# SCREENING AND CHARACTERIZATION OF SOME ANTICANCER COMPOUNDS FROM SALICACEAE, MYRTACEAE, EUPHORBIACEAE AND SOLANACEAE FAMILIES

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**A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirement for the Doctor of Philosophy Degree in Chemistry of Egerton University**

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## DECLARATION AND RECOMMENDATION

**Declaration**

This research thesis is my original work and has not been submitted wholly or in part for any award in any institution

Signature:…… Date:……

Chirchir Denis Kipngeno

**SD11/0342/12**

**Recommendation**

This thesis has been submitted with our approval as University supervisors.

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## DEDICATION

This work is dedicated to the late Prof Peter Kiplagat Cheplogoi.

**ACKNOWLEDGEMENT**

First I thank the Almighty God for the gift of life and strength to undertake this study. I appreciate Egerton University for allowing me to pursue PhD degree in Chemistry. I am thankful to my supervisors; the late Prof P.K Cheplogoi and Prof J. O. Omolo who have natured me to maturity in Natural Product Chemistry. My appreciation also goes to University of Kabianga for allowing me time to pursue PhD and partially funding the project. My deep appreciation goes to Dr. Langat Moses of Surrey University, Guild Ford UK for immense support in NMR spectra acquisition for the isolated compounds. I also acknowledge the support of my PhD colleagues; Dr. Alice Njue and Mrs Regina Kemunto Mayaka for their moral support, brilliant comments and constructive critics that shaped my work. Lastly my sincere appreciation goes to my lovely family, my parents and brothers for their encouragement, moral and material support.

## ABSTRACT

The chemistry of natural products is very important since it has been used in the search for bioactive compounds for management of various human diseases including cancer. The increase in the incidence of cancer coupled with the undesirable side effects observed with chemotherapic agents urges the discovery of new agents from natural sources. In this study the four ethnomedicinal plants; *Dovyalis abyssinica* (Salicaceae), *Solanum mauense* (Solanaceae), *Syzigium guinense* (Myrtaceae) and *Croton dichogamous* (Euphorbiaceae) were investigated for their unvalidated anticancer activities**.** Crude extracts for stem bark of *S. guinense*, fruits of *S. mauense,* and roots of both *D. abbysinica* and roots of *C. dichogamous* were prepared via cold extraction method. The crude extracts were purified by repeated column chromatography and Thin Layer Chromatography. This resulted in various pure compounds which were analysed by use of 1D NMR, 2D NMR spectroscopic techniques and MS spectrometry. The NMR spectral data obtained together with MS data were interpreted, the structures of the compounds elucidated and their chemical structures proposed. A total of twelve compounds were isolated, purified, their chemical structures proposed and four of these compounds were evaluated for their anticancer activity. Two previously reported compounds; *β*-sitosterol (**47**) and betulinic acid (**21**), were obtained from stem bark extracts of *S. guinense a*s well as from the fruits extracts of *S. mauense*. Two previously reported compounds; Tremulacin (**29**) and Benzoic acid (**48**) were isolated from the roots extract of *D. abyssinica*. Eight compounds were isolated from roots extract of *C. dichogamous;* Acetyl aleuritolic acid (**49**), 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (**50**), 3β, 4β:15, 16-diepoxy-13(16), 14-*ent*-clerodadien-17,12*S*-olide (**51**), 15,16-epoxy-5, 13(16), 14-*ent*-halimatriene-3-ol (**52**), crotodichogamoin A (**53**), crotohaumanoxide (**54**), crotodichogamoin B (**55**) and Cadin-1(6),2,4,7,9-penta-ene (**56**)**.** Selected compounds were evaluated for their anticancer activity by use of cancer cell lines. Betulinic acid (**21)** was screened against 57 cell lines and only 25 gave positive results. Acetyl aleuritolic acid (**49**), 15,16-epoxy-5,13(16),14-*ent*-halimatriene- 3-ol (**52**) and crotodichogamoin A (**53**) were also evaluated for their anticancer activity and and their one dose mean value percentage growth at 15 µg/ml were 97.86, 99.39 and 100.6, respectively. The mean values of growth inhibition of the three compounds tested against one dose NCI cell line panel did not meet the standards for further testing against the five-dose NCI cell line panel. The study recommends toxicological studies be done on the medicinal plants extracts to enhance their full exploitation.

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| | |
|---|---|
| **$^{13}$C NMR** | Carbon 13-nuclear magnetic resonance |
| **COSY** | Correlated spectroscopy |
| ***J*** | Coupling constant |
| **$CDCl_3$** | Deuterated chloroform |
| **DEPT** | Distortionless enhancement of polarization transfer |
| **d** | Doublet |
| **dd** | Doublet of doublet |
| **DCM** | Dichloromethane |
| **DMSO** | Dimethyl sulphoxide |
| **Hz** | Hertz |
| **HRMS** | High resolution mass spectrometry |
| **HMBC** | Heteronuclear multiple bond coherence |
| **HSQC** | Heteronuclear single quantum coherence |
| **LC50** | 50 % Lethal concentration |
| **m** | Multiplet |
| **Nm** | Nanometre |
| **NMR** | Nuclear magnetic resonance |
| **NOESY** | Nuclear overhauser effect spectroscopy |
| **1D** | One dimension |
| **2D** | Two dimensions |
| **ppm** | Parts per million |
| **$^{1}$H NMR** | Proton nuclear magnetic resonance |
| **q** | Quartet |
| **s** | Singlet |
| **TLC** | Thin layer lhromatography |
| **t** | Triplet |
| **UV** | Ultraviolet |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background Information

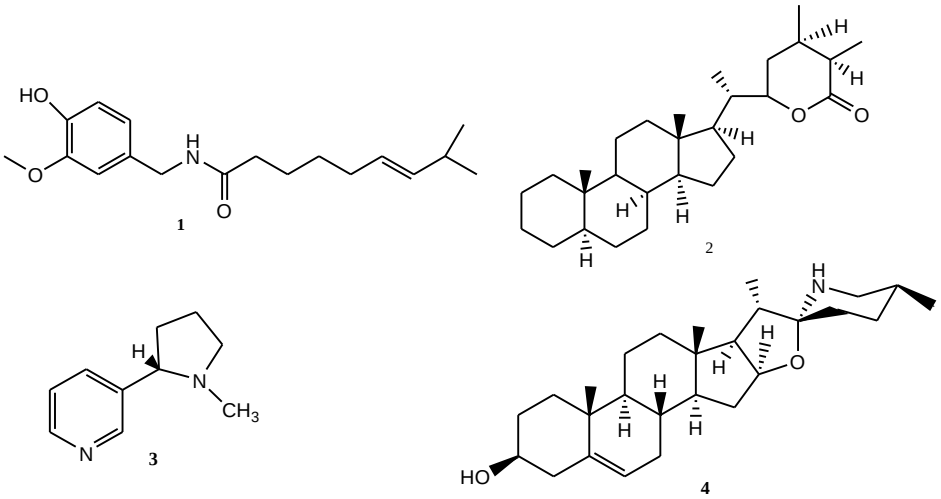
The chemistry of natural products is very important and can be used in the exploration for bioactive compounds (Asif, 2015). The natural products have been used almost in all cultures as a major source of medicament. This usage has been associated with the occurrence of natural products present in them with medicinal properties. The availability of medicinal plants and their cheaper cost in comparison to modern therapeutic agents makes them more attractive as therapeutic agents (Sharma *et al.*, 2010). Ethnobotany and ethno-medicinal studies are today documented as the most viable methods of detecting new medicinal plants or refocusing on those earlier reported for bioactive constituents. Scientific investigations of medicinal plants have been carried out in many places because of their usefulness and contributions to health care. The continual search for natural plant products, for use as medicines has encouraged exploration of methodologies involved in obtaining the required plant materials and hence searching for their constituents for possible medicinal usage.

The Myrtaceae family of medicinal plants is a large, definite family, with about 140 genera and about 4,000 species (Asif, 2015). The whole family is characterized by leathery glandular leaves which have viscous aromatic terpenoid and polyphenolic substances and flowers with several stamens. Numerous *Syzygium* species were reported to have antibacterial, antifungal, anti-inflammatory and antioxidant activities (Ratnam *et al.*, 2015). The phytochemical studies reported for this species has shown that only flavonoids and terpenoids were available from the leaf and that the plant material has been untapped much for detailed studies (Pulla *et al.*, 2005).

*Syzygium guineense* (Myrtaceae) is a small tree with edible fruits (Djoukeng *et al.*, 2005). It is prevalent in Sub-saharan Africa where the bark is utilised traditionally to treat stomachache and diarrhea. This medicinal plant has been used etnobotanically by the Kipsigis and Ogiek communities in Kenya for treatment of various ailments including cancer. The anticancer property of this medicinal plant is unvalidated. In our search for previously undiscovered secondary metabolites with anticancer properties from plants, the dichloromethane extract from the bark of *S. guineense* was investigated.

The genus Solanaceae encompasses a large number of species with both toxic and pharmacological properties in them (Vieira *et al.*, 2013). The Solanaceae are the third most significant plant taxon economically and the most treasured in terms of vegetable crops, and

are the most variable of crops species in terms of agricultural utility. Many *Solanum* species have previously been explored for their cytotoxicity, antioxidant and antiviral activities, and management of protozoal infections. Some of the anticancer compounds isolated from Solanaceae family are Capsaicin (**1**), Withnolide (**2**), Nicotine (**3**) and Solasodine (**4**). It is reported that decoction from the ground seeds of *Solanum mauense* are used by the Ogiek community in Kenya to expel worms, as purgative, manage tuberculosis and to treat chest ailments (Amuka *et al.*, 2014). The ripe fruits, orange or reddish brown in colour, of *S. maunse* are used traditionally by the Kipsigis community to manage bacterial diseases and cancer.




Salicaceae family is known to produce metabolites which have bacteriocidal properties (Amuka *et al.*, 2014). *Dovyalis abyssinica* (A. Rich), commonly called African gooseberry is common in Africa (de Rosso and Mercadante, 2007) and is from the small genus *Dovyalis* (Flacourtiaceae), comprising 16 species. Cyanogenic tribes of Flacourtiaceae were detached in the family Achariaceae, and the noncyanogenic tribes, comprising *Dovyalis* and *Homalium*, were integrated with Salicaceae (Rasmussen *et al.*, 2006). *D. abyssinica* is native to Ethiopia, Eritrea and Somalia in the North through Kenya and Tanzania to Malawi in the South and propagates in upland rainforest, dry evergreen forest, on riverbanks and occasionally in more open woodland (Kiamba *et al.*, 2009).

Literature data on ethnobotanical uses of *D. abyssinica* have been reported (Jeruto *et al.*, 2008). Among these is management of gonorrhea, brucellosis and teeth problems in humans and to treat mastitis in animals. Other studies have reported that decoction from the roots have been used to manage typhoid and diarrhea amongst the Maasai tribe in Kenya (Nyang'au *et al.*, 2017). The roots of *D. abyssinica* aqueous extracts are used by the Kipsigis Community, in folk medicine, as anticancer agent besides managing other ailments.

Euphorbiaceae family is the prevalent families of flowering plants, that has over 300 genera and 8,000 species (Mwine and Van Damme, 2011).The family is widespread in range, composed of all categories of plants ranging from big woody trees through climbing lianas to simple weeds that grow horizontal to the ground. Members are broadly distributed all around the world. Many family members are found in tropical climates surviving hot dry desert conditions while others are found in rainforest trees and herbs. Previous studies have reported the Euphorbiaceae plants as a rich source of diterpenoids and triterpenoids (Benmerache *et al.*, 2017; Gvazava *et al.*, 2017). Diterpenoids have been suggested as the main chemical constituent responsible for the biological activities of *Euphorbia* species such as the antitumour, antiproliferative, cytotoxic, anti-inflammatory, antiviral and multidrug resistance (Gvazava *et al.*, 2017).

The genus name "Croton" was derived from a Greek word "kroton", meaning thick, in reference to thick smooth seeds, a common feature of most *Croton* species which belong to the subfamily Crotonoideae of the family Euphorbiaceae (Maroyi, 2017). *Croton* is an extensive genus comprising around 1,300 species (Santos *et al.*, 2017). Studies of bioactive compounds from the genus *Croton* have revealed various classes of compounds. The major class of bioactive compounds in the genus *Croton* are terpenoids and mainly diterpenoids, which encompasses acyclic or cyclic diterpenoids, such as clerodane, halimane, kaurane, secokaurane and labdane classes (Salatino *et al.*, 2007).

*Croton dichogamus* is a tree with a pyramidal crown or shrub, the leaves are usually glabrous, membranous, covered with silvery scales and the upper surface is yellowish-brown. The flowers are monoecious, the flower stamens have six sepals and twenty stamens; the flower pistils have five sepals, petals are absent, segments are glabrous and linear, styles are bipartite and the ovary is covered with round membranous scales. The plant has been recorded to occur in Ethiopia, Kenya, Madagascar, Mozambique, Tanzania and Somalia. *C. dichogamus* has been used as a dietary additive to milk or meat soup by the Maasai and Batemi of Kenya and Tanzania, respectively. This species is known as "Mhand" in Tanzania, and it has been used there to cure respiratory disease, by inhalation of smoke of the plant leaves (Mohagheghzadeh *et al.*, 2006). Moreover, this plant has been used to treat chest complaints, malaria and stomach upset in Samburu (Kenya), where it is commonly known as "l-akirding'ai" (Fratkin, 1996) while in another part of Kenya, the Loitoktok district, it is known as "Oloibor benek", and it is used to treat arthritis and gonorrhea (Muthee *et al.*, 2011). Pharmacological studies have shown that the crude extracts of the root bark, stem bark and leaves of *C. dichogamus* do not show significant antifungal, antibacterial and nematicidal

activities. From literature survey the anticancer property of the four medicinal plants under study has not been validated.

The term cancer, malignant neoplasm (neoplasm means new growth) and malignant tumor are synonyms. Cancer is a general term applied to a series of malignant diseases which may affect many parts of the body (Berry *et al.*, 2005). This disease is characterized by a rapid and uncontrolled cell proliferation leading to abnormal growth or tumor. If abnormal growth is not arrested it may progress to death of the patient. Surgery, radiotherapy and chemotherapy are the options currently available for the treatment of cancer (Chudzik *et al.*, 2015). Chemotherapeutic agents can provide temporary relief but cause serious side effects like bone marrow toxicity, neurotoxicity. Surgery is also not possible in all cases. There is urgent need for effective and safe anticancer drugs. A large number of bioactive compounds exist in various plant species. Various plants/extracts have been screened for their anticancer properties in *vitro* and in *vivo* test models. Novel anticancer agents are being sought from traditional medicines. Among bioactive compounds, an important group is that of triterpenes, which show cytotoxic properties against tumor cells at low activity toward normal cells (Zuco *et al.*, 2002). The present study therefore, was aimed at exploring the traditional knowledge and cultural practices in a bid to evaluate medicinal plants for diversity and utilization pattern and also to protect from disappearance of this treasure.

**1.2 Statement of the Problem**

Cancer is a major cause of ill health and death worldwide with about 14 million new cases reported in 2012 (Ministry of Health, 2017). In Kenya, cancer is the 3$^{rd}$ primary cause of death after communicable and cardiovascular diseases. In 2012, there was a projected 37,000 new cancer cases and 28,500 cancer deaths in Kenya. There are numerous conventional treatments of managing cancer nonetheless most of these are faced with challenges such as lack of operational drugs, high cost of chemotherapeutic agents, side effects, harmfulness of anticancer drugs and multi drug unaffected cancer. Thus there is need to search for active naturally occurring anticarcinogenic drugs that can either avert, slow or reverse cancer progress. Natural products are the lead molecules for many of the drugs that are in use. *Dovyalis abyssinica*, *Solanum mauense*, *Syzigium guinense* and *Croton dichogamous* have not been explored for their anticancer property. Therefore, there is need for phytochemical profiling to ascertain their anticancer property.

## 1.3 Objectives

### 1.3.1 General Objective

To investigate anticancer compounds in selected plants from Salicaceae, Myrtaceae, Euphorbiaceae and Solanaceae families.

### 1.3.2 Specific Objectives

i. To obtain bioactive compounds from *Syzigium guinense*, *Dovyalis Abyssinica*, *Croton dichogamus* and *Solanum mauense*.
ii. To determine the structures of the isolated compounds using data obtained from 1D NMR, 2D NMR and mass spectrometry.
iii. To evaluate the anticancer activities of bioactive compounds extracted from from *Syzigium guinense*, *Dovyalis Abyssinica*, *Croton dichogamus* and *Solanum mauense*.

## 1.4 Justification

Cancer is amongst the primary causes of death in the world and management typically consists of different combinations of surgical treatment, radiothrerapy and chemotherapy but notwithstanding these therapeutic choices cancer remains linked with high death rate. The usage of medicinal plants in treatment of cancer is entrenched in the human cultural fabric ever since the times of early man. The four medicinal plants selected in this study are used ethno botanically in managing various cases of cancer by some communities in Kenyan. However, scientific studies have not been undertaken to justify its use. Information of the chemical components of plants is required, not only for the unearthing of these therapeutic agents, but because such data is helpful in availing different chemical resources as well as lead compounds for drug finding. Thus there is necessity to obtain and characterize anticancer compounds in them to enhance the full utilization of selected medicinal plants in managing human diseases.

# CHAPTER TWO

# LITERATURE REVIEW

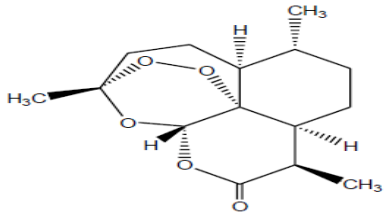
## 2.1 The Traditional Medicine in Drug Discovery

Natural products obtained from medicinal plants, either as uncontaminated compounds or as homogeneous extracts, provide infinite opportunities for previously undicovered drug leads because of the unsurpassed availability of chemical assortment (Sakarkar and Deshmukh, 2011). Many natural products can be categorized into a limited groups only: acetogenins along with propanogenins, terpenoids, byproducts of aminoacids, and aromatic compounds (Croteau *et al.*, 2000). Numerous plant terpenoids are poisons and feeding deterrents to herbivores or are attractants, and many have pharmacological property. Tannins, lignans, flavonoids, and other simple phenolic compounds function as defenses against herbivores and pathogens. Lignins make stronger cell walls, and countless flavonoid pigments are vital attractants for pollinators and seed dispersers. Particular phenolic compounds are associated with allelopathic action and may unfavorably influence the progression of neighboring plants. All over evolution, plants have established defenses against herbivory and microbial outbreak and produced other natural products to nurture attractiveness. The better defended, competitive plants have produced more descendants, and so the capacity to yield and safely stock such ecologically useful metabolites has become widely proven in the plant kingdom.

The research in natural products has yielded nemerous rewards. It has contributed to the discovery of a several array of useful drugs for the management of diverse ailments and led to the development of chromatography and technology, spectroscopic methods of structure determination and various synthesis that now make up the basics of analytical organic chemistry (Newman and Cragg, 2012). One of the significant areas of use of natural products is in the management of human and veterinary diseases. Although the use of natural products as medicinal agents seemingly precedes the first recorded history as the earliest humans used many, but precise plants to treat diseases, the handling of diseases with pure pharmaceutical agents is a fairly modern occurrence. However, the role of traditional medicine in the unearthing of potent chemicals is quite essential.

Amongst certain successes in developing drugs originating in natural products, one can reference the discovery of the antimalarial drugs such as the Cinchona tree alkaloids, analgesics such as the morphine alkaloids as well as the discovery of aspirin.

An effective antimalarial agent, a sesquiterpenoid endoperoxide, known as artemisinin (**5**) was obtained from *Artemisia annua* as a medication against the multidrug resistant strains of *Plasmodium*, after the reported long use of this plant material as an antimalarial agent in the traditional Chinese medicine. By use of the basic structure of artemisinin, semisynthetic agents were made with the objective of improving the pharmacology of the original molecule which lead to the synthesis of artemether (**6**) and dihydroartemisinin (**7**) as potent antimalarial agents that are currently in an extensive application around the world.



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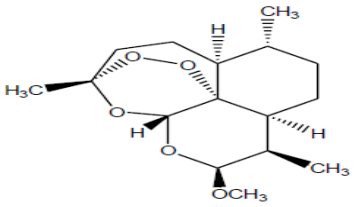

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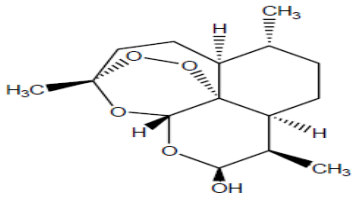

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These limited explanations highlight not only the conceivable of natural products as origin of drugs as well as the solid bridge between the folk medicine and drug development but also the essential of natural products research. Nawadays, drugs obtained from natural products must be pure and completely categorized compounds. Structures are determined primarily by spectroscopic techniques and the amplification of the stereochemistry is a vital feature of the representations.

Plants have shaped the foundation of traditional medicine systems for many years in several countries; China, India and Africa (Newman and Cragg, 2012). Plants utilized in traditional medicine have a varied range of substances that may be used to manage chronic as well as infectious diseases (Duraipandiyan *et al.*, 2006). Their usage in several other cultures has likewise been reported. Due to the development of antagonistic effects and microbial resistance to the chemically prepared drugs, it has led to ethnopharmacognosy. Secondary metabolites seems to function chiefly in defense from predators and pathogens and in giving reproductive benefit as intraspecific and interspecific attractants (Croteau *et al.*, 2000). Several valuable biological activities for instance anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were described (Sakarkar and Deshmukh, 2011). These plant-derived systems continue to contribute an important role in health care, and it has been predicted by the World Health Organization that about 80% of the world's population dpend on traditional medicines for their primary health care (Newman and Cragg, 2012). Medicinal plant drug unearthing offers novel and significant leads against numerous pharmacological targets including cancer, HIV, Alzheimer's and

malaria (Balunas and Kinghorn, 2005). The leading steps to exploit the biologically active compounds obtained from plant resources are extraction, pharmacological screening, isolation and characterization of target compound, toxicological evaluation and clinical evaluation (Sakarkar and Deshmukh, 2011). A summary of the general methods in extraction, isolation and characterization of target compound from plants extract is illustrated figure 2.1.

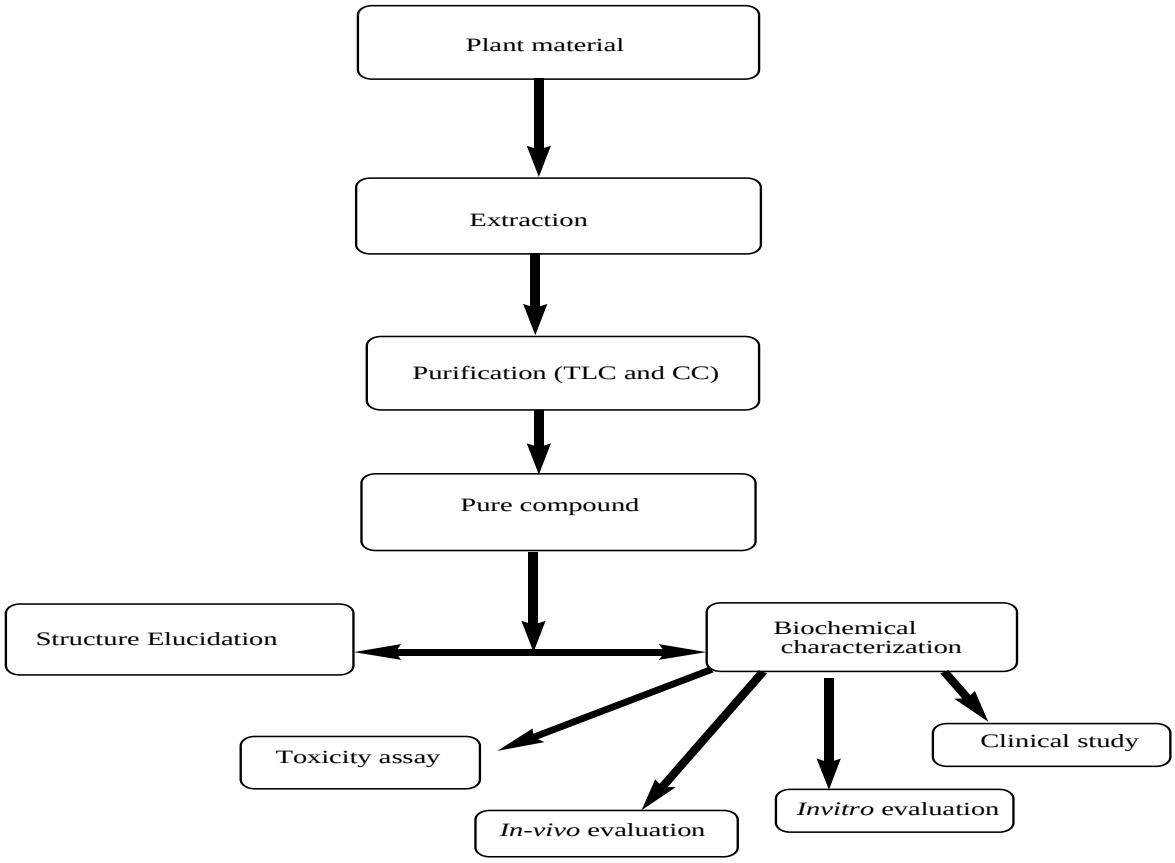



**Figure 2.1: General isolation scheme of bioactive compounds**

Extraction is the fundamental first stage in the examination of medicinal plants, since it is vital to extract the preferred chemical constituents from the plant materials for separation and characterization (Sakarkar and Deshmukh, 2011). The elementary operation encompasses steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a standardized sample and often refining the kinetics of analytic extraction and also amassing the contact of sample surface with the solvent system. Correct actions must be taken to guarantee that probable active components are not lost, distorted or damaged during the preparation of the extract from plant samples. If the plant was chosen based on traditional

uses (Fabricant and Farnsworth, 2001), then there is need to make the extract as outlined by the traditional healer in order to mimic as meticulously as possible the 'herbal' drug. The choice of solvent system mainly depends on the exact nature of the target compound. Diverse solvent systems are accessible to obtain the bioactive compound from natural products. The isolation of hydrophilic compounds entails utilization of polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some cases, isolation with hexane is used to eradicate chlorophyll (Cos *et al.*, 2006).

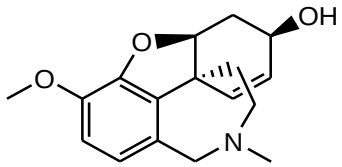
As the target molecules may be non-polar to polar and thermally labile, the appropriateness of the methods of isllation must be taken into account. Various approaches, such as sonification, heating under reflux, soxhlet extraction and others are widely used (Lee *et al.*, 2002) for the plant samples extraction. In addition, plant isolates are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

Owing to the fact that extracted compounds from plants usually occur as a blend of many type of compounds or phytochemicals with diverse polarities, their separation is still a big challenge for the method of identification and characterization of bioactive compounds. Noramlly the isolation of these bioactive compounds is embedded in a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, which should be used to obtain pure compounds. The uncontaminated compounds obtained are then used for the elucidation of structure and biological activity.

TLC is a modest, fast, and cheap procedure that gives the investigator a rapid answer as to how many constituents are in a mixture. TLC is also used to support the characteristics of a compound in a mixture when the retardation factor of a compound is compared with the retardation factor of an identified compound. Further tests include the spraying of phytochemical screening reagents, which effect color changes according to the phytochemicals existing in a plants extract; or by viewing the plate under the UV light. This has also been used for confirmation of purity and identity of isolated compounds. Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound.

Isolating the compounds of importance from the non-soluble medium in which they are implanted needs numerous issues to be considered (Bucar *et al.*, 2013).These comprise the polarity and stability of the etracts and the solvent, the poisonousness, volatility, viscosity and pureness of the extraction solvent, the probability of artefact formation during the extraction process, and the quantity of bulk material to be extracted. In medicinal plants, secondary metabolites typically are originate inside cells, thus crushing of the raw material and breaking tissue and cell integrity before extraction increases extraction yield.

Most of isolation procedures still exploit simple extraction measures with organic solvents of different polarity, water and their mixtures. The methods include maceration, percolation, Soxhlet extraction, ultrasound-assisted extraction and turbo-extraction. Maceration is carried out at room temperature by soaking the material with the solvent with eventual stirring. This is associated with the advantage of moderate extraction conditions although it suffers from high solvent use, extended extraction times and low extraction yields. Several drugs that are derived from different natural sources have been reported; artemisinin **(5)** galanthamine **(8)** and nitisinone **(9)** (Chin *et al.*, 2006; Ganesan, 2008).

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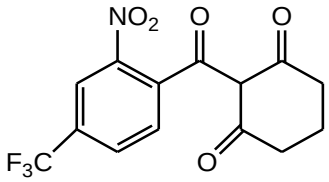


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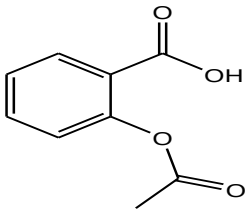
Synthetic drug that was inspired by a natural product is aspirin **(10)** and was synthesized from salicylic acid **(11)** which had been isolated from willow bark. The structure of salicylic acid was employed in design of non-steroidal anti-inflammatory drugs (NSAIDs) for instance acetaminophen **(12)**, ibuprofen **(13)** and naproxen **(14)** (Rishton, 2008)**.** This demonstrates that natural products isolated from medicinal plants and their synthetic derivatives have provided several clinically used medicines. Isolation of natural products from medicinal plants or their synthetic derivatives as drugs requires constant need to investigate several medicinal plants (Butler, 2004).

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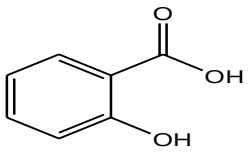


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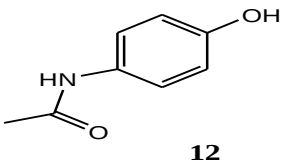


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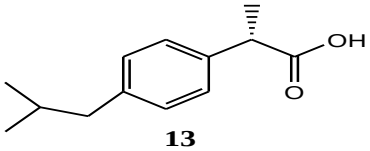


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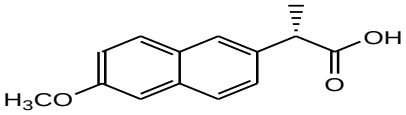


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## 2.2 Plants With Anticancer Activity

Cancer refers in this context to a series of malignant diseases that may upset diverse parts of body. These ailments are characterized by a fast and unrestrained formation of abnormal cells, which may build together to form a growth or tumor, or multiply throughout the body, starting abnormal development at other sites. If the process is not controlled, it may develop until it leads the death of the organism (Sakarkar and Deshmukh, 2011). This disease is the chief public health problem and the second principal cause of death around the world with an apparent rate of more than 2.6 million reports per year (Gali-Muhtasib *et al.*, 2015). Cancer is one of the major causes of death worldwide and only modest development has been made in curbing the illness and death associated with this disease (Bhanot *et al.*, 2011). Cancers may be triggered in one of three ways, explicitly incorrect diet, genetic predisposition, and via the environment. As many as 95% of all cancers are triggered by life style and might take as long as 20–30 years to progress. In addition, research shows that most cancers are triggered by a dysfunction of several genes coding for proteins such as growth factors, growth factor receptors, antiapoptotic proteins, transcription factors, and tumor suppressors, all of which is a target for cancer treatment (Gali-Muhtasib *et al.*, 2015).

The chief methods of management for cancer in humans are surgery, radiation and drugs (cancer chemotherapeutic agents). Cancer chemotherapeutic agents can often give temporary relief of symptoms, elongation of life, and rarely cures (Sakarkar and Deshmukh, 2011). An effective anticancer drug would kill or harm cancer cells without triggering excessive impairment to normal cells. This ideal is hard, or perhaps impossible, to achieve and that is why cancer patients often suffer unpleasant side effects when under-going disease management. The upsurge in the occurrence of cancer along with the unwanted side effects witnessed with chemotherapy demands for the discovery of novel agents from natural sources (Gali-Muhtasib *et al.*, 2015).

Historically, plants have been used in the treatment of cancer and it is noteworthy that over 60% of presently used anti-cancer drugs come from natural sources. The plant kingdom has given an infinite source of medicinal plants that were earlier used as herbal medicines in their unpolished forms as syrups, infusions, and ointments (Ansari and Inamdar, 2010). Natural products continue being an important source of new drugs, new drug leads and new chemical entities (Bhanot *et al.*, 2011). The plant based drug discovery occasioned mainly in the development of anticancer agents including plants (vincristine, vinblastine, etoposide, paclitaxel, camptothecin, topotecan and irinotecan), marine organisms (citarabine, aplidine and dolastatin 10) and micro-organisms (dactinomycin, bleomycin and doxorubicin). Secondary metabolites obtained from plants potentially embody an unlimited source of chemicals for the search of new drugs (Gali-Muhtasib *et al.*, 2015). The ideal compound extracted from the plant may not directly serve as the drug but lead compounds for the development of prospective novel drugs. More than half of all anti-cancer agents allowed internationally were either natural products or their end product and were established on the basis of knowledge gained from small molecules or macromolecules that exist in nature (Bhanot *et al.*, 2011).

Cancer causes significant ill health and death and is a main health problem international (Das *et al.*, 2009). Billions of shillings have been used up on cancer research and until now it is not understood (Nahar *et al.*, 2012). Free radicals, one of the major grounds for the alteration of normal cell to cancerous cells are made as a consequences of a number of endogenous metabolic methods involving redox enzymes and bioenergetics electron transfer and exposure to a surplus of exogenous chemicals (Rajkumar *et al.*, 2011).

Research has described that overproduction of free radical and reactive oxygen species (ROS) would attack important biological molecules such as DNA, protein or lipid leading to many degenerative diseases, such as cancer, Alzheimer's, arthritis and ischemic reperfusion (Suja *et al.*, 2004). More evidence proposes that this potentially cancer inducing oxidative damage might be prevented or limited by antioxidant. Antioxidant may mediate their effect by directly reacting with ROS, quenching them or chelating the catalytic metal ions (Sun *et al.*, 2002). It has been shown that the antioxidant rich diets can reduce oxidative damage to DNA, thus preventing a critical step in the onset of carcinogenesis and the impact of antioxidants on mutagenesis and carcinogenesis has been well established (Zhang *et al.*, 2005; Meyskens and Szabo, 2005)

Plants have a long history of use in the treatment of cancer. A high percentage of anticancer agents are derived in one one way or another from natural sources, including

plants, marine organism and microorganisms (Newman and Cragg, 2012). A number of plants have been accepted a one of the main source of cancer chemoprevention drug discovery and development (Gonzales and Valerio, 2006) due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Gupta *et al.*, 2008; Dahiru and Obidoa, 2008). A huge reservoir of bioactive compounds exists in many species of plants of earth, only a small percentage of which have been examined and continued to be an important source of anticancer agents (Dhanamani *et al.*, 2011). With the decline in the number of new molecular entities from the pharmaceutical industry, novel anticancer agents are being sought from traditional medicines (Akinmoladun *et al.*, 2007). These medicinal plants were used based on ethno botanical evidence as being safer, acceptable, affordable, culturally compatible and suitable for chronic treatment. Phytochemical screening of these plants has revealed that they contain bioactive chemical substances such as alkaloids, tannins, saponin and others with therapeutic potentials (Dhanamani *et al.*, 2011). In traditional medicine, many plants have provided valuable clues for being used as potentially antiparasitic, antimalarial, antileishmania, antitumorous, antifungal and antibacterial compounds (Swapna *et al.*, 2011). Hence, the natural products now have been contemplated of exceptional value in the development of effective anticancer agents with minimum host cell toxicity.

**2.3 Plant Derived Anticancer Agents in Clinical Use**

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally as foods of plant origin (Jing *et al.*, 2010). Flavonoids are important for human health because of their high pharmacological activities as radical scavengers. These compounds possess a common phenyl benzopyrone structure (C6-C3-C6) and they are categorised according to the saturation level and opening of the central pyran ring, mainly into flavones, flavanols, isoflavones, flavonols, flavanones and flavanones (Farquar, 1996; Lopez-Lazaro, 2002).

Flavonoids are a large class of phytochemicals which are omnipresent in human diets, found for example in fruit, vegetables, tea, chocolate and wine and to which a number of beneficial effects on human health, such as antioxidant, anti-inflammatory, antiallergic, antiviral and anticarcinogenic activities. While some flavonoids exhibit potential for anti-human immunodeficiency virus functions (Yao *et al.*, 2004). They are considered as potent agents against cancer, microbes and tumours (Farquar, 1996; Lopez-Lazaro, 2002).

Flavonoids are capable of preventing oxidative cell damage and have strong anticancer activity (Salah *et al.*, 1995; Benavente-García *et al.*, 1997; Okwu and Okwu, 2004).

Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi *et al.*, 1992) and may contribute directly to antioxidative action (Duh *et al.*, 1999). These compounds are very important constituents of plants and their free radical scavenging ability is due to their hydroxyl groups (Hatano *et al.*, 1989).

Tannins are also reported to have a strong inhibition of tumors. Tannins have astringent properties, hasten healing of wounds and inflamed mucous membrane. Likewise, cumarins and their derivatives are another class of cytotoxic compounds showing activity in plants (Cao *et al.*, 1998). Saponins have the property of precipitating and coagulating red blood cells (Okwu and Okwu, 2004).

Alkaloids are some of the most important groups of secondary metabolites due to the great number of isolated products and their pharmacological activity (Pérez-Amador *et al.*, 2007). They have restricted distribution and are readily affected by plant growth location and atmospheric conditions. The isolation of the vinca alkaloids; vinblastine (**15**) and vincristine (**16**) from the Madagascar periwinkle, *Catharanthus roseus* introduced a new era of the use of plant material as anticancer agents (Shoeb, 2006). They were the first agents to advance into clinical use for the treatment of cancer. Vinblastine and vincristine are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers and Kaposi's sarcoma (Newman and Cragg, 2012).

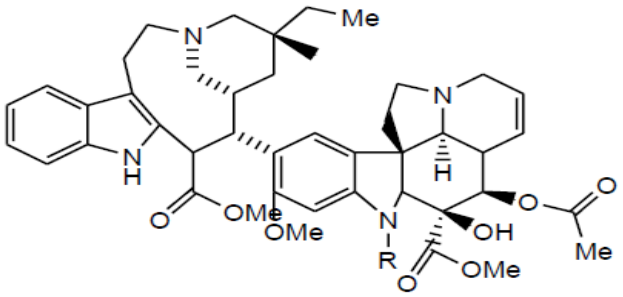
The isolation of paclitaxel (Taxol®, **17**) from the bark of the Pacific Yew, *T. brevifolia* Nutt. (Taxaceae), is an additional indication of the achievement in natural product drug discovery(Shoeb, 2006). Several parts of *T. brevifolia* and numerous taxus species have been utilised by several native American tribes for the management of some non-cancerous incidents (Newman and Cragg, 2012). Reports of *T. baccata* being used in Indian Ayurvedic medicine for the management of cancer has been documented. Paclitaxel is pointedly active against ovarian cancer, progressive breast cancer, small and non-small cell lung cancer (Rowinsky *et al.*, 1992). Camptothecin (**18**), extracted from the Chinese ornamental tree *Camptotheca acuminate* Decne (Nyssaceae), was forwarded to clinical trials by NCI in the 1970s but was stopped due to severe bladder toxicity (Shoeb, 2006). The semi-synthetic

derivatives of camptothecin; Topotecan (**19**) and irinotecan (**20**) are used for the management of ovarian and small cell lung cancers, and colo-rectal cancers, in that order.

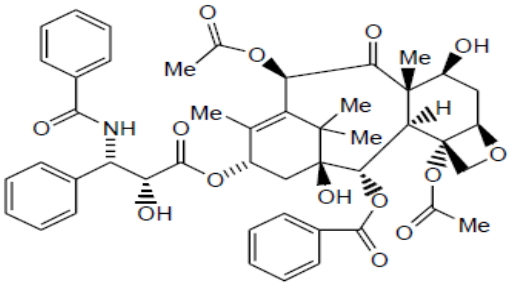
### 2.4 Plant-Derived Anticancer Agents for Future Development

Betulinic acid (**21**) is a pentacyclic triterpene that is a common secondary metabolic product of plants, mainly from *Betula* species (Betulaceae) (Cichewicz and Kouzi, 2004). It was documented having been isolated from *Zizyphus* species (Pisha *et al.*, 1995). Pervilleine A (**22**) was extracted from the roots of *Erythroxylum pervillei* Baill. (Erythroxylaceae) (Shoeb, 2006). Pervilleine A was selectively cell toxic on a multidrug resistant (MDR) oral epidermoid cancer cell line (KB-VI) in the alongside the anticancer agent vimblastine (Mi *et al.*, 2001). Pervilleine A underwent preclinical trial (Mi *et al.*, 2003). Silvestrol (**23**) was first extracted from the mature fruits of *Aglaila sylvestre* (Meliaceae) and it showed cytotoxicity against lung and breast cancer cell lines (Newman and Cragg, 2012).

Two alkaloids; schischkinin (**24**) as well as Montamine (**25**) were isolated from the seeds of *Centaurea schischkinii* and *Centaurea montana* (Shoeb, 2006). Both alkaloids displayed noteworthy cytotoxicity against human colon cancer cell lines. The exceptional structural features of **24** and **25** can be used as template for generating compounds with improved activity.



**15** R=Me
**16.** R=CHO



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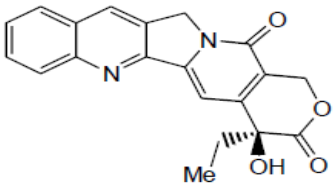

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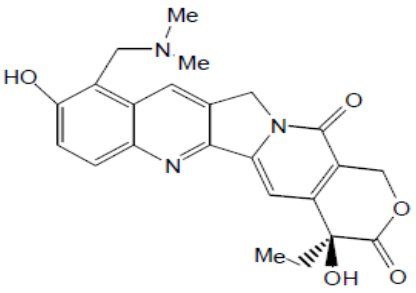

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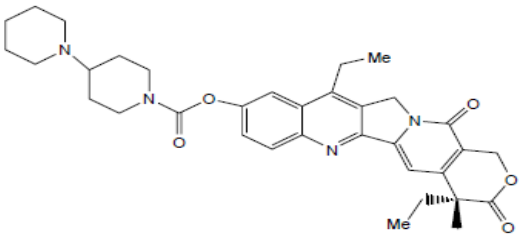

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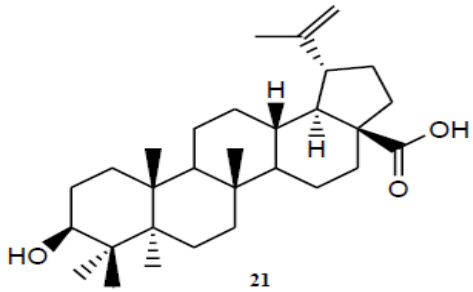

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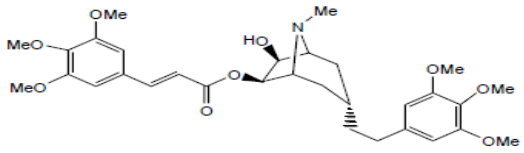


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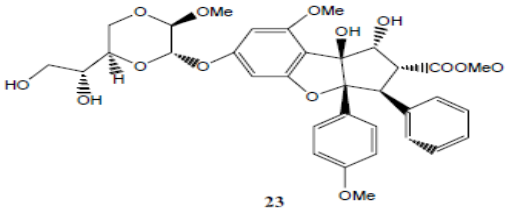


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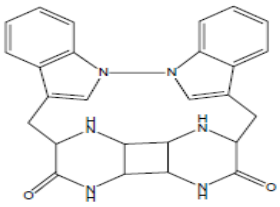


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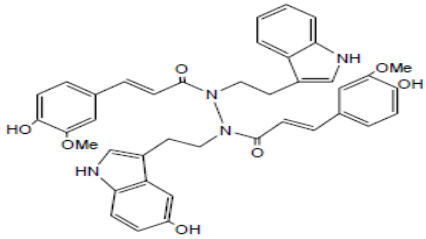



25

## 2.5 The Chemistry of Euphorbiaceae Family.

It is estimated that about 2000 *Euphorbia* species (Euphorbiaceae) all over the world, going from annual weeds to trees widely distributed, mainly in Africa, and Central and South America (Benmerache *et al.*, 2017; Gvazava *et al.*, 2017). Numerous Euphorbiaceae are well recognized in diverse parts of the world as poisonous and/or medicinal (Salatino *et al.*, 2007). Complexity in habitation range and diversity in morphology and genetics has made Euphorbiaceae classification complex. Consequently, (Webster, 1994) reported that there is no single feature that can be used to describe the Euphorbiaceae. Its diverse medicinal acticities are related to their widespread distribution which is buttressed by their survival characteristic adaptations such as succulence. The high complexity of the stated effects is a response of the high chemical diversity of this plant group (Salatino *et al.*, 2007). The exposure to a wide range of habitats prompts them to unavoidably high transformation loads (accruing from stressful habitats) and a wide range of environmental stimuli hence the need to develop a wide battery variety of protective secondary metabolites. *Euphorbia* plants are a rich source of diterpenoids and triterpenoids (Benmerache *et al.*, 2017; Gvazava *et al.*, 2017) . Diterpenoids have been reported as the main chemical components associated with the activities of *Euphorbia* species such as the antitumour, antiproliferative, cytotoxic, anti-inflammatory, antiviral and multidrug resistance (Wang *et al.*, 2016; Gvazava *et al.*, 2017)

Croton is an extensive genus of Euphorbiaceae, consisting of around 1,300 species of trees, shrubs and herbs spread in tropical and subtropical regions of the earth hemispheres (Salatino *et al.*, 2007). The *Croton* genus is categorised into the Crotoneae species of the Inaperturate Crotonoideae clade (Wurdack *et al.*, 2005) and it is one of the main genera of the

Euphorbiaceae family comprising of more than 800 species distributed in the tropics and subtropics, with most being found in America (Webster, 1994). The main morphological features of the *Croton* genus, which vary from other genera, are that the petals and pistillate flowers can either be reduced or absent, the filament in the staminate flowers are inflexed in the bud and the terminal flowers in the thyrses are usually pistillate, while distal flowers in cymules are staminate (Webster, 1993).This genus with extensive range of biologically active compounds has been found to apply vasorelaxant action (Baccelli *et al.*, 2007). Earlier phytochemical surveys show that this genus has alkaloids, flavonoids triterpenoids and steroids and a huge number of diterpenoids (Santos *et al.*, 2017). The genus is rich in components with biological propertries, mainly diterpenoids such as phorbol esters, clerodane, labdane, kaurane, trachylobane, pimarane (Salatino *et al.*, 2007; Mulholland *et al.*, 2010; Langat *et al.*, 2012; Ndunda *et al.*, 2016; Abebayehu *et al.*, 2016; Maroyi, 2017);

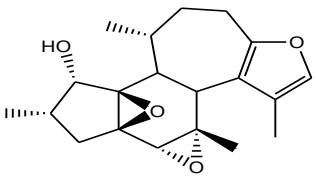
**2.6 Chemical Constituents of the *Croton* Genus**

Studies done on phytoconstituents of the *Croton* genus have shown numerous classes of compounds. The major category of compounds in the *Croton* genus is terpenoids (Chávez *et al.*, 2013), and mostly diterpenoids, which comprises acyclic or cyclic diterpenoids, such as clerodane, halimane, kaurane, secokaurane and labdane classes (Salatino *et al.*, 2007; Chávez *et al.*, 2013, Mulholland *et al.*, 2010; Langat *et al.*, 2012; Ndunda *et al.*, 2016*;* Abebayehu *et al.*, 2016, Maroyi, 2017). It has been widely described that a countless diversity of structurally assorted diterpenoids such as abietanes, neoclerodanes, labdanes, *ent*-kauranes, cembranoids and *nor*-Crotofolane-type diterpenoids are often found in *Croton* species (Vigor *et al.*, 2002; Santos *et al.*, 2009; Yang *et al.*, 2009; Mulholland *et al.*, 2010).

*Croton dichogamus* is a tree that has pyramidal crown or shrub. Its leaves are typically glabrous, membranous, covered with silvery scales and the upper surface is yellowish-brown. The flowers are monoecious, the flower stamens have six sepals and twenty stamens; the flower pistils have five sepals, have no petals, segments are glabrous and linear, styles are bipartite and the ovary is covered with round membranous scales. The plant has been documented to occur in Ethiopia, Kenya, Madagascar, Mozambique Tanzania and Somalia.

*C. dichogamus* has been exploited as a dietary additive to milk or meat soup by the Maasai and Batemi of Kenya and Tanzania in that order. This species is known as "Mhand" in Tanzania, and it has been used there as a remedy to respiratory disease, by inhalation of smoke of the plant leaves (Mohagheghzadeh *et al.*, 2006). Furthermore, this plant has been

used to manage chest complaints, malaria and stomach upset in Samburu (Kenya), where it is commonly known as "l-akirding'ai" while in another part of Kenya, the Loitoktok district, it is known as "Oloibor benek", and it is used to manage arthritis and gonorrhea (Muthee *et al.*, 2011). Nevertheless, pharmacological research have shown that the crude extractives of the root bark, stem bark and leaves of *C. dichogamus* do not exhibit noteworthy antifungal, antibacterial and nematicidal activities. The chemical constituents of *C. dichogamus* have not been screened since (Jogia *et al.*, 1989), who isolated two previously undescribed crotofolane diterpenoids: crotoxide A (**26**) and crotoxide B (**27**) from the leaves of the species collected in Kenya. (Chávez *et al.*, 2013) additionally established the presence of crotofolanes in *C. dichoganous.*



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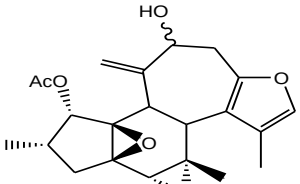

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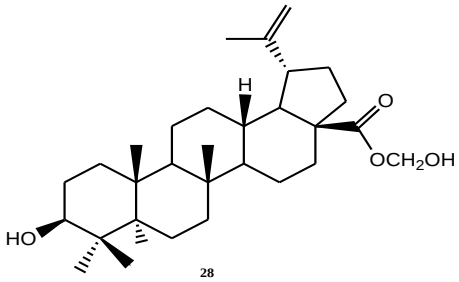
## 2.7 Compounds Isolated from Myrtaceae Family

The myrtaceae is a large family of plants comprising of trees and shrubs originating in the tropics and subtropics and encompasses about 150 genera and 3600 species (Yadav *et al.*, 2011). *S. cumini* L. is a significant member of this family. Few reports have pointed out the potential of *S. cumini* fruits to combat cancer. (Nazif, 2007) extracted four antocyanins from acidic alcoholic extracts of *S. cumini* fruits in Egypt and the crude extract was reported to exhibit effective cytotoxic activity on several types of human cancer cell lines.(Barh and Viswanathan, 2008) indicated that the crude extract of *S. cumini* fruits repressed growth and prompted apoptosis in cervical cancer cell lines HeLa and SiHa in a dose and time-dependent manner.

The family Myrtaceae is associated with tannins and flavonols as the chief chemical components. *Syzygium guineense* is a member of the family Myrtaceae characterized as an evergreen water loving dicotyledon which propagates to a height of 8 – 15 metres (Abok and Manulu, 2017). In Africa, the plant is found in Nigeria, Senegal, Eritrea, Ethiopia, Somalia, Zaire, Rwanda, Zambia, Malawi, Zimbabwe, Namibia, Uganda, Swaziland, Cameroon, and South Africa. The leaf and bark of this plant are used for the management of tuberculosis, chronic diarrhea, cough, dysentery, malaria, amenorrhea, wounds, ulcers, rheumatism and infections (Abok and Manulu, 2017).

The species of the genus *Syzygium* are well reported for their antimicrobial, anti-inflammatory, antimalarial and larvicidal effect in mosquito vector. Different species of Syzygium have exhibited encouraging antimalarial activity *in vitro* (Tadesse and Wubneh, 2017). Secondary metabolites isolated from *S. guineense* responsible for their antimalarial activity include: Terpenoids, Phenols, Anthraquinones and Flavonoids. *S. guineense* leaves are also recognized by their immunomodulatory, anti-oxidant, and anti-inflammatory properties (Tadesse and Wubneh, 2017).

Isolation of two antimycobacterial lupane type-isoprenoids was reported from the stem bark of *S. guinense*; Betulinic acid (**21**) and Betulinic acid methylenediol ester (**28**) (Oladosu *et al.*, 2017). There is no previous studies that have had been done on the Kenya's species from literature survey.



28

**2.8 Compounds from Salicaceae Family.**

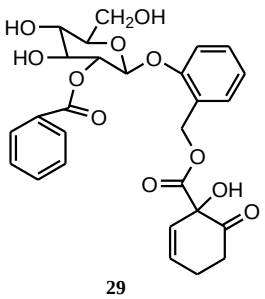
The genus *Dovyalis* has previously been categorized in the family Flacourtiaceae both with *Homalium*, a genus producing spermine-type alkaloids. Flacourtiaceae has long been documented as a family containing a highly adjustable and provocative circumscription. Lately, cyanogenic tribes of Flacourtiaceae were detached in the family Achariaceae, and the noncyanogenic tribes, comprising *Dovyalis* and *Homalium*, were combined with Salicaceae (Rasmussen *et al.*, 2006).

*Dovyalis abyssinica* (A. Rich), commonly called African gooseberry which is native to Africa (de Rosso and Mercadante, 2007) and locally known as "Koshim" in Amharic, belongs to the small genus *Dovyalis,* comprising 16 species (Rasmussen *et al.*, 2006). Although alkaloids are generally uncommon in this family, two alkaloids have been identified in *D. caffra* (Stanstrup *et al.*, 2010) . Besides, (Stærk *et al.*, 2003) indicated the presence of a different class of spermidine-type alkaloids, dovyalicins A-D, in the leaves of *D. macrocalyx*, with dovyalicin A as the chief alkaloid. (Rasmussen *et al.*, 2006) examined the phytochemistry of *D. macrocalyx*, *D. abyssinica,* and *D. hebecarpa* and described the presence of dovyalicin-type alkaloids. Phytochemical analysis doned on the leaf extracts of *D. abyssinica* showed the presence of sapponins, phenolic compounds, flavonoids, and

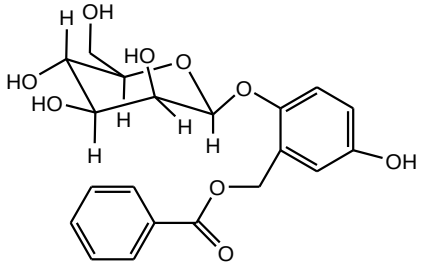
steroids (Tadesse *et al.*, 2015). Alkaloids are were reported as absent in salicaceae but the phenol glucosides such as tremulacin (**29**) and the closely related salicortin (a debenzoyl derivative of tremulacin) are recognized to be specific markers of Salicaceae (Rasmussen *et al.*, 2006). Salicinoids are a category of phenolic glycosides that are the signature secondary metabolites of the Salicaceae (Boeckler *et al.*, 2011). These compounds consist of glucosylated variations of salicyl alcohol, with the simplest one known as salicin (Rubert-Nason *et al.*, 2018).

Phenolglycoside byproducts of 2-acyloxy salicyl alcohol are prevalent in plants of the family Salicaceae. Flacourtiaceae currently allotted to Salicaceae are a number in which secondary metabolic profiles are categorized by the occurrence of phenolic glycosides founded on hydroxylated derivatives of benzyl alcohol alike to, and in some cases indistinguishable to compounds isolated from Salicaceae (Mosaddik *et al.*, 2007). Many phenolglycosides of this family have salicin moiety (2-(b-D-glucopyranosyloxy)-benzyl alcohol) (Belyanin *et al.*, 2012). These ordinary compounds encompass a large range of biological activity. Phenolic glycosides are some of the most plentiful secondary metabolites found in plant tissues, and play an important activity as anti-herbivore defenses in the Salicaceae. Salireposide (**30**) has antiviral activity, and antitumor activity. It could be useful for arthritis therapy and exhibit an inhibitory activity against snake-venom phosphodiesterase.

Reported studies have documented that the dichloromethane extract of *D. abyssinica* leaves were the most active trypanocidal compounds with a high selectivity index (SI = 125.0) (Nibret and Wink, 2011). *D. abyssinica* contains dovyalicin-type spermidine alkaloids which in part resemble the chemical structures of standard drugs such as pentamidine, and hence these components may be responsible for the biological action of the plant. Other studies has documented this medicinal plant to have shown antibacterial and antifungal activities (Tadesse and Wubneh, 2017).




**29**

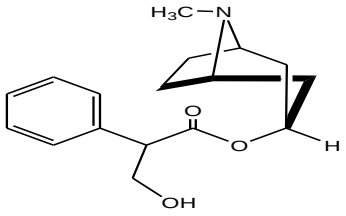



**30**

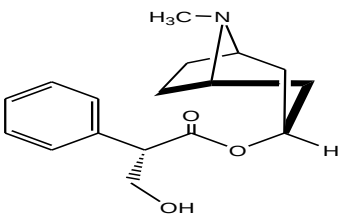
## 2.9 Compounds from Solanaceae Family

The Solanaceae comprises of about 98 genera and 2,700 species with a great variety of habitats, morphology and ecology (Yadav *et al.*, 2016). Eight genera, *Solanum, Lycianthes, Cestrum, Nolana, Physalis, Lycium, Nicotiana, Brunfelsia* comprise more than 60% of the species. The name Solanaceae comes from the genus *Solanum*, "the nightshade plant". The etymology of the Latin word is vague. The name may originate from a supposed similarity of certain solanaceous flowers to the sun and its rays. At least one species of *Solanum* is known as the "sunberry". Alternatively, the name could come from the Latin verb *solari*, meaning "to soothe".

Solanaceae is one of the leading families of drug-producing plants used not only in contemporary medicine but also in traditional and herbal medicine for the management of a varied range of diseases (Mohammed *et al.*, 2016). Solanaceae are documented having a wide range of alkaloids. To humans, these alkaloids can be necessary, deadly, or both. The tropanes are the utmost well-reported of the alkaloids found in the Solanaceae. Tropane alkaloids such as atropine (**31**), hyoscyamine (**32**) and scopolamine (**33**), are of pronounced concern for the pharmaceutical industry as they are extensively utilised for their mydriatic, antispasmodic, anesthetic, bronchodilators and antiasthmatic characteristics.




