UNIVERSITY OF NAIROBI

PHYTOCHEMISTRY AND BIOACTIVITY INVESTIGATIONS OF THREE KENYAN *CROTON* SPECIES

BY

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I80 / 80060 / 08

A Thesis Submitted in Fulfilment of the Requirement for the Award of Doctor of Philosophy Degree in Chemistry at the Department of Chemistry, University of Nairobi

2014

DECLARATION

This is original work by the author except where reference is made. It has never been submitted anywhere for award of any degree or diploma.

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I dedicate this work to my children (Evans, Lewis and Michelle Mbithi).

ACKNOWLEDGEMENTS

To God be the glory for the grace that has enabled me complete this training. The same grace had many individual persons and institutions contribute immensely to the validation of my dream of presenting this thesis report. First and foremost are my supervisors Prof. Jacob O. Midiwo, Dr. Moses K. Langat and Dr. Leonidah Kerubo Omosa. I will forever be grateful for your patience as you guided, supported and encouraged me through the challenges of research and academic work. Prof. Dulcie Mulholland (Surrey University-UK), thank you for giving me the chance to learn in your research laboratory under your commendably insightfull and well co-ordinated supervision.

To all my colluagues in the Department of Chemistry-University of Nairobi, thank you for the concern which you never kept hidden and willingness to help whenever needed. Prof. Abiy Yenesew and the entire Natural Products Research group, your enthusiasm and accomplishments in research are a motivator worth emulating. Prof. Amir Yusuf (Chairman, Department of Chemistry- University of Nairobi), thank you for supporting me when I needed time away to go and gain more experience and knowledge that saw the completion of this work. The University of Nairobi through the Office of the DVC (Administration and Finance), International Foundation of Science (IFS), Organization of Prevention of Chemical Weapons (OPCW) and Internationanal Science Program (ISP-Sweden) through KEN 02 are acknowledged for financial support. Natural Products Research network for East and Central Africa (NAPRECA) and Pan African Chemistry Network (PACN) are acknowledged for training sponsorships. Prof. Illias Muhammad (School of Pharmacy- University of Mississipi), Dr. Christine Bii (KEMRI) and Prof. S.P. Dhanabal (Principal- JSS college of Pharmacy, Ooty-Tamil Nadu, India) are acknowledged for collaborative work.

Dr Linda Langat, your good nature supplemented by Kenzo boosted the tutorials Moses was giving me. Prof. Angela De Namor and my colleagues at Surrey University-UK, Dr. Francis Machumi (Muhimbili Research Center- Tanzania), Mr Partick Mutiso (Taxonomist), Theophillus Mbithi (my invaluable “co-parenter”), Antonia Mwikali (my personal assistant), Dr. Vincent Bagire and Dr. Levi Kabagambe (Makerere University, Bussiness school), Emily Sumbeiywo and Dr. Pius Kigamwa (University of Nairobi Health Services) and my children (Evans, Lewis and Michelle Mbithi), I appreciate the special roles that each one of you played in my life over the study period.

ABSTRACT

Three Kenyan *Croton* species, *C. megalocarpoides* Friis and Gilbert, *C. alienus* Pax and *C. sylvaticus* Hochst were investigated for their phytochemistry and biological activity relevancies. Anti-microbial activity evaluation was done on aqueous and methanol crude plant extracts to enable selection of most active parts. Documented procedures were used to profile the selected extracts for their phytochemical concentrations followed by fractionation using column chromatography. The phytochemicals obtained were identified using NMR spectroscopic techniques and subjected to various biological activity tests. Forty one compounds (fifteen of them new) were isolated. *C. megalocarpoides* roots produced twenty diterpenoids belonging to, *ent*-clerodane (thirteen, twelve new), abietane (three, one new) and *ent*-trachylobane (four known) series. Two known triterpenoids (lupeol and acetyl aleuritolic acid) and common phytosterols (stigmasterol and sitosterol) were also isolated. Two novel compounds (alienusolin, a 4α-deoxyphorbol ester and crotonimide C, a glutarimide alkaloid derivative) and nine known compounds (an alkaloid, six methylcyclohexane derivatives of crotepoxide, a triterpenoid and a phytosterol were isolated from *C. alienus* leaves and roots. From *C. sylvaticus* roots, seven diterpenoids belonging to clerodane (four, one new), halimane (two known) and labdane (one known) series and a phytosterol were isolated.

Anti-microbial activity tests were done using different strains of bacteria and fungi. *Candida albicans* was the most susceptible micro-organism to the crude plant extracts. *C. alienus* and *C. sylvaticus* (root and stem bark aqueous extracts) were active at the lowest concentration tested (25 mg / mL). *C. sylvaticus* stem bark (methanol extract) was the only crude extract that inhibited the growth of a bacteria strain (*Bacillus subtillis*) at a concentration of 10 mg / mL. The compounds that were isolated and assayed from *C. alienus* and *C. megalocarpoides* were inactive to all microorganisms used (IC_{50} > 20µg / mL). *C. alienus* leaves (MeOH: DCM, 1:1 *v / v* extract) is the only crude extract that showed activity against *Leishmania donovanii* (IC_{50} = 80µg / mL). The compounds isolated from it were however inactive against the same, *L. donovanii* (IC_{50} and IC_{90} > 40µg / mL). All the crude extracts and compounds isolated and tested from *C. alienus* and *C. megalocarpoides* were inactive against D6 and W2 strains of *Plasmodium falciparum* (IC_{50} > 4760 ng / mL); VERO (IC_{50} > 4760 ng / mL) and *Aedes aegypti* and *Anopheles gambiae* larvae (LC_{50} and LC_{95} >100 ppm). The methanol extract of *C. megalocarpoides* and *C. sylvaticus* stem barks had a low total phenolic content (1.89 $\pm$ 0.02% -1.14 $\pm$ 0.01% *w / w* equivalent of gallic acid) and anti-oxidant activity (IC_{50} > 1000 µg / mL compared to ascorbic acid, IC_{50} = 9.51 $\pm$ 0.22 µg/mL).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF APPENDICES

LIST OF ABREVIATIONS AND ACRONYMS

ACT Artemisinin based Combination Therapies

CC Column Chromatography

CD Circular Dichroism

COSY Correlation Spectroscopy

DBE Double Bond Equivalence

DCM Dichloromethane

DDT Dichlorodiphenyltrichloroethane

DEPT Distortionless Enhancement by Polarization Transfer

DMSO Dimethylsulfoxide

DPPH 2, 2-Diphenyl-1-picrylhydrazyl

ED_{50} Effective Dose-50: Amount of material required to produce a specified effect on 50% of test animal

EI MS Electron Impact Mass Spectrometry

FT-IR Fourier Transform Infrared Spectroscopy

GPR General Purpose Reagent

HERPES *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid

HIV Human Immunodeficiency Virus

HMBC Heteronuclear Multiple Bond Correlation

HPLC High Performance Liquid Chromatography

HR-EIMS High Resolution Electron Impact Mass Spectrometry

HSQC Heteronuclear Single Quantum Correlation

IC_{50} Inhibition Concentration-50: Concentration of substance that produce 50% inhibition of certain process

IUCN International Union of Conservation of Nature and natural resources

IR Infrared

KEMRI Kenya Medical Research Institute

LC_{50} Lethal Concentration-50: Concentration that kills 50% of test animal

MIC Minimum Inhibition Concentation

MS Mass Spectrometry

NMR Nuclear Magnetic Resonance

NOESY Nuclear Overhauser effect Spectroscopy

***p*LDH** Plasmodium lactate dehydrogenase

PTLC Preparative Thin Layer Chromatography

TLC Thin Layer Chromatography

UV Ultra Violet

UV-VIS Ultra Violet-Visible

WHO World Health Organization

$\boldsymbol{\delta_H}$ Proton Chemical Shift in the Proton NMR spectra

$\boldsymbol{\delta_C}$ Carbon Chemical Shift in the Carbon NMR spectra

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Since time immemorial and in almost all cultures, man has relied on nature for basic needs such as food, shelter, clothing, transportation, fertilizers, flavours, fragrances and medicines (Cragg and Newman, 2005). This is attributed to availability of chemical diversity in animals, minerals and plants, plants parts being the major sources of empirical traditional medicine systems (Verpoorte *et al*., 2005). The medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations whose dosage was developed through experience and experimentation (Balick and Cox, 1997; Samuelsson, 2004). Due to development of separation Chemistry and pharmacological testing, the medicines are nowadays made of active compounds isolated from the plants, or their synthetic equivalents. Information on the specific plants to be used for a particular ailment and the method of application was initially passed down by oral traditional mode but later became documented in herbal pharmacopoeias (Balunas and Kinghorn, 2005). These records are characterised by marked regional differences and healing practices that can be attributed to the rich biological and cultural diversity.

Despite unreliable reports on therapeutic properties attributed to some medicinal plant therapies, there is a lot of historical evidence to their dependability. Hundreds of clay tablets from as early as 2600 BC from Mesopotamia are some of the earliest documented evidence of nature being used as a medicine. The chemical structures of three popular and potent phytochemicals that have been in use since time immemorial have been given in Figure 1.1. Included is morphine (**1**), one of the most potent pain killers to date, reported to have been isolated from opium poppy. Others are oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) and *Papaver somniferum* (poppy juice) all of which are still in use today for management of ailments ranging from coughs and colds to parasitic infections and inflammation (Newman *et al*., 2000; Butler and Buss 2009). Salicylic acid (**2**) was first reported by Hippocrates in the 5th century BC, describing it as a "bitter powder extracted from willow bark that could ease aches, pains and reduce fever" (Fryers, 1982). Ancient Egyptians used *Ammi majus* (Bishops weed) for treatment of vitiligo (a skin condition characterised by loss of pigmentation).

It is from this plant (Bishops weed), *β*-methoxypsoralen (**3**) (7H-furo[3,2-g]chromen-7-one or 7H-furo[3,2-g][1]benzopyran-7-one), a drug used to treat psoriasis and other skin disorders as well as T-cell lymphoma has recently been reported from (Staniszewska *et al.*, 2003; Beissert and Schwarz, 2002).

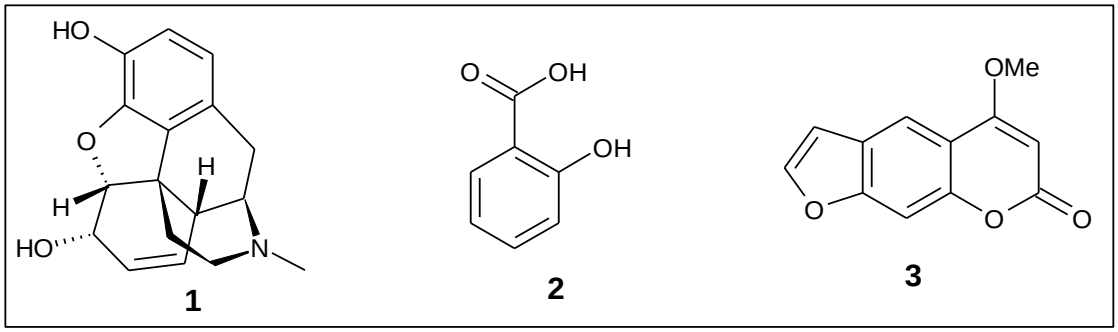


Figure 1.1: Chemical structure of some potent phytochemicals since ancient times

Over the centuries, the Chinese have extensively documented their herbal prescriptions for known illnesses in *Materia Medica*, first records dating back to 1100 BC (Butler and Buss, 2009). Some other well-known medicinal plants found around the globe are given in Table 1.1.

Table 1.1: Popular medicinal plants across the globe

Region	Botanical name (common name)
Africa	*Acacia senegal* (gum arabic), *Agathosma betulina* (buchu), *Aloe ferox* (Cape aloes), *Aloe vera* (North African origin), *Artemisia afra* (African wormwood), *Aspalanthus linearis* (rooibos tea), *Boswellia sacra* (frankincense), *Catha edulis* (khat), *Catharanthus roseus* (rosy periwinkle), *Commiphora myrrha* (myrrh), *Harpagophytum procumbens* (devil's claw), *Hibiscus sabdariffa* (hibiscus, roselle), *Hypoxis hemerocallidea* (African potato), *Prunus africana* (African cherry) (Newman *et al.*, 2000; Neuwinger, 2000).
Australia and South-East Asia	*Croton tiglium* (purging croton), *Duboisia hopwoodii* (pituri), *Eucalyptus globulus* (bluegum), *Melaleuca alternifolia* (tea tree), *Myristica fragrans* (nutmeg or mace), *Piper methysticum* (kava kava), *Strychnos nux-vomica* (strychnine), *Styrax benzoin* (benzoin) and *Syzygium aromaticum* (cloves) (Maher, 1999; Kapoor, 1990; Newman, 2000; Gurib-Fakim, 2006).

Central and South America	*Cinchona pubescens* (Peruvian bark), *Erythroxylum coca* (coca), *Ilex paraguariensis* (mate), *Myroxylon balsamum* (tolu balsam), *Paullinia cupana* (guarana), *Peumus boldus* (boldo), *Psidium guajava* (guava), *Spilanthes acmella* (Brazilian cress), *Tabebuia impetiginosa* (lapacho) and *Uncaria tomentosa* (cat's clow) (Fabricant and Farnsworth, 2001; Gurib-Fakim, 2006).
North America	*Echinacea purpurea* (Echinacea) and *Hydrastis canadensis* (Goldenseal) (Pieroni, 2000; Gurib-Fakim, 2006)
China	*Angelica polymorpha* var. *sinensis* (dang gui), *Artemisia annua* (qing hao), *Ephedra sinica* (ma huang), *Paeonia lactiflora* (bai shao yao), *Panax ginseng* (ren shen) and *Rheum palmatum* (da huang) (Magner, 1992; Padua de *et al*., 1999; Gurib-Fakim, 2006).
India	*Azandirachta indica* (neem), *Centella asiatica* (gotu kola), *Cinnamomum camphora* (camphor), *Elettaria cardamomum* (ela or cardamomum), *Rauwolfia serpentina* (Indian snake root), *Santalum album* (sandalwood), *Terminalia* species (myrobolan) and *Withania somnifera* (aswargandha) (Kapoor, 1990; Magner, 1992; Padua de, 1999; Gurib-Fakim, 2006).
Middle East and Egypt	*Allium cepa* (onion), *Astracantha gummifera* (tragacanth), *Carthamus tinctorius* (safflower), *Carum carvi* (caraway), *Ferula assafoetida* (asafoetida), *Lawsonia inermis* (henna), *Papaver somniferum* (opium poppy), *Peganum harmala* (syrian rue), *Prunus dulcis* (almond), *Punica granatum* (pomegranate), *Rosa damascene* (damask rose), *Ricinus communis* (castor oil plant), *Salvadora persica* (toothbrush tree), *Senna alexandrina* (senna), *Sesamum indicum* (sesame), *Trachyspermum ammi* (ajowan), *Trigonella foenum-graecum* (fenugreek) and *Vitis vinifera* (grape) (Padua de, 1999; Neuwinger, 2000; Gurib-Fakim, 2006).

1.1.1 Natural products and their place in modern drugs

Natural products and their derivatives represent more than 50% of all the drugs in clinical use with higher plants contributing about 25% to this number (Fransworth *et al.*, 1985; Cragg and Newman, 2005) and 11% of those considered basic and essential by WHO (Rates, 2001). A lot of other products of natural origin are used as tools in pharmacological, physiological and biochemical studies. Three of the major sources of anti-cancer drugs on the market or completing clinical trials are from North American plants used by the American natives against ovarian cancer (papaw, *Asimina* spp and western yew tree, *Taxus brevifolia*), leukemia, lymphoma lung and testicular cancer (mayapple, *Podophyllum peltatum*) (Gurib-Fakim, 2006).

Two other good anti-cancer agents are vincristine (**4**) and vinblastine (**5**) [Figure 1.2**]**, alkaloids reported from Rosy Periwinkle (*Catharanthus roseus*) formerly known as *Vinca rosea*, a Madagascan medicinal plant, used by the natives to treat diabetes and fever (Newman *et al*., 2000, Gurib-Fakim, 2006). Other notable medicinal plants in use in modern medicine include *Dioscorea* species (diosgenin) from which all anovulatory contraceptive agents have been derived; *Rauwolfia* species, a source of reserpine and other anti-hypertensive and tranquilizing alkaloids; a group of South American trees belonging to the *Pilocarpus* spp of the Citrus family from where pilocarpine that is used to treat glaucoma and "dry mouth" is derived; *Cassia* spp, a source of laxative agents and *Digitalis* spp., a source of cardiotonic agent that is used to treat heart failure (Newman *et al.*, 2000). There are many other indigenous botanical drugs whose active constituents have found their way into useful modern drugs summarised in Table 1.2; Figure 1.2 (Babu *et al*., 2003; Gurib-Fakim, 2006).

Table 1.2: Sources of some bio-active phytochemicals used in modern drugs

Botanical name (common name)	**Region of origin**	**Indigenous use**	**Biomedical uses**	**Bio-active phytochemicals** [Figure 1.2]
Adhatoda vasica	India, Sri Lanka	Antispasmodic, antiseptic, insecticide, fish poison	Antispasmodic, oxytocic, cough suppressant	Vasicine (**6**)

Artemisia annua L.	China	Treat fever	Anti-malarial	Artemisinin (**7a**) Artesunate (**7b**)*[1] Arteether (**7c**)* Artemether (**7d**)*
Cinchona succuriba	South America	Treat fever	Anti-malarial	Quinine (**8**)
Condrodendron tomentosum	Brazil, Peru	Arrow poison	Muscular relaxation	d-Tubocurarine (**9**)
Gingko biloba (Gingko)	Eastern China	Asthma, Anthelmintic (the fruit)	Dementia, cerebral deficiencies	Ginkgolides A-C, J, M (Five terpene trilactones **10**-**14**)
Harpagophytum procumbens (devil's claw)	Southern Africa	Fever, Inflammatory conditions	Pain, Rheumatism	Harpagoside (**15**), Caffeic acid (**16**)
Piper methysticum (Kava Kava)	Pacific Island	Ritual stimulant, Tonic	Anxiolytic, Mild stimulant	Kava pyrones (kavain (**17**); 7,8-dihydrokavain (**18**); methysticin (**19**); 7,8-dihydromethysticin (**20**); yangonin (**21**); desmethoxyyangonin (**22**)
Podophyllum peltatum (May apple)	North America	Laxative, Skin infections	Cancer chemotherapy, warts	Podophyllotoxin (**23**)
Silybum marianum (Milk thistle)	England	Liver diseases	Hepatic toxicity	Silibinin (**24**)
Mentha arvensis	Central Asia	Digestive problems, gall bladder and coughs	Coughs, sore throats, topical analgesis	Menthol (**25**)

1 * Artemisinin derivatives that are more effective anti-malarial drugs

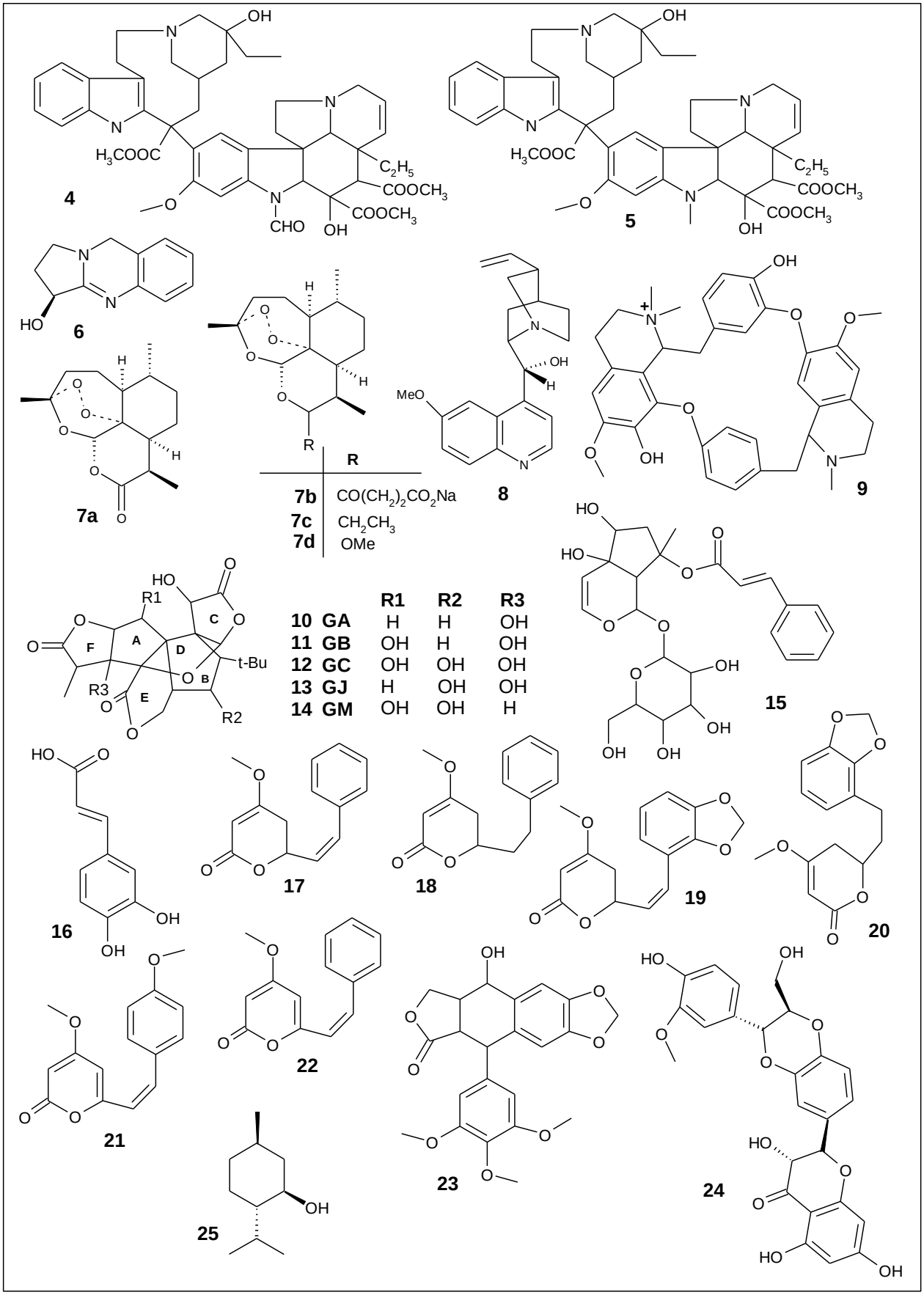


Figure 1.2: Chemical structures of some bio-active phytochemicals in modern drugs

1.1.2 Pharmacological activity screening of medicinal plants

There is enormous potential of finding phytochemicals with therapeutic properties from plants as evidenced by the various reports accessed. Due to the diversity of medical uses of plants, their development into drugs involves a multidisciplinary approach, one of them being biological screening of the extracts in pharmacologically relevant assays. An inclusive evaluation of plant species belonging to genera that are reputed for their medicinal value has been hailed as of great value in solving some of the challenges facing health care needs of mankind. This approach has led to a reservoir of potential chemotherapeutic agents and starting points for the development of new drugs from nature, the first step in the lengthy drug development process (Reichert 2003; Dickson and Gagnon, 2004).

Plant species belonging to *Croton* genus were investigated in this study. *Croton* is one of the largest genera of the Euphorbiaceae family, members of which are well known for producing compounds of diverse medicinal uses and toxicity (Caruzo *et al*., 2011; Berry *et al*., 2005). Microbial infections and parasitic diseases, some of whose successful therapy can be traced from natural sources account for 26.2% of the global causes of death, the vast majority being from developing countries (WHO, 2003). Based on the aforementioned, this study intended to evaluate the phytochemistry and bioactivity of the chosen Kenyan *Croton* species. The ultimate goal was to support potential formulations of new drugs that could help in management of microbial infections, malaria and neglected tropical diseases. In addition, it was the intention of the investigators to provide scientific data that would give credible support to their conservation and cultivation for medicinal value if found to have any.

1.1.3 Phytochemistry and biological activity reports on Kenyan *Croton* species

So far, only four of the fifteen Kenyan *Croton* species have had their phytochemistry reported. These are *C. dichogamus* Pax. (Jogia *et al*., 1989); *C. macrostachyus* Del., A. Rich (Kapingu *et al*., 2000); *C. megalocarpus* Hutch (Addae-Mensah *et al.,* 1989) and *C. sylvaticus* Hochst (leaves) (Mwangi *et al.,* 1998). This leaves us with scanty information about the ethno-pharmacological relevancies and chemical constituents of eleven Kenyan *Croton* species (*C. alienus*; *C. bonplandianus* Pax (Syn. *C. sparsiflorus*)-Originally a South American (Argentina) species which is now a common weed in Kenya; *C. megalocarpoides* Pax; *C. menyhartii* Pax.; *C. polytrichus* Pax.; *C. pseudopulchellus* Pax.; *C. talaeporos* Radc-Smith.; *C. scheffleri* Pax.; *C. somalensis* Vatke and Pax and *C. zambesicus* Mull.Arg).

This knowledge gap, backed by observed folkloric uses of the family Euphorbiaceae justified this study on the phytochemistry and pharmacological relevancies of three of the Kenyan *Croton* species, *C. alienus* Pax. *C. megalocarpoides* Friis and Gilbert and *C. sylvaticus* Hochst (Krauss).

C. alienus is a moderate sized tree that is threatened with extinction and is endemic to central Kenya (IUCN, 1993). It is distributed in the humid, evergreen mountainous regions near Nairobi, often found in association with *Brachylaena hutchinsii* Hutch and *C. megalocarpus* Hutch (Beentje, 1994). Its leaves are silvery-white shiny on the underside, turning orange-red with age and its flowers are greenish white [Figure 1.3]. Literature reviewed gave only one ethno-medicinal use of *C. alienus* (treatment of body weakness) (Gachathi, 2007) and isolation of only one compound, crotepoxide (Chhabra *et al*., 2007).

Figure 1.3: *Croton alienus* plant[2] and twigs

C. megalocarpoides is a monoecious shrub or tree, growing up to 8 meters tall in rocky places of semi-evergreen coastal bush land or forest of Kenya and South Somalia (Beentje, 1994). Just like *C. alienus*, this plant is listed by IUCN among plant species that are threatened with extinction (IUCN, 1993). Its taxonomic relationship with other African *Croton* species is demonstrated by its semblance to *C. megalocarpus* (a plant it has often been confused with), *C. mayumbensis* and *C. mubango* by possession of grey scaly bark, silvery beneath leaves and *tri*-lobed fruits [Figure 1.4]. No ethno-medicinal use and / or phytochemical report were accessed by the investigators by the commencement of this study.

[2] *C.alienus* plant in its natural habitation at Ngong forest in Nairobi City County

Figure 1.4: *Croton megalocarpoides* plant and fruits

C. sylvaticus is a plant found mainly in Africa at an altitude of 350-1750 m, spreading from Ethiopia in the North to the Eastern Cape in South Africa, more widely in Gabon to Angola (Venter and Venter, 1996). In Kenya, it is found in the Coastal regions (Kokwaro, 1993; Kokwaro, 2009; Beentje, 1994). *C. sylvaticus* tree is monoecious, growing up to 30 meters tall with a dense spreading crown, bole straight up to 1 meter in diameter and bark smelling of black pepper. Its leaves are broadly ovate and flowers are greenish-cream producing orange or red *tri*-lobed fruits [Figure 1.5].

Figure 1.5: *Croton sylvaticus* flowering buds and fruits

In Kenya, *C. sylvaticus* is used in ethno-medicine as a wash for body swellings caused by kwashiorkor and purgative (leaves), oral remedy for tuberculosis (stem bark) and poultices for swellings (roots) (Kokwaro, 1993 and 2009). Other reports on its ethno-medicinal uses in various regions in Africa include: - treatment of gall-sickness in cattle; abdominal pains; indigestion; pleurisy; rheumatism; chest pains; inflammation; malaria and fish poison(Watt, 1962; Neuwinger, 1996; Neuwinger, 2000; Neuwinger, 2004; Beentje, 1994).

Water and methanol extracts of *C. sylvaticus* (unspecified part, concentration and species locality) are reported to have exhibited very promising 5-lipoxygenase inhibitory activity (Frum and Viljoen, 2005). Another report indicated absence of anti-microbial activity at 500 µg / mL by the stem bark extract of the Eastern Africa species (Taniguchi and Kubo, 1993). Reported phytochemical constituents of *C. sylvaticus* are given in Figure 1.6 and include toxalbumin crotin (**32**), a glycoprotein molecule attached to crotin, a dihydrochalcone isolated from its roots (Watt, 1962).

Hydro-distillation of the leaves of the Eastern Africa *C. sylvaticus* species showed presence of over fifty-two components (Mwangi *et al.,* 1998), a few of which were isolated and characterized [sitosterol; caryophyllene oxide (**33**); α-humulen-1,2-epoxide (**34**); penduliflaworosin (**35**); hardwickic acid (**36**); lupeol (**37**); stigmasterol (**38**) and julocrotine (**39**)]. Fourteen phytochemicals were isolated from the stem bark and leaves of the Southern Africa *C. sylvaticus* species (Langat, 2009) [a phytosterol, sitosterol; one acyclic diterpenoid, *trans*-phytol (**40**); three *trans*-*ent*-clerodane diterpenoids [15, 16 – dihydroxy-*trans*-*ent*-cleroda-3, 13-diene (**41**), 15-acetoxy-2-oxo-*trans*-*ent*-cleroda-3,13- diene (**42**) and t*rans*-annonene (**43**)]; two *trans*-clerodane diterpenoid [15-acetoxy-*trans*-cleroda-3, 13-diene (**44**) and 15-hydroxy-*trans*-cleroda-3, 13-dien-15-ol (**45**)]; one *trans*-*ent*-clerodane nor diterpenoid, 19-nor-clerodane, sylvaticinol (**46**); three triterpenoids [lupenone (**47**), 3*β*-acetoxylup-20(29)-ene (**48**) and *β*-amyrin (**49**)]; a *nor*-cyclo-farnesene sesquiterpenoid, (+) – [5*R*, 6*S*, 9R] - 4, 5 – dihydroblumenol A (**50**); a ferulate derivative, lignoceryl *trans* –ferulate (**51**) and a lignan, (+) – syringaresino (**52**)].

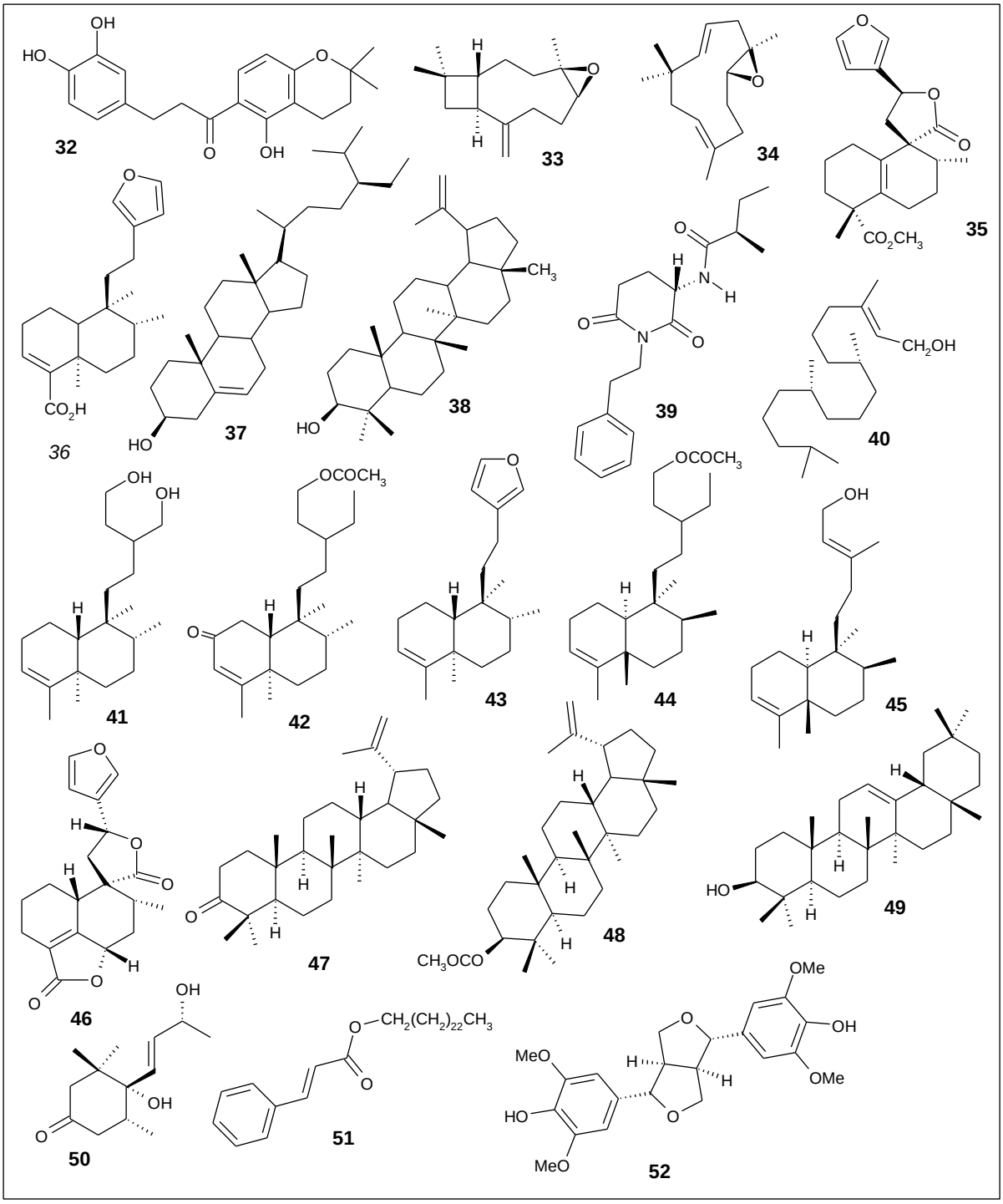


Figure 1.6: Compounds reported from Eastern and Southern Africa *Croton sylvaticus* species

1.2 Statement of the problem

Despite the tremendous progress made in medicine, diseases have continued to terrorise mankind and threaten human health for centuries in all ages, races and sexes. Bacterial, viral, protozoan, helminthic and fungal invaders are the major threats according to WHO (WHO, 2008). The burden is however felt more in developing countries due to poverty, unavailability of medicines and the emergence of widespread resistance of pathogens to the available drugs (Okeke *et al.,* 2005). The majority poor in developing countries still use natural products of plant origin based on accumulated explicit and implicit wealth of knowledge and belief in tribal medical systems (WHO, 2008). Other reasons adduced for the impressive use of native medical systems are social-cultural acceptability; ease of availability hence affordability; eco-friendliness and the belief that, being from natural origins, they are free from side effects. Despite all the arguments in support of herbal based drugs use, folk medicine will continue to be folk medicine unless they are scientifically validated to give their pharmacological-toxicological profiles (efficacy, safety of therapy and raw materials and interaction with other drugs).

In Africa alone, close to 50% of the population does not have access to essential medicines CFA (2005), yet, the continent is home to various plant species that are of medicinal value. Some of these African medicinal plants are endangered by extinction because of the rapid loss of their natural habitants due to uncontrolled human activities (IUCN, 1993). The impact of this loss cannot be under estimated because of the high endemism of some of the plant species in the African continent. There is therefore great need for urgent documentation of their phytochemistry and pharmacological values (Green and Sussman, 1990).

Considering the high diversity of the *Croton* genus (over 1300 species), the number studied for their ethno-pharmacological relevancies' are rather few. American and Asian *Croton* species lead in chemistry and pharmacology reports. Plaunotol, the active ingredient in a drug currently dispensed in most pharmacies and hospitals in the world for the treatment of peptic ulcer was isolated from an Asian *Croton* plant, *C. sublyratus* Kurz, later renamed, *C. stellatopilosus* H (Luzbetak *et al*., 1979). The same compound has been found to have anti-cancer properties (Kawai *et al*., 2005). The seeds of another Asian *Croton* plant, *C. tiglium* have been found to be a source of "Croton oil", established to be a tumor promoter (co-carcinogen) and anti-HIV-1 phorbol esters have also been isolated from it (El-Mekkawy *et al*., 2000).

1.3 General objective of the study

To investigate the phytochemistry and bioactivity potential of Kenyan *C. alienus, C. megalocarpoides* and *C. sylvaticus*.

1.3.1 Specific objectives of the study

1. To isolate phytochemicals from the selected *Croton* plants
2. To characterize the isolated phytochemicals from the selected *Croton* plants
3. To screen the crude plant extracts and isolated phytochemicals for *in vitro* anti-plasmodial, anti-bacterial, anti-fungal, anti-leishmanial and mosquito larvicidal activities
4. To assess the cytotoxicity of biologically active compounds

1.4 Justification of the study

At present, interest in herbal medicines is enjoying a renaissance with a seeming emergence of a new culture of "return to nature" among pharmaceutical companies and other stake holders. A positive, rational and non-prejudicial approach in scientifically evaluating the potential of reputed medicinal plants as chemotherapeutics is a more realistic response to global health burden. This was the driving force behind the serendipitous, random and multidisciplinary screening approaches that were used in this study. *Croton* plants have an historical application in folk medicine for management of a wide array of ailments with terpenoids, alkaloids and flavonoids being the major classes of phytochemicals reported from them (Salatino *et al*., 2007). Some of these compounds from *Croton* species and other sources have been found to be pharmacologically useful. Others have been used in studies as chemical models or templates for the design and total synthesis of new drug entities.

There are reports on therapeutic effects of *Croton* plants originally not described in the texts of traditional systems thus making them new chemical entities. Isolation of a large number of chemical compounds having toxic and inhibitory effects to the growth of micro-organisms from some of these plants has also been reported. Notable examples were the cytotoxic, antimycobacterial and antimalarial effects of secokaurane diterpenes of *C. kongensis*, the cytotoxicity of taspine, the hypolipidemic and hypoglycaemic effects of *C. urucurana* and the cytotoxicity of trachylobane diterpenes of *C. zambesicus* (Salatino *et al*., 2007). Over 70% of the *Croton* species reported in ethnomedicinal treatment of malaria and tested for anti-plasmodial activities were found to be active, an indication of the potential of these species in the fight against malaria. Included were *C. argyratus* (aerial parts, inactive; roots, active (Horgen *et al*., 2001)), *C. californicus* (leaves and stem, weakly active (Chavez *et al.,* 1982)) , *C. capitatus* (aerial parts, weakly active (Spencer *et al.,* 1947)), *C. geayi* (stem bark, active (Rasoanaivo, 1999)) , *C. guatemalensis* (stem bark, active (Franssen *et al*., 1997)), *C. hovarum* (leaves, active (Krebs and Ramiarantosa 1996 and 1997; Rasoanaivo *et al*., 1999), *C. lobatus* (entire plant, active (Attioua *et al.,* 2007)) , *C. leiophyllus* (roots, active (Horgen *et al*., 2001)), *C. tonkinensis* (entire plant, active (Be and Truong, 1991) and *C. urucurana* (entire plant, in active (Brandao *et al*., 1985)).

CHAPTER TWO
LITERATURE REVIEW

2.1 Background information on microbial infections and parasitic diseases

Since the isolation of penicillin by Alexander Flemings (1929) and its subsequent successful clinical application as anti-biotic, a number of penicillin derivatives with similar properties have been synthesised (Bahl and Bahl 2011). These derivatives [Figure 2.1] have the same skeletal structure (**26**) but differ in the character of the side chain, R. Other synthetic anti-biotics in current use include streptomycin (**27**), tetracyclin (**28**) and its 7-chloro derivative, aureomycin (**29**) and 5-hydroxy derivative, terramycin (**30**) (Bahl and Bahl 2011).

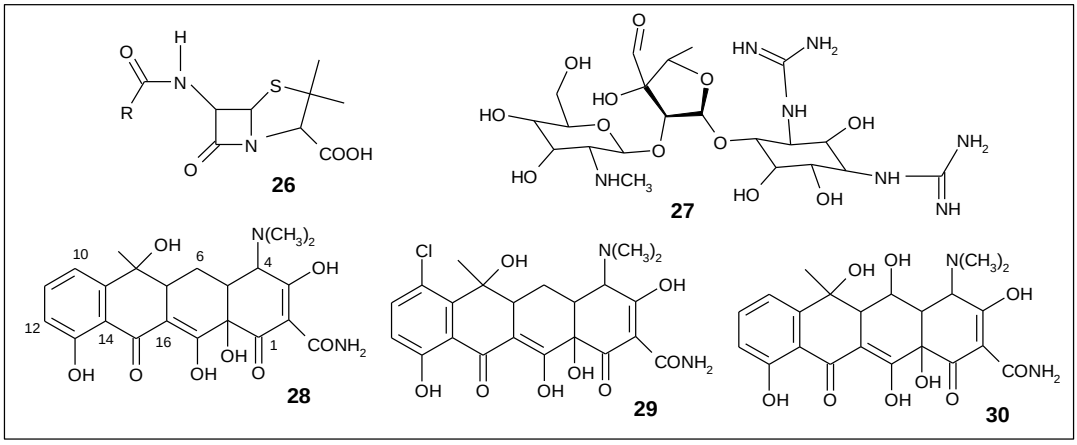


Figure 2.1: Structures of chemical constituents in commonly used anti-biotics

Parasitic infections cause a tremendous burden of disease in both the tropics and subtropics as well as in more temperate climates and developed countries, including the USA (CDC, 2013). The use of natural products for treatment of parasitic diseases is well documented, stemming from the fact that some natural products are biosynthesized as defence agents against plant pathogens (Kaur *et al*., 2009). Cutaneous leishmaniasis, the most common form of leishmaniasis is one of the severe neglected parasitic diseases. It is caused by a sandfly bite and manifests itself as a sore at the bite site that takes a few months to a year to heal, leaving a disfiguring (ugly) scar (MedicinNet, 2013; James *et al*., 2006; CDCa,b 2013;WHO, 2013) [Figure 2.2]. A number of drugs that are used to treat leishmaniasis include paromomycin (**31**), liposomal amphotericin B, ketoconazole and berberine (from a plant source, a *Berberis* species (Kumar, 1997)). Seeds of *Phytolacca maricana* are reported to produce antiviral proteins that are anti-leishmanial (Kokate, 2013).

Figure 2.2: Cutaneous leishmaniasis[3] and chemical constituent of paromomycin

Malaria is one of the most fatal parasitic diseases which despite continuous control measures continue to be a major concern in sub-Saharan Africa. About 40% of world population lives in areas at risk of malaria infection [Figure 2.3] with Africa bearing over 90% of the global disease burden (WHO, 2003; WHO, 2011; UNEP, 2001). WHO recommends integrated management of malaria and a scale up of prevention campaigns and / or measures. Quinine (**8**), isolated from *Cinchona succuriba* in 1820 was the first successful malaria drug therapy from a natural source but reports of toxicity associated with its use had the therapy change to sulfadoxine-pyrimethamine (SP) based drugs. The malaria parasites' development of resistance to the SP based drugs necessitated a change to the current first line treatment, the artemesinin-containing combination therapy (ACTs). The active ingredients in these ACTs are the artemisinin (**7a**) derivatives, artesunate (**7b**), arteether (**7c**) and artemether (**7d**). Artemisinin (**7a**) is a sesquiterpene lactone isolated from a Chinese herb, *Artemisia annua* L. (Asteraceae) that has activity comparable to that of quinine (WHO, 2003; Babu *et al*., 2003).

Vector control is reported to be the best preventive measures of malaria spread. WHO still recommends the use of DDT for indoor residual spraying (IRS) using "best application practices" until locally appropriate and cost-effective alternatives are availed for a suitable transition (WHO, 2011; UNEP, 2001).

[3] Cutaneous leishmaniasis in the hand of a Central American adult (Picture by CDC Dr. DS Martins (CDCa, 2013) and face of a Kenyan Child (Picture from Kenyan Nation Newspaper of 6th May 2014)

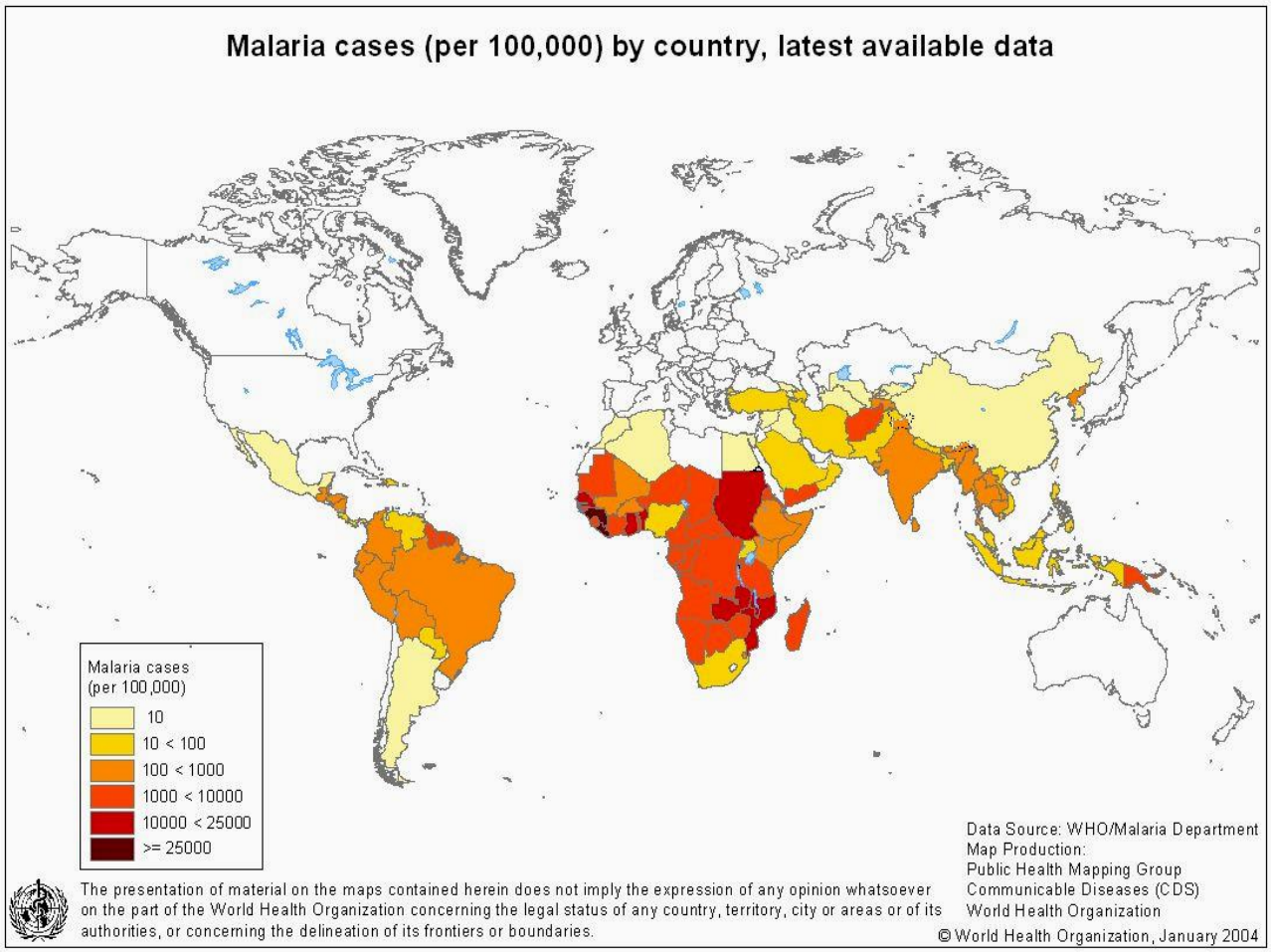


Figure 2.3: Global malaria distribution (WHO global atlas, 2005)

2.2 Botanical information on *Croton* genus

The name "Croton" is a Greek word referring to thick smooth seeds, a common feature of most *Croton* plants which belong to the Crotonoideae subfamily of the Euphorbiaceae family.

2.2.1 The Euphorbiaceae family

The Euphorbiaceae is a very large family with about 300 genera, comprising of 7,500 species that are distributed in its five sub-families which were originally Acalyphoideae, Crotonoideae, Euphorbioideae, Oldfieldioideae and Phyllanthoideae (Govaerts *et al*, 2000). The Phyllanthoideae subfamily has recently become the new family of Phyllanthaceae while the Oldfieldioideae has become Picrodendraceae family (Wurdack *et al*., 2005). Eight genera of Euphorbiaceae family have more than 100 species, making them significantly large (*Euphorbia* > 1600; *Croton* > 1300; *Acalypha* > 430; *Glochdion* > 280; *Macaranga* >240; *Manchot* >160; *Jatropha* >150 and *Tragia* >140).

Members of Euphorbiaceae family are well known in different parts of the world as toxic and / or medicinal which is a reflection of their high chemical diversity. The plants are characterized by the frequent occurrence of milky sap that is rich in secondary metabolites, mainly alkaloids and terpenoids (Palgrave, 1990 and 2002).

2.2.2 The *Croton* genus

Croton genus consists of over 1300 species of monoeceous and dioeceous trees, shrubs and herbs. Included are well known medicinal plants such as *C. tiglium*, *C. schiedeanus* and *C. zambesicus* (Caruzo *et al*., 2011; Berry *et al*., 2005).The plants usually have stellate hairs, rounded scales and flowers that are usually spikes or racemes with separate sexes on the same tree. The leaves are alternate, sometimes opposite, rarely whorled, simple and usually with two glands at the top of the petiole. Contact with some of these plants leaves can cause dermatitis. Their fruits occur as three lobed capsules while seeds of others are reported to be tumor promoters (Palgrave, 1990 and 2002; Mabberley, 2009).

2.2.3 Geographical distribution of *Croton* species

Croton plants are mainly found in the warm tropical regions and to some extent in the temperate regions of the Northern and Southern hemispheres. Tropical America, India and Africa are the major centers of distribution [Figure 2.4]. Extreme diversity is reported in Madagascar, West Indies and Southern Brazil (Caruzo *et al*., 2011; Berry *et al*., 2005; Mabberley, 2009).

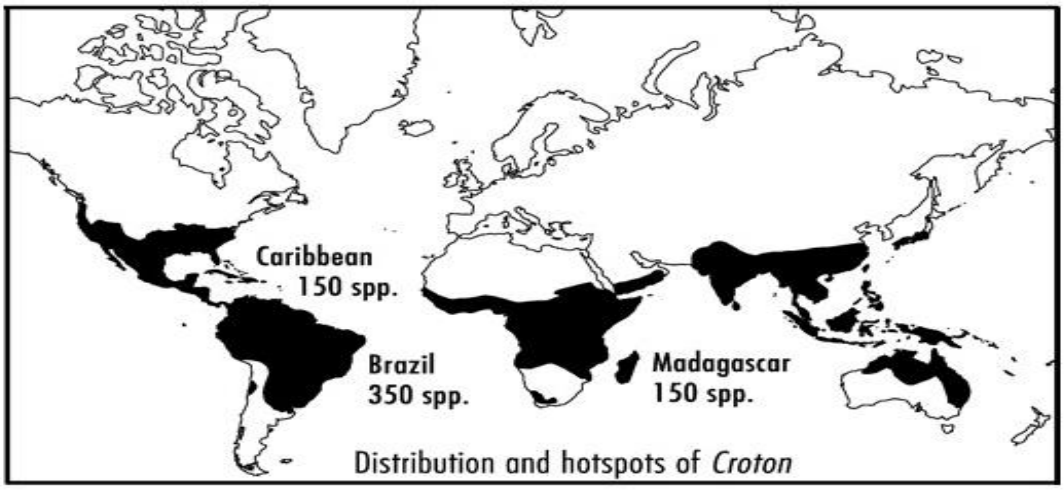


Figure 2.4: Geographical distribution of *Croton* genus[4]

[4] Dark shaded regions represent areas of *Croton* species distribution

2.3 Ethnomedicinal uses of *Croton* species

Croton plants have been used widely and variedly in folk medicine all over the world. A notable example is *sangre de drago*, a sap from a number of American *Croton* species including *C. lechleri* Muell.-Arg which is marketed as an herbal remedy for diarrhea, inflammation, insect bites, viral infections and wounds (Cai *et al*., 1993a, b; Chen *et al*., 1994). Common ethno-medicinal uses of *Croton* plants include treatment of: - cancer, constipation, diabetes, digestive problems, dysentery, external wounds, fever, hypercholesterolemia, hypertension, inflammation, intestinal worms, malaria, pain, ulcers and weight-loss (Salatino *et al*., 2007). Specific ethno-medicinal applications of various species across the globe are given in Table 2.1.

Table 2.1: Ethnomedicinal uses of *Croton* species

Name of species (other names, region)	Plant part	Condition managed
C. alienus Pax (Kenya)	Unspecified	Body weaknesses (Gachathi, 2007)
C. antanosiensis Leandri (Syn. *Croton antanosiensis* var. *basaltorum* Leandri) (Madagascar)	Stem bark	Induce virility during circumcision ceremonies, Ordeal poison in ancient times (Schmelzer and Gurib-Fakim, 2008)
	Leafy branches	Fumigate houses in case of epidemic diseases (Schmelzer and Gurib-Fakim, 2008)
C. antisiphiliticus (Brazil)	Entire plant	Stimulant, Wound healing, Veneral diseases, Rheumatic fever (Elisabetsky *et al.*, 1992)
C. arboreous Millsp. ("Cascarillo", Mexico)	Aerial parts	Auxiliary anti-inflammatory in respiratory ailments (Aguilar-guadarrama and Rios, 2004)
C. argyratus (Malaysia)	Dried flowers	Purgative (Ilham *et al.*, 1995)
C. aubrevillei J. Leonard (Cote d'ivoire, Ghana, Cameroon and Central African Republic)	Leaves and Stem bark	Stomach-aches, Constipation and Female fertility, Guinea-

	infusion Stem bark and stem Roots, leaves and stem bark infusion	worm infection. Pain, toothbrush, aid sleep in babies High blood pressure and stomach-aches(Schmelzer and Gurib-Fakim, 2008)
C. barorum Leandri (Madagascar)	A decoction of stem and root barks Aromatic leafy branches	Malarial fever, Cough, Diarrhea, Leukaemia (Schmelzer and Gurib-Fakim, 2008) and Breast Cancer (Rakotonandrasana *et al.*, 2010) Insect Repellent (lice) and Perfumery in soap (Schmelzer and Gurib-Fakim, 2008)
C. bonplandianus Baill (Argentina although it has gotten its way into Kenya where it is found as a common weed)	Entire plant	Antiseptic (Bandoni *et al.*, 1976)
C. cajucara Benth. ("Sacaca" , Peru and Brazil)	Stem bark and Leaves (in form of tea or pills)	Diabetes, Diarrhea, Malaria, High Blood Cholesterol Levels, Gastrointestinal disturbances, Hepatic disturbances, weight loss (Duke, 1984; Duke, 1994; Campos *et al*., 2002; Grassi-Kassisse *et al*., 2003)
C. californicus Mueller Arg. (California, U.S.A.)	Leaves	Rheumatism, Malaria, Pain reliever (Williams *et al*., 2001; Chavez *et al*., 1982; Wilson *et al.*, 1976; Farnsworth *et al*., 1969)
C. capitatus Mitchx	Unspecified	Malaria (Farnsworth *et al*., 1969)

C. caudatus (Indonesia, India)	Stem bark	Stomach disorders, Malaria (Banerji *et al*., 1988)
C. celtidifolius Baill. ("Sangue-de- adave", Brazil)	Stem bark and Leaf infusions	Inflammatory diseases, Leukemia, Ulcers and Rheumatism (Nardi *et al*., 2003)
C. ciliatoglandulifer (Syn. *C. ciliato-glandulosus*, Mexico)	Entire plant	Purgative (Farnsworth *et al*., 1969)
C. cortesianus (Mexico)	Aerial parts	Veneral diseases and Wound healing (Dominguez and Alcorn, 1985)
C. corymbulosus (U.S.A)	Aerial parts	Purgative (Coon, 1974)
C. decaryi Leandri (Madagascar)	Leafy branches Decoction from aerial parts	Mattress filler to Repel Lice Calm patients suffering from Paranoid Psychosis (Schmelzer and Gurib-Fakim, 2008)
C. dichogamus Pax (Kenya, Uganda, Tanzania, Rwanda and Ethiopia)	Leaves, Roots Whole plant decoction	Fever, Chest ailments, Stomach diseases, Tuberculosis, Impotence (Kokwaro, 1993 and 2009) Malaria (Jeruto *et al*., 2011)
C. draco Cham. & Schltdl. (one of the "sangre-de-drago" plants, bearing a red sap widely used in traditional medicine in Mexico and Central America)	Aerial parts	Fever, Tumors, Bleeding, Cough, Flu, Diarrhoea and Stomach ulcers, Topically as wound healing for cuts, open sores, herpes, Anti-septic after tooth extraction and Oral sores (Gupta *et al.*, 1996; Murillo *et al*., 2001)
C. draconoides (Peru)	Latex	Cancer, Wounds, Inflammation (Piacente *et al*., 1998)

C. eluteria Bennett ("Cascarilla", Syn. *C. eluteria* (L.) Wright, West Indies and Northern South America-Bahama Island)	Stem bark (used as substitute for *Chinchona* and *Cascara*,Vigor *et al.*, 2001)	Dysentry, Dyspepsia (Duke, 1984), Malaria, Fever, Bronchitis,Tonic and Bitters, Flavoring for liqueurs and Scenting tobacco
C. flavens L. (Curacao, Venezuela)	Leaves	Rheumatism, Fever, Menstrual pains(Flores and Ricalde, 1996)
C. fragilis (Mexico)	Entire plant	Stomach-aches, Hepatic pains (Hecker, 1984)
C. geayi Leandri (Madagascar)	Infusion of its Leafy twigs	Fevers, Coughs, Asthma and Constipation in new-born babies(Schmelzer and Gurib-Fakim, 2008; Palazzino *et al*., 1997)
C. glabellus (Mexico)	Leaves	Ulcers (Flores and Ricalde, 1996)
C. glandulosus (Mexico)	Entire plant	Stomach-aches (Heinrich *et al.*, 1992)
C. goudotii Baill (Syn. *C. mollivelus* Baill, Madagascar)	Unspecified Leaves Stem bark	Chronic blennorrhea, Cough and an Aphrodisiac (Rakotonandrasana *et al*., 2010) Malaria, Chronic gonorrhea (Schmelzer and Gurib-Fakim, 2008)
C. gratissimus Burch (Syn. *C. microbotryus* Pax, *C. antunesii* Pax, *C. welwitschianus* Mull. Arg. and *C. zambesicus* Muell. Arg. (Syn. *C. amabilis* Muell. Arg.; Western and Southern Regions of Africa)	Leaves Stem bark	Rheumatism, Perfume, Dropsy, Fever, Bleeding gum, Perfume (Farnsworth *et al*., 1969) Carthatic, Eruptive irritant, Respiratory condition, Intercostals neuralgia, Dropsy,

		Indigestion, Pleurisy, Uterus disorder (Wattand Breyer-Brandwijk, 1962), Fish poison (Farnsworth *et al*., 1969)
C. gubouga S. Moore (Syn. *C. megalobotyrs* Mull. Arg.; South Africa, Tanzania , Botswana, Caprivi strip, Malawi, Zambia and Zimbabwe; Goodson and Clewer, 1919; Kew, 2012 and 2013)	Seed and stem bark	Emesis, Pugartive, Febrifuge, Fish poison, Laxative, Malaria (Watt and Breyer-Brandwijk,1962; Neuwinger,1996, 2000 and 2004)
C. guatemalensis (Guatemala)	Stem bark and Leaves	Malaria (Franssen *et al.*, 1997)
C. haumanianus (Congo)	Stem bark, Leaves	Blennoragy, Gastric diseases, Hypertension, Epilepsy (Tchissambou *et al.*, 1990)
C. hovarum Leandri (Madagascar)	Stem bark - Aerial parts Leaves	Fish poison (Krebs and Ramiarantosa, 1996) Molluscicidal (Schmelzer and Gurib-Fakim, 2008) Colic and Acute Body Weakness (Krebs and Ramiarantsoa, 1997)
C. humilis (Jamaica)	Entire plant	Insecticide (Asprey and Thornton, 1955)
C. insularis (Caledonia, Pacific Islands-East Australia)	Entire plant	Abortifacient (Rageau, 1973)
C. jatrophoides Pax (Tanzania)	Roots	Colds, Intestinal worms and Stomachache (Schmelzer and Gurib-Fakim, 2008; Kokwaro, 2009)
C. joufra Roxb. ("Plau Noi"; Thailand)	Stem bark Decoction of Leaves and	Blood purification Anti-dysentery and Peptic promoter

	Stem bark Decoction of the flowers	Anthelmintic (Mokkhasmit *et al.,* 1971; Sutthivaiyakit *et al*., 2001)
C. kongensis Gagnep. ("Plao Ngeon" or "Plau Noi"; Thailand; China)	Entire plant	Sores (Pei, 1985)
C. lechleri L.(one of the "sangre-de-drago" plants; Ecuador and Peru; Cai *et a*l., 1993a, b and 1991)	Latex from stem bark	Wound healing, Cancer, Stomach ulcers, Rheumatism (Duke, 1994)
C. lobatus Linne (Senegal, Eritrea and Ethiopia; Carribean, South America and The Arabian Peninsula)	Leaves (Ivory Coast) Leaves combined with seeds and bark of "fufusuf bigor"(Senegal) Fresh leaves juice Leaf macerate Leaf decoction (Togo) Leaves and Roots (Benin) Leaves + leaves of *Hildegardia barteri*	Malaria, Pregnancy troubles, Dysentery, Rheumatic pain Whooping Cough, Convulsions, Mouth infections Eye diseases, un consciousness (Neuwinger, 1996, 2000and 2004; Attioua *et al.*,2007) Lotion for female sterility Purgative (Schmelzer and Gurib-Fakim, 2008) Antispasmodic in case of threatening miscarriage and hiccups Anti-hypertensive medication (Neuwinger, 1996, 2000 and 2004)
C. longiracemosus (Gabon)	Roots	Antheimintic, Anti-Inflammatory (Akendengue and Louis,1994)
C. macrostachys Hoscht. *ex* A. Rich *ex* Delile (Syn. *C. macrostachys* var. *mollissimus* Chiov.;	Entire plant and Seeds	Malaria, Dysentry, Rheumatism, Taenacide,

Madagascar, Somali, Sudan, Eritrea, East Africa, Angola Guinea, Liberia, Malawi, Zambia and Zimbabwe (Kew, 2012 and 2013)	decoctions (Schmelzer and Gurib-Fakim, 2008; Klauss and Adala, 1994; Mazzanti *et al*., 1987)	Venereal diseases, Conjuctivitis, Purgative, blood clotting, mumphs, skin rashes Anthelmintic, vermifuge, Female infertility, Constipation, Stomach pains, Chest pains, Bloat, wound healing, Diabetics
C. malabaricus (India)	Fresh shoots	Joint Pains, Rheumatic Arthritis (Pushpangadan and Atal, 1984)
C. malambo Karsten ("Palomatias", "Torco"; Venezuela and Colombia)	Stem bark infusion	Diabetes, Diarrhoea, Rheumatism, Gastric Ulcer, Anti-Inflammatory, Analgesic (Suárez *et al.*, 2003)
C. mayumbensis J. Leonard (Gabon, Cameroon and The Central African Republic)	Stem bark and Leaves	Microbial Infections, Human Parasitic Diseases such as Amoebiasis (Yamale *et al*., 2009)
C. mauritianus (Reunion Island)	Entire plant	Fever (Vera *et al*., 1990)
C. megalobotrys (Zimbabwe)	Stem bark, Roots, Seeds	Purgative, Malaria, Abortion, Tape worms (Nyazema, 1984)
C. megalocarpus Hutch (Kenya Eastwards to The Democratic Republic of Congo and Southwards to Mozambique, Malawi and Zimbabwe (Kew, 2012 and 2013)	Entire plant Stem bark decoction Root decoction Sap issuing from its leaves	Gall bladder problems, Chest pains, Internal swellings, Malaria (John *et al*., 1994) Anthelmintic, Whooping Cough Pneumonia Bleeding Wounds (Kokwaro, 2009)
C. membranaceus Mull Arg.(West Africa)	Root and Leaf extracts	Aromatize tobacco (Bahamas), Improve Digestion (Nigeria), Benign Prostate Hyperplasia

	Essential oils from the Stem bark	and Measles (Ghana) Aromatherapy to treat cough, Fever, Flatulence, Diarrhoea and Nausea (Asare *et al*., 2011; Adesogan, 1981)
C. menyhartii (Eastern Africa, Somalia)	Roots	Malaria, Dymenorrhea, Intestinal obstruction, Influenza (Kokwaro, 1993 & 2009)
C. mongue Baill (Syn. *C. mongue* var. *vatambensis* Leandri.; Madagarscar)	Stems and seeds Stem	Toxic Match manufacturing (Ralison *et al.*, 1986)
C. mubango Mill. (Congo, Ivory Coast, Angola)	Entire Plant	Female sterility, Spiritual madness, Asthma, Paralysis, Hepatalgia, Sleeping Sickness, Diarrhea, Furgative, Vermifuge (Watt and Breyer-Brandwijk, 1962; Bossard *et al*., 1993; Bouquet and Debray, 1974; Otshudi *et al*., 2000)
C. mucronifolius (Brazil)	Leaves	Syphilis, Rheumatism, Influenza (Lemos *et al*., 1992)
C. nepetaefolius Baill. ("Marmeleirovermelho". Brazil)	Infusions or decoctions of the stem bark and leaves	Antispasmodic properties, Relieve flatulence, Increase appetite, Sedative (Santos *et al*., 2008)
C. oblongifolus Roxb. ("Chucka"; India, Thailand and China)	Entire plant and seeds	Sores, Ringworm, Migraine, Leprosy, Dysentery, Diarrhea, Purgative, Insecticide, Blood Purification, Anti-Pyretic, Gastric Ulcers, Liver enlargement and remittent

		fever, Hepatitis (Pei, 1985; Sommit *et al*., 2003; Ngamrojnavanich *et al*., 2003)
C. onacrostachyus (Kenya)	Entire tree	Psychotherapeutic effect on muphs-"ngumbu" (Kokwaro, 2009)
C. palanostigma Klotzsch (Peru)	Stem bark latex, Leaves,	Boils and sores, Uterine ulcers, Wounds, Snake bites, Gastro-intestinal cancer (Lahlou *et al*., 2000)
C. penduliflorus Hutch (Sierra Leone Eastwards to Nigeria , Central African Republic and Gabon (Schmelzer and Gurib-Fakim, 2008)	Roots, Seeds, Stem bark Leaf infusion Seed extract	Purgative, Stomach-aches, Labor pains, Headaches, Impotence (Anika and Shetty, 1983) Menstrual disorders(Cote d'Ivoire), Fever (Ghana) Uterine tumors and Stomach complaints (Nigeria) (Adesogan, 1981)
C. polytrichus (Kenya)	Roots	Headache and labour pains (Kokwaro, 2009)
C. pseudopulchellus Pax (Mali, Nigeria, Somalia, Kenya, Ethiopia, Angola, Zimbabwe, Mozambique and South Africa)	Unspecified Leaves Roots Stem	Anthrax, Insecticide (Hedberg *et al*., 1983) Syphilitic ulcers, Chest infections, Tuberculosis (Tanzania) Asthma, Colds, Viral and Tissue infections Condiment, Burnt and smoke used to flavour fresh milk (Kenya-Coastal region) (Langat *et al*., 2012)
C. regelianus var. *matosii* ("Velame de Cheiro";	Leaf Infusion	Rheumatism, Malignant

Brazil)		tumors, Stomach aches (Torres *et al*., 2010)
C. repens (Mexico)	Entire plant	Dysentery, Diarrhea (Heinrich *et al.*, 1992)
C. roxburghii (India)	Entire plant	Antivenin, Clear bowels, Malaria, Cardiotonic (Selvanayahgam *et al*., 1994)
C. ruizianus (Peru)	Leaves	Anti-spasmodic, Vulnerary (Piacente *et al*., 1988)
C. sakamaliensis Leandri (Syn. *C. sakamaliensis* var. *microphyllus* Leandri, Madagascar)	Stem bark infusion	Diarrhea, Cough, Fever, Purgative (to remove intestinal worms; Radulovic *et al*., 2006)
C. salutaris (Peru)	Leaves	Fever (Brandao *et al*., 1985)
C. scheffleri Pax (Tanzania)	Roots	Insanity, Remedy for miscarriage (Watt and Breyer-Brandwijk, 1962; Mathias, 1982)
C. schiedeanus Schlecht. ("Almizclillo", Columbia)	-	Hypertension (Guerrero *et al*., 2004; Guerrero *et al*., 2002; Guerrero *et al*., 2001)
C. soliman (Mexico)	Latex	Skin infections, Warts (Zamora-martinez and Pola, 1992)
C. steenkampianus Gerstner ("Marsh fever-berry" and "Tonga *Croton*"; Tanzania, Mozambique and Southern Africa)	Fresh leaves Vapor inhalation	Relieve body pains (Schmelzer and Gurib-Fakim, 2008; Adelekan *et al*., 2008)
C. sublyratus Kurz, renamed *C. stellatopilosus* H. and *C. longissimus* Airy Shaw ("Plau noi"; South-Eastern Asian Countries and Thailand)	Its mixture with *C. oblongifolius* Stem bark	Gastric ulcers and gastric cancer (Kawai *et al*., 2005) Anthelmintic and dermatological problems (Vongchareonsathit and De-Eknamkul, 1998; Ogiso *et al*., 1981)

C. sylvaticus Hochst (Syn. *C. verdickii* De Wild, *C. oxypetalus* Mull. Arg. and *C. stuhlmannii* Pax; Distributed from Ethiopia in the Northern parts of Africa to the Eastern Cape in South Africa, more widely found in Gabon to Angola (Venter and Venter, 1996).	Stem bark Roots Unspecified Leaves decoction Leaves infusion	Abdominal disorders (Venter and Venter, 1996; Mc Gaw *et al*., 2000),Tuberculosis (Kokwaro, 2009), Chest pains, Rheumatism, Fish poison Gall sickness in cattle (Watt and Breyer-Brandwijk, 1962; Neuwinger, 1996, 2000 & 2004), Indigestion, Pleurisy, Poultices for swellings / wash for body swellings caused by kwashiokor (Kokwaro, 2009), Malaria and Purgative (Beentje, 1994)
C. texensis (U.S.A., India)	Leaves, Roots	Laxative, Antivenin (Moore, 1979)
C. tiglium L.(Asia)	Fruits, Roots	Fish poison, Abortifacient, Tumors, Laxative, Gout, Contraceptive, Insecticide, Cancerous sores, Purgative (Gimlette, 1929; Chang *et al*., 1981)
C. tonkinensis Gagnep ("Kho sam Bac Bo"; A Vietnam)	Leaves	Digestive disorders, Abdominal pains, Dyspepsia, abscesses, Impetigo, Gastric and duodenal ulcers, Malaria, Urticaria, Leprosy, Psoriasis, Genital organ prolapse (Giang *et al*., 2003; Minh *et al*., 2003)
C. trinitatis (Nicaragua)	Entire plant	Cough, Bleeding gum, Influenza (Duke, 1994; Kuo *et al*., 2007)
C. urucarana Baill. (Syn. *C. ururucana* Baill.;	Red latex of	Cancer, Diarrhea, Respiratory

Brazil and Argentina)	stem bark ("Sangre-de-drago")	and Urinary tract infection, Wound healing, Rheumatism (Perez and Anesini, 1994; Perez *et al*., 1997 &1998)
C. zambesicus Muell.Arg. (Syn.*C. amabilis* Muell.Arg.; Originally a Guineo-Congolese species but now Widespread in Tropical Africa)	Roots Leave decoction (externally) (internally) Mixture of the leaves and *Grewia villosa*	Menstrual pains(Sudan) Aperient, Anti-malarial, Anti-diabetic (Sierra Leon and Nigeria) Wash for fevers Dysentery and Convulsions (Sierra Leon and Nigeria) Hypertension and Urinary infections (Benin), Anti-microbial, Fever associated with malaria (El-hamidi, 1970; Mohamed *et al*., 2009; Ngadjui *et al*., 1999; Baccelli *et al*., 2007; Okokon *et al*., 2005 & 2013) Body strengthening medicine (Watt and Breyer-Brandwijk, 1962)
C. zehntneri Pax. *et* Hoffm.("Canelade-cunhã"; Brazil)	Leaves and Stem bark	Seizures, Insomnia, Anxiety, Sedative, Appetite stimulating, Gastro-intestinal disturbances, Food and drinks sweetener (Coelho-de-souza *et al*., 1997&1998; Batatinha *et al*., 1995)

2.4 The Phytochemistry of *Croton* genus

The phytochemistry of *Croton* genus is considerably diverse, comprising of many classes of natural products mainly, alkaloids, flavonoids, terpenoids and essential oils containing mono and sesquiterpenoids. The sections which follow here in will capture each class of compounds reported from *Croton* genus.

2.4.1 Alkaloids from *Croton* genus

Alkaloids are nitrogenous compounds classified according to the nature of the nitrogen containing carbon skeleton. The alkaloids reported from *Croton* genus are made up of the basic carbon skeletons given in Table 2.2 with specific examples given in Tables 2.3-2.5 and Figures 2.5-2.7.

Table 2.2: Carbon skeletons of alkaloids reported from *Croton* genus

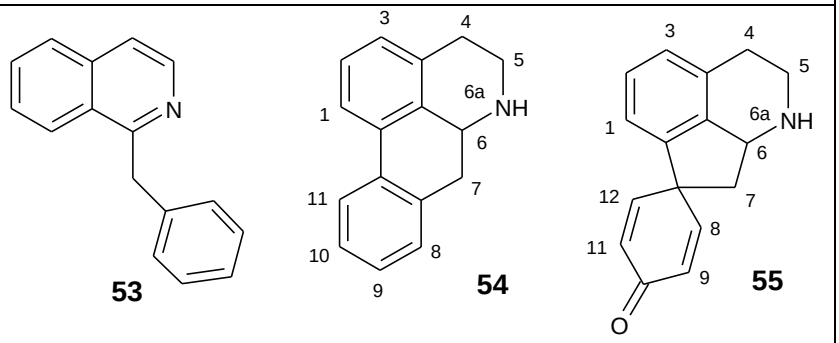
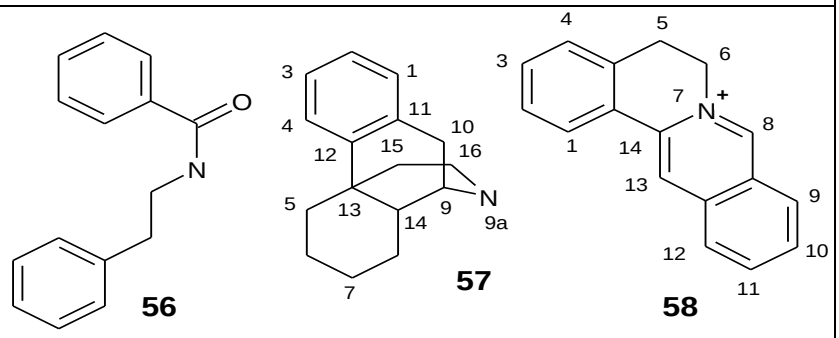
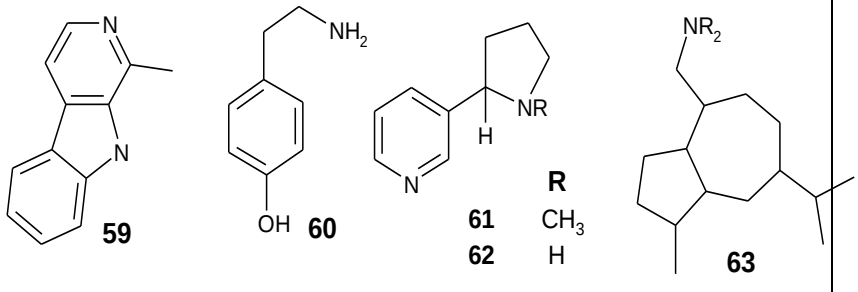
Basic skeleton	Structure
Benzylisoquinoline (**53**) Aporphine (**54**) Proaporphine (**55**)	
Peptide derived alkaloids (**56**) Morphinane (**57**) Protoberberine (**58**)	
Harman (**59**) Tyramine (**60**) Nicotine (**61**) Anabasine (**62**) Guaiane (**63**)	

Table 2.3: Benzylisoquinoline-derived alkaloids possessing aporphine, proaporphine and morphinane skeletons

Code	Skeleton	Name	Source
64	Aporphine	Glaucine (Milanowski *et al*., 2002; Dos Santos *et al*., 2001)	*C. lechleri*
65		Thaliporphine (Milanowski *et al*., 2002)	
66		Norisoboldine (Berry *et al*., 2005)	
67		Isoboldine (Amaral and Barnes, 1997)	
68		Magnoflorine (Milanowski *et al*., 2002)	*C. celtidifolius*
60		Sparsiflorine	*C. sparsiflorus* (Bhakuni *et al*., 1970)
70		N-methyl-sparsiflorine	
71		Wilsonirine	*C. wilsonii* (Stuart and Chambers, 1967)
71		Hernovine	
73		*N*-methylhernovine	
74		10-*O*-Methylhernovine	
75		*N*,*O*-Dimethylhernovine	
76		*O*,*O*-Dimethylhernovine	*C. hemiargyeus* (Wen-han *et al*., 2003)
77		Isocorydine	
78		*S*(+)-Magnoflorine bromide (Casagrande *et al*., 1975)	*C. turumiquirensis*
79	Abnormal aporphine	Hemiargine B	*C. hemiargyeus* (Wen-han *et al*., 2003)
80		Norcorydine	
81		*O*,*O*-Dimethylhernovine	
82		Nornuciferine	*C. sparsiflorus* (Bhakuni *et al*., 1979)
83		Nuciferine	
84	Proaporphine	Linearisine	*C. linearis* (Farnsworth *et al*., 1969; Haynes *et al*., 1966; Piacente *et al*., 1998)
85		Homolinearisine	
86		Pronuciferine	
87		Base E	
88		Jacularine	

89	Proaporphine	Crotsparine/Crotoflorine	*C. sparsiflorus* (Bhakuni *et al*., 1970; Casagrande *et al*., 1975; Bhakuni and Dhar, 1968; Chatterjee and Majumder, 1968)
90		*N*-methylcrotsparine	
91		*N*,*O*-Dimethylcrotsparine	
92		Amuronine (Charris *et al*., 2000)	*C. flavens*
93		Crotonosine (Farnsworth *et al*., 1969; Haynes *et al*., 1966)	*C. linearis*
94		*N*,*O*-Dimethylcrotonosine (Stuart, 1970)	*C. plumieri*
95		Methylcrotonosine	*C. discolor* (Stuart, 1970)
96		Discolorine	
97		Jaculadine	
98	8, 9-Dihydro proaporphine	Crotsparinine	*C. sparsiflorus* (Casagrande *et al*., 1975; Bhakuni *et al*., 1979; Bhakuni and Dhar, 1969)
99		*N*,*O*-Methylcrotsparinine	
100	Morphinane Dienone	Salutaridine (Barnes and Soeiro, 1981; Bracher *et al*., 2004; Eisenreich *et al*., 2003; Sanchez and Sandoval, 1982)	*C. flavens*
101		Norsalutaridine (Barnes and Soeiro, 1981)	*C. salutaris*
102		8,14-Dihydrosalutaridine	*C. linearis* (Farnsworth *et al*., 1969; Sanchez and Sandoval, 1982; Haynes *et al*., 1968)
103		8,14-Dihydronorsalutaridine	
104		Flavinine (Bhakuni *et al*., 1979; Stuart *et al*., 1968 &1969)	*C. flavens*

105	Morphinane dienone	*O*-Methylflavinantine (Farnsworth *et al*., 1969; Eisenreich *et al*., 2003)	*C. ruizianus*
106		Salutarine (Eisenreich *et al*., 2003)	*C. flavens*
107		Flavinantine (Piacente *et al*., 1998; Eisenreich *et al*., 2003; Stuart *et al*., 1969; Chambers and Stuart, 1968; Bittner *et al*., 1997)	*C. chilensis*
108		Isosalutaridine (Bittner *et al*., 1997)	
109		Norsinoacutine	*C. lechleri* (Charris *et al*., 2000; Stuart *et al*., 1969; Carlin *et al*., 1995)
110		Sinoacutine	
111		4,5-Dihydroxymorphinandien-7-one (Tiwari *et al*., 1981)	*C. bonplandianum*
112	Biarylic *bis*-morphinane dienone	Saludimerine A	*C. flavens* (Bracher *et al*., 2004)
113		Saludimerine B	

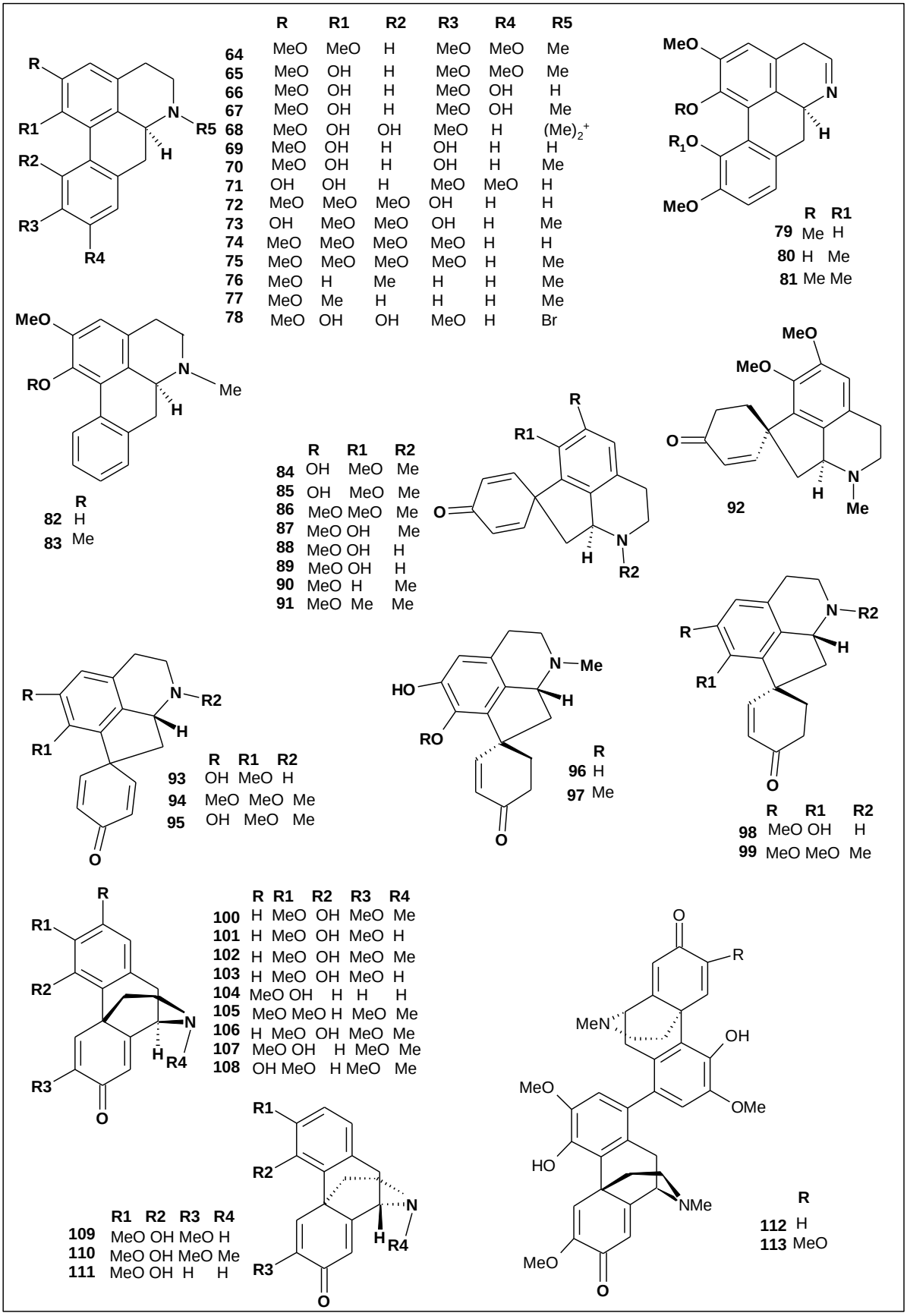


Figure 2.5: Benzylisoquinoline-derived alkaloids possessing aporphine, proaporphine and morphinane carbon skeletons

Table 2.4: Tetrahydroprotoberberine, glutarimide, guaiane, harman, tyramine and other benzylisoquinoline type alkaloids from *Croton* species

Code	Type	Name	Source
114	Tetrahydro protoberberine	Hemiargyrine (Amaral and Barness, 1998)	*C. hemiargyeus*
115		Tetrahydropalmatrubine (Wen-han *et al*., 2003)	
116		Xylopinine (Wen-han *et al*., 2003)	
117		Corytenchine	*C. tonkinensis* (Pham *et al*., 2004)
118		Corytenchirine	
119		Coreximine	*C. flavens* (Eisenreich *et al*., 2003)
120		Scoulerine	
39 / 121	Glutarimide	Julocrotine (Mwangi *et al*, 1998; Aboagye *et al*., 2000; Bayor *et al*., 2009)	*C. sylvaticus* *C. membranaceus*
122		Crotonimide A (*N*-[2, 6-dioxo-1-(2-phenylethyl)-3-piperidinyl] propanamide)	*C. pullei* (Barbosa *et al*., 2007)
123		Crotonimide B (*N*-[2, 6-dioxo-1-(2-phenylethyl)-3-piperidinyl] methylpropanamide)	
124 / 125		Julocrotone / Isojulocrotol	*C. cuneatus* (Suarez *et al*., 2004)
126		Julocrotol	
127	Guaiane	Muscicapine A	*C. muscicapa* (De Araujo-Junior *et al*., 2005)
128		Muscicapine B	
129		Muscicapine C	
130	Harman	2-Ethoxycarbonyltetrahydroharman	*C. moritibensis* (De Araujo-Junior *et al*., 2004)
131		6-Hydroxy-2-methyltetrahydroharman	
132	Tyramine	*N*-methyltyramine	*C. humilis* (Stuart and Byfield, 1971)
133		*N*-methylhomotyramine	

134	Benzylisoquinoline	Laudanidine (Amaral and Barnes, 1997)	*C. celtidifolius*
135		Reticuline (Milanowski *et al*., 2002)	*C. lechleri*
136		Norlaudanosine (Wen-han *et al*., 2003)	*C. hemiargyeus*

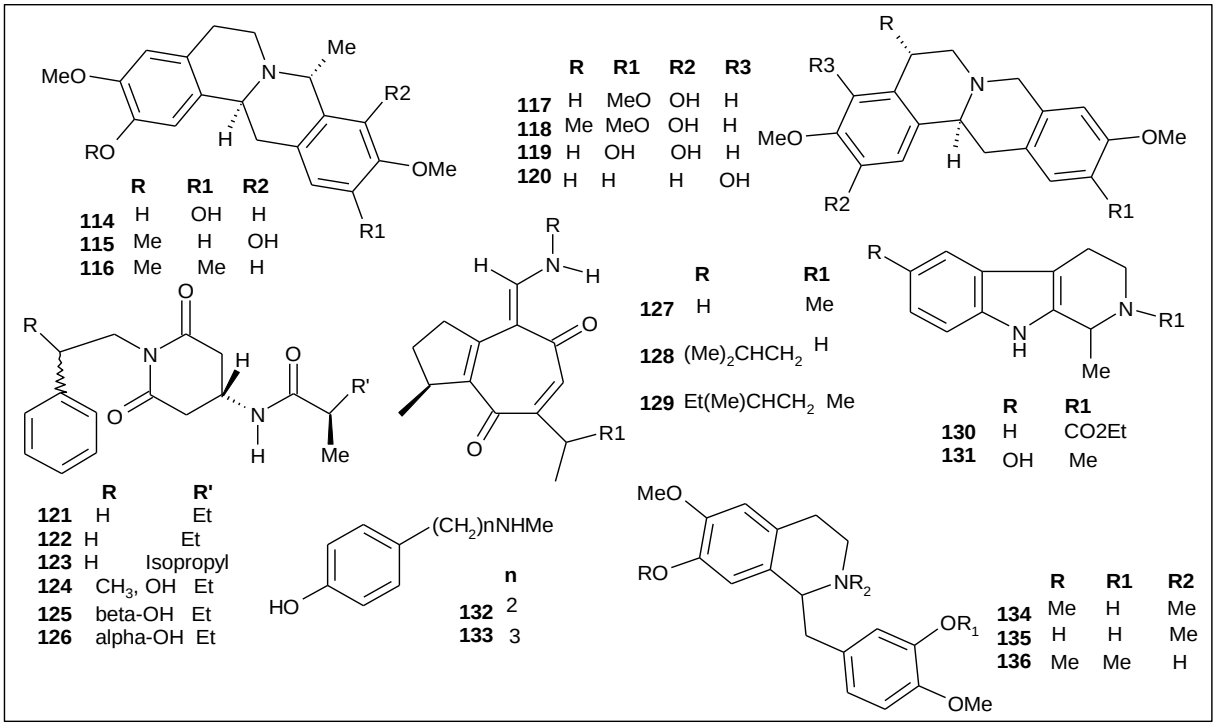


Figure 2.6: Tetrahydroprotoberberine, glutarimide, guaiane, harman, tyramine and other benzylisoquinoline type alkaloids

Table 2.5: Peptide derived alkaloids and other types of alkaloids from *Croton* species

Code	Type	Name	Source
137	Peptide derivative	*N*-benzoylphenylalaninol	*C. hieronymi* (Catalan *et al*., 2003)
138		Aurentiamide acetate	
139		*N*-benzoylphenylalaninyl-*N*-benzoylphenylalaninate	
140	Unspecified	Taspine	*C. lechleri, C. draco, C. campestris* (Milanowski *et al*., 2002; Risco *et al*., 2003; Tsacheva *et al*., 2004; Ribeiro Prata *et al*., 1993)
141	Isoquinoline	Hemiargine D	*C. hemiargyeus*

142	Phenanthrene	Hemiargine C	(Wen-han *et al*., 2003)
143	Proaporphine	1, 2, 10-Trihydroxycrotosinoline -*N*-oxide	*C. campestris* (Ribeiro Prata *et al*., 1993)
144	Nicotine derivative	Anabasine	*C. muscicapa* (De Araujo-Junior *et al*., 2005)
145	Pyrrolidine	4-Hydroxyhygrinic acid	*C. hovarum* (Krebs and Ramiarantosa, 1996 & 1997)

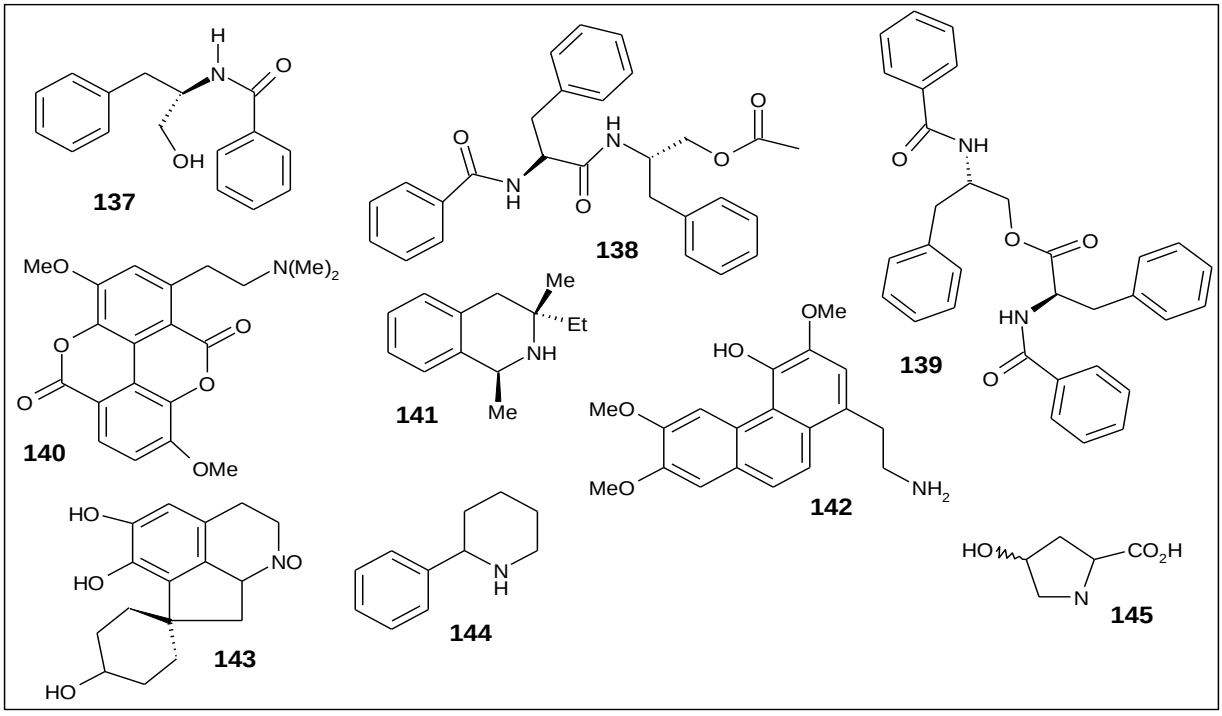


Figure 2.7: Peptide derived alkaloids and other types of alkaloids from *Croton* species

2.4.2 Flavonoids from *Croton* genus

Flavonoids are phenolic derivatives that occur naturally as water-soluble glycosides. Their classification is based either on their biosynthetic origin and / or molecular size. Some flavonoids are both intermediates in biosynthesis as well as end-products which can accumulate in plants. Ayanin, vitexin, tilirosine, rutin and quercetrin are some of the common flavonoids isolated from *Croton* genus [Table 2.6; Figure 2.8].

Table 2.6: Flavonoids reported from *Croton* species

Code	Name	Source
146	Ayanin	*C. schiedeanus* (Puebla *et al*., 2005; De Garcia *et al*., 1986)
147	Quercetin-3,7-dimethyl ether	
148	5-Hydroxy-7,4′-dimethoxyflavone	*C. betulaster* (Barbosa *et al*., 2003)
149	Kaempferol -3-*O*-rutinoside	*C. cajucara* (Capasso *et al*., 1998 & 2000)
150	Kaempferol-3,4′,7-trimethylether	*C. menthodorus* (Maciel *et al*., 2000)
151	Tiliroside	*C. tonkinensi; C. hovarum* and *C. zambesicus* (Wagner *et al*., 1970; Capasso *et al*., 2000; Phan *et al*., 2004; Krebs and Ramiarantosa, 1996 & 1997; Pham *et al*., 2004)
152	Vitexin	
153	Isovitexin	
154	Kaempferol-3,7-dimethylether	*C. cajucara* (Maciel *et al*., 2000)
155	Rutin	*C. menthodorus* (Capasso *et al*., 2000)
156	Quercitrin	*C. glabellus* (Novoa *et al*., 1985)
157	Quercetin	*C. steenkampianus* (Schmelzer and Gurib-Fakim, 2008; Adelekan *et al*., 2008)
158	Taxmarixetin	
159	Eriodictyol	

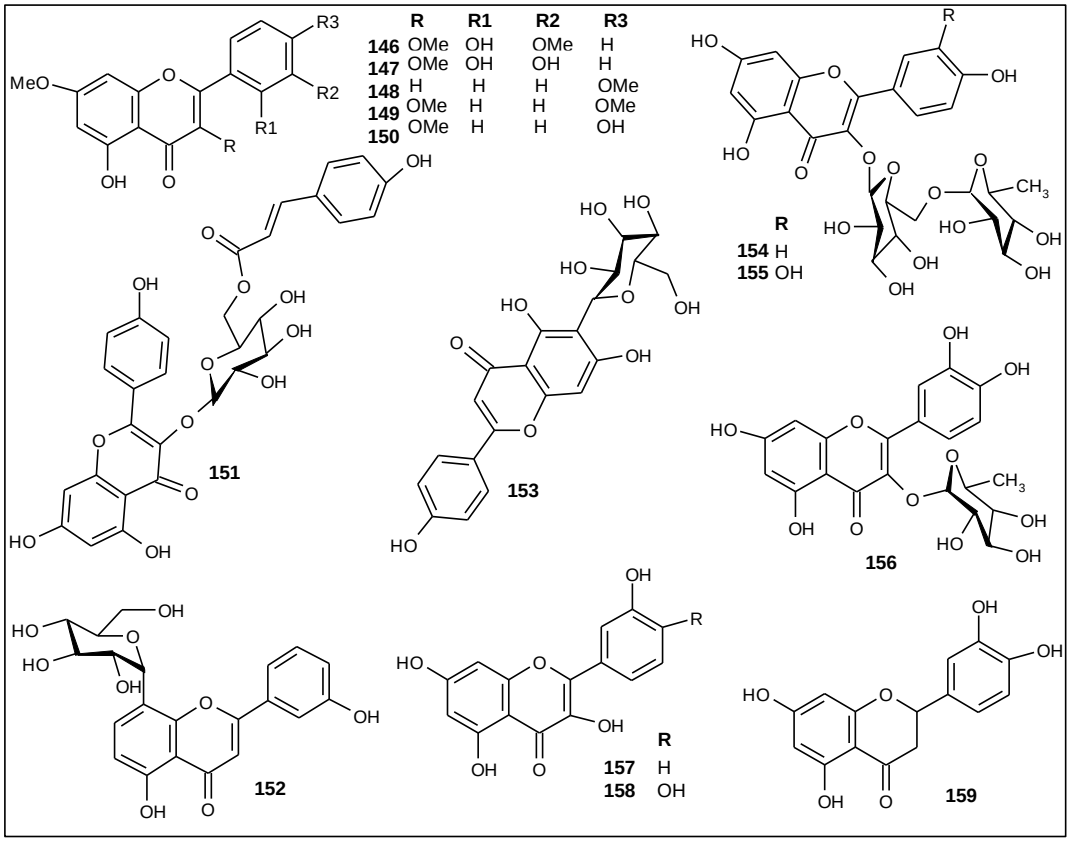


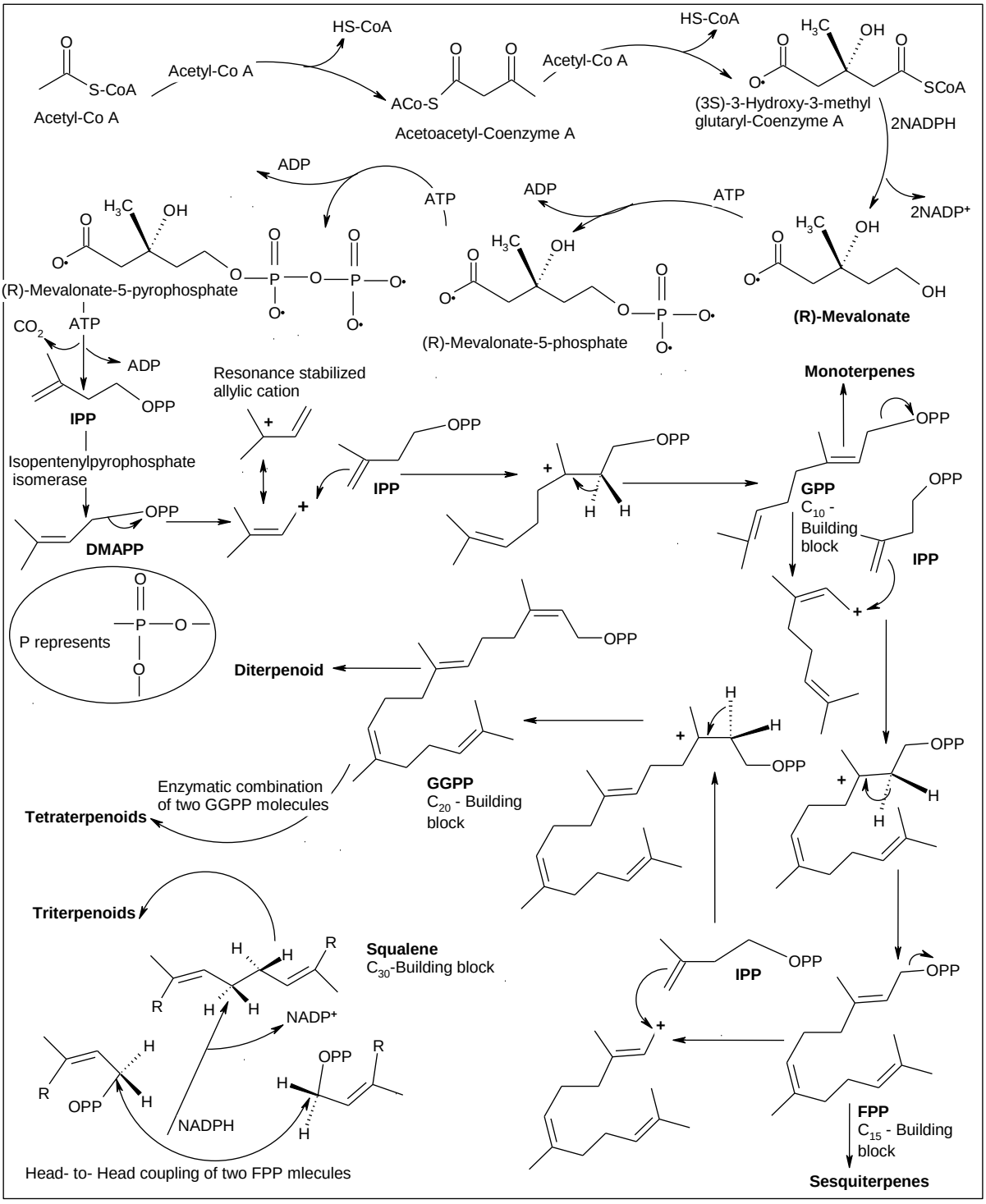
Figure 2.8: Flavonoids reported from *Croton* species

2.4.3 Terpenoids from *Croton* genus

Terpenes are hydrocarbon components of resins and turpentine produced from resins. They constitute a large and structurally diverse family of natural products derived from C_5-isoprene units. Chemical modifications through oxidation and re-arrangement of their carbon skeletons produce terpenoids. *Mono-*, *sesqui-*, *di-*, *tri*-terpenoids and phytosterols have been reported from *Croton* genus. Only three compounds of all those characterised in this study were alkaloids. The rest were terpenoids, majority being diterpenoids of *ent*-clerodane series. The sections which follow here will therefore focus on the general biosynthetic pathway for terpenes. Details of biosynthesis of diterpenes in order to provide a background to the study will also be discussed.

2.4.3.1 Biosynthesis of terpenes

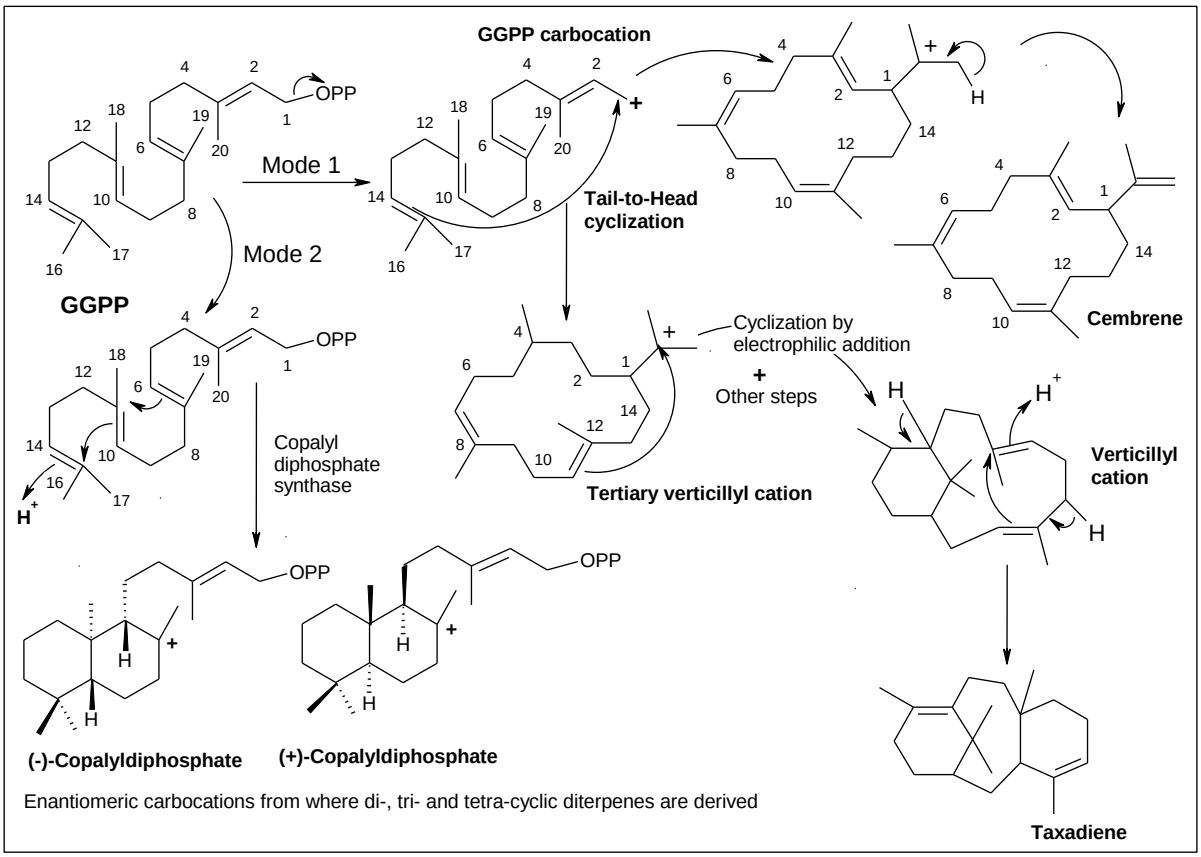
A simple direct head-to- tail coupling reaction is not applicable in the linking of the C_5 isoprene units to produce terpenes. The process is rather complex, involving a sequence of enzymatic reactions (with very few exceptions) that can be accounted for using chemical analogies based on established chemical principles and mechanisms. Plants predominantly use the Mevalonate pathway to synthesise terpenes. The process starts from a single acetyl-coenzyme A (Acetyl-CoA). Three of these Acetyl-CoA molecules go through various steps to generate (R)-mevalonate from where the fundamental building blocks of terpenes which are two isomers, isopentenyl-diphosphate / isopentenyl pyrophosphate (**IPP**) and dimethylallyldiphosphate (**DMAPP**) are derived [Scheme 1]. The conversion of **IPP** to **DMAPP** is catalysed by isopentenylpyrophosphate isomerase. Further enzymatic catalysed combinations of **IPP** and **DMAPP** results to precursor molecules from where various terpenes are derived. Monoterpenes are derived from geranyl diphosphate (**GPP**) which is formed as result of combinations between **IPP** and **DMAPP**. Combination of **IPP** and **GPP** results to formation of farnesyldiphosphate (**FPP**) from which sesquiterpenes are derived. Diterpenes are derived from geranylgeranylpyrophosphate (**GGPP**) which is a product of combining **IPP** and **FPP**. Squalene is the parent carbon skeleton from where triterpenoids are derived and is as a result of enzymatic combination of two **FPP** molecules in a tail to tail manner. Combinations of two **GGPP** molecules results to formation of tetraterpenes. In all these reactions, the role of the enzyme is to activate the pyrophosphate groups to become better leaving groups in order to generate an allylic-tertiary carbocation through an $\mathbf{S_N1}$ reaction mechanism [Scheme 1] which is the first step in the combination processes (Dewick, 2002).



Scheme 1: Biosynthesis of terpenoids from acetyl-Co A

2.4.3.2 Biosynthesis of diterpenes

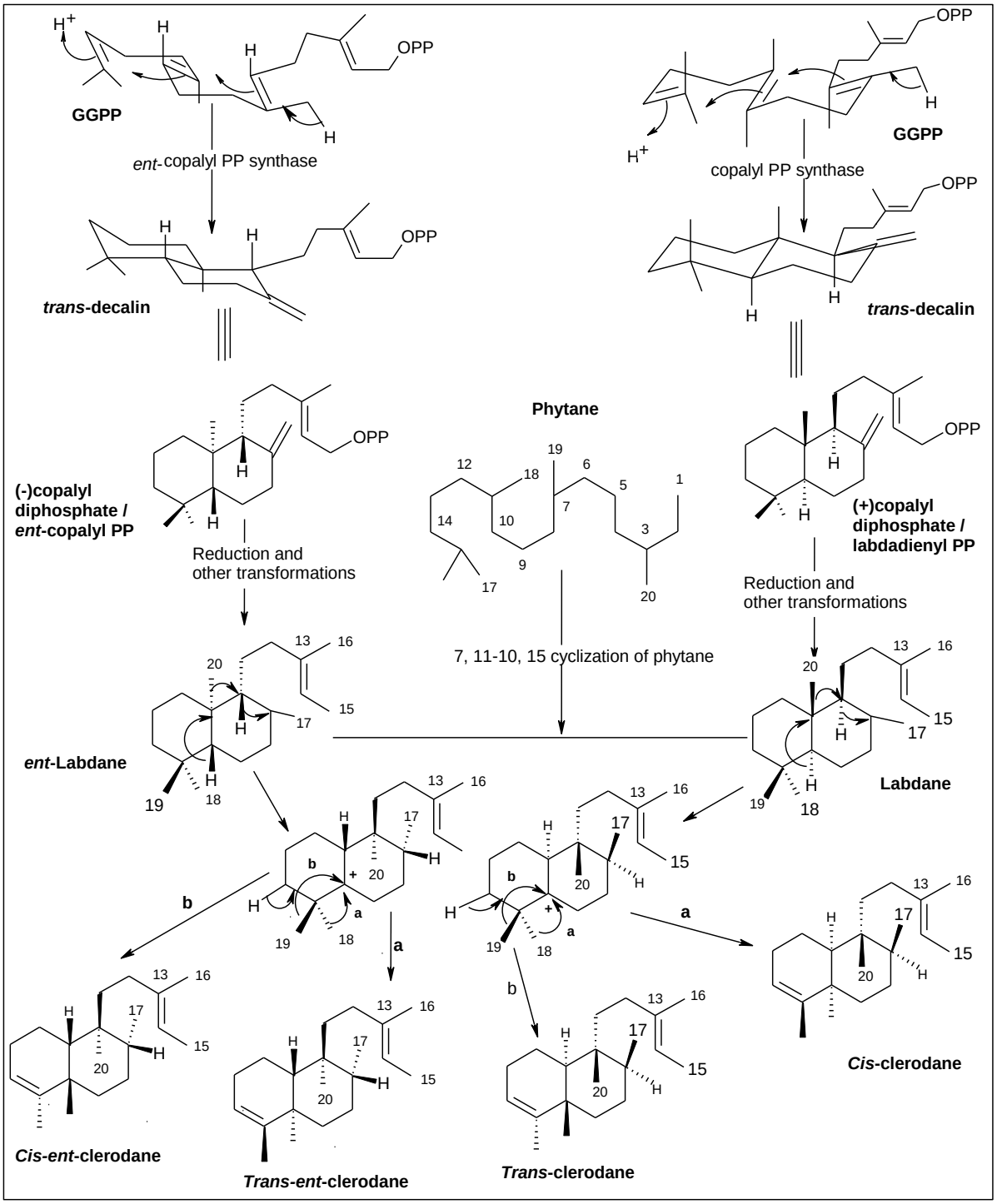
Diterpenes are C_{20} molecules derived from four isoprene units joined head to tail to the parent hydrocarbon, phytane (3, 7, 11, 15-tetramethylhexadecane). They are non-volatile in nature and are richly found in Conifer and Angiosperm resins and in appreciable quantities in Labiatae, Ranunculaceae and Euphorbiaceae. They are also found in marine animals (Coelentrates) like soft corals and sea fans (Dewick, 2002). As was illustrated in Scheme 1, **GGPP** is the building block of all diterpenes. Its allylic pyrophosphate group with the assistance of Mg^{2+} acts as a good leaving group to generate a carbocation which initiates a variety of different reaction paths. Depending on the bound conformation of the active site of each enzyme, a series of other reactions (addition to double bonds, Wagner-Meerwein re-arrangements, hydride shifts as well as de-protonation) follows the carbocation formation. Simple enzymatic reduction of **GGPP** leads to formation of acyclic diterpenoids while protonation of a double bond can initiate cyclization reactions through two main modes as illustrated in Scheme 2.



