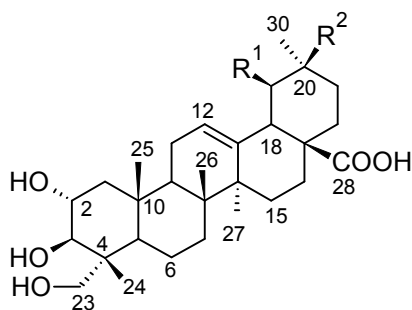
An inseparable mixture of an isomeric pair of triterpenoids (**3.65** and **3.66**) was isolated. It showed a pseudo-molecular ion peak at *m/z* 511.3399 [M+Na]$^+$ in the HRESIMS spectrum which is in agreement with a molecular formula of C$_{30}$H$_{48}$O$_5$. The percentage composition of asiatic acid and arjunolic acid in the mixture was determined to be 50:50% by NMR spectral data. The ^{1}H NMR spectrum (Plate 16a) of **3.65** and **3.66** showed signals of olefinic protons at δ_H 5.26 (m, H-12). Oxygen-bearing methylene protons appeared at δ_H 3.29 (d, *J* = 11.1 Hz, H-23) and 3.52 (d, *J* = 11.1 Hz, H-23), which is characteristic of both asiatic acid (**3.65**) and arjunolic acid (**3.66**). ^{1}H NMR and ^{13}C NMR spectra (Plate 16b) also showed two oxygen-bearing methine protons at δ_H 3.38/ δ_C 69.7 and δ_H 3.72/ δ_C 78.3. Cross peaks were observed in ^{1}H-^{1}H COSY spectrum (Plate 17c) between these signals, and thus were assigned as H-2 and H-3.

Two set of six methyl signals were observed in the ^{1}H and ^{13}C NMR spectra. In one set, four methyl signals were singlets while two were doublets (Table 3.2), characteristic of the ursane-type triterpene (asiatic acid). In second set of methyl signals, all of them were singlets, suggesting oleanane-type skeleton (arjunolic acid). Of note, there was only a slight difference in the chemical shift for most methyl signals for compounds **3.65** and **3.66**. A significant difference in δ_C values (>5 ppm) was observed for C-29 (Table 3.2). This was expected since C-29 is attached to a methine carbon (C-19) in compound **3.65**, whereas it is attached to a tertiary carbon (C-20) in compound **3.66**. The signals highlighted in green in Table 3.2 also showed slight differences in chemical shifts.

The ^{1}H and ^{13}C NMR spectra also showed a doublet at δ_H 2.22/ δ_C 54.4 and a doublet of doublets at δ_H 2.87/ δ_C 42.8. These signals were assigned to H-18 in compound **3.65** (asiatic acid) and arjunolic acid (**3.66**), respectively. Other signals observed in the ^{1}H and ^{13}C NMR spectra are collated in Table 3.2. From the HMBC spectrum (Plate 16f), correlations were observed between H-23, C-24, C-4 and C-3; H-24, C-23, C-3, C-4 and C-5; H-26, C-7, C-14, C-5; and H-25, C-10 and C-9. These correlations confirmed the proposed partial structure shown in Figure 3.12. Notably, the same HMBC correlations were observed for both compound **3.65** and **3.66**.
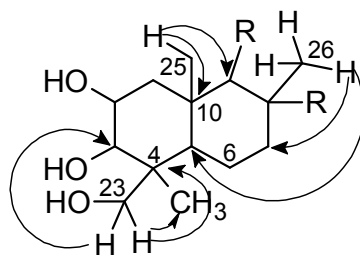
Figure 3.12: HMBC correlations in a partial structure of compound **3.65** and **3.66**

From the other half of the structure, ^{1}H/^{13}C long-range correlations existed between H-12, C-18 and C-27; H-29, C-27 and C-18; and H-27 correlated to C-15, C-14 and C-13 (Fig. 3.13). These were in agreement with the proposed structures for compound **3.65** and **3.66**.
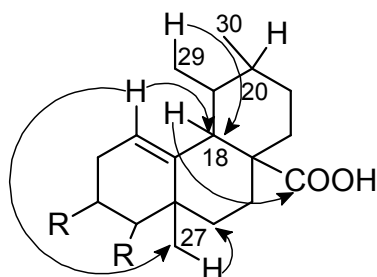
Figure 3.13: HMBC correlations in a partial structure of compound **3.65**

The experimental NMR data of compounds **3.65** and **3.66** was in agreement with the literature data of asiatic acid and arjunolic acid respectively (Aguirre et al., 2006). The

structures of asiatic acid (**3.65**) and arjunolic acid (**3.66**) are closely related and it was also observed by other researchers that a mixture of these compounds is inseparable by chromatographic methods (Bag et al., 2008). This is the first report on the isolation of both asiatic acid (**3.65**) and arjunolic acid (**3.66**) from the genus *Rhoicissus*. Asiatic acid was previously isolated from the aerial parts of *Cissus repens* (Vitaceae) (Wang et al., 2006).

Table 3.2: ^{1}H and ^{13}C NMR spectroscopic data (CD$_3$OD) of compounds **3.65** and **3.66**

Position	3.65 δ_C	3.65 δ_H (*J* in Hz)	3.66 δ_C	3.66 δ_H (*J* in Hz)
1a	47.3	1.68, m	47.3	1.68, m
1b		1.68, m		1.68, m
2	69.7	3.72, m	69.7	3.72, m
3	78.3	3.38, dd (9.6, 1.7)	78.3	3.38, dd (9.6, 1.7)
4	44.1		44.1	
5	47.8	3.32	47.8	3.32
6a	19	1.44, m	19	1.44, m
6b		1.44, m		1.44, m
7a	33.7	1.54, m	33.6	1.54, m
7b		1.75, m		1.75, m
8	40.8		40.4	
9	48	1.29, m	48	1.29, m
10	38.9		38.9	
11	23.9	1.11, m	24.1	1.96
12	126.7	5.26, m	126.7	5.26, m
13	138.6		138.6	
14	43		43	
15a	29.2	1.09, m	29.2	1.09, m
15b		1.96, m		1.96, m
16a	25.3	1.64, m	25.3	1.64, m
16b		1.64, m		1.64, m
17	48		48	
18	54.4	2.22, d (11.0)	42.8	2.87, dd (14.3, 3.7)
19a	40.4	0.98, m	47.9	0.92, m
19b				1.96, m
20	40.4	0.98, m	31.8	

Position	3.65 δ_C	(continued) δ_H (*J* in Hz)	3.66 δ_C	(continued) δ_H (*J* in Hz)
21a	31.6	1.34	31.6	1.34
21b		1.51		1.51
22a	38.1	1.67, m	38.1	1.67, m
22b		1.34, m		1.34, m
23	66.4	3.52, d (11.1)	66.4	3.52, d (11.1)
		3.29, d (11.1)		3.29, d (11.1)
24	13.9	0.72, s	13.8	0.72, s
25	17.8	1.07	17.5	1.06
26	17.9	0.83	17.7	0.83
27	24	1.15, s	26.5	1.20, s
28	181.7		181.7	
29	17.6	0.92, d (6.4)	33.4	0.93, s
30	23.7	0.96, m	23.8	0.96, s

3.6.7 β-Sitosterol (3.22)
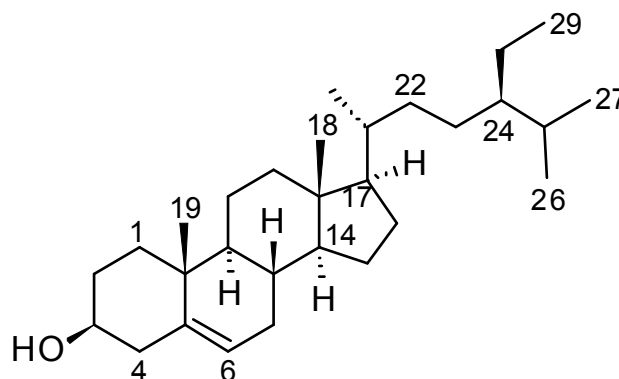
3.22

Compound **3.22** showed a molecular ion peak at m/z 414.7067 [M-H]⁻ in the LRMS spectrum, which is in agreement with the molecular formula of $C_{29}H_{50}O$. In the ¹H and ¹³C NMR spectra (Plates 17a and 17b), six upfield methyl signals were observed at δ_H 0.68 (3H, s, Me-18)/ δ_C 11.9; δ_H 0.83 (9H, m, Me-26, Me-27, Me-29)/ δ_C 19.8, 19.4, 11.9; δ_H 0.92 (4H, d, *J* = 6.5 Hz, Me-21, H-9)/ δ_C 18.8; and δ_H 1.00 (3H, s, Me-19)/ δ_C 19.0. This is consistent with the 6 methyl groups in the structure of β-sitosterol. A

doublet appeared downfield at δ_H 5.35/ δ_C 121.7 which was indicative of the vinyl proton (H-6). The H-3α proton resonated downfield as a multiplet at δ_H 3.51 in the ^{1}H NMR experiment and C-3 at δ_C 71.8 in the ^{13}C NMR experiment. In the ^{13}C NMR spectrum, additional signals observed included 11 methylene, 7 methine and 3 quaternary carbons. In combination with the ^{1}H NMR data, it was concluded that compound **3.22** was β-sitosterol.

The HMBC spectrum (Plate 17f) was used to confirm the structure of compound **3.22**. Amongst the correlations observed in the HMBC spectrum were correlations between Me-18, C-13 at δ_C 42.3, C-14 at δ_C 56.8 and C-17 at δ_C 56.1; Me-27/29 and C-24 at δ_C 45.9; as well as between H-4, C-2 at δ_C 31.7, C-5 at δ_C 140.8, C-6 at δ_C 121.7 and C-10 at δ_C 36.5 (Fig. 3.14). Further confirmation of compound **3.22** as β-sitosterol was achieved by comparing the experimental NMR data of this compound with literature data (Ahmad et al., 2010). β-Sitosterol was previously isolated from the roots of *R. tridentata* and in the same study, it was evaluated for *in vitro* uterotonic activity using rat uterine smooth muscle (results from these assays will be discussed in Section 3.7).
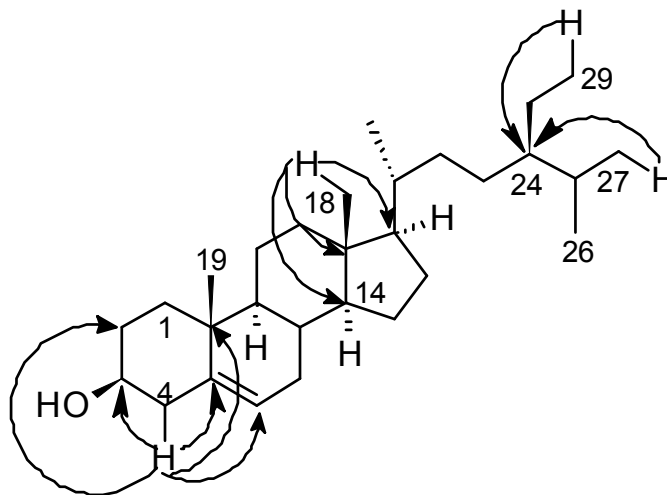
Figure 3.14: HMBC correlations in compound **3.22**

β-Sitosterol was previously isolated from *R. tridentata*, as well as several other Vitaceae plants such as *Ampelocissus latifolia* (Brookes and Katsoulis, 2006; Raman et

al., 2014), *Cissus quadrangularis*, *Tetrastigma erubescens* Planch. (Dao et al., 2013) and *Cissus sicyoides (*Beltrame et al., 2002). This compound showed antibacterial activity against *Bacillus subtilis* with an MIC of 50 µg/mL and it has been isolated from numerous plants showing analgesic and anti-inflammatory activities (Nirmal et al., 2012; Sengupta et al., 2012).

3.6.8   Linoleic acid (3.18)

3.18
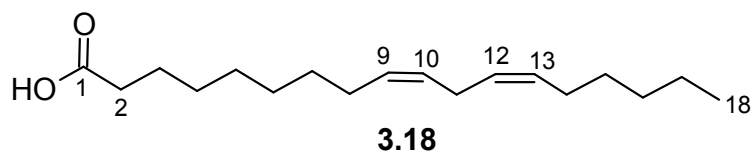
Compound **3.18** was isolated from the non-polar fraction of the root methanol extract, with an R_f value of 0.8 (DCM-EtOAc, 8:2). The TLC plate treated with anisaldehyde stain and heated, showed compound **3.18** as a purple spot. A molecular ion peak at m/z 281.2538 [M-H]⁻ observed in the LREIMS spectrum was in agreement with the molecular formula of $C_{18}H_{32}O_2$.

In the ¹H NMR spectrum (Plate 18a), four deshielded olefinic protons were observed at δ_H 5.35 (4H, m, H-9, H-10, H-12, H-13). Cross peaks were observed in the ¹H-¹H COSY NMR spectrum (Plate 18c) between the olefin protons and a signal at δ_H 2.78 (2H, m, H-11) and at δ_H 2.05 (4H, m, H-8 and H-14). A triplet appeared at δ_H 2.35 in the ¹H NMR spectrum which is characteristic of C-2 protons in a fatty acid. These protons showed a ¹H-¹H correlation to a multiplet at δ_H 1.63, which was readily assigned as H-3. The terminal methyl protons resonated at δ_H (0.89, t, $J = 6.8$), and a ¹H-¹H COSY spectrum revealed a connection between these protons and a methylene signal at δ_H (1.29, m, 14H).

The ¹³C NMR spectrum (Plate 18b) showed the presence of 13 carbon signals, consisting of acid carbonyl carbon at δ_C 179.9, four olefin carbon signals at δ_C 127.9, 128.1, 130.0 and 130.2, one methyl carbon at δ_C 14.0, and twelve methylene carbon signals at δ_C 22.6, 24.7, 25.6, 27.2, 27.2, 29.0, 29.1, 29.1, 29.3, 29.6, 31.5 and 34.0. These spectral data suggested

that compound **3.18** was an unsaturated fatty acid, and after comparison with the literature data, the structure of this compound was determined as linoleic acid (Marwah et al., 2007).

Linoleic acid (**3.18**) has never been reported from the genus *Rhoicissus*, however, this compound is present in several oxytocic plants. Linoleic acid (**3.18**) was isolated from the supercritical fluid extract of the roots of *Clivia miniata* L. and was found to induce the contractility of the uterine smooth muscle (Sewram et al., 2001).

3.6.1   Conclusion

Investigation of the acetone fraction of *R. tridentata* root methanol extract using bioassay-guided isolation technique afforded six compounds [quercetrin (**3.61**), morin 3-*O*-α-L-rhamnopyranoside (**3.62**), catechin (**3.63**), *trans*-resveratrol glucoside (**3.64**) and an inseparable mixture of asiatic acid (**3.65**) and arjunolic acid (**3.66**)]. The non-polar (DCM) fraction of the root methanol extract was also investigated and two compounds [β-sitosterol (**3.22**) and linoleic acid (**3.18**)] were isolated.

Except for β-sitosterol (**3.22**) and catechin (**3.63**), all isolated compounds are reported for the first time from *R. tridentata*; however these compounds are common in the family Vitaceae. We have not isolated any of the other compounds reported by Brookes et al. (2006) from the HPLC analysis of the methanol extract of the root of *R. tridentata*. The isolated compounds were assayed for their uterotonic activity to provide rationale for the use of this plant by local communities to facilitate labour during childbirth. Results obtained from the uteroactivity investigations are discussed in the next section.

3.7 Results and discussion: Biological activity

3.7.1 Introduction

The uterotonic effects of the methanol extracts of the leaves and roots of *R. tridentata*, the root aqueous extract of *Gunnera perpensa* and the isolated pure compounds from *R. tridentata* were evaluated using uterine muscle strips obtained from stilboestrol-primed Sprague-Dawley rats. The uterine muscle contractility effect of the aqueous extract of the root of *R. tridentata* was previously reported, but in that study the polar compounds were not isolated (discussed in Section 3.5.2). In the present study, the uterotonic activity of *R. tridentata* crude polar extracts was re-investigated and the activity of the pure isolated compounds was also evaluated.

Uterotonic assays were performed by Ms. S. Dube in the laboratory of Prof. C. T. Musabayane (School of Laboratory Medicine and Medical Science, University of KwaZulu-Natal, Westville). Ethical clearance for all experiments was obtained by Prof. Musabayane. Briefly, portions of the uterine muscle tissue were harvested from the stilboestrol-primed rats. The tissues were mounted in an organ bath containing a buffer solution at room temperature. A control experiment, in which the muscle strip was mounted in a drug-free buffer solution, was run for each experiment. The crude extracts and pure compounds were dissolved in a small amount of DMSO and diluted with a buffer solution to give solutions of different concentrations. Each experiment was performed by subjecting the uterine tissue to a solution with a specific concentration and the uterine contractions were electronically recorded using a labograph. Results obtained are presented as a graph of % contractions versus sample concentration. Values in each plot are presented as means ± SEM (n=6 for each concentration), ✴ $p < 0.05$ when compared with the control group.

3.7.2   Uteroactivity assays of the crude *R. tridentata* extracts

The uterine muscle strips were subjected to various solutions of *R. tridentata* leaves, with concentrations ranging from 0.24 mg/mL to 62.08 mg/mL. The lower doses (0.24 mg/mL-1.94 mg/mL) induced a gradual increase in both the force and the rate of uterine muscle contractility; however, these results were not significantly different compared to the control (Fig. 3.15). A statistically significant increase in the force of contractility was observed at a concentration of 3.88-62.08 mg/mL. The maximum response (80%) to the force of contractility was observed at 15.50 mg/mL and this force remained constant with increasing concentration (Fig. 3.15).

The leaves extract also caused an increase in the rate of uterine muscle contractions, with a maximal response (30%) (Fig. 3.15). These results suggest that the leaves contain uteroactive agents and can be used as a replacement to the roots for the induction of labour during pregnancy. The uterotonic activity of the leaves of *R. tridentata* was previously reported by Brookes and Katsoulis (2006).
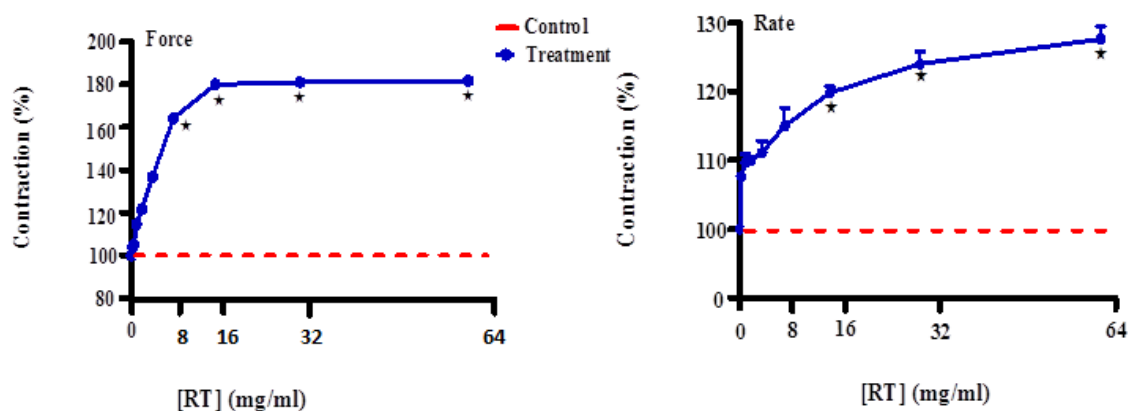
Figure 3.15: Effects of *R. tridentata* leaves crude extract on the force and rate of uterine muscle contractility.

The root methanol extract directly stimulated a concentration dependent contraction of the uterus, with the maximal response (80%) at 15.50 mg/mL. The higher dose (62.08 mg/mL) of extract caused a significant decrease in the force of uterine contractions. An increase in the rate of uterine muscle contractions was observed after subjecting the tissue to the root methanol extract of *R. tridentata*, with a maximum of response of 20% at 15.50 mg/mL (Fig. 3.16).
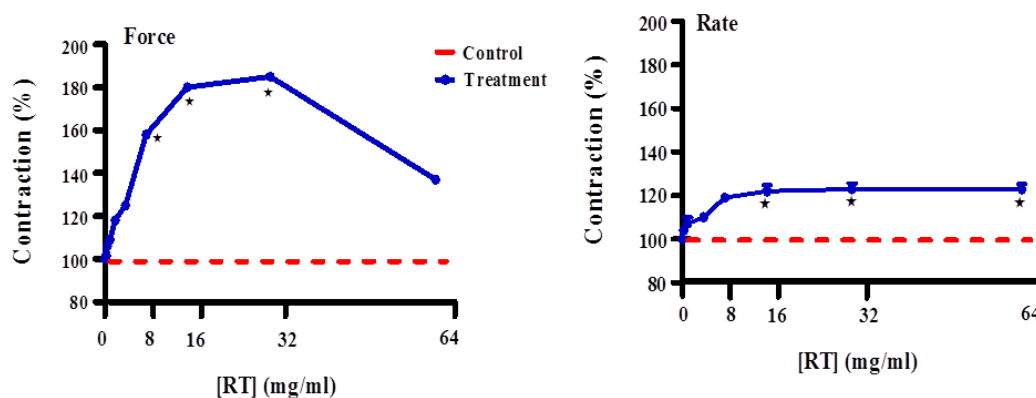
Figure 3.16: Effects of *R. tridentata* root crude extract on the force and rate of uterine muscle contractions.

The methanol extract of the root of *R. tridentata* was separated using column chromatography to give the non-polar (DCM) fraction, acetone fraction and the very polar (methanol) fraction. The uterotonic activity of the acetone and methanol fractions was investigated. The acetone fraction caused an increase (75%) in the force of uterine contractility at a dose of 1.06 mg/mL (Fig. 3.17). On the other hand, a maximal response (60%) in the force of contractions was observed at a concentration of 4.0 mg/mL of the methanol fraction.

The acetone and methanol fractions also caused an increase in the rate of uterine contractions. The highest change (35%) in contraction rate was exhibited by the acetone fraction at a dose of 2.13 mg/mL. The methanol fraction caused a maximal response of 30% at a concentration of 4.0 mg/mL (Fig. 3.17). These results indicate that the very polar (methanol) fraction is less potent than the moderately polar fraction. In this research, the

phytochemistry of the acetone fraction from the methanol extract of the root of *R. tridentata* was studied (Section 3.6). The pure isolated compounds were assayed for uterotonic activity and the results are discussed in Section 3.7.3. Furthermore, the chemical composition of the methanol and DCM fractions from the methanol extract was also investigated. The major compounds (**3.18** and **3.22**) isolated from the DCM fraction were also assayed for uterotonic activity.
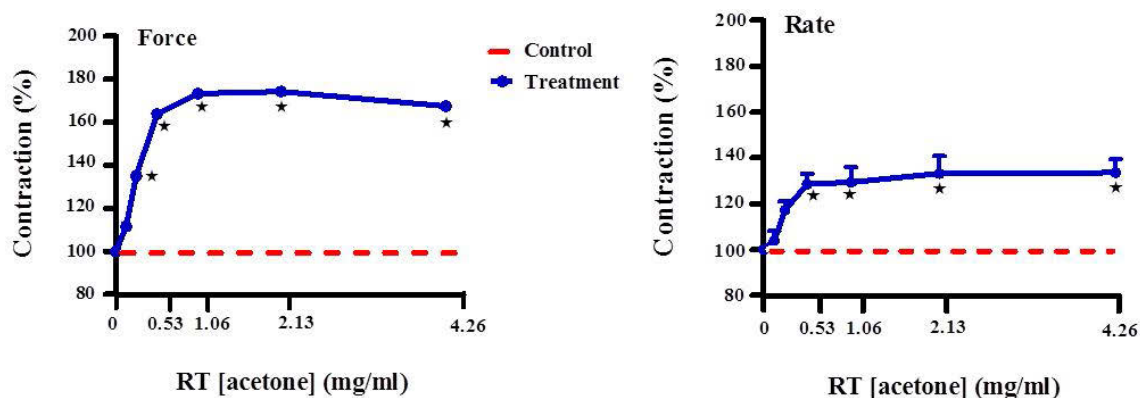
Figure 3.17: Uterine muscle contractility effects of the acetone fraction from the roots of *R. tridentata*.
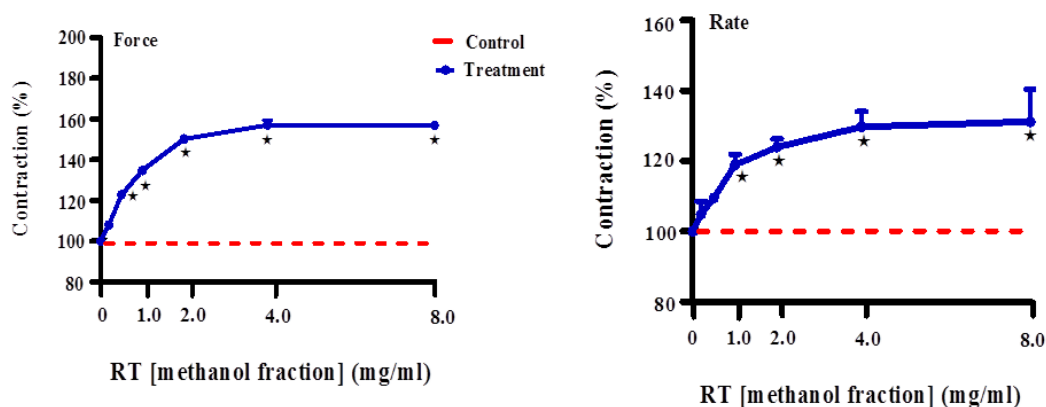
Figure 3.18: Uterine muscle contractility effects of the methanol fraction from the roots of *R. tridentata*.

3.7.3 Uteroactivity assays of the isolated compounds

The isolated compounds (quercetrin, morin 3-*O*-L-rhamnopyranoside, catechin, *trans*-resveratrol glucoside, a mixture of asiatic and arjunolic acid and β-sitosterol) were evaluated for uteroactivity effects on isolated uterine muscle strips. An inseparable mixture of asiatic-arjunolic acid (**3.65** and **3.66**) stimulated the direct contractile response of the uterus. The maximum response in both the force and rate of contractions was observed at a concentration of 1.23 μg/mL and this response remained unchanged with higher sample concentrations (6.14 μg/mL) (Fig. 3.19). This is a first report on the uterotonic activity of asiatic acid (**3.65**) and arjunolic acid (**3.66**).

Cumulative addition of β-sitosterol (**3.22**) (from 0.09 μg/mL to 57.10 μg/mL) to the organ bath, caused stimulation of uterine muscle contractility, with the highest response (40%) in the force of contractility observed at 57.1 μg/mL (Fig. 3.20). The rate of uterine contractility was increased by 20% at a dose of 11.4 μg/mL and the rate remained constant at higher concentrations (57.1 μg/mL). The contractility effect of β-sitosterol (**3.22**) on isolated uterine smooth muscle was previously investigated. Compound **3.22** was isolated (along with other uterotonic compounds) from *Ekebergia capensis* and it showed no *in vitro* uterotonic activity against both pregnant and non-pregnant guinea pig uterine smooth muscle in this study (Sewram et al., 2000). In another study, β-sitosterol (**3.22**) was isolated from *R. tridentata* and was reported to stimulate uterine contractions at higher concentrations of acetylcholine (Brookes and Katsoulis, 2006). The results obtained in the present study are in agreement with those reported by Brookes and Katsoulis (2006) since the maximal response in uterine contractions was observed at higher sample concentrations (Fig. 3.20).
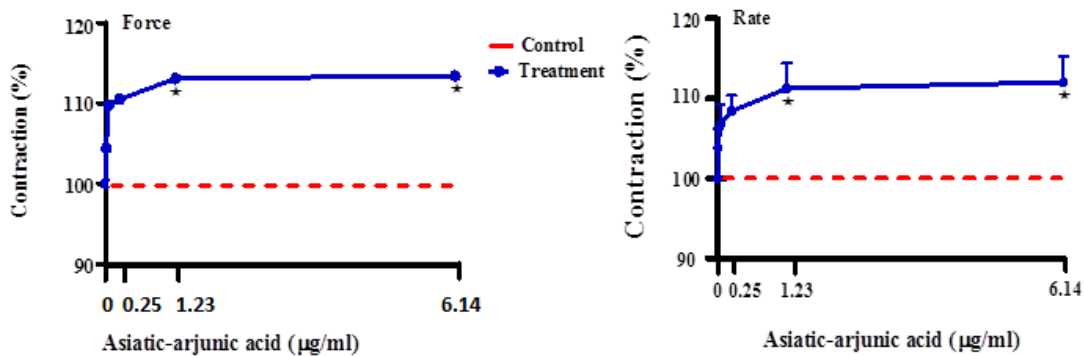
Figure 3.19: Effect of the mixture of asiatic acid and arjunolic acid on the force and rate of uterine muscle contractility.
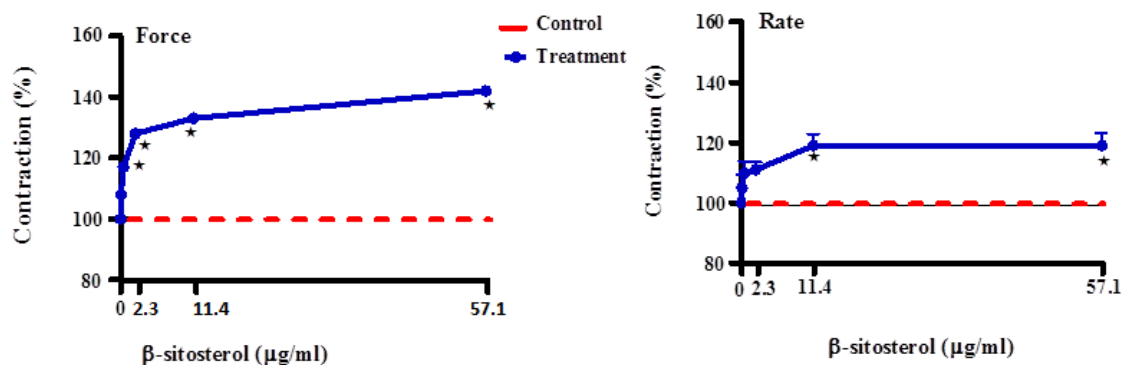
Figure 3.20: Effect of β-sitosterol on the force and rate of uterine muscle contractility.

Interestingly, morin 3-*O*-α-L-rhamnopyranoside (**3.62**) and *trans*-resveratrol glucoside (**3.64**) caused a relaxation of the isolated uterine smooth muscle when added to the organ bath (Fig. 3.21 and Fig 3.22). Both the force and rate of uterine contractility were reduced below the control, which suggested that compound **3.62** and compound **3.64** had inhibitory effects on the uterine muscle contractility. These findings suggest that the *R. tridentata* extract possesses opposing activities (oxytocic and tocolytic). It is well known that biological activities of plant extracts depend on the preparation, composition and dosage. In traditional medicine, boiled aqueous root extract of *R. tridentata* are administered orally to treat different conditions; however, information about the dosage used in each case is not

well known. It is possible that this extract possesses oxytocic and tocolytic effects at different dosage.

This is the first report of the relaxation effect of morin-3-*O*-α-L-rhamnopyranoside (**3.62**) and *trans*-resveratrol glucoside (**3.64**) on uterine muscle. The inhibitory effect shown by compound **3.64** is in agreement with the data reported in literature for resveratrol. Resveratrol showed inhibitory effects on non-pregnant rat uterine contractility. Specifically, this compound inhibited prostaglandin $F_{2\alpha}$, oxytocin, acetylcholine, carbachol and high K^+ concentration-induced uterine contractions as well as Ca^{2+} channel activator (Hsia et al., 2011; Novakovic et al., 2013). The addition of quercetrin (**3.61**) and catechin (**3.63**) into the organ bath completely stopped the uterine muscle contractions. It was concluded that these compounds were cytotoxic to the uterine tissue cells.
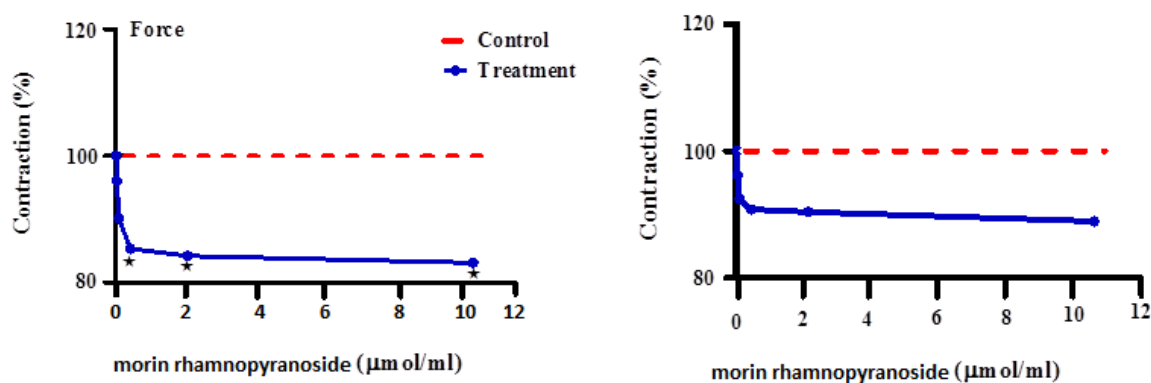
Figure 3.21: Effect of morin 3-*O*-α-L-rhamnopyranoside on the force and rate of uterine muscle contractility.
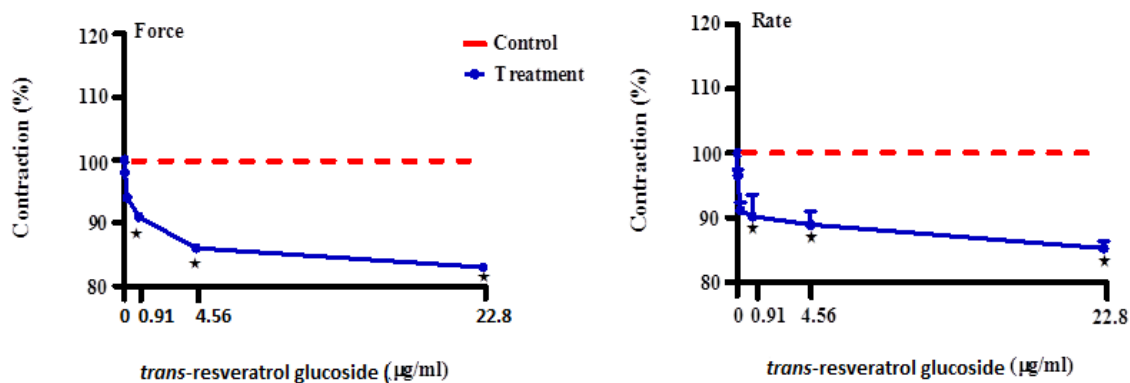
Figure 3.22: Effect of *trans*-resveratrol glucoside on the force and rate of uterine muscle contractility.

3.7.4   Uteroactivity assays of *Gunnera perpensa*

The effect of aqueous root extract of *Gunnera perpensa* stimulated a direct contractile response on the isolated uterine muscle strips. The force of uterine muscle contractions gradually increased between the doses of 0.95 mg/mL to 7.76 mg/mL, with the maximal response observed at a concentration of 7.76 mg/mL. Concentration-dependant stimulation in the rate of uterine contractility was observed at 0.95 mg/mL to 3.88 mg/mL. The higher concentrations (7.76 mg/mL) inhibited the rate of uterine muscle contractions (Fig. 3.23).

The observed uterotonic activity of *G. perpensa* is in agreement with the results reported in literature (Kaido et al., 1997; Khan et al., 2004). The results from the present study showed that the crude polar extract of *G. perpensa* was more active than the extract of *R. tridentata*. Lower concentrations of *G. perpensa* (7.76 mg/mL and 3.88 mg/mL) were required to stimulate maximal force and rate of uterine contractions when compared to the 15.50 mg/mL required for the *R. tridentata* extract. The observed lower activity of the *R. tridentata* when compared to *G. perpensa* extract may be attributed to the occurrence of compounds with opposing effects (oxytocic and tocolytic) in this extract. The phytochemistry of *G. perpensa* was previously investigated and the isolation of pure compounds was not repeated in this study.
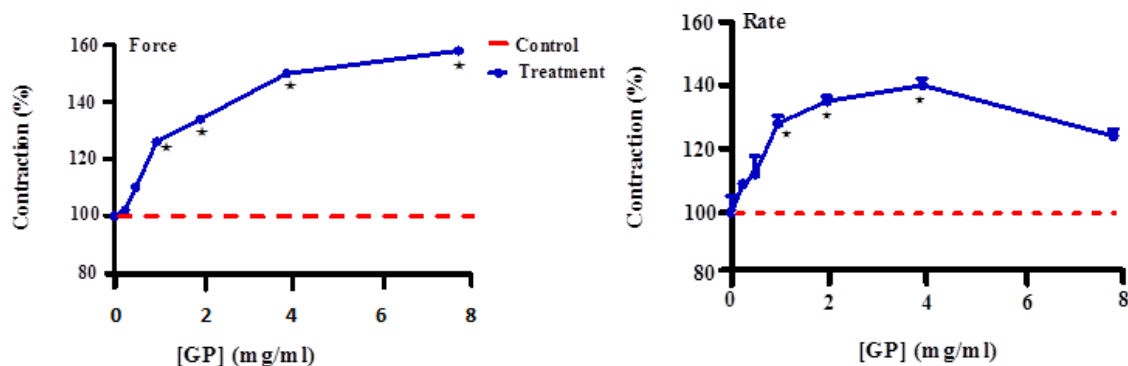
Figure 3.23: Effect of *G. perpensa* root aqueous extract on the force and rate of uterine muscle contractility.

3.8   Conclusion

The methanol root extract of *R. tridentata* stimulated a concentration-dependent contractility of the isolated uterine smooth muscle. However, the uterotonic activity of *R. tridentata* was lower than the activity of the aqueous extract of the root of *G. perpensa* (one of the six most frequently cited plants used to facilitate labour during childbirth). The acetone fraction obtained from the methanol extract of *R. tridentata* displayed efficacy at much lower doses than the methanol fraction and this fraction was further purified to yield compounds **3.61-3.66**. Compounds **3.18** and **3.22** were obtained from the DCM fraction of the methanol extract.

The uterotonic effects of the isolated compounds, with the exception of linoleic acid (**3.18**), were assayed. Linoleic acid (**3.18**) and β-sitosterol (**3.22**) were previously reported to exhibit uterotonic activity. The results from this study confirmed the uterotonic activity of β-sitosterol (**3.22**), with the highest response in the force and the rate of uterine contractions at higher doses. An inseparable mixture of asiatic and arjunolic acid (**3.65** and **3.66**) elicited a muscle response at much lower doses compared to β-sitosterol (**3.22**), therefore it can be concluded that the mixture of asiatic-arjunolic acid (**3.65** and **3.66**) is the active principle responsible for the uterotonic properties of the methanol extract of the root

of *R. tridentata*. This is the first report on the uterotonic activity of the mixture of asiatic acid and arjunolic acid.

Morin 3-*O*-α-L-rhamnopyranoside (**3.62**) and *trans*-resveratrol glucoside (**3.64**) caused a relaxation of the uterus, which is consistent with the use of this plant in early pregnancy for threatened abortion and for treating dysmenorrhea. It is speculated that this plant possesses the oxytocic and tocolytic activities at different doses, however, further studies are required to determine the correct dosage associated with each activity.

3.9 Experimental

3.9.1 General experimental procedure

The general experimental procedure is discussed in Chapter 2 of this thesis.

3.9.2 Plant material

Plant material was collected from two sources:

1. *Rhoicissus tridentata subsp. cuneifolia* (leaves, branches and root) was collected from Silverglen nursery, Durban in December 2012. The plant was identified by Mrs. Allyson Young from the University of KwaZulu-Natal Botanical Gardens and a voucher specimen (van Heerden 2) was deposited in the Bews Herbarium, University of KwaZulu-Natal (Pietermaritzburg).

2. *Rhoicissus tridentata subsp. cuneifolia* (root) was collected from the vendors in the Warwick Triangle, Durban on the 26th of June 2013, and a voucher specimen (van Heerden 20) was deposited in the Bews Herbarium, University of KwaZulu-Natal (Pietermaritzburg).

3.9.3   Extraction of the leaves

The dried leaves and branches (143.4 g) of *R. tridentata* (collected from Silverglen nursery) were extracted consecutively with dichloromethane (DCM), methanol (MeOH), and water at room temperature for 48 hours. After evaporating the solvent, 7.89 g MeOH extract, 2.89 g DCM extract and 4.02 g H_2O extract were obtained. The HPLC chromatogram of the water and MeOH extract showed the same chemical profile, therefore these extracts were combined and analysed for uterotonic activity.

3.9.4   Extraction and isolation of the roots

The oven-dried (30 °C) roots and tubers of *R. tridentata* were milled into a brown powder (2.5 kg). This powder was extracted consecutively at room temperature with DCM and MeOH for 48 hours. After evaporating the solvents, DCM (13 g) and MeOH (72 g) extracts were obtained. The MeOH extract was fractionated by silica gel column chromatography into non-polar (DCM 2), intermediate polar (acetone) and very polar (MeOH 2) fractions. After evaporating the solvents, each fraction was submitted for uterotonic activity testing.

DCM 2 fraction (0.90 g) was further purified on a Chromatotron with DCM: EtOAc (8:2) resulting in the isolation of 3 mg of compound **3.18** and 293 mg of compound **3.22**. The acetone fraction (9.96 g) was fractionated on silica gel column using DCM: EtOAc (1:1) into 5 fractions (A1-A5), where the last fraction was a methanol wash. Fraction A1 showed a similar chemical profile to DCM 2 fraction when analyzed with TLC [eluting with DCM: EtOAc (8:2)]. Fraction A2 was purified with a Chromatotron eluting with DCM: EtOAc (1:1) to afford **3.63** (2.0 mg) and a mixture of **3.65** and **3.66** (26.7 mg). Purification of fraction A3 on a Chromatotron using DEE: MeOH (9:1) resulted in the isolation of **3.61** (21.8 mg) and **3.62** (13.6 mg). Purification of fraction

A4 on a Chromatotron with DCM: MeOH (8.5:1.5) afforded 61 mg of **3.64**. The flow diagram for the isolation of compounds from *R. tridentata* roots is shown below.

The flow diagram for the extraction of *R. tridentata* roots

72 g MeOH extract

DCM 0.90 g — Combined

9.96g Acetone

MeOH 61.24 g

Combined

Silica gel 7:3Hex/Et, chromatotron 8:2 DCM/ EtOAc

A1 2.52 g

A2 0.63 g

A3 1.34 g

A4 0.76 g

A5 0.98g

Silica gel, 8.5: 1.5 DCM: MeOH

3.22 293 mg

3.18 3 mg

Chromatotron DEE: MeOH (9:1)

3.64 61 mg

Silica gel, DCM: EtOAc (1:1)

3.63 2 mg

3.65 & **3.66** 27 mg

3.61 21.8 mg

3.62 13.6 mg
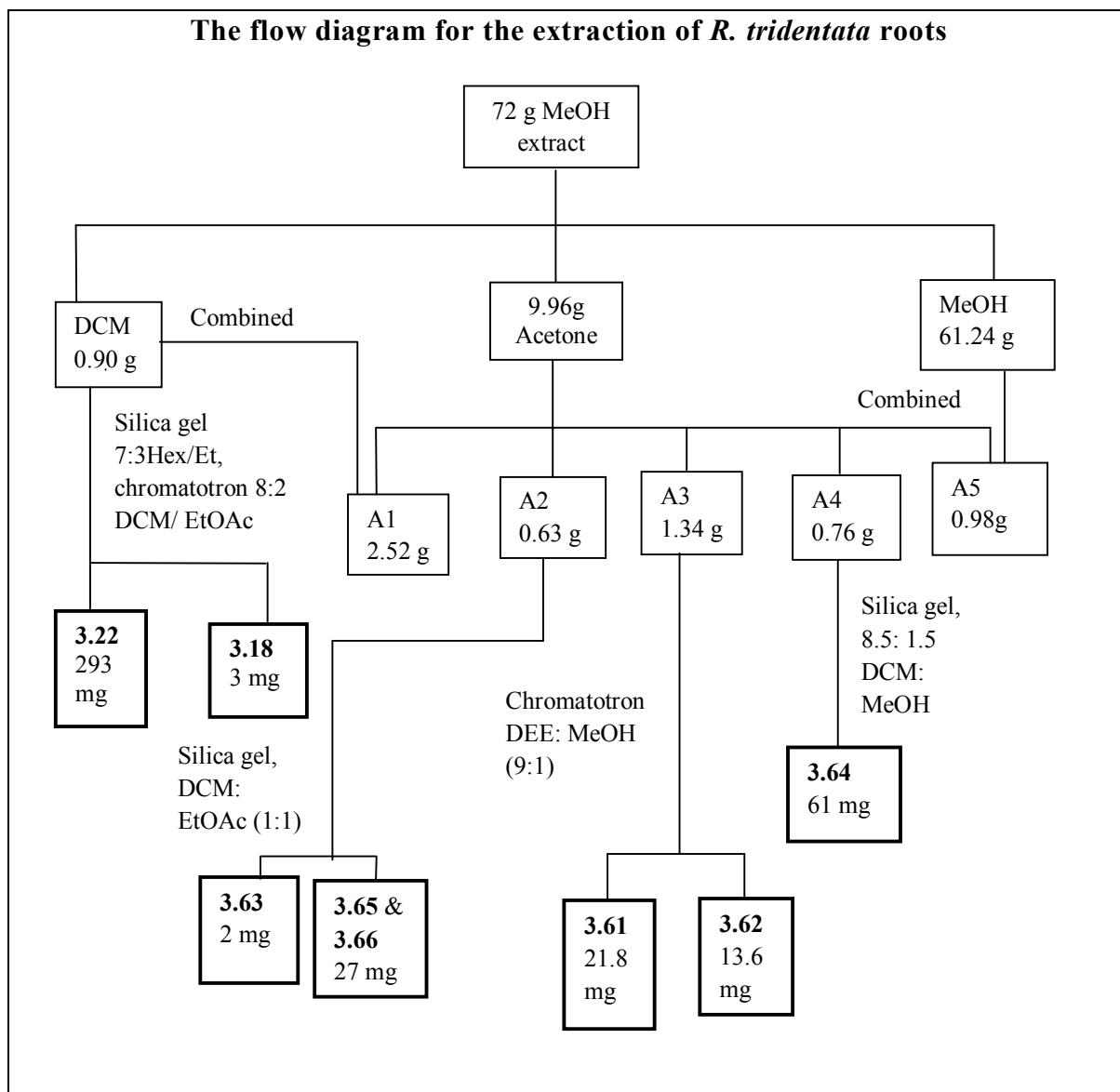
3.9.5   Physical data of the isolated compounds

Quercetrin (3.61) was isolated as a light yellow fine powder, mp 178-182 °C. ^{1}H NMR (400 MHz, CD$_3$OD) δ_H 0.96 (3H, d, 5.9, 5"-OMe), 3.44 (1H, dd, 5.9, 5.9, H-5"), 4.24 (1H, m, H-2"), 3.77 (1H, dd, 9.4, 3.4, H-3"), 3.37 (1H, m, H-4"), 6.40 (1H, d, 2.3, H-8), 6.23 (1H, d, 2.3, H-6), 5.37 (1H, d, 1.7, H-1"), 6.94 (1H, d, 8.2, H-5'), 7.33 (1H, dd, 8.2, 2.26, H-6'), 7.36 (d, 2.3, H-2') (Plate 12a); ^{13}C NMR (125 MHz, CD$_3$OD) δ_C 179.7 (C, C-4), 166.0 (C, C-7), 163.4 (C, C-5), 159.3 (C, C-2), 158.6 (C, C-8a), 149.8 (C, C-3'), 146.4 (C, C-4'), 136.2 (C, C-3), 123.0 (C, C-1'), 122.9 (CH, C-6'), 116.97 (CH, C-2') 116.4 (CH, C-5'), 105.9 (C, C-4a), 103.6 (CH, C-1"), 99.9 (CH, C-6), 94.7 (CH, C-8), 73.3 (CH, C-4"), 72.2 (CH, C-3"), 72.0 (CH, C-2"), 71.9 (CH, C-5"), 17.7 (CH$_3$, 5"-OMe) (Plate 12b). HRESIMS (positive ionization mode, m/z 471.3709 [M+Na]$^+$ (calculated for C$_{21}$H$_{20}$O$_{11}$Na 471.3712); λ_{max} 257 and 352 nm.

Morin 3-*O*-α-L-rhamnopyranoside (3.62) was isolated as a light yellow fine powder, mp 271-276 °C. ^{1}H NMR (400 MHz, CD$_3$OD) δ_H 0.92 (3H, d, 6.2, 5"-OMe), 3.50 (1H, dd, 6.2, 6.03, H-5"), 4.25 (1H, m, H-2"), 3.83 (1H, dd, 9.6, 3.7, H-3"), 3.29 (1H, m, H-4"), 6.22 (1H, d, 2.0, H-8), 6.08 (1H, d, 2.0, H-6), 5.29 (1H, d, 1.7, H-1"), 6.73 (d, 8.2, H-6'), 7.13 (1H, d, 2.0, H-3'), 7.21 (dd, 8.2, 2.0, H-5') (Plate 13a); ^{13}C NMR (125 MHz, CD$_3$OD) δ_C 178.8 (C, C-4), 162.7 (C, C-7), 159.1 (C, C-5), 153.2 (C, C-2), 159.0 (C, C-8a), 121.9 (C, C-3'), 156.7 (C, C-4'), 135.3 (C, C-3), 121.1 (C, C-1'), 159.9 (C, C-2'), 109.2 (CH, C-6') 109.5 (CH, C-5'), 103.8 (C, C-4a), 103.4 (CH, C-1"), 102.0 (CH, C-6), 96.5 (CH, C-8), 73.4 (CH, C-4"), 72.1 (CH, C-3"), 72.0 (CH, C-2"), 72.1 (CH, C-5"), 17.7 (CH$_3$, 5"-OMe) (Plate 13b). HRESIMS (positive ionization mode, m/z 417.3710 [M+Na]$^+$ (calculated for C$_{21}$H$_{20}$O$_{11}$Na 417.3712); $[\alpha]_D^{28}$ -22.1° (*c* 0.1), CH$_3$OH) (lit., $[\alpha]_D$ -20.8, *c* 0.003) (Yen et al., 2009); λ_{max} 257 and 352 nm

Catechin (3.63) was isolated as a light brown solid, mp 175-177 °C. ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 2.53 (1H, dd, 8.2, H-4β), 2.86 (1H, dd, 15.9, 5.6, H-4α), 3.99 (1H, m, H-3), 4.59 (1H, d, 7.3, H-2), 5.88 (1H, d, 2.3, H-8), 5.95 (1H, d, 2.3, H-6), 6.75 (1H, dd, 8.2, 1.9, H-6'), 6.79 (1H, d, 8.2, H-5'), 6.86 (1H, d, 1.9, H-2') (Plate 14a); ^{13}C

NMR (100 MHz, CD$_3$OD) δ_C 28.5 (CH$_2$, C-4), 68.8 (CH, C-3), 82.9 (CH, C-2), 95.5 (CH, C-8), 96.3 (CH, C-6), 100.8 (C, C-4a), 115.3 (CH, C-2'), 116.1 (CH, C-5'), 120.0 (CH, C-6'), 132.2 (C, C-1'), 146.2 (C, C-3'), 146.3 (C, C-4'), 156.9 (C, C-8a), 157.6 (C, C-5), 157.8 (C, C-7) (Plate 14b). HRESIMS (positive ionization mode, m/z 313.2611 [M+Na]$^+$ (calculated for C$_{15}$H$_{14}$O$_6$Na 313.2620); λ_{max} 278 nm

***Trans*-resveratrol glucoside (3.64)** was isolated as a brown solid, mp 223-225 °C. ^{1}H NMR (500 MHz, CD$_3$OD): δ_H 3.42-3.73 (11H, m, H-2", H-3", H-4", and H-5"), 3.76 (1H, dd, 11.9, 2.3, H-6b"), 3.95 (1H, dd, 11.9, 2.3, H-6a"), 4.91 (2H, d, 7.5, H-1"), 6.47 (1H, m, H-4), 6.64 (1H, m, 1.70, H-6), 6.79 (2H, d, 8.7, H-3', 5'), 6.80 (1H, m, 1.7, H-2), 6.87 (1H, d, 16.3, H-7), 7.04 (1H, d, 16.3, H-8), 7.39 (2H, d, 8.7, H-2', 6') (Plate 15a); ^{13}C NMR (125 MHz, CD$_3$OD) δ_C 62.6 (CH$_2$, C-6"), 71.5 (CH, C-4"), 73.9 (CH, C-2"), 78.1 (CH, C-3"), 78.3 (CH, C-5"), 102.5 (CH, C-1"), 104.1 (CH, C-4), 107.1 (CH, C-2), 108.4 (CH, C-6), 116.5 (CH, C-3',5'), 126.7 (CH, C-7), 128.9 (CH, C-2', 6'), 130.0 (CH, C-8), 131.4 (C, C-1'), 141.4 (C, C-1), 158.5 (C, C-4'), 159.6 (C, C-5), 160.5 (C, C-3) (Plate 15b). LRESIMS (positive ionization mode, m/z 413.1199 [M+Na]$^+$ (calculated for C$_{20}$H$_{22}$O$_8$Na 413.1212); λ_{max} 310 nm

Asiatic (3.65) and arjunolic (3.66) acid mixture was isolated as light yellow solid. ^{1}H NMR (Plate 16a) and ^{13}C NMR (Plate 16b) (125 MHz, CD$_3$OD) data is shown in Table **3.2**. HRESIMS (positive ionization mode, m/z 511.3396 [M+Na]$^+$ (calculated for C$_{30}$H$_{48}$O$_5$Na 511.3399).

β-sitosterol (3.22) was isolated as white crystals (recrystallized from methanol), mp 139-140 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 0.68 (3H, br.s, Me-18), 0.83 (10H, m, Me-26, Me-27 & Me-29, H-24), 0.92 (5H, d, 6.5, H-21 & H-9), 1.00 (4H, br.s, Me-19, H-17), 1.22 (14H, m, H-1, H-12, H-14, H-15, H-16, H-20, H-22, H-23 & H-28), 1.57 (9H, m, H-8, H-11, H-15b, H-22b, H-25), 1.84 (3H, m, H-1b, H-2 & H-16b), 1.99 (2H, m, H-7 & H-12b), 2.22 (2H, m, H-4), 3.51 (1H, m, H-3), 5.35 (1H, d, 4.9, H-6) (Plate 17a); ^{13}C NMR (100 MHz, CDCl$_3$) δ_C 11.7 (CH$_3$, C-18), 11.9 (CH$_3$, C-29), 18.8 (CH$_3$, C-21), 19.0 (CH$_3$, C-19), 19.4 (CH$_3$, C-27), 19.8 (CH$_3$, C-26), 21.1 (CH$_2$, C-11), 23.1 (CH$_2$, C-28), 24.3 (CH$_2$, C-15), 26.1 (CH$_2$, C-23), 28.3 (CH$_2$, C-16) , 29.2 (CH, C-25),

31.6 (CH$_2$, C-2), 31.7 (CH, C-8), 31.9 (CH$_2$, C-7), 33.9 (CH$_2$, C-22), 36.2 (CH, C-20), 36.5 (C, C-10), 37.3 (CH$_2$, C-1), 39.8 (CH$_2$, C-12), 42.33 (CH$_2$, C-4), 42.34 (C, C-13), 45.9 (CH, C-24), 50.2 (CH, C-9), 55.9 (CH, C-17), 56.8 (CH, C-14), 71.8 (CH, C-3), 121.7 (CH, C-6), 140.8 (C, C-5) (Plate 17b). LRESIMS (negative ionization mode, m/z 414.7067 [M-H]$^-$.

Linoleic acid (3.18) was isolated as off-white solid, ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 0.89 (3H, t, 6.8, H-18), 1.29 (14H, m, H-4, H-5, H-6, H-7, H-15, H-16, H-17), 1.63 (3H, m, H-3), 2.04 (4H, m, H-8, H-14), 2.35 (3H, t, 7.4, H-2), 2.78 (2H, m, H-11), 5.35 (4H, m, H-9, H-10, H-12, H-13) (Plate 18a); ^{13}C NMR (100 MHz, CDCl$_3$): δ_C 179.9 (C, C-1), 130.2 (CH, C-13), 130.0 (CH, C-9), 128.1 (CH, C-10), 127.9 (CH, C-12), 34.0 (CH$_2$, C-2), 31.5 (CH$_2$, C-16), 29.6 (CH$_2$, C-7), 29.3 (CH$_2$, C-15), 29.1 (CH$_2$, C-5), 29.1 (CH$_2$, C-6), 29.0 (CH$_2$, C-4), 27.2 (CH$_2$, C-8), 27.2 (CH$_2$, C-14), 25.6 (CH$_2$, C-11), 24.7 (CH$_2$, C-3), 22.6 (CH$_2$, C-17), 14.0 (CH$_3$, C-18) (Plate 18b). LRESIMS (negative ionization mode, m/z 281.2530 [M-H]$^-$.

3.9.6   Uteroactivity assays

Uterotonic activity assays were performed by Ms. S. Dube in the laboratory of Prof. C.T. Musabayane (School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Westville, RSA). The protocol for these assays is reported in the MSc. thesis of Ms. Dube, which was submitted at UKZN in December 2014.

After preparation of the leaves and roots aqueous crude extracts from our laboratory, 1.00 g of each extract was submitted for the assaying of uterotonic activity by Ms. Dube. Various amounts (depending on the yield) of the pure isolated compounds were also submitted for biological assays.

CHAPTER 4: Conclusions

This thesis reports the phytochemical investigation of two Zulu medicinal plants, namely, *E. rhinocerotis* (Asteraceae) and *R. tridentata* (Vitaceae). *E. rhinocerotis*, commonly known as *renosterbos*, became popular for the treatment of influenza and fever in the flu epidemic of 1918. This plant is also used in the treatment of colic, wind, diarrhoea, indigestion, dyspepsia, gastric ulcers, stomach cancer and the infusions of the twigs are used as a tonic drink to improve appetite (Hutchings et al., 1996; Watt and Breyer-Brandwijk, 1962). The phytochemistry of this species was previously studied by Dekker et al. (1988) and Proksch and co-workers (1982). In this study, the isolation and identification of compounds from the aerial parts of *E. rhinocerotis* was re-investigated to confirm the occurrence of the previously reported compounds and to isolate new chemical constituents. In addition, the variation in chemical profiles of *E. rhinocerotis* plants collected from different geographic locations was studied.

Two labdane diterpenes, namely, (+)-13-*epi*-labdanolic acid (**2.57**), (+)-labdanolic acid (**2.62**) were isolated as major compounds from the ethyl acetate extract of the aerial parts of *E. rhinocerotis*. This extract also yielded (+)-labdanolic acid methyl ester (**2.64**), (+)-labdanediol (**2.66**), as well as several flavonoids. The isolation of labdane diterpenes is of taxonomical importance as this type of compounds very rarely occurs in the plants in the Asteraceae family. Flavonoids abundantly occur in this plant and this is not surprising since this species usually grows in high-altitude areas where it uses flavonoids for protection against high UV-B radiation exposure (Paula and Virginia, 2005). At high altitudes, UV-B solar radiation exposure increases as there is less air mass, greater transparency of the atmosphere to shorter wavelength radiations, and the recent damage of stratospheric ozone. As a result, plants occurring in these habitats have developed mechanisms (such as, accumulating UV-absorbing compounds in their leaf surfaces) to prevent cellular damage caused by exposure to UV-B radiation (Paula and Virginia, 2005).

The reported anticancer activity of *E. rhinocerotis* extract in traditional medicine may be attributed to the flavonoids found in this extract. In addition, literature indicates that C-13 epimers of the labdane diterpenes isolated in the present study exhibit promising anti-inflammatory activity (Girón et al., 2010). Inflammation has been linked to the initiation and progression of cancer and several studies have shown that the use of anti-inflammatory agents decreased the incidence, recurrence of various cancers, and improved the prognosis for patients (Rayburn et al., 2009). A reverse-phase HPLC method which separated the chemical components of *E. rhinocerotis* was successfully developed and this method was used to analyse the variations in the chemical compositions of three *E. rhinocerotis* plants collected from different locations in a farm in Western Cape. Although there were significant variations in the chemical profiles of the branches collected from different locations in the farm, the overall chemical constituents of the aerial parts (leaves and branches) collected from different locations were similar. The plants used in this study were collected during the same season; therefore, further investigation is required to evaluate the seasonal variations in the chemical composition of *E. rhinocerotis*.

The phytochemistry of the acetone fraction from the methanol extract of the roots of *R. tridentata* was studied. This plant, commonly known as *Isinwazi* (Zulu) is one of the six most frequently cited plants used in the preparation of *Isihlambezo*, a herbal drink taken during the 3rd trimester of pregnancy to induce or facilitate labour and tone the uterus after childbirth (Brookes and Katsoulis, 2006; Hutchings et al., 1996; Veale et al., 1992). In this study, the isolation and identification of compounds from the root MeOH extract of the plant, followed by screening of the isolated compounds for their uterotonic activity was investigated.

Eight known compounds, namely, quercetrin (**3.61**), morin 3-*O*-α-L-rhamnopyranoside (**3.62**), catechin (**3.63**), *trans*-resveratrol glucoside (**3.64**), an inseparable mixture of asiatic acid (**3.65**) and arjunolic acid (**3.66**), β-sitosterol (**3.22**) and linoleic acid (**3.18**) were isolated. Except for catechin and β-sitosterol, all the isolated compounds are reported for the first time from *Rhoicissus* plants and the occurrence of morin 3-*O*-α-L-

rhamnopyranoside in the family Vitaceae is reported for the first time (Burns et al., 2002; Joshi et al., 2013; Wang et al., 2006).

The compounds (β-sitosterol, an inseparable mixture of asiatic acid and arjunolic acid and linoleic acid) showed promising uterotonic activity. The uterotonic activity of the inseparable mixture of asiatic acid (**3.65**) and arjunolic acid (**3.66**) is reported for the first time and this mixture was determined as the active principle in the methanol extract of *R. tridentata*. Therefore, we have successfully validated the uterotonic activity of the polar extract of the root of *R. tridentata* and the uterotonic component in this plant was determined.

Two compounds (morin 3-*O*-α-L-rhamnopyranoside and *trans*-resveratrol glucoside) caused a relaxation of the uterine smooth muscle contractility and this could explain the use of *R. tridentata* extract in traditional medicine in the prevention of miscarriage and dysmenorrhea (Hutchings et al., 1996). Therefore, this plant showed opposing activities (oxytocic and tocolytic), and further studies are required to determine how these activities are modulated. It is assumed that the extract exerts different activities at different dosages, since the preparation method is similar. In addition, future investigations are essential to determine the mechanism of action by which this plant exerts the tocolytic effect.

References

Abad, M.J., Bedoya, L.M., Apaza, L., Bermejo, P., 2012. The *Artemisia* L. genus: a review of bioactive essential oils. Molecules 17, 2542-2566.

Abe, F., Yamauchi, T., 2000. 5,11-Epoxymegastigmanes from the leaves of *Asclepias fruticosa*. Chemical and Pharmaceutical Bulletin 48, 1908-1911.

Afolayan, A., Meyer, J., 1997. The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. Journal of Ethnopharmacology 57, 177-181.

Agrawal, P.K., 1989. Carbon-13 NMR of flavonoids. Elsevier Science Publishers B.V., Amsterdam, Netherlands.

Aguirre, M.C., Delporte, C., Backhouse, N., Erazo, S., Letelier, M.E., Cassels, B.K., Silva, X., Alegría, S., Negrete, R., 2006. Topical anti-inflammatory activity of 2α-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*. Bioorganic & Medicinal Chemistry 14, 5673-5677.

Ahmad, F., Ali, M., Alam, P., 2010. New phytoconstituents from the stem bark of *Tinospora cordifolia* Miers. Natural Product Research 24, 926-934.

Aiyegoro, O.A., Van Dyk, S., 2013. The antioxidant properties, cytotoxicity and monoamine oxidase inhibition abilities of the crude dichloromethane extract of *Tarchonanthus camphoratus* L. leaves. African Journal of Biotechnology 10, 17297-17304.

Alakurtti, S., Mäkelä, T., Koskimies, S., Yli-Kauhaluoma, J., 2006. Pharmacological properties of the ubiquitous natural product betulin. European Journal of Pharmaceutical Sciences 29, 1-13.

Amabeoku, G.J., Green, I., Eagles, P., Benjeddou, M., 2000. Effects of *Tarchonanthus camphoratus* and *Eriocephalus africanus* on nociception in mice and pyrexia in rats. Phytomedicine 7, 517-522.

Amaro-Luis, J., Hung, P., 1988. Phytochemical studies on the Venezuela Andean flora, IV. Structure of labd-13(*Z*)-en-8-alpha-ol-15-oic acid, a component from *Stevia lucida Lagasca*. Acta Cientifica Venezolana 39, 21-24.

Amaro, L.J.M., Adrian, R.M., 1982. Diterpenoids of *Oxylobus glanduliferus* (Sch-Bip) Gray. Revista Latinoamericana de Química 13, 110-113.

Andrade-Cetto, A., Heinrich, M., 2005. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. Journal of Ethnopharmacology 99, 325-348.

Antunes-Ricardo, M., Moreno-García, B., Gutiérrez-Uribe, J., Aráiz-Hernández, D., Alvarez, M., Serna-Saldivar, S., 2014. Induction of apoptosis in colon cancer cells treated with isorhamnetin glycosides from *Opuntia ficus-indica* pads. Plant Foods for Human Nutrition 69, 331-336.

Aremu, A.O., Ndhlala, A.R., Fawole, O.A., Light, M.E., Finnie, J.F., Van Staden, J., 2010. *In vitro* pharmacological evaluation and phenolic content of ten South African medicinal plants used as anthelmintics. South African Journal of Botany 76, 558-566.

Arnold, T.H., Prentice, C., Hawker, L., Snyman, E., Tomalin, M., Crouch, N., Pottas-Bircher, C., 2002. Medicinal and Magical Plants of Southern Africa: An Annotated Checklist. National Botanical Institute, Pretoria, South Africa.

Arwa, P., Onyango, J., Nyunja, R., 2008. Phytochemical compounds and antimicrobial activity of extracts of *Rhoicissus* plant (*Rhoicissus revoilli*) (Planch). Plant Sciences Research 1, 68-73.

Atawodi, S., 2005. Antioxidant potential of African medicinal plants. African Journal of Biotechnology 4, 128-133.

Attah, A.F., O'Brien, M., Koehbach, J., Sonibare, M.A., Moody, J.O., Smith, T.J., Gruber, C.W., 2012. Uterine contractility of plants used to facilitate childbirth in Nigerian ethnomedicine. Journal of Ethnopharmacology 143, 377-382.

Avula, B., Wang, Y.-H., Smillie, T.J., Mabusela, W., Vincent, L., Weitz, F., Khan, I.A., 2009. Quantitative determination of flavonoids by high-performance liquid chromatography with mass spectrometry and ultraviolet absorption detection in *Artemisia afra* and comparative studies with various species of *Artemisia* plants. An International Journal of Analytical Science 92, 633-644.

Bag, B.G., Dey, P.P., Dinda, S.K., Sheldrick, W.S., Oppel, I.M., 2008. A simple route for renewable nano-sized arjunolic and asiatic acids and self-assembly of arjuna-bromolactone. Beilstein Journal of Organic Chemistry 4, 24.

Barnett, C.J., Cullinan, G.J., Gerzon, K., Hoying, R.C., Jones, W.E., Newlon, W.M., Poore, G.A., Robison, R.L., Sweeney, M.J., 1978. Structure-activity relationships of dimeric *Catharanthus* alkaloids. Deacetyl vinblastine amide (vindesine) sulfate. Journal of Medicinal Chemistry 21, 88-96.

Barreda, V.D., Palazzesi, L., Katinas, L., Crisci, J.V., Tellería, M.C., Bremer, K., Passala, M.G., Bechis, F., Corsolini, R., 2012. An extinct Eocene taxon of the daisy family (Asteraceae): evolutionary, ecological and biogeographical implications. Annals of Botany 109, 127-134.

Barrett, B., 2003. Medicinal properties of *Echinacea*: a critical review. Phytomedicine 10, 66-86.

Beltrame, F.L., Pessini, G.L., Doro, D.L., Dias Filho, B.P., Bazotte, R.B., Cortez, D.A.G., 2002. Evaluation of the antidiabetic and antibacterial activity of *Cissus sicyoides*. Brazilian Archives of Biology and Technology 45, 21-25.

Beltrame, F.L., Sartoretto, J.L., Bazotte, R.B., Cuman, R.N., Cortez, D.A.G., Fernandes, L.C., Tchaikovski, O., 2001. Phytochemical study and evaluation of the antidiabetic potential of *Cissus sicyoides* L. (Vitaceae). Quimica Nova 24, 783-785.

Bjamer, K., Ferguson, G., Melville, R., 1968. The configuration at C (l3) in labdanolic and eperuic acids by an X-ray crystal structure analysis of the *p*-bromophenacyl ester of labdanolic acid. Acta Crystallographica Section B: Structural Crystallography and Crystal Chemistry 24, 855-865.

Bohlmann, F., Zdero, C., 1977. Neue norkauren- und thymol-derivate aus *Athrixia*-arten. Phytochemistry 16, 1773–1776.