**31**

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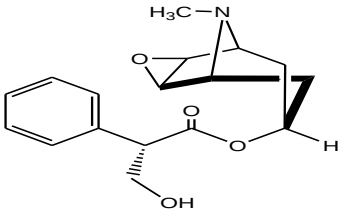
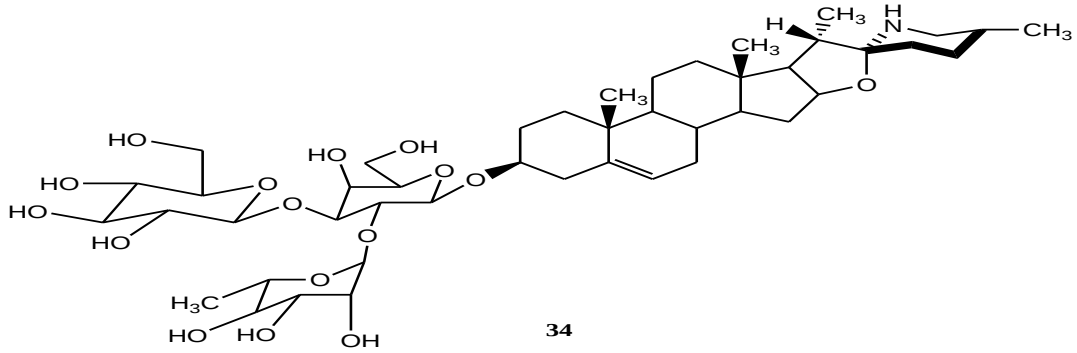



**33**

The Solanaceae family is a cradle of steroidal alkaloids and bioassay-guided isolation of *S. panduriforme* and *S. aculeatissimum* gave solasonine (**34**), (Khorombi *et al.*, 2006)




**34**

## 2.10 Biosythetic Pathways

Biosynthetic pathway of selected class or compounds isolated in this study has been given in this section

### 2.10.1 Biosynthesis of Diterpenoids

The precursor of diterpenoids is geranylgeranyl diphosphate **(GGPP)**. GGPP is formed by adding an IPP unit to FPP (Figure **2.2)** (Dewick, 2002). Loss of the pyrophosphate group leads to carbocation formation, which is followed by cyclization and Wagner-Meerwein rearrangements to form the cyclic diterpenoids. Since some of the compounds isolated in this study are clerodane crotofolane diterpenoids, their mechanism of formation is illustrated.

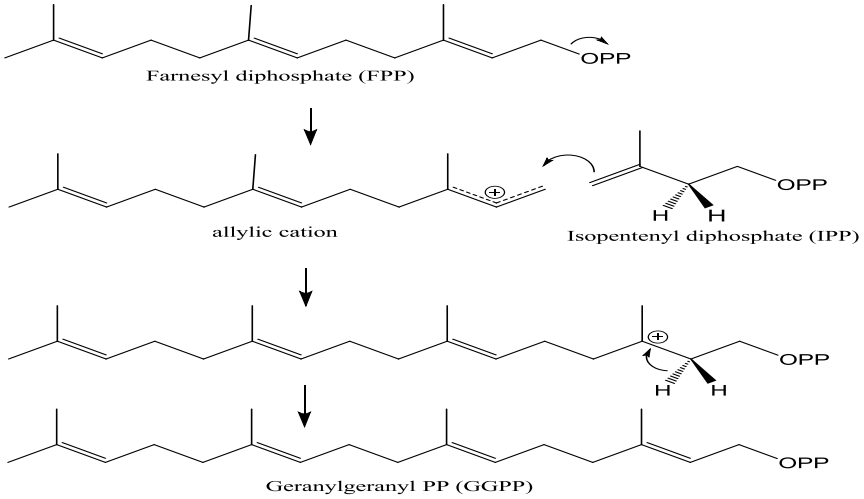



**Figure 2.2: Biosynthetic of GeranylGeranyl Diphosphate (GPPP)**

The biosynthesis of crotofolene bioactive compounds starts with the loss of the diphosphate from GGPP to form a carbocation which then generate a cembrene dierpenoid (Dewick, 2002). A 2,15-cyclization of this product produces the casbene diterpentoid skeleton structure. This is then followed by 6,10-cyclization to forming lathyrene which undergoes 5,13 cyclization to give Jatropholene compound. The opening of the C - 14/C - 15 cyclopropane ring of Jatropholene produces crotofolene bioactive compound, while the breakage of the C - 1/C - 15 bond produce isocrotofolene ( figure 2.3) (Kawakami *et al.*, 2015).

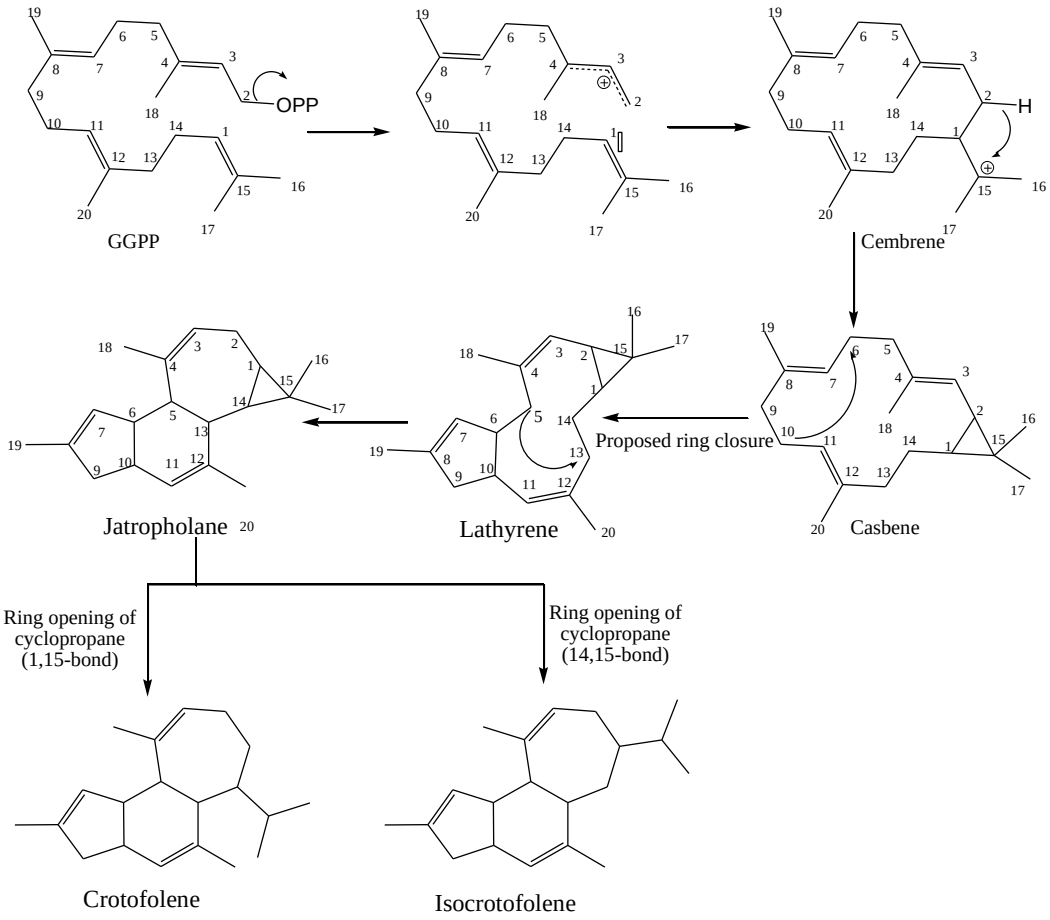
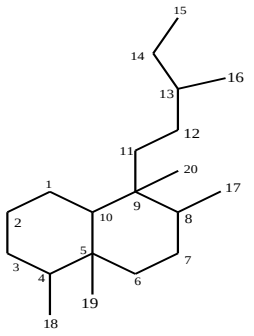


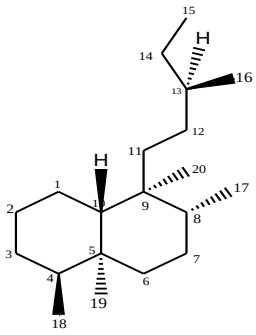

**Figure 2.3: Biosynthesis of Crotofolene**

In this study one of the classes of compounds isolated is Clerodane diterpenoids. They are a huge cluster of natural secondary metabolites originating from several hundreds of plant species (Li *et al.*, 2016). These compounds have aroused interest due to wide array of biological activities reported in the literature by various scholars such as antitumor, antifungal, NGF-potentiating, antibiotic, anti-peptic ulcer, antiplasmodial, as well as hypoglycemic, hypolipidemic, and anti-thrombin inhibitory activity.

Clerodane diterpenes are bioactive compounds that have two rings fused and their basic skeleton has two components: a fused ring decalin moiety having 10 carbon atoms and a side chain that has six-carbon attached to C - 9 (C11 – C16, with C - 16 attached at C – 13 (3-methylpentyl) (Li *et al.*, 2016). The remaining four carbon atoms (C17–C20) are attached to decalin ring at C8, C4, C5, and C9, in that order. Therefore there are three structures arising from this arrangement; clerodane skeleton (**35**), neo-clerodane (**36**) and entneo-clerodane (**37**) as shown below.



**35**



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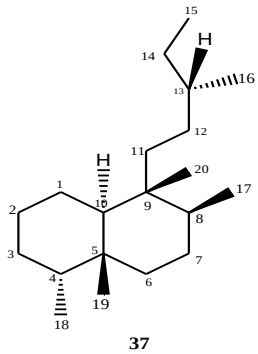


**37**

About 25 % of clerodanes have a 5: 10 cis ring structure and the other 75% of clerodanes have a 5 : 10 trans ring structure (Li *et al.*, 2016). Those structures having a 5 : 10 cis ring junction are commonly isolated from medicinal plants from specific families: Euphorbiaceae, Flacourtiaceae (Salicaceae) and Menispermaceae families. Additionally to the comparative configuration of the trans or cis junction of the fused rings, these bioactive compounds are classified based on their relative configurations at C-8 and C-9. Therefore four types of clerodane skeletons can be identified in relation to the arrangement at the point of fused atoms and of the substituents at C-8 and C-9: trans–cis (TC), trans–trans (TT), cis–cis (CC), and cis–trans (CT). In the mainstream of clerodanes, the C-17 and C-20 substituents on C-8 and C-9 are cis. Documented reports in literature gives an account of varied stereochemistry of clerodanes which are categorized as neo-Clerodanes (formerly ent-clerodanes) and their enantiomers entneo-clerodanes (Li *et al.*, 2016).

### 2.10.2 Biosynthesis of Triterpenoids

Triterpenes are a big group of compounds having carbon skeleton with six isoprene units. These class of bioactive compounds are common in plants and animals and are characterized by C-30 carbon skeleton. Majority of these are known to originate from squaline (**38**) due to various cyclizations, loss of small molecules, ring expansions, or contractions. The different types of triterpenoids arises due to varied folding pattern of the squalene chain. There are about 20 different groups of triterpenoids bioactive compounds documented. First the cyclizations of chair-boat-chair-boat conformation of squalene give a protostane cation and lanostrane skeleton is derived from this cation, which forms the biological precursor for most steroids found in animals. The cycloartane skeleton is also formed from the protostane cation by cyclization between C9 - C19 carbon atoms. Most plants biosynthesize their triterpenes from the cycloartane skeleton (Buckingham, 1997) and these triterpenoids are commonly called phytosterols. On the other hand triterpenoids may be considered as products of rearrangement of squalene epoxide which is believed to have been

biosynthesized by the mevalonate pathway, although the route via the deoxyxylulose phosphate pathway (DXP) is reported to be more widely accepted (Dewick, 2002). The main precursor of terpenoids is the isoprene unit, which is formed *via* isopentyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the latter being formed from IPP with the isomerase enzyme ( figure **2.4**).

Most triterpenoids have methyl groups at the C10 and C13 positions and an alkyl side chain at position C17. It is estimated that 2500 triterpenoids have been documented, however, very few of them have had their biological importance investigated. Other studies have reported that several triterpenoids have diverse pharmacological properties such as anti-fungal (Zaidi and Crow 2005) anti-bacterial and anti-mutagenic activity (Lin *et al.*, 2003). Their documented mode of action is the inhibition of multi-drug resistance (MDR) protein, the activity of which leads to the failure of several potential anticancer agents (Molnár *et al.*, 2006).

The distribution of triterpenoids has been reported as being widespread in nature and are highly abundant in in different parts of medicinal plants especially in the leaves, bark, fruits and seeds of the herbs. Classification of triterpenoids is based on the number of isoprene units; they can be acyclic, mono-, bi-, tri-, tetra- and pentacyclic. Triterpenoids are based on a 30-carbon skeleton consisting of five to six membered rings or four to six membered rings and a five membered ring. Pentacyclic triterpenes have six isoprene units with a basic formula of $C_{30}H_{48}$. These class of compounds are reported as being synthesized in plants by cyclization of squalene. Notably the most studied triterpenoids are the tetracyclic triterpenoids which encompasses cycloartanes, dammaranes, euphanes and protostanes. On the other hand pentacyclic triterpenes have been extensively studied and these in includes gammaceranes, hopanes, lupanes, oleananes and ursanes (Laszczyk, 2009).

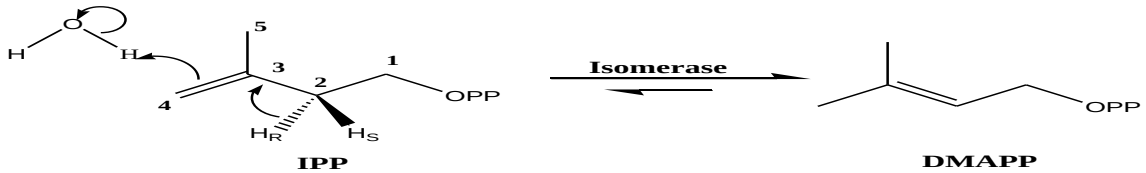



**Figure 2.4: Isomerization of IPP to DMAPP**

In plants, the enzymes from the mevalonate pathway are found in the cytosol and therefore triterpenoids and steroids (cytosolic products) are considered being formed through this pathway, whereas in the deoxyxylulose phosphate, the enzymes are found mainly in the chloroplasts where the other terpenoids are derived (Dewick, 2002). IPP undergoes isomerism to DMAPP through the isomerase enzyme. This results in removal of the *pro-R* proton from C – 2 stereospecifically and the incorporation of a proton from water onto C - 4. Though the isomerization reaction of IPP to DMAPP is reversible, the equilibrium favours the formation of DMAPP.

When the phosphate (a good leaving group) of DMAPP leaves, it gives an allylic carbocation which is stabilised by charge delocalization thus making it electrophilic. IPP on the other hand is a strong nucleophile due to the terminal double bond. This differing reactivity of DMAPP and IPP forms the basis of terpenoid biosynthesis work in the linkage of isoprene units in a head-to-tail manner (Dewick, 2002).

The combination of DMAPP and IPP through prenyl transferase mediation, leads to the formation of geranyl diphosphate (GPP). GPP is the fundamental precursor for monoterpenoid ($C_{10}$) compounds synthesis. The combination involves ionisation of DMAPP to the allylic cation to which the double bond of IPP adds, followed by the stereochemical loss of the *pro-R* proton. GPP possesses the reactive allyl diphosphate group and the reaction mediated by prenyl tranferase continues by addition of an IPP unit to the geranyl cation which leads to the formation of farnesyl diphosphate (FPP), the sesquiterpenoid ($C_{15}$) and geranyl geranyl diphosphate (GGPP), the diterpenoid precursor.

Triterpenoids are formed by joining two FPP units in a tail-to-tail resulting in the hydrocarbon squalene (**38**). These are formed from the precursor presqualene diphosphate by a reaction which is considered to go through different pathways. However the mechanism for its biosynthesis from squalene (**38**) is not clear though it has been resolved when presqualene diphosphate was isolated from rat liver (Dewick, 2002). This formation of presqualene diphosphate (Figure **2.5**) is considered to occur via an attack of the $\alpha^2$ double bond of one FPP molecule on the farnesyl cation (formed by enzymatic ionisation of the second FPP molecule). The tertiary cation obtained as an intermediate is discharged by loss of a proton resulting in the formation of a cyclopropane ring to yield presqualene diphosphate. This is followed by loss of diphosphate to form an unstable primary cation that undergoes rearrangement to generate a more favourable secondary carbocation and less strained cyclobutane ring. Bond cleavage produces an allylic cation, stabilised by charge

delocalisation, which is quenched by attack of a hydride ion from NADPH to form squalene (**38**), an important precursor of cyclic triterpenoids (figure **2.6**).

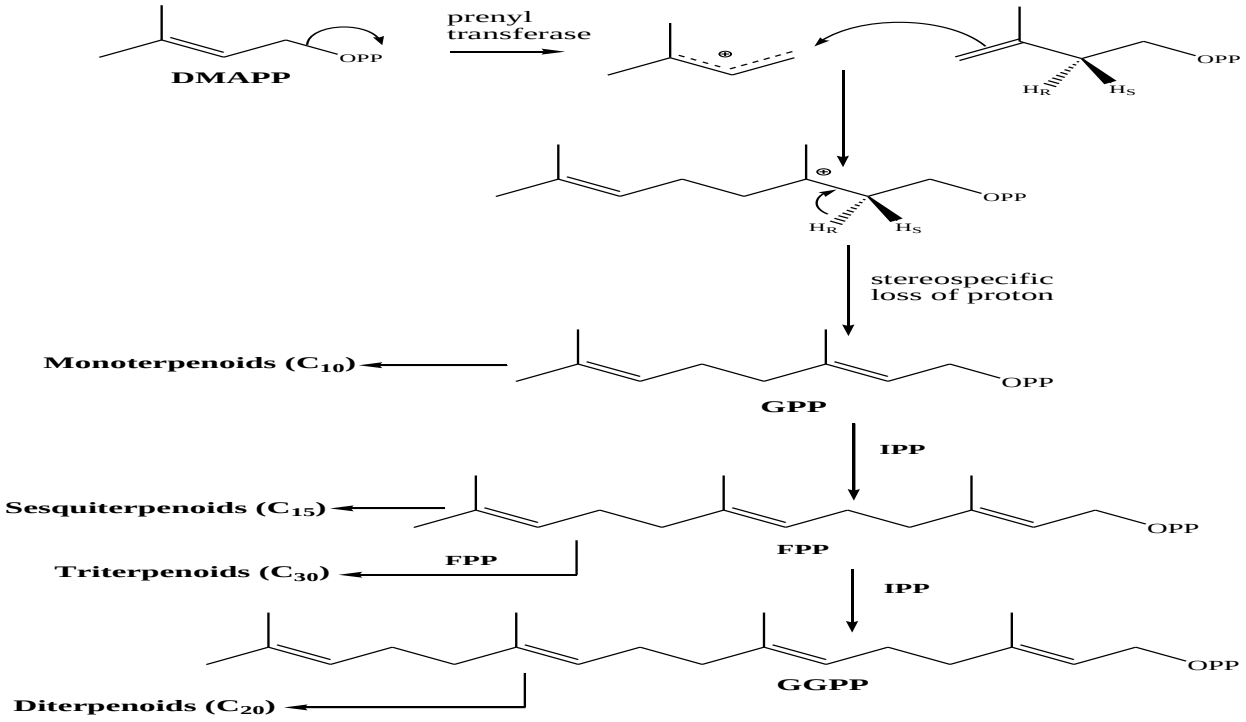



**Figure 2.5: Formation of Triterpenoid Precursors GPP, FPP and GGPP**

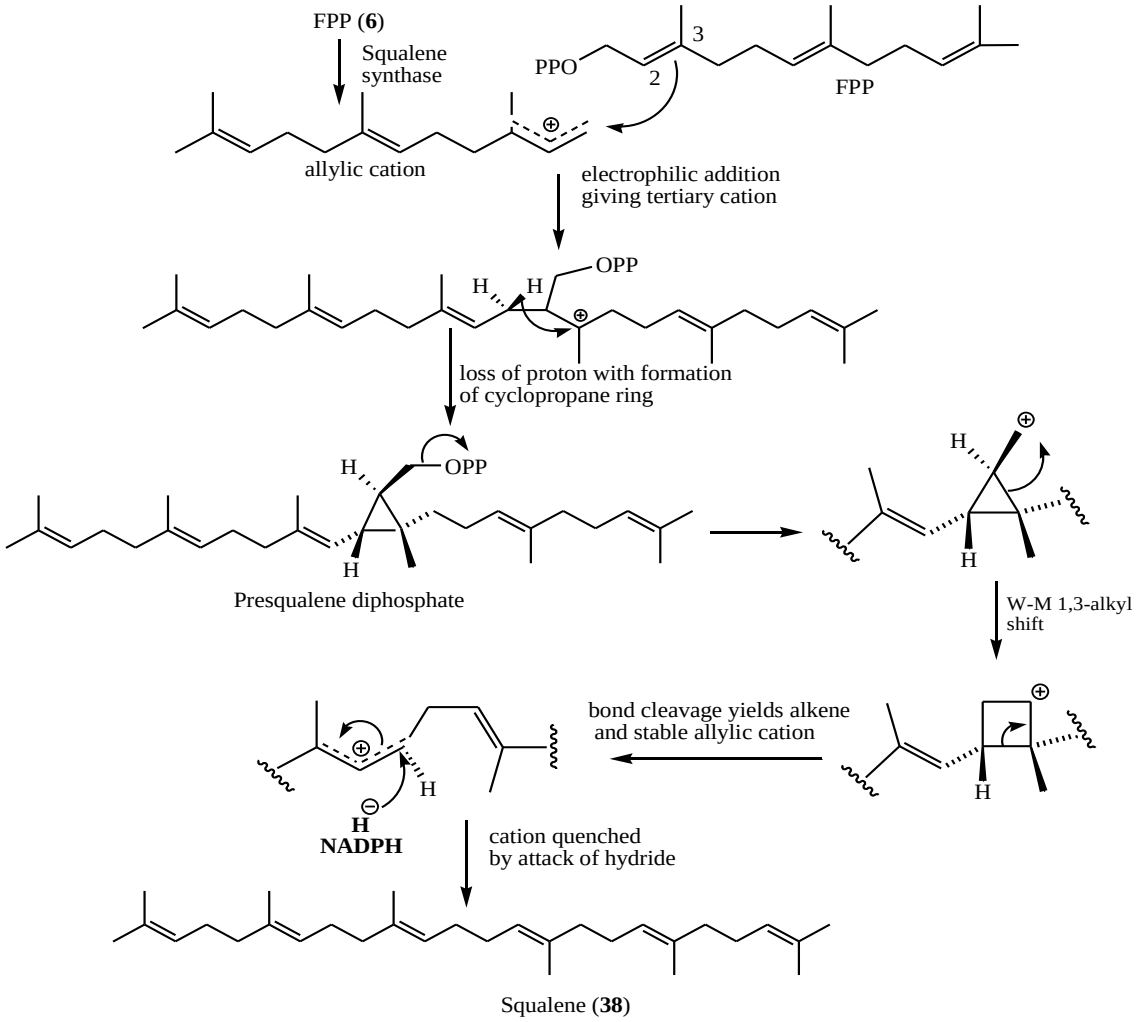



**Figure 2.6: Formation of Squalene from Farnesyl Diphosphate (FPP)**

Cyclisation of squalene (**38**) is possible through the intermediate squalene-2, 3-oxide (**39**) which is produced in a reaction catalysed by a flavoprotein in the presence of $O_2$ and NADPH cofactors, resulting in the epoxidation of the terminal double bond in squalene (**38**). If squalene-2,3-oxide (**39**) is folded onto a chair-chair-chair-boat conformation on the enzyme surface, the transient dammarenyl cation (**40**) is produced through a series of cyclizations followed by a sequence of Wagner-Meerwein rearrangements of 1,2-hydride and 1,2-methyl migrations to form the C-20 epimers, euphol (**41**) and tirucallol (**42**) (figure **2.7**) (Dewick, 2002).

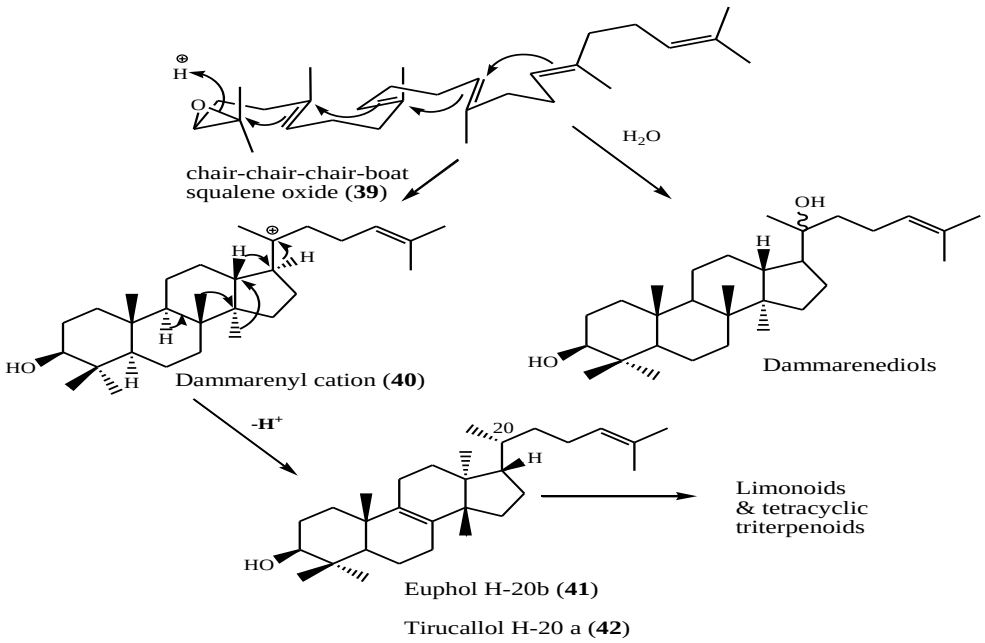



**Figure 2.7: Formation of Triterpenoid Precursors**

### 2.10.3 Lupane Triterpenoids

One of the compounds isolated from this study is betulinic acid which is reported to originate from lupane. The lupane triterpenoids are characterised by four six-membered rings with a fifth five-membered ring to which an isopropyl group is attached. Their biosynthesis has been documented in literature reports as being formed by a 1,2-alkyl shift in the dammarenyl cation (**40**) which results in the bacharenyl cation (**43**) and then cyclisation onto ring D leading to the formation of lupenyl cation (**44**). This in the end forms lupeol (**45**) by the loss of a proton (figure **2.8**).

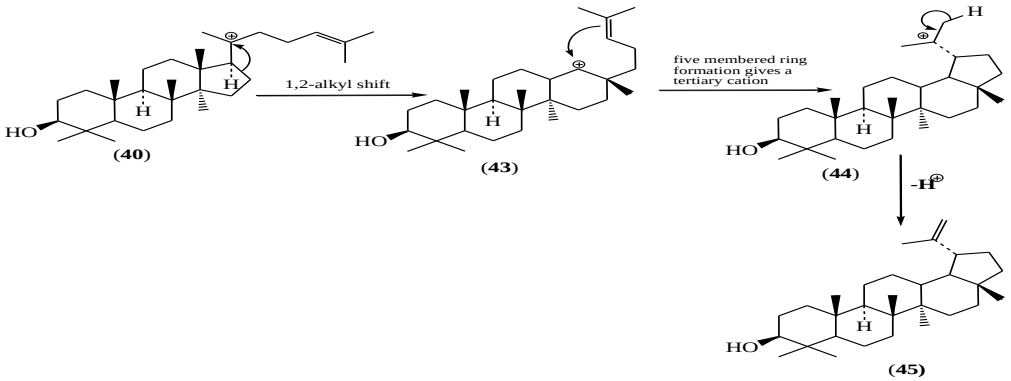



**Figure 2.8: Formation of Lupeol**

### 2.10.4 Synthesis of Betulinic Acid

Betulin (**46**) has extensively been explored by research scientist due to its anticancer properties and its bioactive derivatives. Betulinic acid (**21**) is noted for its anti-HIV activity (Boparai *et al.*, 2017). Betulin is obtained from linear hydrocarbon squalene (**38**) and noted for its three most prominent positions where chemical derivatisation can be accomplished: primary hydroxyl group at position located at C-28, secondary hydroxyl group at located at position C - 3, and alkene moiety at position C - 20. These bio-chemical derivatisation at positions C - 28 of the parent structure of betulin produces betulinic acid (Figure **2.9**) (Drag *et al.*, 2009).

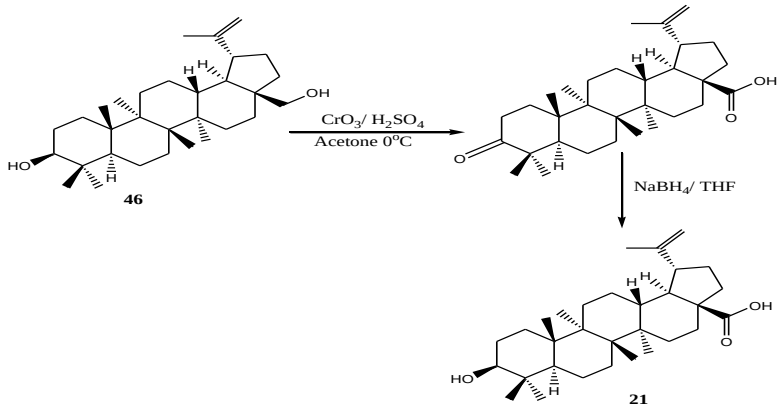



**Figure 2.9: Synthesis of Betulinic Acid from Betulin**

### 2.10.5 Biosynthesis of Sitosterol

Phytosterols are a subgroup of the steroids and this is an important class of bioorganic molecules, common in plants, animals, marines as well as fungi and have similarity to cholesterol in structure (Saeidnia *et al.*, 2014). Among phytosterols, β-sitosterol is usually used for heart disease, hypercholesterolemia, modulating the immune system, prevention of cancer, as well as for rheumatoid arthritis, tuberculosis, cervical cancer, hair loss and benign prostatic hyperplasia. δ - sitosterol is the dominant phytosterol, which may undergo oxidative process just like cholesterol, resulting in β-sitosterol oxides (Saeidnia *et al.*, 2014).

The biosynthesis of the plant sterols is regulated during membrane biogenesis (Dewick, 2009). The literaturesupports that β-sitosterol is biologically synthesized from both mevalonate and deoxyxylulose pathways. The mechanism of β-sitosterol biosynthesis has been studied using $^{13}C$-labeling approach and although varies found according to the organism used, cycloarteol has been identified as an initial substrate (Saeidnia *et al.*, 2014). Actually, one molecule of isopentenyl-diphosphate (IPP) joins to two molecules of dimethylallyldiphosphate (DMAPP) to produce farnesyldiphosphate (FPP). Two of the later

molecule (FPP) are then combined tail-to-tail to result in formation of squalene, as a triterpene and finally cycloartenol (Dewick, 2009)

### 2.10.6 Furan Ring Formation via Side-Chain Oxidation

The furan ring is biosynthesized through stepwise oxidation of the protolimonoid's side-chain to produce a hydroxyl group at C - 23, a 24, 25 - epoxy group, and an aldehyde at C - 21, followed by nucleophilic cyclisations from the oxygen at C - 23 to the carbonyl group at C - 21 to form the hemiacetal ring. Opening of the C - 24, 25 epoxide ring followed by oxidation produces a ketone at C - 24, which then undergoes Baeyer-Villiger oxidative cleavage of the C -23,24 bond to give a dihydrofuran ring, which finally forms the furan ring, with the loss of four carbon atoms (figure **2.10**).

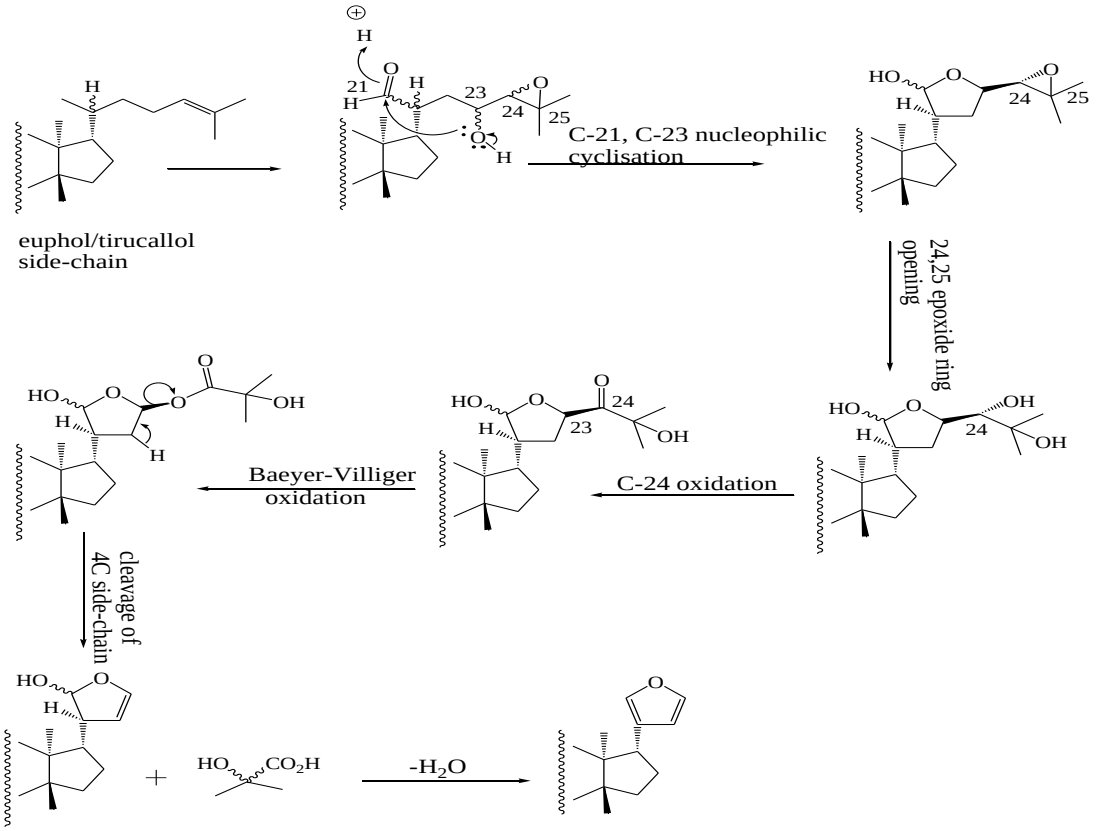



**Figure 2.10: Furan Ring Formation via Side-Chain Oxidation**

## 2.11 Thin Layer Chromatography (TLC)

TLC involves the use of a particulate sorbant spread on an inert sheet of glass, plastic, or metal as a stationary phase (Kaczmarski *et al.*, 2001). The mobile phase is allowed to travel up the plate carrying the sample that was initially spotted on the sorbant just above the solvent. Depending on the nature of the stationary phase, the separation can be either partition or adsorption chromatography. The advantage of TLC is that the samples do not have to undergo the extensive cleanup steps, and the ability to detect a wide range of compounds, using reactive spray reagents (Kaczmarski *et al.*, 2001; Altemimi *et al.*, 2017). Non-destructive detection (fluorescent indicators in the plates, examination under a UV lamp) also makes it possible for purified samples to be scraped off the plate and be analyzed by other techniques (Zhang *et al.*, 2005).

## 2.12 Column Chromatography (CC)

Column chromatography consists of a column of particulate material such as silica or alumina that has a solvent passed through it at atmospheric, medium or low pressure (Zhang *et al.*, 2005; Altemimi *et al.*, 2017). The separation can be liquid/solid (adsorption) or liquid/liquid (partition). The columns are usually glass or plastic with sinter frits to hold the packing (Guiochon, 2001). Most systems rely on gravity to push the solvent through, but medium pressure pumps are commonly used in flash CC. The sample is dissolved in solvent and applied to the front of the column (wet packing), or alternatively adsorbed on a coarse silica gel (dry packing). The solvent elutes the sample through the column, allowing the components to separate. Normally, the solvent is non polar and the surface polar, although there are a wide range of packings including chemically bound phase systems. Bonded phase systems usually utilize partition mechanisms. The solvent is usually changed stepwise, and fractions are collected according to the separation required, with the eluting products usually monitored by TLC (Zhang *et al.*, 2005). The technique is not efficient, with relatively large volumes of solvent being used, and particle size is constrained by the need to have a flow of several mls/min. The advantage is that no expensive equipment is required, and the technique can be scaled up to handle sample sizes approaching gram amounts.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 General Experimental Methods

All solvents used in the study were either of analytical grades or were distilled before use. Silica gel (0.063–0.200 mm, Merck 9385) and size exclusion (Sephadex LH-20) chromatography were used as the adsorbents for column chromatography. Thin layer chromatography (TLC) was performed on aluminium-backed TLC plates (Merck 554, 20 x 20 cm, silica gel 60 $F_{254}$-coated) with compounds visualized by spraying with 1.5% (v/v) *p*-anisaldehyde in 96% methanol and 2.5% (v/v) sulphuric acid before heating in an oven. TLC plates were analyzed under UV light (254 nm and 366 nm) before being sprayed with anisaldehyde spray reagent and then heated in an oven. Spectroscopic and spectrometry techniques including NMR and MS were used to elucidate the chemical structures of the isolated compounds

## 3.2 Collection of S*yzigium guinense*

The bark of a mature S. *guinense* (Figure **3.1**) was collected at Olenguruone in the South Western Mau forest (0°33'5.62" N 35°28'41.49" E), Nakuru County Kenya (Figure **3.2**). Purposive sampling was done guided by the ethnobotanical data on its use. The leaves of the same medicinal plant were also collected to aid in its taxanomic identification. They were transported to Egerton University Chemistry research laboratory. The bark was washed in tap water to remove dirt, chopped into small pieces and air dried inside the laboratory to avoid direct sunlight that could degrade some of the compounds in the samples. They were spread out and regularly turned over to avoid fermenting and rotting. This was done for a period of about one month. The dry samples were ground to fine powder using a heavy duty electric blender (Model; 24CB10C, 230VAC, 50/60 Hz, 1500W, manufacturer; Waring commercial, Torrington Connecticut 06790 assembled in USA). The powder was then weighed, packed and labeled in sample bags and stored at room temperature in the research laboratory. About 2 kg of the ground sample was obtained.

**Figure 3.1: A photo of *S. guinense***

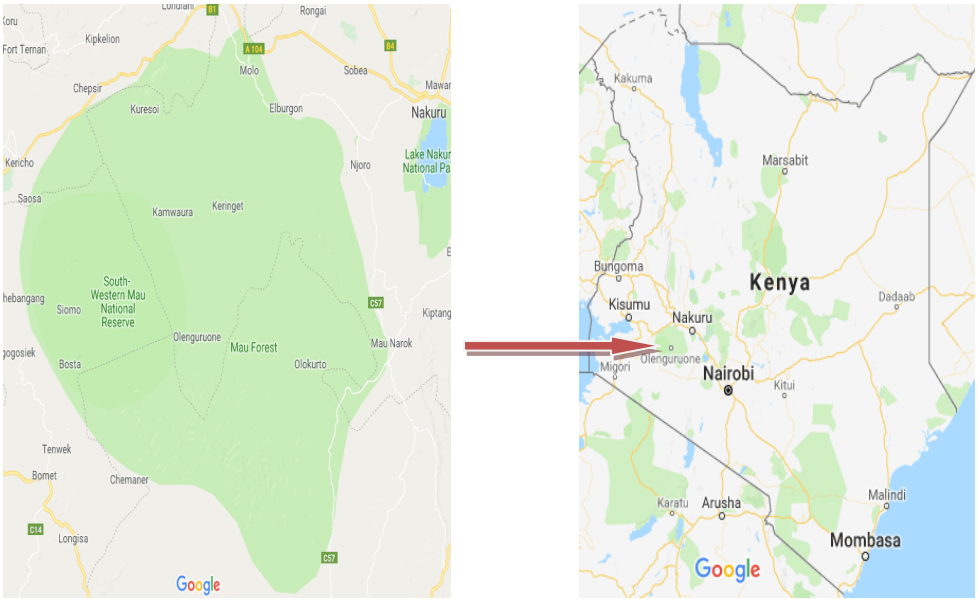



**Figure 3.2: Map of South Western Mau Forest Source (google map)**

### 3.3 Collection of *Dovyalis abyssinica*

The roots of a mature *D. abyssinica* (figure **3.3**) as well as a sample leaves were collected at Olenguruone in the South Western Mau forest (0°33'5.62" N 35°28'41.49" E), Nakuru County Kenya (Figure **3.2**). The roots were carefully cut from a mature medicinal plant to avoid killing it. They were transported to Egerton University chemistry research laboratory. The leaves were identified by a botanist. The root samples were washed in tap water to remove dirt, chopped into small pieces and air dried inside the laboratory to avoid direct sunlight that could degrade some of the compounds in the samples. They were spread out and regularly turned over to avoid fermenting and rotting. This was done for a period of about two months. The dried samples were ground to fine powder using a blender (Model; 24CB10C, 230VAC, 50/60 Hz, 1500W, manufacturer; Waring commercial, Torrington Connecticut 06790 assembled in USA). About 1.5 kg of the sample was stored in sampling bags for further use.

**Figure 3.3: A photo of *D. abyssinica***

### 3.4 Collection of *Solanum mauense*

The fruits of *S. mauense* (figure **3.4**) were collected from Olenguruone in the South Western Mau forest (0°33'5.62" N 35°28'41.49" E), Nakuru County Kenya (Figure **3.2**). Leaves were also collected to aid in taxonomic identification. About 2 kg of fruits were taken to Chemistry Research Laboratory, Egerton University. They were cleaned, cut to small pieces and air dried inside the research laboratory. This was to avoid direct sunlight that could degrade some of the compounds in the samples. They were then spread out and regularly turned over to avoid attack by moulds. This was done for four weeks till they dried. The dried samples were ground to fine powder using a heavy duty electric blender (Model; 24CB10C, 230VAC, 50/60 Hz, 1500W, manufacturer; Waring commercial, Torrington Connecticut 06790 assembled in USA). The powder was then weighed, packed and labeled in

sample bags and stored at room temperature in the research laboratory. About 1.5 kg of the ground sample was stored.

**Figure 3.4: A photo of *S. mauense***

### 3.5 Collection of *Croton dichogamus*

The root of *Croton dichogamus* Pax (figure 3.5) had been collected in Wamunya, within Machakos county of Kenya (1.41 $^{0}$S, 37.62 $^{0}$E) and the sample identification was done by Mr. Patrick Mutiso of the University Herbarium, School of Biological Studies, University of Nairobi, Kenya. A voucher specimen (BN 2010/ 13) was deposited at the Herbarium, School of Biological Studies, University of Nairobi.

**Figure 3.5: A photo of *C. dichogamus***

### 3.6 Extraction of Phytochemicals

Cold extraction method was used for all the powdered plant materials that were prepared in the section 3.2 above. Exact amount of the the dried plant materials (1.5 Kg) were weighed and then soaked in 1.5 L hexane for 72 hours at room temperature with frequent shaking after every 12 hours. The solvent-containing extracts were then decanted and filtered using filter paper (Whatmann No.1) to obtain crude hexane extract. The remaining residue was serially extracted by solvents of increasing polarity and in each step the residue was

dried before introducing the next solvent. The solvents that were used were dichloromethane (1.5 L), ethyl acetate (1.5 L) and lastly methanol (1.5 L) each for 72 hours. In each step, the filtrate was concentrated under a reduced pressure to a minimum volume of 5 ml by use of a Rotavapor (Büchi Labortechnik AG, Switzerland). The concentrated crude extracts were allowed to dry to constant weight at room temperature in pre-weighed screw capped vials before the weight of the extract was determined. The general extraction scheme used in this study is outlined Figure **3.6**

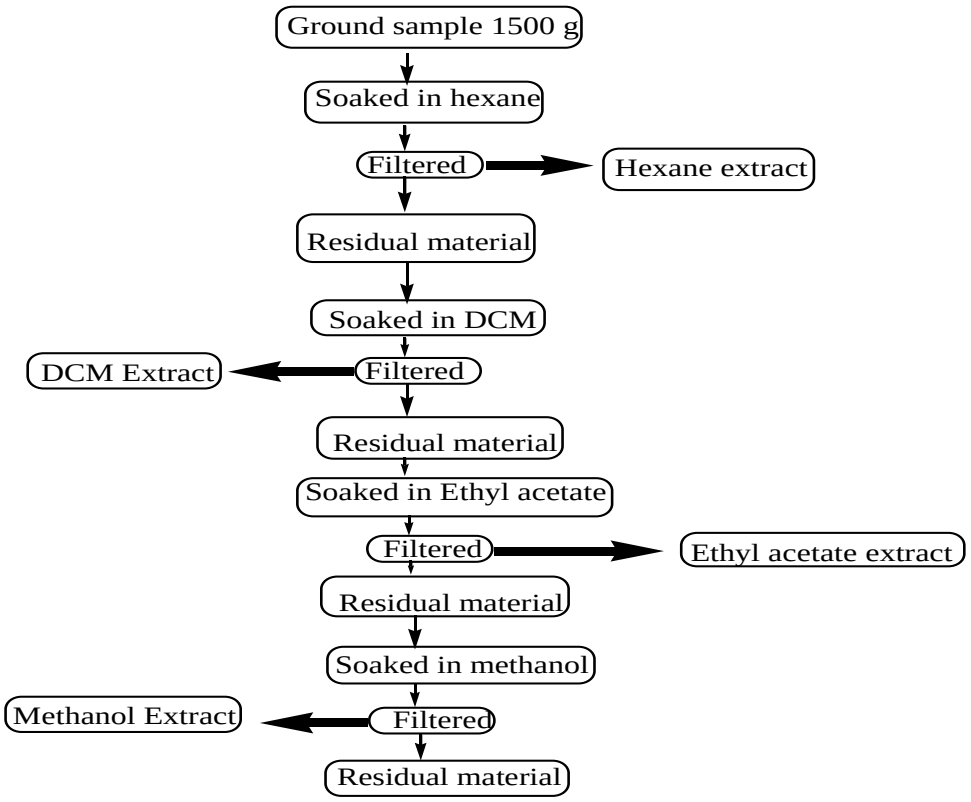



**Figure 3.6: Extraction Scheme of the Bioactive Compounds**

### 3.7 Isolation of Compounds from Crude Extracts

The general purification process of the crude extracts from the sampled plants is outlined in this section. Column chromatography was performed on silica gel (0.063-0.100 mm, Merck). Thin Layer Chromatography (TLC) was performed on silica gel plates (Merck 0.20 mm silica gel 60 with fluorescence indicator, $UV_{254}$). The solvents used for extraction in this research and chromatographic fractionation were either of analytical grades or they were distilled before use.

First, the column was cleaned, air dried and a piece of cotton wool was slowly placed at the bottom of the column by use of a glass rod. The column was clamped vertically to a tripod stand and hexane solvent was filled to the mark. Meanwhile about 8 g of silica gel (70-230 mesh, 0.063-0.2 mm particle size) was weighed into a beaker and mixed with hexane to form slurry, degassed and packed into the column slowly until the column stabilised. Once the column chromatography was packed crude extract was separately adsorbed in silica gel using hexane as the solvent and then dried until it was free flowing. This mixture was poured on top of the packed column. The mobile phase was slowly introduced and the tap was opened to allow separation of the crude extracts which were collected as eluents.

The crude extracts prepared from dichloromethane solvent extraction stage were separated by column chromatography using a step gradient with solvents of varied polarity.The elution process started with 100 % hexane stepped to 10 %, 20 %, 30 %, 50 %, 80 % and 100 % dichloromethane, followed by 20 % and 30 % ethyl acetate in dichloromethane. Several fractions of about 50 mL were collected in each step. For each fraction collected, the solvent was distilled off by exposing in fumehood overnight. The concentrated fraction was subjected to TLC analysis. On each of the TLC plates, (20 cm x 5 cm) were prepared and on it a base line of 0.5 cm was drawn. A sample from each of the fractions was spotted onto a silica gel pre-coated aluminium TLC plate using a capillary tubes. The distance between the spots were maintained at least 1 cm. The TLC developing tank was saturated with 5 mL of dichloromethane and it was covered with a lid and swirled gently. It was allowed to stand and saturate before the remaining solvent was poured out. About 5 mL of established solvent system was then poured into the chamber ready for use. The TLC plates were then placed slowly into chromatographic tank and left to develop in the suitable solvent system. The developed TLC plate were visualised by use of UV lamp pre-set at two fixed wavelengths, $\lambda$=254 nm and 365 nm and they were later sprayed with a newly prepared *p*–anisaldehyde (prepared by adding: 2 mL $H_2SO_4$, 4 mL *p*-anisaldehyde and 200

mL cold methanol) spraying reagents before heating in an oven at about 110 °C. The position of visible compounds on the TLC was determined by calculating the retardation factors ($R_f$), which corresponds tp the distance that the compound has moved relative to the distance the solvent moved. The information obtained enabled pooling of like fractions into major fraction. Further fractionation of the pooled fractions using different solvent system was done till pure compounds were obtained.

### 3.7.1 Isolation of Compounds from *S. guinense* Extract

Purification of stem-bark Dichloromethane (DCM) crude extract of *S. guinense was* accomplished by use of repeated column chromatography and Thin Layer Chromatography (TLC). Column chromatography was done and various fractions were collected which were subjected to TLC analysis. From the TLC analysis, various fractions were pulled together based on their retardation factor (RF) values and coded as shown in figure 3.7. Suitable solvent systems were determined and used in further purification until pure compounds were obtained.The isolated compounds were analysed using NMR (1D and 2D) spectroscopy and mass spectrometry. The spectroscopic data obtained were interpreted and the information obtained from these was used in proposing chemical structures of the isolated compounds.

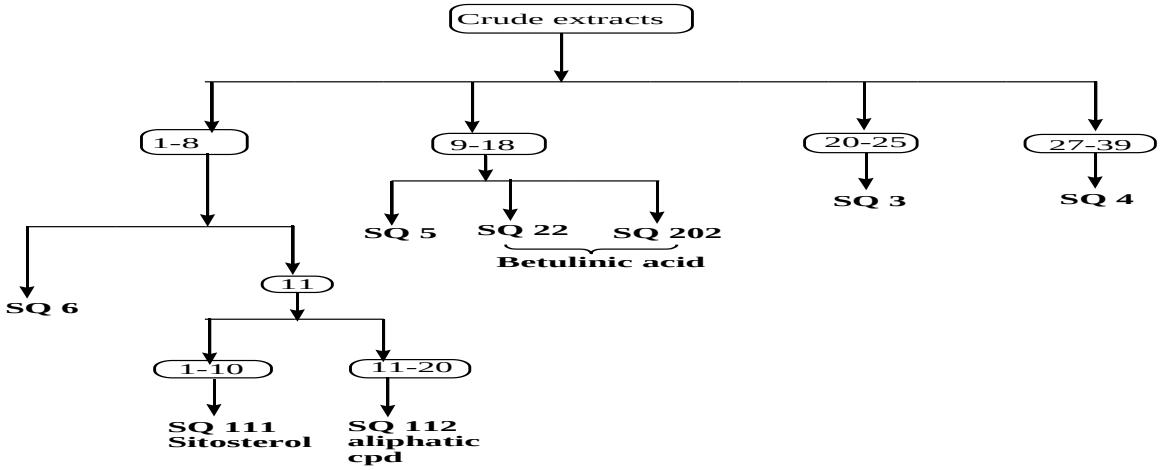



**Figure 3.7: Isolation Scheme of *S. guinense* Stem Bark DCM Extract**

### 3.7.2 Isolation of Compounds from *S. mauense* Extract

Purification of dichloromethane (DCM) crude extract from the roots of *S. mauense was* accomplished by use of repeated Column Chromatography and Thin Layer Chromatography (TLC). The dichloromethane extract from the roots was separated with a hexane: ethyl acetate step gradient starting from 100% hexane to 80% ethyl acetate in

hexane. The fractions obtained were combined and purified further using a different suitable solvent systems. The isolation scheme is shown in figure **3.8**.

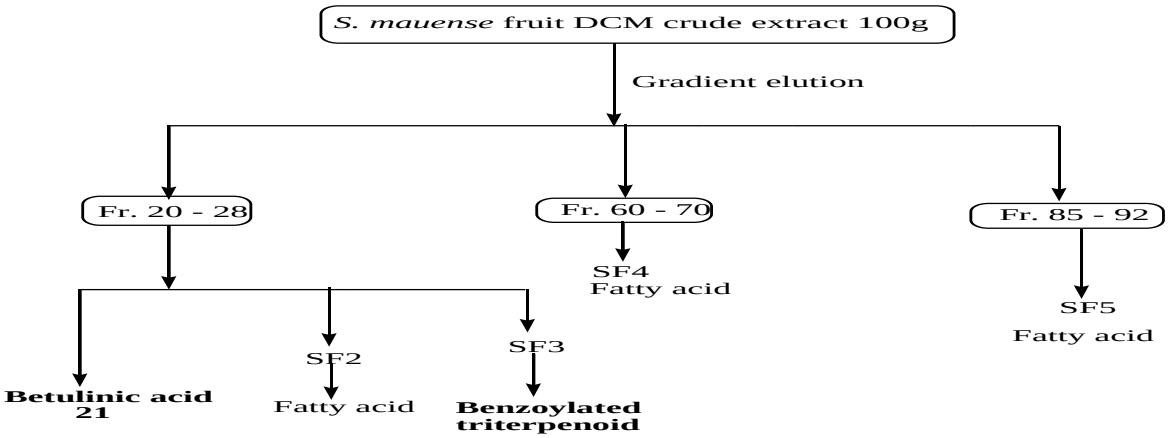



**Figure 3.8: Isolation Scheme of *S. mauense* Fruit Extract**

### 3.7.3 Isolation of Compounds from *D. abyssinica* Extract

About 200 g of crude extract was fractionated by Column chromatography and gradient elution of solvents from hexane, dichloromethane, ethyl acetate and methanol to obtain 70 fractions. The fractions obtained were subjected to TLC analysis and the results obtained led to pooling together the fractions based on their retardation factors.

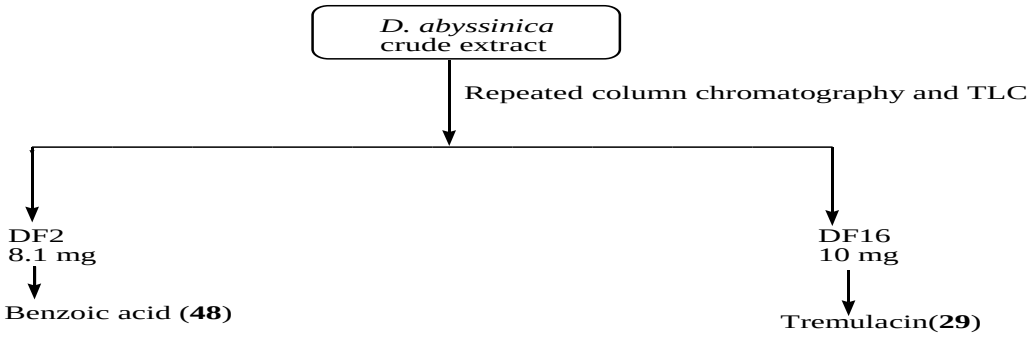



**Figure 3.9: Isolation Scheme for *D. abyssinica* Extracts**

### 3.7.4 Isolation of Compounds from *C. dichogamus* Extract

The dichloromethane crude extract fromthe roots of *C. dichogamus* (200 g) were subjected to silica gel column chromatography using gradient elution with solvents of varied polarity; *n*-hexane and then increasing the concentration of ethyl acetate from 10% to 80% in

*n*-hexane and 60 fractions of 100 ml were collected. The isolation process is summarised in figure 3.10. From the TLC analysis it was evident that some fractions were almost pure.

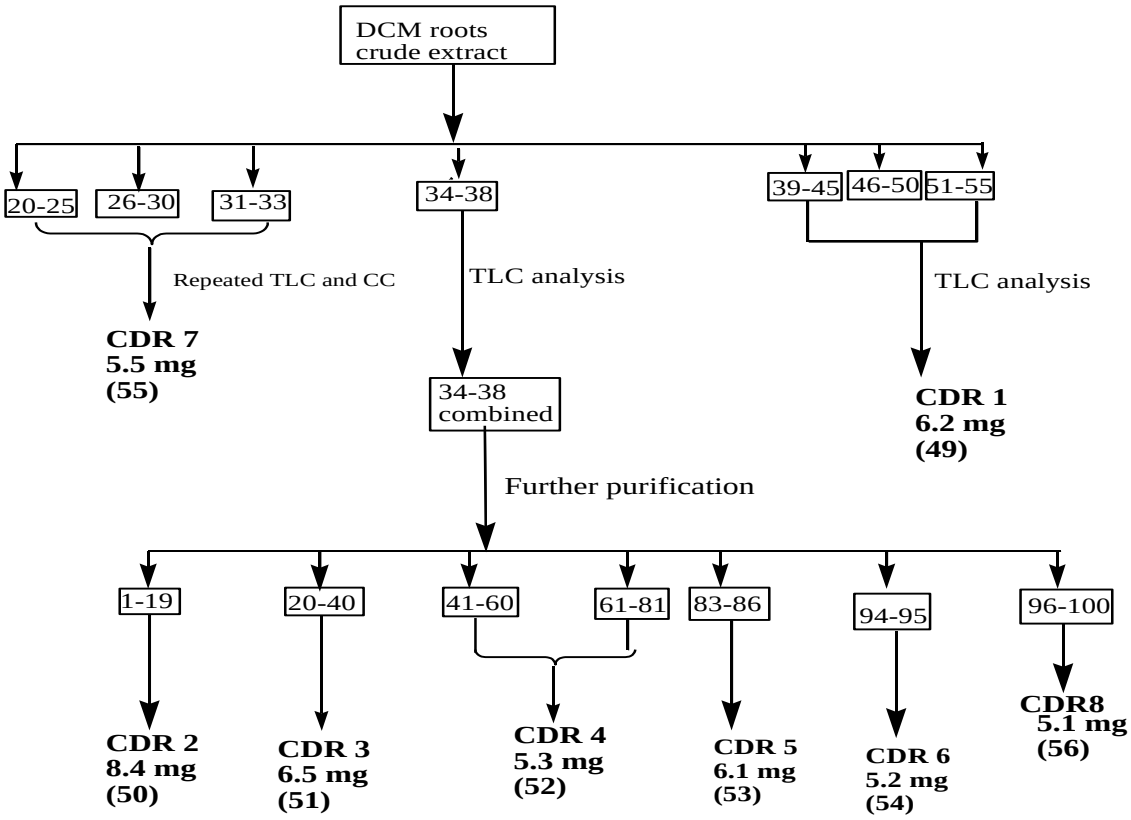



**Figure 3.10: Isolation scheme for *C. dichogamus* root extract**

### 3.8 Nuclear Magnetic Resonance Spectroscopy (NMR)

The pure compounds obtained from section 3.7 above, were sent to the University of Surrey, United Kingdom, for 1D nmr and 2D NMR spectroscopic analysis. The NMR experiments were done using a 500 MHz Bruker AVANCE NMR spectrophotometer. Deuterated chloroform ($CDCl_3$) or deuterated methanol ($CD_3OD$) or deuterated pyridine ($C_5H_5N$). The deuterated chloroform was referenced to central line at δH = 7.26 for the $^1H$ NMR spectrum and at δC = 77.23 for the $^{13}C$ NMR spectrum. In the case of deuterated methanol, it was referenced according to central line at δH = 4.87 and at δH = 3.31 in the $^1H$ NMR spectrum and at δC = 49.15 in the $^{13}C$ NMR spectrum. The deuterated pyridine ($C_5H_5N$) was referenced according to three central lines at δH = 8.74, δH = 7.58 and δH = 7.22 in the $^1H$ NMR spectrum and at δC = 150.3, δC = 135.9 and δC = 123.9 in the $^{13}C$ NMR spectrum.

### 3.9 Mass Spectrometry (MS)

Low resolution electron impact mass spectra were acquired by using a Micromass Quadruple time of flight Mass Spectrometer. In this case samples were dissolved in either acetonitrile (LC-MS grade) or methanol (LC-MS grade) depending on solubility so as to give a 100 ng/ml concentration. From this a 100 µl sample was measured by either a Micromass Q-TOF in ESI+ mode, with a flow rate of 100 µl/min or was done by direct infusion. The results from this experiment were recorded at the Department of Chemistry, University of Surrey, Guild Ford, UK, on a QTOF Premier–Water Corp.

