UNIVERSITY OF NAIROBI

PHYTOCHEMICAL INVESTIGATION OF *TURRAEA ROBUSTA*, *TURRAEA NILOTICA* AND *EKEBERGIA CAPENSIS FOR* ANTIPLASMODIAL AND CYTOTOXIC COMPOUNDS.

BY

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2014

DECLARATION

I declare tha t this thesis is my original work and has not been submitted elsewhere for examination or award of a degree.

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DEDICATION

This thesis is dedicated to my husband **Kimani Maina** for enduring this long process with me, always offering support and love.

our children

Maina Kimani

and

 Nyawira Kimani

Though too young to understand what mum was going through, you supported me in your own little ways. I pray that this will serve as an encouragement as you walk through your academic pathways.

ACKNOWLEDGEMENT

ABSTRACT

In an effort to identify antiplasmodial and/or cytotoxic secondary metabolites, three African medicinal plants, *Ekebergia capensis*, *Turraea nilotica* and *Turraea robusta* were investigated for antiplasmodial and cytotoxic compounds. Except for *T. nilotica*, the other plants were selected on the basis of previous reports on antiplasmodial activities of crude extracts. A combination of different chromatographic techniques including preparative HPLC was employed in isolation of compounds. The characterization of compounds was done using 1D and 2D NMR as well as MS analyses. The crude extracts and the isolated compounds were evaluated for antiplasmodial activity against the chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* using a semiautomated micro dilution technique which measures the ability of the compounds to inhibit the incorporation of (G-^{3}H) hypoxanthine into the malaria parasite. They were also evaluated for cytotoxicity properties against African green monkey kidney (vero), using a rapid colourimetric assay that employs 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as a viability indicator. Selectivity index (SI) defined as IC$_{50}$ (Vero cells) / IC$_{50}$ (*P. falciparum*) was also determined.

 A total of twenty six compounds were isolated. From the stem bark of *Turraea robusta* seven compounds were isolated of which acetoxy-7-deacetylazadirone (**28**) and 11-*epi*-toonacilin (**62**) were new to the species whereas azadironolide (**192**) was new to the genus. *Turraea nilotica* leaves, root and stem bark yielded twelve compounds of which five [mzikonone (**17**), azadirone (**19**), acetoxy-7-deacetylazadirone (**28**), 1α,3α-diaacety-7α-tigloylvilasinin (**40**) and hipidol B (**96**)] were new to the species and four [toonapubesins (**194**) and phytosterols (**195**-**197**)] were new to the genus. From the root bark and leaves of *Ekebergia capensis* ten compounds were isolated with two glycoflavonoids (**199**-**200**) being new to the genus and a new natural product, 3-oxo-12β-hydroxy-oleanan-28,13β-olide (**198**).

Azadironolide (**192**) displayed the highest antiplasmodial activity with an IC$_{50}$ of 1.1 and 2.4 μM against W2 and D6 strains respectively, with a SI > 10.5. This compound can be evaluated further for its antimalarial activity in a mouse model. The rest of the compounds displayed moderate to low antiplasmodial activities with IC$_{50}$ ranging 14.4-205 μM against the two *P. falciparum* strains with low selectivity index (< 10). The low SI values indicate that the observed moderate antiplasmodial activity may be due to general cytotoxicity rather than the activity against the parasites. This motivated further cytotoxicity investigation on cancerous cell lines, mouse breast cancer (4T1), human larynx carcinoma (HEp2) and human breast cancer (MDA-MB-231). Six compounds were cytotoxic to cell lines 4T1 and HEp2 (IC$_{50}$ < 20 μM) with oleanonic acid (**160**) being the most cytotoxic to HEp2 cell line with an IC$_{50}$ value of 1.4 μM.

Interaction of oleanonic acid (**160**) isolated as a major compound from *E. capensis*, with five triterpenoids; 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18- tetracosatetraene (**151**), 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18 tetracosatetraene (**152**), ekeberin A (**158**), oleanolic acid (**159**) and 3-*epi*-oleanolic acid (**161**) was evaluated against vero and HEp2 cell lines. No appreciable synergism was observed.

Structure-Activity–Evaluation by acetylating niloticin (**25**), piscidinol A (**27**) and oleanolic acid (**159**) was carried out. The derivatives were evaluated for cytotoxicity activity where niloticin acetate (**201**) and piscidinol A diacetate (**203**) were of lower activity than the parent molecules while oleanolic acid acetate (**202**) was seven folds more active than oleanolic acid.

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LIST OF ABBREVIATIONS and ACRONYMS

∑FIC   Sum of fractional inhibition concentration

4T1   Mouse breast cancer

CC   Column chromatography

gCOSY   Gradient correlation spectroscopy

CQ   Chloroquine

DEPT   Distortionless Enhancement by Polarization Transfer

DDT   Dichlorodiphenyltrichloroethane

DMSO   Dimethyl sulfoxide

FBS   Fetal bovine serum

gHMBC   Gradient Heteronuclear Multiple Bond Coherence

gHSQC   Gradient Heteronuclear Single QuantumCoherence

gNOESY   Gradient Nuclear Overhauser effect Spectroscopy

HEp2   Human larynx carcinoma

HPLC   High Performance liquid chromatography

EIMS   High resolution- Mass spectrometry

HR-EIMS   High Resolution Electron Impact Mass Spectrometry

IC_{50} Drug Concentration causing 50 % to inhibition of the desired
                activity

LC   Liquid chromatography

mp   Melting point

MS   Mass spectrometry

MTT   3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide

nm   nanometer

ng/ml   Nanogram/mililitre

NOE	Nuclear Overhauser effect
NMR	Nuclear magnetic resonance
OD	Optical density
PBS	Phosphate buffer
PC	Percentage cytotoxicity
PTLC	Preparative thin layer chromatography
RP-HPLC	Reverse phase –high performance liquid chromatography
TLC	Thin layer chromatography
UV	Utra violet
WHO	World health organisation

CHAPTER ONE

INTRODUCTION

1.1. GENERAL OVERVIEW

Plants have been used as a source of important products with nutritional and therapeutic values. In early days, before the 'synthetic era' medicinal plants were used in their crude form as teas, powders, tinctures and other formulations (Balunas and Kinghorn, 2005; McChesney *et al*., 2007). Knowledge on the use of herbal remedies was orally passed from one generation to another. In countries such as China and India, well developed systems of traditional medicine are in practice and are very popular (Yuan and Lin, 2000). The use of medicinal plants as crude extracts has gradually changed to using purified compounds in drug discovery and development.

Medicinal plants have served as a significant source of new drugs, drug templates and new chemical entities for pharmaceutical development. Though there has been a lot of interest in high throughput synthesis, combinatorial chemistry and other synthetic techniques that produce large libraries of compounds, medicinal plants still remain an unbeaten reservoir of new molecules with unique structural features that have potential for application in drug discovery (Rates, 2001). In recent times, there has been a greater need for new drugs to fight emerging and re-emerging diseases such as malaria and cancer (Wells and Poll, 2010; Zheng *et al*., 2010) and so the search for drug leads from higher plant continues.

Malaria is a parasitic disease caused by *Plasmodium* species. It causes great global public health problems especially in sub-Saharan Africa despite being a preventable and curable disease (Kaur *et al*., 2009). According to WHO there were about 207 million cases of malaria in 2012 and an estimated 627 000 deaths (WHO malaria fact sheet, 2014). In Kenya, malaria is a leading cause of morbidity and mortality. It accounts for 30-50% of all outpatient attendance and 20% of all admissions to health facilities (Division of Malaria Control, 2011). Several malaria control measures such as environmental improvements, use of insecticide impregnated nets, residual indoor spraying, early case detection and treatment with Artemisinin Combination Therapy (ACT) has led to a reduction in malaria burden in many areas (Barnes *et al*., 2005; Bhattarai *et al*., 2007). However, the adaptation of the mosquitoes and *Plasmodium* parasite to insecticides and drugs respectively, is a drawback to these interventions. Thus, the mortality and morbidity due to malaria is still high and the challenge for the future remains significant.

Antimalarial drugs play a significant role in the control and elimination of malaria. Quinine an alkaloid isolated from *Cinchona succiruba* is the oldest antimalarial drug and it is still in use today. It has served as a template for the development of drugs such as chloroquine and amodiaquine (Schlitzer, 2008). Chloroquine was widely used as a first-line antimalarial drug for many years but the emergence of resistant parasites rendered it ineffective (Payne, 1987).

Plasmodium falciparum resistance to chloroquine and sulphadoxine–pyrimethamine led to adoption of Artemisinin-based Combination Therapies (ACTs) as the first line treatment against malaria (Eastman and Fidock, 2009). However, recent reports indicate a decline in efficacy of artesunate monotherapy and artesunate-mefloquine along the Thai-Cambodia border, a site historically known for emerging antimalarial

drug resistance (Phyo *et al.*, 2012). The spread of artemisinin derivative resistant parasites to other malaria endemic areas is therefore not a question of if but when. Without an apparent class of antimalarial drugs ready to replace the artemisinin derivatives, the search for new lead compounds has to continue. In this regard, screening of plants especially those used to treat malarial traditionally is promising.

In the *in vitro* screening of plants and compounds, often positive activity is due to cytotoxicity. Although such cytotoxic compounds have no potential as antimalarial agents, they could be developed into anticancer drug leads. Cancer is a leading cause of death accounting for 8.2 million deaths in 2012 according to WHO Cancer fact sheet 297 of February 2014 (WHO Cancer Fact sheet, 2014). Drug discovery from natural products has played a key role in management and treatment of cancer. This is evidenced in a review by Newman and Cragg (2012) which indicates that of the 175 anticancer drugs approved worldwide, 131 (74.9%) were nature inspired agents. In Kenya, cancer treatment is expensive and not readily accessible to majority of the patients. Also, effectiveness of most anticancer drugs is limited by toxicity to normal rapidly growing cells. Further more, cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug (Zheng *et al.*, 2010). These emphasize the need to get better alternatives. Medicinal plants have played a significant role in anticancer drug discovery and development. Infact, there are a number of anticancer drugs in clinical use directly derived from plants. These include. vinblastine and vincristine from *Cantharanthus roseus*, paclitaxel from *Taxus brevifolia* and camptothecin from *Camptotheca acuminata* (Jacobs *et al.*, 2004; Oberlies and Kroll, 2004).

In our efforts to search for promising cytotoxic or antimalarial compounds, *Ekebergia capensis*, *Turraea nilotica* and *Turraea robusta* were investigated. There is a report

on antiplasmodial secondary metabolites isolated from the stem bark of *Ekebergia capensis*, a medicinal plant traditionally used by Abagusii community in Kenya to treat malaria (Murata *et al*., 2008; Muregi *et al*., 2004). Also, documented is antiplasmodial and antimalarial activity of crude stem bark extract whereas there are no reports on antiplasmodial and cytotoxicity of *E. capensis* leaves and root bark (Muregi *et al*., 2004; 2007). Antiplasmodial and antimalarial potential of *T. robusta* root bark is reported (Gathirwa *et al*., 2008: Irungu *et al*., 2007). Though there are no reports indicating antiplasmodial activity of *T. nilotica* it was included in this study since it has been shown that plants belonging to the same genus have related compounds and activities. Plant parts with no documented antiplasmodial and cytotoxicity activities were selected. Use of combination therapy in treatment of diseases such as cancer and malaria, is used to delay resistance and also increase activity of two or more compounds that act synergistically. Hence, interaction studies on selected compounds were carried out. Also, carried out is the structure-activity-evaluation of selected compounds.

1.2. Statement of the Problem

Emergence and spread of drug resistant parasites, absence of a vaccine, lack of systematic vector control strategies and limitations of the existing drugs (such as high cost, non compliance, low efficacy and toxicity) are some of the factors contributing to the escalating prevalence and distribution of malaria. Hence, in the absence of a widely readily available vaccine and the inevitable emergence of drug resistant parasites, search for promising antiplasmodial compounds from plants that are documented to be used traditionally in treatment of malaria and/ or those that are shown to possess antiplasmodial activity continues.

Cancer is one of the leading causes of death in the world and is still increasing, particularly in developing countries where treatment is expensive and inaccessible to majority of patients. In addition, majority of the available drugs have associated side effects. Medicinal plants are useful sources of clinically relevant antitumour compounds. Kenya has a rich heritage of indigenous knowledge on the use of traditional medicinal plants that have been used to treat several diseases. In spite of the success of natural-products approach to anticancer drug discovery, scientific reports on medicinal plants with anticancer activity are rare in Kenya. As a result, extracts and compounds found cytotoxic to normal cells were evaluated further against cancerous cell lines.

1.3. Objectives

1.3.1. *General Objective*

To establish antiplasmodial and cytotoxic potential of extractives from *Ekebergia capensis, Turraea nilotica* and *Turraea robusta*.

1.3.2. *Specific objectives*

1. To determine the antiplasmodial and cytotoxic properties of extracts from *E. capensis*, *T. nilotica* and *T. robusta.*

2. To isolate and characterize secondary metabolites from *E. capensis*, *T. nilotica* and *T. robusta.*

3. To establish antiplasmodial and cytotoxic properties of isolated compounds.

4. To evaluate combination effects of selected secondary metabolites.

5. Carry out structural activity –evaluation on selected compounds.

1.4. Justification

Medicinal plants have served as a source of compounds for discovery and development of new drugs for several diseases. Previously, they provided compounds and/or templates for drug development, some of which have been employed clinically in the treatment of malaria and cancer. Continuing search for natural products that can be developed into clinically relevant compounds has offered secondary metabolites with either potent antiplasmodial and cytotoxic effects and with structural diversity (Batista *et al*., 2009; Hideji *et al*., 1992; Nogueira and Lopes, 2011; Tan and Luo, 2011). They remain an unbeaten reservoir for compounds with diverse structures and biological activities that can serve as leads for development of new drugs.

Three plants from family Meliaceae *Turraea nilotica*, *Turraea robusta* and *Ekebergia capensis* were investigated in this work. Previous phytochemical work on *T. nilotica* and *T. robusta* leaves room for more research as not all parts were investigated. Moreover, there are no reports on antiplasmodial and cytotoxic properties of isolated compounds. There are previous reports on antiplasmodial activity of triterpenoids isolated from stem bark of *Ekebergia capensis*. However, there are no reports on cytotoxicity. Additionally, there are no reports on phytochemical investigation of the root and leaves of this plant. It is expected that at least one hit compound with either potential antiplasmodial or cytotoxicity activity will be isolated and characterized.

CHAPTER TWO

LITERATURE REVIEW

2.1. Background on Malaria

Malaria is a life-threatening disease caused by parasites of *Plasmodium* species and transmitted to people through the bites of infected female *Anopheles* mosquitoes. It claims more than 600,000 lives every year, mostly children under the age of five and pregnant women who lack protective immunity. Most of these deaths occur in sub Saharan-Africa where it imposes a heavy economic burden on individuals and nations (White *et al*., 2011; WHO Malaria Fact Sheet, 2014).

There are four species of *Plasmodium* causing human malaria with distinct disease pattern; *Plasmodium falciparum, P. malariae*, *P. ovale* and *P. vivax.* A simian parasite, *P. knowlesi*, occasionally infect human (Flannery *et al*., 2013; Schlitzer, 2008). Of these *P. falciparum* is responsible for most of severe and fatal cases. Furthermore, resistance development of this parasite to most antimalarial drugs has reached an alarming level (Na-Bangchang and Karbwang, 2009).

Malaria was once found in many parts of the world including North America and Europe. The WHO led malaria eradication campaign of the 1950's and 1960's, through the use of the synthetic insecticide, dichlorodiphenyltrichloroethane (DDT) and synthetic drugs such as chloroquine and sulfadoxine-pyrimethamine, managed to get rid of malaria from vast endemic areas (Baird, 2005; Flannery *et al*., 2013).

In later years however, following development of resistance to these drugs and restriction on the use of DDT, malaria re-emerged in many areas (Baird, 2005). In response to the call for widespread control of malaria and the challenge to meeting millennium development goal 6, various interventions have been scaled up. These include the use of insecticide treated bednets, improved diagnosis using rapid diagnostic tests and use of artemisinin combination therapy (White *et al.*, 2011). Though there are reports on reduction in malaria burden in many areas (Barnes *et al.*, 2005; Bhattarai *et al.*, 2007), deaths associated with malaria are still high.

2.2.   Antimalarial Drugs

Antimalarial drugs play a key role in the fight against malaria. These drugs act at different stages of the malaria life cycle, with most of them targeting the intraerythrocytic phases of the parasite (Delfino *et al.*, 2002).

2.2.1. *Artemisinin and its derivatives*

Artemisinin (**1**), a sesquiterpene lactone was isolated from a Chinese herb, *Artemisia annua*. Dihydroartemisinin (**2**), artemether (**3**) and artesunate (**4**) are semi synthetic derivatives of the endo-peroxide artemisinin, retaining the peroxide functionality (Taylor and White, 2004; Schlitzer, 2008). The drugs are active against all species of *Plasmodium* and are able to clear blood stage parasites and reduce fever rapidly. However, they are limited by their short half-lives, which is why they are used in combination with longer lasting drugs (Schlitzer, 2008; Flannery *et al.*, 2013; Burrows *et al.*, 2014). Thus, Artemisinin Combination Therapy (ACT) is currently recommended as first line drugs for treatment of malaria (Burrows *et al.*, 2014; WHO Malaria Fact Sheet, 2014). Artemether-lumefantrine, a fixed combination oral drug combines the fast onset of action of artemether in terms of parasite clearance with a

high cure rate of lumefantrine in treatment of acute uncomplicated *P. falciparum* malaria (Alkadi, 2007). Unfortunately, recent reports indicate the emergence of resistance to artemisinin derivatives along Thai–Cambodia border (Dondorp *et al*., 2010; Maude *et al*., 2010). There are chances that resistant will evantually spread to other malaria endemic areas as it previously happened with chloroquine. Hence, new drugs, that are cheap, readily available and active against artemisinin resistant parasites will be needed.
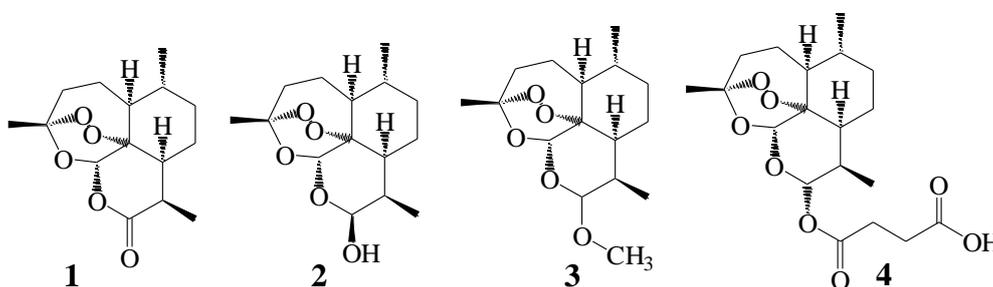
Figure 2-1: Structure of artemisinin and its derivatives

2.2.2. *4-Aminoquinolines*

Chloroquine (**5**), a 4-aminoquinoline derivative was the mainstay treatment for malaria both for prophylaxis and treatment for many years. It was cheap, safe and adequate for outpatient use before resistant strains began to emerge in the 1960's. It is no longer in use due to reduced parasite sensitivity (Payne, 1987; Schlitzer, 2008). Incorporation of an aromatic structure into chloroquine's side chain resulted in amodiaquine (**6**), as chloroquine it was considered to be safe for use in pregnancy despite the availability of limited data on its toxicity (Thomas *et al*., 2004). However its use is severely limited by cross resistance with chloroquine in *P. falciparum* (Nosten *et al*., 2006).

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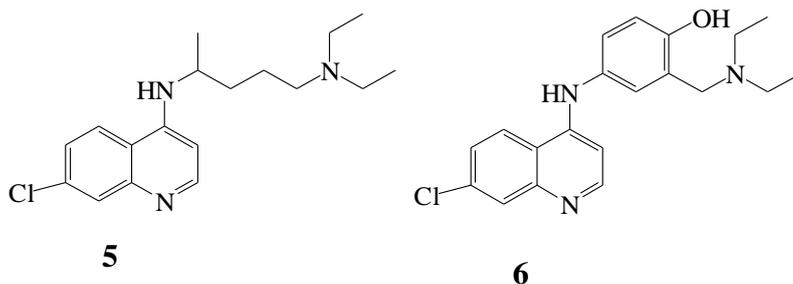
Figure 2-2: Structure of chloroquine and amodiaquine

2.2.3. *Arylaminoalcohols*

Quinine (**7**) a natural compound isolated from *Cinchona* trees is one of the oldest drugs used to treat malaria. There are no well documented cases of high grade resistance to quinine treatment (Pukrittayakamee *et al*., 1994; White, 1998). However, diminished sensitivity of *P. falciparum* to quinine has been widely documented in Asia and South America (Mayxay *et al*., 2007; Legrand *et al*., 2008). It is still relied upon for treatment of complicated malaria (Sinclair *et al*., 2012).

Mefloquine (**8**) structurally analogous to quinine, is an effective antimalarial drug. It is an effective prophylactic drug when used appropriately (Taylor and White, 2004). However, its use is limited by its prohibitive cost (Delfino *et al*., 2002; Nosten *et al*., 2006). Mefloquine was widely used in Asia until *Plasmodium* parasites resistant to it were reported along Thai-Cambodia border (Schlitzer, 2008).

Halofantrine (**9**) is an expensive drug active against chloroquine resistant *Plasmodium* strains. However, it has been withdrawn from the market in several countries due to its toxicity (Delfino *et al*., 2002; Schlitzer, 2008).

7   **8**   **9**
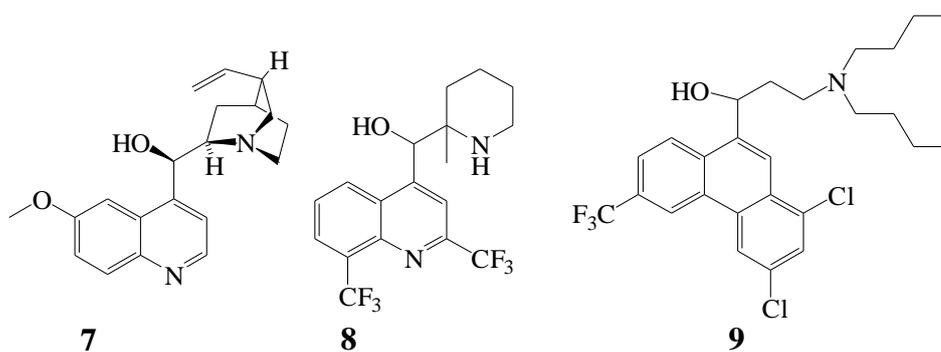
Figure 2-3: Structures of arylaminoalcohols quinine, mefloquine and halofantrine

2.2.4. *8-Aminoquinolines*

Primaquine (**10**), the only 8-aminoquinoline in use is different from other drugs as it is active in the liver and sexual blood stages of different *Plasmodia* species (Schlitzer, 2008). It is primarily used to achieve cure of *P. vivax* and *P. ovale*. Most antimalarial drugs target the intraerythrocytic phases of the parasite (Schlitzer, 2008). Unfortunately use of primaquine is limited by toxicity (Taylor and White, 2004). A drug that targets sexual stages of the parasite is needed so as to block the transmission of the parasites from the mosquitos to humans.

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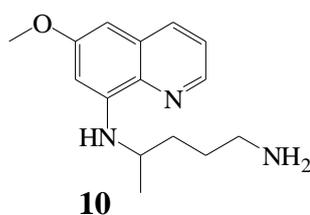
Figure 2-4: Structure of primaquine

2.2.5. *Antifolates: Sulfadoxine-Pyrimethamine*

Combination of sulfadoxine (**11**) and pyrimethamine (**12**) was known under its brand name fansidar. Kenya recognized sulphadoxine-pyrimethamine (SP) as a first line treatment of malaria in 1997 following a number of CQ treatment failures (Ogutu *et al*., 2000). Fansidar did not last long as it was lost to resistance in many malaria

endemic areas (Jelinek *et al*., 1999; Ogutu *et al*., 2000). The risk of resistance to antimalarial drugs means that search for new a drugs continues.
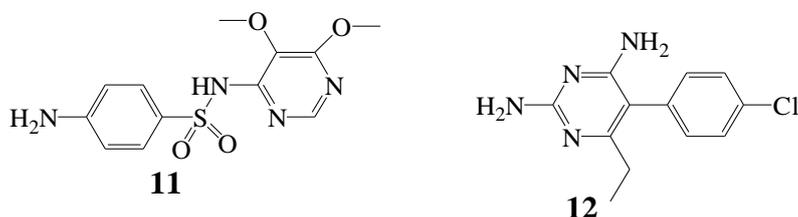
Figure 2-5: Structure of sulfadoxine and pyrimethamine

2.3.   Medicinal plants as a source of antiplasmodial agents

The appearance of resistant *P. falciparum* strains to chloroquine and thereafter fansidar (sulfadoxine and pyrimethamine) which were cheap and readily available antimalarial drugs and most recently indications of emergence of parasites resistant to artemisinin derivatives, used in combination with other drugs (Artemisinin Combination therapy), has promoted research on development of new drug leads. Natural products have played a major role in discovery and development of antimalarial drugs (Batista *et al*., 2009). It is anticipated that medicinal plants would still serve as a source of new drug leads given their chemodiversity (Batista *et al*., 2009). There are reports on antiplasmodial activity of extracts and pure compounds from a large number of plant species against different strains of *P. falciparum*. A review by Batista and co-workers gave details on antiplasmodial and antimalarial activities of crude extracts and non alkaloidal natural products, a total of 126 extracts and 194 compounds were included in this review (Batista *et al*., 2009). A review by Nogueira and Lopes highlighted 360 natural products displaying moderate to high antiplasmodial activity (Nogueira and Lopes, 2011). Kaur and co-workers published a review on 266 antiplasmodial compounds isolated from both plant and marine extracts (Kaur *et al*., 2009) whereas Saxena and co-workers published a review which

detailed antiplasmodial activities of crude extracts and 250 compounds against different *P. falciparum* strains (Saxena *et al*., 2003). Though none of the above reviews reported the discovery of a new antimalarial drug, they provided a positive outlook on continuing investigation of plants as a source of antiplasmodial agents having new chemical entities that can serve as templates in drug discovery and development.

2.4. Natural products as a souce of anticancer drugs

Cancer is one of the leading causes of death worldwide accounting for 8.2 million deaths in 2012 according to WHO (WHO Cancer Fact sheet, 2014). Lung, liver, stomach, colorectal and breast cancers cause most cancer deaths each year (WHO Cancer Fact sheet, 2014). Conventional cancer treatment consists of radiotherapy and chemotherapy alone or in combination. Unfortunately, none of the cancer treatment is 100% effective (Gottesman, 2002). Also, a single "cure" for cancer has proved elusive since there are over 100 different types of cancer. Moreover, effectiveness of many anticancer drugs is limited by their toxicity to normal and rapidly growing cells (Basu and Lazo, 1990).

Natural products have played a leading role in cancer chemotherapy. According to a review by Newman and Cragg, of the 206 anticancer drugs approved worldwide 131 are naturally inspired agents (Newman and Cragg, 2012). Some of the plant-derived antitumour compounds include vinblastine (**13**), vincristine (**14**), camptothecin (**15**) and epipodophyllotoxin (**16**) (Balunas and Kinghorn, 2005).
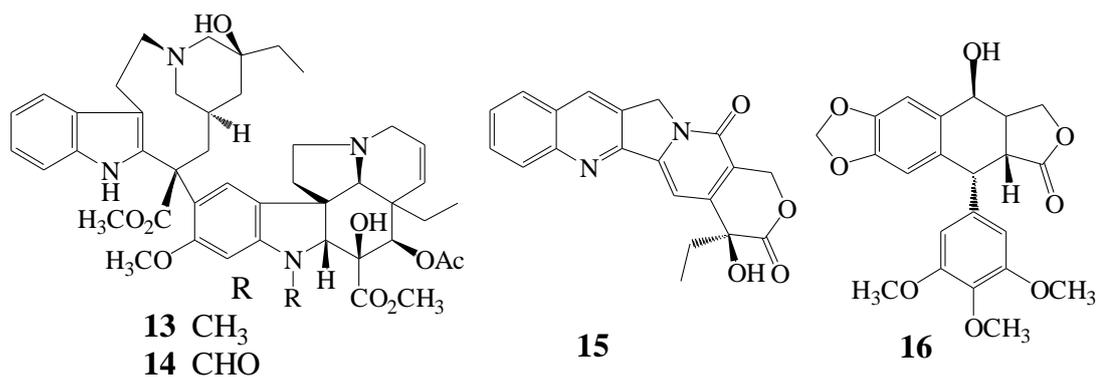
Figure 2-6: Structures of anticancer compounds derived from plants

Plant secondary metabolites and their semi-synthetic derivatives play a key role in anticancer drug discovery and development. In 2008, there were 56 plant derived anticancer compounds on clinical trials (Saklani and Kutty, 2008). In a review by Pan and co-workers, they documented 20 anticancer compounds derived from higher plants on clinical trials (Pan *et al*., 2010). These two reviews demonstrate that plants will continue to be an important source of compounds for anticancer drug discovery and development.

2.5. Ethnobotanical, Ethnopharmacological and Phytochemical Information on *Turraea robusta*, *Turraea nilotica* and *Ekebergia capensis.*

2.5.1. *The Genus Turraea*

The genus Turraea (family Meliaceae) consists of some 60-70 species of shrubs and small trees widely distributed in Eastern Africa. There are about 11 species growing in Kenya some of which have been used traditionally to manage various illnesses such as coughs, diarrhoea and stomach-pains (Beentje, 1994; Kokwaro, 2009).

The genus *Turraea* is rich in different classes of limonoids. The word "limonoid" originated from the bitterness of lemon or other citrus fruits (Tan and Luo, 2011). Structurally, limonoids are formed by loss of four terminal carbons of the side chain in the apotirucallane or apoeuphane skeleton and then cyclized to form the 17β-furan

ring and thus limonoids are also known as tetranortriterpenoids (Tan and Luo, 2011). Hence, a common feature in all limonoids is the presence of a furan ring characterized by three resonances in the ^{1}H NMR ranging δ_H 7.30-6.30 ppm for H- 21, H-22 and H-23 and in ^{13}C NMR spectrum signals appear at *ca.* δ_C 110 (C-22), δ_C 120 (C-20) and δ_C 142-140 (C-21 and C-23). Oxidation and skeletal rearrangements can occur, which lead to various classes of limonoids.

Different classes of limonoids are reported in the genus *Turraea* with each species synthesizing more than one class of limonoids (Bentley *et al.*, 1992, 1995; Adul *et al.*, 1993; McFarland *et al.*, 2004; Owino *et al.*, 2008). Classes of limonoids isolated from *Turraea* species include ring intact limonoids of the (a) azadirone class which are characterized by a ketone at C-3 and a C-1/C-2 double bond; (b) vilasinin class characterized by a 6α-28 ether bridge with rings A-D intact; (c) havanensin class that bears a substituent at C-1, C-2 and C-3 and the degree of oxidation at C-28 varying from methyl to carbonyl (Tan and Luo, 2011). Ring intact limonoids are reported in eight *Turraea* species which include *robusta*, *nilotica*, *cornucopia*, *parvifolia*, *floribunda*, *holstii*, *wakefieldii* and *pubescens* (Bentley *et al.*, 1992, 1995; Ndung'u *et al.*, 2004; Owino *et al.*, 2008; Yuan *et al.*, 2013a).

Various ring *seco* limonoids are isolated; (a) ring A-B *seco* limonoids of the prieurianin class that arises from cleavages of C-3/C-4 and C-7/C-8 and formation of 3 (4)-lactone with substitution of a formyl oxy or acetoxy group at C-11 (b) ring B *seco* limonoids characterized by cleavage of C-7/C-8 and formation of C-8/C-30 double bond (c) ring C *seco* limonoids of azadirachtin and nimbolinin class characterized by cleavage of C-12/C-13 and oxidation of ring C (C-12/C-13) (d) Ring A *seco* limonoids characterized by loss of methyl 25 to form C-1/C-25 bond, a ketone at C-1 and oxidation of ring A (C-3/C-4). So far this latter class of ring *seco* limonoids

has only been isolated from *T. wakefieldii* (Ndung'u *et al*., 2003). Ring B *seco* limonoids are reported in species *floribunda, holstii* and *pubescens* (Mulholland *et al*., 1998b, 1999; McFarland *et al*., 2004; Yuan *et al*., 2013a). Ring A-B *seco* are reported in *T. mombassana* and *T. obtusifolia* while ring C *seco* are reported in *T. holstii* and *T. pubescens* (Adul *et al*., 1993; Sarker *et al*., 1997; Mulholland *et al*., 1998b; Yuan *et al*., 2013a). Interestingly, there are no reports on ring D *seco* limonoids from genus Turraea.

Terpenoid derivatives characterized by absence of a furan ring with either rings A-D intact or ring B *seco* are also reported in species *robusta*, *parvifolia*, *floribunda* and *holstii* (Bentley *et al*., 1992; Mulholland *et al*., 1999; Cheplogoi and Mulholland, 2003b; McFarland *et al*., 2004).

2.5.2. *Biosynthesis of Limonoids*

Limonoids are modified triterpenes with, or derived from a precursor with a 4, 4, 8-trimethyl-17-furanylsteroid skeleton. They are biogenetically thought to originate from Δ^7 tirucallol [20 (S) or Δ^7- euphol (20R)]. The bond Δ^7 is epoxidized to a 7-epoxide and is then opened inducing a Wagner-Meerwein shift of Me-14 to C-8 which leads to formation of 7-OH and introduction of a double bond at C-14/15 (Figure 2-7). This scheme accounts for both the ubiquitous presence of oxygen at C-7 and correct stereochemistry of the C-30 methyl group. Oxidative degradation of C-17 side chain results in loss of four carbon atoms and formation of the 17 β-furan. Successive oxidations and skeletal rearrangements of one or more of the four rings, which are designated as A, B, C and D, gives rise to different groups of limonoids (Figure 2-8). That the latter step is accomplished after formation of 4,4,8-trimethyl-steroidal skeleton is indicated by the occurrence of several protolimonoids (Champagne *et al*., 1992; Tan and Luo, 2011).
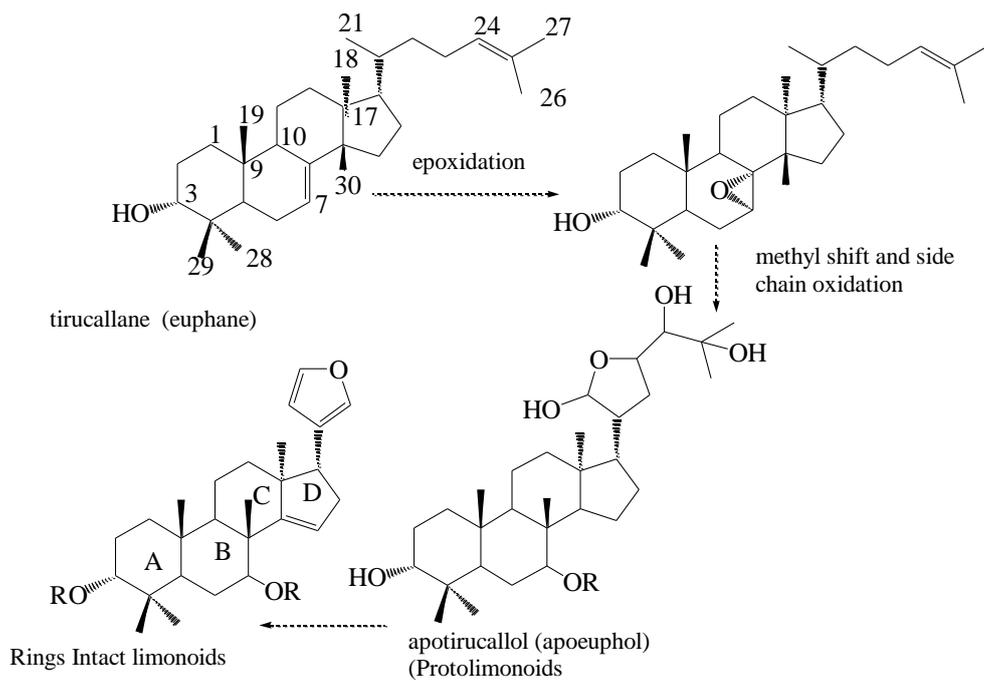
Figure 2-7: Biosynthetic pathway leading to formation of a basic limonoid skeleton (Champagne *et al*., 1992)
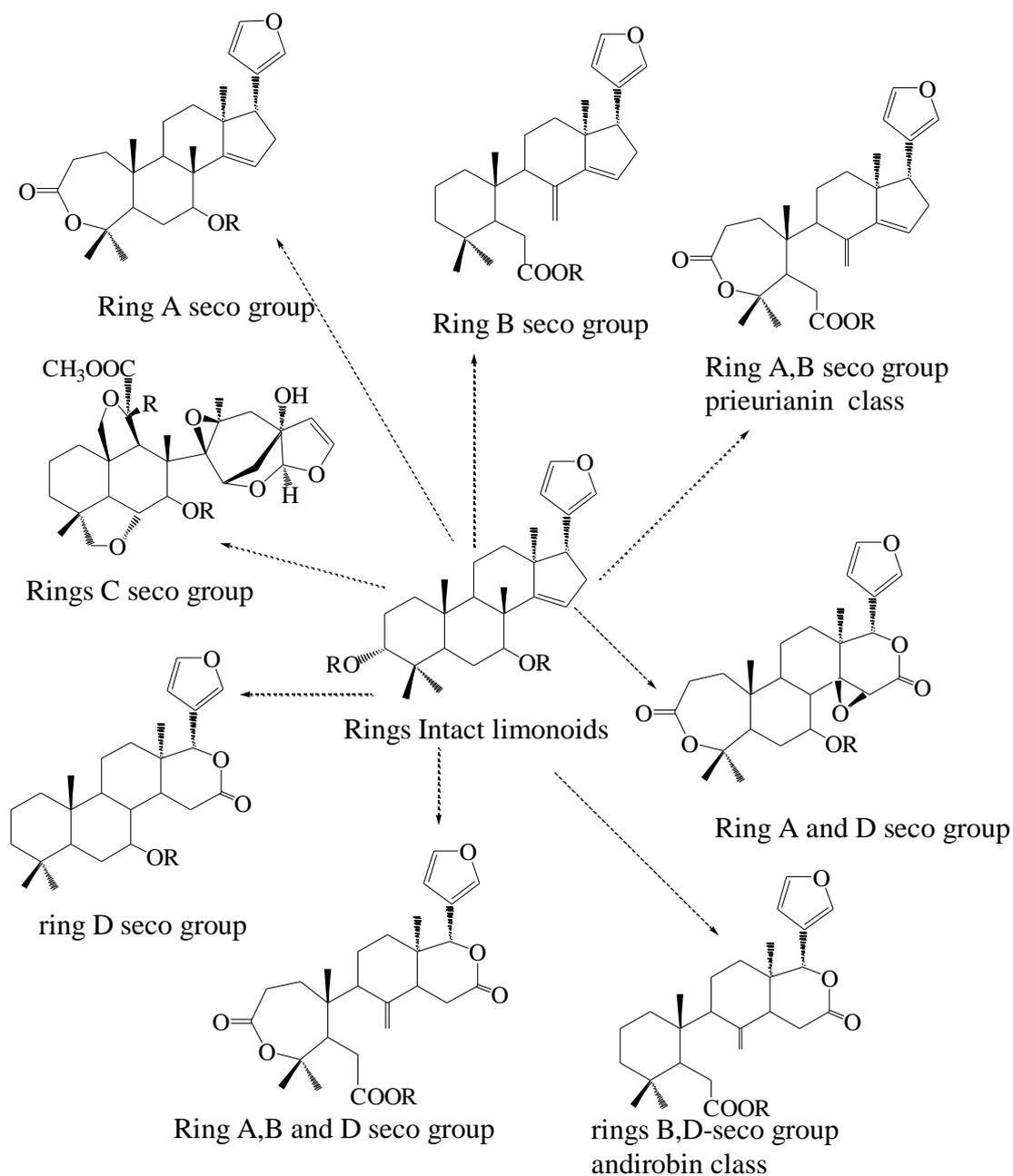
Figure 2-8: Biogenetic map showing classification of meliaceous limonoids (Tan and Luo, 2011).

2.5.3. *Compounds isolated from Turraea species*

Turraea robusta Guerke is a small tree found in East Africa. It is a medicinal plant traditionally used to treat diarrhoea and other stomach associated troubles (Kokwaro, 2009). Antiplasmodial and antimalarial activities of the crude methanol root bark extract was previously reported (Irungu *et al*., 2007; Gathirwa *et al*., 2008). The

extract is also reported to be safe in mice with an oral LD_{50} value > 5000 mg/kg body weight (Gathirwa *et al*., 2008). Previous phytochemical investigation on the root bark led to isolation of limonoids [mzikonone (**17**), mzikonol (**18**), azadirone (**19**) 1,2-dihydroazadirone (**20**) and nimbolinin B (**21**)]; triterpenoids [turranolide (**22**) and butyrospermol (**23**)] (Rajab *et al*., 1988; Bentley *et al*., 1992). Except for azadirone (**19**), antiplasmodial and cytotoxicity activity of the other compounds is not reported. Furthermore, there are no phytochemical reports on the stem bark.
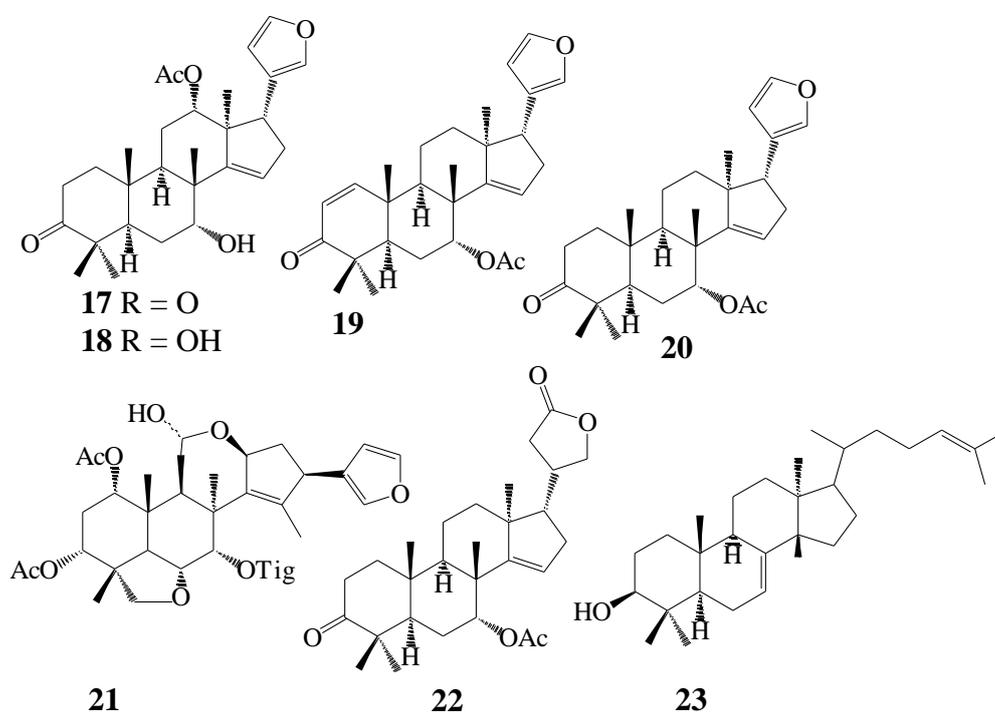
Figure 2-9: Limonoids and triterpenoids isolated from *T. robusta* root bark

Turraea nilotica Kotschy and Peyr is a shrub or tree growing along the coastal region in Kenya. In traditional medicine a decoction from the root is used for stomach upset (Kokwaro, 2009). Previous phytochemical investigation on the root and stem bark led to isolation of limonoid nilotin (**24**) and protolimonoids niloticin (**25**), dihydroniloticin (**26**) and piscidinol A (**27**) (Mulholland and Taylor, 1988; Bentley *et al*., 1995). Cytotoxic properties of compounds **24** and **27** are documented (Hideji *et*

al., 1992; Mitsui *et al*., 2007). However, there are no reports on antiplasmodial activity.
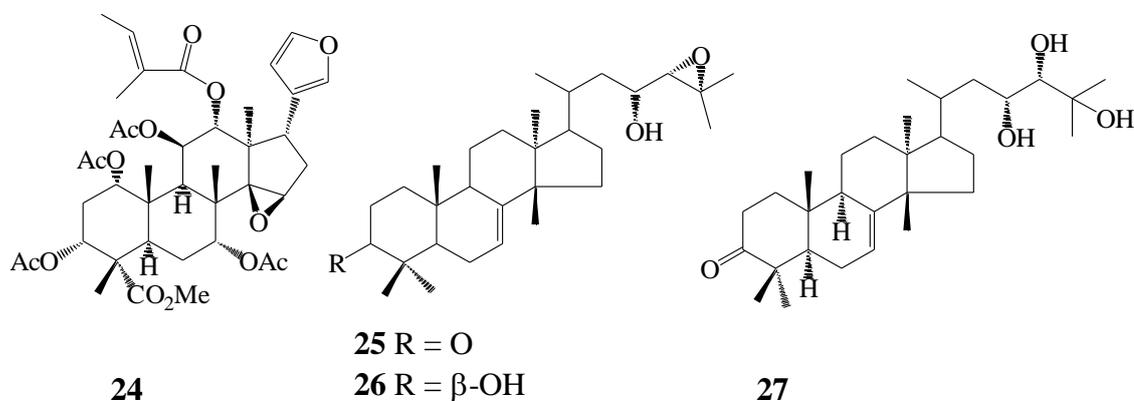
Figure 2-10: Limonoid and protolimonoids isolated from *T. nilotica*

Other *Turraea* species have been phytochemically investigated and a number of limonoids, protolimonoids and triterpenoids isolated. From *Turraea cornucopia* the limonoids mzikonone (**17**), 12α-acetoxy-7-deacetylazadirone (**28**) and 1α,12α - diacetoxy-7-deacetyl-1,2-dihydro-3α -hydroxyazadiron (**29**) were reported (Owino *et al*., 2008).
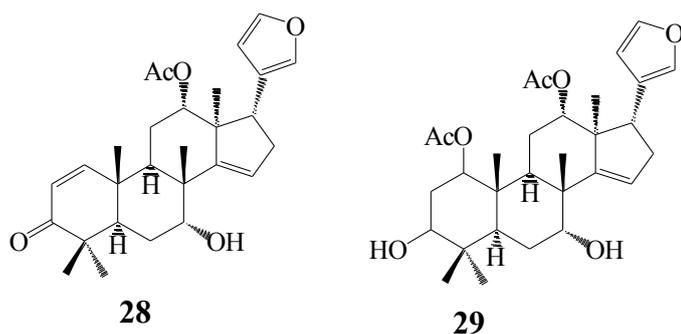
Figure 2-11: Limonoids isolated from *T. cornucopia*

From the root bark of *Turraea mombassana* C. DC, a shrub whose roots are traditionally used for the treatment of excess bile, an emetic for malaria and other

fevers (Kokwaro, 2009), the limonoids mombasone (**30**) and mombasol (**31**) were isolated (Adul *et al*., 1993).
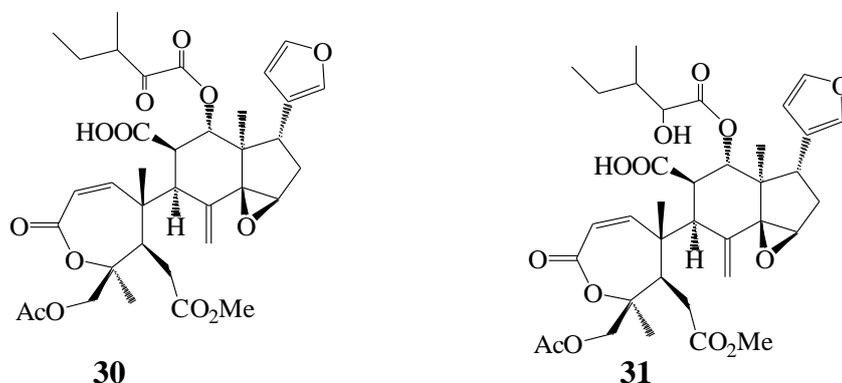
Figure 2-12: Limonoids isolated from *T. mombassana*

From the seeds and roots of *T. parvifolia* previous phytochemical investigation led to isolation of triterpenoids [turraparvin A (**32**), 12α-acetoxyazadironolide (**33**), turraparvin B (**34**), turraparvin C (**35**), 11-*epi*-hydroxytoonacilide (**36**), 11-*epi*-23-hydroxytoonacilide (**37**) and turraparvin D (**38**)]; limonoids mzikonone (**17**) 1α-acetyl-3α-propionylvilasinin (**39**), 1α,3α-diacetyl-7α-tigloyvilasinin (**40**), 12α-acetoxy-1,2-dihydroazadirone (**41**) (Cheplogoi and Mulholland, 2003a, 2003b).

32 R = H
33 R = Ac

34 R = H
35 R = Ac

36

37
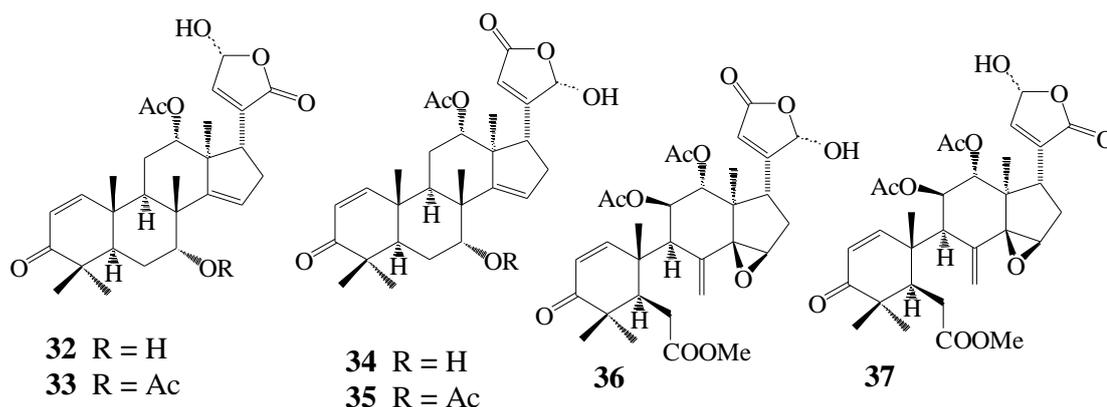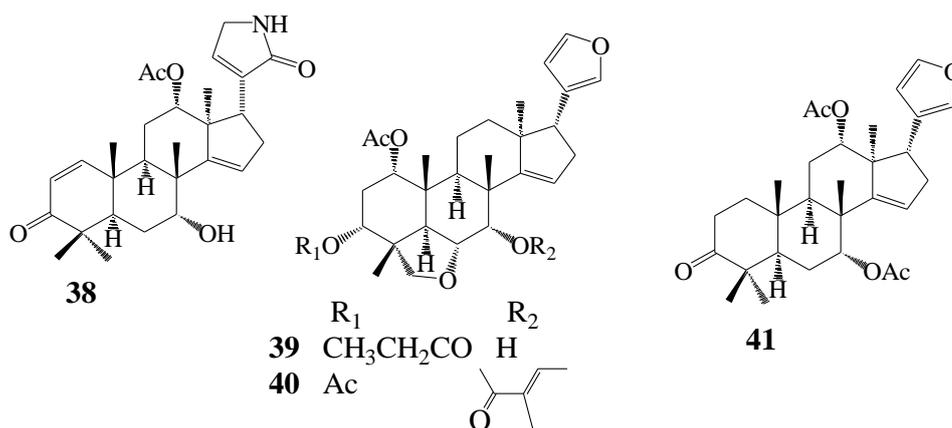
Figure 2-13: Triterpenoids and limonoids from isolated *T. parvifolia*

Previous phytochemical investigation of *T. floribunda* seeds yielded limonoids turraflorin A (**42**), turraflorin B (**43**), turraflorin C (**44**), turraflorin G (**45**), turraflorin H (**46**) and turraflorin I (**47**) (McFarland *et al*., 2004). From the root bark limonoids 1α,7α,11β-triacetoxy-4α-carbomethoxy-12α-(2-methylpropanoyloxy)-14β,15β epoxyhavanensin (**48**), 1α,7α,11β-triacetoxy-4α-carbomethoxy-12α-(2-methylbutanoyloxy)-14β,15β-epoxyhavanensin (**49**), 1α,11β-diacetoxy-4α-carbomethoxy-7α-hydroxy-12α-(2-methylpropanoyloxy)-15-oxohavanensin (**50**), 28-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy) 14,15deoxyhavanensin-1,7-diacetate (**51**), 28-nor-4α-carbomethoxy-11β-hydroxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate (**52**), 18-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate (**53**), 28-nor-α-carbomethoxy-7-deoxy-7-oxo-11β-acetoxy-12α(2-methylbutanoyloxy)-14,15-deoxyhavanensin-l-acetate (**54**), 11β-acetoxy-3,7-diacetyl-4α-carbomethoxy-12trisobutyryloxy-28-nor-1-tigloyl-havanensin (**55**), 11β-acetoxy-7α-acetyl-12α-hydroxy-1,2-dihydroneotrichilenone (**56**), 12α-acetoxy-7-acetyl-1,2-dihydroneotrichilenone (**57**); triterpenoids turraflorin D (**58**), turraflorin E

(**59**) and turraflorin F (**60**) (Torto *et al*., 1995; Mulholland *et al*., 1998b; McFarland *et al*., 2004; Ndung'u *et al*., 2004).

42 R = Ac
43 R = H

44

45

46

47

	R_{11}	R_{12}
48	OAc	$OCOCH(CH_3)_2$
49	OAc	$OCOCH(CH_3)CH_2CH_3$

$R_{12} = OCOCH(CH_3)_2$

50

	R_1	R_2
51	OAc	OAc
52	O H	OH
53	OAc	OH
54	OAc	O

55

	R_1	R_2	R_3
56	Ac	β-OAc	OH
57	Ac	H	OAc

58
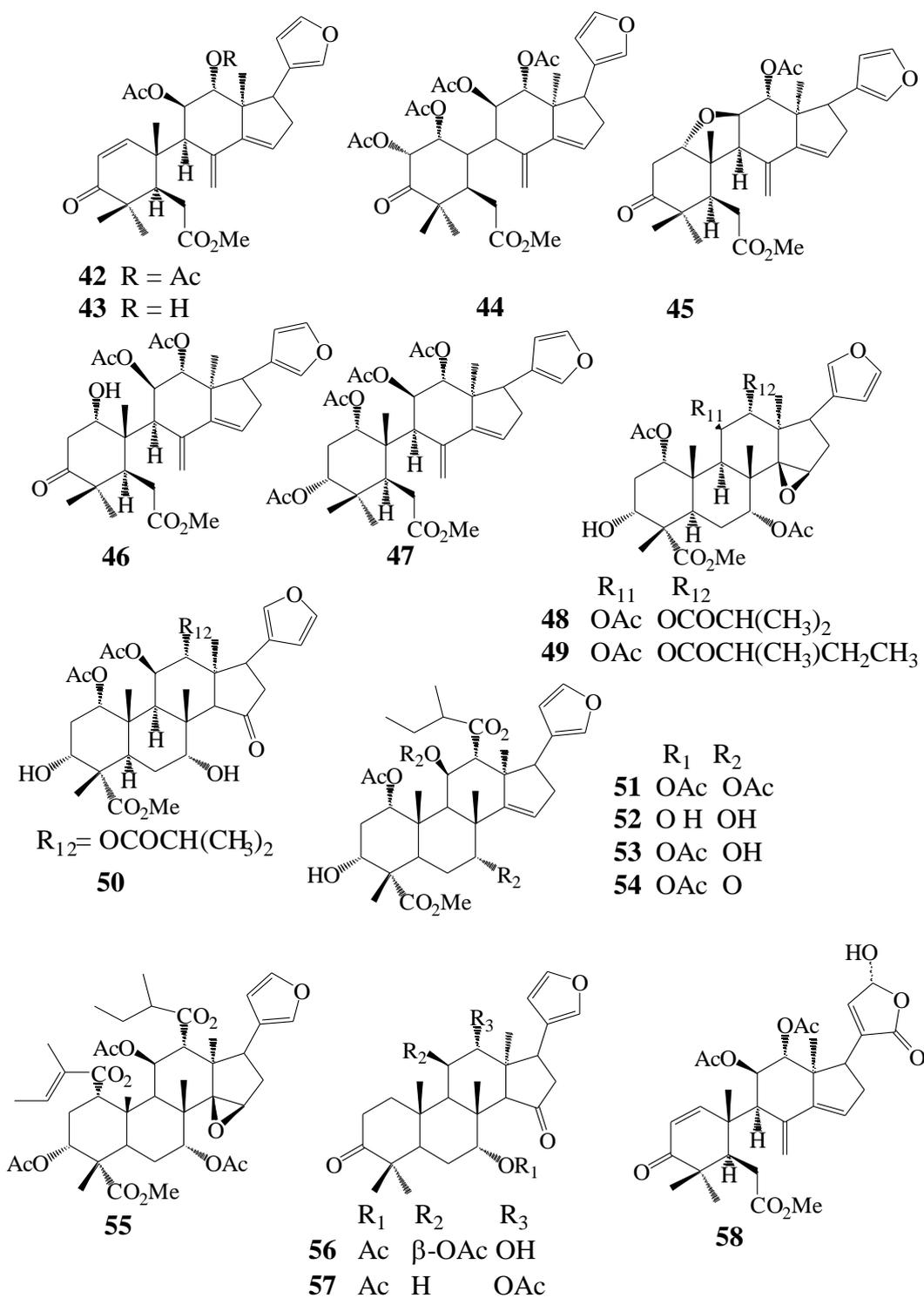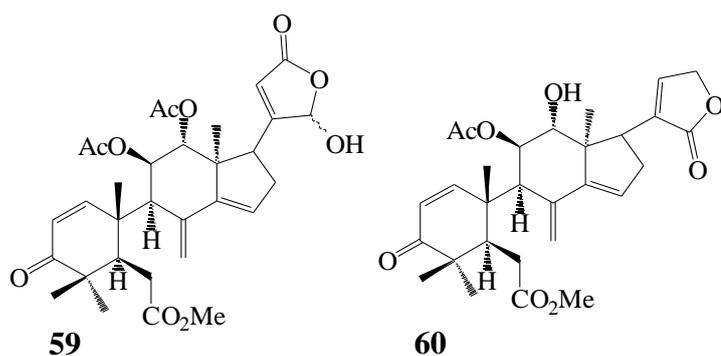
Figure 2-14: Limonoids and triterpenoids isolated from *T. floribunda*

Limonoids reported from the root and stem bark of T. *holstii* are 1α,3α-diacetylvilasinin (**61**), 11-*epi*-toonacilin (**62**), 11β,12α-diacetoxycedrelone (**63**), 12-O-methylnimbolinin (**64**), 12α-acetoxyneotrichilenone (**65**), 12α-acetoxy-7-acetyl-1,2-dihydroneotrichilenone (**66**), 12α-acetoxy-1,2-dihydroneotrichilenone (**67**) and 11β-acetoxy-7-acety-12α-hydroxy-1,2-dihydroneotrichilenone (**68**): triterpenoids holstinone A (**69**), holstinone B (**70**) and holstinone C (**71**) (Mulholland *et al*., 1998b, 1999).

65

	R_1	R_2	R_3
66	Ac	H	Ac
67	H	H	Ac
68	Ac	OAc	H

	R_1	R_2	R_3	R_4
69	OH	OH	Me	α-OMe
70	OH	OH	H	β-OMe
71	OH	OH	H	α-OMe
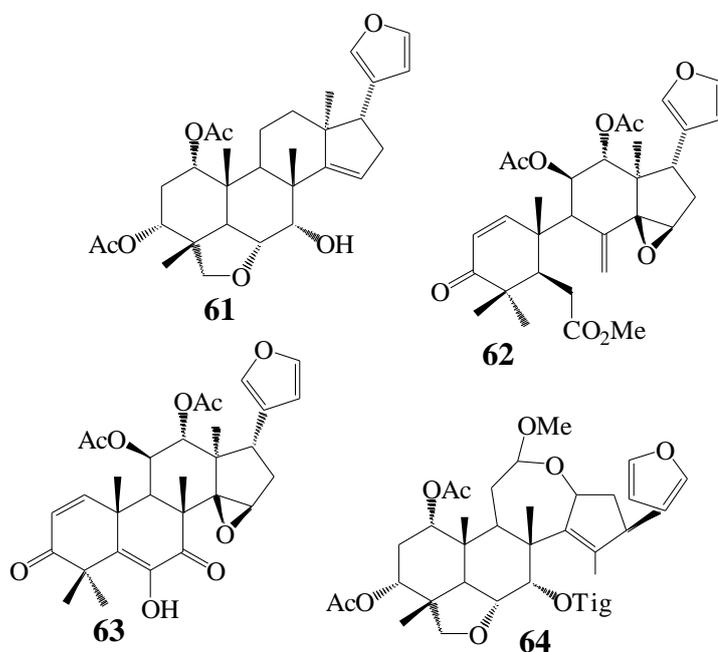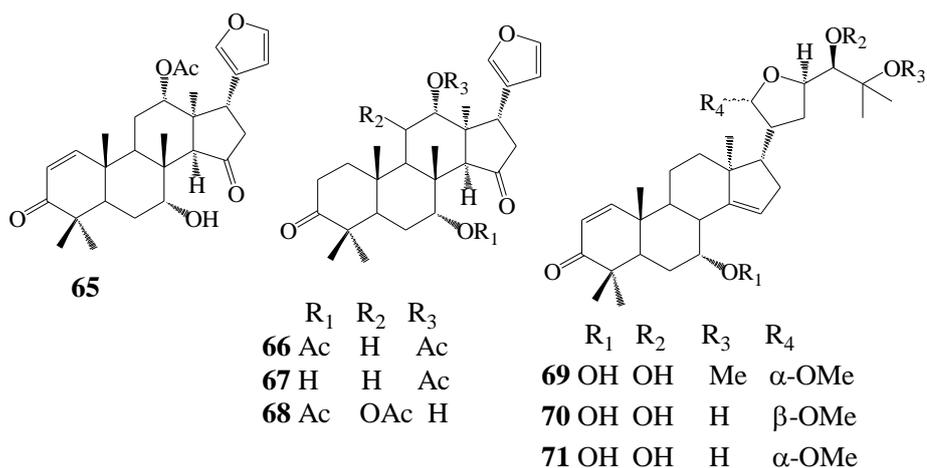
Figure 2-15: Limonoids and triterpenoids from *T. holstii*

From the root bark of *T. wakefieldii* limonoids 11β, 12α-diacetoxyneotecleanin (**72**), 11β,12α-diacetoxy-14β,15β-epoxyneotecleanin (**73**), 7α,12α-diacetoxy-14β,15β-epoxy-11β-hydroxyneotecleanin (**74**), 7α,12α-diacetoxy-11β-hydroxyneotecleanin (**75**), 11β,12α-diacetoxy-1-deoxo-14β,15β-epoxy-3β-hydroxy-2-oxo-neotecleanin (**76**) and 1α-acetoxy-3α-propanoyloxyvilasinin (**77**) are reported (Ndung'u *et al*., 2003, 2004) .

73 R = H
76 R = OH

$R_1 = OCOCH_2CH_3$

72   **74**   **75**   **77**
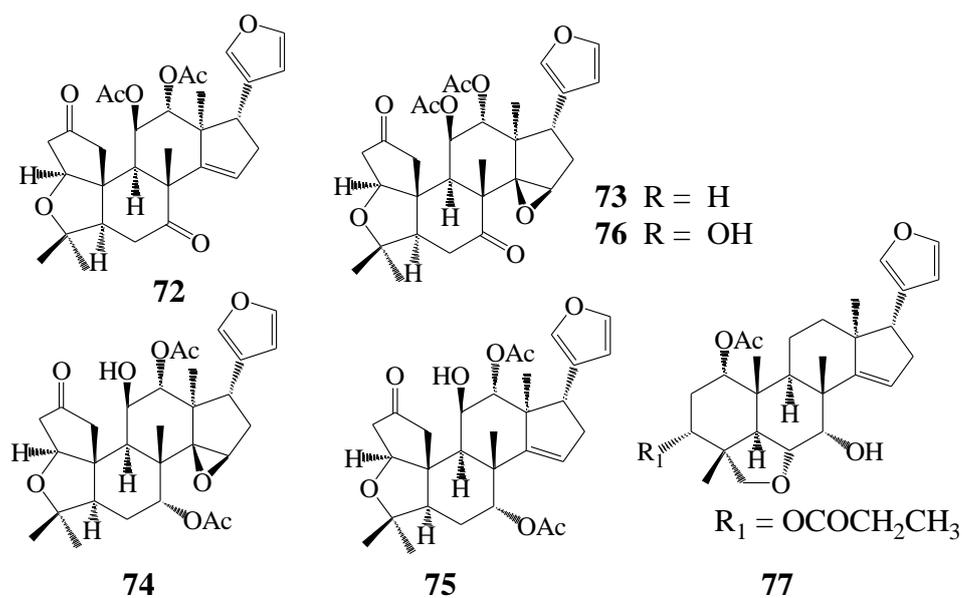
Figure 2-16: Limonoids isolated from *T. wakefieldii*

From the twigs and leaves of *T. pubescens* several limonoids, protolimonoids and triterpenoids are reported. Limonoids include mzikonone (**17**), turraflorin A (**42**), 11-*epi*-toonacilin (**62**), 12α-acetoxy-7-deacetylazadirone (**28**), 1α,acetoxy-3α-propanoyloxyvilasinin (**77**), turrapubin A-G, I-K (**78-87**), turrapubesin A and C (**88-89**), nimonol (**90**) and azadirachtin (**91**). Protolimonoids dihydroniloticin (**26**), piscidinol A (**27**), turrapubin H (**92**), 7-deacetylbruceajavanin B (**93**), 7-deacetyl-21α-methoxydihydrobruceajavanin (**94**), dihydrosapelin E acetate (**95**), hispidol B (**96**), mesendanin T (**97**), mesendanin U (**98**), turrapubesols A–C (**99-101**), bourjotinolone B (**102**), grandifoliolenone (**103**), hispidone (**104**), bourjotinolone A (**105**) and 3-episapelin A (**106**). Other triterpenoids isolated are 11-*epi*-23-hydroxytoonacilide (**37**), 2β,3β,5β-trihydroxy-pregn-20-en-6-one (**107**), 3β-hydroxy-5α-pregn-7,20-dien-6-one (**108**), and 3β-acetoxy-5α-pregn-7,20-dien-6-one (**109**), turrapubesin B, D-G (**110, -114**), turrapubesic acid A-C (**115-117**), guaidiol (**118**), turranin A-F (**119-124**), 2β,3β,5β-trihydroxypregn-20-en-6-one (**125**), 1α,4αdihydroxyeudesman-11-ene (**126**), cyperusol C (**127**), 1β,4βdihydroxyeudesman-11-ene (**128**), clovandiol (**129**) and caryolane-1,9β-diol (**130**) (Wang *et al*., 2006a, 2006b, 2007, 2008; Yuan *et al*., 2013a, 2013b).

78 R=Ac
79 R=A
80 R=B

81

82 R=H
83 R=A
84 R=B

85

86

87 R=C

A

B

C

88

89

90

91

92

93

94

95

96

97

98

99

27

100 **101** **102**

103

104

105 O
106 β-OH

R

107

R =

108 R=OH
109 R=OAc

110

CO₂Me

	R₁	R₂	R₃
111	COCH₂Ph	O	OH
112	COCH₂Ph	OH	O
113	iBu	OH	O
114	Piv	OH	O

115 R=acetyl
116 R=isobutanoyl
117 R=2-methylbutanoyl

118

	R₁	R₂
119	H	OH
125	OH	OH

120

121

122

123

124

28
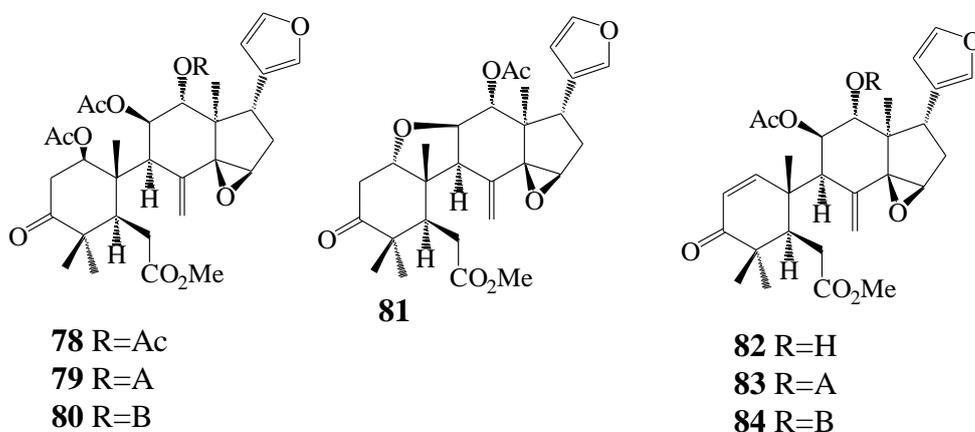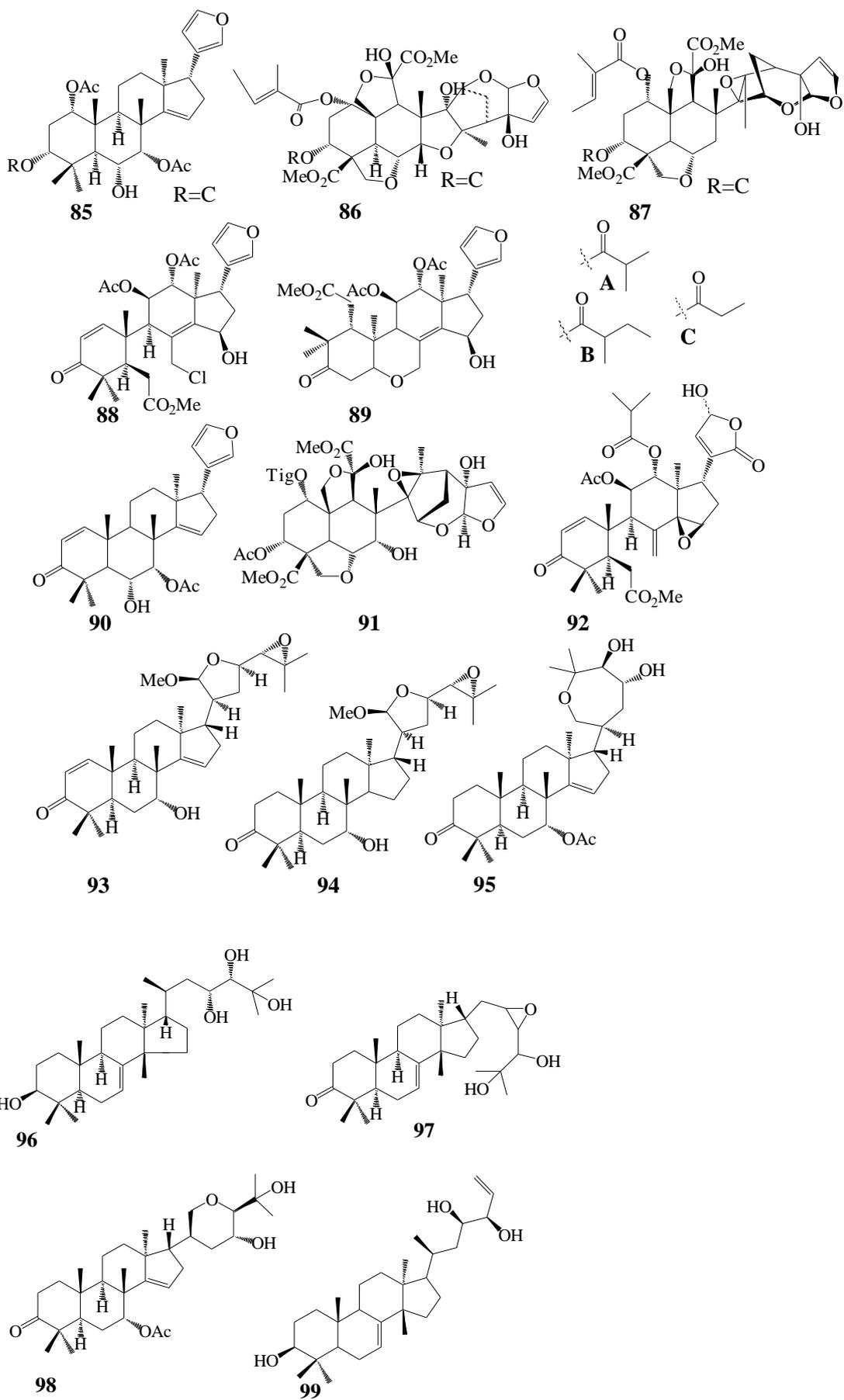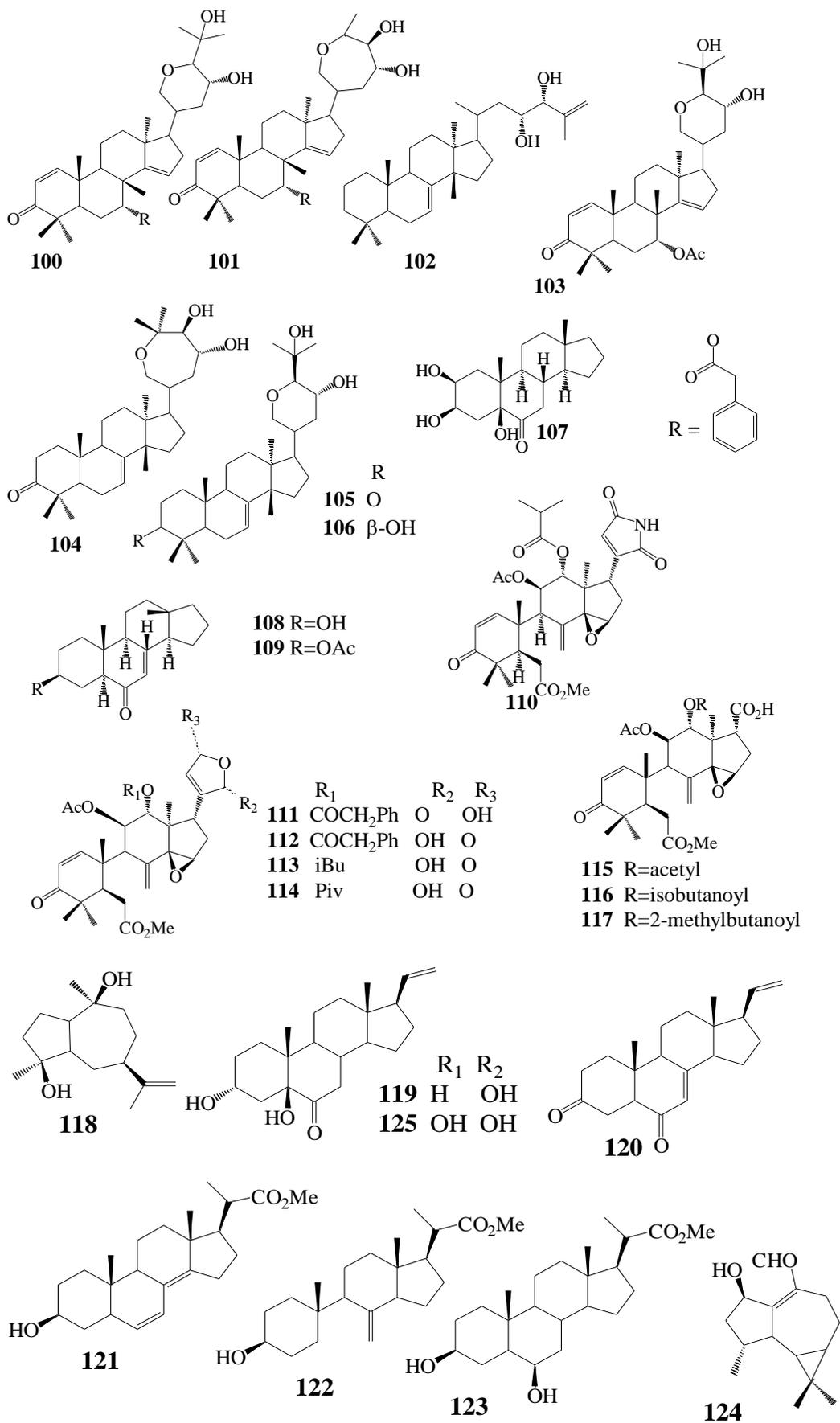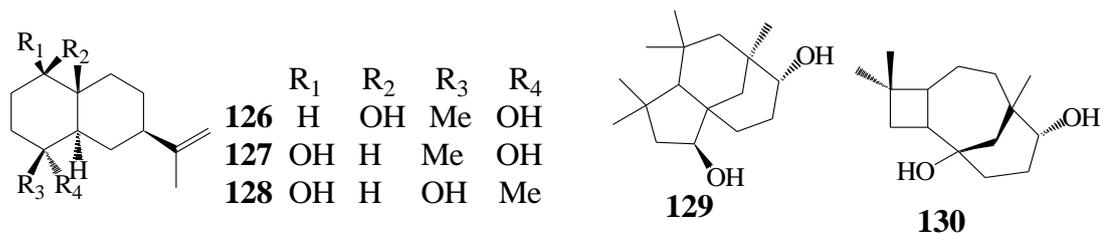
Figure 2-17: Limonoids and protolimonoids isolated from *T. pubescens*

From the seeds of *Turraea obtusifolia* the limonoids prieurianin (**131**), rohitukin (**132**) and rubrin E (**133**) were isolated (Fraser LA *et al*., 1995; Sarker *et al*., 1997). A pregnane steroid villosterol (**134**) is reported from aerial parts of *Turraea villosa* (Chiplunkar *et al*., 1993).
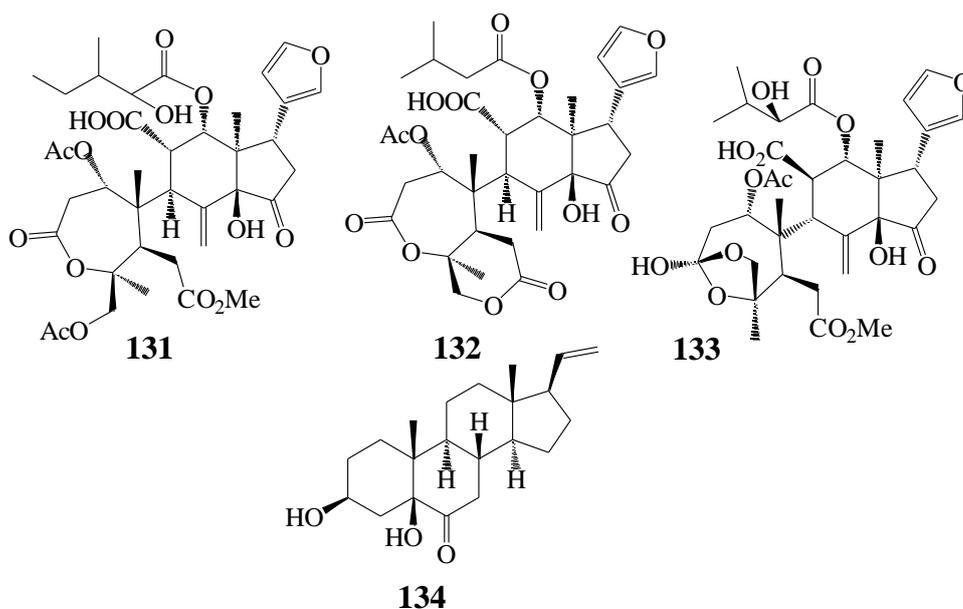
Figure 2-18: Limonoids isolated from *T. obtusifolia*

2.5.4. *Antiplasmodial and cytotoxic activities of compounds isolated from Genus Turraea*

Cytotoxicity properties of protolimonoids niloticin (**24**), dihydroniloticin (**26**), hispidol B (**96**), turrapubesol B-C (**100-101**), grandifoliolenone (**103**), hispidonone (**104**) and bourjitinolone (**105**) and limonoids turrapubesin A (**88**) and azadirone (**19**)

isolated from *Turraea pubescens*, *T. robusta* and *T. nilotica* are reported to be cytotoxic to P-388 cell lines with IC_{50} values of less than 10 μM (Hideji *et al*., 1992; Mitsui *et al*., 2007; Wang a *et al*., 2006a, 2006b;). Thus, triterpenoids obtained from this work were tested for cytotoxicity and since cytotoxic compounds can be useful anticancer drug leads, they were also tested for cytotoxicity against cancerous cell lines. There are no reports on antiplasmodial activitity of Turraea triterpenoids except for azadirone (**19**) (Chianese *et al*., 2010). Antiplasmodial activity of several triterpenoids isolated from plants belonging to family Meliaceace is reported (Bickii *et al*., 2000; Chianese *et al*., 2010; Mohamad *et al*., 2009). It is for this reason that secondary metabolites isolated in this work were tested for antiplasmodial activity.