Bohm, B.A., Stuessy, T.F., 2001. Flavonoids of the sunflower family (Asteraceae). Springer, Vienna, Austria.

Borchardt, J.K., 2002. The beginnings of drug therapy: ancient mesopotamian medicine. Drug News & Perspectives 15, 187-192.

Bory, S., Lederer, E., 1957. Partial synthesis of labdanolic acid from sclareol. Croatica Chemica Acta 29, 157-161.

Bracken, H., Mundle, S., Faragher, B., Easterling, T., Haycox, A., Turner, M., Alfirevic, Z., Winikoff, B., Weeks, A., 2014. Induction of labour in pre-eclamptic women: a randomised trial comparing the foley balloon catheter with oral misoprostol. BMC Pregnancy and Childbirth 14, 308.

Bremner, P., Meyer, J., 2000. Prenyl-butyrylphloroglucinol and kaurenoic acid: two antibacterial compounds from *Helichrysum kraussii*. South African Journal of Botany 66, 115-117.

Bremner, P.D., Meyer, J., 1998. Pinocembrin chalcone: an antibacterial compound from *Helichrysum trilineatum*. Planta Medica 64, 777-777.

Brevoort, P., 1998. Booming US botanical market: a new overview. HerbalGram.

Brickell, C. 2008. RHS A-Z encyclopedia of garden plants. In RHS A-Z encyclopedia of garden plants, Brickell, C., ed. (United Kingdom, Dorling Kindersley), 1136.

Bringmann, G., Saeb, W., Assi, L.A., Francois, G., Sankara, N.A., Peters, K., Peters, E.-M., 1997. Betulinic acid: isolation from *Triphyophyllum peltatum* and *Ancistrocladus heyneanus*, antimalarial activity, and crystal structure of the benzyl ester. Planta Medica 63, 255-257.

Brookes, K.B., Doudoukina, O.V., Katsoulis, L.C., Veale, D.J.H., 1999. Uteroactive constituents from *Combretum kraussii*. South African Journal of Chemistry 52, 127.

Brookes, K.B., Dutton, M.F., 2007. Bioactive components of the uteroactive medicinal plant, *Gunnera perpensa* (or *ugobo*). South African Journal of Science 103, 187-189.

Brookes, K.B., Katsoulis, L.C., 2006. Bioactive components of *Rhoicissus tridentata*: a pregnancy-related traditional medicine. South African Journal of Science 102, 267-272.

Brookes, K.B., Smith, A.N., 2003. Cytotoxicity of pregnancy-related traditional medicines. South African Medical Journal 93, 359-361.

Bullough, C.H., Leary, W.P., 1982. Herbal medicines used by traditional birth attendants in Malawi. Tropical and Geographical Medicine 34, 81-85.

Burn, J.H., Withell, E.R., 1941. A principle in raspberry leaves which relaxes uterine muscle. The Lancet 238, 1-3.

Burns, J., Yokota, T., Ashihara, H., Lean, M.E.J., Crozier, A., 2002. Plant foods and herbal sources of resveratrol. Journal of Agricultural and Food Chemistry 50, 3337-3340.

Buss, A.D., Cox, B., Waigh, R.D., Abraham, D.J. 2003. Natural Products as Leads for New Pharmaceuticals. In Burger's Medicinal Chemistry and Drug Discovery, Abraham, D., ed. (New York, John Wiley & Sons, Inc.), 847-900.

Butler, M.S., 2004. The role of natural product chemistry in drug discovery. Journal of Natural Products 67, 2141-2153.

Céspedes, C.L., Avila, J.G., Martínez, A., Serrato, B., Calderón-Mugica, J.C., Salgado-Garciglia, R., 2006. Antifungal and antibacterial activities of Mexican Tarragon (*Tagetes lucida*). Journal of Agricultural and Food Chemistry 54, 3521-3527.

Chagnon, M., 1984. General pharmacologic inventory of Rwandese medicinal plants. Journal of Ethnopharmacology 12, 239-251.

Chandramu, C., Manohar, R.D., Krupadanam, D.G., Dashavantha, R.V., 2003. Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. Phytotherapy Research 17, 129-134.

Chen, C.-X., Kwan, C.-Y., 2001. Endothelium-independent vasorelaxation by leonurine, a plant alkaloid purified from Chinese motherwort. Life Sciences 68, 953-960.

Chen, I., Manchester, S.R., 2007. Seed morphology of modern and fossil *Ampelocissus* (Vitaceae) and implications for phytogeography. American Journal of Botany 94, 1534-1553.

Cheung, H.A., Chiu, F.C., Watson, T.R., Wells, R.J., 1983. Cardenolide glycosides of the Asclepiadaceae. New glycosides from *Asclepias fruticosa* and the stereochemistry of uscharin, voruscharin and calotoxin. Journal of the Chemical Society, Perkin Transactions 1, 2827-2835.

Chinou, I., 2005. Labdanes of natural origin-biological activities (1981-2004). Current Medicinal Chemistry 12, 1295-1317.

Cocker, J., Halsall, T., 1956. The chemistry of gum labdanum. Part II. The structure of labdanolic acid. Journal of Chemical Society, 4262-4271.

Cocker, J.D., Halsall, T.G., Bowers, A., 1956. The chemistry of gum labdanum. I. Some acidic constituents. Journal of Chemical Society, 4259-4262.

Cragg, G.M., Newman, D.J., 2005. Plants as a source of anti-cancer agents. Journal of Ethnopharmacology 100, 72-79.

Cragg, G.M., Newman, D.J., 2013. Natural products: a continuing source of novel drug leads. Biochimica et Biophysica Acta - General Subjects 1830, 3670-3695.

Cuadrado, I., Cidre, F., Herranz, S., Estevez-Braun, A., de las Heras, B., Hortelano, S., 2012a. Labdanolic acid methyl ester (LAME) exerts anti-inflammatory effects through inhibition of TAK-1 activation. Toxicology and Applied Pharmacology 258, 109-117.

Cuadrado, I., Cidre, F., Herranz, S., Estevez-Braun, A., de, l.H.B., Hortelano, S., 2012b. Labdanolic acid methyl ester (LAME) exerts anti-inflammatory effects through inhibition of TAK-1 activation. Toxicology and Applied Pharmacology 258, 109-117.

Dale, H.H., 1909. The action of extracts of the pituitary body. Biochemical Journal 4, 427.

Dao, P.T.A., Le Quan, T., Mai, N.T.T., 2013. Some compounds from stem of *Tetrastigma erubescens* Planch. (Vitaceae). Journal of Engineering Technology and Education 9, 54-57.

Davis, A.L., Cai, Y., Davies, A.P., Lewis, J.R., 1996. ^{1}H and ^{13}C NMR assignments of some green tea polyphenols. Magnetic Resonance in Chemistry 34, 887-890.

Dawson, R., Jarvis, M., Jefferies, P., Payne, T., Rosich, R., 1966. Acidic constituents of *Dodonaea lobulata*. Australian Journal of Chemistry 19, 2133-2142.

de Boer, H.J., Cotingting, C., 2014. Medicinal plants for women's healthcare in southeast Asia: a meta-analysis of their traditional use, chemical constituents, and pharmacology. Journal of Ethnopharmacology 151, 747-767.

de Groot, A.J.A., van Dongen, P.J., Vree, T., Hekster, Y., van Roosmalen, J., 1998. Ergot alkaloids. Drugs 56, 523-535.

De Las Heras, B., Navarro, A., Díaz-Guerra, M.J., Bermejo, P., Castrillo, A., Boscá, L., Villar, A., 1999. Inhibition of NOS-2 expression in macrophages through the inactivation of NF-κB by andalusol. British Journal of Pharmacology 128, 605-612.

de Oliveira, C.B., Comunello, L.N., Lunardelli, A., Amaral, R.H., Pires, M.G., da Silva, G.L., Manfredini, V., Vargas, C.R., Gnoatto, S.C., de Oliveira, J.R., 2012. Phenolic enriched extract of *Baccharis trimera* presents anti-inflammatory and antioxidant activities. Molecules 17, 1113-1123.

De Vries, P., Dien, T., 1996. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. Drugs 52, 818-836.

de Wet, H., Ngubane, S.C., 2014. Traditional herbal remedies used by women in a rural community in northern Maputaland (South Africa) for the treatment of gynaecology and obstetric complaints. South African Journal of Botany 94, 129-139.

Dekker, T., Fourie, T., Matthee, E., Snyckers, F., Vanderschyf, C., Boeyens, J., Denner, L., 1988. Studies of South African medicinal plants. Part 7. Rhinocerotinoic acid: a labdane diterpene with anti-inflammatory properties from *Elytropappus rhinocerotis*. South African Journal of Chemistry 41, 33-35.

den Hertog, C.E.C., de Groot, A.N.J.A., van Dongen, P.W.J., 2001. History and use of oxytocics. European Journal of Obstetrics & Gynecology and Reproductive Biology 94, 8-12.

Denis, J.N., Greene, A.E., Guenard, D., Gueritte-Voegelein, F., Mangatal, L., Potier, P., 1988. Highly efficient, practical approach to natural taxol. Journal of the American Chemical Society 110, 5917-5919.

Dev, S. 2001. Ancient-Modern Concordance in Ayurvedic Plants: Some Examples, In: Saxena, P.K. (Ed.) Development of Plant-Based Medicines: Conservation, Efficacy and Safety. Springer Netherlands, 47-67.

Dilika, F., Bremner, P.D., Meyer, J.J.M., 2000. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia 71, 450-452.

Dong, T.T.X., Cui, X.M., Song, Z.H., Zhao, K.J., Ji, Z.N., Lo, C.K., Tsim, K.W.K., 2003. Chemical assessment of roots of *Panax notoginseng* in China:. regional and seasonal variations in its active constituents. Journal of Agricultural and Food Chemistry 51, 4617-4623.

Dorchin, N., Gullan, P.J., 2007. A new genus and species of a lasiopterine gall midge (*Diptera*: Cecidomyiidae) from bud galls on renosterbos, *Elytropappus rhinocerotis* (Asteraceae), in South Africa. African Entomology 15, 233-240.

Drewes, S.E., van Vuuren, S.F., 2008. Antimicrobial acylphloroglucinols and dibenzyloxy flavonoids from flowers of *Helichrysum gymnocomum*. Phytochemistry 69, 1745-1749.

du Vigneaud, V., Ressler, C., Swan, J.M., Roberts, C.W., Katsoyannis, P.G., Gordon, S., 1953. The synthesis of an octapeptide amide with the hormonal activity of oxytocin. Journal of American Chemistry Society 75, 4879-4880.

Efferth, T. 2009. Artemisinin: A Versatile Weapon from Traditional Chinese Medicine, In: Ramawat, K.G. (Ed.) Herbal Drugs: Ethnomedicine to Modern Medicine. Springer Berlin Heidelberg, 173-194.

Efferth, T., Dunstan, H., Sauerbrey, A., Miyachi, H., Chitambar, C.R., 2001. The anti-malarial artesunate is also active against cancer. International Journal of Oncology 18, 767-773.

El Tahir, K.E.H., 1991. Pharmacological actions of magnoflorine and aristolochic acid-1 isolated from the seeds of *Aristolochia bracteata*. Pharmaceutical Biology 29, 101-110.

Enwerem, N., Okogun, J., Wambebe, C., Okorie, D., Akah, P., 2001. Anthelmintic activity of the stem bark extracts of *Berlina grandiflora* and one of its active principles, Betulinic acid. Phytomedicine 8, 112-114.

Ersoz, T., Harput, U.S., Saracoglu, I., Calis, I., 2002. Phenolic compounds from *Scutellaria pontica*. Turkish Journal of Chemistry 26, 581.

Escandón-Rivera, S., González-Andrade, M., Bye, R., Linares, E., Navarrete, A., Mata, R., 2012. α-Glucosidase inhibitors from *Brickellia cavanillesii*. Journal of Natural Products 75, 968-974.

Eshiet, E.R., Zhu, J., Anderson, T.A., Smith, E.E., 2014. Chemical characterization of *Brickellia cavanillesii* (Asteraceae) using gas chromatographic methods. Food Science & Nutrition 2, 105-113.

Fakhfakh, J.A., Martin, M.T., Damak, M., 2005. 2D NMR of lignans and flavonoids isolated from *Centaurea furfuracea* (Asteraceae). Journal of Societe Chimique de Tunisie 7, 11-18.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology 94, 205-217.

Ferreres, F., Gil-Izquierdo, A., Vinholes, J., Grosso, C., Valentão, P., Andrade, P.B., 2011. Approach to the study of C-glycosyl flavones acylated with aliphatic and aromatic acids from *Spergularia rubra* by high-performance liquid chromatography-photodiode array detection/electrospray ionization multi-stage mass spectrometry. Rapid Communications in Mass Spectrometry 25, 700-712.

Flamini, G., Antognoli, E., Morelli, I., 2001. Two flavonoids and other compounds from the aerial parts of *Centaurea bracteata* from Italy. Phytochemistry 57, 559-564.

Flom, M.S., Doskotch, R.W., Beal, J.L., 1967. Isolation and characterization of alkaloids from *Caulophyllum thalictroides*. Journal of Pharmaceutical Sciences 56, 1515-1517.

Florão, A., Budel, J.M., Duarte, M.d.R., Marcondes, A., Rodrigues, R.A.F., Rodrigues, M.V.N., Santos, C.A.d.M., Weffort-Santos, A.M., 2012. Essential oils from *Baccharis* species (Asteraceae) have anti-inflammatory effects for human cells. Journal of Essential Oil Research 24, 561-570.

Flores, S., Herrán, J., 1960. Atanasin: a new flavone isolated from *Brickelia squarrosa* (Cav.) Robinson. Chemistry and Industry, 291-291.

Flores, S.E., Herrán, J., 1958. The structure of pendulin and penduletin: a new flavonol glucoside isolated from B*rickelia pendula*. Tetrahedron 2, 308-315.

Forgo, P., Zupkó, I., Molnár, J., Vasas, A., Dombi, G., Hohmann, J., 2012. Bioactivity-guided isolation of antiproliferative compounds from *Centaurea jacea* L. Fitoterapia 83, 921-925.

Fouche, G., Cragg, G.M., Pillay, P., Kolesnikova, N., Maharaj, V.J., Senabe, J., 2008. In *vitro* anticancer screening of South African plants. Journal of Ethnopharmacology 119, 455-461.

Fulda, S., Debatin, K.-M., 2000. Betulinic acid induces apoptosis through a direct effect on mitochondria in neuroectodermal tumors. Medical and Pediatric Oncology 35, 616-618.

Funk, R.L., Yost, K.J., 1996. Preparation and Diels-Alder cycloaddition of 2-acyloxyacroleins. Facile synthesis of functionalized taxol A-ring synthons. The Journal of Organic Chemistry 61, 2598-2599.

Funk, V.A., Susanna, A., Stuessy, T.F., Robinson, H., 2009. Classification of Compositae. International Association for Plant Taxonomy, Vienna, Austria.

Gakuba, E., 2010. Isolation and characterisation of secondary metabolites of two asteraceae species, *Artemisia afra* and *Elytropappus rhinocerotis*. MSc. Thesis [Unpublished]. University of KwaZulu-Natal, Pietermaritzburg,

Gao, H.-f., Zheng, B.-b., Wang, J.-h., Shan, T.-j., Zhao, S.-f., Zhou, L.-g., 2011. Flavonol Derivatives from *Caragana leucophloea* and their antimicrobial and antioxidant activities. Natural Product Research & Development 23, 853-930.

Gao, J., Sun, N., Wang, F., 1999. Effects of castor oil-diet on the synthesis of prostaglandin E2 in pregnant rats. Zhonghua fu chan ke za zhi 34, 147-149.

Gao, J., Sun, N., Wang, F., Hao, N., 1998. Effect of castor oil-diet on the initiation of labor of pregnant rat. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. Acta Academiae Medicinae Sinicae 20, 367-370.

García-Sánchez, E., Ramírez-López, C.B., Talavera-Alemán, A., León-Hernández, A., Martínez-Muñoz, R.E., Martínez-Pacheco, M.M., Gómez-Hurtado, M.A., Cerda-García-Rojas, C.M., Joseph-Nathan, P., del Río, R.E., 2014. Absolute configuration of (13*R*)- and (13*S*)-labdane diterpenes coexisting in *Ageratina jocotepecana*. Journal of Natural Products 77, 1005-1012.

Gené, R.M., Cartaña, C., Adzet, T., Marín, E., Parella, T., Cañigueral, S., 1996. Anti-inflammatory and analgesic activity of *Baccharis trimera*: identification of its active constituents. Planta Medica 62, 232-235.

Gerrath, J.M., Wilson, T., Posluszny, U., 2004. Morphological and anatomical development in the Vitaceae. VII. Floral development in *Rhoicissus digitata* with respect to other genera in the family. Canadian Journal of Botany 82, 198-206.

Girón, N., Pérez-Sacau, E., López-Fontal, R., Amaro-Luis, J.M., Hortelano, S., Estevez-Braun, A., de las Heras, B., 2010. Evaluation of labdane derivatives as potential anti-inflammatory agents. European Journal of Medicinal Chemistry 45, 3155-3161.

Girón, N., Través, P.G., Rodríguez, B., López-Fontal, R., Boscá, L., Hortelano, S., de las Heras, B., 2008. Supression of inflammatory responses by labdane type diterpenoids. Toxicology and Applied Pharmacology 228, 179-189.

Gomes, R.F., Silva dos Santos, H., Albuquerque, M.R.J.R., Pessoa, O.D.L., Lotufo, L.V.C., Pessoa, C.d.O., Odorico de Moraes, M., Rodrigues, F.A.R., 2010. *Blainvillea rhomboidea*: chemical constituents and cytotoxic activity. Quimica Nova 33, 1122-1125.

González, A.G., Francisco, C.G., Freire, R., Hernández, R., Salazar, J.A., Suárez, E., 1975. 9(11)-Dehydroagapanthagenin, a new spirostan sapogenin from *Agapanthus africanus*. Phytochemistry 14, 2259-2262.

González, A.G., Freire, R., Francisco, C.G., Salazar, J.A., Suárez, E., 1974. 7-Dehydroagapanthagenin and 8(14)-dehydroagapanthagenin, two new spirostan sapogenins from *Agapanthus africanus*. Phytochemistry 13, 627-632.

Goto, M., Noguchi, T., Watanaba, T., Ishikawa, I., Komatsu, M., Aramaki, Y., 1957. Studies on uterus contracting ingredients on plants. Takeda Kenkyusho Nempo 16, 21-27.

Govindaraj, S., Kumari, B.D.R., Cioni, P.L., Flamini, G., 2008. Mass propagation and essential oil analysis of *Artemisia vulgaris*. Journal of Bioscience and Bioengineering 105, 176-183.

Gran, L., Sletten, K., Skjeldal, L., 2008. Cyclic peptides from *Oldenlandia affinis* DC. Molecular and biological properties. Chemistry & Biodiversity 5, 2014-2022.

Green, E., Samie, A., Obi, C.L., Bessong, P.O., Ndip, R.N., 2010. Inhibitory properties of selected South African medicinal plants against *Mycobacterium tuberculosis*. Journal of Ethnopharmacology 130, 151-157.

Gruber, C.W., O'Brien, M., 2011. Uterotonic plants and their bioactive constituents. Planta Medica 77, 207.

Guenard, D., Gueritte-Voegelein, F., Potier, P., 1993. Taxol and taxotere: discovery, chemistry, and structure-activity relationships. Accounts of Chemical Research 26, 160-167.

Ha, D., Chen, Q., Hung, T., Youn, U., Ngoc, T., Thuong, P., Kim, H., Seong, Y., Min, B., Bae, K., 2009. Stilbenes and oligostilbenes from leaf and stem of *Vitis amurensis* and their cytotoxic activity. Archives of Pharmacal Research 32, 177-183.

Ha, S.-Y., Youn, H., Song, C.-S., Kang, S.C., Bae, J.J., Kim, H.T., Lee, K.M., Eom, T.H., Kim, I.S., Kwak, J.H., 2014. Antiviral effect of flavonol glycosides isolated from the leaf of *Zanthoxylum piperitum* on influenza virus. Journal of Microbiology 52, 340-344.

Hadden, W.L., Watkins, R.H., Levy, L.W., Regalado, E., Rivadeneira, D.M., van Breemen, R.B., Schwartz, S.J., 1999. Carotenoid composition of Marigold (*Tagetes erecta*) flower extract used as nutritional supplement. Journal of Agricultural and Food Chemistry 47, 4189-4194.

Hammoda, H., Ela, A., El-Lakany, A., El-Hanbali, O., Zaki, C., Ghazy, N., 2008. New constituents of *Artemisia monosperma* Del. Die Pharmazie - An International Journal of Pharmaceutical Sciences 63, 611-614.

Hamza, O.J.M., van den Bout-van den Beukel, C.J.P., Matee, M.I.N., Moshi, M.J., Mikx, F.H.M., Selemani, H.O., Mbwambo, Z.H., Van der Ven, A.J.A.M.,

Verweij, P.E., 2006. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. Journal of Ethnopharmacology 108, 124-132.

Hanawa, F., Tahara, S., Mizutani, J., 1992. Antifungal stress compounds from *Veratrum grandiflorum* leaves treated with cupric chloride. Phytochemistry 31, 3005-3007.

Harborne, J.B., 1993. The Flavonoids: Advances in Research Since 1986, Vol 4. CRC Press, Boca Raton, Florida.

Henrick, C.A., Jefferies, P.R., Rosich, R.S., 1964. Enantiomers of labdanolic and 13-*epi*-labdanolic acids. Tetrahedron Letters, 3475-3480.

Herz, W., Govindan, S.V., 1980. Eucannabinolide and other constituents of *Schkuhria virgata*. Phytochemistry 19, 1234-1237.

Hillis, W.E., 1966. Variation in polyphenol composition within species of *Eucalyptus l'herit*. Phytochemistry 5, 541-556.

Hofmeyr, G., Gülmezoglu, A., Pileggi, C., 2009. Vaginal misoprostol for cervical ripening and induction of labour. The Cochrane database of systematic reviews, CD000941-CD000941.

Holton, R.A., Kim, H.B., Somoza, C., Liang, F., Biediger, R.J., Boatman, P.D., Shindo, M., Smith, C.C., Kim, S., 1994. First total synthesis of taxol. 2. Completion of the C and D rings. Journal of the American Chemical Society 116, 1599-1600.

Hsia, S.-M., Wang, K.-L., Wang, P.S., 2011. Effects of resveratrol, a grape polyphenol, on uterine contraction and Ca^{2+} mobilization in rats *in vivo* and *in vitro*. Endocrinology 152, 2090-2099.

Huang, K.C., 2010. The Pharmacology of Chinese Herbs, Second Edition. CRC Press, Boca Raton, Florida.

Hutchings, A., Haxon-Scott, A., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants: An inventory. University of Natal Press, Pietermaritzburg.

Ieven, M., Vlietinick, A.J., Berghe, D.A.V., Totte, J., Dommisse, R., Esmans, E., Alderweireldt, F., 1982. Plant antiviral agents. III. Isolation of alkaloids from *Clivia miniata* Regel (Amaryllidaceae). Journal of Natural Products 45, 564-573.

Iinuma, M., Roberts, M.F., Matlin, S.A., Stacey, V.E., Timmermann, B.N., Mabry, T.J., Brown, R., 1985. Synthesis and revised structure of the flavone brickellin. Phytochemistry 24, 1367-1368.

Ijeh, I.I., Ejike, C.E., 2011. Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del. Journal of Medicinal Plants Research 5, 1051-1061.

Imamura, P.M., Marsaioli, A.J., Barata, L.E.S., Ruveda, E.A., 1977. Carbon-13 NMR spectral analysis of eperuane diterpenes. Phytochemistry 16, 1842-1844.

Ivancheva, S., Stancheva, B., 1997. Flavonoid aglycons from *Tanacetum macrophyllum* Willd. (Asteraceae). Dokladi na Bulgarskata Akademiya na Naukite 50, 53-54.

Jakupovic, J., Klemeyer, H., Bohlmann, F., Graven, E.H., 1988. Glaucolides and guaianolides from *Artemisia afra*. Phytochemistry 27, 1129-1133.

Jang, S.I., Kim, Y.-J., Kim, H.J., Lee, J.-C., Kim, H.-Y., Kim, Y.-C., Yun, Y.-g., Yu, H.-H., You, Y.-O., 2006. Scoparone inhibits PMA-induced IL-8 and MCP-1 production through suppression of NF-κB activation in U937 cells. Life Sciences 78, 2937-2943.

Jansen, R.K., Palmer, J.D., 1987. A chloroplast DNA inversion marks an ancient evolutionary split in the sunflower family (Asteraceae). Proceedings of the National Academy of Sciences of the United States of America 84, 5818-5822.

Jin, R., Chen, Z., Chen, C., Zhang, H., 2003. Effects of motherwort on dysmenorrhea. The Chinese Pharmaceutical Association 21, 90-93.

Johnson, S.A., Harper, P., Hortobagyi, G.N., Pouillart, P., 1996. Vinorelbine: an overview. Cancer Treatment Reviews 22, 127-142.

Joshi, A., Tari, P., Bhobe, M., 2013. Phytochemical investigation of the roots of *Leea indica* (Burm. F.) Merr. International Journal of Research in Pharmaceutical and Biomedical Sciences 4

Joubert, E., Gelderblom, W.C.A., Louw, A., de Beer, D., 2008. South African herbal teas: *Aspalathus linearis*, *Cyclopia spp*. and *Athrixia phylicoides*—a review. Journal of Ethnopharmacology 119, 376-412.

Kaido, T.L., Veale, D.J.H., Havlik, I., Rama, D.B.K., 1997. Preliminary screening of plants used in South Africa as traditional herbal remedies during pregnancy and labour. Journal of Ethnopharmacology 55, 185-191.

Kamatenesi-Mugisha, M., 2004. Medicinal plants used in reproductive health care in Western Uganda: documentation, phytochemical and bioactivity evaluation. PhD Thesis. Makerere University, Kampala, Uganda.

Kamatenesi-Mugisha, M., Makawiti, D.W., Oryem-Origa, H., Nganga, J., 2007. The oxytocic properties of *Luffa cylindrica* (L.) M. Roem. and *Bidens pilosa* L., traditionally used medicinal plants from western Uganda. African Journal of Ecology 45, 88-93.

Kamatenesi-Mugisha, M., Oryem-Origa, H., Odyek, O., Makawiti, D. 2005. Ethno-pharmacological screening of *Vernonia amygdalina* and *Cleome gynandra* traditionally used in childbirth in western Uganda. In: 11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar, 110-122.

Kanwal, U., 2011. *Cyphostemma glaucophilla* may serve as a cheap protectant of liver and kidney. Pakistan Journal of Biological Sciences 14, 912-913.

Kapoor, L., 1989. CRC Handbook of Ayurvedic Medicinal Plants. CRC press, Boca Raton, Florida.

Katsoulis, L.C., Veale, D.J., Havlik, I., 2000. The pharmacological action of *Rhoicissus tridentata* on isolated rat uterus and ileum. Phytotherapy Research 14, 460-462.

Katsoulis, L.C., Veale, D.J.H., Havlik, I., 2002. Seasonal variation in uterotonic activity of *Rhoicissus tridentata* extracts. South African Medical Journal 92, 375-377.

Keelan, J.A., Blumenstein, M., Helliwell, R.J.A., Sato, T.A., Marvin, K.W., Mitchell, M.D., 2003. Cytokines, prostaglandins and parturition - a review. Placenta 24, Supplement A, S33-S46.

Kellerman, T., Coetzer, J., Naudé, T., 1988. Plant Poisonings and Mycotoxicoses of Livestock in Southern Africa. Oxford University Press, Cape Town.

Khan, F., Peter, X.K., Mackenzie, R.M., Katsoulis, L., Gehring, R., Munro, O.Q., van Heerden, F.R., Drewes, S.E., 2004. Venusol from *Gunnera perpensa*: structural and activity studies. Phytochemistry 65, 1117-1121.

Khan, R., Saif, A.Q., Quradha, M.M., Ali, J., Rauf, A., 2015. Phytochemical analysis, antimicrobial, antioxidant and urease inhibitory potential of *Cyphostemma digitatum* Lam. Natural Product Research 29, 466–468.

Kingston, D.G.I., 2010. Modern natural products drug discovery and its relevance to biodiversity conservation. Journal of Natural Products 74, 496-511.

Kinoshita, K., Akiba, M., Saitoh, M., Ye, Y., Koyama, K., Takahashi, K., Kondo, N., Yuasa, H., 1998. Antinociceptive effect of triterpenes from cacti. Pharmaceutical Biology 36, 50-55.

Knowles, C.-L., 2005. Synergistic effects of mixtures of the kresoxim-methyl fungicide and medicinal plant extracts *in vitro* and *in vivo* against *Botrytis cinerea*. MSc (Herbal Science and Medicine). University of the Western Cape, Retrieved from http://etd.uwc.ac.za/xmlui/handle/11394/1373 (accessed: 27-10-2014),

Koehbach, J., O'Brien, M., Muttenthaler, M., Miazzo, M., Akcan, M., Elliott, A.G., Daly, N.L., Harvey, P.J., Arrowsmith, S., Gunasekera, S., Smith, T.J., Wray, S., Göransson, U., Dawson, P.E., Craik, D.J., Freissmuth, M., Gruber, C.W., 2013. Oxytocic plant cyclotides as templates for peptide G protein-coupled receptor ligand design. Proceedings of the National Academy of Sciences 110, 21183-21188.

Koekemoer, M., 2002. Systematics of the *Metalasia* group in the *Relhaniinae* (Asteraceae-Gnaphalieae). PhD. Botany [Unpublished]. University of Johannesburg, Retrieved from https://ujdigispace.uj.ac.za (accessed: 27-10-2014),

Kohler, I., Jenett-Siems, K., Kraft, C., Siems, K., Abbiw, D., Bienzle, U., Eich, E., 2002. Herbal remedies traditionally used against malaria. Part 7. Herbal remedies traditionally used against malaria in Ghana: Bioassay-guided fractionation of *Microglossa pyrifolia* (Asteraceae). Z. Naturforsch., C: J. Biosci. 57, 1022-1027.

Kong, Y.C., Yeung, H.W., Cheung, Y.M., Phil, M., Hwang, J.C., Chan, Y.W., Law, Y.P., Ng, K.H., Yeung, C.H., 1976. Isolation of the uterotonic principle from *Leonurus artemisia,* the Chinese Motherwort. The American Journal of Chinese Medicine 04, 373-382.

Kraft, C., Jenett-Siems, K., Siems, K., Jakupovic, J., Mavi, S., Bienzle, U., Eich, E., 2003. In *vitro* antiplasmodial evaluation of medicinal plants from Zimbabwe. Phytotherapy Research 17, 123-128.

Kundodyiwa, T.W., Alfirevic, Z., Weeks, A.D., 2009. Low-dose oral misoprostol for induction of labor: a systematic review. Obstetrics & Gynecology 113, 374-383.

Lai, H., Singh, N.P., 1995. Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. Cancer Letters 91, 41-46.

Lakornwong, W., Kanokmedhakul, K., Kanokmedhakul, S., 2014. Chemical
constituents from the roots of *Leea thorelii Gagnep*. Natural Product Research
28, 1015-1017.

Lall, N., Hussein, A.A., Meyer, J.J.M., 2006. Antiviral and antituberculous activity of
Helichrysum melanacme constituents. Fitoterapia 77, 230-232.

Lee, K.-H., 2010. Discovery and development of natural product-derived
chemotherapeutic agents based on a medicinal chemistry approach. Journal of
Natural Products 73, 500-516.

Levinson, W., Jawetz, E., 2002. Medical Microbiology and Immunology: Examination
and Board Review, 7 Edition. McGraw Hill Companies, New York.

Levyns, M.R., 1926. A preliminary note on the Rhenoster Bush (*Elytropappus
rhinocerotis*) and the germination of its seed. Transactions of the Royal Society
of South Africa 14, 383-388.

Levyns, M.R., 1935. Notes on the nomenclature in some members of Compositae.
Transactions of the Royal Society of South Africa 23, 91-95.

Li, J.W.-H., Vederas, J.C., 2009. Drug discovery and natural products: end of an era or
an endless frontier? Science 325, 161-165.

Lim, H., Son, K.H., Chang, H.W., Bae, K., Kang, S.S., Kim, H.P., 2008. Anti-
inflammatory activity of pectolinarigenin and pectolinarin isolated from *Cirsium
chanroenicum*. Biological and Pharmaceutical Bulletin 31, 2063-2067.

Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jager,
A.K., van Staden, J., 1999. Preliminary screening of some traditional Zulu
medicinal plants for anti-inflammatory and antimicrobial activities. Journal of
Ethnopharmacology 68, 267-274.

Lindenmeyer, F., Li, H., Menashi, S., Soria, C., Lu, H., 2001. Apigenin acts on the
tumor cell invasion process and regulates protease production. Nutrition and
Cancer 39, 139-147.

Lindsey, K., Jäger, A.K., Raidoo, D.M., van Staden, J., 1998. Screening of plants used
by southern African traditional healers in the treatment of dysmenorrhoea for
prostaglandin-synthesis inhibitors and uterine relaxing activity. Journal of
Ethnopharmacology 64, 9-14.

Liu, X.H., Pan, L.L., Zhu, Y.Z., 2012. Active chemical compounds of traditional Chinese medicine herba leonuri: implications for cardiovascular diseases. Clinical and Experimental Pharmacology and Physiology 39, 274-282.

Lourens, A.C.U., Viljoen, A.M., van Heerden, F.R., 2008. South African *Helichrysum* species: A review of the traditional uses, biological activity and phytochemistry. Journal of Ethnopharmacology 119, 630-652.

Lu, Y., Foo, L.Y., 1997. Identification and quantification of major polyphenols in apple pomace. Food Chemistry 59, 187-194.

Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer-Verlang, New York.

Marie, D., Gurib-Fakim, A., Gray, A., Waterman, P., 2006. Constituents of *Psiadia terebinthina* A. J. Scott, an endemic Asteraceae from Mauritius. Natural Product Research 20, 1169-1175.

Markham, K.R., 1982. Techniques of Flavonoid Identification, Vol 31. Academic press London.

Marles, R.J., Farnsworth, N.R., 1995. Antidiabetic plants and their active constituents. Phytomedicine 2, 137-189.

Marwah, R.G., Fatope, M.O., Deadman, M.L., Al-Maqbali, Y.M., Husband, J., 2007. Musanahol: a new aureonitol-related metabolite from a *Chaetomium sp.* Tetrahedron 63, 8174-8180.

Mashimbye, M.J., Mudau, F.N., Soundy, P., Van Ree, T., 2006. A new flavonol from *Athrixia phylicoides* (bush tea). South African Journal of Chemistry 59, 1-2.

Masoko, P., Mokgotho, M., Mbazima, V., Mampuru, L., 2008. Biological activities of *Typha capensis* (Typhaceae) from Limpopo province (South Africa). African Journal of Biotechnology 7, 3743-3748.

Mata, R., Cristians, S., Escandón-Rivera, S., Juárez-Reyes, K., Rivero-Cruz, I., 2013. Mexican antidiabetic herbs: valuable sources of inhibitors of α-glucosidases. Journal of Natural Products 76, 468-483.

Mathekga, A.D.M., Meyer, J.J.M., Horn, M.M., Drewes, S.E., 2000. An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. Phytochemistry 53, 93-96.

Matsuda, H., Morikawa, T., Sakamoto, Y., Toguchida, I., Yoshikawa, M., 2002. Labdane-type diterpenes with inhibitory effects on increase in vascular

permeability and nitric oxide production from *Hedychium coronarium*. Bioorganic & Medicinal Chemistry 10, 2527-2534.

McGaw, L.J., Lall, N., Meyer, J.J.M., Eloff, J.N., 2008. The potential of South African plants against *Mycobacterium* infections. Journal of Ethnopharmacology 119, 482-500.

Merfort, I., 1984. Methylated flavonoids from *Arnica montana* and *Arnica chamissonis*. Planta Medica 50, 107-108.

Meyer, J., Afolayan, A., Taylor, M., Erasmus, D., 1997. Antiviral activity of galangin isolated from the aerial parts of *Helichrysum aureonitens*. Journal of Ethnopharmacology 56, 165-169.

Mierendorff, H.-G., Stahl-Biskup, E., Posthumus, M.A., Beek, T.A.v., 2003. Composition of commercial Cape chamomile oil (*Eriocephalus punctulatus*). Flavour and Fragrance Journal 18, 510-514.

Milner, R.T., Hubbard, J.E., Wiele, M., 1945. Sunflower and safflower seeds and oils. Oil & Soap 22, 304-307.

Mishra, B.B., Tiwari, V.K., 2011. Natural products: an evolving role in future drug discovery. European Journal of Medicinal Chemistry 46, 4769-4807.

Mitchell, M., Romero, R., Edwin, S., Trautman, M., 1995. Prostaglandins and parturition. Reproduction, Fertility and Development 7, 623-632.

Mjiqiza, S.J., 2006. Evaluation of the pulmonary effects of the traditionally prepared *Artemisia afra*: steam inhalation and nebulized aqueous extract and the possible involvement of flavonoid luteolin. Msc. Thesis. University of the Western Cape, Retrieved from etd.uwc.ac.za (accessed: 2-2-2015),

Mjiqiza, S.J., Syce, J.A., Obikeze, K.C., 2013. Pulmonary effects and disposition of luteolin and *Artemisia afra* extracts in isolated perfused lungs. Journal of Ethnopharmacology 149, 648-655.

Moghaddam, M.G., Ahmad, F.B.H., Samzadeh-Kermani, A., 2012. Biological Activity of betulinic acid: a review. Pharmacology & Pharmacy 03, 5.

Moiseev, D., Buzuk, G., Shelyuto, V., 2011. Identification of flavonoids in plants by HPLC. Pharmaceutical Chemistry Journal 45, 47-50.

Moore, M.O., Giannasi, D.E., 1994. Foliar flavonoids of eastern north American *Vitis* (Vitaceae) north of Mexico. Plant Systematics and Evolution 193, 21-36.

Mpofu, S.J., Arotiba, O.A., Hlekelele, L., Ndinteh, D.T., Krause, R., 2014.
Determination of catechins from *Elephantorrhiza elephantina* and *Pentanisia prunelloides* using voltammetry and UV spectroscopy. Natural Product Communications 9, 41-43.

Muganga, R., 2007. Luteolin levels in selected folkloric preparations and the bioavailability of luteolin from *Artemisia afra* aqueous extract in the vervet monkey. Etudes Rwandaises 14, 100-111.

Mugo, J., 1977. Biological activity of cordifene: on myosin: a model for conjugated alpha-methylene ketone group's cardiac toxicity. Agressologie 18, 143-147.

Mukherjee, P.K., Saha, K., Das, J., Pal, M., Saha, B., 1997. Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera*. Planta Medica 63, 367-369.

Mukhtar, M., Arshad, M., Ahmad, M., Pomerantz, R.J., Wigdahl, B., Parveen, Z., 2008. Antiviral potentials of medicinal plants. Virus Research 131, 111-120.

Mukinda, J.T., Syce, J.A., Fisher, D., Meyer, M., 2010. Effect of the plant matrix on the uptake of luteolin derivatives containing *Artemisia afra* aqueous extract in CaCO-2 cells. Journal of Ethnopharmacology 130, 439-449.

Naidoo, V., Chikoto, H., Bekker, L., Eloff, J., 2006. Antioxidant compounds in *Rhoicissus tridentata* extracts may explain their antibabesial activity: research in action. South African Journal of Science 102, p. 198-200.

Nair, J.J., van Staden, J., 2013. Pharmacological and toxicological insights to the South African Amaryllidaceae. Food and Chemical Toxicology 62, 262-275.

Nalule, A., Mbaria, J., Kimenju, J., Olila, D., 2012. Ascaricidal activity of *Rhoicissus tridentata* root-tuber ethanolic and water extracts. Livestock Research for Rural Development 24.

Nalule, S.A., 2011. Ethnopharmacology, phytochemical composition and toxicity of medicinal plants used against livestock helminths in drylands. PhD Thesis. University of Nairobi, Kenya.

Naude, P., 2012. Phylogenetic analysis of Vitaceae based on plastid sequence data. MSc. Thesis. University of Johannesburg, South Africa.

Nazaruk, J., 2009. Flavonoid compounds from *Cirsium palustre* (L.) Scop. flower heads. Biochemical Systematics Ecology 37, 525-527.

Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B., Van Staden, J., 2013. Toxicology of some important medicinal plants in southern Africa. Food and Chemical Toxicology 62, 609-621.

Neuwinger, H.D., 2000. African Traditional Medicine: a Dictionary of Plant Use and Applications. With Supplement: Search System for Diseases. Medpharm.

Newman, D.J., Cragg, G.M., 2012. Natural Products As Sources of New Drugs over the 30 Years from 1981 to 2010. Journal of Natural Products 75, 311-335.

Nie, Z.-L., Sun, H., Chen, Z.-D., Meng, Y., Manchester, S.R., Wen, J., 2010. Molecular phylogeny and biogeographic diversification of *Parthenocissus* (Vitaceae) disjunct between Asia and North America. American Journal of Botany 97, 1342-1353.

Nikolajsen, T., Nielsen, F., Rasch, V., Sørensen, P.H., Ismail, F., Kristiansen, U., Jäger, A.K., 2011. Uterine contraction induced by Tanzanian plants used to induce abortion. Journal of Ethnopharmacology 137, 921-925.

Nirmal, S., Pal, S., Mandal, S., Patil, A., 2012. Analgesic and anti-inflammatory activity of β-sitosterol isolated from *Nyctanthes arbortristis* leaves. Inflammopharmacology 20, 219-224.

Njenga, E.W., Viljoen, A.M., 2006. In vitro 5-lipoxygenase inhibition and anti-oxidant activity of *Eriocephalus* L. (Asteraceae) species. South African Journal of Botany 72, 637-641.

Nogueira, N.P.A., Reis, P.A., Laranja, G.A.T., Pinto, A.C., Aiub, C.A.F., Felzenszwalb, I., Paes, M.C., Bastos, F.F., Bastos, V.L.F.C., Sabino, K.C.C., Coelho, M.G.P., 2011. In *vitro* and in *vivo* toxicological evaluation of extract and fractions from *Baccharis trimera* with anti-inflammatory activity. Journal of Ethnopharmacology 138, 513-522.

Novakovic, R., Ilic, B., Beleslin-Cokic, B., Radunovic, N., Heinle, H., Scepanovic, R., Gojkovic-Bukarica, L., 2013. The effect of resveratrol on contractility of non-pregnant rat uterus: the contribution of K^+ channels. Journal of Physiology and Pharmacology, 14.

Nthambeleni, R., 2008. Isolation and characterisation of cytotoxic compounds from *Anthospermum hispidulum* and *Eriocephalus tenuifolius*. MSc degree. University of KwaZulu-Natal, Retrieved from

http://researchspace.ukzn.ac.za/xmlui/handle/10413/6524/search (accessed 16-02-2015), Pietermaritzburg, South Africa.

O'Sullivan, M.D., Hehir, M.P., O'Brien, Y.M., Morrison, J.J., 2010. 17-Alpha-hydroxyprogesterone caproate vehicle, castor oil, enhances the contractile effect of oxytocin in human myometrium in pregnancy. American Journal of Obstetrics and Gynecology 202, 453/451-e453/454.

Okem, A., Finnie, J.F., Van Staden, J., 2012. Pharmacological, genotoxic and phytochemical properties of selected South African medicinal plants used in treating stomach-related ailments. Journal of Ethnopharmacology 139, 712-720.

Omino, E.A., Kokwaro, J.O., 1993. Ethnobotany of Apocynaceae species in Kenya. Journal of Ethnopharmacology 40, 167-180.

Opoku, A.R., Geheeb-Keller, M., Lin, J., Terblanche, S.E., Hutchings, A., Chuturgoon, A., Pillay, D., 2000. Preliminary screening of some traditional Zulu medicinal plants for antineoplastic activities versus the HepG2 cell line. Phytotherapy Research 14, 534-537.

Opoku, A.R., Maseko, N.F., Terblanche, S.E., 2002. The *in vitro* antioxidative activity of some traditional Zulu medicinal plants. Phytotherapy Research 16 Suppl 1, S51-56.

Opoku, A.R., Ndlovu, I.M., Terblanche, S.E., Hutchings, A.H., 2007. In vivo hepatoprotective effects of *Rhoicissus tridentata* subsp. *cuneifolia*, a traditional Zulu medicinal plant, against CCl4-induced acute liver injury in rats. South African Journal of Botany 73, 372-377.

Otterstätter, G., 1999. Coloring of Food, Drugs, and Cosmetics. CRC Press, Marcel Dekker, New York.

Overton, K., Renfrew, A.J., 1967. The configuration at C-13 in labdanolic and eperuic acids. Journal of the Chemical Society C: Organic, 931-935.

Page, J.E., Balza, F., Nishida, T., Towers, G.H.N., 1992. Biologically active diterpenes from *Aspilia mossambicensis*, a chimpanzee medicinal plant. Phytochemistry 31, 3437-3439.

Panda, S., Kar, A., 2007. Apigenin (4',5,7-trihydroxyflavone) regulates hyperglycaemia, thyroid dysfunction and lipid peroxidation in alloxan-induced diabetic mice. Journal of Pharmacy and Pharmacology 59, 1543-1548.

Patel, J.G., Patel, N.M., Patel, A.A., Patel, A.J., Patel, S.A., 2013. Pharmacognosy and preliminary phytochemical screening of *Ampelocissus latifolia* (ROXB.) root. Pharmeutical Letters 5, 271-275.

Pathomwichaiwat, T., Ochareon, P., Soonthornchareonnon, N., Ali, Z., Khan, I.A., Prathanturarug, S., 2015. Alkaline phosphatase activity-guided isolation of active compounds and new dammarane-type triterpenes from *Cissus quadrangularis* hexane extract. Journal of Ethnopharmacology 160, 52-60.

Paul, E.L., Lunardelli, A., Caberlon, E., de Oliveira, C.B., Santos, R.C.V., Biolchi, V., Bastos, C.M.A., Moreira, K.B., Nunes, F.B., Gosmann, G., 2009. Anti-inflammatory and immunomodulatory effects of *Baccharis trimera* aqueous extract on induced pleurisy in rats and lymphoproliferation in *vitro*. Inflammation 32, 419-425.

Paula, C., Virginia, W., 2005. Differential accumulation of maysin and rhamnosylisoorientin in leaves of high-altitude landraces of maize after UV-B exposure. Plant, Cell and Environment 28, 788–799.

Percival, S.S., 2000. Use of *Echinacea* in medicine. Biochemical Pharmacology 60, 155-158.

Perez G, R.M., Cervantes C, H., Zavala S, M.A., Sanchez A, J., Perez G, S., Perez G, C., 2000. Isolation and hypoglycemic activity of 5,7,3′-trihydroxy-3,6,4′-trimethoxyflavone from *Brickellia veronicaefolia*. Phytomedicine 7, 25-29.

Piccaglia, R., Marotti, M., Grandi, S., 1998. Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. Industrial Crops and Products 8, 45-51.

Pisha, E., Chai, H., Lee, I.-S., Chagwedera, T.E., Farnsworth, N.R., Cordell, G.A., Beecher, C.W.W., Fong, H.H.S., Kinghorn, A.D., Brown, D.M., Wani, M.C., Wall, M.E., Hieken, T.J., Das Gupta, T.K., Pezzuto, J.M., 1995. Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nature Medicine 1, 1046-1051.

Pool, E., Klaasen, J., Shoko, Y., 2009. The environmental toxicity of *Dicerothamnus rhinocerotis* and *Galenia africana*. African Journal of Biotechnology 8.

Potier, P., 1989. The synthesis of navelbine prototype of a new series of vinblastine derivatives. Seminars in Oncology 16, 2-4.

Power, F.B., Salway, A.H., 1913. XXIII.-The constituents of the rhizome and roots of Caulophyllum thalictroides. Journal of the Chemical Society, Transactions 103, 191-209.

Pretorius, E. 2008. African plants and herbs in asthma therapy. In Botanical Medicine in Clinical Practice, Ronald Ross Watson, V.R.P., ed. (Wallingford, UK, CAB International), 20-28.

Proksch, P., Proksch, M., Rundel, P.W., Rodriguez, E., 1982. Ecological significance of the chemistry of the leaf resin of *Elytropappus rhinocerotis*. Biochemical Systematics and Ecology 10, 49-53.

Rader, J., Pawar, R., 2013. Primary constituents of blue cohosh: quantification in dietary supplements and potential for toxicity. Analytical and Bioanalytical Chemistry 405, 4409-4417.

Rahman, M.A., Moon, S.-S., 2007. Antimicrobial phenolic derivatives from *Dendranthema zawadskii* var. *latilobum* kitamura *(*Asteraceae). Archives of Pharmacal Research 30, 1374-1379.

Rajeshkumar, P., Hosagoudar, V., 2012. Mycorrhizal fungi of *Artemisia japonica*. Bulletin of Basic and Applied Plant Biology 2, 7-10.

Rakuambo, N., Meyer, J.J.M., Hussein, A., Huyser, C., Mdlalose, S., Raidani, T., 2006. *In vitro* effect of medicinal plants used to treat erectile dysfunction on smooth muscle relaxation and human sperm. Journal of Ethnopharmacology 105, 84-88.

Raman, B., Pednekar, P.A., Kulkarni, V., 2014. High perfomance liquid chromatographic method development and validation for the simultaneous determination of gallic acid and beta sitosterol in *Ampelocissus latifolia* (ROXB.) Planch. Asian Journal of Pharmaceutical and Clinical Research 7, 86-89.

Ramírez, M., B, Tellez, A., N, Torrenegra, R., D, 2000. Chemical study of *Espeletiopsis muiska* Cuatr (Cuatr) and evaluation of its antimicrobial activity. Revista Latinoamericana de Química 28, 119-124.

Ramirez, M., Tellez, N., Torrenegra, R., 1998. (+)-8-Hydroxy-labdan-17-oic acid. Molecules 3, M48.

Ramos, S., 2007. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. The Journal of Nutritional Biochemistry 18, 427-442.

Rampedi, I., Olivier, J., 2005. The use and potential commercial development of *Athrixia phylicoides*. Acta Academica-University of the Free State 37, 165.

Rao, B.M. 2010. Powder of the roots of *Ampelocissus latifolia* Roxb plant used for treatment of gynecological problem, Appl., I.P., ed. (India), 8pp.

Rashmi, T.R., Francis, M.S., Soumya, M., 2014. Determination of artemisinin in selected *Artemisia* L. species by HPLC. Indo American Journal of Pharmaceutical Research 4, 2637-2644.

Rayburn, E.R., Ezell, S.J., Zhang, R., 2009. Anti-inflammatory agents for cancer therapy. Molecular and Cellular Pharmacology 1, 29-43.

Ren, W., Qiao, Z., Wang, H., Zhu, L., Zhang, L., 2003. Flavonoids: promising anticancer agents. Medicinal Research Reviews 23, 519-534.

Rijke, E.d., Out, P., Niessen, W.M.A., Ariese, F., Gooijer, C., Brinkman, U.A.T., 2006. Analytical separation and detection methods for flavonoids. Journal of Chromatography A 1112, 31–63.

Rishton, G.M., 2008. Natural products as a robust source of new drugs and drug leads: past successes and present day issues. The American Journal of Cardiology 101, 43D-49D.

Rivero-Cruz, B., Rivero-Cruz, I., Rodríguez, J.M., Cerda-García-Rojas, C.M., Mata, R., 2006. Qualitative and quantitative analysis of the active components of the essential oil from *Brickellia veronicaefolia* by nuclear magnetic resonance spectroscopy. Journal of Natural Products 69, 1172-1176.

Rivière, C., Pawlus, A.D., Mérillon, J.-M., 2012. Natural stilbenoids: distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. Natural Products Reports 29, 1317-1333.

Robin, V., Boustie, J., Amoros, M., Girre, L., 1998. In-vitro antiviral activity of seven *Psiadia* species, Asteraceae: isolation of two antipoliovirus flavonoids from *Psiadia dentata*. Pharmacy and Pharmacology Communications 4, 61-64.

Rodríguez-López, V., Moreno-Escobar, J.A., Ávila-Torres, O., Tlahuext, H., 2006. 6-Acetyl-5-hydroxy-2,2-dimethyl-2H-chromene. Acta Crystallographica Section E 62, 3616-3617.

Rogers, C., Verotta, L. 1996. Chemistry and biological properties of the African Combretaceae, In: K. Hostettmann, F.C., M. Maillard and J.-L. Wolfender (Ed.)

Chemistry, Biological and Pharmacological Properties of African Medicinal Plants. University of Zimbabwe Publications, Zimbabwe, 121-141.

Rojo, A.L., Palacios, P.S., Acevedo, C., Spegazzini, E.D., Debenedetti, S.L., 2004. 6-Methoxyflavonoids from *Heterotheca latifolia* (Asteraceae). Biochemical Systematics and Ecology 32, 351-353.

Sadava, D., Phillips, T., Lin, C., Kane, S.E., 2002. Transferrin overcomes drug resistance to artemisinin in human small-cell lung carcinoma cells. Cancer Letters 179, 151-156.

Saether, O., Craik, D.J., Campbell, I.D., Sletten, K., Juul, J., Norman, D.G., 1995. Elucidation of the primary and three-dimensional structure of the uterotonic polypeptide kalata B1. Biochemistry 34, 4147-4158.

Salie, F., Eagles, P.F.K., Leng, H.M.J., 1996. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology 52, 27-33.

Samie, A., Obi, C., Bessong, P., Namrita, L., 2005. Activity profiles of fourteen selected medicinal plants from rural Venda communities in South Africa against fifteen clinical bacterial species. African Journal of Biotechnology 4.

Schmidt, T.J., Willuhn, G., 2000. Sesquiterpene lactone and flavonoid variability of the *Arnica angustifolia* aggregate (Asteraceae). Biochemical Systematics and Ecology 28, 133-142.

Schou, J., Kristiansen, U., Stafford, G., van Heerden, F., Jäger, A., 2010. Effect of *Rhoicissus digitata* extracts on acetylcholine-mediated contraction in rat uterus. Planta Medica 76, P097.

Schuster, D., Wolber, G., 2010. Identification of bioactive natural products by pharmacophore-based virtual screening. Current Pharmaceutical Design 16, 1666-1681.

Scott, G., Springfield, E.P., Coldrey, N., 2004. A pharmacognostical study of 26 South African plant species used as traditional medicines. Pharmaceutical Biology 42, 186-213.

Sengupta, R., Sheorey, S.D., Hinge, M.A., 2012. Analgesic and anti-inflammatory plants: an updated review. International Journal of Pharmaceutical Sciences Review and Research 12, 114-119.

Sewram, V., Raynor, M.W., Mulholland, D.A., Raidoo, D.M., 2000. The uterotonic activity of compounds isolated from the supercritical fluid extract of *Ekebergia capensis*. Journal of Pharmaceutical and Biomedical Analysis 24, 133-145.

Sewram, V., Raynor, M.W., Mulholland, D.A., Raidoo, D.M., 2001. Supercritical fluid extraction and analysis of compounds from *Clivia miniata* for uterotonic activity. Planta Medica 67, 451-455.

Sewram, V., Raynor, M.W., Raidoo, D.M., Mulholland, D.A., 1998. Coupling SFE to uterotonic bioassay: an on-line approach to analysing medicinal plants. Journal of Pharmaceutical and Biomedical Analysis 18, 305-318.

Shah, S.A., Sander, S., White, C.M., Rinaldi, M., Coleman, C.I., 2007. Evaluation of *Echinacea* for the prevention and treatment of the common cold: a meta-analysis. The Lancet Infectious Diseases 7, 473-480.

Sharp, H., Hollinshead, J., Bartholomew, B.B., Oben, J., Watson, A., Nash, R.J., 2007. Inhibitory effects of *Cissus quadrangularis* L. derived components on lipase, amylase and α-glucosidase activity *in vitro*. Natural Product Communications 2, 817-822.

Shen, Y.-C., Chen, C.-F., Chiou, W.-F., 2002. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. British Journal of Pharmacology 135, 399-406.

Shetty, A., Danielian, P., Templeton, A., 2001a. A comparison of oral and vaginal misoprostol tablets in induction of labour at term. An International Journal of Obstetrics & Gynaecology 108, 238-243.

Shetty, A., Danielian, P., Templeton, A., 2001b. A comparison of oral and vaginal misoprostol tablets in induction of labour at term. BJOG: An International Journal of Obstetrics & Gynaecology 108, 238-243.

Shode, F.O., Mahomed, A.S., Rogers, C.B., 2002. Typhaphthalide and typharin, two phenolic compounds from *Typha capensis*. Phytochemistry 61, 955-957.

Silbernagel, E., Spreitzer, H., Buchbauer, G., 1990. Nichtflüchtige Inhaltsstoffe von *Artemisia afra*. Monatshefte für Chemie / Chemical Monthly 121, 433-436.

Simionatto, E., Ilha, V., Mallmann, A.S., Porto, C., Dalcol, I.I., Morel, A.F., 2008. Chemical composition and antimicrobial activity of the volatile oil from *Baccharis articulata* (Lam.) Pers. Journal of Essential Oil Research 20, 366-368.

Singh, N.P., Lai, H., 2001. Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. Life Sciences 70, 49-56.

Soejima, A., Wen, J., 2006. Phylogenetic analysis of the grape family (Vitaceae) based on three chloroplast markers. American Journal of Botany 93, 278-287.

Soon-Ho, Y., Hyun Jung, K., Ik-Soo, L., 2003. A polyacetylene and flavonoids from *Cirsium rhinoceros*. Archives of Pharmacal Research 26, 128-131.

Steck, W., Bailey, B.K., 1969. Characterization of plant coumarins by combined gas chromatography, ultraviolet absorption spectroscopy, and nuclear magnetic resonance analysis. Canadian Journal of Chemistry 47, 3577-3583.

Steenkamp, V., Nkwane, O., Van Tonder, J., Dinsmore, A., Gulumian, M., 2013. Evaluation of the phenolic and flavonoid contents and radical scavenging activity of three southern African medicinal plants. African Journal of Pharmaceutical Pharmacology 7, 703-709.

Stolz, E.D., Müller, L.G., Trojan-Rodrigues, M., Baumhardt, E., Ritter, M.R., Rates, S.M.K., 2014. Survey of plants popularly used for pain relief in Rio Grande do Sul, Southern Brazil. Revista Brasileira de Farmacognosia 24, 185-196.

Street, R.A., Stirk, W.A., Van Staden, J., 2008. South African traditional medicinal plant trade—challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology 119, 705-710.

Stuessy, T., 2010. The rise of sunflowers. Science 329, 1605-1606.

Sun, I.C., Wang, H.-K., Kashiwada, Y., Shen, J.-K., Cosentino, L.M., Chen, C.-H., Yang, L.-M., Lee, K.-H., 1998. Anti-AIDS agents. 34. synthesis and structure−activity relationships of betulin derivatives as anti-HIV agents. Journal of Medicinal Chemistry 41, 4648-4657.

Sun, J., Huang, S.H., Zhu, Y.C., Whiteman, M., Wang, M.J., Tan, B.K.H., Zhu, Y.Z., 2005. Anti-oxidative stress effects of herba leonuri on ischemic rat hearts. Life Sciences 76, 3043-3056.

Sutton, L., 1965. Special Publication No. 18. London: The Chemical Society.

Tang, H.Q., Hu, J., Yang, L., Tan, R.X., 2000. Terpenoids and flavonoids from *Artemisia* species. Planta Medica 66, 391-393.

Teguo, P.W., Decendit, A., Vercauteren, J., Deffieux, G., Mérillon, J.-M., 1996. *Trans*-resveratrol-3-O-β-glucoside (piceid) in cell suspension cultures of *Vitis vinifera*. Phytochemistry 42, 1591-1593.

Thornbush, M.J., 2012. Tracking the use of climbing plants in the urban landscape through the photoarchives of two Oxford colleges, 1861–1964. Landscape Research 38, 312-328.

Thring, T., Weitz, F., 2006. Medicinal plant use in the Bredasdorp/Elim region of the Southern Overberg in the Western Cape Province of South Africa. Journal of Ethnopharmacology 103, 261-275.

Ticktin, T., Dalle, S.P., 2005. Medicinal plant use in the practice of midwifery in rural Honduras. Journal of Ethnopharmacology 96, 233-248.

Torres, T., da Silva, I., de Castro, É., dos Santos, E., da Cunha, R., Lima, J., 2014. Catalase inhibition affects glyoxylate cycle enzyme expression and cellular redox control during the functional transition of sunflower and safflower seedlings. Journal of Plant Growth Regulation 33, 272-284.

Toselli, F., Matthias, A., Gillam, E.M.J., 2009. *Echinacea* metabolism and drug interactions: the case for standardization of a complementary medicine. Life Sciences 85, 97-106.

Tröndle, D., Schröder, S., Kassemeyer, H.-H., Kiefer, C., Koch, M.A., Nick, P., 2010. Molecular phylogeny of the genus *Vitis* (Vitaceae) based on plastid markers. American Journal of Botany 97, 1168-1178.

Tuwangye, I., Olila, D., 2006. The anthelmintic activity of selected indigenous medicinal plants used by the Banyankole of western Uganda. Journal of Animal and Veterinary Advances 5, 712-717.

Urones, J.G., Basabe, P., Marcos, I.S., Diez Martin, D., Sexmero, M.J., Peral, M.H., Broughton, H.B., 1992. Chemistry of labdanediol from *Cistus ladaniferus* L. Synthesis of 12-nor-ambreinolide and α- and β-levantenolides. Tetrahedron 48, 10389-10398.

Urzua, A., 2004. Secondary metabolites in the epicuticle of *Haplopappus foliosus* DC. (Asteraceae). Journal of the Chilean Chemical Society 49, 137-141.

Valant-Vetschera, K.M., Wollenweber, E., 1995. Flavonoid aglycons from the leaf surfaces of some *Artemisia spp.* (Compositae - Anthemideae). Zeitschrift fuer Naturforsch., C: Biosciences 50, 353-357.

Van der Kooi, R., Theobald, S., 2006. Traditional medicine in late pregnancy and labour: perceptions of *Kgaba* remedies amongst the Tswana in South Africa.

African Journal of Traditional, Complementary, and Alternative Medicines 3 11-22.

Van Dongen, P.W., de Groot, A.N., 1995. History of ergot alkaloids from ergotism to ergometrine. European Journal of Obstetrics & Gynecology and Reproductive Biology 60, 109-116.

Van Puyvelde, L., De Kimpe, N., Costa, J., Munyjabo, V., Nyirankuliza, S., Hakizamungu, E., Schamp, N., 1989. Isolation of flavonoids and a chalcone from *Helichrysum odoratissimum* and synthesis of helichrysetin. Journal of Natural Products 52, 629-633.

Van Wyk, B.-E., Gericke, N., 2000. People's Plants: A Guide to Useful Plants of Southern Africa. Briza Publications.

Van Wyk, B.E., 2008a. A broad review of commercially important Southern African medicinal plants. Journal of Ethnopharmacology 119, 342-355.

Van Wyk, B.E., 2008b. A review of Khoi-San and Cape Dutch medical ethnobotany. Journal of Ethnopharmacology 119, 331-341.

Van Wyk, B.E., 2011. The potential of South African plants in the development of new medicinal products. South African Journal of Botany 77, 812-829.

Varga, C.A., Veale, D.J.H., 1997. *Isihlambezo*: Utilization patterns and potential health effects of pregnancy-related traditional herbal medicine. Social Science & Medicine 44, 911-924.

Vasudevan, P., Kashyap, S., Sharma, S., 1997. *Tagetes*: a multipurpose plant. Bioresource Technology 62, 29-35.

Veale, D., Havlik, I., Katsoulis, L., Kaido, T., Arangies, N., Olive, D., Dekker, T., Brookes, K., Doudoukina, O., 1998. The pharmacological assessment of herbal oxytocics used in South African traditional medicine. Biomarkers and Environment 2, 42-45.

Veale, D.J.H., Furman, K.I., Oliver, D.W., 1992. South African traditional herbal medicines used during pregnancy and childbirth. Journal of Ethnopharmacology 36, 185-191.

Veale, D.J.H., Havlik, I., Oliver, D.W., Dekker, T.G., 1999. Pharmacological effects of *Agapanthus africanus* on the isolated rat uterus. Journal of Ethnopharmacology 66, 257-262.

Veale, D.J.H., Oliver, D.W., Arangies, N.S., Furman, K.I., 1989. Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. Journal of Ethnopharmacology 27, 341-346.

Veale, D.J.H., Oliver, D.W., Havlik, I., 2000. The effects of herbal oxytocics on the isolated "stripped" myometrium model. Life Sciences 67, 1381-1388.

Veitch, N.C., Grayer, R.J., 2011. Flavonoids and their glycosides, including anthocyanins. Natural Product Research 28, 1626-1695.

Venditti, A., Bianco, A., Tomassini, L., Nicoletti, M., 2014. A C-methylated resacetophenone from *Cistus monspeliensis* L. Fitoterapia 95, 182-185.

Vigneaud, V.d., Ressler, C., Swan, C.J.M., Roberts, C.W., Katsoyannis, P.G., Gordon, S., 1953. The synthesis of an octapeptide amide with the hormonal activity of oxytocin Journal of the American Chemical Society 75, 4879-4880.

Villa-Ruano, N., Betancourt-Jiménez, M.G., Lozoya-Gloria, E., 2009. Biosynthesis of uterotonic diterpenes from *Montanoa tomentosa* (zoapatle). Journal of Plant Physiology 166, 1961-1967.

Waibel, R., Achenbach, H., Torrenegra, R., Pedrozo, J., Robles, J., 1992. Diterpenes from *Gnaphalium pellitum* and *Gnaphalium graveolens*. Phytochemistry 31, 2415-2418.

Waithaka, J., 2004. The evaluation of markers for quality control studies of flavonoid-containing medicinal preparations. University of the Western Cape,

Wang, L.-j., Zhang, L., Wang, Y., Wang, J., 2009. Anti-inflammatory and analgesic effects of herba leonuri. Lishizhen Medicine and Materia Medica Research 3, 523-537.

Wang, Y., Zhang, Z., He, H., Gao, S., Kong, N., Ding, M., Hao, X., 2006. Lignans and triterpenoids from *Cissus repens* (Vitaceae). Acta Botanica Yunnanica 28, 433-437.

Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P., McPhail, A.T., 1971. Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. Journal of the American Chemical Society 93, 2325-2327.

Watanabe, Y., Novaes, P., Varela, R.M., Molinillo, J.M.G., Kato-Noguchi, H., Macias, F.A., 2014. Phytotoxic potential of *Onopordum acanthium* L. (Asteraceae). Chemistry & Biodiversity 11, 1247-1255.

Wathes, D.C., Swann, R.W., 1982. Is oxytocin an ovarian hormone? Nature 297, 225-227.

Watt, J., Breyer-Brandwijk, M., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa 2nd Edn. Edition. Livingstone, Edinburgh, London

Weiss, E.A., 2000. Oilseed Crops. Blackwell Science, Oxford.

Westfall, R., 2001. Herbal medicine in pregnancy and childbirth. Advances in Therapy 18, 47-55.

Whitehouse, B., 1941. Fragarine: an inhibitor of uterine action. British medical journal 2, 370.

Wollenweber, E., Christ, M., Dunstan, R.H., Roitman, J.N., Stevens, J.F., 2005. Exudate flavonoids in some Gnaphalieae and Inuleae (Asteraceae). Zeitschrift fur Naturforschung C-Journal of Biosciences 60, 671-678.

Wollenweber, E., Dorr, M., Fritz, H., Papendieck, S., Yatskievych, G., Roitman, J.N., 1997a. Exudate flavonoids in Asteraceae from Arizona, California, and Mexico. Zeitschrift fuer Naturforschung, C: Biosciences 52, 301-307.

Wollenweber, E., Dorr, M., Fritz, H., Valant-Vetsghera, K., 1997b. Exudate flavonoids in several *Asteroideae* and *Cichorioideae* (Asteraceae). Zeitschrift fur Naturforschung C-Journal of Biosciences 52, 137-143.

Wollenweber, E., Fischer, R., Dorr, M., Irvine, K., Pereira, C., Stevens, J.F., 2008. Chemodiversity of exudate flavonoids in *Cassinia* and *Ozothamnus* (Asteraceae, Gnaphalieae). Zeitschrift für Naturforschung, C: Journal of Biosciences 63, 731.

Wollenweber, E., Schober, I., Dostal, P., Hradetzky, D., Arriaga-Giner, F.J., Yatskievych, G., 1986a. Flavonoids and terpenoids from the exudates of some Baccharis species. Z. Naturforsch., C: Biosci. 41, 87-93.

Wollenweber, E., Schober, I., Dostal, P., Hradetzky, D., Arriaga-Giner, F.J., Yatskievych, G., 1986b. Flavonoids and terpenoids from the exudates of some *Baccharis* species. Zeitschrift fuer Naturforschung, C: Journal of Biosciences 41, 87-93.

Wrolstad, R.E., Culver, C.A., 2012. Alternatives to those artificial FD & C food colorants. Annual Review of Food Science and Technology 3, 59-77.

Xie, D.-Y., Dixon, R.A., 2005. Proanthocyanidin biosynthesis – still more questions than answers? Phytochemistry 66, 2127-2144.

Yen, C.-T., Hsieh, P.-W., Hwang, T.-L., Lan, Y.-H., Chang, F.-R., Wu, Y.-C., 2009. Flavonol glycosides from *Muehlenbeckia platyclada* and their anti-inflammatory activity. Chemical and Pharmaceutical Bulletin 57, 280-282.

