PHYTOCHEMICAL INVESTIGATION OF FOUR KENYAN PLANTS FROM FAMILIES VERBENACEAE AND ASTERACEAE FOR THEIR ANTIPLASMODIAL, ANTILEISHMANIAL AND ANTIOXIDANT ACTIVITIES

Francis Machumi

Department of Chemistry, University of Nairobi

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PhD Thesis by

Francis Machumi
Department of Chemistry, University of Nairobi

Supervised by

Prof. Jacob O. Midiwo
Department of Chemistry, University of Nairobi

Prof. Abiy Yenesew
Department of Chemistry, University of Nairobi

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DECLARATION

This is the original work of the author, except where reference is made, carried out at University of Nairobi between November 2007 and June 2010. It has never been submitted anywhere for award of any degree or diploma.

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Prof. Jacob O. Midiwo

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Prof. Abiy Yenesew

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malaria complications such as cerebral and pulmonary oedema, anemia and poor eyesight [Taoufiq *et al.*, 2006].

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Clerodendrum eriopyllum (aerial parts)	8.8	8.8

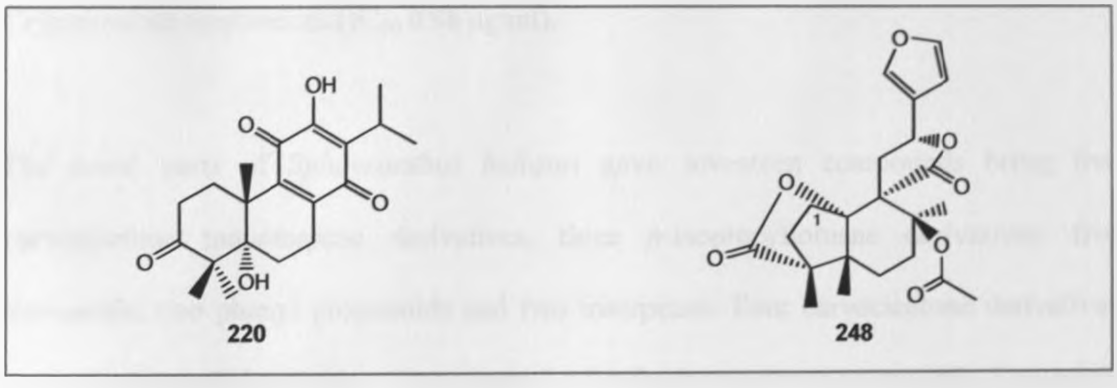
LIST OF ABBREVIATIONS AND ACRONYMS

ACT	Artemisinin based Combination Therapies
CC	Column Chromatography
CD	Circular Dichroism
COSY	Correlation Spectroscopy
DBE	Double Bond Equivalence
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ED_{50}	Effective Dose-50: Amount of material required to produce a specified effect on 50% of test animal
EI MS	Electron Impact Mass Spectrometry
FAB MS	Fast Atom Bombardment Mass Spectrometry
FT-IR	Fourier Transform Infrared Spectroscopy
GDP	Gross Domestic Product
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
HR-EIMS	High Resolution Electron Impact Mass Spectrometry
HSQC	Heteronuclear Single Quantum Correlation

IC_{50}	Inhibition Concentration-50: Concentration of substance that produce 50% inhibition of certain process
IR	Infrared
LC_{50}	Lethal Concentration-50: Concentration that kills 50% of test animal
MIC	Minimum Inhibition Concentation
MS	Mass Spectrometry
NAPRALERT	Natural Product Alert Database
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect Spectroscopy
pLDH	Plasmodium lactate dehydrogenase
PTLC	Preparative Thin Layer Chromatography
RBM	Roll Back Malaria
ROS	Reactive Oxygen Species
SAR	Structure Activity Relationship
TLC	Thin Layer Chromatography
UNICEF	United Nation Children Fund
UV	Ultra Violet
UV-VIS	Ultra Violet-Visible
WHO	World Health Organization

ABSTRACT

In an effort made to address the problem of malaria and its associated complications, four Kenyan plants used to treat malaria were investigated for compounds with anti-plasmodial and anti-oxidant activities. The plants are *Clerodendrum eriophyllum* (Verbenaceae), *Sphaeranthus bullatus* (Asteraceae), *Microglossa pyrifolia* (Asteraceae) and *Vernonia galamensis* (Asteraceae). A total of forty-two compounds were isolated from the plants, two being new, namely 12-hydroxy-8,12-abietadiene-3,11,14-trione (**220**) from *Clerodendrum eriophyllum* and 8-acetoxyisochiliolide lactone (**248**) from *Microglossa pyrifolia*. The antifungal, antibacterial and antileishmanial activity of compounds were also investigated.



220 248

Separation of compounds was carried out using chromatographic methods and their identification done by spectroscopic methods. The *in vitro* anti-plasmodial tests were done by a colorimetric assay that determined the parasitic lactate dehydrogenase (pLDH) activity in 96-well microplate. Antioxidants were analysed by UV-VIS spectrometry method employing the stable radical compound DPPH.

From the roots of *Clerodendrum eriophyllum*, fifteen compounds were isolated, being twelve abietane diterpenoids, two triterpenoids and a long chain ester of tyrosol. Three abietane diterpenoids had anti-plasmodial activities, these are taxodione (**222**), 6-hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**), with IC_{50} of 1.2 μg/ml, 1.8 μg/ml and 3 μg/ml respectively against chloroquine sensitive D6 strain, 1.2 μg/ml, 2.5 μg/ml and 4.8 μg/ml respectively against chloroquine sensitive W2 strains of *P. falciparum*. Anti-oxidant activities were observed on four abietane diterpenoids, taxodione (**222**), 6-hydroxysalvinolone (**226**), 6,16-dihydroxysalvinolone (**227**) and Nellionol (**228**) with IC_{50} of 31.5±0.8, 17.7±0.4, 20.5±0.5 and 10.2±0.5 respectively. Antifungal activitivies were observed on taxodione (**222**), 6-hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**), the best activity observed with taxodione (**222**) on *Cryptococcus neoformans* (IC_{50} 0.58 μg/ml).

The aerial parts of *Sphaeranthus bullatus* gave seventeen compounds being five carvotacetone monoterpene derivatives, three *p*-isopropyltoluene derivatives, five flavonoids, two phenyl propanoids and two triterpenes. Four carvotacetone derivatives had anti-plasmodial activities. These were 3,5,7-trihydroxycarvotacetone (**85**), 3-acetoxy-5,7-dihydroxycarvotacetone (**106**), 3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**) and 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**), with IC_{50} of 3.4 μg/ml, 0.6 μg/ml, 0.8 μg/ml and 1.4 μg/ml respectively against chloroquine sensitive D6 strain as well as 2.8 μg/ml, 0.7 μg/ml, 0.9 μg/ml and 2.0 μg/ml respectively against chloroquine sensitive W2 strains of *P. falciparum*. This is a first encounter of antiplasmodial activity on carvotacetone derivatives. The two phenyl propanoids, caffeic acid (**240**) and

coniferaldehyde (**241**) showed anti-oxidant activities, with IC_{50} of 2.6±0.3 and 14.1±0.6 respectively.

The aerial parts of *Microglossa pyrifolia* afforded six compounds. These were two rearranged clerodane diterpenoids, two steroids, one triterpenoid and one eudesman sesquiterpene. The compounds from this plant showed no activities.

The aerial parts of *Vernonia galamensis* gave seven compounds; five flavanols, one lignan and one benzenoid. The compounds showed no anti-plasmodial activitiy, but showed anti-oxidant activities, with IC_{50} of 6.0±0.2 for quercetin (**235**), 6.6±0.3 for quercetin 3-methylether (**244**), 10.4±0.5 for crysoplenol D (**238**), 9.3±0.4 for quercetin 3-*O*-*β*-galactopyranoside (**212**), 12.9 ± 0.4 for quercetin 3-*O*-*β*-rhamnopyranoside (**245**), 8.3 ± 0.4 for Syringic acid (**247**) and 21.0 ± 0.5 µg/ml for syringaresinol (**246**).

Anti-microbial activities were observed with the abietane diterpenoids taxodione (**222**), ferruginol (**224**), 6-hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**). Best antifungal activity was shown by taxodione (**222**) and 6-hydroxysalvinolone (**226**) (IC_{50} 0.58 and 0.96 µg/ml respectively against *C. neoformans*) where as best antibacterial activity was shown by ferruginol (**224**) on methicillin-resistant *Staphylococcus aureus* (MRS) (IC_{50} 0.96 µg/ml). The best antileishmanial activity was shown by taxodione (**222**) (IC_{50} 0.08 µg/ml against *L. donovani*).

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Plants are known to perform photosynthesis whereby they absorb carbon dioxide, water and light energy to produce monosaccharides, which in turn undergo further chemical metabolism to give an enormous variety of metabolites. The metabolites vary from primary metabolites which are essential for plant growth and development, to secondary metabolites, also referred to as Natural Products, which fulfill ecological functions, aiding interaction between plants and their biotic and abiotic environments. Secondary metabolites/natural products therefore serve as defense compounds against pathogens and herbivores, as flower pigments that attract pollinators, and as hormone or signal molecules [Osbourn and Lanzotti, 2009].

In addition to their function in plants, the natural products have great impact on human and animal life. Mankind has throughout the ages used natural products for flavors, fragrances, dyes and most important, for medicine. The use of natural products for treatment of parasitic diseases is well known and documented since ancient times, and stems from the fact that some of these natural products are biosynthesized as defense agents against plant pathogens [Kaur *et al.*, 2009]. Initially these medicines took a form of crude extracts contained in herbal formulations like teas, tinctures, poultices and powders [Balunas and Kinghorn, 2005]. With development of separation chemistry and pharmacological testing, the medicines now are made of active compounds isolated from the plants, or their synthetic equivalents.

Currently, with progress in synthetic and computational chemistry, some medicinal drugs are designed through molecular modeling process [Meyer *et al.*, 2000]. However, plants still constitute an important source of pharmaceuticals. Without them there would be a significant therapeutic deficit in several important clinical areas, such as, neurodegenerative disease, cardiovascular disease, most solid tumours and immune-inflammatory diseases such as rheumatoid arthritis [Nisbet and Mooret, 1997]. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world, including the treatment of malaria [Gurib-Fakim, 2006].

Malaria chemotherapy is one of the medicinal fields that are known to use pharmaceuticals originating from natural products research, examples are quinine from *Cinchona succiriba* and artemisinin from *Artemisia annua*. Many more compounds with significant anti-plasmodial activities have been isolated from plants and efforts are being made to develop some of these into future drugs [Kaur *et al*, 2009]. In underdeveloped communities, crude natural products made in the form of herbal formulations are still used for malaria therapy. Several plants with traditional use in curing malaria by different communities have been documented [Muthaura *et al.*, 2007a; Gessler *et al.*, 1995; Kvist *et al.*, 2006].

The use of natural product-derived drugs and drugs from other sources in combating malaria has however been faced with several challenges, including the emergence of drug resistance parasites to drugs world-wide as well as complications arising from oxidative stress during malaria infections. In fact, oxidative stress has been linked to several

malaria complications such as cerebral and pulmonary oedema, anemia and poor eyesight [Taoufiq *et al.*, 2006].

The emergence of resistance has made many of the first line drugs such as chloroquine not effective. The need for new drugs, preferably with new mode of action is therefore strongly felt. In this regard, several plants are being screened for anti-plasmodial activity. In collaboration programme between University of Nairobi and University of Mississippi, 140 plants were screened out of which four plants namely *Clerodendrum eriophyllum* (Verbenaceae), *Sphaeranthus bullatus* (Asteraceae), *Microglossa pyrifolia* (Asteraceae) and *Vernonia galamensis* (Asteraceae) showed activities shown in Table 1:1. These activities were interpretated as good to moderate activities, based on WHO guidence on activity, which stipulates that extracts with IC_{50} of less than 10 µg/ml have good activity, where as those with IC_{50} of 11-50 µg/ml have modearate activity.

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The supremacy of medicinal plants is encounted in widespectram of diseases. It is therefore becoming a common practice to test the extracts and compounds from medicinal plants against several pathogens. In this regard, this work has also investigated antileishmanial, antibacterial and antifungal properties.

1.2 Statement of the problem

Malaria is a major contributor to the global burden of disease and a significant impediment to socioeconomic development in poor countries. It is estimated that 300 to 660 million clinical attacks of malaria occur globally each year (Geissbühler *et al.*, 2007) and result in over a million deaths yearly (Hetzel *et al.*, 2007), over 80% of these deaths occurring in Africa (Geissbühler *et al.*, 2007). Most of fatalities are linked to oxidative stress, caused by eleveted production of radicals in host cells [Taoufiq *et al.*, 2006]. Efforts to combat the disease are hampered by growing resistance of malaria parasites to the readily available drugs. There is, therefore, a need for continued efforts in the search of antioxidants and compounds that are active against malaria parasites, that can be developed to new and more effective drugs.

1.3 Justification of the research

Research on medicinal plants represents a major strategy for the discovery of compounds that can be developed to new anti-malarial drugs. The most important antimalarial compounds that have been isolated from traditional medicinal plants are quinine (**1**) from *Cinchona succiriba* (Rubiaceae), and artemisinin (**4**) from the *Artemesia annua*

(Asteraceae). Both compounds have or are being used as molecular templates in the syntheses of more potent antimalarial drugs.

The plants investigated under this study [*Clerodendrum eriophyllum* (Verbenaceae), *Sphaeranthus bullatus* (Asteraceae), *Microglossa pyrifolia* (Asteraceae) and *Vernonia galamensis* (Asteraceae)] are used traditionally to treat malaria in Kenya [Beentje, 1994; Kokwaro, 1976] and their extracts have shown anti-plasmodial activities [Midiwo *et al.*, unpublished results]. This makes them good candidates for investigation for compounds with anti-malarial compounds.

The previous phytochemical work on these plants leave a room for further research since not all parts were studied for compounds present. For *Clerodendrum eriopyllum*, no phytochemical work has been done at all. Moreover, compounds isolated from these plants have never been tested for antimalarial and anti-oxidant activities. It is therefore important that the plants should be investigated further for metabolites and tested for anti-plasmodial and anti-oxidant activities.

1.4 Objectives

1.4.1 General objective

To identify anti-plasmodial and anti-oxidant lead structures from plants used in traditional medicine to cure malaria.

1.4.2 Specific objectives

1. To isolate metabolites from four Kenyan plants used traditionally to treat malaria; namely *Clerodendrum eriopyllum*, *Sphaeranthus bullatus*, *Microglossa pyrifolia* and *Vernonia galamensis* by use of chromatographic methods.
2. To elucidate the structures of the isolated metabolites using spectroscopic methods.
3. To establish the *in vitro* anti-plasmodial activities of pure compounds and extracts
4. To establish the radical scavenging activities of pure compounds
5. To establish antimicrobial and antileshmanial activities of the compounds
6. To predict structure/activity relationships for anti-plasmodial and ant-oxidant activities of some compounds isolated.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background on malaria

Malaria is a global disease that is predominant in the tropics and caused by blood parasites *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium vivax* [Odugbemi *et al*., 2007]. The parasites are transmitted to humans by female *Anopheles* mosquito. Clinical malaria is manifested by a range of symptoms such as fever, vomiting, joint pain and convulsions [Nkuo-Akenji and Menang, 2005]. Besides contributing to over a million deaths yearly, malaria is known to be a cause of anaemia and its various complications, miscarriages, brain damage, decreased cognition and irreversible disabilities [Rugemalila *et al*., 2006].

In terms of economy, malaria is known to be both a disease and a cause of poverty. It is estimated that malaria is responsible for a GDP economic growth penalty of up to 1.3% per year in some African countries [Sachs and Malaney, 2002]. When compounded over the years, this penalty leads to substantial differences, totaling US$ 12 billion annually in GDP between countries without malaria and countries with malaria, and severely restrains the economic growth of entire Africa region [Kamau, 2006].

Efforts to overcome the problem are hindered by two obstacles. One is the growing resistance of malaria parasite *Plasmodium falciparum* to chloroquine and other commonly used cheap synthetic drugs, as well as the growing resistance of the vector *Anopheles* to DDT and other insecticides [Bilia, 2006]. The second obstacle is limited

availability, coupled with higher cost and greater toxicity of alternative drugs [Saidu *et al.*, 2000].

In recognition of the gravity of the malaria problem, WHO and UNICEF launched the Roll Back Malaria (RBM) partnership in 1998, with the aim of reducing malaria burden by at least 50% by the year 2010 through application of evidence based interventions and strengthening health delivery services [Kamau, 2006]. This was supported by the Abuja declaration in 2000 where African heads of state agreed on the concerted effort to reduce malaria burden on the continent and endorsed the goals of RBM partnership of halving the number of malaria deaths by the year 2010 [Hetzel *et al.*, 2007]. Currently, concerted efforts promote three core malaria control interventions: use of insecticide treated nets, intermittent preventive treatment during pregnancy and infancy, and effective treatment of clinical cases with artemisinin based combination therapies (ACT) [Kamau, 2006].

In Kenya, like other tropical countries, malaria is endemic and resistance of malaria parasites to readily available and cheap drugs like chloroquine is growing fast. According to the Ministry of Health, twenty four million Kenyans are at risk of malaria infections and majority cannot afford the costs of the adopted artemisinin based combination therapy. The government therefore found it necessary to provide free ACT therapy in health facilities alongside expansion of healthcare provision [Muthaura *et al.*, 2007b; World Report, 2007]. Despite this effort however, many regions remain underserved and subsequently the communities use herbal remedies and other cheaply available alternatives [Muthaura *et al.*, 2007b; Kirira *et al.*, 2006].

2.2 The Use of Plants and Natural Products in Chemotherapy of Malaria

Plants and natural products have had a great contribution to the fight against malaria since time immemorial. In Kenya and other developing countries, the use of herbal remedies in treatment of malaria is well known as evidenced by overwhelming literature on plants used traditionally to cure malaria [Kokwaro, 1976; Muregi *et al.*, 2003; Kirira *et al.*, 2006; Koch *et al.*, 2005; Muthaura *et al.*, 2007a; Muthaura *et al.*, 2007b]. Apart from their use in traditional medicine, plants and natural products have also contributed to modern treatment of malaria, being the source of some commonly used anti-malarial drugs like quinine (**1**) and artemisinin (**2**) from Cinchona and Artemisia respectively and their synthetic modifications and analogues (Figure 2.1).

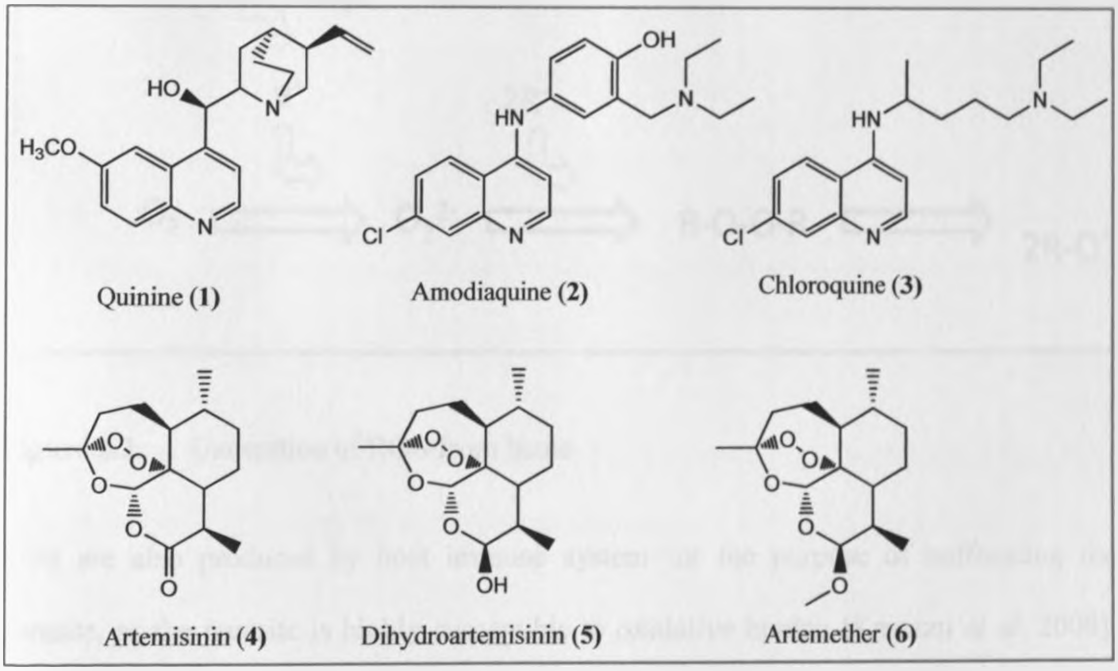


Figure 2.1: Some of the Natural Products and synthetic modifications used against malaria.

2.3 Malaria and oxidative stress

Malaria is normally accompanied by oxidative stress caused by elevated production of reactive oxygen species (ROS). Such oxidative stress will lead to tissue damages and are associated with several pathological phenomenon such as cerebral and pulmonary oedema, poor eyesight, atherosclerosis, cardiac ischemies, rheumatic diseases and cancer [Bahorun *et al.*, 1996]. The major cause of elevated ROS production is heme, a byproduct of hemoglobin digestion degradation by *Plasmodium*, whereby the iron II in heme is oxidized to iron III releasing an electron that is used to ionize oxygen molecule and subsequently form peroxide which is easily broken to radicals (Figure 2.2).

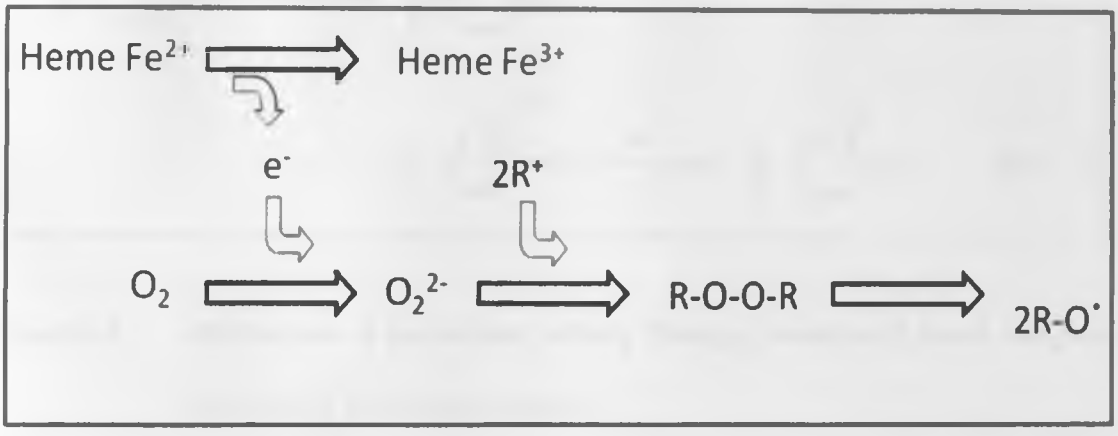


Figure 2.2: Generation of ROS from heme

ROS are also produced by host immune system for the purpose of suffocating the parasite, as the parasite is highly susceptible to oxidative burden [Kawazu *et al*, 2008]. Malaria parasites are therefore equipped with anti-oxidant defenses that are meant to establish redox equilibrium for their survival. Such defenses are the ones targeted in malaria control strategies, some of the anti-malarials like chloroquine and artemether are known to act by increasing the production of ROS [Taoufiq *et al.*, 2006].

The effect of ROS to malaria patient therefore, though beneficial as it suffocates the parasite, it is also detrimental since it leads to the damage of some cells and hence bringing about more complications to the patient. It is therefore important that the cells are protected from oxidative burden through the use of effective antioxidants.

The antioxidants act by either suppressing the enzymes responsible for superoxide production, or chelate metal ions that induce free radical formation, or reduce the radicals by donation of hydrogen atoms (Figure 2.3) [Pieta, 2000].

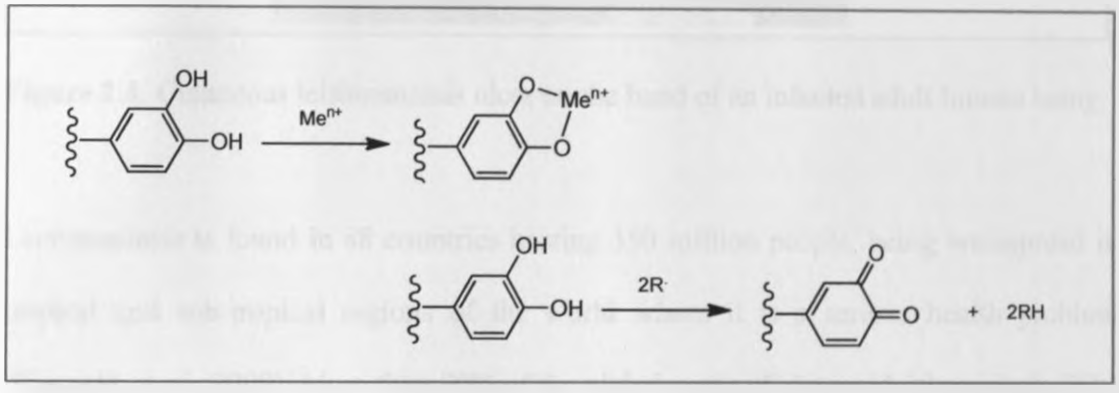


Figure2.3: Mechanisms of antioxidant activity through chelation of metal ions and reduction by hydrogen donation.

Antioxidants are usually taken in through diet of plants and plant products. Plants therefore make a good target to hunt for antioxidants.

2.4 Leishmaniasis

Leishmaniasis is a parasitic disease transmitted by a bite of some species of sand flies which infect the blood with parasites of the genus *Leishmania* [Tonui 2006]. Two common forms of leishmaniasis are known; cutaneous leishmaniasis (CL) which causes

sore (ulcers) at the bite site (Figure 2.4) and visceral leishmaniasis (VL) which affects vital organs.

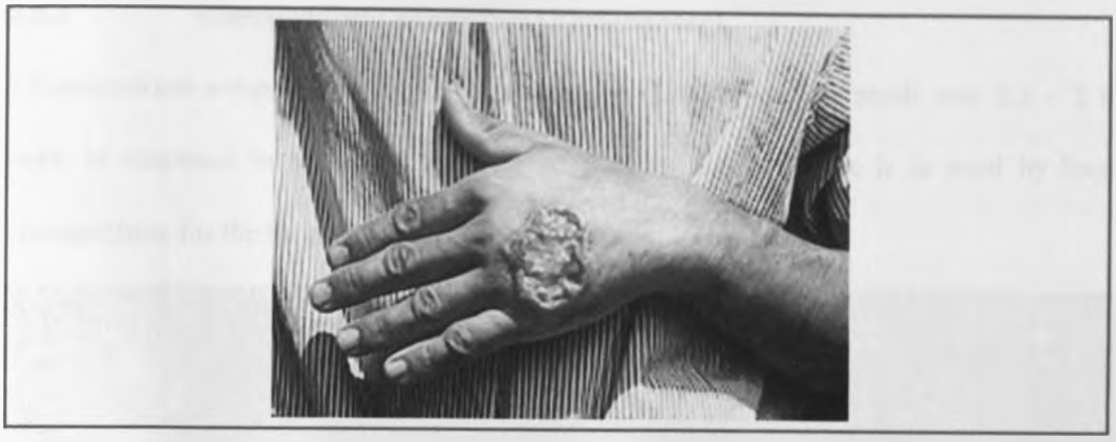


Figure 2.4: Cutaneous leishmaniasis ulcer on the hand of an infected adult human being.

Leishmaniasis is found in 88 countries hosting 350 million people, being widespread in tropical and sub-tropical regions of the world where it is a serious health problem (Kigondu *et al.*, 2009). More than 90% of the global cases of visceral leishmaniasis (VL) occur in India, Bangladesh, Nepal, Brazil and Africa (particularly East and North Africa including Sudan). Globally, the number of new cases of cutaneous leishmaniasis (CL) and VL is estimated at 1.5million and 500,000 annually, respectively. People of all ages are at risk of infection with leishmaniasis if they live or travel where the disease is endemic. In Kenya, the disease has been reported in Baringo, Kitui, Machakos, Meru, West Pokot, Elgeyo Marakwet and Turkana districts, which are knownto be endemic foci for kala-azar (VL) (Kigondu *et al.*, 2009). Currently, the recommended drugs for both VL and CL are pentavalent antimonials: sodium stibogluconate (pentosam®) and meglumine antimoniate (glucantime®). Both drugs require long courses of parenteral administration and have toxic side effects (Kigondu *et al.*, 2009).

2.5 Ethnobotanical, ethnopharmacological and phytochemical information on selected plants

2.5.1 *Clerodendrum eriopyllum* (*Verbenaceae*)

Clerodendrum eriophyllum Gürke [Verbenaceae (Lamiaceae)], a small tree 0.5 – 2 m high, is scattered in the dry bushlands of Eastern Kenya where it is used by local communities for the treatment of malaria [Kokwaro, 1976].

Figure 2.4: *Clerodendrum eriophyllum*

The plant has no record of previous phytochemical analysis. However, the methanol extract of its root bark is reported to have good *in vitro* activity against *Plasmodium falciparum* D6 and W2 clones (IC_{50} 9.51- 10.56 μg/mL); its methanol and aqueous

extracts exhibited significant *in vivo* chemosuppression (i.e., 90.1% and 61.5%, respectively) against *P. berghei* infected mice treated intraperitoneally at a dose of 100 mg/kg body weight [Muthaura *et al.*, 2007c].

The genus, *Clerodendrum* L. (Verbenaceae) is widely distributed in tropical and subtropical regions of the world. It is comprised of small trees, shrubs and herbs making more than five hundred species and varieties [Bashwira and Hootele, 1988]. The genus has a wide spectrum of folk and indigenous medicinal uses, with more than fifty species reported in the treatment of diseases like malaria, fevers, inflammatory diseases, venereal infections, skin diseases and internal body aches [Shrivastava and Patel, 2007].

Clerodendrum species used traditionally in treatment of malaria are *C. colebrookianum* whose leaves are used for fever and malaria in India [Rao and Jamir, 1982], *C. cyrtophyllum* which is used for malaria and common colds in China [Tian *et al.*, 1993], *C. eriophyllum*, *C. johnstonii* and *C. myricoides* used for malaria in Kenya [Kokwaro, 1976], *C. heterophyllum* used for malaria and fever in Reunion Islands [Vera *et al.*, 1990] as well as *C. indicum* and *C.serratum* used in China [Pei, 1985].

