EVALUATION OF ANTICONVULSANT ACTIVITY OF EXTRACTS AND ISOLATES FROM *MAYTENUS HETEROPHYLLA*

BY

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DECLARATION

This thesis is my original work and has not previously been presented for a degree of Maseno University or any other university. Any work quoted herein is indicated and acknowledged by means of a comprehensive referencing.

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This thesis has been submitted in partial fulfilment of Masters of Science degree at Maseno University with our approval as supervisors.

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This thesis is dedicated to
all the seekers of truth and realities
who see things as they really are
and
who endure sincerely for the benefit of mankind
past, present and future

ABSTRACT

Convulsion is a chronic neurological disorder that develops in up to 5% of the world's population with prevalence of nearly 88% occurring in Sub-Saharan Africa, prompting serious medical, social, health related stigma and discrimination. Although a number of antiepileptic drugs (AED) are available for patients, most of the drugs have been associated with adverse side effects, dose-related neurotoxicity and teratogenic effects besides the AED therapy failures reported in approximately 20% of the patients. Since AED therapies have draw backs restricting their clinical utility, herbal medicines may offer therapeutic options due to their accessibility, and less or no side effects. *Maytenus heterophylla* is one such plant that has been in use by the Teso communities in western Kenya to manage convulsions. However, there is no scientific evidence to validate the application. Therefore, the current study was intended to screen the extracts for anticonvulsant activity, isolate the pure compounds and test them for anticonvulsant activity. A bioassay-guided isolation and characterization was designed to evaluate the antiepileptic potential of the various parts of *M. heterophylla.* Solvent extraction followed by chromatographic separation and spectroscopic techniques were used to isolate and characterize the compounds from the active fractions. Picrotoxin-induced convulsion assays in white albino Swiss mice was used to evaluate the *in-vivo* anticonvulsion potential of the extracts and compounds. Stem bark methanol extract (200 mg/kg b.w) significantly ($P<0.05$) decreased convulsions compared to control animals against Picrotoxin–induced seizure and relatively better than the leaf and root extract. The stem bark extract also offered up to 62.5% protection against seizure at 200 mg/kg which was significant ($P<0.05$) compared to diazepam (87.5%). Two new triterpenes; 3-methoxy-4-decarboxydihydrozeylasterone (**55**) and 3,4-*seco*-1-hydroxy-21-oxo-olean-3,11-olide (**56**) together with three known compounds; 3-acetoxy-28-hydroxylupe-20(29)-ene (**54**), Oleanolic acid (**58**) and a monoterpene, 3,5-dihydroxycamphanoate (**57**) were isolated from the stem bark of *M. heterophylla.* The isolates showed weak to moderate activities with compounds **55** and **56** showing the highest activities at 62.5% ($P<0.05$) and 75% ($P<0.05$) protection respectively, against convulsion compared to the distilled water (Negative control) at concentrations of 50 mg/Kg b.w. The results suggest that *Maytenus heterophylla* contains bioactive compounds against Picrotoxin-induced convulsion and the plant's stem bark may be beneficial in management of epilepsy. Compounds **55** and **56** should therefore be explored further for potential to manage convulsions. These results lend credence to the ethno-medical claim for the use of the plant in traditional medicine.

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ABBREVIATIONS AND ACRONYMS

2D	2-Dimension
AED	Antiepileptic Drugs
ANOVA	Analysis of Variance
br s	Broad singlet
CAM	Complementary and Alternative Medicine
$CDCl_3$	Deuterated chloroform
CNS	Central Nervous System
COSY	Correlated spectroscopy
d	Doublet
dd	Double doublet
DEPT	Distortionless Enhancement by Polarization Transfer
ESIMS	Electron Spin Impact Mass Spectroscopy
EtOAc	EtOAc
EtOH	Ethanol
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Pressure Liquid Chromatography
HRESIMS	High Resolution Electron Spin Impact Mass Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
i.p	Intraperotonial
IR	Infrared Spectroscopy
LD_{50}	Lethal dosage against 50% of population
m	Multiplet
m/z	Mass to charge ratio
MHz	Megahertz
MIC	Minimum Inhibitory Concetration
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance Spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy
p.o	Per oral
PTX	Picrotxin
PWE	People with Epilepsy
s	Singlet
S.E.M.	Standard error of mean
t	Triplet
TLC	Thin Layer Chromatography
TMS	Trimethyl Silane
UV-Vis	Ultraviolet-Visible spectroscopy
λ_{max}	UV absorption maxima

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CHAPTER ONE: INTRODUCTION

1.1. Background Information

Epilepsy is a common and diverse set of chronic neurological disorders characterized by seizures (Chang and Lowenstein, 2003). A seizure is the physical change in behaviour that occur after an episode of abnormal electrical activity in the brain. The term "seizure" is oftenly used interchangeably with convulsion, a condition characterized by rapid shakes of the patient's body uncontrollably and unconsciously (Fisher *et al.*, 2005). Symptoms of epileptic seizures includes dizziness, loss of consciousness, abrupt falling down, frothing from the mouth, loss of memory, biting of the tongue either at the tip or on the sides, confusion, and restlessness (Duncan *et al.*, 2006; Engel, 2008). Epilepsy becomes more common as people age (Holmes and Browne, 2008) and up to 5% the world population develop epilepsy in their lifetime (Sander and Shorvon, 1996) which makes epilepsy to remain a major medical and social problem. It usually begins in childhood, potentially impeding education, employment, social relationships and development of a sense of self-worth (Warren, 2003). The disease therefore needs serious attention in terms of modifications, addition and improvements of the various forms of therapies.

Prompt and accurate diagnosis of epilepsy with appropriate social and medical management optimizes the situation (Lerman, 1977). There is always need to carry out clear diagnosis since the management of convulsions with antagonistic drugs can cause adverse effects on the patient (Sander *et al.*, 1990). For instance, sedation and dizziness are common complaints of patients starting anti-epileptic drugs (AED) therapy (Richens and Rowe, 1970). Polytherapy is probably associated with more cognitive side effects than monotherapy such as osteopenia, osteomalacia and increased risk of hip fracture which have been associated with AED (Richens and Rowe, 1970). Approximately one third of people with epilepsy (PWE) have drug-resistant seizures (Kwan and Brodie, 2000; Kwan *et al.*, 2010). Surgery is

highly effective and safe for selected patients (since a comprehensive family history of epilepsy should be taken and expert advice on the genetics of epilepsy should be available as required) with treatment-resistant focal epilepsy (Choi *et al.*, 2008; Engel, 2008), but is still underutilized, even in high-income countries because a single site of origin of their seizures cannot be localized or exists within complex regions of the cortex (Boon *et al.*, 2009). With these myriad limitations to the use of AED, management of convulsions may lead to severe problems than the epileptic conditions, which necessitates further investigation of alternative remedies that may efficiently control the seizure conditions and manage the problems associated with epilepsy.

About 150 million people worldwide have epilepsy and nearly 88% of epilepsy occurs in Sub-Saharan Africa (WHO, 2012), with prevalence estimated to be two or three times higher than in the developed world (Njamnshi *et al.*, 2010). Due to such high prevalence, the disease is rated as a health priority in Africa by social health workers forum in Africa (WHO, 2004). However, progress towards proper management has been slow due to lack of specialized personnel, insufficient support materials, lack of drugs or high cost and cultural interpretation of the disease (WHO, 2004). These factors have contributed to over-reliance on traditional phytotherapy to manage epilepsies among the very low income populations (Njamnshi *et al.*, 2010). However, little is understood of the efficacies of most of the traditional phyto-therapeutic remedies due to lack of effective scientific validation of these therapeutic procedures.

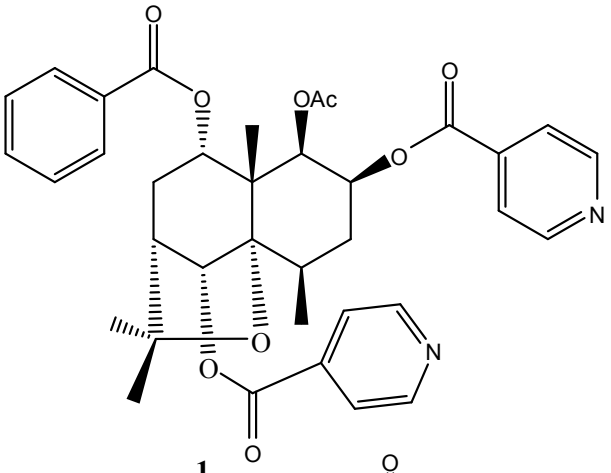
The main benefits for the use of herbal remedies have been their safety, offering powerful therapeutic options with less documented adverse effects and readily available treatment (Iwu, 1993). In most developing countries, people with epilepsy (PWE) take herbal extracts or engage in other forms of complementary and alternative medicine (CAM) to manage

seizures or to alleviate symptoms of co-morbidities or side effects of antiepileptic medications. For example, in well developed medical systems, such as traditional Chinese Medicine (TCM) and *Ayurveda* of India, properly developed epileptic therapies are often used on PWE (Dana and Steven, 2010). Some plants from the family Celestraceae, such as *Catha edulis* and *Celastrus paniculatus* have long historical application towards palliation of diseases related to the brain like agitations, anxiety, convulsions, dizziness, headaches, insomnia, pain and schizophrenia according to traditional healers and literature reports (Nutt *et al.*, 2007; Bhanumathy *et al.*, 2010).

An ethnobotanical survey among traditional healers of the Teso communities in western Kenya indicated the application of boiled root and stem of *Maytenus heterophylla* (Celestraceae) to manage epilepsy (Personal communication; Okello *et al.,* 2010). It is in this line that *Maytenus heterophylla* has been identified as being used empirically by traditional healers in western Kenya. However, the use of this plant in the management of convulsion has not scientifically been validated and hence not documented.

Previous pharmacological and phytochemical studies on *M. heterophylla* reported the isolations of a dihydroagarofuran alkaloid, 1β-acetoxy-9α-benzoyloxy-2β,6α-dinicotinoyloxy-β-dihydroagarofuran (**1**) together with β-amyrin (**2**), maytenfolic acid (**3**), 3α-hydroxy-2-oxofriedelane-20α-carboxylic acid (**4**), lupeol (**5**), lup-20(29)-ene-1β,3β-diol (**6**), (-) - 4′-methylepigallocatechin (**7**) and (-)epicatechin (**8**) from ethanol (EtOH) extracts of the aerial parts (Orabi *et al.*, 2001). Most of the compounds were biologically active. For instance, pristimerin (**9**) isolated from *M. heterophylla* showed potent anticytomegalovirus properties against human cytomegalovirus (HCMV) (Murayama *et al.*, 2007) whereas maytenfolic acid (**3**) showed moderate antimicrobial activity (Orabi *et al.*, 2001). The few investigations on *M. heterophylla* reported antimicrobial and antiviral activities. From ethno-medicinal information, a wide spectrum of biological activities may be expected from the

compounds of *M. heterophylla*. Since biological activities of the compounds from *M. heterophylla* against pathophysiological conditions such as convulsion is not validated, establishment of medicinal efficacies towards such an application remains unknown, thus validation of the claim by traditional healers remains a non clear venture.



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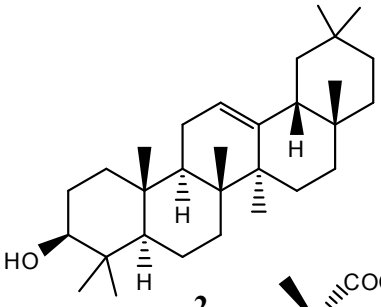

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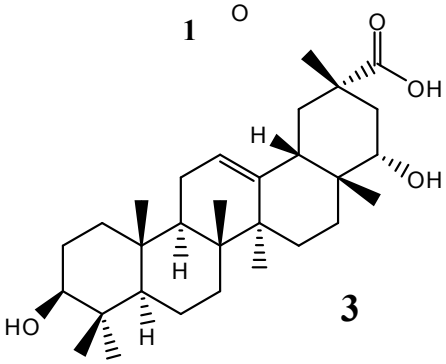

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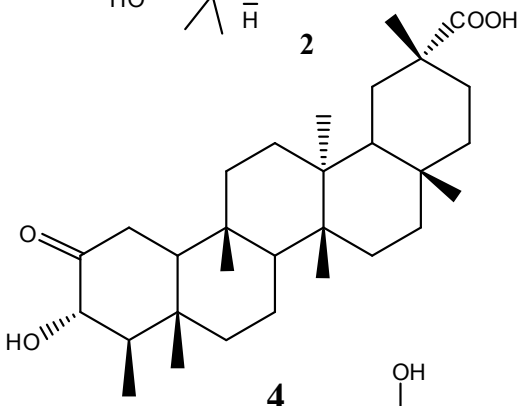

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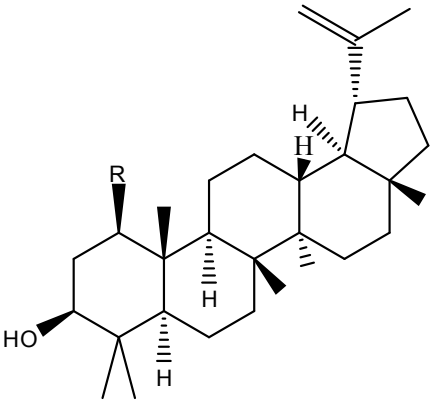

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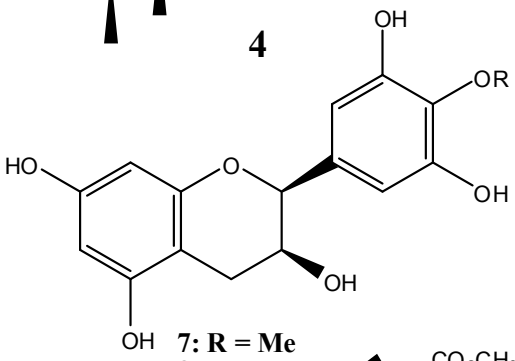

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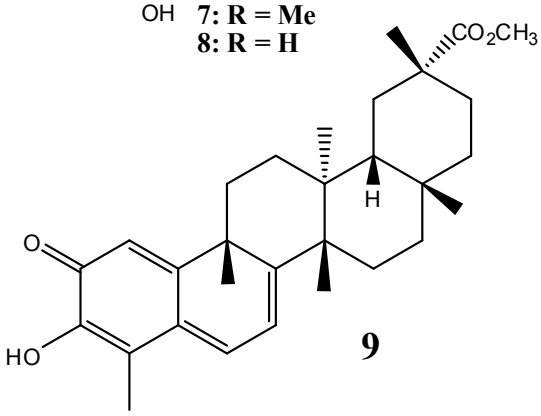

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1.2 Statement of the Problem

Convulsion is a neuropathophysiological condition affecting both the effluent and poor families/societies. The generalised management of the condition is by use of synthetic anticonvulsant agents which are life-long, continuous use and can have major adverse effects on quality of life. However, most of the management therapies have myriad side effects. Ethnomedicines have been noted to offer complementary and alternative management therapies. However, a lot of the ethno-medicinal materials such as the herbal remedies have no clear scientific evidence to validate their efficacies. For instance, the use of *M. heterophylla* by the Teso community in western Kenya may be well accepted among them but scientific validation and evaluation of action towards epileptic condition remains unclear. It is not established, if the extracts have the anticonvulsant activity and which compounds, if any, are the active principles against epilepsy.

1.3 Research Objectives

1.3.1 General Objective

To evaluate the ethno-medical application of the plant *Maytenus heterophylla* against convulsion through bioassay guided isolation of the active chemical entities elaborated by the plants.

1.3.2 Specific Objectives

i). To determine the anti-convulsant activity of extracts from the leaves, stem and root bark of *Maytenus heterophylla*.

ii). To isolate and characterize chemical compounds from the anti-convulsant extracts of *Maytenus heterophylla*.

iii). To determine the anti-convulsant potential of the compounds from the active extracts from the plant

1.4 Null Hypotheses

i). Extracts from *Maytenus heterophylla* do not exhibit anti-convulsant activity.

ii). Secondary metabolites from the plant do not exhibit anti-convulsant activity

1.5 Justification of the Study

Notwithstanding the limitations of using AEDs like osteopenia, osteomalacia, sedation and dizziness, the ease of use and ready availability of medications, as well as the prompt reversibility of dose-related side effects, AEDs remain the mainstay of epilepsy treatment for the foreseeable future unless new remedies are found. Therefore, new drug therapies with efficacy against drug-resistant seizures, minimal side effects profiles, especially in regard to neurological and psychiatric effects, and if possible, low costs to patients and good accessibility are clearly needed.

1.6 Significance of the Study

Therefore, the use of natural products from plants such as *M. heterophylla* which are indigenous, effective and with low mammalian toxicity are therefore necessary for the management of epilepsy. Besides, sub-Saharan Africa still boasts of a wide variety of indigenous plant species, with possibility of discovering new and very interesting natural products that can be developed for potential therapeutic values, hence such studies are necessary to document the relevance of such plants like *M. heterophylla* which are rare.

1.7 Limitations of the Study

The plant materials were obtained far from where the laboratory work was done. It was therefore difficult to account for the biochemical changes that may have been due to transportation, drying, grinding and solvent extraction of the plant extract.

CHAPTER TWO: LITERATURE REVIEW

Convulsion is a major neurological disorder and up to 5% of the world population develops convulsions in their lifetime (Sander and Shorvon, 1996). It is the second most common neurological disorder attended to by neurologists (Sridharan, 2002). Convulsion may lead to oxidative stress as result of free radicals production and membrane lipid peroxidation, which cause tissue damage (Tripathi, 2008). Despite the introduction of many new antiepileptic drugs (AEDs), 30% of epilepsy is refractory to treatment (WHO, 2001) and a significant percentage of patients with epilepsy continue to experience seizures. Hence, there continues to be an unmet clinical need for more effective and less toxic anti-epileptic drugs (Bhatacharjee, 2001).

Many people in developing countries may not receive basic treatment due to high cost, unavailability and adverse effects associated with the available antiepileptic drugs (Sander *et al.*, 1990). These limitations have resulted into continuous reliance on traditional healing practices including the use of medicinal plants for their basic management of epilepsy in developing countries (Boon *et al.*, 2009), a practice well encouraged by the WHO (Amos *et al.*, 2001). Medicinal plants are the important source for the new chemical substances with potential therapeutic effects. Several plants are used for the treatment of epilepsy in the system of traditional medicine and many such plants are yet to be scientifically investigated (Tripathi, 2008).

2.1 Causes of Epilepsy

The human brain is the source of human epilepsy. Although the symptoms of a seizure may affect any part of the body, the electrical events that produce the symptoms occur in the brain (Engel, 2001). The location of that event, the extent of its reach to the tissue of the brain, and how long it lasts all have profound ill health effects (Salinsky *et al.*, 1987). Some of the main

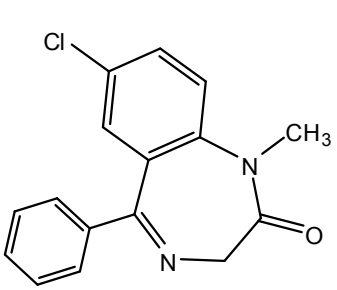
causes of epilepsy include; low oxygen during birth, head injuries, brain tumours, genetic conditions that result in brain injury, such as tuberous sclerosis, infections such as meningitis or encephalitis, stroke or any other type of damage to the brain and abnormal levels of substances such as sodium or blood sugar (Chang and Lowenstein, 2003).

2.2 Management of Epilepsy

The management of epilepsy with drugs requires a clear diagnosis of the type of epilepsy (Sander *et al.*, 1990). One or several individual drugs are then tested initially as monotherapy and in combination if necessary until efficacy is established (Brodie and Dichter, 1996). In a minority of patients, seizures are controlled by combinations of antiepileptic drugs (Brodie and Yuen, 1997). Approximately 20% of patients have treatment-resistant epilepsy which, in some cases, will respond to surgical treatment (Sander *et al.*, 1990). Antiepileptic drugs (AEDs) should not be given until the diagnosis of epilepsy has been confirmed. If there is uncertainty, a period of observation usually clarifies the epilepsy syndrome and confirms the need for treatment (Sander *et al.*, 1990).

Comparative, randomised, double-blind trials in patients with newly-diagnosed partial and generalised tonic-clonic seizures suggest similar efficacy for diazepam (**10**), phenytoin (**11**), sodium valproate (**12**), lamotrigine (**13**) and oxocarbazepine (**14**) (Mattson *et al.*, 1985; Dam *et al.*, 1989). The newer AEDs, lamotrigine (**13**) and oxocarbazepine (**14**) seem to be better tolerated and may produce fewer long term side effects and adverse interactions (Brodie *et al.*, 1995; Brodie and Kwan, 2001). Sodium valproate (**12**) and lamotrigine (**13**) also have efficacy for absence and myoclonic seizures but lamotrigine (**13**) can worsen myoclonus in some cases (Brodie and Dichter, 1996; Brodie and French, 2000) despite being well tolerated with favourable cognitive and behavioural profile (Biton *et al.*, 2001). Ethosuximide (**15**) has been used for seizures in children for many decades but has adverse side effects (Brodie and

Dichter, 1996). With such side effects, alternative application of natural remedies may offer a solution up on proper investigation to establish their efficacies. However, there have not been results of natural remedies which possess similar or almost similar chemical compounds as those used in AEDs.



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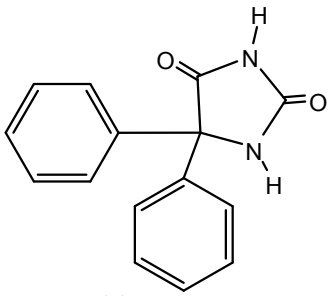

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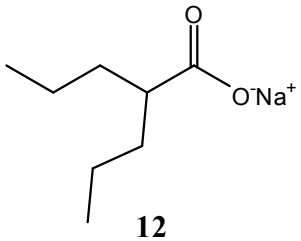

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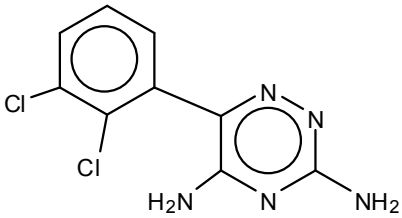

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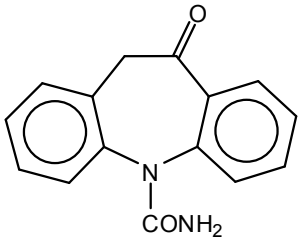

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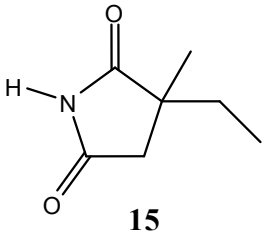

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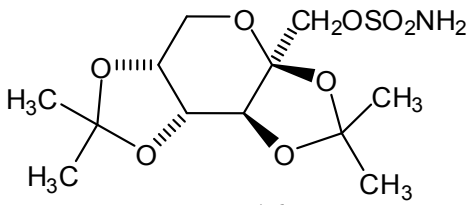
2.3 Management of Drug-Resistant Epilepsy

The effect of most of anti-epileptic agents is to enhance the response to GABA (gamma amino butyric acid), by facilitating the opening of GABA-activated chloride channels (Nagaraja *et al.*, 2012). $GABA_A$ receptors are involved in epilepsy and their direct activation would have anti-epileptic effect. It is well documented that PTX-induced seizures are produced due to diminution of GABA level in the brain (Nishino *et al.*, 2008).

Drug-resistant epilepsy is defined as a continuation of seizures despite optimal monotherapy with two successive first-line AEDs or with one monotherapy and one combination regimen (Duncan *et al.*, 2006). The majority of patients with newly-diagnosed epilepsy respond well

to AEDs. Failure to respond may be due to; an incorrect diagnosis of epilepsy (Smith *et al.*, 1999), an inappropriate choice of AED for the epilepsy syndrome (Perucca *et al.*, 1998), failure to take the prescribed AED or an underlying cerebral neoplasm and covert drug or alcohol abuse (Duncan *et al.*, 2006). Given a correct diagnosis of epilepsy, failure to control seizures completely with the first well tolerated AED is a powerful predictor of drug-resistant epilepsy (Dlugos *et al.*, 2001; Kwan *et al.*, 2010).

When two AEDs have failed as monotherapy, the chance of seizure-freedom with further monotherapy is very low (Brodie and Dichter, 1996). Improvement in seizure control may be obtained by combining AEDs (Brodie and Yuen, 1997).. Choice of AED combinations should be guided by side effects profile and drug interactions (Brodie and Kwan, 2001). There is some evidence that combining AEDs which have different mechanisms of action may enhance effectiveness, for instance, lamotrigine (**13**) in combination with sodium valproate (**12**) is more effective but not in combination with carbamazepine (**10**) or phenytoin (**11**) (Brodie and Yuen, 1997). However, there are chronic side effects associated with AEDs such as weight gain which is seen with many AEDs though weight gain is associated with sodium valproate (**12**) (Biton *et al.*, 2001). Topiramate (**16**) can cause weight loss (Reife *et al.*, 2000). Sedation and dizziness are common complaints of patients starting AED therapy but usually resolve with time (Richens and Rowe, 1970). It is on such premise that use of herbal remedies with a number of chemical components may elicit effective actions with no side effects as AED. However, the side effects of the natural compounds may still not be understood because their chemical structures are not yet known.



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2.4 The use of Plant Materials and Extracts in Management of Convulsion

In Africa, phytotherapy in traditional medicine still plays an important role in the management of diseases, mainly among populations with low income (Geoffrey and Kirby, 1996). Phytotherapy relies on the use of a wide variety of plant species that are used empirically in traditional medicine to manage epilepsy and diseases related to the brain like agitations, anxiety, convulsions, dizziness, headaches, insomnia, migraines, pains and schizophrenia (Abbiw, 1990). For instance, the decoction of mature leaves of *Annona muricata* (Linn.) (Annonaceae) is used in central African traditional medicine to control fever and convulsive seizures (N'Gouemo *et al.*, 1997). In South Africa traditional medicine, several *Searsia* species (Anacardiaceae), including *S. dentata* and *S. pyroides*, are used to manage epilepsy (Marchetti *et al.*, 2011).

A cross-sectional study performed in Temeke District (Dar es Salaam, Tanzania) showed that 5.5% of the traditional healers have knowledge for the management of epilepsy. *Abrus precatorius* L. (Leguminosae), *Clausena anisata* (Willd.) Oliv. (Rutaceae) and *Hoslundia opposita* Vahl (Lamiaceae) are among the plants mentioned, have proven anticonvulsant activity, while a few other species on their list have been reported to be useful in the management of epilepsy (Moshi *et al.*, 2005). Biological testing of these plants, using different models of convulsions has however, not been done.

In India, retrospective reports revealed that the phytoconstituents such as flavonoid and saponin moieties in the leaf extract of *Cynodon dactylon* are responsible for anticonvulsant activity of the plant (Venkateswarlu *et al.*, 2012). In the traditional west African medicine, the roots of *Cnestis ferruginea* Vahl ex DC. (Connaraceae) are useful in the treatment of infantile illness, epilepsy, dysmenorrhoea and cough (Ishola *et al.*, 2014). Some other medicinal plants (Table 1) being used successfully as remedy against epilepsy are described below (Saba *et al.*, 2012).

Table 1: Some medicinal plants used to manage epilepsy

	Name of plant	**Part of plant**	**Diseases**
1	*Citrus sinensis* (Rutaceae)	Leaves, flowers, barks, roots	Epilepsy, Insomnia, malaria, schizophrenia, headache, anxiety
2	*Datura stramonium* (Solanaceae)	Fruits, leaves	Asthma, epilepsy, cough,
3	*Ricinus communis* (Euphorbiaceae)	Leaves, flowers	Epilepsy, convulsions, diarrhea, asthma
4	*Terminalia glaucescen* (Combretaceae)	Leaves, barks, roots	Malaria, stomach aches, hepatitis, leucorrhoea, epilepsy
5	*Tetrapleura tetraptera* (Leguminosae-Mimosoideae)	Barks, fruits, roots	Epilepsy, fever, convulsions, malaria
6	*Senna singuena* (Leguminosae – Caesalpinioideae)	Leaves, flowers, barks, roots	Fever, conjunctivitis, convulsions, epilepsy, syphilis, constipation
7	*Jatropha gossypiifolia* (Euphorbiaceae)	Leaves, roots	Convulsions, hypertension, fever
8	*Mentha cardifolia* (Lamiaceae)	Leaves	Insomnia, muscle relaxant

Source*:* (Saba *et al.*, 2012)

Recent studies (Dana and Steven, 2010) involving bioassay-guided isolation of active compounds have revealed anticonvulsant potential of some medicinal plants which are more rational. The future outlook for the development of new anti-epileptic drugs derived from these medicinal plants is therefore positive. Thus adopting an approach to evaluate the potential in *Maytenus heteropylla*, more rational and confimatory results may be envisaged.

2.5 The Family Celastraceae

The family Celastraceae comprises more than 90 genera and nearly 1300 species (Simmons *et al.*, 2008), the vast majority of which are exclusively tropical, with the exceptions of the

widely distributed genera *Celastrus* and *Euonymus* (Simmons *et al.*, 2008). Many species belonging to the family have been extensively studied due to their worldwide use in traditional medicine (Muñoz *et al.*, 1995; González *et al.*, 1996; Alvarenga and Ferro, 2006). The family has a long history in traditional medicine since they produce variety of bioactive metabolites of medical interest such as triterpenoid quinonemethides or phenolic triterpenes (González, 2000a; Alvarenga and Ferro, 2006).