2.6. *Ekebergia capensis*

Ekebergia capensis Sparrm (family Meliaceae) is a deciduous tree attaining 30 m high and is widely distributed in Central and Nyanza regions of Kenya (Beentje., 1994; Gacathi., 2007). It is also widespread in South Africa, Swaziland, Zimbabwe, Uganda and Ethiopia. The Zulu community in South Africa uses the wood to facilitate childbirth (Sewram *et al*., 2000). In Kenya, the Sabaot community uses leaf macerations internally or externally to treat headache, fever, cough and skin complaints while the Agĩkũyũ community uses the stem bark to treat diarrhoea (Gacathi, 2007; Okello *et al*., 2010). Pharmacological studies have showed that the crude *E. capensis* extracts have antiplasmodial, anti-inflammatory, hypotensive effects, uterotonic, cardiovascular effects and antituberculosis activities (Lall and Meyer, 1999; Sewram *et al*., 2000; Muregi *et al*., 2004; Kamadyaapa *et al*., 2009; Mulaudzi *et al*., 2013). Previous investigations on seeds and stem bark led to isolation of the limonoids capensolactone 1, (**135**) capensolactone 2a (**136**), capensolactone 2b (**137**), capensolactone 3a (**138**), capensolactone 3b (**139**), methyl

3α-hydroxy-3-deoxyangolensate (**140**), ekebergin (**141**), methylangolensate (**142**), ekeberins C1-C3 (**143-145**), 7-deacetoxy-7-oxogedunin (**146**), 7-acetylneotrichilenone (**147**), proceranolide (**148**), mexicanolide (**149**) and swietenolide (**150**) (Taylor, 1981; Mulholland and Iourine, 1998; Murata *et al*., 2008). Also isolated from the stem bark are acyclic triterpenoids 2,3,22,23,-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**151**), 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene (**152**), ekeberins D1-D5 (**153-157**); triterpenoids ekeberin A (**158**), oleanolic acid (**159**), oleanonic acid (**160**), 3-*epi*-oleanolic acid (**161**), 3,11-dioxoolean-12-en-28-oic acid (**162**), melliferone (**163**) and 3-*oxo*-11,13(18)-oleandien-28-oic acid (**164**) ; coumarins ekersenin (**165**), 4,6-dimethoxy-5-methylcoumarin (**167**) and 7-hydroxy-6-methoxycoumarin (**167**); pregnanes ekeberin B (**168**) and (Z)-volkendousin (**169**) (Nishiyama *et al*., 1996a; Sewram *et al*., 2000; Murata *et al*., 2008).

	R_1	R_2	R_3
135	OH	$(CH_3)_2CHCO_2$	OH
136	ONicotinyl	$CH_3CH_2CH(CH_3)CO_2$	OAc
137	$CH_3CH_2CH(CH_3)CO_2$	ONicotinyl	OAc

	R_1	R_2	R_3
138	ONicotinyl	$(CH_3)_2CHCO_2$	OAc
139	$(CH_3)_2CHCO_2$	ONicotinyl	OAc

	R_1	R_2	R_3
140	OH	$(CH_3)_2CHCO_2$	OAc
141	H	OH	H
142	H	O	H

143

R₁   **R₂**
144 H   β O-angelate
145 OCOCH₃ O

146

147

    R₁   R₂   R₃
148 H   OH H
149 H   O   H
150 H   H   OH

R
151 CH₃
152 CH₂OH

153

    R₁   R₂
154 β-CH3   α-OH
155 α-CH3   β-OH

156

157

32
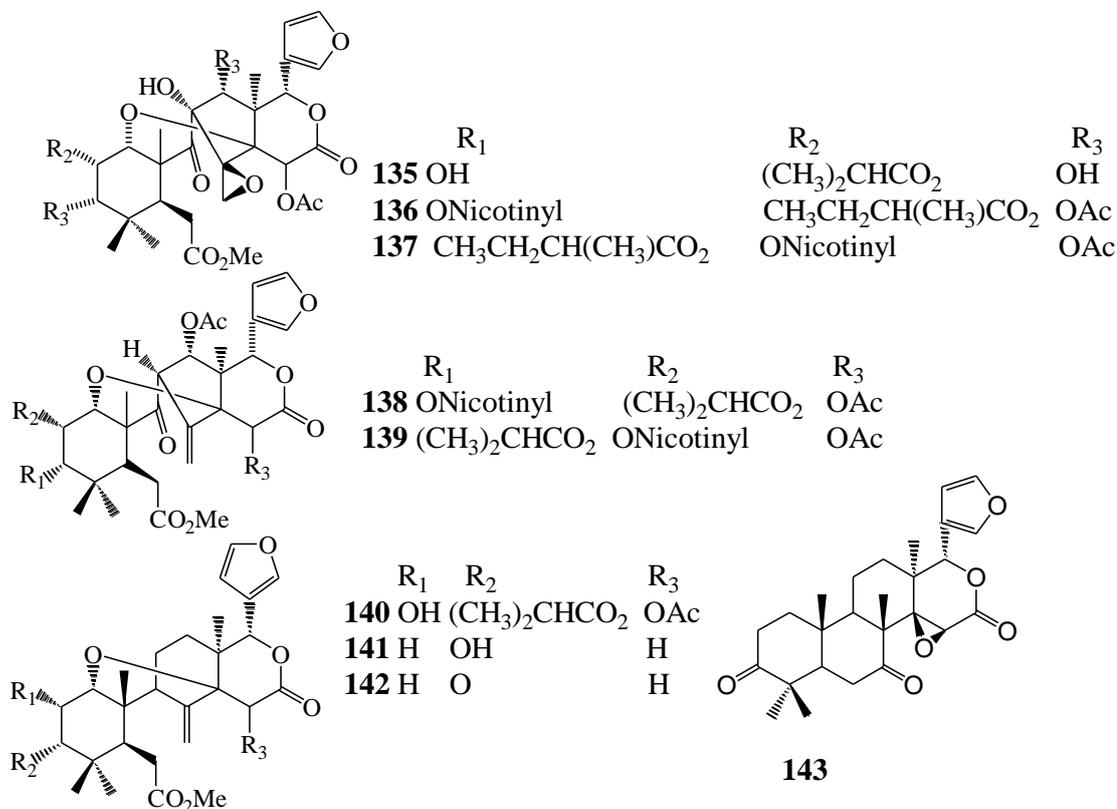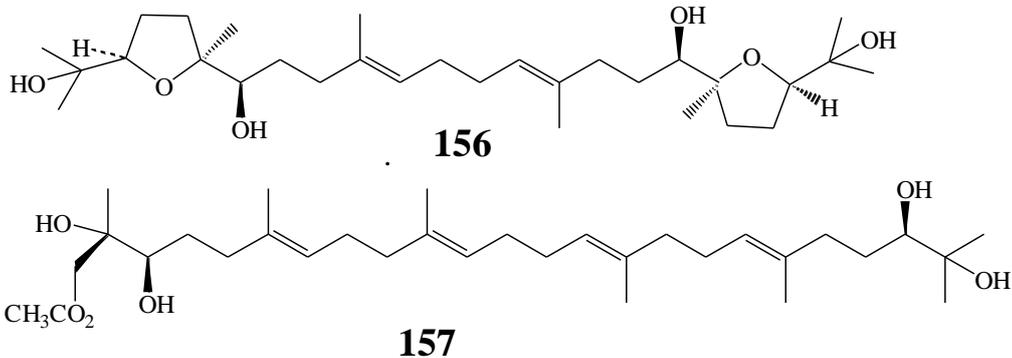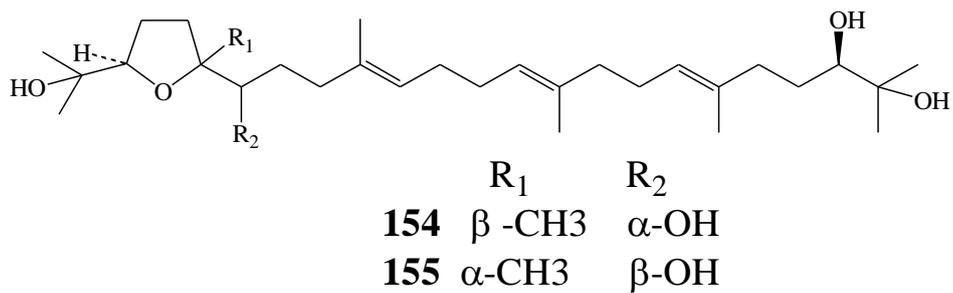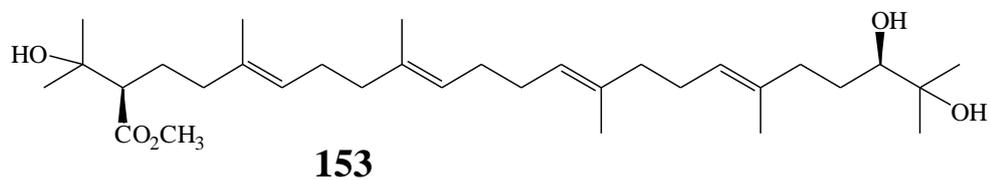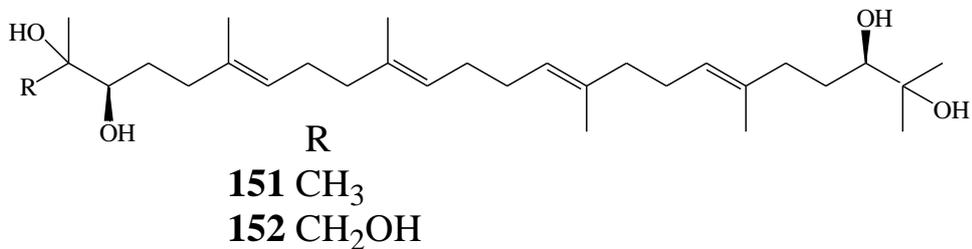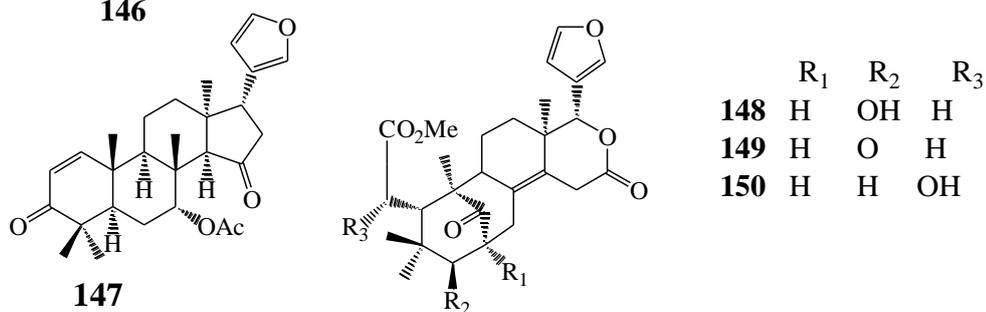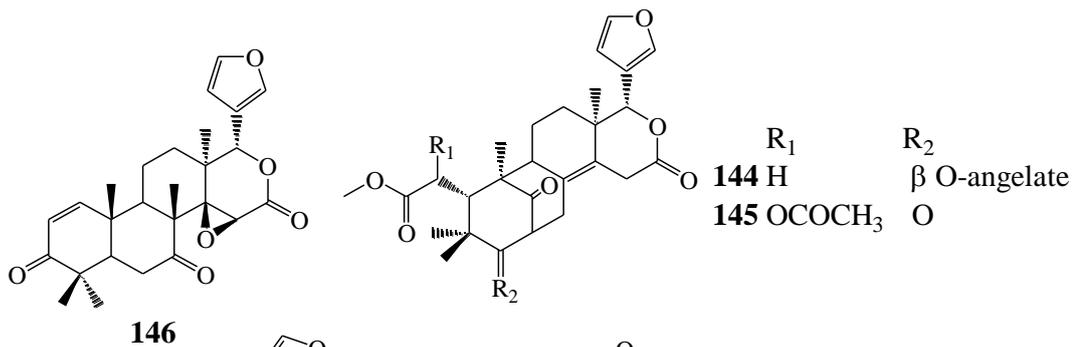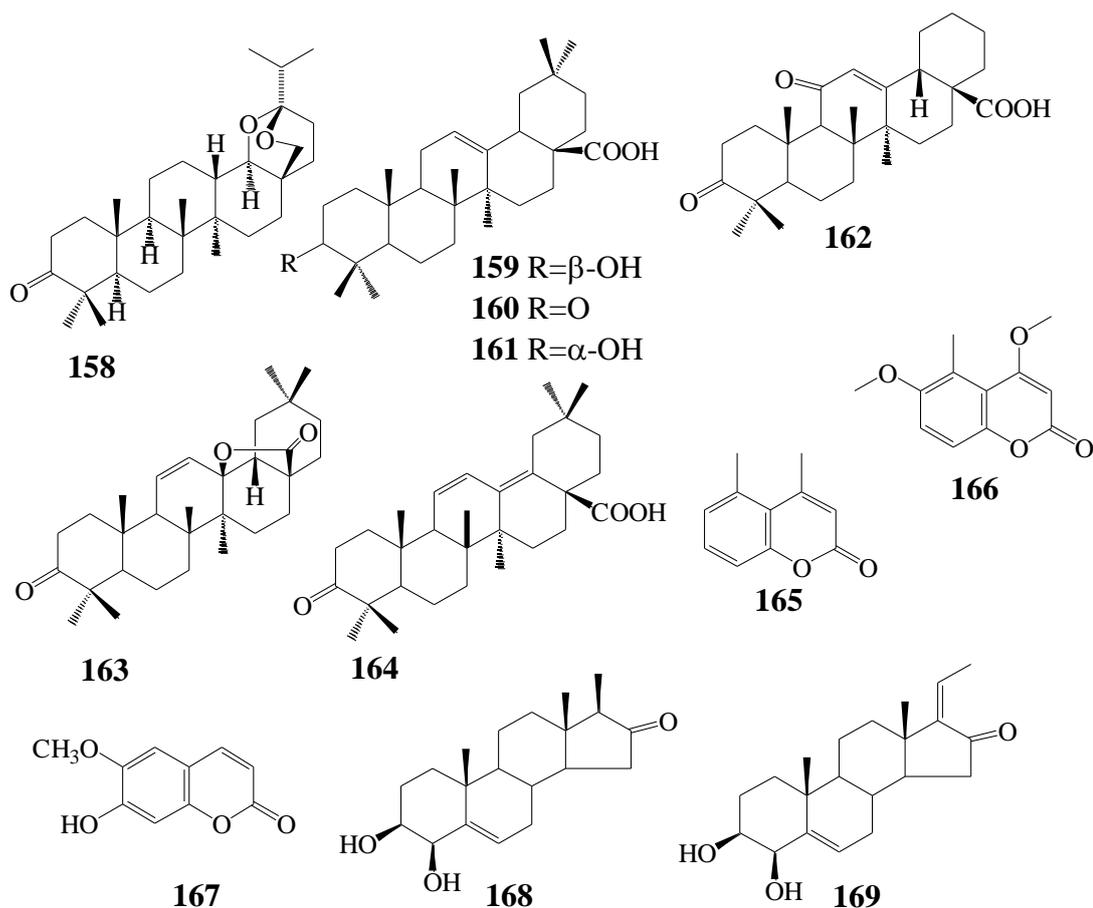
Figure 2-19: Compounds previously isolated from *E. capensis*

Phytochemical investigation on the seeds of *E. pterophylla* led to isolation of the limonoids EP1-EP6 (**170-175**) (Taylor and Taylor, 1984; Kehrli *et al*., 1990). The bark yielded atraric acid (**176**), β-amyrin (**177**), β-amyrone (**178**), β-sitosterylacetate (**179**), β-sitosterol (**180**), pterophyllin 1 (**181**) and pterophyllin 2 (**182**) while extractive from the wood yielded pterophylin 3-5 (**183-185**). Lupeol (**186**) was isolated from the leaves (Mulholland *et al*., 1998a).

	R$_1$	R$_2$	R$_3$
170	OAc	H	H
171	H	Ac	OAc
172	OAc	OAc	H
175	OTig	H	OH
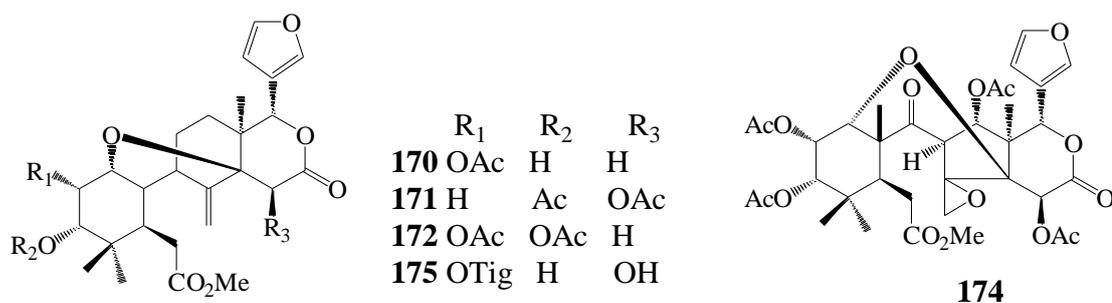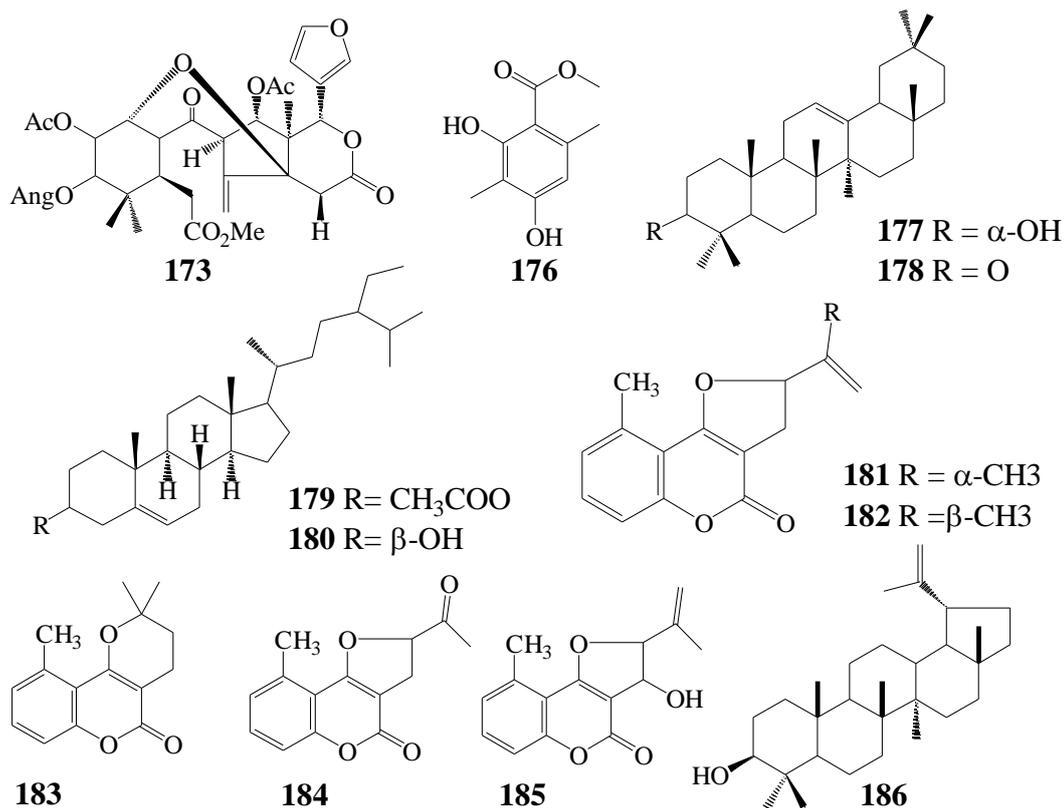
Figure 2-20: Compounds isolated from *E. pterophylla*

From the root bark of *E. benguelensis* coumarins 4-methoxy-5-hydroxymethylcoumarin (**187**) and stilbenes (**188**-**191**) were isolated (Jonker *et al.*, 1997; Chávez *et al.*, 2001).

188 R=H
189 R=Glc

187

190 R=Me
191 R=H
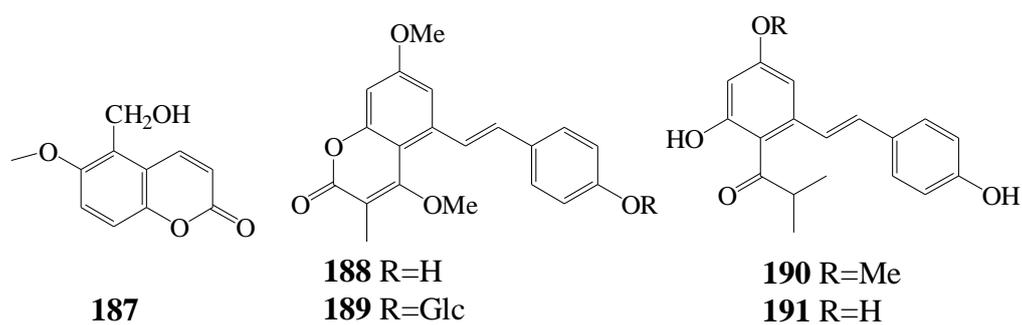
Figure 2-21: Compounds isolated from *E. benguelensis*

2.6.1. *Antiplasmodial and cytotoxic activities of compounds isolated from Genus*

   Ekebergia

Besides the use of *E. capensis* in traditional medicine to treat malaria, crude extract from the stem bark is reported to possess antiplasmodial activity (Muregi *et al*., 2004, 2008). Secondary metabolites from the stem bark were isolated and tested for antiplasmodial activity with compounds 7-deacetoxy-7-oxogedunin (**146**) and 2-hydroxymethyl-2,3,22,23-tetrahydroxy-2-6,10,15,19,23-pentamethyl-6,10,14,18 tetracosatetraene (**152**) displaying the highest activity against FCR-3 and K-1 *P. falciparum* strains. Lupeol (**186**) is another compound isolated from *E. pterophylla* whose antiplasmodial activity is documented (Fortie *et al*., 2006).

 Triterpenoids have previously been shown to be cytotoxic (Kim *et al*., 2012; Leal *et al*., 2013). Infact, two triterpenoid isolated from Ekebergia species, oleanolic acid (**159**) and lupeol (**186**) are reported to possess anticancer activity (Liu 2005; Saleem *et al*., 2008; Siddique *et al*., 2011). Other triterpenoids isolated from Ekerbergia reported to have cytotoxic activities include oleanonic acid (**160**), 3-epi-oleanolic acid (**161**), 3,11-dioxoolean-12-en-28-oic acid (**162**) and 7-deacetoxy-7-oxogedunin (**146**) (Kwon *et al* ., 1997; Pudhom *et al*., 2009; Sakai *et al*., 2004). The above reported antiplasmodial and cytotoxic properties on secondary metabolites isolated from Ekebergia species motivated the investigation of root bark and leaves of *E. capensis* whose secondary metabolites and antiplasmodial and cytotoxicity activities are not documented.

CHAPTER THREE

Materials and Methods

3.1.   General Overview on Chromatography and Spectroscopic Techniques

Column chromatography (CC) on Scharlau silica gel 60, 0.06-0.2 mm (70-230 mesh ASTM) and Fluka Sephadex^R LH-20; preparative thin layer chromatography (PTLC) on locally made plates, 20 x 20 cm glass, using Macherey-Nagel silica gel G/UV 254 for thin layer chromatography (TLC). TLC on Fluka silica on TLC alu foils and visualized under UV light (254 or 366 nm) followed by spraying with 1% vanillin in sulphuric acid. LC-ESI-MS spectra on a Perkin Elmer PE SCIEX API 150EX instrument equipped with a Turbolon spray ion source and a Gemini 5 mm C-18 110Å HPLC column using water acetonitrile gradient (80:20 to 20:80) at 30 eV.

High resolution mass spectral analysis (Q-TOF-MS) was done by Stenhagen Analyslab, using a micromass QTOF micro instrument with lockmass-ESI source and negative ion detection. Preparative HPLC was run on a Waters 600E HPLC system using the Chromulan software (Pikron Ltd., Praha, Czech Republic), a Kromasil C-8 250 $\times$ 25 mm C-8 column and water-acetonitrile eluent mixture. For structure elucidation gCOSY, gNOESY, gHSQC and gHMBC NMR spectra were acquired on Varian 800, 500 and 400 MHz spectrometers.

3.2. Plant Materials

The stem bark of *Turraea robusta* Guerke (BN/2011/1) was collected from University of Nairobi, Chiromo Campus in July 2011 (01°16$^-$31.34$^-$ $^-$S; 036°38$^-$64$^-$ $^-$E) and while *Turraea nilotica* Kotschy and Peyr leaves, stem and root bark (BN/2012/1) were collected in February 2012 in Mombasa Diani area (039°38$^-$17.06$^-$ $^-$E; 04°19$^-$04.72$^-$ $^-$S). The root bark and leaves of *Ekebergia capensis* Sparrm (BN/2013/1) were collected from Gakoe forest Kiambu County in April, 2013(01°04$^-$33.78$^-$ $^-$S; 36°14$^-$46.74$^-$ $^-$E). The plants were authenticated by Mr. Patrick Mutiso of the Herbarium, School of Biological Science, University of Nairobi where voucher specimens are deposited.

3.3. Extraction and Isolation

3.3.1. *Turraea robusta*

Air dried and ground *T. robusta* stem bark (1.4 kg) was extracted with MeOH/CH$_2$Cl$_2$ (1:1) for 48 hours (x2) at room temperature. The filtrate was evaporated using a rotary evaporator to yield 144 g of dark red oil. A portion (79 g) was fractionated on CC by silica gel with gradient elution using petroleum ether (40-60 ^{o}C) and ethyl acetate in the following ratios: 100:0, 99:1, 98:2, 94:6, 92:8, 90:10, 88:12, 86:14, 8:2, 15:5, 7:3, 6:3, 1:1, 5:15, 0:100. A total of 41 eluents (*ca*. 250 ml each) were collected and combined into 11 fractions (A-K) on the basis of TLC profiles. Fraction D (1.17 g) was re-chromatographed over silica gel column eluting with petroleum ether and acetone (95:5) to yield azadirone (**19**, 32.4 mg). Fraction G (4 g) was further fractionated on Sephadex^R LH-20 CC eluting with methanol to obtain fraction G1 (415.3 mg) which was purified on PTLC eluting with petroleum ether/chloroform/methanol (16:2:1) to yield 12α-acetoxy-7-deacetylazadirone (**28**, 7 mg) and mzikonone (**17**, 6.3 mg). Fraction H (3 g) was re-chromatographed on silica

gel CC eluting with petroleum ether/ethylacetate (9:1) to obtain fraction H1. This subfraction was purified on PTLC eluting with petroleum ether/acetone (7:4) to yield azadironolide (**192**, 16.5 mg). Fraction I (80 mg) was crystallized in acetone to yield stigmasterol (**193**, 6.2 mg). Fraction J (145.2 mg) was subjected to further separation on PTLC eluting with petroleum ether/chloroform/methanol (10:2:1) yielding 11-*epi*-toonacilin (**62**, 3 mg). Fraction K (331.9 mg) was subjected to PTLC eluting with petroleum ether/chloroform/methanol (12:2:1) to yield turranolide (**22**, 17.7 mg). Fraction A, B C, E and F which contained mostly oils and phytosterols were not investigated.

3.3.1.1. *Physical and spectroscopic data of compounds isolated from Turraea robusta*

Mzikonone (**17**); white amorphous solid, mp (100-101 ^{o}C); ^{1}H NMR, (acetone-d$_{6}$) δ (600 MHz, ppm), Appendix 1: 7.47 (1H, t, J=1.2 Hz, H-23), 7.35 (1H, t, J=1.2 Hz, H-21), 6.36 (1H, m, H-22), 5.70 (1H, dd, J=3.6, 1.8 Hz, H-15), 5.06 (dd, J=7.2, 9 Hz, H-12), 4.05 (brs, 1H, H-7), 3.01 (1H, dd, 7.8, 10.8 HZ, H-17), 2.59 (1H, m, CH$_{2}$-16a), 2.56 (1H, m, CH$_{2}$-2a), 2.4 (1H, m, CH$_{2}$-16b), 2.35 (1H, m, CH$_{2}$-2b), 2.23 (1H, m, H-9), 2.19 (1H, m, CH$_{2}$-11a), 2.12 (1H, dd, J=2.4, 12.6, Hz, H-5), 1.95 (1H, m, CH$_{2}$-6a), 1.81 (1H, m, CH$_{2}$-1a), 1.7 (1H, m, CH$_{2}$-6b), 1.52 (1H, m, CH$_{2}$-1b), 1.43 (1H, m, CH$_{2}$-11b), 1.88 (3H, s, Ac-Me), 1.17 (3H, s, Me-30), 1.07 (3H, s, Me-19), 1.06 (3H, s, Me-18), 1.03 (3H, s, Me-29), 1.02 (3H, s, Me-28); ^{13}C NMR, (Acetone-d$_{6}$); δ (150 MHz, ppm) Appendix 1A: 216.5 (C-3), 171.4 (Ac-CO), 159.5 (C-14), 143.7 (C-23), 141.9 (C-21), 126.5 (C-20), C-123.6 (C-15), 113.4 (C-22), 79.1 (C-12), 73.1 (C-7), 52.8 (C-13), 51.9 (C-17), 48.1 (C-8), 48.0 (C-5), 45.1 (C-4), 44.0 (C-9), 40.0 (C-1), 38.4 (C-10), 38.1 (C-16), 35 (C-2), 28.6 (C-30), 27.2 (C-28), 27 (C-11), 26.9 (C-6), 22.1 (Ac-

Me), 22.0 (C-18), 16.2 (C-19), 15.9 (C-29); ESI-MS (30 eV): m/z 455.5 [M+H]$^+$, Appendix 1B.

Azadirone (**19**); yellowish gum; ^{1}H NMR, (CD$_2$Cl$_2$) δ (600 MHz, ppm), Appendix 2 : 7.39 (1H, t, J=3 Hz, H-23), 7.18 (1H, d, J=10.2 Hz, H-1), 7.16 (1H, m, H-21), 6.32 (1H, m, H-22), 5.79 (1H, d, J=10.2 Hz, H-2), 5.36 (1H, dd, J=1.2, 3, Hz, H-15), 5.25 (1H, dd, J=1.8, 3 Hz, H-7), 2.82 (1H, dd, J = 7.8, 11.4 Hz, H-17), 2.45 (1H, ddd, J = 1.8, 11.4, 25.6 Hz, CH$_2$-16a), 2.32 (1H, ddd, J=3.6, 7.2, 18.6 Hz, CH$_2$-16b), 2.23 (1H, dd, J= 2.4, 13.2 Hz, H-5), 2.22 (1H, m, H-9), 2.0 (1H, m, CH$_2$-6a), 1.98 (1H, m, CH$_2$-11a), 1.93 (1H, m, CH$_2$-12a), 1.92 (3H, s, Ac-Me), 1.8 (1H, m, CH$_2$-6b), 1.79 (1H, m, CH$_2$-11b), 1.65 (1H, m, CH$_2$-12b), 1.20 (6H, s, Me-30, Me-19), 1.06 (3H, s, Me-29), 1.05 (3H, s, Me-28), 0.7 (3H, s, Me-18); ^{13}C NMR (CD$_2$Cl$_2$); δ (150 MHz, ppm), , Appendix 2A: 204.6 (C-3), 170.1 (Ac-CO), 159.3 (C-14), 158.6 (C-1), 142.7 (C-23), 140.0 (C-21), 125.4 (C-2), 125.1 (C-20), 119.2 (C-15), 111.4 (C-22), 74.6 (C-7), 51.8 (C-17), 47.4 (C-13), 46.3 (C-5), 44.3 (C-4), 43.1 (C-8), 40.2 (C-10), 38.9 (C-9), 34.6 (C-16), 33.2 (C-12), 27.3 (C-30), 27.0 (C-29), 24.0 (C-6), 21.3 (C-28), 21.2 (Ac-Me), 20.6 (C-18), 19.1 (C-19), 16.7 (C-11). EIMS: m/z 436, Appendix 2B.

Turranolide (**22**); whitish amorphous powder, mp 198-200 ^{o}C: ^{1}H NMR (acetone-d$_6$) δ (600 MHz, ppm) Appendix 5: 5.26 (1H, dd, J=11.8, 3.6 Hz, H-15), 5.17 (1H, dd, J=11.8, 3.6, Hz, H-7), 4.96 (1H, t, J=8.4 Hz, CH$_2$-21a), 3.93 (1H, dd, J=19, 10.2 Hz, CH$_2$-21b), 2.8 (1H, m, H-20), 2.61 (1H, m, CH$_2$-2a), 2.44 (1H, dd, J=17.8, 16.8 Hz, CH$_2$-22a), 2.3 (1H, m, CH$_2$-2b), 2.28 (1H, dd, J=11.4, 16.8 Hz, CH$_2$-22b), 2.15 (2H, m, CH$_2$-16), 2.13 (1H, m, H-9), 1.99 (1H, m, CH$_2$-16a), 1.94 (1H, m, H-5), 1.92 (3H, s, Ac-Me), 1.8 (1H, m, CH$_2$-11a), 1.78 (1H, m, H-17), 1.76 (2H, m, H-12), 1.68 (1H, m, CH$_2$-11b), 1.69 (1H, m, CH$_2$-16b), 1.19 (3H, s, Me-18), 1.1 (6H, s, Me-19, Me-

30), 1.02 (3H, s, Me-29), 0.98 (3H, s, Me-28); ^{13}C NMR, (acetone-d$_6$) δ (150 MHz, ppm), Appendix 5A: 216.1 (C-3), 177.6 (C-23), 171.1 (Ac-CO), 161.0 (C-14), 119.8 (C-15), 76.1 (C-7), 73.5 (C-21), 59.6 (C-17), 49.6 (C-5), 48.1 (C-10), 48 (C-13), 44.1 (C-9), 43.5 (C-8), 40.1 (C-1), 38.9 (C-20), 38.4 (C-4), 35.9 (C-16), 35.1 (C-22), 35 (C-12), 34.9 (C-2), 28.0 (C-18), 26.8 (C-28), 25.5 (C-6), 22.0 (C-29), 21.8 (Ac-Me), 20.5 (C-30), 17.7 (C-11), 16.0 (C-19); EIMS *m/z* 456, Appendix 5B.

12α-acetoxy-7-deacetylazadirone (**28**); whitish amorphous powder: ^{1}H NMR, (acetone-d$_6$) δ (600 MHz, ppm) Appendix 3: 7.47 (1H, t, J=3 Hz, H-23), 7.36 (1H, m, H-12), 7.17 (1H, d, J=10.2, Hz, H-1), 6.37 (1H, m, H-22), 5.74 (1H, m, H-2), 5.73 (1H, d, J=2.4, Hz, H-15), 5.12 (1H, dd, J=7.2, 9 Hz, H-12), 4.09 (1H, brs, H-7), 3.05 (1H, dd, J=7.8, 11.4 Hz, H-17), 2.6 (1H, m, CH$_2$-16a), 2.51 (1H, dd, J=7.2, 9 Hz, H-9), 2.45 (1H, dd, J=3, 13.2 Hz, H-5), 2.4 (1H, m, CH$_2$-16b), 2.35 (1H, m, CH$_2$-11a), 2.04 (1H, m, CH$_2$-6a), 1.90 (3H, s, Ac-Me) 1.78 (1H, m, CH$_2$-6b), 1.75 (1H, m, CH$_2$-11b), 1.22 (3H, s, Me-30), 1.12 (3H, s, Me-29), 1.11 (3H, s, Me-28), 1.06 (3H, s, Me-19), 1.05 (3H, s, Me-18); ^{13}C NMR, (acetone-d$_6$), δ (150 MHz, ppm), Appendix 3A: 205.9 (C-3), 171.4 (Ac-CO), 159.4 (C-1, C-14), 143.7 (C-23), 142.0 (C-21), 126.4 (C-2, C-20), 123.7 (C-15), 113.4 (C-22), 79.0 (C-12), 72.7 (C-7), 52.8 (C-13), 52.0 (C-17) 46.1 (C-5), 45.8 (C-8), 45.4 (C-4), 41.5 (C-10), 39.8 (C-9), 38.1 (C-16), 29.1 (C-30), 28.1 (C-28), 26.9 (C-6), 26.7 (C-11), 22.5 (Ac-Me), 22.0 (C-18), 20.0 (C-29), 16.3 (C-19). EIMS (observed m/*z* 452.2555, calcd 452.2557) Appendix 3B.

11-*epi*-toonacilin (**62**); White and crystalline, mp 117-122 °C: ^{1}H NMR, (acetone-d$_6$) δ (600 MHz, ppm): Appendix 4: 7.51 (1H, dd, J=10.8Hz, H-1), 7.47 (1H, m, H-23), 7.25 (1H, m, H-21), 6.29 (1H, m, H-22), 6.02 (1H, d, J=10.2 Hz, H-2), 5.75 (1H, d, J=10.8 Hz, H-12), 5.56 (1H, dd, J=7.8, 10.8 Hz, H-11), 5.51 (1H, d, J=0.6 Hz, H-30a), 5.48 (1H, d, J=1.2 Hz, 30b), 4.07 (1H, brs, H-15), 3.62 (3H, s, OMe), 3.11 (1H, d,

J=7.2 Hz, H-5), 3.08 (1H, dd, J=7.8, 10.8 Hz, H-9), 2.96 (1H, dd, J=7.2, 10.8 Hz, H-17), 2.58 (1H, dd, J=2.4, 16.8 Hz, CH_2-6a), 2.47 (1H, J=2.4, 16.8 Hz, CH_2-6b), 2.21 (1H, m, CH_2-16a), 1.96 (1H, m, CH_2-16b), 1.86 (3H, s, Ac-Me), 1.70 (3H, s, Ac-Me), 1.10 (3H, s, Me-29), 1.04 (3H, s, Me-19), 0.96 (3H, s, Me-28), 0.95 (3H, s, Me-18); ^{13}C NMR, (acetone-d_6); δ (150 MHz, ppm), Appendix 4A: 204.2 (C-3), 175.6 (C-7), 153.8 (C-1), 171.1 (Ac-C0), 170.2 (Ac-CO), 144.2 (C-23), 142.0 (C-21), 138.6 (C-8), 124.2 (C-20), C-30 (C-30), 113.0 (C-22), 76.8 (C-12), 72.7 (C-11), 61.3 (C-15), 54.4 (C-9), 52.7 (OMe), 47.6 (C-5), 46.6 (C-5), 46.5 (C-13), 43.5 (C-10), 39.8 (C-17), 34.7 (C-16), 32.4 (C-6), 24.1 (C-28), 23.7 (C-29), 22.4 (C-19), 21.4 (Ac-Me), 21.3 (Ac-Me), 14.6 (C-18). EIMS (observed m/z 554.2506, calcd 554.2510), Appendix 4B.

Azadironolide (**192**); a   clear gum: ^1H NMR (acetone-d_6), δ (600 MHz, ppm);, Appendix 6: 7.27 (1H, d, J=10.2 Hz, H-1), 7.18 (1H, d, J=6.6 Hz, H-22), 6.18 (1H, s, H-23), 5.76 (1H, d, J=10.2 Hz, H-2), 5.36 (1H, s, H-15), 5.32 (1H, d, J=3.2 Hz, H-7), 2.76 (1H, m, H-17), 2.27 (1H, m, H-5), 2.24 (1H, m, H-5), 1.94 (3H, s, Ac-Me), 1.3 (3H, s, Me-30), 1.24 (3H, s, Me-19), 1.06 (3H, s, Me-28), 1.03 (3H, s, Me-29), 0.94 (3H, s, Me-18); ^{13}C NMR (acetone-d_6); δ (150 MHz, ppm), Appendix 6A: 204.6 (C-3), 173.0, 172.8 (C-21), 170.7 (Ac-CO), 160.6 (C-14), 159.7 (C-1), 148.8 (148.5) (C-22), 138.1 (C-20), 126.4 (C-2), 119.9(199.8) (C-15), 98.6 (98.4) (C-22), 75.7 (C-7), 54.5 (C-17), 52.3 (C-13), 47.8 (C-5), 45.3 (C-4), 41.4 (C-10), 40.2 (C-8), 40.1(C-9), 35.1 (C-16), 34.7 (C-12), 28.4 (C-30), 28.0 (C-29), 25.1 (C-6), 22.3 (C-28), 22.0 (C-18), 20.1 (C-19), 17.7 (C-11).

Stigmasterol (**193**); Whitish crystals, mp 175-278 °C: ^1H NMR (acetone-d_6) δ (600 MHz, ppm) Appendix 7; 5.31 (1H, d, J=5.4 Hz, H-6), 5.21 (1H, dd, 9, 15, Hz, H-22), 5.08 (1H, dd, 8.4, 15 Hz, H-23), 3.39 (1H, m, H-3), 2.20 (1H, m, H-4), 2.01 (1H, m, CH2-7a), 2.00 (1H, m, CH_2-1a), 1.90 (1H, m, CH_2-2a), 1.80 (1H, m, CH_2-7b), 1.75

(1H, m, CH$_2$-1b), 1.63 (1H, m, CH$_2$-15a), 1.62 (3H, m, CH$_2$-28a, CH$_2$-11), 1.50 (1H, m, CH$_2$-8), 1.42 (1H, m, H-20),1.40 (1H, m, CH$_2$-12a), 1.34 (1H, m, CH$_2$-2b), 1.24 (2H. m, CH$_2$-16), 1.20 (1H, m, H-14), 1.19 (1H, m, H-17), 1.18 (1H, m, CH$_2$-18b), 1.17 (1H, m, CH$_2$-15b), 1.06 (3H, s, Me-21), 1.04 (3H, s, Me-19), 0.87 (3H, s, Me-27), 0.84 (3H, s, Me-26), 0.83 (3H, s, Me-29), 0.75 (3H, s, Me-18): ^{13}C NMR (acetone-d$_6$); δ (150 MHz, ppm). Appendix 7A: 143 (C-5), 140 (C-22), 130.7 (C-23), 122.2 (C-6), 72.4 (C-3), 58.3 (C-14), 57.6 (C-17), 52.8 (C-24), 51.9 (C-9), 44.0 (C-4), 42.1 (C-13), 37.6 (C-20), 35.3 (C-12), 33.4 (C-1, C-10), 33.3 (C-8, C-7, C-15), 29.6 (C-2), 27.4 (C-16), 25.6 (C-28), 24.4 (C-11, C-15), 22.5 (C-21, C-26), 20.5 (C-19,C-27), 12.9 (C-29).

3.3.2. *Turraea nilotica*

Air dried and ground stem bark of *Turraea nilotica* (1.16 kg) was extracted as described in section 3.3.1. The filtrate was dried *in vacuo* using a rotary evaporator to yield 59 g of a dark gum. A portion (58 g) of the extract was fractionated by CC on silica gel eluting with petroleum ether (40-60 ^{o}C) and ethyl acetate in the following ratios: 100:0, 98:2, 96: 4, 92:8, 9:1, 88:12, 86:14, 8:2, 7:3, 1:1, 5:15, 0: 100. A total of 50 eluents (*ca*. 250 ml each) were collected and combined to 20 fractions (A-T) after TLC. Fraction L (1.35 g) was fractionated further by CC on Sephadex^R LH-20 eluting with methanol to yield fractions L1-L3. Subfraction L2 (300 mg) was re-chromatographed (silica gel CC eluting with petroleum ether/acetone, 95:5) yielding fractions L2 (A–D). Fraction L2D (131.6 mg) was purified on PTLC eluting with petroleum ether/chloroform/methanol (18:2:1) to yield niloticin (**25**, 9.3 mg). Fractions M, N, O and T were combined (7 g) and re-chromatographed on silica gel gradient CC eluting with petroleum ether and acetone in the following ratios: 98:2, 95:5, 9:1, 85:15, 8:2, 7:3, 1:1. Eleven eluents of *ca.* 100 ml each (MNOT 1-11) were

collected. Fraction MNOT 11 (619.8 mg) was re-chromatographed on Sephadex^R LH-20 column (eluting with methanol) to yield fractions MNOT 11 (A-C). Fraction MNOT 11B (215.2 mg) was crystallized from acetone/dichloromethane mixture to yield toonapubesins F (**194**, 20.1 mg). Fraction P (3 g) was fractionated on silica gel CC eluting with petroleum ether/acetone ratios: 98:2, 96:4, 9:1, 85:15. Twenty fractions (*ca*. 100 ml each) were collected and combined to 3 fractions (P1-P3) after TLC. Fraction P2 was crystallized from acetone to yield piscidinol A (**27**, 775.6 mg). Fraction R was crystallized from mixture of methanol and dichloromethane to yield hispidol B (**96**, 84.2 mg). Fractions A-K were difficult to fractionate with the available chromatographic techniques while Q and S were similar to R and hence were not worked on.

The root bark of *Turraea nilotica* (837 g) was extracted as described in section 3.3.1 above. The filtrate was dried *in vacuo* yielding 13 g of a yellowish gum. A portion of the extract (11 g) was fractionated by CC over silica gel eluting with petroleum ether (40-60 ^{0}C) and acetone in the following ratios: 99.9: 0:1, 99.8: 0.2, 99.6: 0.4, 99.4:0.6, 99.2: 0.8, 99:1, 98.8:0.2, 98.6:0.4, 98.2: 0.8; 98:2, 97.8:0.2, 97.4: 0.6, 97.2:0.8, 97:3, 96.5:3.5, 96:4, 95.5:4.5, 95:5, 94.5:5.5, 94:6, 93.5:6.5, 93:7, 92.5:7.5, 92:8, 91.5: 8.5 9:1. Forty six eluents (*ca*. 100 ml each) were collected and combined into 12 fractions (A-L) after TLC. Fraction G (140.7 mg) was purified on PTLC eluting with petroleum ether/acetone (9:1) to yield azadirone (**19**, 8.3mg). Fraction L (366 mg) was subjected to RP-HPLC (CH$_3$OH/water) yielding 12α-acetoxy-7-deacetylazadirone (**28**, 18.5 mg) and mzikonone (**17**, 4.4 mg). Fraction K (358 mg) was subjected to further CC on Sephadex^R LH-20 eluting with methanol and dichloromethane (1:1). Sixteen eluents (*ca*. 100 ml each) were collected and combined into 3 fractions (K1-K3) after TLC. Fraction K1 was further purified on

PTLC eluting with petroleum ether/chloroform/methanol (10:2:1) to yield 1α,3α-diacetyl-7α-tigloyvilasinin (**40**, 11.6 mg). There was no phytochemical investigation carried out on Fractions A-F, H, I and J as the amounts were inadequate.

Dried and ground leaves of *T. nilotica* (500g) were extracted with MeOH/CH$_2$Cl$_2$ (1:1) for 48 hours (x2) at room temperature yielding 30 g of a dark green gum. A portion (20 g) was fractionated on CC over silica gel eluting with petroleum ether/acetone in the following ratios: 100:0, 9.75: 0.25, 9.25:0.75, 9:1, 8.75:1.25, 8.5:1.75, 8:2. A total of fifty eight eluents (ca. 100 ml each) were collected and combined into 12 fractions (A-L) after TLC. Fraction C was crystallized from acetone giving stigmasterol (**193**) and β-sitosterol (**180**) as a mixture (4.72 mg). Fraction J crystallized from acetone yielding sitosterol 3-*O*-β-D-glucopyranoside acetate (**195**) and stigmasterol-3-*O*-β-D-glucopyranoside acetate (**196**) as a mixture (9.8 mg). Fraction L crystallized in acetone to yield sitosterol-3-*O*-β-D-glucopyranoside (**197**, 4.7 mg). Fraction A-B and contained oils while fractions D-I were heavily loaded with chlorophyll and were not investigated.

3.3.2.1. *Physical and spectroscopic data of compounds isolated from Turraea nilotica*

Niloticin (**25**); white crystals, mp (138-142 $^{\circ}$C); ^{1}H NMR (acetone-d$_6$), δ (600 MHz, ppm), Appendix 27; 5.35 (1H, dd, J=7.2, 3 Hz, H-7), 3.49 (1H, dd, J=13.8, 6.6 Hz, H-23), 2.81 (1H, dt, J=14.4, 5.4 Hz, CH$_2$-2a), 2.56 (1H, d, J=8.4 Hz, H-24), 2.4 (1H, m, H-9), 2.13 (1H, dt, J=5.4, 14.4 Hz, CH$_2$-2b), 2.1 (2H, m, CH$_2$-6), 2.08 (1H, m, CH$_2$-16a), 2.02 (1H, ddd, J=3, 5.4, 13.2 Hz, CH$_2$-1a), 1.87 (1H, m, CH$_2$-12a), 1.75 (1H, dd, J=6, 17 Hz, H-5), 1.67 (1H, m, CH$_2$-12b), 1.64 (1H, m, CH$_2$-22a), 1.63 (2H, m, CH$_2$-11), 1.60 (1H, m, CH-17), 1.56 (2H, m, CH$_2$-15), 1.51 (1H, m, CH-20), 1.45

(1H, m, CH$_2$-1b), 1.36 (1H, m, CH$_2$-22b), 1.26 (3H, s, Me-19), 1.25 (3H, s, Me-27), 1.10 (3H, s, Me-29), 1.06 (3H, s, Me-30), 1.05 (3H, s, Me-18), 1.00 (3H, s, Me-28), 0.99 (3H, s, Me-21), 0.87 (3H, s, Me-26); ^{13}C NMR (acetone-d$_6$),; δ (150 MHz, ppm), Appendix 27A: 214.3 (C-3), 145.8 (C-8), 117.9 (C-7), 69.2 (C-23), 68.4 (C-24), 58.1 (C-25), 53.4 (C-17), 52.2 (C-5), 51.1 (C-14), 48.4 (C-9), 47.4 (C-4), 43.5 (C-13), 41.1 (C-22), 38.2 (C-1), 34.9 (C-10), 34.5 (C-2), 33.9 (C-15), 33.6 (C-20), 33.3 (C-12), 28.5 (C-16), 27.0 (C-30), 24.3 (C-28), 24.2 (C-6, C-27), 21.3 (C-29), 20.9 (C-26), 19.8 (C-21), 19.3 (C-19), 18.1 (C-11), 12.2 (C-18). ESI-MS (30 eV): 457.6 [M+H]$^+$Appendix 27B.

Piscidinol A (**27**); white crystals, mp (81-82 ^{o}C); ^{1}H NMR (CD$_2$Cl$_2$) δ (600 MHz, ppm), Appendix 8: 5.32 (1H, m, H-7), 4.08 (1H, dd, J=8.6, 5 Hz, H-23), 3.14 (1H, d, J=5.6 Hz, H-24), 2.76 (1H, td, J=5.5, 14.5 Hz, CH$_2$-2a), 2.39 (1H, m, H-9), 2.18 (1H, td, J=14.2, 7.1 Hz, CH$_2$-2b), 2.10 (2H, m, CH$_2$-6), 2.02 (1H, m, CH$_2$-16a), 2.00 (1H, m, CH$_2$-1a), 1.84 (1H, m, CH$_2$-22a), 1.83 (1H, m, CH$_2$-12a), 1.72 (1H, dd, J=10.9, 6.7 Hz, H-5), 1.66 (1H, m, CH$_2$-12b), 1.56 (2H, m, CH$_2$-11), 1.50 (1H, m, H-17), 1.49 (2H, m, CH$_2$-15), 1.40 (1H, m, CH$_2$-1b), 1.36 (1H, m, H-20), 1.30 (1H, m, CH$_2$-16b), 1.26 (6H, s, Me-26, Me-27), 1.15 (1H, m, CH$_2$-22b), 1.10 (3H, s, Me-29), 1.01 (6H, s, Me-19, Me-30), 0.92 (3H, d, Me-21), 0.82 (3H, s, Me-18); ^{13}C NMR (CD$_2$Cl$_2$), δ (150 MHz, ppm) Appendix 8A: 216.5 (C-3), 145.8 (C-8), 118.0 (C-7), 74.9 (C-24), 74.1 (C-25), 69.6 (C-23), 53.1 (C-17), 52.3 (C-5), 51.2 (C-14), 48.5 (C-9), 47.5 (C-4), 43.5 (C-13), 40.5 (C-22), 38.5 (C-1), 35.0 (C-10), 34.9 (C-2), 34.0 (C-20), 33.7 (C-12, C-15), 28.4 (C-160, 27.1 (C-27, C-30), 26.1 (C-26), 24.4 (C-6), 24.3 (C-28), 21.7 (C-18), 21.4 (C-29), 18.5 (C-21), 18.3 (C-11). ESI-MS (30 eV): *m/z* 475.5 [M+H]$^+$ Appendix 8B.

1α, 3α-diacety-7α-tigloyvilasinin (**40**); white amorphous powder; mp 196-197 $^{\circ}$C: ^{1}H NMR (CD$_2$Cl$_2$), δ (600 MHz, ppm), Appendix 12; 7.28 (1H, t, J=3.4 Hz, H-23), 7.15 (1H, m, H-21), 6.80 (1H, qq, J=1.5, 7.1 Hz, H-3'), 6.20 (1H, m, H-22), 5.51 (1H, d, J=2.8 Hz, H-7), 5.42 (1H, dd, J=1.5, 3.2 Hz, H-15), 4.82 (1H, t, J=5.9 Hz, H-3), 4.62 (1H, t, J=5.8 Hz, H-1), 4.10 (1H, dd, J=2.8, 12.6 Hz, H-6), 3.35 (1H, m, H-28), 2.71 (1H, dd, J=10.9, 7.4 Hz, H-17), 2.59 (2H, m, H-5, H-9), 2.3 (1H, ddd, J=1.7, 11, 2.2 Hz, CH$_2$-16a), 2.22 (1H, ddd, J=3.5, 7.4, 15.3 Hz, CH$_2$-16b), 2.15 (1H, dt, J=6.5, 16.6 Hz, CH$_2$-2a), 2.02 (1H, dt, J=5.2, 16.6 Hz, CH$_2$-2b), 1.92 (3H, s, Ac-Me), 1.88 (3H, s, Ac-Me), 1.78 (3H, 2, Me-5'), 1.78 (3H, s, Me-4'), 1.70 (1H, m, CH$_2$-12a), 1.56 (1H, CH$_2$-11a), 1.50 (1H, m, CH$_2$-12b), 1.26 (1H, m, CH$_2$-11b), 1.11 (3H, s, Me-30), 1.10 (3H, s, Me-29), 0.95 (3H, s, Me-19), 0.67 (3H, s, Me-19): ^{13}C NMR (CD$_2$Cl$_2$); δ (150 MHz, ppm), Appendix 12A: 169.8 (Ac-CO), 169.5 (Ac-CO), 166.5 (C-1'), 158.1 (C-14), 142.5 (C-23), 139.7 (C-21), 136.6 (C-3'), 128.8 (C-2'), 124.9 (C-20), 120.3(C-15), 111.1 (C-22), 77.7 (C-28), 73.9 (C-7), 72.7 (C-6), 72.4 (C-1), 71.7 (C-3), 51.5 (C-17), 47.3 (C-13), 44.6 (C-8), 42.1 (C-4), 41.4 (C-5), 39.2 (C-10), 34.8 (C-9), 34.2 (C-16), 32.7 (C-12), 27.4 (C-2), 26.0 (C-30), 21.1 (C-18), 21.0 (Ac-Me), 20.8 (Ac-Me), 19.1 (C-29), 15.2 (C-11), 14.1 (C-4'), 11.9 (C-9'). EIMS: *m/z* 594 [M]$^{+}$ Appendix 12B.

Hispidol B (**96**); White crystals, mp 133-135°C. ^{1}H NMR (DMSO-d$_6$) δ (600 MHz, ppm), Appendix 10, ; 5.22 (1H, d, J=3.6 Hz, H-7), 4.37 (1H, d, J=4.8 Hz, H-23), 4.15 (1H, d, J=6 Hz, H-3), 4.11 (1H, d, J=8.4 Hz, H-24), 2.12 (1H, m, H-9), 2.06 (1H, m, CH$_2$-6a), 1.90 (2H, m, CH$_2$-6b, CH$_2$-16a), 1.35 (3H, m, CH$_2$-15, CH$_2$-16b), 1.23 (1H, m, H-5), 1.11 (3H, s, Me-27), 1.08 (3H, s, Me-26), 0.94 (3H, s, Me-30), 0.86 (6H, s, Me-28, Me-21), 0.77 (3H, s, Me-19), 0.75 (3H, s, Me-29), 0.68 (3H, s, Me-18): ^{13}C NMR (DMSO-d$_6$); δ (150 MHz, ppm), Appendix 10A:146.0 (C-8), 118.0 (C-7), 77.4

(C-3), 75.9 (C-24), 72.9 (C-25), 67.9 (C-23), 53.9 (C-17), 51.2 (C-14), 50.6 (C-5), 48.9 (C-9), 43.5 (C-13), 41.8 (C-22), 37.2 (C-1), 34.9 (C-10), 34.0 (C-15), 33.8 (C-12), 33.6 (C-20), 28.4 (C-16, C-2), 28.2 (C-28), 28.0 (C-27), 27.6 (C-30), 26.4 (C-26), 24.0 (C-6), 22.2 (C-19), 19.4 (C-21), 18.1 (C-11), 15.4 (C-29), 13.4 (C-18). EIMS m/z 476.3868, calcd 476.3860), Appendix 10B.

Toonapubesins F (**194**); white armophous powder: ^{1}H NMR (CD$_2$Cl$_2$) δ (600 MHz, ppm), Appendix 11; 5.67 (1H, m, C-7), 4.09 (1H, m, H-23), 4.08 (1H, d, J=10 Hz, CH$_2$-29a), 3.60 (1H, d, J=10.9 Hz, CH$_2$-29b), 3.14 (1H, s, H-24), 2.69 (1H, td, J=5.8, 14.6 Hz, CH$_2$-2a), 2.30 (1H, td, J=3.2, 4.4, 14.6 Hz, CH$_2$-2b), 2.34 (1H, m, H-9), 2.16 (1H, m, CH$_2$-6a), 2.05 (1H, m, CH$_2$-6b), 2.02 (1H, m, CH$_2$-1a), 2.00 (2H, m, CH$_2$-16), 1.88 (1H, m, CH$_2$-22a), 1.87 (1H, m, H-5), 1.83 (1H, m, CH$_2$-12a), 1.66 (1H, m, CH$_2$-12b), 1.52 (1H, m, CH$_2$-1b), 1.51 (1H, m, H-17), 1.50 (4H, m, CH$_2$-11, CH$_2$-15), 1.40 (1H, m, H-20), 1.27 (6H, s, Me-26, Me-27), 1.12 (1H, m, CH$_2$-22b), 1.12 (3H, s, Me-28), 1.02 (3H, s, Me-30), 0.98 (3H, s, Me-19), 0.82 (3H, s, Me-18): ^{13}C NMR (CD$_2$Cl$_2$); δ (150 MHz, ppm), Appendix 11A: 215.7 (C-3), 146.0 (C-8), 118.0 (C-7), 74.9 (C-24), 74.1 (C-25), 69.6 (C-23), 65.7 (C-29), 53.7 (C-17), 53.4 (C-5), 53.1 (C-4), 51.2 (C-14), 48.3 (C-9), 43.5 (C-13), 40.5 (C-22), 37.8 (C-1), 35.6 (C-2), 35.0 (C-10), 34.0 (C-15), 33.7 (C-12, C-20), 28.4 (C-16), 27.2 (C-30, C-27), 26.0 (C-26), 24.7 (C-6), 21.9 (C-18), 20.1 (C-28), 18.8 (C-21), 18.5 (C-11), 13.4 (C-19). ESI-MS (30 eV): m/z 491.6 [M+H]$^{+}$ Appendix 11B.

β-sitosterol and **stigmasterol** (**180**); white crystals, mp 129-131 ^{o}C. ^{1}H NMR (CDCl$_3$) δ (800 MHz, ppm), Appendix 15; 5.35 (1H, m, H-6), 3.52 (1H, m, H-3), 0.96 (1H, d, J=6.5 Hz, H-24), 0.94 (1H, d, J=6.5 Hz, H-9), 1.03 (3H, s, Me-19), 0.87 (3H, t, Me-29), 0.85 (3H, d, Me-26), 0.84 (3H, d, Me-27), 0.70 (3H, s, Me-18): ^{13}C NMR (CDCl$_3$); δ (200 MHz, ppm), Appendix 15A: 140.7 (140.6) (C-5), 121.73, 121.7 (C-

6), 71.8 (C-3), 56.8 (C-14), 56.1, 56.0 (C-17), 50.1 (C-9), 45.9, (51.3) (C-24), 42.3 (C-4), 42.2 (C-13), 39.7, 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.2 (40.5) (C-20), 34.0 (C-22), 31.6 (C-2), 31.9 (C-7, C-8), 29.3 (C-25), 29.2, 28.3, (C-16), 26.1 (C-23), 24.4 , 24.3 (C-15), 21.2, 21.1 (C-11), 23.1 (C-28), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.0 (C-29), 11.9 (C-18),

Sitosterol 3-*O*-*β*-D-glucopyranoside acetate (195) and **stigmasterol 3-*O*-*β*-D-glucopyranoside** (**196**); White amorphous powder: ^{1}H NMR (CDCl$_3$) δ (800 MHz, ppm), Appendix 13; 5.37 (1H, s, H-6), 4.50 (1H, dd, J=12, 4.8 Hz, CH$_2$-6'a), 4.25 (1H, dd, J=12, 4.8 Hz, CH$_2$-6'b), 4.38 (1H, d, J=8 Hz, H-1'), 3.56 (2H, m, H-3, H-3'), 3.46 (1H, m, H-5'), 3.37 (2H, H-2', H-4'), 1.01 (3H, s, Me-19), 0.92 (3H, Me-21), 0.84 (6H, s, Me-26, Me-29), 0.83 (3H, s, Me-27), 0.68 (3H, s, Me-18):^{13}C NMR (CDCl$_3$); δ (200 MHz, ppm), Appendix 13A: 175.1 (Ac-CO), 140.2 (C-5), 122.1 (C-6), 101.5 (C-1'), 79.5 (C-3), 76.2 (C-3'), 74.3 (C-5'), 73.9 (C-2'), 70.3 (C-4'), 63.4 (C-6'), 56.7 (C-14), 56.0 (C-17), 50.5 (C-9), 46.2 (C-24), 42.6 (C-13), 40.1 (C-12), 39.2 (C-4), 37.6 (C-1), 37.0 (C-10), 36.5 (C-20), 34.5 (C-22), 32.4 (C-7), 32.3 (C-8), 29.8 (C-2), 29.4 (C-25), 28.6 (C-16), 26.4 (C-23), 24.6 (C-15), 23.0 (C-28), 21.4 (C-11), 20.1 (C-26), 19.7 (C-19), 19.3 (C-27), 19.0 (C-21), 12.3 (C-29).

sitosterol-3-*O*-*β*-D-glucopyranoside (**197**); white amorphous powder: ^{1}H NMR (CDCl$_3$) δ (800 MHz, ppm), Appendix 14; 5.37 (1H, s, H-6), 4.43 (1H, d, J=8 Hz, H-1'), 3.92 (1H, m, CH2-6'a), 3.81 (1H, m, CH$_2$-6'b), 3.61 (1H, m, H-4'), 3.60 (1H, m, H-3'), 3.59 (1H, m, H-3), 3.42 (1H, m, H-5'), 3.36 (1H, m, H-2'), 1.01 (3H, s, Me-19), 0.95 (2H, d, J=6.4 H-9, H-24), 0.92 (3H, d, Me-21), 0.84 (3H, d, Me-26), 0.83 (3H, t, Me-29), 0.81 (3H, d, Me-27), 0.68 (3H, s, Me-18): ^{13}C NMR (CDCl$_3$); δ (200 MHz, ppm), Appendix 14A: 140.6 (C-5), 121.4 (C-6), 100.9 (C-1'), 77.1 (C-3), 76.9

(C-3', C-5'), 73.6 (C-2'), 70.2 (C-4'), 61.2 (C-6'), 55.6 (C-17), 56.3 (C-14), 49.7 (C-9), 45.3 (C-24), 42.0 (C-13), 40.1 (C-12), 38.4 (C-4), 37.0 (C-1), 36.4 (C-10), 35.6 (C-20), 33.5 (C-22), 31.6 (C-7), 31.5 (C-8), 29.4 (C-2), 28.8 (C-25), 27.9 (C-16), 25.3 (C-23), 24.0 (C-15), 22.8 (C-28), 20.7 (C-11), 19.9 (C-27), 19.3 (C-26), 19.1 (C-19), 18.8 (C-21), 11.9 (C-29), 11.8 (C-18).

3.3.3. *Ekebergia capensis*

The air dried and ground root bark (600 g) of *E. capensis* was extracted as described in section 3.3.1 above. The filtrate was dried *in vacuo* to yield a blackish gum (97 g). A 30 g portion of the extract was fractionated in a gradient CC eluting with petroleum ether (40-60 ^{0}C) and ethyl acetate in the following ratios: 100:0; 19:1; 9:1; 4:1; 3:2; 1:1; 2:3; 1:4; 0:100. A total of 86 eluents (*ca.* 250 ml each) were collected and combined into 22 fractions (labeled A to V) based on TLC profiles. Fraction B was crystallized from acetone to yield oleanonic acid (**160**, 2.7 g). Supernatant from fraction B was re-chromatographed over Sephadex^R LH-20 eluting with methanol to yield fraction B1 which was crystallized from acetone to yield ekeberin A (**158**, 2.1 mg). Fraction C was crystallized from acetone to yield whitish crystals C1 (762.8 mg) which were further fractionated on RP-HPLC (CH$_3$OH/water) to yield 3-*epi*-oleanolic acid (**161**, 7.4 mg). Whitish amorphous powder settled in fraction D yielding 3-oxo-12α- hydroxy-oleanan-28,13β-olide (**198**, 2.3 mg). Fraction G yielded oleanolic acid (**159**, 284 mg) as whitish powder. Fractions I and M were separated on PTLC eluting with petroleum ether and acetone (7:3) to yield 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene (**152**, 30.3 mg) and 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosate (**151**,

90.6 mg) respectively. The rest of the fractions were not investigated as they were small in quantity.

Dried and ground leaves of *E. capensis* (500 g) were extracted with MeOH/CH$_2$Cl$_2$ (1:1) to yield 21 g of a greenish gum. A 20 g portion was fractionated on CC over silica gel eluting with petroleum ether and acetone as follows: 100:0, 9.75: 0.25, 9.5:0.5, 9.25:0.75, 9:1, 8.75:1.25, 8.5:1.5, 8.25:1.75, 8:2, 7:3, 1:1, 0:100. Total of 81 eluents (*ca.* 100 ml each) were collected and combined into 22 fractions (A-V) after TLC. Fraction Q was further fractionated with RP-HPLC (CH$_3$OH/water) to yield subfractions Q2 and Q6. Fraction Q2 was purified on preparative TLC eluting with *iso*-hexane and acetone (4:1) to yield proceranolide (**148**, 5.7 mg). Fractions T and W were separately re-fractionated with RP-HPLC (CH$_3$OH/Water) to yield kaempferol-3-*O*-*β*-D-glucopyranoside (**199**, 3.5 mg) and quercetin-3-*O*-*β*-D-glucopyranoside (**200**, 10.1 mg) respectively. The rest of the fractions were having large amounts of chlorophyll and were not investigated.