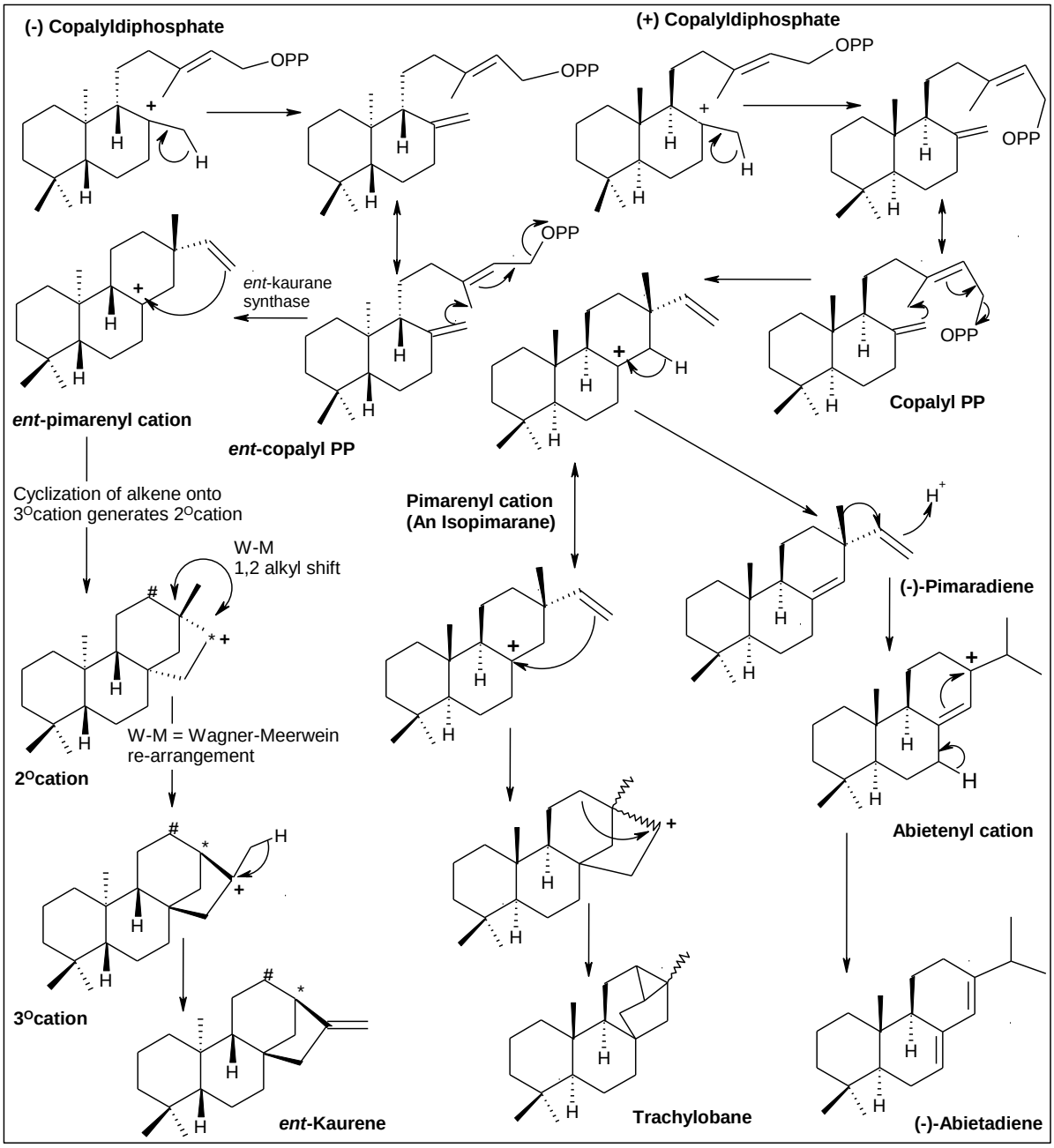
Scheme 2: Cyclization of GGPP during biosynthesis of cyclic diterpenes

Bicyclic diterpenoids are a product of enzymatic C-14 double bond protonation of **GGPP** followed by the anti-parallel additions of the C-10 and C-6 double bonds and eventually the loss of a proton from the methyl group to give a double bond. The cyclization process is terminated by generation of a *trans*-decalin intermediate which undergoes more enzymatic modifications involving folding of **GGPP** on the surface of copalyl and *ent*-copalyl synthase to form the two bicyclic enantiomers, (+)-copalyl PP (labdadienyl PP) and (-)-copalyl PP (*ent*-copalyl PP). Further enzymatic modifications and reduction processes generates labdane and *ent*-labdane series of diterpenoids. Labdanes are basically 7, 11-10, 15-cyclophytanes containing the decalin bicycle as a core structure which also defines the usually accepted numbering system [Scheme 3] (Dewick, 2002). Normal *cis*- and *trans*- isomers of clerodane and their *ent*- epimers arise from two methyl migrations in *ent*- and normal labdanes respectively (Dewick, 2002; Kubo *et al*., 1982; DNP, 2007). Further cyclization of the (+)-copalyl PP and (-)-copalyl PP gives rise to tri-, tetra- and penta-cyclic diterpenes through loss of the pyrophosphate group followed by Wagner-Meerwein shifts [Scheme 4] (Dewick, 2002).

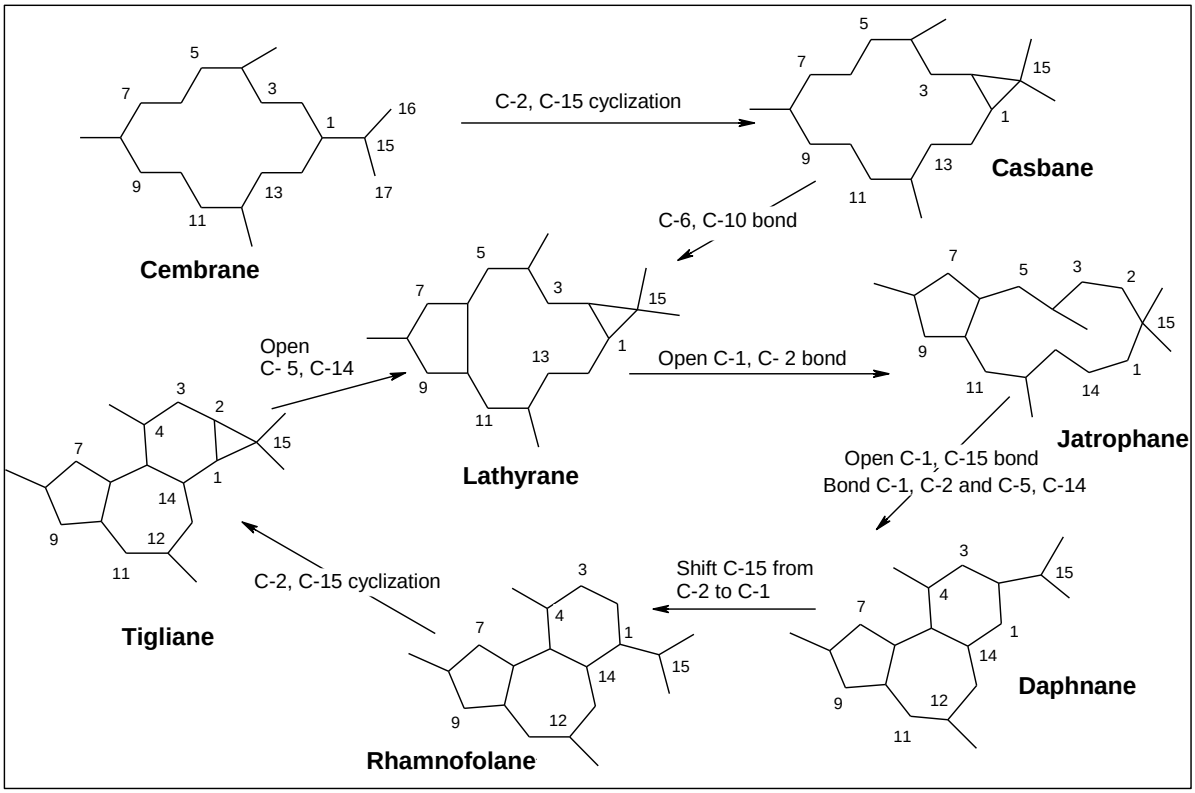
Plants from Euphorbiaceae are reported to provide novel diterpenoids based on casbane and its cyclization products. Biosynthetically, the process begins with a cembrane molecule which is a reduction product of the cembrene molecule whose biosynthesis was illustrated in Scheme 2. The process proceeds with various *bi*- and *tri*-cyclic diterpenoids formation [Scheme 5] including a jatrophane skeleton whose name stems from *Jatropha gossypiifolia* (Euphorbiaceae) reported to have the antineoplastic and antileukemic (+) - jatrophone (Frum and Viljoen, 2005). Various differently substituted jatrophanes are reported from Euphorbiaceae such as the esulones from *Euphorbia esula* and the euphormines from *E. helioscopia* and *E. maddeni* (Frum and Viljoen, 2005).



Scheme 3: Biosynthesis of bicyclic diterpenoids



Scheme 4: Biosynthesis of *tri-* , *tetra-* and *penta*-cyclic diterpenes



Scheme 5: Cembrane as a precursor skeleton of other diterpenoids

2.4.4 Essential and fixed oils from *Croton* genus

Perhaps, one of the great values of the *Croton* genus is the discovery of *C. megalocarpus* seeds as a potential source of fixed oils that could be a suitable alternative bio-diesel. Linoleic acid (a fixed oil common in seeds) was found to be the major fatty acid, constituting 74.3% of all the fatty acids present in the oil (Wu *et al*., 2013). Earlier reports on the same oil had indicated that it possessed Epstein-Barr virus-activating potency (Wu *et al*., 2013). The seeds of *C. macrostachys* were found to contain 48% oils (linoleic acid (80%), palmitic acid (12%), stearic acid (6%) and myristic acid (2%)). The purgative and inflammatory activities of these oils have been demonstrated rationalizing the ethno-botanical use of *C. macrostachys* as a purgative (Mazzanti *et al.*, 1987). *C. penduliflorus* seeds produced essential oils that were found to be hypocholesterolemic but could predispose anaemia (Ojokuku *et al*., 2011). From *C. stellulifer* [Syn. *C. stelluliferus*], oils having anti-microbial activities except against *Aspergillus niger* were isolated (Martins *et al*., 2000). Other reported sources of oils from *Croton* genus are given in Table 2.7; Figure 2.9.

Table 2.7: Essential oils reported from *Croton* species

Source	Plant part (% essential oil)	Phytochemical constituents of the essential oil (% composition)
C. antanosiensis	Dried aerial parts (0.25)	Monoterpenes (73.07) (α-pinene (**160**), β-pinene (**161**) and limonene (**162**)) (Radulovic *et al*., 2006)
C. aubrevillei	Dried stem bark (0.19) (Menut *et al*., 1995)	Monoterpenes (α-pinene (**160**) (0.1), β-pinene (**161**) (2.0), linalool (coriander oil (**163**) (34.6) and β-caryophyllene (**164**) (11.9))
C. decaryi	Leaves (0.29) Stem bark (0.19) (Radulovic *et al*., 2006)	Leaf oil (sesquiterpenes (61.31)) Stem bark oil (monoterpenes (74.72)) Both the leaf and stem bark oils (low amounts of aliphatic compounds of non-terpenic origin)
C. geayi	Dried aerial parts (0.32) (Radulovic *et al*., 2006)	Sesquiterpenes (45.74) (caryophyllene oxide (**166**), β-caryophyllene (**167**), γ-cadinene (**168**) and α-cadinene) and Monoterpenes (36.87)
C. sakamaliensis	Leaves (0.32) Stem bark (0.15) (Radulovic *et al*., 2006)	Leave oil (sesquiterpenes (70.69)) Stem bark oil (monoterpenes (96.25)) Both leaf and stem bark oils (low amounts of aliphatic compounds of non-terpenic origin)
C. stellulifer	Stem bark (Martins *et al*., 2000)	Monoterpenes (α-phellandrene, α–pinene, ρ-cymene (**165**) and linalool)
C. zambesicus	Species from various localities in Africa	Monoterpenes, Sesquiterpenes and Aliphatic compounds (Boyom *et al*., 2002)

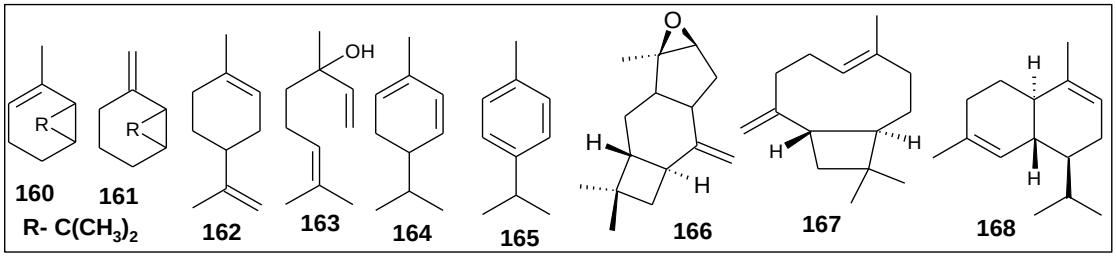
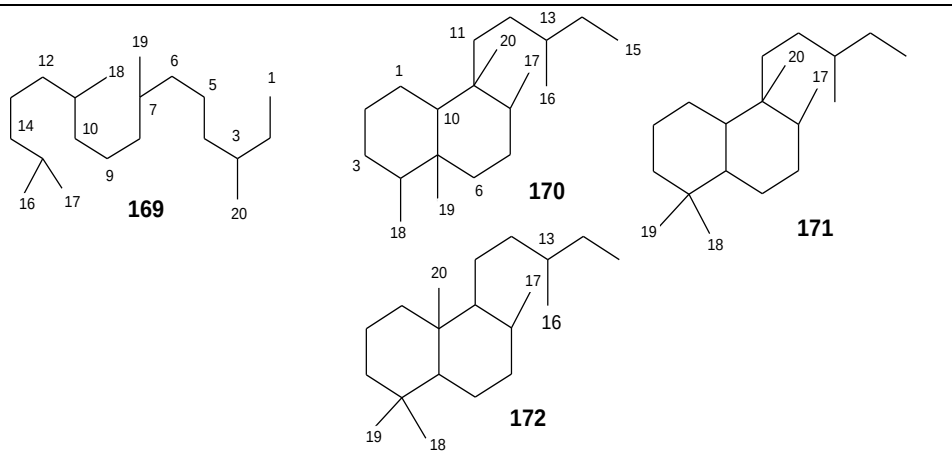
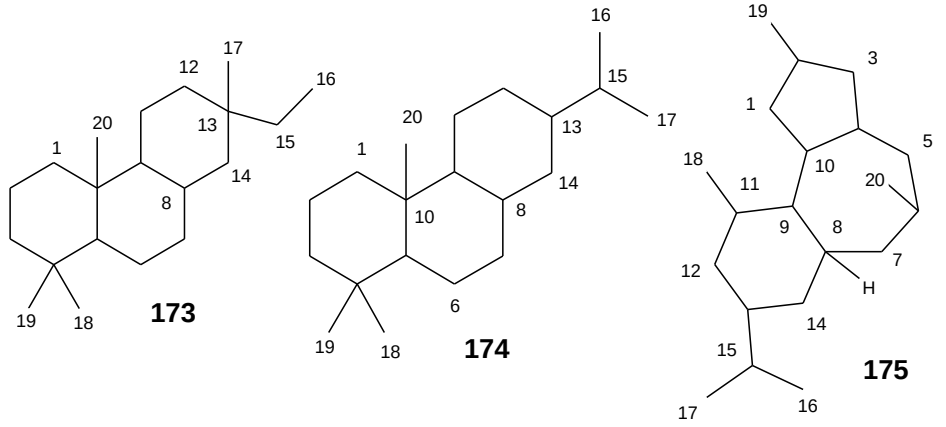
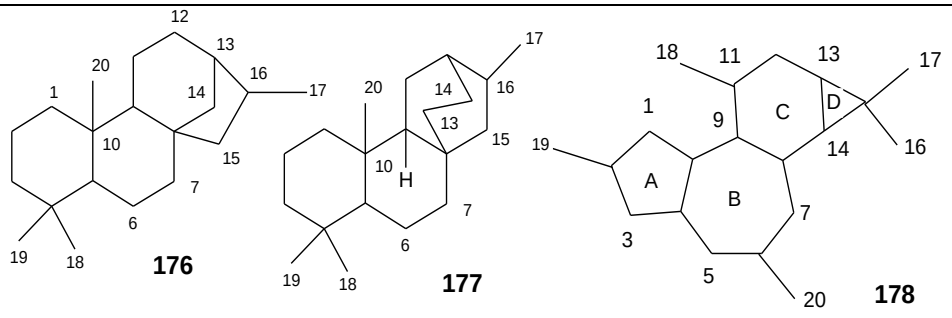


Figure 2.9: Monoterpenes and sesquiterpenes reported from *Croton* species

2.4.5 Diterpenoids reported from *Croton* genus

Acyclic and cyclic diterpenoids are the most abundant natural products to have been isolated from *Croton* genus. Acyclic diterpenoids are linear and may have cyclic or lactone groups included while the cyclic ones are categorised according to the number of rings they possess (*di-*, *tri-*, *tetra-* and *penta-*) as summarised in Table 2.8. The cyclic ones are additionally classified into two distinctive enantiomeric groups referred to as a "normal" and "*ent-*" series with opposite configurations at C-5, C-9 and C-10 as captured in Table 2.9.

Table 2.8: Carbon skeletons of diterpenoids from *Croton* genus

Type / Name	Basic carbon skeleton
Acyclic Phytane (**169**) Bicyclic Clerodane (**170**) Halimane (**171**) Labdane (**172**)	
Tricyclic Pimarane (**173**) Abiatane (**174**) Daphnane (**175**)	
Tetracyclic Kaurane (**176**) Atisane (**177**) Tigliane (**178**)	

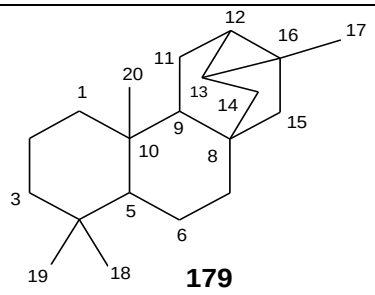
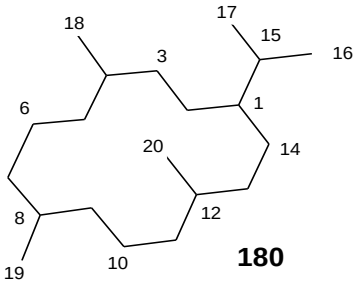
Pentacyclic Trachylobane (**179**) Macrocyclic Cembrane (**180**)	 **179**  **180**

Table 2.9: Enantiomeric diterpenes and their distinguishing parameters

Class of diterpene	Series	Specific rotation	Selected NOESY resonance correlations
Abiatane Labdane	Normal	+	H-5α and H-9α; H-5α and 3H-18; 3H-19 and 3H-20
	ent-	-	H-5β and H-9β; H-5β and 3H-18; 3H-19 and 3H-20
Isopimaranes	Normal	+	H-5α and H-9α; H-5α and 3H-18; 3H-19 and 3H-20; 3H-20 and 3H-17
	ent-	-	H-5β and H-9β; H-5β and 3H-18; 3H-19 and 3H-20
Pimaranes	Normal	+	H-5α and H-9α; H-5α and 3H-18; 3H-19 and 3H-20
	ent-	-	H-5β and H-9β; H-5β and 3H-18; 3H-19 and 3H-20; 3H-20 and 3H-17

2.4.5.1 Acyclic diterpenoids reported from *Croton* genus

Phytol (**181**) is the simplest acyclic diterpenoid that easily gets biosynthetically oxidised to plaunotol (**182**) (2, 6, 10, 14-phytatetraene-1, 19-diol) [Figure 2.10], the chief constituent of the leaves of Thai medicinal plant *C. sublyratus*, later renamed *C. stellatopilosus*. This phytochemical is marketed as "Plau noi" or "Kelnac" that is used as an anti-ulcerative (Wungsintaweekul and De-Eknamkul, 2005). Other acyclic phytanes from *Croton* genus include:- 3, 12-dihydroxy-1, 10, 14-phytatriene-5, 13-dione (**183**) from *C. salutaris* (Tansakul and De-Eknamkul, 1998); *trans*-phytol and isomers of phytol (**181**) from *C. zambesicus* (Catalan *et al.*, 2003; Block *et al.*, 2004) and geranylgeraniol (**184**), from *C. lobatus* (Attioua *et al*.,2007; Chabert *et al*., 2006).

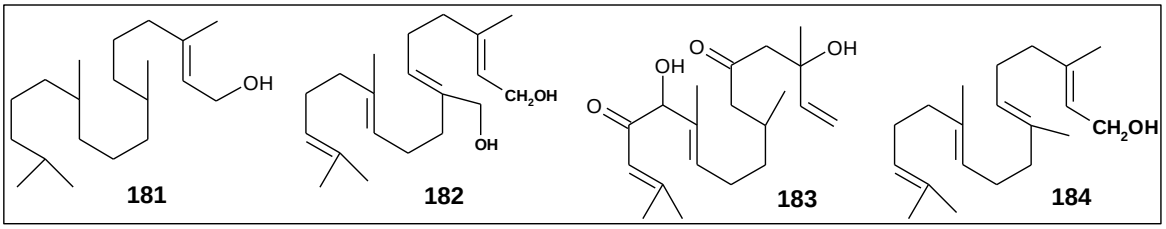


Figure 2.10: Acyclic diterpenoids from *Croton* species

2.4.5.2 Bicyclic diterpenoids reported from *Croton* genus

Clerodanes, labdanes, halimanes and an indane derivative are some of the bicyclic diterpenoids reported from *Croton* genus, clerodane and labdane being the major classes.

2.4.5.2.1 Clerodanes

Clerodane diterpenoids are the most prevalent compounds reported from *Croton* genus. These compounds have been tested for many pharmacological principles and have been found to be potentially useful as anti-tumour, anti-viral, anti-microbial, anti-peptic ulcer, anti-fungal and psychotropic agents. Their anti-feedant and insecticidal properties have also been reported. Specific examples and their reported biological activities are given in Table 2.10 and Figures 2.11 and 2.12.

Table 2.10: Clerodanes from *Croton* genus and their reported biological activities

Code	Name	Source (Biological activities)
185	*trans*-dehydrocrotonin, a nor-*ent*-clerodane diterpenoid	Amazonian *C. cajucara* (**185**) *C. schieddeanus* (**185** and **186**) (both epimers have ability to lower blood glucose and triglyceride in rats, Insect growth-inhibition, anti-inflammatory, anti-nociceptive (stops pain), anti-ulcerogenic (stops ulceration), anti-tumour against sarcoma 180 and Ehrlich carcinoma ascetic tumours in rats, cytotoxicity, anti-genotoxicity (Maciel *et al*., 1997 and 2000; Babili *et al*., 1998; Merritt and Levy, 1992; Rodriguez *et al*., 2004; Grynberg *et al*., 1999)
186	*cis*-dehydrocrotonin	
187	Derivatives of *trans*-dehydrocrotonin	*C. sonderianus* (Agner *et al*., 2001)
188		**188** and **189** from *C. schieddeanus* (its ethanolic extract was found to decrease pressure and have vasorelaxant effect

189	5β-hydroxy-*cis*-dehydrocrotonin (*12R*)-12-hydroxycascarillone	(Maciel *et al*., 2006). **188** and **189** in addition to the flavonoids, 3, 7-dimethylquercetin and ayanin, had synergestic role in the total vasodilator response induced by the plant (Guerrero *et al*., 2004).
190 **191, 192** **193**	*ent*-clerodanes Crotocorylifuran - Corylifuran -	*C. zambesicus* (Ngadjui *et al*., 1999) and *C. haumanianus* (Tchissambou *et al*.,, 1990) *C. corylifolius* (Tchissambou *et al*., 1990 and Burke *et al*., 1976)
194 **195**		Brazilian *C. campestris* (Babili *et al*., 1998)
Furano - *ent* – clerodanes **196** **197** **198**	Cascallin, Cascarillone, Cascarillin A Cascarillin B Cascarillin C Cascarillin D	All these cascallin derivatives are reported from *C. eluteria* (stem bark extract was found to be balsamic, digestive, hypotensive, narcotic, stomachic and tonic, (Vigor *et al*., 2001))
199 **200** **201**	Sonderianin - 12-*epi*-methyl-barboscoate	*C. ururucana* (Puebla *et al*., 2003)
202	Clerodane diterpenoid	*C. cajucara* (Maciel *et al*., 1997)
203	Furano-clerodane, crotomembranafuran	*C. membraneaceus* (Bayor *et al*., 2009)
204-207		*C. hovarum* (Krebs and Ramiarantosa, 1996 &1997)
208	Isoteucvin	**208-211** are reported from *C. jatrophoides* (Mbwambo *et al*., 2009). **211** is in addition reported from *Mallotus* sp.
209	Jatropholdin	

210	Teucvin derivative	(Euphorbiaceae) and *Teucrium* sp. (Labiateae) and has been showed to be amoebicidal, have root development inhibition property (Mbwambo *et al*., 2009) and anti-feedant activity against the colorado potato beetle, *Leptinotarsa decemlineata* (Say), an economically important pest with developed resistance to most classes of synthetic insecticides (Chen *et al*., 2008).
211	Teucvin	
212	Chiromodine	*C. megalocarpus* (Addae-mensah *et al*., 1989; Marko *et al*., 1999)
213	Epoxy-chiromodine	
214	Crotepoxide, Crotomacrine, Floridoline, Hardwickiic 12-Oxo-hardwickiic acid	*C. macrostachys* (Addae-mensah *et al*., 1989; Kapingu *et al*., 2000)

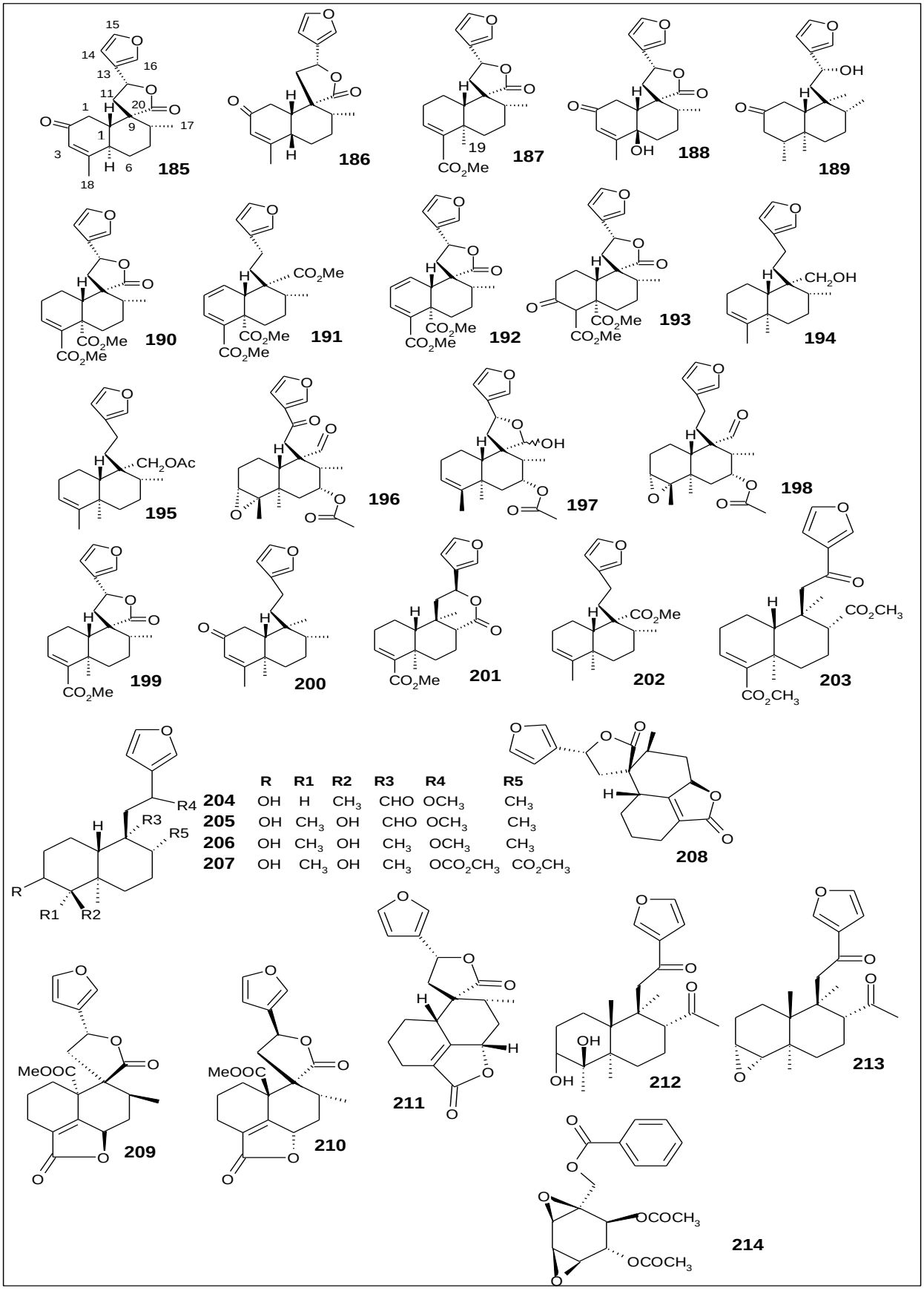


Figure 2.11: Clerodane diterpenoids from *Croton* species

Many other reports pointing to the fact that clerodane diterpenoids not only from *Croton* plants but also from many other plants are important bioactive molecules were accessed. A few notable examples include clerocidin (**215**) from *Oidiodendron truncatum* (Moniliales) that has shown antibiotic potential (Kapingu *et al*., 2000). Kolavenic acid (**216**) reported from *Polyalthia longifolia* var. *pendulla* (Annonaceae) and many other sources (Aristolochiaceae, Caesalpiriaceae and Compositae) is reported to possesses anti-bacterial activity to most bacteria and anti-fungal activity against the kanamycin resistant fungal strains, *Asperigillus fumigatus* and *Candida albicans* (Andersen and Rasmussen, 1984). Terpentecin (**217**) isolated from *Kitasatosporia* sp. (Actinomyes) has been found to have anti-microbial and anti-tumour properties (Rashid *et al*., 1996). *Tinospora cordifolia* Miers (Menispermaceae) used in Ayurvedic medicine produced compound (**218**), used against jaundice, urinary disease and rheumatism (Isshiki *et al*., 1985). Compound (**219**) was isolated from *Casearia sylvetris* (Flacourtiaceae) and has been found to have anti-tumour potential against sarcoma in mice (Hanuman *et al*., 1988).

Salvinoron (**220**), isolated from *Salvia divinorum* (Labiateae), has been reported as possessing psychotropic activity (Itokawa *et al*., 1988). Solidago lactone which is reported from *Solidago sp.* (Compositae) has been used as a piscicidal agent (Valdes *et al*., 1984). Ajugarin 1 (**221**) with anti-feedant activity towards the African army worm (*Spodoptera exempta*) and the African desert locust (*Schistocerca gregaria*)(Merritt and Levy, 1992) and ajugarin IV (**222**) having insecticidal activity against the silkmoth, *Bombyx mori* (Nishino *et al*., 1984) have been reported from *Ajuga remota* (Labiateae).

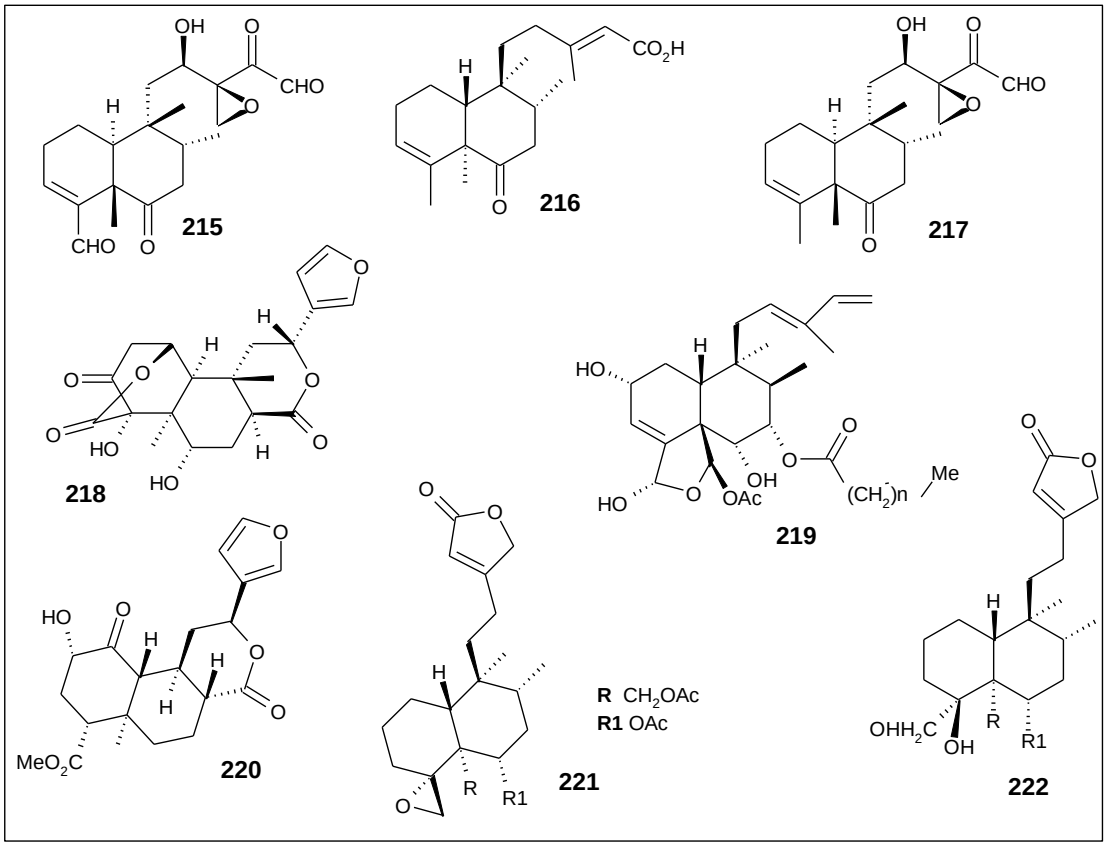


Figure 2.12: Bioactive clerodane diterpenoids from other plants

2.4.5.2.2 Halimanes and an Indane derivative

Biosynthetically, halimane diterpenoids possessing the halimane carbon skeleton (**171**) lie between the labdanes (**172**) and clerodanes (**170**) in their general structure. Halimane diterpenoids that have been reported from *Croton* genus include [Figure 2.13]:- centrafine 1 (**223**) from *C. membranaceous,* penduliflaworosin (**224**) from *C. jatrophoides* (Mbwambo *et al*., 2009), *C. penduliflorus* Hutch (Adesogan, 1981) and *C. sylvaticus* leaves (Schneider *et al*., 1995), (**225**) from *C. hovarum* (Krebs and Ramiarantosa, 1996 and 1997) and neoclerodane-5, 10-en-19, 6β, 20,12-diolide (**226**) from *C. macrostachys* (Addae-mensah *et al*., 1989). An indane derivative (**227**) from *C. steenkampianus* (Adelekan *et al*., 2008) is another of the bicyclic phytanes reported from *Croton* species.

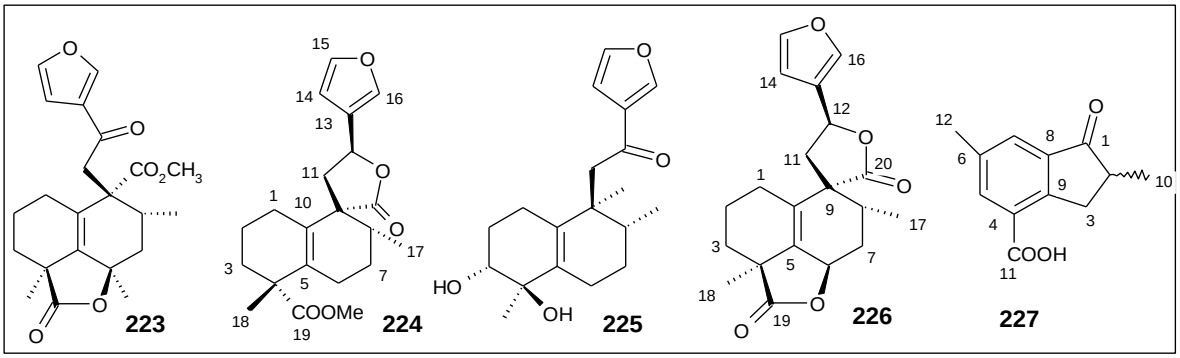


Figure 2.13: Halimane diterpenoids and an Indane derivative from *Croton* species

2.4.5.2.3 Labdanes

Hundreds of labdanes and their pharmacological values have been reported from higher plants. Their reports from *Croton* species have been summarised in Table 2.11; Figure 2.14.

Table 2.11: Labdanes from *Croton* species and their reported biological values

Code	Name	Source (biological value)
228	2α,3α–Dihydroxylabda-8(17),12,14-triene	**228** and **229** from *C. ciliatoglanduliferus* (both inhibit photophosphorylation, electron transport (basal, phosphorylating and uncoupled) and have partial reactions of both photosystems in spinach thylakoids (Nabeta *et al*., 1995)
229	2α-acetoxy-3α–dihydroxylabda-8(17),12,14-triene	
230	Labdane-8α, 15-diol	*C. eluteria* (Vigor *et al*., 2001)
231	15-acetoxylabdan-8α-ol	
232	Austroinulin	*C. glabellus* (Morales-Flores *et al*., 2007)
233	6-*O*-acetylaustroinulin	
234	Labda-7,12(*E*),14-trien-17-oic acid	**234-241** from *C. oblongifolius* (with an exception of **241**, all are reported to have non-specific and moderate cytotoxicity against five human tumour cell lines (Sommit *et al*., 2003; Garcia *et al*., 2006)
235	Labda-7,12 (E),14-trien-17-al	
236	17-hydroxylabda-7,12,14-Triene	
237	17-acetoxylabda-7,12,14-triene	
238	labda-7,13-dien-17,12-olide	
239	15-hydroxylabda-7,13-diene-17,12-olide	

240	12,17-dihydroxylabda-7,13-diene	
241	*Ent*-3α-hydroxymanoyl oxide	
-	Labda-7,12 (E),14-triene	
242	Crotonadiol	*C. zambesicus* (Ngadjui *et al*., 1999)
243	Maruvic acid	*C. matourensis* (Chaichantipyuth *et al*., 2005)
-	2,3-dihydroxy-labda-8(17),12(13), 14(15)-triene	*C. joufra* (weakly cytotoxic) (Sutthivaiyakit *et al.,* 2001)
244	Gomojoside H	*C. membraneaceus* (roots have anti-microbial activity and cytotoxic activities against human cancer cell line (Asare *et al*., 2011). **244** had antimicrobial activities equal to the activity of gentamycin (Bayor *et al*., 2009)
245		*C. zambesicus* (Ngadjui *et al*., 1999)
246	Geayinine (*ent*-8,13-epoxylabd-14-enes)	*C. geayi* (Radulovic *et al*., 2006)
247	Isogeayinine	
248	Crotomachlin	*C. macrostachyus* (Addae-mensah *et al*., 1989)
249		*C. pseudopulchellus* (Langat *et al*., 2012)

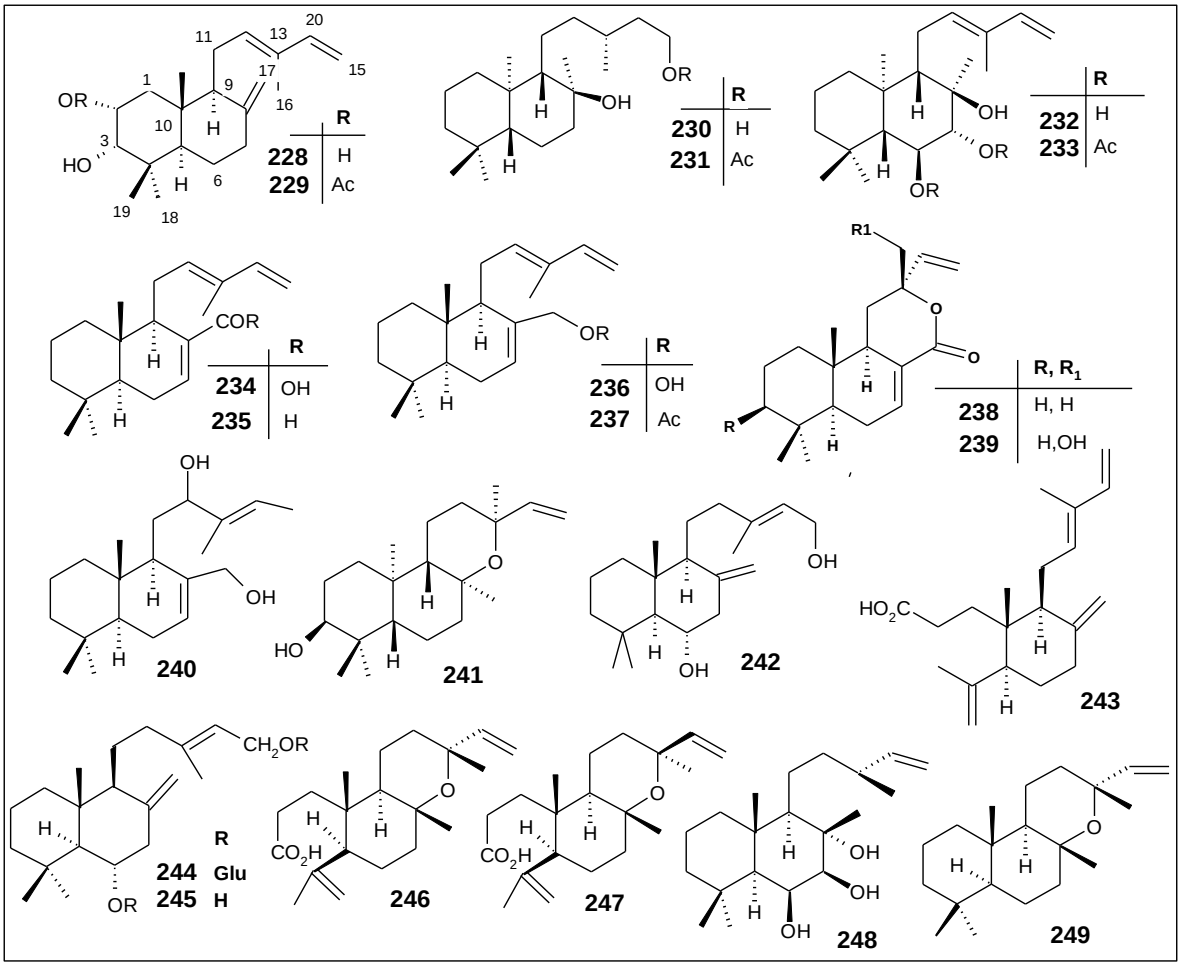


Figure 2.14: Labdane diterpenoids from *Croton* species

2.4.5.3 Tricyclic Diterpenoids from *Croton* genus

Tricycloditerpenoids reported from *Croton* genus include abiatanes, daphnanes, pimaranes, and isopimaranes.

2.4.5.3.1 Abietanes

Migration of the methyl group, C-17 from C-13 to C-15 in pimaranes (**173**) results to formation of abietane diterpenoids (**174**) [Table 2.8]. However, in plants, they are formed by cyclization of geranylgeranylpyrophosphate, **GGPP** [Scheme 4]. Related parent diterpene hydrocarbons include [Figure 2.15]: - 13, 16-cycloabiatanes (**250**); 17 (15-16)-*abeo*-abietanes (**251**) in which the methyl group, C-17 has shifted from C-15 to C-16 and totaranes (**252**) which arise from abietane when the isopropyl group migrates from C-13 to C-14. African *C. zambesicus* is the only *Croton* species reported to have produced abietane diterpenoids but their names were not included in the report accessed (Aiyar and Seshadri, 1970).

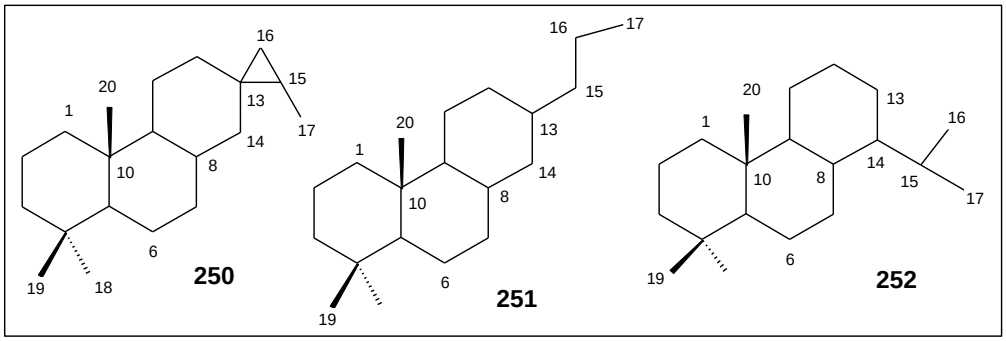


Figure 2.15: Abietane related parent diterpene hydrocarbons

2.4.5.3.2 Daphnanes

Included in this category is rhamnofolanes such as (-)-20-acetoxy-9-hydroxy-1, 6, 14-ramnofolatriene-3, 13- dione reported from *C. rhamnifolius* (Breitmaier, 2006). Daphnanes are similar in structure to rhamnofolanes, differing only in the position of the isopropyl group, C-15 where by, in daphnanes, it is on C-2 while in rhamnofolane, it is on C-1 [Scheme 5]. However, rhamnofolanes and other constituents from *Jatropha* species rarely occur in plants. Instead, daphnanes are more frequently found ((Breitmaier, 2006). Two daphnanes, steenkrotin B (**253**) and its triacetyl derivative (**254**) have been reported from *C. steenkampianus* (Adelekan *et al*., 2008) [Figure 2.16].

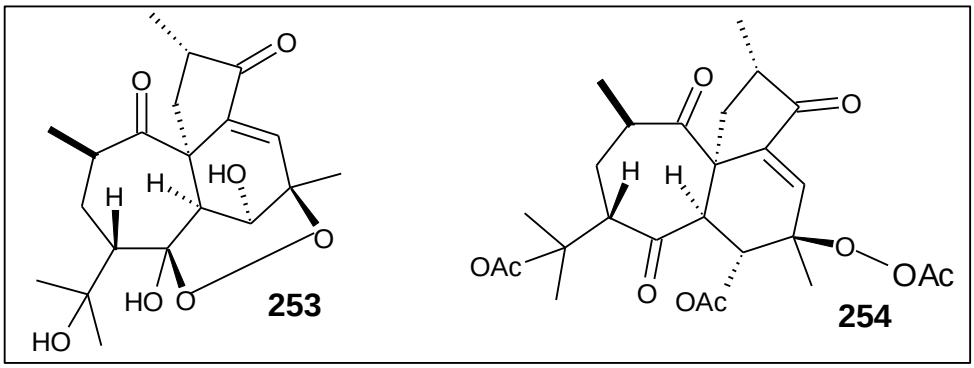


Figure 2.16: Daphnane diterpenoids from *Croton steenkampianus*

2.4.5.3.3 Pimaranes and Isopimaranes

Pimaranes (**173**) and isopimaranes are 13-14, 8-cyclolabdanes [Figure 2.11] with the perhydrophenanthrene basic skeleton, differing only in their configuration at C-13 [Figure 2.17]. *Ent*-isopimarane, yucalexin P-4 (**255**) has been reported from Argentinian *C. sarcopetalus* (Mwangi *et al*., 1998; De Heluani *et al*., 2000). 3β-hydroxy-19-acetoxy-*ent*-isopimara-8, 15-dien-7-one (**256**), plaunol A and C, swassin and 3β-hydroxy-19-*O*-acetyl-pimara-8(9), 15-dien-7-one which has been found to be weakly cytotoxic are reported from Thai *C. joufra* (Sutthivaiyakit *et al.*, 2001 Neuwinger, 2000).

From Asian *C. oblongifolius*, *ent*-pimara-7, 15 – dien – 19 – oic acid (**257**) was isolated (De Heluani *et al*., 2000) while from African *C. zambesicus*, three isopimaranes, isopimara-7, 15-dien-3β-ol (**258**), (**259**) and (**260**) are reported (Block *et al*., 2004).

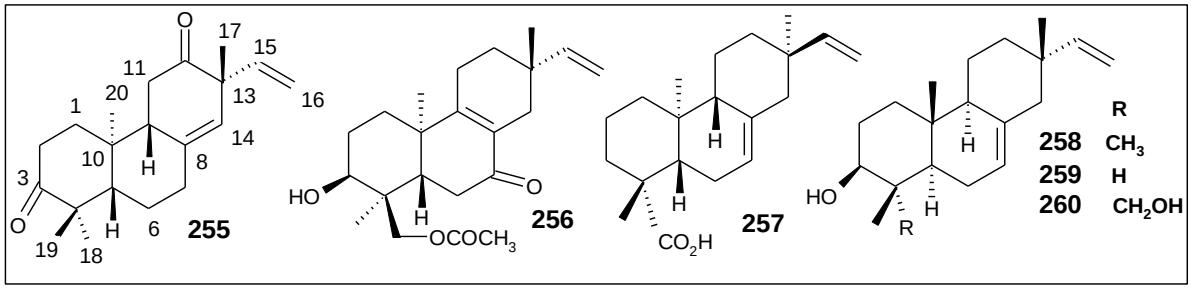


Figure 2.17: Pimarane diterpenoids from *Croton* species

2.4.5.4 Tetracyclic diterpenoids from *Croton* genus

Atisanes, kauranes and tiglianes are the reported tetracyclic diterpenoids from *Croton* genus. The bio-synthesis of kauranes and tiglianes was discussed in Schemes 4 and 5 respectively.

2.4.5.4.1 Atisanes

Atisane is the basic carbon skeleton of various diterpene alkaloids (aconitum-alkaloids) found in the plant families of Rhanunculaceae and Garryaceae ((Breitmaier, 2006). Two 3, 4-*seco*-atisane diterpenoids with cytotoxic potency [Figure 2.18], crotobarin (**261**) from *C. barorum* and crotogaudin (**262**) from *C. goudotii* have been reported (Rakotonandrasana *et al.*, 2010).

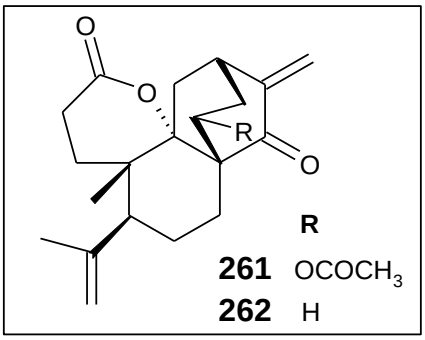


Figure 2.18: Atisane diterpenoids from *Croton* species

2.4.5.4.2 Kauranes

Kauranes are the commonest class of the tetracyclic diterpenoids reported from *Croton* genus [Table 2.12; Figure 2.19].

Table 2.12: Kauranes from *Croton* genus

Code	Name	Source
263 -274	Twelve kauranes and *ent*-kauranes	Vietnamese *C. tonkinensis* (crude extract significantly cytotoxic (Kuo *et al*., 2007)
275 -290	Fifteen *ent*-kauranes from the leaves only (Minh *et al*., 2003; Ngadjui *et al*., 2002; Giang *et al*., 2005)	
291	Argyrophilic acid, a stereoisomer of cunabic acid found to be active against gram positive bacteria *in vitro* (Giang *et al*., 2004)	*C. argyrophylloides*
292	*Ent* –15 -oxokaur – 16– en – 18 – oic acid (Fernandes *et al*., 1974)	
293	*Ent*-16β, 17-dihydroxykaurane	Japanese *C. sublyratus* (Monte *et al*., 1988)
294	Two *ent*-kauranes including this one	Asian *C. kongensis* (Kitazawa and Ogiso, 1981)
-	*Ent*-kauran-16β, 17-diol	*C. hutchinsonianus* (Chen *et al*., 2007)
-	*ent*-kauran-16β, 17, 19-triol	
295-297	Three *ent*-kauranoids	*C. lacciferus* (Li *et al*., 1990)
298	Geayine	*C. geayi* (Radulovic *et al*., 2006)
299	7-Oxogeayine	
300	-	*C. zambesicus* (Aiyar and Seshadri, 1970)
301-307	-	*C. pseudopulchellus* (Langat *et al*., 2012)

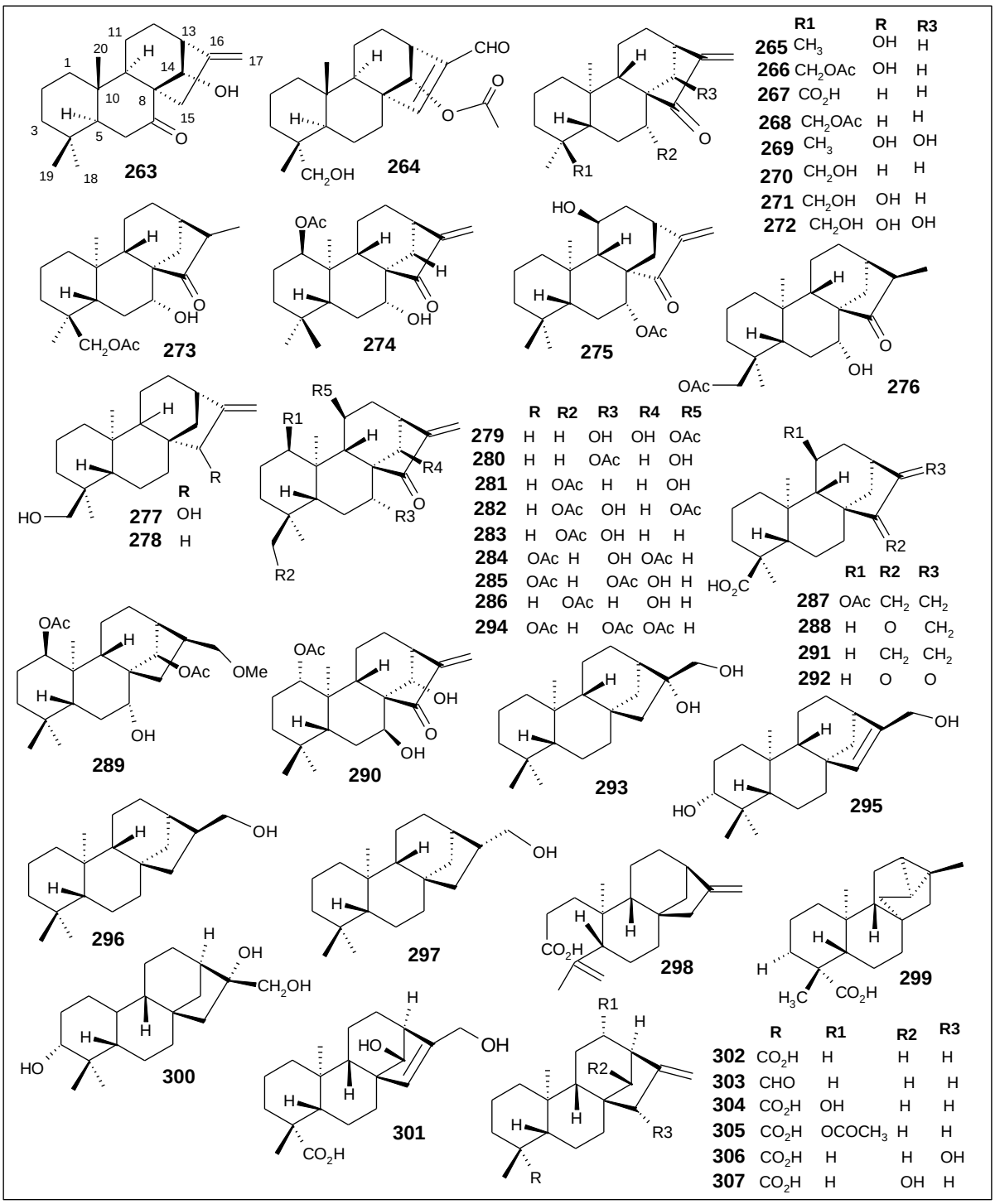


Figure 2.19: Kaurane diterpenoids from *Croton* species

2.4.5.4.3 Tiglianes

Polyhydroxylated tiglianes (**308**) esterified with linoleic and palmitic acid is among the irritant and co-carcinogenic (tumor-promoting) constituents of various members of the Euphorbiaceae family (Dewick, 2002). Phorbol (**309**) and isophorbol (**310**) diterpenoids are C- 4 epimers obtained upon hydrolysis of their esters. Hydrolysis of prostratin (**311**), isolated from *Pimela prostrata* yields 12- deoxyphorbol (**312**). Fatty acid esters of 12- deoxyphorbol occur in various members of Euphorbiaceae [Figure 2.20]. The main irritant component of *C. tiglium* seeds is 12-*O*-tetradecanoylphorbol-13-acetate (**313**), a tumor promoter used in experimental mice cancer research (Bandara et *al*., 1988). Other phorbol esters of *C. tiglium* seeds include 13-*O*-acetylphorbol-20-linoleate, 13-*O*-tigloylphorbol-20-linoleate, 12-*O*-acetylphorbol-13-tigliate, 12-*O*-decanoylphorbol-13-(2-methylbutyrate), 12-*O*-tigloylphorbol-13-(2-methylbutirate) and 12-*O*-acetylphorbol-13-decanoate89, 12-O-tetradecanoylphorbol-13-acetate and 12-*O*-(2-methylbu t i r o y l) - p h o r b o l - 1 3 - d o d e c a n o a t e (Glaser *et al*., 1988). Small amounts of a phorbol ester were detected in flowers of *C. draco* (Murillo *et al.*, 2001).

A nitrogenous phorbol ester (**314**) with inhibitory effects on cyclo-oxygenase which is responsible for production of prostaglandins from arachidonic acid has been reported from *C. ciliatoglandulifer* (El-mekkawy *et al.,* 2000). Another phorbol derivative with anti-plasmodial activity, steenkrotin A (**315**) from *C. steenkampianus* has been reported (Adelekan *et al*., 2008). Also included in this category are some crotofolane diterpenoids: -crotoxide A and B (**316** and **317**) reported from *C. dichogamus* (Rios and Aguilar-Guadarrama, 2006); crotofolins A, B, C and E (**318**, **319**, **320** and **321** from *C. corylifolius*, a Jamaican species closely related to *C. dichogamus* (Rios and Aguilar-Guadarrama, 2006) and crotohaumanoxide (**322**) from *C. haumanianus* (Tchissambou, 1990). Hydrolysis of a methanol soluble extract of essential oils obtained from *C. macrostachys* seeds showed presence of phorbol esters upon comparison with a hydrolyzed product of commercially available 12-*O*-tetradecanoyl-phorbol-13-acetate (**313**) (Mazzanti *et al*., 1987).

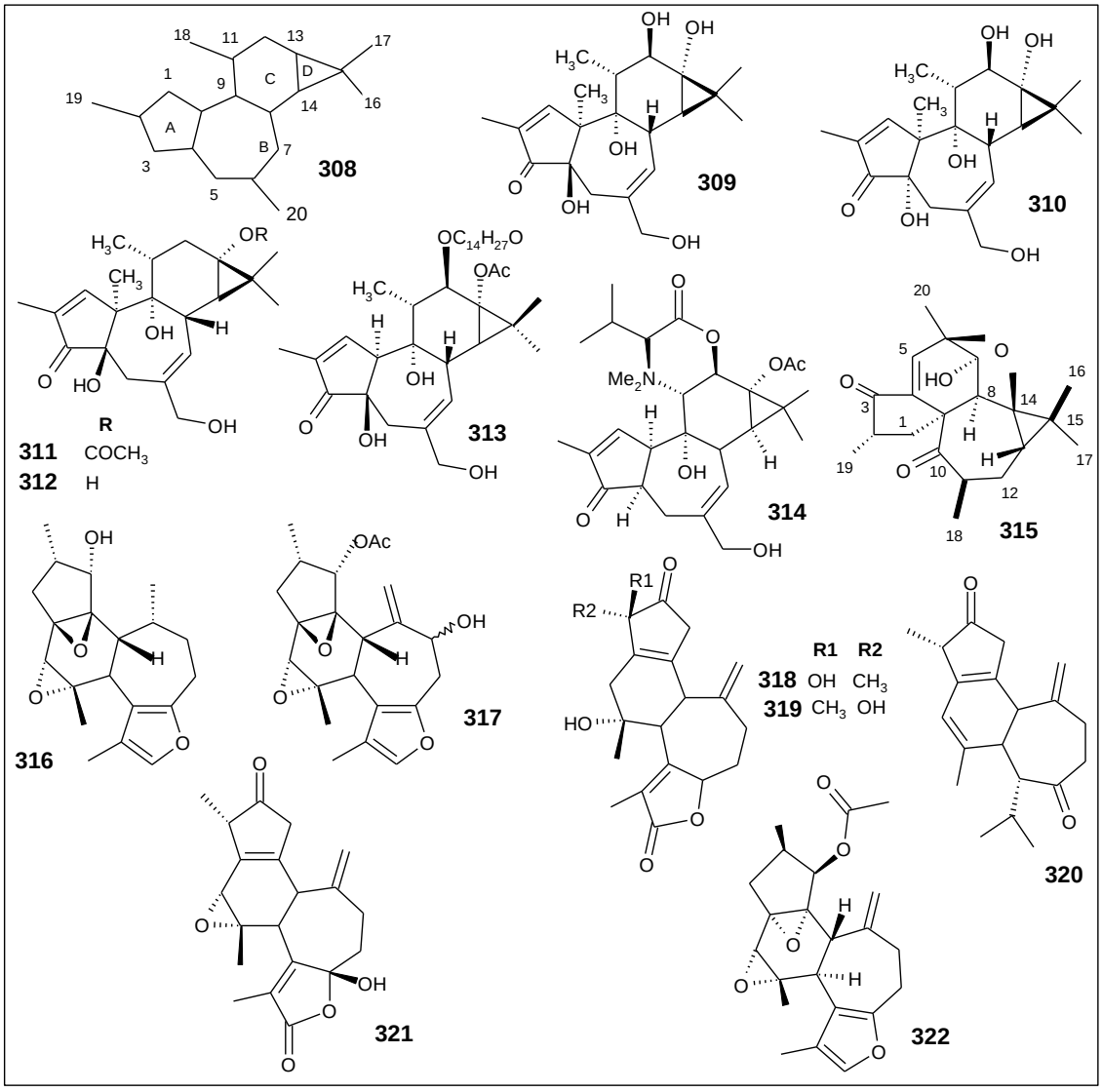


Figure 2.20: Tiglianes and Phorbolesters from *Croton* species

2.4.5.5 Pentacyclic diterpenoids from *Croton* genus

In this category, only trachylobanes are reported from two African *Croton* species [Figure 2.21]. From Beninian *C. zambesicus*, *ent*-18-hydroxy-trachyloban-3-one (**323**) and its vaso-relaxant properties (Jogia *et al*., 1989), *ent*-trachyloban-3-one (**324**), **325**, **326**, *ent*-trachyloban-3β-ol (**327**) and **328** are reported (Ngadjui *et al*., 1999; Block *et al*., 2004; Aiyar and Seshadri, 1970). Cameroonian *C. zambesicus* is reported to have produced compounds **329**, **330**, 7β-acetoxytrachyloban-18-oic (**331**) and trachyloban-7β-18-diol (**332**) (Ngadjui *et al*., 1999). Compounds **333**, **334**, trachyloban-18-oic acid (**335**), trachyloban-19-oic acid (**336**), 3α, 19- dihydroxytrachylobane (**337**), 3α, 18, 19-trihydroxytrachylobane (**338**), 3β,19 – dihydroxytrachylobane (**339**) and 3β,18,19 – trihydroxytrachylobane (**340**) are reported from Eastern Africa *C. macrostachyus* (Addae-mensah *et al*., 1989; Kapingu *et al*., 2000).

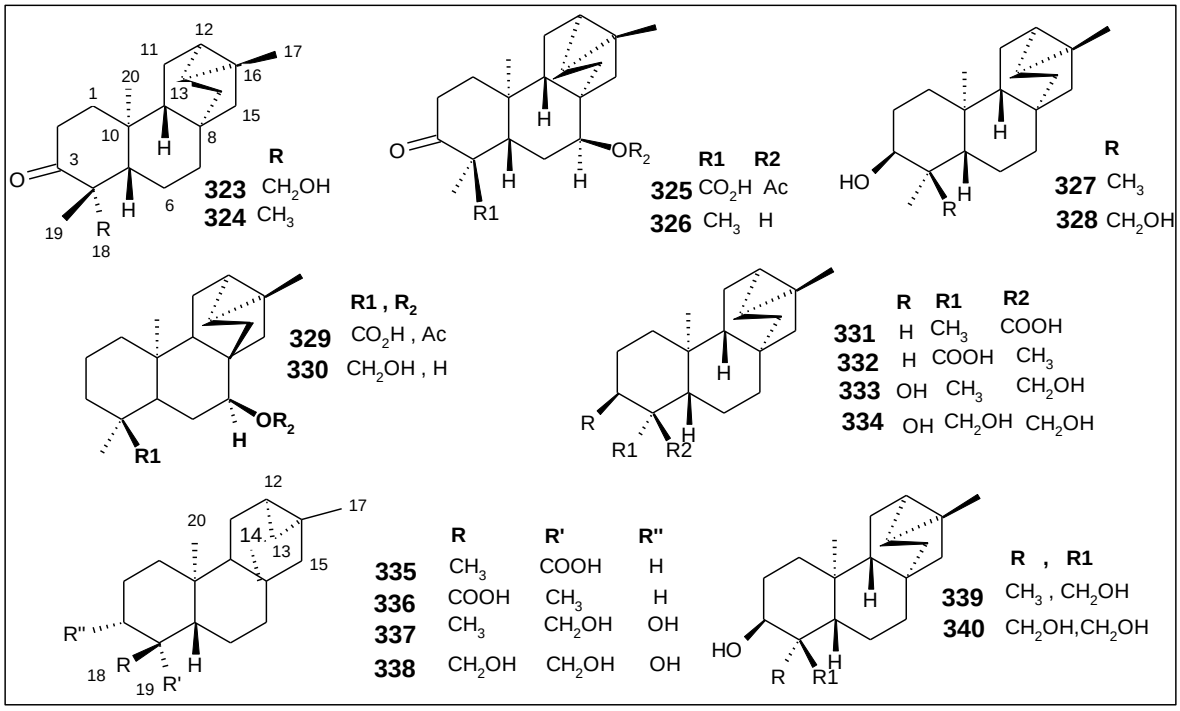


Figure 2.21: Trachylobanes from *Croton* species

2.4.5.6 Macrocyclic diterpenoids from *Croton* genus

Cembranoids are the macrocyclic diterpenoids reported from *Croton* genus [Table 2.13; Figure 2.22]. *C. zambesicus* is a tropical African medicinal plant whose phytochemistry has extensively been studied. It is reported in world plant check list database as being a synonym of *C. gratissimus* var. *gratissimus*, *C. amabilis* Muell. Arg. and *C. welwitschianus* Muell. Arg. (Kew plant data base, 2012 and 2013). A wide range of compounds including, labdane, clerodane, and trachylobane diterpenoids and flavone-C-glycosides have been reported from *C. zambesicus* (Ngadjui *et al*., 1999; Aiyar and Seshadri, 1970). This section however reports *C. gratissimus* as having predominantly yielded cembrane diterpenoids (Pudhom *et al*., 2007; Mulholland *et al*., 2010). The remarkable difference in the chemical constituents between *C. zambesicus* and *C. gratissimus* var. *gratissimus* is therefore a sharp contrast in their acclaimed synonymy. Jatrophone (**358**) reported from *Euphorbia* species is included in this category because it is an intermediate skeleton during the biosynthesis of many important diterpenoids from cembrane molecules [Scheme 5].

Table 2.13: Cembranoids from *Croton* species

Code	Name	Source (biological activities)
341	Neocrotocembranal (Baccelli *et al*., 2007)	**341-343** from the stem bark of *C. oblongifolius* **344** from *C. poilanei*. Compound **341** was found to inhibited platelet aggregation induced by thrombin (IC_{50} 47.21 μg/ml) and have cytotoxicity against P-388 cells *in vitro* (IC_{50} value of 6.48 μg/ml). Compounds **341-344** were studied for their inhibitory activities against cAMP phosphodiesterase. Those with carboxylic acid functional groups showed higher activity (Roengsumran *et al*., 1998)).
342	Crotocembranoic acid (Roengsumran *et al*., 1999)	
343	Neocrotocembranoic acid (Roengsumran *et al*., 1999)	
344	Poilaneic acid (Roengsumran *et al*., 2002)	
345, 346 and **347**	Furano-cembranoids	*C. oblongifolius* (they all showed broad cytotoxic activities against five cell lines-BT474, CHAGO, Hep-G2, KATO-3, and SW-620 by the MTT [3-(4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide] colourimetric method (Roengsumran *et al*., 1998; Sato *et al*., 1981)
348	Lactonized cembranoid	
349	(+)-[1*R*,10*R*]-cembra-2*E*,4*E*,7*E*,11*Z*-tetraen-20,10-olide	**349-357** were all isolated from Southern Africa *C. gratissimus* (methanol and water extracts of this plant showed scavenging ability of hydroxyl radicals (Langat *et al*., 2011) and 5-lipoxygenase inhibitory activity (Steenkamp *et al*., 2005). Compounds **350** and **352** had lower potency than paclitaxel when subjected to PEO1 and PEO1-TaxR ovarian cancer cell lines. Their sensitivity to taxane sensitive and taxane resistant cells was however similar (Pudhom *et al*., 2007; Mulholland *et al*., 2010). The isomer of compound **356**, (+)-[10*R*]-cembra-1*Z*, 3*Z*, 7*E*, 11*Z*, 15-penten-20, 10-olide was isolated from the leaves (Mulholland *et al*., 2010).
350	(+)-[1*R*,4S,10*R*]-4-hydroxycembra-2*E*, 7*E*,11*Z*-trien-20,10-olide	
351	(-)-[1*R*,4*R*,10*R*]-4-hydroxycembra-2*E*, 7*E*, 11*Z*-trien-20, 10-olide	
352	(+)-[1*R*,2*S*,7*S*,8*S*,12*R*]-7,8-epoxy-2,12-cyclocembra-3*E*,10*Z*-dien-20,10-olide	

353 & 354 (epimers at C-7)	(+)-[1*S*, 4*S*, 7*R*, 10*R*]-1,4,7-trihydroxycembra-2*E*, 8 (19),11*Z*-trien-20, 10-olide	
355 (hydroxyl derivative of **350**)	(-)-[1*S*, 4*S*, 10*R*]-1, 4-Dihydroxycembra-2*E*, 7*E*, 11Z-trien-20, 10-olide	
356	(+)-[10*R*]-cembra-1*E*, 3*E*, 7*E*,11*Z*,15-penten-20,10-olide	
357	(+)-[1*S*, 4*R*, 8*S*, 10*R*]-1, 4, 8-Trihydroxycembra-2*E*,6*E*,11*Z*-trien-20, 10-olide	

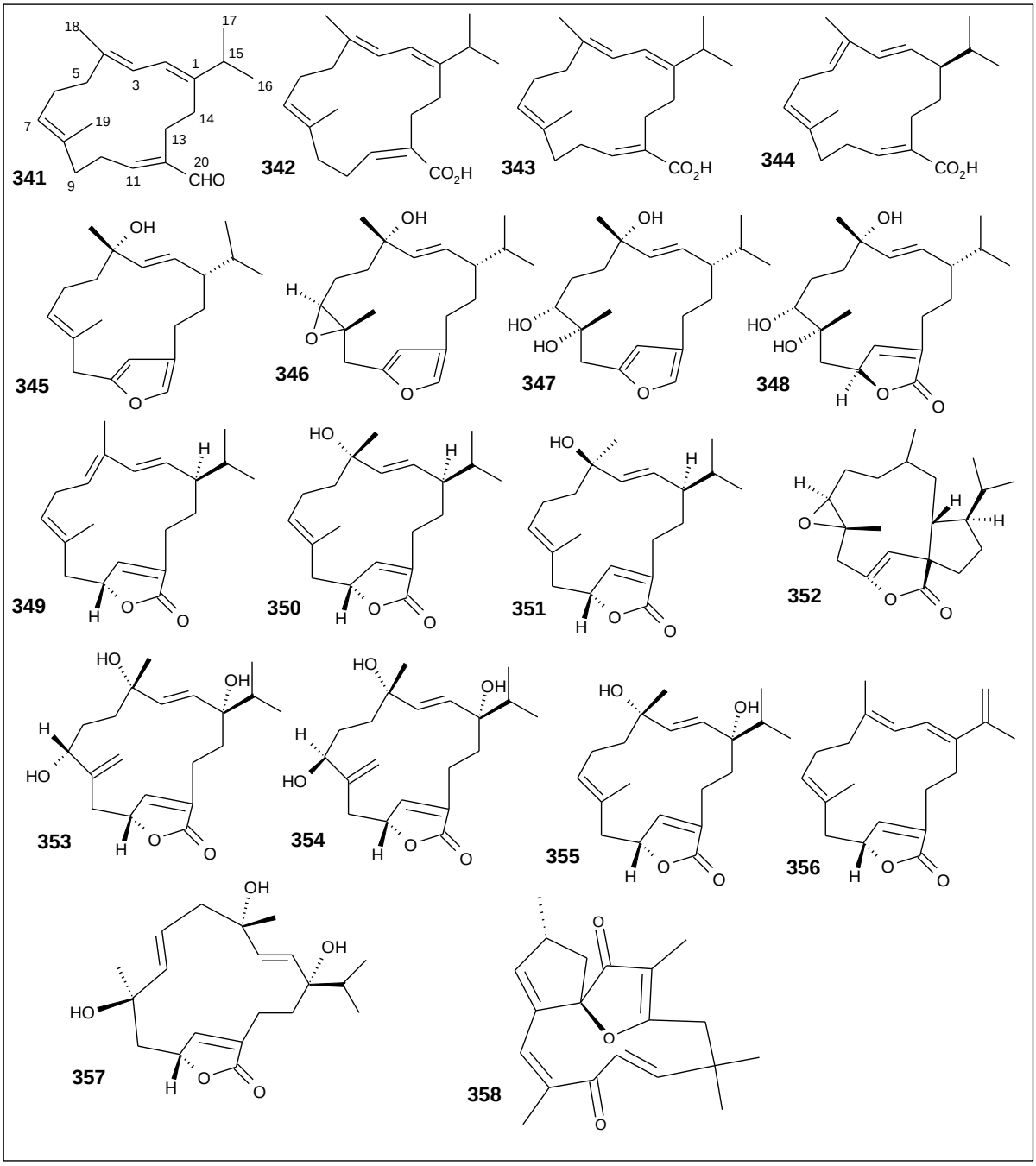


Figure 2.22: Cembranoids from *Croton* species and jatrophone from *Euphorbia* species

2.4.5.7 Limonoids from *Croton* genus

Only one research group has reported the isolation of limonoids from a *Croton* plant, *C. jatrophoides* (Kubo *et al*., 1990; Nihei *et al*., 2002, 2005 and 2006). This report is however highly doubted because it is the first and the only group reporting a member of the Euphorbiaceae family as producing several limonoids that are known to be restricted to the Meliaceae, Simaroubaceae, Rutaceae, Cneoraceae and Flacourtiaceae families (Langat, 2009). A specimen of the *C. jatrophoides* plant studied and reported in one of their publications to have yielded the limonoids, was not recorded as having been deposited in any herbarium (Kubo *et al*., 1990). However, in subsequent papers published by the same research group (Nihei *et al*., 2002, 2004, 2005 and 2006) it is reported that the plant specimen (AC 76-134) was deposited at the University of Nairobi Herbarium but a spot check did not confirm it.

The above aluded observations raise doubts on the true identity of the plant that yielded the limonoids. *C. jatrophoides* is not listed by any of the authority books on Kenyan plant species (Kokwaro, 2009; Beentje, 1994). It is listed as a Tanzanian *Croton* species (Kokwaro, 2009) and there are phytochemical reports on isolation of five diterpenoids from it four clerodanes, isoteucvin, an isomer of teucvin and another teucvin derivative, one halimane, penduliflaworosin and jatropholdin (Mbwambo *et al*., 2009). Until other members of the *Croton* genus are shown to yield limonoids, the correct identification of the *C. jatrophoides* worked on by this research group (Kubo *et al*., 1990; Nihei *et al*., 2002, 2004, 2005 and 2006) remains questionable.

The chemical structures of the limonoids that were reported supposedly from *C. jatrophoides* by Kubo *et al*., 1990 and Nihei *et al*., 2002, 2004, 2005 and 2006 are given in Figure 2.23 (Lemos *et al*., 1992; Santos *et al*., 2008; Sommit *et al*., 2003; Ngamrojnavanich *et al*., 2003). Their names are: - dumsin (**359**); zumsin (**360**); zumketol (**361**); zumsenin (**362**); zumsenol (**363**); dumnin (**364**); dumsenin (**365**); musidunin (**366**) and musiduol (**367**). Compounds **364** - **367** showed potent anti-feedant activity ($PC_{50} \leq 2.0$ µg/mL) against the larvae of the pink bollworm, *Pectinophora gossypiela* and fallworm, *Spodoptera frugiperda* (Nihei *et al*., 2004 and 2006)).

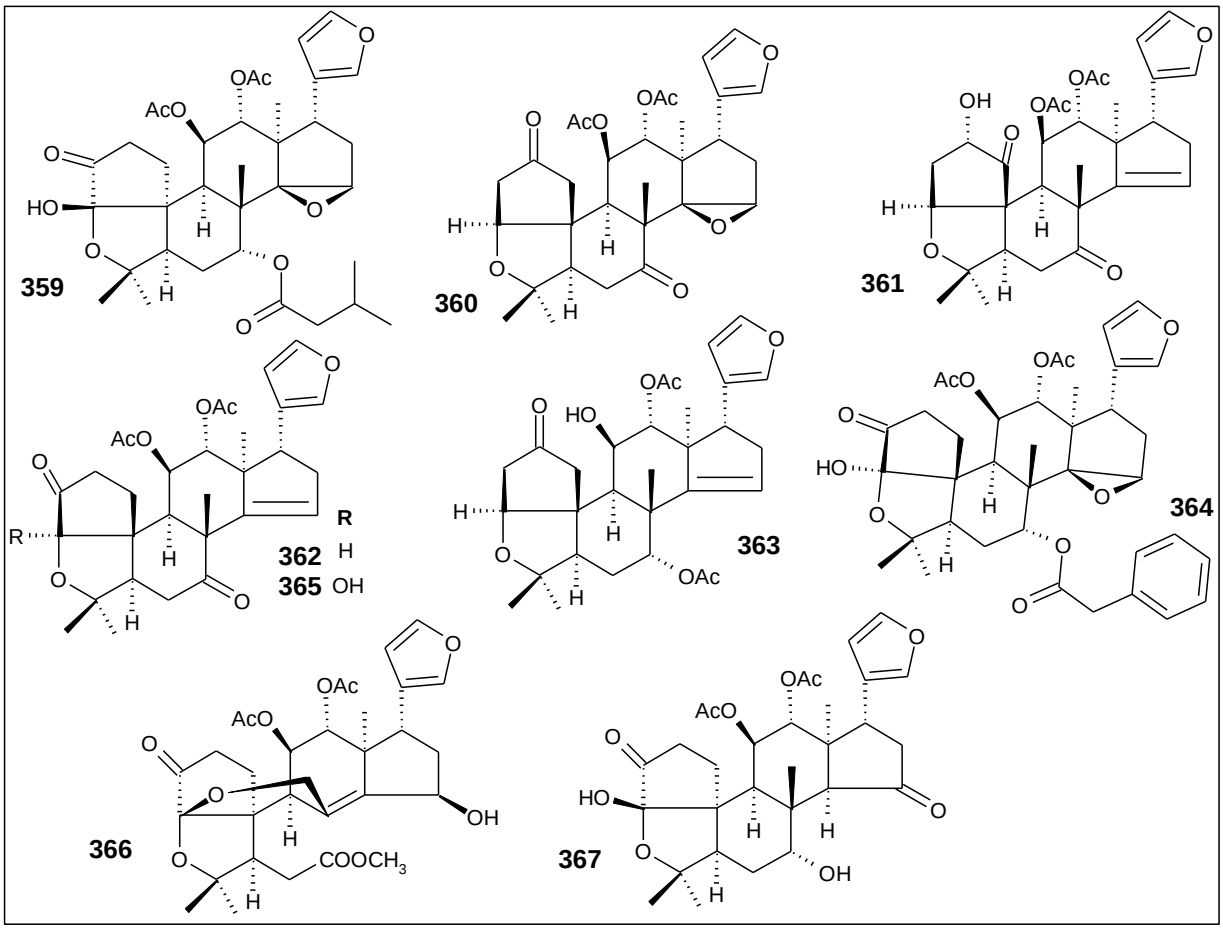


Figure 2.23: Limonoid diterpenoids reported supposedly from *Croton jatrophoides*

2.4.6 Triterpenoids and Phytosterols

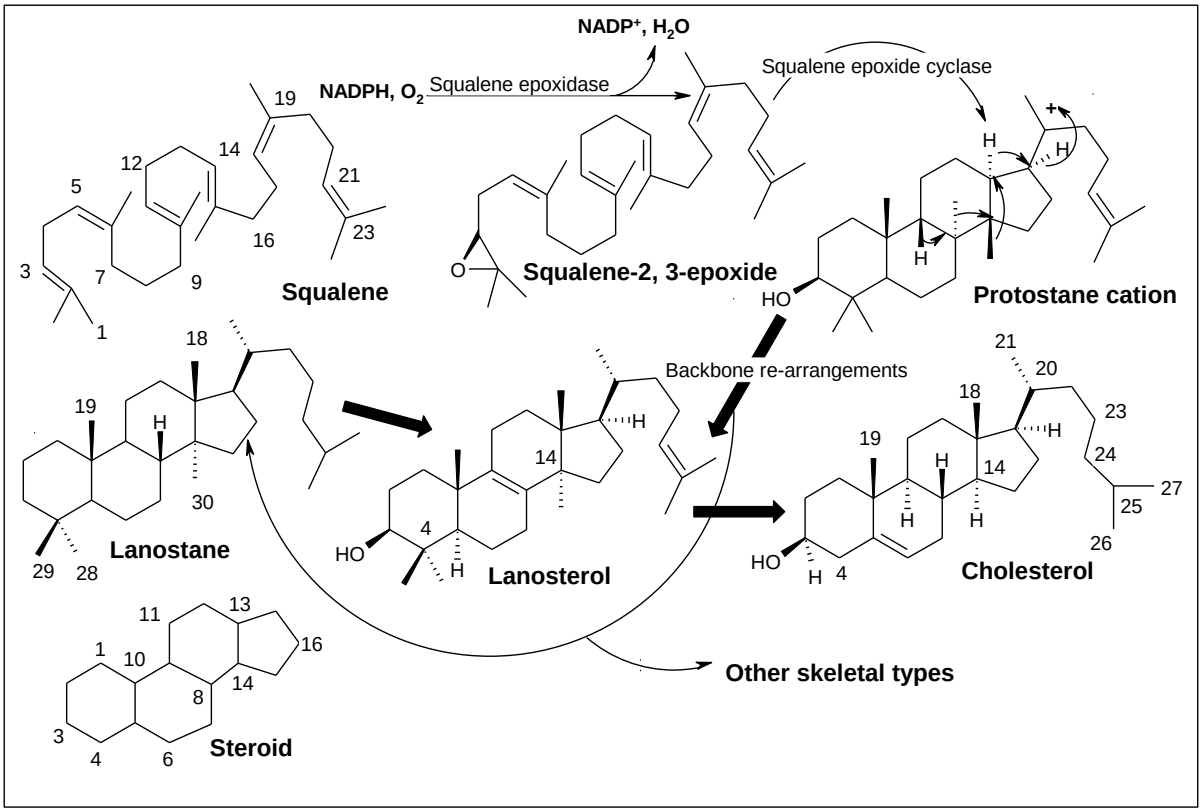
Triterpenoids are C_{30} compounds derived from six isoprene units and are widely distributed in plant kingdom in a free state or as esters or glycosides. They are further sub-grouped into tetracyclic and pentacyclic triterpenoids. Phytosterols are degraded forms of terpenoids. A number of tritepenoids and phytosterols were isolated from the plants that were being investigated in this study. Consequently, the section which follows here will describe their biosynthesis.

2.4.6.1 Biosynthesis of Triterpenoids and Phytosterols

Biosynthetically, triterpenoids are derived from squalene [Scheme 2]. The 3β-hydroxytriterpenoids, however, originates from the 3*S*-isomer of squalene 2, 3-epoxide. Cyclisation of the chair-boat-chair-boat conformation of squalene 2, 3-epoxide gives the protostane cation [Scheme 6].

A series of 1, 2-hydride and methyl migrations, commonly called backbone rearrangements occurs in the protostane (protosteryl) cation, to give a variety of triterpenoid skeletal types, lanostane (from where steroids are made) being one of them (Frum and Viljoen, 2005; Dewick, 2002).

Steroids are modified triterpenoids containing the tetracyclic ring system of lanosterol but lacking the three methyl groups at C-4 and C-14 (C-28, 29, 30 in the lanostane numbering). A wide range of biologically important natural products, steroids included, are derived from a cholesterol basic structure with modifications especially to the side-chain. The functional groups attached to the steroid nucleus give them the profound biological activities used in routine medicine. Most natural triterpenoids and steroids contain a 3-hydroxyl group arising from the original epoxide oxygen of oxidosqualene with the C-10 methyl and H-5 sharing an anti-axial relationship. For steroids, majority have one or two methyl groups present at the bridgehead positions C-10 and C-13 with their methyl carbon atoms numbered C-19 and C-18 respectively as shown in the structure of cholesterol [Scheme 6].



Scheme 6: Biosynthesis of triterpenoids and phytosterols

2.4.6.2 Triterpenoids from *Croton* genus

Triterpenoids of various carbon skeletons have been reported from the *Croton* genus [Table 2.14; Figure 2.24].

Table 2.14: Triterpenoids from *Croton* species

Code	Name	Carbon skeleton	Source
368	Acetylealeuritolic acid	Taraxerane	*C. cajucara*, *C. tonkinesis*, *C. megalocarpus*, *C. hovarium*, *C. urucarana* (Addae-mensah *et al*., 1989; Maciel *et al*., 1997; Krebs and Ramiarantosa, 1996 and 1997; Puebla *et al*., 2003; Pham and Pham, 2002)
369	Lupeol (Ngadjui *et al*., 1999; Addae-mensah *et al*., 1989; Mulholland *et al*., 2010; Tschissambou, 1990)	Lupane	*C. zambesicus*, *C. megalocarpus* , *C. gratissimus* and *C. haumanianus*
370	3β-*O*-Acetoacetyl lupeol		*C. megalocarpus* (Addae-mensah *et al*., 1989)
371	Betulin		
372	Lupenone (Barbosa *et al*., 2003)		*C. betulaster*
373	20-Hydroxylupan-3-one		
374	Friedelin	Friedelane	*C. hovarum* (Krebs and Ramiarantosa, 1996 and 1997)
375	β-Amyrin	Oleanane	
376	3-Oxo-olean-12-en-28-oic acid		*C. betulaster* (Barbosa *et al*., 2003)
377	3-Oxo-olean-18-en-28-oic acid		
378	α-Amyrin (Block *et al*., 2004)	Ursane	*C. hieronymi*
379	α-Amyrin acetate		*C. hieronymi*, *C. tonkinensis* (Addae-mensah *et al*., 1989; Pham and Pham, 2002)
380	3-Oxo-20β-hydroxytarastane	Taraxastane	*C. betulaster* (Barbosa *et al*., 2003)
381	3-Oxo-22-hydroxyhopane	Hopane	
382	Hop-22-(29)-en-3β-ol		*C. hieronymi* (Risco *et al*., 2003)

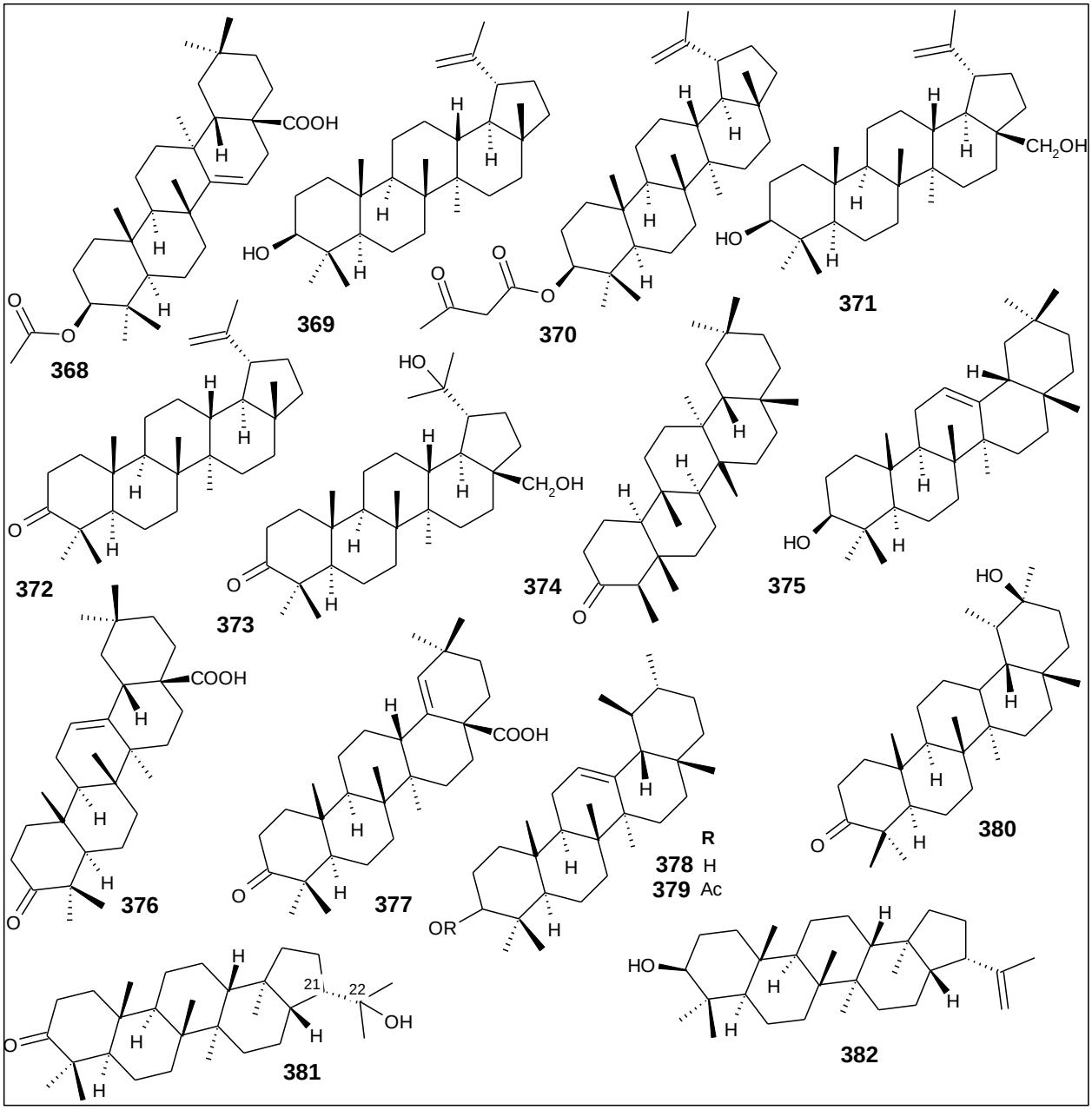


Figure 2.24: Triterpenoids from *Croton* species

2.4.6.3 Phytosterols from *Croton* genus

Quite a number of phytosterols [Figure 2.25] have been reported from *Croton* genus. Included is:- sitosterol (**383**) from *C. zambesicus* (Ngadjui *et al*., 1999) and *C. membranaceus* (Bayor *et al*., 2009); sitosterol -3-D-glucoside (**384**) , DL- threitol (**385**) (Bayor *et al*., 2009) and ethylcholesta 4, 22-diene-3-one (**386**) from *C. gratissimus* (Mulholland *et al*., 2010); cholestan-5,7-dien-3-ol (**387**), 3-hydroxycholest-5-en-7-one (**388**), cholestan-3-one (**389**) and ergosterol (**390**) from *C. pseudopulchellus* (Langat *et al*., 2012). Others are stigmasterol, campesterol, 3 – oxocycloart – 24*E* – en – 26 – oic acid, 22 –dihydrobrassicasterol, cholesterol, ergosta – 4, 22 – dien – 3 – one, cholest – 8(14) – en - 3β-ol, gramisterol, lophenol, isofucosterol, cholest – 4-en – 3 – one and β-sitostenone.

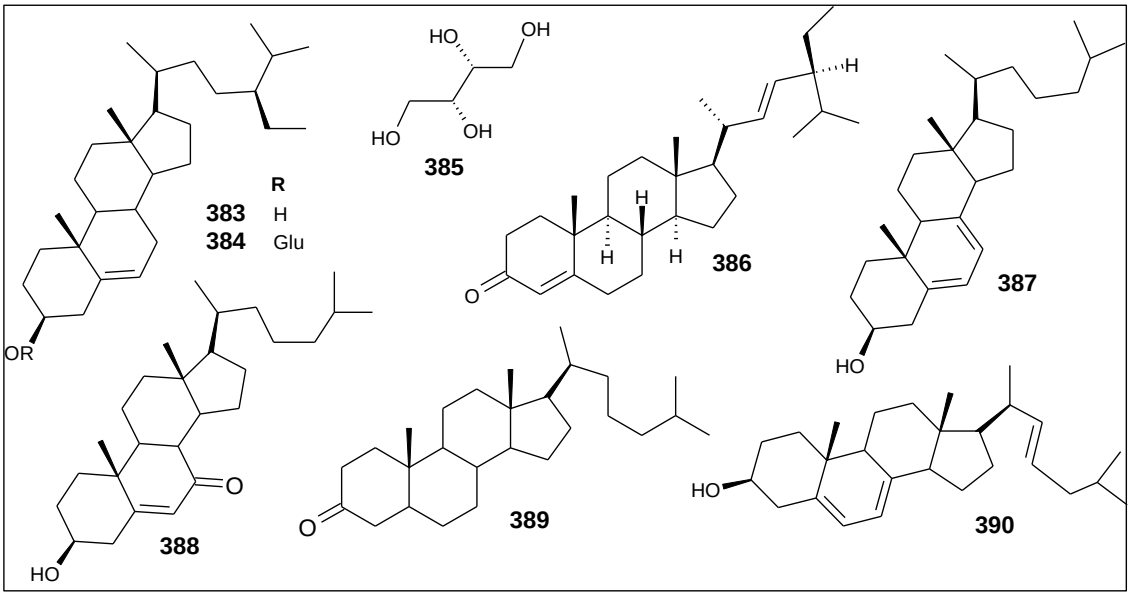


Figure 2.25: Phytosterols from *Croton* species

CHAPTER THREE
METHODOLOGY

3.1 General experimental procedure

Infra Red (IR) spectra were recorded using a Perkin-Elmer (2000) FTIR spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$ on a 500 MHz Bruker AVANCE NMR instrument at room temperature. Chemical shifts, δ, were expressed in ppm and referenced against the solvent resonances at 7.28 and 77.23 ppm for ^{1}H and ^{13}C- NMR respectively. Mass Spectra were recorded on a GC-MS Bruker MicroToF Mass Spectrometer by direct injection using a Bruker Bioapex-FTMS with electrospray ionization. GC-MS spectra were recorded on an Agilent 7890A instrument (University of Oxford). The above analysis was done at the Department of Chemistry, Faculty of Engineering and Physical Sciences, Surrey University-UK.

Column chromatographies were done at the Department of Chemistry, University of Nairobi and Department of Chemistry, Faculty of Engineering and Physical Sciences, Surrey University-UK. Merck Silica gel 60 (0.063-0.200 mm) and Fluka Sephadex LH-20 as stationary phases and analytical TLC using factory prepared aluminium plates (0.25 mm) coated with silica gel (high-purity grade (Merck Grade 9385), pore size 60 Å, 230–400 mesh particle size) were used. Compounds were visualized by observation under UV light at 254 or 365 nm, followed by spraying with 1% vanillin-sulphuric acid spray reagent and warming.

3.2 Plant sources

C. alienus plant parts (leaves, stem and roots) were collected in September 2007 from Ngong forest in Nairobi City County, *C. sylvaticus* in May 2009 from Taita Hills and *C. megalocarpoides* in July 2009 from the Kenyan Coastal region. The plants were identified at the University of Nairobi herbarium in the School of Biological Studies and voucher specimens, BN 2007/12 for *C. alienus*, BN 2008/6 for *C. sylvaticus* and BN 2008/8 for *C. megalocarpoides* deposited there.

3.3 Extracting plant parts for preliminary screening

The plant parts were dried under shade for 4 weeks after which they were ground into a fine powder. Distilled water was used to extract 10 g of the powder by boiling (3 x 20 minutes), cooling, filtering and freeze drying the filtrates. Similarly, 10 g of the powder was extracted using methanol by cold percolation (3 x 72 hrs) at room temperature followed by filtration and concentration of the combined extracts under reduced pressure below 50 ^{O}C using a rotary evaporator.

3.4 Phytochemical and antioxidant activity screening of crude plant extracts

Qualitative phytochemical screening was done at the Center for Traditional Medicine Research in Kenya Medical Research Institute (KEMRI). Documented standard procedures for presence of alkaloids, anthraquinones, flavanoids, phenolic compounds, steroids and terpenoids were used on both aqueous and methanol plant extracts (Harborne, 1984; Peter and Amala, 1998).

Total Phenolic Content (TPC) and anti-oxidant potential assessment of the crude plant extracts was done at JSS College of Pharmacy in Ooty-Tamil Nadu state, India. Folin-Ciocalteu reagent was used to estimate the total phenolic content (TPC) of the extracts A 0.1 mL suspension of 1 mg / mL extract in distilled methanol in an Erlenmeyer flask was made up to 50 mL using distilled water to produce a 2M solution. A solution of 10% Folin-Ciocalteu reagent in distilled water was made and 1 mL of it added to the plant extract suspension followed by 3 mL of 0.7M sodium carbonate solution three minutes later. The mixture was thoroughly shaken for 2 hrs at room temperature and its absorbance taken at 760 nm using a spectrophotometer. A serially diluted gallic acid monohydrate standard solution (250 µg / mL to 25 µg / mL) was used to prepare the standard curve. The TPC in the extract was expressed as % *w / w* gallic acid equivalent.

DPPH radical scavenging method was used to evaluate the anti-oxidant potential of the extracts using ascorbic acid as a standard. The assay was carried out in a 96 well microtiter plate. 100µM DPPH solution (200 µL) was added to 10 µL of test sample (prepared by dissolving weighed sample in DMSO and serially diluting it to give a range of concentrations, 1,000 µg / mL to 1.95 µg / mL). The plates were then incubated at 37^{O}C for 20 minutes and the absorbance of each well measured at 490 nm, using microtiter plate reader (ELISA) against the corresponding test and standard blanks.

The remaining DPPH of the test sample was compared with that of the standard (ascorbic acid) by expressing it as IC_{50} (concentration of the sample required to scavenge 50% of DPPH free radicals, calculated as, % inhibition = Absorbance of [(Control- Sample) / Control] x 100%).

3.5 Biological activity screening of crude plant extracts and isolated compounds

Anti-microbial activity tests of the crude plant extracts were done at the Center for Microbiology Research- KEMRI. Mosquito larvicidal activity assay was done at the School of Biological Sciences, University of Nairobi. The National Center for Natural Products Research, School of Pharmacy- University of Mississippi in collaboration with Prof. L. Walker and Prof. Illias Muhammad conducted the anti-leishmanial, anti-plasmodial and general cytotoxicity activity tests and the anti-microbial activity test of the isolated compounds. In all these assays, standard procedures were followed as alluded to in the sections following below.

3.5.1 Anti-microbial screening procedure

The antimicrobial tests of the crude plant extracts were done using American Type Culture Collection (Manassas, VA) organisms, referenced as ATCC in this text except where otherwise indicated. Aqueous and methanol crude extracts were assayed using sterile filter paper disc diffusion method. Different strains of bacteria (*Bacillus subtillis*, local isolate; *Escherichia coli*, ATCC 25922 and *Staphylococcus aureus*, ATCC 25923) and fungi (*Aspergillus niger*, local isolate; *Cryptococcus neoformans*, ATCC 90113 and *Candida albicans*, ATCC 90028). The extracts were tested at high concentrations of 100 mg / mL, 50 mg / mL, 25 mg / mL and 10 mg / mL. Negative control (DMSO) and positive controls (gentamycin for bacteria and nystatin for fungi assays) were included in each assay.

The isolated compounds were assayed at high concentrations of 20 µg / mL on a variety of sampled fungi and bacteria strains (*Candida albicans* , ATCC 90028 (Ca); *Candida glabrata*, ATCC 90030 (Cg); *Candida krusei*, ATCC 6258 (Ck); *Aspergillus fumigates*, ATCC 90906 (Afu); *Cryptococcus neoformans*, ATCC 90113 (Cn); *Staphylococcus aureus*, ATCC 29213 (Sa); Methicillin-resistant *S. aureus*, ATCC 33591 (MRS); *Escherichia coli*, ATCC 35218 (Ec); *Pseudomonas aeruginosa*, ATCC 27853 (Pa) and *Mycobacterium intracellulare*, ATCC 23068 (Mi)).

Susceptibility testing was done using a modified version of the CLSI methods as described in literature (Samoylenko *et al*., 2009). Drug controls ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included in each assay.

3.5.2 *In vitro* anti-leishmanial

In vitro anti-leishmanial activity was evaluated using a culture of *Leishmania donovani* promastigotes in two phases (primary for selecting those to undergo secondary screening) with pentamidine and amphotericin B as positive controls. High concentrations of 80 µg/mL (for primary assay) and 40 µg/mL (for secondary assay), appropriately diluted were added to the *Leishmania* promastigotes culture (2 x 106 cells / mL) in triplicates. The plates were then incubated at 26 ^{O}C for 72 hrs. Growth of *Leishmania* promastigotes in each test sample was determined by alamar blue assay and IC_{50} values computed from the growth inhibition curve (Samoylenko *et al*., 2009).

3.5.3 *In vitro* anti-plasmodial

Crude extracts and pure compounds were tested for their *in vitro* anti-plasmodial activity by a modified assay that determines the parasitic lactase dehydrogenase (pLDH) activity (Peter and Amala, 1998; Makler *et al*., 1993) using two *Plasmodium falciparum* strains, D6 (chloroquine-sensitive) and W2 (chloroquine-resistant). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method as described in literature (Trager and Jensen, 1976). Full dose-response curves were generated by plotting percent growth of the *P. falciparum* protozoan against test concentrations to determine concentration inhibiting 50% of parasite growth (IC_{50}-values) relative to negative (DMSO) and positive controls (artemisinin and chloroquine drugs).The test protocol involved two stages, primary and secondary screening.

Primary screening involved testing the crude extracts against the D6 *P. falciparum* strain at 47,600 ng / mL in duplicate. Inhibitions (% inh.) were calculated relative to the negative and positive controls. Extracts showing ≥ 50% inhibition were selected for secondary screening in which dissolved samples (crude extracts, some column fractions and pure compounds) were tested at 47600, 15867, and 5289 ng / mL and IC_{50} reported.

3.5.4 *In vitro* cytotoxicity

General cytotoxicity on animal cell viability was studied using monkey kidney fibroblasts (VERO) obtained from the American Type Culture Collection (ATCC, Rockville, MD). The assay was performed in a 96-well tissue culture-treated micro-plate in which cells were seeded at a density of 25000 cells / well and incubated for 24 hrs. Samples at different concentrations were added and plates again incubated for 48 hrs. The number of viable cells was determined using neutral red according to a modified procedure described in literature (Barbosa *et al*., 2007). Doxorubicin and DMSO were used as positive and negative controls respectively and selectivity indices (SI) ratio of VERO to D6 and W2 calculated and expressed as IC_{50}.

3.5.5 Mosquito larvicidal assays

Crude extracts and isolated compounds were tested against two species of mosquito larvae, *Anopheles gambiae* s.s. and *Aedes aegypti* L. (Diptera Culicidae) according to standard WHO bioassays for larvicidal activity (WHO, 1996). Standard *w/v* concentrate of each test material in DMSO was made in three replicates. Twenty late third-instar larvae were transferred into each of the test and control solutions (azadirachtin and DMSO). Larval mortality was recorded after 24 hrs. Dead larvae in the three replicates were combined and expressed as a percentage mortality of each concentration using a computerized log-probit analysis at 95% confidence intervals. Lethal dosages (LC_{50} and LC_{95}) were used to measure the potency of test samples and < 100 ppm potencies recorded.

3.6 Extraction and isolation of compounds from *Croton megalocarpoides*

The air dried root bark of *C. megalocarpoides* (500 g) was sequentially extracted by cold percolation at room temperature (3 x 2L solvent, 24 hrs each). The extracts were concentrated using rotary evaporator, combined and left to dry yielding 9 g (1.8%) *n*-hexane, 47 g (9.4 %) dichloromethane (DCM) and 16 g (3.2 %) methanol extracts. From the DCM extract, 30 g were adsorbed in 30 g silica gel and subjected to CC on a silica gel column (300 g, 5×35 cm). Fractionation of CC was done using *n*-hexane with gradual increase of polarity using ethyl acetate solvent and monitored using analytical TLC plates. Purification of the fractions was done using DCM / diethyl ether solvent system of varying ratios to afford two phytosterols, sitosterol (4.5 mg) and stigmasterol (4.1 mg) and the compounds given in Table 3.1 below.

Table 3.1: Compounds isolated from the roots of *Croton megalocarpoides*

Code	Name	Mass (mg)
391	Crotocorylifuran	58.80
392	12-*Epi*-crotocorylifuran	13.40
393	8-Hydroxycrotocorylifuran	3.50
394	2-Ketocrotocorylifuran	3.50
395	7, 8-Dehydrocrotocorylifuran	5.10
396	Megalocarpoidolide F	38.50
397	12-*Epi*-megalocarpoidolide F	14.30
398	Megalocarpoidolide E	50.90
399	Megalocarpoidolide G	23.30
400	Megalocarpoidolide H	16.80
401	Megalocarpoidolide I	84.40
402	Megalocarpoidolide J	6.70
403	Megalocarpoidolide K	16.70
404	Isolophanthin A	6.10
405	Isolophanthin E	10.90
406	Abietic acid	4.30
407	3α, 18-dihydroxytrachylobane	13.0
408	*Ent*-trachyloban-18-ol	4.10
409	*Ent*-trachyloban-18-oic acid	3.80
410	*Ent*-3β-hydroxytrachyloban-18-al	6.50
411	Acetylaleuritolic acid	12.90
412	Lupeol	3.60

3.7 Extraction and isolation of compounds from *Croton alienus*

The leaves (1.3 kg) were dried under shade for 4 weeks, ground to fine powder and extracted by cold percolation at room temperature starting with 3 × 3 L of *n*-hexane, dichloromethane (DCM), 5% MeOH in DCM, and MeOH. The solvents were then removed under reduced pressure and the extracts obtained each chromatographed on sephadex (LH-20) packed columns using MeOH: DCM (1:1 *v/v*) to remove chlorophyll. The chlorophyll free extracts were weighed and 19.6 g (0.015%) *n*-hexane, 61.3 g (0.047%) DCM, 36.6 g (0.028%) 5% MeOH / DCM and 77.1 g (0.059%) MeOH extracts obtained.

The *n*-hexane and DCM extracts were combined due to similarities on their silica TLC compound profiles and chromatographed (40 g) over silica gel packed CC using a step gradient elution (*n*-hexane with increasing amounts of DCM). Fractions (75mL each) were collected from the initial column and subsequently purified using suitable solvent systems. The following compounds were obtained and weighed: - crotepoxide (**416**; 565.7 mg); monodeacetylcrotepoxide (**417**; 32.4 mg); dideacetylcrotepoxide (**418**; 174.3 mg); α-senepoxide (**419**; 36.8 mg); β-senepoxide (**420**; 61.7 mg) and (+)-(2*S*, 3R)-diacetoxy-1-benzoyloxymethylenecyclohex-4, 6-diene (**421**; 10.0 mg). Extraction of the roots of *C. alienus* (1.5 kg) was done using 3L of MeOH: DCM (1:1, *v/v*) and the solvent removed under reduced pressure to yield 184.1 g (12%) of crude extract. The extract (50 g) was chromatographed over silica gel and eluted in the same way as the leaves above, giving acetylaleuritolic acid (**411**; 33.0 mg); alienusolin (**413**; 8.6 mg); julocrotine (**414**; 132.3 mg); crotonimide C (**415**; 86.5 mg); crotepoxide (**416**; 174.7 mg) and D_4-stigmasterone (**422**; 65.4 mg).