Plants of the Celetraceae family have been reported to have several activities against pathophysiological conditions of the central nervous system. *Catha edulis* (Khat) is an important example known for its monoamine alkaloids with amphetamine-like stimulant effects, causing moderate psychological dependence and euphoria (Nutt *et al.*, 2007). *Celastrus paniculatus* is another plant widely used in the 'Ayurveda' medicine as an anxiolytic, analgesic, sedative, tranquillizer and has anti-epileptic properties (Bhanumathy *et al.*, 2010). The plant is also exhibits a number of compounds including sequiterpene polyesters characterized with benzoyloxydihydro-*β*-agarofuran nuclei (Borbone *et al.*, 2007), sesquiterpene alkaloids (Wagner and Heckel, 1975), terpenoids and steroids (Gamlath *et al.*, 1990) in the same way as *Maytenus* genus. Besides, the family elaborates quinonemethide triterpenes which have been reported as anticancer compounds (Bavovada *et al.*, 1990), and anti-ulcer agents (Souza-Formigoni *et al.*, 1991) among other biological relevance. However, potential anticonvulsant principles among these plants have not been identified.

2.5.1 Botanical Information of the Genus *Maytenus* (Synonym: *Gymnosporia heterophylla*)

Maytenus is a genus of flowering plants including more than 225 species in the family Celastraceae. Members of the genus are distributed throughout Central and South America, South East Asia, Australia, the Indian Ocean and Africa. They grow in a wide variety of

climates, from tropical to subpolar regions. *Maytenus* spp. are the most wide spread representatives of this Celastraceae family and are an evergreen shrubs, trees or, more rarely, a shrublet, often spreading or straggling, which grows up to 9m high. The leaves are petiolated, alternated or often fascicled (Fig. 1). The lamina of *M. heterophylla* is pale to deep green, often with a pale midrib, petiole up to 10 mm long (Fig. 1). Spathulated, oblanceolated to ovated or elliptic margins, up to 9.5 cm × 5 cm, irregularly serrulated to entire leaf (Hutchings *et al.*, 1996).

Figure 1; Aerial parts of *Maytenus heterophylla* in its natural habitat

2.5.2 Ethnopharmacological uses of the Genus *Maytenus*

The plants of the genus *Maytenus* are widely used in folk medicine as an antiseptic, anti-asthmatic, fertility regulating agents, antitumor, as well as for managing stomach problems (Flores, 1984; Ghazanfar, 1994). The genus *Maytenus* is notably used in Brazilian traditional medicine for the treatment of gastric ulcers (De Andrade *et al.*, 2008), inflammation and

diarrhea (Santos *et al.*, 2007), as antimicrobial (González *et al.*, 1996), antitumor (González *et al.*, 2000) and insecticidal agent (Avilla *et al.*, 2001). *Maytenus heterophylla* and *M. Senegalensis* are two African medicinal plants used to manage painful and inflammatory diseases (Da Silva *et al.*, 2011), a claim confirmed through an in *vivo* experiment using mice that showed a significant anti-inflammatory activity of both *M. heterophylla* and *M. senegalensis* leaf extracts (Da Silva *et al.*, 2011). *M. heterophylla* is further claimed by different African communities to be used in management of malaria (Muthaura *et al.*, 2007); sexually transmitted diseases (antimicrobial effects); convulsions, breathing difficulties and chest pains (Okello *et al.*, 2010), livestock diarrhea (Watt and Breyer-Brandwijk, 1962); and removal of ticks from animals (Wanzala *et al.*, 2012). However, the efficacies for these applications of *M. heterophylla* root and stem bark have not been validated due to lack of scientific experimental evidence.

Plants of the genus *Maytenus* are extensively investigated for bioactive compounds as they are widely used in folk medicine as an antiseptic, antiasthmatic, fertility regulating agent, antitumor, as well as for stomach problems (Flores, 1984; Ghazanfar, 1994). In Mozambique, leaves, stems and roots of some species are used by traditional healers to control dysentery, snake bites, wounds and respiratory diseases (Da Silva *et al.*, 2011). The same traditional uses are also common in other African countries like Benin, Kenya, Zambia, Tanzania, Senegal and Zimbabwe (Hutchings *et al.*, 1996; Muller and Mechler, 2005). These plants are used as muscle relaxants and analgesics, for relief from arthritis, rheumatism, hemorrhoids, kidney swelling, skin eruptions, as well as skin cancer prevention, and the treatment of colds, dysentery, bronchitis, against worms (Revilla, 2002), as aphrodisiacs (Silva *et al.*, 1977), stimulants and tonics (Duke and Vasquez, 1994). In Brazil, plants from *Maytenus genus* are used in the traditional Brazilian medicine for the treatment of gastric ulcers (De Andrade *et*

al., 2008), inflammations, and diarrhea (Santos *et al.*, 2007), antitumor (Shirota *et al.*, 1994; González *et al.*, 2000b) and as insecticidal agents (Kiem *et al.*, 2004).

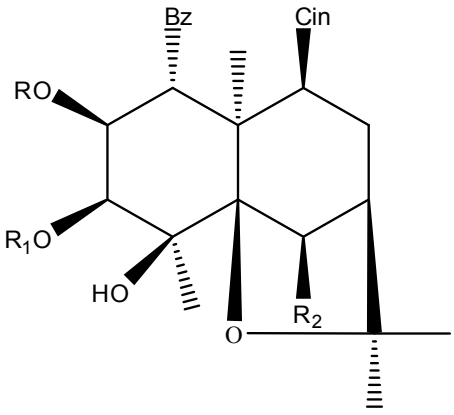
In Kenya, *M. heterophylla* root bark decoction is known to be used against malaria by the Meru community (Muthaura *et al.*, 2007). The root bark decoction is used against sexually transmitted diseases, breathing difficulty and chest pains by the Sabaot community of western Kenya (Okello *et al.*, 2010); the bark and leaf decoction are reported to be used by the Zulu community of South Africa to treat diarrhea among the livestocks (Watt and Breyer-Brandwijk, 1962) and the leaves, fruits, stem bark and roots of the plant are applied on the animal's body surface by the Bukusu community of western Kenya to remove livestock ticks (Wanzala *et al.*, 2012). The application of *M. heterophylla* by several communities in Kenya and other African countries against several ailments, convulsion included, has however not been validated.

2.5.3 Phytochemistry and pharmacological activities of the genus *Maytenus*

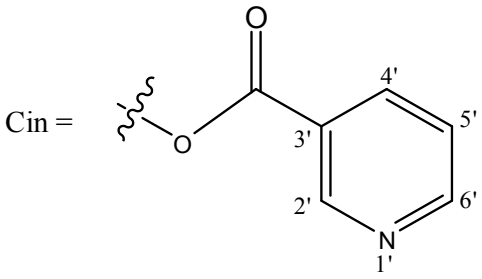
Plants of the genus *Maytenus* are rich sources of diverse types of secondary metabolites, including triterpenes (Shirota *et al.*, 1996), oligo-nicotinated sesquiterpenes and sesquiterpene pyridine alkaloids (Corsino *et al.*, 1998), phenolic glycosides (Sannomiya *et al.*, 1998) and agarofuran sesquiterpenes (Orabi *et al.*, 2001). Most of these compounds display broad spectrum of biological activities including antitumor (González *et al.*, 1996; Chavez *et al.*, 1998), antimicrobial (Orabi *et al.*, 2001) and insecticidal activities (Núñez *et al.*, 2004) etc. A general overview of compounds-activity relationship indicates that sesquiterpene alkaloids exhibit the cytotoxic and insecticidal or antifeedant properties of genus *Maytenus* (Kuo *et al.*, 1990; Kuo *et al.*, 1994; Núñez *et al.*, 2004) whereas the phenolic-type are antimicrobial compounds active as antiseptic, or disinfectants (McDonnell and Russell, 1999). The insecticidal and antifeedant properties (Núñez *et al.*, 2004) make these plants to be free from

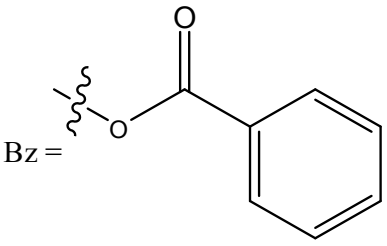
insects and other browsers infestations. Dihydro-β-agarofuran sesquiterpenes and sesquiterpene alkaloids have been isolated from other plants of the family Celasteraceae which are particularly used as anticonvulsants (Wagner and Heckel., 1975; Borbone *et al.*, 2007). It is however, not yet established if these are the compounds responsible for anticonvulsant effects in *Maytenus heterophylla*.

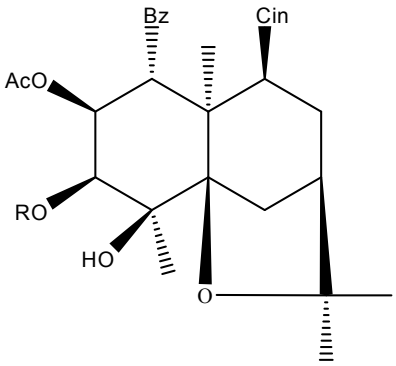
Highly oxygenated sesquiterpenes with a dihydro-*β*-agarofuran skeleton are widespread within the family Celastraceae. For instance four β-agarofuran polyesters were isolated from the seeds of *Maytenus boaria, Maytenus spinosa, Maytenus jelskii, and Maytenus putterlickoides* (Alarcon *et al.*, 1995). Most of these sesquiterpenes have alkaloid moiety in their structure and have been associated with cyctoxocity and cancer cells prevention. For instance dihydro-β-agarofuran sesquiterpenes (**17-22**) from *Maytenus jelskii* were shown to be potential cancer chemopreventive agents against Epstein-Barr virus antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Perestelo *et al.*, 2010). Sesquiterpenoids isolated from *Maytenus spinosa* were tested for anti-HIV activity, but only, β-dihydroagarofuran (**23**), was found to be active (Gutiérrez-Nicolás *et al.*, 2014).



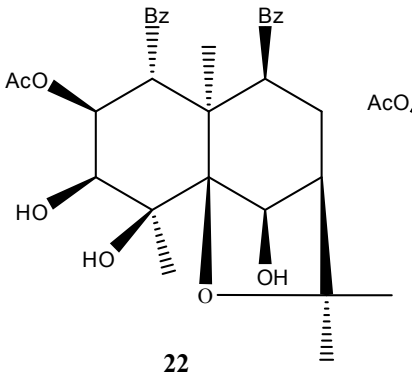
17 R = Ac; R_1 = H; R_2 = H_2
18 R = Ac; R_1 = Ac; R_2 = OH
19 R = H; R_1 = H; R_2 = OH







20 R = Ac
21 R = H



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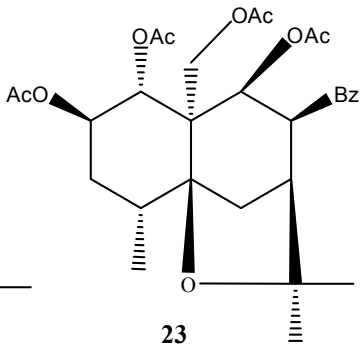
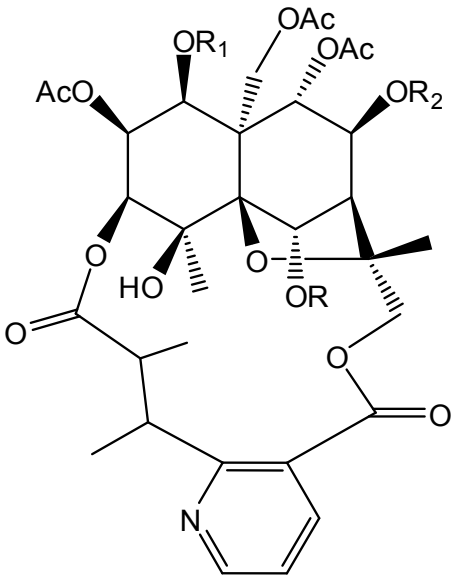
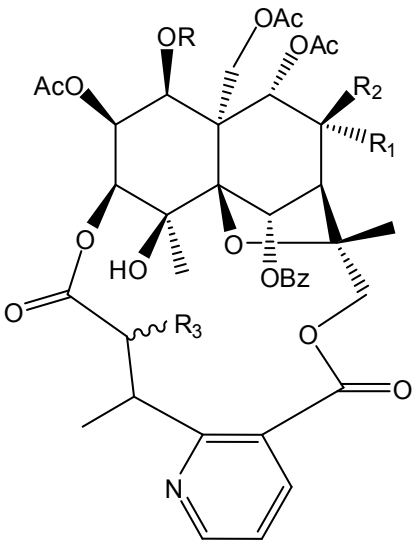


23

A bioprospecting investigation of *Maytenus ilicifolia* root bark for antiprotozoal agents led to the isolation of four sesquiterpene alkaloids, ilicifoliunine A (**24**), aquifoliunine E-I (**25**) and mayteine (**26**) (Santos *et al.*, 2012), which were tested against *Leishmania chagasi* and *Trypanosoma cruzi in vitro*, however, only aquifoliunine E-I (**25**) showed activity with an IC_{50} values of 1.4 and 41.9 µM, respectively and low cytotoxicity of IC_{50} of 1.8 mM against murine peritoneal macrophages (Santos *et al.*, 2012). Investigation of *Maytenus mekongensis* roots reported the isolation of over eight sesquiterpene alkaloids (**27-31**), which showed moderate to potent antiplasmodial activity against *Plasmodium falciparum*, K1 strains and low cytotoxicity using a panel of cell lines (Lhinhatrakool *et al.*, 2011).

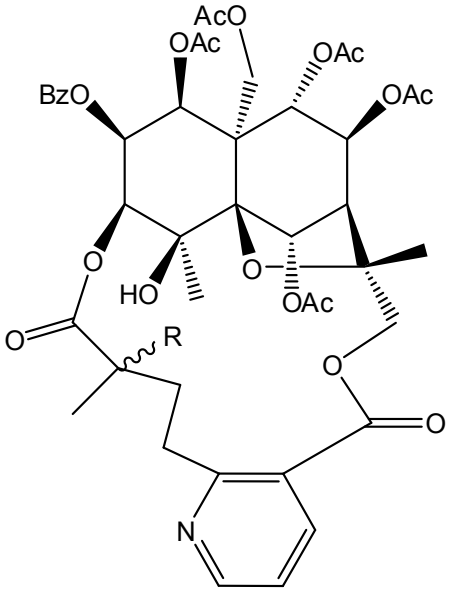


24 R = H; R_1 = Ac; R_2 = Bz
25 R = Ac; R_1 = Ac; R_2 = Bz
26 R = Ac; R_1 = Bz; R_2 = Ac

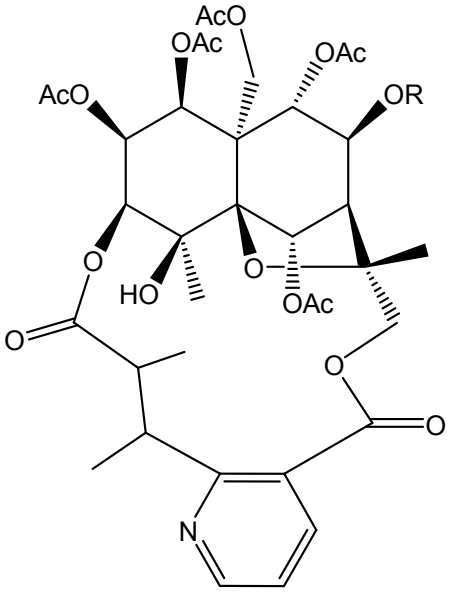


	R	R_1	R_2	R_3
27	Ac	H	OAc	OAc
28	Ac	OAc	H	OAc
29	Bz	H	OAc	OAc
30	Ac	H	OAc	H
31	Bz	H	OAc	H

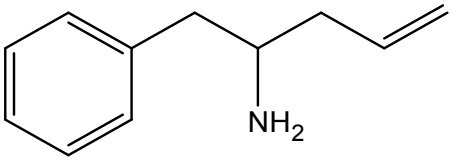
Sesquiterpene pyridine alkaloids from *Maytenus* genus have also been implicated to have antifeedant properties such as sesquiterpene pyridine alkaloids mayteine (**26**), wilfordine (**32**), euonine (**33**), euonymine (**34**), 4-hydroxy-7-epi-chuchuhuanine E-V (**35**), alatamine (**36**) and forestine (**37**), from the leaves of *Maytenus chiapensis* that showed good antifeedant properties (Núñez *et al.*, 2004). However, only wilfordine (**32**), and euonine (**33**) exhibited strong antifeedant activity against *Spodoptera littoralis* (Núñez *et al.*, 2004).



32: R = OH
33: R = H



34: R = H
35: R = Ac



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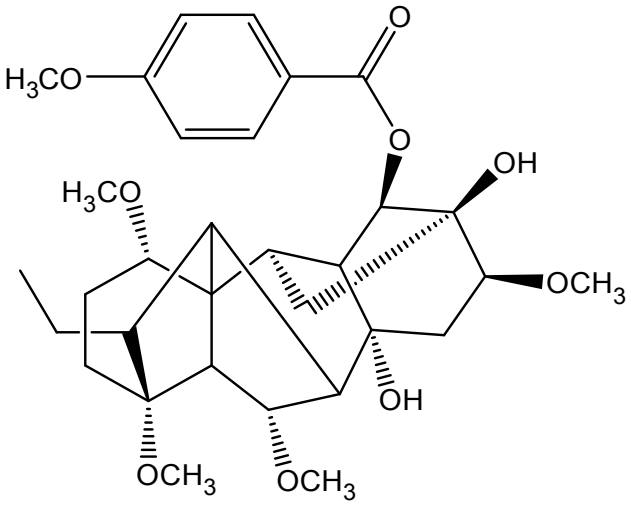
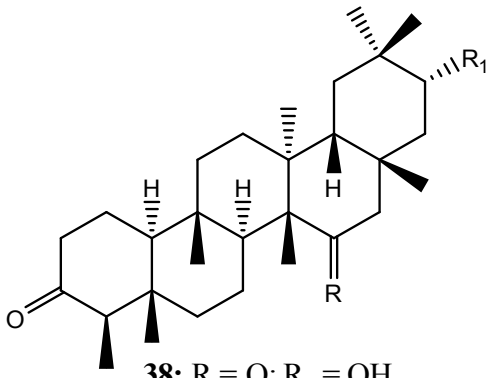


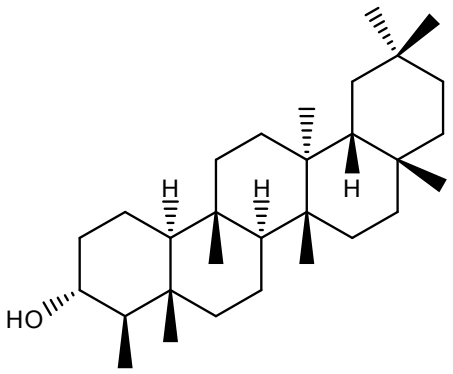
37

The triterpenes have been the active compounds against the microbial growth (Orabi *et al.*, 2001) as well as showing anticytomegalovirus properties (Murayama *et al.*, 2007), however, the biological activities of the other compounds such as the sesquiterpene alkaloids and minor compounds from this plant against other human pathophysiological condition such as convulsion are not known.

Pentacyclic triterpenes are also important bioactive compounds encountered within plants of the genus *Maytenus.* For instance, previous investigation on *Maytenus robusta* led to the isolation of antinociceptive (Niero *et al.*, 2006) and antiulcerogenic 3,15-dioxo-21-hydroxyfriedelane (**38**) among other active triterpenes (de Andrade *et al.*, 2008). Further phytochemical investigation lead to the isolation of acetylcholinesterase inhibitory compounds from the leaves led to the characterization of eleven compounds including friedelin (**39**), β-friedelinol (**40**), 3 -oxo-21*β*-*H*-hop-22(29)-ene (**41**), 3,4-*seco*-olean-3,11*β*-olide (**42**), 3*β*-hydroxy-21*β*-*H*-hop-22(29)-ene (**43**), 3,4-*seco*-21*β*-*H*-hop-22(29)-en-3-oic acid (**44**), 3,4-*seco*-friedelan-3-oic acid and (**45**) (Sousa *et al.*, 2012). Such results indicated the ability of *Maytenus* compounds are involved in the control of nervous activities which by extension may be envisaged to have anticonvulsant effects.



38: R = O: R_1 = OH
39: R = H_2; R_1 = H



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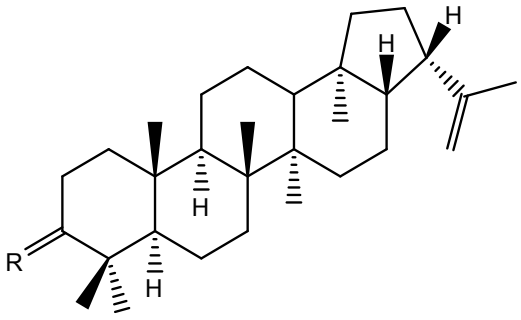
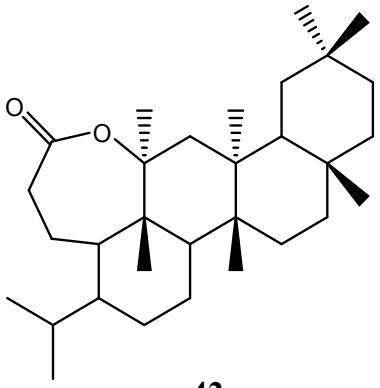


41: R = O
43: R = OH/H



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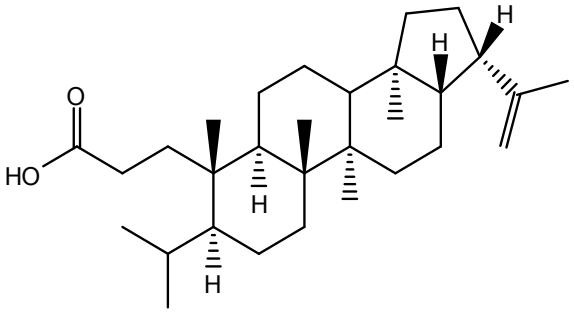
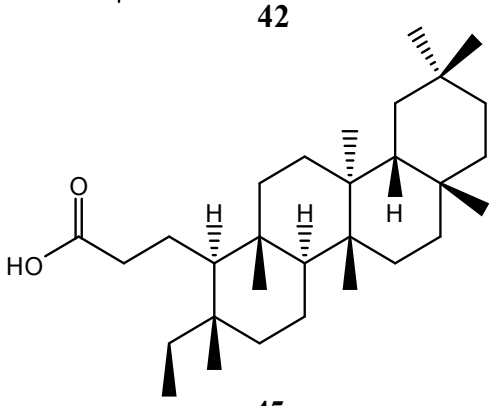


44



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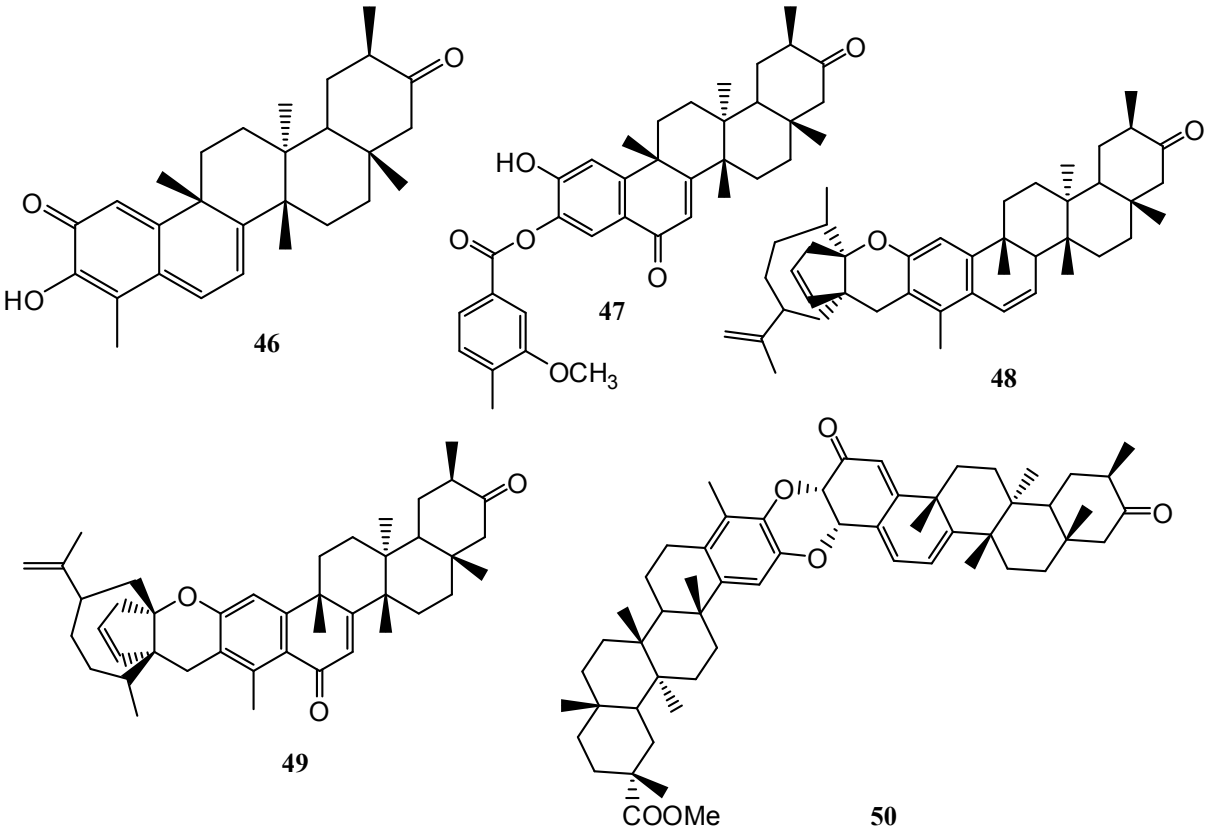
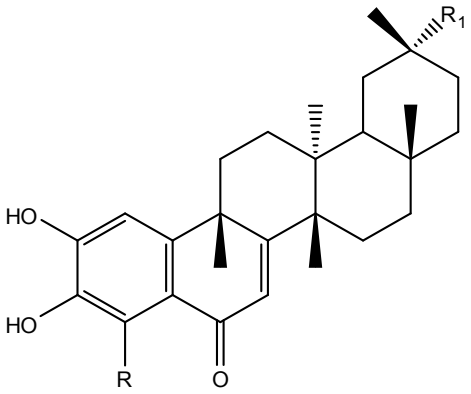
A study on the antiprotozoal activities of the *M. ellicifolia* root bark led to the isolation of two quinonemethide triterpenes namely maytenin (**8**) and pristimerin (**9**) (Dos Santos *et al.*, 2013) which showed potent leishmanicidal activity with an IC_{50} lower than 0.3 nM against *Trypanosoma cruzi* epimastigotes and selectivity indexes (SI) of 243.65 and 46.61 for (**8**) and 193.63 and 23.85 for (**9**) based on BALB/c macrophages for *L. amazonensis* and *L. chagasi*

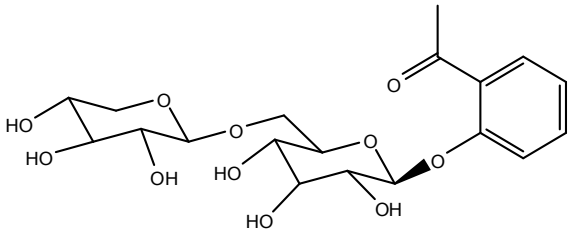
indicating that both compounds presented high selectivity for *Leishmania* spp. (Dos Santos *et al.*, 2013). Additionally, dimeric quinonemethide triterpenes (**46-50**) were isolated from the same plant following an improved centrifugal partition chromatography (Gutiérrez-Nicolás *et al.*, 2014). However, their biological activities were not established.



Triterpenes from the genus *Maytenus* have also been reported to show potent antibacterial activities. For instance, two 6-oxophenolic triterpenoids [zeylasterone (**51**) and demethylzeylasterone(**52**)] isolated from *Maytenus blepharodes* showed bactericidal activity against *Staphylococcus aureus* (De Leo′n *et al.*, 2010). A bioautography-guided examination of *M. heterophylla* and *M. arbutifolia* led to the isolation of dihydroagarofuran alkaloid (**1**) as well as the triterpenes maytenfolic acid (**3**), 3α-hydroxy-2-oxofriedelane-20α-carboxylic acid (**4**) and lup-20(29)-ene-*1β,3β*-diol (**6**) (Orabi *et al.*, 2001). However, only maytenfolic acid

(**3**) showed moderate antimicrobial activity by inhibiting the growth of *Candida albicans, Crytococcus neoformans, Staphylococcus aureus* and *Pseudomonas aeruginosa* (Orabi *et al.*, 2001). Pristimerin (**9**), a triterpene isolated from the Kenyan *M. heterophylla* alongside lupeol (**5**) and 2-acetylphenol-1β-D-glucopyranosyl (1 --> 6)-β-D-xylpyranoside (**53**) showed potent anticytomegalovirus properties against human cytomegalovirus (HCMV) (Murayama *et al.*, 2007). Considering the structural diversity of these triterpenoids from *Maytenus* in relation to their biological activities, it is not known as yet whether they may be the novel molecules acting as anticonvulsants in *Maytenus heterophylla*.