3.3.3.1. *Physical and spectroscopic data of compounds isolated from Ekebergia capensis*

Proceranolide (148); Yellowish gum: ^{1}H NMR (CDCl$_3$) δ (799.88 MHz, ppm), Appendix 26: 0.73 (3H, s, Me-29), 0.81 (3H, s, Me-28), 1.03 (3H, s, Me-18), 1.12 (3H, s, Me-19), 2.38 (1H, m, CH$_2$-6a), 2.38 (1H, m, CH$_2$-6b), 3.05 (1H, ddd, J=10.7, 6.0, 2.7, Hz, CH-2), 3.19 (1H, dd, J=14.5, 3.0 Hz, CH-30), 3.24 (1H, dd, J=11.4, 2.7 Hz, CH-5), 3.74 (1H, m, CH-3), 3.70 (3H, s, OMe), 4.05 (1H, dt, J=21.4, 2.12, 2.12 Hz, CH-15), 5.58 (1H, s, CH-17), 6.49 (1H, d, J=2.3 Hz, CH-22), 7.39 (1H, s, CH-23), 7.56 (1H, s, CH-21); ^{13}C NMR (CDCl$_3$); δ (201.20 MHz, ppm), Appendix 26A: 17.2 (C-19), 19.1(C-11), 20.5 (C-29), 24.1 (C-28), 28.9 (C-12), 33.4 (C-15), 33.6 (C-

6), 33.9 (C-30), 38.2 (C-13), 39.6 (C-5), 50.3 (C-2), 52.1 (OMe), 52.3 (C-9), 53.9 (C-10), 77.5 (C-3), 80.5 (C-17), 110.4 (C-22), 121.1 (C-20), 128.5 (C-8), 131.7 (C-14), 142.0 (C-21), 142.9 (C-23), 171.7 (C-16), 174.7 (C-7), C-1 (218.3); ESI-MS (30 eV): m/z 471.9 [M+H]$^+$ Appendix 26B.

2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene (152):colourless oil; ^{1}H NMR (DMSO-d$_6$) δ (799.88 MHz, ppm), Appendix 22: 0.93 (3H, s, Me-25), 0.96 (3H, s, Me-24), 0.99 (3H, s, Me-30), 1.50 (12H, s, Me-26, 27, 28, 29), 3.03 (1H, d, J=10.2 Hz, CH-22), 3.20 (1H, d, J=10.8 Hz, CH$_2$-1a), 3.24 (1H, d, J=10.4 Hz, CH-3), 3.32 (1H, d, J=10.8 Hz, CH$_2$-1b), 5.04 (4H, br s, CH-7, 11, 14, 18); ^{13}C NMR (DMSO-d$_6$); δ (201.20 MHz, ppm), Appendix 22A: 16.33, 16.34 16.4, 16.5, (Me- 26, 27, 28, 29), 21.2 (C-25), 25.2 (C-24), 26.0 (C-30), 26.65, 26.7 (C-17 and C-8), 28.3 (C-11/C-12), 29.8 (C-4), 30.1 (C-21), 36.9 (C-5), 37.1 (C-20), 39.82 (C-9), 39.83 (C-16), 67.2 (C-1), 72.4 (C-23), 74.5 (C-2), 74.6 (C-3), 124.0 (C-11/C-14), 124.4 (C-7), 124.44 (C-18), 134.8 (C-6), 134.9 (C-10/C-15), 134.9 (C10/C15), 135.0 (C19); ESI-MS (30 eV): m/z 495.7 [M+H]$^+$ Appendix 22B.

2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosate (151); Yellowish oil: ^{1}H NMR (CDCl$_3$) δ (500 MHz, ppm), Appendix 23: 1.13 (6H, s, Me-1, 24), 1.18 (3H, s, Me-25, 30), 1.58 (6H, Me-27, Me-28), 1.60 (6H, s, Me-26, 29), 3.33 (2H, dd J=10.5, 1.9 Hz), 5.13 (2H, m, CH-11, 14) 5.17 (1H, t, J=6.4 Hz, CH-7); ^{13}C NMR (CDCl$_3$) δ (125.00 MHz, ppm), Appendix 23A: 16.3 (C-27/C-28), 16.4 (C-26/C-29), 23.6 (C-1/C-24), 26.78 (C-8), 26.8 (C-17), 26.9 (C-25), 28.6 (C-12/C-13), 30.1 (C-4/C-21), 37.2 (C-5/C-20), 40.0 (C-9/C-16), 73.4 (C-2/C-23), 78.6 (C-3/C-22), 124.8 (C-11/C-14), 125.4 (C-7/C-18), 135.2 (6, 10, 15,19), 135.3(6, 10, 15,19); ESI-MS (30 eV): m/z 479.4 [M+H]$^+$ Appendix 23B.

Ekeberin A (158); white and crystalline, ^{1}H NMR (CDCl$_3$) δ (500 MHz, ppm), Appendix 17: 0.88 (3H, s, Me-30), 0.89 (3H, s, Me-29), 0.90 (3H, s, Me-27), 0.96 (3H, s, Me-25), 1.03 (3H, s, Me-24), 1.06 (3H, s, Me-26), 1.07 (3H, s, Me-23), 1.42 (1H, d, J=2.6 Hz, CH-9), 2.03 (1H, ddd, J=13.1, 5.4, 2.5 Hz, CH$_{2a}$-12), 2.44 (1H, ddd, J=11.3, 7.8, 3.9 Hz, CH$_{2a}$-2), 2.51 (1H, ddd, J=15.7, 9.5, 7.6 Hz, CH$_2$-2b), 3.44 (1H, dd, J =8.5, 1.7 Hz, CH$_{2a}$-28), 3.55 (1H, dd, J=10.3, 1,7 Hz, CH-18), 4.25 (1H, dd, J=8.5, 3.2 Hz, CH$_2$-28b); ^{13}C NMR (CDCl$_3$) δ (125.00 MHz, ppm), Appendix 17A: 14.8 (C-27), 16.1 (C-26), 16.8 (C-25), 17.4 (C-29), 17.7 (C-30), 20.1 (C-6), 21.5 (C-24), 21.8 (C-11), 24.7 (C-12), 27.2 (C-23), 28.0 (C2-1), 26.9 (C-15), 29.0 (C-16), 31.9 (C-17), 33.3 (C-22), 33.4 (C-7), 34.6 (C-2), 37.5 (C-10), 35.9 (C-20), 39.9 (C-13), 40.3 (C-1), 41.2 (C-8), 41.9 (C-14), 47.8 (C-4), 50.8 (C-9), 55.5 (C-5), 69.6 (C-28), 79.3 (C-18), 98.0 (C-19), 218.6 (C-3).

3-β-hydroxyolean-12-en-28-oic acid (Oleanolic acid) 159; white crystals, mp 308-311°C: ^{1}H NMR (CDCl$_3$) δ (500 MHz, ppm), Appendix 18: 0.77 (3H, s, Me-26), 0.79 (3H, s, Me-24), 0.92 (3H, s, Me-29), 0.93 (3H, s, Me-25), 0.95 (3H, s, Me-30), 1.00 (3H, s, Me-23), 1.15 (3H, s, Me-27), 2.83 (1H, dd, J=13.8, 4.5 Hz, CH-18), 3.24 (1H, dd, J=10. 5 Hz, CH-3), 5.28 (1H, s, CH-12); ^{13}C NMR (CDCl$_3$) δ (125.00 MHz, ppm), Appendix 18A: 15.5 (C-24), 15.3 (C-25), 17.1 (C-26), 18.3 (C-6), 22.9 (C-11), 23.4 (C-16), 23.6 (C-30), 25.9 (C-27), 27.2 (C-2), 28.1 (C-23), 30.7 (C-20), 32.4, (C-22), 32.6 (C-29), 33.1 (C-7), 33.8 (C-21), 37.1 (C-10), 38.4 (C-1), 38.8 (C-4), 39.3 (C-8), 41.0 (C-18), 41.6 (C-14), 45.9 (C-19), 47.6 (C-9), 55.2 (C-5), C-3 (79.0), 122.6 (C-12), 143.6 (C-13), 182.9 (C-28).

3-Oxo-olean-12-en-28-oic acid (Oleanonic acid) 160; White crystals, mp185-189 °C: ^{1}H NMR (DMSO-d$_6$) δ (799.88 MHz, ppm):, Appendix 20: 0.77 (3H, s, Me-26), 0.88 (6H, Me-29, 30), 0.94 (3H, s, Me-24), 0.97 (3H, s, Me-25), 1.00 (3H, s, Me-23),

1.11 (3H, s, Me-27), 1.38 (1H, m, CH$_2$-1a), 1.77 (1H, ddd, J=11.8, 7.4, 3.8 Hz, CH$_2$-1b), 2.30 (1H, ddd, J=15.9, 7.1, 3.7 Hz, CH$_2$-2a), 2.75 (1H, dd, J=14, 4.5 Hz, CH-18), 5.19 (1H, s, CH-12); ^{13}C NMR (DMSO-d$_6$): δ (201.20 MHz, ppm), Appendix 20A. 15.1 (C-25), 17.2 (C-26), 19.6 (C-6), 21.6 (C-24), 23.1 (C-11), 23.5 (C-16), 23.8 (C-8), 25.9 (C-27), 26.7 (C-23), 27.7 (C-15), 30.9 (C-20), 32.3 (C-7), 32.5 (C-22), 33.3 (C-29), 33.8 (C-21), 34.1 (C-2), 36.7 (C-10), 38.9 (C-1), 29.3 (C-8), 41.4 (C-18), 41.9 (C-14), 46.0 (C-19), 46.1 (C-9), 46.6 (C-17), 47.1 (C-4), 54.8 (C-5), 121.9 (C-12), 144.3 (C-13), 179.1 (C-28), 216.9 (C-3); ESI-MS (30 eV): *m/z* 455.4 [M+H]$^{+}$ Appendix 20B.

3-α-hydroxyolean-12-en-28-oic acid (3-epi-Oleanolic acid) 161; White crystals: ^{1}H NMR (DMSO-d$_6$) δ (799.88 MHz, ppm), Appendix 21: 0.71 (3H, s, Me-26), 0.75 (3H, s, Me-24), 0.83 (3H, s, Me-23), 0.85 (3H, s, Me-25), 0.86 (6H, s, Me-29), 1.09 (3H, s, Me-27), 2.77 (1H, dd, J=15, 5, CH-18), 3.17 (1H, s, CH-3), 5.08 (1H, s, CH-12); ^{13}C NMR (DMSO-d$_6$): δ (201.20 MHz, ppm), Appendix 21A: 15.0 (C-25), 17.0 (C-26), 17.8 (C-6), 22.3 (C-24), 22.9 (C-11), 23.5, (C-16), 23.6 (C-30), 25.2 (C-2), 25.7 (C-27), 27.3 (C-15), 28.7 (C-23), 30.5 (C-20), 32.3 (C-1), 32.5 (C-7), 32.6 (C-21), 32.7 (C-22), 33.0 (C-29), 36.7 (C-10), 36.9 (C-4), 41.0 (C-8), 41.4 (C-18), 45.5 (C-14), 46.1 (C-19), 46.9 (C-17), 48.3 (C-9), 48.6 (C-5), 73.8 (C-3), 120.5 (C-12), 144.1 (C-13), 178.3 (C-28).

3-oxo-12β-hydroxy-oleanan-28,13β-olide (**198**); White and crystalline: ^{1}H NMR (DMSO-d$_6$) δ (800 MHz, ppm), Appendix 16: 3.91 (1H, d, J=3.3 Hz, H-12), 2.53 (1H, ddd, J=4.4, 9.8, 15.7 Hz, CH$_2$-2a), 2.45 (1H, ddd, J=4.4, 7.6, 15.7 Hz, CH$_2$-2b), 2.14 (1H, ddd, J=5.9, 13.3, 13.3 Hz, CH$_2$-16a), 2.06 (1H, m, CH$_2$-11a), 2.05 (1H, dd, J=3.5, 13.4 Hz, H-18), 2.01 (1H, dd, J=13.1, 13.4 Hz, CH$_2$-19a), 1.94 (1H, ddd, J=4.4, 7.6, 12.5 Hz, CH$_2$-1b), 1.88 (1H, ddd, J=2.6, 2.6, 12.4 Hz, CH$_2$-15a), 1.87 (1H, dd,

J=3.5, 13.1 Hz, CH$_2$-19b), 1.72 (1H, dd, J=2.3, 13.1 Hz, H-9), 1.64 (2H, m, CH$_2$-22), 1.60 (1H, m, CH$_2$-7a), 1.55 (1H, m, CH$_2$-6a), 1.48(1H, m, CH$_2$-6b), 1.47 (1H, ddd, J=7.6, 9.8, 12.5 Hz, CH$_2$-1a), 1.46 (1H, m, CH$_2$-11b), 1.39 (1H, dd, J=2.7, 12.0 Hz, H-5), 1.38 (1H, m, CH$_2$-21a), 1.32 (3H, s, Me-27), 1.30 (1H, m, CH$_2$-7b), 1.29 (1H, m, CH$_2$-16b), 1.27 (1H, m, CH$_2$-21b), 1.20 (1H, m, CH$_2$-15b), 1.20 (3H, s, Me-26), 1.10 (3H, s, Me-23), 1.05 (3H, s, Me-24), 0.99 (6H, s, Me-25, Me-29), 0.91 (3H, s, Me-30). ^{13}C NMR (DMSO-d$_6$); δ (200 MHz, ppm), Appendix 16A: 217.7 (C-3), 179.9 (C-28), 90.6 (C-13), 76.2 (C-12), 55 (C-5), 51.3 (C-18), 47.5 (C-4), 44.9 (C-17), 44.0 (C-9), 42.3 (C-8, C-14), 39.8 (C-1), 39.7 (C-19), 36.3 (C-10), 34.3 (C-21), 34.1 (C-2), 33.5 (C-7), 33.4 (C-29), 31.8 (C-20), 29.3 (C-11), 28.2 (C-15), 27.6 (C-22), 26.7 (C-23), 24.0 (C-30), 21.2 (C-24), 21.3 (C-16), 19.2 (C-6), 18.6 (C-27), 18.4 (C-26), 16.4 (C-25). HR (ESI)MS observed [M+H]$^+$, *m/z* 471.3386, calcd 471.3474), Appendix 16F.

Kaempferol-3-*O*-β-D-glucopyranoside (**199**); Yellow amorphous powder, mp 230-232 ^{o}C. White amorphous powder: ^{1}H NMR (DMSO-d$_6$) δ (799.88 MHz, ppm), Appendix 24: 3.08 (2H, s, H-4", H-5"), 3.13 (1H, m, CH$_{2a}$-6"), 3.17 (1H, s, H-2"), 3.21 (1H, s, H-3"), 3.56 (1H, m, CH$_2$-6"b), 5.46 (1H, d, J=7.2 Hz, H-1"), 6.20 (1H, s, H-6), 6.42 (1H, s, H-8), 6.88 (2H, m, CH-3', H-5') 8.04 (2H, d, J=7.2 Hz, H-2', CH-6'); ^{13}C NMR (DMSO-d$_6$) δ (201.20 MHz, ppm), Appendix 24A: 61.3 (C-6"), 70.4 (C-4"), 74.7 (C-2"), 76.9 (C-3"), 78.0 (C-5"), 94.1 (C-8), 99.2 (C-6), 101.3 (C-1"), 104.4 (C-10), 115.6 (C-3'/C-5'), 121.4 (C-1'), 131.3 (2'/6'), 133.6 (C-3), 156.1 (C-2), 156.9 (C-9), 160.4 (4'), 161.9 (C-5), 177.9 (C-4); ESI-MS (30 eV): *m/z* 449.1 [M+H]$^+$, Appendix 24B.

Quercetin-3-*O*-*β*-D-glucopyranoside (200); Yellow amorphous powder, mp 225-227 °C. ^{1}H NMR (DMSO-d$_6$) δ (800 MHz, ppm), Appendix 25: 3.08 (2H, s, CH-4"/H-5"), 3.22 (2H, m, H-2"/H-3"), 3.33 (1H, m, CH$_2$-6"a), 3.58 (1H, dd, J=11.9, 4 Hz, CH$_2$-6"b), 5.46 (1H, d, J=7.4 Hz, H-1"), 6.20 (1H, d, J=2.0 Hz, H-6), 6.40 (1H, d, J=2.0 Hz, CH-8), 6.84 (1H, d, J=10.0 Hz, CH-5'), 7.57 (1H, m, CH-2'), 7.58 (1H, m, H-6'); ^{13}C NMR (DMSO-d$_6$); δ (201.20 MHz, ppm), Appendix 25A: 61.0 (C-6"), 69.9 (C-4"), 74.1 (C-2"), 76.5 (C-3"), 77.6 (C-5"), 93.5 (C-8), 98.6 (C-6), 100.8 (C-1"), 104.0 (C-10), 115.2 (C-5'), 116.2 (C-2'), 121.2 (C-1'), 121.6 (C-6'), 133.3 (C-3), 144.8 (3'), 148.5 (C-4'), 156.2 (C-2), 156.3 (C-9), 161.2 (C-5), 164.1 (C-7), 177.4 (C-4); ESI-MS (30 eV): *m/z* 465.1 [M+H]$^+$ Appendix 25B.

3.4. Acetylation

Method described by Mulholland and co-workers (1999) was adopted. Briefly, 20 mg portion of compound to be acetylated was dissolved in 1 ml of pyridine followed by addition of 1 ml acetic anhydride. The mixture was left to stand overnight. Methanol was added to the reaction mixture and the solvents removed under vacuum. The acetate was precipitated by addition of water while stirring briskly. The acetate was filtered and dried (Mulholland *et al*., 1999). Niloticin (**25**), oleanolic acid (**159**) and piscidinol A (**27**) were acetylated to yield niloticin acetate (**201**), oleanolic acid acetate (**202**) and piscidinol A diacetate (**203**) respectively.

3.4.1.1. *Physical and spectroscopic data of acetate derivatives*

Niloticin acetate (201): white amorphous powder, mp 157-159 °C. ^{1}H NMR (CDCl$_3$) 200 (MHz, ppm), Appendix 28; 5.32 (1H, d, J=3 Hz, H-7), 4.86 (1H, m, H-23), 2.76 (1H, d, J=9.6 Hz, H-24), 2.08 (3H, s, Ac-Me), 1.37 (3H, s, Me-19), 1.34 (3H, s, Me-27), 1.12 (3H, s, Me-29), 1.06 (6H, s, Me-18, Me-30), 1.01 (6H, s, Me-28, Me-21),

0.81 (3H, s, Me-36). ^{13}C NMR (CDCl$_3$); δ (50 MHz ppm), Appendix 28A: 217.2 (C-3), 170.6 (Ac-CO), 145.9 (C-8), 118.3 (C-7), 72.3 (C-25), 65.6 (C-24), 59.6 (C-25), 53.2 (C-17), 52.5 (C-5), 51.5 (C-14), 48.7 (C-9), 48.1 (C-4), 43.8 (C-13), 38.7 (C-1, C-22), 35.2 (C-2, C-10), 34.1 (C-15), 33.8 (C-20), 33.5 (C-12), 29.0 (C-16), 27.6 (C-30), 24.9 (C-28), 24.6 (C-6), 24.6 (C-27), 22.0 (Ac-Me), 21.8 (C-29), 21.5 (C-26), 20.1 (C-21), 19.8 (C-19), 18.5 (C-11), 13.0 (C-18). ESI-MS (30 eV): *m/z* 499.8 [M+H]+, Appendix 28B

Oleanolic acid acetate (202); white amorphous powder; ^{1}H NMR (CD$_2$Cl$_2$) 600 (MHz, ppm), Appendix 19; 5.27 (1H, t, J=3.7 Hz, H-12), 4.47 (1H, m, H-3), 1.14 (3H, s, Me-27), 0.95 (3H, s, Me-23), 0.92 (3H, s, Me-30), 0.90 (3H, s, Me-25), 0.85 (3H, s, Me-29), 0.86 (3H, s, Me-24), 0.75 (3H, s, Me-26); ^{13}C NMR (CD$_2$Cl$_2$) (150 MHz, ppm), Appendix 19A; 184.2 (C-28), 171.3 (Ac-CO), 144.3 (C-13), 123.1 (C-12), 81.3 (C-3), 55.8 (C-5), 47.1 (C-9), 46.3 (C-19), 42.1 (C-14), 41.6 (C-18), 39.8 (C-8), 38.6 (C-4), 38.1 (C-1), 37.5 (C-10), 34.3 (C-7, C-21), 33.3 (C-29), 33.0 (C-22), 31.1 (C-20), 28.3 (C-23), 26.2 (C-2), 24.1 (C-27), 24.0 (C-30), 23.9 (C-16), 23.4 (C-11), 21.6 (Ac-Me), 18.7 (C-6), 17.7 (C-26), 17.0 (C-25), 15.4 (C-24).

Piscidinol A diacetate (203): white amorphous solid ^{1}H NMR (CD$_2$Cl$_2$) 600 (MHz, ppm), Appendix 9; 5.37 (1H, ddd, J=9.3, 5.1, 1.7 Hz CH-23), 5.35 (1H, m, CH-7), 4.86 (1H, d, J 1.6 Hz, H-24), 1.20 (Me-27), 1.15 (Me-26), 1.01 (Me-28), 0.99 (Me-19, Me-30), 0.94 (Me-21), 0.80 (Me-18). ^{13}C NMR (CD$_2$Cl$_2$) (150 MHz, ppm), Appendix 9A: 216 (C-3), 170.54 (C-24 Ac-CO), 170.2 (C-23;Ac-CO), 146.3 (C-8), 118.6 (C-7), 77.2 (C-24), 72.8 (C-25), 70.6 (C-23), 53.9 (C-17), 52.8 (C-5), 51.7 (C-14), 50.0 (C-9), 48.2 (C-4), 39.0 (C-22), 38.4 (C-1), 35.5 (C-1), 25.4 (C-2), 34.5 (C-20), 34.4 (C-20), 33.6 (C-12), 28.4 (C-16), 27.7 (C-30), 27.4 (C-27), 26.7 (C-26), 24.9

(C-6, C-28), 22.2 (C-18), 22.0 (C-29), 21.8 (C-23; Ac-Me), 21.2 (C-24; Ac-Me), 18.8 (C-21), 18.6 (C-11), 13.1 (C-19).

3.5. Cytotoxicity assay

Rapid colorimetric assay was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) (Mosmann, 1983; Prayong *et al*., 2008). This assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and thereby form dark blue formazan crystals, which are largely impermeable to cell membranes, resulting in their accumulation within healthy cells. The amount of generated formazan is directly proportional to the number of cells (Mosmann, 1983). In this assay, the mammalian cell lines African monkey kidney (vero), mouse breast cancer (4T1) and human larynx carcinoma (HEp2) were used. Cells were maintained in Eagle's Minimum Essential Medium (MEM) containing 10% fetal bovine serum (FBS). A cell density of 20,000 cells per well in 100 µl were seeded on 96-well plates and incubated for 12 hours at 37 °C and 5% CO_2 to attach to the surface. After 12 hours, the medium was replaced with maintenance medium containing the appropriate drug concentrations (0.14 to 100 µg/ml) or vehicle control ($\leq$ 1.0% v/v DMSO). After 48 hours incubation, cell viability was measured by addition of 10 µL of MTT reagent (5 mg MTT in 1 ml of PBS). The plates were incubated for additional 4 hours at the same conditions. Next, all media was removed from the plates and 100 µl DMSO added to dissolve the formazan crystals. The plates were read on a Multiskan EX Labsystems scanning multi-well spectrophotometer at 562 nm and 620 nm as reference. The results were recorded as optical density (OD) per well at each drug concentration. Data was transferred into the software Microsoft Excel 2007 and expressed as percentage of the

untreated controls. Percentage cytotoxicity (PC) as compared to the untreated controls was calculated using the following equation:

$$PC = \left[\frac{A-B}{A}\right] \times 100 \qquad (1)$$

Where A is the mean OD of the untreated cells and B is the mean OD at each drug concentration (Prayong *et al.*, 2008). The drug concentration required for 50% inhibition of cell growth, using nonlinear regression analysis of the dose-response curve is reported.

Cytotoxicity tests on MDA-MB-231 cells were carried out following a previously described procedure (Abdissa *et al.*, 2014). MDA-MB-231 human breast cancer cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units / ml penicillin and 100 μg/ml streptomycin at 37 °C in humidified 5% CO_2. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density (10,000 cells per well) to ensure exponential growth for the duration of the assay. After a 24 hrs preincubation growth, the medium was replaced with experimental medium containing the appropriate drug concentrations or vehicle controls (0.1% or 1.0% v/v DMSO). After 72 hours incubation, cell viability was measured using Alamar Blue reagent (Invitrogen Ab, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as the mean ± standard error for six replicates as a percentage of vehicle control (taken as 100%). Experiments were performed independently at least six times. Statistical analyses were performed using a two-tailed Student's t-test. $P < 0.05$ was considered to be statistically significant.

The interaction of oleanonic acid (**160**) with other triterpenoids was studied using fixed concentration ratios; oleanonic acid versus 'other triterpenoid.' Following ratios were adopted; 0:1, 1:3, 1:1, 3:1, 1:0. The vero cell cytotoxicity assay was used, as described above, to evaluate the cytoxicity of the mixtures. To determine whether there was synergy, additive effect or antagonism, the sum of fractional inhibition concentration ($\sum$FIC) was calculated using the following formula:

$$K = \frac{Ax}{Ay} + \frac{Bx}{By} \quad (2)$$

Where K is $\sum$FIC, A_x and B_x are the IC_{50} values when the substances are used in combination, and A_y and B_y are the IC_{50} values when the substances are used alone. The data was scored with the scale $\sum FIC < 1$ = synergism, $2 > \sum FIC \geq 1$ = additive, $4 > \sum FIC \geq 2$ = slight antagonism, $\sum FIC \geq 4$ = marked antagonism (Gupta *et al*., 2002).

3.6. Antiplasmodial bioassay

Continuous *in vitro* cultures of asexual erythrocytic stages of *P. falciparum* strains (W2 and D6) were maintained following previously described procedures (Trager and Jensen, 1976; Kigondu *et al*., 2009). Drug assay was carried out following a modification of the semiautomated micro dilution technique which measures the ability of the extracts to inhibit the incorporation of (G-^{3}H) hypoxanthine into the malaria parasite (Desjardins *et al*., 1979). Plates were harvested onto glass fibre filters and (G-^{3}H) hypoxanthine uptake determined using a micro-beta trilux liquid scintillation and luminescence counter (Wallac, MicroBeta TriLux) and results recorded as counts per minute (cpm) per well at each drug concentration. Data was transferred into a graphic programme (Microsoft Excel 2007) and expressed as

percentage of the untreated controls. Results were expressed as the drug concentration required for 50% inhibition of (G-^{3}H) hypoxanthine incorporation into parasite nucleic acid, using non linear regression analysis of the dose-response curve. Criterion for scoring activity described by Batista and co-workers was adopted (Batista *et al*., 2009): $IC_{50} < 1$ μM, highly active; $IC_{50} \geq 1$ and <20 μM, Active; $IC_{50} \geq 20$ and <100 μM, moderate activity; $IC_{50} > 100$ inactive.

3.7. X-ray diffraction

All crystals were selected and mounted under a stereo microscope on to a glass fiber and transferred to a Rigaku R-AXIS IIc image plate system. Diffracted intensities were measured using graphite-monochromated Mo Kα (λ = 0.710 73 Å) radiation from a RU-H3R rotating anode operated at 50 kV and 40 mA. Using the R-AXIS IIc detector, 90 oscillation photos with a rotation angle of 2° were collected and processed using the CrystalClear software package. An empirical absorption correction was applied using the REQAB program under CrystalClear. All structures were solved by direct methods (SIR 97) (Altomare *et al*., 1999) and refined using full-matrix least-squares calculations on *F*2 (SHELXL-97) (Sheldrick, 2007) operating in the WinGX program package (Farrugia, 2012). Anisotropic thermal displacement parameters were refined for all the non-hydrogen atoms. Hydrogen atoms were included in calculated positions and refined using a riding model. Displacement ellipsoids are drawn with ORTEP-3 for Windows (Farrugia, 1997) under WinGX.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Secondary metabolites isolated from the stem bark of *Turraea robusta*

The stem bark of *Turraea robusta* Guerke afforded three ring intact limonoids; mzikonone (**17**), azadirone (**19**), 12α-acetoxy-7-deacetylazadirone (**28**) and a ring B *seco* limonoid 11-*epi*-toonacilin (**62**). It also afforded three triterpenoids turranolide (**22**), azadironolide (**192**) and stigmasterol (**193**). Secondary metabolites present in *T. robusta* stem bark are similar to those reported from other Turraea species where limonoids are the main constituents. Compounds (**17**), (**22**) and (**19**) were previously isolated from the root bark of *Turraea robusta* (Rajab *et al*., 1988; Bentley *et al*., 1992) whereas (**28**) and (**62**) are reported from this species for the first time there is no previous report on compound (**192**) from genus Turraea.

4.1.1. *Rings intact limonoids from Turraea robusta stem bark*

4.1.1.1. *12α-Acetoxy-1,2-dihydro-7-deacetylazadirone (17) (mzikonone)*

17
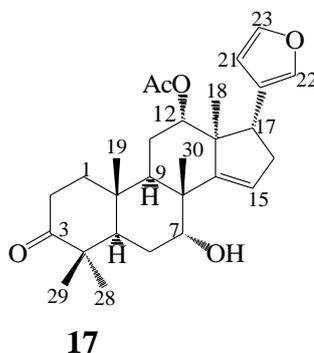
Mzikonone (**17**) was isolated as a whitish amorphous solid. The limonoid nature of this compound was indicated from the ^{13}C NMR spectrum (Tabel 4.1; Appendix 1A) and by presence of five tertiary methyls at δ_H 1.02, 1.03, 1.06, 1.07 and 1.17 in the ^{1}H NMR spectrum (Appendix 1). Resonances at δ_H 7.47 (*t*, *J* = 1.2 Hz), 7.35 (*t*, *J* = 1.2 Hz) and 6.36 (*m*) and the corresponding ^{13}C NMR signals at δ_C 141.9, 113.5, 143.7 were characteristic of a furan ring (Akinniyi *et al*., 1986; Rajab *et al*., 1988). Resonances at δ_C 216.5 and δ_C 171.4 in the ^{13}C NMR spectrum indicated presence of a ketone and an acetyl group respectively. A ketone group was assigned to C-3 following HMBC correlation of Me-28 (δ_H 1.02, *s*), Me-29 (δ_H 1.03, *s*), CH$_2$-2a (δ_H 2.35, *m*) and CH$_2$-2b (δ_H 2.56, *m*) with C-3 (δ_C 216.5) while the acetyl was assigned to C-12. Placement of acetyl group to C-12 was confirmed by HMBC correlation of Me-18 (δ_H 1.05, *s*) with C-12 (δ_C 79.1) and H-12 (δ_H 5.06) with Ac (δ_C 171.4). Presence of a double bond at C-14/C-15 was indicated by resonances at δ_C 159.5 (C-14) and 123.6 (C-15) in the ^{13}C NMR spectrum and a proton resonance at δ_H 5.70 assigned to H-15. This was further confirmed by HMBC correlation of Me-30 (δ_H 1.17, *s*) and Me-18 (δ_H 1.06, *s*) with C-14 (δ_C 159.5). A hydroxyl group was placed at C-7 following HMBC correlation of Me-30 (δ_H 1.17, *s*) with C-7 (δ_C 73.1). The relative configuration at C-7 and C-12 was established from the NOESY spectrum where H-12 and H-7 correlated with Me-30 which is in β orientation hence α-orientation of the hydroxyl and acetyl groups. ESI-MS (30 eV) indicated pseudo molecular ion peak at *m/z* 455.5 [M+H]$^+$ (Appendex 1B). This molecular ion peak together with ^{1}H and ^{13}C NMR data led to the suggestion of the molecular formula $C_{28}H_{38}O_5$ for this compound, consequently it was identified as mzikonone previously isolated from the root bark of *T. robusta*, *T. cornucopia* and *T. parvifolia* (Rajab *et al*., 1988; Cheplogoi and Mulholland, 2003a; Owino *et al*., 2008).

Table 4.1: [1]H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **17** in Acetone-d$_6$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1 α	1.81 *m*	40.0	14	-	159.5
1 β	1.52 *m*		15	5.70 *dd* (3.6, 1.8)	123.6
2	2.56 *m*	35.0	16	2.40 *m*	38.1
	2.35 *m*			2.59 *m*	
3	-	216.5	17	3.01 *dd* (7.8, 10.8)	51.9
4	-	45.1	18	1.06 *s*	22.0
5	2.12 *dd* (2.4, 12.6)	48.0	19	1.07 *s*	16.2
6	1.95 *m*	26.9	20	-	126.5
	1.70 *m*		21	7.35 *t* (1.2)	141.9
7	4.05 *br s*	73.1	22	6.36 *m*	113.4
8	-	48.1	23	7.47 *t* (1.2)	143.7
9	2.34 *m*	44.0	28	1.02 *s*	27.2
10	-	38.4	29	1.03 *s*	15.9
11	1.43 *m*	27.0	30	1.17 *s*	28.6
	2.19 *m*		Ac (Me)	1.88 *s*	22.1
12	5.06 *dd* (7.2, 9)	79.1	Ac (CO)	-	171.4
13	-	52.8			

4.1.1.2. *Azadirone (**19**)*

19
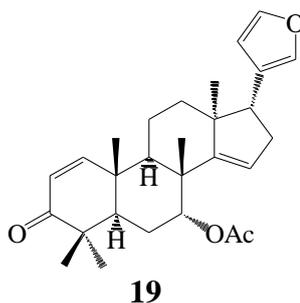
Compound **19** was isolated as a yellowish gum with EIMS at *m/z* 436 (Appendix 2C) consistent with molecular formula $C_{28}H_{36}O_4$. The ^{1}H and ^{13}C NMR (Table 4.2, Appendices 2 and 2A) spectra showed the compound to be a limonoid (Bordoloi *et al*., 1993; Mulholland *et al*., 1999) with resonances at δ_H 7.36 (*m*), 6.31 (*m*) and 7.38 (*t*, *J* = 3 Hz) and the corresponding carbon signals appearing at δ_C 140.0 (C-21), 111.4 (C-22) and 142.8 (C-23) characteristic of a furan ring (Siddiqui *et al*., 2000). The ^{1}H and ^{13}C NMR spectra further indicated the presence of a 1-en-3-one system where a pair of AB doublets at δ_H 7.18 (*d*, *J* = 10.2 Hz) and 5.79 (*d*, *J* = 10.2 Hz) and carbon resonances at δ_C 158.6 (C-1), 125.4 (C-2) and 204.6 (C-3) were observed (Siddiqui *et*

al., 1999, 2000). Also present in the ^{1}H NMR spectrum were five tertiary methyls at

δ_H 0.79, 1.05, 1.07, 1.20 and 1.23 common with limonoids having rings A-D intact

(Bordoloi *et al*., 1993; Mulholland *et al*., 1999). The presence of an acetyl group was

also indicated in the ^{1}H and ^{13}C NMR spectra by an ester resonance at δ_C 170.1; δ_H

1.93 (s, 3H) and placed at C-7 with the deshielded proton of the acetoxy methine

appearing at δ_H 5.25 (*dd*, *J* = 3, 1.8 Hz) and corresponding carbon (C-7) at δ_C 74.6. A

doublet of doublets at δ_H 5.36 (*J* =1.2, 3 Hz) was due to a vinyl proton ascribable to

H-15. The above data is in agreement that this compound is azadirone (**19**) previously

isolated from the root bark of *Turraea robusta* and *Melia toosendan* (Bentley *et al*.,

1992; Zhou *et al*., 1997).

Table 4.2: [1]H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **19** in CD_2Cl_2

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	7.18 *d* (10.2)	158.6	14	-	159.3
2	5.79 *d* (10.2)	125.4	15	5.36 *dd* (1.2, 3)	119.2
3	-	204.6	16α	2.45 *m*	34.6
4	-	44.3	β	2.32 *m*	
5	2.23 *dd* (2.4, 13.2)	46.3	17	2.82 *dd* (7.8, 11.4)	51.8
6 α	2.00 *m*	24.0	18	0.70 *s*	20.6
6 β	1.80 *m*		19	1.20 *s*	19.1
7	5.25 *dd* (1.8, 3)	74.6	20	-	125.1
8	-	43.1	21	7.26 *m*	140.0
9	2.22 *m*	38.9	22	6.32 *m*	111.4
10	-	40.2	23	7.39 *t* (3)	142.7
11 α	1.79 *m*	16.7	28	1.05 *s*	21.3
11 β	1.98 *m*		29	1.06 *s*	27.0
12α	1.65 *m*	33.2	30	1.20 *s*	27.3
12β	1.93 *m*		Ac(Me)	1.92 *s*	21.2
13	-	47.4	Ac(CO)	-	170.1

4.1.1.3. *12α-Acetoxy-7-deacetylazadirone (28)*
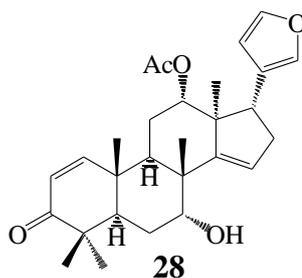
Compound **28** was isolated as a white amorphous powder. EIMS suggested molecular formula $C_{28}H_{36}O_5$ (observed *m/z* 452.2555, calcd 452.2557, Appendex 3B). Both the ^1H and ^{13}C NMR spectra (Table 4.3, Appendices 3 and 3A) established a limonod skeleton whose furan ring resonances appeared at δ_H 7.36 (*m*, H-21), 6.37 (*m*, H-22), 7.47 (*t*, H-23, *J* = 3 Hz) and δ_C 142.0, 113.4 and 143.7 corresponding to C-21, C-22 and C-23 respectively. Five singlets at δ_H 1.05, 1.05, 1.08, 1.21 and 1.22 each integrating for three protons indicated presence of five tertiary methyls groups. Spectroscopic data for this compound is very similar to those of mzikonone (**19**) except that compound **28** has extra vinylic protons resonating at δ_H 7.17 (*d*, *J* =10.2 Hz, H-1) and 5.74 (*m*, H-2) while ^{13}C NMR spectrum had signals at δ_C 159.4 (C-1), 126.4 (C-2) and 204.9 (C-3) indicating presence of a 1-en-3-one system functionality in ring A (Siddiqui *et al*., 1999). Resonances at δ_C 159.4 and δ_C 123.7 were typical of a C-14/C-15 double bond while the signal at δ_H 5.73 (*m*) was assigned to H-15 (Bentley *et al*., 1992). Carbonyl resonance at δ_C 171.4 was assigned to C-12 based on HMBC correlation of H-12 (δ_H 5.12, *dd*, *J* = 7.2, 9 Hz) with C-18 (δ_C 16.3) and C-17 (δ_C 52.0) and also H-11 (δ_H 1.75; 2.35) with C-12 (δ_C 79.0). A carbinol proton at δ_H 4.09 (*brs*) was ascribable to C-7 and the corresponding carbon at δ_C 72.6. The relative orientation of the hydroxyl and acetoxyl group at C-7 and C-12 were established by NOESY whereby H-7 correlated with Me-30 which is in β-orientation while H-12 correlated with H-17 which is also in β orienation. Thus the acetoxyl and hydroxyl group are in α orientation. From the above data, the compound was elucidated as 12α-acetoxy-7-deacetylazadirone previously isolated from *Turraea cornucopia* (Owino *et al*., 2008).

Table 4.3: ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **28** in acetone-d_6

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	7.17 *d* (10.2)	159.4	14	-	159.4
2	5.74 *m*	126.4	15	5.73 *d* (2.4)	123.7
3	-	204.9	16α	2.60 *m*	38.1
4	-	45.4	β	2.40 *m*	
5	2.45 *dd* (3, 13.2)	46.1	17	3.05 *dd* (7.8, 11.4)	52.0
6 α	2.04 *m*	26.9	18	1.05 *s*	22.0
6 β	1.78 *m*		19	1.06 *s*	16.3
7	4.09 *br s*	72.7	20	-	126.4
8	-	45.8	21	7.36 *m*	142.0
9	2.51 *dd* (7.2, 9)	39.8	22	6.37 *m*	113.4
10	-	41.5	23	7.47 *t* (3)	143.7
11 α	1.75 *m*	26.7	28	1.11 *s*	28.1
11 β	2.35 *m*		29	1.12 *s*	20.0
12	5.12 *dd* (7.2, 9)	79.0	30	1.22 *s*	29.1
13	-	52.8	Ac(Me)	1.90 *s*	22.5
			Ac(CO)	-	171.4

4.1.2. *Ring B seco limonoid from the stem bark of Turraea robusta*

4.1.2.1. *11-epi-Toonacilin (**62**)*

62
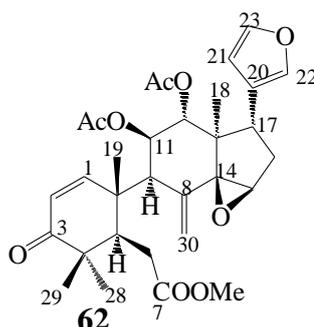
EIMS data of compound **62** suggested the molecular formula $C_{31}H_{38}O_9$ (observed *m/z* 554.2506, calcd 554.2510, Appendix 4B). The ^1H NMR (Table 4.4, Appendix 4) indicated resonances at δ_H 7.47 (*m*, H-21), 6.29 (*m*, H-22) and 7.25 (*m*, H-23) and the corresponding ^{13}C NMR (Appendix 4A) resonances appearing at δ_C 144.2, 113.0 and 141.2 respectively, being characteristic of the furan ring of limonoids in this genus (Yuan *et al*., 2013a). A 1-en-3-one system in ring A was indicated by presence of a pair of doublets at δ_H 7.51 (H-1, *d*, *J* = 10.8 Hz) and 6.02 (H-2, *d*, *J* = 10.2 Hz) and ^{13}C NMR resonances at δ_C 153.8, 126.7 and 204.2 for C-1, C-2 and C-3 respectively. The presence of an exocyclic 8-30 double bond was indicated by a pair of doublets at

δ_H 5.51 (*s*) and 5.48 (*d*, *J* = 1.2 Hz) ascribable to methylene group at C-30 and ^{13}C NMR resonances at δ_C 138.6 (C-8) and 122.5 (C-30). The open nature of ring B in this compound was confirmed by the ^{1}H NMR spectrum that showed four tertiary methyls at δ_H 0.95 (6H), 1.04 and 1.10 instead of the usual five methyls and a three proton singlet at δ_H 3.62 (δ_C 52.7) with a carbonyl resonace at δ_C 175.6 ascribable to a methyl ester. Two singlets in the ^{1}H NMR spectrum at δ_H1.86 (3H) and 1.70 (3H) and ^{13}C NMR resonances at δ_C 171.1 and 170.2 indicated presence of two acetoxyl groups placed at C-11 and C-12. This was based on the ^{1}H-^{1}H COSY spectrum whereby H-9 (δ_H 3.11, *d*, *J* = 7.2 Hz) coupled with H-11 (δ_H 5.56, *dd*, *J* = 7.8, 10.8 Hz) which also coupled with H-12 (δ_H 5.75, *d*, *J* = 10.8 Hz). The placement of the acetates at C-11 and C-12 was confirmed by HMBC correlation of H-9 (δ_H 3.08) and Me-18 (δ_H 0.95) with C-11 (δ_C 72.7) and C-12 (δ_C 76.8). It has been shown that in limonoids with an open ring B, the 12-α acetyl protons (δ_H 1.7) are usually up field shifted (Fraser *et al*., 1995; Mulholland *et al*., 1998). The relative configuration of C-11 and that of C-12 was confirmed by NOESY spectrum which showed correlation of H-5 (δ_H 3.11), H-9 (δ_H 3.08) and Me-18 (δ_H 0.95) with H-11 (δ_H 5.56) and H-17 (δ_H 2.96) with H-12 (δ_H 5.75). A broad singlet at δ_H 4.07 attached to a tertiary carbon at δ_C 61.3, a quaternary carbon at δ_C 72.7 were characteristic of a C-14/C-15 epoxide (Fraser *et al*., 1994; Ndung'u *et al*., 2004). Overall the spectral data for this compound compared well with those reported for 11-*epi*-toonacilin previously isolated from the stem bark of *Turraea holstii* (Mulholland *et al*., 1998b).

Table 4.4: [1]H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **62** in acetone-d_6

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	7.51 *dd* (10.8)	153.8	β	2.21 *m*	
2	6.02 *d* (10.2)	126.8	17	2.96 *dd* (7.2, 10.8)	39.8
3	-	204.2	18	0.95 *s*	14.6
4	-	47.6	19	1.04 *s*	22.4
5	3.11 *d* (7.2)	46.6	20	-	124.2
6 α	2.47 *dd* (7.8, 16.8)	32.4	21	7.25 *m*	142.0
6 β	2.58 *dd* (2.4, 16.8)		22	6.29 *m*	113.0
7	-	175.6	23	7.47 *m*	144.2
8	-	138.6	28	0.96 *s*	24.1
9	3.08 *dd* (2.4, 7.8)	54.4	29	1.10 *s*	23.7 -
10	-	43.5	30	5.51 *s*	122.5
11	5.56 *dd* (7.8, 10.8)	72.7		5.48 *d* (1.2)	
12	5.75 *d* (10.8)	76.8	OMe	3.62 *s*	52.7
13	-	46.5	C-11 Ac(Me)	1.86 *s*	21.4 or 21.3
14	-	72.8	C-11 Ac(CO)	-	170.2
15	4.07 *brs*	61.3	C-12 Ac(Me)	1.70 *s*	21.4or 21.3
16α	1.96 *m*	34.7	C-12 Ac(CO)	-	171.1

4.1.3. *Triterpenoids from the stem bark of Turraea robusta*

4.1.3.1. *Turranolide (22)*

22
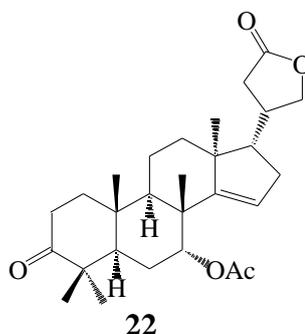
Compound **22** was isolated as white amorphous powder with EIMS showing molecular ion at *m/z* 456 (Appendix 5B) consistent with the molecular formula $C_{28}H_{40}O_5$. The ^{13}C NMR spectrum (Table 4.5, Appendix 5A) indicated presence of three carbony carbons *vis* ketonic carbonyl (δ_C 216.1), an ester carbonyl (δ_C 170.7) and lactonic carbonyl (δ_C 177.6), two vinyl carbons (δ_C 161.0, 119.8) and two oxygenated carbons (δ_C 76.1, 73.5). The ketone group was placed at C-3 following

HMBC correlation of Me-28 (δ_H 0.98) and Me-29 (δ_H 1.02) with C-3 (δ_C 216.1). In the ^{1}H NMR spectrum (Appendix 5) the chemical shifts ascribable to H-7 (δ_H 5.17) and H-15 (δ_H 5.26) were deshielded indicating the presence of an acetoxy group (δ_H 1.93) at C-7 and a double bond at C-14 (Cheplogoi and Mulholland, 2003a). Hence, resonances at δ_C 76.1, 161.0, 119.8 were assigned to C-7, C-14 and C-15 respectively while the signal at δ_C 170.7 was assigned to the acetyl carbon. Relative orientation of the acetyl group at C-7 was confirmed by NOESY whereby correlation of Me-30 (δ_H 1.19, *s*) which is β-oriented with H-7 (δ_H 5.17) were observed. Hence, α-orientation of acetyl group. ^{1}H NMR spectrum indicated tetracyclic nature of this compound where the precence of five tertiary methyl signals at δ_H 1.19, 1.10, 1.11, 1.02 and 0.96 were observed.

The presence of a lactone was established by both the ^{1}H and ^{13}C NMR spectra, in which a pair of doublet of doublets at δ_H 4.96 and 3.93 were assigned to CH_2- 21 and the corresponding carbon appearing at δ_C 73.5. The other signals for the lactone ring were at δ_C 177.5 (C-23), 38.9 (C-20) and 35.1 (C-21).

The carbon resonances for methylenes, CH_2-16 and CH_2-12 and the methyl Me- 29 and the acetate methyl have been distinguished and correctly assigned for the first time in this work by use of HMBC. This compound was hence elucidated as turranolide previously isolated from the root bark of *T. robusta* (Bentley *et al*., 1992).

Table 4.5: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **22** in acetone –d$_6$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1 β	1.94 *m*	40.1	14	-	161.0
1α	1.54 *m*		15	5.26 *dd* (11.8, 3.6)	119.8
2a	2.61 *m*	34.9	16	2.15 *m*	35.9
b	2.30 *m*		17	1.78 *m*	59.6
3	-	216.1	18	1.10 *s*	28.0
4	-	38.4	19	1.10 *s*	16.0
5	1.94 *m*	49.6	20	2.80 *m*	38.9
6 α	1.99 *m*	25.5	21α	3.93 *dd* (1.9, 10.2)	73.5
6 β	1.69 *m*	"	21β	4.96 *t* (8.4)	"
7	5.17 *dd* (11.8, 3.6)	76.1	22α	2.28 *dd* (11.4, 16.8)	35.1
8	-	43.5	22β	2.44 *dd* (17.8, 16.8)	"
9	2.13 *m*	44.1	23	-	177.6
10	-	48.1	28	0.98 *s*	26.8
11 α	1.80 *m*	17.7	29	1.02 *s*	22.0
11 β	1.68 *m*	"	30	1.19 *s*	20.5
12α	1.55 *m*	35.0	Ac(Me)	1.92 *s*	21.8
12β	1.76 *m*	"	Ac(CO)	-	171.7
13	-	48.0			

4.1.3.2. *Azadironolide (192)*
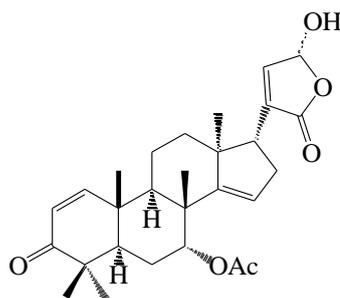
192

The ^{1}H NMR spectrum (Table 4.6, Appendix 6) of **192** indicated that the compound is a triterpenoid with five tertiary methyl resonances at δ_H 0.93, 0.94, 1.03, 1.06 and 1.24 indicating that rings A-D are intact. The presence of a 1-en-3-one system in ring A was indicated by a pair of doublets (*J* = 10.2 Hz) at δ_H 7.27 (H-1) and 5.76 (H-2) with the corresponding ^{13}C NMR (Appendix 6A) resonances appearing at δ_C 159.7 (159.5), 126.4 and 204.6 for C-1, C-2 and C-3, respectively. This was further confirmed by HMBC correlation of H-1 (δ_H 7.27) with C-3 (δ_C 204.6). A double bond at C-14/C-15 was indicated by the presence of a singlet δ_H 5.36 assigned to H-15 while the ^{13}C NMR signals resonated at δ_C 160.7 (160.6) and δ 119.9 (119.8) for C-14

and C-15 respectively.The presence of an ester group was indicated by ^{13}C NMR resonances at δ_C 170.0 (Ac-CO) and 75.7 (C-7) and by the corresponding ^{1}H NMR signals at δ_H 5.32 (H-7, *d*, *J* = 1.3 Hz) and 1.94 (*s*, Ac-Me). The relative orientation of acetyl was confirmed by NOESY where correlation of Me-30 (δ_H 1.3) and H-7 (δ_H 5.32) was observed. Placement of the acetate group at C-7 was confirmed by HMBC correlation of Me-30 (δ_H 1.23) with C-7 (δ_C 75.7). Characteristic resonances associated with a furan ring were conspicuously missing. Instead resonances typical of a 23-hydroxyl-21,23 butenolide moiety were present; a lactonic carbonyl resonance at δ_C 173.0 (172.8) and two vinylic carbons at δ_C 138.1 and 148.8 (148.5) ascribable to C-21, C-20 and C-22 respectively. The presence of a hemiacetal carbon was indicated by a resonance at δ_C 98.6 (98.4) ascribable to C-23 (Cheplogoi and Mulholland, 2003b; McFarland *et al*., 2004). The presence of the butenolide moiety was further confirmed by HMBC correlation of H-17 (δ_H 2.76) with C-20 (δ_C 138.1) and C-22 (δ_C 148.8, 148.5), H-22 (δ_H 7.18) with C-21 (δ_C 173.01, 172.82), C-20 (δ_C 138.0) and C-23 (98.81, 98.39). The doubling of some of the NMR signals was an indication that this compound occurred as an epimeric mixture and this is a common phenomenon in limonoids having a hemiacetal functionality in place of a furan ring (Siddiqui *et al*., 1999; Cheplogoi and Mulholland, 2003b; McFarland *et al*., 2004). The compound was therefore identified as azadironolide, previously isolated from fruit coats of *Azadirachta indica* (Siddiqui *et al*., 1999).

Table 4.6: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **192** in acetone-d$_6$

Position	δ$_H$ (J in Hz)	δ$_C$	Position	δ$_H$ (J in Hz)	δ$_C$
1	7.27 *d* (10.2)	159.7	14	-	160.6
2	5.76 *d* (10.2)	126.4	15	5.36 *s*	119.9 (119.8)
3	-	204.6	16α	2.60-2.68 *m*	35.1
4	-	45.3	β	2.22-2.29 *m*	
5	2.24 *m*	47.8	17	2.76 *m*	54.5
6 α	2.05-2.10 *m*	25.1	18	0.94 *s*	22.0
6 β	1.75-1.80 *m*		19	1.24 *s*	20.1
7	5.32 *d* (1.3)	75.7	20	-	138.1
8	-	40.2	21	-	173.0 (172.8)
9	2.27 *m*	40.1	22	7.18 *d* (6.6)	148.8(148.5)
10	-	41.4	23	6.18 *s*	98.6 (98.4)
11 α	1.75-2.10 *m*	17.7	28	1.06 *s*	22.3
11 β	2.00-2.05 *m*		29	1.03 s	28.0
12α	1.99-2.02 *m*	34.7	30	1.30 *s*	28.4
12β	1.74-1.78 *m*		Ac(Me)	1.94 *s*	21.8
13	-	52.3	Ac(CO)	-	170.7

4.1.3.3. *Stigmasterol (**193**)*

193
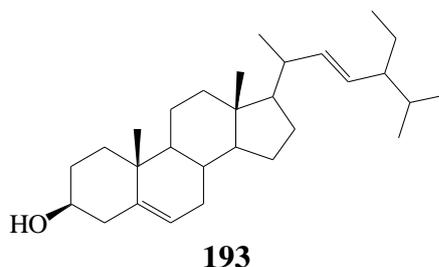
Compound **193** was isolated as white crystals. ^{13}C NMR (Table 4.7, Appendix 7A) indicated presence of four olefinic carbons at δ$_C$ 143.0, 122.2, 140.0 and 130.7 ascribable to C-5, C-6, C-22 and C-23 respectively and their corresponding proton resonances at δ$_H$ 5.31 (H-6, *d*, *J* = 1.3 Hz), 5.21 (H-22, *dd*, *J* = 9, 15 Hz) and 5.08 (H-23, *dd*, *J* = 8.4, 15 Hz) on a skeloidal skeleton. A carbinol proton at δ$_H$ 3.30 (*m*) ascribable to H-3 and its corresponding carbon signal at δ$_C$ 72.4. ^{1}H NMR also indicated presence of six methyl protons at δ$_H$ 0.75, 0.83. 0.84, 0.87, 1.04 and 1.06. These data is in agreement with literature data for stigmasterol a ubiquitous compound in plants (Kojima *et al*., 1990).

Table 4.7: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **193** in acetone-d$_6$:

Position	δ$_H$ (*J* in Hz)	δ$_C$	Position	δ$_H$ (*J* in Hz)	δ$_C$
1	2.00 *m*	33.4	14	1.07 m	58.3
	1.75 *m*		15	1.63 *m*	24.4
2	1.90 *m*	29.6		1.17*m*	
	1.34 *m*		16	1.24 *m*	27.4
3	3.39 *m*	72.4	17	1.19 *m*	57.6
4	2.20 *m*	44.0	18	0.75 *s*	12.9
5	-	143.0	19	1.04 *s*	20.5
6	5.31 *d* (5.4)	122.2	20	1.42 *m*	37.6
7	2.01 *m*	33.3	21	1.06 *s*	22.5
	1.80 *m*		22	5.21 *dd* (9.0, 15.0)	140.0
8	1.50 *m*	33.3	23	5.08 *dd* (8.4, 15)	130.7
9	0.96 *m*	51.9	24	1.20 *m*	52.8
10	-	33.4	25	1.50 *s*	33.3
11	1.62 *m*	24.4	26	0.84 *s*	22.5
12	1.40 *m*	35.3	27	0.87 *s*	20.5
				1.62 *m*	
	1.10 *m*		28	1.18 *m*	25.6
13	-	42.1	29	0.83 *s*	12.9

4.2. Secondary metabolites isolated from *Turraea nilotica*

Four limonoids mzikonone (**17**), azadirone (**19**), acetoxy-7-deacetylazadirone (**28**), 1α,3α-diacety-7α-tigloyvilasinin (**40**) were isolated from the roots of *Turraea nilotica* Kotschy & Peyr; Limonoids **17**, **19** and **28** were also isolated from the stem bark of *T. robusta* in this work and are discussed in Sections 4.1.1.1- 4.1.1.3. Also isolated are four protolimonoids niloticin (**25**), hispidol B (**96**), piscidinol A (**27**), toonapubesins F (**194**) from the stem bark; phytosterols; a mixture of stigmasterol and β-sitosterol (**180**), mixture of sitosterol-3-*O*-β-D-glucopyranoside acetate (**195**) and stigmasterol-3-*O*-β-D-glucopyranoside acetate (**196**) and sitosterol-3-*O*-β-D-glucopyranoside (**197**) from the leaves. Compounds **17**, **19**, **28**, **40**, **96**, are reported from this species for the first time while there are no previous reports on compounds **194**, **195**, **196** and **197** from genus Turraea. The root, stem and leaves of *Turraea nilotica*, were found to synthesize different secondary metabolites; limonoids are present in the roots,

protolimonoids in the stem and phytosterols in the leaves. Limonoids, protolimnoids and steroids have a tetracyclic triterpenoid skeleton. However, they differ in the side chain where limonoids and protolimonoids have different oxidation states while steroids have an alkyl chain. From the previously proposed biosynthetic pathway of limonoids (Champagne *et al*., 1992; Tan and Luo, 2011) compounds from *T. nilotica* may be proposed to follow the following pathway phytosterols (leaves) protolimonoids (stem bark) and limonoids (root bark).

4.2.1. *Protolimonoids from the stem bark of Turraea nilotica*

4.2.1.1. *Niloticin (25)*
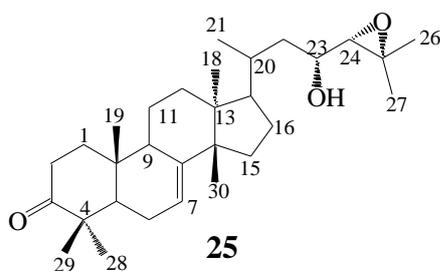
Compound **25** was isolated as a white amorphous powder. The ^{1}H and ^{13}C NMR, HSQC and HMBC were used to characterize the structure. ESI-MS (30 eV) showed an *m/z* at 457.6 [M+H]$^+$ (Appendix 27B). The ^{1}H NMR data (Table 4.8, Appendix 27) showed signals for seven tertiary methyls at δ_H 1.26, 1.25, 1.10, 1.07, 1.05, 1.00 and 0.87 (3H each), a secondary methyl at δ_H 0.99 (3H), an olefinic proton at δ_H 5.35 (*dd*, *J* = 3, 7.2 Hz) and a carbinol proton at δ_H 3.49 (*dd*, *J* = 6.6, 13.8 Hz) indicating that this compound possessed a tirucallane protolimonoid skeleton (Wang *et al*., 2011). Also present were characteristic resonances at δ_H 2.81 (*td*, *J* = 5.4, 14.4 Hz) and 2.13 (*dt*, *J* = 7.2, 14.4 Hz) for H-2 in a 3-oxo triterpene. The ^{13}C NMR spectrum (Table 4.8, Appendix 27A) indicated presence of 30 carbons, that were classified together with HSQC experiment as a ketone (δ_C 214.3), one trisubstituted double bond (δ_C 145.8, 117.9), eight methyls, eight *sp^3* methylenes, five *sp^3* methines and five *sp^3*

quaternary carbons. The presence of epoxide group was deduced from the doublet at δ_H 2.56 (J = 8.4 Hz) which was placed in the side chain at C-24 and C-25 based on HMBC correlation of H-23 (δ_H, 3.49) with C-24 (δ_C 68.4); H-24 with C-23 (δ 69.2), C-25 (δ_C 58.1); Me-27 (δ_H 1.35) with C-24 (δ_C 68.4) and C-25 (δ_C 58.1). The compound was thus identified as niloticin through comparison of spectroscopic data with that reported in literature (Gray *et al*., 1988; Mulholland and Taylor, 1988).

201
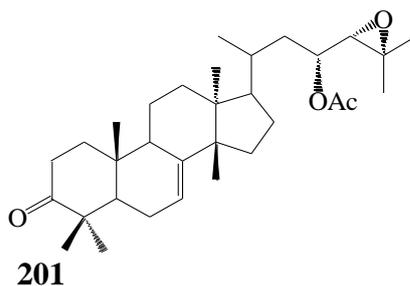
Compound **25** was acetylated to give niloticin acetate (**201**). ESI-MS (30 eV) indicated a molecular ion peak at *m/z* 499.8 [M+H]$^+$ (Appendix 28B) corresponding to molecular formula $C_{32}H_{50}O_4$. That niloticin formed a monoacetate (**201**) was established by the presence of acetate carbon signal at δ_C 170.6 (ester carbonyl) and δ_C 22.0 (acetate methyl carbon) with methyl protons at δ_H 2.08 (*s*), a deshielded C-23 acetoxymethine proton at δ_H 4.86 (*m*) and its corresponding carbon signal at δ_C 72.3 (Table 4.9, Appendices 28 and 28A).

Table 4.8: ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for niloticin **25** in acetone- d_6

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1a	2.02 *ddd* (3, 5.4, 13.2)	38.2	16a	*2.08 m*	28.5
b	1.45 *m*		b	1.31 *ddd* (3, 5.4, 8.4)	
2a	2.81 *td* (5.4, 14.4)	34.5	17	1.60 *m*	53.4
b	2.13 *dt* (7.2, 14.4)		18	1.05 *s*	12.2
3	-	214.3	19	1.26 *s*	19.3
4	-	47.4	20	1.51 *m*	33.6
5	1.75 *dd* (6,17)	52.2	21	0.99 *d*	19.8
6	2.10 *m*	24.2	22a	1.64 *m*	41.1
7	5.35 *dd* (3, 7.2)	117.9	b	1.36 *m*	
8	-	145.8	23	3.49 *dd* (6.6, 13.8)	69.2
9	2.40 *m*	48.4	24	2.56 *d* (8.4)	68.4
10	-	34.9	25	-	58.1
11	1.63 *m*	18.1	26	0.87 *s*	20.9
12a	1.87 *m*	33.3	27	1.25 *s*	24.2
b	1.67 *m*		28	1.00 *s*	24.3
13	-	43.5	29	1.10 *s*	21.3
14	-	51.1	30	1.06 *s*	27.0
15	1.56 *m*	33.9			

Table 4.9: ^1H (200 MHz) and ^{13}C NMR (50 MHz) chemical shifts for the side chain for compounds **25** and **201**

Position	δ_H (*J* in Hz) (201)	δ_C (201)	δ_C (25)
20		33.5	33.6
21		19.8	19.8
22		38.7	41.1
23	4.86 *m*	72.3	69.2
24	2.76 *d* (9.2)	65.6	68.4
25	-	59.6	58.1
Ac (CO)		170.6	-
Ac(Me)	2.08 *s*	22.0	

4.2.1.2. *Piscidinol A (27)*
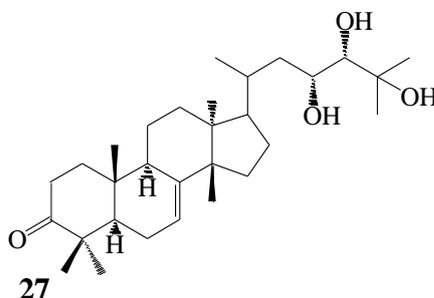
Compound **27** was isolated as white crystals. ^1H and ^{13}C NMR, HSQC, DEPT and ESI-MS (30 eV) indicating an *m/z* at 475.5 [M+H]$^+$ (Appendix 8C) suggested molecular formula $C_{28}H_{38}O_5$. The ^{13}C NMR spectrum (Table 4.10, Appendix 8A)

showed the presence of 30 carbons that were classified with DEPT and HSQC experiments as a ketone [δ_C 216. 5 (C-3)], a trisubstituted double bond [δ_C 145.8 (C-8), 118.0 (C-7)], three oxygenated carbons [δ_C 74.9 (C-24), 74.2 (C-25) and 69.6 (C-23)], eight sp^3 methylenes, four sp^3 methines, three sp^3 quaternary carbons and eight methyls. In addition, ^{1}H NMR data indicated presence of seven tertiary methyls at δ_H 1.26 (6H), 1.01, 1.03, 1.01 (6H) 0.82 and a secondary methyl (at δ_H 0.92), two carbinol protons [H-23, δ_H 4.08 (*dd*, $J = 5$, 8.6 Hz), 3.14 (H-24, *d*, $J = 5.6$ Hz)] and an olefinic proton [H-7, *m*, δ_H 5.32]. The above data is consistent with that of a tirucallane protolimonoid skeleton with a 3-ketone and a 7-8 double bond (Sang *et al*., 2009).

The nature of the side chain was established from a deshielded singlet at δ_H 1.26 integrating for six protons ascribable to Me-26 and Me-27. This was further supported by HMBC correlation of Me-26 and Me-27 with C-25 (δ_C 74.1). The signals at δ_C 69.6 and 74.9 were assigned oxymethine carbons C-23 and C-24 following coupling of H-23 (δ_H 4.08) with H-24 (δ_H 3.14) in ^{1}H-^{1}H COSY spectrum. With the aid of HSQC, HMBC, ^{13}C NMR/ DEPT and ^{1}H-^{1}H COSY compound **27** was identified as piscidinol A, a compound previously isolated from the stem bark of *Turraea nilotica* (Mulholland and Taylor, 1988). Its single-crystal x-ray structure is as shown in Figure 4-1.
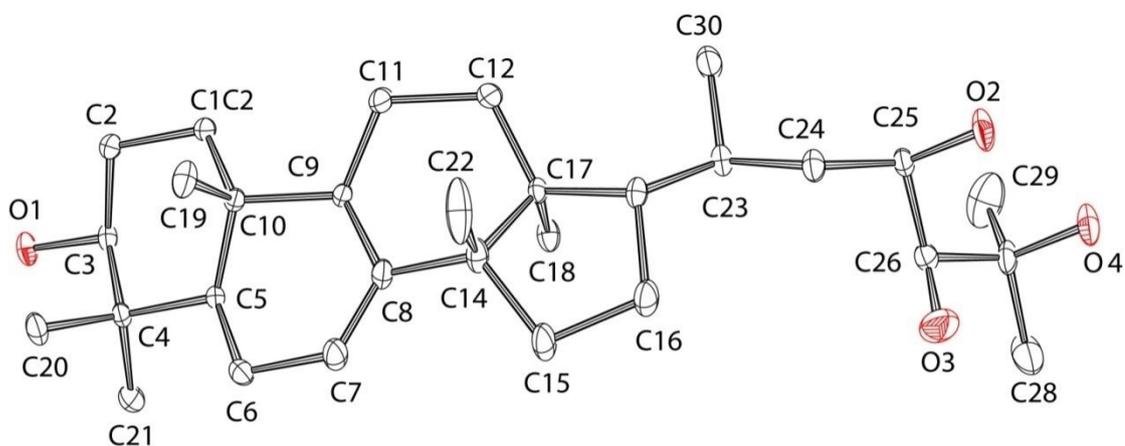
Figure 4-1: Single–crystal X-ray structure of piscidinol A (**27**)

203
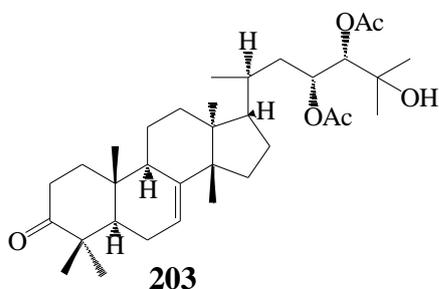
Compound **27** was acetylated to give piscidinol A diacetate (**203**). The ^{13}C NMR spectrum (Table 4.11, Appendix 9A) indicated the presence of 34 resonances (instead of 30 as in compound **27**) of which two carbonyl resonances (δ_C 170.5 and 170.2), two vinyl carbons (δ_C 146.3, 118.6), two oxygenated methines (δ_C 77.2 and 70.6) and a quartenary carbon (δ_C 70.6) were apparent. These data indicated that the two hydroxyl groups at C-23 and C-24 are acetylated. The presence of two acetate methyls at (δ_H 2.16 and 2.20) which correlated with the carbonyl signals (δ_C 170.5 and 170.2) in the HMBC spectrum further confirmed double acetylation. HMBC correlation of terminal methyls Me-26 (δ_H 1.15) and Me-27 (δ_H 1.20) with δ_C 76 and δ_C 72.8 led to assignment of these signals to C-24 and C-25 respectively. In the ^{1}H NMR, a vinly proton at δ_H 5.38 was ascribable to H-7 and its corresponding carbon signal at δ_C 118.6. A

resonance at δ_H 5.38 (*ddd*, $J = 9.3, 5.1, 1.6$ Hz) was ascribable to H-23 while that at δ_H 4.86 (*d*, $J = 1.58$ Hz) to H-24. This was further confirmed by HMBC correlation of H-24 (δ_H 4.86) with C-23 (δ_C 70.6).

Table 4.10: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **27** in CD$_2$Cl$_2$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1α	1.40 *m*	38.5	16α	2.02 *m*	28.4
β	2.00 *m*		β	1.30 *m*	
2α	2.18 *td* (7.1, 14.2)	34.9	17	1.50 *m*	53.1
β	2.76 *td* (5.5, 14.5)		18	0.82 *s*	21.7
3	-	216.5	19	1.01 *s*	12.5
4	-	47.5	20	1.36 *m*	34.0
5	1.72 *dd* (10.9, 6.7)	52.3	21	0.92 *d* (6.6)	18.5
6	2.10 *m*	24.4	22α	1.84 *m*	40.5
7	5.32 *m*	118.0	β	1.15 *m*	
8		145.8	23	4.08 *dd* (5, 8.6)	69.6
9	2.39 *m*	48.5	24	3.14 *d* (5.6)	74.9
10		35.0	25		74.1
11	1.56 *m*	18.3	26	1.26 *s*	26.1
12α	1.83 *m*	33.7	27	1.26 *s*	27.1
β	1.66 *m*		28	1.02 *s*	24.3
13		43.5	29	1.10 *s*	21.4
14		51.2	30	1.01 *s*	27.1
15	1.49 *m*	33.7			

Table 4.11: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) chemical shifts for the side chain for compounds **27** and **203**

Position	δ_H (*J* in Hz)(203)	δ_C (203)	δ_C (27)
20	-	34.4	34.0
21		18.8	18.5
22		39.0	40.5
23	5.37*ddd* (9.3, 5.1, 1.7)	70.6	69.6
24	4.86 *d* (1.6)	77.2	74.9
25	-	72.8	74.1
C-24 Ac(CO)		170.5	
C-23 Ac(CO)		170.2	
C-23 Ac(Me)	2.07 *s*	21.8	
C-24 Ac(Me)	2.20 *s*	21.2	

4.2.1.3. *Hispidol B (96)*

96
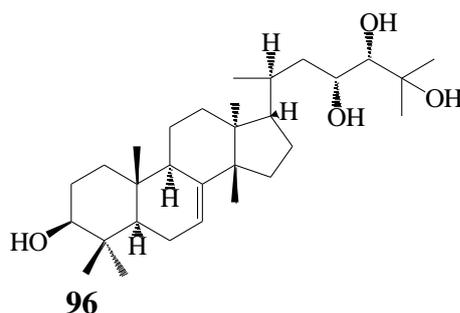
Compound **96** was isolated as white crystals, was insoluble in common organic solvents except DMSO. EIMS suggested molecular formula $C_{30}H_{52}O_4$ (observed *m/z* 476.3868, calcd 476.3860, Appendix 10B). ^{1}H and ^{13}C NMR spectra (Table 4.12, Appendices 10 and 10A) were similar to those of piscidinol A (**27**) except that the ketone at C-3 is replaced by a hydroxyl group. Other characteristic resonances observed in ^{13}C NMR spectrum were three oxygenated carbons [δ_C 75.7 (C-24), 72.9 (C-25) and 67.9 (C-23)] and two olefinic carbons [δ_C 146.0 (C-8) and 118.0 (C-7)]. The ^{1}H NMR indicated characteristic seven tertiary methyls resonances at δ_H 0.62, 0.75, 0.77, 0.86, 0.94, 1.08. 1.12; a secondary methyl at δ_H 0.84; three oxymethines at δ_H 4.11 (H-24, *d*, *J* = 8.4 Hz), 4.15 (H-3, *d*, *J* = 6 Hz), 4.37 (H-23, *d*, *J* = 4.8 Hz) and an olefinic proton at δ_H 5.22 (H-7, *d*, *J* = 2.6 Hz). Thus, the NMR data is consistent with a tirucallane protolimonoid skeleton with a 7-8 double bond.   Relative orientation of hydroxyl group at C-3 was established by NOESY whereby H-3 correlates with Me-28. ^{1}H and ^{13}C NMR, HSQC, HMBC and ^{1}H-^{1}H COSY were carefully used to assign both carbon and proton signals. The compound was identified as hispidol B previously isolated from *Trichilia hispida* (Jolad *et al*., 1981).