### 3.10 Cytotoxicity Assay

The anti-cancer screening of some selected compounds isolated in this study was performed in two ways. First by use of the neutral red assay which was used for the estimation of cell cytotoxicity against Caco-2 (Human Colorectal Adenocarcinoma) cell lines.The second method entailed submitting selected compounds to the Development Therapeutics Program (DTP) of the National Cancer Institute (NCI). About $5x10^4$ Caco-2 cells/ml were seeded into 96-well plates and incubated at appropriate conditions ($CO_2$, temperature and humidified atmosphere) for 48 hours. After incubation, the growth of cells was checked by microscope. 100 mM of every compound was prepared as a stock solution by dissolving the material in sterile DMSO. From this, five concentrations were prepared for every compound 1, 3, 10, 30, 100 µM, by dilution of the stock solution in medium (Minium Essential Media Eagel M2279). The culture medium from the plates was removed by pipette, and 200 µl of each prepared concentration was added in three replicates to the wells.

In addition, 200 µl of medium without the compound of interest and 200 µl of medium with equivalent amounts of solvent that were used to prepare stock solution (1µl DMSO/ml medium) were added in three replicates to the wells plate as negative control. Actinomycin D was used as a positive control in this experiment at a concentration of 10 µg/ml medium, and was added in three replicates to the well. The plates were incubated at appropriate conditions for 48 hours. Meanwhile, 40 µg/ml of neutral red working solution was prepared and incubated overnight under the same conditions as the plates. Later, the plates were examined under microscope, and the neutral red solution was centrifuged for 10 min. 100 µl of neutral red medium was added to each well of the plates after removing all medium from the wells. Then the plates were incubated at appropriate culture conditions for 2 hours, and then checked under the microscope. The neutral red medium was removed and

the cells were washed with 150 µl PBS (Phosphate Buffer Saline) per well. Then the PBS was removed and 150 µl of neutral red distain solution was added per well. The plates were shaken on a microtiter plate shaker for about 10 minutes. The Optical Density (OD) of the neutral red extract was measured by a microtiter plate reader spectrofluorimeter at 540 nm. The experiment was repeated three different times. The percentage of cell death for each concentration of the tested compounds was calculated with the formula 100 - [($Abs_{540}$ treated sample/ $Abs_{540}$ untreated sample) x 100].

The $IC_{50}$ (the concentration producing 50% inhibition of Neutral Red uptake) was calculated for promising compounds by using the Graphepad software programme. In addition to the above, measuring Apoptosis was also done. Caspase - 3/7 reagent was prepared to a final concentration of 5 µM in the desired medium formulation. The 96 - well plates of culture cells were prepared by the Neutral red assay method, followed by the same steps of preparing and adding the positive and negative control, and 100 µM of tested compound. Before incubating the plates, 1 µl of caspase-3/7 reagent was added to each well of the plates. Then, the plates were incubated within a microplate tray into the Incu Cyte FLR. The NCI 60 cancer screening method used was obtained from the following website for cancer screening; https://dtp.cancer.gov/branches/btb/ivclsp.html

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Compounds Isolated from *S. guinense.*

Two compounds; β-sitosterol (**47**) and Betulinic acid (**21**) were obtained from *S. guinense* extract. Betulinic acid was sent for anticancer screening using the NCI 60 human cancer cell line panel. Structure elucidation of β-sitosterol and Betulinic acid is expalined in section **4.1.1** and section **4.1.2** in that order.

### 4.1.1 Structure Elucidation of β-Sitosterol (47)

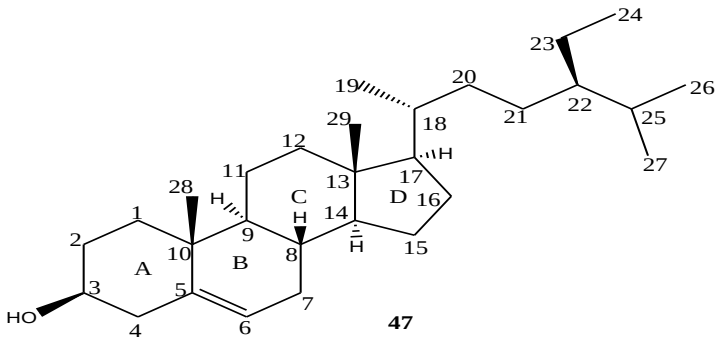
β-sitosterol was isolated as a white powder compound and its complete spectral assignment was made based on $^{1}H$ NMR, $^{13}C$ NMR, COSY, HSQC and HMBC, spectroscopic data. The $^{13}C$ NMR (125 MHz) (Appendix **2**) together with 2D NMR (COSY, HSQC and HMBC) showed twenty - nine carbon signals which were categorized as six methyl, eleven methylene, nine methyne and three quaternary carbons atoms. $^{13}C$ NMR also exhibited a signal at δ = 140.96 and δ = 121.93 which was for C - 5 = C - 6 double bond in that order and δ = 72.02 assigned to C - 3 which bears *β* - hydroxyl group.

From the $^{1}H$ NMR spectrum (500 MHz, $CDCl_3$) (Appendix **1**) of compound **47,** there was evidence of six methyl signals that were categorized as two methyl singlets at δ = 0.68, and δ =1.01; three methyl doublets that appeared at δ = 0.81, δ = 0.83 and δ = 0.93; and a methyl triplet at δ = 0.84. There is evidence of one $SP^2$ carbon proton at δ = 5.36. This was assigned the typical H - 6 of the steroidal skeleton since it appeared as a triplet for one proton. It further showed a peak corresponding to the proton that is connected to the C - 3 hydroxy group and it appeared as a triplet of doublet of doublets at δ = 3.53. This location as well as its multiplicity indicated the H - 3 of the steroid nucleus. The HSQC showed a correlation between C - 6 (δ = 121.93) with a proton at 5.36 ppm, C - 3 (δ = 72.02) with a proton at 3.53 ppm and C - 13 (δ = 42.53) with proton at 2.30 ppm. From these spectral information, β-sitosterol **(47)** was characterized and its identity was ascertained by comparison of its spectral data with those reported values in literature (Chaturvedula and Prakash, 2012). These spectral data is summarized in table **4.1** below.

**Table 4.1: NMR spectral data of β-sitosterol (47)**

| Position | $^{13}$C Isolated Compound | $^{13}$C literature value (Chaturvedula and Prakash, 2012) | $^{1}$H NMR (500 MHz, $CDCl_3$) J=Hz | DEPT |
|---|---|---|---|---|
| 1 | 37.5 | 37.5 | | $CH_2$ |
| 2 | 31.9 | 31.9 | | $CH_2$ |
| 3 | 72.0 | 72.0 | 3.53 tdd (J = 4.5, 4.2, 3.8) | CH |
| 4 | 42.5 | 42.5 | | $CH_2$ |
| 5 | 141.0 | 140.9 | - | C |
| 6 | 121.9 | 121.9 | 5.36 t (J = 6.4 ) | CH |
| 7 | 32.1 | 32.1 | | $CH_2$ |
| 8 | 32.1 | 32.1 | | CH |
| 9 | 50.3 | 50.3 | | CH |
| 10 | 36.7 | 36.7 | - | C |
| 11 | 21.3 | 21.3 | | $CH_2$ |
| 12 | 40.0 | 39.9 | | $CH_2$ |
| 13 | 42.5 | 42.6 | - | C |
| 14 | 57.0 | 56.9 | | CH |
| 15 | 26.3 | 26.3 | | $CH_2$ |
| 16 | 28.5 | 28.5 | | $CH_2$ |
| 17 | 56.3 | 56.3 | | CH |
| 18 | 36.5 | 36.3 | | CH |
| 19 | 19.3 | 19.2 | 0.93 d (J = 6.4 ) | $CH_3$ |
| 20 | 34.2 | 34.2 | | $CH_2$ |
| 21 | 24.5 | 26.3 | | $CH_2$ |
| 22 | 46.1 | 46.1 | | CH |
| 23 | 23.3 | 23.3 | | $CH_2$ |
| 24 | 12.2 | 12.2 | 0.84 (t) (J = 7.2) | $CH_3$ |
| 25 | 29.4 | 29.4 | | CH |
| 26 | 20.0 | 20.1 | 0.83 d (J = 6.4 ) | $CH_3$ |
| 27 | 19.6 | 19.6 | 0.81 d (J = 6.4 ) | $CH_3$ |
| 28 | 19.0 | 19.0 | 0.68 (s) | $CH_3$ |
| 29 | 12.1 | 12.0 | 1.01 (s) | $CH_3$ |

The chemical structure of β-sitosterol (**47**) is shown below;




**47**

*β*-sitosterol (**47**) is a known as a common chemical component of most medicinal plants and is mostly refered to as phytosterols. These class of compounds are composed of a tetracyclic cyclopenta [α] phenanthrene ring and has a lengthy flexible side chain attached to C-17 carbon atom. It has four rings (A, B, C, D, from left to right) which have *trans* ring junctures and it forms a flat α system (Subhadhirasakul and Pechpongs, 2005). Biological activities of *β*–Sitosterol have been reported extensively in literature. For instance *β*–Sitosterol and fatty acids obtained from *Mallotus peltatus* leaf extract were reported to show antibacterial and anti-inflammatory activities (Chattopadhyay *et al.*, 2002). Literature reports of β-Sitosterol reducing carcinogen-induced cancer of the colon among other biological activities has been documented; antiinflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and inhibition of spermatogenesis (Patra *et al.*, 2010).

Reports of isolation of *β*–Sitosterol from Myrtaceae family has been documented in literature. It was isolated from leaf hexane extract of *Syzigium cumini* and reported to exhibit antidiabetic effect (Alam *et al.*, 2012). Its isolation was also reported from methanol extracts of *S. cumini* (Sikder *et al.*, 2012). This literature supports the isolation of this compound from *S. guinense* which belongs to myrtaceae family of medicinal plants.

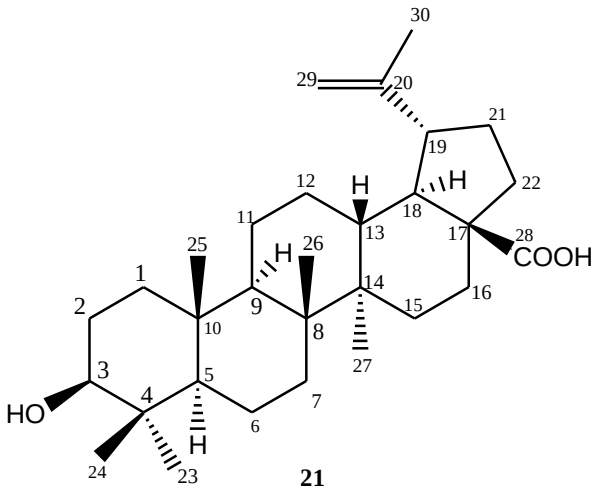
Cytotoxicity assay of β-sitosterol could not be done since it is known compound whose anticancer activity has been done previousely and extensively reported. (Chai *et al.*, 2008) reported that *β* – Sitosterol repressed the increase of MCF-7 cells (Breast cancer cell) which led to caspase-induced apoptosis. Other studies documented antiproliferative and apoptosis actions in human leukemic U937 cells by triggering of caspase-3 and Bax/Bcl-2 ratio (Awad *et al.*, 2000; Ju *et al.*, 2004; Osvena *et al.*, 2004; Park *et al.*, 2007) and Inhibition of the HT-29 cell line (colon carcinoma) (Manayi *et al.*, 2013).

### 4.1.2 Structure Elucidation of Betulinic acid (21)

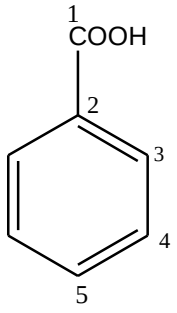
Betulinic acid (**21**) was obtained from *S. guinense* as a white crystal compound. The $^{1}H$ NMR spectrum (appendix **3**) of betulinic acid showed various peaks that were noted as corresponding to the methyl groups at around $\delta = 0.80$ to $\delta = 1.74$ and a pair of $SP^2$ protons at $\delta = 4.61$ to $\delta = 4.73$, which is a feature associated with an exocyclic methylene group. The chemical shift at $\delta = 3.16$ was associated with the carbinolic proton attached to C-3.

The $^{13}C$ NMR spectrum (Appendix **4**) of betulinic acid showed characteristic peaks of a lupeol-type triterpene derivative. The pair of $sp^2$ carbon atoms of lupeol was observed as peaks at $\delta = 152.1$ and $\delta = 110.3$. Oxygenated carbon shifts for C-3 was observed at $\delta = 79.8$. The $^{13}C$ NMR experiments showed six methyl singlet groups at $\delta = 28.1$ (C - 23), $\delta = 16.3$ (C-24), $\delta = 16.7$ (C-25), $\delta = 16.9$ (C-26), $\delta = 15.3$ (C-27), and $\delta = 19.6$ (C-30). The signals due to eleven methylene, five methine and five quaternary carbons were assigned with the aid of the DEPT experiment. The the structure of compound **21** was confirmed by the use of its 2D NMR experimental data; COSY and HMBC. The deshielded signal at $\delta = 79.8$ was associated with C-3 having a hydroxyl group attached to it. Confirmation was evident from the NOESY correlations seen between H - 3α resonance ($\delta = 3.16$) with 3H-23 ($\delta = 0.96$) and H-5 ($\delta = 0.75$) resonances. In the HMBC spectrum, the 3 - H resonance also showed correlations with a methyl carbon signals ($\delta = 28.1$, C -23) and ($\delta = 16.3$, C-24). The two vinyl methy proton $H_2$-29 with resonances $\delta_H = 4.56$ and $\delta_H = 4.69$ showed correlation in the HMBC spectrum with C-19 resonance ($\delta = 49.7$) and C-18 resonance ($\delta = 49.6$).

The NOESY spectrum 2H-29 showed correlations with the H-30 resonance $\delta = 1.96$. The $\delta = 180.2$ (C-28) was confirmed by HMBC correlation with $\delta = 1.38$ (H-16) and $\delta = 1.76$ (H- 22). The spectra showed a compound that has thirty carbon atoms (a number equivalent to the total number of carbon atoms of a triterpenoid). These carbon atoms comprised; six methyl groups, eleven methylenes, six methines, and seven quaternary carbons. The most deshielded carbon (carboxylic acid) was assigned as C-28 and it appeared at around $\delta = 180.2$ ppm which is also in supported by reported literature. These spectral data helped in identifying the compound as betulinic acid (**21**). Further confirmation of the structure was done by comparison of spectral data with literature values (Prakash and Prakash, 2012). The spectral data has been summarized in table **4.2** below.




**21**




(**48)**

**Table 4.2: NMR spectral data for Betulinic acid (21)**

| Position | $^{13}$C Isolated compound (δ) | $^{13}$C Literature value (δ) (Prakash and Prakash, 2012) | $^{1}$H NMR (500 MHz) In $CD_3OD$ | DEPT Isolated compound |
|---|---|---|---|---|
| 1 | 40.2 | 39.1 | 0.99 m | $CH_2$ |
| 2 | 28.2 | 28.3 | 1.90 m | $CH_2$ |
| 3 | 79.8 | 78.3 | 3.16 dd (1H , J= 4.8 Hz) | CH |
| 4 | 39.0 | 39.6 | - | C |
| 5 | 57.0 | 56.2 | 0.75 m | CH |
| 6 | 19.6 | 18.8 | 1.53 m | $CH_2$ |
| 7 | 35.7 | 34.5 | 1.62 m | $CH_2$ |
| 8 | 40.1 | 40.8 | - | C |
| 9 | 50.6 | 51.0 | 1.58 m | CH |
| 10 | 38.5 | 37.3 | - | C |
| 11 | 22.2 | 21.3 | 1.93 m | $CH_2$ |
| 12 | 27.0 | 26.2 | 1.93 m | $CH_2$ |
| 13 | 39.8 | 38.7 | 2.71 | CH |
| 14 | 42.1 | 42.9 | - | C |
| 15 | 30.9 | 31.3 | 1.54 m | $CH_2$ |

| | | | | |
|---|---|---|---|---|
| 16 | 31.9 | 32.6 | 1.96 m | $CH_2$ |
| 17 | 57.6 | 56.4 | - | C |
| 18 | 48.6 | 47.8 | 2.32 m | CH |
| 19 | 50.6 | 49.8 | 3.04 m | CH |
| 20 | 152.1 | 152.1 | - | C |
| 21 | 31.8 | 30.6 | 1.49 m | $CH_2$ |
| 22 | 38.2 | 37.4 | 1.76 m | $CH_2$ |
| 23 | 28.1 | 28.5 | 0.93 s | $CH_3$ |
| 24 | 16.3 | 16.2 | 0.77 s | $CH_3$ |
| 25 | 16.7 | 16.8 | 0.81 s | $CH_3$ |
| 26 | 16.9 | 16.4 | 0.95 s | $CH_3$ |
| 27 | 15.3 | 15.0 | 0.96 s | $CH_3$ |
| 28 | 180.2 | 178.4 | - | C=O |
| 29 | 110.3 | 109.4 | 4.60 (1H, br, s)<br>4.72 (1H, br, s) | $CH_2$ |
| 30 | 19.7 | 19.3 | 1.96 s | $CH_3$ |

Betulinic acid (**21**) is a pentacyclic triterpenoid which is documented to originate from lupane (Chudzik *et al.*, 2015). Lupane is a group of pentacyclic triterpenoids characterized by cytotoxic activities, which may be obatined from plants such as *Spirostachys africana*) or synthetisized (Mathabe *et al.*, 2008). Pentacyclic triterpenes are biosynthesized by cyclisation of squalene epoxide (Figure **2.7**, figure **2.8** and figure **2.9**) and contains a 30-carbon skeleton having a five six-membered rings (ursanes and lanostanes) or four six-membered rings and one five-membered ring (lupanes and hopanes) (Patocka, 2003).They are widely found in the plant kingdom all over the world and are obatined from the outer bark of a assortment of tree species (Alakurtti *et al.*, 2006). Triterpenes not only prevent the life of neoplastic cell lines, but also prompt apoptosis of cancer cells, to cause their "suicidal" death, without affecting normal cells of the body. Such characteristics (selectivity of triterpenes' activity) present them as options in cancer management and control. Betulinic acid (**21**) has been studied

extensively owing to its selective antitumor activity against human melanoma cell culture and anti - HIV activity (Sharma *et al.*, 2010). Betulinic acid has also been reported as catalytic inhibitor of Topo II with IC value of 56.12 μM comparable to 52.38 μM for a classic Topo II inhibitor etoposide (Moghaddam and Javitt, 2012).

Betulinic acid (**21)** was screened against NCI 57 cell lines and only 25 gave positive results. It inhibited leukemia CCRF - CEM and SR human cancer cell line (32.49 and 29.03 % respectively). It also showed moderate inhibition against colon cancer HCT-116 (17.06 %) and non small lung cancer cell line A-549/ATCC (16.88 %). The data summarized in table **4.3** shows an overview of the results of the single dose anti-tumour screen. It shows the number of cell lines studied and mean graph midpoint (MG-MID) giving an average activity parameter for all the cancer cell lines screened. From the screened results betulinic acid (**21)** was moderately reactive. Based on the result of NCI's analysis, it is proposed that this compound might have the potential to be a candidate for anticancer drugs development. The results from this study supports previous studies done on betulinic acid and reported.

**Table 4.3: Growth Percentage of Compound 21 in the NCI *In vitro* 60-Cell Drug Screen Program at $10^{-5}$ M**

| **Panel/Cell Lines** | **COMPOUND 21** |
|---|---|
| **Leukemia** | **% Growth** |
| CCRF-CEM | 67.51 |
| HL-60(TB) | 116.70 |
| K-562 | 95.15 |
| MOLT-4 | 84.20 |
| RPMI-8226 | 92.69 |
| SR | 70.69 |
| A549/ATCC | 100.94 |
| HOP-62 | 114.44 |
| HOP-92 | 91.29 |
| NCI-H226 | 98.72 |
| NCI-H23 | 98.36 |
| NCI-H322M | 110.99 |
| NCI-H460 | 101.32 |
| NCI-H522 | 83.12 |

| | |
|---|---|
| **Colon Cancer** | |
| COLO 205 | 106.12 |
| HCC-2998 | 112.29 |
| HCT-116 | 82.94 |
| HCT-15 | 87.95 |
| HT29 | 103.80 |
| KM12 | 106.51 |
| SW-620 | 108.01 |
| **CNS Cancer** | |
| SF-268 | 108.65 |
| SF-295 | 106.14 |
| SF-539 | 100.19 |
| SNB-19 | 106.38 |
| SNB-75 | 99.44 |
| U251 | 99.85 |
| | |
| **Melanoma** | |
| LOX IMVI | 85.57 |
| MALME-3M | 107.29 |
| M14 | 99.35 |
| MDA-MB-435 | 101.47 |
| SK-MEL-2 | 109.02 |
| SK-MEL-28 | 104.72 |
| SK-MEL-5 | 85.99 |
| UACC-257 | 98.48 |
| UACC-62 | 91.73 |
| **Ovarian Cancer** | |
| IGROV1 | 117.30 |
| OVCAR-3 | 111.75 |
| OVCAR-4 | 107.21 |
| OVCAR-5 | 129.12 |
| OVCAR-8 | 94.38 |
| NCI/ADR-RES | 101.65 |

| | |
|---|---|
| SK-OV-3 | 122.14 |
| **Renal Cancer** | |
| 786-0 | 104.19 |
| A498 | 93.40 |
| ACHN | 102.50 |
| CAKI-1 | 106.93 |
| XF 393 | NT |
| SN12C | 102.55 |
| TK-10 | 129.20 |
| UO-31 | 97.96 |
| **Prostate Cancer** | |
| PC-3 | 78.18 |
| DU-145 | 108.39 |
| **Breast Cancer** | |
| MCF7 | 79.94 |
| MDA-MB-231/ATCC | 91.67 |
| HS 578T | 96.16 |
| T-47D | NT |
| MDA-MB-468 | 100.01 |
| BT-549 | 96.26 |

Isolation of betulinic acid (**21**) from *S. guinense* has been previously reported (Oladosu *et al.*, 2017) who studied its anti-tuberculosis effects and found it to be active with an MIC of 0.6 mg/ml.

### 4.2 Compounds Isolated from *S. mauense* Crude Extracts

Betulinic acid (**21**) was isolated and characterized. The $^{13}C$ NMR spectrum of compound **21** confirmed a lupeol-type triterpene derivative. The characteristic pair of $sp^2$ carbons comprising the double bond of lupeol was observed as shifts at $\delta$ = 150.6 and $\delta$ = 109.8. Oxygenated carbon shifts for C - 3 was observed at $\delta$ = 79.2. In all, the spectra revealed a compound with six methyl groups, thirty carbon atoms (which is equivalent to the total number of carbon atoms in triterpenoid). Interpretation of $^{1}H$ NMR spectrum of SF1 led to the conclusion that it was betulinic acid (**21**) which was also isolated from *S. guinense* and whose chemical structure has been extensively explained in section **4.1.2**.

### 4.3 Compounds Isolated from *D. abyssinica* Crude Extract

Two compounds of interest were obtained from *D. abyssinica* crude extracts; Benzoic acid (**47**) and Tremulacin (**29**). The other compounds weigh less than 2 mg hence could not be sent for NMR spectroscopic analysis. The chemical structures of compounds; Benzoic acid (**48**) and Tremulacin (**29**) were proposed based on their NMR spectral data. The two compounds were found to have been described prevously. Their structure elucidation is explained in section **4.3.1** and **4.3.2** respectively.

#### 4.3.1 Structure Elucidation of Compound Benzoic acid (48)

Compound **48** was isolated as a white crystalline solid compound and its NMR spectral data helped in the structure elucidation. The $^{1}H$ NMR spectrum (appendix **5**) of this compound showed two triplets at $\delta$ = 7.47 and $\delta$ = 7.63 representing two protons and one proton, respectively. A doublet was also observed at $\delta$ = 8.13 representing two protons. A broad downfield signal was also seen at $\delta$ = 11.2 indicating the presence of carboxylic acid proton. The $^{13}C$ NMR spectrum (appendix **6**) of benzoic acid also exhibited signals at $\delta$ = 128.7, $\delta$ = 129.5, $\delta$ = 130.4, $\delta$ = 134 and $\delta$ = 172.1. It was evident from DEPT NMR spectrum of this compound that the three signals at $\delta$ = 128.7, $\delta$ = 130.4 and $\delta$ = 134 are typical aromatic methine carbons. From these spectroscopic data (table **4.4**) the compound was proposed to be benzoic acid (**48**).

**Table 4.4: NMR spectral data for Benzoic acid (48)**

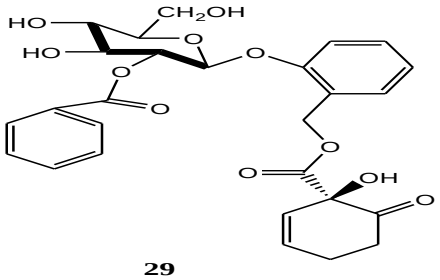
| Position | $^{13}C$ | $^{1}H$ | DEPT |
|---|---|---|---|
| 1 | 172.1 | - | - |
| 2 | 129.5 | - | - |
| 3/7 | 130.4 | 8.13 | -CH- |
| 4/6 | 128.7 | 7.47 | -CH- |
| 5 | 134 | 7.63 | -CH- |

Benzoic acids (**48**) are aromatic carboxylic acids having seven carbon atoms. They serve as precursors for a wide assortment of natural products bioactive compounds and plays vital roles in plant fitness. Plant benzoic acids (BAs) are the building blocks in biosynthesis and acts as crusial elements for many primary metabolites such as plant hormones, cofactors, protection compounds, pollination attractants and seed dispersers (Widhalm and Dudareva,

2015). Numerous natural productsoriginate from plant BA's or containing benzoyl or benzyl moieties are also of medicinal or nutritional value to humans.

### 4.3.2 Structure Elucidation of Trumelacin (29)

The structure of tremulacin (**29**) was proposed based on its NMR spectral data and comparison with the documented literature values. The $^{1}H$ NMR spectrum (appendix **7**) of compound **29** showed a pattern that is characteristic of a phenolic ester glycoside and this was obvious from group of peaks at $\delta$ = 3.80 - $\delta$ = 5.34. The existence of aromatic protons was apparent from peaks at around $\delta$ = 7.03 – $\delta$ = 8.13 which potrayed the existence of substituted Benzene ring. The $^{13}C$ NMR spectrum (appendix **8**) of this compound showed the presence of O - linked carbon atoms of the sugar portion and carbinol carbon atom of the phenolic aglycone. The anomeric carbon and the carbonyl carbon of an ester showed a resonance at $\delta$ = 100.8 and the two carbonyl carbons of an ester at $\delta$ = 167.2 and $\delta$ = 171.2, in that order. The aromatic carbon atoms were evident in the region $\delta$ = 116.7 – $\delta$ = 156.5. Its NMR spectral data is summarized in the table **4.5** below. It is commonly known as Tremulacin.




**29**

Tremulacin is characterised as phenolic glycoside and literature reports that curative properties of plants are associated with a number of compounds, including phenolic secondary metabolites which are the most abundant (Đurđević *et al.*, 2013). Phenolic glycoside are have a sugar portion or moiety connected by a special bond to one or non-sugar portions (Kabera *et al.*, 2014) . Many plants store bioactive compounds in the form of glycosides, which can be triggered by enzyme hydrolysis and thus most glycosides may be categorised as prodrugs since their activity is triggered by hydrolysis in the large intestine necessitating the release of the aglycone, which is the right active component (Kabera *et al.*, 2014). Tremulacin can be thought of as benzoic acid derived and such compounds have been documented to have numerous biological properties. They are known to show anti-sickling characterics *in vitro* (Pierre *et al., 2015).* Other properties associated with it are anticancer, expectorant, sedative and digestive (Zhou and Zhang, 2013).

Phenolic bioactive metabolites acts as antioxidants and helps in adaptation of plantsto their biological environment (Đurđević *et al.*, 2013). Isolation of phenolic glycosides from Salicaceae family of medicinal plants have been reported in literature and its role as anti-herbivore defense (Boeckler *et al.*, 2011). Many phenolic bioactive compounds have been discovered a wide number of plant species, and noted as widely distributed group of secondary plant natural products. They are biosynthesised from the shikimate and acetate-malonate pathways. Phenolic compounds are known as radical scavengers to quench oxygen-derived radicals by giving out its hydrogen atom (Nguyen *et al.*, 2016) and they have been shown to be quashing free radicals. There are other biological activities of *D. abyssinica* which have been reported in literature. For instance the leaf aqueous extract was tested for its cytotoxicity and recorded a $CC_{50}$ (the cytotoxic concentration causing 50% cell lysis and death) value which was greater than 90 ug/ml and it was concluded that the extract was non-toxic. Another study established the antitrypanosomal activity of the extract of *D. abyssinica* which were tested for its invivo acticity and found to be active (Tadesse *et al.*, 2015).

**Table 4.5: NMR spectral data for tremulacin (29)**

| Position | $^{13}$C Isolated compound (δ) | $^{13}$C Literature value (δ) (Sharma *et al.*, 2010) | $^{1}$H NMR 500 MHz, $CD_3$0D |
|---|---|---|---|
| 1 | 156.5 | 156.0 | - |
| 2 | 129.2 | 127.5 | - |
| 3 | 131.0 | 129.5 | 7.14 |
| 4 | 123.8 | 123.1 | 6.97 |
| 5 | 131.2 | 130.1 | 7.23 |
| 6 | 116.7 | 115.6 | 7.02 |
| 7 | 64.02 | 63.6 | 4.95 |
| 1’ | 78.4 | 78.3 | - |
| 2’ | 129.6 | 127.5 | 5.68 |
| 3’ | 133.3 | 132.1 | 6.01 |
| 4’ | 27.2 | 26.6 | 2.41 |
| 5’ | 36.8 | 35.3 | 2.52 |
| 6’ | 207.4 | 207.5 | - |
| 7’ | 171.2 | 170.0 | - |
| 1’’ | 100.8 | 99.5 | 5.15 |
| 2’’ | 75.9 | 74.0 | 5.30 |
| 3’’ | 75.5 | 74.8 | 3.86 |
| 4’’ | 71.6 | 69.9 | 3.86 |
| 5’’ | 76.0 | 76.1 | 3.01 |
| 6’’ | 62.5 | 61.3 | 3.86 |
| 1’’’ | 167.2 | 166.0 | - |
| 2’’’ | 130.2 | 129.5 | - |
| 3’’’ | 130.9 | 129.9 | 7.99 |
| 4’’’ | 129.7 | 128.4 | 7.34 |
| 5’’’ | 134.4 | 133.3 | 7.49 |

### 4.4 Purification of Extract from *C. dichogamus*

Eight compounds : acetyl aleuritolic acid (**49**), 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (**50**), 3β, 4β:15,16-diepoxy-13(16), 14-*ent*-clerodadien-17,12*S*-olide (**51**), 15,16-epoxy-5,13(16), 14-*ent*-halimatriene-3-ol (**52**), crotodichogamoin A (**53**), crotohaumanoxide (**54**), crotodichogamoin B (**55**) and cadin-1(6),2,4,7,9-penta-ene (**56**) were isolated from the root extracts of *C. dichogamus*. Their structure elucidation is explained in sections **4.4.1** to **4.4.8**

#### 4.4.1 Structure Elucidation of Acetyl Aleuritolic acid (49)

Compound acetyl aleuritolic acid (3β-*O*-acetyl-D-friedoolean-14-en-28-oic-acid) (**49**) was isolated as a white crystalline bioactive compound from the dichloromethane extract of the roots of *C. dichogamus*. The LREIMS spectrum (appendix **9**) for Acetyl aleuritolic acid gave a molecular ion peak at *m/z* 497.3829 which indicated a molecular formula of $C_{32}H_{50}O_4$ and this helped in determination of a double bond equivalence of eight.

The $^{13}C$ NMR spectrum (appendix **11**), showed thirty-two carbon atom resonances, which included nine quartenary carbons; δ = 37.9 (C - 4), δ = 39.0 (C - 8), δ = 37.9 (C - 10), δ = 37.7 (C - 13), δ = 160.6 (C - 14), δ = 51.4 (C - 17), δ = 29.3 (C - 20), δ = 183.2 (C - 28) and δ = 171.0 (AC - C). There was evidence of five tertiary carbon atoms, ten secondary carbon atoms and eight primary carbon atoms resonances. The HSQC - DEPT (appendix **14**) and $^{13}C$ NMR spectra also revealed two carbonyl carbon atom resonances at δ = 171.0 and δ = 183.2, two $SP^2$ carbon atoms resonances at δ = 116.8 (C - 15) and δ = 160.6 (C - 14). The signals at δ = 183.2 and δ = 171.0 potrayed the presence of carboxylic acid functional group and acetoxy carbonyl functional group. The position of acetoxy group was ascertained from HSQC-DEPT spectrum (appendix **14**), which showed that protons were found to be related with C - 3 and it could be ascertained from HMBC spectrum (appendix **15**) of quaternary carbon atom at acetoxy group. Position of carboxylic acid group was confirmed from HMBC spectrum, which revealed that carbon on carbonyl groups and C - 17 were found to correlate to protons at C-16 and C-22. The double bond (C = C) showed deshielding condition at δ = 160.6 and δ = 116.8, which is a typical C14-C15 double bond of taraxerane skeleton. Position of this double bond was determined from the HMBC spectrum (appendix **15**), which confirmed that H-15 correlate to C-15 and was seen by carbon C-14.

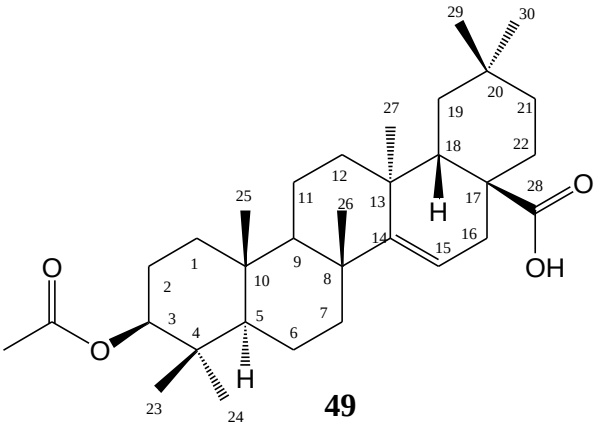
The $^{1}H$ NMR (appendix **10**) spectrum showed seven singlet primary carbon groups of proton resonances at δ = 0.85(3H-23), δ = 0.88 (3H-24), δ = 0.92 (3H-30), δ = 0.93 (3H-27),

δ = 0.94 (3H-29), δ = 0.95 (3H-25), δ = 0.96 (3H-26) and one acetyl methyl group proton resonances at δ = 2.05. These data buttressed a triterpenoid structure. $^{1}$H NMR spectrum showed a characteristic singlet at δ = 2.05 assigned to acetoxy protons, a doublet doublet proton at 5.54 (*J* = 7.77, 3.19 Hz) indicating a proton on the trisubstituted double bond adjacent to methylene function. Another doublet of doublet proton resonated at δ = 4.46 (*J* = 9.18 and *J* = 6.54 Hz) assigned to the ubiquitous 3 α methane proton germinate to the acetoxy function of triterpene skeleton. Seven tertiary methyls appeared as singlets in the region δ = 0.85 to δ = 0.96.

The oxygenated carbon resonance at δ = 80.9 was assigned as C-3 due to its correlations with two methyl singlet proton resonances at δ = 0.85 (3H - 23) and δ = 0.88 (3H - 24) in the HMBC spectrum and coupling with the 2H - 2 (δ = 1.62) resonance in the COSY spectrum (appendix 13). The fully substituted carbon resonance δ = 160.6 was assigned to C-14 due to the correlations with the two methyl singlet proton resonances at δ = 0.96 (3H - 26) and δ = 0.93 (3H - 27) in the HMBC spectrum.

The presence of thirty-two resonances showed a pentacyclic triterpene skeleton. Acetyl aleuritolic acid showed the same $^{13}$C NMR chemical shifts to those of the known acetyl aleuritolic acid which has been extensively reported previously. From these spectral data Acetyl aleuritolic acid was found to be the common triterpenoid, acetyl aleuritolic acid. Its spectroscopic data is summarized in table **4.6** below.

Acetyl aleuritolic acid has been obtained previously from other medicinal plants in the family Euphorbiaceae; *C. cajucara* , *C. cascarilloides* (Cuong *et al., 2002)* and *Spirostachys africana* (Mathabe *et al.,* 2008*)*. It has been isolated from the ethanolic extract of stem and roots of *C. zehntineri* among other bioactive compounds Santos *et al.,* (2017). Acetyl aleuritolic acid has been obtained from numerous Euphorbiaceae plants roots and barks such as *Aparisthmium cordatum* (Juss.) Baill (Sousa *et al.,* 2015) which is an American variety often mistaken with *A. cordifolia*.The anti-inflammatory property of this bioactive compound is broadly documented in other studies (Santos *et al.,* 2009). Its isolation was reported from the root bark of *A. cordifolia* and it was documented as possessing acceptable antiinflamatory activity (Mavar-Manga *et al.,* 2006). It was further reported to be effective at 50 mg/kg on rat paw oedema induced by carrageenin, dextrin and histamine, after intraperitoneal injection.




**49**

Cytotoxicity of acetyl aleuritolic acid was evaluated against the NCI59 developmental therapeutics program 59 cancer cell line screens at one dose of 1 x $10^{-5}$M and gave a mean growth percentage of 97.86 (appendix 24). However this did not meet the standards for further testing.

Cytotoxic properties of acetyl aleuritolic acid have been reported comprehensively in literature. Acetyl aleuritolic acid isolated from *Aleurites moluccana* is noted to prevent lymphocytic leukemia tumor, inhibition of DNA topoisomerase II, lung carcinoma and cytotoxic activity (Prabowo *et al.*, 2013; Alimboyoguen *et al.*, 2014). It was also obtained from *Spirostachys Africana* and documented as being active against *Vibrio cholera, Escherichia coli, Shigella dysentery, Staphylococcus aureus, Salmonella typhimurium* (Mathabe *et al.*, 2008; Alimboyoguen *et al.*, 2014). It was isolated from *Discoglypremna caloneura* and reported to possess antifilarial against *Onchocerca gutturosa* (Nyasse *et al.*, 2006). Cytotoxicity activity against human cancer central nervous system (U251, Glia) cell line, with IC50 8.4 $\mu$M was documented for Acetyl aleuritolic acid obtained from *Garcia parviflora* (Reyes *et al.*, 2010) and HIV-I reverse transcriptase activity was noted for it from *Homonoia riparia* by (Viswanadh *et al.*, 2006).

Table 4.6: NMR data for Acetyl aleuritolic acid (49)

| Position | $^{13}$C NMR (δ) | DEPT | $^{1}$H NMR |
|---|---|---|---|
| 1 | 37.4 | $CH_2$ | 1.61 |
| 2 | 23.5 | $CH_2$ | 1.62 |
| 3 | 80.9 | CH | 4.46 |
| 4 | 37.9 | C | - |
| 5 | 55.6 | CH | 0.86 |
| 6 | 18.7 | $CH_2$ | 1.64 |
| 7 | 40.8 | $CH_2$ | 1.96 |
| 8 | 39.0 | C | - |
| 9 | 49.1 | CH | 1.42 |
| 10 | 37.9 | C | - |
| 11 | 17.3 | $CH_2$ | 1.59 |
| 12 | 33.3 | $CH_2$ | 1.77 |
| 13 | 37.7 | C | - |
| 14 | 160.6 | C | - |
| 15 | 116.8 | CH | 5.54 |
| 16 | 29.7 | $CH_2$ | 1.96 |
| 17 | 51.4 | C | - |
| 18 | 41.5 | CH | 2.28 |
| 19 | 35.3 | $CH_2$ | 1.26 |
| 20 | 29.3 | C | - |
| 21 | 33.7 | $CH_2$ | 1.18 |
| 22 | 31.0 | $CH_2$ | 1.43 |
| 23 | 28.0 | $CH_3$ | 0.85 |
| 24 | 16.6 | $CH_3$ | 0.88 |
| 25 | 15.6 | $CH_3$ | 0.95 |
| 26 | 26.2 | $CH_3$ | 0.96 |
| 27 | 22.4 | $CH_3$ | 0.93 |
| 28 | 183.2 | C | - |
| 29 | 31.9 | $CH_3$ | 0.94 |
| 30 | 28.7 | $CH_3$ | 0.92 |
| AC-M | 21.3 | $CH_3$ | 2.05 |
| AC | 171.0 | C | - |

**AC-M** : Acetyl methyl

**AC-**Acetyl

### 4.4.2 Structure Elucidation of 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)

Compound **50** was isolated as a yellow powdered compound. The $^{1}H$ NMR spectrum (Appendix **17**) exhibited four primary carbon atom proton resonances at δ = 0.82 (3H-17), δ = 1.21 (s) (3H-18), δ = 0.68 (s) (3H-19) and δ =1.09 (s) (3H-20), along with the reocgnisable resonances for a β-substituted furan ring at δ = 6.26, δ = 7.36 and δ = 7.21. The furan ring proton resonances were noted to be coupled in the COSY spectrum (appendix **19**) and were assigned as H-14, H-15 and H –16 in that order. The fully substituted carbon atom resonance at δ = 125.6 was assigned as C-13 due to the perceived correlations in the HMBC spectrum (appendix **22**) between this carbon resonance and proton resonances of H-14 (δ = 6.26) and H-15 (δ = 7.36) and the two proton resonances (δ = 2.15 and δ = 2.34) for H-12.

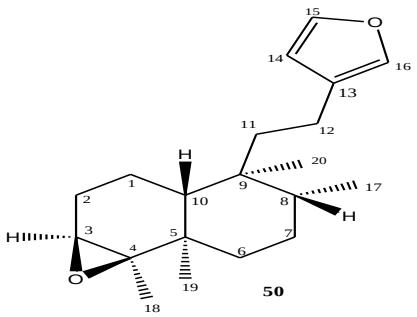
The $^{13}C$ NMR spectrum (appendix **18**) showed the presence of twenty carbon atom resonances for compound **50**, which included four primary carbon atom carbon resonances at δ = 16.0 (C-17), at δ = 19.7 (C-18), at δ = 18.6 (C-19) and at δ = 16.8 (C-20), six secondary carbon atoms, six tertiary carbon atoms including four olefinic carbon resonances at δ = 125.6 (C-13), δ = 111.0 (C-14), δ =142.8 (C-15) and δ = 138.4 (C-16) and four fully substituted carbon resonances. The $^{13}C$ NMR spectrum showed the presence of an oxirane ring carbon resonances at δ = 62.2 and δ = 66.5 for C-3 and C-4, respectively. The proton resonance of H-3 at δ = 2.95 showed a correlation in the HMBC spectrum (appendix **22**) with carbon resonances at δ = 15.4 (C-1), δ = 28.3 (C-2) and δ = 19.7 (C-18). Furthermore, correlations between the H-3 proton resonance at δ = 2.95 and 3H - 18 methyl group proton resonance at δ = 1.21 in the NOESY (appendix **23**) spectrum, and between 3H - 18 and 3H-19 methyl group proton resonance at δ = 0.68 in the spectrum were observed. These observations are in agreement with the presence of an oxirane ring between C-3 and C-4 of compound.

In order to confirm the relative configuration for compound **50**, the NOESY was examined. The NOESY spectrum displayed correlations between the proton resonance of the 3H-20 group at δ= 1.09 (s) and the proton resonances of 3H-17 at δ = 0.82 (d), 3H-19 at δ = 0.68, (s) and H-11 at δ = 1.53. This confirmed that 3H-17, 3H-19, 3H-20 and one of the H-11 were on the same face of compound **50** and were assigned as α. Moreover, the 3H-18 proton resonances at δ = 0.68 showed correlations in the NOESY spectrum with the H-3 resonance at δ = 2.95 and one of the H-6 proton resonance at δ = 1.67. The relative configuration of compound **50** was assigned based on the above NOESY experiment, in which the correlations of H-3/3H - 18, H-3/3H-19, 3H-18/3H-19, 3H-20/3 – H-17, and H-19 confirmed the assigned

α-orientations. The ROESY correlation between H-10 and H-8 confirmed their β-orientation. These NMR data is summarized in table **4.7**

The compound **50** belonged to the *ent*-clerodane series. The cytotoxicity activity of this compound has been documented in the literature. A similar clerodane that was isolated from *Croton oblongifolius* was tested in *vitro* for cytotoxic activity, against the five human cancer cell lines, human liver hepatoblastoma (HEP-G2), human colon adenocarcinoma (SW-620), lung carcinoma (CHAGO), human gastric carcinoma (KATO-3) and human breast carcinoma (BT 474), it exhibited cytotoxicity on the cancer cell lines tested with IC50 values of 7.59, 5.20, 7.83, 6.71 and 4.77 µg/ml in that order (Pudhom and Sommit, 2011).

Clerodane diterpenes are a vast collection of naturally occurring bioactive compounds found in many medicinal plant from numerous families and in organisms such as fungi, bacteria, and marine sponges (Li *et al.*, 2016). They are noted as bicyclic class of diterpenoids. Clerodane diterpenes are extensively spread within the genus Croton, and noted to have antifeedant, cytotoxic and antiprotozoal properties (Guetchueng *et al.*, 2018). The chemotaxonomic implications as well as the distribution of clerodane diterpenes have been reported (Ndunda *et al.*, 2016). Diterpenoids are known to give inconstant skeletons having high oxidative functional moieties or acylated branching groups (Jian *et al.*, 2018). These chemical and structural characteristics of diterpenoids have aroused the interest in the search of bioactive molecules.




**50**

**Table 4.7: NMR Data for 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)**

| Carbon position | $^{13}$C NMR (δ ) | DEPT | $^{1}$H NMR |
|---|---|---|---|
| 1 | 15.4 | $CH_2$ | 1.3 (m) |
| 2 | 28.3 | $CH_2$ | 1.72 (m) |
| 3 | 62.2 | CH | 2.95(s) |
| 4 | 66.5 | C | - |
| 5 | 37.2 | C | - |
| 6 | 37.3 | $CH_2$ | 1.67(m) |
| 7 | 29.7 | $CH_2$ | 1.49(m) |
| 8 | 36.3 | CH | 1.55(m) |
| 9 | 39.1 | C | - |
| 10 | 47.9 | CH | 1.07(m) |
| 11 | 38.6 | $CH_2$ | 1.61(m) |
| 12 | 18.3 | $CH_2$ | 2.34(m) |
| 13 | 125.6 | C | - |
| 14 | 111.0 | CH | 6.26(s) |
| 15 | 142.8 | CH | 7.36(s) |
| 16 | 138.4 | CH | 7.21(s) |
| 17 | 16.0 | $CH_3$ | 0.82 (d) J=6.2 |
| 18 | 19.7 | $CH_3$ | 1.21(s) |
| 19 | 18.6 | $CH_3$ | 0.68(s) |
| 20 | 16.8 | $CH_3$ | 1.09 (s) |

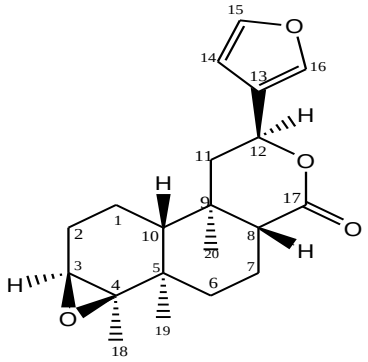
### 4.4.3 Structure Elucidation of (3β, 4β:15,16-diepoxy-13(16),14-*ent*-clerodadien-17,12*S*-olide) (51)

Compound **51** was obatined as a colourless crystalline solid and was found to be a clerodane-type diterpenoid. The $^{1}$H NMR spectrum (appendix **25**) showed the typical proton resonances of three methyl group proton resonances at δ = 1.18 (s) for 3H- 18, δ = 1.11 (s) for 3H - 19 and δ = 1.01 (s) for 3H - 20, along with the characteristic proton resonances for a β-substituted furan ring at δ = 6.40 (H - 14), δ = 7.40 (H - 15) and δ = 7.43 (H - 16). These three proton resonances at δ = 6.40, δ = 7.40 and δ = 7.43 were seen to be coupled in the COSY spectrum (appendix **30**) and were assigned as H - 14, H - 15 and H – 16, in that order.

The $^{13}C$ NMR spectrum (appendix **26**) exhibited the presence of twenty carbons, including three methyl carbon atom resonances that were evident at δ =19.6 (C-18), δ = 17.3 (C-19) and δ = 14.9 (C-20), four $SP^2$ carbon resonances at δ = 126.2 (C-13), δ = 108.7 (C-14), δ = 144.0 (C-15) and δ = 139.5 (C -16) and one ester carbonyl carbon atom resonance at δ = 172.5 (C-17). The fully substituted carbon resonance at δ = 126.2 was assigned as C-13 due to the observed correlations in the HMBC spectrum (appendix **29**) between this resonance and the assigned H-14, H-15 and H-16 proton resonances. Additionally, the C-13 carbon resonance showed further correlations in the HMBC spectrum with the proton resonances at δ = 5.50 that was assigned as H-12. Correlations between the ester carbonyl C-17 resonance (C = 172.5) and C-11 (C = 44.1) with H-12 were also noted in the HMBC spectrum. The NOESY spectrum (appendix **31**) of compound **51** displayed that one of the H-11, H-12, and 3H-20 resonances were on the same face of the molecule and were assigned as α whereas one of the H-2, H-8, H-10 and the other H-11 were relatively assigned as β.

From the $^{13}C$ NMR spectrum, it was evident than an oxirane ring was present between C-3 and C-4 and was supported by the chemical shifts of C-3 at δ = 61.9 and C-4 at δ = 66.2 which indicated the presence of an epoxide. Additionally, the presence of an oxirane ring was confirmed by correlations in the HMBC (appendix **29**) spectrum between the H-3 proton resonance at δ = 2.93 and carbon atoms resonances of C-1 (δ = 15.6), C-2 (δ = 28.3), C - 4 (δ = 66.2) and C-18 (δ = 19.6). Correlations in NOESY spectrum (appendix **31**) were evident between the H-3 and 3H-18 proton resonances. These information is summarized in table **4.8** below.

Reports on *in-vitro* cytotoxic activity of this compound against the five human cancer cell lines, human liver hepatoblastoma (HEP-G2), human colon adenocarcinoma (SW-620), lung carcinoma (CHAGO), human gastric carcinoma (KATO-3) and human breast carcinoma (BT-474) has been documented in literature. It was reported that this compound did not show any detectable cytotoxicity against the five cell lines tested (Pudhom *et al.*, 2011).



**51**

**Table 4.8: NMR spectral data for compound 51**

| Carbon position | $^{13}$C NMR (δ ) | DEPT | $^{1}$H NMR |
|---|---|---|---|
| 1 | 15.6 | $CH_2$ | 1.29 (M) |
| 2 | 28.3 | $CH_2$ | 2.19 (M) |
| 3 | 61.9 | CH | 2.93 (b) |
| 4 | 66.2 | C | - |
| 5 | 36.8 | C | - |
| 6 | 36.2 | $CH_2$ | 1.84 |
| 7 | 19.3 | $CH_2$ | 1.73 |
| 8 | 51.3 | CH | 2.12 |
| 9 | 37.6 | C | - |
| 10 | 54.2 | CH | 0.92 |
| 11 | 44.1 | $CH_2$ | 2.30 |
| 12 | 72.0 | CH | 5.5 |
| 13 | 126.2 | C | - |
| 14 | 108.7 | CH | 6.40 (s) |
| 15 | 144.0 | CH | 7.40 (s) |
| 16 | 139.5 | CH | 7.43 (s) |
| 17 | 172.5 | C | - |
| 18 | 19.6 | $CH_3$ | 1.18 (s) |
| 19 | 17.3 | $CH_3$ | 1.11 (s) |
| 20 | 14.9 | $CH_3$ | 1.01 (s) |

**4.4.4 Structure Elucidation of 15,16-epoxy-5, 13(16),14-*ent*-halimatriene- 3-ol (52)**

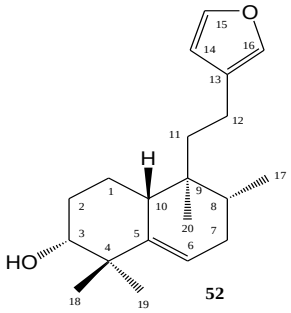
Compound **52** was isolated as yellow oil from the dichloromethane extract of the roots of *C. dichogamus*. The MS spectrum (appendix **32**) of Compound **52** gave a $[M + Na]^+$ ion at *m/z* 325.2138 which indicated a molecular formula of $C_{20}H_{30}O_2$ for the compound. The $^1H$ NMR spectrum (appendix **33**) showed a characteristic resonances for a $\beta$ – substituted furan ring present in compound **52.** This was exhibited by three coupled proton resonances at δ = 6.28 (H-14), δ = 7.35 (H-15) and at δ = 7.22 (H-16) in the COSY spectrum (appendix **38**). Three singlet proton resonances at δ = 1.07 (3H -18), δ = 1.15 (3H-19) and δ = 0.71 (3H-20) and one doublet proton resonance at δ = 0.88 (d, *J* = 6.7, 3H-17) in the $^1H$ NMR spectrum confirmed the presence of four methyl groups. In addition, one olefinic methine proton resonance at δ = 5.56 (H-6) and one oxymethine proton resonance at δ = 3.49 (H-3) were seen in the $^1H$ NMR spectrum**.**

The $^{13}C$ NMR spectrum (appendix **34**), displayed twenty carbon atom resonances, including four carbon resonances at δ = 111.2 (C-14), δ = 125.9 (C-13), δ = 138.6 (C-16) and δ = 143.0 (C-15) for a furan ring, a secondary alcohol carbon resonance at δ= 76.6 (C-3) and double bond carbon resonances at δ = 142.5 (C-5) and δ = 121.0 (C-6). The double bond and the furan ring accounted for four out of six units of double bond equivalence and the remaining two indicated the presence of two additional rings. The secondary oxymethine resonance at δ = 76.6 was assigned as C - 3 due to its correlations in the HMBC spectrum (appendix **37**) with the two singlet methyl group proton resonances at δ = 1.07 (H-18) and δ = 1.15 (H-19), methylene proton resonances at δ = 1.68 and δ = 1.55 (2H-1) and one proton of the methylene proton resonances at δ =7.2 (H- 2).

The coupling in the COSY spectrum between δ = 3.49 (H-3) and the H-2 (δ = 1.72) proton resonances confirmed the placement of the hydroxyl group at C-3. The placement of a double bond between C-5 (δ = 142.5) and C-6 (δ = 121.0) was due to the correlations in the HMBC spectrum and coupling in the COSY spectrum. The two methylene proton resonances at 2H-1 (δ = 1.55 and δ = 1.69) and 2H-7 (δ = 1.85 and δ = 1.90) showed correlations in the HMBC spectrum with the C-5 (δ = 142.5) carbon atom resonances. Moreover, the correlations in the HMBC spectrum between the H-6 (δ = 5.56) proton resonances and the C-4 (δ = 41.5), C-7 (δ = 32.0), C -8 (δ = 33.7) and C-10 (δ = 39.0) carbon atom resonances, and coupling seen in the COSY spectrum between the H-6 and 2H-7 resonances confirmed the position of the double bond. The two methylene proton resonances at δ = 1.56 (H-11) and δ = 2.36 (H-12) showed correlations in the HMBC spectrum with the furan ring carbon atom

resonances, C-13 (δ = 125.9), C-14 (δ = 111.3) and C-16 (δ = 138.6). From these NMR data the Compound **52** was found to be an halimane-type diterpenoid; 15, 16-epoxy-5,13(16),14-*ent*-halimatriene-3-ol. The NMR spectral data for compound **52** is summarized in table **4.9** below.

Cytotoxicity activity of Compound **52** was evaluated against one dose NCI60 cell line panel and its one dose mean value percentage growth 15μg/ml was 99.39 (appendix **40**). This result did not meet the standards for further testing against the five-dose NCI60 cell line panel.




**52**

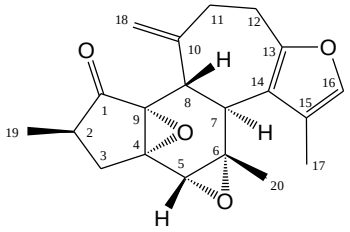
**Table 4.9: NMR data for 15,16-epoxy-5,13(16), 14-*ent*-halimatriene- 3-ol (52)**

| Carbon position | $^{13}$C NMR (δ ) | DEPT | $^{1}$H NMR |
|---|---|---|---|
| 1 | 18.53 | $CH_2$ | 1.55 |
| 2 | 28.5 | $CH_2$ | 1.72 |
| 3 | 76.5 | CH | 3.49 |
| 4 | 41.5 | C | - |
| 5 | 142.5 | C | - |
| 6 | 121.0 | CH | 5.56 |
| 7 | 32.0 | $CH_2$ | 1.85 |
| 8 | 33.7 | CH | 1.62 |
| 9 | 37.4 | C | - |
| 10 | 39.0 | CH | 2.31 |
| 11 | 20.3 | $CH_2$ | 1.56 |
| 12 | 18.5 | $CH_2$ | 2.36 |
| 13 | 125.9 | C | - |
| 14 | 111.2 | CH | 6.28 |
| 15 | 143.0 | CH | 7.35 |
| 16 | 138.6 | CH | 7.22 |
| 17 | 15.3 | $CH_3$ | 0.88 |
| 18 | 28.6 | $CH_3$ | 1.07(s) |
| 19 | 25.7 | $CH_3$ | 1.15(s) |
| 20 | 16.4 | $CH_3$ | 0.71(s) |

### 4.4.5 Structure Elucidation of Crotodichogamoin A (53)

Compound **53** was isolated as a yellow gum from the DCM extract of the roots of *C. dichogamus*. The MS spectrum (appendix **41**) of Compound **53** gave a $[M+H]^+$ ion at *m/z* 327.1590 which indicated a molecular formula of $C_{20}H_{22}O_4$. The $^{13}$C NMR (appendix **43**) and DEPT spectra (appendix **44**) displayed twenty carbon resonances, including one keto carbonyl resonance at δ = 206.1, four oxygenated carbon atom resonances at δ = 56.2 (C-5), δ = 61.5 (C-6), δ = 65.8 (C-4) and δ = 66.1 (C-9), three methyl carbon atom resonances at δ = 8.9 (C-17), δ = 16.0 (C-19) and δ = 20.3(C-20) and six aromatic/olefinic carbon resonances, four of them for the furan ring at δ = 117.0 (C-15), δ = 121.8 (C-14), δ = 137.0 (C-16) and δ = 150.4 (C-13), one fully substituted and a methylene olefinic carbon resonances at δ = 141.9

(C-10) and δ= 116.9 (C -18), in that order. From these spectral data compound **53** was characterized as a crotofolane-type diterpenoid commonly identified as crotodichogamoin A. This information is summarized in table **4.10** below.




**53**

Cytotoxicity activities of crotodichogamoin A was evaluated against the NCI59 developmental therapeutics programcancer cell line screening at one dose of 1 x $10^{-5}$M (appendix **49**) but it did not meet the standards for further testing.