Other species of the genus used for malaria are *C. multiflorum* whose leaves are used for fever and malaria in India [Sikarwar and Kaushik, 1993], *C. pleiosciadium* whose leaves are used in Tanzania [Gessler *et al.*, 1994], *C. splendens* used for cerebral malaria in Guinea [Akendengue, 1992] and *C. viscosum* whose leaves and roots are used for malaria and fever in India [Bhandary *et al.*, 1995]. The extracts of *C. inerme* and *C. serratum*

have been found to posses anti-oxidant activities [Narayanan *et al.*, 1999; Masuda *et al.*, 1999].

Other than their therapeutic use, some of the species of this genus such as *C. inerme*, *C. thomsonae*, *C. indicum* and *C. speciosum* are also cultivated and used as ornamental plants [Shrivastava and Patel, 2007].

Efforts have been made by various researchers to isolate and identify biologically active principles and other major chemical constituents from various species of the genus. Compounds reported include abietane diterpenoids, *ent*-clerodane diterpenoids, iridoid glucosides, phenylpropanoid glycosides, macrocyclic alkaloids, flovones, steroids and triterpenes.

Previously reported abietane diterpenoids (Figure 2.5) are bungone A (**7**), bungone B (**8**) and sugiol (**9**) from *C. bungei* [Fan *et al.*, 1999a]; cyrtophyllone A (**12**) and cyrtophyllone B (**10**) from *C. cyrtophyllum* [Tian *et al.*, 1993]; mandarone A (**11**), mandarone B (**13**), mandarone D (**18**), mandarone E (**14**), mandarone F (**15**), mandarone G (**16**) and mandarone H (**17**) from *C. mandarinorum* [Fan et al., 1999b]; uncinatone (**19**) from *C. uncinatum* [Dorsaz *et al.*, 1985]; clerodendrone (**20**) from *C. indicum* [Ravindranath *et al.*, 2003] and royleanone (**21**) from *C. inerme* [Singh and Prakash 1983].

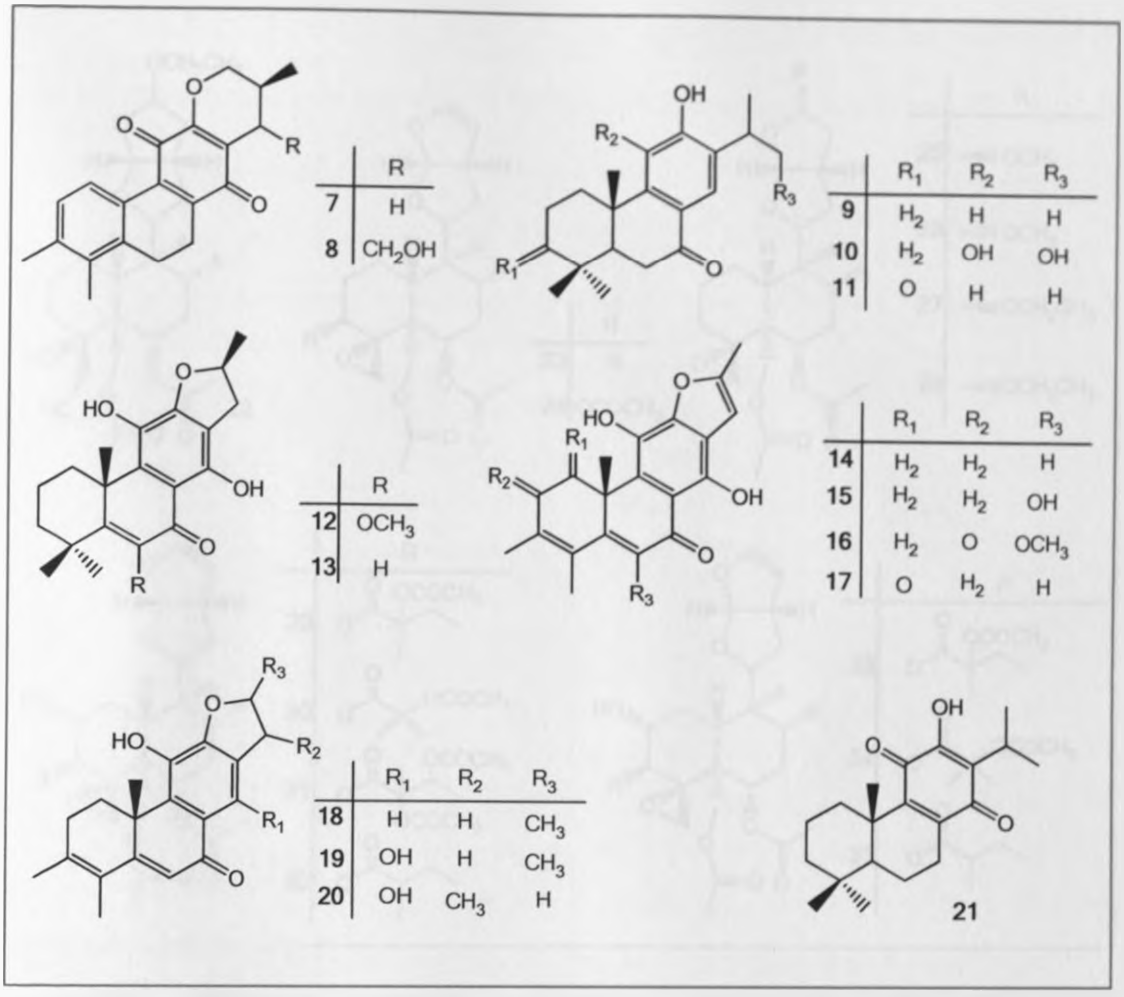


Figure 2.5: Abietane diterpenoids previously reported from the genus *Clerodendrum*

Reported *ent*-clerodane diterpenoids (Figure 2.6) from the genus *Clerodendrum* are clerodiol (**22**), clerodin (**23**), clerodinin A (**25**), clerodinin B (**26**), clerodinin C (**27**) and clerodinin D (**28**) from *C. brachyanthum* [Lin *et al.*, 1989a, 1989b]; caryoptin (**24**) from *C. calamitosum* [Vigneron 1978]; clerodendrin A (**29**), clerodendrin B (**33**), clerodendrin C (**34**), clerodendrin D (**35**), clerodendrin E (**30**), clerodendrin F (**31**) and clerodendrin G (**32**) from *C. trichotomum* [Nishida *et al.*, 1989].

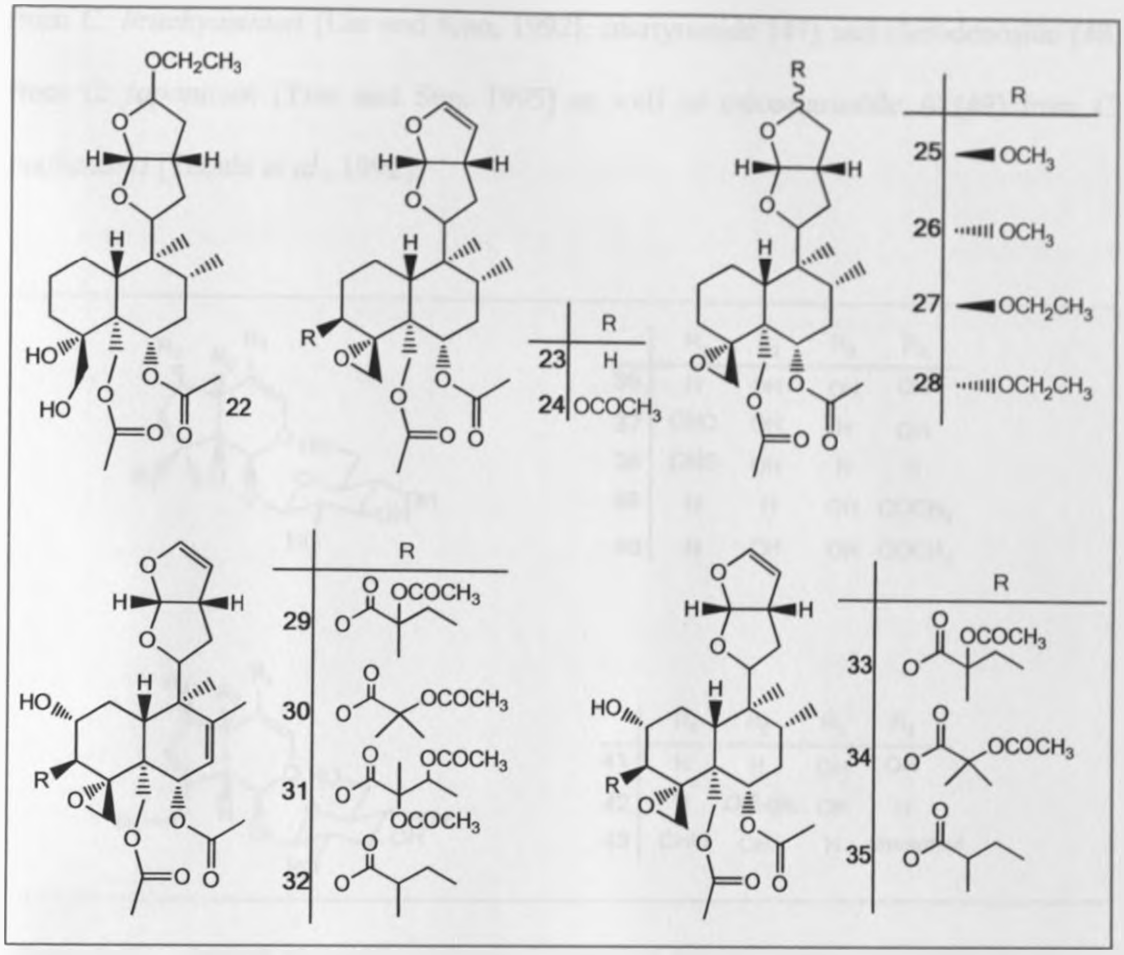
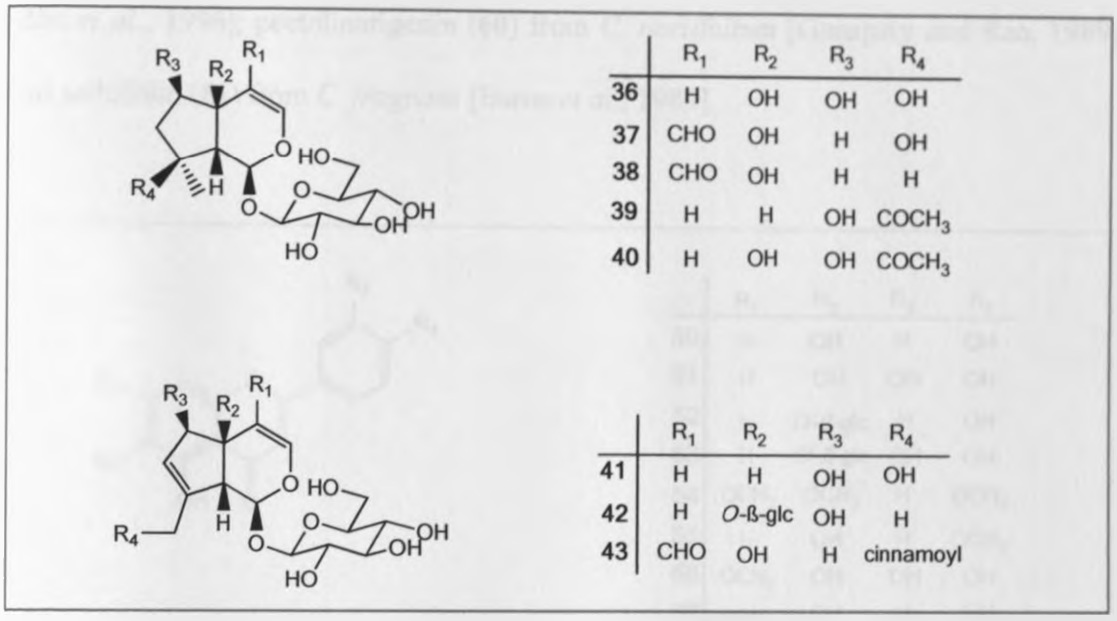


Figure 2.6: Previously reported *ent*-clerodane diterpenoids from the genus *Clerodendrum*

The iridoid glucosides previously reported (Figure 2.7) are harpagide (**36**) isolated from *C. indicum* and *C. colebrookianum* [Jacke and Rimpler, 1983]; euphroside (**37**) and plantarenaloside (**38**) from *C. incisum* [Stenzel *et al.*, 1986]; ajugoside (**39**), reptoside (**40**), aucubin (**41**) and melittoside (**42**) from *C. thomsonae* [Jacke and Rimpler, 1983] as well as serratoside B (**43**) from *C. serratum* [Yang *et al.*, 2000a].

Phenylpropanoid glycosides isolated from the genus (Figure 2.8) are acteoside (**44**) and leucosceptoside A (**45**) from *C. inerme* [Kanchanapoom *et al.*, 2001]; brachynoside (**46**)

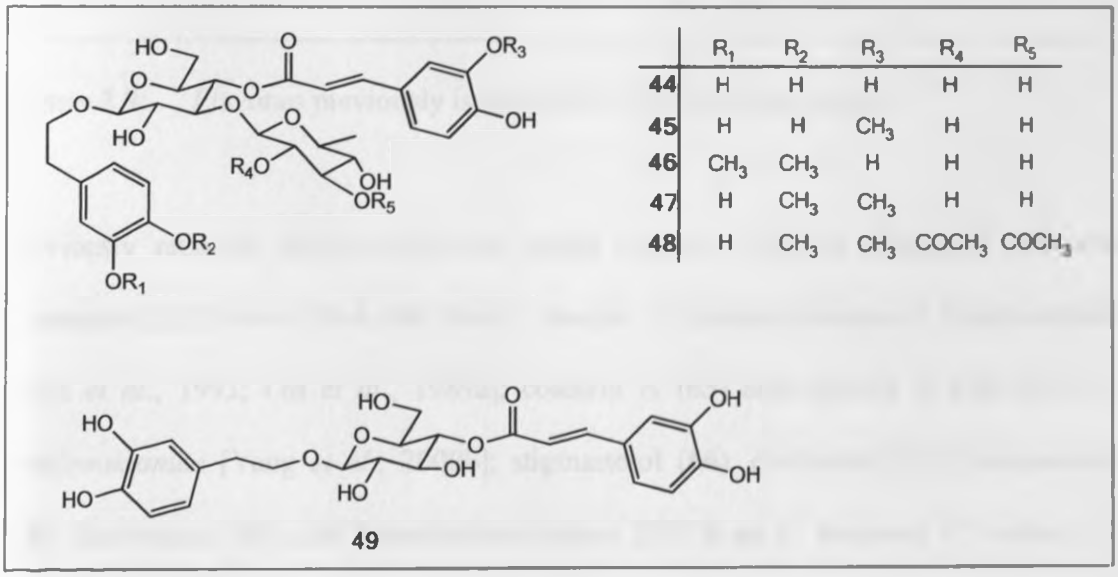
from *C. brachyanthum* [Lin and Kuo, 1992]; martynoside (**47**) and clerodenoside (**48**) from *C. japonicum* [Tian and Sun, 1995] as well as calceolarioside A (**49**) from *C. buchananii* [Taoubi *et al.*, 1992].



	R_1	R_2	R_3	R_4
36	H	OH	OH	OH
37	CHO	OH	H	OH
38	CHO	OH	H	H
39	H	H	OH	$COCH_3$
40	H	OH	OH	$COCH_3$

	R_1	R_2	R_3	R_4
41	H	H	OH	OH
42	H	*O*-ß-glc	OH	H
43	CHO	OH	H	cinnamoyl

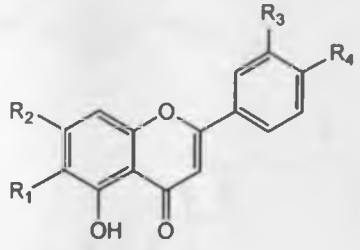
Figure 2.7: Iridoid glucosides previously reported from the genus *Clerodendrum*



	R_1	R_2	R_3	R_4	R_5
44	H	H	H	H	H
45	H	H	CH_3	H	H
46	CH_3	CH_3	H	H	H
47	H	CH_3	CH_3	H	H
48	H	CH_3	CH_3	$COCH_3$	$COCH_3$

Figure 2.8: Previous phenylpropanoid glycosides from the genus *Clerodendrum*

Flavones isolated from *Clerodendrum* species (Figure 2.9) are apigenin (**50**), luteolin (**51**), cosmosiin (**52**), cynacoside (**53**), salvigenin (**54**) and acacetin (**55**) from *C. inerme* [El-Shamy *et al.*, 1996; Raha *et al.*, 1989]; eupafolin (**56**), hispidulin (**57**) and seutellarein (**58**) from *C. indicum* [Tian and Sun, 1999]; cirsimaritin (**59**) from *C. mandarinorum* [Zhu *et al.*, 1996]; pectolinarigenin (**60**) from *C. neriifolium* [Ganapaty and Rao, 1989] and sorbifolin (**61**) from *C. fragrans* [Barua *et al.*, 1989].



	R_1	R_2	R_3	R_4
50	H	OH	H	OH
51	H	OH	OH	OH
52	H	*O*-*ß*-glc	H	OH
53	H	*O*-*ß*-glc	OH	OH
54	OCH_3	OCH_3	H	OCH_3
55	H	OH	H	OCH_3
56	OCH_3	OH	OH	OH
57	OCH_3	OH	H	OH
58	OH	OH	H	OH
59	OCH_3	OCH_3	H	OH
60	OCH_3	OH	H	OCH_3
61	OH	OCH_3	H	OH

Figure 2.9: Flavones previously isolated from *Clerodendrum* species

Previously reported steroids from this genus (Figure 2.10) are clerosterol (**62**) and stigmasta-5,22,25-trien-3*β*-ol (**64**) from *C. bungei*, *C. cyrtophyllum* and *C. brachyanthum* [Tian *et al.*, 1993; Lin *et al.*, 1989a]; colebrin A (**63**) and colebrin B (**65**) from *C. colebrookianum* [Yang *et al.*, 2000b]; stigmasterol (**66**), cholesterol (**67**), campesterol (**68**), cholestanol (**69**) and 24-methylcholestanol (**70**) from *C. fragrans*, *C. inerme*, *C. infortunatum*, *C. scandens* and *C. serratum* [Akihisa *et al.*, 1989].

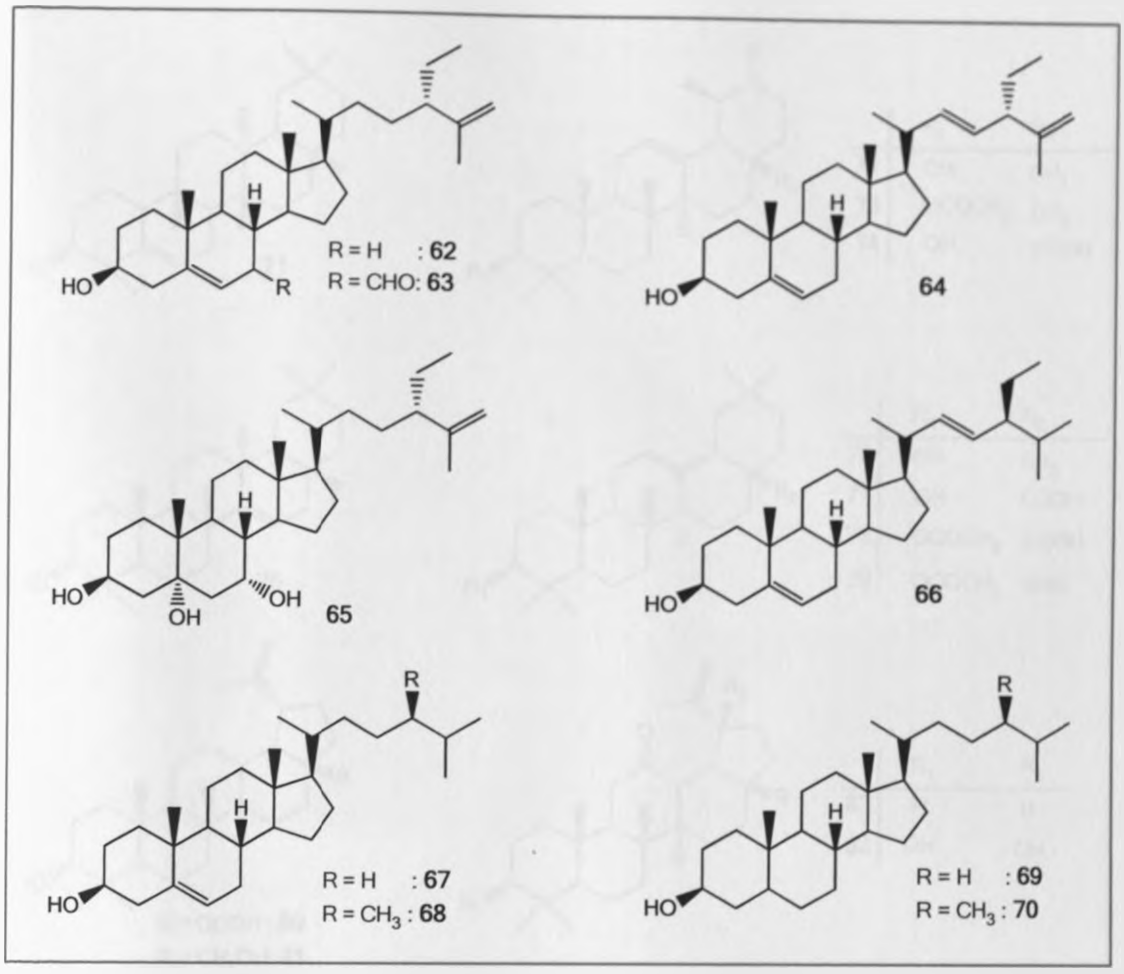


Figure 2.10: Previously reported steroids from *Clerodendrum* species

Triterpenes reported from the genus *Clerodendrum* (Figure 2.11) are glutinol (**71**) from *C. brachyathum* [Lin *et al.*, 1989a]; α-amyrin (**72**), 3-acetoxy-α-amyrin (**73**), β-amyrin (**76**) and betulin (**81**) from *C. inerme* [Singh *et al.*, 1983]; Ursolic acid (**74**) from *C. japonicum* [Tian and Sun, 1995]; taraxerol (**75**), betulinic acid (**80**) and clerodone (**82**) from *C. bungei* [Yang *et al.*, 2002; Dong *et al.*, 1999; He *et al.*, 1997]; 3-acetoxyoleanoic acid (**78**) and 3-acetoxyoleanoic aldehyde (**79**) from *C. indicim* [Ravindranath *et al.*, 2003] and Clerodolone (**83**) *C. cyrtophyllum* [Tian *et al.*, 1993] and *C. colebrookianum* [Joshi *et al.*, 1979].

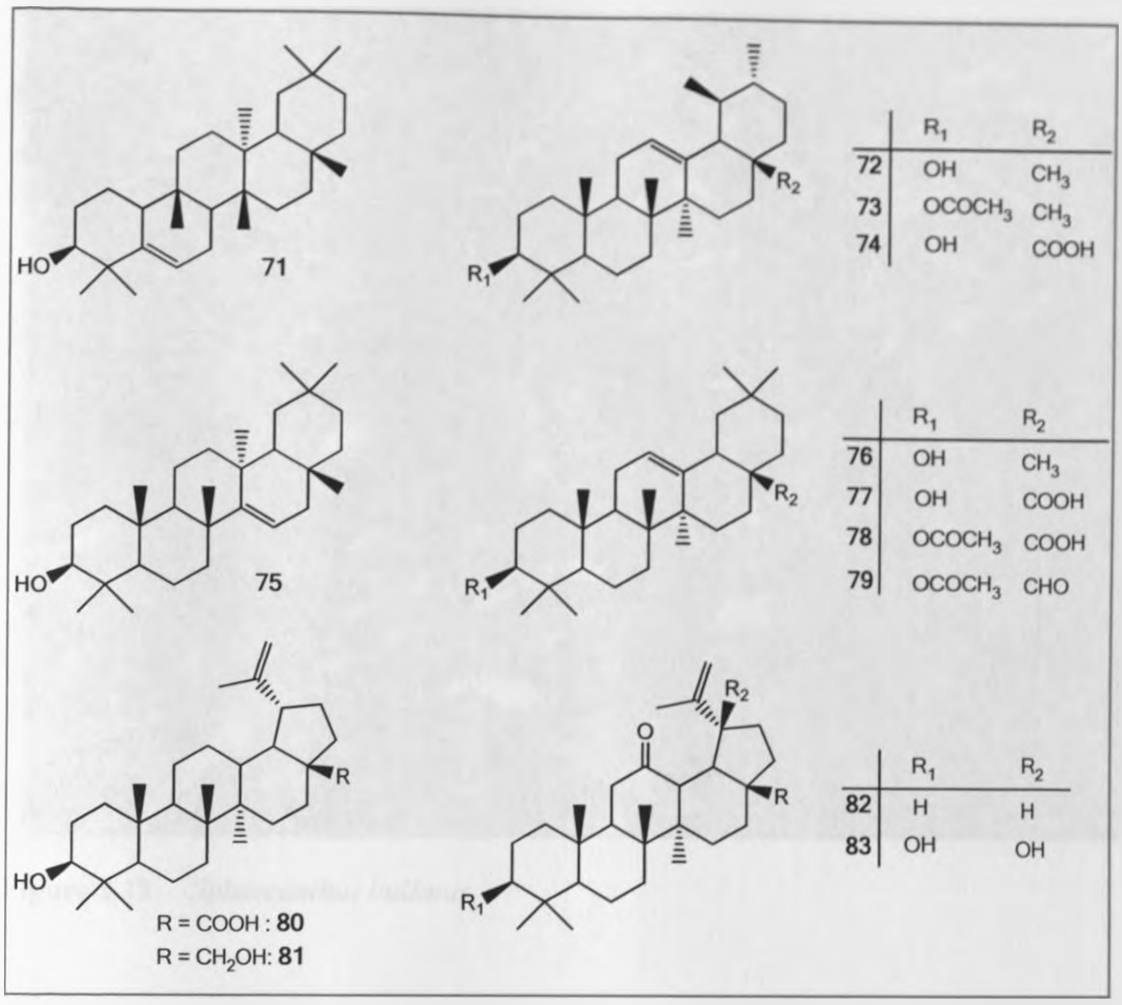


Figure 2.11: Previously reported triterpenes from *Clerodendrum* species

2.5.2 *Sphaeranthus bullatus* (*Asteraceae*)

Sphaeranthus bullatus is an annual or perennial herb, 0.2–1 m tall with aromatic leaves and purple flowers, native to tropical East Africa [Beentje, 1994]. The leaves of this plant are used against malaria [Kokwaro, 1976] and the 1:1 MeOH/CH_2Cl_2 extract of its aerial parts showed antiplasmodial activity [Midiwo *et al.*, unpublished results].

Figure 2.12: *Sphaeranthus bullatus.*

Previously reported phytochemicals from this plant are carvotacetone derivatives (**84 – 90** and **94**), derivatives of *p*-cymene (**91 – 93**), thiophene derivatives (**95 – 96**), a sesquiterpene α-humulene (**97**) and a triterpene squalene (**98**) [Jakupovic *et al.*, 1990] (Figure 2.13).

The genus comprises of about forty species distributed mainly in tropical areas of Africa, Southern Asia and Australia and [Jakupovic *et al.*, 1990].

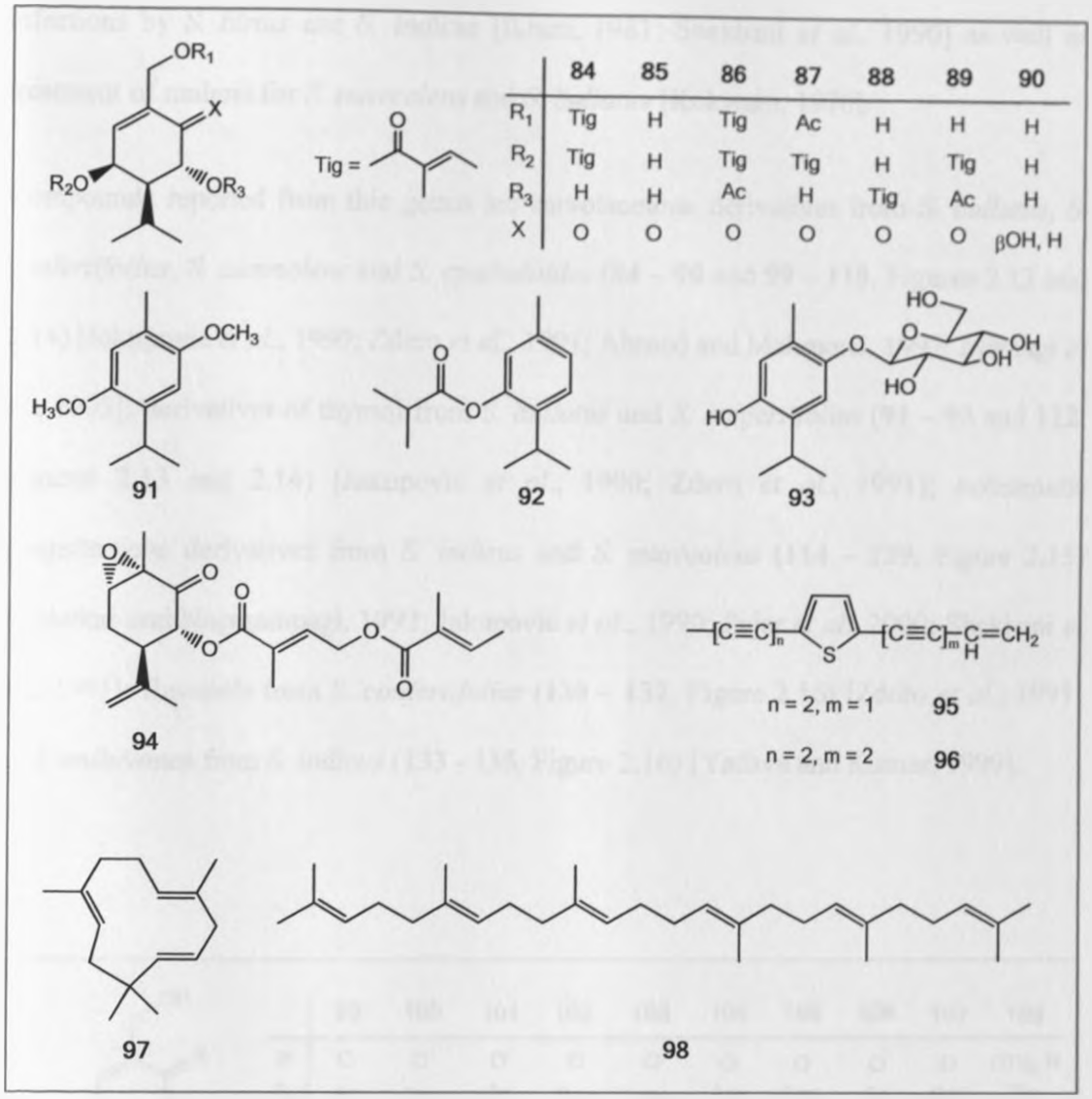
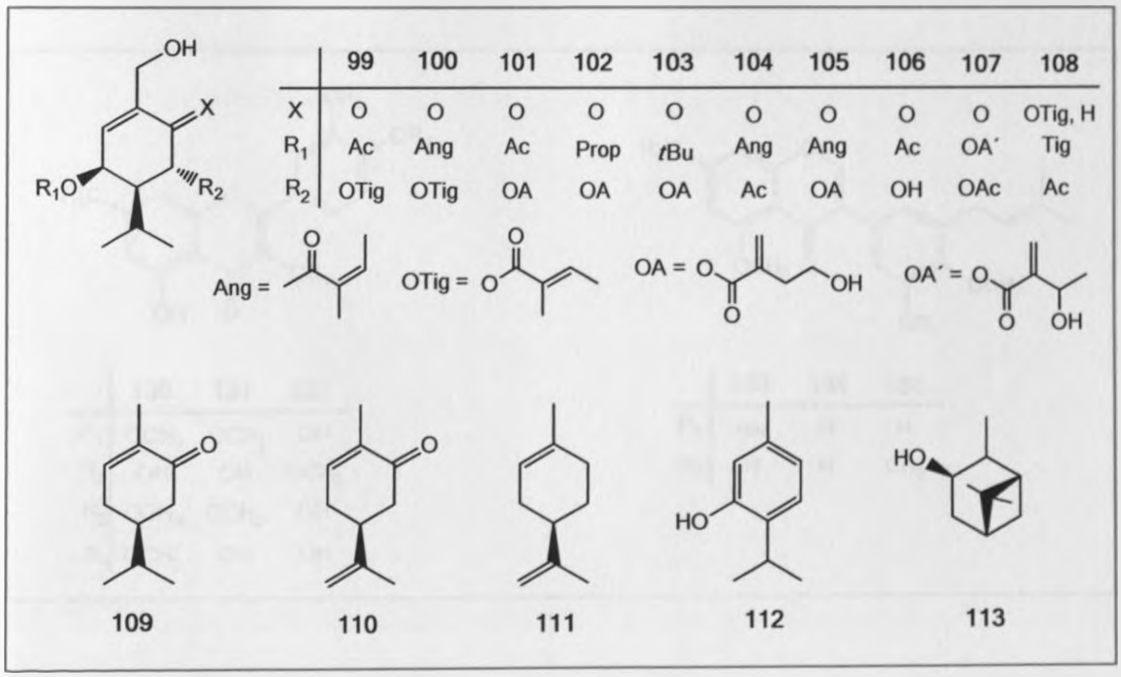


Figure 2.13: Previous compounds from *Sphaeranthus bullatus*.