3.8 Extraction and isolation of compounds from *Croton sylvaticus*

The air-dried and powdered roots bark (460 g) were extracted by cold percolation at room temperature using MeOH: DCM (1:1, *v / v*) solvent mixture (3 x 1L, 24 h each). The filtrates were then concentrated under reduced pressure using a rotary evaporator and combined to give 126.9 g (27.6%) yield of extract. The extract was re-extracted using various solvents and % yields determined as follows: - *n*-hexane (15.4 g; 12.1%), DCM (31.2 g; 24.6%) and MeOH (55.5 g; 43.7%). DCM and *n*-hexane extracts were combined due to their TLC compounds profile similarity. The combined extract (50 g) was adsorbed in 50 g silica gel, chromatographed over silica gel (500 g, 10 x 60 cm column) and step gradient eluted with *n*-hexane in increasing amounts of DCM. Fractions (75 ml each) were obtained and combined based on their TLC compound profiles. Purification of the fractions was done by re-chromatographing them over silica gel using DCM: diethyl ether (34:1 *v/v*) solvent system.

Eight compounds were obtained from this column:- stigmasteroid (5 mg); hardwickiic acid (**423**; 20.5 mg); *ent*-3,13*E*-clerodadiene-15-ol (**424**; 10.4 mg); 15-acetoxy-*ent*-3, 13*E*-clerodadiene (**425**; < 2 mg); 3, 8 (17),13*E*-clerodatriene-16-ol (**426**; < 2 mg); 15-formate-*ent*-3, 13*E*-clerodadiene (**427**; < 2 mg); crotohalimaneic acid (**428**; 5.8 mg); penduliflaworosin (**429**; 12.4 mg) and labda-13*E*-ene-8α, 15-diol-7 (**430**; 10.1 mg).

CHAPTER FOUR

RESULTS AND DISCUSIONS

4.1 Phytochemistry Investigations Results

A total of fourty one compounds was isolated from the three Kenyan *Croton* species investigated in this study.

4.1.1 The Phytochemistry of *Croton megalocarpoides*

The compounds isolated from the roots of *C. megalocarpoides*are are described in this section. They include compounds belonging to *ent*-clerodane (**391 – 403**), abietane (**404 – 406**) and trachylobane (**407 – 410**) classes of diterpenoids. Triterpenoids (**411** – **412**) and common phytosterols, sitosterol and stigmasterol were also isolated.

4.1.1.1 *Ent*-clerodane diterpenoids from *Croton megalocarpoides*

Thirteen clerodane diterpenoids whose chemical structures are shown in Figure 4.1 below were isolated from the roots of *C. megalocarpoides*. Compounds **392-403** were new clerodane derivatives. The CD spectra for compounds **401** and **402 [**Appendices 11a and 12a] showed negative Cotton effect at 240 nm that was empirically similar to that for laevinoid reported before as '*ent*-clerodane' (Wang *et al*., 2013). Consequently, these clerodanes (**401** and **402**) and by extension all the others from this plant were assigned as '*ent*-clerodanes'.

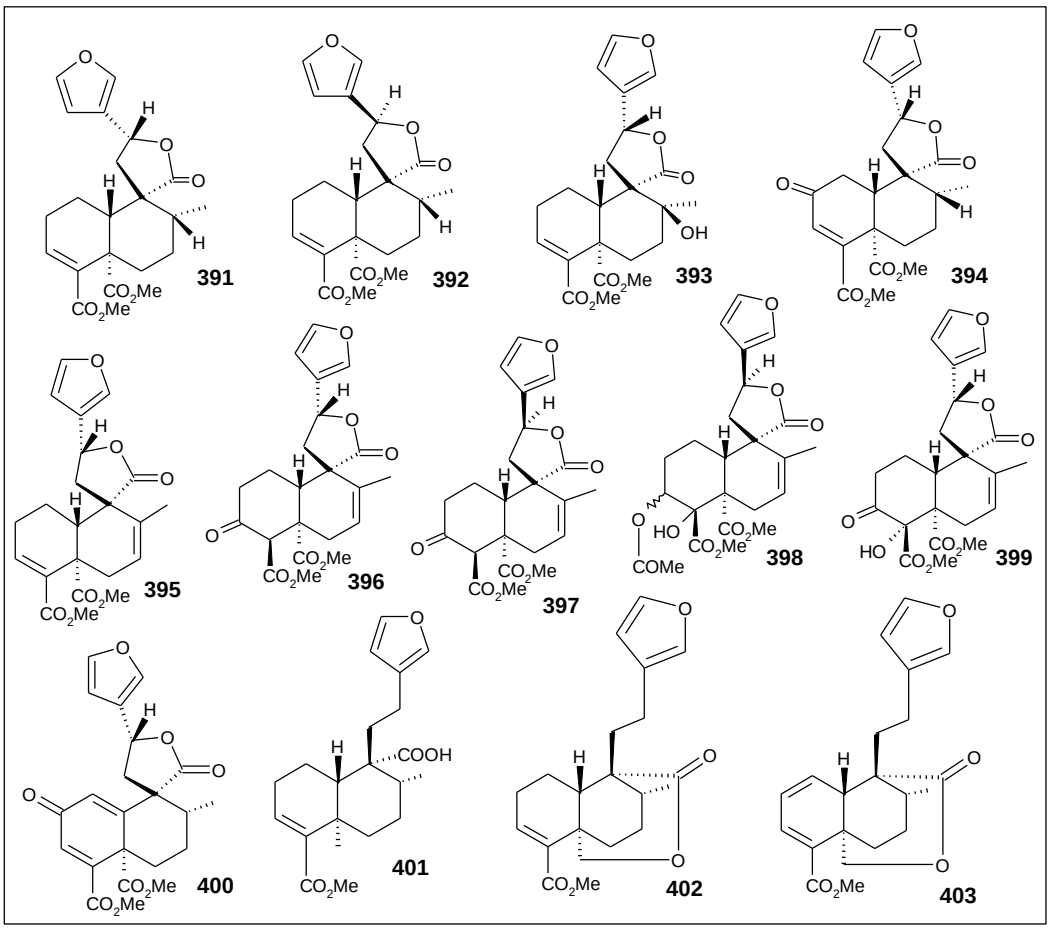
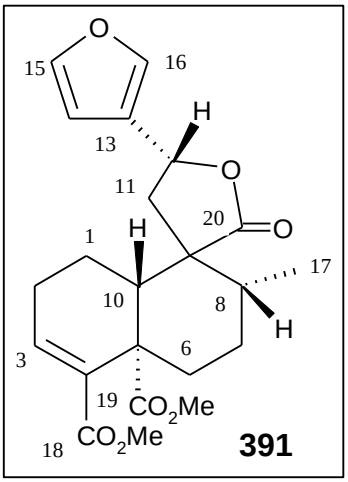


Figure 4.1: *Ent*-clerodane derivatives Isolated from *Croton megalocarpoides*

4.1.1.1.1 Crotocorylifuran (391)

Compound **391** was isolated as white crystals and its mass spectrum [Appendix 1a] found to have a quasi-molecular ion peak at *m/z* 425.45 for [M + Na^+]. This was consistent with the proposed molecular formula, $C_{22}H_{26}O_7$ and a calculated DBE of 10.



The ^{1}H NMR spectrum [Appendix 1b**]** showed resonances of four olefinic protons, three of them characteristic of a *β*- substituted furanyl ring at δ_H 6.38 *d* (*J* = 0.95 Hz), 7.44 *t* (*J* = 1.68 Hz) and 7.45 *s* (Tchissambou *et al*., 1990). The fourth olefinic proton at δ_H 6.84 *t* (*J* = 3.26 Hz) was taken to be of a *tri*-substituted carbon-carbon double bond. A doublet at δ_H 1.01 (*J* = 6.80 Hz) integrating to three protons indicated presence of a secondary methyl group. Additional resonances of three-proton singlets were observed at δ_H 3.70 and 3.75 and were taken to be of two ester methyl groups. An oxymethine proton doublet of doublet resonance integrating for one proton was also observed at δ_H 5.39 (*J* = 8.14, 9.18 Hz). The ^{13}C NMR spectrum [Appendix 1c**]** showed resonances of 22 carbons associated with a diterpenoid. Included were resonances of four sp^2 carbons of a *β*-substituted furanyl ring, three of them methine carbons at δ_C108.3, 144.3, 139.6 and a fully substituted carbon at δ_C 125.6. Additional resonances of two sp^2 carbons associated with a *tri*-substituted double bond were also observed (one fully substituted at δ_C 136.5 and a methine one at δ_C140.3). Other significant resonances observed included: - three carbonyl carbons at δ_C 167.0, 173.1 and 176.1; an oxymethine carbon at δ_C72.0; five methylene, two methine and one methyl group carbons [Table 4.1].

Examination of DEPT spectrum together with 2D NMR experiments indicated that compound **391** possessed a *di*-carbocyclic decalin ring- *β*-furan-*γ*-lactone system of an *ent*-clerodane type diterpenoid (Tchissambou *et al*., 1990). The observed correlations in the HMBC experiment [Appendix 1d] that helped confirm the proposed structure included a correlation between the olefinic proton triplet at $\delta_{\mathbf{H\text{-}3}}$ 6.84 with carbon resonances at $\delta_{\mathbf{C\text{-}1,\ 2,\ 3}}$ 19.2, 26.5, 136.5; $\delta_{\mathbf{H\text{-}8}}$ 1.58 with $\delta_{\mathbf{C\text{-}\ 6,\ 7,\ 10,\ 17,\ 20}}$ 32.5, 28.0, 52.0, 17.2, 176.4 and an oxymethine proton resonance at $\delta_{\mathbf{H\text{-}12}}$ 5.39 with $\delta_{\mathbf{C\text{-}11,\ 13,\ 14,\ 16,\ 20}}$ 42.5, 125.6, 108.3, 139.6, 176.4. Coupling in the COSY spectrum were also observed and have been summarized in Figure 4.2.

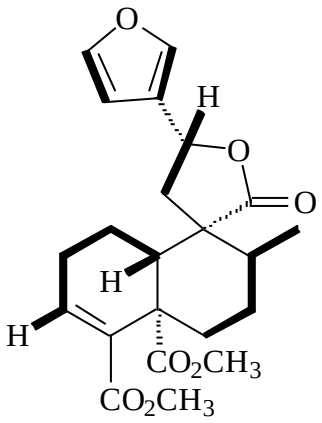


Figure 4.2: Bold lines showing COSY couplings in compound 391

NOESY spectrum [Appendix 1d] was used to assign the relative configuration for this compound and included were correlations between $\delta_{\mathbf{H\text{-}1\alpha}}$ 1.80 *m* with $\delta_{\mathbf{H\text{-}12}}$ 5.39 *t*; $\delta_{\mathbf{H\text{-}10}}$ 1.76 *dd* with $\delta_{\mathbf{H\text{-}11\beta}}$ 2.30 *m*; $\delta_{\mathbf{H\text{-}10}}$ 1.76 *dd* with $\delta_{\mathbf{H\text{-}8}}$ 1.56 *m*; $\delta_{\mathbf{H\text{-}14}}$ 6.38 *d* with $\delta_{\mathbf{3H\text{-}17}}$ 1.01 *d* and $\delta_{\mathbf{H\text{-}16}}$ 7.45 *s* with $\delta_{\mathbf{3H\text{-}17}}$ 1.01 *d*, confirming H-12 was α-configured. A literature search for a compound with the above structural characteristics indicated that, compound **391** was the known crotocorylifuran isolated previously from *C. zambesicus* (Ngadjui *et al*., 2002) and *C. haumanianus* (Tchissambou *et al*., 1990). This compound is reported to be a derivative, resulting from reduction of the known corylifuran (**193**) previously isolated from *C. coryliforious* in 1976 (Tchissambou *et al*., 1990; Burke *et al*., 1976).

Table 4.1: NMR (500 MHz) spectroscopic data of crotocorylifuran (391)

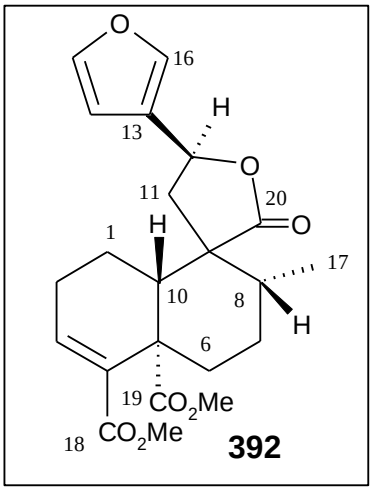
Pstn	δ_C		δ_H (*m, J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
	Lit.[5]	Experi-mental				
1	19.1	19.2	1.89-1.93 (*m*; H_α) 2.54- 2.60 (*m*; H_β)	2, 3, 5, 10 2, 3, 9, 10	1, 2, 10 1, 2	1β, 2α, 10, 12 1α, 10
2	42.3	26.5	2.54-2.60 (*m*; H_α) 2.30-2.45 (*m*; H_β)	1, 3, 4, 10 3, 4	2, 3 1β, 2, 3	3 1β, 3
3	139.8	140.3	6.84 (*t*, 3.26; H)	1, 2,4, 5,18, 19	2α,β	2α / β
4	136.4	136.5				
5	51.7	46.3				
6	32.2	32.3	1.08 (*m*; H_α) 2.93 (*dt*, 13.20, 3.20; H_β)	4, 5, 7, 8, 9, 19 4, 5, 7, 8, 10, 19	6β, 7β 6α	6β, 10 6α, 7α, β

[5] (Tchissambou *et al*., 1990)

7	27.9	28.0	2.30-2.45 (*m*; H_α) 1.56-1.59 (*m*; H_β)	5, 8	8, 6α	8 6β
8	40.0	40.2	1.56-1.59 (*m*; H)	6, 7, 10, 17, 20	7, 17	7β
9	46.3	51.4				
10	51.5	52.0	1.76 (*dd* , 10.73, 2.40; H)	1, 2, 4, 5, 6, 8, 9, 11		1β, 6α, 7α, 8, 11α / β, 17
11	26.3	42.5	2.30-2.45 (*m*; 2H)	9, 12, 13, 20	12	12
12	71.8	72.0	5.39 (*t* , 9.18, 8.41; H)	11, 13, 14, 16, 20	11α, β, 16	11α, β, 1α
13	125.5	125.6				
14	108.1	108.3	6.38 (*d* , 0.95; H)	12, 13, 15, 16	15	15, 17
15	144.0	144.3	7.44 (*t*, 1.68; H)	13, 14, 16	14	14
16	139.4	139.6	7.45 (*s*; H)	13, 14, 15	12	
17	17.0	17.2	1.01 (*d*, 6.80; 3H)	7, 8, 9	8	8, 10, 14, 19-acetoxy
18	166.7	167.0				
19	172.8	173.1				
20	176.0	176.4				
18-**acetoxy**	51.3	51.8	3.70 (*s*; 3H)	18		19-acetoxy
19-**acetoxy**	51.4	51.8	3.75 (*s*; 3H)	19		18-acetoxy

4.1.1.1.2 12-*epi*-crotocorylifuran (392)

Compound **392** was isolated as white crystals. Just like compound **391**, the MS spectrum of compound **392** [Appendix 2a] had a quasi-molecular ion peak at *m/z* 425.45 for [M + Na^+], consistent with a molecular formula, $C_{22}H_{26}O_7$.



392

The ^{1}H NMR and ^{13}C NMR spectroscopic data for **392** [Table 4.2; Appendix 2b] was similar to that of **391** except for minor differences in C-1, C-8, C-9, C-10; H-8 and H-11 that did not result to structural changes. However, there were key differences in their NOESY correlations [Appendix 2c].

Compound **392** had NOESY cross peaks [Figure 4.3] at $\delta_{\mathbf{H\text{-}1\beta}}$ 2.43 *m* with $\delta_{\mathbf{H\text{-}14}}$ 6.39 *br s*; $\delta_{\mathbf{H\text{-}10}}$ 1.59 *m* with $\delta_{\mathbf{H\text{-}11\beta\,/\,1\beta\,/\,2\beta}}$ 2.43 *m;* $\delta_{\mathbf{H\text{-}12}}$ 5.43 *t* with $\delta_{\mathbf{3H\text{-}17}}$ 1.12 *d* and $\delta_{\mathbf{H\text{-}16}}$ 7.45 *s* with $\delta_{\mathbf{H\text{-}11\beta/\,1\beta}}$ 2.43 *m* indicating that **392** was a C-12 epimer of **391**. This compound has not been reported before and was named 12-*epi*-crotocorylifuran.

Table 4.2: NMR (500 MHz) spectroscopic data of 12-*epi*-crotocorylifuran (392)

Position	δ_C	δ_H (*m, J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	18.7	1.59-1.72 (*m*; H_α)	2, 3, 5,9,10	1β	1β, 2α, β, 11
		2.43-2.47 (*m*; H_β)	2, 3, 5, 9,10	1α, 10	1α, 11, 14
2	27.0	2.26-2.35(*m*; H_α)	1, 3, 4,10	2β, 3	1α, 2β
		2.43-2.47 (*m*; H_β)	1, 3, 4,10	2α, 3	1α, 2α, 10
3	140.7	6.80 (*t*, 2.78, 4.17; H)	1, 2, 4, 5, 18, 19	2α, β	
4	137.4				
5	46.4				
6	32.3	1.07-1.13 (*m*; H_α)	4, 7, 8,10, 19	7β, 6β	6β, 7α, 8
		2.95 (*dt*, 12.99, 3.25; H_β)	4, 5, 7, 8, 10, 19	7α, β, 6α	6α, 7
7	28.4	1.56 (*m*; H_α)		6 α,β, 7α	6α, β, 8
		2.26-2.35 (*m*; H_β)	6, 8, 10	7β	
8	43.0	2.43-2.47 (*m*; H)			6α,7β

9	52.1				
10	49.9	1.59-1.72 (*m*; H)	1, 2, 4, 5, 11, 19, 20	1β	1β, 12, 17
11	42.7	1.67 (*s*; H_α) 2.43-2.47 (*m*; H_β)	8, 12, 13, 20	11β 11α, 12	1, 11β 11α, 12
12	72.1	5.43 (*t*, 8.27, 8.50; H)	11, 13, 14, 16, 20	11β, 16	11β, 16, 17
13	126.0				
14	108.2	6.39 (*br s*; H)	12, 13, 15, 16	15	1α
15	144.3	7.45 (*d* , 0.92; H)	13, 14, 16	14	
16	139.3	7.45 (*s*; H)	15	12	1, 12, 17
17	17.6	1.12 (*d* ,7.48; 3H)			10, 12, 16
18	167.0				
19	173.5				
20	176.7				
18-acetoxy	51.9	3.70 (*s*; 3H)	4		
19-acetoxy	51.6	3.78 (*s*; 3H)	5		

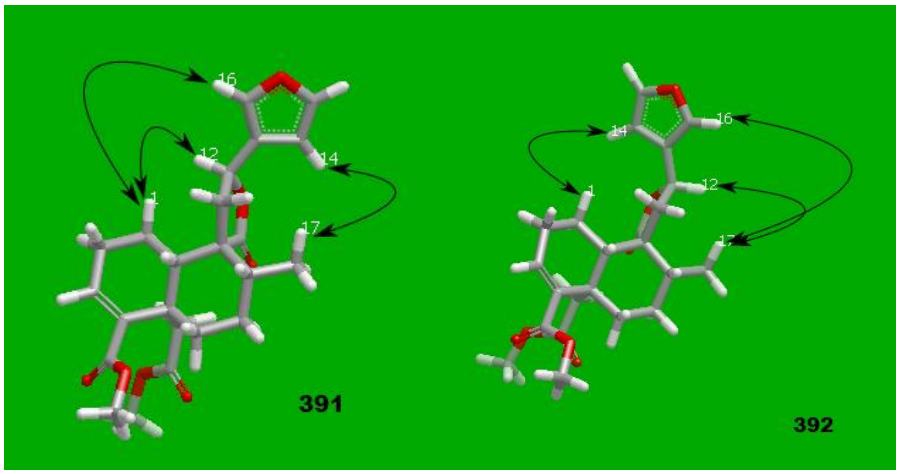
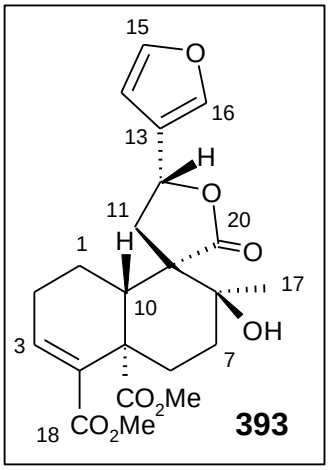


Figure 4.3: Key NOESY correlation illustrations for compounds (391) and (392)

4.1.1.1.3 8-Hydroxycrotocorylifuran (393)

Compound **393** was isolated as white crystals. Its MS spectrum [Appendix 3a] had a molecular ion peak at *m/*z 441.45 for [M + Na^+] consistent with the proposed molecular formula, $C_{22}H_{26}O_8$ and a calculated DBE of 10.



The NMR spectroscopic data of **393** [Table 4.3] was similar to that of **391** except for a resonance associated with an oxygenated sp^3carbon at δ_C 72.7 that was assigned to C-8 based on observed correlations in 2D NMR experiments. The ^{1}H NMR spectrum [Appendix 3b] had a doublet of a doublet at δ_H 4.82 (*J* = 6.94, 7.20 Hz) leading to a deduction that, a hydroxyl group, that was proposed to be a substituent at C-8 was α-configured.

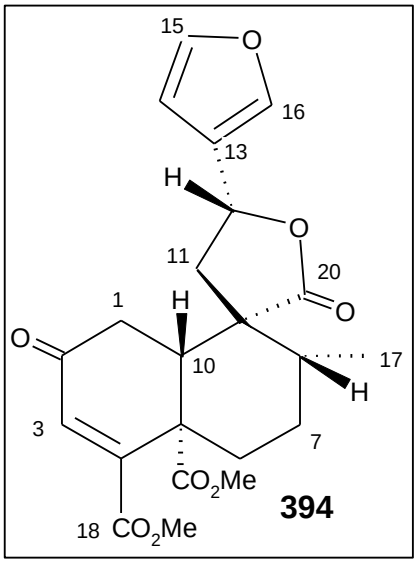
A three proton singlet observed at δ_H 1.27 was deduced to be of a tertiary methyl group substituent and was taken to be 3H-17. ^{13}C NMR spectrum [Appendix3b] showed a resonance at δ_C 26.4 taken to be of a methyl carbon assigned to C-17. HMBC spectrum [Appendix 3c] showed cross peaks between the $\delta_{\mathbf{3H\text{-}17}}$ 1.27 *s* and the oxygenated quaternary carbon at $\delta_{\mathbf{C\text{-}8}}$ 72.5. COSY spectrum had the hydroxy proton of 8-OH sharing cross peaks with the three proton singlet at $\delta_{\mathbf{3H\text{-}17}}$ 1.27. Key NOESY correlations observed [Appendix 3c] were cross peaks of the proton at $\delta_{\mathbf{H\text{-}12}}$ 5.42 *t* with the one at $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.60 *m* implying that the configuration at C-12 of **393** was similar to that in **391**. Additional NOESY correlation that supported the above deduction was observed at $\delta_{\mathbf{3H\text{-}17}}$ 1.27 *s* with $\delta_{\mathbf{H\text{-}7\beta,\ 6\beta,\ 19\text{-}acetoxy}}$ 1.57 *dt*, 2.85 *dt* and 3.76 *s*. Other correlations in 2D NMR experiments that confirmed the proposed structure are shown in Table 4.13. Compound **393** was subsequently deduced to be a new derivative of crotocorylifuran (**391**) and was given the name 8-hydroxycrotocorylifuran.

Table 4.3: NMR (500 MHz) spectroscopic data of 8-hydroxycrotocorylifuran (393)

Position	δ_C	δ_H (*m, J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	19.0	2.60 (*m*; H_α)	10	1β, 10, 2	1β,3, 12
		1.87 (*t* , 9.38, 7.83; H_β)	10,	1α, 10, 2	1α, 2β
2	26.5	2.60 (*m*; H_α)	10, 1	3, 1, 2β	
		2.42 (*m*; H_β)	10	3, 1, 2α	1β
3	140.8	6.88 (*t*, 4.29, 3.03; H)	18, 5	2α,β	1α
4	136.0				
5	46.2				
6	27.1	1.48 (*m*; H_α)	7, 10	7	
		2.85 (*dt*,3.21, 3.33, 13.29; H_β)	5, 10		17
7	34.2	2.93 (*dd*, 3.90, 0.68; H_α)		7β, 6α,β	
		1.57 (*dt*, 3.39, 14.77; H_β)	19, 5	7α	17
8	72.7				
8-OH		4.82 (*dd*, 6.94, 7.29; H)		17	
9	55.8				
10	46.6	2.27 (*dd*, 2.43,10.65; H)		1α, β	
11	39.3	2.42 (*m*; H_α)	20, 9, 10,	11β, 12	12
		2.72 (*m*; H_β)	13, 12, 9	10, 11α, 12	
12	72.2	5.42 (*t*, 8.62; H)	16, 13, 14, 14	11 α, β	1α, 11α
13	125.5				
14	108.4	6.42 (*d*, 0.98; H)	15, 16	15	
15	144.3	7.46 (*t*, 1.68; H)	16, 13,	14	
16	139.7	7.48 (*d*, 0.75; H)	15, 14		
17	26.6	1.27 (*s*; 3H)	8, 9, 7, 6	8 -OH	6β,7β, 19-acetoxy
18	166.8				
19	172.0				
20	176.4				
18-acetoxy	52.0	3.72 (*s*; 3H)	18		
19-acetoxy	51.8	3.76 (*s*; 3H)	19		17

4.1.1.1.4 2-Ketocrotocorylifuran (394)

Compound **394** was isolated as white crystals. Its mass spectrum [Appendix 4a] had a quasi-molecular ion peak at *m/*z 439.43 for [M + Na^+] consistent with the proposed molecular formula, $C_{22}H_{24}O_8$ and a calculated DBE of 11.



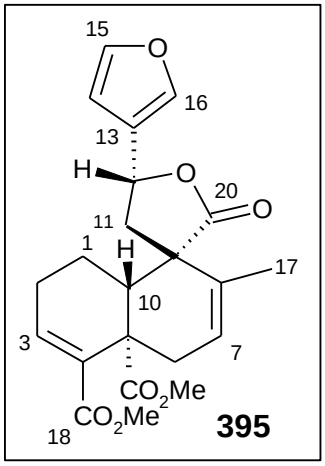
The ^{1}H NMR and ^{13}C NMR data of **394** [Table 4.4; Appendix 4b] was similar to that of **391** except for a resonance of a ketone carbon at δ_C198.0 that was placed at position 2 based on correlations observed in 2D NMR experiments. The ketone carbon had an HMBC correlation [Appendix 4c] with a proton at δ_H 2.72 assigned to H-1β which in turn correlated with carbons at δ_C 50.3 and 131.9 assigned to C-10 and C-3 respectively. NOESY cross peaks [Appendix 4c] were observed between $\delta_{\mathbf{H\text{-}12}}$ 5.41 with $\delta_{\mathbf{H\text{-}10}}$ 2.40 implying that the relative configuration at C-12 of **394** and the known crotocorylifuran (**391**) were the same. Other NOESY correlations supporting the deduced structure were observed at $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.72 with $\delta_{\mathbf{H\text{-}1\beta}}$ 3.37; $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.72 with $\delta_{\mathbf{C\text{-}10}}$ 2.40; $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.72 with $\delta_{\mathbf{H\text{-}12}}$ 5.41 and $\delta_{\mathbf{H\text{-}10}}$ 2.40 with $\delta_{\mathbf{H\text{-}7\alpha}}$ 1.68 and $\delta_{\mathbf{H\text{-}8}}$ 1.65. Compound **394** was proposed to be another new derivative of crotocorylifuran (**391**) and was named 2-ketocrotocorylifuran.

Table 4.4: NMR (500 MHz) spectroscopic data of 2-ketocrotocorylifuran (394)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY
1	36.7	2.72 (*dd* , 4.70, 15.96; H_α) 3.37 (*t*, 15.92; H_β)	2, 10 3, 10	1β 1α
2	198.0			
3	131.9	6.48 (*s*; H)	1, 5, 19	
4	153.3			
5	47.8			
6	31.3	2.88 (*d* ,1 3.15; H_α) 1.31 (*m*; H_β)	 19	6β 6α, 7α
7	27.3	1.68 (*m*; H_α) 2.47 (*m*; H_β)		7β, 8, 10 6β, 7α, 8
8	39.3	1.65(*m*; H)		7, 10, 17
9	51.2			
10	50.3	2.40 (*d*, 4.12; H)	1, 4, 5, 8, 9, 19	7α, 8
11	41.5	2.43 (*m*; 2H)	8, 9, 10, 12, 13	12
12	72.0	5.41 (*t*, 8.79; H)	13, 14, 16	11
13	125.0			
14	108.0	6.40 (*s*; H)	13, 15, 16	15
15	144.5	7.46 (*s*; H)	13, 14, 16	14
16	140.0	7.47 (*s*; H)	15	
17	16.9	1.06 (*d*, 6.19; 3H)	7, 8, 9	
18 19 20	166.1 170.0 175.7			
18-acetoxy	52.8	3.78 (*s*; 3H)	19	
19-acetoxy	52.8	3.83 (*s*; 3H)	18	

4.1.1.1.5 7, 8-Dehydrocrotocorylifuran (395)

Compound **395** was isolated as white crystals. Its mass spectrum [Appendix 5a] had a quasi-molecular ion peak at *m/z* 423.43 for [M + Na^+] consistent with the proposed molecular formula, $C_{22}H_{24}O_7$ and a calculated DBE of 11.



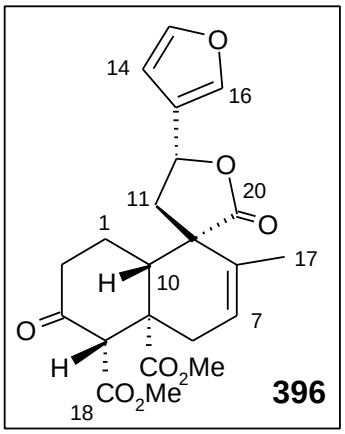
The NMR spectroscopic data for compound **395** [Table 4.5; Appendix 5b] was similar to the one of compound **391** except for a resonance associated with an extra carbon-carbon double bond in **395** (δ_H 6.98 *dd* (*J* = 2.04, 2.63 Hz); δ_C 140.7 and 135.3) that was placed at position **7** using correlations observed in the 2D NMR experiments. HMBC correlations [Appendix 5c] were observed between the olefinic proton at $\delta_{\mathbf{H\text{-}7}}$ 6.98 with sp^3 carbons at $\delta_{\mathbf{C\text{-}5}}$ 45.6 (quaternary) and $\delta_{\mathbf{C\text{-}17}}$ 19.6 (a tertiary methyl group). More HMBC correlations were observed between the two methylene protons at $\delta_{\mathbf{H\text{-}6}}$ 1.59 / 1.77 and 2.35 / 249 with $\delta_{\mathbf{C\text{-}8,\ 7}}$ 135.3, 140.2. COSY spectrum showed coupling between $\delta_{\mathbf{2H\text{-}6}}$ 1.59 / 1.77 and 2.35 / 249 with $\delta_{\mathbf{H\text{-}7}}$ 6.98 further confirming the proposed chemical structure. Key NOESY cross peaks [Appendix 5c] were observed at $\delta_{\mathbf{H\text{-}12}}$ 5.50 with $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.24; $\delta_{\mathbf{H\text{-}1\beta}}$ 2.24 with $\delta_{\mathbf{H\text{-}11\beta}}$ 2.69; $\delta_{\mathbf{H\text{-}12}}$ 5.50 with $\delta_{\mathbf{H\text{-}11}}$ 2.24 / 2.69; $\delta_{\mathbf{H\text{-}12}}$ 5.50 with $\delta_{H\text{-}16}$ 7.45 indicating that compound **395** had similar relative configuration at C-12 with crotocorylifran (**391**). Compound **395** was subsequently identified as a new derivative of crotocorylifuran (**391**) and was given the name 7, 8-dehydrocrotocorylifuran.

Table 4.5: NMR (500 MHz) spectroscopic data of 7, 8-dehydrocrotocorylifuran (395)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY
1	19.2	2.24-2.44 (*m*; H_α) 1.59-1.77 (*m*; H_β)	10 9 , 3, 4	1β, 2 α, 10 2β, 1α
2	26.5	2.56 (*t*, 5.21,4.89; H_α) 1.59-1.77 (*m*; H_β)	4, 10 1, 3, 4	1α, 3 3
3	127.1	5.83 (*d*, 6.41; H)	1, 5	
4	130.4			
5	45.6			
6	33.2	1.59-1.77 (*m*; H_α) 2.24-2.44 (*m*; H_β)	7,19,4 4, 8, 7	6β,7 6α, 7, 19-acetoxy
7	140.2	6.98 (*dd*, 2.44, 2.84; H)	17, 5	6 β
8	135.3			
9	52.8			
10	50.3	2.00 (*dd*, 2.28, 10.26; H)	20, 19, 9, 5, 2, 1	1α, 11α
11	42.2	2.24-2.44 (*m*; H_α) 2.69 (*dd*, 7.82, 6.52; H_β)	13, 12, 9, 10, 20 20, 12, 9, 10, 13	10, 11β, 12 11α
12	72.0	5.50 (*t*, 8.05; H)	11, 16, 13, 14	11α, β
13	125.6			
14	108.0	6.39 (*d*, 1.31; H)	15, 16, 13	15
15	144.2	7.44 (*t*,1.90, 1.63; H)	16, 13,	14
16	139.5	7.45 (*s*; H)	14, 15	
17	19.6	1.25 (*s*; 3H)		
18	166.5			
19	172.0			
20	175.9			
18-acetoxy	51.7	3.70 (*s*; 3H)	18	
19-acetoxy	52.2	3.71 (*s*; 3H)	19	6β

4.1.1.1.6 Megalocarpoidolide F (396)

Compound **396** was isolated as white crystals. Its LC-MS spectrum [Appendix 6a] had a molecular ion peak at *m/z* 439.43 for [M + Na^+] supporting the proposed molecular formula, $C_{22}H_{24}O_8$. The FTIR spectrum [Appendix 6a] had peaks at 1720.0 and 1748 cm^{-1} representing carbonyl and lactone functionalities. Other peaks observed at 2364.0, 2917.8 and 1174 cm^{-1} were associated with carbon-carbon double bond, ester carbonyl and carbon-oxygen atom stretches respectively.



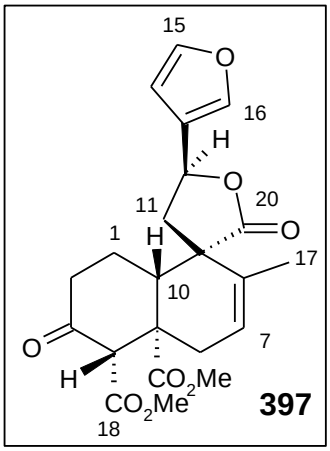
The NMR spectroscopic data of **396** [Table 4.6] was similar to that of crotocorylifuran except for a resonances of a keto carbonyl at δ_C 200.9 that was observed in **396**, absent in **391**. ^1H NMR spectrum [Appendix 6b] had a doublet of an olefinic proton at δ_H 5.81(J = 5.82 Hz) assigned to H-7 and a three proton singlet at δ_H 1.69 assigned to a tertiary methyl group, 3H-17. ^{13}C NMR spectrum [Appendix 6b] had resonances of a keto carbonyl carbon at δ_C 200.9 assigned to C-3 and sp^2 carbons at δ_C 127.4 and 131.0 assigned to C-7 and C-8 respectively. A down field shifted carbon resonance at δ_C 67.0 was assigned to C-4. HMBC spectrum [Appendix 6c] had ^1H-^{13}C cross peaks between $\delta_{\mathbf{H\text{-}1\beta,\ 2\delta,\ 2\beta,\ 4}}$ 2.20, 2.78, 2.54, 3.26 and $\delta_{\mathbf{C\text{-}3}}$ 200.9; $\delta_{\mathbf{H\text{-}7}}$ 5.81 and $\delta_{\mathbf{C\text{-}5,\ 6,\ 9,\ 17}}$ 49.5, 33.7, 53.0, 20; $\delta_{\mathbf{H\text{-}2\alpha,\ 6\alpha,\ 6\beta,\ 10}}$ 2.78, 2.78, 1.96, 2.37 and $\delta_{\mathbf{C\text{-}4}}$ 67.0. COSY spectrum [Appendix 6c] had ^1H-^1H cross peaks at $\delta_{\mathbf{H\text{-}7}}$ 5.76 with $\delta_{\mathbf{H\text{-}6\alpha,\ \beta}}$ 2.78, 1.96 further confirming the proposed chemical structure. NOESY spectrum [Appendix 7c] had cross peaks at $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.45 with $\delta_{\mathbf{H\text{-}12}}$ 5.50 confirming the α-configuration of H-12 as in crotocorylifuran. Other NOESY correlations were at $\delta_{\mathbf{H\text{-}4}}$ 3.26 with $\delta_{\mathbf{H\text{-}10}}$ 2.37 and $\delta_{\mathbf{H\text{-}14}}$ 6.41 with $\delta_{\mathbf{3H\text{-}17}}$ 1.69 that alongside correlations in other 2D NMR experiments led to the confirmation of the proposed structure. Compound **396** was deduced to be a new compound and was given the IUPAC name 18, 19-dimethoxycarbonyl-3-keto-15, 16-epoxy-cleroda-7, 13 (16), 14-triene-12, 20-olide and trivial name, megalocarpoidolide F.

Table 4.6: NMR (500 MHz) spectroscopic data of megalocarpoidolide F (396)

Postn	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	24.6	2.45 (*m*; H_α)		10	2 α, 12
		2.20 (*m*; H_β)	2, 3, 10,9	10	
2	39.7	2.78 (*m*; H_α)	3, 4, 1, 10		1α, 2β
		2.54 (*m*; H_β)	3,1,10		2α
3	200.9				
4	67.0	3.26 (*br s*; H)	19,18,3,5,6		10
5	49.5				
6	33.7	2.78 (*m*; H_α)	4,19,8,10	7, 6β	7, 6β
		1.96 (*m*; H_β)	4,19, 5,8, 10	7, 6α	6α
7	127.4	5.81 (*d*, 5.82; H)	5, 6, 9, 17,	6α, β, 17	6 α
8	131.0				
9	53.0				
10	49.5	2.37 (*m*; H)	20, 4, 9, 19,20,5,6, 2,11	1α, β	4
11	42.1	2.74 (*m*; H_α)	20, 9, 13, 8,12, 10	12	11β
		2.53 (*m*; H_β)	8,10, 20, 13, 12, 9	12	12, 11α
12	72.4	5.50 (*t*, 8.15; H)	20,11, 13, 16,14	11α / β, 16	1α, 11β
13	125.5				
14	108.1	6.41 (*s*; H)	12, 13, 16, 15	15	17
15	144.5	7.46 (*d*,1.33; H)	16, 13	14	
16	139.8	7.48 (*s*; H)	15, 13	12	
17	20.0	1.69 (*s*; 3H)	8, 9, 7	7	14
18	168.0				
19	171.1				
20	176.0				
18-acetoxy	52.5	3.75 (*s*; 3H)	18, 4		
19-acetoxy	52.5	3.76 (*s*; 3H)	19, 4		

4.1.1.1.7 12-*Epi*-megalocarpoidolide F (397)

Compound **397** was isolated as white crystals. Its LC-MS spectrum [Appendix 7a] had a quasi-molecular ion peak at *m/z* 439.43 for [M + Na^+] as compound **396**, consistent with the proposed molecular formula, $C_{22}H_{24}O_8$. The FTIR spectrum [Appendix 7a] had absorption bands similar to those observed in **396** implying that they had similar functional groups.



The ^1H and ^{13}C NMR spectroscopic data for **397** [Table 4.7; Appendix 7b] was similar to that of compound **396** except for slight variations at positions 1 and 10 that did not result to structural changes. NOESY spectrum of **397** [Appendix 7c] was used to assign relative configuration at positions 4 and 12. NOESY correlations were observed at $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.02 with $\delta_{\mathbf{H\text{-}14}}$ 6.42 and $\delta_{\mathbf{H\text{-}12}}$ 5.53 with $\delta_{\mathbf{3H\text{-}17}}$ 1.87 leading to the deduction that, H-12 was β-configured **[**Figure 4.4**]**. The α-configuration of $\delta_{\mathbf{H\text{-}4}}$ 3.21 was deduced from its NOESY correlation with $\delta_{\mathbf{H\text{-}10}}$ 2.18. Compound **397** was therefore deduced to be a C-12 epimer of compound **396** that has not been reported before. It was given the IUPAC name 12-*epi*-18, 19-dimethoxycarbonyl-3-keto-15, 16-epoxy-cleroda-7, 13 (16), 14-triene-12, 20-olide and trivial name 12-*epi*-megalocarpoidolide F.

Table 4.7: NMR (500 MHz) spectroscopic data of 12-*epi*-megalocarpoidolide F (397)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	23.5	2.02 (*m*; H_α)	3, 5	1β, 2α	1β, 2α, 10, 14
		2.41 (*m*; H_β)	2, 3	1α	1α
2	40.0	2.41 (*m*; H_α)	1, 3	1α	1α,
		2.74 (*m*; H_β)	1, 3, 4		
3	201.0				
4	67.3	3.21 (*s*; H)	3, 6, 10, 18		2α, 6α, 10
5	49.5				
6	33.7	1.94 (*m*; H_α)	17, 19	7	7, 10
		2.78 (*m*; H_β)	4, 7, 8, 10, 19	7	7
7	124.5	5.76 (*d*, 6.71; H)	5, 6, 9, 17	6α, β	6α, β
8	131.0				
9	52.4				
10	47.4	2.18 (*m*; H)	1, 5, 9, 11, 19		1α, 4, 6α, 11α, 17
11	42.4	2.79 (*m*; H_α)	8, 9, 20	11β, 12	12
		2.35 (*m*; H_β)	7, 8, 9,10, 12	11α, 12	12
12	72.4	5.53 (*t*, 8.69; H)	11, 13, 14, 16	11α, β,16	11α, β, 17
13	125.2				
14	108.1	6.42 (*s*; H)	13, 15, 16	15	1β
15	144.6	7.48 (*t*, 1.76; H)	13, 14, 16	14	
16	139.5	7.50 (*br s*; H)	13, 14, 15	12	1β, 10, 12
17	20.0	1.87 (*s*; 3H)	7, 8, 9		12
18	167.9				
19	171.0				
20	175.4				
18-acetoxy	52.6	3.77 (*s*; 3H)	18		
19-acetoxy	52.6	3.77 (*s*; 3H)	19		

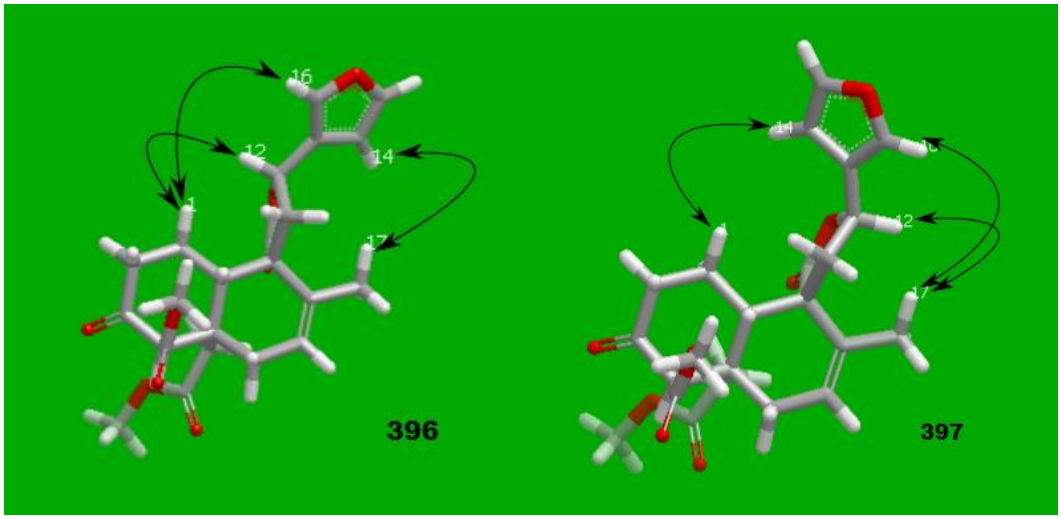
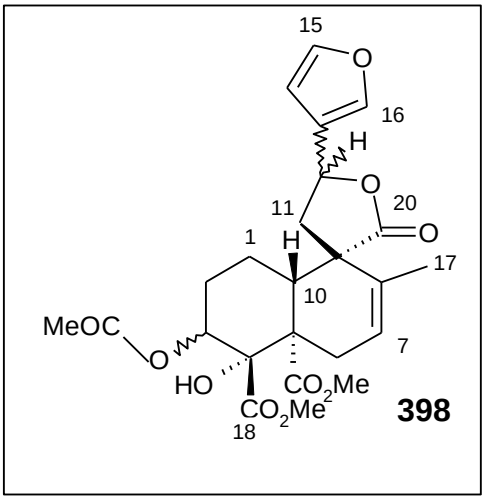


Figure 4.4: Key NOSEY correlation illustrations for megalocarpoidolide F (396) and 12-*epi*-megalocarpoidolide F (397)

4.1.1.1.8 Megalocarpoidolides E (398)

Compound **398** was isolated as white crystals. Its LC-MS spectrum [Appendix 8a] had a quasi-base peak at *m/*z 499.43 for [M + Na^+] consistent with a proposed molecular formula of $C_{24}H_{28}O_{10}$.



The ^1H and ^{13}C NMR spectroscopic data for **398** [Table 4.8; Appendices 8c and 8d] was similar to that of compound **397** except for resonances of an oxymethine at C-3 (δ_H 5.10 *br s*; δ_C 70.5) in place of a ketone at C-3 as was the case in **397**. An oxygenated quaternary sp^3 carbon was observed at δ_C 76.1 and was placed at position 4. In addition, resonances attributed to an acetate methyl group substituent (-$OOCCH_3$; δ_H 2.03 *s*; δ_C 173.2 and 21.3) were observed and placed at position 3.

HMBC spectrum [Appendix 8e] had correlations that helped confirm the proposed structure at $\delta_{\mathbf{H\text{-}3}}$ 5.10 with $\delta_{\mathbf{C\text{-}2,\,4,\,5,\,18}}$ 21.0, 76.1, 52.1, 173.2; $\delta_{\mathbf{H\text{-}7}}$ with $\delta_{\mathbf{C\text{-}5,\,6,\,9,\,17}}$ 52.1, 29.9, 53.4, 19.6; the three proton singlets at δ_H 3.74 and 3.72 with the carbonyls at $\delta_{\mathbf{C\text{-}19}}$ 170.3 and $\delta_{\mathbf{C\text{-}18}}$ 173.2 respectively; methylene protons at $\delta_{H\text{-}2\alpha,\,\beta}$ 2.64, 2.03 with $\delta_{C\text{-}3,\,4}$ 70.5, 76.1 and the hydroxyl proton at $\delta_{H\text{-}4\text{-}OH}$ 5.00 with $\delta_{C\text{-}4}$ 76.1. Compound **398** was deduced to be a new derivative of compound **397** that was named megalocarpoidolide E.

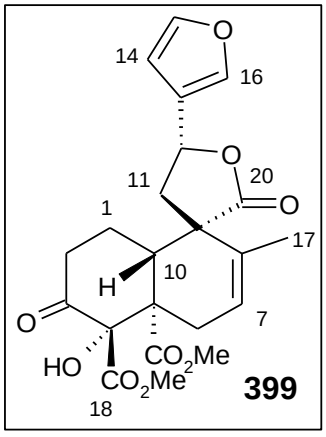
Table 4.8: NMR (500 MHz) spectroscopic data of megalocarpoidolide E (398)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	**HMBC** (H→C)	**NOESY**
1	27.3	2.22(*m*; 2H)	3, 9	12
2	21.0	2.64(*m*; H_α) 2.03(*br s*; H_β)	3, 4, 10 3, 4	 3
3	70.5	5.10 (*br s*; H)	2, 4, 5, 18	2β
4 4-OH	76.1	 5.00 (*br s*; H)	 4	
5	52.1			
6	29.9	2.36 (*m*; H_α) 1.26 (*m*; H_β)	7, 4, 8, 9, 10	
7	125.3	5.67 (*d*, 6.87; H)	5, 6, 9, 17	
8	132.2			
9	53.4			
10	46.3	2.62 (*m*; H)	20	
11	45.1	2.55 (*m;* H_α) 2. 52 (*m*; H_β)	8, 10, 20 8, 10, 20	11β, 12 11α, 12
12	71.7	5.47 (*t*, 8.16; H)	11, 13, 14, 16	1α, 11α, 11β
13	125.6			
14	108.3	6.41 (*s*; H)	12, 13, 15, 16	
15	144.3	7.44 (*s*; H)	13, 14, 16	

16	139.9	7.48 (*s*; H)	13, 14, 15
17	19.6	1.67 (*s*; 3H)	7, 8, 9
18	173.2		
19	170.3		
20	175.4		
18-acetoxy	53.4	3.72 (*s*; 3H)	18
19-acetoxy	53.7	3.74(*s*; 3H)	19
3-OOCMe	171.3		
3-OOCCH_3	21.3	2.03 (*s*; 3H)	3-OOCMe, 3

4.1.1.1.9 Megalocarpoidolide G (399)

Compound **399** was isolated as white crystals. Its LC- MS spectrum [Appendix 9a] had a molecular ion peak at *m/*z 455.43 for [M + Na^+] supporting a proposed molecular formula, $C_{22}H_{24}O_9$. The FTIR spectrum [Appendix 9a] had a peak at 3353.0 cm^{-1} associated with a hydroxyl group stretch that was the only peak missing in the FTIR of **396** and **397.**



The NMR spectroscopic data [Table 4.9; Appendix 9b] was similar to that of **396** and **397**except for resonances of an oxygenated quaternary sp^3 carbon at δ_C 82.0 and an exchangeable proton at δ_H 4.20 *s* that were assigned to position 4 of an *ent*-clerodane molecule.

HMBC correlations [Appendix 9c] supporting the proposed chemical structure were observed between the proposed hydroxyl group proton at $\delta_{\mathbf{OH\text{-}4}}$ 4.20 with $\delta_{\mathbf{C\text{-}5,\ 4,\ 18,\ 3}}$ 53.1, 82.0, 171.1, 201.8; olefinic proton at $\delta_{\mathbf{H\text{-}7}}$ 5.80 with $\delta_{\mathbf{C\text{-}5,\ 9,\ 19}}$ 53.1, 53.5, 170.5 and the methyl singlet at $\delta_{\mathbf{3H\text{-}17}}$ 1.69 with $\delta_{\mathbf{C\text{-}9,\ 7,\ 8}}$ 53.5, 125.5, 130.1. NOESY spectrum [Appendix 9c] had ^{1}H-^{1}H cross peaks at $\delta_{\mathbf{H\text{-}1\alpha}}$ 2.14 with $\delta_{\mathbf{H\text{-}12}}$ 5.49 supporting a relative α- configuration of H-12. Compound **399** was consequently identified as a new derivative of the new megalocarpoidolide F (**396**). It was subsequently given the IUPAC name 18, 19-dimethoxycarbonyl-4hydroxy-3-keto-15, 16-epoxy-cleroda-7, 13 (16), 14-triene-12, 20-olide and trivial name, megalocarpoidolide G.

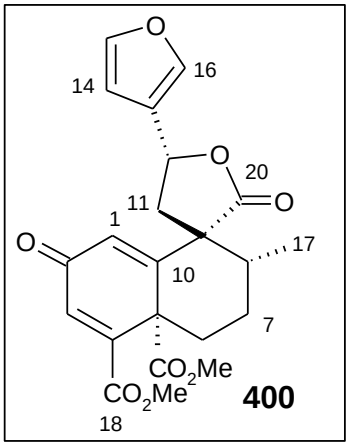
Table 4.9: NMR (500 MHz) spectroscopic data of megalocarpoidolide G (399)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	23.6	2.14 (*d*, 2.45; H_α) 2.35 (*d*, 6.00; H_β)	3,2 2	10, 2, 1β 2α,10, 1α	12
2	34.9	2.59 (*m*; H_α) 3.00 (*m*; H_β)	3,10 3,1	 1α,β, 2β	10
3	201.8				
4	82.0				
4-OH		4.20 (*s*; H)	3,18,4, 5		
5	53.1				
6	26.4	2.38 (*m*; H_α) 2.17 (*m*; H_β)	5,7,8,10 5,7,8,10	17 7, 17	10, 17
7	125.5	5.80 (*d*, 6.80; H)	9,5,19	17, 6	17
8	130.1				
9	53.5				
10	43.1	2.81 (*dd*, 4.59, 8.71; H)	20, 19, 4,5,9,11,2	1α,β	2α, 6α, 11α
11	41.8	2.59 (*m*; H_α) 2.75 (*d*, 8.36; H_β)	8, 9, 10, 12,13 8, 9,10, 12, 13, 20	12 12	12 12, 17
12	72.3	5.49 (*t*, 8.53; H)	16,13,11	11, 16	1α, 11α, β
13	125.3				

14	108.0	6.42 (*d*, 0.98; H)	15,16, 13	15	17
15	144.3	7.47 (*t*, 1.72,1.66; H)	16,13	14	
16	139.6	7.48 (*d*, 0.57; H)	15,13	12	
17	19.9	1.69 (*t* , 1.20,1.15; 3H)	8, 7, 9	7	6α, 7, 11β, 14
18	171.1				
19	170.5				
20	176.3				
18-acetoxy	54.0	3.88 (*s*; 3H)	18		
19-acetoxy	52.1	3.74 (*s*; 3H)	19		

4.1.1.1.10 Megalocarpoidolide H (400)

Compound **400** was isolated as white crystals. Its LC-MS spectrum [Appendix 10a] had a quasi-molecular ion peak at *m/z* 437.43 for [M + Na^+], supporting the proposed molecular formula, $C_{22}H_{22}O_8$. FTIR spectrum [Appendix 10a] had bands at 2917.9, 1769.7, 1730.0, 1663.3, 1246.5 and 1156.55 cm^{-1}.



400

The NMR data of compound **400** [Table 4. 10] had very minor variations to that of crotocorylifuran (**391**). The ^1H NMR spectrum [Appendix 10b] had resonances showing singlets of olefinic protons at δ_H 6.78 and δ_H 6.88 assigned to H-1 and H-3. The ^{13}C NMR spectrum [Appendix 10b] had resonances associated with sp^2 carbons at δ_C 128.0, 131.6, 150.7 and 155.5 that were assigned to C-1, C-3, C-4 and C-10 respectively.

A resonance of a carbonyl carbon was observed at δ_C 185.9 and assigned to C-2 based on correlations observed in the 2D NMR experiments [Table 4.10]. HMBC spectrum [Appendix 10c] had ^{1}H-^{13}C cross peaks at $\delta_{\mathbf{H\text{-}1}}$ 6.78 with $\delta_{\mathbf{C\text{-}9,\ 3,\ 20}}$ 55.1, 131.4, 172.0 and $\delta_{\mathbf{H\text{-}3}}$ 6.88 with $\delta_{\mathbf{C\text{-}5,\ 1,\ 18}}$ 53.6, 128.0, 165.4 further supporting the proposed chemical structure. NOESY spectrum [Appendix 10c] had ^{1}H-^{1}H cross peaks at $\delta_{\mathbf{H\text{-}1\alpha}}$ 6.78 with $\delta_{\mathbf{H\text{-}12}}$ 5.55 supporting α-configuration of H-12. Compound **400** was deduced to be a new clerodane derivative of crotocorylifuran and was given the IUPAC name 18, 19-dimethoxycarbonyl-4-hydroxy-3-keto-15, 16-epoxy-cleroda-7, 13 (16), 14-triene-12, 20-olide and the trivial name megalocarpoidolide H.

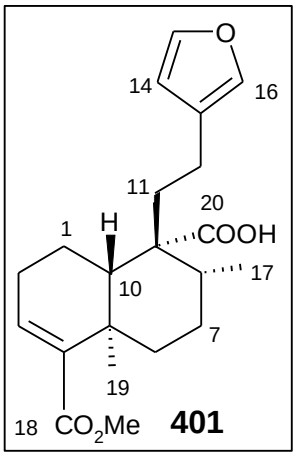
Table 4.10: NMR (500 MHz) spectroscopic data of megalocarpoidolide H (400)

Postn	**δ_C**	**δ_H** (*m*, *J* Hz; Integral)	**HMBC** (H→C)	**COSY**	**NOESY**
1	128.0	6.78 (*s*; H)	3, 9, 20		11α, 12
2	185.9				
3	131.6	6.88 (*s*; H)	1, 5, 18		
4	150.7				
5	53.6				
6	33.2	1.45 (*d*, 4.25; H_α) 3.12 (*dd*, 13.50, 3.05; H_β)	 7, 8, 10	6β, 7β 6α	6β, 8 6α, 7β
7	26.4	2.66 (*d*, 2.66; H_α) 2.80 (*d*, 5.18; H_β)	8, 9 8, 9	7β 6α, 7α, 8	7β 6β, 7α
8	39.4	1.73(*m*; H)	7, 9, 20	7, 17	6α, 11α
9	55.1				
10	155.5				
11	39.1	2.77 (*d*, 4.94; H_α) 2.68 (*s*; H_β)	9, 10, 20 8, 9, 10, 12, 13	11β,12 11α	1, 8, 11β, 12 11α
12	71.4	5.55 (*dd*, 5.46, 4.96; H)	13, 14, 16	11α, β	1, 11α
13	123.6				
14	108.2	6.45 (*s*; H)	13, 15, 16	15	

15	144.5	7.48 (*s*; H)	13, 14, 16	14
16	140.6	7.55 (*s*; H)	13, 14, 15	
17	17.1	1.18 (*d*, 6.34; 3H)	7, 8, 9	8
18	165.4			
19	166.4			
20	172.4			
18-acetoxy	53.1	3.85 (*s*; 3H)	18	
19-acetoxy	53.3	3.66(*s*; 3H)	19	

4.1.1.1.11 Megalocarpoidolide I (401)

Compound **401**was isolated as colourless oil and a molecular formula, $C_{21}H_{28}O_5$ proposed for it. The FTIR spectrum [Appendix 11a] displayed absorption bands for carbonyl and free carboxylic acid stretches at 1713 and 1695 cm^{-1} respectively. A peak observed at 1251.06 cm^{-1} was attributed to carbon - oxygen bond stretch. Other peaks were seen at 2923.4, 2853 and 2400 cm^{-1}. CD spectrum [Appendix 11a] showed negative Cotton effect at 240 nm, similar to one shown by laevinoid that has been reported to be an *ent*-clerodane (Wang *et al*., 2013) and therefore, by extension the clerodane molecules deduced were assigned '*ent*' series.



401

The ^{1}H NMR spectrum [Appendix 11b] had resonances integrating for three protons at δ_H 1.18 *d* and 1.24 *s* that were associated with secondary and tertiary methyl groups respectively. Another three proton singlet was observed down field at δ_H 3.70 indicative of a methoxy group functionality. Resonances of four olefinic protons were also observed at δ_H 6.29 *d*, 6.70 *q*, 7.25 *br s* and 7.37 *t* in addition to other resonances associated with methylene and methine protons. The ^{13}CNMR [Appendix 11b] had resonances of twenty one carbons indicating that **401** was a diterpenoid molecule. Observed were resonances associated with two carbonyl carbons at δ_C167.9 and 182.9 and six olefinic carbons, four of them taken to be of a furan ring (δ_C111.0, 138.9 and 143.1 for methine carbons and δ_C 124.7 for a fully substituted carbon) and two for a *tri*-substituted carbon-carbon double bond (δ_C 137.6 and 141.5 for a methine and a quaternary carbon respectively). Resonances of three methyl carbons were observed, two of them up field at δ_C 16.7 and 18.3 and the remaining one, down field at δ_C 51.4 associated with a methoxy group. The remaining resonances were of six methylene and two methine carbons.

HMBC spectrum [Appendix 11c] had ^{1}H-^{13}C cross peaks at $\delta_{\mathbf{H\text{-}14}}$ 6.29 with a methylene carbon at $\delta_{\mathbf{C\text{-}12}}$ 18.1 and three furan ring carbons at $\delta_{\mathbf{C\text{-}13,\ 16,\ 15}}$ 124.7, 138.8 and 143.1. The methines of the furan ring were mutually coupled [Table 4.11]. The olefinic proton at $\delta_{\mathbf{H\text{-}3}}$ 6.70 had cross peaks with the carbonyl carbon at $\delta_{\mathbf{C\text{-}18}}$167.9 which was in addition correlated by the three proton singlet at $\delta_{\mathbf{3H\text{-}18\text{-}acetoxy}}$ 3.70. The other carbonyl carbon at $\delta_{\mathbf{C\text{-}20}}$ 182.9 had a correlation with a multiplet at $\delta_{\mathbf{H\text{-}10}}$ 1.61/1.63. The methyl carbon at $\delta_{\mathbf{C\text{-}19}}$ 18.3 correlated with the methylene protons at $\delta_{\mathbf{2H\text{-}6}}$ 1.94- 2.00 and 2.25-2.38.

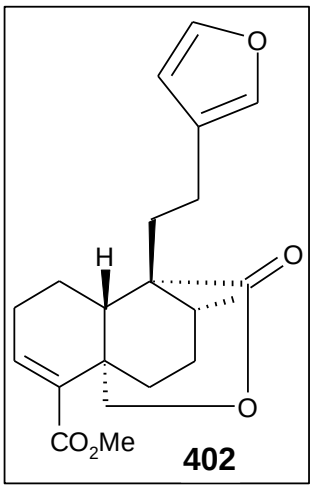
From the above spectral data features and other correlations from other 2D NMR experiments [Table 4.11], **401** was deduced to be a clerodane molecule having a *tri*-substituted C=C at position 3 of the *bi*-carbocyclic ring, an acetoxy group substituent at position 4, a carboxylic acid functionality at position 20 and a β-substituted furan ring at position 13. NOESY spectrum [Appendix 11c] showed ^{1}H-^{1}H cross peaks between $\delta_{\mathbf{H\text{-}8,\ 10}}$ 1.61/1.63 with $\delta_{\mathbf{H\text{-}11\alpha,\ 12\alpha}}$ 1.21-1.23 and no correlation between H-10 and 3H-19 indicating that, **401** was an *ent*-clerodane molecule. The proposed chemical structure were deduced to be of a new compound given the IUPAC name18-methoxycarbonyl-15, 16-epoxycleroda-3, 13 (16), 14-trien-20-oic acid and trivial name megalocarpoidolide I.

Table 4.11: NMR (500 MHz) spectroscopic data of megalocarpoidolide I (401)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	19.6	1.80 (*m*; H_α)			12α
		1.94-2.00 (*m*; H_β)	3, 4, 10	2β	
2	27.3	1.45 (*dd*, 2.68, 10.73; H_α)			
		2.25-2.38 (*m*; H_β)	3, 4	1β, 3	3
3	137.6	6.70 (*q* 2.82, 2.35; H)	1, 2, 4, 5, 18	2β	2β
4	141.5				
5	38.5				
6	34.1	1.94-2.00 (*m*; H_α)	7, 8, 10, 19, 17	6β	6β
		2.25-2.38 (*m*; H_β)	7, 8, 19	6α	6α
7	27.7	2.15 (*m*; H_α)			8
		2.25-2.38 (*m*; H_β)	6, 8		
8	37.3	1.61-1.63 (*m*; H)	10, 17		7α, 11α, 12α
9	50.0				
10	48.7	1.61-1.63 (*m*; H)	2, 8, 20		11α, 12α
11	36.7	1.21-1.23 (*m*; H_α)	10, 12		8, 10
		2.25-2.38 (*m*; H_β)		14	
12	18.1	1.21-1.23 (*m*; H_α)			1α, 10
		2.25-2.38 (*m*; H_β)	9,13, 14,	14, 16	16
13	124.7				
14	111.0	6.29 (*d*, 0.80; H)	12, 13, 15, 16	12β, 15	15
15	143.1	7.37 (*t*, 1.55, 1.66; H)	13, 14, 16	14	14
16	138.8	7.25 (*br s*; H)	13, 14, 15	12β	12β
17	16.7	1.18 (*d*, 6.88; 3H)	8, 9		
18	167.9				
19	18.3	1.24 (*s*; 3H)			
20	182.9				
18-acetoxy	51.4	3.70 (*s*; 3H)	18		

4.1.1.1.12 Megalocarpoidolide J (402)

Compound **402** was isolated as white crystals and a molecular formula, $C_{21}H_{26}O_5$ proposed for it and therefore a calculated DBE of 9. The FTIR [Appendix 12a] had stretches at 2927.9, 2867.8, 2381.3, 1700 and 1200 cm^{-1} as was observed in the FTIR of compound **391** except for free carboxylic acid bond stretch at 1695 cm^{-1} that was notably absent.



402

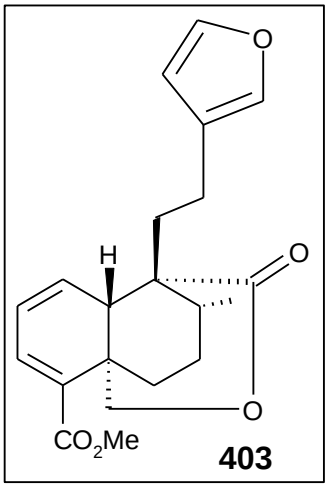
The spectroscopic data of compound **402** [Table 4.12; Appendix 12b] was similar to that of **401** except for resonances of an oxymethylene group at δ_H 4.40 *dd*, 4.84 *d* and δ_C 75.7 which was placed at position 19 in place of a methyl group as was the case in **401**. HMBC spectrum [Appendix 12c] had ^1H-^{13}C cross peaks for the methylene protons at $\delta_{\mathbf{H\text{-}19}}$ 4.40, 4.84 with $\delta_{\mathbf{C\text{-}6,\ 10,\ 4,\ 20}}$ 35.4, 43.7, 136.4, 173.2 and the methine proton at $\delta_{\mathbf{H\text{-}10}}$1.80 with $\delta_{\mathbf{C\text{-}19,\ 20}}$ 73.2, 173.2 confirming the proposed structure. COSY spectrum [Appendix 12c] had ^1H-^1H cross peaks between the methylene proton at $\delta_{\mathbf{H\text{-}19\alpha}}$ 4.40 and $\delta_{\mathbf{H\text{-}6\alpha}}$ 1.37 further confirming the proposed chemical structure. NOESY cross peaks [Appendix 12d] at $\delta_{\mathbf{H\text{-}8}}$ 1.93 with $\delta_{\mathbf{H\text{-}10}}$ 1.80 and $\delta_{\mathbf{H\text{-}6\alpha,\ \beta}}$ 1.37, 2.53 with $\delta_{\mathbf{H\text{-}19\alpha,\ \beta}}$ 4.40, 4.84 were also supportive of the proposed structure. It was opined that, compound **402** formed when hydrolysis occurred between the requisite substituents at positions **19** and **20** of an *ent*-clerodane molecule that was very similar in structure to compound **401**. Consequently, compound **402** was deduced to be a derivative of **401** that is also new and was given the IUPAC name 18-methoxycarbonyl-15, 16-epoxy-cleroda-3, 13 (16), 14-triene-19, 20-olide and trivial name megalocarpoidolide J.

Table 4.12: NMR (500 MHz) spectroscopic data of megalocarpoidolide J (402)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	19.6	1.40 (*m*; H_α)	2, 5	1β , 2α,β, 10	1β, 10
		1.93 (*m*; H_β)	3	1α	1α
2	26.4	2.27(*m*; H_α)		3, 1β	
		2.40(*m*; H_β)	3, 4, 10	3, 1α	
3	139.6	6.86 (*dd*, 2.35, 2.82; H)	1, 5, 18	2α, β	
4	136.4				
5	36.5				
6	35.6	1.37 (*m*; H_α)		7, 19α	19α, 6α
		2.53 (*m*; H_β)	5, 8	7	19β, 6β
7	29.7	1.28 (*s*; H_α)	11	6α, β	
		1.60 (*s*; H_β)	8, 17	6α, β	
8	37.2	1.93 (*m*; H)		17	17
9	48.9				
10	43.7	1.80 (*m*; H)	1, 2, 5, 9, 19, 20	1α	1α
11	29.4	1.79 (*m*; H_α)	8, 9, 12, 13		12α
		2.48 (*m*; H_β)	9, 10, 13		
12	17.5	2.20 (*m*; H_α)	11, 13, 14, 16	11α	
		2.38 (*m*; H_β)	11, 13, 14		
13	124.4				
14	111.0	6.30 (*d*, 0.84; H)	13, 15, 16	15	
15	143.2	7.39 (*t* ,1.65; H)	13, 14, 16	14	
16	138.8	7.28 (*s*; H)	13, 14, 15		
17	16.6	0.98 (*d*, 6.92; 3H)	7, 8, 9	8	8
18	167.3				
19	75.7	4.40 (*dd*, 2.40, 10.40; H_α)	4, 6, 20	19β, 6α	6α, 19β
		4.84 (*d* ,12.00; H_β)	6, 10, 20	19α	6β, 19α
20	173.2				
18-acetoxy	51.9	3.75 (*s*; 3H)	18		

4.1.1.1.13 Megalocarpoidolide K (403)

Compound **403** was isolated as white crystals and a molecular formula, $C_{21}H_{24}O_5$ proposed for it and therefore a calculated DBE of 10.



403

The NMR spectroscopic data of compound **403** [Table 4.13; Appendix 13a] was similar to that of **402** except for observed resonances of an additional carbon-carbon double bond at δ_H 6.21 *m* and 6.24 *m* and δ_C 125.7 and 130.3 that was placed at position 1. HMBC spectrum [Appendix 13c] had ^1H-^{13}C cross peaks at $\delta_{\mathbf{H\text{-}1}}$ 6.21 with $\delta_{\mathbf{C\text{-}5,\ 10,\ 9,\ 3}}$ 36.5, 43.9, 48.0, 133.2 and $\delta_{\mathbf{H\text{-}2}}$ 6.24 with $\delta_{\mathbf{C\text{-}10,\ 3,\ 4}}$ 43.9, 133.2, 135.4 confirming the proposed assignments.

COSY spectrum [Appendix 13c] had ^1H-^1H cross peaks at $\delta_{\mathbf{H\text{-}2}}$ 6.24 with $\delta_{\mathbf{H\text{-}3}}$ 6.86 and $\delta_{\mathbf{H\text{-}1}}$ 6.21 with $\delta_{\mathbf{H\text{-}10}}$ 2.77 further confirming the proposed chemical structure as that of a derivative of **402** that was formed through oxidation process by H-loss at C-1 and C-2. Compound **403** was consequently identified as a new compound that was given the IUPAC name 18-methoxycarbonyl-15, 16-epoxy-cleroda-1, 3, 13 (16), 14-tetraen-19, 20-olide and trivial name megalocarpoidolide K.

Table 4.13: NMR (500 MHz) spectroscopic data of megalocarpoidolide K (403)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY	NOESY
1	125.7	6.21 (*m*; H)	3, 5, 9, 10	10	10
2	130.3	6.23(*m*; H)	3, 4, 10	3	3
3	133.2	6.85(*m*; H)	1, 2, 5, 18, 19	2	2
4	135.4				
5	36.5				
6	34.0	1.60 (*m*; H_α) 2.81 (*m*; H_β)	7	6α, 19α 6β, 7α, β, 19β	19α,β; 6β 6α
7	29.9	1.60 (*m*; H_α) 2.55 (*m*; H_β)	5, 6, 9, 17, 19	6β 6β	
8	37.3	1.95 (*m*; H)		17	
9	48.0				
10	43.9	2.77(*s*; H)	2, 4, 5, 6, 9, 19, 20	1	1
11	29.3	1.60(*m*; H_α) 2.55 (*m*; H_β)	 9, 10, 12, 13		11β,12α, β 11α, 12α, β
12	17.6	2.20(*m*; H_α) 2.36 (*m*; H_β)	11, 13, 14, 16 11, 13, 14, 16		11α, β; 12β 11α, β; 12α
13	124.3				
14	111.0	6.30 (*s*; H)	13, 15, 16	15	
15	143.2	7.38 (*t*,1.55/1.62; H)	13, 16	14	
16	138.9	7.30(*s*; H)	14, 15		
17	16.4	0.98 (*s*; 3H)		8	
18	167.4				
19	73.2	4.24 (*dd*, 1.59, 10.09; H_α) 4.48 (*d* , 11.68; H_β)	4, 5, 6 5, 6, 10, 20	6α, 19β 6β, 19α	6α, β
20	173.0				
18-acetoxy	51.9	3.80 (*s*; 3H)	4, 18		

4.1.1.2 Abietane diterpenoids from *Croton megalocarpoides*

Three abietane diterpenoids were isolated from the roots of *C. megalocarpoides* [Figure 4.5**]** two of them, **404** and **406** are known compounds while **405** is new. Their structural elucidation will be discussed in the sections that follow here in.

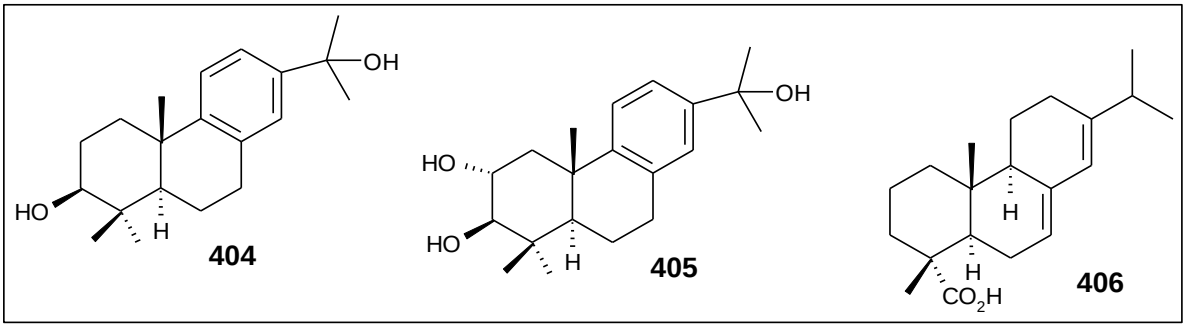
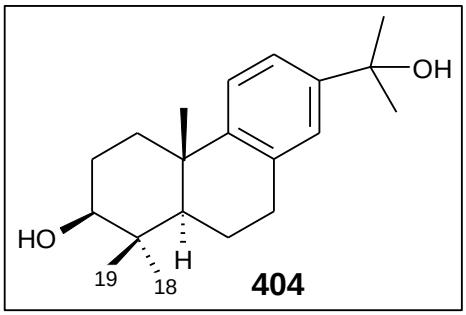


Figure 4.5: Abietane diterpenoids from *Croton megalocarpoides*

4.1.1.2.1 Isolophanthin A (404)

Compound **404** was isolated as white crystals and a molecular formula, $C_{20}H_{30}O_2$ proposed.



The ^{1}H NMR spectrum of compound **404** [Appendix 14a] showed three aromatic protons at δ_H 7.19 *br s*, 7.21 *s* and 7.23 *d* (*J* = 4.44 Hz). Also observed were five methyl group singlets at δ_H 0.91, 1.09, 1.21, 1.58 and 1.58 and other resonances associated with methine and methylene groups. The ^{13}C NMR spectrum [Appendix 14b] had 20 peaks of a diterpene having a *tri*-substituted aromatic ring as evidenced by presence of three protonated carbons at δ_C 122.2, 124.6, 125.0 and three fully substituted ones at δ_C 135.0, 146.2, 148.1. Resonances of oxygenated quaternary and methine carbons at δ_C 72.5 and 79.0 were among resonances of sp^3 carbons observed including five methyl groups at δ_C 15.4, 24.9, 28.4, 31.8 and 31.8. These obserations were supported by DEPT spectrum [Appendix 14c].

HMBC correlations [Appendix 14d] displayed evidence of an isopropyl group attached to an aromatic ring due to observed ^{1}H-^{13}C cross peaks between $\delta_{\mathbf{3H\text{-}16\,/\,17}}$ 1.58 with $\delta_{\mathbf{C\text{-}13}}$ 146.2. The hydroxyl group substitution at position 15 was supported by ^{1}H-^{13}C cross peaks between the three proton singlets at $\delta_{\mathbf{3H\text{-}16\,/\,17}}$ 1.58 with an oxygenated quaternary carbon at $\delta_{\mathbf{C\text{-}15}}$ 72.5. More ^{1}H-^{13}C cross peaks were observed between methyl proton singlets at $\delta_{\mathbf{3H\text{-}19,\,18}}$ 0.91 and 1.09 with the oxymethine carbon at $\delta_{\mathbf{C\text{-}3}}$ 79.0. The aforementioned structural features were consistent with an abietane diterpenoid with ring C being aromatic. A methine proton doublet of a doublet at $\delta_{\mathbf{H\text{-}3}}$ 3.32 led to a deduction of a 3β-OH configuration. Among the observed NOESY correlations [Appendix 14d] were ^{1}H-^{1}H cross peaks at $\delta_{\mathbf{3H\text{-}19}}$ 1.07 with $\delta_{\mathbf{3H\text{-}20}}$ 1.21 and $\delta_{\mathbf{3H\text{-}18}}$ 0.91 with $\delta_{\mathbf{H\text{-}5}}$ 1.31 as expected of a natural abietane (Hirasawa *et al*., 2007).

Literature survey showed compound **404** was the known (3*β*)-abieta-8, 11, 13-triene-3,15-diol, trivial name, isolophanthinA, isolated previously from *Isodon lophanthoides* var. *gerardianus* (Yang *et al*., 2011) and *Vitex rotundifolia* (Lee *et al*., 2013). This is therefore the first report of its isolation from *Croton* genus. Isolophanthin A has been reported to be ineffective against four human tumor cell lines (Yang *et al*., 2011) and not a potential anti-inflammatory agent (Lee *et al*., 2013).

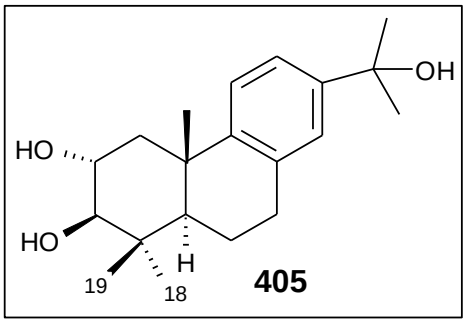
Table 4.14: NMR (500 MHz) spectroscopic data of isolophanthin A (404)

Postn	**δ_C**		**δ_H** (*m*, *J* Hz; Integral)	**HMBC** (H→C)	**NOESY** (H→ H)
	Yang *et al*., 2011	**Experimental**			
1	31.4t	18.9	1.89 (*m*; H_α) 1.75 (*m*; H_β)		
2	25.8t	28.2	1.09 (*s*; H_α) 1.27 (*s*; H_β)	20, 3	19 18, 19
3 3-OH	75.6d	79.0	3.32 (*dd* , 4.53, 6.80; H) 5.32 (*s*; H)		18
4	37.2s	39.2			
5	43.2d	50.0	1.31 (*m*; H)		18
6	18.6t	37.1	2.34 (*m*; H_α) 1.58 (*s*; H_β)	5, 7	
7	30.4t	31.1	1.27 (*s*; H_α) 1.09 (*s*; H_β)	5, 6	

8	134.8s	135.0			
9	148.2s	148.1			
10	37.7s	38.2			
11	124.7d	125.0	7.23 (*d*, 4.44; H)		
12	121.9d	122.2	7.19 (*br s*; H)		
13	145.8s	146.2			
14	124.2d	124.6	7.21 (*s*; H)		
15	72.3s	72.5			
16	31.6q	31.8	1.58 (*s*; 3H)	13, 15, 17	
17	31.6q	31.8	1.58 (*s*; 3H)	13, 15, 16	
18	22.1q	15.4	0.91 (*s*; 3H)	3, 5,	2β, 3, 5, 19
19	28.1q	28.4	1.09 (*s*; 3H)	5, 18	2α, 2β, 18, 20
20	24.6q	24.9	1.21 (*s*; 3H)		

4.1.1.2.2 Isolophanthin E (405)

Compound **405** was isolated as white crystals and a molecular formula, $C_{20}H_{30}O_3$ proposed for it.



405

The NMR spectroscopic data for compound **405** [Table 4.15; Appendix 15a] was similar to that of **404** except for an extra hydroxyl group and a proton at δ_H 4.25 *dd* (*J* = 3.16, 3.48 Hz) that were placed at position 2 based on correlations observed in 2D NMR experiments [Figure 4.6]. The ^{13}C NMR spectrum [Appendix 15a] had a resonance at δ_C 71.5 confirming the oxymethine carbon proposed to be at position 2.

An HMBC correlation [Appendix 15b] was observed between $\delta_{H\text{-}1}$ 2.72 and $\delta_{C\text{-}2}$ 71.5 in addition to COSY correlation observed between H-1 and H-2 further supporting the proposed structure. Key NOESY correlations [Appendix 15b] included $^{1}H\text{-}^{1}H$ cross peaks between the methylene protons at $\delta_{H\text{-}1\alpha,\beta}$ 2.72 and 1.75 with the oxymethine proton at $\delta_{H\text{-}2}$ 4.25 *dd* (*J* = 3.16, 3.48 Hz) implying that H-2 was β-configured and the hydroxyl group α-configured. H-2 in addition had a NOESY correlation with the oxymethine proton at $\delta_{H\text{-}3}$ 3.26 *br s* which had a correlation with the three proton singlet at $\delta_{3H\text{-}18}$ 1.13 indicating that the hydroxyl group at C-3 must be β-configured. The three proton singlets at $\delta_{3H\text{-}19}$ 1.10 and $\delta_{3H\text{-}20}$ 1.46 had a NOESY correlation with one another thus justifying the proposed configuration at C-4. Other long range correlations were similar to those observed of isolophanthin A (**404**). Compound **405** was therefore deduced to be a new derivative of **404** and was given the IUPAC name 2*α*, 3*β*-abietan-8, 11, 13-triene-2,3,15 triol and trivial name isolophanthin E following the naming of isolophanthin A-D (Yang *et al*., 2011; Lee *et al*., 2013).

Table 4.15: NMR (500 MHz) spectroscopic data of isolophanthin E (405)

Position	δ_C	δ_H (*m*, *J* Hz; Integral)	HMBC (H→ C)	COSY	NOESY
1	42.8	2.72 (*dd*, 2.94, 11.16; H_α) 1.75 (*dd*, 3.69, 11.02; H_β)	2, 3, 5, 10, 20 9, 10, 20	1β, 2, 10 1α, 2	2 2
2	71.5	4.25(*dd*, 3.16, 3.48; H)	1, 3, 10	1α / β, 3	1α, β, 3
3	78.4	3.26 (*br s*; H)		2	2, 18
4	38.5				
5	50.0	1.42 (*dd*, 2.82, 8.45; H)	9, 10, 18, 19, 20		
6	18.9	1.93 (*m*; 2H)		7α	7
7	31.1	2.90 (*m*; H_α) 2.98 (*m*; H_β)	6, 8, 9, 14 5, 6, 8, 9, 14	7β, 6 7α	6
8	134.8				
9	148.6				
10	37.0				
11	125.1	7.25 (*d*, 1.16; H)	7, 8, 9, 13, 15		10, 1β
12	122.3	7.25 (*d*, 1.16; H)	9, 13, 11, 15, 10		16

13	146.2			
14	124.8	7.20 (*br s*; H)	7, 9, 12, 15,	17
15	72.5			
16	31.8	1.58 (*s*; 3H)	13, 14, 15, 17	12
17	31.8	1.58(*s*; 3H)	13, 14, 15, 16	14
18	17.2	1.13(*s*; 3H)	3, 4, 5, 19	3
19	29.8	1.10 (*s*; 3H)	2, 3, 4, 5, 18	20
20	26.8	1.46 (*s*; 3H)	1, 5, 9, 10	19

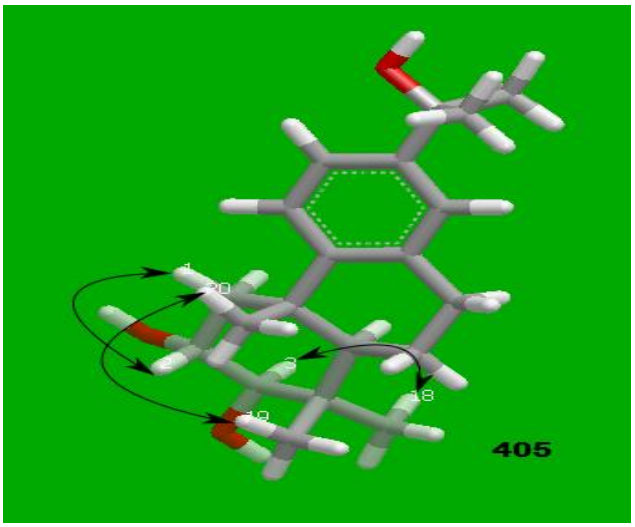
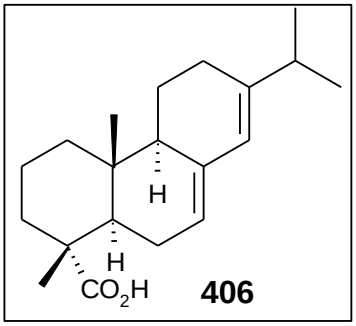


Figure 4.6: Key NOESY correlations of compound 405

4.1.1.2.3 Abietic acid (406)

Compound **406** was isolated as white crystals and a molecular formula, $C_{20}H_{30}O_2$ proposed for it. The FTIR spectrum [Appendix 16a] had a peak at 1705.0 cm^{-1} attributed to a free carboxylic acid group. Other peaks were observed at 2926.2, 2871.1, 2382.0, 2342.0, 1253.4 and 1143.8 cm^{-1}.



The ^{1}H NMR spectrum [Appendix 16b] had resonances of two olefinic singlets integrating for one proton each at δ_H 5.78 and 5.37. Resonances of an isopropyl group (two three-proton doublets at δ_H 1.01 and 1.06 and a septet at δ_H 2.21 (J = 6.8 Hz) and two methyl singlets at δ_H 0.83 and 1.27 were also observed. The ^{13}C NMR spectrum [Appendix 16b] had resonances of 20 carbons of a diterpenoid that included resonance of an isopropyl group at δ_C 20.9, 21.4 and 34.8 and two methyl group carbons at δ_C14.0 and 16.7. Resonances of four sp^2 carbons, two of them methines at δ_C 120.5 and 122.4 and the other two quaternary at δ_C 135.6 and 145.1 and a carbonyl carbon at δ_C 187.2 were observed.

Correlations observed in 2D NMR experiments and literature survey showed that, the spectroscopic data of compound **406** [Table 4.16] was similar to that of the known *syn*-abietic acid (Spessard *et al*., 1995). Abietic acid, also known as rosin acid, is a major component of gum rosin, and is used in the paints and varnishes industry (Atta *et al*., 2004; Zinkel and Landucci, 1991). It is also reported as having anti-allergic (Ulusu *et al*., 2002), anti-inflammatory (Kim *et al*., 2010), phyto alexin-like and anti-convulsant activities (Spessard *et al*., 1995; Talevi *et al*., 2007).

Table 4.16: NMR (500 MHz) spectroscopic data of abietic acid (406)

Position	**δ_C**		**δ_H (*m*, *J* Hz; Integral)**	
	Spessard *et al*., 1995	**Experimental**	**Experimental**	**Spessard *et al*., 1995**
1	38.3	38.3	0.92 (*m*; 2H)	0.93 *dd*
2	18.1	18.1	1.35 (*m*; 2H)	1.40 *m*
3	37.2	37.2	1.59 (*m*; 2H)	1.60 *m*
4	46.3	50.9		
5	44.9	45.0	2.22 (*t*; H)	2.25 *dd*
6	25.6	25.6	2.08 (*s*; 2H)	2.06 *s*
7	120.5	120.5	5.37 (*s*; H)	5.37 *s*
8	135.5	135.6		
9	51.0	46.2	1.83 (*s*; H)	1.83 *s*
10	34.5	34.5		
11	22.5	22.5	1.22 (*m*; H_α)	1.22 *m*
			1.57 (*m*; H_β)	1.61 *m*

12	27.5	27.5	2.22 (*m*; 2H)	2.19
13	145.1	145.4		
14	122.5	122.4	5.78 (*s*; H)	5.77 *s*
15	34.8	34.9	2.21 (*sept*; H)	2.18 *q*
16	20.9	20.9	1.01 (*d*; 3H)	1.02 *d*
17	21.4	21.4	1.06 (*d*; 3H)	1.04 *d*
18	185.4	187.2		
19	16.7	16.8	1.27 (*s*; 3H)	1.24 *s*
20	14.0	14.0	0.83 (*s*; 3H)	0.81 *s*

4.1.1.3 Trachylobane diterpenoids from *Croton megalocarpoides*

Four known trachylobane diterpenoids [Figure 4.7**]** were isolated from the roots of *C. megalocarpoides*.

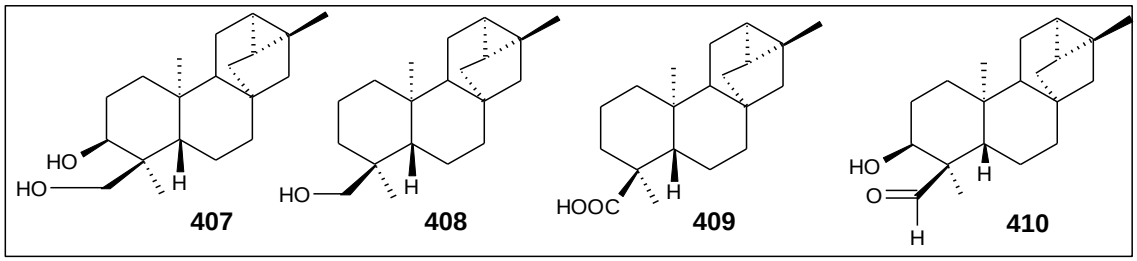
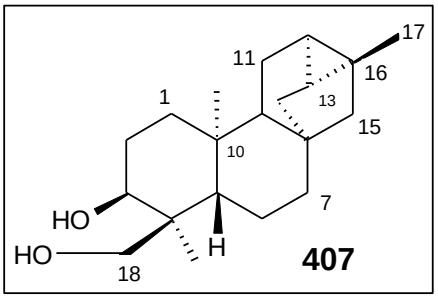


Figure 4.7: Trachylobane diterpenoids from *Croton megalocarpoides*

4.1.1.3.1 3α, 18-Dihydroxytrachylobane (407)

Compound **407** was isolated as white crystals. The LC-MS of compound **407** [Appendix 17a] had a quasi-molecular ion peak at 327.48 for [M + Na^+] consistent with the proposed molecular formula, $C_{20}H_{32}O_2$.



407

The ^{1}H NMR spectrum [Appendix 17b] had five methine protons, two of them up field at δ_H 0.60 *d* and 0.80 *m* which is characteristic of a cyclopropane ring of a tricyclo [3.2.1.0] octane ring system as found in a trachylobane structure (Kapingu *et al*., 2000; Fraga, 1994). Resonances of singlets by three methyl group protons at δ_H 0.90, 1.00 and 1.15 were also observed and in addition, eight methylene proton resonances. The ^{13}C NMR spectrum [Appendix 17b] had resonances of 20 carbons of a diterpenoid. Included were resonances of a cyclopropane of a tricyclo [3.2.1.0] octane ring system at δ_C 20.7, 24.4 and 23.8 (Kapingu *et al*., 2000). Resonances of three methyl group carbons at δ_C 11.5, 15.2 and 20.7 and four sp^3 quaternary carbons at δ_C 22.7, 38.1, 40.7 and 42.1 were also observed. The only resonances observed that are associated to functionalities where of two oxygenated sp^3 carbons, a methine and a methylene carbons at δ_C 77.3 and 72.4 respectively.

From the aforementioned and in consultation with 2D NMR experiments and literature data [Table 4.17], a pentacyclic diterpene, having a carbon skeleton with a tricyclo [3.2.1.0] octane ring system for rings C, D and E (Kapingu *et al*., 2000) and a 2^O and 1^O alcohol substituent on ring A was deduced. HMBC spectrum [Appendix 17c] had ^{1}H-^{13}C cross peaks at $\delta_{\mathbf{3H\text{-}17}}$ 1.15 with $\delta_{\mathbf{C\text{-}12,\ 16,\ 13}}$ 20.7, 22.7, 24.4; $\delta_{\mathbf{H\text{-}3}}$ 3.63 with $\delta_{\mathbf{C\text{-}19}}$ 11.5 and $\delta_{\mathbf{2H\text{-}18}}$ 3.72, 3.42 with $\delta_{\mathbf{C\text{-}19,\ 4,\ 5,\ 3}}$ 11.3, 42.1, 49.9, 77.3. Key NOESY correlations [Appendix 17c] were observed at $\delta_{\mathbf{3H\text{-}19}}$ 0.90 with $\delta_{\mathbf{3H\text{-}20}}$ 1.00 and $\delta_{\mathbf{H\text{-}3}}$ 3.63 with $\delta_{\mathbf{H\text{-}2\alpha,\ \beta}}$ 1.58.

Literature searches indicated that, compound **407** was the known 3α, 18-dihydroxytrachylobane, previously isolated from the roots of *C. macrostachys* (Kapingu *et al*., 2000) and *Mitrephora alba* (Annonaceae) where it is named as *ent*-trachyloban-3β, 18-diol in the report (Rayanil *et al*., 2013). *Ent*-trachyloban-3β, 18-diol was found to have moderate anticancer activity against human small cell lung carcinoma (IC_{50} 49.8 µM) and human carcinoma of the nasopharynx (IC_{50}62.1 µM) but weak activity against human breast adenocarcinoma (IC_{50}106.4 µM).

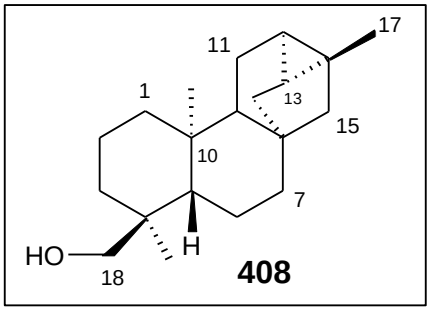
Its C-4 epimer, *ent*-trachyloban-3β, 19-diol was relatively weaker in activity against the same anti-cancer cell lines (IC_{50} > 150, 92.3 and > 150 µM respectively (Rayanil *et al*., 2013)).

Table 4.17: NMR (500 MHz) spectroscopic data of 3*α*, 18-dihydroxytrachylobane (407)

Position	δ_C		δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)
	Kapingu *et al*., 2000	Experimental		
1	36.9	37.3	1.55 (*m*; H $_{\alpha}$) 0.90 (*s*; H $_{\beta}$)	
2	26.6	26.8	1.58 (*m*; H $_{\alpha}$) 1.58(*m*; H $_{\beta}$)	1, 3, 4
3	80.1	77.3	3.63 (*t* , 7.81, 8.33; H)	18
4	40.2	42.1		
5	55.3	49.9	0.85(*m*; H)	
6	19.7	19.9	1.38 (*m*; H $_{\alpha}$) 1.38 (*m*; H $_{\beta}$)	8, 10
7	38.8	33.6	2.06 (*d*, 11.81; H_{α}) 1.16 (*m*; $H_{\beta)}$	13, 15, 16
8	41.8	40.7		
9	52.8	53.3	1.11(*m*; H)	
10	37.4	38.1		
11	19.7	20.3	1.87 (*dt*, 3.63, 11.81; H_{α}) 1.67 (*m*; H_{β})	9, 13
12	20.2	20.7	0.60 (*d*, 7.27; H)	
13	23.8	24.4	0.80 (*m*; H)	
14	33.0	38.8	1.38 (*m*; H_{α}) 1.38 (*m*; H_{β})	8, 9, 10, 12, 16
15	49.9	50.4	1.24 (*s*; H_{α}) 1.40 (*s*; H_{β})	7, 8, 9, 13, 16
16	23.8	22.7		
17	20.2	20.7	1.15 (*s*; 3H)	12, 13, 16
18	64.1	72.4	3.72 (*d*,10.41; H_{α}) 3.42 (*d*, 10.41; H_{β})	3, 4, 5 3, 19
19	22.1	11.5	0.90 (*s*; 3H)	3, 4, 5, 18
20	14.9	15.2	1.00 (*s*; 3H)	1, 5, 9

4.1.1.3.2 *Ent*-trachyloban-18-ol (408)

Compound **408** was isolated as white crystals and a molecular formula, $C_{20}H_{32}O$ proposed for it.



408

The spectroscopic data of compound **408** was similar to that of compound **407** less the substitution at C-3 [Table 4.18; Appendices 18a and 18b] confirmed by resonance of only one functionality at δ_C72.5 in the sp^3 region. Correlations observed in the 2D NMR experiments and literature search showed **408** was the known *ent*-trachyloban-19-ol.

Table 4.18: NMR (500 MHz) spectroscopic data of *ent*-trachyloban-19-ol (408)

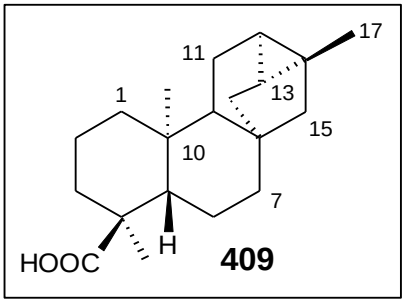
Position	δ_C		δ_H (*m*, *J* Hz; Integral)
	Kapingu *et al.*, 2000[6]	Experimental	
1	36.9	39.0	0.75 (*m*; H_α) 1.44 (*m*; H_β)
2	26.6	19.7	1.46 (*m*; H_α) 1.34 (*m*; H_β)
3	80.1	38.9	1.29 (*m*; H_α) 1.62 (*m*; H_β)
4	40.2	40.9	
5	55.3	49.5	1.00 (*m*; H)
6	19.7	29.2	1.20 (*m*; H_α) 1.62 (*m*; H_β)
7	38.8	33.8	1.34 (*m*; H_α) 1.33 (*m*; H_β)
8	41.8	40.8	

[6] Literature data for 3α, 18-dihydroxytrachylobane (**407**)

9	52.8	53.5	1.16 (*m*; H)
10	37.4	37.6	
11	19.7	20.2	1.83 (*m*; H_α) 1.61 (*m*; H_β)
12	20.2	20.8	0.50 (*m*; H)
13	23.8	24.5	0.90 (*s*; H)
14	33.0	38.7	2.00 (*d*,11.65; H_α) 1.17 (*m*; H_β)
15	49.9	50.8	1.34 (*m*; H_α) 1.20 (*m*; H_β)
16	23.8	22.8	
17	20.2	17.7	1.18 (*s*; 3H)
18	64.1	72.5	5.23 (*s*; 2H)
19	22.1	17.7	1.05 (*s*; 3H)
20	14.9	15.3	0.67 *s*; 3H)

4.1.1.3.3 Tachyloban-18-oic acid (409)

Compound **409** was isolated as white crystals. The LC-MS of compound **409** [Appendix 19a] had a quasi-molecular ion peak at 321.1 for [M + Na^+] consistent with the proposed molecular formula, $C_{20}H_{30}O_2$.



409

The NMR spectroscopic data of compound **409** [Table 4.19; Appendix 19b] was similar to that of compound **407** except for absence of resonance of hydroxyl groups at C-3 and C-18. A resonance of a carboxyllic acid functionality, δ_C 184.0 was observed and placed at position 18 based on correlations seen in the 2D NMR experiments. Comparison of the spectroscopic data with literature values identified compound **409** as the known *ent*-trachyloban-18-oic acid. *Ent*-trachyloban-18-oic acid is reported alongside its C-4 epimer, e*nt*-trachyloban-19-oic acid as having been previously isolated from the Malaysian liverwort, *Mustigophora diclados* (Leong and Harrison, 1997) and *C. macrostachyus* (Kapingu *et al*., 2000).

Ent-trachyloban-19-oic acid has been found to have larval development inhibition of *Homeosoma electullum* (sunflower moth) and the three *Lepidoptera* species *Heliotis virscens*, *H. zea* and *Pectinophera gossypiella* (pink bollworm) (Alliger *et al*., 1976). It has also been found to have antimicrobial activity against methicillin resistant *Staphylococcus aureus* and *Mycobacterium smegmatis* (Zgoda-Pols *et al*., 2002). Both *ent*-trachyloban-19-oic acid and its derivative, *ent*-trachyloban-19-oic methyl ester inhibited the growth of *Streptococcus mutans* (associated with caries) at 8.9 and 70.5 µg/mL respectively and had biofilm formation by the same bacteria at 32.5 and 125.0 µg/mL respectively (Hernández *et al*., 2012). They were however inactive against *Porphyromonas gingivalis* (associated with periodontal disease (Hernández *et al*., 2012).

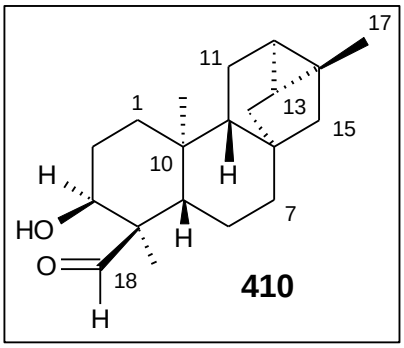
Table 4.19: NMR (500 MHz) spectroscopic data of *ent*-trachyloban-18-oic acid (409)

Postn	δ_C		δ_H (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY
	Leong and Harrison, 1997	Experimental			
1	39.5	37.2	1.78 (*m*; H_α) 1.60 (*m*; H_β)		
2	18.7	22.7	1.43 (*m*; H_α) 1.12 (*m*; H_β)		1β, 2β 2α
3	37.8	38.5	1.37 (*m*; H_α) 1.14 (*m*; H_β)	2, 4, 5 1,2,4,5,18	
4	43.7	41.1			
5	57.0	50.4	1.62 (*m*; H)	9, 10	
6	21.8	17.3	1.58 (*m*; H_α) 1.47 (*m*; H_β)	4, 5, 7, 10, 20	

7	39.2	33.7	2.04 (*m*; H_α) 1.16 (*m*; H_β)	5, 13, 16	7β 7α
8	40.8	47.4			
9	52.2	53.4	1.23 (*m*; H)		14
10	39.8	37.8			
11	19.7	19.7	1.89 (*m*; H_α) 1.67 (*m*; H_β)		9
12	20.5	20.7	0.57 (*m*; H)	9, 17	13
13	24.2	24.4	0.83 (*m*; H)	7, 12	11β, 12
14	33.1	38.6	1.37 (*m*; H_α) 1.14 (*m*; H_β)	9, 10, 12 8, 12, 13	13, 14β 14α
15	50.3	50.4	1.38 (*m*; H_α) 1.26 (*m*; H_β)	 7, 12, 13	14β
16	22.4	23.2			
17	20.6	20.8	1.12 (*m*; 3H)		
18	28.9	184.0			
19	184.7	16.5	1.15 (*s*; 3H)	18	
20	12.5	15.2	0.97 (*s*; 3H)	5, 9, 10	

4.1.1.3.4 3α-Hydroxytrachyloban-18-al (410)

Compound **410** was isolated as white crystals and a molecular formula, $C_{20}H_{30}O_2$ proposed for it.



Comparison of the NMR spectroscopic data of compound **410** [Table 4.19; Appendix20a] with that of **409** showed that their chemical structures were mostly identical. In **410** however, there were resonances of a formyl group at δ_H 9.25 *s* and δ_C 207.1 and an hydroxyl group substituent at δ_C 75.6 with an oxymethine proton at δ_H 3.51 *t* (*J* = 2.80 Hz) unlike in **409** where the only functionality was that of a carboxylic acid group at δ_C 184.0. The formyl group and hydroxyl group in **410** were subsequently placed on C-18 and C-3 respectively using correlations in 2D NMR experiments.

HMBC spectrum [Appendix 20c] had correlations by the formyl hydrogen at $\delta_{\mathbf{H\text{-}18}}$ 9.25 with $\delta_{\mathbf{C\text{-}19,\ 5}}$ 14.2, 39.4; the oxymethine proton at $\delta_{\mathbf{H\text{-}3}}$ 3.51 with $\delta_{\mathbf{C\text{-}1,\ 2,\ 5}}$ 32.8, 32.5, 39.4 and $\delta_{\mathbf{H\text{-}2\beta}}$ 1.95 with $\delta_{\mathbf{C\text{-}19,\ 10,\ 4,\ 3,\ 18}}$ 14.2, 29.9, 49.1, 75.6, 207.1. NOESY spectrum [Appendix 20c] showed correlations between 3H-19 and 2H-6 and a COSY between 3H-19 and H-3. Literature search showed that **410** was the known *ent*-3β-hydroxytrachyloban-18-al previously reported from *Mitrephora alba* (Rayanil *et al*., 2013) making this the first report of its isolation from *Croton* genus. In the same report (Rayanil *et al*., 2013), its anti-cancer activities were recorded as moderate activity (IC_{50} 55.9 µM) against human small cell lung carcinoma and weak activity against human breast adenocarcinoma (92.0µM) and human carcinoma of the nasopharynx (69.4 µM).

Table 4.20: NMR (500 MHz) spectroscopic data of 3*α*-*ent*-hydroxytrachyloban-18-al (410)

Position	δ_C		δ_H	HMBC
	Rayanil *et al*., 2013	Experimental	(*m*, *J* Hz; Integral)	(H→C)
1	37.1	32.8	1.23-1.43 (*m*; H_α) 1.23-1.43 (*m*; H_β)	
2	25.8	32.5	1.23-1.43 (*m*; H_α) 1.95 (*dd*, 2.00, 11.14; H_β)	3, 4, 10, 18, 19
3	72.0	75.6	3.51 (*t*, 2.80; H)	1, 2, 5
3-OH			5.23 (*s*; H)	
4	55.2	49.1		
5	48.0	39.4	2.06 (*m*; H)	
6	22.2	19.4	1.95 (*dd*, 2.00, 11.14; H_α) 1.95 (*dd*, 2.00, 11.14; H_β)	4, 8

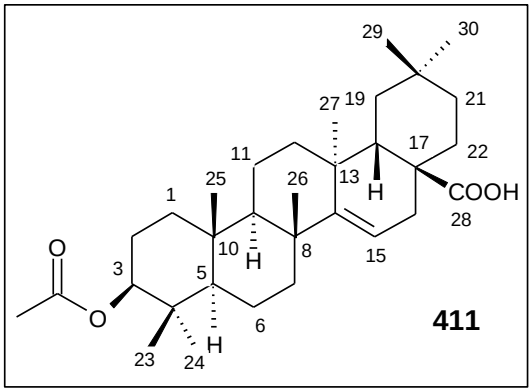
7	38.1	38.2	1.85-1.89 (*m*; H)	9, 15, 16
8	40.7	37.2	-	
9	53.0	47.5	1.57-1.63 (*m*; H)	1, 8, 15, 20
10	36.9	29.9		
11	19.6	16.7	1.57-1.63 (*m*; H_α) 1.57-1.63 (*m*; H_β)	8, 9, 12, 16
12	20.4	20.6	0.62 (*d*; H)	
13	24.2	24.3	0.88-0.95 (*m*; H)	
14	33.4	30.5	1.57-1.63 (*m*; H_α) 1.19 (*s*; H_β)	8, 9, 12, 16 9, 10
15	50.3	45.5	1.43-1.48 (*m*; H_α) 1.43-1.48 (*m*; H_β)	9, 16
16	22.5	23.3		
17	20.5	20.6	1.36 (*s*; 3H)	12, 15, 16
18	207.1	207.1	9.25 (*s*; H)	5, 19
19	8.8	14.2	0.95 (*s*; 3H)	18
20	14.9	15.0	0.95 (*s*; 3H)	

4.1.1.4 Triterpenoids from *Croton megalocarpoides*

Two known pentacyclic triterpenoids, acetylaleuritolic acid (**411**) and lupeol (**412**) were isolated from the roots of *C. megalocarpoides*.

4.1.1.4.1 Acetylaleuritolicacid (411)

Compound **411** was isolated as white crystals and a molecular formula, $C_{32}H_{50}O_4$ proposed for it.



The ^{1}H-NMR [Appendix 21] had seven singlets integrating for three protons each at δ_H 0.86, 0.87, 0.88, 0.91, 0.92, 0.94 and 0.95 representing seven methyl groups instead of the expected eight methyl groups of a triterpene. Another singlet integrating for three protons, that was observed down field at δ_H 2.04 was taken to be of an acetate methyl group substituent. A broad singlet observed at δ_H 11.6 was taken to be of a carbinol proton in a carboxylic acid group substituent. It was then deduced that, a methyl group of a triterpene must have been oxidized to a carboxylic acid during the biosynthetic process. Resonances of an olefinic proton at δ_H 5.52 (*dd*, *J* = 4.0, 8.0 Hz) and an oxymethine proton at δ_H 4.46 (*dd*, *J* = 5.5, 10.0 Hz) were also observed. The ^{13}C NMR spectrum [Appendix 21] had resonances of thirty two carbons including two carbonyl carbons at δ_C 171.2 and 184.3 and two sp^2 carbons at δ_C 117.1 and 160.8.

Comparison of the adduced spectroscopic data with literature identified compound **411** as the acetylated pentacyclic triterpenoid, acetylaleuritolic acid (Carpenter *et al*., 1980) previously isolated from *C. cajucara* (Maciel *et al*., 2000; Pertino *et al*., 2007), *C. urucurane* (Peres *et al*., 1997 and 1998a,b), *C. lacciferus* (Bandara *et al*., 1988) and *C. pseudopulchellus* (Langat *et al*., 2012). Biological activities of acetylaleuritolic acid that are reported include activity against *Salmonella aureus* and *Salmonella typhimurium* (MIC, 0.1 mg / mL (Peres *et al*., 1998a, b), anti-nociceptive effect (analgesic activity, ID_{50} = 21.63 mg / Kg (Peres *et al*., 1998a, b), and gastroprotective effect at 25 mg / Kg (Pertino *et al*., 2007).

