CHEMICAL CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF COMPOUNDS FROM SOME SELECTED MEDICINAL KENYAN *Ganoderma* AND *Trametes* SPECIES

MAYAKA REGINA KEMUNTO

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

EGERTON UNIVERSITY

JUNE 2020

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted for an award in any institution.

Signature: _____ Date: _____

Mayaka Regina Kemunto

SD11/0339/12

Recommendation

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

Signature: _____ Date: _____

Prof. Josiah Ouma Omolo, Professor of Natural Product Chemistry

Chemistry Department

Egerton University

Signature: _____ Date: _____

Prof. Peter Kiplagat Cheplogoi (Posthumously), Professor of Natural Product Chemistry

Chemistry Department

Egerton University

COPYRIGHT

DEDICATION

This work is dedicated to my loving family who gave me moral and material support to attain the highest academic qualification.

ACKNOWLEDGEMENTS

First, and foremost, I thank the Almighty God who saw it good to give me life and chance to complete my PhD study.

Egerton University for allowing me to pursue a degree in PhD Chemistry on a full time programme. The Chemistry department for accommodating me while pursuing my PhD degree. Special thanks goes to all the members of staff for their guidance, support and encouragement during my studies.

My intellectual debt to my advisors Prof. Josiah Ouma Omolo and late Prof. Peter Kiplagat Cheplogoi for their continuous support during my PhD study and research. Their constant motivation, enthusiasm, patience, immense knowledge and guidance helped during my research and writing of this thesis. I could not have imagined having better advisors and mentors for my PhD.

My deepest heartfelt appreciation to Prof. Dulce Mulholland and Dr. Moses Langat of the University of Surrey for their support in acquisition NMR Spectral data.

I would like to express my sincere gratitude to my colleague Dr. Alice Njue for her moral and emotional support during my studies. Without her persistent help and guidance, this thesis development would not have been conceivable. Most sincere gratitude to my officemates Abigael Wambui, Onyiego Kegoncha, colleagues Dennis Chirchir and Fasahat Parker for their continuous encouragement, offering me an ear even when the going was tough.

I would like to express my gratitude to the National Research Fund (NRF) under National Commission for Science, Technology and Innovation (NACOSTI) for funding this research project and Kenya Medical Research Institute for antimicrobial activity tests.

ABSTRACT

Ganoderma polypore species are hard, leathery poroid mushrooms that lack the distinct stipe. They are a unique group of fungi usually ignored by most fungi enthusiasts because of their typical inedibility, unfamiliar habitat and general opacity. In addition to their traditional use, contemporary research has suggested many applications for cancer treatment and boosting of the immune system. Due to increase in bacterial resistance to existing antibiotics infectious diseases have remained a major threat to human health. Hence bioactive compounds are continuously sought for disease prophylaxis and treatment. The main objective of the current work was to evaluate bioactive compounds from medicinal polypore species *Ganoderma adspersum*, *Ganoderma applanatum*, *Ganoderma australe* and *Trametes elegans* collected from Mau, Kericho area, Kabarnet and Kerio valley forests in Kenya. The dried, ground fruiting bodies of the species were extracted with methanol to give methanol crude extract, then consecutively extracted using ethyl acetate solvent to obtain an ethyl acetate extract. Fractionation and purification using column chromatography technique and further purification of some compounds was achieved on sephadex LH20. The chemical structures were determined on the basis of NMR spectroscopic data from ^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H-^{1}H COSY and NOESY experiments, and by comparing obtained results to the values indicated in previous studies. The polypore *Ganoderma adspersum* yielded ergostane compounds namely ergosta-7,22-dien-3-one (**54**), ergosta-7,22-diene-3β -ol (**55**) and ergosta-5,7,22-trien-3-ol (**56**). *Ganoderma applanatum* gave five compounds, **55**, **56**, 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol (**57**), *5α,8α*–epidioxyergosta-6,22-dien-3β-ol (**58**), 24-hydroxy-olean-12-en-3-one (**59**). The fruiting body of *Ganoderma australe* yielded compounds **55**, **56** and **57** and *Trametes elegans* gave **55**, **56**, ergosta-7,22-dien-3,5,6-triol (**60**), lupeol (**61**) and 9,19-cycloartane-3,30-diol (**62**). Antimicrobial activity was assessed against important clinical bacterial and fungal strains and zones of inhibition examined using one-way ANOVA through Tukey's PostHoc test. Most notable inhibition being against *Streptococcus pyogenes* 9.7±0.58 mm by compound **56,** 9.0±0.58 mm by compound **55**, 9.0±0.58 mm by a mixture of **57** and **58** and 8.0±0.33 mm by compound **59.** It was observed that all Gram negative bacteria were insensitive to the treatment of compounds. In conclusion the study has indicated that the isolated compounds have antibacterial properties hence have demonstrated their potential as antibacterial agents. The research has also revealed that our natural indigenous forests still harbours novel natural bioactive substances and strains that needs to be investigated for novel myco-medicines in the future.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBRIEVIATIONS AND ACRONYMNS

ANOVA	Analysis of Variance
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
COSY	Correlation Spectroscopy
D	Doublet
dd	Doublet of doublets
HMBC	Heteronuclear Multiple Bond Coherence
HSQC	Heteronuclear Single Bond Coherence
J	Coupling constant
KEMRI	Kenya Medical Research and Institute
M	Multiplet
MHz	Mega-hertz
MIC	Minimum inhibitory concentration
NADPH	β-nicotinamide adenine dinucleotide phosphate.
NMR	Nuclear magnetic resonance spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy
S	Singlet
SAR	Structure Activity Relationship
SEM	Standard error of mean
sp	Species
T	Triplet
TLC	Thin layer chromatography
UV-VIS	Ultra violet and visible light spectroscopy
WHO	World Health Organization of the United nations
Δ	Chemical shift in ppm

CHAPTER ONE

INTRODUCTION

1.1 Background information

Infectious diseases are caused by various pathogenic microorganisms that attack the body and cause immune responses. They have continued to be a leading threat to human health in both the developed and developing countries. Pathogenic organisms are divided into five classes namely bacteria, protozoa, fungi, viruses, and parasites. (Bannister *et al.*, 2000). A number of these pathogenic microbes have been found to cause diseases never experienced before; others are formerly known pathogens that are spreading to other new areas and affecting new or spreading to other populations for the first time. Hence microbial threats constantly continue to crop up, re-surface and persist (Lederberg *et al.*, 2003). The burden brought about by the infectious diseases has become more compounded as resistance to antimicrobials has grown rampant worldwide (Morens *et al.*, 2004).

Antibiotics are essential for treating pathogenic microbial infections such as meningitis, tuberculosis and pneumonia and also in avoiding infections during surgical treatments and helping persons with immunosuppressed immunity cope in life (Finch, 2007; Herbst *et al.*, 2009). In spite of the importance for novel antibiotics, the invention growth channel is constrained, chiefly those that gear towards significant multidrug-resistant Gram negative bacteria. In addition, antibiotic discovery which was at its apex in the 1940s to 1950s has dropped steeply. Furthermore, the worrying concern is the reality that almost all the antibiotics introduced to the market for the past three decades, have been modifications of the previously developed drugs Most recently, reports indicate that almost all the antibiotics at hand are derivatives of a class invented between 1940 and 1984 (Talkington *et al.*, 2016).

Coinciding with this, is the popping up of antibiotic-resistant microbes that have given rise to life-alarming infections that do not act in response to existing antibiotic therapies. Consequently, the pathogenic bacteria continue to develop resistance as the antibiotics are overused and misused making them less efficient and hence leading public health authorities worldwide to invoke antibiotic resistance as a compelling and viable public health threat (Butler *et al.*, 2013). Studies have indicated that decreasing the unprofessional and needless use of antibiotic drugs can help slow the resistance, but it cannot stop it. Eventually, after a period of time the existing antibiotics will lose their effectiveness, and patients will continue to need new antibiotics and remedies (Renwick *et al.*, 2016).

It is widely known that natural metabolites have been used in the field of anti-infectives, as a number of beneficial antibiotic drugs have been derived from natural sources since the launch of benzylpenicillin (penicillin G) during cancer treatment as an antibacterial agent in the 1940s. In addition, aminoglycosides, tetracyclines, cephalosporins, glycopeptides, macrolides, and rifamycins are other antibacterial classes that have been developed and utilized from natural sources (Demain, 2009). An Antifungal, griseofulvin and ivermectin, a polyene which is an antiparasitic drug, were also obtained of the natural source of the microbial origin (Dewick, 2002; Heindrich *et al.*, 2004; Evans, 2009; Samuelsson and Bohlin, 2009). Metabolites from natural sources have always attracted considerable notice in health promotion and disease treatment especially cancer (Wu *et al.*, 2002; Liu *et al.*, 2015). They have been found to be some of the best efficacious sources of drug leads for the management of many illnesses and diseases bearing in mind the high diversity of both marine and terrestrial organisms (David *et al.*, 2015).

Of these, are *β*-lactam and statin antibiotic classes which are natural components from filamentous fungi that have been established and utilized. The *β*-lactam class evolved from the first antibacterial agent discovered from a species of *Penicillium* by Sir Alexander Fleming hundred years ago (Fleming, 1929). Subsequently this led to more studies and development of the antibiotic penicillin by a group led by Florey (Florey *et al.*, 1949). This further unfolded the path for the development of many more ant infective and curative drugs. Penicillin was signalled as a fascinating drug for infectious and communicable diseases and it nevertheless remains as one of the most effective and least poisonous antibiotic (Demain and Elander, 1999).

Investigation of natural biodiversity for unique biologically active compounds, has been a vibrant area of medicine providing therapeutics or lead compounds of significant pharmacological potential over the past decades (De Silva *et al.*, 2013). Furthermore, the major path leading to establishing therapeutic drugs for numerous diseases is the natural product-based drug discovery. Most health products in existence are obtained naturally from microorganisms, some animals and plants. Often, the natural molecule is not used itself but serves as a lead molecule for manipulation by chemical or genetic means. In the period of 1981 to 2002 almost 80% of the 90 drugs introduced European countries and Japan are of microbial origin (Newman and Cragg, 2007).

Thus from research reports and estimations, amid the 12,000 antibiotics recognized, relatively more than half (55%) are obtained from streptomyces, 22% from filamentous fungi, 11% by other actinomycetes and 12% from other bacteria (Inouye *et al.*, 2004). In the past there has been a 75% drop in the number of freshly approved antibiotics. For example, a drug daptomycin approved in 2003, linezolid in 2000 and mupirocin in 1985 being the only novel antibacterial classes licensed since 1970 (Demain, 2009). Notwithstanding this, only a small number of dominant pharmaceutical companies have been previously researching on the discovery of novel antibiotic agents from natural sources, due to conceivable bacterial resistance against new antibacterial agents and disquiets in regard to rules governing the development of the drug (Butler, 2008). The pharmaceutical industry is generating hardly any new antibiotics due to lack of financial incentives, leading to a high demand for alternative sources of new drugs. Thus, there is an urgent requirement to find new antibacterial compounds from other novel bio diversities (ecological niches other than soil and bacteria) for the treatment of both animal and human diseases due to resistant bacteria (Ali *et al.*, 2018).

The kingdom fungi is classified into four different divisions that is Deuteromycota, Basidiomycota (club fungi), Ascomycota and Zygomycota. The members in the kingdom have been identified to fulfil vital functions in natural ecologies particularly in terrestrial ecologies and are regarded as the leading biodiversity reservoir from both aquatic and terrestrial sources on earth. Previously, the approximate figure of fungal species on the planet is around 1.5 million types, although those characterized worldwide is only nearly 7% of this number (Hawksworth, 2001, 2004). Thus, a very large amount of fungal strains remain obscured and need to be evaluated, identified, protected and made use of for the wellbeing of human kind in particular, the environment and mycobiota.

Thus the diverseness and exploration of therapeutic macro fungi constitute a prominent area for novel medicines (Zhong and Xiao, 2009). Most of the researches have reported that among the divisions of fungi, most studies were directed towards Basidiomycota strains as sources of biological significance *Ganoderma, Grifola,, Auricularia, Trametes, Coprinus Hericium, Flammulina, Laetiporus, Panus, Pleurotus, Tramella, Lentinula* and *Schizophyllum* (Polishchuk and Kovalenko, 2009).

Basidiomycetous fungi represent an unfathomable source of natural compounds of high curative worth and a huge number of bioactive components have been recognized throughout

the world in a number of medicinal species (Wasser and Weis, 1999b; Barros *et al.*, 2007; Kitzberger *et al.*, 2007; Turkoglu *et al.*, 2007; Wasser, 2011). Even though biological and medicinal researches have shown that the Basidiomycetous fungal species are a vast source of pharmacologically active components, quite a small number of these species have been characterized and even tested for beneficial therapeutic values (Lindequist *et al.*, 2005; Blackwell, 2011).

These Basidiomycetous fungi have been found to have various anticancer and immune properties. In addition, they display a diverse range of biological activities inclusive of antifungal, antibacterial, antiviral, ant allergic, immunomodulation, anti-inflammatory, anti-oxidative, cytotoxic, ant depressive, antihyperlipidemic, hypotensive, osteoprotective, antidiabetic, nephroprotective, hepatoprotective, neuroprotective activities and significant health benefits (Gargano *et al.*, 2017). Basidiomycetes fungi are good candidates for the greatly desired antibiotics since the cell glucans and the secondary metabolites from the cellular secretions by the mycelium and the heavy molecular polysaccharides have been found to have active compounds and well known immunodulating activities (Harkonen *et al.*, 2003; Zjawiony, 2004).

In addition, cultivated basidiomycetes contain huge variety of bioactive molecules with nutritive (Kalac, 2009) and some with therapeutic potential (Borchers *et al.*, 2004; Lindequist *et al.*, 2005; Poucheret *et al.*, 2006). After the establishment of new fermentation, isolation, purification and refining technologies, basidiomycetes fungi have received significant awareness as future springs of novel classes of anti infectives (Suay *et al.*, 2000). In addition, these group of fungi are capable of hindering the growth of bacteria, actinomycetes and other organisms, showing that the antimicrobial agents made by them have vital ecological ramification (Sidorova and Velikanov, 2000).

Humans and fungi are both eukaryotes and hence have common microbial enemies such as *Staphylococcus aureus*, *Pseadomonas aeruginosa* and *Escherichia coli*. Fungi are found in tropical habitats that harbour extreme ecological niches capable of unique secondary metabolism hence were first producers of antibiotics and are still regarded as crucial sources of active compounds. This fungi including polypores produce bioactive agents to fight infection from microorganisms, in the microbial contaminated environment and therefore humans can take advantage of these natural defensive strategy given the increasing prevalence of antibiotic resistance (Stamets, 2002). This has therefore prompted the

investigation on three *Ganoderma* and one *Trametes* polypore species for antimicrobial bioactive compounds against clinically important bacterial and fungal strains.

1.2 Statement of the problem

Microbial infectious and communicable diseases such as pneumonia, respiratory, urinary and gastrointestinal, candidiasis and staphylococcal food poisoning remain the major threats to human health worldwide. In addition, there has been a constant increase in antimicrobial resistance, emerging and evolving of new pathogens that have outpaced the development of new chemotherapeutics. Numerous natural and synthetic antimicrobial compounds have been identified and advanced to kill pathogenic microorganisms efficiently, but potential global spread, economic globalization and climate change, have represented contributing factors in the spread of the infectious diseases. Therefore, new bioactive compounds from different ecological sources are constantly pursued for disease prevention, treatment or adjunct therapy. The polypores are less explored compared to their counterparts in the plant kingdom. The enormous diversity, structural complexity and various different functional groups of the compounds from basidiomycetes is a factor in search for antimicrobial compounds. The basidiomycetes are largely endemic and consequently represent an under-explored resource of potentially unique metabolites. Investigation of the three *Ganoderma* species and one *Trametes* species from indigenous Kenyan forests has been prompted by the fact that no research information has been reported especially on the Kenyan polypore basidiomycetes and scientific prospects of obtaining bioactive compounds were evident.

1.3 Objectives

1.3.1 General objective

To investigate antimicrobial compounds from some selected medicinal Kenyan *Ganoderma* and *Trametes* species.

1.3.2 Specific objectives

i) To evaluate bioactive chemical constituents of the polypore species using column chromatographic technique.

ii) To determine the structures of the purified compounds using spectral data from NMR spectroscopic technique.

iii) To assess for the antimicrobial activity of the purified compounds against clinically important strains of bacterial and fungal pathogens.

1. 4 Justification

With a large number of bacteria developing resistance to existing antibiotics, extracts and derivatives from higher fungi hold pronounced potential for new medicines in current times. Due to their extensive chemical and structural diversity, natural biologically active agents are unmatched by any manmade synthetic library, they are evolutionally enhanced as drug-like compounds and might be considered biologically authenticated. The hypothesis, is that basidiomycetes particularly polypores provide a defensive immunological shield against a number of infectious diseases. The polypore genome fundamentally stands out as an untapped niche for new antimicrobials. The decreasing natural Kenyan forest plantations harbour novel polypore species and strains that may give on to novel medicines derived from fungi, conceivably beforehand the chance is lost as natural growth tropical rainforests are transformed into manmade tree plantations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Polypores basidiomycetes

Polypores, are higher macro fungi habituated to a variety of habitats, climates and substrates, they produce rich and diversified secondary metabolites (Schüffler, 2018). They are a major component of the basidiomycete fungi in forest ecosystems, they do not contain gills as most of the familiar mushrooms but pores that hold reproductive spores. They are sometimes called bracket fungi because they are shaped like shelves and not like umbrellas (although some are crust-like). They belong to the phylum, Basidiomycota, order Aphyllophorales, Hymenomycetes and Agaricomycetes classes. A group of tough complex structural terrestrial basidiomycetes. (Ryvarden and Johansen, 1980).

These crust-like fungi have often been observed as a source of wisdom and eternal strength owing to their hard and perennial fruit basidiocarps. They have been incorporated into the pharmacopeia and medicine of native societies worldwide. (Grienke *et al.*, 2014). They decay wood, assisting in the decomposition of dead wood, recycling the nutrients and minerals, hence discharging them after a period of time to be used by other forest organisms (Harmon *et al.*, 1986). Thus, they possess a noteworthy role in recycling of nutrients and producing carbon dioxide in the forest ecosystems.

Nearly all polypore fruiting bodies are perennial with some that ordinarily do not decay readily. Since they resist rot, they sometimes have algae and mosses growing on their crust surfaces. The undecaying nature is attributable to the ability of the polypores producing substances with anti-pathogenic activity in the microbial contaminated environment (Zjawiony, 2004). Majority of the polypores are saprobic and hence get their nutrients from the dead woody plant part, others grow on live trees and they decompose the non-functional heartwood. Some are parasites by invading plant tissues but do not kill the host and a few have a symbiotic relationship because they benefit mutually as they exchange carbon and nutrients with the roots of the plants (Peintner *et al.*, 1998).

Wood inhabiting basidiomycetes from genera *Ganoderma, Fomes, Fomitopsis, Inonotus, Phellinus, Trametes, Schyzophillum* and others have been found to produce different bioactive molecules, such as terpenoids, phenolic compounds and glucans with immune-modulating and antimicrobial activities (Wasser, 2010). Among these, three bioactive

molecules are licensed drugs inclusive of the caspofungin acetate, a pneumocandin B_0 derivative obtained semisythetically, from fermentation of the species *Glarea lozoyensis*. Caspofungin mode of action is by non-competitive inhibition of the β-(1,3)-d-glucan synthase enzyme that is by hindering the production of the β-(1,3)-d-glucan cell wall of the fungi, hence providing both fungicidal and a fungistatic result. The antifungal drug is used for infections by *Candida* species and is administered by slow intravenous infusion (Colombo *et al.*, 2010). Consequently, depicted as safe and important supplements to existing antimicrobial therapies natural product medicines may be used for the control of infectious diseases (Martin and Ernst, 2003).

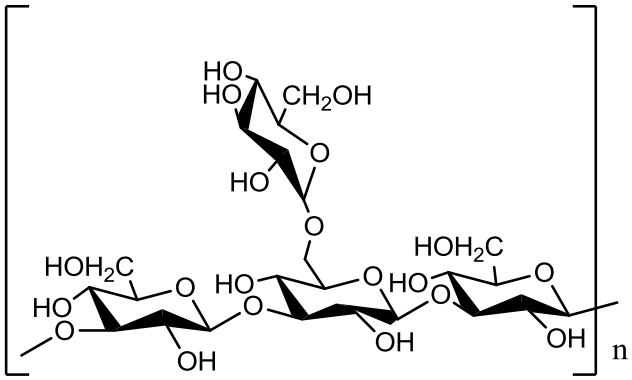
The medical consortium has accomplished a swift step in organ transplant advancement due to a drug cyclosporin that was obtained from a fungus whose host is an insect. The drug suppresses the immune system of the patients undergoing organ transplant hence increasing tissue acceptance rates. In addition to this, some organisms are used to commercially produce beer, cheese, wine, bread, vitamins and organic acids (Chang and Miles, 1989). Also, other natural products such as statins have successfully been advanced as drugs, they are used in lowering the possibility for coronary heart disease and hypercholesterolaemia (Shu, 1998). The first devoloped mevastatin drug in a class of statins, was also discovered from *Penicillium* fungal species (Endo *et al.*, 1976). Other statin medicines such as lovastatin drug from a fungus *Aspergillus terrus* have been isolated and developed (Alberts, 1988).

2.2 Biological activity of polypore basidiomycetes

Reports on research studies on the phylum Basidomycota, polypores are the predominant and divergent fungal sources of pharmacologically active natural products (Zjawiony, 2004). They have been found to be the fundamental sources of medicinal compounds such as antibacterial metabolites (Zjawiony, 2004). By way of illustration, *Fomes fomentarius* commonly known the tinder polypore, was used in the last two centuries in homeostatic bandages and dressings. The tinder polypore in addition to *Piptoporus betulinus*, birch polypore, have had a variety of medicinal uses and were found in 1991 with a 5,300-year-old corpse of an "Ice Man" in Otztal Alps of Italy glacier. *Ganoderma lucidum* (Reishi), *Polyporus umbellatus* and several others are used in oriental medicine, mostly in making tea like extracts. Another polypore of significant importance *Polyporus arcularius* which has

been observed to produce norsesquiterpene derivatives, for example drimanes, possessing antimicrobial effect towards Gram-positive bacteria (Fleck *et al.*, 1996).

A wide range of biological activities namely antiviral, antifungal, cardiovascular, antimicrobial, antioxidant anticancer, immunoenhancing, anti-inflammatory, nematocidal, and other activities have been displayed by secondary metabolites of polypores (Chihara, 1992; Limbird and Hardman, 2001; Hobbs, 2002). Some of the pharmacologically bioactive compounds from Basidiomycota species, including a number from polypores, have found their way to the market. The polypore, *Grifola frondosa*, commonly known as Maitake had previously drew immense attention from the pharmaceutical industry, especially in Korea and Japan, reason attributable to a sizable number of unique compounds being at play. The polypore transforms from gray mounds, labyrinthine folds, petals, and leaflets demonstrating one of great's divinely given nature (Deng *et al.*, 2009). The maitake D-fraction extract, an extract from the polypore has previously displayed strong anticancer activity by increasing immune-competent cell activity and it contains *beta*-1, 6 glucan with *beta*- D-1→3 branched chains (**1**) (Zjawiony, 2004).



1

Another polypore *Trametes versicolor*, is also among the most studied Chinese medicinal polypore in the genus, which is well known to possess a range of pharmacological activities including immune-stimulating activity (Li *et al.*, 2011), antitumor (Standish *et al.*, 2008) and antiviral effects (Teplyakova *et al.*, 2012). A bioactive mushroom extract, PSK, derived from of *T. versicolor* mycelia, has been proved to be effective against carcinogenesis (Fisher and Yang, 2002). The extract ratified by the Japanese Ministry of Health and Welfare in 1977 for use in cancer treatment is a protein-polysaccharide (Moon and Shibamoto, 2009). In addition, several other basidiomycetes, including polypores such as *Grifola frondosa*, *T. versicolor* and

Ganoderma lucidum provided polysaccharide preparations that were previously under clinical trials in China (Zjawiony, 2009).

The different classes of bioactive compounds produced by different polypores are polysaccharides, phenolics, terpenoids, lectins, statins and Muscarine alkaloids. The compounds from polypores have also shown to exhibit cytotoxic, antiviral, and antineoplastic activities. In addition, polysaccharides are other vital components of immense arsenal of compounds obtained from the cell walls of the fungi. The antitumor properties of these polyssacharides is due to their immunostimulating effects, hence they have for years attracted significant attention. Because they possess high molecular weights, the compounds are often called immunopotentiators or biological response modifiers as they stop carcinogenesis, prevent tumour metastasis and show direct anticancer effects (Zjawiony, 2004).

2.3 Compounds from polypores

Owing to the high diversity of various habitats that house polypores they develop rich and very unique secondary metabolites (Schüffler, 2018). The most studied members are of the family Ganodermataceae, species belonging to the most common polypore *Ganoderma lucidum* (Curtis) complex. The genus *Ganoderma* contains important and diverse pharmacological natural agents namely ergosterols, proteins, unsaturated fatty acids, polysaccharides, minerals, ganoderic acids and vitamins (Niu *et al.*, 2002). These compounds contain properties instrumental in stabilizing and balancing the body, therefore improving health and aiding in relief of a number of diseases (Zhou *et al.*, 2007). Notable number of secondary compounds from various chemical investigations on different *Ganoderma* species over the past four decades have been isolated. They include farnesyl hydroquinones, lanostanes, steroids, sesquiterpenoids, alkaloids, benzenoid derivatives, benzopyran-4-one derivatives and prenyl hydroquinone benzofurans (Baby *et al.*, 2015).

Among these isolated compounds, terpenoids have been found to have well-established activity toward various microorganisms such as bacteria, fungi, and protozoa (Cowan, 1999). In view of their well-known pharmacological activities, they had received considerable notable awareness (Zhou *et al.*, 2007). The terpenoids are the dominant collection of the secondary metabolite compounds from the basidiomycetes, especially from their basidiocarps and based on their structural characteristics they can be divided into acyclic, penta-, tetra-, tri-, di-, and monocyclic terpenoids (Dewick, 2002). The ergostane, lanostane, cucurbitane and saponaceolide polycyclic triterpenoids are the four types that have been obtained from

higher fungi. Notably, lanostanes accounting for the largest fraction of basidiomycetes triterpenoids followed by ergostanes. To date, the lanostanes obtained from fungal sources are regarded to be potential anticancer compounds and lanostanes from *Ganoderma* have been explored to the greatest extent (Rios *et al.*, 2012). Reports from various investigators indicate that nearly half of the isolated lanostanes are from the *G. lucidum* medicinal fungus (Liu and Chen, 2017).

These *Ganoderma* triterpenoids have displayed diverse pharmacological effects, including anti-inflammatory antitumor, antiplasmodial, and antiviral properties. So far, most of the pharmacological studies on *Ganoderma* terpenoids were aimed at their properties on their potential anti-inflammatory activity and the spread of tumour cells. Additionally, some of the *Ganoderm*a terpenoids have also been found to inhibit many enzyme activities (Liu and Chen, 2017). *Ganoderma* triterpenes have diversified chemical structures, a range of pharmacological activities and therefore are possible candidates for drug discovery (Xia *et al.*, 2014). These lanostane compounds have the same skeleton (**2**). The rings have 10β, 13β, 14α, 17β substituents and A/B, B/C, C/D a trans-configuration geometry. Furthermore, the parent skeleton nucleus often have substituents at C-3, 7, 11, 12, 15, 22, 23, 24 and 25 positions (Liu and Chen, 2017). The ergostane compounds also possess the same skeleton (**3**).

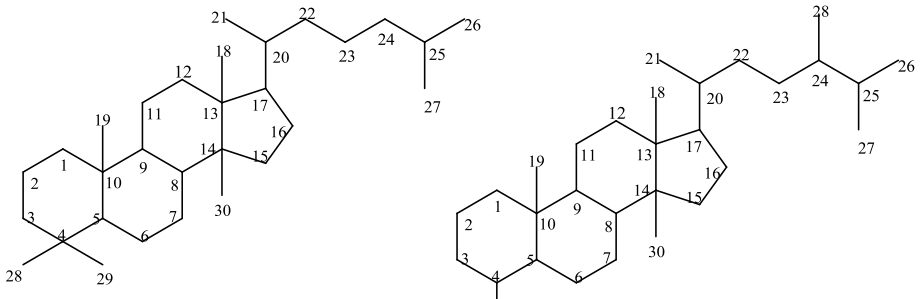


Figure 2.1: Triterpenoid skeletons from higher fungi

2.3.1 Biosynthetic pathway for triterpenoids, ergosteroids and related compounds isolated from polypores

Terpenoid natural products are the most structurally diverse and abundant higher fungi (Yang *et al.*, 2017) and have an immense variety of apparently unrelated structures. They are hydrocarbon derivatives formed from the joining of isoprene units. Terpenoids of different

types have been identified from polypore species and demonstrated therapeutic efficiency. Among all polypore species of importance, the major part of secondary metabolites is composed of triterpenoids, whereas other secondary metabolites classes are synthesized to a smaller extent.

Tetracyclic derivatives are the main group of triterpenoids and their glycosides and are represented by of protostane, dammarane, apotirucallane, tirucallane, euphane, lanostane, cycloartane and cucurbitane and pentacyclic derivatives of baccharane type like oleanane, bauerane, baccharane, lupane, taraxerane, glutinane, multiflorane, friedelane, pachysanane, taraxastene and ursane and hopane type such as neohopane, hopane, adianane, fernane, filicane, gammacerane (Breitmaier, 2006).

Steroids are modified triterpenoids that are biosynthesized from the triterpene lanosterol and have a common tetracyclic carbon skeleton. It is a polycyclic alkane compound containing four cycloalkane rings that are fused to each other in a typical trans-arrangement (Sarker and Nahar, 2007). They are fat soluble compounds and have been found to possess a significant role in living organisms. Cholesterol and cholic acid are examples of initial known natural steroids. The ergosterol steroid is a major sterol in basidiomycetes rather than cholesterol in animal and is a precursor of a number of polyoxygenated ergostane steroids, which possess unmatched structural diversities and extraordinary properties (Sarker and Nahar, 2012).

2.3.2 The mevalonate pathway to triterpenoids and steroid synthesis

The basic structure of lanosterol is the foundation of the chemical structure triterpene, which is the principal intermediate in the biogenesis pathway for triterpenes including steroids in microorganisms and animals (Chang and Buswell, 1999). The various diversities of triterpenoids is due to the difference in stereochemistry of the structures produced. Tetracyclic triterpenoids such as sterols and related compounds occur in tremendous variety in the various taxonomic groups of plants and animals (Sandermann, 1962). The steroid compounds are obtained from the C_{30} squalene oxide, hence are derived from triterpenoids (Dewick, 2002). They have a wide variety of pharmacological functions and many have potential therapeutic applications (Eisenreich *et al.*, 1998). For example, cholesterol, is a crucial constituent of cell membranes of eukaryotic organisms. All terpenoids are assembled biosynthetically from two precursors: isopentenyl pyrophosphate (IPP) (or isopentenyl diphosphate (IDP)) and dimethylallyl pyrophosphate (DMAPP). These two biochemical isoprene precursors are obtained by two pathways, by way of intermediates mevalonic acid

(MVA) or 1-deoxy-Dxylulose 5-phosphate simply deoxyxylulose phosphate (DXP). But fungal sterols, are formed predominantly via mevalonate (**Figure 2.2**) (Shiao, 2003; Nes, 2011).

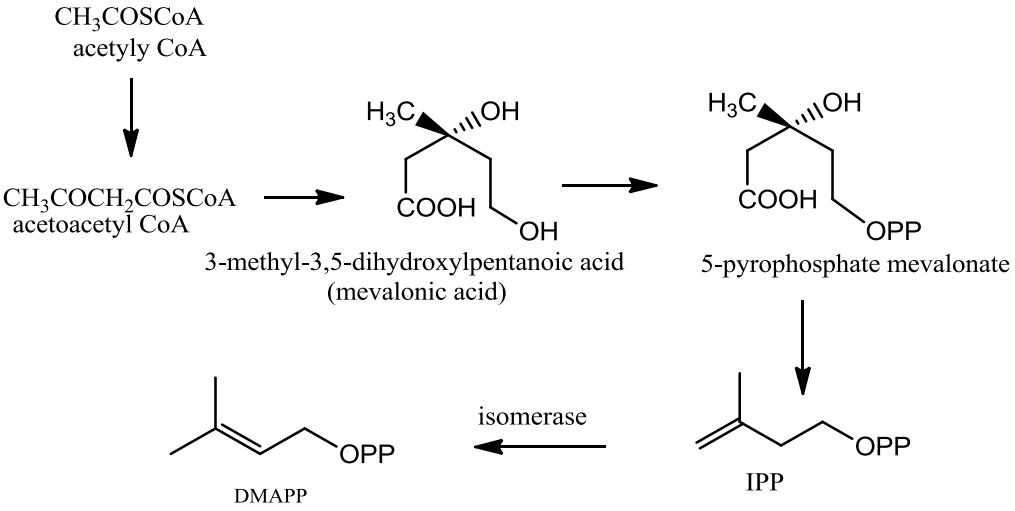


Figure 2.2: Formation of biochemical isoprene units in fungi

When the C_5 isoprene units are linked together in a head to-tail way they form the terpenoids which are the huge and structurally diverse group of natural products. The tetracycles are also produced from these precise arrangements of the building block C_5-isoprenoid, isopentyldiphosphate. Triterpenes are C_{30} compounds which are products of joining six C_5 isoprene units in order to conform to the verifiable isoprene rule (Ruzicka, 1953). Two isoprene units form geranyl diphosphate (GPP). The addition of a C_5 unit to geranyl diphosphate yields farnesyl diphosphate (FPP) (Nes, 2011).

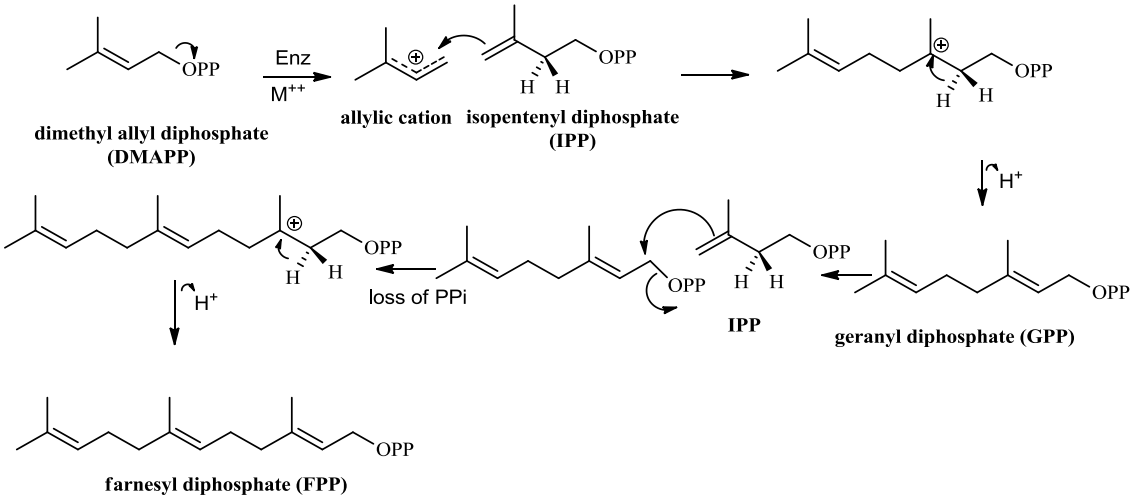


Figure 2.3: Formation of farnesyl diphosphate (FPP)

The biosynthesis of triterpenoids solely occurs in the cytol cytoplasm making use of IPP and its isomer DMAPP that are obtained from acetyl CoA via cytosolic mevalonic acid pathway. An IPP (C_5) molecule condenses with its isomer DMAPP (C_5) to form a monoterpene (C_{10}) called geranyl pyrophosphate (GPP), by action of the enzyme prenyl transferase. The monoterpene GPP is joined with another IPP to form farnesyl pyrophosphate (FPP) which is a sesquiterpene molecule. In both of these above head to tail condensations, the reactions are catalysed by the enzyme prenyl transferase. In the above reactions a carbocation is formed as an inorganic diphosphate (PP_i) is eliminated. Then through the enzyme squalene synthase, two molecules of FPP are linked together to form a triterpene squalene (C_{30}). These triterpenes are formed by two molecules of farnesyl diphosphate joining tail to tail to yield the hydrocarbon squalene and through the extension of the familiar process of adding IPP to the growing chain. Initially, one farnesyl diphosphate molecule undergoes loss of inorganic diphosphate (PP_i) and addition of the other molecule of FPP through the allylic carbocation to the alkene end and accompanied by loss of a hydrogen ion gives presqualene pyrophosphate. Presqualene pyrophosphate (PSPP) loses PP_i and the presumptive cyclopropylcarbinyl cation go through ring opening and reduction by NADPH to squalene in the subsequent step.

The C_{30} symmetric alkene via an NADPH-dependent mono-oxygenase reaction and action of the catalyst squalene epoxidase (SQE), undergoes oxidation to form S-oxidosqualene and the intermediate finally undergoes cyclization by an oxidosqualene sterol synthase to produce lanosterol, a steroidal skeletal structure. The cyclization of squalene-2,3-oxide folds in a chair–boat–chair–boat–conformation producing a 17β-side chain with a 17α-hydrogen at C_{20}. Several subsequent modifications occur to form cholesterol in mammals and after the cyclization of 2,3-oxidosqualene to form lanosterol more tranformations yield ergosterol in fungi. Loss of the C-4 methyl and C-14 methyl groups, the reduction reaction of C-24 double bond and a number of double bond shifts leads to the formation of ergosterol. Meanwhile, the biosynthesis of ergosterol has several other steps that result in a methyl group at C-24 and two more double bonds at C-7 and C-22 of the ergosterol side chain. The most abundant type ergosterol has a hydroxy group at C-3 of the sterol backbone ring and a double bond at C-5. Due to these structural differences and abundance ergosterol is outstandingly suited for fulfilling both the membrane and cellular needs of the fungi (Benveniste, 1986; Nes, 2011).

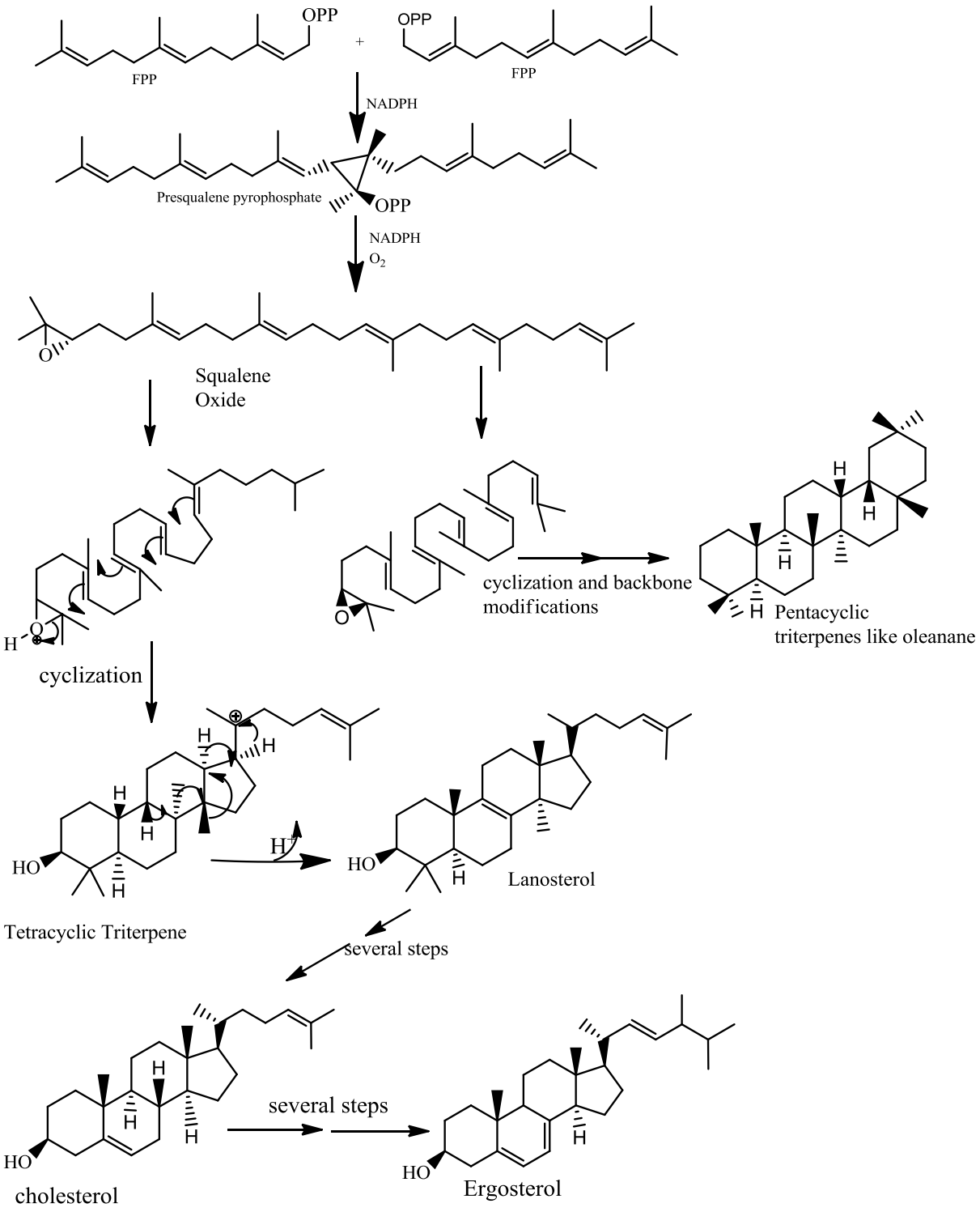


Figure 2.4: Biosynthetic pathway of tetracyclic and pentacyclic triterpenoids from farnesyl pyrophosphate (FPP)

The pentacyclic triterpenoids of baccharene type are the result of another cyclization of the 2,3-epoxysqualene to form a six-membered ring, succeeding rearrangements (Wagner-Meerwein) produces the intermediate cation of tetracyclic triterpene 3β-hydroxybacchar-21-

ene, and finally folds the fifth ring to the pentacyclic 3β-hydroxylupanium ion. Thus, modifications in baccharane structure produce a group of pentacyclic triterpenes. The connection of bond from C-18 to C-21 of baccharane closes the five-membered ring E yields the pentacyclic lupane. The movement of C-21 from C-19 to C-20 leads the formation of oleanane, a six-membered ring E (**Figure 2.4**). Oleanane then may undergo methyl shifts to a variety of other pentacyclic triterpenoids (Breitmaier, 2006; Grishko *et al.*, 2015).

2.4 Antimicrobial activity of polypore extracts

Toxicity and drug resistance and are still the main drawbacks in achieving healing properties even though in the past years, several synthetic antimicrobial compounds have been identified, refined and developed (Gao *et al.*, 2005). The cellular polyssacharide glucans of the polypores have been found to be exhibit immunomodulatory properties, and many of the excreted secondary metabolite components of the mycelium combat bacteria (Deyrup *et al.*, 2007). *Ganoderma* species has been reported as a prolific source of bioactive compounds of antimicrobial properties. The terpenoids, terpenes, and polyketides of farnesyl quoninesare some are the major types of secondary metabolites.

Polar alcoholic solvents such as ethanol and methanol are the preferred solvents for the extraction of antibacterial compounds than other organic solvents. Although other factors such as part of fruiting body and test bacterial organisms used may limit the choice of the solvent (Basnet *et al.*, 2017). Studies that have employed the use alcoholic solvents for extraction have also shown very low minimum inhibitory concentration (MIC) (Li *et al.*, 2012; Shang *et al.*, 2013; Cilerdzic *et al.*, 2016). Compounds from the several studies on the fruiting bodies of *Ganoderma* species affirm that they have the antibacterial properties against different types of Gram positive bacteria, Gram negative bacteria as well as the mycobacteria (Al-Fatimi *et al.*, 2005; Isaka *et al.*, 2015).

Studies done in the early 1950s and several other studies carried in the 1990s reported that a number of polypore fungi had antimicrobial activities. In one of the studies, on thirty four polypore basidiomycetes species, the mycelial extracts of the polypore showed antimicrobial activity against the bacterial strains *Bacillus subtilis*, *B. cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* species and the fungus *Candida albicans* (Coletto and Mondino, 1991). In another report, the antimicrobial properties of the culture and mycelial filtrates of twenty eight polypore basidiomycetes were

also evaluated against various bacterial strains such as *B. subtilis, B. cereus, E. coli, S. aureus, Agrobacterium tumefaciens, Salmonella typhimurium,* and *C. albicans* (Coletto and Striano, 2000). Other studies have also shown that the crude extracts of the polypores such as *Ganoderma applanatum*, *G. adspersum*, *Stereum purpureum*, *S. hirsutum*, *Armillariella mellea*, *Agrocybe praecox*, *Hypholoma sublateritium*, *Macrolepiota procera*, *Clitocybe nebularis* and *Pholiota abstrusa* possess activity towards the strains of both Gram-positive and Gram-negative bacteria.

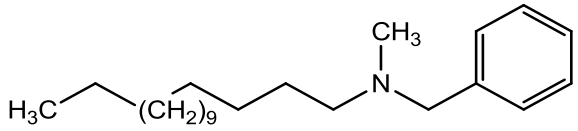
In other earlier researches *Ganoderma lucidum* together with other *Ganoderma* species showed that they possess antimicrobial agents that hinder the growth of both Gram-negative and Gram-positive bacteria (Yoon *et al.*, 1994a). Additionally other studies demonstrated that blending *Ganoderma lucidum* extract with three common antibiotics that is cefazolin, ampicillin and chloramphenicol resulted in a combined effect in all test cases, synergism effect in two occasions, and antagonism in two instances. Synergism effect was observed when *ganoderma* was combined with cefazolin antibiotic against *B. subtilis* and *Klebsiella oxytoca* (Yoon *et al.*, 1994b). From a recent study, two methanol extracts from *Trametes versicolor* and *Ganoderma lucidum* were tested against various bacterial pathogen strains that is *B. cereus S. aureus, S. typhimurium, M. flavus, L. monocytogenes, P. aeruginosa, Saccharomyces cerevisiae, E. coli and E. cloacae* and a fungal yeast. The most notable effect was against *S. cerevisiae* with a MIC_{50} value of 3 ug/mL for *Ganoderma lucidum* extract and MIC_{50} value of 24 ug/mL for *Trametes versicolor* extract (Hleba, 2014).

Furthermore, antibacterial activity of different extracts of *Ganoderma praelongum, G. lucidum,* and *G. resinaceum* were investigated against thirty strains of medical bacteria isolates of methicillin-resistant and sensitive *Staphylococcus aureus*. Most active was the *G. praelongum* ethyl acetate extract and was found to contain sesquiterpenoid compounds that exhibited a maximum activity of 35.67± 0.62 µm and a MIC value of 0.390–6.25 mg/mL (Ameri *et al.*, 2011).

In a most recent study, varying antimicrobial activities with zones of inhibition ranging from 1.50 to 25.50 mm were shown by the different solvent extracts of *Trametes elegans*. The methanol extract exhibited a superior antibacterial activity against the strain *B. cereus* NCIB 6344 with an inhibition zone of 20.5 mm, while the acetone extract of the polypore *T. elegans* exhibited a higher antifungal activity against *Aspergillus fumigates* with a zone of 25.5 mm (Awala and Oyetayo, 2015).

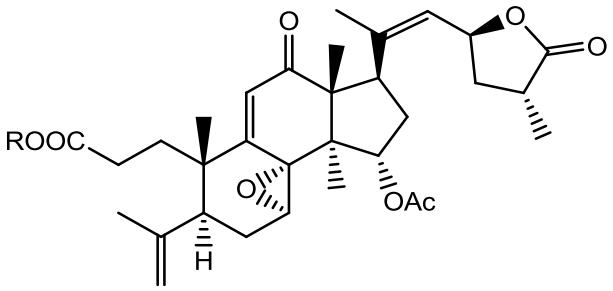
2.4.1 Anti-bacterial compounds from the polypores

In the course of study for antimicrobial metabolites from basidiomycetes, a submerged culture of a polypore *Piptoporus betulinus* Lu 9-1yielded piptamine compound (**4**). Minimum inhibitory concentration (MIC) values were *Bacillus subtillis* ATCC 6633 (1.00), *Staphylococcus aureus* 134.9 (46.25 µg/ml), *S. aureus* SG 511 (0.78 µg/ml), *Kluyveromyces marxianus* IMET 25148 (6.25 µg/ml), *Enterococcus faecalis* 1528 (1.56 µg/ml), *Rhodotorula rubra* IMET 25030 (50.0 µg/ml) and *Escherichia coli* SG 458 (12.5 µg/ml). The study indicated that piptamine is important for managing microbial effects and demonstrated its antibacterial activities, notable MIC results being for *E. faecalis* and for *S. aureus* SG 511 (Schlegel *et al.*, 2000).



4

In search for bioactive agents, compounds methyl australate (**5**) and australic acid (**6**) were isolated from *Ganoderma australe.* The two compounds were shown to be active against fungi and Gram-positive bacteria, the methyl ester being also active against Gram-negative bacteria (Smania *et al.*, 2007).

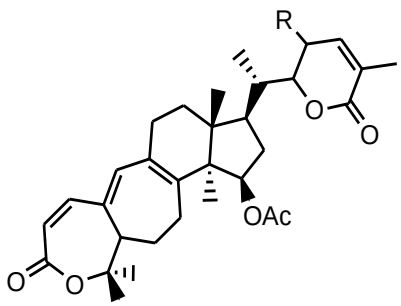


5. R= CH_3
6. R=H

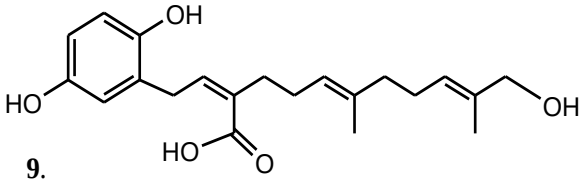
A previous study yielded 23-hydroxycolossolactone E (**7**) and colossolactone E (**8**), these colossolactones-triterpenoids, were found to hinder the growth of *Pseudomonas syringae* and *Bacillus subtilis*. However, the MIC and minimum bactericidal concentration (MBC) of isolated compounds against this bacterium were not determined (Ofodile *et al.*, 2012). Moreover, antibacterial and antifungal activity against a number of Gram-positive and Gram-negative bacteria of two previously undescribed compounds farnesyl hydroquinones named ganomycins B (**9**) and A (**10**) isolated from *Ganoderma pfeifferi* was tested (Mothana *et al.*, 2000). The notable MIC values of compounds **9** and **10** were 2.5 µg/ml against *Micrococcus*

flavus and 25 µg/ml against *Staphylococcus aureus* respectively whereas the positive control ampicillin had 0.05 and 0.25 µg/ml for *S. aureu*s and *M. flavus*, respectively.

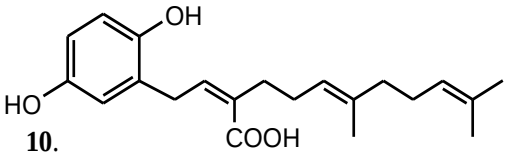
.



7. R=OH
8. R=H

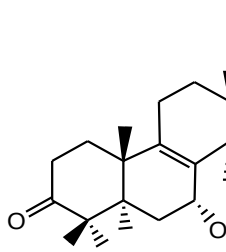


9.