Yff, B.T.S., Lindsey, K.L., Taylor, M.B., Erasmus, D.G., Jäger, A.K., 2002. The pharmacological screening of *Pentanisia prunelloides* and the isolation of the antibacterial compound palmitic acid. Journal of Ethnopharmacology 79, 101-107.

Yoo, Y.-M., Nam, J.-H., Kim, M.-Y., Choi, J., Park, H.-J., 2008. Pectolinarin and pectolinarigenin of *Cirsium setidens* prevent the hepatic injury in rats caused by D-galactosamine via an antioxidant mechanism. Biological and Pharmaceutical Bulletin 31, 760-764.

Yount, S.M., Lassiter, N., 2013. The pharmacology of prostaglandins for induction of labor. Journal of Midwifery & Women's Health 58, 133-144.

Zavada, M., de Villiers, S., 2000. Pollen of the Asteraceae from the Paleocene-Eocene of South Africa. Grana 39, 39-45.

Zhang, W.D., Chen, W.S., Wang, Y.H., Liu, W.Y., Kong, D.Y., Li, H.T., 2000. Studies on flavone constituents of *Erigeron breviscapus* (Vant.) Hand.-Mazz. Zhongguo Zhong Yao Za Zhi. Zhongguo Zhongyao Zazhi. China Journal of Chinese Materia Medica 25, 536-538.

Zheng, G.-h., Yang, J., Feng, Q.-l., 2009. Studies on the Chemical Constituents of *Meicha* (II). Journal of Chinese Medicinal Materials 32, 370-372.

Zheng, X., Wang, W., Piao, H., Xu, W., Shi, H., Zhao, C., 2013. The genus *Gnaphalium* L. (Compositae): phytochemical and pharmacological characteristics. Molecules 18, 8298-8318.

Zhu, Y.Z., Huang, S.H., Tan, B.K.H., Sun, J., Whiteman, M., Zhu, Y.C., 2004. Antioxidants in Chinese herbal medicines: a biochemical perspective. Natural product reports 21, 478-489.

Zu, Y., Li, C., Fu, Y., Zhao, C., 2006. Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. Journal of Pharmaceutical and Biomedical Analysis 41, 714-719.

Zuco, V., Supino, R., Righetti, S.C., Cleris, L., Marchesi, E., Gambacorti-Passerini, C., Formelli, F., 2002. Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer Letters 175, 17-25.

Appendix 1

NMR spectra of isolated compounds

Plate 1a: ^{1}H NMR spectrum of 6,7-dimethoxycoumarin (2.52) in CDCl$_3$

171
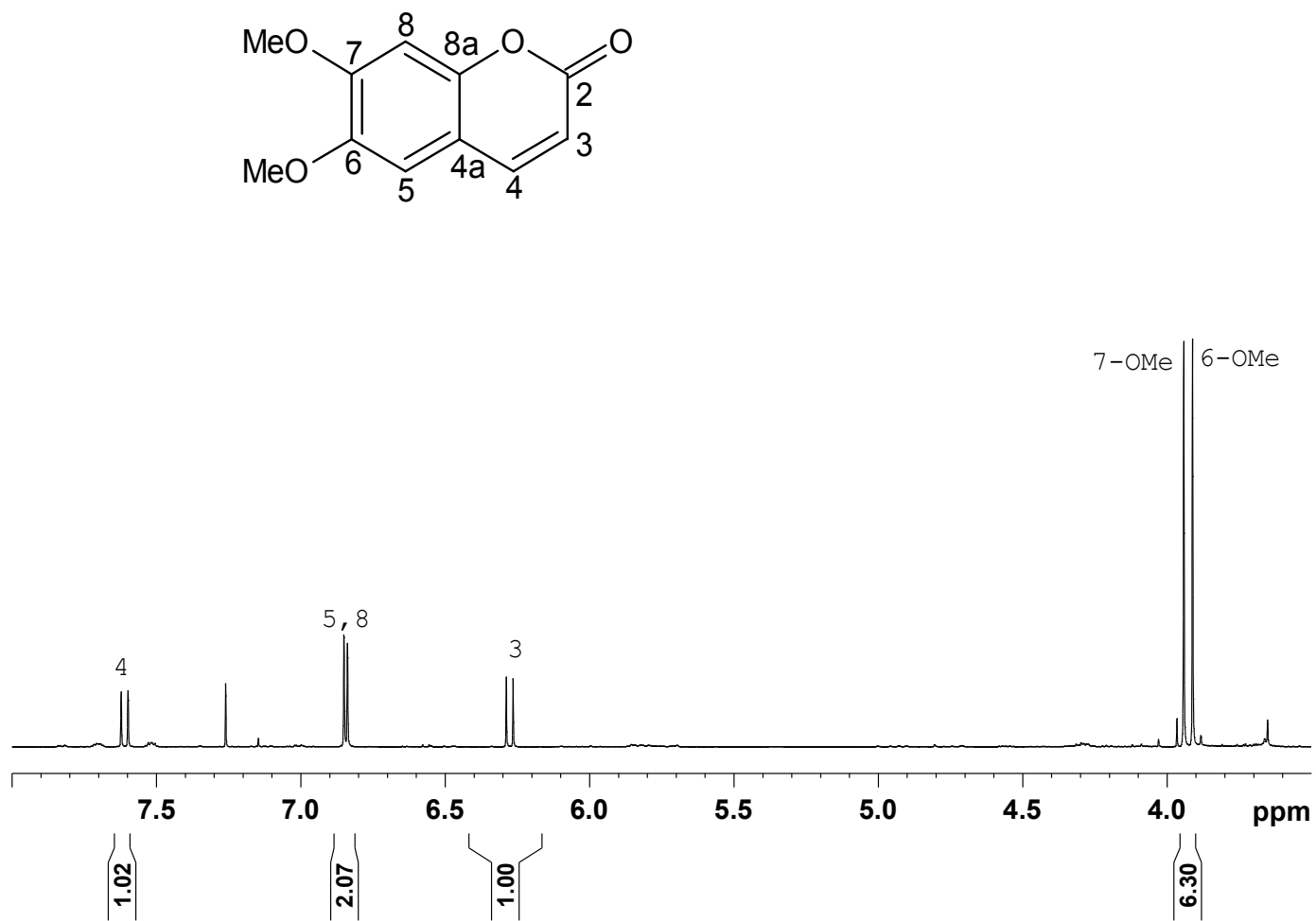
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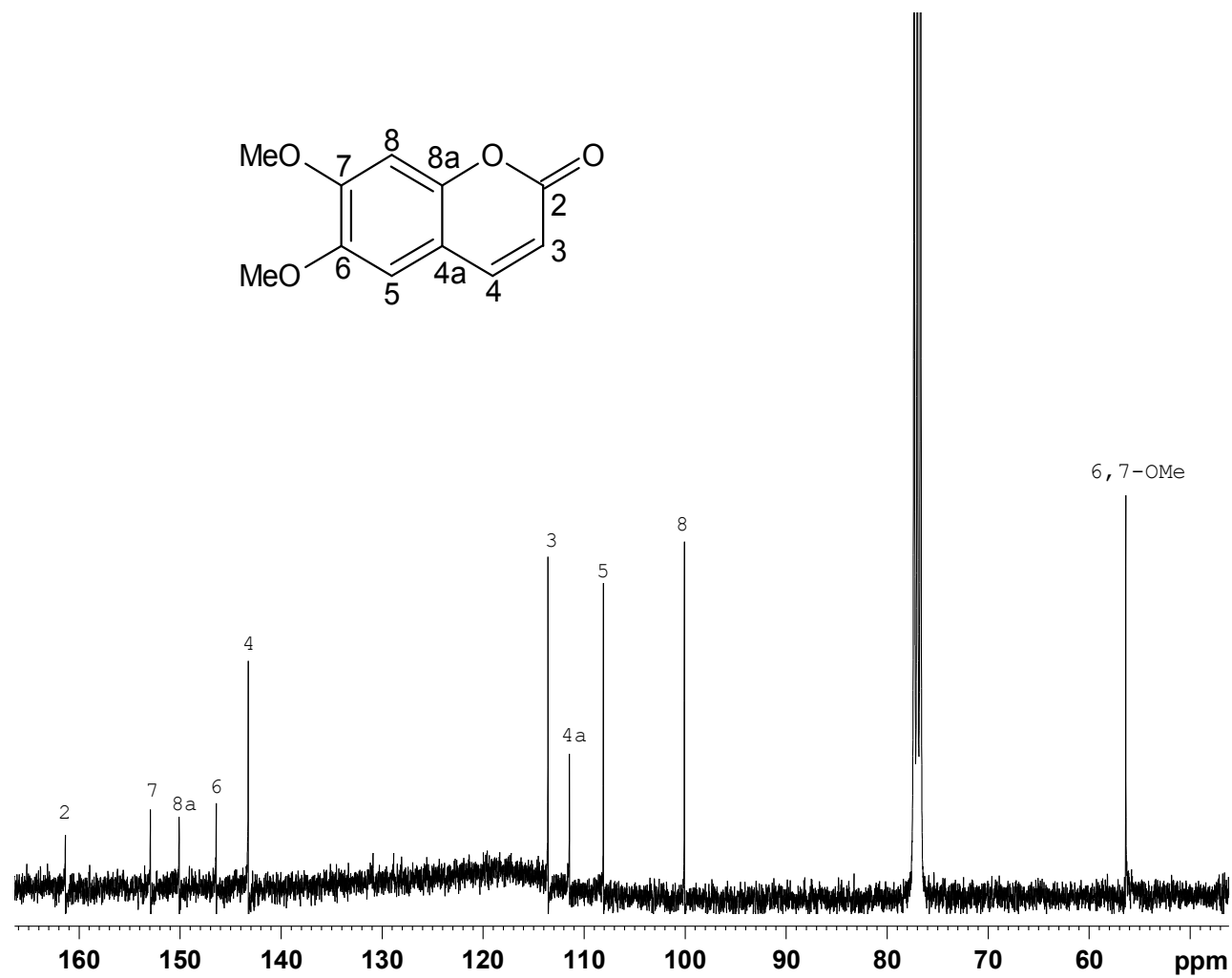
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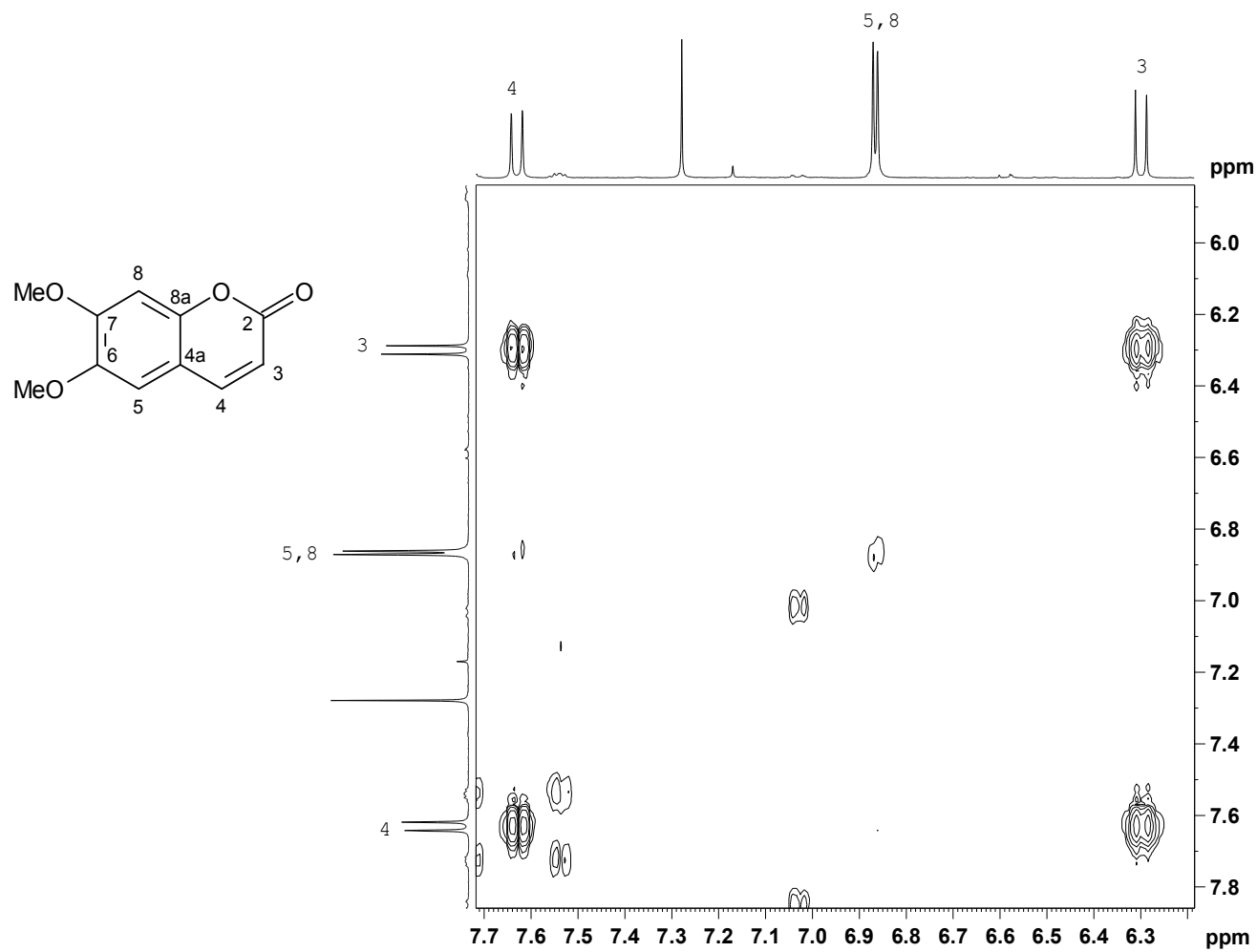
Plate 1d: DEPT 135 NMR spectrum of 6,7-dimethoxycoumarin (2.52) in CDCl$_3$
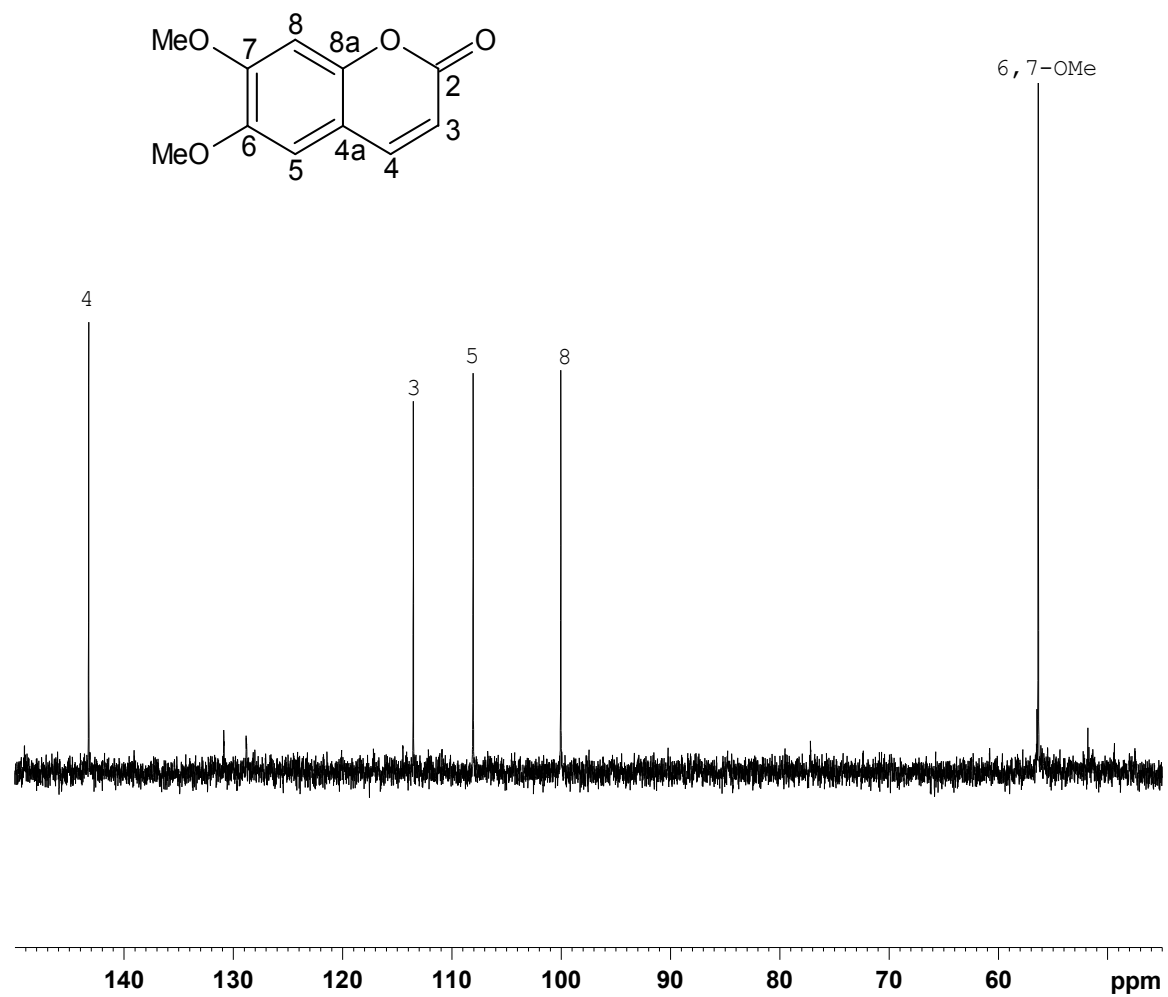
Plate 1e: HSQC NMR spectrum of 6,7-dimethoxycoumarin (2.52) in CDCl$_3$

175
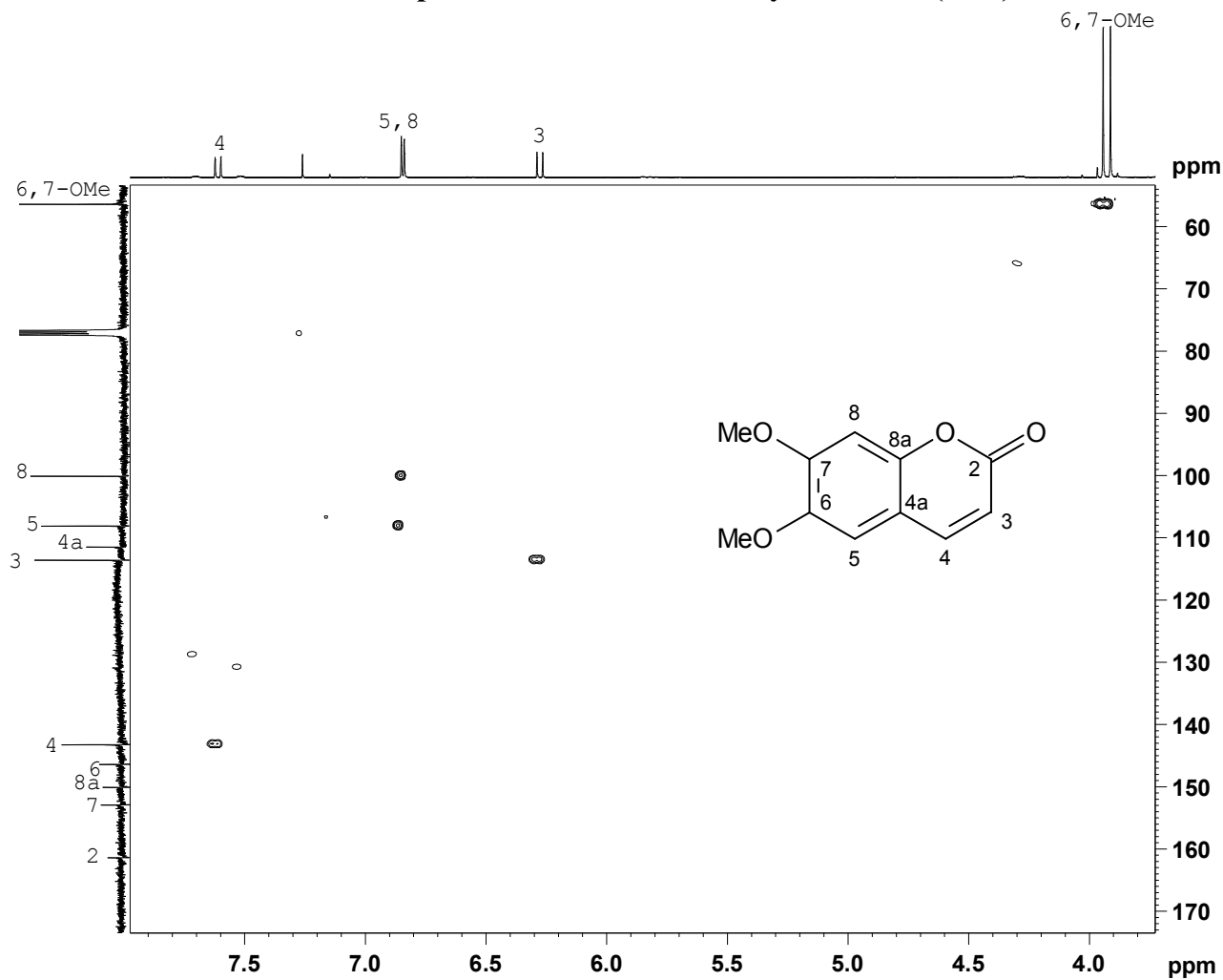
Plate 1f: HMBC NMR spectrum of 6,7-dimethoxycoumarin (2.52) in CDCl₃

176
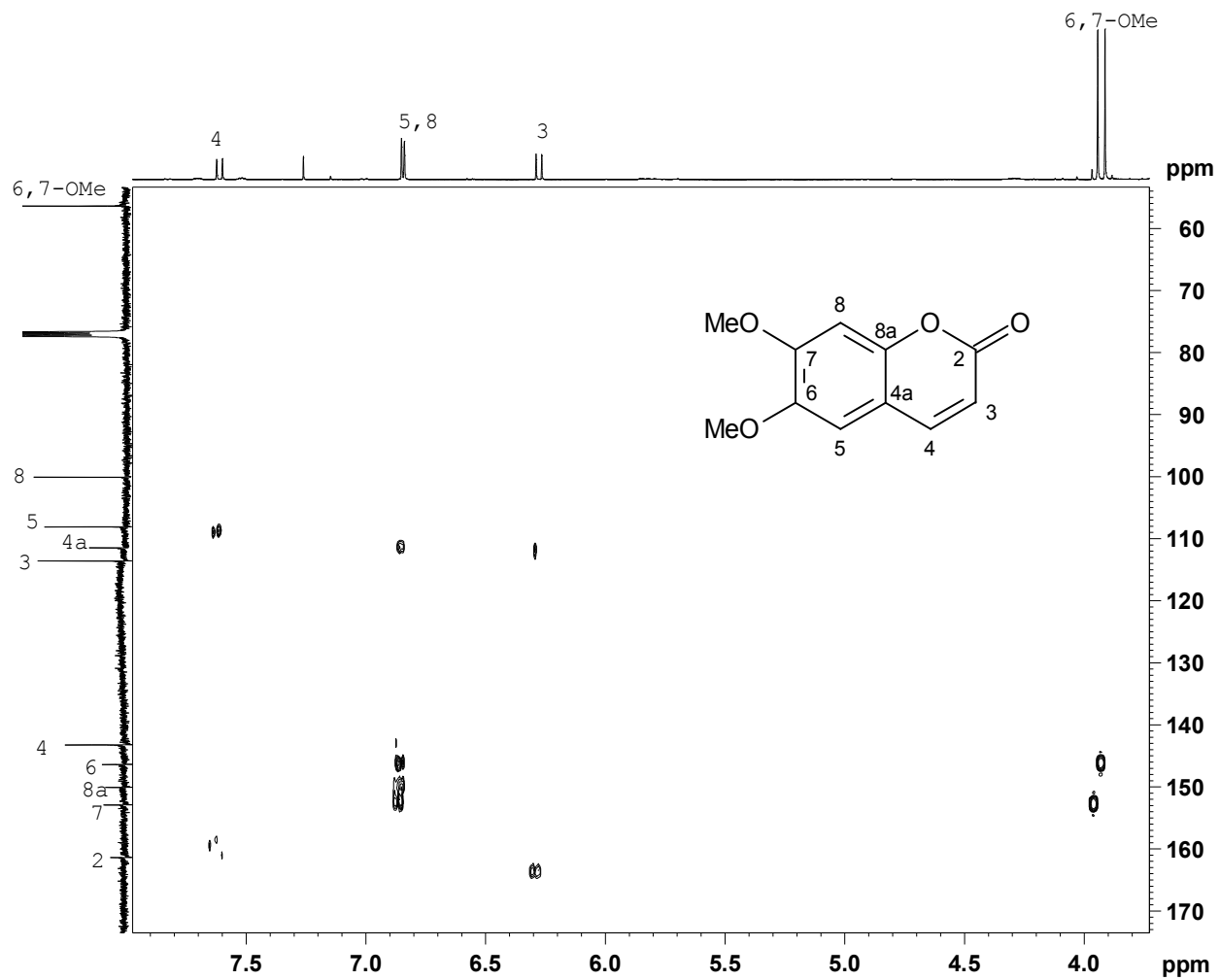
Plate 2a: ¹H NMR spectrum of 5,7,4'-trihydroxyflavone (2.53) in CD₃OD
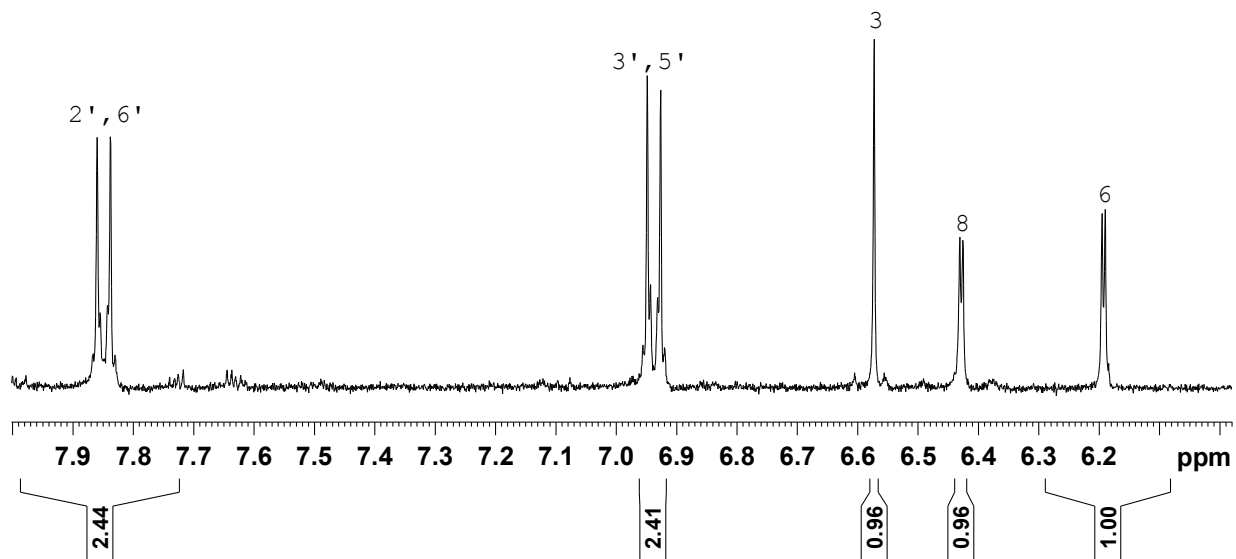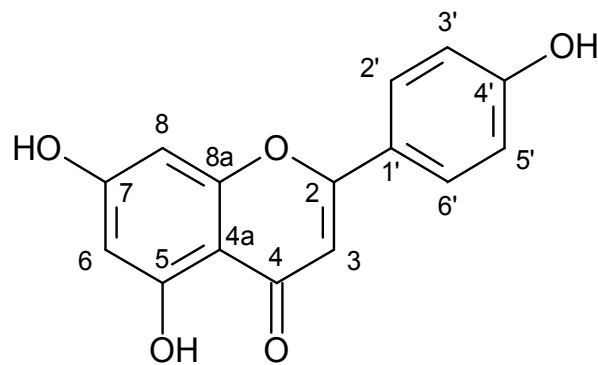
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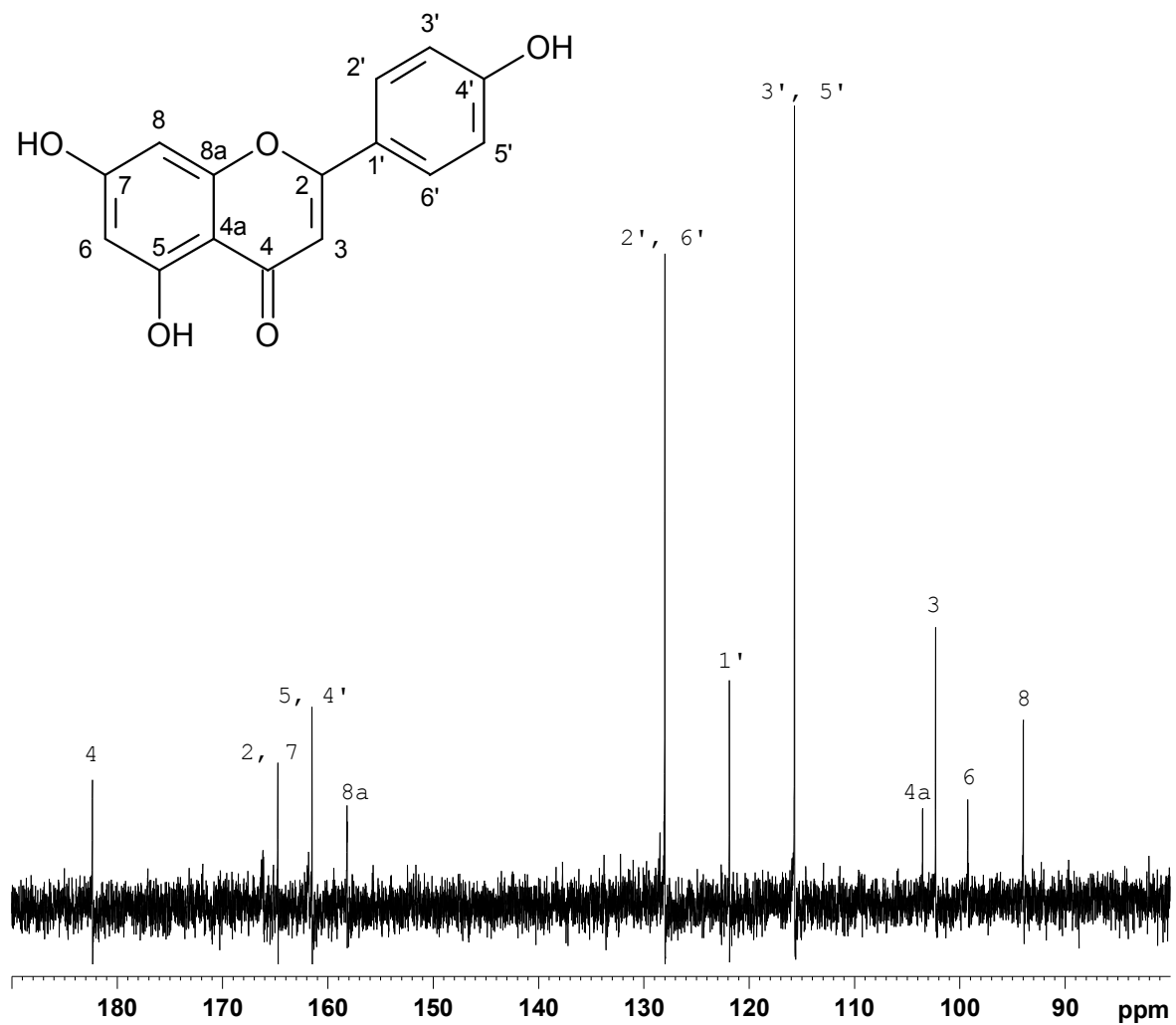
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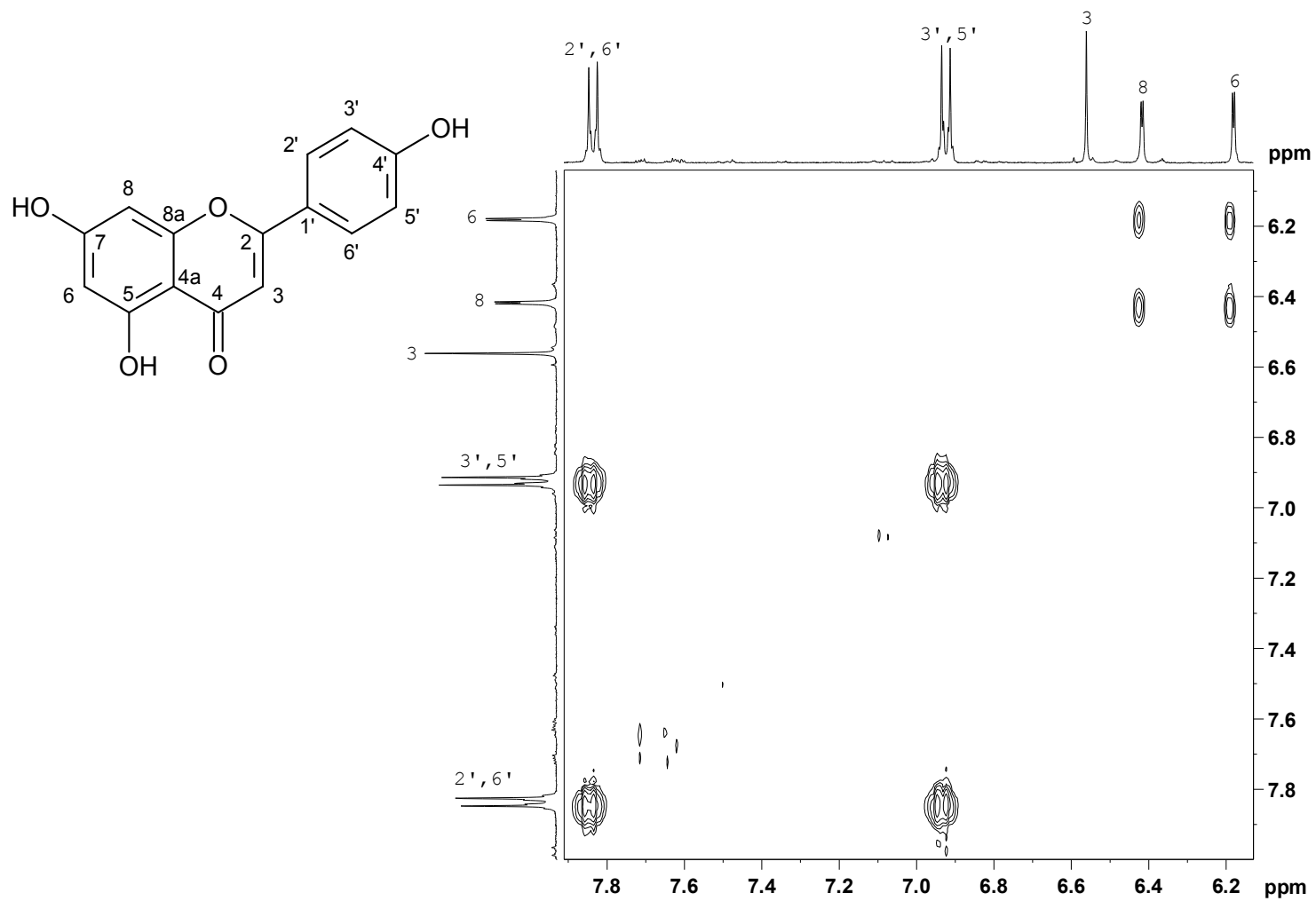
Plate 2d: DEPT 135 NMR spectrum of 5,7,4'-trihydroxyflavone (2.53) in CD₃OD
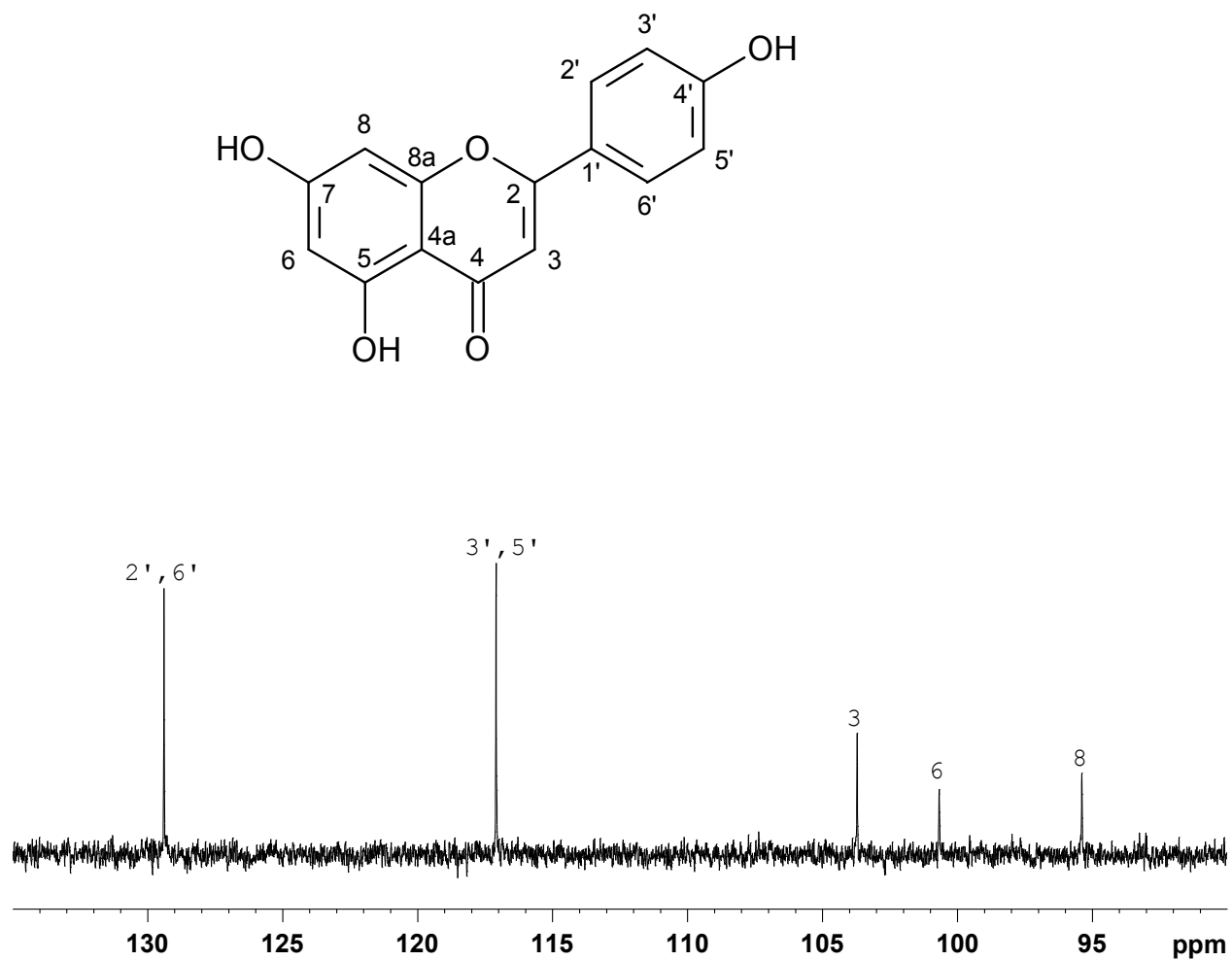
Plate 2e: HSQC NMR spectrum of 5,7,4'-trihydroxyflavone (2.53) in CD₃OD

181
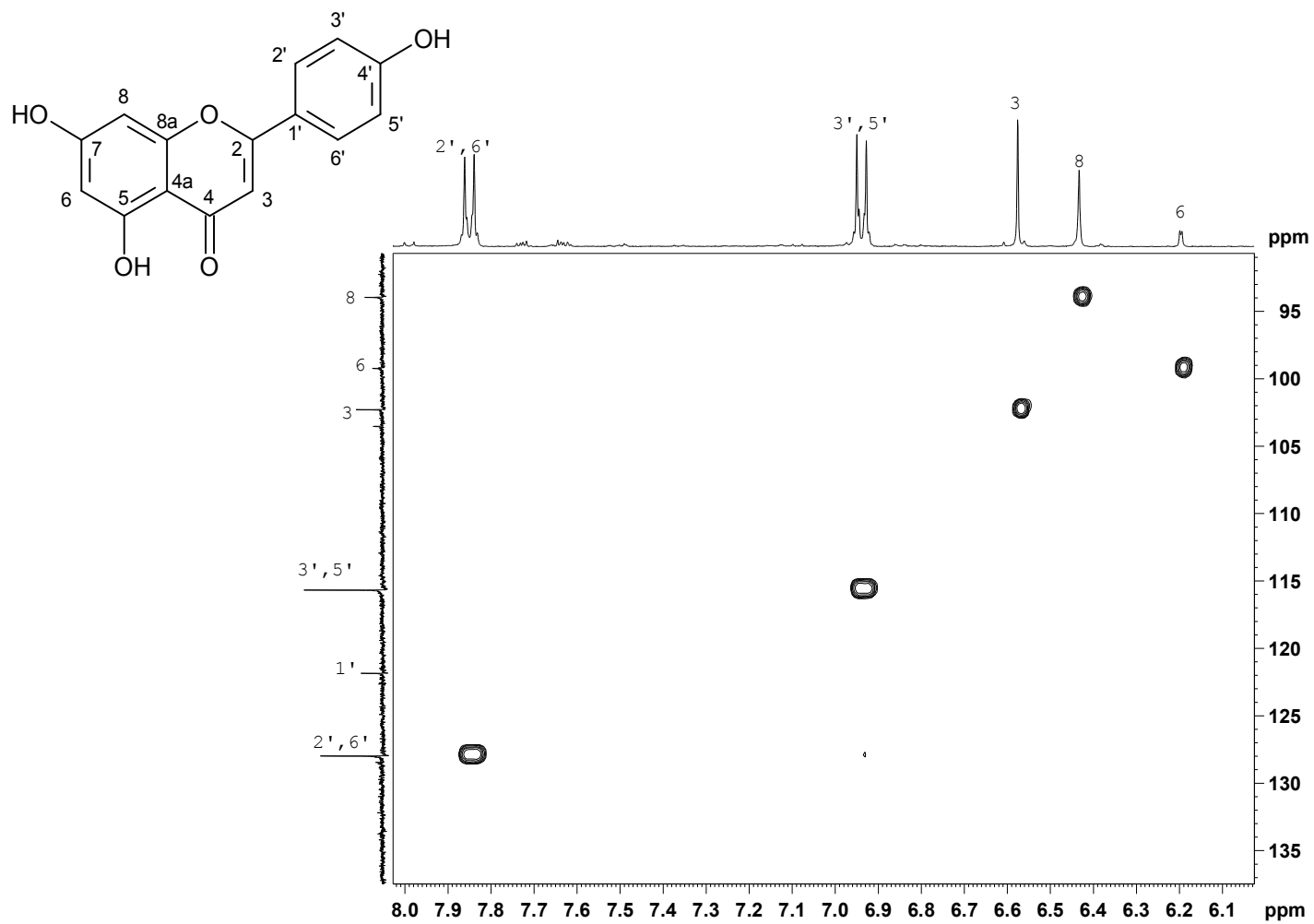
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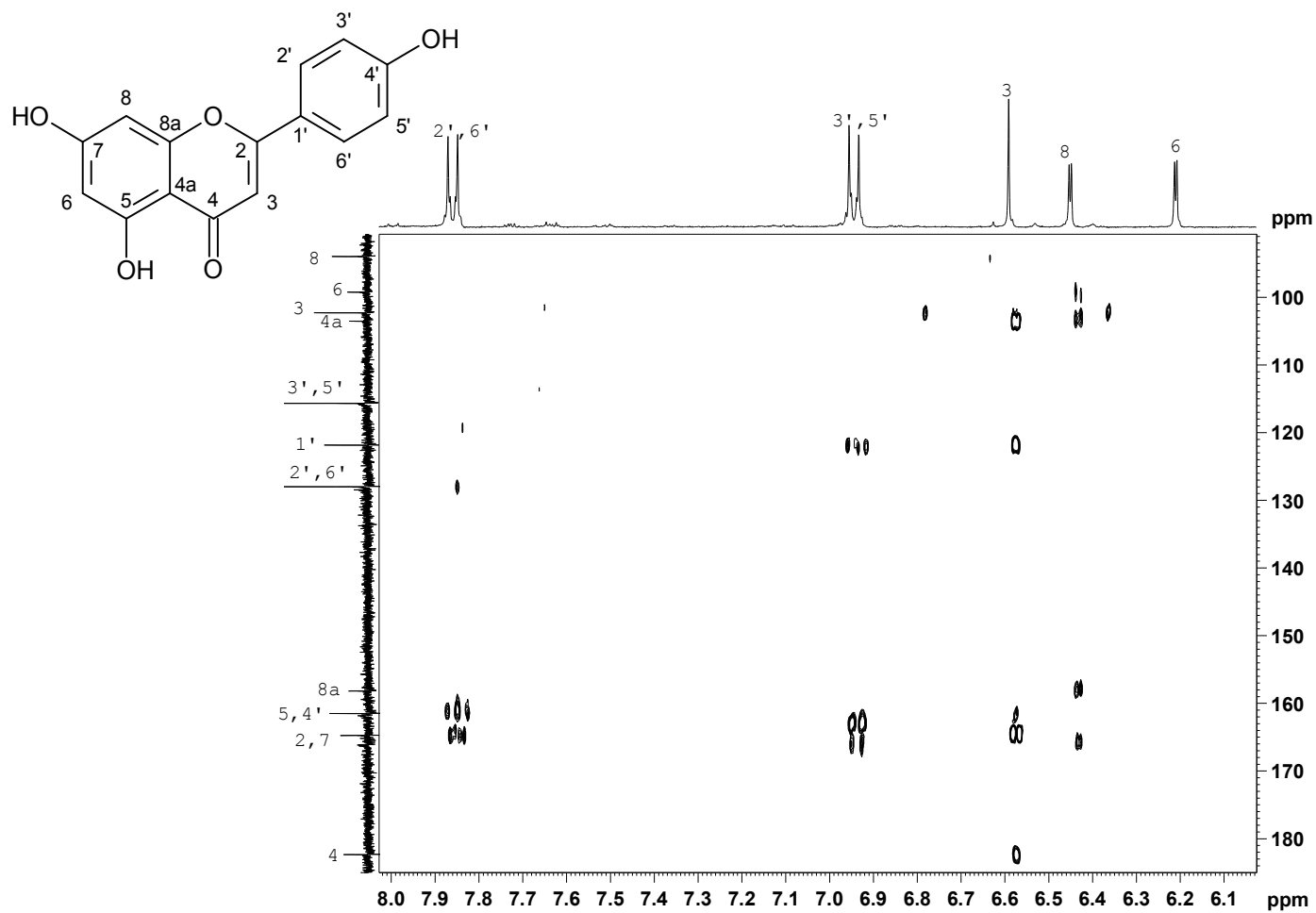
Plate 3a: ^{1}H NMR spectrum of 5,7-dihydroxy-4'-methoxyflavone (2.54) in Acetone-d$_6$
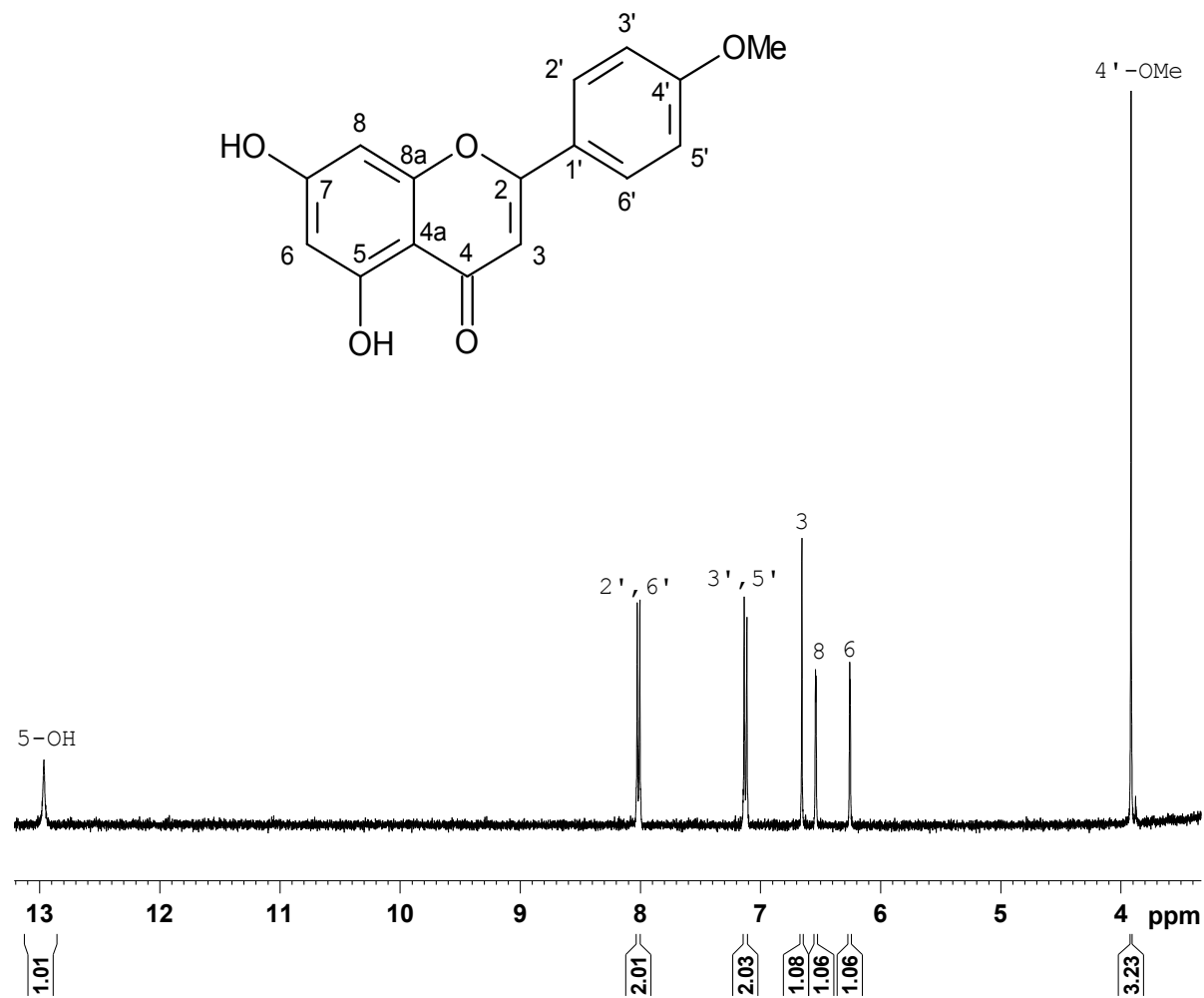
Plate 3b: ^{13}C NMR spectrum of 5,7-dihydroxy-4'-methoxyflavone (2.54) in Acetone-d$_6$
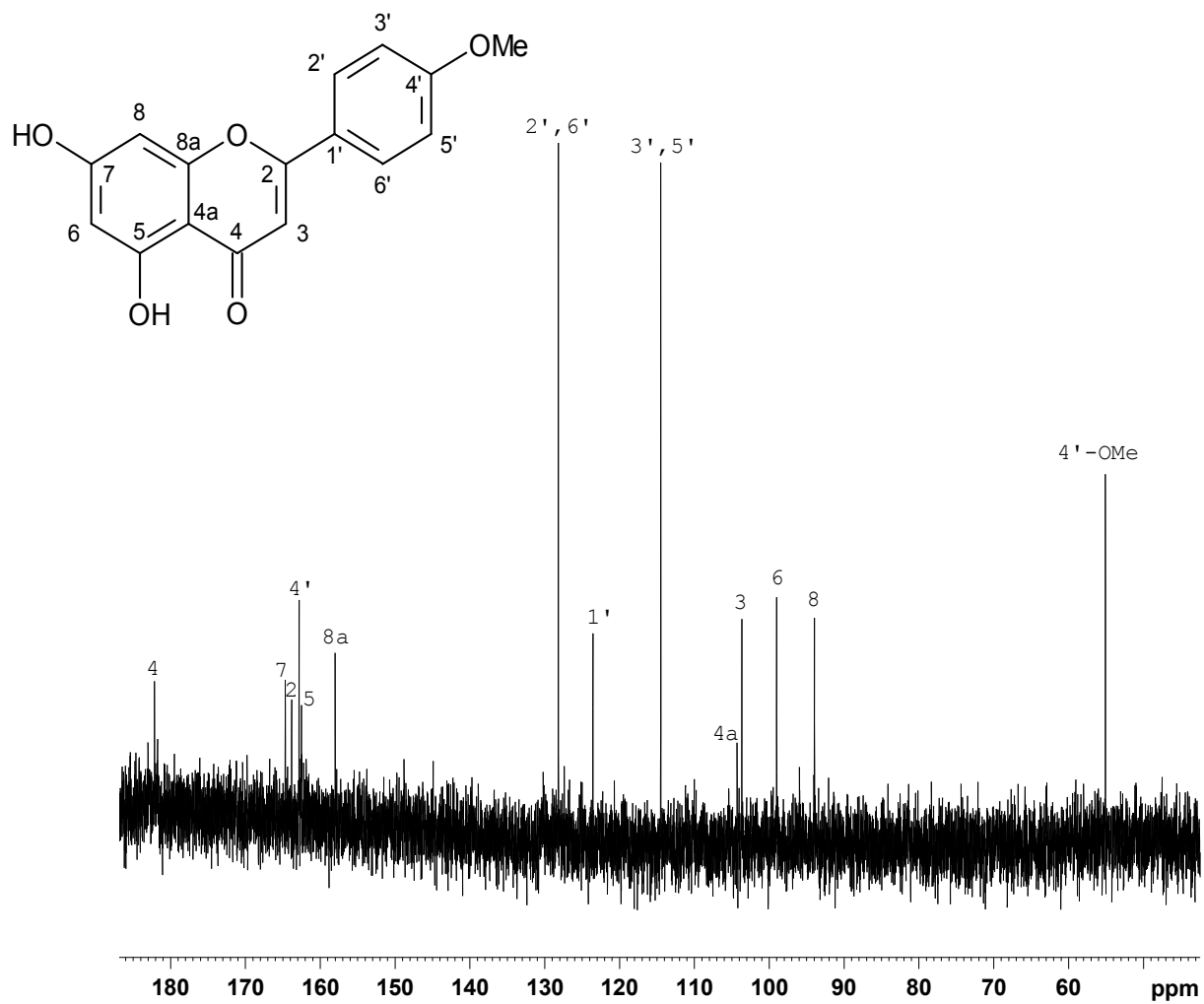
Plate 3c: COSY NMR spectrum of 5,7-dihydroxy-4'-methoxyflavone (2.54) in Acetone-d$_6$
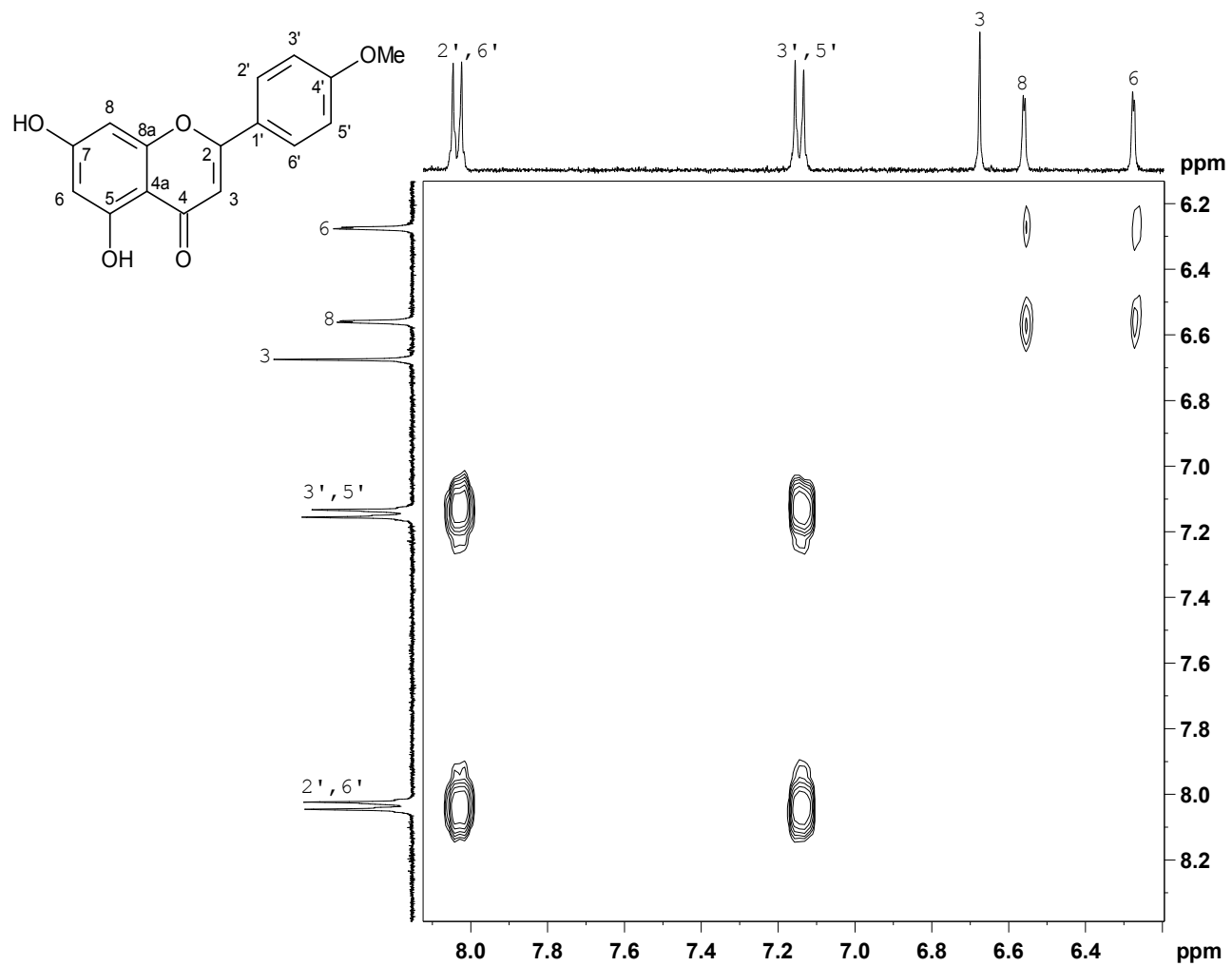
Plate 3d: DEPT NMR spectrum of 5,7-dihydroxy-4'-methoxyflavone (2.54) in Acetone-d$_6$
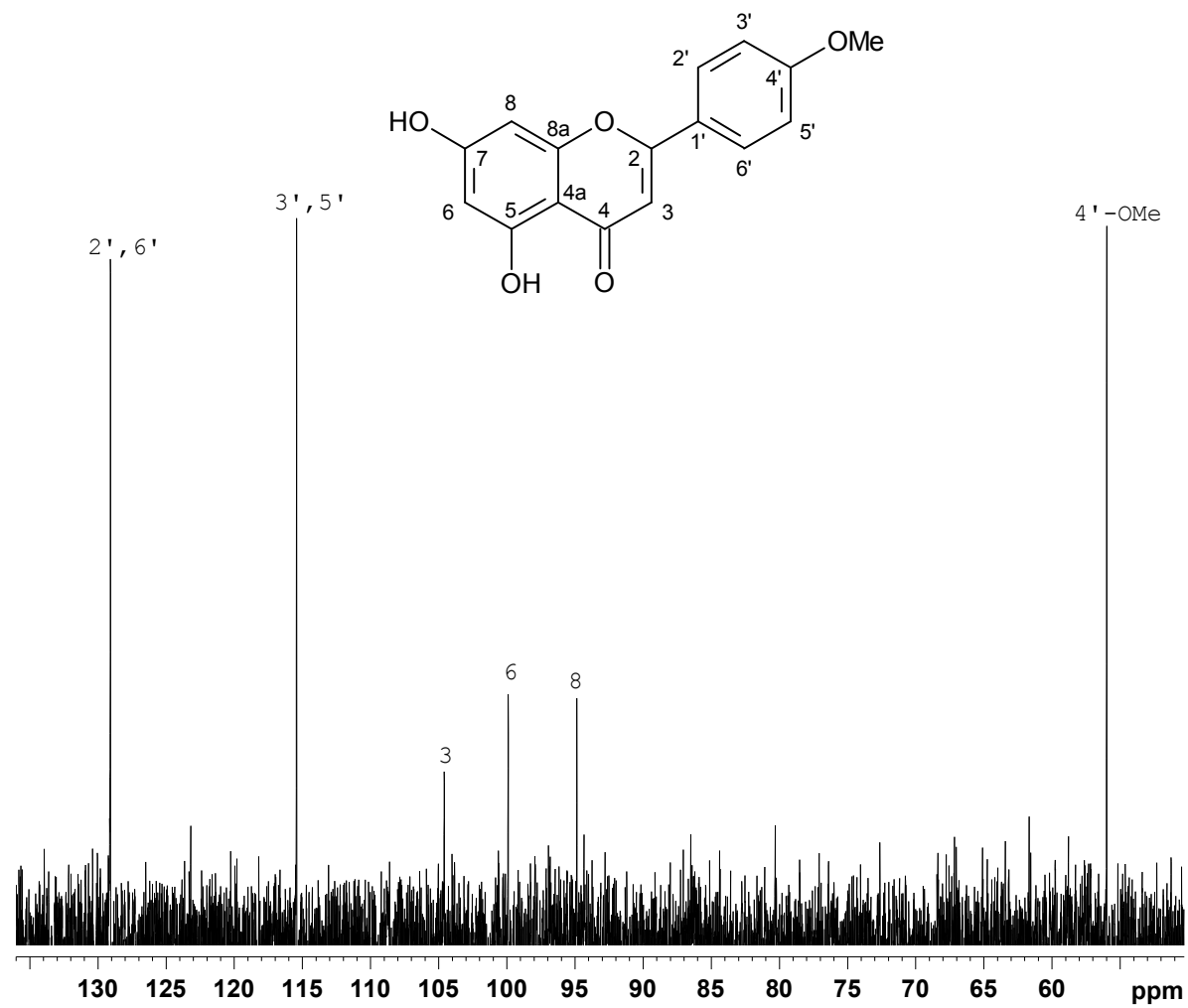
Plate 3e: HSQC NMR spectrum of 5,7-dihydroxy-4'-methoxyflavone (2.54) in Acetone-d₆

187
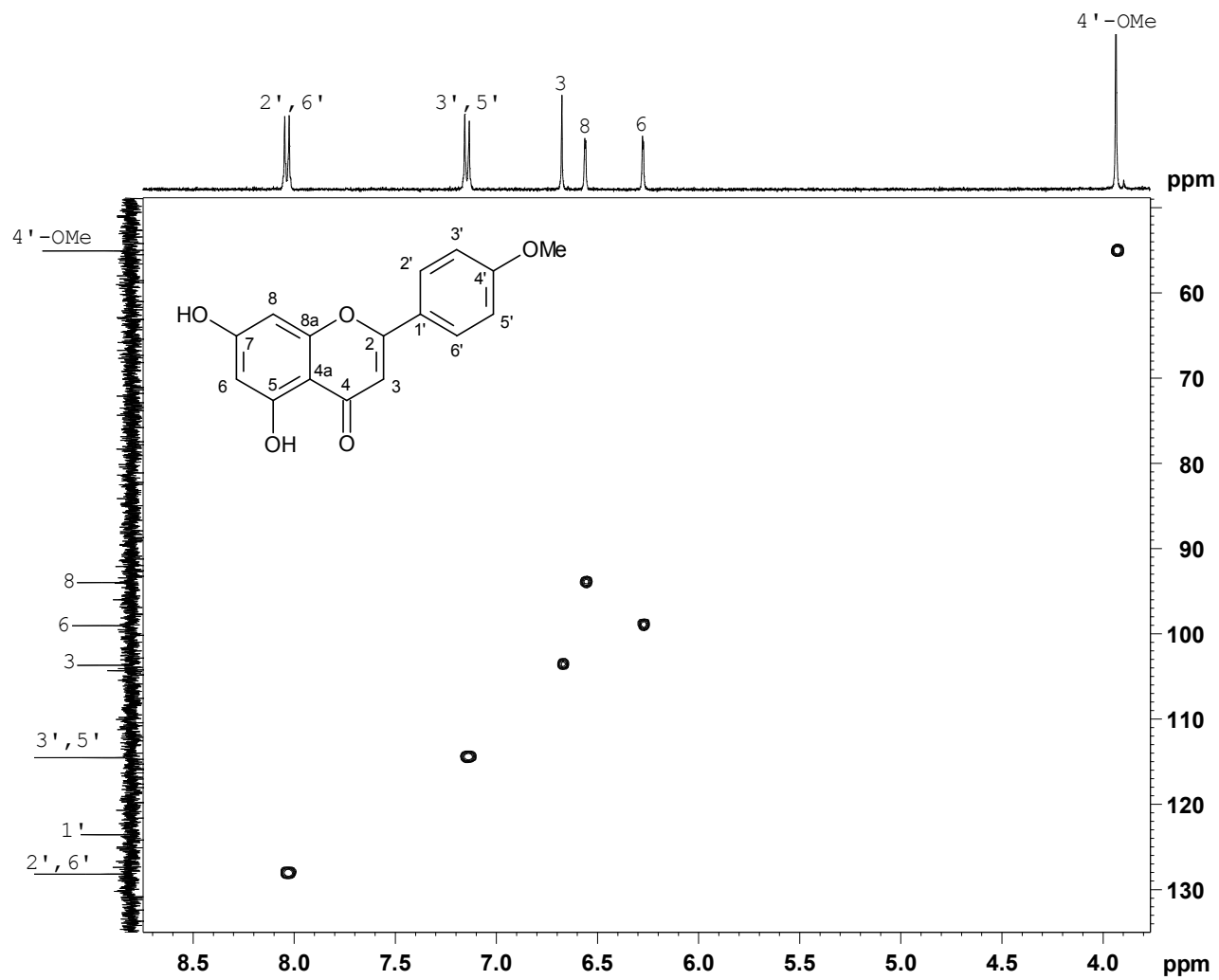
Plate 3f: HMBC NMR spectrum of 5,7-dihydroxy-4'-methoxyflavone (2.54) in Acetone-d$_6$