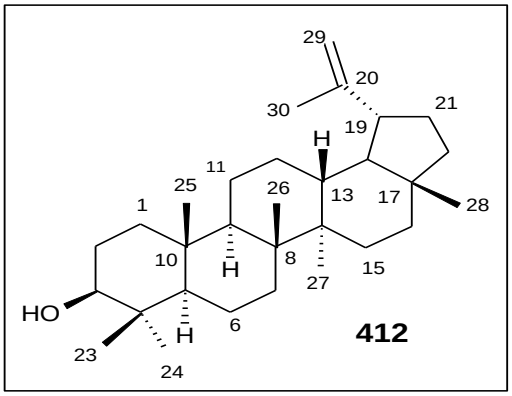
Table 4.21: NMR (500 MHz) spectroscopic data of acetylaleuritolic acid (411)

Position	δ_C		δ_H (*m*, *J* Hz; Integral)
	Carpenter *et al*., 1980	Experimental	
1	37.4	37.5	1.59 (*m*; H_α) 1.03 (*m*; H_β)
2	23.4	23.7	1.62 (*m*; H_α) 1.62 (*m*; H_β)
3	80.8	81.1	4.46 (*dd*, 5.5, 10.0; H)
4	37.6	37.9	
5	55.6	55.8	0.86 (*s*; H)
6	18.7	19.0	1.78 (*m*; 2H)
7	35.3	35.6	1.22 (*m*; H_α) 1.09 (*m*; H_β)

8	39.0	39.3	
9	49.0	41.0	1.41 (*m*; H)
10	37.3	37.6	
11	17.3	17.5	1.62 (*m*; H_α) 1.44 (*m*; H_β)
12	31.2	31.5	2.37 (*q*, 7.33; H_α) 1.91 (*m*; H_β)
13	37.9	38.2	
14	160.5	160.8	
15	116.8	117.1	5.52 (*dd*, 4.0, 8.0; H)
16	30.9	30.9	1.67 (*t*, 14; H_α) 1.41 (*m*; H_β)
17	51.5	51.7	
18	41.6	41.6	2.28 (*m*; H)
19	40.7	41.0	1.96 (*m*; H_α) 1.27 (*m*; H_β)
20	29.3	29.5	
21	33.6	33.9	1.74 (*m*; H_α) 1.06 (*m*; H_β)
22	31.8	32.1	1.06 (*m*; 2H)
23	27.9	28.2	0.86 (*s*; 3H)
24	16.6	16.8	0.88 (*s*; 3H)
25	15.7	15.9	0.94 (*s*; 3H)
26	28.6	28.9	0.88 (*s*; 3H)
27	26.2	26.4	0.95 (*s*; 3H)
28	184.4	184.3	
29	33.3	33.5	0.92 (*s*; 3H)
30	22.4	22.7	0.91 (*s*; 3H)
3-$OOCCH_3$	171.3	171.2	
3-$OOCCH_3$	21.6	21.5	2.04 (*s*; 3H)
-COOH		184.3	11.6 (*br s*; H)

4.1.1.4.2 Lupeol (412)

Compound (**412**) was isolated as a white crystals and a molecular formula, $C_{30}H_{50}O$ proposed for it.



The ^{1}H NMR spectrum [Appendix 22a] had resonances of seven methyl proton singlets at δ_H 0.79, 0.83, 0.88, 0.94, 0.96, 1.08 and 1.68 that corresponded to δ_C 15.4, 18.0, 16.1, 14.6, 28.0, 16.0 and 19.3 in the ^{13}C NMR spectrum [Appendix 22b]. The proton resonance at δ_H 3.19 was taken to be the oxymethine proton at C-3 because it corresponded to a carbon resonating at δ_C 79.1 in the HSQC spectrum. Methylene protons singlets observed in the olefinic region at δ_H 4.57 and 4.69 were taken to belong to the carbon of the terminal C=C. A methine proton resonating at δ_H 2.40 was attached to a carbon adjacent to the C=C, C-19. ^{13}C NMR spectrum had resonances of 30 carbons that were classified using DEPT spectrum into seven methyl, eleven methylene, six methine and six quaternary carbons. The sp^2 carbons were observed at δ_C 151.0 and 109.3.

The physical and spectral data obtained [Table 4.22] corresponded to that reported for the known 3β-hydroxylup-20(29)-ene commonly known as lupeol (Burns *et al*., 2000; Sutomo *et al*., 2013). Lupeol is a very common triterpenoid that has been isolated from many different plant families. It has been reported as having varying biological activities including dead cell stimulant of human leukemic cells (HL-60), an aggressive inhibitor of human metastatic melanoma cells, anti-arthritic, anti-malarial, anti-microbial and anti-inflammatory (Aratanechemuge *et al*., 2004; Agarwal and Rangari, 2003; Gallo and Sarachine 2009; Fotie *et al*., 2006).

Table 4.22: NMR (300 MHz) spectroscopic data of lupeol (412)

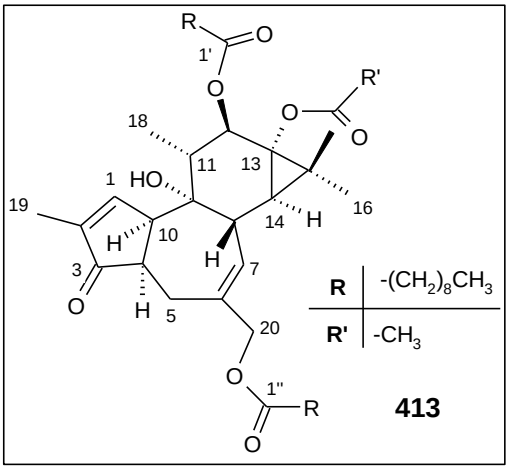
Pstn	δ_C		δ_H (*m*, *J* Hz; Integral)	
	Sutomo *et al*., 2013	Experimental	Experimental	Sutomo *et al*., 2013
1	40.1	38.8	0.96 (*m*; H_α)	0.96 *s*
			1.65 (*m*; H_β)	1.70 *m*
2	28.7	27.5	1.65 (*m*; 2H)	1.63 *m*
3	79.7	79.1	3.19 (*dd*, 11.4, 4.9; H)	3.14 (*dd*, 11.0, 5.3)
4	40.0	38.7		
5	56.9	55.3	0.69 (*d*, 9.25; H)	0.69 *d*
6	19.5	18.3	1.39 (*m*; H_α)	1.42 *m*
			1.58 (*m*; H_β)	1.56 *m*
7	35.6	34.3	1.37 (*m*; H_α)	-
			1.63 (*m*; H_β)	
8	42.1	40.8		
9	51.9	50.4	1.36 (*m*; H)	1.34 *m*
10	38.3	37.2		
11	22.1	20.9	1.34 (*m*; H_α)	1.31 *m*
			1.49 (*m*; H_β)	1.47 *m*
12	26.5	25.1	1.25 (*m*; H_α)	1.20 *m*
			1.74 (*m*; H_β)	1.72 *m*
13	39.7	38.1	1.72 (*m*; H)	1.68 *m*
14	44.0	42.8		
15	28.1	27.4	1.03 (*m*; H_α)	1.01 *m*
			1.61 (*m*; H_β)	1.60 *m*
16	35.6	35.6	1.53 (*m*; 2H)	1.51 *m*
17	44.2	43.0		
18	49.5	48.3	1.46 (*m*; H)	1.43 *m*
19	49.3	48.0	2.40 (*m*; H)	2.40 *m*
20	152.0	151.0		
21	30.8	29.7	1.32 (*m*; 2H)	1.28 *m*
22	41.1	40.0	1.43 (*m*; 2H)	1.43 *m*
23	28.7	28.0	0.96 (*s*; 3H)	0.98 *s*
24	16.2	15.4	0.79 (*s*; 3H)	0.76 *s*
25	16.8	16.1	0.88 (*s*; 3H)	0.86 *s*
26	16.7	16.0	1.08 (*s*; 3H)	1.07 *s*
27	15.1	14.6	0.94 (*s*; 3H)	0.96 *s*
28	18.5	18.0	0.83 (*s*; 3H)	0.83 *s*
29	110.2	109.3	4.57 (*s*; H_α)	4.56 *s*
			4.69 (*s*; H_β)	4.68 *s*
30	19.7	19.3	1.68 (*s*; 3H)	1.69 *s*

4.1.2 The Phytochemistry of Kenyan *Croton alienus*

Eleven compounds were isolated from the roots and the leaves of *C. alienus*. Two of these compounds were new (a 4α-deoxyphorbolester, 12,20-*O*-[*n*-didecanoyl]-4α-deoxyphorbol-13-acetate, given trivial name, alienusolin (**413**) and a glutarimide alkaloid, *N*-[1,3-dioxo-2-(2-phenylethyl)-6-piperidinyl]-phenylanamide, given a trivial name, crotonimide C **(415)**). The other compounds included the known glutarimide alkaloid (julocrotine (**414)**), six methylcyclohexane derivatives including the common crotepoxide (**416)** and five of its derivatives (monodeacetylcrotepoxide (**417)** , dideacetylcrotepoxide (**418)**, α-senepoxide (**419)** , β-senepoxide (**420)** and (+)-(2*S*,3*R*-diacetoxy-1-benzoyloxymethylenecyclohex-4,6-diene (**421**)), the common pentacyclic triterpenoid (acetylaleuritolic acid (**411**) and an α, β-unsaturated phytosterol (24-ethylcholesta-4, 22-dien-3-one (**422**). The work reported in this section / plant has been published and the paper is presented as Appendix 41.

4.1.2.1 A Phorbol ester derivative, alienusolin (413)

A new phorbol ester derivative was isolated as yellow oil from the roots of *C. alienus*. The molecular formula of **413** was deduced to be $C_{42}H_{66}O_8$ from the HRESIMS [Appendix 23a] that had a *m/z* 721.4641 for a quasi-ion $[M+Na]^+$, calc. 721.4650 and $[\alpha]_D$ + 36.1 ($CHCl_3$, c 0.003). This compound displayed diagnostic IR absorptions at 3412 (OH group stretch), 1735 (*br*, 1690-1740) for ester and α, β-unsaturated carbonyl groups.



The ^{1}H NMR spectrum [Appendix 23b] showed a broad singlet at δ_H 1.25 indicative of the presence of a long chain fatty acid moiety. Resonances of protons associated with six methyl groups at δ_H 2.05*s*, 1.75*s*, 1.20*s*, 1.16*s*, 1.05*d* (*J* = 6.3 Hz), and 0.87*t* (*J* = 1.23 Hz), two olefinic protons at δ_H 6.97*s* and 5.47*d* (*J* = 10.4 Hz) and two oxymethylene protons at δ_H 4.46*d* (*J* = 12.5 Hz) and 4.32*d* (*J* =12.5 Hz) were observed.

The ^{13}C NMR spectrum [Appendix 23b] indicated presence of a carbonyl carbon at δ_C 211.2, three ester carbonyls at δ_C 173.9, 173.7 and 173.6, four Sp^2 carbons at δ_C 155.4, 143.5, 133.0 and 128.6 and four oxygenated sp^3 carbons at δ_C 78.0, 75.5, 70.7 and 65.4. Analysis of COSY and HMBC spectra [Appendix 23c] led to a deduction that compound **413** had a tigliane diterpenoid skeleton. However, the presence of only one doublet of a methyl group in its ^1H - NMR spectrum pointed to a likely modification at positions of C-1 and C-6. The upfield methine proton doublet at δ_H 0.80 (J = 5.1 Hz) was assigned to H-14 with its carbon, C-14 resonating at δ_C 37.0. HMBC correlation between C-14 and the two methyl proton singlets at δ_H 1.16 and 1.20 enabled their assignments as 3H-17 (δ_{C-17} 24.3) and 3H-16 (δ_{C-16} 16.6) respectively. The two 3H-16 and 3H-17 methyl group protons further showed correlations in the HMBC spectrum with carbons at δ_C 25.2 and 65.9 assigned to C-15 and C-13 respectively [Figure 4.8**]**. C-15 and C-13 had additional HMBC correlations with a methine proton at δ_H 5.47*d* (J = 10.4 Hz) assigned to H-12. This H-12 was coupled to another methine proton at δ_H 1.66*m* assigned to H-11. H-11 was additionally coupled to a proton at δ_H 1.06*d* (J = 6.3 Hz) that was assigned to 3H-18 [Figure 4.8]. The 3H-18 further had an HMBC correlation with C-9 which also had ^1H-^{13}C cross peaks with H-1, H-4, H-7, H-8 and H-10. The olefinic proton resonance at δ_H 6.97 (*s*) was assigned to H-1 and showed HMBC correlations with C-3, C-4 and C-19. Another key HMBC correlation was observed for H-7 with C-5, C-6 and C-20.

An acetate group and two acyl groups were attached via oxygen to C-13, C-12 and C-20 respectively.The acetate group was deduced to be on C-13 due from correlations observed in the NOESY spectrum between δ_{3H-16} 1.20*s* and acetoxy methyl protons at δ_H 0.87*t* [Figure 4.8]. The second acetate group was placed at position 12 from observance of a resonance of a methine proton downfield at δ_{H-12} 5.47*s* which is characteristic of esters that exhibit presence of an acyl group at C-12 and an acetate group at C-13 (Taylor *et al*., 1981; Thebpatiphat *et al*., 1988). Acid hydrolysis of compound **413** and subsequent analysis of the resulting products using GC / MS indicated that, the acyl groups attached to C-12 and C-20 were the same and were identified to be decanoyl moieties. The suggested relative configuration at positions 4, 8, 11 and 15 was supported by correlations observed in the NOESY spectrum between H-4 with H-10 and H-5β / H-5β with H-4 and H-20; H-8 with H -5α and 3H-17; 3H-18 with H-10 / H-11 with 3H-17 and H-14 with 3H-16 and H-7 respectively [Figure 4.8]. Compound **413** was identified to be a new phorbol ester derivative that was given the IUPAC name, 12, 20-*O*-[*n*-didecanoyl]-4α-deoxyphorbol-13-acetate and trivial name alienusolin.

Table 4.23: NMR spectroscopic data of alienusolin (413)

Position	δ_C (125 MHz)	δ_H (500 MHz) (*m*, *J* Hz; Integral)	HMBC (H→C)	COSY
1	155.3	6.97 (*s*; H)	2, 10, 3, 4, 9, 19	10
2	143.5			
3	211.2			
4	49.0	2.71 (*m*; H)	6	5, 10
5	26.6	2.46 (*m*; H_α) 3.35 (*m*; H_β)	6, 10	4, 5β 5α
6	133.0			
7	128.6	5.14 (*br s*, $W_{1/2}$ = 7.5Hz; H)	6, 5, 9, 14, 20	8
8	41.0	1.96 (*m*; H)	9, 14	7
9	78.0			
10	47.0	3.45 (*m*; H)	1, 9, 2, 8	1, 4
11	43.0	1.66 (*m*; H)	12	12, 18
12	75.4	5.45 (*d*, 10.4; H)	11, 13, 15,18,-OOCR′	11
13	65.4			
14	37.0	0.80 (*d*, 5.1; H)	13, 15, 7	
15	25.2			
16	16.6	1.20 (*s*; 3H)		
17	24.2	1.16 (*s*; 3H)		
18	12.1	1.05 (*d*, 6.3; 3H)	11, 9, 12	
19	10.7	1.75 (*s*; 3H)	2, 1, 3	
20	70.7	4.34, 4.46 (*d*, 12.5; 2H)	6, 5, 7, -OOCR	
-OOCR	173.8			
-OOC-R-	14.4	0.87 (*t*, 1.23; 3H)		
CH_3				
-$OOCCH_2$-	22.8-	2.00-2.42 (*m*; 2H)	-OOCR	
$(CH_2)_n$-R′	34.7			
(-OOCR′)	173.6			
-$OOCCH_3$	21.2	2.05 (*s*; 3H)		

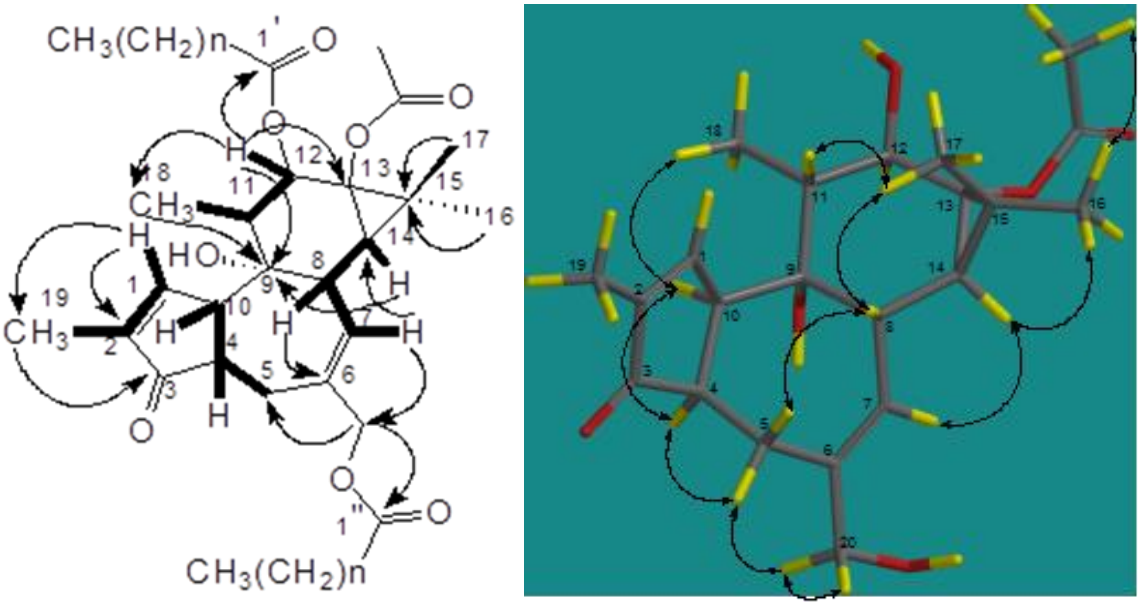


Figure 4.8: COSY, HMBC and NOESY correlations observed in alienusolin (413)

4.1.2.2 Glutarimide alkaloids from *Croton alienus*

Two glutarimide alkaloids [Figure 4.9] were isolated from the roots of *C. alienus* as white crystalline. They were identified as the known julocrotine [**414**] (Aboagye *et al*., 2000; Suarez *et al*., 2004) and crotonimide C [**415**], a new natural product.

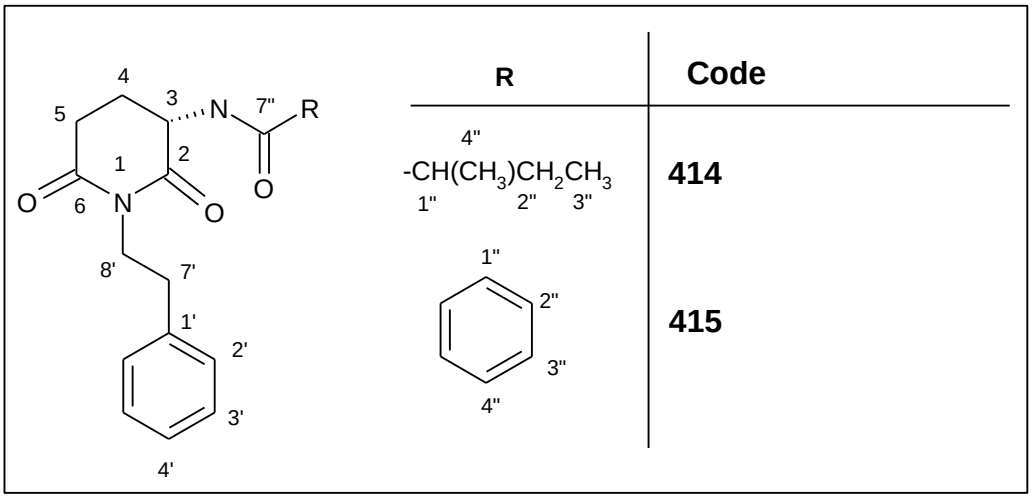


Figure 4.9: Glutarimide Alkaloids from *C. alienus*

4.1.2.2.1 Julocrotine (414)

The MS spectrum of compound **414** had a molecular ion peak at *m/z* 316.20 confirming the proposed molecular formula of $C_{18}H_{24}N_2O_3$. The ^{1}H NMR spectrum [Appendix 24] had resonances of methine protons associated with an aromatic ring at δ_H 7.19 *m* (integrating for three protons) and δ_H 7.26 *m* (integrating for two protons).

Other significant proton resonances observed were of two methyl groups at δ_H 0.92 *t* (*J* = 7.5 Hz) and 1.14 *d* (*J* = 7.0 Hz) respectively and down field shifted protons, a doublet integrating for one at δ_H 6.25 (*J* = 4.5) and a doublet of a triplet of a doublet at δ_H 4.45 (*J* = 2.0, 5.5, 12.5 Hz). The ^{13}C NMR spectrum of 2 [Appendix 24] had eighteen carbons. Included were resonances associated with a mono-substituted aromatic ring (five methine carbons at δ_C 126.5, 128.3 and 128.8 and a fully substituted carbon at δ_C 138.0). Three carbonyl carbon resonances were observed at δ_C 171.2, 172.1 and 177.0 and two methyl carbons at δ_C 12.0 and 17.5. From these spectral data and in consultation with literature, an alkaloid with a phenylethyl-glutarimide ring system and a 2-methylbutanoyl group substituent was deduced (Aboagye *et al*., 2000; Cuong *et al*., 2002). HMBC spectrum had ^1H-^{13}C cross peaks at $\delta_{\mathbf{H\text{-}3}}$ 4.45 with $\delta_{\mathbf{C\text{-}4,\,2,\,7''}}$ 24.3, 172.1 and 177.0; $\delta_{\mathbf{H\text{-}NH}}$ 6.25 with $\delta_{\mathbf{C\text{-}7''}}$ 177.0 and $\delta_{\mathbf{H\text{-}4}}$ 2.50 with $\delta_{\mathbf{C\text{-}5,\,3,\,6,\,2}}$ 31.9, 51.4, 171.2 and 172.1 thus confirming the proposed chemical structure. Other spectral data related to this compound are given in. Compound **414** was subsequently identified to be the known *N*-[1, 3-dioxo-2-(2-phenylethyl)-6-piperidinyl]-2-*N*-(2-methylbutanoylanamide), trivial name, julocrotine. Julocrotine was previously isolated from the dichloromethane extract of the roots of *C. membranaceus* (Aboagye *et al*., 2000), *C. cascarilloides* (Cuong *et al*., 2002) and the stem of *C. pullei* (Suarez *et al*., 2004).

Table 4.24: NMR spectroscopic data of julocrotine (414)

Position	δ_C (125 MHz)		δ_H (500 MHz) (*m*, *J* Hz; Integral)
	Aboagye *et al*., 2000	Experimental	
2	171.7	172.1	
3	51.0	51.4	4.45 (*dtd*, 12.5, 5.5, 2.0; H)
4	24.3	24.6	2.50 (*dtd*, 12.0, 5.5, 2.0, H_α); 1.65 (*m*; H_β)
5	31.6	31.9	2.70 (*m*; 2H)
NH	-	-	6.25 (*d*, 4.5; H)
6	170.9	171.2	
1″	42.8	43.2	2.18 (*m*; H)
2″	27.1	27.4	1.45 (*m*; H_α) 1.65 (*m*; H_β)
3″	11.7	12.0	0.92 (*t*, 7.5; 3H)

4″	17.1	17.5	1.14 (*d*, 7.0; 3H)
7″	178.9	177.0	
1′	138.0	138.3	
6′ / 2′	128.8	129.2	7.19 (*m*; 2H)
5′ / 3′	128.3	128.7	7.26 (*m*; 2H)
4′	126.5	126.8	7.19 (*m*; H)
7′	33.6	34.2	2.78 (*t*, 7.0; 2H)
8′	41.4	41.8	3.98 *m*; 2H)

4.1.2.2.2 Crotonamide C (415)

Compound **415** was isolated as a white crystalline and identified to be a new form of glutarimide alkaloid that was given the trivial name, crotonamide C. Its HRESIMS [Appendix 25a] had a quasi-molecular ion peak at 359.1354 $[M + Na]^+$ (calcd for $C_{20}H_{20}N_2NaO_3$, 359.1366) supporting the proposed molecular formula, $C_{20}H_{20}N_3O_3$. The optical rotation was established to be, $[\alpha]_D$ - 13.0 ($CHCl_3$, c 0.0009). FTIR ν_{max} cm^{-1} (neat): 3392, 3066, 3028, 2962, 2928, 1729, 1680, and 1641.

The NMR spectroscopic data of **415** [Table 4.25; Appendix 25b] was similar to that of julocrotine (**414**) except for resonances of a phenyl ketone / benzamide group substituent observed at δ_C 167.5 for a ketonic carbon and aromatic ring chemical shifts of methine carbons at δ_C 127.3, 128.6 and 132.2 and a fully substituted one at δ_C 133.9 alongside their resonances in the ^{1}H NMR spectrum at δ_H7.48 *dd* (*J* = 1.4, 7.5 Hz), 7.55 *tt* (*J* = 1.5, 7.2 Hz) and 7.83 *td* (*J* = 1.5, 6.9) in place of a 2-methylbutanoyl group substituent as in **414**. Similarity in the configuration of **415** at C-3 with **414** was supported by the coupling constant for both H-3 and H-4β (*J* = 12.5 Hz) indicating a pseudo-axial position for the H-3. Apart from the coupling of the phenyl group carbons and protons that was observed in the HMBC spectrum of **415**, there was coupling between the aromatic protons at δ_H 7.83 with the ketone carbon at δ_C 167.5. This alongside with other 2D NMR correlations supported the proposed structure of **415** which was of a new derivative of julocrotine (**414**). It was also closely related to other reported glutarimide alkaloids, crotonimide **A** and **B** that were isolated previously from the Amazonian *C. pullei* var. glabrior Lanj (Barbosa *et al*., 2007).

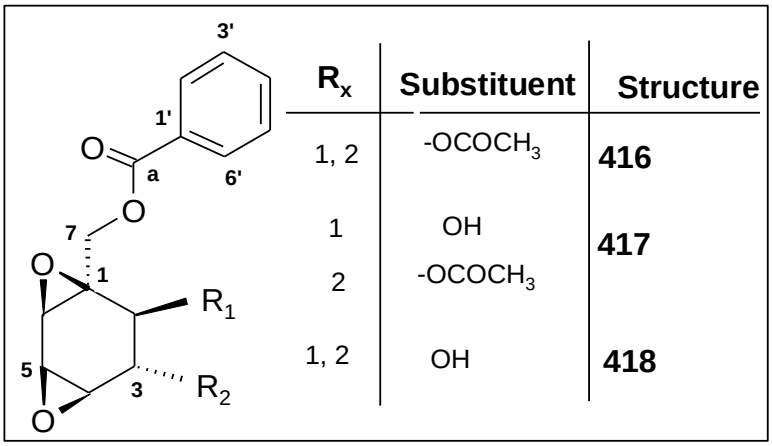
Subsequently, compound **415** was given the IUPAC name, 3-[*N*-benzamide]-*N*-phenylethyl-glutarimide (*N*-[1, 3-dioxo-2-(2-phenylethyl)-6-piperidinyl]-phenylanamide), and trivial name, crotonamide C.

Table 4.25: NMR spectroscopic data of crotonamide C (415)

Position	**δ_C** (75 MHz)	**δ_H** (300 MHz) **(*m*, *J* Hz; Integral)**	**HMBC (H→ C)**	**COSY**
2	171.1			
3	52.1	4.63 (*dt*, 5.4, 12.9; H)	2, 4, 7″	4α, 4β
4	24.6	2.75 (*m*; H_α) 1.78 (*dq*, 5.4, 12.9; H_β)	3 5, 3, 2, 6	4β
5	31.8	2.84 (*m*; 2H)	4, 3	4α
6	172.0			
NH		7.03 (*d*, 4.5; H)	7″	3
1″	133.9			
2″	127.3	7.83 (*td*, 6.9, 1.5; 2H)	3″ , 7″, 4″	3″
3″	128.6	7.48 (*dd*, 7.5, 1.4; 2H)	2″, 4″	
4″	132.2	7.55 (*tt*, 7.2, 1.5; H)	3″, 2″	3″
7″	167.5			
1′	138.2			
2′	129.1	7.26 (*d*, 6.0; 2H)		3′
3′	128.8	7.22 (*t*, 1.5; 2H)	2′, 4′	
4′	126.8	7.30 (*dt*, 6.9,1.5; H)	3′, 2′	3′
7′	34.1	2.84 (*m*; 2H)	8′	
8′	41.9	4.05 (*m*; 2H)	7′	7′

4.1.2.3 Methylcyclohexane derivatives from *Croton alienus*

Seven known methylcyclohexane derivatives, including the rampant crotepoxide (**416**) were isolated as white crystals from both the leaves and roots of *C. alienus*. Three of them were methycyclohexane diepoxide derivatives (**416-418**) [Figure 4.10] and the other three were methylcyclohexene epoxide derivatives (**419-421**) [Figure 4.11].



R_x	Substituent	Structure
1, 2	-$OCOCH_3$	**416**
1	OH	**417**
2	-$OCOCH_3$	
1, 2	OH	**418**

Figure 4.10: Methylcyclohexane diepoxide derivatives from *Croton alienus*

4.1.2.3.1 Crotepoxide (416) and other methylcyclohexanediepoxide derivatives (417 and 418)

The ^{1}H NMR spectrum of compound **416** [Appendix 26] had three resonances that corresponded to five protons of a mono substituted benzene ring at δ_H 7.52, 7.65 and 8.02. Others were of two one proton doublets of an AA′ spin system at δ_H 4.30 and 4.53 (J = 12.3 Hz), two chemically equivalent three proton singlets at δ_H 2.02 and 2.03 and five doublets of methine protons [Table 4.26]. The ^{13}C NMR spectrum of compound **416** [Appendix 26] had eighteen carbon resonances including those of a mono substituted benzene ring at δ_C 128.6, 129.5 and 133.4 for methine carbons and δ_C 129.6 for a fully substituted carbon. Also noted were three ester carbonyls at δ_C 166.0, 169.9 and 170.3; two methyl carbons at δ_C 20.8 and 20.9 and seven sp^3 oxymethine carbons at δ_C 48.2 – 70.6 [Table 4.27]. Consequently, a methylcyclohexane system having two epoxide rings, two acetate residues and a benzoate group substituent was deduced for compound **416**. Comparison of these spectral features with literature identified compound **416** to be the naturally occurring oxirane that is wide spread in the plant kingdom, 4-benzoyloxymethyl-3, 8-dioxatricyclo-octane-5, 6-diyl diacetate, trivial name, crotepoxide.

Crotepoxide is previously been reported from the fruits of *Croton macrostachys* (Kupchan *et al*., 1969), several species of Piperaceae family (*P. clarkia* (Pancharoen *et al*., 1989), *P. futokadzura* (Takahashi, 1969) and *P. cubeb* Cass DC (Nighat *et al*., 2009), two genera of the Zingiberaceae family (*Kaempferia angustifolia* (Pai *et al*., 1970) , *K. rotunda* (Boll *et al*., 1992) and a *Boesenbergia* species (Tantiwachwuttikul *et al*., 1987)) and the Annonaceae family from two *Monanthotaxis* species, *M. caffra* and *M. congoensis* (Mulholland *et al*., 2000). Crotepoxide has been reported as having significant inhibitory activity against Lewis Lung carcinoma in mice (LL), walker intramuscular carcinosarcoma in rats (WM) (Kupchan *et al*., 1969), binding of [3H] platelet-activating factor to human platelets and leukocytes (Shen *et al*., 1989). It has also been shown to have no effect to platelet aggregation induced by collagen and ADP (Ganem and Holbert, 1977).

Compound **417** had similar spectral data with **416** except for resonance of only one methyl proton singlet at δ_H 1.89 and a methyl carbon resonance at δ_C 20.7 [Table 4.26 and 4.27; Appendix 27a]. Since the number of oxymethine carbons was the same for both **416** and **417**, it was deduced that, ester hydrolysis occurred to one of the acetate residues in **416** to produce **417** during the biosynthesis. Using correlations in 2D NMR experiments, the lone acetate residue was placed at position 3 and a hydroxyl group at position 2. Acetylation of **417** yielded crotepoxide [Appendix 27b]. This implied that, the relative configuration at stereo-centers of **417** was similar to that in crotepoxide (**416**). **417** was subsequently identified as the known monodeacetylcrotepoxide, reported previously from the rhizomes of *Kaempferia rotunda* (Pancharoen *et al*., 1996). This is however, its first report from a *Croton* species.

Just like **417**, compound **418** had spectral data that was similar to that of crotepoxide [Table 4.26 and 4.27; Appendix 28]. It however had no methyl proton singlets that could be associated with acetate substituents unlike in **416** and **417**. **418** had the same number of oxymethine carbon resonances as **416** and **417** pointing to the likelihood of ester hydrolysis having occurred in them during the biosynthesis of **418**. Acetylation of **418**, just like that of **417** produced crotepoxide. The relative configuration at the stereo-centers of these three methylcyclohexane derivatives was therefore deduced to be similar. Compound **418** was subsequently identified to be the known dideacetylcrotepoxide, reported from a synthetic process alongside its anti-tumor activity (Kupchan and Sunshine, 1978). This is the first time the compound has been isolated from a natural source.

Table 4.26: ^{1}H NMR spectroscopic data of cyclohexane diepoxides from *Croton alienus* (416-418)

Position	416 Lit.[7]	416 (600 MHz, $(CD_3)_2CO$)	417 (300 MHz, $CDCl_3$)	418 (500 MHz, $CDCl_3$)
2	5.73	5.82 (*d*, 9.6, H)	4.12(*d*, 4.18, H)	4.03 (*t*, 8.0, H)
3	5.01	4.87 (*dd*, 1.2, 9.6, H)	5.16 (*dd*, 2.6, 5.0, H)	4.07 (*m*, H)
4	3.09	3.09 (*dd*, 1.2, 4.2, H)	3.30 (*dd*, 1.1, 4.2, H)	3.23 (*dd*, 2.0, 4.0, H)
5	3.45	3.53 (*dd*, 1.2, 4.2, H)	3.54 (*dd*, 1.7, 3.3, H)	3.48 (*dd*, 3.25, 6.5, H)
6	3.67	3.82 (*d*, 2.4, H)	3.69 (*d*, 3.0, H)	3.61 (*d*, 3.0, H)
7	4.58 4.23	4.53 (*d*,12.6, H_α) 4.30 (*d*,12, H_β)	4.54 (*d*, 12.3, H_α) 4.4 (*d*, 12.3, H_β)	4.74 (*d*, 12.5, H_α) 4.28 (*d*, 12.0, H_β)
2', 6'	8.04	8.02 (*dd*, 1.2,8.4, 2H)	8.05 (*dd*, 1.4, 8.1, 2H)	8.05 (*dd*, 1.25, 8.0, 2H)
3',5'	7.46	7.52 (*t*, 7.8, 2H)	7.46 (*t*, 8.0, 2H)	7.47 (*t*, 7.8, 2H)
4'	7.61	7.65 (*t*, 8.1, H)	7.59 (*tt*, 1.2, 7.2, H)	7.60 (*t*, 7.5, 2H)
CH_3	2.03$^{\Delta 8}$	2.03$^{\Delta}$ (*s*, 3H)		
CH_3	2.12$^{\Delta}$	2.02$^{\Delta}$ (*s*, 3H)	1.89(*s*, 3H)	
2-OH			4.85 *br s*	2.89 (*d*, 8.0, H)
3-OH				2.25 (*d*, 4.5, H)

Table 4.27: ^{13}C NMR spectroscopic data of cyclohexane diepoxide from *Croton alienus* (416-418)

Position	416 Lit.[9]	416 Experimental (75 MHz)	417	418
1	59.3	60.1	56.2	58.0
2	69.4	69.9	66.8	70.1
3	70.1	70.5	70.0	69.2
4	52.3	52.6	51.2	53.5

[7] Thebpatiphat *et al.*, 1988
[8] The pair of resonance marked ▲ and Δ is arbitrary and could be interchanged
[9] Thebpatiphat *et al.*, 1988

5	47.7	48.1	48.2	48.2
6	53.3	53.5	53.9	53.7
7	62.1	62.1	64.8	65.0
1′	129.0	129.6	129.5	129.0
2 ′/ 6′	128.2	129.5	130.0	130.1
3′ / 5′	129.4	128.6	128.8	128.8
4′	133.1	133.4	133.8	133.9
a, C	168.3	165.2	166.3	167.9
b, C	169.6▲	169.6▲		
c, C	169.3▲	169.5▲	170.1	
CH_3	20.2$^{\Delta}$	19.8$^{\Delta}$		
CH_3	20.1$^{\Delta}$	19.7$^{\Delta}$	20.7	

4.1.2.3.2 Methylcyclohexane monoepoxide derivatives (419 - 421)

Two methylcyclohexene monoepoxide derivatives that were C-1 epimers (**419** and **420**) and their pre-cursor molecule (**421**) were isolated as white crystals from *C. alienus* leaves [Figure 4.11].

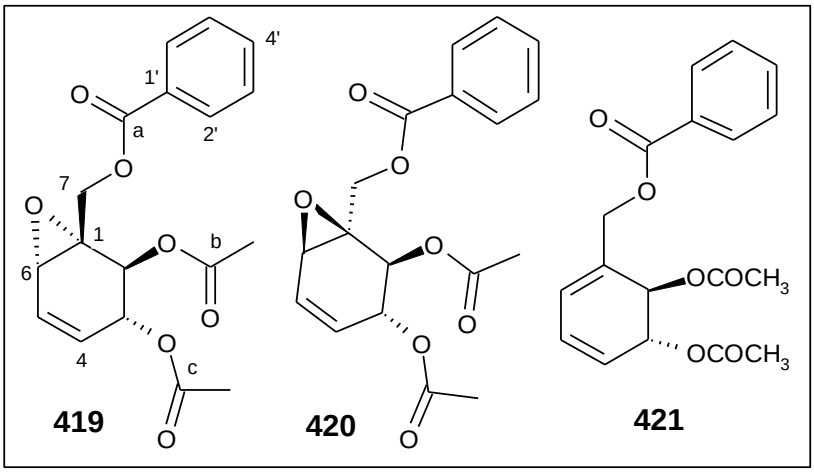


Figure 4.11: Methylcyclohexane monoepoxide derivatives from *Croton alienus*

Compound **419** had a molecular ion peak at *m/z* 347.23 supporting the proposed molecular formula, $C_{18}H_{18}O_7$. Its spectroscopic data [Table 4.28 and 4.29; Appendix 29] was similar to that of crotepoxide (**416**) except for resonances associated to a C=C bond at δ_H 6.37*dd* and δ_C 129.2. Based on correlations observed in 2D NMR experiments, the C=C bond was placed on the cyclohexane ring at position 4.

There was a COSY correlation between protons at δ_H 5.19 and 3.46 that had been assigned to H-2 and H-6 respectively hence the deduction that these two protons were α-configured and the epoxide ring at C-6/C-1 was β-positioned. Literature search identified compound **419** as the known senepoxide (Ogawa and Takagaki, 1987).

Compound **420** had a molecular ion peak at *m / z* 346.23 consistent with the proposed molecular formula $C_{18}H_{18}O_7$. Its spectroscopic data was similar to that of compound 7 [Table 4.28 and 4.29; Appendix 30]. However, there was no COSY correlation observed between H-2 and H-6 as was the case with compound **419**. It was therefore deduced that, the configuration of the epoxide ring at C-6/C-1 was as found in crotepoxide (α-positioned) and hence, compounds **419** and **420** were epimers at C-1. Compound **420** was subsequently identified to be the known β-senepoxide.These monoepoxide epimers (**419** and **420**) have previously been reported from *Uvaria* species (Annonaceae), senepoxide from *U. catocarpa* (Hollands *et al*., 1968) and β-senepoxide from *U. pandensis* and *U. ferruginea* (Nkunya *et al*., 1987). This is however the first report on their isolation from *Croton* species. They have also been reported as having tumor-inhibitory, antileukemic and antibiotic activity properties (Shing and Tam, 1998).

Compound **421** had a molecular ion peak, $[M-2]^{+\bullet}$ At *m / z* 228.43 that was in agreement with the proposed molecular formula, $C_{18}H_{18}O_6$. The spectroscopic data of **421** [Table 4.28 and 4.29; Appendix 31] was similar to that of the monoepoxide epimers **419** and **420** but, in place of the epoxide ring at C-6/C-1, there were resonances of a C=C bond ($\delta_{H\text{-}6}$ 6.28*d*; $\delta_{C\text{-}6,\,1}$ 125.4, 131.1) in compound **421**. Just like compound **420**, compound **421** did not have COSY correlation between H-2 and H-6. It was therefore deduced that, their configuration at C-1 was the same. Literature search identified compound **421** as a diene precursor of β-senepoxide, (+)-(2*S*, 3*R*)-diacetoxy-1-benzoyloxymethylenecyclohex-4, 6-diene (*trans*-5, 6-*di*-acetoxy-1-benzoyloxymethyl-1, 3-cyclohexadiene). This compound **421** is previously reported as an intermediate in the total synthesis of the optically active natural (+)-crotepoxide (Ogawa and Takagaki, 1987). This is the first report of its isolation from natural sources. No biological activity reports on it have been reported.

Table 4.28: ^{1}H NMR data (300 MHz) of methylcyclohexene monoepoxides (419 and 420) and the diene precursor of *β*- Senepoxide (421)

Postn	419	420	421
2	5.19 (*dd*, 2.1,2.7)	5.57 (*dd*, 2.3, 6.0)	5.80 (*d*, 6.0)
3	5.58 (*dd*, 0.75, 1.35)	5.67 (*d*, 8.4)	5.49 (*t*, 5.0)
4	6.37 (*dd*,4.2,9.9)	6.06(*d*, 10.0)	6.16 (*dd*, 1.0, 9.0)
5	6.10 (*dd*, uncalculatable)	5.79(*d*, 10.0)	5.92 (*dd*, 4.5, 9.8)
6	3.46 (*d*, 3.9)	3.57 (*dd*, 1.8,3.8)	6.28 (*d*, 5.5)
7	4.84 (*d*, 12.6) 4.24 (*d*, 12.6)	4.62,4.37 (*d*,12.0)	4.90 (*s*)
2', 6'	8.06 (*dd*, 1.2,7.5)	8.03 (*d*, 7.5)	8.04 (*d*, 7.5)
3', 5'	7.46 (*t*, 6.9)	7.45 (*t*, 7.8)	7.45 (*t*, 7.8)
4'	7.57 (*t*, 7.5)	7.57 (*t*, 7.5)	7.57 (*t*, 7.5)
$OOCCH_3$	2.08 (*s*)	2.13 (*br s*)	2.05 (*s*)
$OOCCH_3$	2.06 (*s*)	2.05 (*s*)	2.02 (*s*)

Table 4.29: ^{13}C NMR data (75 Hz) for methylcyclohexane monoepoxide derivatives (419 and 420) and the diene precursor of *β*- Senepoxide (421)

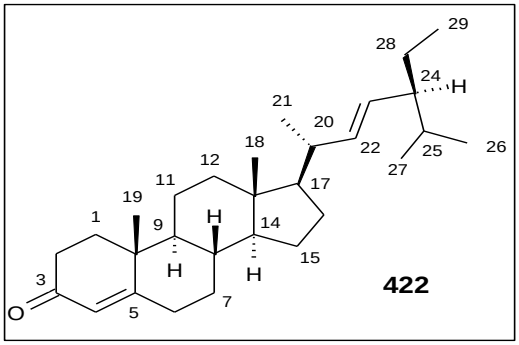
Pstn	419	420	421
1	61.8	58.5	131.1
2	67.5	71.5	70.0
3	67.1	71.5	70.8
4	129.2	124.3	126.0
5	128.9	133.6	125.5
6	49.8	54.7	125.4
7	64.2	62.4	64.9
1′	129.6	129.5	137.6
2′, 6′	130.0	130.0	129.9
3′, 5′	128.7	128.7	128.7
4′	133.6	133.6	133.4
a	166.2	166.0	170.2
b	170.3	170.4	170.4
c	169.5	170.5	170.4
$OOCCH_3$	21.3	21.1	21.2
$OOCCH_3$	20.1	21.0	21.1

4.1.2.4 A triterpenoid and a phytosterol from *Croton alienus*

The known triterpenoid, acetylaleuritolic acid (**411**), also isolated from the roots of *C. megalocarpoides* and the known phytosterol, D_4-stigmasterone (**422**) were isolated from the roots of *C. alienus*. The structural elucidation of the phytosterol is the one going to be discussed in the next section since that of acetylaleuritolic acid was done in Section 4.1.1.4.1.

4.1.2.4.1 D_4-stigmasterone (422)

Compound **422** was isolated as a white crystalline from the hexane extract of the leaves of *C. alienus* and its molecular formula proposed to be $C_{29}H_{46}O$.



422

The ^{1}H NMR spectrum of compound **422** [Table 4.30; Appendix 32] showed resonances of six methyl groups, two of which were doublets at δ_H 0.83 *d* (*J* = 4.5 Hz) and 0.88 *d* (*J* = 4.5 Hz), associated to an isopropyl group substituent. Three double bond resonances of methine protons were observed at δ_H 5.72 *br s*, 5.15 *dd* (*J* = 8.4, 15.2 Hz) and 5.02 *dd* (*J* = 8.4, 15.2 Hz). This is characteristic of H-4, H-22 and H-23 in 24-ethylcholest-4, 22-dien-3-one. The ^{13}C NMR spectrum [Table 4.30; Appendix 32] showed resonances of a ketone carbon at δ_C 199.8 and four olefinic carbons at δ_C 171.9, 138.3, 129.6 and 123.9. Other carbon chemical shifts were found to be similar to those reported of the known 24-ethylcholest-4-en-3-one (Georges *et al*., 2006) and 24-ethylcholest-4, 22-dien-3-one (Chen *et al*., 2008). Compound **422** was subsequently deduced to be the widely known D_4-stigmasterone.

Table 4.30: NMR spectroscopic data of D_4-stigmasterone (422)

Position	δ_C (75 MHz)		δ_H (300 MHz) (*m*, *J* Hz; Integral)
	Georges *et al*., 2006	Experimental	
1	35.9	36.3	2.01(*m*; H_α) 1.69 (*m*; H_β)
2	34.2	34.1	2.28 (*m*; H_α) 2.41 (*m*; H_β)
3	200.6	199.8	
4	124.0	123.9	5.72 (*s*; H)
5	171.9	171.9	
6	32.8	33.1	2.28 (*m*; H_α) 2.35 (*m*; H_β)
7	35.8	32.2	1.85 (*m*; H_α) 1.01(*m*; H_β)
8	35.8	35.8	1.51(*m*; H)
9	54.0	54.0	0.92(*m*; H)
10	39.7	39.7	
11	21.2	21.3	1.50 (*m*; H_α) 1.51 (*m*; H_β)
12	39.8	39.8	1.15 (*m*; H_α) 2.04 (*m*; H_β)
13	42.6	42.5	
14	56.1	56.0	1.11 (*m*; H)
15	24.4	24.3	1.22 (*m*; H_α) 1.28 (*m*; H_β)
16	28.4	28.3	1.10 (*m*; H_α) 1.60 (*m*; H_β)
17	56.2	56.0	1.02 (*m*; H)
18	12.2	12.3	0.73 (*s*; 3H)
19	18.1	17.5	0.83 (*s*; 3H)

20	46.1	46.0	2.27 (*m*; H)
21	20.0	21.3	1.04 (*s*; 3H)
22	138.2	138.3	5.15(*dd*, 8.4, 15.2; H)
23	130.0	129.6	5.02 (*dd*, 8.4, 15.2; H)
24	51.5	51.4	1.53 (*s*; H)
25	32.1	32.2	1.60 (*s*; H)
26	19.2	19.2	0.81 (*d*, 4.5; 3H)
27	21.2	21.2	0.88 (*d*, 4.5; 3H)
28	26.3	26.2	1.20 (*m*; H_α) 1.50 (*m*; H_β)
29	12.2	12.1	0.82 *t*, 4.5; 3H)

4.1.3 The Phytochemistry of Kenyan *Croton sylvaticus*

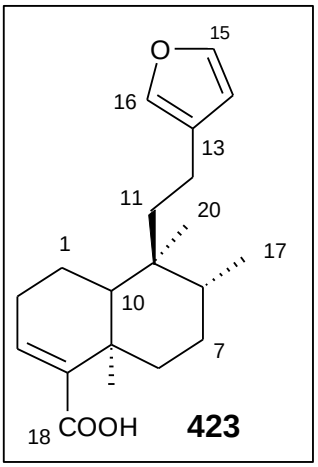
Nine compounds were isolated from the roots of Kenyan *C. sylvaticus*. They included five clerodane (**423- 427**), two halimane (**428** and **429**) and one labdane (**430**) diterpenoids. Also isolated was a phytosterol, sitosterol that had been isolated from *C. megalocarpoides*. The clerodanes had negative specific rotation values and were therefore assigned as *ent*-clerodanes.

4.1.3.1 *Ent*-clerodane diterpenoids from *Croton sylvaticus*

Hardwickiic acid (**423**), a very rampant compound in *Croton* genus and kolavenol and its three derivatives (**424-427**) were the five *ent*-clerodane diterpenoids isolated from the roots of *C. sylvaticus*.

4.1.3.1.1 Hardwickiic acid (423)

Compound **423** was isolated as white crystals and had a molecular ion peak at *m/z* 316.44 supporting the proposed molecular formula, $C_{20}H_{28}O_3$.



423

The ^{1}H NMR spectrum [Appendix 33] had resonances of three olefinic protons that are characteristic of a furan ring at δ_H 7.37 *t* (*J* = 1.63 and 1.65 Hz); δ_H 6.88 *t* (*J* = 3.02 and 4.34 Hz) and δ_H 6.28 *d* (*J* = 0.91 Hz) and another one of a carbon-carbon double bond at δ_H 7.22. Resonances of three methyl protons were also observed at δ_H 1.28 *s*, 0.85 *d* (*J* = 6.4 Hz) and 0.78 *s*. The ^{13}C NMR spectrum [Appendix 33] had resonances of twenty carbons implying that compound **423** was a diterpene. Resonances characteristic of a β- substituted furan ring were observed at δ_C 143.1, 140.6, 126.0 and 111.4 and a carbonyl carbon at δ_C 171.9. There were additionally resonances of two sp^2 carbons at δ_C 138.8 and 141.7 and three methyl group carbons at δ_C 20.9, 17.8 and 16.4. The remaining resonances were of sp^3 carbons of methylene and methine groups [Table 4.31].

The chemical shifts alluded above were consistent with those reported in literature for 15, 16-epoxy-3, 13(16), 14-clerodatrien-18-oic acid also called, hardwickiic acid (McChesney *et al*., 1991). Hardwickiic acid is reported to have been first isolated from *Hardwickia pinnata* (Misra *et al*., 1979) and later from an Asteraceae species, *Solidago rugosa* (Henderson 1973). It is also reported from several *Croton* species among them, *C. aromaticus* of Sri Lanka (Bandara *et al*., 1987), the entire plant of *C. californicus* of U.S.A. (Luzbetak *et al.,* 1979), the stem bark of *C. lechleri* of Brazil (Cai *et al.,* 1993b) and the roots of *C. sonderianus* of Brazil (McChesney *et a*l., 1991; McChesney and Silveira, 1990).

The reported biological activities of hardwickiic acid include:- inactivity against *Mycobacterium tuberculosis* and *Mycobacterium avium* at 100 µg/ml (Lu Tiansheng *et al*., 1995); weak cytotoxic activity against *in vitro* cell culture with a LD_{50} 21.90 µg/ml (Chen *et al.,* 1994) and 62% mortality of adult female aphids after 24 hours post-treatment (Bandara *et al*., 1987).

Table 4.31: NMR spectroscopic data of hardwickiic acid (423)

Position	δ_C (75 MHz)		δ_H (300 MHz) (*m*, *J* Hz; Integral)
	(McChesney *et al.*, 1991)	Experimental	
1	18.6	18.7	(2H)*[10]
2	28.0	27.9	(2H)*
3	138.1	138.8	7.22 (*br s*; H)
4	142.8	141.7	
5	38.3	38.0	
6	37.0	36.7	(2H)*
7	27.1	27.7	(2H)*
8	36.6	36.2	2.19-2.48 (*m*; H)
9	39.7	39.2	
10	47.6	47.0	1.42 (*s*; H)
11	39.5	39.0	1.38-1.74 (*m*; 2H)
12	18.6	18.6	(2H)*
13	126.5	126.0	
14	111.8	111.4	6.28 (*d*, 0.91; H)
15	139.4	140.6	6.88 (*t*, 3.02, 4.34; H)
16	143.1	143.1	7.37 (*t*, 1.63, 1.65; H)
17	16.2	16.4	0.85 (*d*, 6.4; 3H)
18	173.1	171.9	
19	20.9	20.9	1.28 (*s*; 3H)
20	18.2	17.9	0.78 (*s*; 3H)

4.1.3.1.2 Kolavenol and its derivatives

Kolavenol (**424**) and three of its derivatives, among them a novel formate derivative, 15-formate-*ent*-3,13*E*-clerodadiene (**427**) were isolated from the root bark of *C. sylvaticus* as white crystalline compounds [Figure 4.12]. Their optical rotations were negative values indicating that they were *ent*-clerodanes.

[10] * → Protons superimposed on each other

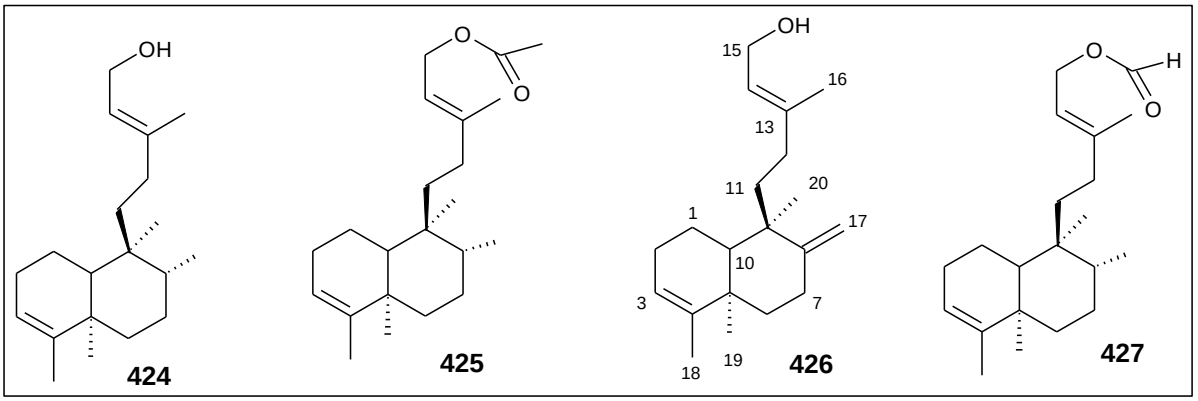


Figure 4.12: Kolavenol and its derivatives from *Croton sylvaticus*

The ^{1}H NMR spectrum of compound **424** [Appendix 34] had resonances of five three proton singlets at δ_H 0.80, 1.00, 1.58, 1.58 and 1.69. Two olefinic protons were observed at δ_H 5.19 *br s* and δ_H 5.40 *t* (*J* = 6.90 Hz) and a doublet that integrated for two protons was observed at δ_H 4.14. The ^{13}C NMR spectrum [Appendix 34] had resonances of twenty carbons, four of them olefinic at δ_C 120.7, 123.0, 141.2 and 144.8; an oxymethylene carbon at δ_C 59.7 and five methyl group carbons at δ_C 20.2, 18.2, 18.2, 16.8 and 16.2. Correlations in 2D NMR experiments and comparison of the spectral data for compound **424** [Tables 4.32 and 4.33] with literature identified it as the known *ent*-3, 13*E*-clerodadien-15-ol, trivial name kolavenol.

Compound **425** had spectroscopic data [Appendix 35] that was similar to that of kolavenol except for resonances of an acetoxy group (a carbonyl carbon at δ_C 171.4 and a methyl group at δ_C 20.3 and δ_{3H} 1.00*s*) that was placed as a substituent at C-15 using 2D NMR experiments correlations. Compound **425** was subsequently identified to be the known 15-acetoxy-*ent*-3,13*E*-clerodadiene. Both kolavenol and compound **425** have been isolated previously from *Solidago canadensis*, *S. elongata*, *S. rugosa*, *Hardwickia pinnata* and many other plant families including Aristolochiaceae, Compositae and Leguminosae (Lu Tiansheng *et al*., 1993 and 1995). This report is therefore the first of their isolation from *Croton* genus. Kolavenol has been found to have anti-feedant activity against the leaf cutter ants, *Atta cephalotes* (Hubert and Wiemer, 1985). Kolavenic acid, a derivative of kolavenol which is reported from *Polyalthia longifolia* var. *pendulla* (Annaceae) and many other plant families including Aristolochiaceae, Caesalpiriaceae and Compositae has been showed to possess anti-microbial activity to most bacteria and anti-fungal activity against kanamycin resistant fungal strains, *Aspergillus fumigatus* and *Candida albicans* (Krebs and Ramiarantosa, 1996).

The spectroscopic data of compound **426** [Table 4.32 and 4.33; Appendix 36] differed from that one of kolavenol by a C=C bond placed at C-8 (17). It was subsequently identified to be a derivative of kolavenol named 3, 8(17), 13*E*-clerodatriene-15-ol. Similarly, the spectroscopic data of compound **427** was very similar to that of kolavenol except for resonances of a formate group (a carbonyl at δ_C 161.3 and an aldehydic proton singlet at δ_H 8.07) [Appendix 37a]. HMBC spectrum [Appendix 37b] showed ^{1}H-^{13}C NMR cross peaks of the allylic methyl group proton at $\delta_{\mathbf{3H\text{-}16}}$ 1.72 *s* with the carbons at $\delta_{\mathbf{C\text{-}12,\ 14,\ 13}}$ 32.9, 117.1, 127.0; oxymethylene protons at $\delta_{\mathbf{2H\text{-}15}}$ 4.69 with the sp^2 carbon and the formate carbonyl at $\delta_{\mathbf{C\text{-}14,\ OCOH}}$ 117.1 and 161.3 respectively; the allylic methyl group protons at $\delta_{\mathbf{3H\text{-}18}}$ 1.57 *s* with $\delta_{\mathbf{C\text{-}3,\ 4,\ 5}}$ 120.5, 144.5, 38.4. Additionally, $\delta_{\mathbf{C\text{-}5}}$ 38.4 had HMBC correlation with $\delta_{\mathbf{3H\text{-}19}}$ 0.98 *s* further supporting the proposed molecular formula of $C_{21}H_{34}O_2$ and a calculated double bond equivalence of 5. The relative configuration for this compound was assigned using NOESY experiment [Appendix 37c] where H-10 showed correlations with H-8 and 2H-11. Other correlations observed in the NOESY were between 3H-19 with 3H-20 and 3H-19 with 3H-17. A specific rotation of -39.2° which was similar to that of kolavenol allowed for the identification of compound **427** as a new formate derivative of kolavenol, 15-formate-*ent*-3,13*E*-clerodadiene.

Table 4.32: ^{1}H NMR spectroscopic data of kolavenol and its derivatives (424-427)

Cpd	**424**	**425**	**426**	**427**	
Pstn		δ_H (300MHz)			HMBC for **427** (H→C)
1	0.70 (*m*; H_α) 1.47 (*m*; H_β)	1.58 (*d*, 1.3; H_α) 1.42(*s*; H_β)	0.76 (*m*, 2H)	0.72 (*s*; H_α) 1.75 (*m*; H_β)	10, 9
2	1.16 (*m*; 2H)	1.35(*s*; H_α) 1.40(*s*; H_β)	1.16 (*m*; 2H)	1.17 (*m*; H_α) 1.17(*m*; H_β)	
3	5.19 (*br s*; H)	5.20 (*br s*; H)	5.17 (*br s*; H)	5.19 (*br s*; H)	
6	1.70 (*m*; H_α) 1.35 (*m*; H_β)	1.70 (*m*; 2H)	1.72 (*m*, 2H)	1.69(*m*; H_α) 1.35(*m*; H_β)	4, 10
7	2.02(*m*; 2H)	2.05(*m*; 2H)	2.02 (*m*; 2H)	2.04(*m*; 2H)	
8	1.45 (*m*; H)	1.69 (*m*; H)		1.45 (*m*; H)	
10	1.36 (*m*; H)	1.35(*m*; H)	1.34 (*m*; H)	1.33 (*m*; H)	

11	1.70 (*m*; 2H)	1.70 (*m*; 2H)	1.69 (*m*; 2H)	1.70 (*m*; 2H)	12, 4
12	1.90 (*m*; 2H)	1.85 (*m*; 2H)	1.83 (*m*; 2H)	1.90 (*m*; 2H)	
14	5.40 (*t*, 6.90; H)	5.32 (*t*, 1.05; H)	5.68 (*t*, 2.12; H)	5.35 (*t*,7.10; H)	
15	4.14 (*d*, 6.85; 2H)	4.57(*d*, 7.1; 2H)	4.36 (*d*, 6.34; H)	4.69(*d*; 6.65; H)	14, (C=O)
16	1.69(*s*; 3H)	1.69(*s*; 3H)	1.70 (*s*; 3H)	1.72(*s*; 3H)	13, 12, 14
17	0.80 (*d*, 6.30; 3H)	0.80 (*d*, 6.20; 3H)	4.56 (*br s*; 2H)	0.87 (*d*, 3.45; 3H)	9
18	1.58 (*s*; 3H)	1.58 (*s*; 3H)	1.58 (*s*; 3H)	1.57 (*s*; 3H)	4, 5
19	1.00 (*s*; 3H)	1.05 (*s*; 3H)	1.00 (*s*; 3H)	0.98 (*s*; 3H)	4
20	1.58 (*s*; 3H)	1.58 (*s*; 3H)	0.74 (*s*; 3H)	1.57 (*br s*; 3H)	
OCOH				8.07 (*s*; H)	
$OCOCH_3$		1.00 (*s*; 3H)			

Table 4.33: ^{13}C NMR spectroscopic data of kolavenol and its derivatives from *Croton sylvaticus*

Position	**424**		**425**	**426**	**427**
	Lu Tiansheng *et al.*, 1993 / 95	**Experimental** (75 MHz)			
1	36.7	18.5	18.5	18.2	18.3
2	26.9	27.7	27.5	26.8	27.6
3	120.4	120.7	120.7	120.4	120.5
4	144.5	144.8	144.8	144.4	144.6
5	38.2	38.4	38.4	38.1	38.4
6	36.8	38.4	36.8	36.8	36.8
7	27.5	27.1	26.9	27.4	26.9
8	36.2	36.5	36.3	143.4	36.3

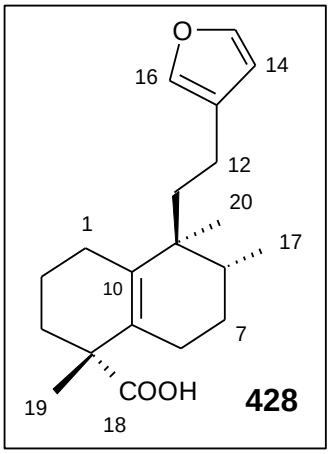
9	38.6	38.8	38.8	38.7	38.8
10	46.4	46.5	46.7	46.4	46.5
11	18.2	38.1	36.5	36.3	38.1
12	32.8	33.0	33.0	33.0	32.9
13	140.9	141.2	143.6	163.8	127.0
14	122.8	123.0	118.0	115.0	117.1
15	59.4	59.7	61.5	59.8	60.9
16	16.5	16.8	17.0	16.5	16.8
17	16.0	16.2	16.2	112.8	16.0
18	18.3	18.2	18.6	18.3	18.9
19	19.9	20.2	21.3	19.9	20.2
20	18.0	18.2	18.2	18.0	18.2
OCOH					161.3
$OCOCH_3$			171.4		
$OCOCH_3$			20.3		

4.1.3.2 Halimane diterpenoids from *Croton sylvaticus*

Two halimane diterpenoids, crotohalimaneic acid (**428**) and penduliflaworosin (**429**) were isolated from the root bark of *C. sylvaticus*.

4.1.3.2.1 Crotohalimaneic acid (428)

Crotohalimaneic acid (**428**) was isolated as a semi-crystalline mixture with hardwickiic acid from the root bark of *C. sylvaticus*.



When the mixture of the two compounds, hardwickiic acid and crotohalimaneic acid (**428**) was purified, hardwickiic acid was the only one that was obtained in a pure form. The remaining sample mixture was insufficient for a further successful purification process in an attempt to get a pure form of the crotohalimaneic acid (**428**). However, using literature data, it was possible to pick out the resonances representing each compound from the spectra of the mixture that had been obtained before the first purification attempt.

The ^{1}H NMR spectrum of the mixture [Appendix 38a] had resonances characteristic of two β-substituted furan rings appearing as un-split doublets at δ_H 6.26 (6.25), 7.35 (7.33) and a singlet at δ_H 7.20 (7.20). Resonances of two singlets each integrating for three protons were observed at δ_H 0.86 (0.86) and 1.28 (1.30) and two doublets each integrating for also three protons at δ_H 0.86 (0.87), taken to represent 3H-20, 3H-19 and 3H-17 respectively in each of the two compounds of the mixture. A triplet observed at δ_H 6.86 was taken to be the olefinic proton at position 3 of the hardwickiic acid (**423**) in the mixture.

The ^{13}C NMR spectrum [Appendix 38b] had 20 carbon resonances which included four furan ring carbons at δ_C 111.0 (111.1), 125.6 (125.9), 138.4 (138.4) and 142.6 (142.7) for C-14, C-13, C-16 and C-15 in each of the two compounds and three methyl carbons at δ_C 16.0 (16.0), 20.5 (18.3) and 22.9 (20.9) representing C-16, C-20 and C-19. Two sp^2 carbons observed at δ_C 131.0 and 137.0 were taken to be C-5 and C-10 respectively of the crotohalimaneic acid (**428**) and at δ_C 140.3 and 141.4 for C-3 and C-4 respectively of the hardwickiic acid (**423**). A resonance of a carbonyl carbon in both compounds was observed at δ_C 183.7 (172.2) and was taken to be for C-18.

All the above observed resonances in the ^{13}C NMR spectrum were confirmed by reported data [Table 4.34] of *ent*-halim-5(10), 13, 14-trien-15, 16-olide-18-oic acid, trivial name, crotohalimaneic acid (**428**) (Kanlayavattanakul *et al*., 2005) and hardwickiic acid (**423**) (McChesney *et al*., 1991). DEPT experiment of the mixture confirmed the chemical shift assignments of five quaternary carbons (two sp^3 and three sp^2 types) for crotohalimaneic acid (**428**) at $\delta_{\mathbf{C\text{-}9,\ 4,\ 13,\ 5,\ 10}}$ 40.9, 47.4, 125.6, 131.0 and 136.0 and four quaternary carbons (two sp^3 and two sp^2 types) for the hardwickiic acid (**423**) at $\delta_{\mathbf{C\text{-}5,\ 9,\ 13,\ 4}}$ 37.6, 38.8, 125.9 and 141.4. Correlations in the HMBC spectrum between the carbonyl carbon at $\delta_{\mathbf{C\text{-}18}}$ 183.7 of crotohalimaneic acid (**428**) with $\delta_{3H\text{-}19}$ 1.30 *s* and the multiplet at $\delta_{\mathbf{H\text{-}3}}$ 1.64-2.02 was observed. There was no HMBC correlation observed between the carbonyl carbon at $\delta_{\mathbf{C\text{-}18}}$ 172.2 of hardwickiic acid (**423**) and the methyl proton singlet at $\delta_{\mathbf{3H\text{-}19}}$ 1.28 further confirming the proposed identities of the two compounds in the sample mixture.

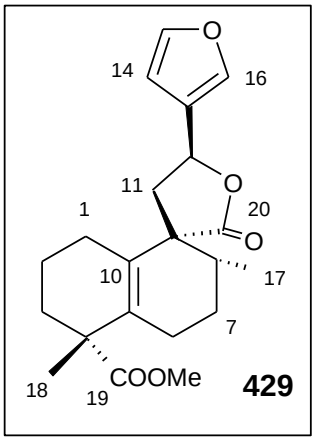
Table 4.34: NMR spectroscopic data of crotohalimaneic acid (428) and hardwickiic acid (423)

	Crotohalimaneic acid **(428)**			Hardwickiic acid **(423)**	
	δ_H (*m*, *J* Hz; Integral)		**δ_C** (300 MHz)		
Pstn	**Kanlayavattanakul *et al*., 2005**		**Experimental**	**McChesney *et al*., 1991**	**Experimental**
1	1.89-2.02 (*m*; H_a) 2.07-2.17 (*m*; H_b)	25.1	25.1	17.9	17.5
2	1.74-1.81 (*m*; 2H)	19.5	19.5	27.9	27.5
3	1.64-1.69 (*m*; H_a) 1.89-2.02 (*m*; H_b)	35.4	35.4	138.8	138.4
4		47.4	47.4	141.7	141.4
5		131.0	131.0	38.0	37.6
6	1.34-1.44 (*m*; H_a) 1.89-2.02 (*m*; H_b)	25.9	25.9	36.7	36.5
7	1.50-1.56 (*m*; 2H)	26.8	26.8	27.7	27.3
8	1.74-1.81 (*m*; H)	33.8	33.3	36.2	36.2
9		40.9	40.9	39.2	38.8
10		136.0	136.0	47.0	46.7
11	1.64-1.69 (*m*; 2H)	36.5	35.8	39.0	38.6

12	2.07-2.17 (*m*; H_a) 2.33-2.40 (*m*; H_b)	19.5	19.5	18.6	18.2
13		125.8	125.6	126.0	125.9
14	6.26 (*dd*, 0.8, 0.8; H)	111.0	111.0	111.4	111.1
15	7.34 (*dd*, 1.5, 1.5; H)	142.6	142.6	143.1	142.7
16	7.20 (*s*; H)	138.4	138.4	140.6	140.3
17	0.87 (*d*, 7.0; 3H)	16.0	16.0	16.4	16.0
18		183.1	183.7	171.9	172.2
19	1.30 (*s*; 3H)	22.9	22.9	20.9	20.9
20	0.86 (*s*; 3H)	20.8	20.5	18.7	18.3

4.1.3.2.2 Penduliflaworosin (429)

Penduliflaworosin (**429**) was obtained as a white crystalline compound from the root bark of *C. sylvaticus*. The MS had a molecular ion peak at *m/z* 359.64 for $[M^+ + 1]^+$ supporting the proposed molecular formula, $C_{21}H_{26}O_5$.



429

The ^{1}H NMR and ^{13} C NMR [Appendices 39a and 39b] had typical resonances of a *β*-substituted furan moiety (δ_H 6.40 *br s*, 7.48 *br s*, 7.47 *br s*; methine carbons at δ_C 108.2, 139.4, 144.1 and a quaternary carbon at δ_C 125.9). A total of 21 resonances were observed in the ^{13}C NMR spectrum indicating that compound **429** was a diterpenoid. The DEPT spectrum showed presence of three methyl groups, one of them, a methoxy functionality at δ_H 3.63 *s* and δ_C 51.7. Resonances of two carbonyl carbons at δ_C 177.8 and 177.1 and a C=C bond at δ_C 128.7 and 134.7 were also observed.

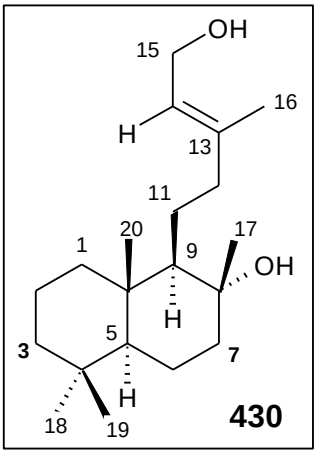
The spectroscopic data adduced [Table 4.35] was identical to that reported for the known furanoid diterpene, *ent*- (12*R*)-methyl-15, 16-epoxy-9, 10-friedolabda-5 (10), 13 (16), 14-trien-19-oate 20, 12-lactone also called, penduliflaworosin, previously isolated from *C. penduliflorus* (Adesogan, 1981) and *C. jatrophoides* (Mbwambo *et al*., 2009).

Table 4.35: NMR (600 MHz) spectroscopic data of penduliflaworosin (429)

Position	δ_C		δ_H (*m*, *J* Hz; Integral)
	Mbwambo *et al*., 2009	Experimental	
1	24.6	24.6	2.06 (*m*; H_α) 1.80 (*m*; H_β)
2	19.0	18.9	1.70 (*m*; 2H)
3	26.5	26.4	2.21 (*dd*, 6.0, 7.8; H_α) 1.94 (*d*, 2.4; H_β)
4	47.5	47.3	
5	134.8	134.7	
6	26.6	26.6	1.82 (*m*; H_α) 1.54 (*m*; H_β)
7	34.9	34.7	1.97 (*m*; H_α) 1.62 (*m*; H_β)
8	37.7	37.7	1.71 (*m*; H)
9	53.2	53.2	
10	128.6	128.7	
11	41.3	41.1	2.78 (*dd*, 8.4, 9.0; H_α) 2.21 (*dd*, 6.0, 7.8; H_β)
12	72.1	72.0	5.44 (*t*, 8.1; H)
13	125.8	125.9	
14	108.2	108.2	6.40 (*br s*; H)
15	144.0	144.1	7.47 (*br s*; H)
16	139.2	139.4	7.48 (*br s*; H)
17	16.2	16.1	1.00 (*br s*; 3H)
18	22.8	22.5	1.31 (*s*; 3H)
19	178.1	177.8	
20	177.4	177.1	
19-acetoxy	51.9	51.7	3.63 (*s*; 3H)

4.1.3.3 A labdane diterpenoid from *Croton sylvaticus*

A known labdane type diterpenoid, labd-13*E*- ene - 8α, 15-diol (**430**) was isolated from the root bark of *C. sylvaticus*.



The molecular formula of compound **430** was proposed to be $C_{20}H_{36}O_2$ from its ^{1}H and ^{13}C NMR data. ^{1}H NMR spectrum [Appendix 40a] had resonances of five methyl proton singlets at δ_H 1.69, 1.13, 0.86, 0.79 and 0.78 and an olefinic proton at δ_H 5.43 *t* (*J* = 3.96 Hz). A quintet (double doublet) integrating for two protons was observed down field at δ_H 4.14 (*J* = 3.86, 7.24 Hz). The ^{13}C NMR spectrum [Appendix 40b] had 20 carbon resonances that included two sp^2 carbons at δ_C 141.0 and 123.2 and two oxygenated sp^3 carbons at δ_C 74.1 and 61.2. Correlations observed in the 2D NMR experiments [Table 4.36] and comparison of the experimental spectral data with literature showed that compound **430** had a labdan-8-hydroxylabdan-13-ene skeleton (Ngadjui *et al*., 1999).

Correlations observed in the NOESY spectrum between 3H-17 and H-7a led to a deduction that the methyl group at position 8 was α-configured while that between H-5 and both 3H-18 and 3H-19 enabled assignment of the relative configuration at C-5. The *E*-geometry of the substituents of the C (13) / C (14) double bond was deduced from the up field resonance of the methyl group at $\delta_{C\text{-}16}$ 16.5. Compound **430** was subsequently identified to be the known labd-13*E*-ene-8α, 15-diol reported previously from *Cistus creticus* subsp. *Creticus* (Koukoulitsa *et al*., 2008). This is the first report of its isolation from a *Croton* species. Its potential cytotoxicity and cytostatic effects against human cancer cell lines have too been reported (Koukoulitsa *et al*., 2008).

Table 4.36: NMR spectroscopic data of labda-13*E*-ene-8*α*, 15- diol (430)

Pstn	δ_C (75 MHz)		δ_H (300 MHz)	HMBC	COSY	NOESY
	Koukoulitsa *et al*., 2008	Experimental	(*m*, *J* Hz; Integral)	(H→ C)		
1	40.0	39.8	1.64 (*br s*; H_α) 0.95 (*d*,1.86; H_β)	2, 3, 5, 9, 10, 20	1β, 2 1α	20
2	18.7	18.5	1.59 (*m*; H_α) 1.43 (*m*; H_β)	1, 3, 4, 10	1, 2β, 3 2α	
3	42.2	42.0	1.14 (*m*; H_α) 1.37 (*m*; H_β)	1, 2, 4, 5, 18, 19	2, 3β 3α	18, 19
4	33.5	33.3				
5	56.3	56.1	0.92 (*br s*; H)	1, 4, 6, 7, 9, 10, 18, 19, 20	6	18, 19
6	20.8	20.6	1.26 (*m*; H_α) 1.64 (*m*; H_β)	4, 5, 7, 8, 10	6β, 7α,β 6α	
7	44.8	44.6	1.86 (*dt*, 1.62; H_α) 1.38 (*m*; H_β)	5, 6, 8, 9, 17	6, 7β 7α	17
8	74.3	74.1				
9	61.5	59.3	1.05 (*t*, 2.01; H)	1, 5, 7, 8, 11, 12, 17, 20	11	
10	39.5	39.3				
11	23.8	23.6	1.54 (*m*; H_α) 1.38 (*m*; H_β)	8, 9, 10, 12, 13	9, 11β, 12 11α	
12	43.1	42.9	2.09 (*t*, 4.41; 2H)	9, 11, 13, 14, 16	11	16
13	141.2	141.0				
14	123.4	123.2	5.43 (*t*, 3.96; H)	12, 16	15	15
15	59.5	61.2	4.14 (*q*, 3.86, 7.24; 2H)	13, 14	14	14
16	16.7	16.5	1.69 (*s*; 3H)	12, 13, 14, 15		
17	24.2	24.0	1.13 (*s*; 3H)	7, 8, 9		7
18	36.6	33.4	0.86 (*s*; 3H)	3, 5, 19		3, 5
19	21.2	21.5	0.78 (*s*; 3H)	3, 4, 5, 18		3, 5
20	15.7	15.5	0.79 (*s*; 3H)	1, 5, 9, 10		1

4.2 Preliminary phytochemical screening results

Phytochemical screening of the aqueous and methanol extracts of *C. alienus*, *C. megalocarpoides* and *C. sylvaticus* showed predominance of terpenoids and sterols. Alkaloids, anthraquinones, tannins, phenolics and flavanoids were found in trace amounts. The methanol extracts of the stem barks of *C. megalocarpoides* and *C. sylvaticus* were found to have very low total phenolic content (TPC; 1.89 $\pm$ 0.02% - 1.14 $\pm$ 0.01% *w/w* equivalent of gallic acid). These two extracts were additionally found to have low antioxidant potential (IC_{50} > 1000 µg / ml compared to ascorbic acid, IC_{50} = 9.51 $\pm$ 0.22 µg/ml).

The above observations were consistent with reports of plant species belonging to *Croton* genus. Asian and African *Croton* species yielded mainly diterpenoids while American *Croton* species tended to yield mainly alkaloids (Salatino *et al.*, 2007; Chapter 2 in this thesis). Results of the phytochemical investigations in this study [Section 4.1] did not show isolation of flavonoids and phenolics. Plant phenolics in general are highly effective in free radical scavenging hence good anti-oxidants (Atanassova *et al*., 2011).

4.3 Biological activity screening results

Anti-microbial activity tests were done using different strains of bacteria and fungi [Table 4.37 and 4.38]. *Candida albicans* was the most susceptible microorganism to the crude plant extracts. The root and stem bark aqueous extracts of *C. alienus* and *C. sylvaticus* were active towards *C. albicans* at the lowest concentration tested (25 mg / mL). Harwickiic acid (**423**), that was isolated from the roots of *C. sylvaticus* was found to inhibit the growth of *C. albicans* (MIC $\leq$ 12.5 µg / mL). Methanol extract of the stem bark of *C. sylvaticus* was the only crude extract that inhibited the growth of a bacteria strain, *Bacillus subtillis* at 10 mg / mL. Penduliflaworosin (**429**) that was isolated from the roots of *C. sylvaticus* showed some anti-bacterial activities towards *B. subtillis* (MIC $\leq$ 12.5 µg / mL). The compounds that were isolated from *C. alienus* and *C. megalocarpoides* and and subjected to anti-microbial activity tests were found be inactive (IC_{50} > 20 µg / mL) when compared to control drugs whose activity is given in Table 4.38.

Table 4.37: Anti-microbial activity test results of crude plant extracts

Extracts Exhibiting Activity	Lowest concentration showing activity (mg/mL)	Micro-organism(s) with Inhibited growth
C. alienus roots		
• Aqueous	25	*C. albicans*
• Methanol	50	
C. alienus stem bark		
• Aqueous	25	*C. albicans*
• Methanol	50	*C. albicans*
	100	*A. niger*
C. megalocarpoides root bark		
• Aqueous and Methanol	50	*C. albicans*
C. megalocarpoides stem bark		
• Aqueous	50	*C. albicans*
C. sylvaticus root bark		
• Aqueous	25	*C. albicans*
• Methanol	50	*B. subtillis*
C. sylvaticus stem bark		
• Aqueous	25	*C. albicans*
• Methanol	10	*B. subtillis*
Gentamycin	3.0	*B. subtillis*
Nystatin	3.0	*C. albicans*
	3.0	*A. niger*
DMSO	-	-

Table 4.38: Anti-microbial test results of control drugs used in secondary screening

Micro-organism	Drug Control	IC_{50}[11] (µg/ml)*	MIC (µg/ml)**	MFC/MBC (µg/ml)***
Candida albicans ATCC 90028 (Ca)	Amphotericin B	0.428	2.500	2.500
Candida glabrata ATCC 90030 (Cg)	Amphotericin B	1.040	2.500	2.500
Candida krusei ATCC 6258 (Ck)	Amphotericin B	1.599	2.500	2.500
Aspergillus fumigatus ATCC 90906 (Afu)	Amphotericin B	0.293	0.625	2.500
Cryptococcus neoformans ATCC 90113 (Cn)	Amphotericin B	0.695	1.250	1.250
Staphylococcus aureus ATCC 29213 (Sa)	Ciprofloxacin	0.082	0.250	0.500
Methicillin-resistant *S. aureus* ATCC 33591 (MRS)	Ciprofloxacin	0.091	0.250	0.500
Escherichia coli ATCC 35218 (Ec)	Ciprofloxacin	0.003	0.008	0.031
Pseudomonas aeruginosa ATCC 27853 (Pa)	Ciprofloxacin	0.053	0.250	0.500
Mycobacterium intracellulare ATCC 23068 (Mi)	Ciprofloxacin	0.485	1.000	-

*IC_{50} (Inhibitory Concentration), the concentration (µg/ml) that affords 50% inhibition of growth

**MIC (Minimum Inhibitory Concentration), the lowest test concentration (µg/ml) that allows no detectable growth

*** MFC/MBC (Minimum Fungicidal / Bactericidal Concentration), the lowest test concentration (µg/ml) that kills the organism

Other biological activity tests the crude plant extracts and compounds isolated were subjected to included anti-leishmanial, anti-plasmodial and larvicidal activity tests. *C. alienus* leaves MeOH: DCM (1:1 *v/v*) is the only extract that showed activity against *Leishmania donovani* (IC_{50} = 80µg/mL). However, the compounds isolated from both its leaves and roots were inactive against the same microbe (*L. donovani*; IC_{50} and IC_{90} > 40µg / mL). The control drugs used, pentamidine and amphotericine B had IC_{50} / IC_{90} 0.85 / 1.75 and 0.12 / 0.15 µg / mL respectively.

All the crude plant extracts were inactive towards D6 and W2 strains of *Plasmodium falciparum* (IC_{50} > 4760 ng/mL). The compounds isolated from the leaves and roots of *C. alienus* and roots of *C. megalocarpoides* that were tested for activity towards D6 and W2 strains of P. *falciparum* were also found to be inactive (IC_{50} > 4760 ng/mL). The crude plant extracts and the compounds subjected to *P. falciparum* were also tested for their cytotoxicity activity. They were all found to be inactive against VERO cells (IC_{50} > 4760 ng/mL). All the crude plant extracts and compounds isolated from *C. alienus* that were subjected to anti-plasmodial assays were additionally tested for their mosquito larvicidal activity. They were all found to be inactive against *Aedes aegypti* and *Anopheles gambiae* larvae (LC_{50} and LC_{95} >100 ppm) compared to azadirachtin, the larvicide control drug used (LC_{50} = 60 ppm).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The root and stem bark extracts of *C. alienus* and *C. megalocarpoides* were active against *Candida albicans* at the lowest concentration tested (25 mg / mL). Methanol extract of the root and stem bark of *C. sylvaticus* showed anti-microbial activity against *C. albicans* and *Bacillus subtillis* (MIC $\leq$ 12.5 µg / mL and 10.0 mg / mL respectively). Two compounds isolated from the roots of *C. sylvaticus*, hardwickiic acid (**423**) and penduliflaworosin (**429**) had activity against *C. albicans* and *B. subtillis* respectively (MIC $\leq$ 12.5 µg / mL). The leave extracts of *C. alienus* showed activity against *Leishmania donovani* at IC_{50} 80 µg / mL. These results support conservation for medicinal value of the three plant species investigated. Loss of synergistic activity by the isolated compounds and trace others in the crude extracts upon purification [Chapter 4; Section 4.3] can be used to explain the apparent lack of bio-activity by the compounds isolated and subjected to biolocigal activity tests.

A wide range of phytochemicals including glutarimide alkaloids, methyl cyclohexane derivatives, diterpenoids (*ent*-clerodanes, abietanes, trachylobanes, halimanes, labdane and a phorbol ester), triterpenoids and phytosterols were isolated from the three plants investigated. Some of the compounds (**417-421, 424-426** and **430**) were being reported for the first time from *Croton* genus, others had not been isolated before from any plant (**392-403, 405, 415** and **415)** while a lot others (**391, 404, 406-414, 414, 416, 422, 423** and **427-429**) have been previously isolated from other *Croton* species. Although many of the compounds isolated were not screened for their bio-activity because of sample limitations, the medicinal potential of the plants investigated is supported by the reported bio-activities of some of the compounds previously isolated.

This study generated the first phytochemical report of *C. alienus* and *C. megalocarpoides* that are endemic to Kenya. *C. alienus* did not produce any '*ent*-clerodane', compounds that have been widely reported from many African *Croton* species. Additionally, it produced both alkaloids and diterpenoids, a not so common finding in many plants. It also produced a phorbol ester derivative, a class of phytochemicals that are not very commonly found in African *Croton* species and that have been reported as having notably reported interesting biological activities.

C. megalocarpoides is the second African *Croton* species after *C. zambesicus* to have produced abietane diterpenoids. The *ent*-clerodane derivatives it produced are highly oxygenated like those reported from *C. zambesicus* (see compounds **190-19**2 in Chapter 2 and compare them with compounds **391-400** in Chapter 4). Additionally, the phytochemical similarity of *C. megalocarpoides* with *C. zambesicus* is further evidenced by their production of *ent*-trachylobanes which are reported from only one other African *Croton* species, *C. macrostachyus*. A report on the Southern Africa *C. sylvaticus* had none of the compounds isolated from the Kenyan species investigated in this study. The phyto-constituents of *C. sylvaticus* are therefore likely to be region specific.

5.2 Recommendations

I. Based on the number of new compounds isolated in this study, there is a possibility of Kenyan *Croton* species having interesting phyto-constituents' behaviour and by extension un-reported pharmacological-toxicological values. All the Kenyan *Croton* species should therefore be evaluated for their phyto-pharmacological relevancies.

II. *C. megaloacrpoides* and *C. zambesicus* were observed to have similar phyto-constituents. They should therefore be investigated further to establish whether the two names refer to the same species.

III. A repeat isolation in large quantities of the compounds obtained in this study should be done to enable:-

(i) Evaluation of all the isolated compounds for their bio-activity potential because sample limitations made some of the compounds isolated in this study not be assayed.

(ii) Structural modification studies of all compounds isolated with an aim of enhancing their bio-activity properties.

IV. Future studies involving *Croton* plant species should:-

(i) Follow all protocols for isolating special classes of phytochemicals such as phorbol ester diterpenoids and alkaloids

(ii) Adhere to bioassay guided fractionation approach and aim at documenting active fractions that can further be developed into useful products rather than the pure isolates that possess no activity

(iii) Investigate synergism, antagonism and additive interactions as a contributor to bio-activity of crude plant extracts.

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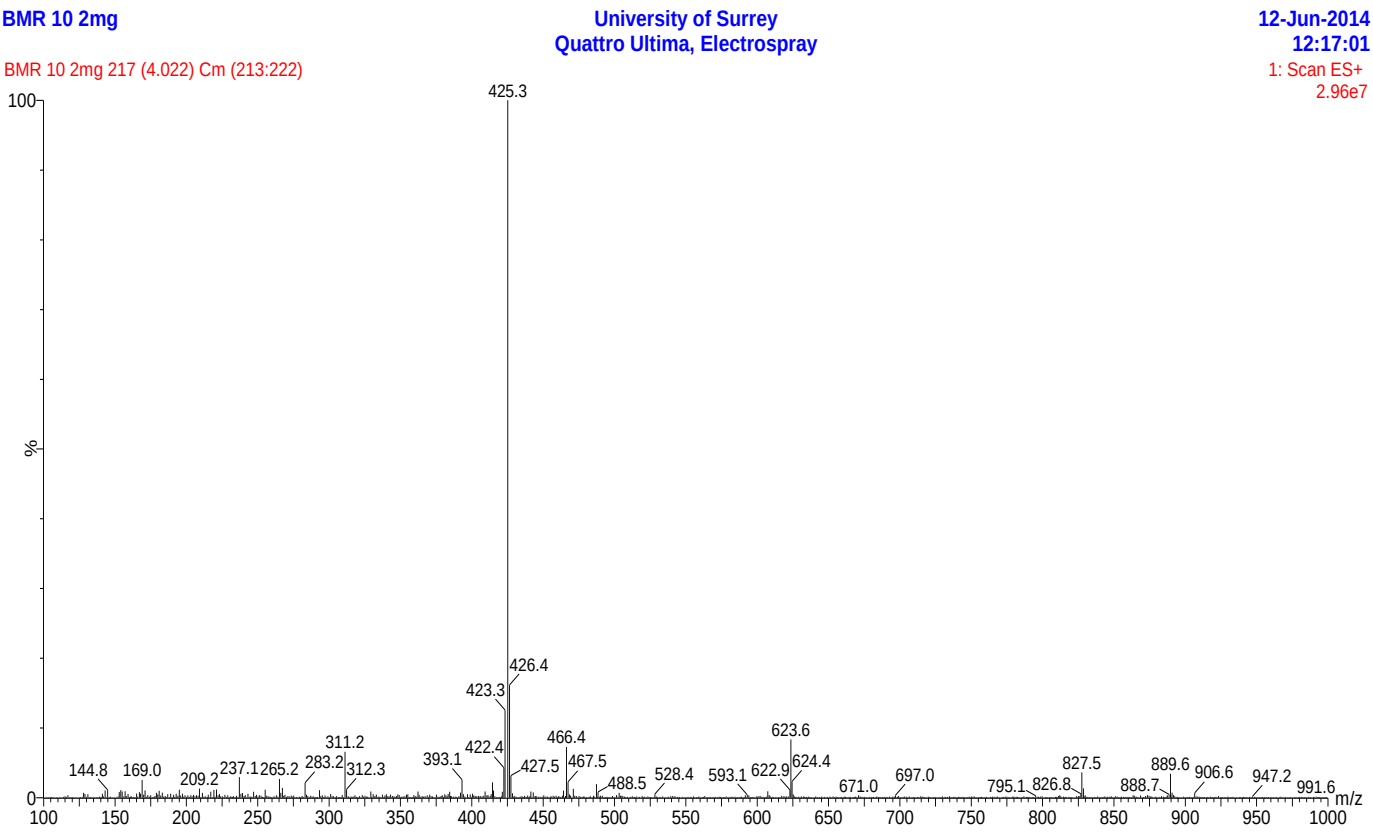
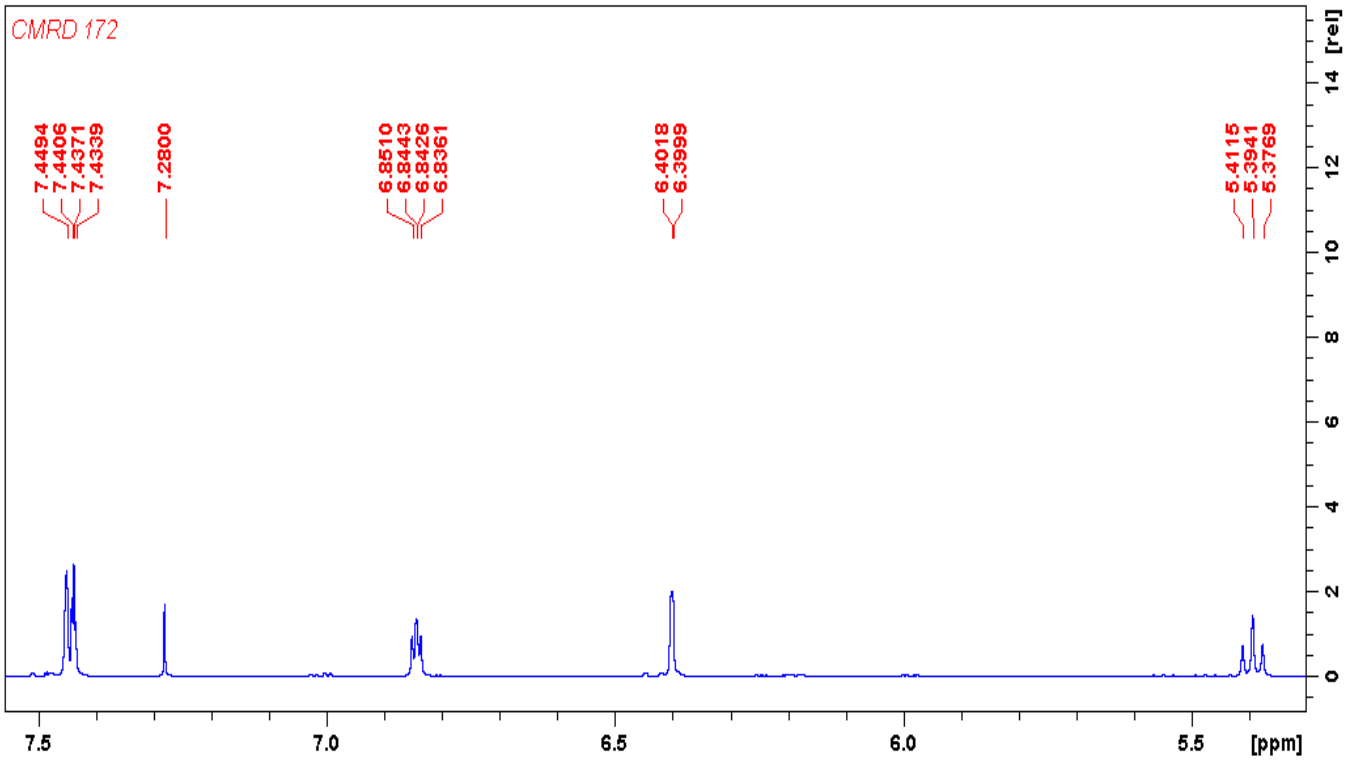
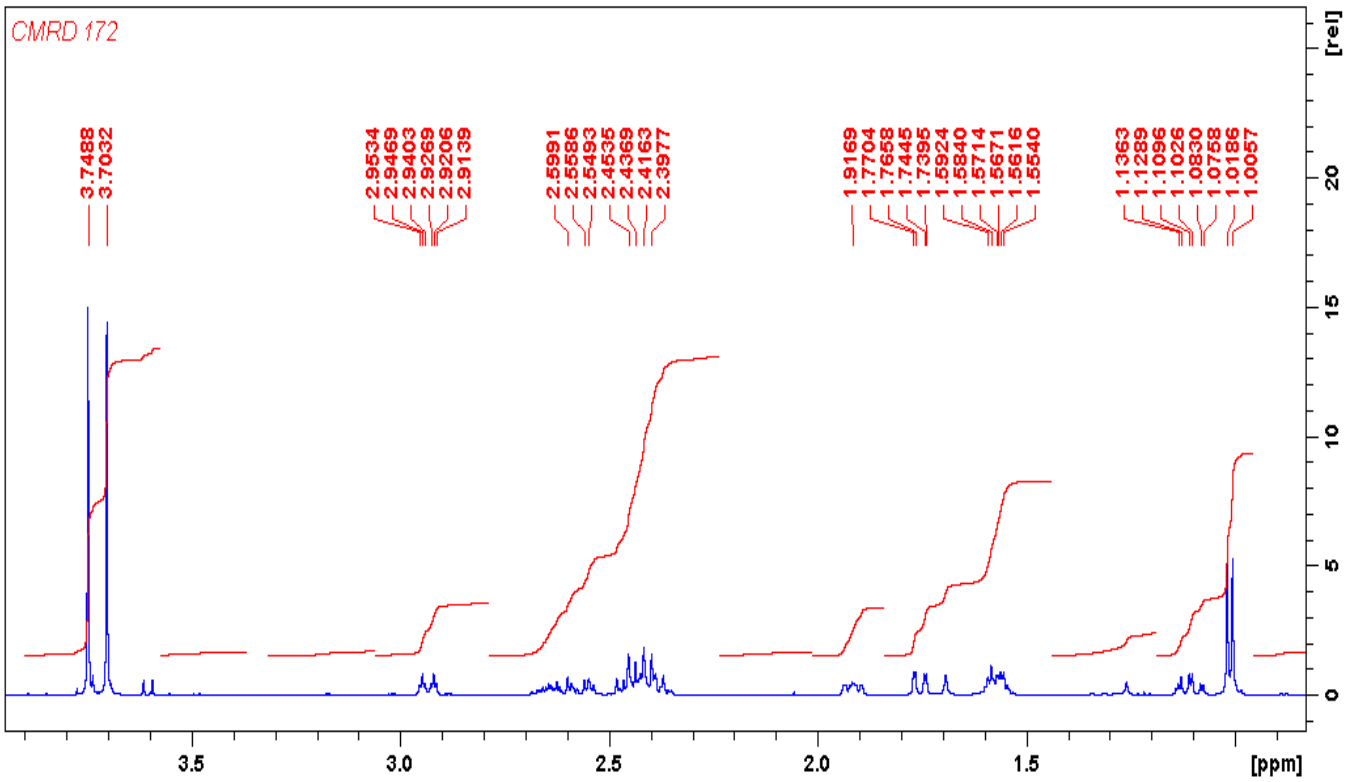
Appendix 1 a: Mass spectrum for crotocorylifuran (391)

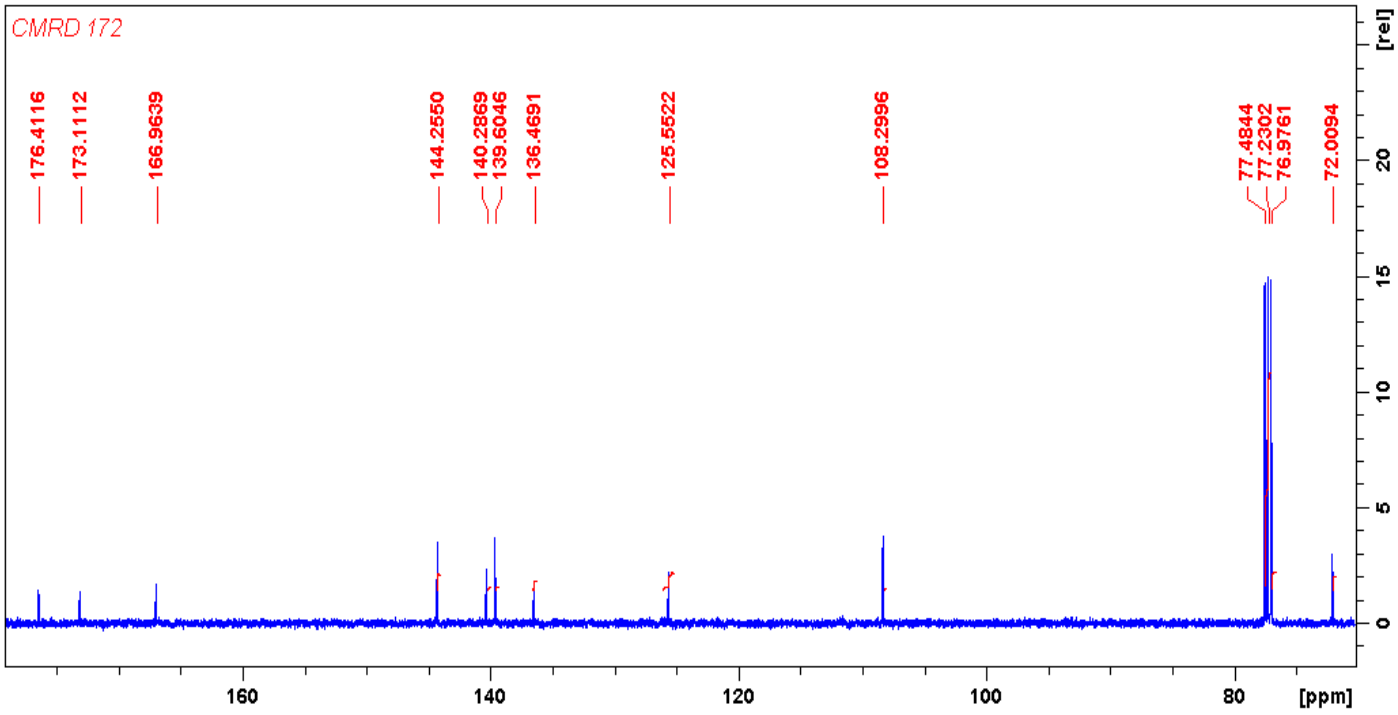


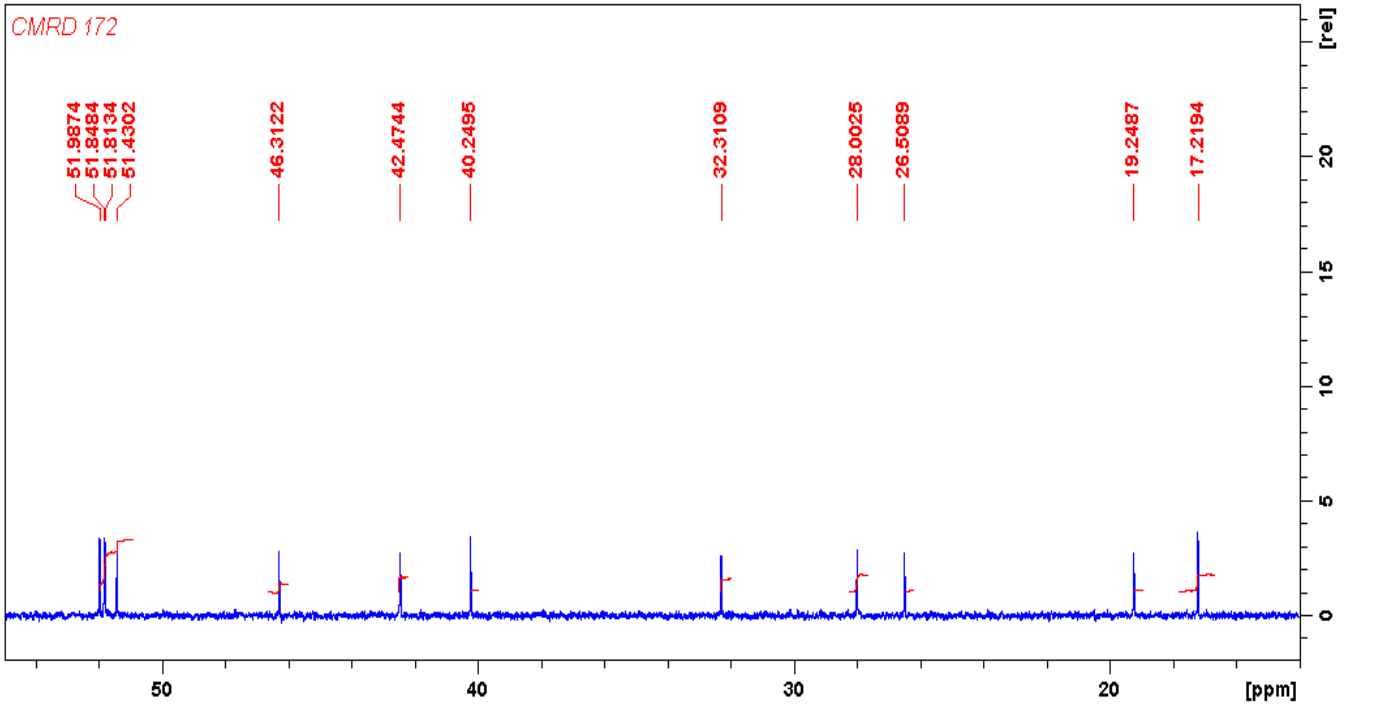
Appendix 1 b: ^{1}H NMR spectrum for crotocorylifuran (391)



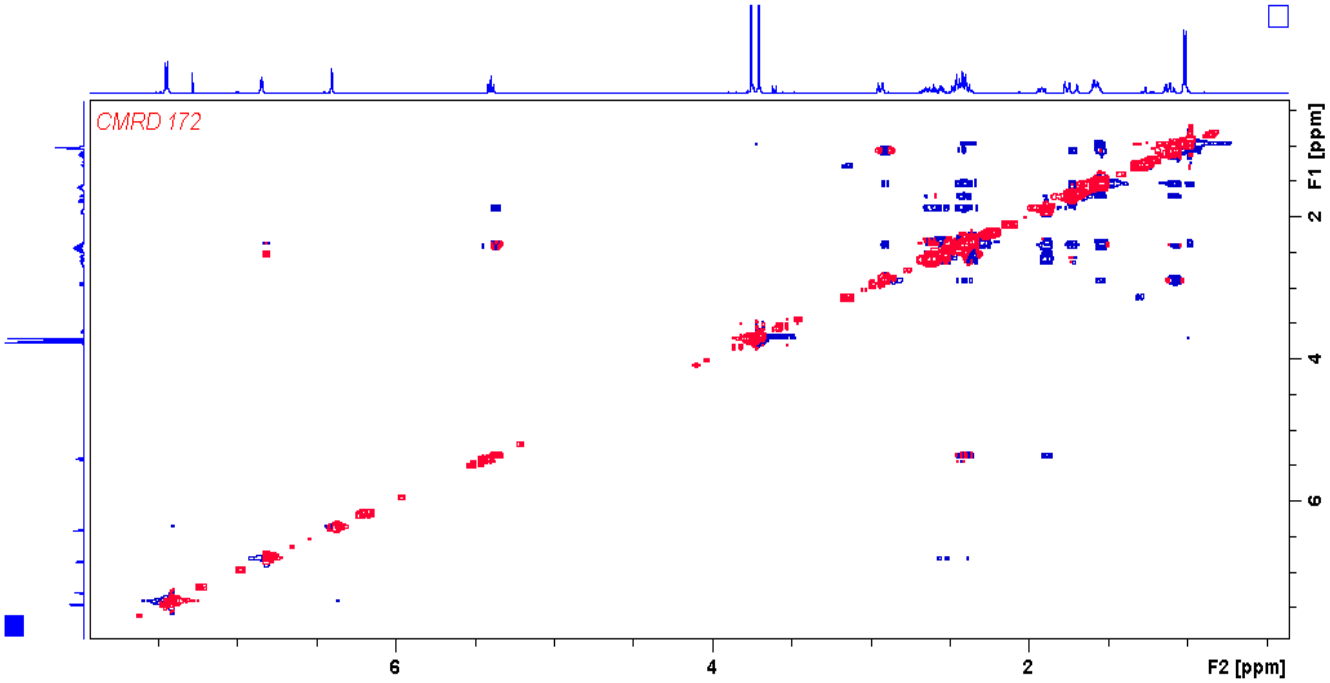


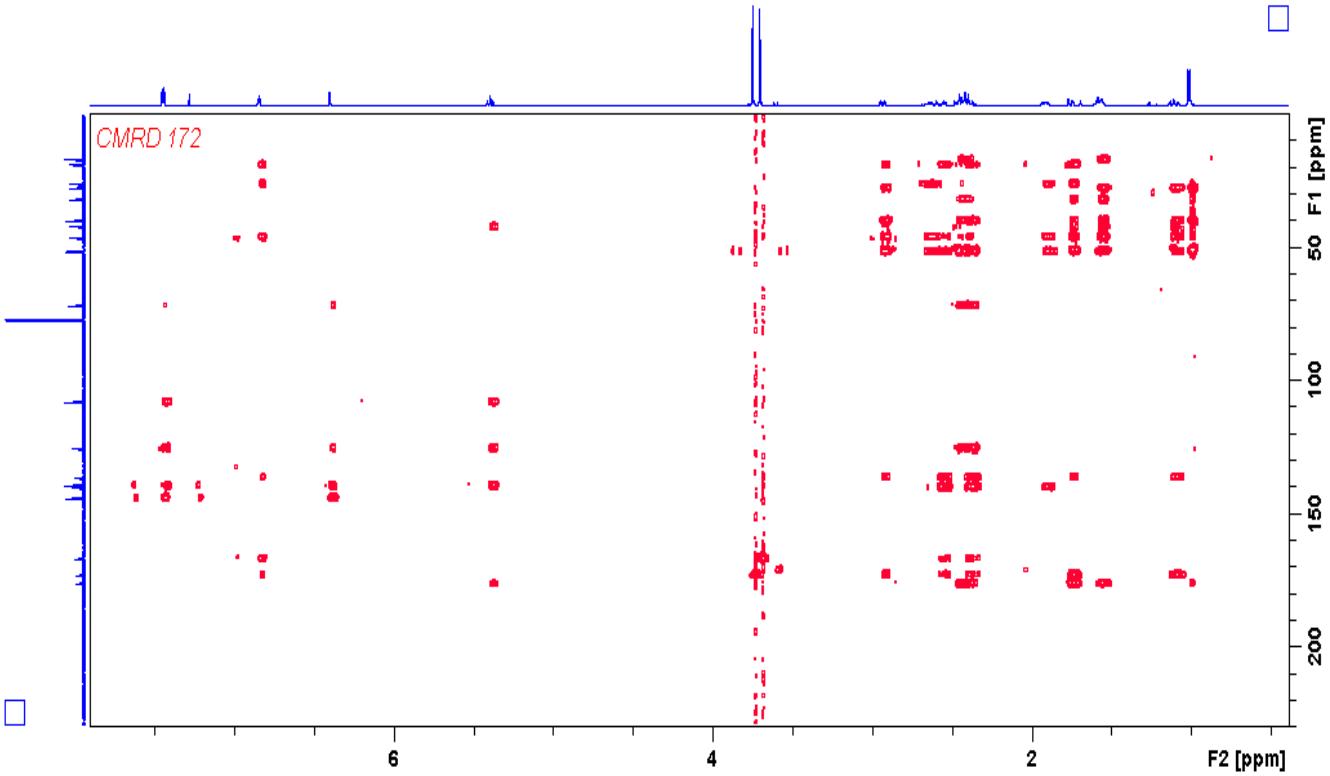
Appendix 1 c: ^{13}C NMR spectrum for crotocorylifuran (391)