53

51: R = COOH; R_1 = COOMe

52: R = COOH; R_1 = COOH

CHAPTER THREE: MATERIALS AND METHODS

3.1 General Instrumentation

All the solvents used for extraction were either of analytical grade or pre-distilled general purpose reagents. Extracts were concentrated using vacuum rotary evaporator (Buchi R-114, Switzerland). Melting points were determined on a Koffler melting point apparatus and are uncorrected. All column chromatographic purifications were performed on silica gel (Merck 60–120 mesh ASTM) whereas all TLC developments were performed on silica gel aluminium pre-coated (Merck Kieselgel 60 F_{254}, 0.2 mm thickness) plates. Detection and visualization was done under UV light (254/365 nm) for the UV-active compounds. For UV-inactive compounds, the chromatogram was sprayed with 4% anaisaldehyde-sulphuric acid reagent and heated with a heat gun for visualization. IR spectra were recorded on a Perkin Elmer 200 FT-IR spectrometer. The EI-MS and HR-MS spectra were recorded on FinniganMat SSQ 7000 direct probe mass spectrometer. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz) spectra were recorded on Bruker's Avance-500 FT 500 MHz and Avance – 600 (Bruker, Switzerland) using deuterated solvents and referenced to residual signal. Standard pulse sequences were used for each experiment

3.2 Plant Materials

The leaves, stem and roots of *M. heterophylla* were collected from Kitale KCC forest (1°01′27.14″N 35°00′55.04″E), Trans Nzoia County in western Kenya and authenticated by Mr Matheng in the National Museum of Kenya where the voucher specimen (MEW/03/2014) was deposited. The materials were then shade dried and powdered using a hand mill.

3.3 Solvent Extraction

Solvent extraction was carried out on 2 kg, 2.6 kg and 1.3 kg of ground – dried leaves, stem back and roots of *Maytenus heterophylla*, respectively using methanol. The powdered plant materials were separately extracted by soaking in methanol (3L×3). The mixture were shaken using an orbital shaker machine at 150 rpm for 12 h and left for extraction to take place through percolation for 2 days. Filtration and concentration under reduced pressure and re-soaking were done until the solvent became clear. The extraction process took 9 days to afford crude extracts (14 g, 72.3 g and 10 g) of leaves, stems and roots, respectively which were stored at 4 ^{0}C awaiting anticonvulsant activity and phytochemical studies.

3.4 Isolation of Compounds from *M. heterophylla* Stem Bark

The stem bark extract which exhibited significantly ($P<0.05$) higher anticonvulsant activity (Table 5) was subjected to chromatographic separation to identify the active principles. The stem bark extract (60 g) was suspended in distilled water and then partitioned successively with *n*-hexane (S-1), CH_2Cl_2 (S-2) and EtOAc (S-3) using a separating funnel. Each fraction was evaporated under reduced pressure to afford 2.2 g, 11.6 g and 23.6 g of extracts, respectively leaving the residual water extract (21.2 g). The *n*-hexane extract did not show a clear spots on TLC and was not followed further.

3.4.1 Fractionation of EtOAc Extract (S-3)

The EtOAc extract (23.6 g) was adsorbed onto silica gel and then subjected to column chromatography (Merck 60–120 mesh ASTM SiO_2 350 g, pressure≈1 bar) using *n*-hexane-EtOAc gradient (from 100% *n*-hexane to 8:2 *n*-hexane: EtOAc) to give 80 fractions which were pooled based on their TLC profiles to afford three major fractions (E_1– E_3). Fraction E_1 (3.74 g) was further separated by *n*-hexane-EtOAc (4:1) system to give five sub-fractions

(E_1a–E_1e). Sub-fraction E_1c (1.69 g) was separated using preparative thin layer chromatography (PTLC) to obtain a colourless amorphous solid of compound **54** (64 mg). Sub-fraction E_1e (1.32 g) was chromatographed on silica gel (230 – 400 mesh, 50 g) using a small column eluted with *n*-hexane – EtOAc (5:1 – 1:1 gradient system) and recrystallized in MeOH to yield a pale yellow amorphous solid of compound **55** (96 mg). Compound **56** (83 mg) was obtained by repeated fractional crystallization of the mother liquor of fraction E_2 (2.1 g) using methanol (1/3, v/v) as a white amorphous solid. Fraction E_3 (1.61 g) was subjected to CC on silica gel (48g) with EtOAc-hexane (1:1, v/v) to give **57** (28 mg) as a white amorphous solid.

3.4.2 Fractionation of CH_2Cl_2 Extract

The crude CH_2Cl_2 extract (23.6 g) was subjected to CC on silica gel (Merck 60–120 mesh ASTM) (230 g), eluting with *n*-hexane – CH_2Cl_2 (3:1 and 2:1) gradient at 20 ml/min and resulted in three fractions (D1-D3) weighing 5.2 g, 6.1 g and 7.9 g, respectively. Upon recrystallization of sub-fraction D1 in CH_2Cl_2–*n*-hexane (1:1) system, additional **55** (25 mg) was obtained. Compound **54** (150 mg) crystallized out in CH_2Cl_2:MeOH from sub-fraction D2 after elution with *n*-hexane-CH_2Cl_2 (4:1 and 7:3). When Fraction D3 was eluted with CH_2Cl_2-MeOH 5:1 solvent system, a white crystalline solid of compound **58** (71 mg) was obtained.

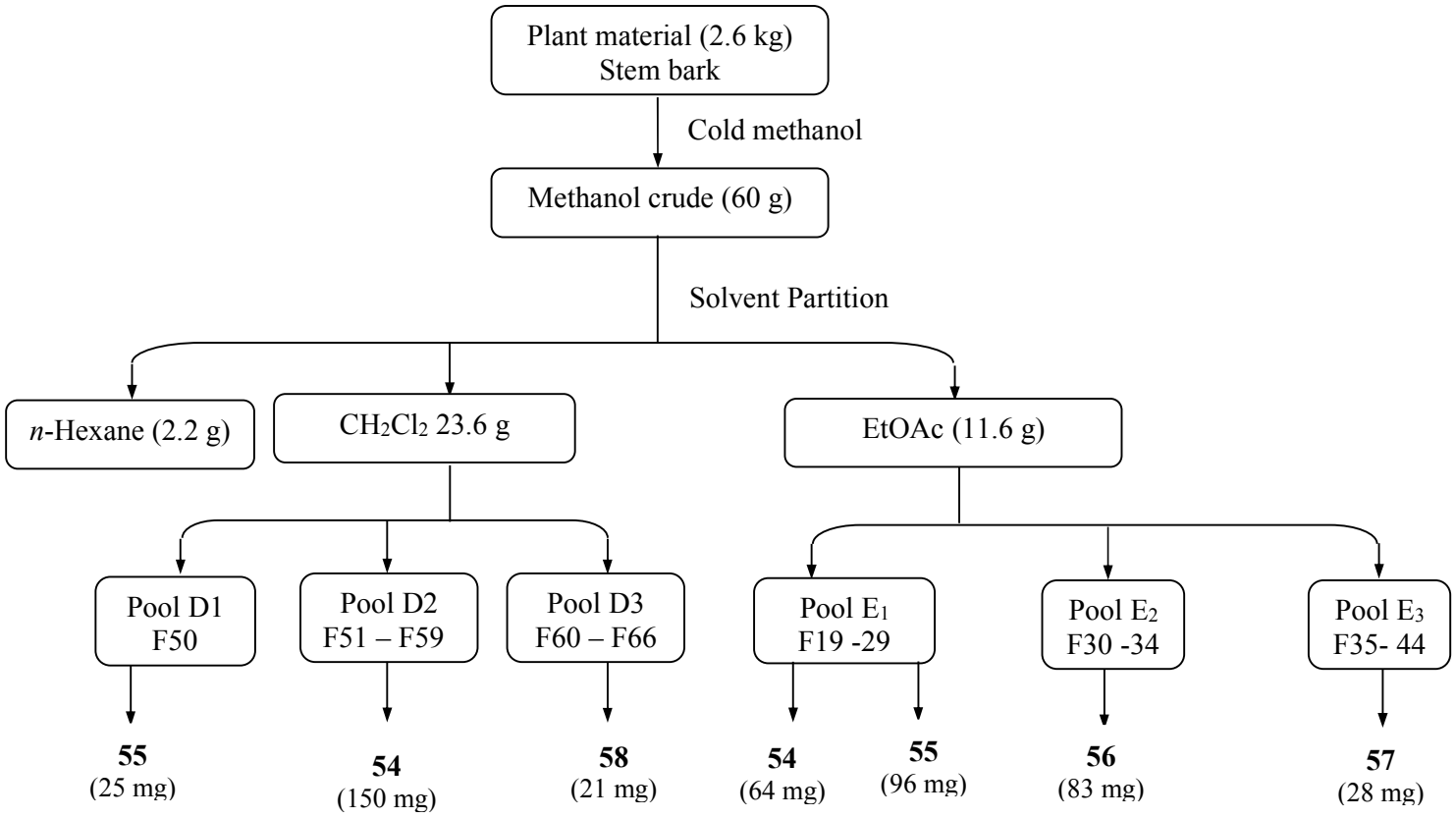


Figure 2: Summary of the isolation procedure of the stem bark of *M. heterophylla*

3.5 Physical and Spectroscopic Data of Isolated Compounds

Compound **54 (**3-acetoxy-28-hydroxylupe-20(29)-ene**):** white amorphous solid, m.p 256-257ºC; IR: ν_{max} (KBr) cm^{-1}: 3468 (O-H stretching), 1729 cm^{-1} (C=O stretch), 883, 976 cm^{-1} (C-H bending), 1643 cm^{-1} (C=C stretch) ^{1}H NMR and^{13}C NMR ($CDCl_3$, 500 MHz and 125 MHz; Table 2 and 3, respectively); HRESIMS for $C_{32}H_{52}O_3$; *m/z*: 485.1872 [M+] Calculated for 485.1950 $[M+H]^+$

Compound **55** (2-Methoxy-4-decarboxydihydrozeylasterone): pale yellow amorphous solid, m.p 252-254°C. TLC (purple spot R_f 1.6) in *n*-hexane-EtOAc 5:1 solvent system. Uncorrected; IR (KBr) ν cm^{-1}: 1727, 1642, 3517 and 1200. UV (MeOH) λ_{max}: 241nm and 304nm; ^{1}H NMR and^{13}C NMR ($CDCl_3$, 600 MHz and 150 MHz; Table 2 and 3 respectively); HRESIMS *m/z* (482.3012 $[M]^+$ calculated for 482.2921.

Compound **56 (**3, 4-*seco*-1-hydroxy-21-oxo-olean-3, 11-olide): white amorphous solid, m.p 268-270°C; ^{1}H NMR and ^{13}C NMR ($CDCl_3$, 600 MHz and 150 MHz; Table 2 and 3 respectively); ESIMS *m/z* 470.68 $[M^+]$

Compound **57** (Oleanolic acid): White amorphous solid, m.p 305-306°C; ^{1}H NMR and ^{13}C NMR ($CDCl_3$, 600 MHz and 150 MHz; Table 2 and 3, respectively)

Compound **58 (**3, 5-dihydroxycamphanoate): white crystalline solid, m.p 121-122°C; ^{1}H NMR and^{13}C NMR ($CDCl_3$, 600 MHz and 150 MHz; Table 4); ESI-MS *m/z* 199.1256 $[M+H]^+$ calculated for 199.1315.

3.6. *In vivo* Anticonvulsion Assays

3.6.1 Laboratory Animals

White albino mice (*Mus musculus* Swiss; 22 ± 2 g; 8 per group) of either sex (Singh *et al.*, 2012) were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos state Nigeria. The animals were housed in well ventilated plastic cages at room temperature with 12/12 h light-dark cycle and free access to commercial food pellets and water *ad libitum* (Hema *et al.,* 2009). They were acclimatized for at least one week before use for all experiments. The study was carried out in accordance with the ethical guidelines of the Animal Ethics and Experimentation, College of Medicine, University of Lagos.

3.6.2 Drugs and Treatment

Diazepam (**10)** (Swipha, Pharmaceutical Ltd, Lagos, Nigeria) was used in the current investigation as a standard drug for treatment of experimental animals and picrotoxin (PTX) (Sigma-Aldrich, St. Louis, USA), to induce convulsions. All drugs were dissolved in distilled water immediately before administration. Doses of 50, 100, and 200 mg/kg of crude extracts and 0.5, 5 and 50 mg/kg of pure compounds were used in distilled water (Sushma *et al*., 2012)

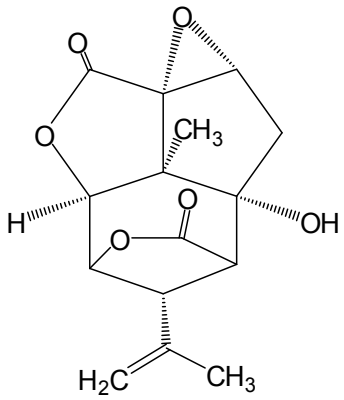
3.7 Screening of crude extracts

Picrotoxin-induced convulsion test was carried out according to the method previously described by Sushma *et al*. (2012). Albino mice of either sex were allotted to 5 groups (n=8). Group I received distilled water (10 ml/kg, p.o.) and served as negative control, three test groups II – IV received different doses of *Maytenus heterophylla* crude extracts (50, 100, 200 mg/kg, p.o), and one more group V that received Diazepam (5 mg/kg, p.o.), a known anticonvulsant compound and served as standard (positive control). Picrotoxin (5 mg/kg, i.p) was injected 60 min post drug treatments. Onset and duration of seizures were recorded

(Table 5). Mice that did not convulse after 30 minutes of picrotoxin administration were considered to be protected (Vogel and Vogel, 1997; Hema et al., 2009).

3.8. Anticonvulsant Acitivity of Isolated Compounds

The tests were done as already described in sections 3.4 and 3.5 above (Sushma et al., 2012). Mice were randomly distributed into treatment groups (8 per group) and isolated compounds **54**, **55**, **56** or **58** administered (0.5, 5, and 50 mg/kg, p.o.), diazepam (5 mg/kg, p.o.) used as standard drug or distilled water (10 ml/kg; p.o.), 60 min prior to the administration of picrotoxin (PTX), **59** (5mg/kg, i.p.) (Table 6). Animals were immediately placed individually in a transparent observation chamber and observed for the expression of convulsions for 30 min after picrotoxin injection (Nagaraja *et al.*, 2012). Compound **57** was not subjected to bioassay studies to small amounts.



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3.9 Statistical Analysis

The latency of clonic PTX seizures were compared using one-way analysis of variance (ANOVA) followed Turkey's Least Significant Difference ($P \leq 0.05$).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Anticonvulsant activity of crude extracts

PTX is used as a substance as well as an acute experimental model in the preliminary screening to test potential anticonvulsant drugs. PTX induces convulsion by antagonizing the ɤ-aminobutyric acid $(GABA)_A$ receptor chloride (Cl)-channel complex to attenuate GABA-dependent inhibition (Sushma *et al*., 2012). Picrotoxin (PTX) produces clonic and tonic convulsions. Prevention of PTX-induced seizures in laboratory animals is commonly used in preliminary screening for identifying potential anticonvulsant drugs. Based on the duration of seizures, there was a significant ($P \leq 0.05$) decrease in time for seizures in groups treated with Diazepam, high dose and medium dose of the stem bark methanol extract of *M. heterophylla* in comparison with control (Table 5).

There was also decreased time for the onset of convulsions groups treated with Diazepam and high doses of *Maytenus heterophylla* extracts compared to control animals. The methanol extracts of *Maytenus heterophylla* increased the threshold of PTX-induced convulsion in mice and offered protection against PTX-induced convulsion. The protection offered against PTX-induced convulsion in mice varied with concentration with leaf extracts offering minimal seizure protection except at high concentrations. The protection offered by stem bark extract against PTX-induced convulsion in mice (62.5% at 50 mg/kg) was high ($P<0.05$) compared to distilled water (87.5%) indicating that constituents of the stem bark have potential anticonvulsant activity.

These results suggest that the methanolic stem bark extract of *Maytenus heterophylla* contains bioactive constituents which possess clinically applicable anticonvulsant activities that may be beneficial in the management of epilepsy and lend credence to the use of the plant's stem bark in the management of epilepsy in traditional medicine.

Furthermore, the study also indicates that *Maytenus heterophylla* extract is useful in overcoming PTX (Picrotoxin) induced convulsions in a dose dependent manner.These results are consistent with previous studies that pointed to the anti-epileptic properties of plants in the family Celasteraceae which are particularly used as anticonvulsants (Wagner and Heckel, 1975; Borbone *et al*., 2007). In other studies, the chloroform extract of *Erythrina variegata* increased the threshold of Pentelyne tetrazole -induced convulsion in rats and offered protection against PTZ-induced convulsion (Vogel and Vogel, 1997). *Ferula Assa Foetida* gum extract is able to reduce seizure duration and its intensity (Kiasalari *et al.,* 2013) In this study, the maximum anti-epileptic response of *Maytenus heterophylla* extract in terms of suppressing seizure attacks, the latency of epileptic response, and the duration of the epileptic statues was observed at the dose of 200 mg/kg. This dose is the most effective dose of the plant tested. Therefore, it seems that these effects are dose dependent.

Table **2**: Effect of methanol extracts of *M. heterophylla* on PTX -induced seizure in Mice

Treatment	Conc. mg/kg	On set of convulsion (sec)	Duration of convulsion (sec)	No convulsed/ No used	% Protection
Distilled water	10	214.00	230.0	8/8	0
Leaf extract	50	210.13	176.0	8/8	0
Leaf extract	100	253.80	172.0	7/8	12.5
Leaf extract	200	310.60	121.0	7/8	12.5
Root extract	50	310.60	142.0	8/8	0
Root extract	100	389.47	110.0	6/8	25
Root extract	200	493.20	107.0	4/8	50
Stem extract	50	284.53	124.0	7/8	12.5
Stem extract	100	347.27	115.0	5/8	37.5
Stem extract	200	491.20	81.5	3/8	62.5
Diazepam	5	668.40	72.6	1/8	87.5
CV (%)		0.3849	0.3426		
LSD, P< 0.05		7.14	4.44		

Values are expressed as means of n=8. Statistical level of significance analysis by one way ANOVA followed Tukey's Least Significant Difference (P<0.05).

4.2 Structure Elucidation of Compounds from the Stem Back of *M. heterophylla*

Phytochemical evaluation of the stem back of *M. heterophylla* led to the isolation of two new compounds named 3-methoxy-4-decarboxydihydrozeylasterone (**54**) and 3,4-*seco*-1-hydroxy-21-oxo-olean-3,11-olide (**55**) together with three known compounds named 3-acetoxy-28-hydroxylupe-20(29)-ene (**56**), oleanolic acid (**57**) and 3,5-dihydroxycamphanoate (**58**). The structures were determined as discussed below.

4.1.1 3-Acetoxy-28-hydroxylupe-20(29)-ene (54)

Compound **54** was isolated as a white amorphous solid with a melting point of 256 - 257°C and its mass spectral data gave a molecular ion peak at *m/z* 485.1872 ($C_{32}H_{52}O_3$; calculated for 485.1950) corresponding to its $[M+H]^+$ from the HRESIMS. This was supported by the ^{13}C NMR spectral data (Table 2) which revealed 32 carbons that were sorted out by DEPT experiments (Appendix 3) as; eight methyls, eleven methylene, six methine and seven quaternary carbons.

IR (KBr) spectrum (Appendix 5) of **54** showed bands at 1729 cm^{-1} suggesting the presence of ester carbonyl, a very intensely broad band at 3468 cm^{-1} was observed for the O-H stretching vibration. The out of plane C-H vibrations of the unsaturated carbon was observed at 883 cm^{-1} and 976 cm^{-1}. The corresponding C=C vibrations shown at 1643 cm^{-1} was weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intence band at 2921 cm^{-1} and medium intensity band at 1461cm^{-1}. The vibration of the methylenic part was shown by the band at 2852 cm^{-1} and the medium band at 1461cm^{-1}. The moderate intense band at 721 cm^{-1} was attributed to the rocking movement of methylenic bonds. The corresponding C-C vibration was shown as weak intense band at 1039 cm^{-1}.

The ^1H NMR spectrum (Appendix 1 showed seven methyl signals (consisting of six tertiary methyl singlets at δ_H 0.81, 0.86, 0.88, 0.90, 0.96 and 1.05 similar to the methyls attached to a

triterpenoid nucleus (Shamma *et al.*, 1962) and acetate methlyl at δ_H 2.01), one methylene group at C-28 as a broad singlet at δ_H 4.53 and an oxygenated methine at δ_H 4.55. It also showed two vinylic protons at δ_H 4.59 and 4.70 representing the exocyclic double bond (C-20 and C-29) (Chaturvedula and Indra, 2012). The ^{13}C NMR spectrum of compound **54** showed 30 carbon resonances for the terpenoid of lupane skeleton (Seung *et al.*, 2013) which included a carbon bonded to the hydroxyl group at C-28 position (δ 68.3). The Δ^{20-29}-functionality of a lupene skeleton was inferred for this compound from the resonance of the sp^2 carbons at C-29 (secondary carbon signal deduced by DEPT pulse sequence at δ_C 107.8) and C-20 (quaternary carbon at δ_C 154.2)

A detailed analysis of the ^1H and ^{13}C NMR spectra of compound **54** confirmed the characteristic features of a triterpenic lupe-20(29)-ene parent structure having an acetoxy group at C-3 which was supported by the reported literature values (Pravat *et al.*, 2010) and compound **54** was thus identified as 3-acetoxy-28-hydroxylupe-20(29)-ene.

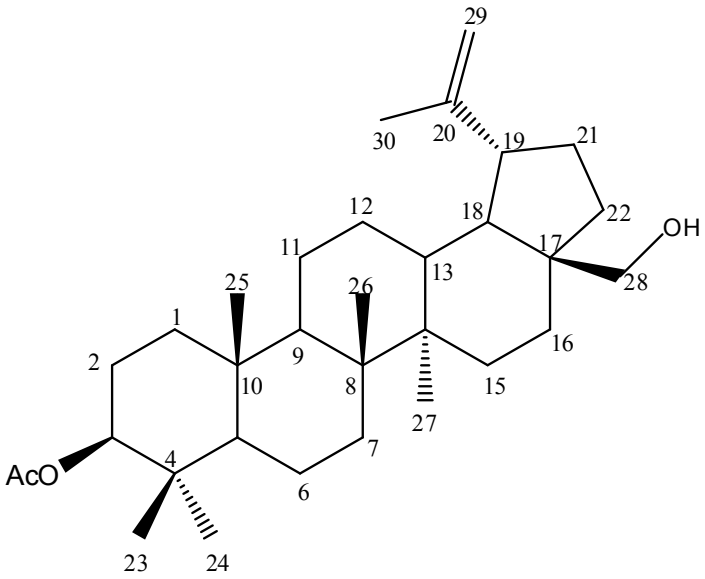

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Table 3: ^{1}H NMR spectral data for compound 54 ($CDCl_3$, 500 MHz), 55, 56 and 57 ($CDCl_3$, 600 MHz), δ(J in Hz)

Atom	54	Literature (Pravat *et al.*, 2010)	55	56	57
1	1.42 (m, 2H)	1.44 (m, 2H)	6.82 s	4.34 dd (4.8, 7.9)	1.55/1.73 (2H, m)
2	1.67 (m, 2H)	1.71 (m, 2H)	–	3.19 dd (7.9, 14.5)/2.79 dd (4.8, 14.5)	1.58/1.63 (2H, m)
3	4.55 (1H, dd, J = 4, 14.5 Hz)	4.60 (1H, dd, J = 4, 14.5 Hz)	–	–	3.14 (1H, dd, *J* =8.4, 5.1 Hz)
4	–	–	7.61 s	2.75 m	–
5	1.42 (m, 1H)	1.44 (m, 1H)	–	2.05 m	0.71 (br s)
6	1.42 (m, 2H)	1.44 (m, 2H)	–	1.69 m / 2.13 dt (3.5, 13.8)	1.52/1.57 (2H, m)
7	1.28 (m, 2H)	1.31 (m, 2H)	2.53/2.14	1.59 / 1.16 t (t)	1.26/1.38 (2H, m)
8	–	–	2.38	–	–
9	1.42 (m, 1H)	1.44 (m, 1H)	–	2.03 d (9.2)	1.58 (1H, t, *J* = 6.7 Hz)
10	–	–	–	–	–
11	1.42 (m, 2H)	1.44 (m, 2H)	1.36 / 2.18	4.54 d (9.2)	1.85/1.89 (2H, m)
12	1.68 (m, 2H)	1.71 (m, 2H)	1.43 / 1.99	5.76 d (2.9)	5.24 (br s)
13	1.68 (m, 1H)	1.71 (m, 1H)	–	–	–
14	–	–	–	–	–
15	1.28 (m, 2H)	1.31 (m, 2H)	1.58 / 1.62	1.57 m / 1.56 m	1.03/1.09 (2H, m)
16	1.28 (m, 2H)	1.31 (m, 2H)	1.43 / 1.56	1.55 m / 1.43 m	1.53/1.61 (2H, m)
17	–	–	–	–	–
18	1.68 (m,1H)	1.71 (m,1H)	1.86	1.60 t (3.5)	2.85 (1H, dd, *J* = 13.6, 3.6Hz)
19	2.55 (dd, J = 5.4, 11.0 Hz)	2.60 (dd, J = 5.4, 11.0 Hz)	1.46 / 1.58	1.65 dd (3.5, 14.6) / 1.48 dd (7.2, 14.6)	1.21/1.36 (2H, m)
20	–	–	–	–	–
21	1.51(1H, ddd, 12.0, 8.5, 15.0 Hz)	1.52(1H, ddd, 12.0, 8.5, 15.0 Hz)	1.47 / 1.24	–	1.21/1.36 (2H, m)
22	1.42 (m, 2H)	1.44 (m, 2H)	1.19 / 1.39	2.36 d (14.5) / 3.01 d (14.5)	1.95/1.58 (2H, m)
23	0.81 (s, 3H)	0.82 (s, 3H)	1.08 s	1.01 d (6.8)	0.72 (3H, s)
24	0.86 (s, 3H)	0.87 (s, 3H)	1.02 s	1.13 d (6.7)	0.92 (3H, s)
25	0.88 (s, 3H)	0.86 (s, 3H)	0.78 s	1.30 s	0.84 (3H, s)
26	0.90 (s, 3H)	0.89 (s, 3H)	0.99 s	1.07 s	1.00 (3H, s)
27	0.96 (s, 3H)	0.95 (s, 3H)	1.16 s	1.22 s	0.80 (3H, s)
28	4.53 (br s, 2H)	4.55 (br s, 2H)	–	1.45 s	–
29	4.59 and 4.70 (d, *J* = 2.0 Hz)	4.60 and 4.70 (d, *J* = 2.0 Hz)	–	1.19 s	1.04 (3H, s)
30	1.05 (s, 3H)	1.07 (s, 3H)	–	1.23 s	0.77 (3H, s)
C=O			–	–	–
$C(O)CH_3$	2.01 (s, 3H)	2.21 (s, 3H)	–	–	–
OCH_3	–	–	3.87	–	–
$COOCH_3$	–	–	3.62	–	–

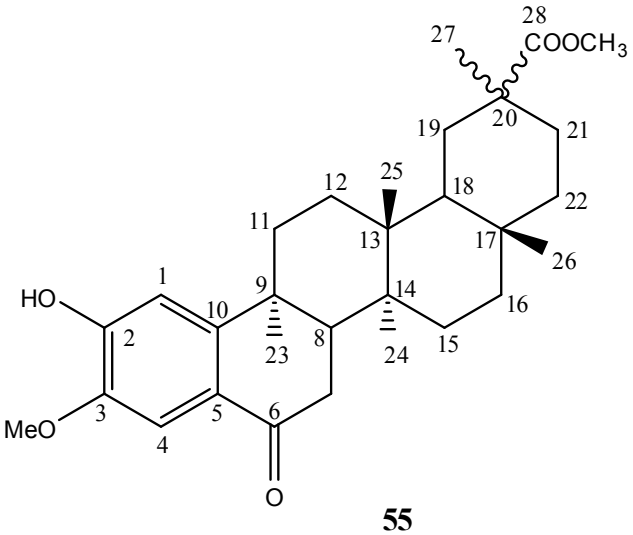
4.1.2 3-Methoxy-4-decarboxydihydrozeylasterone (55)

Compound **55** was isolated as a pale yellow amorphous solid with melting point of 252-254°C. Assignment of the molecular formula $C_{30}H_{42}O_5$ was based on ^{1}H and ^{13}C NMR (Table 2 and 3) and ESIMS observed at *m/z* at 482, confirmed by HRESIMS (482.3012 $[M]^+$, calcd. for 482.2921) suggesting the presence of ten degrees of unsaturation. Its IR (KBr) (Appendix 10) showed bands at 1727 cm^{-1} (ester carbonyl), 1642 cm^{-1} (α, β – unsaturated carbonyl), 3517 cm^{-1} (OH) and 1200cm^{-1} (aromatic C–H stretching frequency). The UV spectrum showed maximum absorptions at 241 nm and 304 nm characteristic of an aromatic nucleus and a conjugated ketone, respectively (Kamal *et al.*, 1983).

^{1}H NMR spectrum (Table 2, Appendix 6) showed the presence of a tetra-substituted benzene ring with two *para* oriented singlet protons at δ_H 6.82 and δ_H 7.61, two methoxy protons and five aliphatic methyl protons. ^{13}C NMR (Table 3) and DEPT spectra (Appendix 7) indicated the presence of seven methyls, eight methylenes, four methine and eleven quaternary carbons. These spectroscopic data showed presence of cyclohexanone moiety comparable to 6-oxophenolic triterpenoid skeleton (Itokawa *et al.*, 1991).

In the HMBC correlation (Appendix 9), the singlet at H-4 (δ_H 7.61) showed ^{3}J correlation with keto carbon at δ_C 201.2 (C-6), thus confirming that the cyclohexanone ring is fused to an aromatic ring. The methoxy proton [δ_H 3.87] showed a correlation with an aromatic carbon at δ_C 152.8 (C-3) indicating connectivity to the aromatic moiety. A three proton singlet at δ_H 3.62 (showing HSQC correlation to carbon at δ_C 51.5) showed HMBC correlation with a carboxyl carbon at δ_C 179.4, indicating the presence of a methyl ester moiety. The placement of this ester group at C-20 was confirmed by the HMBC correlation between the methyl proton (H-27) and the carbonyl carbon, which in turn showed ^{3}J correlation with two sets of methylene protons δ_H 1.46/1.58 and δ_H 1.47/1.58 (H-19 and H-21, respectively).