Several species of the genus are used traditionally as arbortifacients or anti-conceptions and include *S. africanus* [Lal and Lata, 1980], *S. cyanthuloides* [Watt and Breyer-Brandwijk, 1962], *S. gomphrenoides* [Kokwaro, 1976], *S. indicus* [Sharma *et al.*, 1992] and *S. suaveolens* [Amico, 1977]. Other ethnomedical uses of *Sphaeranthus* species includes treatment of stomach pains for *S. angolensis*, *S. indicus* and *S. senegalensis* [Samuelsson *et al.*, 1992; Nagaraju and Rao, 1990; Manandhar, 1993], treatment of skin

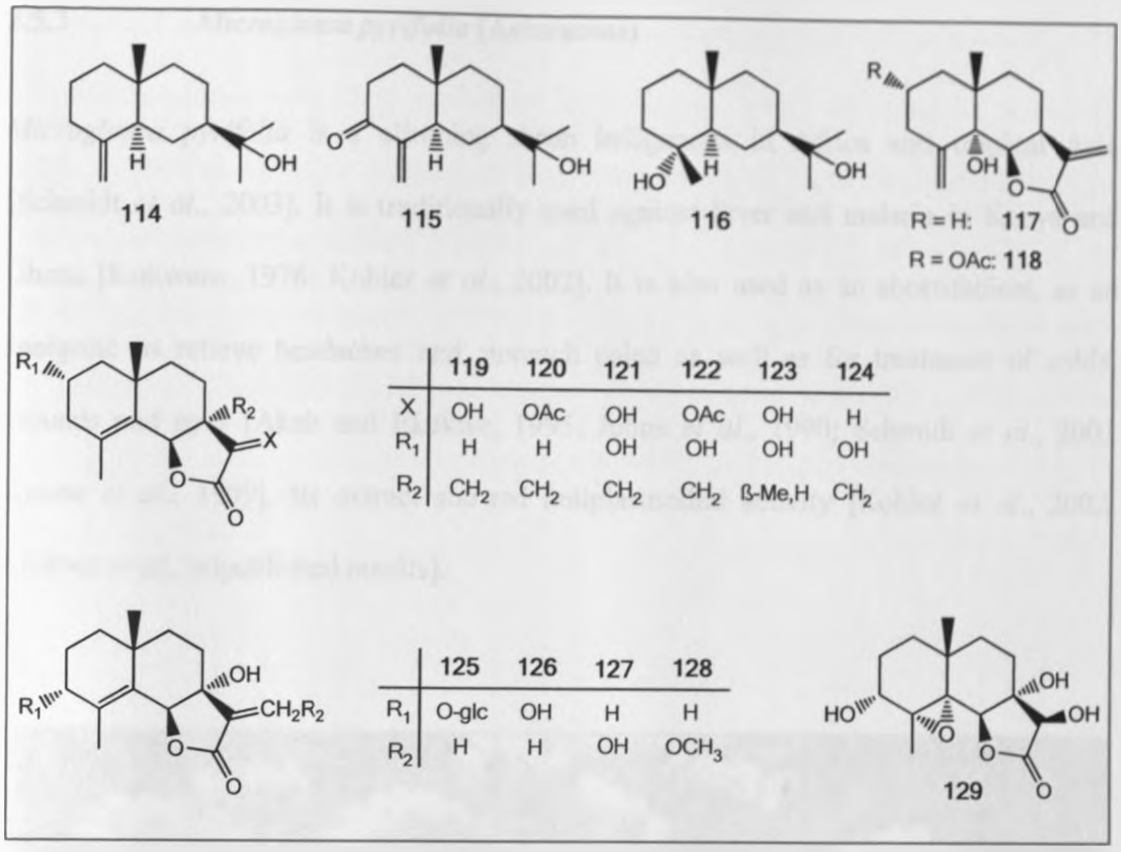
infections by *S. hirtus* and *S. indicus* [Ikram, 1981; Shekhani *et al.*, 1990] as well as treatment of malaria for *S. suaveolens* and *S. bullatus* [Kokwaro, 1976].

Compounds reported from this genus are carvotacetone derivatives from *S. bullatus*, *S. confertifolius*, *S. suaveolens* and *S. cyathuloides* (**84 – 90** and **99 – 110**, Figures 2.13 and 2.14) [Jakupovic *et al.*, 1990; Zdero *et al.*, 1991; Ahmed and Mahmoud, 1997; Mwangi *et al.*, 1995]; derivatives of thymol from *S. bullatus* and *S. confertifolius* (**91 – 93** and **112**, Figures 2.13 and 2.14) [Jakupovic *et al.*, 1990; Zdero *et al.*, 1991]; eudesmane sesquiterpene derivatives from *S. indicus* and *S. suaveolens* (**114 – 129**, Figure 2.15) [Rojatkar and Nagasampagi, 1992; Jakupovic *et al.*, 1990; Pujar *et al.*, 2000; Shekhani *et al.*, 1991]; flavanols from *S. confertifolius* (**130 – 132**, Figure 2.16) [Zdero *et al.*, 1991] and isoflavones from *S. indicus* (**133 - 135**, Figure 2.16) [Yadava and Kumar, 1999].



	99	100	101	102	103	104	105	106	107	108
X	O	O	O	O	O	O	O	O	O	OTig, H
R_1	Ac	Ang	Ac	Prop	*t*Bu	Ang	Ang	Ac	OA´	Tig
R_2	OTig	OTig	OA	OA	OA	Ac	OA	OH	OAc	Ac

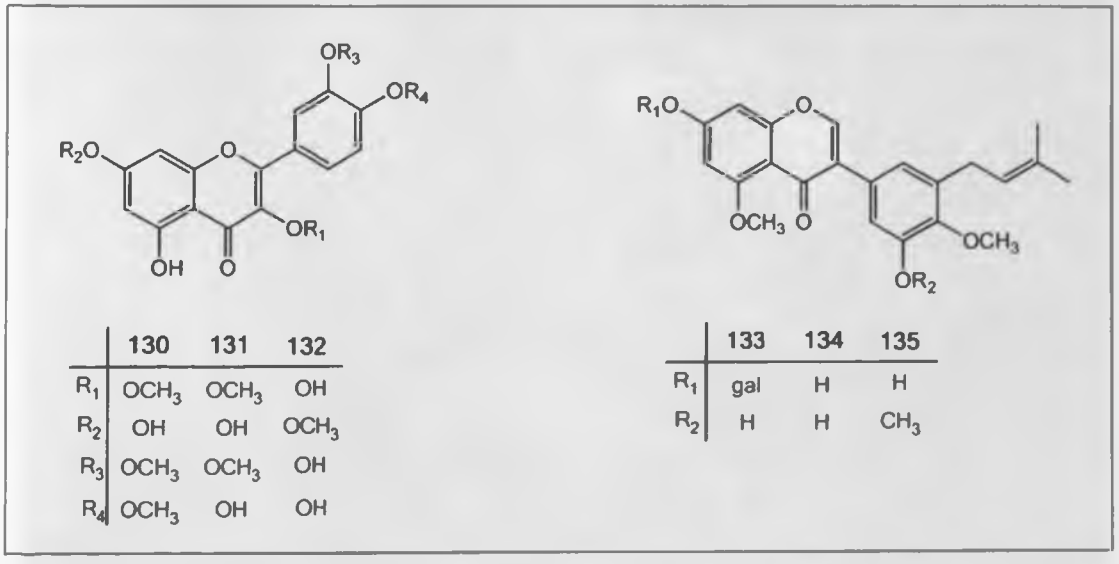
Figure 2.14: Monoterpenes from *Sphaeranthus* species.



	119	120	121	122	123	124
X	OH	OAc	OH	OAc	OH	H
R_1	H	H	OH	OH	OH	OH
R_2	CH_2	CH_2	CH_2	CH_2	ß-Me,H	CH_2

	125	126	127	128
R_1	O-glc	OH	H	H
R_2	H	H	OH	OCH_3

Figure 2.15: Eudesmane sesquiterpnoids from *S. indicus* and *S. suaveolens*



	130	131	132
R_1	OCH_3	OCH_3	OH
R_2	OH	OH	OCH_3
R_3	OCH_3	OCH_3	OH
R_4	OCH_3	OH	OH

	133	134	135
R_1	gal	H	H
R_2	H	H	CH_3

Figure 2.16: Flavanols and isoflovones from *S. confertifolius* and *S. indicus*.

2.5.3 *Microglossa pyrifolia* (Asteraceae)

Microglossa pyrifolia is a climbing shrub indigenous in Africa and tropical Asia [Schmidt *et al.*, 2003]. It is traditionally used against fever and malaria in Kenya and Ghana [Kokwaro, 1976; Kohler *et al.*, 2002]. It is also used as an abortifacient, as an analgesic to relieve headaches and stomach pains as well as for treatment of colds, wounds and eyes [Akah and Ekekwe, 1995; Johns *et al.*, 1990; Schmidt *et al.*, 2003 Kuiate *et al.*, 1999]. Its extract showed antiplasmodial activity [Kohler *et al.*, 2002; Midiwo *et al.*, unpublished results].

Figure 2.17: *Microglossa pyrifolia*

The plant is also called *Conyza pyrifolia* or *Microglossa volubilis* and it belongs to the genus *Microglossa*, with about ten species [Kuiate *et al.*, 1999]. *M. pyrofolia* is the only species reported to have medicinal uses and biological activities. Phytochemical information however is available on *M. pyrifolia*, *M. angolensis*, *M. mespilifolia*, *M. pyrrhopappa* and *M. zeylanica*. Clerodane and *seco*-clerodane diterpenoids have been reported from *M. pyrifolia* (**136** – **145**, Figure 2.18) [Kohler *et al.*, 2002; Zdero *et al.*, 1990a], M. *pyrrhopappa* and *M. angolensis* (**146** – **172**, Figure 2.19) [Zdero *et al.*, 1990b; Tene *et al.*, 2005].

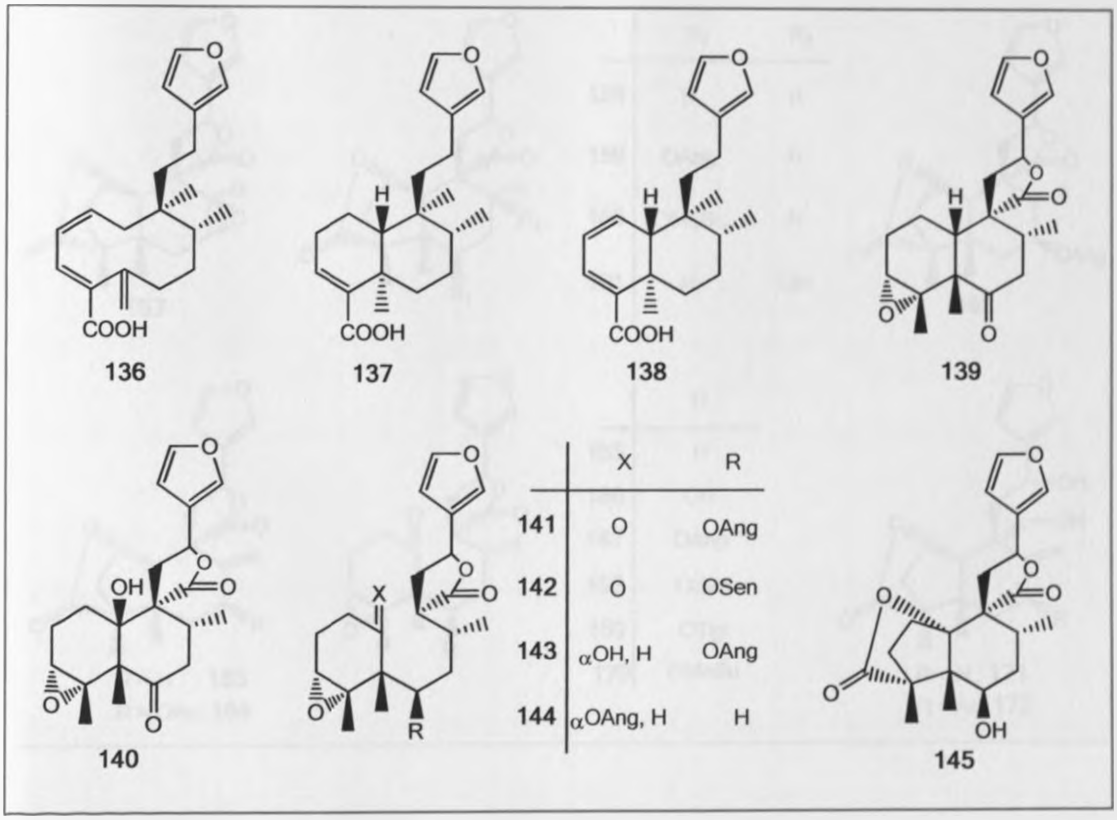


Figure 2.18: Clerodane and *seco*-clerodane diterpenoids from *Microglossa pyrifolia*

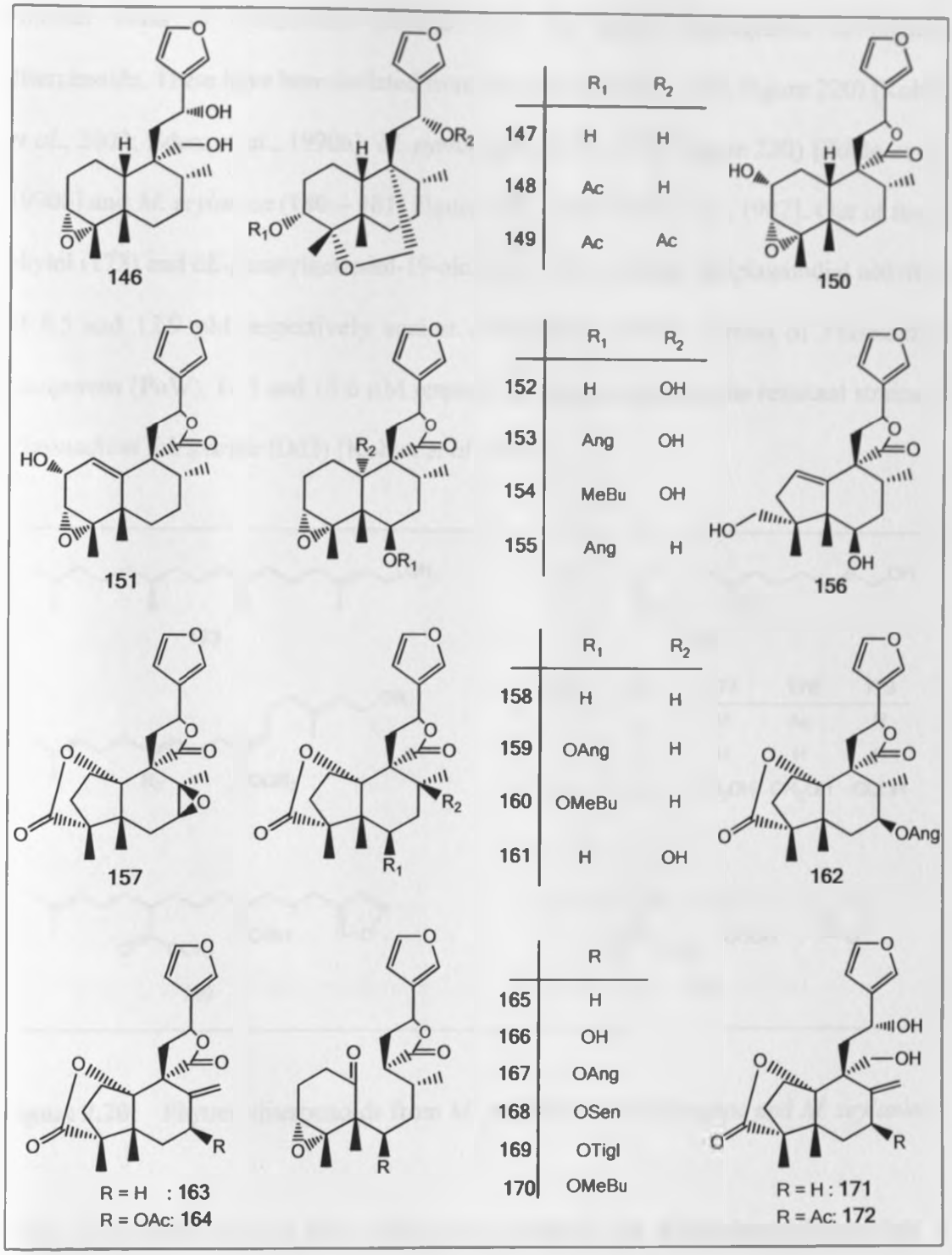


Figure 2.19: Clerodane and *seco*-clerodane diterprnoids from *M. pyrrhopappa* and *M. angolensis*

Another class of compounds isolated from the genus *Microglossa* is phytane diterpenoids. These have been isolated from *M. pyrifolia* (**173 – 178**, Figure 220) [Kohler *et al.*, 2002; Zdero *et al.*, 1990a], *M. pyrhopappa* (**177 – 179**, Figure 220) [Zdero *et al.*, 1990b] and *M. zeylanica* (**180 – 181**, Figure 220) [Gunatilaka *et al.*, 1987]. Out of these, phytol (**173**) and 6*E*-geranylgeraniol-19-oic acid (**175**) exhibited antiplasmodial activities of 8.5 and 12.9 μM respectively against chloroquine sensitive strains of *Plasmodium falciparum* (PoW), 11.5 and 15.6 μM respectively against chloroquine resistant strains of *Plasmodium falciparum* (Dd2) [Kohler *et al.*, 2002].

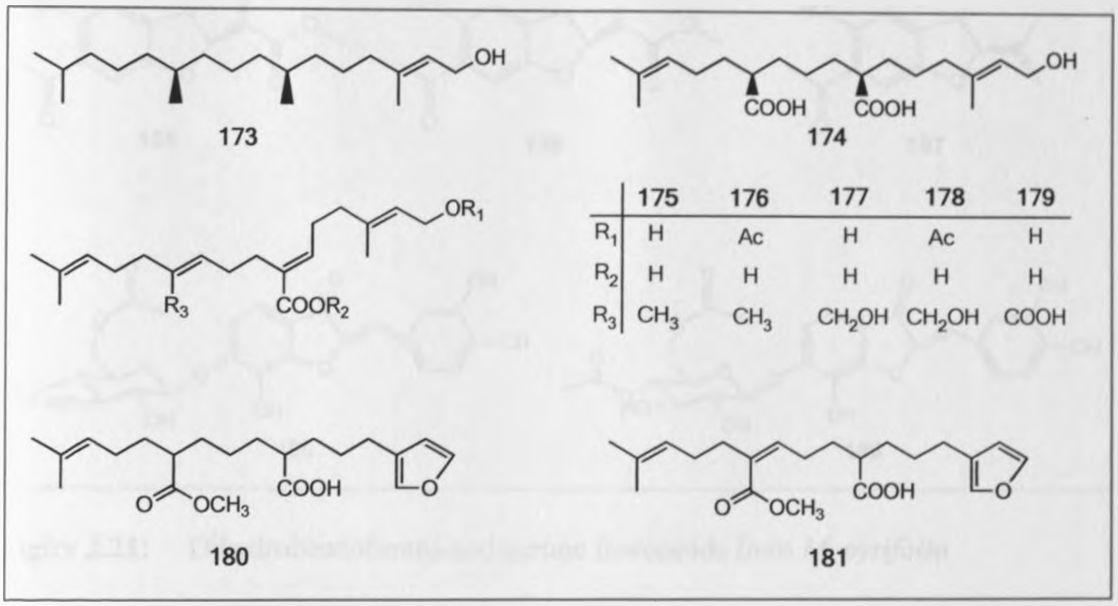
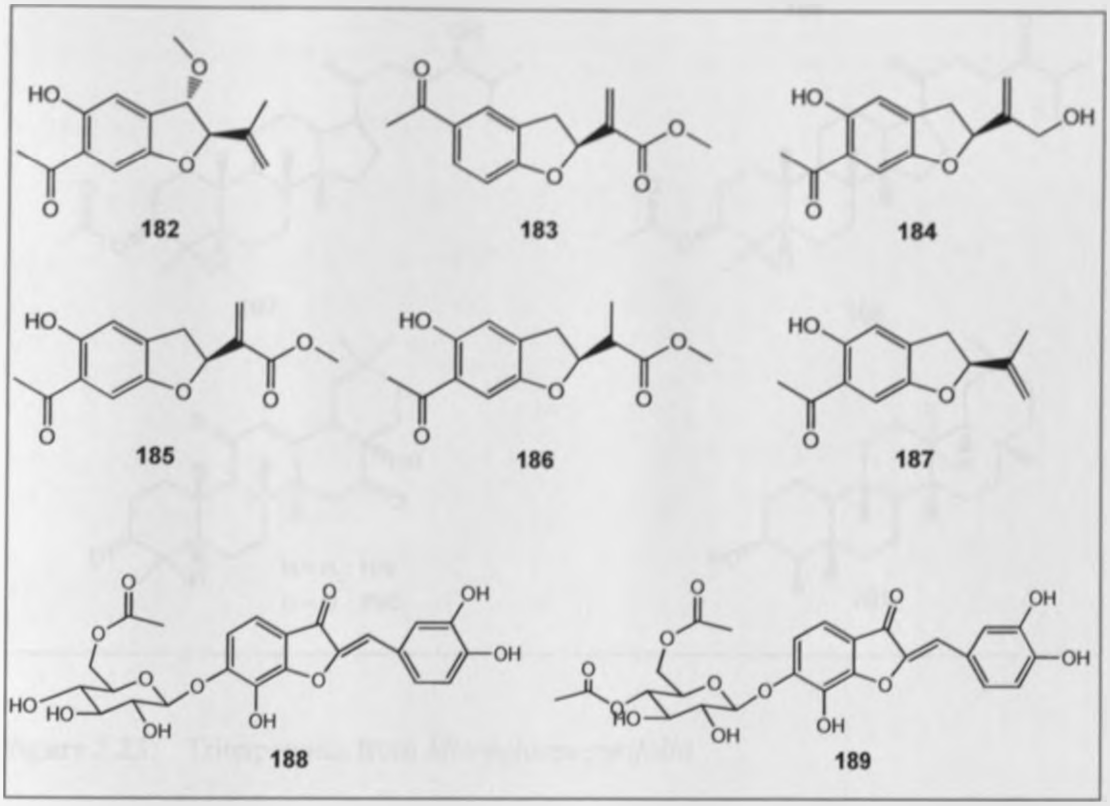


Figure 2.20: Phytane diterpenoids from *M. pyrifolia*, *M. Pyrhopappa* and *M. zeylanica*

Other compounds reported from *Microglossa pyrifolia* are dihydrobenzofurans (**182 – 187**, Figure 2.21), triterpenoids (**195 – 201**, Figure 2.23) [Schmidt *et al.*, 2003], aurone flavonoids (**188 – 189**, Figure 2.21) and sesquiterpenes (**190 – 194**, Figure 2.22) [Ruecker *et al.*, 1992; Kohler *et al.*, 2002; Zdero *et al.*, 1990a] as well as acetylens and acetylenic

glucosides (**202 – 209**, Figure 2.24) [Ruecker *et al.*, 1992]. Compound **203**, namely 2-*β*-*D*-glucopyranosyloxy-1-hydroxytrideca-3,5,7,9,11-pentayne exhibited antibacterial activities against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* [Ruecker *et al.*, 1992].



Figire 2.21: Dihydrobenzofurans and aurone flavonoids from *M. pyrifolia*

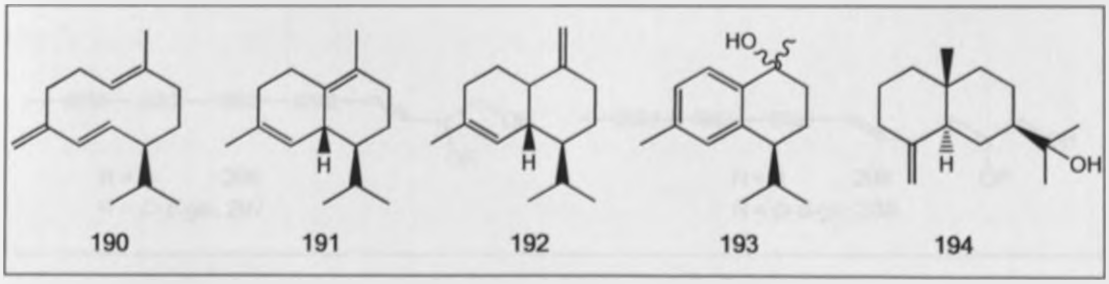


Figure 2.22: Sesquiterpenes from *Microglossa pyrifolia*

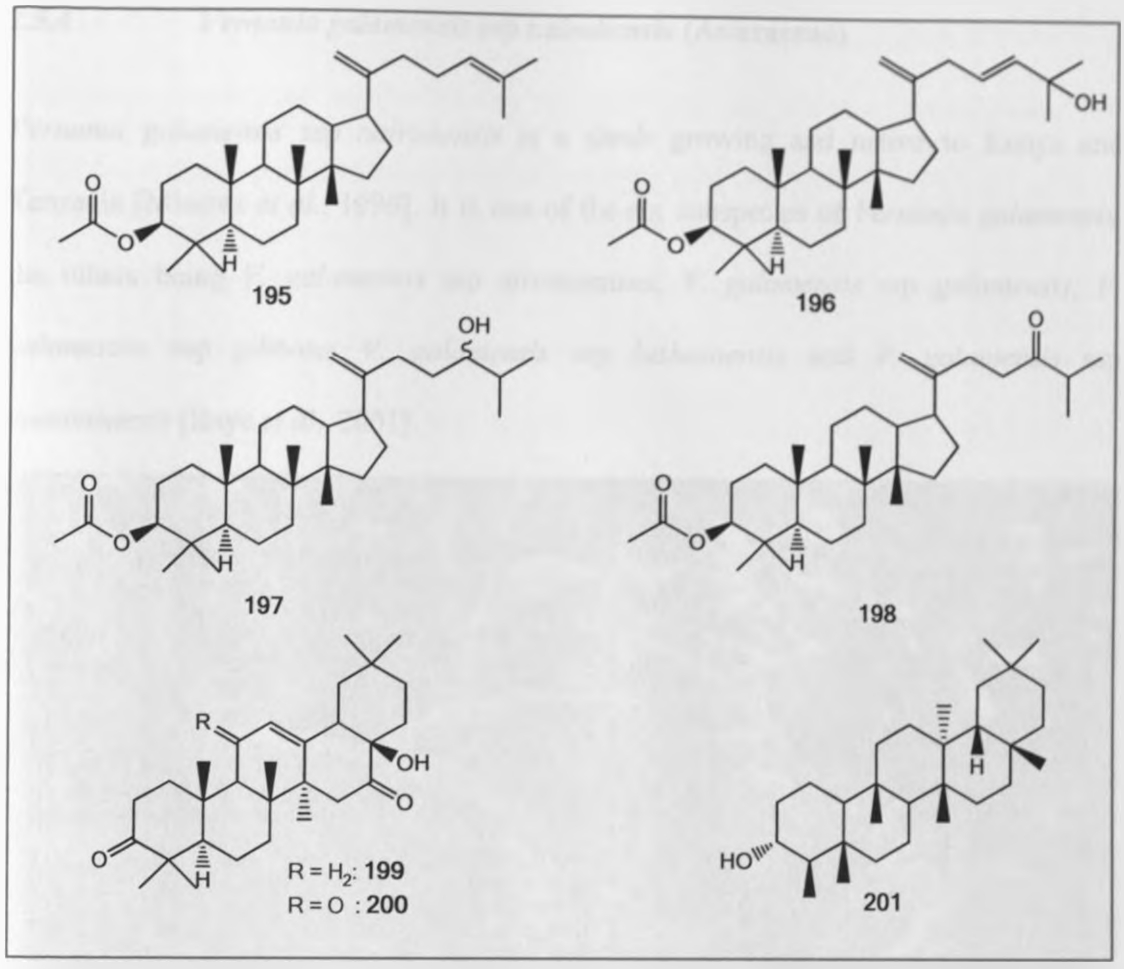


Figure 2.23: Triterpenoids from *Microglossa pyrifolia*

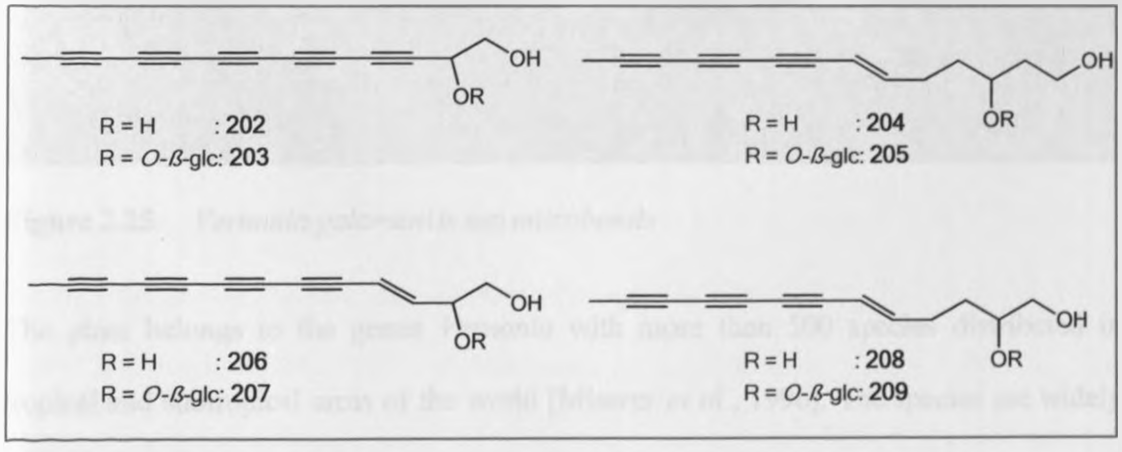


Figure 2.24: Acetylenes and acetylenic glucosides from *Microglossa pyrifolia*

2.5.4 *Vernonia galamensis* ssp *nairobensis* (Asteraceae)

Vernonia galamensis ssp *nairobensis* is a shrub growing and native to Kenya and Tanzania [Miserez *et al.*, 1996]. It is one of the six subspecies of *Vernonia galamensis*, the others being *V. galamensis* ssp *afromontana*, *V. galamensis* ssp *galamensis*, *V. galamensis* ssp *gibbosa*, *V. galamensis* ssp *lushotoensis* and *V. galamensis* ssp *mutomonesis* [Baye *et al.*, 2001].