**Table 4.10: NMR data for Crotodichogamoin A (53).**

| Carbon position | $^{13}$C NMR (δ ) | DEPT | $^{1}$H NMR |
|---|---|---|---|
| 1 | 206.1 | C | - |
| 2 | 38.7 | CH | 2.49 |
| 3 | 31.2 | $CH_2$ | 2.66 dd (J = 8.3, 14.0 Hz) |
| 4 | 65.8 | C | - |
| 5 | 56.2 | CH | 3.295(s) |
| 6 | 61.5 | C | - |
| 7 | 49.0 | CH | 2.49 d (J = 13.0 Hz) |
| 8 | 39.9 | CH | 2.78 d (J = 13.0 Hz) |
| 9 | 66.1 | C | - |
| 10 | 141.9 | C | - |
| 11 | 37.8 | $CH_2$ | 2.53 |
| 12 | 23.1 | $CH_2$ | 2.81 |
| 13 | 150.4 | C | - |
| 14 | 121.8 | C | - |
| 15 | 117.0 | C | - |
| 16 | 137.0 | CH | 7.03(s) |
| 17 | 89.0 | $CH_3$ | 1.96 (s) |
| 18 | 116.9 | $CH_2$ | 5.06 (s) |
| 19 | 16.0 | $CH_3$ | 1.11d (J = 7.5 Hz) |
| 20 | 20.3 | $CH_3$ | 1.09 (s) |

### 4.4.6 Structure Elucidation of Crotohaumanoxide (**54**)

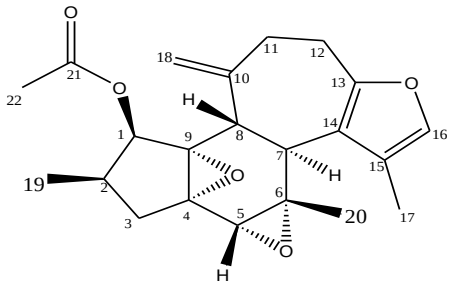
The $^{13}$C NMR spectrum (appendix **51**) displayed twenty carbon atoms resonances, which could be assigned using HSQC - DEPT (appendix **53**), COSY (appendix **55**) and HMBC (appendix **54**) experiments. The C-1 acetate carbon atom resonance was shown as a peak at δ = 75.3. The presence of a furan ring was shown by resonances at δ = 117.5 (C-15), δ = 122.3 (C-14), δ = 137.0 (C-16) and δ = 150.4 (C-13) and the C-14, C-15 and C -16 resonances exhibited correlations in the HMBC spectrum (appendix **54**) with the 3H - 17 resonance (δ = 1.96). The two H - 12 resonances (δ = 2.77, δ = 2.98) showed correlations in

the HMBC spectrum with the C-10 (δ = 145.3), C-11 (δ = 36.5), C-13 (δ = 150.4) and C-14 (δ = 122.3) resonances and correlations in the COSY spectrum with two H-11 resonances (δ = 2.58, δ = 2.47). A pair of exo - methylene proton resonances (δ = 5.01 (b), δ = 4.91 (b), 2 H-18) showed correlations in the HMBC spectrum with the C-8 (δ = 37.1), C–10 (δ = 145.3) and C-11 (δ = 36.5) resonances, confirming its placement at C-10. The C - 18 resonance (δ = 114.1) showed correlations with the H-8 and two H-11 resonances, and the H - 8 resonance showed further correlations with the C-1 (75.3), C-4 (δ = 60.7), C-6 (δ = 57.4), C- 7 (δ = 41.8), C-9 (δ = 69.0), C-10 (δ = 145.3) and C-14 (δ = 122.3) resonances and a correlation in the COSY spectrum with the H-7 resonance (δ = 2.80). The H-7 resonance showed correlations in the HMBC spectrum (appendix **54**) with the C-5 (δ = 57.8), C- 6, C- 8, C- 9, C- 10, C-13, C-14, C-15 and C-20 resonances.

The chemical shifts of the C- 9/C- 4 and C-5/C-6 resonances indicated the presence of epoxides at these positions. The H-5 resonance occurred as a singlet (δ = 3.14) and its assignment was confirmed by correlations with the C-4, C-6, C-9 and methyl C-20 (δ = 20.2) resonances. Both the C-4, C-9 and acetyl carbon resonance showed correlations in the HMBC spectrum (appendix **54**) with the H - 2 (δ = 2.21) and two H - 3 (δ = 2.47 and δ = 1.64) proton resonances. The 3H - 19 resonance (δ = 0.91, d) showed correlations in the HMBC spectrum with the C-2, C-3 and the acetyl carbon resonance, confirming the placement of the acetyl group at C-1. The COSY spectrum showed coupling between the 3H-19/H-2 and H-2/two H-3 resonances. The relative configuration was assigned using the NOESY spectrum. Correlations were seen between the H-5/3H-20, 3H-20/H-8 and H-8/3H-17 resonances, and, they were assigned as β. The proposed chemical structure of compound **54** is shown below and its NMR spectral data is summarized in table **4.11**

**Table 4.11: NMR spectral data for Crotohaumanoxide (54)**

| POSITION | $^{13}$C NMR (125 MHz) | $^{1}$H NMR (500 MHz) | DEPT | HMBC (H → C) |
|---|---|---|---|---|
| 1 | 75.3 | 5.5 d (J = 5.6 Hz) | CH | 2, 3, 4, 8, 9 |
| 2 | 33.8 | 2.21 (m) | CH | 3, 19 |
| 3a | 37.2 | 2.47 dd (J= 13.7, 7.3 Hz) | $CH_2$ | 1, 2, 4, 9 |
| 3b | | 1.64 dd (J= 13.7, 7.3 Hz) | | 1, 2, 4, 5, 19 |
| 4 | 60.7 | - | C | - |
| 5 | 57.8 | 3.14 (s) | CH | 3, 4, 6, 9, 20 |
| 6 | 57.4 | - | C | - |
| 7 | 41.8 | 2.8 d (J = 12.5 Hz) | CH | 5, 6, 8, 9, 10, 13, 14, 15, 20 |
| 8 | 37.1 | 3.06 d (J = 12.5 Hz) | CH | 6, 7, 9, 10, 11, 18 |
| 9 | 69.0 | - | C | - |
| 10 | 145.3 | - | C | - |
| 11 a | 36.5 | 2.58 (m) | $CH_2$ | 8, 10, 12, 18 |
| 11 b | | 2.47 (m) | | 8, 10, 12, 13, 18 |
| 12 a | 22.9 | 2.98 (m) | $CH_2$ | 11, 13, 14 |
| 12 b | | 2.77 (m) | | 10, 11, 13 |
| 13 | 150.4 | - | C | - |
| 14 | 122.3 | - | C | - |
| 15 | 117.5 | - | C | - |
| 16 | 137.0 | 7.0 (b) | CH | 13, 14, 15 |
| 17 | 8.8 | 1.96 (b) | $CH_3$ | 14, 15, 16 |
| 18 a | 114.1 | 4.91 (b) | $CH_2$ | 8, 10, 11 |
| 18 b | | 5.01 (b) | | 8, 10, 11 |
| 19 | 12.8 | 0.91 d (J = 7.1 Hz) | $CH_3$ | 1, 2, 3 |
| 20 | 20.2 | 1.08 (s) | $CH_3$ | 5, 6, 7 |
| 21 | 169.7 | - | C | - |
| 22 | 20.8 | 2.11 (s) | $CH_3$ | 1, 21 |



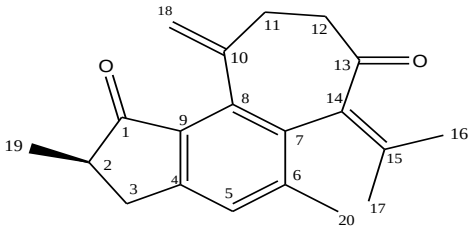
**54**

### 4.4.7 Structure elucidation of Crotodichogamoin B (55)

Crotodichogamoin B, was isolated as a yellow greasy solid from the DCM root extract. It was found to be a crotofolane diterpenoid. The HRMS (appendix **56**) data showed a $[M + H]^+$ ion at m/z 295.1694 indicating a molecular formula of $C_{20}H_{22}O_2$ and ten degrees of unsaturation.

The $^{13}C$ NMR spectrum (appendix **58**) showed twenty carbon resonances, including two keto carbonyl carbon atom resonances at δ = 208.1 (C-1) and δ = 202.5 (C-13). Vinyl methyl carbon atoms resonces were evident at C-16 (δ = 23.5) and C-17 (δ = 22.1) and the HMBC spectrum (appendix **61**) showed correlations between the 3H-16 (δ = 1.49), 3H-17 (δ = 2.13) and C-15 (δ = 147.5) and C-14 (δ = 134.3) alkene carbon resonances. The HMBC spectrum showed correlations between the C-14 and C- 13 (δ = 202.5) keto carbonyl carbon resonance and the two H-12 resonances (δ = 2.27 and δ = 2.43). The C-13 resonance also showed a correlation with the two H-11 resonances (δ = 2.82 and δ = 2.72), which showed correlations with the C-10 (δ = 142.0) and C-18 (δ = 114.8) resonances of the exo methylene group and the C-8 (δ = 139.2) aromatic ring carbon resonance. The 3H-20 resonance showed correlations with C-5 methine (δ = 126.9) and C-6 (δ = 137.9) and C-7 (δ = 143.4) fully substituted carbon atom resonances. The corresponding H-5 singlet resonance (δ = 7.22) showed correlations with the C-3 (δ = 34.4), C-4 (δ = 131.0), C - 6 (δ = 137.9), C - 7 (δ = 143.4), C-9 (δ = 153.3) and C-20 (δ = 20.6) resonances. The C-1 (δ = 208.0) keto carbonyl carbon resonance exhibited correlations with the two H-3 (δ = 2.26, δ = 3.34), the H-2 (δ = 2.72) and 3H-19 (δ = 1.28) proton resonances in the HMBC spectrum. The H-2 resonance showed correlations with the C-4 (δ = 131.0) and C-9 (δ = 153.5) resonances.

The NMR spectral data for compound **55** is tabulated in table **4.12**




**55**

**Table 4.12: NMR spectral data for Crotodichogamoin B (55) (appendices 56 -63)**

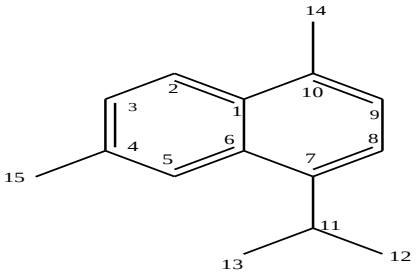
| Position | $^{13}$C NMR (125 MHz) | $^{1}$H NMR (500 MHz) | DEPT | HMBC (H → C) |
|---|---|---|---|---|
| 1 | 208.0 | - | C | - |
| 2 | 42.9 | 2.72 (m) | CH | 1, 3, 9, 19 |
| 3a | 34.4 | 3.34 dd (J = 16.8 Hz, J = 8.0 Hz) | $CH_2$ | 1, 2, 4, 5, 9, 19 |
| 3b | | 2.66 (m) | | 2, 4, 5, 9, 19 |
| 4 | 131.0 | - | C | - |
| 5 | 126.9 | 7.22 (s) | CH | 3, 4, 6, 7, 9, 20 |
| 6 | 137.9 | - | C | - |
| 7 | 143.4 | - | C | - |
| 8 | 139.2 | - | C | - |
| 9 | 153.3 | - | C | - |
| 10 | 142 | - | C | - |
| 11 a | 31.7 | 2.82 (m) | $CH_2$ | 8, 10, 12, 13, 18 |
| 11 b | | 2.72 (m) | | 8, 10, 12, 13, 18 |
| 12 a | 40.9 | 2.27 (m) | $CH_2$ | 10, 11, 13, 14 |
| 12 b | | 2.43 (m) | | 10, 11, 13 |
| 13 | 202.5 | - | C | - |
| 14 | 134.3 | - | C | - |
| 15 | 147.5 | - | C | - |
| 16 | 23.5 | 1.49 (s) | $CH_3$ | 14, 15, 17 |
| 17 | 22.1 | 2.13 (s) | $CH_3$ | 14, 15, 16 |
| 18 a | 114.8 | 5.26 (s) | $CH_2$ | - |
| 18 b | | 4.93 (b) | | - |
| 19 | 16.1 | 1.28 d (J = 7.1 Hz) | $CH_3$ | 1, 2, 3 |
| 20 | 20.6 | 2.21 (s) | $CH_3$ | 8, 10, 11 |

### 4.4.8 Structure Elucidation of Cadin-1(6),2,4,7,9-penta-ene (cadalene) (56)

Compound **56** was isolated as a white oily compound from the dichloromethane extract of the roots of *C. dichogamus*. The $^{13}C$ NMR spectrum (appendix **65**) displayed fifteen carbon resonances, including four methyl, six methine, and five fully substituted carbon resonances. $^{1}H$ NMR spectrum (appendix **64**) revealed two singlet proton resonances at δ = 2.56 (3H-15) and δ = 2.64 (3H-14) for methyl groups, and resonances at δ = 1.38 (6H, d, *J* = 6.9 Hz, 3H-12 and 3H-13) and δ = 3.72 (H, *J* = 6.9 Hz) due to protons of an isopropyl group. The $^{1}H$ and $^{13}C$ NMR spectra also displayed sets of aromatic proton resonances at δ = 7.92 (d, *J* = 8.5 Hz, H- 2), δ = 7.90 (H- 5), δ = 3.5 (H, d, *J* = 8.5 Hz, H - 3), and δ = 7.28 (H, d, *J* = 7.2 Hz, H - 8) and δ = 7.22 (H, d, *J* = 7.2 Hz, H - 9), which corresponded in the HSQC – DEPT (appendix **67**) spectrum to five aromatic carbon resonances at δ = 125.0, δ = 123.1, δ = 127.4, δ = 121.6 and δ = 125.8, respectively.

The H-5 (δ = 7.90) resonance showed correlations, in the HMBC spectrum (appendix **68**), with the aromatic carbon resonances at δ = 131.3 (C-1), δ = 127.4 (C-3), δ = 134.9 (C-4), δ = 131.7 (C-6), δ = 142.3 (C-7) and one methyl group at δ = 22.3 (C-15). The HMBC spectrum showed correlations between the 3H-14 resonance at δ = 2.64 and the carbon resonances at δ = 131.3 (C-1), δ = 125.8 (C-9) and δ = 132.1 (C-10). The other singlet methyl group proton resonance at δ = 2.56 (3H-15) was assigned as C-15 due to its correlations with the carbon resonances at δ = 127.4 (C-3), δ = 134.9 (C-4) and δ = 123.1 (C-5) in the HMBC spectrum. The isopropyl group was placed at C-7 based on HMBC correlations between the doublet methyl group proton resonance δ = 1.38 (3H-12 and 3H-13) and the aromatic carbon resonance at 142.3 (C-7) and the carbon resonances at δ = 28.4 (C-11).

From the spectral data compound **56** was found to be the known sesquiterpenoid called cadin-1(6), 2, 4, 7, 9-penta-ene (**56**), normally known as cadalene. These spectral data is summarized in the table **4.13** below.




**56**

**Table 4.13: NMR spectral data for Cadin-1(6),2,4,7,9-penta-ene (56) (appendices 64 - 70)**

| Position | $^{13}$C NMR (125 MHz) | $^{1}$H NMR (500 MHz) | DEPT | HMBC (H → C) |
|---|---|---|---|---|
| 1 | 131.3 | - | C | |
| 2 | 125.0 | 7.92 d (J= 8.5 Hz) | CH | 1, 3, 4, 6, 10 |
| 3 | 127.4 | 7.35 d (J= 8.5 Hz) | CH | 1, 5, 15 |
| 4 | 134.9 | - | C | |
| 5 | 123.1 | 7.90 (s) | CH | 1, 3, 4, 6, 7, 15 |
| 6 | 131.7 | - | C | |
| 7 | 142.3 | - | C | |
| 8 | 121.6 | 7.28 d (J = 7.2 Hz) | CH | 6, 10, 11 |
| 9 | 125.8 | 7.22 d (J = 7.2 Hz) | CH | 1, 7, 8 |
| 10 | 132.1 | - | C | |
| 11 | 28.4 | 3.72 | CH | 6, 7, 8, 12, 13 |
| 12 | 23.9 | 1.38 d (J = 6.9 Hz ) | $CH_3$ | 7, 11, 13 |
| 13 | 23.9 | 1.38 d (J = 6.9 Hz ) | $CH_3$ | 7, 11, 12 |
| 14 | 19.7 | 2.64 (s) | $CH_3$ | 1, 9, 10 |
| 15 | 22.3 | 2.56 (s) | $CH_3$ | 3, 4, 5 |

This study augments the previous reports made by Sousa *et al.,* (2015) that *Croton* genus is a rich source of several categories of structurally complex bioactive compounds. The compounds obtained in this research are consistent with those investigated and reported for African *Croton* species, which have been shown to produce a wide variety of diterpenoids along with sesquiterpenoids and common triterpenoids (Mulholland *et al.,* 2010; Langat *et al.,* 2011, 2012; Ndunda *et al.,* 2016*;* Abebayehu *et al.,* 2016, Maroyi, 2017).

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

The chemical constituents of four medicinal plants; *D. abbysinica* (Salicaceae), *S. mauense* (Solanaceae), (Myrtaceae) and *C. dichogamous* (Euphorbiaceae) were investigated in this study**.** Crude extracts from stem bark of *S. guinense*, fruits of *S. mauense*, roots of *D. abbysinica* and roots of *C. dichogamous* were prepared via cold extraction method. Purification of isolated compounds was accomplished by use of chromatographic techniques. The chemical structures of isolated compounds from this study were proposed based on their 1D and 2D NMR spectral data and MS where possible.

A total of twelve compounds were characterized in this study. Two previously reported compounds; *β*-sitosterol (**47**) and Betulinic acid (**21**), were isolated from stem bark extracts of *S. guinense*. Betulinic acid (**21**) was also isolated from the fruits extracts of *S. mauense* alongside fatty acids**.** Two known compounds; Tremulacin (**29**) and Benzoic acid (**48**) were isolated from the roots extract of *D. abbysinica*. Eight compounds were isolated from roots extracts of *C. dichogamous;* Acetyl aleuritolic acid (**49**), 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (**50**), 3β, 4β:15, 16-diepoxy-13(16), 14-*ent*-clerodadien-17,12*S*-olide (**51**), 15,16-epoxy-5, 13(16), 14-*ent*-halimatriene- 3-ol (**52**), crotodichogamoin A (**53**), Crotohaumanoxide (**54**), Crotodichogamoin B (**55**) and Cadin-1(6),2,4,7,9-penta-ene (**56**)**.**

Some compounds were evaluated for their anticancer activity using a panel of human cancer cell lines. Betulinic acid (**21)** was screened against 57 cell lines and only 25 gave positive results. It inhibited leukemia CCRF-CEM and SR human cancer cell line (32.49% and 29.03% respectively). It also showed moderate inhibition against colon cancer HCT-116 (17.06%) and non small lung cancer cell line A-549/ATCC (16.88%). Based on the result of NCI's analysis, it was evident that betulinic acid (**21)** is moderately reactive and it might have the potential to be candidate for anticancer drugs development. Acetyl aleuritolic acid (**49**), 15,16-epoxy-5, 13(16), 14-*ent*-halimatriene- 3-ol (**52**) and crotodichogamoin A (**53**) were screened for their anticancer action and and their one dose mean value percentage growth at 15μg/ml were 97.86, 99.39 and 100.6 respectively. The mean values of growth inhibition of the three compounds tested against one dose NCI cell line panel did not attain the criteria for further testing touching the five-dose NCI cell line panel. However, a literature review showed a degree of anticancer activity for most of the isolated compounds in this study.

## 5.2 Recommendations

The following recommendations were made in relation to this study:

i. That there is need to screen the crude extracts against cancer cell lines since isolating one constituent from the others may denature and lessen potency. The crude extracts may exhibit anticancer activity due to synergism.

ii. The yields from the *S. mauense* and *S. guinense* were low thus some compounds were not identified. There is need to collect these plant samples in large quantities and investigate it further for compounds with activity against cancer cell lines.

iii. There is need to undertake in vivo studies of Acetyl aleuritolic acid (**49**), 15,16-epoxy-5, 13(16), 14-*ent*-halimatriene- 3-ol (**52**) crotodichogamoin A (**53**) and betulinic acid (**21**) and evaluate their anticancer potency.

iv. Toxicological studies to be done before this medicinal plant is recommended for use as anticancer.

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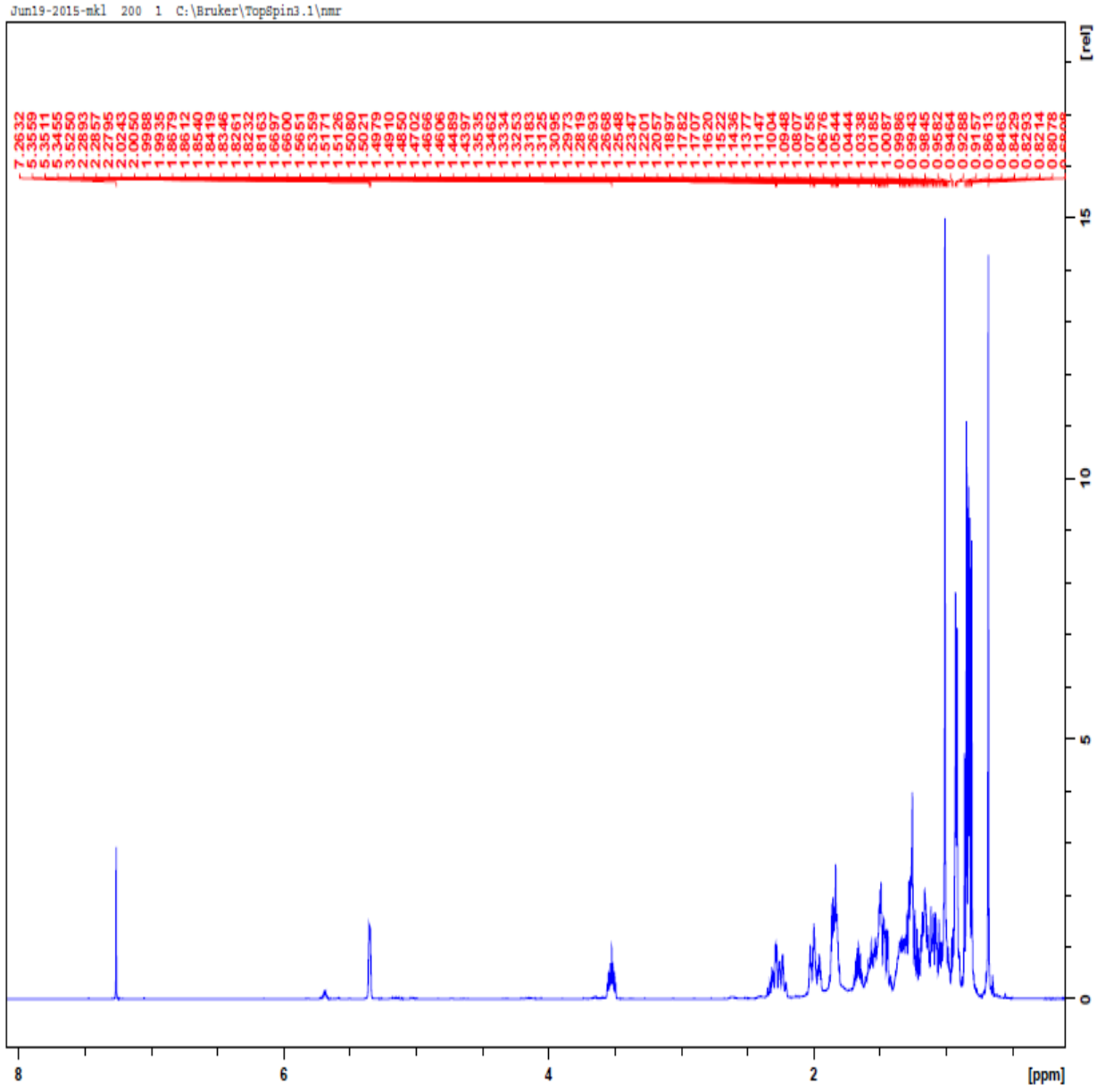
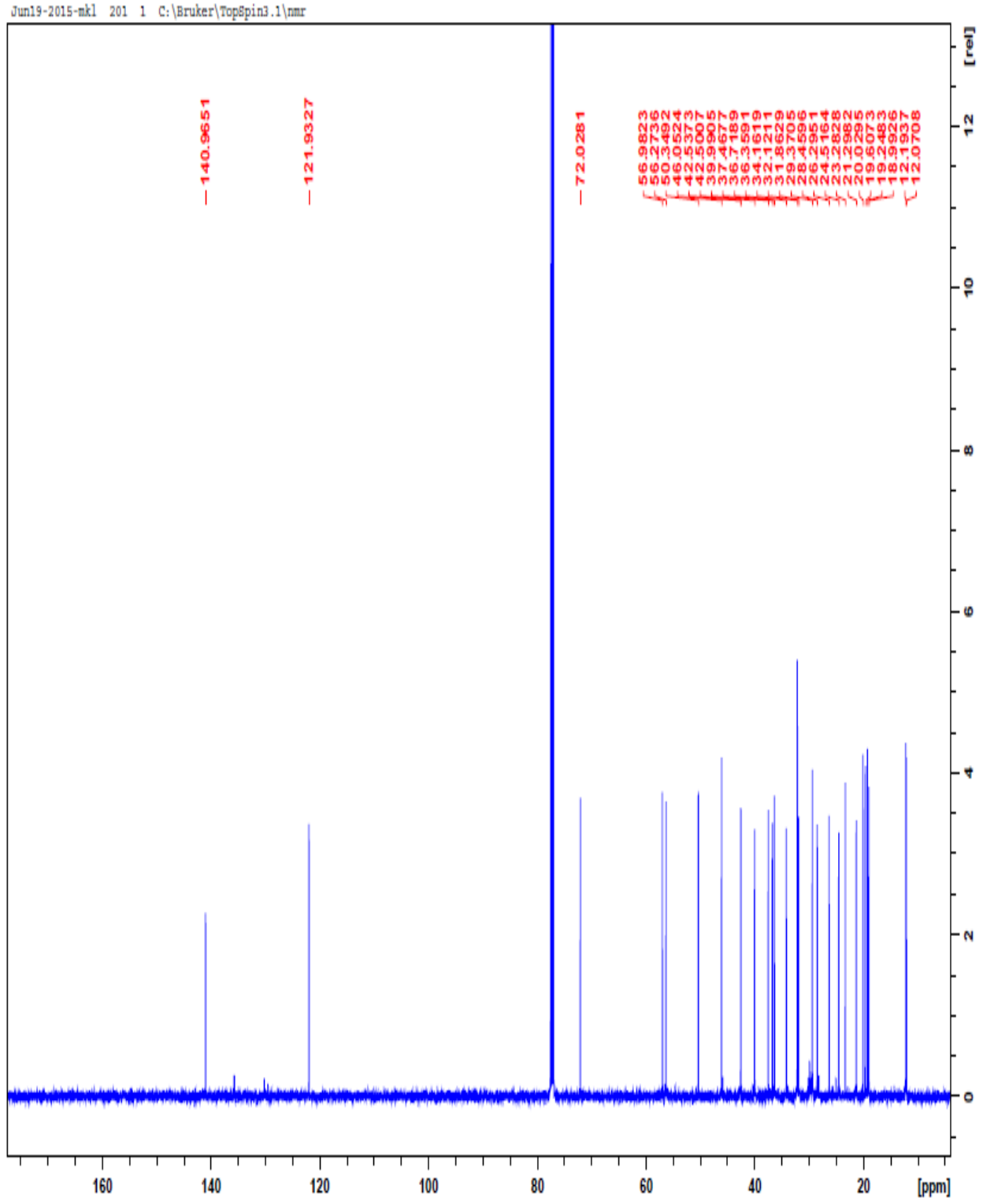
## APPENDICES

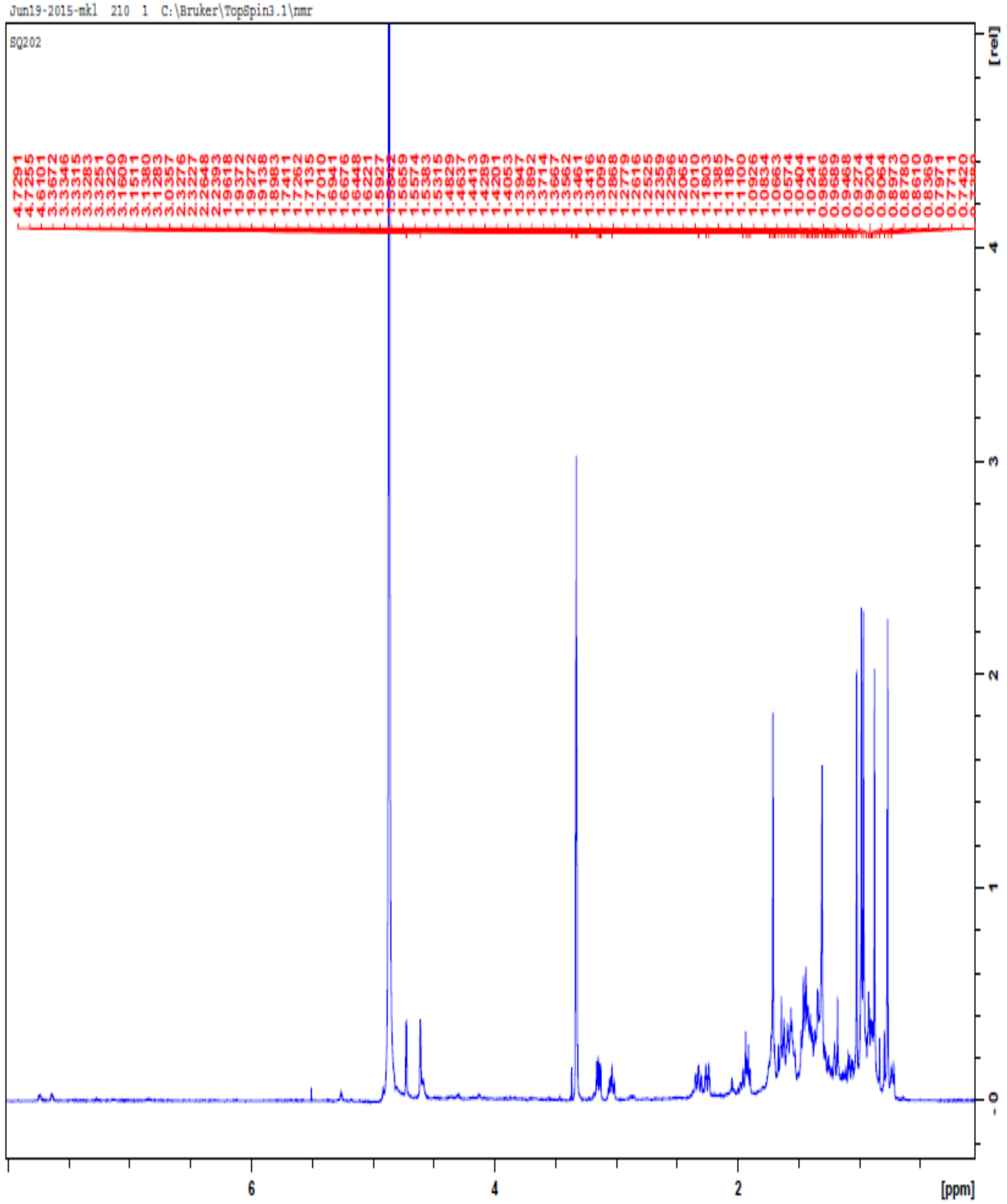
### Appendix 1: $^{1}$H NMR spectrum of β-sitosterol (47)



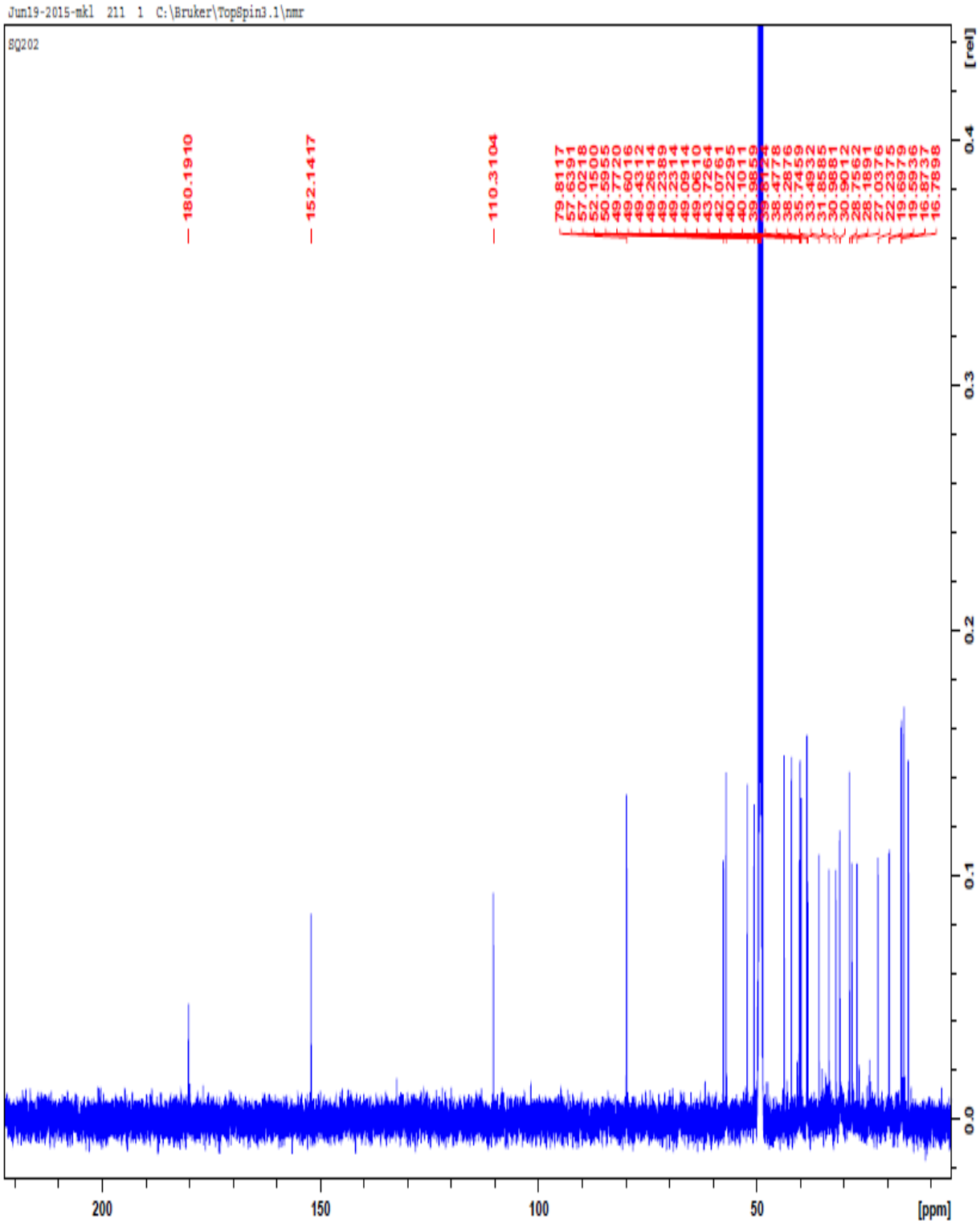
**Appendix 2: $^{13}$C NMR spectrum of β-sitosterol (47)**



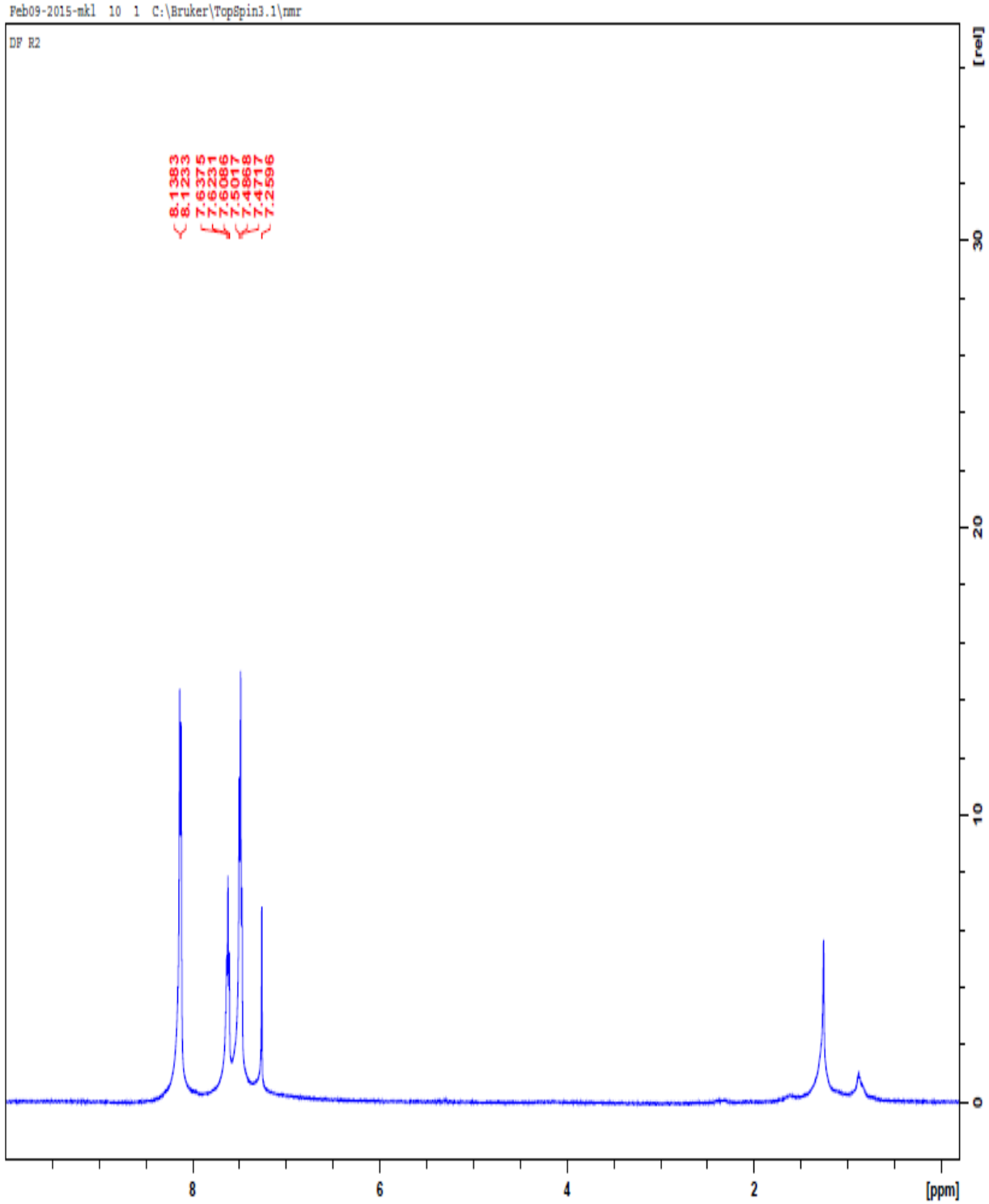
**Appendix 3: $^{1}$H NMR spectrum of Betulinic acid (21)**



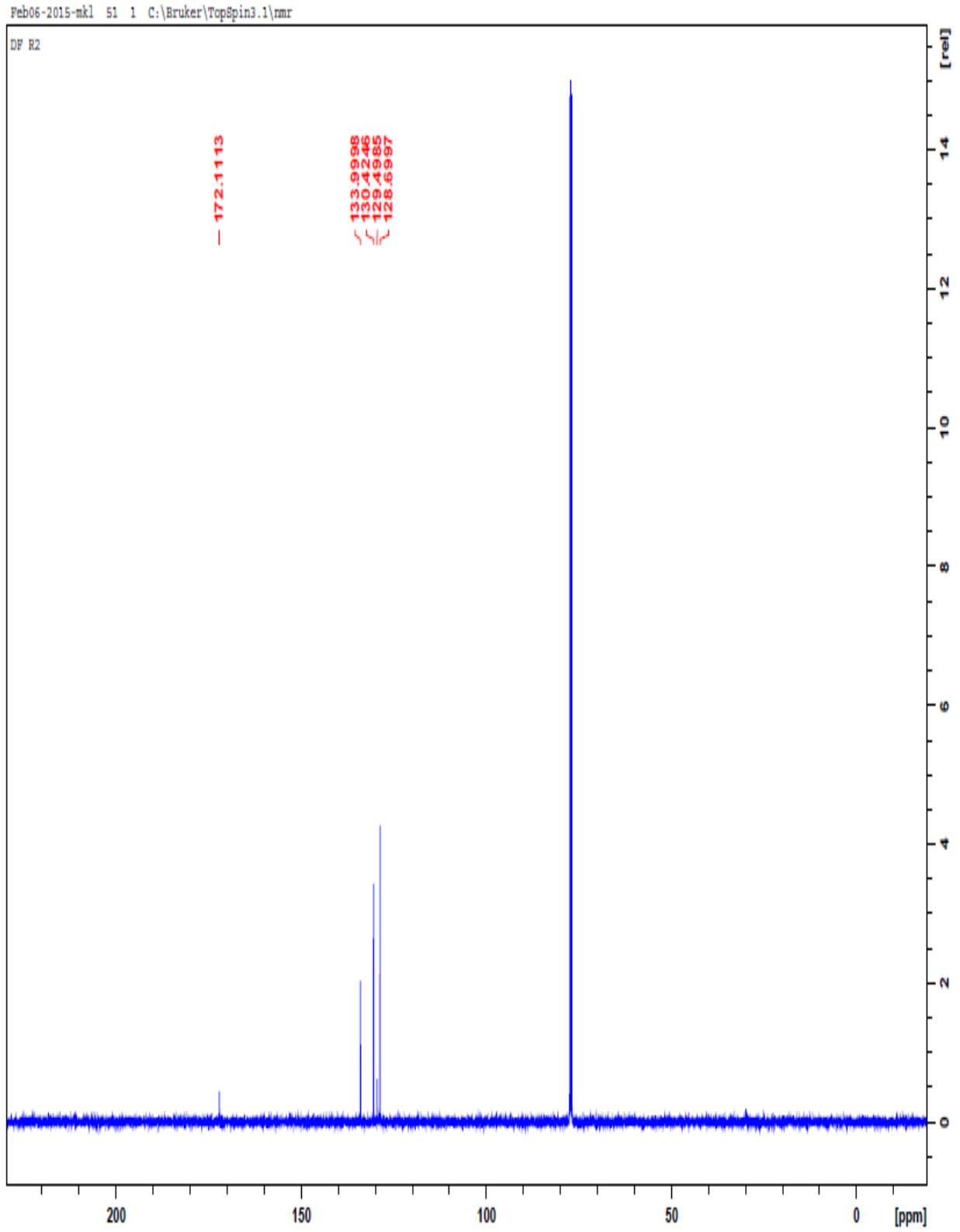
**Appendix 4: $^{13}$C NMR spectrum of Betulinic acid (21)**



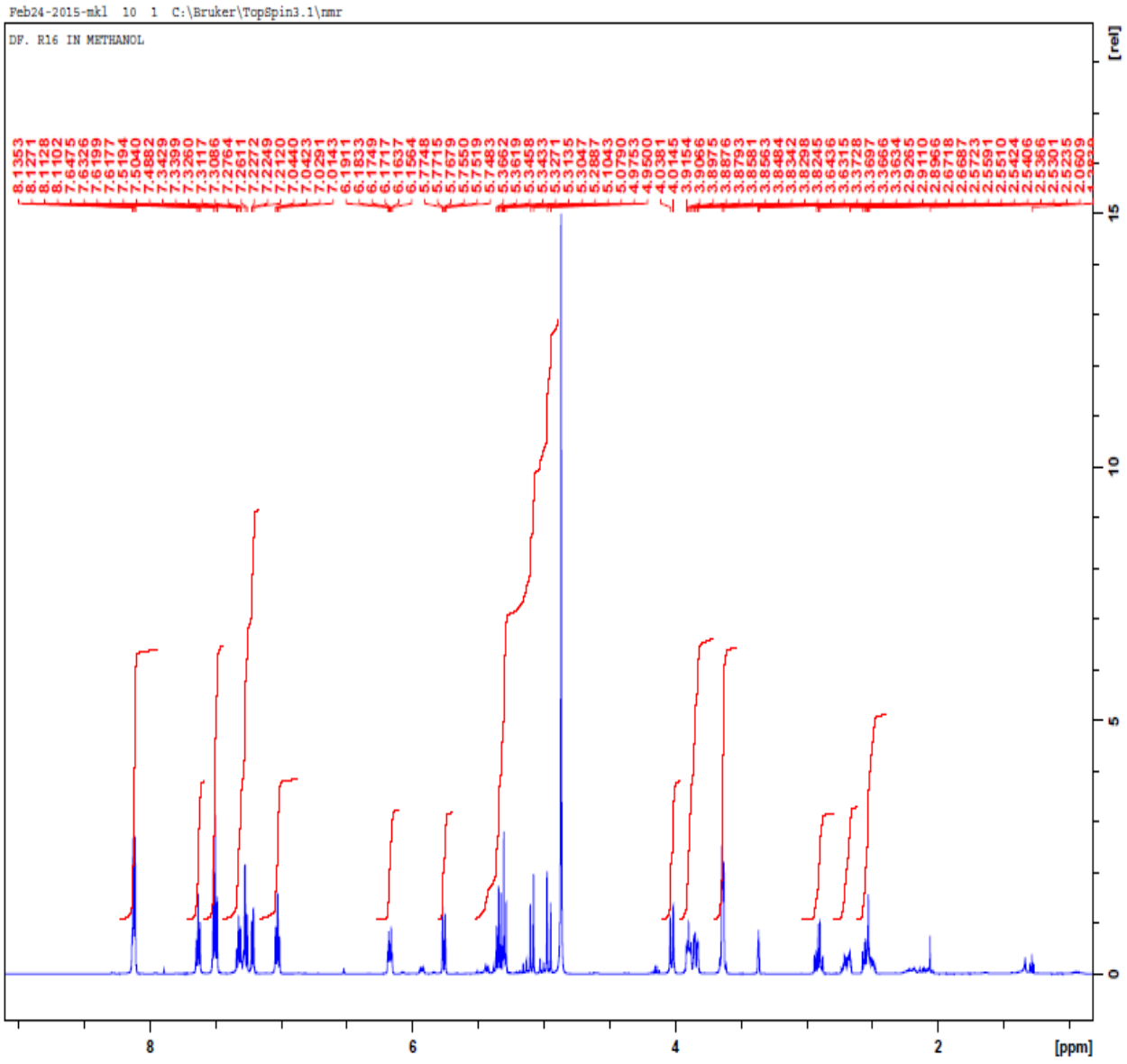
**Appendix 5: $^{1}$H NMR spectrum of Benzoic acid (48)**



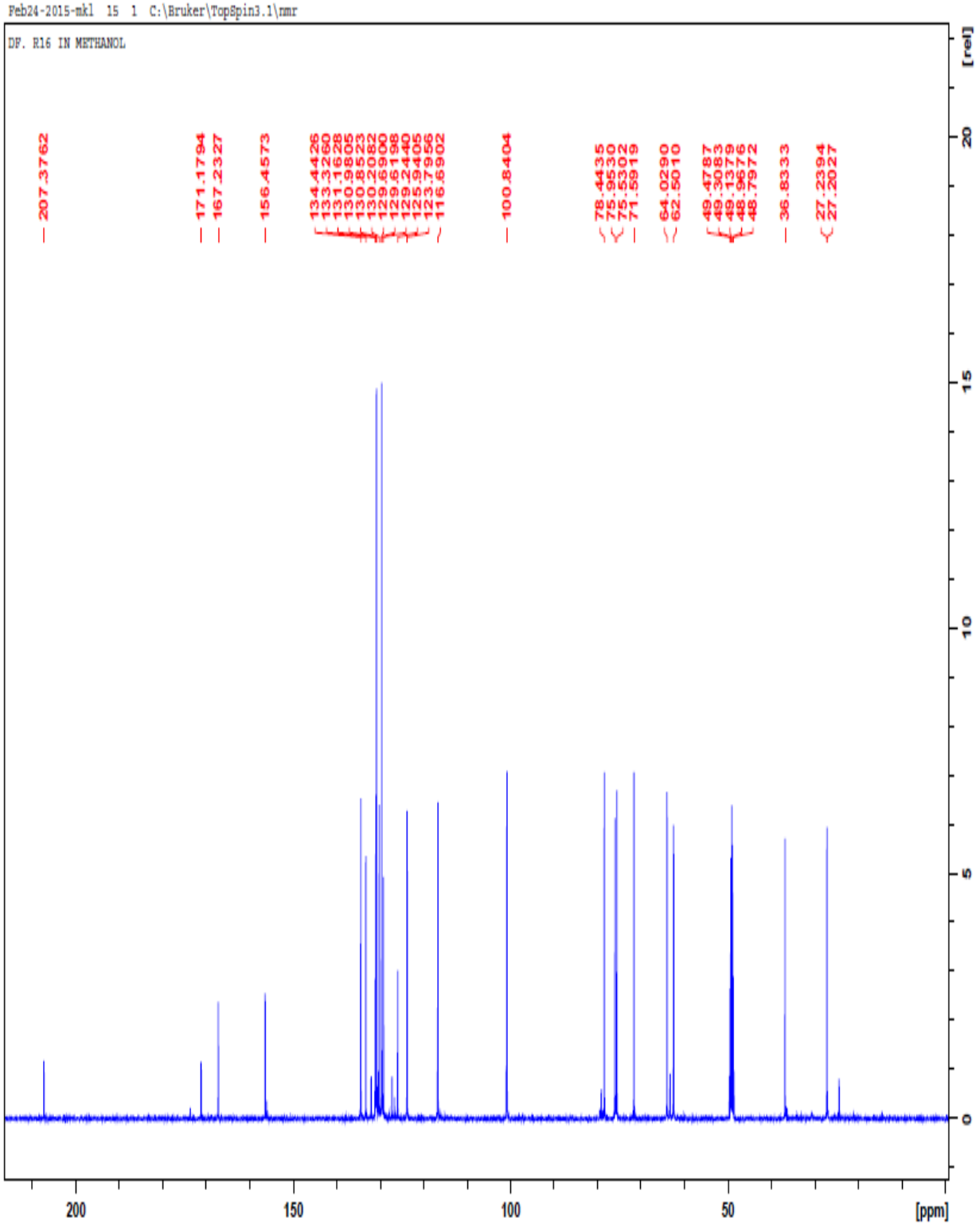
**Appendix 6: $^{13}$C NMR spectrum of Benzoic acid (48)**



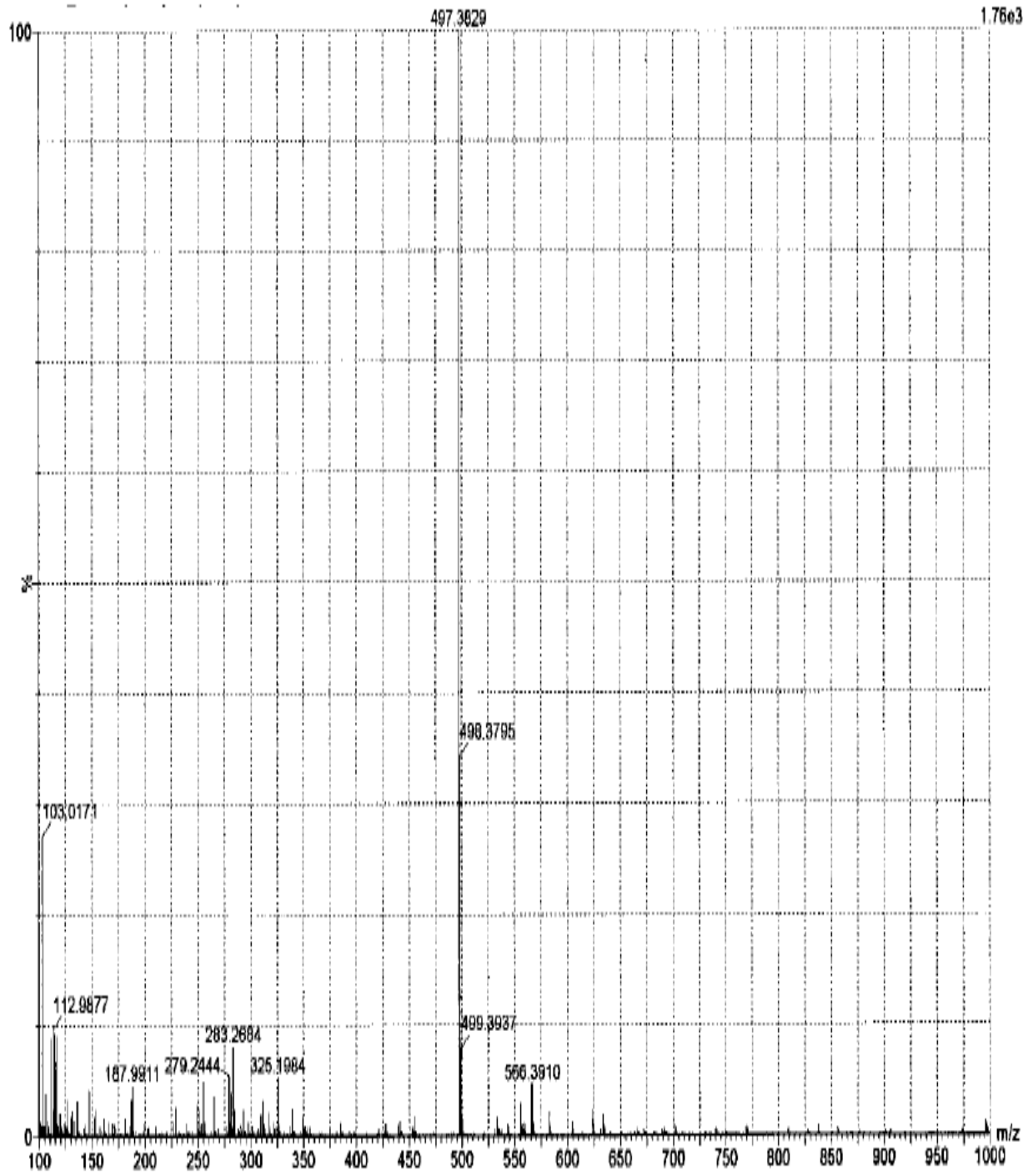
**Appendix 7: $^{1}$H NMR spectrum of Tremulacin (29)**



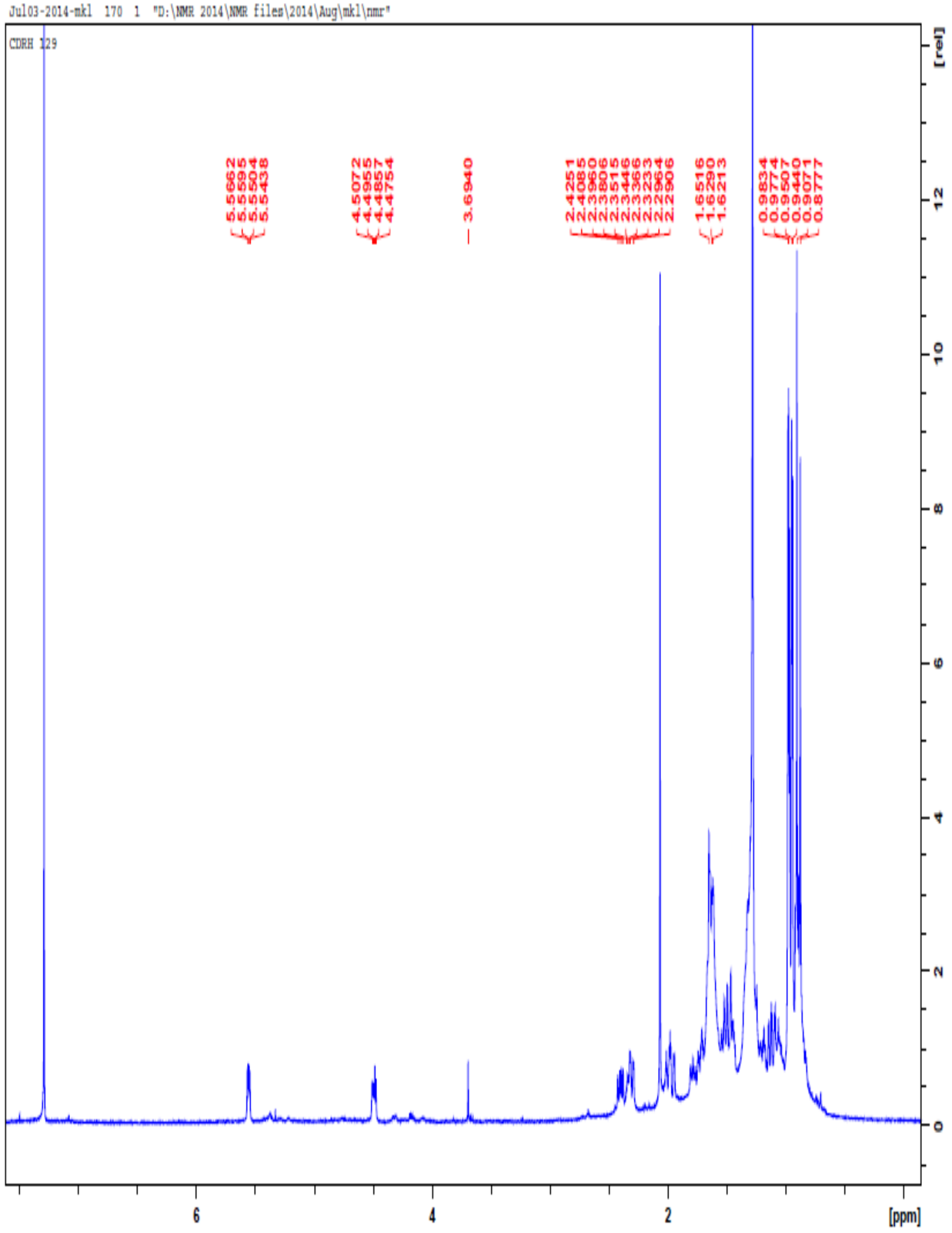
**Appendix 8: $^{13}$C NMR spectrum for Tremulacin (29).**