From these spectroscopic data, the complete connectivity of the functional groups supported by the literature data, confirmed compound **55** to be a 6-oxophenolic triterpenoid named 3-methoxy-4-decarboxydihydrozeylasterone based on the structure of zeylasterone (**51**) isolated from *Maytenus blaphorodes* and *Kokoona zeylanica* (Kamal *et al.*, 1983) and thus was considered a new compound.



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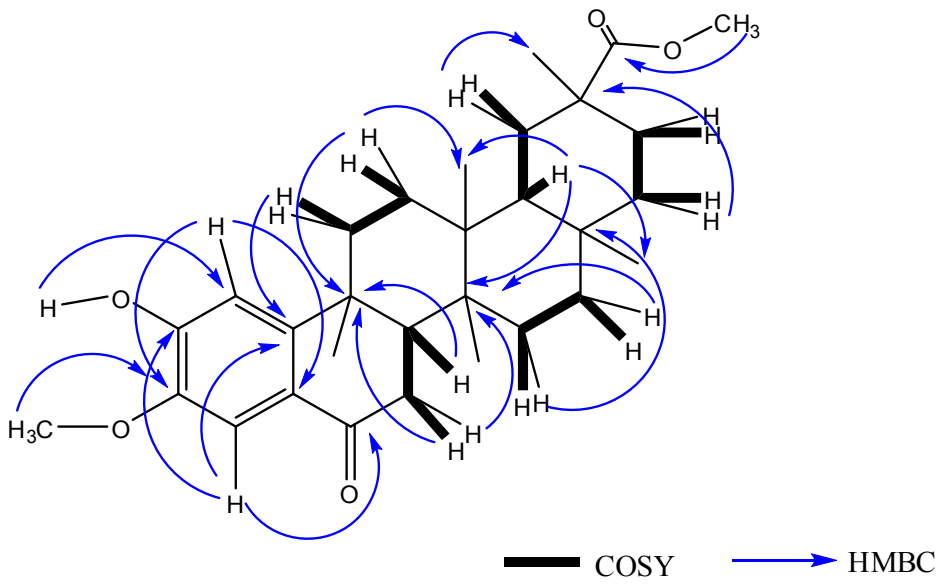


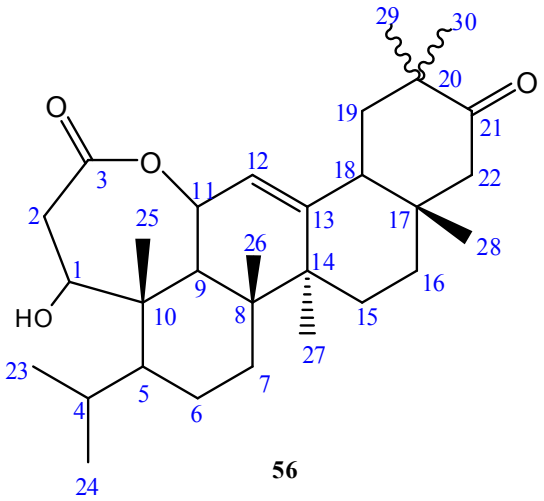
Figure 3: COSY and key HMBC correlations of compound 55

4.1.3 3,4-*seco*-1-hydroxy-21-oxo-olean-3,11-olide (56)

Compound **56** was isolated as white amorphous solid with melting point of 268-270°C and showed an ESIMS molecular mass of 470.68 suggesting a molecular formula of $C_{30}H_{46}O_4$ with eight double bond equivalents supported by the proton and ^{13}C NMR data. The ^{13}C NMR and DEPT spectrum (Appendix 13) showed 30 carbons including eight methyls, seven methylenes and seven methines. From the ^{1}H NMR data (Table 2), out of the seven methines, two were oxy-methines [δ_H 4.34 and δ_H 4.54] and one was a vinylic proton observed at δ_H 5.76. Existence of eight methyl peaks suggesting compound **56** to be a pentacyclic triterpene derivative (Kiem *et al.*, 2004). The observation of two doublets at **δ_H** 1.01(3H) and δ_H 1.13 (3H) both coupled to a multiplet for the methine at δ_H 2.75 (1H) and in turn mutually coupled on COSY indicated the presence of isopropyl group**.** This was supported by COSY correlation between the methyls and the methine proton (δ_H 2.75, m) and further supported by HMBC correlation between the two methyls and the methine carbon, C-4 (δ_C 56.0).

The ^{13}C NMR spectrum indicated the presence of two carbonyl functional groups at δ_C 215.9 and δ_C 172.4, for a ketone and a carboxyl carbon, respectively. However, connectivity of an oxy-methine proton at δ_H 4.54 (C-11) to the carboxyl carbon at δ_C 172.4 (C-3) observed from HMBC spectrum (Appendix 15), indicated carbonyl unit to be part of a lactone moiety. This was confirmed by the fact that the second oxy-methine proton at δ_H 4.34 (H-1) also showed ^{3}J correlation with carbonyl carbon at δ_C 172.4 while methylene protons at δ_H 3.19 and δ_H 2.79 (CH_2-21) showed correlations to C-3, C-1 [oxy-methine carbon at δ_C 79.6 (C-1)] and C-10 (δ_C 44.5). In the COSY spectrum (Fig. 4, Appendix 16) coupling between the oxy-methine proton at δ_H 4.34 and the methylene protons at δ_H 3.19/2.79 and lack of coupling between these protons indicated the presence of a C – CH(OH) – CH_2 – CO – O – CH moiety thus confirming the presence of a lactone.

Based on the structural features of triterpenes exhibited by *Maytenus* plants (Shirota *et al.*, 1998), compound **56** was proposed to have a 3,4–*seco*-oleonolic skeleton (Sousa *et al.*, 2012) and a lactone cyclising with an hydroxyl group at C-11 forming an Ɛ-caprolactone. Furthermore, existence of an oleonolic skeleton was based on the existence of two methyl groups (δ_H 1.19 and δ_H 1.23) which showed mutual HMBC correlation. The two methyl groups further showed a HMBC ^3J correlation to the Keto carbon δ_C 216.1 indicating that the ketone functionality is at either C-19 or C-21. HMBC correlation of the keto carbon to two methylene protons (CH_2-19 and CH_2-22) but not with methine proton at δ_H 1.60 (CH_2-18) is consistent with the placement of the carbonyl at C-21 rather than C-19. The presence of characteristic vinylic functionality of an oleonolic skeleton was confirmed by the presence of one vinylic proton at δ_H 5.76 and to two vinylic carbons, one of which being a quaternary carbon at δ_C 139.8 (C-13) and a methine carbon at δ_C 133.5 (C-12). Following the observed spectroscopic evidence, corroborated by literature data, compound **56** was named as 3, 4-*seco*- 1-hydroxy-21-oxo-olean-3, 11-olide relative to the previously isolated 3,4-*seco*-olean-3,11*β*-olide (**42**) from *Maytenus robusta* (Sousa *et al.*, 2012) and is a new compound.



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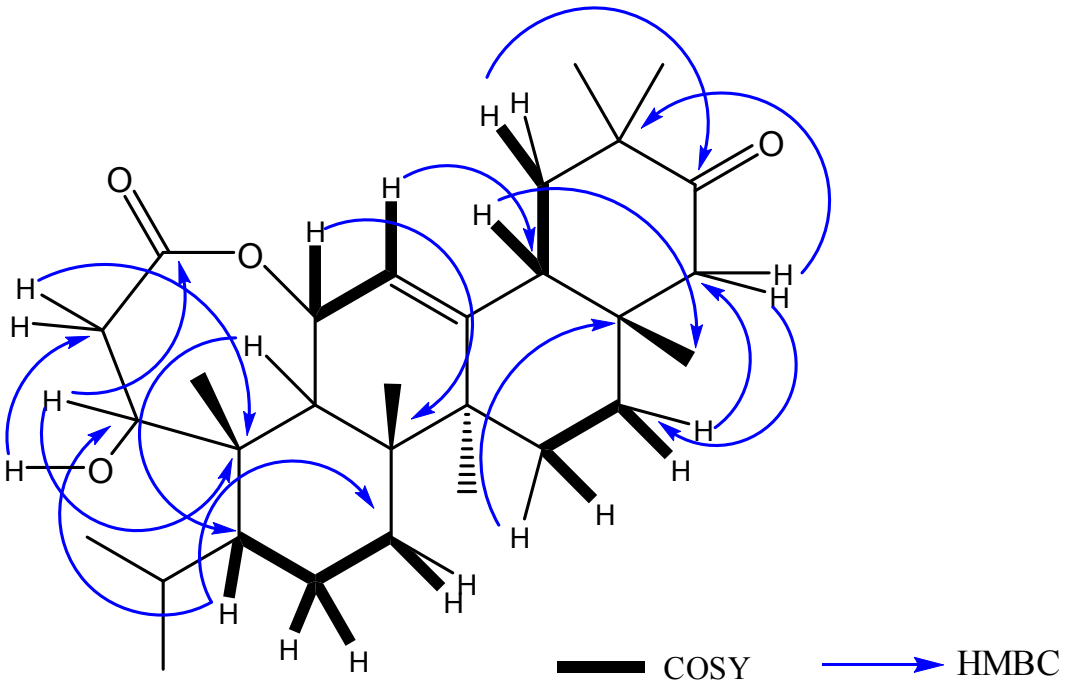

Figure 4: COSY and key HMBC correlations of compound **56**

4.1.5 Oleanolic acid (57)

Compound **57** was isolated from the EtOAc extract of *M. heterophylla* stem bark, as a white solid (melting point 305-306ºC) which appeared as purple spots on TLC when the developed plate was sprayed with *p*-anisaldehyde in Sulphuric acid followed by heating (Asad *et al.*, 2013). The ^{1}H NMR (600 MHz, $CDCl_3$) spectrum (Table 3) of compound **57** (Appendix 20) revealed an olefinic proton at δ_H 5.24 (1H, br s), a carbinolic proton at δ of 3.14 (1H, dd, J = 5.1 Hz and 11.0 Hz) suggesting it's β and α orientation and δ of 2.80 (1H, bd, J = 11.0 Hz) along with seven tertiary methyl groups at δ_H 0.72 s, 0.80 s, 0.84 s, 0.92 s, 1.00 s and 1.04 s (each 3H, s). Signals for seven methyl groups in the ^{1}H NMR spectrum indicated the presence of a 12-oleanene skeleton in the compound and it belongs to an oleanane-type triterpene having a carboxylic acid functionality (Asad *et al.*, 2013; Seebacher *et al.*, 2003). The ^{13}C NMR spectrum (Table 4) revealed the presence of signals due to an oxygenated carbon signal at δ_C 77.6 (C-3), one tri-substituted double bond at δ_C 121.4 (C-12) and 142.9

(C-13), and a carbonyl for carboxylic acid at δ_C 179.8 among aliphatic carbon atoms which were typical of an olean-12-ene derivative (Seebacher *et al*, 2003). The compound was identified unambiguously as oleanolic acid (**57**) by the spectroscopic data with literature reported for oleanolic acid. This was further confirmed by Co-TLC and mixed m.p with an authentic sample (Seebacher *et al.*, 2003).

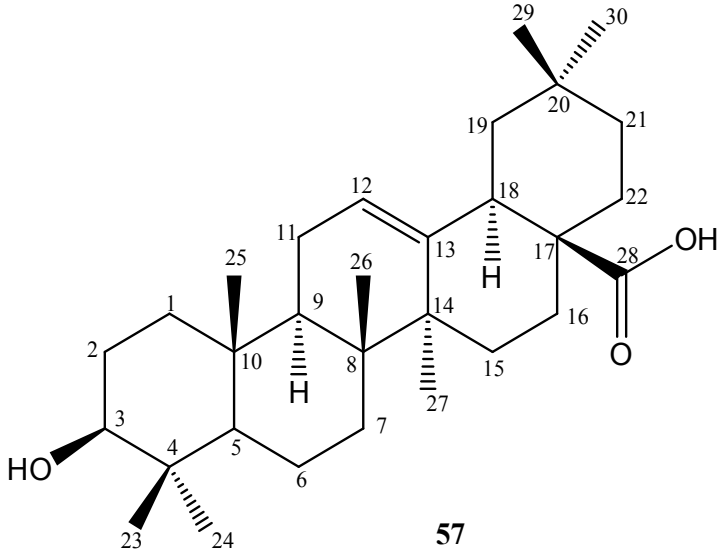

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Table 4: ^{13}C NMR data for compound 54 ($CDCl_3$, 125 MHz) 55, 56 and 57($CDCl_3$, 150 MHz)

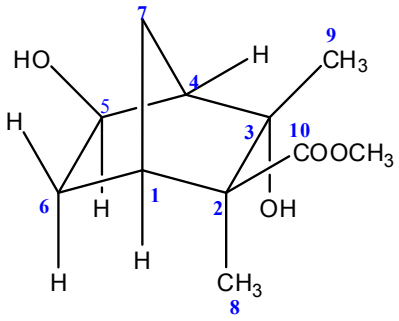
Atom	**54**	Literature, **54** (Pravat *et al.*, 2010)	**55**	**56**	**57**	Literature, **57** (Hak *et al*, 1997)
1	34.2	36.3	109.5	79.6	32.8	32.5
2	27.5	27.9	150.8	44.5	25.8	25.2
3	81.5	82.4	152.8	172.4	77.6	76.2
4	32.8	33.1	113.2	56.0	38.5	37.3
5	55.4	56.3	141.9	85.0	54.7	49.0
6	18.2	19.0	201.2	33.2	17.5	18.2
7	34.2	34.9	43.8	27.6	31.8	32.5
8	42.8	43.0	35.4	43.2	40.7	39.5
9	50.4	52.4	38.6	55.2	48.0	47.4
10	37.1	37.2	123.5	44.5	36.2	37.2
11	21.0	22.0	32.4	67.3	22.6	23.0
12	25.5	26.5	29.9	133.5	121.4	122.7
13	38.1	38.8	36.8	139.8	142.9	143.6
14	43.0	44.1	35.9	42.6	38.3	41.7
15	29.3	30.2	30.3	19.8	26.8	27.6
16	36.5	37.3	–	34.1	22.1	23.3
17	40.8	41.7	35.7	51.4	45.6	46.5
18	48.3	49.1	40.4	37.8	40.6	41.0
19	48.0	49.2	29.5	40.5	37.8	38.9
20	154	156	39.1	47.7	32.9	30.7
21	29.9	30.2	29.6	215.9	29.7	33.8
22	40.0	41.3	28.2	40.4	45.3	44.9
23	19.3	21.2	31.9	21.0	14.3	15.3
24	16.6	16.7	25.3	20.3	26.9	22.2
25	16.1	17.9	16.7	13.8	13.9	15.1
26	16.6	16.3	15.2	19.1	15.8	17.2
27	14.1	14.4	31.5	22.6	24.7	26.1
28	68.3	68.3	179.4	26.8	179.8	183.3
29	107.8	108.6	–	28.4	22.2	23.3
30	28.3	28.4	–	21.3	32.2	32.5
C=O	172.8	171.6	–	–	–	–
$C(O)CH_3$	28.0	28.4	–	–	–	–
OCH_3	–	–	55.7	–	–	–
$COOCH_3$	–	–	51.5	–	–	–

4.1.4 3, 5-Dihydroxycamphanoate (58)

Compound **57** was isolated as a white crystalline compound with a melting point of 121-122°C. The comppound was deduced to have a molecular formula of $C_{11}H_{18}O_4$ from its positive ESI-MS at m/z 214.2582 $[M+H]^+$ with three indices of hydrogen deficiency. The ^1H and ^{13}C NMR spectra (Table 4, appendices 22 and 23, respectively) of **58** showed the presence of three singlet methyls at δ_H 1.17 (H-9; δ_C 19.5), δ_H 1.28 (H-8; δ_C 21.3) and a methoxy at δ_H 3.66 (δ_C 51.4). Furthermore two methines [δ_H 2.03 (1H, d, J = 3.5, H-4) and δ_H 2.51 (1H, m, H-1) attached on carbons at 47.7 (C-4) and 44.2 (C- 1), respectively] were observed alongside two methylenes [δ_H 1.33/1.77 (2H, m, H_2-6) and δ_H 1.22/2.12 (2H, d, J = 10.5, H_2-7)] both attached to the carbon atoms resonating at δ_C 23.2 (C-6) and δ_C 22.9 (C-7), respectively, from the HMQC spectrum (Appendix 24). Slightly downfield were two protons observed as an oxymethine proton [δ_H 3.45 (1H, dt, J = 3.5, 7Hz, H- 5)] and a methoxy proton [3.66 (3H, s)] attached to the carbon signals at δ_C 74.1 and δ_C 51.4, respectively (Appendix 21). The later showed an HMBC correlation with carboxylic carbon at δ_C 174 indicating the presence of a methyl ester functionality whereas the hydroxymethine showed HMBC correlation with the carbon signal at δ_C 47.7 (C-4) which in turn showed ^3J HMBC correlation with methyl protons at δ_H 1.17, signifying a ^3J correlation between the oxymethine proton and C-3 at δ_C 57.3.

According to the degrees of unsaturation and the presence of a carbonyl group, compound **58** was suggested to contain a bicyclic nucleus confirmed by ^1H-^1H COSY spectrum (Appendix 26) showing spin system as H-4 to methylene protons at δ_H 1.22/2.12 (H-7) and methine proton at δ_H 2.51 (H- 1). On the other hand H-4 showed COSY correlation with oxymethine proton (δ_H 3.13) which in turn displayed a spin system with methylene at δ_H 1.33/1.77 (H- 6) whereas H-5 had no COSY correlation with the methylene protons at δ_H 1.22/2.12 (H-7) but showed a correlation to H-6, indicating that CH_2-7 was actually bridged H-4 and H-1. In the

HMBC spectrum, correlations from the two methyl groups CH_3-8 and CH_3-9 to C-1 and C-4, respectively, which in turn showed inverse ^2J correlation to the methylene protons at δ_H 1.22/2.12 (H-7) confirmed the final planar structure of compound **58**. The relative configuration of compound **58** was determined by NOESY correlation of H-5 (δ_H 3.13) to H-6 (δ_H 1.77) which in turn correlated to H-1 (δ_H 2.51) indicated that the oxymethine H-5 was possibly α-oriented and opposite to the methylene bridge (Fig 5). Whereas lack of mutual correlation between the methyl groups indicated *trans* orientation between the two groups (Fig. 5). The structure was thus deduced as 3, 5-dihydroxycamphanoate.



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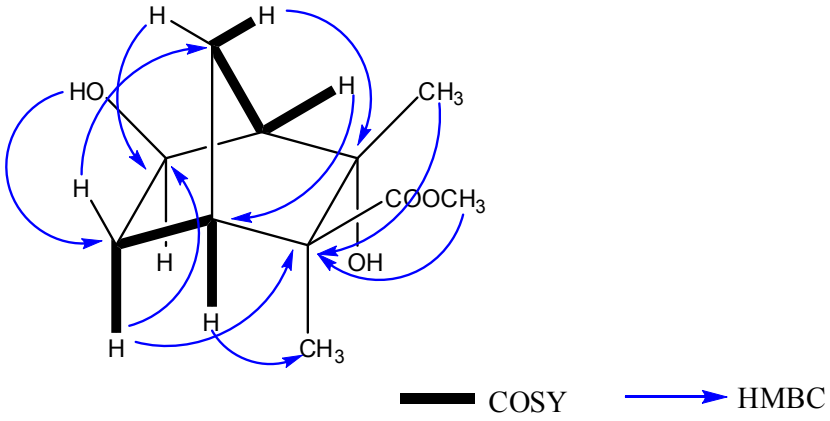

Figure 5: COSY and key HMBC correlations of compound **58**

Table 5: ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) for compound 58

Atom	^{1}H NMR	^{13}C NMR	HMBC
1	2.51 m	44.2	C-2, C-3, C-4, C-8
2	-	57.3	-
3	-	79.1	-
4	2.03 d(3.5)	47.7	C-1, C-5, C-6, C-9
5	3.45 dt (7, 3.5)	74.1	C-6, C-2, C-7
6	1.31 m	23.24	C-1, C-3, C-7
	1.77 m	-	-
7	1.22dd (10.5, 3.0)	22.9	C-1, C-4, C-5, C-6
	2.12 dd (10.5, 3.0)	-	-
8	1.28 s	21.3	C-2, C-10, C-6
9	1.17 s	19.5	C-1, C-3
10	-	174.7	-
OCH_3	3.66 s	51.4	C-10

4.3 Anticonvulsion activity studies of pure compounds

The tests were done as already described in sections 3.8 above (Sushma et al., 2012). The results of this study suggest that the constituents of *Maytenus heterophylla* are bioactive and possess anti-convulsant acivity that may be beneficial in the management of convulsions and lend credence to the use of the plant in the management of epilepsy in traditional medicine. Pre-treatment of mice with the pure compounds in the doses of 0.5, 5 and 50 mg/kg showed varied anticonvulsant activity depending on either latency or duration of seizures as depicted in table 6.

Table 6: Effect of compounds of *M. heterophylla* stem bark on PTX-induced seizure in mice

Treatment	Conc. (mg/kg)	Onset of seizure (sec)	Duration of seizure (sec)	No. Convulsed/ No. Used	% prote
Distilled water	10	103.0	210.0	8/8	0
Compound 54	0.5	90.4	278.0	8/8	0
	5	159.0	193.0	7/8	12.5
	50	285.0	123.0	7/8	12.5
Compound 55	0.5	75.0	211.0	7/8	12.5
	5	116.0	156.0	5/8	37.5
	50	165.0	120.0	3/8	62.5
Compound 56	0.5	71.5	255.0	4/8	50
	5	103.0	191.0	3/8	62.5
	50	117.0	128.0	2/8	75
Compound 58	0.5	133.0	199.0	8/8	0
	5	225.0	120.0	7/8	12.5
	50	366.0	74.6	6/8	12.5
Diazepam	5	557.0	47.8	1/8	87.5
CV		0.4054	0.4496		
LSD, P< 0.05		6.6696	5.1144		

Values are expressed as means of n=8. Statistical level of significance analysis by one way ANOVA followed Tukey's Least Significant Difference (P<0.05).

Intraperitoneal injection of picrotoxin induced tonic clonic seizures with 100% mortality in vehicle-treated control. The pre-treatment of mice with compound **54** (0.5, 5 and 50 mg/kg) failed to prolonged the latency to seizure but significantly ($P<0.05$) reduced the duration of seizure induced by picrotoxin at 50 mg/kg. As expected, diazepam (5 mg/kg) prolonged the onset of seizures and reduced the duration of seizures ($P<0.05$) in comparison with vehicle treated control.

However, the pre-treatment of mice with compound **55** (50 mg/kg) or diazepam (5 mg/kg) prolonged the onset of seizures ($P<0.05$) and reduced the duration of seizures ($P<0.05$) in comparison with vehicle treated control. Moreover, compound **55** and diazepam produced 62.5% (at 50 mg/kg) and 87.5% protection, respectively (Table 6).

Compound **56** (50 mg/kg) produced dose dependent and significant increase in the latency to seizure ($P<0.05$) which was relatively lower than the effect of diazepam ($P<0.05$). Moreover, compound **56** (50 mg/kg) or diazepam (5 mg/kg) produced significant reduction in the duration of seizures ($P<0.05$) in comparison with vehicle treated control. Moreover, compound **56** (50 mg/kg) and diazepam produced 75% (50 mg/kg) and 87.5% protection, respectively (Table **6**).

Compound **58** (0.5, 5 and 50 mg/kg) failed to affect latency and duration of seizure but diazepam treated group showed significant increase in latency to seizure ($P<0.05$) and reduced the duration of seizure induced by picrotoxin ($P<0.05$) in comparison with vehicle treated control.

A few corresponding observations exist in the literature where *Valeriana officinalis* has been used folkloric medicine as an effective anticonvulsant medicinal herb (Mohammad *et al.,* 2010). Furthermore, the study of Hiller and Zetler (1996) showed that ethanol extract of *valerian* could decrease convulsions caused by picrotoxin but not pentylenetetrazole.

CHAPTER FIVE: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The study was intended to provide necessary information on the efficacy and validate the potential behind the use of *Maytenus heterophylla* in the management of convulsions. During the study, the following conclusions were derived from the results which are consistent with the ethnomedical information about this plant.

5.1 Summary

The crude extracts of the leaves, stem and root bark showed anticonvulsant activity in animal models using PTX-induced seizures in-*vivo*. The stem bark was the most active. The results also demonstrated that the EtOAc and CH_2CH_2 of *M. heterophylla* stem bark elaborates triterpenoids of lupane type alongside those of 6-oxophenolic and oleanolic skeleton. Two of the triterpenes; 3-methoxy-4-decarboxyzeylasterone (**55**) and 3, 4-*seco*-1-Hydroxy-21-oxo-olean-3,11-olide (**56**) showed mild ($P \leq 0.05$) protection against convulsion and are reported here as new natural products, together with 3-acetoxy-28-hydroxylupe-20(29)-ene (**54**), oleanolic acid (**57**) and 3,5-dihydroxycamphanoate (**58**). The pure isolates from the stem back, however showed weak to moderate activities with compounds **55** and **56** showing the highest activities at 62.5% ($P<0.05$) and 75% ($P<0.05$) protection against convulsion compared to the standard drug (Diazepam - 5mg/kg) at concentrations of 50 mg/Kg b.w. Compounds **54** and **58** did not show significant ($P<0.05$) activity at same the concentrations to offer protection against seizures induced by picrotoxin.

5.2 Conclusions

1 These results suggest that *Maytenus heterophylla* extract possesses anti-epileptic activity which is useful in overcoming Picrotoxin-induced convulsions. Among the different extracts tested, the stem back was found to be the most active followed by the root. The leaf extract did not show significant activity compared to standard drug.

2 From the active stem bark extract; 3-acetoxy-30-hydroxylupe-20(29)-ene (**54**), 3-methoxy-4-decarboxydihydrozeylasterone (**55**), 3,4-*seco*-1-hydroxy-21-oxo-olean-3,11-olide (**56**), oleanolic acid (**57**) and 3,5-dihydroxycamphanoate (**58**) were isolated, of which compounds **55** and **56** are new natural products.

3 3-methoxy-4-decarboxydihydrozeylasterone, **55** and 3,4-*seco*-1-hydroxy-21-oxo-olean-3,11-olide, **56** showed better activity ($P<0.05$).. However, the activities were mild compared to Diazepam.

5.3 Recommendations

1. The crude extracts of *Maytenus heterophylla* were biologically active and can be used to manage convulsions by local communities.
2. Compounds **54**, **55**, **56** and **58** were mildly active and are recommended as possible candidates for further pharmacological investigation.
3. *Maytenus heterophylla* exhibits anticonvulsant properties and is recommended for use in traditional medicine to manage epilepsy. This plant species should therefore be conserved for medicinal use and scientific research.

5.4 Suggestion for further studies

1. Further phytochemical analysis is necessary for *Maytenus heterophylla* using advanced techniques since separation of the leaf extract was not performed due to low activities against epilepsy but it could have novel compounds that may have other useful medicinal values. The root back was also not investigated.
2. The *Maytenus* species also elaborates alkaloids with structural diversity. Advanced separation techniques should be employed to isolate alkaloids and other constituents.
3. The present study was a preliminary attempt in evaluating the anticonvulsant activity of *Maytenus heterophylla.* Further pharmacological investigations to establish mechanism of action by isolates towards enhancing neuro-transmission by Gamma Amino Butyric Acid (GABA) receptors should be conducted.