Figure 2.25: *Vernonia galamensis* ssp *nairobensis*

The plant belongs to the genus *Vernonia* with more than 500 species distributed in tropical and subtropical areas of the world [Miserez *et al.*, 1996]. The species are widely used as folklore remedies for variety of human ailments. The most documented medicinal use includes treatment of malaria (more than 20 species including *V. galamensis*), fever,

venereal diseases, abdominal pains, wounds, diarrhea and hepatitis. Biological activities commonly encountered on the species are anti-bacterial, anti-yeast, antifungal, anti-inflammatory, anti-oxidant and anti-malarial activities [NAPRALERT, 2009].

Members of the genus are rich in epoxyfats and epoxyfatty acids that constitute vernonia oil used industrially for manufacturing paints and coatings [Baye *et al.*, 2001]. They are also known to produce secondary metabolites like sesquiterpenoidss, triterpenoids, steroids, phenylpropanoids, flavones and flavanols [Erasto *et al.*, 2006; Sanogo *et al.*, 1998; Perdue Jr. *et al.*, 1993].

Compounds isolated from *Vernonia galamensis* ssp *nairobensis* (Figure 2.26) are glaucolide-type sesquiterpene lactones glaucogalamensolide isobutyrate (**210**) and glaucogalamensolide isovalerate (**211**) [Zdero *et al.*, 1990c] as well as flavanols glycosides quercetin 3-*O*-*β*-*D*-galactoside (**212**), quercetin 3-*O*-*β*-*D*-rhamnosyl(1→6)-*β*-galactoside (**213**) quercetin 3-*O*-*β*-*D*-apiosyl(1→2)-*β*-*D*-galactoside, (**214**) and isorhamnetin 3-*O*-*β*-*D*-apio-*D*-furanosyl(1→2)-*β*-*D*-galactopyranoside (**215**) [Miserez *et al.*, 1996].

The other subspecies of *Vernonia galamensis* have been reported to produce vernolic acid, the steroids stigmasterol and *β*-sitosterol, triterpenoids lupeol, lupeol acetate, *α*-amyrin, *β*-amyrin, taraxasterol and taraxasterol acetate [Perdue Jr. *et al.*, 1993]. They also contain prevernocistifolide sesquiterpenoids namely 8-isobutyrateprevernocistifolide (**216**) (*V. galamensis* ssp *galamensis*, *V. galamensis* ssp *gibbosa*), 8-senecioateprevernocistifolide (**217**) (*V. galamensis* ssp *afromontana*, *V. galamensis* ssp *galamensis*, *V. galamensis* ssp *gibbosa*) as well as 14-acetyl-8-

isobutyrateprevernocistifolide (**218**) and 14-acetyl-8-senecioateprevernocistifolide (**219**) from *V. galamensis* ssp *galamensis* var *petiti* (Figure 2.27) [Perdue Jr. *et al.*, 1993].

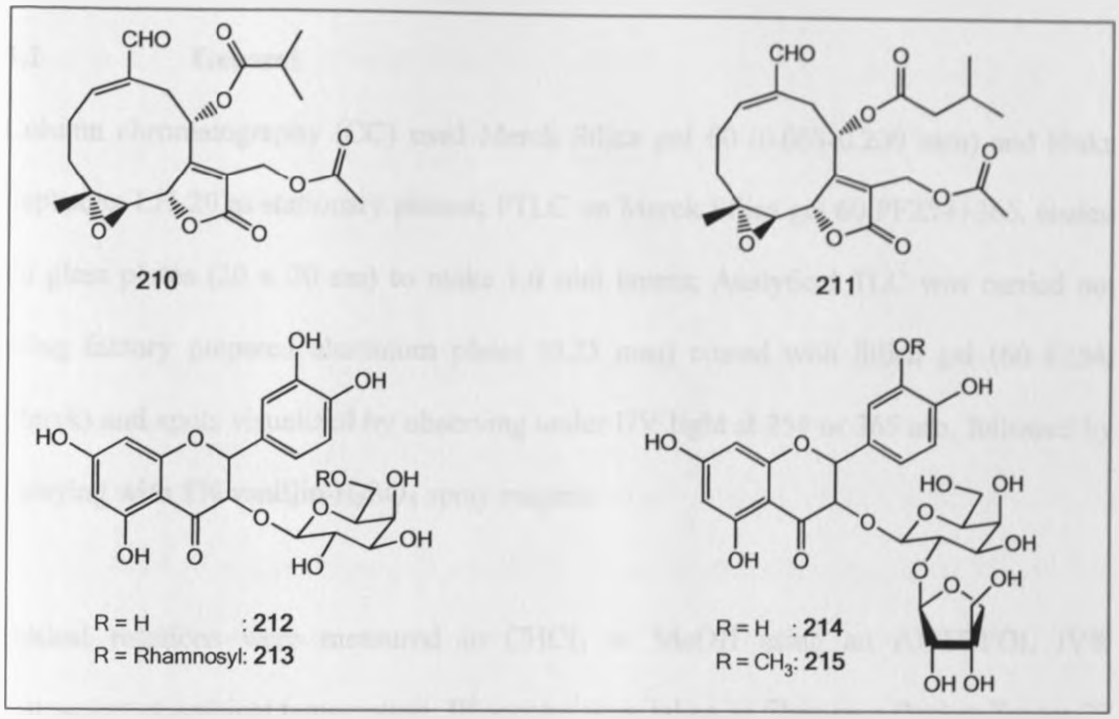


Figure 2.26: Glaucolide-type sesquiterpene lactones and flavanols from *Vernonia galamensis* ssp *nairobensis*

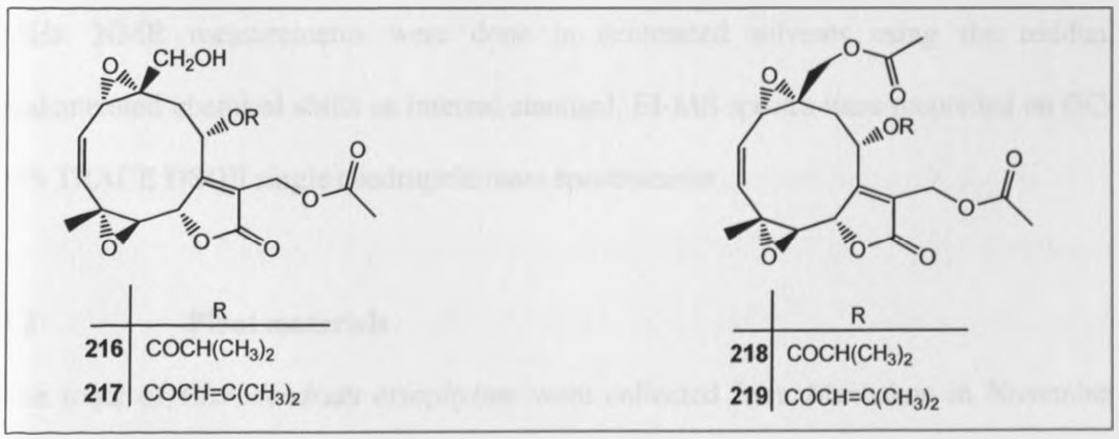


Figure 2.27: Prevernocistifolide sesquiterpenoids from *Vernonia galmensis* subspecies

CHAPTER THREE

EXPERIMENTAL METHODS

3.1 General

Column chromatography (CC) used Merck Silica gel 60 (0.063-0.200 mm) and Fluka Sephadex LH-20 as stationary phases; PTLC on Merck Silica gel 60 PF254+366, coated on glass plates (20 x 20 cm) to make 1.0 mm layers; Analytical TLC was carried out using factory prepared aluminum plates (0.25 mm) coated with Silica gel (60 F254, Merck) and spots visualized by observing under UV light at 254 or 365 nm, followed by spraying with 1% vanillin-H_2SO_4 spray reagent.

Optical rotations were measured in $CHCl_3$ or MeOH using an AUTOPOL IV® instrument at ambient temperature. IR spectra were taken as films on a Bruker Tensor 27 FTIR instrument. UV spectra were obtained in MeCN using a Hawlett- Packard 8453 spectrophotometer; 1D and 2D NMR data were acquired on Bruker Avance 600 MHz or Bruker BioSpin instrument 500 MHz or Bruker Avance 300 MHz or Varian-Mercury 200 MHz. NMR measurements were done in deuterated solvents using the residual undeuterated chemical shifts as internal standard. EI-MS spectra were recoreded on GC-MS TRACE DSQII single quadrupole mass spectrometer.

3.2 Plant materials

The roots of *Clerodendrum eriophylum* were collected from Machakos in November 2007, while the rest of the plants (*Sphaeranthus bullatus*, *Microglossa pyrifolia* and *Vernonia galamensis*) were collected from Ngong forest Nairobi, in November 2007.

Voucher specimens were deposited at the Department of Botany, University of Nairobi (No JMFM/2007/11 *Clerodendrum eriophyllum*, JMFM/2007/12 *Microglossa pyrifolia*, JMFM/2007/13 *Sphaeranthus bullatus* and JMFM/2007/14 *Vernonia galamensis* ssp *nairobensis*). The plants were air dried under shade and pulverized using a Willy mill.

3.3 Extraction and isolation

3.3.1 *Clerodendrum eriophyllum*

The shade air dried and pulverized roots of *C. eriophyllum* (1.8 Kg) were extracted by cold percolation at room temperature using 1:1 CH_2Cl_2/MeOH (3×4 L, 24 h each), followed by 100% methanol (1×4 L, 24 h). These were rotavaped and combined to give 65 g of brown gummy extract, of which 35 g was adsorbed on 40 g of Silica gel and subjected to CC on a silica gel column (300 g, 5×35 cm), eluted with *n*-hexane/ CH_2Cl_2 (19:1, 3.5 L; 9:1, 1.25 L; 3:1, 2 L; 1:1, 3 L; 1:3, 1 L; 100% CH_2Cl_2, 1.5 L) followed by CH_2Cl_2/ MeOH (99:1, 1.25 L; 49:1, 1 L; 19:1, 1 L).

Sixty-two fractions of eluents, collected in 250 mL aliquots, were collected and concentrated using a rotary evaporator with similar fractions combined on the basis of TLC analysis. Fraction 4 (76 mg) was rechromatographed over Silica gel (10 g, 1x12cm) eluted with *n*-hexane/CH_2Cl_2 (19:1) to give *β*-amyrinoctacosanoate (**230**) (21 mg). Combination of fraction 5-9 crystallized in *n*-hexane/CH_2Cl_2 (19:1) to give royleanone (**21**) (260 mg) which had 6,7-dehydroxyroyleanone (**221**) as a minor component. Fractions 11-19 formed crystals (9:1 *n*-hexane/ CH_2Cl_2) which were filtered to give tyrosyloctacosanoate (**231**) (58 mg) and the filtrate was conncentrated (640 mg) and rechromatographed over Silica gel (50 g, 2×30 cm) eluting with *n*-hexane/CH_2Cl_2 (19:1)

to give 11-hydroxy-7,9(11),13-abietatrien-12-one (**223**) (5 mg) after 0.6 L of elution, and ferruginol (**224**) (65 mg) after 1.4 L of elution. The latter was further purified by PTLC developed with *n*-hexane/CH_2Cl_2 (4:1).

Fraction 22-28 (160 mg) was purified on a Sephadex LH-20 column (20 g, 2.5×30 cm), eluted with CH_2Cl_2/MeOH 1:1 (0.3 L), followed by PTLC developed with *n*-hexane/CH_2Cl_2 7:3 to give taxadione (**222**) (28 mg). Fraction 34-45 (600 mg) was subjected to Silica gel CC (50 g, 2.0×30 cm), eluted with *n*-hexane/CH_2Cl_2 (1:4) to give 12-hydroxy-8,12-abietadiene-3,11,14-trione (**220**) (4 mg) after 0.28 L of elution, uncinatone (**19**) (7 mg) after 0.35 L of elution, and sugiol (**9**) (10 g) after 0.8 L of elution.

Fractions 52-57 formed crystals which were filtered to give betulinic acid (**229**) (94 mg) and the mother-liquor was concentrated to 580 mg and rechromatographed over silica gel (50 g, 2×30 cm) eluted with CH_2Cl_2 to yield 6-hydroxysalvinolone (**226**) (7 mg), nellinol (**228**) (42 mg) and 6,16-dihydroxysalvinolone (**227**) (64 mg), both of which crystallized from CH_2Cl_2, after 0.5 L, 0.9 and 1.3 L of elution, respectively. 11-hydroxy-8,11,13-abietatriene-12-*O*-β-xylopyranoside (**225**) (15 mg) was obtained after purifying fractions 58-60 on a Sephadex LH-20 column (20 g, 2.5×30 cm) eluted with CH_2Cl_2/MeOH 1:1 (0.3 L).

3.3.2 *Sphaeranthus bullatus*

The shade dried and pulverized aerial parts of *S. bullatus* (2.3 Kg) were extracted by cold percolation at room temperature using 1:1 CH_2Cl_2/MeOH (3×5 L, 24 h each), followed by 100% methanol (1×4 L, 24 h). The filtrates were rotavaped and combined to give 168

g of black-brown gummy extract, of which 100 g were suspended in methanol/water (2:8) and partioned successively with *n*-hexane, CH_2Cl_2 and EtOAc to give 31.5 g of *n*-hexane extract, 26 g of CH_2Cl_2 extract and 9.2 g of ethyl acetate extract.

The *n*-hexane soluble part (30 g) was adsorbed in 40 g of Silica gel and chromatographed over Silica gel (300 g, 5 x 35 cm) eluted with *n*-hexane/EtOAc mixtures (100:0, 2.0L; 19:1, 2.5 L; 9:1, 2.0L; 4:1, 1.5 L; 3:2, 2.5 L; 1:1, 2.0 L; 2:3, 1.5 L; 1:4, 1.5 L; 0:100, 1.0 L). A total of 65 fractions of 250 ml each were collected and combined on the basis of similarities observed on TLC analysis. Fraction 23-27 (164 mg) were rechromatographed over Silica gel (10 g, 1 x 12 cm) eluted with *n*-hexane/EtOAc 23:2 to give boehmery acetate (**243**) (37 mg) which was recrystillized from methanol. Fraction 29 and 30 gave crystals (*n*-hexane/EtOAc 4:1) which were filtered to make dammaradienyl acetate (**242**) (26 mg). Fractions 32-37 (420 mg) were passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm; 1:1 CH_2Cl_2/MeOH) to remove chlorophylls and then chromatographed over Silica gel (20 g, 2.0 x 12 cm) eluted with *n*-hexane/CH_2Cl_2 (3:2, 1.2 L) to give 5-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**) (114 mg).

The dichloromethane soluble fraction of the original extract (25 g) was adsorbed on 30 g of Silica gel and chromatographed over Silica gel (300 g, 5 x 35 cm) eluted with *n*-hexane/CH_2Cl_2 mixtures (4:1, 2.5 L; 3:2, 2.5 L; 1:1, 2.25 L; 2:3, 2.0 L; 1:4, 1.50 L; 0: 100, 2.0 L) followed by CH_2Cl_2/MeOH mixtures (99:1, 1.50 L; 49:1, 1.25; 19:1, 2.0 L; 9:1, 1.5 L; 4:1, 1.0 L). A total of 80 fractions of 250 ml each were collected and combined on the basis of TLC analysis.

Fraction 9-17 (1.7 g) were rechromatographed over Silica gel (50 g, 2 x 30 cm) eluted with *n*-hexane/CH_2Cl_2 (7:3, 2.0 L) to give 5-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**) (326 mg). Fraction 20-22 (164 mg) was passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm; 1:1 CH_2Cl_2/MeOH) to remove chlorophylls followed by purification with PTLC (three plates) developed with *n*-hexane/CH_2Cl_2 1:1 to give 3,7-dihydroxy-5-tigloyloxycarvotacetone (**106**) (46 mg). Penduletin (**237**) (93 mg) crystallized from fractions 34-36 and was filtered off. The mother liquor was mixed with fractions 37-40 to give 432 g which was chromatographed over silicalgel (30 g, 2 x 20 cm) eluted with CH_2Cl_2/MeOH (19:1, 1.2 L) to give quercetin-3,7-dimethylether (**236**) (42 mg) and 4-hydroxylonchocarpin (**239**) (18 mg). The latter was purified by PTLC (two plates) developed with CH_2Cl_2/MeOH 19:1. Fraction 43-50 (535 mg) was passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm) eluted with 1:1 CH_2Cl_2/MeOH. The major component was concentrated and re-crystallized in CH_2Cl_2 to give chrysoplenol D (**238**) (177 mg). Fraction 54-55 (56 mg) was cleaned by PTLC to give 3,5,7-trihydroxycarvotacetone (**85**) (18 mg). Quercetin (**235**) (12g) was collected as crystals from fraction 61-62 of the major column.

The ethyl acetate extract (9 g) was chromatographed over Silica gel (100 g, 2.5 x 30 cm) eluted with *n*-hexane/CH_2Cl_2 mixtures (1:1, 1.2 L; 1:3, 1.0 L, 100:0, 1.0 L) followed by CH_2Cl_2/MeOH mixtures (99:1, 0.80 L; 98:2, 0.60 L; 95:5, 0.80 L; 9:1, 0.60 L; 8:2, 0.50 L; 7:3, 0.40 L). Sixty-five fractions of 100 ml each were collected.

Fraction 14-17 (87 mg) was passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm) eluted with 1:1 CH_2Cl_2/MeOH and the fractionated eluents concentrated and re-

crystallized in *n*-hexane/CH_2Cl_2 to give coniferaldehyde (**241**) (13 mg) and caffeic acid (**240**) (18 mg). Fraction 21-25 (107 mg) was rechromatographed over silicagel (50g, 2.0 x 30 cm) developed with CH_2Cl_2/MeOH 97:3 to give 5-*O*-*β*-glucopyranosylcarvotacetone (**232**) (8 mg) and thymol 3-*O*-*β*-glucopyranoside (**233**) (28 mg). Zetroside B (**93**) (283 mg) crystallized on fractions 29-36 of the main column and was filtered off. It contained zetaroside A (**234**) as a minor component.

3.3.3 *Vernonia galamensis* ssp *nairobensis*

The pulverized and shade dried aerial parts (1.1 Kg) were extracted with 1:1 CH_2Cl_2/MeOH (3×3 L, 24 h each) followed by 100% methanol (1×3 L, 24 h). The filtrates were concentrated on a rotary evaporator and combined to give 101g of black extract which was dissolved in methanol/water 1:4 and portioned successively with CH_2Cl_2 and ethylacetate to give 23 g of CH_2Cl_2 extract and 8 g of ethyl acetate extract.

The CH_2Cl_2 soluble extract (20 g) was run on column chromatography using Silica gel (200 g, 4 x 30 cm) and eluted with *n*-hexane/CH_2Cl_2 mixtures (100:0, 1.6 L; 9:1, 1.4 L; 4:1, 1.2 L; 7:3, 1.2 L; 1:1, 1.6 L; 1:3, 1.2 L; 0:100, 1.8 L) and CH_2Cl_2/MeOH mixtures (99:1, 1.4 L; 49:1, 1.2 L; 19:1, 1.2 L; 9:1, 1.0 L). Seventy-two fractions of 200 ml each were collected and concentrated on a rotary evaporator. Similar fractions were combined on the basis of TLC analysis.

Fractions 24- 27 (126 mg) was passed through Sephadex LH-20 column (20 g, 2.5 x 30 cm) eluted with 1:1 CH_2Cl_2/MeOH to remove chlorophylls, followed by CC over silicagel (20 g, 2.0 x 12 cm) eluted with *n*-hexane/CH_2Cl_2 3:2 to give syringaresinol

(**246**) (8mg). Quercetin-3,7-dimethyl ether (17 mg) (**236**) crystallized in fractions 27-31 and was filtered off. Fractions 33-38 (242 mg) was chromatographed over Silica gel (20 g, 2.0 x 12 cm) eluted with 100% CH_2Cl_2 followed by 0.5% MeOH in CH_2Cl_2 to give a mixture of two compounds which were separated by PTLC to give quercetin (**235**) (9 mg) and quercetin-3-methyl ether (**244**) (34 mg).

The EtOAc extract (8 g) was chromatographed over Silica gel (100 g, 2.5 x 30 cm) eluted with *n*-hexane/CH_2Cl_2 mixtures (1:1, 1.0 L; 1:3, 1.0 L, 0:100, 0.8L) followed by CH_2Cl_2/MeOH mixtures (99:1, 0.80 L; 49:1, 0.60 L; 19:1, 0.60 L; 9:1, 0.60 L; 4:1, 0.50 L; 7:3, 0.40 L). Fifty-nine fractions of 100 ml each were collected. Syringic acid (**247**) (9 mg) was obtained after CC of fractions 27-31 over Sephadex LH-20 (20 g, 2.5 x 30 cm; 1:1 CH_2Cl_2/MeOH). Fractions 41-45 (*n*-hexane/CH_2Cl_2 19:1) and 48-51 (*n*-hexane/CH_2Cl_2 9:1) gave crystals which were filtered to give quercitrin (**245**) (124 mg) and quercetin-3-*O*-*β*-galactopyranoside (**212**) (92 mg) respectively.

3.3.4 *Microglossa pyrifolia*

The air dried and pulverized aerial parts of *M. pyrifolia* (2.6 Kg) was extracted by cold percolation at room temperature using 1:1 CH_2Cl_2/MeOH (3×5 L, 24 h each), followed by 100% methanol (1×4 L, 24 h). After filtering, the filtrates were concentrated on a rotary evaporator and combined to give 190 g of black gummy extract. Out of this, 120 g was taken and partitioned by suspending it in water/methanol 4:1 and extracted in turns with *n*-hexane and CH_2Cl_2 to give 53 g of *n*-hexane extract and 19 g of CH_2Cl_2 extract.

The *n*-hexane extract (50 g) was chromatographed over Silica gel (350 g, 5 x 40 cm) eluted with *n*-hexane/EtOAc mixtures (100: 0, 2.25 L; 19:1, 2.5 L; 9:1, 2.0 L; 4:1, 1.5 L; 3:2, 2.0 L; 1:1, 2.0 L; 2:3, 1.5 L; 1:4, 1.0 L) to give 55 fractions of 250 ml each. These gave friedelan-3-one (**250**) (25 mg), *β*-sitosterol (**249**) (14 mg) and stigmasterol (**66**) (66 mg) that crystallized in fractions 8-13, 19-22 and 24-36 respectively. The other fractions contained mainly chlorophylls, carotenoids and oils.

The CH_2Cl_2 extract (18 g) 35 g was adsorbed onto 20 g of Silica gel and subjected to CC on a silica gel column (200 g, 4×30 cm) eluted with *n*-hexane/CH_2Cl_2 mixtures (100:0, 1.8 L; 9:1, 1.4 L; 4:1, 1.4 L; 7:3, 1.2 L; 1:1, 1.6 L; 1:3, 1.2 L; 0:100, 1.4 L) and CH_2Cl_2/MeOH mixtures (99:1, 1.6 L; 49:1, 1.2 L; 19:1, 1.0 L; 9:1, 1.0 L) to give 70 fractions of 200 ml each. Repeated CC on fractions 33-41 (348 mg) (silicagel, 20 g, 2.0 x 12 cm) with *n*-hexane/CH_2Cl_2 3:2 followed by *n*-hexane/CH_2Cl_2 1:1 gave epoxyisochiliolide lactone (**157**) (57 mg) which chrystallized in 1:1 *n*-hexane/CH_2Cl_2. The 8-acetoxyisochiliolide lactone (**248**) (97) crystallized in fractions 44-51 of the original column. Fraction 57-65 (183 mg) was chromatographed over sephadex LH-20 (20 g, 2.5 x 30 cm; 1:1 CH_2Cl_2/MeOH) followed by silicagel CC (20 g, 2.0 x 12 cm) to give 1,4-dihydroxy-7(11)-eudesmen-8-one (**251**) (9 mg).

3.4 *In vitro* anti-plasmodial activities

The *in vitro* anti-plasmodial activities ware measured at the National Center for Natural Products Research of School of Pharmacy, University of Mississippi. The method used a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity [Makler *et al.*, 1993; Samoylenko *et al.*, 2009]. The assay was performed in 96-well

microplate and included two *P. falciparum* strains [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. The IC_{50} values were computed from the dose response curves generated by plotting percent growth against test concentrations. DMSO, artemisinin and chloroquine were included in each assay as vehicle and drug controls, respectively.

3.5 Cytotoxicity Assay

These were measured at the National Center for Natural Products Research of School of Pharmacy, University of Mississippi. The activity was determined against monkey kidney fibroblasts (VERO) obtained from the American Type Culture Collection (ATCC, Rockville, MD). The assay was performed in 96-well tissue culture-treated microplates. Cells were seeded at a density of 25000 cells/well and incubated for 24 hours. Samples at different concentrations were added and plates were again incubated for 48 hours. The number of viable cells was determined using Neutral Red according to a modification of the procedure of Borenfreund *et al.* [Borenfreund *et al.*, 1990]. IC_{50} values were determined from dose response curves of percent growth inhibition against test concentrations. Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

3.6 Antioxidant test

Preliminary anti-oxidant test was done by spotting the compounds on a TLC followed by spraying the TLC plate with 0.2 mg/ml DPPH solution to view the active compounds which displayed white or yellowish sport on a purple background.

The active compounds were quantitatively analysed by UV-VIS spectrometry method adopted from Hou *et al.*, (2002) with modifications made on the concentrations of the samples. For each compound, the concentration of the sample was varied by serial dilutions to give concentrations of 160, 80, 40, 20, 10, 5.0, 2.5, 1.25 and 0 µg/ml while the concentration of DPPH was kept constant at 100 µg/ml (Figure 3.1). These solutions were then measured for UV-VIS absorbance at DPPH absorbing wavelength (517 nm) half an hour after adding the DPPH. The absorbance measured at each of these intervals were converted into percentages of scavenged DPPH radicals using the following equation

$$\%\text{ge of scavenged DPPH} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100\%$$

Where A_{blank} is the absorbance of DPPH solution without sample. The percentages of scavenged DPPH were then plotted against concentration of the compound to give graphs from which concentrations at half inhibition (IC_{50}) were determined. The tests were done in triplicates.

Figure 3.1: Samples with varied conc of compound but constant conc of DPPH

3.7 Antimicrobial Assay

The organisms are obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113 and *Aspergillus fumigatus* ATCC 90906 as well as the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed at the National Center for Natural Products Research, School of Pharmacy, University of Mississippi, using a modified version of the CLSI methods as described by Samoylenko *et al* [Samoylenko *et al.*, 2009]. Drug controls ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included in each assay.

3.8 Antileishmanial Assay

Antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes at the National Center for Natural Products Research of School of Pharmacy, University of Mississippi. In a 96 well microplate assay compounds with appropriate dilution were added to the Leishmania promastigotes culture (2×106 cells/mL). The plates were incubated at 26°C for 72 hours and growth of *Leishmania* promastigotes was determined by Alamar blue assay [Mikus and Steverding, 2000]. Pentamidine and amphotericin B were used as standard antileishmanial agents. IC_{50} values for each compound were computed from the growth inhibition curve.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Secondary metabolites isolated from *Clerodendrum eriophyllum*

4.1.1 Abietane diterpenoids

The roots of *Clerodendrum eriophyllum* afforded twelve abietane diterpenoids, which are 3-oxoroyleanone (**220**), royleanone (**21**), 6,7-dehydroroyleanone (**221**), taxodione (**222**), 11-hydroxy-7,9(11),13-abietatrien-12-one (**223**), ferruginol (**224**), 11-hydroxy-8,11,13-abietatriene *12-O-β*-xylopyranoside (**225**), sugiol (**9**), 6-hydroxysalvinolone (**226**), 6,16-dihydroxysalvinolone (**227**), nellionol (**228**) and uncinatone (**19**).