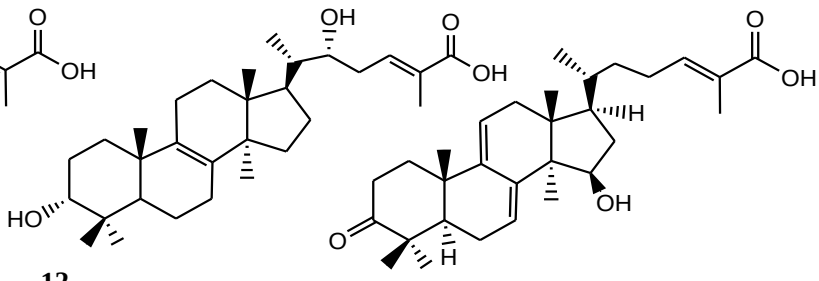


10.

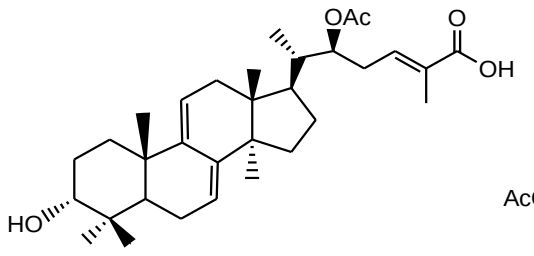
In another previous study, forty two (42) antitubercular triterpenoids of lanostane type, both previously undescribed and known, have been obtained from cultures of the *Ganoderma* polypore species BCC 16642. A study on methanol and ethyl acetate extracts of *Ganoderma* species BCC 16642 gave diverse compounds including ganorbiformin F (**11**), astraodoric acid C (**12**), ganoderic acid TR (**13**), ganoderic acid S (**14**) and ganoderic acid T (**15)** (Isaka *et al.*, 2015). The isolated *Ganoderma* triterpenoids were assessed for anti-TB activity against *Mycobacterium tuberculosis* H37Ra. All the compounds that were screened to determine anti-TB activity against *Mycobacterium tuberculosis* H37Ra were found to be in the range of 0.781–50 µg/ml. During the study the structure−activity relationship of these active compounds was also recommended.



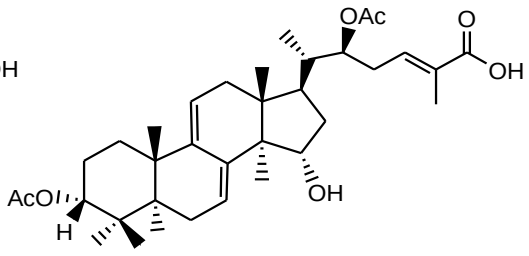
11



12 **13**

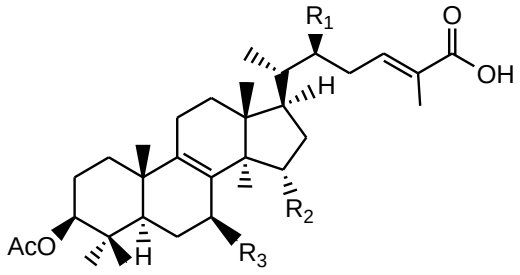


14

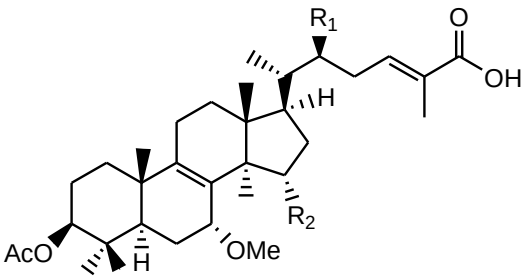


15

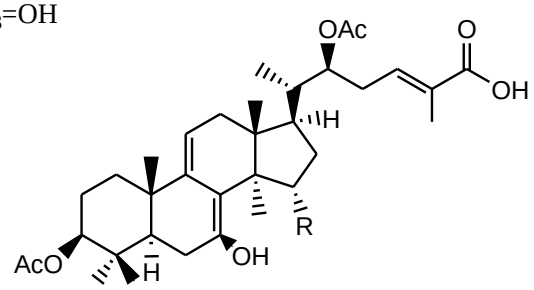
In addition, lanostanoids, (24E)-3β-acetoxy-7α-hydroxylanosta-8,24-dien- 26-oic acid (**16**), (22S,24E)-3β,15α,22-triacetoxylanosta-8,24-dien-26-oic acid (**17**), (22S,24E)-7α-hydroxy-3β,15α,22-triacetoxylanosta-8,24-dien-26-oic acid (**18**), (24E)-3β,15α-diacetoxy-7α-hydroxylanosta-8,24-dien-26-oic acid (**19**), (22S,24E)-7α-methoxy-3β,15α,22 triacetoxylanosta-8,24-dien-26-oic acid (**20**), (22S,24E)-3β,22-diacetoxy-7α-methoxylanosta-8,24-dien-26-oic acid (**21**), (22S,24E)-7α-hydroxy 3β,22-diacetoxylanosta-7,9 (11),24-trien-26-oic acid (**22**) and (22S,24E)-7α-hydroxy 3β,15,22-diacetoxylanosta-7,9 (11),24-trien-26-oic acid (**23**) were also isolated (Isaka *et al.*, 2015). Studies from structure activity relationships among the 3β-acetoxy derivatives were investigated and it was observed that the (3β-OAc) functional group appeared to be vital for the anti-TB activity of the compounds (Isaka *et al.*, 2015).



16 R_1= H,R_2=H,R_3=OH
17 R_1= OAc,R_2=OAc,R_3=H
18 R_1=R_2=OAc, R_3=OH
19 R_1=H,R_2=OAc,R_3=OH



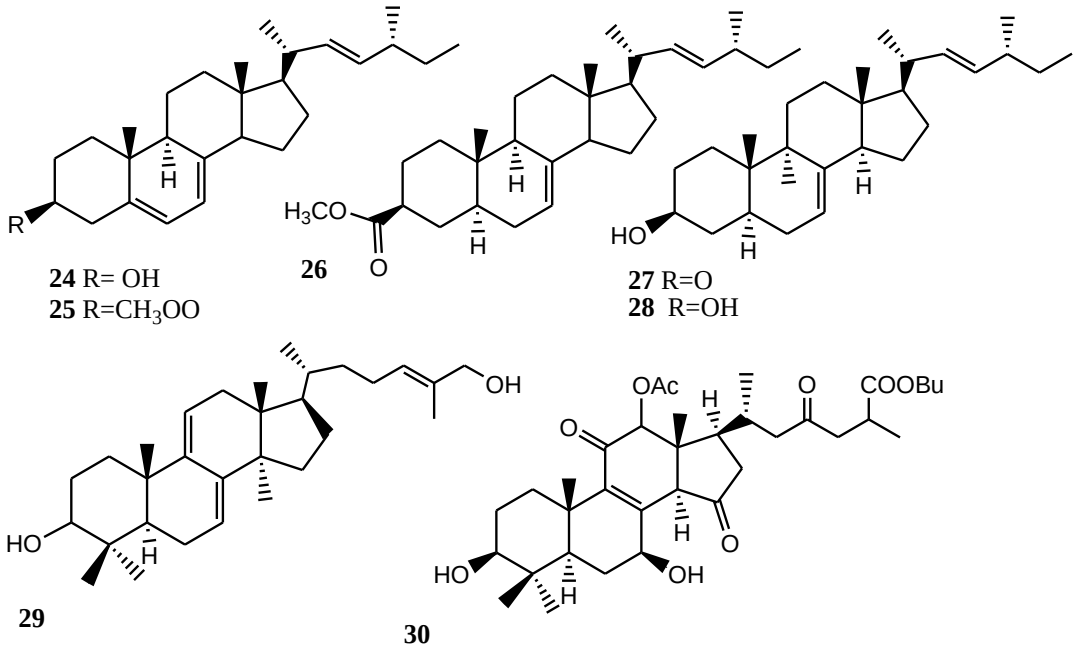
20 R_1=R_2=OAc
21 R_1=OAc, R_2= H



22 R= H
23 R= OAc

Furthermore, egorstane steroid compounds such as ergosta-5,7,22-trien-3β-ol (**24**), ergosta-5,7,22-trien- 3β-yl acetate (**25**), ergosta-7,22-dien-3β-yl acetate (**26**), ergosta-7,22-dien-3-one (**27**), ergosta-7,22-dien-3β-ol (**28**) and ganodermadiol (**29**) were observed to have MIC value ranging from 2.5–5 mg/ml against *Staphylococcus aureus* and *Bacillus subtilis* (Vazirian *et al.*, 2014). In addition, the basidiocarps of *Ganoderma lucidum* gave a bioactive compound 12β-acetoxy-3β,7β-dihydroxy-11,15,23-trioxolanost-8-en-26-oic acid butyl ester (**30**). The compound showed noteworthy inhibition against *S. aureus* and *B. subtilis* with MIC values of 68.5 and 123.8 µM, respectively with the positive control ampicillin having values of 19.3

and 4.1 µM, respectively (Liu *et al.*, 2014). Literature survey from various investigations have shown that a number of the antibacterial studies are conducted on crude extracts with noteworthy results rather than pure compounds (Sa-ard *et al.*, 2015; Zengin *et al.*, 2015; Cilerdzic *et al.*, 2016; Rios and Andujar, 2017). It was previously noted that, its only compounds (**9**) and (**10**) that have been screened against the Methicillin-Resistant *Staphylococcus aureus* (MRSA)-infected mouse in the *in vivo* model (Mikolasch *et al.*, 2016).



24 R= OH
25 R=CH_3OO

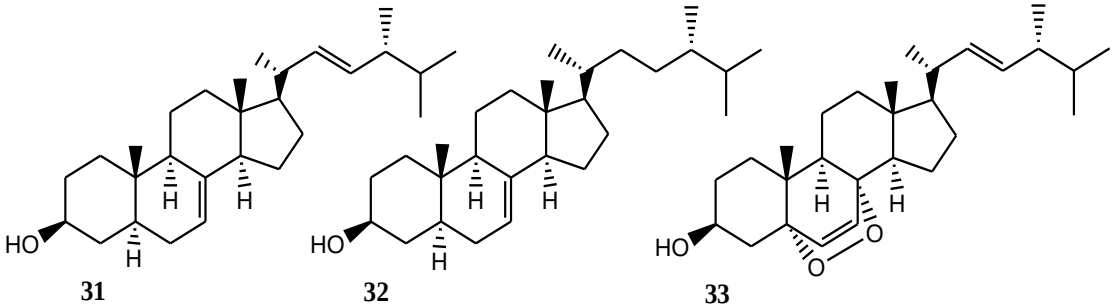
26

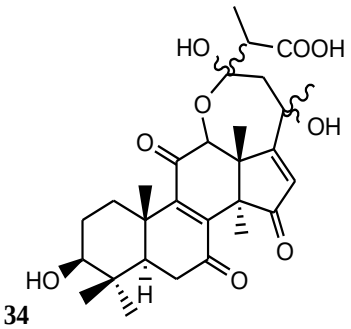
27 R=O
28 R=OH

29

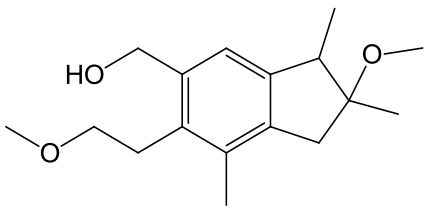
30

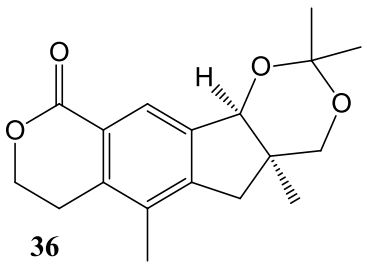
Further, screening of crude extracts of the fruiting bodies of *Ganoderma resinaceum* and *Ganoderma lucidum* showed discriminative activity against *Bacillus subtilis.* Earlier *Ganoderma applanatum*, recognized as the artist's conk, had provided the sterols 5α-ergosta-7,22-dien-3β-ol (**31**), 5α-ergot-7-en-3β-ol (**32**), 5,8-epidioxy-5α, 8α - ergosta-6,22-dien-3β-ol (**33**) and a previously undescribed triterpene lanostanoid (**34**) that were effective largely against Gram- positive bacteria (Smania Jr *et al.*, 1999).





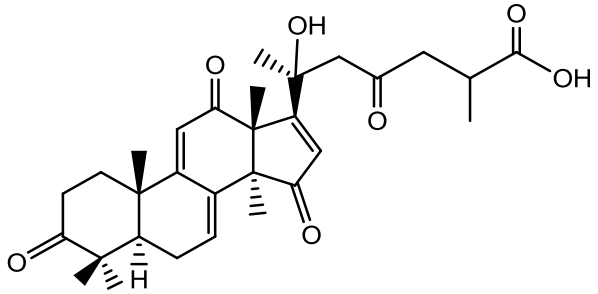
In other later studies steroids 5α-ergosta-7, 22-dien-3β-ol and 5,8-epidioxy-5α, 8α-ergosta-6, 22-dien-3β-ol were also obtained from *G. applanatum* and demonstrated to be weakly effective against many Gram-negative and Gram-positive microorganisms (Lindequist *et al.*, 2005). The ganoderic acid T C-3 epimer of the compound (**15**) was also found to exhibit important anti-TB activity against *mycobacterium tuberculosis* H37Ra (Niedermeyer *et al.*, 2005). Compounds applanatine C (**35**) and applanatine D (**36**) were also observed to suppress the development of *Fusobacterium nucleatum* that causes periodontosis, the bacteria strain being a dominant member that the oral microbioata (Fushimi *et al.*, 2010).





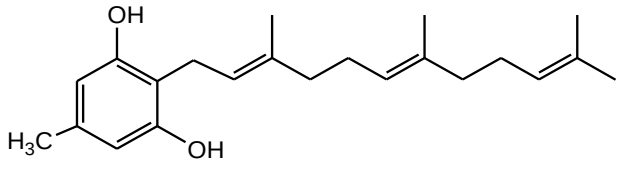
Recently, a previously undescribed lanostane triterpenoid (**37**), among other three known compounds methyl australate (**5**), applanoxidic acid C and applanoxidic acid F, were obtained from the cultivated basidiomycete *Ganoderma australe* fruiting bodies. Compounds **38**, **39** and **40** were evaluated against *M. tuberculosis* H37Ra and were found to be ineffective in these assay at a concentration of 50 μg/mL. The compounds were also ineffective towards a

number bacteria such as *Acinetobacter baumannii, Klebsiella pneumonia, Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Isaka *et al.*, 2017).

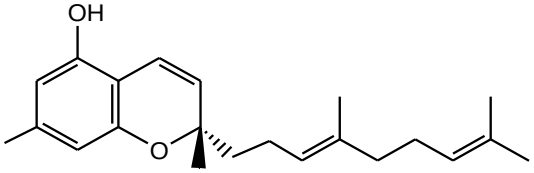


37

In another study previously undescribed compounds from two American polypore species, *Jahnoporus hirtus* and *Albatrellus flettii* were found to be active against a number of bacterial strains. The compounds were a new lanostane-type triterpene, 3,11-dioxolanosta-8, 24(Z)-diene-26-oic acid from *J. hirtus* and from *A. flettii* grifolin (**38**), confluentin (**39**) and neogrifolin . Compound **39** showed good activities against *Bacillus cereus* by 10 μg/mL and *Enterococcus faecalis* by 0.5 μg/mL (Liu *et al.*, 2010b).

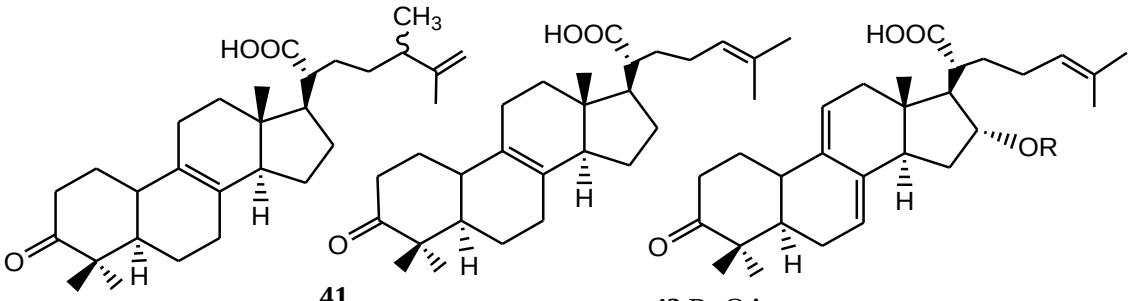


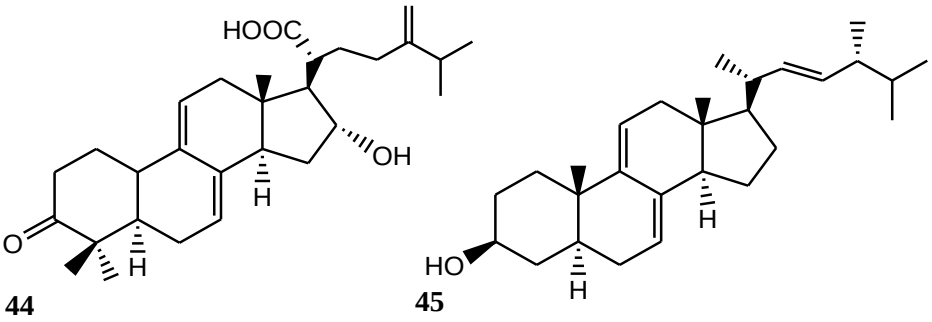
38



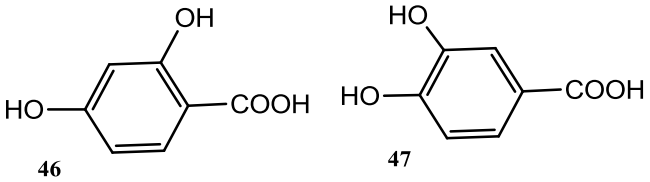
39

Further phytochemical study on a basidiomycete *Fomitopsis pinicola* resulted in a previously undescribed triterpenoid of lanostane type 3-oxo-24-methyl-5α-lanost-8,25-dien-21-oic acid (**40**) and four earlier identified lanostanoids namely 3-oxo-5α-lanost-8,25-dien-21-oic acid (**41**), 16α-acetyloxy-24-methylene-3-oxo-5α-lanost-8-en-21-oic acid(**42**), 16α-hydroxy-24-methylene-3-oxo-5α-lanost-7,9(11)-dien-21-oic acid (**43**), 16α-acetyloxy-24-methylene-3-oxo-5α-lanost-7,9(11)-dien-21-oic acid (**44**). A steroid 22E-5α-ergost-7,9(11),22-trien-3β-ol (**45**) was also isolated. Screening tests for these compounds showed significant anti–*bacillus* activity (Liu *et al.*, 2010a).





Further antibacterial activity of various phenolic compounds had previously evaluated in different basidiomycetes fungi worldwide. Molecular docking studies were performed on the compounds in addition to a structure–activity relationship (SAR) analysis in order to deliver understanding into the mechanism of action of probable antibacterial drugs for resistant micro-organisms. Amid the phenolic compounds tested 2,4-dihydroxybenzoic (**46**) and protocatechuic acid (**47**) were found with greater activity against the majority of Gram negative and Gram positive bacteria. Additionally, they were found to inhibit strongly methicillin-resistant *Staphylococcus aureus* than methicillin-susceptible *Staphylococcus aureus* (Alves *et al.*, 2013).

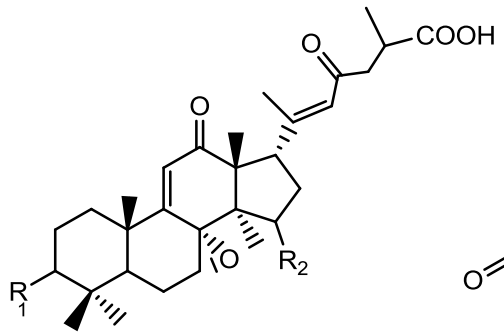


2.4.2 Antifungal activities of extracts and compounds from polypores

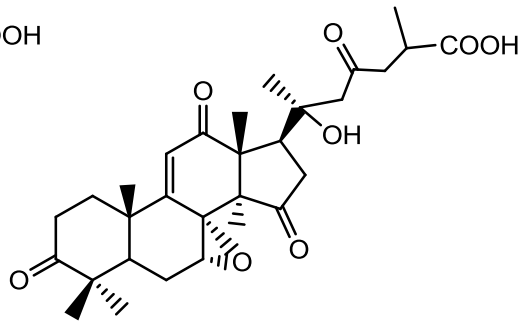
Candidiasis is the most common oral infection in babies and immunocompromised patients. Currently different antifungal components are used and the search is going on for more agents showing anti-candida activities. Various different concentrations of toothpaste consisting *Ganoderma lucidum* extracts were screened using the *in vitro* method for antifungal properties against *Candida. albicans*. The biossay was carried by serial broth

dilution method and was expressed by (MIC). The toothpaste showed antifungal activities against the tested organism of less than 2 µgm /ml ((Nayak *et al.*, 2010).

A protein, ganodermin from a previous study on *Ganoderma lucidum* fruiting bodies was found to hinder the growth of the fungi *Botrytis cinerea, Fusarium oxysporum* and *Physalosporapiricola* with IC_{50} values of 15.2, 12.4 and 18.1 mM, respectively (Wang and Ng, 2006). Triterpenoids obtained from *Ganoderma annulare*, applanoxidic acids A (**48**), C (**49**) and F (**50**) were found to hinder the growth of the fungi *Trichophyton mentagrophytes* and *Microsporum cannis* at values of 500–1000 µg/ml (Smania *et al.*, 2003). Additionally from alternative study, polysaccharide complexes with diverse rare earth metals (RE–CGAP), RE for Eu, La and Yb were synthesised and investigated for their properties against the fungi. It was observed that the metal carboxymethylated *Ganoderma applanatum* polysaccharide mixtures had antifungal activities with EC_{50} value of ranging from 1.01 to 28.48 mg/ml, values of >100 mg/ml were exempted (Xia *et al.*, 2014).

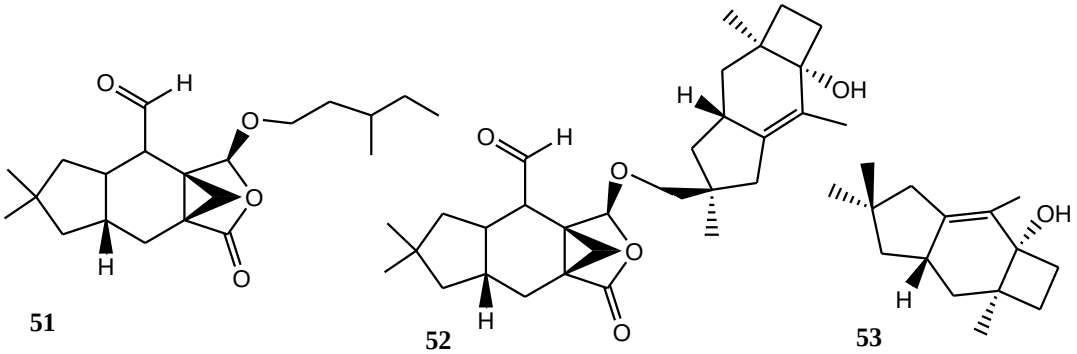


48 R_1=O, R_2= OH
49 R_1=R_2=O



50

Previously three previously undescribed sesquiterpenoids namely udalactaranes A (**51**) and B (**52**) and udasterpurenol A (**53**) were identified from a polypore *Phlebia uda*. These compounds were observed to hinder the germination of the plant spores of *Fusarium graminearum* pathogenic fungus (Schüffler *et al.*, 2012).



51 **52** **53**

2.5 The species in this study from the genus *Ganoderma*

The genus *Ganoderma* is a collection of wood rotting polypores with crust-like fruiting basidiocarps. It is a plenteous producer of unique mycochemicals (Paterson, 2006). Various reports have indicated there folkloric use for the management of different ailments (Smith *et al.*, 2002; Jeong *et al.*, 2008). They have a leathery feel and are woody, in addition an obvious characteristics that distinguishes the polypores from other common types of basidiomycete's mushrooms is the presence of pores.

The fungi classification studies have reported more than 300 species in genus *Ganoderma*, and majority of them are commonly found in the tropical regions (Seo and Kirk, 2000;Richter *et al.*, 2015; Chen and Liu, 2017). Over 250 taxonomic names have been reported worldwide in the genus *Ganoder*ma, including the commonly studied species *G. lucidum, G. applanatum, G. adspersum, G. australe, G. tsugae, G. incrassatum, G. boninense, G. cupreum, G. lipsience, G. lobatum, G. oregonense, G. oerstedii, G. resinaceum, G. pfeifferi, G. platense, G. sinense, G. sessile, G. webrianum* and *G. tornatum* (Moncalvo *et al.*, 1995).

Ganoderma P. Karst. 1881 consist mainly wood rot fungi of diverse conifers and deciduous trees and it is a universal, poroid basidiomycete genus (Ryvarden and Gilbertson, 1993). It is one of the most significant group of polypores due to their economically medicinal effects (Paterson and Russell, 2006; Trigos and Suarez, 2011; Papp *et al.*, 2012) and also since they cause rot in a very wide range of tree species worldwide (Flood *et al.*, 2000; Schwarze and Ferner, 2003). This polypore G*anoderma* is named as *Ganoderma* in US, Reishi in Japan, , and Lingzhi in China (Zhou *et al.*, 2007). *G. lucidum* (Fr.) Karst being considered the representative species of Lingzhi and has been reported to be exceptionally beneficial Chinese traditional medicine since ancient times. *G. lucidum* products have previously been estimated to have a global trade of annual value of 2.5 billion US dollars (Li *et al.*, 2013). In addition to the health benefits and economic importance, cultivation and utilization of *G.lucidum* is also considered an essential part of Chinese native culture (Dai *et al.*, 2017).

Over more than one hundred *Ganoderma* species are scattered all over the world, Japan and China reporting around 98 species and in the midst of them around 18 species are being studied. Furthermore hardly a few species have been subjected to formal biological tests and clinical trials, while many are yet to be studied further. From the reports, about two to three species have been cultivated and utilized commercially (Baby *et al.*, 2015). In the recent past reports indicate that many *Ganoderma* crude constituents are used as remedies for the

treatment of a number of ailments including cancer and are regularly retailed in Chinese herbal medicine markets (Baby *et al.*, 2015).

Ganoderma polypore species have been known for their numerous pharmacological effects. *Ganoderma lucidum*, being extremely categorized in oriental traditional medicine, has been used as a remedy for chronic illnesses such as hypertension, diabetes, asthma, cancer, bronchitis, hepatopathy, arthritis, insomnia and nephritis (Nishitoba *et al.*, 1988; Mizushina *et al.*, 1998; Wasser and Weis, 1999a; Wasser, 2005; Fatmawati *et al.*, 2010). It is a generally recognized crude preparation drug that has long existed in Traditional Chinese Medicine to promote long life and maintain liveliness (Lieu *et al.*, 1992;Wang *et al.*, 2006; Adams *et al.*, 2010). *Ganoderma* have been less investigated for anti-bacterial agents but most intensely on anti-tumour and antiviral agents (Gao *et al.*, 2003).

From the *Ganoderma lucidum* (Kim *et al.*, 1993) and *Ganoderma orgonense* basidiocarp extracts (Brian, 1951), anti-bacterial activity has been previously observed against Gram-positive bacteria. In addition, (Sudirman and Mujiyati, 1997) discovered that seven *Ganoderma* species from Indonesia hindered the development of *Bacillus subtilis*. (Yoon *et al.*, 1994b) also evaluated the combined effect on the effectiveness of a *G. lucidum* aqueous extract with four recognized antibiotics and observed that the anti-bacterial activity was enhanced.

According to (Cheung, 2008), among fungi used in oncology, the most popular species belong to the genus *Ganoderma* that includes *Ganoderma lucidum*, *G. applanatum*, *G. capense* and *G. tsugae* and are also the dominantly known medicinal fungi in the countries of Eastern Asia. Organic extracts of their spores (Fukuzawa *et al.*, 2008), vegetative mycelium (Hu *et al.*, 2002; Choi *et al.*, 2004) and basidiocarps (Takaku *et al.*, 2001) from various species of these Basidiomycota were previously reported to possess cytotoxic activity against cancer cells. Around 25 million persons are afflicted from one of the various manifestations of cancer and 10 million new cases are reported yearly (WHO, 2002). And therefore in view of this, there is an increasing demand for more active anticancerigenous substances and therapies for the different manifestations (Lord and Ashworth, 2010).

.

2.5.1 *Ganoderma adspersum* (Schulz.) Donk

Ganoderma adspersum, a wood rot polypore occurring in various range of tree species inclusive of conifers and deciduous trees around the world. The polypore species has more regularly been recognized in trees growing nearby human habitations, gardens, parks and planted sites (De Simone and Annesi, 2012; Papp and Szabó, 2013). *Ganoderma adspersum* causes white rot it is an infectious microorganism of roots and butts of live trees. Moreover, they can remain and continue to grow feeding on non-living tissue of stumps of felled trees (De Simone and Annesi, 2012). As a result of rot in the butt and base of the trunk, the polypore often kills their hosts and periodically, a diseased tree is wind-thrown or breaks though still alive.

Figure 2.5: Photograph of *Ganoderma adspersum*

2.5.2 *Ganoderma applanatum* (Pers.) Pat

Ganoderma applanatum (Pers.) Pat also called artist's conk, bear bread or artist's bracket, is precisely a perennial, universal, woody shelf fungus, broadly dispersed worldwide and widely found in tropical and temperate regions. The polypore regularly appears on the sides of their peculiar niche, that is hardwood trees, individually or in clusters. The polypore is a very essential decomposer of tree wood material, therefore contribute to the increase in minerals of organic matter largely since they grow on stumps, logs or dying or dead trees. The polypore mycelium spreads into the rotting wood of dead or dying hardwood trees and hence ecologically, a regular cause of death for poplars and beech trees, although it also feeds on several other species of tree, including apple, chestnut, oak, elm, walnut and maple. The fruiting body or conk in most cases, grows out from the base or lower trunk of its host tree.

Ganoderma applanatum is a perennial polypore thus releases hundreds of millions of spores during the course of its annual growth cycle.

Among the numerous polypores, woody polyporaceae *Ganoderma applanatum* is frequently considered to have comparable efficacy as *Ganoderma lucidum* and distinctive in its therapeutic value rather than a source of food. It has been used in the Far East as an Asian traditional medicine for remedy of many ailments in the body. A number of positive effects of this valuable mushroom on human health have been corroborated by intensive modern scientific studies carried out in the modern decades. The fruiting basidiocarps and mycelium of *G. applanatum* contain a variable number of biologically active compounds, such as triterpenoids, polyphenols, polysaccharides, polypeptides and amino acids. Thus, this bioactive agents impart to the pharmacological activities such as immunomodulatory, antitumor and (Smith *et al.*, 2002; Jeong *et al.*, 2008) and antiviral properties.

Figure 2.6: Photograph of *Ganoderma applanatum*

Ganoderma applanatum has a rich, smoky and slightly bitter taste when often consumed as a tea. It is not thought to be as beneficial as it's more well-known relative, *Ganoderma lucidum* but it is used as a medicinal mushroom. This perennial polypore causes the white rot of the base of the stem and more rarely on the upper stem of broadleaved trees. The brackets grows up to 60 cm across, up to 25 cm wide and up to 20 cm thick and they appear all year round. The upper part of the polypore bracket is composed of a hard dark brown lumpy crust that has concentric ridges. The upper part surface is often covered in spore deposits that is like cocoa-like powder. Spores are fine and white with brown spore powder. They have a white like margin on the edge of the bracket leading to a white and lumpy under surface.

Due to the notable curative effects of this fungus, several phytochemical studies have been carried out which have shown the presence of various compounds including steroids, triterpenoids, polysaccharides, phenols, alkaloids, polypeptides, lectins, coumarins and amino acids (Gan *et al.*, 1998; Wang and Liu, 2008; Zhu *et al.*, 2013). In addition, a characteristic attribute of the fruit basidiocarps of *Ganoderma applanatum* is that they are usually narrow than those of *G. adspersum* thickness of 20-60 mm, likened with 40-100 mm at the base. The underside of *Ganoderma adspersum* usually have a decurrent attachment while that of *G. applanatum* comes out sharply at right angles from the host stem. Furthermore, *Ganoderma applanatum* has a lean crust, which can be indented with a fingernail. The pore structure differences can be observed in a radial section with the help of a hand lens, in the older parts of the fruit body pores of *Ganoderma adspersum* remain empty, while those of *G. applanatum* become filled with white mycelium. Previous studies have indicated that fungi in general are a good source of antibacterial and antifungal agents (Janes *et al.*, 2007). But a limited number of reports on the biologically active secondary metabolites of *Ganoderma applanatum* are feasable.

2.5.3 *Ganoderma australe* (Fr.) Pat

Ganoderma australe is a hardwood-decay polypore which is also near in classification to *Ganoderma lucidum*. Considerable amount of work had previously been reported on the chemical agents from *G. lucidum*, but there have been less work on the chemical exploration of the fruiting basidiocarps of *Ganoderma australe* (Leon *et al.*, 2003; Smania *et al.*, 2007). Just as *Ganoderma lucidum* and a number of other several species of *Ganoderma*, the polypore *Ganoderma australe* has been shown to contain compounds of the class lanostane and ergostane triterpenes (Isaka *et al.*, 2017).

Figure 2.7: Photograph of *Ganoderma australe*

2.6 The species in this study in the genus *Trametes*

Trametes, a genus of Polyporaceae (Polyporales, Basidiomycota) was discovered and established by Fries in 1835. They decay wood leaving a white substance therefore known as white rot fungi and are associated with a smooth non-dextrinoid and noamyloid spores, crested pileate basidiocarps, trimitic hyphal systems and lack of hymenial cystidia (Ryvarden, 1991). The genus is widely scattered in nature and some species of *Trametes* have been used as folk medicines in China. They are found frequently on numerous genera of hardwoods throughout temperate forests and in almost all forest ecosystems and are believed to be not host specific in the tropics. Many species of *Trametes* and their related genera have similar structural morphological features hence difficult to identify each species (Gilbertson and Ryvarden, 1987). Just-like their *Ganoderma* counter parts, they are play a central role in natural ecosystems as wood decomposers, bioremediation and biodegradation endeavours, hence both ecologically and economically important. (Hattori *et al.*, 2007).

2.6.1 *Trametes elegans* (Spreng.) Fr

Trametes elegans is a saprobic polypore found on deadwood of hardwoods. They are annual or occasionally perennial, growing alone or sociable together on stumps and logs. They usually spring through fall and are commonly found in the tropics (Ryvarden and Gilbertson, 1994). The cap grows up to 35 cm across and 3 cm thick, it is kidney-shaped, semi-circular, flattened-convex, a lumpy part near the point of attachment, smooth toward the thin margin and with concentric zones of a surface texture. They have a whitish buff texture that occasionally become dark with age, particularly near the point of attachment and along the margin. The pore surface is usually white and vary, ranging from maze-like with slot or gill-like to poroid with round to angular pores. The variable pore surface is a key feature for diagnostics.

Figure 2.8: Photograph of *Trametes elegans*

CHAPTER THREE

MATERIALS AND METHODS

3.1 General experimental methods

Solvents were purchased from Sigma-Aldrich Chemical Co Germany and all were analytical grade. 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-coated TLC plates (Merck 554, 20 cm 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. Data obtained from spectroscopic techniques including NMR (^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H-^{1}H COSY, and NOESY) were used to identify the compounds.

3.2 Collection of *Ganoderma adspersum, G. applanatum* and *G. australe*

A number of polypores are dead-wood dependant organisms, and therefore the three indigenous forests were chosen as sampling areas. Such areas provide ecological niches with unique conditions to support growth of polypores. The three wood decaying fungi were collected from the South West part of Mau Forest, Kericho County in the month of July 2013 from the South west Mau forest near the tea plantations. Located in north-west of Nairobi, the Forest Complex is about 170 kilometres from the city. The forest harbours largest closed-canopy forest ecosystem with the largest water towers. A report from years ago indicated that the forest was the only indigenous forest remaining in Kenya which covers over 400,000 hectares and (Wass, 1995). The forest comprises of blocs namely, Eastern Mau, Western Mau, South-West Mau (Tinet), Oldonyo Purro, Transmara, Southern Mau, and Maasai Mau. The forest area has some of the highest rainfall rates in Kenya and is the largest drainage basin in Kenya. The forest borders four counties, Nakuru to the North, Kericho to the West, Narok to the South, and Bomet to the South-West. Rivers including Southern Ewaso Ng'iro that feeds Lake Natron, Mara and Sondu Rivers feeding Lake Victoria, and Njoro River feeding Lake Nakuru originate from the forest. (Beentje, 1990).

The Mau Forest Complex comprises a diverse types of forests with a variety of indigenous trees, different combinations of vegetation zone pattern from west to east. At the a higher altitude is Juniperus-Podocarpus, Olea forest around the top of the Mau Escarpment, mixed Bamboo/forest/grassland vegetation forest above 2,300 metres and a lower montane forest below 2,300 metres. The forest is abode to uncommon special indigenous trees such as

African olive, cedar, dombeya, shrubs and bamboo. For commercial purposes, the Kenya Forest Department also frequently plants exotic trees such as cypress, pine, grevillea robusta and eucalyptus. The forest receives rainfall of between 1500-2000 mm per annum in the months of March to September with a maximum in April and August. The temperatures are usually between 17.4-23.7ºC on average. The forest has been critical in the supply of medicinal plants, wood fuel, aid biodiversity and honey.

The collected fruiting bodies of the polypores had different morphological features, which indicated that they were different strains. Further mycological identification was done on the basis of micro and macroscopic characteristics existing in literature (Ryvarden and Gilbertson, 1993; Ryvarden and Gilbertson, 1994) and by comparison using coloured field guide books and monographs of coloured mushrooms (Mueller *et al.*, 2004; Lincoff, 2005). The collection was carried out using paper bags that were serialized. The samples were transported to Integrated Biotechnology Research Laboratory (IBRL) at Egerton University and voucher specimen's *Ganoderma adspersum* (JO1302), *Ganoderma applanatum* (JO13033), *Ganoderma australe* (JO13066) were retained in the laboratory.

3.3 Collection of *Trametes elegans* (Spreng:Fr.) Fr

Two samples of the polypore *Trametes elegans* were collected from Kerio Valley, Elgeyo Marakwet County and Kabarnet Forest, Baringo County. The Valley is a narrow long strip high volcanic forest, about 80 km by 10 km wide at its broadest, through which the Kerio River flows. The Elgeyo Escarpment rises more than 6,000 feet (1,830 metres) above the valley. The valley is 1,200 m deep and lies between the Cherengani and the Tugen Hills (Fitzpatrick *et al.*, 2006). It receives between 800-1000 mm per annum in the months of March to September with maximum in May (Fitzpatrick *et al.*, 2006). The most comfortable time of the year for harvest was July and August, when the rains have ended and the temperatures are not excessive (between 18 -26ºC), providing appropriate humidity that allow sprouting of the fruiting bodies of the polypore (Hodd, 2002).

Located near Kabarnet town, Tarambas Tugen hill forest which is at an altitude of 1,815 metres (5,957 feet) on the eastern border of the Kerio Valley, 89 km east of Eldoret through Eldoret-Iten-Kabarnet road and roughly 138 km north of Nakuru town through Nakuru-Marigat-Kabarnet road. Collection of the samples of the fruiting bodies of the polypore *Trametes elegans* were done in the month of August 2013 from Kerio valley and Kabarnet forests. The identification of the species was achieved as described in the literature

(Tomsovsky *et al.*, 2006; Zhang *et al.*, 2006). The collection was carried out using paper bags that were serialized. The samples were transported to Integrated Biotechnology Research Laboratory (IBRL) at Egerton University and a sample specimen *Trametes elegans* (JO 13020/59) was retained in the laboratory.

3.4 Preliminary preparations of the polypore samples

The fruiting bodies of the polypore species were cleaned with a brush to remove the debris that were attached on surface of the collected polypore fruiting bodies. They were sliced into small pieces and were air-dried in shade to a constant weight. They were pulverized into fine powder using a heavy duty blender, model number 24CB10C, serial number 563972 and the weight for each species was obtained. The weights of the various polypores were *Ganoderma adspersum* (823 gm), *Ganoderma applanatum* (1230 gm), *Ganoderma australe* (911 gm) and *Trametes elegans* (400 gm).

3.5 Extraction of the crude extracts

The powdered fruiting bodies of each of the *Ganoderma* species and *Trametes elegans* were extracted with methanol for extended periods (24-72h) at normal ambient temperature (20-25ºC). The subsequent extracts obtained were then concentrated at low temperature under reduced pressure in order not to destroy any thermo-labile antimicrobial agents present. The extracts obtained were a deep brown syrup for the three *Ganoderma* polypore species and light brown syrup for *Trametes elegans*. The extracts were then suspended in water- ethyl acetate partitioning solvents to afford ethyl acetate fractions.

3.5.1 Extraction of *Ganoderma adspersum* (Schulz.) Donk

Dried fruiting basidiocarps of *G. adspersum* (823 gm) were sliced into small pieces, blended, macerated and suspended in methanol (2 L) at ambient room temperature (20-25ºC) for 24-72 hrs. The extracted mixture was filtered and the filtrate was concentrated under reduced pressure to obtain a deep brown gum (methanol extract, 50.2 gm). The residual fungal material was then extracted with ethyl acetate to obtain an ethyl acetate extract, 5.5 gm dark brown gum.

3.5.2 Extraction of *Ganoderma applanatum* (Pers.) Pat

Powdered *Ganoderma applanatum* fruiting bodies (1230 gm) were extracted with methanol three times (each 2.0 L × 3 days) with frequent stirring at room temperature. The filtrate was

concentrated under reduced pressure to yield a methanol crude extract. It was suspended in water then extracted with ethyl acetate to afford an ethyl acetate soluble extract (8.8 gm).

3.5.3 Extraction of *Ganoderma australe* (Fr.) Pat

Ground dried *Ganoderma australe* basidiomycetes (911 gm) were soaked and extracted with methanol for 72 hours. After the solvent being evaporated, the residue (55 gm) was suspended in ethyl acetate: H_2O (9:1) and partitioned to yield ethyl acetate extract (6.0 gm).

3.5.4 Extraction of *Trametes elegans* (Spreng.) Fr

The ground fruiting bodies (400 gm) of *Trametes elegans* were suspended in methanol three times at room temperature for 48 hours. The methanol extract (26 gm) was obtained by removing solvent under reduced pressure. The methanol extract was suspended in water and partitioned with ethyl acetate to yield 4 gm of ethyl acetate extract.

3.6 Fractionation and purification of the compounds using column chromatography

The crude extract was fractionated using column chromatography on silica gel 60 and eluted with solvents of increasing polarity. Repeated column chromatography was carried out in order to obtain pure compounds. The extracts were separated using Merck Art. 9385 Silica Gel as adsorbent with fractions collected after gradient elution of solvent mixtures starting with hexane and increasing proportions of dichloromethane, ethyl acetate and methanol to obtain fractions. The eluents from the column were collected into beakers as sequenced fractions determined by volume. A sample of each of the fractions was loaded onto a silica gel pre-coated aluminium TLC plate using a capillary tube. The plates were then placed in chromatographic tank and left to develop in the selected solvent system. The developed TLC plate were visualised by UV lamp pre-set at fixed wavelengths, λ=254 and 365 nm and later sprayed with a freshly prepared *p*-anisaldehyde solution before heating at 115°C. The visualisation enabled like fractions to be pooled together. The pooled fractions were further fractionated using different solvents in a small column to yield pure compounds.

3.6.1 Fractionation and purification of compounds from *Ganoderma adspersum*

The ethyl acetate extract (5.0 gm) was fractionated on column chromatography (CC) employing silica gel and hexane/dichloromethane solvents via gradient elution 100:0, 80:20, 60:40, 40:60, 20:80, then 0:100 to yield three sub fractions. The three sub fractions were further repeatedly chromatographed on small silica columns and different solvent

combinations to yield pure compounds. The third sub fraction was further purified on sephadex LH20 to yield a pure compound 8 mg (**Figure 3.1**).

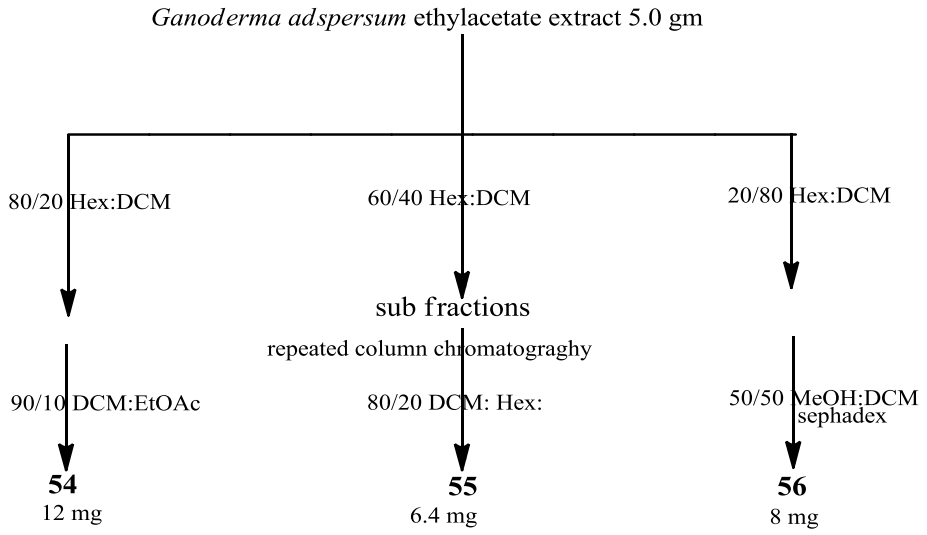


Figure 3.1: Flow chart for the isolation of compounds from *Ganoderma adspersum*

3.6.2 Fractionation and purification compounds from *Ganoderma applanatum*

The ethyl acetate soluble extract (8.8 g) was subjected to silica gel column chromatography with *n*-hexane and diethyl ether solvent gradients to afford fractions. Fractions of similar spot patterns on TLC were pooled together to afford three sub fractions. The three sub fractions were triterpene-rich as visualized by freshly prepared ρ- anisaldehyde spraying reagent followed by heating at 110 °C for 10 min. Each of the sub fractions was subjected to repeated silica gel column chromatography eluted with dichloromethane, ethyl acetate gradients to afford pure compounds. Pooled sub fraction Fr_1 was purified by silica gel column chromatography (eluted with dichloromethane/ethyl acetate 90:10 and to yield two pure compounds 8 mg and 6.0 mg. The fraction Fr_2 was repeatedly purified by small silica gel column chromatography (eluted with dichloromethane/ethyl acetate 80:20 to afford a pure compound of 5.6 mg. The sub-fraction Fr_3 was repeatedly purified by small silica gel column chromatography (eluted with dichloromethane/ethyl acetate 80:20 and finally purified on sephadex LH20 to yield two pure compounds of weight 5.4 and 7.0 mg respectively (**Figure 3.2)**.

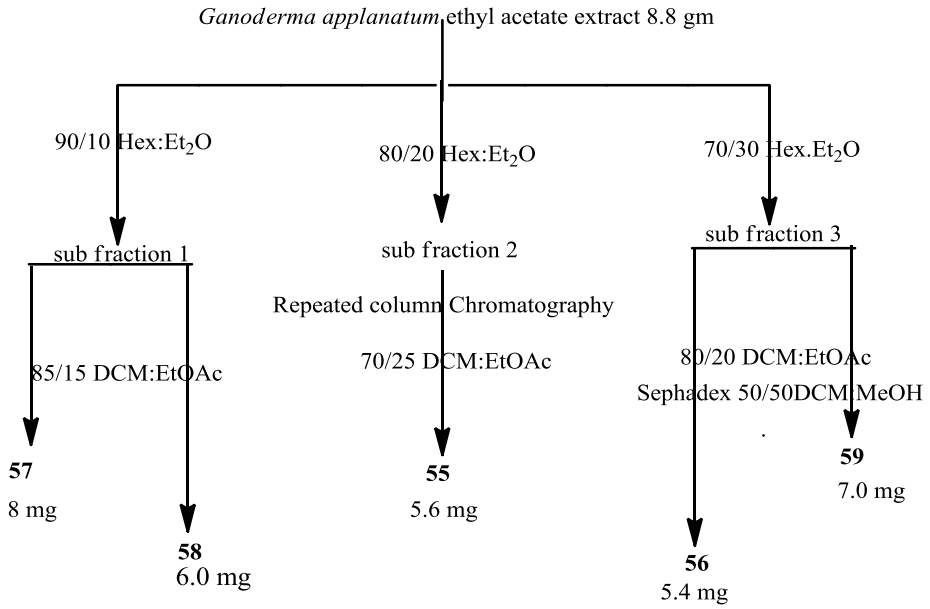


Figure 3.2: Flow chart for the isolation of compounds from *Ganoderma applanatum*

3.6.3 Fractionation and purification of compounds from *Ganoderma australe*

The ethyl acetate extract was fractionated with gradient elution of hexane: dichloromethane solvents, to obtain three sub fractions. Pooled sub fraction 1 was purified by silica gel column chromatography (eluted with dichloromethane/ethyl acetate 90:10) to yield a pure compound (5.3 mg). The second sub-fraction was eluted with dichloromethane/ethyl acetate 85:15 solvent to yield a pure compound of 5.5 mg. The last sub-fraction were repeatedly purified by small silica gel CC eluted with dichloromethane/ethyl acetate 70:30 and finally purified on Sephadex LH20 to yield a pure compound 10 mg (**Figure 3.3)**.

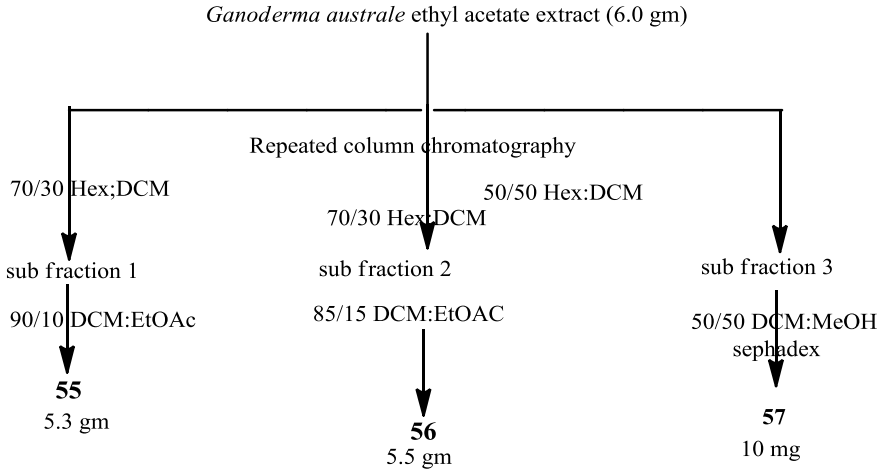


Figure 3.3: Flow chart for the isolation of compounds from *Ganoderma australe*

3.6.4 Fractionation and purification of compounds from *Trametes elegans*

Employing hexane–diethyl ether gradient elution, the ethyl acetate extract (4 gm) was chromatographed and fractionated on silica gel chromatography to yield fractions. They were further subjected to repeated silica gel column chromatography eluted with dichloromethane, ethyl acetate gradients to afford six sub-fractions as displayed in a TLC. Further repeated column chromatography on the sub-fractions yielded the first four pure compounds. The other two were obtained on further purification on Sephadex LH20 (**Figure 3.4**).