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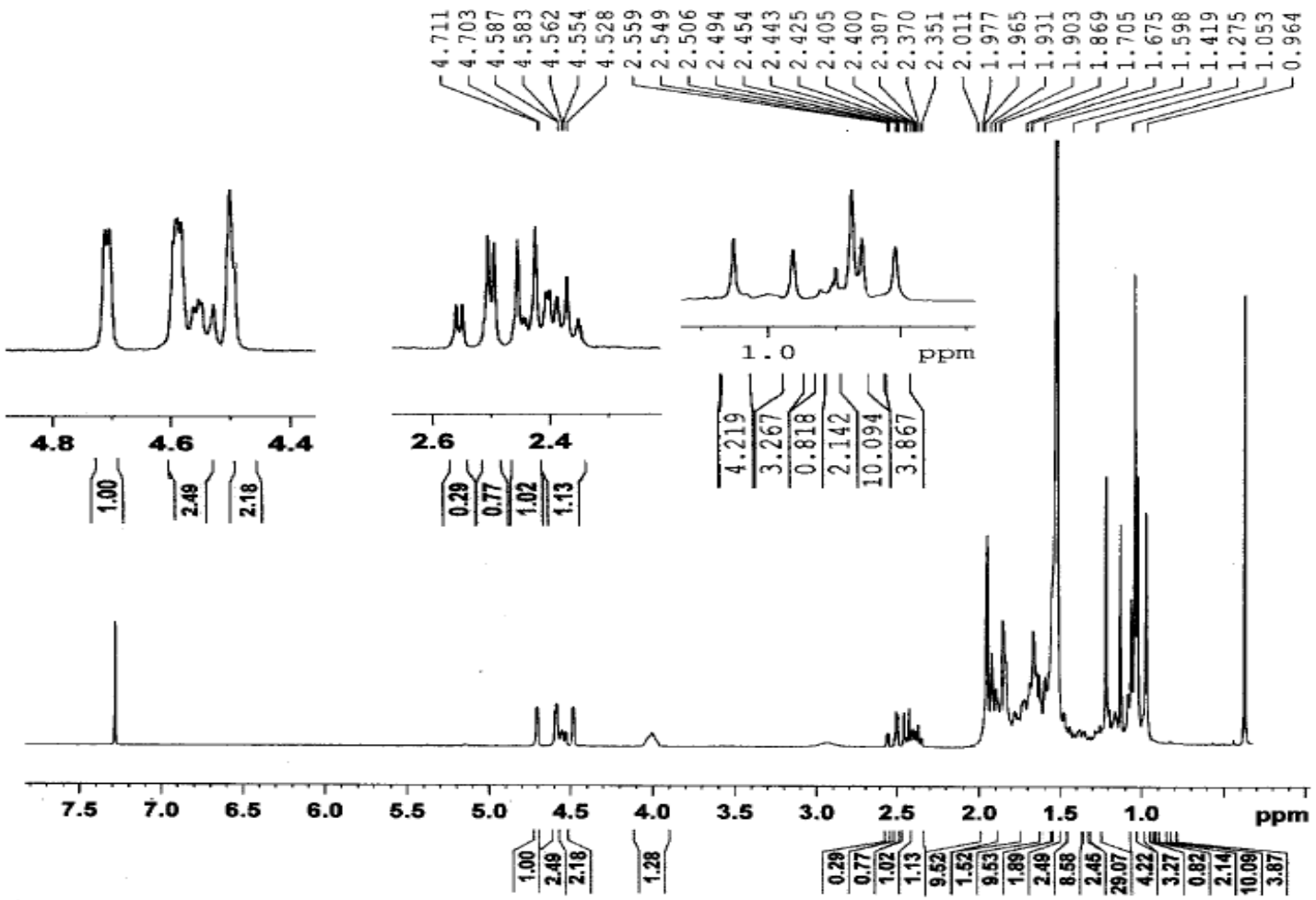
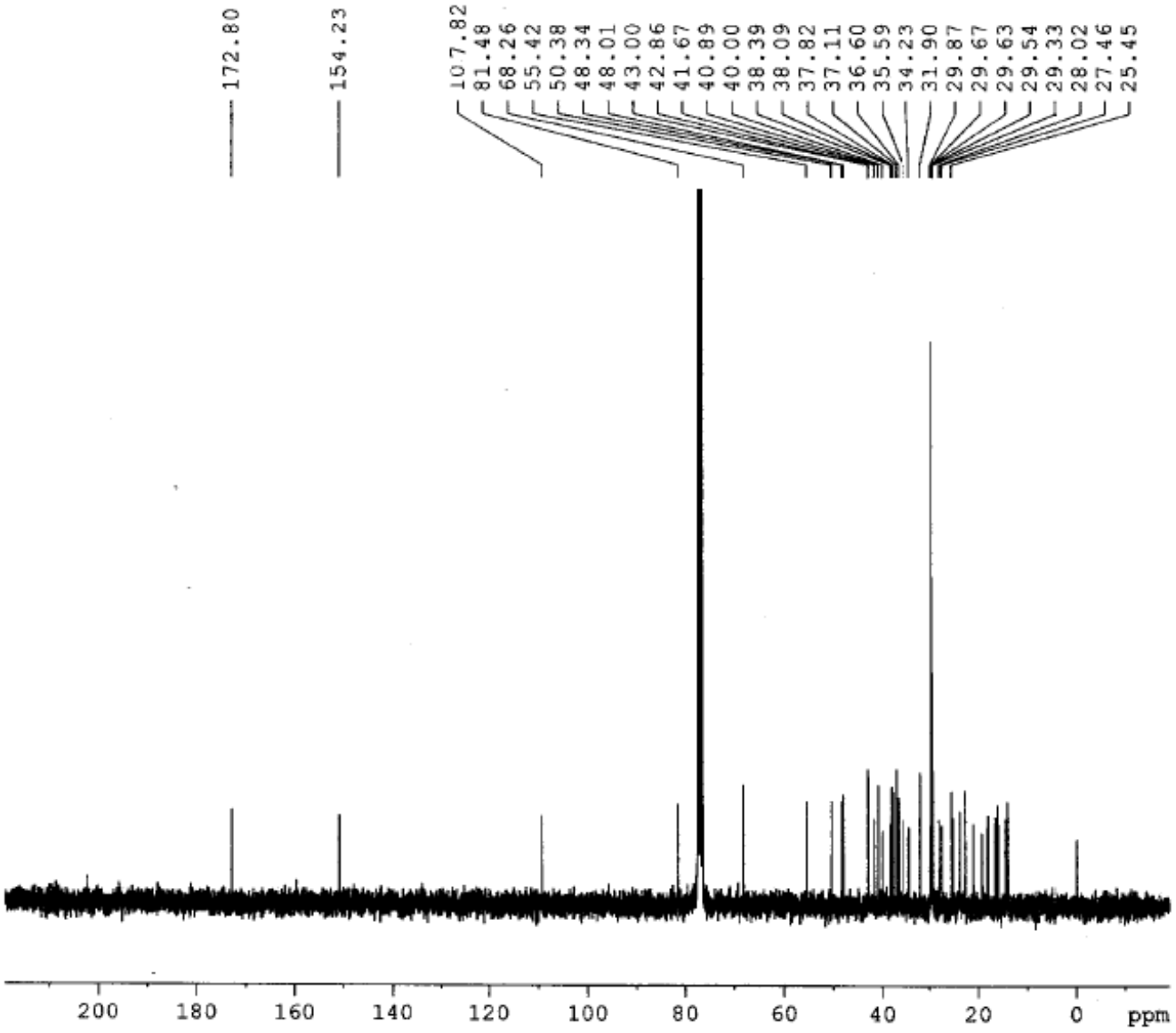
APPENDICES

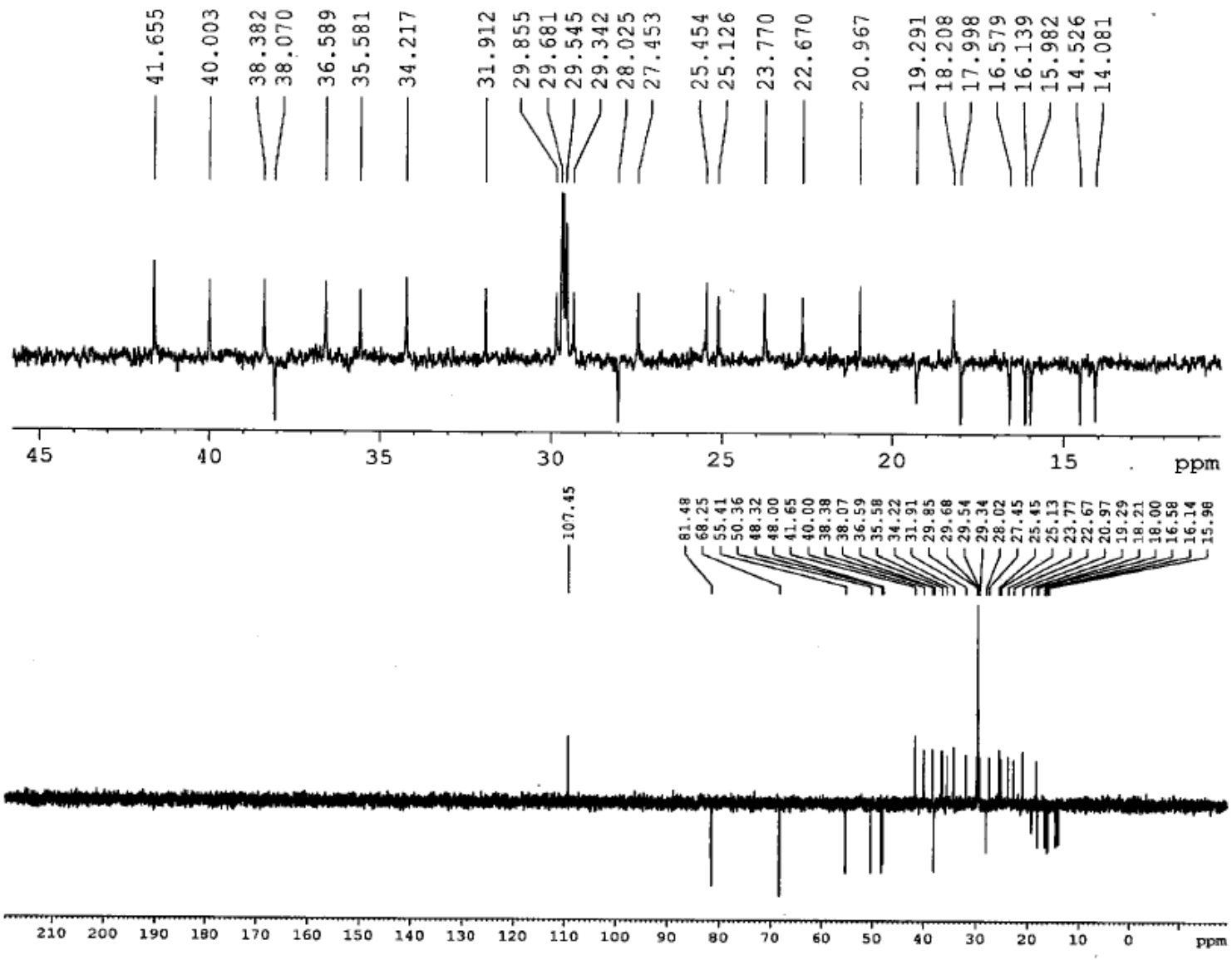
Appendix 1: ^{1}H NMR ($CDCl_3$, 500 MHz) of 3-Acetoxy-28-hydroxylupe-20(29)-ene, **54**



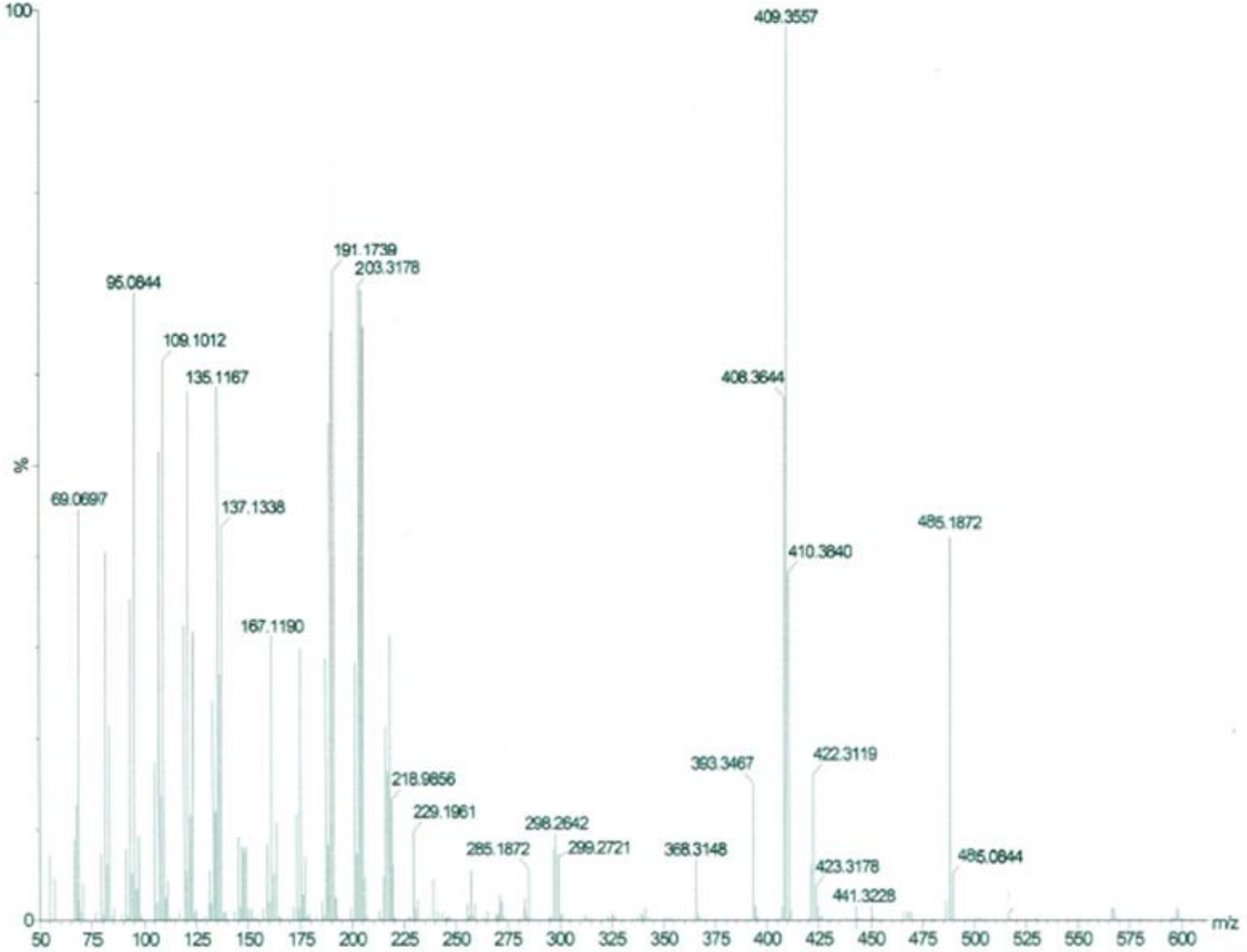
Appendix 2: ^{13}C NMR ($CDCl_3$, 125 MHz) of 3-Acetoxy-28-hydroxylupe-20(29)-ene, **54**



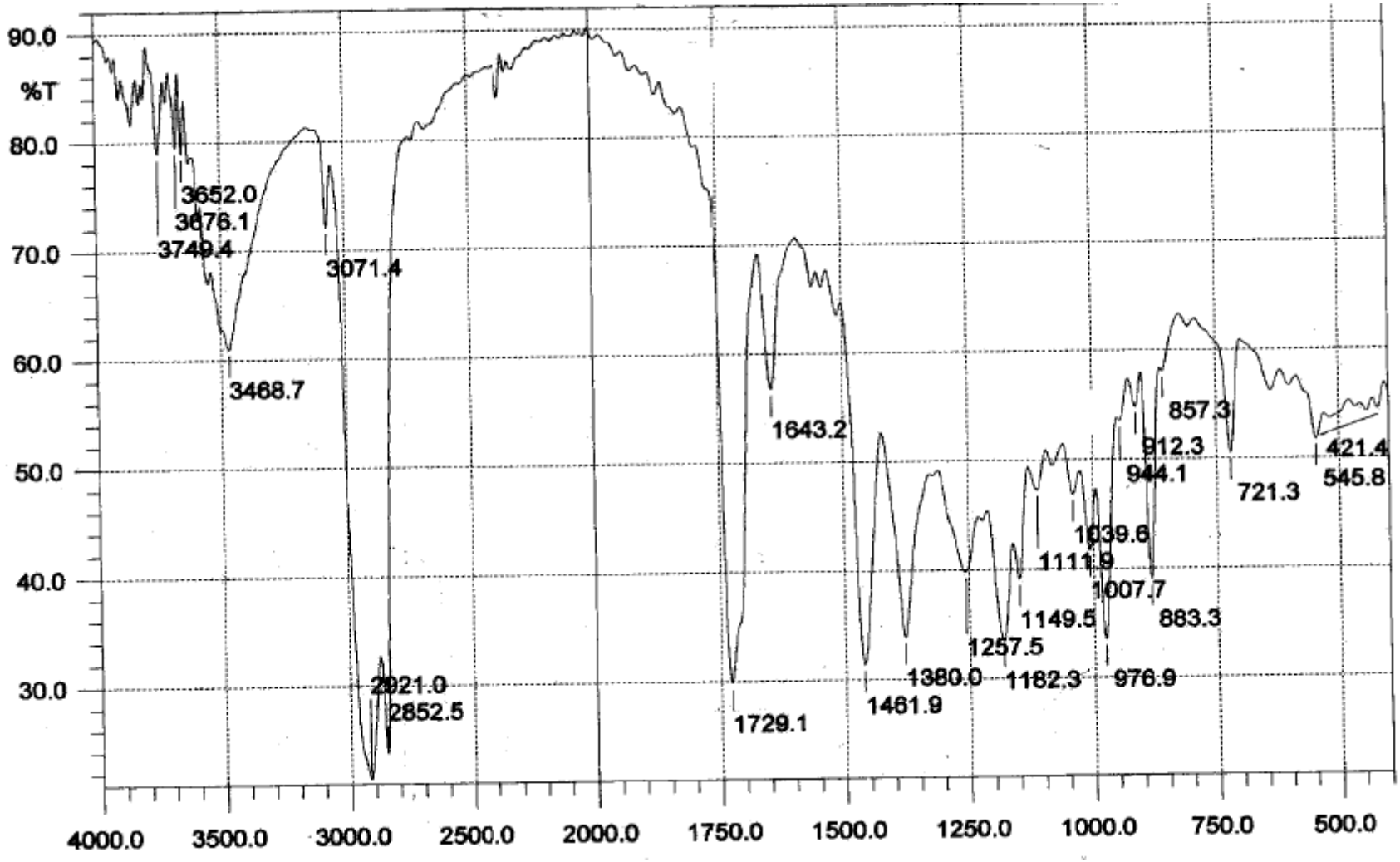
Appendix 3: DEPT spectrum of 3-Acetoxy-28-hydroxylupe-20(29)-ene, **54**



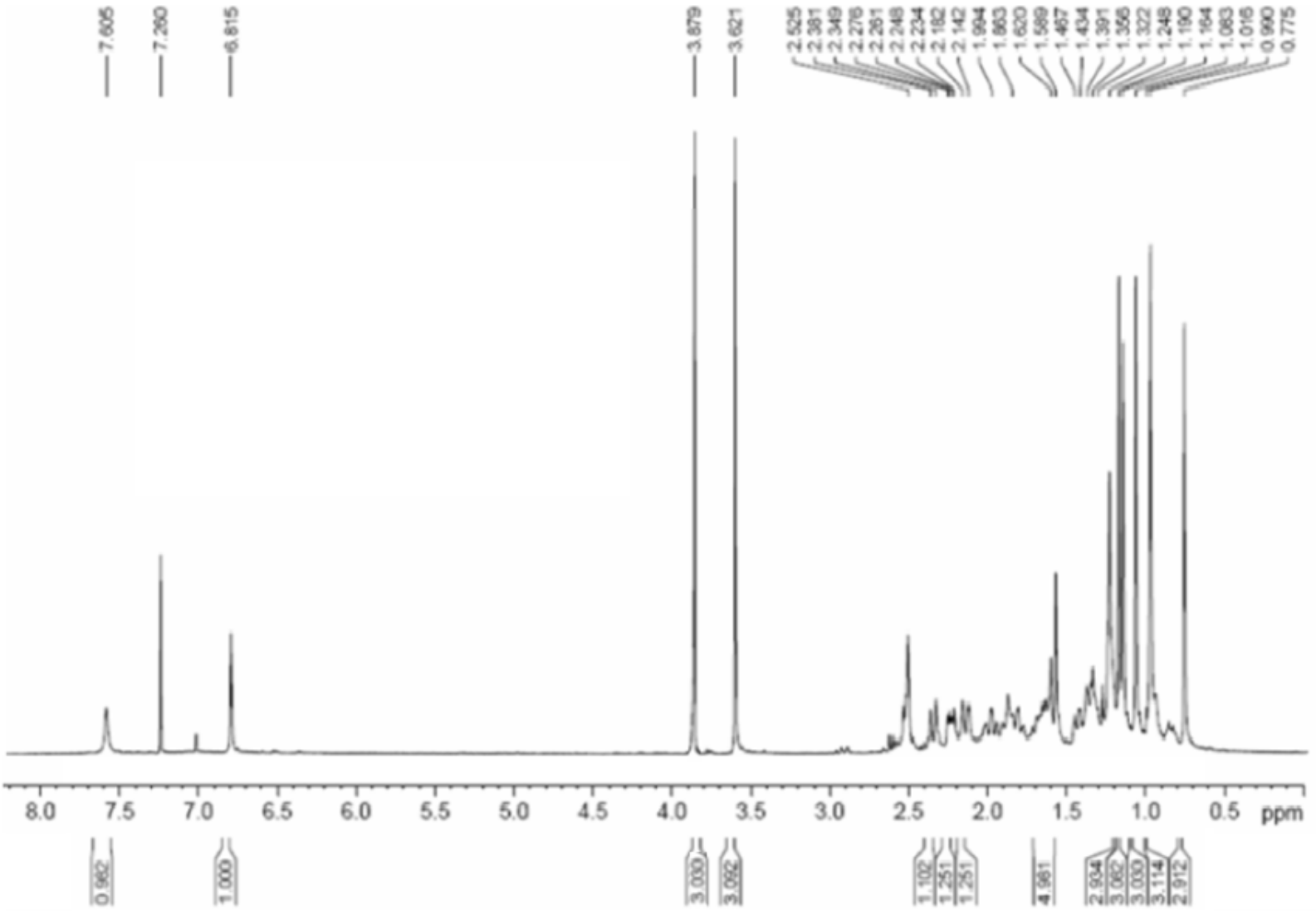
Appendix 4: HRESIMS of 3-Acetoxy-28-hydroxylupe-20(29)-ene, **54**



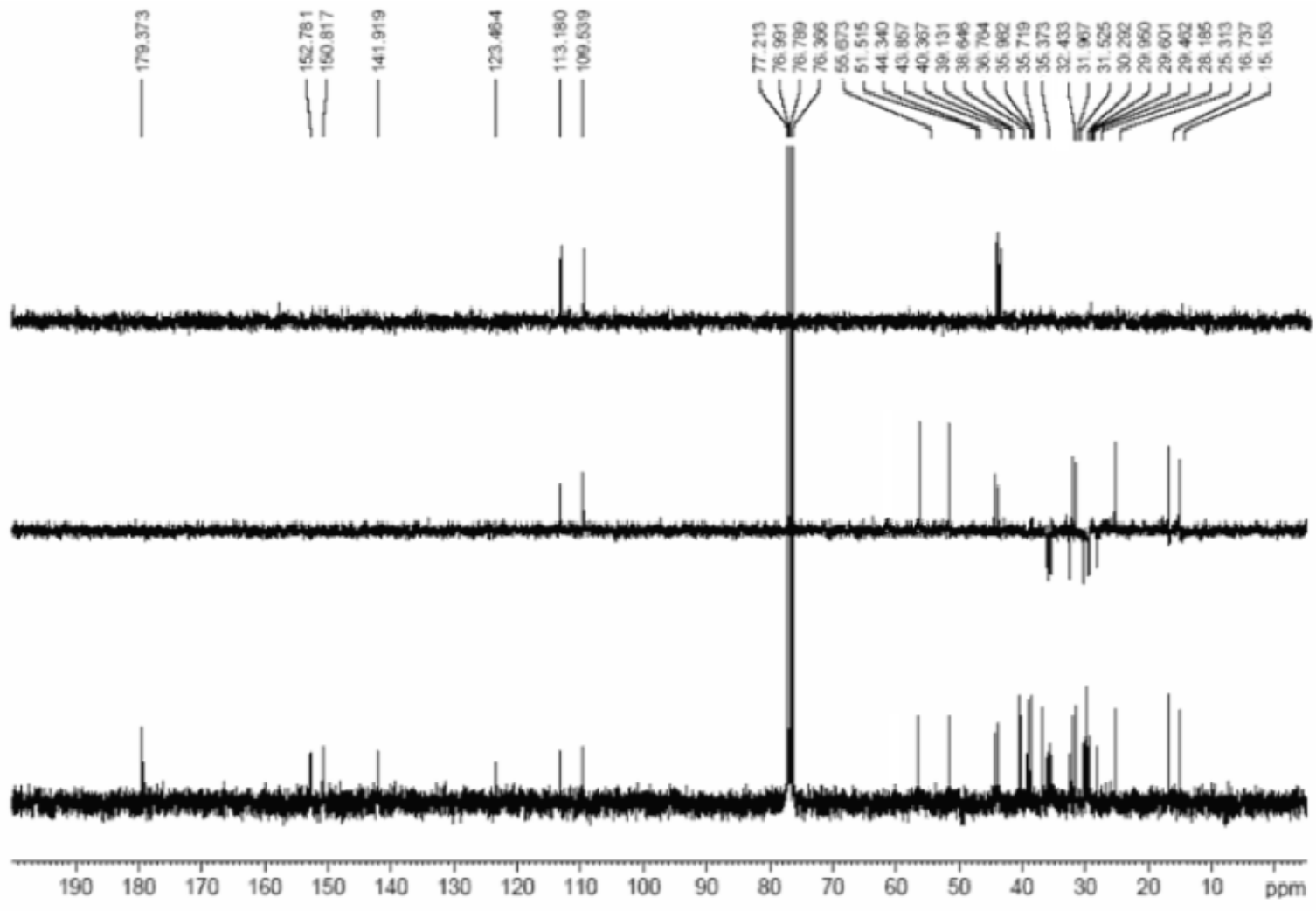
Appendix 5: IR spectrum of 3-Acetoxy-28-hydroxylupe-20(29)-ene, **54**



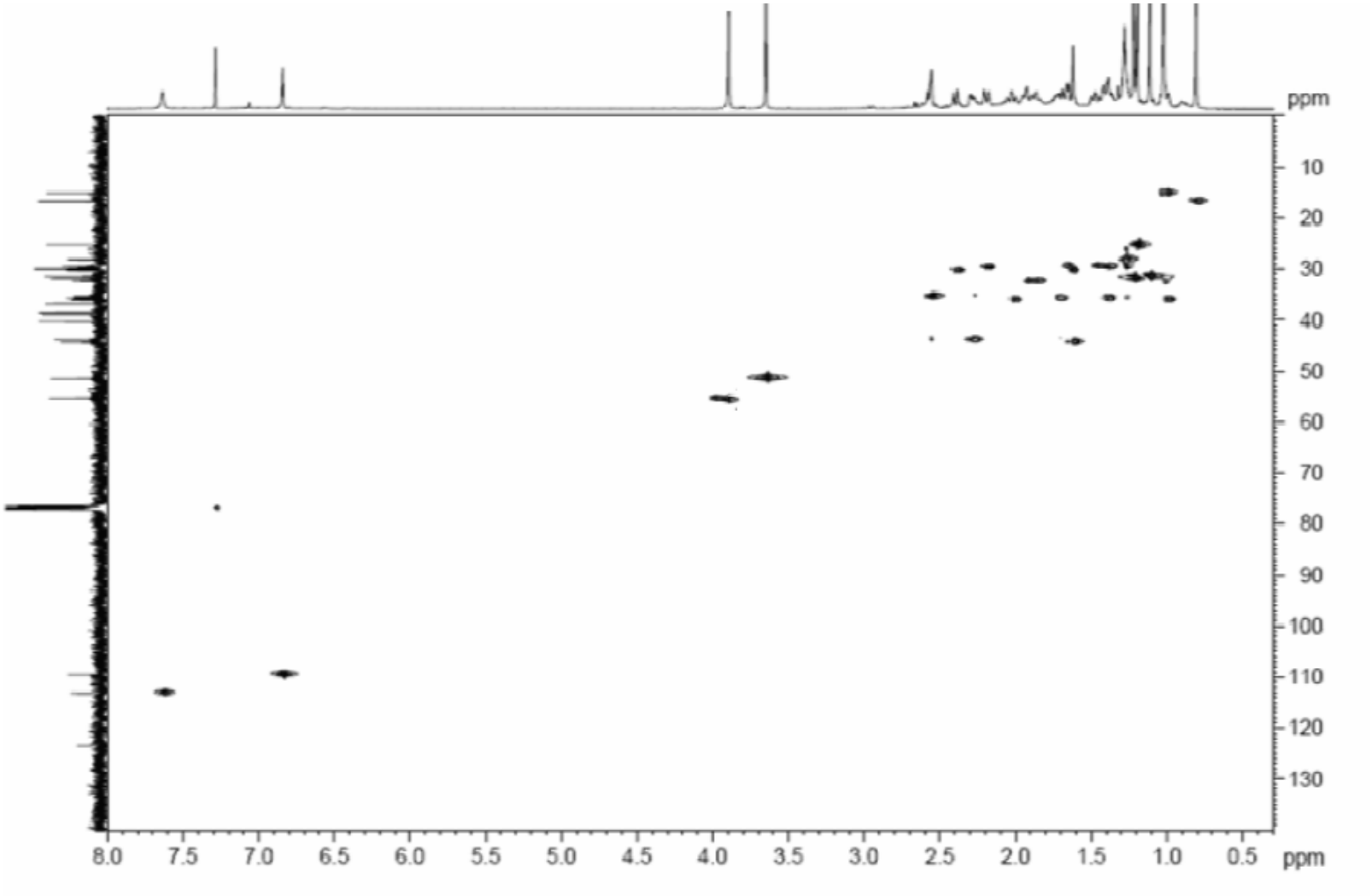
Appendix 6: ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of 3-methoxy-4-decarboxydihydrozeylasterone**, 55**