188
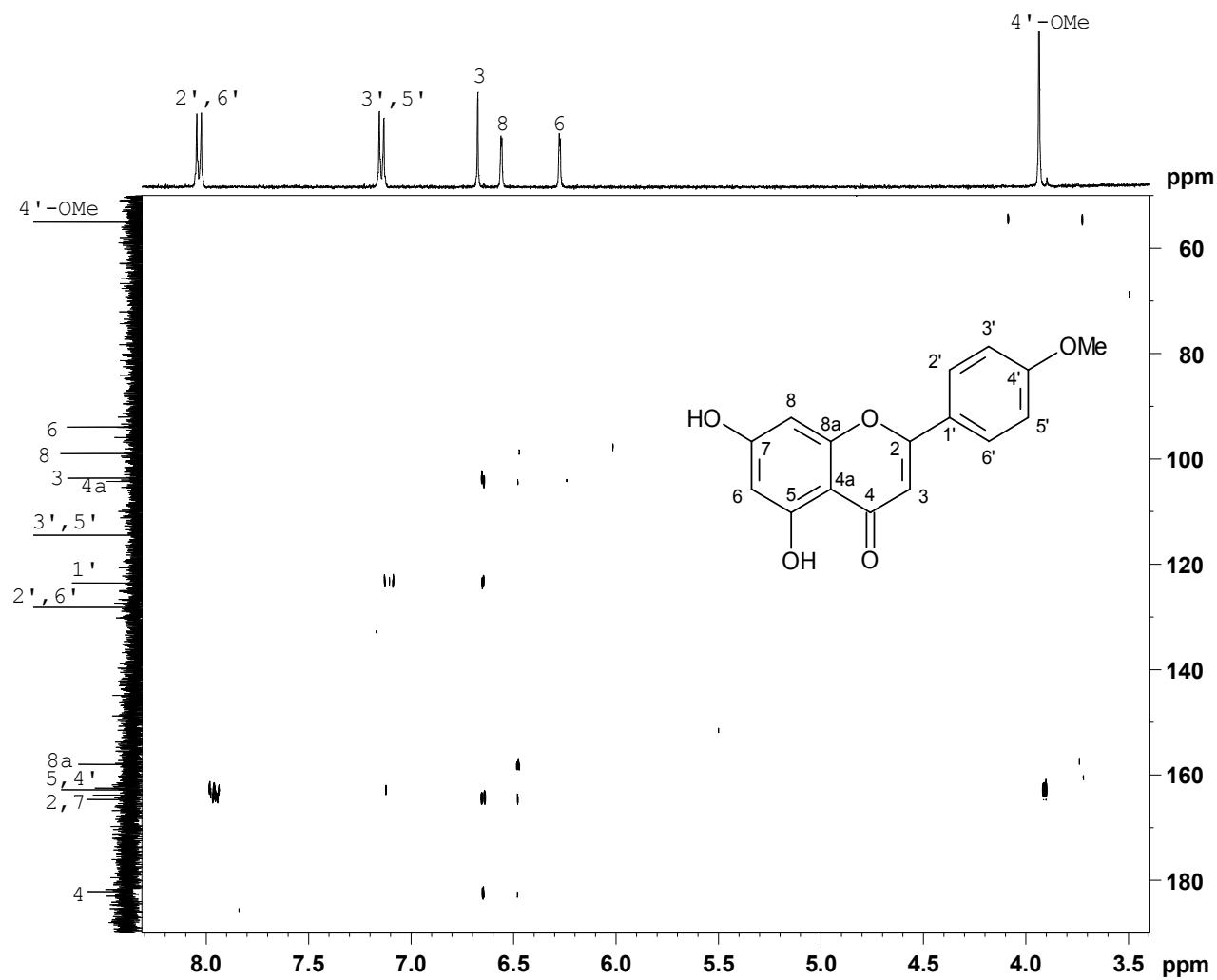
Plate 4a: ^{1}H NMR spectrum of 5,7-dihydroxy-6,4'-dimethoxyflavone (2.55) in CDCl$_3$
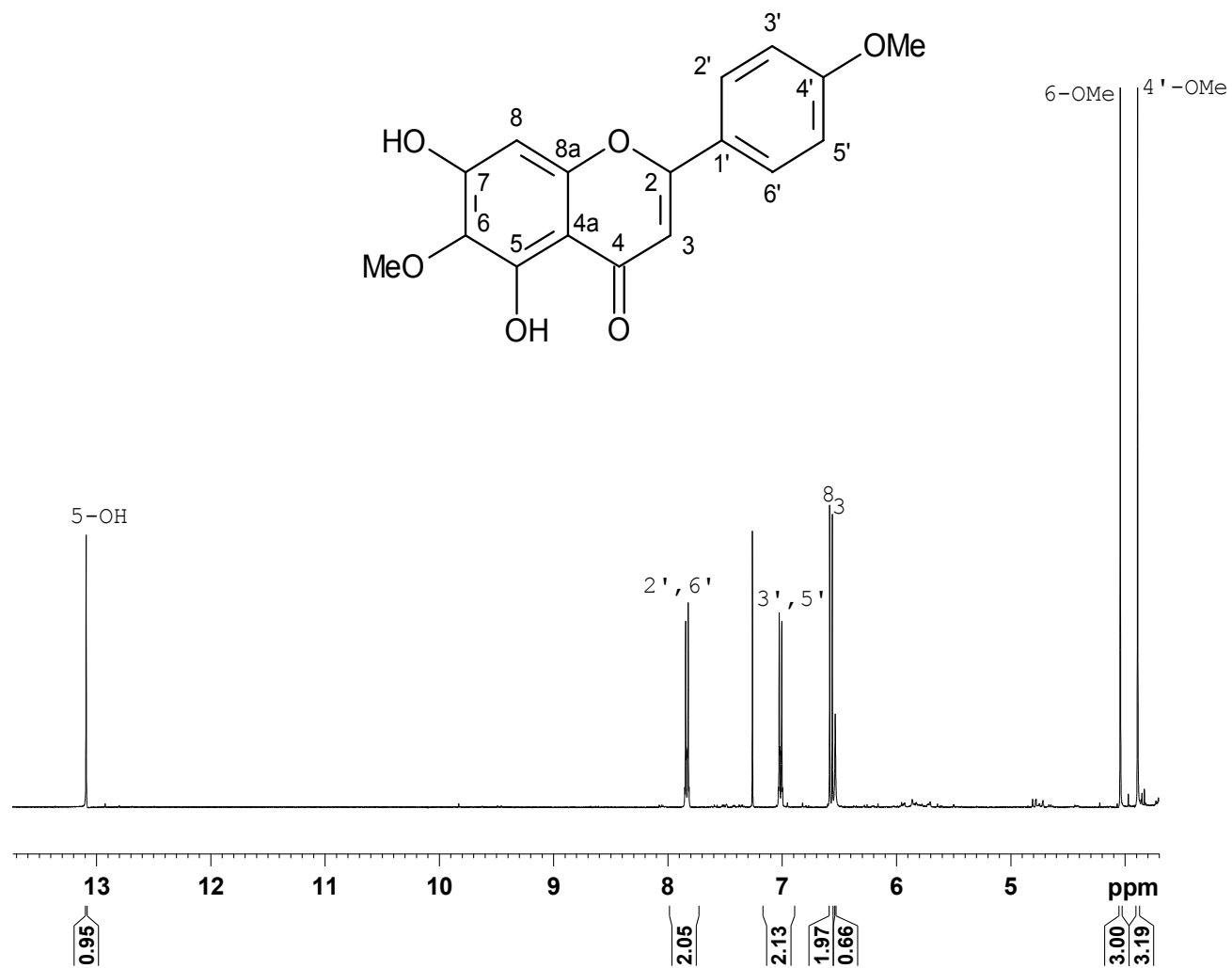
Plate 4b: ^{13}C NMR spectrum of 5,7-dihydroxy-6,4'-dimethoxyflavone (2.55) in CDCl$_3$

190
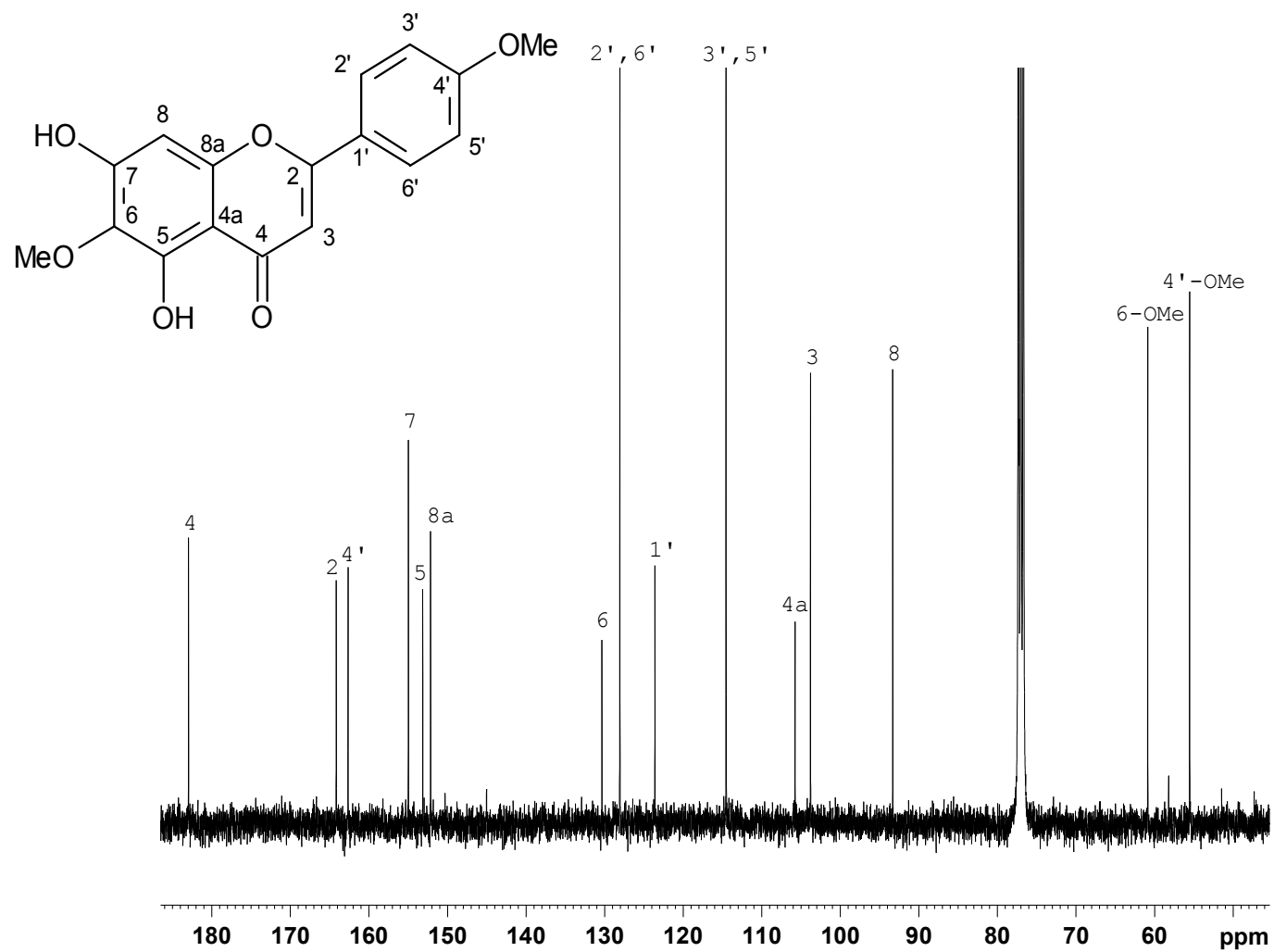
Plate 4c: COSY NMR spectrum of 5,7-dihydroxy-6,4'-dimethoxyflavone (2.55) in CDCl₃
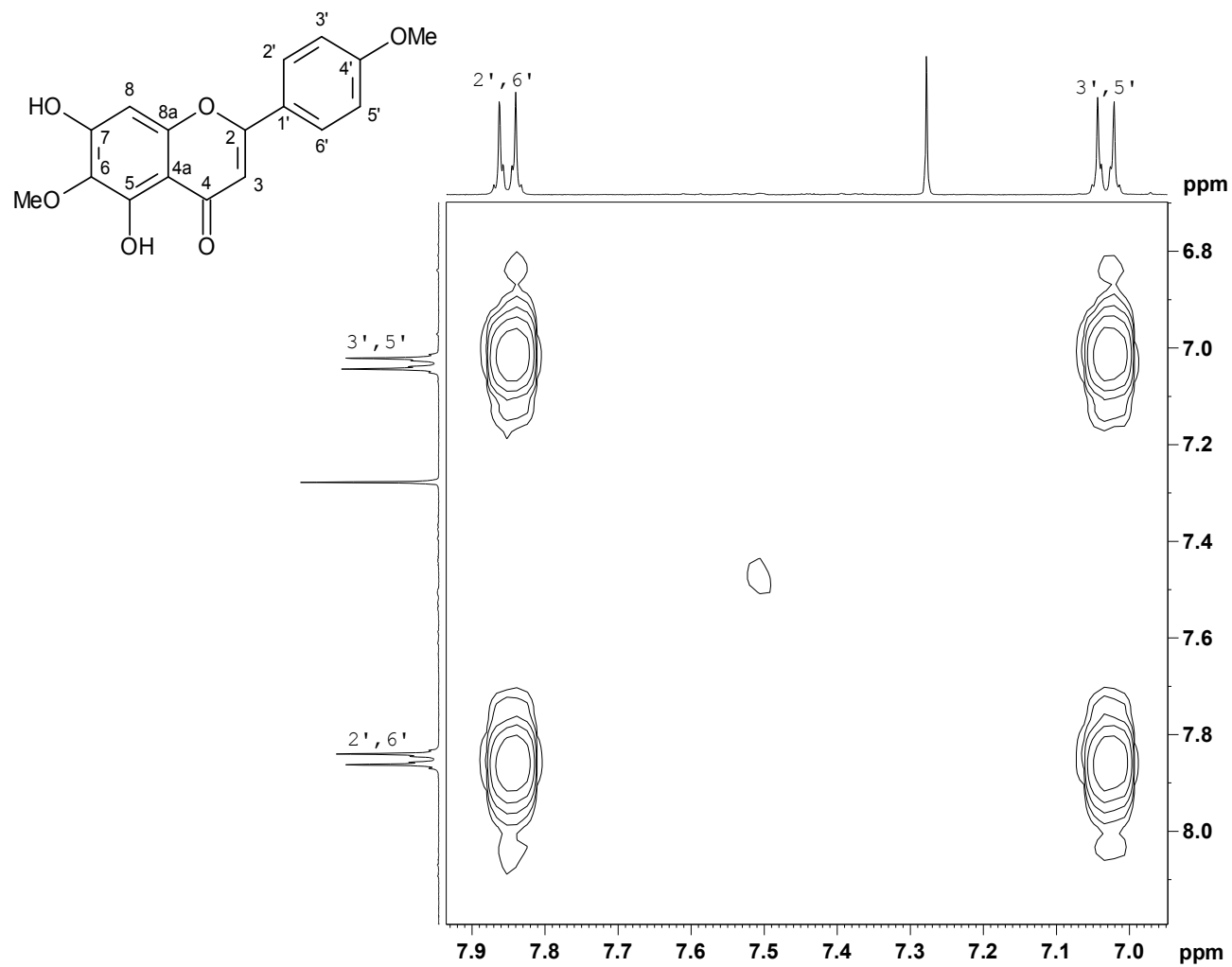
Plate 4d: DEPT 135 NMR spectrum of 5,7-dihydroxy-6,4'-dimethoxyflavone (2.55) in CDCl₃
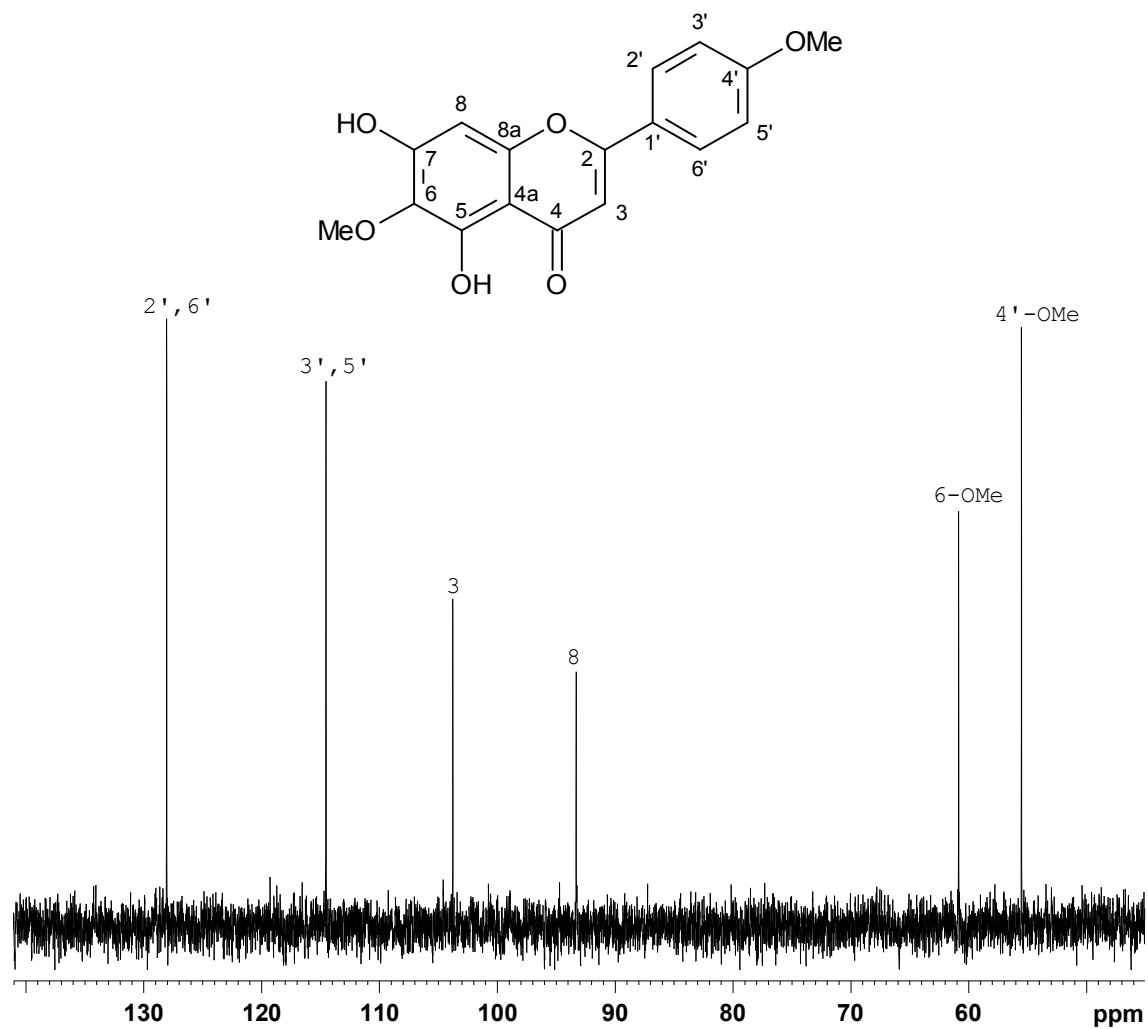
Plate 4e: HSQC NMR spectrum of 5,7-dihydroxy-6,4'-dimethoxyflavone (2.55) in CDCl₃

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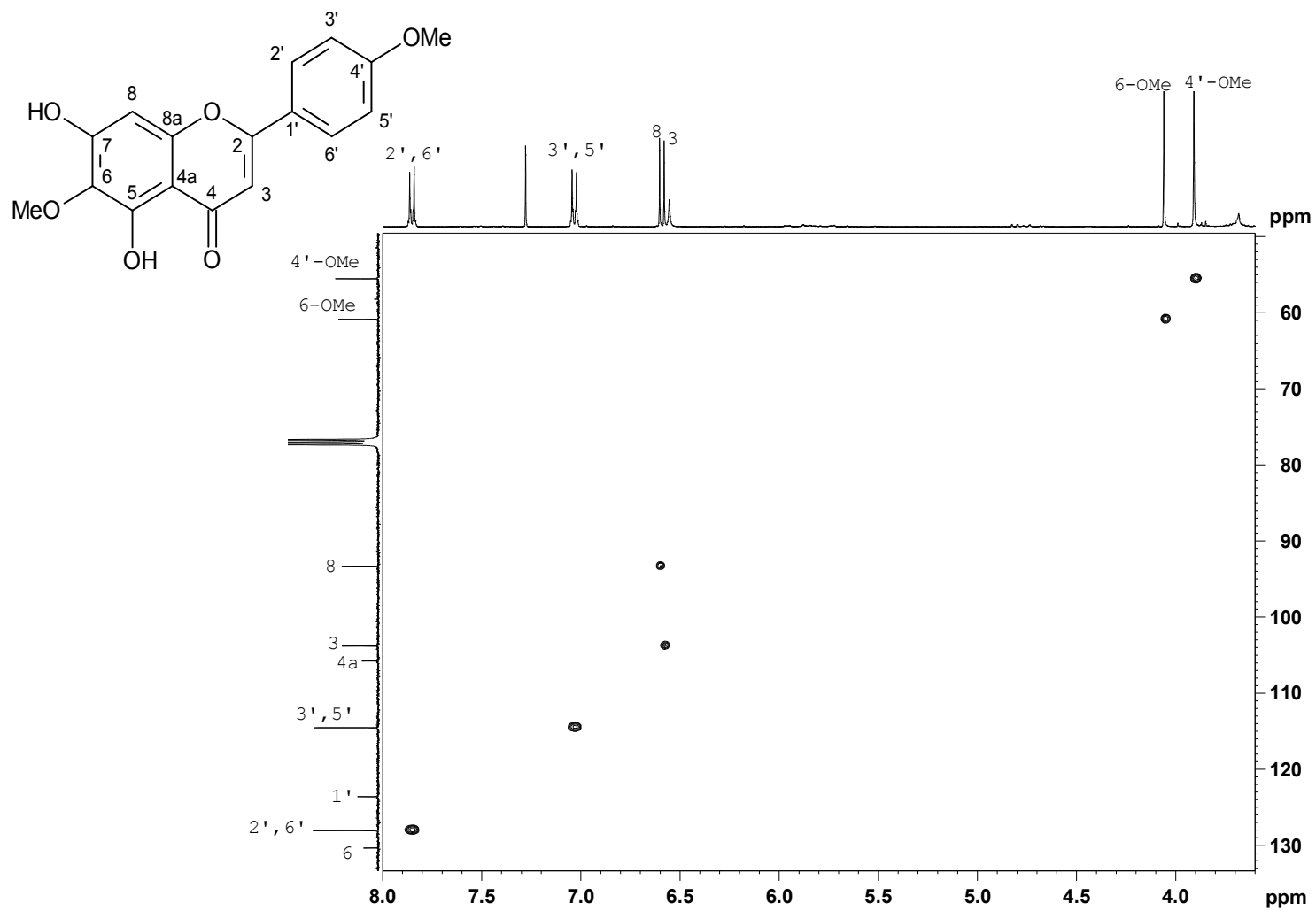
Plate 4f: HMBC NMR spectrum of 5,7-dihydroxy-6,4'-dimethoxyflavone (2.55) in CDCl₃

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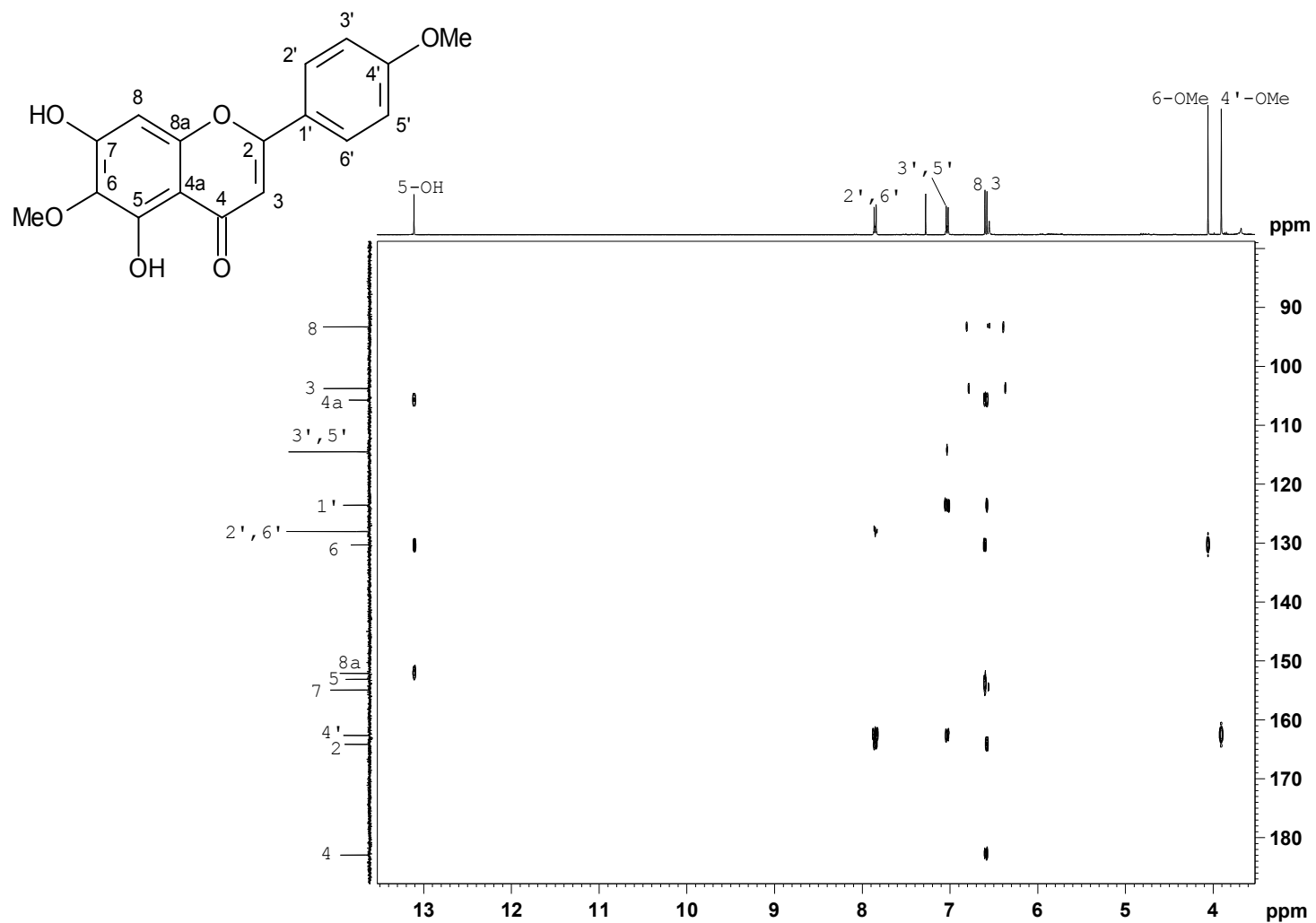
Plate 5a: ^{1}H NMR spectrum of kaempferol 3-methyl ether (2.56) in CD$_3$OD
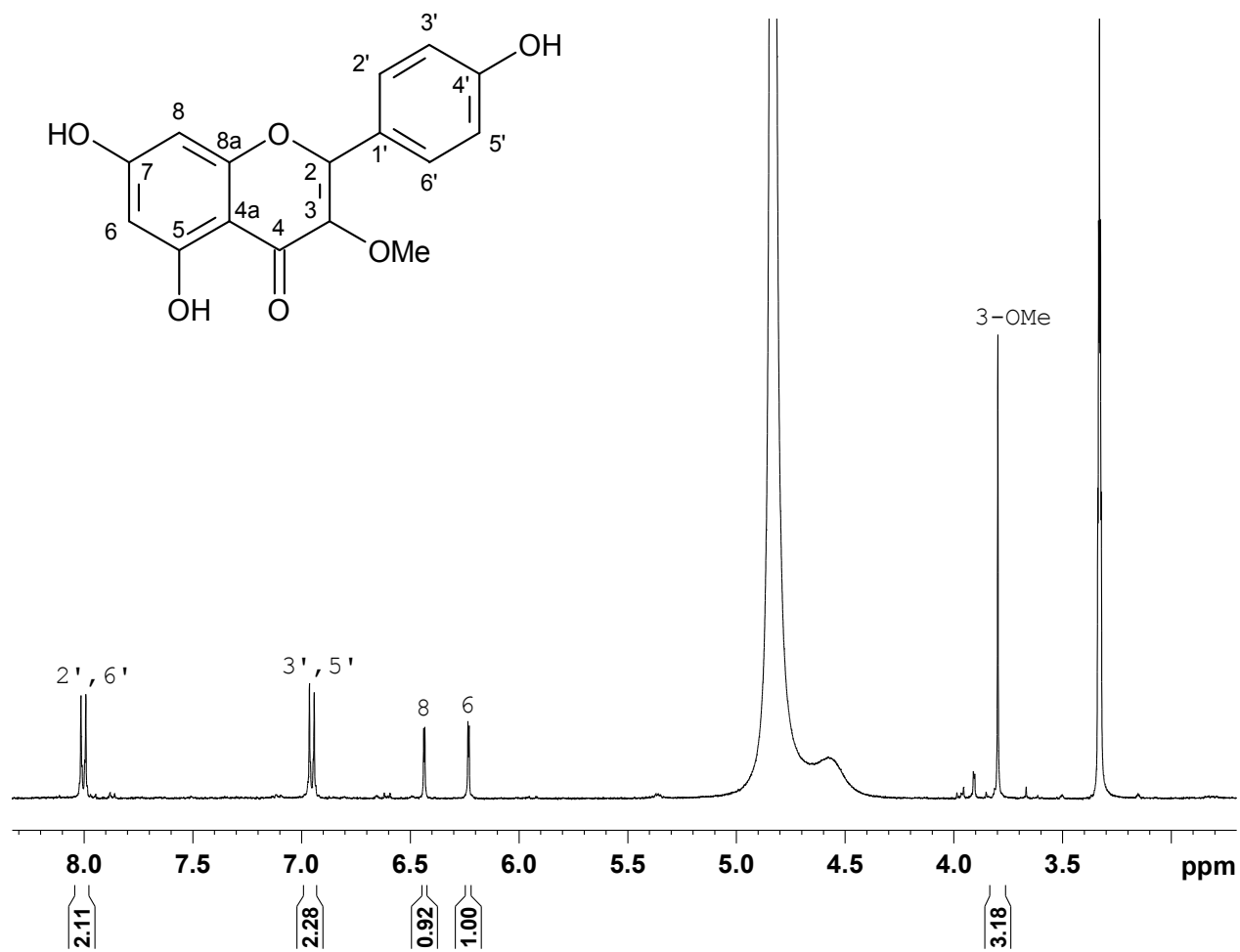
Plate 5b: ^{13}C NMR spectrum of kaempferol 3-methyl ether (2.56) in CD$_3$OD
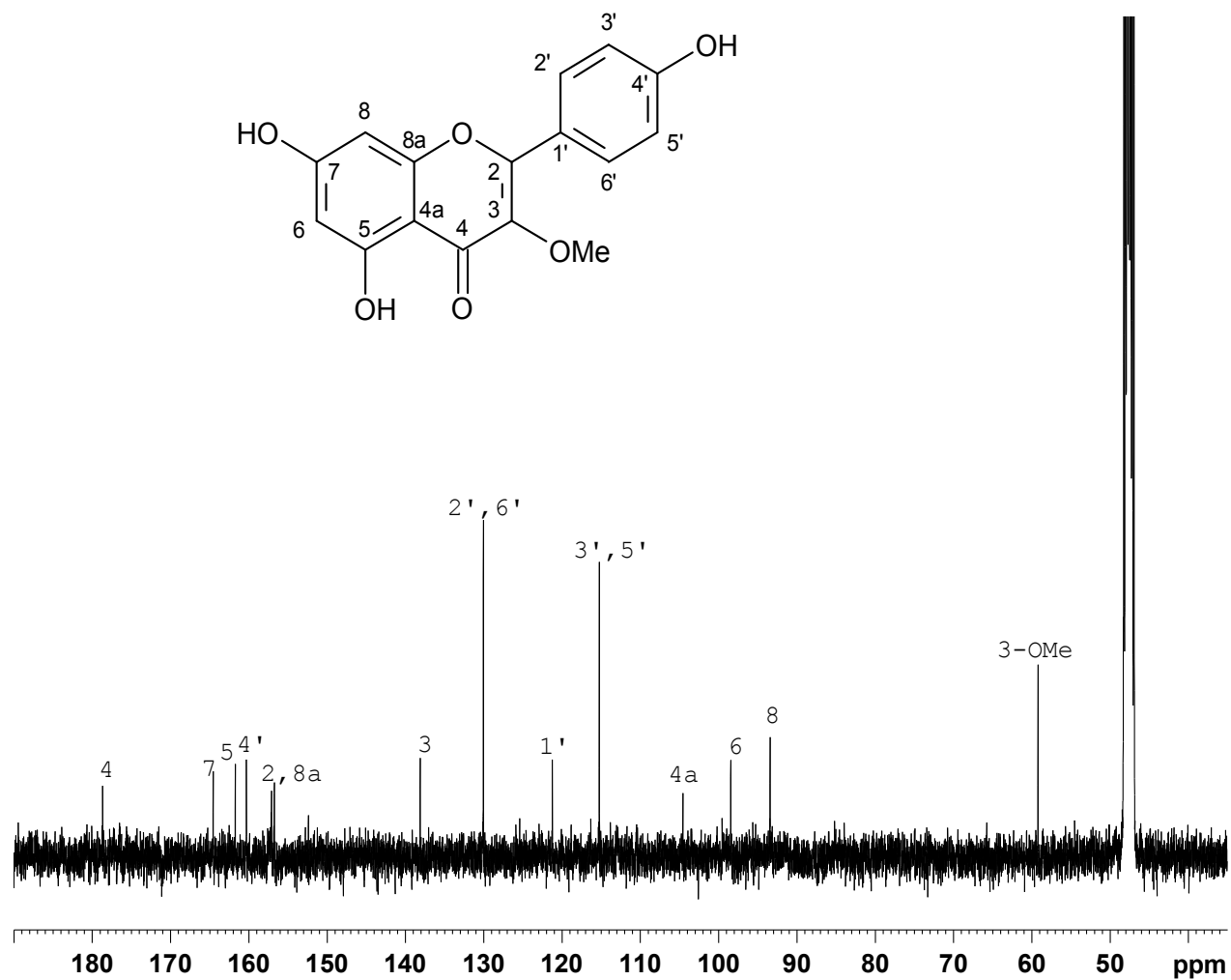
Plate 5c: COSY NMR spectrum of kaempferol 3-methyl ether (2.56) in CD₃OD

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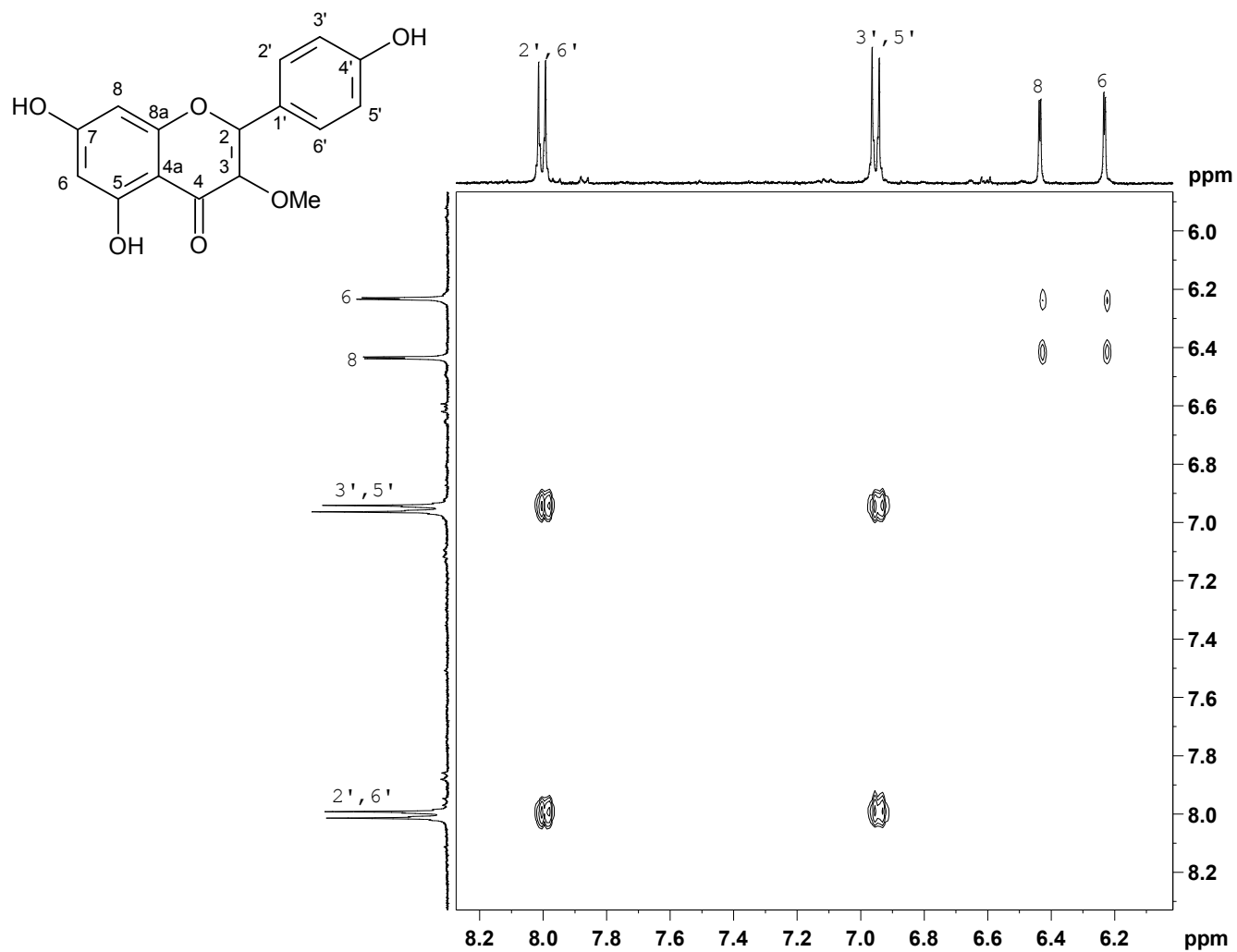
Plate 5d: DEPT 135 NMR spectrum of kaempferol 3-methyl ether (2.56) in CD₃OD
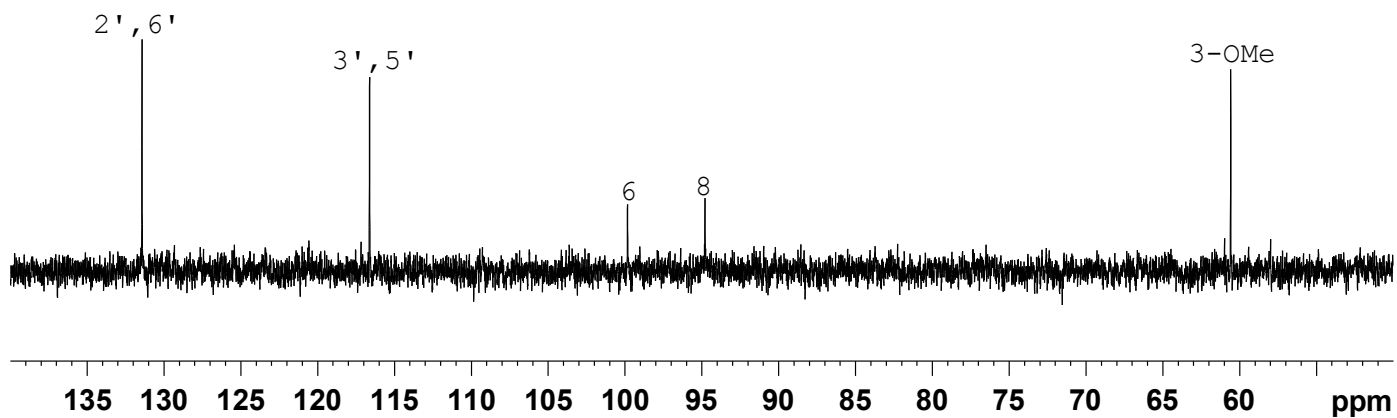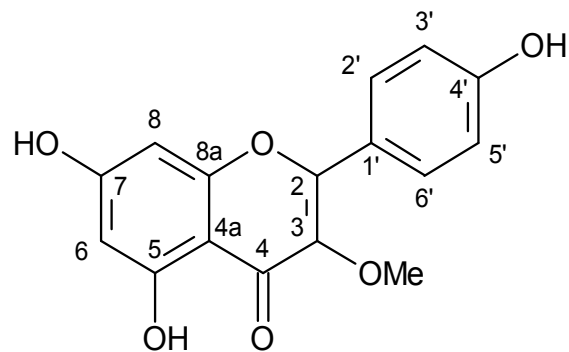
Plate 5e: HSQC NMR spectrum of kaempferol 3-methyl ether (2.56) in CD$_3$OD

199
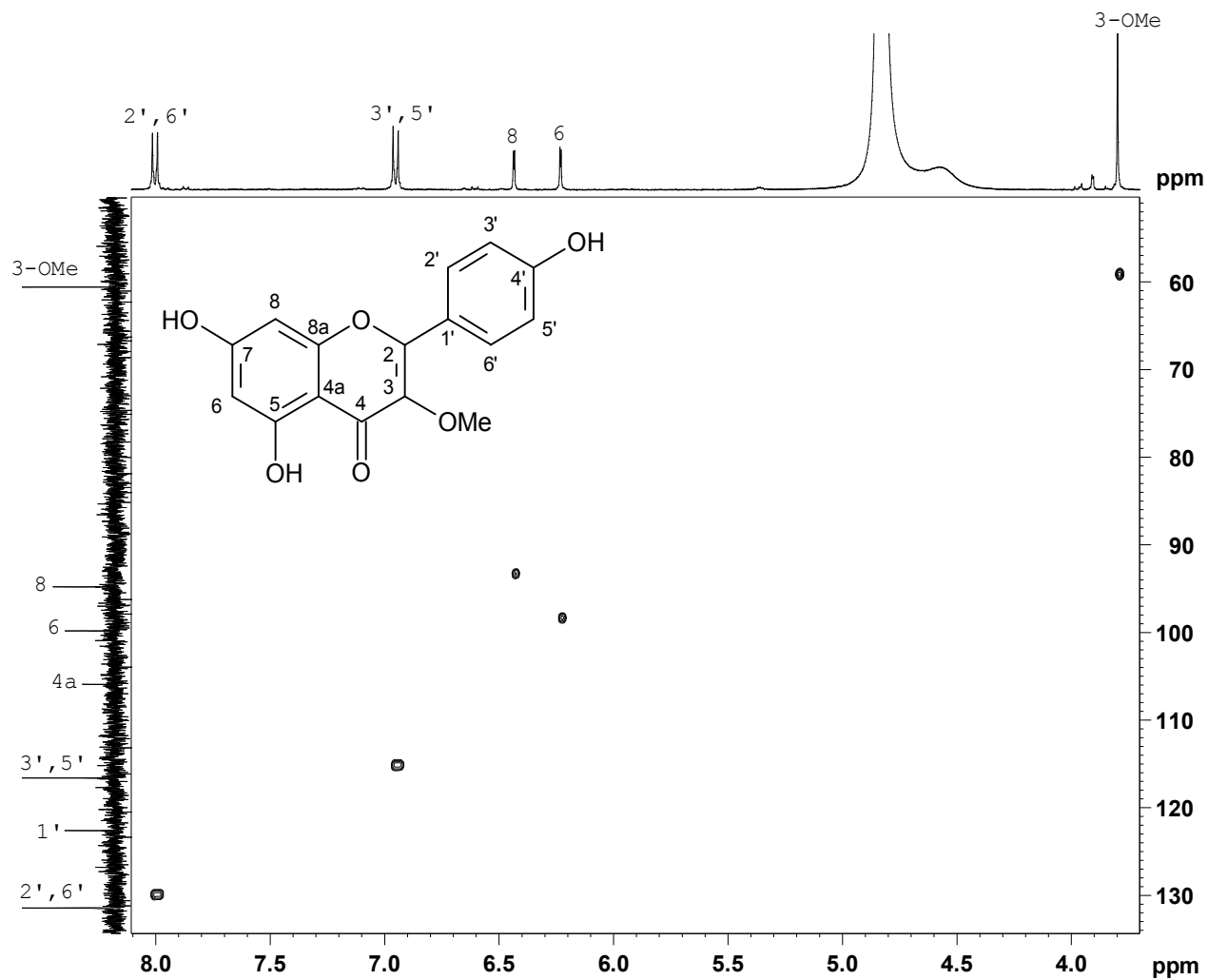
Plate 5f: HMBC NMR spectrum of kaempferol 3-methyl ether (2.56) in CD₃OD
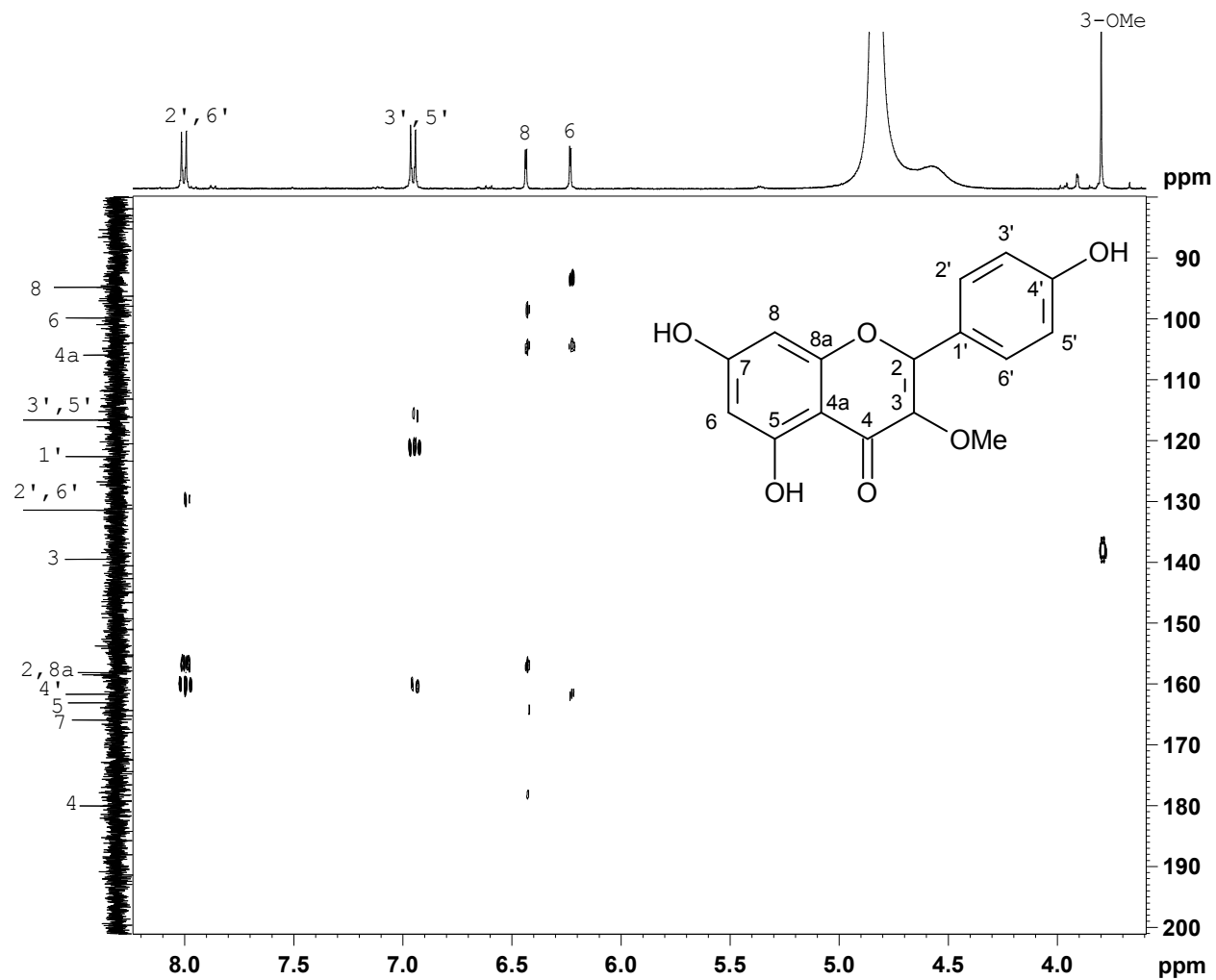
Plate 6a: ^{1}H NMR spectrum of 13-*epi*-labdanolic acid (2.57) in CDCl$_3$
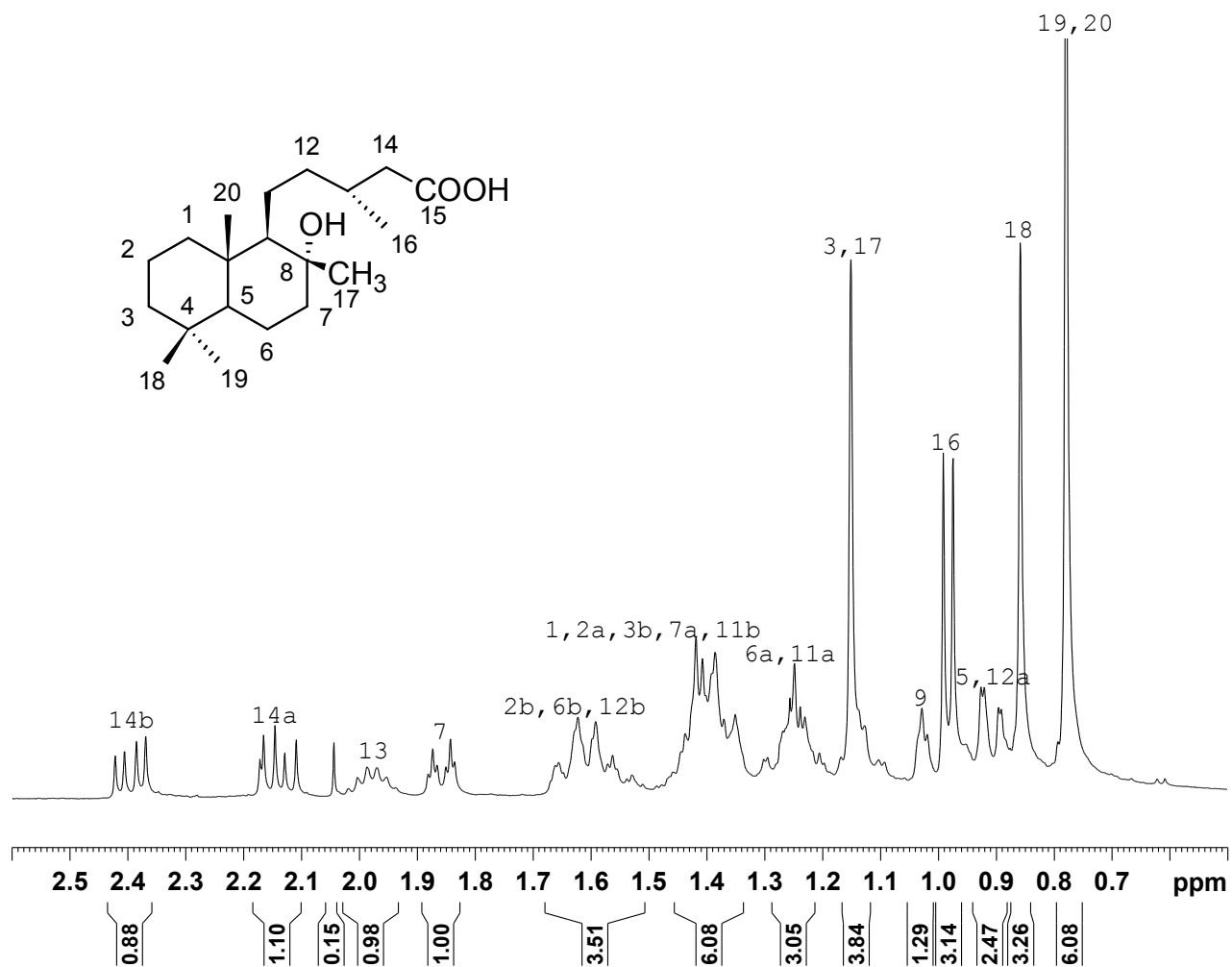
Plate 6b: ^{13}C NMR spectrum of 13-*epi*-labdanolic acid (2.57) in CDCl$_3$

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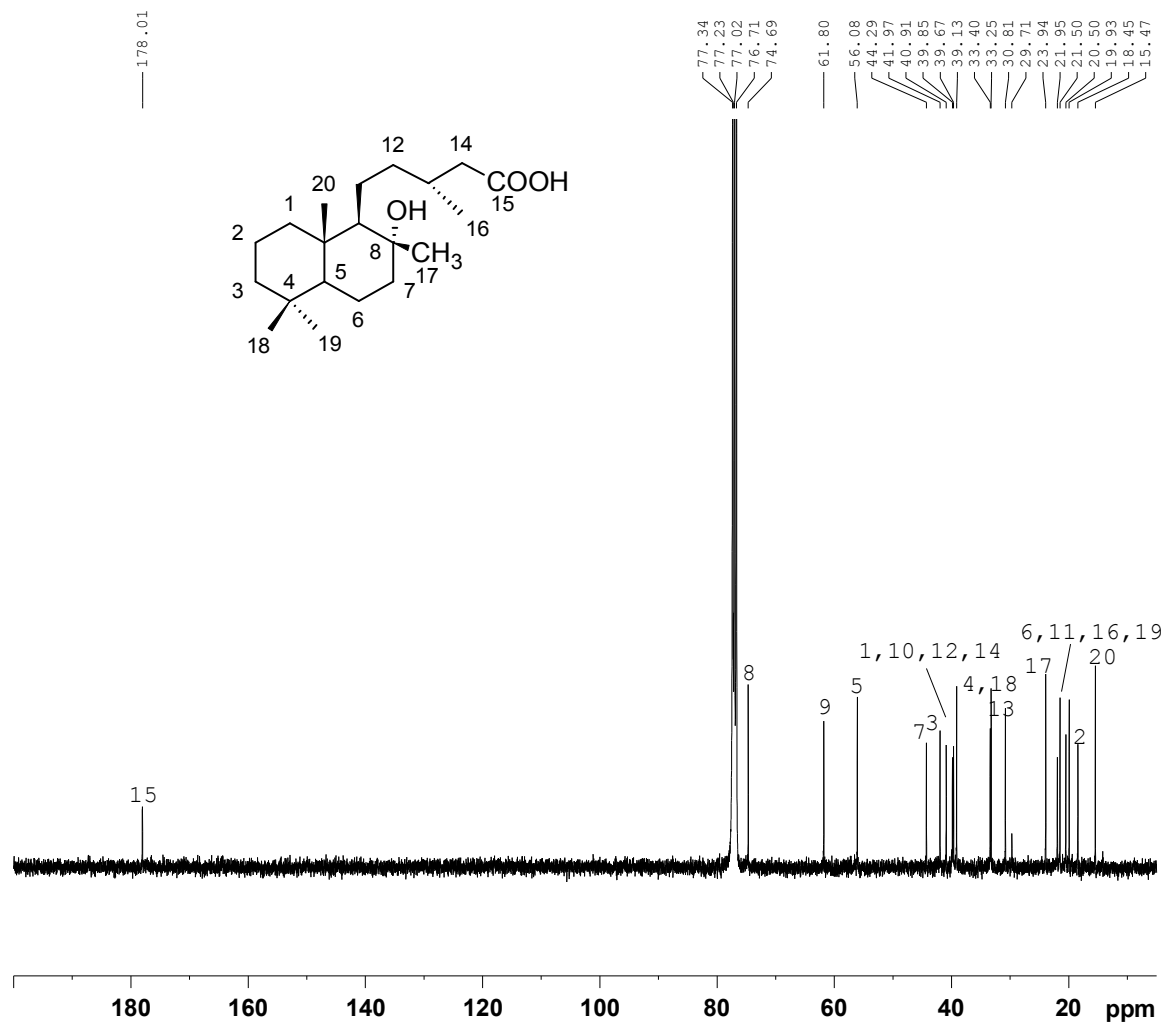
Plate 6c: COSY NMR spectrum of 13-*epi*-labdanolic acid (2.57) in CDCl₃
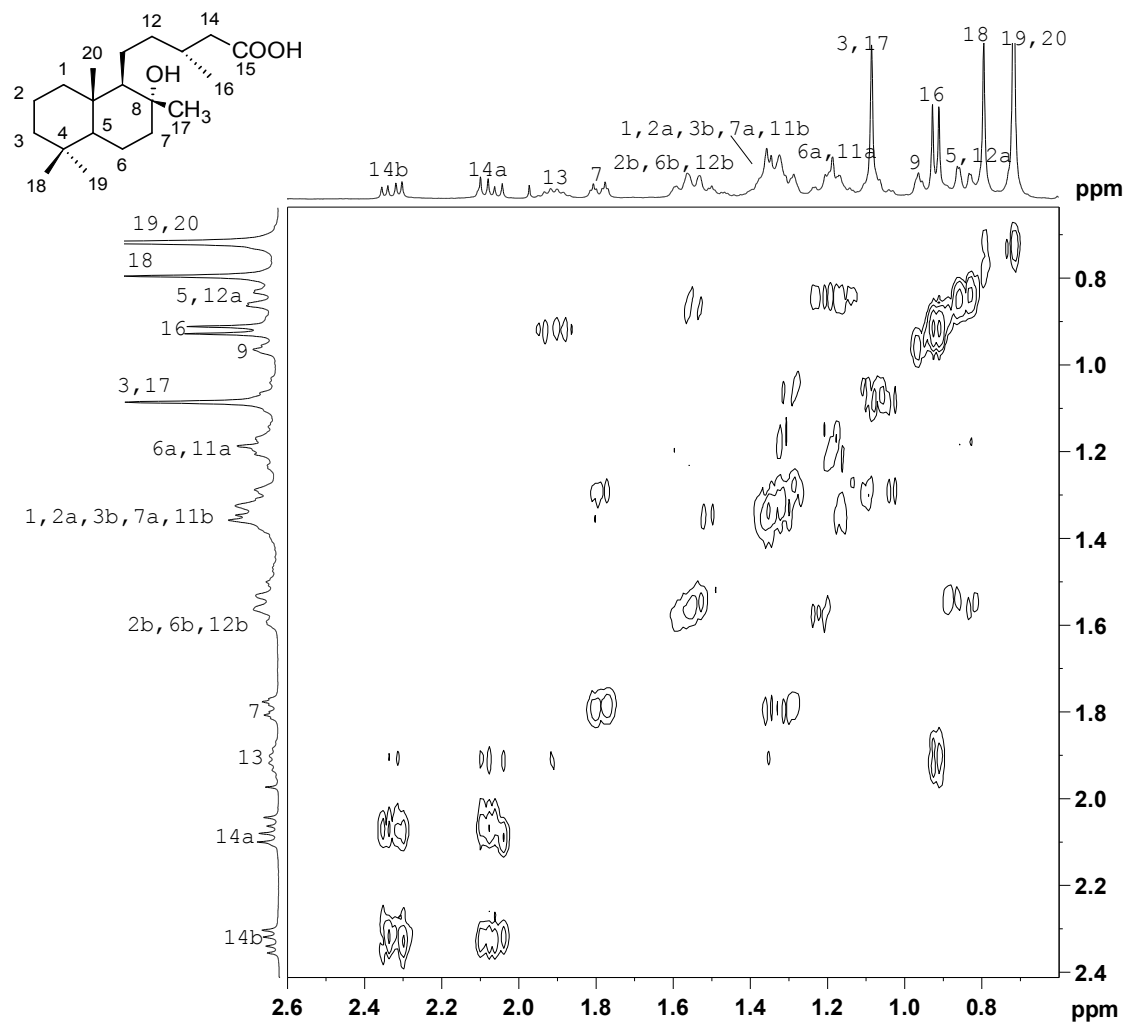
Plate 6d: DEPT 135 NMR spectrum of 13-*epi*-labdanolic acid (2.57) in CDCl₃
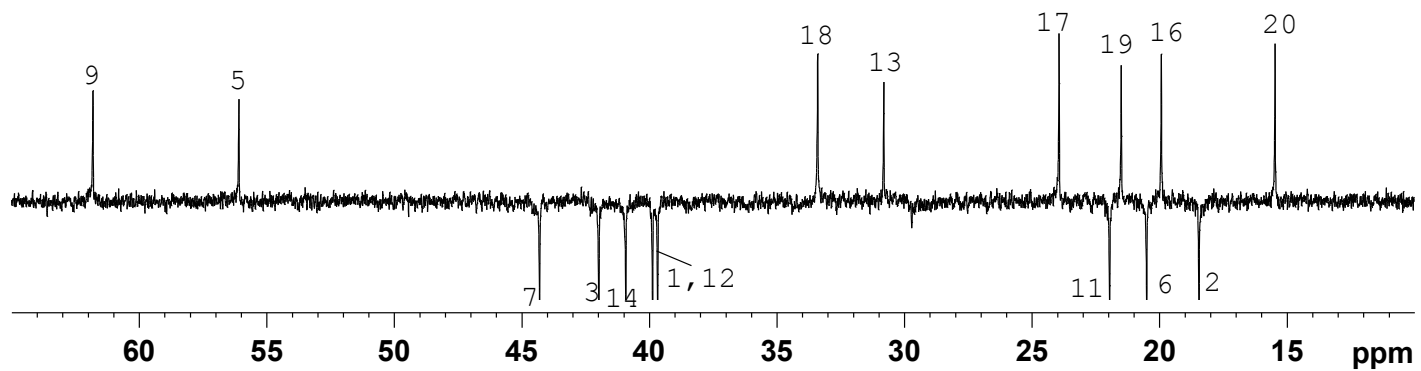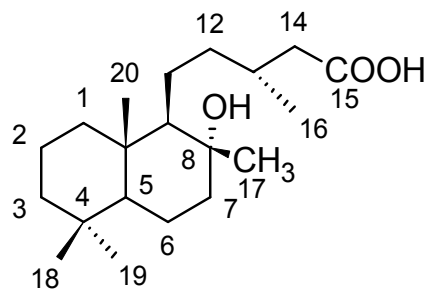
Plate 6e: HSQC NMR spectrum of 13-*epi*-labdanolic acid (2.57) in CDCl₃
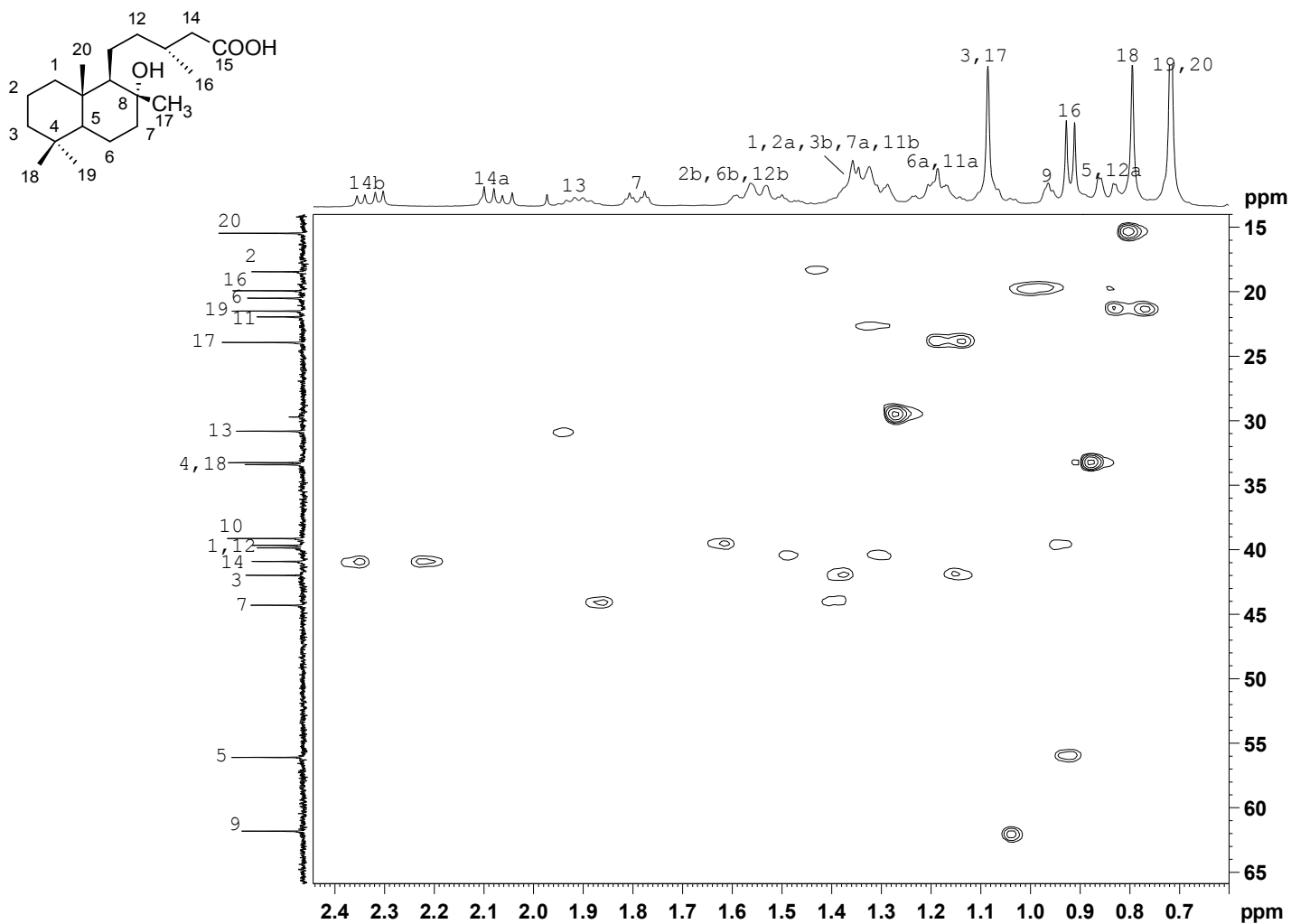
Plate 6f: HMBC NMR spectrum of 13-*epi*-labdanolic acid (2.57) in CDCl₃
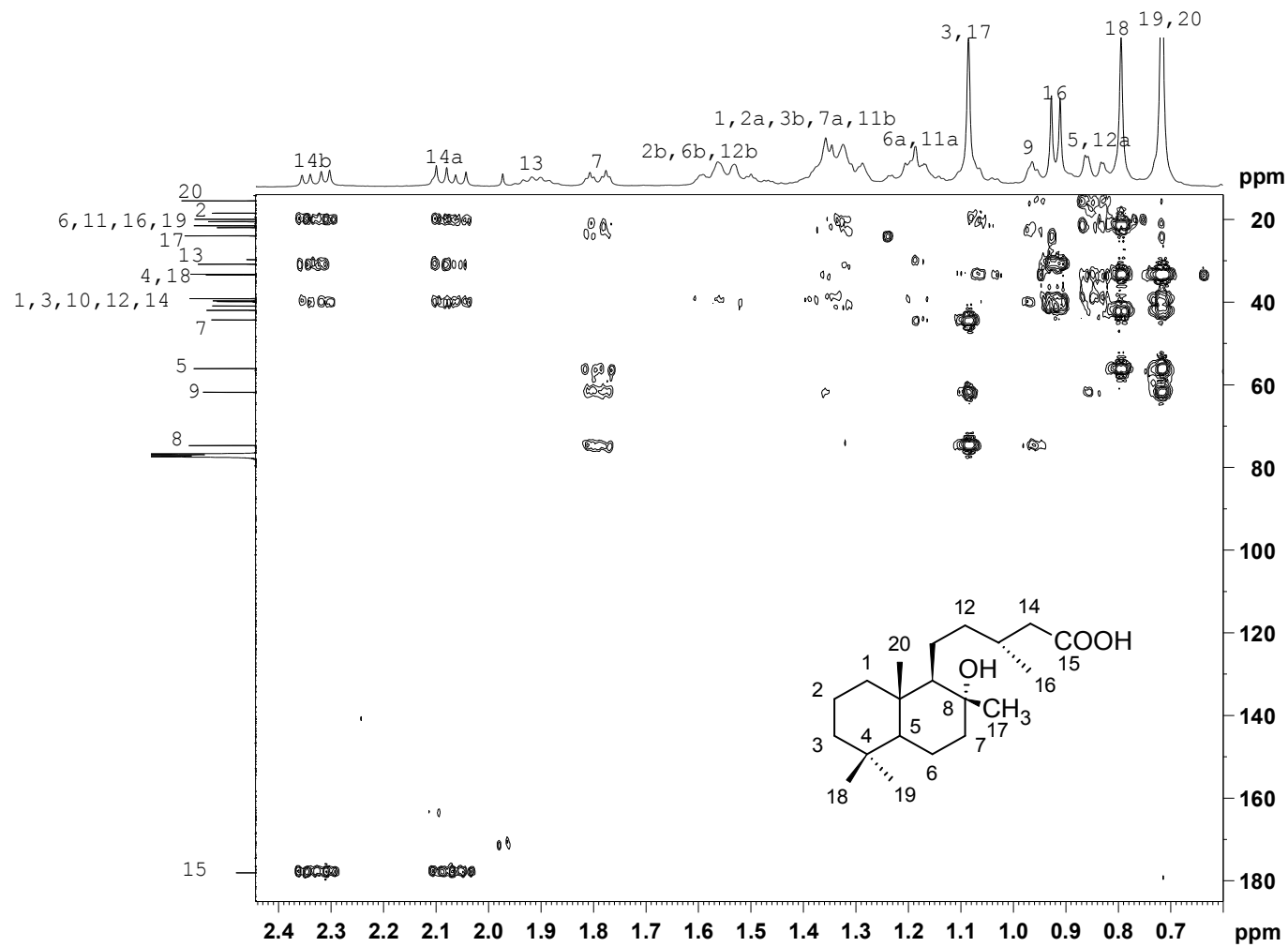
Plate 6g: NOESY NMR spectrum of 13-*epi*-labdanolic acid (2.57) in CDCl₃