Table 4.12: [1]H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **96** in DMSO-d$_6$

Position	δ$_H$ (*J* in Hz)	δ$_C$	Position	δ$_H$ (*J* in Hz)	δ$_C$
1α	1.06 *m*	37.2	16	1.35 *m*	28.4
β	1.27-1.22 *m*			1.90 *m*	
2	1.46-1.40 *m*	28.4	17	1.49-1.40 *m*	53.9
3	4.15 *d* (6)	77.4	18	0.68 *s*	13.4
4	-	39.0	19	0.77 *s*	22.2
5	1.23 *m*	50.6	20	1.49-1.40 *m*	33.6
6α	2.06 *m*	24.0	21	0.86 *t* (6)	19.4
β	1.90 *m*		22β	0.99 *m*	41.8
7	5.22 *d* (3.6)	118.0	α	1.46-1.40 *m*	
8	-	146.0	23	4.37 *d* (4.8)	67.9
9	2.12 *m*	48.9	24	4.11 *d* (8.4)	75.9
10	-	34.9	25	-	72.9
11	1.46-1.40 *m*	18.1	26	1.08 *s*	26.4
12	1.46-1.40 *m*	33.8	27	1.11 *s*	28.0
13	-	43.5	28	0.86 *t* (6)	28.2
14	-	51.2	29	0.75 *s*	15.5
15	1.35 *m*	34.0	30	0.94 *s*	27.6

4.2.1.4. *Toonapubesins F (**194**)*

194
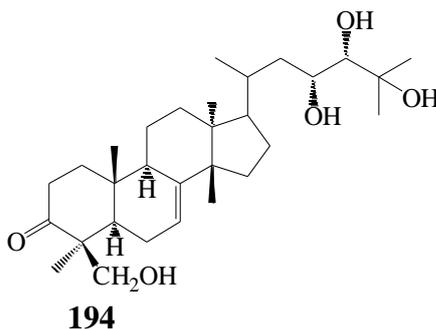
Compound **194** was isolated as white crystals. ESI-MS (30 eV) showed a pseudo molecular ion peak, *m/z* at 491.6 [M+H]$^+$ (Appendix 11B). The NMR data   for this compound (Table 4.13, Appendices 11 and 11A) were very similar to those of piscidinol A (**27**) except for the substituents at C-4. The ^{13}C NMR resonance associated with dimethyl substituted C-4 is at *ca*. δ$_C$ 47 in **27** (Jolad *et al*., 1981; Gray *et al*., 1988; Mulholland and Taylor, 1988; Hideji *et al*., 1992). This downfield shifted to δ$_C$ 53.3 in **194**. Furthermore in the ^{1}H NMR spectrum one of the methyl resonace is replaced by a typical AB type doublet [δ$_H$ 3.60 (*d*, *J* = 10.0 Hz), 4.08 (*d*, *J* = 10.9 Hz)]. This is an indication that one of the methyl groups attached to C-4 is oxidized to an

alcohol. Methyl group at C-29 was the one oxidized. This was established through NOE cross peaks between H-19/H-29a, H-5/H-28, H-5/H-6a, H-19/H-6b and H-5/H-9 and HMBC correlation of Me-28 (δ_H 1.12, *s*) with δ_C 65.7 (C-29). This was confirmed by X-ray crystallography (Figure 4.2, Appendices 11C-F).

The ^{1}H NMR showed the presence of six tertiary methyls resonating at δ_H 0.82, 0.98, 1.02, 1.12, 1.27 (6H) instead of seven tertiary methyls as in **27**. Thus compound **194** was identified as toonapubesin F previously isolated from *Toona ciliate* var *pubescens* (Wang *et al*., 2011).

Table 4.13: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **194** in CD$_2$Cl$_2$.

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1α	1.52 *m*	37.8	15	1.50 *m*	34.0
β	2.02 *m*		16	2.00 *m*	28.4
2α	2.30 *ddd* (3.2, 4.4, 14.6)	35.6	17	1.51 *m*	53.7
β	2.69 *td* (5.8, 14.5)		18	0.82 *s*	21.9
3		215.7	19	0.98 *s*	13.4
4		53.1	20	1.40 *m*	33.7
5	1.87 *m*	53.4	21	0.93 *d* (6)	18.8
6α	2.16 *m*	24.7	22α	1.88 *m*	40.5
β	2.05 *m*		β	1.12 *m*	
7	5.67 *m*	118.0	23	4.09 *m*	69.6
8		146.0	24	3.14 *s*	74.9
9	2.34 *m*	48.3	25		74.1
10		35.0	26	1.27 *s*	26.0
11	1.50 *m*	18.5	27	1.27 *s*	27.2
12α	1.83 *m*	33.7	28	1.12 *s*	20.1
β	1.66 *m*		29a	4.08 *d* (10.0)	65.7
13		43.5	b	3.60 *d* (10.9)	
14		51.2	30	1.02 *s*	27.2
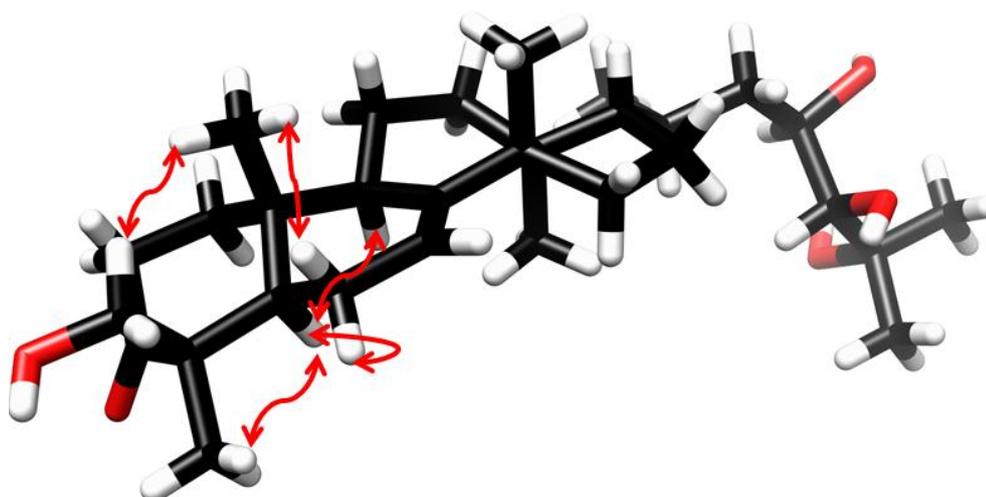
Figure 4-2: Relative stereochemistry for compound (**194**) at C-4 on X-ray

4.2.2. *Limonoids from the root bark of Turraea nilotica*

4.2.2.1. *1α, 3α-Diacety-7α-tigloyvilasinin* (**40**)

40
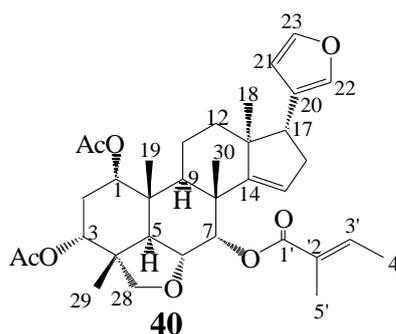
Compound **40** was isolated as a white amorphous powder with EIMS giving an *m/z* at 594 [M]$^+$ (Appendix 12B) corresponding to the molecular formula $C_{35}H_{46}O_8$. The ^{13}C NMR spectrum (Table 4.14, Appendix 12A) showed characteristic resonances associated with a furan ring of limonoids at δ_C 142.5 (C-23), 139.7 (C-21) and 111.1 (C-22) with the corresponding proton resonances appearing at δ_H 7.8 (*t*, *J* = 3.4 Hz), 7.15 (*m*) and 6.20 (*m*), respectively. The presence of a tiglate moiety was indicated by resonances at δ_C 166.5 (C-1'), 136.6 (C-3'), 128.8 (C-2'), 14.1 (C-4') and 11.9 (C-5') (Ntalli *et al*., 2010). In the ^1H NMR spectrum occurence of an olefinic proton at δ_H 6.80 (*qq*, *J* = 1.5, 7.1 Hz) ascribable to H-3' supported the presence of a tiglate moiety.

83

In the HMBC spectrum correlation of H-7 (δ_H 5.51, *d*, *J* = 2.8 Hz) with C-1' (δ_C 166.5), C-14 (δ_C 158.1) and C-6 (δ_C 72.7) confirmed the placement of the tiglate moiety at C-7. A double bond at C-14/C-15 was indicated from ^{13}C NMR [δ_C 158.1 (C-14) and 120.3 (C-15)] and ^1H NMR (H-14, δ_H 5.42 *dd*, *J* = 1.5, 3.2 Hz). HMBC correlation of Me-30 (δ_H 1.11), CH$_2$-16 (δ_H 2.30, 2.22) and H-7 (δ_H 5.51) with C-14 (δ_C 158.1) further confirmed presence of a double bond at C-14/C-15.

The presence of a 6α-28 ether bridge was indicated by the appearance of oxymethylene protons at δ_H 3.35 (CH$_2$-28, *m*), an oxymethine at δ_H 4.10 (H-6) and two oxygenated carbons at δ_C 77.7 (C-28) and δ_C 72.6 (C-6). The appearance of only four tertiary methyls resonating at δ_H 0.67, 0.95, 1.10 and 1.11 instead of five methyls found in ring intact limonoids supports the presence of a 6α-28 ether bridge in this compound (Mulholland and Taylor, 1988; McFarland *et al*., 2004). This was further confirmed by coupling in the ^1H-^1H COSY [showing correlation of H-7 (δ_H 5.51) with H-6 (δ_H 4.10)] and HMBC [showing correlation of H-5 (2.59 *m*) with C-6 (δ_C 72.6) and C-28 (δ_C 77.7)] spectra.

The acetyl substituents; δ_C 169.8; 169.5 (for carbonyls) and 21.0; 20.8 (for methyls) with the corresponding ^1H NMR signals appearing at δ_H 1.88 (*s*) and 1.92 (*s*) were fixed at C-3 and C-1 respectively following HMBC correlation of Me-29 with C-3 (δ_C 71.7) and CH$_2$-2a (2.02, *dt*, *J* = 5.2, 16.6 Hz), CH$_2$-2b (2.15, *dt*, *J* = 6.5, 16.6 Hz) with C-1 (δ_C 72.4) and C-3 (δ_C 71.7). Relative orientations of the tiglate and acetyl groups was established from the NOESY spectrum which showed correlation between H-1 (δ_H 4.62) with Me-19 (δ_H 0.95), CH$_2$-2a (δ_H 2.15) and CH$_2$-11b (δ_H 1.50) and H-7 (δ_H 5.41) with Me-29 (δ_H 1.10) and Me-30 (δ_H 1.11). This compound was therefore characterized as 1α,3α-diacety-7α-tigloyvilasinin (**40**) first isolated from the root bark of *Turraea parvifolia* (Cheplogoi and Mulholland, 2003a).

Table 4.14: [1]H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **40** in CD_2Cl_2

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	4.62 *t* (5.8)	72.4	17	2.71 *dd* (7.4, 10.9)	51.5
2a	2.02 *dt* (5.2, 16.6)	27.4	18	0.67 *s*	21.1
b	2.15 *dt* (6.5, 16.6)		19	0.95 *s*	15.0
3	4.82 *t* (5.9)	71.7	20	-	124.9
4	-	42.1	21	7.15 *m*	139.7
5	2.59 *m*	41.4	22	6.20 *m*	111.1
6	4.10 *dd* (2.8, 12.6)	72.7	23	7.28 *t* (3.4)	142.5
7	5.51 *d* (2.8)	73.9	28	3.35 *m*	77.7
8	-	44.6	29	1.10 *s*	19.1
9	2.59 *m*	34.8	30	1.11 *s*	26.0
10	-	39.2	1'	-	166.5
11α	1.26 *m*	15.2	2'	-	128.8
β	1.56 *m*		3'	6.80 *qq* (1.5, 7.1)	136.6
12α	1.70 *m*	32.7	4'	1.69 *s*	14.1
β	1.50 *m*		5'	1.78 *s*	11.9
13	-	47.3	C1-Ac (Me)	1.88 *s*	20.8
14	-	158.1	C3-Ac(Me)	1.92 *s*	21.0
15	5.42 *dd* (1.5, 3.2)	120.3	C1-Ac(CO)	-	169.8
16α	2.30 *ddd* (1.7, 11, 2.2)	34.2	C3- Ac(CO)		169.5
β	2.22 *ddd* (3.5, 7.4, 15.3)				

4.2.3. *Phytosterols from the leaves of Turraea nilotica*

4.2.3.1. *Sitosterol 3-O-β-D-glucopyranoside acetate (195) and Stigmasterol 3-O-D-glucopyranoside acetate (196)*

195 R = a
196 R = b
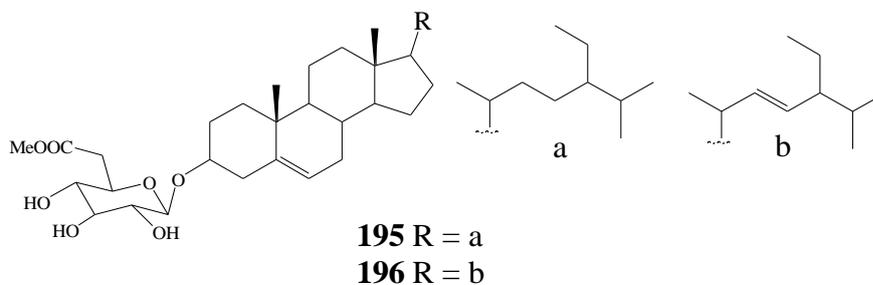
This mixture of glycosides was obtained as white amorphous powder. It consistently gave a single spot using different solvent systems on TLC. Isolation of the glycosides mixtures in their pure form is still unresolved (Zhao *et al*., 1989; Kojima *et al*., 1990). The ^{1}H and ^{13}C NMR (Table 4.15, Appendices 13 and 13A), HSQC, HMBC and literature data were used to identify the aglycones in both compounds as stigmasterol and sitosterol (Zhao *et al*., 1989; Jares *et al*., 1990; Kojima *et al*., 1990). The ^{13}C NMR spectrum indicated two olefinic carbons at δ_C 140.6 (C-5); 122.5 (C-5) and a

corresponding vinyl proton at δ_H 5.37 (H-6, *s*) indicating the glucosterols contain a double bond at C-5/C-6 (Zhao *et al*., 1989; Jares *et al*., 1990). The presence of stigmasterol aglycone was indicated in the ^{1}H NMR spectrum where resonances appeared at δ_H 5.15 (*dd*, *J* = 8.8, 15.2 Hz) and δ_H 5.02 (*dd*, *J* = 8.8, 15.2, Hz). These protons coupled each other in the ^{1}H-^{1}H COSY spectrum. The corresponding carbon signals appeared at δ_C 138.6 (C-22) and 129.6 (C-23). ^{1}H NMR spectrum further indicated six methyl groups at δ_H 0.68, 0.81, 0.83,0.84, 0.88, 0.92, 1.01 that were comparable to literature values of C-24 ethyl phytosterols (Zhao *et al*., 1989; Kojima *et al*., 1990).

Seven well resolved oxygenated carbon resonances were present in the ^{13}C NMR spectrum six of them resonating at δ_C 101.5 (C-1'), 76.2 (C-3'), 74.3 (C-5'), 73.9 (C-2') and 63.4 (C-6') were associated with a sugar moiety while the remaining at δ_C 79.9 was assigned to C-3. The anomeric proton resonated at δ_H 4.38 (H-1', *d*, *J* = 8 Hz) while the rest of the glucose protons resonated between δ_H 3.37-4.50 ppm. The large coupling constant of the anomeric proton suggested that the glucoside is in a β-configuration (Zhao *et al*., 1989; Jares *et al*., 1990). HMBC of anomeric proton H-1' (δ_H, 4.38, *d*, *J* = 8 Hz) with C-3 (δ79.9) confirmed attachment of the sugar moiety to C-3. Both ^{1}H and ^{13}C NMR indicated presence of only one sugar moiety linked to the sterol. Presence of an acetoxyl group was indicated by a resonance at δ_C 175.1. HMBC of CH_2-6a' (δ_H 4.25, *dd*, *J* = 12, 1.6, Hz), CH_2-6b' (δ_H, 4.50, *dd*, 12, 4.8 Hz) with δ_C 175.1 indicated the placement of acetoxyl group to C-6'. From the above data the major compound was identified as sitosterol 3-*O*-β-D-glucopyranoside acetate (**195**) previously isolated from *Prunella vulgaris* (Kojima *et al*., 1990) and the minor compound as stigmasterol 3-*O*-β-D-glucopyranoside acetate (**196**). From ^{1}H NMR spectrum it was concluded that β-sitosterol 3-*O*-β-D-glucopyranoside acetate formed

80% of the mixture. Both ^{1}H and ^{13}C NMR data for sitosterol 3-*O*-*β*-D-glucopyranoside acetate was unambiguously assigned and the values compared well with the literature values (Kojima *et al*., 1990).

Table 4.15: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **195** in CDCl$_3$

Position	δ$_H$ (*J* in Hz)	δ$_C$	Position	δ$_H$ (*J* in Hz)	δ$_C$
1	1.80 *m*	37.6	18	0.68 *s*	12.2
	1.04 *m*		19	1.01 *s*	19.7
2	1.97 *m*	29.8	20	-	36.5
	1.62 *m*		21	0.92 *d* (1.72)	19.0
3	3.56 *m*	79.5	22	1.31 *m*	34.5
4	2.35 *m*	39.2		1.03 *m*	
	2.28 *m*		23	1.18 *m*	26.4
5	-	140.2	24	0.93 *m*	46.2
6	5.37 *s*	122.1	25	1.62 *m*	29.4
7	1.98 *m*	32.4	26	0.84 *s*	20.1
8	1.54 *m*	32.3	27	0.83 *d*	19.3
9	0.92 *d* (6.41)	50.5	28	1.31-1.27 *m*	23.0
10	-	37.0	29	0.84 *t*	12.3
11	1.44 *m*	21.4	Ac (CO)	-	175.1
12	2.00 *m*	40.1	Ac (Me)	1.60 *s*	21.4
	1.16 *m*		1'	4.38 *d* (8)	101.5
13	-	42.6	2'	3.37 *m*	73.9
14	0.97 *m*	56.7	3'	3.56 *m*	76.2
15	1.50 *m*	24.6	4'	3.37 *m*	70.3
	1.17 *m*		5'	3.46 *m*	74.3
16	1.60 *m*	28.6	6'	4.25 *dd* (12, 1.6)	63.4
	1.17 *m*			4.50 *dd* (12, 4.8)	
17	1.01 *m*	56.0			

4.2.3.2. *Sitosterol-3-O-β-D-glucopyranoside (**197**)*
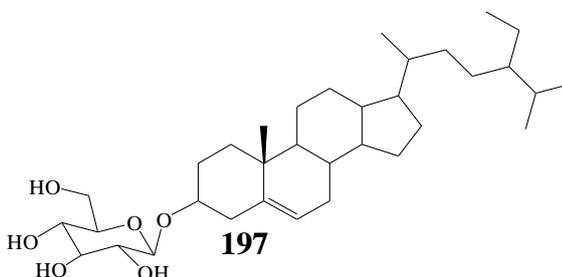
Compound **197** was isolated as white amorphous powder and consistently gave a single spot on TLC though both ^{1}H and ^{13}C NMR (Table 4.16, Appendices 14 and 14A) indicated the presence of minor impurities that did not hinder elucidation of the

major compound. As earlier mentioned, isolation of sterol glycosides in their pure form is still an uphill task (Zhao *et al*., 1989; Kojima *et al*., 1990). The ^{13}C NMR spectrum showed two vinyl carbon signals at δ_C 140.6 (C-5) and 121.4 (C-6) and a vinyl proton at δ_H 5.36 (*s*, H-6) indicating presence of a double bond at C-5/C-6 of the aglycon β-sitosterol (Kojima *et al*., 1990). Presence of a glycoside moiety was indicated by six well resolved carbon resonances at δ_C 100.8 (C-1'), 76.9 (C-5', C-3'), 73.6 (C-2'), 70.2 (C-4') and 61.2 (C-6'). The corresponding proton resonances appeared between δ_H 4.43-3.36 ppm. A doublet at δ_H 4.43 (*J* = 8 Hz) was assigned to anomeric proton H-1'. The large coupling constant suggests that the sugar is *β*-D-glucoside (Zhao *et al*., 1989; Kojima *et al*., 1990).

Six methyl signals at δ_H 0.68, 0.81, 0.83, 0.84, 0.92 and 1.01 (3H each) confirmed that the aglycone is the phytosterol sitosterol (Zhao *et al*., 1989). A carbinol proton at δ_H 3.66 (*m*) and it corresponding carbon resonance at δ_C 77.1 was assigned to C-3. HMBC, HSQC, ^{1}H-^{1}H COSY were carefully used to elucidate the structure. The compound was identified as sitosterol-3-*O*-*β*-D-glucopyranoside previously isolated from *Prunella vulharis* (Kojima *et al*., 1990).

Table 4.16: ¹H (800 MHz) and ¹³C (200 MHz) NMR data for compound **197** in DMSO6

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	1.88 *m*	37.0	17	1.15 *m*	55.6
	1.07 *m*		18	0.68 *s*	11.8
2	1.81 *m*	29.4	19	1.01 *s*	19.1
	1.49 *m*		20	1.33 *m*	35.6
3	3.59 *m*	77.1	21	0.92 *d* (8)	18.8
4	2.55 *m*	38.4	22	1.30 *m*	33.5
	2.38 *m*			1.16 *m*	
5		140.6	23	1.62 *m*	25.6
6	5.37 *s*	121.4		1.16 *m*	
7	1.94 *m*	31.6	24	0.95 *d* (6.4)	45.3
	1.40 *m*		25	1.63 *m*	28.8
8	1.51 *m*	31.5		1.15 *m*	
9	0.95 *d* (6.4)	49.7	26	0.84 *d*	19.3
10		36.4	27	0.81 *d*	19.9
11	1.47 *m*	20.7	28	1.25 *m*	22.8
	1.41 *m*			1.18 *m*	
12	2.00 *m*	40.1	29	0.83 *t*	11.9
	1.18 *m*		1'	4.43 *d* (8)	100.9
13		42.0	2'	3.36 *m*	73.6
14	1.02 *m*	56.3	3'	3.60 *m*	76.9
15	1.55 *m*	24.0	4'	3.61 *m*	70.2
	1.06 *m*		5'	3.42 *m*	76.9
16	1.81 *m*	27.9	6'	3.92 *m*	61.2
	1.24 *m*			3.81 *m*	

4.2.3.3. *β-sitosterol (180) and Stigmasterol (193)*
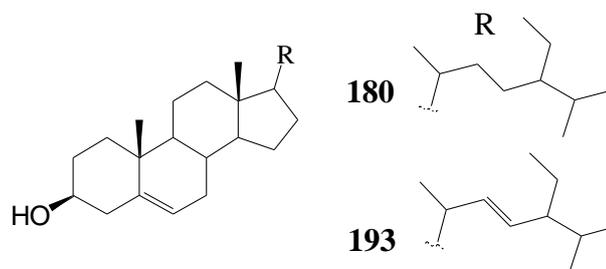
A mixture of of β-sitosterol (**180**) and stigmasterol (**193**) consistently gave a single spot with different solvent systems on TLC. That the sample is a mixture of two was shown from ¹H and ¹³C NMR spectra (Table 4.17, Appendices 15 and 15A). The ¹³C NMR spectrum indicated four vinyl carbons at δ_C 140.8, 140.7 (C-5), 121.73, 121.72 (C6) and a corresponding vinyl proton at δ_H 5.35 (H-6). This indicated that the mixture had C-5/C-6 double bond. Also present were two carbon resonances at δ_C 138.3 (C-22) and 129.3 (C-23)

and corresponding proton signals at δ_H 5.15 (*dd*, *J* = 15.2, 8.7, Hz) and 5.02 (*dd*, *J* = 15.1, 8.7, Hz) comparable to those of stigmasterol (**193**) side chain (Kojima *et al*., 1990). A carbinol proton at δ_H 3.52 (H-3, *m*) and a corresponding carbon signal at δ_C 71.8 (C-3) was also observed. The rest were *sp3* carbons. Careful use of [I]H and ^{13}C NMR, HMBC and HSQC together with literature data indicated that the mixture comprised of β-sitosterol (**180**) and stigmasterol (**193**). Compound **193** was previously discussed under section 4.1.3.3. Isolation of phytosterols as complex mixtures has previously been reported (Zhao *et al*., 1989; Jares *et al*., 1990; Kojima *et al*., 1990).

Table 4.17: [1]H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **180** and **193** in DMSO-d$_6$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	1.87 *m*	37.3		1.09 *m*	
	1.10 *m*		16	1.70 *m*	29.2 (28.3)
2	1.85 *m*	31.6		0.80 *m*	
	1.6-1.5 *m*		17	1.08 *m*	56.0(56.1)
3	3.52 *m*	71.8	18	0.70 *s*	11.9 (12.1)
4	2.32 *ddd* (13.1, 5, 2.3)	42.3	19	1.03 *s*	19.4
	2.26 *m*		20	1.38 *m*	36.2 (40.5)
5		140.7 (140.6)	21	0.92 *d*	18.8 (21.1)
6	5.35 *m*	121.73(121.7)	22	1.34 *m*	34.0 (138.3)
7	2.01 *m*	31.9	23	1.17 *m*	26.1 (129.3)
8	1.51 *m*	31.9	24	0.96 *d* (6.5)	45.9 (51.3)
9	0.94 *d* (6.5)	50.1	25	1.69 *m*	29.3 (31.9)
10		36.5	26	0.85 *d*	19.8 (21.1)
11	1.54-1.47 *m*	21.2 (21.1)	27	0.84 *d*	19.0
12	2.03 *m*	39.7(39.8)	28	1.61 *m*	23.1 (24.3)
	1.18 *m*			1.08 *m*	
13		42.2(42.3)	29	0.87 *t*	12.0 (12.3)
14	1.02 *m*	56.8			
15	1.62 *m*	24.4 (24.3)			

4.3. Chemotaxonomic significance of *Turraea* limonoids

Different classes of limonoids are reported in the genus *Turraea* with each species synthesizing more than one class of limonoids. Compounds **17**, **19**, **28**, **40** and **62** were expectable in that limonoids commonly occur in other species of genus *Turraea*. Except for compound **19** which has been reported in other Meliaceace genus (Arenas and Rodriguez-Hahn, 1990; Zhou *et al*., 1997; Nakatani *et al*., 2001) there are no reports outside genus Turraea for compounds **17**, **28**, **40** and **62** in family Meliaceae. Mzikonone (**17**) is the commonest limonoid and is reported in five species as shown in Table 4.18.

Table 4.18: Occurrence of some limonoids in *Turraea* species

Limonoid	Turraea species isolated from				
Azadirone (**19**)	*nilotica*	*robusta*			
12α-acetoxy-7-deacetylazadirone (**28**)	*robusta*	*cornucopia*	*pubescens*		
Mzikonone (**17**)	*robusta*	*cornucopia*	*parvifolia*	*nilotica*	*pubescens*
11-*epi*-toonacilin (**62**)	*robusta*	*holstii*	*pubescens*	*cornucopia*	
1α,3α-diacety-7α-tigloyvilasinin (**40**)	*nilotica*	*parvifolia*			

4.4. Secondary metabolites from roots and leaves of *Ekebergia capensis*

From the root bark of *Ekebergia capensis* Sparrm seven compounds were isolated of which, one (compound **198**) was new, whereas six were known triterpenoids, namely 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene (**152**), 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**151**), ekeberin A (**158**), oleanolic acid (**159**), oleanonic acid (**160**) and 3-*epi*-oleanolic acid (**161**). From the leaves, proceranolide (**148**) and two glucoflavonoids new to the genus, kaempferol-3-*O*-*β*-D-glucopyranoside (**199**) and quercetin-3-*O*-*β*-D-glucopyranoside (**200**) were identified.

4.4.1. *Pentacyclic triterpenoids from the root bark of Ekebergia capensis*

4.4.1.1. *3-oxo-12β-Hydroxy-oleanan-28,13β-olide* **198**

198
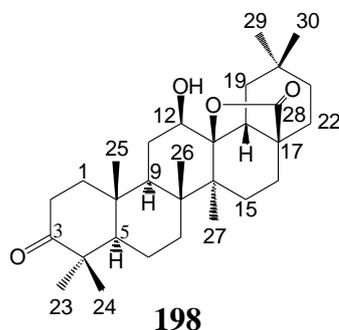
Compound **198** was isolated as a white amorphous powder. Its HR(ESI)MS analysis suggested the molecular formula $C_{30}H_{46}O_4$ (observed $[M+H]^+$, *m/z* 471.3386, calcd 471.3474, Appendix 16F). The presence of seven methyl singlets in its ^1H NMR spectrum at δ_H 1.32, 1.20, 1.10, 1.05, 0.91 integrating for 3H each, a methyl at δ_H 0.99 integrating for 6H (Table 4.19, Appendix 16) and that of thirty ^{13}C NMR signals including an oxygenated methine (δ_C 76.2), a quaternary carbon (δ_C 90.5) and two carbonyls (δ_C 217.5 and 179.8) was compatible with a pentacyclic triterpenoid skeleton (Mahato and Kundu, 1994). This presumption was supported by the high similarity of its NMR data to that of the oleanane triterpenoid 3-oxo-11α-12α-epoxy-oleanan-28,13β-olide, which was isolated from *Cedrela montana* (Castellanos *et al.*, 2002). The HMBC correlations (Table 4.19, Appendix 16C) of the carbonyl carbon at δ_C 217.7 to the methyl protons at δ_H 1.10 (Me-23) and δ_H 1.05 (Me-24) and to the methylene protons at δ_H 2.53 and 2.45 (CH$_2$-2) suggests its C-3 position in the triterpenoid backbone. The placement of a hydroxyl group at C-12 (δ_C 76.2) was established following the ^1H-^1H COSY correlation of H-11 and H-12, and the HMBC correlation of H-12 (δ_H 3.91) with C-9 (δ_C 44.0), C-13 (δ_C 90.6) and C-14 (δ_C 42.3). This was further confirmed by the HMBC correlation of H-11 (δ_H 2.06, 1.46) and H-9 (δ_H 1.72) to C-12 (δ_C 76.2). The relative configuration of the C-12-hydroxyl group

was determined based on the NOE observed between H-12 (δ_H 3.91) and H-27 (δ_H 1.32), revealing their *syn* orientation (Figure 4.3, Appendix 16E). This assignment was further supported by the absence of NOE between H-12 (δ_H 3.91) and Me-26 (δ_H 1.20). Small, comparable $^3J_{H12-H11a}$ and $^3J_{H12-H11b}$ indicate the gauche orientation of H-12 to both CH$_2$-11a and CH$_2$-11b and thus its *pseudo*-equatorial orientation. The ^{13}C NMR shifts δ_C 179.9 (C-28) and δ_C 90.6 (C-13) are typical of a 28,13β-lactone moiety (Castellanos *et al*., 2002). It should be noted that so far all naturally occurring triterpenes possessing a 28,13-lactone moiety were reported to have 28,13β-configuration (Ikuta and Morikawa, 1992; Castellanos *et al*., 2002; Hu *et al*., 2012). The relative orientation of the bridgehead methyl groups and protons was elucidated based on their NOE correlations, shown in Figure 4-3. Hence, the absence of NOE between H-18 and H-27, H-5 and H-25, and H-26 and H-27 is diagnostic for their *anti*-orientation. The observed NOEs revealed that H-5, H-9, H-12 and H-27 are α-oriented, whilst H-18, H-25, and H-26 are β-oriented. The assigned relative configuration is in excellent agreement with previous literature reports (Ikuta and Morikawa, 1992; Castellanos *et al*., 2002; Hu *et al*., 2012). On the basis of the above spectroscopic data, the new compound was characterized as 3-oxo-12β-hydroxy-oleanan-28,13β-olide (**198**).

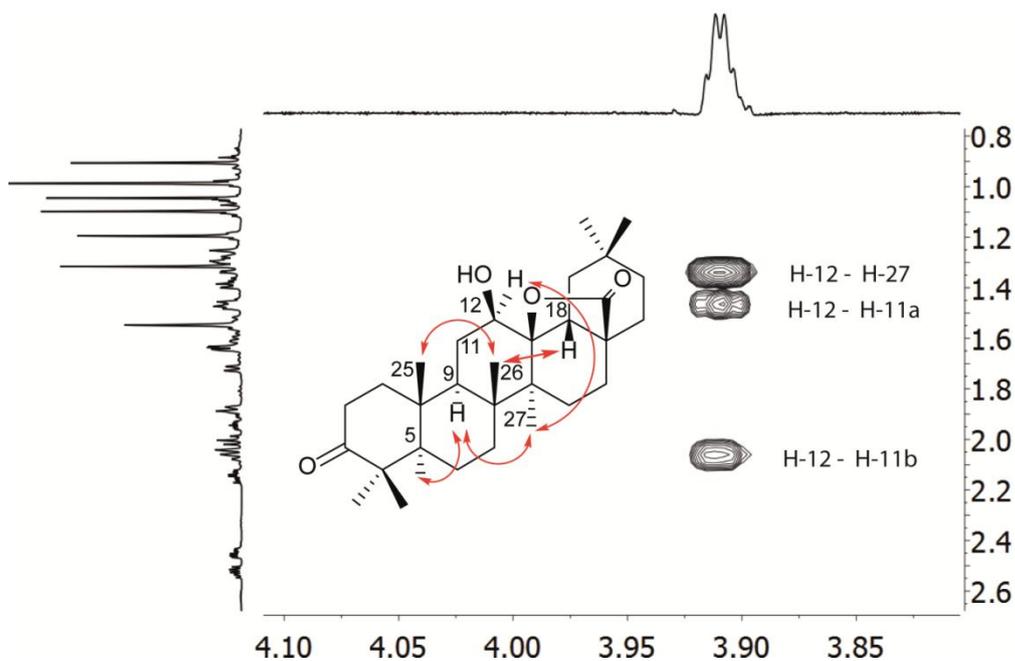**Figure 4-3**: Key NOE correlations observed for compound **198** (mixing time 700 ms, CDCl3, 25 °C, 799.88 MHz) allowing determination of its relative configuration are shown. An expansion of the NOESY spectrum showing the characteristic NOE correlations of H-12 is shown above. The NOE correlation of H-12 and H-27, and the absence of NOE between CH-12 and H-26 indicate the β-orientation of OH-12.

Table 4.19: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **198** in CDCl$_3$.

| Position	δ$_H$ (*J* in Hz)	δ$_C$	HMBC (^2J, ^3J)
1	1.47 *ddd* (7.6, 9.8, 12.5)	39.8	C2, C3, C5, C10, C25
	1.94 *ddd* (4.4, 7.6, 12.5)		
2	2.53 *ddd* (7.6, 9.8, 15.7)	34.1	C1, C3, C4, C10
	2.45 *ddd* (4.4, 7.6, 15.7)		
3		217.7	
4		47.5	
5	1.39 *dd* (2.7, 12.0)	55.0	C4, C6, C7, C9, C10, C23, C25
6	1.55 *m*	19.2	C7, C8, C10, C26
	1.47 *m*		C7, C8, C10, C25, C26
7	1.60 *m*	33.5	C5, C6, C8, C26
	1.30 *m*		C5, C8, C9, C26
8		42.3	
9	1.72 *dd* (2.3, 13.1)	44.0	C1, C5, C8, C10, C11, C25, C26
10		36.3	
11	2.06 *m*	29.3	C8, C9, C10, C12, C13
	1.46 *m*		
12	3.91 *d* (3.3)	76.2	C9, C13, C14
13		90.6	
14		42.3	
15	1.88 *ddd* (2.6, 2.6, 12.4)	28.2	C14, C16, C17, C18, C27
	1.20 *m*		
16	2.14 *ddd* (5.9, 13.3, 13.3)	21.3	C17, C18, C22, C28
	1.29 *m*		
17		44.9	
18	2.05 *dd* (3.5, 13.4)	51.3	C12, C13, C14, C16, C19, C20
19	2.01 *dd* (13.1, 13.4)	39.7	C17, C18, C20, C21, C29, C30
	1.87 *dd* (3.5, 13.1)		
20	-	31.8	
21	1.38 *m*	34.3	C20, C22, C30
	1.27 *m*		
22	1.64 *m*	27.6	C16, C17, C18, C20, C28, C29
23	1.10 *s*	26.7	C3, C4, C5, C24
24	1.05 *s*	21.2	C3, C4, C5, C23
25	0.99 *s*	16.4	C1, C5, C9, C10
26	1.20 *s*	18.4	C7, C9, C13, C14, C27
27	1.32 *s*	18.6	C8, C13, C14, C15
28	-	179.9	
29	0.99 *s*	33.4	C19, C20, C21,C30
30	0.91 *s*	24.0	C19, C20, C21,C29

4.4.1.2. *Ekeberin A (158)*

158
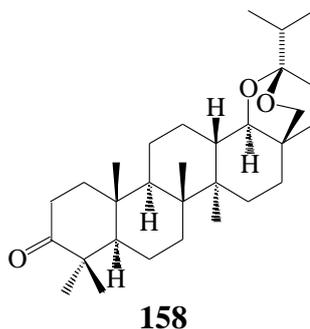
Compound **158** was isolated as needle like crystals. The ^{1}H NMR spectrum (Table 4.20, Appendix 17) indicated seven methyl signals; two doublets at δ_H 0.88 (J = 7 Hz), 0.89 (J = 7 Hz) and five singlets at δ_H 0.90, 0.96, 1.03, 1.06 and 1.07. The ^{13}C NMR spectrum indicated 30 carbon signals including one carbonyl (δ_C 218.1), three oxygenated carbons; quaternary (δ_C 97.6), oxygenated methine (δ_C 78.8) and methylene (δ_C 69.2) compatible with a pentacyclic triterpenoid skeleton. HMBC correlation of CH$_2$-2 (δ_H 2.51; δ_H 2.44), H-1 (δ_H 1.98), Me-23 (δ_H 1.06) and Me-24 (δ_H 1.02) with keto resonance at δ_C 218.1 suggested the position of the latter at C-3 in a pentacyclic skeleton. A carbinol proton at δ_H 3.55 (*dd, J* = 10.3, 1.7 Hz) and its corresponding carbon at δ_C 78.7 was assigned to H-18 following its HMBC correlation with C-12 (δ_C 24.2), C-22 (δ_C 32.8) and C-19 (δ_C 97.6). The assignment of the resonance at δ_C 97.6 to C-19 was confirmed by HMBC correlation of this signal with H-21(δ_H 1.93 *m*, 1.88 *m*), H-20 (δ_H 1.69 *m*) and Me-29 (δ_H 0.89). Relative orientation of H-18 was established by NOE correlation of H-18 with Me-27. The ^{1}H NMR spectrum also indicated downfield shifted double doublets signals [δ_H 4.25 (*dd, J* = 8.5, 3.2 Hz) and δ_H 3.44 (*dd, J* = 8.5, 1.7 Hz)] as shown by their mutual coupling in the ^{1}H-^{1}H COSY spectrum. HMBC correlation of the signals at δ_H 3.44 with δ_C 78.7 (C-18), δ_C 97.6 (C-19) and δ_C 32.8 (C-22) and that of δ_H 4.25 with δ_C 78.7 (C-18) and δ_C 32.8 (C-22) suggested their assignment at CH$_2$-28. The compound was

therefore identified as ekeberin A, first isolated from the stem bark of *E. capensis* (Murata *et al.*, 2008).

Table 4.20: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **158** in DMSO-d$_6$

Position	δ$_H$ (*J* in Hz)	δ$_C$	Position	δ$_H$ (*J* in Hz)	δ$_C$
1	1.45 *m*	39.8	16	1.38 *m*	28.5
	1.98 *m*			1.35 *m*	
2	2.44 *ddd* (11.2, 7.8, 3.9)	34.1	17	-	31.4
	2.51 *ddd* (15.7, 9.5, 7.6)		18	3.55 *dd* (10.29, 1.69)	78.8
3	-	218.1	19	-	97.6
4	-	47.3	20	1.69 *m*	35.4
5	1.34 *m*	55.0	21	1.93 *m*	27.5
6	1.45 *m*	19.5		1.88 *m*	
	1.39 *m*		22	1.64 *m*	32.8
7	1.47 *m*	32.9		1.40 *m*	
8	-	40.7	23	1.07 *s*	26.7
9	1.42 *d* (2.6)	50.3	24	1.03 *s*	21.0
10	-	37.0	25	0.96 *s*	16.3
11	1.35 *m*	21.3	26	1.06 *s*	15.6
	1.53 *m*		27	0.90 *s*	14.3
12	2.03 *ddd* (13.1, 5.4, 2.5)	24.2	28	4.25 *dd* (8.5, 3.21)	69.2
13	1.87 *m*	39.4		3.44 *dd* (8.5, 1.74)	
14	-	41.4	29	0.89 *d* (7)	16.9
15	1.46 *m*	26.4	30	0.88 *d* (7)	17.2
	0.95 *m*				

4.4.1.3. *Oleanolic acid (159)*
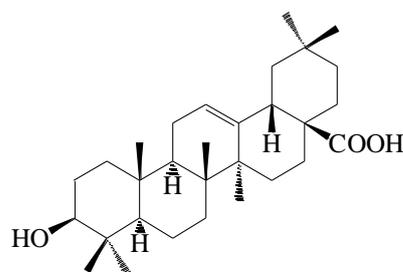
159

Compound **159** was isolated as white crystals. The ^{1}H NMR spectrum (Table 4.21, Appendix 18) indicated the presence of seven tertiary methyl singlets each integrating for three protons at δ$_H$ 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 and 1.13 thirty ^{13}C NMR signals compatible with a pentacyclic triterpenoid skeleton (Seebacher *et al.*, 2003). Two olefinic carbons δ$_C$ 143.6 (C-13) and δ$_C$ 122.7(C-12) with the corresponding vinyl proton appearing at δ$_H$ 5.28 (*d*, *J* = 3.5 Hz) indicated the presence of a C-12/C-13

double bond. HMBC correlation of Me-27 (δ_H 1.13, *s*) with C-13 (δ_C 143.6) confirmed location of the double bond. The presence of a carboxylic acid moiety was established by the presence of carbon resonance at δ_C 182.9 and assigned to C-28 following HMBC correlation of H-18 (δ_H 2.83, *dd*, J = 13.8, 4.5 Hz) with C-28 (δ_C 182.9) while oxymethine at δ_C 79.0 and its corresponding carbinol proton at δ_H 3.24 (H-3 *dd*, J =10, 5 Hz) was assigned to C-3. HMBC correlation of Me-23 and Me-24 with H-3 (δ_H 3.24) confirmed the placement of hydroxyl group to C-3. The relative orientation of hydroxyl group at C-3 was established as equatorial following the large coupling constant between H-3$_{ax}$ and H-2$_{ax}$ (J = 10.0 Hz). NOESY correlation of H-3 (δ_H 3.24) with Me-23, (δ_H 0.98) and Me-26 (δ_H 0.75) showed that they are in the same face (α-orientation) and hence the hydroxyl group is β-oriented. A methine proton at δ_H 2.83 (*dd*, J = 13.8, 4.5 Hz) was assigned to H-18. The relative configuration at C-18 was established through NOE correlation of H-18 (δ_H 2.83) with Me-30 (δ_H 0.93), and H-12 (δ_H 5.28). The ^{13}C NMR assignments were in agreement with literature values reported for oleanolic acid (Mahato and Kundu, 1994; Seebacher *et al*., 2003). However, ^1H NMR for Me-26, Me-30, Me-29 and Me-25 did not corroborate those reported by Seebacher and co-workers (Seebacher *et al*., 2003). HMBC correlation (Figure 4-4) is used here to unequivocally assign the methyls; *vis* correlation of Me-27 (δ_H 1.13) with C-13 (δ_C 143.6); Me-23 (δ_H 0.98) and Me-24 (δ_H 0.77) with C-3 (δ_C 79.0), Me-26 (δ_H 0.75) with C-9 (δ_C 47.6), C-14 (δ_C 41.6) and C-7 (δ_C 33.1); Me-25 (δ_H 0.91) with C-5 (δ_C 55.2), C-4 (δ_C 38.8) and Me-30 and Me-29 with C-20 (δ_C 30.7). The compound oleanolic acid was previously isolated from the stem bark of *E. capensis* (Murata *et al*., 2008).
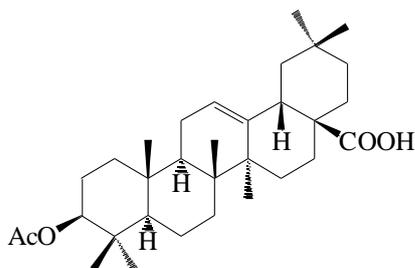
202

Oleanolic acid (**159**) was acetylated to form oleanolic acid acetate (**202**). The [13]C

NMR spectrum (Appendix 19A) displayed 32 carbon signals including an ester

carbon (δ_C 171.3), an acetyl methyl group resonance at δ_C 21.6 and a two ppm down-

field shift (δ_C 81.3) for C-3. The ^{1}H NMR indicated a deshielded proton resonance at

δ_H 4.45 corresponding to H-3. All the other resonances were comparable to those of

159.

Table 4.21: [1]H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **159** in
DMSO-d$_6$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1α	0.98 *m*	38.4	16	1.90 *m*	23.4
β	1.63 *m*			1.60 *m*	
2	1.61 *m*	27.2	17	-	46.5
	1.56 *m*		18	2.83 *dd* (13.8, 4.5)	41.0
3	3.24 *dd* (11.6, 4.3 Hz)	79.0	19α	1.63 *m*	45.9
4	-	38.8	β	1.17 *m*	
5	0.74 *s*	55.2	20	-	30.7
6α	1.57 *m*	18.3	21	1.24 *m*	33.8
β	1.39 *m*			1.34 *m*	
7	1.24 *m*	33.1	22	1.78 *m*	32.4
8	-	39.3		1.60 *m*	
9	1.53 *m*	47.6	23	0.98 *s*	28.1
10	-	37.1	24	0.77 *s*	15.6
11α	2.00 *m*	22.9	25	0.91 *s*	15.3
β	1.63 *m*		26	0.75 *s*	17.1
12	5.28 *d* (3.5)	122.7	27	1.13 *s*	25.9
13	-	143.6	28	-	182.9
14	-	41.6	29	0.90 s	32.6
15α	1.08 *m*	27.7	30	0.93 *s*	23.6
β	1.72 *m*				

Table 4.22: Key ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR resonances for compound **159** (DMSO-d$_6$) and **202** (in CD$_2$Cl$_2$)

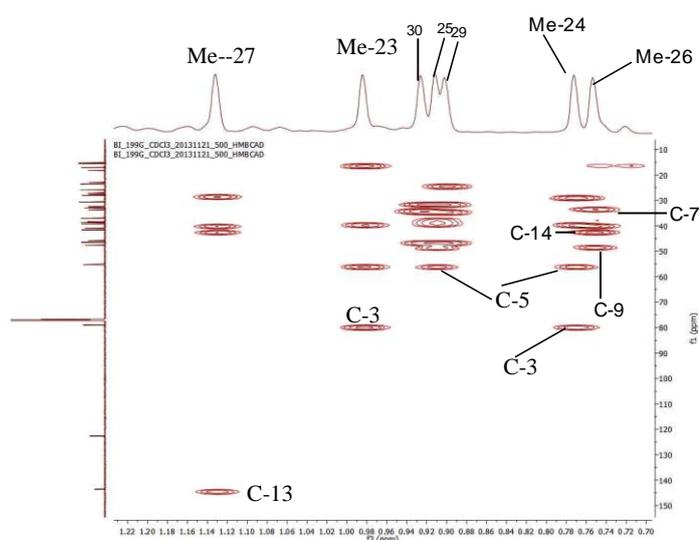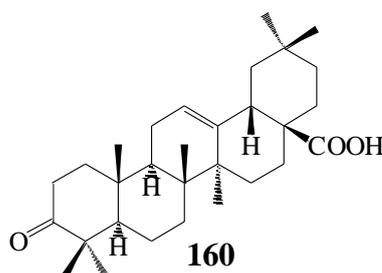|	202			159	
	δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)	δ_C
3	4.47 *m*	81.3	3	3.24 *dd* (11.6, 4.3 Hz)	79.0
12	5.27 t (3.7)	123.1	12	5.28 *d* (3.5)	122.7
13	-	144.3	13	-	143.6
28	-	184.2	28	-	182.9
Ac(CO)	-	171.3		-	-
Ac(Me)		21.6		-	-

Figure 4-4: Key HMBC correlation of Compound **159** methyl groups

4.4.1.4. *Oleanonic acid (160)*

Compound **160** was isolated as white crystals, melting point 153-154°C. ESI-MS (30 eV) indicated [M+H]$^+$ at *m/* 455.4 (Appendix 20B). The ^{13}C NMR spectrum (Table 4.23, Appendix 20) indicated presence of two carbonyls [δ_C 216.2 (C-3), 178.4 (C-28)] and two olefinic carbons [δ_C 121.2 (C-12) and 143.6 (C-13)]. The ^{1}H NMR

spectrum indicated presence of seven tertiary methyls at δ_H 0.77, 0.94, 0.97, 1.00, 1.11(3H each) and 0.88 (6H), olefinic proton; δ_H 5.19 (H-12, *s*) and a methine proton at δ_H 2.75 (H-18, *dd*, *J* = 14, 4.5, Hz). These data are compatible with oleanane type pentacyclic triterpenoid skeleton (Mahato and Kundu, 1994). The ketone group was assigned to C-3 following HMBC correlation of Me-23 (δ_H 1.00) and Me-24 (δ_H 0.94) with δ_C 216.2. Using 1D, 2D NMR and comparison with literature information the compound was identified as oleanonic acid (Kwon *et al*., 1997).

Table 4.23: [1]H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **160** in DMSO-d$_6$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	1.77 *ddd* (11.8, 7.4, 3.8)	38.9	15	1.66 *m*	27.0
	1.38 *m*			1.09 *m*	
2a	2.30 *ddd* (15.9,7.1, 3.7)	34.1	16	1.87 *m*	23.5
b	2.48 *ddd* (15.8, 7.0, 3.7)		17	-	46.4
3	-	216.2	18	2.75 *dd* (14, 4.5)	41.3
4	-	48.4	19	1.04 *m*	45.9
5	1.32 *m*	54.1	20	-	30.2
6	1.43 *m*	20.9	21	1.13 *m*	33.5
	1.29 *m*			1.32 *m*	
7	1.44 *m*	31.9	22	1.62 *m*	32.6
	1.29 *m*			1.32 *m*	
8	-	38.6	23	1.00 *s*	26.1
9	1.62 *m*	46.4	24	0.94 *s*	21.5
10	-	36.1	25	0.97 *s*	14.5
11a	1.91 *m*	22.8	26	0.77 *s*	16.5
b	1.50 *m*		27	1.11*s*	25.3
12	5.19 *s*	121.2	28	-	179.4
13	-	143.6	29	0.88 *s*	33.1
14	-	41.9	30	0.88 *s*	23.1

4.4.1.5. *3-epi- Oleanolic acid (**161**)*
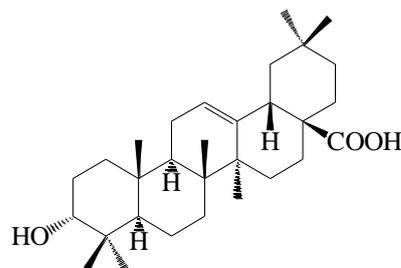

161

Compound **161** was isolated as a white amorphous powder. The ^{1}H NMR spectrum (Table 4.24, Appendix 21) indicated the presence of seven methyl signals all of which attached to saturated carbons at δ_H, 0.71, 0.75, 0.83, 0.85, 0.86 (6H) and 1.09; olefinic proton at δ_H 5.07 (H-12, s); a methine at δ_H 2.77 (H-18, *dd*, J = 15.0, 5.0 Hz) and a carbinolic proton δ_H 3.17 (H-3, *s*). In the ^{13}C NMR spectrum two olefinic carbons; δ_C 121.0 (C-12), 144.6 (C-13), a carbony carbon; 178.2 (C-28) and an oxymethine carbon δ_C 73.8 (C-3) were observed. Both the ^{1}H and ^{13}C NMR data are consistent with an oleanane type triterpene (Mahato and Kundu, 1994; Kwon *et al*., 1997). The position of the double bond at C-12/C-13 was established by HMBC correlation of H-18 (δ_H 2.77, *dd*, J = 15.0, 5.0 Hz) with C-13 (δ_C 144.6). Relative configuration at C-3 was determined by NOE correlation of H-3 (δ_H 3.17, *s*) with Me-24 (δ_H, 0.75, *s*) allowing the placement of the hydroxyl group in α-orientation. This is in agreement with the ^{13}C chemical shift value of C-3 (δ_C 73.8), which is expected to be shielded (δ_C less than δ_C 76) for axially oriented OH (Mahato and Kundu, 1994). This compound is a stereoisomer of compound **159** where they differ in the relative configuration of hydroxyl group at C-3. Compound **159** (β-OH) has H-3 in axial position as evidenced by the large coupling constant (δ_H 3.24, *dd*, J = 11.6, 4.3 Hz) while H-3 in **161** (α-OH) is in equatorial position (δ_H 3.17 *brs*). These data compared well with that of 3-*epi*-oleanolic acid. The compound has previously been isolated from the stem bark of *E. capensis* (Sewram *et al*., 2000; Murata *et al*., 2008).

Table 4.24: [1]H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **161** in DMSO-d$_6$

Position	δ$_H$ (*J* in Hz)	δ$_C$	Position	δ$_H$ (*J* in Hz)	δ$_C$
1	1.58 *m*	32.5		1.82 *m*	
	1.36 *m*		17	-	46.1
2	1.34 *m*	25.2	18	2.77*dd* (15, 5)	41.0
3	3.17 *brs*	73.8	19	1.02 *m*	45.5
4	-	36.9		1.59 *m*	
5	1.12 *m*	48.3	20	30.5 *m*	
6	1.35 *m*	17.8	21	1.40 *m*	33.6
	1.26 *m*			1.59 *m*	
7	1.15 *m*	32.3	22	1.18 *m*	32.7
8	-	39.8	23	0.83 *s*	28.7
9	1.21 *m*	46.9	24	0.75 *s*	22.3
10	-	36.7	25	0.85 *s*	15.0
11	1.45 *m*	22.9	26	0.71 *s*	17.0
12	5.08 *m*	120.0	27	1.09 *s*	25.7
13	-	144.6	28	-	178.8
14	-	41.4	29	0.86 *s*	33.0
15	1.70 *m*	27.3	30	0.86 *s*	23.6
16	1.48 *m*	23.5			

4.4.2. *Acyclic triterpenoids from the root bark of Ekebergia capensis*

4.4.2.1. *2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (151)*
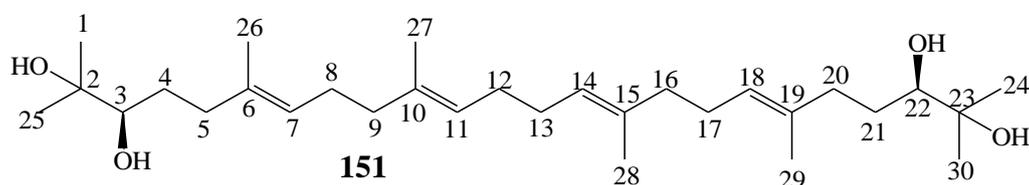

Compound **151** was isolated as yellowish oil with ESI-MS (30 eV) indicating an [M+H]$^+$ peak at *m/z* 479.4 (Appendix 23B). The ^{13}C NMR spectrum (Table 4.25, Appendix 23A) indicated 15 carbons, including four olefinic carbons (δ$_C$ 135.1, 134.9, 125.1, 124.6) and two oxygenated carbons (δ$_C$ 78.63; 73.63). This compound was predicted to be symmetrical acyclic triterpene and hence, only one half of the carbons (15) were observed in the ^{13}C NMR (Nishiyama *et al.*, 1996). ^{1}H NMR spectrum indicated presence of two olefinic protons each integrating for two protons (δ$_H$ 5.17, H-7, H-18, *t*, *J* = 6.4 Hz) ; (δ$_H$ 5.13 H-11, H-14, *s*); a carbinolic proton signal (δ$_H$ 3.33 H-3, H-22, *dd*, *J* =10.5, 1.9 Hz integrating for 2 protons); eight methyls of which

four [δ_H 1.13 (*s*, 6H); 1.18 (*s*, 6H)] attached to an *sp³* and the remaining four [δ_H 1.60 (*s*, 6H), δ_H 1.58 (*s*, 6H) attached to an *sp²* carbon. The ¹H and ¹³C NMR were carefully assigned using HSQC and HMBC. Consequently the molecule was elucidated as 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,15,19,23-tetracosatetraene previously isolated from the stem bark of *E. capensis* (Nishiyama *et al*., 1996).

Table 4.25: ¹H (500 MHz) and ¹³C (125 MHz) NMR data for compound **151** in CDCl₃

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	1.13 *s*	26.5/23.4	16	1.98 *m*	39.8
2	-	73.2	17	2.09 *m*	26.7
3	3.33 *dd* (10.5, 1.9)	78.4	18	5.17 *t* (6.4)	125.1
4	1.39 *m*	29.9	19	-	135.1/134.9
	1.55 *m*		20	2.26 *m*	36.9
5	2.26 *m*	36.9	21	1.39 *m*	29.9
	2.06 *m*			1.55 *m*	
6	-	135.1/ 134.9	22	3.33 *dd* (10.5, 1.9)	78.4
7	5.17 *t* (6.4)	125.1	23	-	73.2
8	2.09 *m*	26.7	24	1.13 *s*	26.5/23.4
9	1.98 *m*	39.8	25	1.18 *s*	26.5/23.4
10	-	135.1/134.9	26	1.60 *s*	16.1
11	5.13 *s*	124.6	27	1.58 *s*	16.06
12	2.00 *m*	28.3	28	1.58 *s*	16.06
13	2.00 *m*	28.3	29	1.60 *s*	16.1
14	5.13 *s*	124.6	30	1.18 *s*	26.5/23.4
15	-	135.1/134.9			

4.4.2.2. *2-Hydroxymethyl-2,3,22,23-tetrahydroxy-2-6,10,15,19,23-pentamethyl-6,10,14,18 tetracosatetraene* (**152**)

152
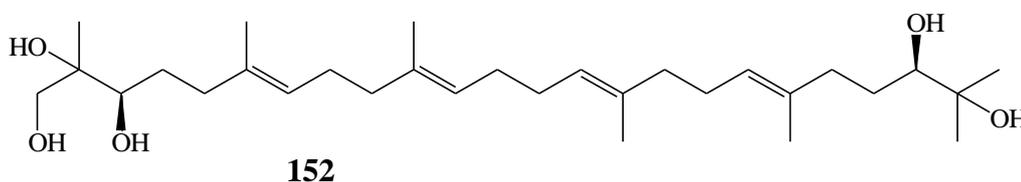
Compound **152** was isolated as an oil. ESI-MS (30 eV) indicated an [M+H]⁺ at *m/z* 495.7 (Appendix 22B). The ¹H NMR spectrum (Table 4.26, Appendix 22) showed the presence of an olefinic proton [δ_H 5.05 integrating for four protons], three methyl

groups [δ_H 0.94 (Me-1), 0.97 (Me-24), 1.00 (Me-30) attached to an sp^3 carbon] and a singlet integrating for 4 methyls [δ_H 1.50 s (Me-26,27,28,29) attached to an sp^2 carbon]. The acyclic triterpene nature of the compound was shown by the ^{13}C NMR spectrum which displayed thirty carbon signals including four olefinic quaternary carbons (δ_C 135.4, 135.4, 134.9, 134.8) and four vinyl methines (δ_C 124.23, 124.2, 123.8, 123.8) (Nishiyama *et al*., 1996). Also present were two oxygenated quaternary carbons [δ_C 74.3 (C-2), 72.2 (23)] and two oxymethines [δ_C 74.4 (C-3), 77.5 (C-22)] whose corresponding carbinol proton (2H) appeared at δ_H 3.23 (H-3, H-22, *dd*, $J = 15$, 5 Hz). An oxymethylene at δ_H 67.0 (C-25) and corresponding AB type protons at δ_H 3.33 (CH$_2$-25a, *d*, $J = 10.4$ Hz) and δ_H 3.04 (CH$_2$-25b, *d*, $J = 10.2$ Hz) were also observed. Placement of oxymethylene to C-25 was confirmed by HMBC correlation of CH$_2$-25 (δ_H 3.33; 3.04) with C-2 (δ_C 74.3) and C-1 (δ_C 20.1). The rest of the signals were carefully assigned by use of HMBC and HSQC spectra. The structure was therefore identified as 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene previously isolated from the stem bark of *E. capensis* (Nishiyama *et al*., 1996).

Table 4.26: [1]H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **152** in DMSO-d$_6$

Position	δ$_H$ (*J* in Hz)	δ$_C$	Position	δ$_H$ (*J* in Hz)	δ$_C$
1	0.90 *s*	20.9	16	1.88 *m*	39.1
			17	1.97 *m*	26.5/26.4
2	-	74.3	18	5.04 *brs*	124.23
3	3.23 *dd* (15, 5)	74.4	19	-	135.4
4	1.23 *m*	29.6	20	2.11 *m*	36.9
5	2.11 *m*	36.7		1.83 *m*	
	1.83 *m*		21	1.16 *m*	29.9
6	-	134.8	22	3.23 *dd* (15, 5)	77.5
7	5.05 *brs*	124.2	23	-	72.2
8	1.97 *m*	26.5/26.4	24	0.97 *s*	25.0
9	1.88 *m*	39.0	25	3.33 *d* (10.8)	67.0
				3.04 *d* (10.8)	
10	-	135.4/134.9	26	1.50 *s*	16.2/16.2
11	5.04 *brs*	123.8			
12	1.90 *m*	28.1	27/3	1.50 *s*	16.1/16.1
13	1.90 *m*	28.1	29	1.50 *s*	16.2/16.2
14	5.04 *brs*	123.8	30	1.00 *s*	25 .9
15	-	135.4/134.9			

4.4.3. *Flavonol glycosides from the leaves of Ekebergia capensis*

4.4.3.1. *Kaemferol-3-O-β-D-glucopyranoside (199)*
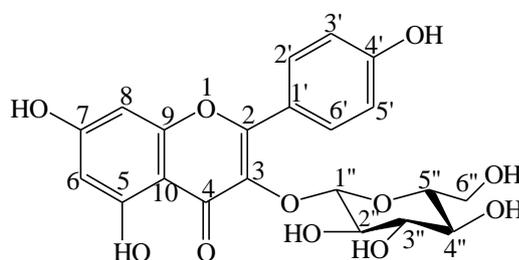
199
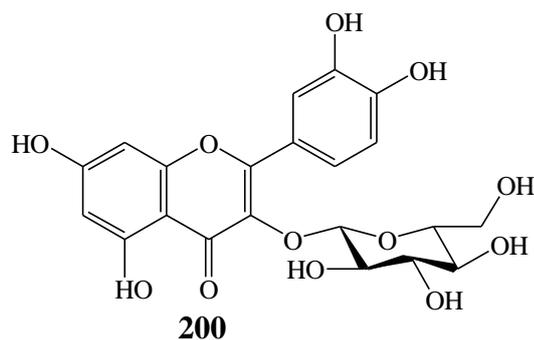
Compound **199** was isolated as a yellow amorphous powder. Based on ^{1}H and ^{13}C NMR spectra as well as ESI-MS (30 eV) which indicated [M+H]$^+$ at *m/z* at 449.1 and a base peak at 287.2 [M+H]$^+$ (Appendix 24B) the molecular formular C$_{21}$H$_{20}$O$_{11}$ was assigned for this compound. The ^{1}H and ^{13}C NMR spectra (Table 4.27, Appendices 24A and B) revealed signals similar to those of the flavonol kaempferol (Kim *et al*., 2004). Hence, two aromatic protons at δ$_H$ 6.41 (*s*) and δ$_H$ 6.20 (*s*) were assigned to H-8 and H-6 respectively, of ring A which is oxygenated at C-5 (OH, due to a broad

singlet at δ_H 12.61) and C-7, as expected biogenetically. The presence of four protons

showing an AA'XX' pattern at δ_H 8.04 (2H, d, $J = 8.5$ Hz) for H-2'/H-6' and δ_H 6.88

(2H, d, $J = 8.5$ Hz) for H-3'/H-5' is typical of 4'-oxygenated ring B. The presence of

a sugar moiety was evident from the ^{13}C NMR spectrum which indicated six

oxygenated signals at δ_C 101.3 (C-1''), 78.0 (C-5''), 76.9 (C-3''), 74.7 (C-2''), 70.4

(C-4'') and 61.3 (C-6''). The anomeric proton (H-1'') resonated at δ_H 5.46 (d, $J = 7.9$

Hz), the large coupling constant of which is consistent with β-orientation of the

aglycone. Attachment of the sugar moiety at C-3 was confirmed by HMBC

correlation of H-1'' (δ_H 5.46) with C-3 (δ_C 133.6). Resonances at δ_H 3.56– 3.08 ppm

were due to protons H-2''- H-6'' of the sugar moiety. The sugar moiety was identified

as glucopyranose. By the use of ^{1}H and ^{13}C NMR, HMBC, HSQC, ^{1}H-^{1}H COSY

consequently the compound was elucidated as kaemferol 3-O-$β$-D-glucopyranoside

previously isolated from *Eucomnia ulmoides* (Kim *et al*., 2004).

Table 4.27: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **199** in CDCl$_3$

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1	-		2'6'	8.04 d (8.5)	131.3
2	-	156.7	3'5'	6.88 d (8.5)	115.6
3	-	133.6	4'	-	160.4
4	-	177.9	1''	5.46 d (7.2)	
5	-	161.7	2''	3.17 s	
6	6.20 s	99.2	3''	3.21 s	101.3
7	-	164.7	4''	3.08 s	74.7
8	6.42 s	94.1	5''	3.08 s	76.9
9	-	156.9	6''	3.56 m	70.4
10	-	104.4		3.13 m	78.0
1'	-	121.4			61.3

4.4.3.2. *Quercetin- 3-O-β-D-glucopyranoside (200)*

200

Compound **200** was isolated as a yellow amorphous powder from the aerial parts of *E. capensis.* ESI-MS (30 eV) which indicated $[M+H]^+$ at *m/z* 465.1 (Appendix 25B) together with the ^1H and ^{13}C NMR (Table 4.28, Appendices 25 and 25A) molecular formular $C_{21}H_{20}O_{12}$ is proposed. This compound is also a flavone glycoside, where a base peak appeared at *m/z* 303.3 $[M+H]^+$ is due to the loss of sugar moiety. The ^1H NMR spectrum showed signals at δ_H 12.61 due to hydroxyl group at C-5; two *meta* coupled protons at δ_H 6.40 (*d*, *J* = 2.03 Hz) and 6.20 (*d*, *J* = 2.03 Hz) assigned to H-8 and H-6 of ring A of flavones with the biogenetic oxygenation at C-5 and C-7. In ring B, the ^{13}C NMR spectrum is consistent with oxygenation at C-3' and C-4' as in quercetin (Sanbongi *et al*., 1998). In agreement with this, aromatic signals at δ_H 7.58 (*m*), 7.57 (*m*) and 6.84 (*d*, *J* = 10 Hz) corresponded to H-6', H-2' and H-5' respectively of ring B. Overall these resonances together with those of ^{13}C NMR are consistent with the aglycone part of this compound to be quercetin (Sanbongi *et al*., 1998). The presence of additional signals in the ^1H NMR spectrum in the range δ_H 5.46 -3.08 ppm indicated presence of a sugar moiety. This was supported by presence of six oxygenated carbons at δ_C 100.8, 77.6, 76.5, 74.1, 69.3 and 61.0. The sugar was established to be *β*-D- glucopyranoside attached to C-3 of the aglycone. This attachment was confirmed by HMBC correlation of H-1" (δ_H 5.46) with C-3 (δ_C 133.3). The β-orientation was indicated by the large coupling constant of the anomeric

proton H-1" (δ_H 5.46 *d*, *J* =7 .4 Hz) (Slimestad *et al*., 1995). This compound was elucidated as quercetin-3-*O-β*-D-glucopyranoside. NMR data was in agreement with that reported for the same compound isolated from *Picea abies* (Slimestad *et al*., 1995).

Table 4.28: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **200** in CDCl$_3$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1			2'	7.57 *m*	116.2
2	-	156.2	3'	-	144.8
3	-	133.3	4'	-	148.5
4	-	177.4	5'	6.84 *d* (10.0)	115.2
5	-	161.2	6'	7.58 *m*	121.6
6	6.20 *d* (2.0)	98.6	1"	5.46 *d* (7.4)	100.8
7	-	164.1	2"	3.22 *m*	74.1
8	6.40 *d* (2.0)	93.5	3"	3.22 *m*	76.5
9	-	156.3	4"	3.08 *s*	69.9
10	-	104.0	5"	3.08 *s*	77.6
1'	-	121.2	6"	3.58 *dd* (11.9, 4.0)	61.0
				3.33 *m*	

4.4.4. *A Limonoid from the leaves of Ekebergia capensis*

4.4.4.1. *Proceranolide (148)*

148
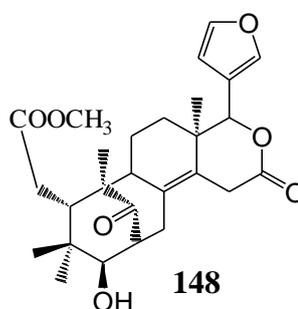
Compound **148** was isolated as a yellowish gum. ESI-MS (30 eV) indicated [M+H]$^+$ at *m/z* 471.9 (Appendix 28B). The ^{1}H and ^{13}C NMR (Tables 4.29, Appendix 28B) indicated characteristic resonances associated with a furan ring δ_H 7.56 (*m,* H-21), 6.49 (*d*, H-22, *J* = 2.3 Hz) and 7.39 (*m,* H-23) and the corresponding carbon signals at δ_C 142.0, 110.4 and 142.9 respectively. Furthermore the presence of carbomethoxy ester at δ_H 3.70 (OMe), δ_C 52.1 (OMe); 174.66 (C-7) and four tertiary methyl groups at δ_H 1.13, 1.03, 0.81 and 0.73 characteristic of mexicanolide class of limonoids (Coombes *et al*., 2005) were also observed. Other notable features were hydroxyl

bearing methine at δ_H 3.74 (H-3) and a lactone δ_H 5.58 (H-17) and the corresponding carbon resonances appearing at δ_C 77.5 and 80.5 respectively. Two quaternary olefinic carbons [δ_C 128.5 (C-8); 131.7 (C-14)] and two carbonyl signals [δ_C 220.1 (C-1); δ 171.73, (C-16)] also featured. Using both 1D and 2D NMR spectra the compound was identified as proceranolide previously isolated from the seeds of *Swietenia mahagoni* and the stem bark of *E. capensis* (Kadota *et al*., 1990; Murata *et al*., 2008).

Table 4.29: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **148** in CDCl$_3$

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	-	218.9	13	-	38.2
2	3.05 *ddd* (10.7, 6, 2.7)	50.3	14	-	131.7
3	3.74 *m*	77.5	15	4.05 *dt* (21.4, 2.1)	33.4
				3.47 *dt* (2.9, 21.3)	
4	-	39.6	16	-	171.7
5	3.24 *dd* (11.4, 2.7)	39.6	17	5.58 *s*	80.5
6	2.34 *m*	33.9	18	1.03 *s*	17.9
	2.38 *m*		19	1.13 *s*	17.2
7	-	174.7	20	-	121.1
8	-	128.5	21	7.56 *m*	142.0
9	1.97 *m*	52.3	22	6.49 *d* (2.3)	110.4
10	-	53.9	23	7.39 *s*	142.9
11	1.78 *m*	19.1	28	0.81 s	24.1
	1.80 *m*		29	0.73 s	20.5
12	1.79 *m*	28.9	30	1.97 *m*	33.6
	1.04 *m*			3.19 *dd* (14.5, 3)	
			OMe	3.70 *s*	52.1

4.5. Antiplasmodial and cytotoxicity activities

The crude extracts from *Turraea nilotica*, *Turraea robusta* and *Ekebergia capensis* were evaluated for antiplasmodial activity against chloroquine resistant (W2) and chloroquine sensitive (D6) *Plasmodium falciparum* strains and also for cytotoxicity against three mammalian cell lines, African green monkey kidney (vero), mouse breast cancer (4T1) and human larynx carcinoma (HEp2). Results are as shown in Table 4.30. Three out of the six extracts tested were classified as active (IC$_{50}$ < 10 µg/ml) with *Turraea robusta* stem bark displaying highest antiplasmodial activity

with IC_{50} values of 2.8±0.02 µg/ml and 2.3±0.05 µg/ml against W2 and D6 respectively. Comparing the IC_{50} values of *T. robusta* (SB) and *T. nilotica* (SB and RB) to those of fruits of *Azadirachta indica* (1.92 µg/ml against W2), a plant that has had a wider use in traditional medicine against malaria, it can be said that these extracts have promising results (Chianese *et al*., 2010). Extracts from *T. robusta* (SB) and *E. capensis* (RB) had promising cytotoxic activities when compared to those reported for other plants such as *Phellinus rimosus, Holarrhena floribunda* and *Warbugia ugandensis* (Ajith and Jonardhanan, 2003; Fotie *et al*., 2006; Irungu *et al*., 2007).

Table 4.30: Antiplasmodial and cytotoxic activities of selected plant parts

	IC_{50} (µg/ml)				
Plant part	**W2**	**D6**	**Vero**	**4T1**	**Hep2**
Turraea robusta (SB)	2.8±0.02	2.3±0.05	21.9±2	5.3±0.6	4.2±1
Turraea nilotica (SB)	7.3±0.05	6.9 ± 0.02	17.70 ±1.3	ND	22.4±4.4
Turraea nilotica (RB)	9.5±1.0	7.9±1	13.7±2	18.6±1.2	27.2±3.6
Turraea nilotica (L)	59.0±3.6	47.4±3	21.5±2	39.1±4	37.4±1.3
Ekebergia capensis (RB)	34.0±2.2	18.2±0.1	2.8±0.1	9.3±0.1	3.4±0.9
Ekebergia capensis (SB)	45.3±2.7	44.9±0.8	97.8±0.8	82.1±5.7	48.6±5.3
Chloroquine	[a]108.0±0	[a]7.7±0.02	43.9±0.5	5701	

Legend: ND; not done, SB; stem bark; RB; root bark, L; leaves, [a]IC_{50}: half maximal inhibitory concentration given in nM for chloroquine: The mean values of at least three independent experiments are reported. Positive control: podophyllum resin, IC_{50} (4T1) = 0.47 ± 0.05 µg/mL; Melarsoprol IC_{50} (Vero) = 0.76 ± 0.01 µg/mL

Previous studies have revealed the antiplasmodial potency of some triterpenoids and limonoids (Murata *et al*., 2008; Ngouamegne *et al*., 2008; Tan and Luo, 2011). Thus, some of compounds isolated in this work were tested for their antiplasmodial activity against D6 and W2. Activity was scored according to the classification of Batista and co-workers (Batista *et al*., 2009). As shown in Table 4.31, of the nineteen compounds tested, two showed good activity with the epimeric mixture azadironolide (**192**), having the best activity (IC_{50} values less than 2.5 µM) while the rest of the compounds had moderate activities against the two strains. The activity of this

compound was comparable to that reported for gedunin (**204**) and neemfruitin A (**205**) triterpenoids from Meliaceae family reported to have good antiplasmodial with IC_{50} values less than 3 μM (Chianese *et al*., 2010). This compound warrants further studies to evaluate it potential as an antimalarial drug lead in an animal model. None of the tested compounds had an activity comparable to that of the standard drug chloroquine whose activity is in the nanomolar range. Due to the low isolated amount, the bioactivity of new compound (**198**) was not evaluated. The antiplasmodial activity of compounds **160** and **19** has previously been reported and the data generated here is comparable with the literature reports (Suksamrarn *et al*., 2003; Chianese *et al*., 2010).