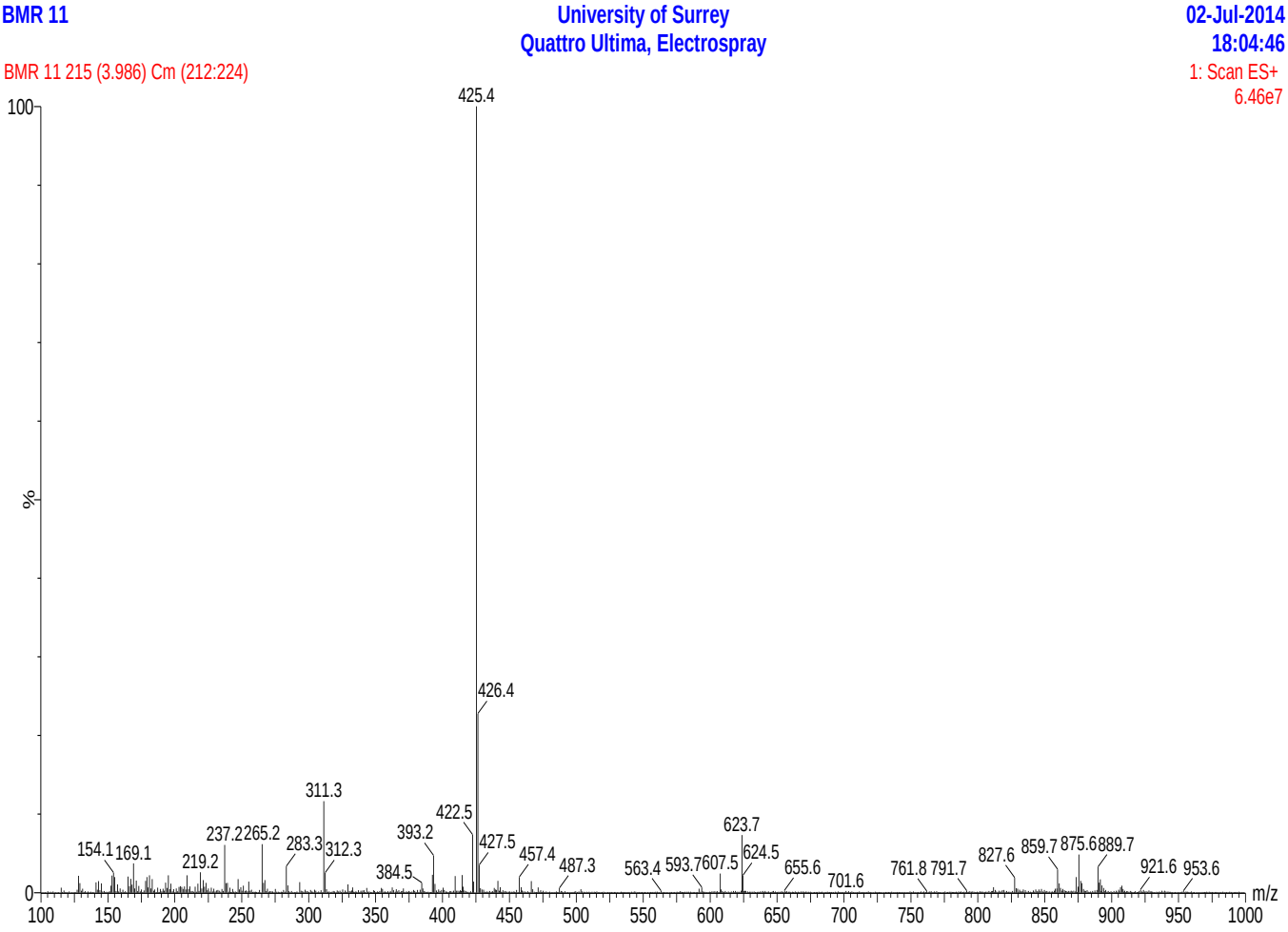


Appendix 1 d: NOESY and HMBC spectra for crotocorylifuran (391)

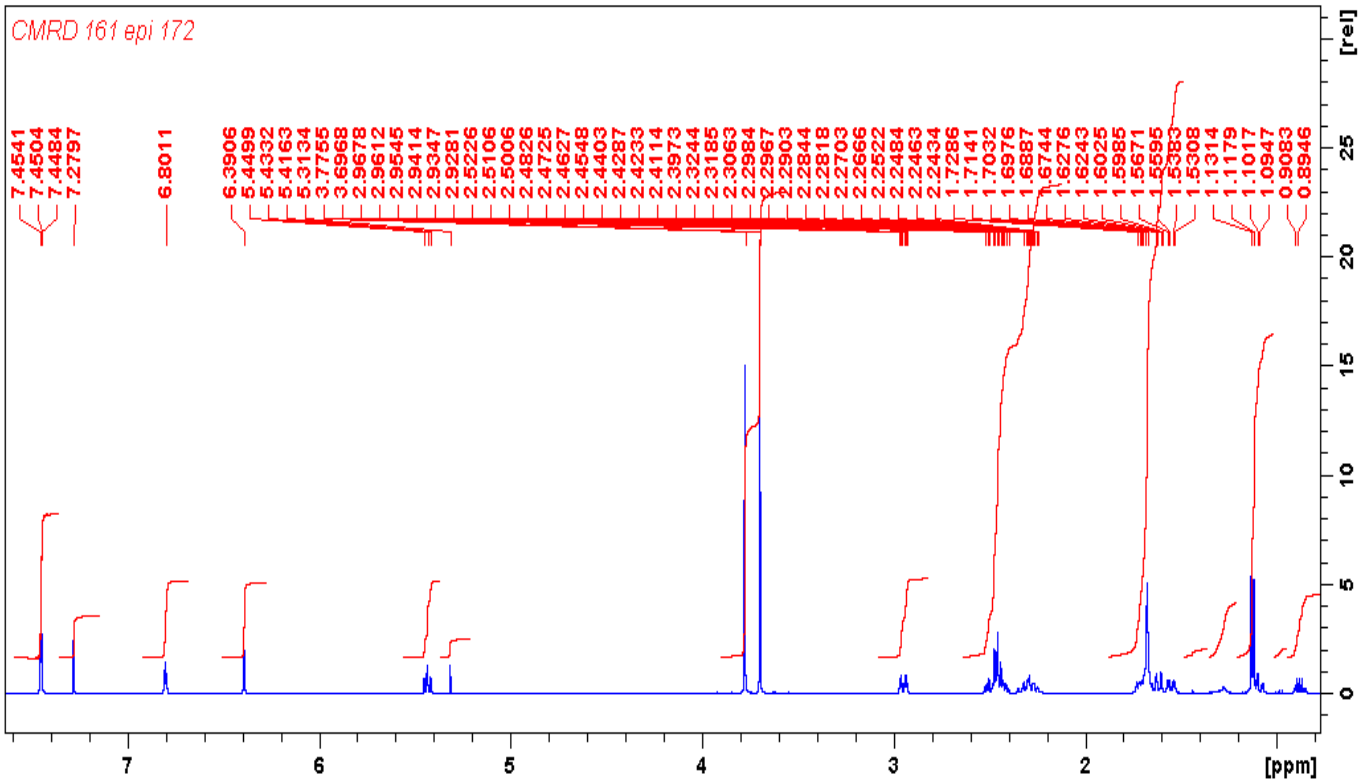


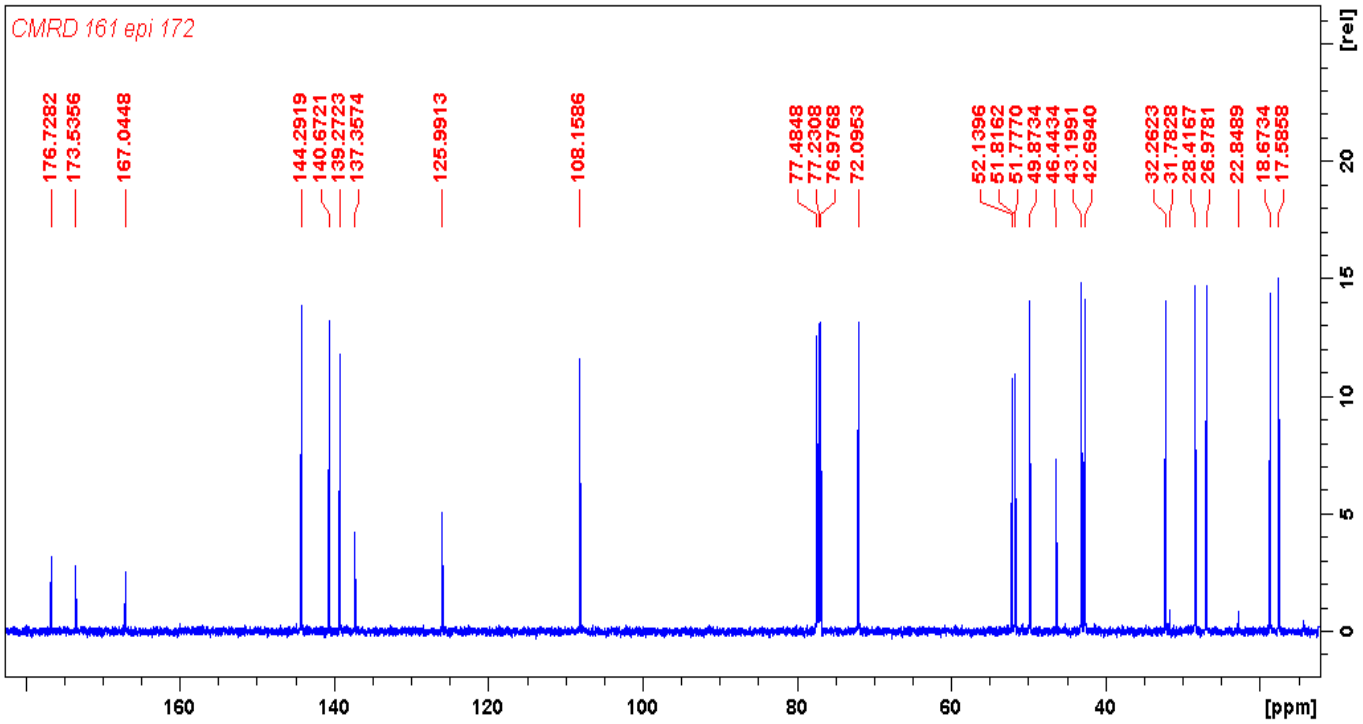


Appendix 2 a: Mass spectrum of 12-*epi*-crotocorylifuran (392)

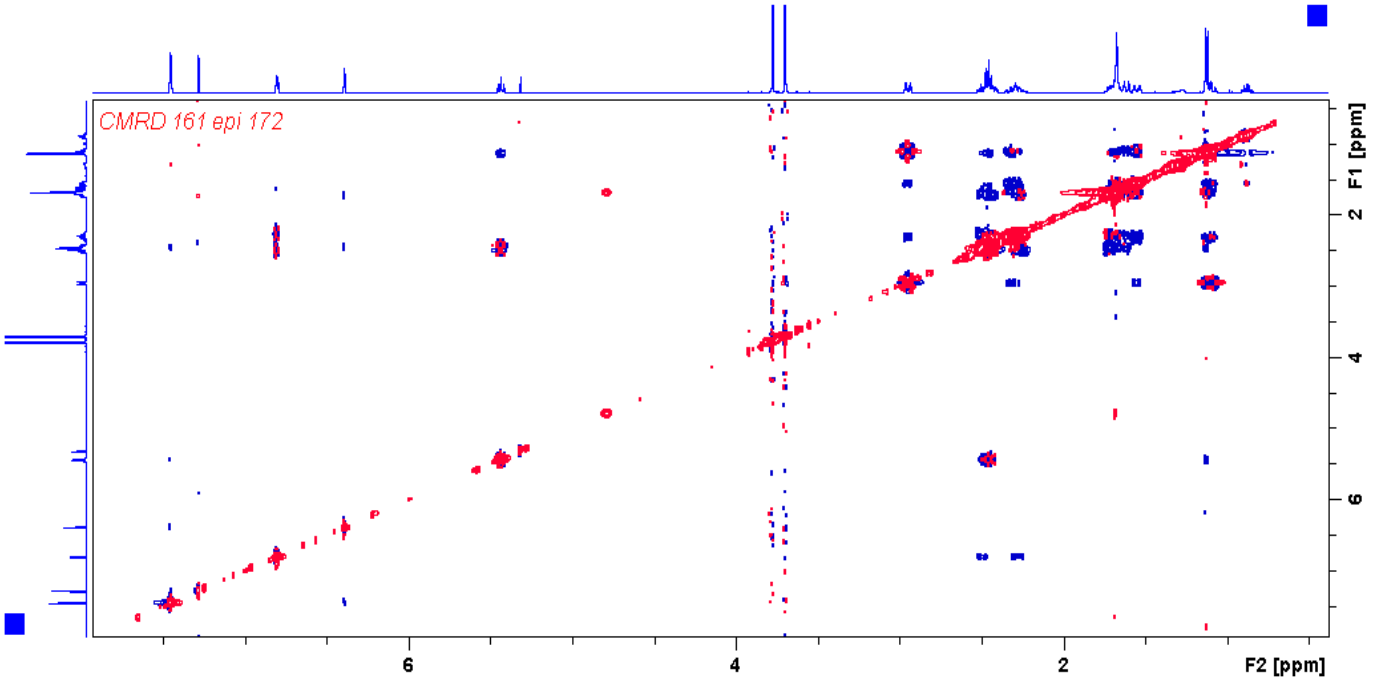


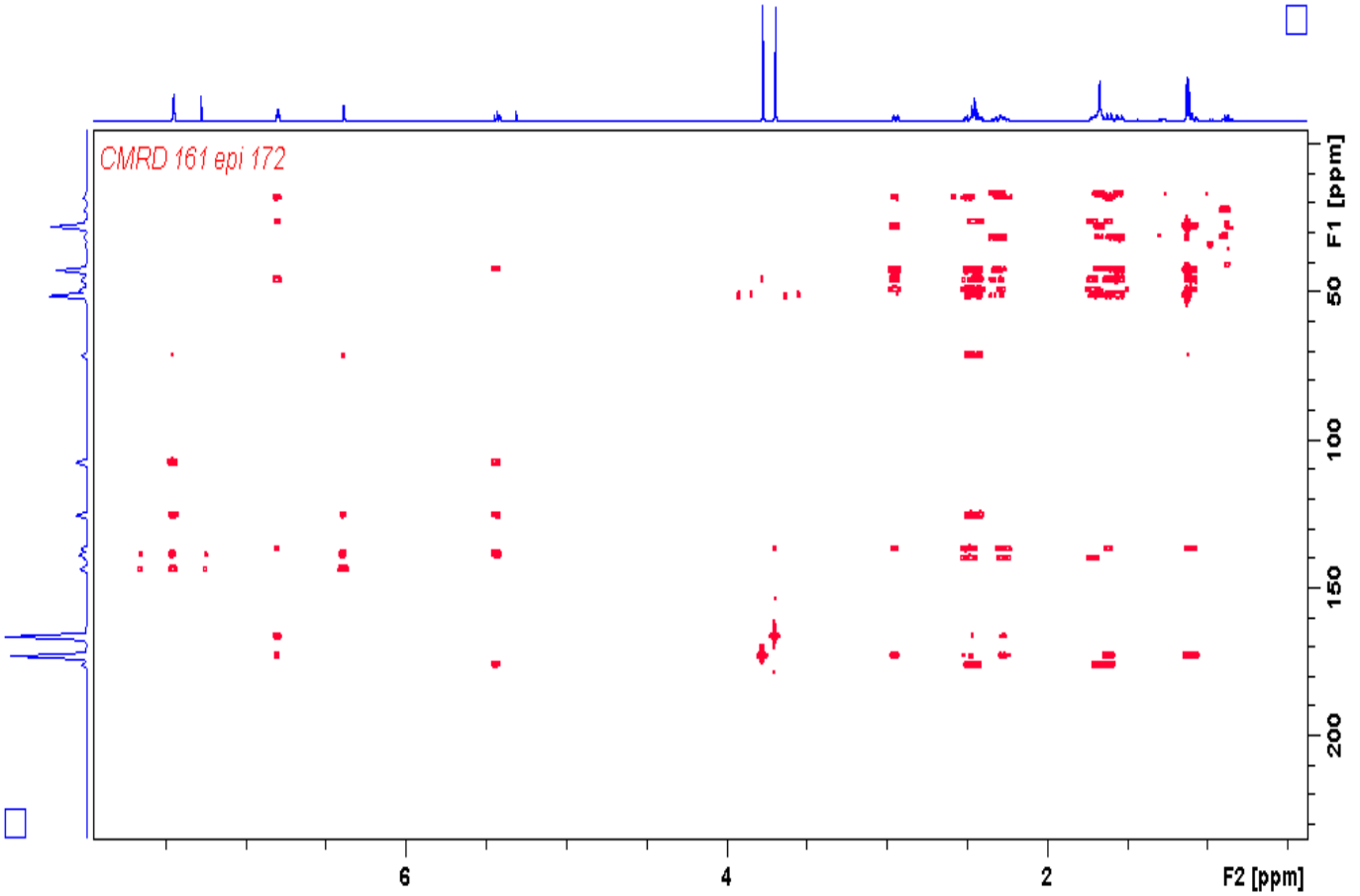
Appendix 2 b: ^{1}H and ^{13}C NMR spectra of 12-*epi*-crotocorylifuran (392)



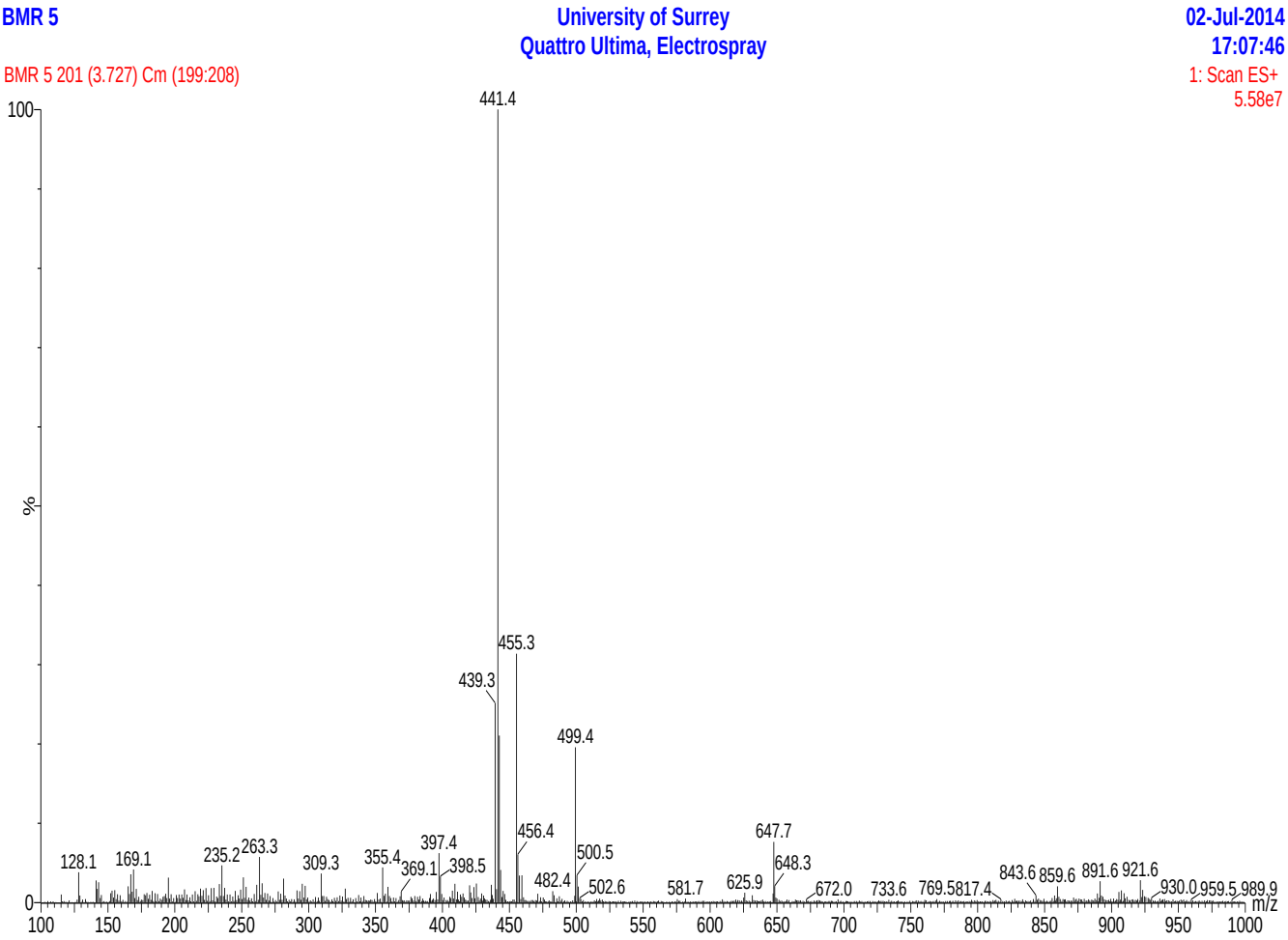


Appendix 2 c: NOESY and HMBC spectra of 12-*epi*-crotocorylifuran (392)

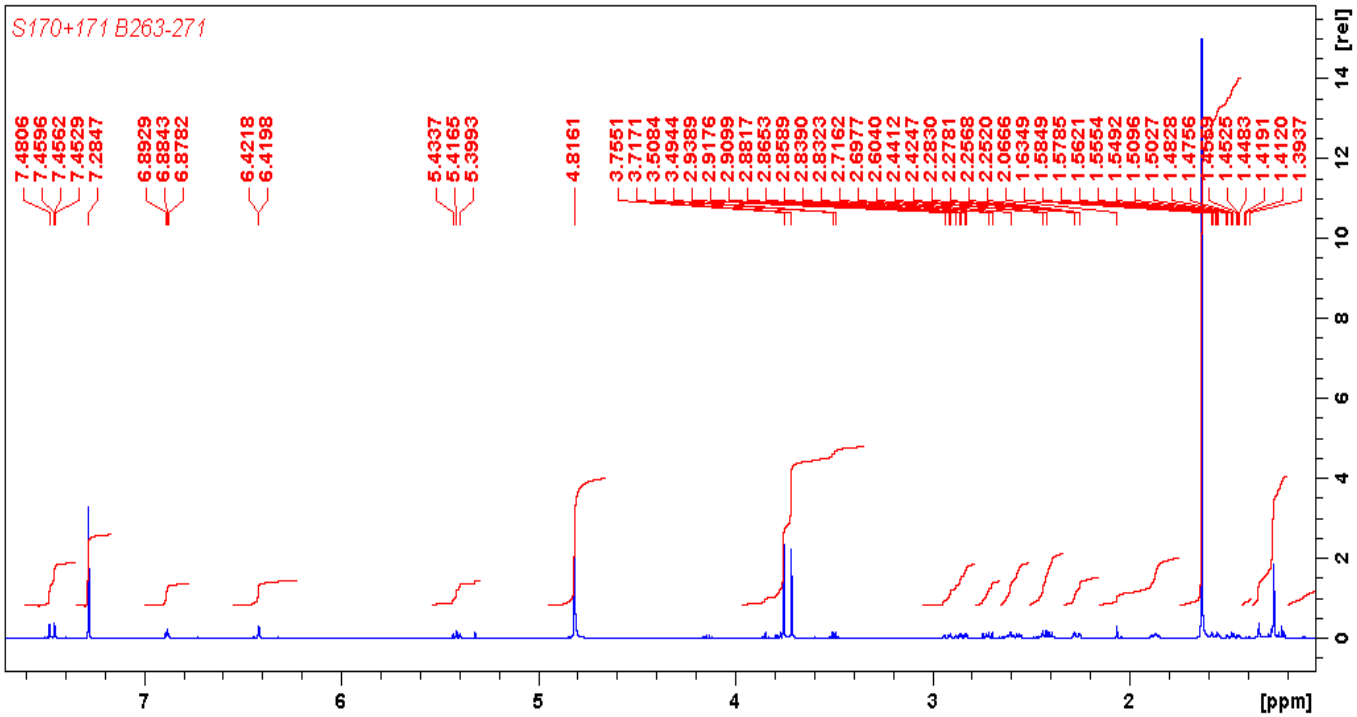


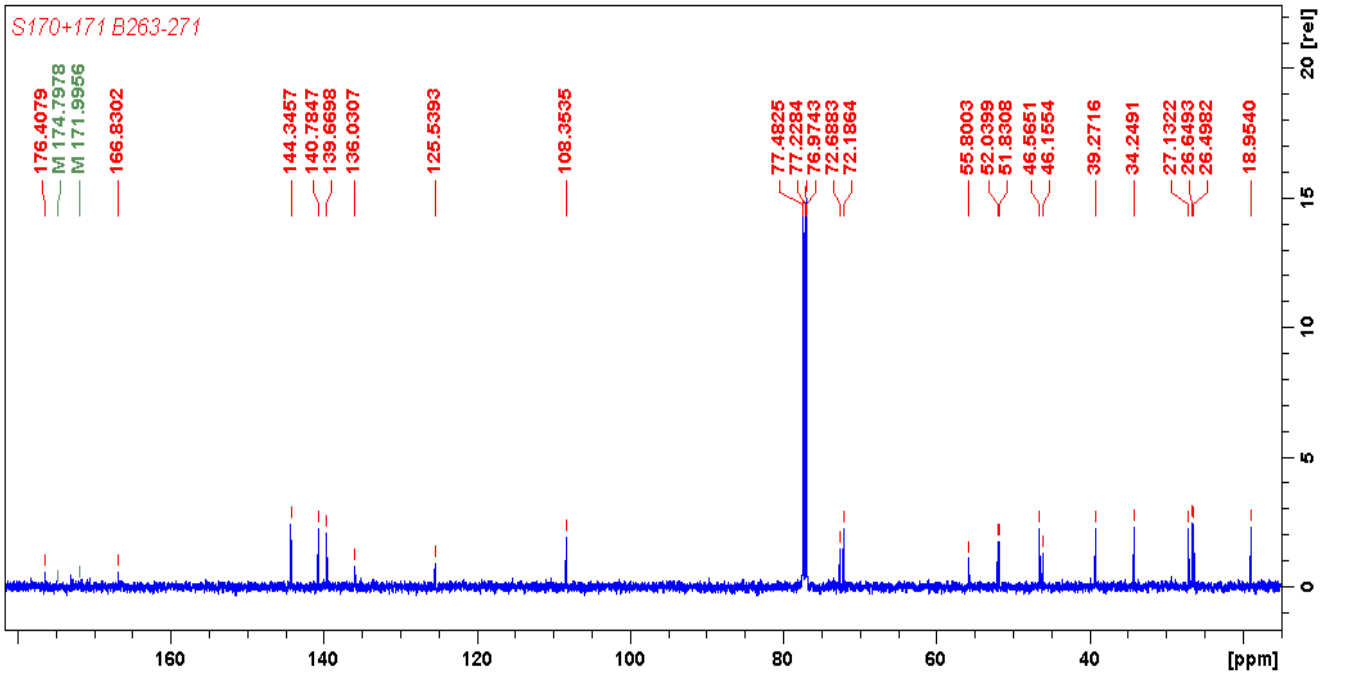


Appendix 3 a: Mass spectrum of 8-hydroxycrotocorylifuran (393)

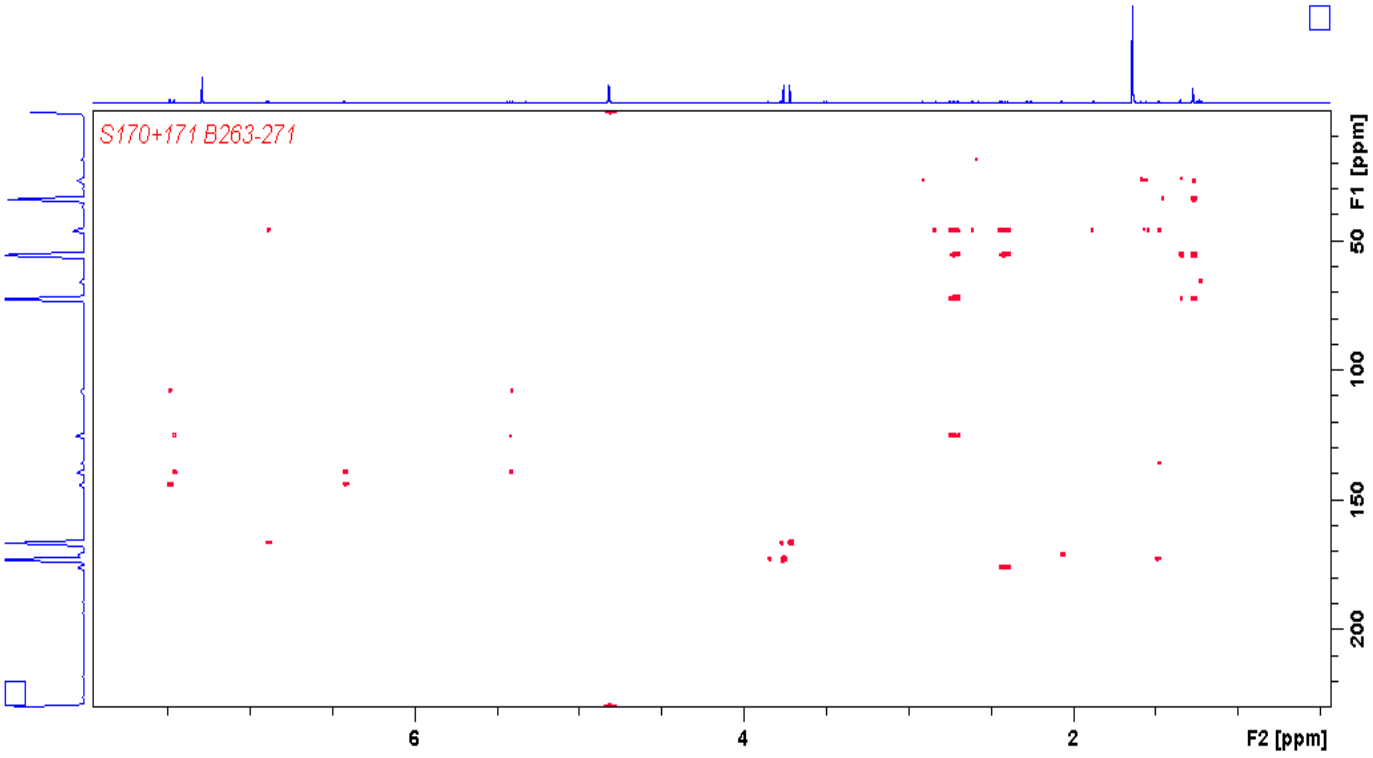


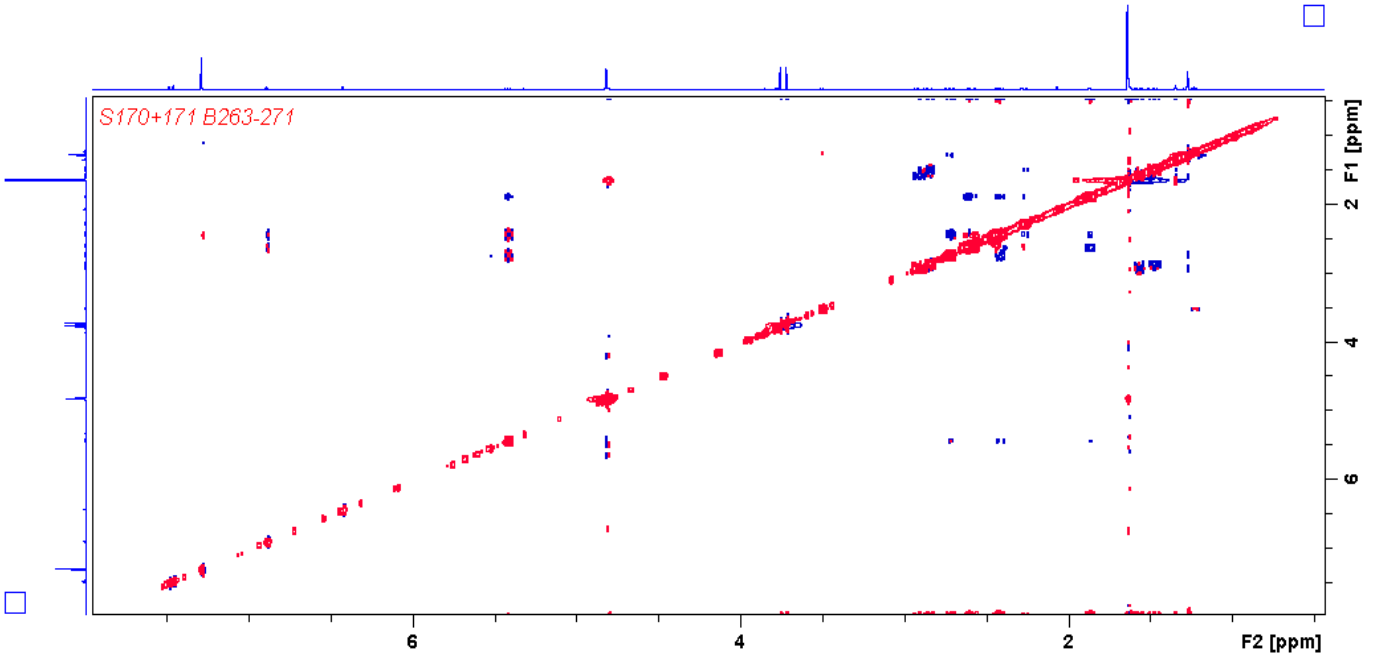
Appendix 3 b: ^{1}H and ^{13}C NMR spectra of 8-hydroxycrotocorylifuran (393)



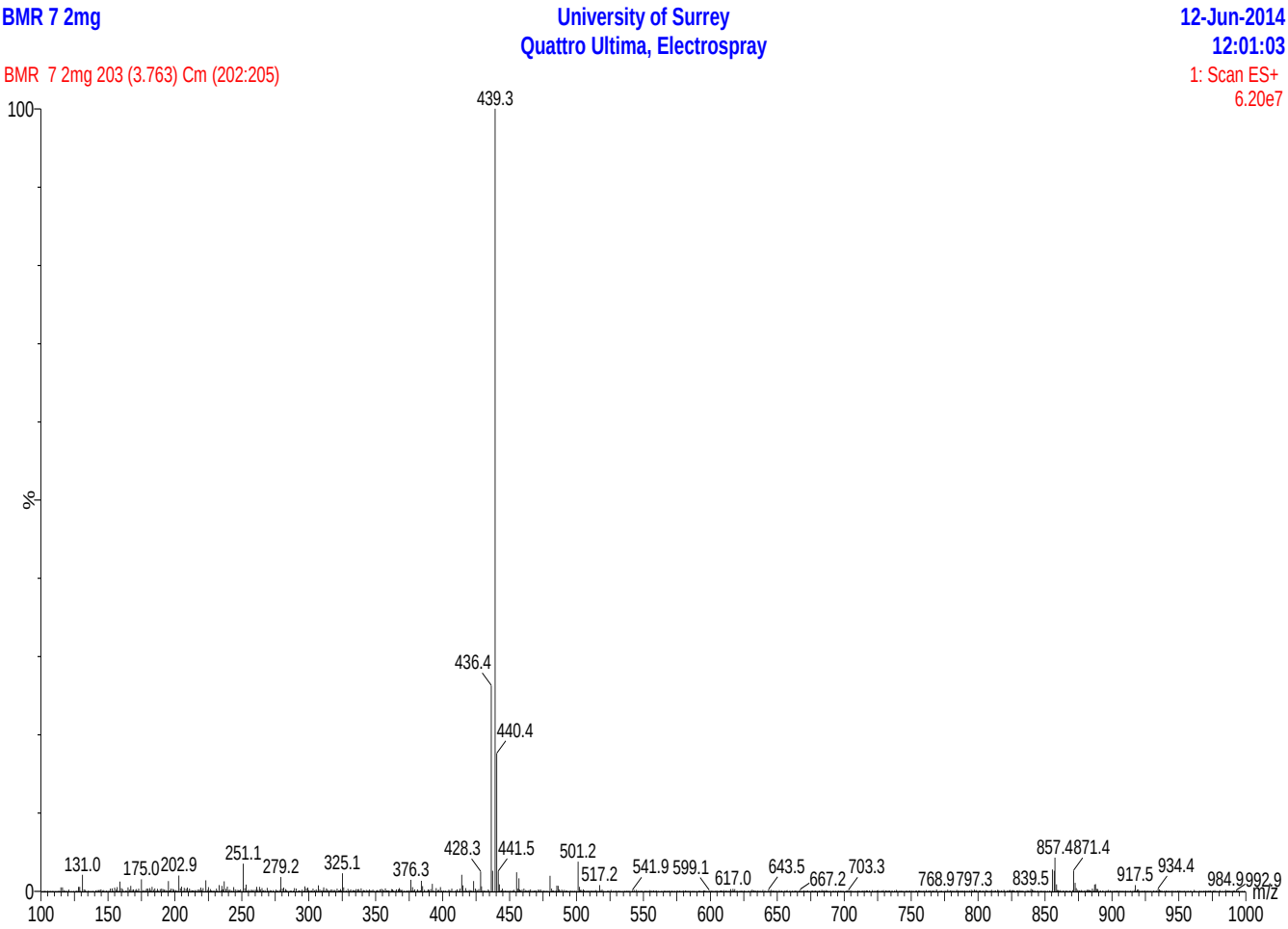


Appendix 3 c: HMBC and NOESY spectra 8-hydroxycrotocorylifuran (393)

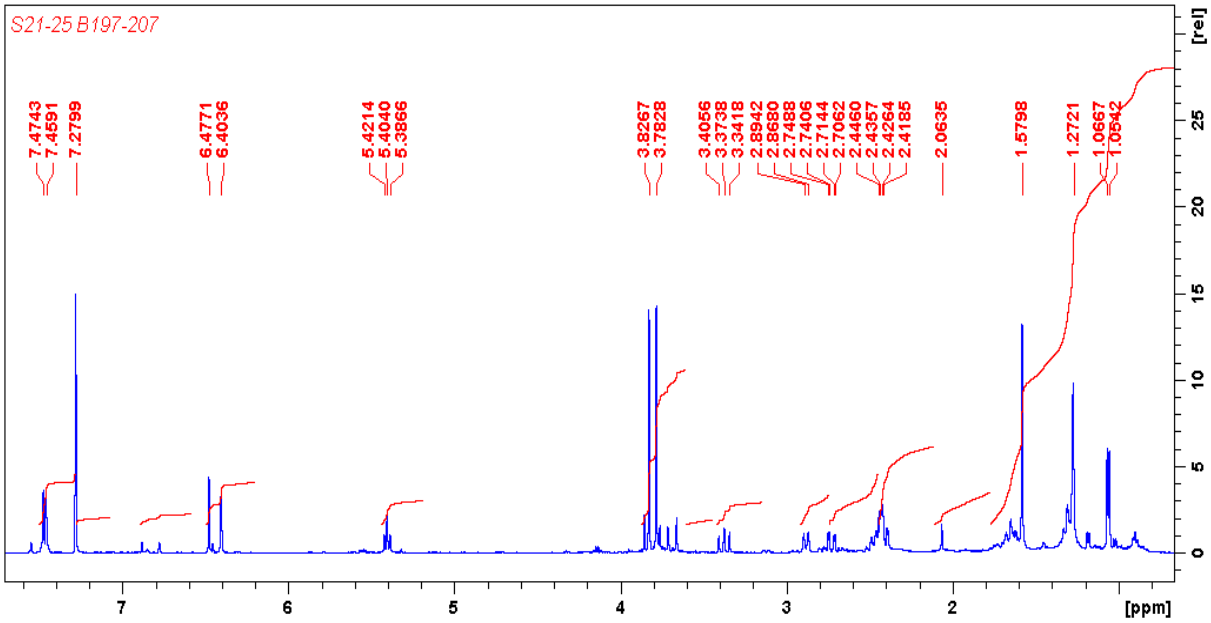


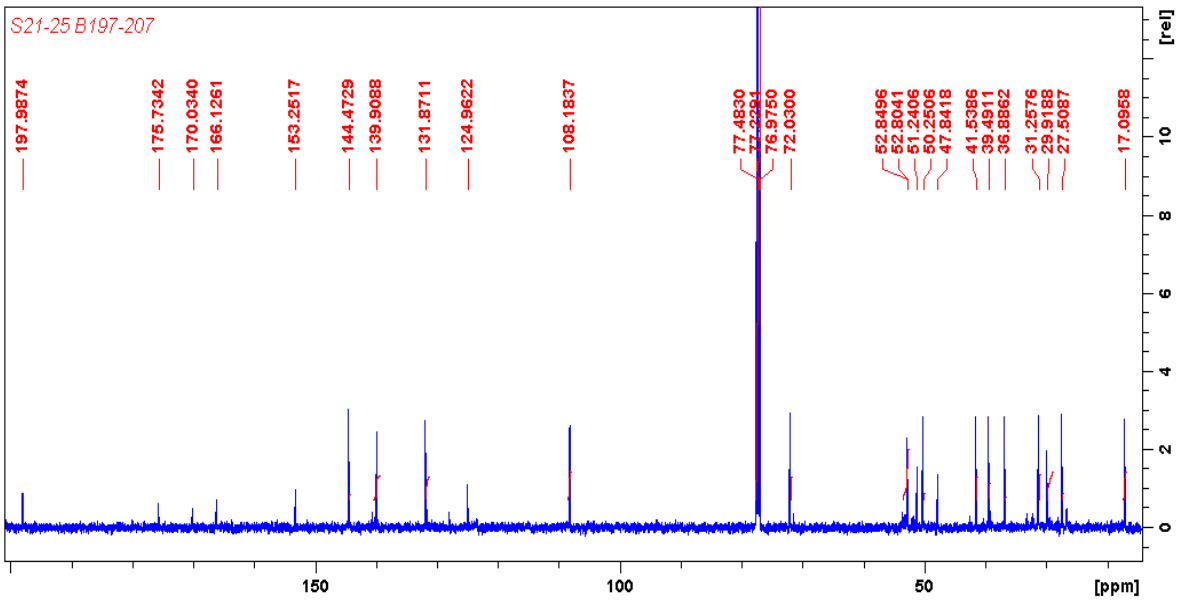


Appendix 4 a: Mass spectrum of 2-ketocrotocorylifuran (394)

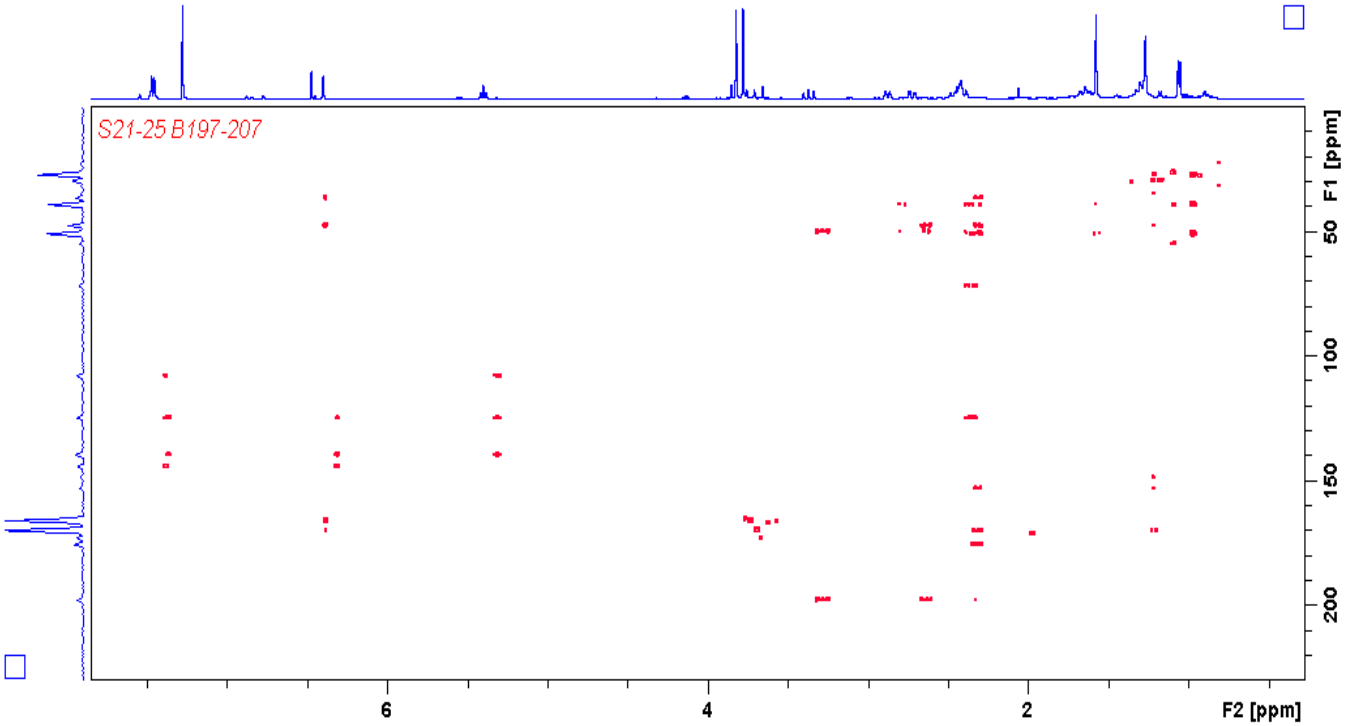


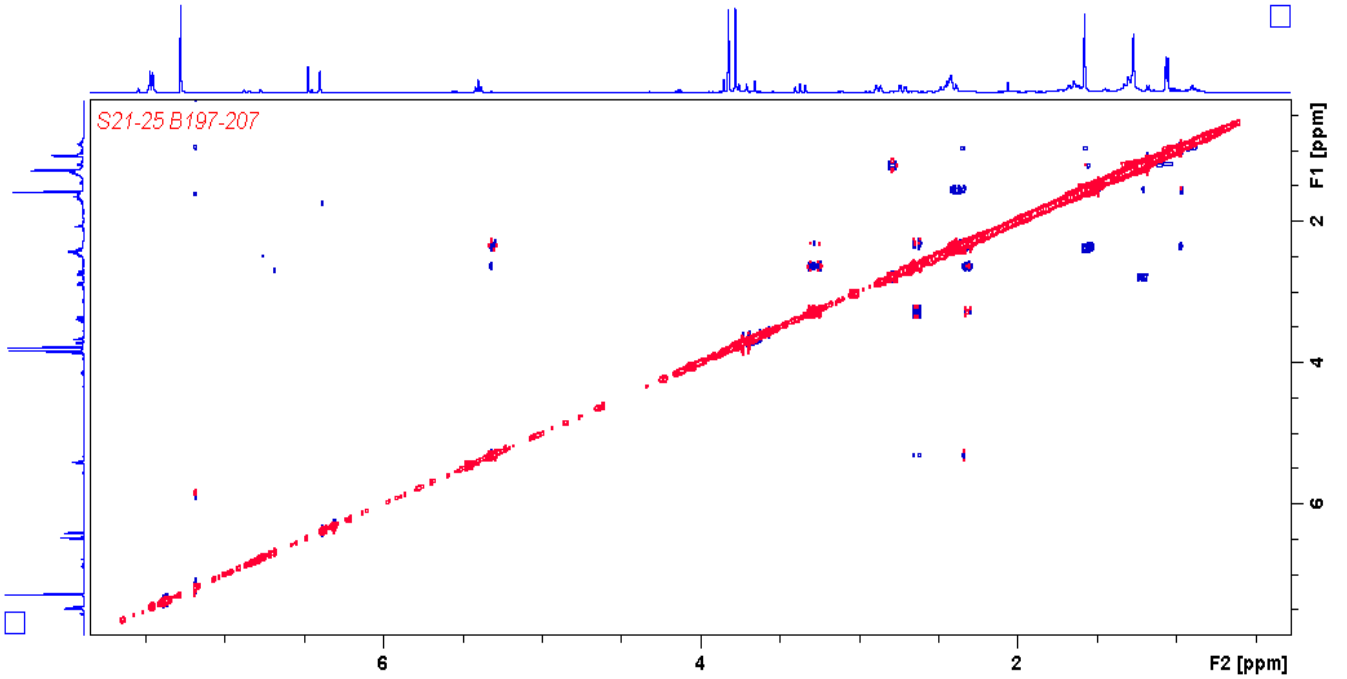
Appendix 4 b: ^{1}H and ^{13}C NMR spectra of 2-ketocrotocorylifuran (394)



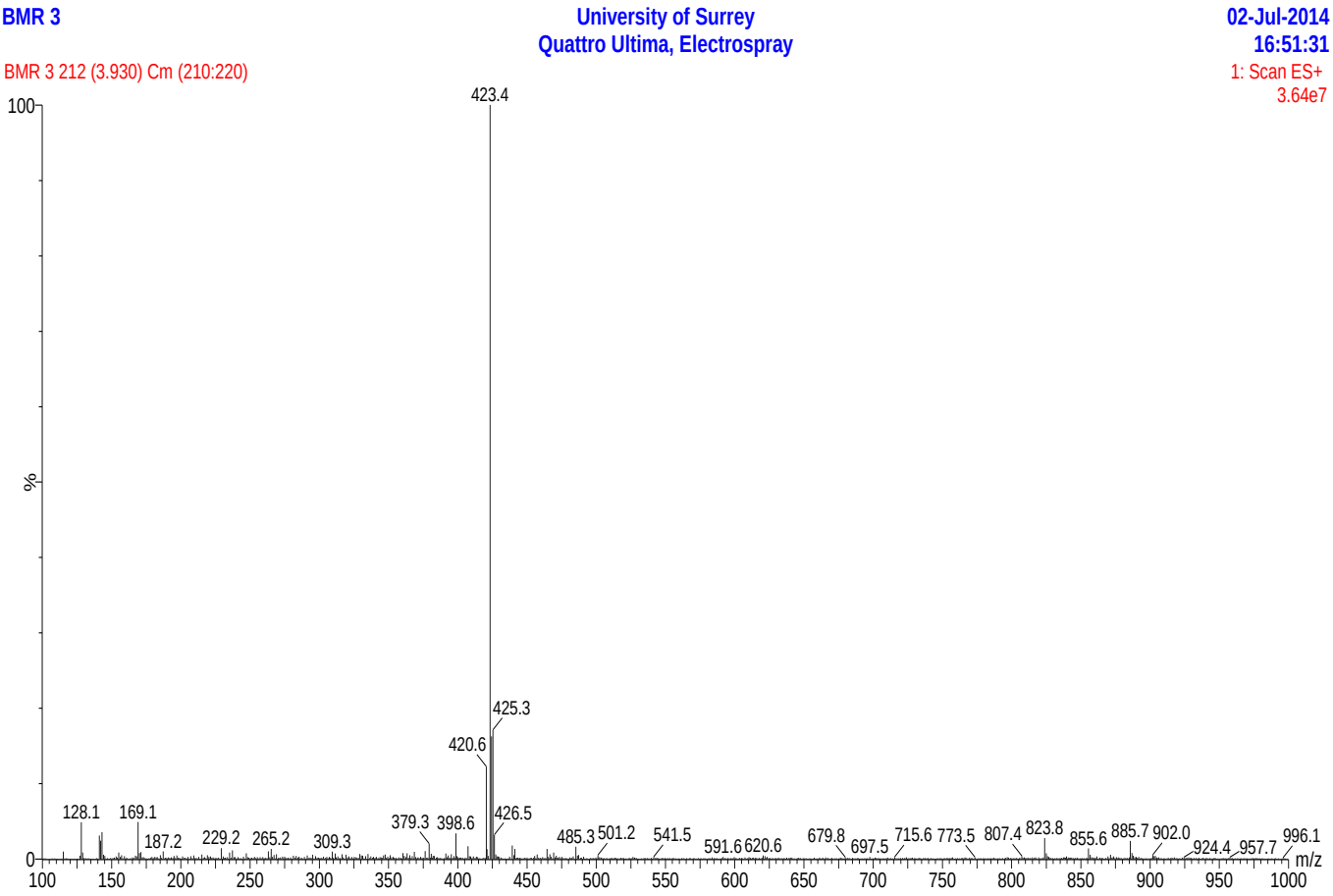


Appendix 4 c: HMBC and NOESY spectra of 2-ketocrotocorylifuran (394)

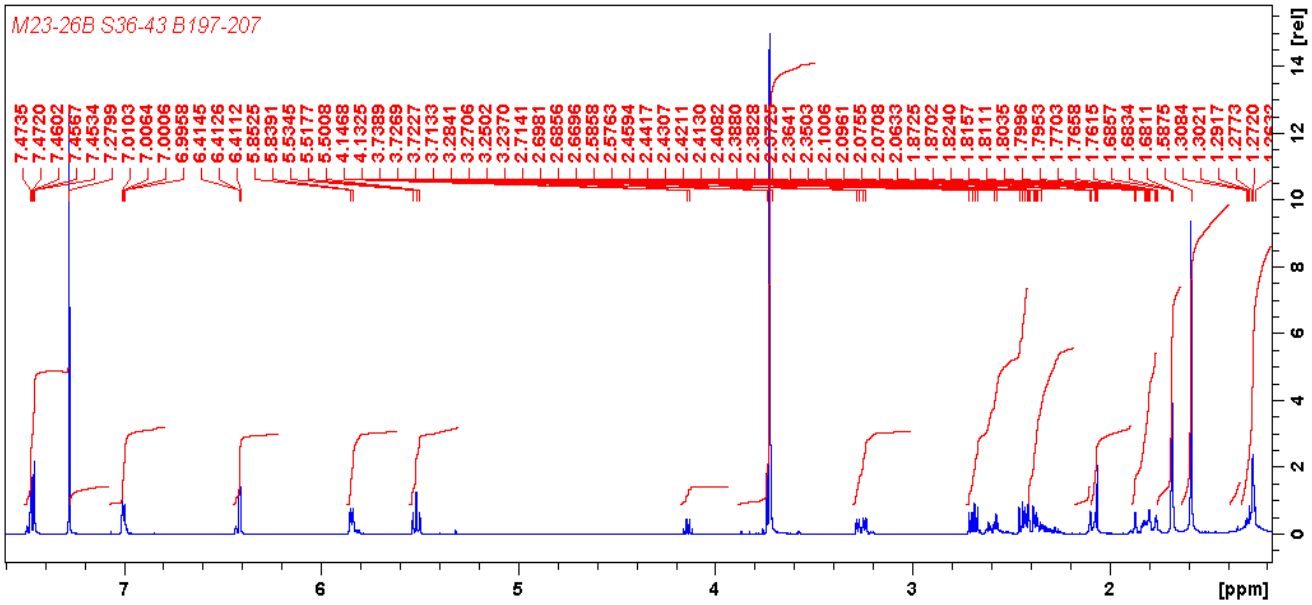


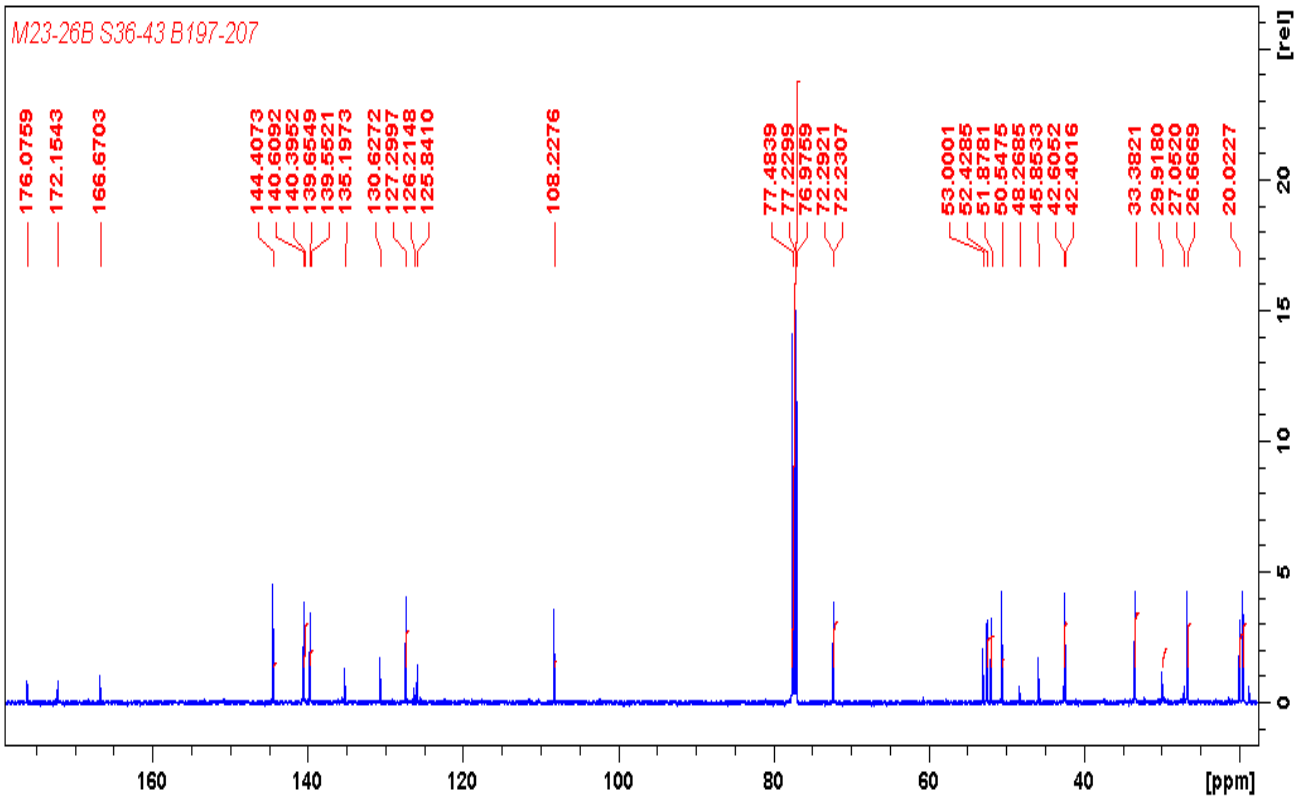


Appendix 5 a: Mass spectrum of 7, 8-dehydrocrotocorylifuran (395)

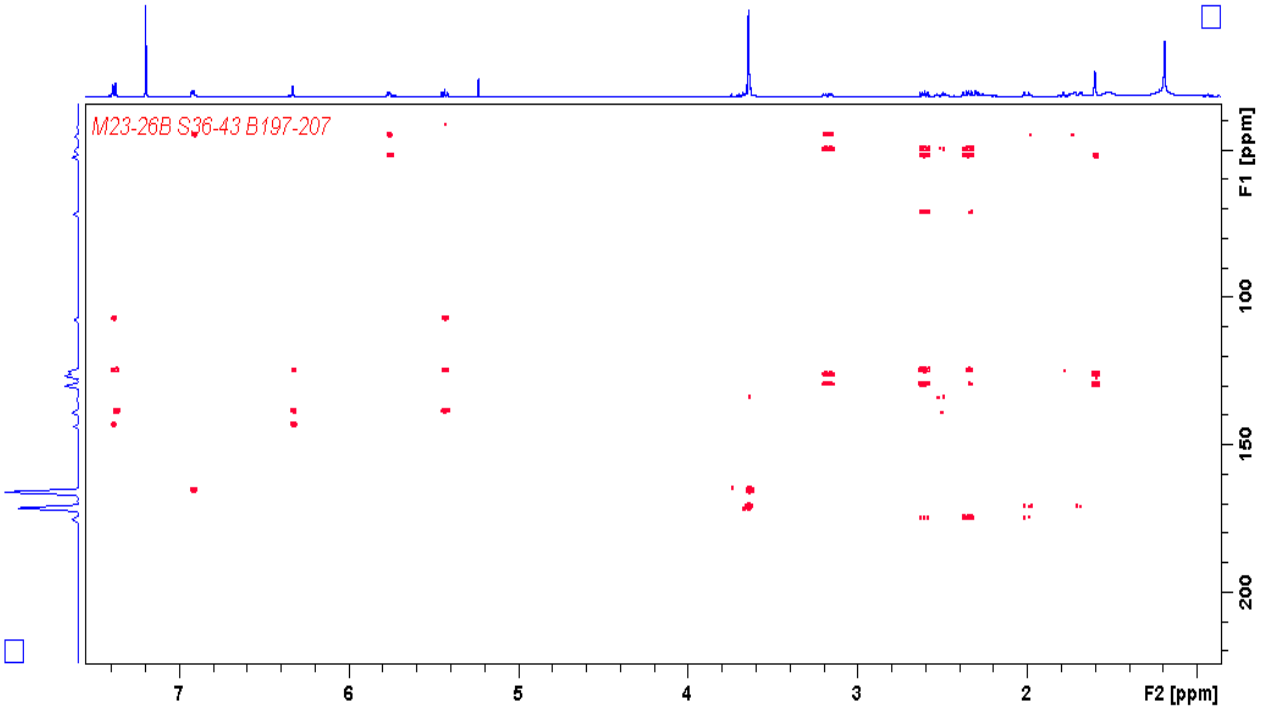


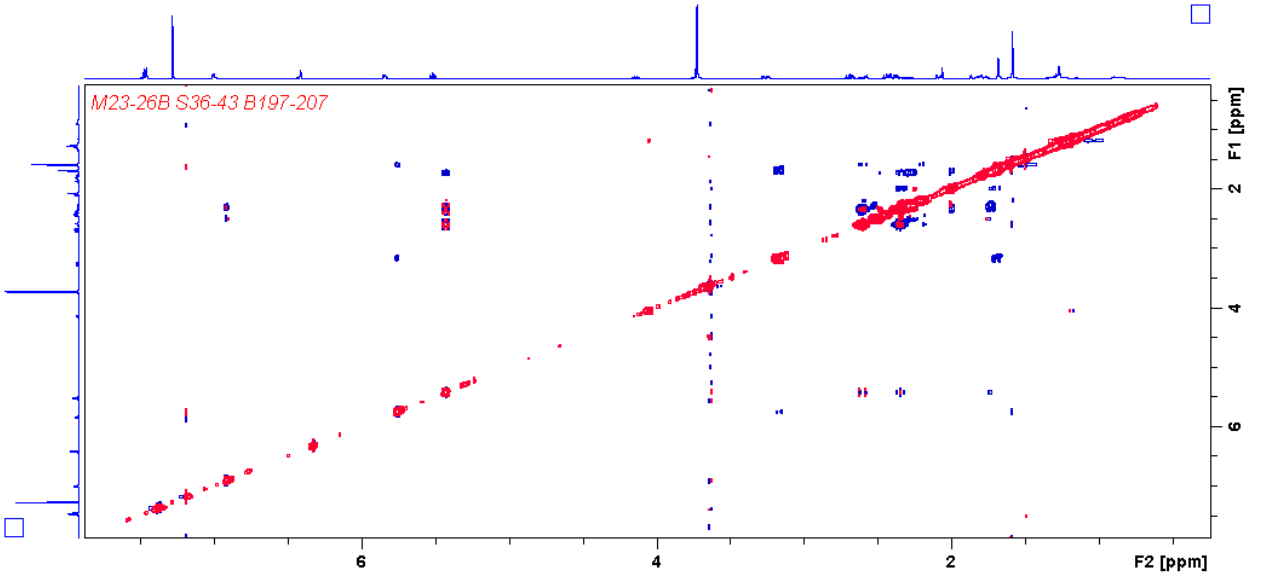
Appendix 5 b: ^{1}H NMR and ^{13}C NMR spectra of 7, 8-dehydrocrotocorylifuran (395)



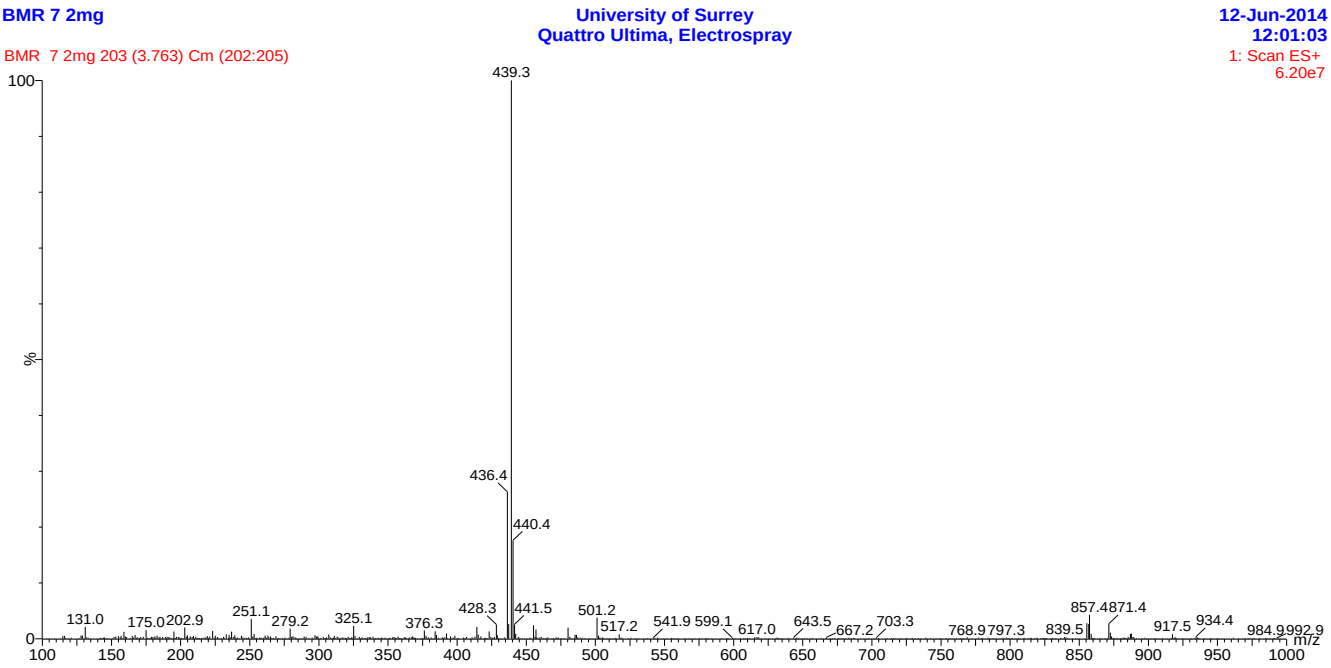


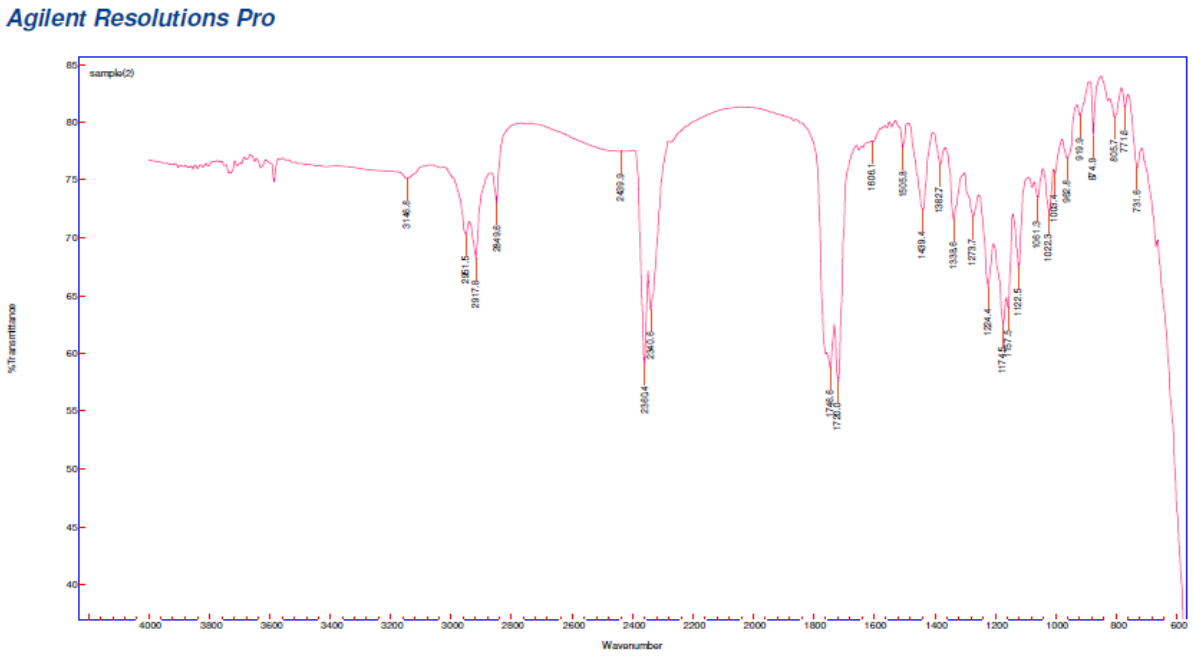
Appendix 5 c: HMBC and NOESY spectra of 7, 8-dehydrocrotocorylifuran (395)



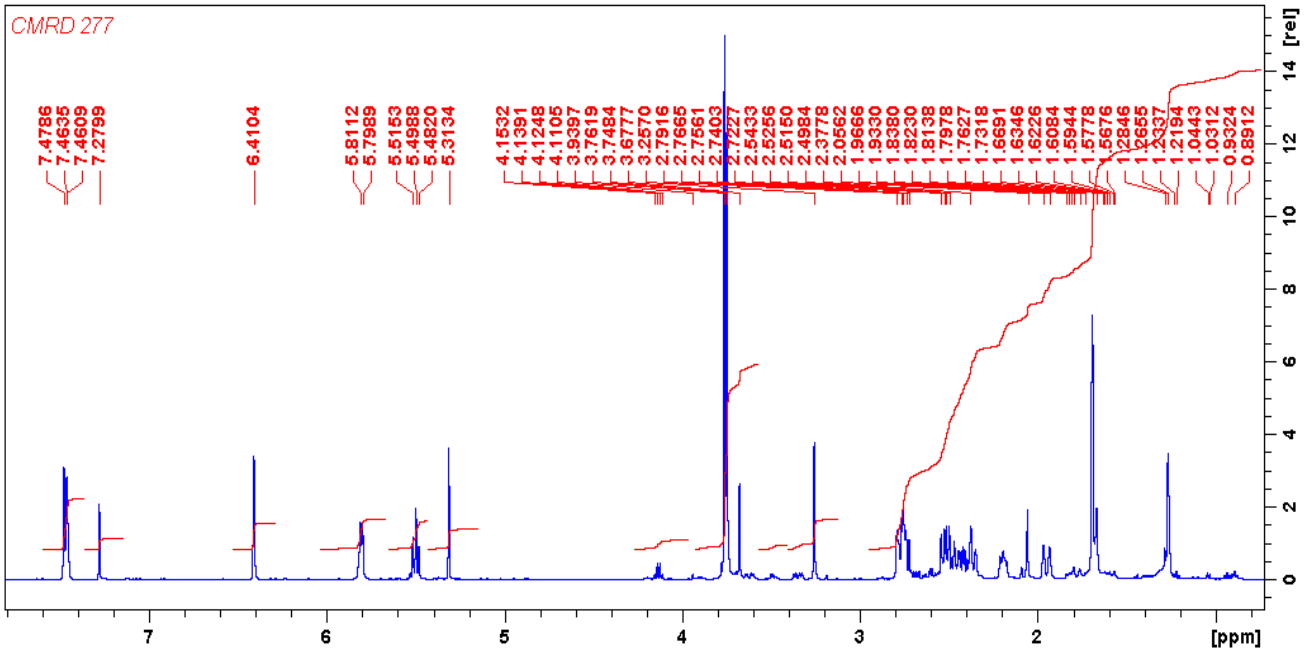


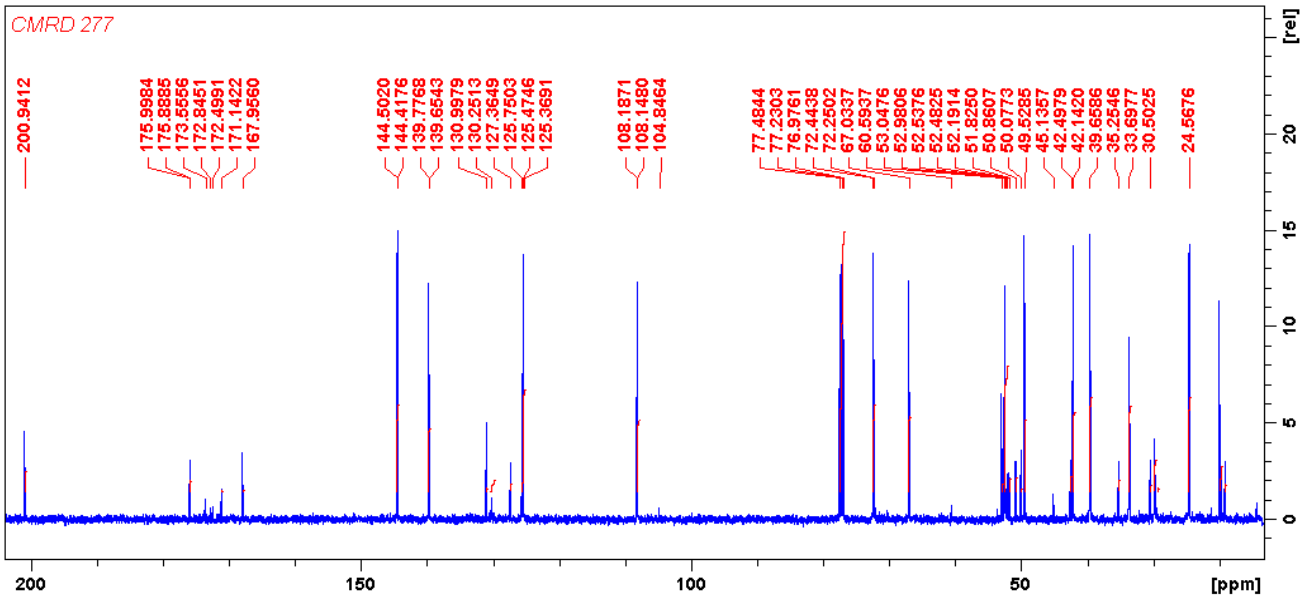
Appendix 6 a: Mass and FTIR spectra of megalocarpoidolide F (396)



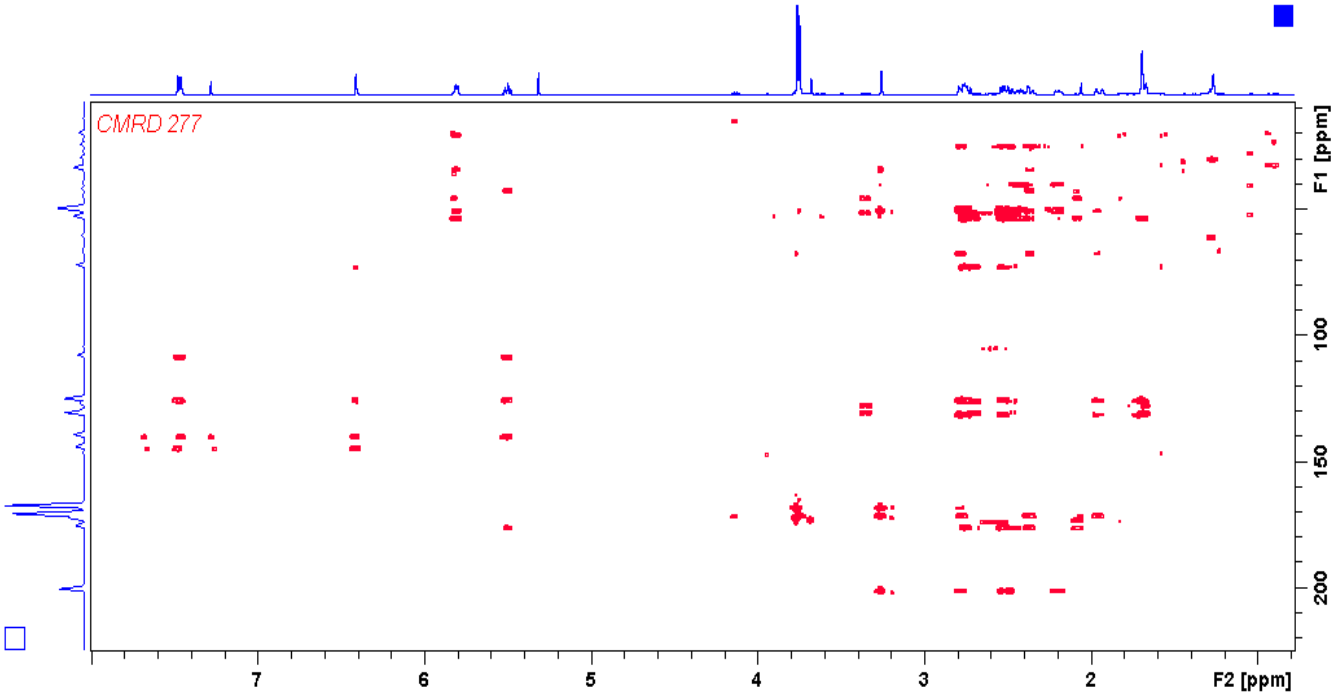


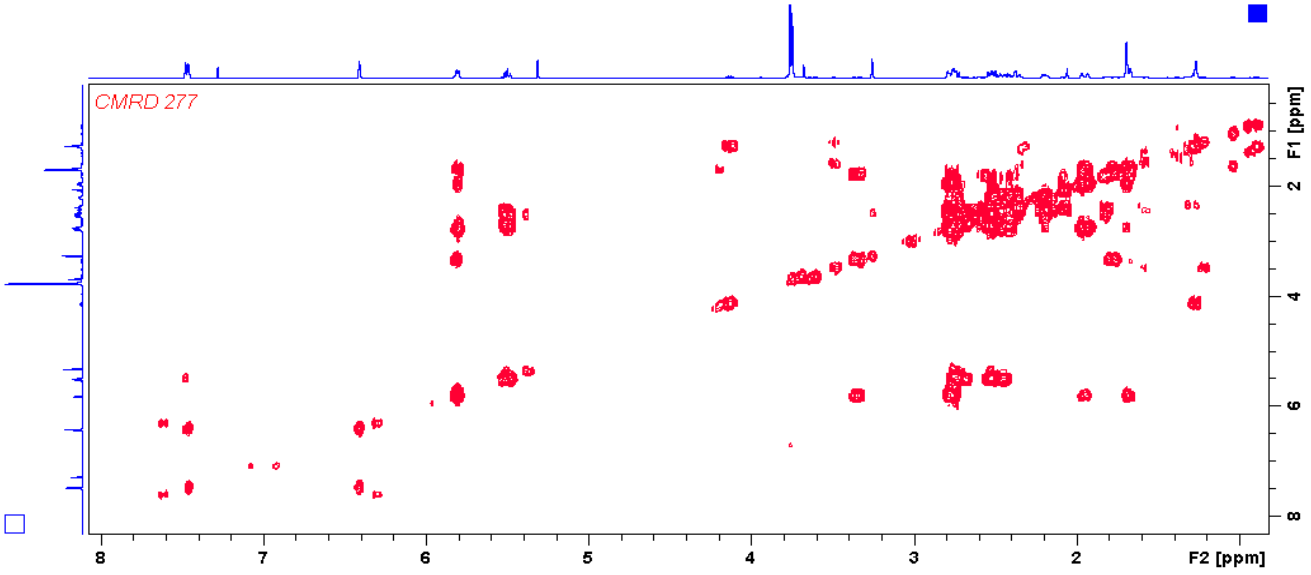
Appendix 6 b: ^{1}H and ^{13}C NMR spectra megalocarpoidolide F (396)



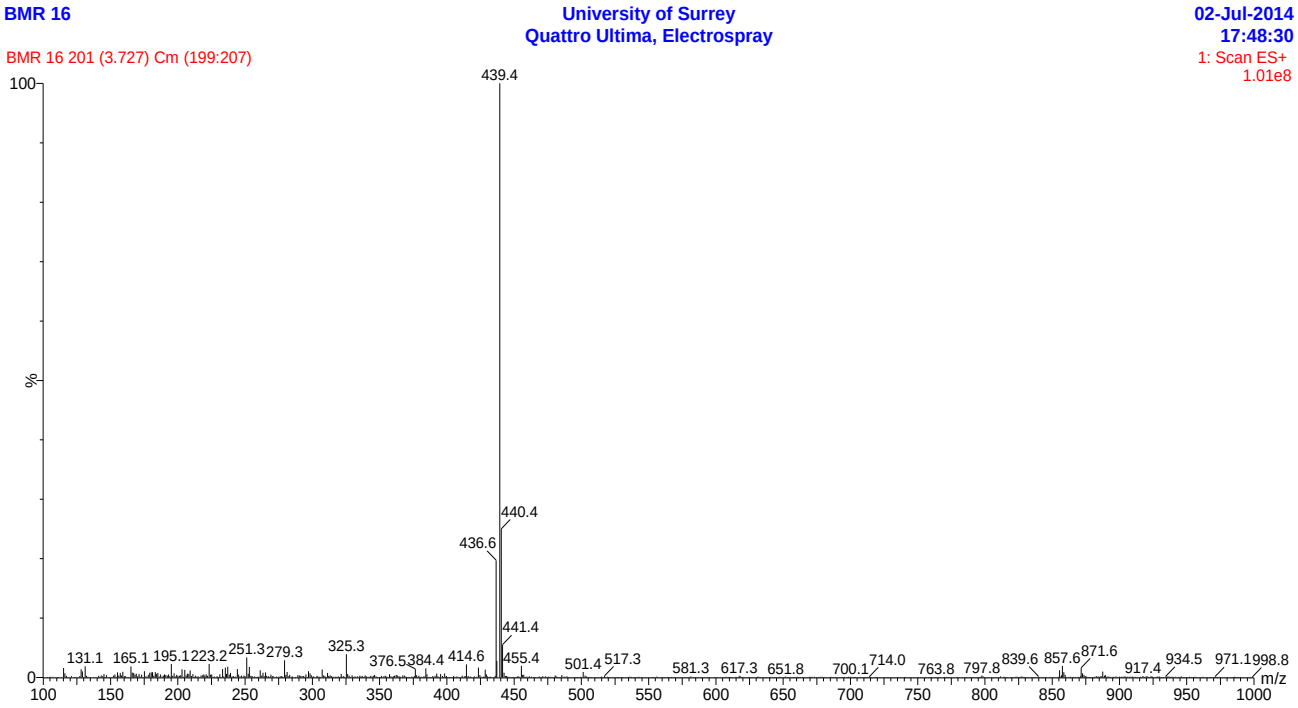


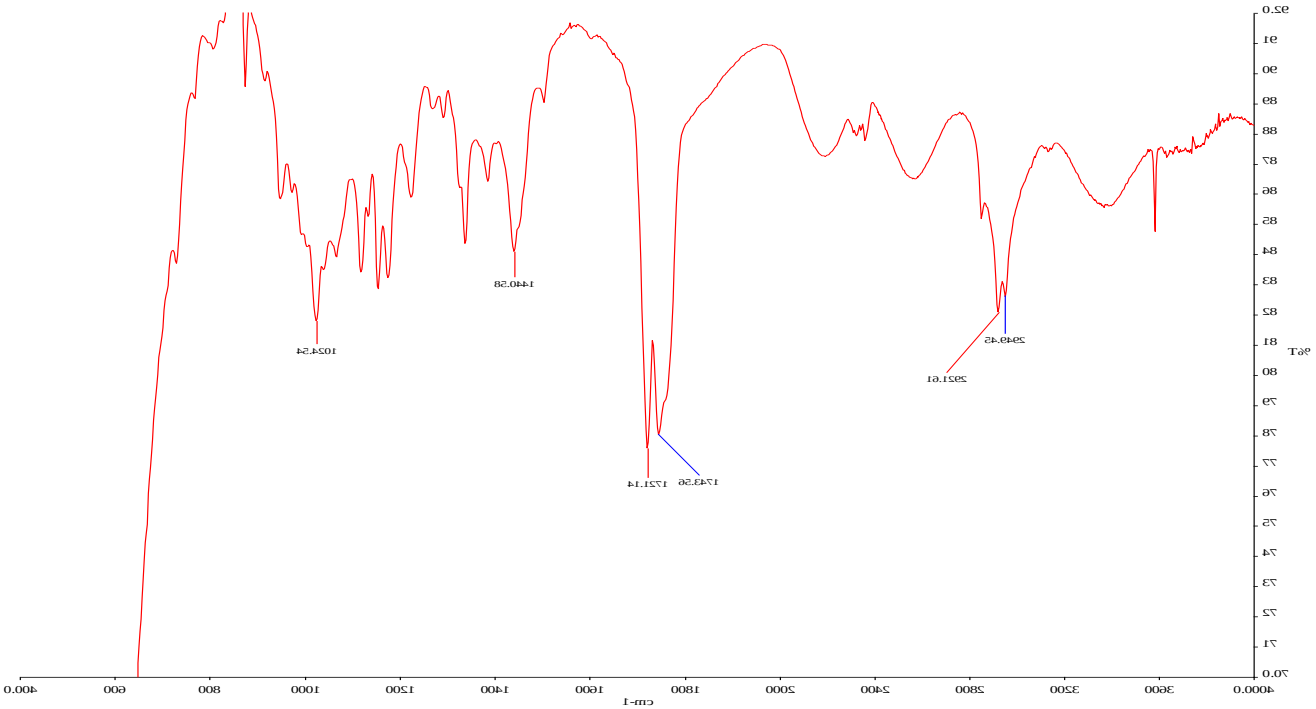
Appendix 6 c: HMBC and COSY spectra for megalocarpoidolide F (396)



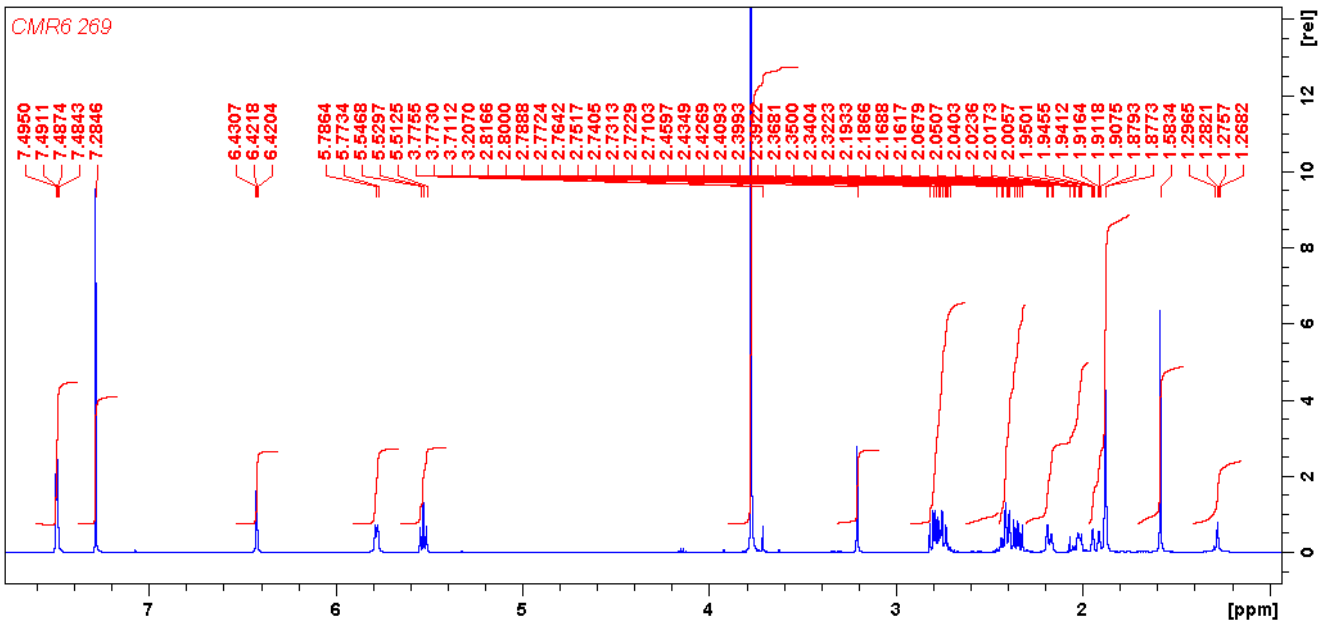


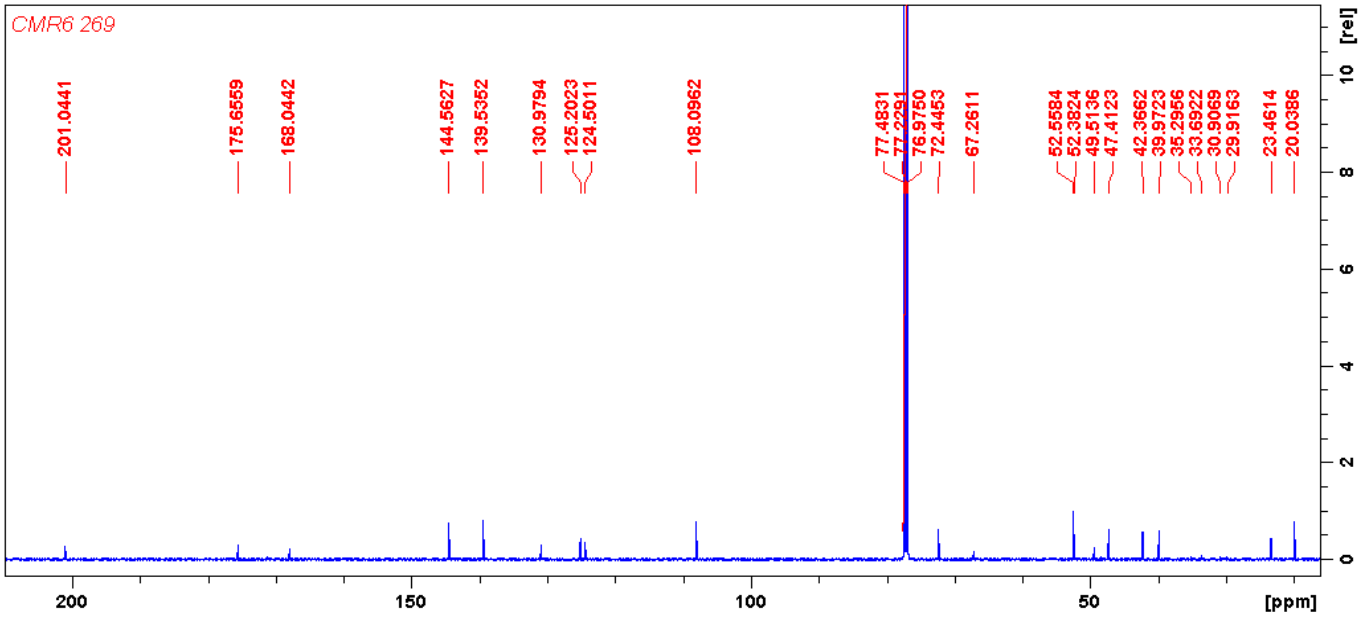
Appendix 7 a: Mass and FTIR spectra of 12-*Epi*-megalocarpoidolide F (397)



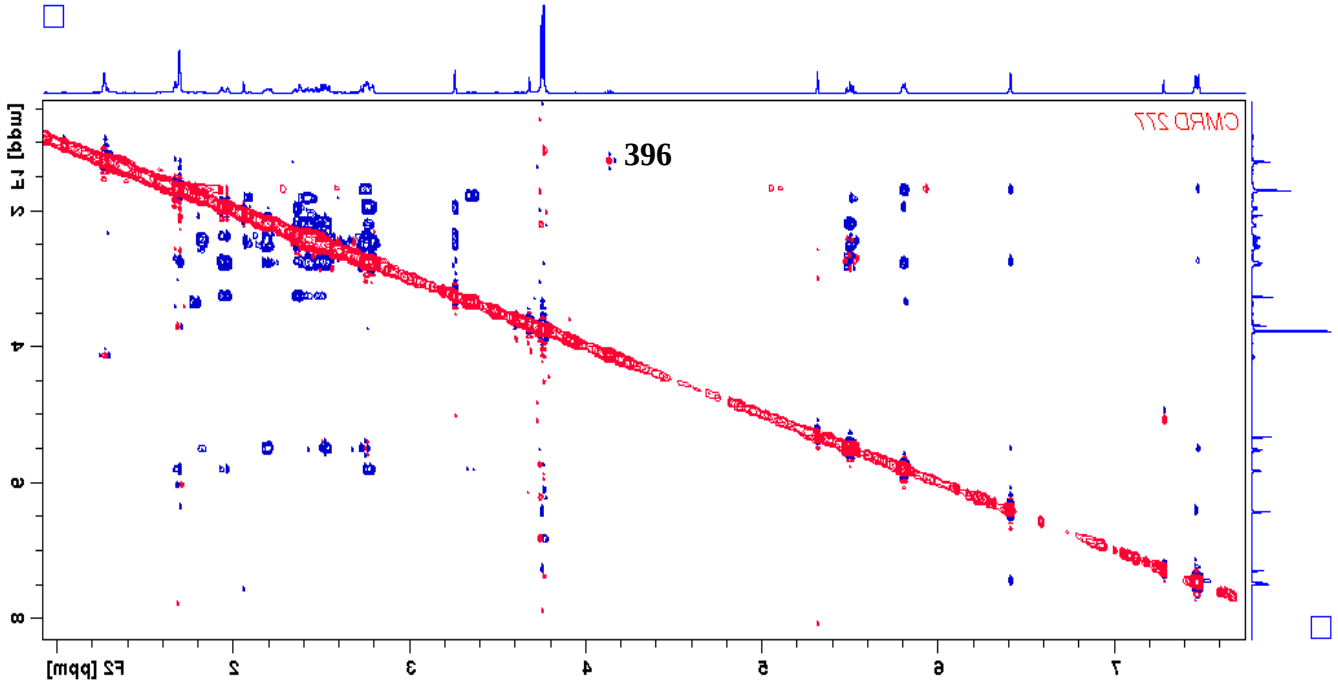


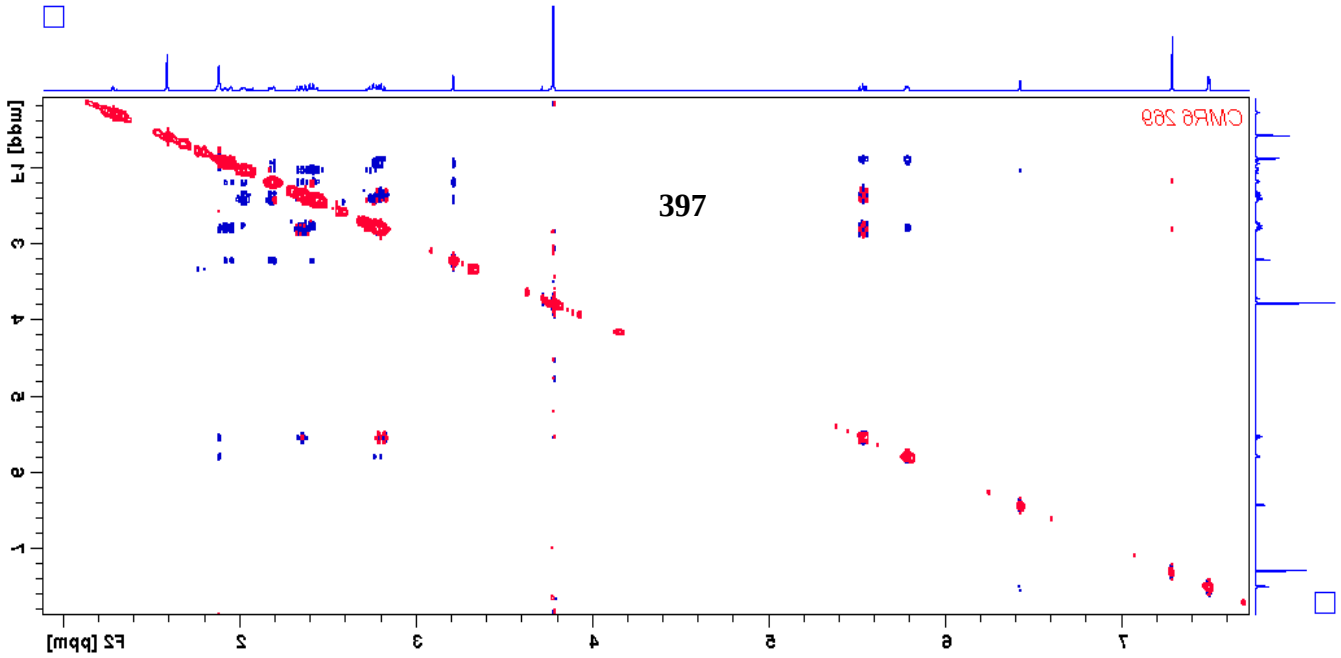
Appendix 7 b: ^{1}H NMR and ^{13}C NMR spectra 12-*Epi*-megalocarpoidolide F (397)



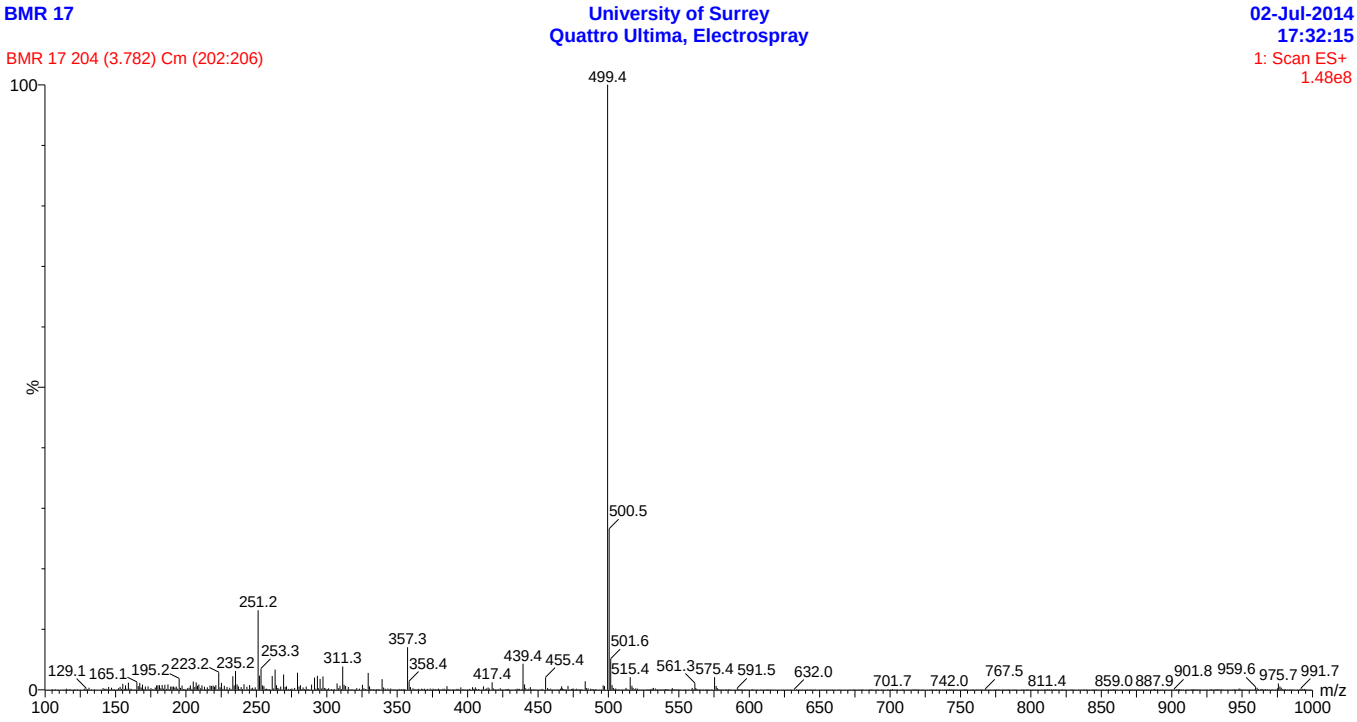


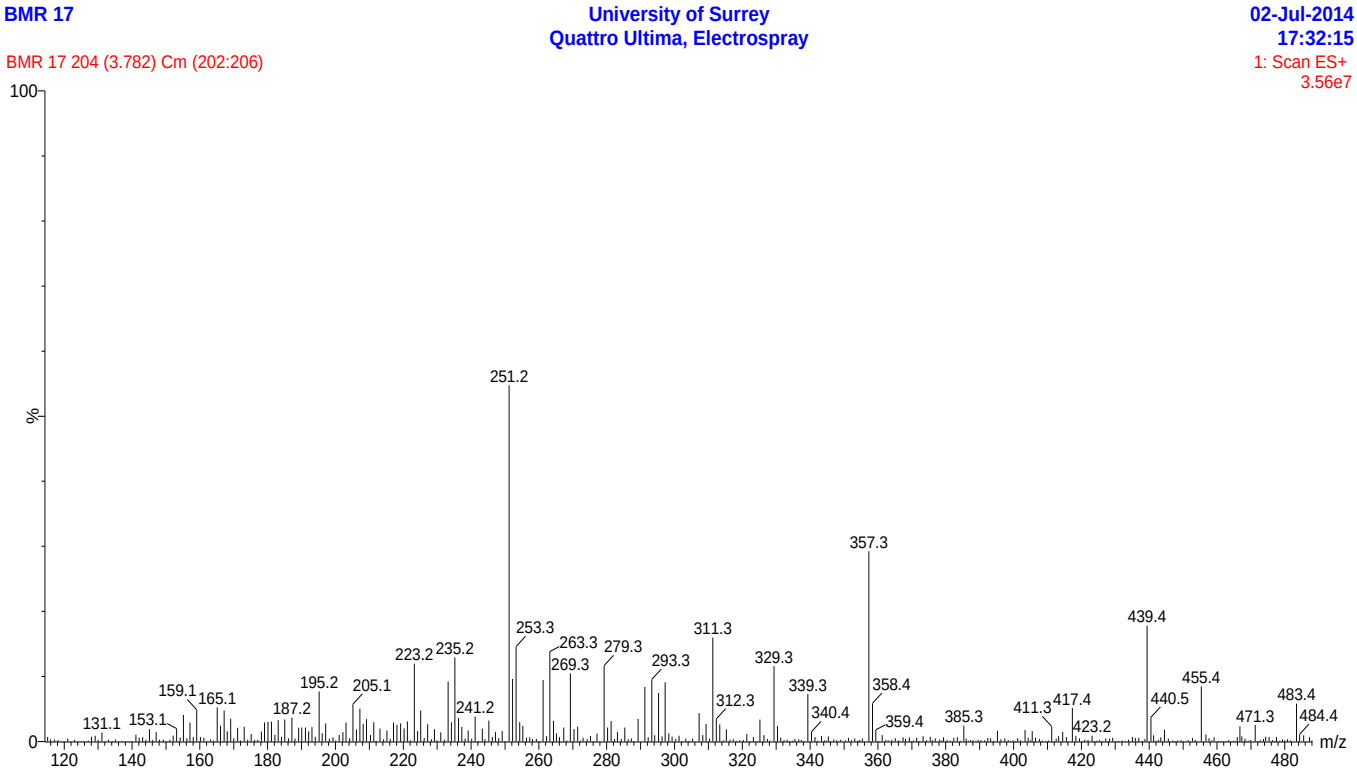
Appendix 7 c: NOESY spectra of megalocarpoidolide F (396) its C-12 epimer (397)



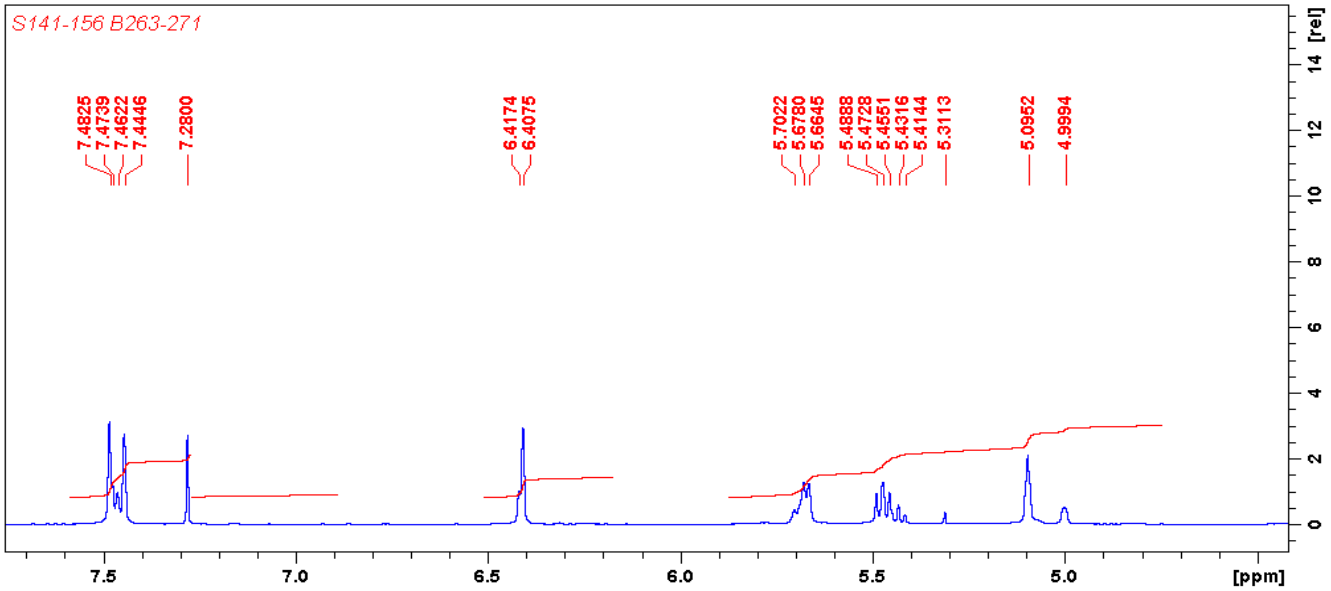


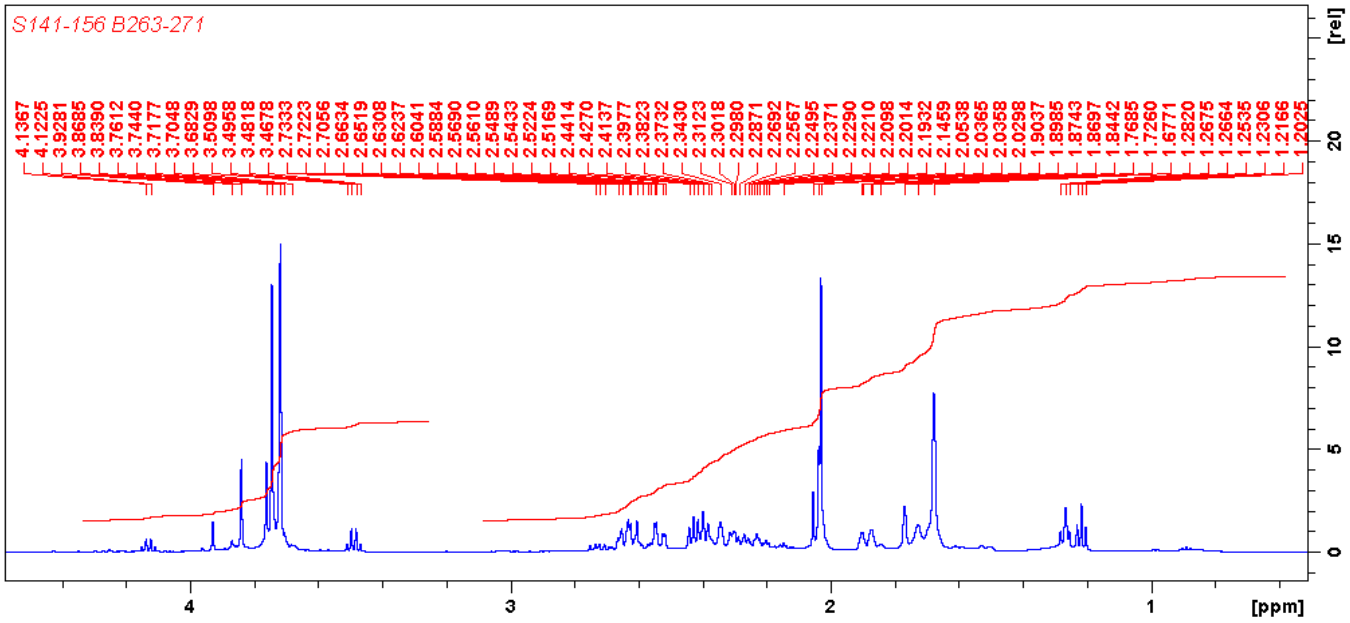
Appendix 8 a: Mass spectrum of megalocarpoidolide E (398)



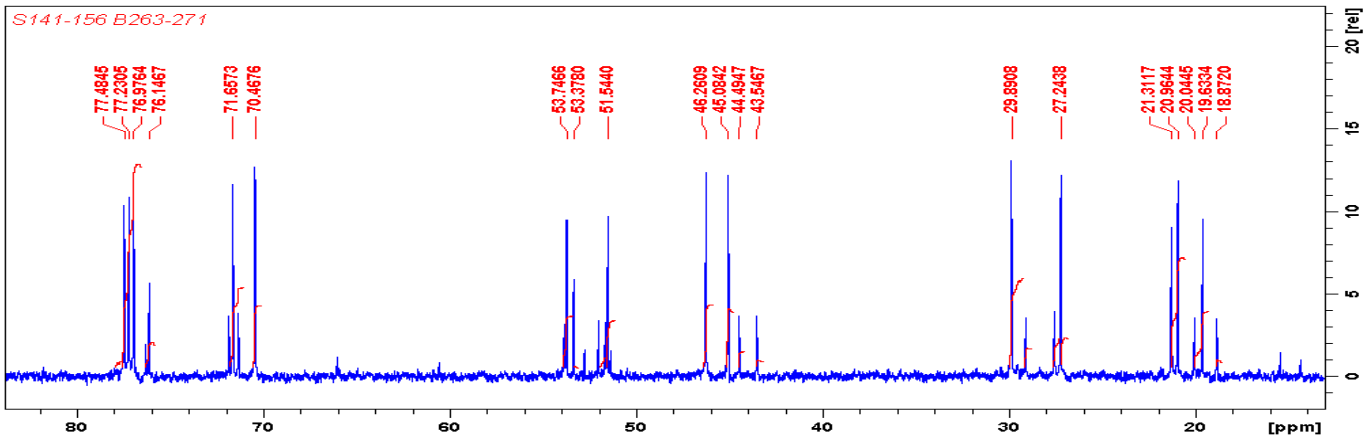


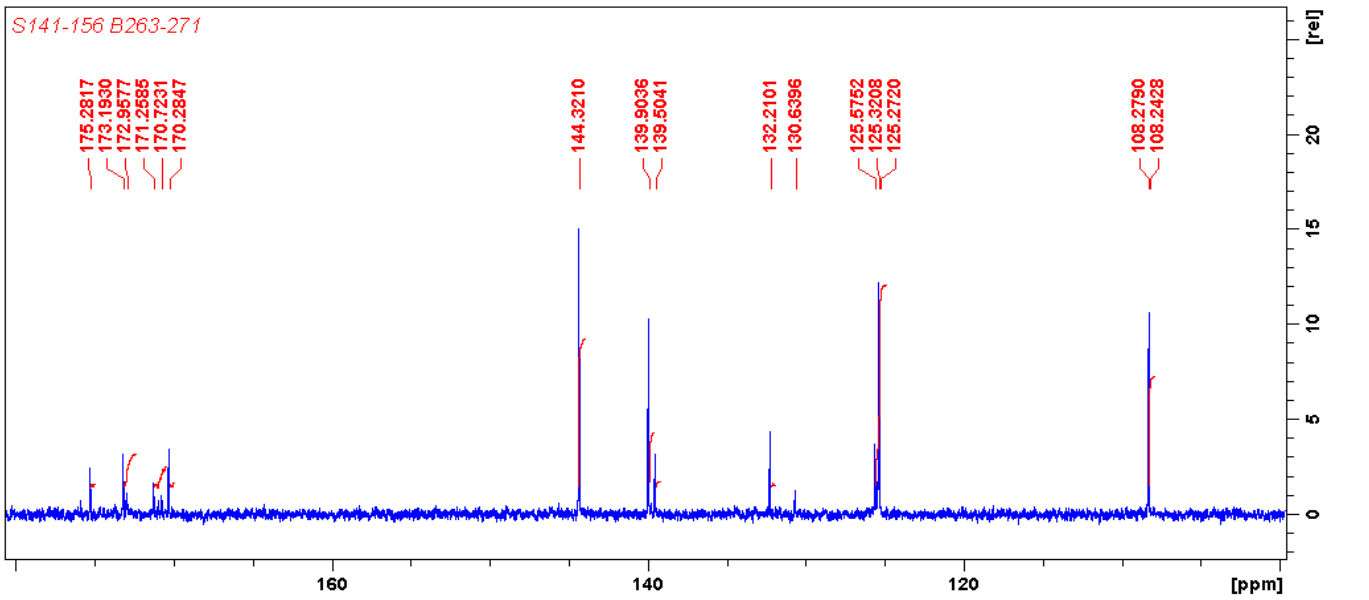
Appendix 8 b: ^{1}H NMR spectrum of megalocarpoidolide E (398)