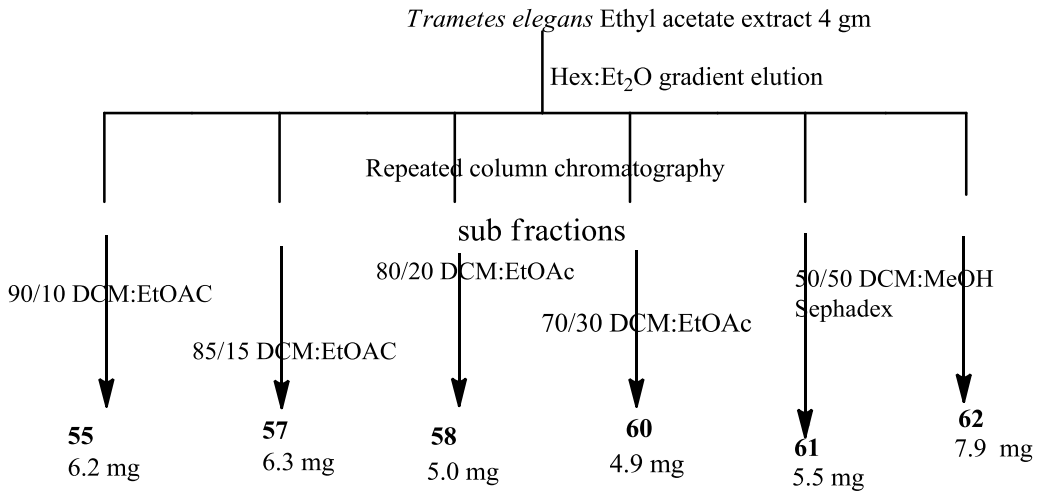


Figure 3.4: Flow chart for the isolation of compounds from *Trametes elegans*

3.7 NMR spectroscopy

The one dimension (1D) ^{1}H, ^{13}C, DEPT and two dimension (2D) HSQC, HMBC, COSY and NOESY nuclear magnetic resonance experiments were carried out on the pure compounds on 500 MHz Bruker AVANCE NMR spectrometer to obtain the spectral data. The solvent used was deuterated chloroform ($CDCl_3$) whose reference solvent signals are δ_H 7.26 and δ_C 77.23 for ^{1}H and ^{13}C experiments respectively. The compounds were dissolved in 5 ml of each of the deuterated solvents in a 5 mm NMR tube and the data processed using TOPSIN software. Tetramethylsilane is used as an internal standard and the chemical shifts are reported in parts per million (ppm) relative to this standard.

The ^{1}H NMR spectra of all the isolated compounds was obtained in $CDC1_3$ solution. A deuterated solvent has no hydrogen and therefore does not give the 'H NMR signal. The solution was put in an NMR tube (thin walled glass) then rotated in a powerful magnetic field. The absorption of electromagnetic radiation of radiofrequency region was measured and the positions of the peaks were read from the scale of the spectrum obtained. Peak

position in Hz or δ enabled the measurement of important valuables, that is coupling constants and chemical shifts (Sinnema and Breitmaier, 1993).

The ^{13}C NMR spectrum was also obtained by putting the compound solution under study in a magnetic field, it was then spun to even out the influence of any variants in the field. The sample NMR tube is then irradiated and the radiation absorbed was recorded as a function of the frequency of radiation. Since the natural carbon is only 1.1%, the ^{13}C NMR spectroscopy gives a spectrum with a high background electronic noise levels. Therefore the machine was scanned repeatedly and rapidly with a one powerful pulse covering the whole frequency range to give repetitive scans accumulated to produce a spectrum. The scans were repetitively done until a significantly robust spectrum was acquired, that was conventionally scanned by a Fourier transform then converted into a readable spectrum (Breitmaier and Sinnema, 1993; Berger, 2008).

Consequently, Distortionless Enhancement by Polarisation Transfer (DEPT-135) experiment was carried out in order to enhance the signal intensity of the spectra. Elucidating the spectra revealed the carbon multiplicities directly that gave the information about the sub-spectra containing CH or CH_2 or CH_3 resonances only.

COSY (correlation Spectroscopy) is 2D homonuclear experiment that provided a way of recognizing mutually coupled protons. The COSY experiment depicts all correlations with a single experiment hence providing an easy way of identifying connectivity between protons when a great amount of coupling linkages are required to be known. It is presented in a contour map of two-dimensions, the contours representing signal intensity and each dimension representing proton chemical shifts. The peaks of interest were identified as the off-diagonal or cross peaks. Each contour represented by a coupling between the protons that were connected by the cross peak.

The 2D heteronuclear HSQC (Heteronuclear Single-Quantum Correlation) experiment is a correlation technique that provided a method of recognizing direct H-C correlations in a compound. The spectra obtained were presented in a similar style to that of COSY, with the vertical dimension of the 2D map representing ^{13}C chemical shifts and horizontal dimension representing ^{1}H chemical shifts. The cross peaks in contour plot depicted the carbons connected to a specific proton (or group of protons).

The 2D HMBC experiment (Heteronuclear Multiple-Bond Correlation) resembles HSQC and work basically in a similar way. But for this case, the connections of extra (two to three) bonds away arise couplings from long-range. The ^{1}H-^{13}C couplings mostly arise with substantial signal strength over only 2 and 3 Hz connected bonds.

The 2D NOESY experiment basically provided a means of identifying the stereochemistry in a compound. Contrary to the other 2D techniques, it depends on the direct, through-space interactions between the proton nuclei and it does not rely on of the presence of through-bond connections. This correlations that occur through-space can be related to the forces experienced when bar magnets are brought towards closely together. From the NMR spectroscopic experiments, structures were proposed based on the interpretation of the spectra and compared with known compounds reported in literature.

3.8 Screening of the compounds for antimicrobial activity

The experiments involving hospital strains of the bacterial and fungal pathogens were carried out at Kenya Medical Research Institute (KEMRI) Laboratories in Nairobi.

3.8.1 Bacterial test organisms

The strains of bacteria included Gram negative strains; *Shigella* (S), *Escherichia coli* (*E. coli*), *Salmonella typhi* (ST), *Klebsiella pneumonia* (KP) and *Citobacter enterocolitica* (CE). Gram- positive bacteria were *Streptococcus pneumonia (*SPN), *Streptococcus pyogenes (*SP), *Entero feacalis* (EF) and *Staphylococcus aureus* (SA).

3.8.2 Fungal test organisms

The antifungal test organisms were *Candida albicans* (CA) and *Cryptococcus neoformans* (CN).

3.8.3 Antibacterial and antifungal testing by disc diffusion method

Circular paper discs of of equal size (diameter 6 mm) were impregnated with compounds broth (50 mg/ml) and air dried. The discs treated with compounds were placed on plates where the organisms were cultured according to (Kirby-Bauer disk diffusion susceptibility test protocol) with the aid of sterile forceps and a wire loop (Hudzicki, 2009). The cover slip was placed on the petri dishes then incubated at 37ºC for 24 hours. To guarantee the reproducibility of the results, individual experiments were performed in triplicates. The zone of inhibition values were evaluated in accordance to (CLSI, 2012). Absence of bacteria growth around the impregnated disc indicated antimicrobial activity of the test compounds.

The level of inhibition was measured as the distance between the bacterial growth and the discs which was measured using a Vernier calliper and expressed in millimetres. The antibiotic ciprofloxacin and phosphate buffered saline water were employed as the positive and negative controls respectively.

3.9 Data management and analysis

The zones inhibited by the antimicrobial compounds were measured, recorded and tabulated in Microsoft excel® spreadsheet. The assessed data was transferred to Minitab statistical software v18.0 upon which descriptive statistics were derived and expressed as mean ± standard error of mean (SEM). One-way ANOVA was used to determine the significant difference between the means of different treatment groups. Tukey's posthoc test was then carried out for pairwise comparison of means and the values of $p \leq 0.05$ were considered.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Extracts from the different polypore species in the study

The percentage yields of ethyl acetate extract crude are shown in Table 4.1 and was based on the weight of dried and ground polypore materials. The percentage yield ranged from 0.6 to 1%, indicating that extractable compounds by the used solvents are in low amounts. The yields were low but were in agreement with extraction yields of previous works (Ren *et al.*, 2006; Adeyelu *et al.*, 2018).

Table 4.1: Amount in grams of different extracts and percent yield

Polypore species	Dry sample material (gm)	Methanol crude extract (gm)	Ethyl acetate crude extract (gm)	Yield of extract (%)
G. adspersum	823	50.2	5.0	0.60
G. applanatum	1230	70.0	8.8	0.72
G. australe	911	55.2	6.0	0.67
T. elegans	400	26.0	4.0	1.00

4.2 Structure elucidation of compounds from *Ganoderma adspersum* (Schulz.) Donk

Chemical constituents of the dried fruiting bodies of *Ganoderma adspersum* were isolated using chromatographic techniques and yielded three ergostane type compounds. The compounds were identified as ergosta-7,22-dien-3-one (**54**), ergosta-7,22-diene-3-ol (**55**) and ergosta-5,7,22 trien-3-ol (**56**) **Figure 4.1**. These compounds had previously been isolated (Protiva *et al.*, 1980; Kwon *et al.*, 2002; Lee *et al.*, 2006) and elucidation of the structures of the compounds was achieved by spectroscopic analysis and identified by comparison of their spectral data with literature reports.

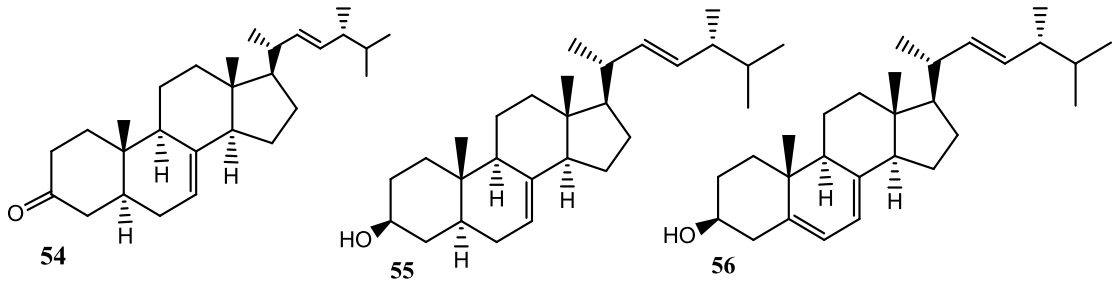
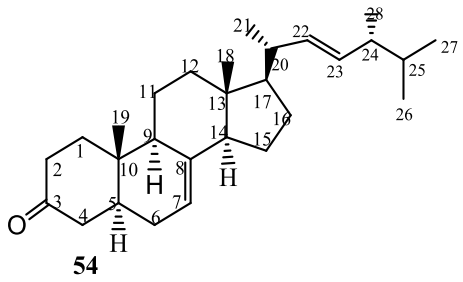


Figure 4.1: Structures of compounds isolated from *Ganoderma adspersum*

4.2.1 Structural elucidation of ergosta-7,22-dien-3-one (54)

Compound **54** was isolated as a white amorphous powder solid (12 mg). The structure of compound ergosta-7,22-dien-3-one was elucidated using NMR (1D, 2D NMR) [**Table 4.2,**

Appendices 1-7]. The ^{1}H-NMR- spectra of **54** displayed key vinylic resonance signal was at δ_H 5.18-5.20 Hz (3H). The spectrum showed six characteristic ergostane-type steroidal methyl signals at δ_H 1.03 (d, *J* =6.2 Hz, 1H-21), 1.02 (s H-19), 0.93 (d, *J* =6.8 Hz, 3H-28), 0.83 (d, *J*= 6.4 Hz, 3H-26), 0.83 (d, *J*= 6.4 Hz, 3H-27) and δ_H 0.58 (s, 3H-18). The ^{13}C NMR spectrum of the compound **54** displayed 28 carbons, including carbonyl carbon signal at δc 212.0 (C-3), and four olefin carbon signals at δc 139.9 (C-8), 117.2 (C-7), 132.8 (C-23) and 135.5 (C-22). Three methylenes (C-1, 2, 4) and the carbonyl carbon at (C-3) δ_C 212.0 were assigned to a linear sequence of carbon atoms (from C-1 to C-4) on the basis of ^{1}H–^{1}H COSY. The carbonyl group at δ_C 212.0 C-3 was confirmed by key HMBC correlations from proton signals at δ_H 2.42 (H-2), δ_H 2.23 (H-1), δ_H 2.12 (H-4). The proton signal at δ_H 5.19 (1H, t) was assigned to H-7 due to correlations seen in HMBC to carbon C-5 resonance δ_C 43.1. The H-5 correlations were confirmed by ^{1}H-^{1}H COSY correlations with the protons at δ_H 2.24 (2H-4).



54

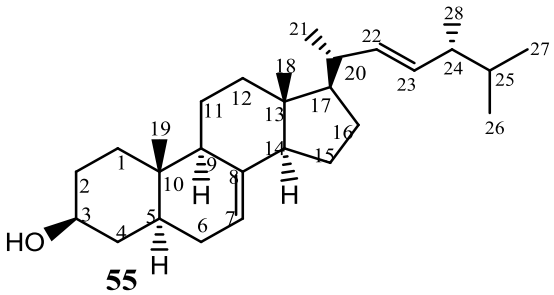
The Carbon C-8 (δ_C 139.7) also had correlation with proton δ_H 1.64 (H-11) in HMBC. The 2H-2 (δ_H 2.42/2.28 dd) had ^{1}H-^{1}H NOESY cross peak correlations to proton signals at δ_H 1.02 (3H-19) and H-1 (δ_H 2.13). There were no cross peak correlations between methyl proton signals at δ_H 1.02 (3H-19) and the proton at δ_H 1.28 (1H-9) supporting their β and α orientations, respectively. The alpha orientation of the proton at δ_H 1.28 (1H-9) was confirmed by cross peak from proton at δ_H 1.83 (1H-5) and at δ_H 1.84 (1H-14). The coupling constant between the protons at δ_H 5.19 (dd, *J*=15.3, 8.3 Hz, 1H) and 5.21 (dd, *J*=15.3, 7.8 Hz, 1H) was consistent with a trans-configuration of the C-(22) =C-(23) double bond. The germinal methyl-26 and 27 values in the compound had mutual HMBC correlations in agreement with ergostane type sterol. The complete assignment of the protons and carbons was accomplished as shown in **Table 4.2**. The structure of compound **54** was resolved as an ergosta- 7,22-dien-3-one and it was also confirmed by relating its spectral data with the literature (Protiva *et al.*, 1980).

Table 4.2: NMR data for ergosta-7,22-dien-3-one (54) in $CDCl_3$

No	^{13}C NMR (125 MHz)	^{13}C NMR (75 MHz) (Protiva *et al.*, 1980)	^{1}H NMR (500 MHz)	Coupling constants (*J* Values)	^{1}H NMR (300 MHz) (Protiva *et al.*, 1980)
1α	39.0 CH_2	38.7	2.13 m		
1β			1.46 m		
2α	38.4 CH_2	38.1	2.42 m		
2β			2.28 m		
3	212.0 C	211.9			
4α	44.5 CH_2	44.2	2.24 m		
4β					
5	43.5 C	42.8	1.83		
6	30.3 CH	29.6	1.85		
			1.26		
7	117.2 CH	117.0	5.19 m	*J*=7.8 Hz	5.14-5.25 t
8	139.7 C	139.6			
9	49.2 CH	48.9	1.75 m		
10	34.6 C	34.4			
11α	21.9 CH_2	21.7	1.64 m		
11β			1.55 m		
12α	39.6 CH_2	39.2	2.00 m		
12β			1.27 m		
13	43.5 C	43.3			
14	55.2 CH	55.9	1.84 m		
15α	23.1 CH_2	22.9	1.64 m		
15β			1.56 m		
16α	28.3 CH_2	28.1	1.74 m		
16β			1.30 m		
17	56.2 CH	55.9	1.25 m		
18	12.4 CH_3	12.1	0.58 s		0.58 s
19	12.7 CH_3	12.3	1.02 s		1.03 s
20	40.6 CH	40.4	2.03 m		
21	21.3 CH_3	21.0	1.03 d	*J*=6.5 Hz	1.08 d *J*=6.5 Hz
22	135.8 CH	135.5	5.19 dd	*J*=8.3, 15.4 Hz	5.14-5.25
23	132.3 CH	132.0	5.20 dd	*J*=7.5, 15.4 Hz	5.14-5.25 dd,
24	43.0 CH	42.8	1.85 m		1.80
25	33.3 CH	33.0	1.48 m		
26	20.2 CH_3	19.6	0.83 d	*J*=6.8 Hz	0.78 d, *J*= 6.8 Hz
27	19.9 CH_3	19.9	0.83 d	*J*=6.9 Hz	0.87 d, *J*= 6.7 Hz
28	17.7 CH_3	17.5	0.90 d	*J*=6.8 Hz	0.97 d *J*=7.2 Hz

4.2.2 Structural elucidation of ergosta-7,22-diene-3-ol (55)

Compound **55** was obtained as a white amorphous powder (6.4 mg). The structure of the compound ergosta-7,22-dien-3-ol was elucidated using NMR (1D, 2D NMR) experiments **[Appendices 8-14]**. Analysis of its ^{13}C NMR spectra showed four typical olefin carbon resonances at δ_c 117.7. 132.1, 135.8 and 139.8 and a methine oxygenated carbon at δ_c 71.2. A ^{13}C and DEPT spectra revealed 28 carbon signals, comprising six methyl groups, eight methylene groups, eleven methine carbon atoms (three sp^2 methine, and one oxygenated methine) and three quaternary carbon atoms. The key proton signals were observed at δ_H 5.18-5.22 (1H-7, 22, 23) and δ_H 3.60 (1H-3). Characteristic signals of the side chain as identified in the ^1H NMR were of olefinic hydrogens at δ_H 5.18 dd *J*=7.3, 15 Hz (H-22), δ_H 5.19 dd *J*= 7.4, 15.1 (H-23) and four doublet methyls δ_H 1.02 *J*=6.7 (H-21), δ_H 0.83 *J*=6.8, δ_H 0.83 *J*=6.7 and δ_H 0.91 *J*=6.7 (H-28) were observed.



55

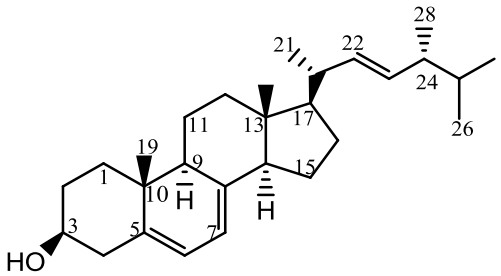
The methyl groups at positions 21-, 26-, 27- and 28- together with vinylic protons at H-22 and H-23 had the appropriate chemical shifts and multiplicities in the ^1H NMR spectrum that were consistent the occurrence of an ergosterol side chain. The hydroxyl proton had a β configuration, which was supported by lack NOESY cross-peak between a proton at δ_H 3.6 H-3, and δ_H0.80, 3H-19. The α proton at δ_H 3.60 m (1H-3) had ^1H-^1H NOESY correlations to proton signals at δ_H 1.08 (H-1), δ_H 1.72 (H-2), δ_H 1.80 (H-4), and proton at δ_H 1.39 (H-5). In addition, NOESY correlations from a proton δ_H 1.64 (H-9) to protons at δ_H 1.39 (H-5) and at δ_H 1.80, (H-14) were observed hence confirming their α orientations in relation with literature. The key HMBC correlations were δc 71.3 (C-3) with protons at δ_H 1.08/1.82 (2H-1), δ_H 1.26/1.72 (2H-2), δ_H 1.39(1H-5) and δc 49.7 (C-9) with δ_H 0.80 (3H-18) and δ_H 2.00/1.26 (2H-12). The carbon at δc 34.4 (C-10) correlated with δ_H 1.83/1.08 (H-1), δ_H 1.72/1.26 (H-2) and δ_H 0.80 (3H-19). Comprehensive interpretations of 2D NMR data including ^1H–^1H COSY, NOESY, HSQC, and HMBC allowed for the complete assignments of the structure. By comparison of the spectral data of the structure with the literature the compound was identified as ergosta-7,22-dien-3-ol (Lee *et al.*, 2006).

Table 4.3: NMR data for ergosta-7, 22-dien-3-ol (55) in $CDCl_3$

No.	^{13}C NMR (125 MHz)	^{13}C NMR 75.5 MHz (Lee *et al.*, 2006)	^{1}H NMR (500 MHz)	Coupling constants *J* values	^{1}H NMR (300 MHz) (Lee *et al.*, 2006)	
1α	37.6 CH_2	37.1	1.82 m			
1β			1.08 m			
2α	28.3 CH_2	29.2	1.72 m			
2β			1.25 m			
3	71.3 CH	71.1	3.60 m		3.60 m	
4α	38.2 CH_2	38.0	1.80 m			
4β			1.28 m			
5	40.5 C	40.3	1.39			
6	29.9 CH_2	29.6	1.76 m			
			1.26 m			
7	117.2 CH	117.5	5.19 m	*J*=7.5 Hz		
8	139.7 C	139.5				
9	49.7 CH	49.5	1.64 m			
10	34.4 C	34.2				
11α	21.8 CH_2	21.8	1.58 m			
11β			1.49 m			
12α	39.7 CH_2	39.5	2.00 m			
12β			1.23 m			
13	43.5 C	43.3				
14	55.3 CH	55.1	1.81 m			
15α	23.1 CH_2	22.9	1.58 m			
15β			1.38 m			
16α	31.7 CH_2	29.7	1.80 m			
16β			1.40 m			
17	56.2 CH	56.0	1.23 m			
18	12.3 CH_3	12.1	0.54 s		0.54 s	
19	13.2 CH_3	13.0	0.80 s		0.80 s	
20	40.7 CH	40.5	2.02 m			
21	21.3 CH_3	21.1	1.02 d	*J*=6.7 Hz	1.02 d	*J*=6.9 Hz
22	135.9 CH	135.7	5.18 dd	*J*=8.3, 15.4 Hz	5.19 dd	*J*=7.6, 15.4
23	132.3 CH	131.9	5.19 dd	*J*=7.5, 15.4 Hz	5.19 dd	*J*=7.9, 15.3
24	43.0 CH	42.8	1.84 m			
25	33.3 CH	33.0	1.47 m			
26	20.2 CH_3	19.9	0.83 d	*J*=6.8 Hz	0.82 d	*J*=6.9
27	19.9 CH_3	19.6	0.83 d	*J*=6.9 Hz	0.84 d	*J*=6.6
28	17.7 CH_3	17.6	0.91 d	*J*=6.7 Hz	0.91 d	*J*=6.9

4.2.3 Structural elucidation of ergosta-5,7,22-trien-3β-ol (56)

A compound **56** was found as a white solid (8 mg). The structure of compound ergosta-5,7,22-trien-3-ol was elucidated using NMR (1D, 2D NMR) [**Table 4.4, Appendices 15-19]**. The ^{1}H NMR spectrum of compound was indicative of two tertiary methyl groups (δ_H, 0.94, 0.63) and four secondary methyl groups δ_H 0.83, 0.84, 0.91 and 1.03. The other key proton resonances are vinyl protons at δ_H 5.57, 5.39 and 5.19-5.21. A broad deshielded proton signal at δ_H 3.64 was also observed. Analysis of the ^{13}C-NMR spectrum and with DEPT-135 showed 28 carbon resonances that involved a one methine carbon that was oxygenated at δc 70.7 (C-3), six olefinic carbon signals δ_C 116.6 C-7, 119.8 C-6, 132.2 C-23, 135.8 C-22, 140.3 C-8 and 141.8 C-5. The HMBC spectrum showed correlation of H-3 resonance (δ_H 3.64) with the wholly substituted C-5 carbon signal (δ_C 140.3). Further interpretation of the HMBC spectrum revealed more correlations from H-3 to C-2, C-1 and C-4; 3H-18 to C-12, C-13, C-14 and C-17; 3H-19 to C-1, C-5, C-9 and C-10; 3H-26 and 3H-27 to C24 and C-25; 3H-28 to C-22, C-25 and location of the hydroxyl carbon at δ 70.7 (C-3) was assigned on the basis of the HMBC correlations from H-3 to C-1, C-2, C-4 and C-5.



56

The HMBC spectrum also showed correlations between C-5 and H-6 (δ_H 5.57) and H-7 (δ_H 5.39). The ^{1}H-^{1}H COSY spectrum showed coupling between the H-6 and H-7 resonances with *J*=5.2 Hz, hence is conjugated. One of the double bond was placed between C-5 (δ_C 141.8) and C-6 (δ_C 119.8) while the other double bond was placed between C-7 (δ_C 116.6) and C-8 (δ_C 140.3). There were NOESY correlations between at δ_H 5.57 (1H-6) and δ_H 2.47 (4Hα) and at δ_H 5.37 (1H-7) and δ_H 1.70/140 (2H-15). The proton at δ_H 3H-19 had a cross peak correlation with proton δ_H 2.30 (4Hβ) confirming their β orientation from the skeleton biosynthesis. The rest of the molecule was same as other sterol molecules with the normal ergostane side chain. In addition, on the basis of the further comparison with reported values, the structure of the compound was identified as ergosta-5,7,22-trien-3β-ol (ergosterol) and one of the important pharmaceutically pertinent compound, a vitamin D precursor. The compound had previously been reported from *Lentinula edodes, Tricholoma matsutake,*

Paecilomyces sp. J300 (Ohnuma *et al.*, 2000; Kwon *et al.*, 2002) and from *Aspergillus awamori* a Mangrove Fungus (Gao *et al.*, 2007).

Table 4.4: NMR data for ergosta-5,7,22-trien-3β-ol (56) in $CDCl_3$

No.	^{13}C NMR (125 MHz)	^{13}C NMR (125 MHz) (Kwon *et al.*, 2002)	^{1}H NMR (500 MHz)	Experimental coupling Constants (*J* values)	^{1}H NMR (500 MHz) (Kwon *et al.*, 2002)	Literature Coupling Constants (*J* values)
1α	38.6 CH_2	39.1	1.89 m			
1β			1.33 m			
2α	32.2 CH_2	32.7	1.52 m			
2β			1.91 m			
3	70.7 CH	71.2	3.65 m		3.65 m	
4α	41.0 C	41.5	2.48 m		1.74-1.82 m	
4β			2.30 m		2.11-2.20 m	
5	141.6 C	142.1				
6	119.8 CH	120.3	5.57 d	*J*=5.3 Hz	5.57 d	*J*=5.1 Hz
7	116.6 C	116.3	5.39 d	*J*=5.4 Hz	5.38 d	*J*=5.2 Hz
8	140.3 C	140.8				
9	46.6 CH	46.9	1.96 m			
10	37.2 C	37.7				
11α	21.3 CH	21.1	1.62 m			
			1.75 m			
12α	39.3 CH_2	39.0	1.28 m			
12β			2.05 m			
13	43.1 C	43.5				
14	54.8 CH	55.3	1.91 m			
15α	23.2 CH_2	21.8	1.40 m			
15β			1.70 m			
16α	29.9 CH_2	29.8	1.77 m			
16β			1.25 m			
17	56.0 CH	56.4	1.29 m		1.29 m	
18	12.3 CH_3	12.8	0.63 s		0.63 s	
19	16.5 CH_3	17.0	0.94 s		0.95 s	
20	40.6 CH	41.1	2.05 m		2.06 m	
21	21.3 CH_3	20.3	1.03 d	*J*=6.5 Hz	1.04 d	*J*=7 Hz
22	135.8 CH	136.7	5.19 dd	*J*=8.0, 15.4 Hz	5.20 dd	*J*=7.5, 15.5 Hz
23	132.2 CH	132.7	5.20 dd,	*J*=7.5, 15.4 Hz	5.22 dd	*J*=7.0, 15.5 Hz
24	43.1 CH	43.0	1.88 m			
25	33.4 CH	33.8	1.49 m			
26	20.1 CH_3	20.6	0.84 d	*J*=6.8 Hz	0.82 d	*J*= 6.6 Hz
27	19.9 CH_3	23.7	0.84 d	*J*=6.9 Hz	0.82 d	*J*= 6.5 Hz
28	17.8 CH_3	18.3	0.92 d	*J*=6.8 Hz	0.92 d	*J*=7.0 Hz

4.3 Structure elucidation compounds from *Ganoderma applanatum* (Pers.) Pat

The dried fruiting basidiocarps of *Ganoderma applanatum* produced five compounds. On the basis spectra from NMR spectroscopic experiments their structures were identified as, ergosta-7,22-diene-3-ol **(55),** ergosta-5,7,22-trien-3-ol (**56**) 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol (**57**), *5α,8α*–epidioxyergosta-6,22-dien-3β-ol (**58)** and 24-hydroxy-olean-12-en-3-one (**59**) (**Figure 4.2**). All the five compounds had previously been isolated hence their structures were confirmed by comparison with spectroscopic data from literature.

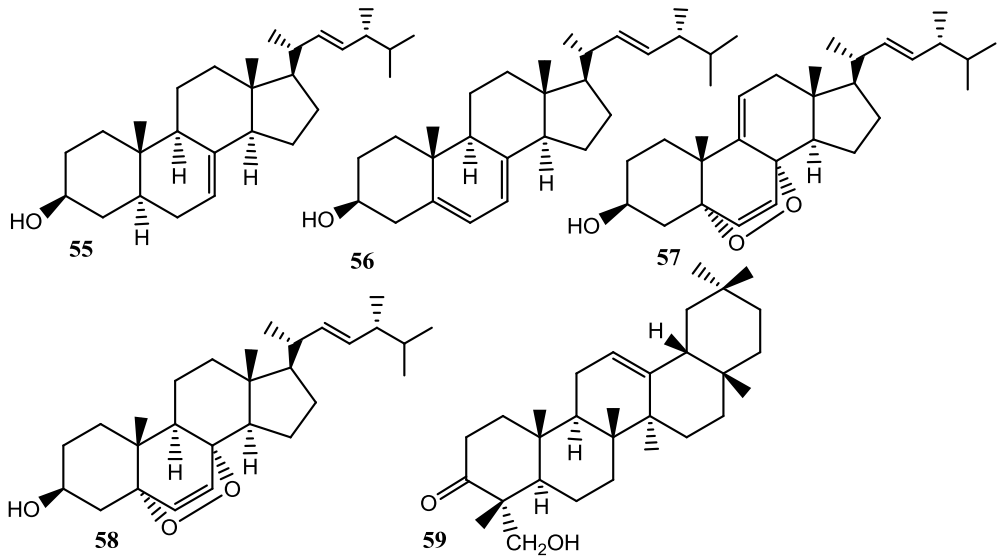
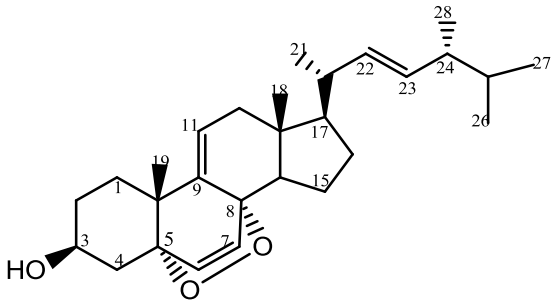


Figure 4.2: Structures of compounds isolated from *Ganoderma applanatum*

4.3.1 Structural elucidation of 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol (57)

Compound **57** was isolated as white crystalline needles from the ethyl acetate crude extract. Interpretation of its ^{13}C NMR and DEPT spectra showed 28 carbon resonances that included six methyl groups, six methylene, eleven methine (five sp^2 methine, and one oxygenated methine), five quartenary carbon atoms (two oxygenated carbon atoms). There were characteristic olefin carbon atoms at δ_c 119.9, 131.0, 132.7, 135.3, 135.6 and 142.8 ppm. Key oxygenated carbons were observed at δ_c 78.6 (C-8), 82.9 (C-5) and 66.6 (C-3) ppm. The ^{1}H-NMR spectra exhibited signals at δ_H 0.74 (3H, s, 18-CH_3), δ_H 0.83 (3H, d, *J* = 6.9 Hz, 27-CH_3), 0.83 (3H, d, *J* = 6.8 Hz, 26-CH_3), δ_H 1.08 (3H, s, 19-CH_3), 0.91 (3H, d, *J* = 6.6 Hz, 28-CH~), 1.00 (3H, d, *J* = 6.2 Hz, 21-CH_3), 4.02 (1H, m, H-3), 5.17 (1H, dd, *J* = 8.3, 15.3 Hz, H-22), 5.23 (1H, dd, *J* = 6.9, 15.3 Hz, H-23), 6.23 (1H, d, *J*= 8.1 Hz, H-6), 6.60 (1H, d, *J* = 8.4 Hz, H-7). The ^{1}H NMR exhibited vinyl proton signals at δ_H 5.40 (H-11), deshielded coupled H6–H7 pair at δ_H 6.29 and 6.60 and H22–H23 pair at δ_H 5.17 and 5.23. The ^{1}H–^{1}H COSY correlations of the proton signals at δ_H 4.02 (1H-3) to δ_H 1.92 (H-2) and δ_H 2.12 (H-4) were also observed. In addition, analysis of the cross-peaks in the NOESY and ^{1}H–^{1}H COSY

spectra confirmed further correlations. The oxygenated methine carbon was assigned to C-3 due to the HMBC correlation between a proton signal at δ_H 4.02, 1H-3 with the C-5 resonance (δ_C 82.5). This was confirmed by HMBC correlations between 3H-19 resonances (δ_H 1.09) with the C-5 resonance (δ_C 82.9).



57

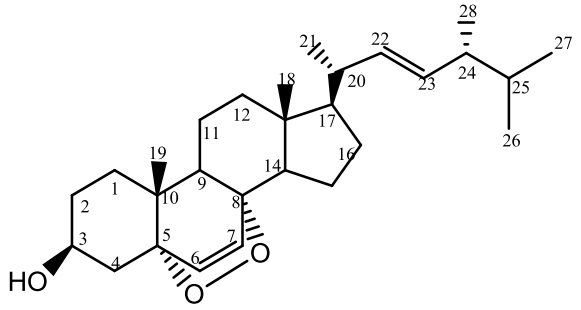
Two other oxygenated quaternary carbons were attributed to the peroxide carbons due to chemical shift values in ^{13}C-NMR that were shifted down field than for primary or secondary carbons with hydroxyl groups. Two of the doubly bonded carbon signals at δc 135.7 and 130.9 respectively were attributable to C-6 and C-7. Their corresponding deshielded protons were at δ_H 6.23 (d, *J* = 8.4 Hz, H-6) and 6.60 (d, *J* = 8.4 Hz, H-7). The carbon at δc 82.9 (C-5) was assigned based on HMBC from H-19, H-1, H-6, H-7 and δc 119.9 (C-11) based on ^{1}H-^{1}H COSY from H-12. The position of the double bond at C9(11) was confirmed by ^{1}H-^{1}H COSY coupling of the δ_H 5.40 (H-11) signal with H-12 and HMBC correlations from H-7, H-12 and H-19. It was identified as 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol. More correlations in the ^{1}H-^{1}H COSY, NOESY and HMBC spectra **Appendices A20-24,** further confirmed its structure. The structure was determined as a sterol and it was also confirmed by comparing its spectral data with the literature (Kobori *et al.*, 2006; Fangkrathok *et al.*, 2013).

Table 4.5: NMR data for 5α,8α-epidioxy-22E-ergosta-6,9(11),22-trien-3β-ol (57) in $CDCl_3$

No.	^{13}C NMR (125 MHz) in $CDCl_3$	^{13}C NMR in $CDCl_3$ (75.5 MHz) (Fangkrathok *et al.*, 2013)	^{1}H NMR (500 MHz)		^{1}H NMR (500 MHz) (Fangkrathok *et al.*, 2013)	
1α	32.8 CH_2	34.9	2.07 m			
1β			1.68 m			
2α	30.8 CH_2	30.7	1.92 m			
2β			1.56 m			
3	66.6 CH	66.7	4.00 m		4.00	
4α	36.3 CH_2	37.2	2.12 m			
4β			1.92 m			
5	82.9 C	83				
6	135.7 CH_2	135.5	6.23 d	*J*=8.4	6.26 d	*J*=8.5
7	130.9 CH	131.0	6.60 d	*J*=8.5	6.40 d	*J*=8.5
8	78.5 C	78.4				
9	142.8 C	142.5				
10	38.2 C	36.1				
11	119.9 CH_2	119.7	5.40 m			
12α	41.4 CH_2	41.2	2.25 m			
12β			2.09 m			
13	43.8 C	43.7				
14	48.3 CH	48.7	1.84 m			
15α	21.1 CH_2	21.3	1.69 m			
15β			1.58 m			
16α	28.8 CH_2	29.0	1.79 m			
16β			1.34 m			
17	56.1 CH	55.9	1.34 m			
18	13.2 CH_3	13.0	0.74 s		0.72 s	
19	25.7 CH_3	25.5	1.09 s		1.08 s	
20	40.1 CH	40.2	2.04 m			
21	20.9 CH_3	20.7	1.00 d	*J*=6.7 Hz	1.00 d	*J*=6.7
22	135.3 CH	135.1	5.17 dd	*J*=7.5, 15.4 Hz	5.16 dd	*J*= 15.2, 8.6
23	132.7 CH	132.5	5.22 dd	*J*=7.5, 15.4 Hz	5.24 dd	*J*= 15.2, 7.8
24	43.8 CH	42.8	1.85 m			
25	33.3 CH	33.1	1.47 m			
26	20.1 CH_3	20.0	0.84 d	*J*=6.9 Hz	0.82 d	*J*=7
27	19.8 CH_3	19.7	0.81 d	*J*=6.9 Hz	0.80 d	*J*=6.9
28	17.7 CH_3	17.5	0.90 d	*J*=6.8 Hz	0.91 d	*J*=7

4.3.2 Structural elucidation of 5α,8α–epidioxyergosta-6,22-dien-3β-ol (58)

Compound **58** was isolated as colourless needles. The ^{13}C-NMR (**Figure 4.15, Table 4.6**) spectrum revealed 28 carbon signals the for C_{28} ergostane type sterol skeleton. Compound **58** was almost similar to **57** except for an extra double bond exhibited by compound **57**. The ^{13}C NMR and DEPT-135 spectrum exhibited six methyl groups, seven methylenes, eleven methine carbons including four olefinic resonances at δc 135.6, 135.4, 132.5, 130.9, and one oxygenated methine at δc 66.7 and four quaternary C-atoms including two oxygenated at δc 82.4 and δc 79.6. One oxygenated methine carbon was assigned to C-3 (δc 66.7) due to the observed HMBC correlation between the 1H-3 (δ_H 4.10) signal with the C-5 signal (δ_C 82.4). The C-3 position was confirmed by HMBC correlations between 3H-19 signals (δ_H 0.88) with the C-5 resonance (δ_C 82.4). The oxygenated quaternary carbons were attributed to the epoxy ring carbons due to chemical shift values in ^{13}C-NMR that were shifted down field than for primary or secondary carbons with hydroxyl groups.



58

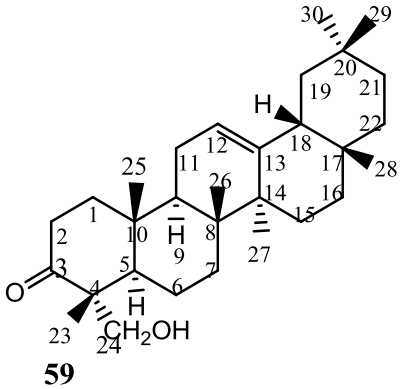
HMBC experiments indicated that δ_H 1.95, 1.69 (2H-1) had ^{1}H-^{13}C long-range correlations with δc 30.2 (C-2) δc 66.7 (C-3), δc 37.2 (C-4) and δc 18.3 (C-19). The carbon δc 79.6 (C-8) had HMBC correlations with δ_H 6.50 (H-6), 6.23 (H-7), 1.95/1.23 (2H-12) and 1.56 (H-14) and 0.88 (H-19). Moreover, the carbon at δc 82.4 (C-5) had cross correlations with δ_H 6.50 (H-6), 6.23 (H-7), 1.91 (H-4), 1.68 (H-1) and 0.88 (3H-19). In addition, the δc 66.7 had HMBC correlations with δ_H 2.11/1.91 (2H-4), 6.50 (1H-6), 1.69 (H-1) and 1.53 (H-2). The correlations were supported by cross peaks in ^{1}H-^{1}H-COSY spectrum. More correlations in the ^{1}H-^{1}H COSY, NOESY and HMBC spectra **Appendices A25-30** and on basis of the further comparison with reported values compound **58** was identified as 5α,8α–epidioxyergosta-6,22-dien-3β-ol (Kobori *et al.*, 2006; Lee *et al.*, 2006).

Table 4.6: NMR data for 5α,8α-epidioxy-22E-ergosta-6,22-dien-3β-ol (58) in $CDCl_3$

No.	^{13}C NMR (125 MHz)	^{13}C NMR (125 MHz) in $CDCl_3$ (Kobori *et al.*, 2006)	^{1}H NMR (500 MHz)	Coupling constants *J* values	^{1}H NMR (500 MHz) (Kobori *et al.*, 2006)
1α	34.9 CH_2	34.7	1.95 m		1.95 m
1β			1.69 m		1.70 m
2α	30.2 CH_2	30.1	1.84 m		1.84 m
2β			1.53 m		1.54 m
3	66.7 CH	66.7	4.00 m		3.97 m
4α	37.2 CH_2	37.0	2.11 m		2.11 m
4β			1.91 m		1.91 m
5	82.4 C	82.2			
6	135.6 CH_2	135.4	6.23 d	*J*=8.5	6.24 d *J*=8.5
7	130.9 CH	130.8	6.50 d	*J*=8.5	6.40 d *J*=8.5
8	79.6 C	79.4			
9	51.3 CH	51.1	1.49 m		1.50 m
10	37.1 C	37.0			
11α	23.6 CH_2	23.4	1.74 m		1.53 m
11β			1.35 m		1.23 m
12α	39.5 CH_2	39.4	1.95 m		1.96 m
12β			1.23 m		1.23 m
13	44.8 C	44.6			
14	51.9 CH	51.7	1.56 m		1.56 m
15α	20.8 CH_2	20.6	1.59 m		1.60 m
15β			1.40 m		1.40 m
16α	28.8 CH_2	28.7	1.74 m		1.75 m
16β			1.35 m		1.35 m
17	56.4 CH	56.2	1.23 m		1.22 m
18	13.1 CH_3	12.9	0.80 s		0.82 s
19	19.8 CH_3	19.7	0.81 s		0.81 s
20	40.0 CH	39.7	2.02 m		2.02
21	21.2 CH_3	20.9	0.99 d	*J*=6.7 Hz	1.00 d *J*=6.7
22	135.2 CH	135.7	5.16 dd	*J*=7.5, 15.4 Hz	5.14 dd *J*= 15.2, 8.6,
23	134.3 CH	134.1	5.22 dd	*J*=7.5, 15.4 Hz	5.22 dd *J*= 15.2, 7.8
24	43.0 CH	42.8	1.85 m		1.85 m
25	33.3 CH	33.1	1.46 m		1.47 m *J*= 13.5, 6.8
26	20.1 CH_3	20.0	0.84 d	*J*=6.9 Hz	0.83 d *J*=6.8
27	19.8 CH_3	19.7	0.81 d	*J*=6.9 Hz	0.81 d *J*=6.9
28	17.7 CH_3	17.7	0.90 d	*J*=6.8 Hz	0.91 d *J*=6.7

4.3.3 Structural elucidation of 24-hydroxy-olean-12-en-3-one (59)

Compound **59** was obtained as a white armophous powder 12 mg. Analysis of its ^{13}C NMR and DEPT-135 spectra (**Table 6**) showed 30 carbon resonances, including seven methyls, eleven methylenes including one oxygenated methylene, four methines inclusive of one sp^2 methines, and eight quartenary carbons together one carbonyl carbon. The ^{13}C NMR spectrum confirmed the presence of double bond signals at δ_C 144.0 and 122.9 ppm which were assigned to C-12 and C-13, respectively, of an olean-12-ene-type. The carbon skeleton was same as that of *beta*-amyrin except for a carbonyl carbon at position C-3 and an oxygenated methylene carbon at position C-24. The ^1H NMR (500 MHz, $CDCl_3$) spectrum showed the presence of seven singlet methyl group proton resonances at δ_H 1.02 (s, 3H-23), 0.90 (s, 3H-30), 0.84 (s, 3H-26), 0.93 (s, 3H-29), 1.14 (s, 3H-25) 1.15 (s, 3H-27) and 0.91(s, 3H-28). The ^1H NMR spectrum of compound **59** was characteristic of pentacyclic triterpenoid of β amyrin displaying signals for one vinylic proton at δ_H 5.22 (H-12). In addition there was a quartet like signal for hydroxyl methylene protons at δ_c 67.3 and (δ_H 3.65/3.40 2H, d, J= 11.5 Hz). HMBC spectrum showed correlations between C-5 (δ_C 49.3) with the H-25 (δ_H 1.15) and H-24 (δ_H 3.64) proton resonances. In addition there were HMBC correlations from the carbonyl carbon C-3 (δ_C 220.0) to the protons at (δ_H 1.02, H-23) and at (δ_H 3.64/3.44, H-24). The ^1H-^1H COSY of **59** showed correlation peaks from H-1 to H-2, H-6 to H-7 and H-11 to H-12. The correlation between the resonance at δ_H 1.15 (3H-27) and δ_c144.0 (C-13) in the HMBC spectrum confirmed the placement for the alkene group. In addition, it was observed that there were NOESY correlations between H-6 (δ_H 1.40) and 2H-24 (δ_H 3.65/3.40) and H-5 to 2H-24.



59

Furthermore, NOESY correlations between 2H-24 (δ_H 3.65) resonance and H-5 (δ_H1.62) resonance were also observed. The deshielded proton 1H-5 at (δ_H 1.62) was due to through

space correlations with the hydroxyl group of the oxymethylene carbon-24 at δ_c 67.2 compared a proton at δ_H 0.68 for a β amyrin triterpene compound, hence both have the α configuration. The H-9 resonance also showed correlations in the NOESY spectrum with the 3H-27 resonance (δ_H 1.15). All these protons (1H-5 and H-9) are in the α-orientation according to the biosynthesis, while the H-18 (δ_H 2.8), 3H-23 (δ_H1.02), H-25((δ_H 1.15) and 3H-26 (δ_H 0.88) resonances, which showed correlations with each other in the NOESY spectrum, are in the β- orientation. The compound 24-hydroxyolean-12-en-3-one had earlier been isolated from the stem bark of *Symplocos racemose* (Ali *et al.*, 1990) but due to scanty spectroscopic data for the compound it was compared with spectroscopic data for the compound β-amyrin pentacyclic triterpenoid. The complete assignment of the protons and carbons was accomplished as shown in Table 5 and other correlations (**Figure 4.3**) in the ^{1}H-^{1}H COSY, NOESY and HMBC spectra **Appendices A31-36.** Based on these evidence compound **59** was named as 24-hydroxyolean-12-en-3-one.

Table 4.7: NMR data for 24-hydroxy-olean-12-en-3-one (59) in $CDCl_3$

No.	^{13}C NMR (125 MHz)	^{1}H NMR (500 MHz)	^{13}C NMR (125 MHz) (Hernandez *et al.*, 2012)	^{1}H NMR (500 MHz) (Hernandez *et al.*, 2012)
1α	35.4 CH_2	2.68 m	38.7	
1β		2.28 m		
2	24.8 CH_2	1.61 m	27.2	
3	219.6 C		79.3	3.15 dd
4	52.1 C		38.5	
5	49.5 CH	1.62 m	55.1	0.68
6α	19.4 CH_2	1.54 m	18.6	
6β		1.40 m		
7α	32.1 CH_2	1.33 m	32.4	
7β		1.26 m		
8	37.2 C		39.8	
9	47.3 CH	1.69 t *J*=3.5 Hz	47.6	
10	55.4 C		36.9	
11α	39.0 CH_2	1.93 m	23.6	
11β		1.40 m		
12	122.9 CH	5.30 t *J*=3.6 Hz	121.7	5.12 t *J*=3.2
13	144.0 C		145.2	
14	42.2 C		41.7	
15α	27.9 CH_2	1.72 m	26.2	1.89
		1.13 m		
16	29.3 CH_2	1.30 m	26.1	
17	31.2 C		32.6	
18	41.4.0 CH	2.80 m	47.2	
19	46.0 CH_2	1.15 m	46.8	1.93
19		1.64 m		
20	34.0 C		31.0	
21α	32.6 CH_2	1.78 m	34.7	
21β		1.25 m		
22α	33.8 CH_2	2.34 m	37.1	1.64.
22β		1.25 m		1.16
23	17.2 CH_3	1.02 s	28.0	0.77
24	67.2 CH_2	3.65 d *J*=11.3 Hz	15.4	0.90
		3.44 d *J*=11.0 Hz		
25	15.9 CH_3	1.15 s	15.4	0.73
26	17.4 CH_3	0.84 s	16.8	0.93
27	26.1 CH_3	1.15 s	25.9	1.19
28	15.8 CH_3	0.91 s	28.4	1.07
29	33.3 CH_3	0.91 s	33.8	0.87
30	23.8 CH_3	0.93 s	23.7	0.80

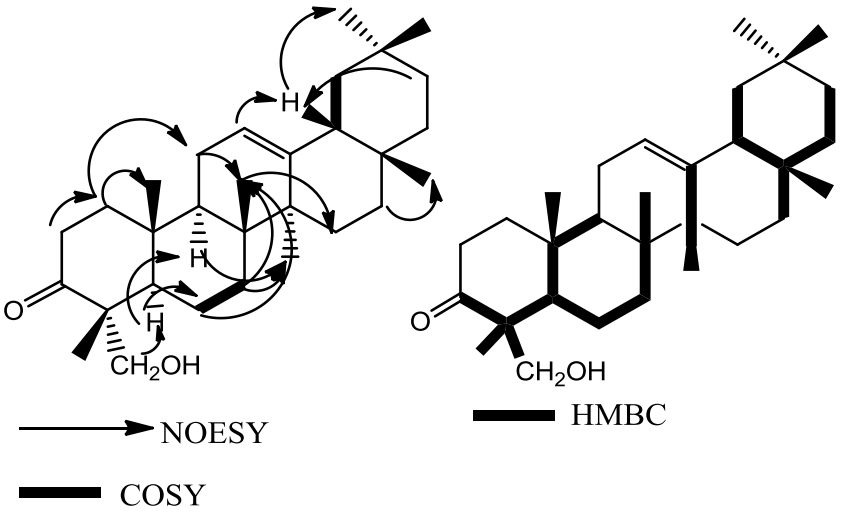


Figure 4.3: COSY, NOESY and HMBC correlations of 24-hydroxyolean-12-en-3-one (**59**)

4.4 Compounds from *Ganoderma australe (*Fr.) Pat

Chemical investigation of the dried fruiting bodies of *Ganoderma australe* afforded three ergostane type compounds **Figure 4.4**. All the compounds have previously been isolated from other sources. By employing detailed NMR spectroscopic analysis, as well as by comparison with literature data, the structures of these compounds were identified.

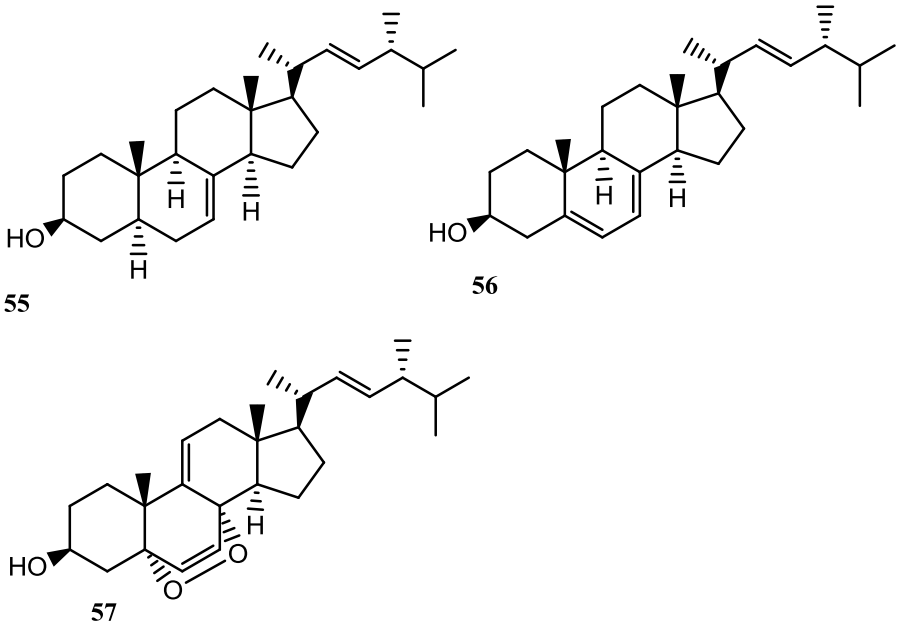


Figure 4.4: Structures of compounds isolated from *Ganoderma australe*

4.5 Compounds from *Trametes elegans*

Isolation of the *Trametes elegans* extract yielded six compounds **Figure 4.5**. All the six compounds had previously been isolated and identified. Interpretation of the spectra of the isolated compounds and comparison with literature data achieved the structures of the compounds below.