The isolated compounds were also tested for their cytotoxicity against the mammalian cell line African green monkey kidney (vero cells). Most of the compounds had moderate cytotoxicity ($IC_{50} > 20$ μM). However, selectivity index, $[IC_{50}(vero)/IC_{50}(D6)]$ for most of the compounds was low (SI < 10). This indicates that the moderate antiplasmodial activity observed for most of the compounds is probably due to cytotoxicity rather than activity against the parasite themselves. Notably, compounds **62** and **192** classified as active, had high selectivity index (SI) of 25 and >10.5 respectively. It is worth noting that though the cytotoxicity against vero cells for oleanonic acid (**160)**, 3-*epi*-oleanolic acid (**161**), 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl 6,10,14,18-tetracosatetraene (**152**) and hispidol B (**96**) was comparable to that of chloroquine it has a high selectivity for malaria parasite with a selectivity index of 5702.
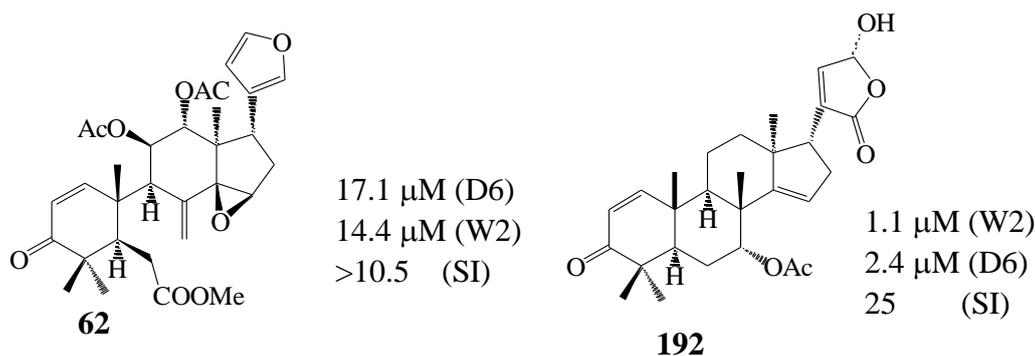
Figure 4-5: Compounds classified as active with IC_{50}'s and SI values
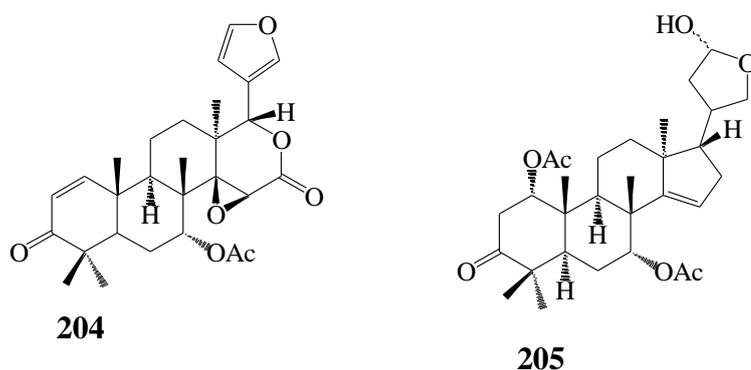
Figure 4-6: Triterpenoids reported to have good antiplasmodial activity (Chianese *et al*., 2010)

Some triterpenoids and limonoids isolated from other plants were previously reported to possess substantial cytotoxicity (Kim *et al*., 2010; Tan and Luo, 2011). Hence, the high to moderate cytotoxic activities of the crude extracts and isolated compounds against 'normal' cell line (vero) motivated the evaluation of their cytotoxicity activities against cancerous cell lines 4T1, HEp2 and MDA-MB-231. The results are as summarized in Table 4.31. Compounds **25**, **27**, **19**, **160** and **192** (Figure 4.7) had high cytotoxicity against 4T1 and HEp2 cell lines with IC_{50} values of less than 20 μM. None of the tested compounds was cytotoxic against MDA-MB-231. Of interest, these compounds had low to moderate cytotoxicity against 'normal cells', whereas cytotoxicity against 4T1 and HEp2 was high. This indicates some degree of selectivity. These compounds may be promising anticancer drug leads. Anticancer

drugs should demonstrate inhibitory activity and specificity for cancer cells without causing excessive damage to normal cells (Diantini *et al*., 2012).

19
14.4 μM (4T1)
12.8 μM (HEp2)

25
14.5 μM (4T1)
6.9 μM (HEp2)

27
8.0 μM (4T1)
8.4 μM (HEp2)

96
7.4 μM (HEp2)

160
13.3 μM (4T1)
2.3 μM (HEp2)

192
14.7 μM (4T1)
8.5 μM (HEp2)
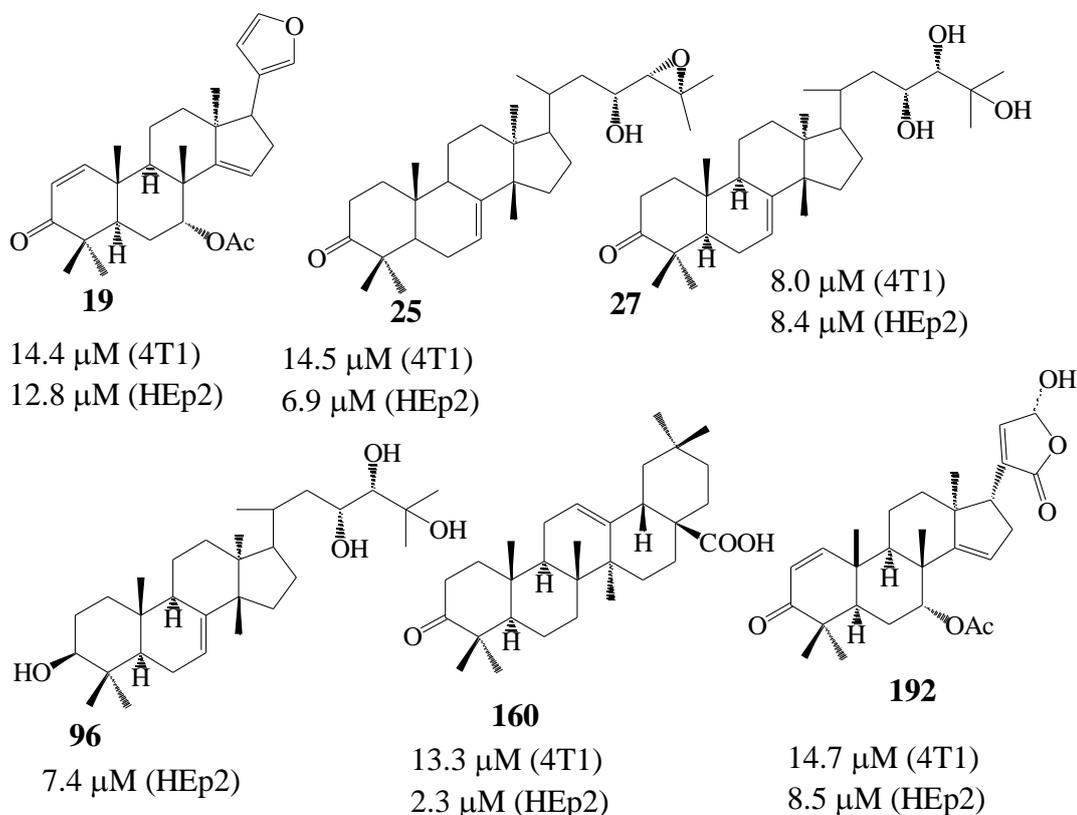
Figure 4-7*:* Compounds cytotoxic to 4T1 and HEp2 cell lines

Compound **159** and **161** are stereoisomers differing in relative stereochemistry of hydroxyl group at C-3. This difference appears to affect their cytotoxicity. Compound **160** (α-OH) had higher cytotoxicity than **158** (β-OH) against the four cell lines tested.

Ester derivatives (**201** and **203**) were of lower cytotoxicity when compared to the parent molecules. Infact, compound **201** was 18 folds less active than the parent molecule (**25**). This loss of activity may suggest that the pharmacophoric portion is probably in the side chain. However, more structural activity evaluations are required before drawing a conclusion on this observation. Activity of oleanolic acid acetate (**202**) increased by seven folds in comparison to the parent molecule (**159**). This

observation is in agreement with previous reports that have shown that oleanolic acid derivatives have a higher activity activity than the parent molecule (Astudillo *et al.*, 2002; Bednarczyk–Cwynar *et al.*, 2012).

Table 4.31: Antiplasmodial and cytotoxity activity of isolated triterpenes and limonoids

Compound	IC50 (µM)						
	D6	W2	Vero cells	SI	4T1	HEp2	MDA-MB-231
azadironolide (**192**)	2.4 ±0.05	1.1±0.01	27.6±0.6	25	14.7±0.2	8.5±0.5	ND
11-*epi*-toonacilin (**62**)	17.1±0.2	14.4±0.5	>180.5	>10.5	88.6±3.2	68.1±1.3	ND
azadirone (**19**)	23.4±0.2	29.6±1.0	>229.4	>9.8	14.4±0.01	12.8±0.02	ND
2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene(**152**)	27.1±0.4	66.9±0.6	35.7±2.1	1.3	30.2±1.3	19.0 ±0.8	74±0.04
12α-acetoxy-7-deacetylazadirone (**28**)	31.0±0.2	30.2±0.5	134.1±2.9	4.3	104.6±7.1	40.3±2.2	ND
mzikonone (**17**)	36.6±0.8	40.5±3.7	139.6±4.7	3.8	38.8±0.4	59.3±1.0	69.2±0.03
hispidol B (**96**)	36.8±2.0	37.2±3.2	130±3.1	3.5	21.7±3.2	7.4±0.7	>210.1
piscidinol A (**27**)	37.6±1.4	36.3±4.4	41.1±5.8	1.1	8.0±0.03	8.4±0.01	97.2±0.03
oleanonic acid (**160**)	38.8±0.5	76.7±4.0	35.8±1.3	0.9	13.3±0.2	1.4±0.1	82±0.5
quercetin-3-*O*-β-D-glucopyranoside (**200**)	42.9±0.3	105.8±1.0	>216	>5.0	>216	>216	> 216
niloticin (**25**)	48.2±2.3	77.0±5.7	14.5 ± 0.4	0.3	14.5±0.5	6.9±0.6	ND
oleanolic acid (**159**)	49.6±2.3	82.7±2.0	112.0±5.1	2.3	117.6±2.6	90.2±0.7	87±0.03
2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**151**)	56.1±0.4	64.3±1.0	24.7±1.8	0.4	22.5±3.2	19.0±3.1	> 209
niloticin acetate (**201**)	68.3±5.3	172.9±4.5	65.5±0.4	1.0	116.5±0.01	121.9±0.08	ND
proceranolide (**148**)	84.7±0.8	150.2±3.0	>213	>2.5	>213	>213	n.d.
kaempferol-3-O-β-D-glucopyranoside (**199**)	97.1±1.0	105.8±0.5	>223	>2.3	>223	>223	> 223
ekeberin A (**158**)	182.2±6.0	>219	>219	>1.2	163.2±4.3	>219	n.d.
3-*epi*-oleanolic acid (**161**)	205.0±3.0	179.4±6.0	58.0±5.2	0.3	30.3±2.6	29.8±0.3	80±0.02
oleanolic acid acetate (**202**)	ND	ND	ND	ND	ND	15.7±2.5	ND
piscidinol A diacetate (**203**)	ND	ND	>179.2		ND	11.6±0.8	ND
toonapubesins F (**194**)	ND	ND	>204				
Chloroquine	7.7±0.02	108.0±0	43.9±0.5	5701			

Legend for Table 4.31 [a]IC_{50}: half maximal inhibitory concentrationgiven in nM for chloroquine. The mean values of at least three independent experiments are reported. SI: selectivity index (IC_{50} vero / IC_{50} (D6); Positive control: podophyllum resin, IC_{50} (4T1) = 0.47 ± 0.05 µg/ml; Melarsoprol IC_{50} (Vero) = 0.76 ± 0.01 µg/ml

Use of combination therapy in treatment of diseases such as cancer and malaria, is a commonly employed to delay resistance and also enhance activity of two or more compounds that act snynergistically. Hence, possible interaction of oleanonic acid (**160**), isolated as a major compound and also the most cytotoxic metabolite isolated from *E. Capensis,* with triterpenoids **151**, **152**, **158**, **159** and **161** against vero and HEp2 cells was evaluated. Interaction against vero cells indicated that 3-*epi*-oleanolic acid (**161**) markedly antagonized the cytotoxic effects of **160** at all concentrations tested, whereas its stereoisomer (**159**) showed slight antagonistic effect (Table 4.32). The cytotoxicity of oleanonic acid (**160**) was antagonized by high concentrations of ekeberin A (**158**), but at lower relative concentrations it enhanced the toxicity of **160**. Triterpenoids **151** and **152** showed weak additive effects.

Interaction of the compounds against HEp2 cell indicated that compounds **161**, **159** and **158** markedly antagonizes activity of **160** at high concentrations and at lower concentrations additive effects were observed (Table 4.33). Acyclic triterpenoids **151** and **152** had additive effects in all the three ratio tested except at a ratio of 1:3 where **152** antagonized **160.** Overall, no appreciable synergistic effect was observed against both cell lines.

Table 4.32: Interaction of oleanonic acid (**160**) with compounds **152**, **153**, **158**, **159** and **161** from root bark extract of *E. capensis* against vero cells

Compound	$\sum$FIC			
	0:1[a]	**1:3**[a]	**1:1**[a]	**3:1**[a]
161	22.5[b]	9.8[c]	5.2[c]	5.8[c]
159	40.3[b]	3.3[c]	4.7[c]	3.6[c]
158	>100[b]	<4[c]	<1.4[c]	<1.3[c]
152	13.6[b]	2.2[c]	2.2[c]	2.3[c]
151	11[b]	2[c]	1.9[c]	2.2[c]

[a]Ratio of oleanonic acid (**160**) versus various constituents of the root extract. [b]IC_{50}

(µg/ml) in the absence of **160** [c]$\sum$FIC; IC_{50} of **160** = 14.8 µg/ml

Table 4.33: Interaction of oleanonic acid (**160**) with compounds **152**, **153**, **158**, **159** and **161** of the root bark extract of *E. capensis* against Hep2 cells.

Compound	$\sum$FIC			
	0:1[a]	**1:3**[a]	**1:1**[a]	**3:1**[a]
161	13.8[b]	5.0[c]	3.0[c]	1.7[c]
159	41.2[b]	16.0[c]	2.7[c]	1.9[c]
158	>100[b]	<7.1[c]	<4.2[c]	<1.4[c]
152	9.4[b]	2.5[c]	1.1[c]	1.1[c]
151	9.1[b]	1.7[c]	1.1[c]	1.0[c]

[a]Ratio of oleanonic acid (**160**) versus various constituents of the root extract. [b]IC_{50} (µg/ml) in the absence of **160**, [c]$\sum$FIC; IC_{50} of **160** = 1.06 µg/ml

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In this work extracts from three plants, *Ekebergia capensis*, *Turraea nilotica* and *Turraea robusta* were investigated. The extracts were initially screened for antiplasmodial and cytotoxic properties. *Turraea robusta* stem bark, *T. nilotica* stem and root bark indicated high antiplasmodial activities ($IC_{50} < 10$ μg/ml) against W2 and D6 *Plasmodium falciparum* strains. They also had moderate cytotoxicities ($IC_{50} > 20$ μg/ml) against vero cells. For *E. capensis*, the root bark had high cytotoxicity and moderate antiplasmodial activity while leaves of *T. nilotica* and *E. capensis* had moderate activities in both antiplasmodial and cytotoxic assays.

The root bark extract of *E. capensis* possesses high toxicity against 'normal' (2.8 μg/ml) cell line. Although *in vitro* data cannot be directly extrapolated to *in vivo* toxicity, results herein underscores the need for careful use of *E. capensis* extracts in traditional medicine

Turraea robusta stem bark yielded seven compounds of which azadironolide (**192**) is new to the genus whereas acetoxy-7-deacetylazadirone (**28**) and 11-*epi*-toonacilin (**62**) are new to the species.

From the leaves, root and stem bark of *Turraea nilotica* twelve compounds were isolated. Toonapubesins F (**194**), mixture of sitosterol 3-*O*-*β*-D-glucopyranoside

acetate (**195**), stigmasterol-3-*O-β*-D-gluconopyranoside acetate (**196**) and sitosterol-3-*O-β-D*-glucopyranoside (**197**) are new to the genus while mzikonone (**17**), azadirone (**19**), acetoxy-7-deacetylazadirone (**28**), 1α,3α-diaacety-7α-tigloylvilasinin (**40**) and hipidol B (**96**) are new to the species. It appears that roots, stem and leaves synthesize different secondary metabolites. Limonoids were present in the roots have limonoids, protolimonoids in the stem bark while phytosterols were in the leaves.

E. capensis root bark yielded seven triterpenoids of which five were pentacyclic and two acyclic. Pentacyclic triterpenoid, 3-oxo-12β-hydroxy-oleanan-28,13β-olide (**198)** is a new natural product. From the leaves, two flavanol glycosides new to the genus [Kaemferol-3-*O-β*-D-glucopyranoside (**199**) and quercetin- 3-*O-β*-D-glucopyranoside (**200**)] and one limonoid were isolate. Triterpenoid content present in the root bark is also present in the stem bark.

Two compounds displayed good antiplasmodial activity, azadironolide (**192**) [2.4 μM (D6) ; 1.1 μM (W2)] and 11-*epi*-toonacilin (**62**) [IC_{50} 17.1 μM (D6); 14.4 μM (W2)] with minimal cytotoxicity to vero cells. The rest of the compounds displayed moderate antiplasmodial activity against the two strains with low selectivity indices (SI < 10), revealing their limited applicability for antimalarial drug development.

Six compounds azadirone (**19**), niloticin (**25**), piscidinol A (**27**), hispidol B (**96**), oleanonic acid (**160**) and azadironolide (**192**) were cytotoxic to HEp2 and 4T1 cells (IC_{50} < 20 μM).

No appreciable synergism on the cytotoxicity of oleanonic acid (**160**) with other triterpenoids isolated from *E. capensis* root bark against HEp2 and vero cell lines was detected.

5.2. Recommendations

1) 11-*epi*-toonacilin (**62**) and azadironolide (**192**) that displayed good antiplasmodial activity warrants further investigation to establish antimalarial activity and explore possibilities of developing them into antimalarial drug leads.

2) Compounds **19**, **17**, **25**, **96**, **160** and **192** which displayed cytotoxicity against cancerous cell lines should be evaluated for their potential as anticancer leads in an *in vivo* model.

3) *E. capensis* (RB) and *Turraea robusta* (SB) extracts were cytotoxic and should be evaluated further for their potential as ancancer agents in an *in vivo* model.

4) Evaluate bioactivity of compound **198**, the new natural product.

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6.0 APPENDICES

Appendix 1: ^{1}H NMR spectrum of mzikonone (**1**)
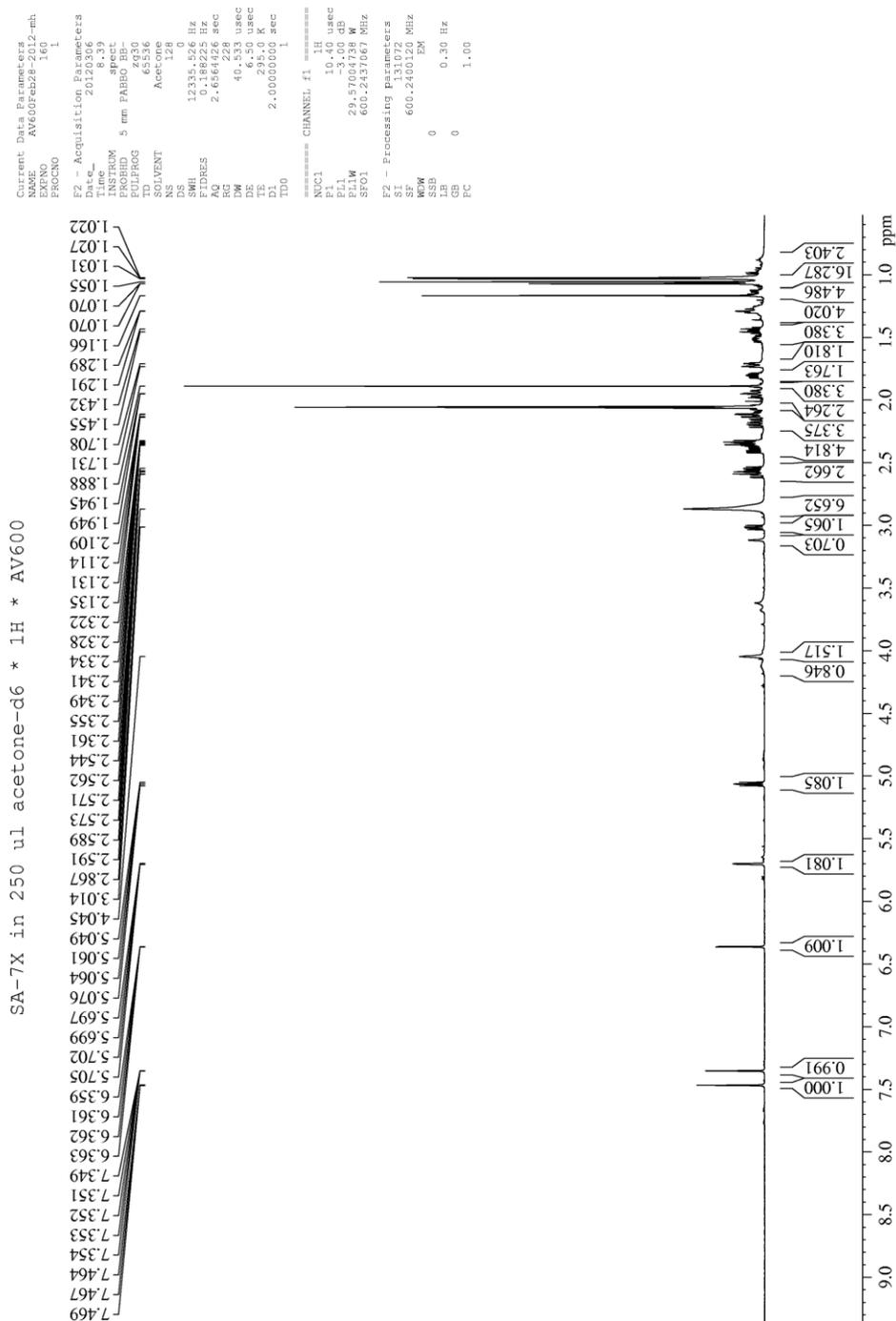
Appendix 1A : ^{13}C NMR spectrum of mzikonone (**17**)

27.049
27.157
28.644
31.007
34.985
38.092
38.449
39.952
44.008
45.078
47.951
48.060
51.855
52.815
73.081
79.097
113.448
123.619
126.451
141.920
143.653
159.529
171.398
216.500

SA-7X in 250 ul acetone-d6 * 13C * AV600
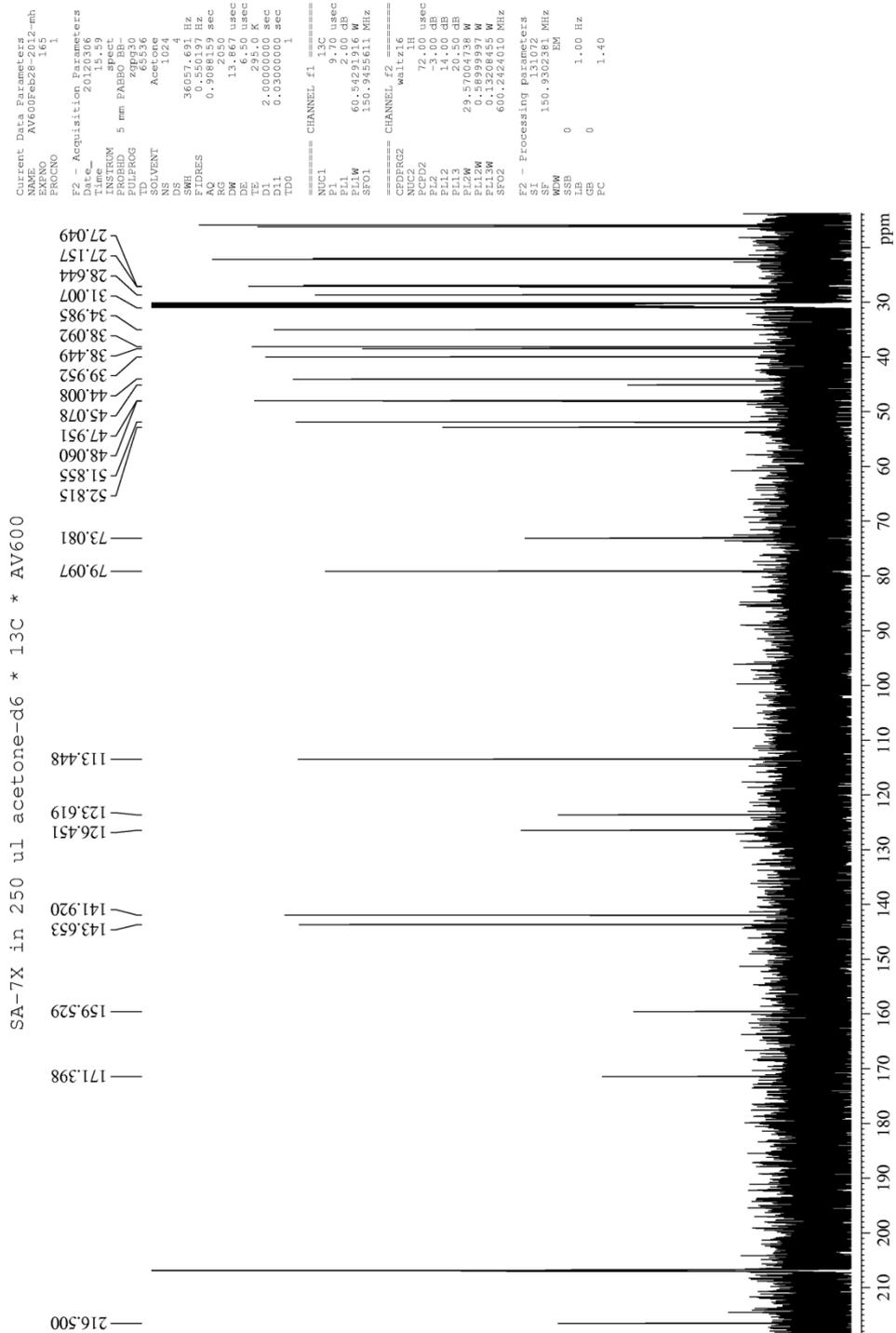
Appendix 1B: LC-MS spectrum of mzikonone (**17**)
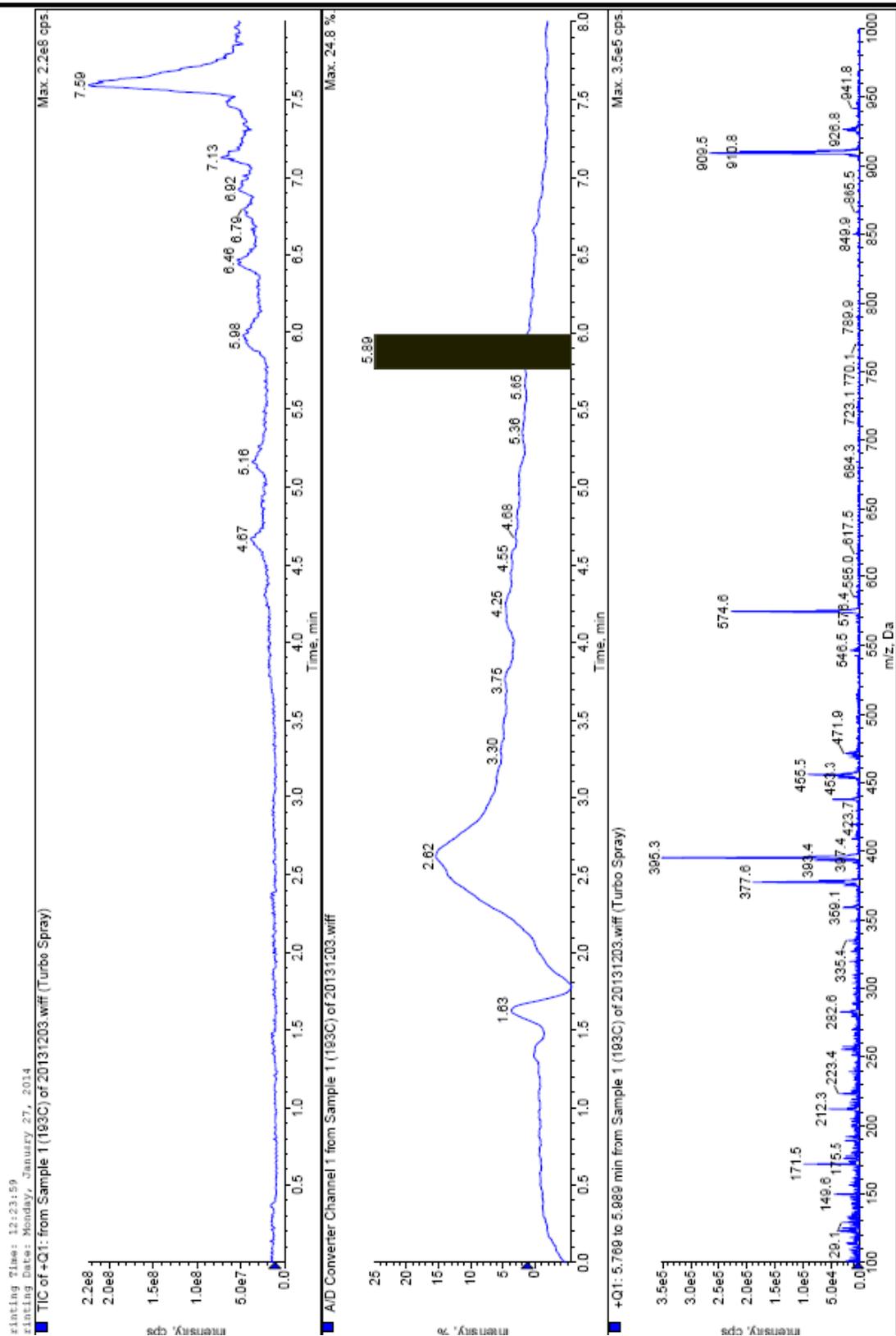
Appendix 2: ^{1}H NMR spectrum of azadirone (**19**)
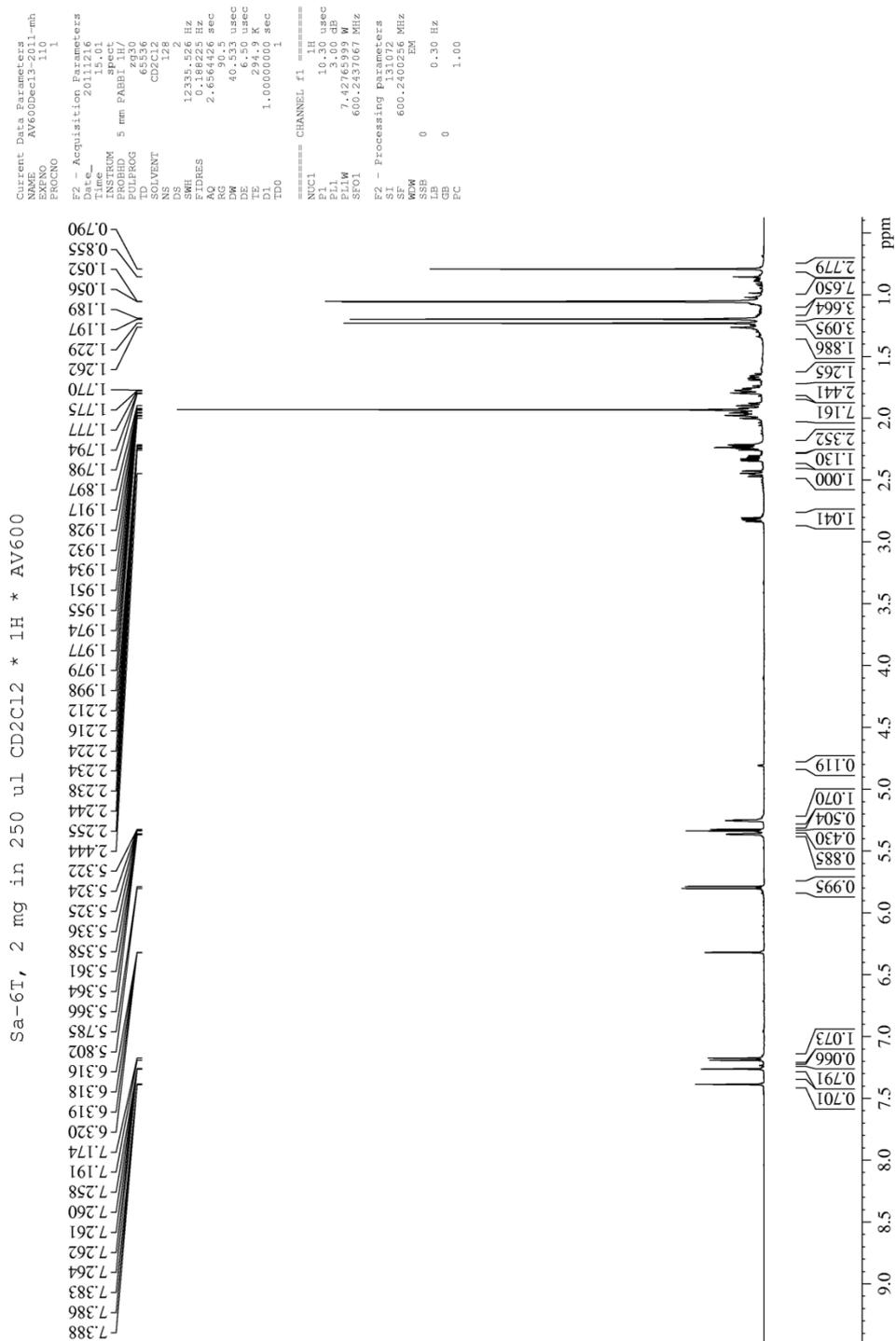
Appendix 2A: ^{13}C NMR spectrum of azadirone (**19**)

Sa-6T, 2 mg in 250 ul CD2Cl2 * 13C * AV600

Peaks:
21.343
23.976
27.015
27.328
33.238
34.573
38.889
40.190
43.057
44.268
46.304
47.371
51.820
74.622
111.368
119.164
125.053
125.348
139.967
142.749
158.587
159.252
170.138
204.567
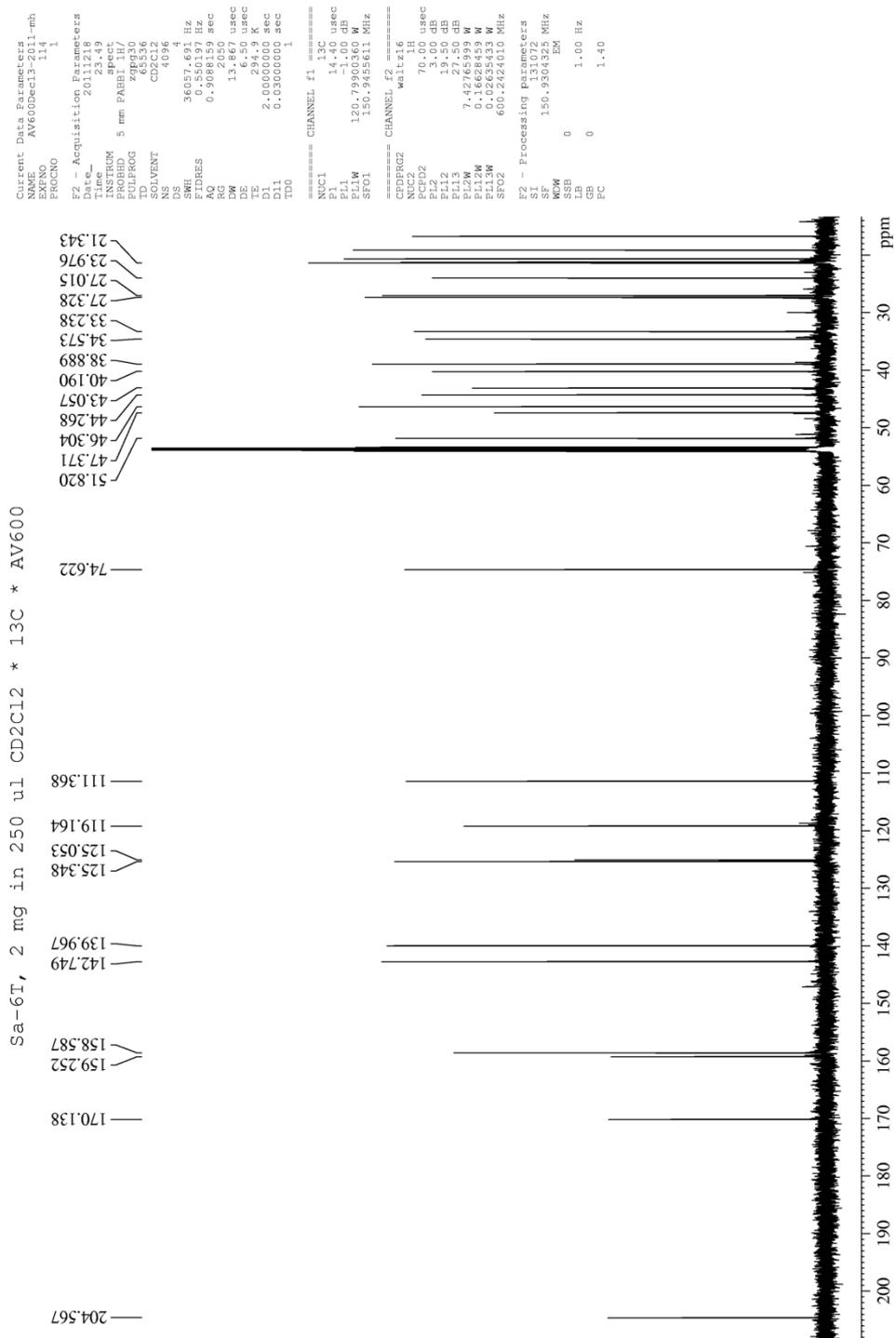
Appendix 2B : MS spectrum of azadirone (**19**)
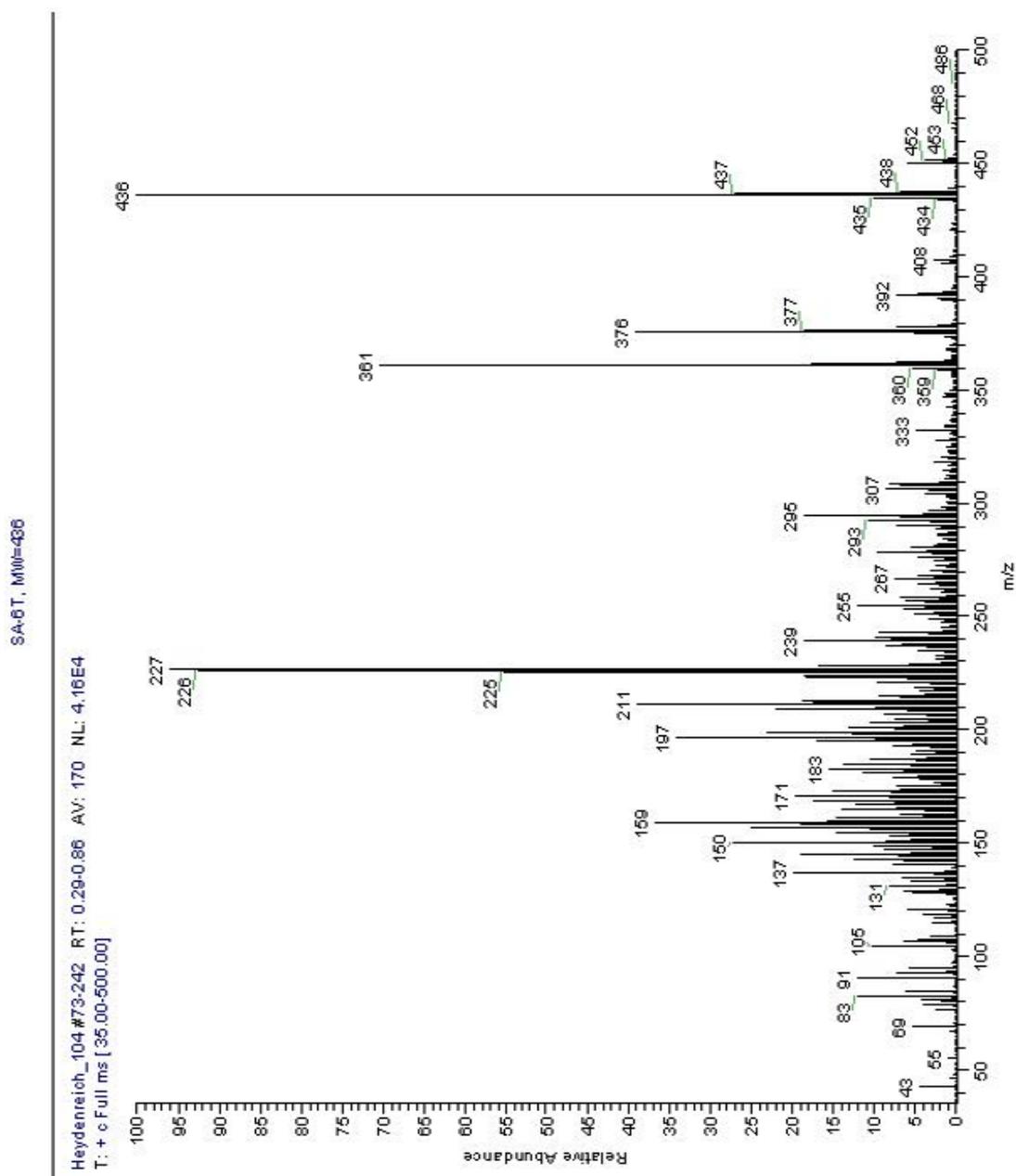
Appendix 3: ^{1}H NMR spectrum of 12α-Acetoxy-7-deacetylazadirone (**28**)
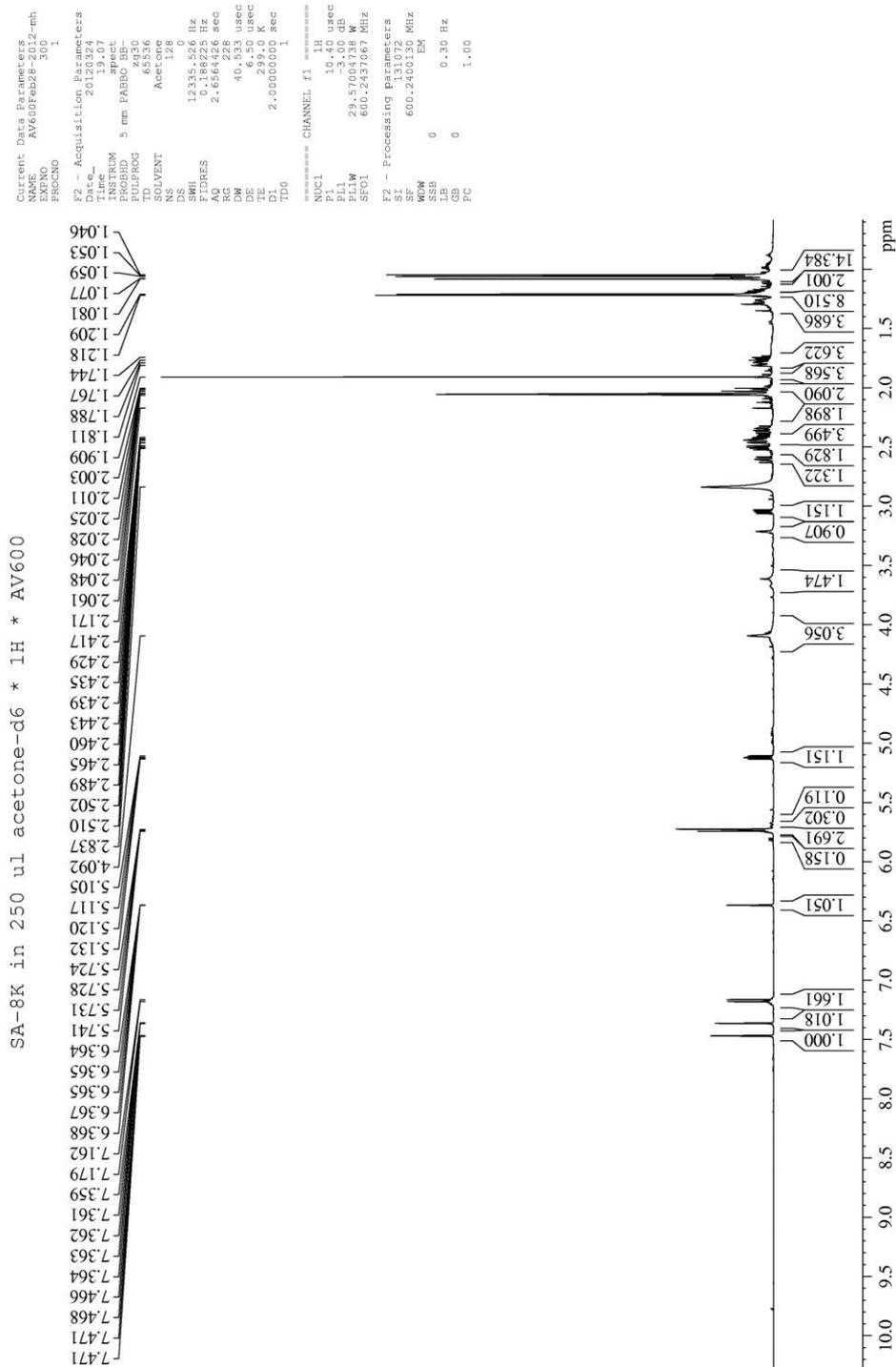
Appendix 3A: ^{13}C NMR spectrum of 12α-Acetoxy-7-deacetylazadirone (**28**)
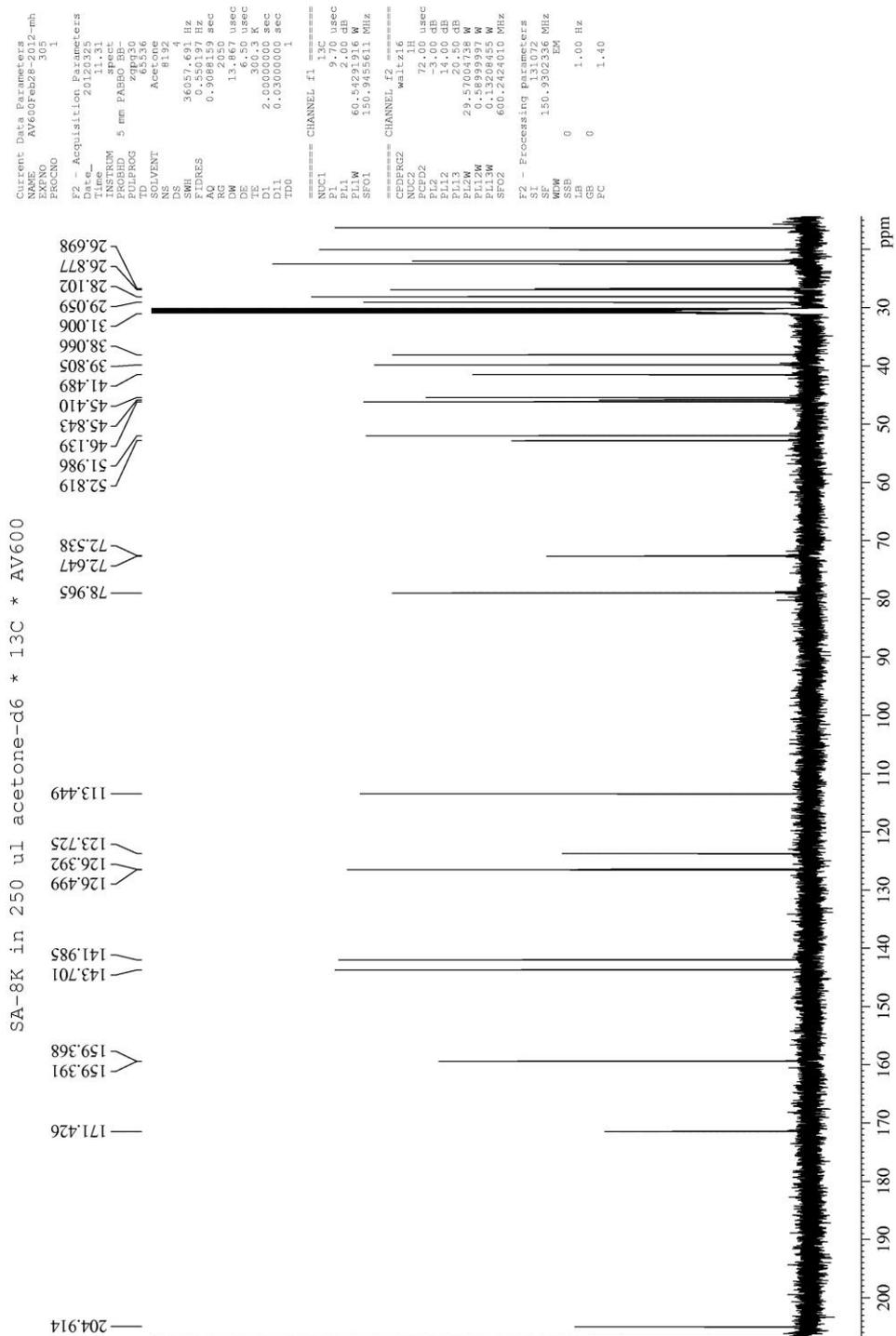
Appendix 3B: MS spectrum of 12α-Acetoxy-7-deacetylazadirone (**28**)

141
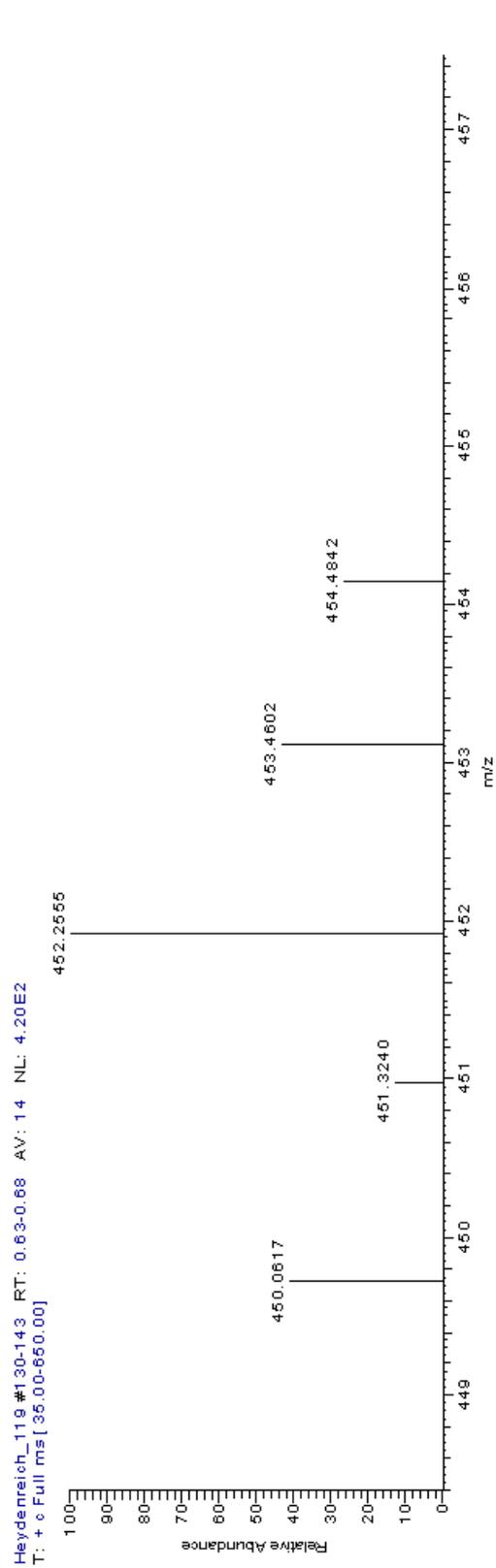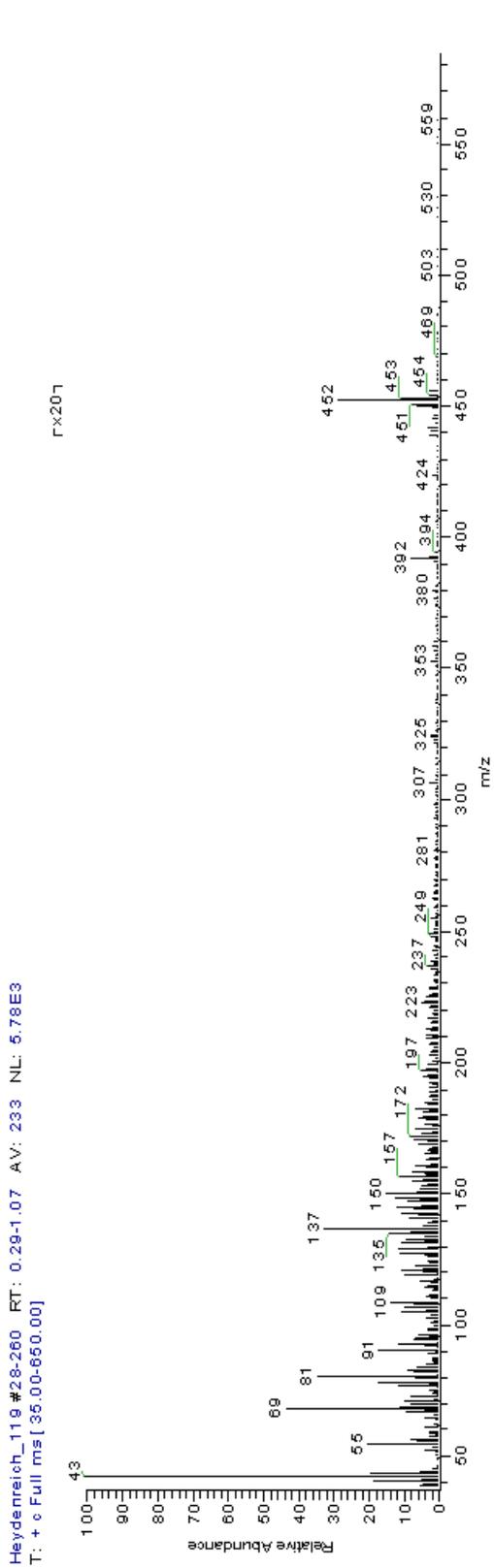
Appendix 4: ^{1}H NMR spectrum of 11-*epi*-toonacilin (**62**)
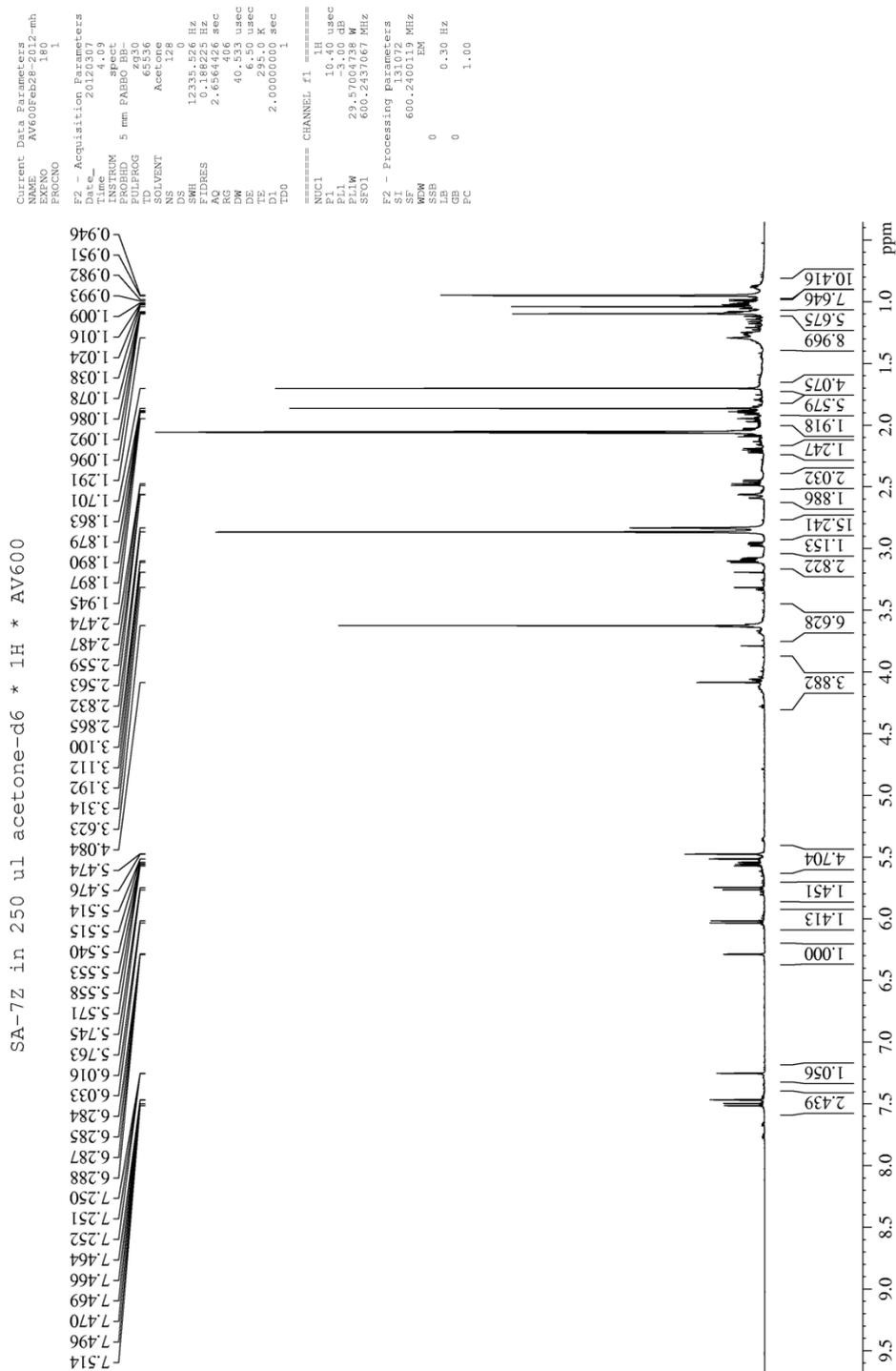
Appendix 4A: ^{13}C NMR spectrum of 11-*epi*-toonacilin (**62**)
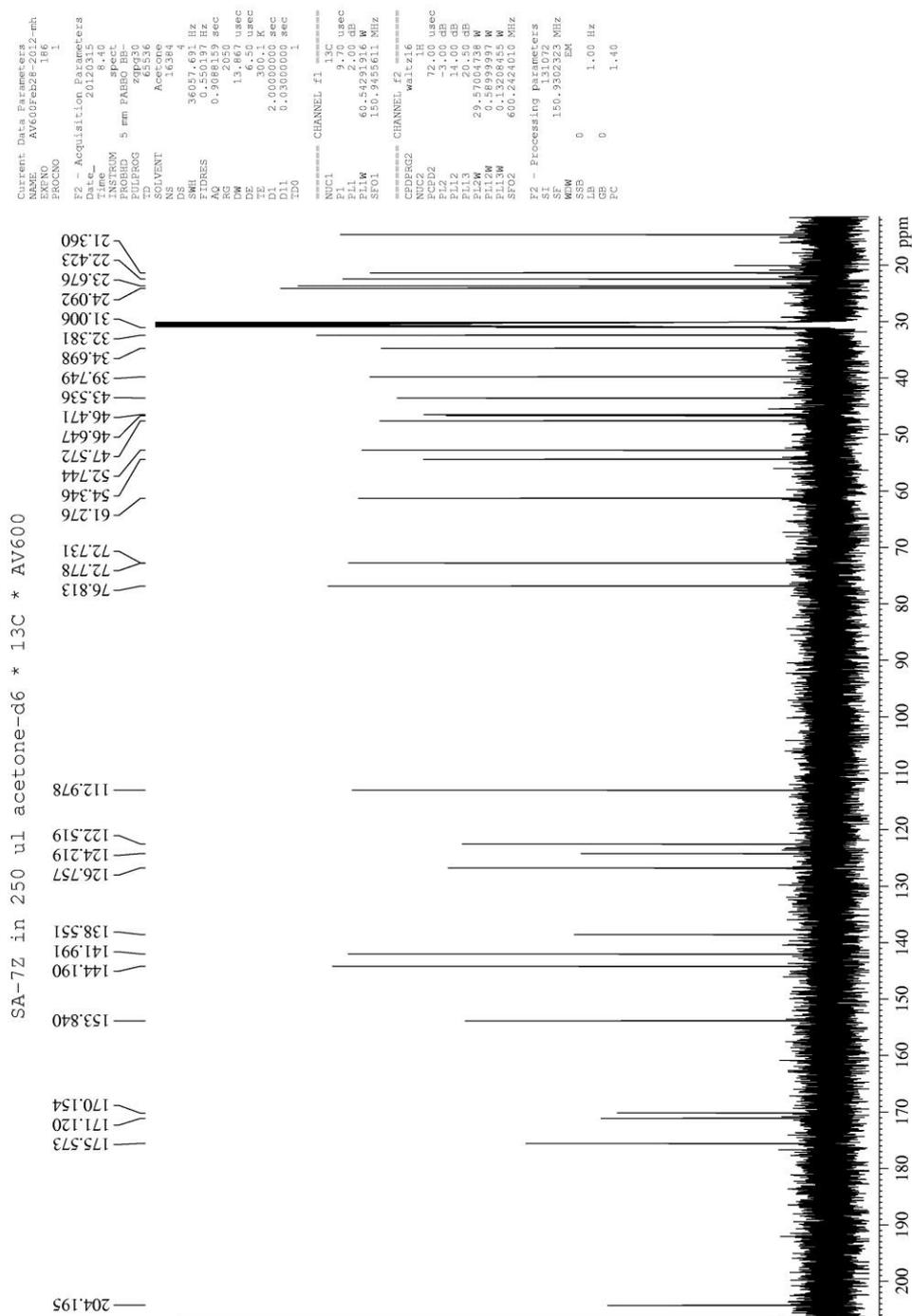
Appendix 4B: MS spectrum of 11-*epi*-toonacilin (**62**)
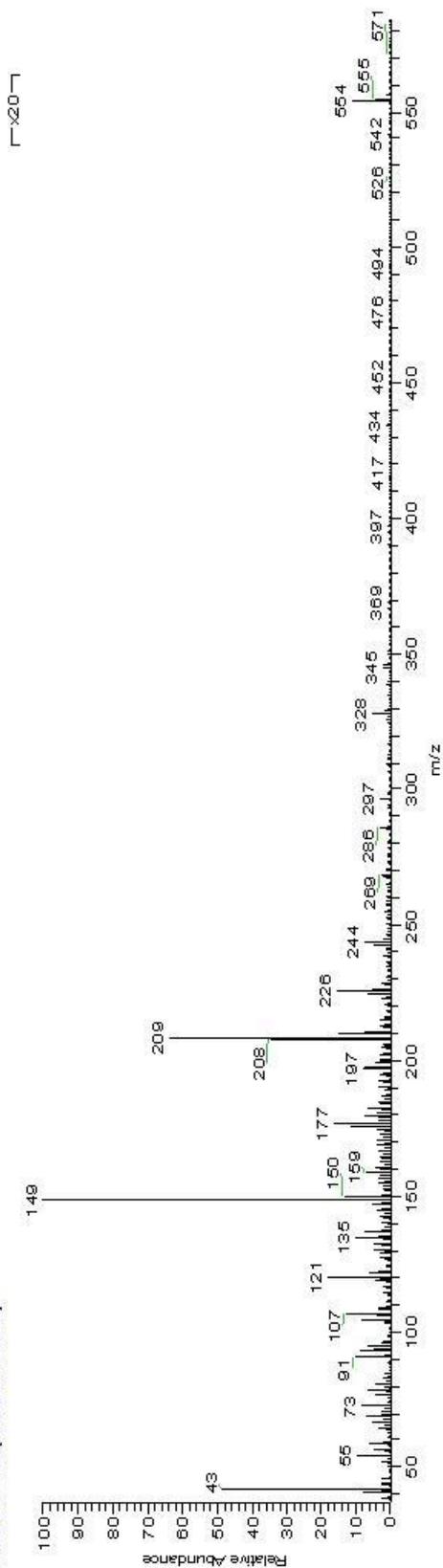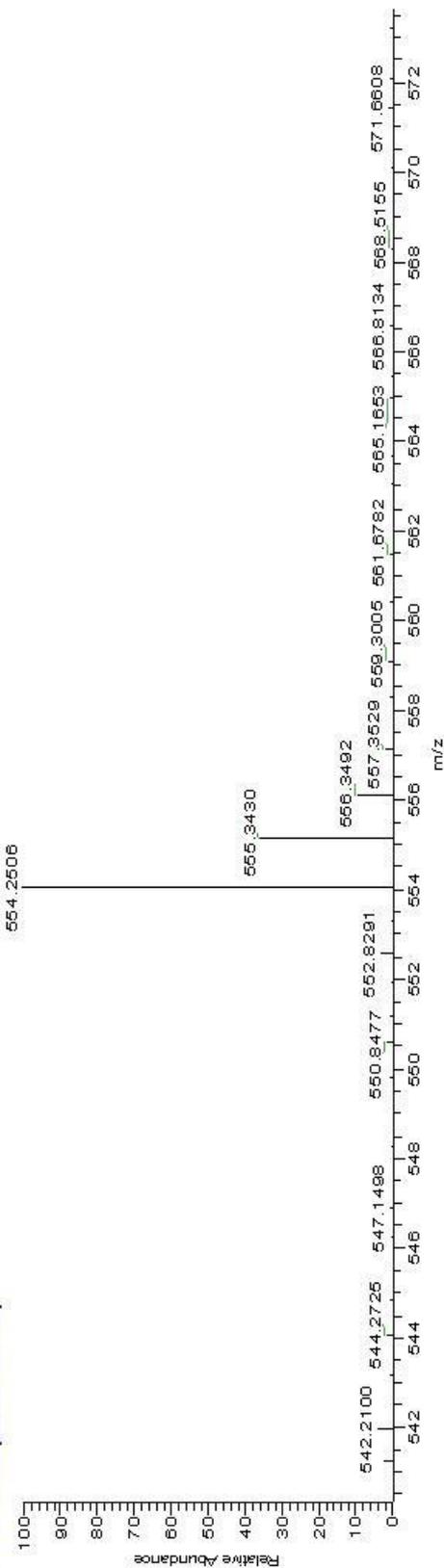
Appendix 5: ^{1}H NMR spectrum of Turranolide (**22**)
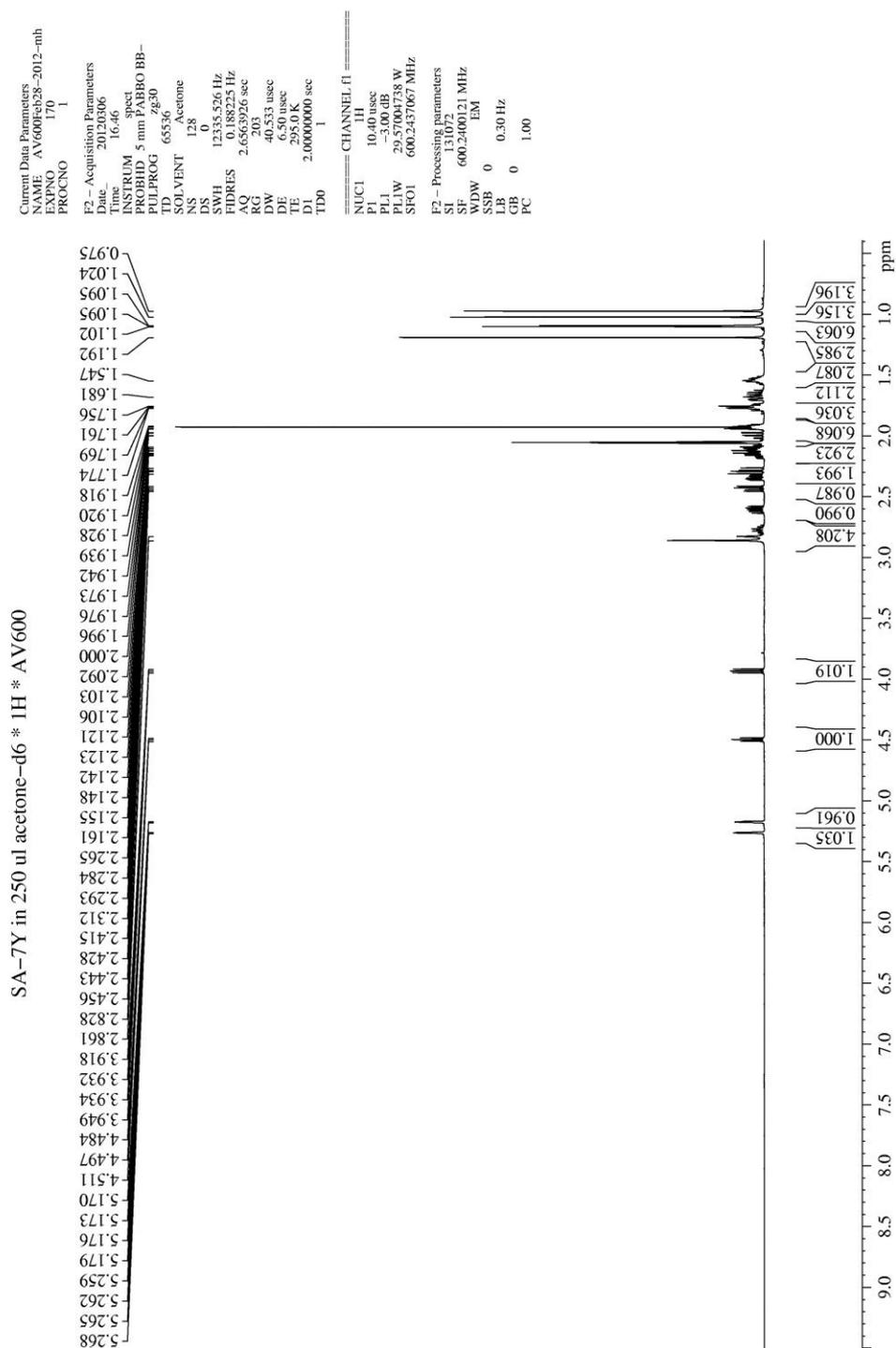
Appendix 5A: ^{13}C NMR spectrum of Turranolide (**22**)
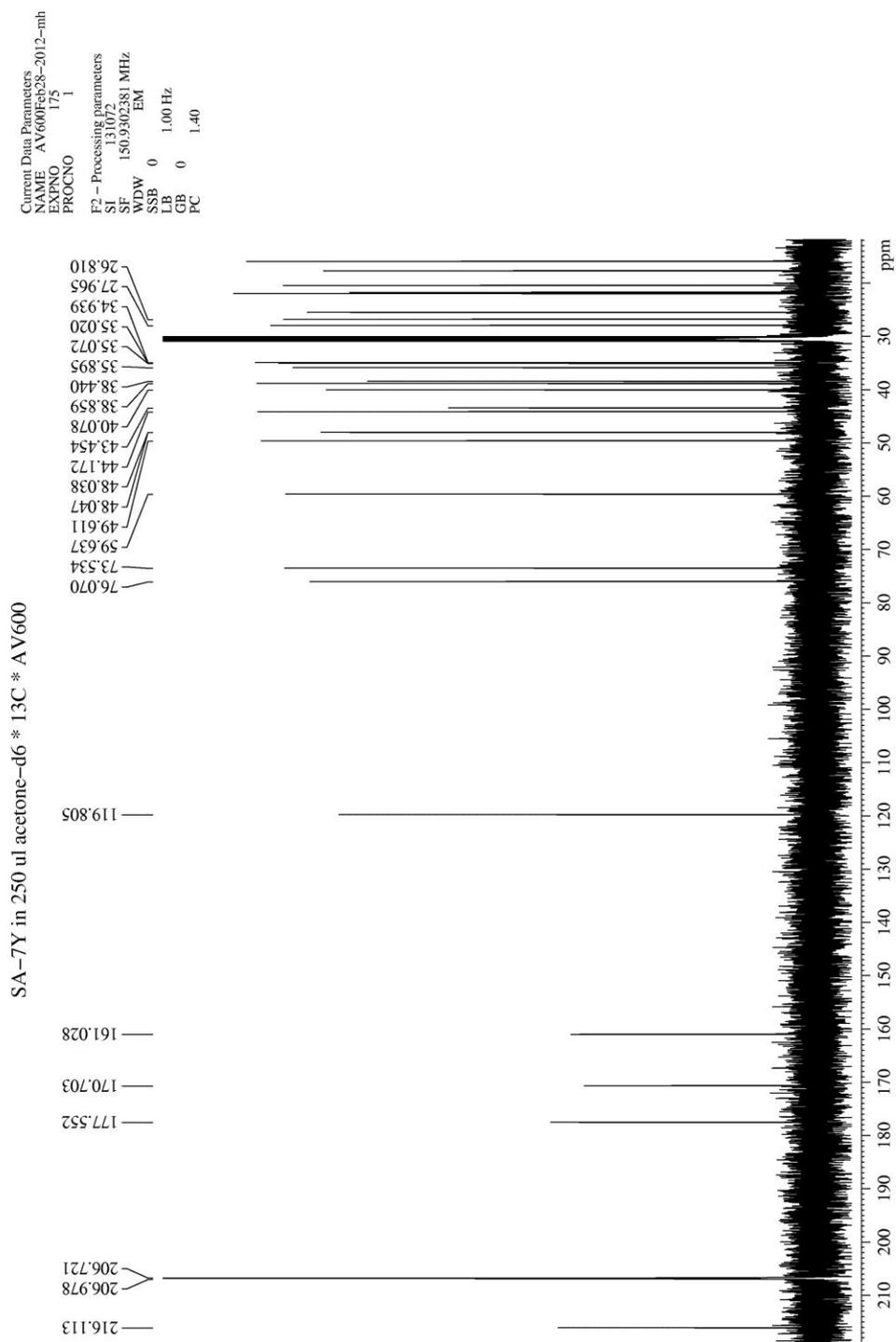
Appendix 5B: MS Turranolide (**22**)