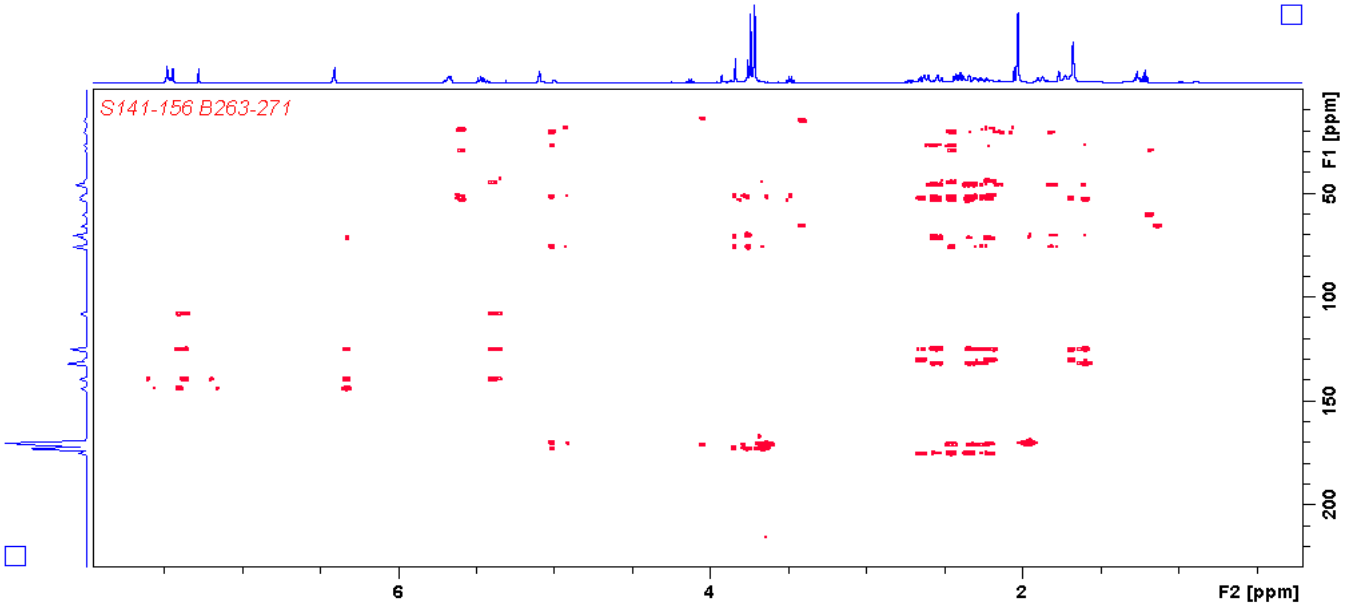


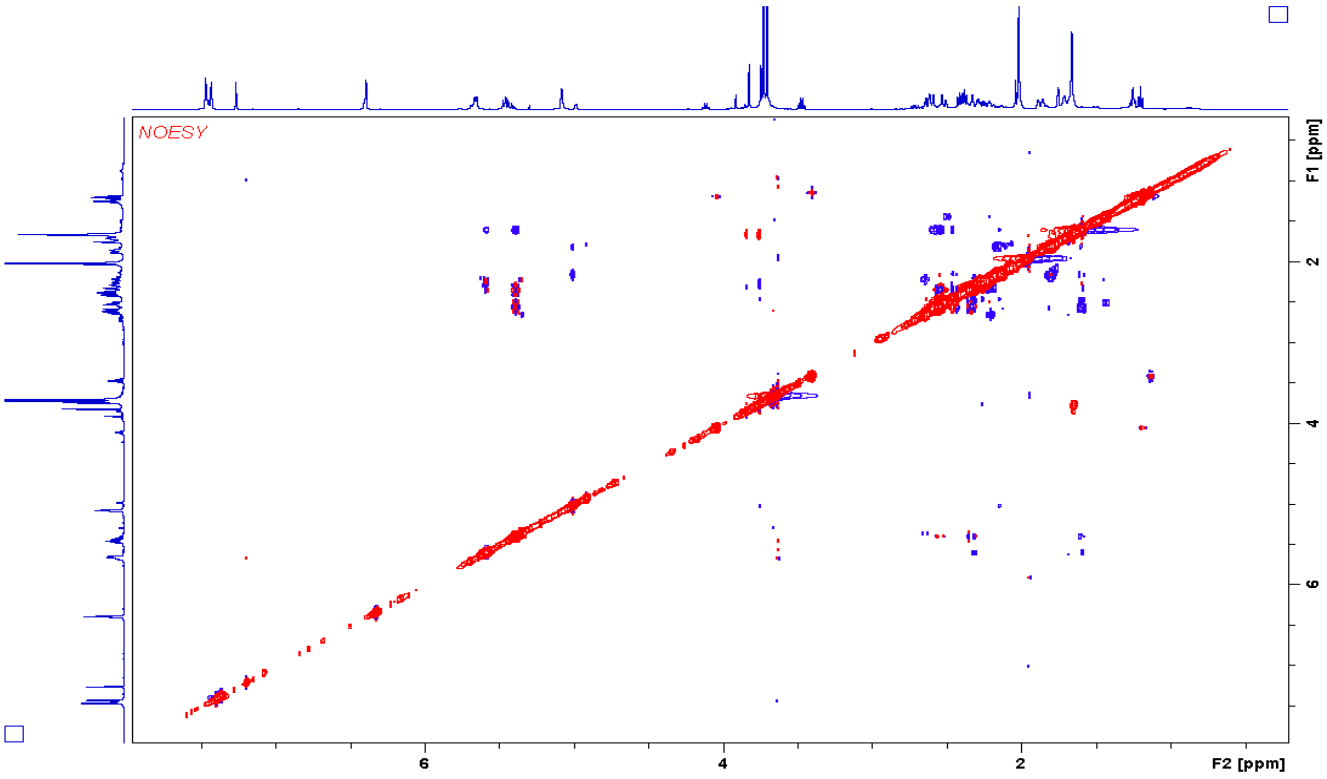
Appendix 8 c: ^{13}C NMR spectrum of megalocarpoidolide E (398)



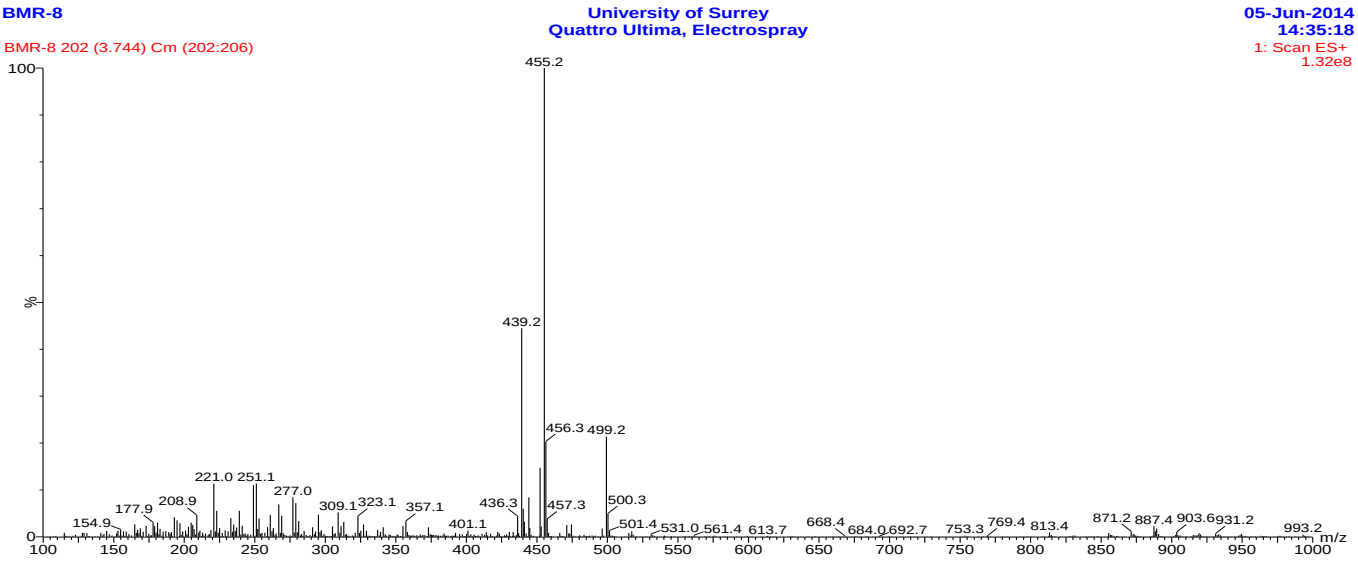


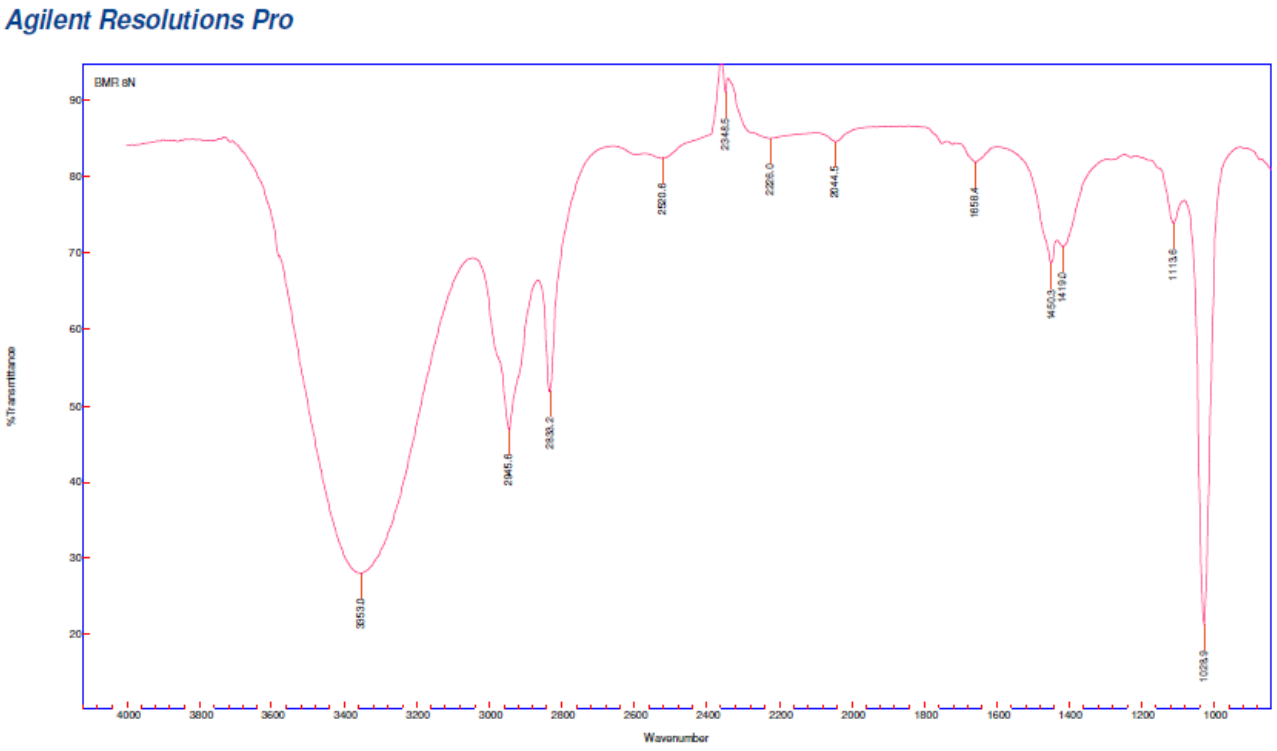
Appendix 8 d: HMBC and NOESY spectra of megalocarpoidolide E (398)



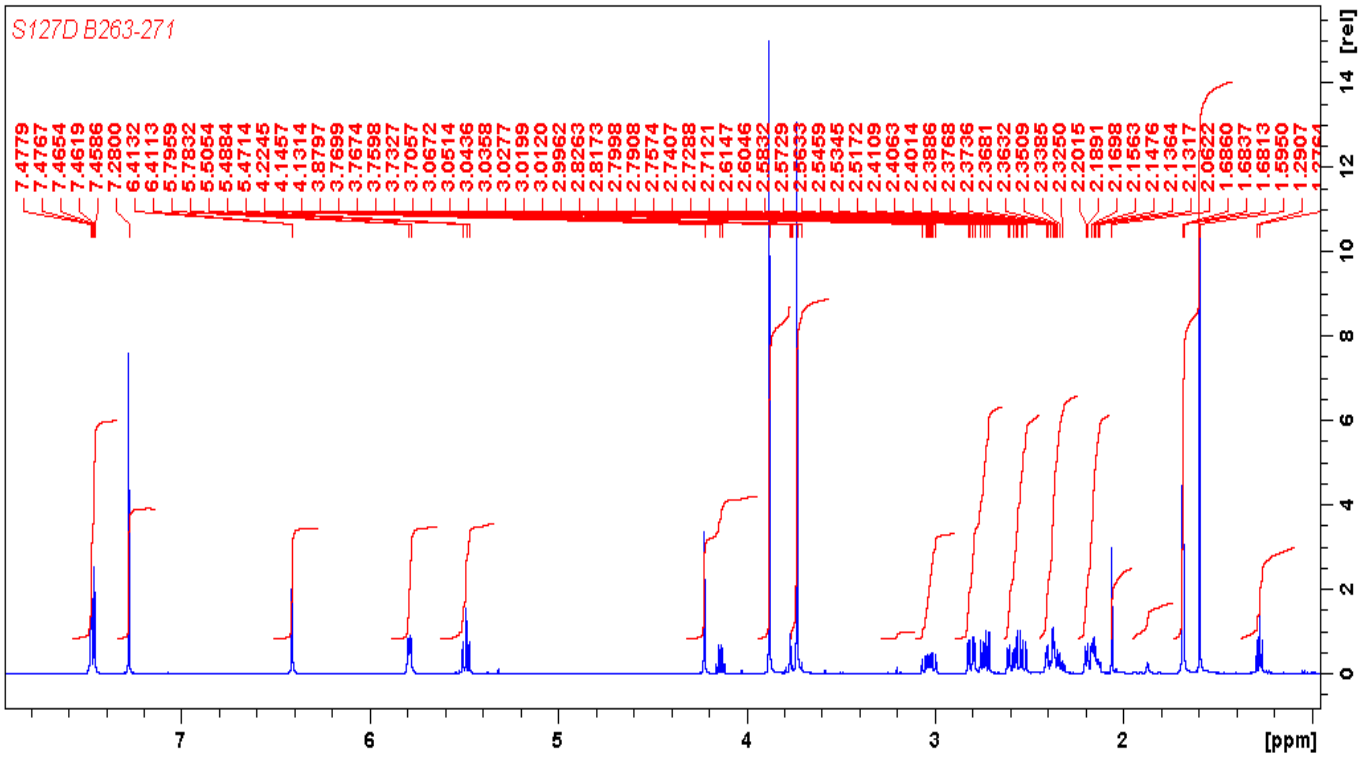


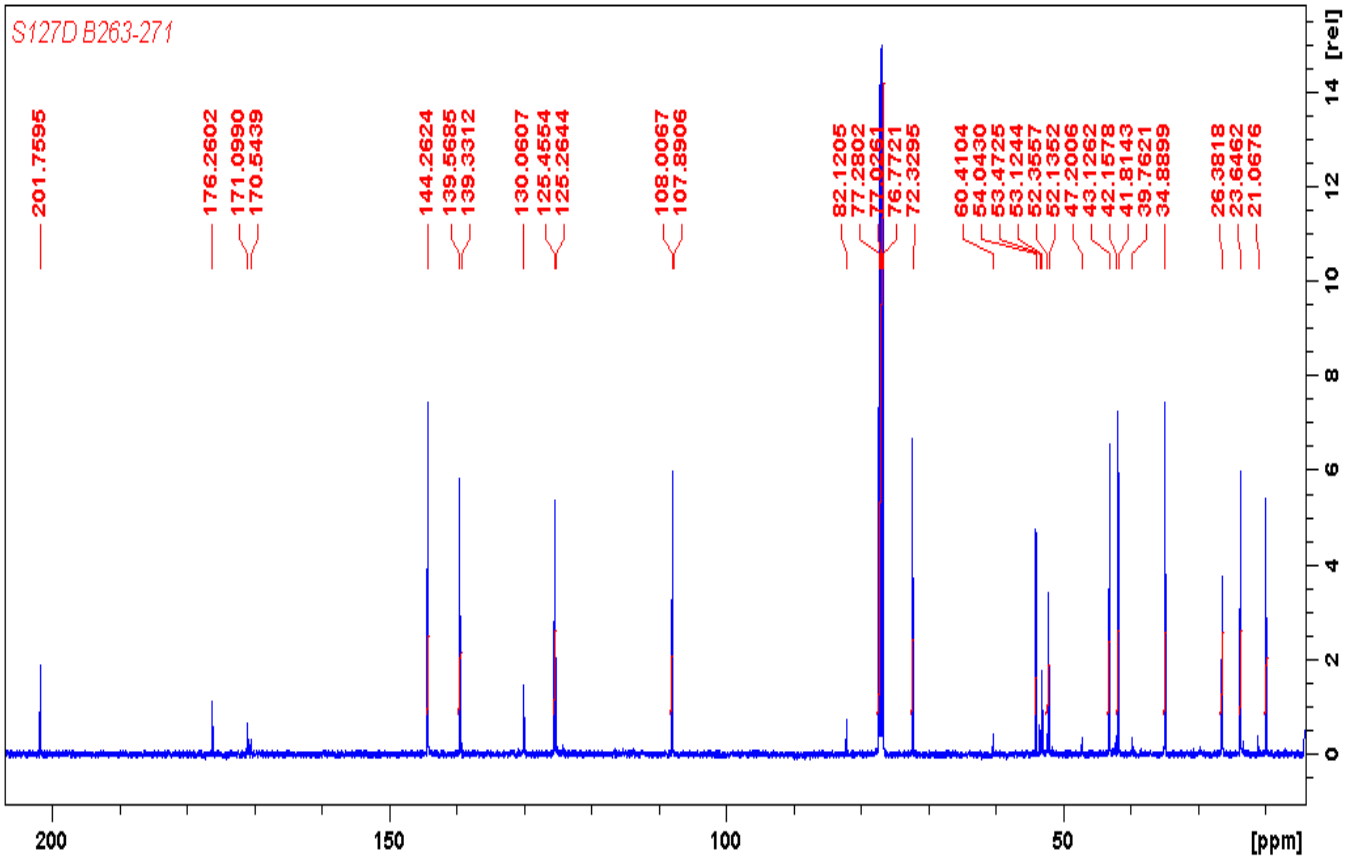
Appendix 9 a: Mass spectrum and FTIR spectra of megalocarpoidolide G (399)



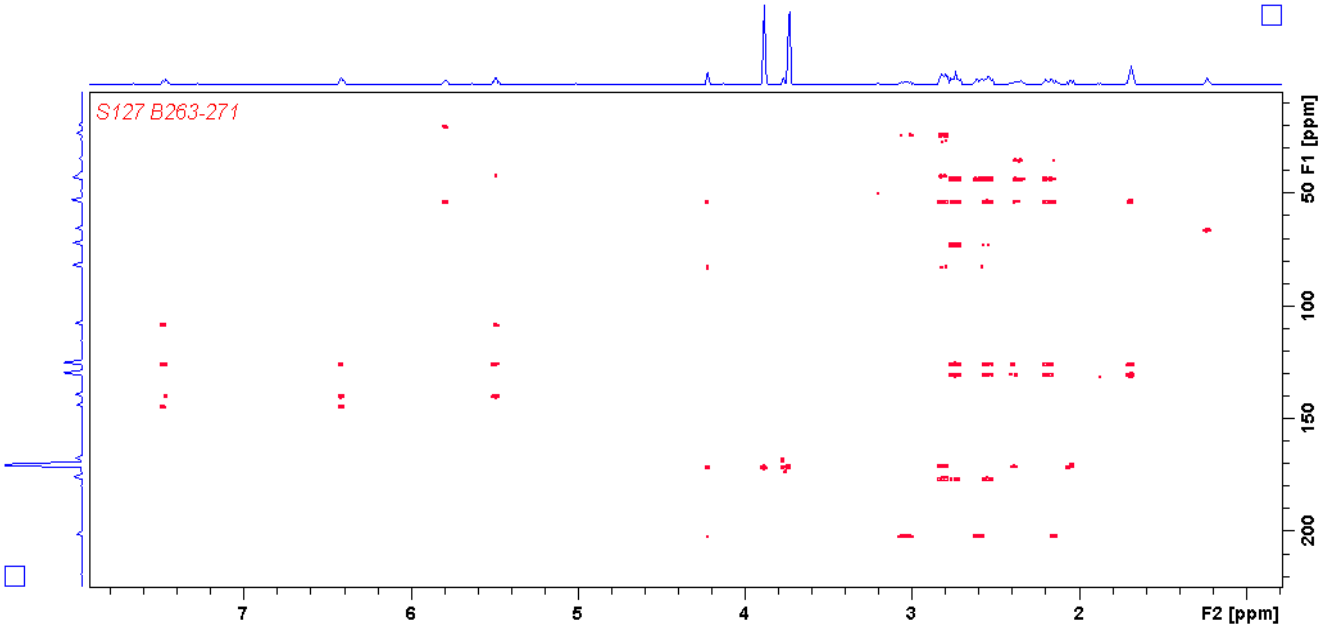


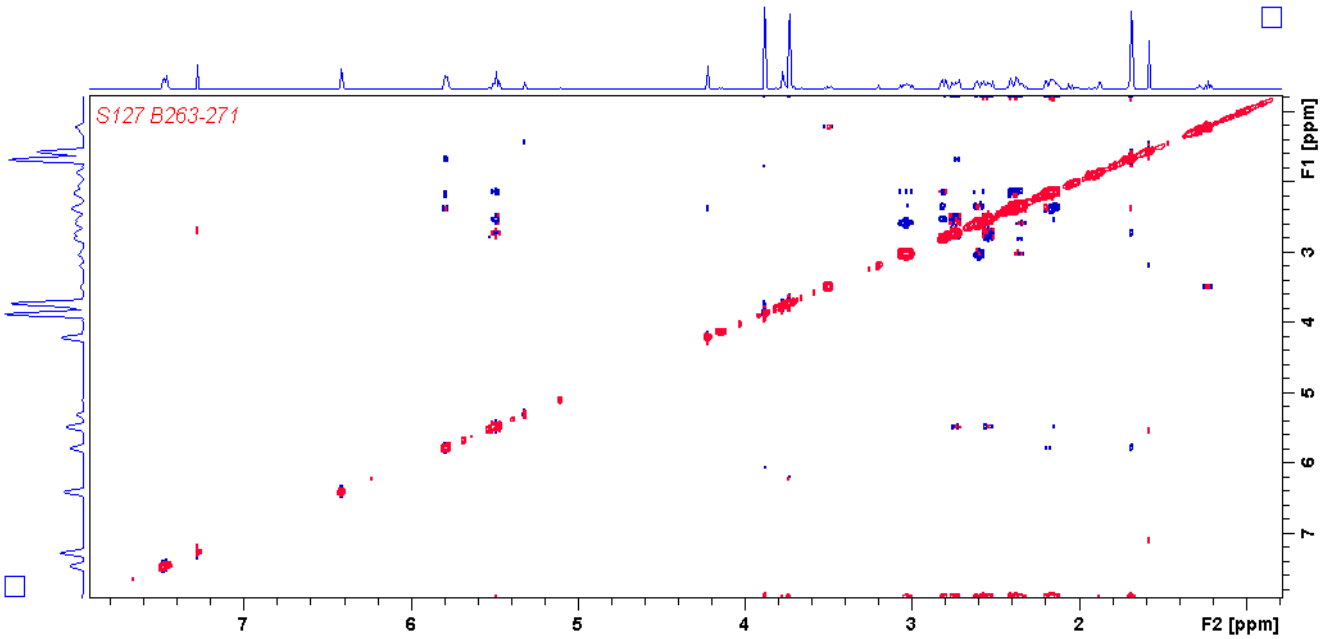
Appendix 9 b: ^{1}H NMR and ^{13}C NMR spectra of megalocarpoidolide G (399)



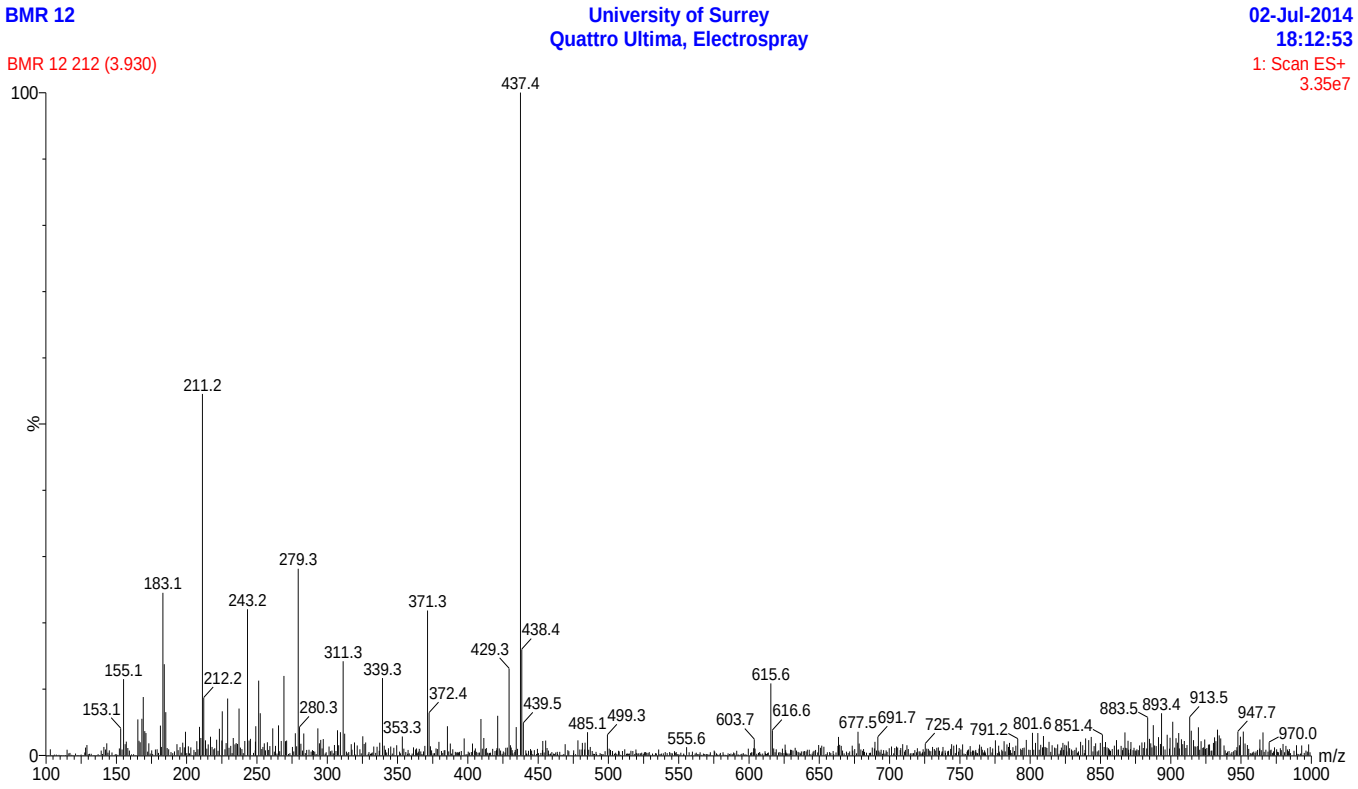


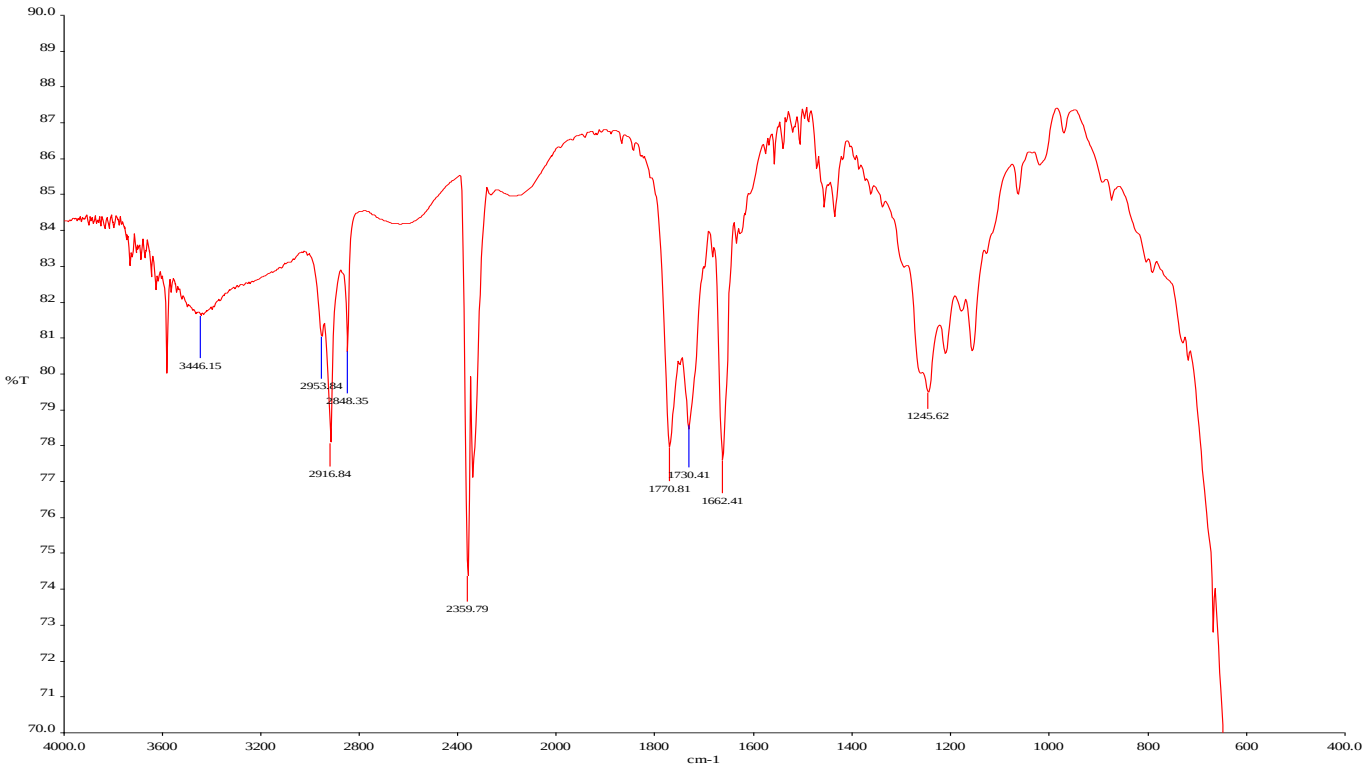
Appendix 9 c: HMBC and NOESY spectra of megalocarpoidolide G (399)



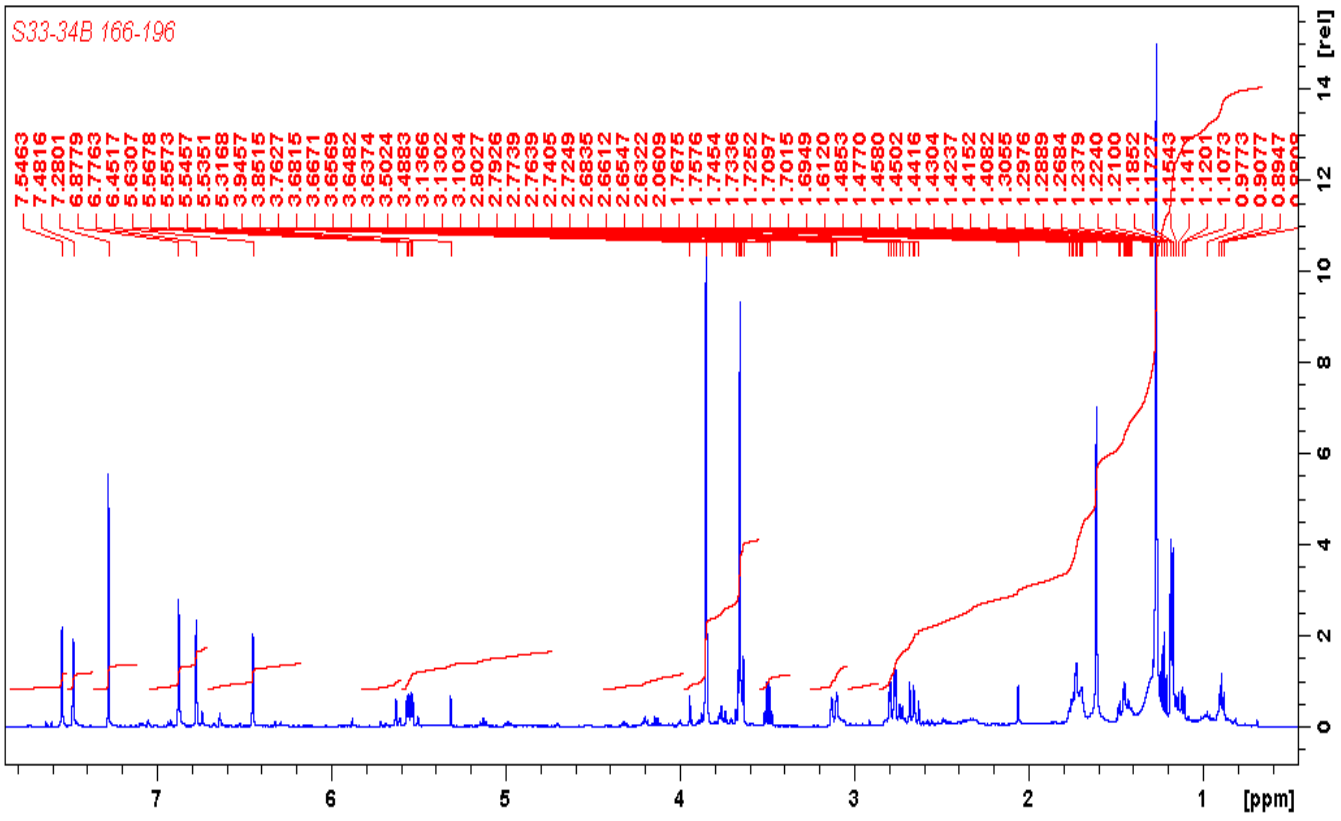


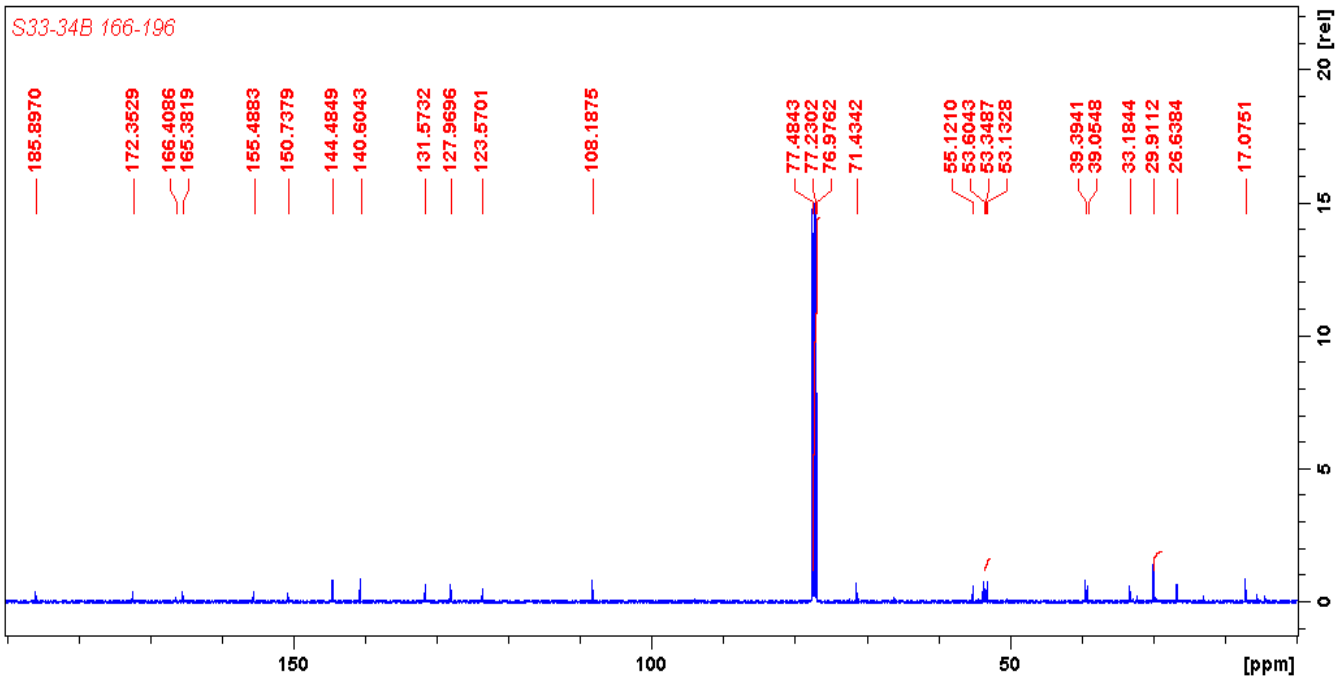
Appendix 10 a: Mass and FTIR spectra of megalocarpoidolide H (400)



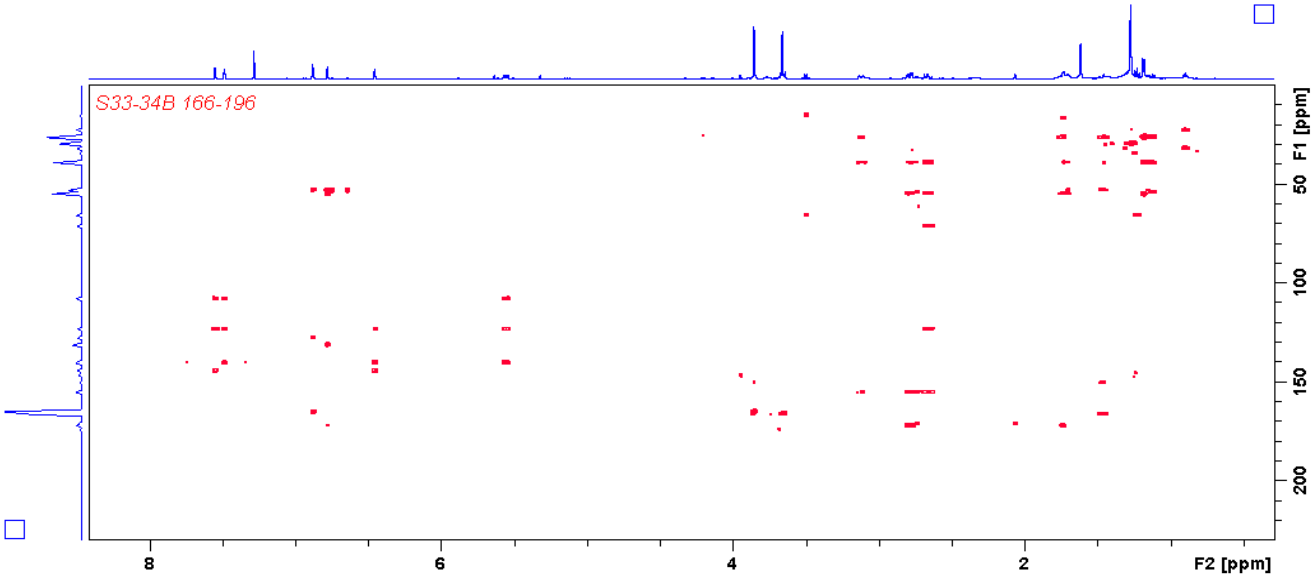


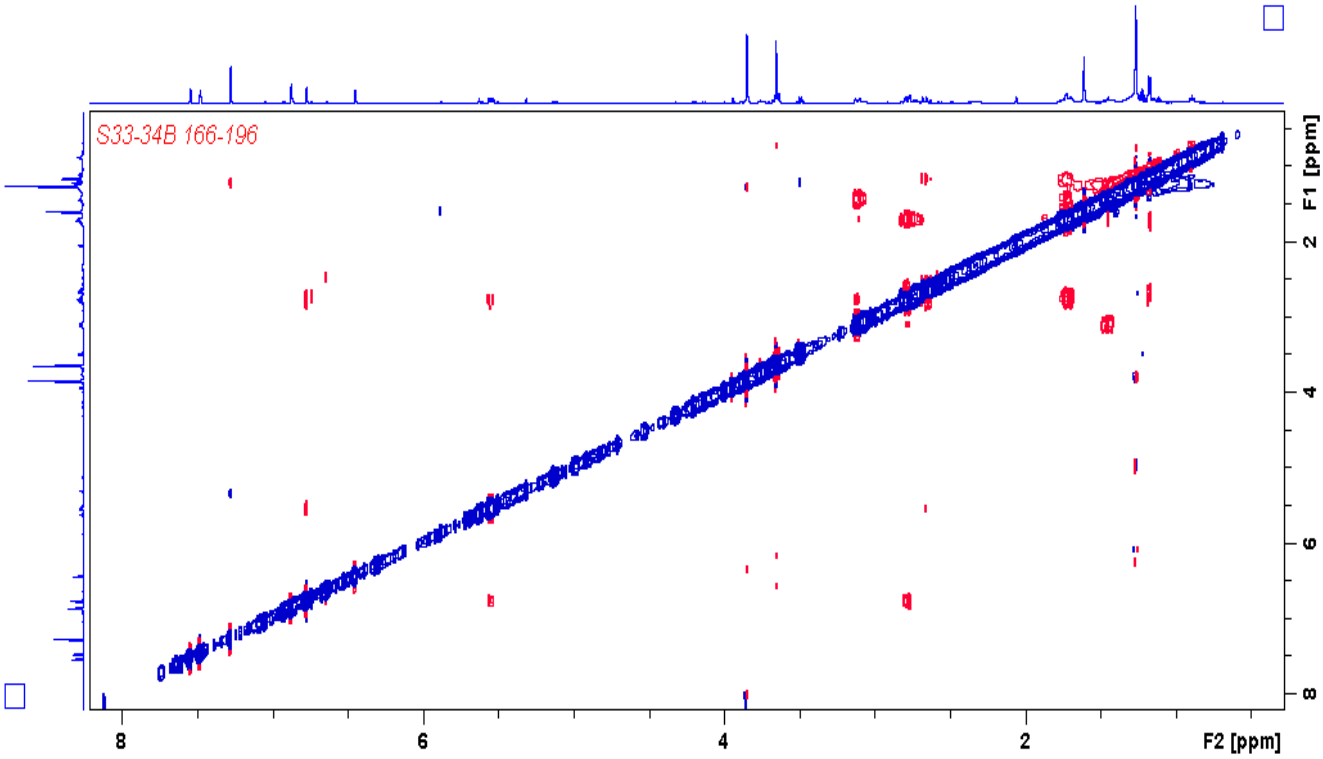
Appendix 10 b: ^{1}H NMR and ^{13}C NMR spectra of megalocarpoidolide H (400)



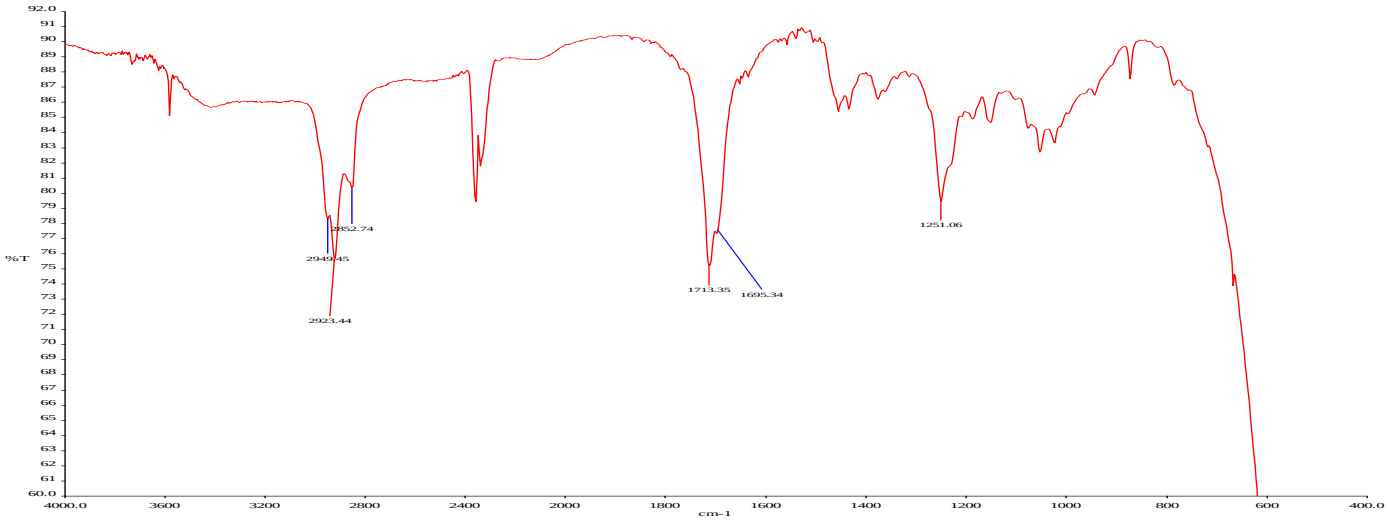


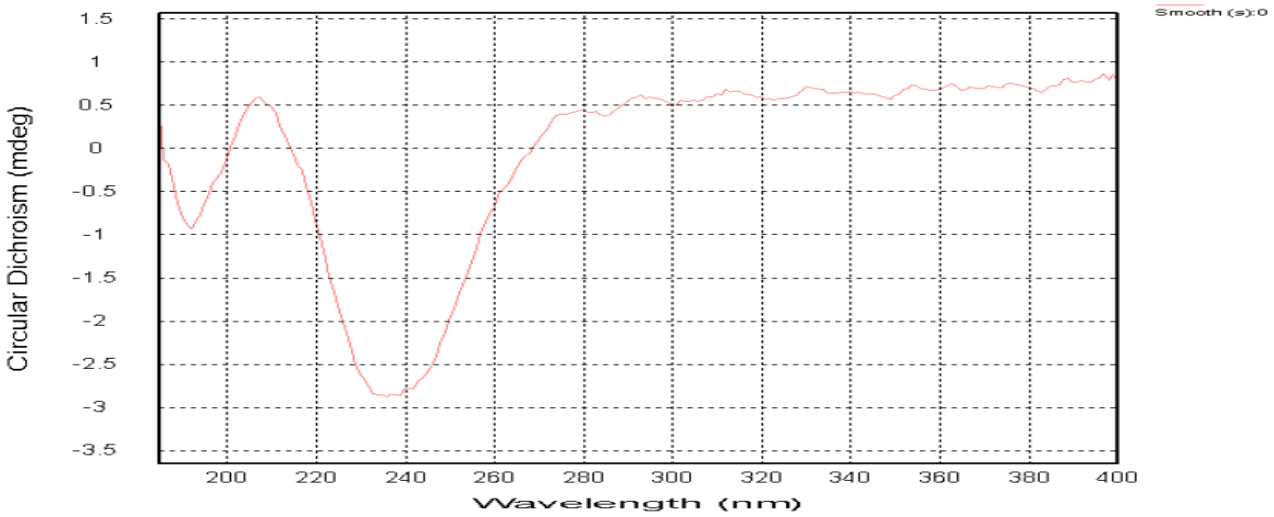
Appendix 10 c: HMBC and NOESY Spectra of Megalocarpoidolide H (400)



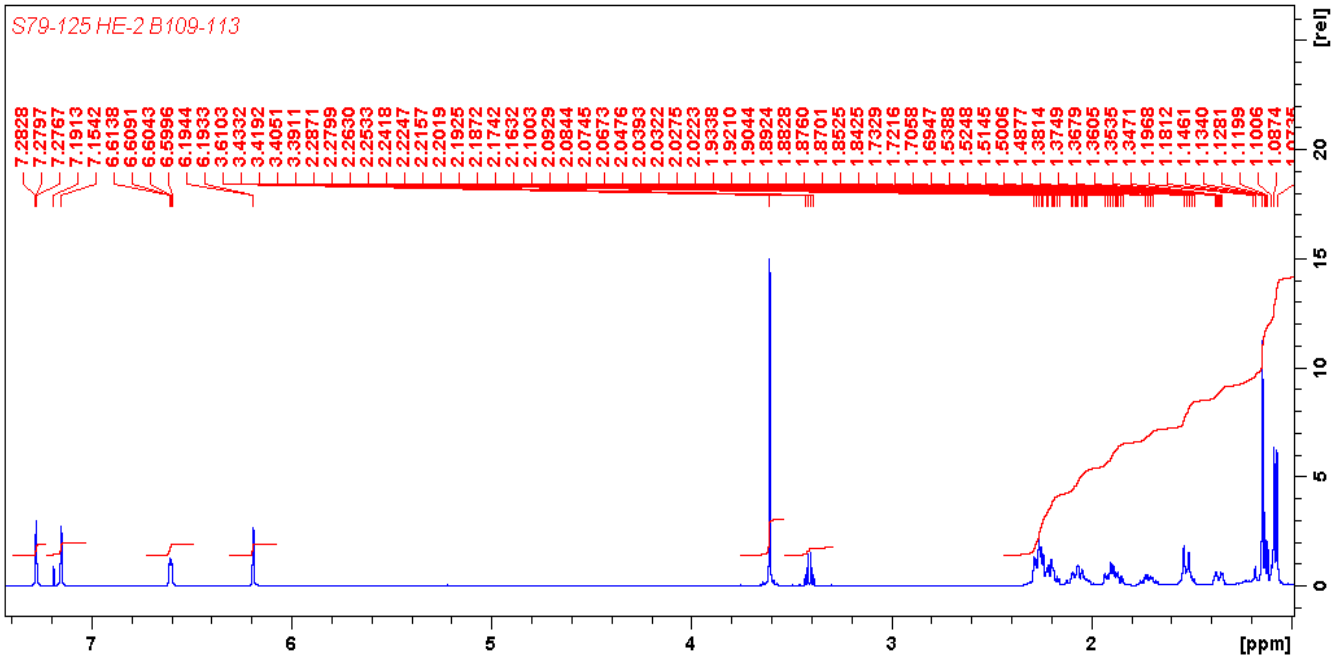


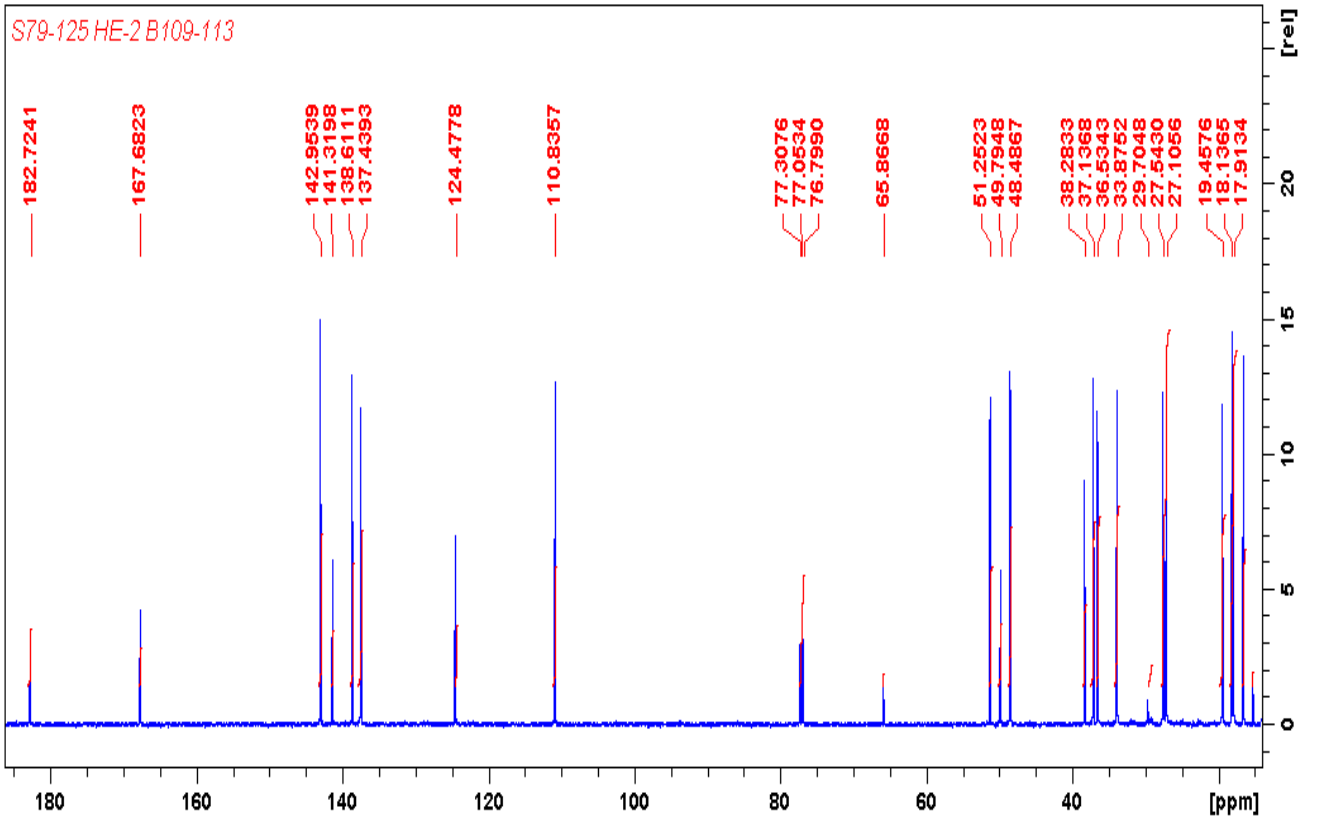
Appendix 11 a: FTIR and CD spectra of megalocarpoidolide I (401)



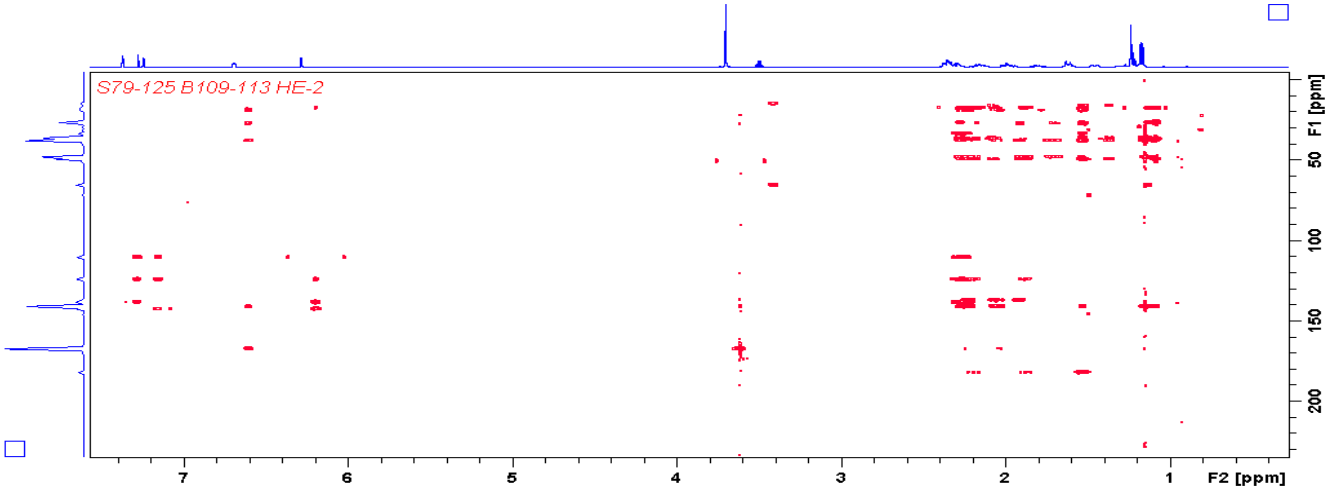


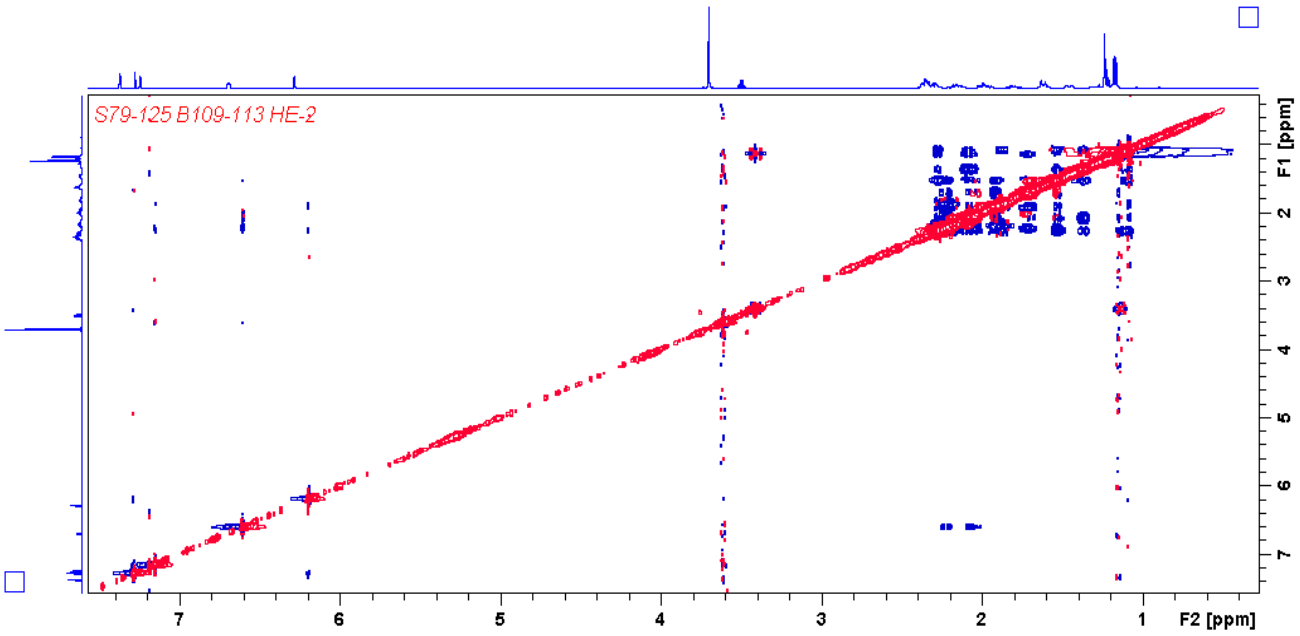
Appendix 11 b: ^{1}H and ^{13}C NMR spectra of megalocarpoidolide I (401)



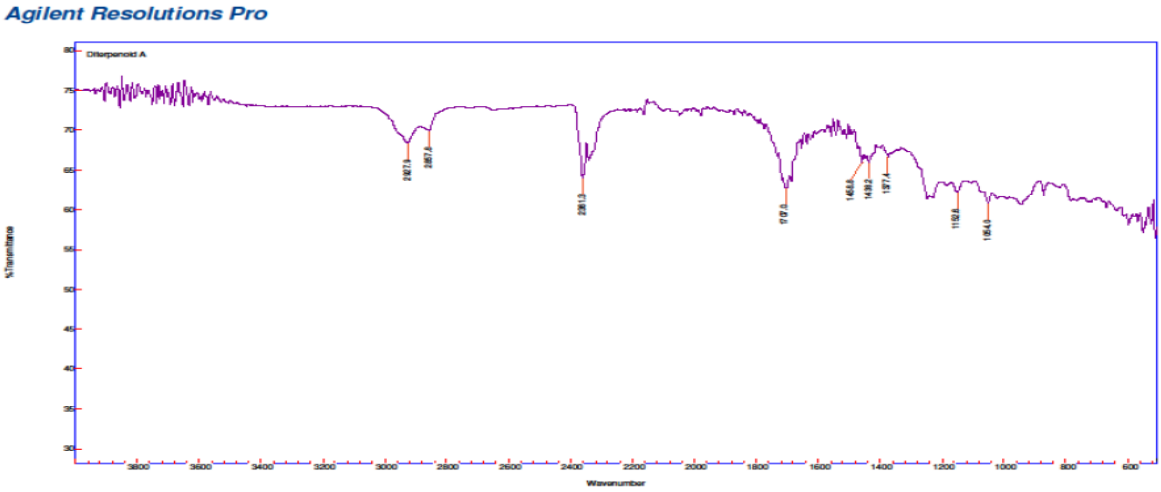


Appendix 11 c: HMBC and NOESY spectra of megalocarpoidolide I (401)

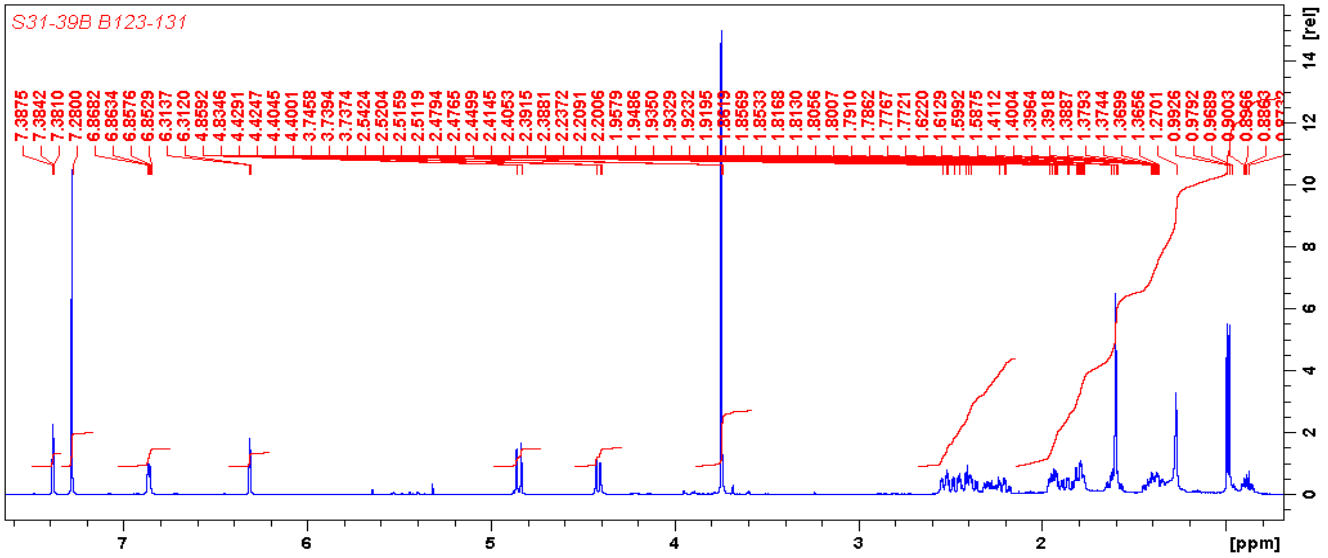


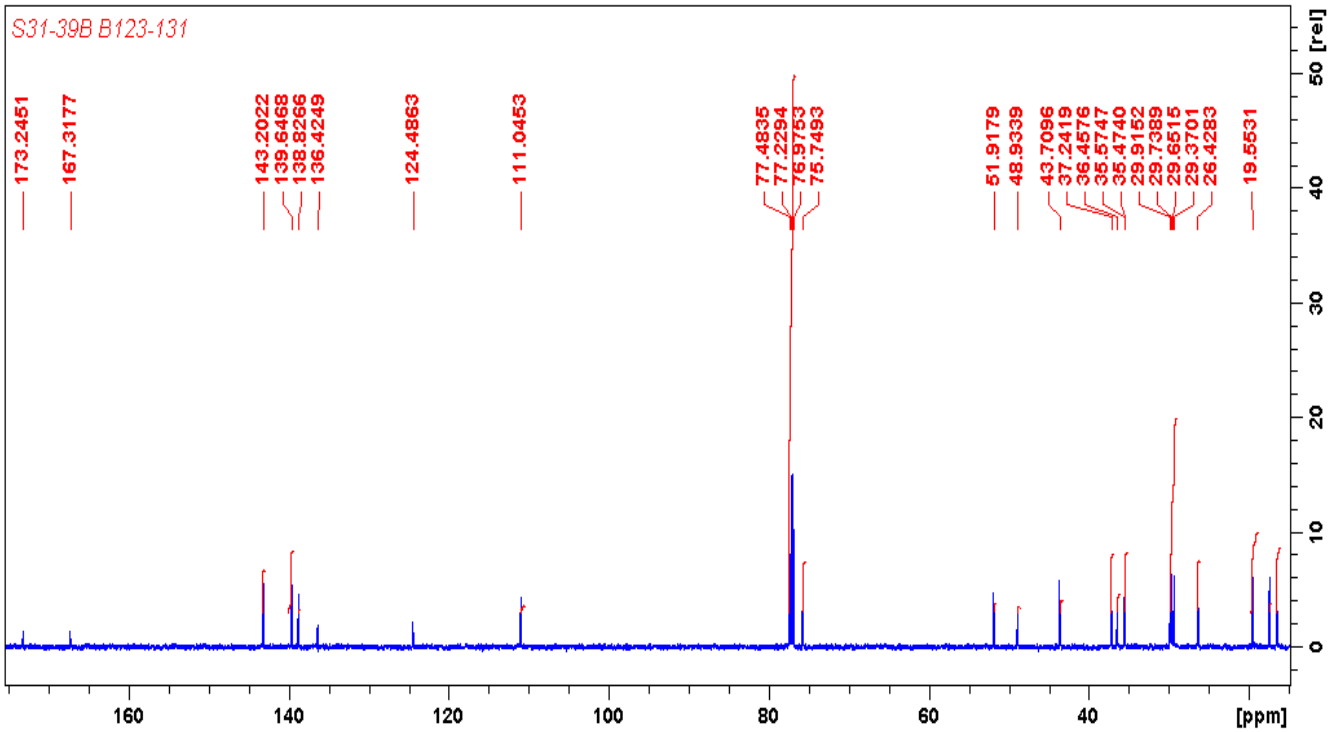


Appendix 12 a: FTIR and CD spectra of megalocarpoidolide J (402)

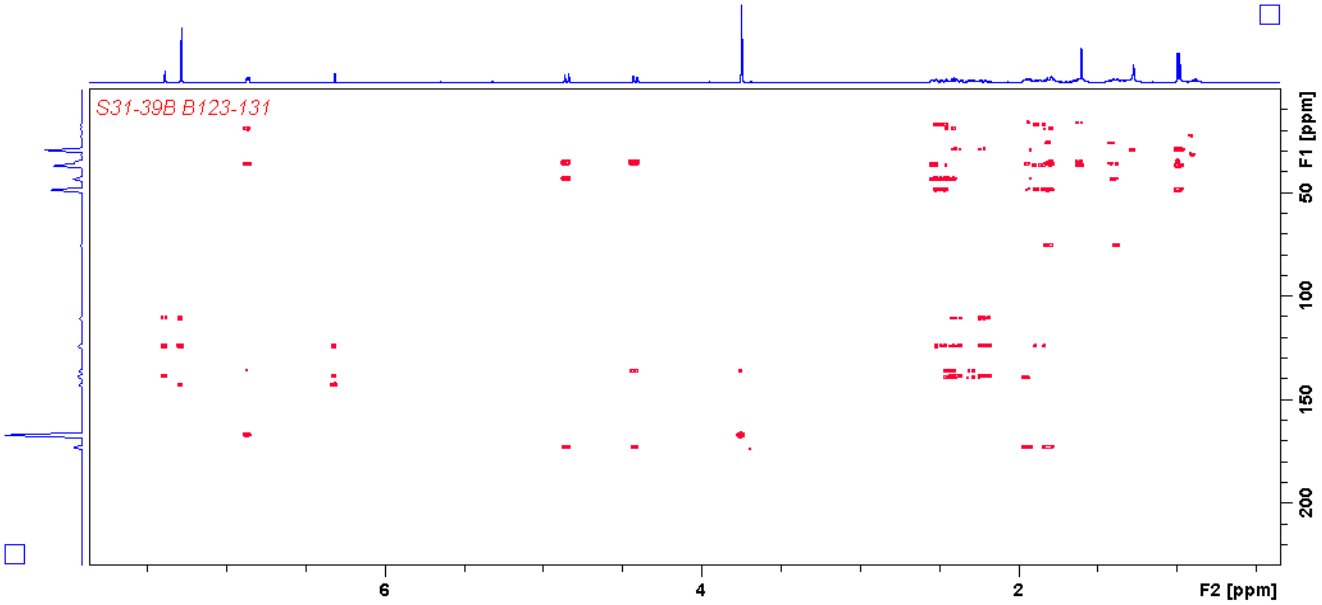


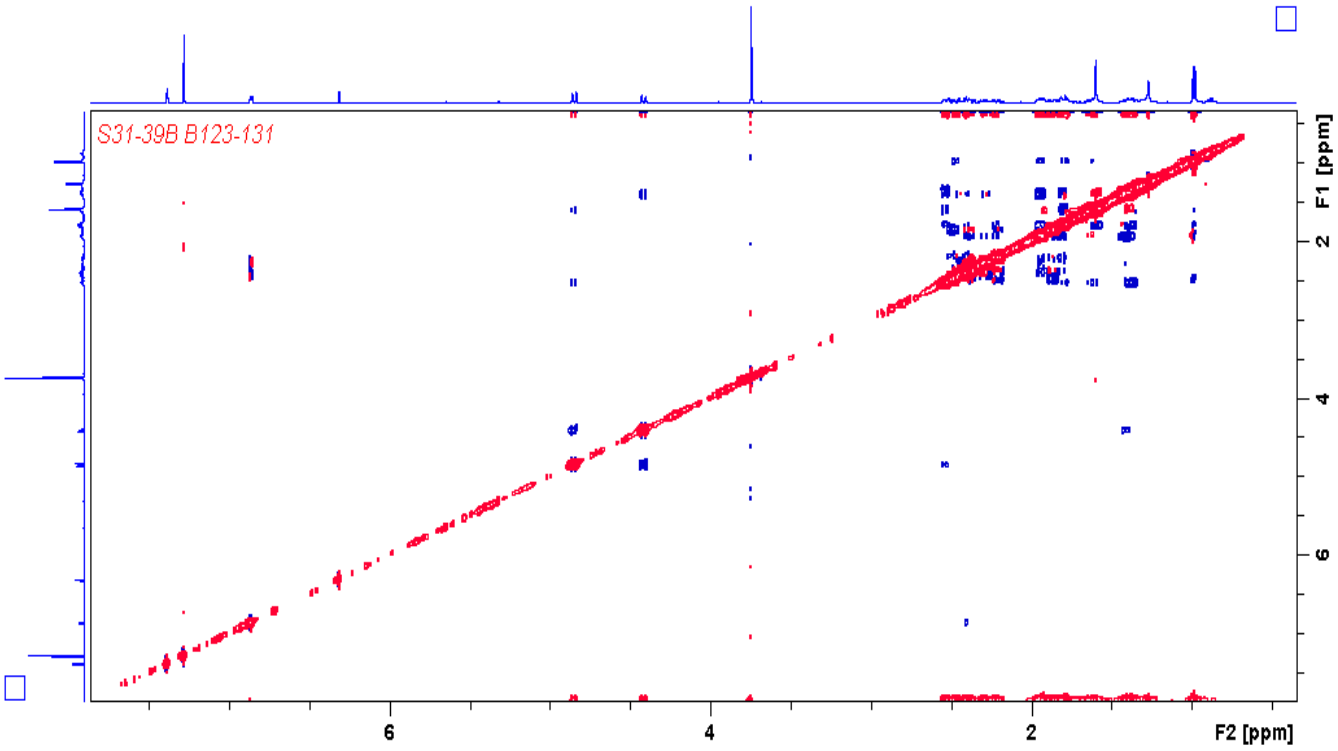
Appendix 12 b: ^{1}H and ^{13}C NMR spectra of megalocarpoidolide J (402)



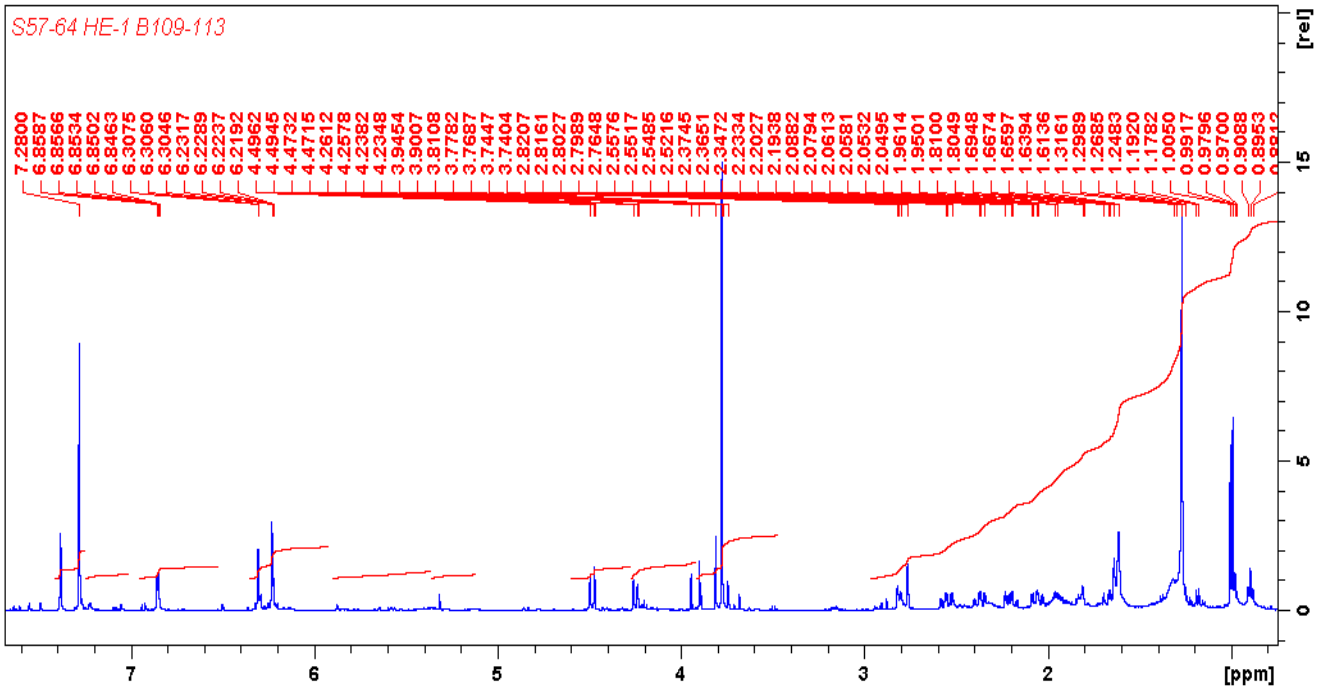


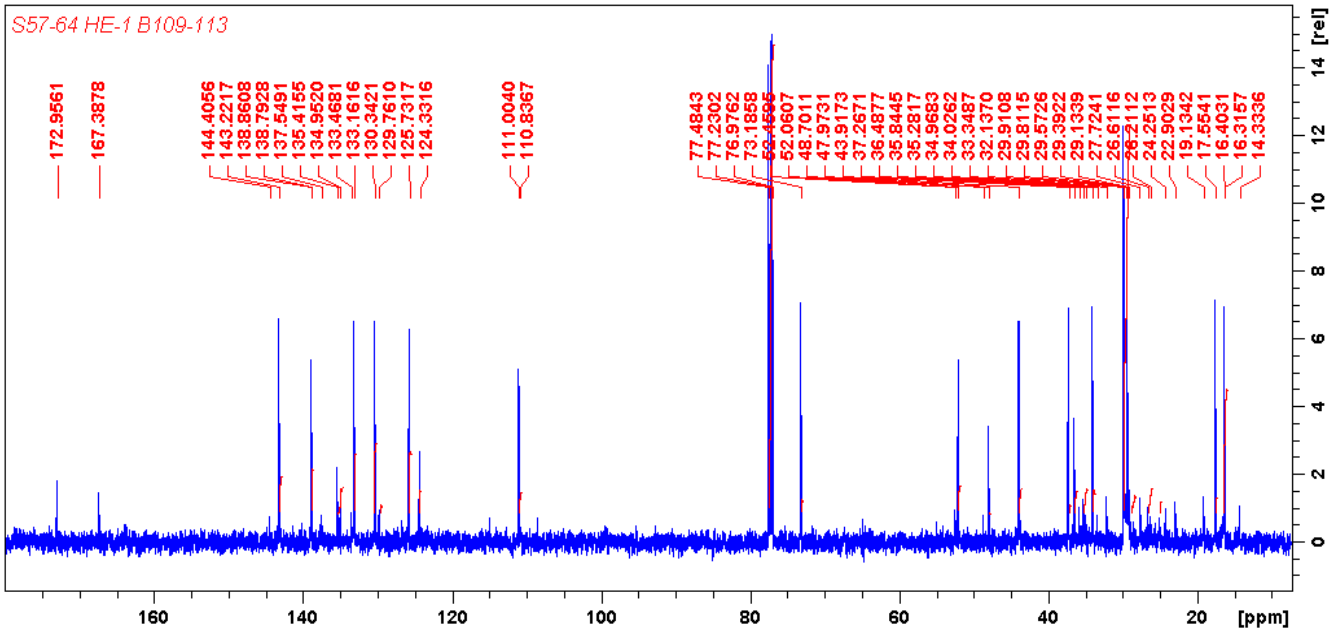
Appendix 12 c: HMBC and NOESY spectra of megalocarpoidolide J (402)



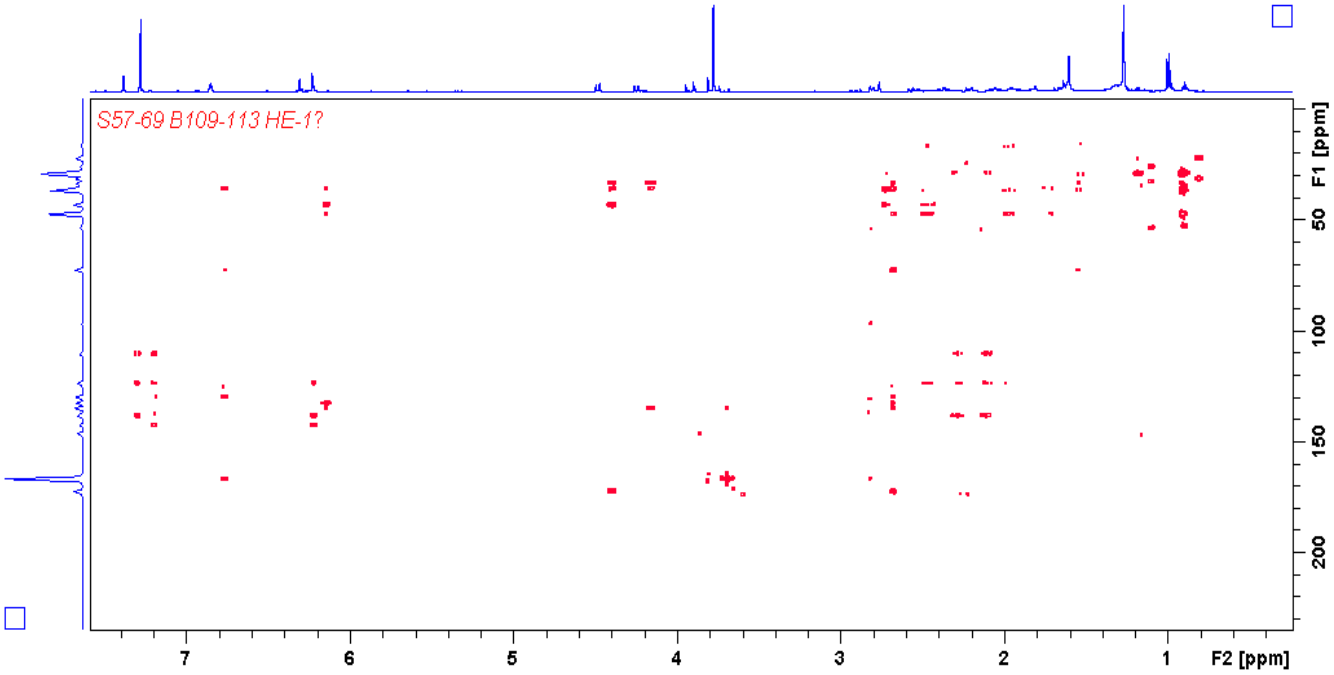


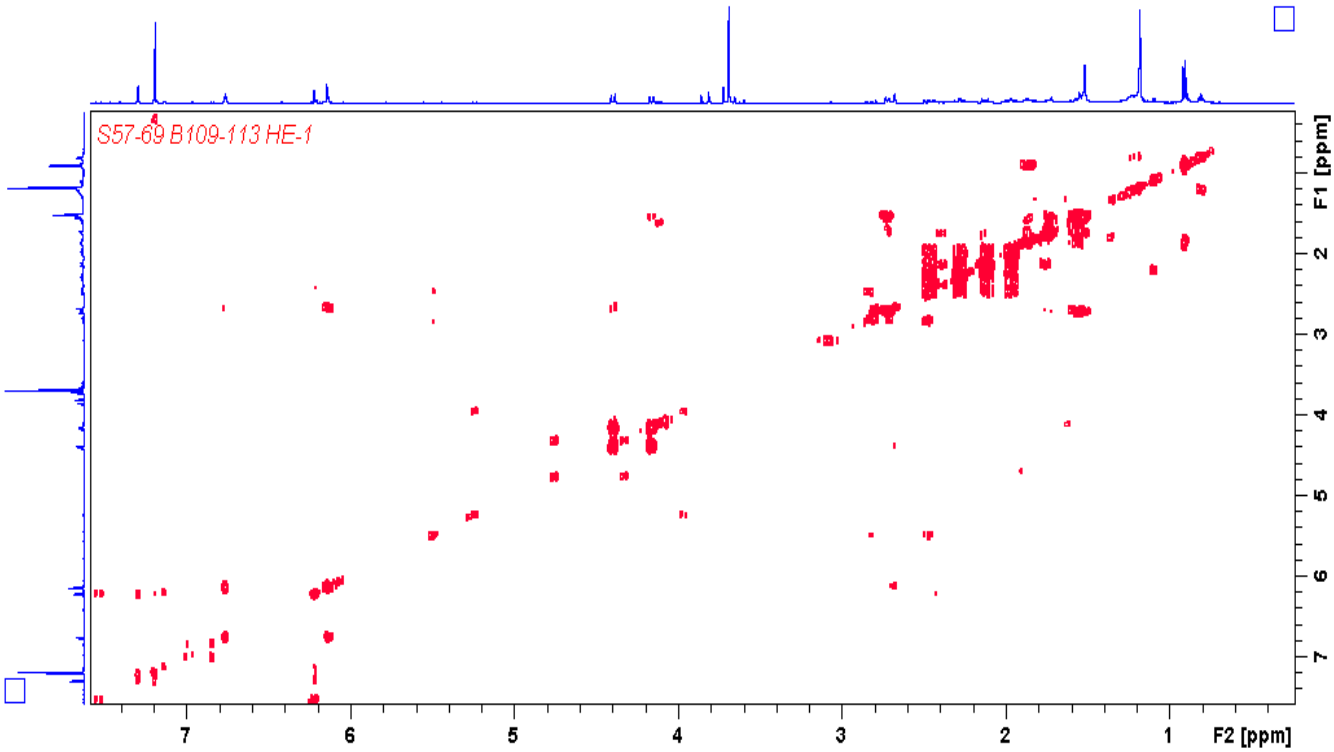
Appendix 13 a: ^{1}H and ^{13}C NMR spectra of megalocarpoidolide K (403)



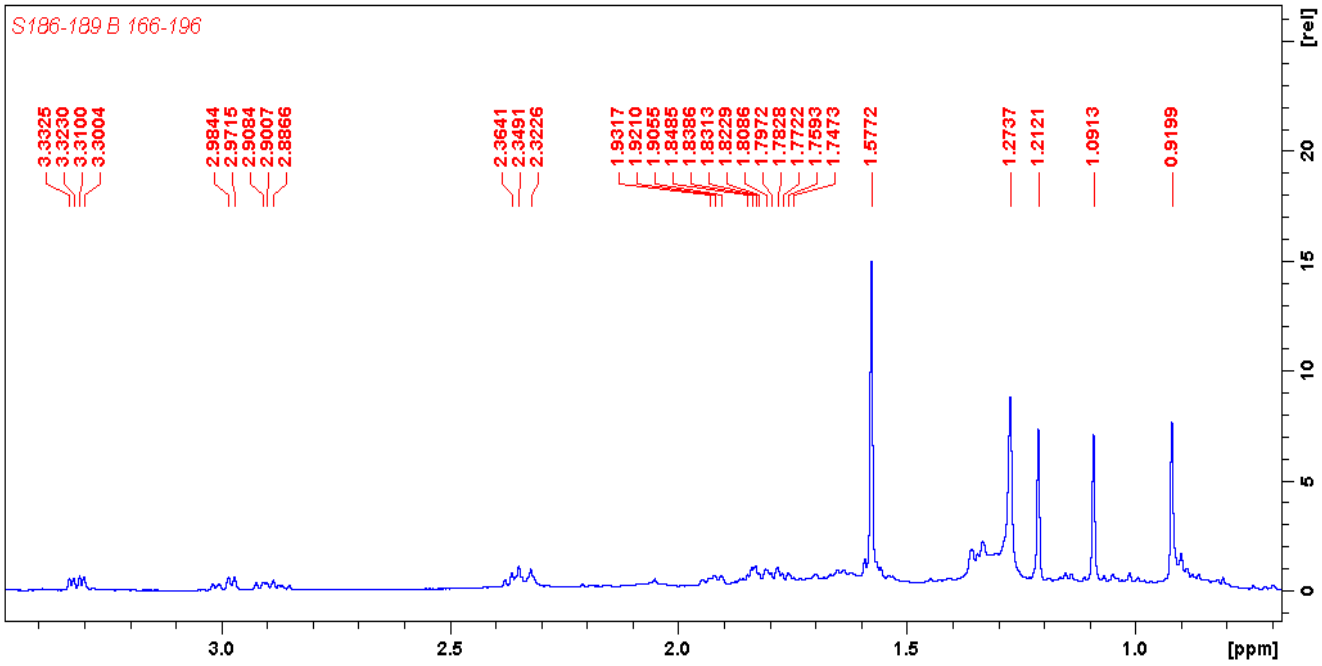


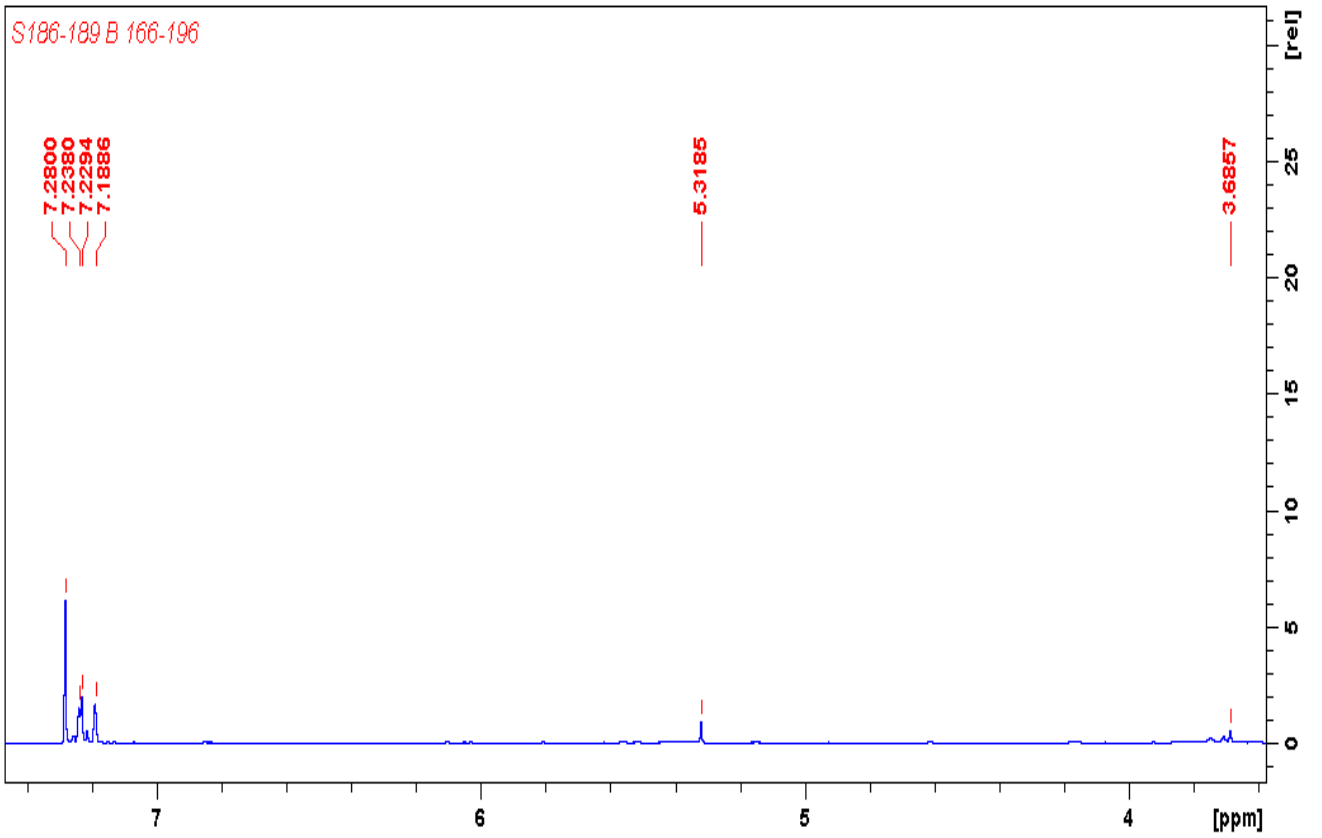
Appendix 13 b: HMBC and COSY spectra of megalocarpoidolide K (403)



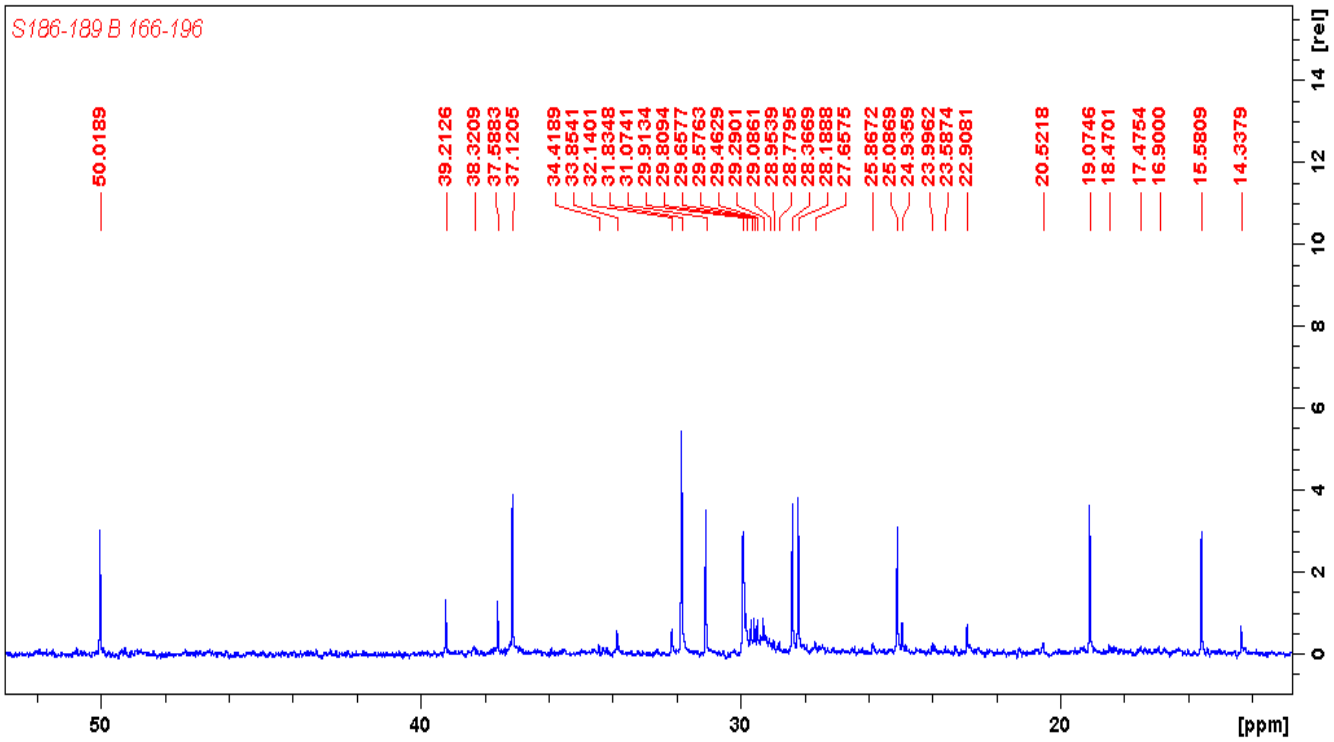


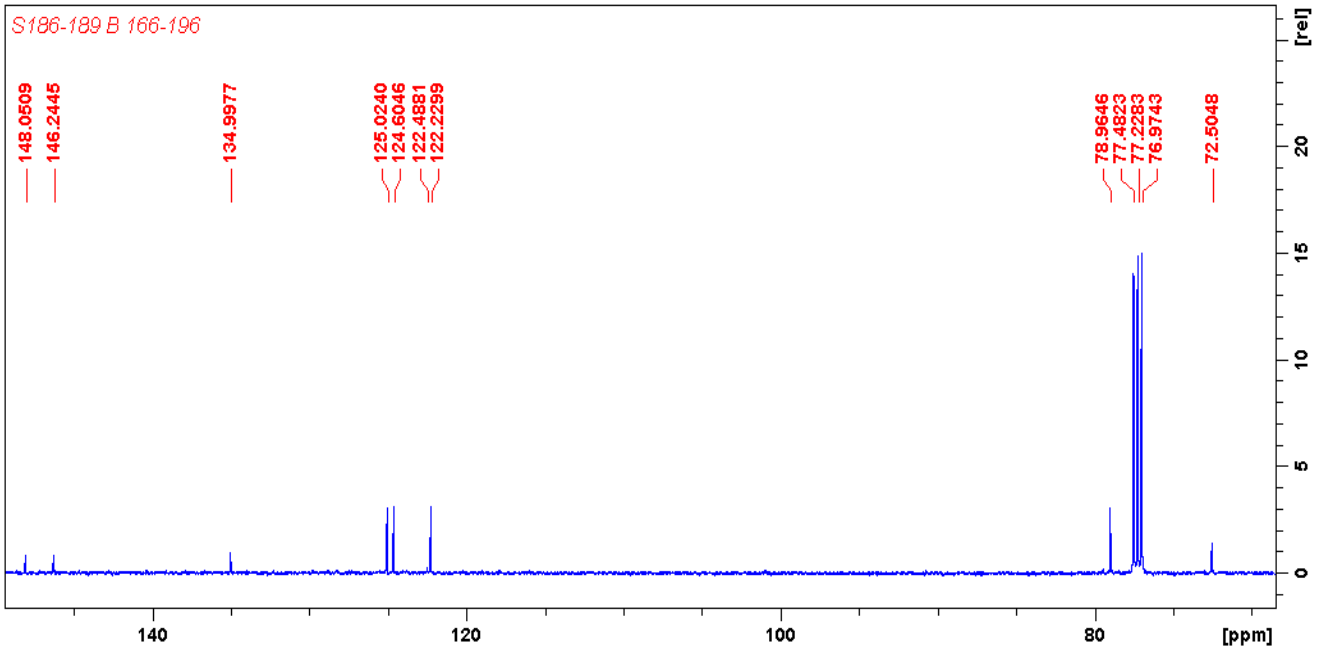
Appendix 14 a: ^{1}H NMR spectrum of isolophanthin A (404)



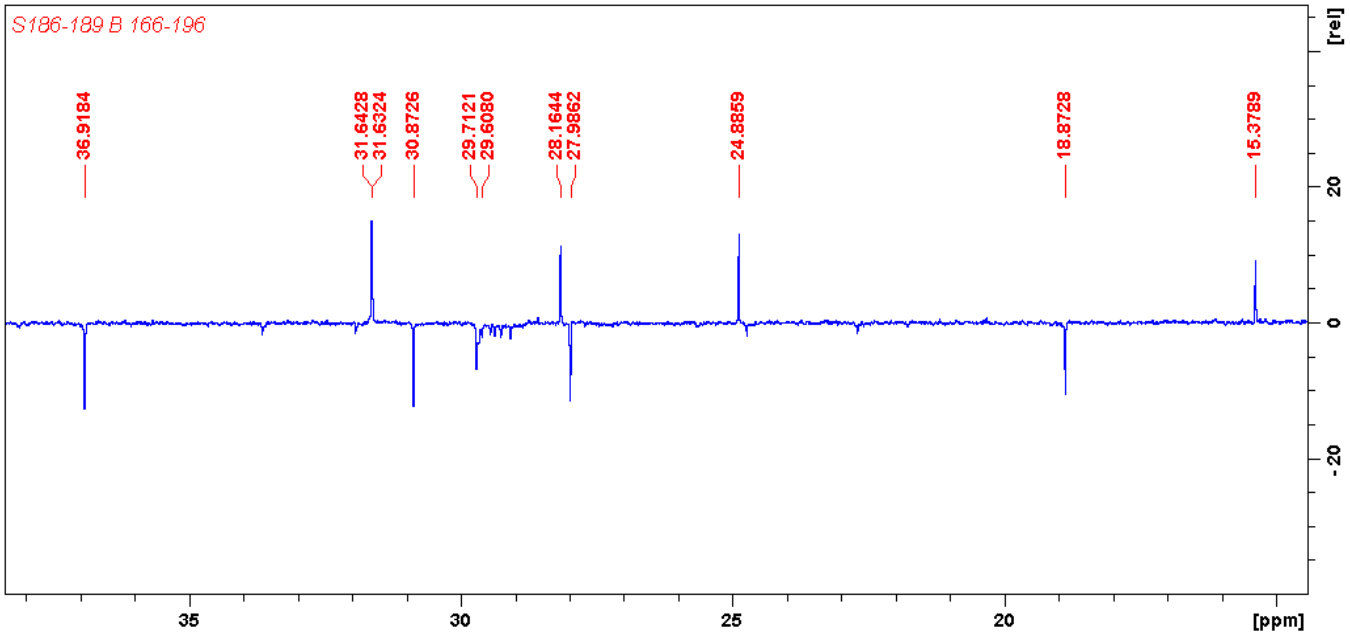


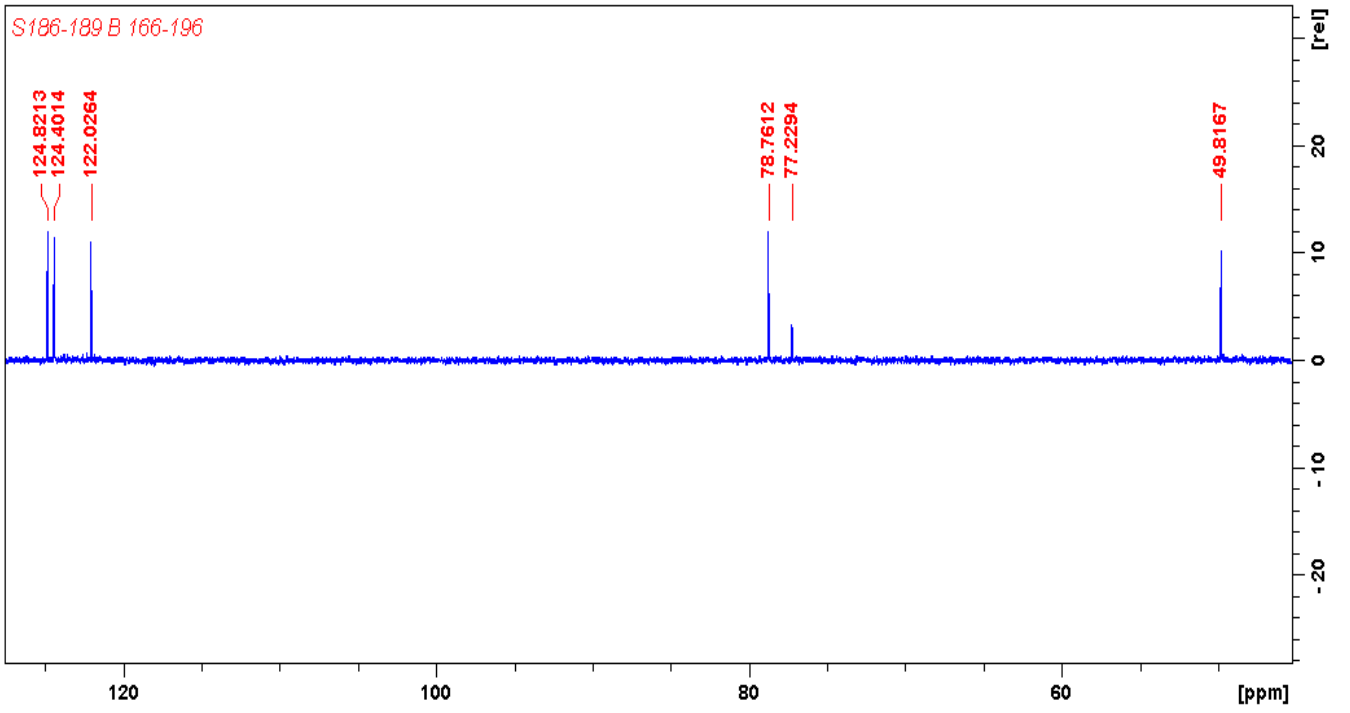
Appendix 14 b: ^{13}C NMR spectrum of isolophanthin A (404)



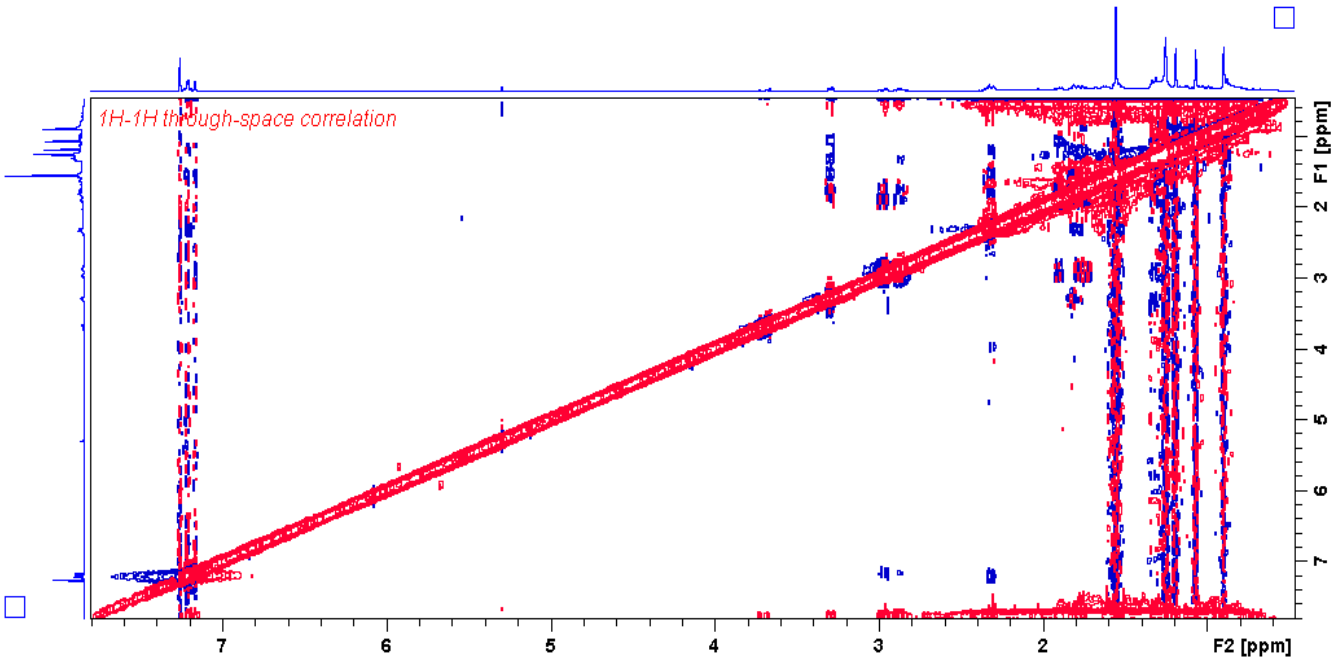


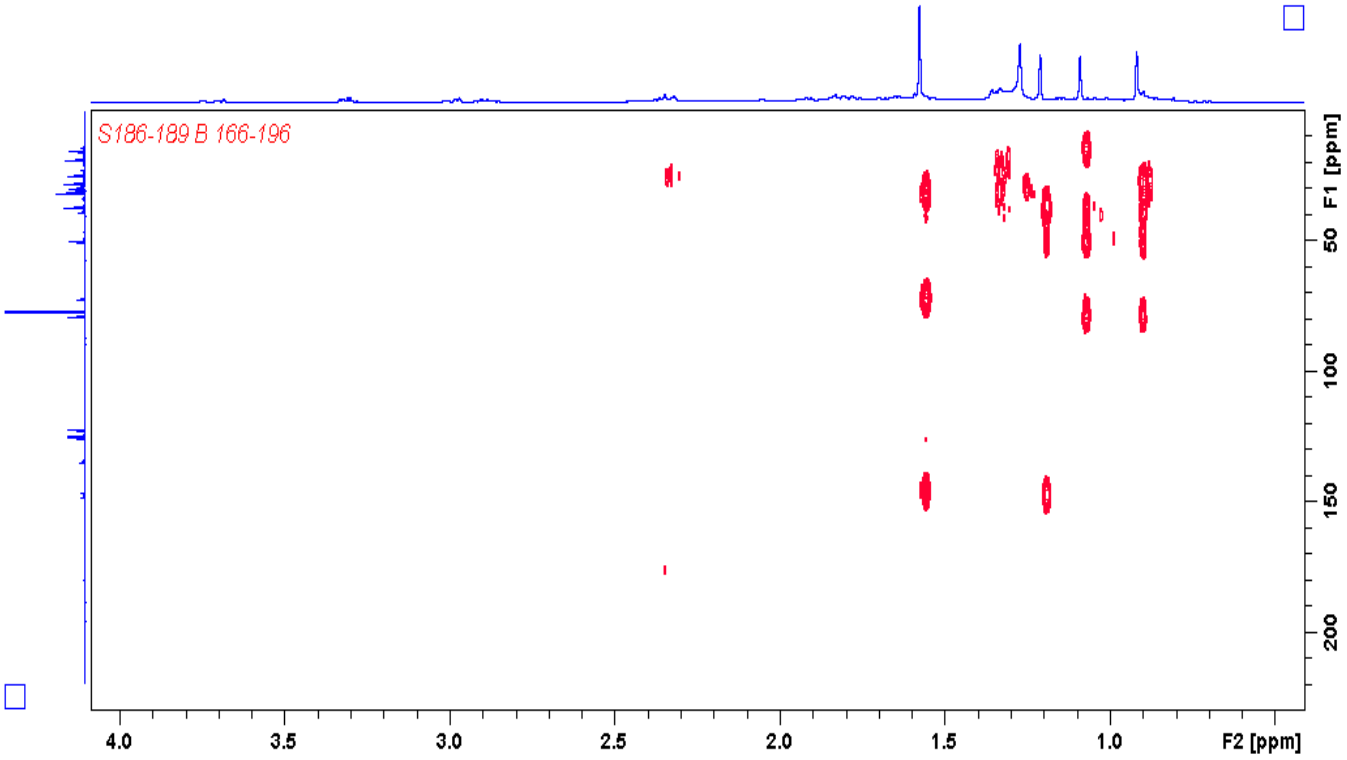
Appendix 14 c: DEPT spectrum of isolophanthin A (404)