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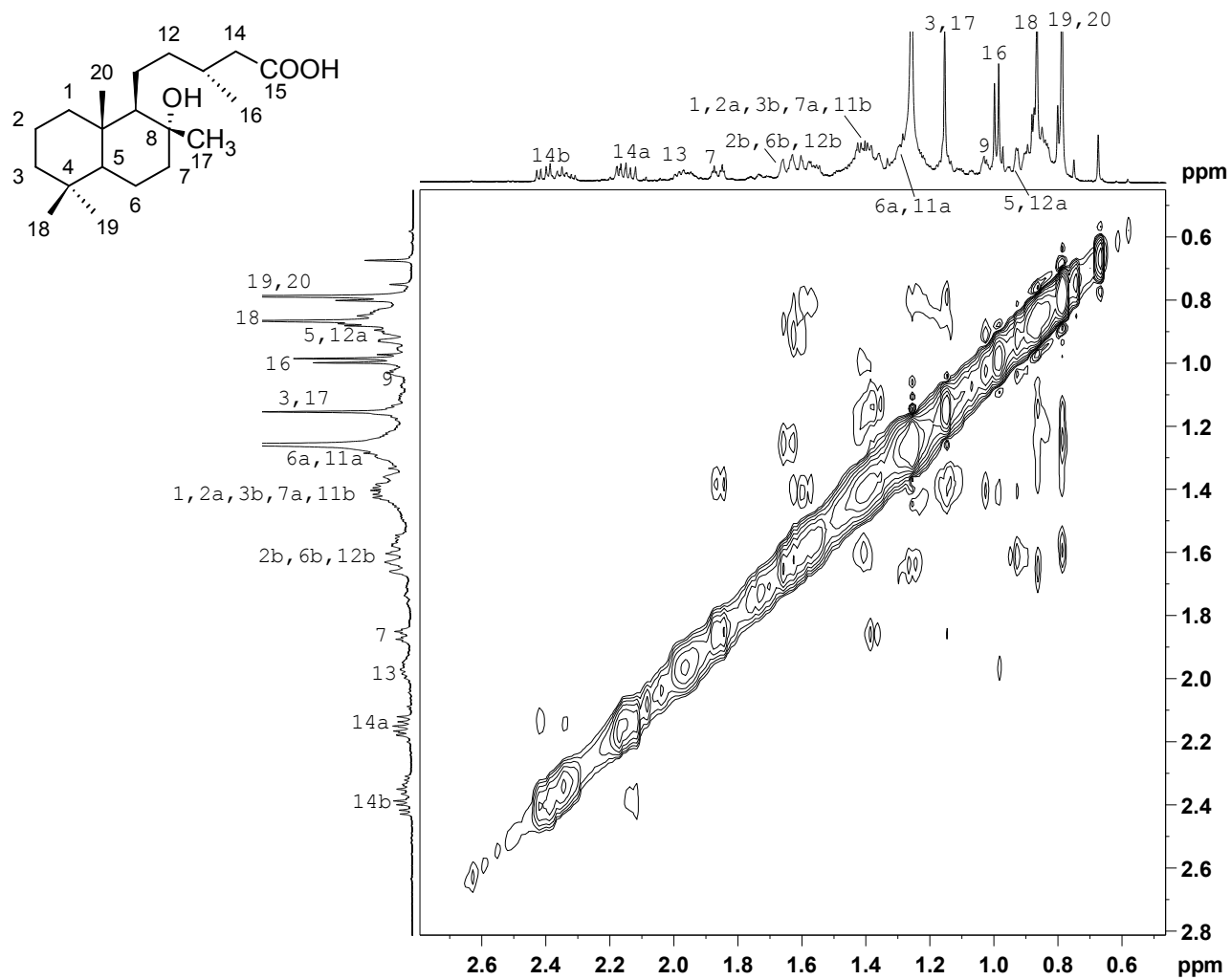
Plate 7a: ^{1}H NMR spectrum of *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61) in CDCl$_3$
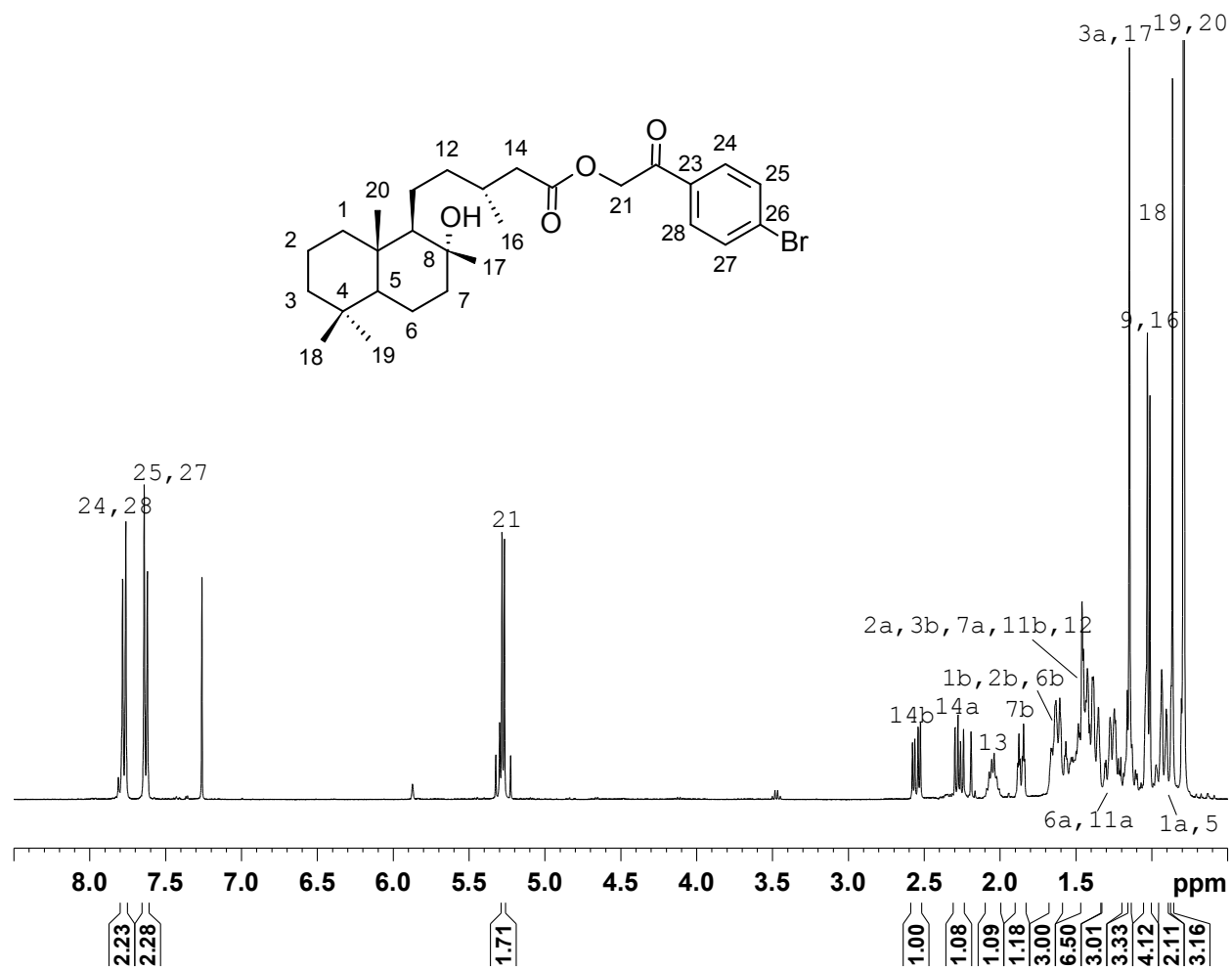
Plate 7b: ^{13}C NMR spectrum of *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61) in CDCl$_3$
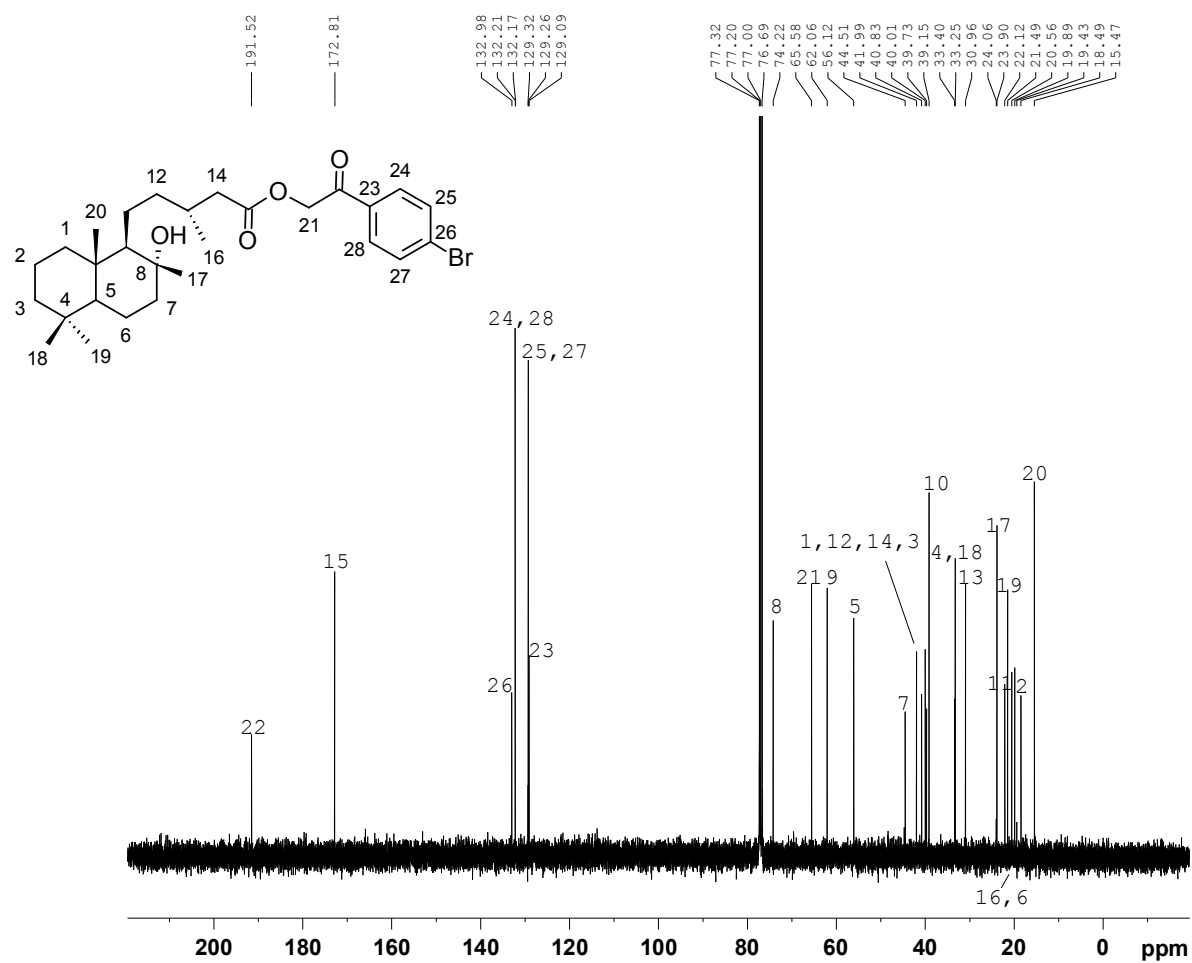
Plate 7c: COSY NMR spectrum of *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61) in CDCl₃
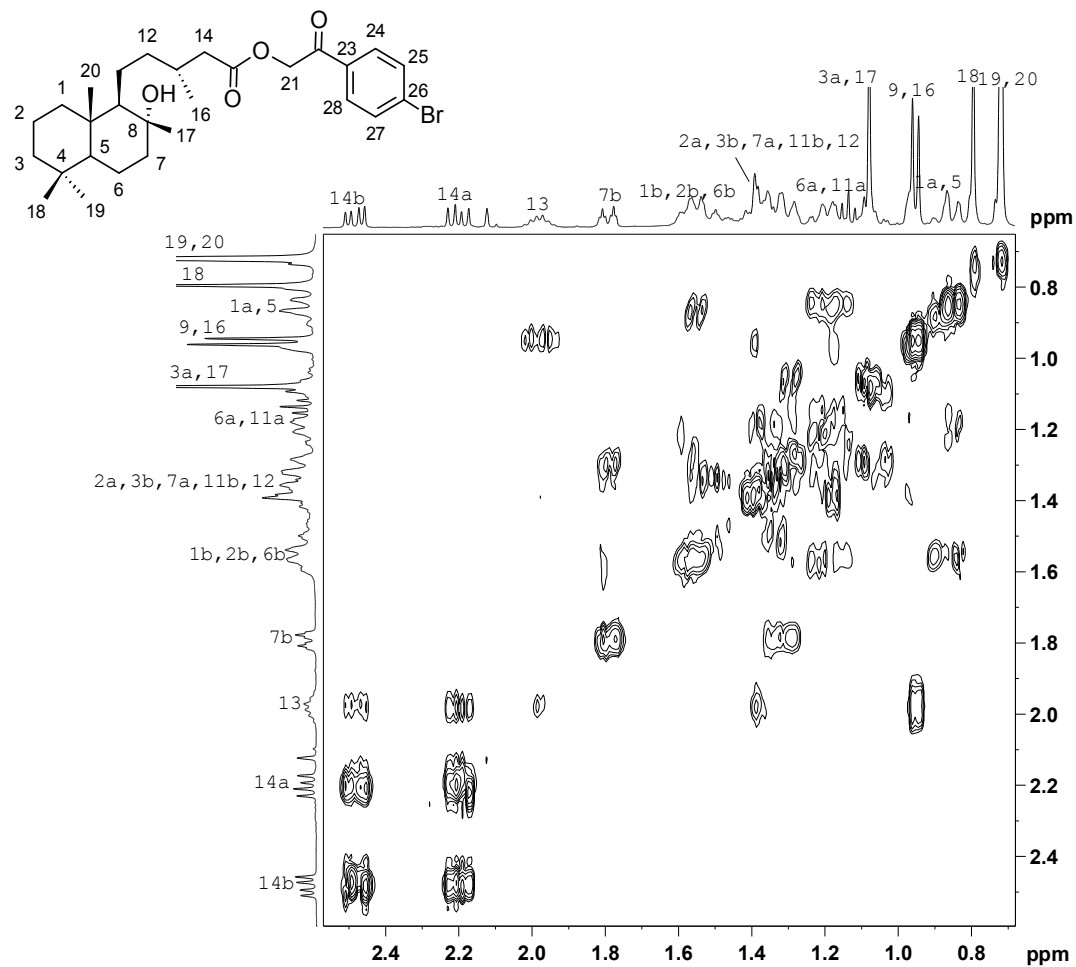
Plate 7d: DEPT 135 NMR spectrum of *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61) in CDCl₃
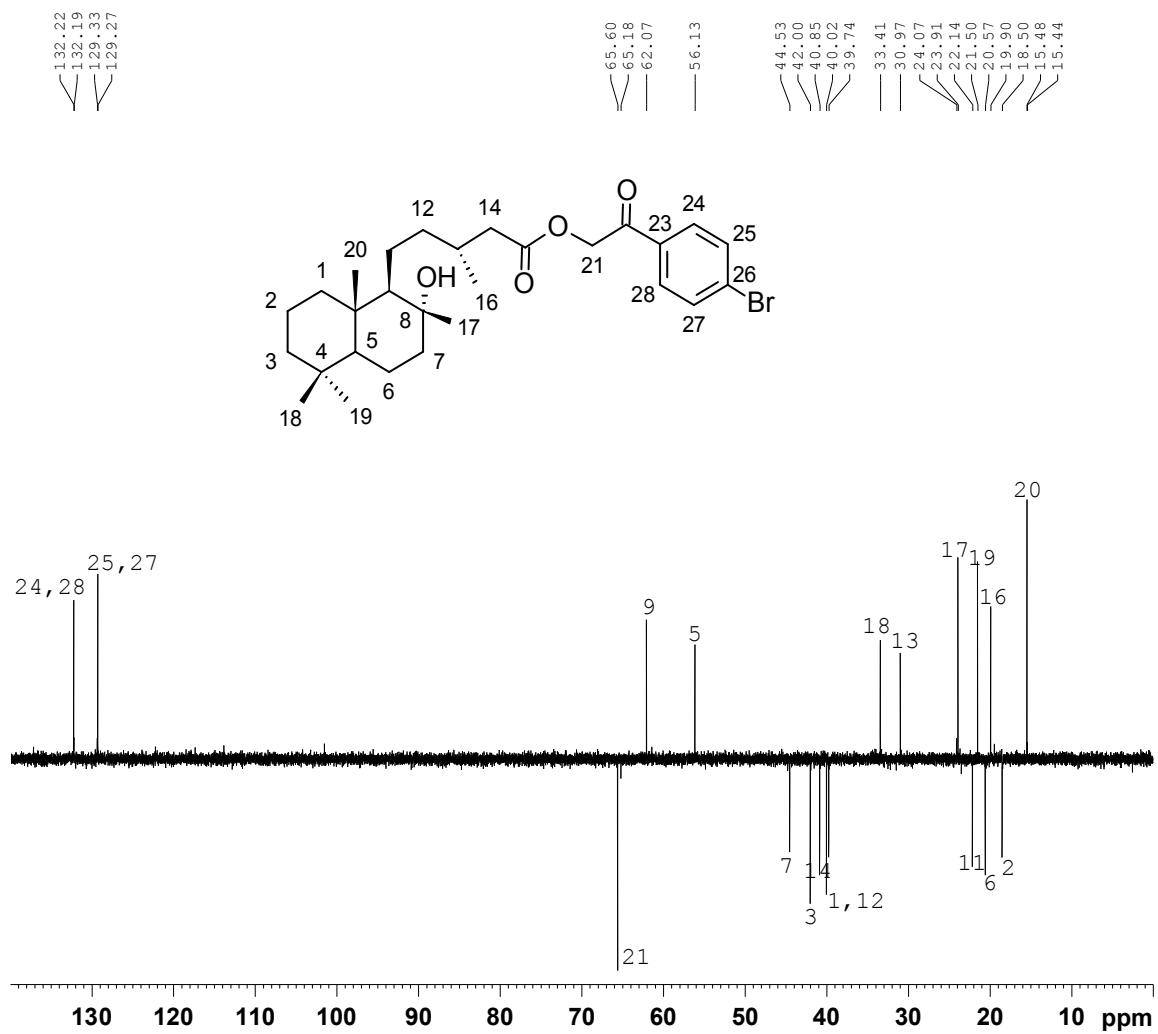
Plate 7e: HSQC NMR spectrum of *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61) in CDCl₃

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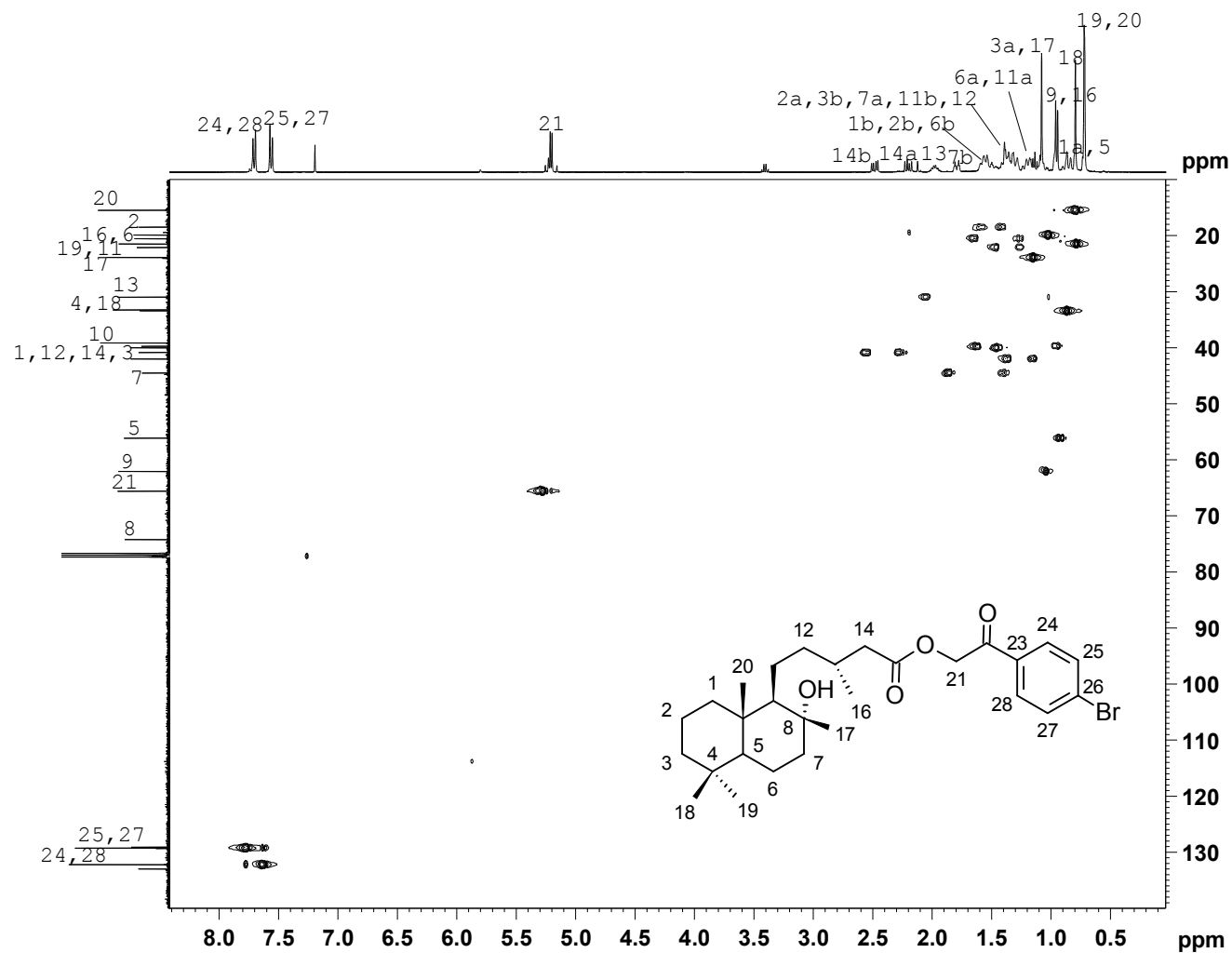
Plate 7f: HMBC NMR spectrum of *p*-bromophenacyl ester of (+)-13-*epi*-labdanolic acid (2.61) in CDCl₃
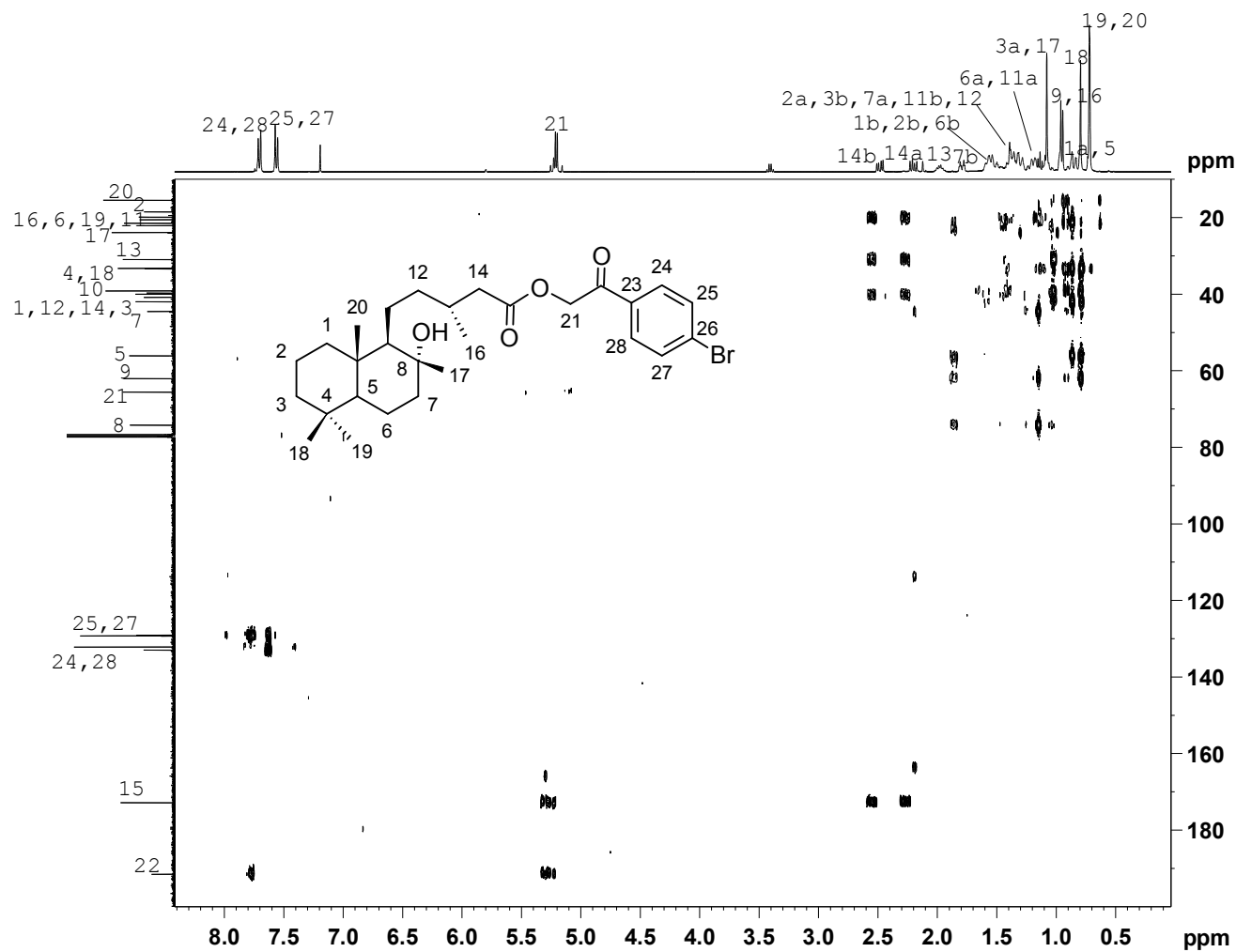
Plate 8a: ^{1}H NMR spectrum of (+)-labdanolic acid (2.62) in CDCl$_3$
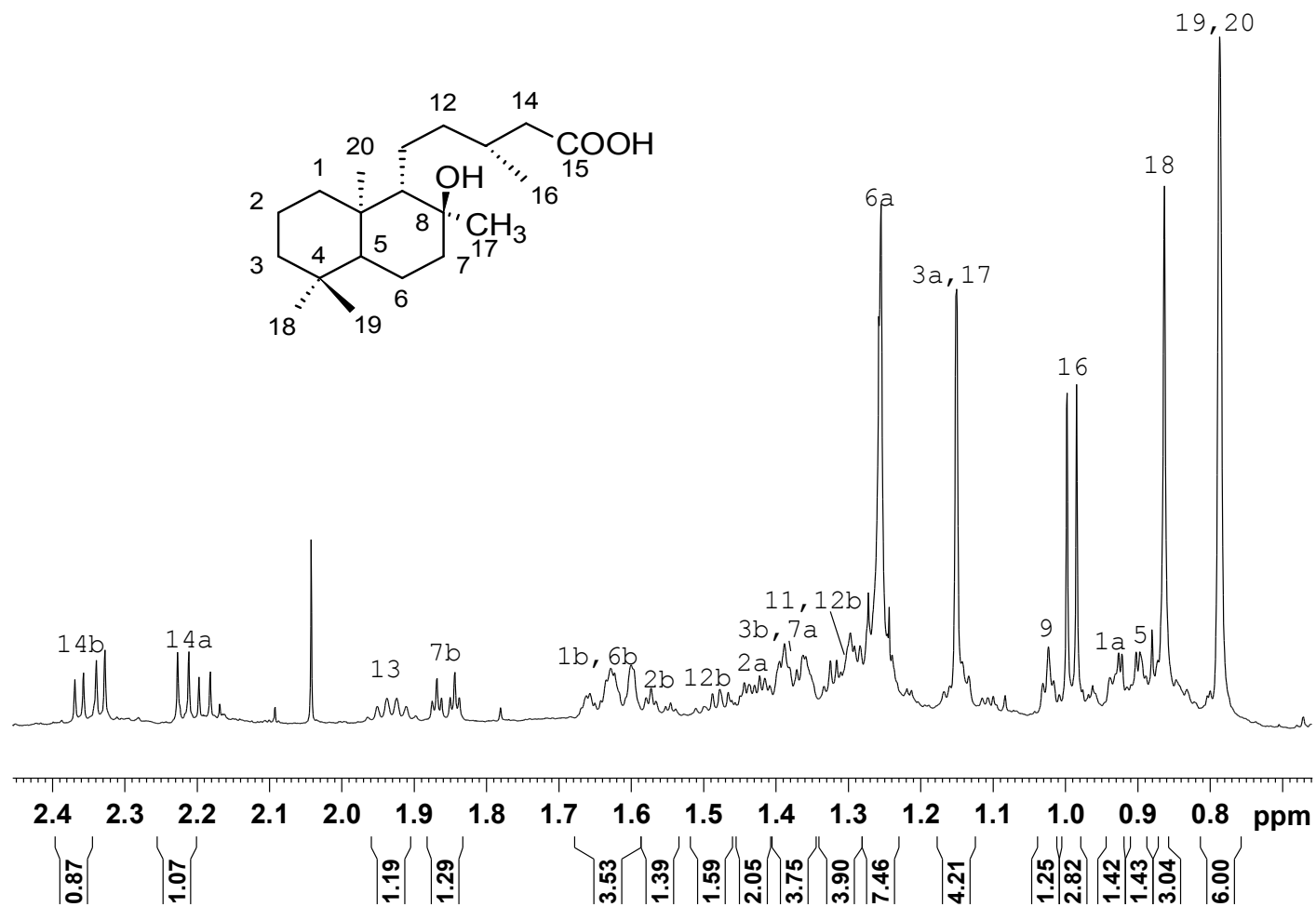
Plate 8b: ^{13}C NMR spectrum of (+)-labdanolic acid (2.62) in CDCl$_3$

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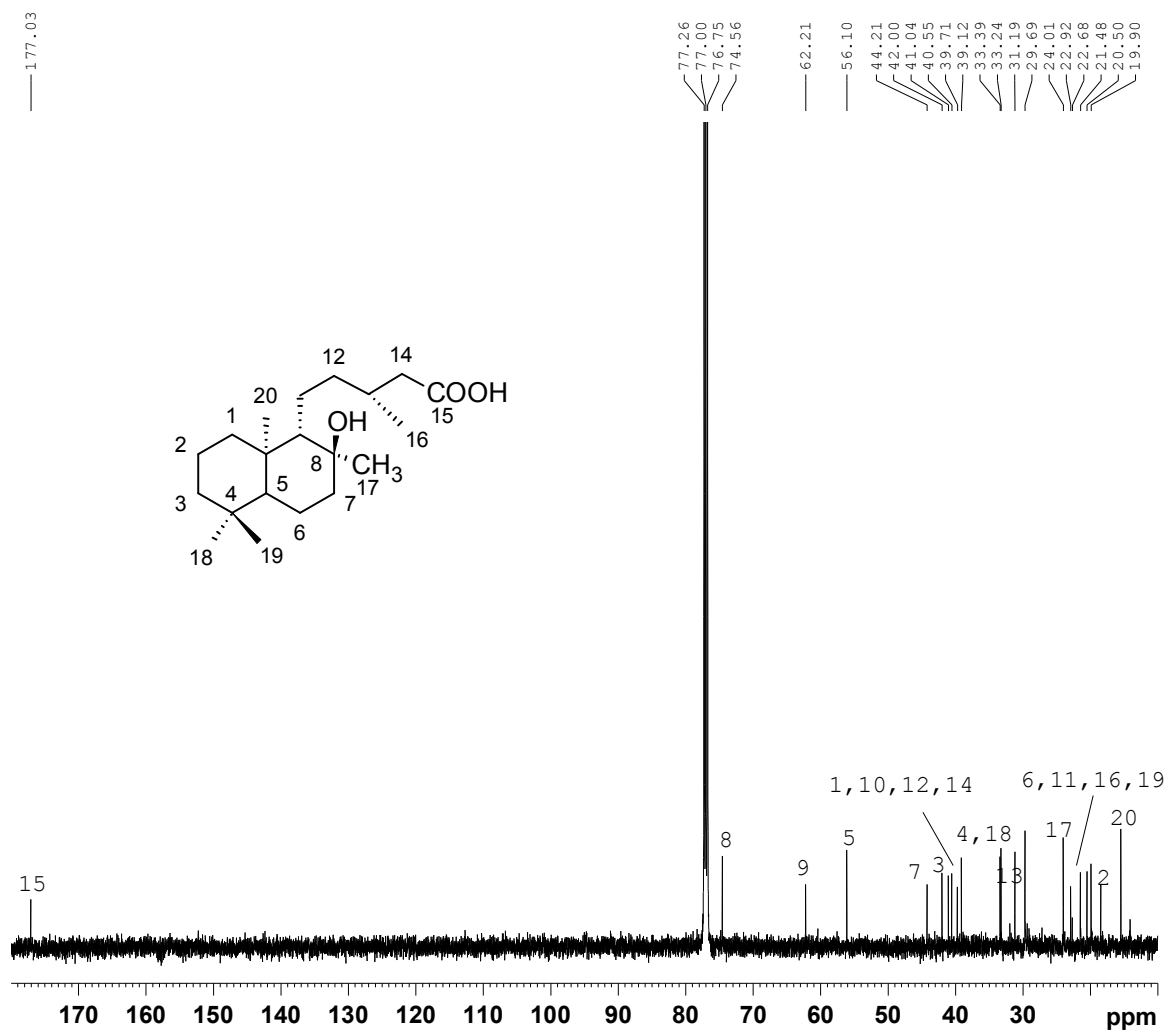
Plate 8c: DEPT 135 NMR spectrum of (+)-labdanolic acid (2.62) in CDCl₃
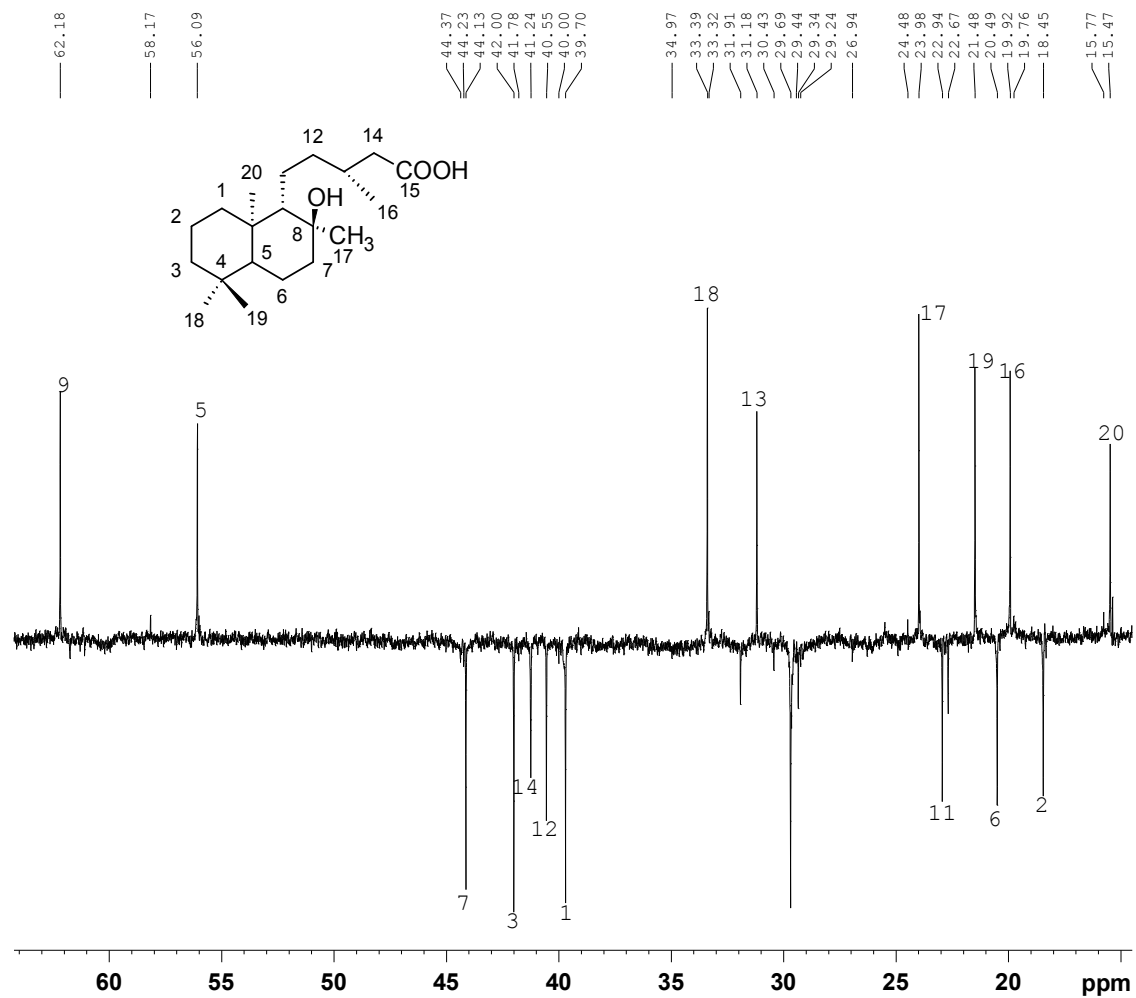
Plate 8d: HSQC NMR spectrum of (+)-labdanolic acid (2.62) in CDCl₃
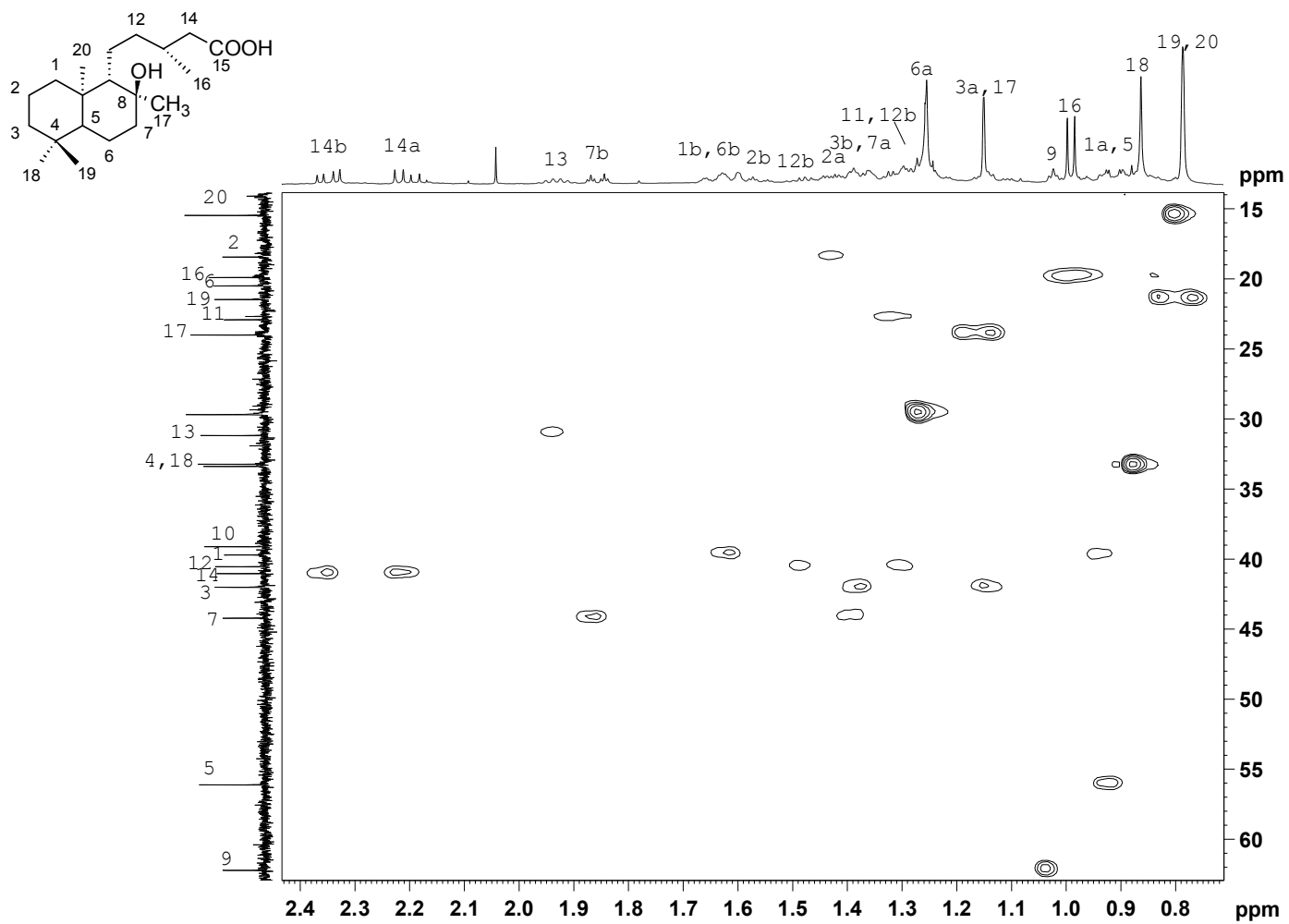
Plate 8e: HMBC NMR spectrum of (+)-labdanolic acid (2.62) in CDCl₃
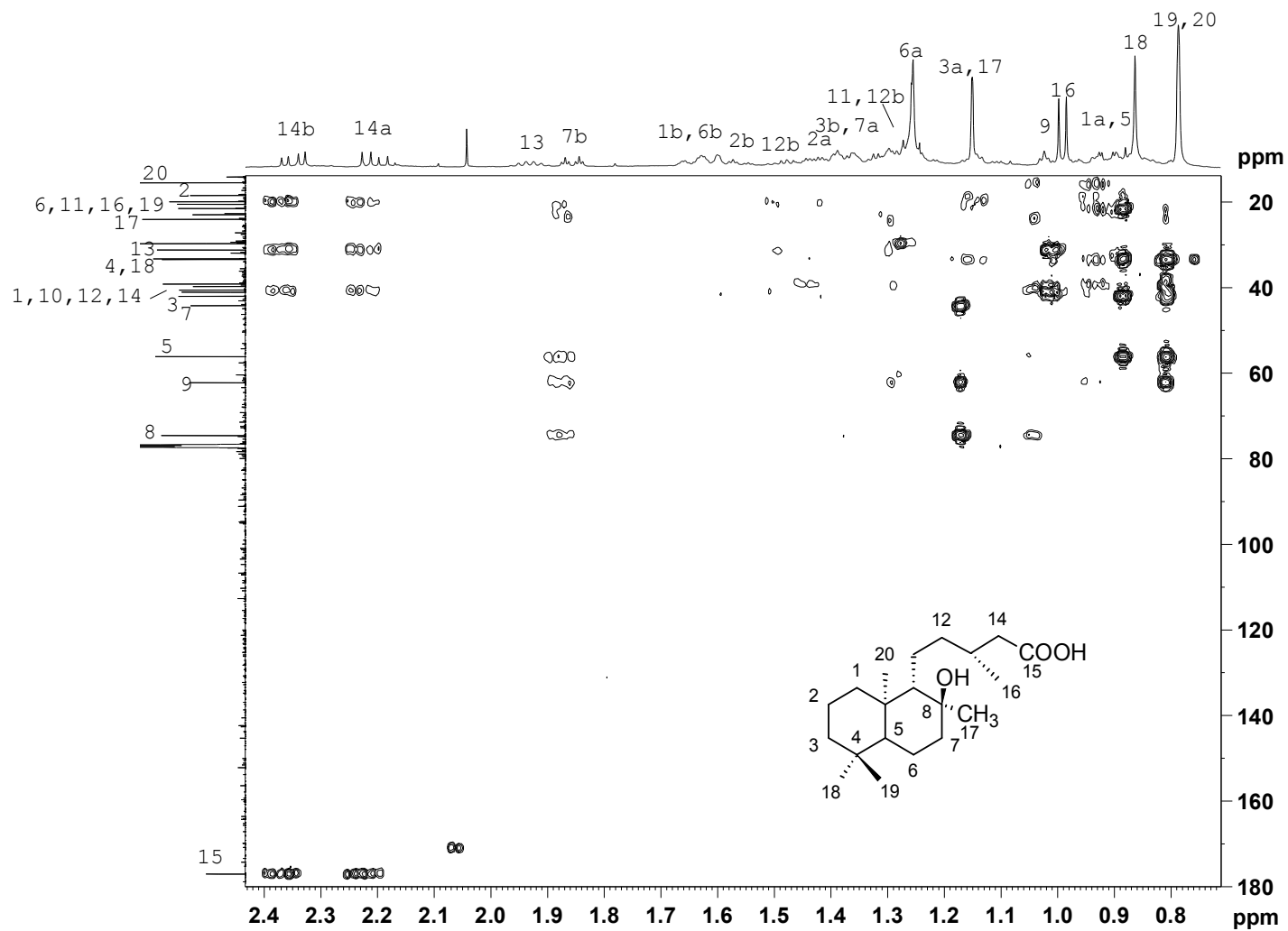
Plate 8f: NOESY NMR spectrum of (+)-labdanolic acid (2.62) in CDCl₃

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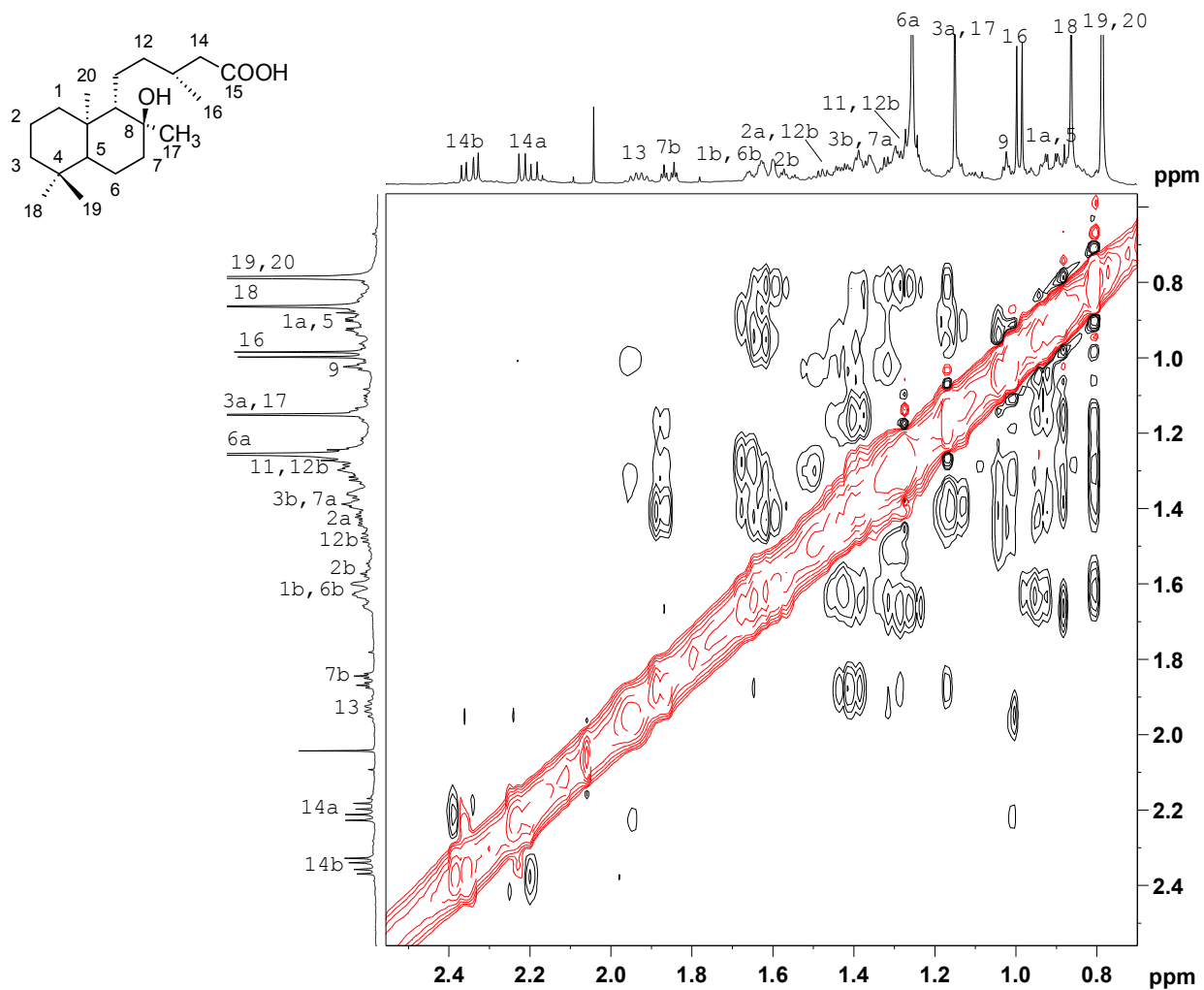
Plate 9a: ¹H NMR spectrum of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63) in CDCl₃
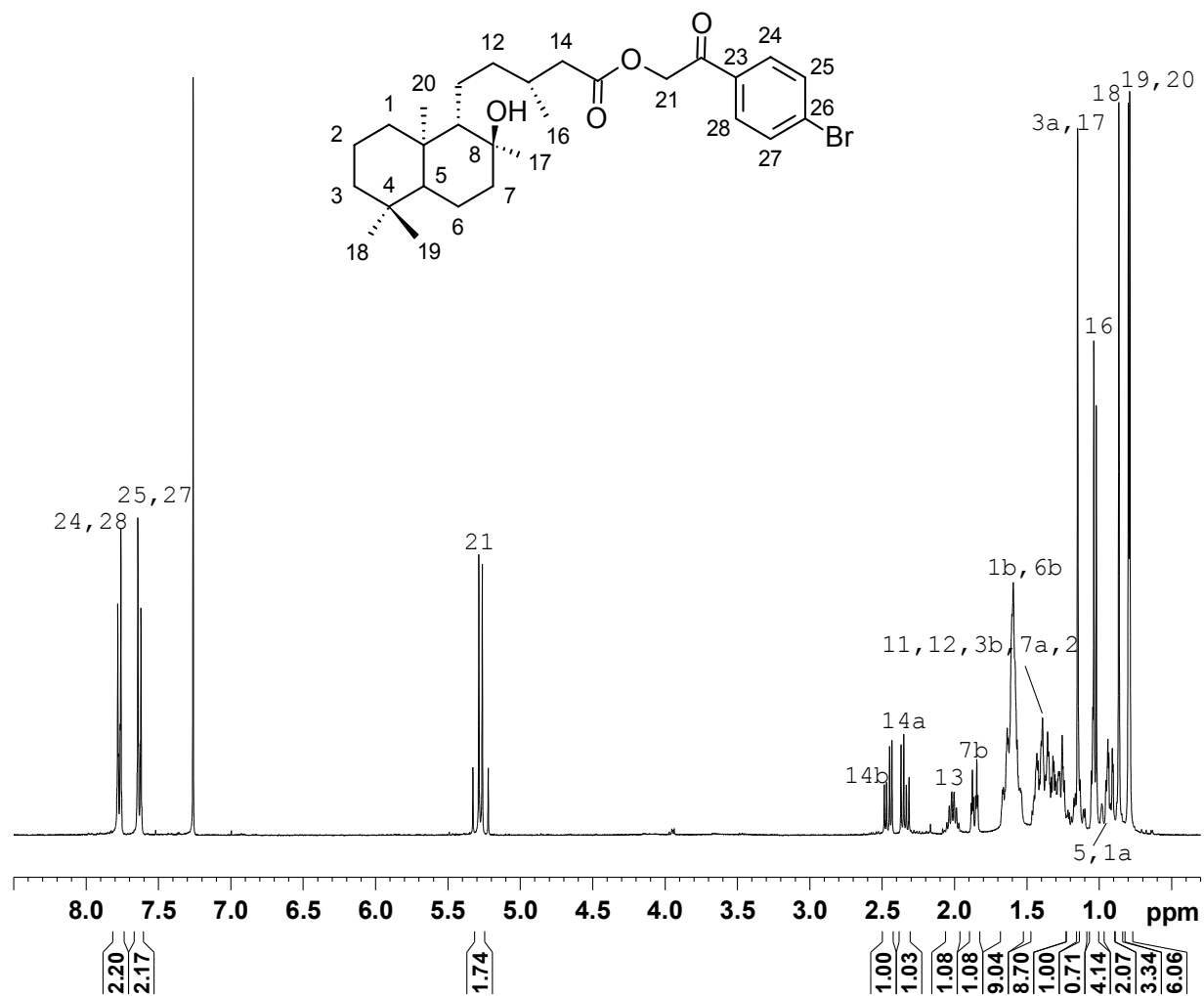
Plate 9b: ^{13}C NMR spectrum of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63) in CDCl$_3$

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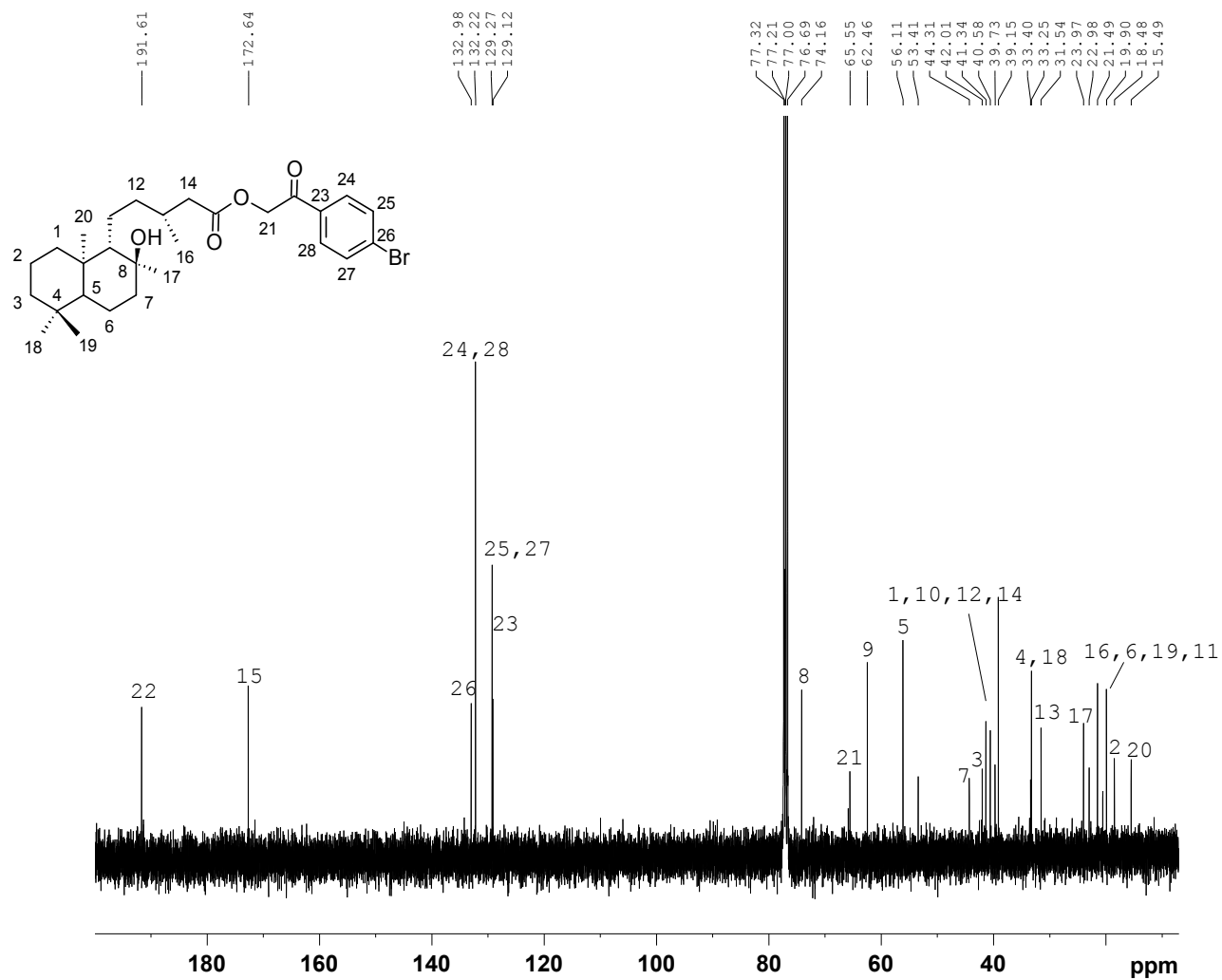
Plate 9c: COSY NMR spectrum of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63) in CDCl₃
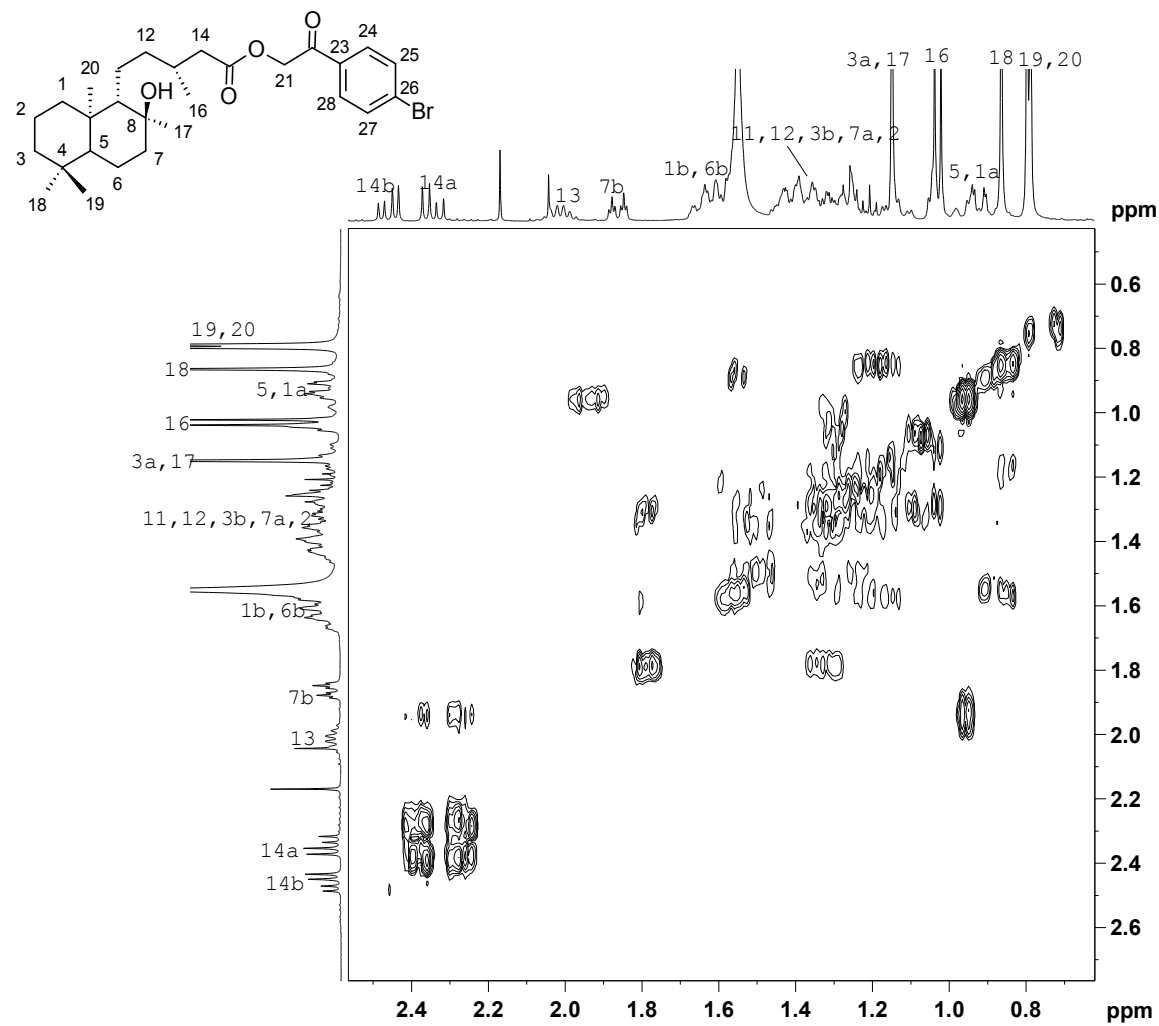
Plate 9d: DEPT 135 NMR spectrum of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63) in CDCl₃
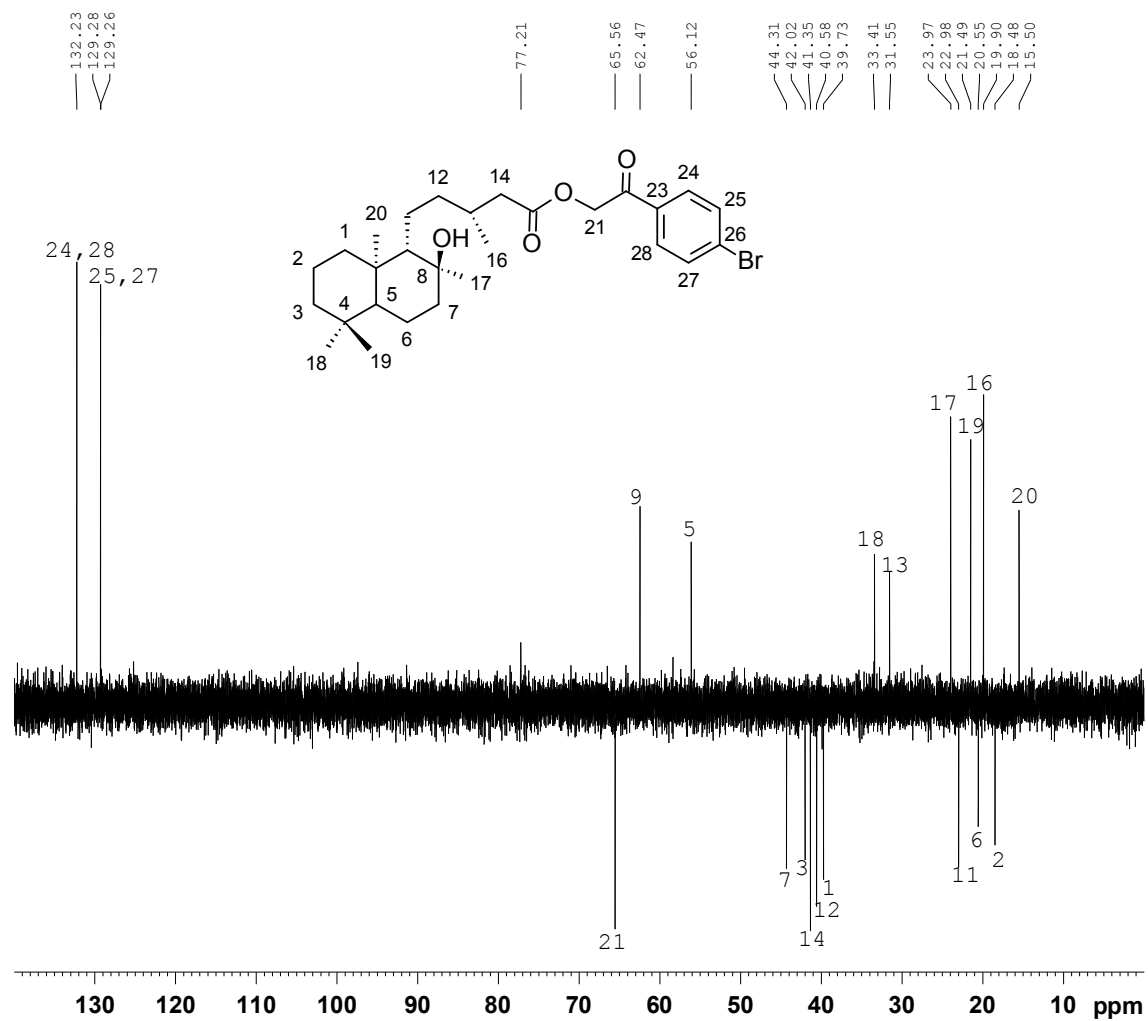
Plate 9e: HSQC NMR spectrum of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63) in CDCl₃
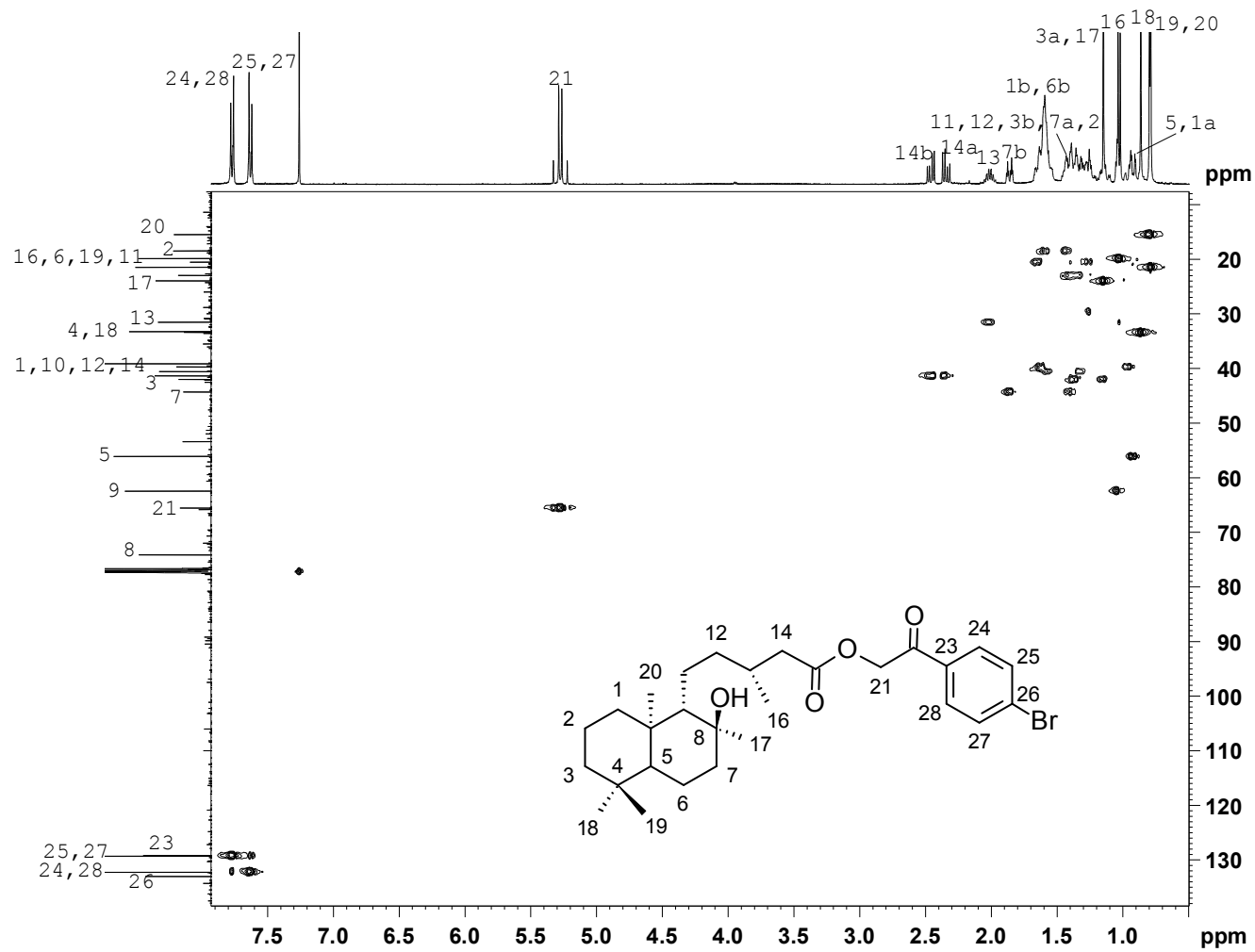
Plate 9F: HMBC NMR spectrum of *p*-bromophenacyl ester of (+)-labdanolic acid (2.63) in CDCl₃
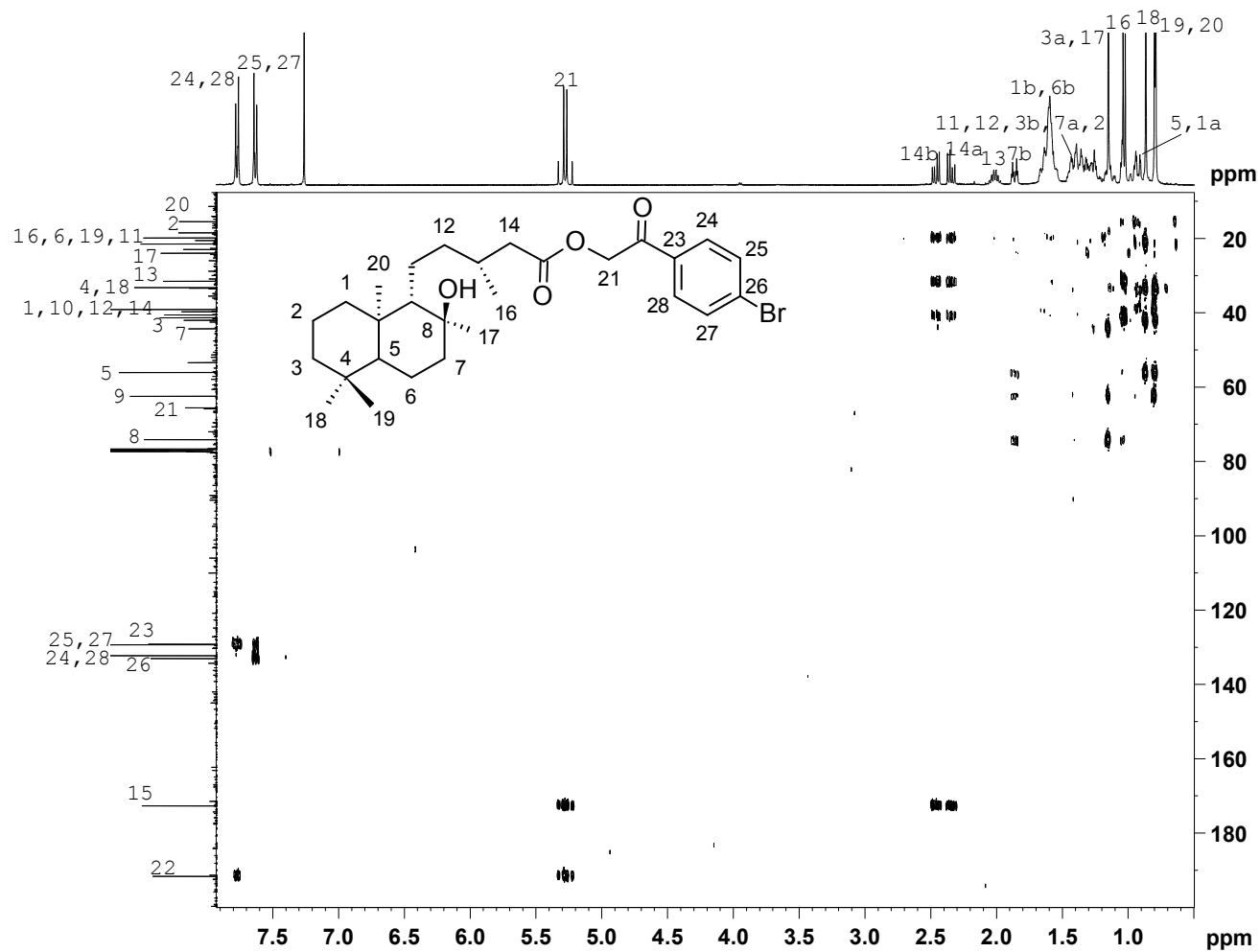
Plate 10a: ¹H NMR spectrum of (+)-labdanolic acid methyl ester (2.64) in CDCl₃
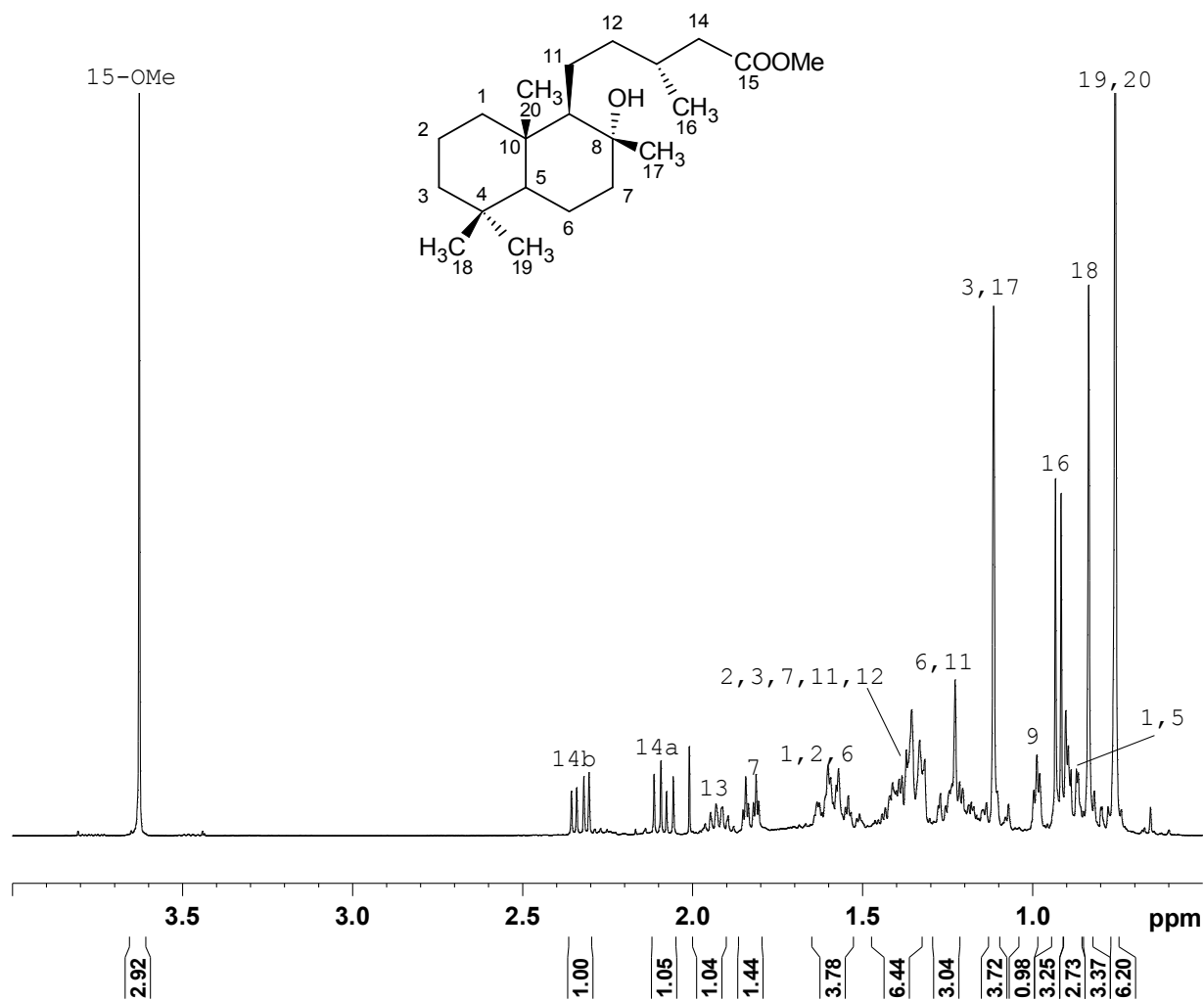
Plate 10b: ^{13}C NMR spectrum of (+)-labdanolic acid methyl ester (2.64) in CDCl$_3$