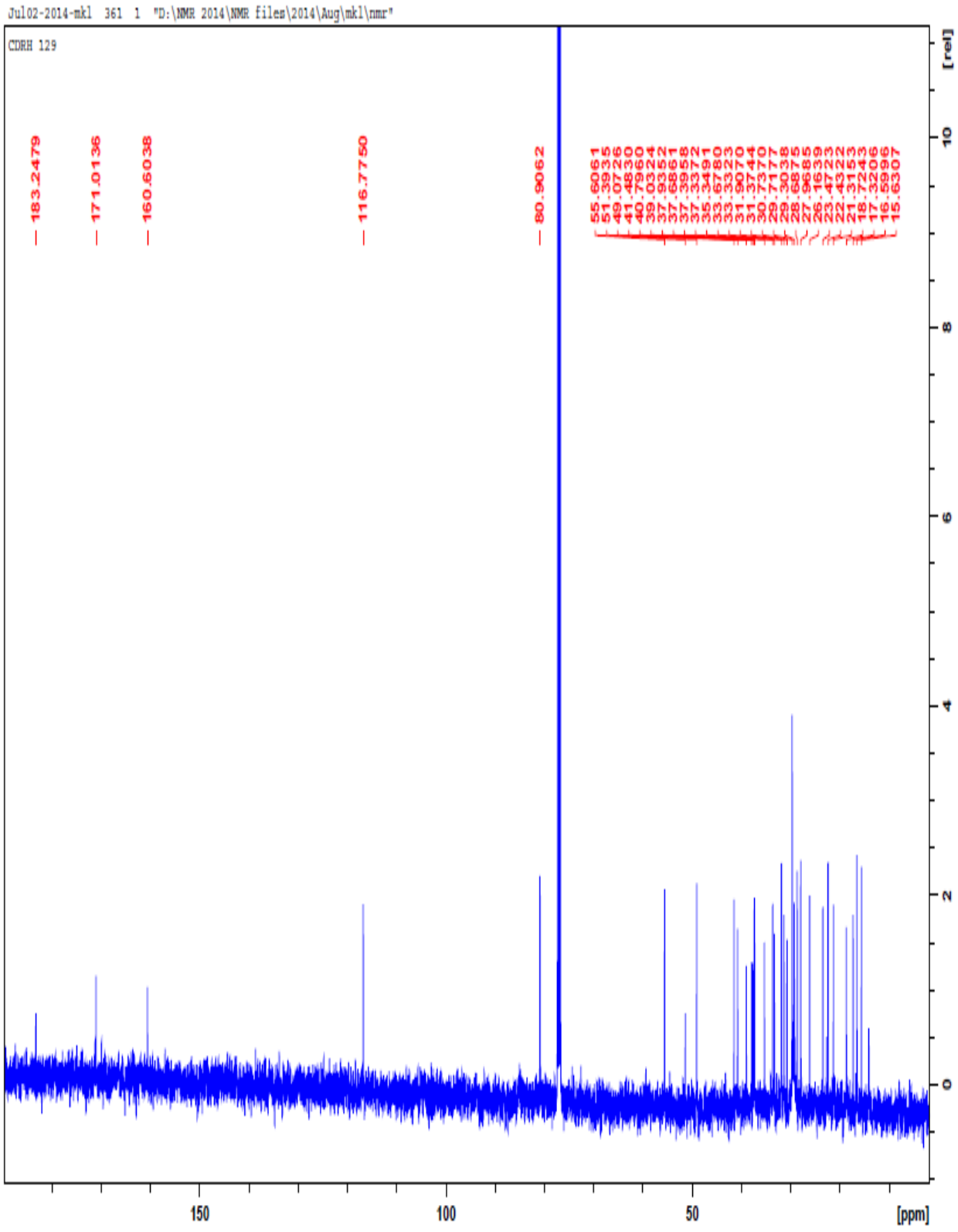
**Appendix 9: The MS spectrum of Acetyl aleuritolic acid (49)**



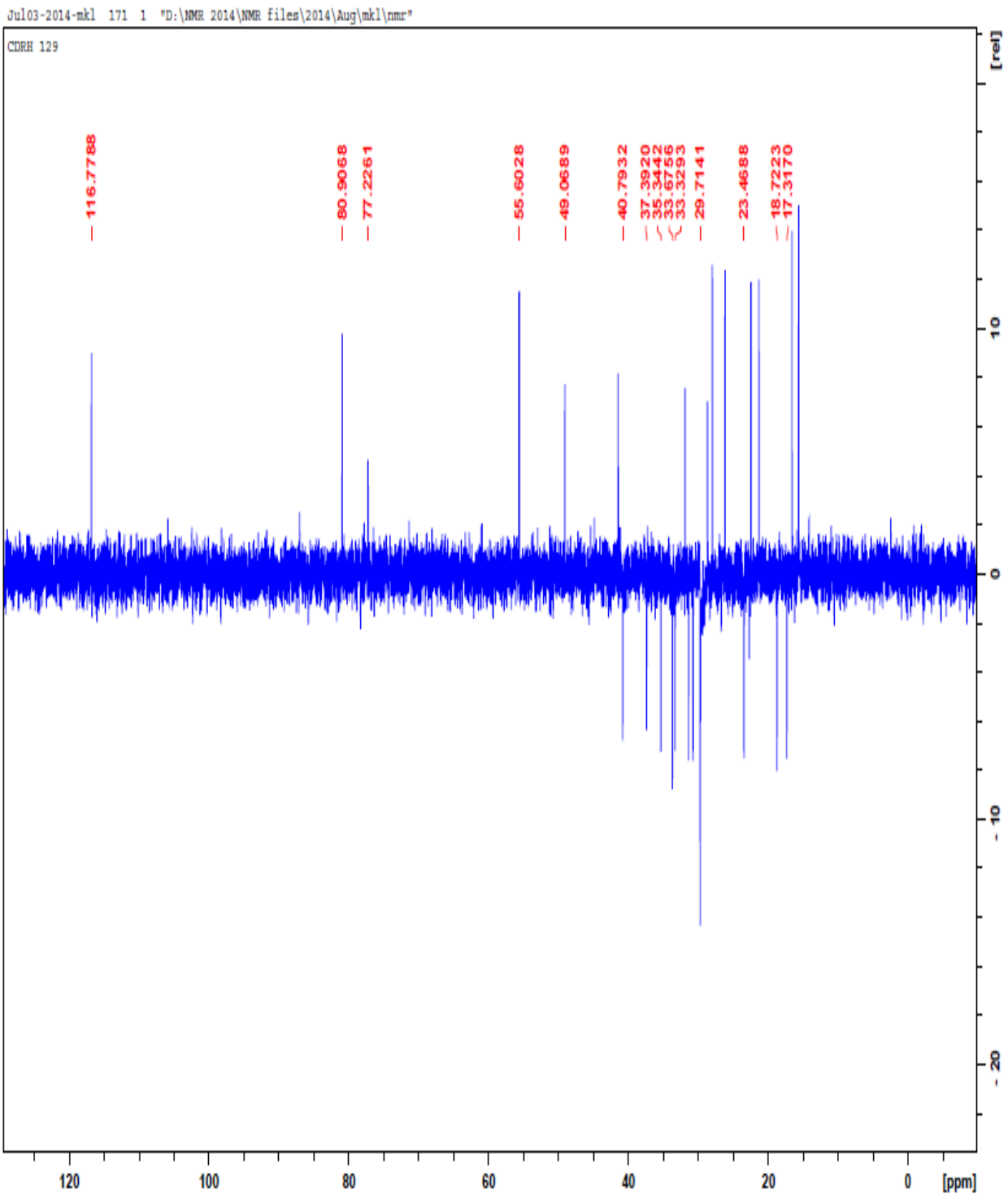
**Appendix 10: $^{1}$H NMR spectrum of Acetyl aleuritolic acid (49)**



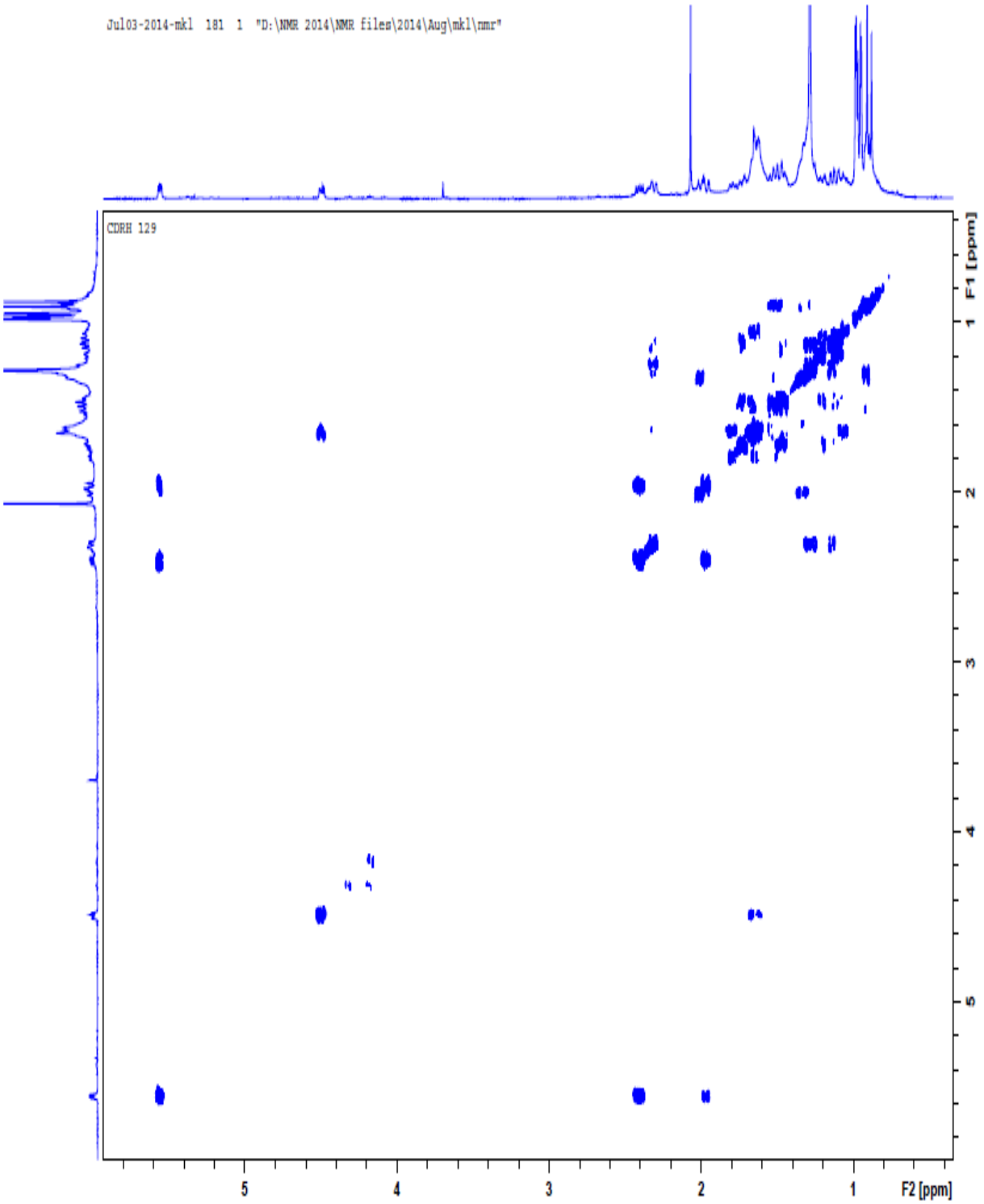

**Appendix 11: $^{13}$C NMR of Acetyl aleuritolic acid (49)**



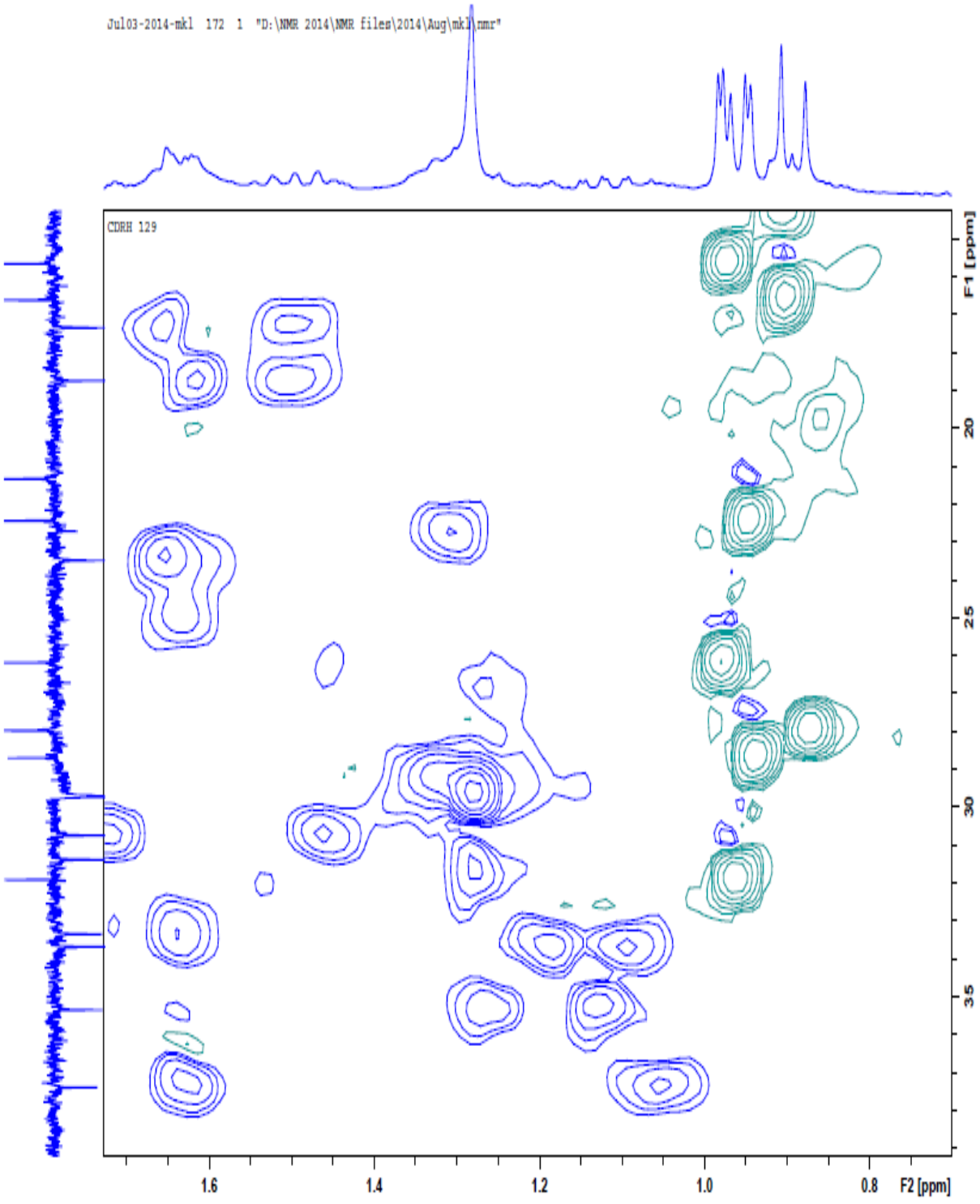

**Appendix 12: DEPT NMR for Acetyl aleuritolic acid (49)**


Jul03-2014-mkl 171 1 "D:\NMR 2014\NMR files\2014\Aug\mkl\nmr"
CDRH 129
116.7788
80.9068
77.2261
55.6028
49.0689
40.7932
37.3920
35.3442
33.6756
33.3293
29.7141
23.4688
18.7223
17.3170
[rel]
10
0
- 10
- 20
120
100
80
60
40
20
0
[ppm]

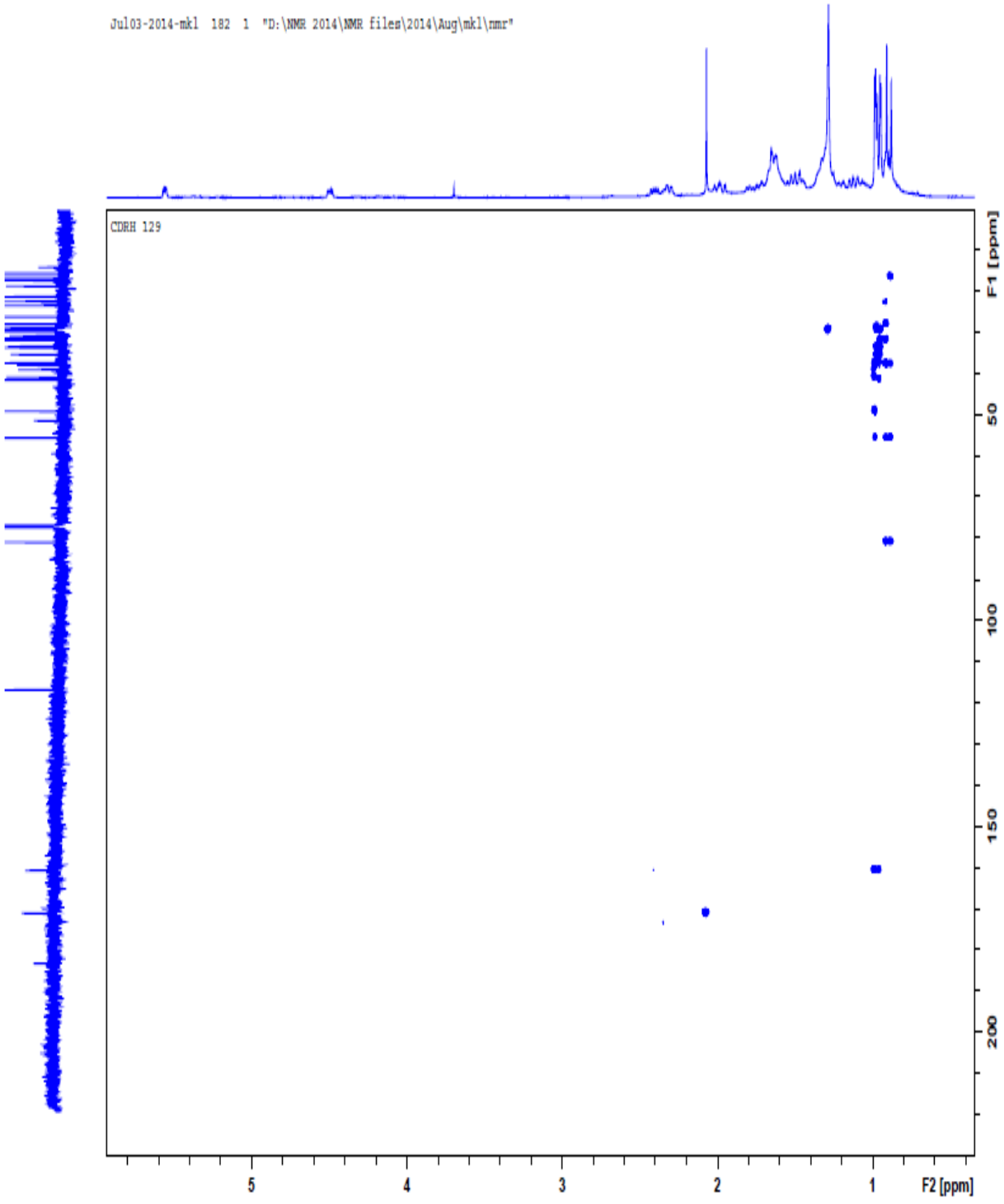
**Appendix 13: COSY spectrum of Acetyl aleuritolic acid (49)**



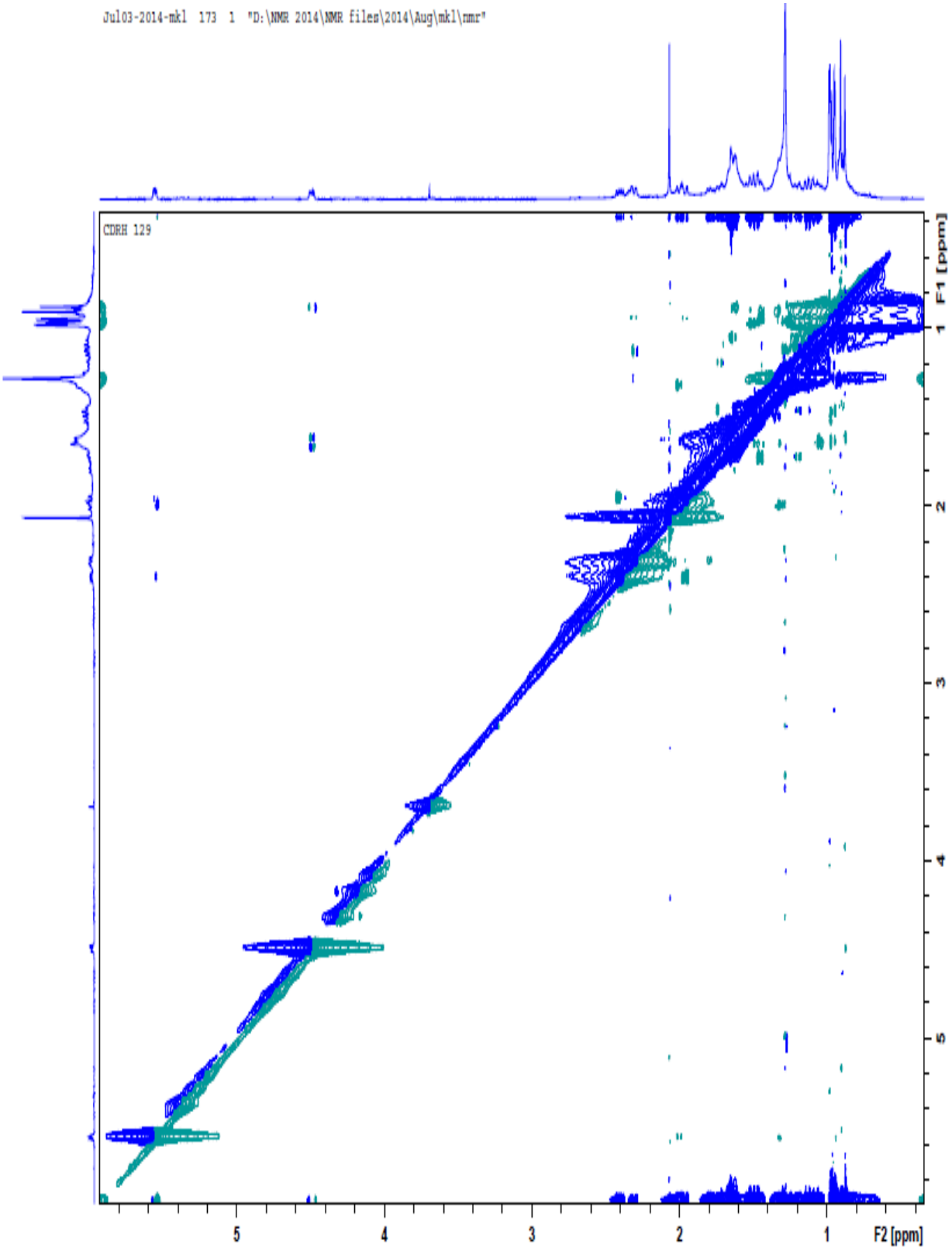
**Appendix 14: HSQC-DEPT spectrum for Acetyl aleuritolic acid (49)**



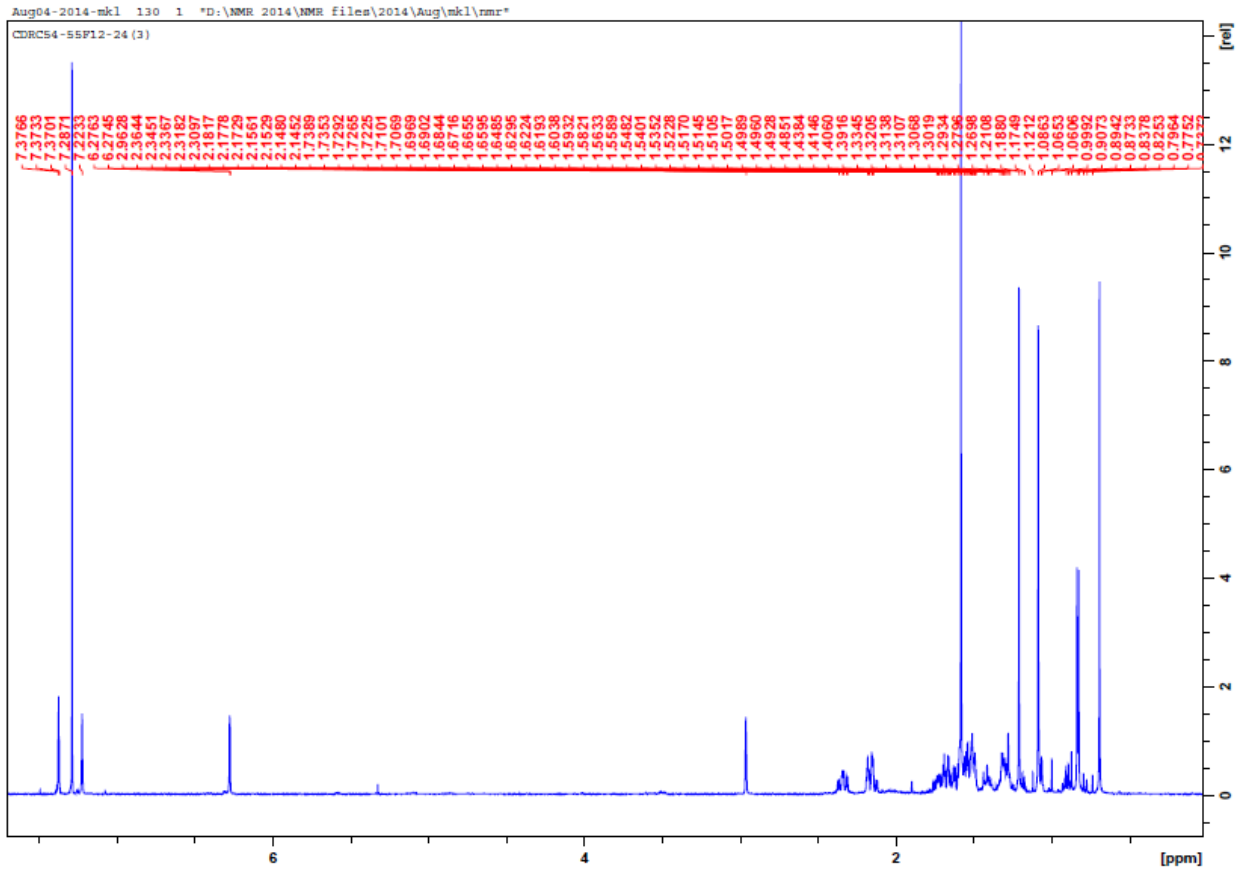
**Appendix 15: HMBC spectrum for Acetyl aleuritolic acid (49)**



**Appendix 16: NOESY spectrum for Acetyl aleuritolic acid (49)**




**Appendix 17: $^{1}$H NMR compound 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)**


Aug04-2014-mkl 130 1 "D:\NMR 2014\NMR files\2014\Aug\mkl\nmr"
CDRC54-55F12-24(3)
7.3766
7.3733
7.3701
7.2871
7.2763
6.2745
2.9628
2.3644
2.3451
2.3367
2.3182
2.3097
2.1817
2.1778
2.1729
2.1561
2.1529
2.1480
2.1452
1.7389
1.7353
1.7292
1.7265
1.7225
1.7101
1.7069
1.6969
1.6902
1.6844
1.6716
1.6655
1.6595
1.6485
1.6295
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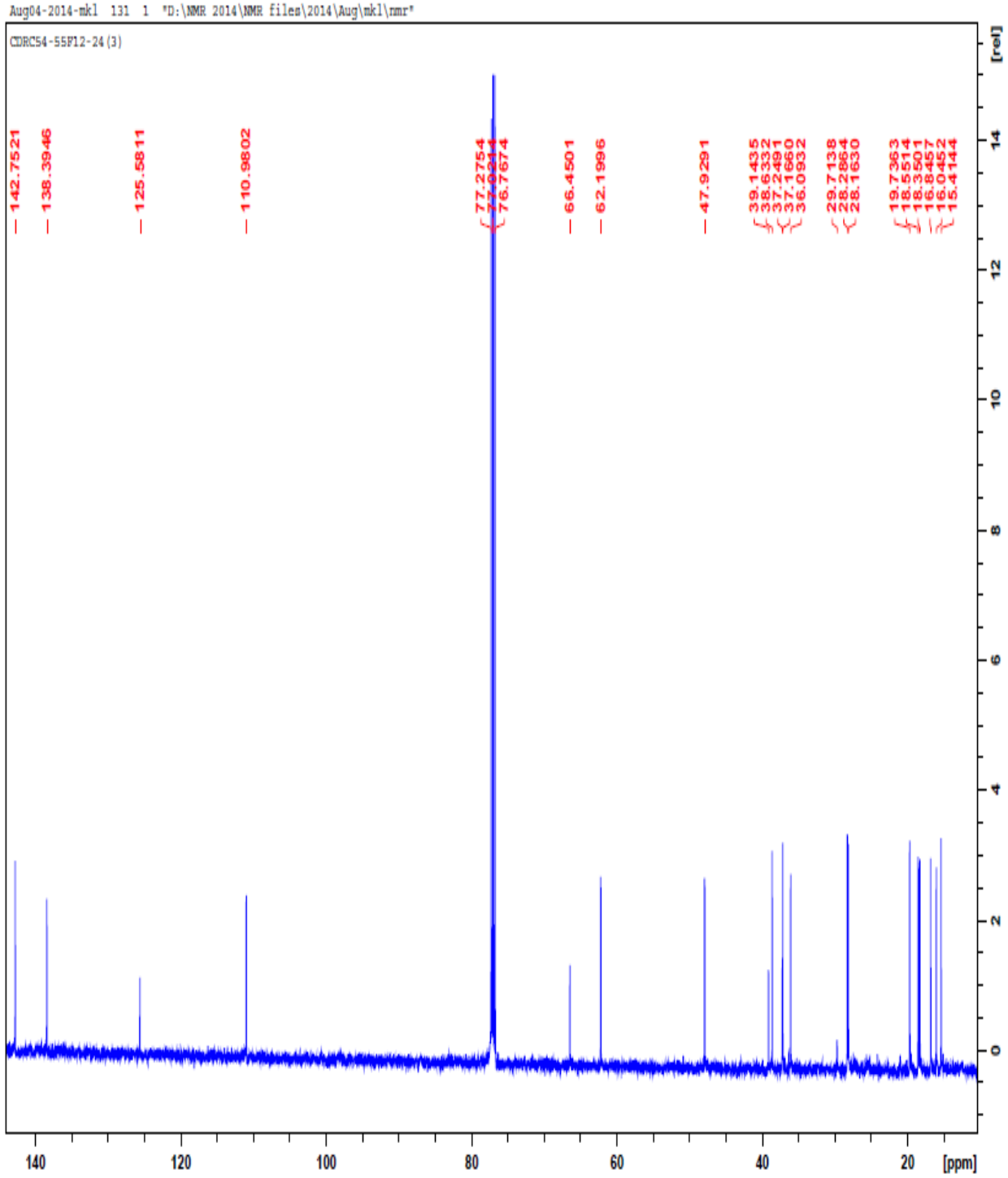
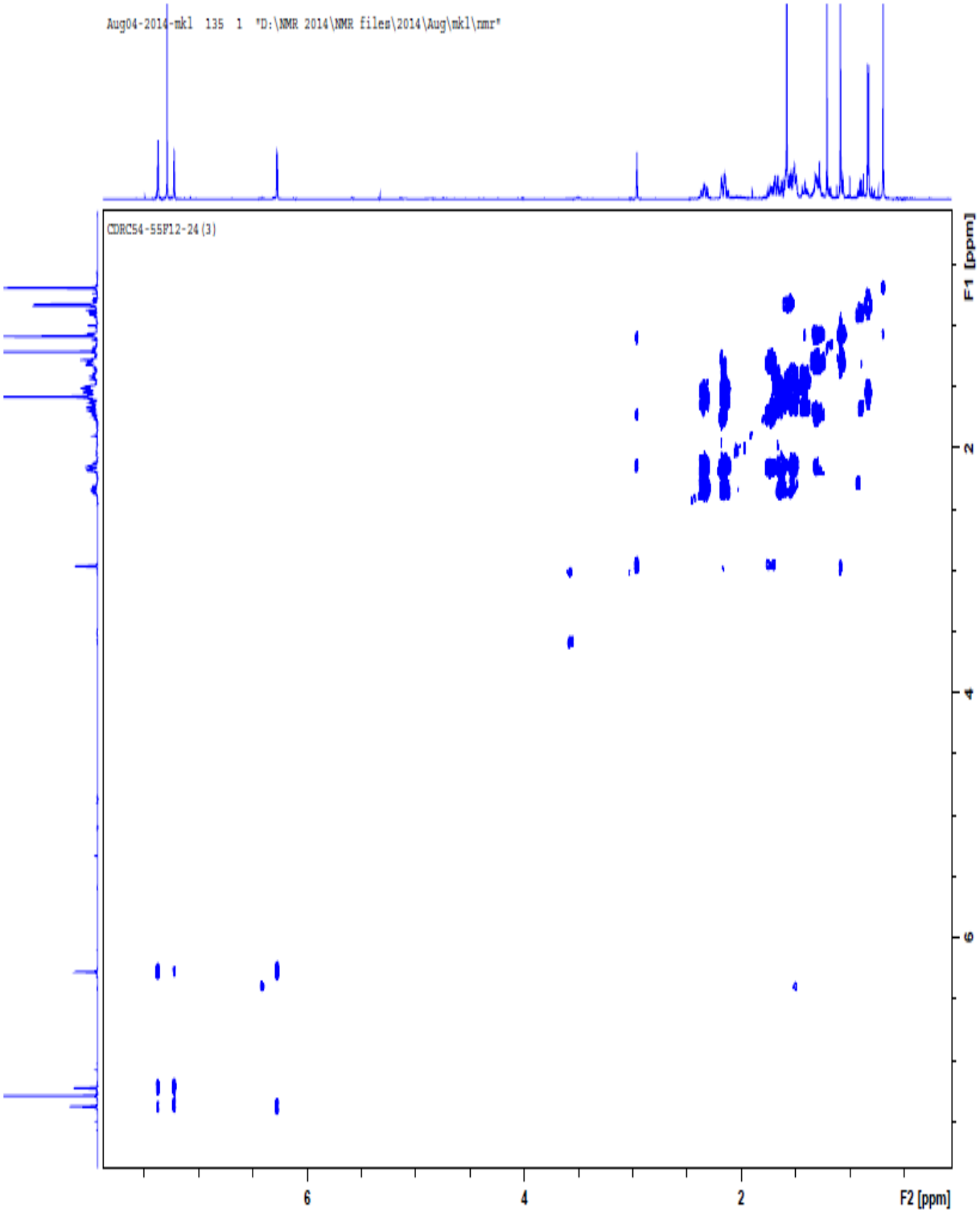
1.6193
1.6038
1.5932
1.5821
1.5633
1.5589
1.5482
1.5401
1.5352
1.5228
1.5170
1.5145
1.5105
1.5017
1.4989
1.4960
1.4928
1.4851
1.4384
1.4146
1.4060
1.3916
1.3345
1.3205
1.3138
1.3107
1.3068
1.3019
1.2934
1.2796
1.2698
1.2108
1.1880
1.1749
1.1212
1.0863
1.0653
1.0606
0.9992
0.9073
0.8942
0.8733
0.8378
0.8253
0.7964
0.7752
[rel]
12
10
8
6
4
2
0
6
4
2
[ppm]

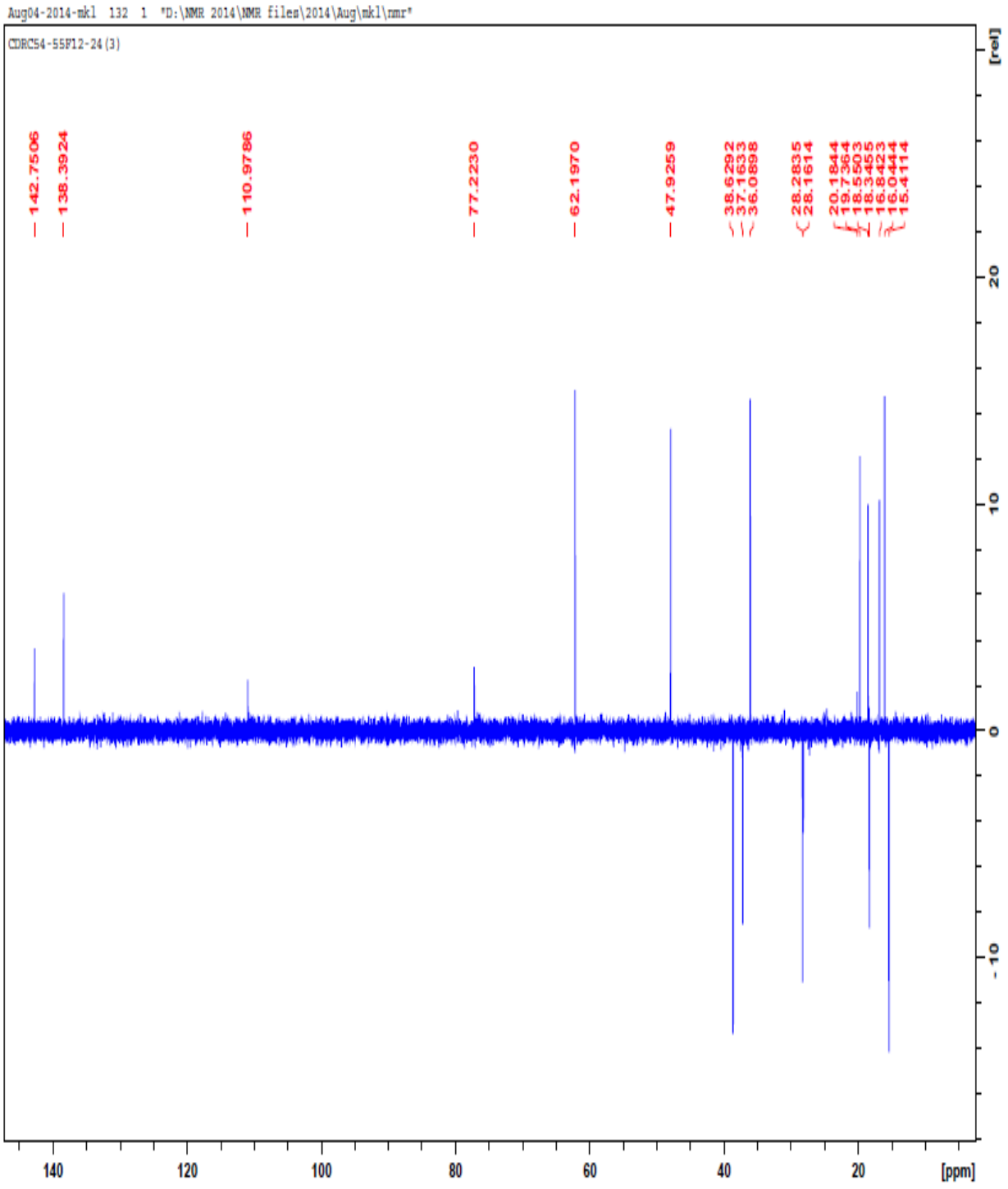
**Appendix 18: $^{13}$C NMR spectrum of 3β,4β:15,16-diepoxy-13(16),14-ent-clerodadiene (50)**



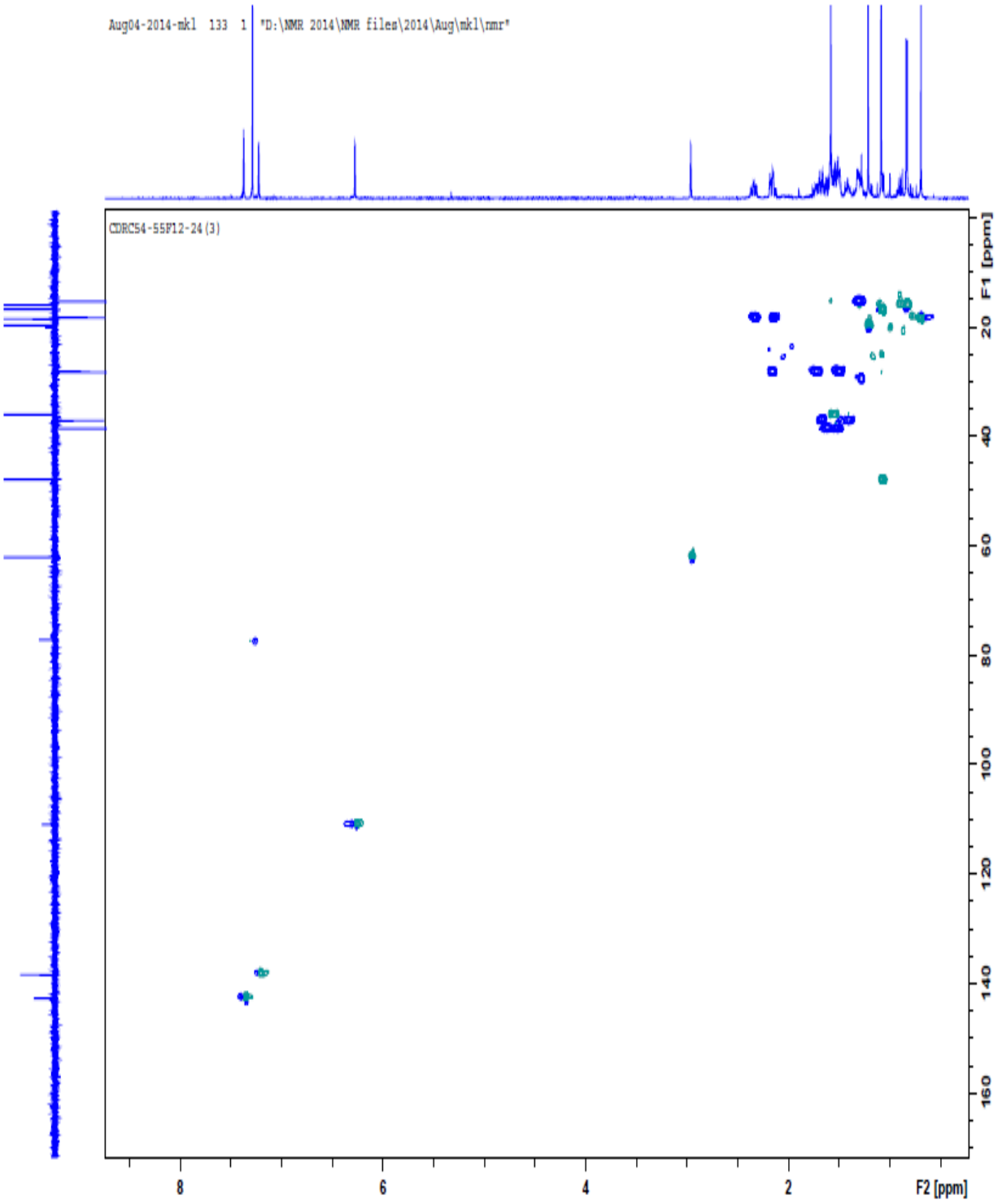
**Appendix 19: Cosy spectrum of 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)**



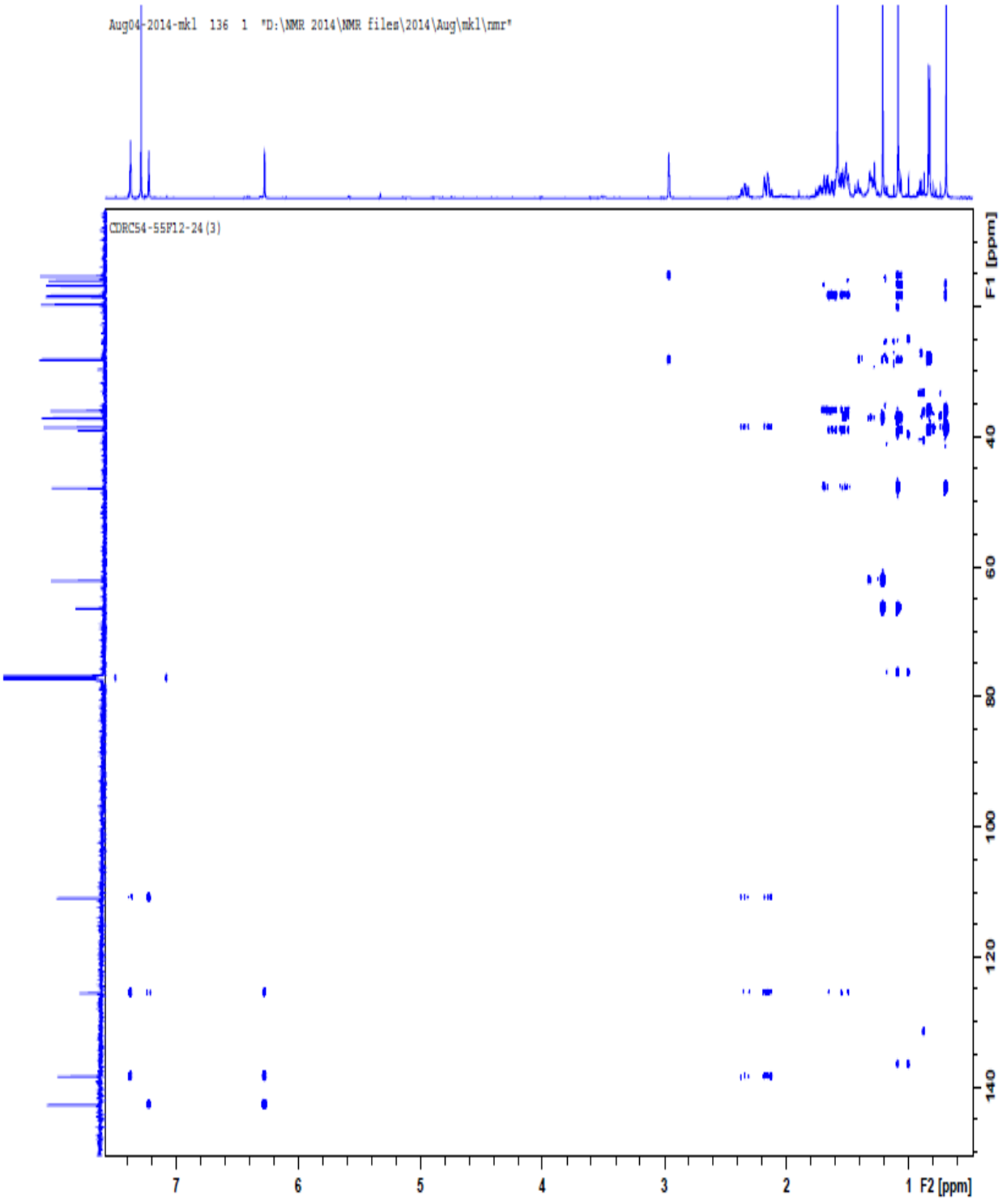
**Appendix 20: DEPT Spectrum of 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)**



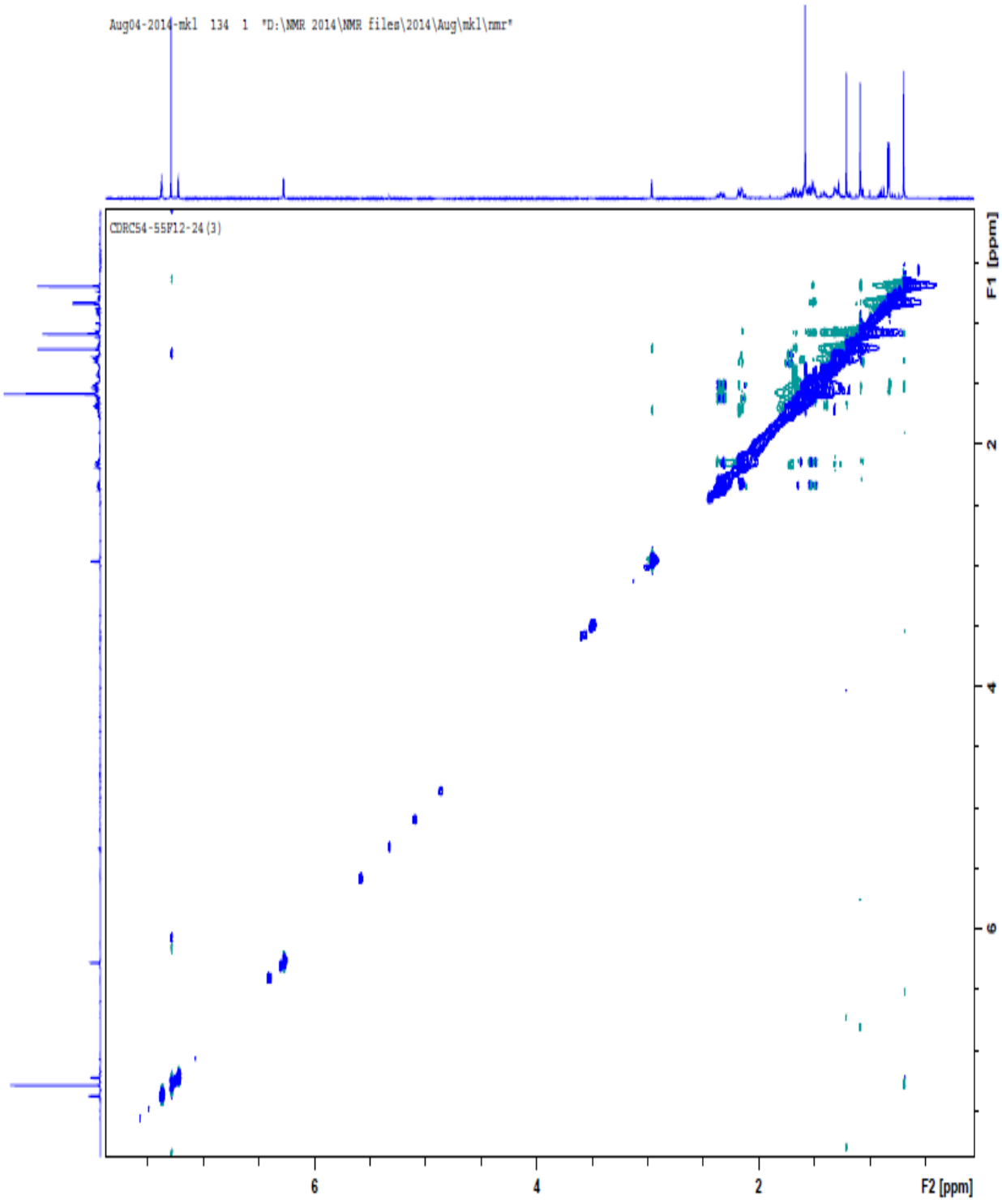
**Appendix 21: HSQC spectrum of 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)**



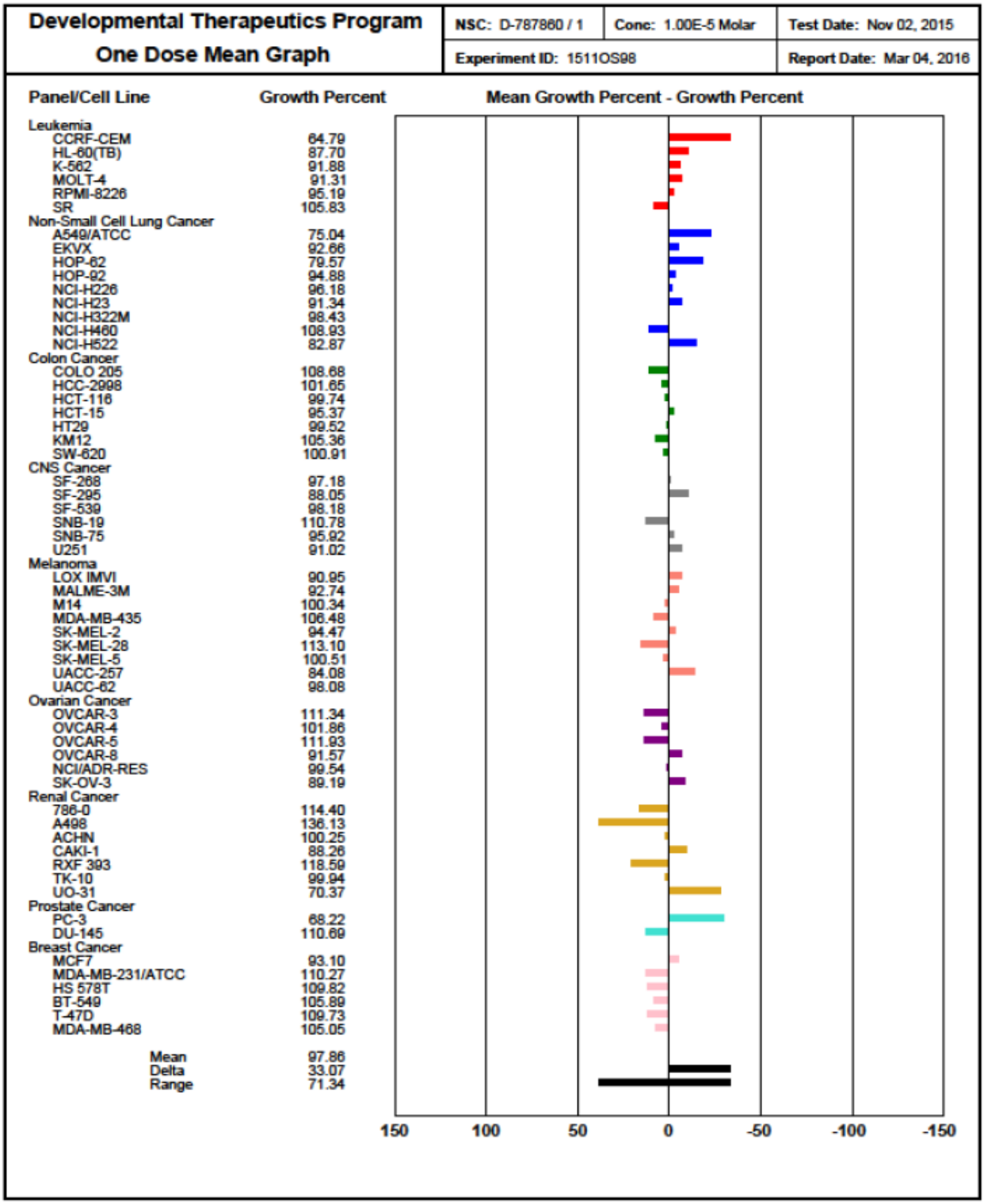
**Appendix 22: HMBC spectrum of 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)**



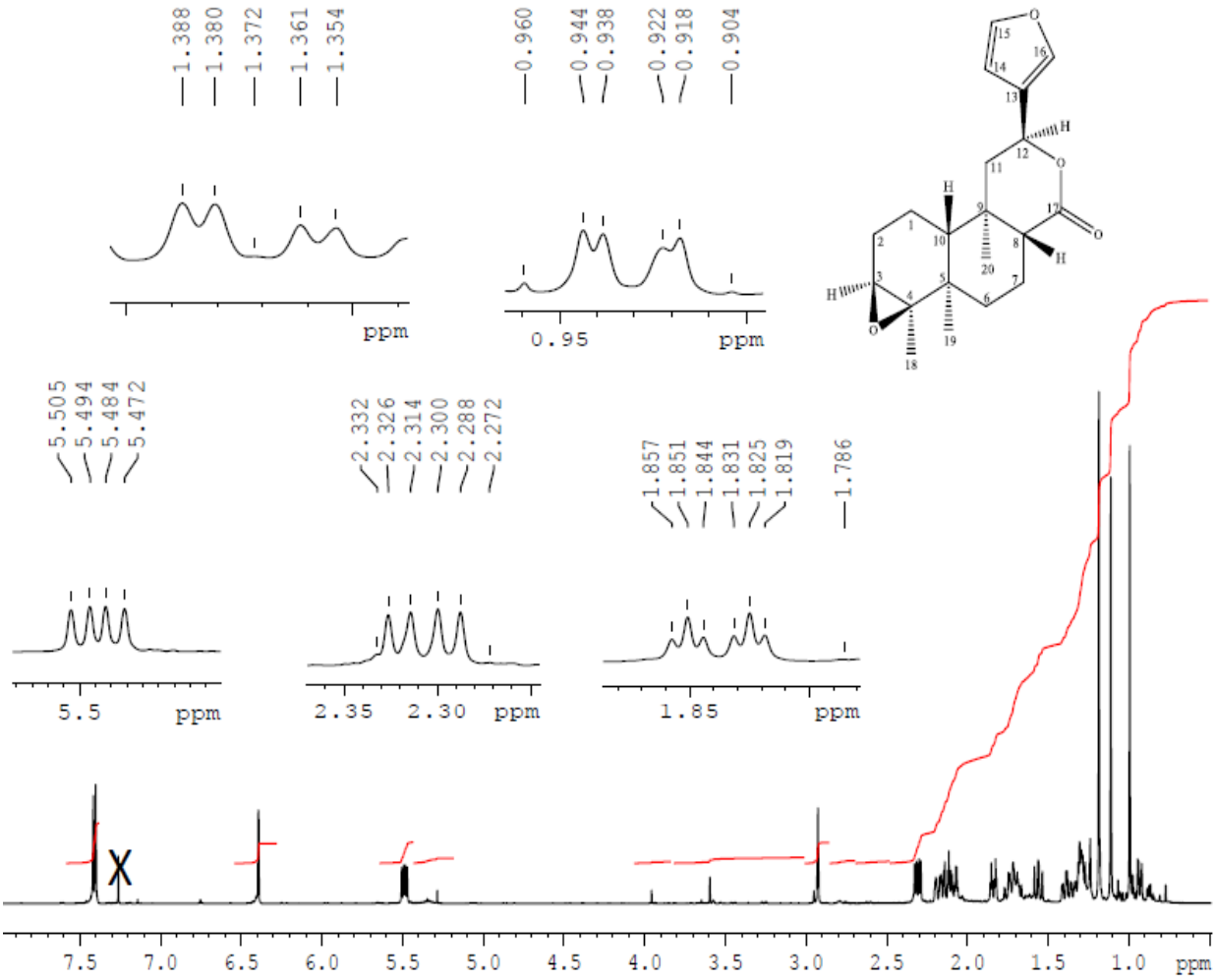
**Appendix 23: NOESY spectrum of 3β,4β:15,16-diepoxy-13(16),14-*ent*-clerodadiene (50)**



**Appendix 24: One dose mean graph for acetyl aleuritolic acid (49).**

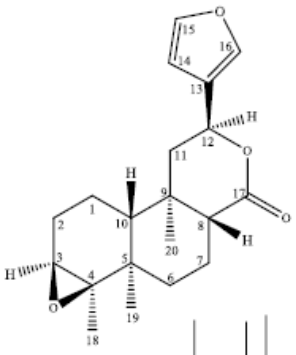


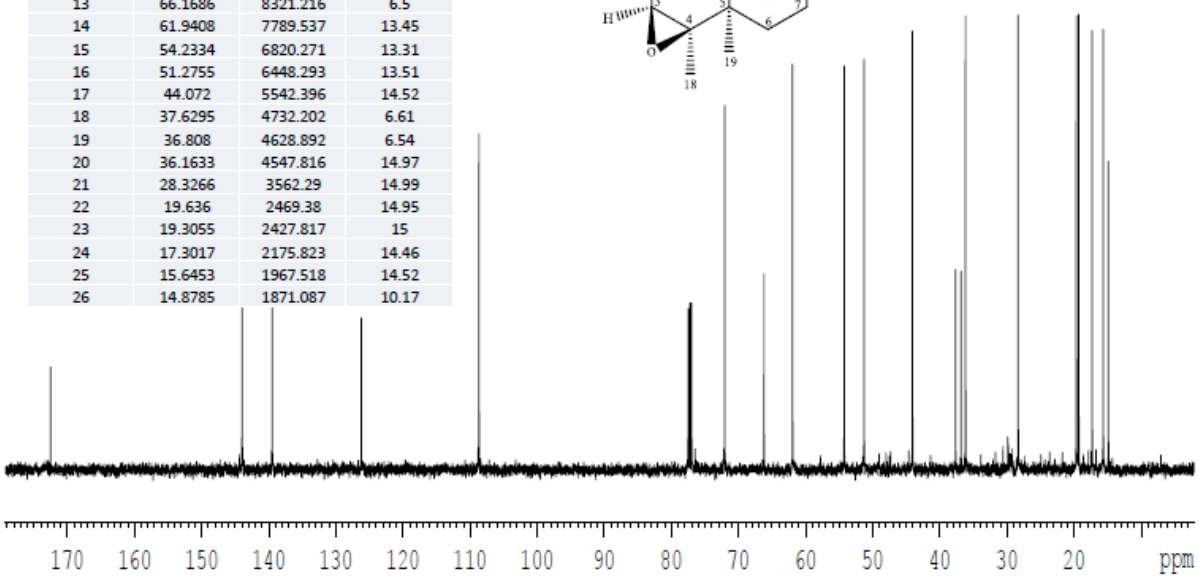
**Appendix 25: $^{1}$H NMR spectrum for compound 51**