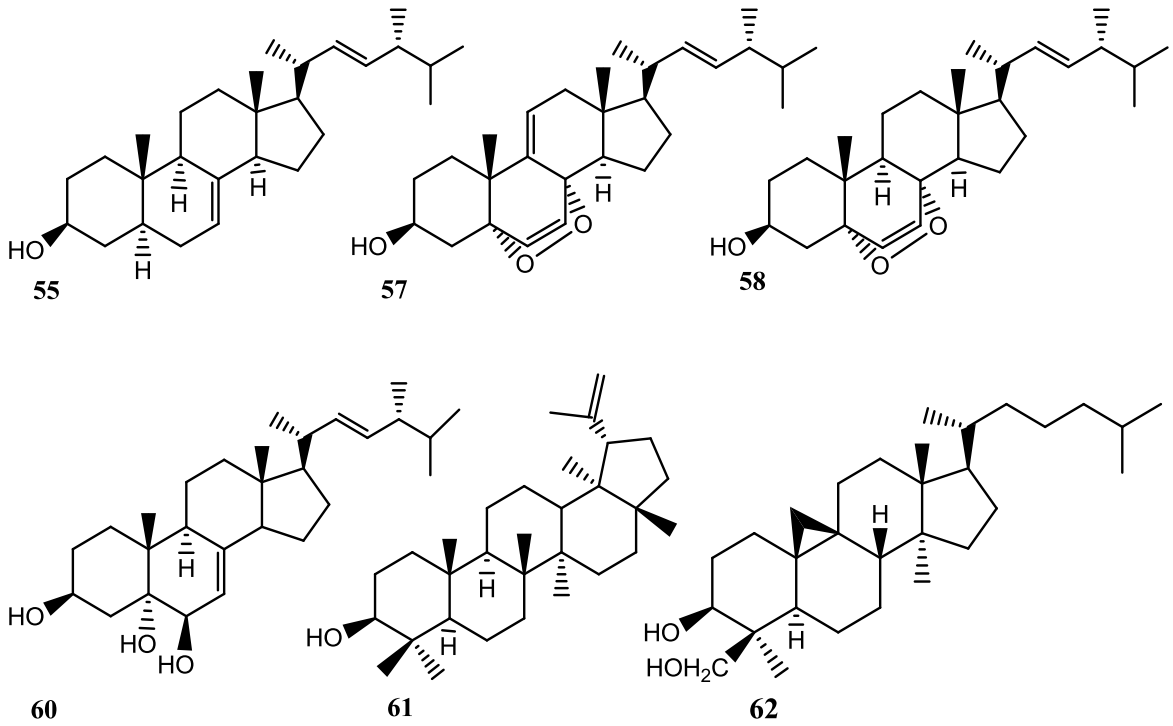
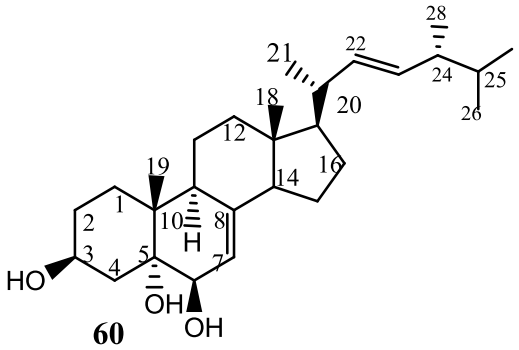


Figure 4.5: Structures of compounds isolated from *Trametes elegans*

4.5.1 Structural elucidation of ergosta-7,22-dien-3β,5α,6β-triol (60)

The compound ergosta-7,22-dien-3β,5α,6β-triol (**60**) was isolated as a white amorphous powder and had previously been identified from *Spongionella gracilis*, a sponge fungal species (Piccialli and Sica, 1987), *Aspergillus ochraceus* 43 (Li *et al.*, 2005), mangrove fungus *Aspergillus awamor*i (Gao *et al.*, 2007) *Ganoderma applanatum* liquid culture (Lee *et al.*, 2011). The ^{1}H NMR exhibited resonance of six methyl groups including two tertiary methyl groups at δ_H 0.92 (3H, s, H-18) and 1.08 (3H, s, H-19). The existence of two double bonds were designated by the proton signals in the ^{1}H NMR spectrum for three vinyl protons between 5.30 and 5.17 ppm (including one trans double bond at δ_H 5.17 1H, dd, *J*= 8.4, 15.3 Hz, H-22). The 'H NMR spectrum also contained one-proton signals at δ_H 4.07 and 3.63 ppm, consistent with the presence of two oxygenated methane protons. The unusual downfield signal of δ_H 4.08 (1H-3α) was due to through space interaction with the hydroxyl group at C-5 typical of 3β-hydroxysterols bearing a 5α-hydroxyl group. In addition the 'H

NMR spectrum showed, an olefinic proton at δ_H 5.30 *J*=5.8 Hz coupled with the broad singlet at δ_H 3.63. The spectrum also contains a doublet for the vinyl protons H-7 (5.3 ppm, = 5.8 Hz), and doublet of doublets H-22 (5.16 ppm, *J* = 15.39 Hz. *J*, = 8.09 Hz), and H-23 (5.23 ppm, *J* = 15.39 Hz, *J* = 7.04 Hz). The ^{13}C and the DEPT-135 spectra revealed the presence of 28 carbon atoms including four quaternary carbon atoms (with one oxygenated at δc 76.2, eleven mithine groups (three olefinic and two oxygenated), seven methylene groups and six methyl groups. The ^{l3}C-NMR spectrum of **60** showed the presence of three sp^3 carbons attached to oxygen atoms, two secondary at δ_c 68.0 and 73.8 and one tertiary at δ_c 76.2. The three carbon signals at δc 68.0, 76.2 and 73.8 in the ^{13}C NMR spectrum were assigned to C-3, C-5 and C-6, respectively. The key HMBC cross correlations were from δc 76.2 (C-5) to δ_H 1.08 (3H-19) and δc 56.2 (C-17) with δ_H 0.60 (3H-18) and δ_H 1.02 (3H-21).



60

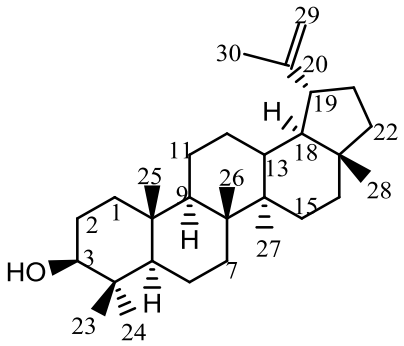
The signals for the vinylic carbon atoms C-7 (117.7 ppm), C-8 (143.2 ppm), C-22 (135.5 ppm), and C-23 (132.3 ppm) are also important for proving the structure. There were ^1H-^1H COSY correlations at δ_H 4.07 to δ_H 1.83 (H-2), δ_H 2.14 (H-4) and also δ_H 3.60 (H-6) with δ_H 5.30 (H-7). There were observed HMBC cross correlations from H-4 to C-5 and C-6 and from H-7 and H-19 to C-5 this was supported by ^1H-^1H COSY correlations from H-6 to H-7. The other correlations in the ^1H-^1H COSY, NOESY and HMBC spectra **Table 4.9, Appendices A37-41** further confirmed the structure of compound **61,** and by comparison of its spectroscopic data with those reported in the literature (Cafieri *et al.*, 1985; Piccialli and Sica, 1987; Gao *et al.*, 2001; Li *et al.*, 2005; Lee *et al.*, 2006) confirmed the structure of the compound.

Table 4.8: NMR data for ergosta-7,22-dien-3β,5α,6β-triol in $CDCl_3$ against reference values

No.	^{13}C NMR (125 MHz)	^{13}C NMR (125 MHz) in $CDCl_3$ (Li *et al.*, 2005)	^{1}H NMR (500 MHz)	Experimental coupling constant *J* in Hz	^{1}H NMR (500 MHz) in $CDCl_3$	
1α	33.2 CH_2	33.0	1.61 m			
1β			1.54 m			
2α	31 CH_2	30.9	1.86 m			
2β			1.44 m			
3	68.0 CH	67.7	4.07 m		4.08 m	
4α	39.6 CH_2	39.6	2.14 m			
4β			1.78 m			
5	76.2 C	76.0				
6	73.8 CH_2	73.8	3.60 s		3.62 s	
7	117.7 CH	117.6	5.30 d	*J* = 5.8 Hz	5.35 d	*J* = 4.9 Hz
8	144.2 C	144.0				
9	43.6 CH	42.9	1.26 m			
10	37.2 C	37.2				
11α	22.3 CH_2	22.1	1.78 m			
11β			1.26 m			
12α	39.4 CH_2	39.3	2.06 m			
12β			1.31 m			
13	43.9 C	43.8				
14	55.0 CH	54.8	1.95 m			
15α	23.1 CH_2	22.9	1.45 m			
15β			1.29 m			
16α	28.1 CH_2	27.9	1.75 m			
16β			1.34 m			
17	56.2 CH	56.1	1.23 m			
18	12.5 CH_3	12.3	0.60 s		0.60 s	
19	19.9 CH_3	18.8	1.08 s		1.08 s	
20	40.7 CH	40.3	2.02 m			
21	21.3 CH_3	21.1	1.02 d	*J*=6.9 Hz	1.03 d	*J*=6..6 Hz
22	135.9 CH	135.4	5.18 dd	*J*=8.3, 15.4 Hz	5.16 dd	*J*=15.2, 7.2 Hz
23	132.3 CH	132.3	5.19 dd	*J*=7.5, 15.4 Hz	5.22 dd	*J*=15.5, 7.9 Hz
24	43.0 CH	43.6	1.84 m			
25	33.3 CH	33.1	1.46 m			
26	20.2 CH_3	19.6	0.82 d	*J*=6.8 Hz	0.82 d	*J*=7.0 Hz
27	19.9 CH_3	19.9	0.82 d	*J*=6.9 Hz	0.84 d	*J*=7.0 Hz
28	17.7 CH_3	17.6	0.91 d	*J*=6.7 Hz	0.92 d	*J*=6.6 Hz

4.5.2 Structural elucidation of Lupeol (61)

The compound lupeol (**61**) was obtained as white solid powder. The ^{1}H NMR spectrum showed the presence of seven tertiary methyl protons at δ 0.76, 0.78, 0.82, 0.93, 0.95, 1.02 and 1.65 (3H each). A sextet proton resonance at δ_H 2.35 referable to H-19 which is typical to the lupeol molecule was observed. In addition a multiplet resonance signal at δ_H 3.18 was assigned to H-3 while a pair of doublets at δ 4.56 and δ 4.67 (J_2= 2.4, 1H each) were typical of olefinic non-equivalent germinal protons at (2H-29). Further, the ^{13}C NMR experiment showed seven methyl groups at 14.7 (C-27), 15.6 (C-24), 16.1 (C-26), 16.3 (C-25), 18.2 (C-28), 19.5 (C-30) and δc 28.2 (C-23) typical of the lupeol molecule. There were signals due to an exomethylene group at δc 109.5 (C-29) and 151.2 (C-20), ten methylene, five methine and five quaternary carbon atoms that were assigned with the aid of DEPT-135 experiment. The spectra showed a deshielded signal at δc 79.2 that was due to C-3 with a hydroxyl group attached to it.



61

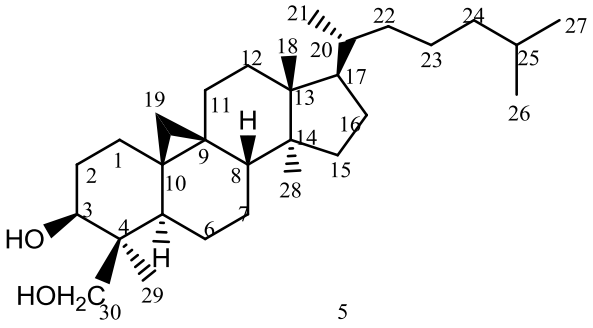
From the HMBC spectrum, there were cross peaks correlations between a methine proton signal at δ_H 3.18 (H-3) to methyl carbon signals at δc 28.2 (C-23) by J_2 correlation and a methyl carbon signal δc 18.5, (C-6) by J_3 correlation. In addition the methine proton signal at δ_H 2.35 (H-19) showed cross peaks correlations to a methine carbon signal δc 48.2 (C-18), a methyl carbon signal δc 19.5 (C-30) and a quaternary carbon signal δc 151.2 (C-20), two methylene carbon signals at δc 30.0 (C-21) and δc 109.5 (C-29). The olefinic protons at δ_H 4.56 and 4.67 also showed cross peaks correlations with a methine carbon signal at δc 48.0 (C-19) and δc 19.5 (C-30) by J_3 correlation. These structural assignments for this compound were in good agreement for the lupeol molecule. Other assignments were accomplished through the 2D NMR experiments (COSY and HMBC) in **Appendices A42-45** and then the structure confirmed by comparing the data reported from literature (Burns *et al.*, 2000; Jamal *et al.*, 2008; Jain and Bari, 2010).

Table 4.9: NMR data for lupeol in $CDCl_3$ compared against reference values

No.	^{13}C NMR (125 MHz)	^{13}C NMR (100 MHz) (Burns *et al.*, 2000)	^{1}H NMR (500 MHz)	1H NMR (500 MHz) (Burns *et al.*, 2000)
1α	38.9 CH_2	38.9	0.99 m	0.90 m
1β			1.65 m	1.67 m
2α	27.4 CH_2	29.6	1.60 m	1.60 m
2β			1.56 m	1.56 m
3	79.2 CH	79.0	3.19 dd *J*=11.3, 4.8 Hz	3.19 (1H, dd)
4	38.9 C	39.1		
5	55.5 CH	55.5	0.68 d *J*=9.25 Hz	0.68 (1H, d)
6α	18.5 CH_2	18.5	1.46 m	1.51 m
6β			1.38 m	1.39 m
7α	34.5 CH_2	34.3	1.38 m	1.39 m
8	41.0 C	40.8		
9	50.6 CH	50.6	1.25 m	1.27 m
10	37.4 C	37.1		
11α	21.1 CH_2	20.9	1.38 m	1.41 m
11β			1.21 m	1.23 m
12α	25.3 CH_2	25.2	1.06 m	1.07 m
12β			1.67 m	1.67 m
13	38.3 CH	37.2	1.65 m	1.66 m
14	43.0 C	42.9		
15α	27.7 CH_2	27.5	1.00 m	1.00 m
15β			1.67 m	1.68 m
16α	35.8 CH_2	35.8	1.36 m	1.37 m
16β			1.46 m	1.47 m
17	43.0 C	43.2		
18	48.3 CH	48.5	1.36 m	1.36
19	48.2 CH	48.0	2.36 m	2.39 m
20	151.2 C	150.9		
21α	30.0 CH_2	29.9	1.32 m	1.32 m
21β			1.92 m	1.92 m
22α	40.2 CH_2	40.0	1.17 m	1.23 m
22β			1.38 m	1.41 m
23	28.2 CH_3	28.2	0.95 s	0.97 s
24	15.6 CH_3	15.6	0.76 s	0.76 s
25	16.1 CH_3	16.3	0.82 s	0.83 s
26	16.0 CH_3	16.2	1.02 s	1.03 s
27	14.8 CH_3	14.7	0.93 s	0.96 s
28	18.0 CH_3	18.2	0.78 s	0.79 s
29	109.0 CH_2	109.5	4.56 s,br *J*=2.4 Hz	4.56 (1H, br, s)
29			4.69 s, br *J*=2.3 Hz	4.67 (1H, br, s)
30	19.5 CH_3	19.5	1.65 s	1.68 s

4.5.3 Structural elucidation of 9,19-cycloartane-3,30-diol (62) steroid

Compound **62** was isolated as a colourless solid. The ^{13}C NMR displayed thirty carbon atoms. The ^{1}H and ^{13}C NMR, DEPT, and HSQC data supported the presence of eight methine carbons including an oxymethine (δ_C 76.8/δ_H 3.22), twelve methylenes including one oxygenated (δ_C 63.4/δ_H 3.63) and four sp3 quaternary carbons. ^{1}H-NMR spectrum showed signals due to three secondary methyl groups at 0.86, 0.88 and 0.86, three tertiary methyl singlet signals at 0.98, 0.96 and 0.89 and a characteristic non-equivalent proton doublets of cyclopropane methylene at δ_H 0.38 (*J*=3.98 Hz) and at δ_H 0.14 (*J*=4.16 Hz). The H-20 resonance at δ_H 1.36 coupled with the H-21 resonance at δ_H 0.86 (d, *J*= 6.5 Hz) in the COSY spectrum.



62

The two geminal methyl group proton resonances (δ_H 0.86 and 0.87, *J*= 6.8 Hz), were assigned to H-26 and H-27, due to coupling with the H-25 resonance (δ_H 1.53 septet, *J*=6.8 Hz), seen in the COSY spectrum. The resonances at δ_H 3.84 and 3.65, in the primary oxygenated carbon region, were assigned to the two H-30 proton which showed correlations with the C-5 (δ_c 43.6) resonance and C-29 resonance (δ_c 14.6) in the HMBC spectrum. From the HMBC spectrum, the methine proton signal at δ_H 3.22 (H-3) showed cross peaks with a oxymethylene carbon signal (δc 63.5, C-30) by J_2 correlation. The methyl protons at δ_H 0.98 (H-29) showed cross peaks with a δc 76.8 (C-3), δc 40.4 (C-4) and 44.8 (C-5). The confirmation of the structure of 9,19-cyloartane-3,30-diol was accomplished through the 2D NMR experiments (COSY and HMBC) appendices **A46-49** and data reported from literature (Khan *et al.*, 1994).

Table 4.10: NMR data for 9,19-cycloartane-3,30-diol (62) in $CDCl_3$ against Literature data

No.	^{13}C NMR (125 MHz)	^{13}C NMR (75.4 MHz) (Khan *et al.*, 1994)	^{1}H NMR (500 MHz)	^{1}H NMR (500 MHz)
1α	30.0 CH_2	31.9	1.26 m	
1β			1.16 m	
2α	35.0 CH_2	31.0	1.98 m	
2β			1.43 m	
3	76.8 CH	78.6	3.22 dd *J*=11.6, 4.8 Hz	3.27 dd *J*=11.2, 4.3 Hz
4	40.4 C	39.6		
5	43.5 CH	47.1	1.20 m	
6α	24.9 CH_2	21.0	1.67 m	
6β			0.58 m	
7α	28.3 CH_2	28.3	1.89 m	
7β			1.58 m	
8	47.1 CH	47.8	1.27 m	
9	23.1 C	20.1		
10	25.9 C	26.2		
11α	25.4 CH_2	26.0	1.31 m	
11β			1.06 m	
12	35.6 CH	35.6	1.27 m	
13	45.6 C	45.1		
14	49.1 C	48.8		
15	33.1 CH	32.7	1.60 m	
16α	27.2 CH_2	26.5	1.22 m	
16β			1.95 m	
17	52.1 CH	52.2	1.55 m	
18	18.0 CH_3	17.9	0.96 s	0.95 s
19α	27.5 CH_2	29.8	0.38 d *J*=4.1 Hz	0.56 d *J*=4.18
19β			0.14 d *J*=4.1 Hz	0.33 d *J*=4.26
20	36.7 CH	36.1	1.36 m	
21	18.6 CH_3	18.3	0.86 d *J*=6.5 Hz	0.87 d *J*=6.72 Hz
22α	35.6 CH	36.4	1.27 m	
23	24.3 CH_2	24.0	1.34 m	
			1.15	
24	39.8 CH	39.4	1.12 m	
25	28.2 CH	28.2	1.53	
26	22.8 CH_3	22.5	0.87 d *J*=6.8 Hz	0.90 d *J*=7.18
27	23.1 CH_3	22.7	0.86 d *J*=6.8 Hz	0.90 d *J*=7.18
28	17.9 CH_3	19.3	0.89 s	0.94 s
29	14.6 CH_3	22.0	0.98 s	0.95 s
30	63.5 CH_2	63.1	3.64 d *J*=12.4 Hz	3.64 d *J*=12.8 Hz
			3.64 d *J*=12.4 Hz	3.64 d *J*=12.8 Hz

4.6 Susceptibility of bacterial and fungal strains to the compounds

In the current study, antibacterial activity of eight compounds namely, **54**, **55**, **56**, **57**, **58**, **59** **60** and mixture of **57** and **58** was determined against various pathogenic bacteria as described in section 3.8.2 and the results summarised in **Table 4.11**

Table 4.11: Zones of inhibition in millimetres by different compounds against various bacteria strains

BS CPD	Negative control	Ciprofloxacin	54	55	56	57 & 58	57	58	59	60
S. Pyogenes	6.0±0.00^{c}	31.0±0.58^{a}	6.0±0.00^{c}	**9.7±0.33^{b}**	**9.0±0.58^{b}**	**9.0±0.58^{b}**	6.0±0.00^{c}	6.0±0.00^{c}	**8.0±0.58^{b}**	6.0±0.00^{c}
S.pneumonia	6.0±0.00^{c}	30.0±0.58^{a}	**7.7±0.67^{b}**	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}
K.Pneumonia	6.0±0.00^{b}	32.7±1.45^{a}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}
E.Feacalis	6.0±0.00^{c}	24.0±0.58^{a}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	**7.7±0.67^{b}**	6.0±0.00^{c}
S. aureus	6.0±0.00^{c}	19.0±0.58^{a}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	6.0±0.00^{c}	**8.0±0.58^{b}**
C.enterocolitica	6.0±0.00^{b}	22.0±1.15^{a}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}
Shigella	6.0±0.00^{b}	24.7±0.88^{a}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}
E. Coli	6.0±0.00^{b}	22.3±0.88^{a}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}
C.albicans	6.0±0.00^{b}	27.7±0.33^{a}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}	6.0±0.00^{b}

Data are reported as mean±SEM.
^{a,b} represents mean values row-wise followed by the same superscript are not significantly different at ($p<0.05$).

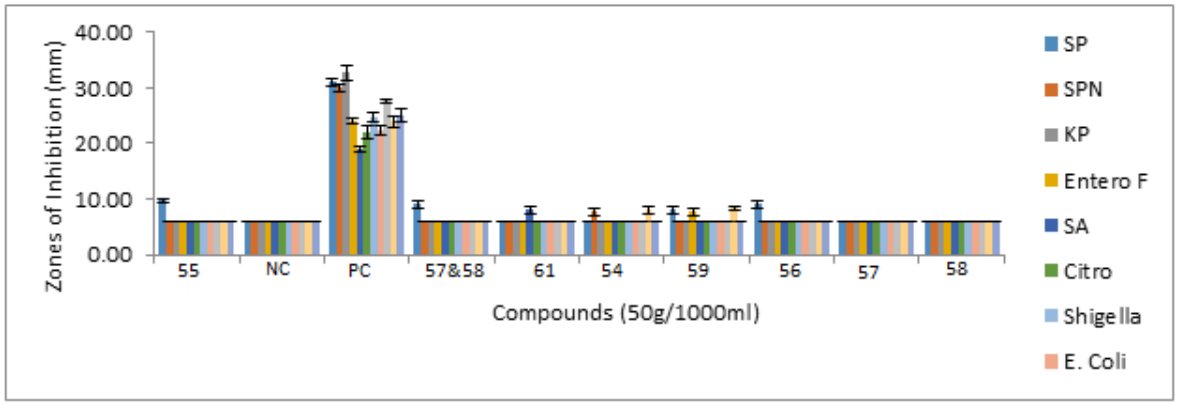


Figure 4.6: Zone of inhibition by different compounds against various bacteria strains

It was observed that **55, 56, 59** and mixture of **57** and **58** had weak inhibitory activity than the negative control against Gram positive *Streptococcus pyogenes* at 9.7±0.33, 9.0±0.58, 8.0±0.58 and 9.0±0.58 mm respectively (*p≤0.05;* **Table 4.11**). *Streptococcus pneumonia* was significantly inhibited by **54** (7.67±0.67) mm more than the negative control. Further, it was observed that **54** inhibited the fungus *Crytococcus neoformas* signicantly the negative control. The bacteria *Entero feacalis* was also significantly inhibited by **59** (7.7±0.67 mm) than the negative control. Compound **60** was also found to inhibit *Staphylococcus aureus* by 8.0±0.58 mm. However, for all the compounds, none was significantly active than the positive control (ciprofloxacin). From all the tested compounds it was observed that compound **54** and **59** inhibited the fungus *Cryptococcus neoformans* by 8.0±0.58 mm and 8.0±0.33 mm**,** respectively. Compound 24-hydroxy-olean-12-en-3-one (**59**), a *beta* amyrin derivative was found to be the most active as it inhibited two of the Gram positive strains and one fungus supporting the documented information about the antimicrobial activity of the *beta* amyrin compounds.

The present study reveals that compounds isolated from polypore basidiomycetes can have efficacy to fight infectious microbes. Compounds **54**, **55**, **56, 59**, a mixture of **57** and **58** and **60** demonstrated weak antimicrobial activities against clinically important Gram positive bacteria and fungus. Compounds **57** and **58** did not inhibit individually any of the tested strains but a mixture of the two compounds inhibited *Streptococcus pyogenes* probably due to synergism. This study has pinpointed the potential of these compounds as antimicrobial agents. Ciprofloxacin the positive control showed more potent antimicrobial than the tested compounds. Therefore, the compounds can be used as templates for development of various antimicrobial agents.

Due to differences in the cell wall of Gram-positive and Gram-negative bacteria, all the Gram-negative bacteria were largely found to be more resistant to antimicrobial compounds than Gram-positive bacteria. Gram-negative bacteria have a double lipid bilayer between the peptidoglycan and the lipopolysaccharide outer layer and therefore resulting in a low degree of permeability for lipophilic small drug compounds compared to Gram-positive bacteria that contain a porous layer of peptidoglycan and a single lipid bilayer outer layer (Sharma *et al.*, 2011). The outer membrane of Gram negative bacteria plays a significant role connected to resistance various antibiotics that are extremely effective, for example rifamycin, macrolides, lincomycin, novobiocin, fusidic acid and clindamycin (Sperandio *et al.*, 2013).

The compounds from the polypore species under study showed variable antimicrobial activities and a number of factors might be at play. From studies, Basidiomycetes extracts are documented to have changeable antimicrobial activity, reliant upon the nature of environment, genetic nature of the basidiomycete's species, test organisms, media in which the test organism grows, solvent used for extraction and dissimilarities in physical and biochemical nature of the antimicrobial mushroom components. (Iwalokun *et al.*, 2007; Ramesh and Pattar, 2010).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study evaluated three *Ganoderma* (*Ganoderma adspersum, Ganoderma applanatum, Ganoderma australe*) and *Trametes elegans* species of wild basidiomycetes fungi collected from the rift valley forests of Kenya. The crude extracts prepared from these polypores had a percentage yield ranging from 0.6 % to 1%. The yield was based on dry sample material. It was noted that the percentage yields were low, nevertheless the results were in agreement with other previous studies. From *Ganoderma adspersum* three compounds were obtained, *Ganoderma applanatum* gave five compounds and *Ganoderma australe* gave three compounds. Similarly from *Trametes elegans* six compounds were obtained.

The NMR experiments gave spectra that were interpreted with the help a TOPSPIN software to obtain the structures of the compounds. Compounds from *Ganoderma adspersum* ergosta-5,22-dien-3-one (**54**), ergosta-7-22-dien-3-ol (**55**), ergosta-5,7,22-trien-3β-ol (**56**) were elucidated. Compounds **55**, **56**, 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol (**57**), *5α,8α–* epidioxyergosta-6,22-dien-3β-ol (**58)** and 24-hydroxy-olean-12-en-3-one (**59**) were identified from *Ganoderma applanatum.* It was noted that compound **59** is being reported for the first time from *G. applanatum.* The polypore *Ganoderma australe* gave compounds **55**, **56**, **57** and finally from *Trametes elegans* the compounds **55**, **57**, **58**, ergosta-7,22-dien-3β,5α,6β-triol (**60**), Lupeol (**61**) and 9,19-cycloartane-3,30-diol (**62**) were also identified. It was worthy to note that isolation of compounds from *Trametes elegans* were being reported for the first time.

In conclusion, we can state that the compounds isolated from *G. adspersum, G. applanatum, G. australe* and *Tramete elegans* displayed weak antimicrobial activity against Gram positive *Streptococcus pyogenes* and *Streptococcus pneumonia.* Similarly, lower antimicrobial activity against a fungus *Cryptococcus neoformans* was observed. From the results antimicrobial activity of the compounds against a fungus *Candida albicans* and Gram-negative bacteria was not observed. The current study has demonstrated that the selected medicinal Kenyan *Ganoderma* and *Trametes* species have antimicrobial activity. Compounds **54**, **55**, **56**, **59** and **60** were found to contribute to the observed antimicrobial activity hence can serve as potential antimicrobials.

5.2 Recommendations

i) There is need for sufficient amounts of the polypore material and optimized extraction properties to enhance the extraction efficiency.

ii) More elaborate equipment (HPLC) should be used for fractionation and purification as little sample will be lost during the process and the yield of the pure compounds will be enhanced.

iii) One concentration of 50 mg/ml used in agar diffusion assay hence need for further assays to obtain minimum inhibitory concentration (MIC).

iv) More research should be carried out on other Kenyan polypore basidiomycetes in order to document their activity.

REFERENCES

Adams M., Christen M., Plitzko I., Zimmermann S., Brun R., Kaiser M. and Hamburger M. (2010). Antiplasmodial lanostanes from the *Ganoderma lucidum* mushroom. *Journal of Natural Products,* **73**: 897-900.

Adeyelu A. T., Oyetayo V. O., Onile T. A. and Awala S. I. (2018). Anticandidal Effect of Extracts of Wild Polypore, *Trametes elegans,* on *Candida* Species Isolated from Pregnant Women in Selected Hospitals in Southwest Nigeria. *Annals of Complementary and Alternative Medicine,***1**:4-8.

Al-Fatimi M., Wurster M., Kreisel H. and Lindequist U. (2005). Antimicrobial, cytotoxic and antioxidant activity of selected basidiomycetes from Yemen. *Die Pharmazie-An International Journal of Pharmaceutical Sciences,* **60**: 776-780.

Alberts A. W. (1988). Discovery, biochemistry and biology of lovastatin. *The American Journal of Cardiology,* **62**: J10-J15.

Ali M., Bhutani K. K. and Srivastava T. N. (1990). Triterpenoids from *Symplocos racemosa* bark. *Phytochemistry*, **29**: 3601-3604.

Ali S. M., Siddiqui R. and Khan N. A. (2018). Antimicrobial discovery from natural and unusual sources. *Journal of Pharmacy and Pharmacology*, **70**: 1287-1300.

Alves M. J., Ferreira I. C. F. R., Froufe H. J. C., Abreu R. M. V., Martins A. and Pintado M. (2013). Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *Journal of Applied Microbiology,* **115**: 346-357.

Ameri A., Vaidya J. G. and Deokule S. S. (2011). In vitro evaluation of anti-staphylococcal activity of *Ganoderma lucidum, Ganoderma praelongum* and *Ganoderma resinaceum* from Pune, India. *African Journal of Microbiology Research,* **5**: 328-333.

Awala S. I. and Oyetayo V. O. (2015). The Phytochemical and Antimicrobial Properties of the Extracts Obtained from *Trametes elegans* Collected from Osengere in Ibadan, Nigeria. *Jordan Journal of Biological Sciences,* **147**: 1-11.

Baby S., Johnson A. J. and Govindan B. (2015). Secondary metabolites from *Ganoderma*. *Phytochemistry*, **114**: 66-101.

Bannister B. A., Beg N. T. and Gillespie S. H. (2000). Antimicrobial Chemotherapy. *Infectious Disease* **3**: 51-80.

Barros L., Baptista P., Correia D. M., Casal S., Oliveira B. and Ferreira I. C. (2007). Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chemistry*, **105**: 140-145.

Basnet B. B., Liu L., Bao L. and Liu H. (2017). Current and future perspective on antimicrobial and anti-parasitic activities of *Ganoderma* sp.: an update. *Mycology,* **54**: 1-14.

Beentje H. J. (1990). The forests of Kenya. *Mitteilungen aus dem Institut für Allgemeine Botanik Hamburg* **23**: 265-286.

Benveniste P. (1986). Sterol biosynthesis. *Annual review of plant physiology,* **37**: 275-308.

Berger S. (2008). Terence N. Mitchell, Burkhard Costisella: NMR–From spectra to structures. An experimental approach. *Analytical and Bioanalytical Chemistry,* **390**: 1681-1682.

Blackwell M. (2011). The Fungi: 1, 2, 3… 5.1 million species? *American Journal of Botany,* **98**: 426-438.

Borchers A. T., Keen C. L. and Gershwin M. E. (2004). Mushrooms, tumors, and immunity: an update. *Experimental Biology and Medicine,* **229**: 393-406.

Breitmaier E. (2006). *Terpenes: flavors, fragrances, pharmaca, pheromones*: John Wiley & Sons pp 53-93.

Breitmaier E. and Sinnema A. (1993). Structure elucidation by NMR in organic chemistry. John and Wiley Sons, pp 72-85

Brian P. W. (1951). Antibiotics produced by fungi. *The Botanical Review,* **17**: 357-430.

Burns D., Reynolds W. F., Buchanan G., Reese P. B. and Enriquez R. G. (2000). Assignment of ^{1}H and ^{13}C spectra and investigation of hindered side- chain rotation in lupeol derivatives. *Magnetic Resonance in Chemistry,* **38**: 488-493.

Butler M. S. (2008). Natural products to drugs: natural product-derived compounds in clinical trials. *Natural Product Reports,* **25**: 475-516.

Butler M. S., Blaskovich M. A. and Cooper M. A. (2013). Antibiotics in the clinical pipeline in 2013. *The Journal of Antibiotics,* **66**: 571.

Cafieri F., Fattorusso E., Gavagnin M. and Santacroce C. (1985). 3β, 5α, 6β-Trihydroxysterols from the Mediterranean *bryozoan Myriapora truncata*. *Journal of Natural Products,* **48**: 944-947.

Chang S. T. and Buswell J. A. (1999). *Ganoderma lucidum* (Curt.: Fr.) P. Karst.(Aphyllophoromycetideae)− A Mushrooming Medicinal Mushroom. *International Journal of Medicinal Mushrooms,* **1**: 3-11.

Chang S. T. and Miles P. G. (1989). Edible mushrooms and their cultivation. *Edible Mushrooms and their Cultivation.*CRC press, USA pg: 345-400.

Chen H. and Liu J. (2017). Secondary Metabolites from Higher Fungi. In *Progress in the Chemistry of Organic Natural Products*, **106**: 1-201.

Cheung P. C. K. (2008). Nutritional value and health benefits of mushrooms. *Mushrooms as Functional Foods*, **2**: 71-109.

Chihara G. (1992). Immunopharmacology of lentinan, a polysaccharide isolated from *Lentinus edodes*: its application as a host defense potentiator. *International Journal of Oriental Medicine,* **17**: 450-459.

Choi Y. H., Huh M. K., Ryu C. H., Choi B. T. and Jeong Y. K. (2004). Induction of apoptotic cell death by mycelium extracts of *Phellinus linteus* in human neuroblastoma cells. *International Journal of Molecular Medicine,* **14**: 227-232.

Cilerdzic J., Stajic M. and Vukojevic J. (2016). Potential of submergedly cultivated mycelia of *Ganoderma* spp. as antioxidant and antimicrobial agents. *Current Pharmaceutical Biotechnology*, **17**: 275-282.

CLSI. (2012). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. *CLSI document M02-A11 (ISBN 1-56238-781-2 [Print]; ISBN 1-56238-782-0 [Electronic]).*

Coletto B. M. A. and Mondino P. (1991). Antibiotic activity in Basidiomycetes: V. *Antibiotic activity of mycelia and cultural filtrates. Allionia (Turin),* **30**: 61-64.

Coletto M. A. B. and Striano B. (2000). Antibiotic activity in Basidiomycetes. XIII. Antibiotic activity of mycelia and cultural filtrates. *Allionia,* **37**: 253-255.

Colombo A. L., Ngai A. L., Bourque M., Bradshaw S. K., Strohmaier K. M., Taylor A. F., Lupinacci R. J. and Kartsonis N. A. (2010). Caspofungin use in patients with invasive candidiasis caused by common non-*albicans Candida* species: review of the caspofungin database. *Antimicrobial Agents and Chemotherapy*, **54**: 1864-1871.

Cowan M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews,* **12**: 564-582.

Dai Y. C., Zhou L. W., Hattori T., Cao Y., Stalpers J. A., Ryvarden L., Buchanan P., Oberwinkler F., Hallenberg N. and Liu P. G. (2017). *Ganoderma* lingzhi (Polyporales, Basidiomycota): the scientific binomial for the widely cultivated medicinal fungus Lingzhi. *Mycological Progress,* **16**: 1051-1055.

David B., Wolfender J. L. and Dias D. A. (2015). The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Reviews*, **14**: 299-315.

De Silva D. D., Rapior S., Sudarman E., Stadler M., Xu J., Alias S. A. and Hyde K. D. (2013). Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. *Fungal Diversity*, **62**: 1-40.

De Simone D. and Annesi T. (2012). Occurrence of *Ganoderma adspersum* on Pinus pinea. *Phytopathologia Mediterranea*, **40**: 374-382.

Demain A. L. (2009). Antibiotics: natural products essential to human health. *Medicinal Research Reviews,* **29**: 821-842.

Demain A. L. and Elander R. P. (1999). The β-lactam antibiotics: past, present, and future. *Antonie van Leeuwenhoek,* **75**: 5-19.

Deng G., Lin H., Seidman A., Fornier M., D'Andrea G., Wesa K., Yeung S., S. C.-R., Vickers A. J. and Cassileth B. (2009). A phase I/II trial of a polysaccharide extract from *Grifola frondosa* (Maitake mushroom) in breast cancer patients: immunological effects. *Journal of Cancer Research and Clinical Oncology,* **135**: 1215-1221.

Dewick P. M. (2002). *Medicinal natural products: a biosynthetic approach*: John Wiley & Sons.pp 113-120

Deyrup S. T., Gloer J. B., O'Donnell K. and Wicklow D. T. (2007). Kolokosides A− D: Triterpenoid Glycosides from a Hawaiian Isolate of *Xylaria* sp. *Journal of Natural Products*, **70**: 378-382.

Eisenreich W., Schwarz M., Cartayrade A., Arigoni D., Zenk M. H. and Bacher A. (1998). The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chemistry & Biology*, **5**: R221-R233.

Endo A., Kuroda M. and Tsujita Y. (1976). ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by *Penicillium citrinum*. *The Journal of Antibiotics,* **29**: 1346-1348.

Evans W. C. (2009). *Trease and Evans' Pharmacognosy E-Book*: Elsevier Health Sciences Amsterdem, Netherlands.

Fangkrathok N., Sripanidkulchai B., Umehara K. and Noguchi H. (2013). Bioactive ergostanoids and a new polyhydroxyoctane from *Lentinus polychrous* mycelia and their inhibitory effects on E2-enhanced cell proliferation of T47D cells. *Natural Product Research,* **27**: 1611-1619.

Fatmawati S., Shimizu K. and Kondo R. (2010). Ganoderic acid Df, a new triterpenoid with aldose reductase inhibitory activity from the fruiting body of *Ganoderma lucidum*. *Fitoterapia,* **81**: 1033-1036.

Finch R. (2007). Innovation—drugs and diagnostics. *Journal of Antimicrobial Chemotherapy*, **60**: 79-82.

Fisher M. and Yang L. X. (2002). Anticancer effects and mechanisms of polysaccharide-K (PSK): implications of cancer immunotherapy. *Anticancer Research*, **22**: 1737-1754.

Fitzpatrick M., Parkinson T. and Ray N. (2006). *East Africa*: Lonely Planet, Oakland, Califonia, USA.

Fleck W. F., Schlegel B., Hoffmann P., Ritzau M., Heinze S. and Gräfe U. (1996). Isolation of isodrimenediol, a possible intermediate of drimane biosynthesis from *Polyporus arcularius*. *Journal of Natural Products*, **59**: 780-781.

Fleming A. (1929). On the antibacterial action of cultures of a *penicillium*, with special reference to their use in the isolation of *B. influenzae*. *British Journal of Experimental Pathology*, **10**: 226.

Flood J., Bridge P. D. and Holderness M. (2000). *Ganoderma Diseases of Perennial rops*: CABI, Oxfordshire, England pp 113-116

Florey H. W., Chain E., Heatley N. G., Jennings M. A., Sanders A. G., Abraham E. P. and Florey M. (1949). Antibiotics. A survey of penicillin, streptomycin, and other antimicrobial substances from fungi, actinomyeetes, bacteria, and plants. Volume II. *Antibiotics. A survey of penicillin, streptomycin, and other antimicrobial substances from fungi, actinomyeetes, bacteria, and plants. Volume II.*

Fukuzawa M., Yamaguchi R., Hide I., Chen Z., Hirai Y., Sugimoto A., Yasuhara T. and Nakata Y. (2008). Possible involvement of long chain fatty acids in the spores of *Ganoderma lucidum* (Reishi Houshi) to its anti-tumor activity. *Biological and Pharmaceutical Bulletin*, **31**: 1933-1937.

Fushimi K., Horikawa M., Suzuki K., Sekiya A., Kanno S., Shimura S. and Kawagishi H. (2010). Applanatines A–E from the culture broth of *Ganoderma applanatum*. *Tetrahedron*, **66**: 9332-9335.

Gan K. H., Kuo S. H. and Lin C. N. (1998). Steroidal Constituents of *Ganoderma applanatum* and *Ganoderma neo-japonicum*. *Journal of Natural Products*, **61**: 1421-1422.

Gao H., Hong K., Zhang X., Liu H. W., Wang N. L., Zhuang L. and Yao X. S. (2007). New Steryl Esters of Fatty Acids from the Mangrove Fungus *Aspergillus awamori*. *Helvetica Chimica Acta*, **90**: 1165-1178.

Gao J. M., Dong Z. J. and Liu J. K. (2001). A new ceramide from the basidiomycete *Russula cyanoxantha*. *Lipids*, **36**: 175-181.

Gao Y., Tang W., Gao H., Chan E., Lan J., Li X. and Zhou S. (2005). Antimicrobial activity of the medicinal mushroom *Ganoderma*. *Food Reviews International,* **21**: 211-229.

Gao Y., Zhou S., Huang M. and Xu A. (2003). Antibacterial and antiviral value of the genus *Ganoderma* P. Karst. species (Aphyllophoromycetideae): a review. *International Journal of Medicinal Mushrooms,* **5**: 56-65.

Gargano M. L., van Griensven L. J., Isikhuemhen O. S., Lindequist U., Venturella G., Wasser S. P. and Zervakis G. I. (2017). Medicinal mushrooms: Valuable biological resources of high exploitation potential. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology,* **151**: 548-565.

Gilbertson R. L. and Ryvarden L. (1987). North American Polypores Vol. 2. Megasporoporia-Wrightoporia. *North American Polypores Vol. 2. Megasporoporia-Wrightoporia.*: 437-885.

Grienke U., Zall M., Peintner U. and Rollinger J. M. (2014). European medicinal polypores. A modern view on traditional uses. *Journal of Ethnopharmacology,* **154**: 564-583.

Grishko V., Tolmacheva I. A. and Pereslavtseva A. V. (2015). Triterpenoids with a five-membered A-ring: distribution in nature, transformations, synthesis, and biological activity. *Chemistry of Natural Compounds,* **51**: 1-21.

Harkonen M., Niemela T. and Mwasumbi L. (2003). *Tanzanian mushrooms. Edible, harmful and other fungi*: Luonnontieteellinen keskusmuseo, Kasvimuseo (Finnish Museum of Natural History, Botanical Museum), Finland.

Harmon M. E., Franklin J. F., Swanson F. J., Sollins P., Gregory S. V., Lattin J. D., Anderson N. H., Cline S. P., Aumen N. G. and Sedell J. R. (1986). Ecology of coarse woody debris in temperate ecosystems. *Advances in Ecological Research,* **15**: 133-302.

Hattori T., Rashid N. M. N. and Salmiah U. (2007). Basidiomycota: diversity of Malaysian polypores. *Malaysian fungal diversity. Mushroom Research Centre, University of Malaya and Ministry of Natural Resources and Environment, Malaysia* **11**: 55-68.

Hawksworth D. L. (2001). The magnitude of fungal diversity: the 1· 5 million species estimate revisited. *Mycological Research,* **105**: 1422-1432.

Hawksworth D. L. (2004). Fungal diversity and its implications for genetic resource collections. *Studies in Mycology,* **50**: 9-18.

Heindrich M., Barnes J., Gibbons S. and Williamson E. M. (2004). Fundamentals of pharmacognosy and phytotherapy. *Australian Journal of Medical Herbalism,* **16**: 72-78.

Herbst C., Naumann F., Kruse E. B., Monsef I., Bohlius J., Schulz H. and Engert A. (2009). Prophylactic antibiotics or G- CSF for the prevention of infections and improvement of survival in cancer patients undergoing chemotherapy. *Cochrane Database of Systematic Reviews,* **1**: 11-34.

Hernandez V. L., Palazon J., A. N. O. and Rao D. V. (2012). The Pentacyclic Triterpenes,- amyrins: A review of sources and Biological Activities. *Phytochemicals, A Global Perspective of the Role in Nutrition and Health*, 487-501.

Hleba L. (2014). Antimicrobial activity of crude methanolic extracts from *Ganoderma lucidum* and *Trametes versicolor*. *Scientific Papers Animal Science and Biotechnologies,* **47**: 89-93.

Hobbs C. (2002). *Medicinal mushrooms: an exploration of tradition, healing, and culture*: Book Publishing Company,Tennessee, USA.

Hodd M. (2002). *East Africa*: Footprint Handbooks- the travel guide. Footprint Travel Guides Publisher, United Kingdom.

Hu H., Ahn N. S., Yang X., Lee Y. S. and Kang K. S. (2002). *Ganoderma lucidum* extract induces cell cycle arrest and apoptosis in MCF- 7 human breast cancer cell. *International Journal of Cancer,* **102**: 250-253.

Hudzicki J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol.

Inouye S., Abe S. and Yamagushi H. (2004). Fungal terpenoid antibiotics and enzyme inhibitors. *Handbook of Fungal Biotechnology, New York, USA.*

Isaka M., Chinthanom P., Mayteeworakoon S., Laoteng K., Choowong W. and Choeyklin R. (2017). Lanostane triterpenoids from cultivated fruiting bodies of the basidiomycete *Ganoderma australe*. *Natural Product Research*, **23**: 1-6.

Isaka M., Chinthanom P., Sappan M., Danwisetkanjana K., Boonpratuang T. and Choeyklin R. (2015). Antitubercular Lanostane Triterpenes from Cultures of the Basidiomycete *Ganoderma* sp. BCC 16642. *Journal of Natural Products,* **79**: 161-169.

Iwalokun B. A., Usen U. A., Otunba A. A. and Olukoya D. K. (2007). Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *African Journal of Biotechnology,* **6**: 34-39.

Jain P. S. and Bari S. B. (2010). Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria*. *Asian Journal of Plant Sciences,* **9**: 163.

Jamal A. K., Yaacob W. A. and Din L. B. (2008). A chemical study on *Phyllanthus reticulatus*. *Journal of Physical Science,* **19**: 45-50.

Janes D., Kreft S., Jurc M., Seme K. and Štrukelj B. (2007). Antibacterial activity in higher fungi (mushrooms) and endophytic fungi from Slovenia. *Pharmaceutical Biology,* **45**: 700-706.

Jeong Y. T., Yang B. K., Jeong S. C., Kim S. M. and Song C. H. (2008). *Ganoderma applanatum*: a promising mushroom for antitumor and immunomodulating activity. *Phytotherapy Research,* **22**: 614-619.

Kalac P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry,* **113**: 9-16.

Khan M. A., Nizami S. S., Khan M. N. I., Azeem S. W. and Ahmed Z. (1994). New triterpenes from *Mangifera indica*. *Journal of Natural Products,* **57**: 988-991.

Kim B. K., Cho H. Y., Kim J. S., Kim H. W. and Choi E. C. (1993). Studies on constituents of higher fungi of Korea (LXVIII). Antitumor components of the cultured mycelia of *Ganoderma lucidum*. *Korean Journal of Pharmacolology,* **24**: 203-212.

Kitzberger C. S. G., Smania A., Pedrosa R. C. and Ferreira S. R. S. (2007). Antioxidant and antimicrobial activities of shiitake (*Lentinula edodes*) extracts obtained by organic solvents and supercritical fluids. *Journal of Food Engineering,* **80**: 631-638.

Kobori M., Yoshida M., M. O. K., Takei T. and Shinmoto H. (2006). 5α, 8α-Epidioxy-22E-ergosta-6, 9 (11), 22-trien-3β-ol from an edible mushroom suppresses growth of HL60 leukemia and HT29 colon adenocarcinoma cells. *Biological and Pharmaceutical Bulletin,* **29**: 755-759.

Kwon H. C., Zee S. D., Cho S. Y., Choi S. U. and Lee K. R. (2002). Cytotoxic ergosterols from *paecilomyces* sp. J300. *Archives of Pharmacal Research,* **25**: 851-855.

Lederberg J., Hamburg M. A. and Smolinski M. S. (2003). *Microbial threats to health: emergence, detection, and response*: National Academies Press.

Lee S. H., Shim S. H., Kim J. S. and Kang S. S. (2006). Constituents from the fruiting bodies of *Ganoderma applanatum* and their aldose reductase inhibitory activity. *Archives of Pharmacal Research,* **29**: 479-483.

Lee S. Y., Kim J. S., Lee S. H. and Kang S. S. (2011). Polyoxygenated ergostane-type sterols from the liquid culture of *Ganoderma applanatum*. *Natural Product Research,* **25**: 1304-1311.

Leon F., Valencia M., Rivera A., Nieto I., Quintana J., Estevez F. and Bermejo J. (2003). Novel cytostatic lanostanoid triterpenes from *Ganoderma australe*. *Helvetica Chimica Acta,* **86**: 3088-3095.

Li F., Wen H., Zhang Y., Aa M. and Liu X. (2011). Purification and characterization of a novel immunomodulatory protein from the medicinal mushroom *Trametes versicolor*. *Science China Life Sciences,* **54**: 379-385.

Li G., Li B., Liu G. and Zhang G. (2005). Sterols from *Aspergillus ocharceus* 43. *Chinese Journal of Applied Enviromental Biology,* **11**: 67-70.

Li P., Deng Y. P., Wei X. X. and Xu J. H. (2013). Triterpenoids from *Ganoderma lucidum* and their cytotoxic activities. *Natural Product Research,* **27**: 17-22.

Li W. J., Nie S. P., Liu X. Z., Zhang H. N., Yang Y., Yu Q. and Xie M. Y. (2012). Antimicrobial properties, antioxidant activity and cytotoxicity of ethanol-soluble acidic components from *Ganoderma atrum*. *Food and Chemical Toxicology,* **50**: 689-694.