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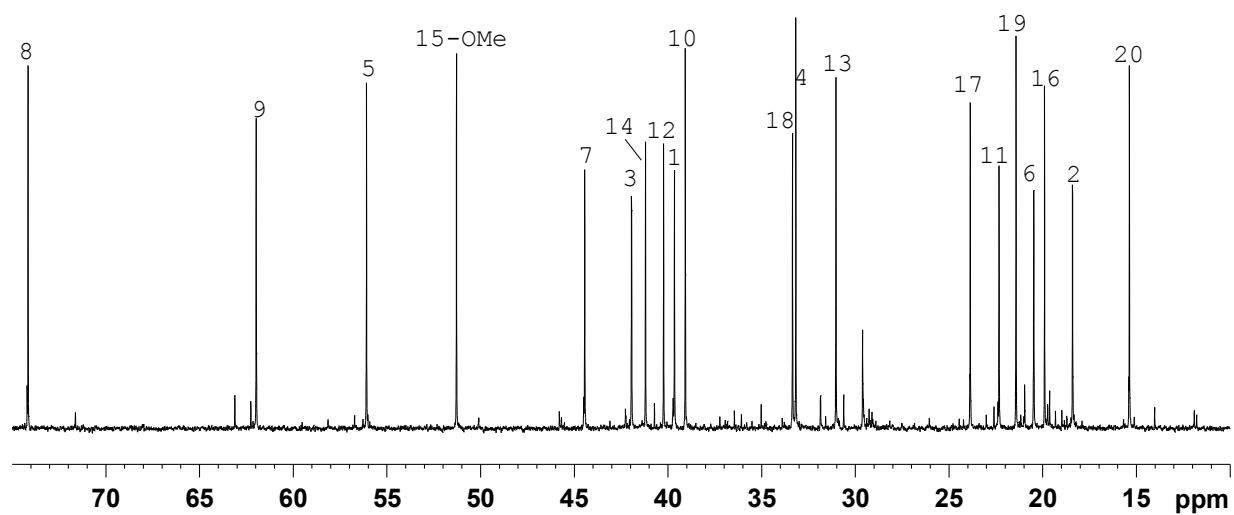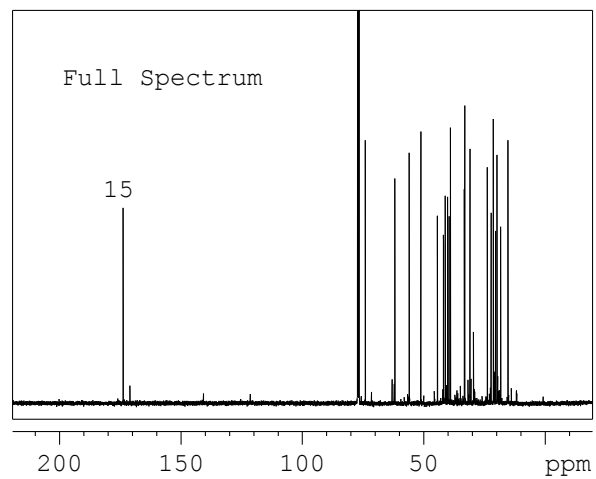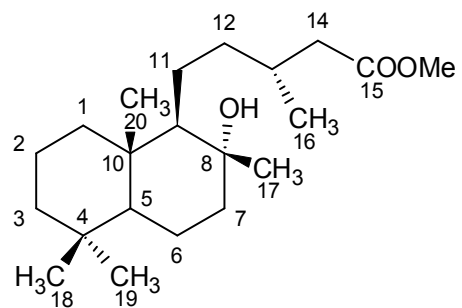
Plate 10c: COSY NMR spectrum of (+)-labdanolic acid methyl ester (2.64) in CDCl₃
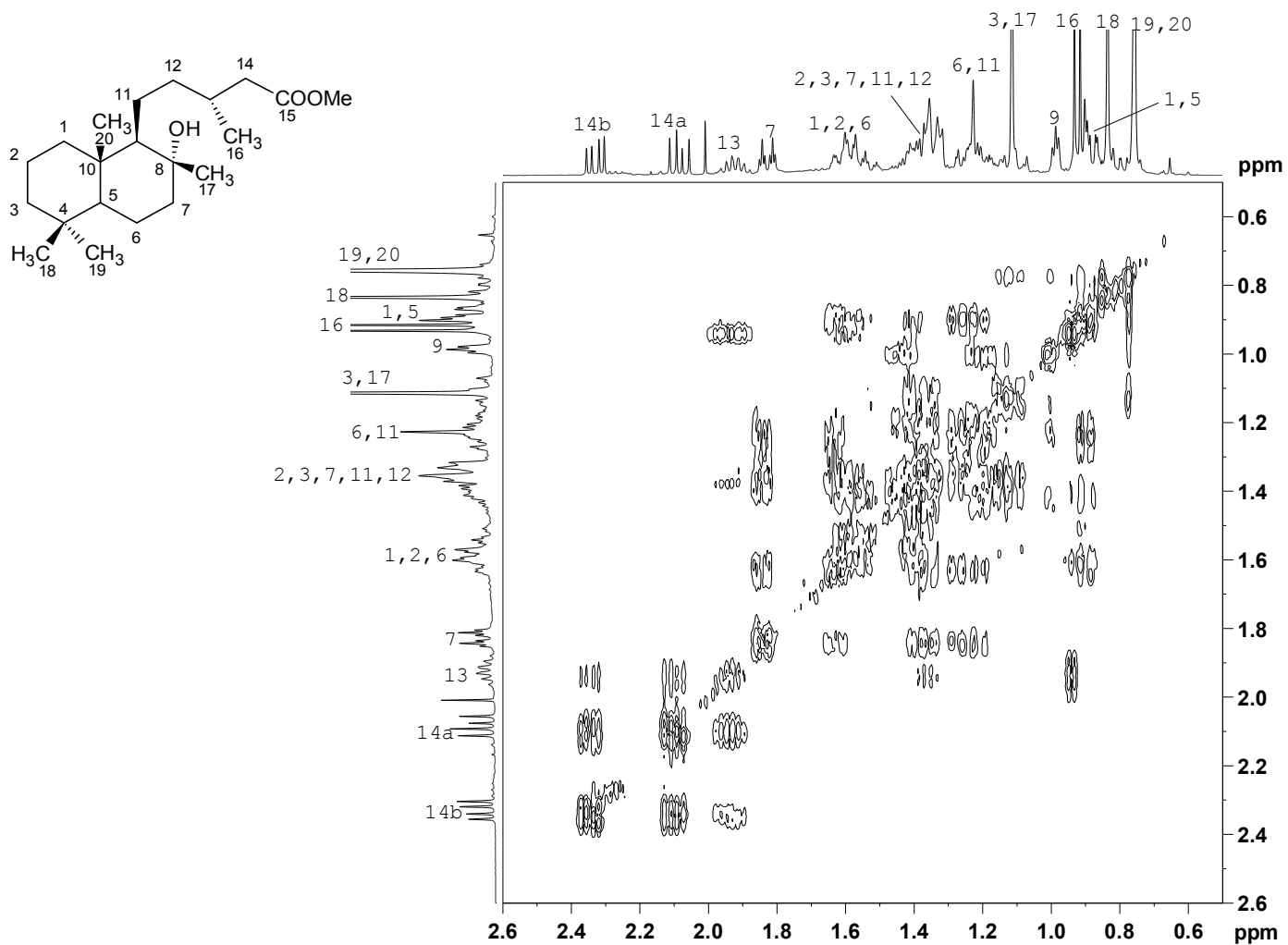
Plate 10d: DEPT 135 NMR spectrum of (+)-labdanolic acid methyl ester (2.64) in CDCl₃
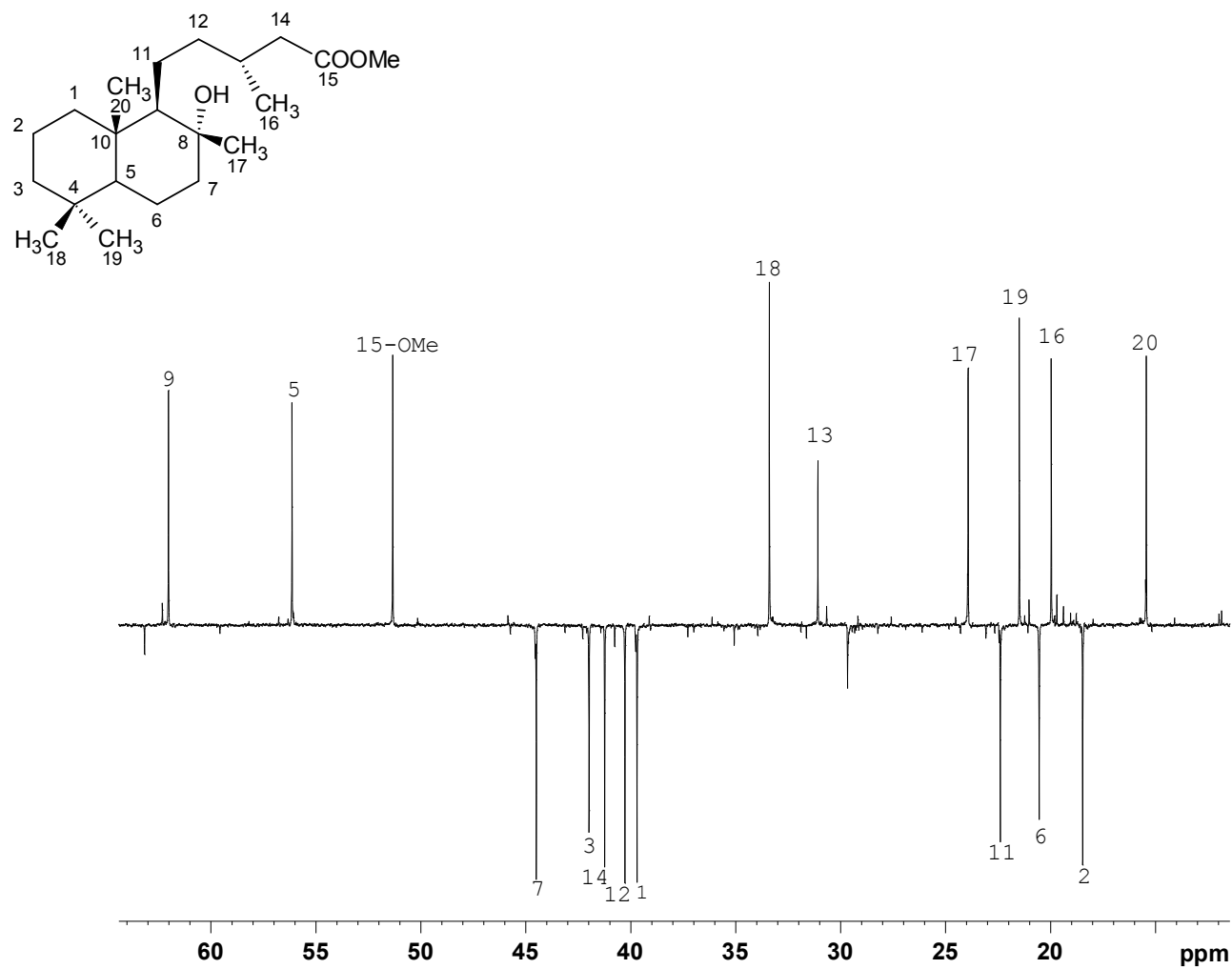
Plate 10e: HSQC NMR spectrum of (+)-labdanolic acid methyl ester (2.64) in CDCl₃

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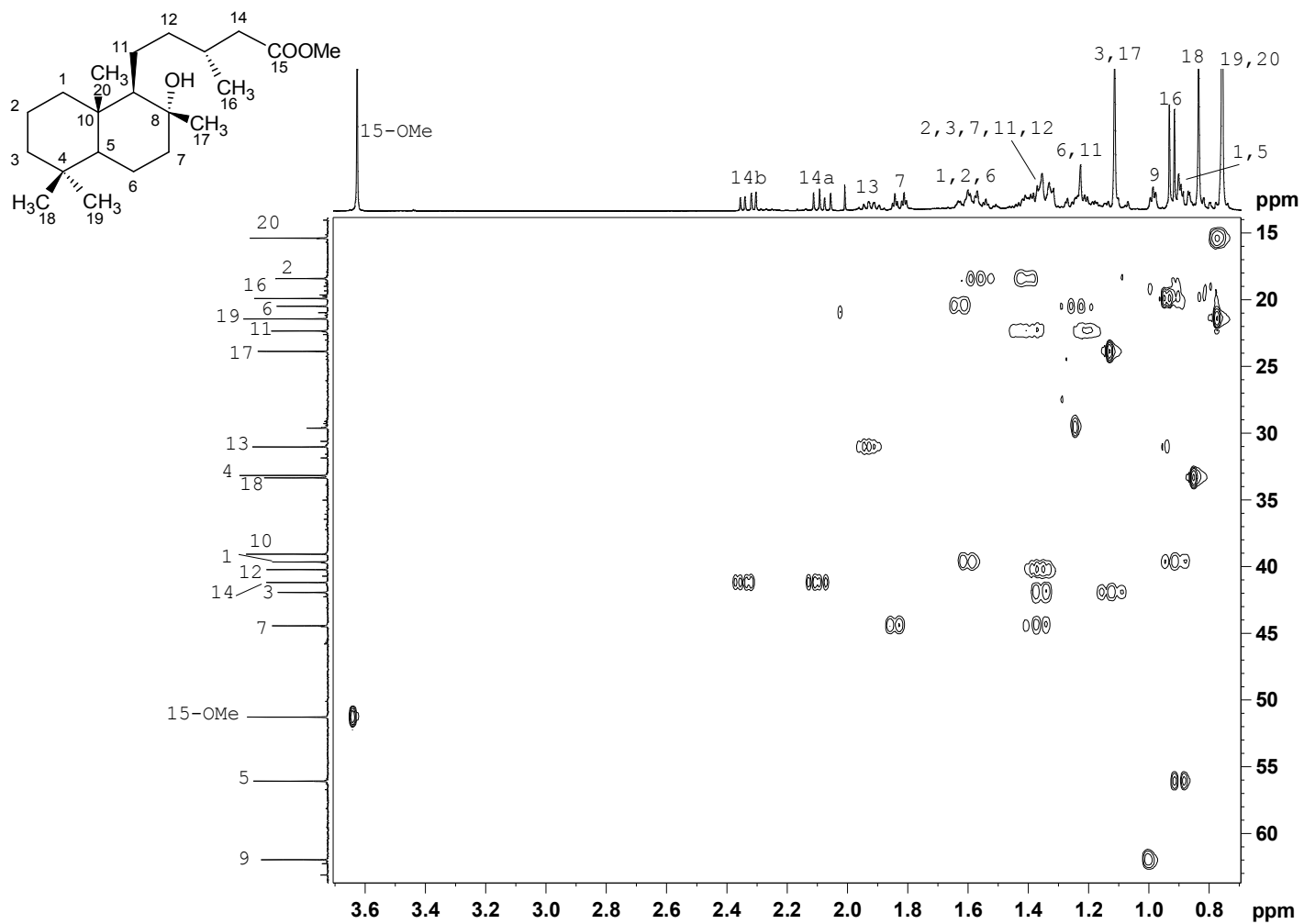
Plate 10f: HMBC NMR spectrum of (+)-labdanolic acid methyl ester (2.64) in CDCl₃
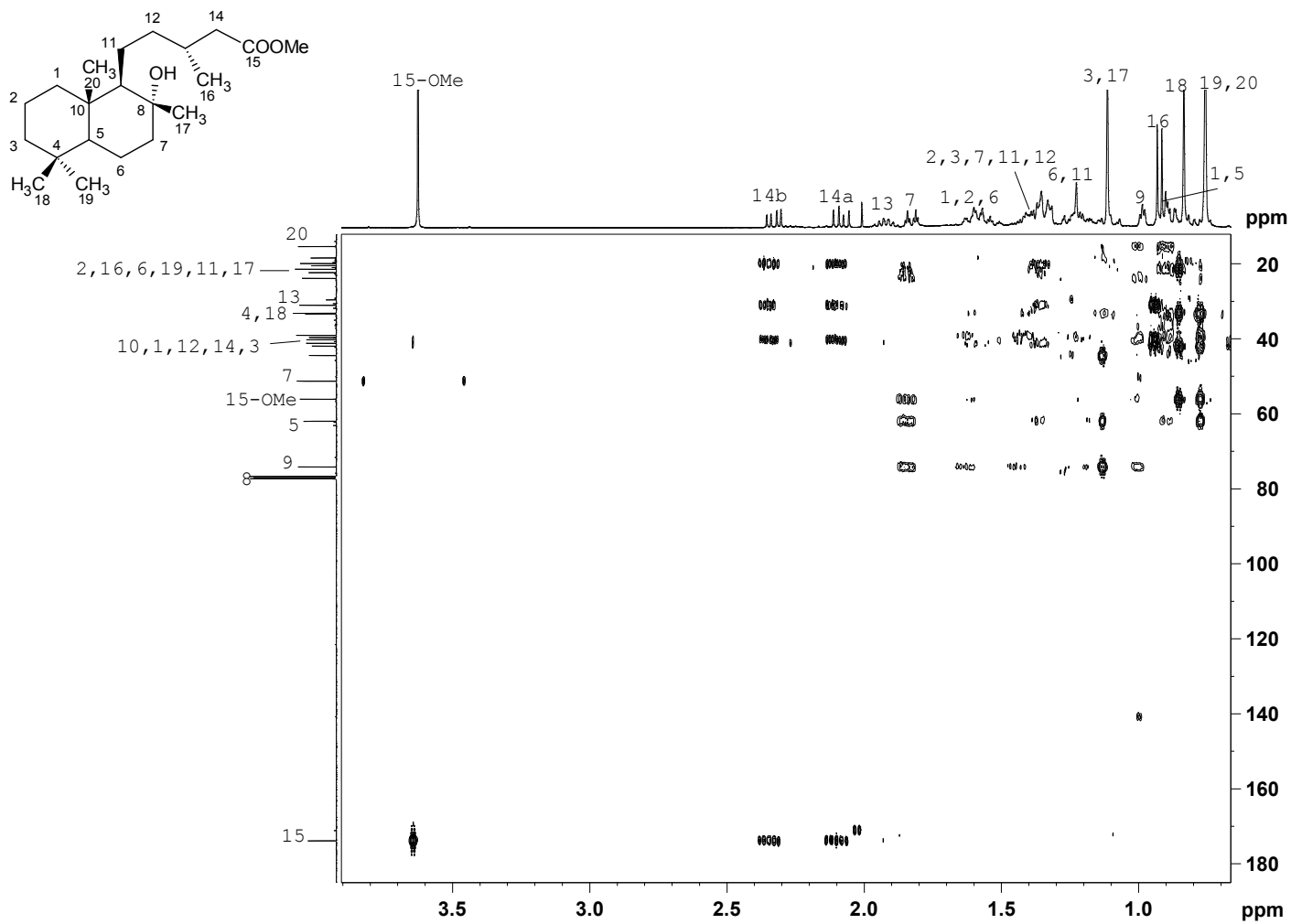
Plate 10g: NOE NMR spectrum of (+)-labdanolic acid methyl ester (2.64) in CDCl₃
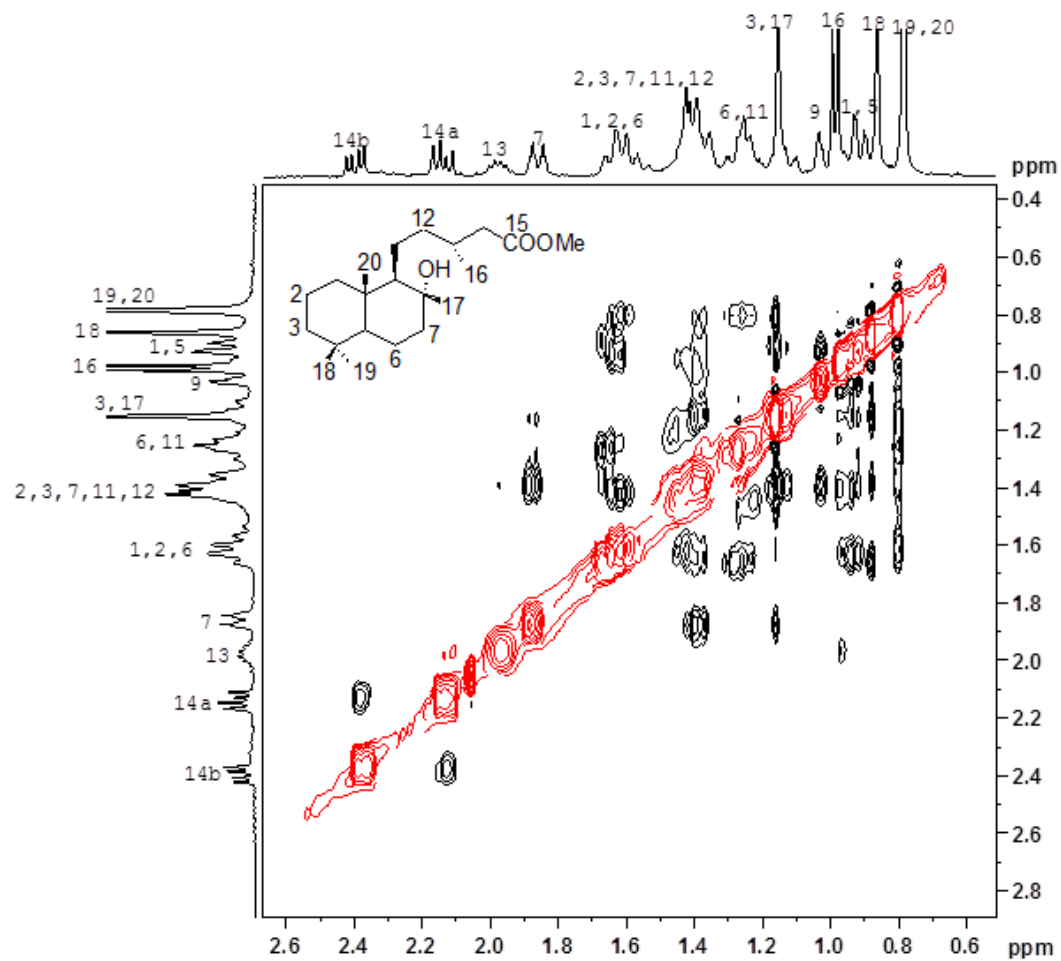
Plate 11a: ^{1}H NMR spectrum of (+)-labdanediol (2.66) in CDCl$_3$
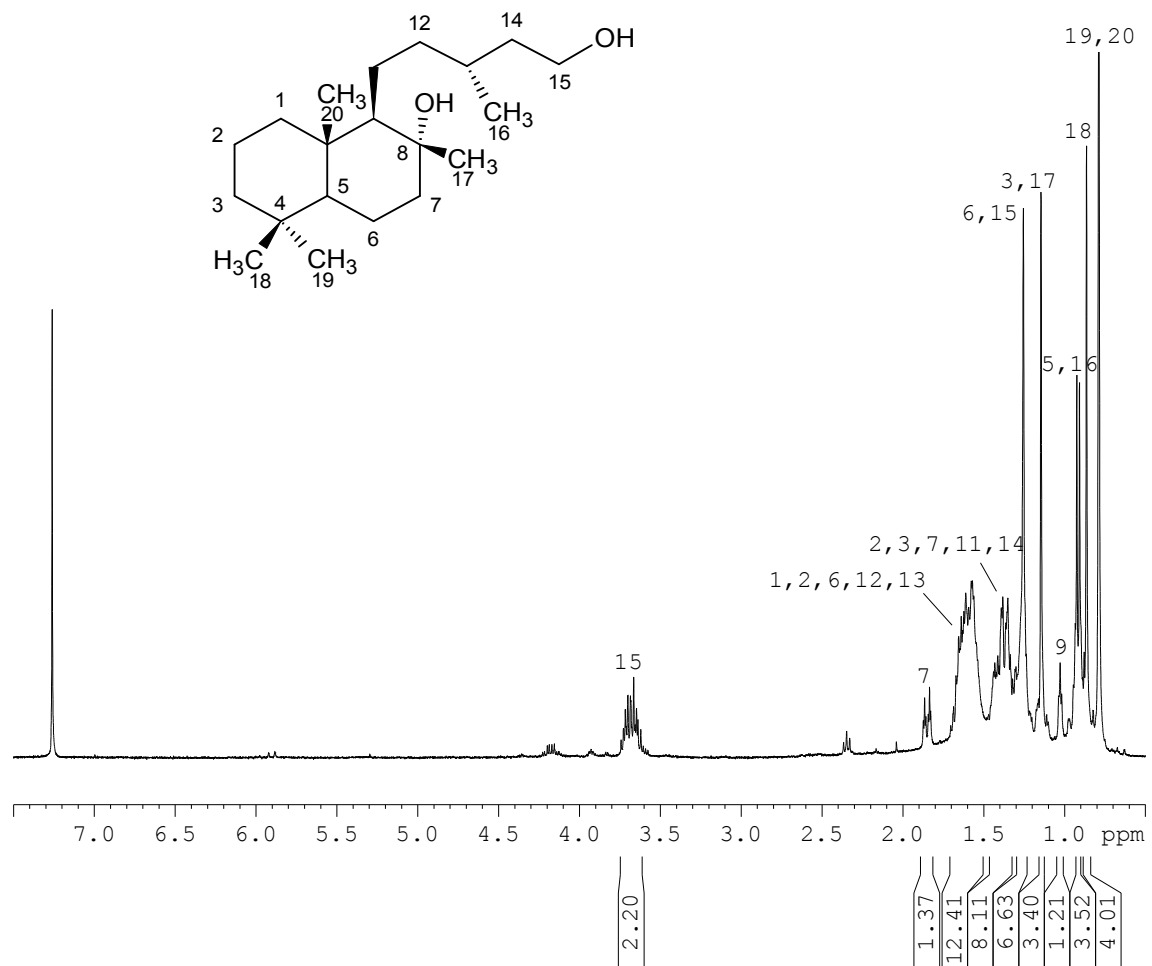
Plate 11b: ^{13}C NMR spectrum of (+)-labdanediol (2.66) in CDCl$_3$

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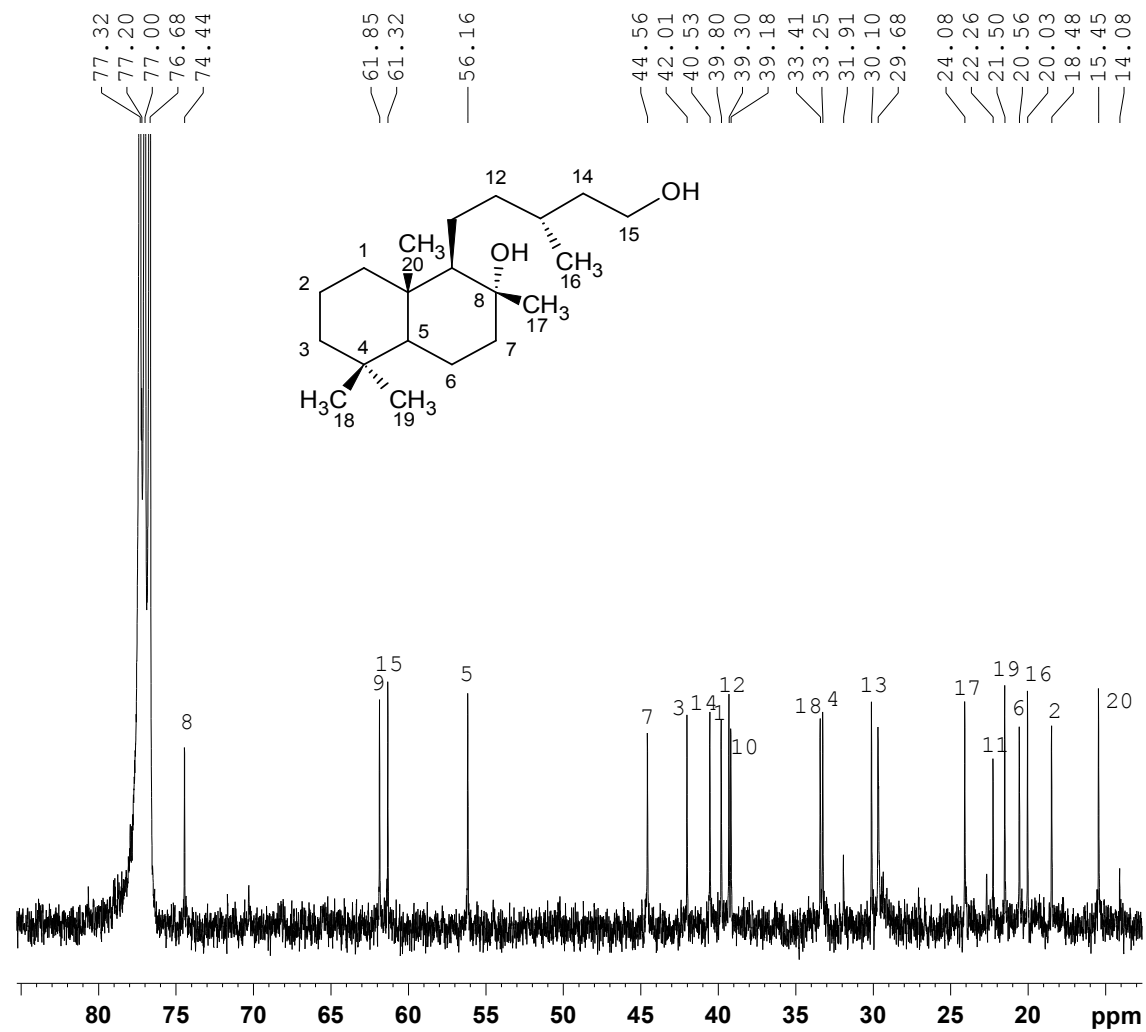
Plate 11c: COSY NMR spectrum of (+)-labdanediol (2.66) in CDCl₃
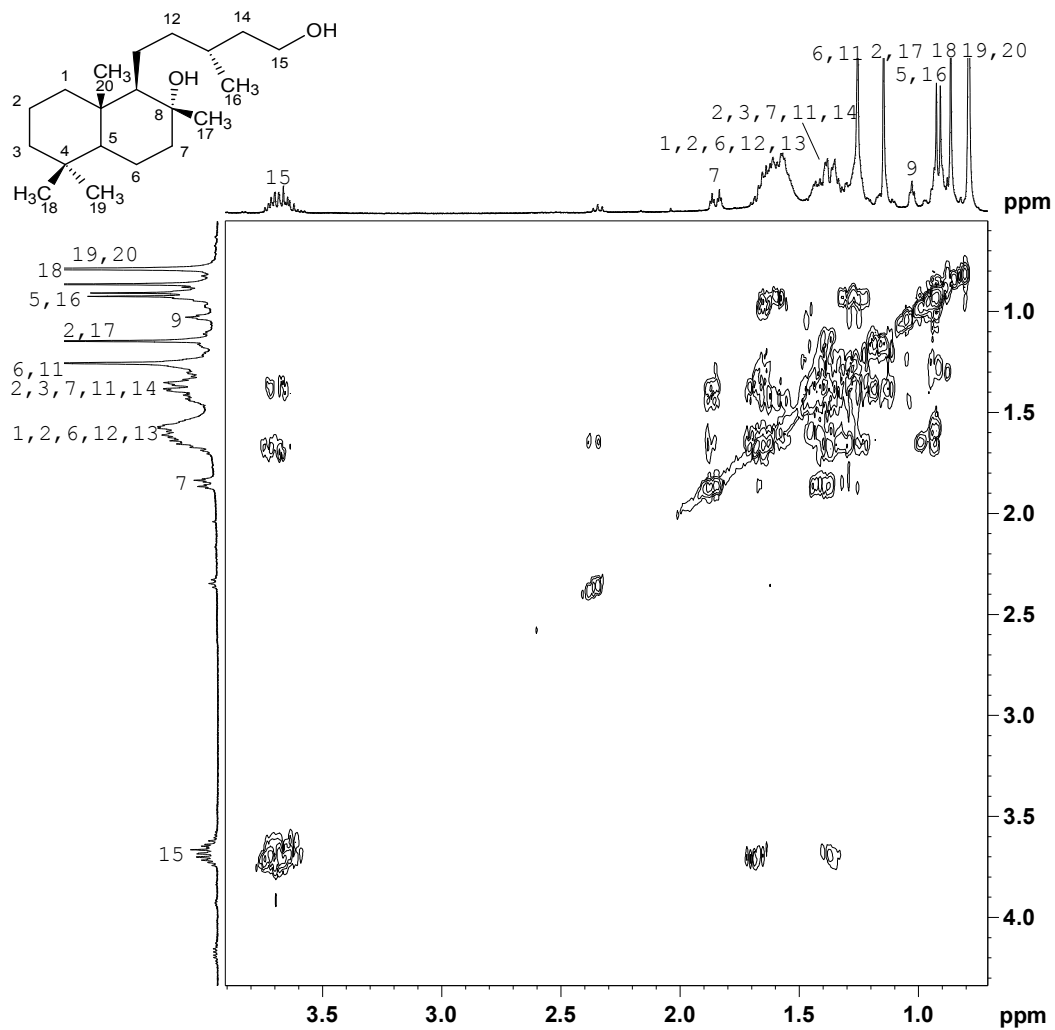
Plate 11d: DEPT 135 NMR spectrum of (+)-labdanediol (2.66) in CDCl₃
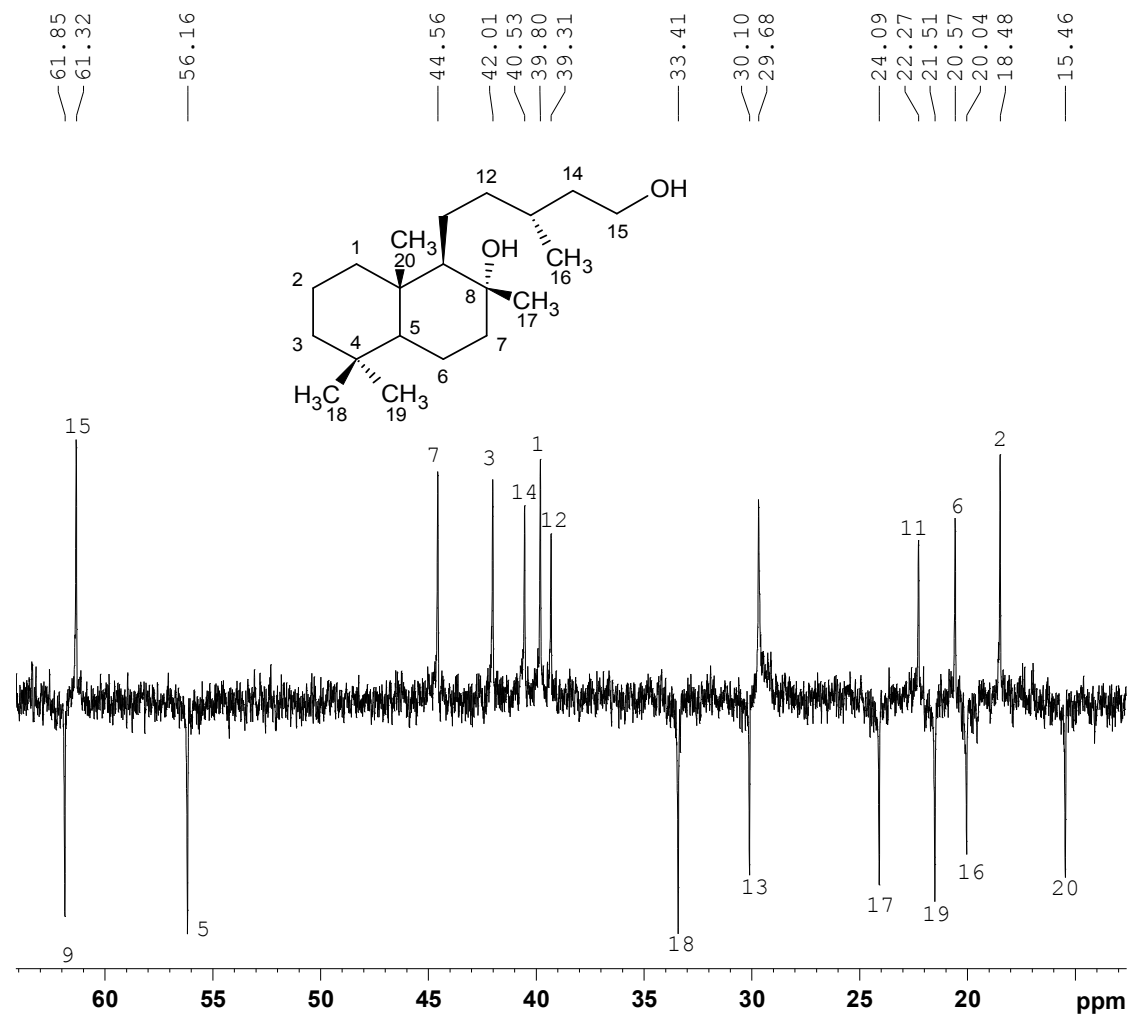
Plate 11e: HSQC NMR spectrum of (+)-labdanediol (2.66) in CDCl$_3$
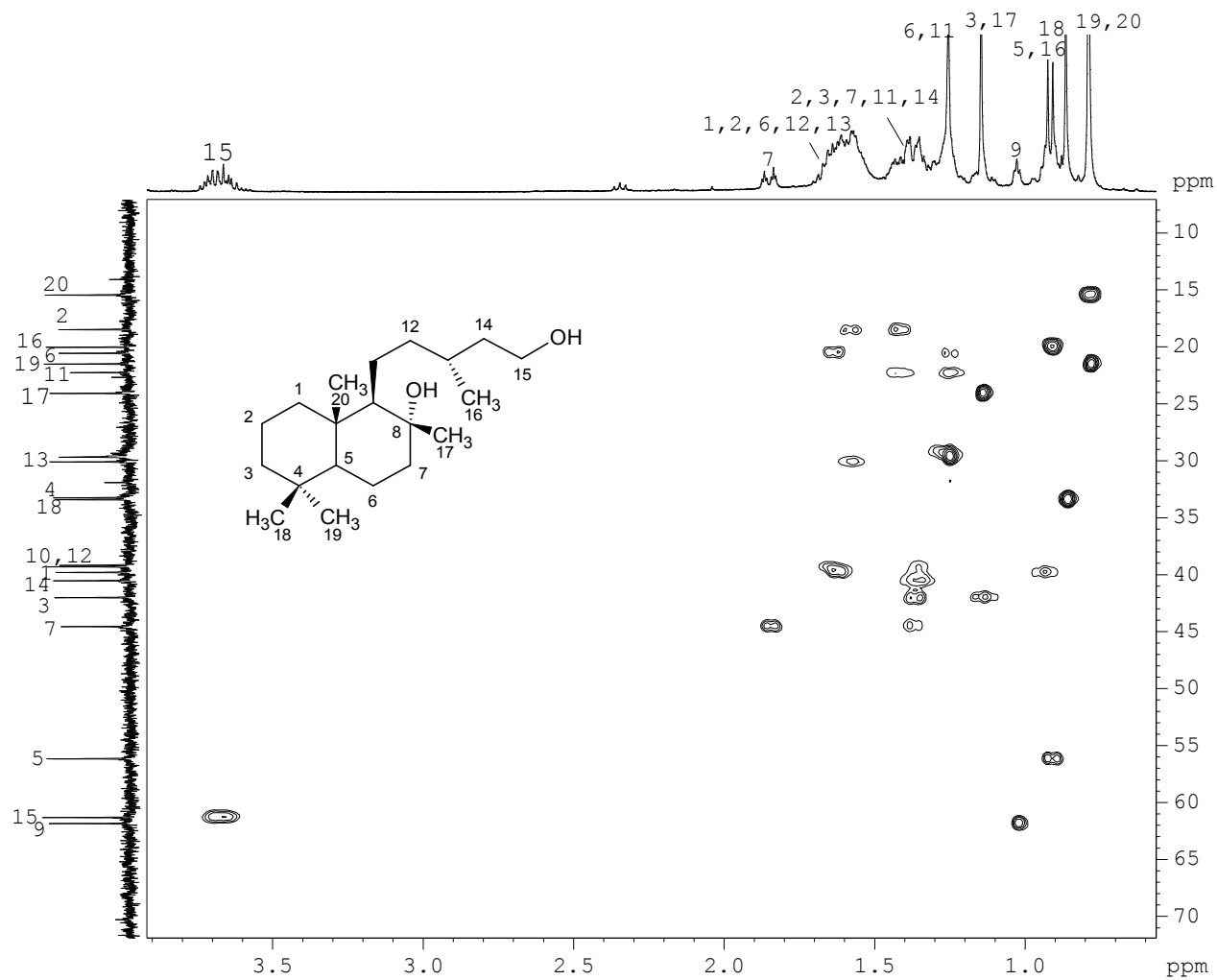
Plate 11f: HMBC NMR spectrum of (+)-labdanediol (2.66) in CDCl₃
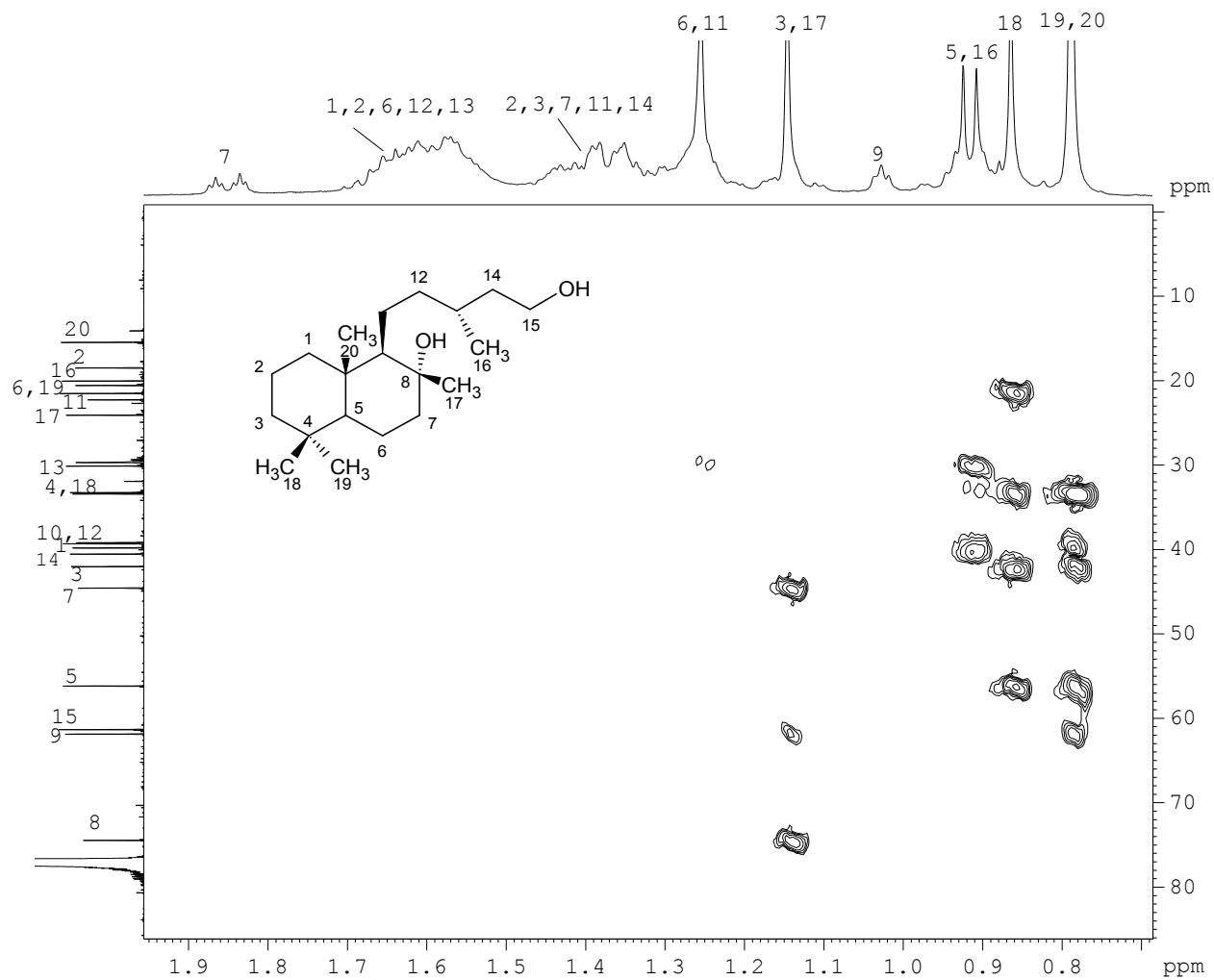
Plate 12a: ^{1}H NMR spectrum of quercetrin (3.61) in CD$_3$OD
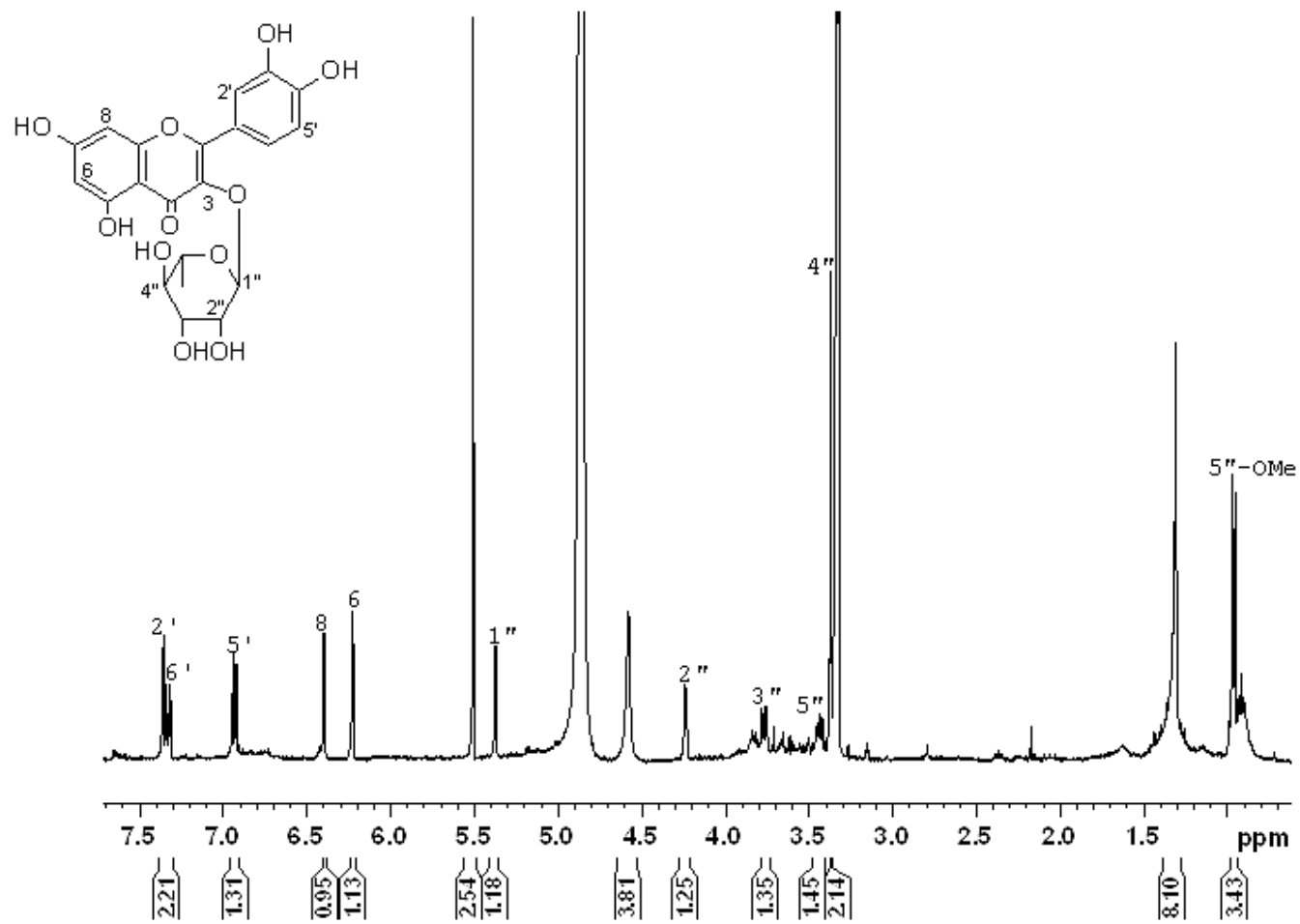
Plate 12b: ^{13}C NMR spectrum of quercetrin (3.61) in CD$_3$OD
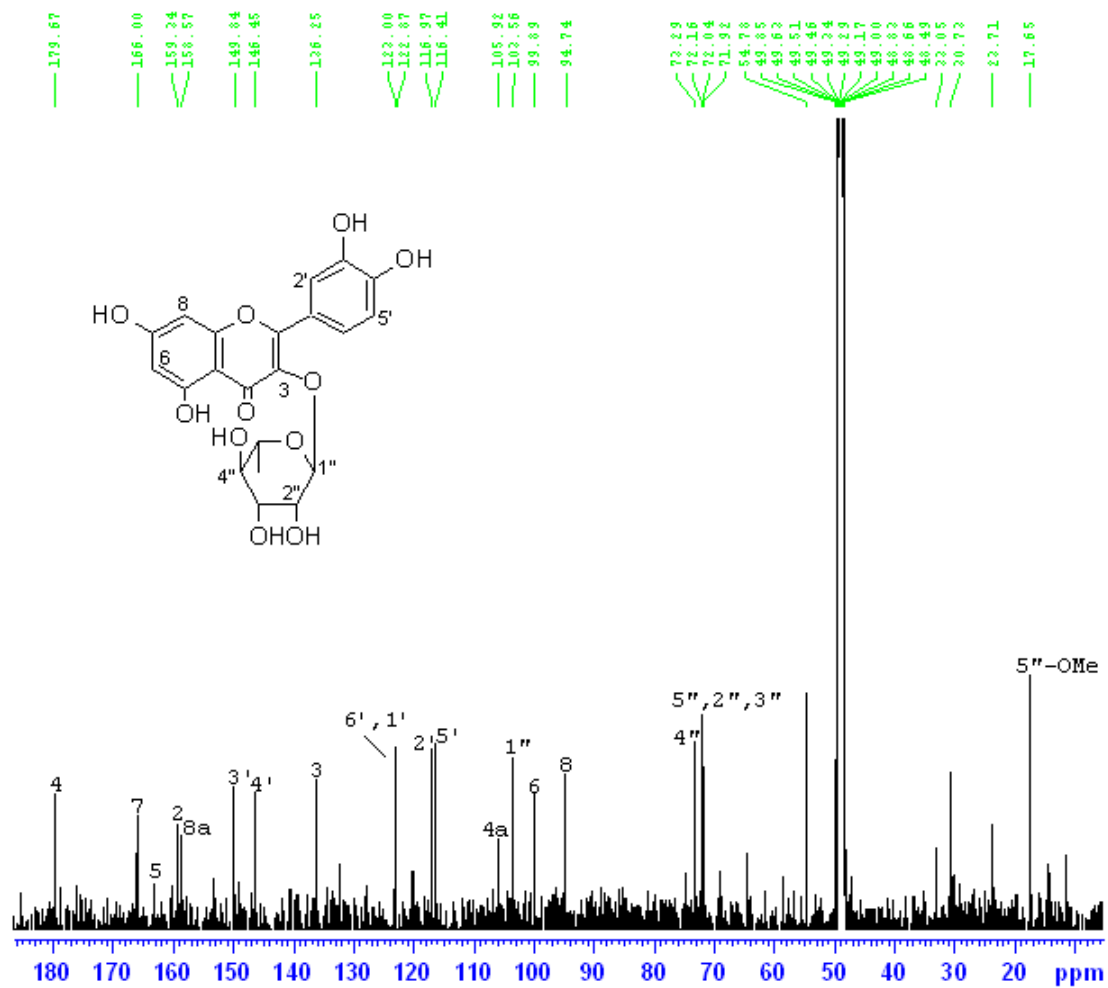
Plate 12c: COSY NMR spectrum of quercetrin (3.61) in CD₃OD
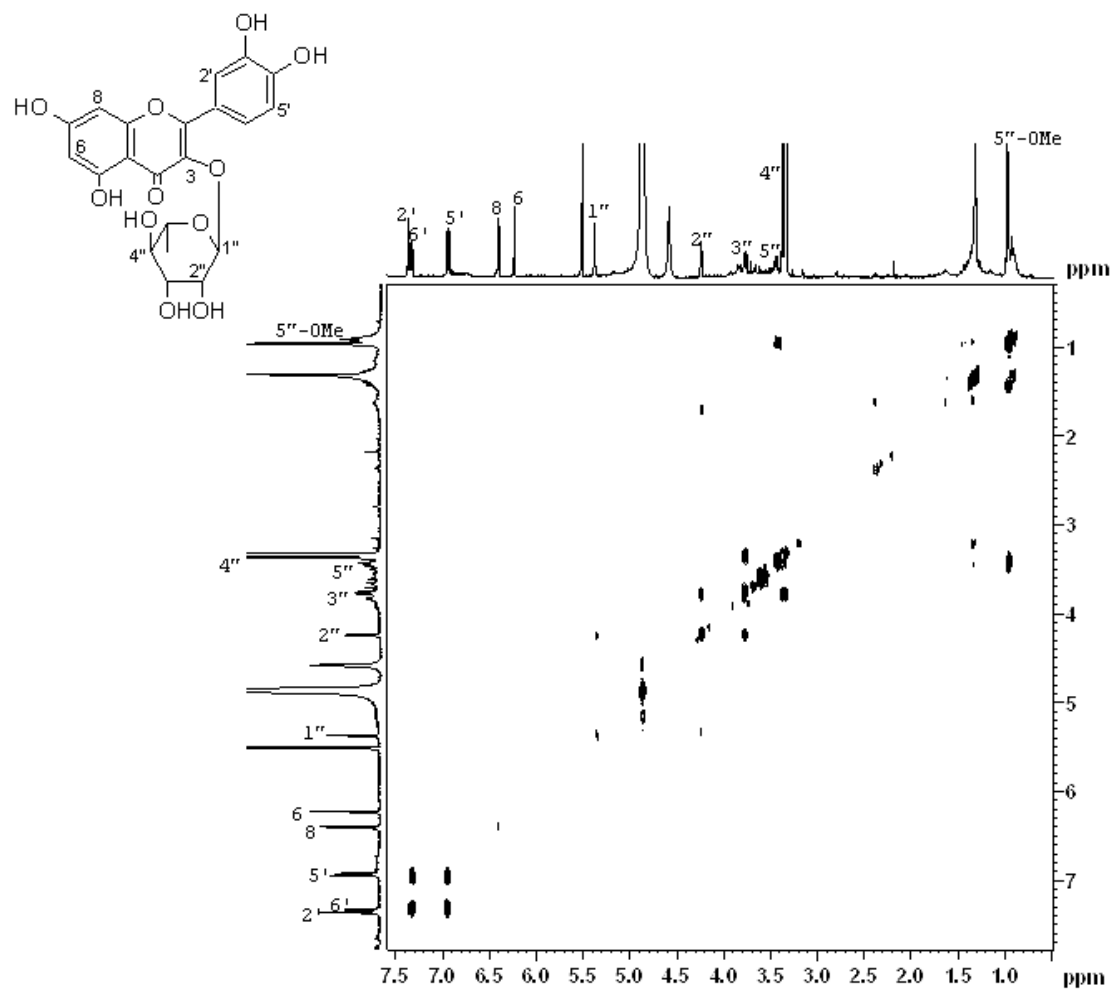
Plate 12d: DEPT NMR spectrum of quercetrin (3.61) in CD₃OD
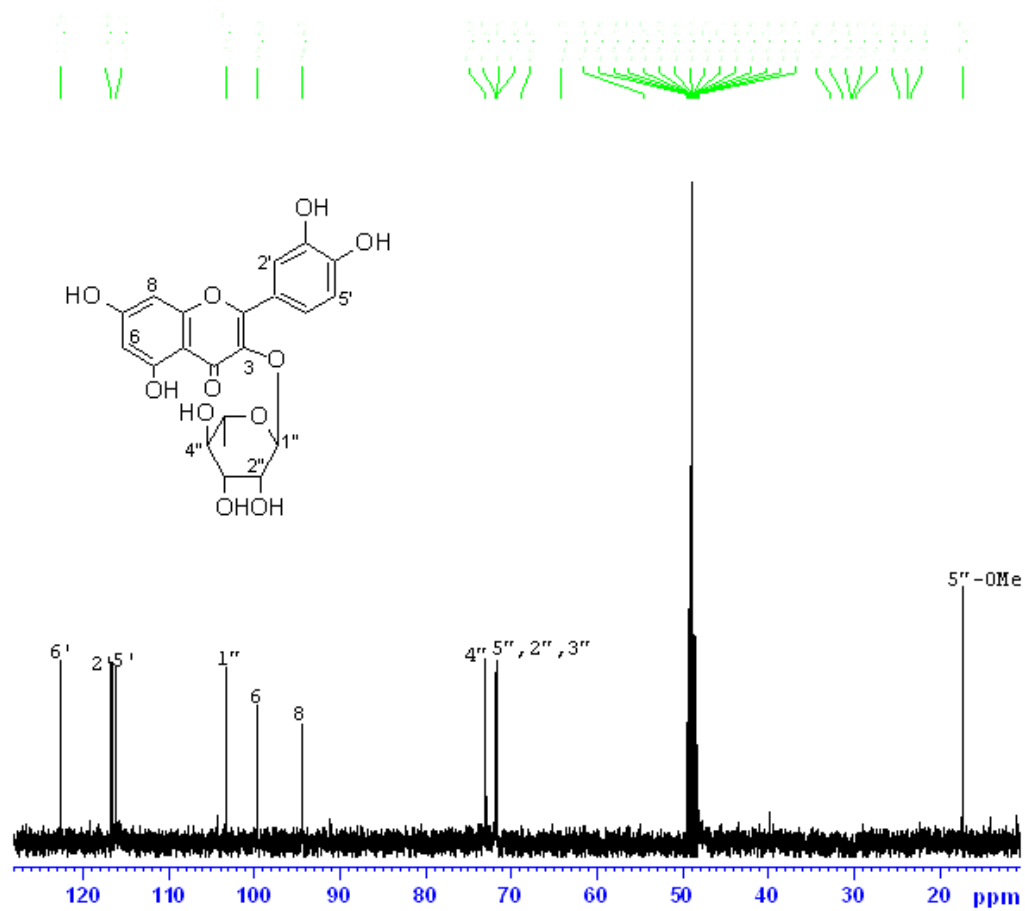
Plate 12e: HSQC NMR spectrum of quercetrin (3.61) in CD₃OD
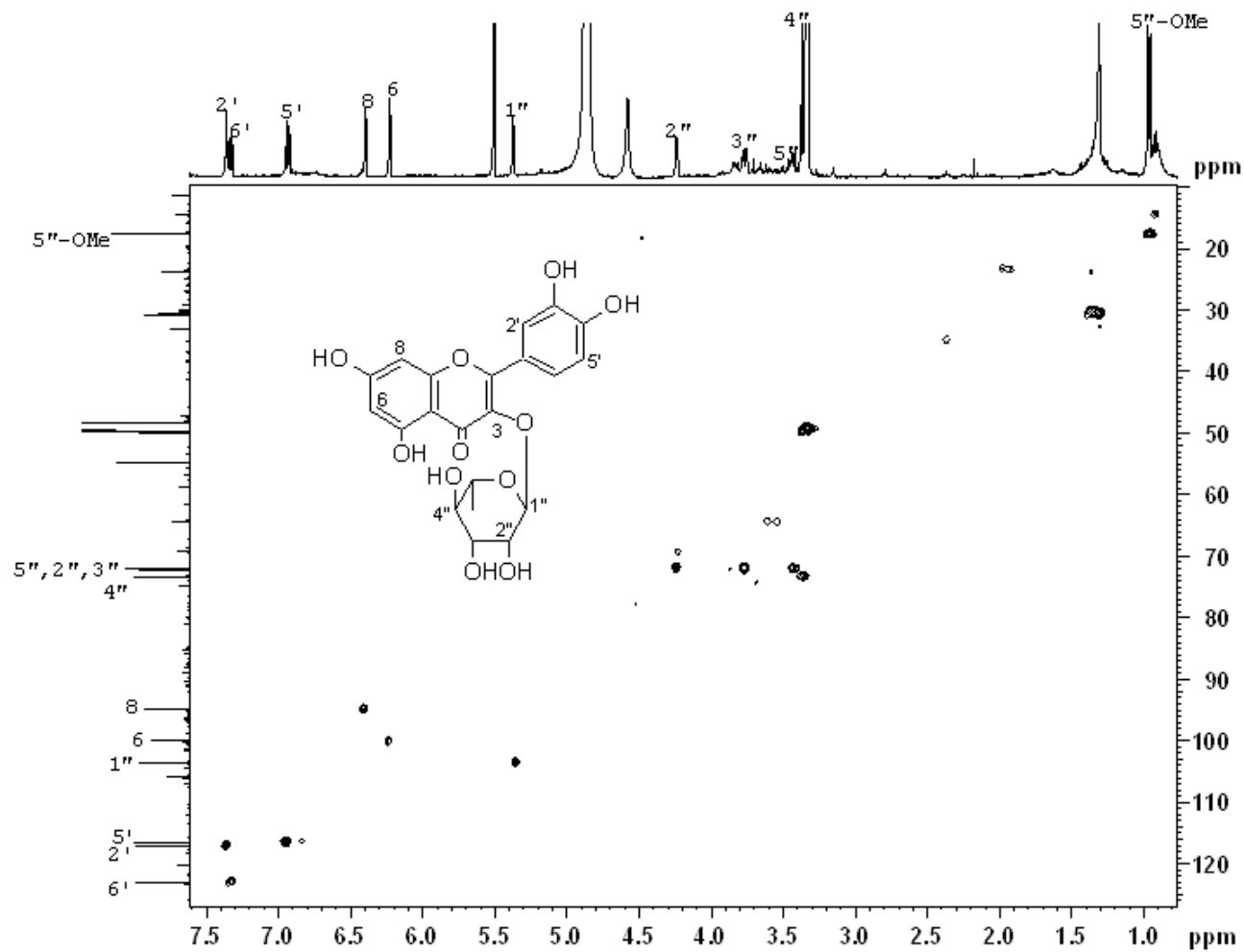
Plate 12f: HMBC NMR spectrum of quercetrin (3.61) in CD₃OD