SA-7Y, MW=456

Heydenreich_105 #73-257 RT: 0.29-0.91 AV: 185 NL: 3.50E5
T: + c Full ms [35.00-500.00]
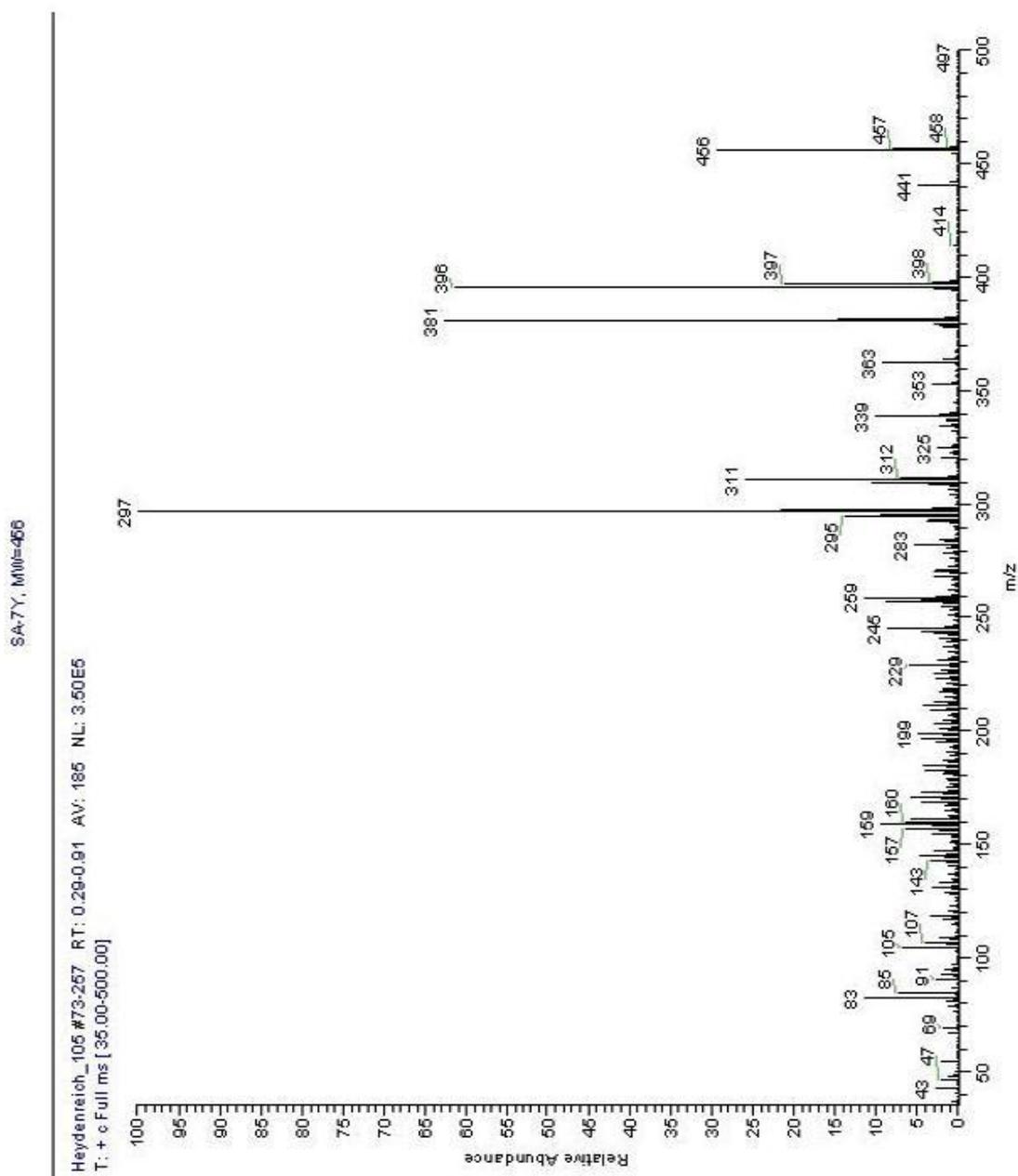
Appendix 6: ^{1}H NMR spectrum of azadironolide (**192**)
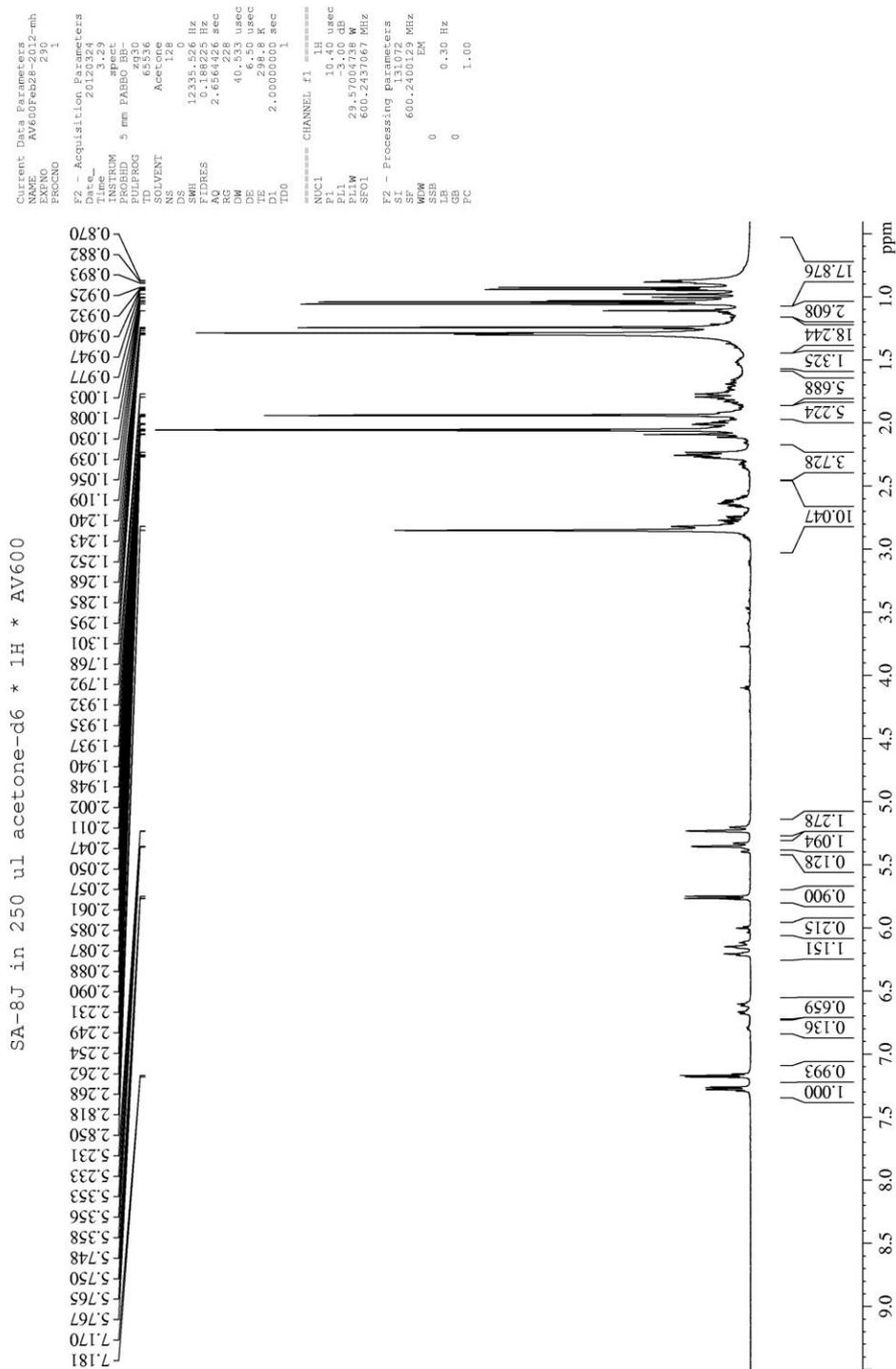
Appendix 6A: ^{13}C NMR spectrum of azadironolide (**192**)

SA-8J in 250 ul acetone-d6 * 13C * AV600

15.017
15.978
17.738
17.814
20.087
21.714
21.753
21.799
21.975
21.994
22.033
22.254
22.304
23.992
25.086
25.568
26.981
28.000
28.091
28.442
31.007
33.303
34.534
34.669
35.087
35.111
35.293
38.497
40.081
40.108
41.423
44.478
45.347
47.818
48.062
48.713
48.901
49.701
52.318
52.333
75.713
76.197
98.390
98.608
119.830
119.904
126.443
148.506
148.769
159.645
160.560
160.699
170.719
172.818
173.007
204.565

Appendix 7: ^{1}H NMR of stigmasterol (**193**)

Appendix 7A: ^{13}C NMR of stigmasterol (**193**)
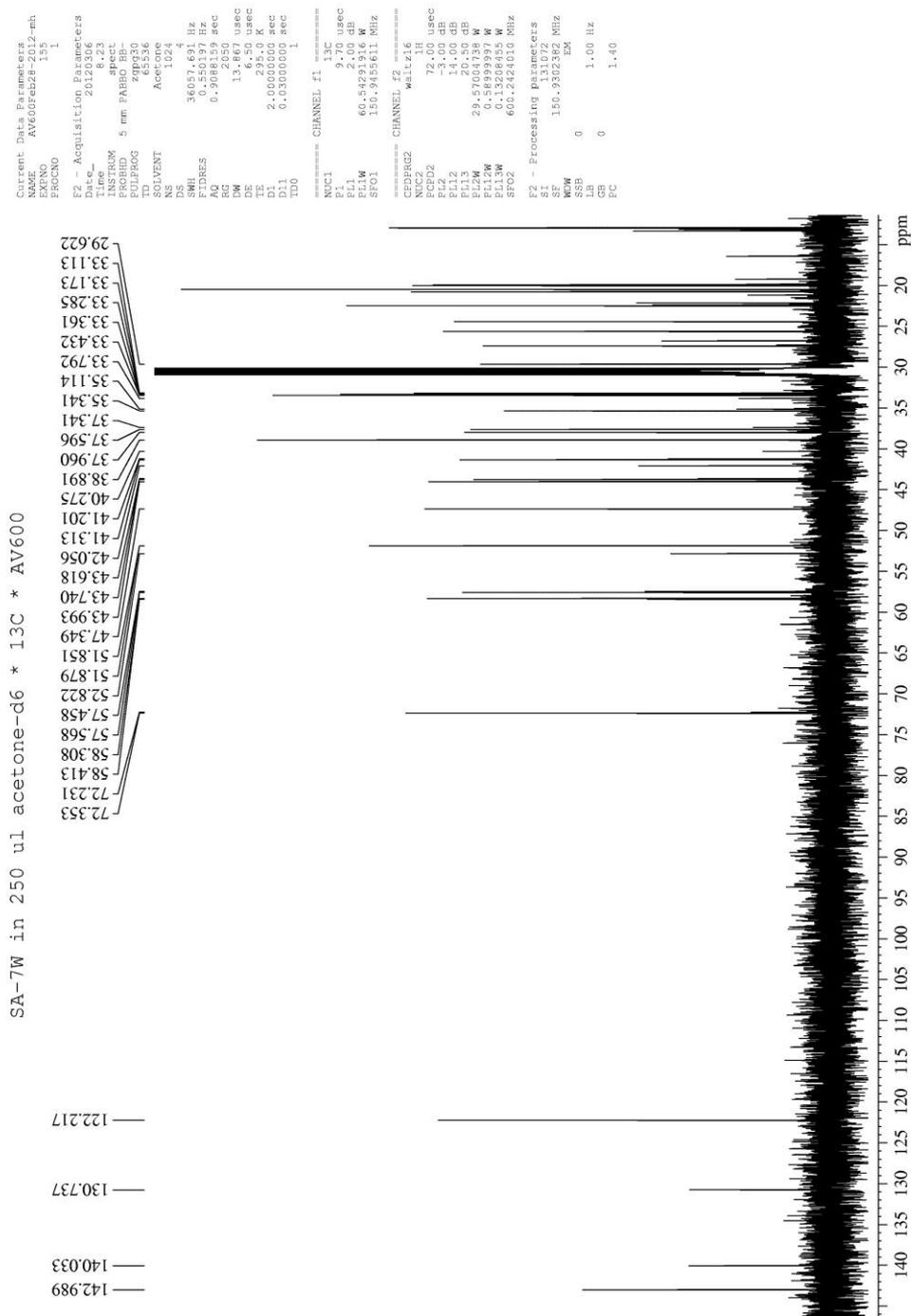
Appendix 8: ^{1}H NMR spectrum of Piscidinol A (**27**)
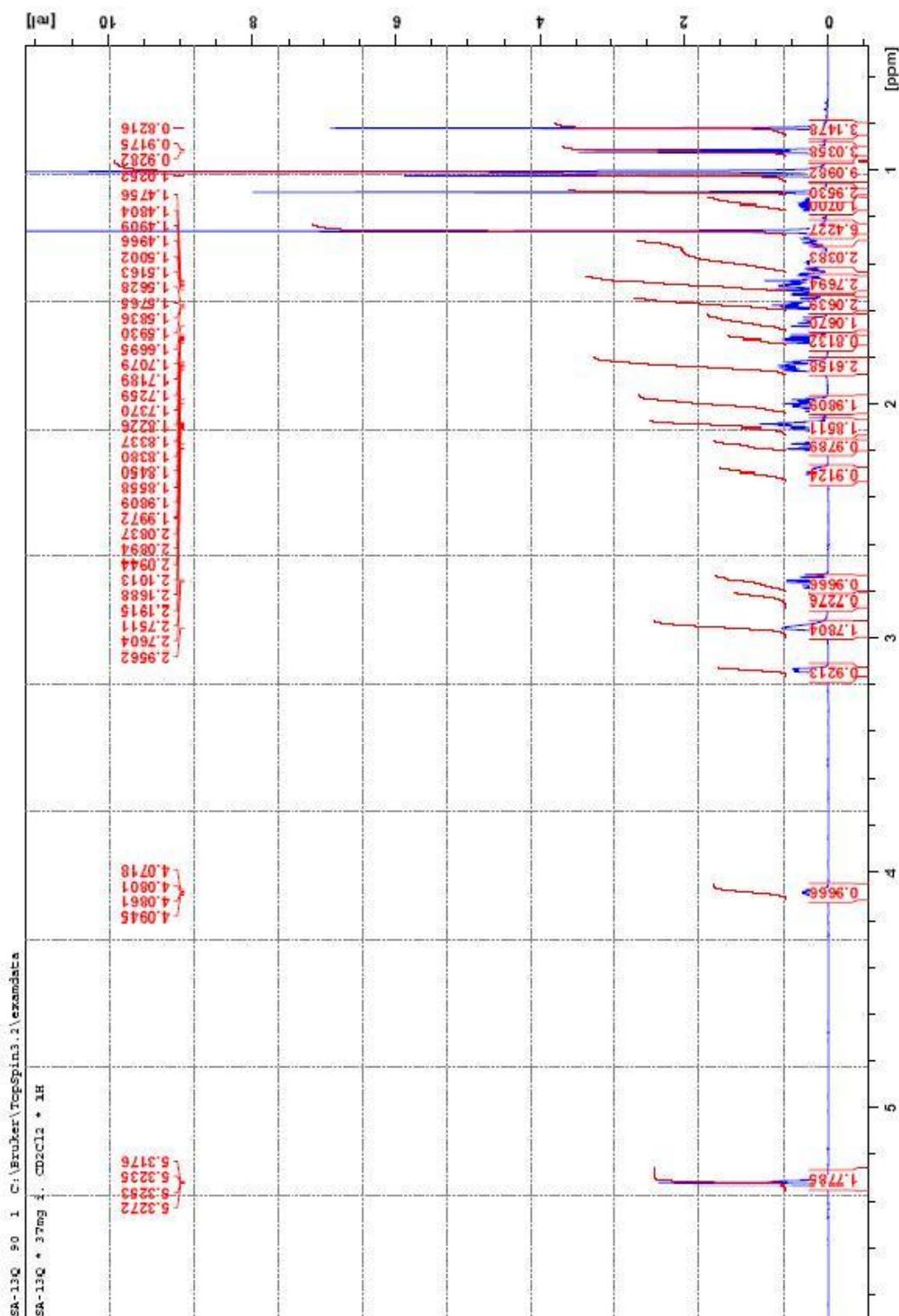
Appendix 8A: ^{13}C NMR spectrum piscidinol A (**27**)
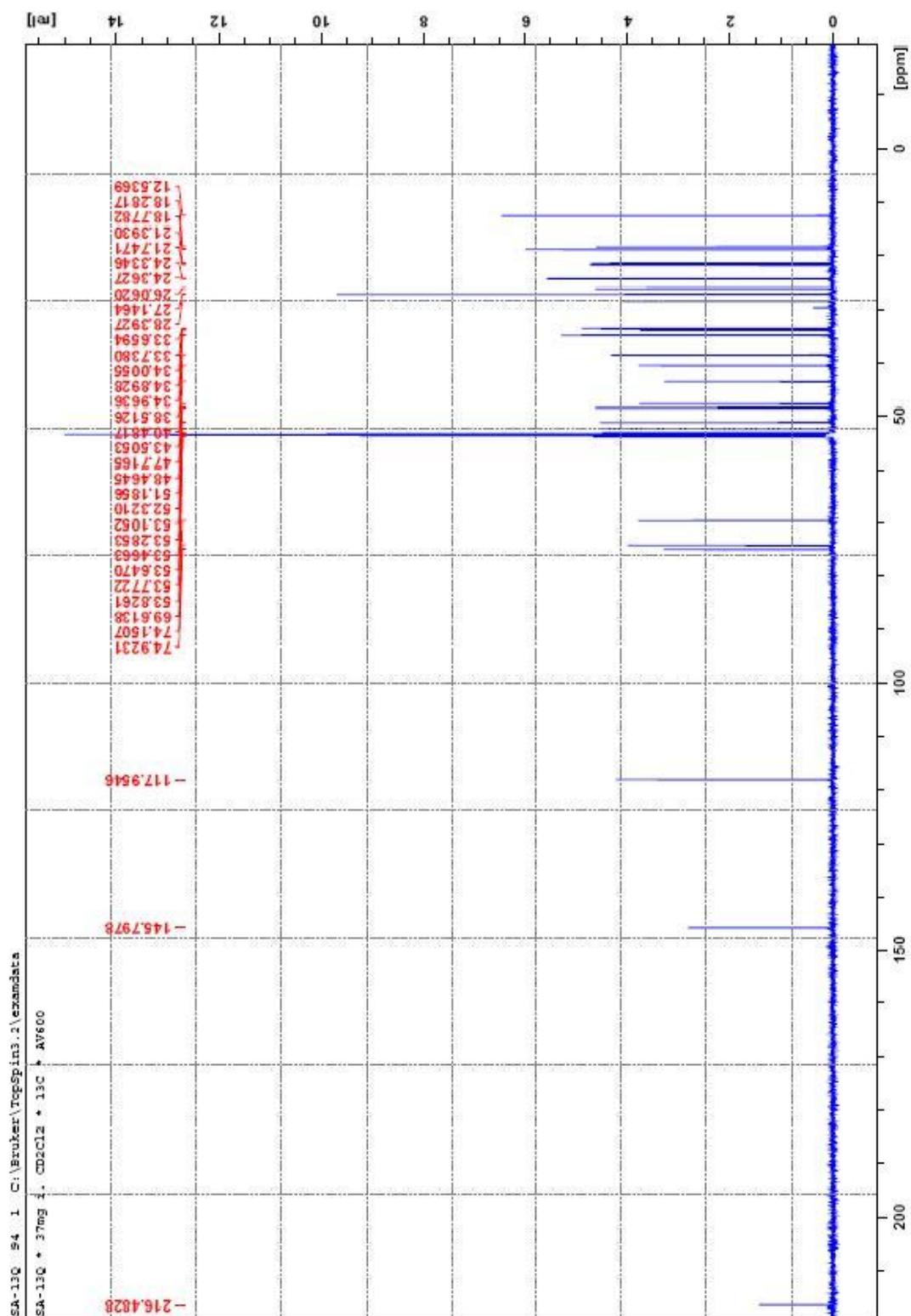
Appendix 8B: LC/MS spectrum of Piscidinol A (**27**)
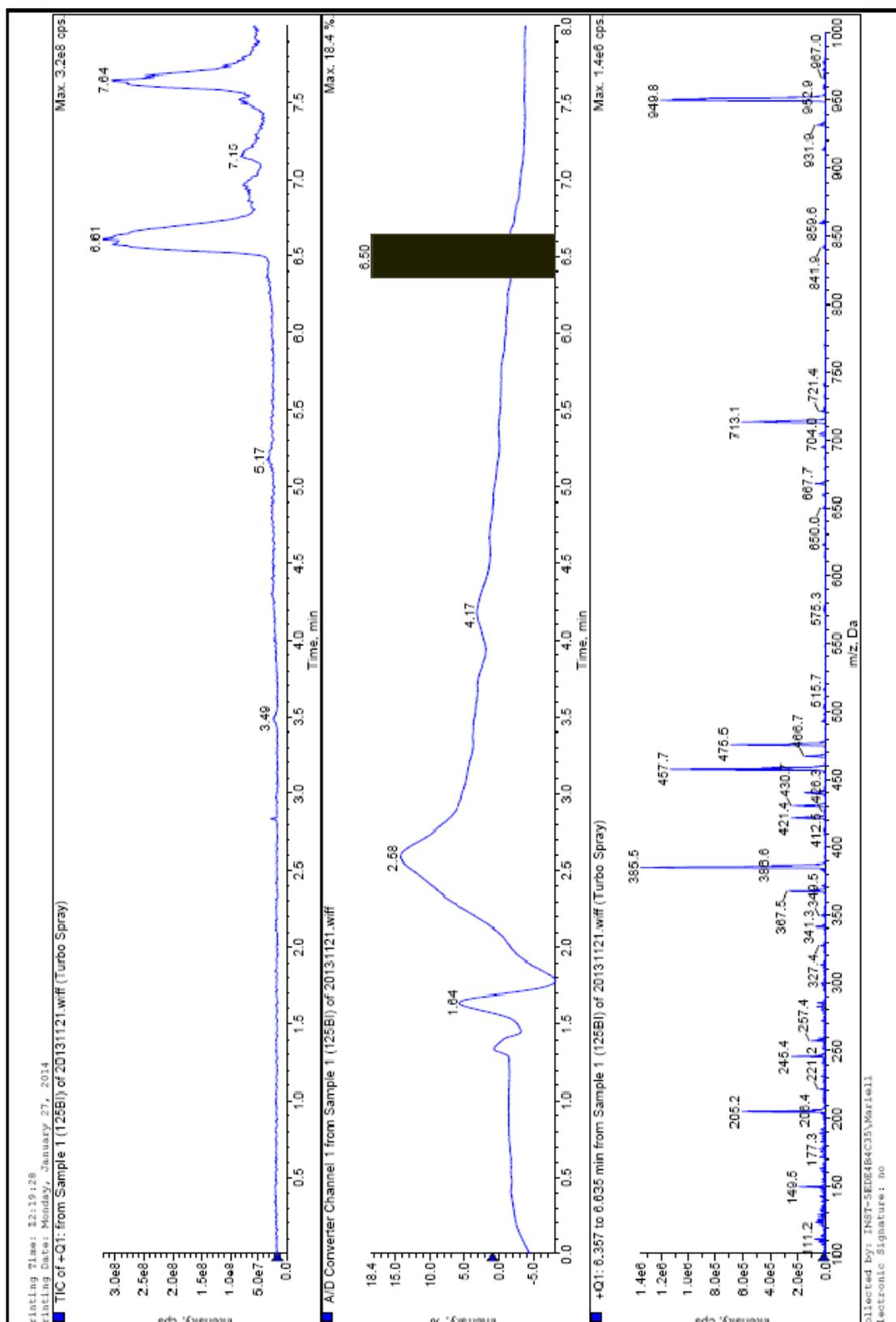
Appendix 9: LC/MS spectrum of Piscidinol A diacetate (**203**)
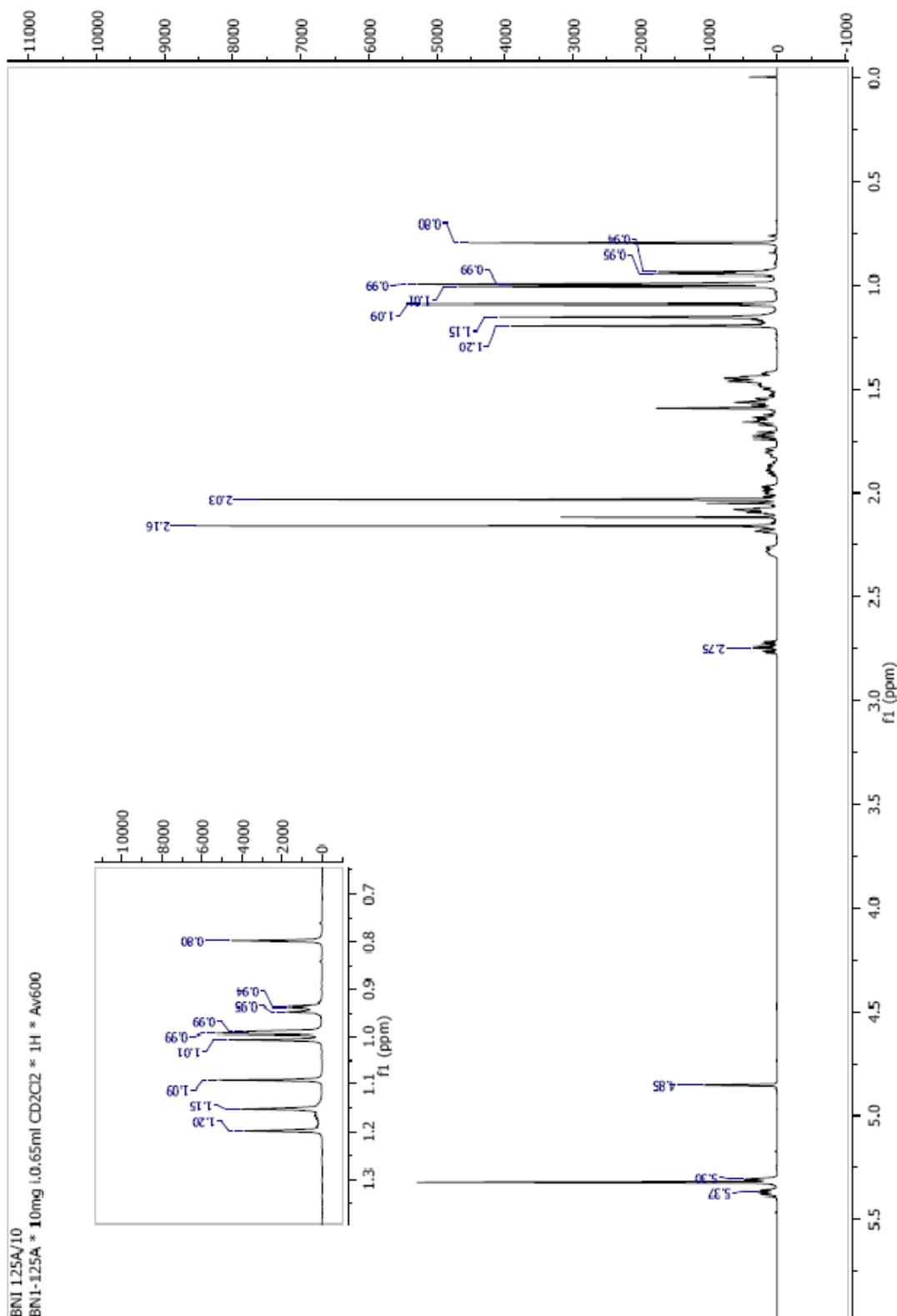
Appendix 9A: LC/MS spectrum of Piscidinol A diacetate (**203**)
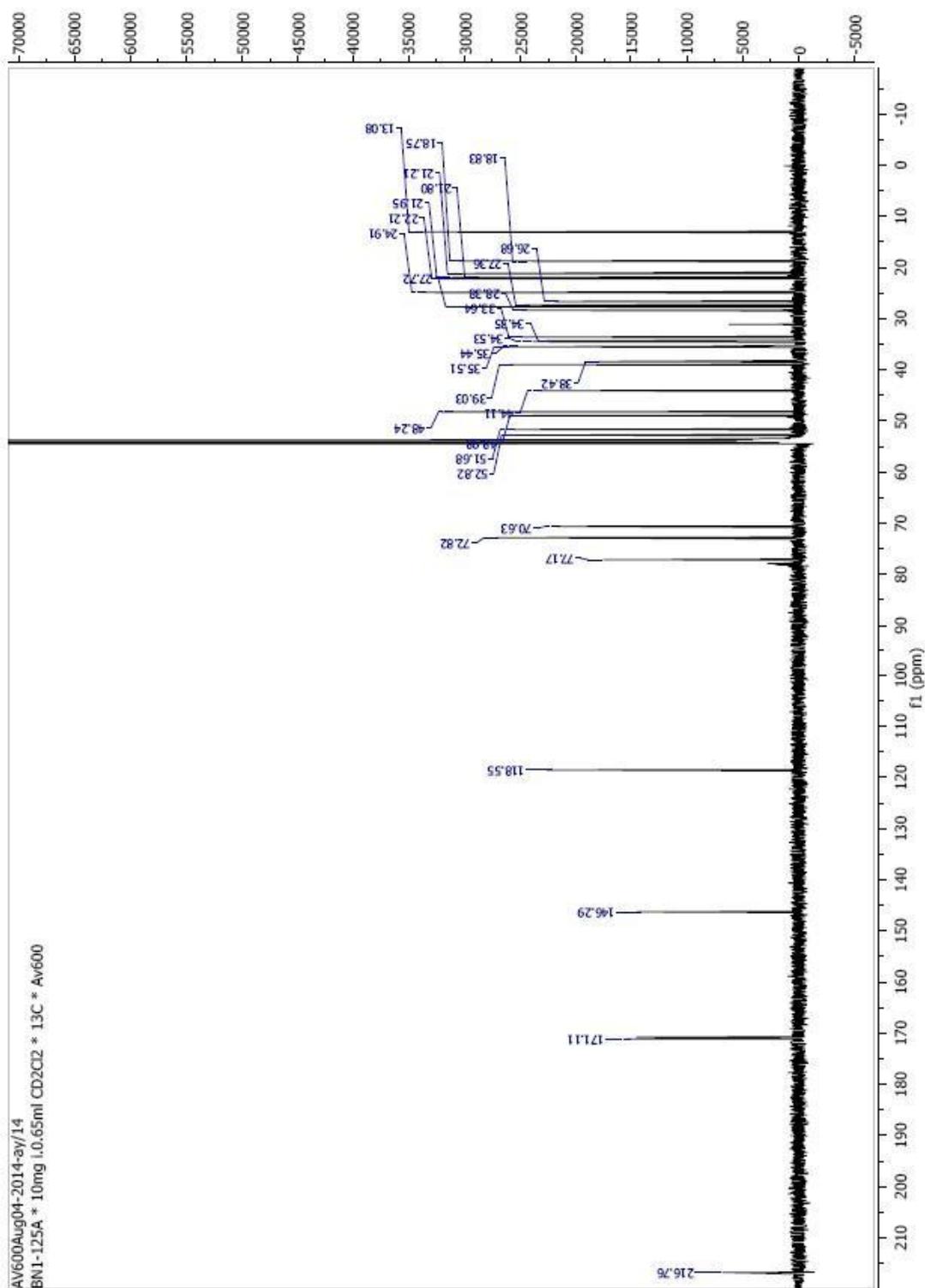
Appendix 10: ^{1}H NMR spectrum of hispidol B (**96**)
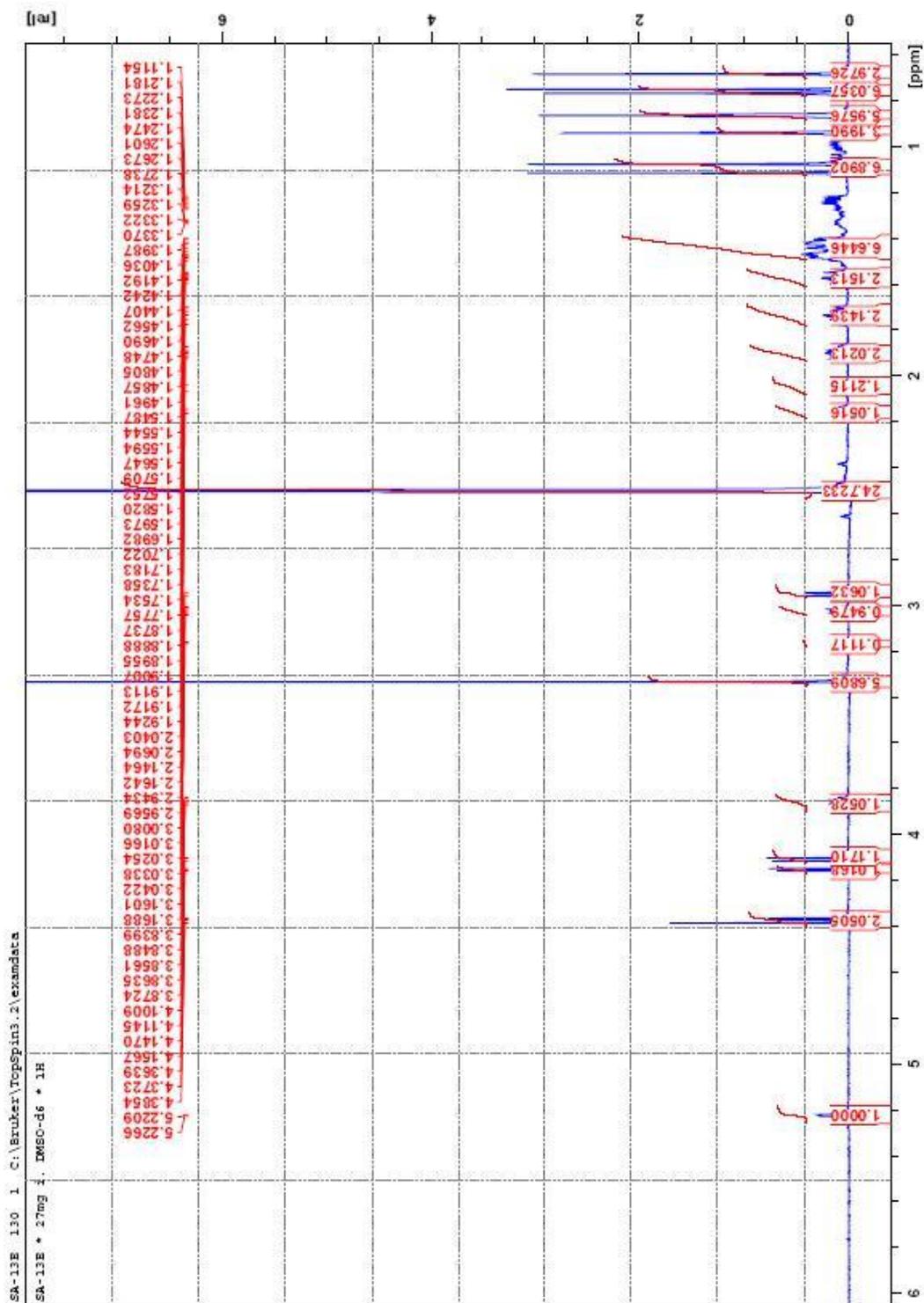
Appendix 10A: ^{13}C NMR spectrum of hispidol B (**96**)
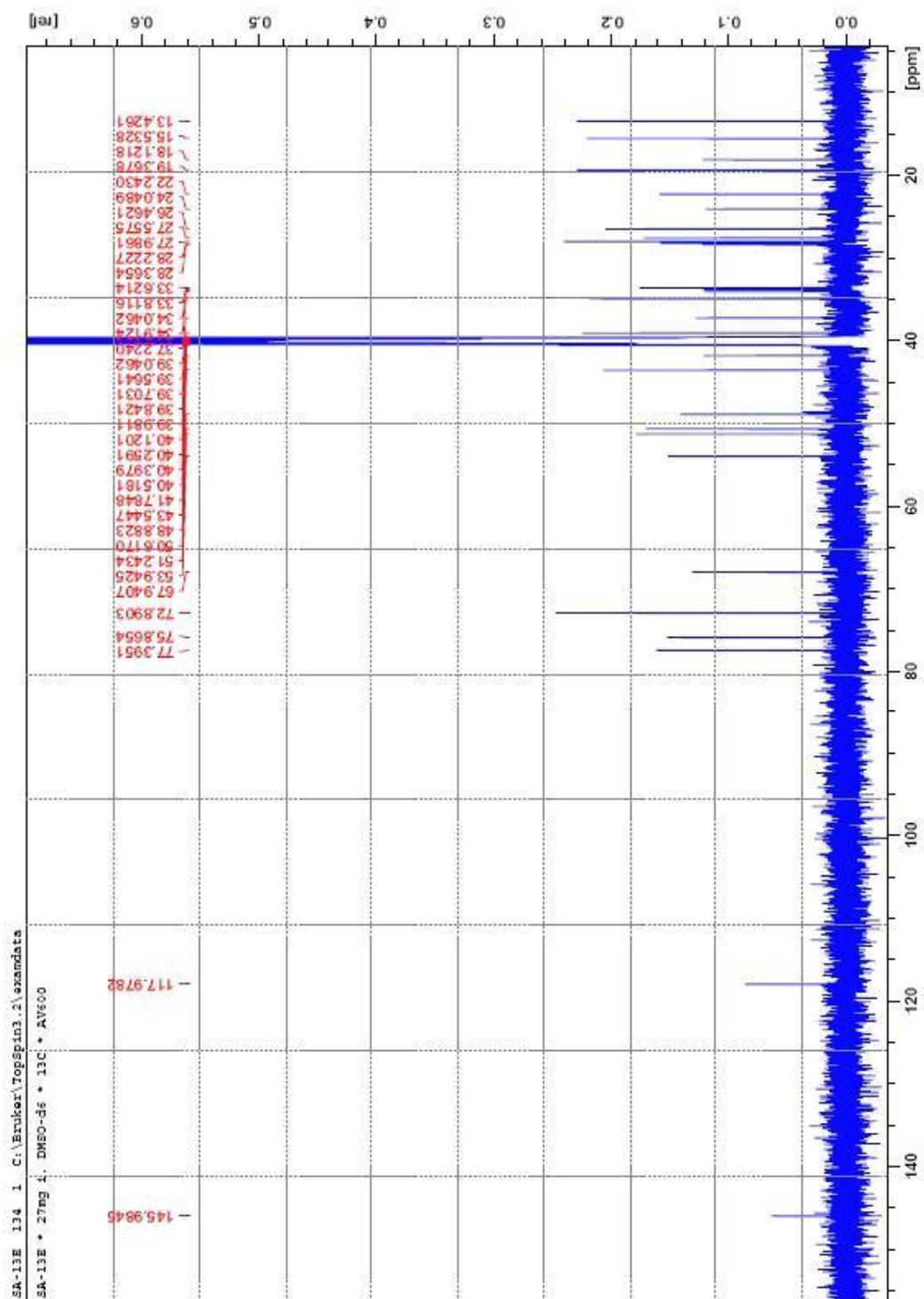
Appendix 10B: MS spectrum of hispidol B (**96**)
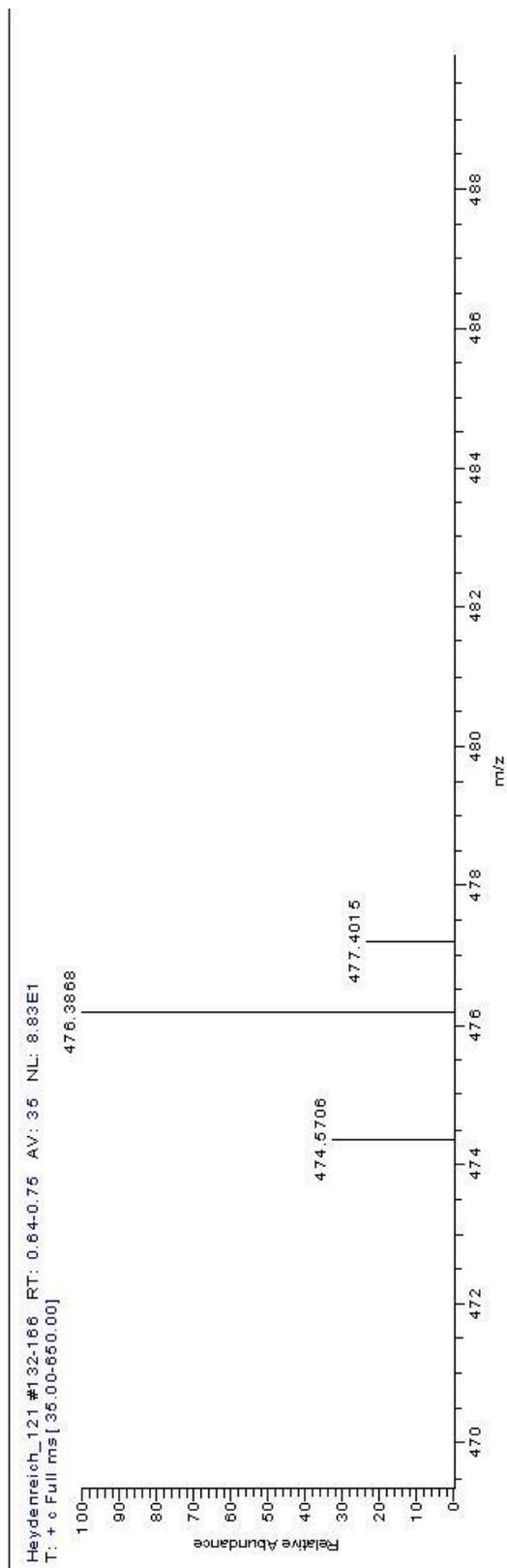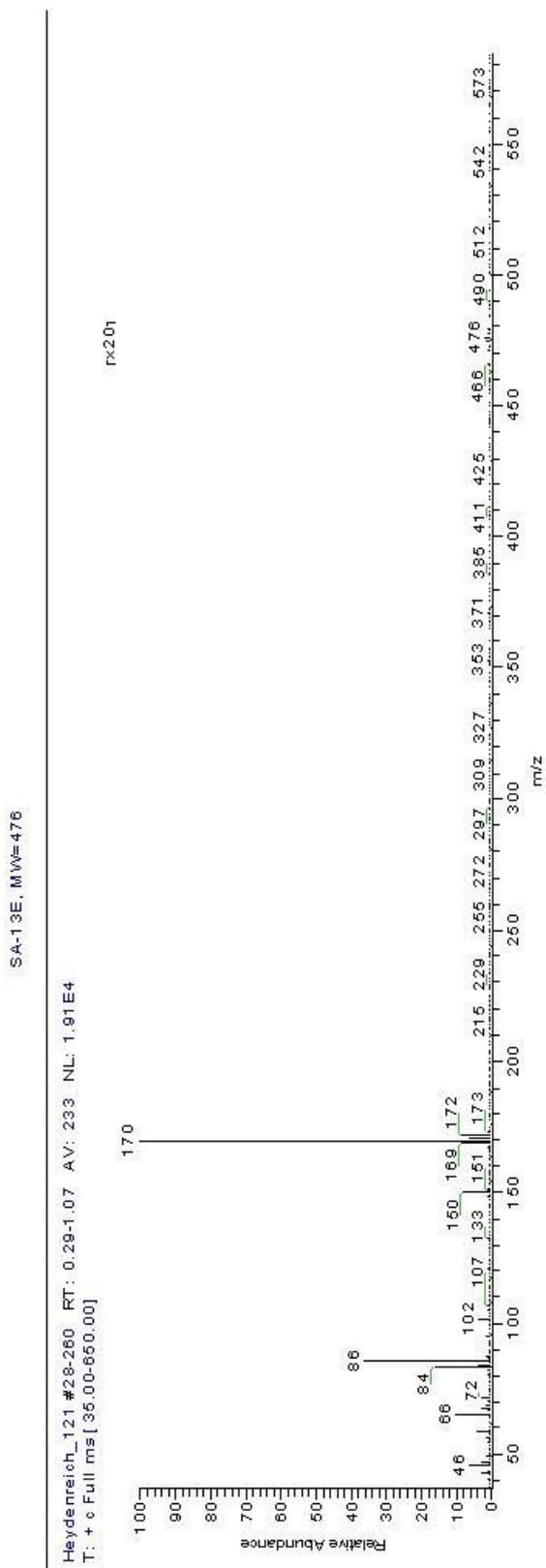
Appendix 11: ¹H NMR spectrum of Toonapubesins F (**194**)
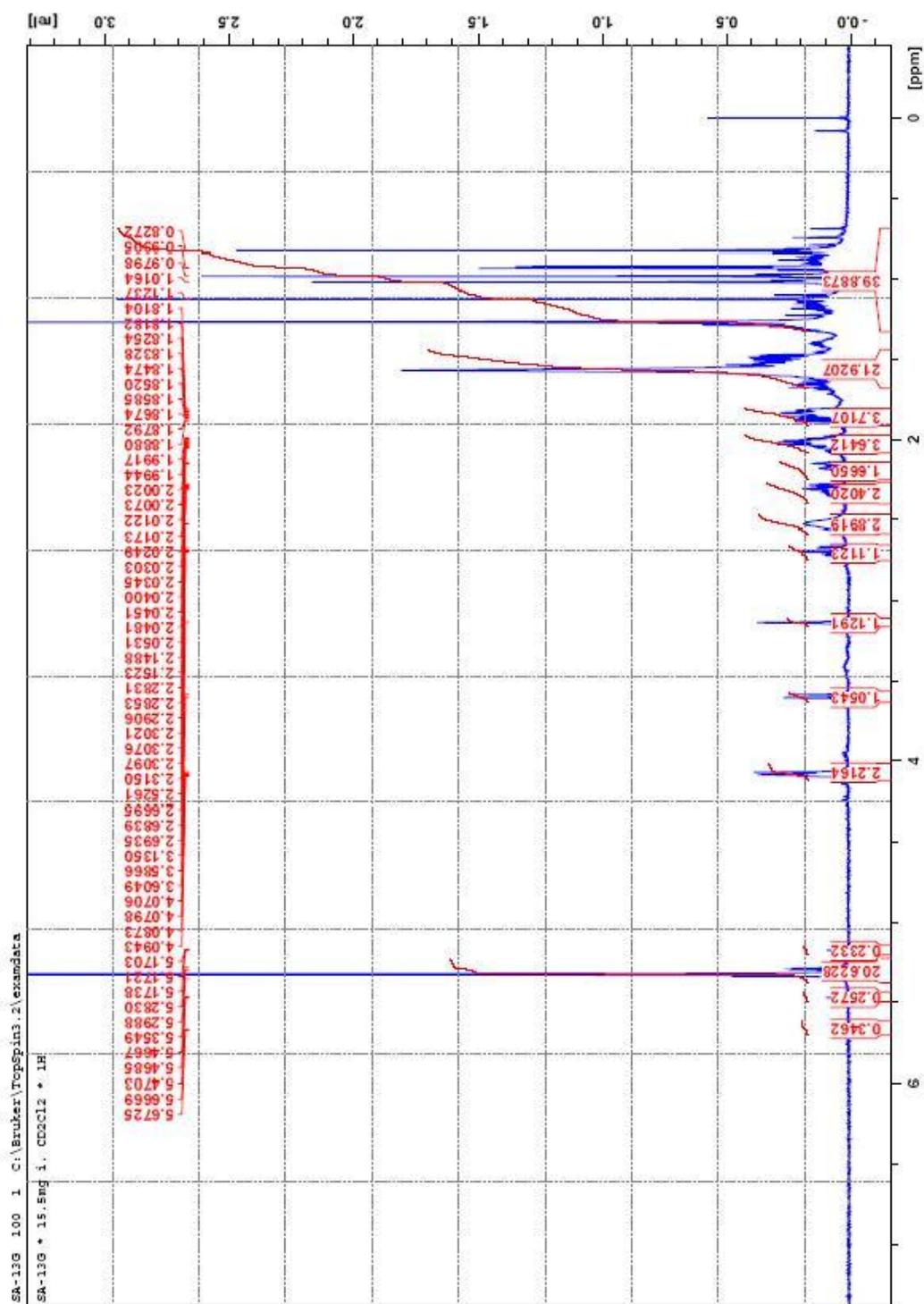
Appendix 11A: ^{13}C NMR spectrum Toonapubesins F (**194**)
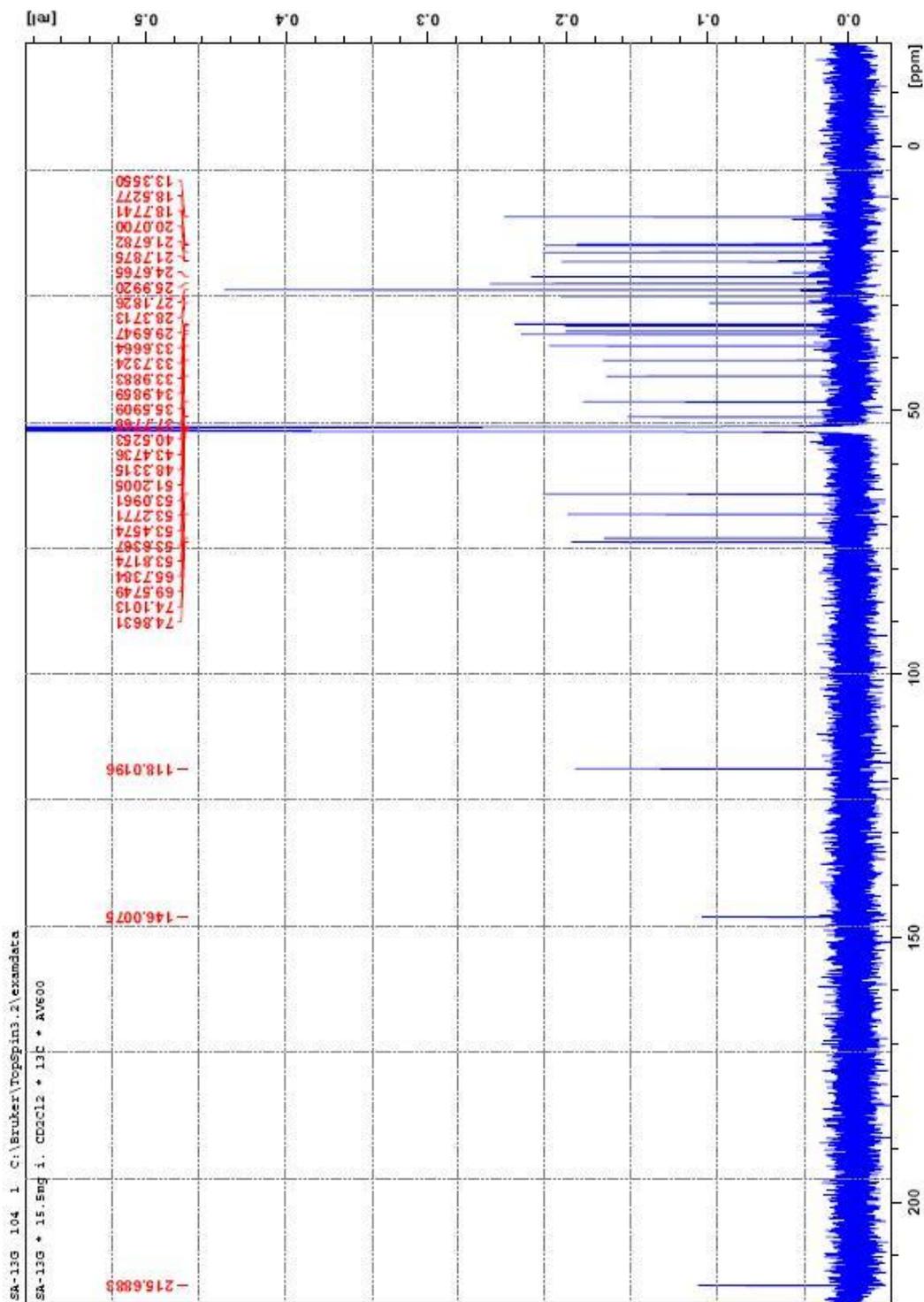
Appendix 11B: MS spectrum Toonapubesins F (**194**)
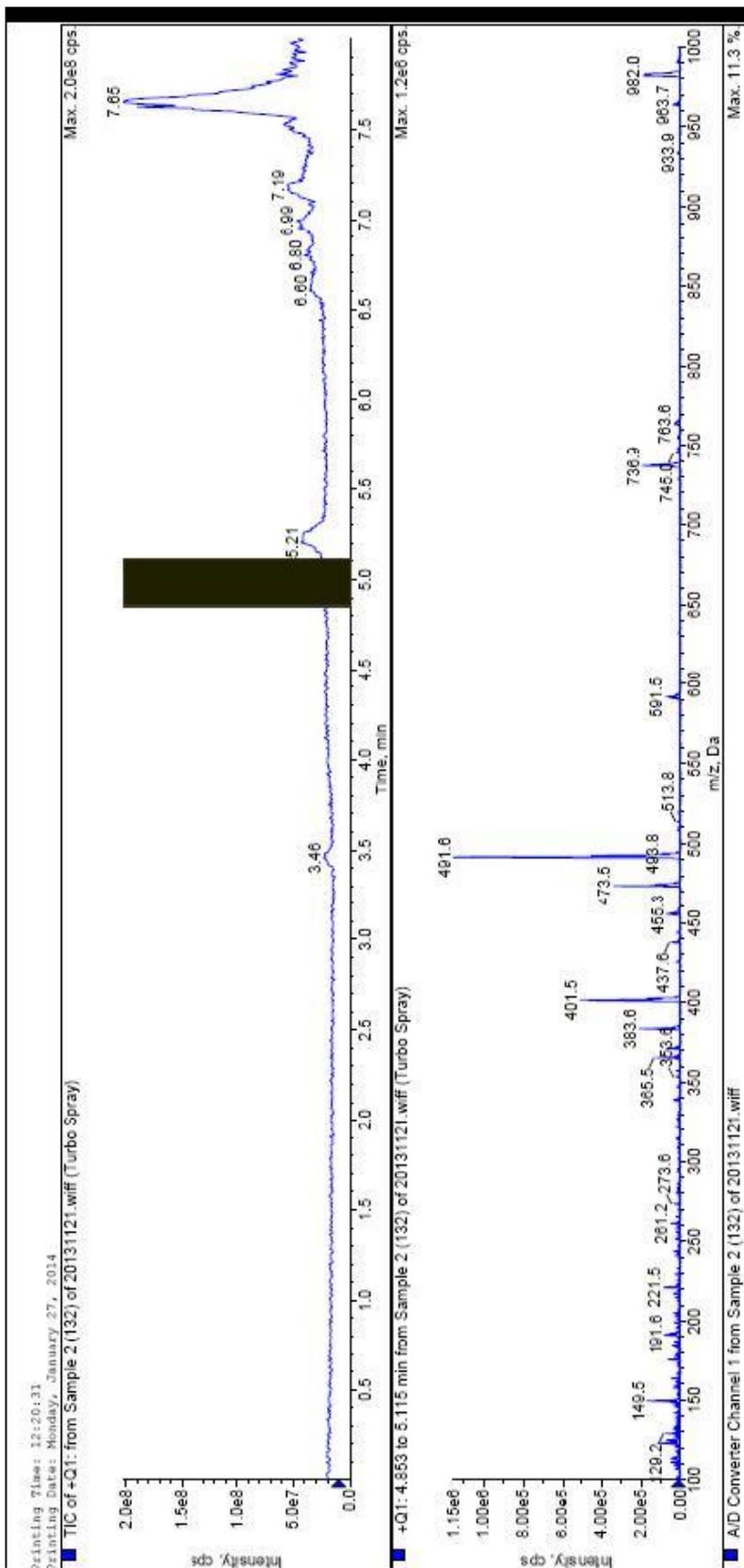
Appendix 11C: Crystal data and structure refinement for toonapubesin F (**194**)

Empirical formula	C30 H50 O5
Formula weight	490.70
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	C 2
Unit cell dimensions	$a = 29.412(10)$ Å $\qquad a = 90°$.
	$b = 7.2554(16)$ Å $\qquad b = 134.122(7)°$.
	$c = 18.168(6)$ Å $\qquad g = 90°$.
Volume	2783.1(14) Å^3
Z	4
Density (calculated)	1.171 Mg/m^3
Absorption coefficient	0.077 mm^{-1}
F(000)	1080
Crystal size	0.2 x 0.2 x 0.05 mm^3
Theta range for data collection	1.929 to 25.996°.
Index ranges	-34<=h<=34, -8<=k<=8, -22<=l<=22
Reflections collected	9862
Independent reflections	5295 [R(int) = 0.0435]
Completeness to theta = 25.242°	96.9 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5295 / 1 / 331
Goodness-of-fit on F^2	1.085
Final R indices [I>2sigma(I)]	R1 = 0.0631, wR2 = 0.1409
R indices (all data)	R1 = 0.0737, wR2 = 0.1469
Largest diff. peak and hole	0.324 and -0.244 e.Å^{-3}

Appendix 11D: Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2x 10^3) for toonapubesin F (**194**)

(U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.)

	x	y	z	U(eq)
C(4)	3698(2)	5537(6)	-709(3)	19(1)
C(5)	3728(2)	5818(6)	187(3)	18(1)
C(10)	4262(2)	4805(6)	1231(3)	19(1)
C(1)	4320(2)	2807(6)	1025(3)	22(1)
C(2)	4391(2)	2677(7)	260(3)	23(1)
C(3)	3808(2)	3503(6)	-733(3)	22(1)
C(6)	3693(2)	7861(6)	372(3)	21(1)
C(7)	3674(2)	8060(6)	1178(3)	21(1)
C(8)	3818(2)	6687(6)	1810(3)	17(1)
C(9)	4020(2)	4783(6)	1769(3)	16(1)
C(11)	4469(2)	3868(7)	2833(3)	23(1)
C(12)	4191(2)	3717(6)	3307(3)	20(1)
C(13)	3671(2)	5159(6)	2886(3)	16(1)
C(14)	3822(2)	6994(6)	2648(3)	18(1)
C(15)	3324(2)	8316(6)	2409(3)	21(1)
C(16)	3290(2)	7743(6)	3195(3)	23(1)
C(17)	3613(2)	5821(6)	3638(3)	18(1)
C(24)	3045(2)	6116(6)	-1735(3)	23(1)
C(25)	4207(2)	6607(7)	-561(3)	24(1)
C(26)	4913(2)	5782(7)	1925(3)	24(1)
C(28)	4482(2)	7814(7)	3606(3)	25(1)
C(27)	3008(2)	4453(6)	1896(3)	20(1)
C(18)	3264(2)	4567(6)	3796(3)	19(1)
C(19)	3246(2)	5455(6)	4554(3)	19(1)
C(20)	2707(2)	4766(6)	4439(3)	18(1)
C(21)	2055(2)	5513(6)	3468(3)	19(1)
C(22)	1467(2)	4722(6)	3194(3)	20(1)
C(23)	868(2)	5453(7)	2148(3)	26(1)
C(30)	1432(2)	5098(6)	3978(3)	24(1)
C(29)	3551(2)	2635(7)	4170(3)	26(1)
O(2)	4257(2)	5897(5)	-1245(2)	29(1)
O(3)	2062(1)	7506(4)	3528(2)	22(1)
O(4)	2849(1)	5337(4)	5347(2)	22(1)
O(5)	1465(1)	2758(4)	3058(2)	22(1)
O(1)	3425(2)	2536(5)	-1508(2)	32(1)

Appendix 11E: Bond lengths [Å] and angles [°] for toonapubesin A (**194**)

C(4)-C(3)	1.518(6)	C(26)-C(10)-C(5)	113.6(3)
C(4)-C(24)	1.535(6)	C(9)-C(10)-C(5)	104.7(3)
C(4)-C(25)	1.536(6)	C(10)-C(1)-C(2)	112.6(4)
C(4)-C(5)	1.579(5)	C(3)-C(2)-C(1)	107.4(3)
C(5)-C(6)	1.540(6)	O(1)-C(3)-C(2)	120.9(4)
C(5)-C(10)	1.568(6)	O(1)-C(3)-C(4)	122.7(4)
C(10)-C(1)	1.535(6)	C(2)-C(3)-C(4)	116.3(4)
C(10)-C(26)	1.548(6)	C(7)-C(6)-C(5)	111.0(3)
C(10)-C(9)	1.561(5)	C(8)-C(7)-C(6)	123.7(4)
C(1)-C(2)	1.547(5)	C(7)-C(8)-C(9)	122.0(3)
C(2)-C(3)	1.499(5)	C(7)-C(8)-C(14)	121.4(4)
C(3)-O(1)	1.239(5)	C(9)-C(8)-C(14)	116.5(3)
C(6)-C(7)	1.509(5)	C(8)-C(9)-C(11)	110.7(3)
C(7)-C(8)	1.346(6)	C(8)-C(9)-C(10)	112.6(3)
C(8)-C(9)	1.526(6)	C(11)-C(9)-C(10)	115.0(3)
C(8)-C(14)	1.529(5)	C(9)-C(11)-C(12)	113.8(3)
C(9)-C(11)	1.538(6)	C(13)-C(12)-C(11)	113.6(3)
C(11)-C(12)	1.551(5)	C(12)-C(13)-C(27)	111.2(3)
C(12)-C(13)	1.546(5)	C(12)-C(13)-C(14)	109.9(3)
C(13)-C(27)	1.555(5)	C(27)-C(13)-C(14)	109.4(3)
C(13)-C(14)	1.557(5)	C(12)-C(13)-C(17)	116.6(3)
C(13)-C(17)	1.566(5)	C(27)-C(13)-C(17)	107.3(3)
C(14)-C(15)	1.539(6)	C(14)-C(13)-C(17)	101.9(3)
C(14)-C(28)	1.564(6)	C(8)-C(14)-C(15)	117.6(3)
C(15)-C(16)	1.558(5)	C(8)-C(14)-C(13)	110.2(3)
C(16)-C(17)	1.559(6)	C(15)-C(14)-C(13)	102.2(3)
C(17)-C(18)	1.543(5)	C(8)-C(14)-C(28)	107.9(3)
C(25)-O(2)	1.445(5)	C(15)-C(14)-C(28)	106.1(3)
C(18)-C(29)	1.531(6)	C(13)-C(14)-C(28)	112.9(3)
C(18)-C(19)	1.554(5)	C(14)-C(15)-C(16)	103.4(3)
C(19)-C(20)	1.531(6)	C(15)-C(16)-C(17)	107.8(3)
C(20)-O(4)	1.453(4)	C(18)-C(17)-C(16)	112.1(3)
C(20)-C(21)	1.540(6)	C(18)-C(17)-C(13)	118.3(3)
C(21)-O(3)	1.449(5)	C(16)-C(17)-C(13)	102.8(3)
C(21)-C(22)	1.539(6)	O(2)-C(25)-C(4)	109.9(4)
C(22)-O(5)	1.445(5)	C(29)-C(18)-C(17)	112.9(3)
C(22)-C(30)	1.523(5)	C(29)-C(18)-C(19)	109.6(3)
C(22)-C(23)	1.528(6)	C(17)-C(18)-C(19)	111.0(3)
C(3)-C(4)-C(24)	110.0(3)	C(20)-C(19)-C(18)	114.5(3)
C(3)-C(4)-C(25)	107.1(3)	O(4)-C(20)-C(19)	107.4(3)
C(24)-C(4)-C(25)	108.5(3)	O(4)-C(20)-C(21)	110.1(3)
C(3)-C(4)-C(5)	107.2(3)	C(19)-C(20)-C(21)	112.8(3)
C(24)-C(4)-C(5)	110.0(3)	O(3)-C(21)-C(22)	110.5(3)
C(25)-C(4)-C(5)	114.0(3)	O(3)-C(21)-C(20)	108.9(3)
C(6)-C(5)-C(10)	110.5(3)	C(22)-C(21)-C(20)	117.6(3)
C(6)-C(5)-C(4)	112.7(3)	O(5)-C(22)-C(30)	110.0(3)
C(10)-C(5)-C(4)	118.5(3)	O(5)-C(22)-C(23)	105.5(3)
C(1)-C(10)-C(26)	110.3(4)	C(30)-C(22)-C(23)	109.4(4)
C(1)-C(10)-C(9)	108.6(3)	O(5)-C(22)-C(21)	107.0(3)
C(26)-C(10)-C(9)	109.8(3)	C(30)-C(22)-C(21)	115.0(3)
C(1)-C(10)-C(5)	109.6(3)	C(23)-C(22)-C(21)	109.6(3)

Symmetry transformations used to generate equivalent atoms:

Appendix 11F: Anisotropic displacement parameters ($\mathring{A}^2$x 10^3)for toonapubsins F. The anisotropic displacement factor exponent takes the form: $-2p^2[$ $h^2a*^2U^{11}$ + ... + 2 h k a* b* U^{12}] (**194**)

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(4)	19(2)	24(2)	15(2)	1(2)	12(2)	2(2)
C(5)	19(2)	22(2)	16(2)	2(2)	13(2)	0(2)
C(10)	16(2)	26(2)	14(2)	-1(2)	10(2)	0(2)
C(1)	25(2)	25(2)	22(2)	3(2)	19(2)	4(2)
C(2)	23(2)	28(2)	16(2)	3(2)	13(2)	6(2)
C(3)	21(2)	28(2)	17(2)	0(2)	13(2)	5(2)
C(6)	30(2)	20(2)	22(2)	4(2)	21(2)	3(2)
C(7)	27(2)	19(2)	21(2)	1(2)	18(2)	1(2)
C(8)	17(2)	21(2)	16(2)	-1(2)	12(2)	1(2)
C(9)	18(2)	19(2)	16(2)	2(2)	13(2)	1(2)
C(11)	23(2)	31(2)	16(2)	3(2)	15(2)	4(2)
C(12)	22(2)	23(2)	17(2)	7(2)	14(2)	5(2)
C(13)	18(2)	19(2)	14(2)	1(2)	11(2)	1(2)
C(14)	18(2)	20(2)	16(2)	-2(2)	13(2)	-3(2)
C(15)	29(2)	18(2)	23(2)	2(2)	21(2)	3(2)
C(16)	29(2)	23(2)	27(2)	2(2)	23(2)	3(2)
C(17)	20(2)	18(2)	17(2)	-2(2)	14(2)	-1(2)
C(24)	20(2)	29(2)	15(2)	2(2)	11(2)	5(2)
C(25)	25(2)	31(2)	20(2)	3(2)	17(2)	2(2)
C(26)	19(2)	38(3)	16(2)	0(2)	12(2)	-1(2)
C(28)	28(2)	27(2)	21(2)	-6(2)	17(2)	-9(2)
C(27)	22(2)	24(2)	18(2)	-4(2)	15(2)	-5(2)
C(18)	25(2)	19(2)	18(2)	-3(2)	16(2)	-2(2)
C(19)	23(2)	23(2)	16(2)	-3(2)	15(2)	-2(2)
C(20)	24(2)	21(2)	14(2)	-2(2)	14(2)	0(2)
C(21)	26(2)	15(2)	16(2)	-1(2)	15(2)	0(2)
C(22)	25(2)	19(2)	17(2)	-2(2)	15(2)	0(2)
C(23)	23(2)	27(2)	21(2)	3(2)	13(2)	5(2)
C(30)	24(2)	30(3)	22(2)	-5(2)	17(2)	-5(2)
C(29)	38(2)	22(2)	28(2)	4(2)	27(2)	2(2)
O(2)	29(2)	43(2)	21(2)	7(1)	20(2)	9(2)
O(3)	32(2)	16(2)	20(2)	2(1)	19(1)	2(1)
O(4)	29(2)	28(2)	16(1)	0(1)	18(1)	-2(1)
O(5)	28(2)	17(2)	18(1)	2(1)	16(1)	1(1)
O(1)	37(2)	32(2)	22(2)	-5(2)	19(2)	1(2)

Appendix 12: ^{1}H NMR spectrum of 1α,3α-diacetyl-7α-tigloyvilasinin (**40**)
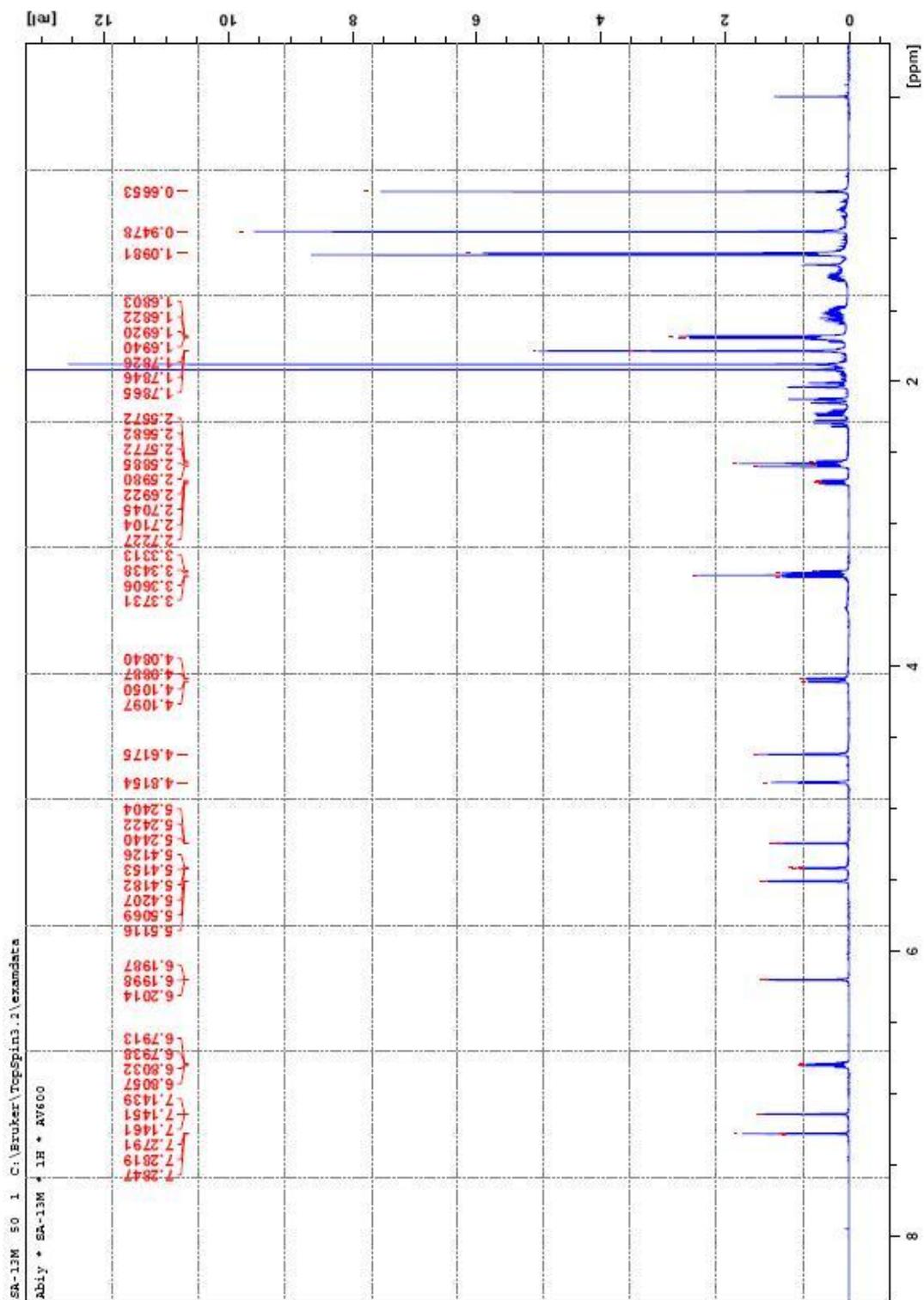
Appendix 12A: ^{13}C NMR spectrum of 1α,3α-diacetyl-7α-tigloyvilasinin (**40**)
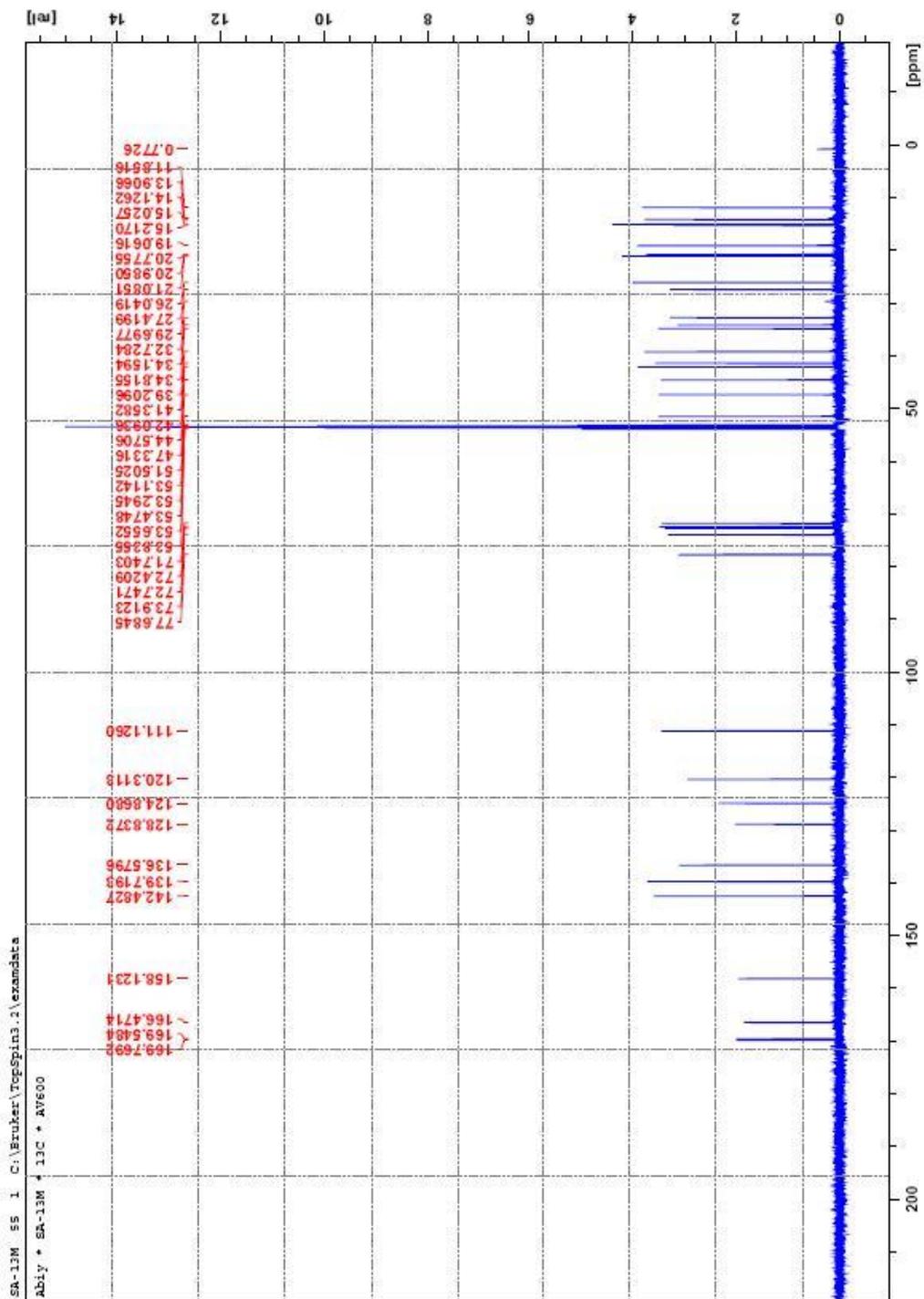
Appendix 12B: MS spectrum of 1α,3α-diacetyl-7α-tigloyvilasinin (**40**)