Lieu C. W., Lee S. S. and Wang S. Y. (1992). The effect of *Ganoderma lucidum* on induction of differentiation in leukemic U937 cells. *Anticancer Research,* **12**: 1211-1215.

Limbird L. E. and Hardman J. G. (2001). Goodman & Gilman's the pharmacological basis of therapeutics. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*.

Lincoff G. H. (2005). *Field Guide to Mushrooms*: National Audubon Society, New York.

Lindequist U., Niedermeyer T. H. J. and Jülich W. D. (2005). The pharmacological potential of mushrooms. *Evidence-Based Complementary and Alternative Medicine,* **2**: 285-299.

Liu D. Z., Zhu Y. Q., Li X. F., Shan W. G. and Gao P. F. (2014). New triterpenoids from the fruiting bodies of *Ganoderma lucidum* and their bioactivities. *Chemistry & Biodiversity,* **11**: 982-986.

Liu J. and Chen H. P. (2017). Secondary Metabolites from Higher Fungi. In *Progress in the Chemistry of Organic Natural Products* **106**: 1-201.

Liu T., Men Q., Wu G. S., Yu C., Huang Z., Liu X. H. and Li W. (2015). Tetrandrine induces autophagy and differentiation by activating ROS and Notch1 signaling in leukemia cells. *Oncotarget,* **6**: 7992.

Liu X. T., Winkler A. L., Schwan W. R., Volk T. J., Rott M. and Monte A. (2010a). Antibacterial compounds from mushrooms II: lanostane triterpenoids and an ergostane steroid with activity against *Bacillus cereus* isolated from *Fomitopsis pinicola*. *Planta Medica,* **76**: 464-466.

Liu X. T., Winkler A. L., Schwan W. R., Volk T. J., Rott M. A. and Monte A. (2010b). Antibacterial compounds from mushrooms I: a lanostane-type triterpene and prenylphenol derivatives from *Jahnoporus hirtus* and *Albatrellus flettii* and their

activities against *Bacillus cereus* and *Enterococcus faecalis*. *Planta Medica,* **76**: 182-185.

Lord C. J. and Ashworth A. (2010). Biology-driven cancer drug development: back to the future. *BMC Biology,* **8**: 1.

Martin K. W. and Ernst E. (2003). Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. *Journal of Antimicrobial Chemotherapy,* **51**: 241-246.

Mikolasch A., Hildebrandt O., Schlüter R., Hammer E., Witt S. and Lindequist U. (2016). Targeted synthesis of novel β-lactam antibiotics by laccase-catalyzed reaction of aromatic substrates selected by pre-testing for their antimicrobial and cytotoxic activity. *Applied Microbiology and Biotechnology,* **100**: 4885-4899.

Mizushina Y., Hanashima L., Yamaguchi T., Takemura M., Sugawara F., Saneyoshi M., Matsukage A., Yoshida S. and Sakaguchi K. (1998). A mushroom fruiting body-inducing substance inhibits activities of replicative DNA polymerases. *Biochemical and Biophysical Research Communications,* **249**: 17-22.

Moncalvo J. M., Wang H. F. and Hseu R. S. (1995). Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. *Mycological Research,* **99**: 1489-1499.

Moon J. K. and Shibamoto T. (2009). Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry,* **57**: 1655-1666.

Morens D. M., Folkers G. K. and Fauci A. S. (2004). The challenge of emerging and re-emerging infectious diseases. *Nature,* **430**: 242.

Mothana R. A. A., Jansen R., Jülich W. D. and Lindequist U. (2000). Ganomycins A and B, new antimicrobial farnesyl hydroquinones from the basidiomycete *Ganoderma pfeifferi*. *Journal of Natural Products,* **63**: 416-418.

Mueller M. G., Bills F. J. and Foster S. M. (2004). *Biodiversity of fungi: Inventory and Monitoring Methods*. : Elsevier Academics Press, New York, USA.

Nayak A., Nayak R. N. and Bhat K. (2010). Antifungal activity of a toothpaste containing *Ganoderma lucidum* against *Candida albicans*-an in vitro study. *Journal of International Oral Health,* **2**: 51-57.

Nes W. D. (2011). Biosynthesis of cholesterol and other sterols. *Chemical Reviews,* **111**: 6423-6451.

Newman D. J. and Cragg G. M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products,* **70**: 461-477.

Niedermeyer T. H. J., Lindequist U., Mentel R., Gördes D., Schmidt E., Thurow K. and Lalk M. (2005). Antiviral Terpenoid Constituents of *Ganoderma pfeifferi*. *Journal of Natural Products,* **68**: 1728-1731.

Nishitoba T., Oda K., Sato H. and Sakamura S. (1988). Novel triterpenoids from the fungus *Ganoderma lucidum*. *Agricultural and Biological Chemistry,* **52**: 367-372.

Niu J. F., Fang Z., Wang H. J. and Wang G. C. (2002). The research advance on the effective constituents of *Ganoderma* spp. *Journal of Agricultural University of Hebei,* **25**: 51-54.

Ofodile L. N., Uma N., Grayer R. J., Ogundipe O. T. and Simmonds M. S. J. (2012). Antibacterial compounds from the mushroom *Ganoderma colossum* from Nigeria. *Phytotherapy Research,* **26**: 748-751.

Ohnuma N., Amemiya K., Kakuda R., Yaoita Y., Machida K. and Kikuchi M. (2000). Sterol constituents from two edible mushrooms, *Lentinula edodes* and *Tricholoma matsutake*. *Chemical and Pharmaceutical Bulletin,* **48**: 749-751.

Papp V., Geösel A. and Erős-Honti Z. (2012). Native *Ganoderma* species from the carpathian basin with the perspective of cultivation review. *Acta Alimentaria,* **41**: 160-170.

Papp V. and Szabó I. (2013). Distribution and Host Preference of Poroid Basidiomycetes in Hungary-*Ganoderma*. *Acta Silvatica et Lignaria Hungarica,* **9**: 71-83.

Paterson R. and Russell M. (2006). *Ganoderma*–a therapeutic fungal biofactory. *Phytochemistry*, **67**: 1985-2001.

Peintner U., Poder R. and Pumpel T. (1998). The iceman's fungi. *Mycological Research,* **102**: 1153-1162.

Piccialli V. and Sica D. (1987). Four new trihydroxylated sterols from the sponge *Spongionella gracilis*. *Journal of Natural Products,* **50**: 915-920.

Polishchuk E. N. and Kovalenko A. G. (2009). Biological activity of glycopolymers from Basidiomycetes mushrooms. *Biopolymer Cell,* **25**: 181-193.

Poucheret P., Fons F. and Rapior S. (2006). Biological and pharmacological activity of higher fungi: 20-year retrospective analysis. *Cryptogamie Mycologie,* **27**: 311.

Protiva J., Skorkovska H., Urban J. and Vystrčil A. (1980). Triterpenes and steroids from *Ganoderma applanatum*. *Collection of Czechoslovak Chemical Communications,* **45**: 2710-2713.

Ramesh C. and Pattar M. G. (2010). Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghats of Karnataka, India. *Pharmacognosy Research,* **2**: 107.

Ren G., Liu X. Y., Zhu H. K., Yang S. Z. and Fu C. X. (2006). Evaluation of cytotoxic activities of some medicinal polypore fungi from China. *Fitoterapia*, **77**: 408-410.

Renwick M. J., Brogan D. M. and Mossialos E. (2016). A systematic review and critical assessment of incentive strategies for discovery and development of novel antibiotics. *The Journal of Antibiotics*, **69**: 73.

Richter C., Wittstein K., Kirk P. M. and Stadler M. (2015). An assessment of the taxonomy and chemotaxonomy of *Ganoderma*. *Fungal Diversity*, **71**: 1-15.

Rios J. L. and Andujar I. (2017). Lanostanoids from Fungi as Potential Medicinal Agents. *Fungal Metabolites*, **40**: 931-964.

Rios J. L., Andujar I., Recio M. C. and Giner R. M. (2012). Lanostanoids from fungi: a group of potential anticancer compounds. *Journal of Natural Products*, **75**: 2016-2044.

Ruzicka L. (1953). The isoprene rule and the biogenesis of terpenic compounds. *Experientia*, **9**: 357-367.

Ryvarden L. (1991). *Genera of polypores: nomenclature and taxonomy*: Lubrecht & Cramer Ltd.

Ryvarden L. and Gilbertson R. L. (1993). *European polypores: Part 1: Abortiporus-Lindtneria*: Fungiflora A/S.

Ryvarden L. and Gilbertson R. L. (1994). European polypores. *Synopsis Fungorum*, **7**: 388-743.

Ryvarden L. and Johansen I. (1980). *Preliminary polypore flora of East Africa* (Vol. 1). Oslo, Norway: synopsis fungorum special.

Sa-ard P., Sarnthima R., Khammuang S. and Kanchanarach W. (2015). Antioxidant, antibacterial and DNA protective activities of protein extracts from *Ganoderma lucidum*. *Journal of Food Science and Technology*, **52**: 2966-2973.

Samuelsson G. and Bohlin L. (2009). *Drugs of natural origin: A treatise of pharmacognosy*: Stockholm, SE: Swedish Academy of Pharmaceutical Sciences.

Sandermann W. (1962). Terpenoids: structure and distribution. In *Comparative Biochemistry*, **3**: 503-590, Elsevier.

Sarker S. and Nahar L. (2007). *Chemistry for pharmacy students: general, organic and natural product chemistry*: John Wiley & Sons.

Sarker S. and Nahar L. (2012). *Steroid dimers: chemistry and applications in drug design and delivery*: John Wiley & Sons.

Schlegel B., Luhmann U., Haertl A. and Graefe U. (2000). Piptamine, a new antibiotic produced by *Piptoporus betulinus* Lu 9-1. *The Journal of Antibiotics,* **53**: 973-974.

Schüffler A. (2018). Secondary Metabolites of Basidiomycetes. *In Physiology and Genetics* pp. 231-275.

Schüffler A., Wollinsky B., Anke T., Liermann J. C. and Opatz T. (2012). Isolactarane and sterpurane sesquiterpenoids from the basidiomycete *Phlebia uda*. *Journal of Natural Products,* **75**: 1405-1408.

Schwarze F. and Ferner D. (2003). *Ganoderma* on trees—differentiation of species and studies of invasiveness. *Arboricultural Journal,* **27**: 59-77.

Seo G. S. and Kirk P. M. (2000). Ganodermataceae: nomenclature and classification. *Ganoderma Diseases of Perennial Crops* **10**: 3-22.

Shang X., Tan Q., Liu R. X., Yu K., Li P. and Zhao G., P. (2013). In vitro anti-*Helicobacter pylori* effects of medicinal mushroom extracts, with special emphasis on the Lion's Mane mushroom, *Hericium erinaceus* (higher Basidiomycetes). *International Journal of Medicinal Mushrooms,* **15**: 11-18.

Sharma D., Sharma B. and Shukla A. K. (2011). Biotechnological approach of microbial lipase: a review. *Biotechnology,* **10**: 23-40.

Shiao M. S. (2003). Natural products of the medicinal fungus *Ganoderma lucidum*: occurrence, biological activities, and pharmacological functions. *The Chemical Record,* **3**: 172-180.

Shu Y. Z. (1998). Recent natural products based drug development: a pharmaceutical industry perspective. *Journal of Natural Products,* **61**: 1053-1071.

Sidorova I. I. and Velikanov L. L. (2000). Bioactive substances of agaricoid basidiomycetes and their possible role in regulation of myco-and microbiota structure in soils of forest ecosystems. II. Antibiotic activity in cultures of litter saprotrophic mushroom *Lepista nuda*. *Mikologiya i Fitopatologiya,* **34**: 10-16.

Sinnema A. and Breitmaier E. (1993). Structure Elucidation by NMR in Organic Chemistry John Wiley, Chichester. *Recueil des Travaux Chimiques des Pays‐Bas,* **112**: 630-650.

Smania E. F. A., Delle Monache F., Smania Jr A., Yunes R. A. and Cuneo R. S. (2003). Antifungal activity of sterols and triterpenes isolated from *Ganoderma annulare*. *Fitoterapia,* **74**: 375-377.

Smania E. F. A., Delle Monache F., Yunes R. A., Paulert R. and A. S. J. (2007). Antimicrobial activity of methyl australate from *Ganoderma australe*. *Revista Brasileira de Farmacognosia,* **17**: 14-16.

Smania Jr A., Monache F. D., Smania E. F. A. and Cuneo R. S. (1999). Antibacterial activity of steroidal compounds isolated from *Ganoderma applanatum* (Pers.) Pat.(Aphyllophoromycetideae) fruit body. *International Journal of Medicinal Mushrooms,* **1**:211-217.

Smith J. E., Rowan N. and Sullivan R. (2002). *Medicinal mushrooms: their therapeutic properties and current medical usage with special emphasis on cancer treatments*: Cancer Research UK London.

Sperandio F. F., Huang Y. Y. and M. R. H. (2013). Antimicrobial photodynamic therapy to kill Gram-negative bacteria. *Recent Patents on Anti-infective Drug Discovery,* **8**: 108-120.

Stamets P. (2002). Novel antimicrobials from mushrooms. *Herbal Gram,* **54**: 28-33.

Standish L. J., Wenner C. A., Sweet E. S., Bridge C., Nelson A., Martzen M., Novack J. and Torkelson C. (2008). *Trametes versicolor* mushroom immune therapy in breast cancer. *Journal of the Society for Integrative Oncology,* **6**: 122.

Suay I., Arenal F., Asensio F. J., Basilio A., Cabello M. A., Diez M. T., García J. B., Del Val A. G., Gorrochategui J. and Hernandez P. (2000). Screening of basidiomycetes for antimicrobial activities. *Antonie van Leeuwenhoek,* **78**: 129-140.

Sudirman L. I. and Mujiyati S. (1997). *Preliminary detection of antimicrobial activity of fruiting bodies' extracts of tropical Ganoderma sp.* Paper presented at the Proceedings of the 1st International Symposium on *Ganoderma lucidum* in Japan. Toyo-Igaku-sha Company Limited., Tokyo.

Takaku T., Kimura Y. and Okuda H. (2001). Isolation of an antitumor compound from *Agaricus blazei Murill* and its mechanism of action. *The Journal of Nutrition,* **131**: 1409-1413.

Talkington K., Shore C. and Kothari P. (2016). A scientific roadmap for antibiotic discovery. *The Pew Charitable Trust: Philadelphia, PA, USA,* **1**:33-39.

Teplyakova T. V., Psurtseva N. V., Kosogova T. A., Mazurkova N. A., Khanin V. A. and Vlasenko V. A. (2012). Antiviral activity of polyporoid mushrooms (higher Basidiomycetes) from Altai Mountains (Russia). *International Journal of Medicinal Mushrooms,* **14**: 1000-1010.

Tomsovsky M., Kolarík M., Pazoutova S. and Homolka L. (2006). Molecular phylogeny of European *Trametes* (Basidiomycetes, Polyporales) species based on LSU and ITS (nrDNA) sequences. *Nova Hedwigia,* **82**: 269-280.

Trigos A. and Suarez M. J. (2011). Biologically active metabolites of the genus *Ganoderma:* Three decades of myco-chemistry research. *Revista Mexicana de Micología*, **34**.

Turkoglu A., Duru M. E. and Mercan N. (2007). Antioxidant and Antimicrobial Activity of *Russula delica* Fr: An Edidle Wild Mushroom. *Eurasian Journal of Analytical Chemistry*, **2**:233-239.

Vazirian M., Faramarzi M. A., Ebrahimi S. E. S., Esfahani H. R. M., Samadi N., Hosseini S. A., Asghari A., Manayi A., Mousazadeh S. A. and Asef M. R. (2014). Antimicrobial effect of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (higher Basidiomycetes) and its main compounds. *International Journal of Medicinal Mushrooms,* **16**: 234-238.

Wang F. and Liu J. K. (2008). Highly oxygenated lanostane triterpenoids from the fungus *Ganoderma applanatum*. *Chemical and Pharmaceutical Bulletin,* **56**: 1035-1037.

Wang H. J. and Ng T. B. (2006). Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. *Peptides*, **27**: 27-30.

Wang X. M., Yang M., Guan S. H., Liu R. X., Xia J. M., Bi K. S. and Guo D. A. (2006). Quantitative determination of six major triterpenoids in *Ganoderma lucidum* and related species by high performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis,* **41**: 838-844.

Wass P. (1995). *Kenya's indigenous forests*: IUCN, Gland, Switzerland, and Cambridge, UK in collaboration with ODA.

Wasser S. P. (2005). Reishi or Ling zhi (*Ganoderma lucidum*). *Encyclopedia of Dietary Supplements,* **1**: 603-622.

Wasser S. P. (2010). Medicinal mushroom science: history, current status, future trends, and unsolved problems. *International Journal of Medicinal Mushrooms,* **12**: 23-30.

Wasser S. P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Applied Microbiology and Biotechnology,* **89**: 1323-1332.

Wasser S. P. and Weis A. L. (1999a). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (review). *International Journal of Medicinal Mushrooms,* **1**: 112-116.

Wasser S. P. and Weis A. L. (1999b). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. *Critical Reviews™ in Immunology*, **19**:322-326.

WHO. (2002). *The world health report 2002: reducing risks, promoting healthy life*: World Health Organization.

Wu J., Wu Y. and Yang B. B. (2002). Anticancer activity of *Hemsleya amabilis* extract. *Life Sciences,* **71**: 2161-2170.

Xia Q., Zhang H. N., Sun X., Zhao H., Wu L., Zhu D., Yang G., Shao Y., Zhang X. Q. and Mao X. (2014). A comprehensive review of the structure elucidation and biological activity of triterpenoids from *Ganoderma* spp. *Molecules,* **19**: 17478-17535.

Yang Y. L., Tao Q. Q., Han J. J., Bao L. and Liu H. W. (2017). Recent Advance on Bioactive Compounds from the Edible and Medicinal Fungi in China. In *Medicinal Plants and Fungi: Recent Advances in Research and Development*, **4**: 253-312.

Yoon S. Y., Eo S. K., Kim Y. S., Lee C. K. and Han S. S. (1994a). Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics. *Archives of Pharmacal Research,* **17**: 438-442.

Yoon S. Y., Eo S. K., Kim Y. S., Lee C. K. and Han S. S. (1994b). Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics. *Archives of Pharmacal Research,* **17**: 438-442.

Zengin G., Sarikurkcu C., Gunes E., Uysal A., Ceylan R., Uysal S., Gungor H. and Aktumsek A. (2015). Two *Ganoderma* species: profiling of phenolic compounds by HPLC–DAD, antioxidant, antimicrobial and inhibitory activities on key enzymes linked to diabetes mellitus, Alzheimer's disease and skin disorders. *Food & Function,* **6**: 2794-2802.

Zhang X., Yuan Jiao Y., Hong Y. and Tang C. (2006). A primary studies on molecular taxonomy of *Trametes* species based on the ITS sequences of rDNA. *Mycosystema,* **25**: 23-30.

Zhong J. J. and Xiao J. H. (2009). Secondary metabolites from higher fungi: discovery, bioactivity, and bioproduction. *In Biotechnology in China* **1**: 79-150.

Zhou X., Lin J., Yin Y., Zhao J., Sun X. and Tang K. (2007). Ganodermataceae: natural products and their related pharmacological functions. *The American Journal of Chinese Medicine,* **35**: 559-574.

Zhu K., Nie S., Li C., Lin S., Xing M., Li W., Gong D. and Xie M. (2013). A newly identified polysaccharide from *Ganoderma atrum* attenuates hyperglycemia and hyperlipidemia. *International Journal of Biological Macromolecules,* **57**: 142-150.

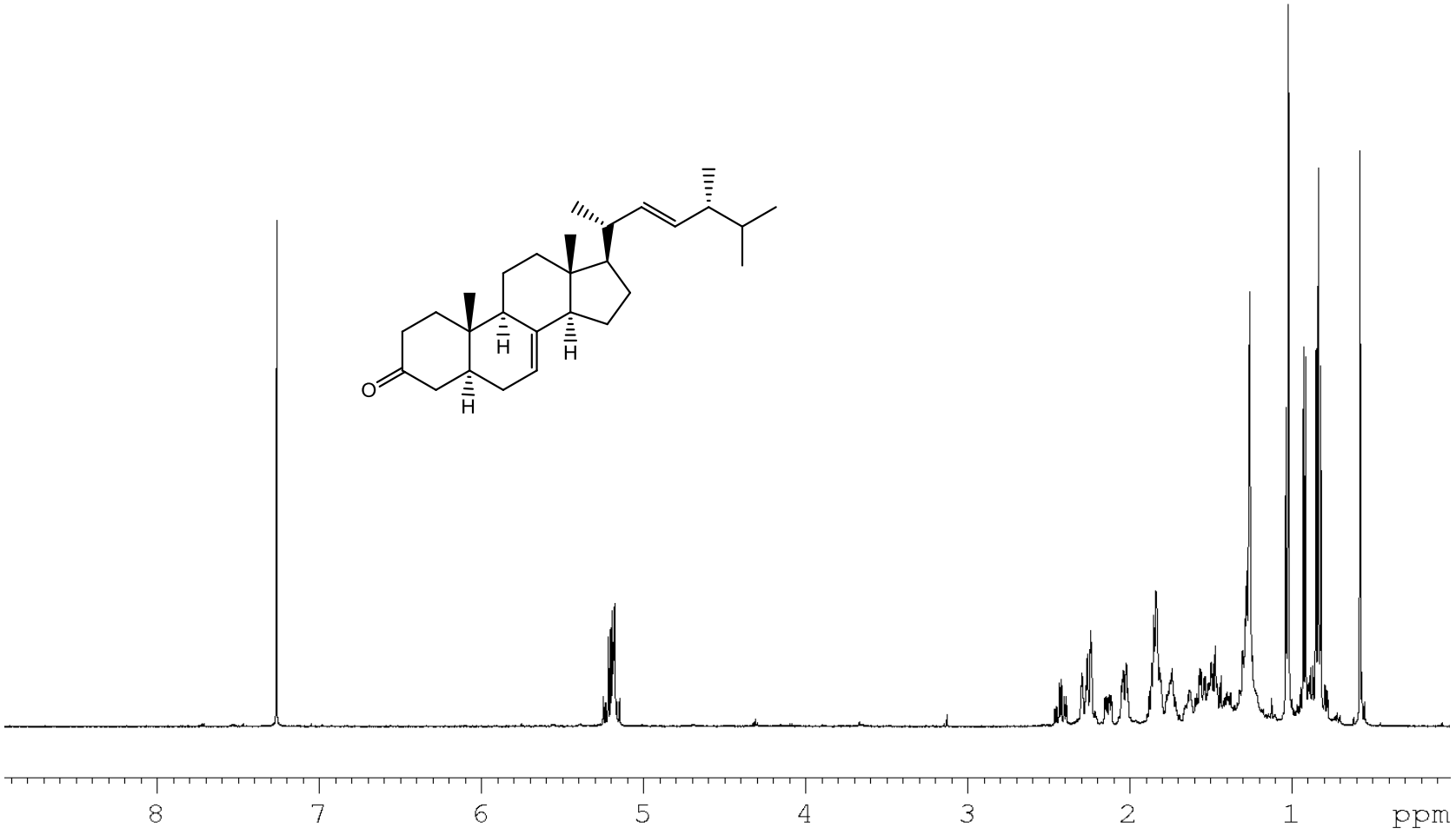
Zjawiony J. K. (2004). Biologically Active Compounds from Aphyllophorales (Polypore) Fungi. *Journal of Natural Products,* **67**: 300-310.

Zjawiony J. K. (2009). Antimicrobial and antiviral metabolites from polypore fungi. *Novel Therapeutic Agents from Plants. Science Publishers, Enfield, New Hampshire,* **10**: 36-59.

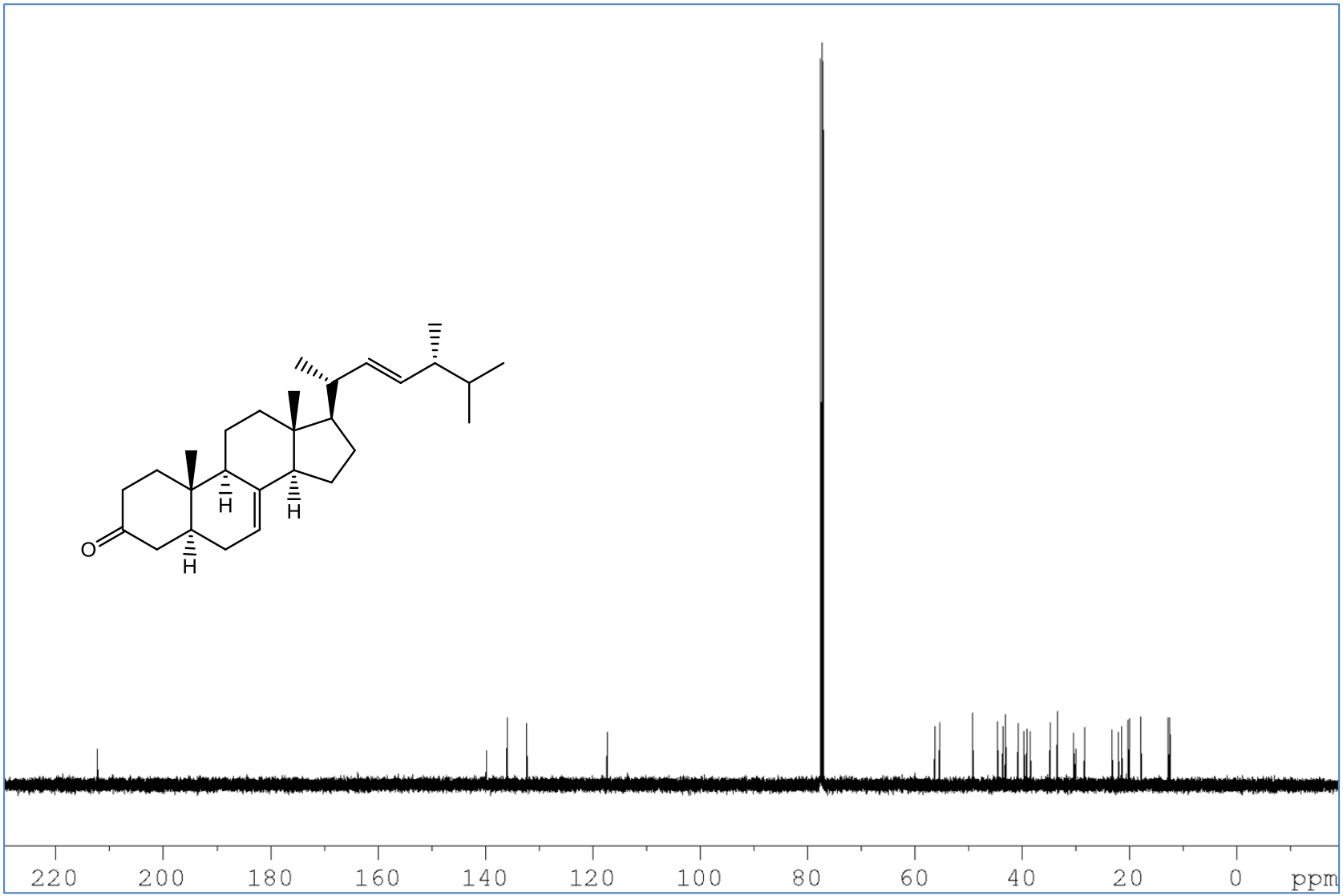
APPENDICES

Appendix Ⅰ: Key Data Analysis Outputs

A1

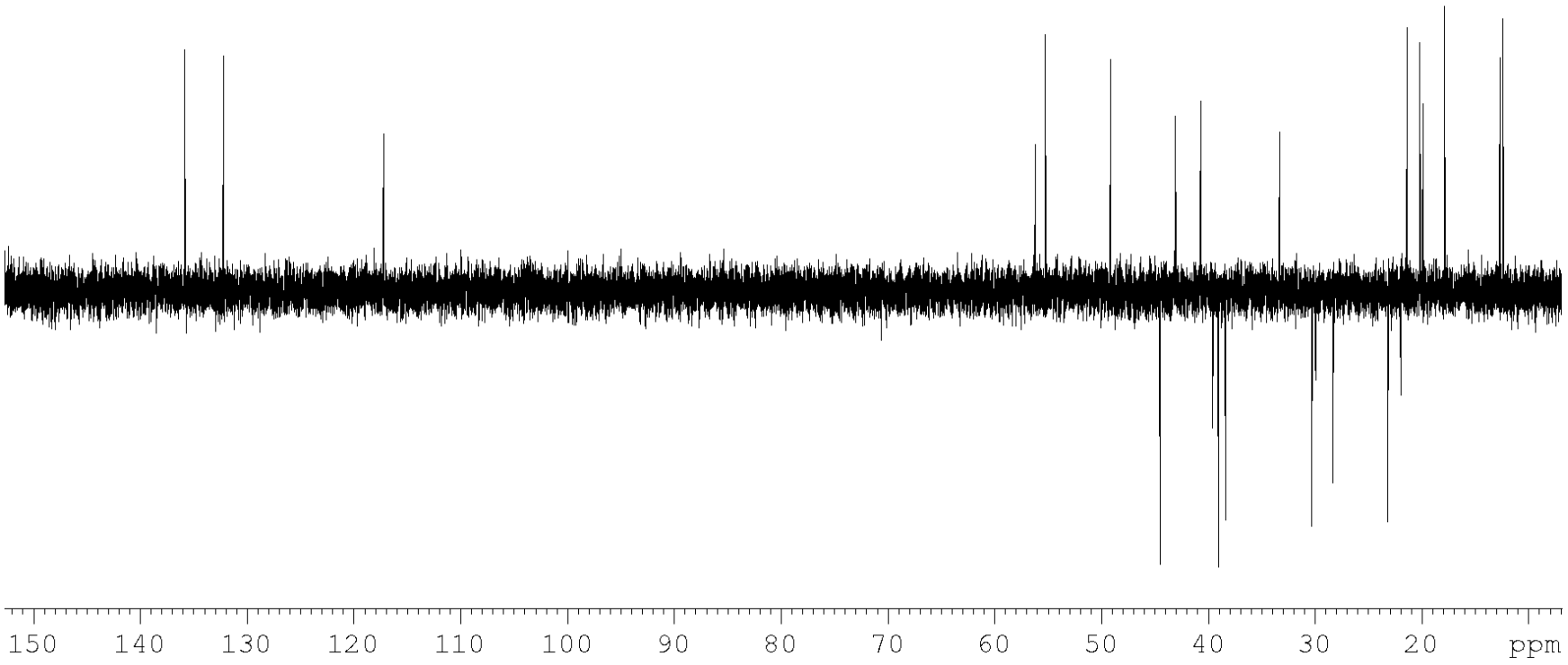


^{1}H NMR spectrum for compound 54 in $CDCl_3$



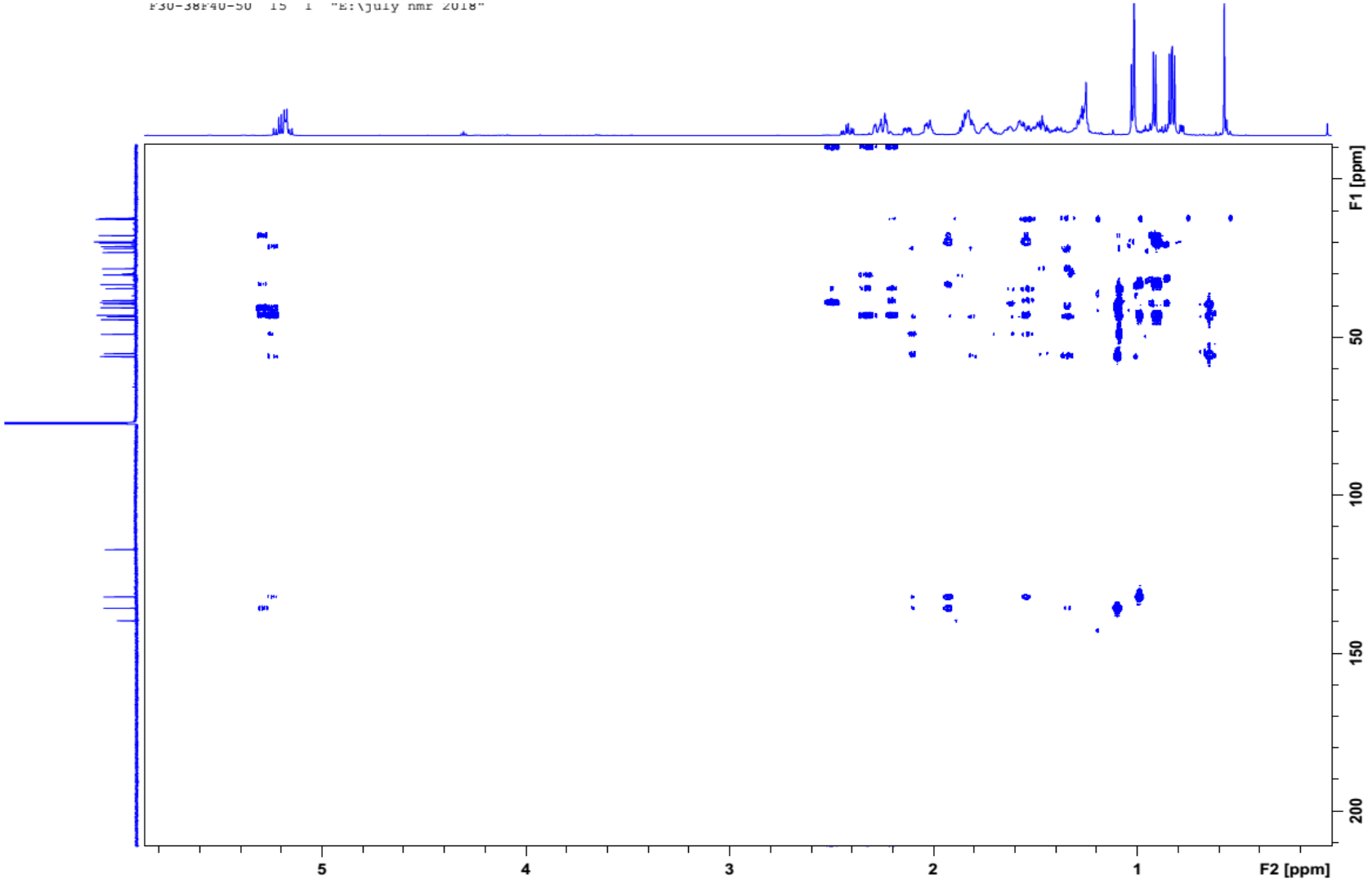
^{13}C NMR spectrum for compound 54 in $CDCl_3$

A3



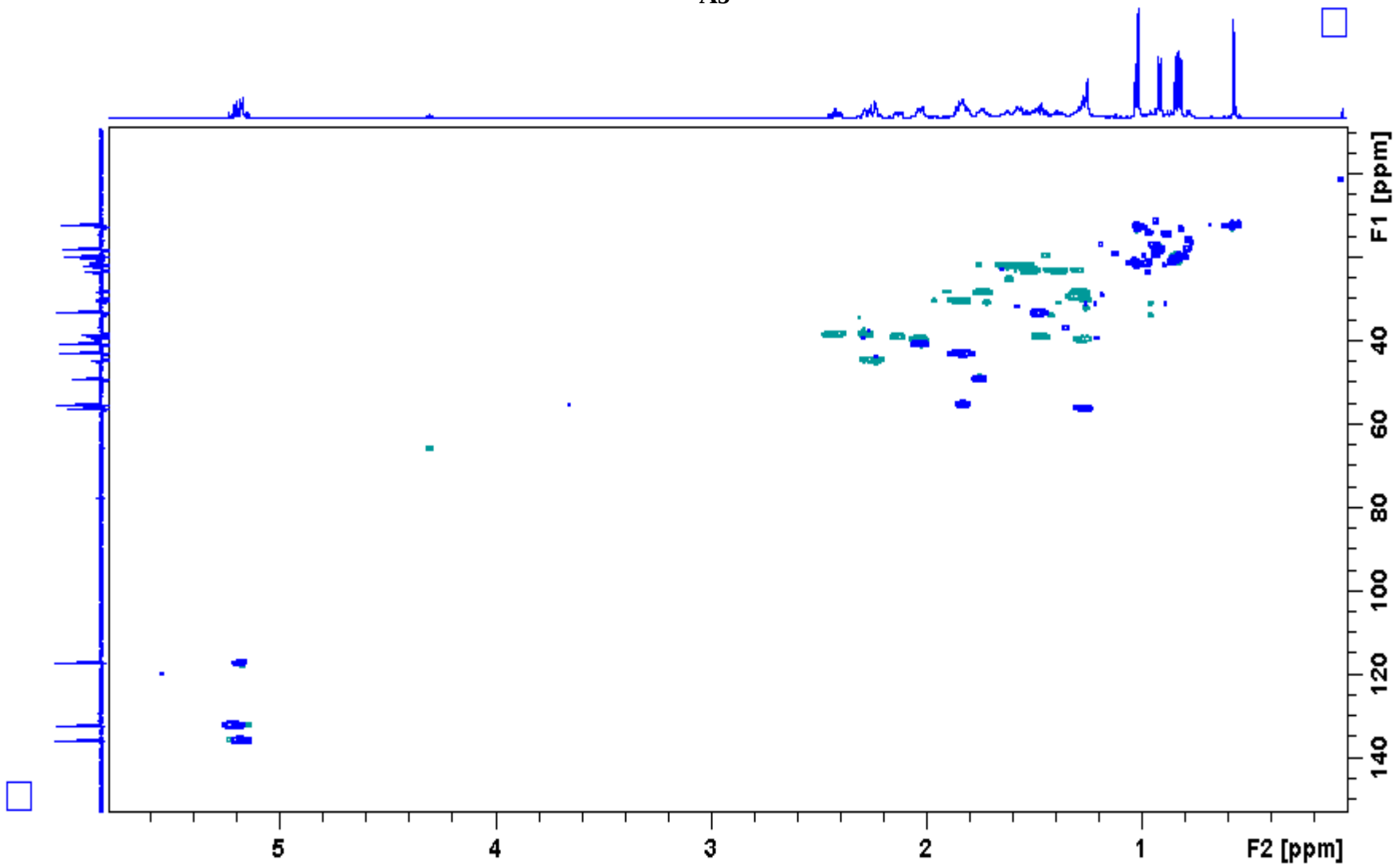
DEPT spectrum for compound 54 in $CDCl_3$

A4

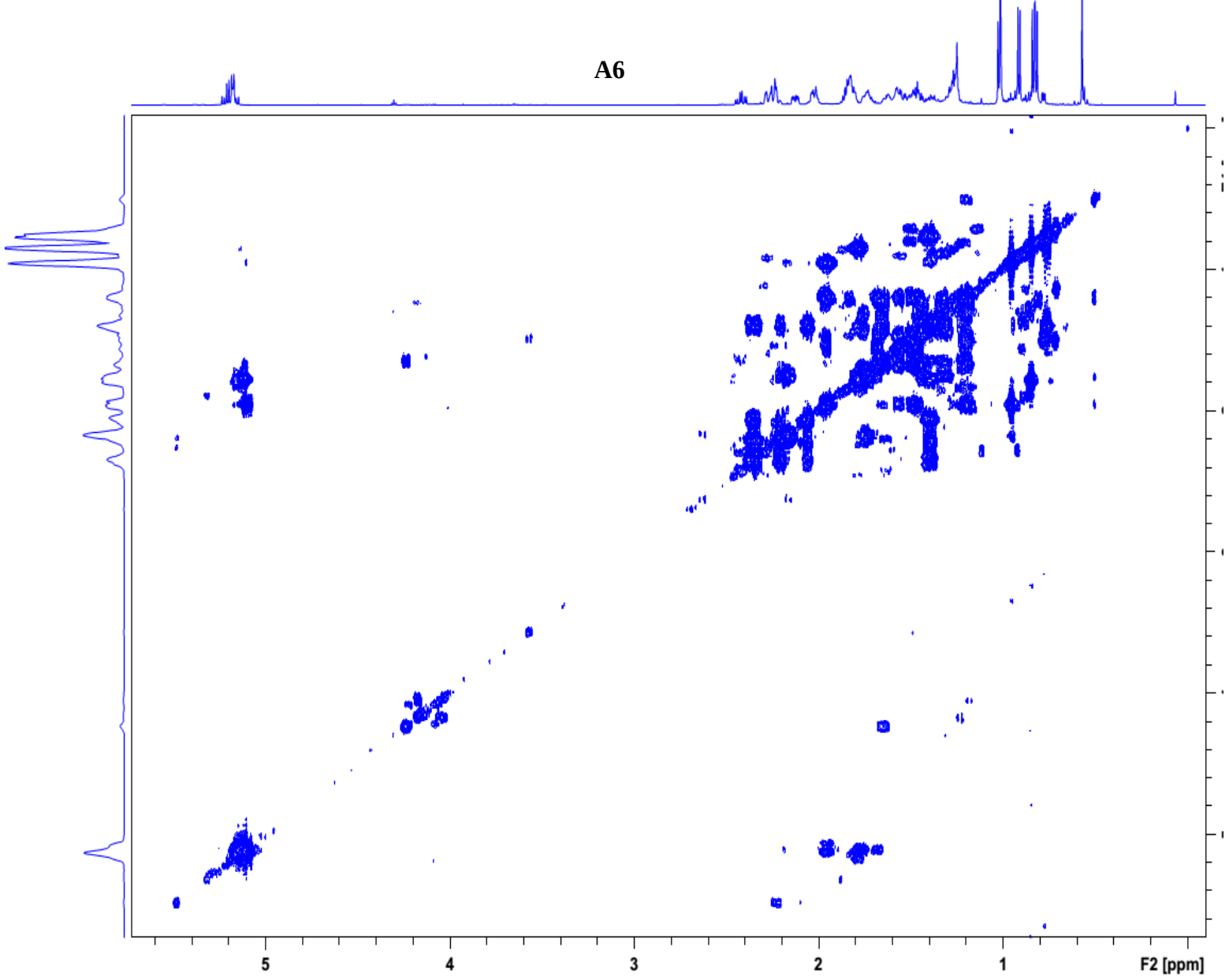


HMBC spectrum for compound 54

A5

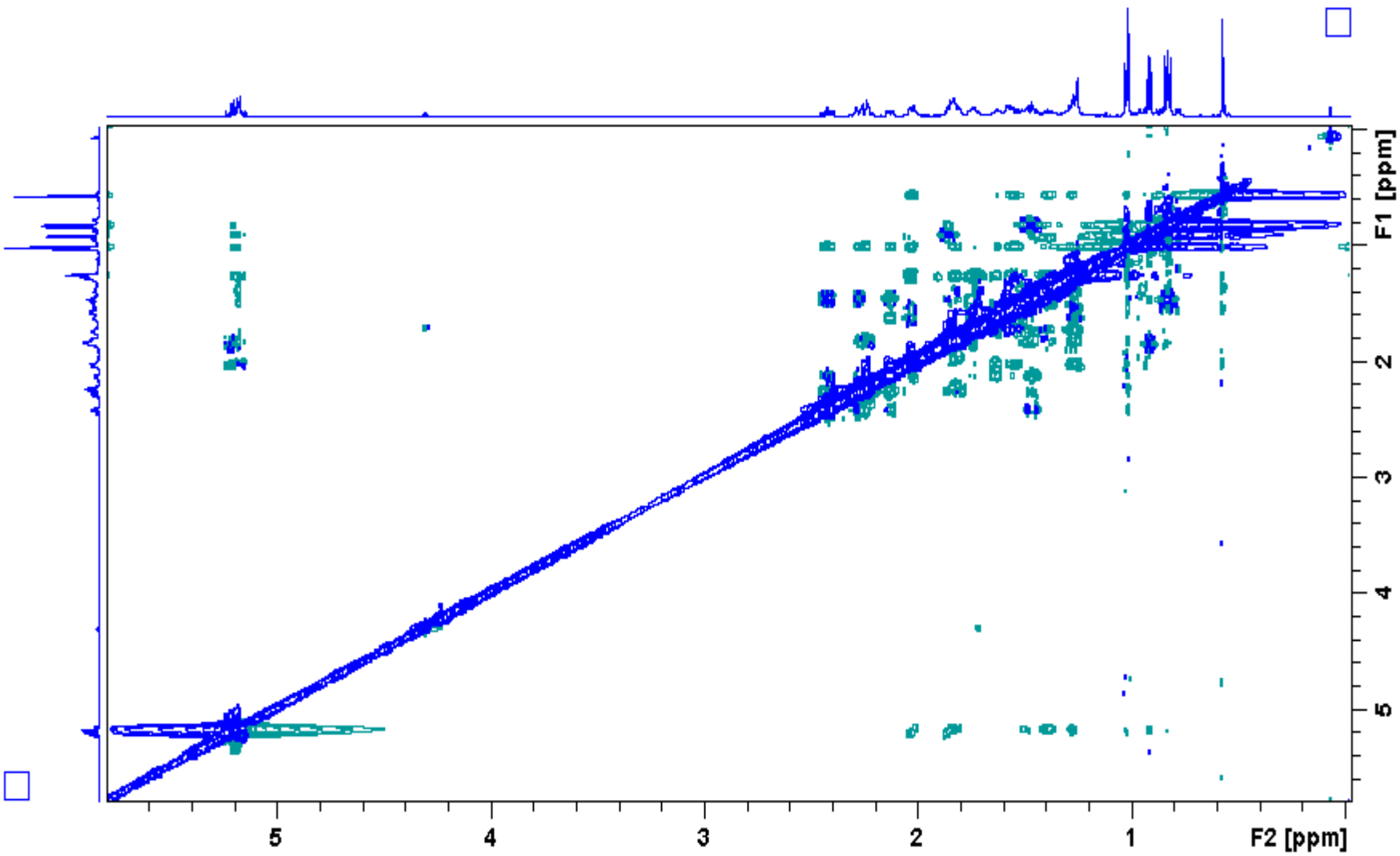


HSQC spectrum for compound 54 in $CDCl_3$



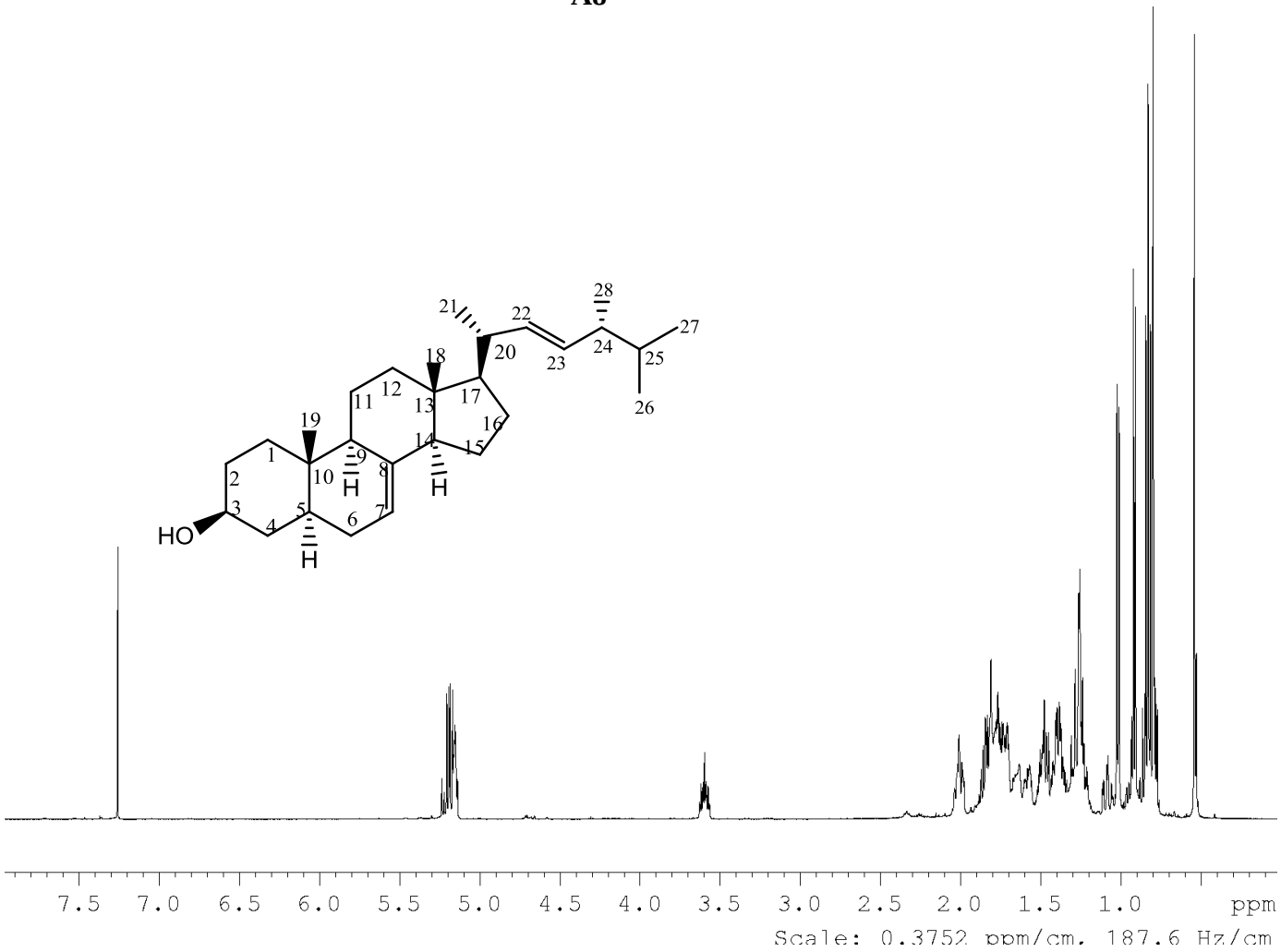
COSY NMR spectrum for compound 54

A7



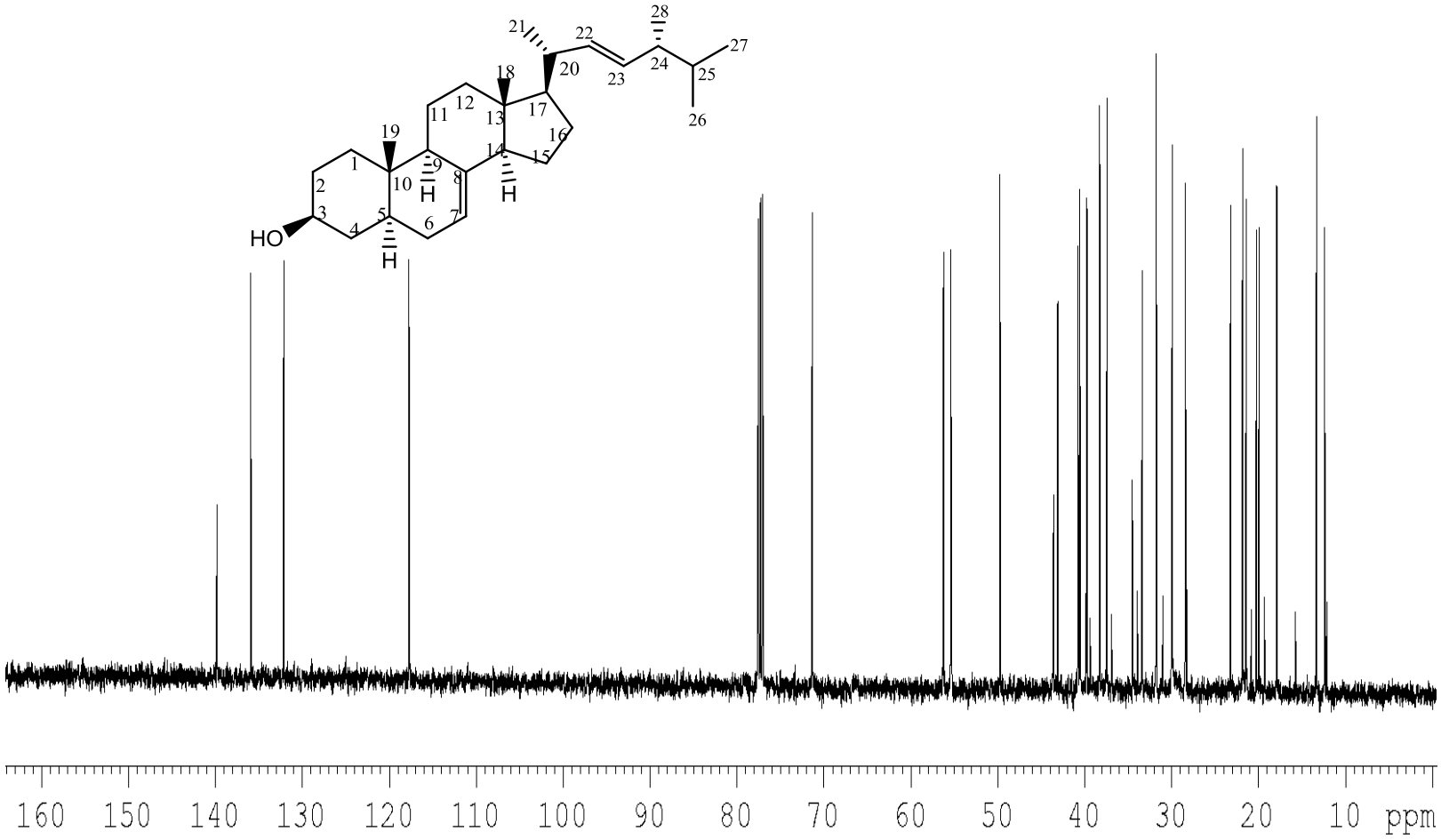
NOESY spectrum for compound 54

A8



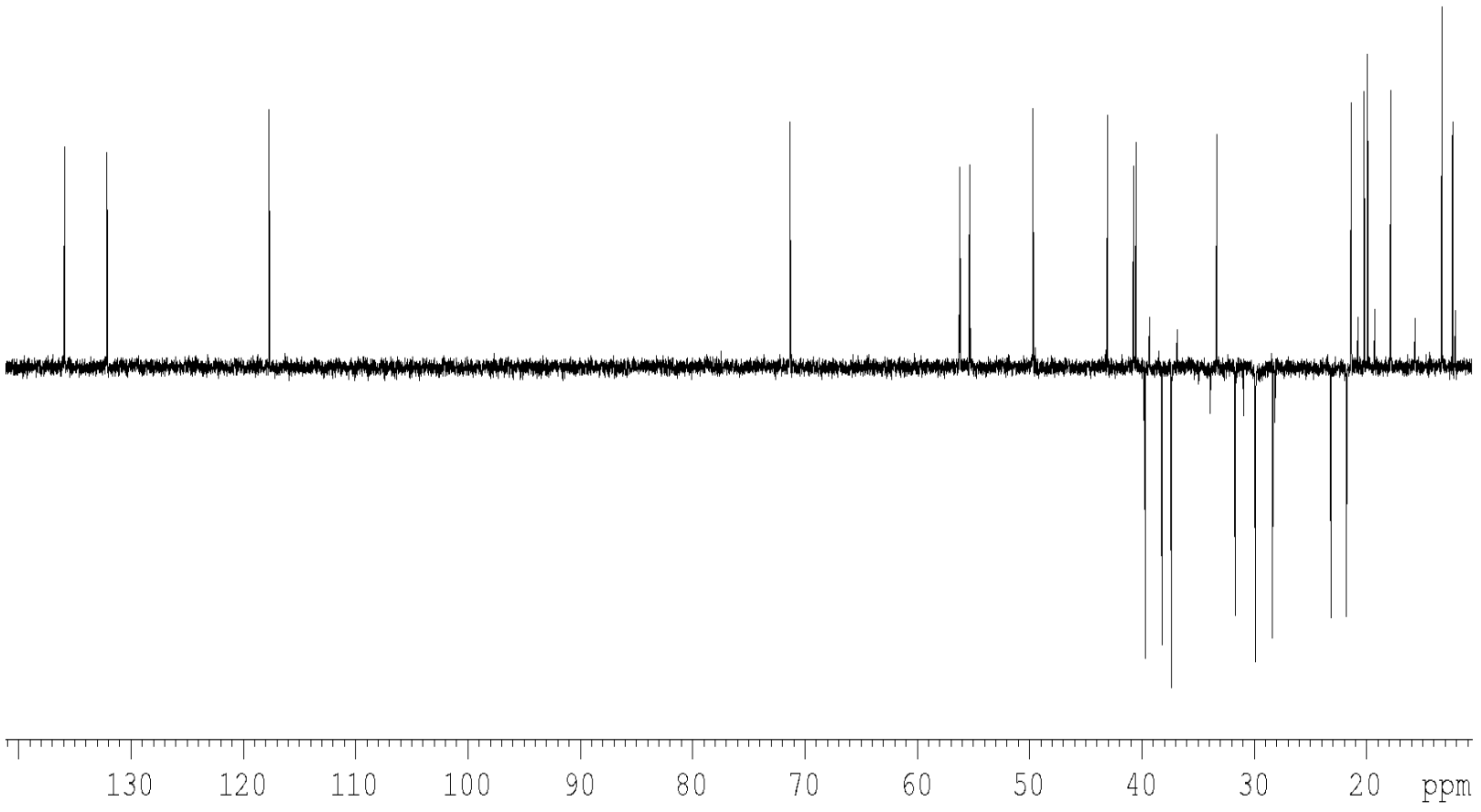
^{1}H NMR spectrum for compound 55 in $CDCl_3$