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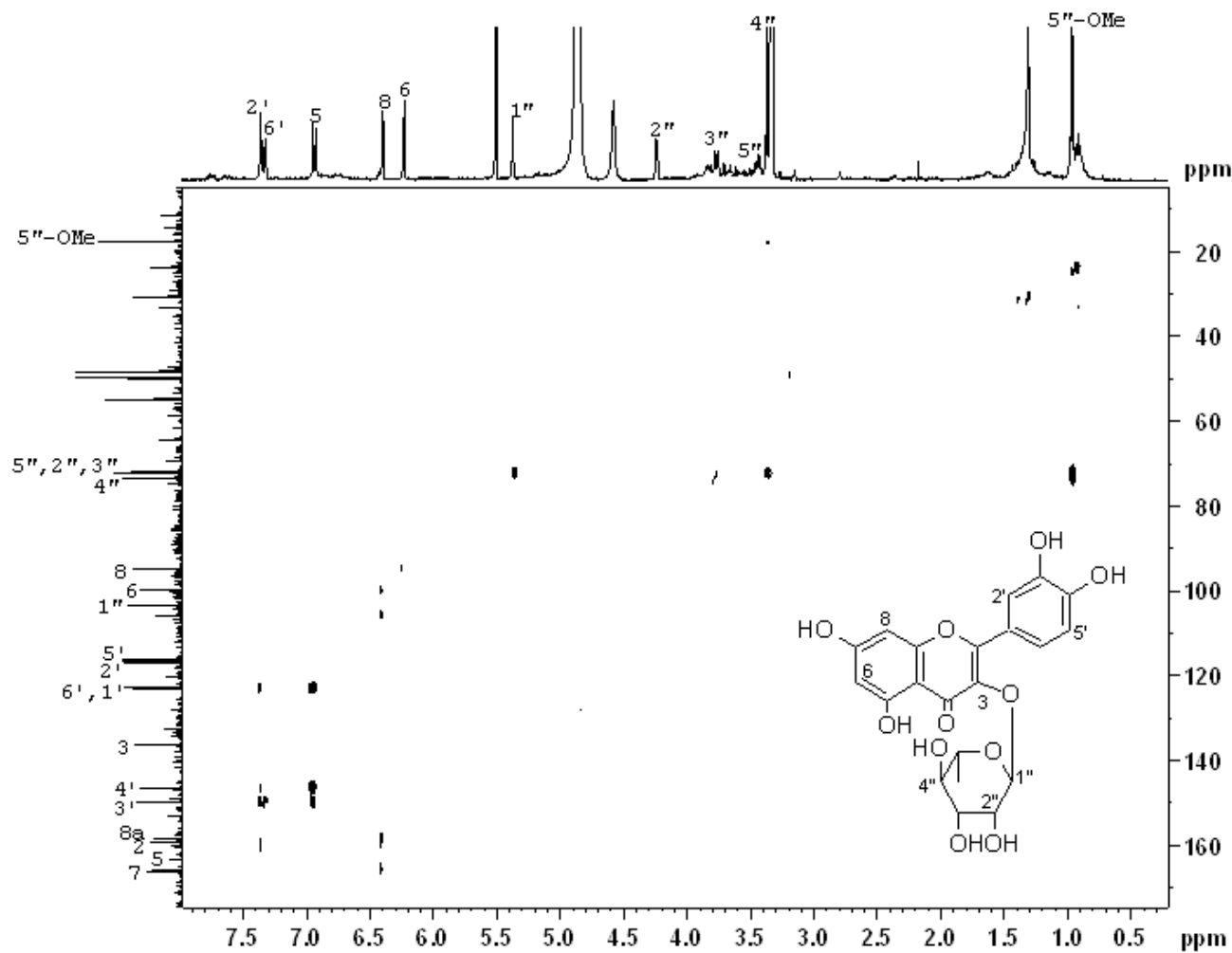
Plate 13a: ^{1}H NMR spectrum of morin 3-*O*-α-L-rhamnopyranoside (3.62) in CD₃OD
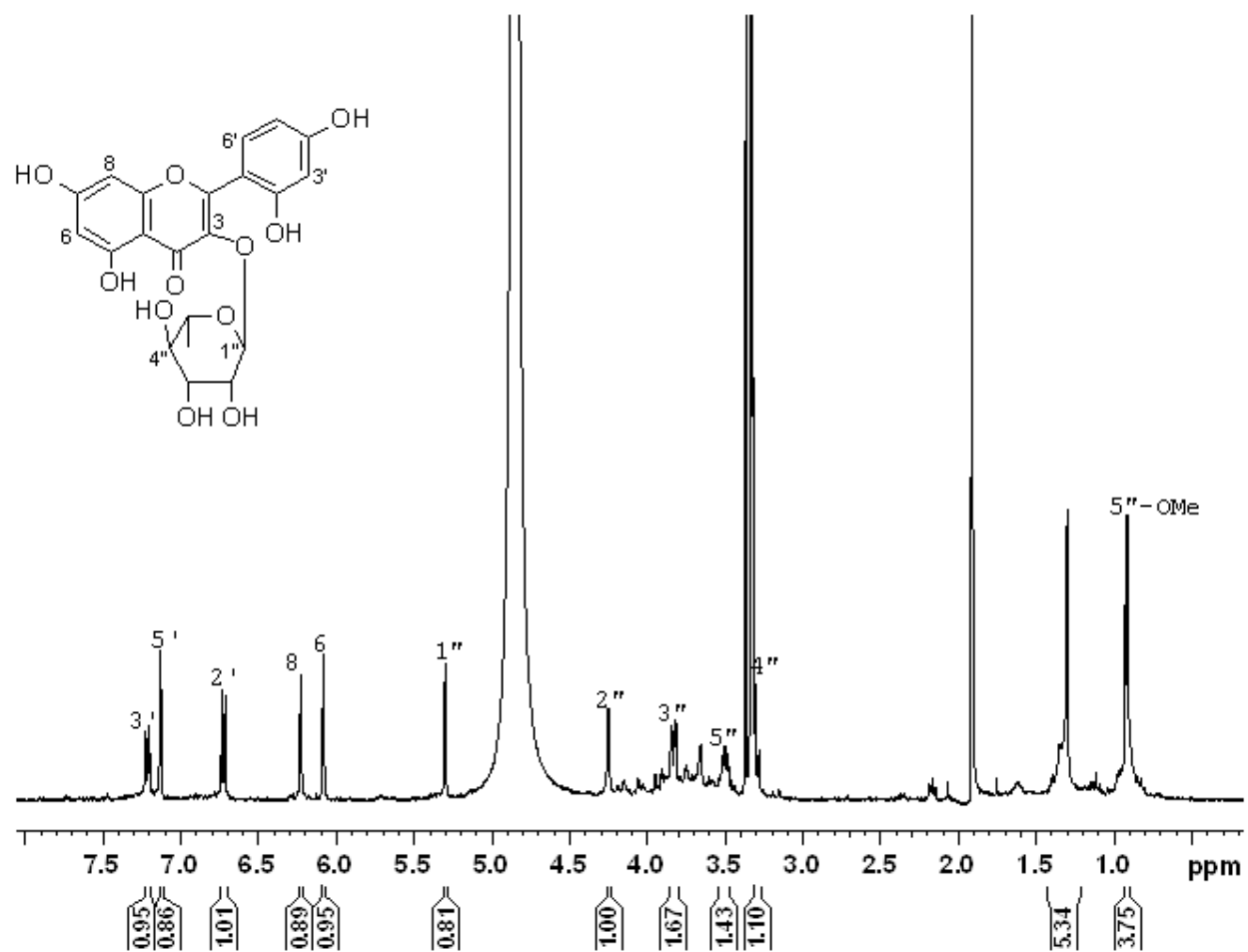
Plate 13b: ^{13}C NMR spectrum of morin 3-O-α-L-rhamnopyranoside (3.62) in CD$_3$OD
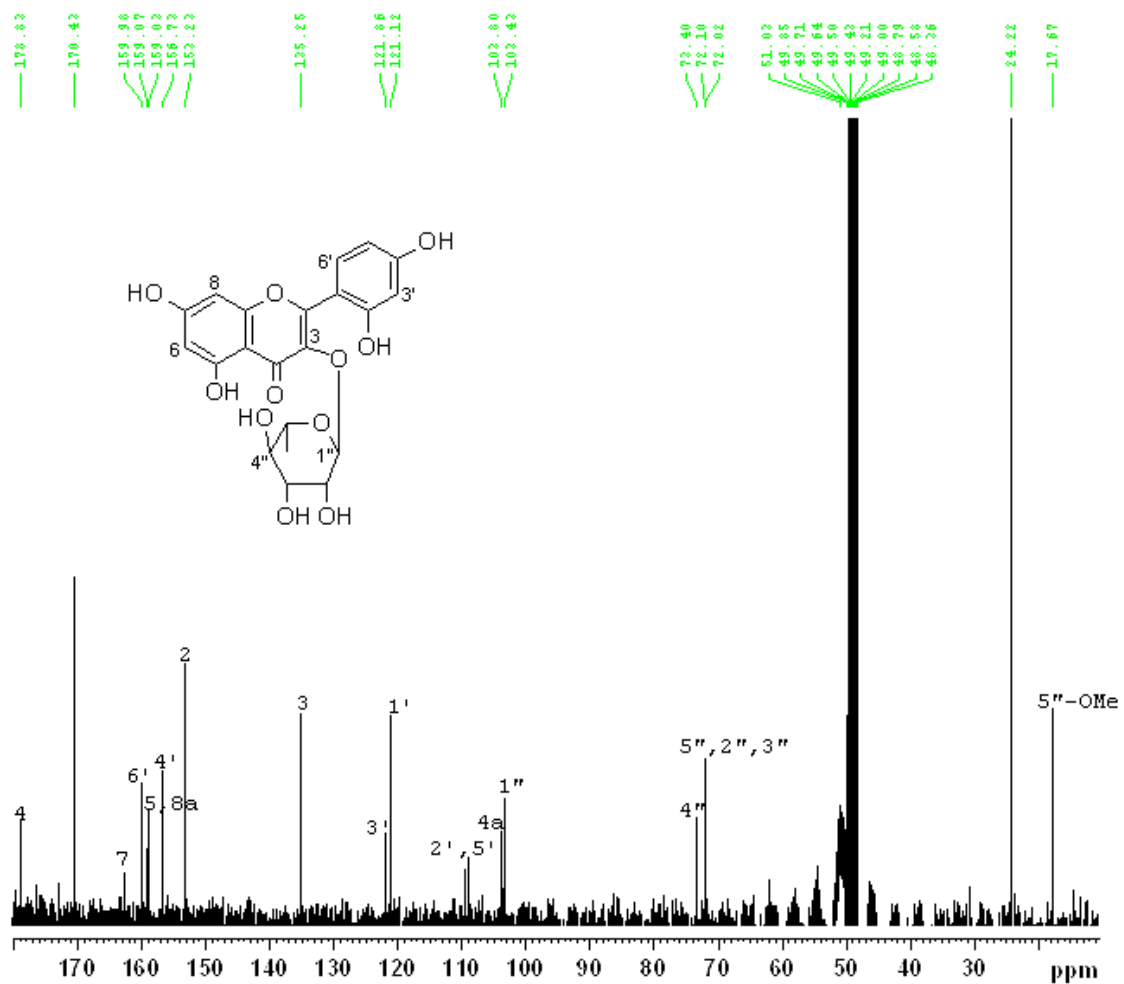
Plate 13c: COSY NMR spectrum of morin 3-*O*-α-L-rhamnopyranoside (3.62) in CD₃OD
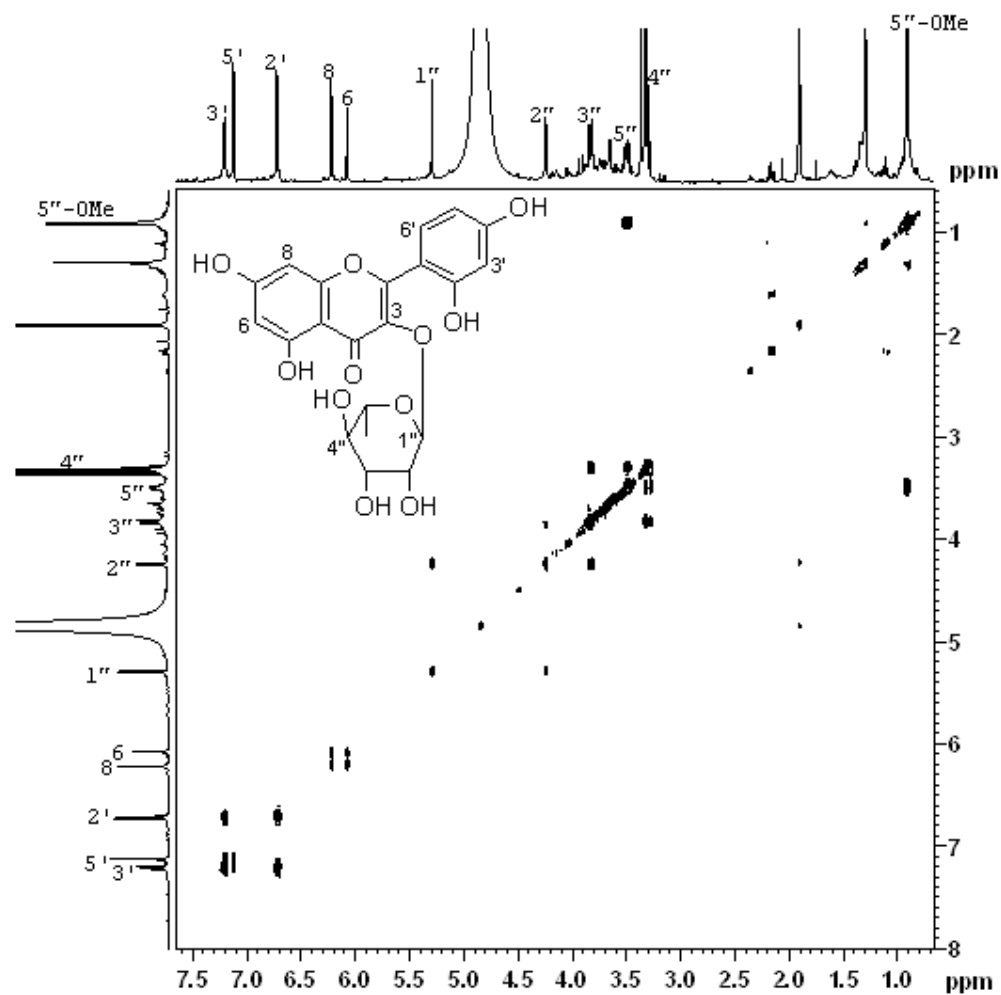
Plate 13d: DEPT NMR spectrum of morin 3-*O*-α-L-rhamnopyranoside (3.62) in CD₃OD
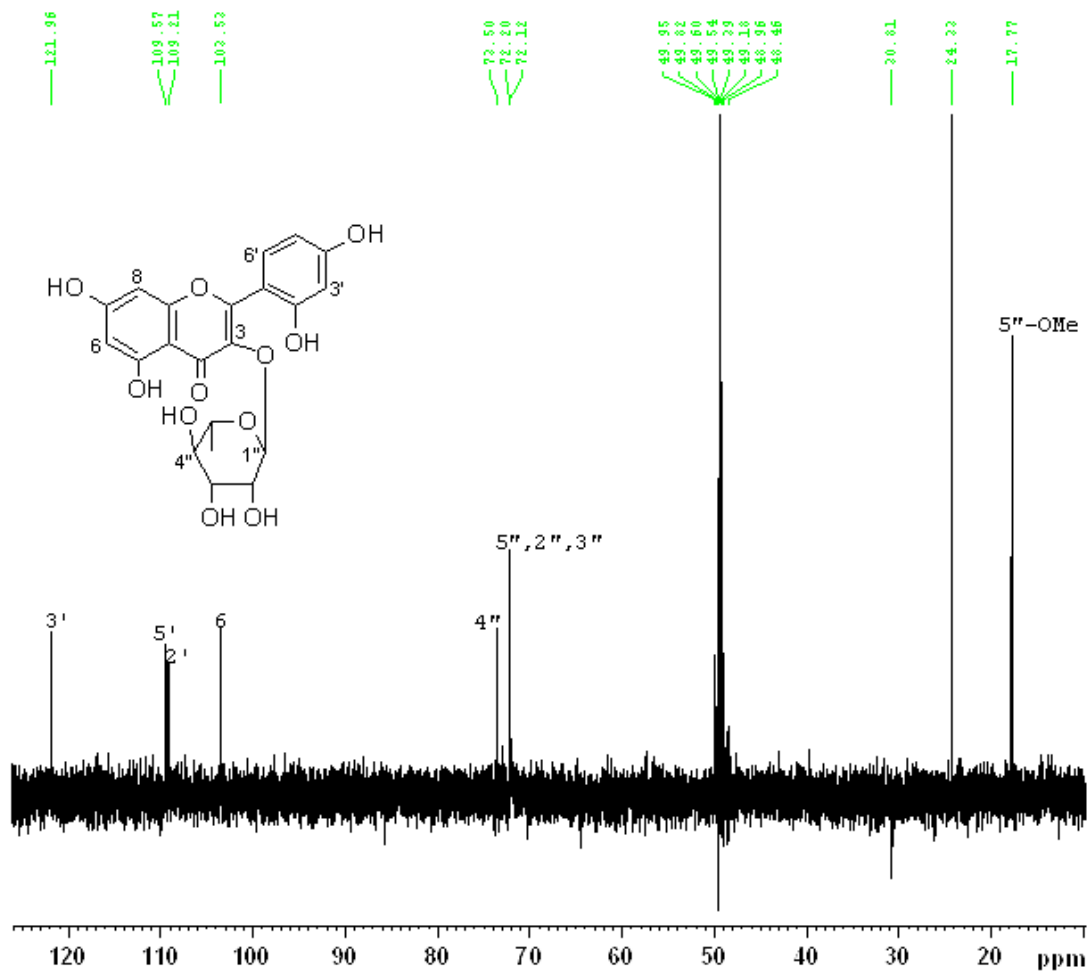
Plate 13e: HSQC NMR spectrum of morin 3-*O*-α-L-rhamnopyranoside (3.62) in CD₃OD
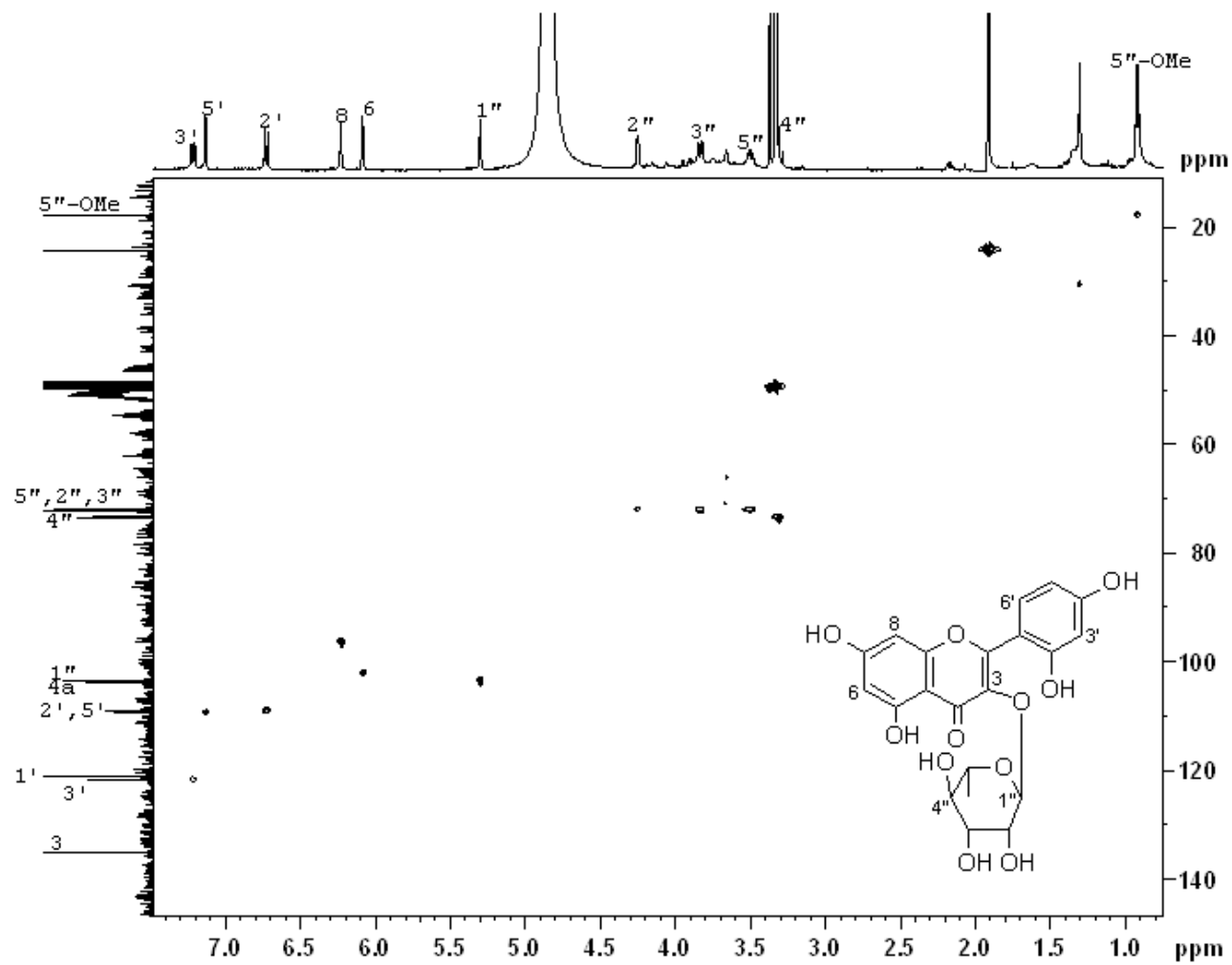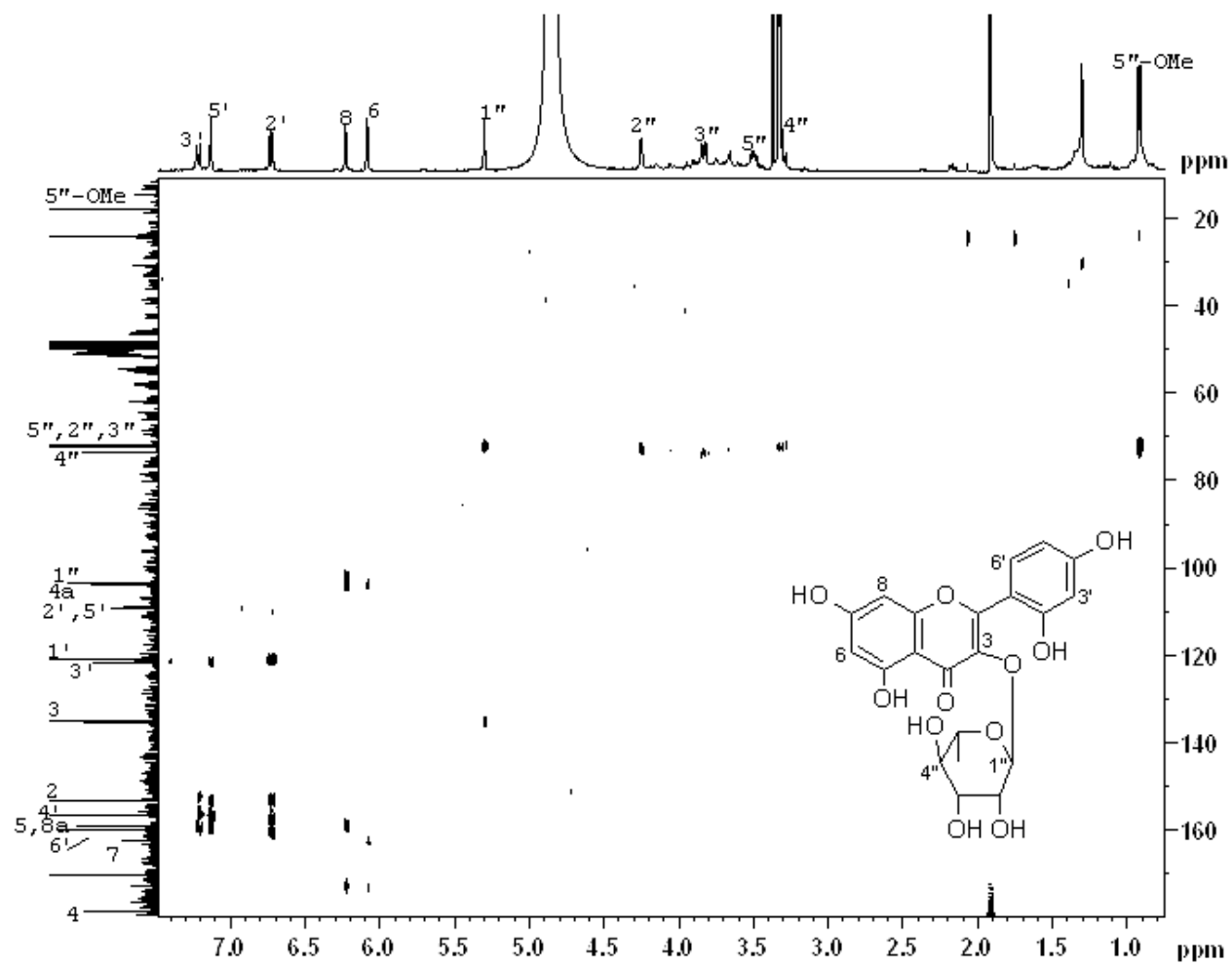
Plate 14a: ^{1}H NMR spectrum of catechin (3.63) in CD$_3$OD
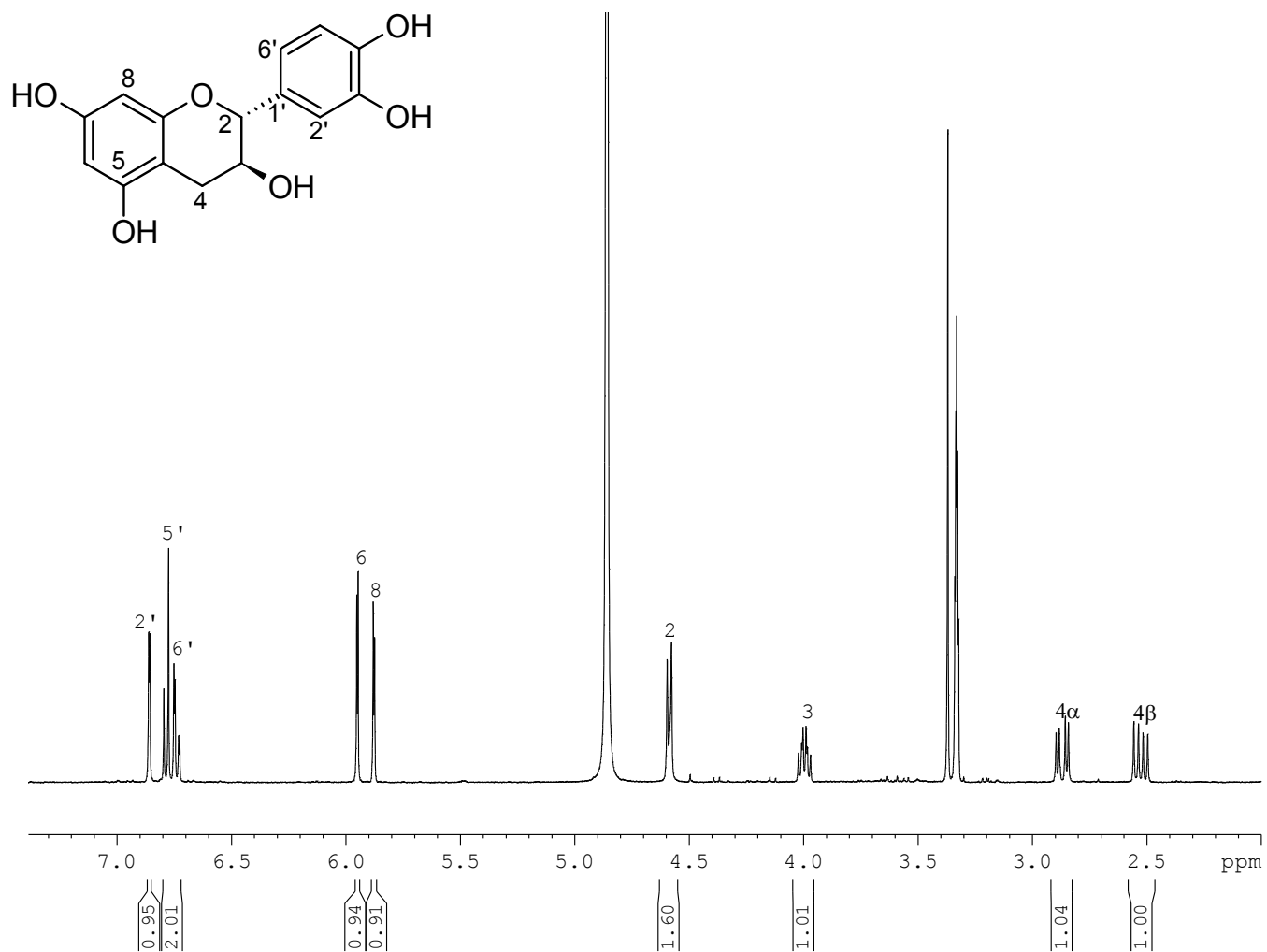
Plate 14b: ^{13}C NMR spectrum of catechin (3.63) in CD$_3$OD
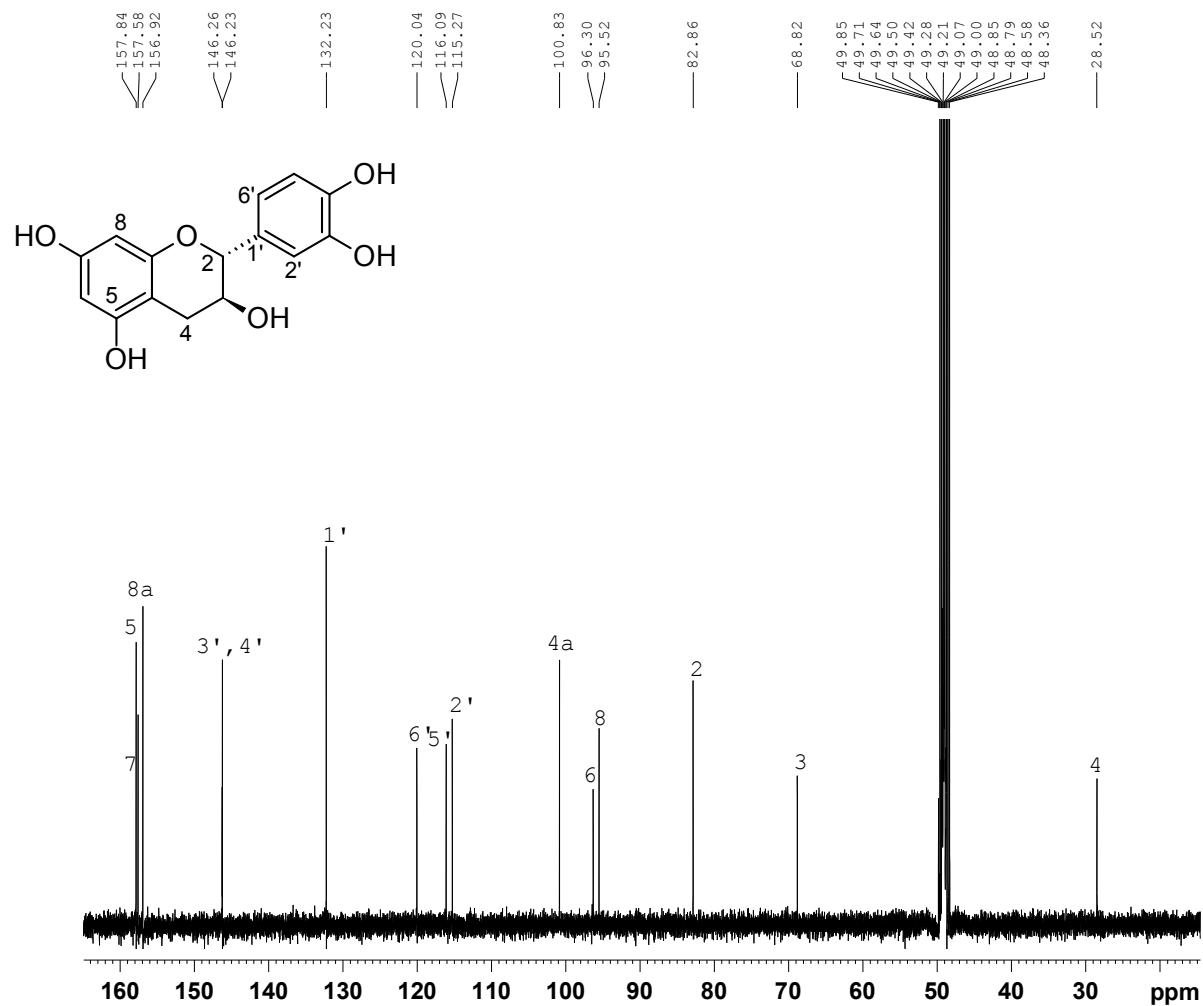
Plate 14c: COSY NMR spectrum of catechin (3.63) in CD₃OD

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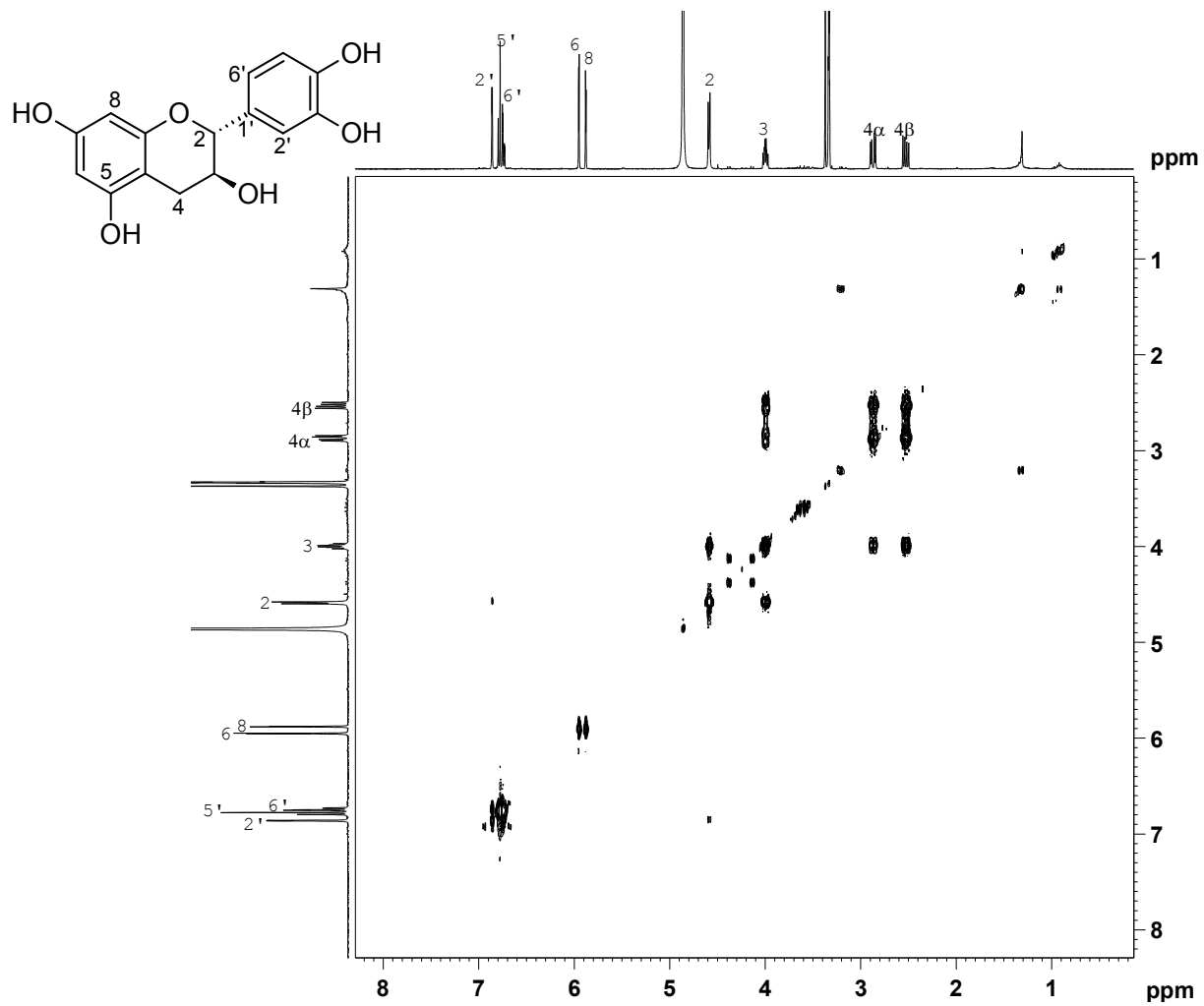
Plate 14d: DEPT NMR spectrum of catechin (3.63) in CD₃OD
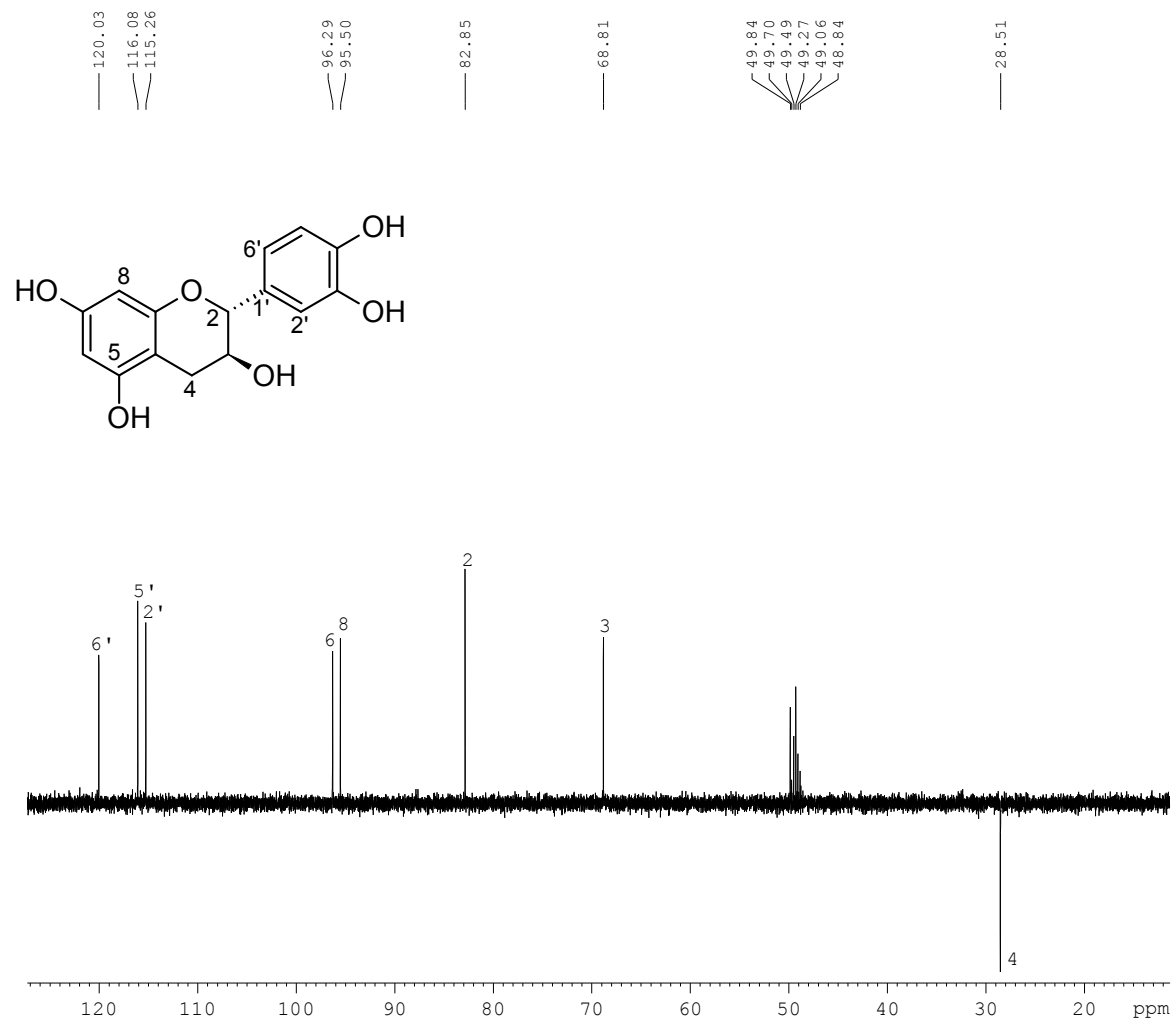
Plate 14e: HSQC NMR spectrum of catechin (3.63) in CD$_3$OD

Plate 14f: HMBC NMR spectrum of catechin (3.63) in CD₃OD
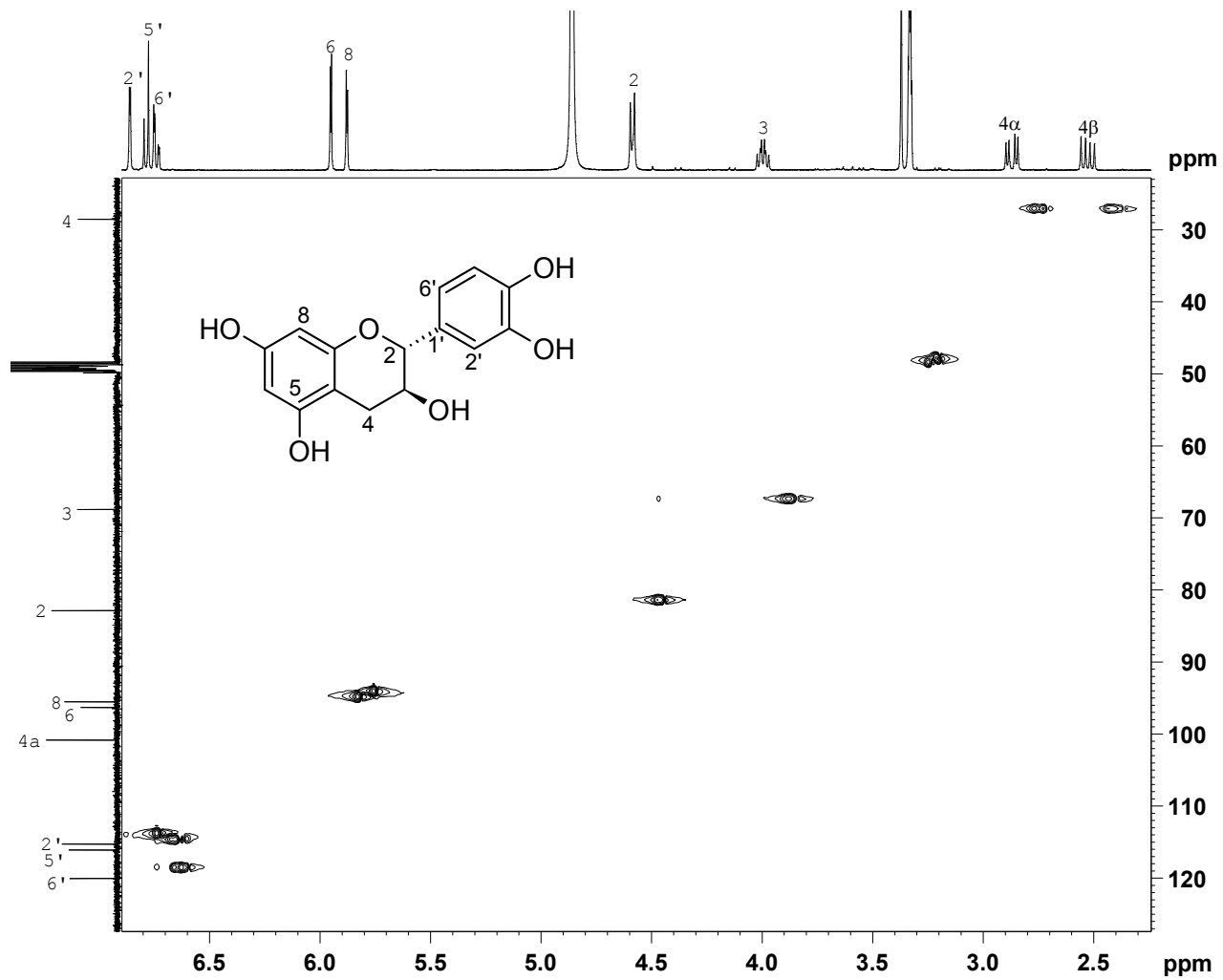
Plate 14f: HMBC NMR spectrum of catechin (3.63) in CD$_3$OD
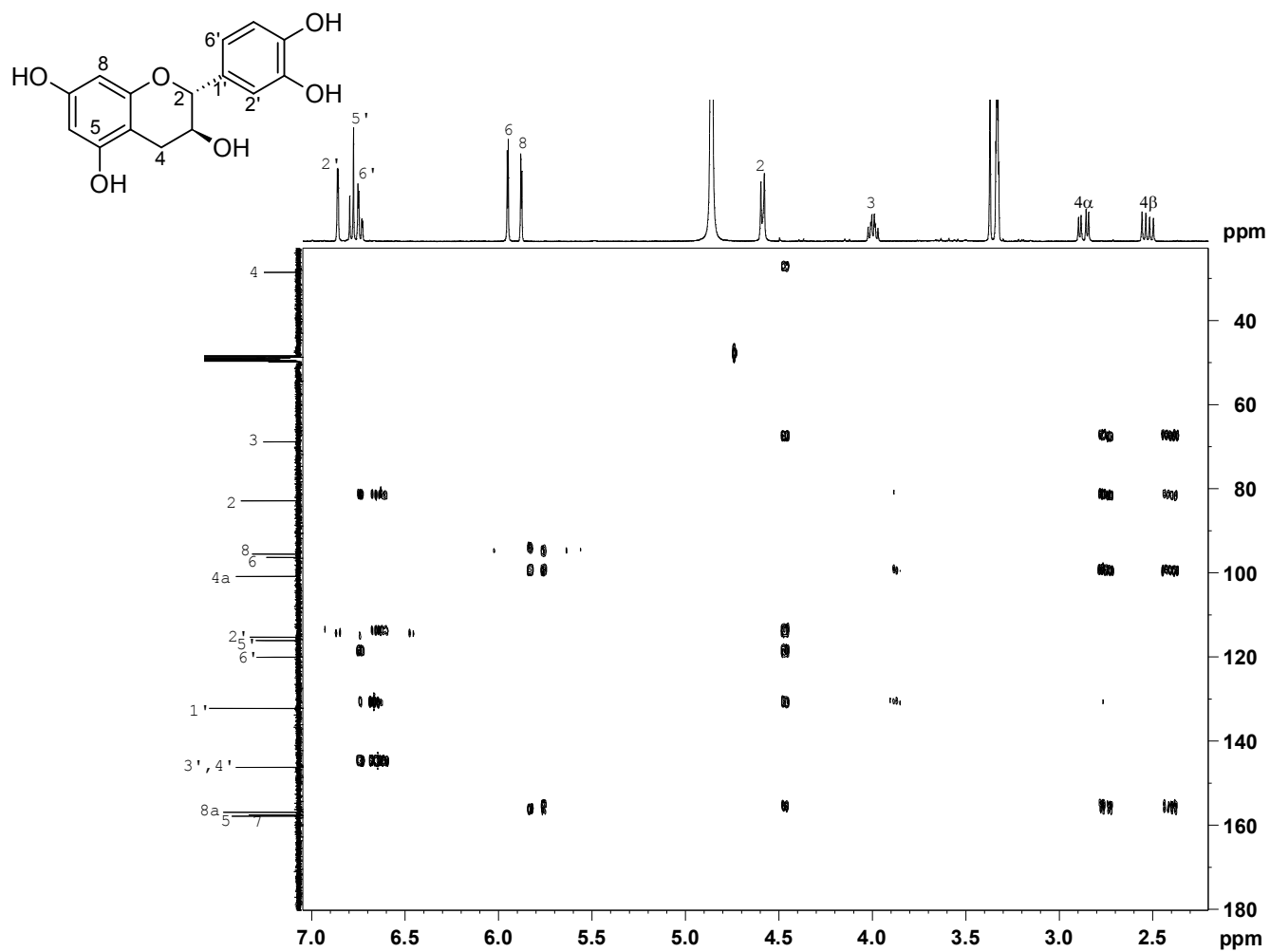
Plate 15a: ^{1}H NMR spectrum of *trans*-resveratrol glucoside (3.64) in CD$_3$OD
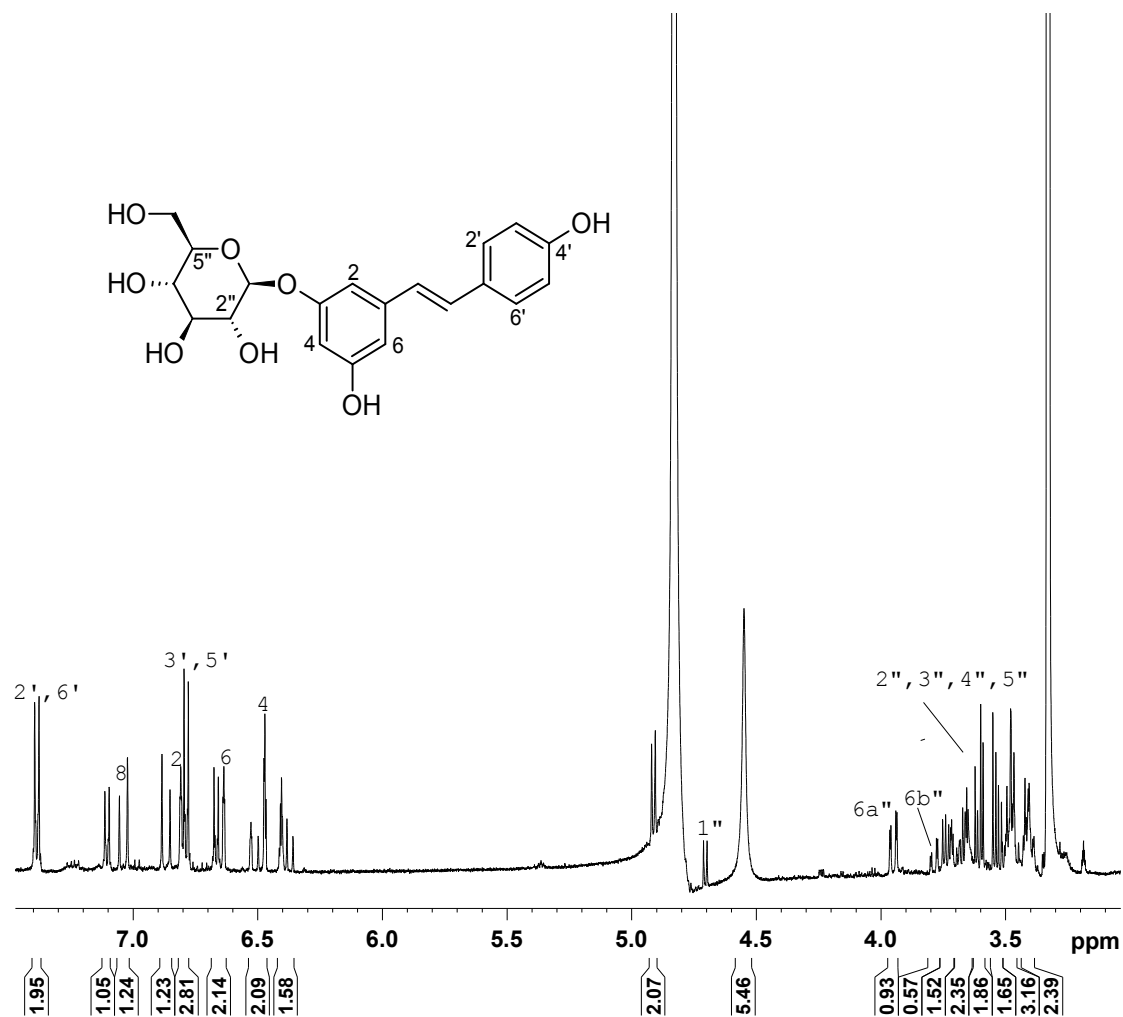
Plate 15b: ^{13}C NMR spectrum of *trans*-resveratrol glucoside (3.64) in CD$_3$OD
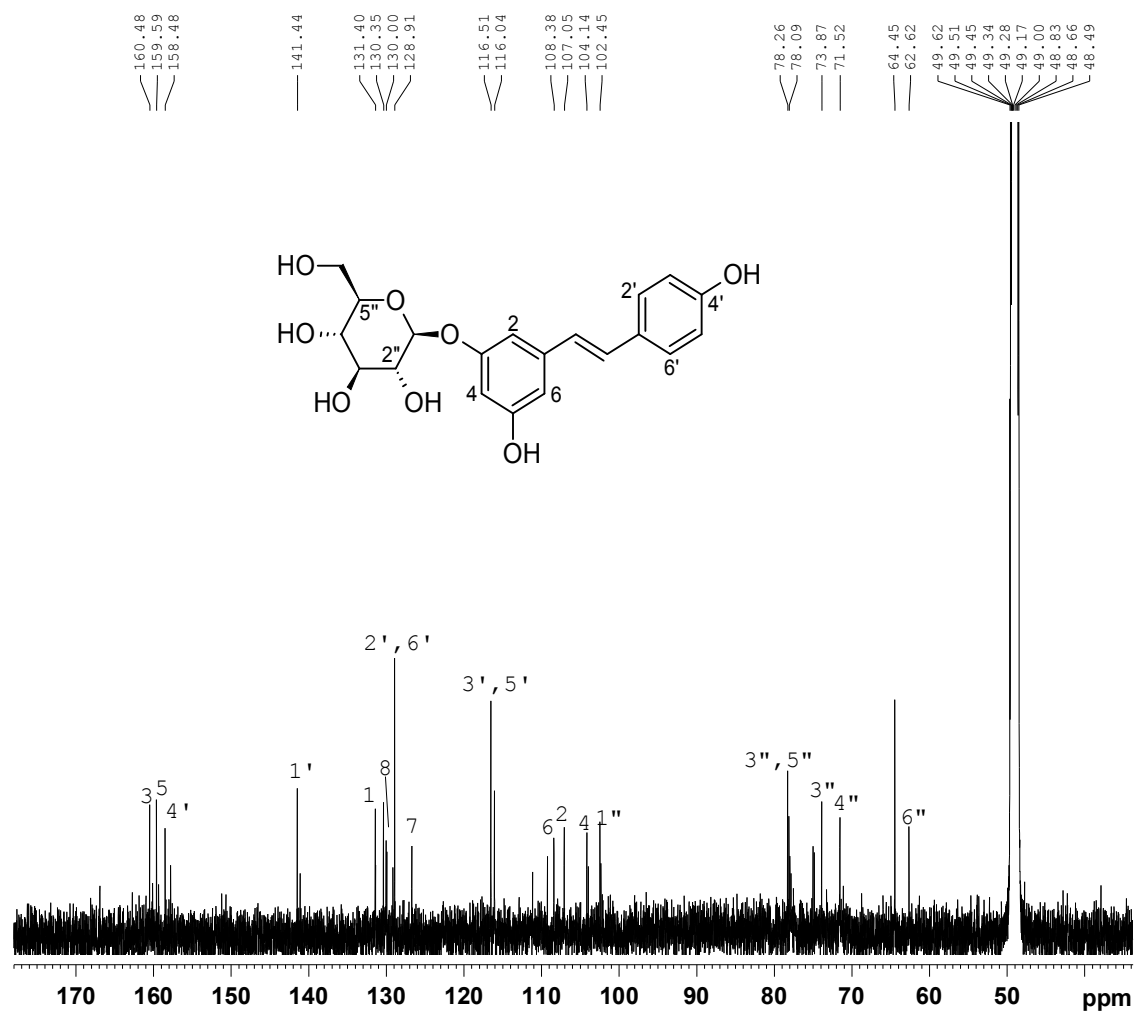
Plate 15c: COSY NMR spectrum of *trans*-resveratrol glucoside (3.64) in CD₃OD
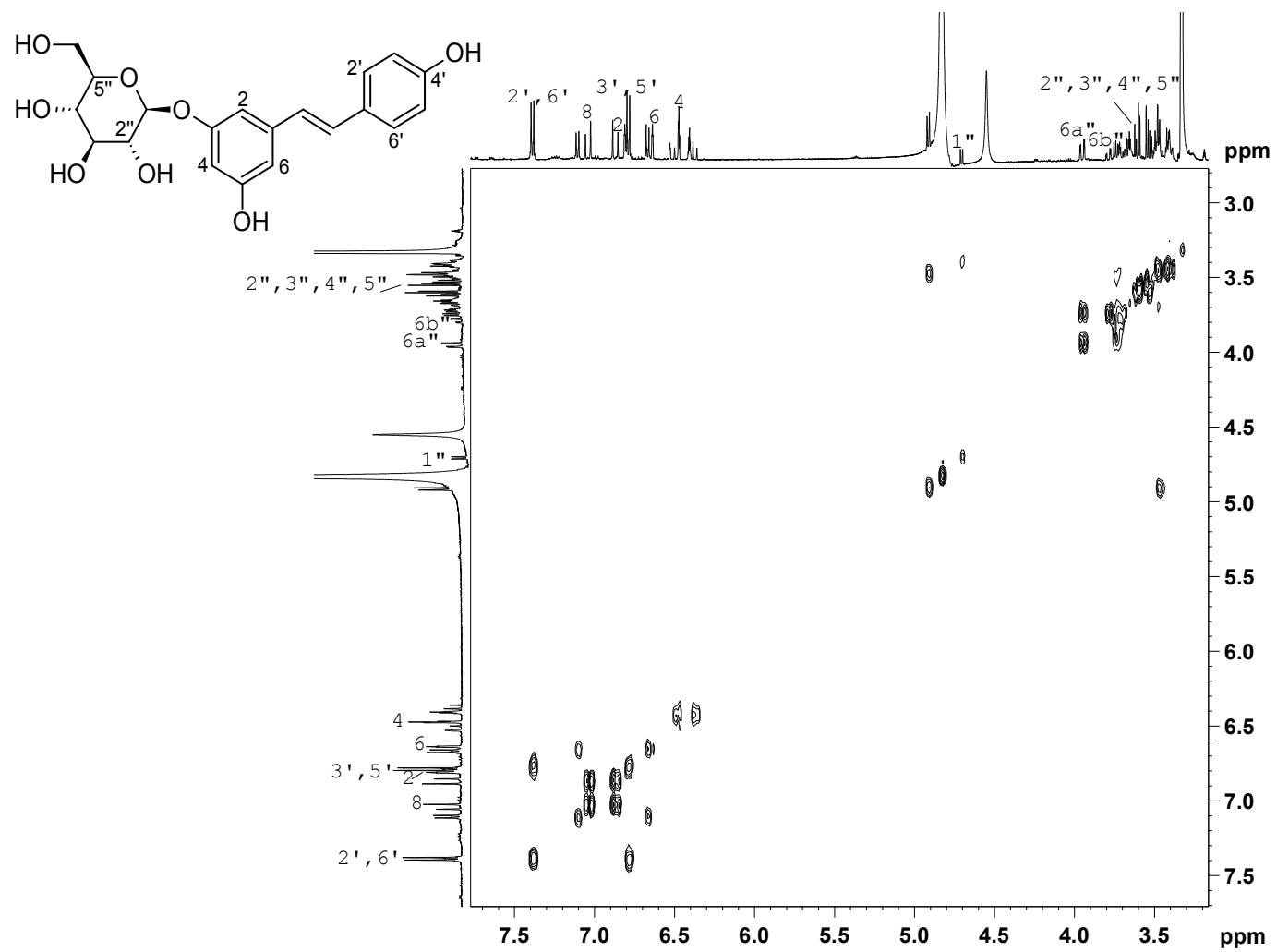
Plate 15d: DEPT NMR spectrum of *trans*-resveratrol glucoside (3.64) in CD₃OD
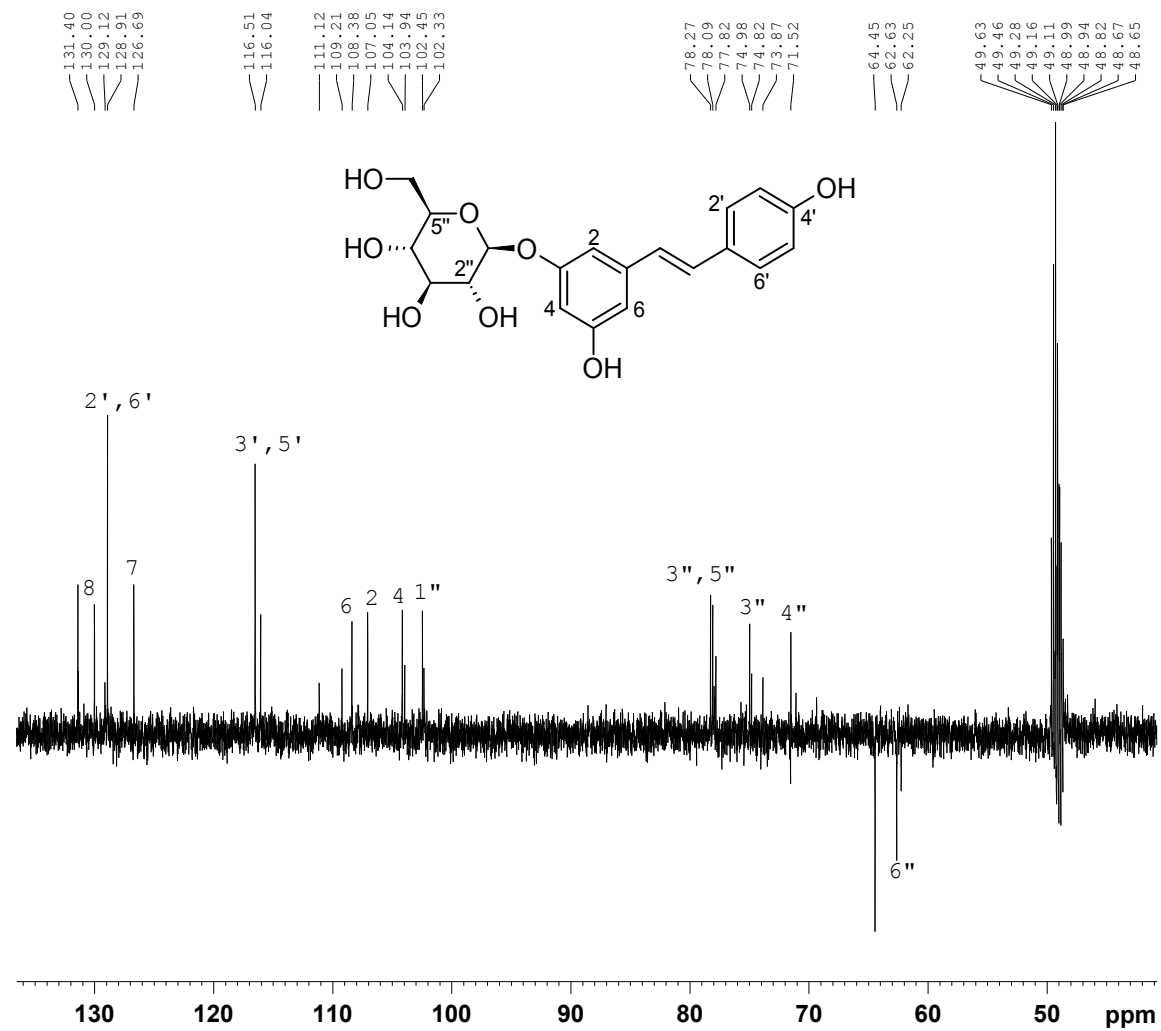
Plate 15e: HSQC NMR spectrum of *trans*-resveratrol glucoside (3.64) in CD₃OD
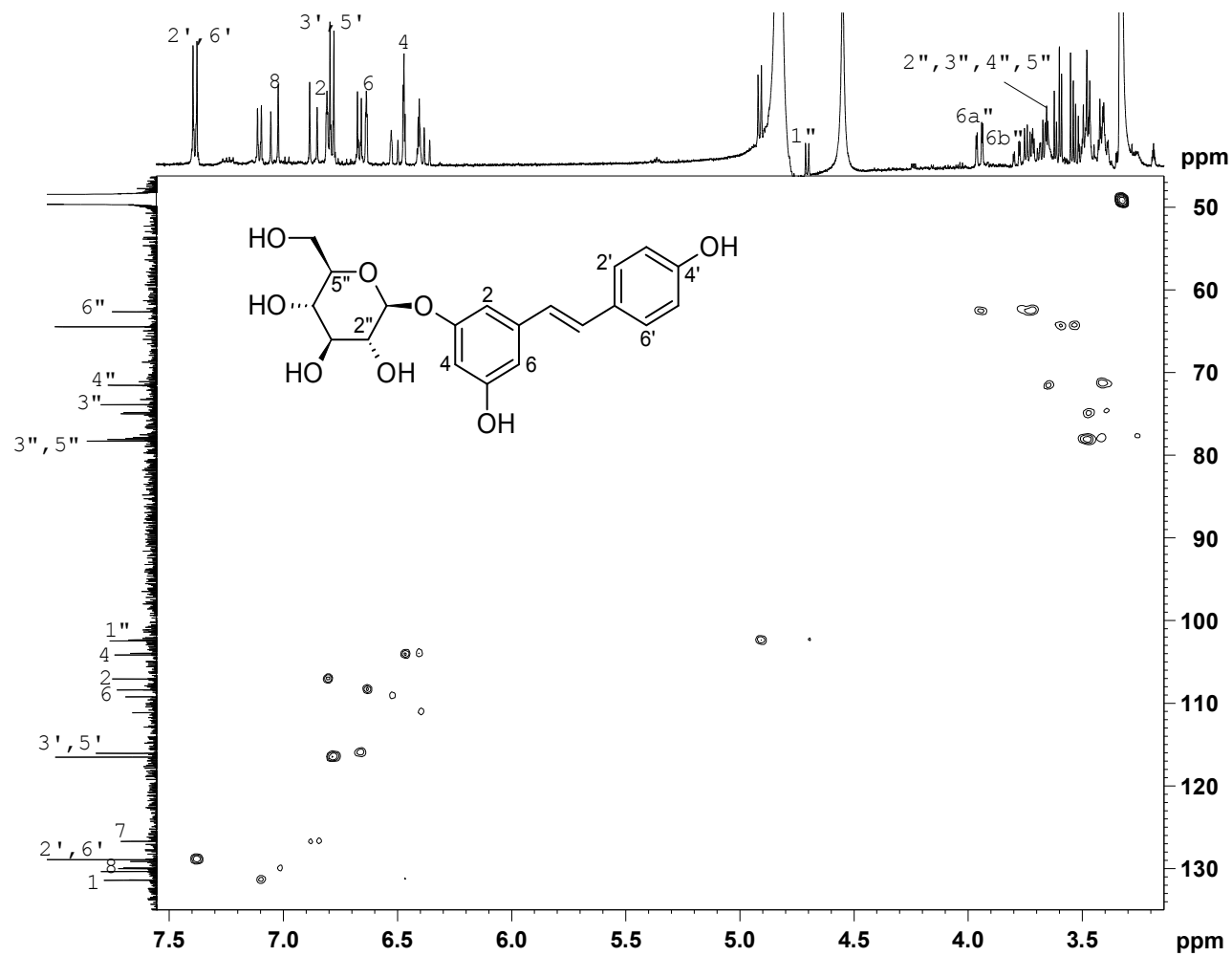
Plate 15f: HMBC NMR spectrum of *trans*-resveratrol glucoside (3.64) in CD₃OD
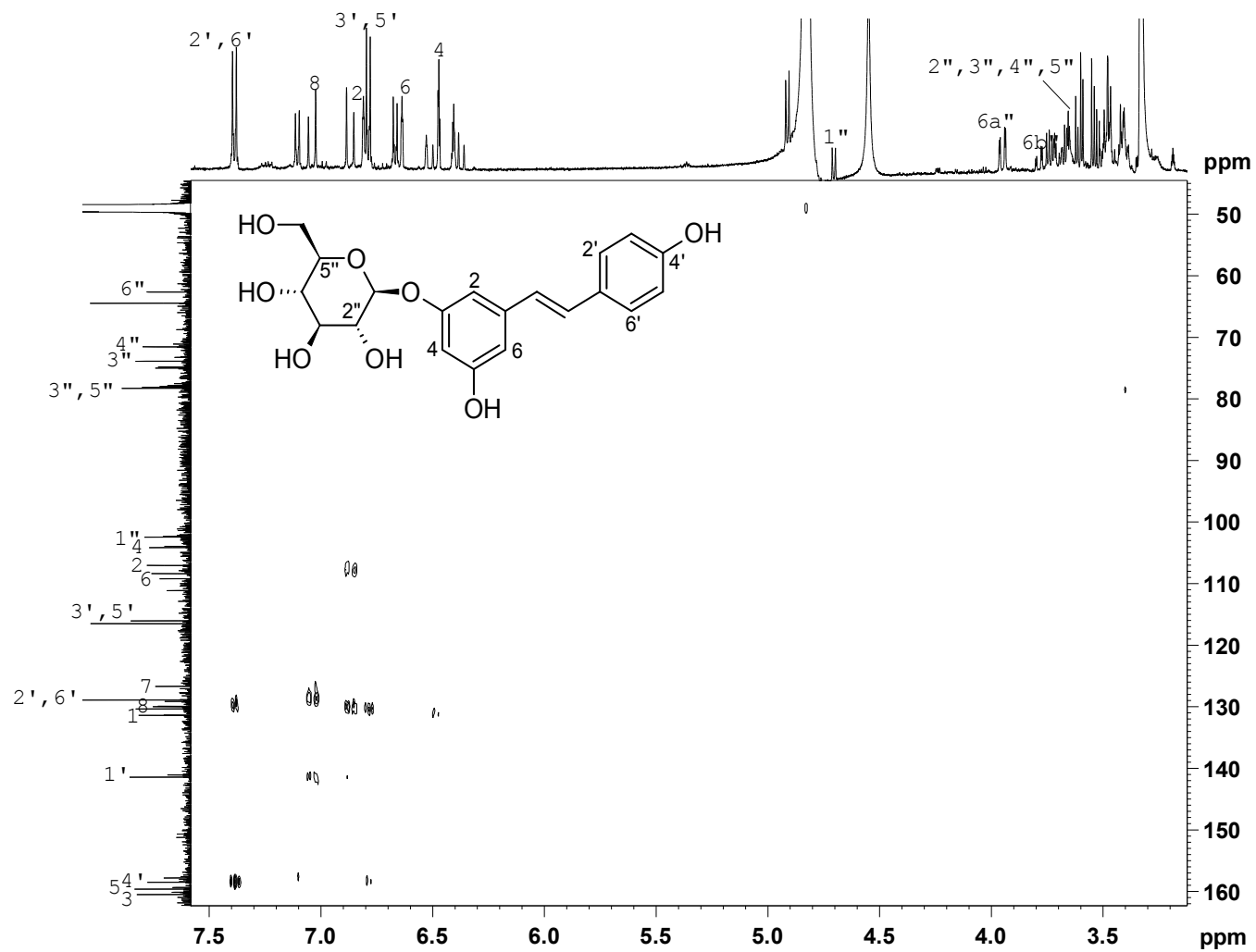
Plate 16a: ^{1}H NMR spectrum of asiatic acid-arjunolic acid mixture (3.65 & 3.66) in CD$_3$OD
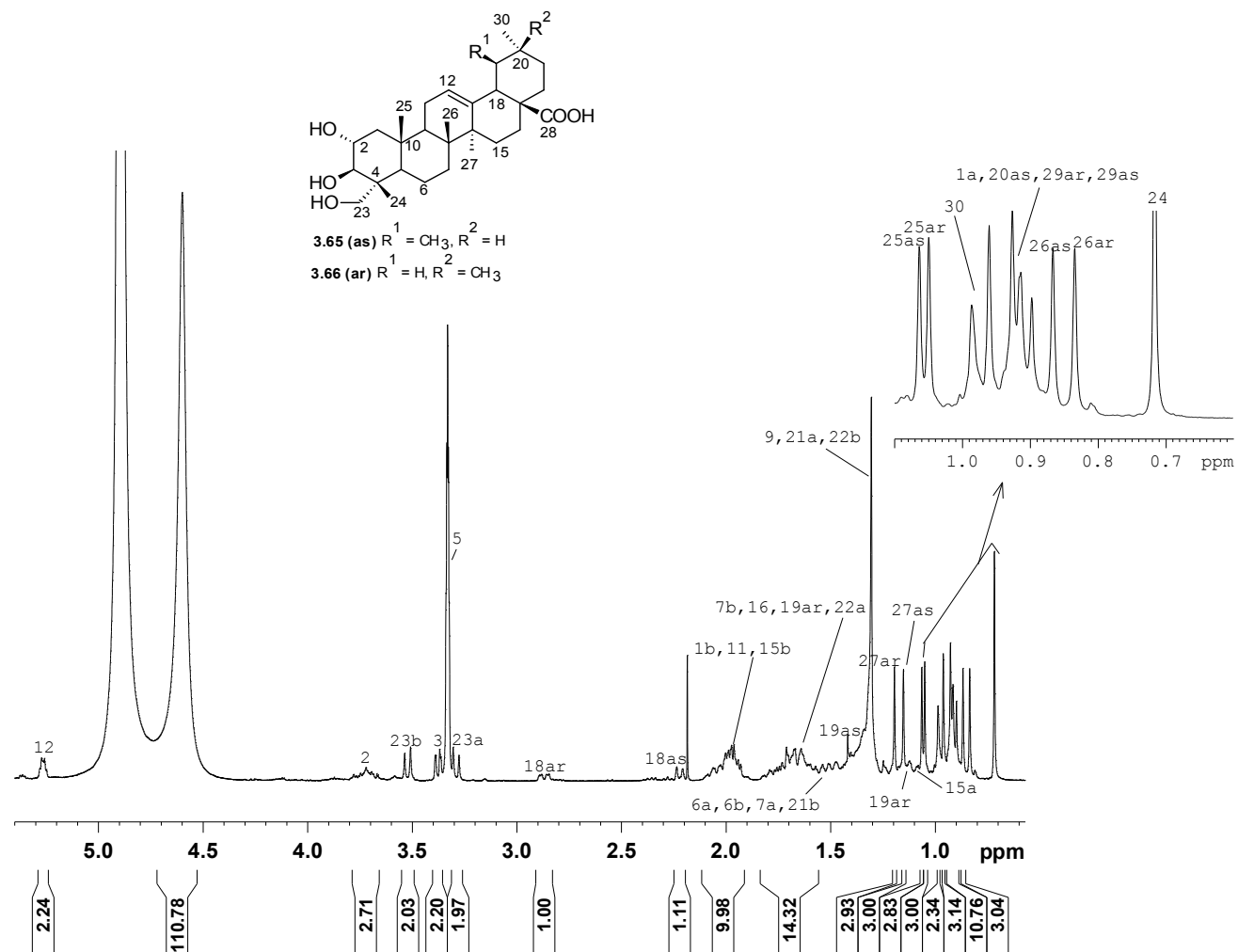
Plate 16b: ^{13}C NMR spectrum of asiatic acid-arjunolic acid (3.65 & 3.66) mixture in CD$_3$OD