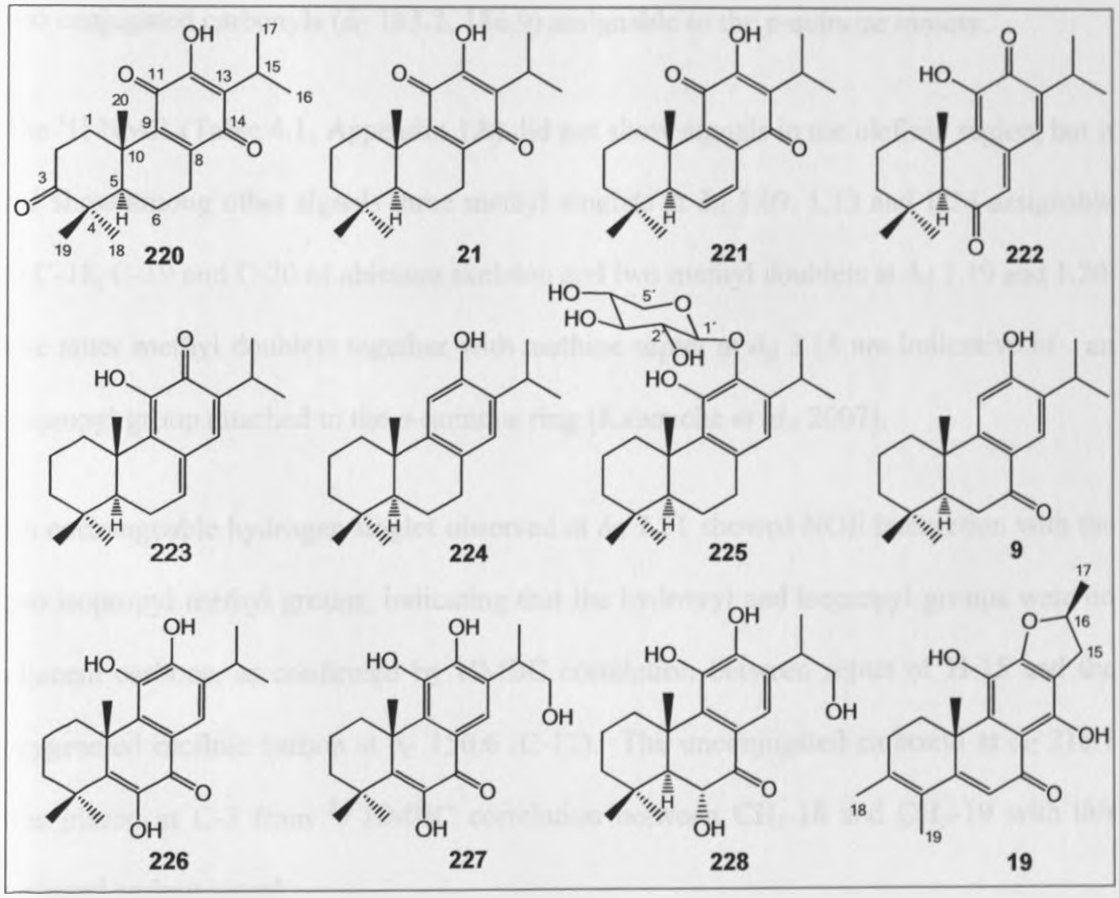


Figure 4.1: Abietane diterpenoids isolated from the roots of *C.eriophyllum*

4.1.1.1 12-Hydroxy-8,12-abietadiene-3,11,14-trione (3-oxoroyleanone)

Compound **220** was isolated as pale yellow amorphous solid. Its molecular formula was established by HRESI to be $C_{20}H_{26}O_4$, *m/z* 331.1910 $[M+H]^+$ requiring 331.1909. Its UV absorption maxima at 275 and 405 nm closely correlated that reported for a *p*-quinone chromophore of royleanone [Edwards *et al.*, 1962].

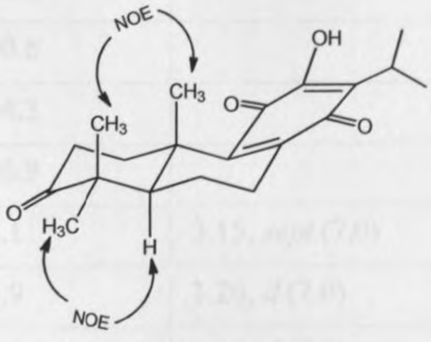
The IR spectrum indicated presence of hydroxyl group (3080-3430 cm^{-1}), unconjugated carbonyl (1704 cm^{-1}) together with olefinic and conjugated carbonyl absorptions of a *p*-quinone moiety (1609, 1633 and 1650 cm^{-1}). The ^{13}C NMR (Table 4.1, Appendix 1B) showed 20 signals with four sp^2 quaternary carbons (δ_C 124.1, 144.1, 145. 8, 150.6) and two conjugated carbonyls (δ_C 183.2, 186.9) assignable to the *p*-quinone moiety.

The ^1H NMR (Table 4.1, Appendix 1A) did not show signals in the olefinic region, but it did show among other signals three methyl singlets at δ_H 1.09, 1.13 and 1.24 assignable to C-18, C-19 and C-20 of abietane skeleton and two methyl doublets at δ_H 1.19 and 1.20. The latter methyl doublets together with methine septet at δ_H 3.15 are indicative of an isopropyl group attached to the *p*-quinone ring [Kabouche *et al.*, 2007].

An exchangeable hydrogen singlet observed at δ_H 7.21 showed NOE interaction with the two isopropyl methyl groups, indicating that the hydroxyl and isopropyl groups were on adjacent carbons, as confirmed by HMBC correlation between septet of H-15 and the oxygenated olefinic carbon at δ_C 150.6 (C-12). The unconjugated carbonyl at δ_C 216.7 was placed at C-3 from ^3J HMBC correlation between CH_3-18 and CH_3-19 with this carbonyl carbon signal.

The realative configurations at C-5 and C-10 were established as follows; H-5 and CH_3-20 were in *axial-axial* orientation, as shown by NOESY experiments which displayed NOE correlations between H-5 and CH_3-18 as well as between CH_3-19 and CH_3-20 and no correlation between H-5 and CH_3-20 (Figure 4.2). This relative configuration at C-5 and C-10 is what is reported for natural abietane diterpenoids [Hirasawa *et al.*, 2007] and was the same for all of the isolated abietane diterpenoids.

Comparison of ^{13}C NMR spectral data of **220** with those of known compound royleanone showed close correlation except for replacement of the methylene at C-3 with carbonyl and the corresponding deviations for neighbouring carbon signals, $\Delta\delta_C$ -1.7, 16.4, 13.4 and -5.7 for C-1, C-2, C-4 and C-19 respectively. Thus, spectral evidence indicated compound **220** to be 12-hydroxy-8,12-abietadiene-3,11,14-trione (3-oxoroyleanone), which is a new compound.



220

Figure 4.2: NOE correlations used to establish relative stereochemistry at C- 5 and C-10.

Table 4.1: NMR data for compound **220** (CDCl3; 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR)

H/C	δ_C	δ_H, *m* (J in Hz)	HMBC
1	34.5	1.76, *m* 2.81, *m*	C-2, C-3, C-5, C-10, C-20 C-2, C-3, C-5, C-10, C-20
2	33.8	2.59, *ddd* (15.6, 9.2, 5.4); 2.51, *dt* (15.6, 7.2)	C-1, C-3, C-4, C-10 C-1, C-3, C-4, C-10
3	216.7		
4	46.9		
5	50.8	1.76, *m*	C-1, C-3, C-7, C-19, C-20
6	18.6	1.46, *ddd* (21.8, 12.0, 4.5); 1.76, *m*	C-4, C-5, C-7, C-8, C-10 C-4, C-5, C-7, C-8, C-10
7	26.0	2.31, *ddd* (19.6, 12.0, 6.0); 2.84, br *dd* (19.6, 5.4)	C-5, C-6, C-8, C-9. C-14 C-5, C-6, C-8, C-9. C-14
8	145.8		
9	144.1		
10	37.3		
11	183.2		
12	150.6		
13	124.3		
14	186.9		
15	24.1	3.15, *sept* (7.0)	C-12, C-13, C-14, C-16, C-17
16	19.9	1.20, *d* (7.0)	C-13, C-15, C-17
17	19.8	1.19, *d* (7.0)	C-13, C-15, C-16
18	27.7	1.13, *s*	C-3, C-4, C-5, C-19
19	20.0	1.09, *s*	C-3, C-4, C-5, C-18
20	20.5	1.24, *s*	C-1, C-5, C-9, C-10
12-OH		7.21, *s*	C-11, C-12, C-13

4.1.1.2 12-Hydroxy-8,12-abietadiene-11,14-dione (royleanone)

Compound **21** was isolated as orange crystals. Its EI-MS spectrum (Appendix 2C) gave a molecular ion at *m/z* 316 ($C_{20}H_{28}O_3$). The ^{13}C-NMR (Table 4.4, Appendix 2B) showed twenty carbon signals, among them four quaternary olefinic and two conjugated carbonyl carbons representing a *p*-quinone moiety (δ_C 123.0, 145.5, 145.9, 149.9, 182.8, 187.0). The presence of an isopropyl (δ_H 0.57 and 0.58, both 3H doublets with J = 7.0 and δ_H 2.49, 1H septet with J = 7.0) attached to *p*-quinone moiety was apparent and hydroxyl group (exchangeable ^{1}H singlet at δ_H 6.60). The two groups were adjacent to each other from HMBC correlations.

The rest of the carbons constituted of three methyls, five methylenes and two methines which completed an abietane skeleton, as from HMBC, with ring C being the *p*-quinone moiety. The EI-MS retro Diels-Alder fragmentation at *m/z* 231 and 84 (Figure 4.3) gave further proof for the structure. The compound was therefore characterized as 12-hydroxy-8,12-abietadiene-11,14-dione (royleanone, **21**) previously reported from *Inula royleana* [Edwards *et al.*, 1962].

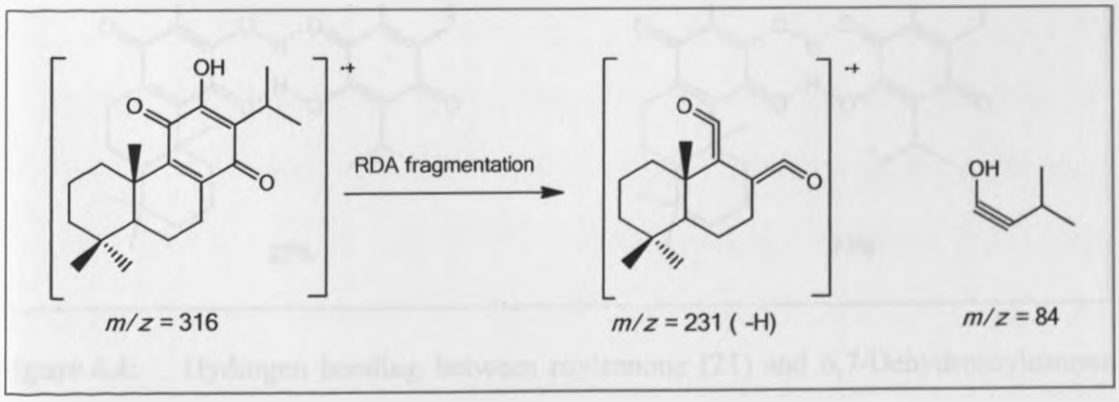


Figure 4.3: Fragmentation pattern for royleanone (**21**)

4.1.1.3 6,7-Dehydroroyleanone

Compound **221** was isolated as a minor component of a sample containing royleanone (**21**). The ^{1}H-NMR (Appendix 2A) indicated that this compound made about 30% of the major compound. The difference between the two compounds was dehydrogenation at position 6 and 7 to form a double bond, which was observed by shift of carbon-13 chemicals shifts from 16.4 and 26.2 to 139.5 and 120.4 respectively for the two positions.

The olefinic protons were observed at δ_H 5.86 and 6.15, both being double boublets with coupling constants of 9.7 and 3.0 Hz. The mixture was consistently giving a single spot on a TLC over different solvent systems, a property that might have been attributed to hydrogen bonding (Figure 4.4), as it is observed with other similar organic molecules [Csehi and Palinko, 2004].

The minor compound was therefore 6,7-dehydroroyleanone, previously isolated from *Plectranthus sp.* [Hensch *et al.*, 1975].

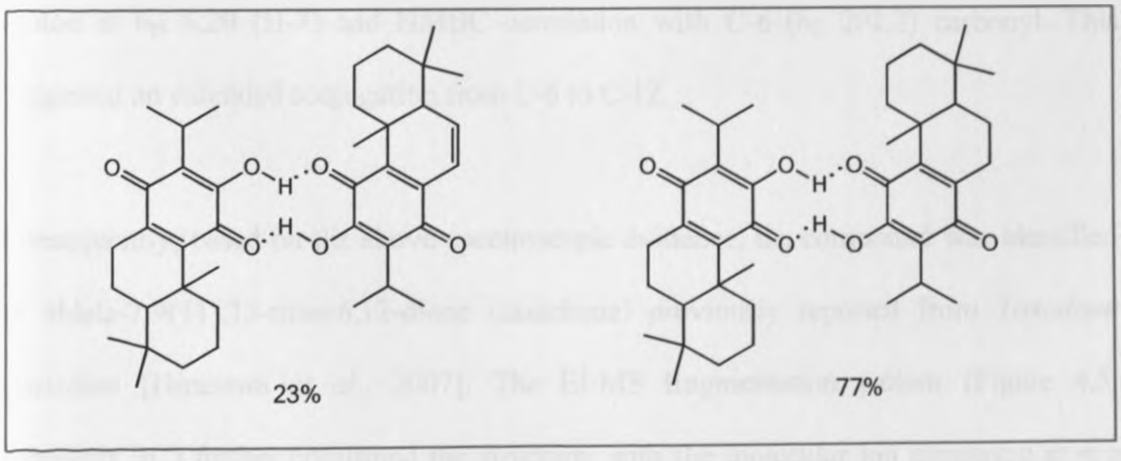


Figure 4.4: Hydrogen bonding between royleanone (**21**) and 6,7-Dehydroroyleanone (**221**) molecules

4.1.1.4 Taxodione

Compound **222** was isolated as yellow solid and gave a molecular ion peak of 314 on EI-MS analysis. The ^{1}H-NMR (Table 4.2, Appendix 3A) showed among other signals two singlet olefinic protons (δ_H 6.20 and 6.92, both 1H singlets), signals for isopropyl unit (two 3H doublets at δ_H 1.15 & 1.16 and 1H septet at δ_H 3.05, J = 6.8) as well as three methyl singlets at δ_H 1.10, 1.249 and 1.253.

The ^{13}C-NMR (Table 4.4, Appendix 3B) gave twenty signals for twenty carbons of an abietane diterpenoid, which included isopropyl signals (δ_C 21.4*q*, 21.7*q* and 27.6*d*) and three other methyls (δ_C 22.0*q*, 22.2*q* and 33.4*q*). Two conjugated carbonyls were observed at δ_C 182.1 and 201.2. The carbonyl at 182.1 had HMBC correlation with the isopropyl 1H septet and an exchangeable proton of 11-OH suggesting that the carbonyl was at C-12 where as the carbonyl at 201.2 had HMBC correlation with a characteristic H-5 singlet at δ_H 2.60 implying that the carbonyl was at C-6. The olefinic proton at δ_H 6.92 (H-14) had HMBC correlation with C-12 (δ_C 182.1) carbonyl where as the olefinic proton at δ_H 6.20 (H-7) had HMBC correlation with C-6 (δ_C 201.2) carbonyl. This suggested an extended conjugation from C-6 to C-12.

Consequently, based on the above spectroscopic evidence, the compound was identified as abieta-7,9(11),13-trien-6,12-dione (taxadione) previously reported from *Taxodium distichum* [Hirasawa *et al.*, 2007]. The EI-MS fragmentation pattern (Figure 4.5, Appendix 3C) further confirmed the structure, with the molecular ion appearing at *m/z* 314 ($C_{20}H_{26}O_3$), loss of isopropyl giving a fragment at *m/z* 271 and *retro* Diels-Alder fragmentation resulting to the base peak at *m/z* 149.

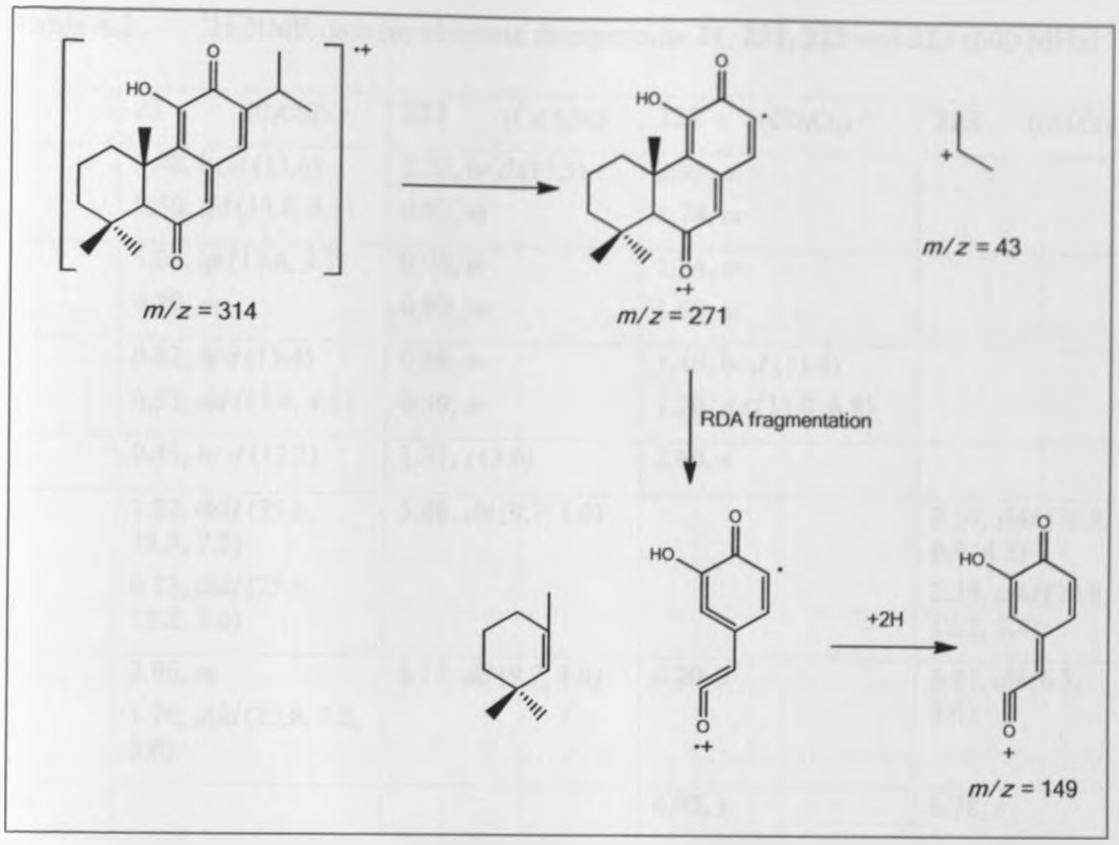


Figure 4.5: Fragmentation pattern for taxadione (**222**)

4.1.1.5 11-Hydroxy-7,9(11),13-abietatrien-12-one

Compound **223** was isolated from the roots of *Clerodendrum eriophyllum* as pale yellow crystals. Its NMR (Tables 4.2 and 4.4) features resembled those of taxadione (**222**), the difference being absence of a carbonyl at C-6 which resulted to major difference in ^{13}C-NMR value at this carbon (δ_C 201.2 for **222** and 26.1 for **223**) as well as differences for ^{13}C-NMR values for C-5, C-7 and C-8 (Table 4.4). The compound was therefore identified as 11-hydroxy-7,9(11),13-abietatrien-12-one. These spectroscopic data are identical to those previously reported for the same compound isolated from *Plectranthus elegans* [Dellar *et al.*, 1996].

Table 4.2: ^{1}H-NMR data for abietane diterpenoids **21**, **221**, **222** and **223** (600 MHz)

	21 ($C_2Cl_4D_2$)	**221** ($C_2Cl_4D_2$)	**222** (CD_2Cl_2)	**223** (CD_2Cl_2)
1	2.08, br *d* (13.6) 0.50, *dd* (13.6, 4.1)	2.22, br *d* (13.3) 0.83, *m*	2.93, *m* 1.74, *m*	
2	1.07, *qt* (13.6, 3.3) 0.89, *m*	0.98, *m* 0.89, *m*	1.74, *m* 1.58, *m*	
3	0.82, *brd* (13.4) 0.53, *dd* (13.4, 4.1)	0.86, *m* 0.59, *m*	1.40, br *d* (11.4) 1.20, *dd* (11.0, 6.8)	
5	0.45, br *d* (12.2)	1.51, *t* (3.0)	2.60, *s*	
6	1.22, *ddd* (25.0, 13.3, 7.2) 0.73, *ddd* (25.0, 12.2, 5.6)	5.86, *dd* (9.7, 3.0)		2.59, *ddd* (20.8, 6.6, 4.2) 2.38, *ddd* (20.8, 12.0, 3.4)
7	2.06, *m* 1.70, *ddd* (20.8, 7.2, 5.6)	6.15, *dd* (9.7, 3.0)	6.20, *s*	6.81, *dd* (6.8, 3.4)
14			6.92, *s*	6.78, *s*
15	2.49, *sept* (7.0)	2.49, *sept* (7.0)	3.05, *sept d* (6.8, 1.0)	3.10, *m*
16	0.58, *d* (7.0)	0.59, *d* (7.0)	1.15, *d* (6.8)	1.13, *d* (6.8)
17	0.57, *d* (7.0)	0.58, *d* (7.0)	1.16, *d* (6.8)	1.15, *d* (6.8)
18	0.29, *s*	0.35, *s*	1.10, *s*	0.93, *s*
19	0.26, *s*	0.38, *s*	1.25, *s*	0.97, *s*
20	0.61, *s*	0.40, *s*	1.25, *s*	1.18, *s*
11-OH			7.60, *s*	
12-OH	6.60, *s*	6.71, *s*		

4.1.1.6 Ferruginol

Compound **224** was isolated as pale yellow oil, having a molecular ion peak at *m/z* 286 ($C_{20}H_{30}O$, Appendix 5C). The ^{13}C-NMR (Table 4.4, Appendix 5B) and DEPT spectra showed showed twenty signals of which five were methyls, five methylenes, four

methines and six quaternary carbons. Six of the twenty carbons appeared on the aromatic region (δ_C 111.5, 127.2, 127.9, 132.1, 149.4 and 151.4) assigned to an aromatic ring with two protonated carbons (111.5 and 127.2). The ^{1}H-NMR (Table 4.3, Appendix 5A) showed two aromatic singlets (δ_H 6.60 and 6.81) implying that the two were *para* protons. An isopropyl group attached to aromatic ring was evident from two 3H doublets at δ_H 1.19 and 1.21 as well as a 1H septet at 3.10. Three singlet methyls were observed from 3H singlets at δ_H 0.92, 0.94 and 1.15.

It was evident that this was an abietane diterpenoid with ring C being aromatic and C-12 substituted with a hydroxyl group observed from an exchangeable proton signal (δ_H 4.67) showing HMBC correlation with C-12 and C-13. The compound was therefore identified as 12-hydroxyabieta-8,11,13-triene (ferruginal) previously reported from *Cryptomeria japonica* [Kondo *et al.*, 1963] and *Salvia syriaca* [Ulubelen *et al.*, 2000]. The structure was further confirmed by the EI-MS fragmentation pattern (Figure 4.6).

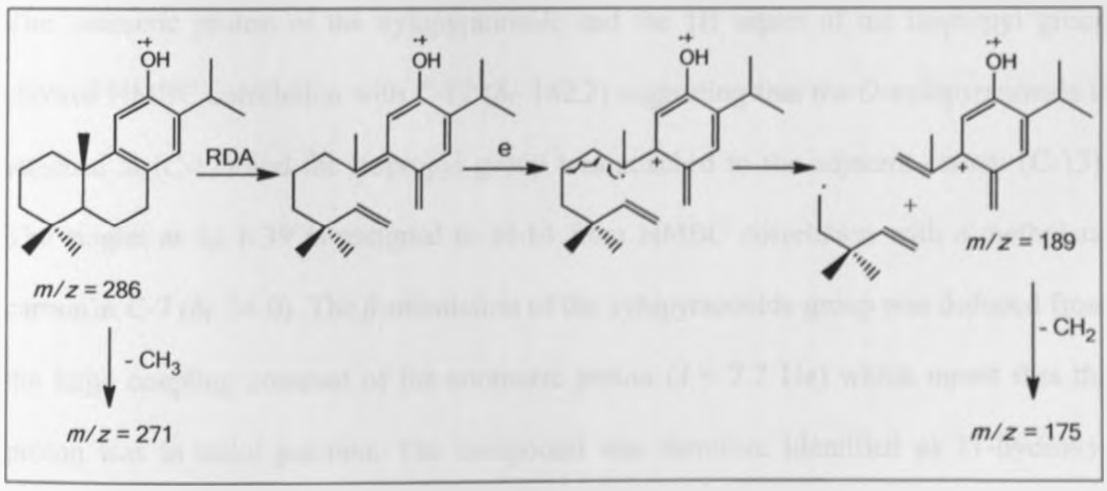


Figure 4.6: Fragmentation pattern for ferruginal (**224**)

4.1.1.7 11-Hydroxy-8,11,13-abietatriene-12-O-β-xylopyranoside (225)

Compound **225** was isolated as white crystals and gave a weak EI-MS molecular ion peak at *m/z* 435 [M+H] ($C_{25}H_{38}O_6$). The ^{13}C-NMR (Table 4.4, Appendix 6B) showed twenty-five carbon signals among them signals for a five-carbon sugar unit at δ_C 68.2, 70.1, 73.0, 74.6 and 108.6. The sugar unit was deduced to be xylopyranoside through comparison of NMR data with literature values [Han et al., 2008].

The remaining twenty carbon signals included six in the aromatic region. The signals at δ_C 142.2 and 149.0 implied adjacent oxygenated sp^2 carbons of benzene ring. The ^{1}H-NMR (Table 4.3, Appendix 6A) had one proton singlet in the aromatic region, implying that only one of the aromatic carbons was protonated. The ^{1}H-NMR also had signals for isopropyl unit on the aromatic ring (two 3H doublets at δ_H 1.10 and 1.11 as well as 1H septet at 3.61, J = 6.9 Hz) in addition to three methyl singlets (δ_H 0.94, 0.96, 1.30) being indicative of abietane diterpenoid with ring C being aromatic.

The anomeric proton of the xylopyranoside and the 1H septet of the isopropyl group showed HMBC correlation with C-12 (δ_C 142.2) suggesting that the *O*-xylopyranoside is attached at (C-12) and the isopropyl group was attached to the adjacent carbon (C-13). The singlet at δ_H 6.39 is assigned to H-14 from HMBC correlation with a methylene carbon at C-7 (δ_C 34.0). The *β*-orientation of the xylopyranoside group was deduced from the large coupling constant of the anomeric proton (J = 7.7 Hz) which meant that the proton was in *axial* position. The compound was therefore identified as 11-hydroxy-8,11,13-abietatriene 12-*O*-*β*-xylopyranoside previously reported from *Avicennia marina*

[Han *et al.*, 2008]. The EI-MS fragment at *m/z* 302 represented the aglycone (Figure 4.7, Appendix 6C).

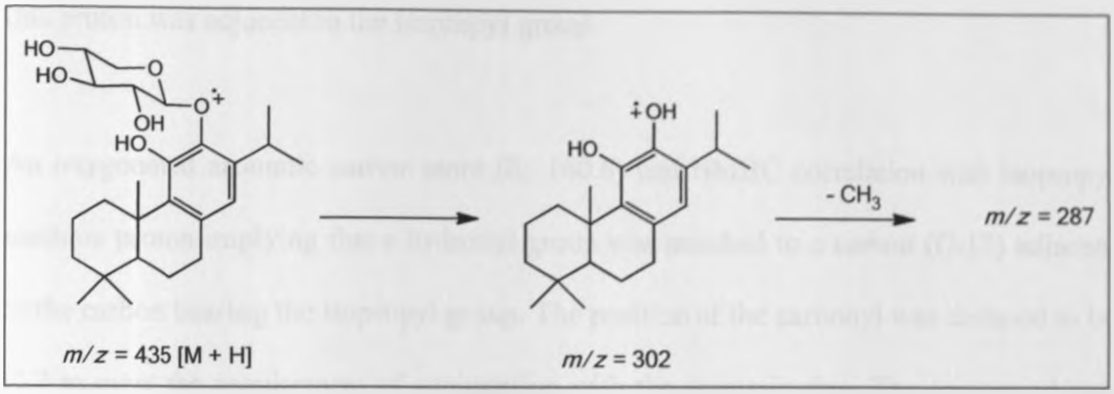


Figure 4.7: Fragmentation pattern for 11-hydroxy-8,11,13-abietatriene 12-*O*-*β*-xylopyranoside (**225**)

4.1.1.8 Sugiol

Compound **9** was isolated as white crystals; analysis by EI-MS showed a molecular ion peak at *m/z* 300 which also happened to be the base peak signifying the stability of the molecule. The ^{13}C-NMR spectrum (Table 4.4, Appendix 7B) had twenty carbon signals for a diterpenoid, amoung them a carbonyl at δ_C 197.1 and six signals in the aromatic region. The chemical shift of the carbonyl (δ_C 197.1) is suggestive of conjugation and its HMBC correlation with an aromatic proton (δ_H 7.80, *s*) imply that it is conjugated to the aromatic ring.

That this compound had an abietane skeleton was deduced from the ^{1}H-NMR isopropyl signals (two 3H doublets at δ_H 1.21 and 1.23 as well as 1H septet at δ_H 3.26, J = 6.9) accompanied by three methyl singlets of for H-18, H-19 and H-20 (δ_H 0.95, 1.10 and 1.22

respectively). The aromatic region of the ^{1}H-NMR spectrum had two singlets (δ_H 6.87 *s* and 7.80 *s*) signaling *para* protons of a tetrasubstituted benzene ring. The signal at δ_H 7.80 proton had ^{3}J HMBC correlation with the isopropyl methine carbon suggesting that this proton was adjacent to the isopropyl group.

An oxygenated aromatic carbon atom (δ_C 160.6) had HMBC correlation with isopropyl methine proton implying that a hydroxyl group was attached to a carbon (C-12) adjacent to the carbon bearing the isopropyl group. The position of the carbonyl was deduced to be C-7 to meet the requirement of conjugation with the aromatic ring. The compound was therefore identified as abieta-8,11,13-trien-7one (trival name sugiol) with molecular formula $C_{20}H_{28}O_2$ consistent with EI-MS molecular ion. The compound was previously reported from *Caryopteris incana* [Gao and Han, 1997].