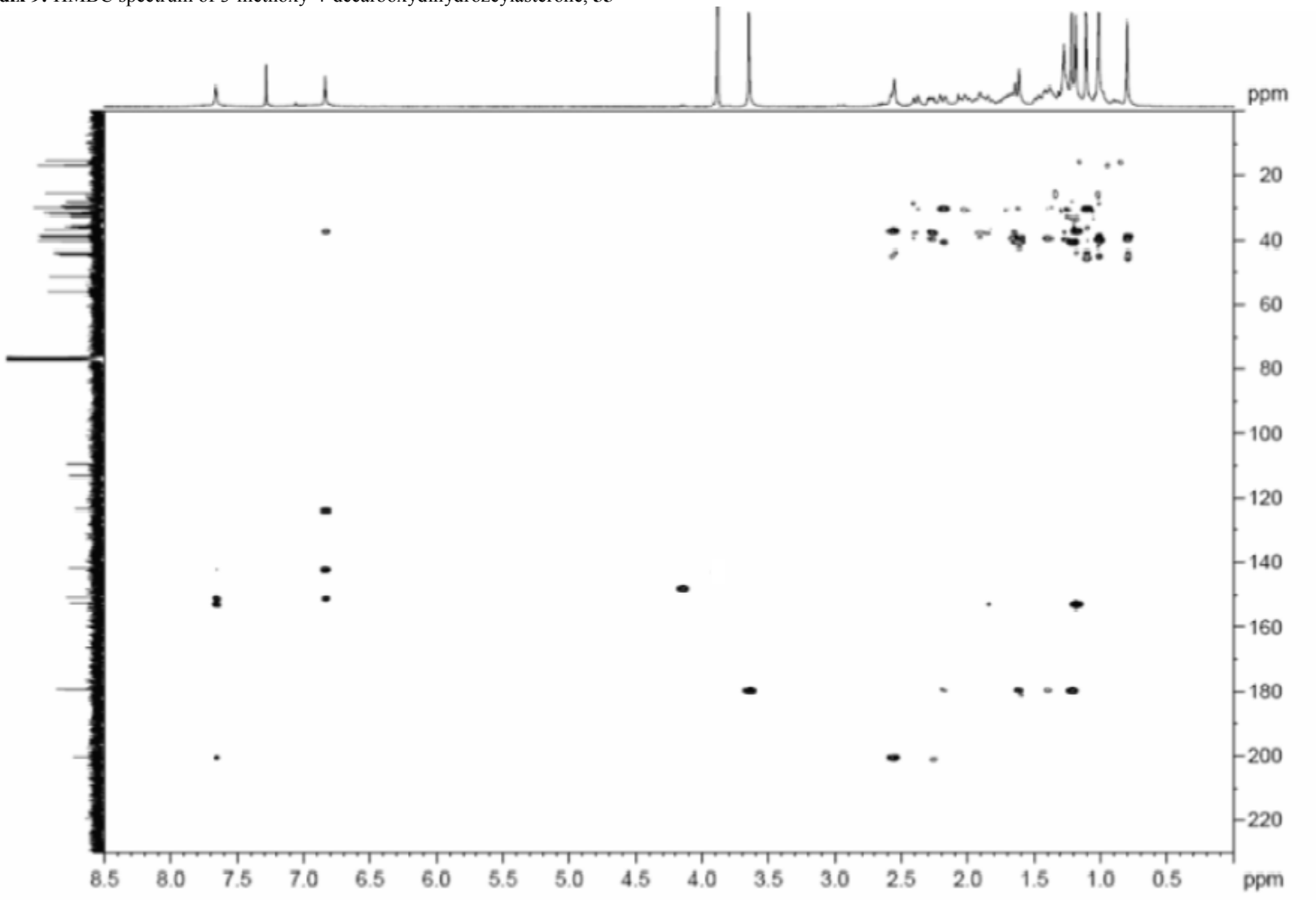
Appendix 7: ^{13}C NMR ($CDCl_3$, 150 MHz) and DEPT spectrum of 3-methoxy-4-decarboxydihydrozeylasterone, **55**



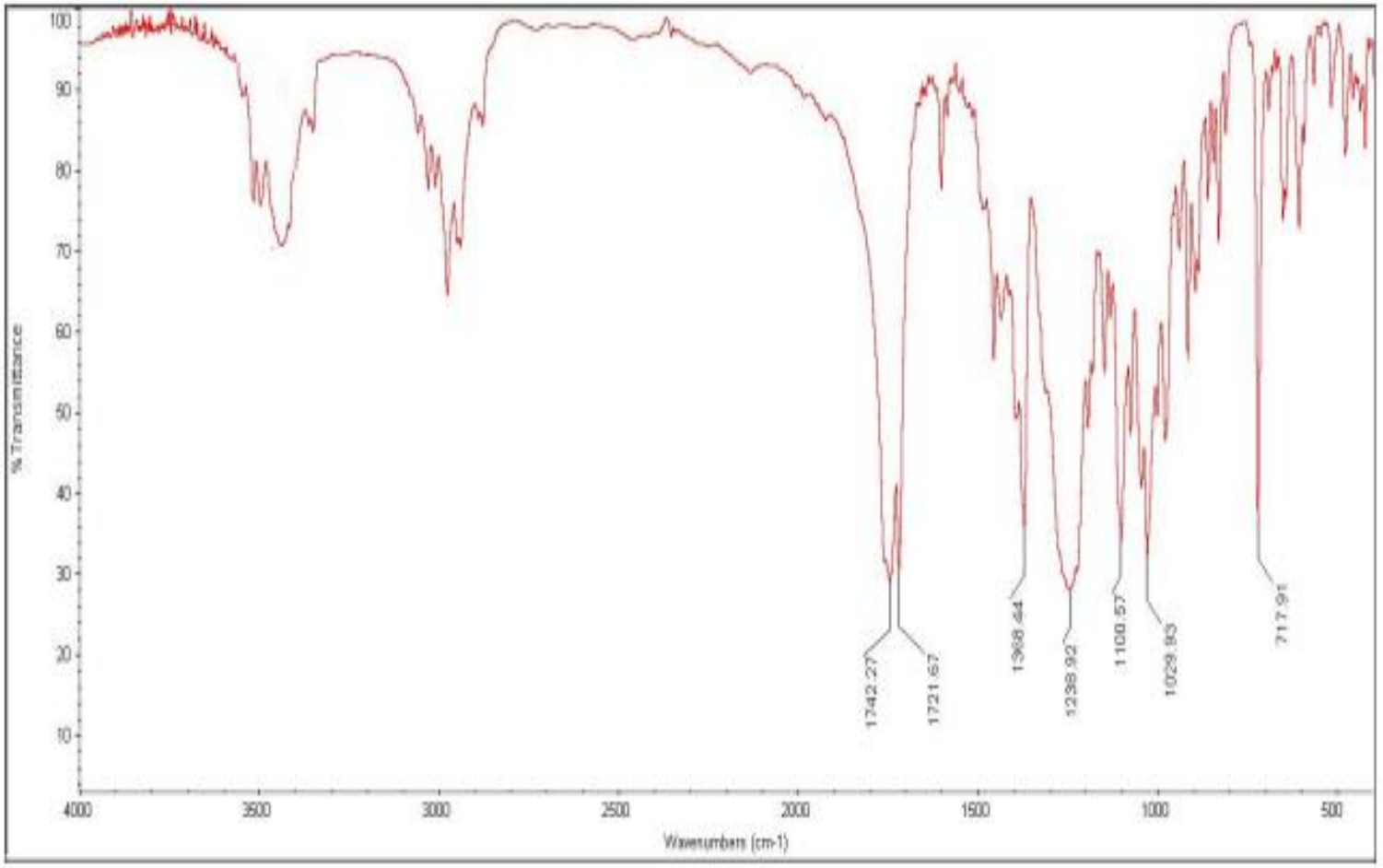
Appendix 8: HSQC spectrum of 3-methoxy-4-decarboxydihydrozeylasterone, **55**



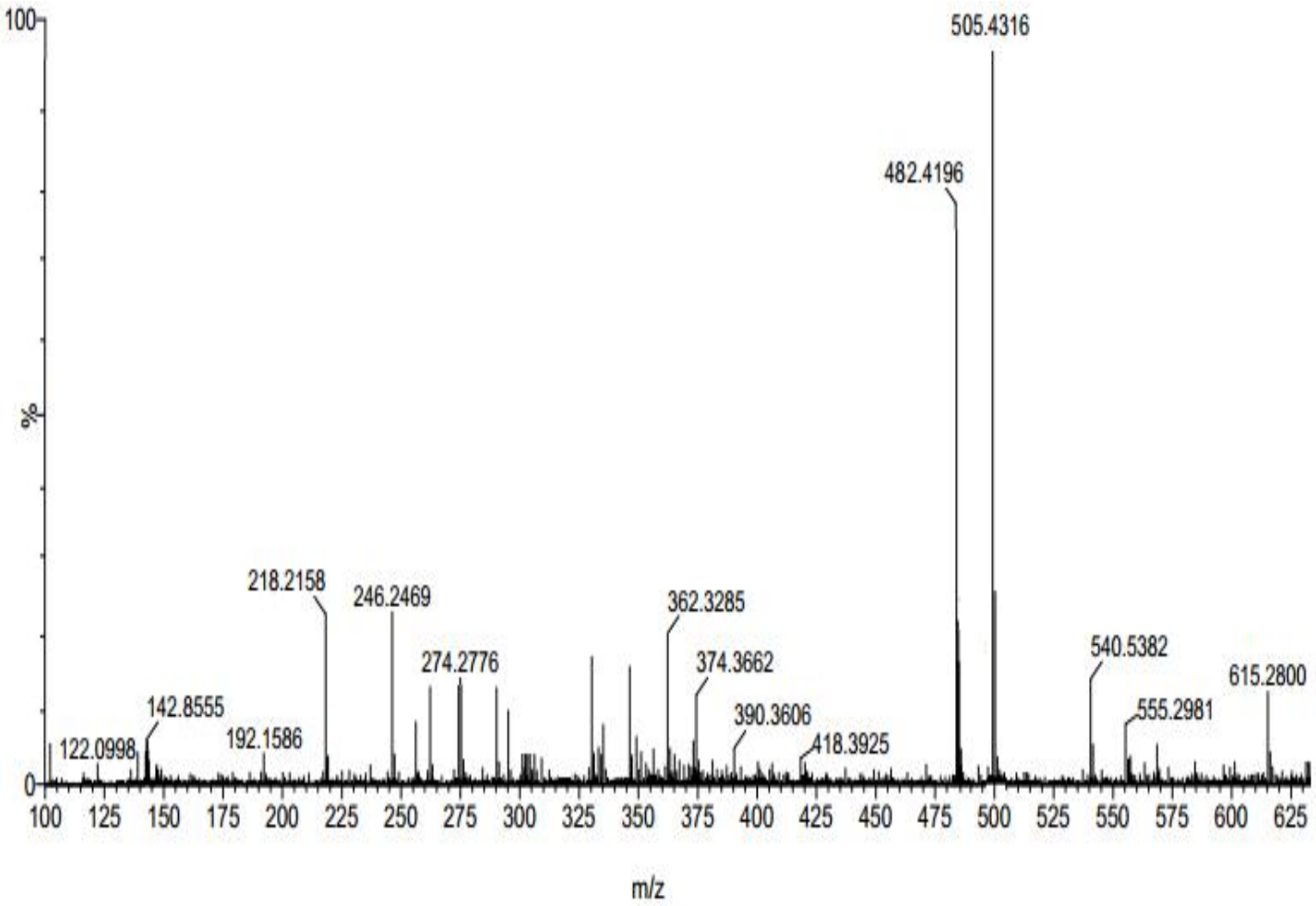
Appendix 9: HMBC spectrum of 3-methoxy-4-decarboxydihydrozeylasterone, **55**



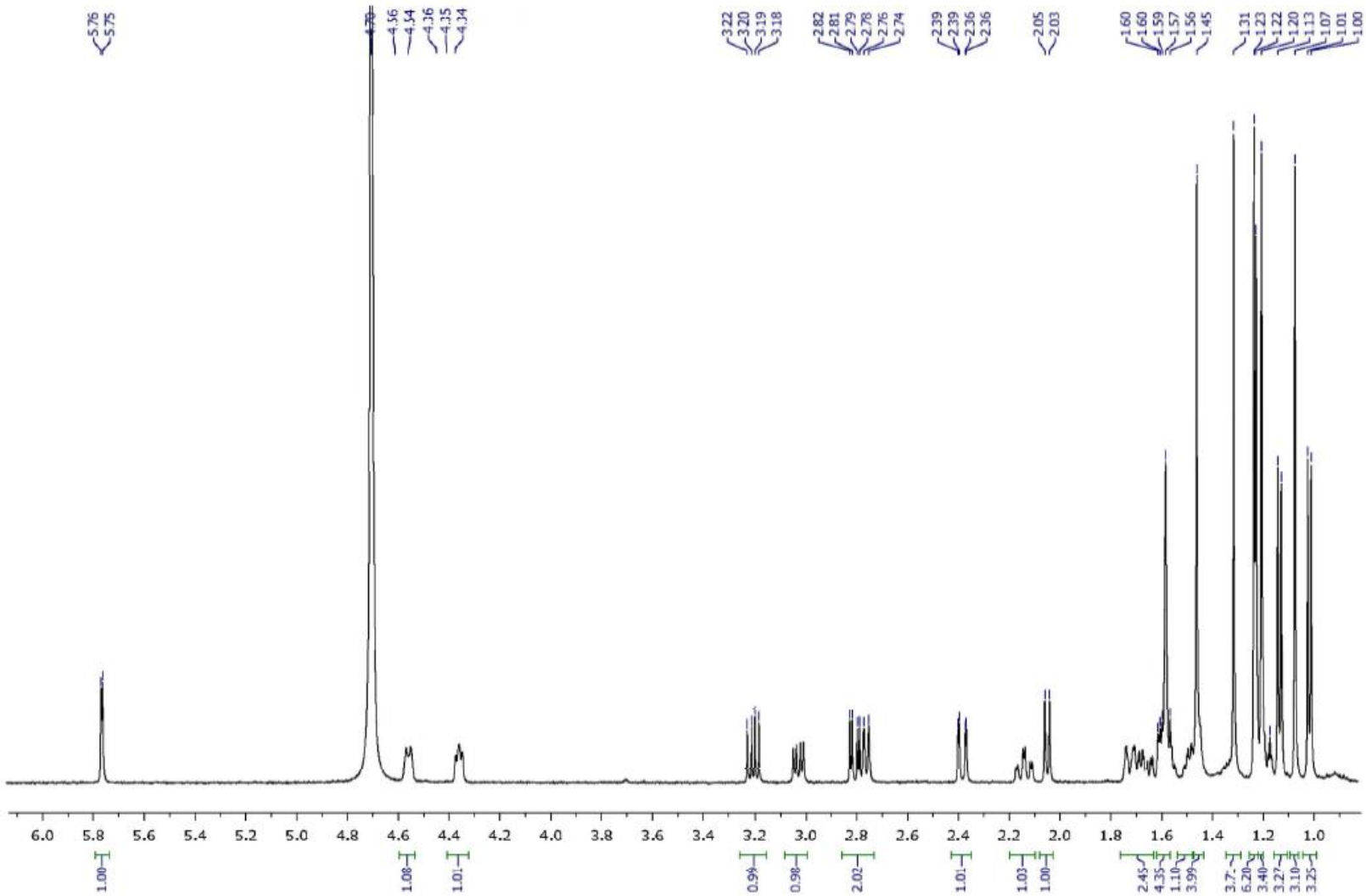
Appendix 10: IR spectrum of 3-methoxy-4-decarboxydihydrozeylasterone, **55**



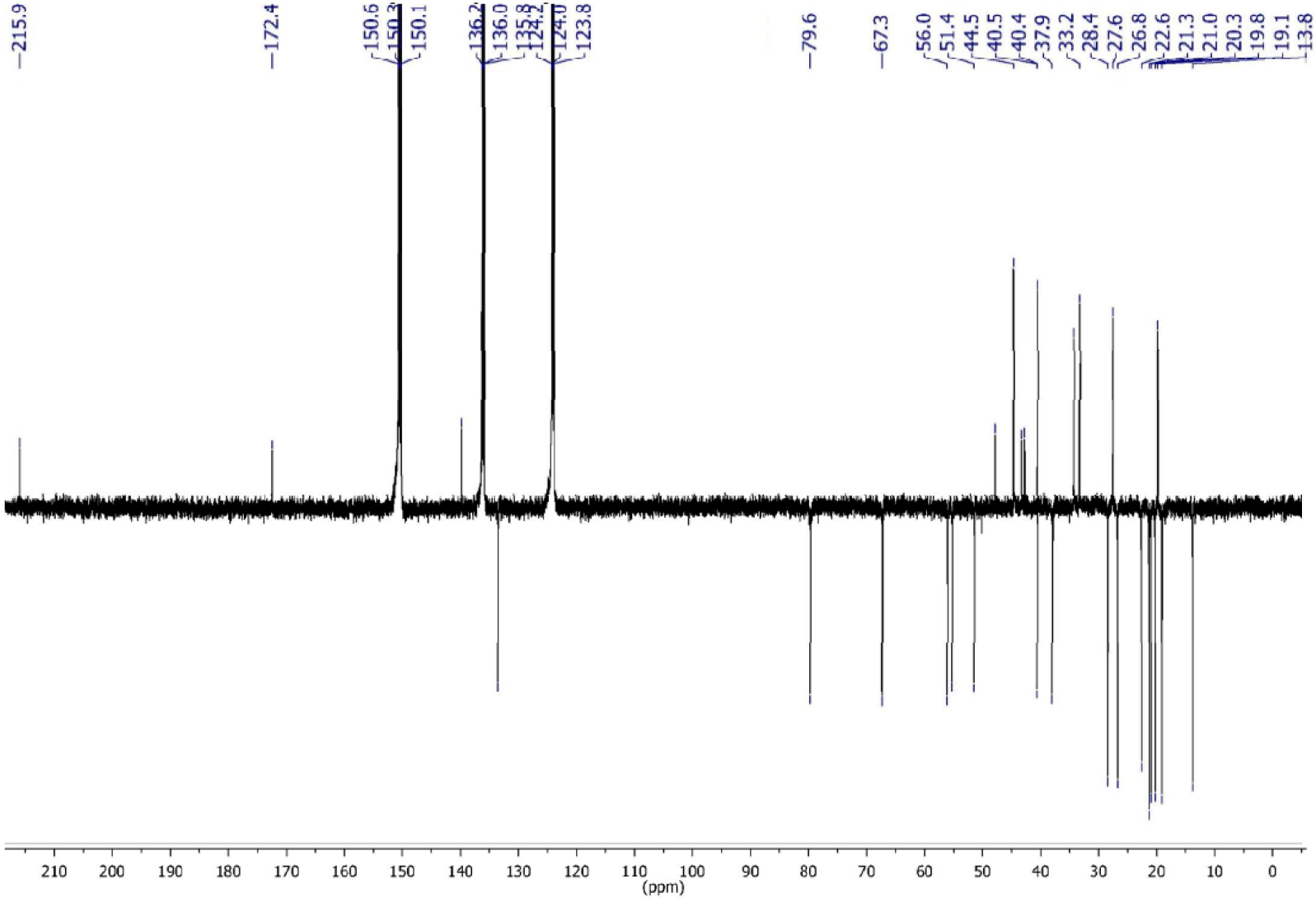
Appendix 11: MS spectrum of 3-methoxy-4-decarboxydihydrozeylasterone, **55**



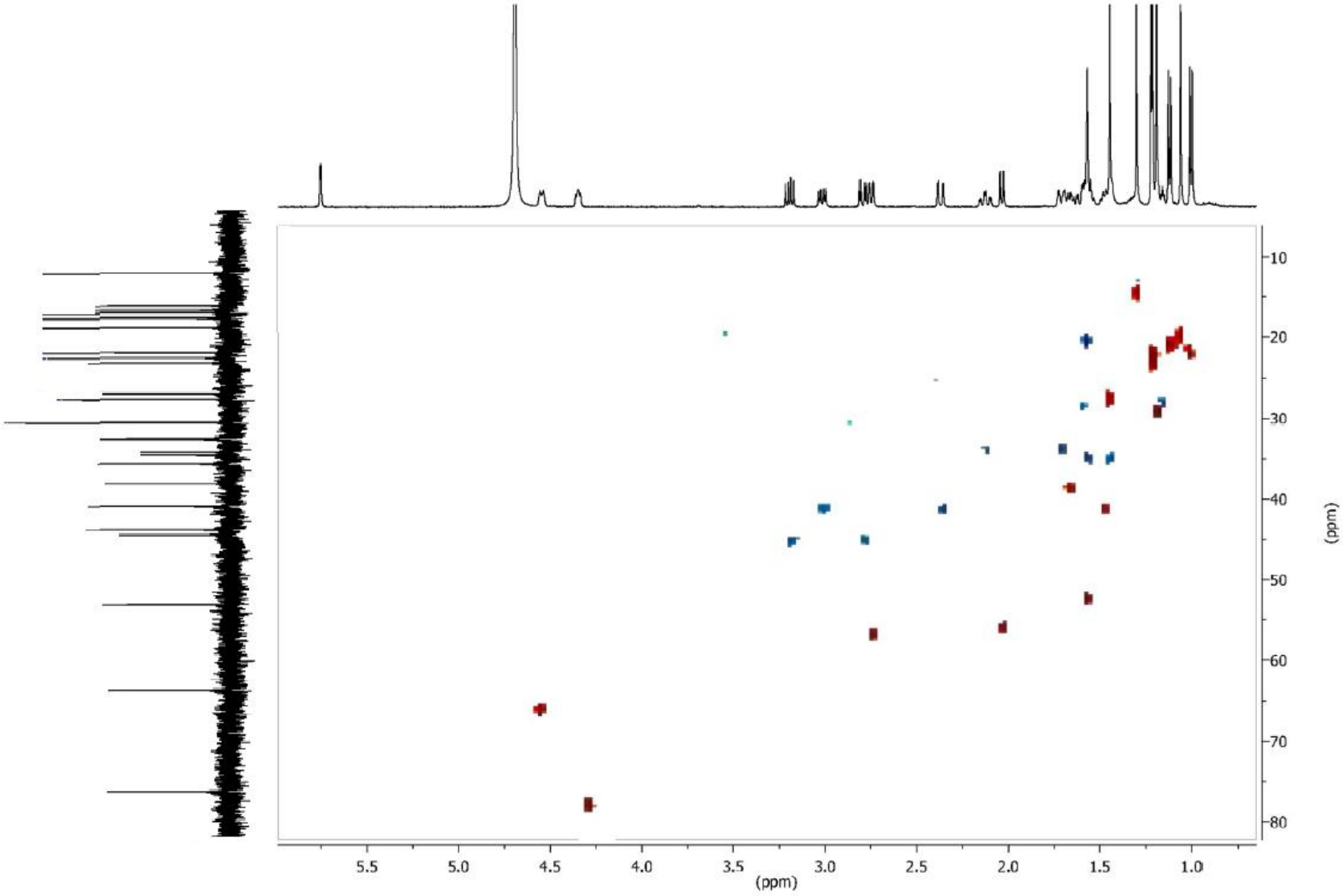
Appendix 12: ^{1}H NMR ($CDCl_3$, 600 MHz) of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3, 11-olide, **56**



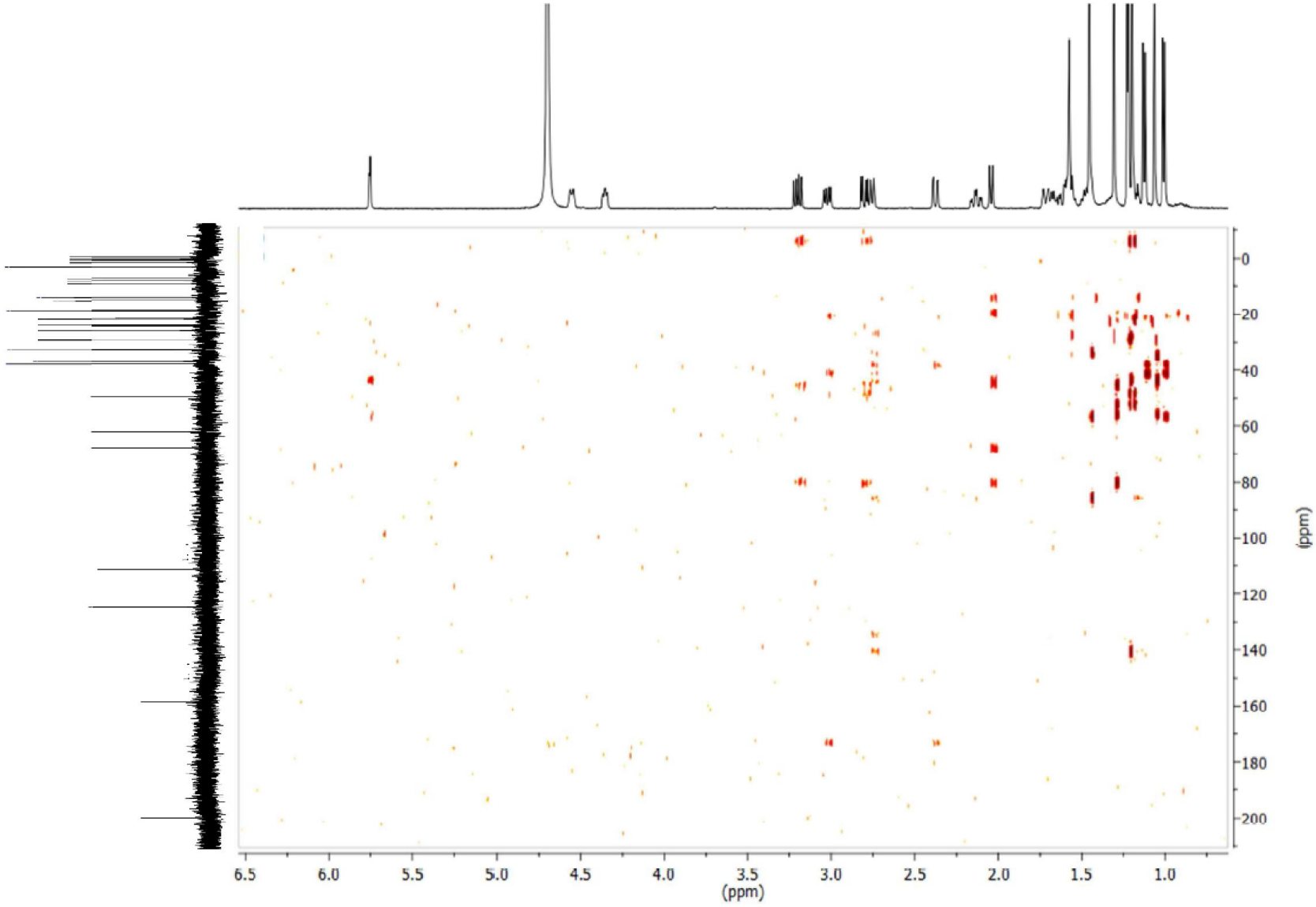
Appendix 13: DEPT 135 spectrum of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3, 11-olide, **56**



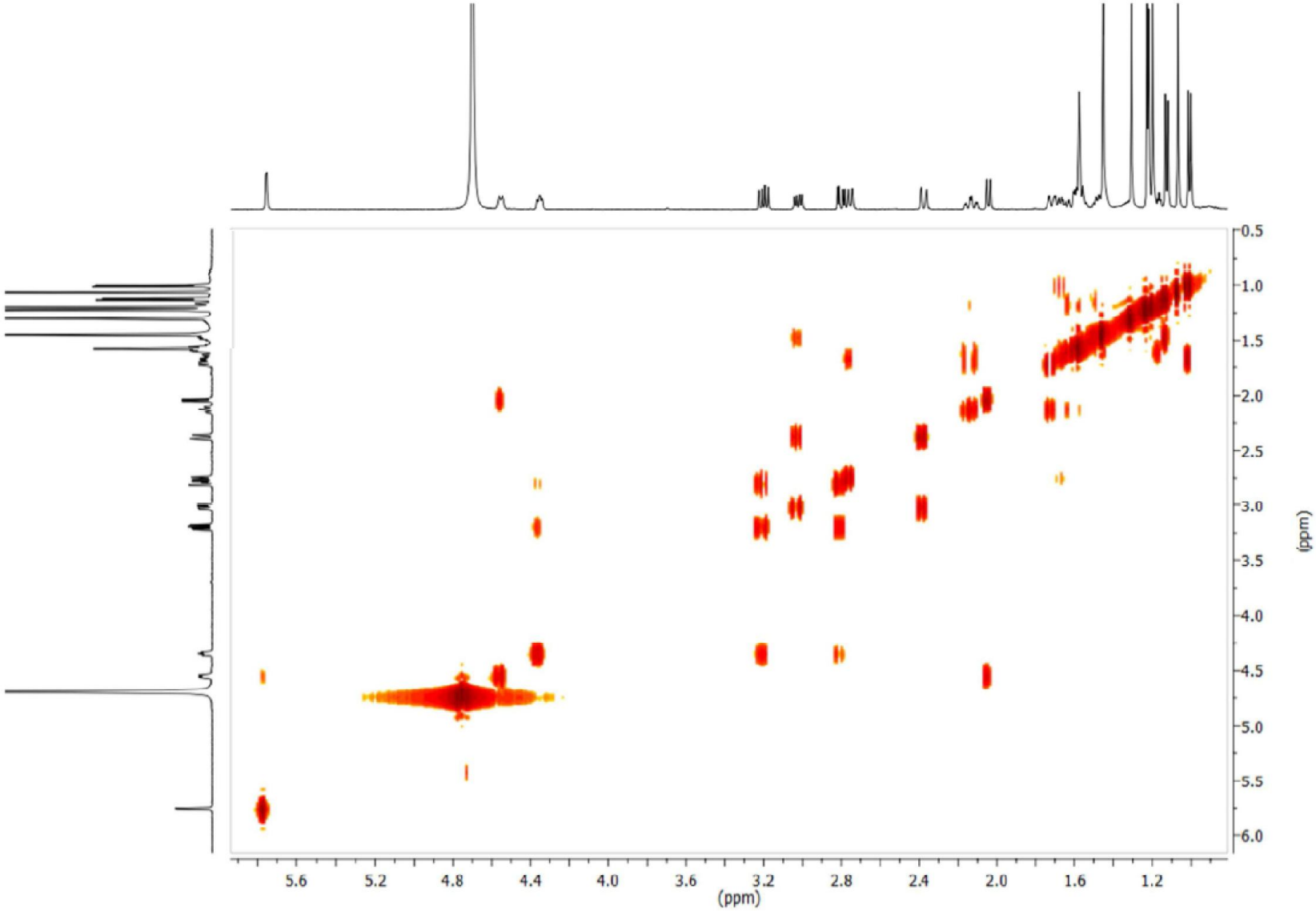
Appendix 14: HSQC spectrum of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3, 11-olide, **56**