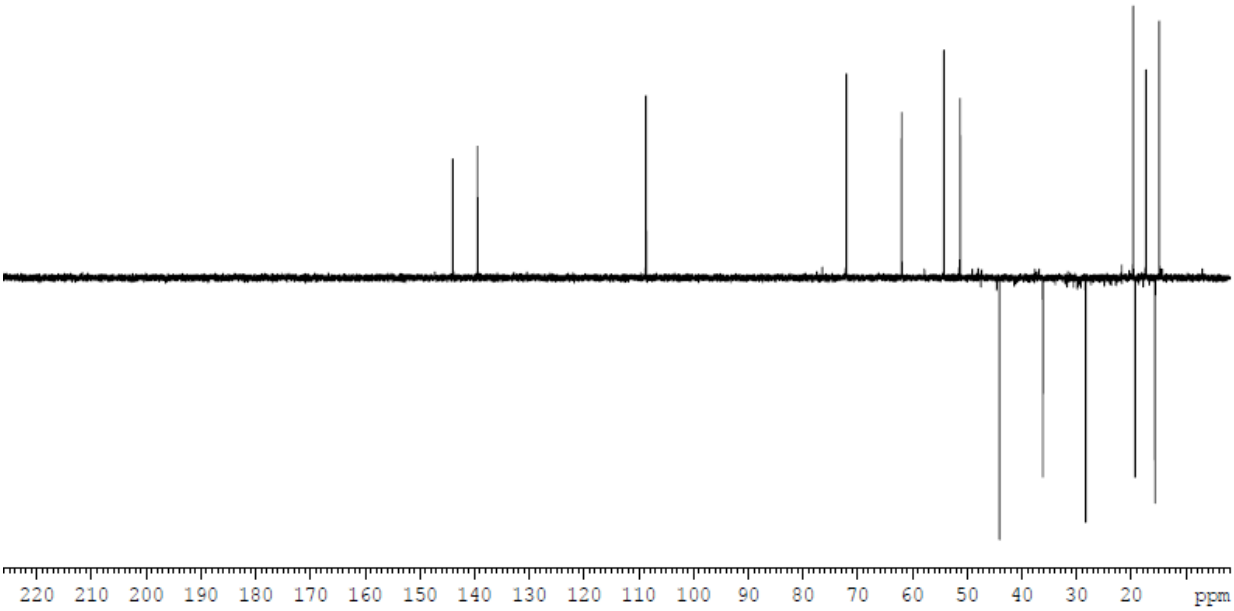
**APPENDIX 26: $^{13}$C NMR spectrum for compound 51**

| Peak | v(F1) [ppm] | v(F1) [Hz] | Intensity [rel] |
|---|---|---|---|
| 1 | 172.4958 | 21692.69 | 3.4 |
| 2 | 143.9745 | 18105.91 | 13.51 |
| 3 | 143.8606 | 18091.59 | 0.72 |
| 4 | 139.4697 | 17539.4 | 10.97 |
| 5 | 126.2107 | 15871.98 | 5.01 |
| 6 | 108.7637 | 13677.88 | 0.72 |
| 7 | 77.4843 | 9744.253 | 5.3 |
| 8 | 77.2297 | 9712.235 | 5.48 |
| 9 | 76.9755 | 9680.267 | 5.5 |
| 10 | 76.4596 | 9615.389 | 0.69 |
| 11 | 72.1825 | 9077.51 | 0.69 |
| 12 | 72.043 | 9059.967 | 11.99 |
| 13 | 66.1686 | 8321.216 | 6.5 |
| 14 | 61.9408 | 7789.537 | 13.45 |
| 15 | 54.2334 | 6820.271 | 13.31 |
| 16 | 51.2755 | 6448.293 | 13.51 |
| 17 | 44.072 | 5542.396 | 14.52 |
| 18 | 37.6295 | 4732.202 | 6.61 |
| 19 | 36.808 | 4628.892 | 6.54 |
| 20 | 36.1633 | 4547.816 | 14.97 |
| 21 | 28.3266 | 3562.29 | 14.99 |
| 22 | 19.636 | 2469.38 | 14.95 |
| 23 | 19.3055 | 2427.817 | 15 |
| 24 | 17.3017 | 2175.823 | 14.46 |
| 25 | 15.6453 | 1967.518 | 14.52 |
| 26 | 14.8785 | 1871.087 | 10.17 |

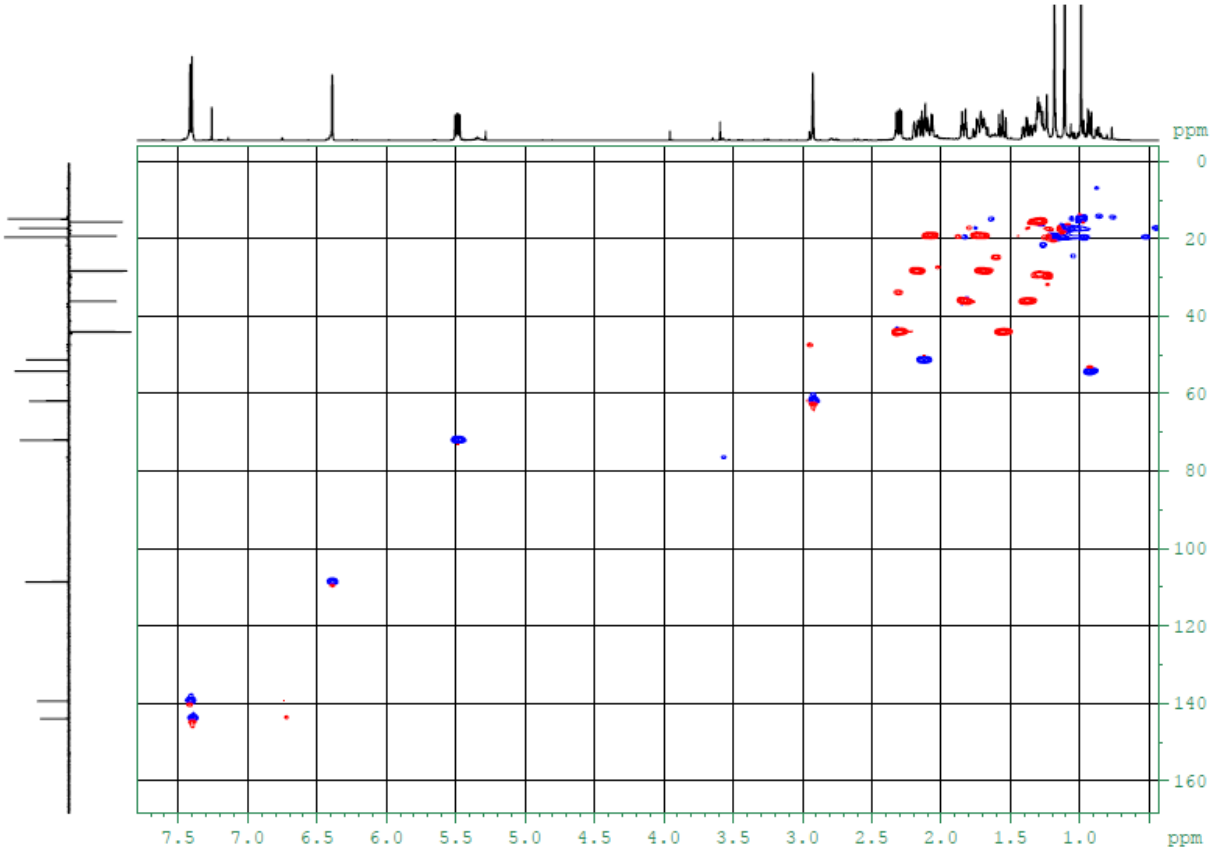




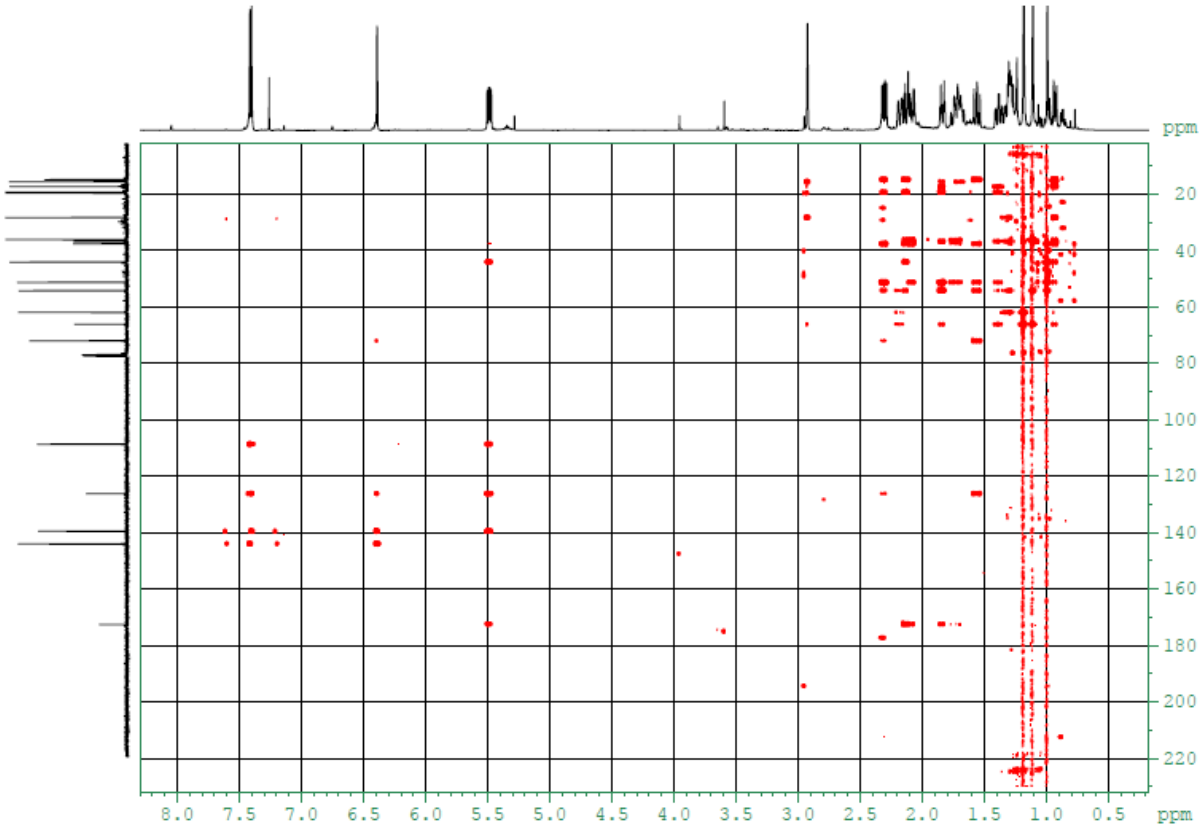
**APPENDIX 27: DEPT spectrum for compound 51 in $CDCl_3$**



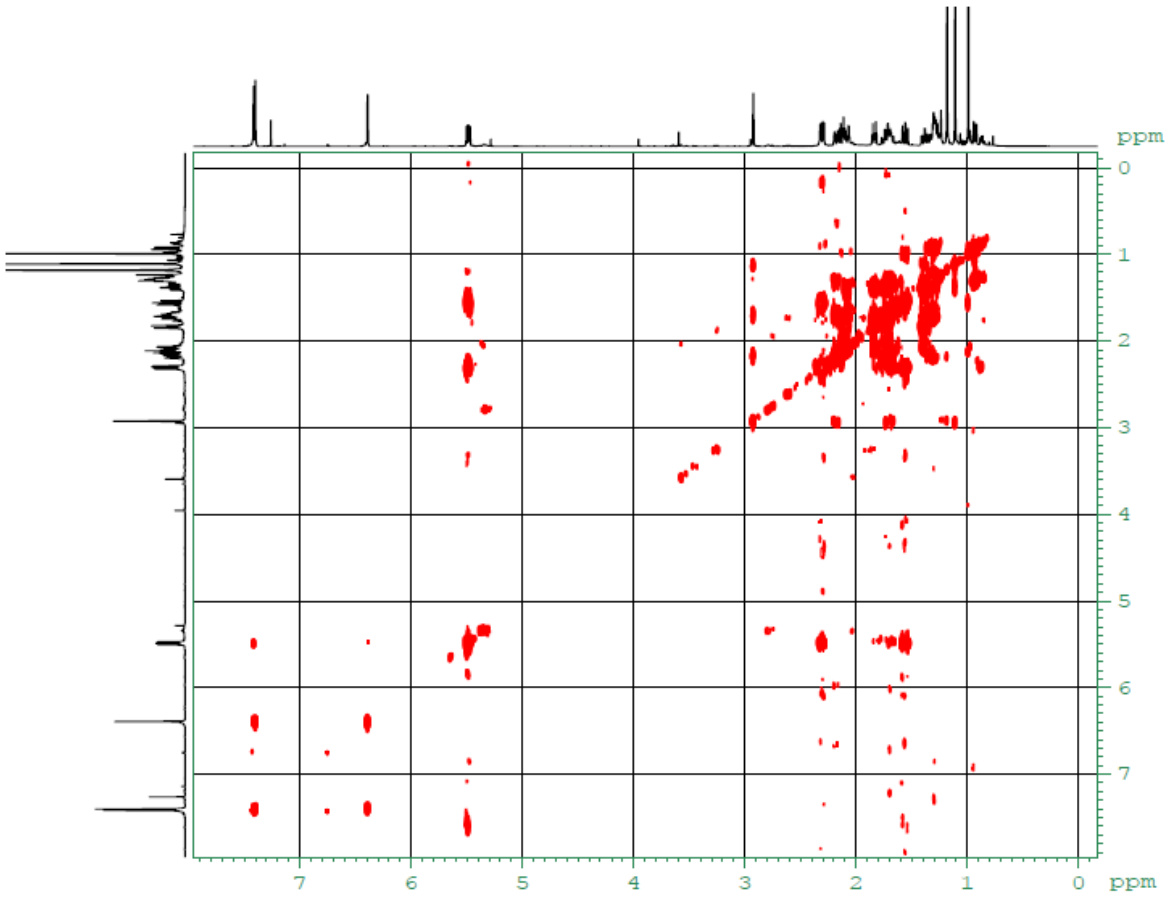
**APPENDIX 28: HSQC-DEPT spectrum for compound 51 in $CDCl_3$**



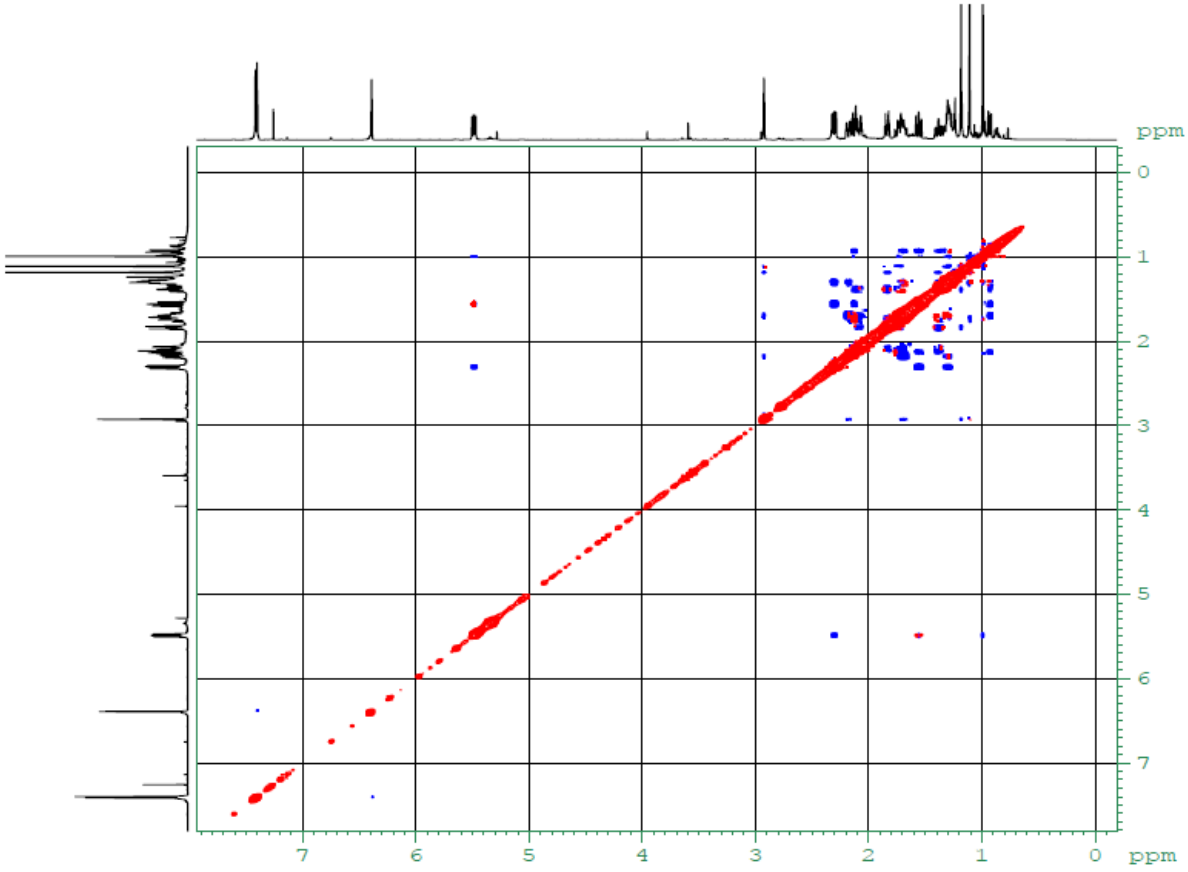
**APPENDIX 29: HMBC spectrum for compound 51 in CDCl3**



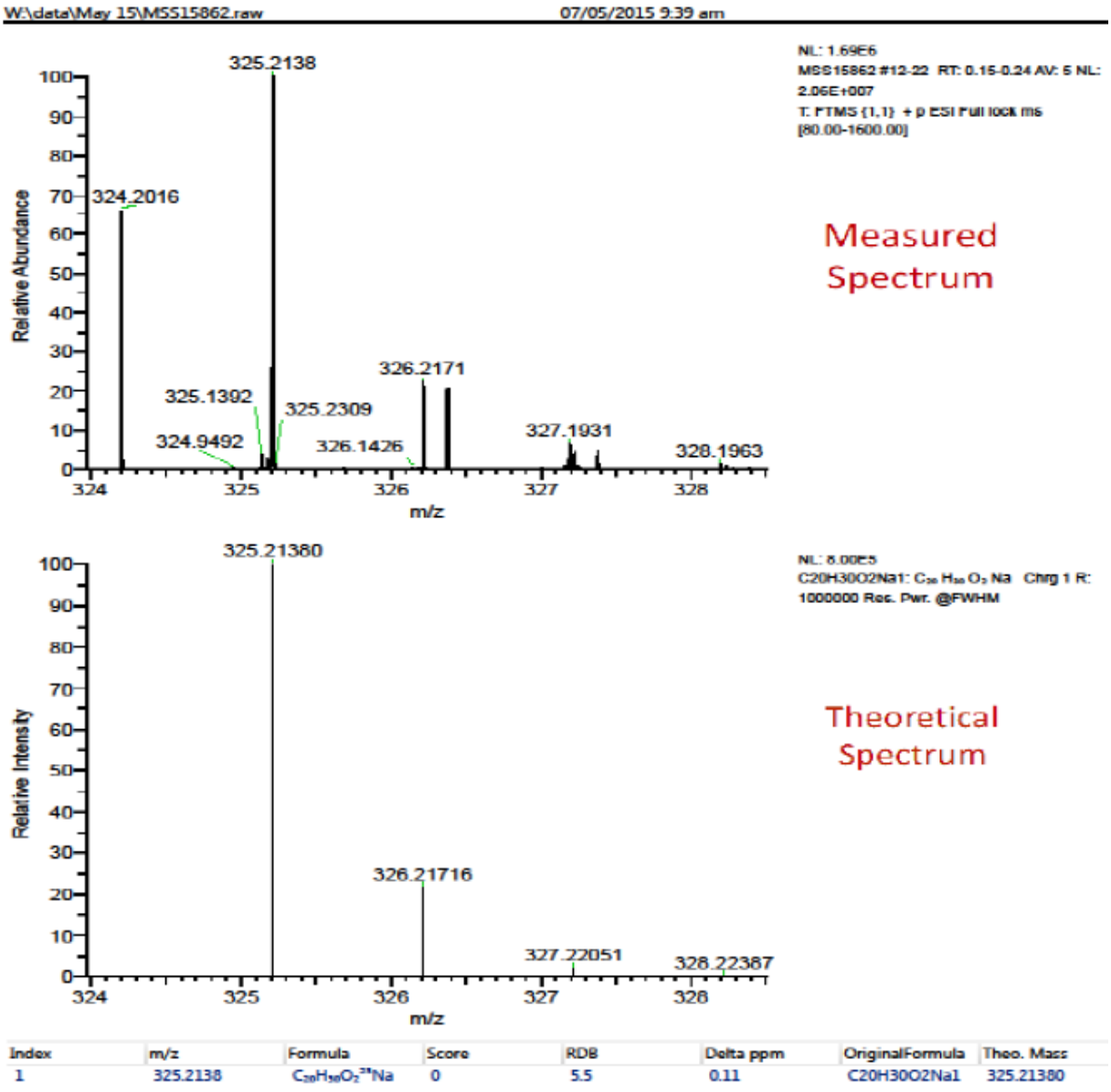
**APPENDIX 30: COSY spectrum for compound 51 in CDCl3**



**APPENDIX 31: NOESY spectrum for compound 51 in CDCl3**

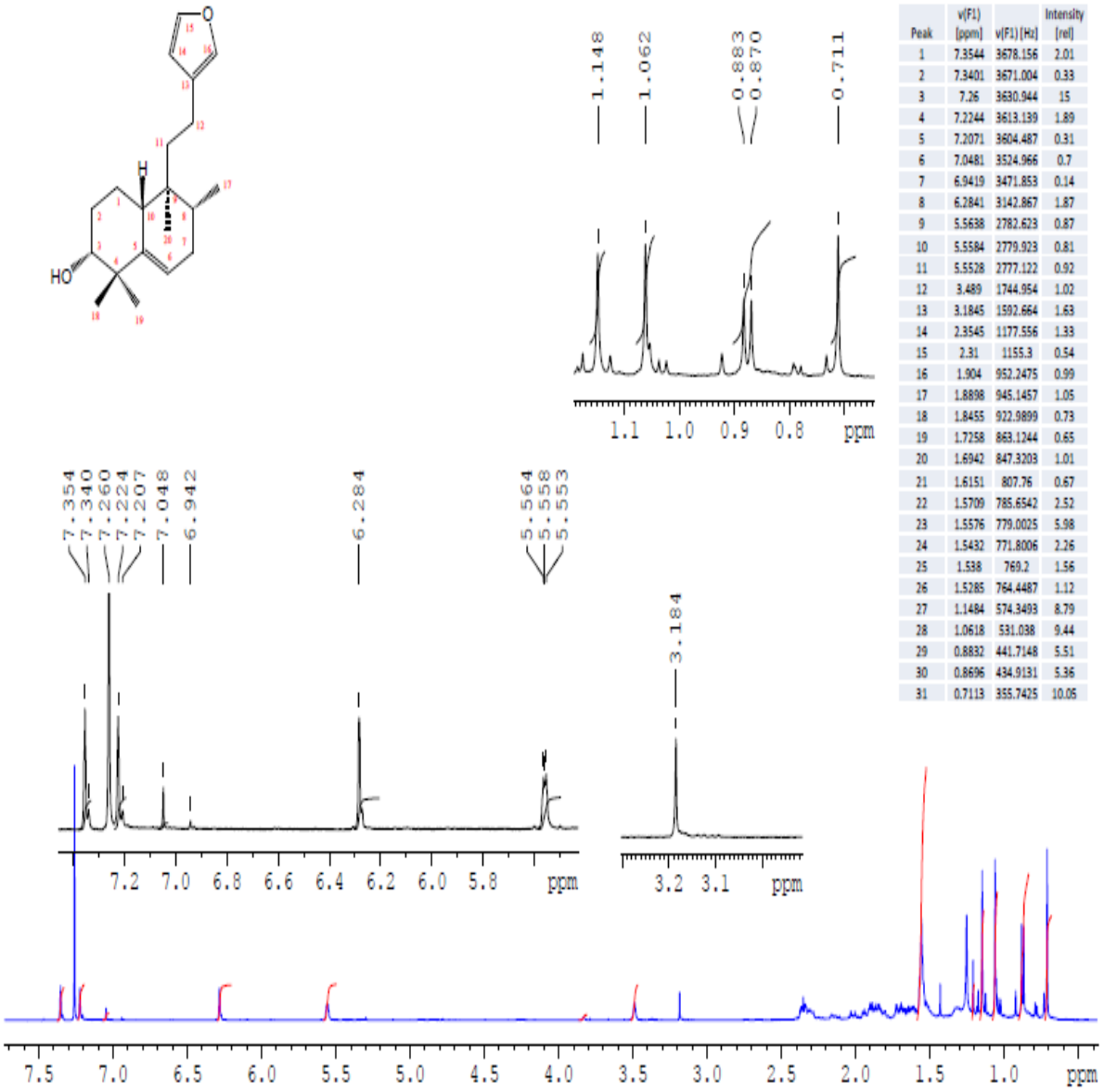


**Appendix 32: Ms spectrum of compound 52**



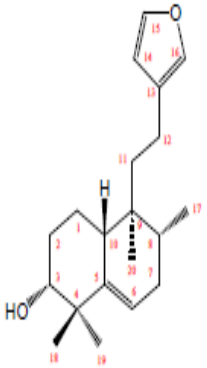

| Index | m/z | Formula | Score | RDB | Delta ppm | OriginalFormula | Theo. Mass |
|---|---|---|---|---|---|---|---|
| 1 | 325.2138 | $C_{20}H_{30}O_2{}^{23}Na$ | 0 | 5.5 | 0.11 | C20H30O2Na1 | 325.21380 |

**Appendix 33: $^{1}$H NMR spectrum of compound 52**

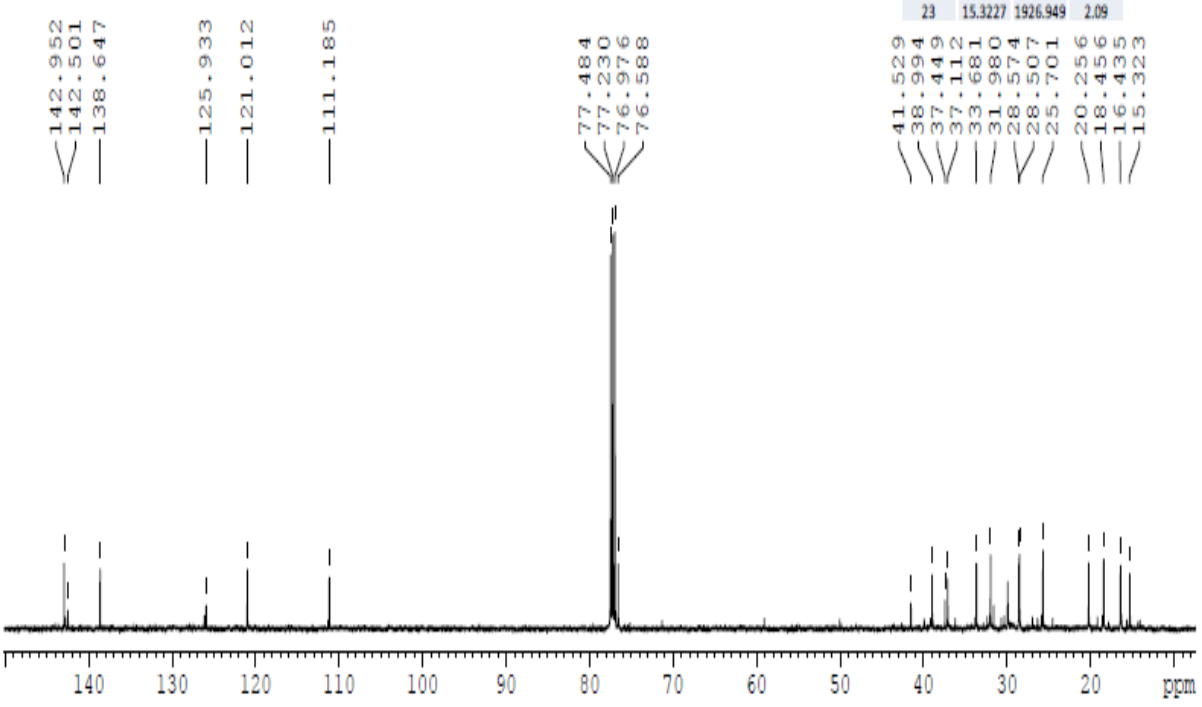



| Peak | v(F1) (ppm) | v(F1) (Hz) | Intensity (rel) |
|---|---|---|---|
| 1 | 7.3544 | 3678.156 | 2.01 |
| 2 | 7.3401 | 3671.004 | 0.33 |
| 3 | 7.26 | 3630.944 | 15 |
| 4 | 7.2244 | 3613.139 | 1.89 |
| 5 | 7.2071 | 3604.487 | 0.31 |
| 6 | 7.0481 | 3524.966 | 0.7 |
| 7 | 6.9419 | 3471.853 | 0.14 |
| 8 | 6.2841 | 3142.867 | 1.87 |
| 9 | 5.5638 | 2782.623 | 0.87 |
| 10 | 5.5584 | 2779.923 | 0.81 |
| 11 | 5.5528 | 2777.122 | 0.92 |
| 12 | 3.489 | 1744.954 | 1.02 |
| 13 | 3.1845 | 1592.664 | 1.63 |
| 14 | 2.3545 | 1177.556 | 1.33 |
| 15 | 2.31 | 1155.3 | 0.54 |
| 16 | 1.904 | 952.2475 | 0.99 |
| 17 | 1.8898 | 945.1457 | 1.05 |
| 18 | 1.8455 | 922.9899 | 0.73 |
| 19 | 1.7258 | 863.1244 | 0.65 |
| 20 | 1.6942 | 847.3203 | 1.01 |
| 21 | 1.6151 | 807.76 | 0.67 |
| 22 | 1.5709 | 785.6542 | 2.52 |
| 23 | 1.5576 | 779.0025 | 5.98 |
| 24 | 1.5432 | 771.8006 | 2.26 |
| 25 | 1.538 | 769.2 | 1.56 |
| 26 | 1.5285 | 764.4487 | 1.12 |
| 27 | 1.1484 | 574.3493 | 8.79 |
| 28 | 1.0618 | 531.038 | 9.44 |
| 29 | 0.8832 | 441.7148 | 5.51 |
| 30 | 0.8696 | 434.9131 | 5.36 |
| 31 | 0.7113 | 355.7425 | 10.05 |

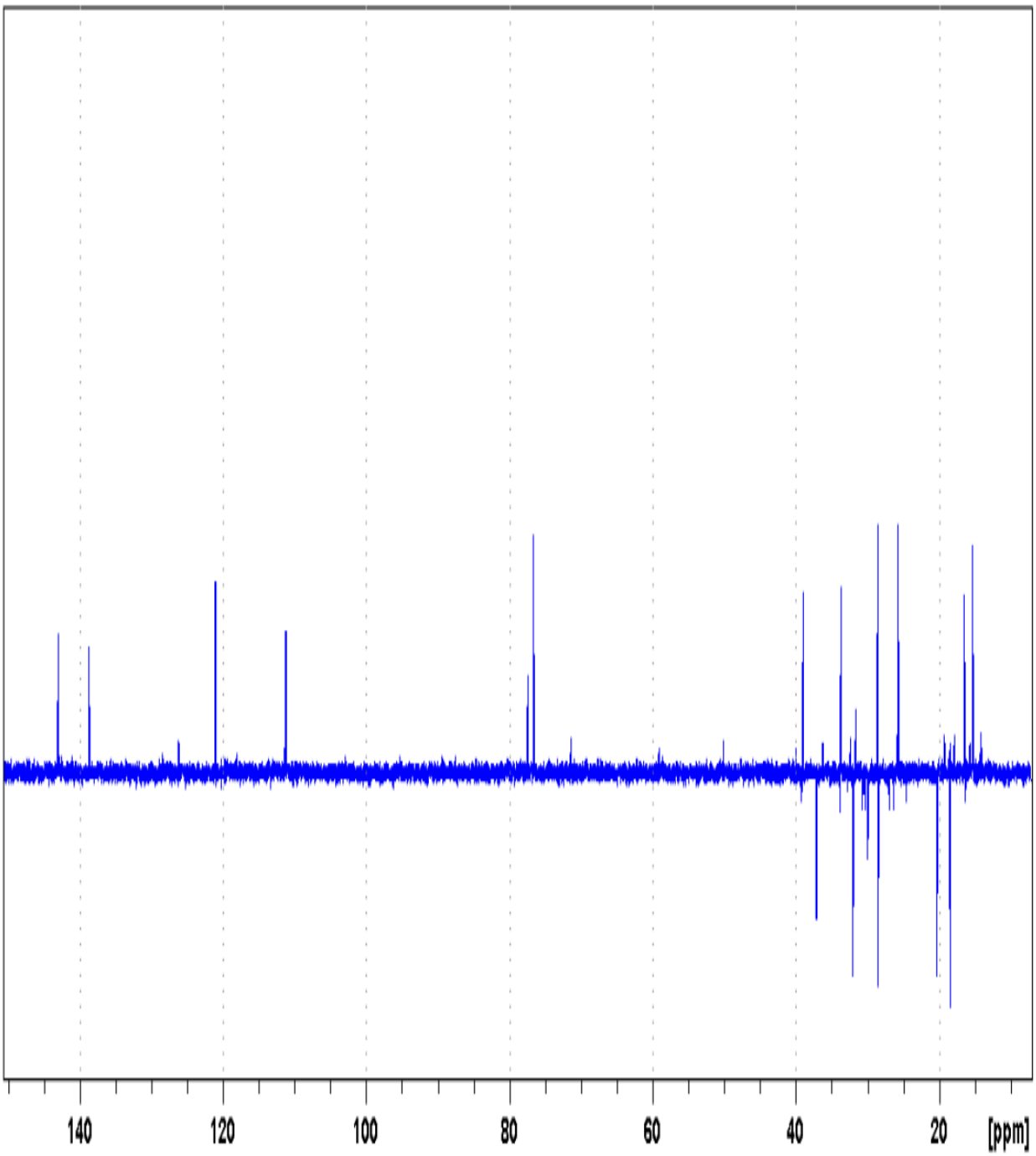
**Appendix 34: $^{13}$C NMR spectrum of compound 52**



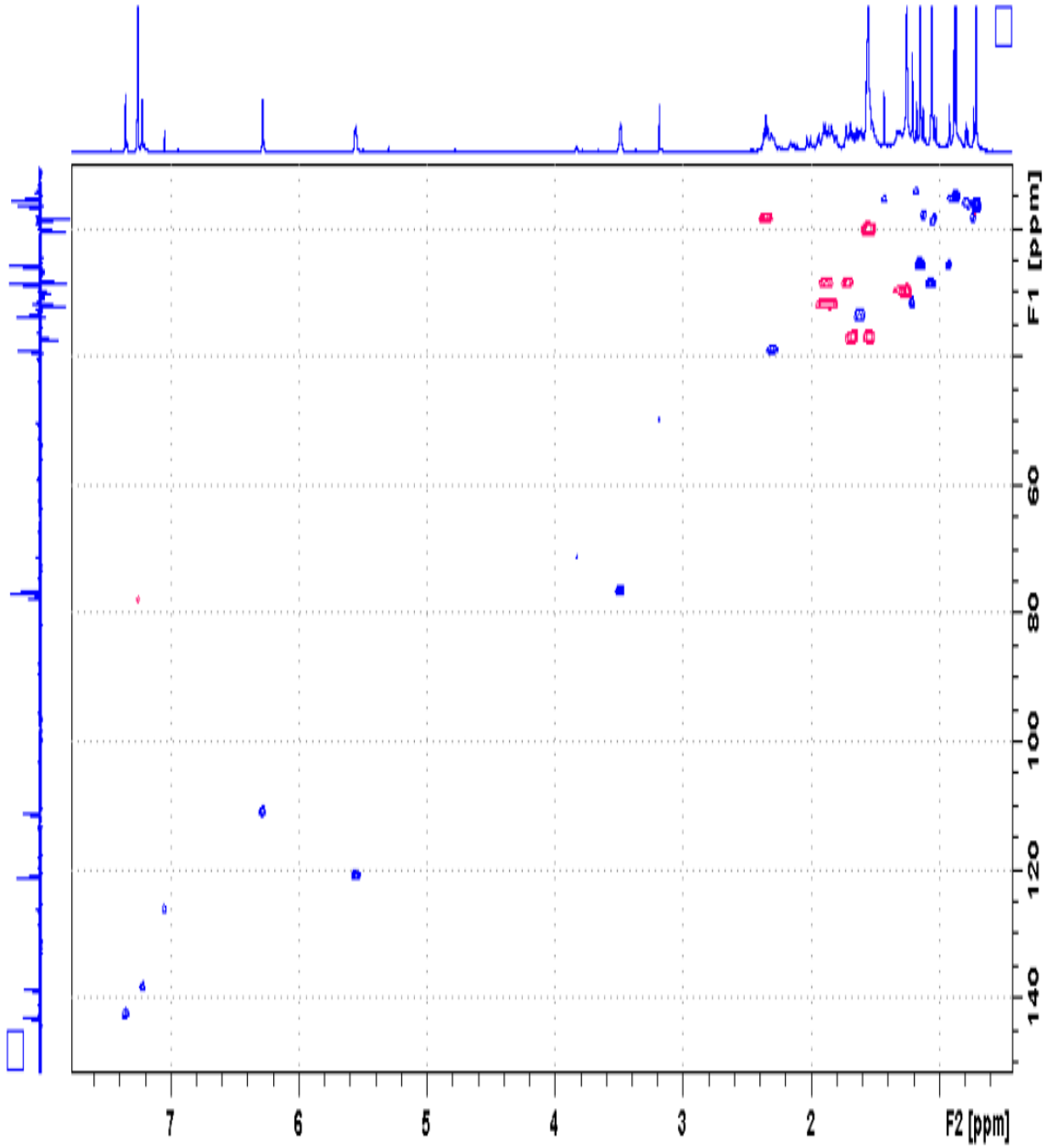

| Peak | v(F1) [ppm] | v(F1) [Hz] | Intensity [rel] |
|---|---|---|---|
| 1 | 142.9522 | 17977.35 | 2.46 |
| 2 | 142.501 | 17920.61 | 0.7 |
| 3 | 138.6474 | 17435.99 | 2.23 |
| 4 | 125.9325 | 15836.99 | 0.89 |
| 5 | 121.0121 | 15218.21 | 2.23 |
| 6 | 111.1854 | 13982.43 | 1.95 |
| 7 | 77.4843 | 9744.252 | 14.15 |
| 8 | 77.2304 | 9712.322 | 14.88 |
| 9 | 76.9763 | 9680.367 | 15 |
| 10 | 76.5883 | 9631.573 | 2.43 |
| 11 | 41.5286 | 5222.544 | 0.96 |
| 12 | 38.9944 | 4903.849 | 2.05 |
| 13 | 37.4494 | 4709.553 | 1.08 |
| 14 | 37.1118 | 4667.097 | 1.88 |
| 15 | 33.6809 | 4235.635 | 2.46 |
| 16 | 31.9796 | 4021.683 | 2.78 |
| 17 | 28.5739 | 3593.39 | 2.66 |
| 18 | 28.5073 | 3585.014 | 2.8 |
| 19 | 25.701 | 3232.1 | 2.96 |
| 20 | 20.2558 | 2547.324 | 2.49 |
| 21 | 18.456 | 2320.985 | 2.62 |
| 22 | 16.4353 | 2066.867 | 2.38 |
| 23 | 15.3227 | 1926.949 | 2.09 |



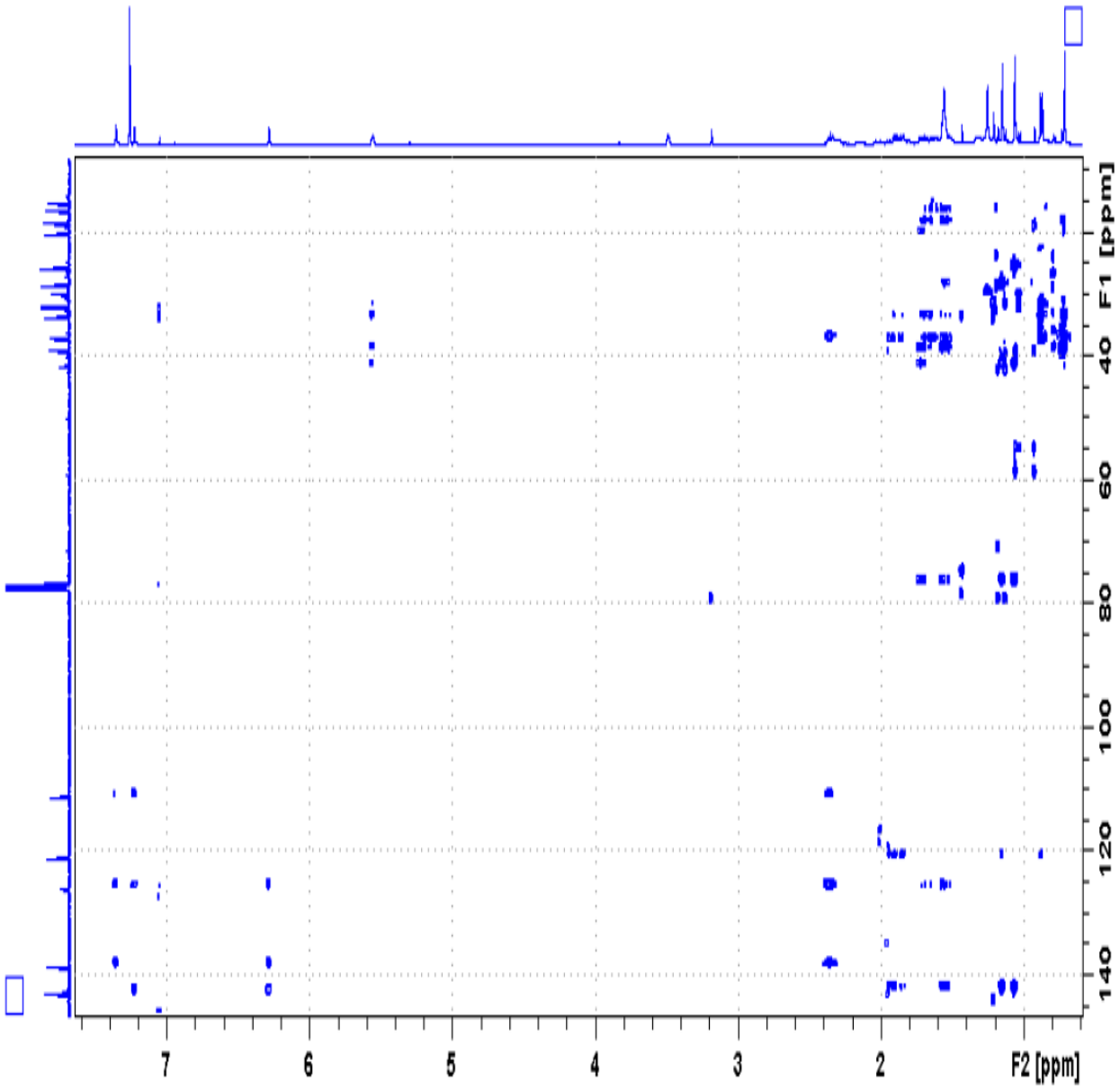
**Appendix 35: DEPT NMR spectrum of compound 52**



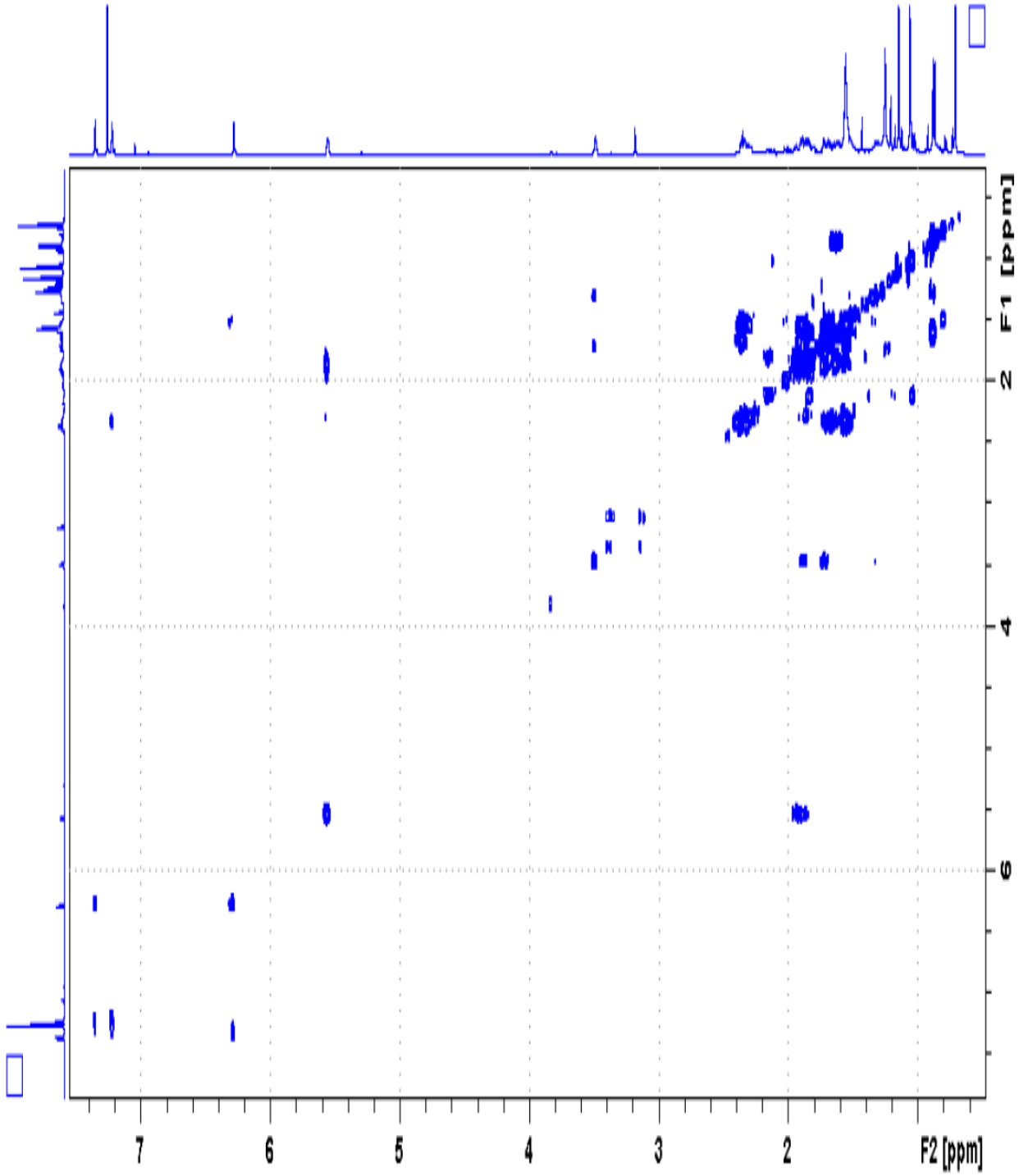
**Appendix 36: HSQC DEPT NMR spectrum of compound 52**



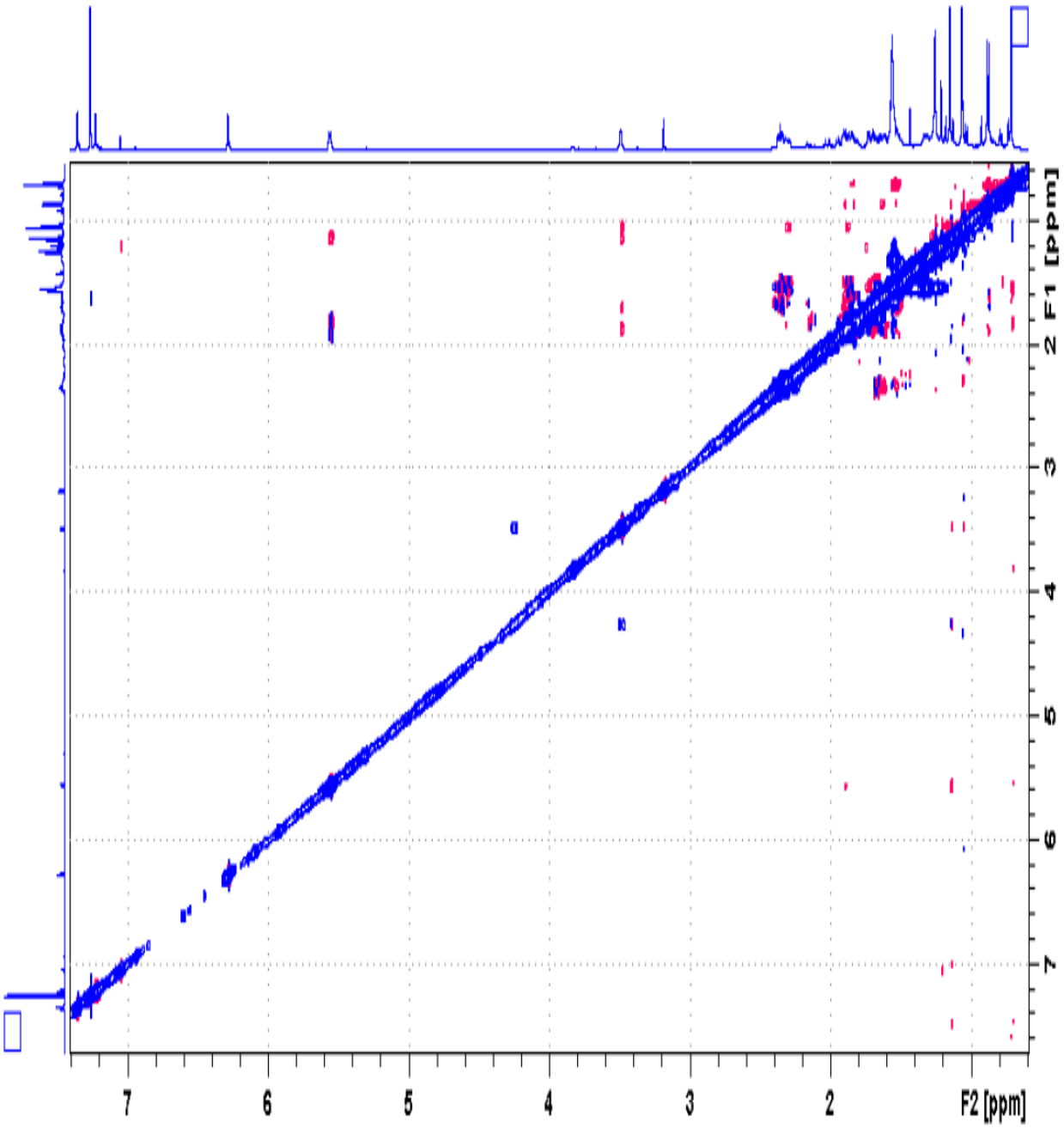
**Appendix 37: HMBC spectrum of compound 52**



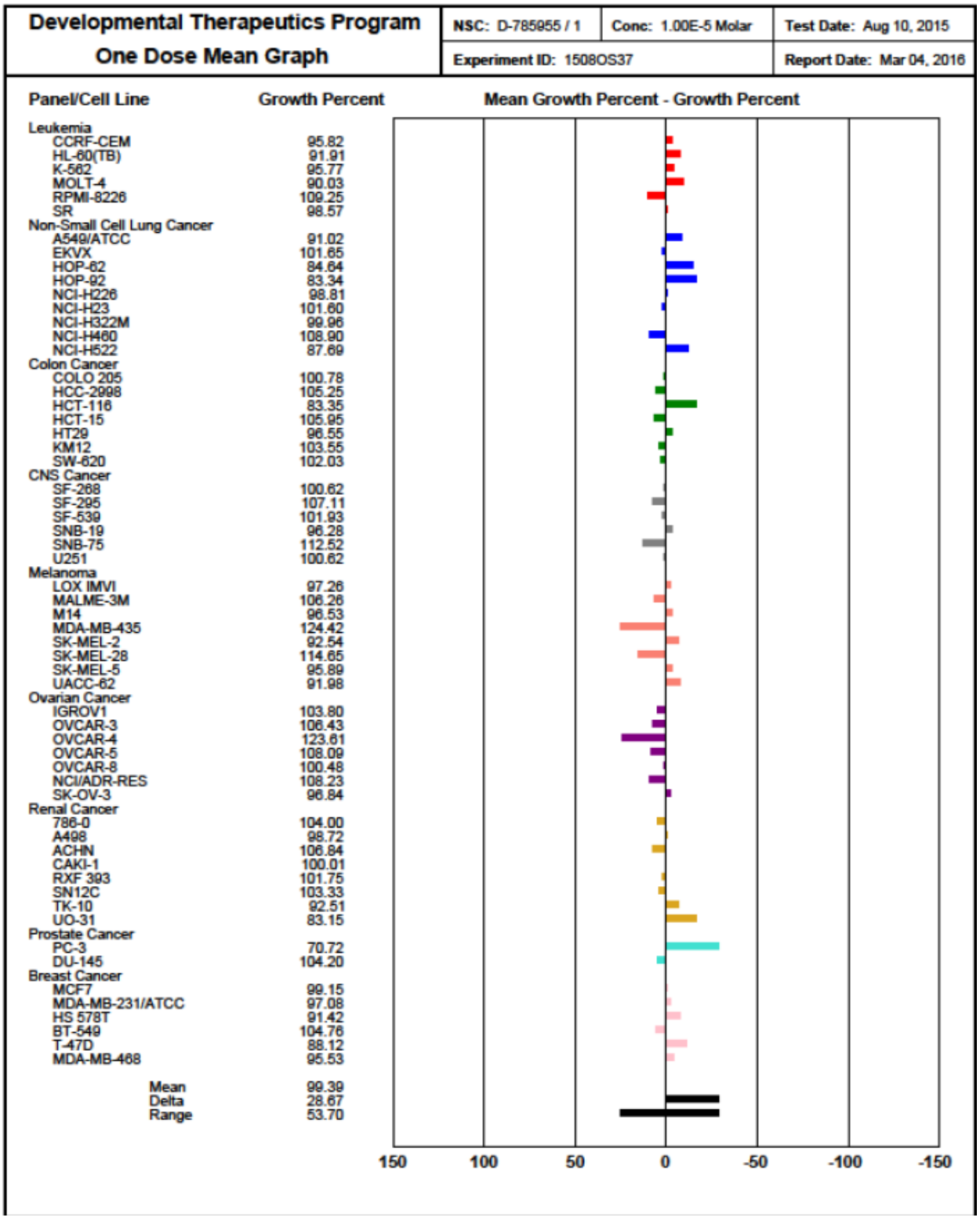
**Appendix 38: COSY spectrum of compound 52**



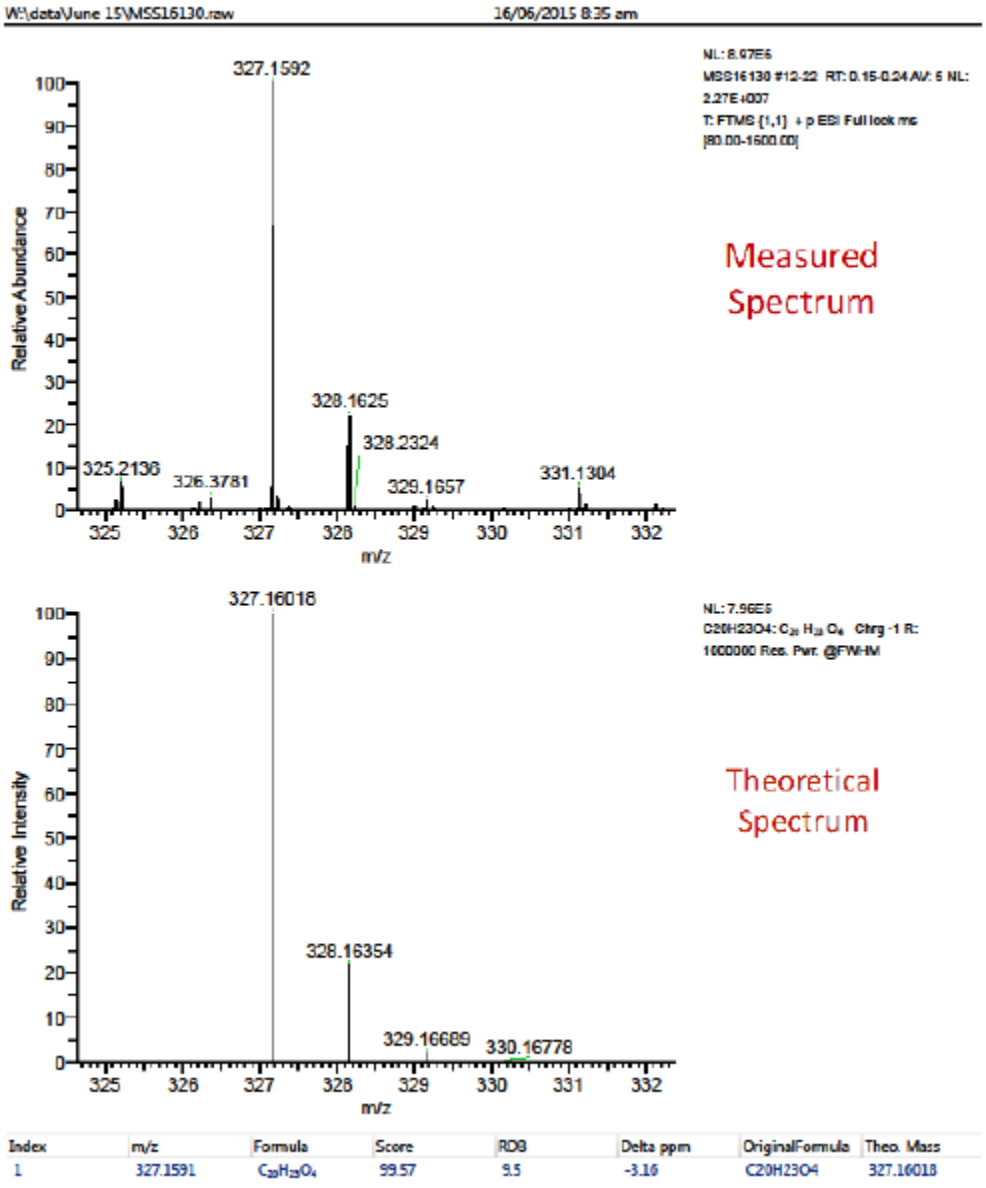
**Appendix 39: NOESY spectrum of compound 52**