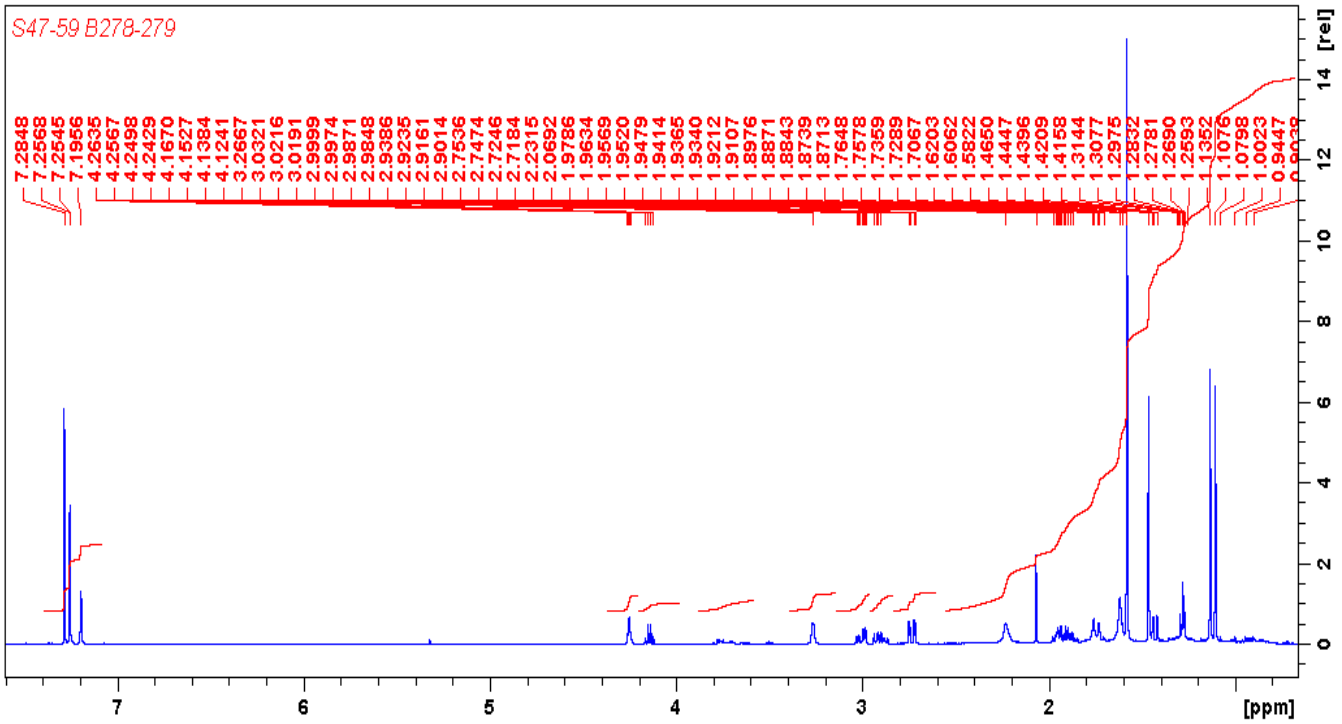


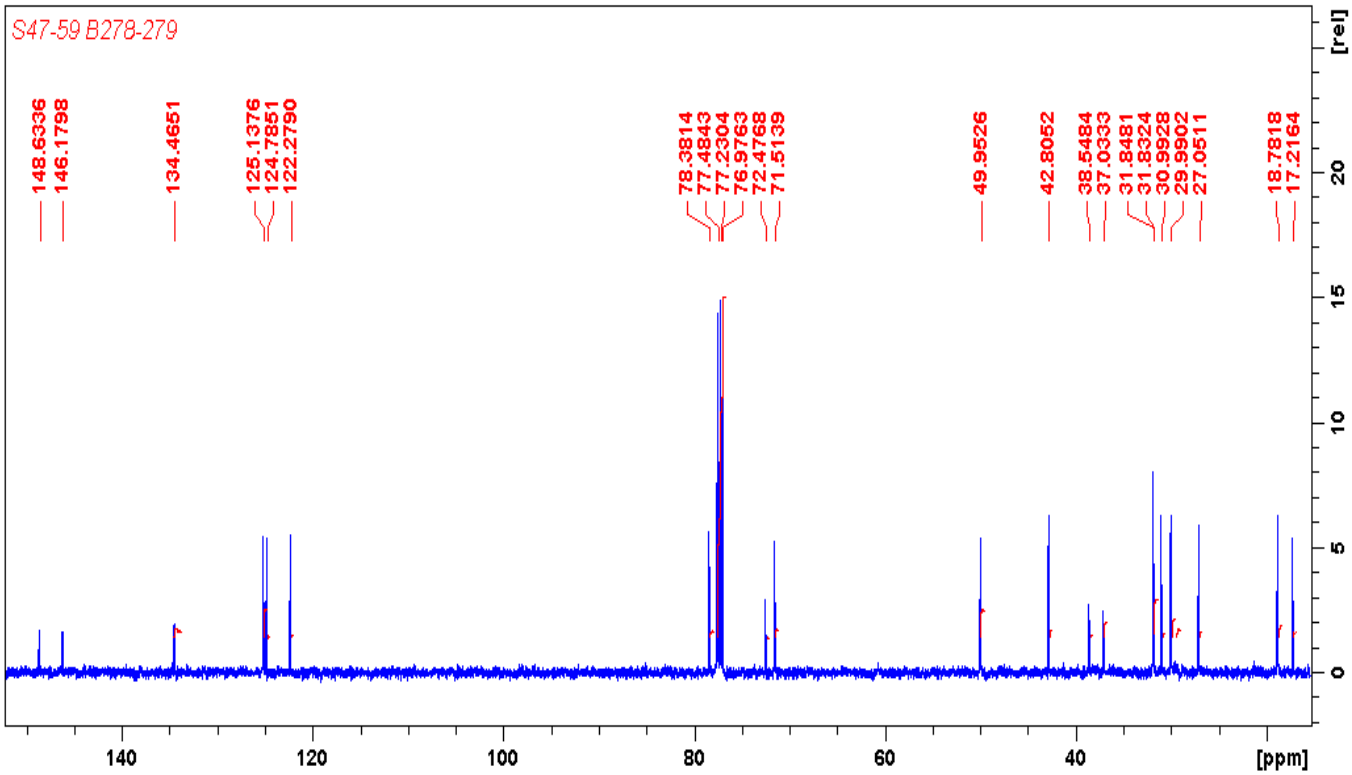
Appendix 14 d: NOESY and HMBC spectra of isolophanthin A (404)



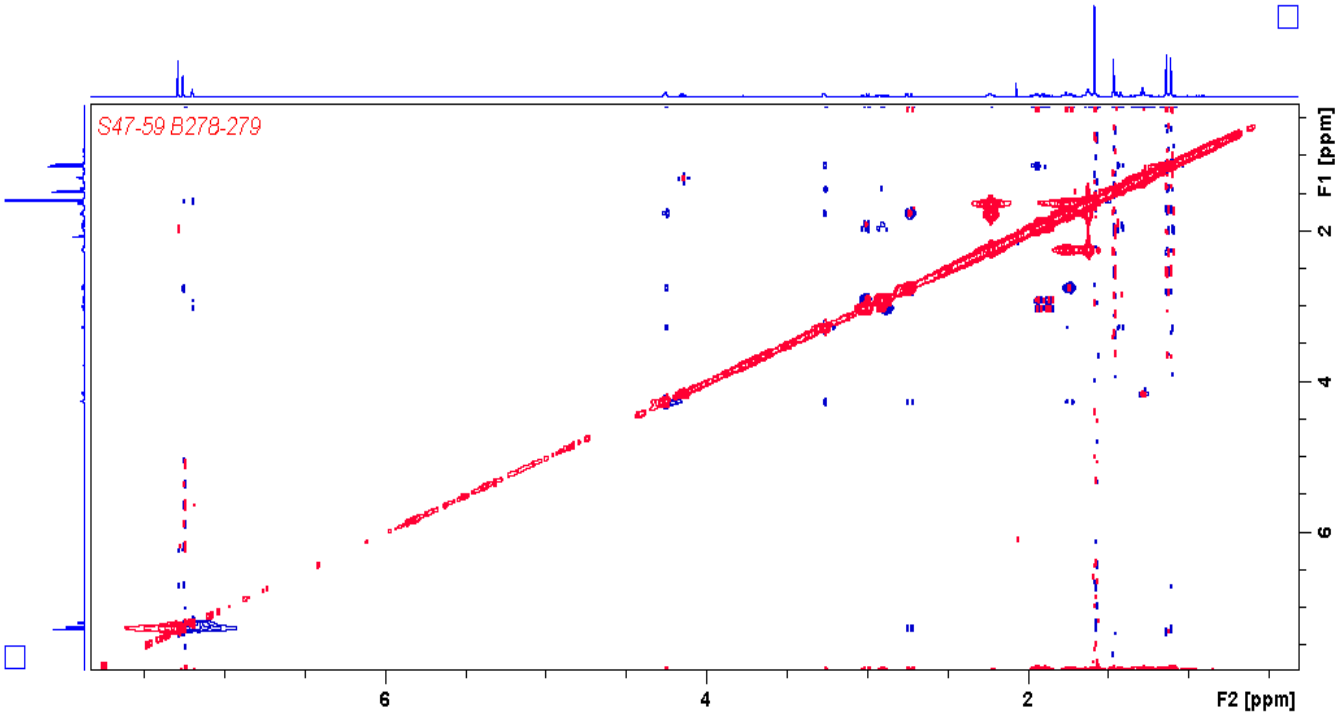


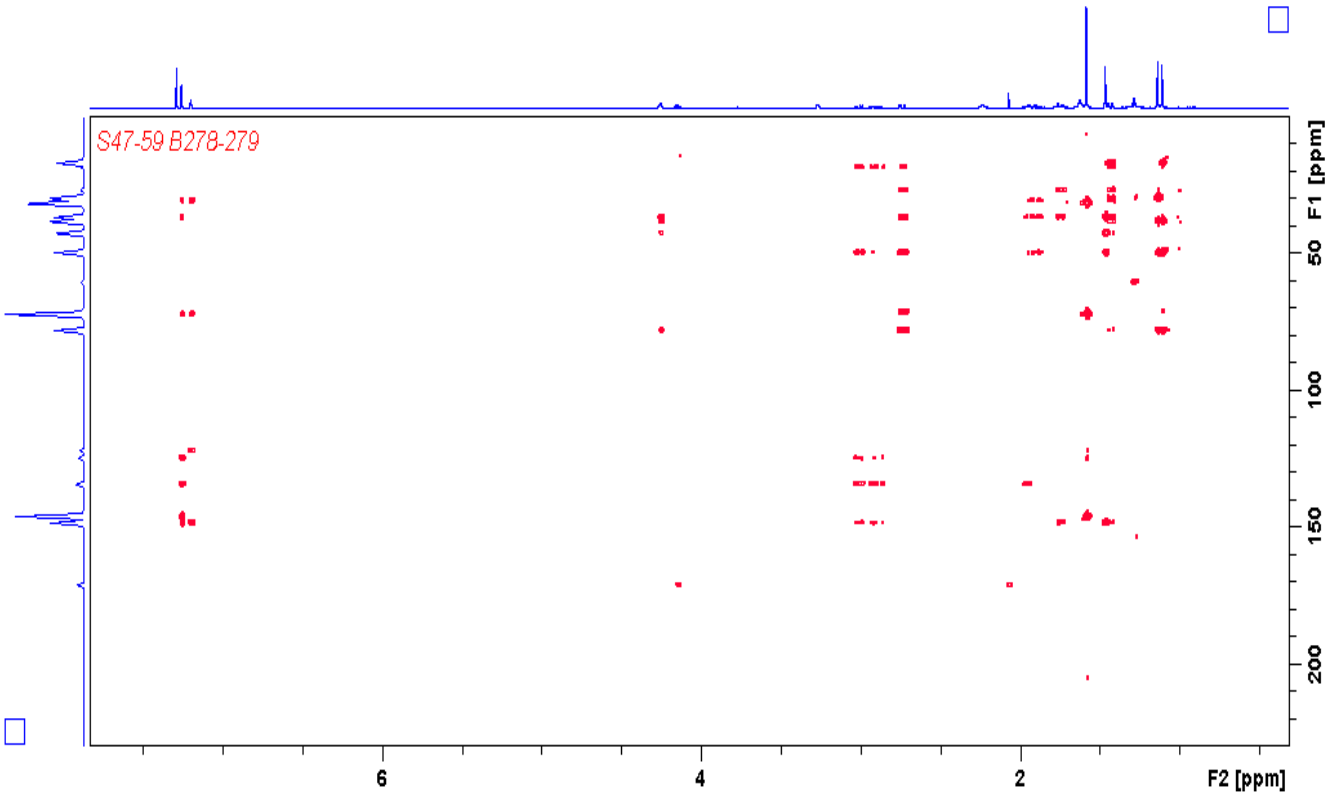
Appendix 15 a: ^{1}H and ^{13}C NMR spectra of isolophanthin E (405)



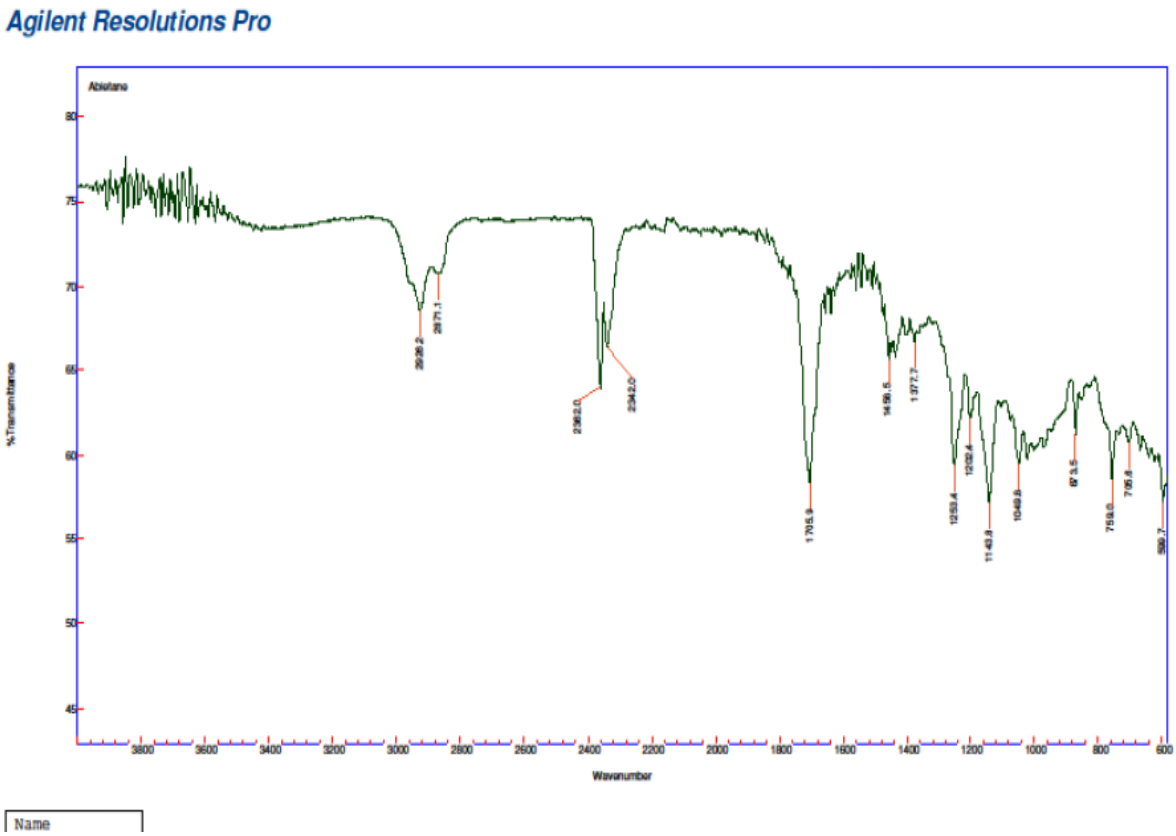


Appendix 15 b: HMBC and NOESY spectra of isolophanthin E (405)

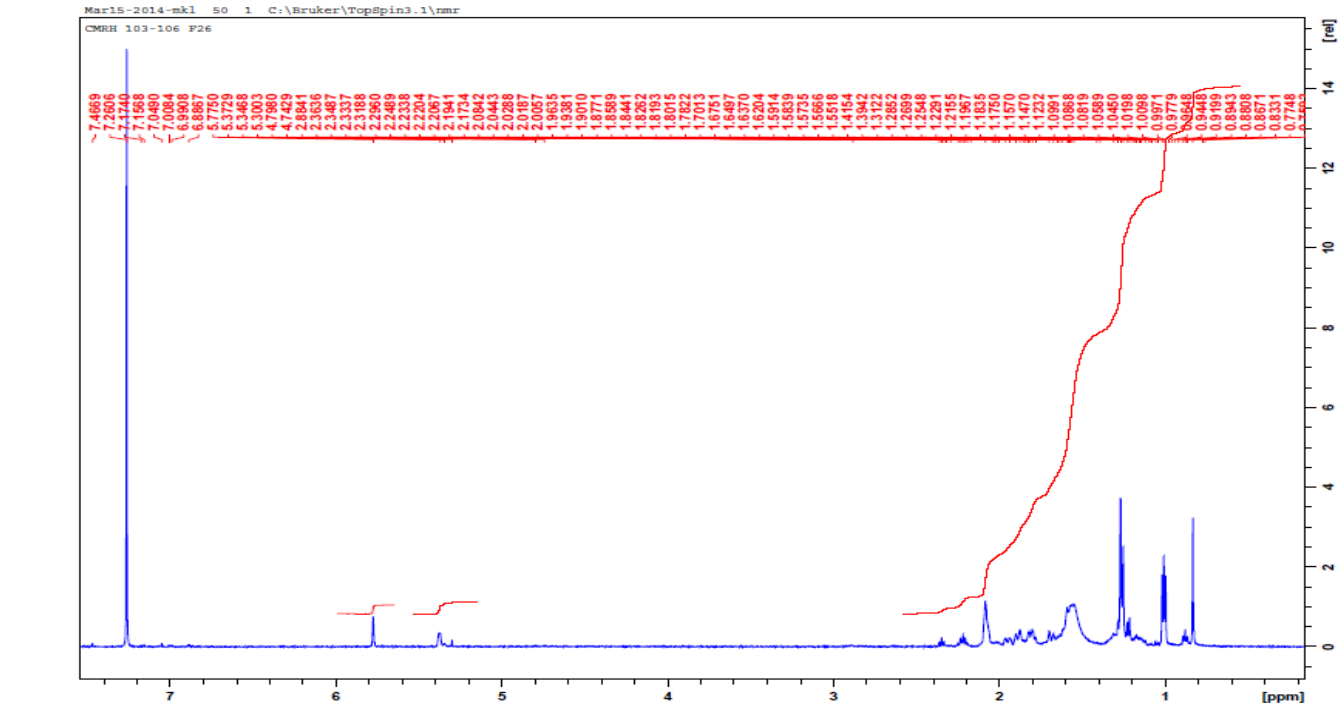


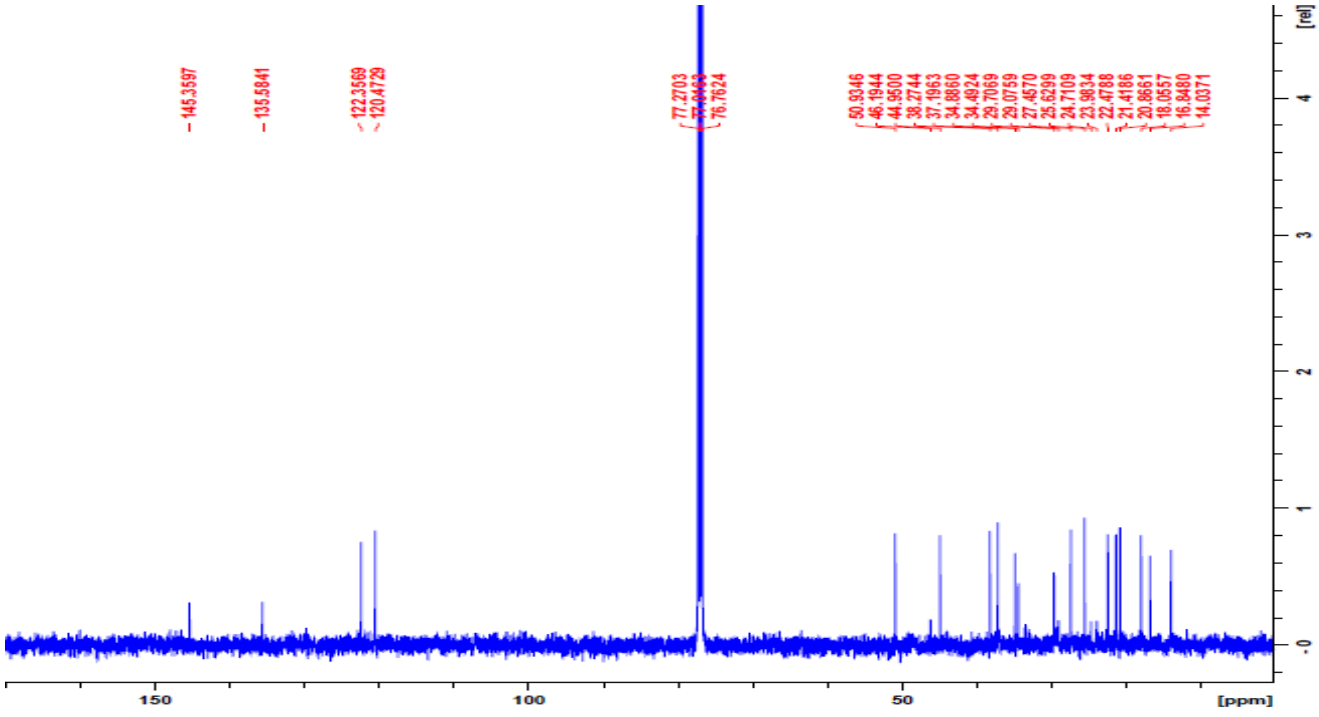


Appendix 16 a: Mass and FTIR spectra of abietic acid (406)

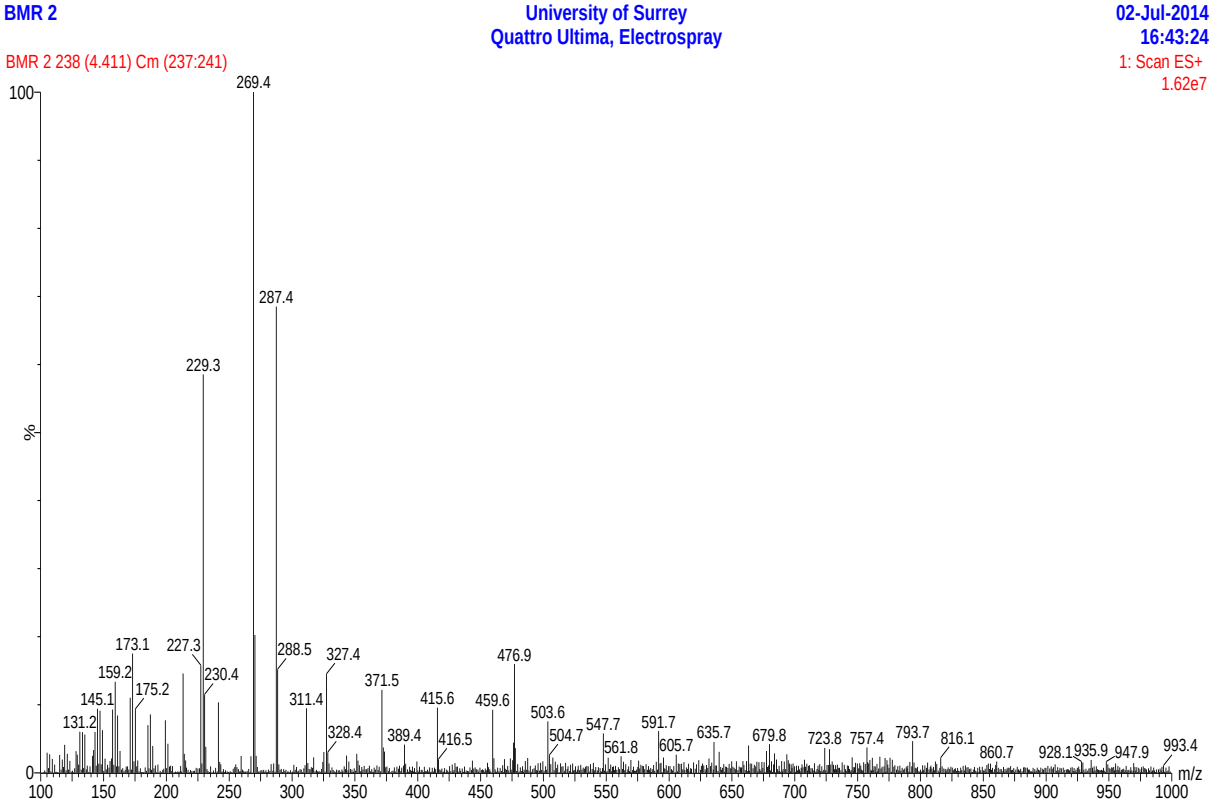


Appendix 16 b: ^{1}H and ^{13}C spectra of abietic acid (406)

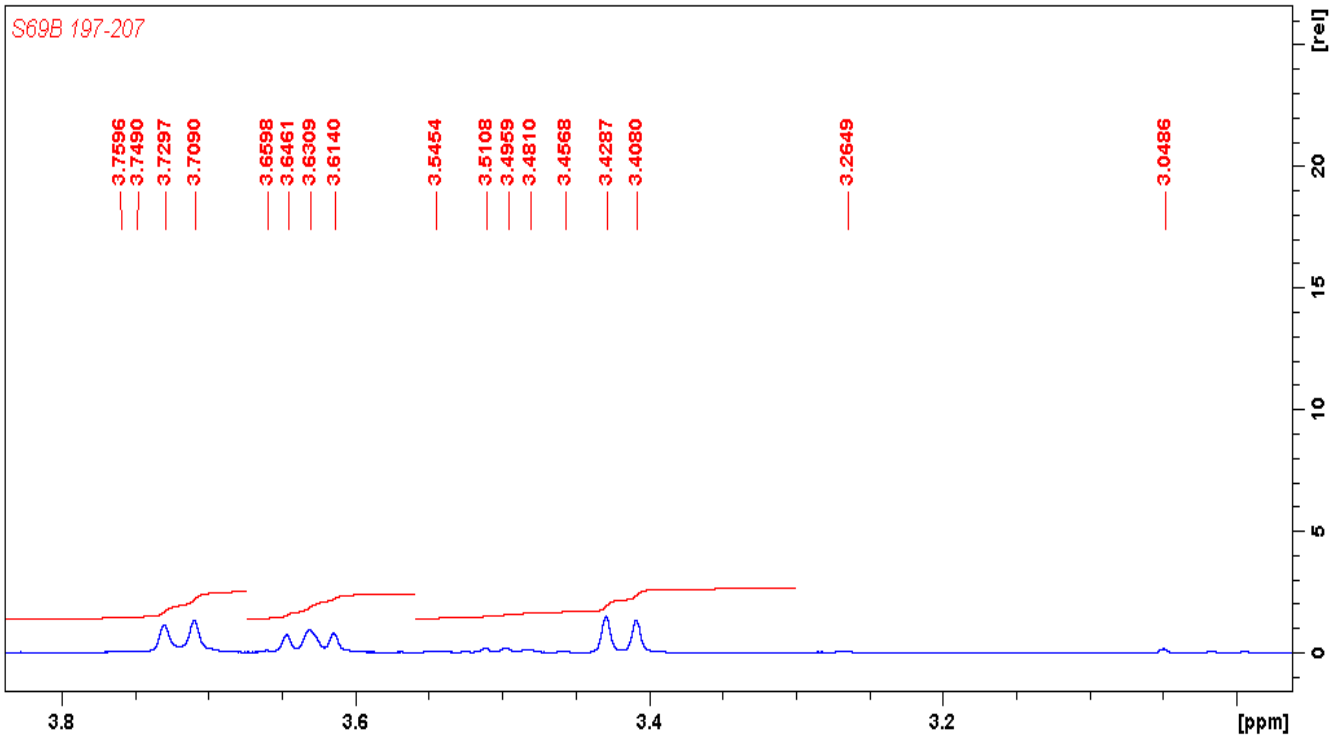


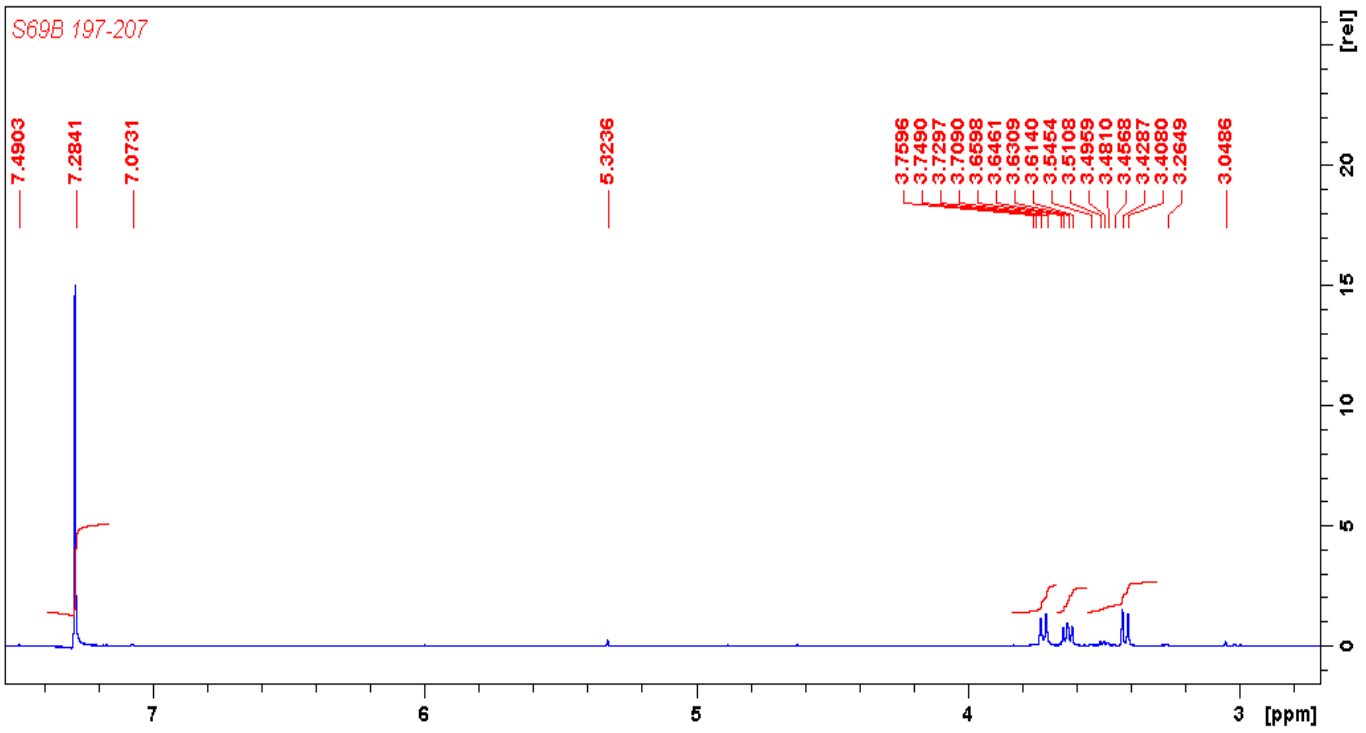


Appendix 17 a: Mass spectrum of 3α, 18-dihydroxytrachylobane (407)

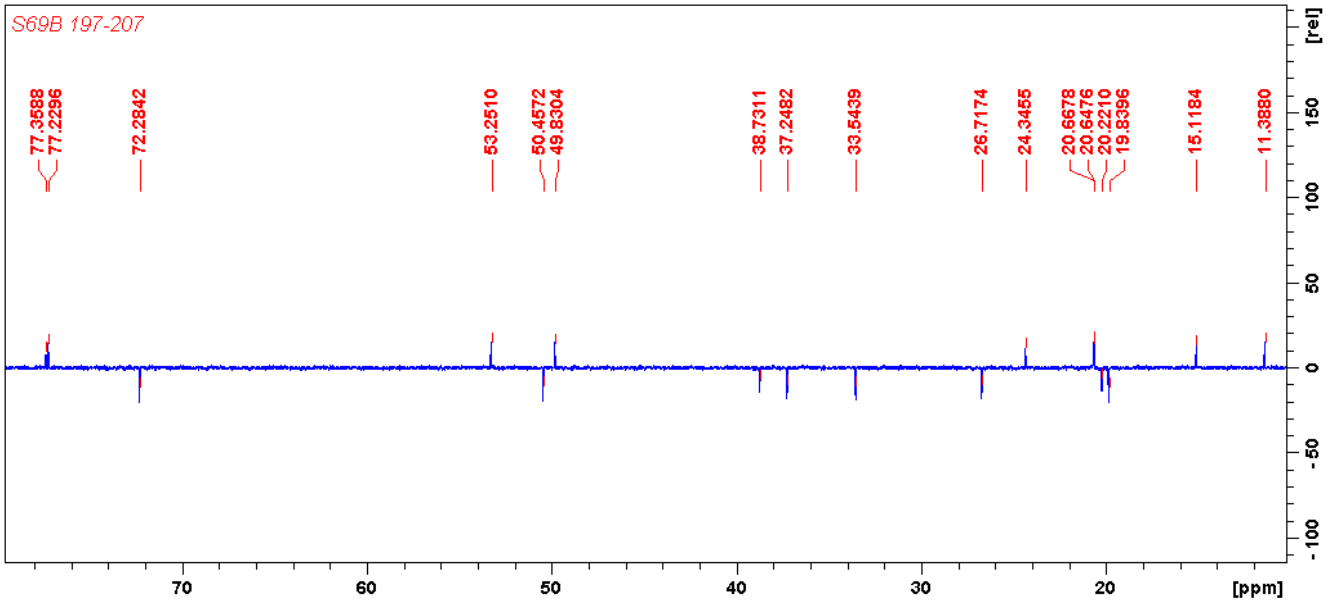


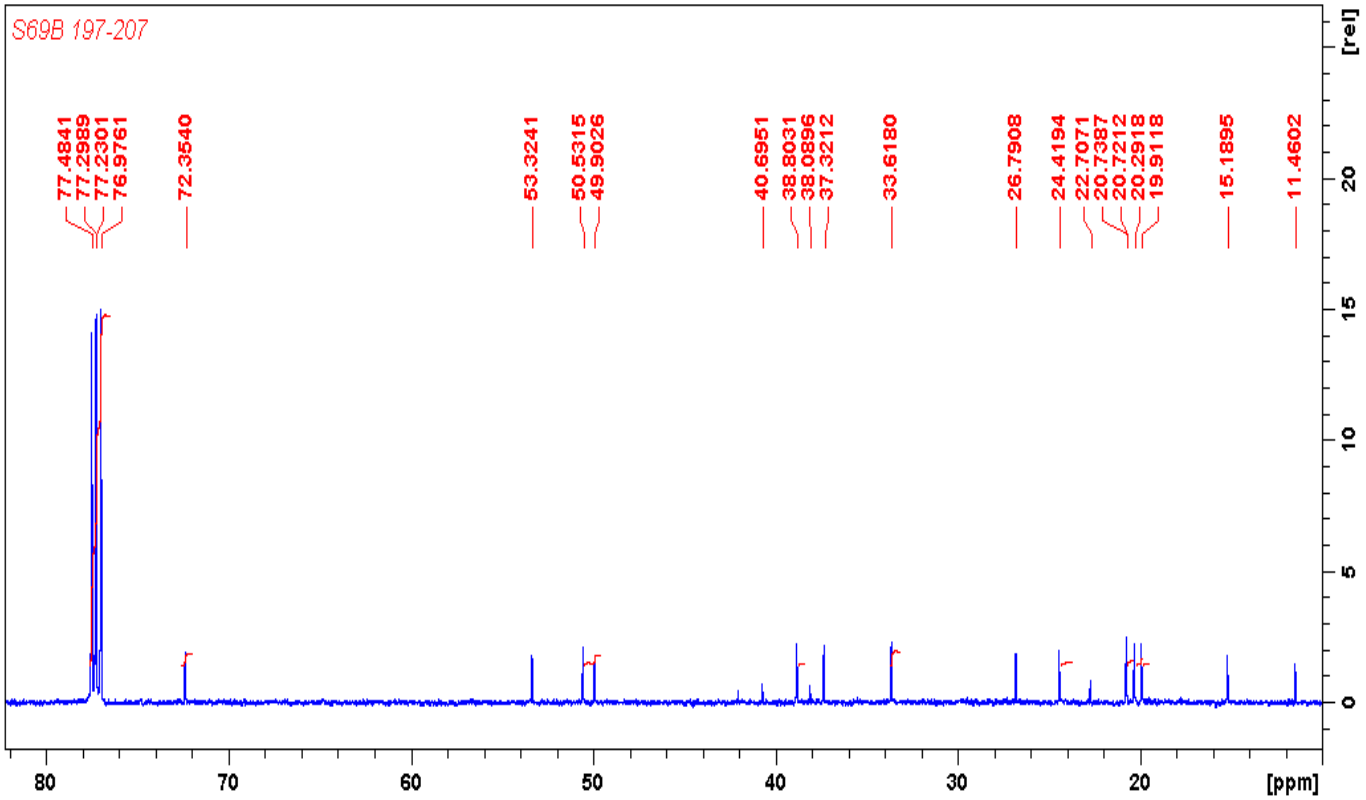
Appendix 17 b: ^{1}H NMR spectrum of 3α, 18-dihydroxytrachylobane (407)



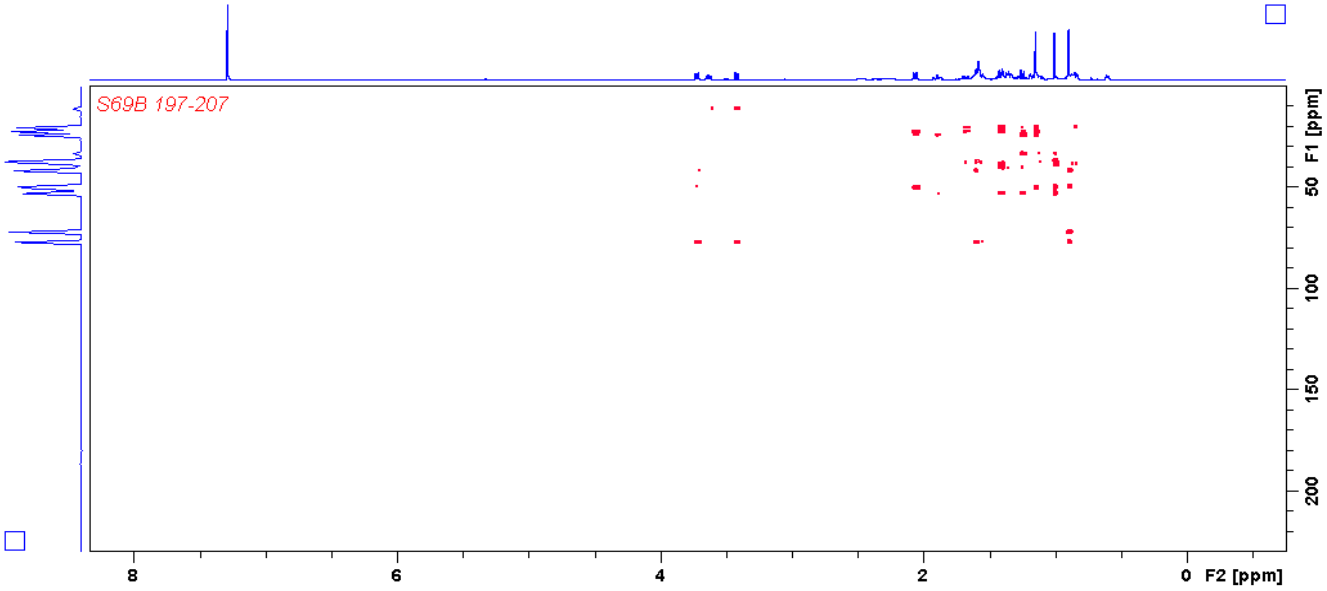


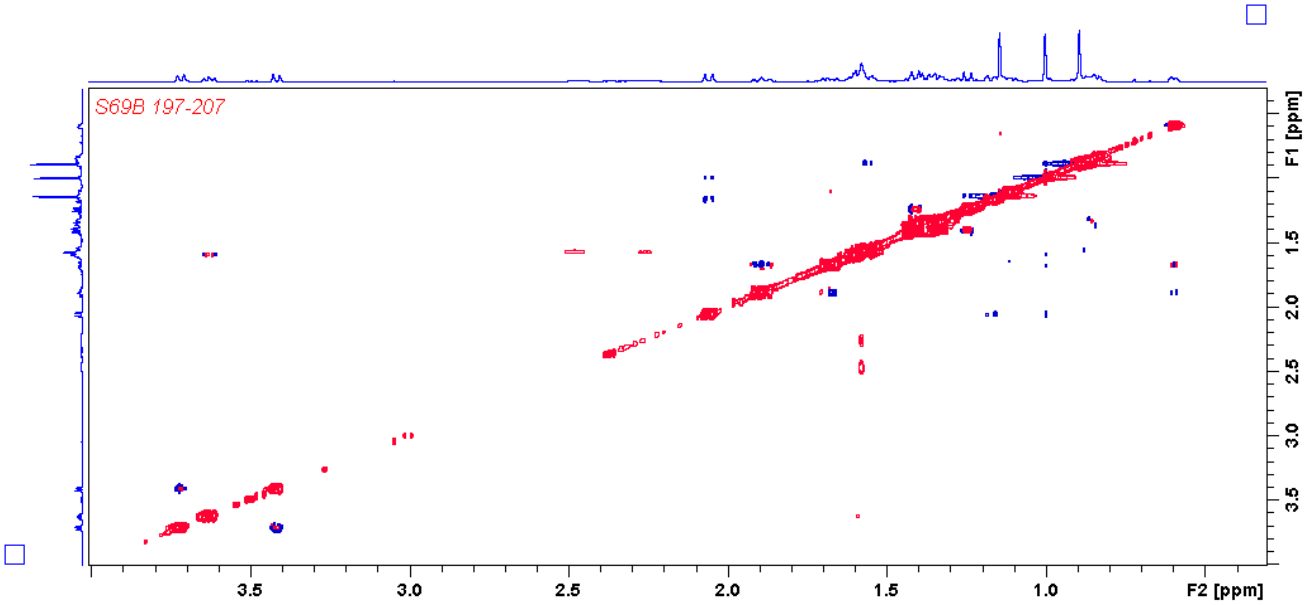
Appendix 17 c: DEPT and ^{13}C NMR spectra of 3α, 18-dihydroxytrachylobane (407)



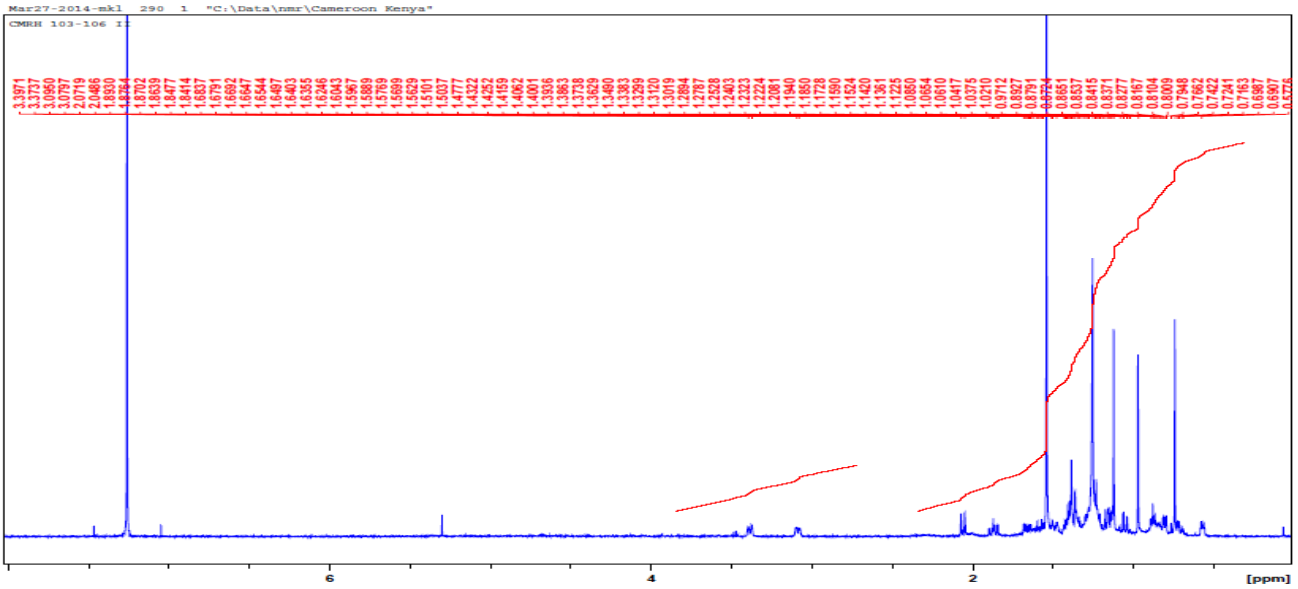


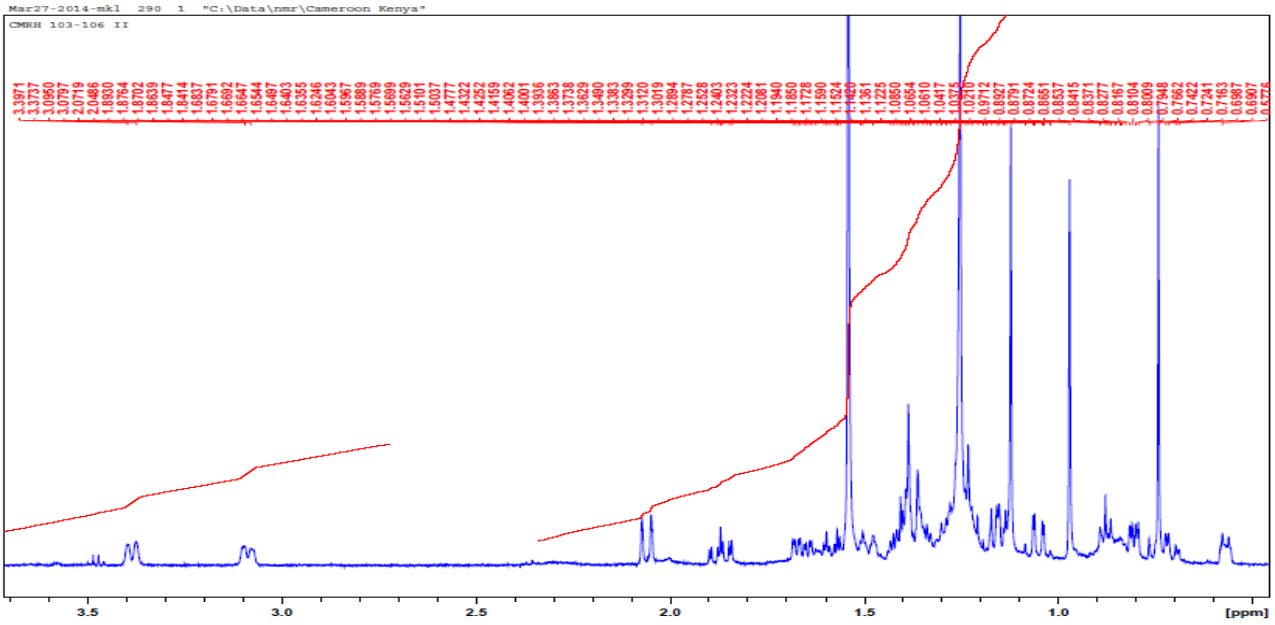
Appendix 17 d: HMBC and NOESY spectra of 3α, 18-dihydroxytrachylobane (407)



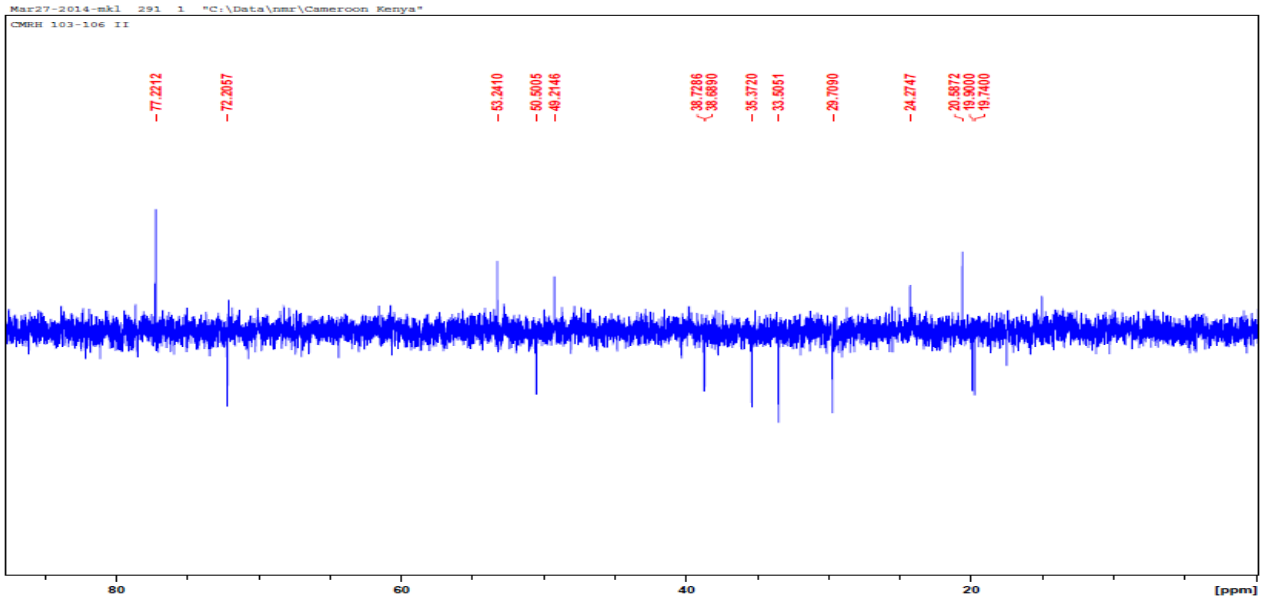


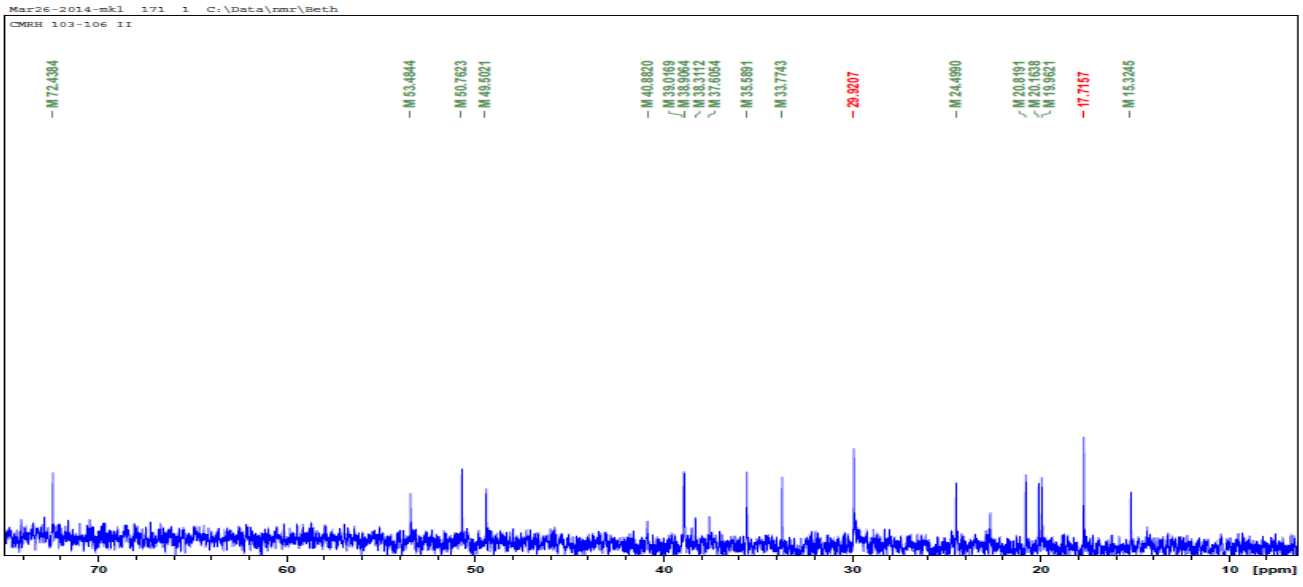
Appendix 18 a: ^{1}H NMR spectrum of *Ent*-trachyloban-19-ol (408)



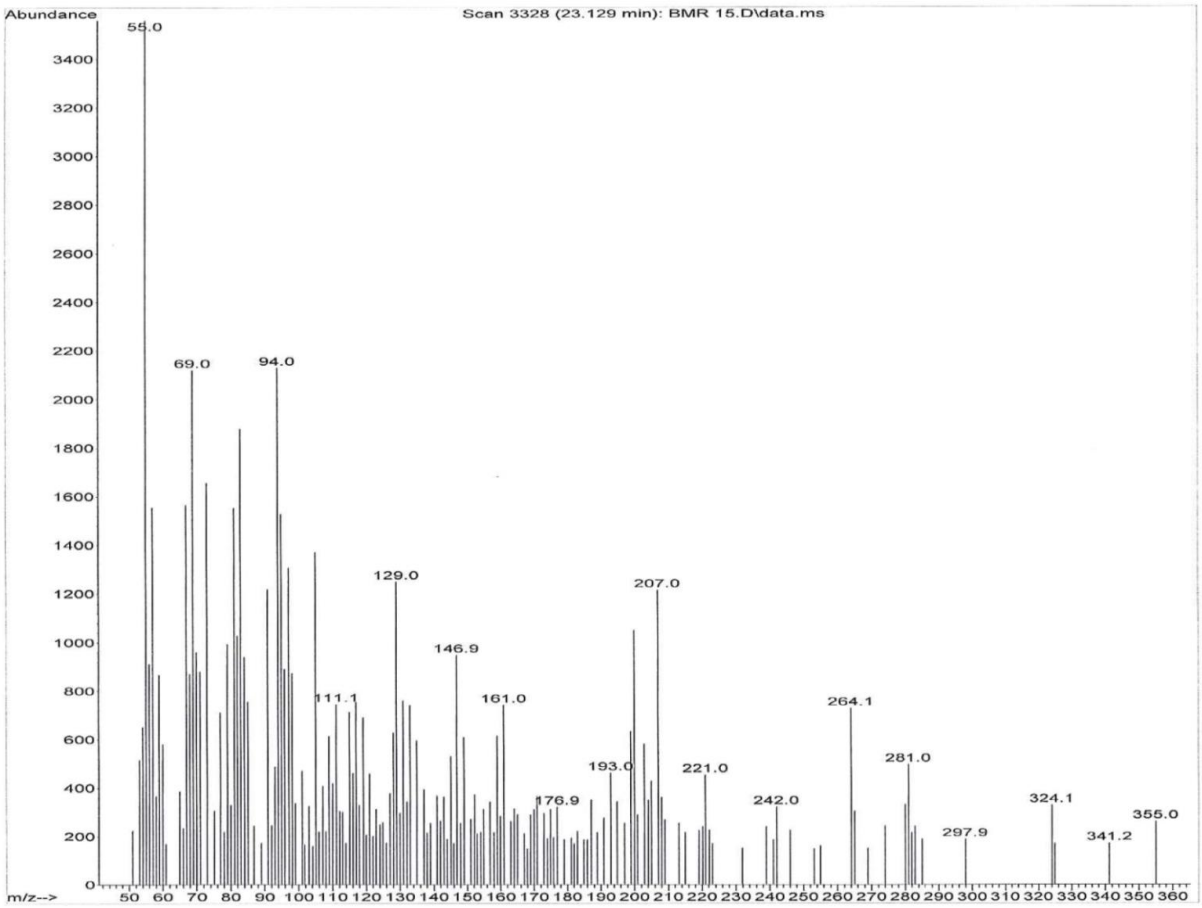


Appendix 18 b: DEPT and ^{13}C NMR spectra of *ent*-trachyloban-19-ol (408)

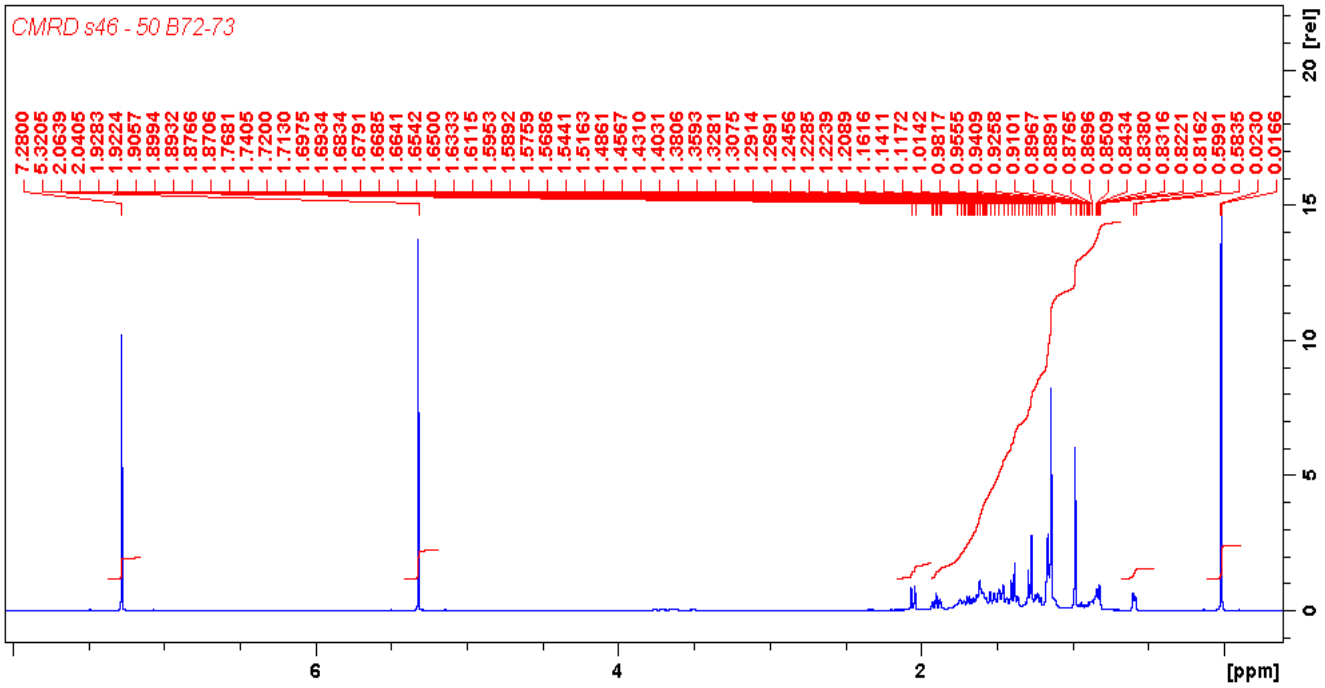


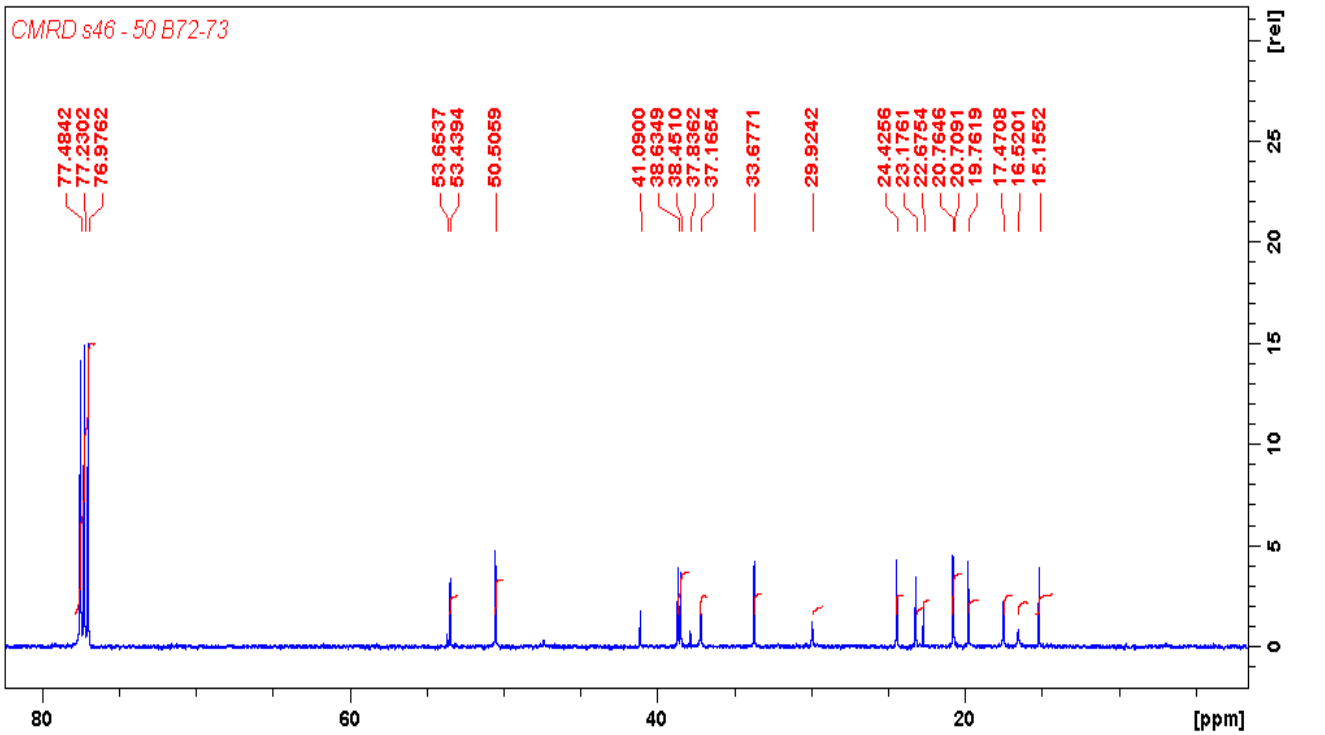


Appendix 19 a: Mass and FTIR spectra for *ent*-trachyloban-18-oic acid (409)

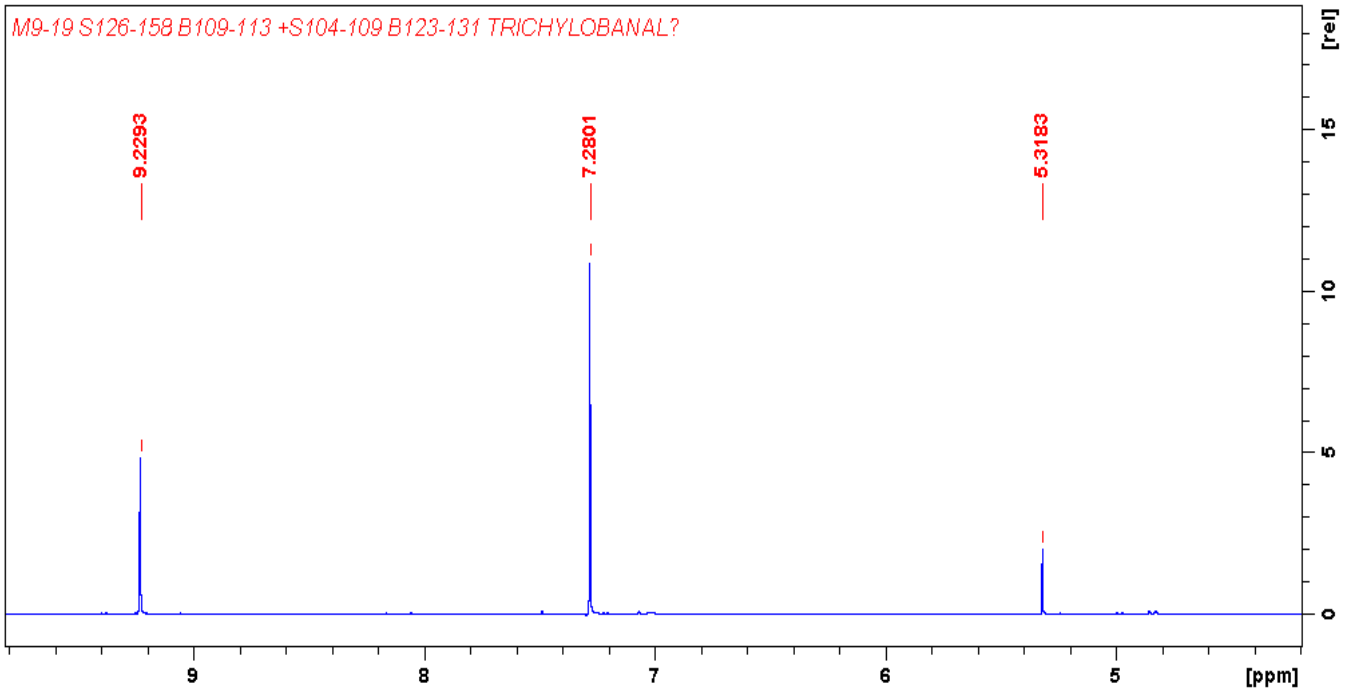


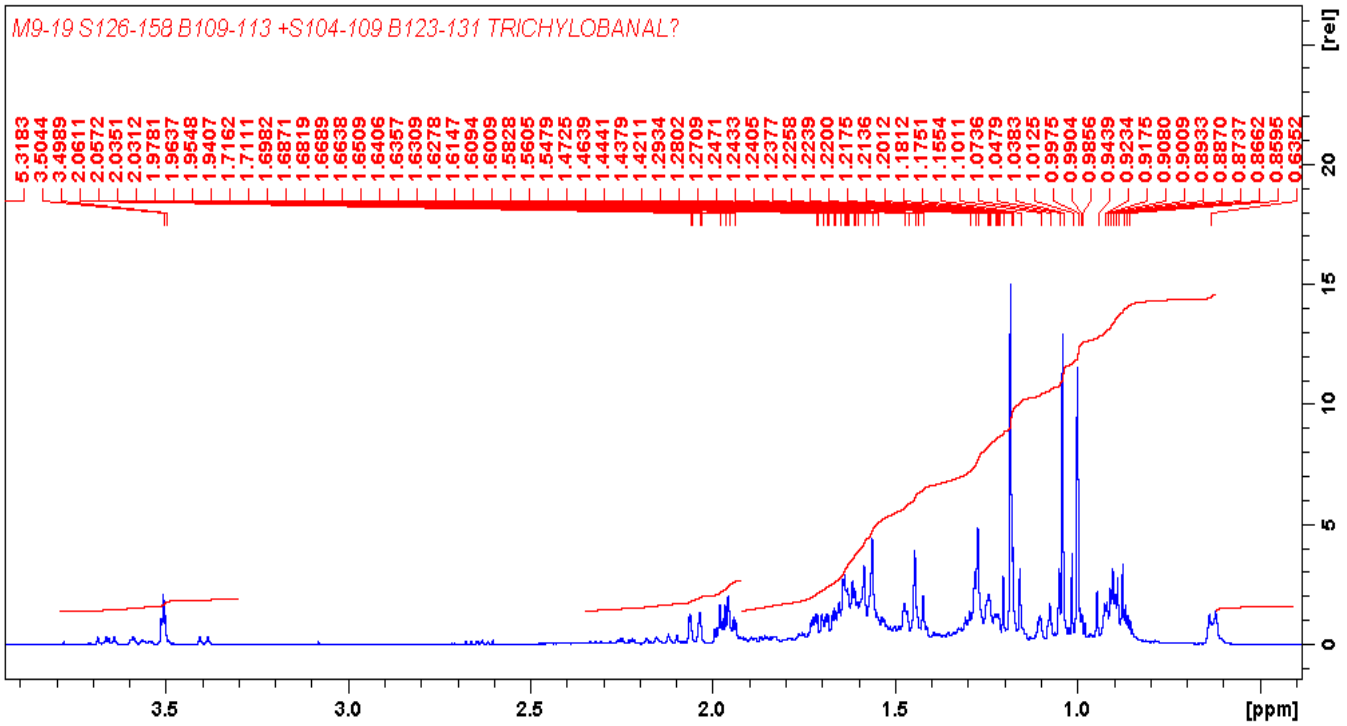
Appendix 19 b: ^{1}H and ^{13}C NMR spectra of *ent*-trachyloban-18-oic acid (409)



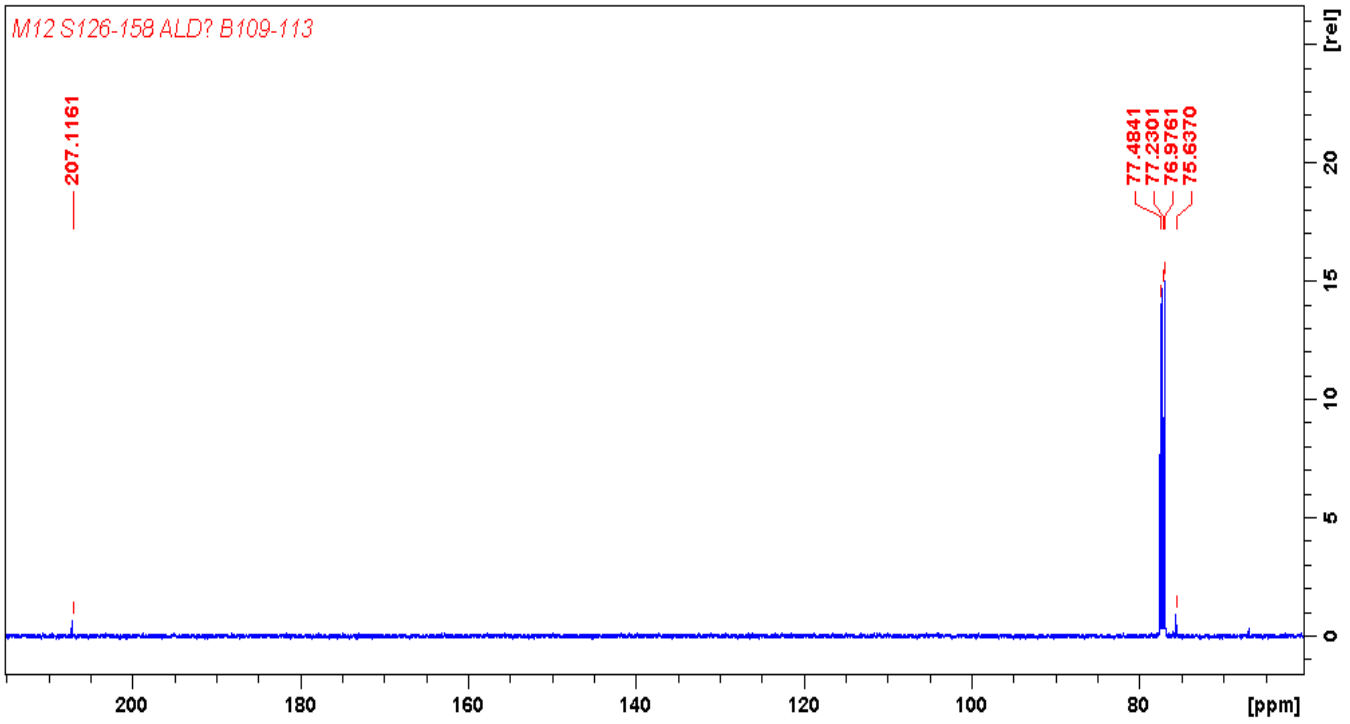


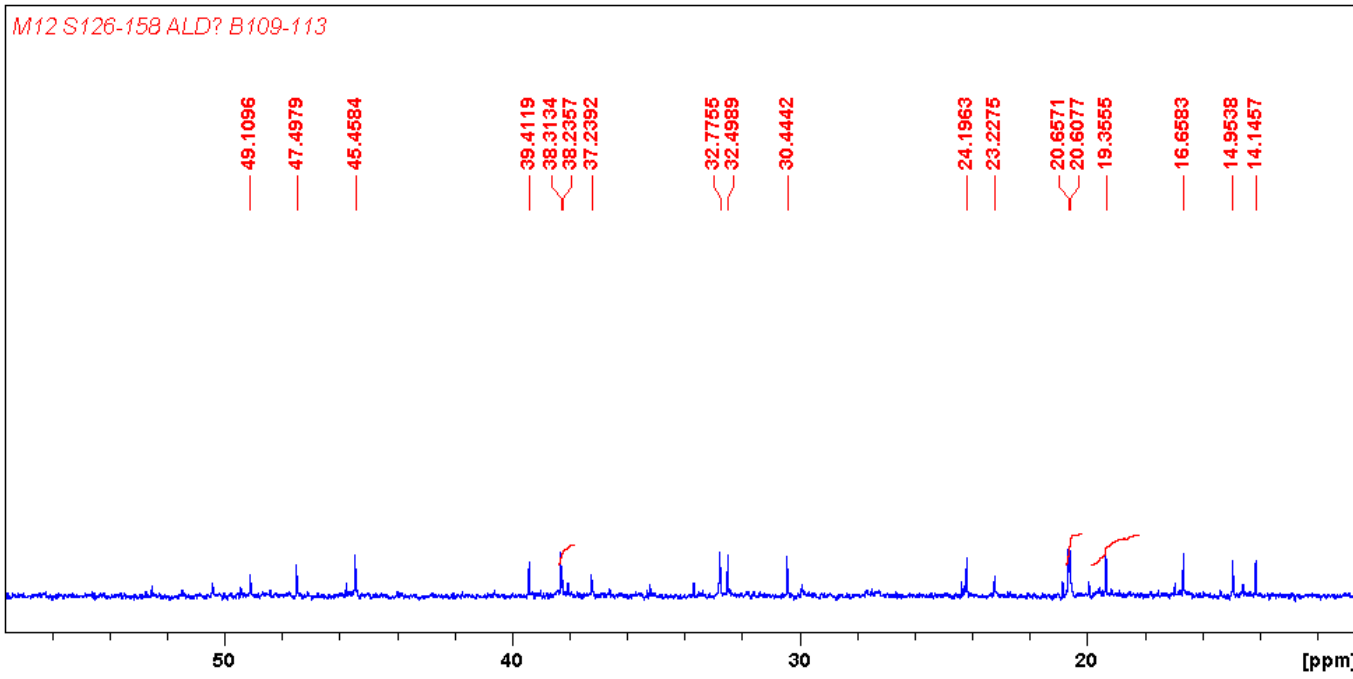
Appendix 20 a: ^{1}H NMR spectrum of 3*α*-*ent*-hydroxytrachyloban-18-al (410)



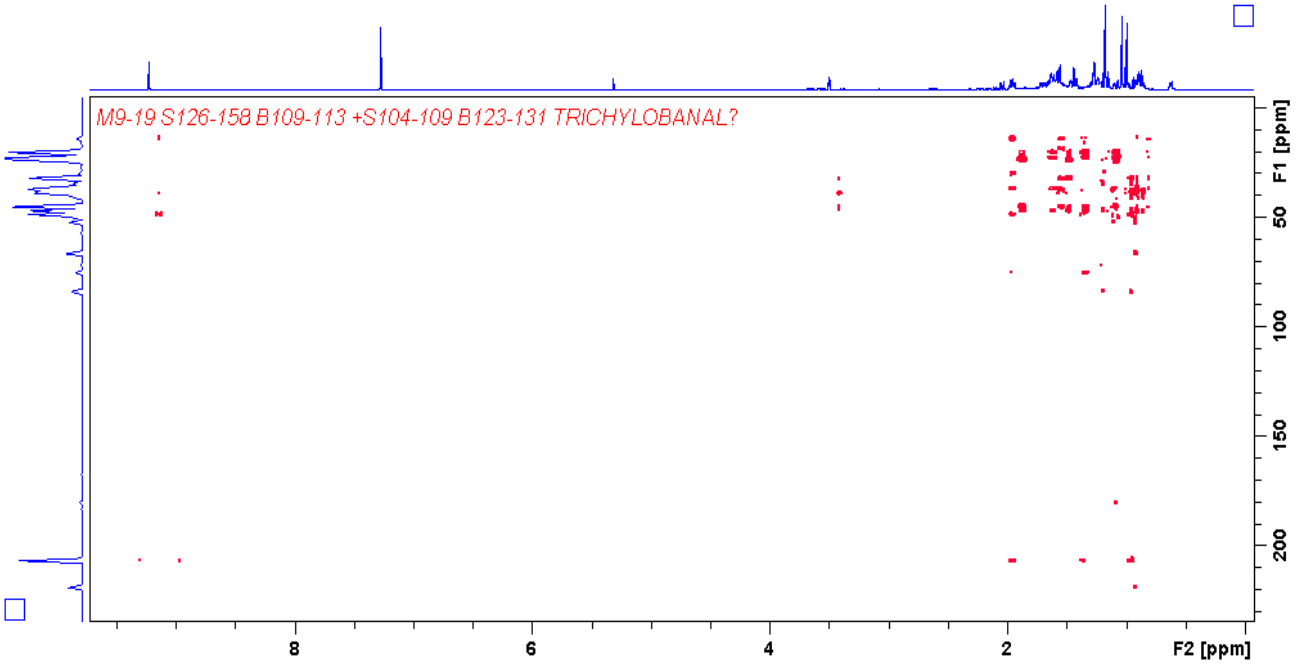


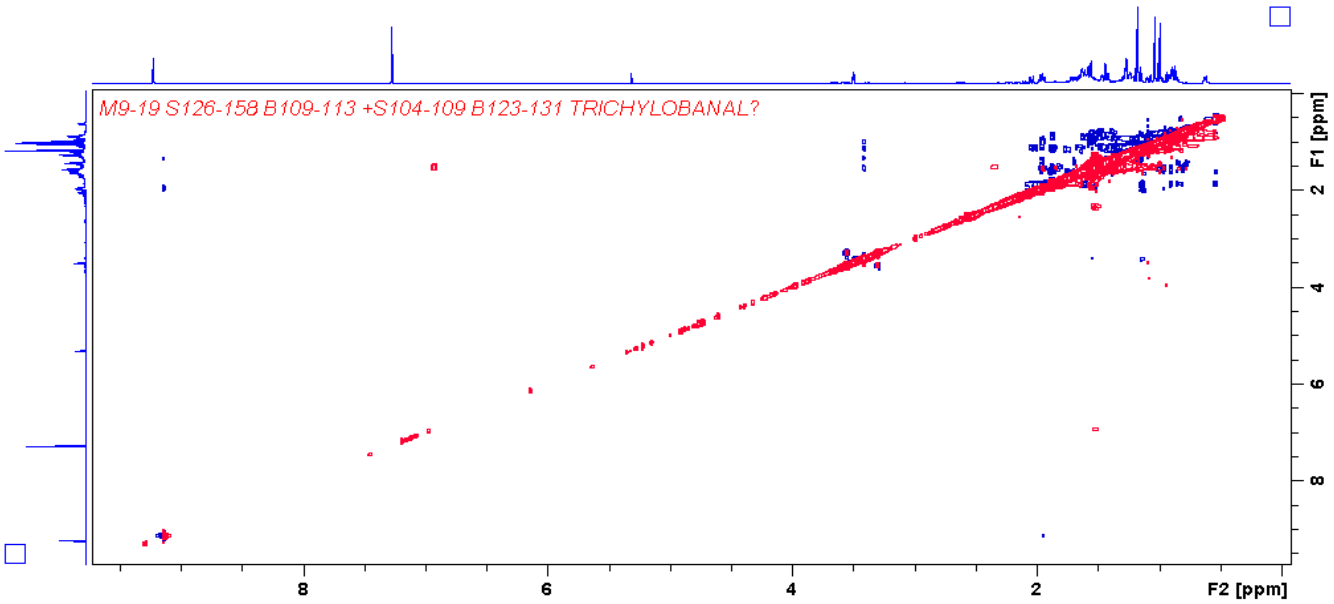
Appendix 20 b: ^{13}C NMR spectrum of 3α-*ent*-hydroxytrachyloban-18-al (410)



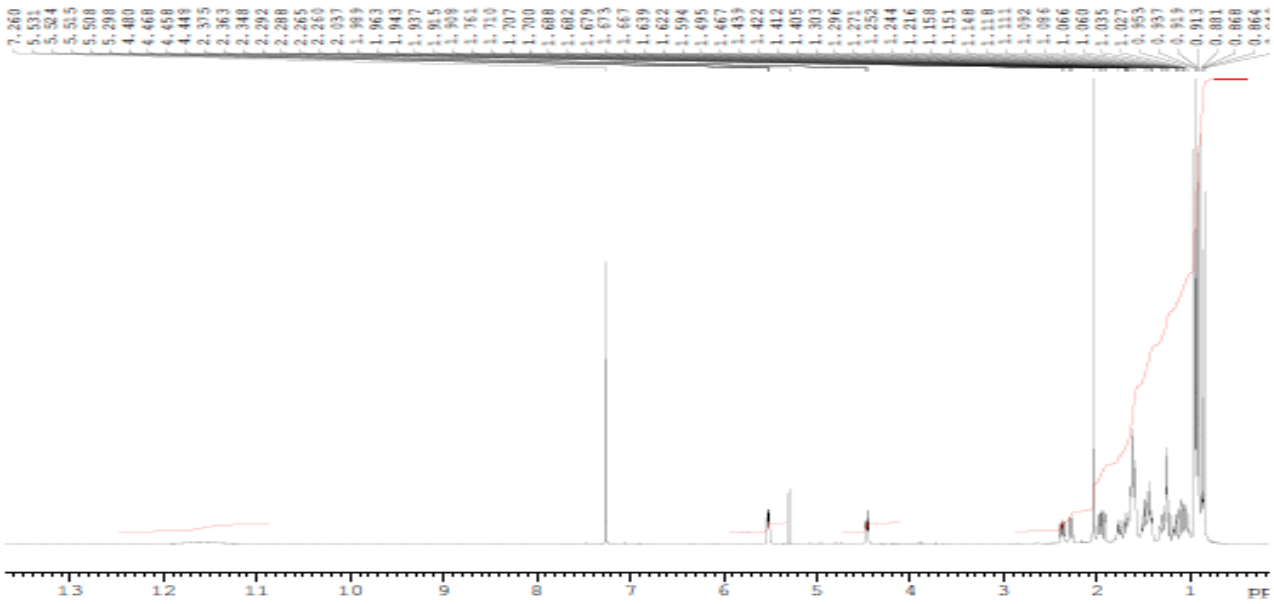


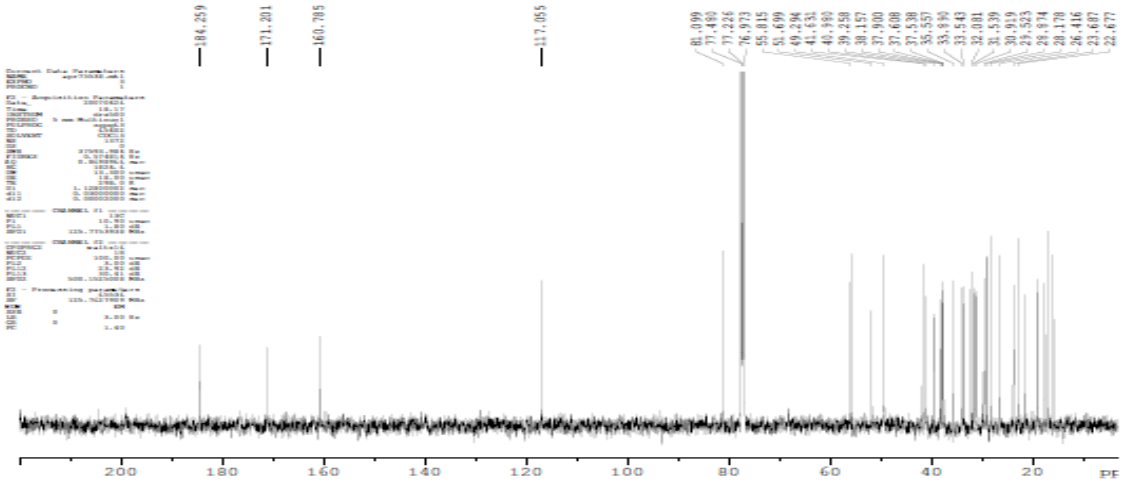
Appendix 20 c: HMBC and NOESY spectra of 3α-ent-hydroxytrachyloban-18-al (410)



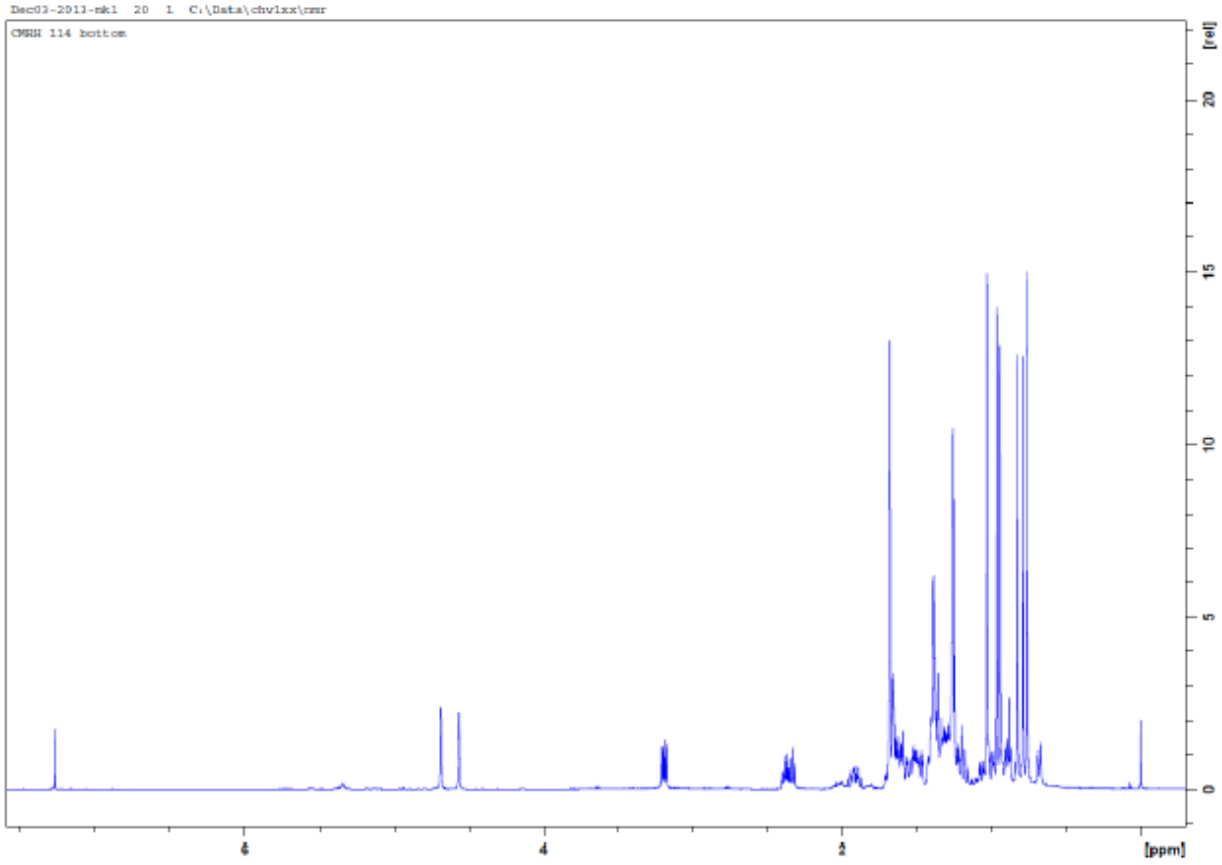


Appendix 21: ^{1}H NMR and ^{13} C NMR spectra of acetylaleuritolic acid (411)

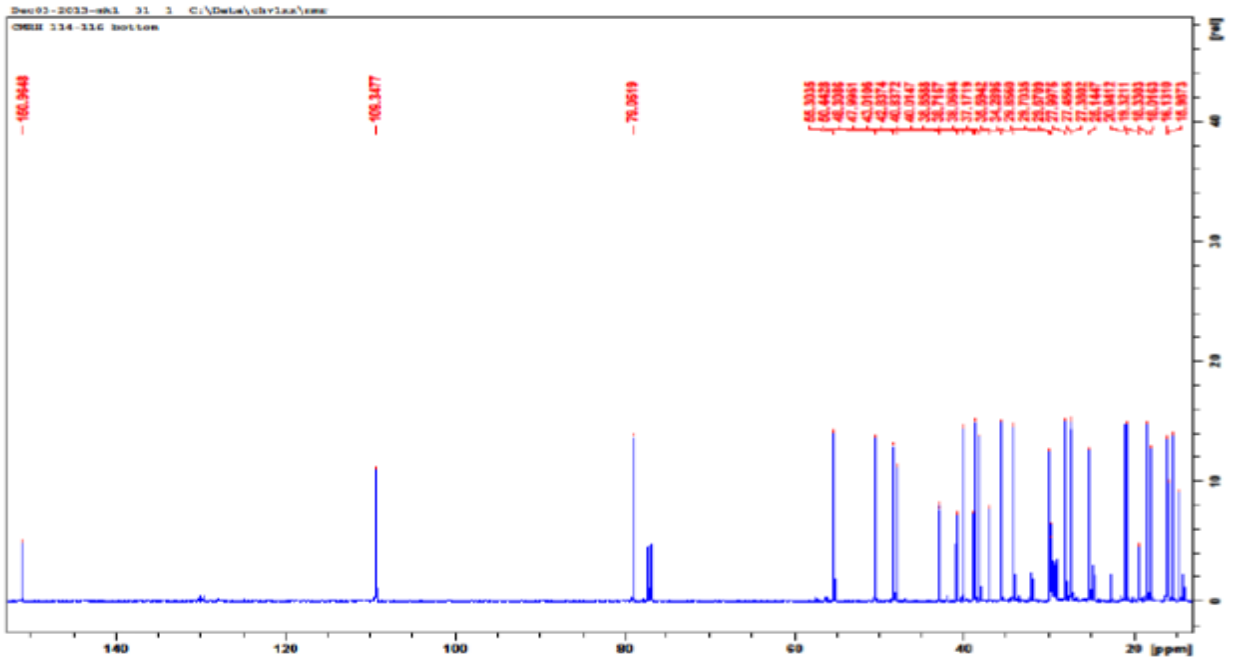


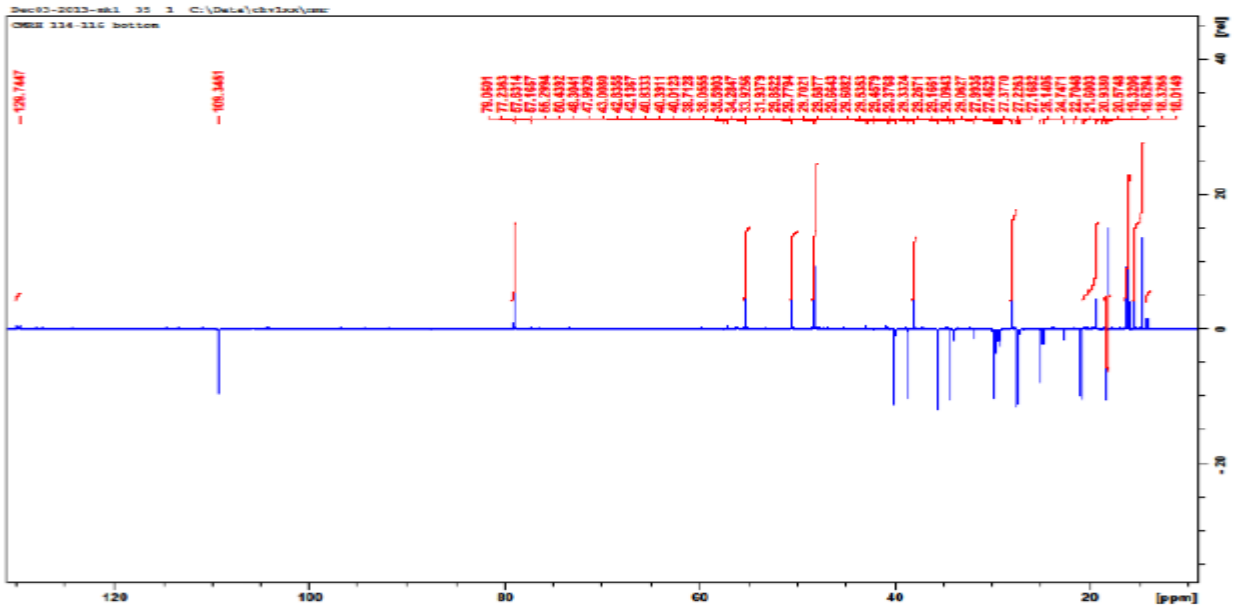


Appendix 22 a: ^{1}H NMR spectrum of lupeol (412)



Appendix 22 b: ^{13}C and DEPT NMR spectra of lupeol (412)





Appendix 23 a: Mass spectrum of alienusolin (413)

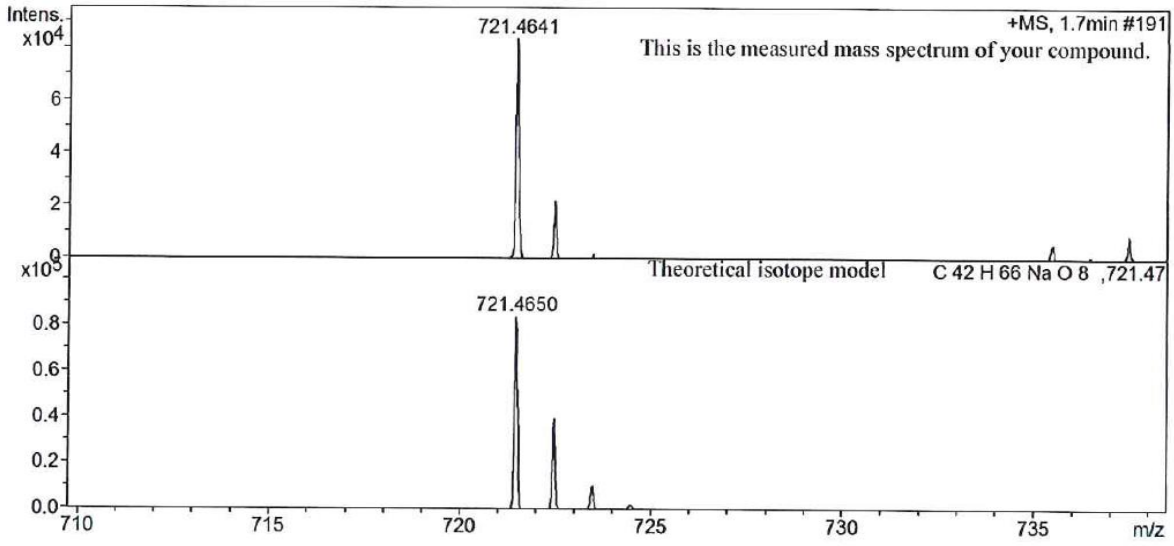
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	Z:\Dec 12\MSS11806_41_01_1407.d	Acquisition Date	10/12/2012 3:10 pm
Method	2.5min_cal_sample_pos_naf_11-10-10.m	Operator	Mass Spec
Sample Name	MSS11806	Instrument / Ser#	micrOTOF 92
Comment			

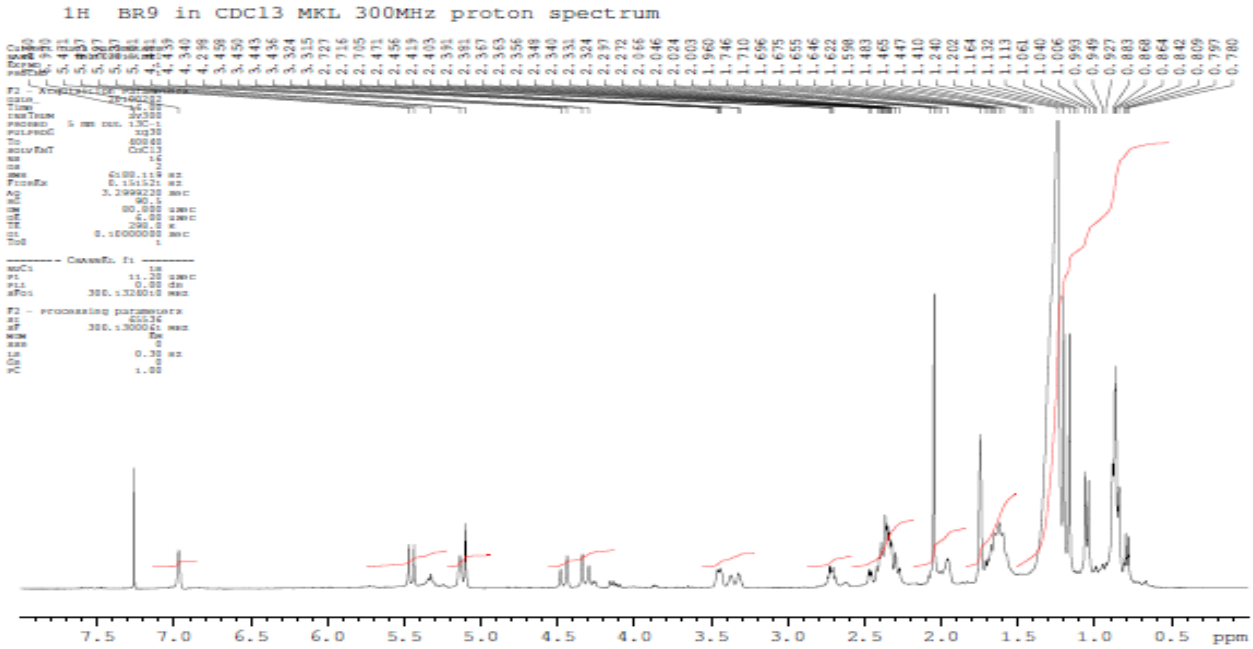
Acquisition Parameter

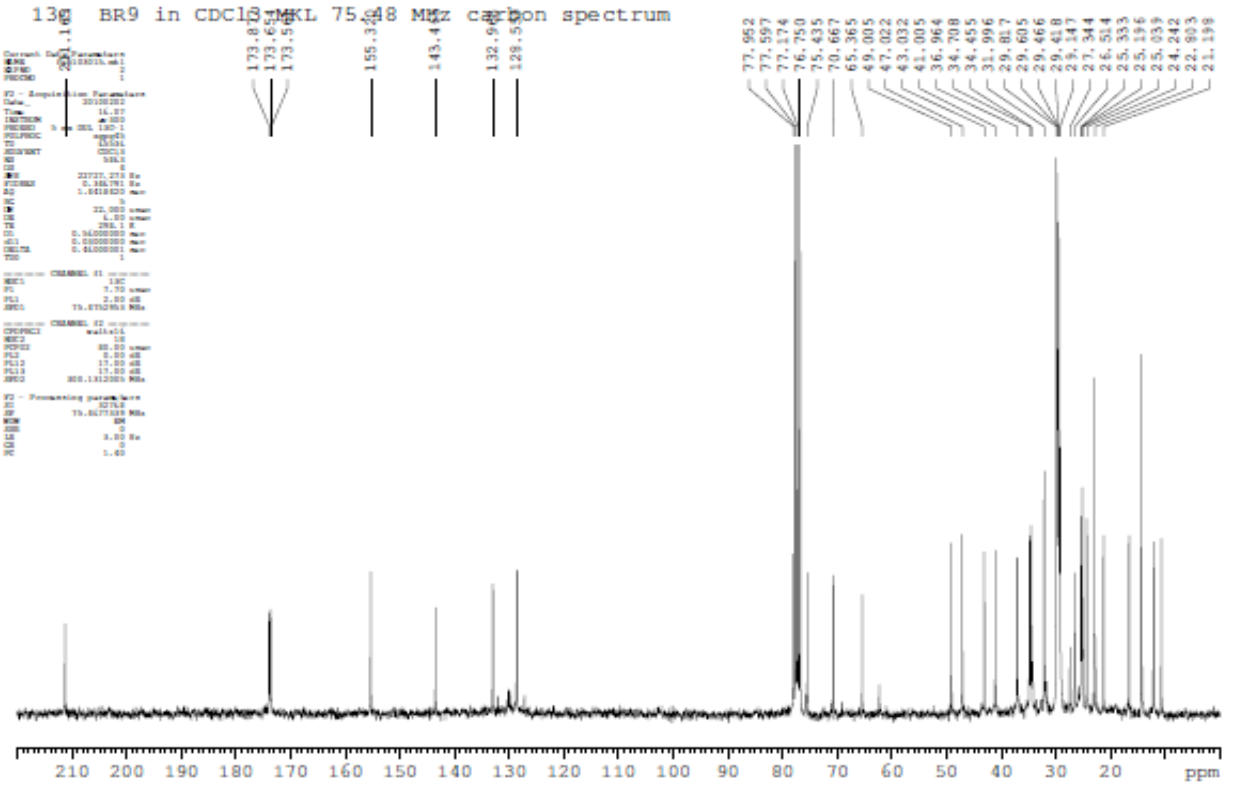
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.0 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	10.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



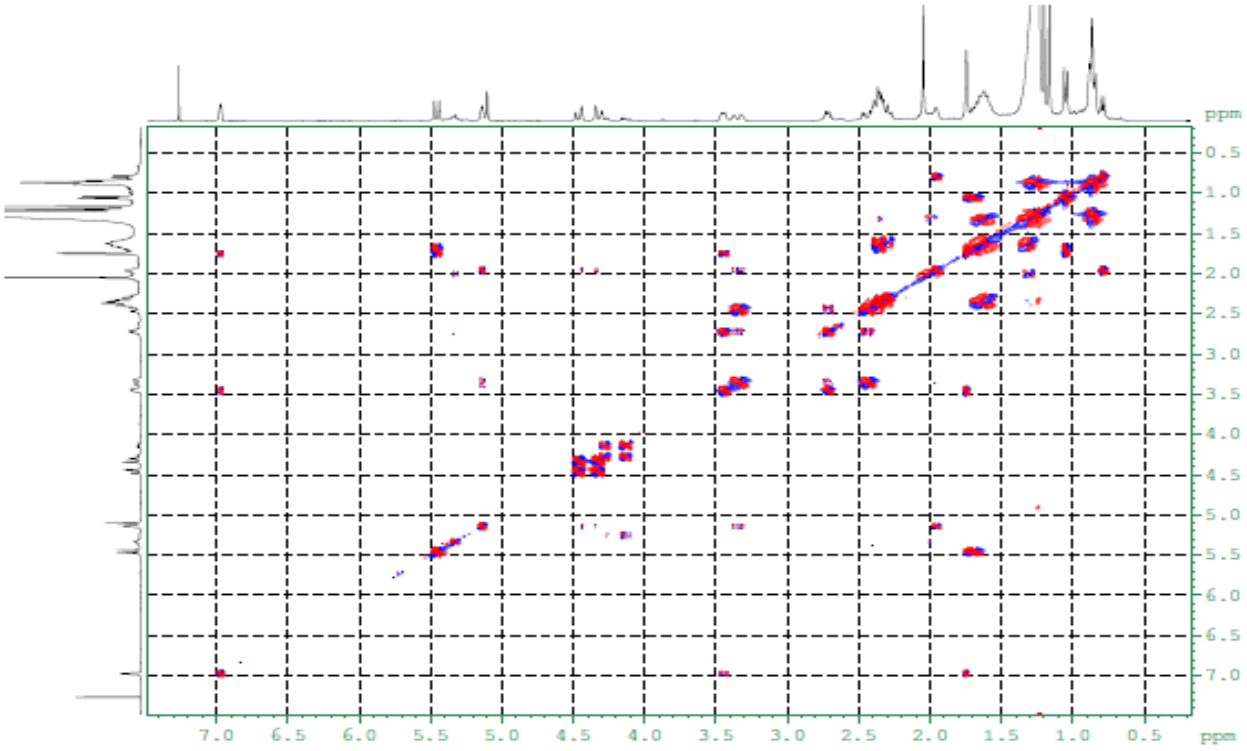
Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	e⁻ Conf	mSigma
721.4641								
	1	C 42 H 66 Na O 8	721.4650	1.3	1.3	9.5	even	111.08

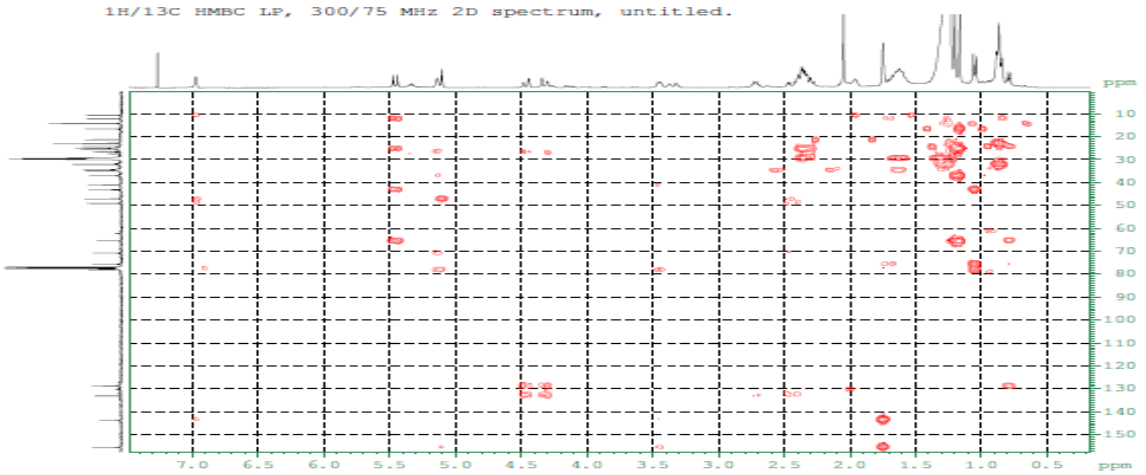
Appendix 23 b: ^{1}H NMR and ^{13}C NMR spectra of alienusolin (413)



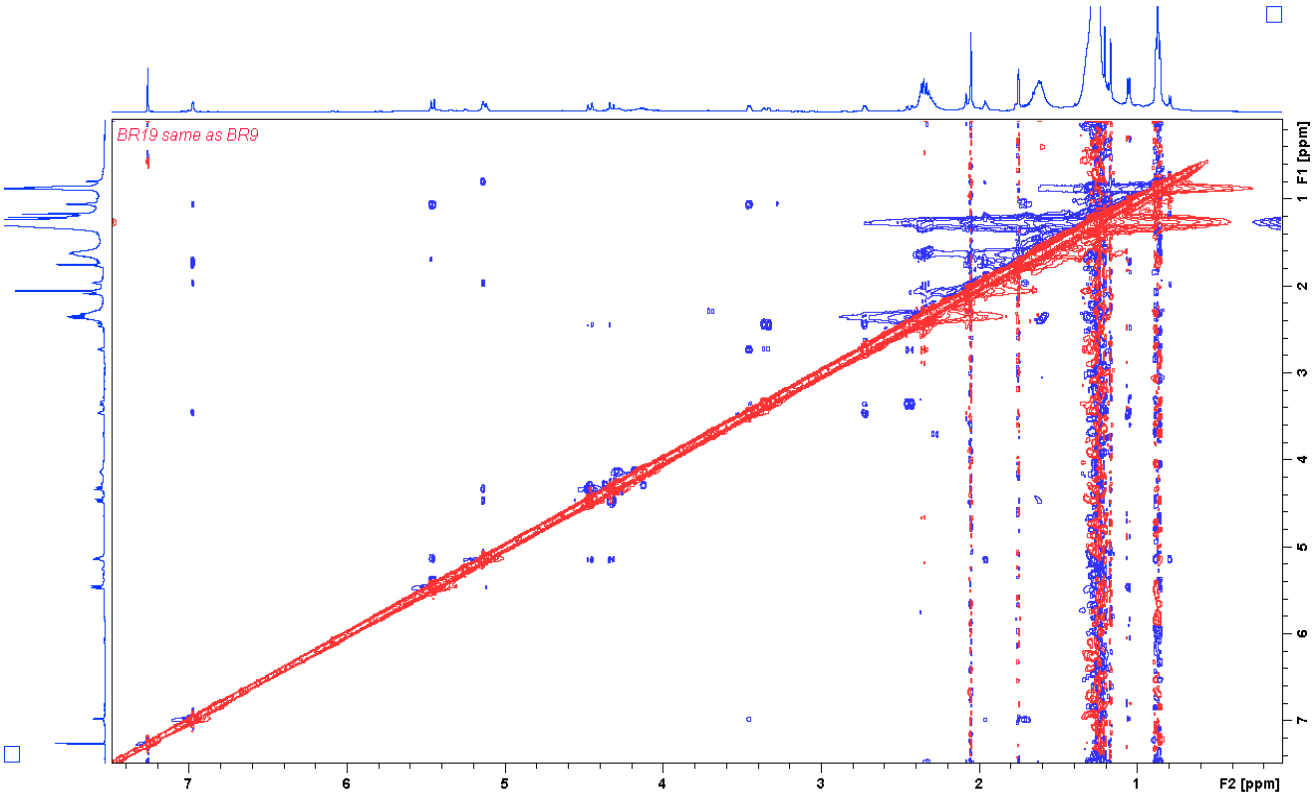


Appendix 23 c: COSY and HMBC spectra for alienusolin (413)

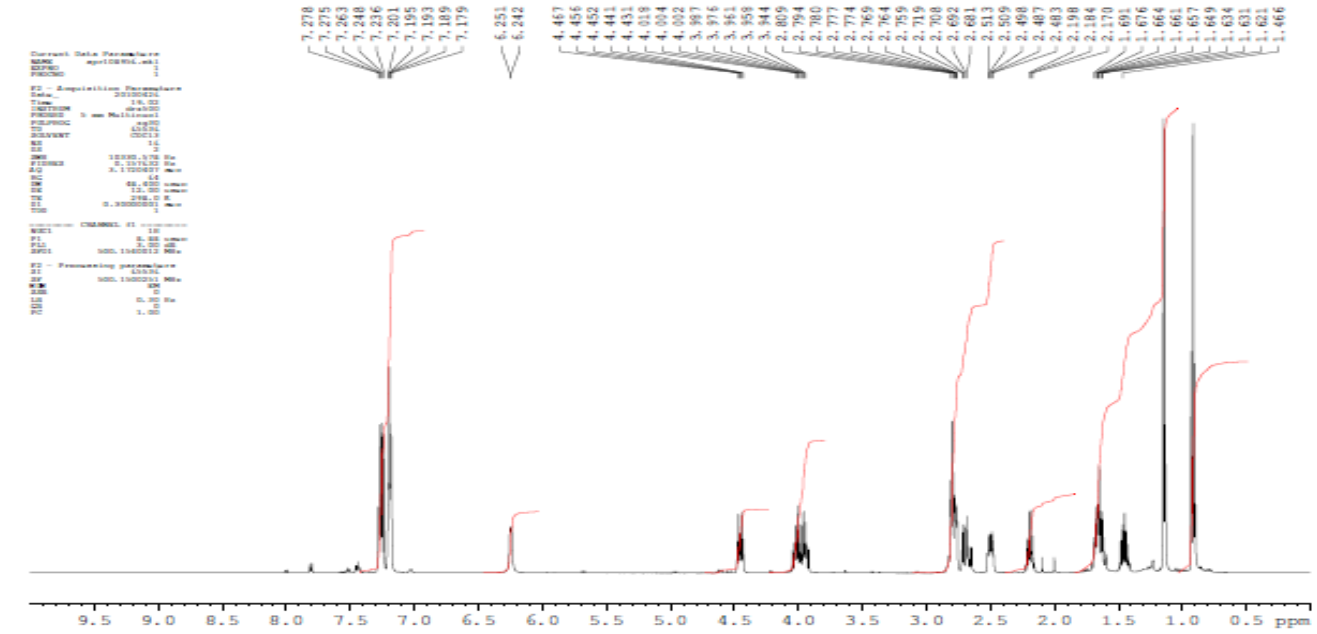


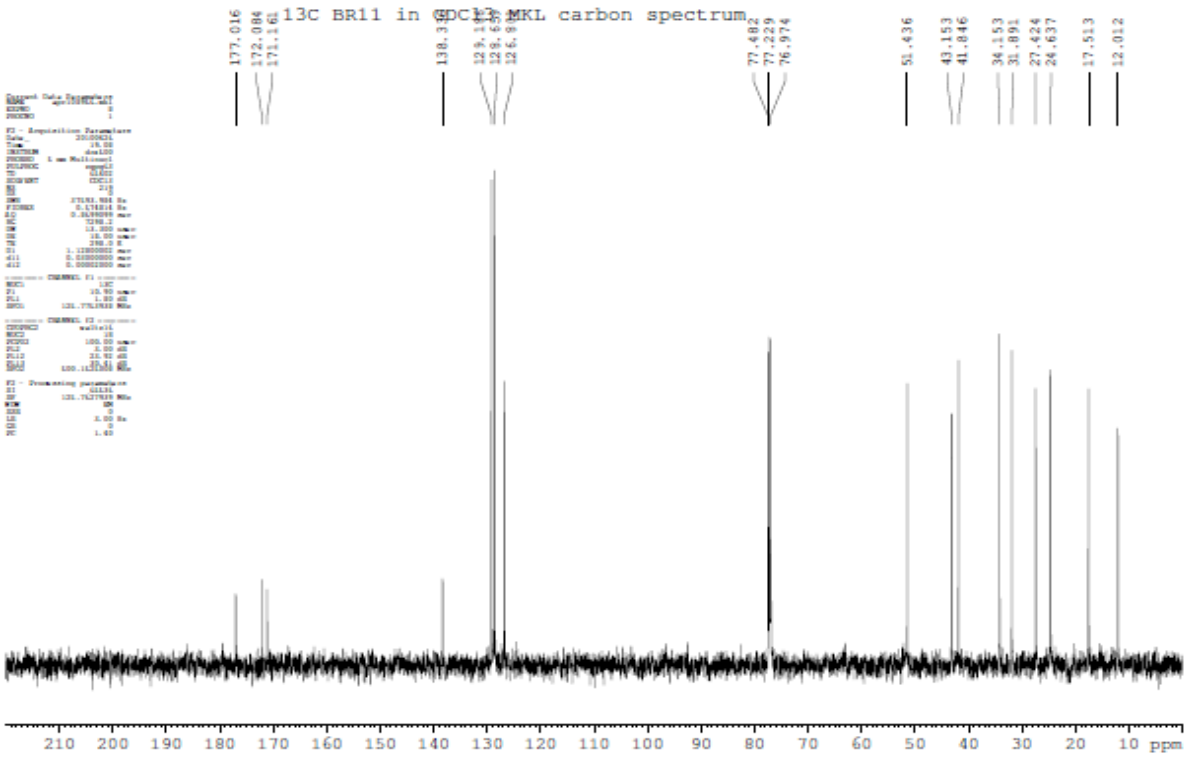


Appendix 23 d: NOESY spectrum of alienusolin (413)