A9



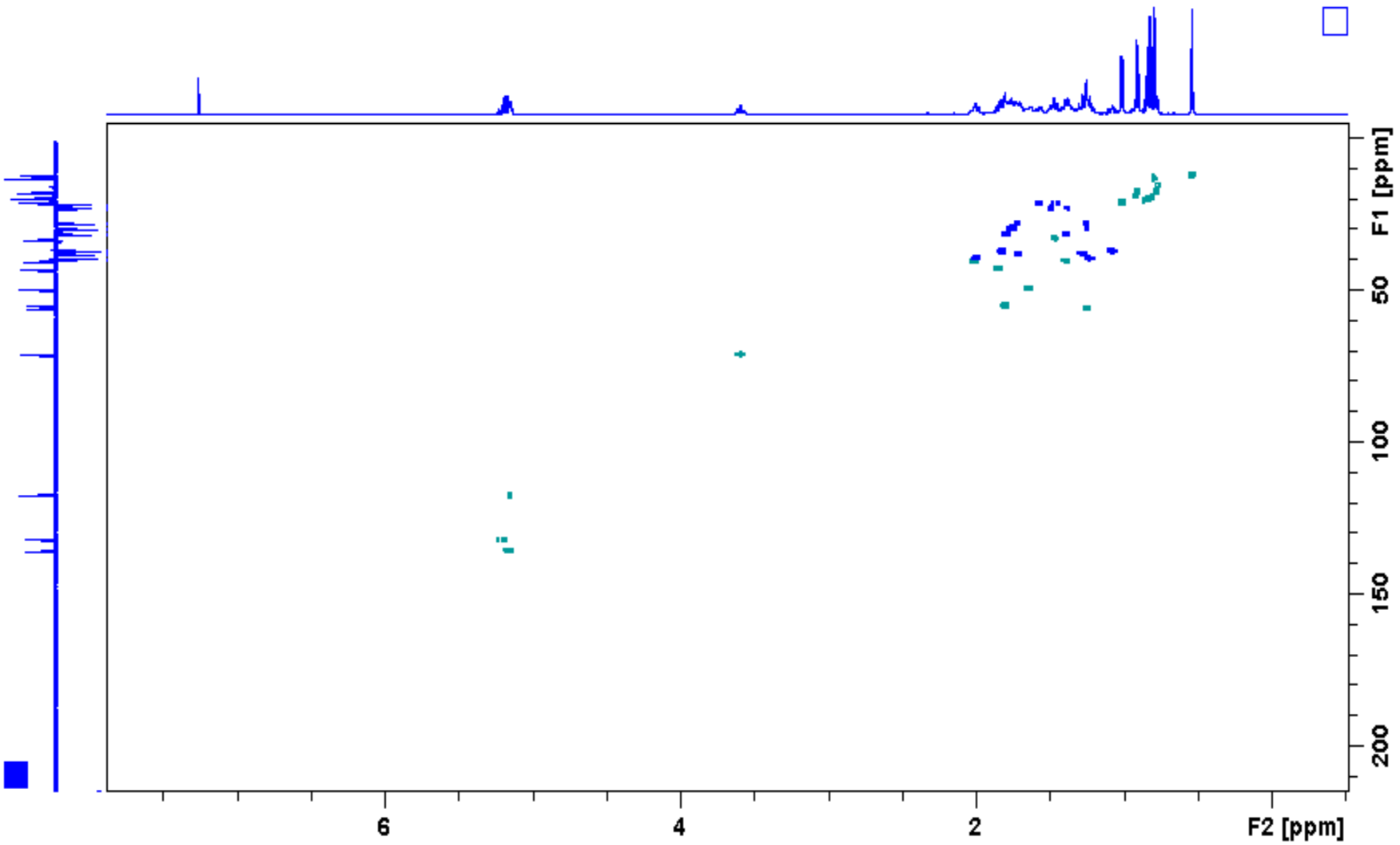
^{13}C NMR spectrum for compound 55 in $CDCl_3$

A10



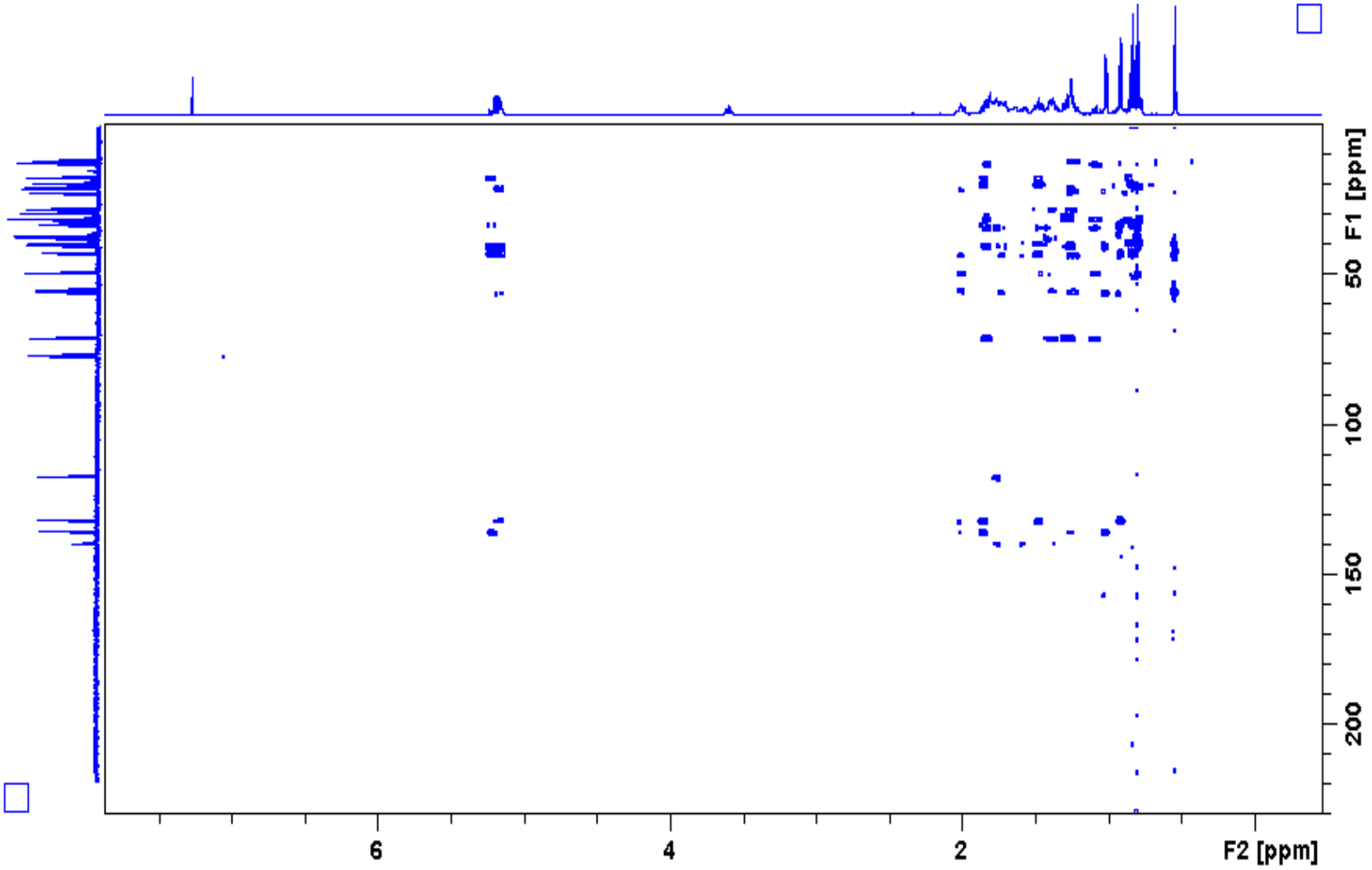
DEPT spectrum for compound 55 in $CDCl_3$

A11



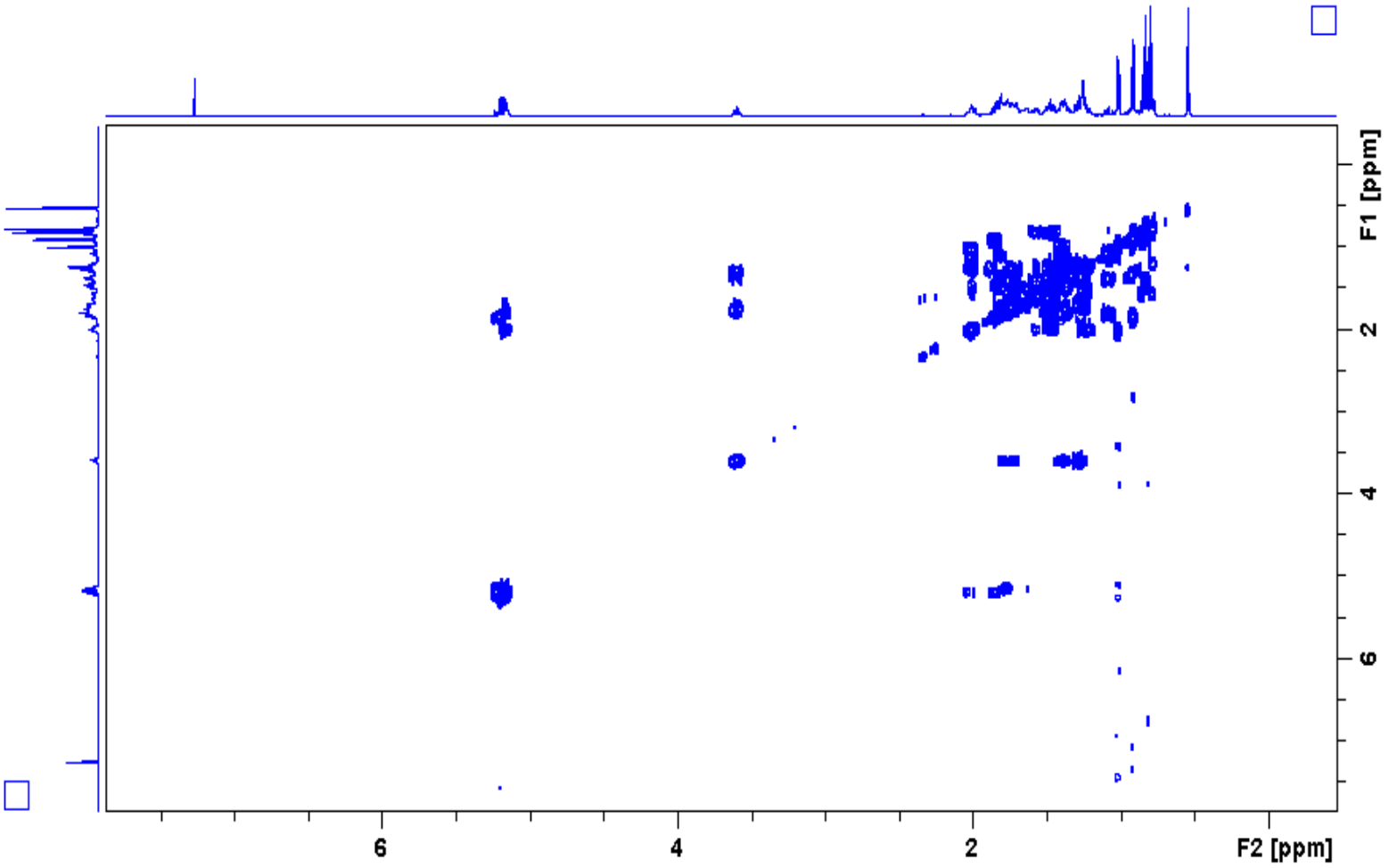
HSQC spectrum for compound 55 in $CDCl_3$

A12



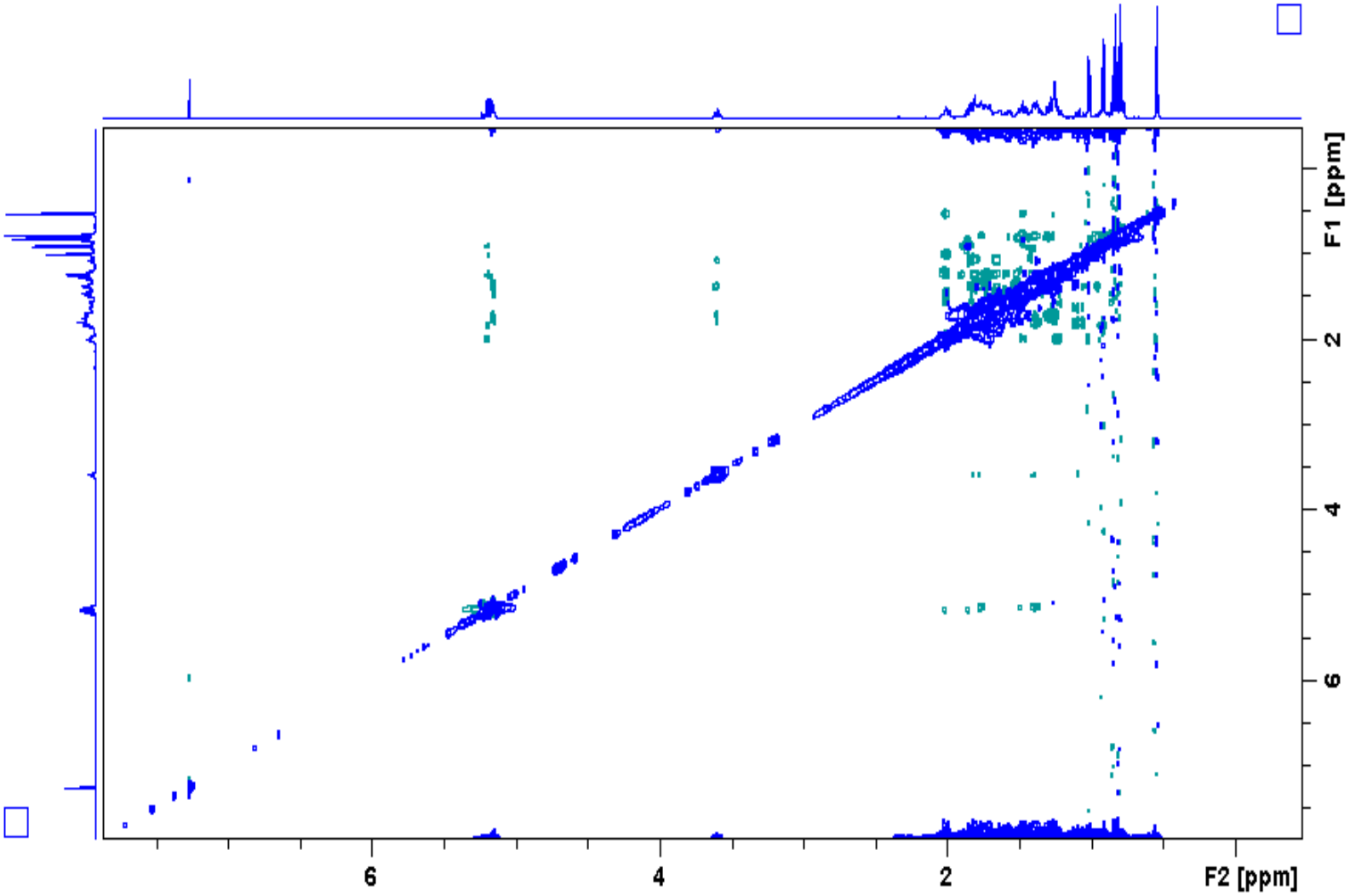
HMBC spectrum for compound 55

A13

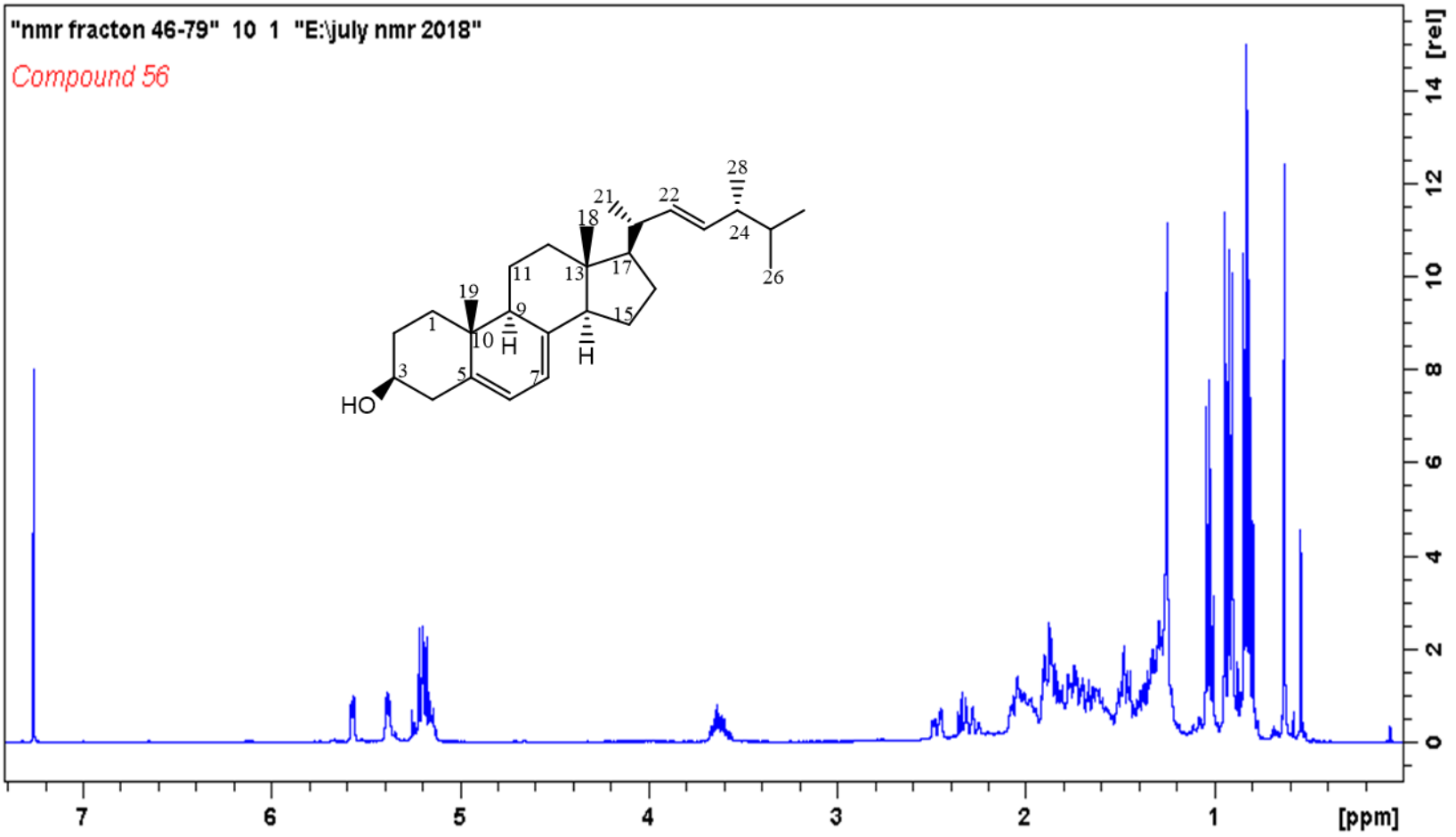


COSY NMR spectrum for compound 55

A14

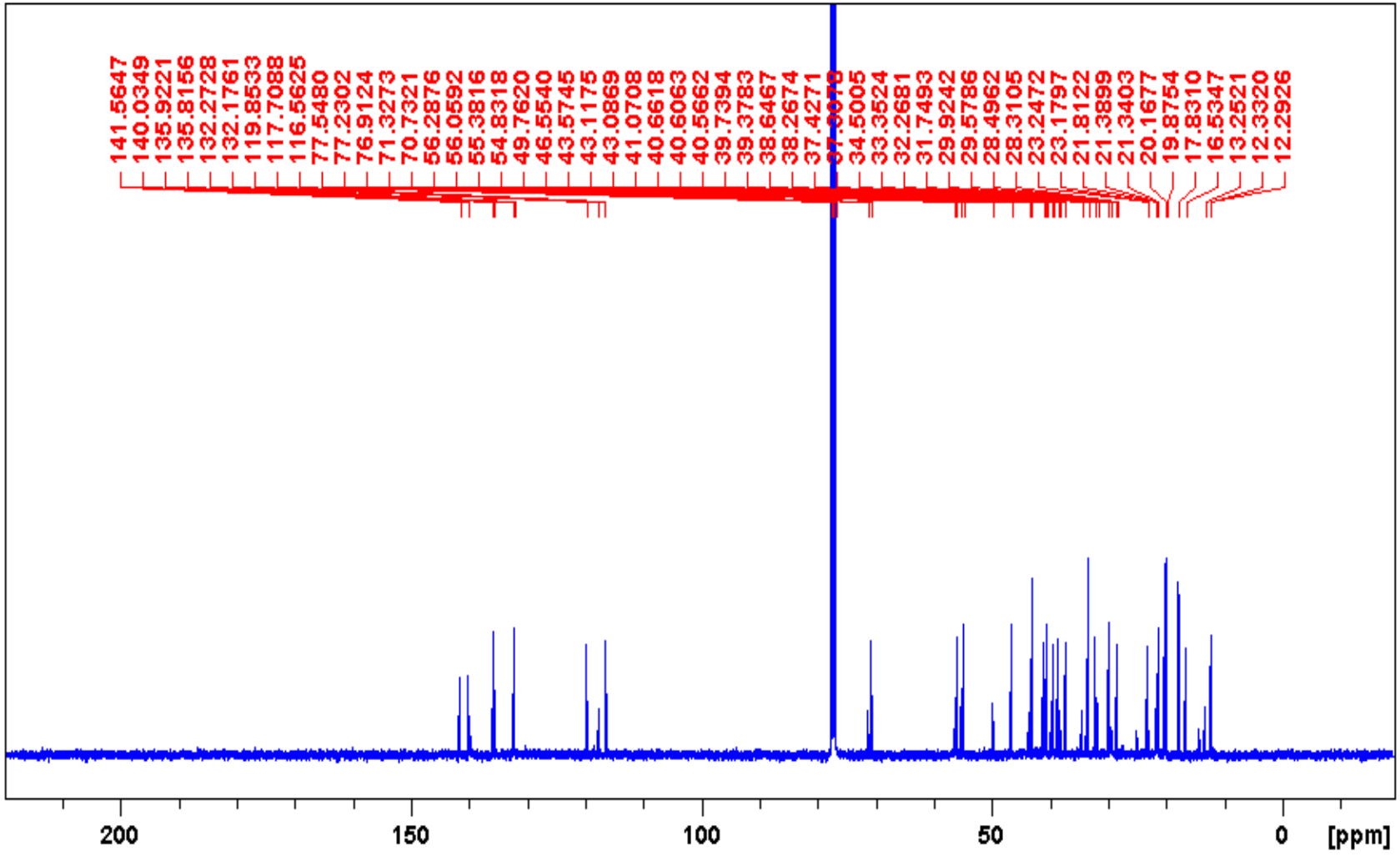


NOESY spectrum for compound 55



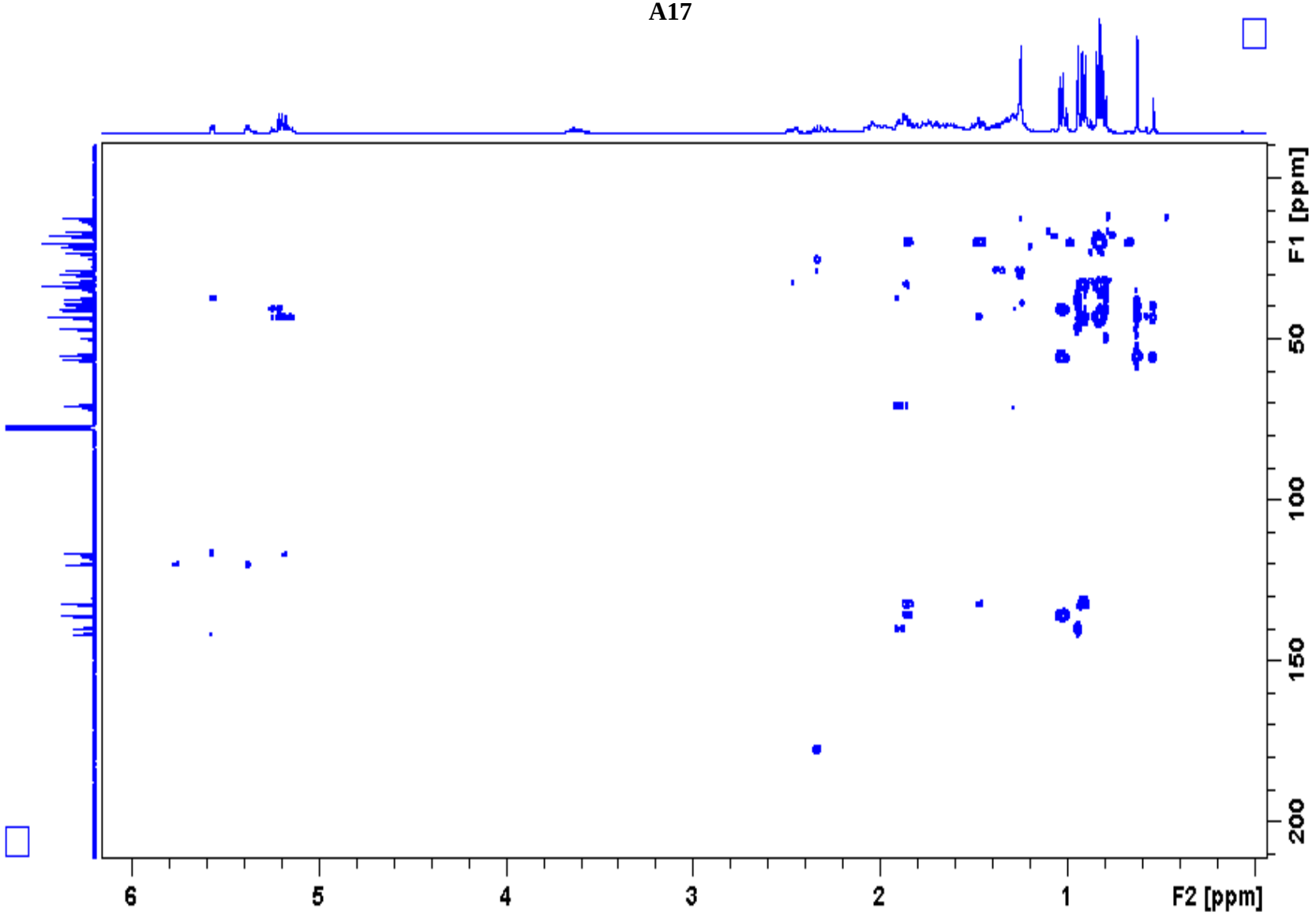
^{1}H NMR spectrum for compound 56 in $CDCl_3$

A16



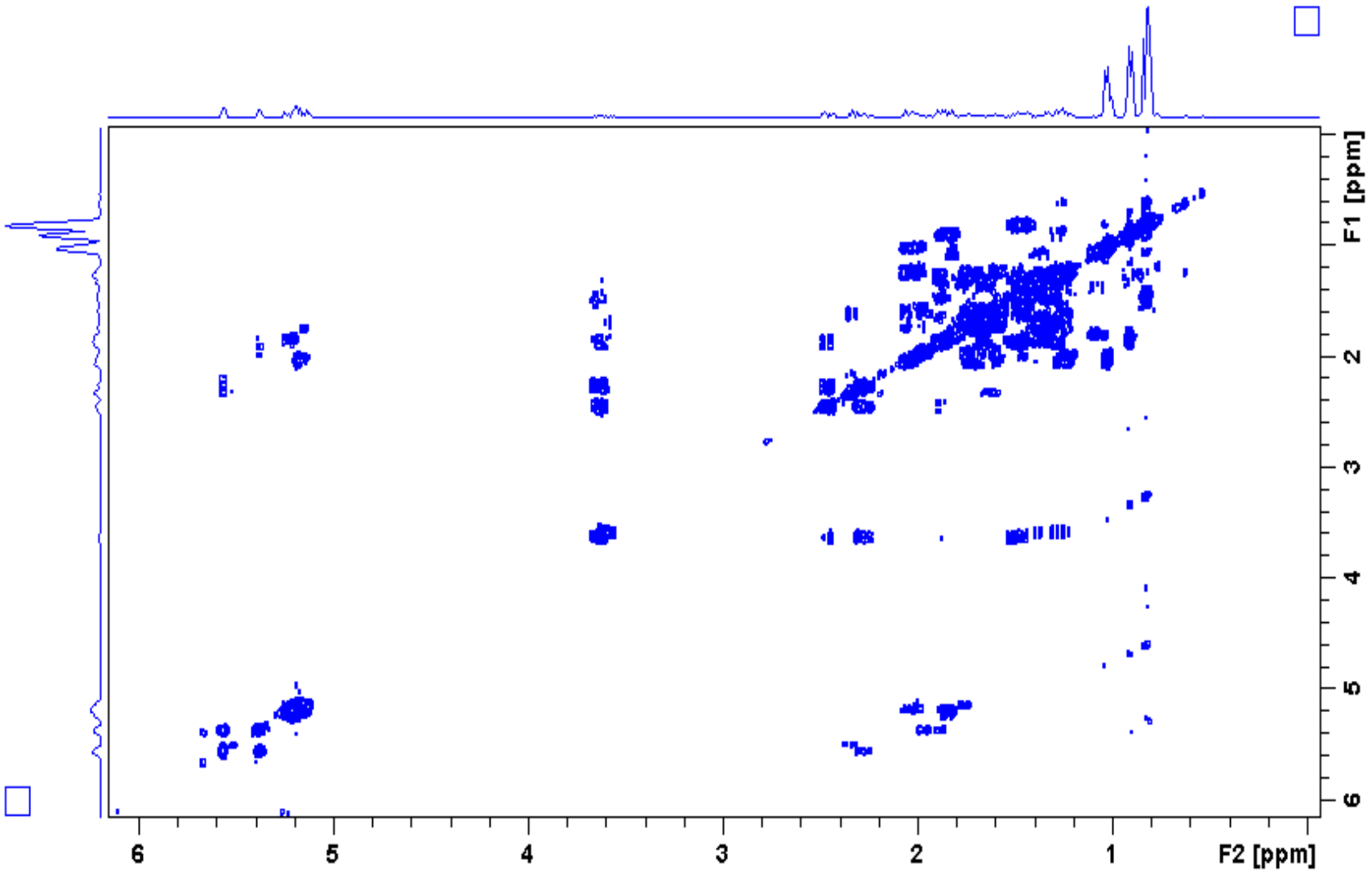
^{13}C NMR spectrum for compound 56 in $CDCl_3$

A17



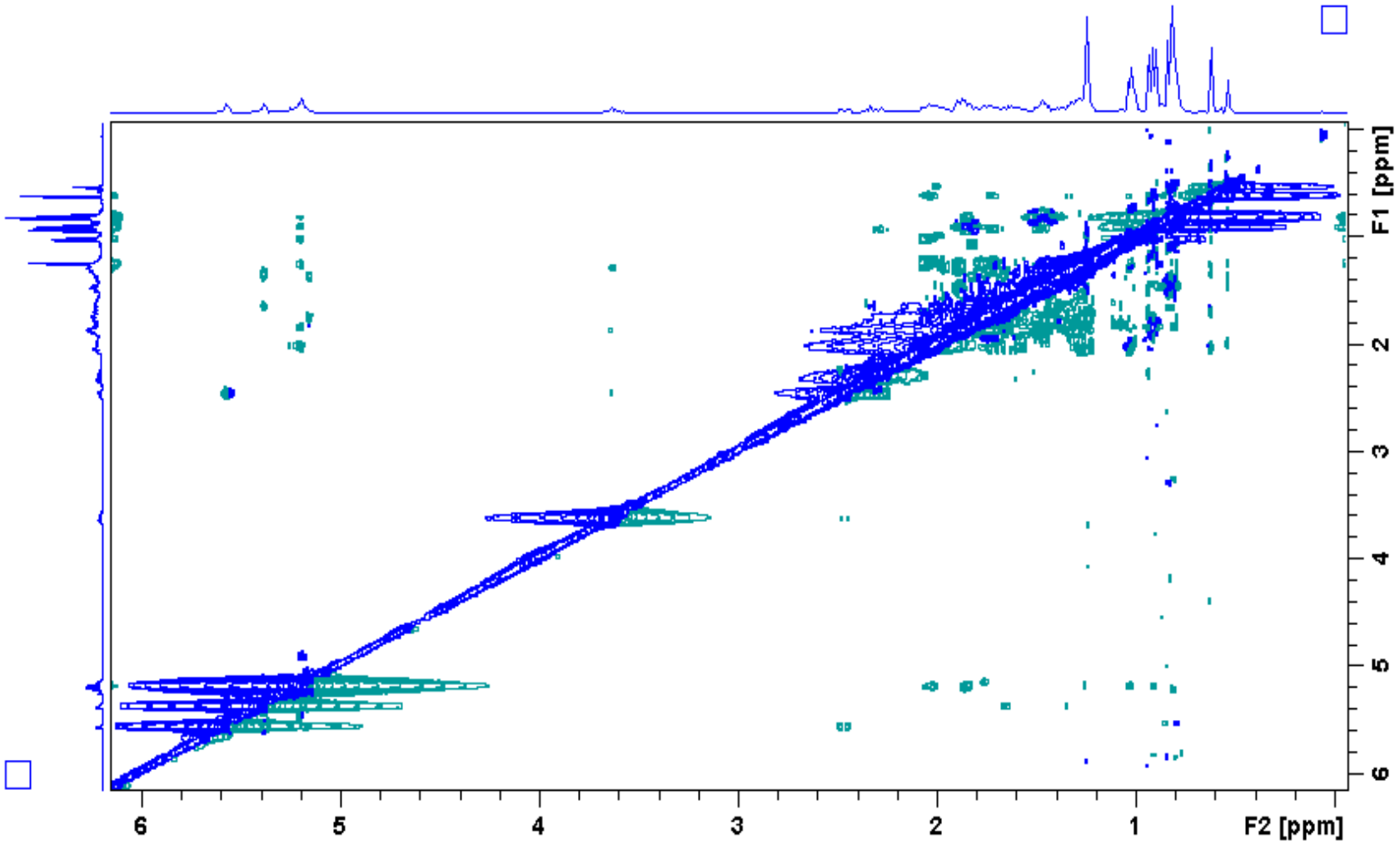
HMBC spectrum for compound 56 in $CDCl_3$