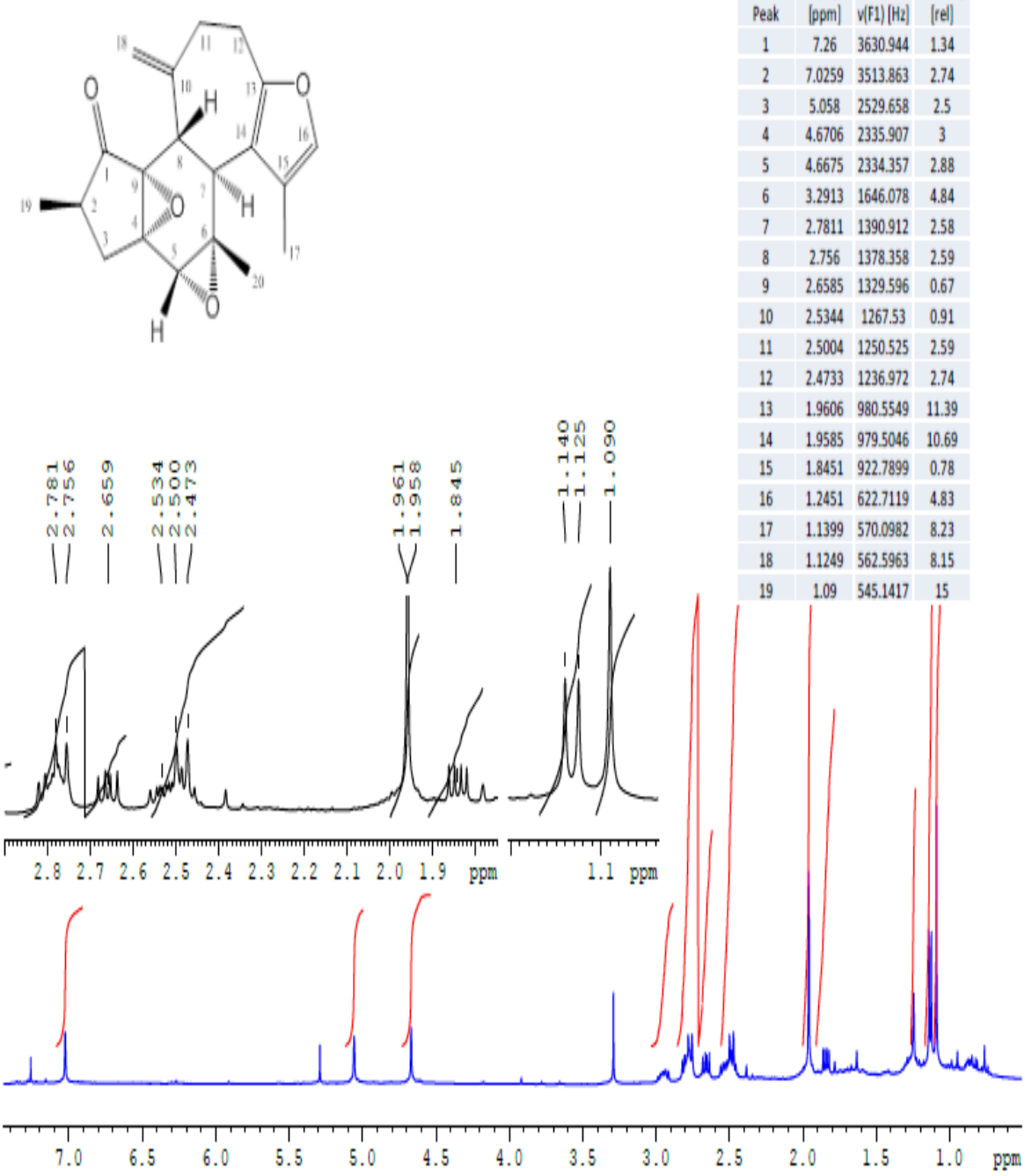
**Appendix 40: One dose mean graph of halimane-type diterpenoid compound 52**



**APPENDIX 41: MS spectrum of crotodichogamoin A (53)**

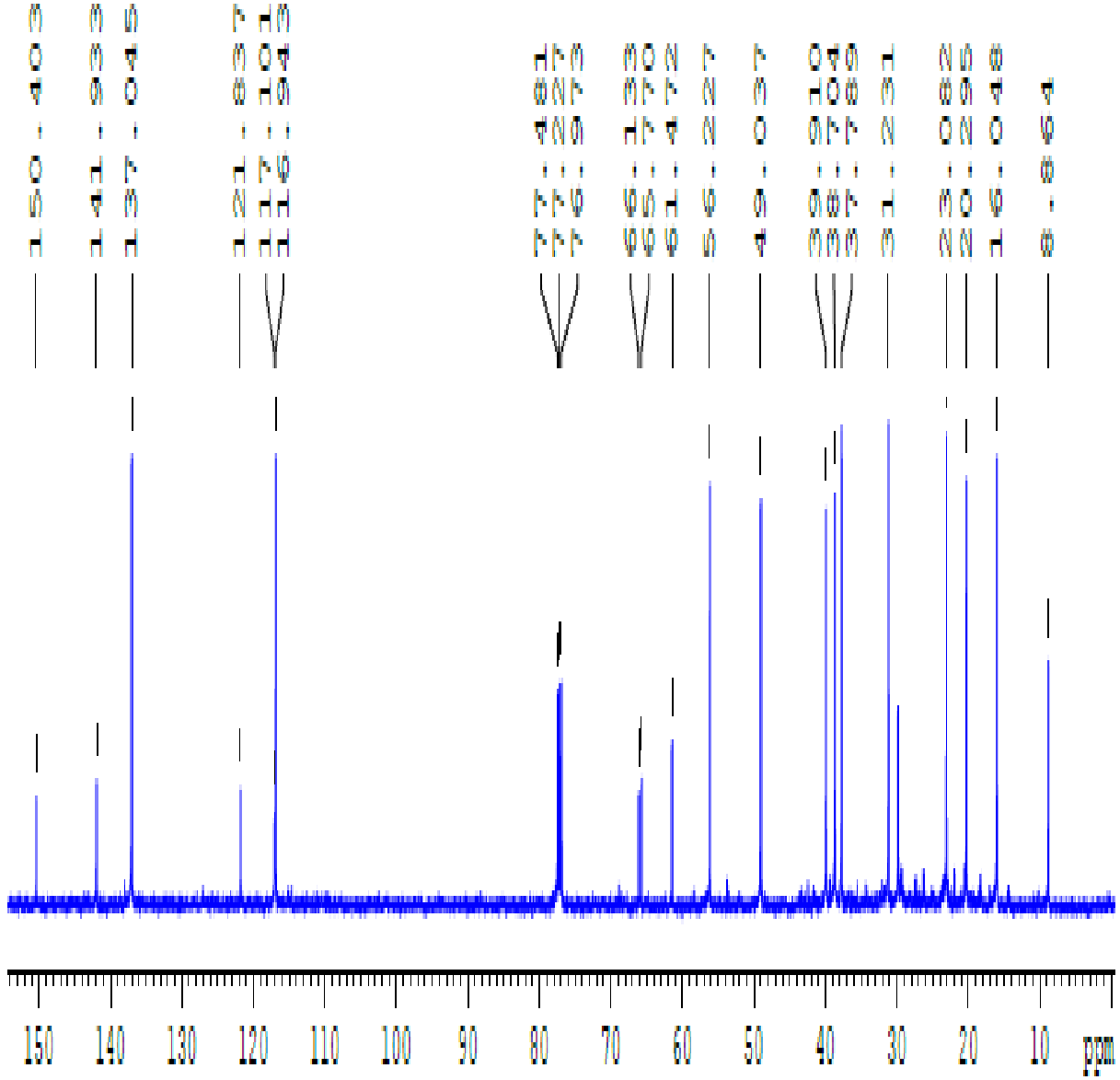


**Appendix 42: $^{1}$H NMR spectrum of crotodichogamoin A (53)**

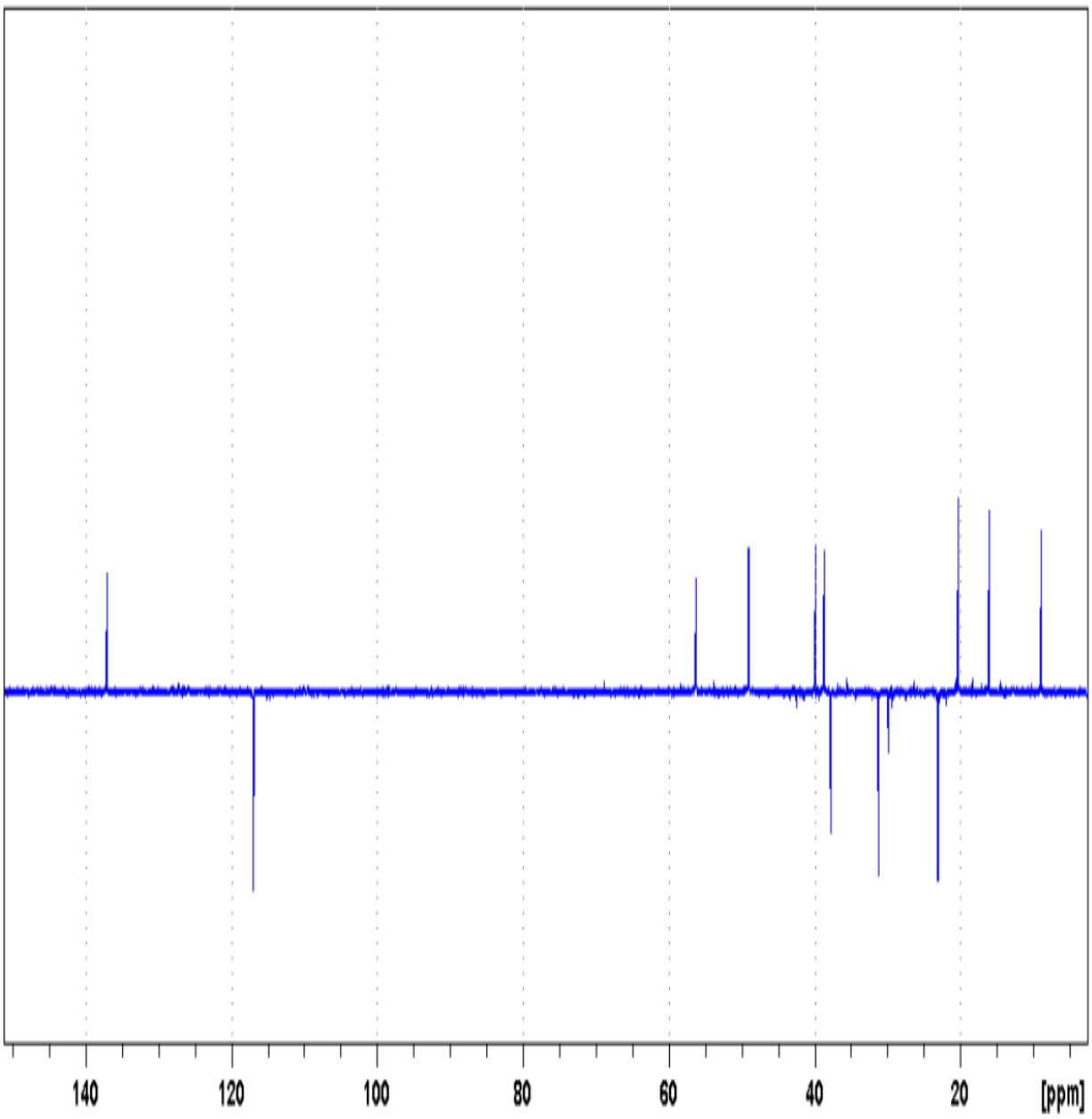



| Peak | v(F1) [ppm] | v(F1) [Hz] | Intensity [rel] |
|---|---|---|---|
| 1 | 7.26 | 3630.944 | 1.34 |
| 2 | 7.0259 | 3513.863 | 2.74 |
| 3 | 5.058 | 2529.658 | 2.5 |
| 4 | 4.6706 | 2335.907 | 3 |
| 5 | 4.6675 | 2334.357 | 2.88 |
| 6 | 3.2913 | 1646.078 | 4.84 |
| 7 | 2.7811 | 1390.912 | 2.58 |
| 8 | 2.756 | 1378.358 | 2.59 |
| 9 | 2.6585 | 1329.596 | 0.67 |
| 10 | 2.5344 | 1267.53 | 0.91 |
| 11 | 2.5004 | 1250.525 | 2.59 |
| 12 | 2.4733 | 1236.972 | 2.74 |
| 13 | 1.9606 | 980.5549 | 11.39 |
| 14 | 1.9585 | 979.5046 | 10.69 |
| 15 | 1.8451 | 922.7899 | 0.78 |
| 16 | 1.2451 | 622.7119 | 4.83 |
| 17 | 1.1399 | 570.0982 | 8.23 |
| 18 | 1.1249 | 562.5963 | 8.15 |
| 19 | 1.09 | 545.1417 | 15 |

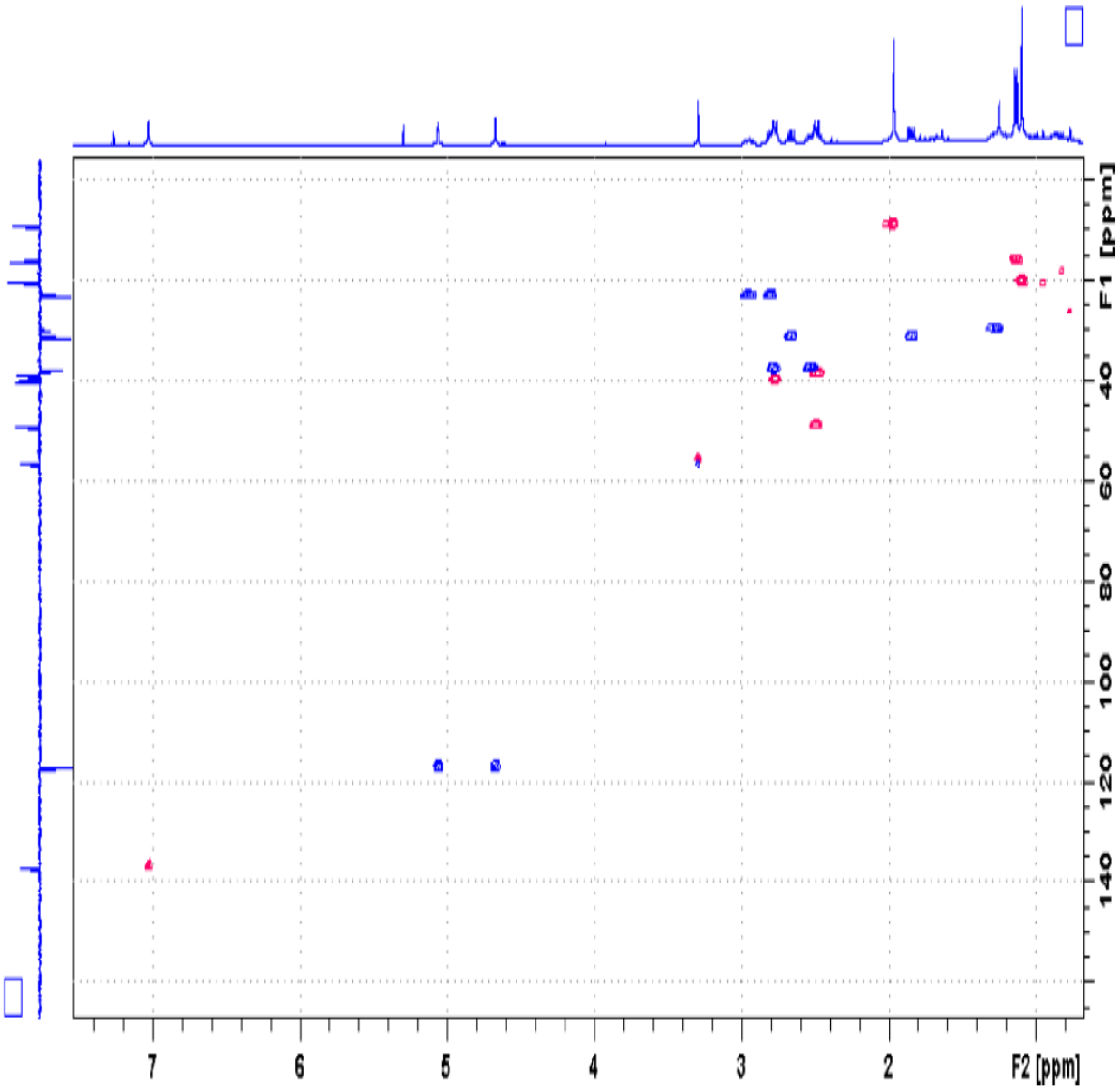
**Appendix 43: $^{13}$C NMR spectrum of crotodichogamoin A (53)**



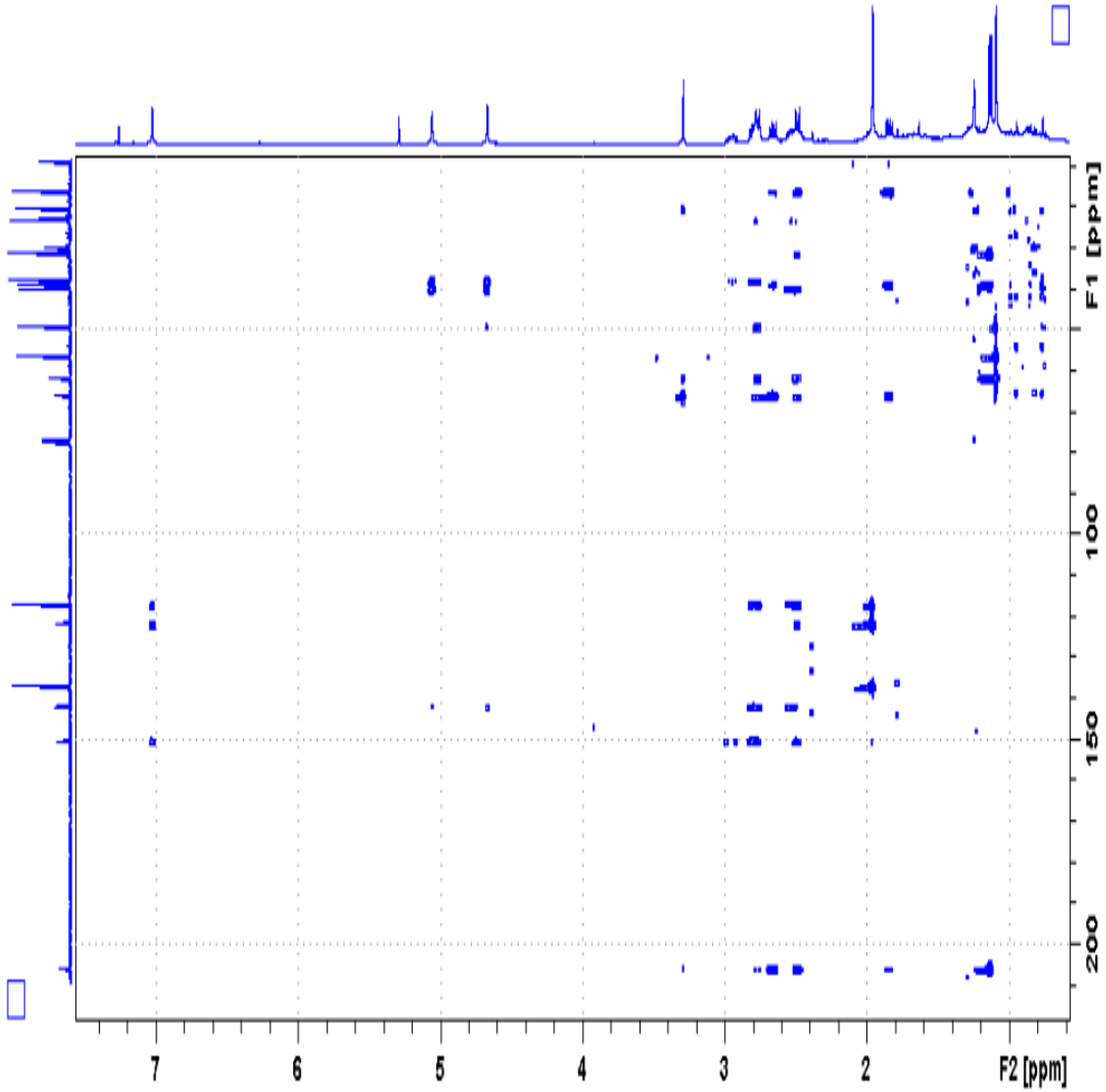
**Appendix 44: DEPT NMR spectrum of crotodichogamoin A (53)**



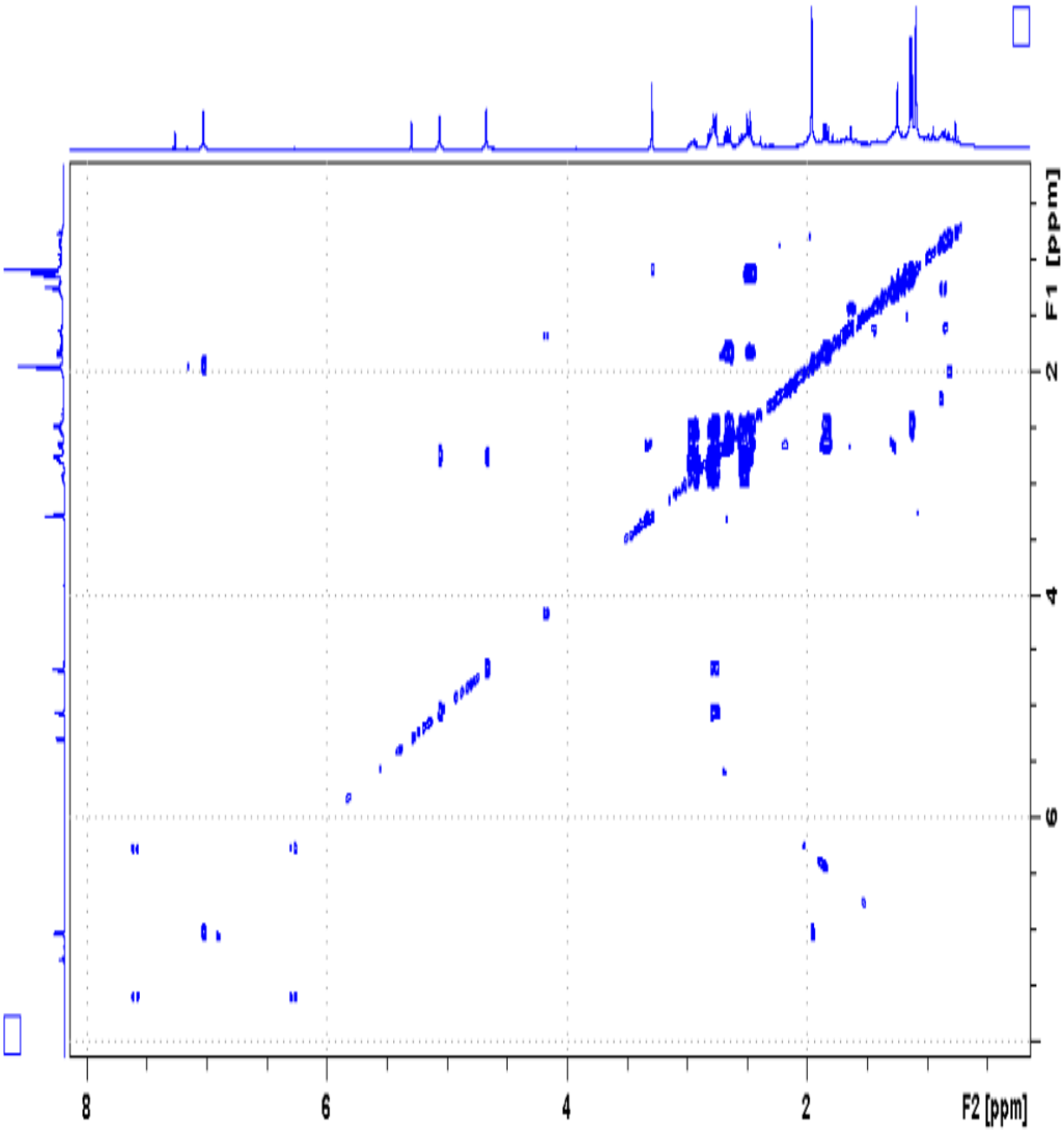
**Appendix 45: HSQC-DEPT NMR spectrum of crotodichogamoin A (53)**



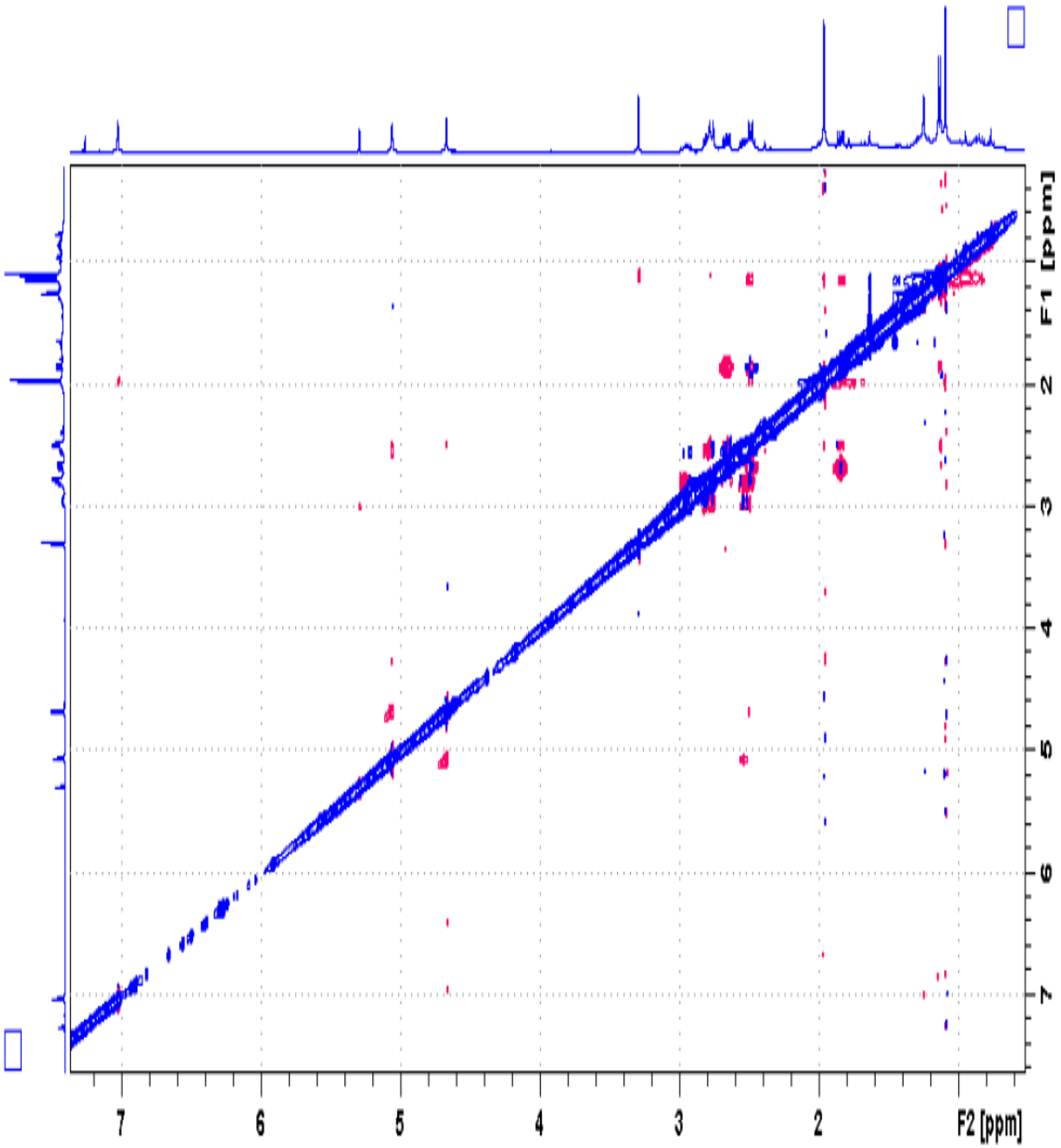
**Appendix 46: HMBC spectrum of crotodichogamoin A (53)**



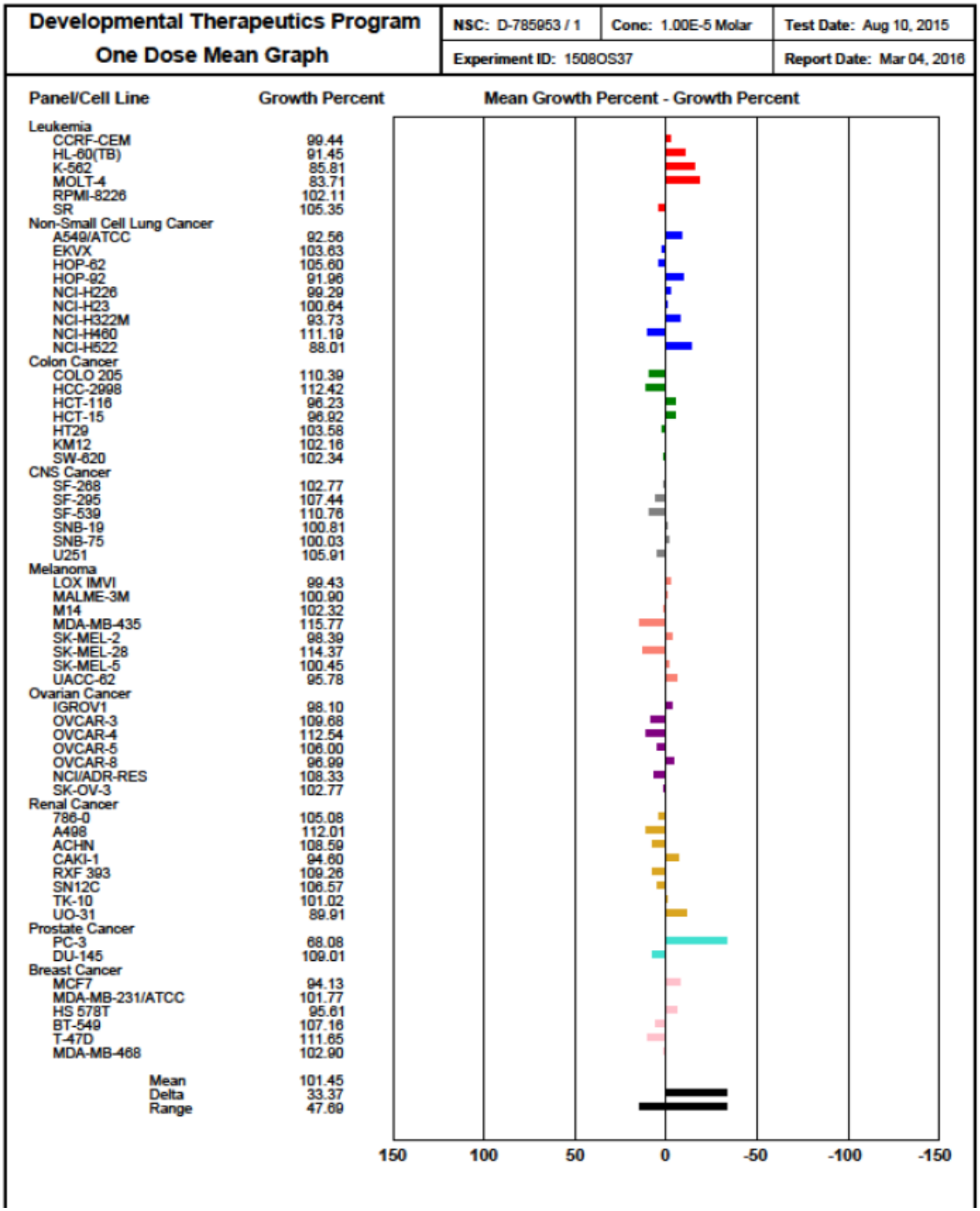
**Appendix 47: COSY Spectrum of crotodichogamoin A (53)**



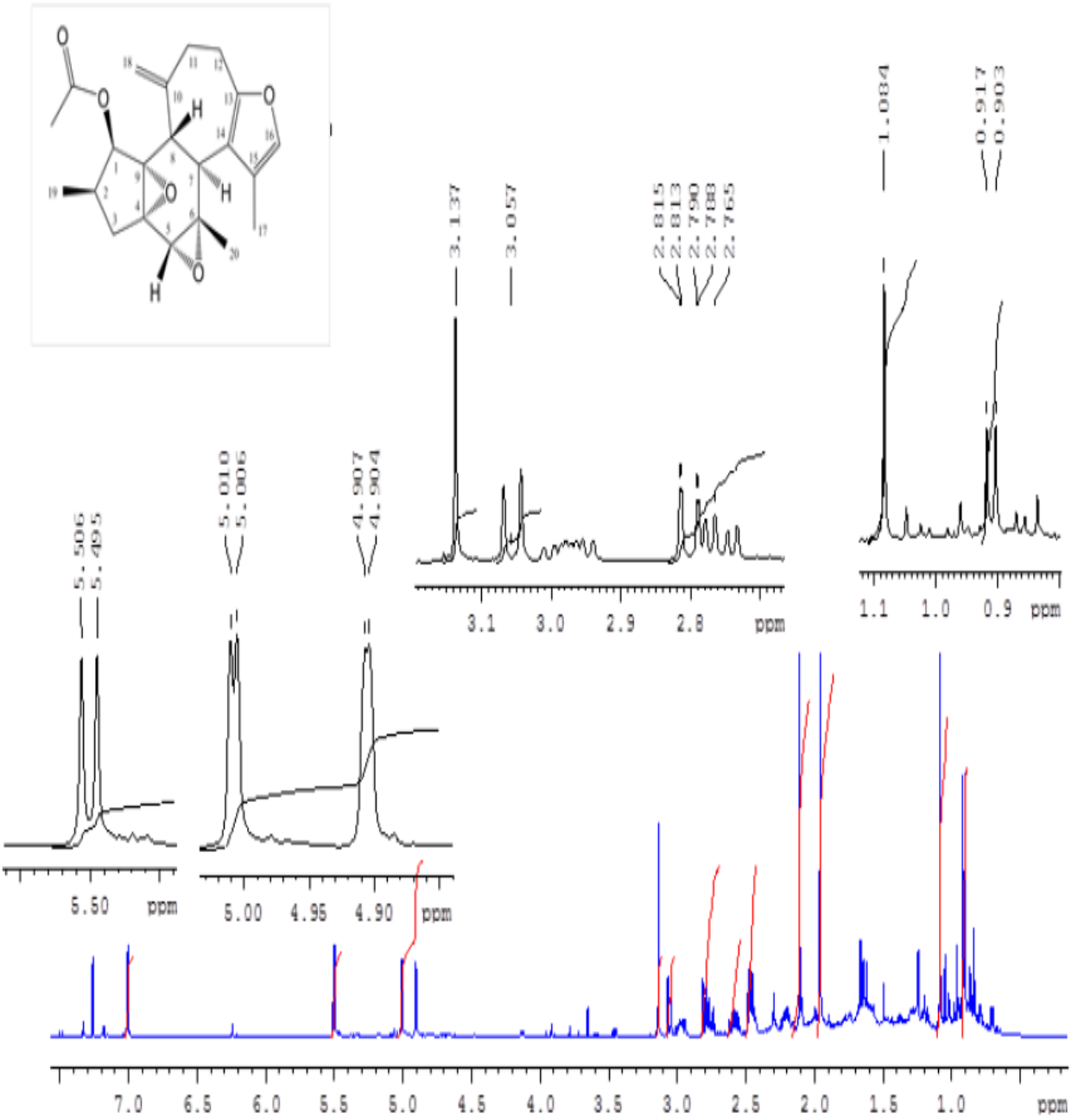
**Appendix 48: NOESY spectrum of crotodichogamoin A (53)**



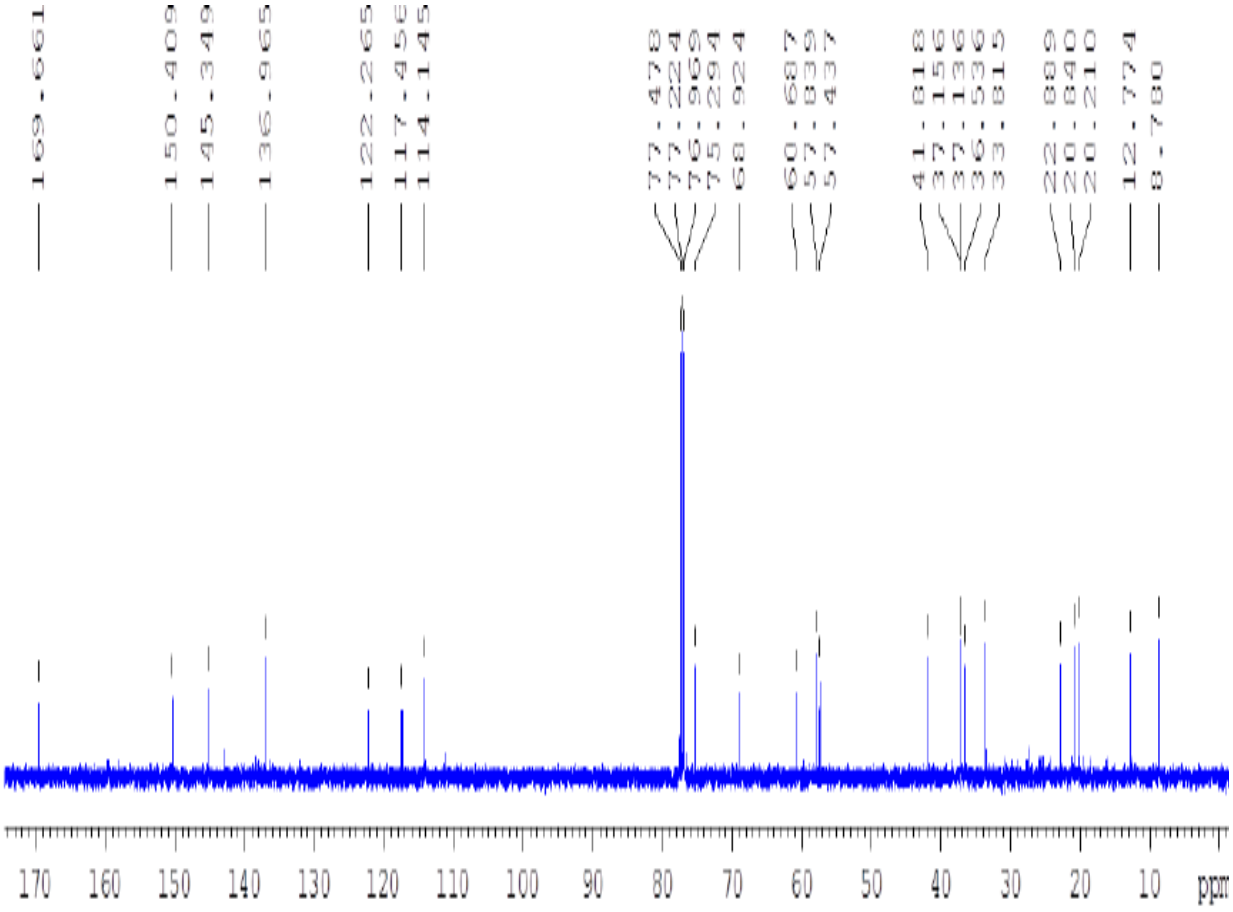
**Appendix 49: One dose mean graph of Crotodichogamoin A (53)**



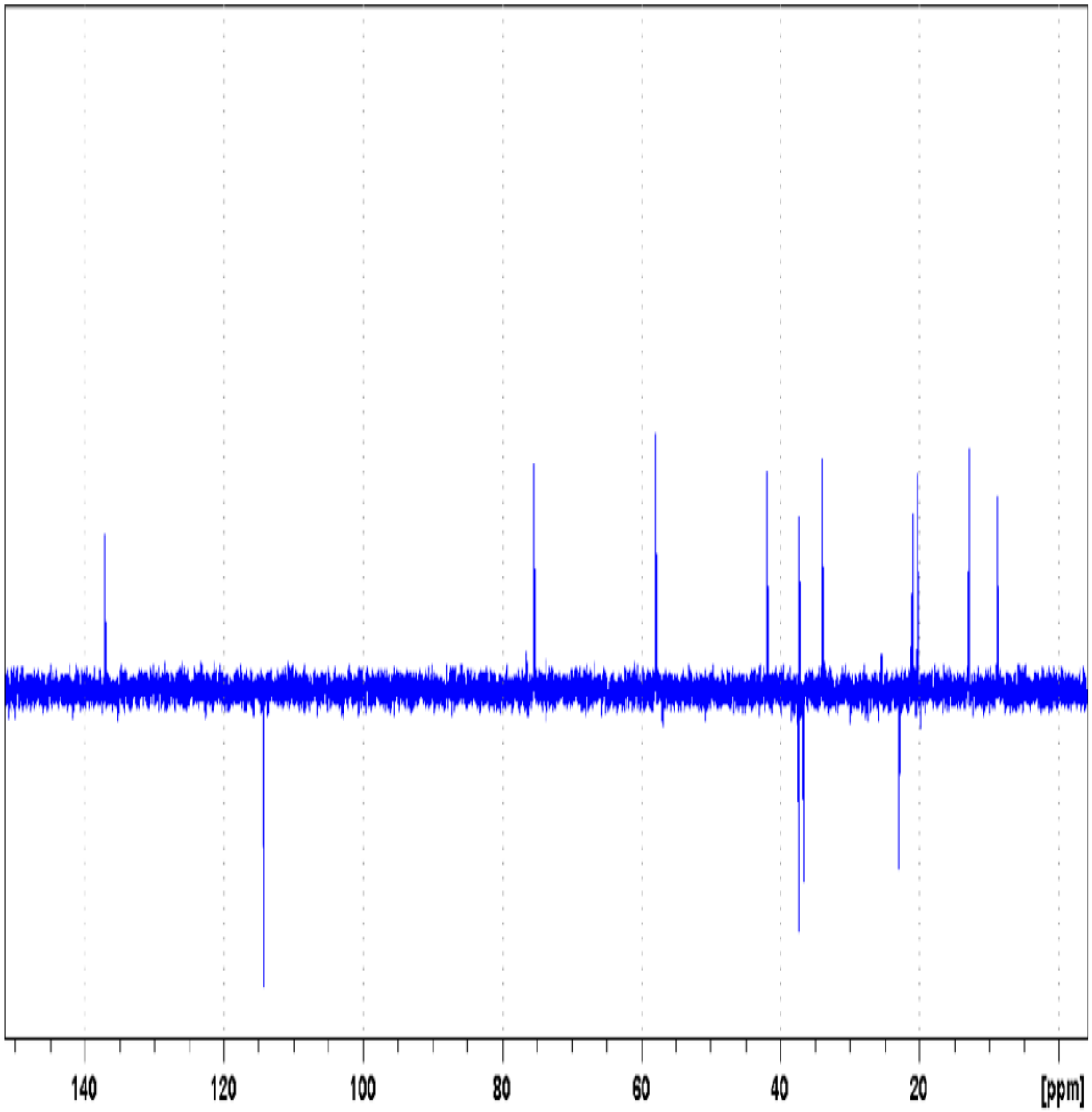
**APPENDIX 50: $^{1}$H NMR spectrum of Crotohaumanoxide (54)**



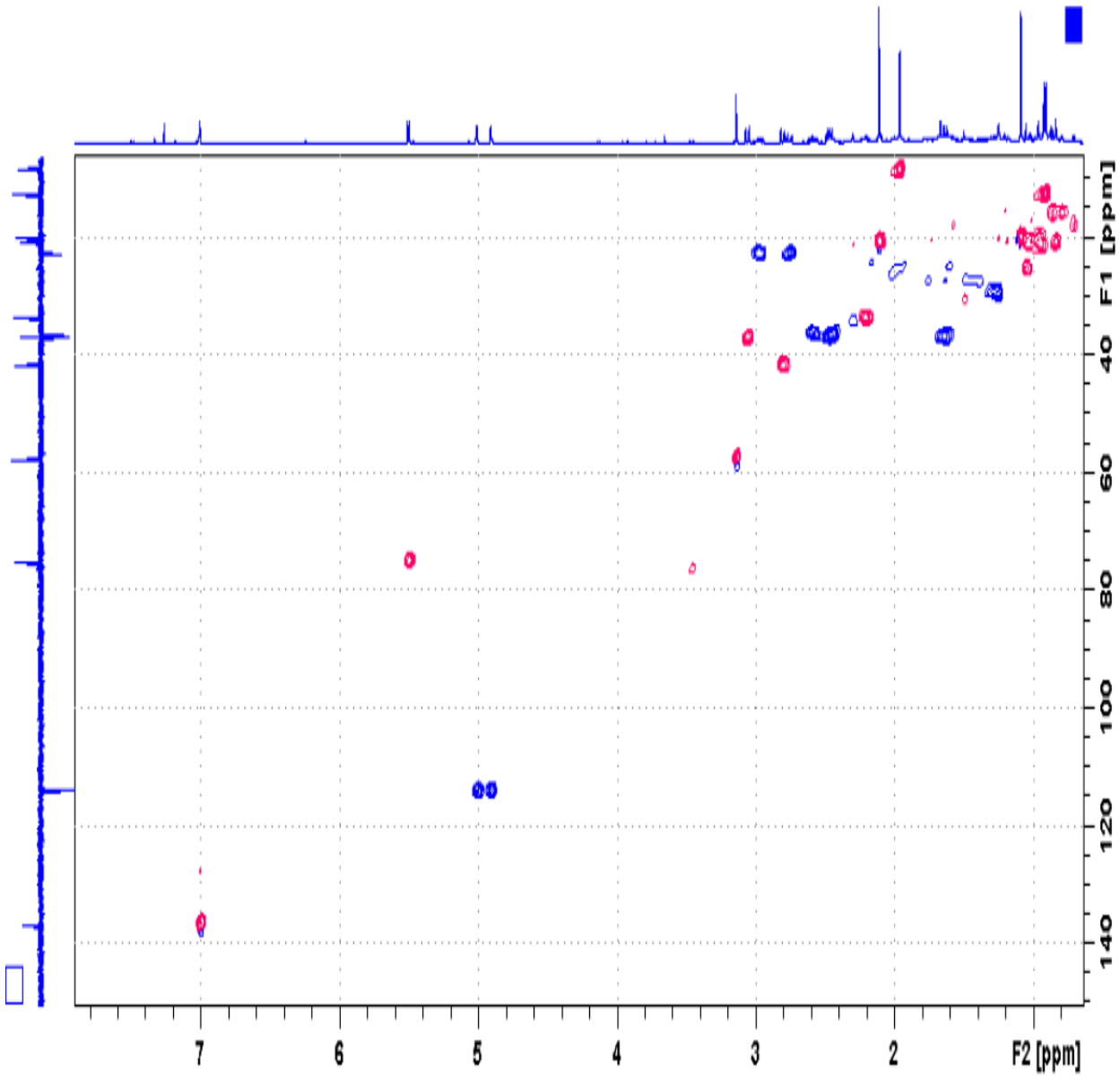
**APPENDIX 51: $^{13}$C NMR spectrum of Crotohaumanoxide (54)**



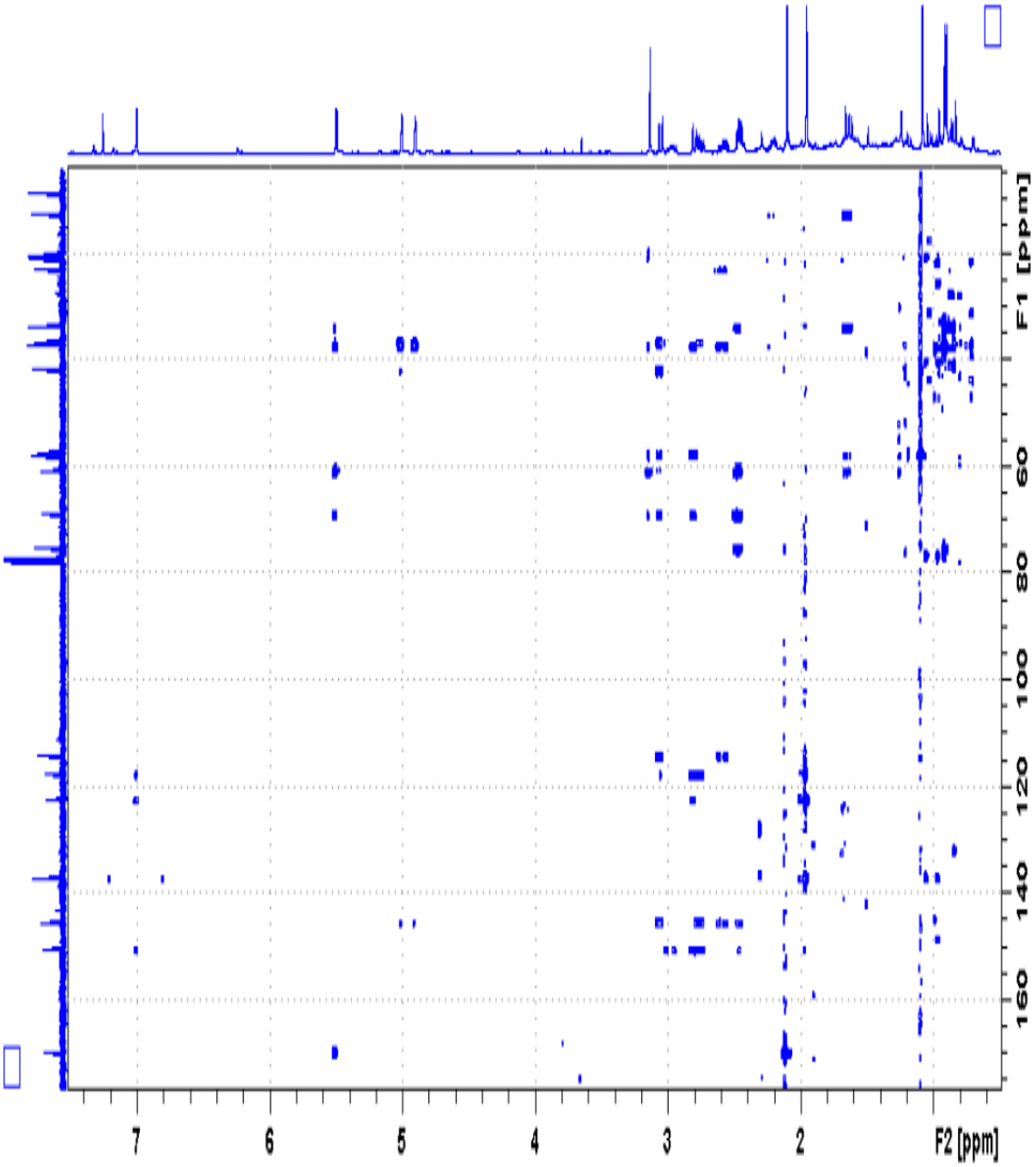
**APPENDIX 52: DEPT spectrum of Crotohaumanoxide (54)**



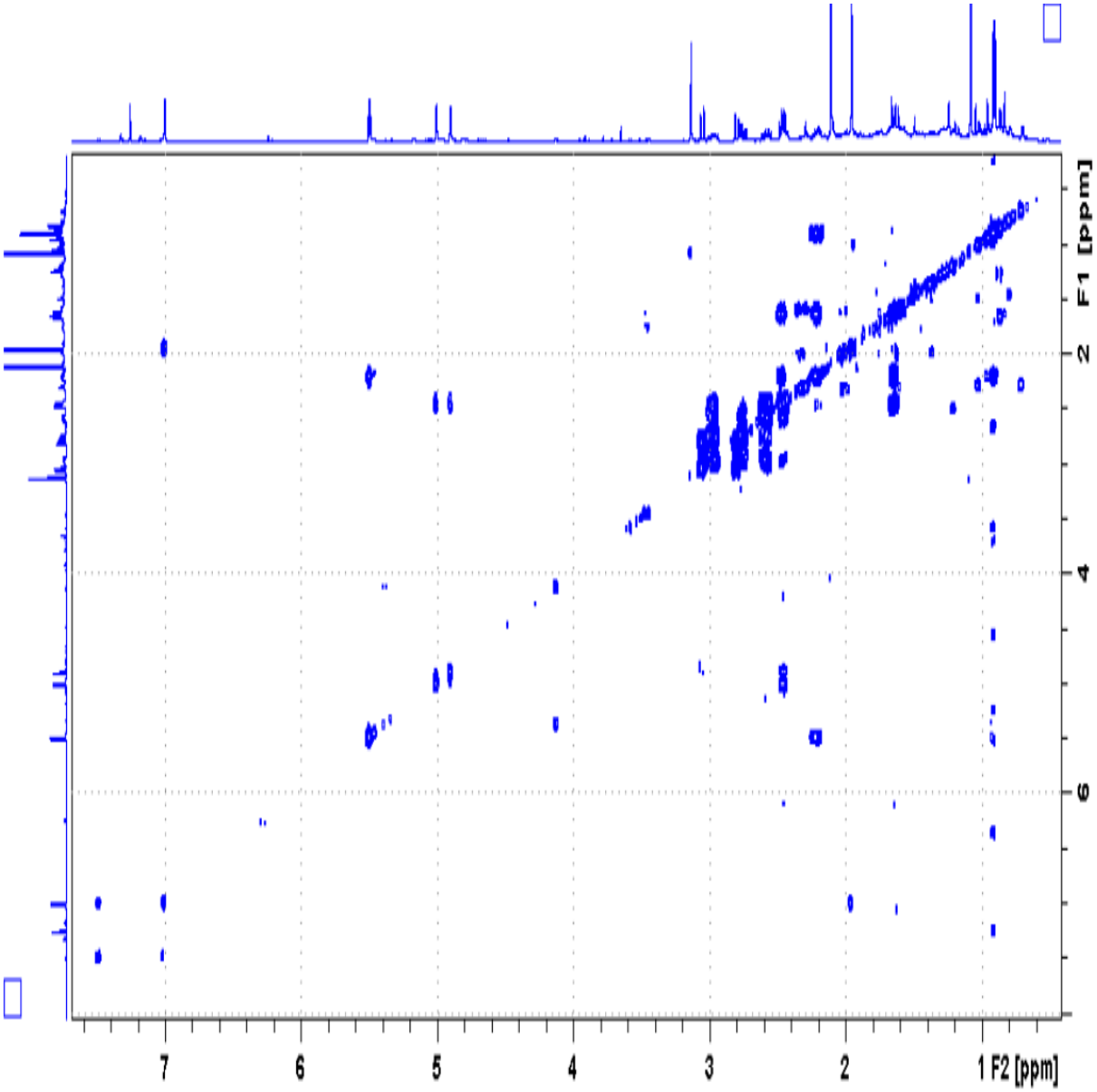
**APPENDIX 53: HSQC DEPT NMR spectrum of Crotohaumanoxide (54)**



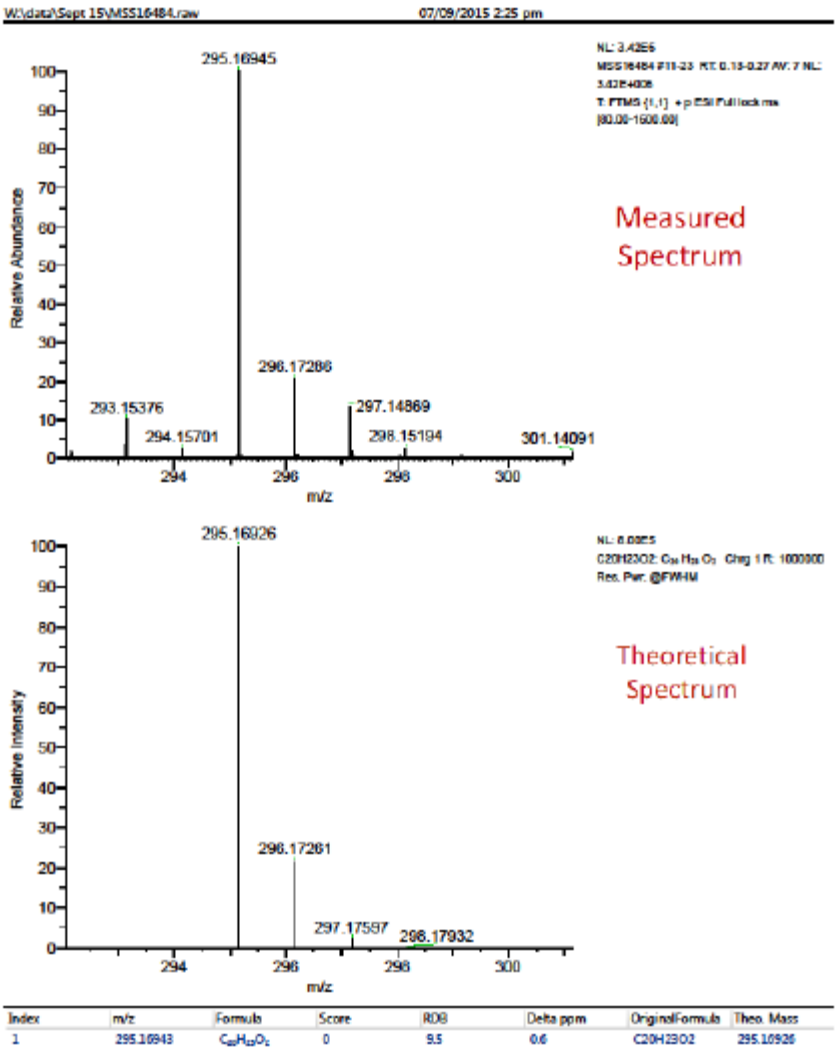
**APPENDIX 54: HMBC spectrum of Crotohaumanoxide (54)**



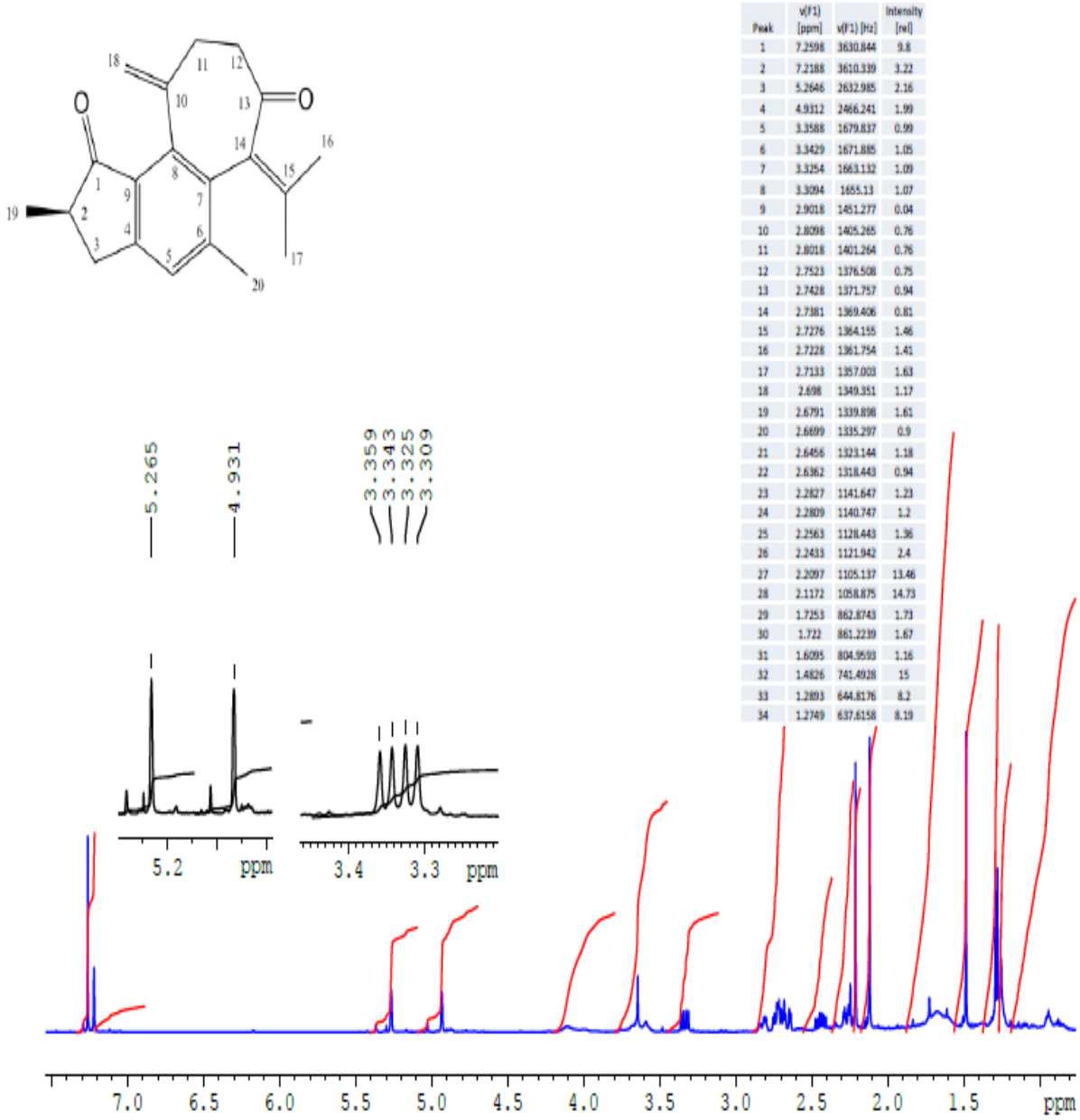
**APPENDIX 55: COSY spectrum of Crotohaumanoxide (54)**