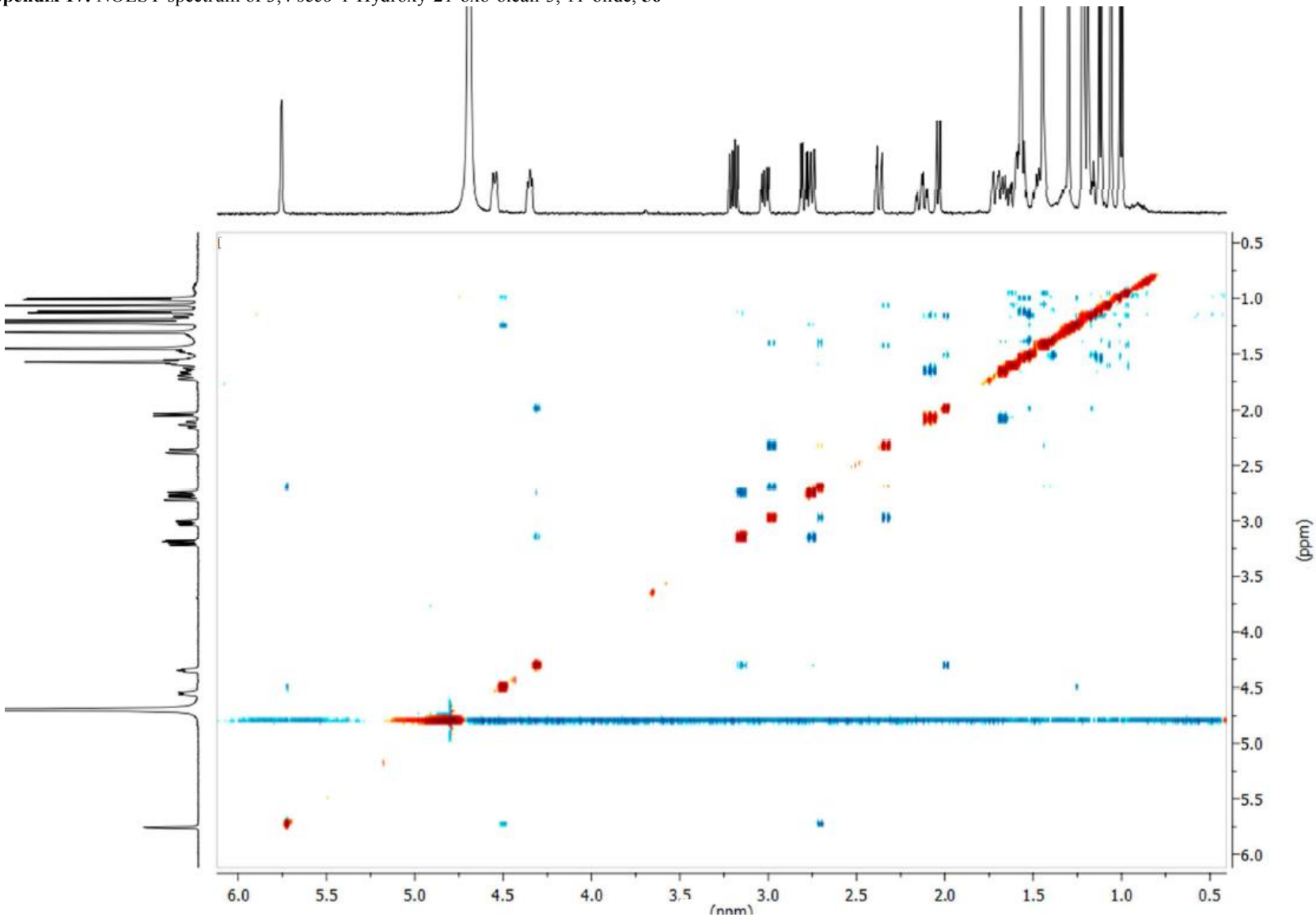
Appendix 15: HMBC spectrum of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3, 11-olide, **56**



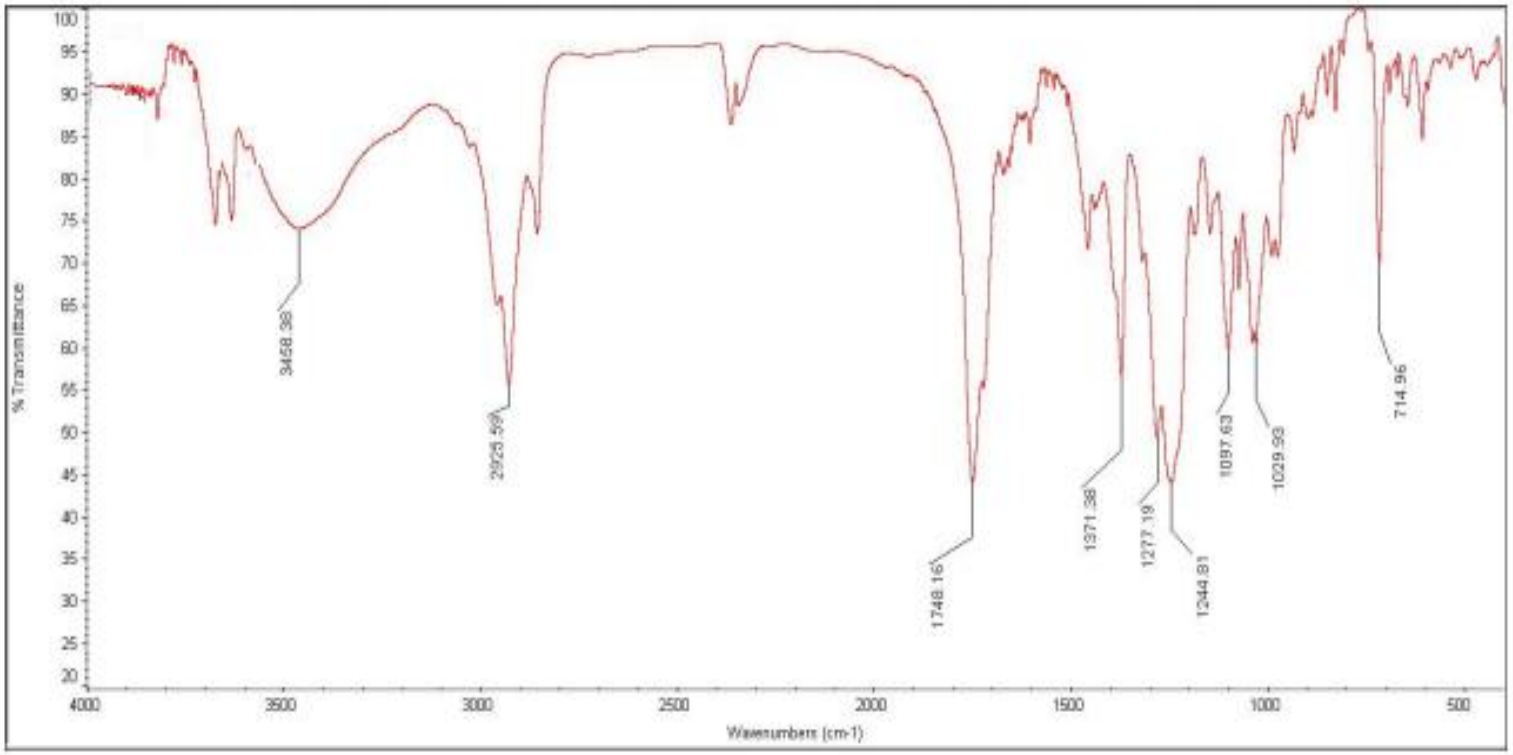
Appendix 16: COSY spectrum of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3, 11-olide, **56**



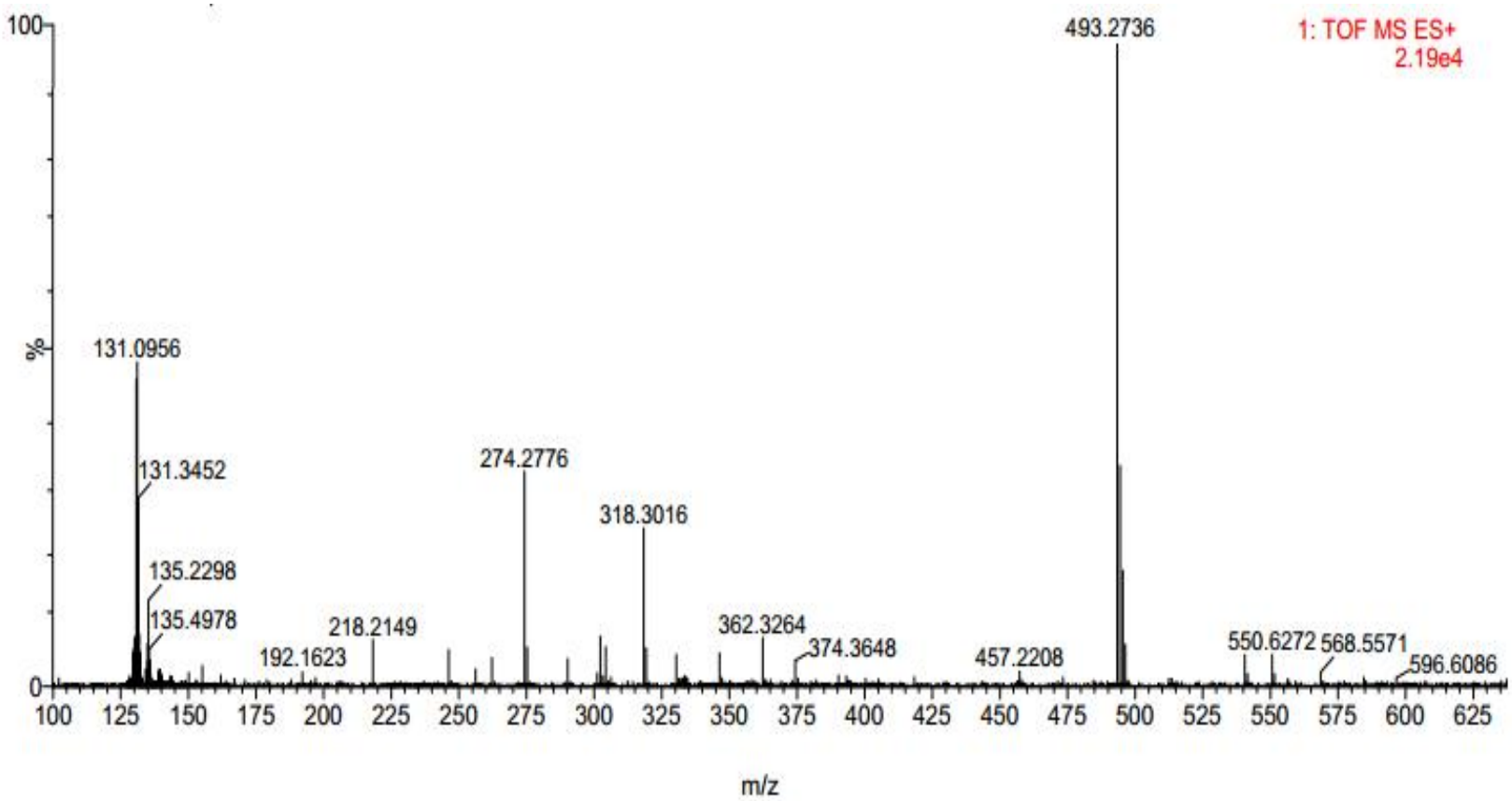
Appendix 17: NOESY spectrum of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3, 11-olide, **56**



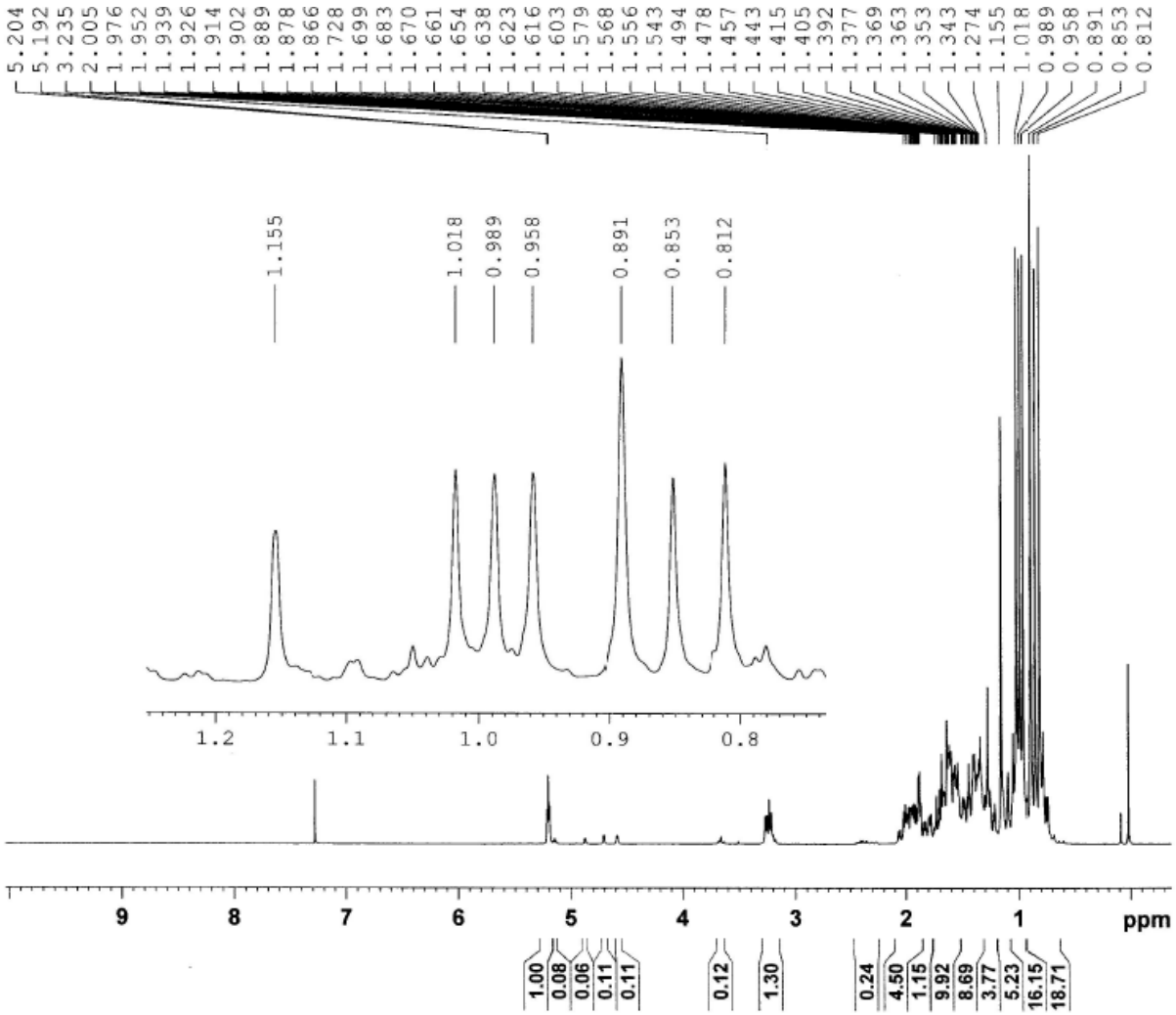
Appendix 18: IR spectrum of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3,11-olide, **56**



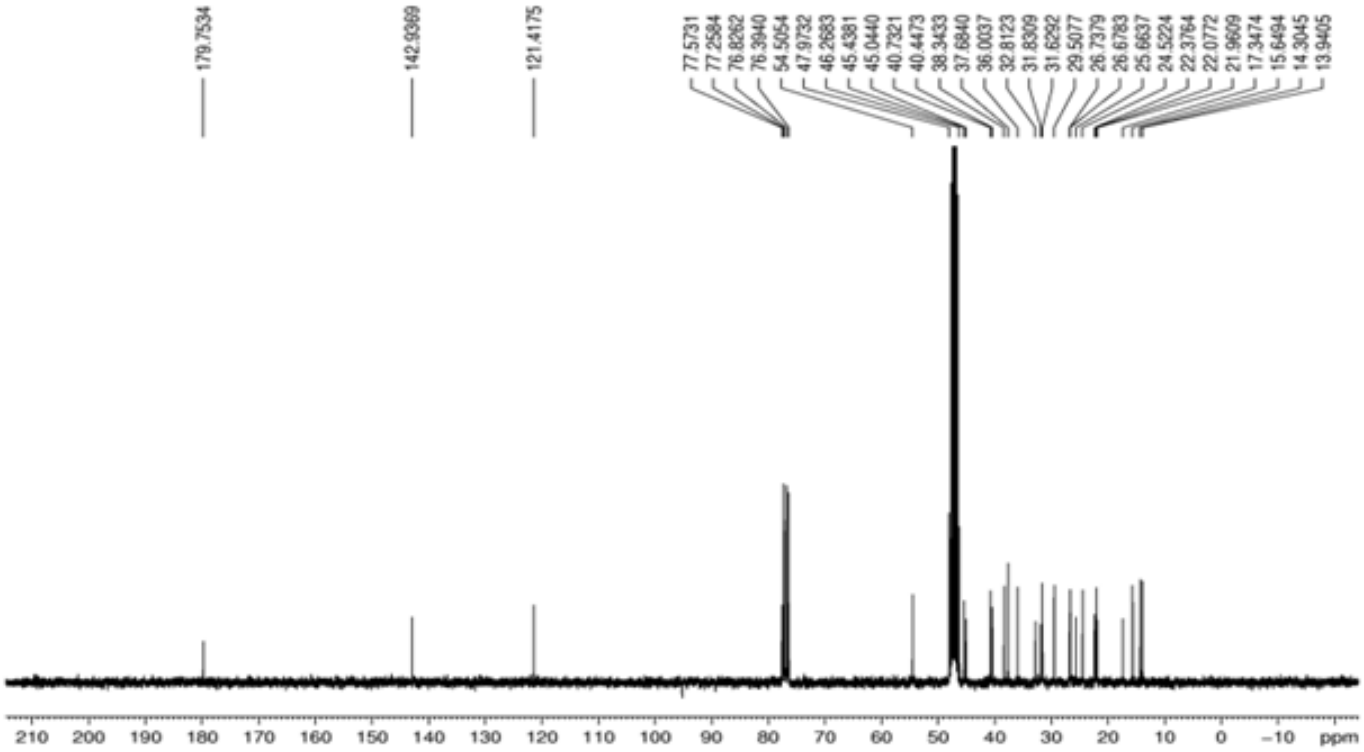
Appendix 19: MS spectrum of 3,4-*seco*-1-Hydroxy-21-oxo-olean-3,11-olide, **56**



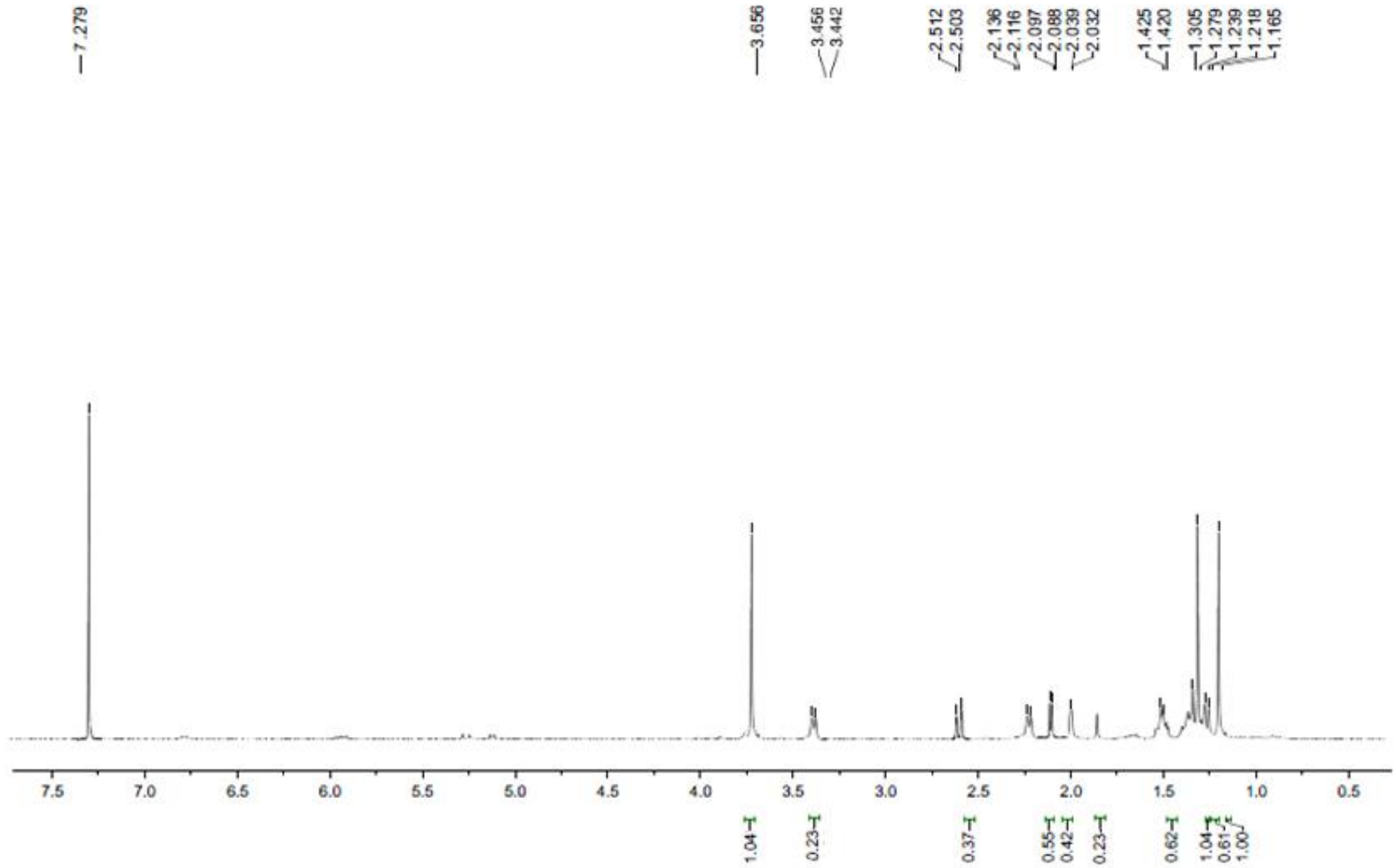
Appendix 20: ^{1}H NMR ($CDCl_3$, 600 MHz) of Oleanolic acid, **57**



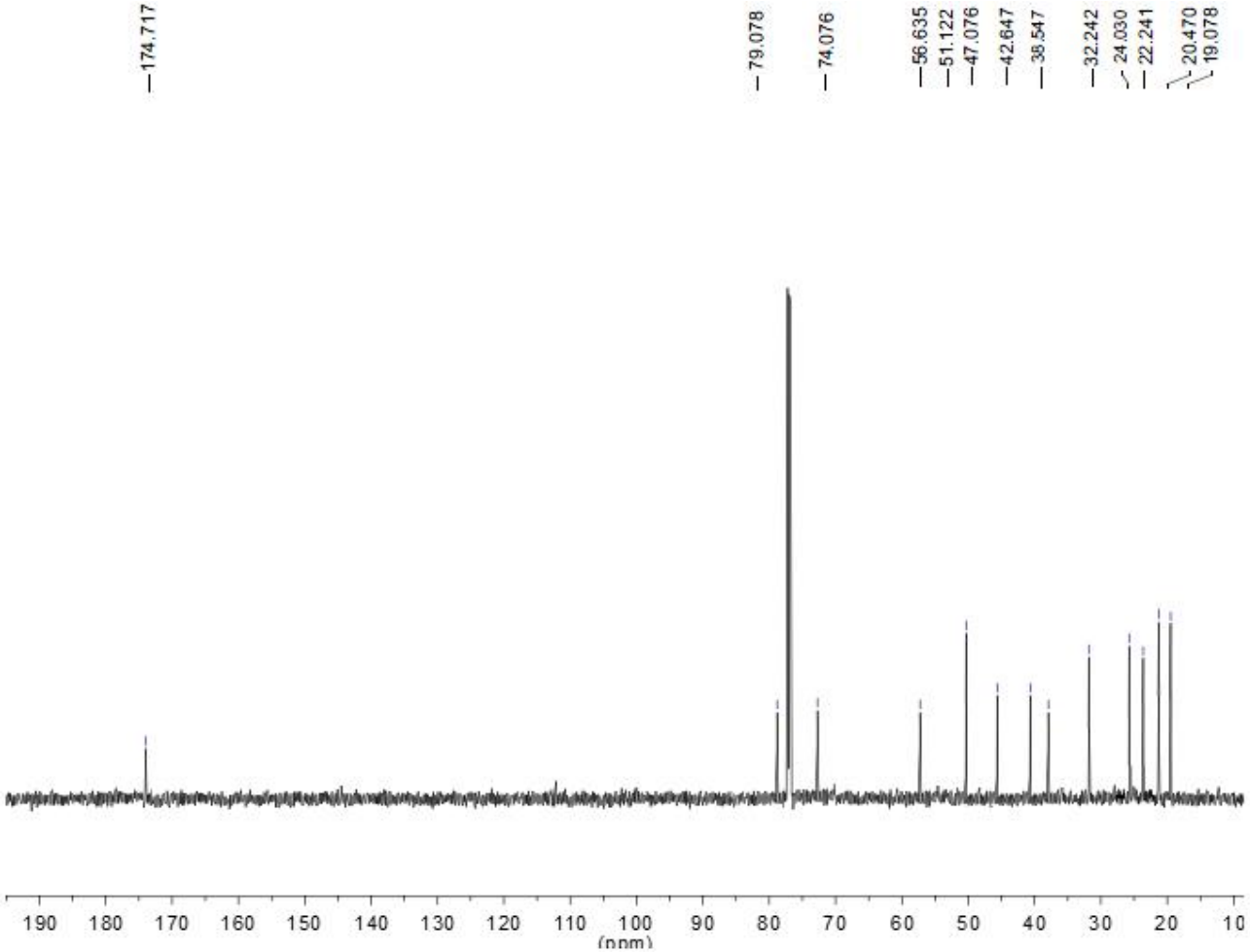
Appendix 21: ^{13}**C** NMR ($CDCl_3$, 150 MHz) of Oleanolic acid, **57**



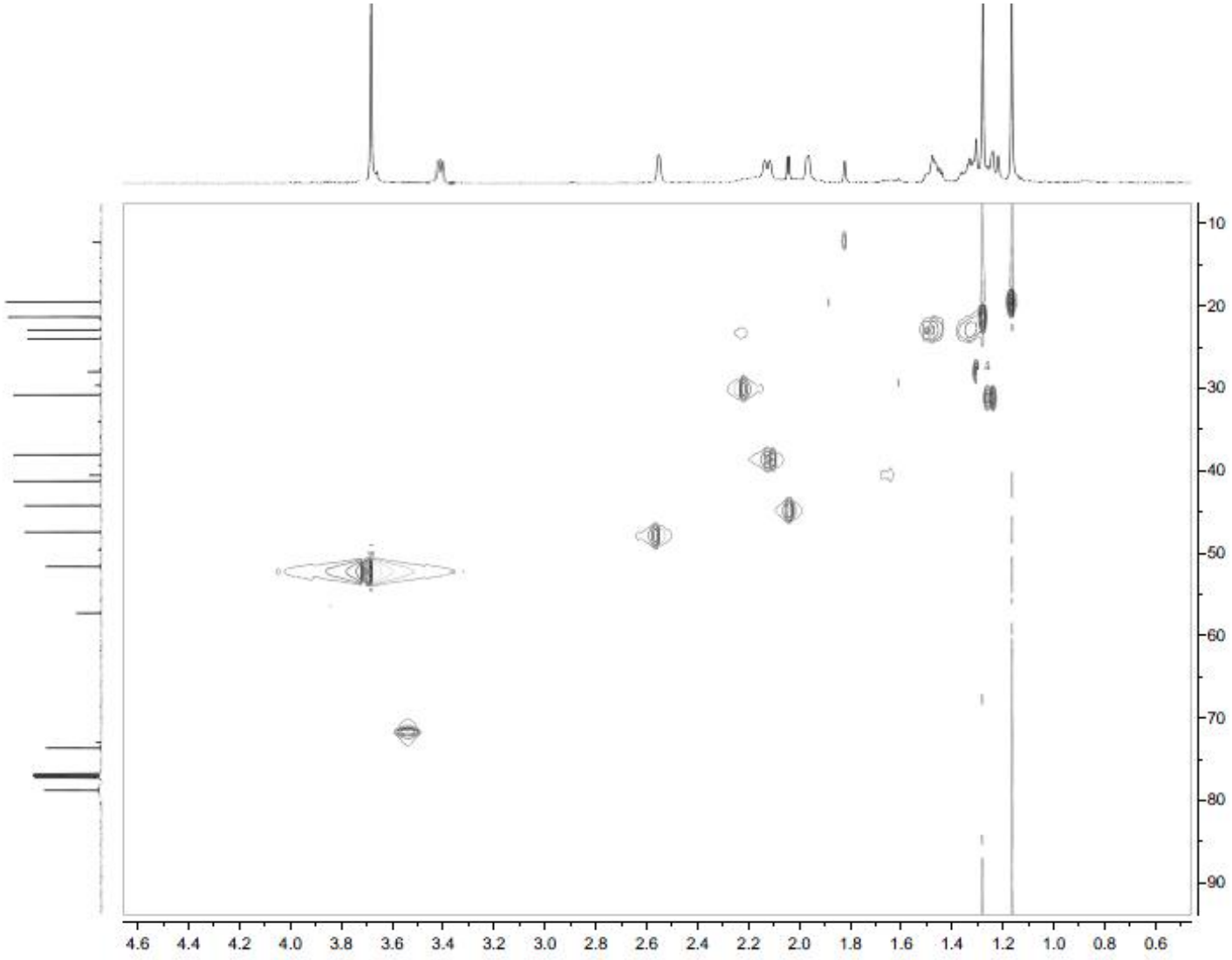
Appendix 22: ^{1}H NMR ($CDCl_3$, 600 MHz) of 3,5-Dihydroxycamphanoate, **58**