3.65 (as) R^1 = CH$_3$, R^2 = H

3.66 (ar) R^1 = H, R^2 = CH$_3$
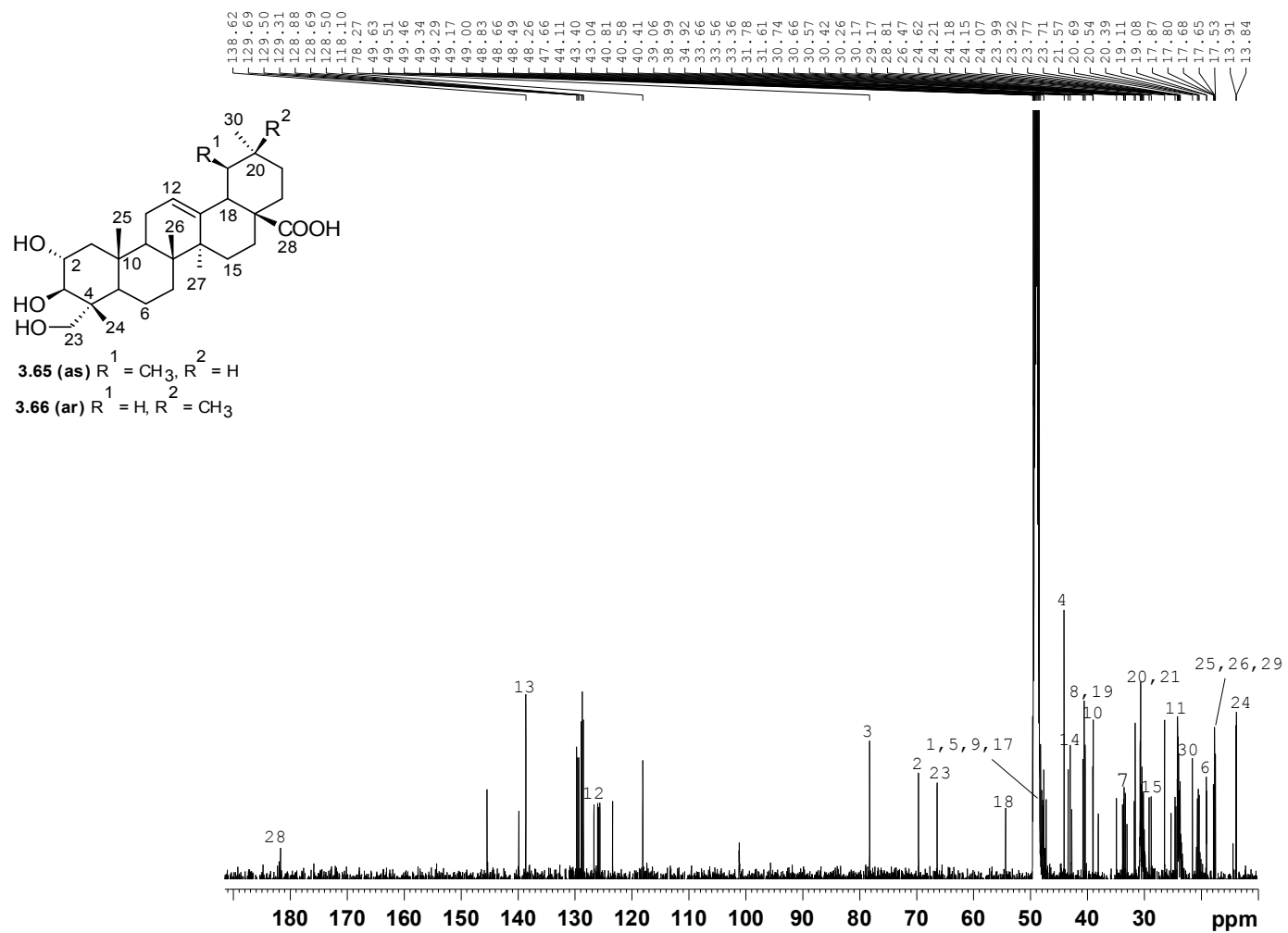
Plate 16c: COSY NMR spectrum of asiatic acid-arjunolic acid (3.65 & 3.66) mixture in CD₃OD
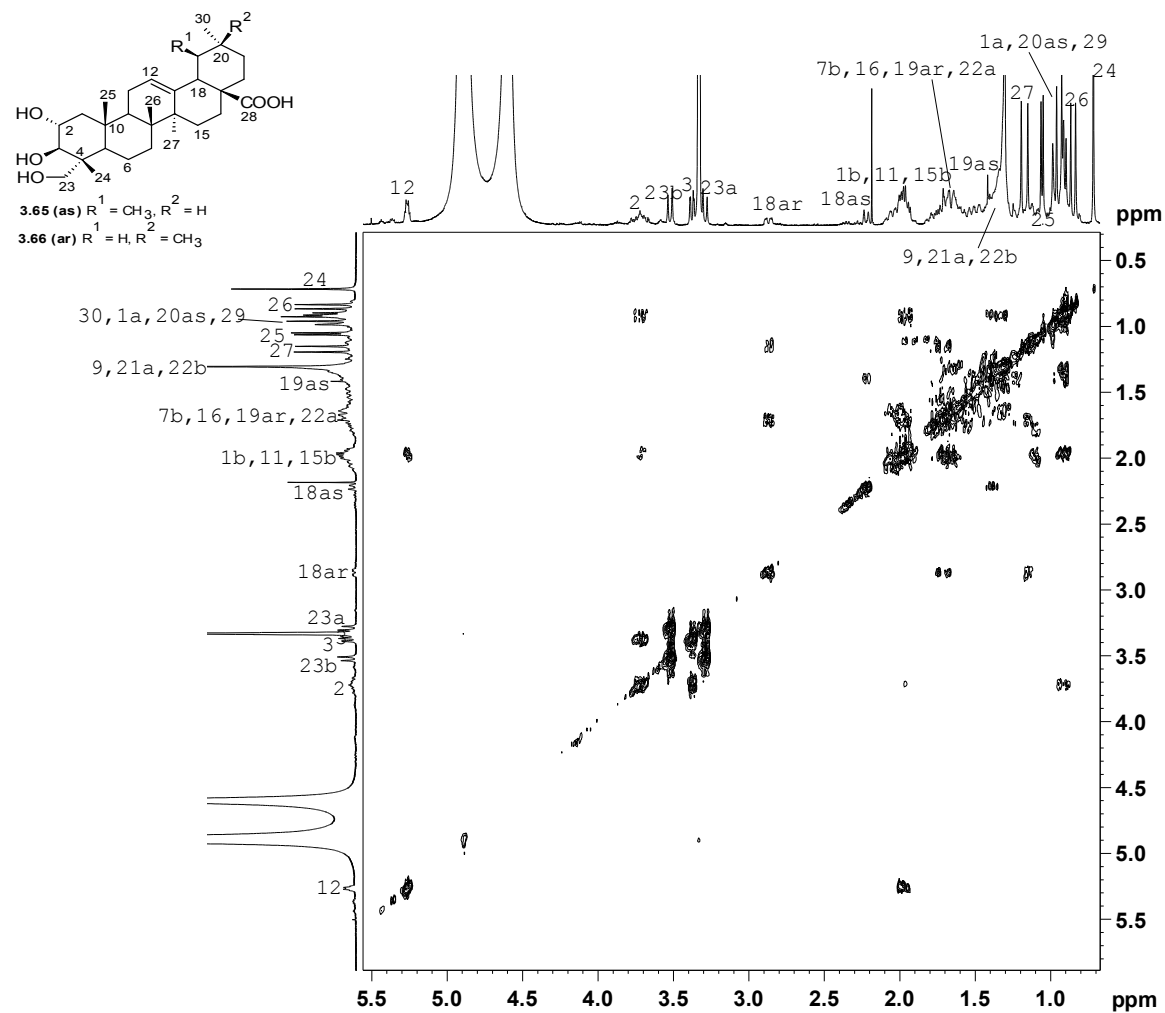
Plate 16d: DEPT NMR spectrum of asiatic acid-arjunolic acid (3.65 & 3.66) mixture in CD₃OD

3.65 (as) R^1 = CH$_3$, R^2 = H

3.66 (ar) R^1 = H, R^2 = CH$_3$
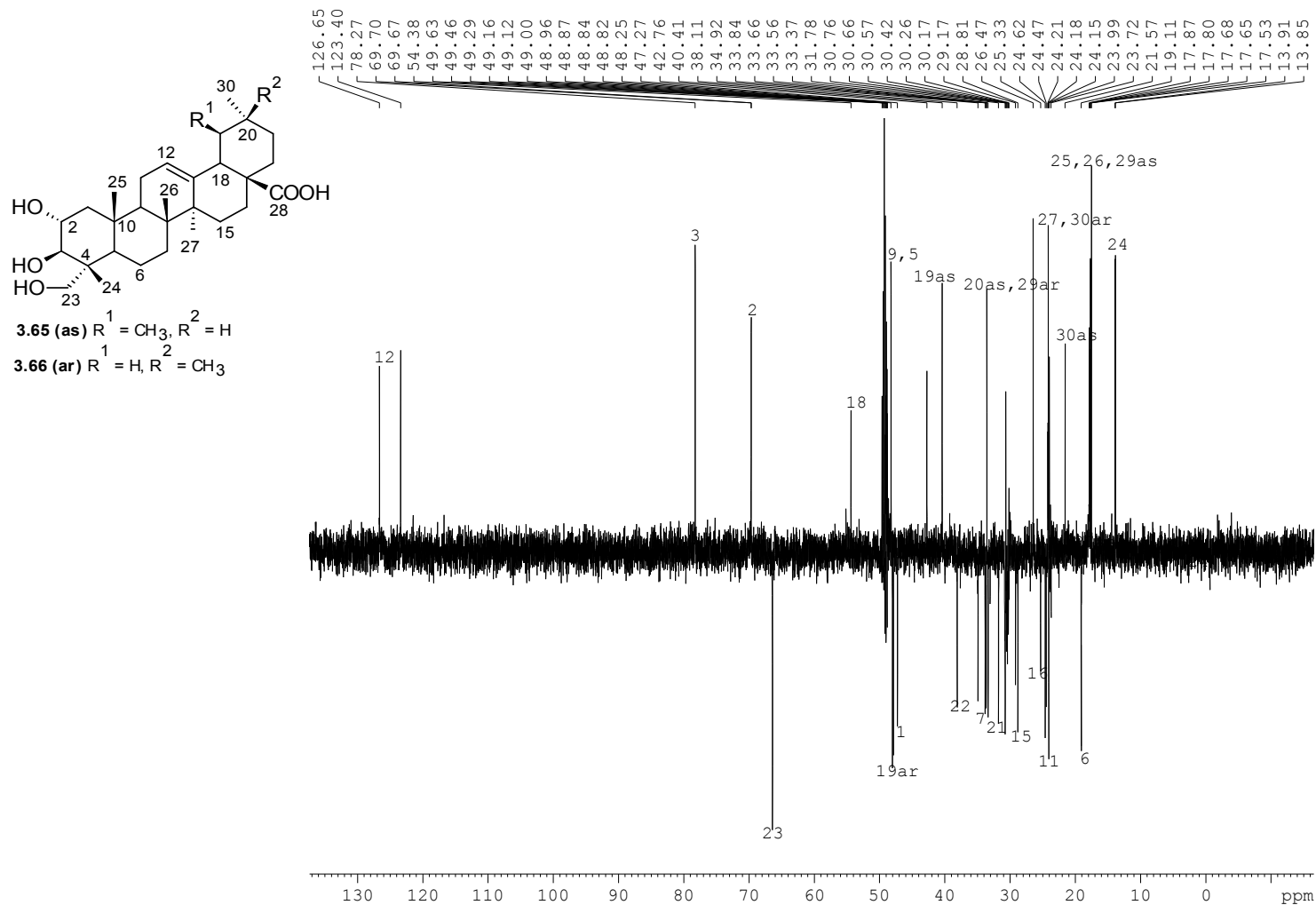
Plate 16e: HSQC NMR spectrum of asiatic acid-arjunolic acid (3.65 & 3.66) mixture in CD₃OD

3.65 (as) R^1 = CH$_3$, R^2 = H
3.66 (ar) R^1 = H, R^2 = CH$_3$
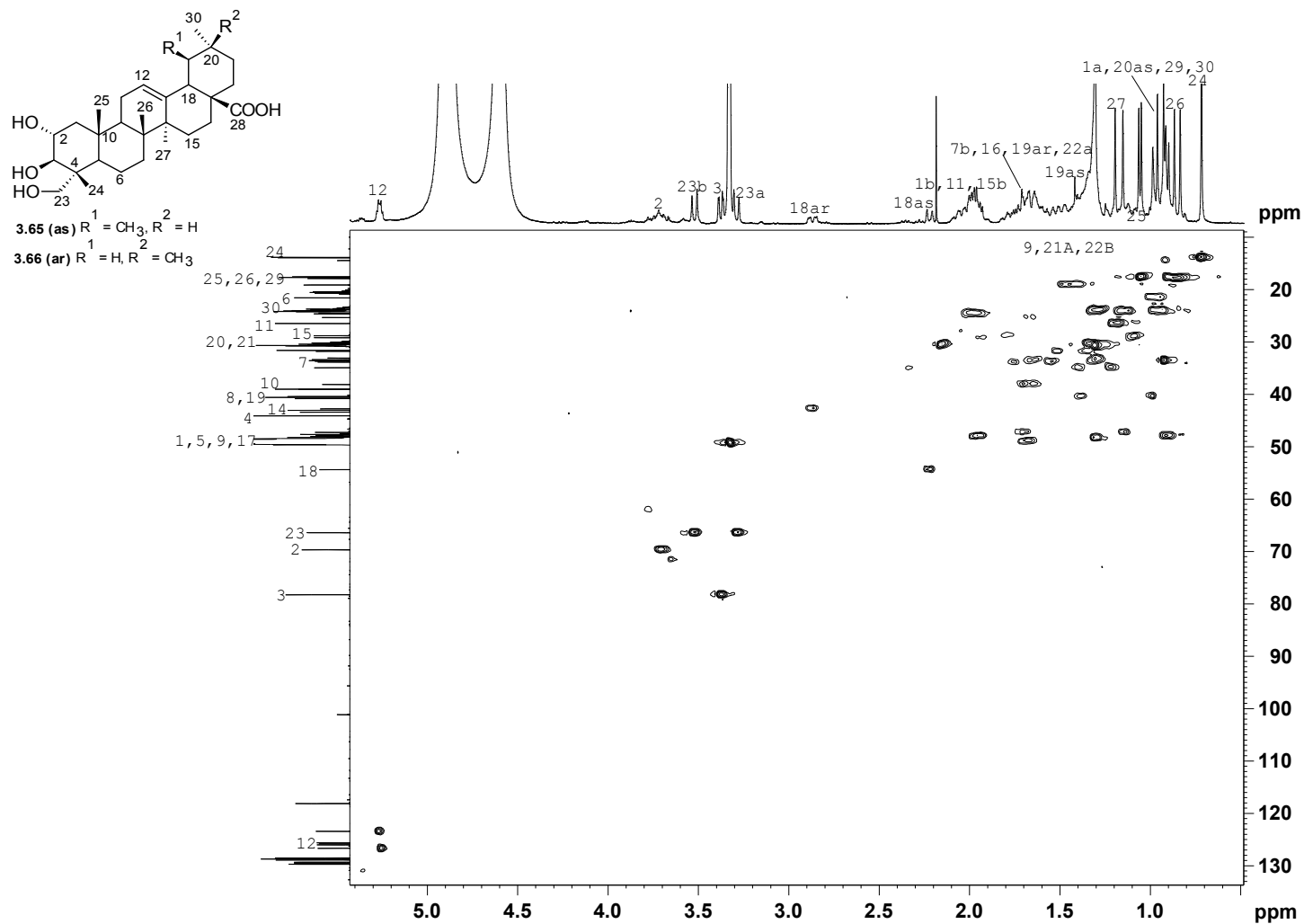
Plate 16f: HMBC NMR spectrum of asiatic acid-arjunolic acid (3.65 & 3.66) mixture in CD₃OD

3.65 (as) R¹ = CH₃, R² = H

3.66 (ar) R¹ = H, R² = CH₃
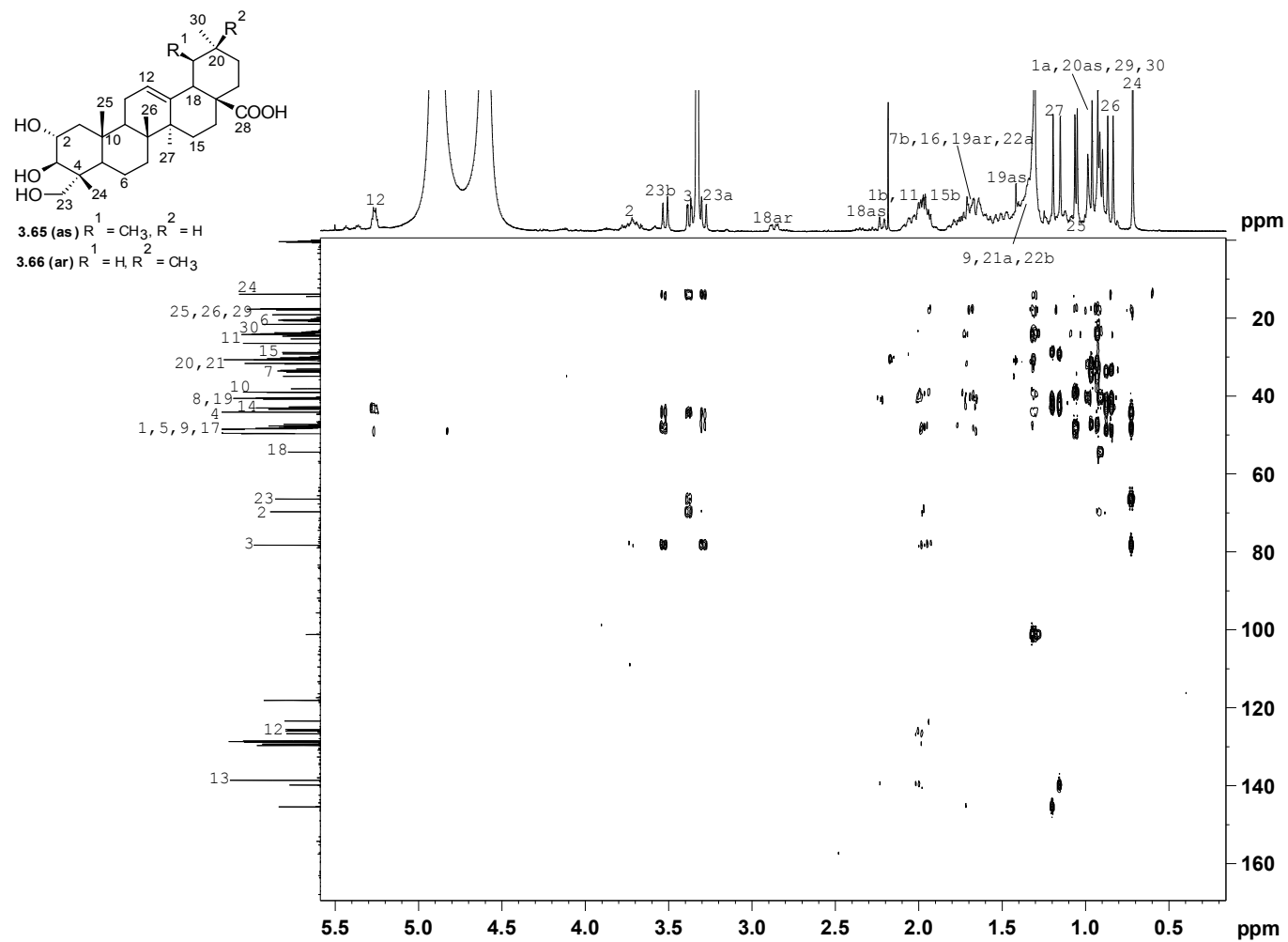
Plate 17a: ^{1}H NMR spectrum of β-sitosterol (3.22) in CDCl$_3$
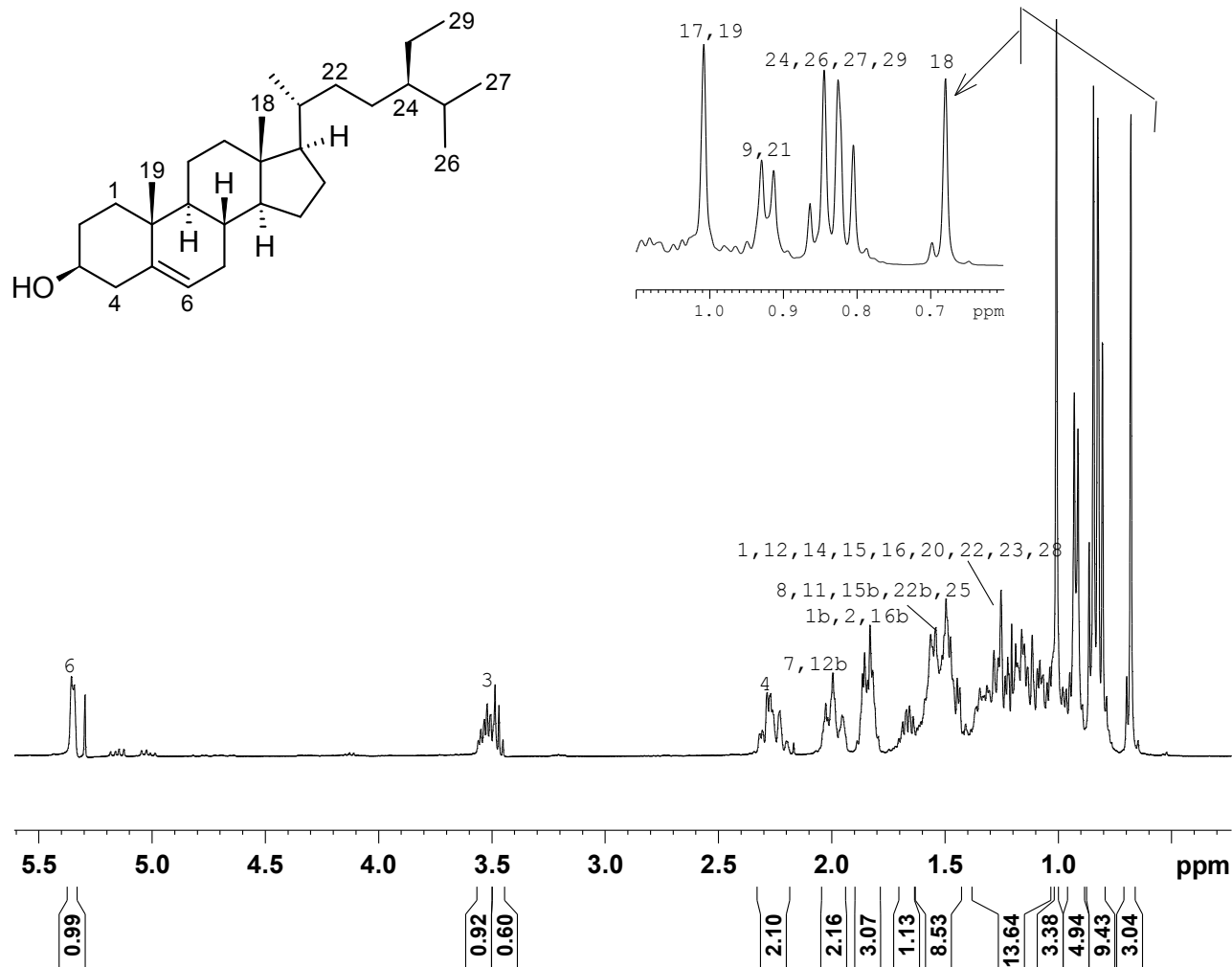
Plate 17b: ^{13}C NMR spectrum of β-sitosterol (3.22) in CDCl₃
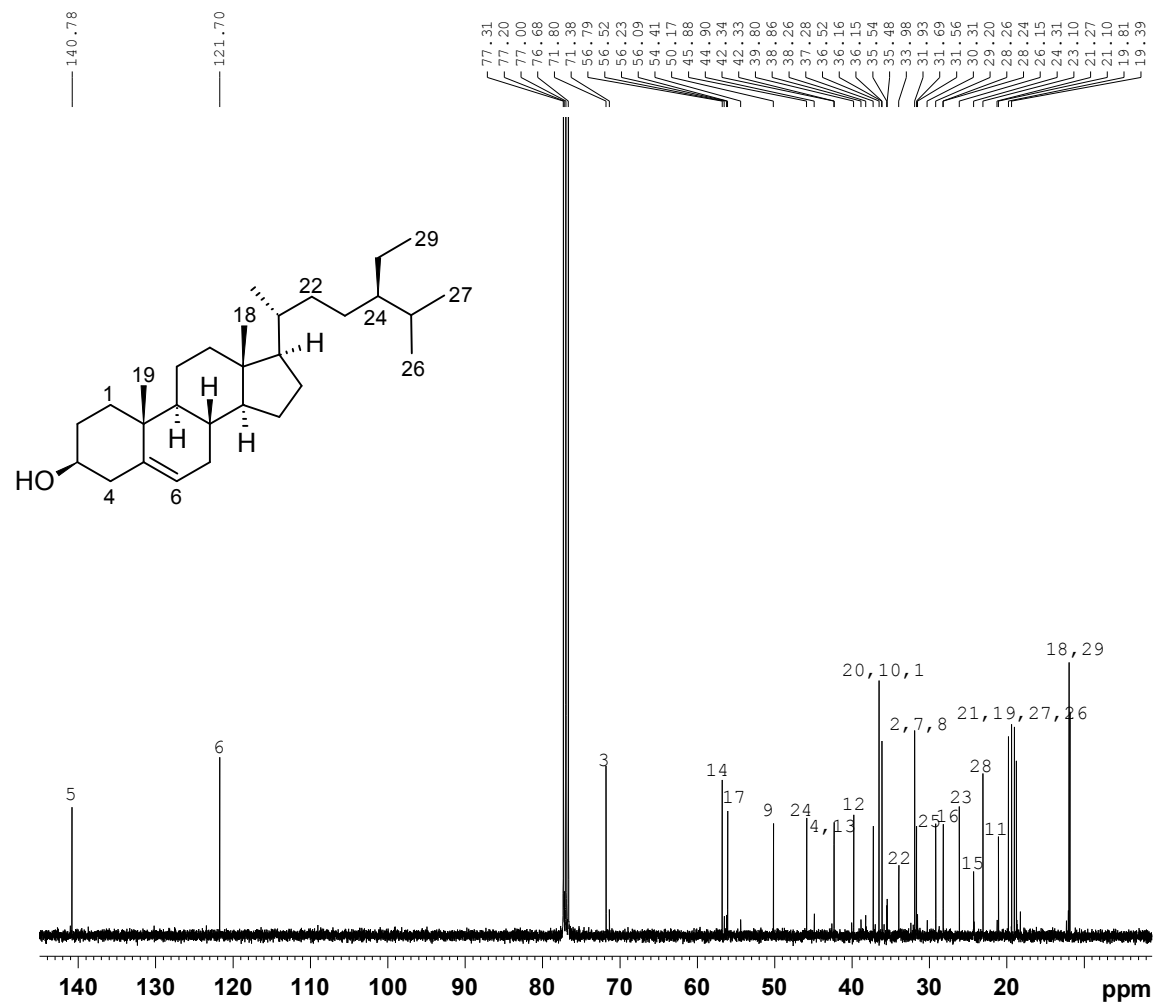
Plate 17c: COSY NMR spectrum of β-sitosterol (3.22) in CDCl₃

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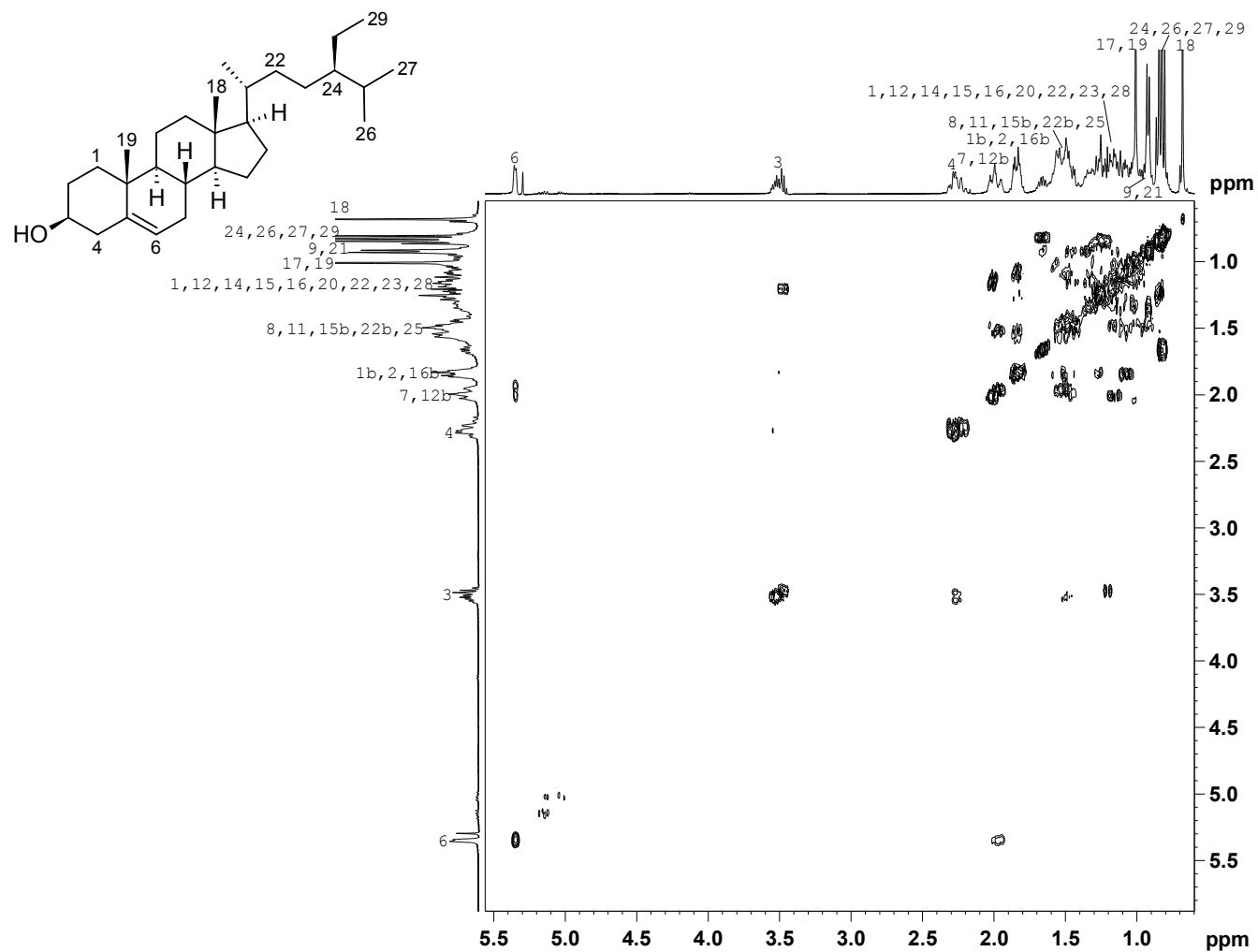
Plate 17d: DEPT NMR spectrum of β-sitosterol (3.22) in CDCl₃
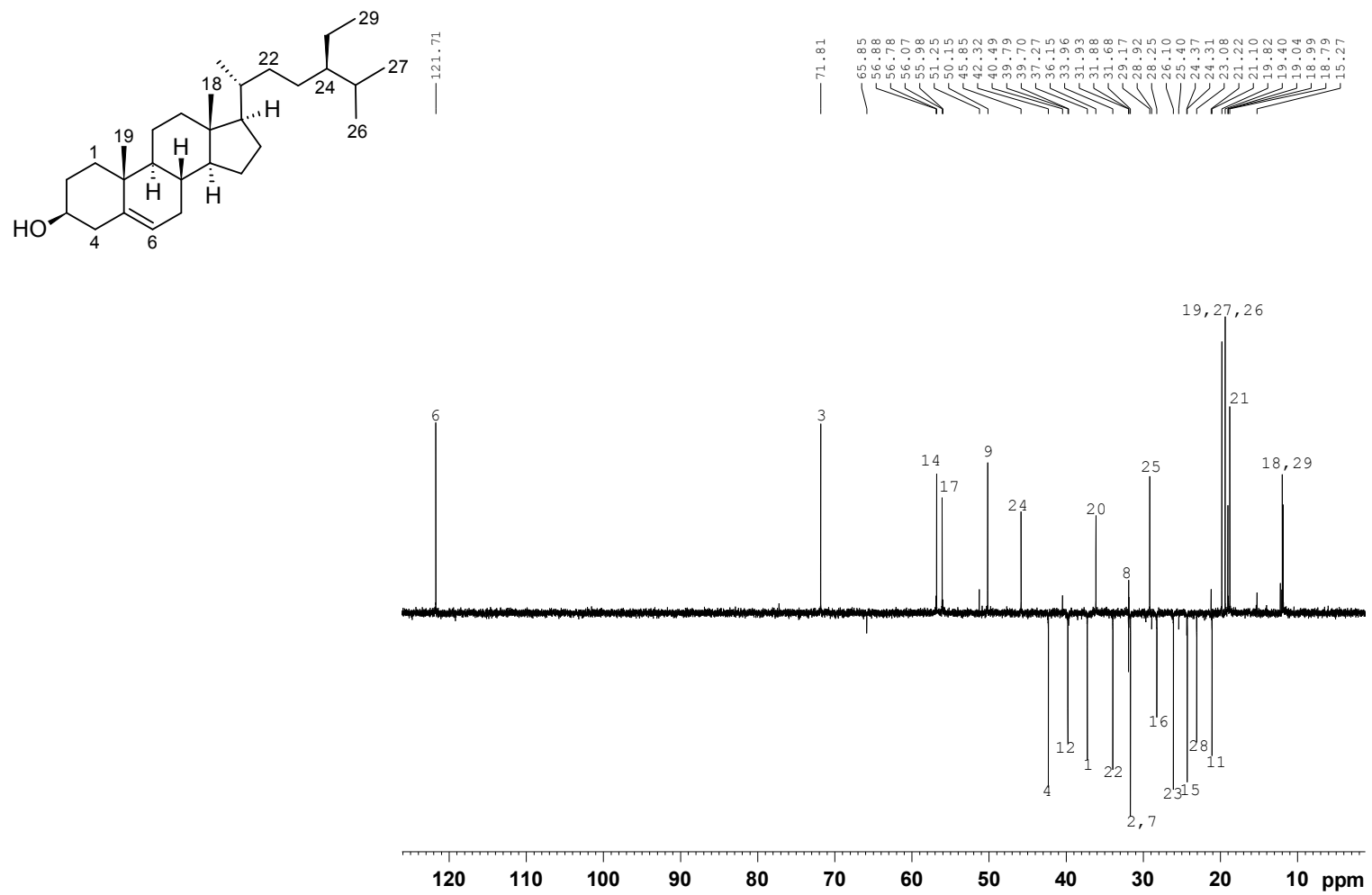
Plate 17e: HSQC NMR spectrum of β-sitosterol (3.22) in CDCl₃
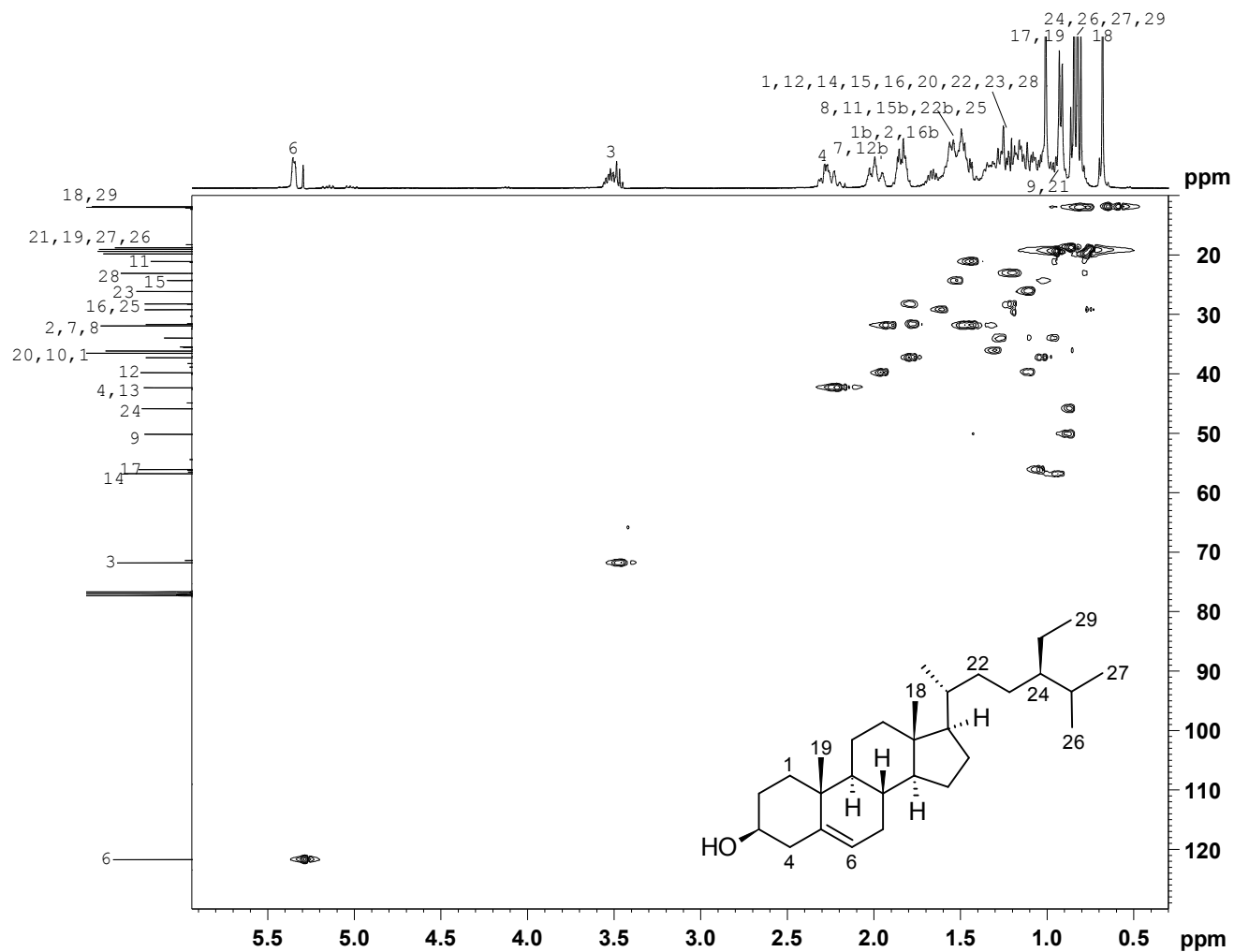
Plate 17f: HMBC NMR spectrum of β-sitosterol (3.22) in CDCl₃
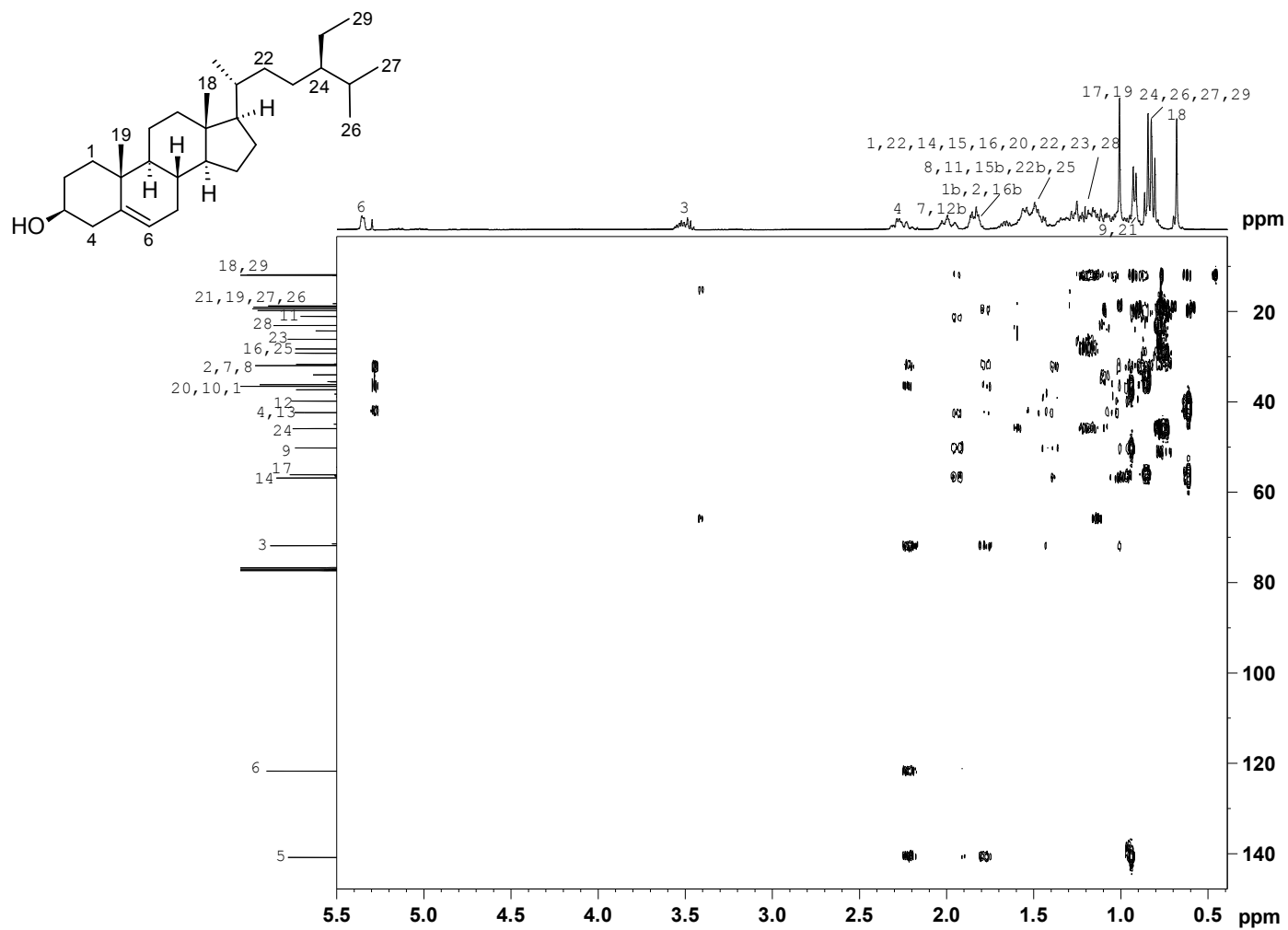
Plate 18a: ^{1}H NMR spectrum of linoleic acid (3.18) in CDCl$_3$
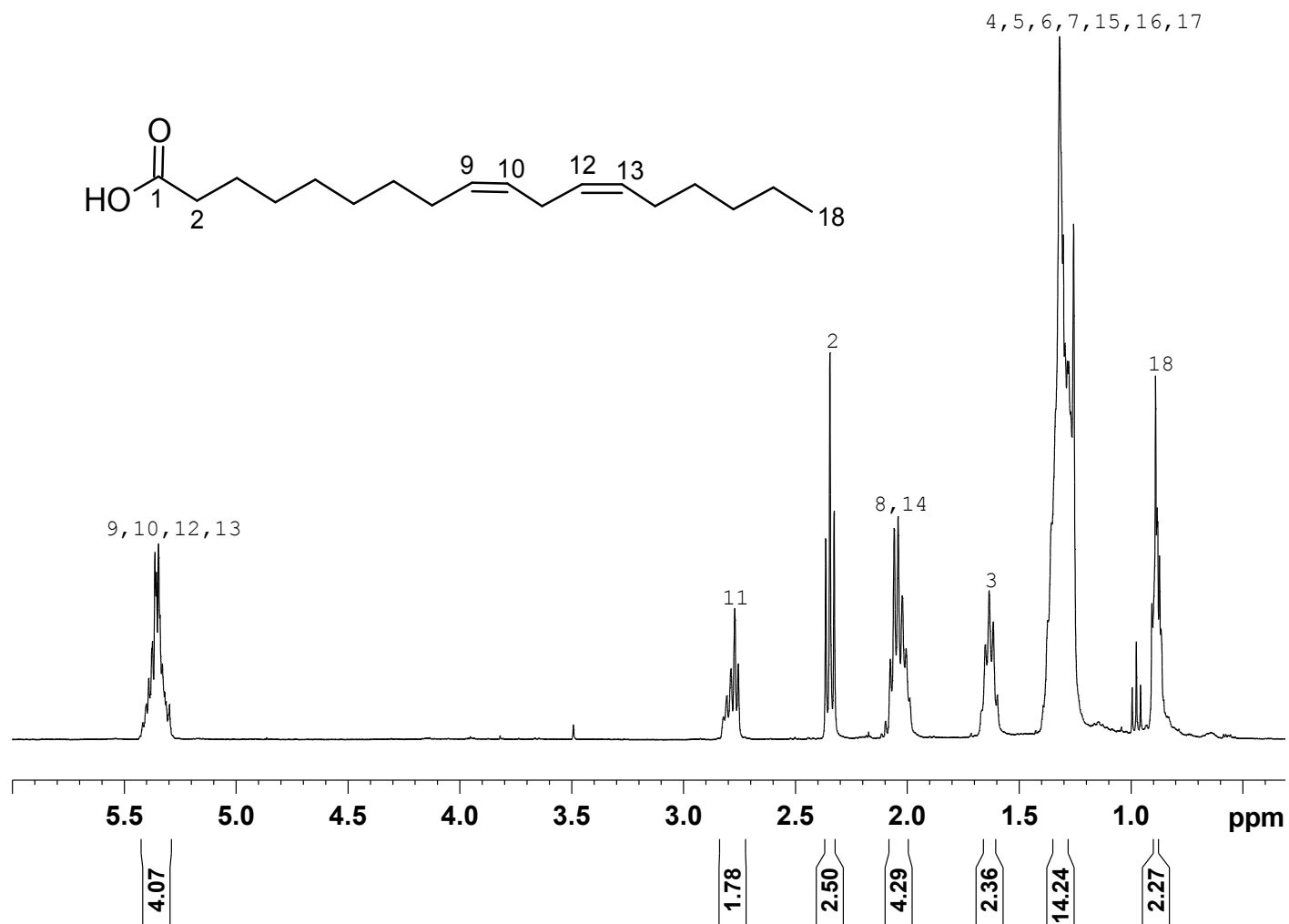
Plate 18b: ^{13}C NMR spectrum of linoleic acid (3.18) in CDCl$_3$
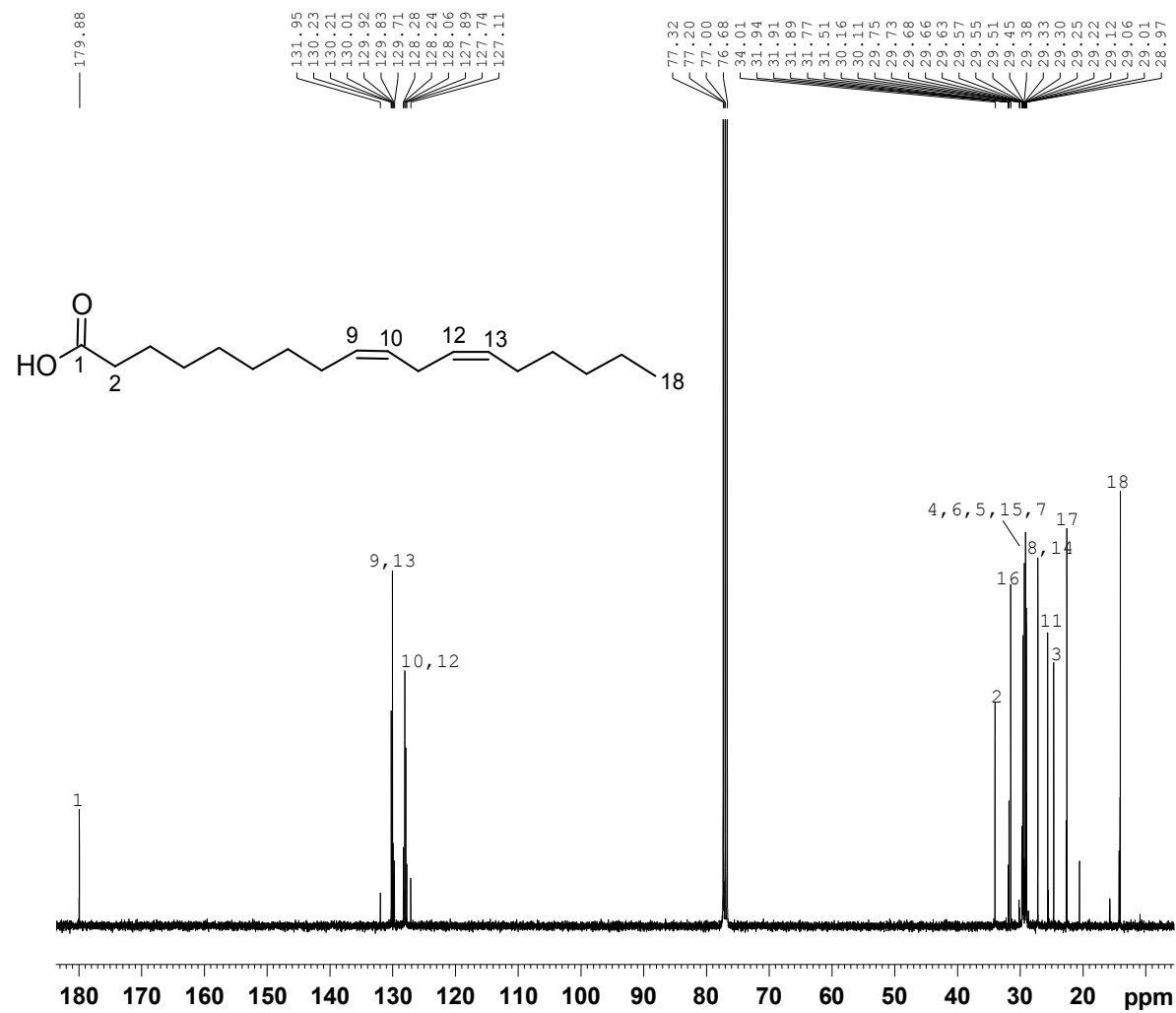
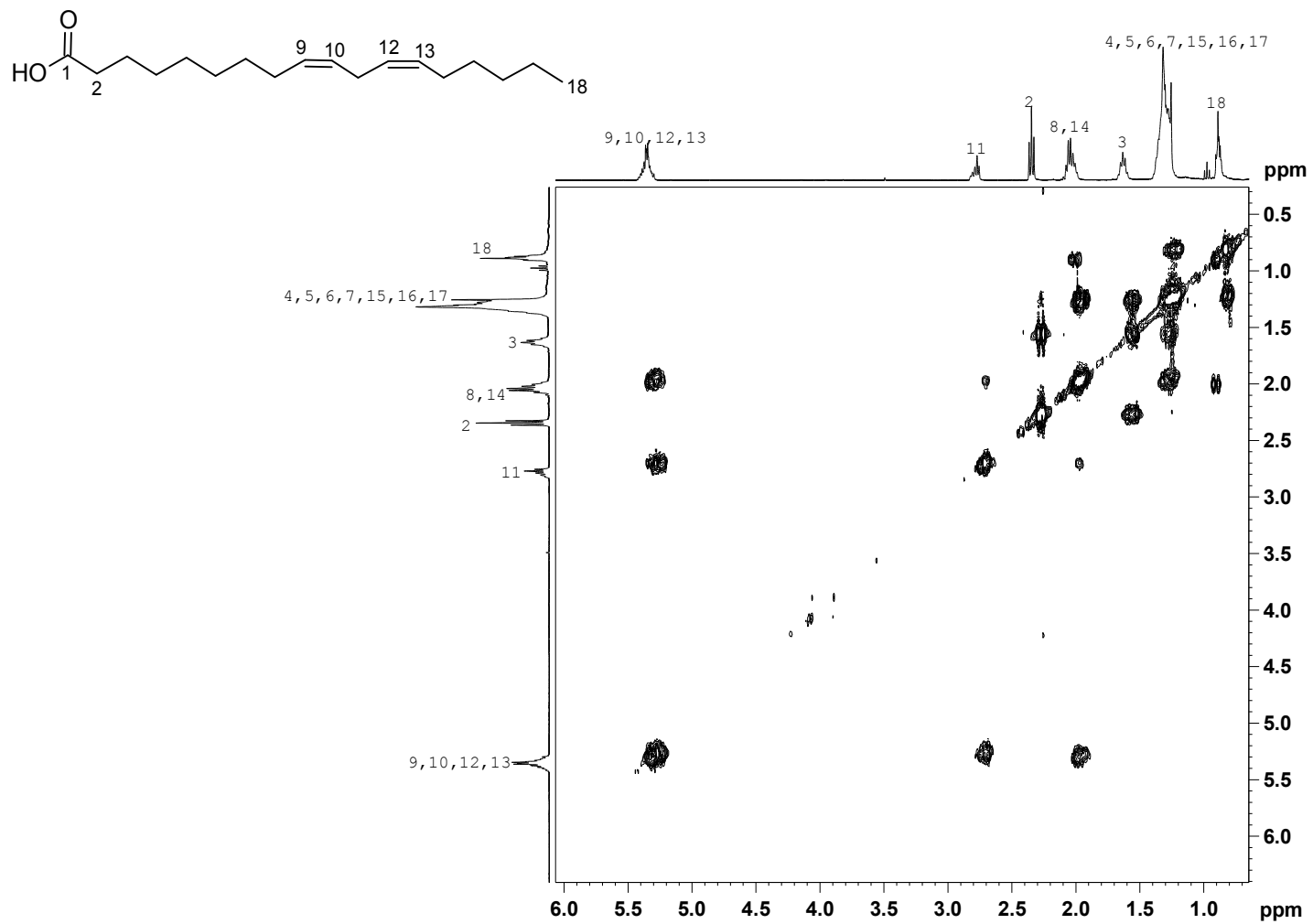# Plate 18c: COSY NMR spectrum of linoleic acid (3.18) in CDCl₃

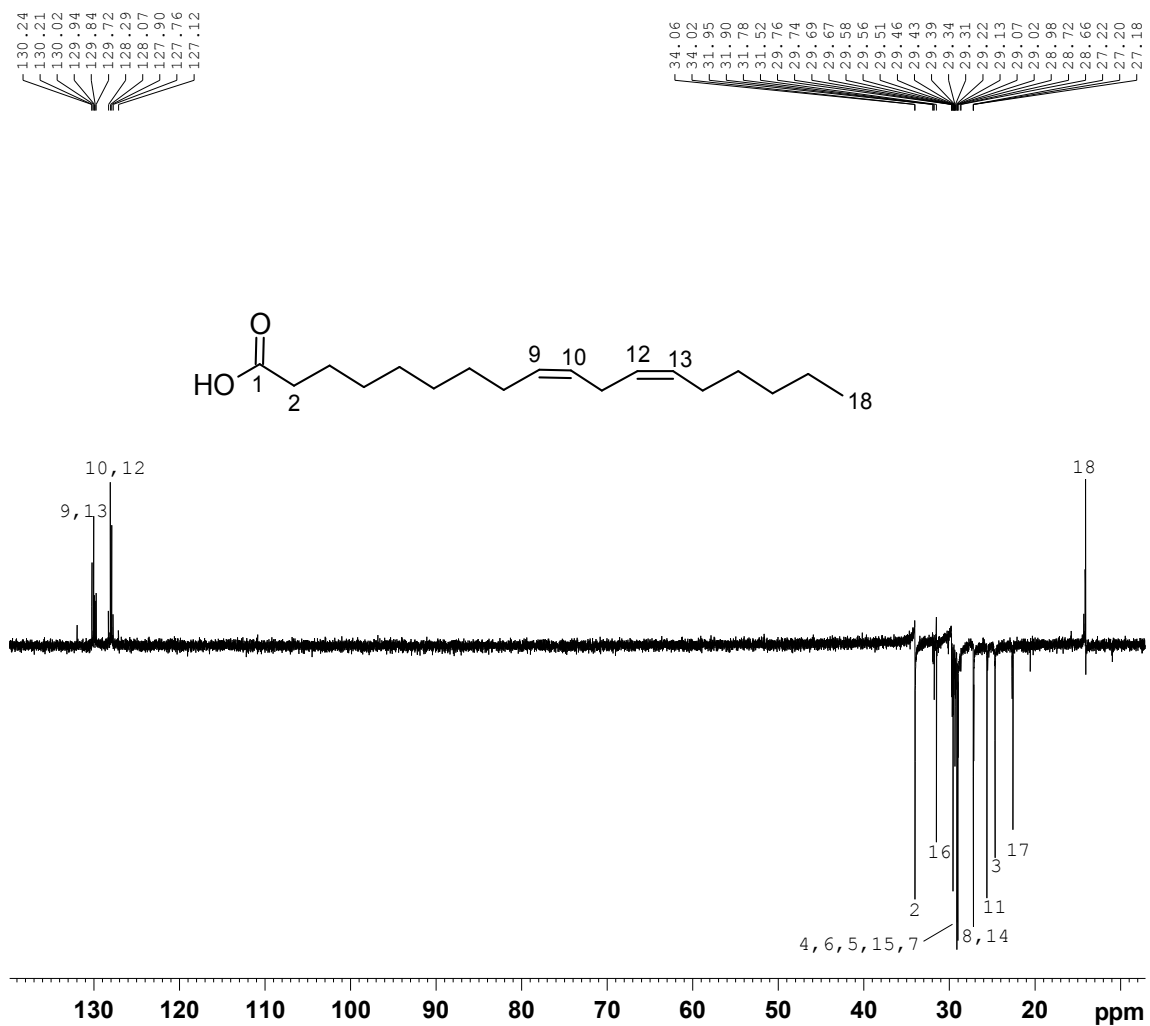# Plate 18d: DEPT NMR spectrum of linoleic acid (3.18) in CDCl₃

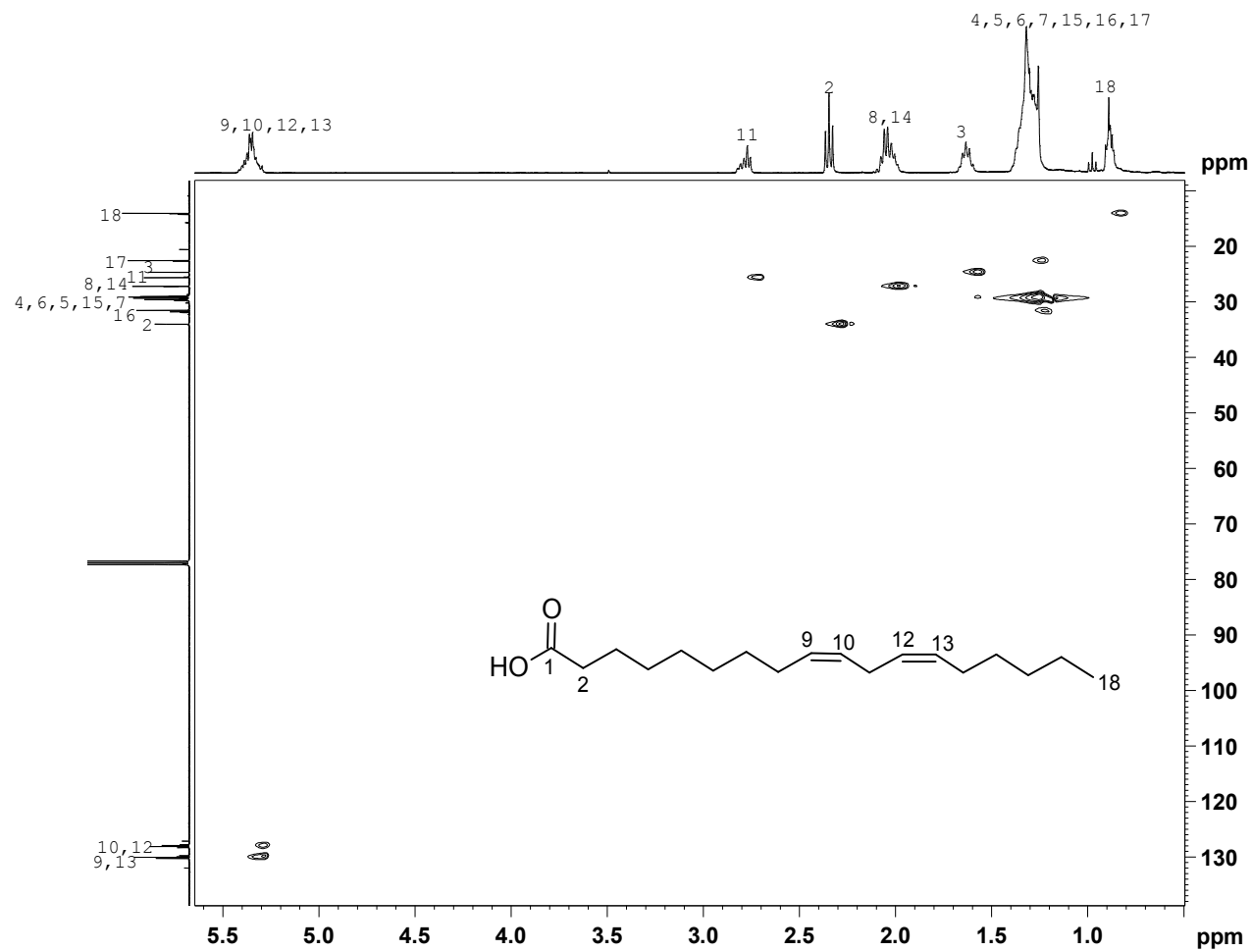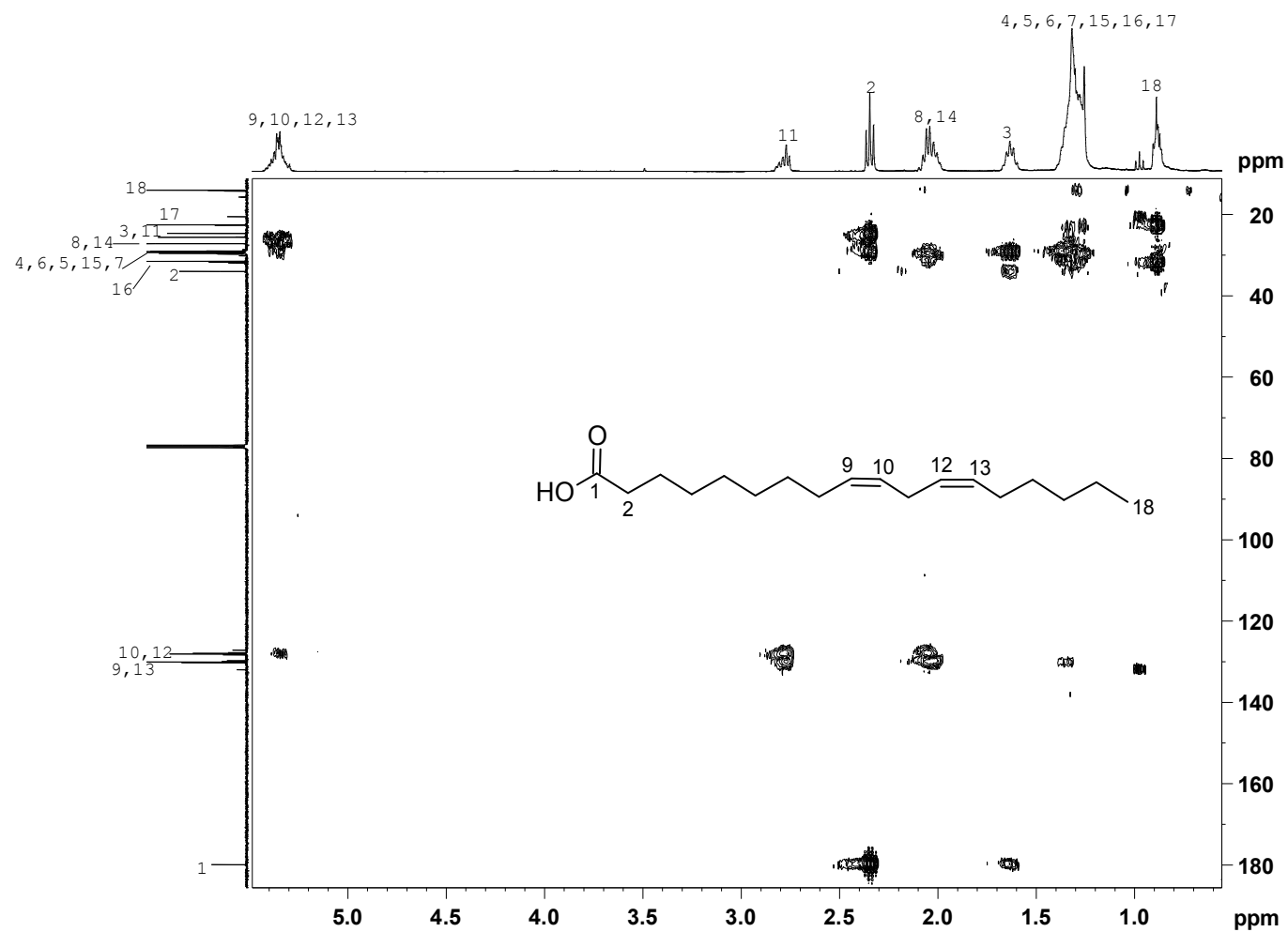# Plate 18e: HSQC NMR spectrum of linoleic acid (3.18) in CDCl₃

Plate 18f: HMBC NMR spectrum of linoleic acid (3.18) in CDCl₃

Appendix 2

Conference presentations

2013   12th International Chemistry Conference Africa (7-12 July, University
            of Pretoria, South Africa).

            Poster presentation: Flavones and Diterpenes from *Elytropappus*
            rhinocerotis.

2014   2nd International Symposium on Natural Products (23-25 September,
            Cape Town, South Africa)
            Oral presentation: Chemical constituents from *Rhoicissus tridentata*:
            structural and activity studies (Certificate received for one of the three
            best presentations).