**Appendix 56: MS spectrum of Crotodichogamoin B (55)**

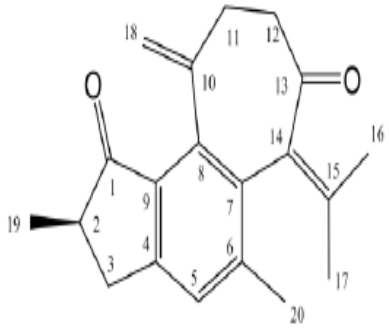


**Appendix 57: $^{1}$H NMR spectrum of Crotodichogamoin B (55)**

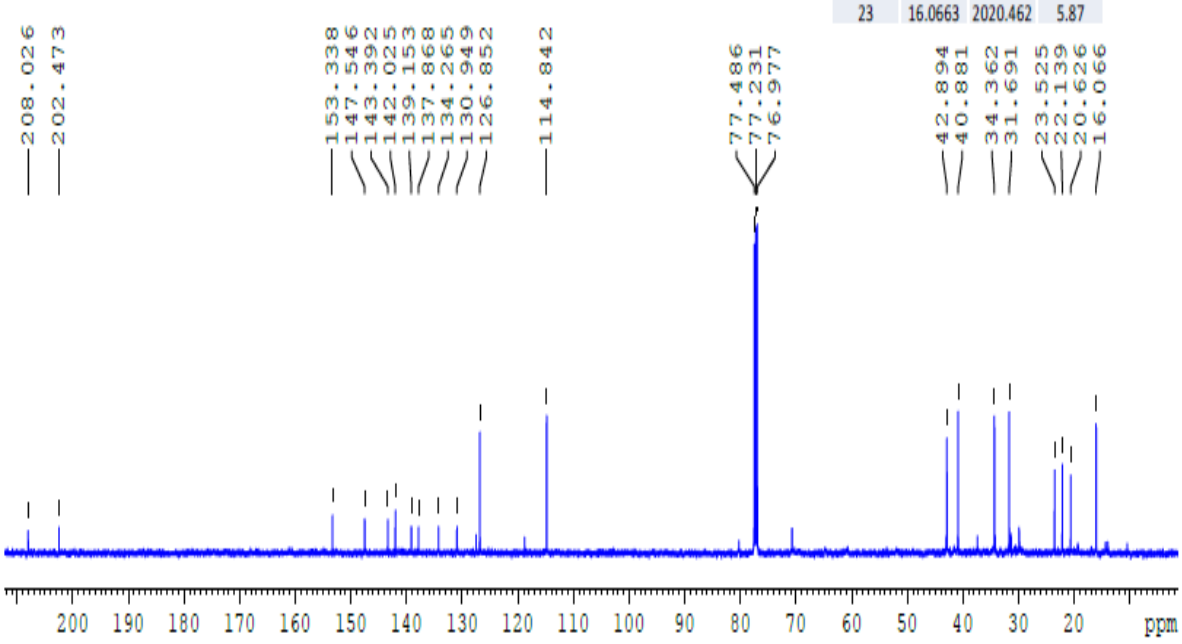



| Peak | v(F1) [ppm] | v(F1) [Hz] | Intensity [rel] |
|---|---|---|---|
| 1 | 7.2598 | 3630.844 | 9.8 |
| 2 | 7.2188 | 3610.339 | 3.22 |
| 3 | 5.2646 | 2632.985 | 2.16 |
| 4 | 4.9312 | 2466.241 | 1.99 |
| 5 | 3.3588 | 1679.837 | 0.99 |
| 6 | 3.3429 | 1671.885 | 1.05 |
| 7 | 3.3254 | 1663.132 | 1.09 |
| 8 | 3.3094 | 1655.13 | 1.07 |
| 9 | 2.9018 | 1451.277 | 0.04 |
| 10 | 2.8098 | 1405.265 | 0.76 |
| 11 | 2.8018 | 1401.264 | 0.76 |
| 12 | 2.7523 | 1376.508 | 0.75 |
| 13 | 2.7428 | 1371.757 | 0.94 |
| 14 | 2.7381 | 1369.406 | 0.81 |
| 15 | 2.7276 | 1364.155 | 1.46 |
| 16 | 2.7228 | 1361.754 | 1.41 |
| 17 | 2.7133 | 1357.003 | 1.63 |
| 18 | 2.698 | 1349.351 | 1.17 |
| 19 | 2.6791 | 1339.898 | 1.61 |
| 20 | 2.6699 | 1335.297 | 0.9 |
| 21 | 2.6456 | 1323.144 | 1.18 |
| 22 | 2.6362 | 1318.443 | 0.94 |
| 23 | 2.2827 | 1141.647 | 1.23 |
| 24 | 2.2809 | 1140.747 | 1.2 |
| 25 | 2.2563 | 1128.443 | 1.36 |
| 26 | 2.2433 | 1121.942 | 2.4 |
| 27 | 2.2097 | 1105.137 | 13.46 |
| 28 | 2.1172 | 1058.875 | 14.73 |
| 29 | 1.7253 | 862.8743 | 1.73 |
| 30 | 1.722 | 861.2239 | 1.67 |
| 31 | 1.6095 | 804.9593 | 1.16 |
| 32 | 1.4826 | 741.4928 | 15 |
| 33 | 1.2893 | 644.8176 | 8.2 |
| 34 | 1.2749 | 637.6158 | 8.19 |

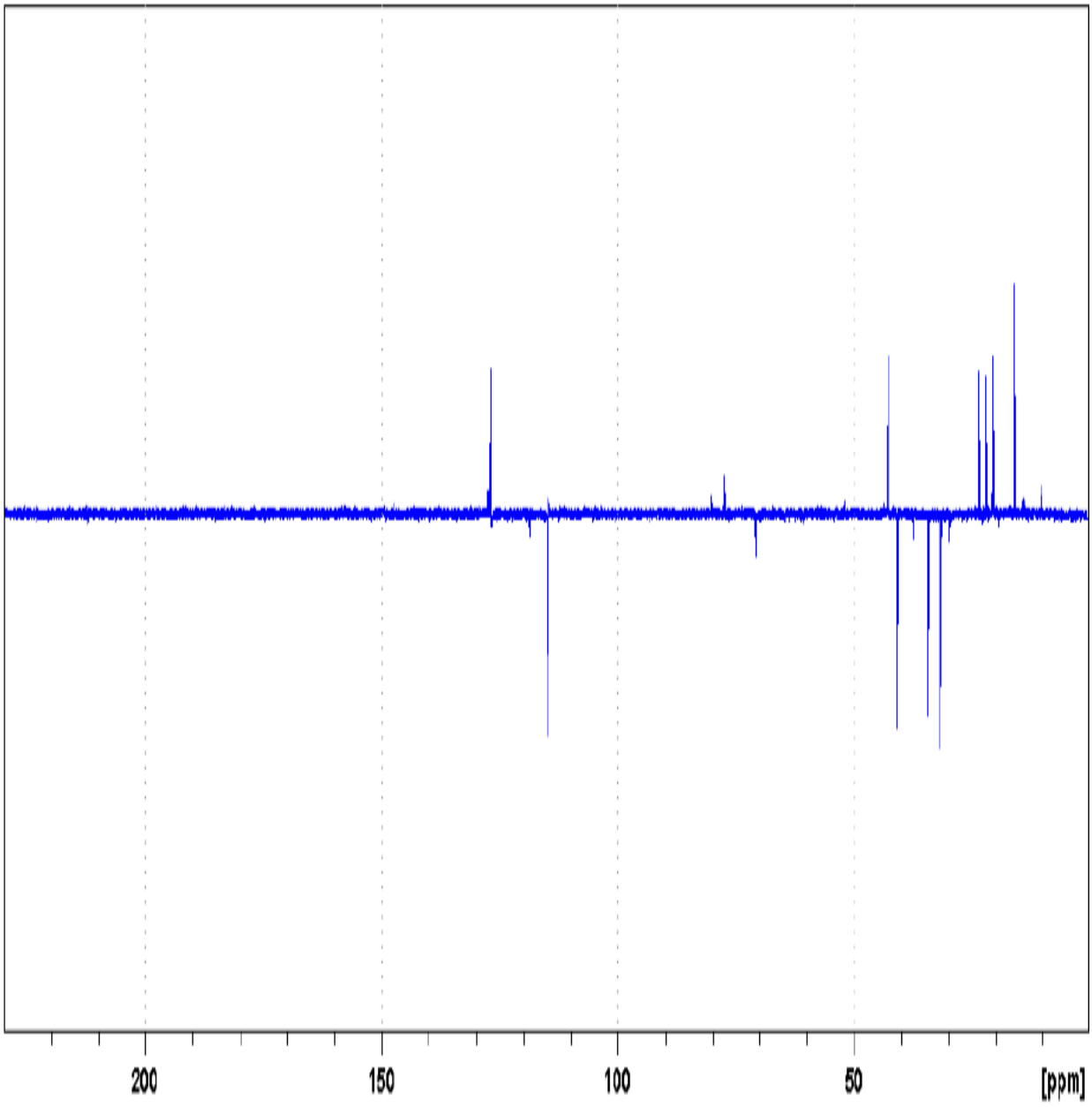
**Appendix 58: $^{13}$C NMR spectrum of Crotodichogamoin B (55)**



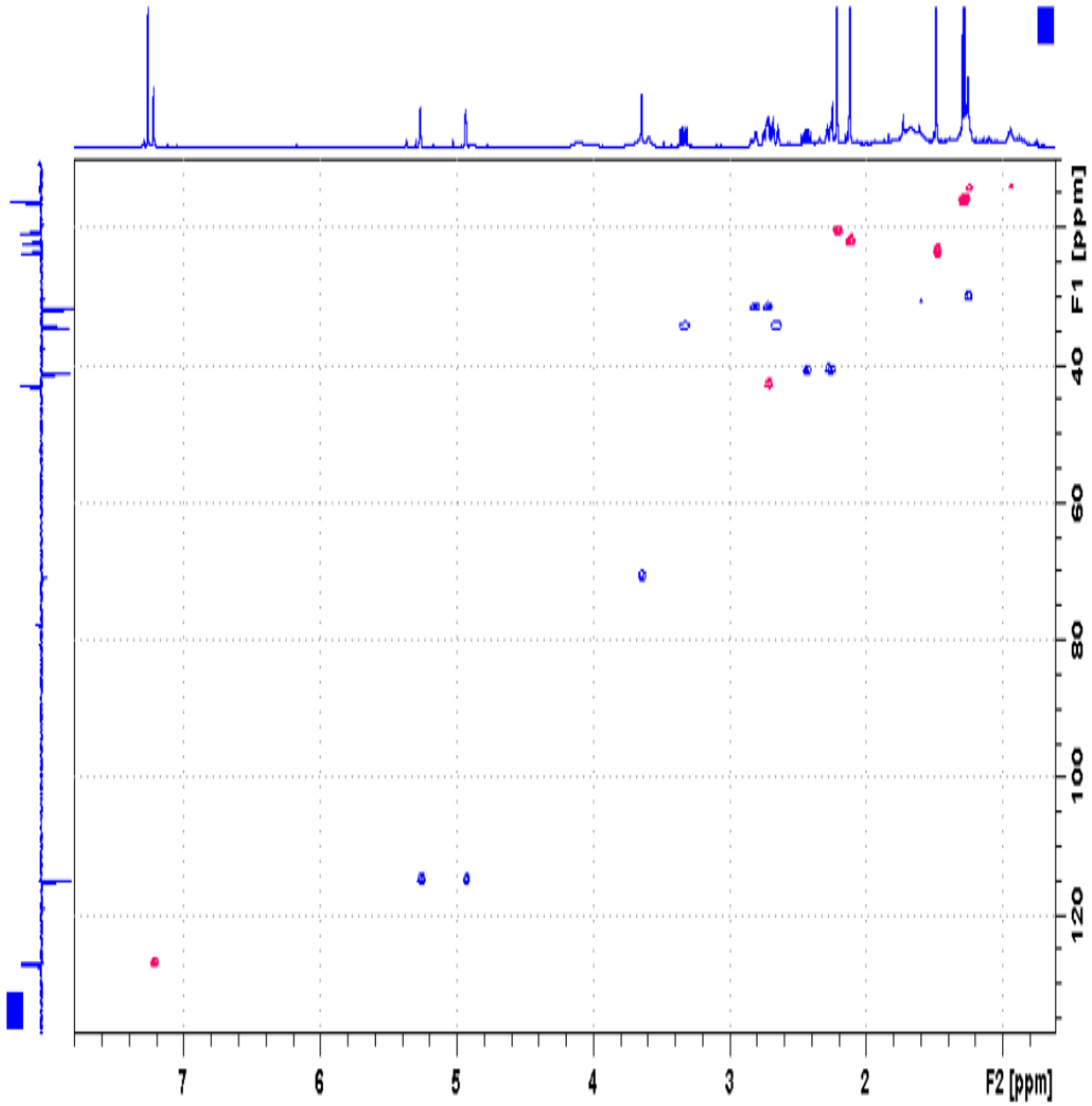

| | | | |
|---|---|---|---|
| 1 | 208.026 | 26160.88 | 1.01 |
| 2 | 202.4725 | 25462.49 | 1.15 |
| 3 | 153.3378 | 19283.42 | 1.71 |
| 4 | 147.5457 | 18555.02 | 1.55 |
| 5 | 143.3919 | 18032.64 | 1.51 |
| 6 | 142.0254 | 17860.8 | 1.93 |
| 7 | 139.1531 | 17499.58 | 1.21 |
| 8 | 137.8675 | 17337.91 | 1.17 |
| 9 | 134.265 | 16884.87 | 1.19 |
| 10 | 130.9491 | 16467.87 | 1.19 |
| 11 | 126.8518 | 15952.6 | 5.51 |
| 12 | 114.842 | 14442.27 | 6.23 |
| 13 | 77.4855 | 9744.403 | 14.08 |
| 14 | 77.2315 | 9712.461 | 14.83 |
| 15 | 76.9773 | 9680.493 | 15 |
| 16 | 42.8938 | 5394.228 | 5.22 |
| 17 | 40.8806 | 5141.053 | 6.43 |
| 18 | 34.3617 | 4321.251 | 6.25 |
| 19 | 31.6909 | 3985.377 | 6.43 |
| 20 | 23.5245 | 2958.389 | 3.77 |
| 21 | 22.1393 | 2784.189 | 4.05 |
| 22 | 20.6259 | 2593.867 | 3.53 |
| 23 | 16.0663 | 2020.462 | 5.87 |



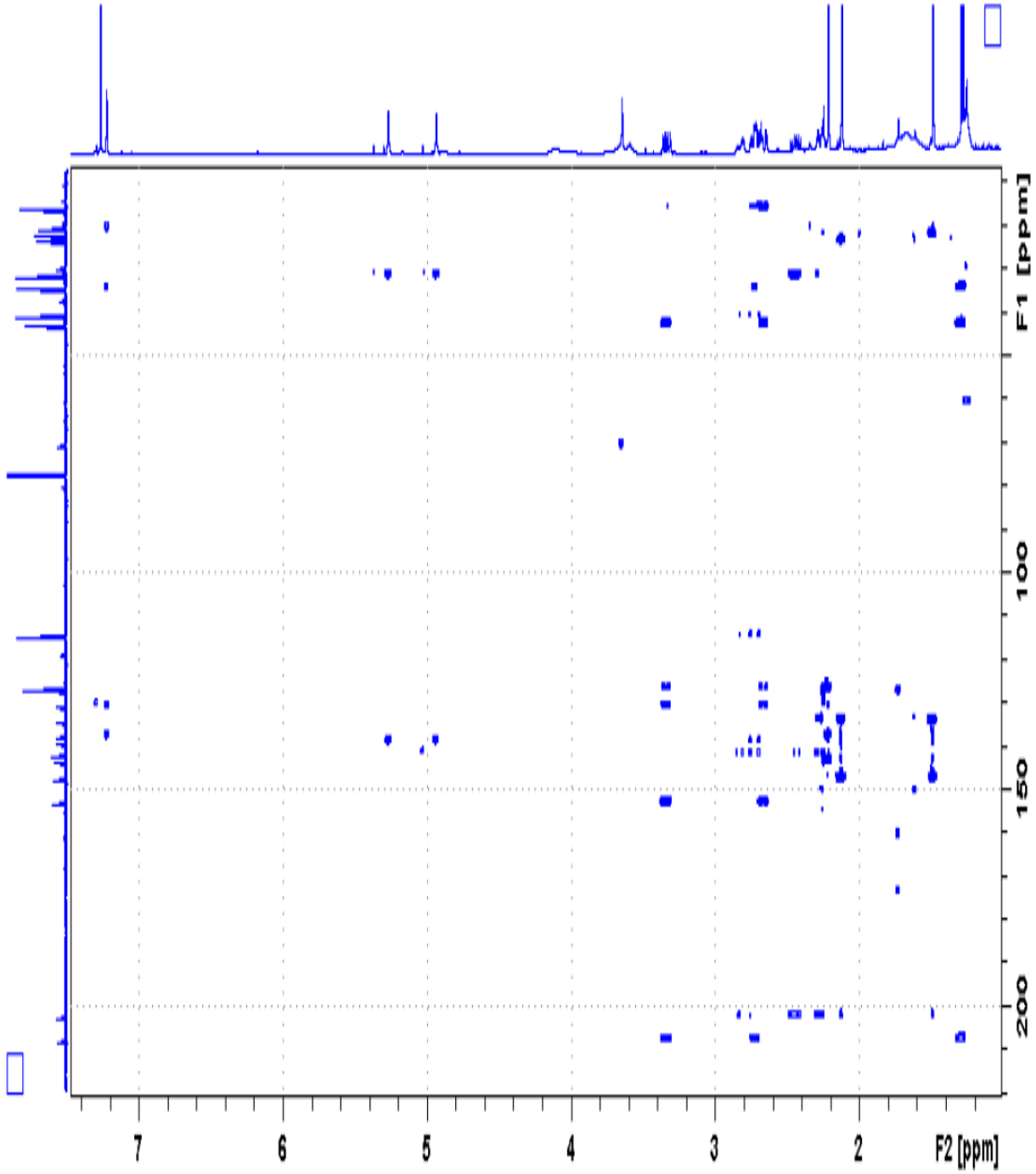
**Appendix 59: DEPT spectrum of Crotodichogamoin B (55)**



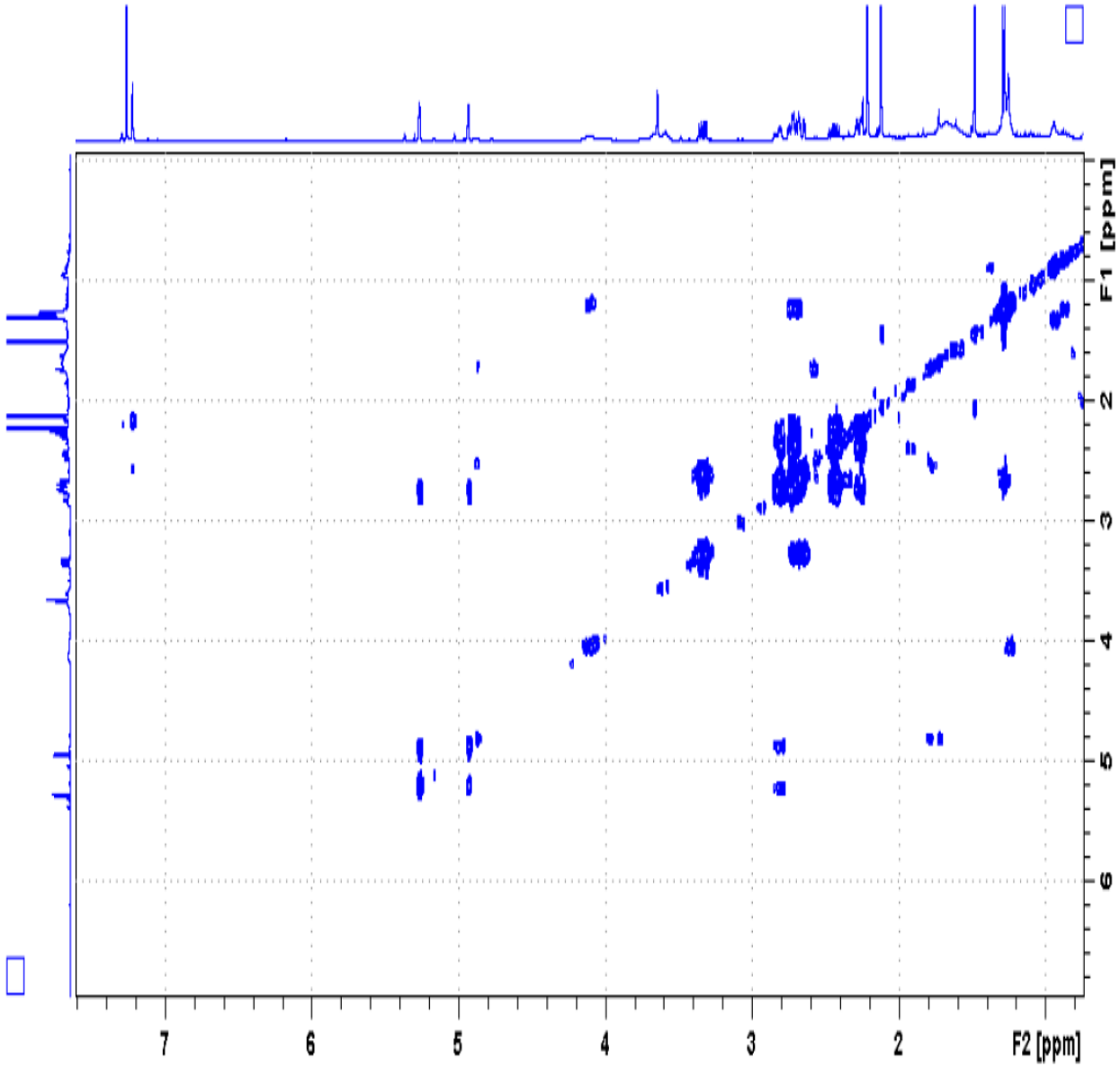
**Appendix 60: HSQC-DEPT NMR spectrum of Crotodichogamoin B (55)**



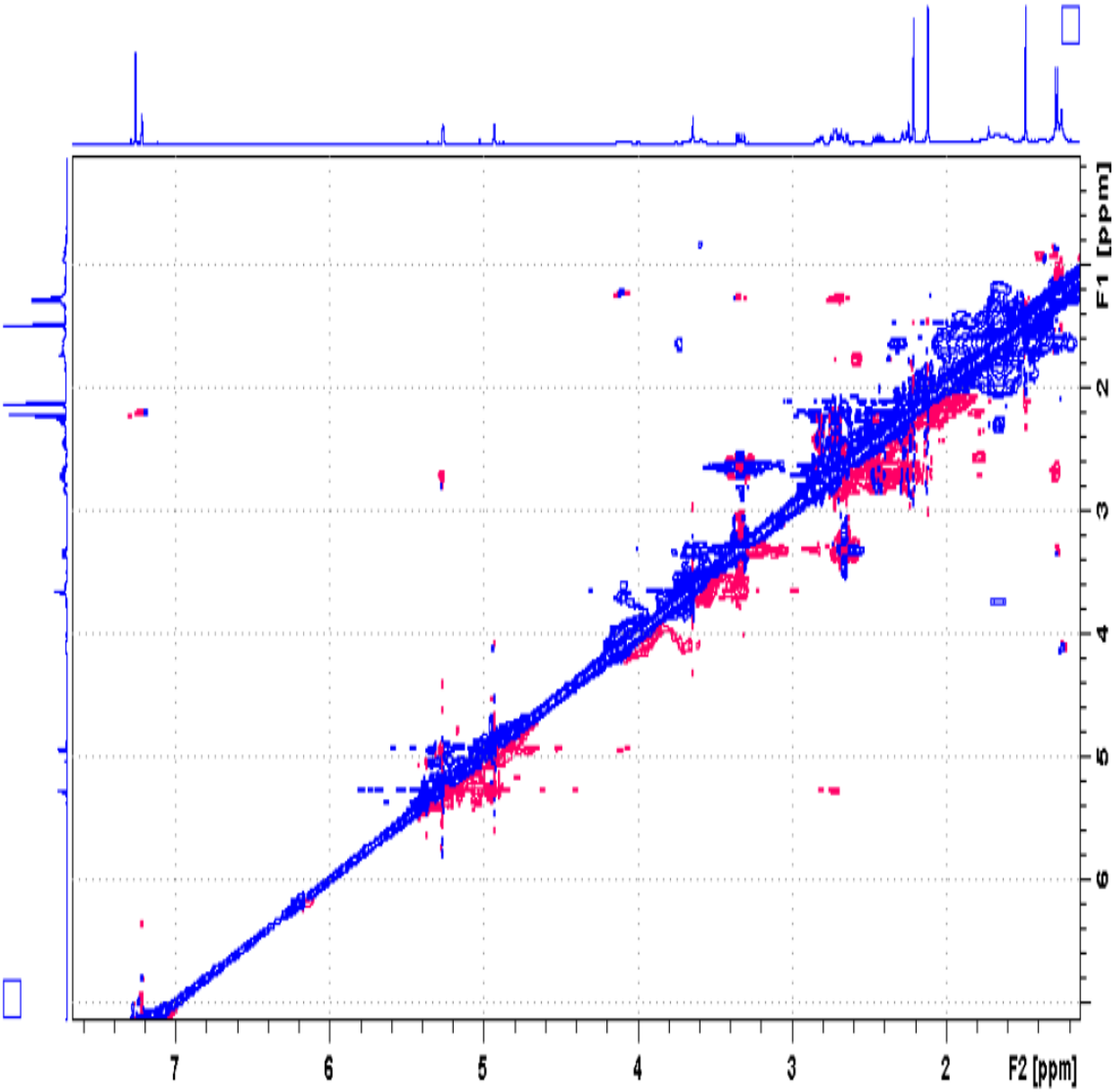
**Appendix 61: HMBC spectrum of Crotodichogamoin B (55)**



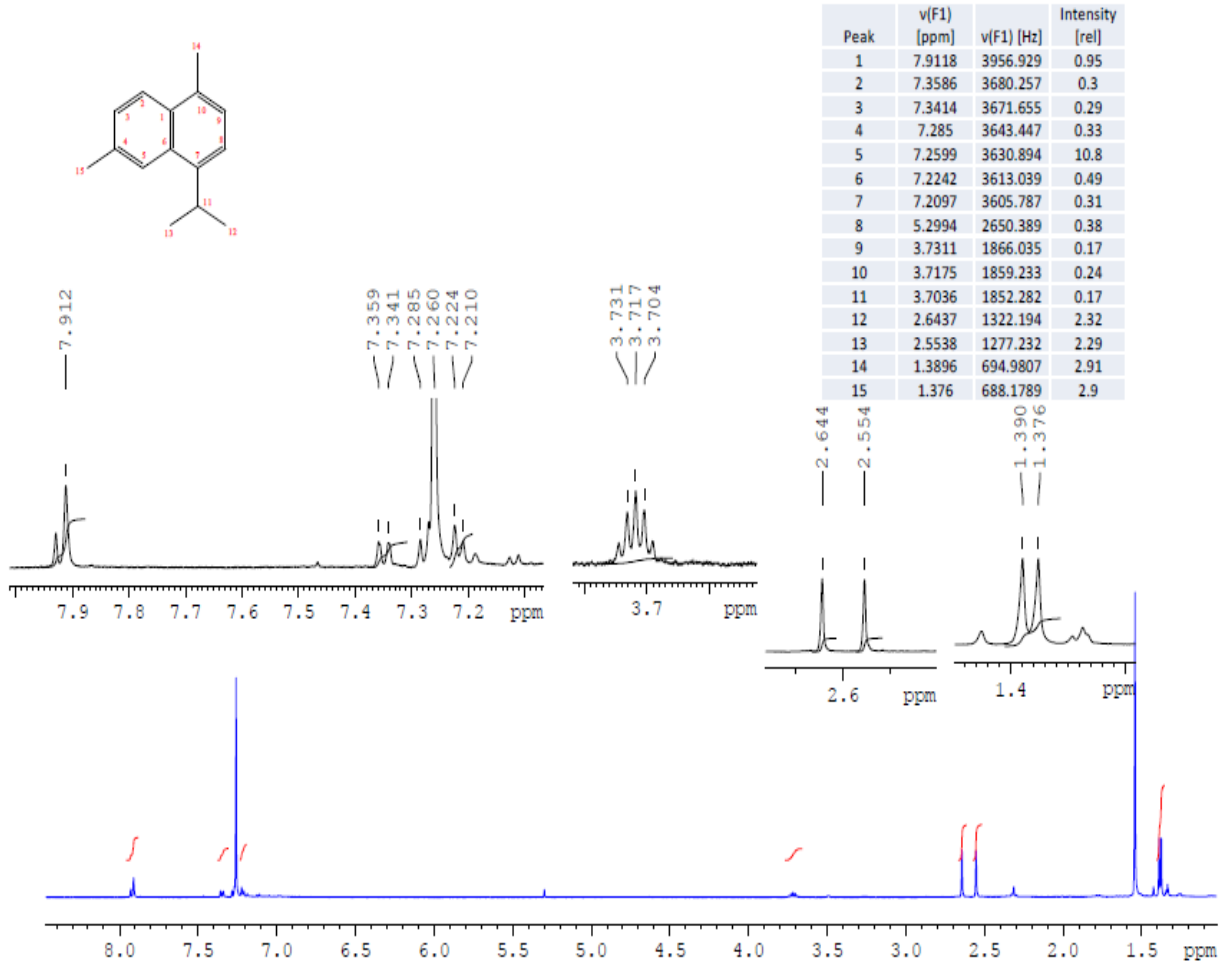
**Appendix 62: COSY spectrum of Crotodichogamoin B (55)**



**Appendix 63: NOESY spectrum of Crotodichogamoin B (55)**

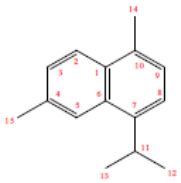


**APPENDIX 64: $^{1}$H NMR spectrum for Cadin-1(6),2,4,7,9-penta-ene (56) in $CDCl_3$**

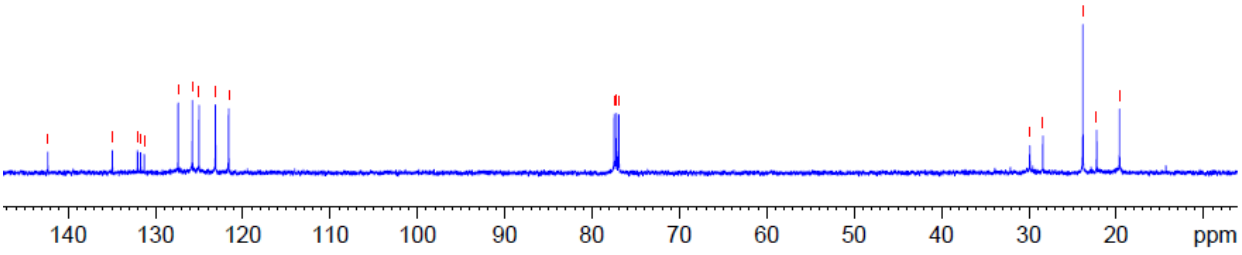



| Peak | v(F1) [ppm] | v(F1) [Hz] | Intensity [rel] |
|---|---|---|---|
| 1 | 7.9118 | 3956.929 | 0.95 |
| 2 | 7.3586 | 3680.257 | 0.3 |
| 3 | 7.3414 | 3671.655 | 0.29 |
| 4 | 7.285 | 3643.447 | 0.33 |
| 5 | 7.2599 | 3630.894 | 10.8 |
| 6 | 7.2242 | 3613.039 | 0.49 |
| 7 | 7.2097 | 3605.787 | 0.31 |
| 8 | 5.2994 | 2650.389 | 0.38 |
| 9 | 3.7311 | 1866.035 | 0.17 |
| 10 | 3.7175 | 1859.233 | 0.24 |
| 11 | 3.7036 | 1852.282 | 0.17 |
| 12 | 2.6437 | 1322.194 | 2.32 |
| 13 | 2.5538 | 1277.232 | 2.29 |
| 14 | 1.3896 | 694.9807 | 2.91 |
| 15 | 1.376 | 688.1789 | 2.9 |

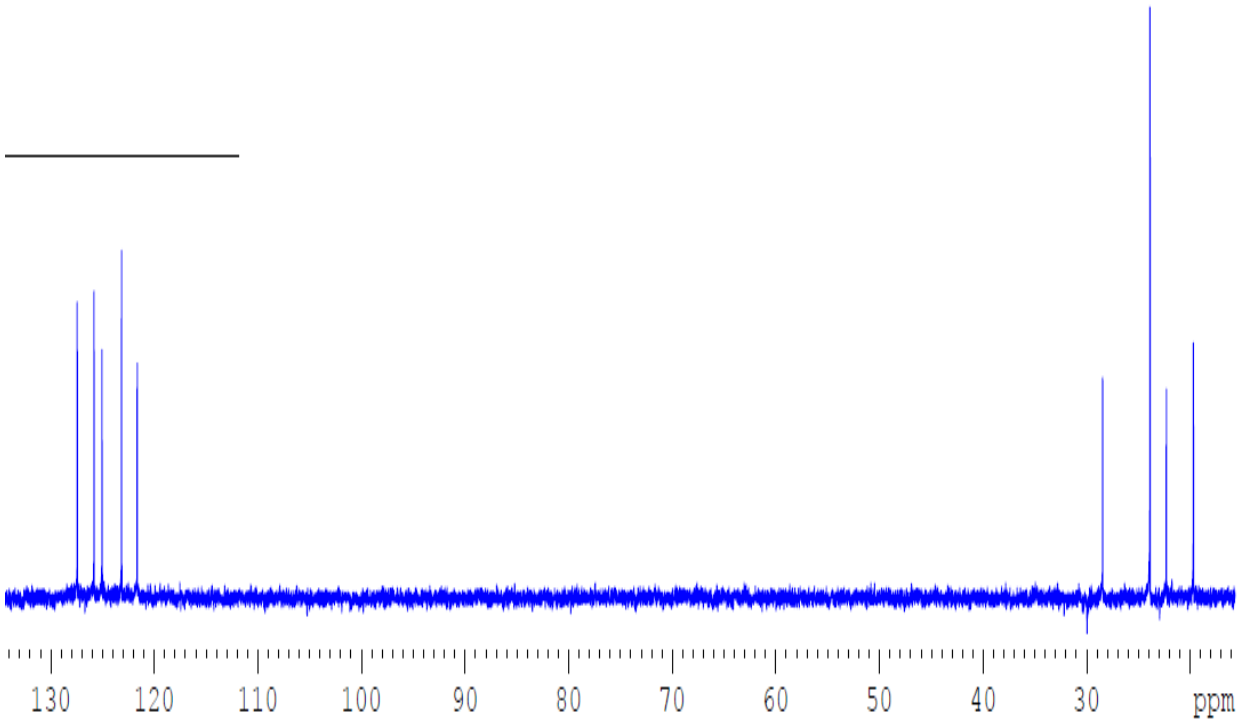
**APPENDIX 65: $^{13}C$ NMR spectrum for Cadin-1(6),2,4,7,9-penta-ene (56) in $CDCl_3$**



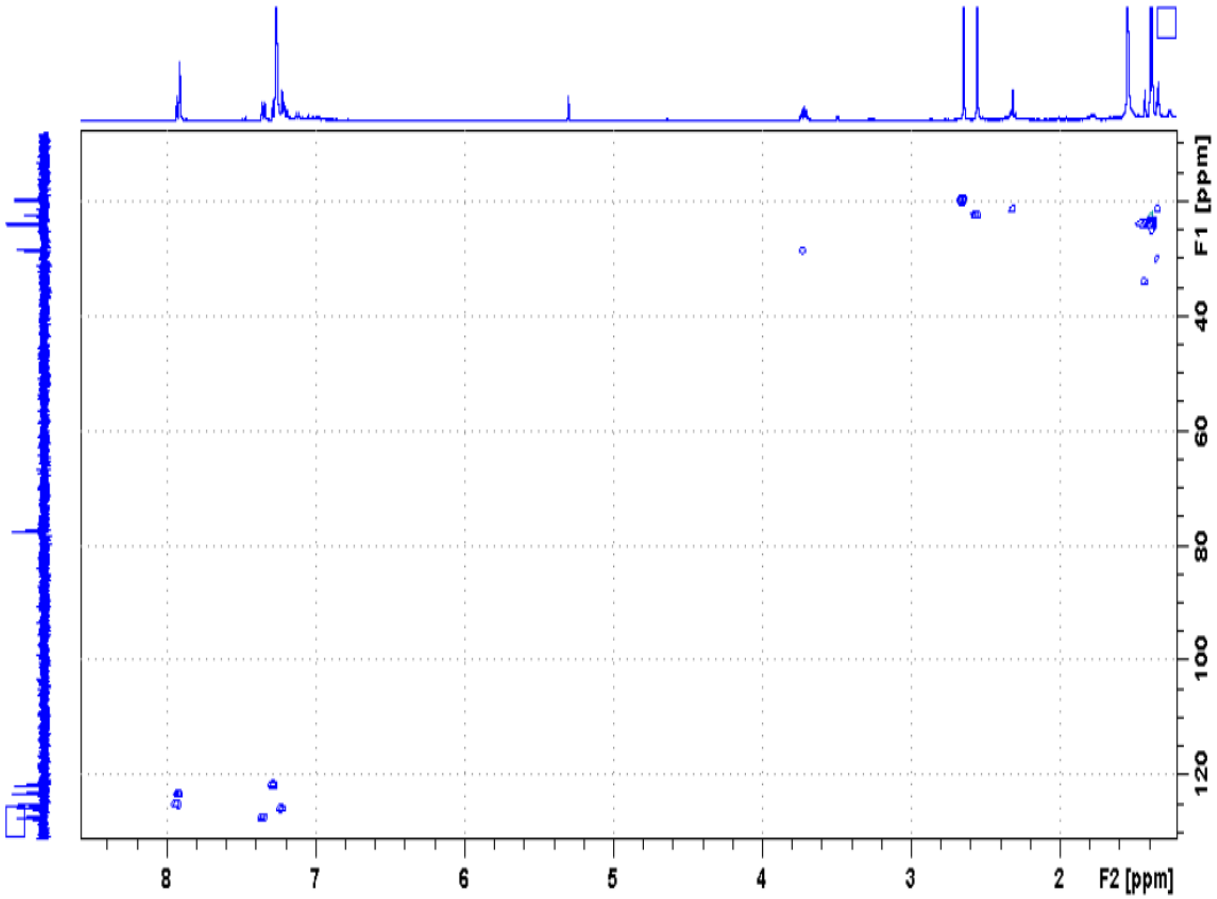
| Peak | v(F1) [ppm] | v(F1) [Hz] | Intensity [rel] |
|---|---|---|---|
| 1 | 142.3464 | 17901.16 | 0.2 |
| 2 | 134.8622 | 16959.97 | 0.01 |
| 3 | 132.0723 | 16609.12 | 0.25 |
| 4 | 131.7381 | 16567.09 | 0.18 |
| 5 | 131.3171 | 16514.14 | 0.18 |
| 6 | 127.424 | 16024.56 | 0.81 |
| 7 | 125.799 | 15820.2 | 0.93 |
| 8 | 125.0302 | 15723.52 | 0.8 |
| 9 | 123.1578 | 15488.05 | 0.83 |
| 10 | 121.6432 | 15297.58 | 0.77 |
| 11 | 77.4839 | 9744.202 | 14.11 |
| 12 | 77.23 | 9712.272 | 14.83 |
| 13 | 76.9759 | 9680.317 | 15 |
| 14 | 28.4489 | 3577.67 | 0.42 |
| 15 | 23.8704 | 3001.888 | 1.78 |
| 16 | 22.2805 | 2801.946 | 0.44 |
| 17 | 19.6631 | 2472.787 | 0.74 |



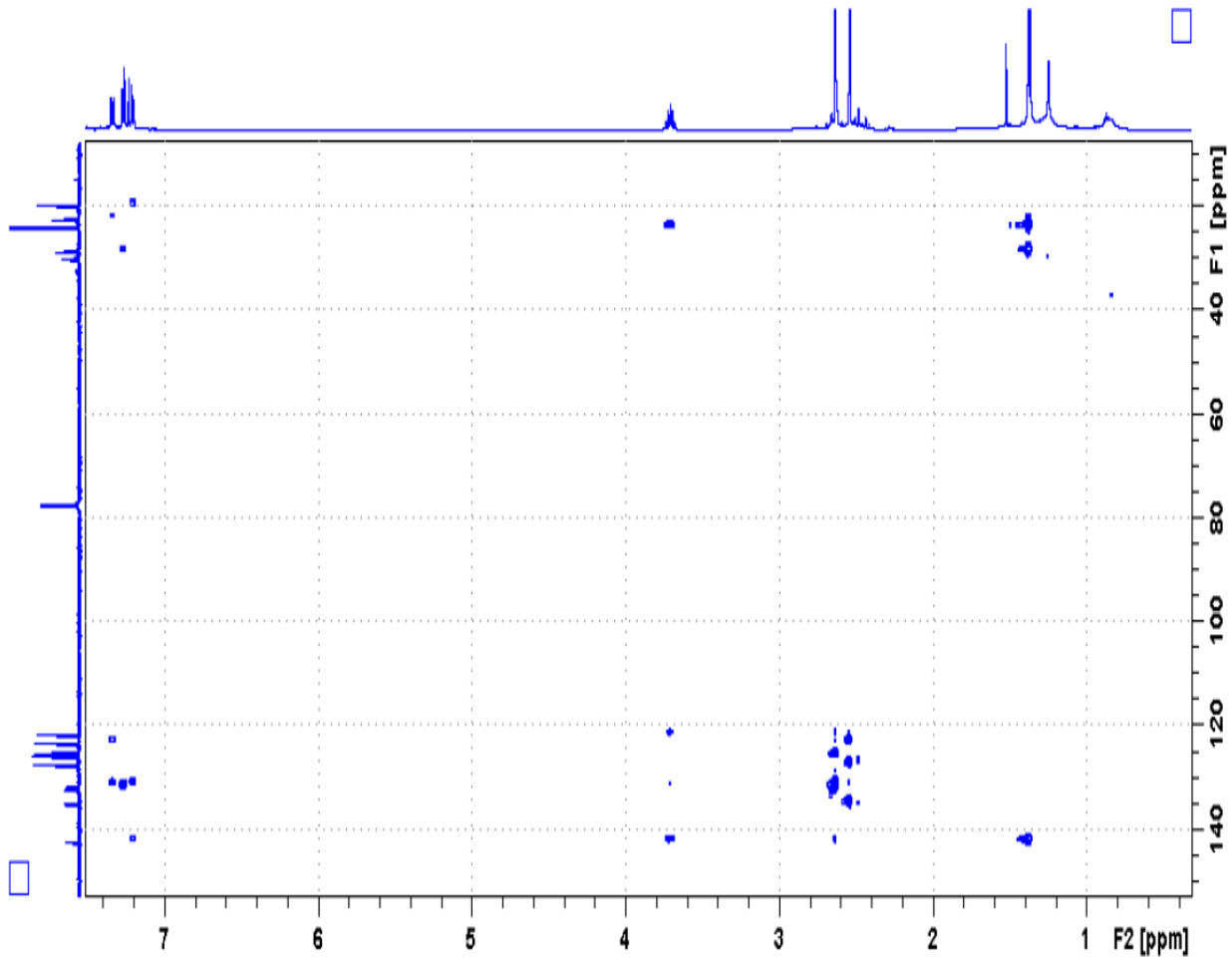
**APPENDIX 66: DEPT spectrum for Cadin-1(6),2,4,7,9-penta-ene (56) in $CDCl_3$**



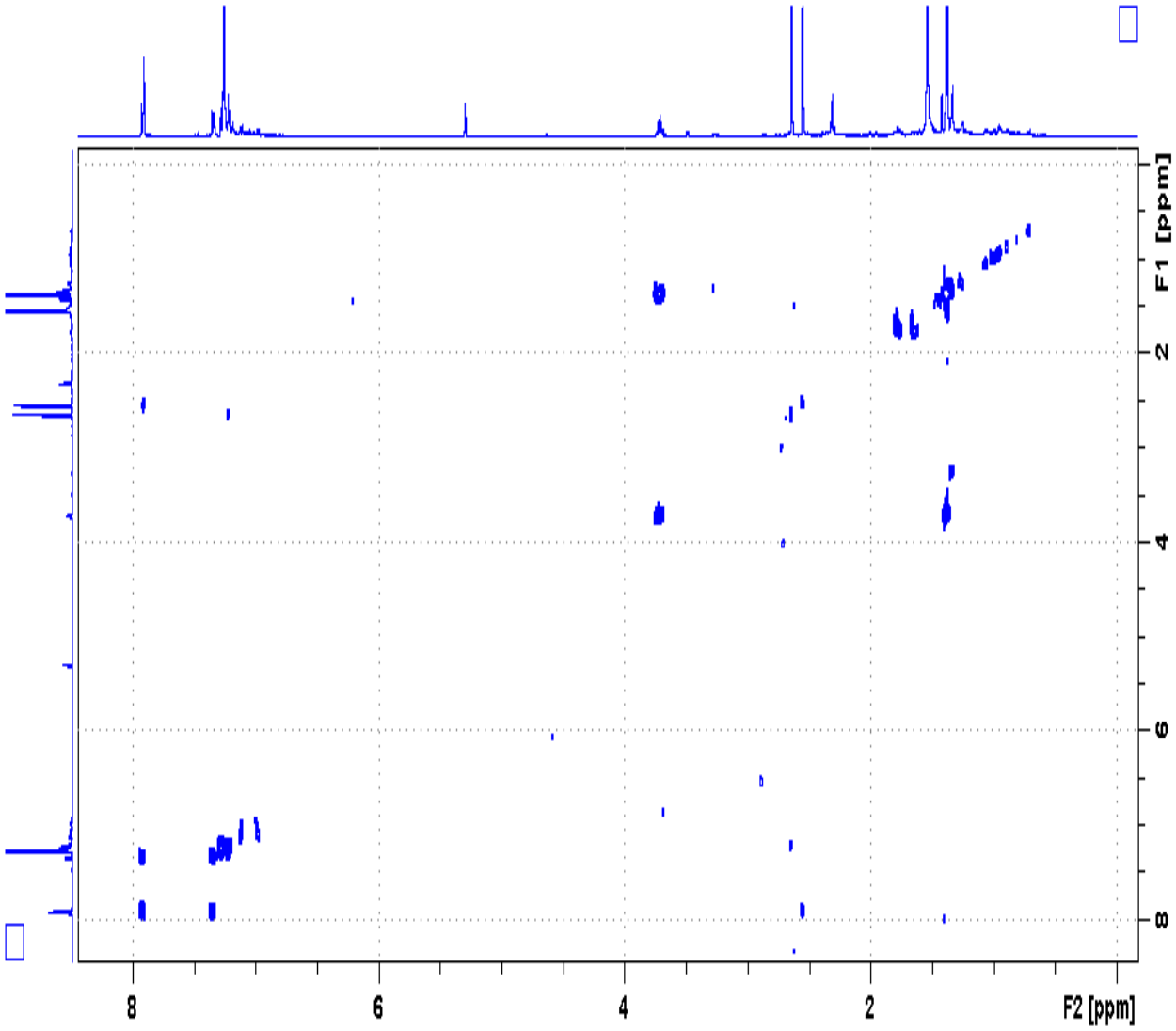
**APPENDIX 67: HSQC - DEPT spectrum for Cadin-1(6),2,4,7,9-penta-ene (56) in $CDCl_3$**



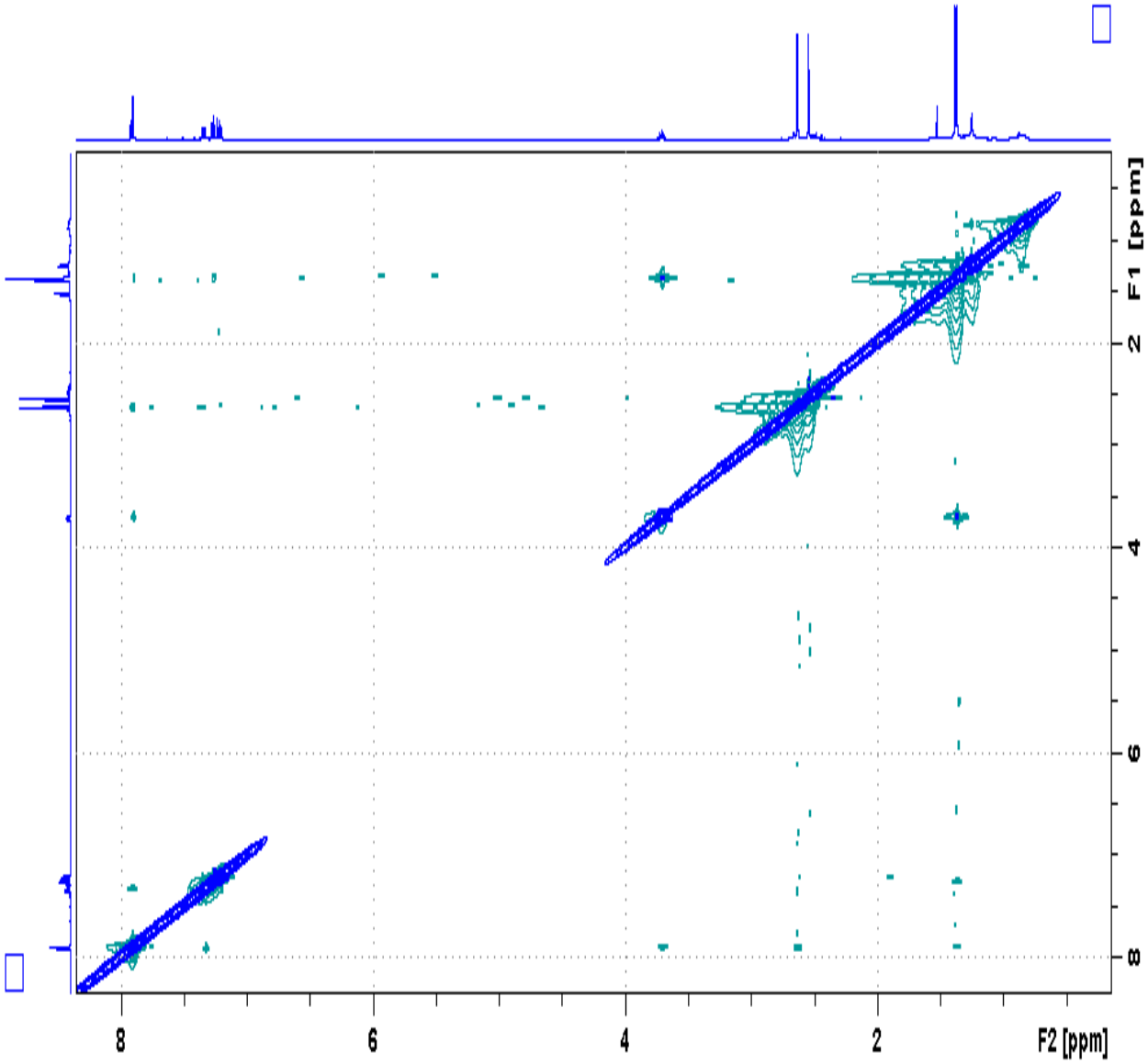
**APPENDIX 68: HMBC spectrum for Cadin-1(6),2,4,7,9-penta-ene (56) in $CDCl_3$**



**APPENDIX 69: COSY spectrum for Cadin-1(6),2,4,7,9-penta-ene (56) in $CDCl_3$**



**APPENDIX 70: NOESY spectrum for Cadin-1(6),2,4,7,9-penta-ene (56) in $CDCl_3$**



**Appendix : Research Permit**

THIS IS TO CERTIFY THAT:
*MR. DENIS KIPNGENO CHIRCHIR*
of EGERTON, 0-20115 EGERTON,has
been permitted to conduct research in
*Nakuru County*

on the topic: *SCREENING AND CHARACTERIZATION OF SOME ANTICANCER COMPOUNDS FROM SALICACEAE, MYRTACEAE AND SOLANACEAE FAMILIES*

for the period ending:
*18th July,2018*

.....
*Applicant's Signature*

Permit No : NACOSTI/P/17/76571/12820
Date Of Issue : 18th July,2017
Fee Recieved :Ksh 2000

.....
*Director General*
*National Commission for Science, Technology & Innovation*

## Appendix : Research Publication

Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 12(2): 999-1007, April 2018

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

*Original Paper* http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

# Chemical constituents of *Solanum mauense* (Solanaceae) and *Dovyalis abyssinica* (Salicaceae)

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**ABSTRACT**

Profiling the chemical constituents of medicinal plants used in folk medicine is vital in enhancing their full exploitation and utilization in modern medicine. The objective of the study was to characterize anticancer compounds from two medicinal plants, *Dovyalis abyssinica* and *Solanum mauense* that are used in folk medicine by the Kipsigis community, in Kenya, in managing various ailments. There is unvalidated folk medicine claim that the decoctions from the roots of *D. abyssinica* and fruits of *S. mauense* have anticancer property among other uses. This study reports on phytochemistry of *D. abbysinica* and *S. maunse.* Cold extraction method via soaking in solvents was used to prepare the dichloromethane crude extracts, which were later fractionated and purified using chromatographic techniques. Betulinic acid, benzoylated triterpenoid and fatty acids were isolated from dichloromethane extract of *S. mauense.* Similarly, two compounds, benzoic acid and tremulacin, were isolated from *D. abbysinica* alongside fatty acid. Anticancer property of betulinic acid, benzoic acid derivative and tremulacin has been reported previously in literature. This study gives the scientific basis for the use of these medicinal plants in folk medicine.


**Keywords**: Medicinal plants, *Solanum mauense, Dovyalis abbysisnica,* Betulinic acid and tremulacin.

## INTRODUCTION

Cancer is a general term applied to a series of malignant diseases that may affect different parts of a body (Sakarkar and Deshmukh, 2011). These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. Cancers may be caused in one of three ways, namely incorrect diet, genetic predisposition, and via the environment (Bhanot et al., 2011). The major treatment regime for cancer includes chemotherapy, radiotherapy and surgery (Owoeye et al., 2010).The synthetic anticancer remedies are beyond the reach of common man because of cost factors. Higher

 
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## Appendix : Research Publication

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# Chemical characterization of *Syzygium guineense* (Myrtaceae) stem bark extracts

**Denis K Chirchir, Peter K Cheplogoi and Josiah O Omolo**

**Abstract**
Medicinal plants used in the folk medicine may be an interesting and largely unexplored source for the development of potential new compounds. This study reports on the Phytochemistry and anticancer activity of *Syzygium guineense* (Myrtaceae). *S. guineense* is a medicinal plant that is traditionally used by the Kipsigis and the Ogiek communities in Kenya in the management of various human diseases. Decoctions from the bark of this plant is reported to have been used as a purgative, anthelmintic, antituberculosis, anticancer and treatment of chest ailments. Cold extraction method was used to prepare the crude extracts which were later fractionated and purified using chromatographic techniques (TLC and CC). Two previously established compounds, β-Sitosterol and Betulinic acid, whose anticancer activity has been reported were isolated alongside fatty acids. This study gives a scientific basis for the use of the medicinal plant in the traditional folklore as an anticancer agent.

**Keywords:** Medicinal plants, anticancer, *Syzygium guineense,* β-Sitosterol and Betulinic acid

## Introduction
The chemistry of natural products is very important and can be used in the search for bioactive compounds (Asif, 2015) [2]. Medicinal plants have been used practically in all cultures as a major source of medicament. This usage has been traced to the availability of secondary metabolites with medicinal properties. The availability of medicines plants and their cheaper cost in comparison to modern therapeutic agents makes them more attractive as therapeutic agents (Sharma *et al.* 2010) [18]. Ethno botany and ethno-medicinal studies are recognised as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents. Scientific investigations of medicinal plants have been initiated in many places because of their contributions to health care.
The Myrtaceae is a large, well-defined family, with about 140 genera and about 4000 species (Asif, 2015) [2]. The whole family is characterized by leathery glandular leaves containing viscous aromatic terpenoid and polyphenolic substances and flowers with numerous stamens. Several *Syzygium Syzygium* species were reported to possess antibacterial, antifungal, anti-inflammatory and antioxidant activities (Kamsala *et al.* 2014) [10]. The phytochemical studies of this species has revealed that only flavonoids and terpenoids were reported from the leaf and that the plant material has been unexploited much for detailed studies (Pulla Reddy *et al.* 2005) [16].
*Syzygium guineense* (Myrtaceae) is a small tree with edible fruits (Djoukeng *et al.* 2005) [9]. It is widespread in Sub-saharan Africa where the bark is traditionally used to treat stomachache and diarrhea. This medicinal plant has been used in traditional folk medicine by the Kipsigis and Ogiek communities in Kenya for managing various ailments. However there is unvalidated claim that it has anticancer properties.
The term cancer, malignant neoplasm (neoplasm means new growth) and malignant tumor are synonyms. Cancer is a general term applied to a series of malignant diseases which may affect many parts of the body (Berry *et al.* 2005) [3]. This disease is characterized by a rapid and uncontrolled cell proliferation leading to abnormal growth or tumor. If abnormal growth is not arrested it may progress to death of the patient.
Surgery, radiotherapy and chemotherapy are the options currently available for the treatment of cancer (Chudzik *et al.* 2015) [8]. Chemotherapeutic agents can provide temporary relief but cause serious side effects like bone marrow toxicity, neurotoxicity. Surgery is also not possible in all cases. There is urgent need for effective and safe anticancer drugs. A large number of bioactive compounds exist in various plant species. Among bioactive compounds, an important group is that of triterpenes, which show cytotoxic properties against tumor cells at low activity toward normal cells (Zuco *et al.* 2002) [21]. In our quest for novel bioactive