Appendix 13: ^{1}H NMR spectrum of sitosterol 3-*O*-*β*-D-glucopyranoside acetate (**195**) and stigmasterol 3-*O*-*β*-D-glucopyranoside mixture (**196**)

Appendix 13A: ^{13}C NMR spectrum of sitosterol 3-*O*-β-D-glucopyranoside acetate (**195**) and stigmasterol 3-*O*-β-D-glucopyranoside mixture (**196**)
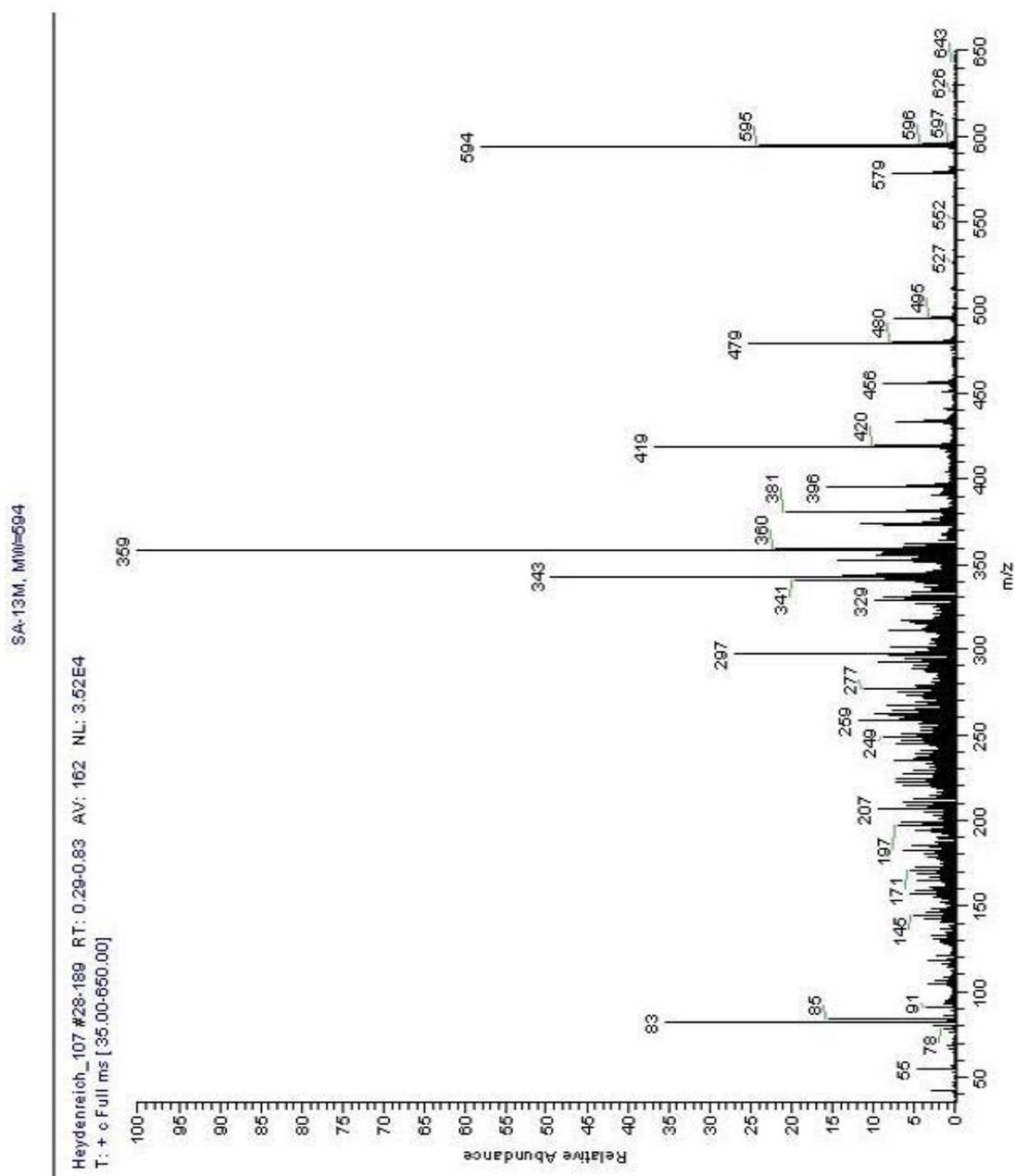
Appendix 14: ^{1}H NMR spectrum of sitosterol 3-O-β-D-glucopyranoside (**197**)
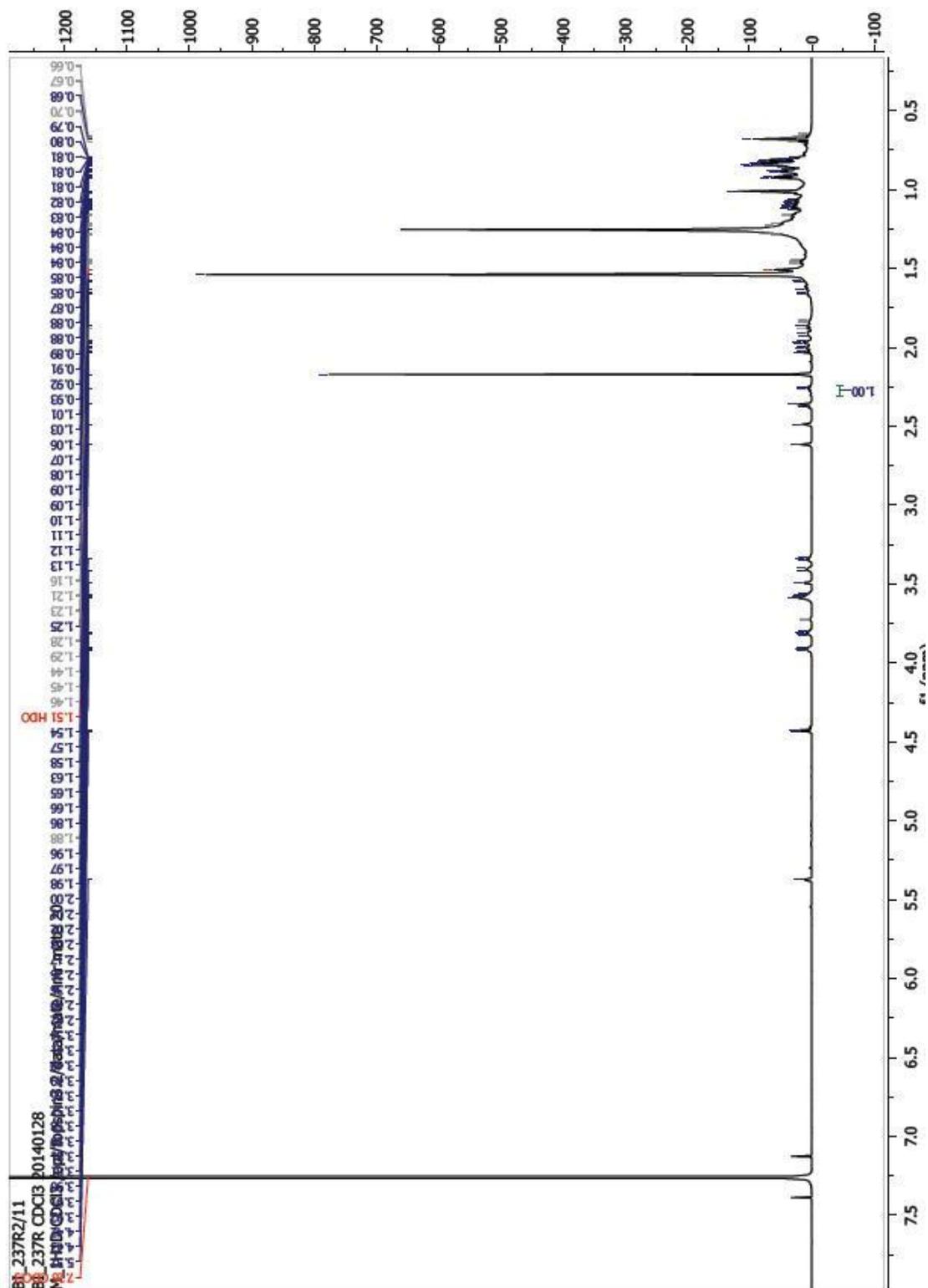
Appendix 14A: ^{13}C NMR spectrum of sitosterol 3-*O*-*β*-D-glucopyranoside (**197**)
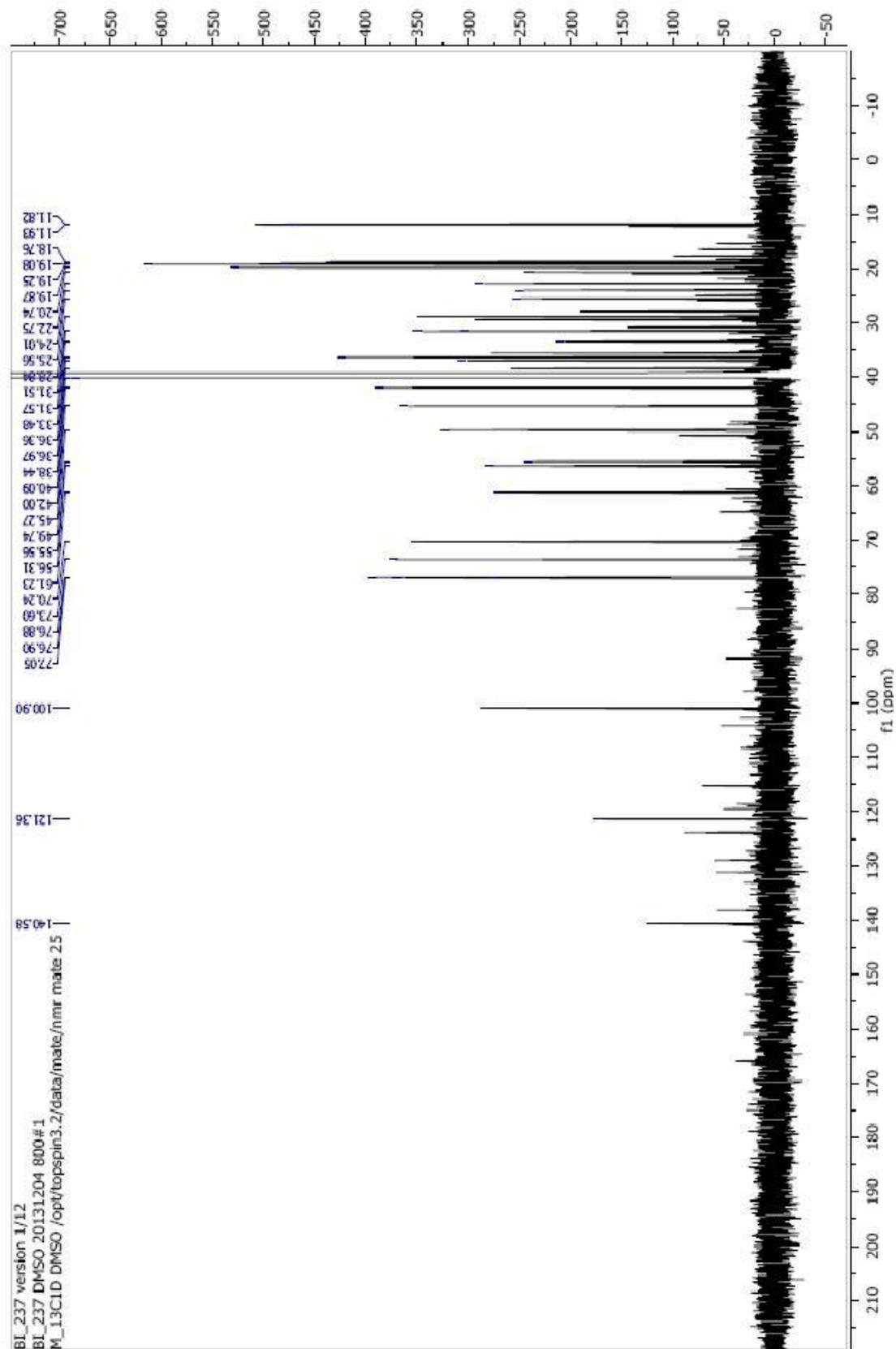
Appendix 15: ¹H NMR spectrum of β-sitosterol (**180**) and stigmasterol mixture (**193**)

Appendix 15A: ^{13}C NMR spectrum of β-sitosterol (**180**) and stigmasterol mixture (**193**)

Appendix 16: ^{1}H NMR spectrum of 3-oxo-12β-hydroxy-oleanan-28,13β olide (**198**)
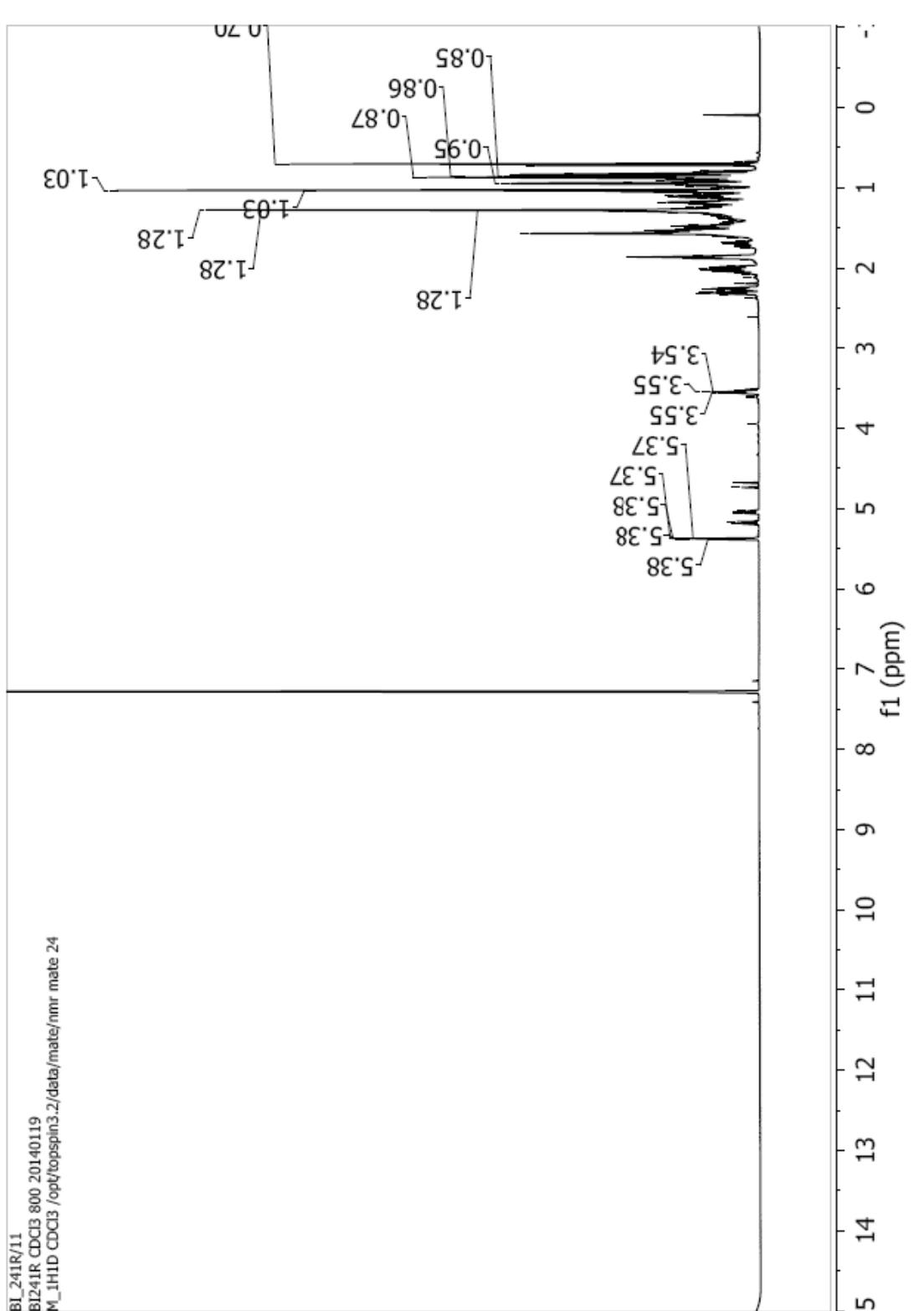
Appendix 16A: ^{13}C NMR spectrum of 3-oxo-12β-hydroxy-oleanan-28,13β olide (**198**)
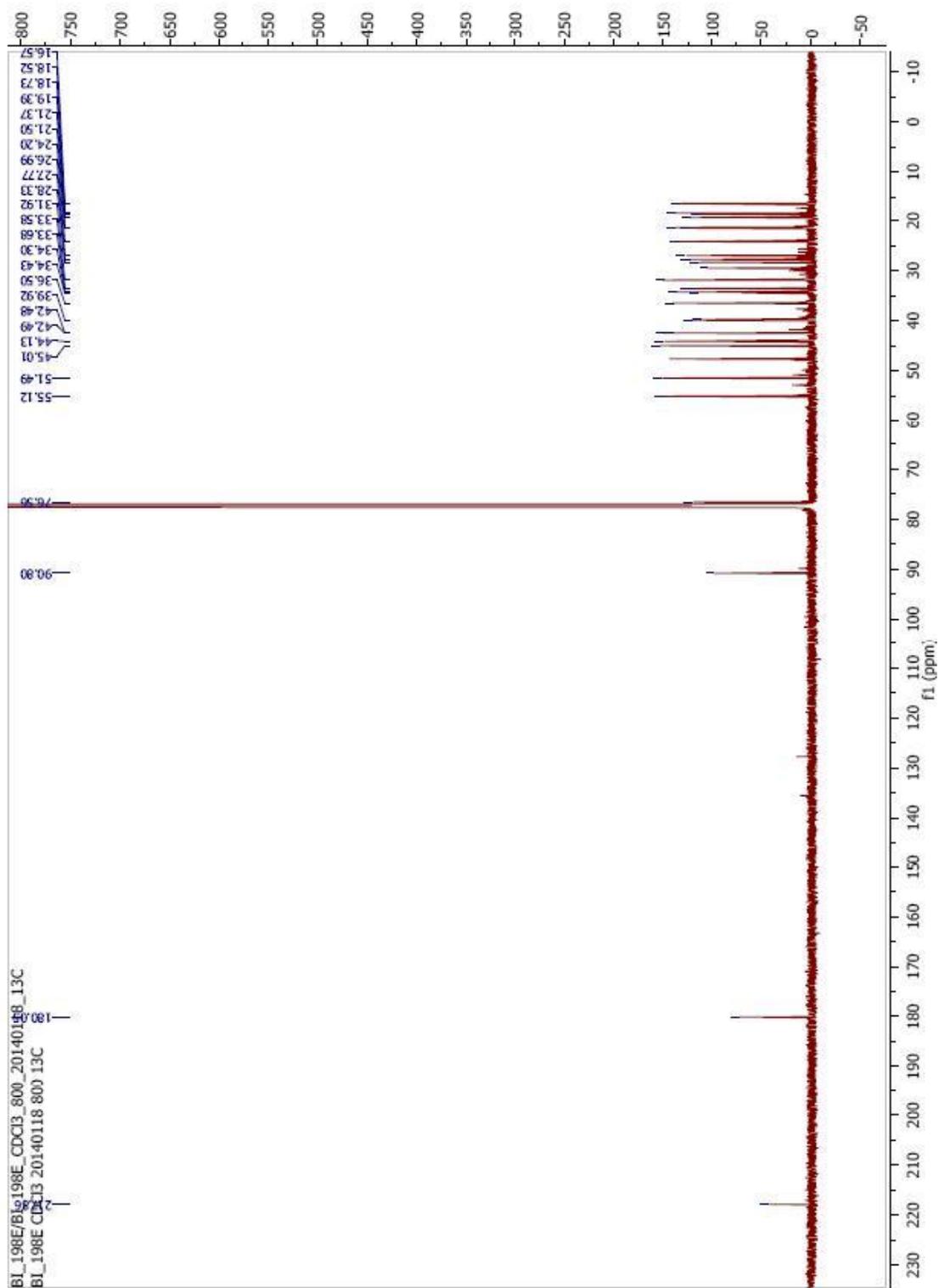
Appendix 16B: HSQC spectrum of 3-oxo-12β-hydroxy-oleanan-28,13β olide (**198**)
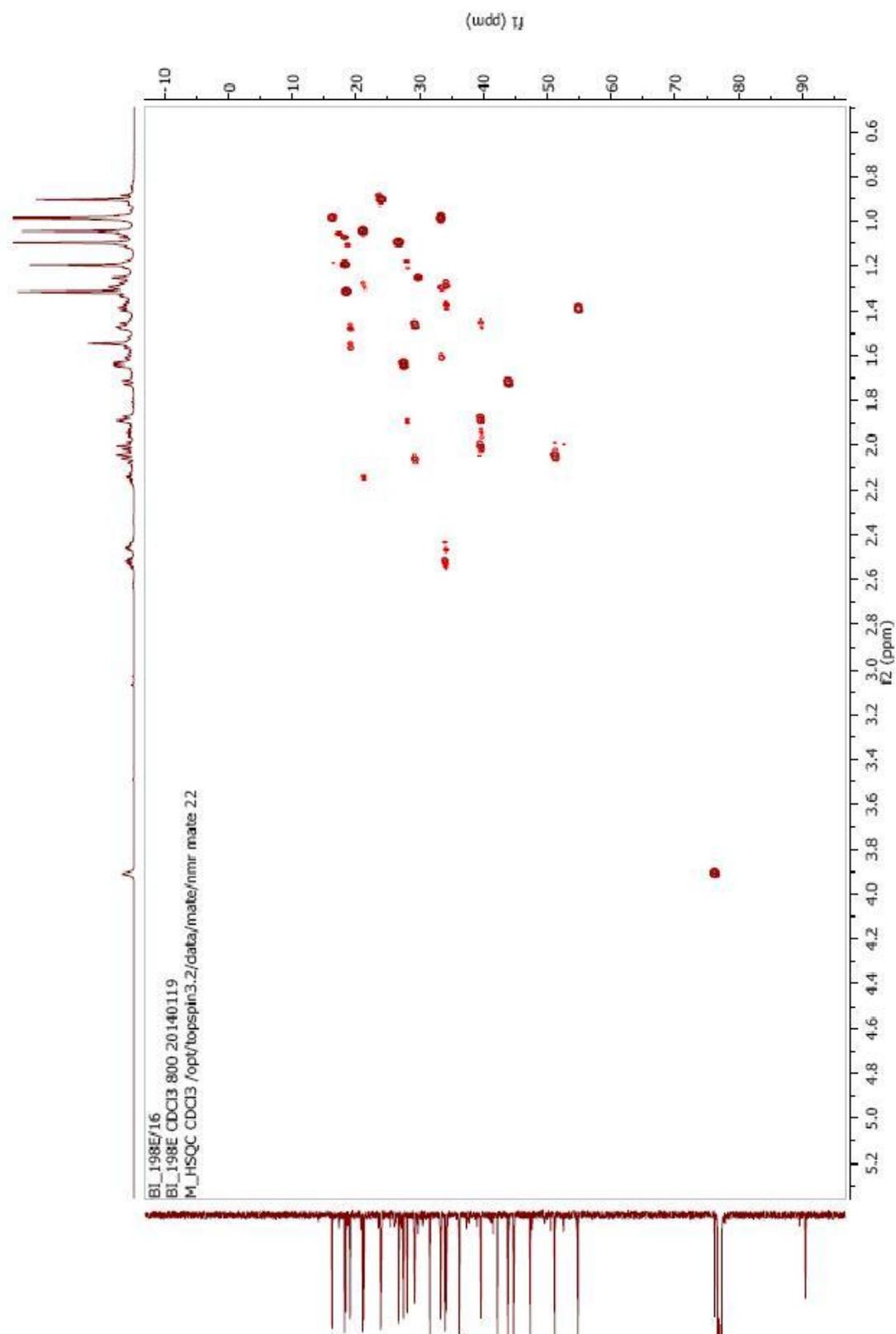
Appendix 16C: ^{1}HMBC spectrum of 3-oxo-12β-hydroxy-oleanan-28,13β olide (**198**)

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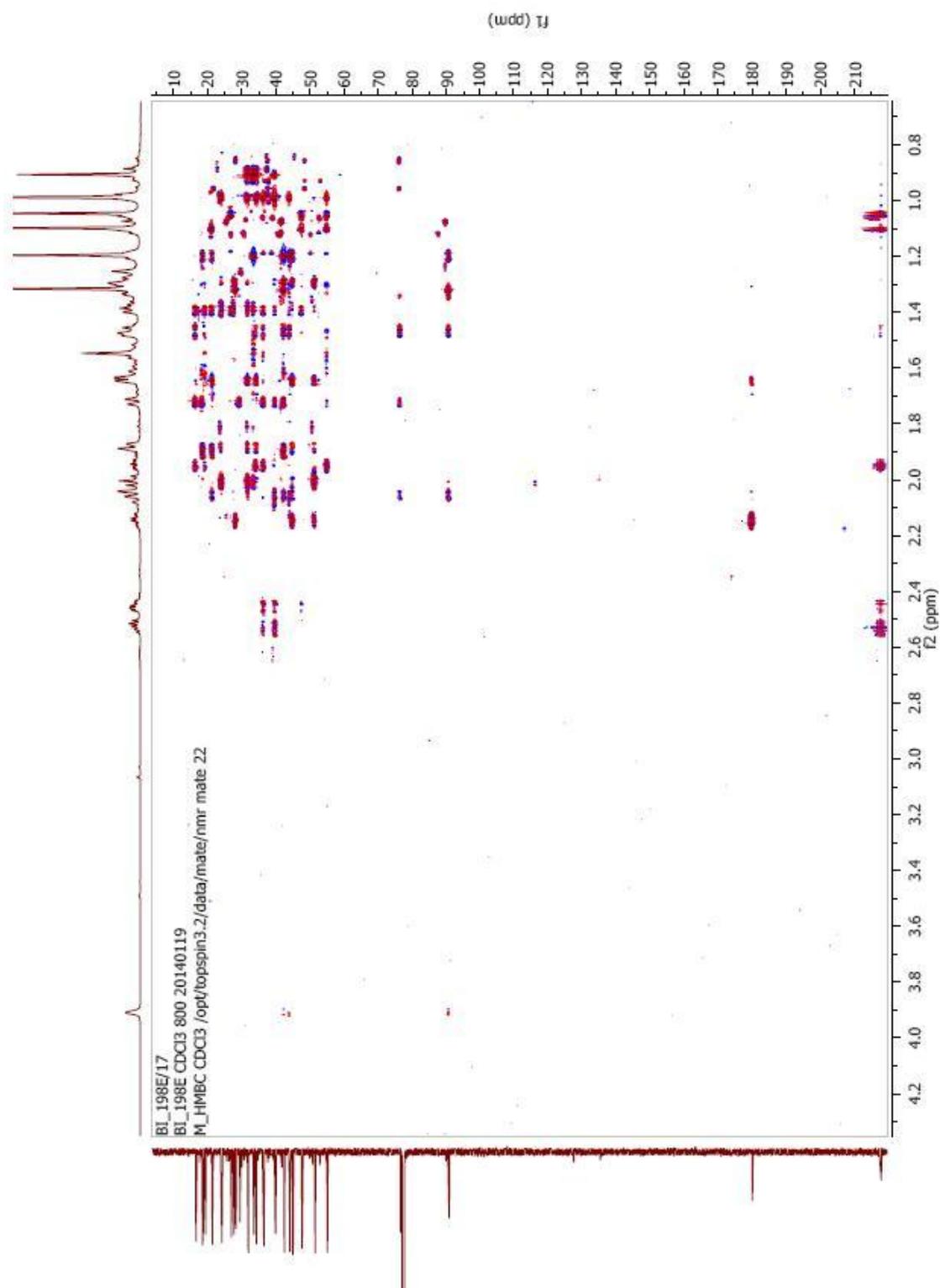
Appendix 16D: ^{1}H-H COSY spectrum of 3-oxo-12β-hydroxy-oleanan-28,13β olide (**198**)
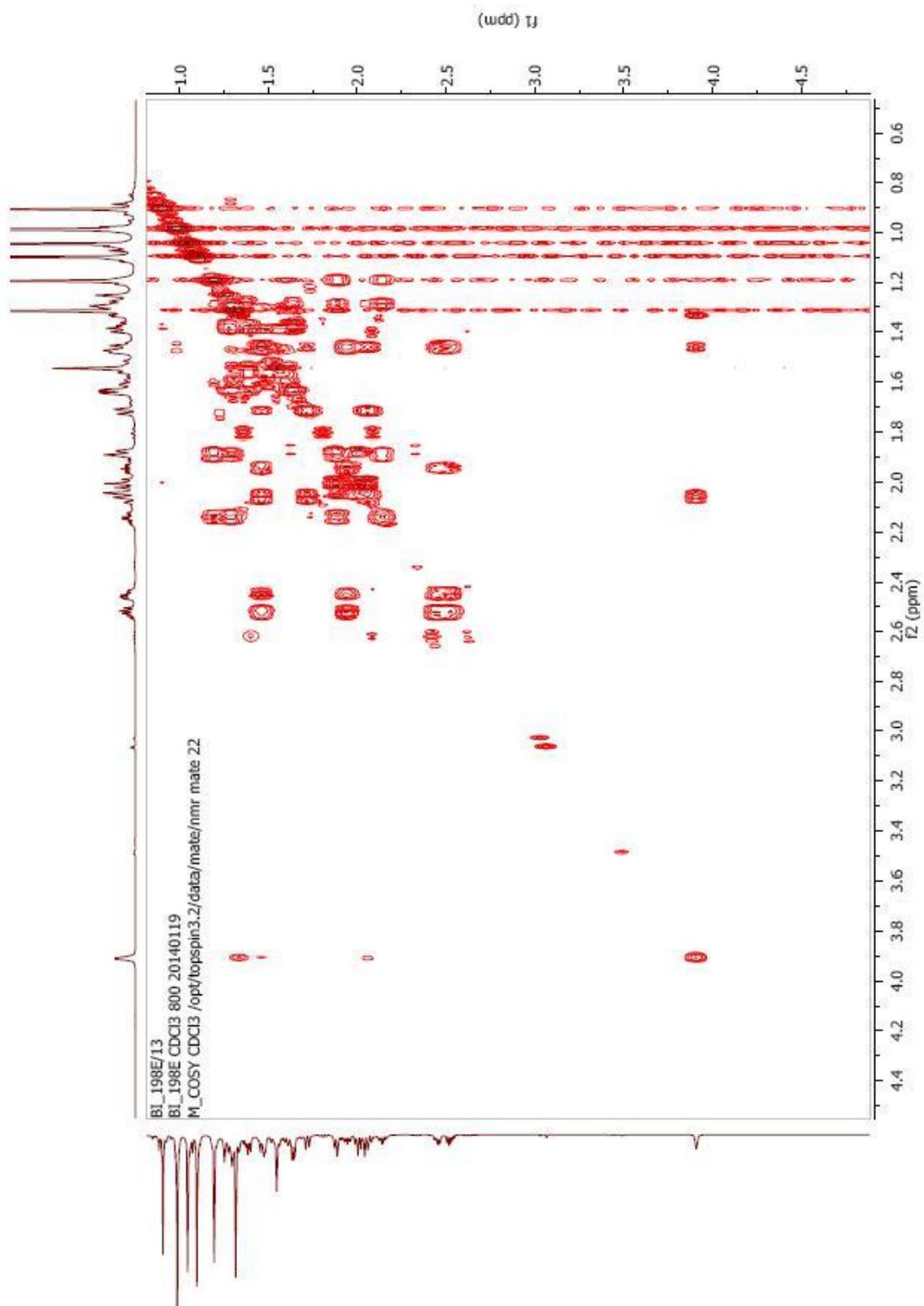
Appendix 16E: NOESY spectrum of 3-oxo-12β-hydroxy-oleanan-28,13β olide (**198**)
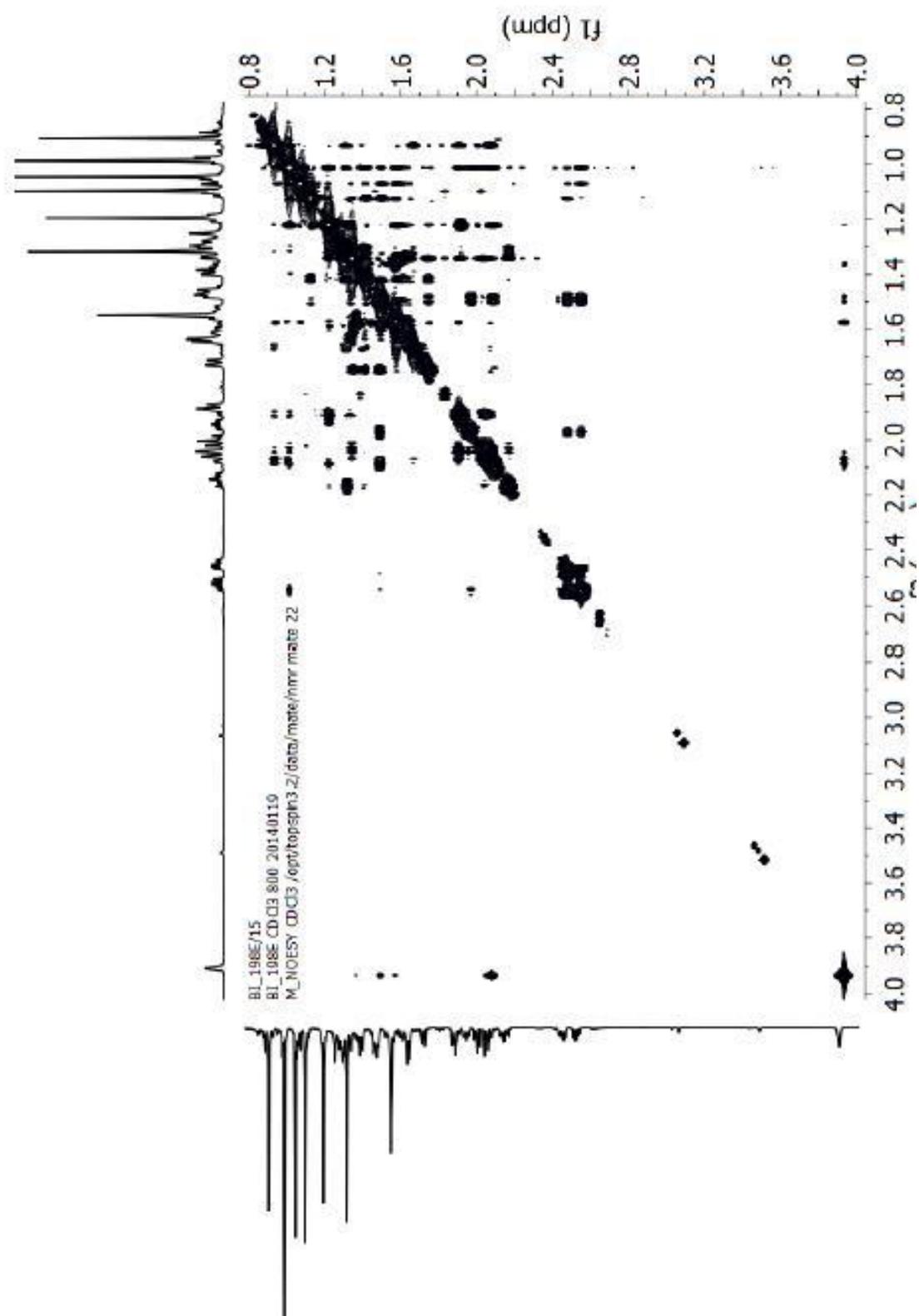
Calc $C_{30}H_{46}O_4$ M+H 471.3474

B) 198E
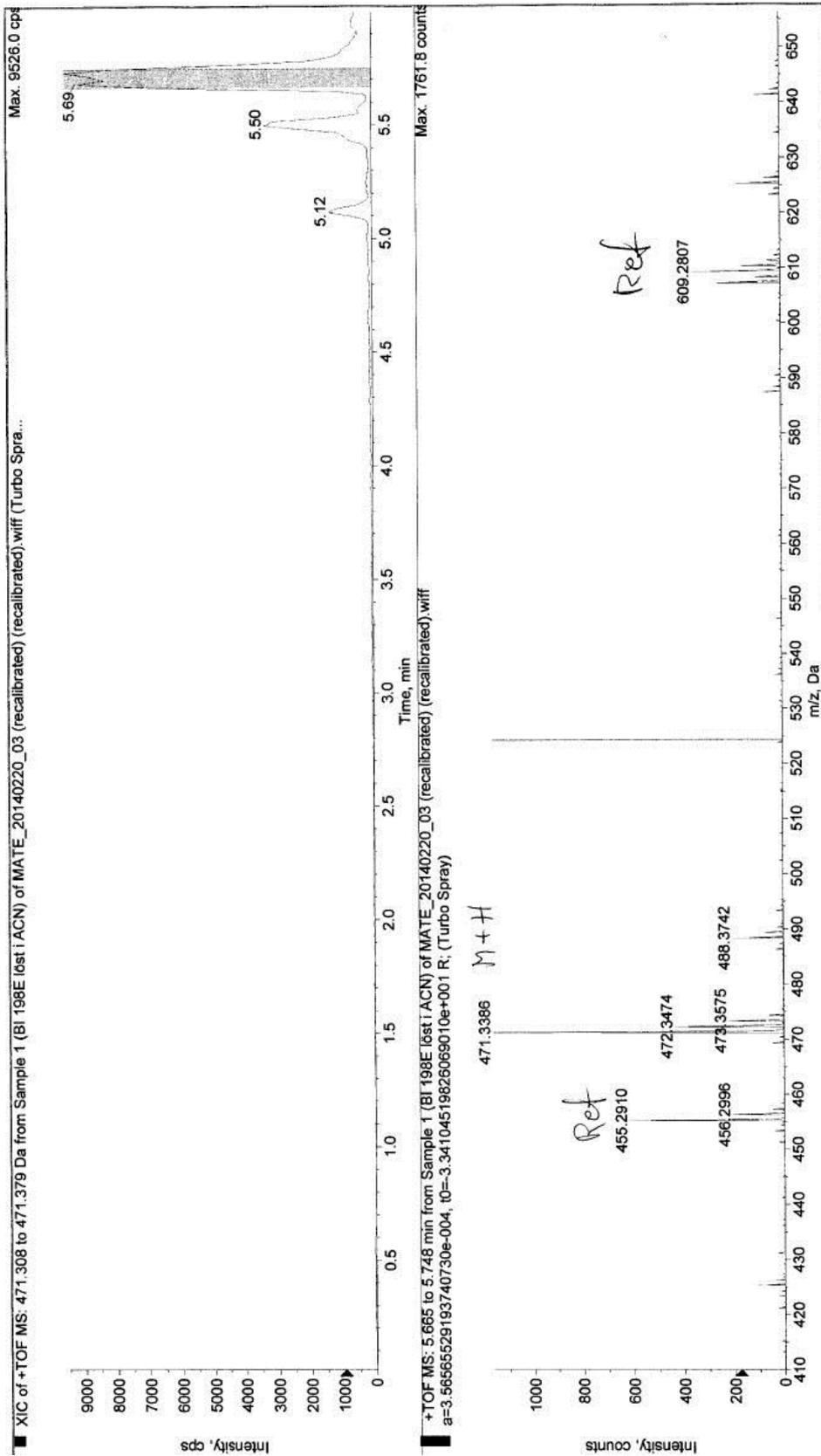
Appendix 17A: 13C NMR spectrum of ekeberin A (**158**)
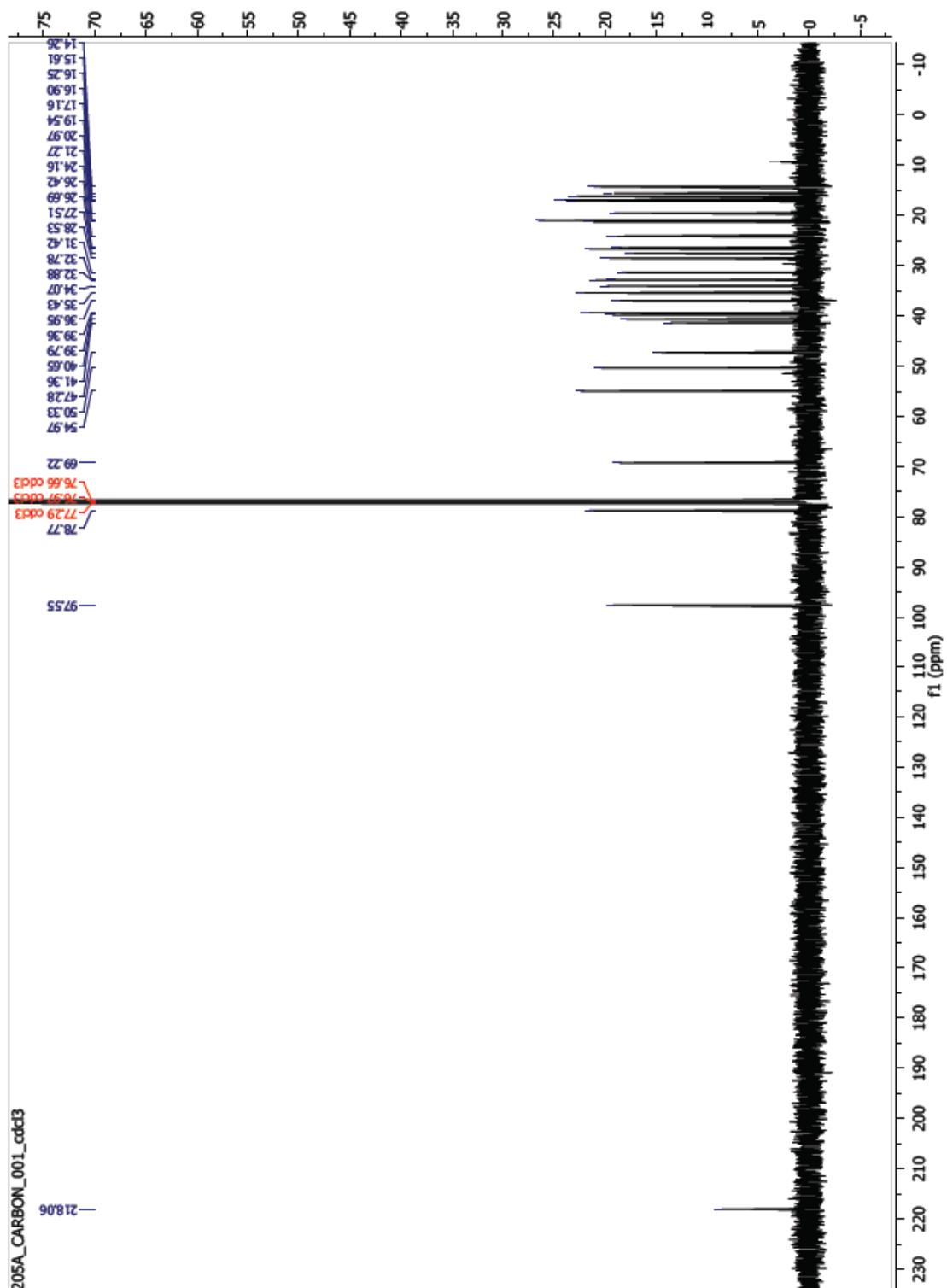
Appendix 18: ^{1}H NMR spectrum of oleanolic acid (**159**)
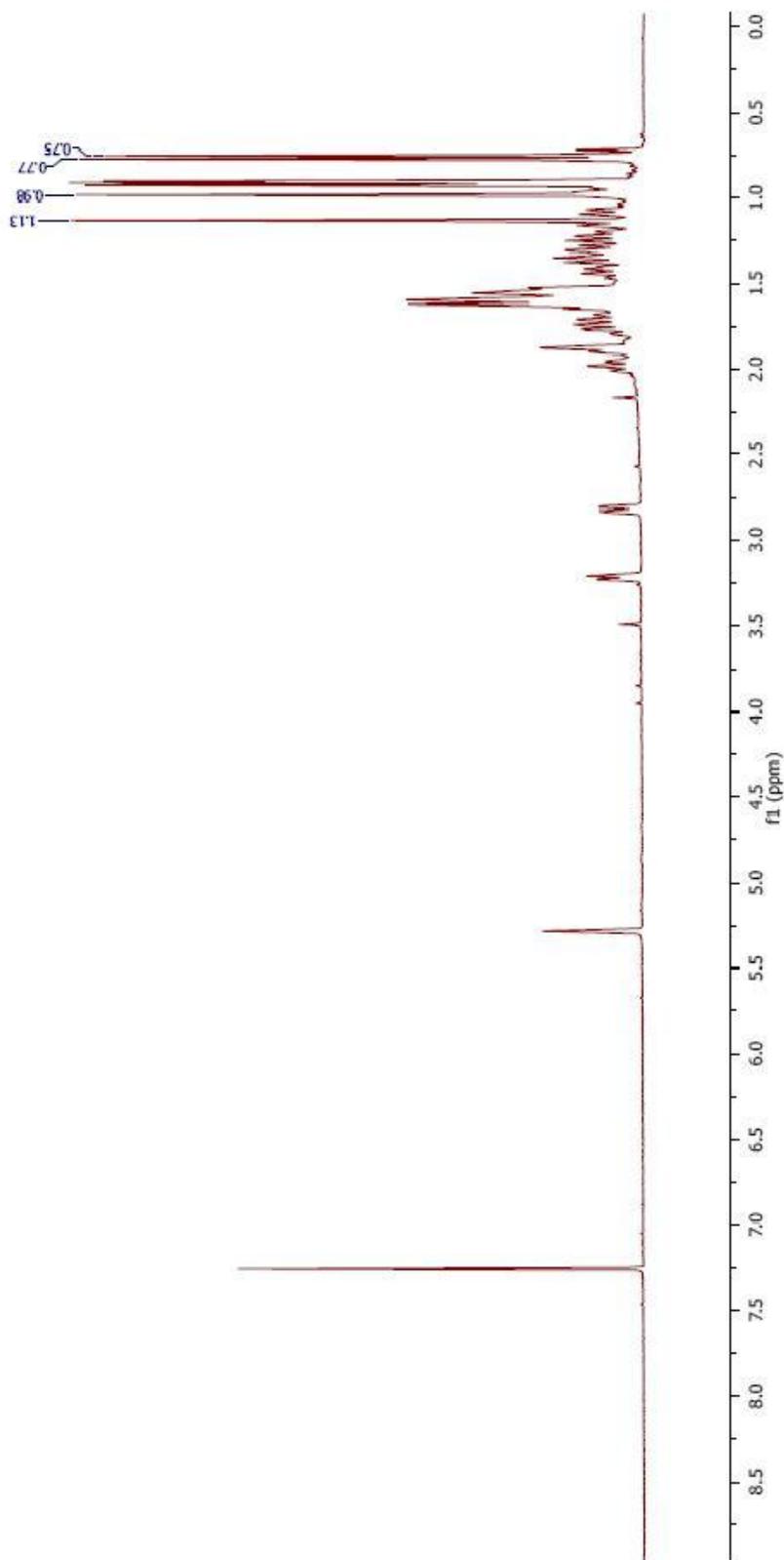
Appendix 18A: ^{13}C NMR spectrum of oleanolic acid (**159**)
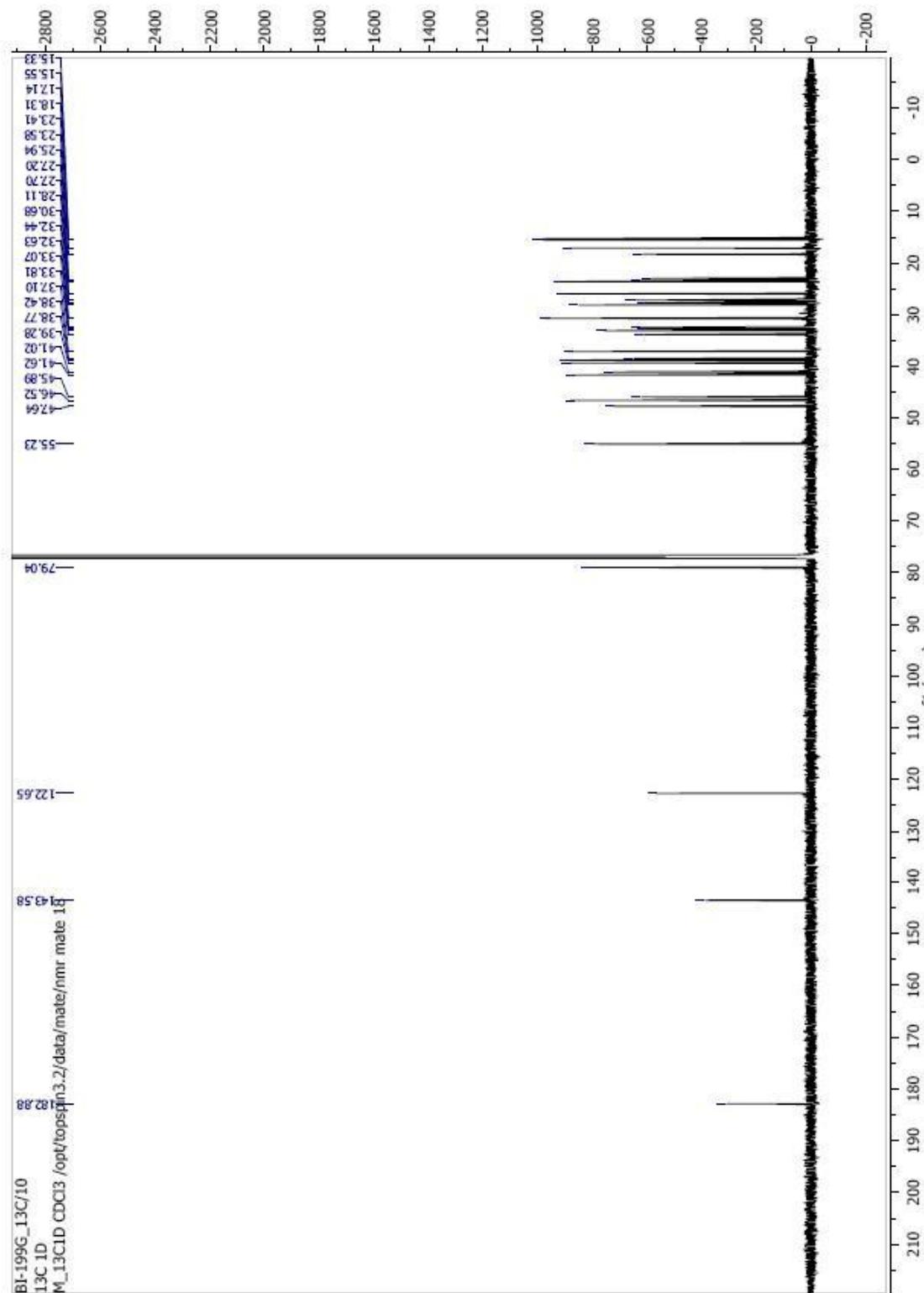
Appendix 19: ^{1}H NMR spectrum of oleanolic acid acetate (**202**)
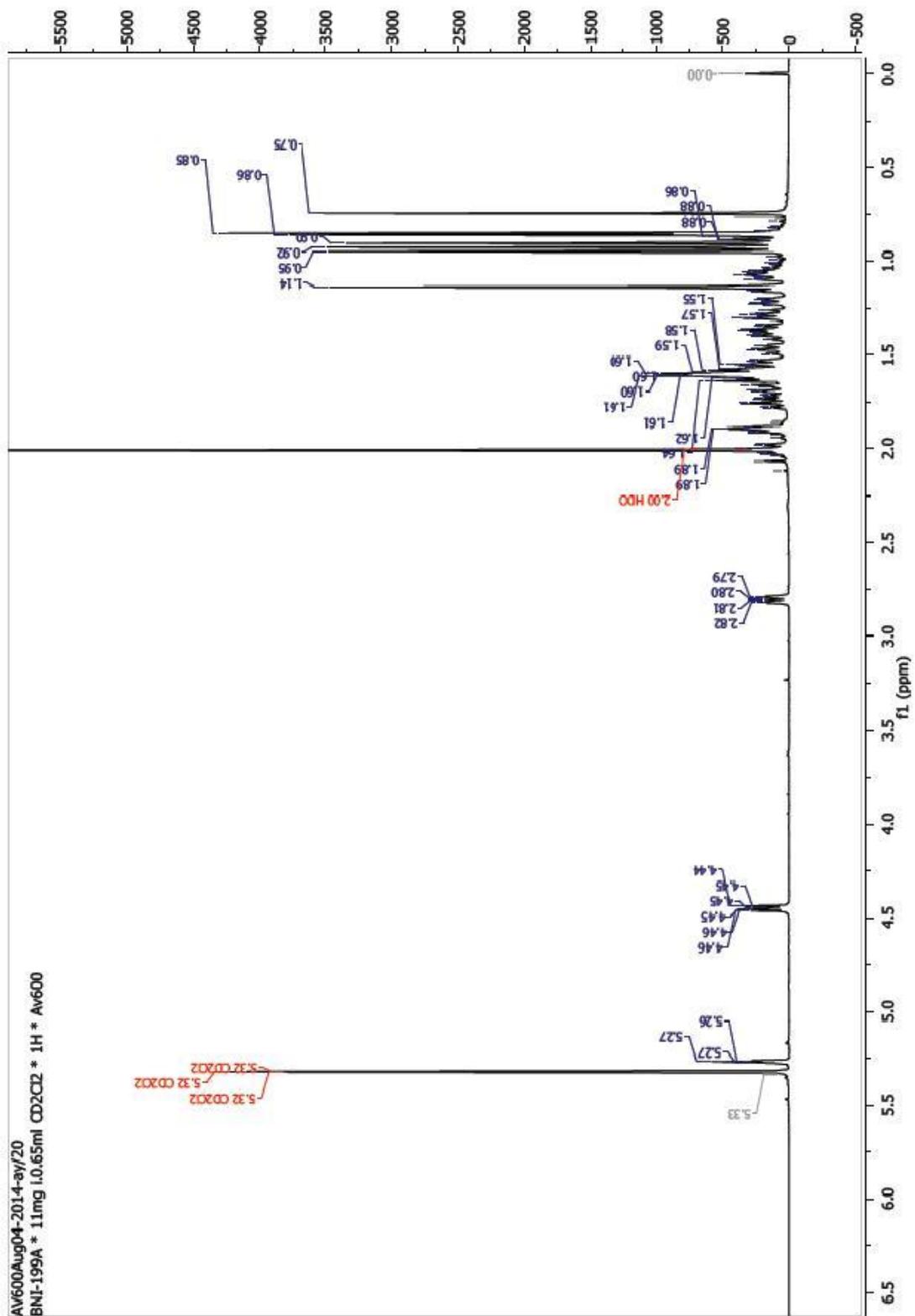
Appendix 19A: ^{13}C NMR spectrum of oleanolic acid acetate (**202**)

Appendix 20: ^{1}H NMR spectrum of oleanonic acid (**160**)
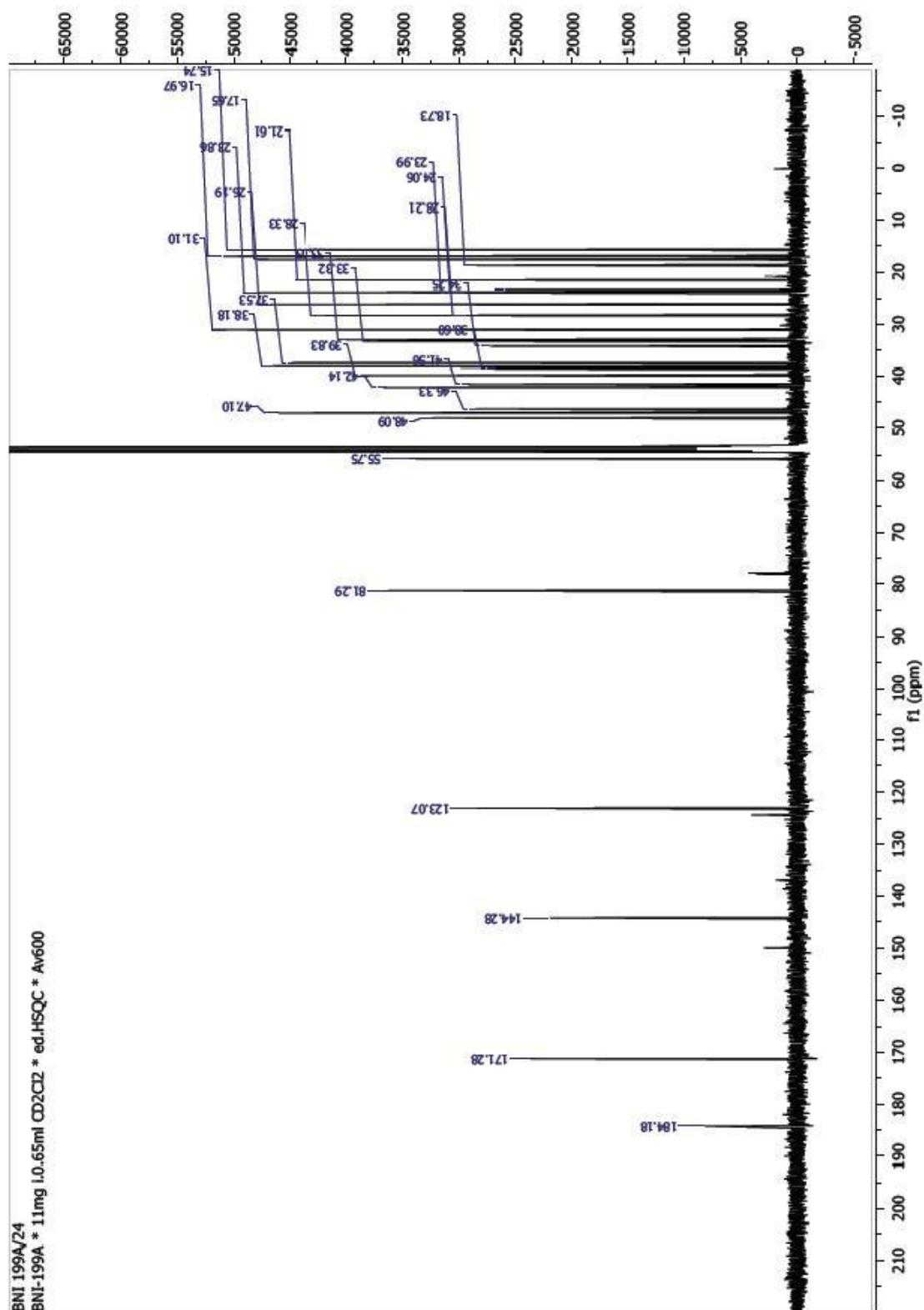
Appendix 20A: ^{13}C NMR spectrum of oleanonic acid (**160**)
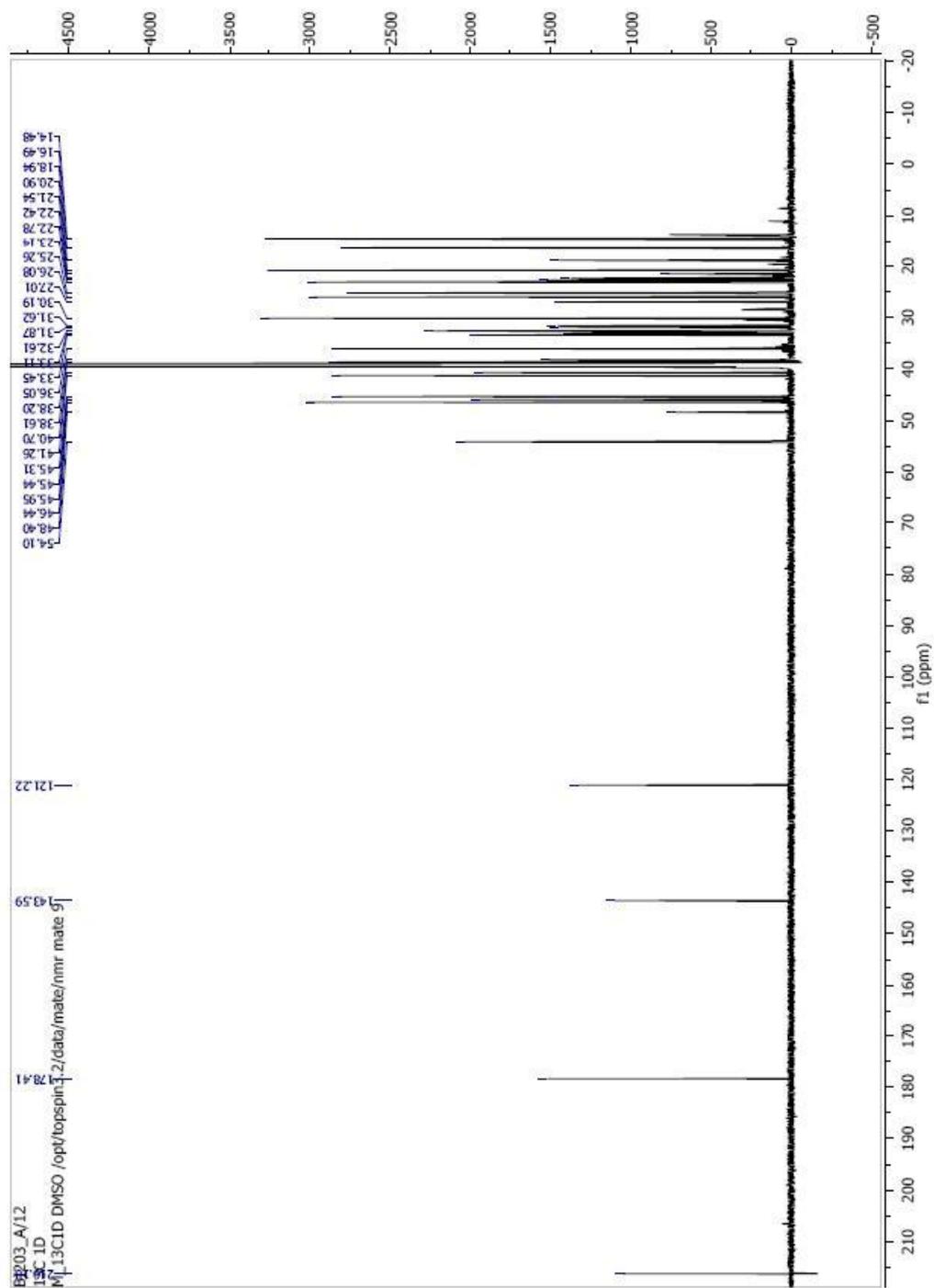
Appendix 20B: MS spectrum of oleanonic acid (**160**)
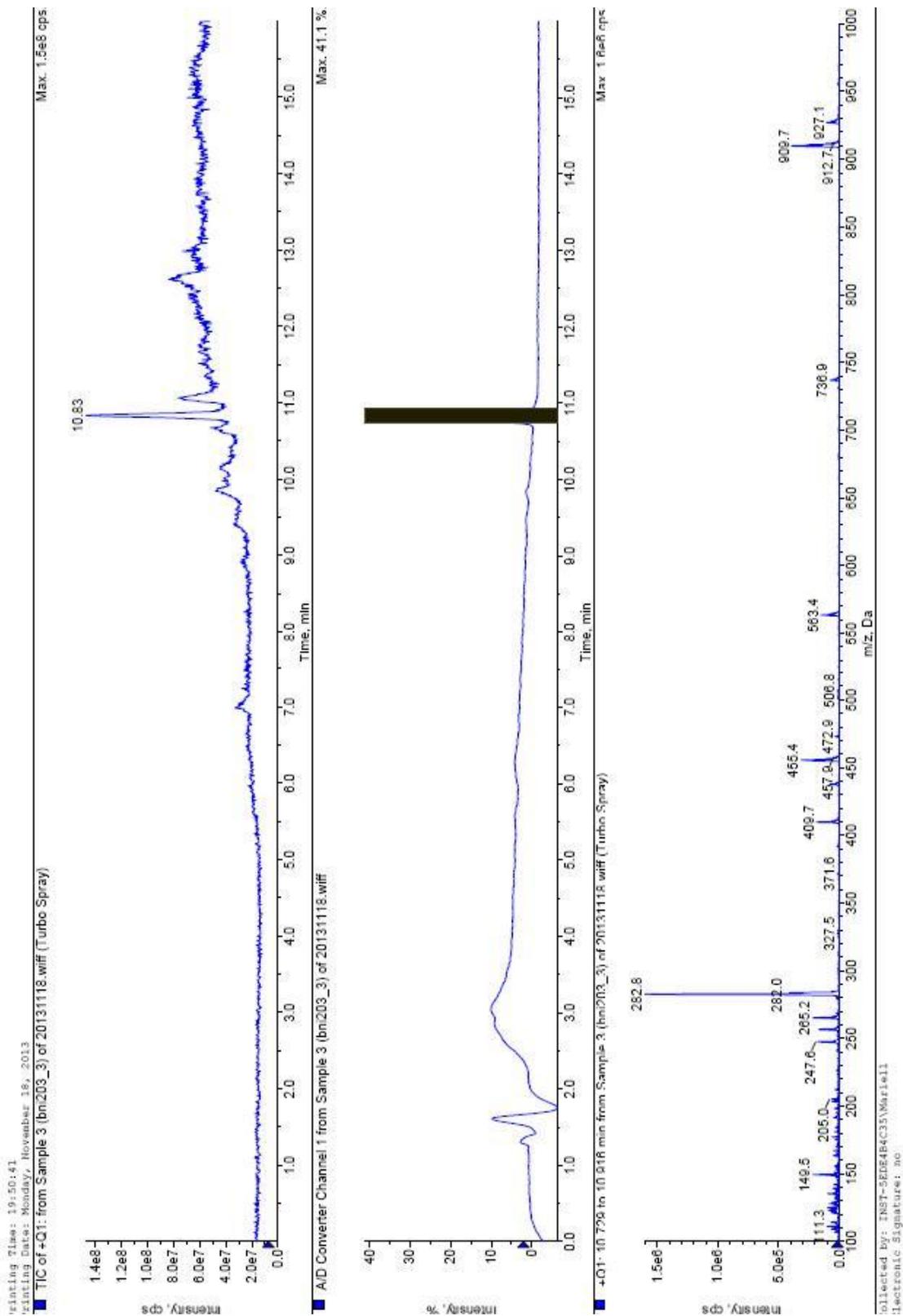
Appendix 21: ^{1}H NMR spectrum of 3-epi-oleanolic acid (**161**)
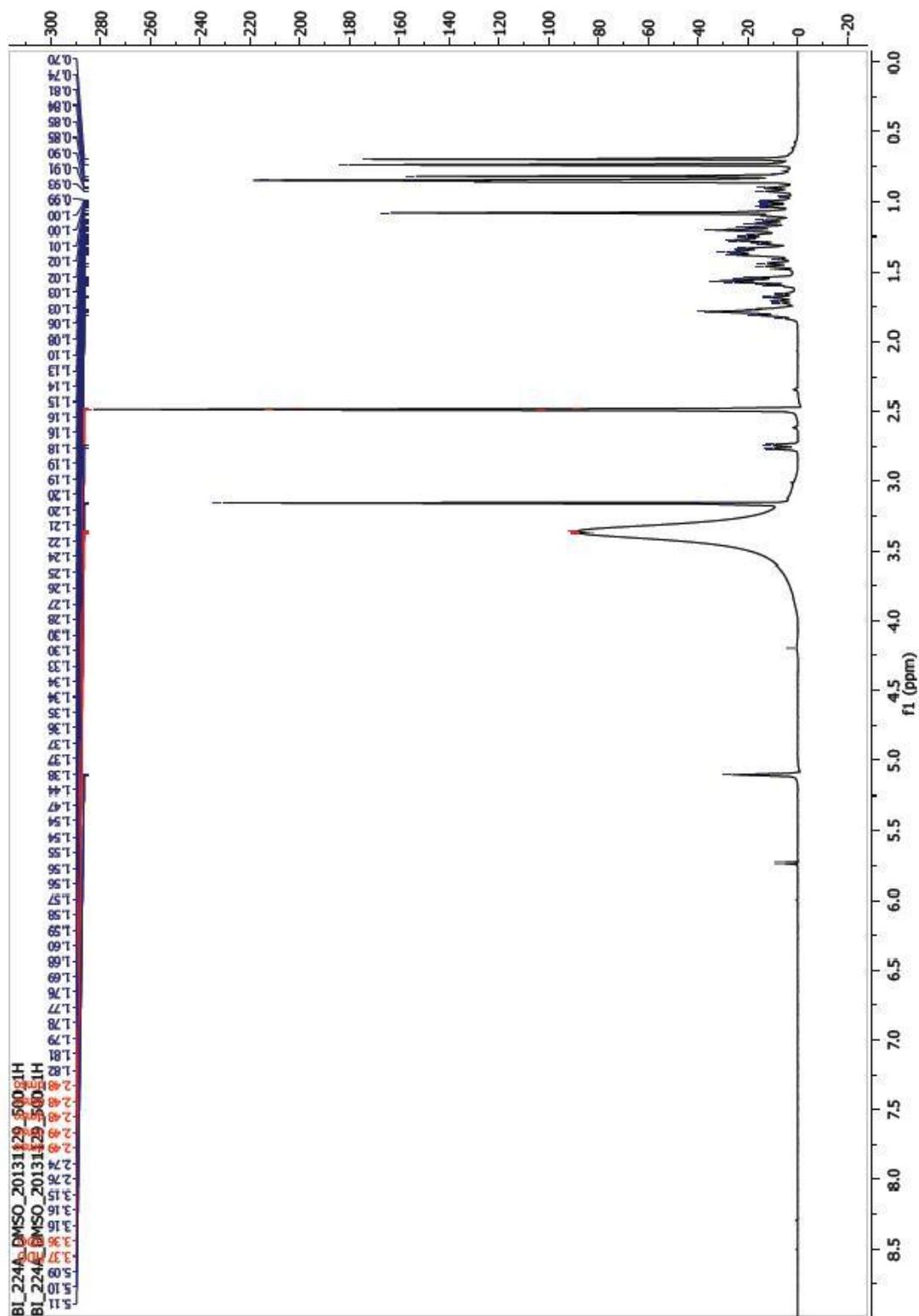
Appendix 21A: ^{13}C NMR spectrum of oleanolic acid (**161**)
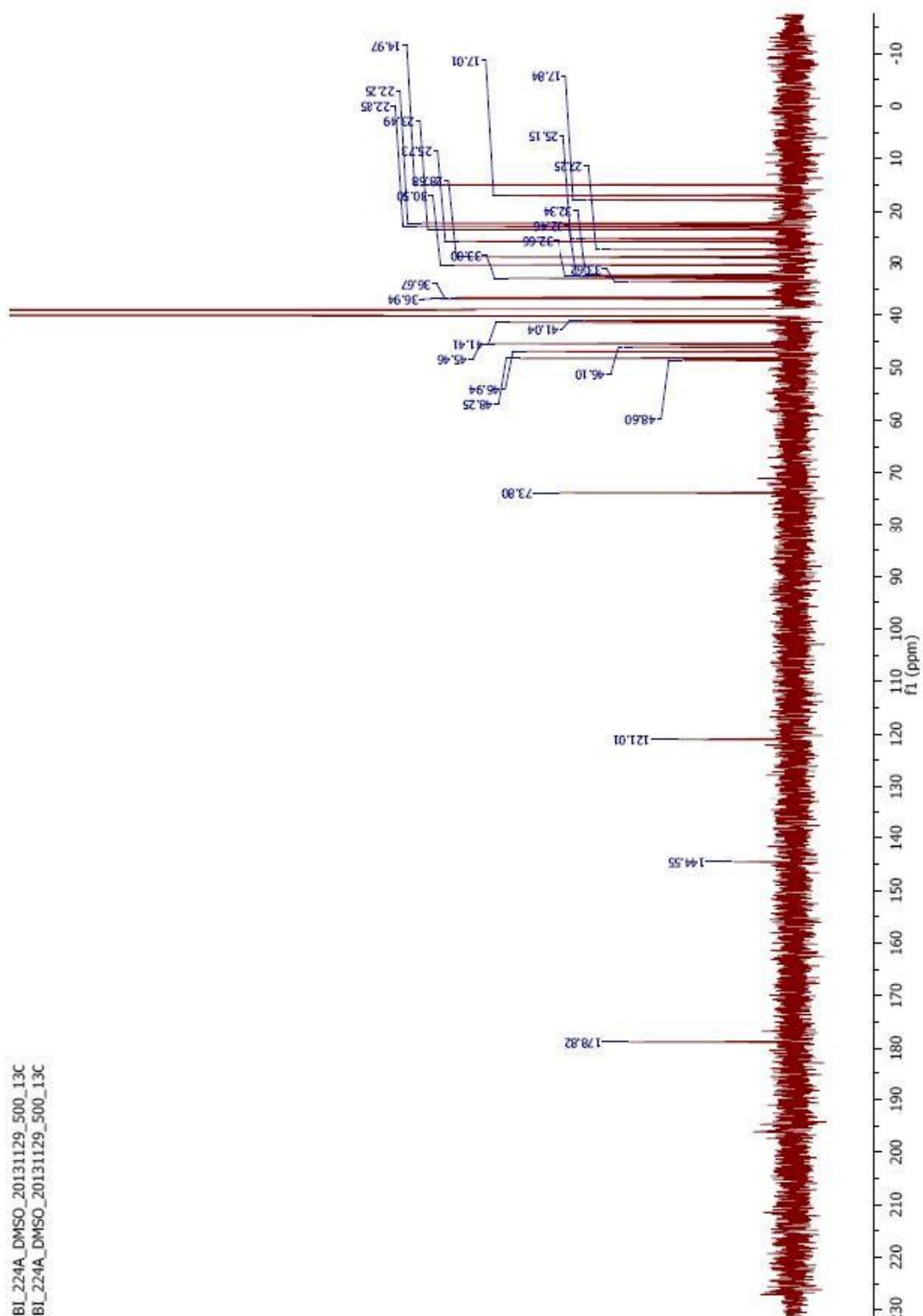
Appendix 22: ^{1}H NMR spectrum of compound **152**
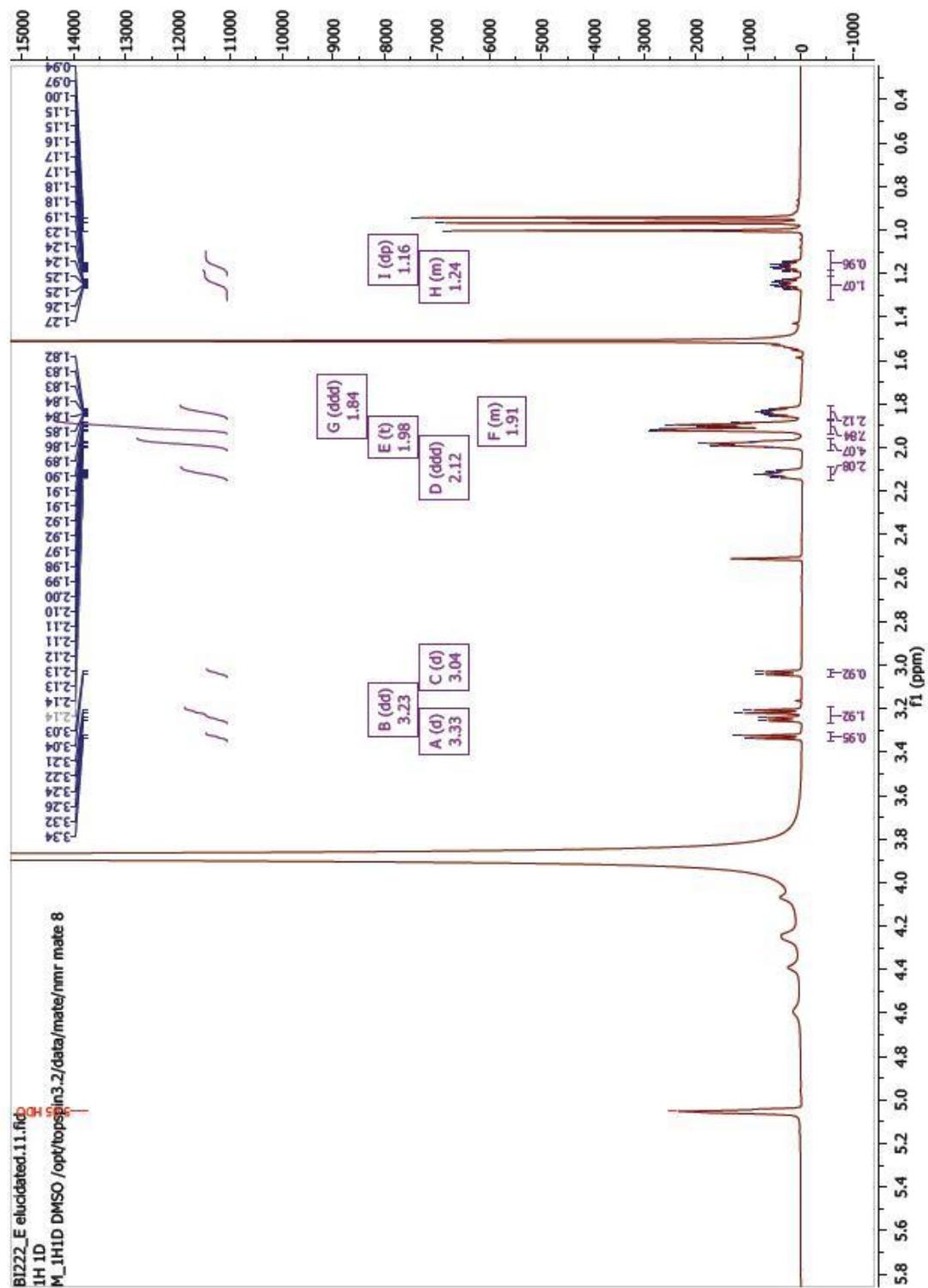
Appendix 22A: ^{13}C NMR spectrum of compound **152**
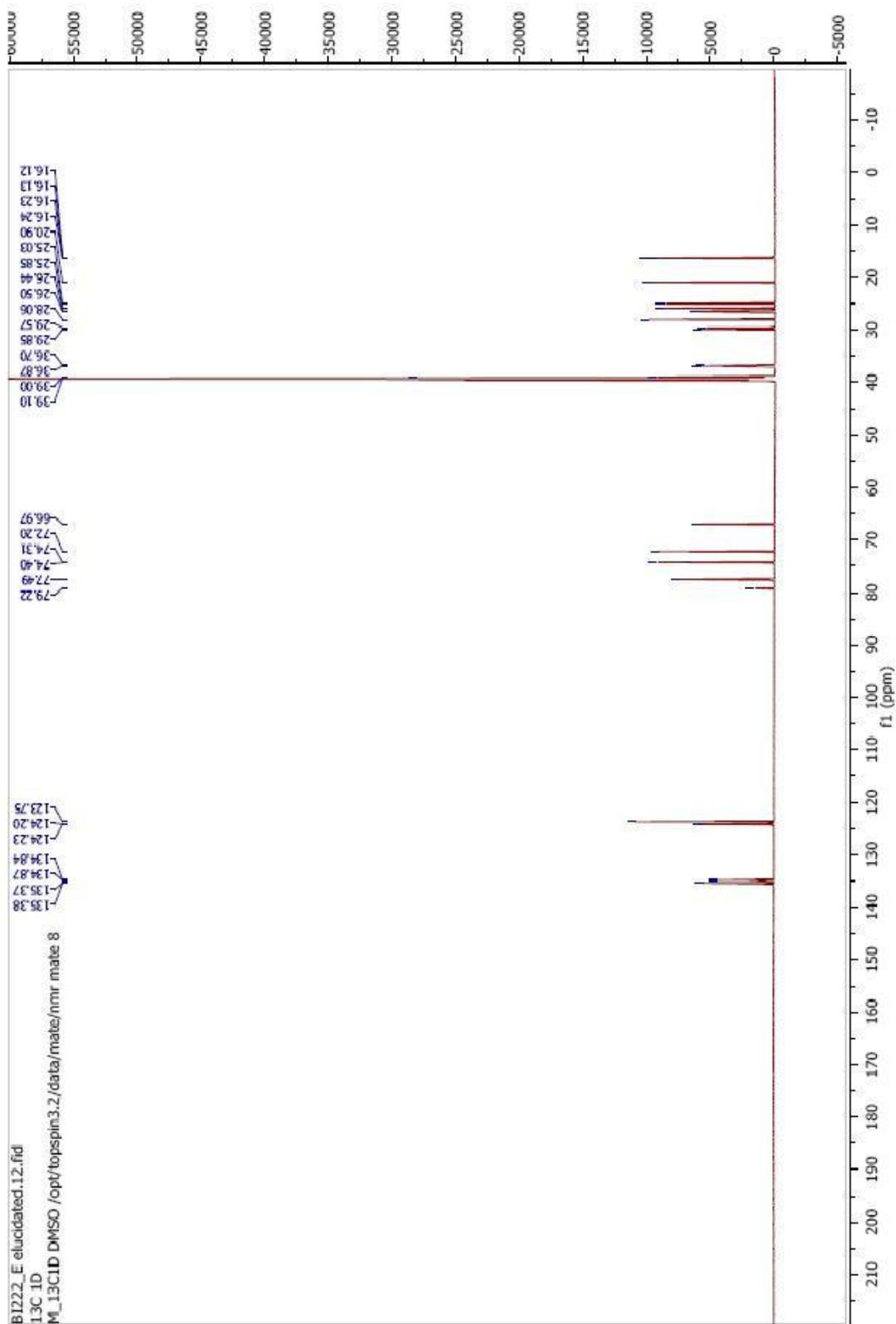
Appendix 22B: LC/MS spectrum of compound **152**