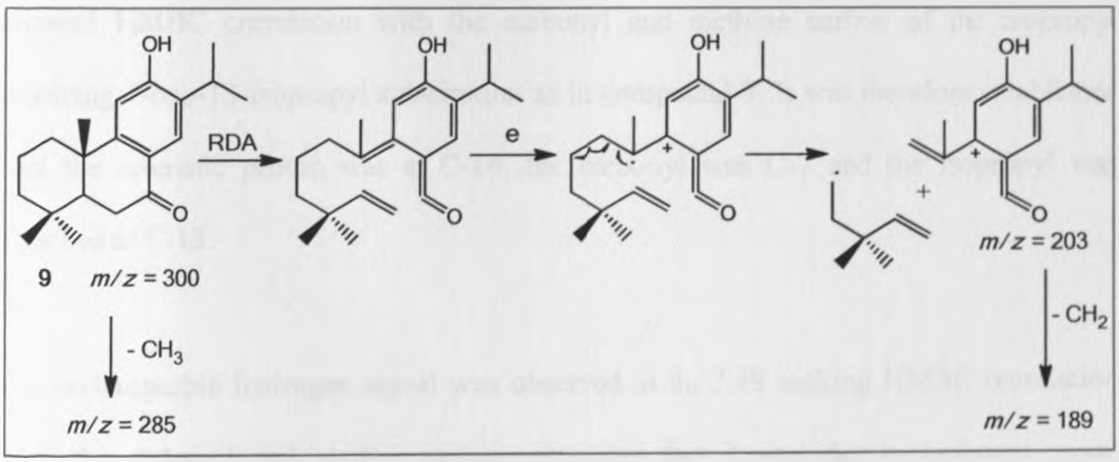


Figure 4.8: Fragmentation pattern for sugiol (**9**)

4.1.1.9 6-Hydroxysalvinolone

Compound **226** was isolated as pale yellow crystals from the roots of *Clerodendrum eriophyllum* with molecular ion peak at *m/z* 330 on EI-MS spectrum (Appendix 8C). The ^{13}C-NMR spectrum of the compound (Table 4.4, Appendix 8B) had twenty signals for a diterpene constititing an aromatic ring (δ_C 116.4, 121.7, 135.1, 139.2, 143.4, 148.1), two olefinic carbons (δ_C 142.8, 143.8) conjugated to a carbonyl (δ_C 180.5) and the rest being sp^3 carbons including five methyls. From HSQC experiment, all the aromatic carbons were quartenary except for one carbon (δ_C 116.4, δ_H 7.66 s).

The ^{1}H-NMR spectrum (Table 4.3, Appendix 8A) showed among other signals the aromatic proton (δ_H 7.66 s), an isopropyl unit attached to aromatic ring (δ_H 1.24 *d J* = 7.0, 1.26 *d J* = 7.0, 3.35 *sept J* = 7.0) and three more singlet methyls (δ_H 1.45, 1.47, 1.70) all of which are indicative of abietane diterpenoid. The aromatic proton (H-14, δ_H 7.66 *s*) showed HMBC correlation with the carbonyl and methine carbon of the isopropyl implying 7-oxo-13-isopropyl substitution as in compound **9**. It was therefore established that the aromatic proton was at C-14, the carbonyl was C-7 and the isopropyl was attached at C-13.

An exchangeable hydrogen signal was observed at δ_H 7.49 making HMBC correlation with the carbonyl and olefinic carbons implying that it was due to hydroxyl group attached at C-6 and that C-5 and C-6 were the olefinic carbons. Two more hydroxyl groups were on the aromatic ring at C-11 and C-12 of δ_C 143.4 and 148.1 respectively. The compound was therefore identified as 6,11,12-trihydroxyabieta-5,8,11,13-tetraene-7-one (6-hydroxysalvinolone) previously reported from the roots of *Salvia monthretii*

[Topcu and Ulubelen., 1996]. The structure of the compound was confirmed by EI-MS hypothetical fragmentation pattern shown in (Figure 4.9)

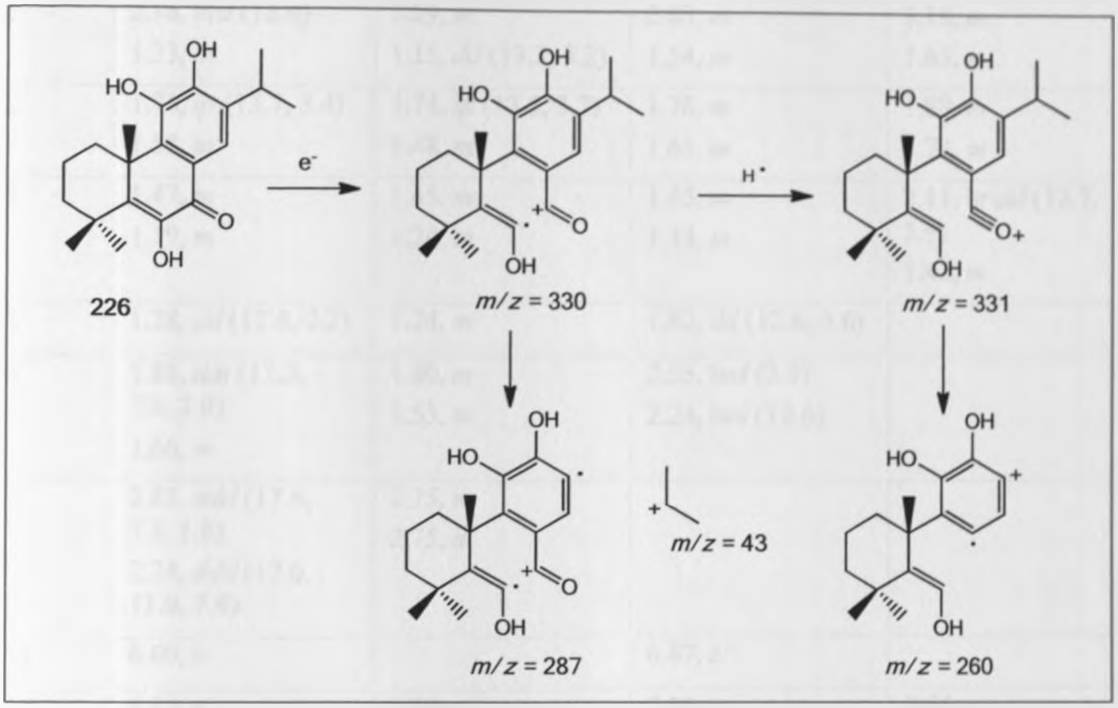


Figure 4.9: Fragmentation pattern for 6-hydroxysalvinolone (**226**)

Table 4.3: ^{1}H-NMR data for abietane diterpenoids **224**, **225**, **9** (600 MHz) and **226** (300 MHz)

	224 (CD_2Cl_2)	**225** (MeOD)	**9** (Acetone-d_6)	**226** (Acetone-d_6)
1	2.16, br*d* (12.6) 1.33, *m*	3.29, *m* 1.15, *dd* (13.3, 3.2)	2.83, *m* 1.54, *m*	3.18, *m* 1.63, *m*
2	1.74, *qt* (13.7, 3.4) 1.58, *m*	1.74, *qt* (13.5, 3.3) 1.48, *m*	1.76, *m* 1.65, *m*	1.89, *m* 1.74, *m*
3	1.47, *m* 1.19, *m*	1.45, *m* 1.24, *m*	1.45, *m* 1.34, *m*	2.11, br *dd* (12.7, 5.9) 1.42, *m*
5	1.28, *dd* (12.4, 2.2)	1.24, *m*	1.82, *dd* (12.6, 3.6)	
6	1.85, *ddt* (13.3, 7.4, 2.0) 1.66, *m*	1.80, *m* 1.53, *m*	2.55, br*d* (3.5) 2.24, br*d* (12.6)	
7	2.83, *ddd* (17.6, 7.4, 1.8) 2.74, *ddd* (17.6, 11.0, 7.4)	2.75, *m* 2.75, *m*		
11	6.60, *s*		6.87, *s*	
14	6.81, *s*	6.39, *s*	7.80, s	7.66, *s*
15	3.10, *sept* (6.9)	3.61, *sept* (6.9)	3.26, *sept* (6.9)	3.35, *sept* (7.0)
16	1.21, *d* (6.9)	1.10, *d* (6.9)	1.21, *d* (6.9)	1.26, *d* (7.0)
17	1.19, *d* (6.9)	1.11, *d* (6.9)	1.23, *d* (6.9)	1.24, *d* (7.0)
18	0.94, *s*	0.96, *s*	0.95, *s*	1.45, *s*
19	0.92, *s*	0.94, *s*	1.10, *s*	1.47, *s*
20	1.15, *s*	1.30, *s*	1.22, *s*	1.70, *s*
1′		4.30, *d* (7.7)		
2′		3.82, *dd* (9.5, 7.7)		
3′		3.57, *dd* (9.5, 3.5)		
4′		3.81, *dd* (3.5, 1.5)		
5′		3.91, *dd* (12.7, 2.2) 3.51, *dd* (12.7, 1.2)		
6-OH				7.49, *s*
12-OH	4.67, br*s*			

Table 4.4: ^{13}C-NMR data for abietane diterpenoids from *C. eriophyllum*

	21[a] $C_2D_2Cl_4$	**221**[a] $C_2D_2Cl_4$	**222**[a] CD_2Cl_2	**223**[d] $CDCl_3$	**224**[a] CD_2Cl_2	**225**[a] MeOD	**9**[a] Acetone	**226**[c] Acetone	**227**[a] Acetone	**228**[a] Acetone	**19**[b] $CDCl_3$
1	35.7	34.6	37.4	37.0	39.6	37.8	38.8	30.6	30.2	37.3	30.3
2	18.4	18.1	18.9	19.1	20.0	20.4	19.7	18.4	18.2	19.6	29.2
3	40.7	39.9	42.8	41.9	42.3	42.9	42.2	37.2	37.0	43.2	136.2
4	32.9	32.6	33.1	33.8	34.0	34.8	33.9	37.0	36.9	34.7	125.3
5	51.0	51.4	63.1	50.7	51.1	54.9	50.6	142.8	142.6	56.2	165.4
6	16.4	139.5	201.2	26.1	19.8	20.4	36.5	143.8	143.7	73.5	118.5
7	26.2	120.4	134.3	147.4	30.4	34.0	197.1	180.5	180.3	200.3	190.1
8	145.5	137.9	140.3	131.8	127.9	135.0	124.6	121.7	121.1	122.4	109.3
9	145.9	139.9	125.9	127.7	149.4	134.4	157.0	139.2	138.6	138.7	140.5
10	37.8	38.6	43.2	38.9	38.1	40.7	38.7	41.5	41.4	41.8	39.6
11	182.8	185.6	145.6	144.1	111.5	149.0	110.4	143.4	144.0	144.5	131.0
12	149.9	150.6	182.1	181.6	151.4	142.2	160.6	148.1	148.7	149.9	154.1
13	123.0	121.9	145.4	140.7	132.1	140.4	133.8	135.1	131.1	130.6	111.0
14	187.0	182.8	136.6	136.3	127.2	117.7	126.5	116.4	118.4	119.8	153.8
15	23.5	23.5	27.6	26.8	27.4	26.7	27.5	27.5	39.1	39.0	34.5
16	19.4	19.4	21.7	21.7	23.0	24.3	22.7	23.0	69.0	68.9	83.0
17	19.5	19.5	21.4	22.0	23.1	23.9	22.8	23.2	16.0	15.9	22.0
18	32.8	32.1	33.4	33.5	33.7	34.4	33.0	28.5	28.3	36.3	20.7
19	21.2	22.3	22.2	22.3	22.0	22.7	21.7	27.6	27.5	22.9	14.9
20	19.5	14.6	22.0	18.7	25.1	20.0	23.6	28.0	27.6	19.2	22.1
1′						108.6					
2′						73.0					
3′						74.6					
4′						70.1					
5′						68.2					

Key: a = 150 MHz, **b** = 125 MHz, **c** = 75 MHz, **d** = 50 MHz

4.1.1.10 6,16-Dihydroxysalvinolone

Compound **227** was isolated from the roots of *Clerodendrum eriophyllum* as pale yellow crystals with EI-MS molecular ion at *m/z* 346 consistent with molecular formula $C_{20}H_{26}O_5$. NMR data (Tables 4.4, 4.5) were similar to those discussed for 6-hydroxysalvinolone (**226**), the only difference being on the isopropyl group where one of two methyl groups was oxidized to methylene (δ_C 69.0; δ_H 3.99 *dd J* = 10.2 Hz & 3.0 Hz, 3.82 *dd J* = 10.2 Hz & 7.0 Hz). The methylene protons showed HMBC correlation with C-15 (δ_C 39.1), C-15 (δ_C 16.0) and C-13 (δ_C 131.1), implying that it was the methyl group of an isopropyl that was oxidized to hydroxymethylene.

Therefore the compound was 6,11,12,16-tetrahydroxyabieta-5,8,11,13-tetraen-7-one (6,16-dihydroxysalvinolone). The loss of oxidized isopropyl group and carbonyl gave a fragment at *m/z* 259 (Figure 4.10, Appendix 9C). The compound was previously reported from *Avicennia marina* [Han *et al.*, 2008].

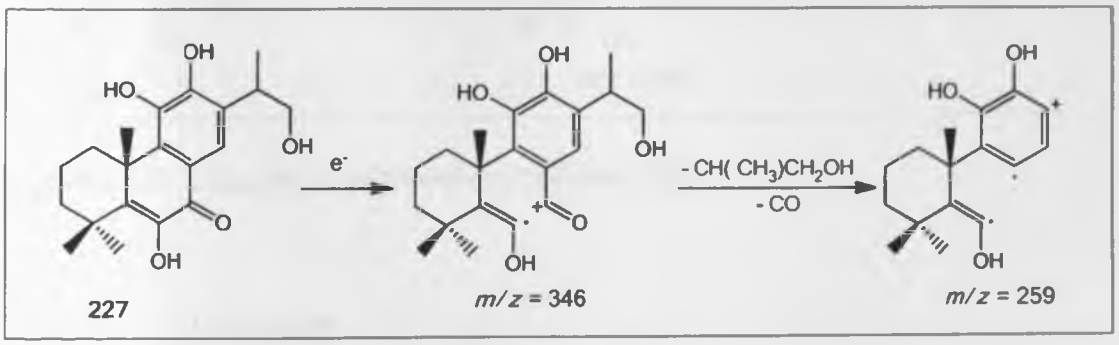


Figure 4.10: Fragmentation pattern for 6,16-dihydroxysalvinolone (**227**)

4.1.1.11 Nellionol

Compound **228** was isolated as white crystals and had almost similar NMR features with 6,16-dihydroxysalvinolone (**227**), except that the signals of olefinic group at 5 and 6 were replaced with signals for saturated carbons (δ_C 56.2 and 73.5) with their respective protons appearing at δ_H 1.76 (*d*, 13.4 Hz) and 4.58 (*dd*, 13.4 and 2.6 Hz). The coupling constant of 13.4 for H-5 and H-6 meant that two protons were in axial positions, a fact used to establish the relative stereochemistry at position 6. The EI-MS molecular ion peak at *m/z* 348 (Appendix 10C) was 2H units more than that of **227**, giving further proof that this compound is a 5,6-dihydroderivative of **227**. The compound was therefore identified as 6,11,12,16-tetrahydroxyabieta-8,11,13-trien-7-one (trival name nellionol) previously reported from *Premna latifolia* [Rao *et al.*, 1979].

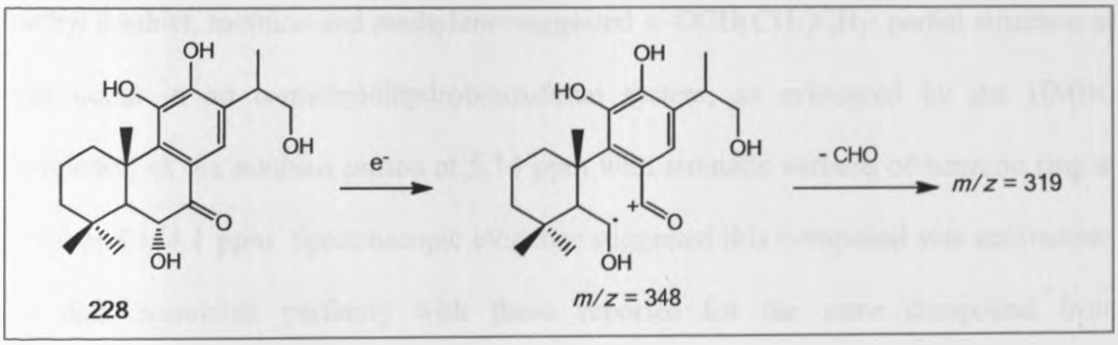


Figure 4.11: Fragmentation pattern for nellionol (**228**)

5.1.1.12 Uncinatone

Compound **19** was isolated as orange crystals. A total of twenty signals were present in the ^{13}C-NMR spectrum (Table 4.4, Appendix 11B) suggesting a diterpenoid, amoung them signals for an α-methyldihydrobenzofuran, ie six quartenary carbons of benzene ring and signals at 83.0*d*, 34.5*t* and 22.0*q* for a -OCH(CH_3)CH_2- moiety, which was

suspected to be a result of rearrangement and cyclization of isopropyl group observed in abietane diterpenoids. A conjugated carbonyl was observed at 190.1 ppm, established to be at C-7 of abietane skeleton from chelation with 14-OH as well as HMBC correlation with the olefinic H-5 at 6.24 ppm.

The ^{1}H-NMR spectrum (Table 4.5, Appendix 11A) showed a strongly H-bonded OH group at δ_H 13.74 ppm, another OH group at δ_H 4.97 ppm, an olefinic proton signal at δ_H 6.24 ppm, together with signals at δ_H 1.90 and 1.93 ppm, typical for non-equivalent vinylic CH_3 groups established by HMBC to be on adjacent carbons. Further two CH_3 groups were observed, one at δ_H 1.52 ppm and the other giving rise to a 6.5 Hz doublet at δ_H 1.54 ppm coupling to a 1H methine proton at 5.14 ppm, which was in turn coupling with two methylene protons at δ_H 2.91 and 3.42 ppm. The connection of the above methyl doublet, methine and methylene suggested a -$OCH(CH_3)CH_2$- partial structure as may occur in an α-methyldihydrobenzofuran system, as evidenced by the HMBC correlation of the methine proton at 5.14 ppm with aromatic carbons of benzene ring at 111.0 and 154.1 ppm. Spectroscopic evidence suggested this compound was uncinatone, the data correlated perfectly with those reported for the same compound from *Clerodendrum uncinatum* [Dorsaz *et al.*, 1985].

Table 4.5: ^{1}H-NMR for abietane diterpenoids **227**, **228** (600 MHz) and **19** (500 MHz)

	227 Acetone-d_6	**228** Acetone-d_6	**19** Acetone-d_6
1	3.20, *ddd* (15.6, 9.4, 6.1) 1.62, *ddd* (15.0, 9.4, 5.8)	3.33, *m* 1.39, *m*	3.26, *dd* (13.0, 5.0) 1.59, *dd* (13.0, 6.0)
2	1.88, *m* 1.72, *m*	1.76, *m* 1.54, *m*	2.52, *m* 2.23, *dd* (18.5, 4.5)
3	2.09, *m* 1.43, *ddd* (14.0, 6.2, 3.0)	1.50, *m* 1.31, *m*	
5		1.76, *d* (13.4)	
6		4.58, *dd* (13.4, 2.6)	6.24, *s*
14	7.54, *s*	7.41, *s*	
15	3.23, *qd* (7.0, 3.0)	3.18, *m*	3.42, *dd* (15.5, 9.0) 2.91, *dd* (15.5, 7.5)
16	3.99, *dd* (10.2, 3.0) 3.82, *dd* (10.2, 7.0)	3.97, *dd* (10.0, 3.0) 3.81, *dd* (10.0, 6.5)	5.14, *m*
17	1.35, *d* (7.0)	1.33, *d* (7.2)	1.54, *d* (6.5)
18	1.45, *s*	1.21, *s*	1.93, *s*
19	1.47, *s*	1.25, *s*	1.90, *s*
20	1.69, *s*	1.58, *s*	1.52, *s*
OH	7.50, *s*	4.04, *d* (2.6)	4.97, *s*; 13.7, *s*

4.1.2 Triterpenoids

Two triterpenoids were isolated from the roots of *C. eriophyllum*. These are betulinic acid (**229**) and *β*-amyrin octacosanoate (**230**).

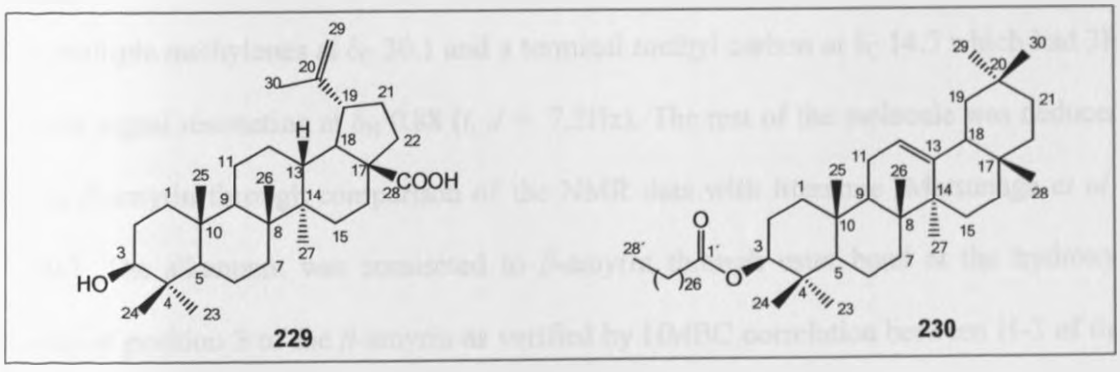


Figure 4.12: Triterpenoids from *Clerodendrum eriophyllum*

4.1.2.1 Betulinic acid

Compound **229** was isolated as white crystals from the roots of *Clerodendrum eriophyllum*. Its EI-MS molecular ion peak at *m/z* 456 corresponded to molecular formula of $C_{30}H_{48}O_3$ (Appendix 12C). The ^{13}C-NMR (Table 4.6, Appendix 12B) gave thirty signals for a triterpene including a carboxylic carbonyl (δ_C 177.2) and terminal alkene (δ_C 150.3 *s*, 109.6 *t*). The ^{1}H-NMR (Appendix 12A, Table 4.6) showed six methyl groups, all singlets, two protons in the olefinic region for the terminal alkene carbon (δ_H 4.68 *d J* = 1.8, 4.55 br*s*) and a characteristic H-3 proton (δ_H 2.95 m) of triterpenes with OH at C-3.

Using combination of 1D ^{1}H-NMR, ^{13}C-NMR and DEPT as well as 2D HMBC, HSQC and COSY, the compound was identified as betulinic acid, widely distributed in the Verbenaceae family. The spectroscopic data correlated with those reported for the same compound from leaves of *Nerium oleander* [Siddiqui *et al.*, 1988].

4.1.2.1 β-amyrin octacosanoate

Compound **230** was isolated as white solids from the roots of *Clerodendrum eriophyllum*. The ^{13}C-NMR (Table 4.7, Appendix 13B) had more than forty signals; the presence of a long alkanoate chain being evident from the ester carbonyl at δ_C 173.7 ppm, intense peak for multiple methylenes at δ_C 30.1 and a terminal methyl carbon at δ_C 14.3 which had 3H proton signal resonating at δ_H 0.88 (*t*, *J* = 7.2Hz). The rest of the molecule was deduced to be *β*-amyrin through comparison of the NMR data with literature [Matsunaga *et al.*, 1988]. The alkanoate was connected to *β*-amyrin through ester bond at the hydroxyl group of position 3 of the *β*-amyrin as verified by HMBC correlation between H-3 of the *β*-amyrin at δ_H 4.46 (dd, J = 10.0, 5.8 Hz) and the alkanoate carbonyl at δ_C 173.7.

The alkanoate was identified to be octacosanoate by EI-MS molecular ion at *m/z* 833 $[M+H]^+$, *retro* Diels-Alder fragmentation at *m/z* 614 and acyllium ion at *m/z* 408 (Figure 4.13 and Appendix 13C). The compound was therefore identified as *β*-amyrin octacosanoate previously found in bast fibres of industrial hemp *Cannabis sativa* [Gutierrez *et al.*, 2006].

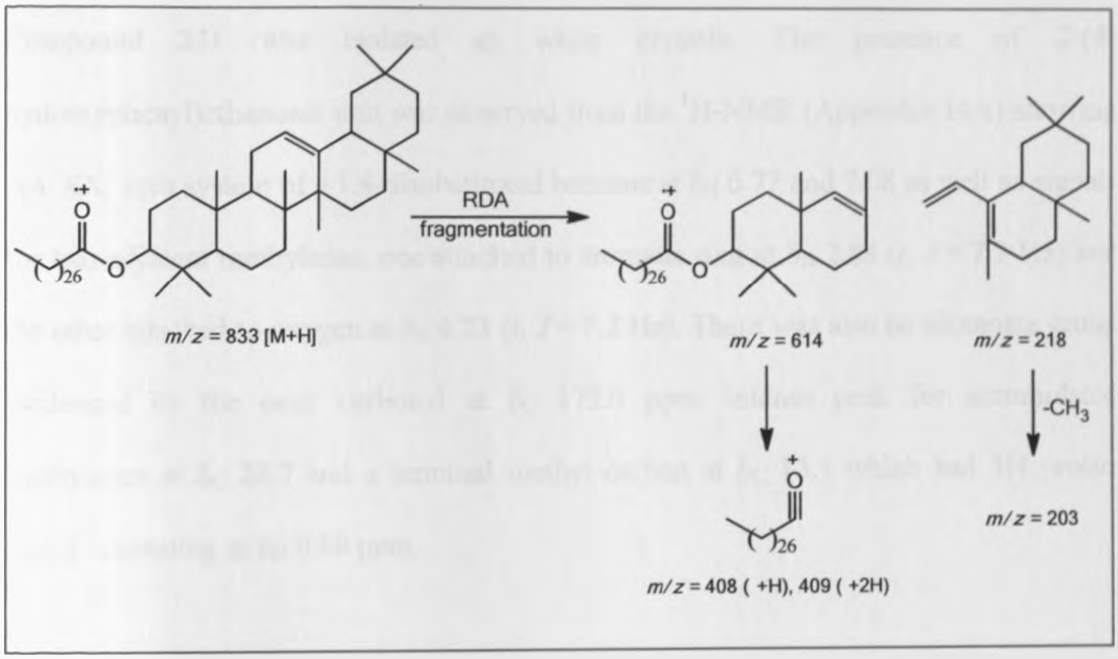
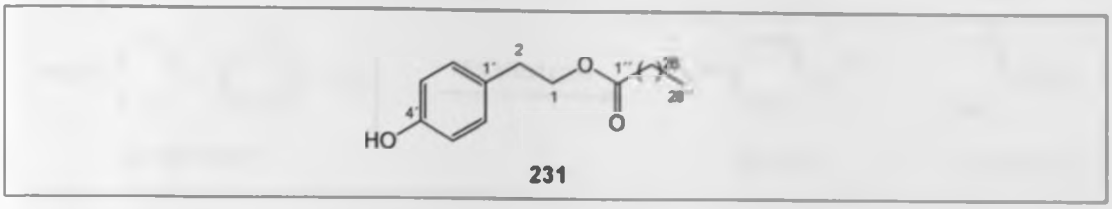


Figure 4.13: Fragmentation pattern for *β*-amyrin octacosanoate **(230)**

4.1.3 Tyrosol derivative

One tyrosol derivative was isolated from *C. eriophyllum*, namely tyrosyl octacosanoate (bongardol) **(231)**



231

Figure 4.14: Structure of bongordol (**231**) from *Clerodendrum eriophyllum*

4.1.3.1 Bongardol

Compound **231** was isolated as white crystals. The presence of 2-(4-hydroxyphenyl)ethanoate unit was observed from the ^{1}H-NMR (Appendix 14A) showing AA′XX′ spin system of a 1,4-disubstituted benzene at δ_H 6.77 and 7.08 as well as signals for two adjacent methylenes, one attached to aromatic ring at δ_H 2.88 (*t*, *J* = 7.2 Hz) and the other attached to oxygen at δ_H 4.23 (*t*, *J* = 7.2 Hz). There was also an alkanoate group evidenced by the ester carbonyl at δ_C 173.0 ppm, intense peak for accumulated methylenes at δ_C 28.7 and a terminal methyl carbon at δ_C 13.1 which had 3H proton signal resonating at δ_H 0.88 ppm.

That 2-(4-hydroxyphenyl)ethanoate was esterified to the alkanoate was established from HMBC correlation of methylene proton signal at δ_H 4.23 and the ester carbonyl. The alkanoate was identified as octacosanoate from EI-MS weak molecular ion peak at *m/z* 545 $[M+H]^+$ as well as a peak for octacosanoic acid at *m/z* 425 (+H) resulting from McLaffert rearrangement (Figure 4.15 and Appendix 14C). The compound was therefore identified as 2-(4-hydroxyphenyl)ethanoyloctacosanoate (bongardol), previously from *Bongardia chrysogonum* [Alfatafta *et al.*, 1989].