A18



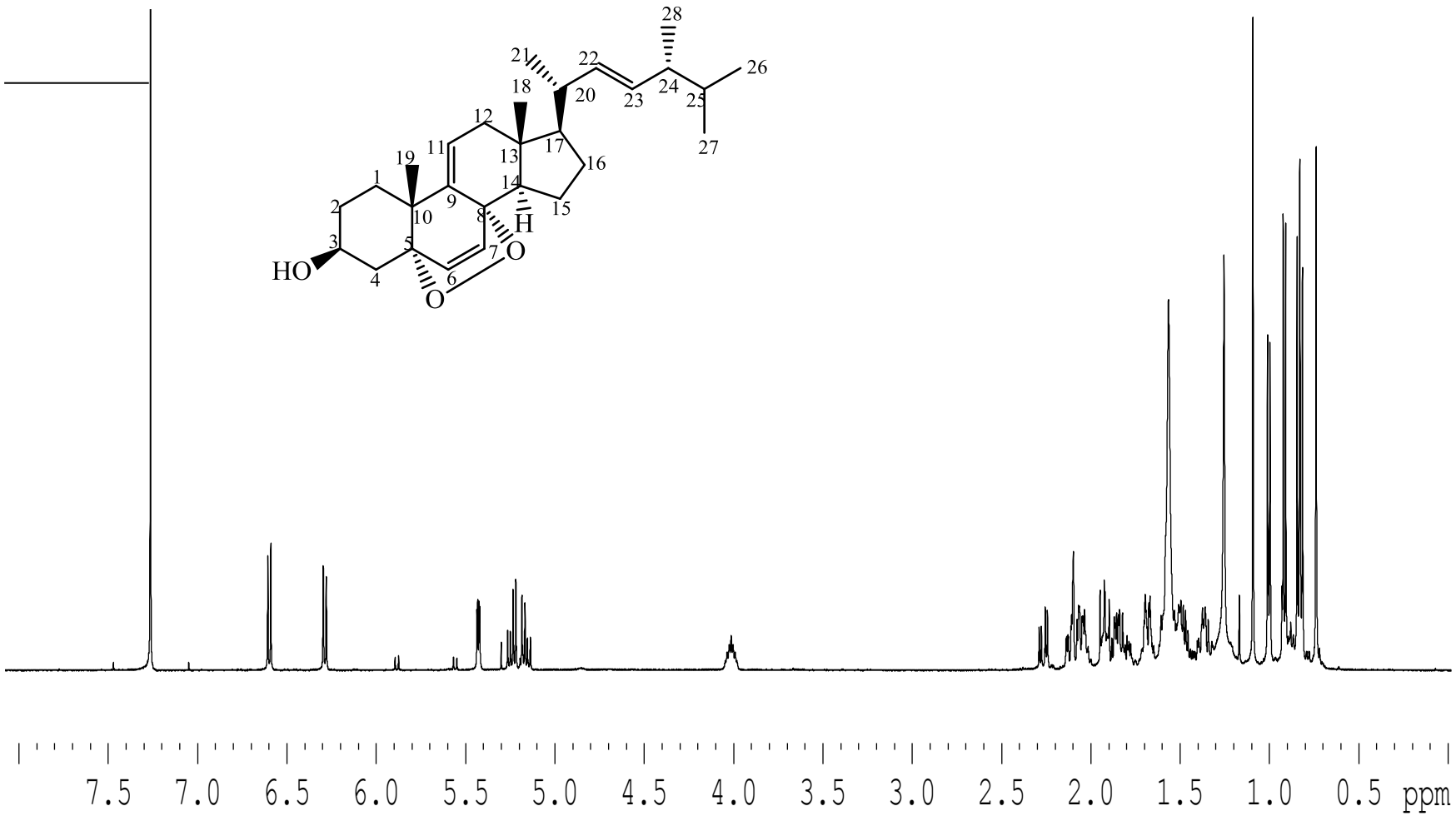
COSY spectrum for compound 56

A19



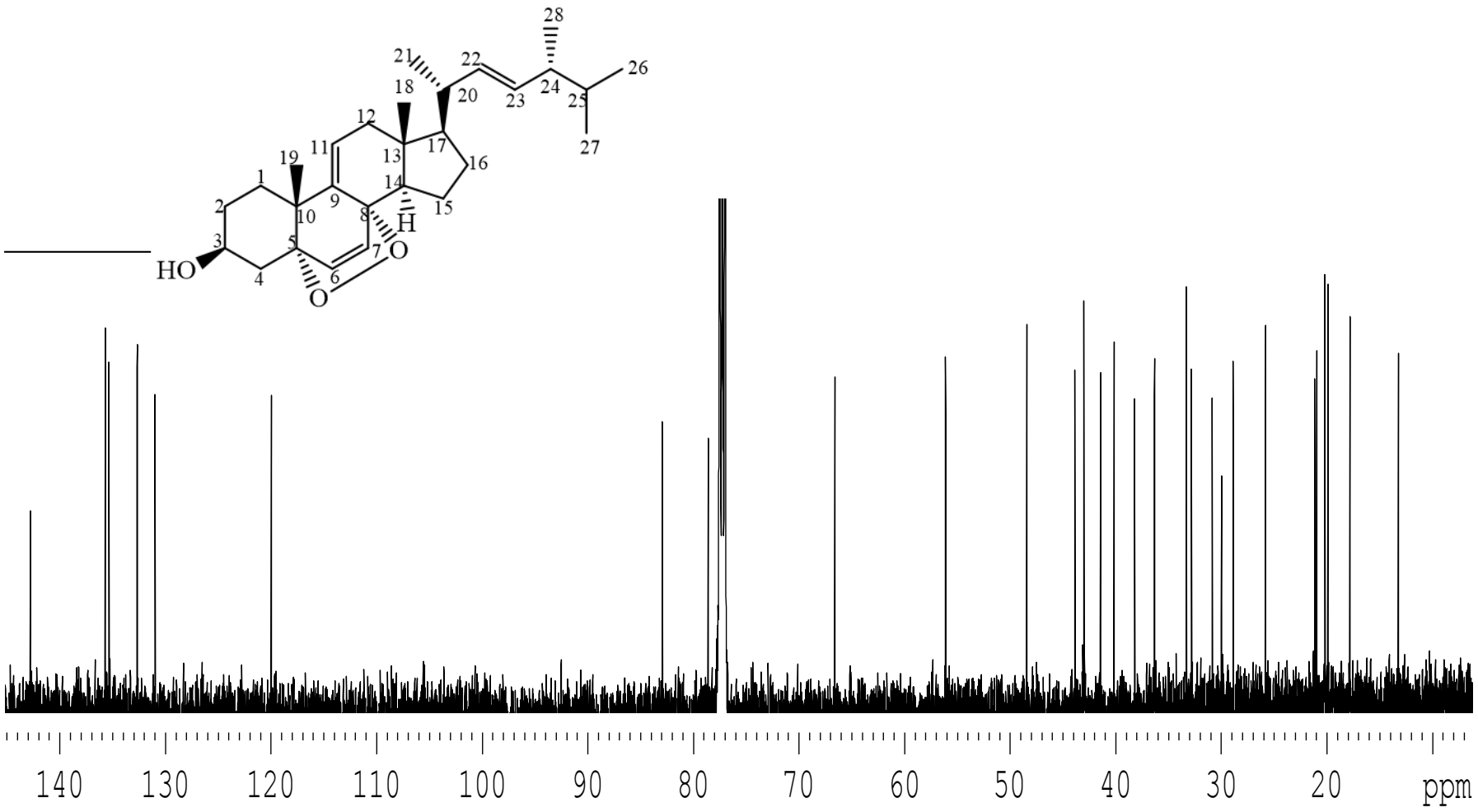
NOESY spectrum for compound 56

A20

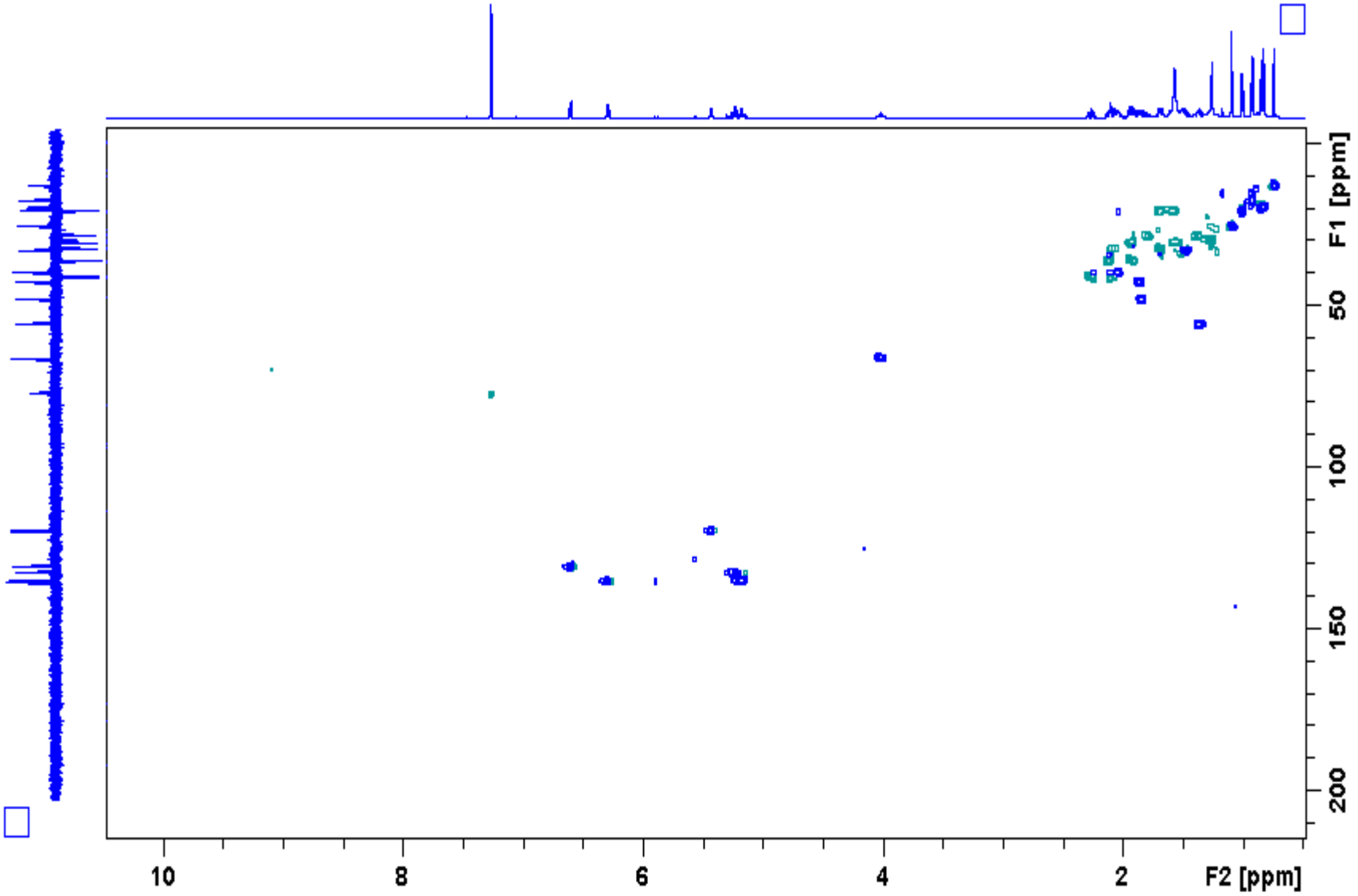


^{1} H NMR spectrum for compound 57 in $CDCl_3$

A21

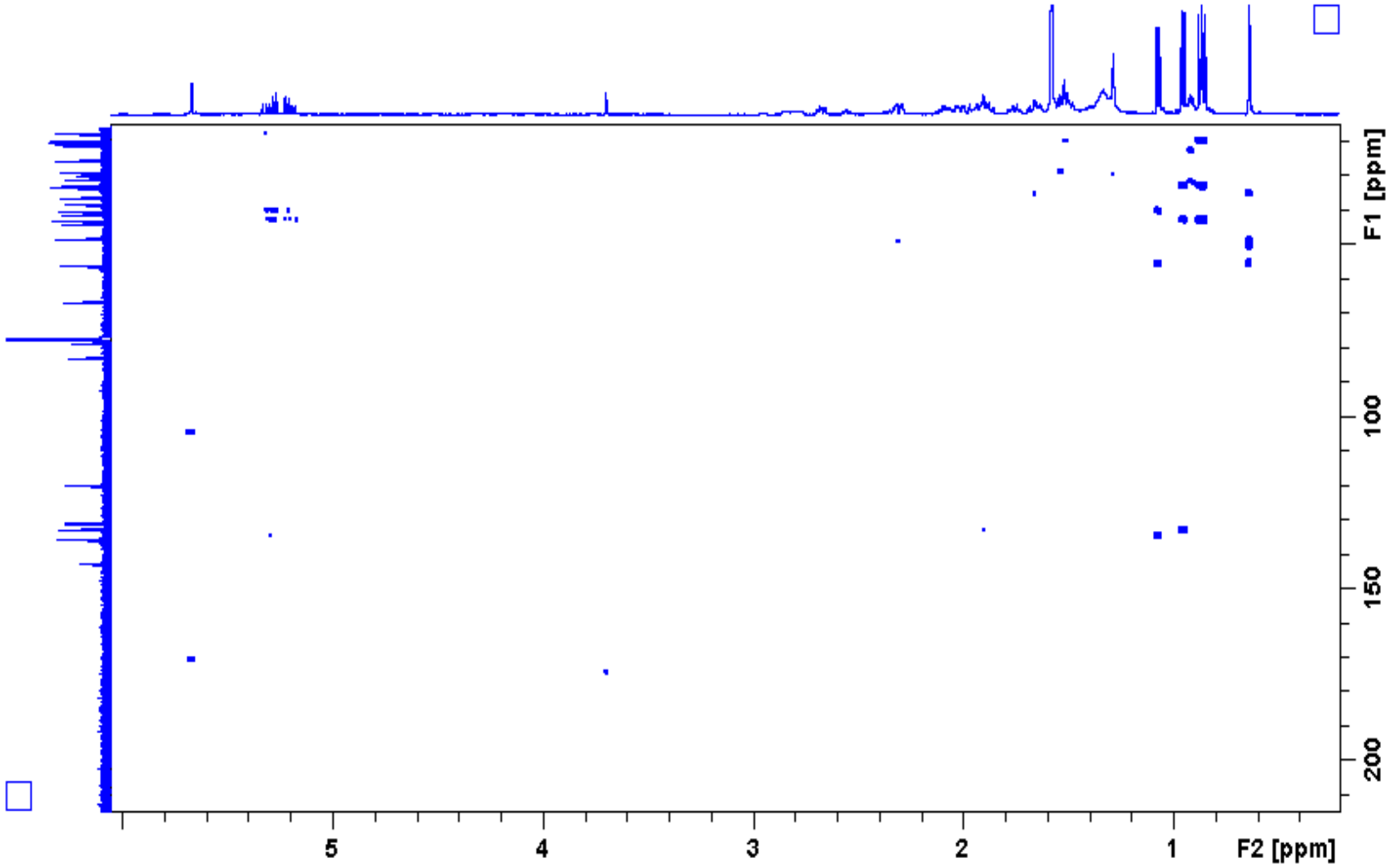


^{13} C NMR spectrum for compound 57 in $CDCl_3$



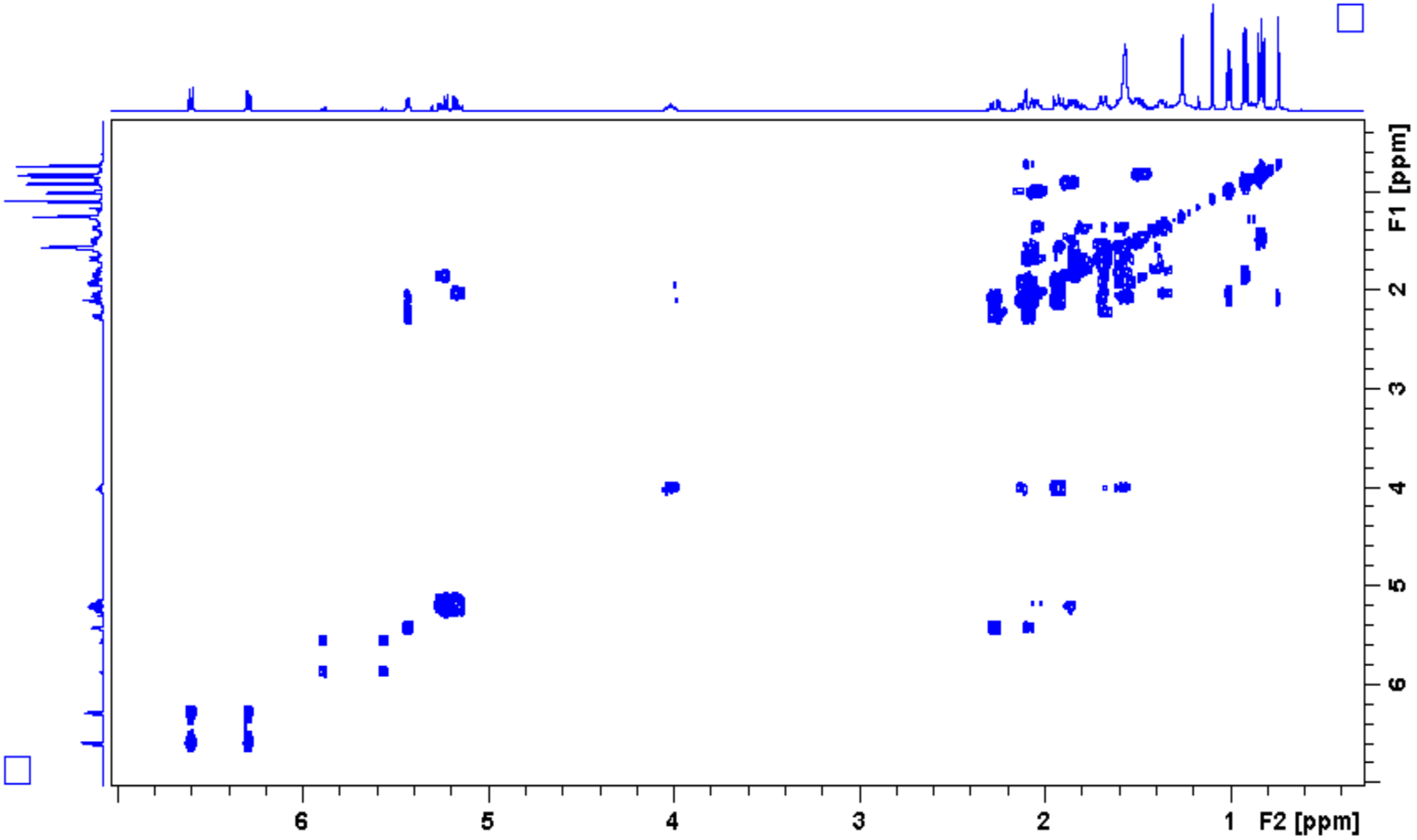
HSQC spectrum for compound 57

A23



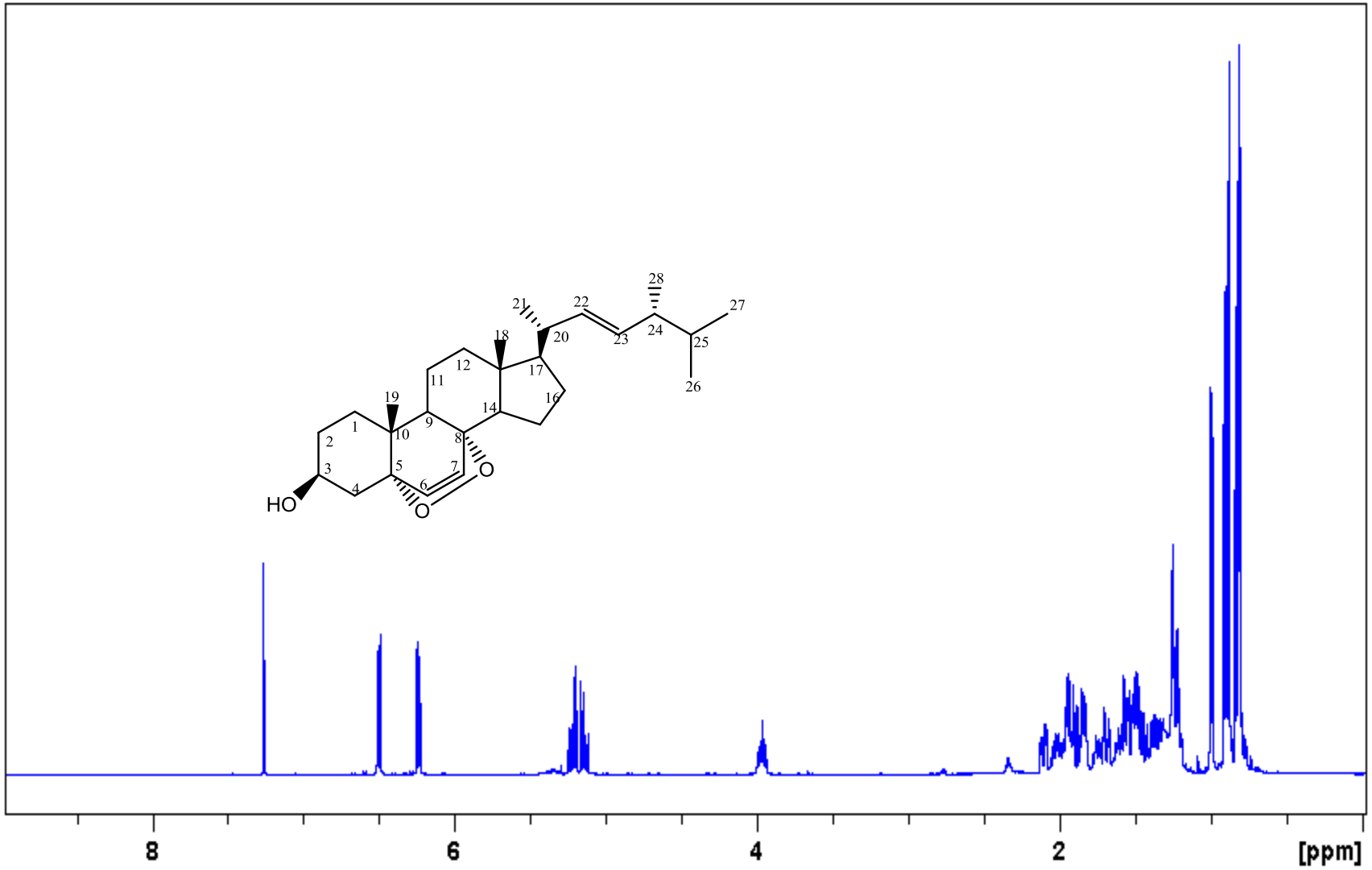
HMBC spectrum for compound 57 in $CDCl_3$

A24



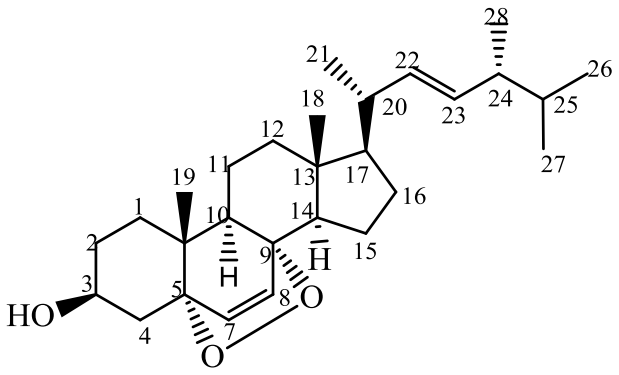
COSY spectrum for compound 57 in $CDCl_3$

A25



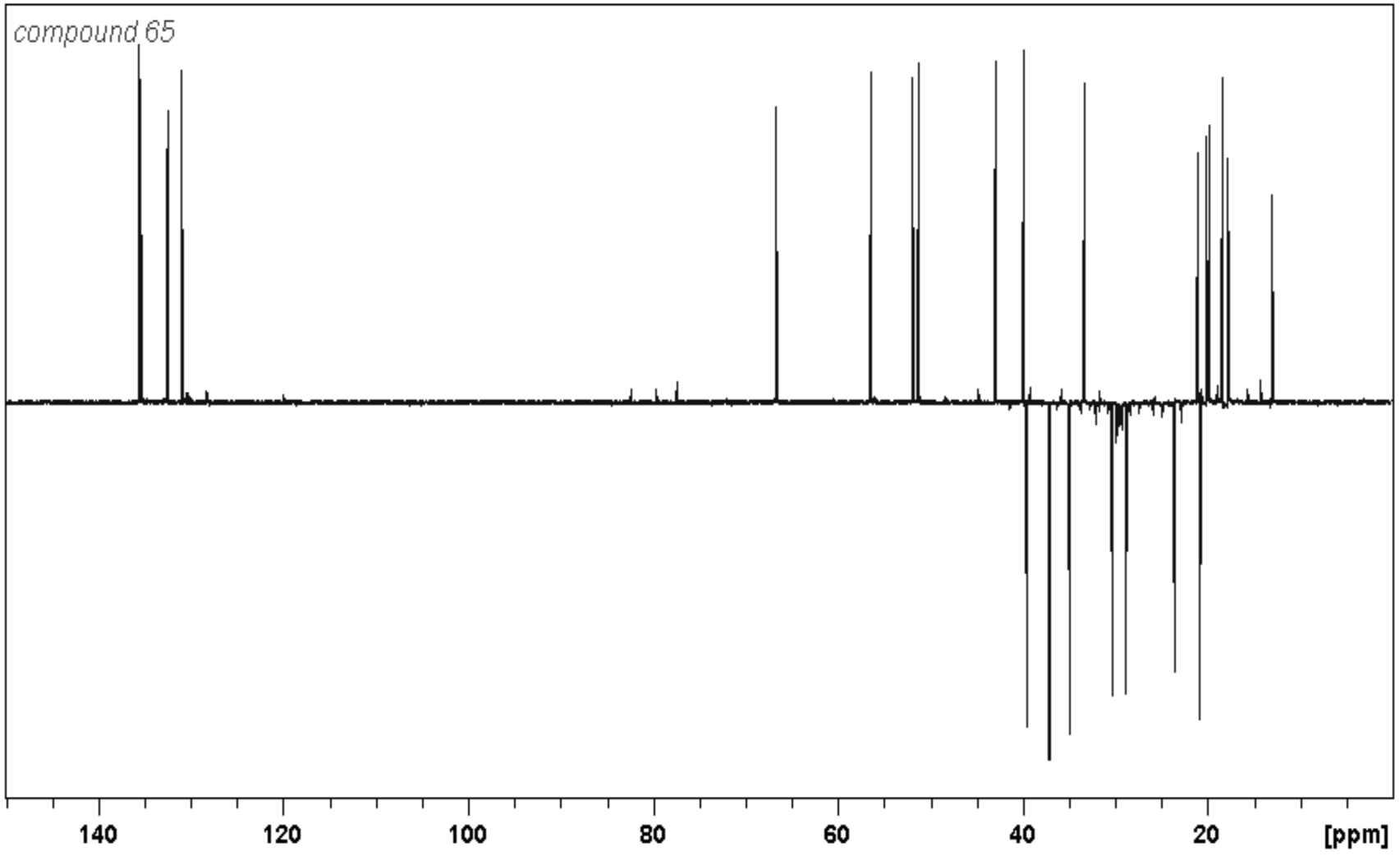
^{1}H spectrum spectrum for compound 58

A26



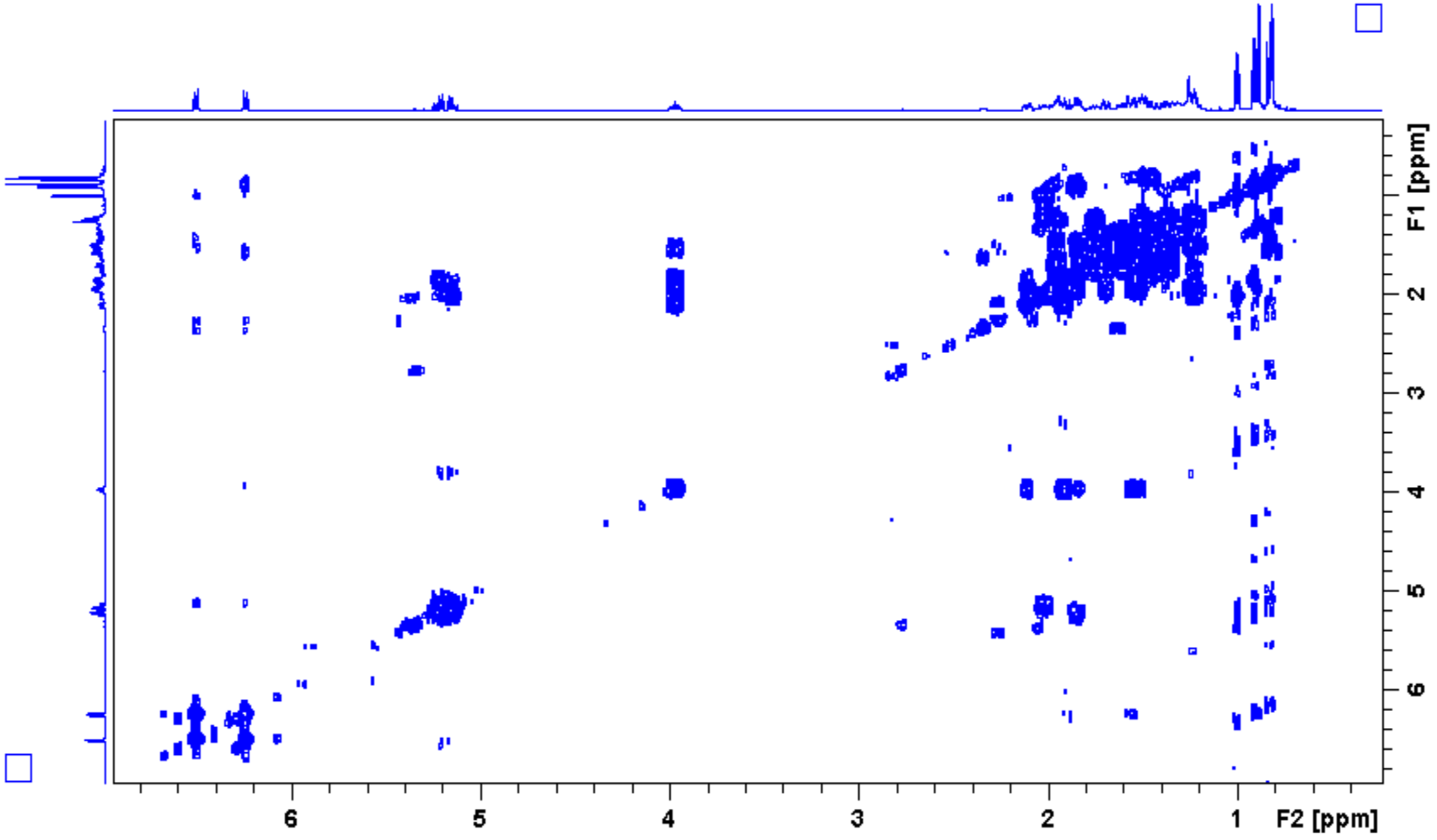
^{13} C NMR spectrum and chemical shift for compound 58 in $CDCl_3$

A27



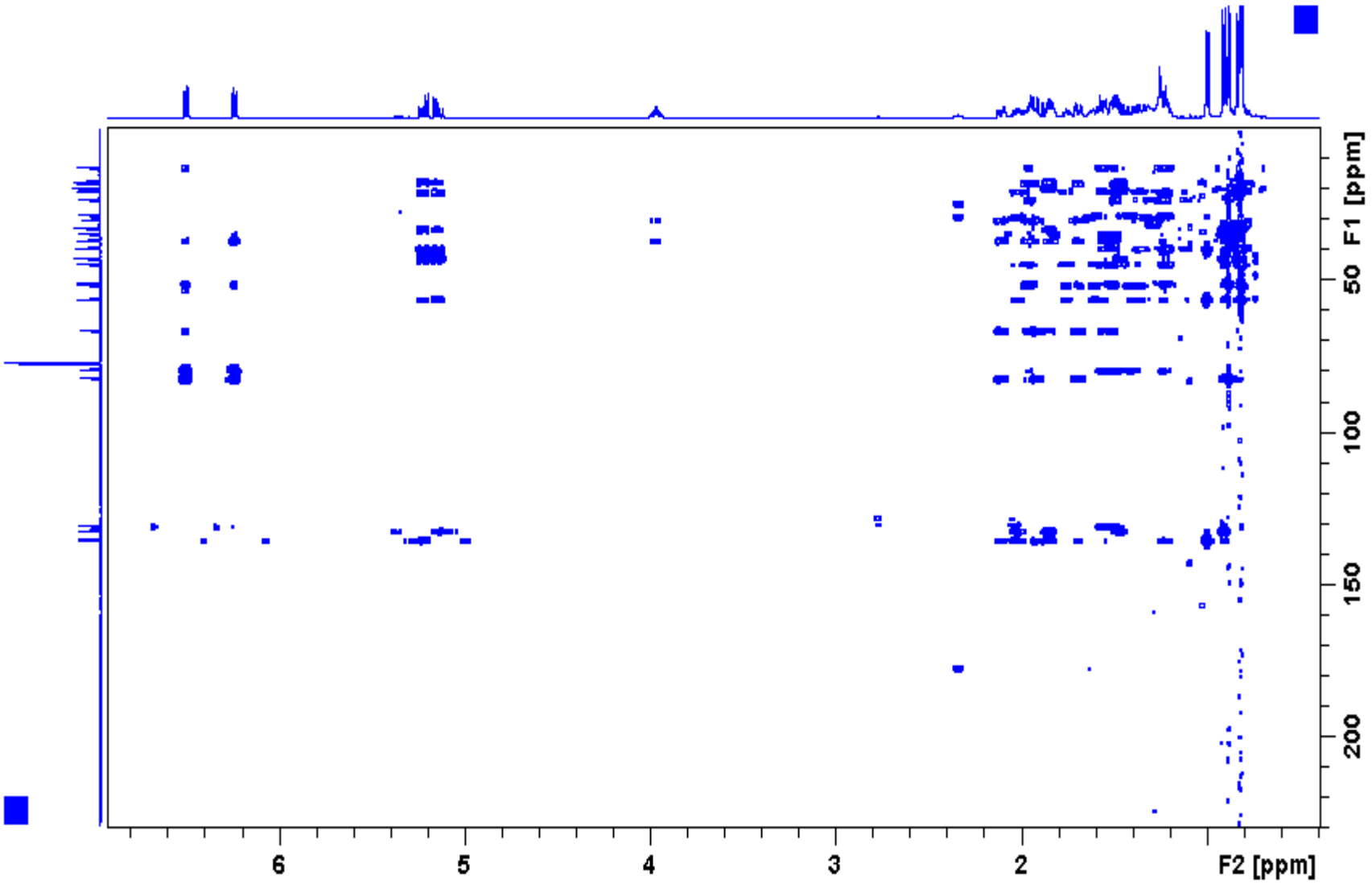
DEPT -135 spectrum for compound 58

A28



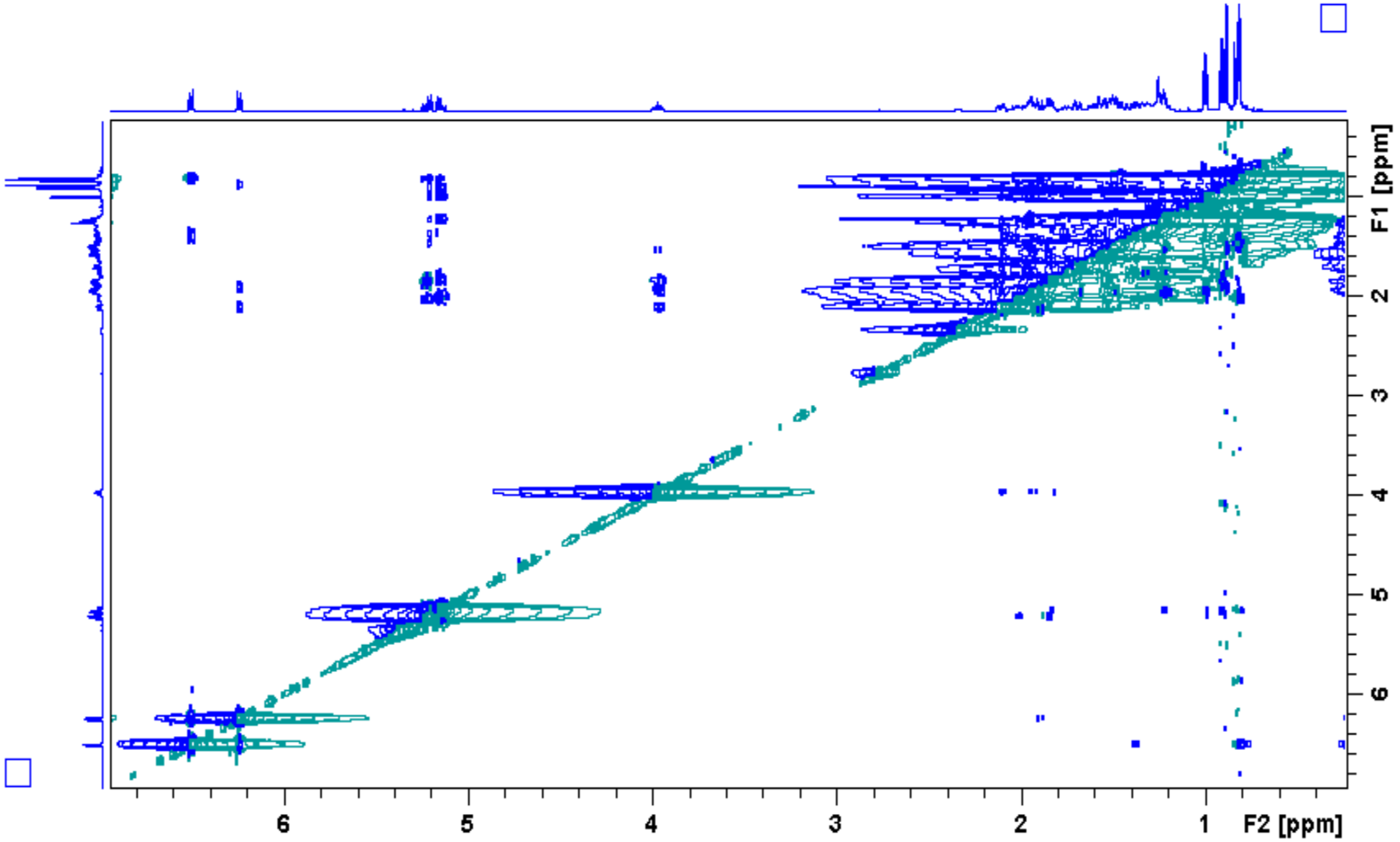
COSY spectrum for compound 58

A29

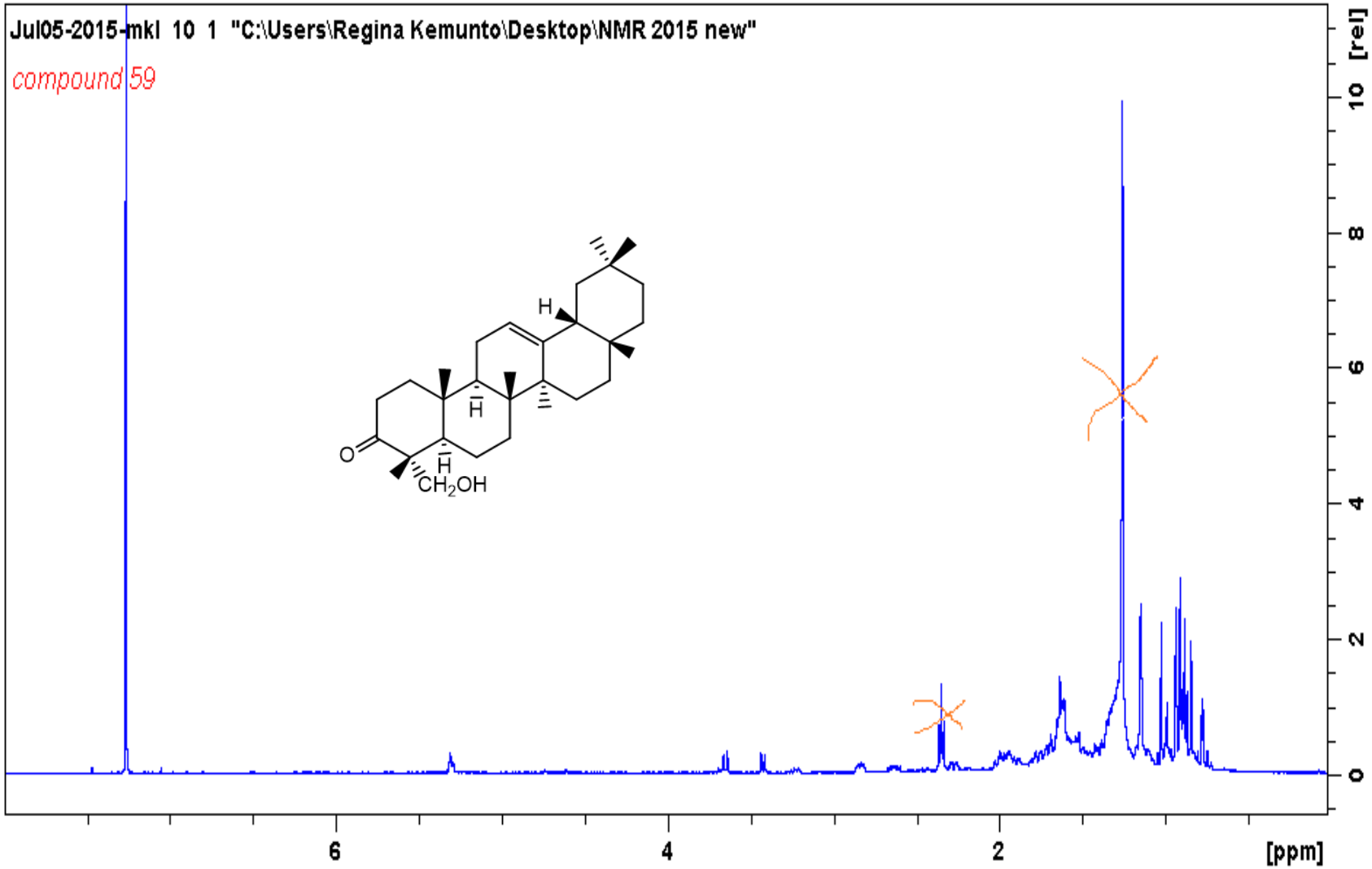


HMBC spectrum for compound 58 in $CDCl_3$

A30

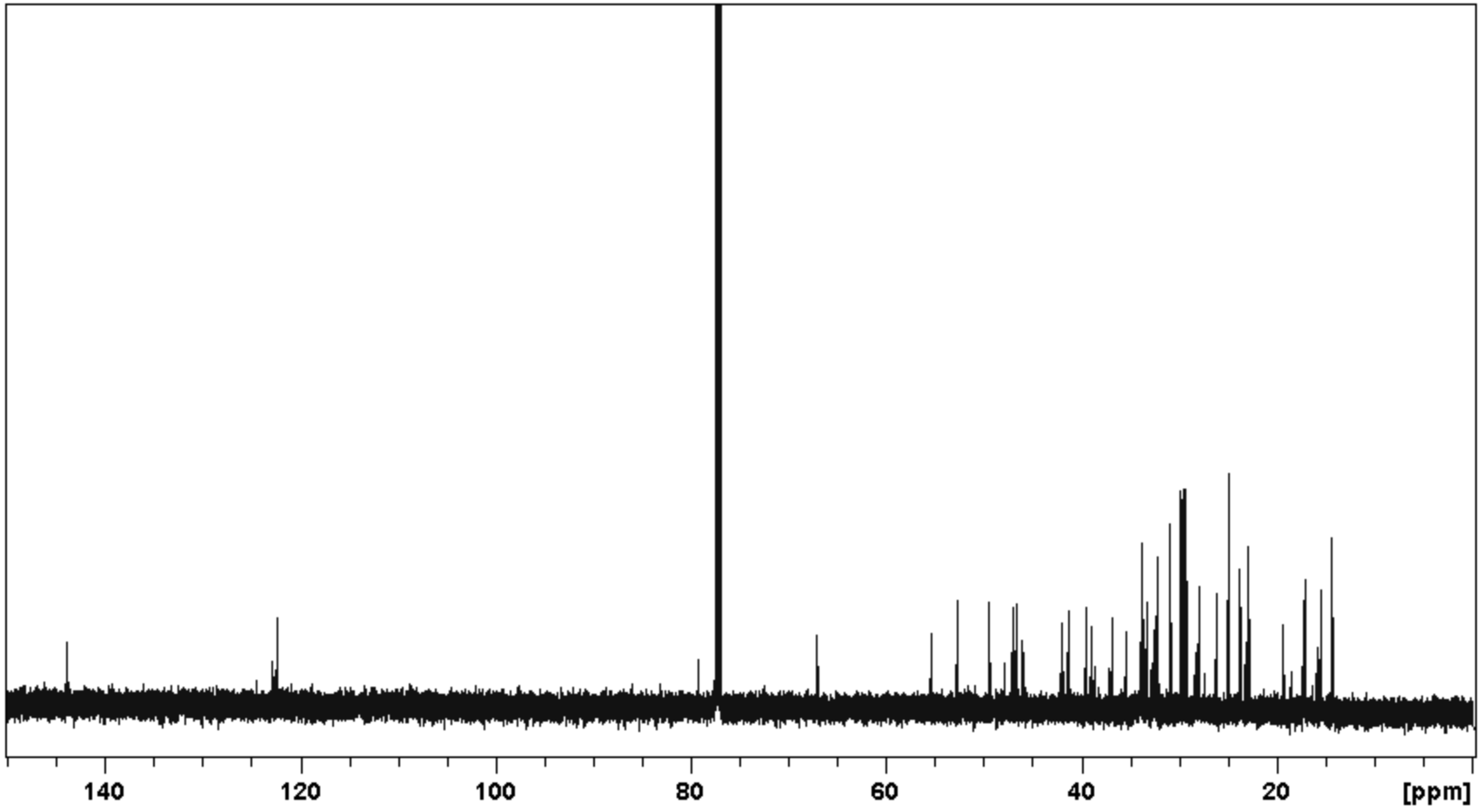


NOESY spectrum for compound 58



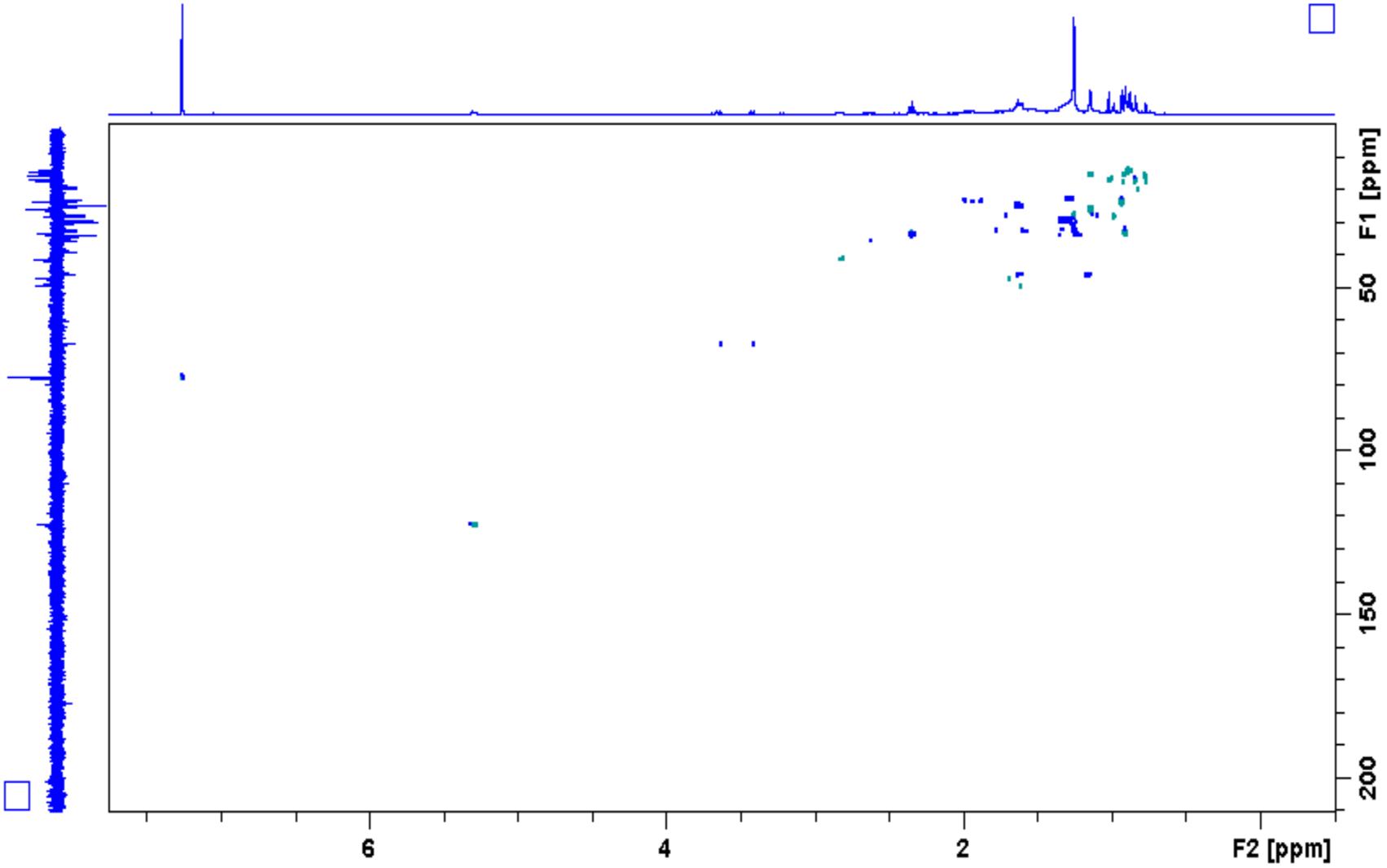
^{1}H NMR spectrum for compound 59 in $CDCl_3$