Appendix 24 1: ^{1}H NMR and ^{13}C NMR spectra of julocrotine (414)





Appendix 25 a: HRESIMS spectrum of crotonamide C (415)

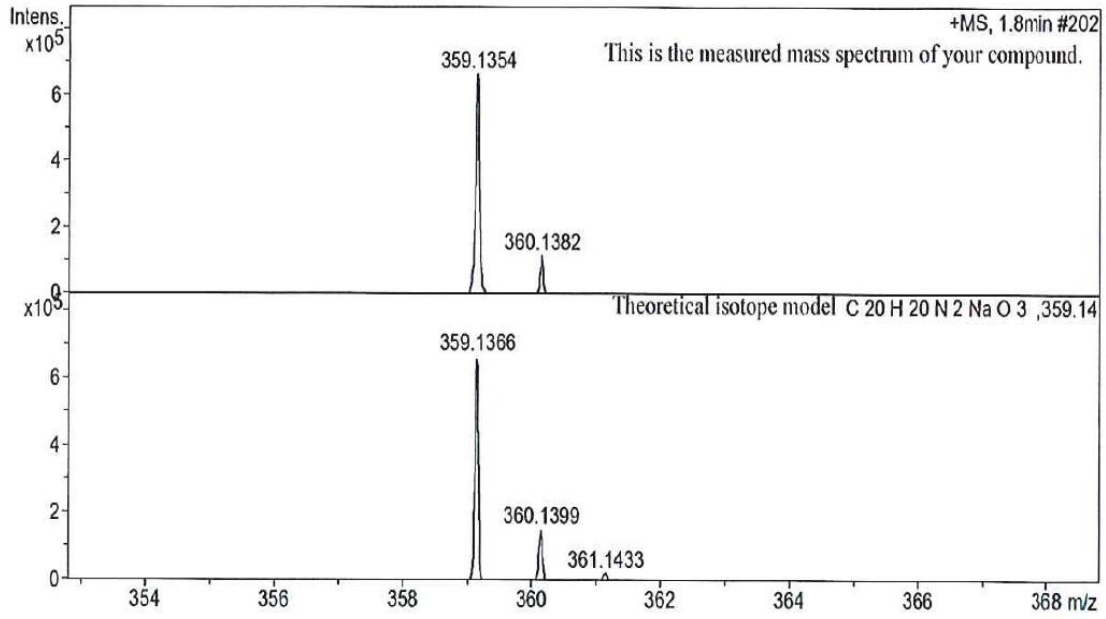
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	Z:\Dec 12\MSS11807_42_01_1408.d	Acquisition Date	10/12/2012 3:14 pm
Method	2.5min_cal_sample_pos_naf_11-10-10.m	Operator	Mass Spec
Sample Name	MSS11807	Instrument / Ser#	micrOTOF 92
Comment			

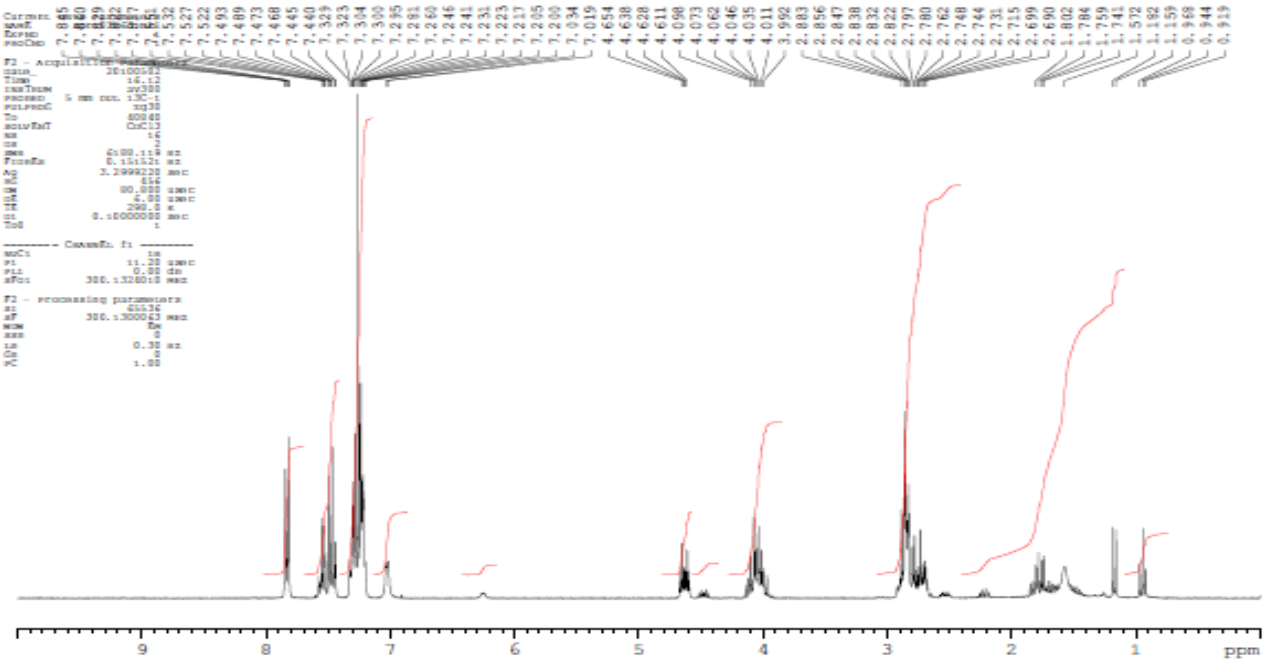
Acquisition Parameter

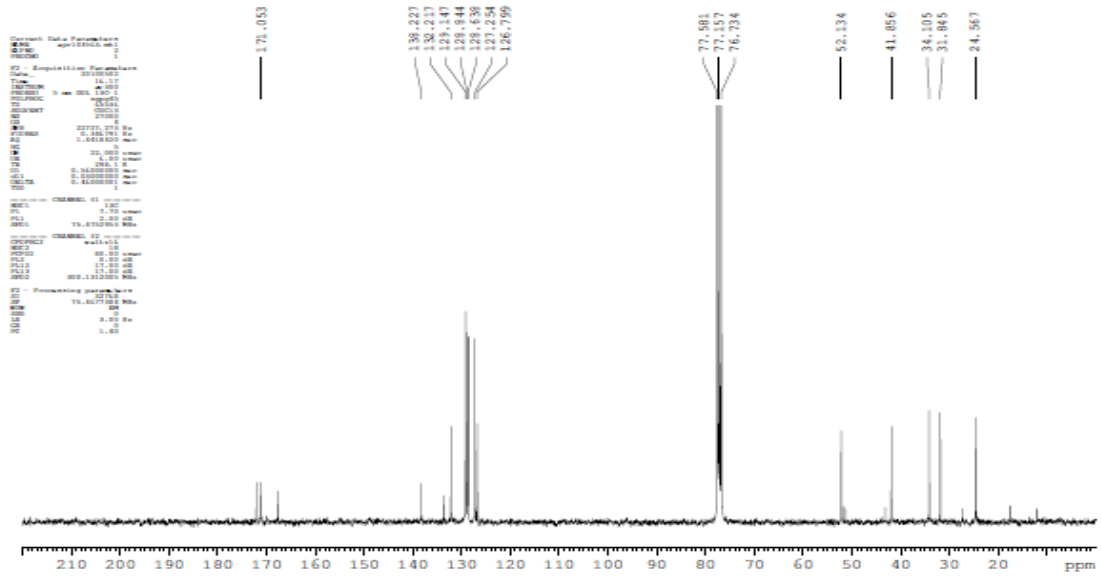
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.0 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	10.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



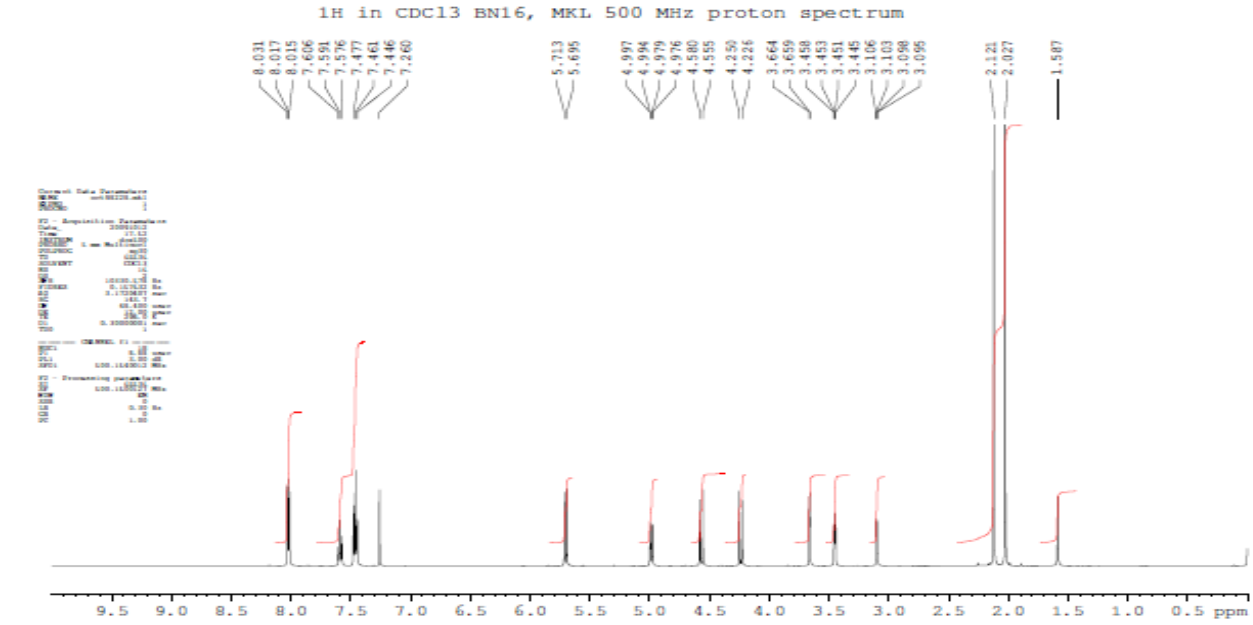
Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	e⁻ Conf	mSigma
359.1354								
	1	C 20 H 20 N 2 Na O 3	359.1366	3.4	3.6	11.5	even	31.88

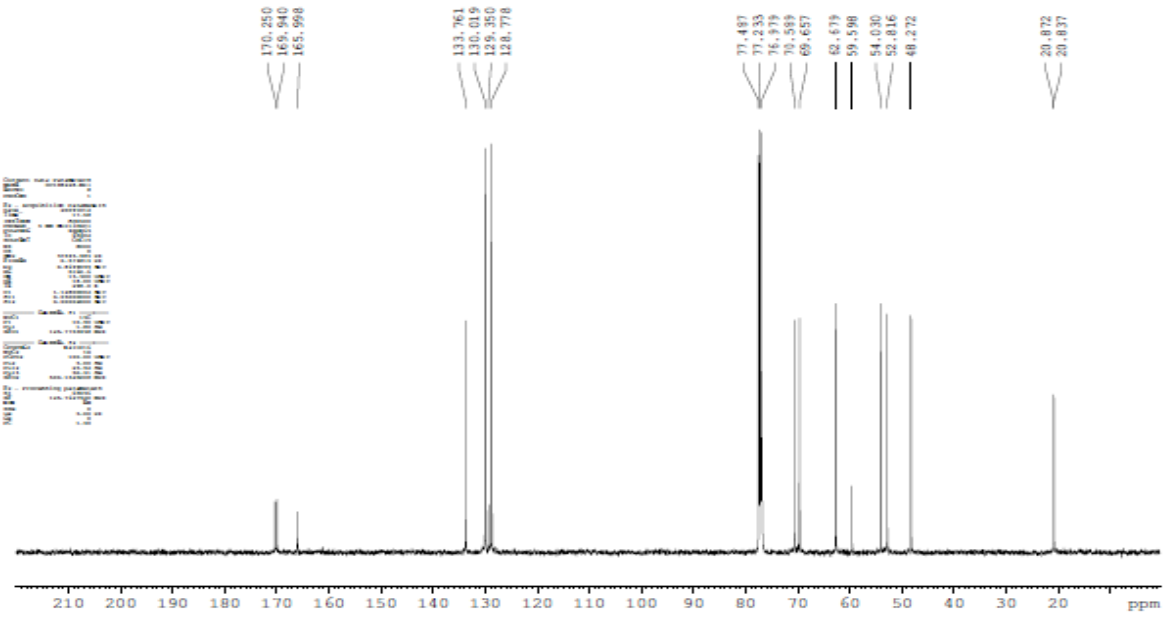
Appendix 25 b: ^{1}H NMR and ^{13}C NMR spectra for crotonimide C (415)



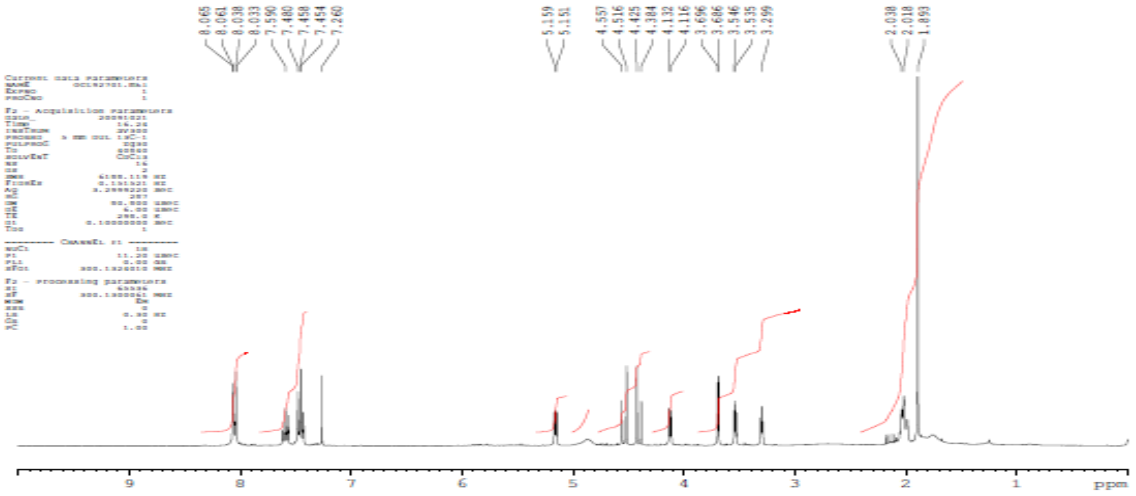


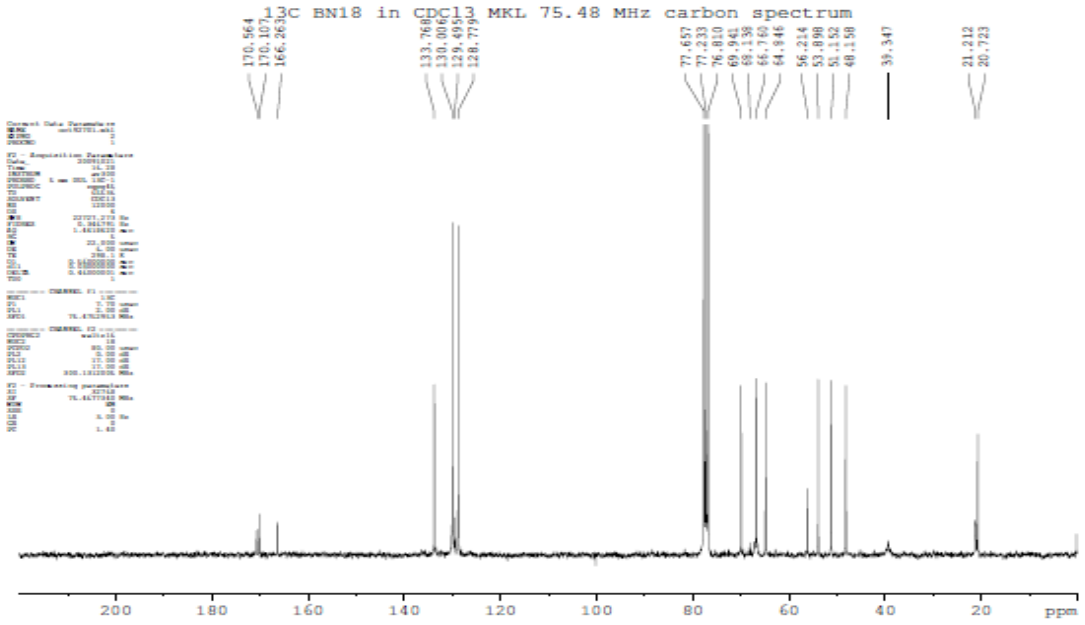
Appendix 26 1: ^{1}H NMR and ^{13}C NMR spectra of crotepoxide (416)



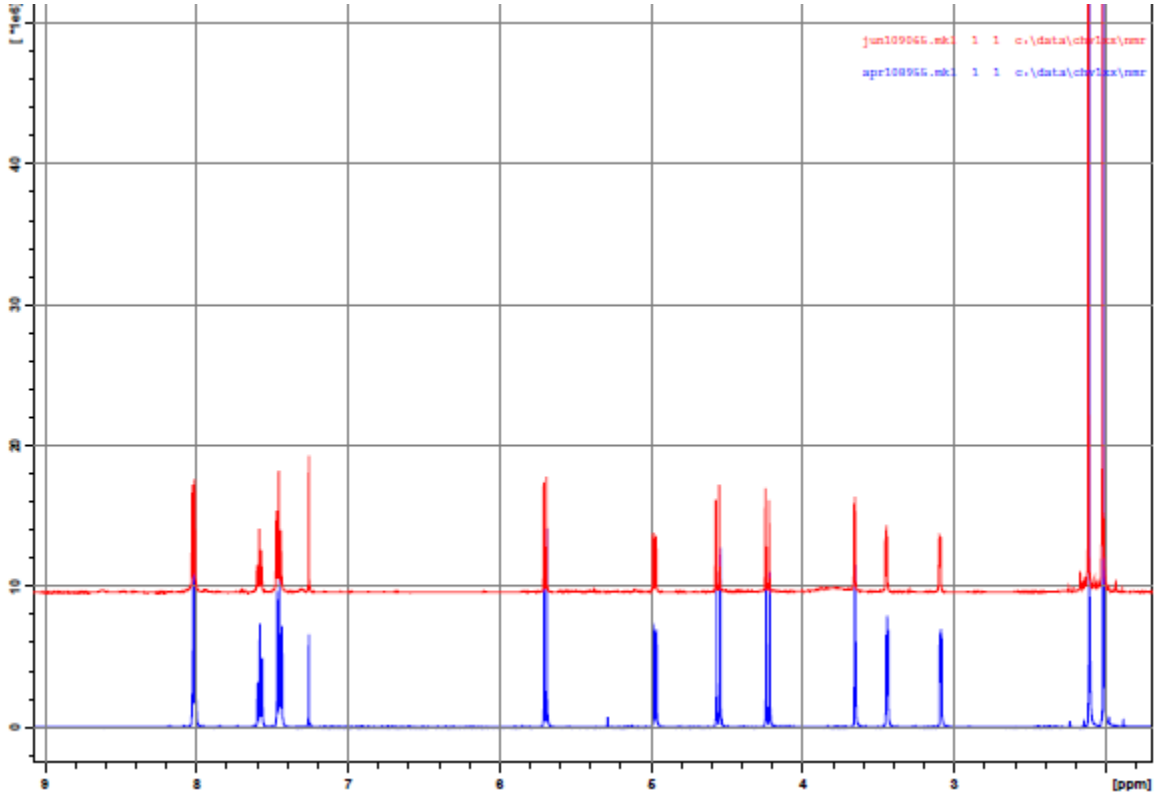


Appendix 27 a: ^{1}H NMR and ^{13}C NMR spectra of monodeacetylcrotepoxide (417)

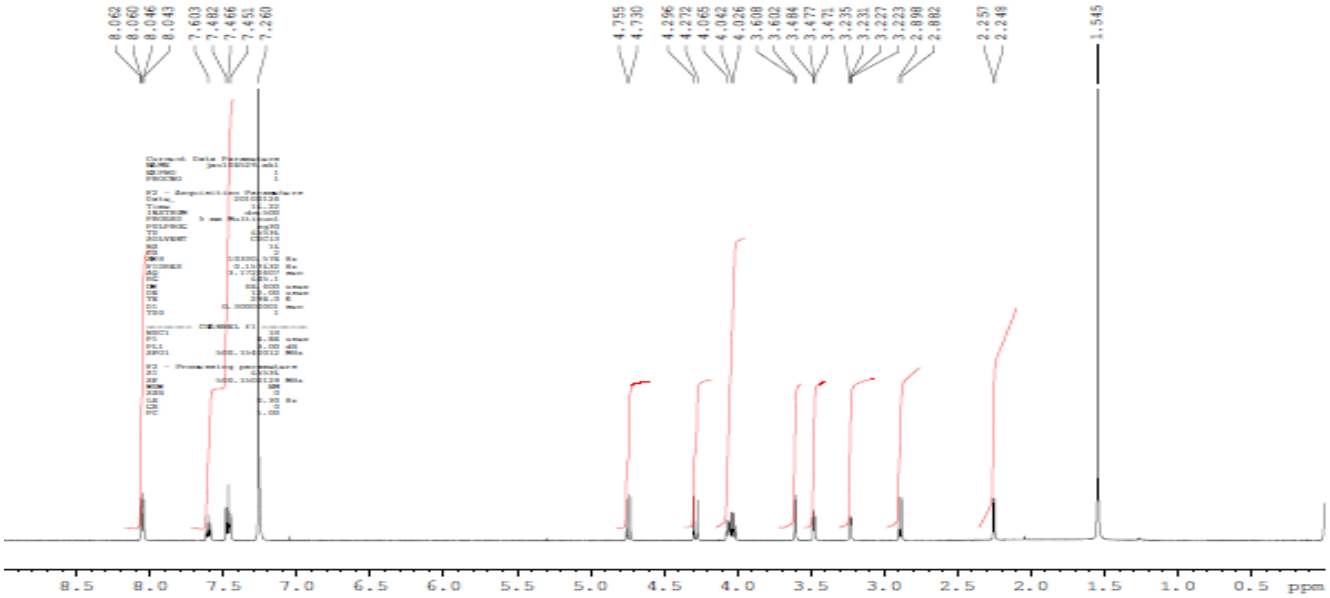


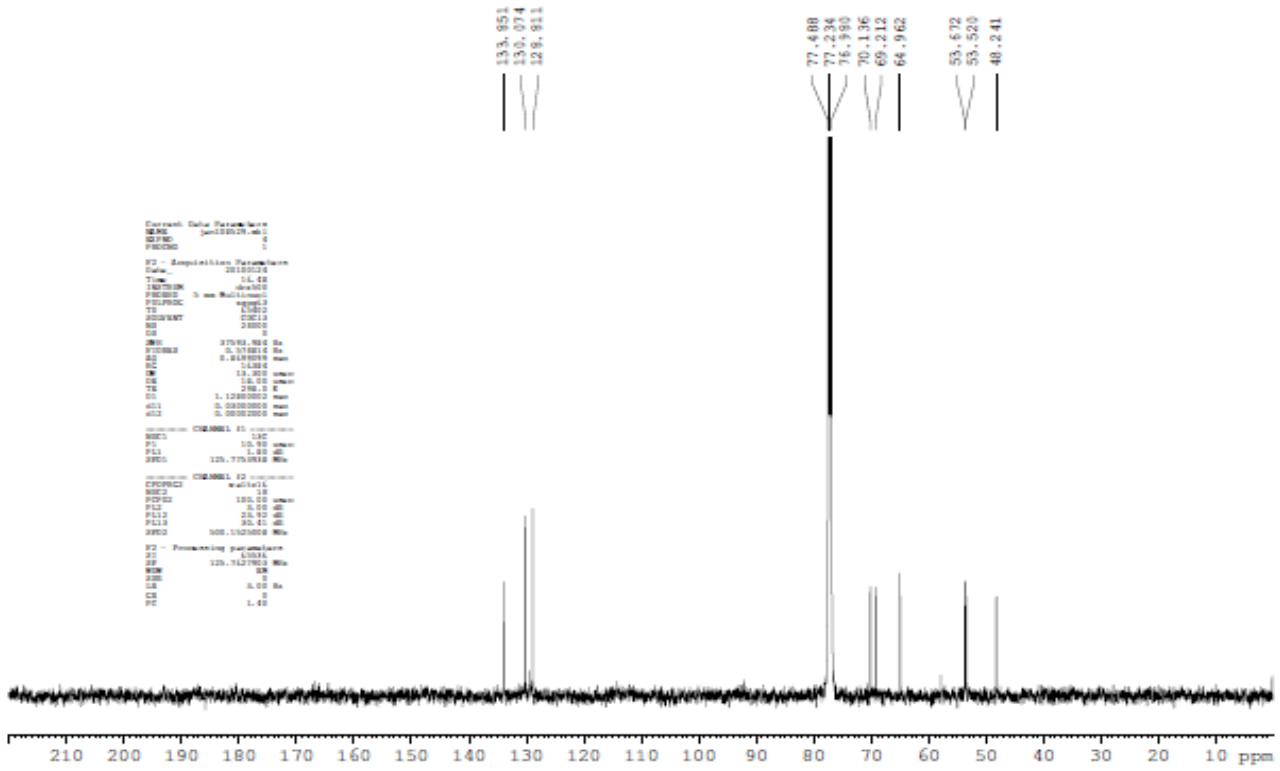


Appendix 27 b: Overlaid ^{1}H spectra of 416 & acetylated 417

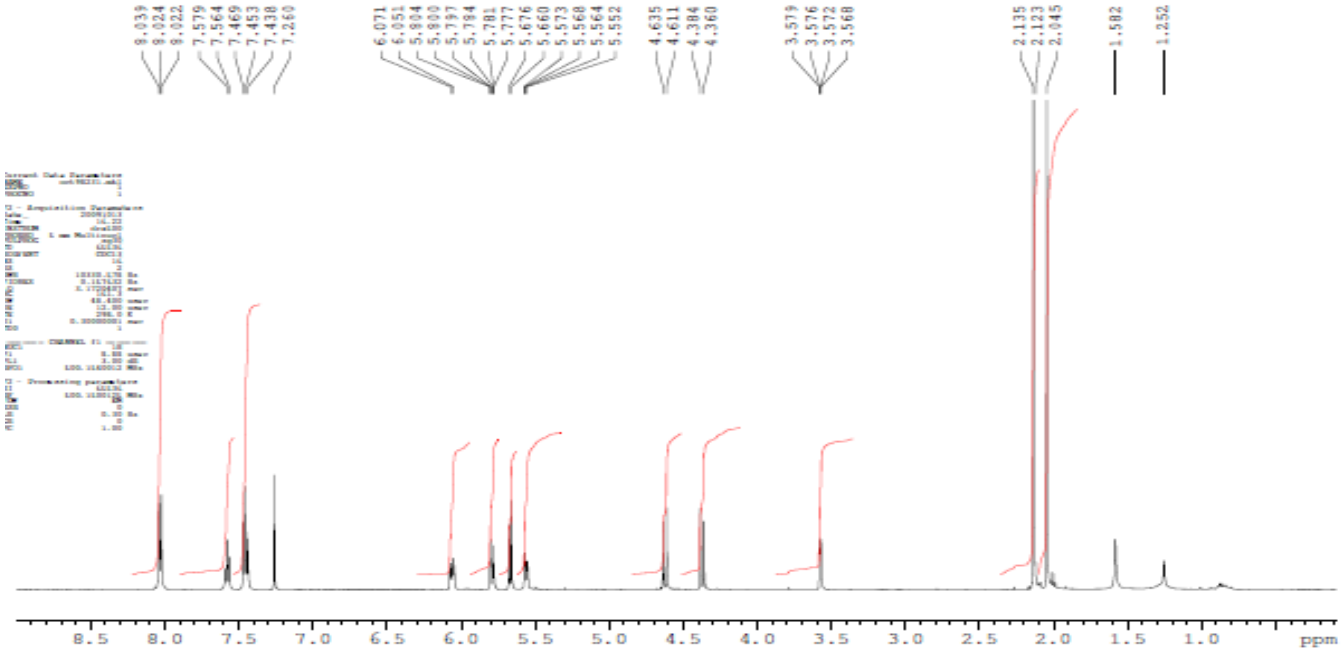


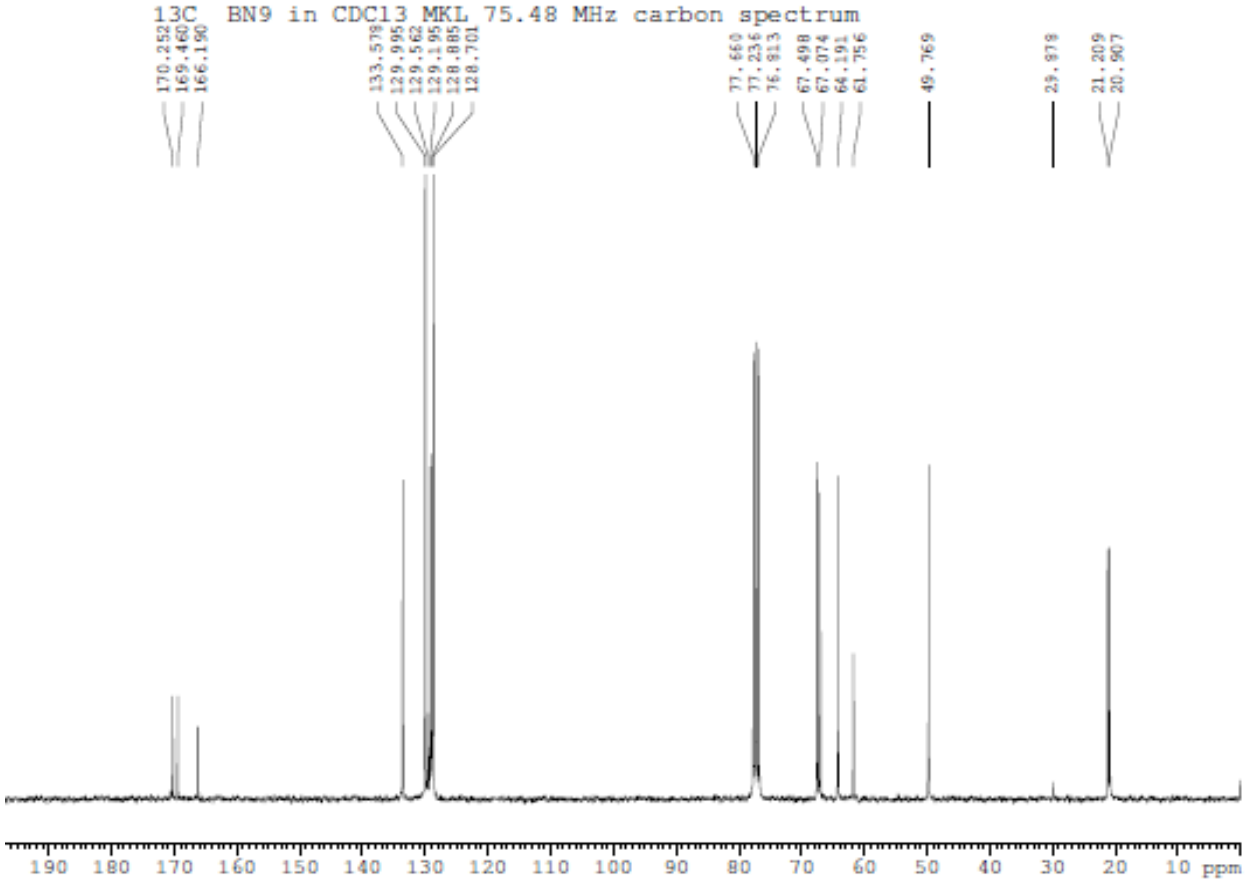
Appendix 28 1: ^{1}H NMR and ^{13}C NMR spectra of dideacetylcrotepoxide (418)



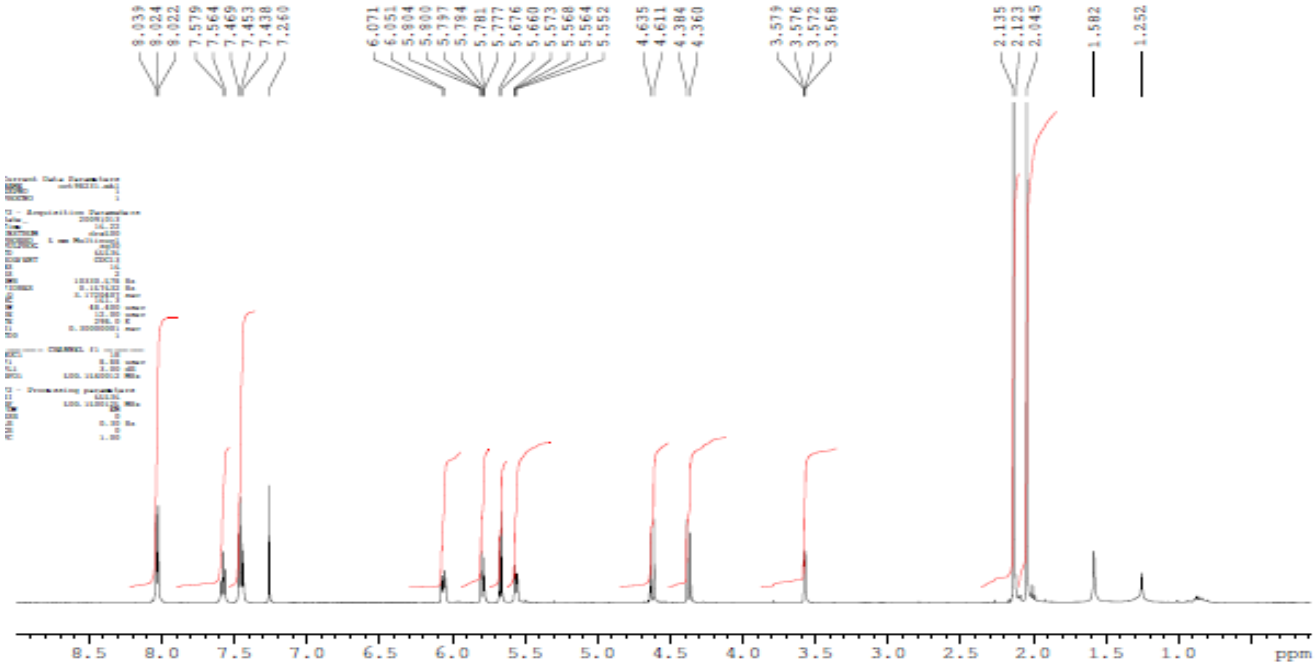


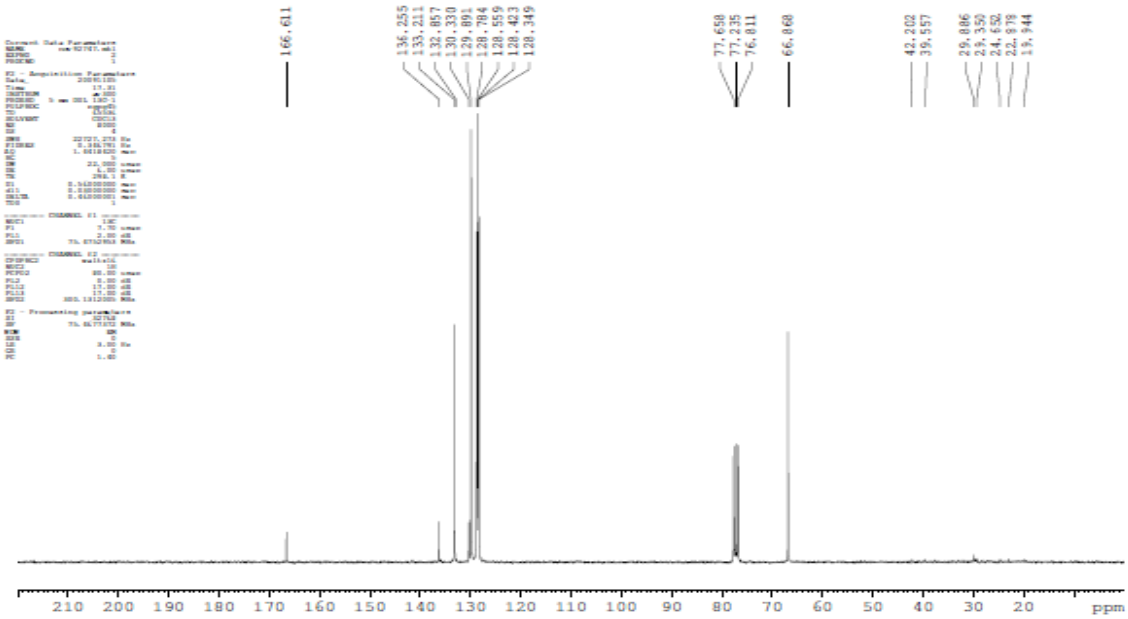
Appendix 29 1: ^{1}H NMR and ^{13}C NMR spectra of senepoxide (419)



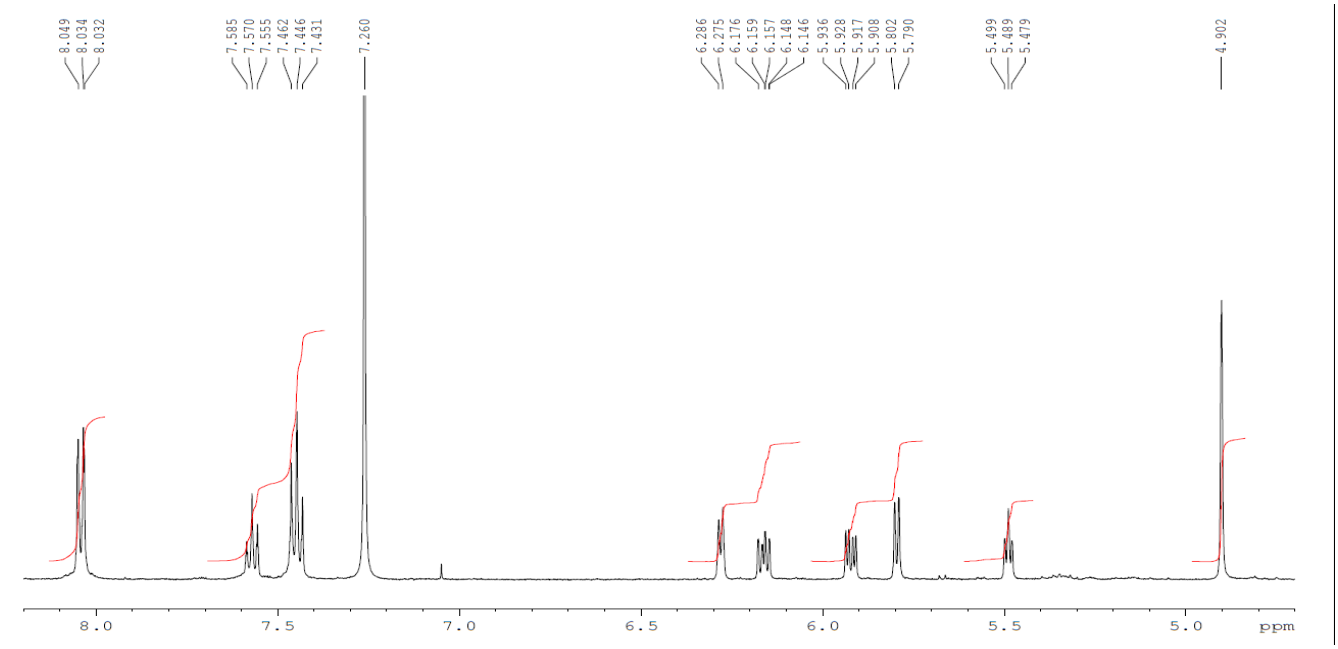


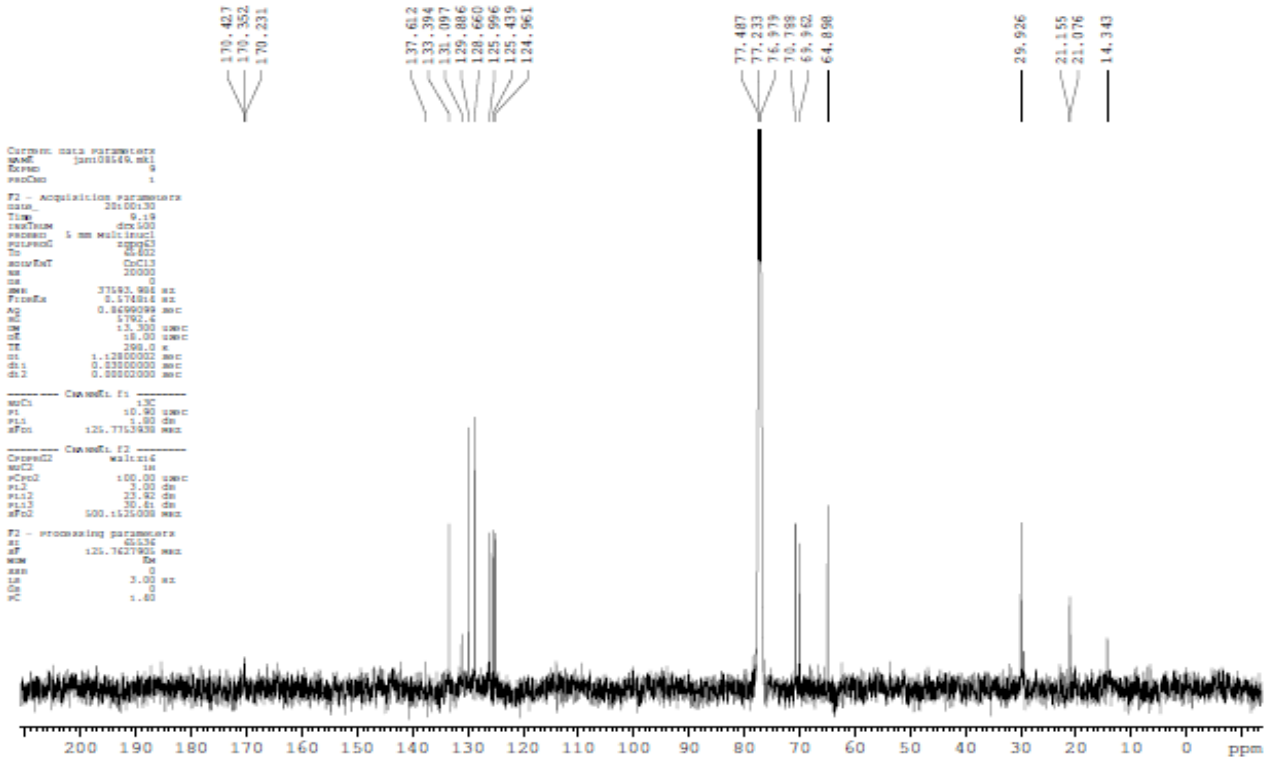
Appendix 30 1: ^{1}H NMR and ^{13}C NMR spectra for β-Senepoxide (420)



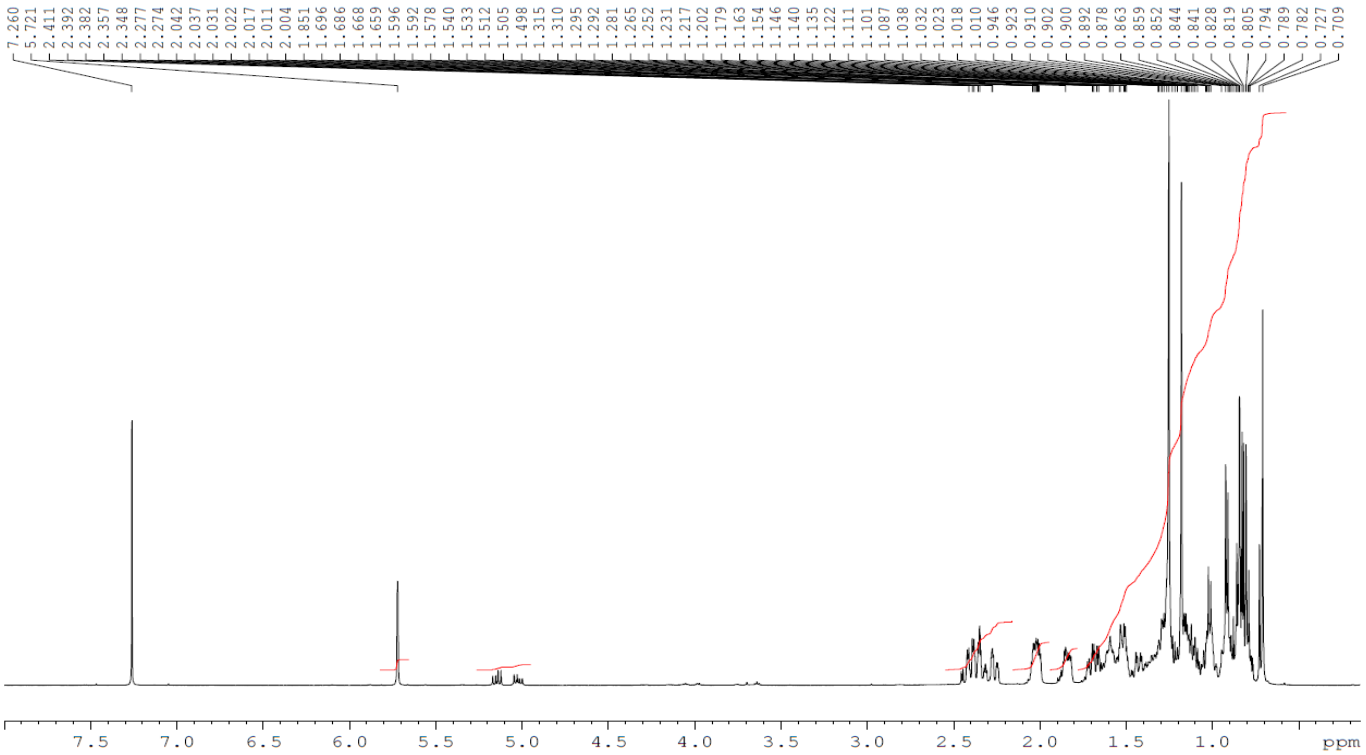


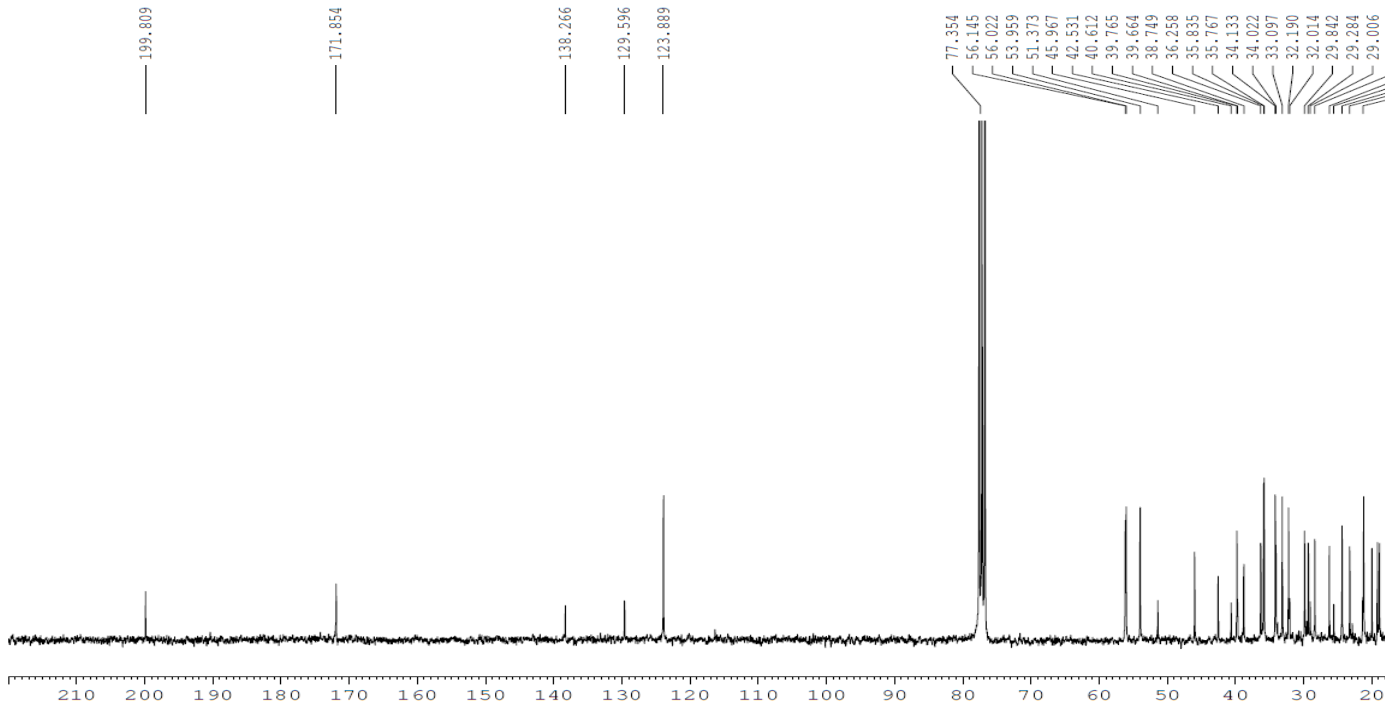
Appendix 31 1: ^{1}H NMR and ^{13}C NMR spectra of diacetyldiene molecule (421)



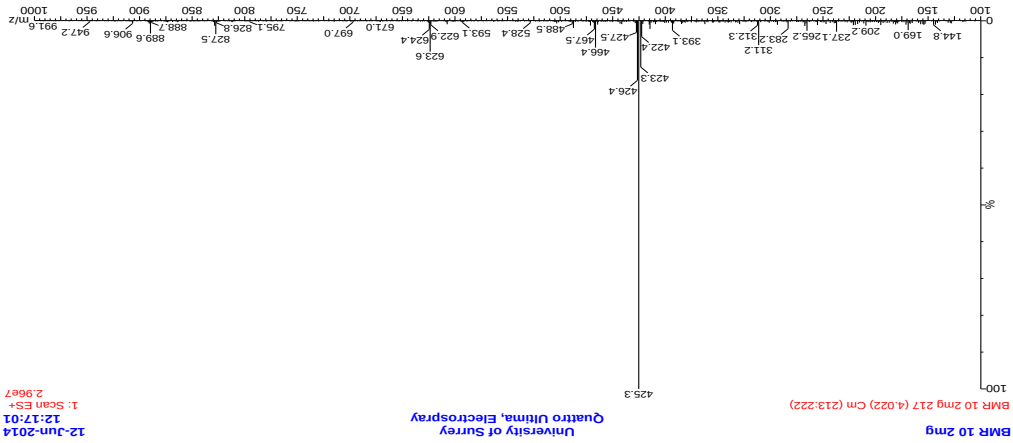


Appendix 32 1: ^{1}H NMR and ^{13}C NMR spectra of D_4-stigmasterone (422)

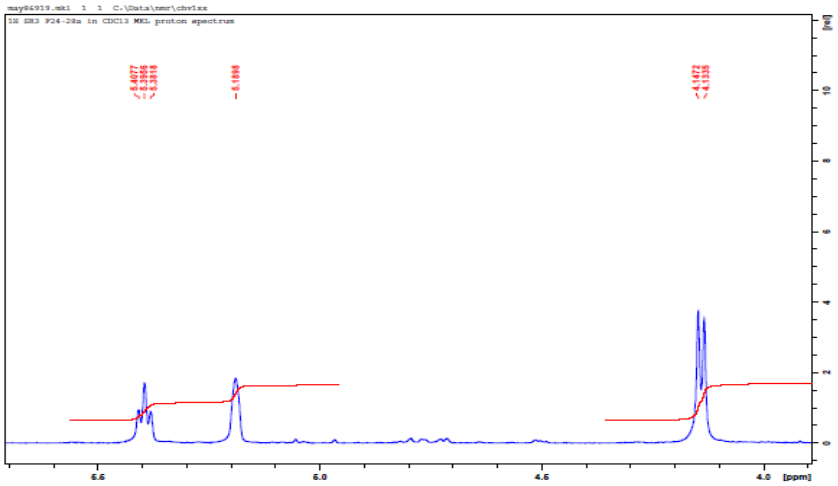


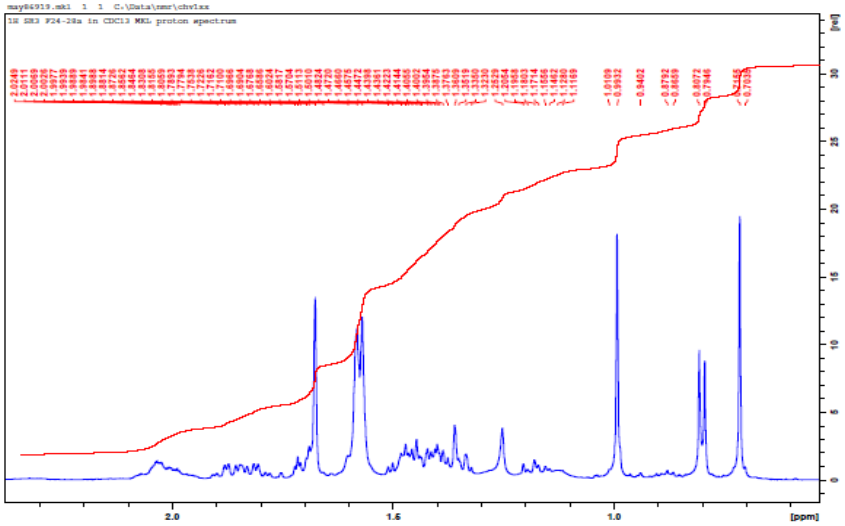


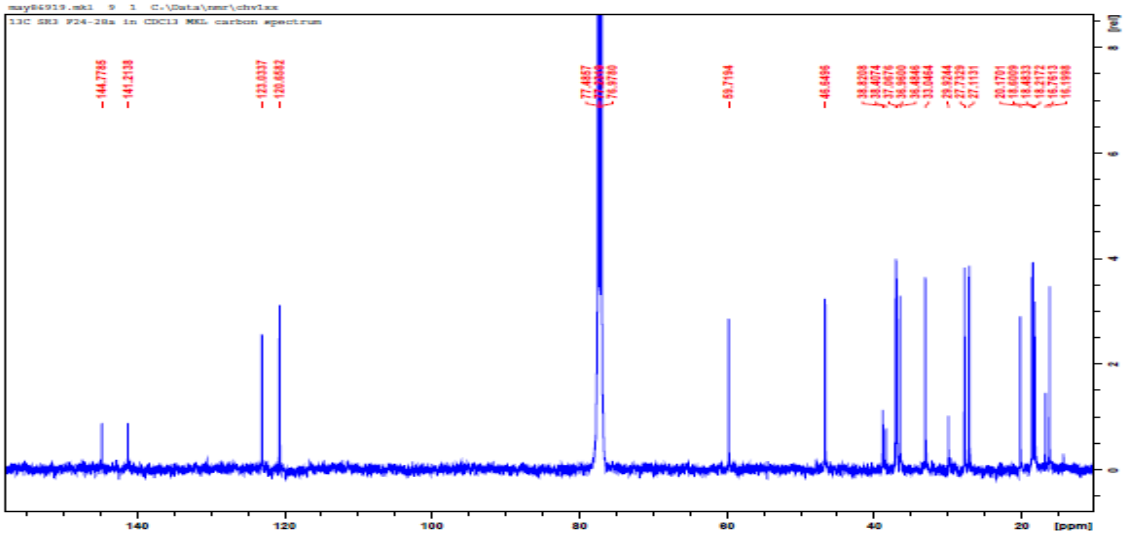
Appendix 33 1: ^{1}H NMR and ^{13}C NMR spectra of hardwickiic acid (423)



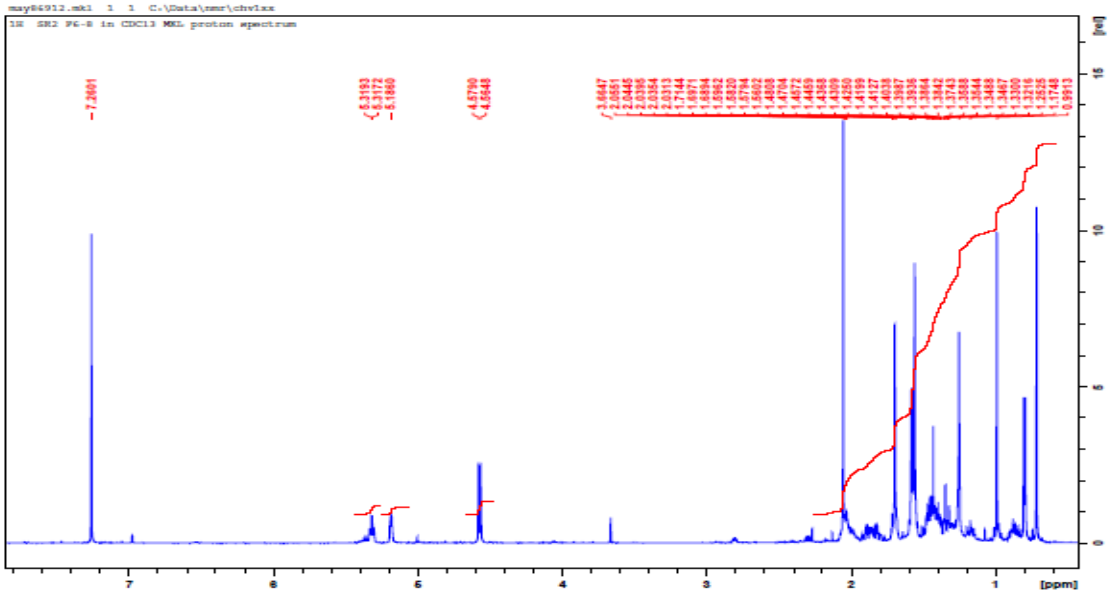
Appendix 34 1: ^{1}H NMR and ^{13}C NMR spectra of kolavenol (424)

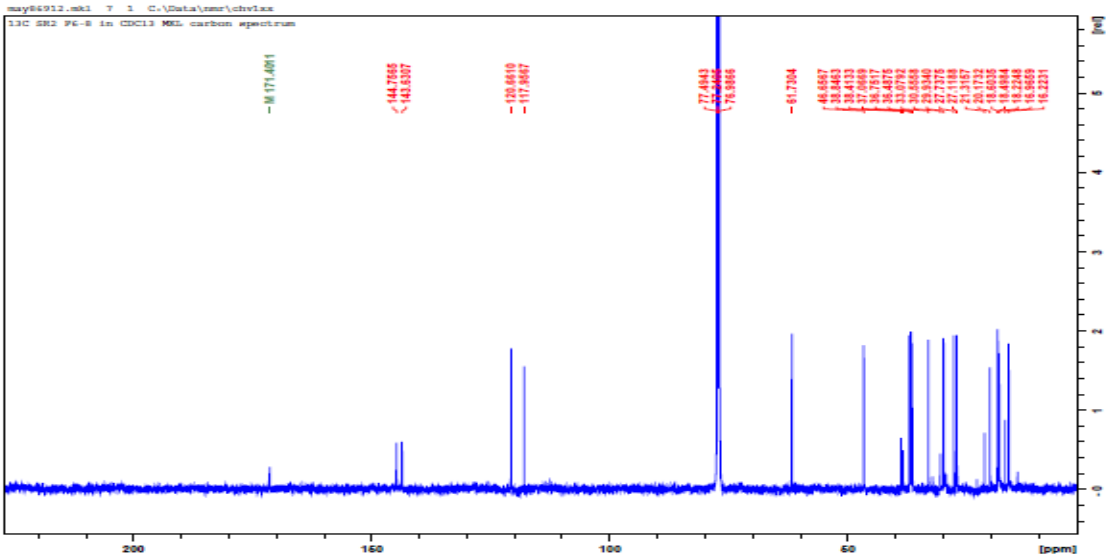




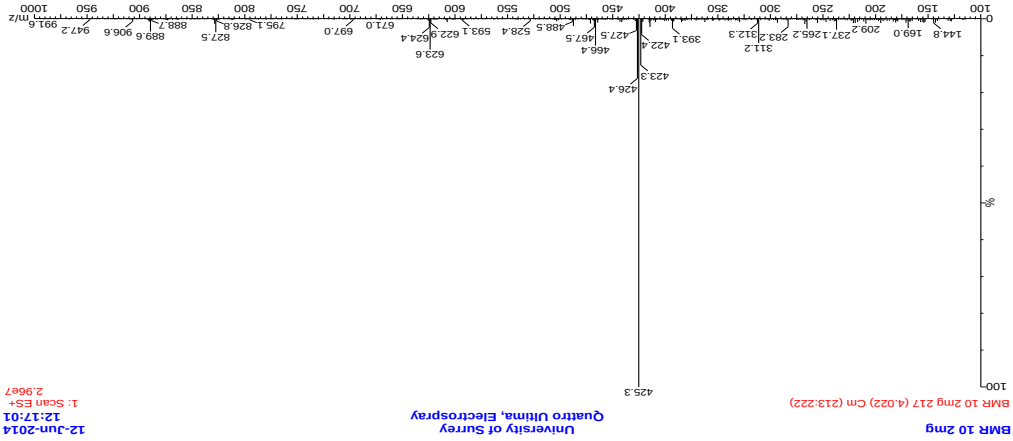


Appendix 35 1: ^{1}H NMR and ^{13}C NMR spectra of 15-acetoxy-*ent*-3,13*E*-clerodadiene (425)

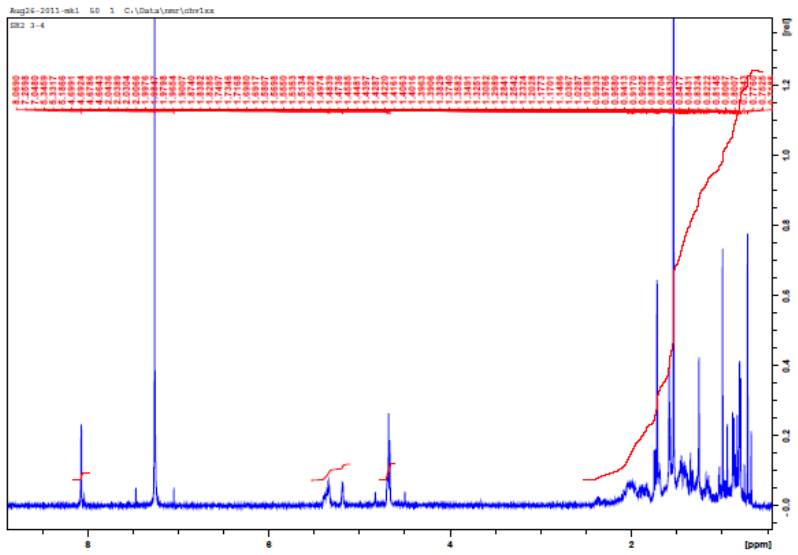


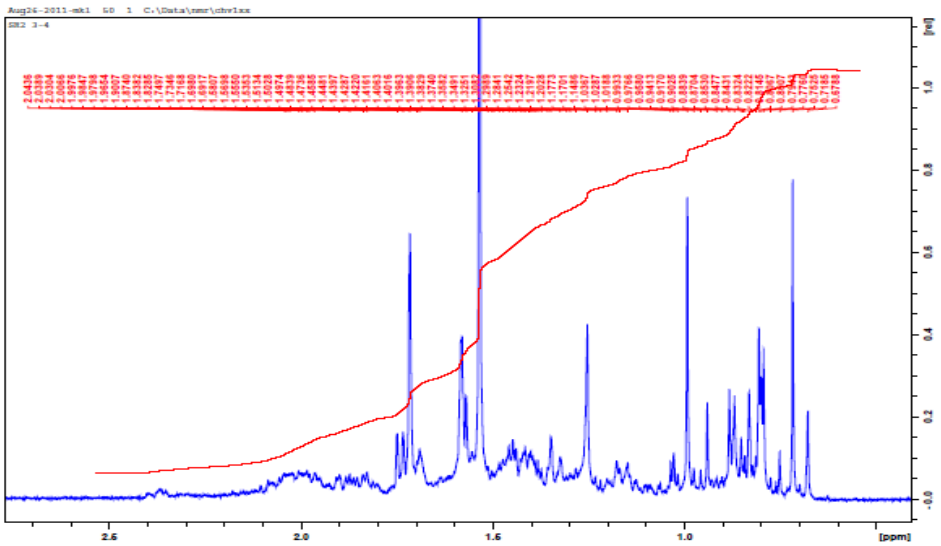


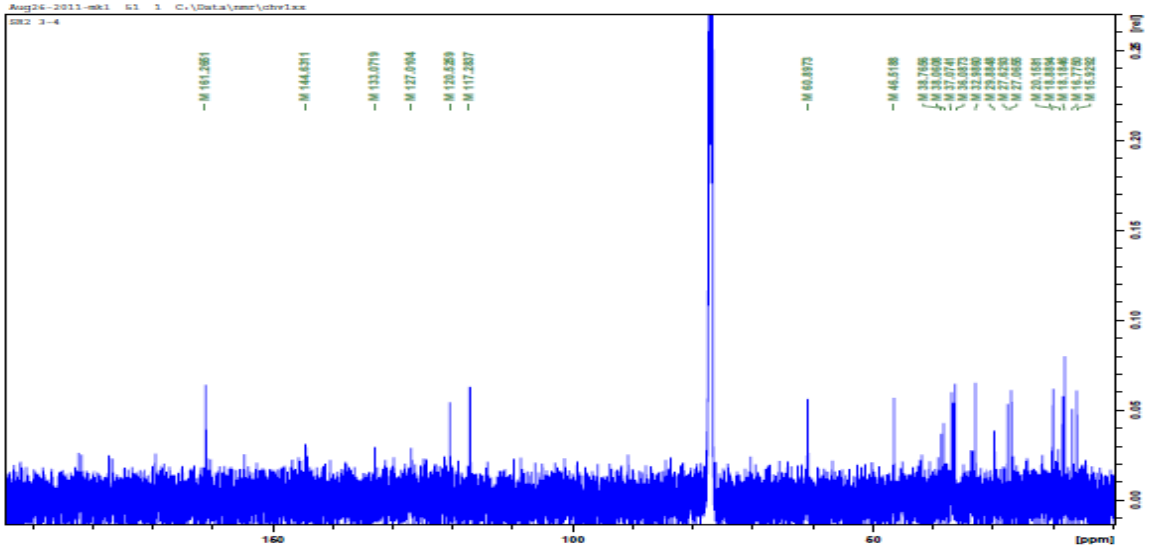
Appendix 36 1: ^{1}H NMR and ^{13}C NMR spectra of 3, 8(17), 13E-clerodatriene-15-ol (426)



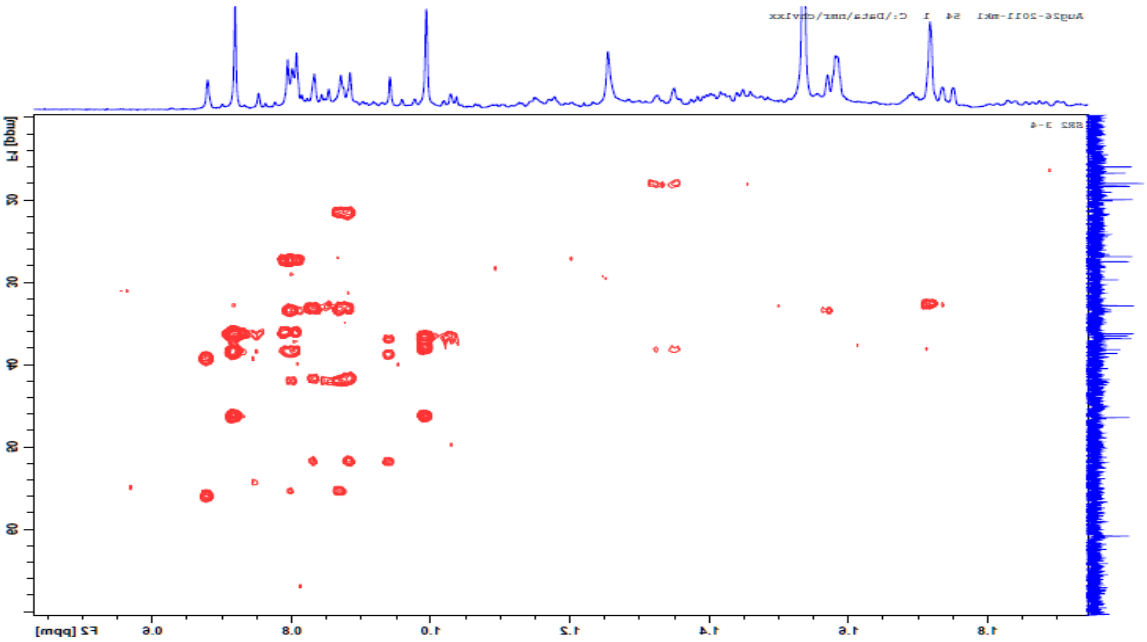
Appendix 37 a: ^{1}H NMR and ^{13}C NMR spectra of 15-formate-ent-3,13E-clerodadiene (427)

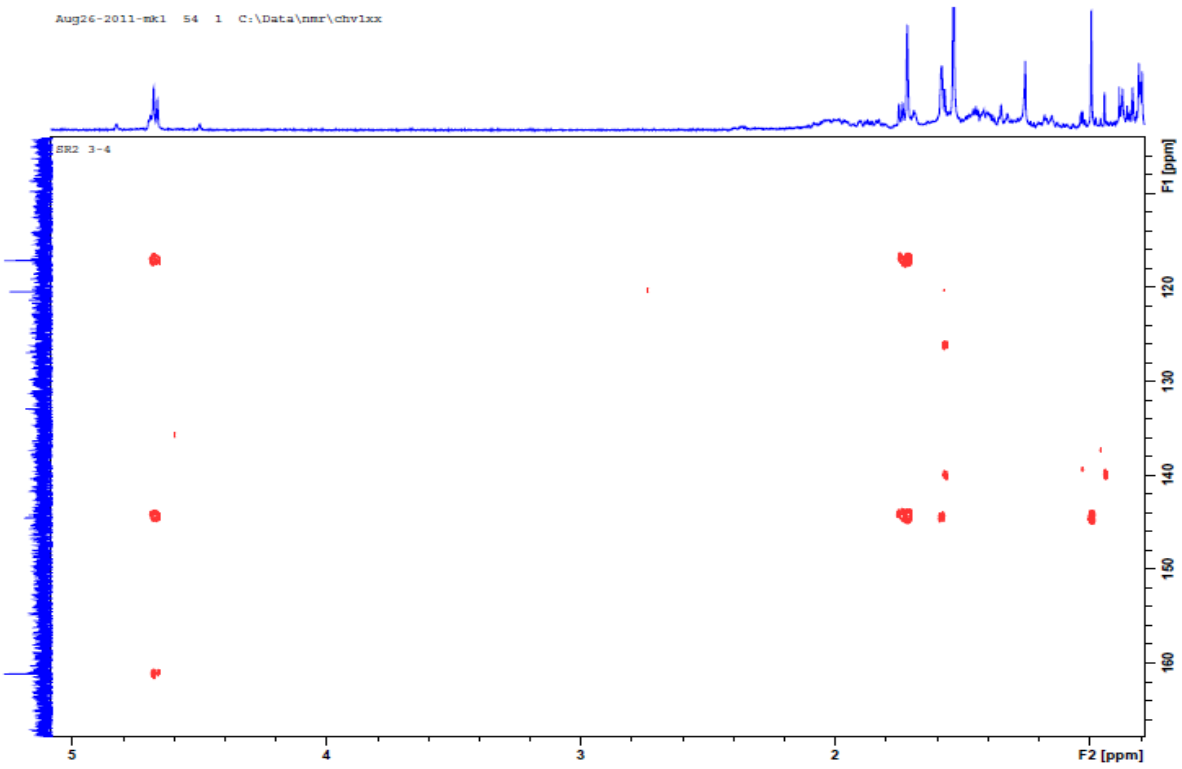




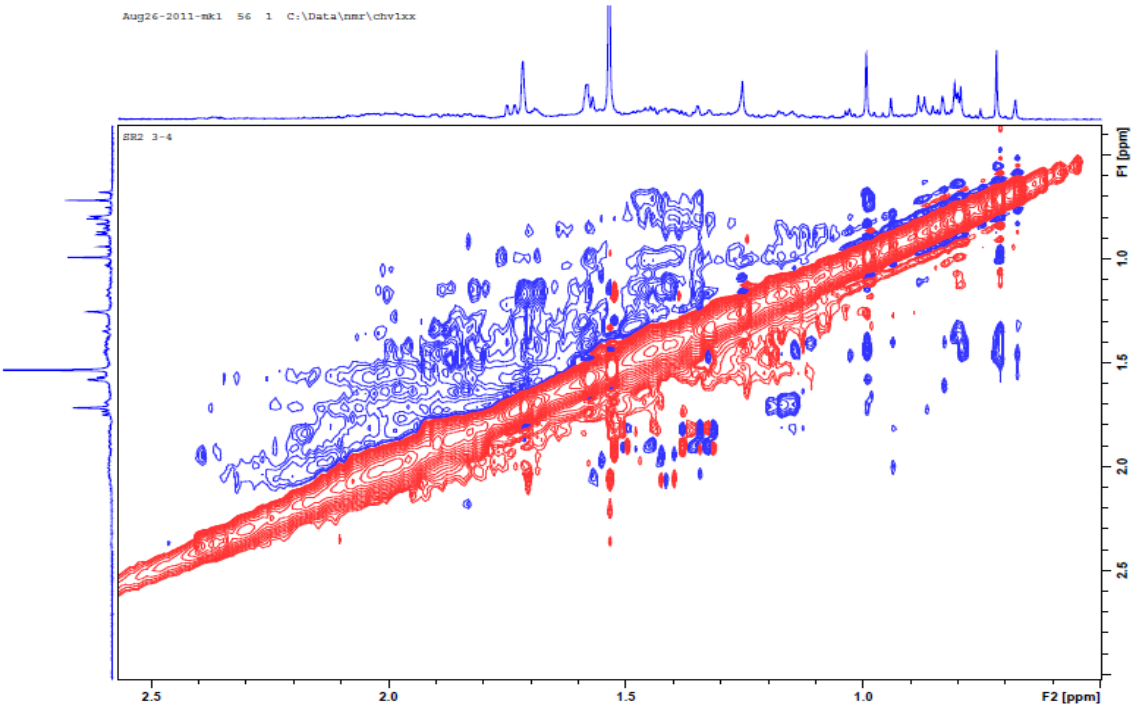


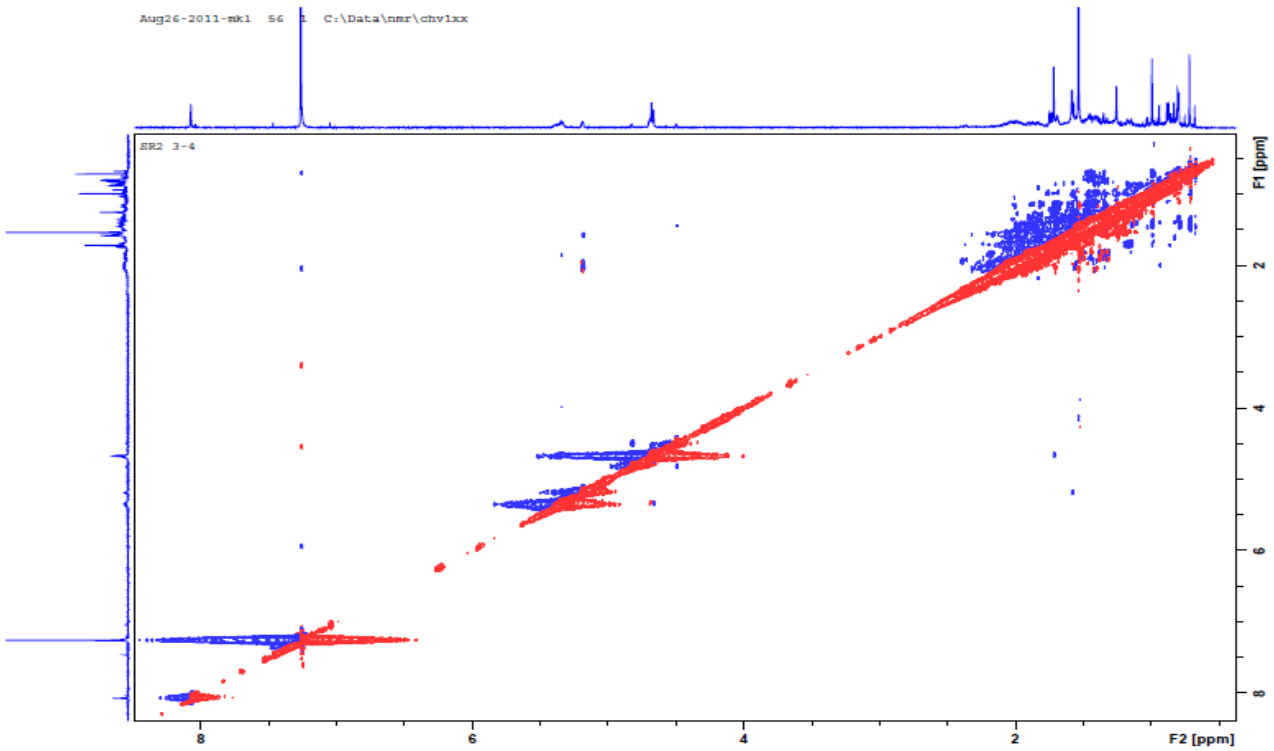
Appendix 37 b: HMBC spectrum of 15-formate-*ent*-3,13*E*-clerodadiene (427)



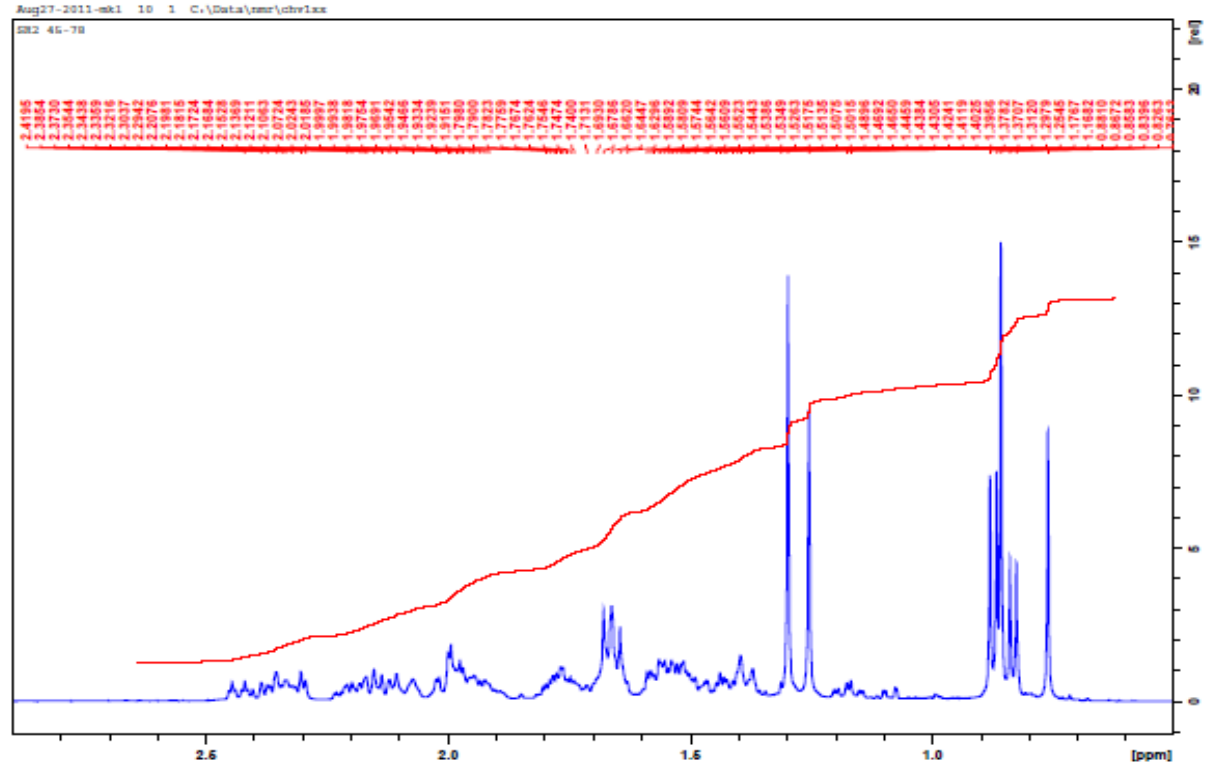


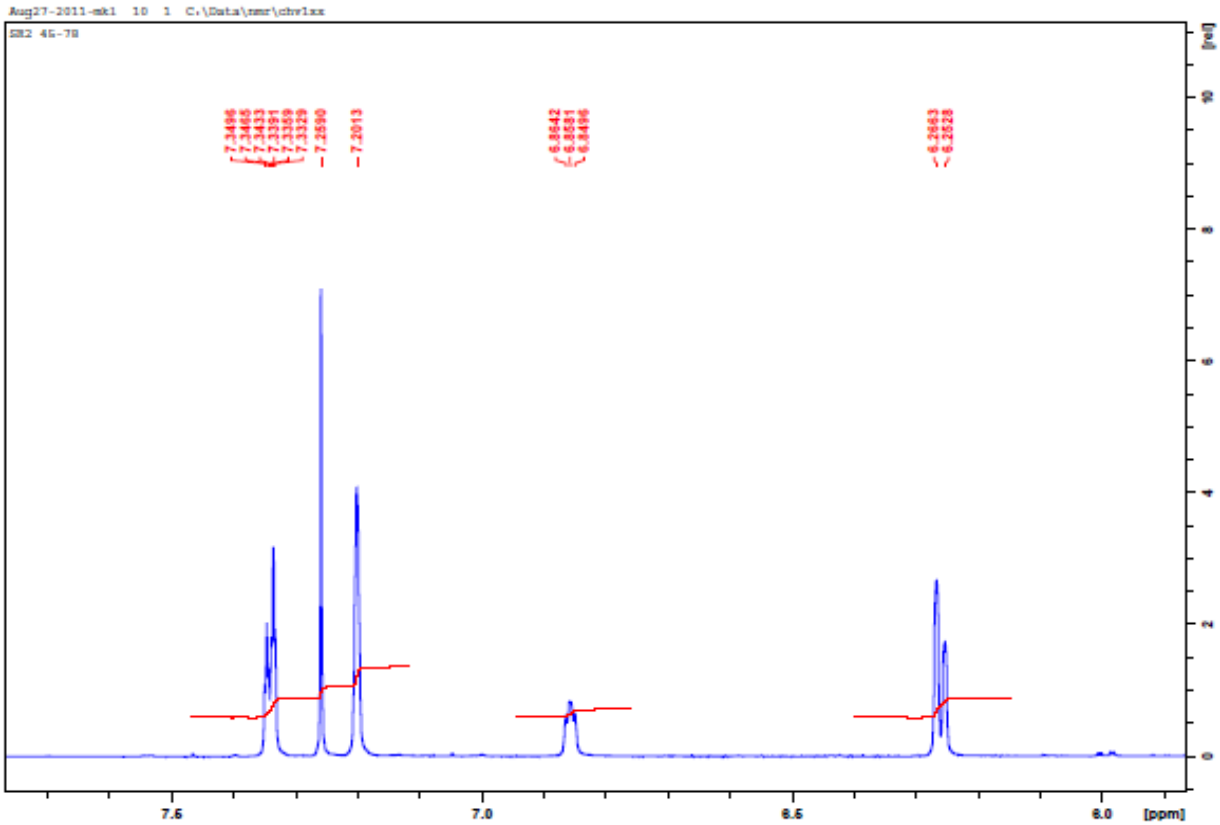
Appendix 37 c: NOESY spectrum of 15-formate-*ent*-3,13*E*-clerodadiene (427)



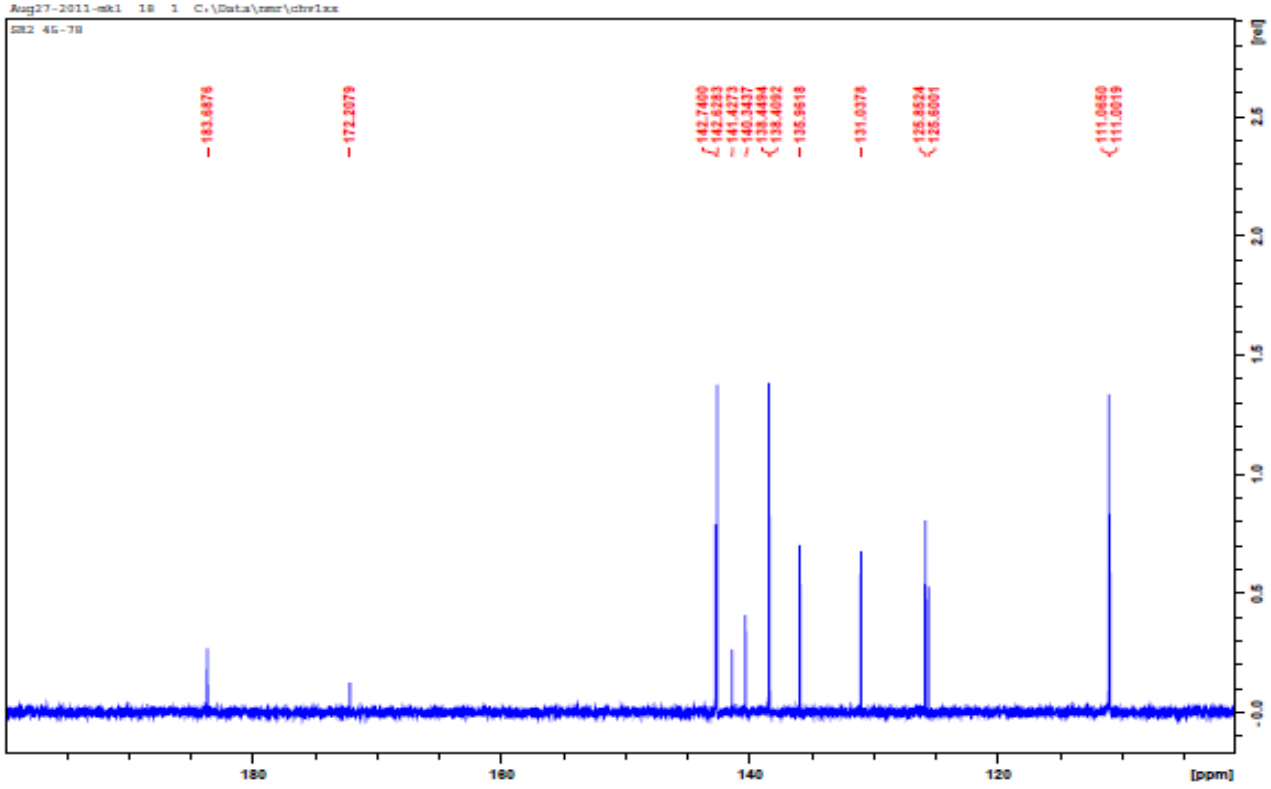


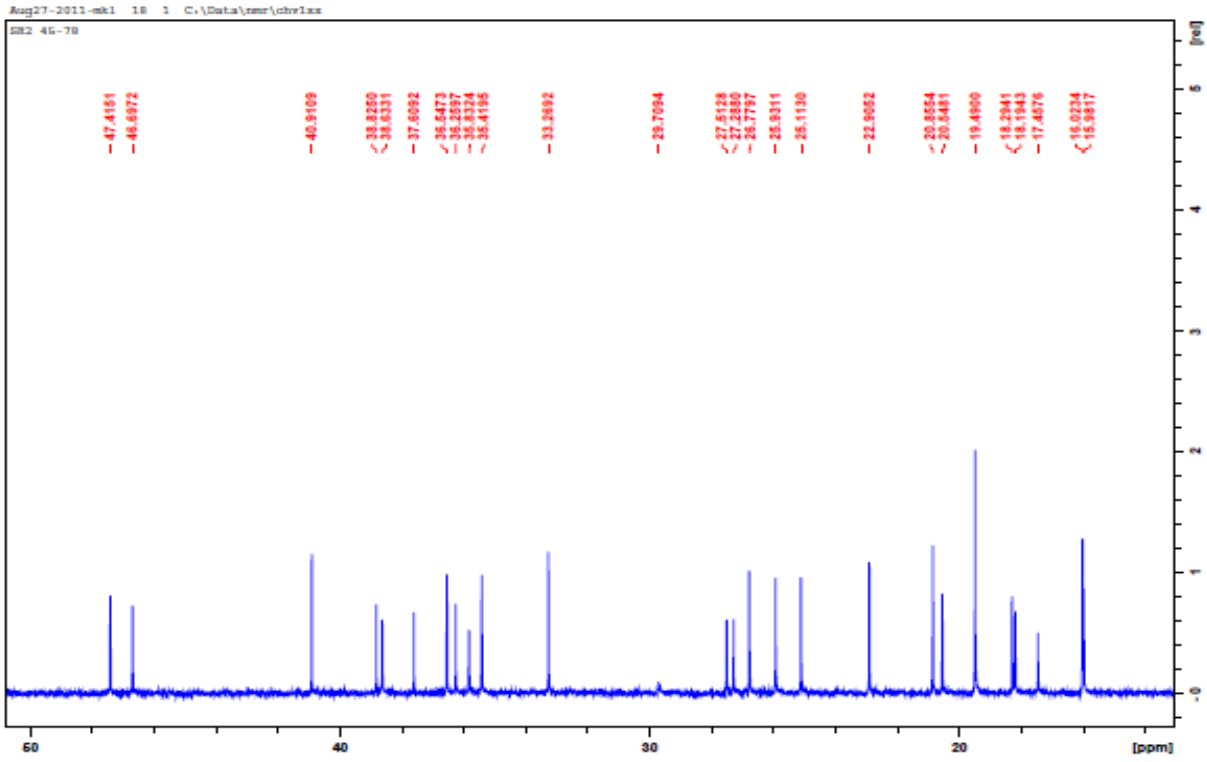
Appendix 38 a: ^{1}H NMR spectra of hardwickiic acid (423) and crotohalimaneic acid (428)



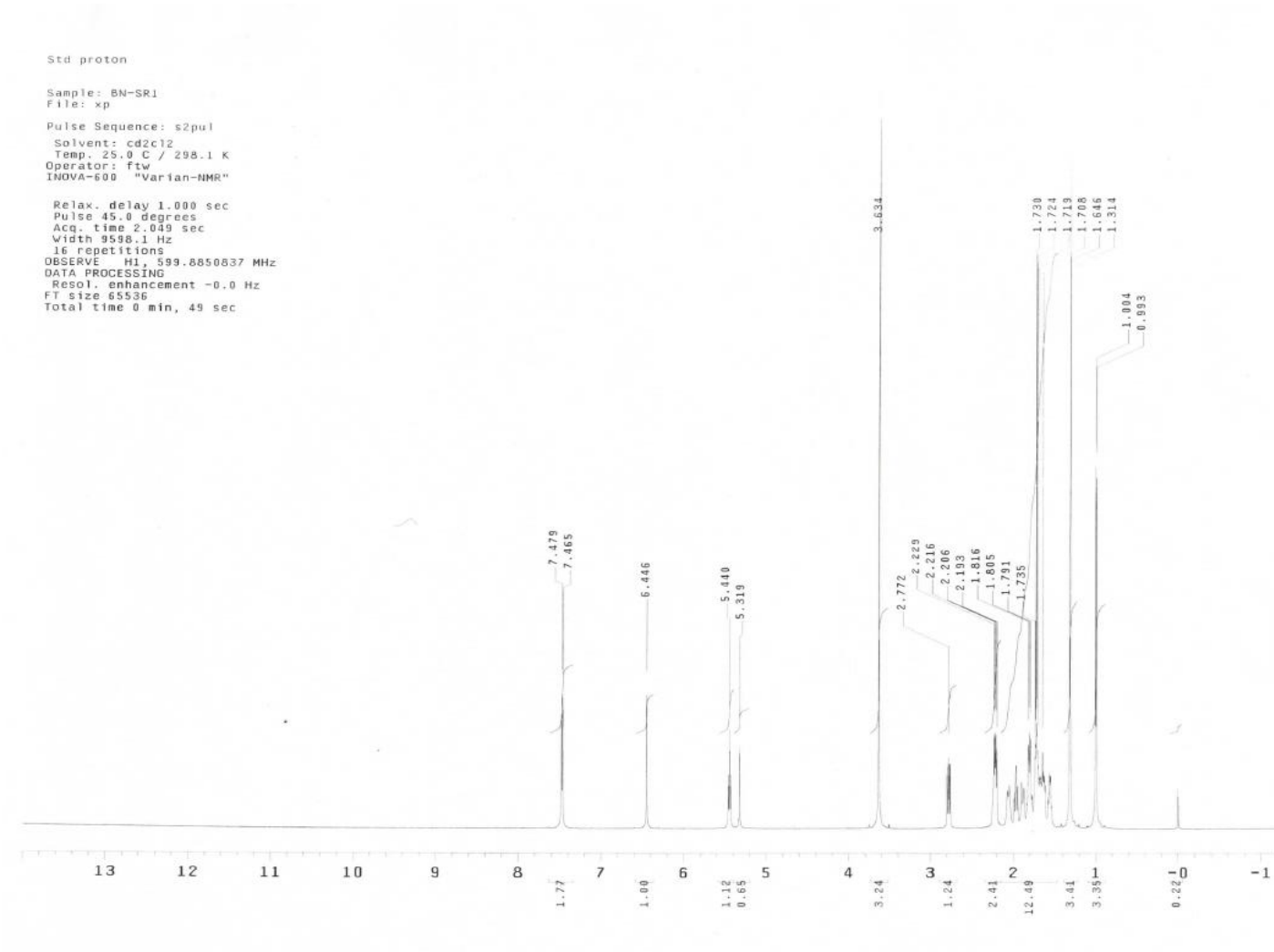


Appendix 38 b: ^{13} C NMR spectra of hardwickiic acid (423) & crotohalimaneic acid (428)

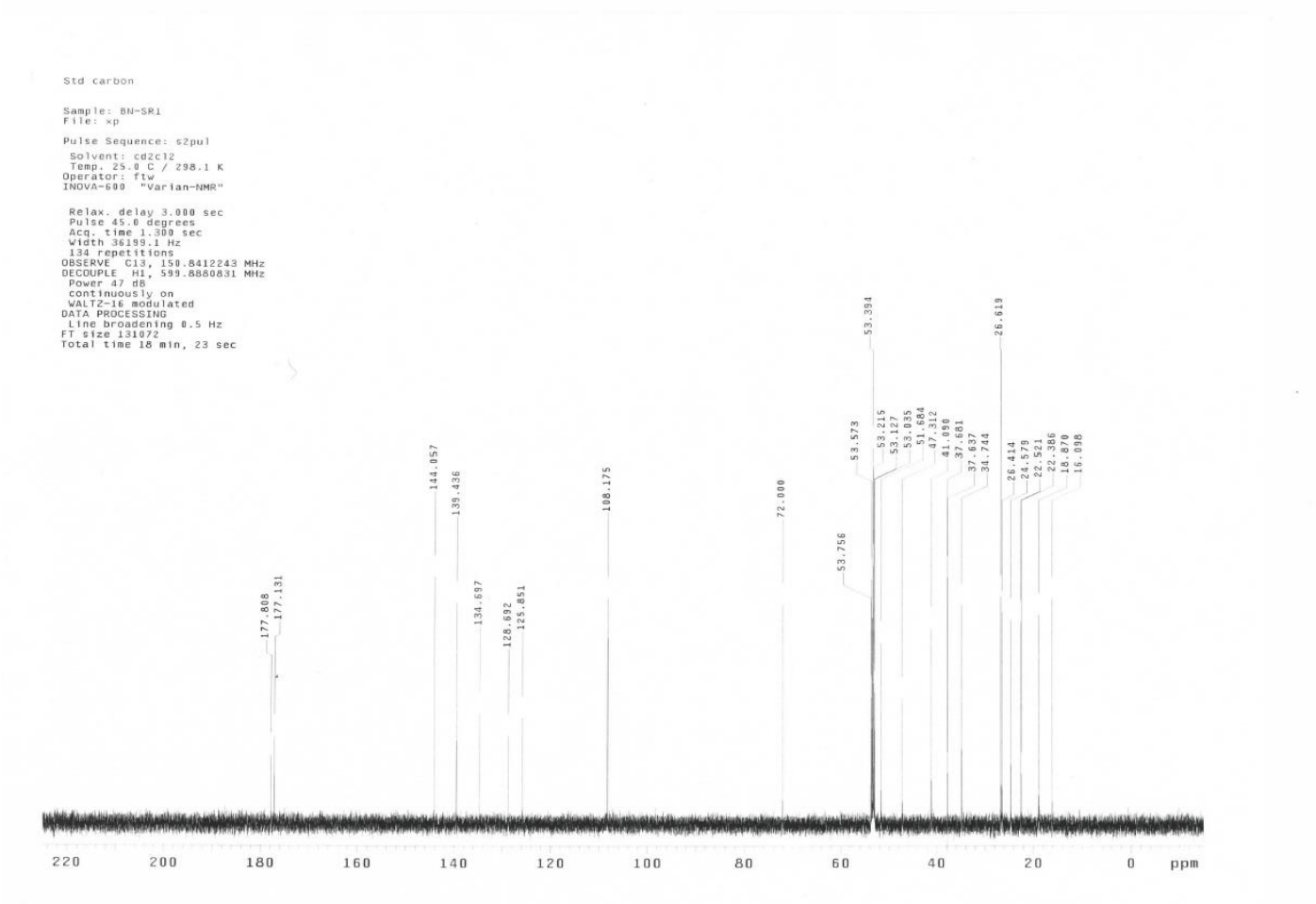




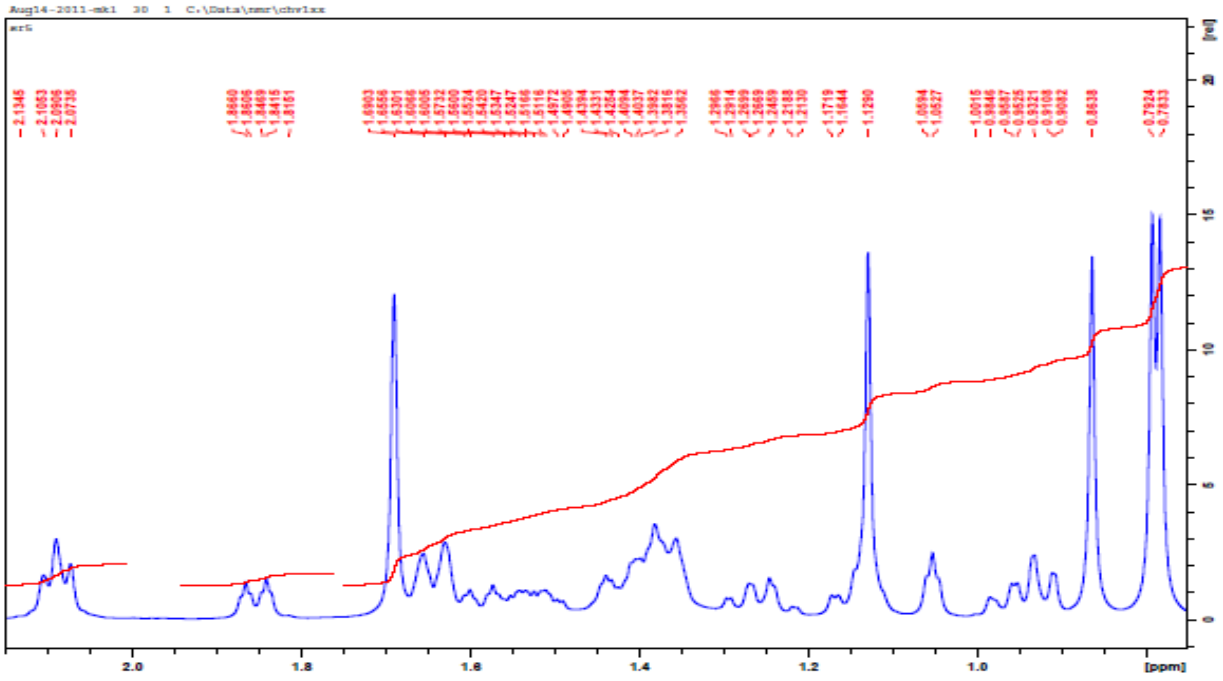
Appendix 39 a: ^{1}H NMR spectrum of penduliflaworosin (429)

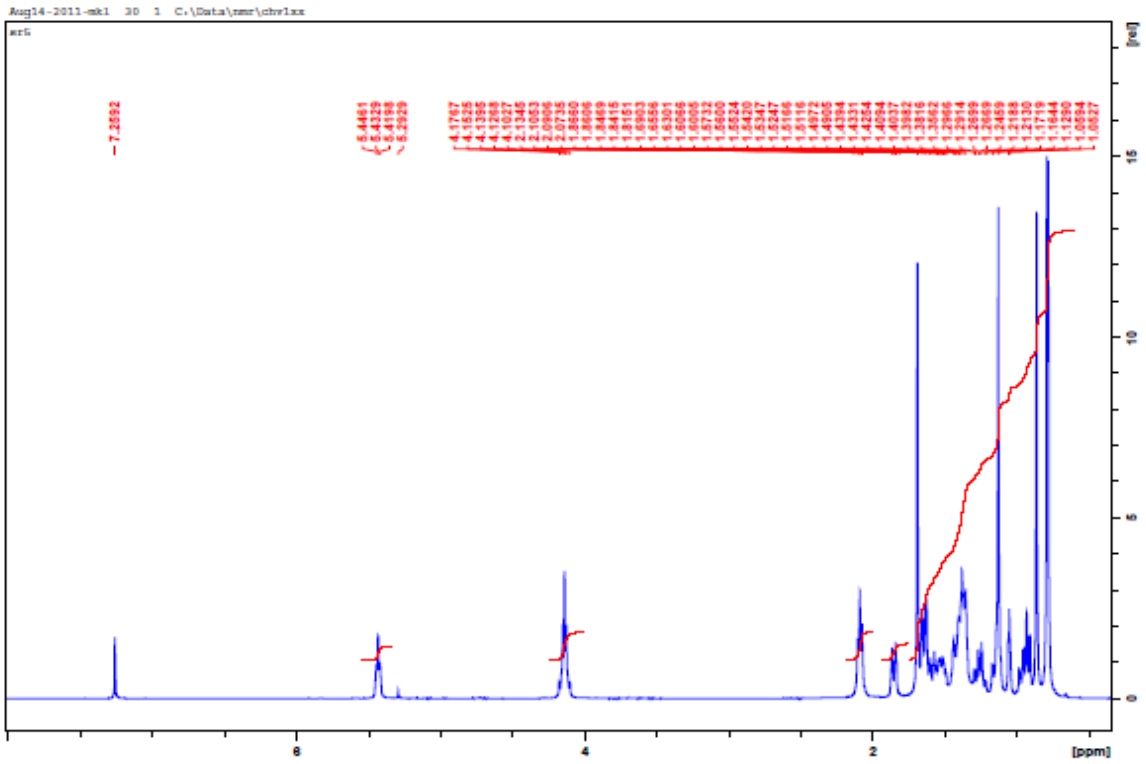


Appendix 39 b: ^{13}C NMR spectrum of penduliflaworosin (429)

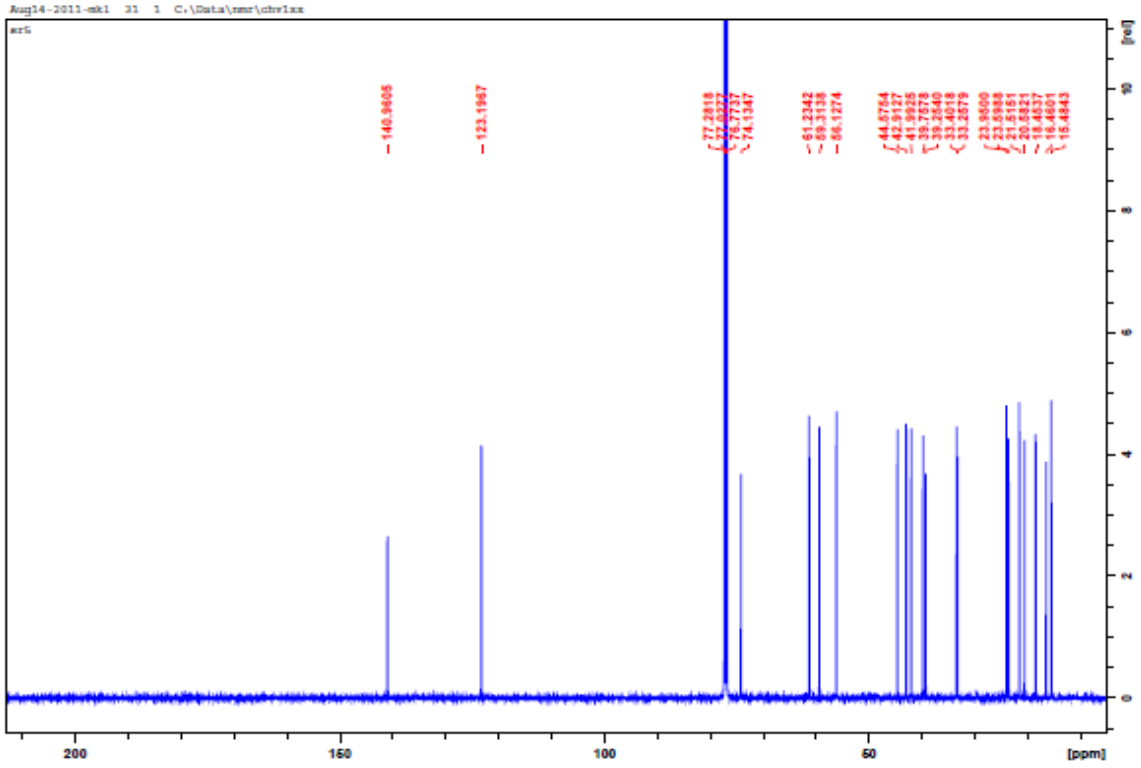


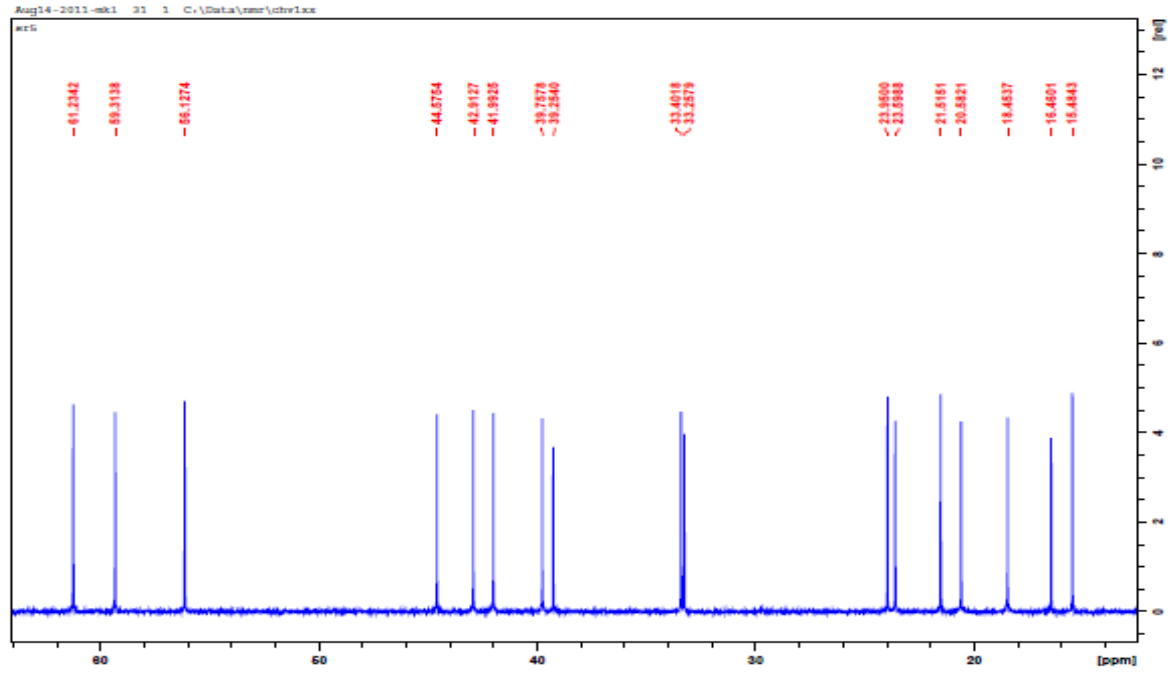
Appendix 40 a: ^{1}H NMR spectrum of Labd-13*E*- ene -8*α*, 15-diol (430)





Appendix 40 b: ^{13}C NMR spectrum of labd-13*E*- ene -8*α*, 15-diol (430)





Appendix 41 : Ndunda, B., Langat, M., K., Wanjohi, J., M., Midiwo, J., O. and Kerubo, L., O. (2013) Alienusolin, a New 4α-Deoxyphorbol Ester Derivative, and Crotonimide C, a New Glutarimide Alkaloid from the Kenyan *Croton alienus*. *Planta medica* **79**: 1762-1766