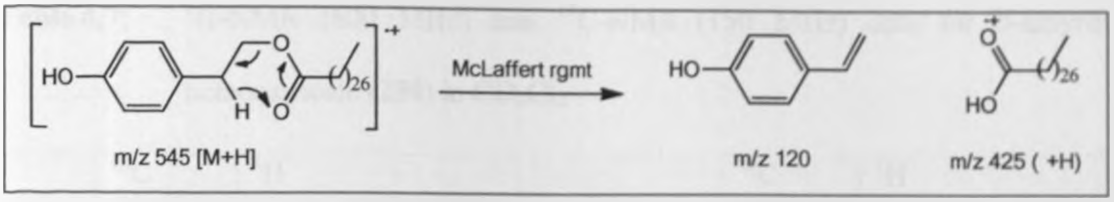


Figure 4.15: Fragmentation pattern for Bongardol (**231**)

Table 4.6: ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data for betulinic acid (**229**) in DMSO

	^{13}C	^{1}H		^{13}C	^{1}H
1	38.3	1.55, *m* 0.82, *m*	16	31.7	2.11, *m* 1.35, *m*
2	27.2	1.41-1.46, *m* 1.41-1.46, *m*	17	55.4	
3	76.8	2.95, *m*	19	48.6	1.51, *t* (11.4, 11.2)
4	38.5		19	46.6	2.95, *m*
5	54.9	0.62, *m*	20	150.3	
6	18.0	1.44, *m* 1.32, *m*	21	30.1	1.80, *m* 1.30, *m*
7	33.9	1.32, *m* 1.32, *m*	22	36.3	1.80, *m* 1.41, *m*
8	40.3		23	28.1	0.86, *s*
9	49.9	1.24, *dd* (12.8, 2.4)	24	15.8	0.64, *s*
10	36.7		25	16.0	0.76, *s*
11	20.5	1.36, *dd* (13.6, 2.6) 1.14, *m*	26	15.7	0.86, *s*
12	25.1	1.61, *m* 0.97, *td* (13.0, 4.4)	27	14.4	0.92, *s*
13	37.6	2.21, *td* (12.6, 3.4)	28	177.2	
14	42.0		29	109.6	4.68, *d* (1.8) 4.55, br*s*
15	29.2	1.39, *m* 1.08, br*d* (12.2)	30	19.0	1.63, *s*
			3-OH		4,27, *d* (5.2)

Table 4.7: ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data for *β*-amyrin octacosanoate (**230**) in CD_2Cl_2

	^{13}C	^{1}H		^{13}C	^{1}H
1	38.6	1.61, *m* 1.07, *m*	20	31.4	
2	23.9	1.90, *m* 1.61, *m*	21	35.1	1.34, *m* 1.10, *m*
3	80.7	4.46, *dd* (10.0, 5.8)	22	37.5	1.43, *dt* (13.6, 3.9) 1.22, *dt* (13.6, 3.2)
4	38.1		23	28.2	0.86, *s*
5	55.5	0.88, *m*	24	16.9	0.86, *s*
6	18.6	1.53, *m* 1.43, *td* (13.8, 3.9)	25	15.7	0.97, *s*
7	32.9	1.55, *m* 1.34, *m*	26	17.0	0.98, *s*
8	40.2		27	26.1	1.14, *s*
9	47.9	1.61, *m*	28	28.6	0.83, *s*
10	37.2		29	33.5	0.87, *s*
11	24.0	1.87, *m* 1.61, *m*	30	23.8	0.87, *s*
12	122.1	5.19, *t* (3.7)	1'	173.7	
13	145.5		2'	35.1	2.27, *td* (7.4, 2.2)
14	42.1		3'	25.5	1.60, *m*
15	26.1	1.77, *td* (13.6, 4.6) 0.97, *m*	4'- 25'	29.5-30.1	1.26, *s* -1.31
16	27.3	2.01, *td* (13.6, 4.6) 0.80, *m*	26'	32.4	1.26, *m*
17	32.8		27'	23.1	1.29, *m*
18	47.6	1.95, *dd* (13.6, 4.6)	28'	14.3	0.88, *t* (7.2)
19	47.1	1.68, *t* (13.6) 1.01, *ddd* (13.6, 4.6, 2.4)			

5.1.4 General observations on compounds of *C. eriophyllum*

The phytochemistry of the plant coincides with that of other species of the genus, giving abietane diterpenoids as the major class of metabolates (twelve abietane diterpenoids). These are biosynthesized through mevalonate pathway, involving cyclization of geranylgeranyipyrophosphate as in Figure 4.16. Out of those, one was a new compound (12-hydroxy-8,12-abietadiene-3,11,14-trione, **220**). Eleven compounds were new to the genus, these are 6,7-dehydroroyleanone (**221**), taxodione (**222**), 11-hydroxy-7,9(11),13-abietatrien-12-one (**223**), ferruginol (**224**), 11-hydroxy-8,11,13-abietatriene *12-O-β*-xylopyranoside (**225**), 6-hydroxysalvinolone (**226**), 6,16-dihydroxysalvinolone (**227**), nellionol (**228**), betulinic acid (**229**), *β*-amyrin octacosanoate (**230**) and bongardol (**231**).

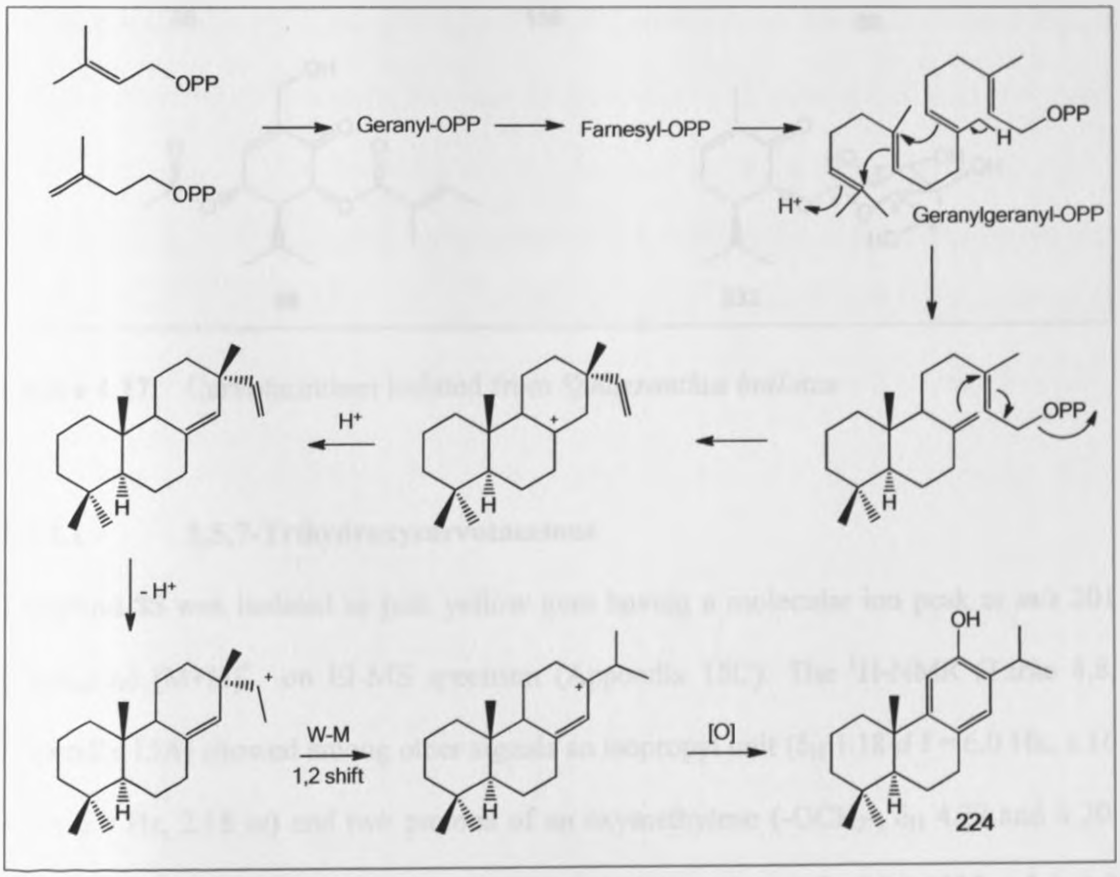


Figure 4.16: Biosynthetic route for abietane diterpenoids

4.2 Secondary metabolites isolated from *Sphaeranthus bullatus*

4.2.1 Carvotacetone derivatives

The aerial parts of *Sphaeranthus bullatus* afforded a total of seventeen compounds, among them five carvotacetone derivatives, which are 3,5,7-trihydroxycarvotacetone (**85**), 3-acetoxy-5,7-dihydroxycarvotacetone (**106**), 3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**), 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**) and 5-*O*-*β*-glucopyranosylcarvotacetone (**232**).

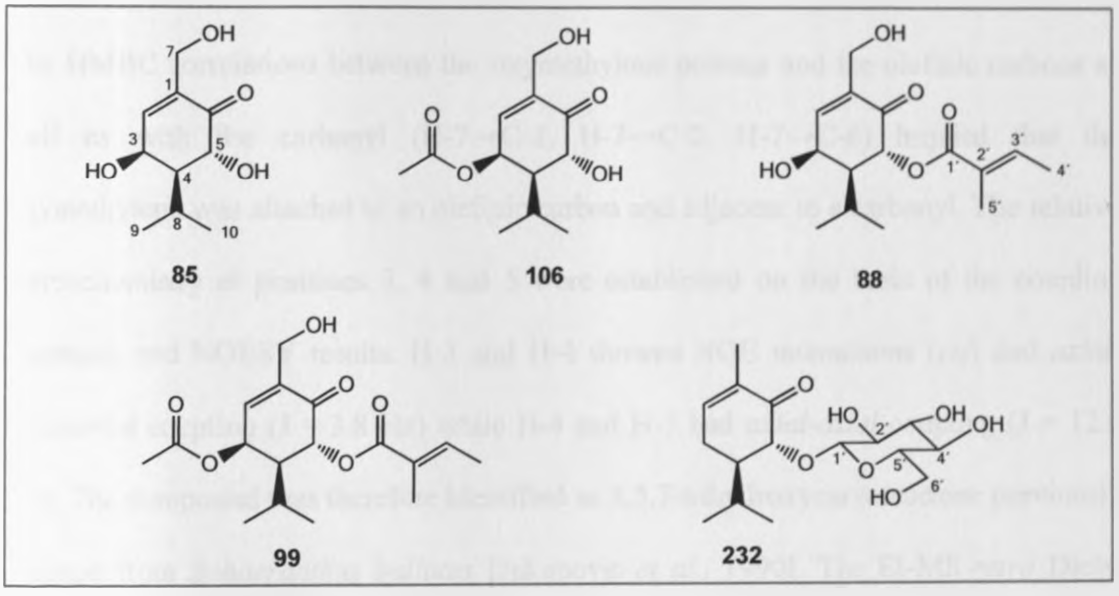


Figure 4.17: Carvotacetones isolated from *Sphaeranthus bullatus*

4.2.1.1 3,5,7-Trihydroxycarvotacetone

Compund **85** was isolated as pale yellow gum having a molecular ion peak at *m/z* 201 ($C_{10}H_{16}O_4$) $[M+H]^+$ on EI-MS spectrum (Appendix 15C). The ^{1}H-NMR (Table 4.8, Appendix 15A) showed among other signals an isopropyl unit (δ_H 1.18 *d* J = 6.0 Hz, 1.16 *d* J = 6.0 Hz, 2.18 *m*) and two protons of an oxymethylene (-OCH_2-, δ_H 4.27 and 4.20, both doublets with J =15.0 Hz) as well as an olefinic proton at δ_H 6.96 (*dd* J = 5.4, 1.8

Hz). The ^{13}C-NMR (Table 4.8, Appendix 15B) had ten signals for ten carbon atoms, including three for the isopropyl unit (δ_C 20.3 *q*, 20.8 *q*, 29.2 *d*) and an oxy-methylene (-OCH_2-, δ_C 59.1). These two groups were connected to 1,4 positions of a six carbon ring containing a carbonyl conjugated to an a double bond (δ_C 202.1, 143.6, 137.1) forming a carvotacetone moiety hydroxylated at positions 3 and 5. The methine proton of the isopropyl group made HMBC correlation with the two hydroxylated carbons (H-8→C-3, H-8→C-5) implying that the isopropyl unit was positioned between hydroxyl groups.

The HMBC correlations between the oxymethylene protons and the olefinic carbons as well as with the carbonyl (H-7→C-1, H-7→C-2, H-7→C-6) implied that the oxymethylene was attached to an olefinic carbon and adjacent to a carbonyl. The relative stereochemistry at positions 3, 4 and 5 were established on the basis of the coupling constants and NOESY results. H-3 and H-4 showed NOE interactions (*cis*) and *axial-equatorial* coupling (J = 3.8 Hz) while H-4 and H-5 had *axial-axial* coupling (J = 12.0 Hz). The compound was therefore identified as 3,5,7-trihydroxycarvotacetone previously isolated from *Sphaeranthus bullatus* [Jakupovic *et al.*, 1990]. The EI-MS *retro* Diels-Alder fragment ions at *m/z* = 114, 96 and 68 (Figure 4.18) confirmed the structure.

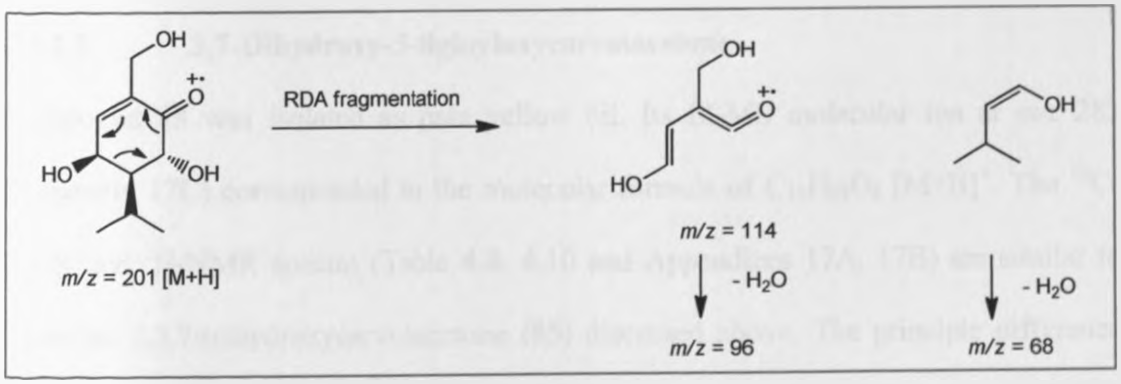


Figure 4.18: Fragmentation pattern for 3,5,7-trihydroxycarvotacetone (**85**)

4.2.1.2 3-Acetoxy-5,7-dihydroxycarvotacetone

Compund **106** was isolated as pale yellow gum. Spectroscopic features resemble those of 3,5,7-trihydroxycarvotacetone (**85**) except for additional acetyl signals at δ_C 170.5 and 21.3 ppm as well as δ_H 2.07 (3H, *s*) making an acetoxy derivative of **85**. The acetoxy group was fixed at C-3 from HMBC correlation of the de-shielded H-3 (δ_H 5.57, *dd* J = 5.8, 3.6 Hz) and the acetyl carbonyl. The presence of acetyl group was also evidenced by EI-MS fragment ion which is also a base peak at *m/z* 43 corresponding to the acetyl group (Figure 4.19, Appendix 16C). The compound was therefore identified as 3-acetoxy-5,7-dihydroxycarvotacetone previously isolated from *Sphaeranthus suaveolens* [Ahmed and Mahmoud, 1997].

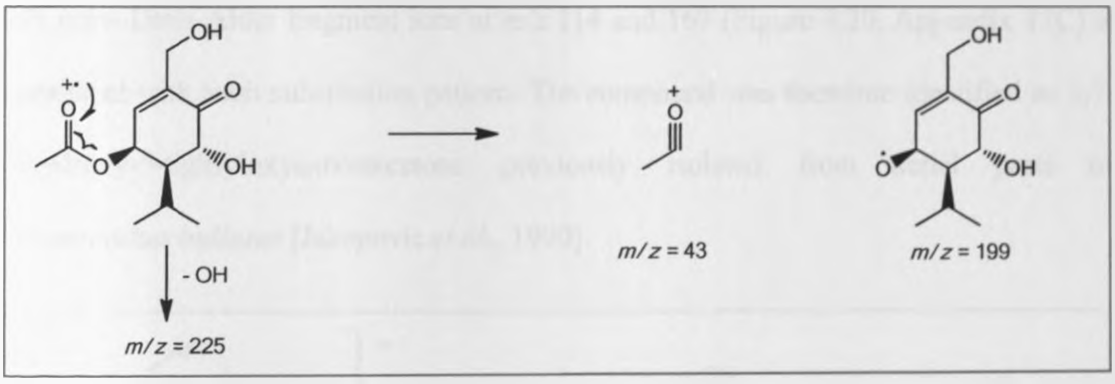


Figure 4.19: Fragmentation pattern for 3-acetoxy-5,7-dihydroxycarvotacetone (**106**)

4.2.1.3 3,7-Dihydroxy-5-tigloyloxycarvotacetone

Compound **88** was isolated as pale yellow oil. Its EI-MS molecular ion at *m/z* 283 (Appendix 17C) corresponded to the molecular formula of $C_{15}H_{22}O_5$ $[M+H]^+$. The ^{13}C-NMR and ^1H-NMR spectra (Table 4.8, 4.10 and Appendices 17A, 17B) are similar to those for 3,5,7-trihydroxycarvotacetone (**85**) discussed above. The principle difference was the presence of additional signals for five more carbon atoms at δ_C 167.7, 138.9,

128.0, 14.4 and 11.9 constituting two sp^3 carbons and a carbonyl conjugated to two olefinic carbons.

The corresponding ^{1}H-NMR indicated that the additional signals constituted two methyl groups (1.82, 3H, *dd*, *J* = 7.2, 1.0 Hz; 1.86, 3H, *d*, *J* = 1.2 Hz) as well as an olefinic proton (6.93, *dq*, *J* = 7.2, 1.2 Hz). These data suggested either a tigloyl group with *cis* methyls or an angeloyl group with *trans* methyls [Zdero *et al.*, 1991]. The NOESY interaction between the two methyls suggested that they were *cis* to each other and hence a tigloyl group. The HMBC correlation of the doublet at 5.76 (H-5) with the tigloyl carbonyl (C-1′, δ_C 167.7) indicated that the tigloyl group was connected to C-5. The EI-MS *retro* Diels-Alder fragment ions at *m/z* 114 and 169 (Figure 4.20, Appendix 17C) is consistent with such substitution pattern. The compound was therefore identified as 3,7-dihydroxy-5-tigloyloxycarvotacetone previously isolated from aerial parts of *Sphaeranthus bullatus* [Jakupovic *et al.*, 1990].

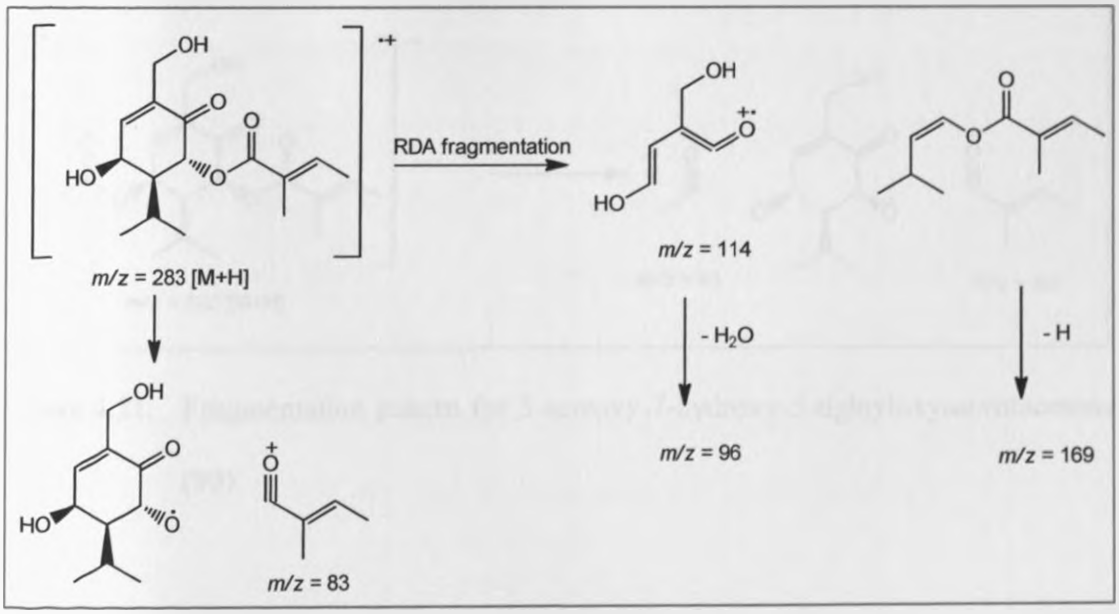


Figure 4.20: Fragmentation pattern for 3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**)

4.2.1.4 3-Acetoxy-7-hydroxy-5-tigloyloxycarvotacetone

Compound **99** was isolated as pale yellow oil with EI-MS $[M+H]^+$ at *m/z* 325 ($C_{17}H_{24}O_6$). The NMR spectroscopic data (Tables 4.8 and 5.10) resembled those for 3,7-dihydroxy-5-tigloyloxycarvotacetone **88** discussed above. There was however additional signals for an acetyl group (δ_C 170.3, 20.9; δ_H 2.08, 3H, *s*). The acetyl carbonyl made HMBC correlation with H-3 (5.65, *dd*, J = 6.0, 3.8 Hz) implying that the acetoxy group was connected to C-3.

The compound was therefore identified as 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone previously isolated from *Sphaeranthus confertifolius* [Zdero *et al.*, 1991]. The presence of acetyl and tiglyl was confirmed by EI-MS fragmentations which gave acyllium ions of the acetyl and tiglyl at *m/z* 43 and 83 respectively (Figure 4.21, Appendix 18C).

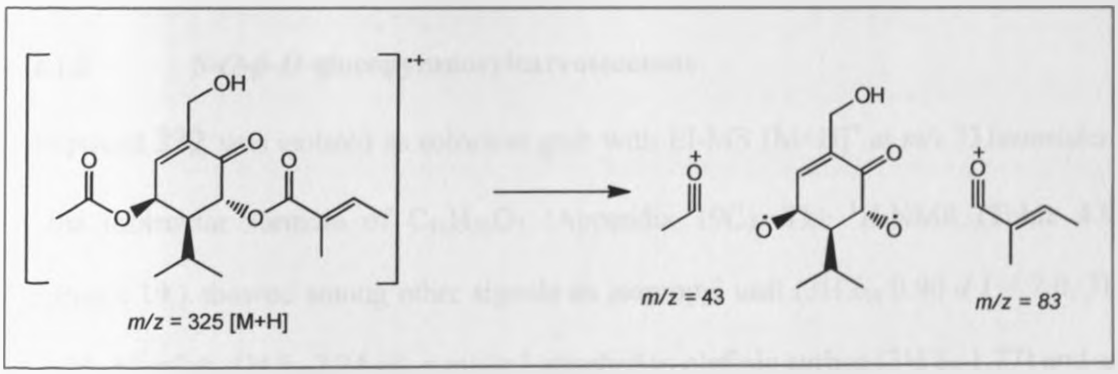


Figure 4.21: Fragmentation pattern for 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone **(99)**

Table 4.8: ^{1}H-NMR data for compounds **85**, **106**, **88** and **99** (600MHz)

	85 Acetone-d_6	**106** CD_2Cl_2	**88** CD_2Cl_2	**99** CD_2Cl_2
2	6.96, *dt* (5.4, 1.8)	6.95, *dt* (5.8, 1.5)	6.90, br*d* (5.6)	6.97, *d* (6.0)
3	4.56, br*s*	5.57, *dd* (5.8, 3.6)	4.58, *dd* (5.6, 3.8)	5.65, *d* (6.0, 3.8)
4	1.78, *ddd* (12.1, 4.5, 3.8)	2.04, *ddd* (12.2, 4.7, 3.6)	2.22, *dt* (12.8, 3.8)	2.48, *dt* (12.6, 3.8)
5	4.51, *d* (12.0)	4.56, *d* (12.2)	5.76, *d* (12.8)	5.75, *d* (12.6)
7	4.27, *d* (15.0) 4.20, *d* (15.0)	4.33, *dd* (14.7, 1.5) 4.30, *dd* (14.7, 1.5)	4.26, *d* (14.4) 4.16, *d* (14.4)	4.22, *dd* (15.2, 1.5) 4.27, *d* (15.2)
8	2.18, *m*	2.16, *septd* (7.0, 4.7)	2.06, *m*	2.03, *m*
9	1.18, *d* (6.0)	1.07, *d* (7.0)	1.07, *d* (7.0)	1.02, *d* (7.2)
10	1.16, *d* (6.0)	1.09, *d* (7.0)	1.04, *d* (7.0)	0.98, *d* (7.2)
3′			6.93, *dq* (7.2, 1.2)	6.93, *td* (7.2, 1.2)
4′			1.82, *dd* (7.2, 1.0)	1.82, *dd* (7.2, 1.0)
5′			1.86, *d* (1.2)	1.87, *t* (1.0)
3-OAc		2.07, *s*		2.08, *s*

4.2.1.5 5-*O*-*β*-*D*-glucopyranosylcarvotacetone

Compound **232** was isolated as colorless gum with EI-MS $[M+H]^+$ at *m/z* 331consistent to the molecular formula of $C_{16}H_{26}O_7$ (Appendix 19C). The ^{1}H-NMR (Table 4.9, Appendix 19,) showed among other signals an isopropyl unit (3H δ_H 0.90 *d* J = 7.0, 3H δ_H 0.95 *d* J = 7.0, 1H δ_H 2.24 *m*), a methyl attached to olefinic carbon (3H δ_H 1.77) and an olefinic proton at δ_H 6.87. The ^{13}C-NMR had 16 signals including six in the sugar region (δ_C 105.3, 77.6, 76.1, 74.7, 70.5 and 62.8) assignable to a glucopyranosyl moiety [Yang et al., 2007].

There were also ten carbons forming a carvotacetone moiety defined by the isopropyl unit (δ_C 15.4, 20.2, 26.2) and the methyl group (δ_C 15.6) attached to the opposite ends of a six carbon ring containing a carbonyl conjugated to an olefinic group (δ_C 201.8, 147.2, 134.1) [Onayade *et al.*, 1990]. The sp^3 carbon attached to an oxygen at δ_C 83.5 was established by HMBC (H-5→C-6) to be adjacent the carbonyl group and was the point of attachment for the glucopyranosyl group (HMBC H-1'→C-5). The link of the glucopyranosyl was established to be *β*-linkage from the coupling constant of the anomeric proton which was 7.8 Hz. H-4 and H-5 had *axial-axial* coupling (J = 13.0), a fact used in establishing the relative stereochemistry at position 4 and 5.

The structure was confirmed by HMBC correlations to be 5-*O*-*β*-*D*-glucopyranosylcarvotanacetone and the NMR data closely correlated to those reported for the same compound from aerial parts of *Laggera pterodonta* [Yang et al., 2007].

4.2.2 *p*-Cymeme derivatives

Three *p*-cymene derivatives were isolated from the aerial parts of *Sphaerantus bullatus*, these are thymol 3-*O*-*β*-glucopyranoside (**233**), zetaroside B (**93**) and zetaroside A (**234**).

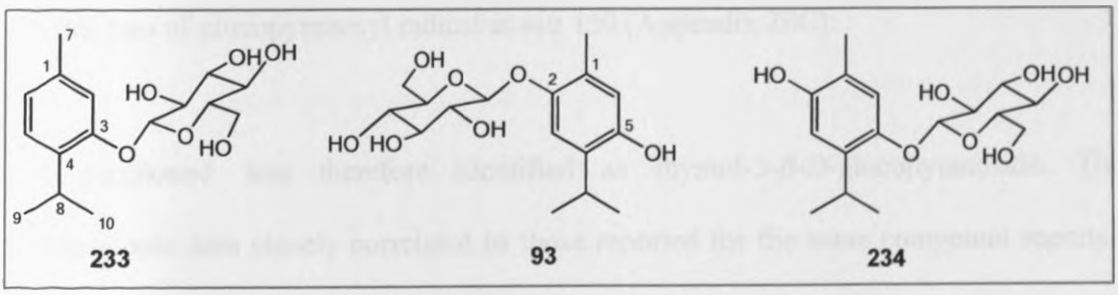


Figure 4.22: *p*-Cymene derivatives isolated from *Sphaeranthus bullatus*

4.2.2.1 Thymol-3-*β*-*D*-glucopyranoside

Compound **233** was isolated as a white solid having a molecular ion peak at *m/z* 312 ($C_{16}H_{24}O_6$). The ^{13}C-NMR (Table 4.10, Appendix 20B) showed signals corresponding to sixteen carbons. The aromatic ring was evident from the ^{13}C-NMR spectrum which showed six signals in the aromatic region (δ_C 116.9, 123.5, 126.3, 135.4, 136.9 and 155.8) and ^{1}H-NMR signals for a 1,3,4 trisubstituted benzene ring (δ_H 7.08 1H *d J* = 7.8 Hz, 6.78 1H br*d J* = 7.8 Hz and 6.99 1H *d J* = 0.6 Hz). Attached to the benzene ring were *β*-*O*-*D*-glucopyranosyl (δ_C 102.4, 78.1, 77.6, 74.8, 71.4, 62.6); an isopropyl (δ_H 1.17 3H *d J* = 7.2 Hz, 1.16 3H *d J* = 7.2 Hz, 3.44 1H *sept J* = 7.2 Hz) and a methyl group (δ_C 21.3, δ_H 2.25 3H *s*).

The HMBC spectrum revealed that the methyl group at C-1 and the isopropyl group were *para* to each other constituting a *p*-cymene skeleton. Thus the *O*-glucopyranosyl at C-3 was *ortho* to the isopropyl group evidenced by the HMBC correlations of glucosidic anomeric proton and isopropyl *septet* with C-3 (δ_C 155.8). The identity of the sugar was confirmed by hydrolyzing 1 mg of the sample in 0.1 M HCl and comparing the products with authentic sample of *D*-glucose on the TLC, as well as presence of EI-MS fragment ion due loss of glucopyranosyl radical at *m/z* 150 (Appendix 20C).

The compound was therefore identified as thymol-3-*β*-*D*-glucopyranoside. The spectroscopic data closely correlated to those reported for the same compound reported from aerial parts of *Jasonia montana* [Ahmed and Jakupovic, 1990] as well as from *Sphaeranthus bullatus* [Jakupovic *et al*., 1990].

4.2.2.2 Zataroside B

Compound **93** was isolated from the aerial parts of *Sphaeranthus bullatus* as white solid, with a weak molecular ion peak observed in the EI-MS spectrum at *m/z* 329 ($C_{16}H_{24}O_7$) $[M+H]^+$ (Appendix 21C). The ^{13}C-NMR (Table 4.10, Appendix 21B) had sixteen signals corresponding to sixteen carbons, six of those being for an aromatic ring. The aromatic carbons consisted of two protonated carbons, (δ_C 115.9 and 117.7) two *C*-substituted carbons (δ_C 127.0 and 134.0) and two *O*-substituted carbons (δ_C 150.4 and 150.5). The other six carbons were oxygen-substituted sp^3 carbons of the glucose unit (δ_C 62.5, 71.4, 75.0, 77.9, 78.1 and 104.3). The remaining four carbons were three methyl carbons and a methine carbon.

The ^{1}H-NMR (Table 4.9, Appendix 21A) showed two aromatic singlets at δ_H 6.51 and 6.98 implying a tetrasubstituted benzene ring with two *para* protons. The methyl doublets at δ_H 1.17 and 1.18 coupling with a septet at 3.17 were indicative of an isopropyl unit attached to the aromatic ring. A methyl singlet also attached to the aromatic ring was observed at δ_H 2.18. HMBC correlations established that the methyl and isopropyl unit were *para* to each other, constituting a monoterpenoid skeleton of *p*-cymene.

The *O*-glucopyranoside was attached to C-2 (δ_C 150.5), adjacent to the methyl group since both the methyl protons and anomeric proton were making HMBC correlation with C-2. The 7.8 Hz coupling constant of the anomeric proton implied that the glucosidic link was β-linkage.

Compound **93** was therefore identified as 2-*O*-glucopyranosyl-5-hydroxy-4-isopropyltoluene (Zataroside B) previously from *Zataria multiflora* [Ali *et al.*, 1999].