A32

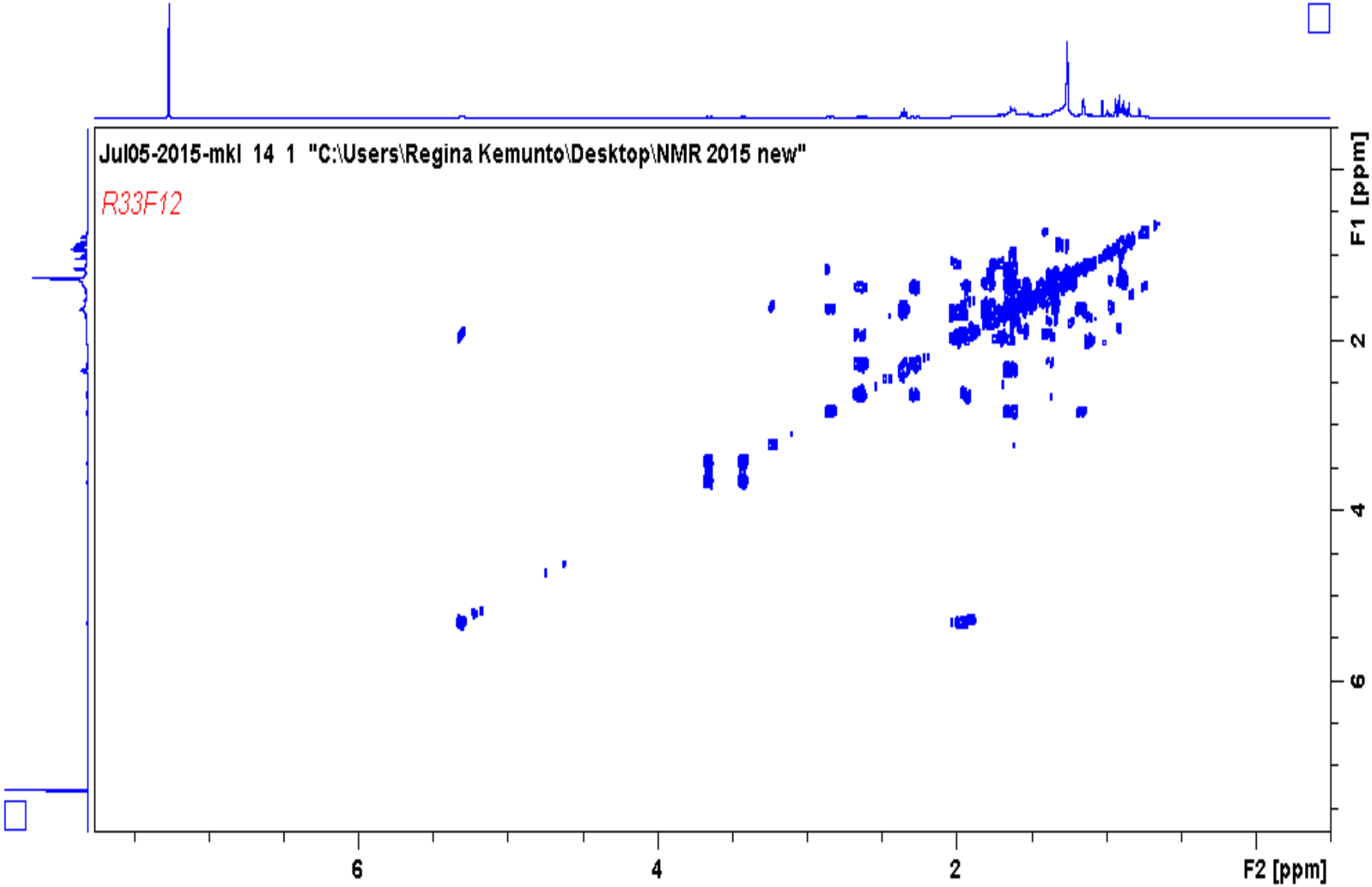


^{13}C NMR spectrum for compound 59 in $CDCl_3$

A33

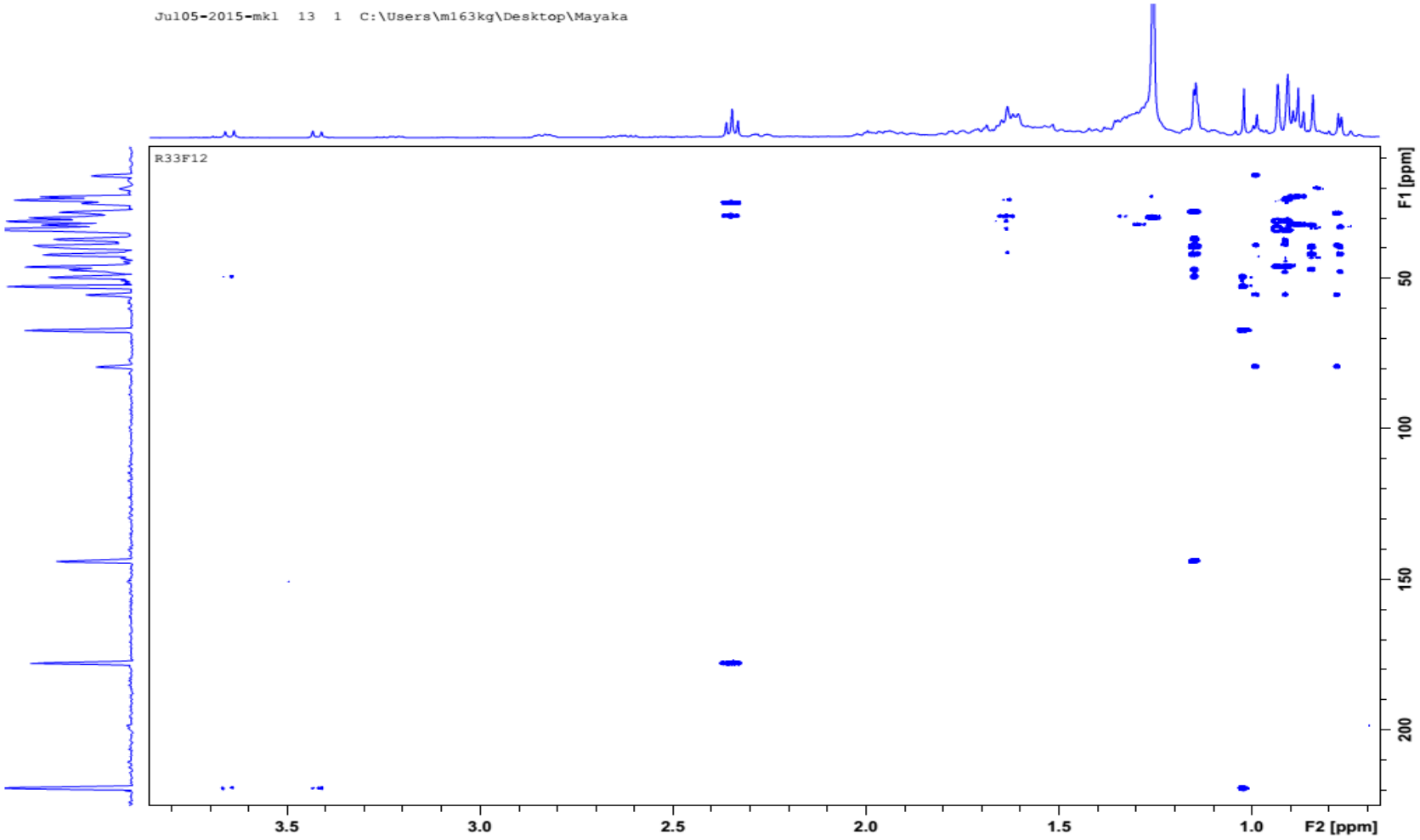


HSQC spectrum for compound 59 in $CDCl_3$



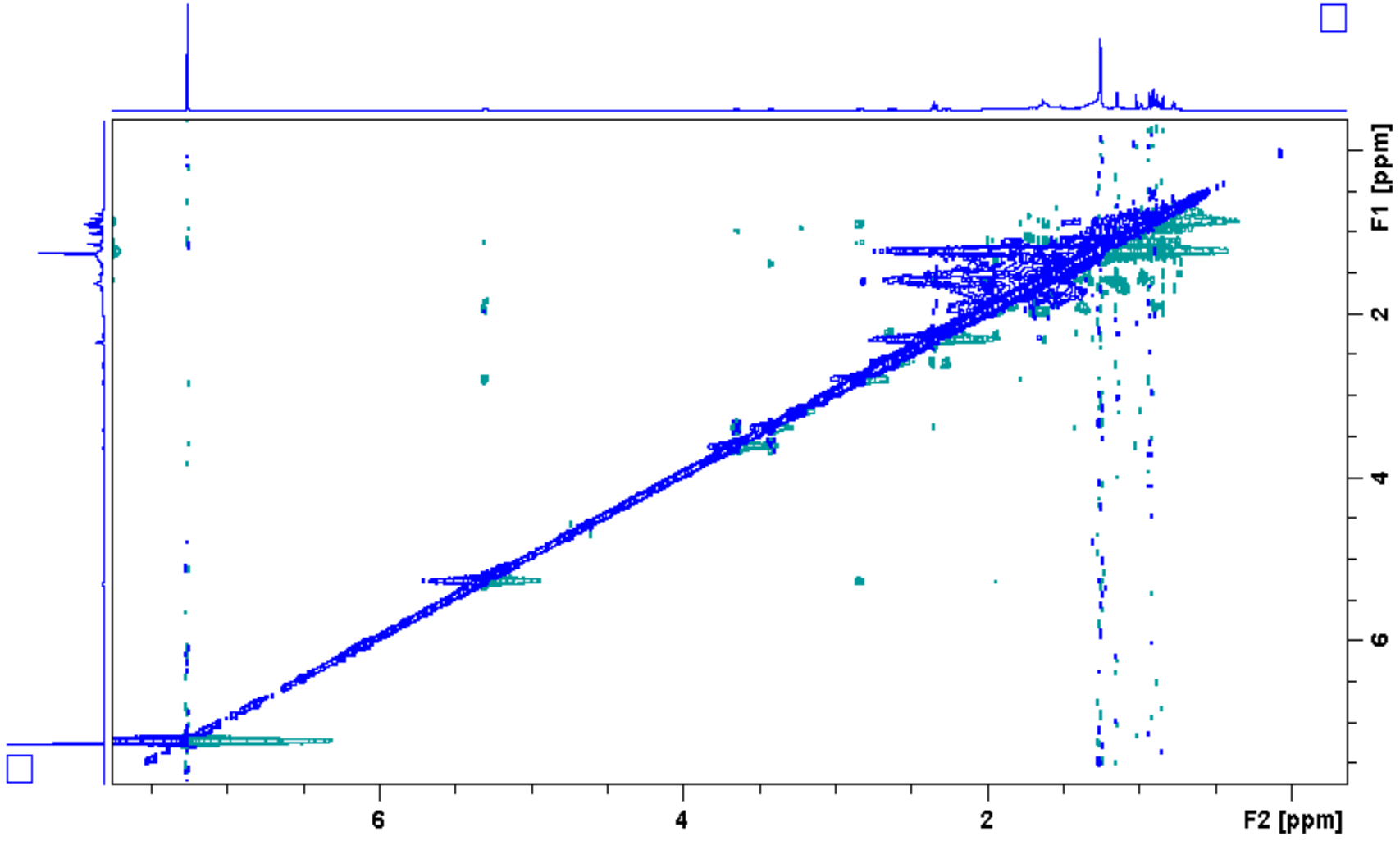
COSY spectrum for compound 59

A35



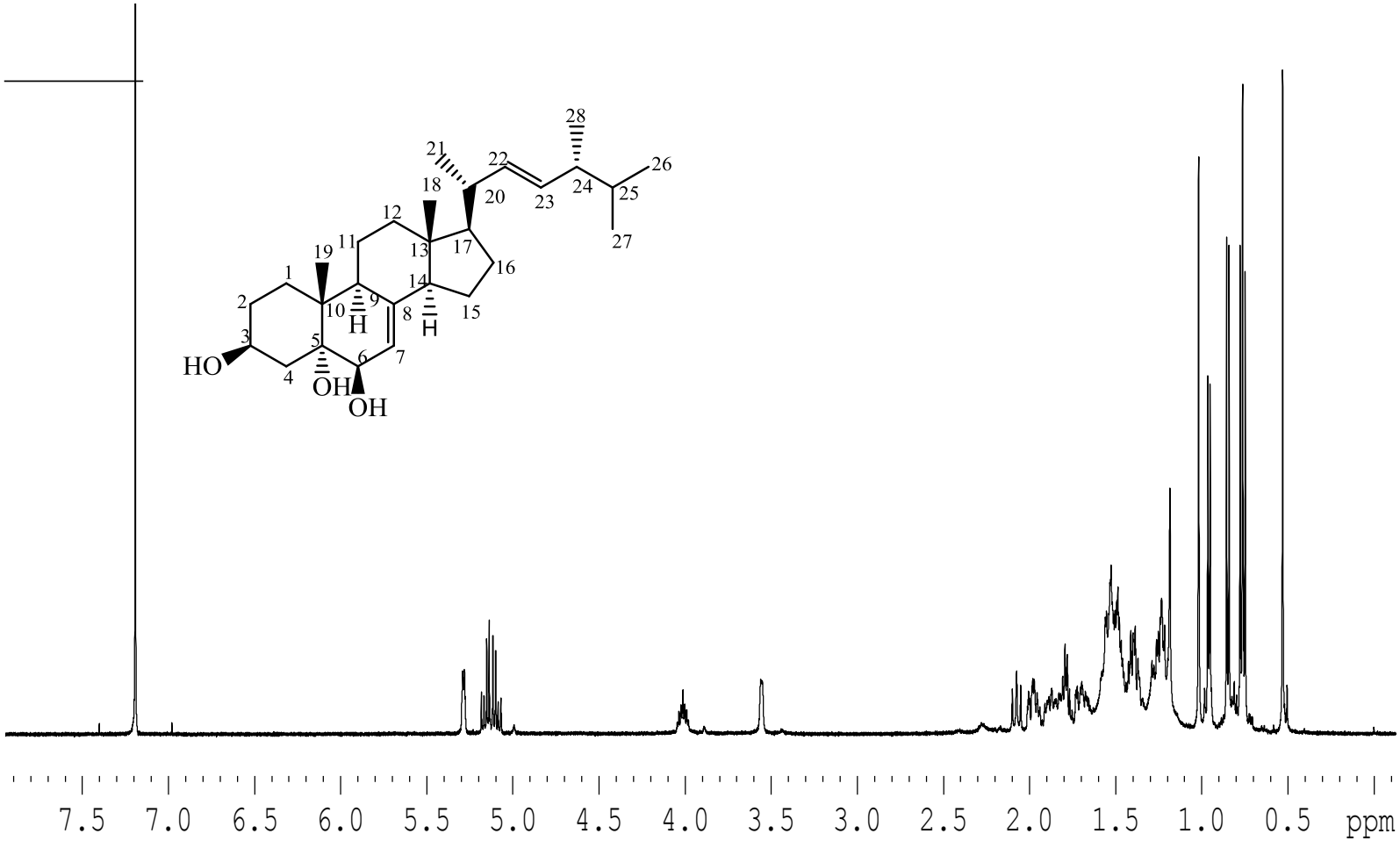
HMBC spectrum for compound 59

A36



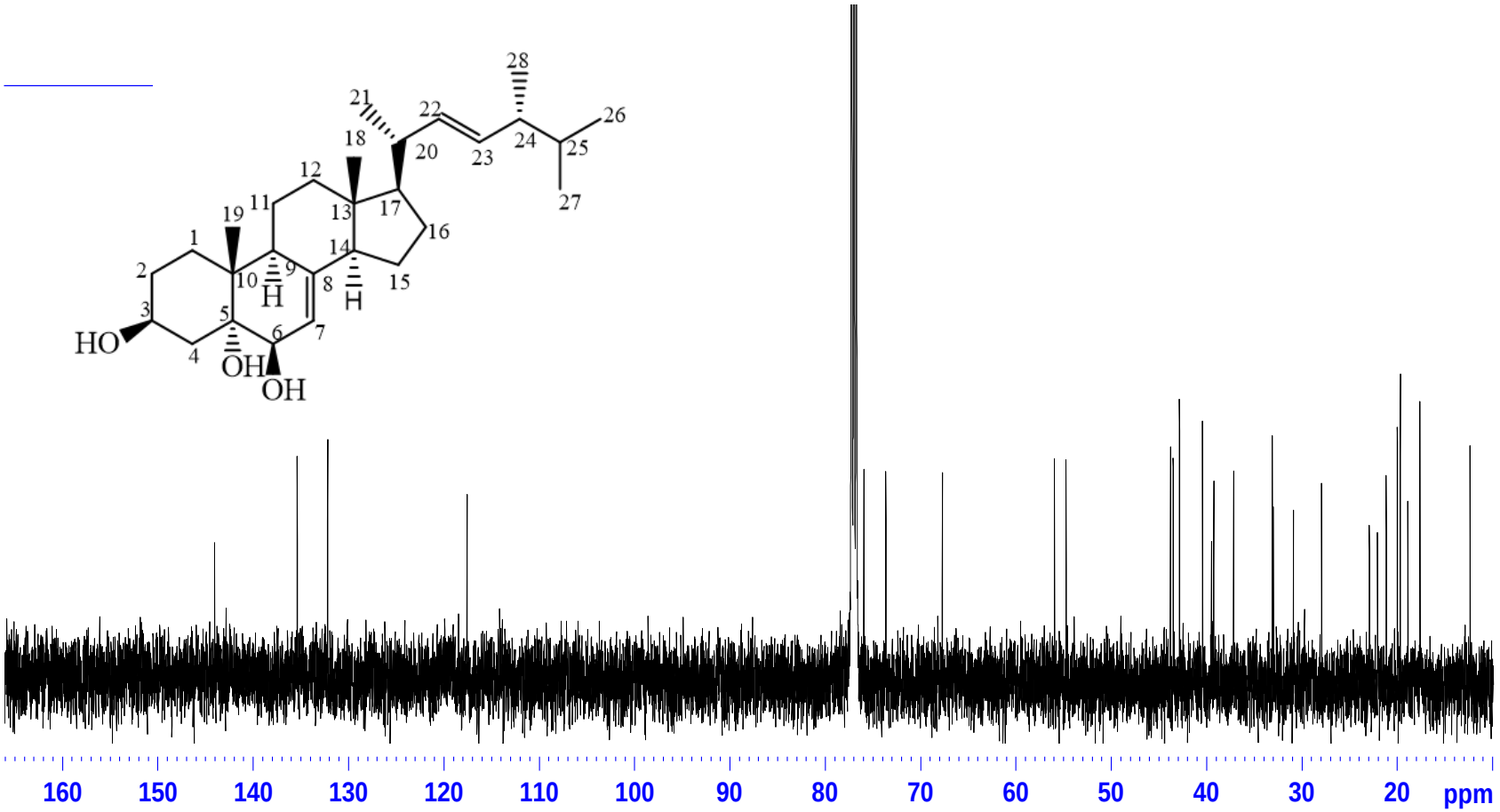
NOESY spectrum for compound 59

A37

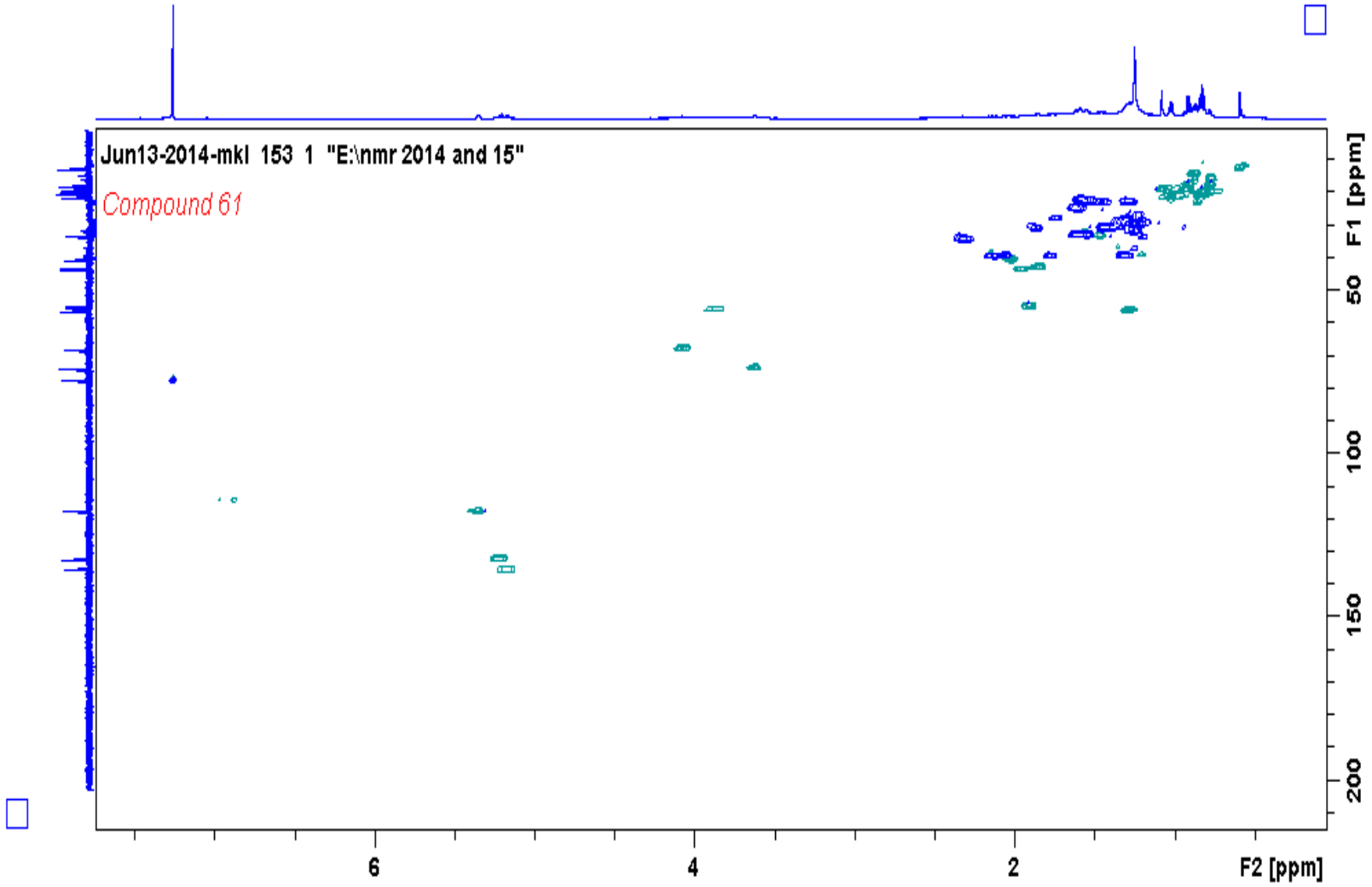


^{1} H NMR spectrum for compound 60 in $CDCl_3$

A38

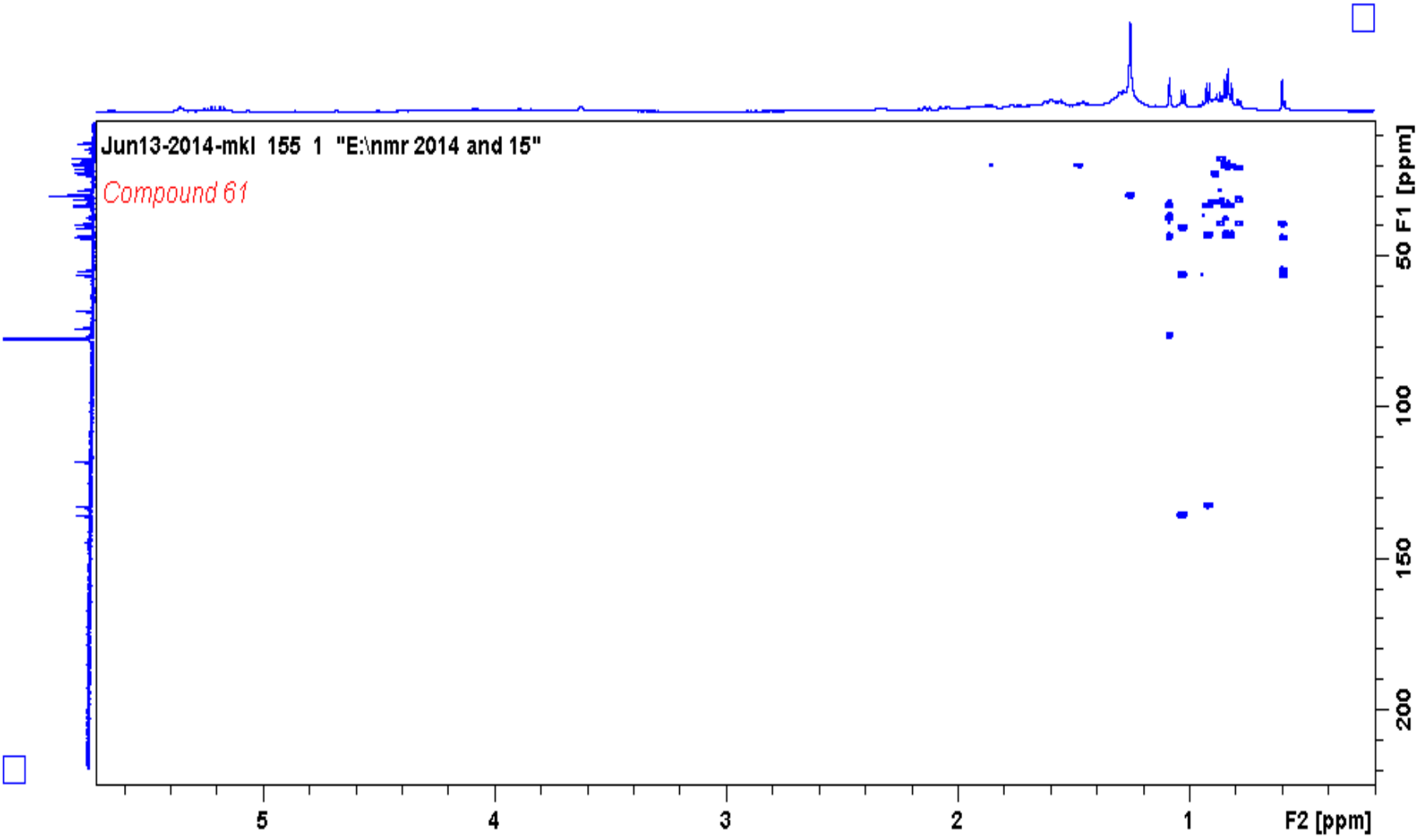


^{13} C NMR spectrum for compound 60 in $CDCl_3$



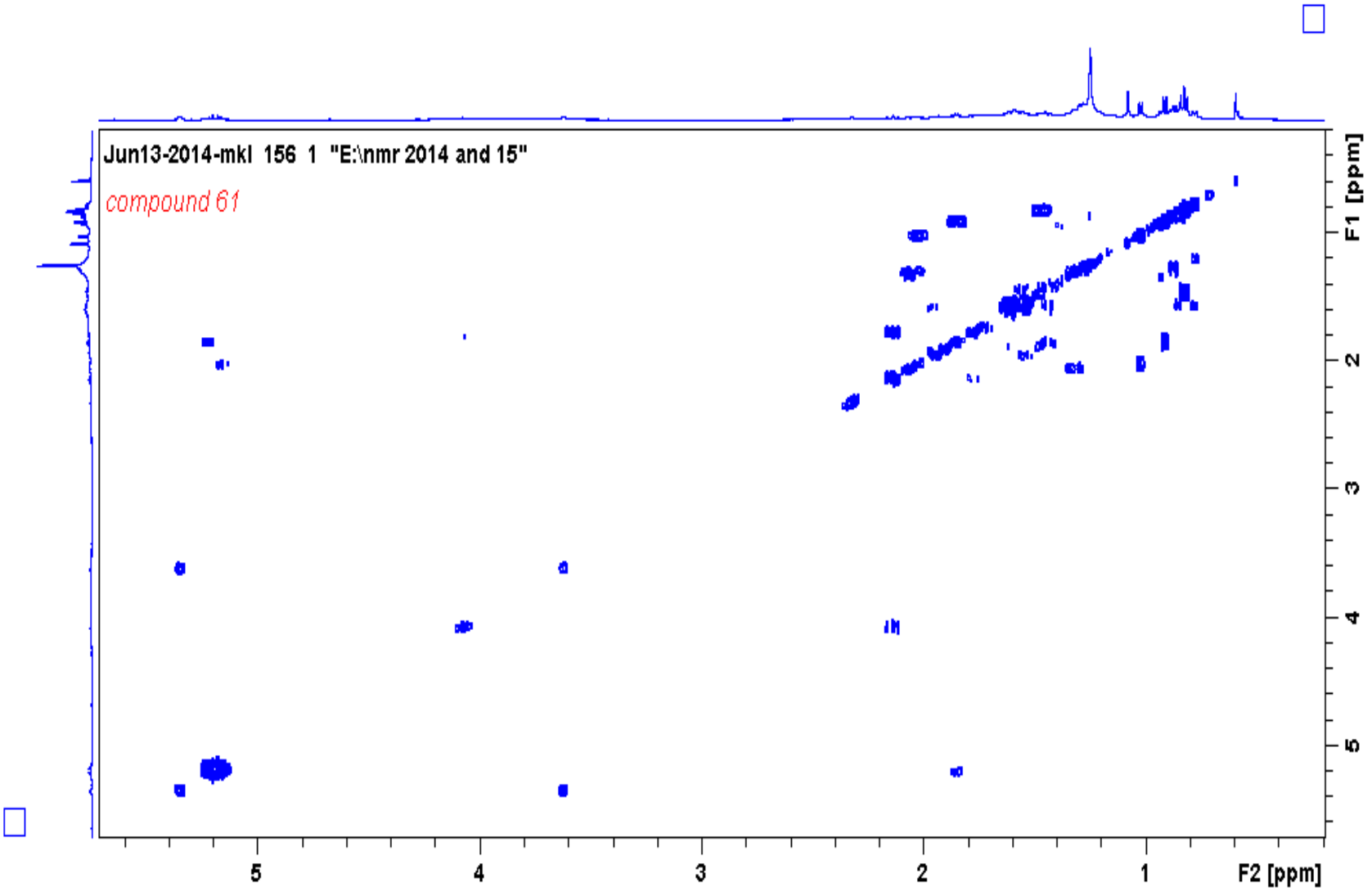
HSQC spectrum for compound 60

A40



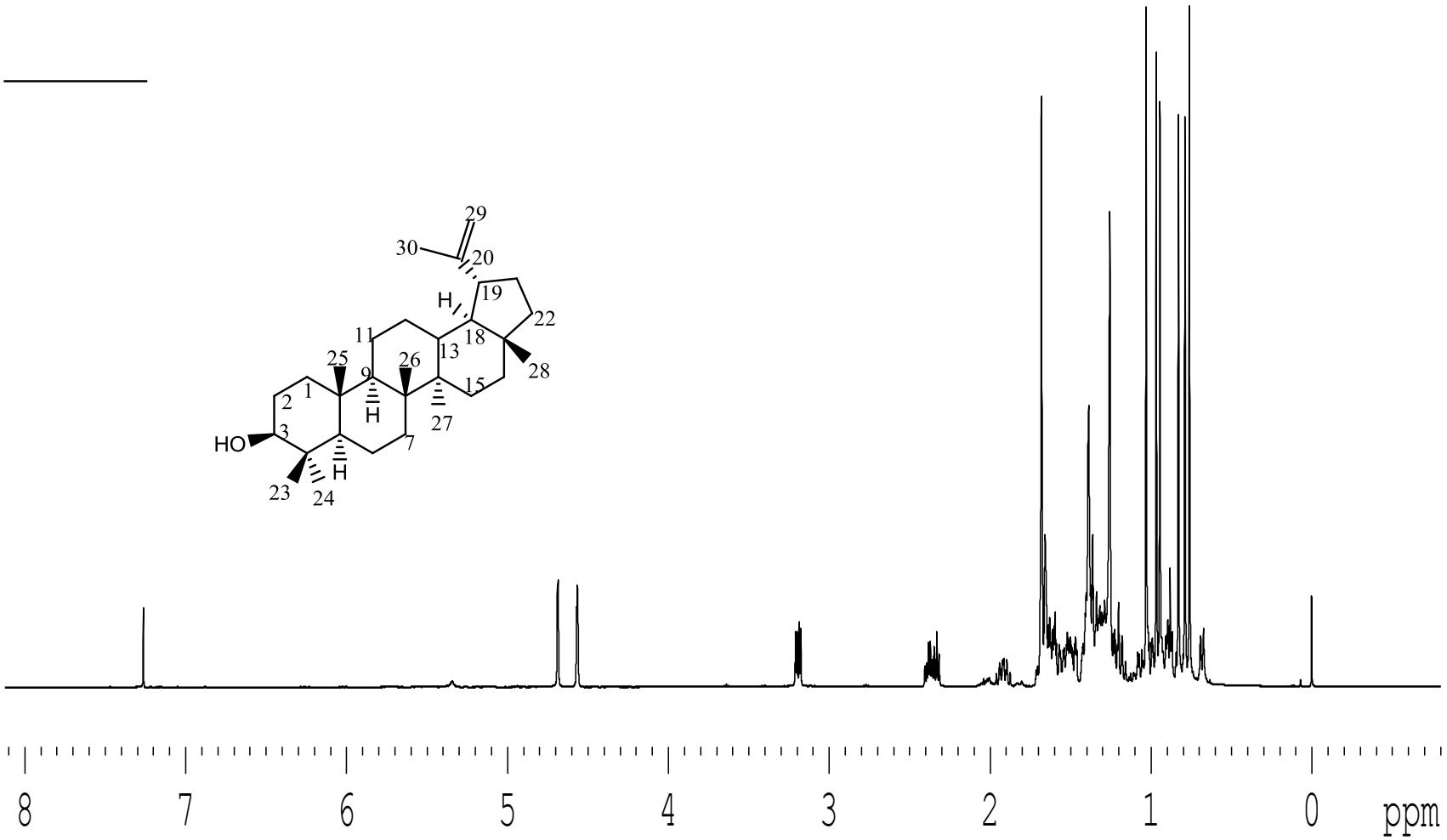
HMBC Spectrum for compound 60

A41

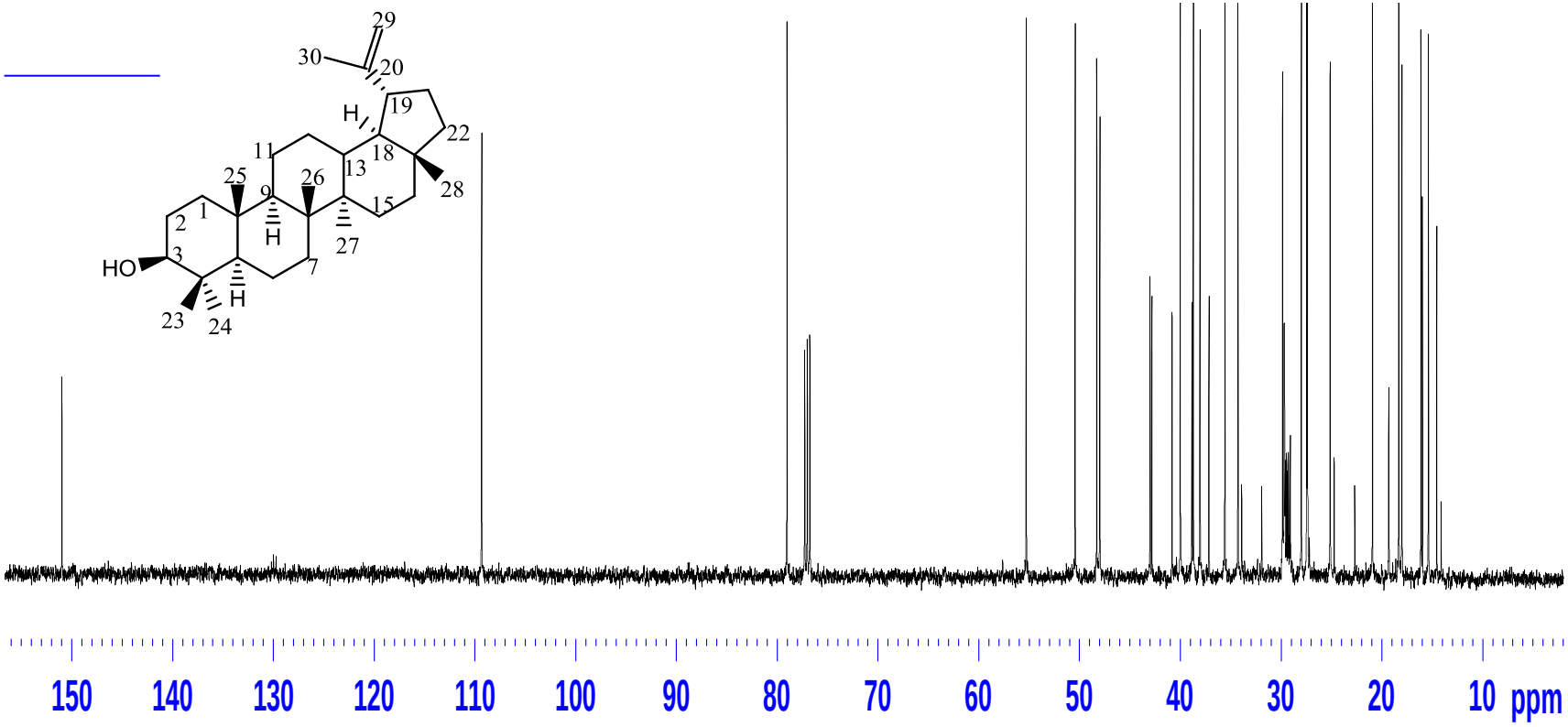


COSY spectrum for compound 60

A42

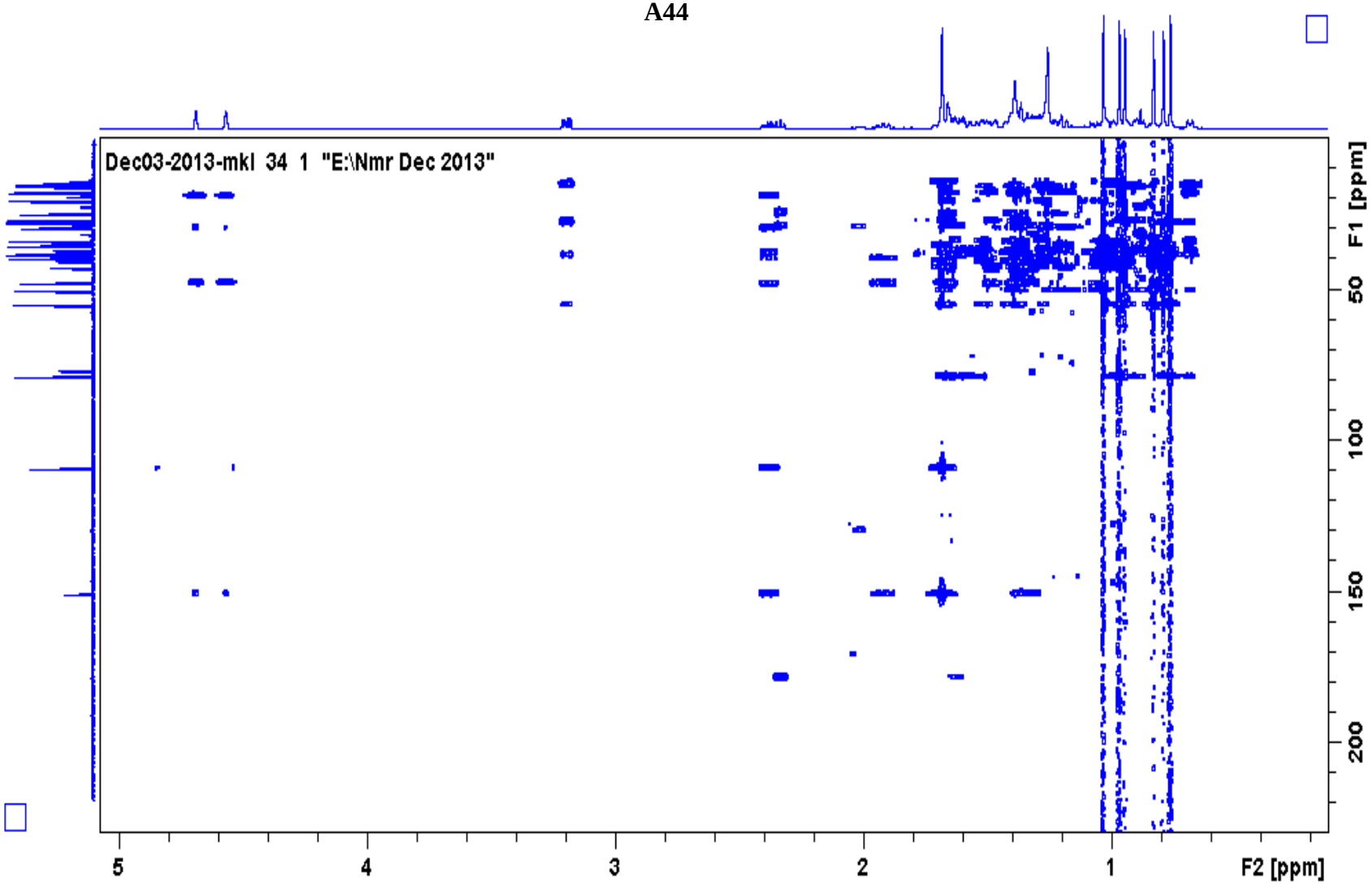


^{1} H NMR spectrum for compound 61 in $CDCl_3$



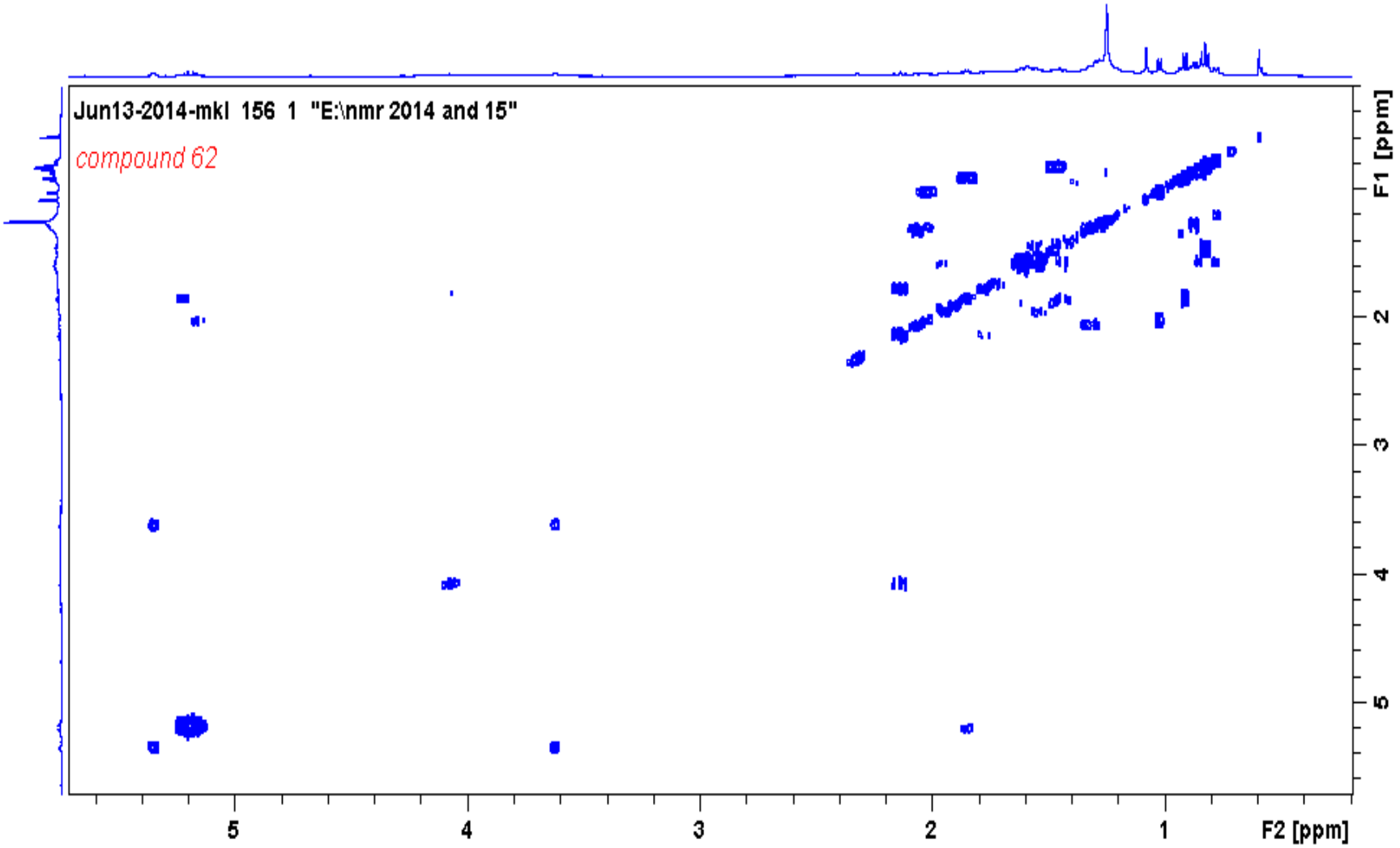
^{13} C NMR spectrum for compound 61 in $CDCl_3$

A44

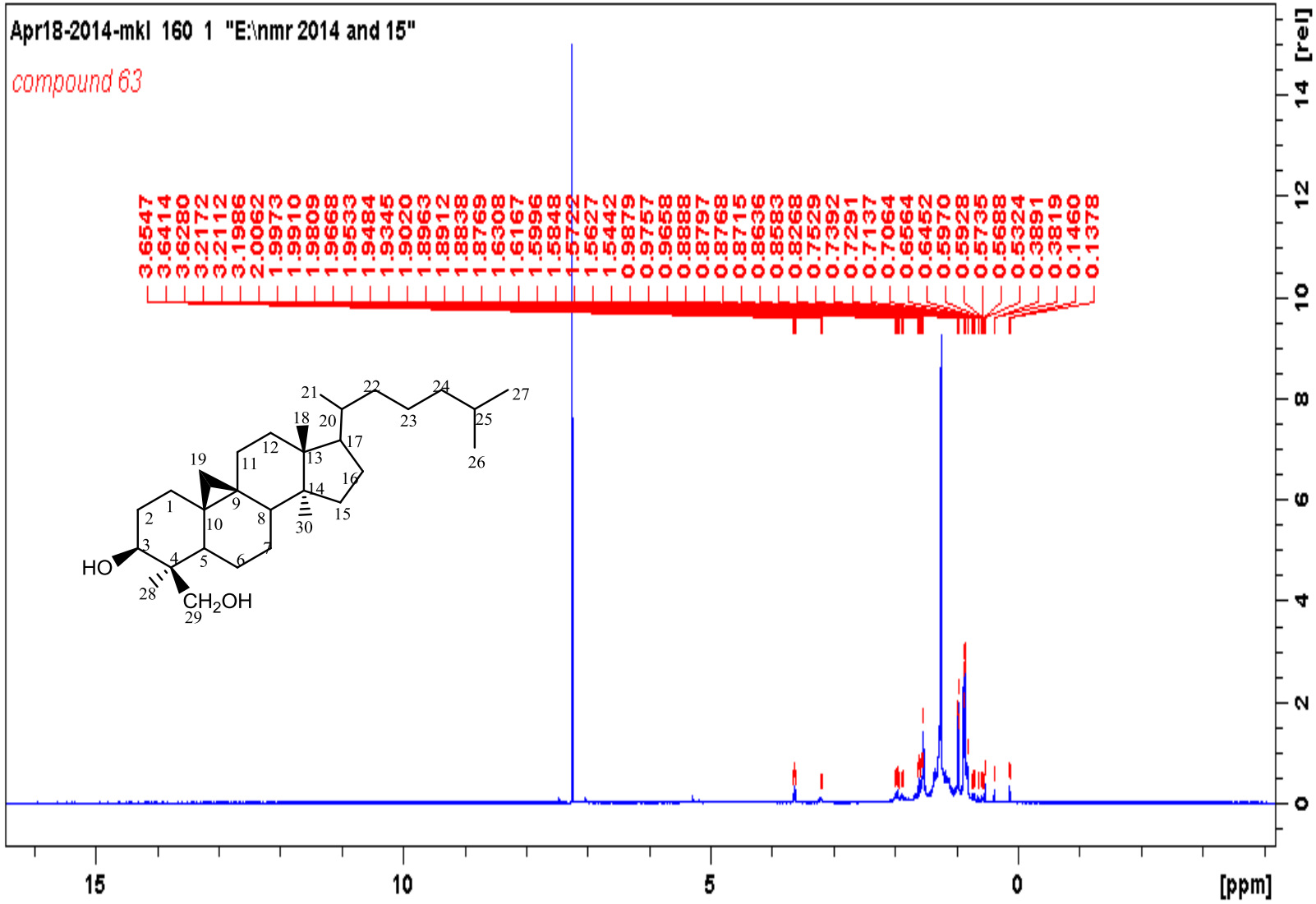


HMBC Spectrum for compound 61

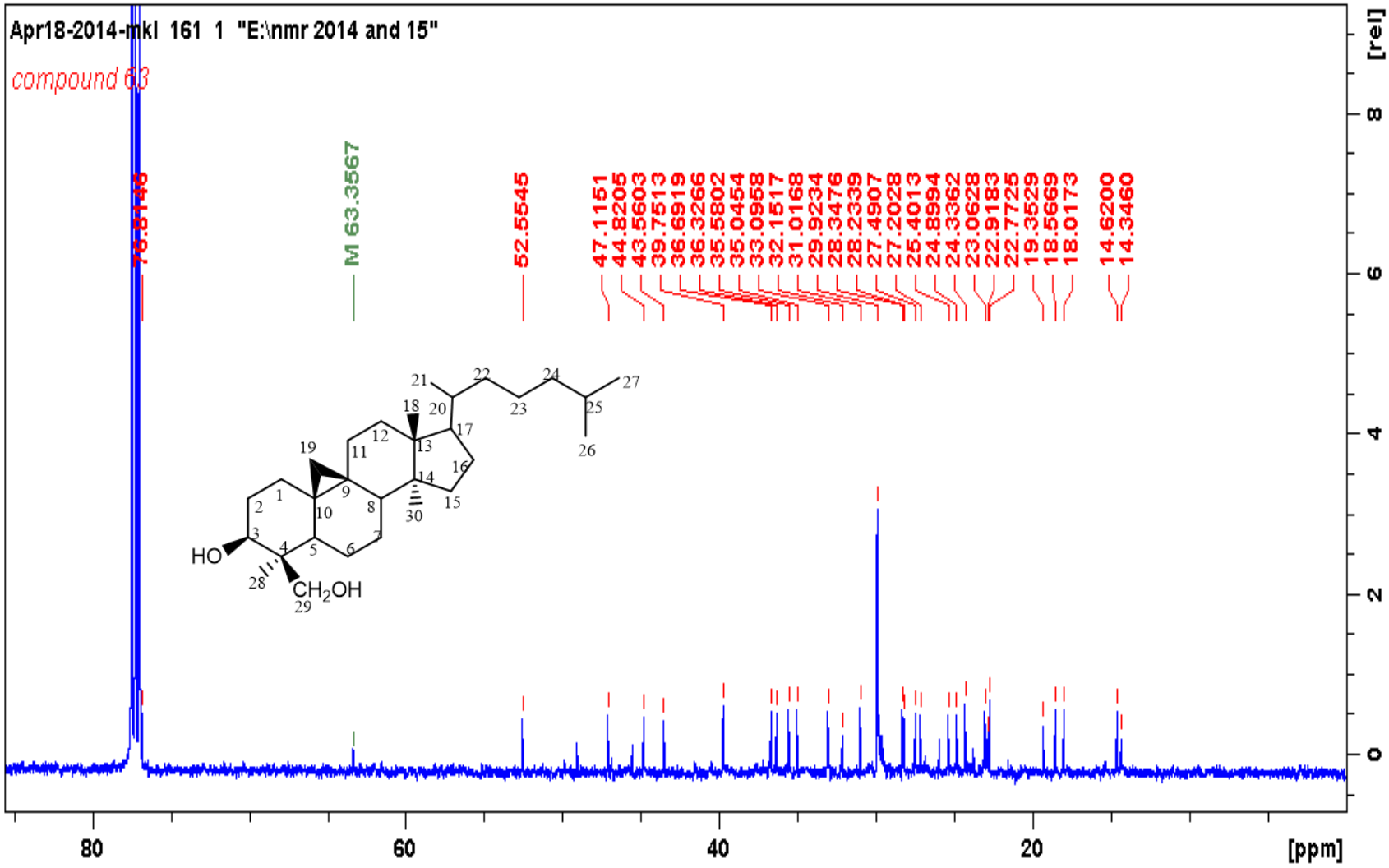
A45



COSY spectrum for compound 62

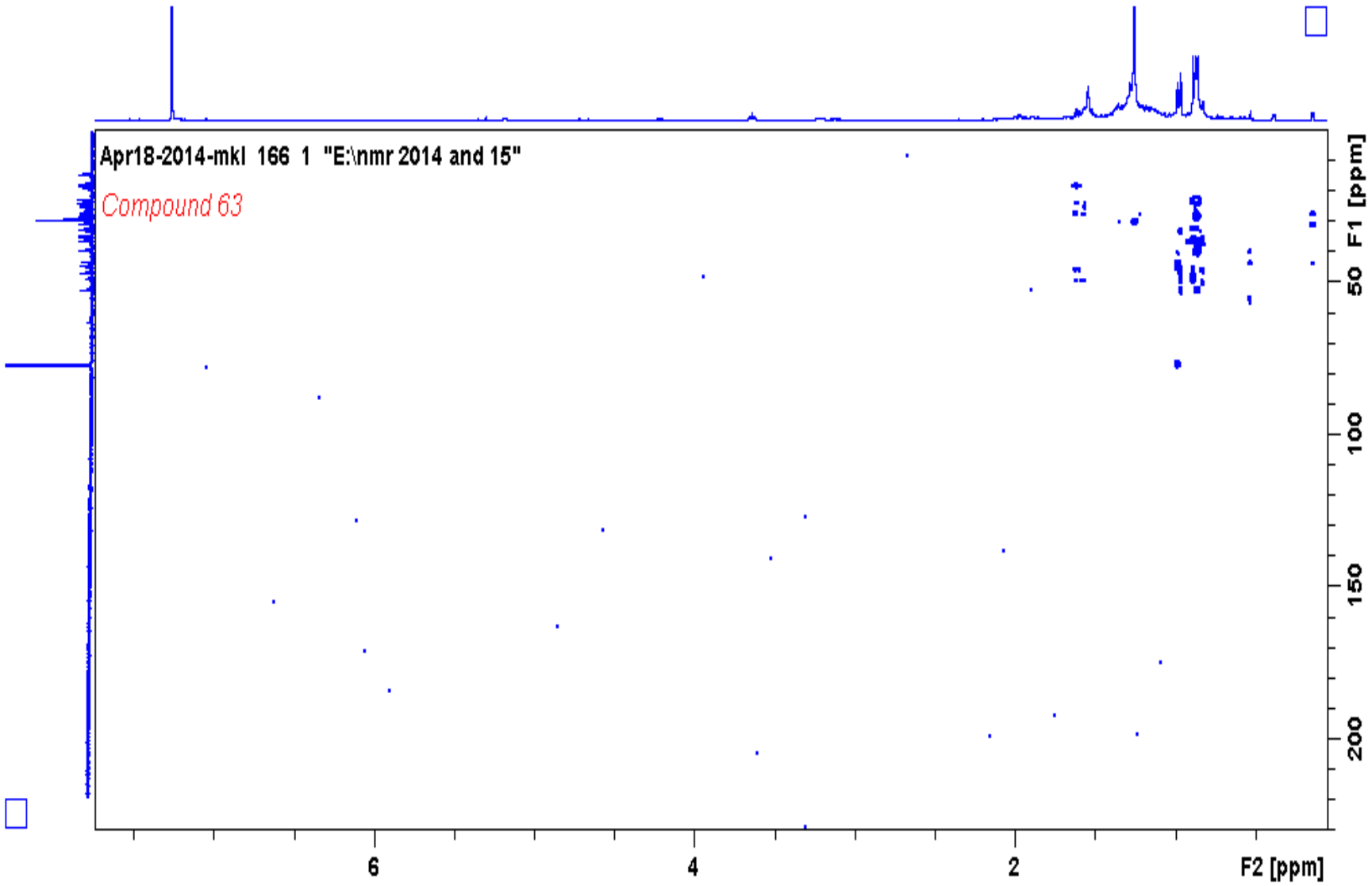


H NMR of compound 62 in $CDCl_3$



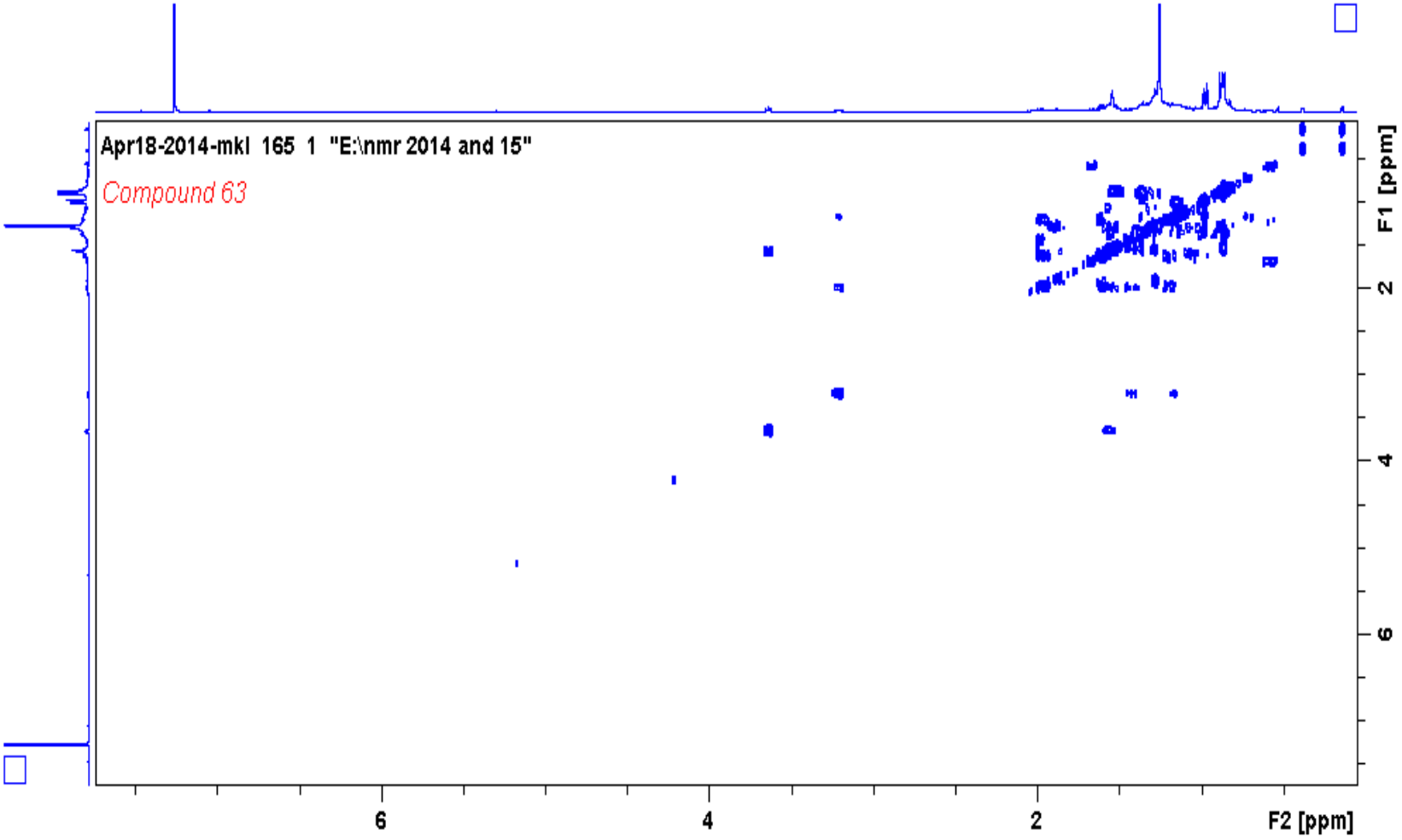
[3] C NMR spectrum for compound 62 in $CDCl_3$

A48



HMBC Spectrum for compound 62

A49



COSY spectrum for compound 62

Appendix Ⅱ: Abstract Page of Publication

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

Int. J. Biol. Chem. Sci. 13(4): 2352-2359, August 2019

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 compounds from the Kenyan polypore *Trametes elegans* (Spreng:Fr.) Fr (Polyporaceae) and their antimicrobial activity

Regina Kemunto MAYAKA[1], Moses Kiprotich LANGAT[2], Alice Wanjiku NJUE[1], Peter Kiplagat CHEPLOGOI[1] and Josiah Ouma OMOLO[1*]

[1]*Department of Chemistry, Egerton University, P.O Box 536-20115 Njoro, Kenya.*
[2]*Natural Product Chemistry in the Chemical Ecology and In Vitro Group at the Jodrell Laboratory, Kew, Richmond, UK.*
[*]*Corresponding author; E-mail: jomolo@egerton.ac.ke.*

ACKNOWLEDGEMENTS

The authors are grateful to the Kenya National Research Fund (NRF)-NACOSTI for the financial assistance for the present work.

ABSTRACT

Over the years, natural products have been used by humans in tackling infectious bacteria and fungi. Higher fungi have potential of containing natural product agents for various diseases. The aim of the study was to characterise the antimicrobial compounds from the polypore *Trametes elegans*. The dried, ground fruiting bodies of *T. elegans* were extracted with methanol and solvent removed in a rotary evaporator. The extract was suspended in distilled water, then partitioned using ethyl acetate solvent to obtain an ethyl acetate extract. The extract was fractionated and purified using column chromatographic method and further purification on sephadex LH20. The chemical structures were determined on the basis of NMR spectroscopic data from ^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H-^{1}H COSY, and NOESY experiments. Antimicrobial activity against clinically important bacterial and fungal strains was assessed and zones of inhibition were recorded. The polypore yielded six known compounds namely ergosta-5,7,22 trien-3-ol (**1**) 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol (**2**), *5α,8α*–epidioxyergosta-6,22-dien-3β-ol (**3**), ergosta-7,22-dien-3β,5α,6β-triol (**4**), Lupeol (**5**) and 9,19-cycloartane-3,30-diol (**6**). From this study, the isolated compounds of *T. elegans* displayed varying antimicrobial activities with zones of inhibition ranging from 8.0±0.58 to 9.7± 0.33 mm at ($p \leq 0.05$). Thus, *Trametes elegans*, could be considered as a potential source of natural antimicrobials.

Keywords: Higher fungi, triterpenoids, disc diffusion assay.

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

Int. J. Biol. Chem. Sci. 13(7): 3390-3397, December 2019

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

Antimicrobial compounds from the Kenyan *Ganoderma adspersum* (Schulz.) Donk species

Regina Kemunto MAYAKA[1], Alice Wanjiku NJUE[1] Moses Kiprotich LANGAT[2] Peter Kiplagat CHEPLOGOI[1] and Josiah Ouma OMOLO[1*]

[1] *Department of Chemistry, Egerton University, P.O Box 536-20115 Njoro, Kenya.*
[2] *Natural Product Chemistry in the Chemical Ecology and In Vitro Group at the Jodrell Laboratory, Kew, Richmond, UK.*
[*] *Corresponding author; E-mail: jomolo@egerton.ac.ke; Tel: +254 722 488821*

ABSTRACT

The emergence of antibiotic resistant pathogens has continuously increased, leading to a growing worldwide health threat due to infectious diseases. And therefore in our search for antibacterial and antifungal compounds from the polypore *Ganoderma adspersum*, the dried, ground fruiting bodies of *G. adspersum* were extracted with methanol and solvent removed in a rotary evaporator. The extract was suspended in distilled water, then partitioned using ethyl acetate solvent to obtain an ethyl acetate extract. The extract was fractionated and purified using column chromatographic method and further purification on sephadex LH20. The chemical structures were determined on the basis of NMR spectroscopic data from ^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H-^{1}H COSY, and NOESY experiments. Antimicrobial activity against clinically important bacterial and fungal strains was assessed and zones of inhibition were recorded. Compound (**1**), ergosta-7,22-dien-3-one weakly inhibited the growth of Gram positive bacteria *Streptococcus pneumonia* and a fungus *Cryptococcus neoformans*. Compounds ergosta-7,22-dien-3-ol (**2**) and ergosta-5,7,22-trien-3-ol (**3**) also inhibited gram positive *Streptococcus pyogenes* bacteria.

Keywords: Polypores, steroid compounds, antimicrobial activity.

Appendix Ⅲ: Research Permit