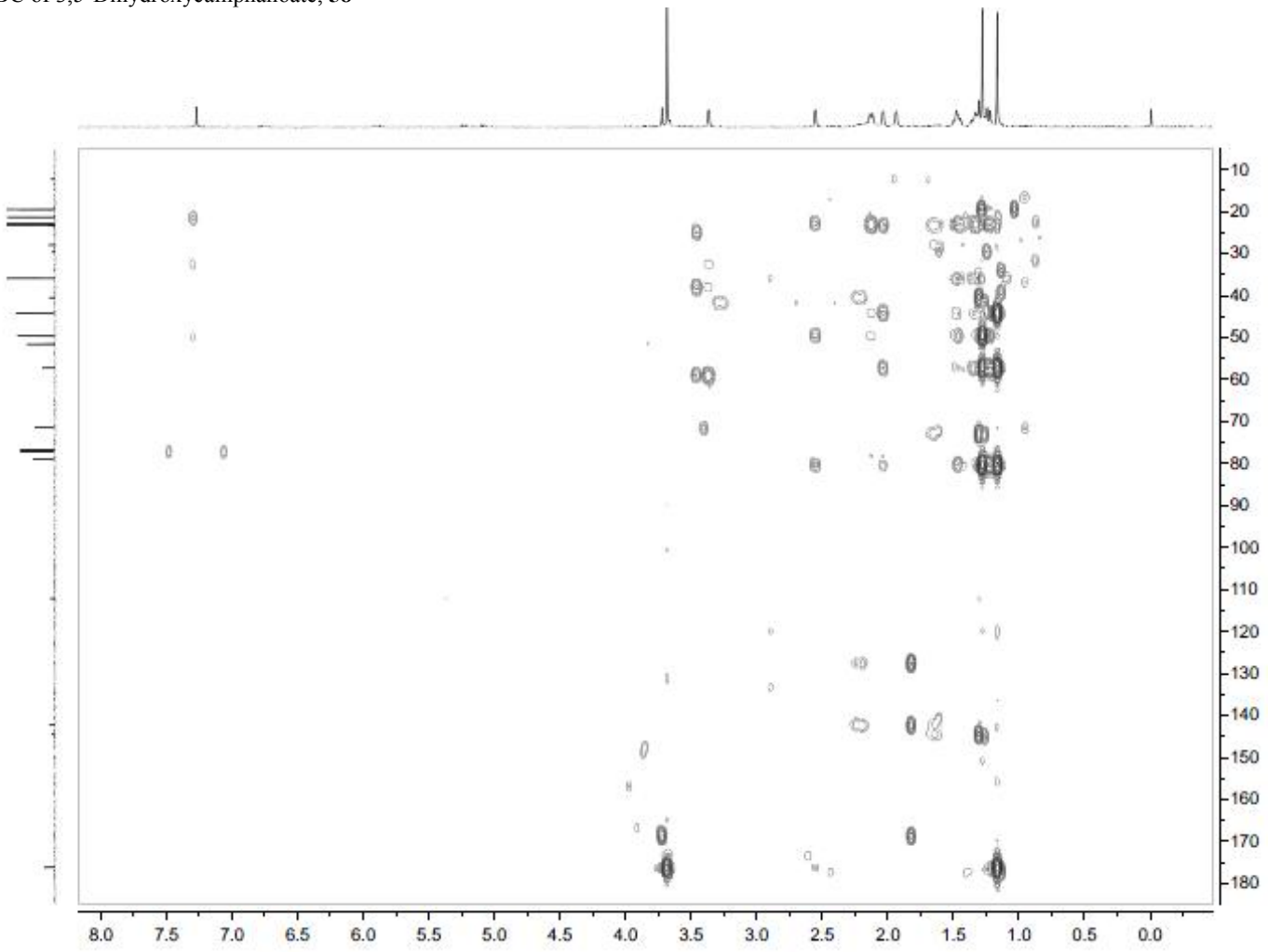
Appendix 23: ^{13}C NMR ($CDCl_3$, 150 MHz) of 3,5-Dihydroxycamphanoate, **58**



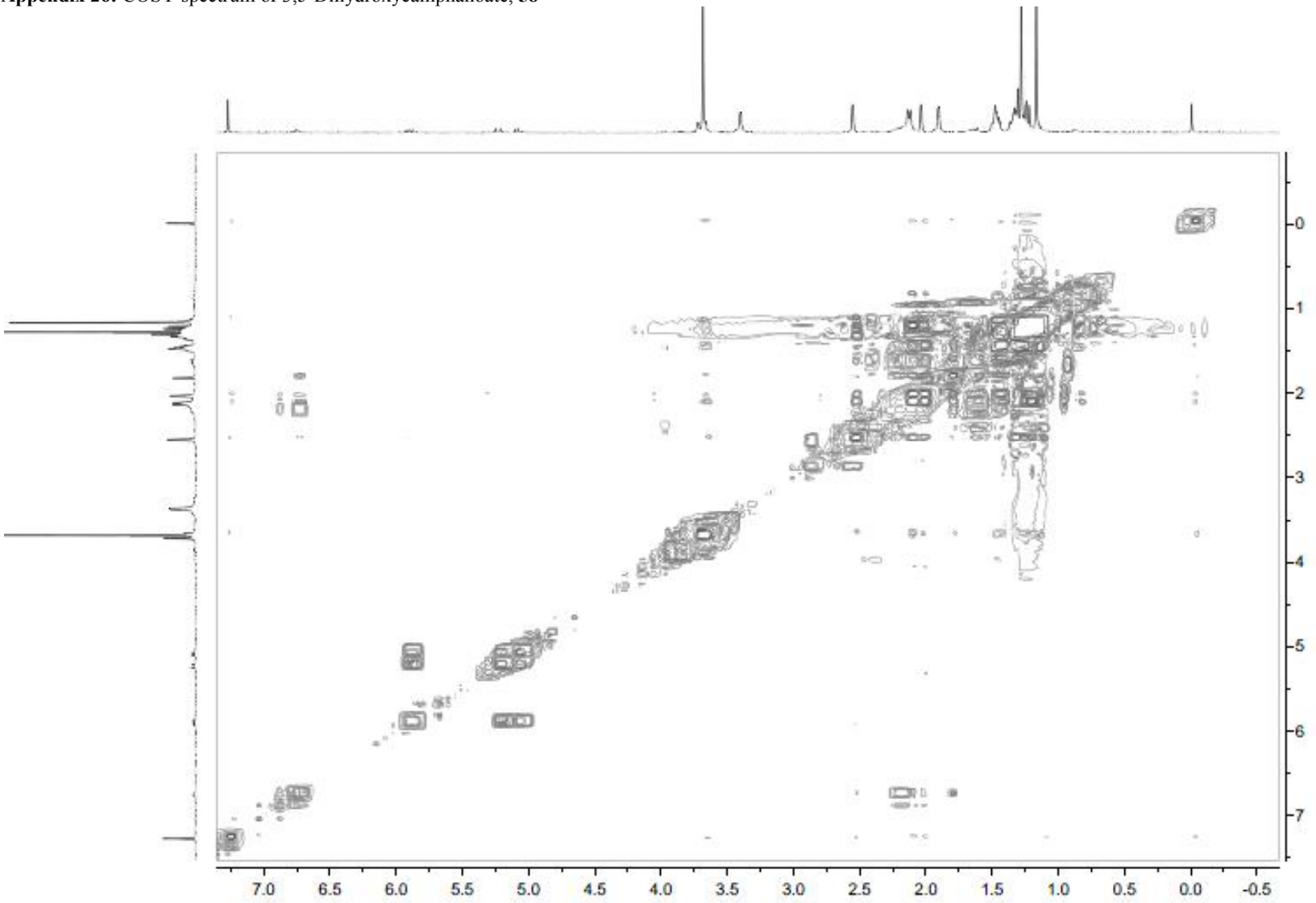
Appendix 24: HSQC spectrum of 3,5-Dihydroxycamphanoate, **58**



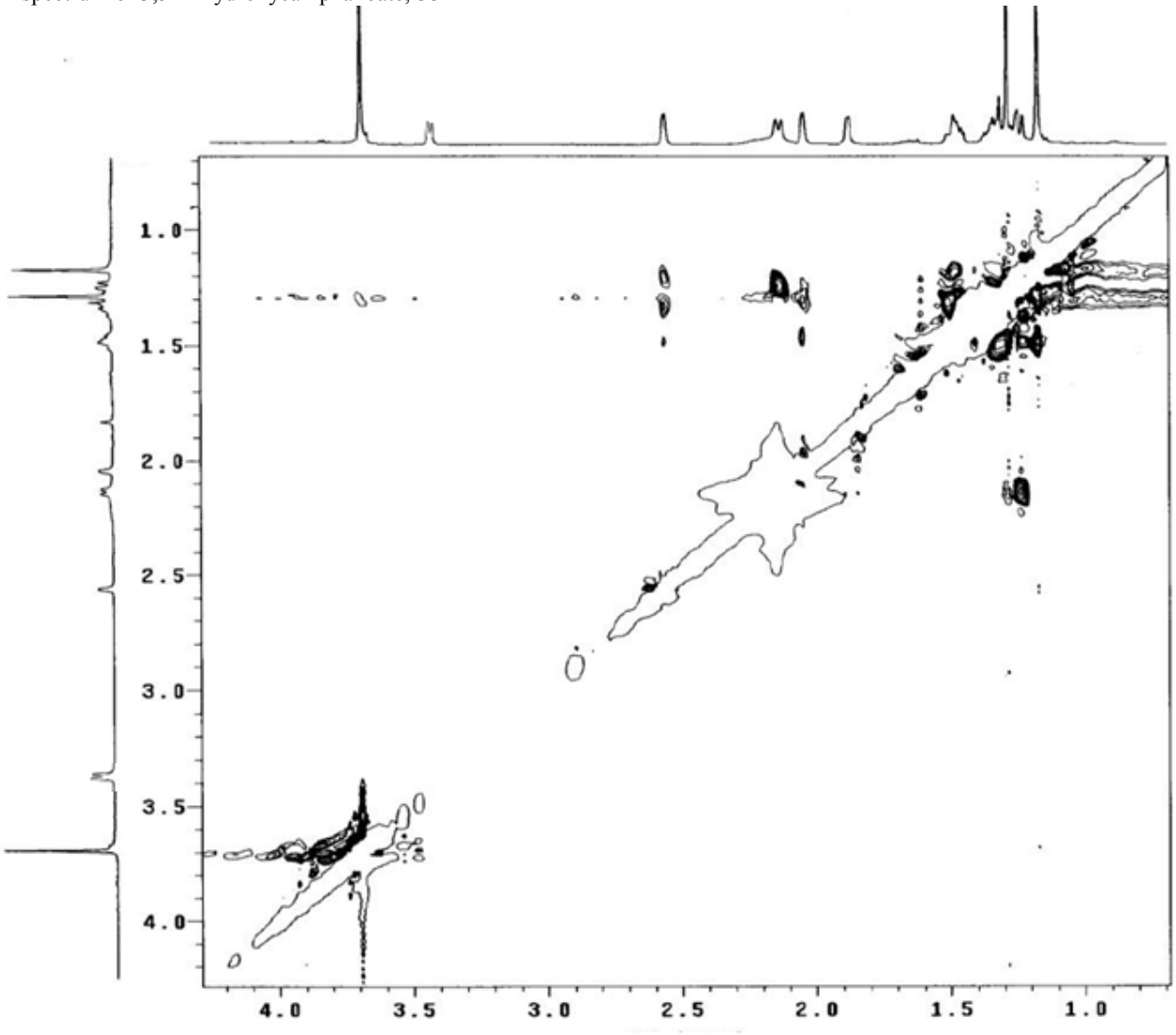
Appendix 25: HMBC of 3,5-Dihydroxycamphanoate, **58**



Appendix 26: COSY spectrum of 3,5-Dihydroxycamphanoate, **58**



Appendix 27: NOESY spectrum of 3,5-Dihydroxycamphanoate, **58**



Appendix 28: Statiatical results for Analyais of Variance

ANOVA: Duration of seizure results for crude extracts

The results of a ANOVA statistical test performed at 09:14 on 16-AUG-2016

Source of Variation	Sum of Squares	d.f.	Mean Squares	F
between	6.2913E+04	10	6291.	58.76
error	2355.	22	107.1	
total	6.5268E+04	32		

The probability of this result, assuming the null hypothesis, is 0.000
Coefficient of variation = 0.3426
Results
Least significant difference (Tukey's Honestly Significant Difference):
for $p<0.05$ =4.4389
for $p<0.01$ =5.3958

Group A (vehicle/control): Number of items= 3
220. 230. 240.
Mean = 230.
95% confidence interval for Mean: 217.8 thru 242.6
Standard Deviation = 9.75
Hi = 240. Low = 220.
Median = 230.
Average Absolute Deviation from Median = 6.50

Group B (Leaf extract 50): Number of items= 3
167. 170. 191.
Mean = 176.
95% confidence interval for Mean: 163.6 thru 188.4
Standard Deviation = 13.1
Hi = 191. Low = 167.
Median = 170.
Average Absolute Deviation from Median = 8.00

Group C (leaf extract 100): Number of items= 3
157. 176. 183.
Mean = 172.
95% confidence interval for Mean: 159.6 thru 184.4
Standard Deviation = 13.5
Hi = 183. Low = 157.
Median = 176.
Average Absolute Deviation from Median = 8.67

Group D (leaf extract 200): Number of items= 3
110. 120. 132.
Mean = 121.
95% confidence interval for Mean: 108.4 thru 133.2
Standard Deviation = 10.8
Hi = 132. Low = 110.
Median = 120.
Average Absolute Deviation from Median = 7.20

Group E (root extract 50): Number of items= 3
132. 141. 152.
Mean = 142.
95% confidence interval for Mean: 129.2 thru 153.9
Standard Deviation = 10.0
Hi = 152. Low = 132.
Median = 141.
Average Absolute Deviation from Median = 6.67

Group F (root extract 100): Number of items= 3
101. 107. 121.
Mean = 110.
95% confidence interval for Mean: 97.28 thru 122.1
Standard Deviation = 10.3
Hi = 121. Low = 101.
Median = 107.
Average Absolute Deviation from Median = 6.67

Group G (root extract 200): Number of items= 3
93.9 105. 121.
Mean = 107.
95% confidence interval for Mean: 94.23 thru 119.0
Standard Deviation = 13.6
Hi = 121. Low = 93.9
Median = 105.
Average Absolute Deviation from Median = 9.04

Group H (stem extract 50): Number of items= 3
120. 121. 131.
Mean = 124.
95% confidence interval for Mean: 111.8 thru 136.6
Standard Deviation = 5.92
Hi = 131. Low = 120.
Median = 121.
Average Absolute Deviation from Median = 3.50

Group I (stem extract 100): Number of items= 3
111. 113. 121.
Mean = 115.
95% confidence interval for Mean: 102.5 thru 127.3
Standard Deviation = 5.42
Hi = 121. Low = 111.
Median = 113.
Average Absolute Deviation from Median = 3.44

Group J (stem extract 200): Number of items= 3
73.0 80.4 91.0
Mean = 81.5
95% confidence interval for Mean: 69.09 thru 93.87
Standard Deviation = 9.05
Hi = 91.0 Low = 73.0
Median = 80.4
Average Absolute Deviation from Median = 6.00

Group K (DZP): Number of items= 3
65.0 70.7 82.0
Mean = 72.6
95% confidence interval for Mean: 60.17 thru 84.95
Standard Deviation = 8.66
Hi = 82.0 Low = 65.0
Median = 70.7
Average Absolute Deviation from Median = 5.67

ANOVA: Latency of seizure results for crude extracts

The results of a ANOVA statistical test performed at 10:04 on 16-AUG-2016

Source of Variation	Sum of Squares	d.f.	Mean Squares	F
between	5.8949E+05	10	5.8949E+04	212.7
error	6096.	22	277.1	
total	5.9558E+05	32		

The probability of this result, assuming the null hypothesis, is less than .0001
Results
Least significant difference (Tukey's Honestly Significant Difference):
for $p<0.05$ =7.1417
for $p<0.01$ =8.6812
Coeeffecient of variation = 0.3849

Group A (vehicle/control): Number of items= 3
209. 215. 220.
Mean = 214.73
95% confidence interval for Mean: 194.8 thru 234.7
Standard Deviation = 5.60
High = 220.2 Low = 209.0
Median = 215.0
Average Absolute Deviation from Median = 3.73

Group B (leaf extract 50): Number of items= 3
205. 210. 215.
Mean = 210.13
95% confidence interval for Mean: 190.2 thru 230.1
Standard Deviation = 5.20
High = 215.4 Low = 205.0
Median = 210.0
Average Absolute Deviation from Median = 3.47

Group C (leaf extract 100): Number of items= 3
230. 250. 281.
Mean = 253.80
95% confidence interval for Mean: 233.9 thru 273.7
Standard Deviation = 25.9
High = 281.4 Low = 230.0
Median = 250.0
Average Absolute Deviation from Median = 17.1

Group D (leaf extract 200): Number of items= 3
300. 312. 320.
Mean = 310.60
95% confidence interval for Mean: 290.7 thru 330.5
Standard Deviation = 9.97
High = 319.8 Low = 300.0
Median = 312.0
Average Absolute Deviation from Median = 6.60

Group E (root extract 50): Number of items= 3
300. 312. 320.
Mean = 310.60
95% confidence interval for Mean: 290.7 thru 330.5
Standard Deviation = 9.97
High = 319.8 Low = 300.0
Median = 312.0
Average Absolute Deviation from Median = 6.60

Group F (root extract 100): Number of items= 3
378. 380. 410.
Mean = 389.47
95% confidence interval for Mean: 369.5 thru 409.4
Standard Deviation = 18.2
High = 410.4 Low = 378.0
Median = 380.0
Average Absolute Deviation from Median = 10.8

Group G (root extract 200): Number of items= 3
471. 489. 520.
Mean = 493.20
95% confidence interval for Mean: 473.3 thru 513.1
Standard Deviation = 24.6
High = 519.6 Low = 471.0
Median = 489.0
Average Absolute Deviation from Median = 16.2

Group H (stem extract 50): Number of items= 3
278. 281. 295.
Mean = 284.53
95% confidence interval for Mean: 264.6 thru 304.5
Standard Deviation = 8.85
High = 294.6 Low = 278.0
Median = 281.0
Average Absolute Deviation from Median = 5.53

Group I (stem extract 100): Number of items= 3
321. 359. 362.
Mean = 347.27
95% confidence interval for Mean: 327.3 thru 367.2
Standard Deviation = 22.8
High = 362.0 Low = 321.0
Median = 358.8
Average Absolute Deviation from Median = 13.7

Group J (stem extract 200): Number of items= 3
472. 491. 511.
Mean = 491.20
95% confidence interval for Mean: 471.3 thru 511.1
Standard Deviation = 19.3
High = 510.6 Low = 472.0
Median = 491.0
Average Absolute Deviation from Median = 12.9

Group K (DZP): Number of items= 3
653. 670. 682.
Mean = 668.40
95% confidence interval for Mean: 648.5 thru 688.3
Standard Deviation = 14.7
High = 682.2 Low = 653.0
Median = 670.0
Average Absolute Deviation from Median = 9.73

ANOVA: Duration of seizure results for compounds

The results of a ANOVA statistical test performed at 05:06 on 16-AUG-2016

Source of Variation	Sum of Squares	d.f.	Mean Squares	F
between	2.8340E+05	13	2.1800E+04	20.88
error	5.8457E+04	56	1044.	
total	3.4186E+05	69		

The probability of this result, assuming the null hypothesis, is 0.000

Results

Least significant difference (Tukey's Honestly Significant Difference):

for $p<0.05$ =5.1144

for $p<0.01$ =6.0551

Coefficients of variation

0.5 mg/kg	0.4201
5 mg/kg	0.428
50 mg/kg	0.5007

Group A (Vehicle control): Number of items= 5

121. 210. 221. 240. 256.

Mean = 210.

95% confidence interval for Mean: 180.7 thru 238.6

Standard Deviation = 52.9

Hi = 256. Low = 121.

Median = 221.

Average Absolute Deviation from Median = 33.2

Group B compound 54 (0.5mg/kg): Number of items= 5

230. 245. 260. 297. 356.

Mean = 278.

95% confidence interval for Mean: 248.7 thru 306.5

Standard Deviation = 50.4

Hi = 356. Low = 230.

Median = 260.

Average Absolute Deviation from Median = 35.6

Group C compound 54 (5 mg/kg): Number of items= 5

164. 174. 182. 189. 254.

Mean = 193.

95% confidence interval for Mean: 163.7 thru 221.5

Standard Deviation = 35.6

Hi = 254. Low = 164.

Median = 182.

Average Absolute Deviation from Median = 21.0

Group D compound 54 [50mg/kg]: Number of items= 5
98.0 109. 116. 145. 147.
Mean = 123.
95% confidence interval for Mean: 94.05 thru 151.9
Standard Deviation = 22.0
Hi = 147. Low = 98.0
Median = 116.
Average Absolute Deviation from Median = 17.0

Group E compound 55[0.5mg.kg]: Number of items= 5
176. 185. 198. 241. 256.
Mean = 211.
95% confidence interval for Mean: 182.3 thru 240.1
Standard Deviation = 35.3
Hi = 256. Low = 176.
Median = 198.
Average Absolute Deviation from Median = 27.2

Group Fcompound 55 [5mg/kg]: Number of items= 5
119. 125. 160. 165. 210.
Mean = 156.
95% confidence interval for Mean: 126.9 thru 184.7
Standard Deviation = 36.5
Hi = 210. Low = 119.
Median = 160.
Average Absolute Deviation from Median = 26.2

Group G compound 55 [50mg/kg]: Number of items= 5
102. 110. 119. 125. 145.
Mean = 120.
95% confidence interval for Mean: 91.25 thru 149.1
Standard Deviation = 16.4
Hi = 145. Low = 102.
Median = 119.
Average Absolute Deviation from Median = 11.6

Group H compound 56 [0.5mg.kg]: Number of items= 5
213. 221. 246. 285. 312.
Mean = 255.
95% confidence interval for Mean: 226.5 thru 284.3
Standard Deviation = 42.3
Hi = 312. Low = 213.
Median = 246.
Average Absolute Deviation from Median = 32.6

Group I compound 56[5mg/kg]: Number of items= 5
151. 174. 195. 214. 221.
Mean = 191.
95% confidence interval for Mean: 162.1 thru 219.9
Standard Deviation = 28.9
Hi = 221. Low = 151.
Median = 195.
Average Absolute Deviation from Median = 22.0

Group J compound 56[50mg/kg]: Number of items= 5
97.0 102. 118. 158. 165.
Mean = 128.
95% confidence interval for Mean: 99.05 thru 156.9
Standard Deviation = 31.6
Hi = 165. Low = 97.0
Median = 118.
Average Absolute Deviation from Median = 24.8

Group K compound 58 [0.5 mg/kg]: Number of items= 5
179. 186. 198. 213. 217.
Mean = 199.
95% confidence interval for Mean: 169.7 thru 227.5
Standard Deviation = 16.5
Hi = 217. Low = 179.
Median = 198.
Average Absolute Deviation from Median = 13.0

Group L compound 58 [5mg/kg]: Number of items= 5
107. 110. 112. 114. 158.
Mean = 120.
95% confidence interval for Mean: 91.25 thru 149.1
Standard Deviation = 21.3
Hi = 158. Low = 107.
Median = 112.
Average Absolute Deviation from Median = 11.0

Group M compound 58 [50 mg/kg]: Number of items= 5
65.0 68.0 69.0 74.0 97.0
Mean = 74.6
95% confidence interval for Mean: 45.65 thru 103.5
Standard Deviation = 12.9
Hi = 97.0 Low = 65.0
Median = 69.0
Average Absolute Deviation from Median = 7.60

Group N standard DZP : Number of items= 5
34.0 38.0 45.0 60.0 62.0
Mean = 47.8
95% confidence interval for Mean: 18.85 thru 76.75
Standard Deviation = 12.7
Hi = 62.0 Low = 34.0
Median = 45.0
Average Absolute Deviation from Median = 10.0

ANOVA: Latency of seizure results for compounds

The results of a ANOVA statistical test performed at 05:37 on 16-AUG-2016

Source of Variation	Sum of Squares	d.f.	Mean Squares	F
between	1.2161E+06	13	9.3546E+04	52.70
error	9.9413E+04	56	1775.	
total	1.3155E+06	69		

The probability of this result, assuming the null hypothesis, is 0.000
Results
Least significant difference (Tukey's Honestly Significant Difference):
for $p<0.05$ =6.6696
for $p<0.01$ =7.8963
Coefficient of variation
0.5 mg/kg 1.047
5.0 mg/kg 0.795
50 mg/kg 0.6566

Group A compound 54 [0.5mg/kg] : Number of items= 5
78.0 85.0 86.0 98.0 105.
Mean = 90.4
95% confidence interval for Mean: 52.65 thru 128.1
Standard Deviation = 10.9
Hi = 105. Low = 78.0
Median = 86.0
Average Absolute Deviation from Median = 8.00
CV = 1.047

Group B compound 54 [5 mg/kg]: Number of items= 5
120. 128. 165. 185. 196.
Mean = 159.
95% confidence interval for Mean: 121.1 thru 196.5
Standard Deviation = 33.8
Hi = 196. Low = 120.
Median = 165.
Average Absolute Deviation from Median = 26.6

Group C compound 54 [50mg/kg]: Number of items= 5
195. 240. 241. 360. 389.
Mean = 285.
95% confidence interval for Mean: 247.2 thru 322.7
Standard Deviation = 84.5
Hi = 389. Low = 195.
Median = 241.
Average Absolute Deviation from Median = 62.8

Group D compound 55 [0.5mg/kg]: Number of items= 5
56.0 65.0 73.0 89.0 92.0
Mean = 75.0
95% confidence interval for Mean: 37.25 thru 112.7
Standard Deviation = 15.4
Hi = 92.0 Low = 56.0
Median = 73.0
Average Absolute Deviation from Median = 12.0

Group E compound 55[5mg/kg]: Number of items= 5
85.0 102. 118. 120. 154.
Mean = 116.
95% confidence interval for Mean: 78.05 thru 153.5
Standard Deviation = 25.6
Hi = 154. Low = 85.0
Median = 118.
Average Absolute Deviation from Median = 17.4

Group F compound 55[50mg/kg]: Number of items= 5
121. 132. 145. 174. 254.
Mean = 165.
95% confidence interval for Mean: 127.5 thru 202.9
Standard Deviation = 53.4
Hi = 254. Low = 121.
Median = 145.
Average Absolute Deviation from Median = 35.0

Group G compound 56[0.5 mg/kg]: Number of items= 5
55.0 56.2 63.4 85.0 98.0
Mean = 71.5
95% confidence interval for Mean: 33.76 thru 109.3
Standard Deviation = 19.1
Hi = 98.0 Low = 55.0
Median = 63.4
Average Absolute Deviation from Median = 14.4

Group H compound 56 [5 mg/kg]: Number of items= 5
87.0 96.0 101. 112. 117.
Mean = 103.
95% confidence interval for Mean: 64.85 thru 140.3
Standard Deviation = 12.1
Hi = 117. Low = 87.0
Median = 101.
Average Absolute Deviation from Median = 9.20

Group I compound 56[50mg/kg] : Number of items= 5
89.0 114. 119. 125. 137.
Mean = 117.
95% confidence interval for Mean: 79.05 thru 154.5
Standard Deviation = 17.8
Hi = 137. Low = 89.0
Median = 119.
Average Absolute Deviation from Median = 11.8

Group J compound 58[0.5mg/kg]: Number of items= 5
99.0 112. 119. 152. 182.
Mean = 133.
95% confidence interval for Mean: 95.29 thru 170.8
Standard Deviation = 33.8
Hi = 182. Low = 99.0
Median = 119.
Average Absolute Deviation from Median = 24.6

Group K compound 58[5mg/kg]: Number of items= 5
186. 195. 219. 256. 271.
Mean = 225.
95% confidence interval for Mean: 187.6 thru 263.1
Standard Deviation = 37.1
Hi = 271. Low = 186.
Median = 219.
Average Absolute Deviation from Median = 29.1

Group L compound 58[50mg/kg]: Number of items= 5
312. 321. 385. 397. 418.
Mean = 366.
95% confidence interval for Mean: 328.8 thru 404.2
Standard Deviation = 47.2
Hi = 418. Low = 312.
Median = 385.
Average Absolute Deviation from Median = 36.3

Group M: Number of items= 5
90.1 96.6 102. 106. 121.
Mean = 103.
95% confidence interval for Mean: 65.48 thru 141.0
Standard Deviation = 11.6
Hi = 121. Low = 90.1
Median = 102.
Average Absolute Deviation from Median = 8.11

Group N: Number of items= 5
459. 485. 565. 624. 650.
Mean = 557.
95% confidence interval for Mean: 518.9 thru 594.3
Standard Deviation = 83.6
Hi = 650. Low = 459.
Median = 565.
Average Absolute Deviation from Median = 66.0