Appendix 23: ^{1}H NMR spectrum of compound **151**
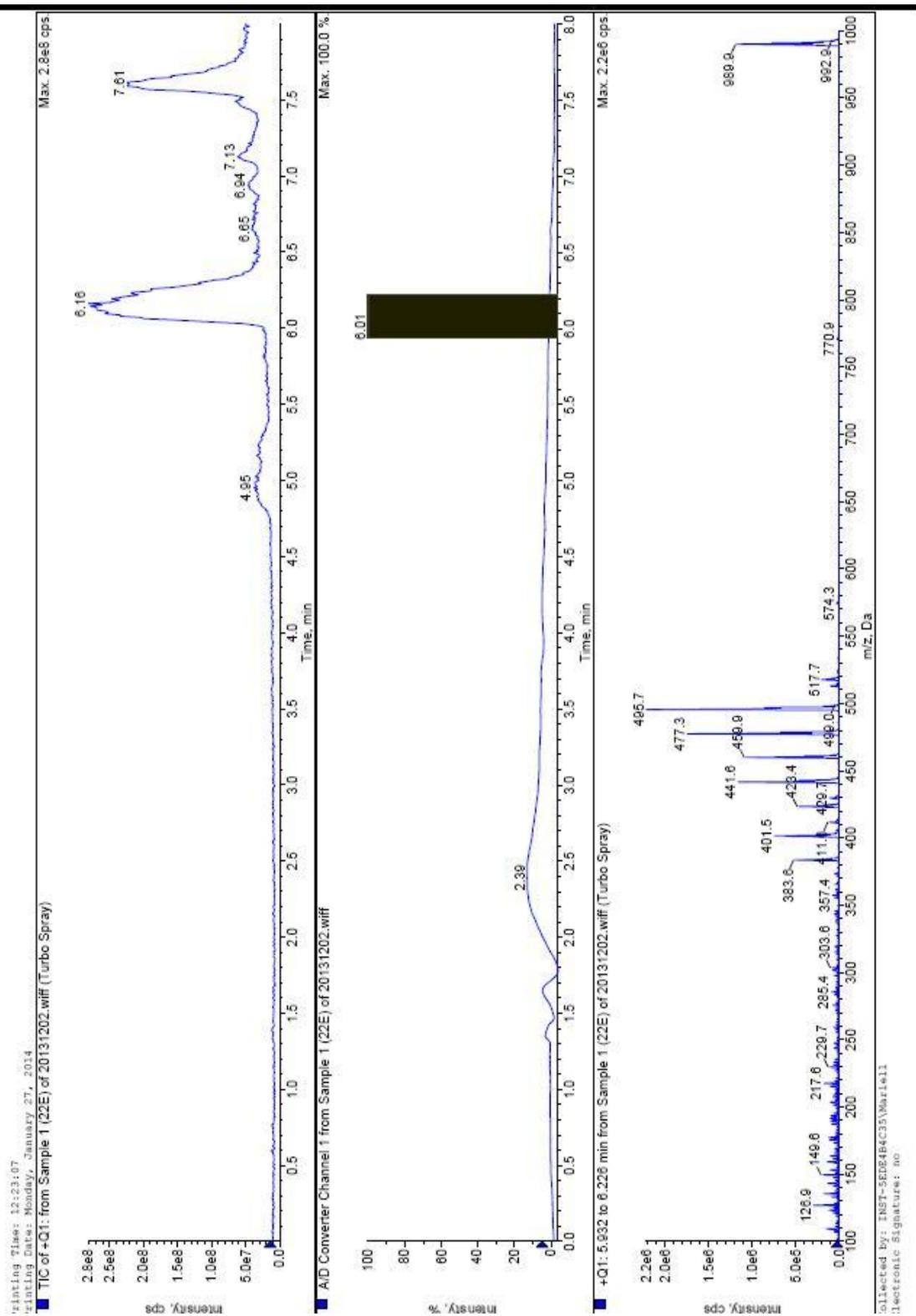
Appendix 23A: ^{13}C NMR spectrum of compound **151**
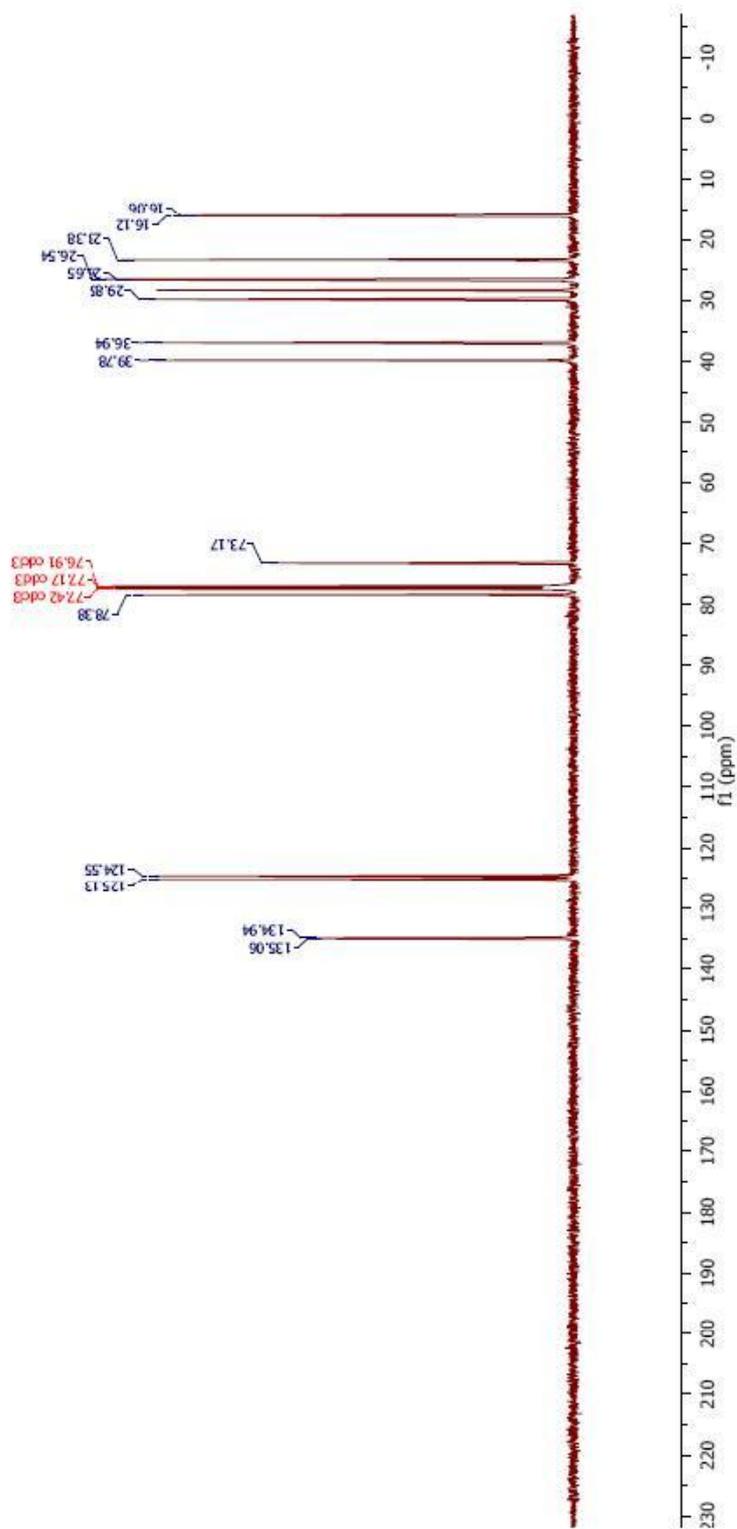
Appendix 23B: LC/MS spectrum of compound **151**
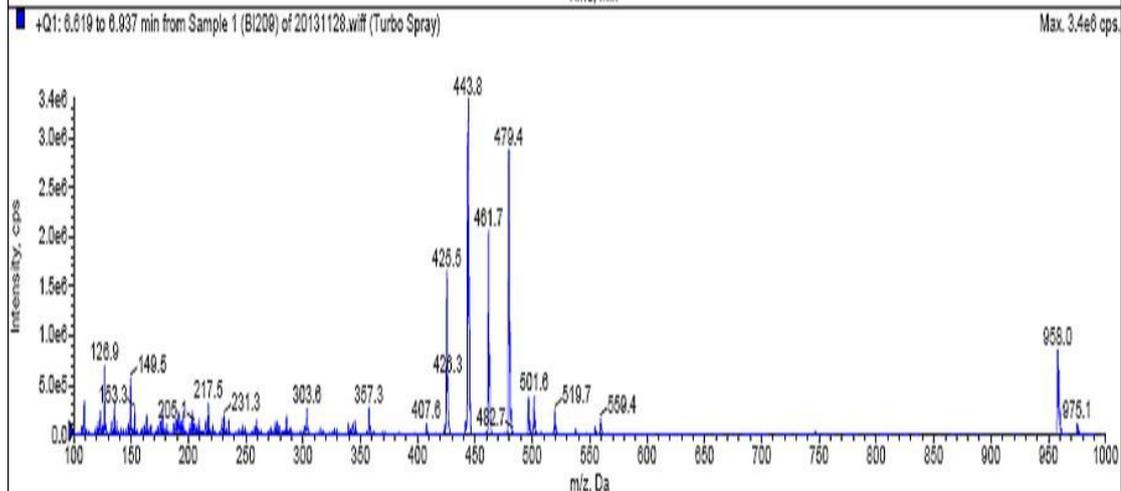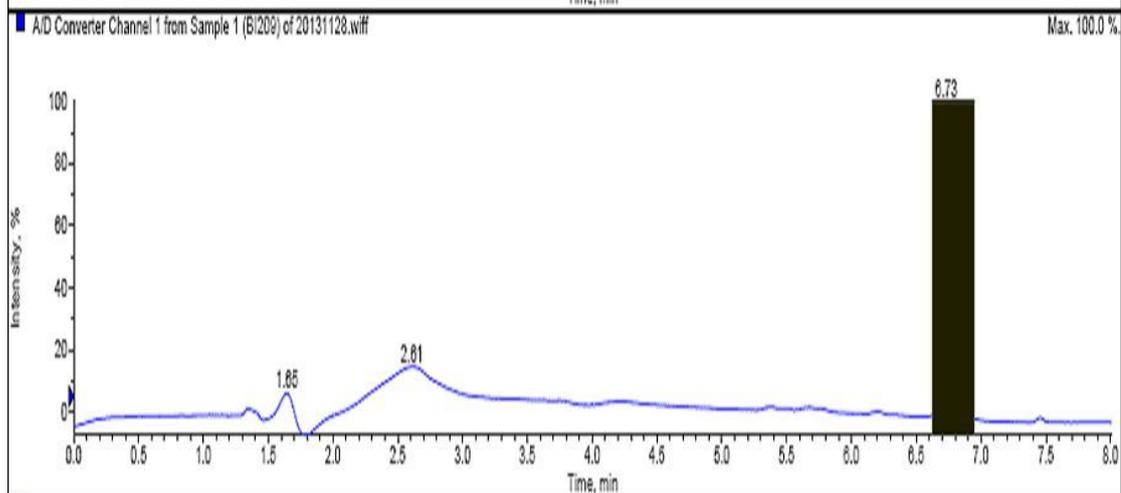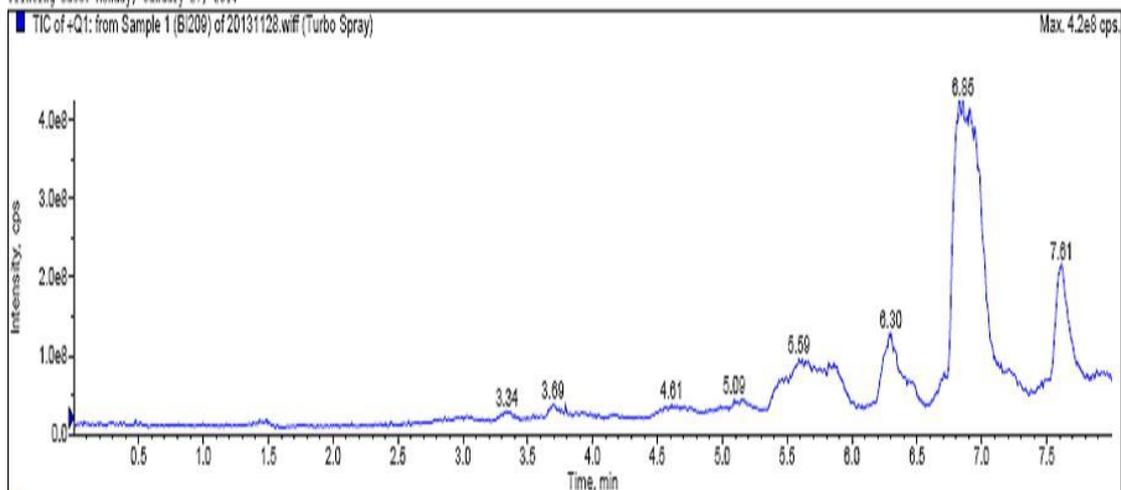
Appendix 24: ^{1}H NMR spectrum of kaemferol-3-*O*-*β*-D-glucopyranoside (**199**)

Appendix 24A: ^{13}C NMR spectrum of kaemferol-3-*O*-β-D-glucopyranoside (**199**)
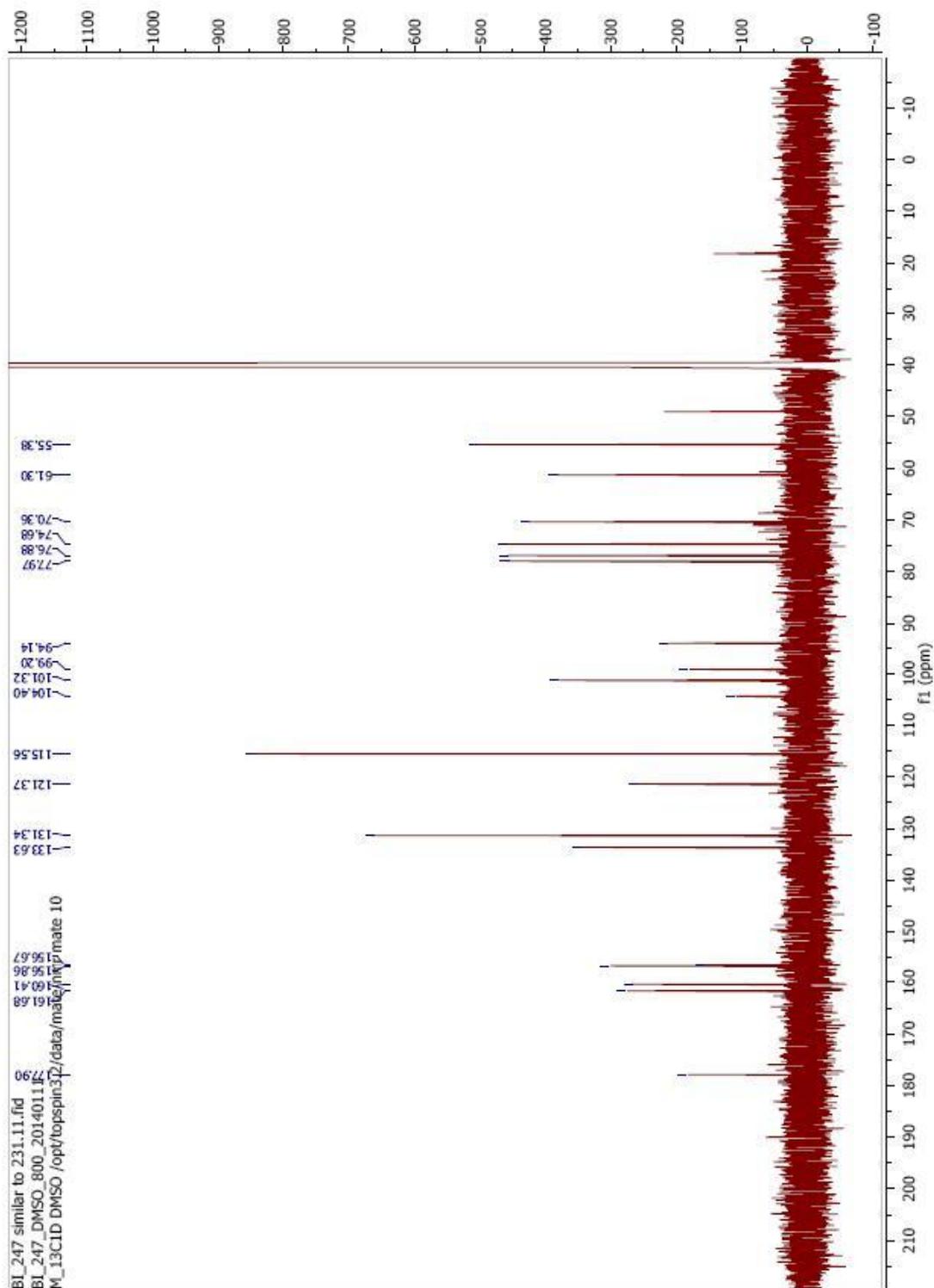
Appendix 24B: LC/MS spectrum of kaemferol-3-*O*-**β**-D-glucopyranoside (**199**)
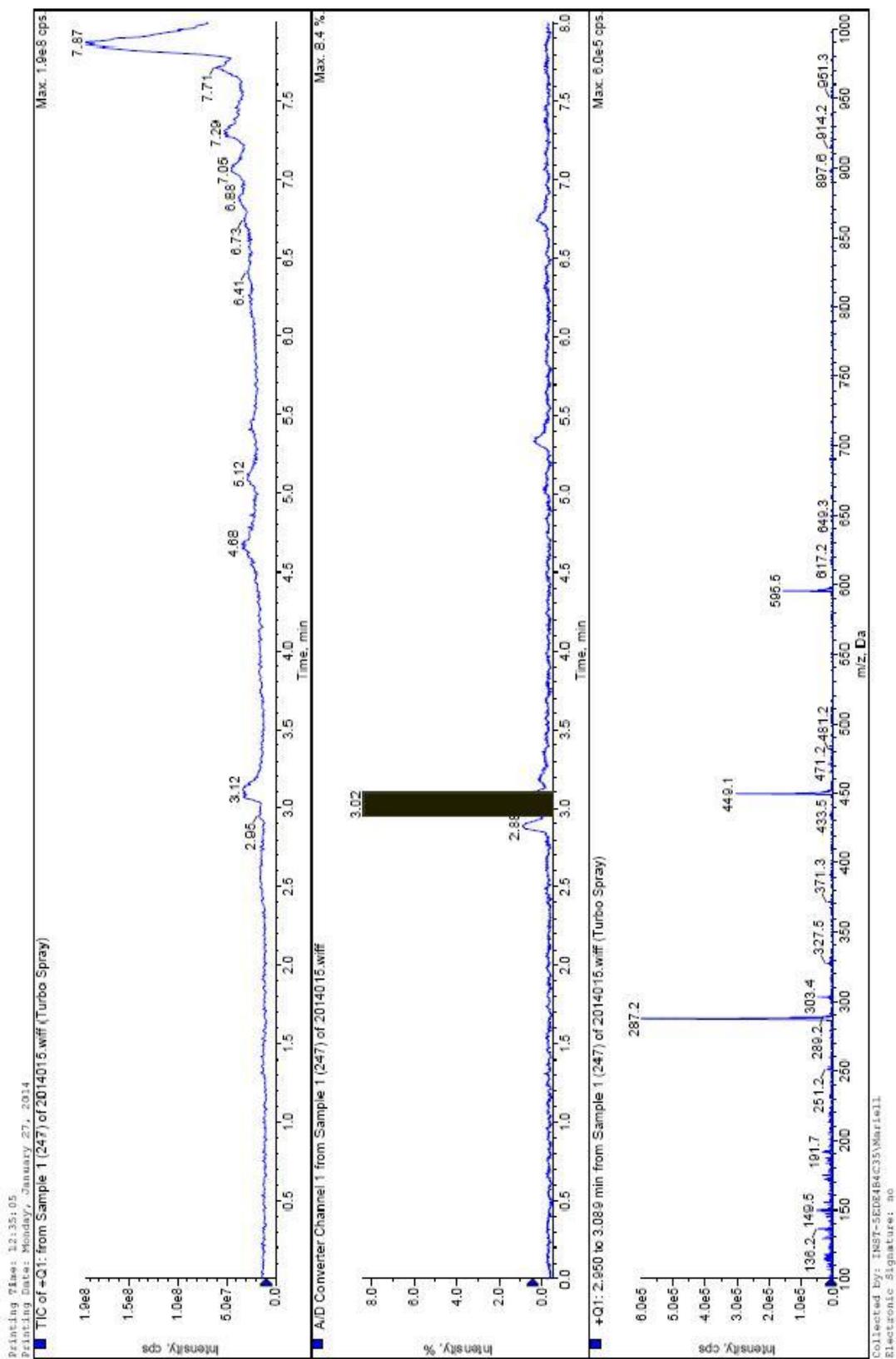
Appendix 25: ¹H NMR spectrum of quercetin-3-*O*-*β*-D-glucopyranoside (**200**)
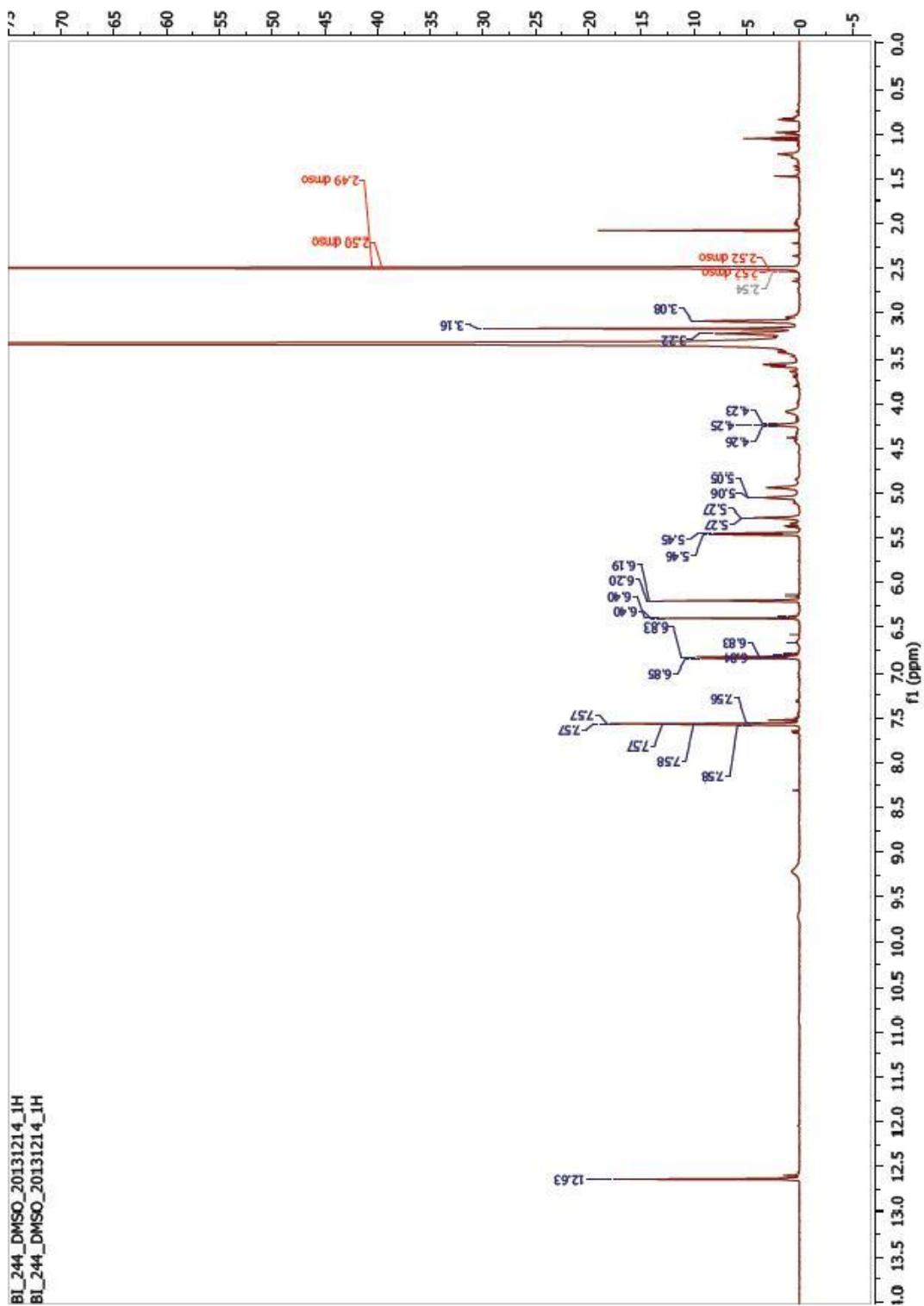
Appendix 25A: ^{13}C NMR spectrum of quercetin-3-*O*-*β*-D-glucopyranoside (**200**)
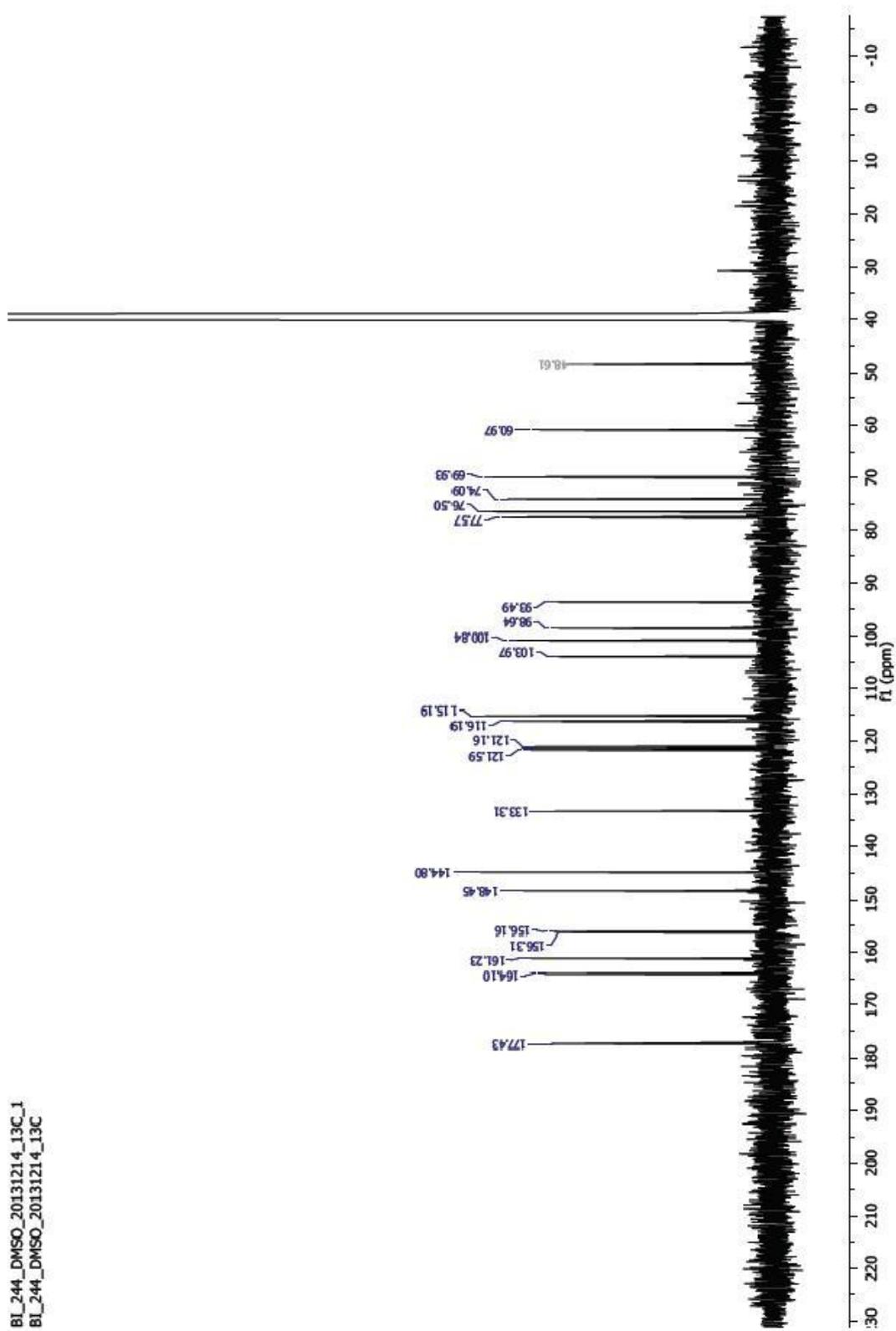
Appendix 25B: LC/MS spectrum of quercetin-3-O-β-D-glucopyranoside (**200**)
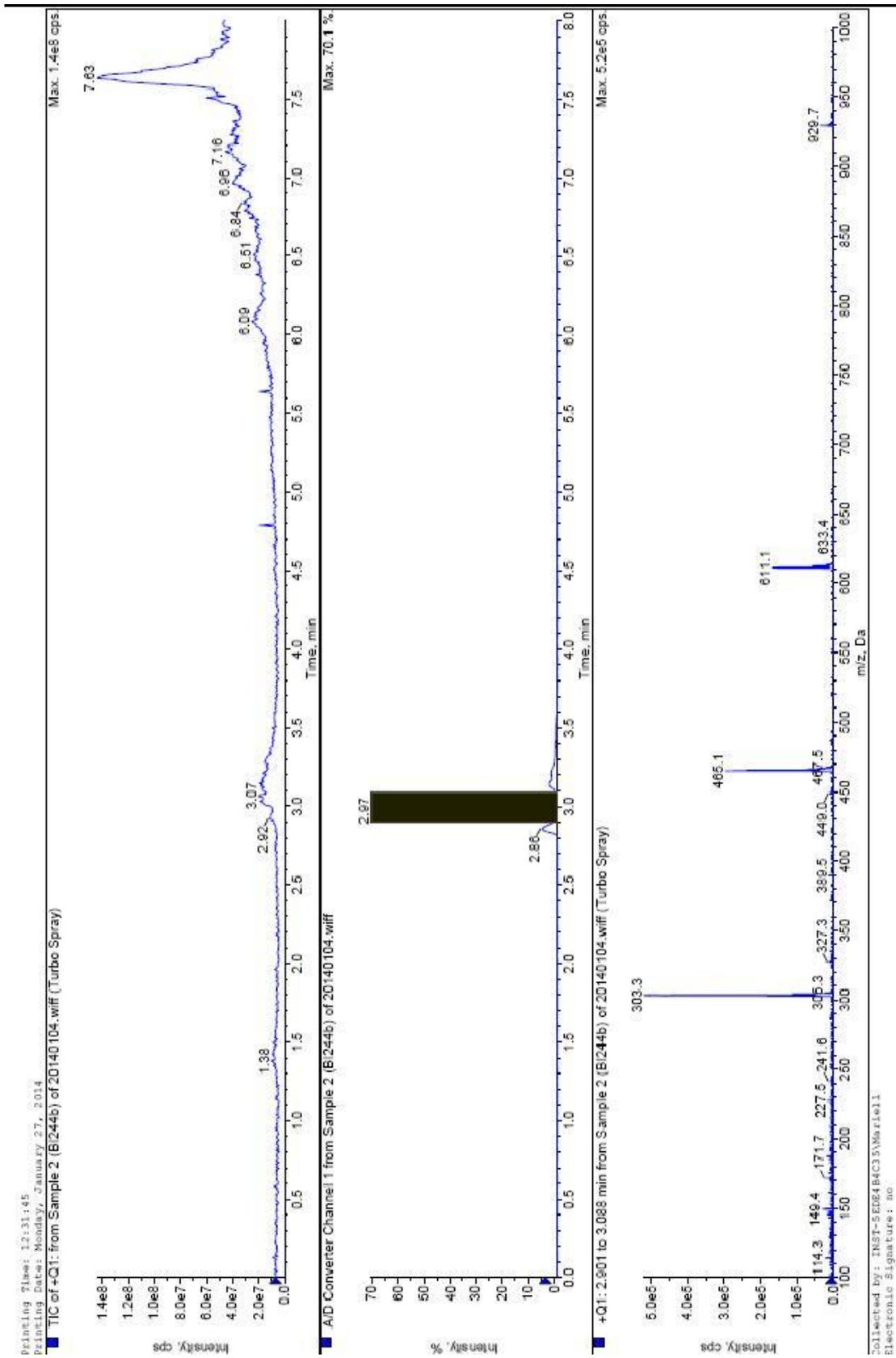
Appendix 26: ^{1}H NMR spectrum of proceranolide (**148**)
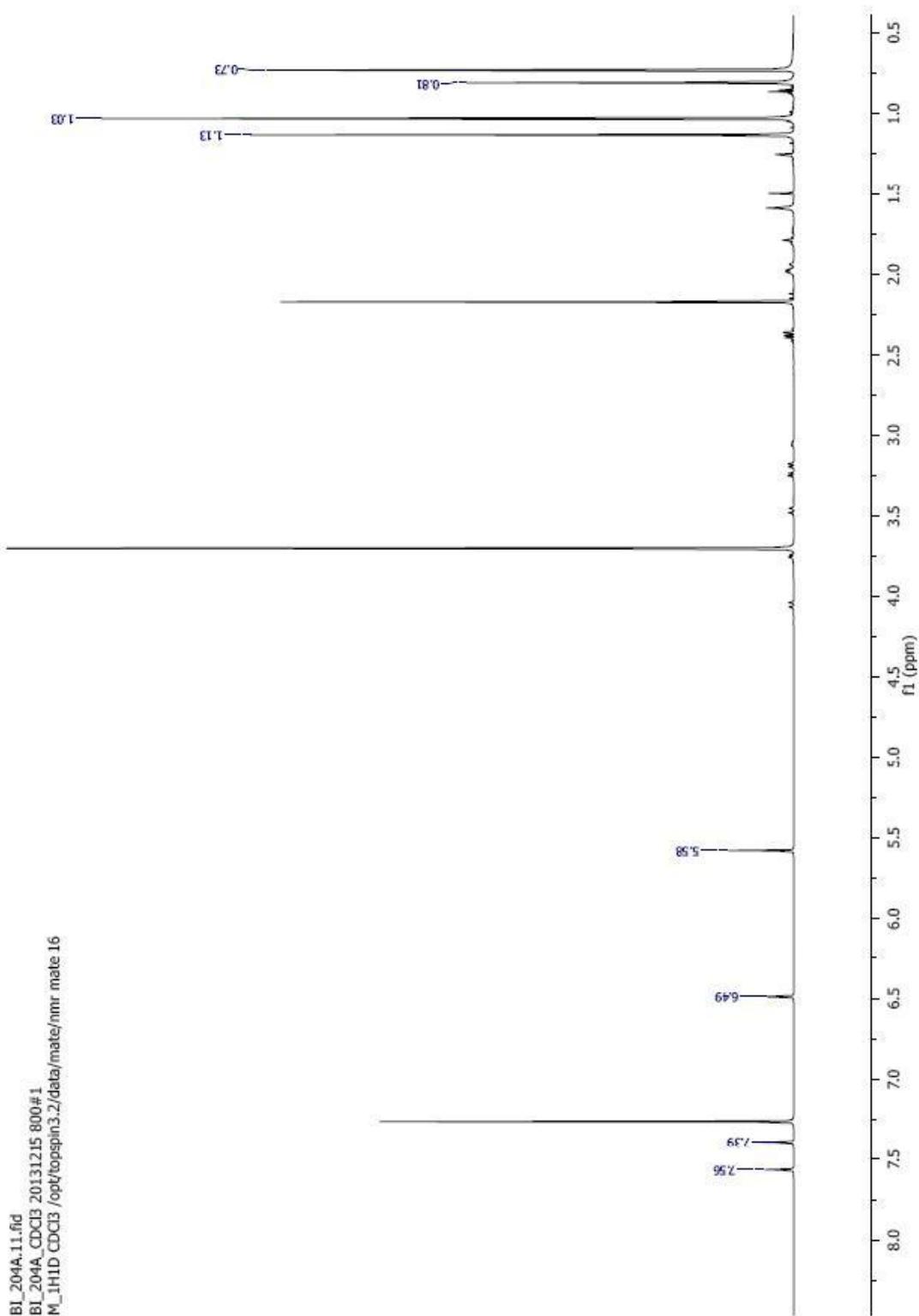
Appendix 26A: ^{13}C NMR spectrum of proceranolide (**148**)
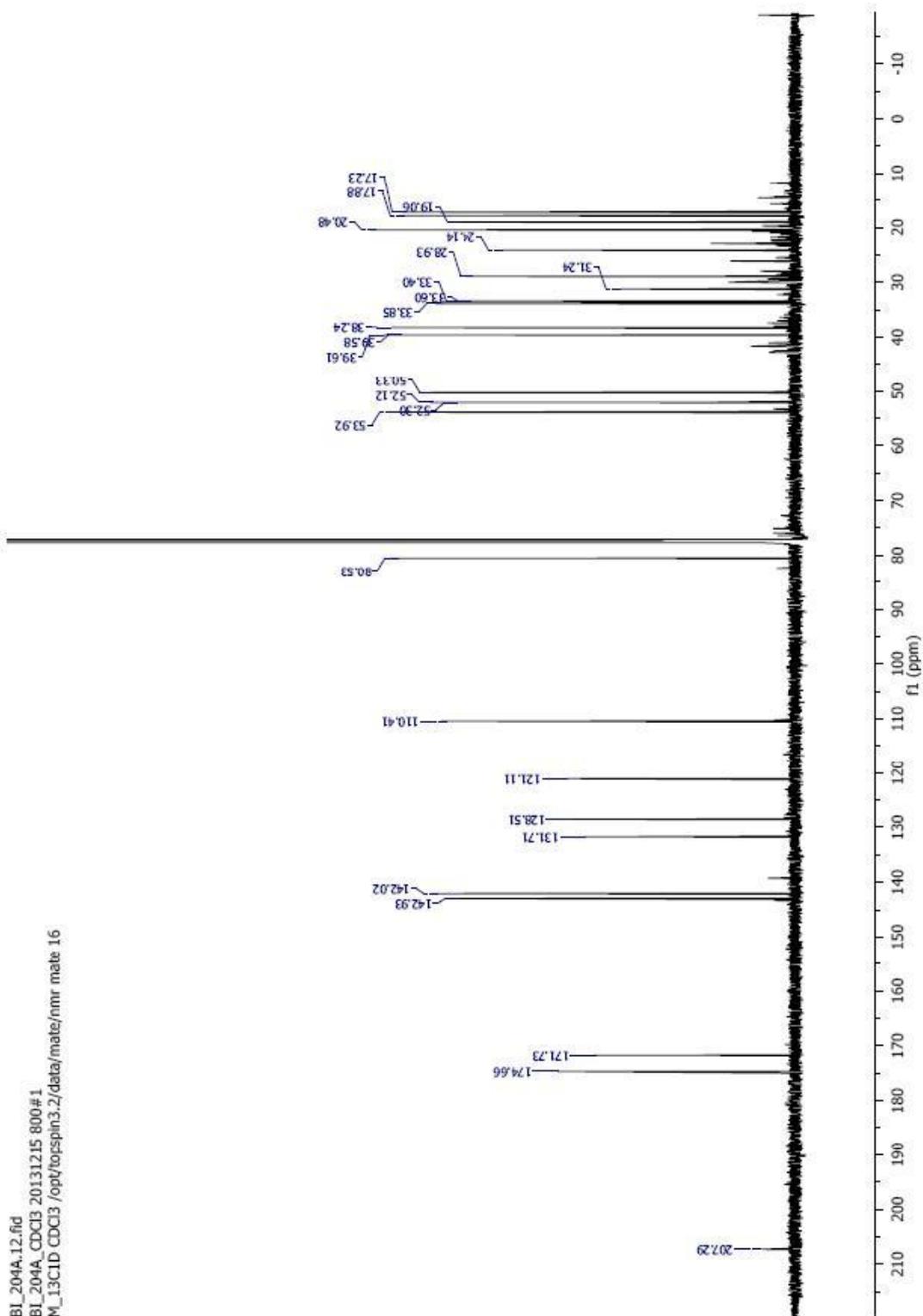
Appendix 26B: LC/MS spectrum of proceranolide (**148**)
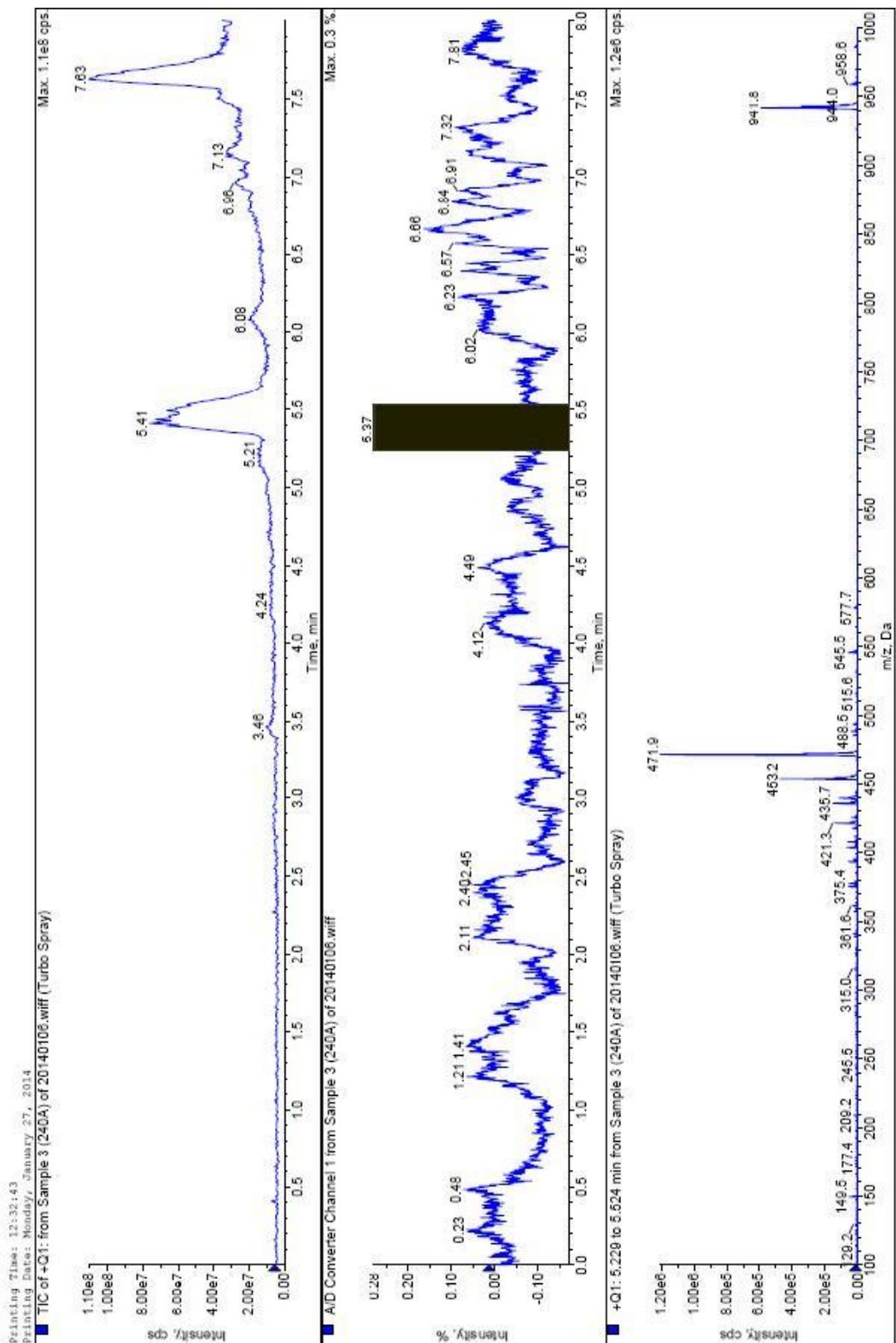
Appendix 27: ^{1}H NMR spectrum of niloticin (**25**)
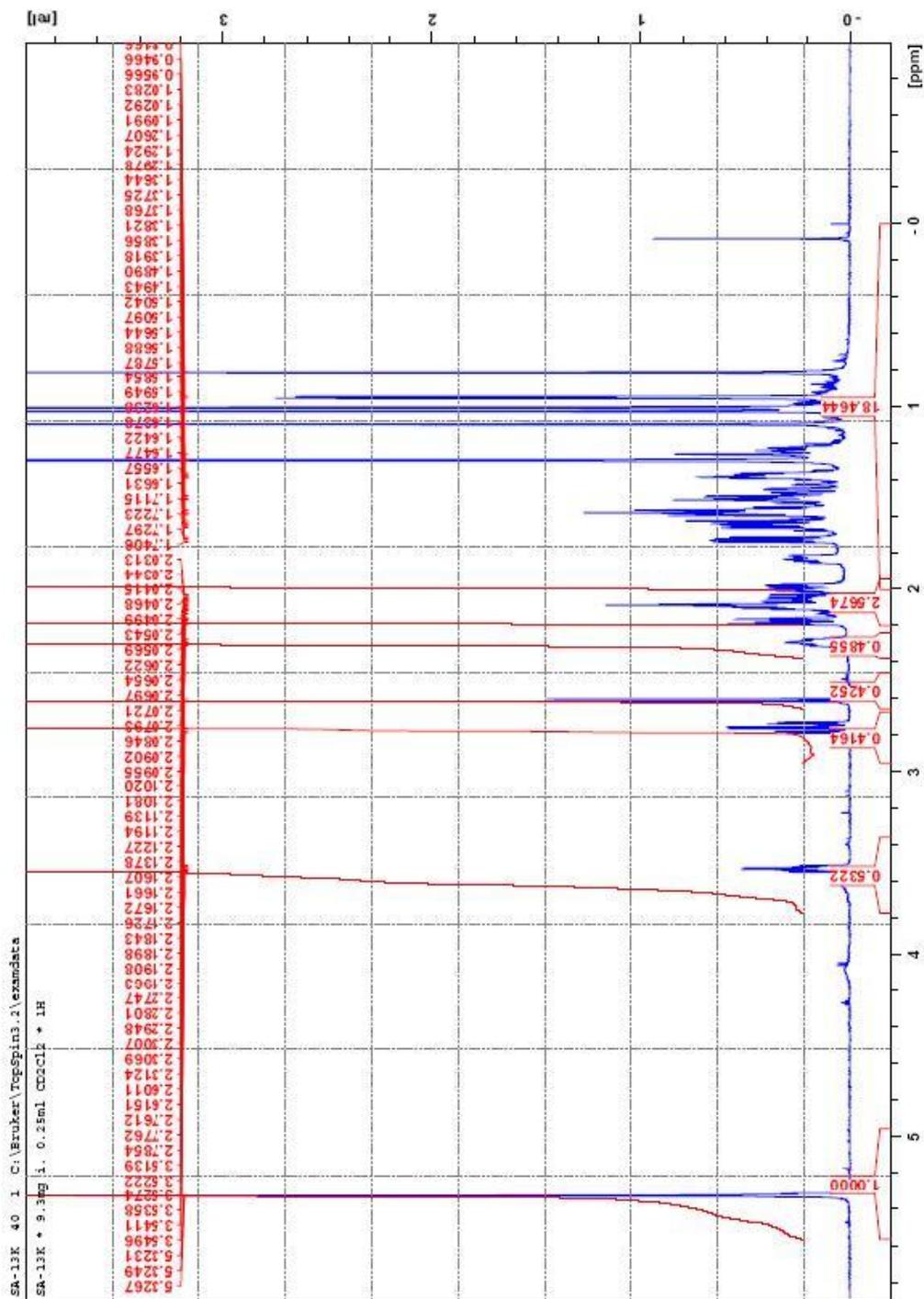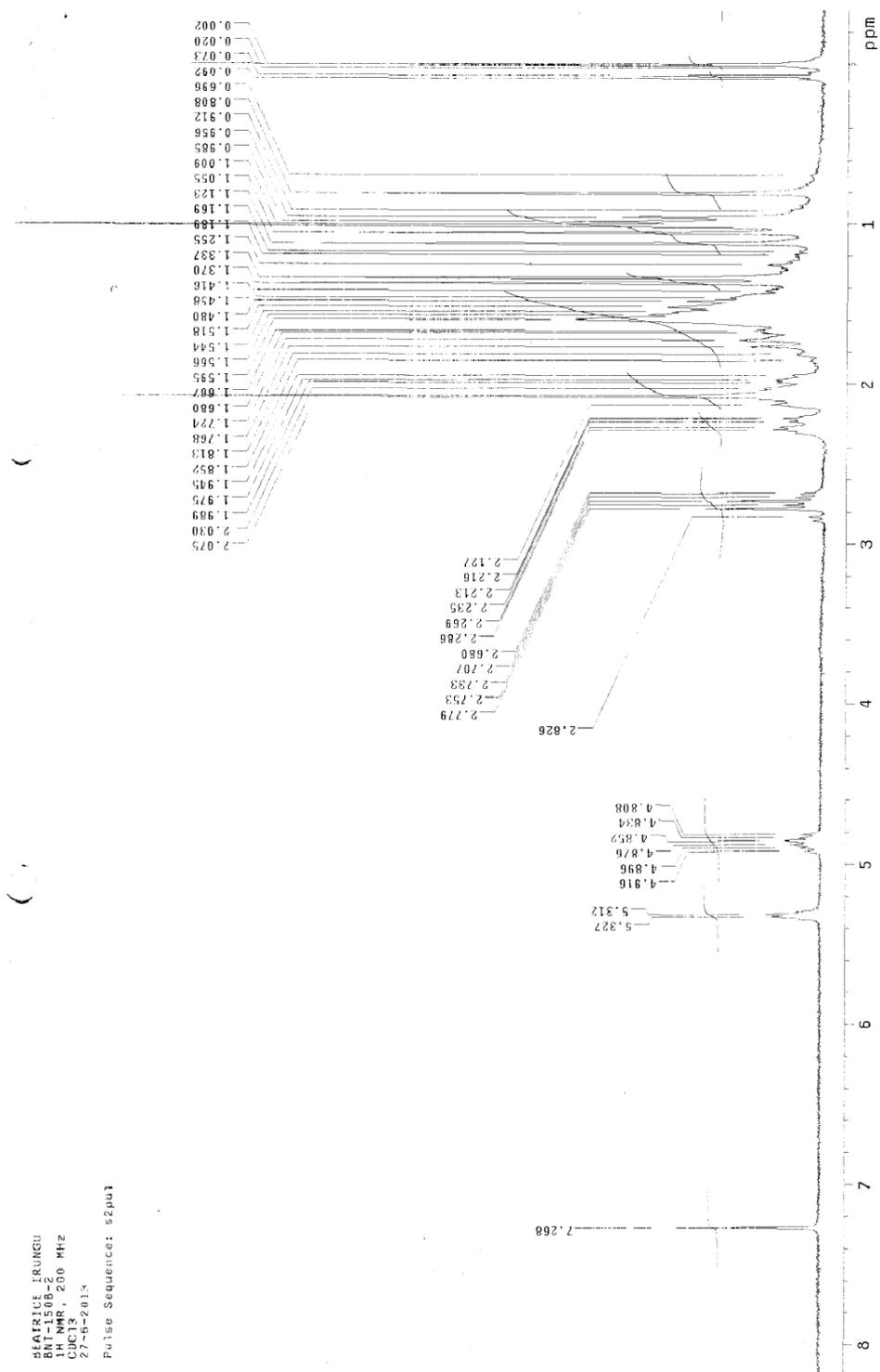
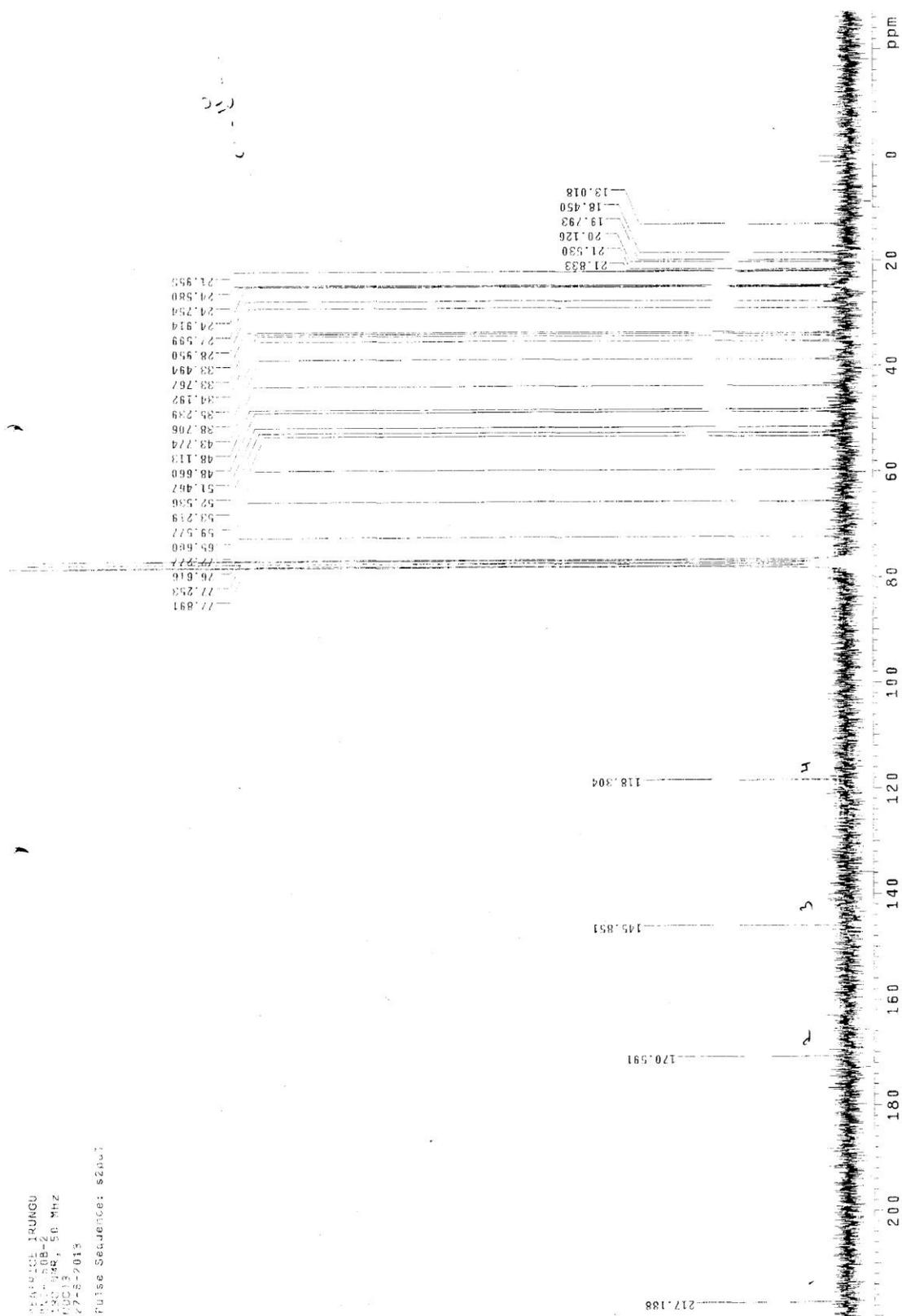Appendix 27A: ¹³C NMR spectrum of niloticin (**25**)
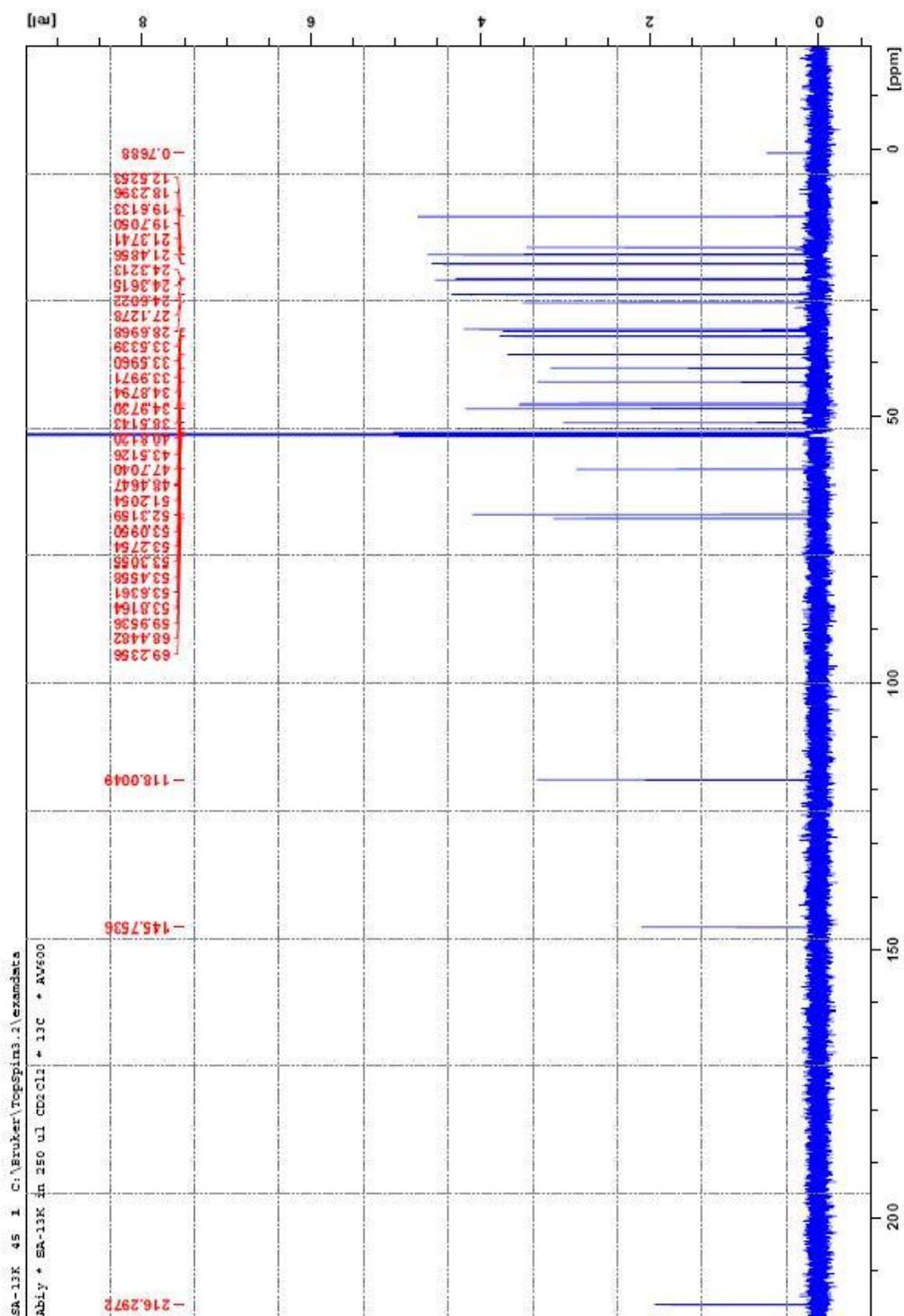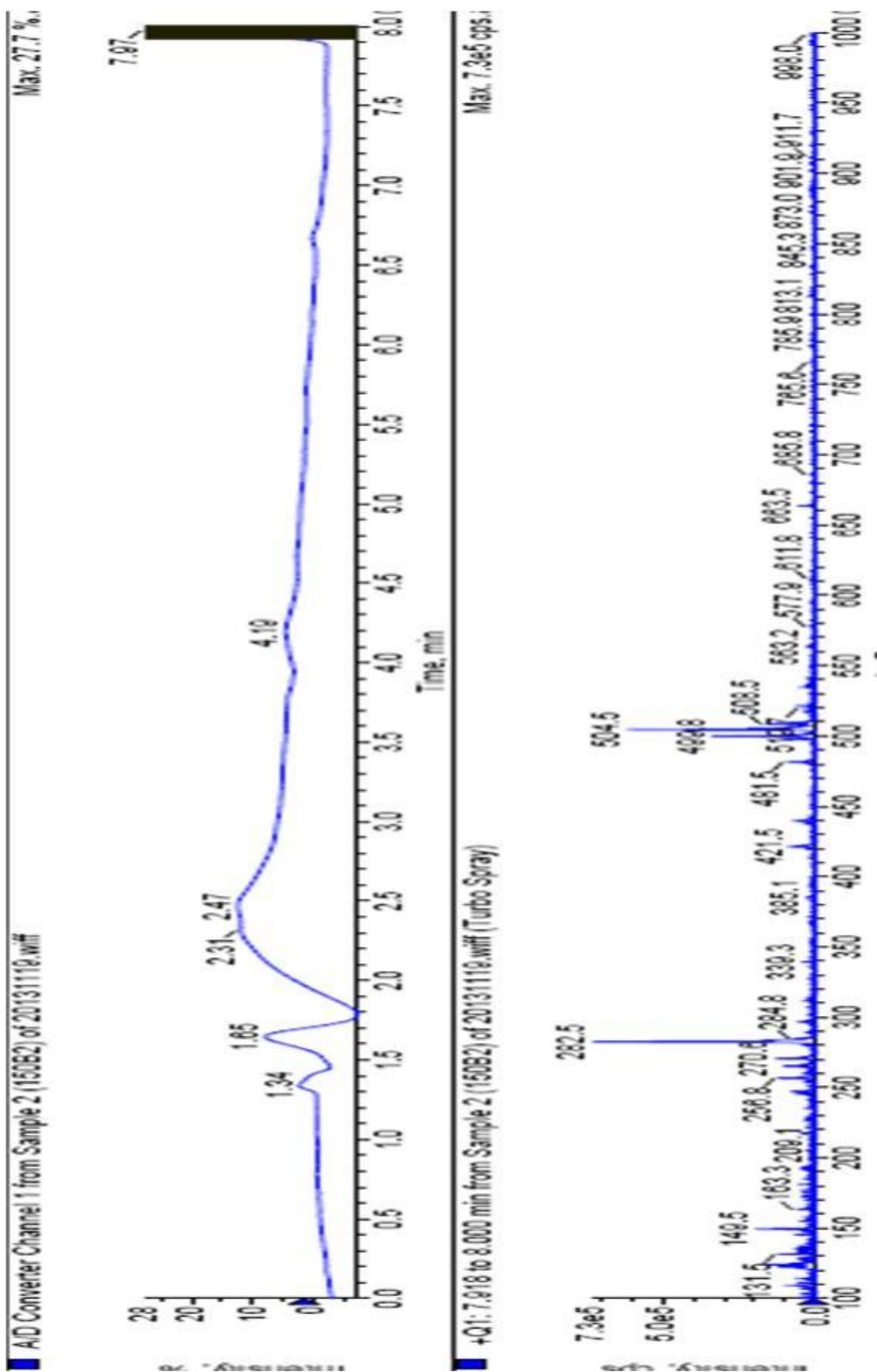
Appendix 27B: LC/MS spectrum of niloticin (**25**)
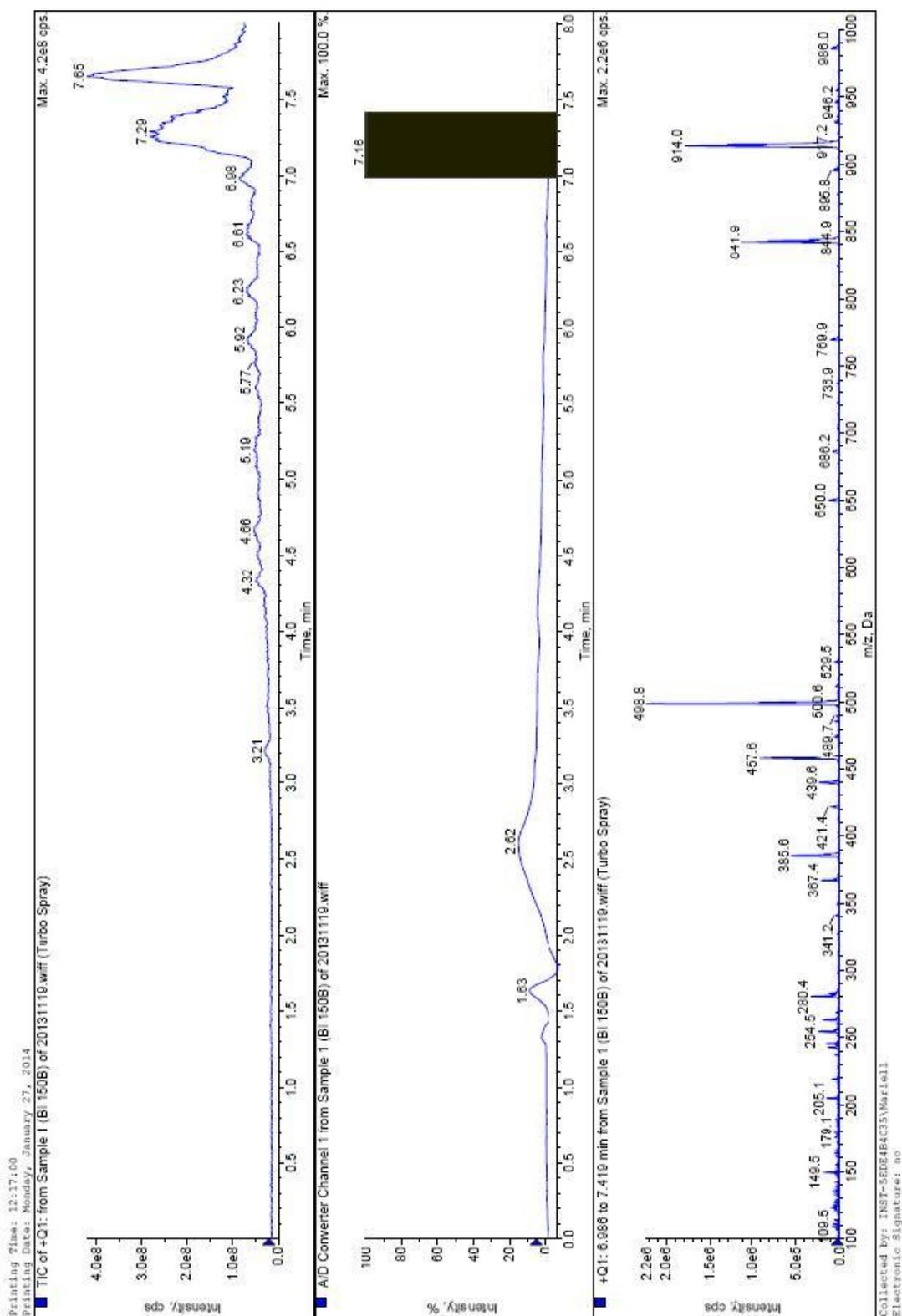
Appendix 28: ^{1}H NMR spectrum of niloticin acetate (**201**)

Appendix 28A: ^{13}C NMR spectrum of niloticin acetate (**201**)

Appendix 28B: LC/MS spectrum of niloticin acetate (**201**)

Appendix 29: Manuscript resulting from this work

Article

Constituents of the Roots and Leaves of *Ekebergia capensis* and Their Potential Antiplasmodial and Cytotoxic Activities

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