Table 4.9: ^{1}H-NMR data for compounds **232**, **233**, **93** and **234** (600 MHz)

	232 CD_2Cl_2	**233** Acetone-d_6	**93** MeOD	**234** MeOD
2	6.87, d(5.7)	6.97, *d* (0.6)	6.98, *s*	6.61, *s*
3	2.36, *dt* (19.0, 4.9) 2.24, *m*			
4	2.11, *m*			
5	4.13, *d* (13.1)	7.08, *d* (7.8)		
6		6.78, br *d* (7.8)	6.51, *s*	6.91, *s*
7	1.77, *t* (1.0)	2.25, *s*	2.18, *s*	2.12, *s*
8	2.24, *m*	3.44, *sept* (7.2)	3.19, *sept* (7.2)	3.19, *sept* (7.2)
9	0.90, *d* (7.0)	1.16, *d* (7.2)	1.18, *d* (7.2)	1.15, *d* (7.2)
10	0.95, *d* (7.0)	1.17, *d* (7.2)	1.17, *d* (7.2)	1.15, *d* (7.2)
1′	4.43, *d* (7.8)	4.92, *d* (7.8)	4.66, *d* (7.8)	4.66, *d* (7.8)
2′	3.41, *t* (8.3)	3.52, *m*	3.43, *m*	3.43, *m*
3′	3.57, *t* (9.0)	3.53, *t* (6.6)	3.43, *m*	3.43, *m*
4′	5.54, *t* (9.1)	3.47, *m*	3.38, *m*	3.38, *m*
5′	3.34, *m*	3.48, *m*	3.33, *ddd* (9.6, 5.4, 2.4)	3.33, *ddd* (9.6, 5.4, 2.4)
6′	3.83, *dd* (11.8, 3.3) 3.77, *dd* (11.8, 4.7)	3.70, br *d* (11.4) 3.86, br *d* (11.4)	3.70, *dd* (12.0, 5.4) 3.86, *dd* (12.0, 2.4)	3.70, *dd* (12.0, 5.4) 3.86, *dd* (12.0, 2.4)

Table 4.10: ^{13}C-NMR data for carvotacetones and *p*-cymene derivatives (150 MHz)

	85 Acetone	**106** CD_2Cl_2	**88** CD_2Cl_2	**99** CD_2Cl_2	**232** CD_2Cl_2	**233** Acetone	**93** MeOD	**234** MeOD
1	137.1	138.7	137.0	139.7	134.1	136.9	127.0	123.0
2	143.6	139.4	142.8	137.5	147.2	116.9	150.5	151.7
3	66.0	67.8	65.8	67.6	25.0	155.8	115.9	112.8
4	50.8	49.1	47.2	46.0	46.6	135.4	134.0	138.0
5	72.2	72.1	73.4	73.1	83.5	126.3	150.4	149.0
6	202.4	202.0	195.8	194.9	201.8	123.5	117.7	120.1
7	59.1	60.4	59.7	59.4	15.6	21.3	15.9	15.9
8	29.2	28.3	28.2	27.8	26.2	26.7	27.8	26.8
9	20.3	19.9	19.5	19.3	15.4	23.2	23.02	23.4
10	20.8	19.8	19.8	19.5	20.2	23.4	22.98	23.6
1′			167.7	167.0	105.3	102.4	104.3	104.3
2′			128.0	128.0	74.7	74.8	75.0	75.0
3′			138.9	138.7	77.6	78.1	78.1	78.1
4′			14.4	14.3	70.5	71.4	71.4	71.4
5′			11.9	11.9	76.1	77.6	77.9	77.9
6′					62.8	62.6	62.5	62.5
OAc-CO		170.5		170.3				
OAc-Me		21.3		20.9				

4.2.2.3 Zataroside A

Compound **234** was isolated as a minor component of zatroside B (**93**), accounting for about 8% of the mixture. The NMR features (Tables 4.9 and 5.10) were similar to those of **93**, the difference being the point of attachment of the *O*-glucopyranosyl unit, where in

this case it was at position 5, as evidenced by HMBC correlation of anomeric proton (δ_H 4.66, *d* J = 7.8) with C-5 (δ_C 149.0) The compound was therefore identified as 5-*O*-glucopyranosyl-2-hydroxy-4-isopropyltoluene (Zataroside A). The NMR data were compared to those of the same compound reported from *Zataria multiflora* [Ali *et al.*, 1999] and they matched.

4.2.3 Flavonoids

From the aerial parts of *S. bullatus*, four flavanols and one chalcone were isolated (Figure 4.23). The flavanols are quercetin (**235**), quercetin-3,7-dimethylether (**236**), penduletin (**237**), crysoplenol D (**238**) and the chalcone 4-hydroxylonchocarpin (**239**).

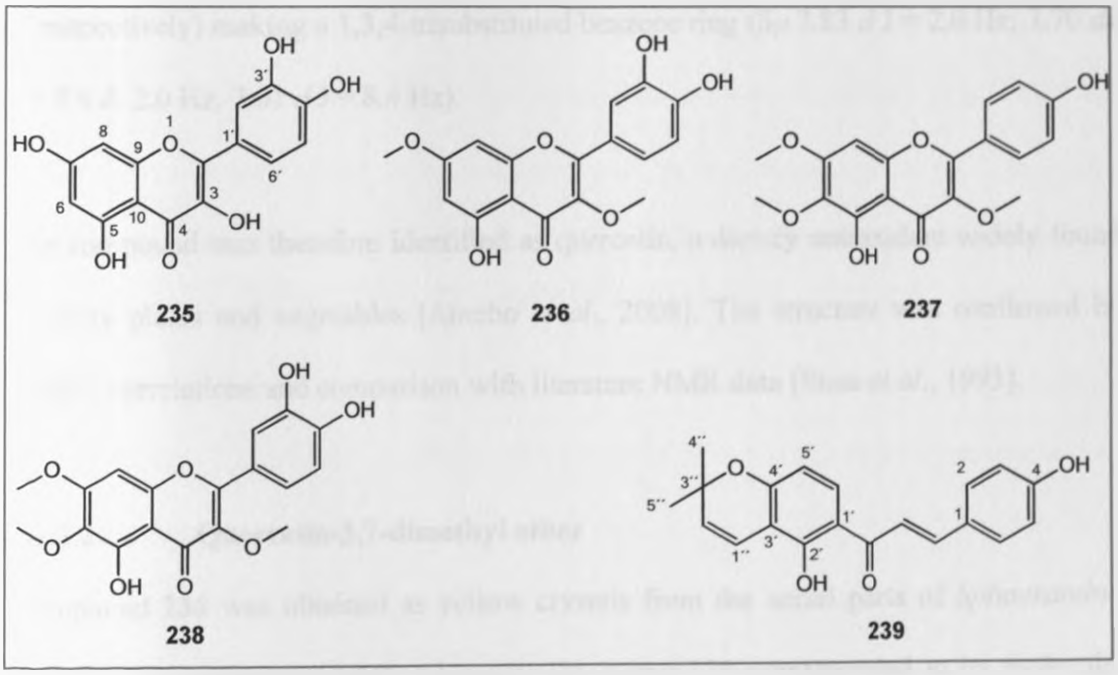


Figure 4.23: Flavonoids isolated from *Sphaeranthus bullatus*

4.2.3.1 Quercetin

Compound **235** was isolated as yellow crystals from the aerial parts of *Sphaeranthus bullatus*. The ^{13}C-NMR (Appendix 22B, Table 4.12) gave fifteen signals of a flavonoid, being twelve aromatic carbons for the two benzene rings and two quaternary olefinic carbons conjugated to a carbonyl. The chemical shifts for C-2, C-3 and C-4 (δ_C 147.0, 136.7 and 176.6 respectively) suggested a flavanol, with C-3 bearing a hydroxyl group.

Ring A had hydroxyl group at C-5 (chelated proton of hydroxyl group at δ_H 12.17, *s*) and another hydroxyl group at C-7, leaving two protonated carbon atoms at *meta* positions (δ_H 6.56 *d* J = 2.2 Hz, 6.28 *d* J = 2.2 Hz). Ring B had *ortho* hydroxyl groups as observed from adjacent oxygenated sp^2 carbons of benzene ring (δ_C 145.9 and 148.5; C-3′ and C-4′ respectively) making a 1,3,4-trisubstituted benzene ring (δ_H 7.83 *d* J = 2.0 Hz, 7.70 *dd* J = 8.4 & 2.0 Hz, 7.01 *d* J = 8.4 Hz).

The compound was therefore identified as quercetin, a dietary antioxidant widely found in fruity plants and vegetables [Ameho *et al.*, 2008]. The structure was confirmed by HMBC correlations and comparison with literature NMR data [Shen *et al.*, 1993].

4.2.3.2 Quercetin-3,7-dimethyl ether

Compound **236** was obtained as yellow crystals from the aerial parts of *Sphaeranthus bullatus*. The observed EI-MS molecular ion at *m/z* 330 corresponded to its molecular formula $C_{17}H_{14}O_7$. The ^{1}H and ^{13}C-NMR spectra (Tables 4.11, 4.12 and Appendices 23A, 23B) resembled those of quercetin (**235**), the difference being additional signals for two methoxyl groups (δ_H 3.82 3H *s* and 3.91 3H *s*; δ_C 60.4 and 56.3). The methoxyl group at

δ_H 3.82 and δ_C 60.4 was placed at C-3 from HMBC correlation between this signal and C-3 (δ_C 139.6). The second methoxyl group (δ_H 3.91, δ_C 56.3) was at C-7 from HMBC correlation between the methoxy signal (δ_H 3.91) and C-7 (δ_C 167.2) as well as NOE interactions between the methoxy protons with H-6 and H-8.

The compound was therefore identified as quercetin-3,7-dimethyl ether. The NMR data matched those reported in literature for the synthesized compound [Bouktaib et al., 2002]. It is previously reported from *Haplopappus integerrimus* var. *punctatus* and other many other plants of the family Asteraceae [Ayanoglu *et al.*, 1981].

4.2.3.3 Penduletin

Compound **237** was isolated from the aerial parts of *Sphaeranthus bullatus* as yellow crystals. The EI-MS molecular ion was observed at *m/z* 344 ($C_{18}H_{16}O_7$). The ^{13}C-NMR (Table 4.12, Appendix 24B) showed signals for a flavanol as compounds **235** and **236**, differences observed in substitution patterns and the number of methoxyl groups where in this case was three (δ_C 57.0, 60.5 and 61.1).

The ^{1}H-NMR (Table 4.11, Appendix 24A) showed two signals of a symmetrical 1,4-disubstituted benzene ring at δ_H 8.02 and 6.93 (both 2H doublets with J = 9.0 Hz) as well as an aromatic 1H singlet at δ_H 6.76 and three methoxy proton signals at δ_H 3.79, 3.82 and 3.96. The 1,4-disubstituted benzene was ring B from HMBC correlation of H-2′ and H-6′ (δ_H 8.02 *d* J = 9.0 Hz) with C-2 (δ_C 158.5). Ring A had one protonated carbon (δ_H 6.76 *s*, δ_C 92.1) established to be H-8 from HMBC correlations of this proton with C-7,

C-9 and C-10. Positions 5, 6 and 7 were therefore bearing oxygenated carbons, with δ_C 154.0, 133.4 and 160.6 respectively.

The methoxyl groups were at C-3, C-6 and C-7, from HMBC correlations of δ_H 3.79 with C-3 (δ_C 139.5); δ_H 3.82 with C-6 (δ_C 133.4) and δ_H 3.96 with C-7 (δ_C 160.6). The compound was therefore identified as 5,4'-dihydroxy-3,6,7-trimethoxyflavone (penduletin). NMR data matched those reported for a synthesized compound [Horie *et al.*, 1998].

4.2.3.4 Crysoplenol D

Compound **238** (M^+ 260, $C_{18}H_{16}O_8$) was isolated from aerial parts of *Sphaeranthus bullatus* as yellow crystals. NMR features (Tables 4.11, 4.12, Appendices 25A, 25B) resembled those of penduletin (**237**), differences being on the substitution pattern of ring B where in this case there were signals for a 1,3,4-trisubstituted benzene ring (δ_H 7.00 *d* J = 8.4 Hz, 7.61 *dd* J = 8.4, 2.0 Hz and 7.73 *d* J = 2.0 Hz). It was therefore established that ring B had hydroxyl groups at C-3' and C-4' (δ_C 145.9 and 149.1 respectively). Substitution pattern in ring A and C as well as the position of three metoxyl groups were as in **237**.

The compound was therefore identified to be 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (crysoplenol D) previously isolated from species of the genus *Crysoplenium* [Jay *et al.*, 1976].

4.2.3.5 4-Hydroxylonchocarpin

Compound **239** was isolated as yellow solid from *Sphaeranthus bullatus*. The ^{13}C-NMR showed two aromatic rings (twelve signals in the aromatic region) and carbonyl carbon (δ_C 192.4) conjugated to olefinic carbons (δ_C 118.3 and 144.3) with *trans* coupled protons (δ_H 7.47 *d* J = 15.2 Hz and 7.83 *d* J = 15.2 Hz) characteristic of a chalcone skeleton.

The ^{1}H-NMR (Appendix 26A, Table 4.11) had signals for a symmetrical 1,4-disubstituted benzene ring (δ_H 7.60 *d* J = 8.4 Hz and 6.90 *d* J = 8.4 Hz) assigned as ring B from HMBC correlation of H-2 and H-6 (δ_H 7.60 *d* J = 8.4 Hz) with *β*-carbon (δ_C 144.3). The chemical shift of C-4 (δ_C 158.6) suggested that it was oxygenated, bearing a hydroxyl group as there were no alkoxyl signals.

Ring A was1,2,3,4-tetrasubstituted as observed from two ortho coupled protons (δ_H 6.38 *d* J = 8.8 Hz and 7.75 *d* J = 8.8 Hz). A cylized prenyl (δ_H 1.45 s, 5.62 *d* J = 9.8 Hz, 6.72 *d* J = 9.8 Hz) was attached to ring A as confirmed by the EI-MS acyllium ion fragment at *m/z* 203 (Figure 4.24, Appendix 26C). The carbon at C-2′ had hydroxyl group that gave rise to a chelated proton signal (δ_H 13.81 *s*). HMBC correlation of H-1′′ (δ_H 6.72 *d* J = 9.8 Hz) with C-3′ (δ_C 109.7) and C-2′ (δ_C 161.3) implied that the prenyl was connected at C-3′ and cyclizing at C-4′.

The compound was therefore identified as 4,2'-dihydroxy-4',3'-(2,2-dimethylpyrano)chalcone (4-hydroxylonchocarpin) with molecular formula of $C_{20}H_{18}O_4$ and molecular weight of 322 as indicated by EI-MS molecular ion. Physical and

spectroscopic data closely correlated to those reported for the same compound from *Mundulea sericea* [Luyengi *et al.*, 1994].

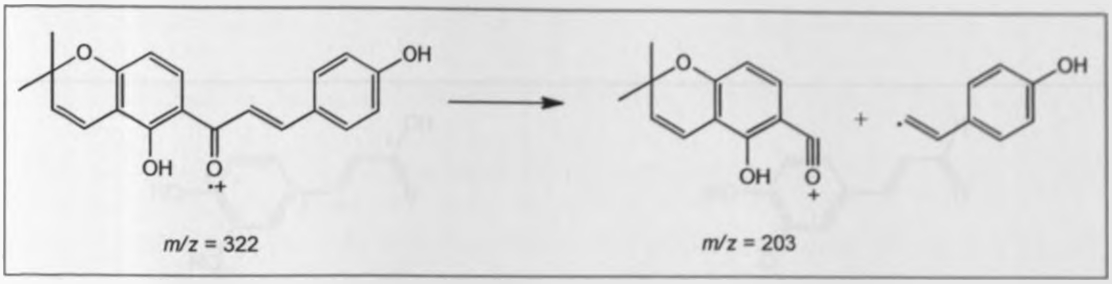


Figure 4.24: Fragmentation pattern for 4-hydroxylonchocarpin (**239**)

Table 4.11: ^{1}H-NMR data for flavonoids from *S.bullatus* (600 MHz)

	235 Acetone-d_6	**236** MeOD	**237** MeOD	**238** Acetone-d_6	**239** CD_2Cl_2
2					7.60, d (8.4)
3					6.90, d (8.4)
5					6.90, d (8.4)
6	6.28, *d* (2.2)	6.36, *d* (1.8)			7.60, d (8.4)
8	6.56, *d* (2.2)	6.62, *d* (1.8)	6.76, *s*	6.81, *s*	
2′	7.83, *d* (2.0)	7.68, br *s*	8.02, *d* (9.0)	7.73, *d* (2.0)	
3′			6.93, *d* (9.0)		
5′	7.01, *d* (8.4)	6.93, *d* (8.4)	6.93, *d* (9.0)	7.00, d (8.4)	6.38, d (8.8)
6′	7.70, *dd* (8.4, 2.0)	7.59, br *d* (8.4)	8.02, *d* (9.0)	7.61, dd (8.4, 2.0)	7.75, d (8.8)
1″					6.72, d (9.8)
2″					5.62, d (9.8)
4″					1.45, s
5″					1.45, s
α					7.47, d (15.2)
β					7.83, d (15.2)
3-OMe		3.82, *s*	3.79, *s*	3.88, s	
6-OMe			3.82, *s*	3.80, s	
7-OMe		3.91, *s*	3.96, *s*	3.99, s	

4.2.4 Phenyl propanoids

Two phenyl propanoids were isolated, caffeic acid (**240**) and coniferaldehyde (**241**).

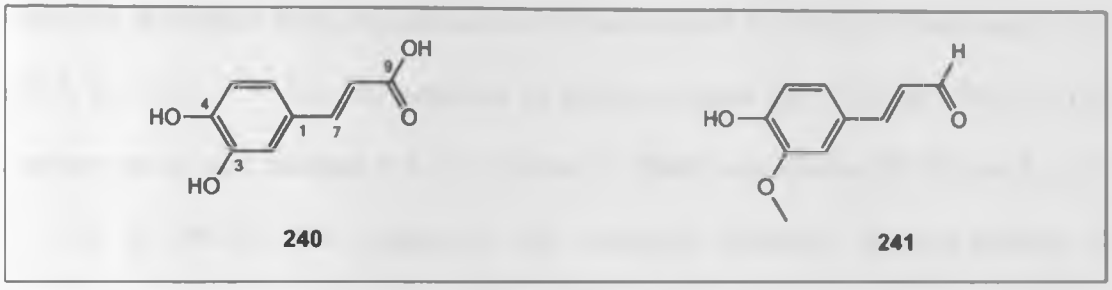


Figure 4.25: Phenyl propanoids isolated from *Sphaeranthus bullatus*

4.2.4.1 Caffeic acid

Compound **240** was isolated as pale brownish crystals. The ^{13}C-NMR (Table 4.12, Appendix 27B) had a total of nine signals for a phenyl propanoid, being six signals for an aromatic ring and two for olefinic carbons conjugated carboxylic acid carbonyl at δ_C 168.3 ppm. The *ortho* hydroxyl groups were evident from the adjacent oxygenated aromatic carbons (δ_C 146.3 and 148.7).

The ^{1}H-NMR (Appendix 27A) had signals for a 1,3,4-trisubstituted benzene ring (δ_H 6.88 *d* J = 8.2 Hz, 7.05 *dd* J = 8.2, 1.8 Hz, 7.17 *d* J = 1.8 Hz) and signals for *trans* olefinic protons (δ_H 6.07 *d* J = 15.8 Hz, 7.55 *d* J = 15.8 Hz). The compound was therefore identified as 3-(3,4-dihydroxyphenyl)-2-propenoic acid (caffeic acid), an antioxidant commonly consumed through coffee beverages [Andueza *et al.*, 2009].

4.2.4.2 Coniferaldehyde

Compound **241** (M^+ 178, $C_{10}H_{10}O_3$) was isolated as light yellow crystals. Its NMR spectral features (Table 4.12, Appendices 28A and 28B) resembled those of caffeic acid (**240**), the difference being replacement of carboxylic acid by aldehyde functionality (δ_C 192.7, δ_H 9.63, d = 7.8) and presence of methoxy signal (δ_C 55.3, δ_H 3.93, s). The methoxy group was attached to C-3 as shown by HMBC correlation (H-OMe at δ_H 3.93 → C-3 at 146.3). The compound was therefore identified as 3-(4-hydroxy-3-methoxyphenyl)-2-propenal (coniferaldehyde). The NMR data matched those reported for the same compound isolated from *Artemisia annua* [Sy and Brown, 1999].

Table 4.12: ^{13}C-NMR data for **235**, **236**, **237**, **238**, **240** and **241** (150 MHz)

	235 Acetone-d_6	**236** MeOD	**237** MeOD	**238** Acetone-d_6	**240** Acetone-d_6	**241** CD_2Cl_2
1					127.8	126.0
2	147.0	158.2	158.5	156.9	115.2	108.8
3	136.7	139.6	139.5	139.2	146.3	146.3
4	176.6	180.0	180.3	179.8	148.7	148.2
5	162.3	162.7	154.0	153.6	116.4	113.9
6	99.2	98.8	133.4	133.1	122.6	123.1
7	165.1	167.2	160.6	160.1	146.0	152.2
8	94.5	92.9	92.1	91.7	115.9	125.6
9	157.8	158.2	153.4	153.1	168.3	192.7
10	104.1	106.6	107.3	107.0		
1′	123.8	122.2	122.5	123.0		
2′	115.9	116.4	131.6	116.3		
3′	145.9	146.4	116.6	145.9		
4′	148.5	149.9	161.9	149.1		
5′	116.4	116.3	116.6	116.4		
6′	121.5	122.6	131.6	122.1		
3-OMe		60.4	60.5	60.1		55.3
6-OMe			61.1	60.5		
7-OMe		56.3	57.0	56.8		

4.2.5 Triterpenoids

Two triterpenoids were isolated from the aerial parts of *S. bullatus*, dammara-20,24-dienylacetate (**242**) and boehmery acetate (**243**).

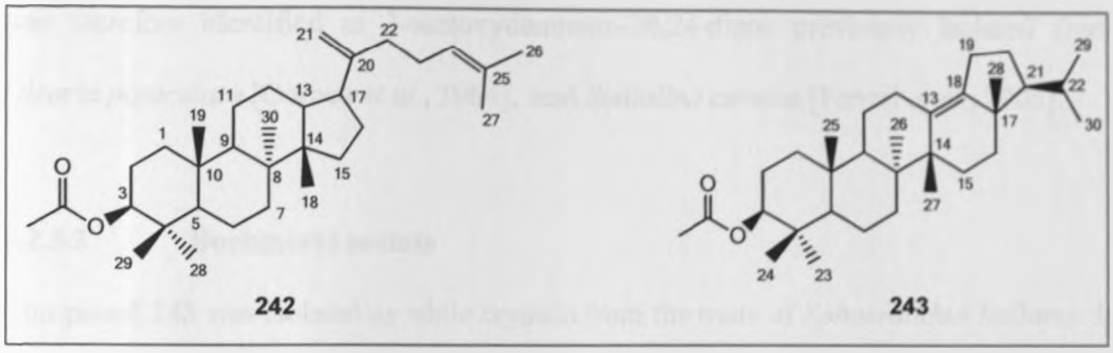


Figure 4.26: Triterpenoids isolated from *Sphaeranthus bullatus*

4.2.5.1 Dammara-20,24-dienylacetate

Compound **242** was isolated as white crystals from the roots of *Sphaeranthus bullatus*. The thirty-two carbon signals in the ^{13}C-NMR (Table 4.13, Appendix 29B) which included acetoxy carbons (δ_C 171.0, 21.4) suggested that it was a triterpene with an acetoxy group. The olefinic region had four carbon signals, two of them being quaternary (δ_C 150.2, 131.7), one methine (δ_C 124.9, δ_H 5.13 *t* J = 7.2) and one methylene (δ_C 107.7, δ_H 4.70 and 4.74, both boublets with J = 1.0 Hz). The sp^3 region of ^{1}H-NMR showed, among other signals, eight methyl groups which were all singlets as well as a characteristic down field shifted H-3 signal (δ_H 4.44 *dd* J = 10.0, 6.6 Hz) of triterpenes having acetoxy group at C-3.

The molecular formula of the triterpene ($C_{32}H_{52}O_2$) determined from MS (*m/z* 468) and DEPT spectra corresponds to DBE of seven, suggesting that it was a tetracyclic triterpene with two double bonds and a carbonyl which belonged to the acetoxy group. The carbon

connectivity was established using combination of HMBC, HSQC and COSY experiments to give a dammarane skeleton with doublebonds at positions 20(21) and 24(25) [Leong and Harrison, 1999], as well as an acetoxy group at C-3. The compound was therefore identified as 3-acetoxydammara-20,24-diene previously isolated from *Olearia paniculata* [Corbett *et al.*, 1964], and *Santolina corsica* [Ferrari *et al.*, 2005].

4.2.5.2 Boehmeryl acetate

Compound **243** was isolated as white crystals from the roots of *Sphaeranthus bullatus*. It had thirty-two carbons from the ^{13}C-NMR (Table 4.13 Appendix 30B), being two acetoxy carbons (δ_C 171.1, 21.4), two olefinic quartenary carbons (δ_C 131.6, 142.1) and twenty-eight sp^3 carbons. The connectivity of carbons was followed using HSQC, HMBC and COSY spectra to give a neohopane triterpene [Mahato and Sen, 1997] having an acetoxy at C-3 and double bond at C-13(18) (δ_C 131.6, 142.1) [Chakravarty, 1994].

The ^{1}H-NMR (Table 4.13, Appendix 30A) showed a total of nine methyl groups, seven being singlets and two doublets (δ_H 0.90 *d* J = 6.6 Hz, 0.94 *d* J = 6.6 Hz) assignable to an isopropyl unit attached at C-21. It also showed a characteristic deshielded signal of H-3 proton (δ_H 4.47 *dd* J = 11.6, 5.3 Hz) for a triterpenes oxygenated at C-3. This proton (H-3) was making HMBC correlation with acetoxy carbonyl (δ_C 171.1) suggesting that the acetoxy is at C-3. The triterpene was therefore identified as boehmeryl acetate, with molecular formula $C_{32}H_{52}O_2$ consistent with EI-MS molecular ion peak at *m/z* 468. It was previously reported from *Pluchea lanceolata* [Chawla *et al.*, 1990].

Table 4.13: ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data for triterpenoids from *S. bullatus* (CD_2Cl_2)

	242		**243**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	39.1	1.71, *dt* (13.2, 3.6); 1.05, *m*	33.3	1.40, *m*; 1.46, *m*
2	24.1	1.62, *m*; 1.62, *m*	25.7	1.64, *dd* (12.2, 4.3); 1.67, *m*
3	81.1	4.44, *dd* (10.0, 6.6)	81.2	4.47, *dd* (11.6, 5.3)
4	38.2		38.5	
5	56.3	0.86, *m*	48.6	1.42, *m*
6	18.5	1.52, *m*; 1.46, *m*	19.2	1.53, *dd* (13.0, 6.8); 1.21, *m*
7	35.7	1.60, *m*; 1.28, *dt* (12.8, 3.10)	35.2	1.31, *m*; 2.01, *m*
8	40.9		41.8	
9	51.2	1.35, *dd* (12.8, 3.0)	46.6	1.58, *dd* (13.1, 3.0)
10	37.5		37.4	
11	21.7	1.52, *m*; 1.22, *ddd* (25.7, 12.8, 4.5)	23.0	1.26, *m*; 1.43, *m*
12	25.3	1.55, *m*; 1.08, *m*	26.8	1.89, *m*; 1.30, *m*
13	45.6	1.66, *m*	131.6	
14	49.8		42.9	
15	31.7	1.60, *m*; 1.10, *m*	30.7	1.27, *m*; 1.47, *m*
16	29.3	1.38, *m*; 1.89, *m*	37.9	1.36, *m*; 1.57, *m*
17	48.3	2.20, *ddd* (17.8, 10.6, 6.8)	43.1	
18	16.1	0.88, *s*	142.1	
19	39.1	0.85, *s*	26.7	2.18, br *dd* (16.9, 10.7); 2.30, *m*
20	150.2		28.0	1.37, *m*; 1.84, *m*
21	107.7	4.74, *d* (1.0); 4.70, *d* (1.0)	59.5	1.05, *m*
22	34.5	1.97, *dd* (13.2, 7.4)	30.2	1.55, *m*
23	27.5	2.12, *q* (7.4)	29.1	0.86, s
24	124.9	5.13, *t* (7.2)	17.3	0.85, *s*
25	131.7		23.1	0.97, *s*
26	25.8	1.68, *s*	25.9	1.02, *s*
27	17.8	1.61, *s*	26.9	1.07, *s*
28	28.1	0.85, *s*	28	0.79, *s*
29	16.4	0.88, *s*	23.1	0.94, *d* (6.6)
30	15.8	0.98, *s*	23.2	0.90, *d* (6.6)
OAc-CO	171.0		171.1	
OAc-Me	21.4	2.00, *s*	21.4	2.00, *s*

4.2.6 General observations on compounds of *S. bullatus*

Out of the seventeen compounds isolated from *Sphaeranthus bullatus*, five were known to the genus, being the carvotacetones **85**, **88**, **99** and **106** as well as the *p*-cymene **93**. Twelve compounds were thus new to the genus. These are 5-*O*-*β*-glucopyranosylcarvotacetone (**232**), thymol 3-*O*-*β*-glucopyranoside (**233**), zetaroside A (**234**), quercetin (**235**), quercetin-3,7-dimethylether (**236**), penduletin (**237**), crysoplenol D (**238**), 4-hydroxylonchocarpin (**239**), caffeic acid (**240**), coniferaldehyde (**241**), dammara-20,24-dienylacetate (**242**) and boehmery acetate (**243**).

For the carvotacetones, the relative stereochemistry at positions 3, 4 and 5 were the same, as established by NOESY experiments, with H-3 and H-4 showing NOE intaractions indicating that the two protons are *cis* to each other, as supported by their *axial-equatorial* coupling constants in the range of 3.4 – 4.0 Hz. H-4 and H-5 had no NOE interactions, implying that they were *trans* to each other as indicated by their *axial-axial* coupling constants in the range of 12 – 13 Hz.

4.3 Secondary metabolites isolated from *Vernonia galamensis* ssp *nairobensis*

4.3.1 Flavanols

Chromatographic separations on the extract of aerial parts of *Vernonia galamensis* gave seven compounds, five of them being flavanols of quercetin (**235**) and derivatives of quercetin namely quercetin 3-methylether (**244**), quercetin 3,7-dimethylether (**236**), quercetin 3-*O*-*β*-*D*-galactoside (**212**) and quercetin 3-*O*-*α*-rhamnoside (**245**).

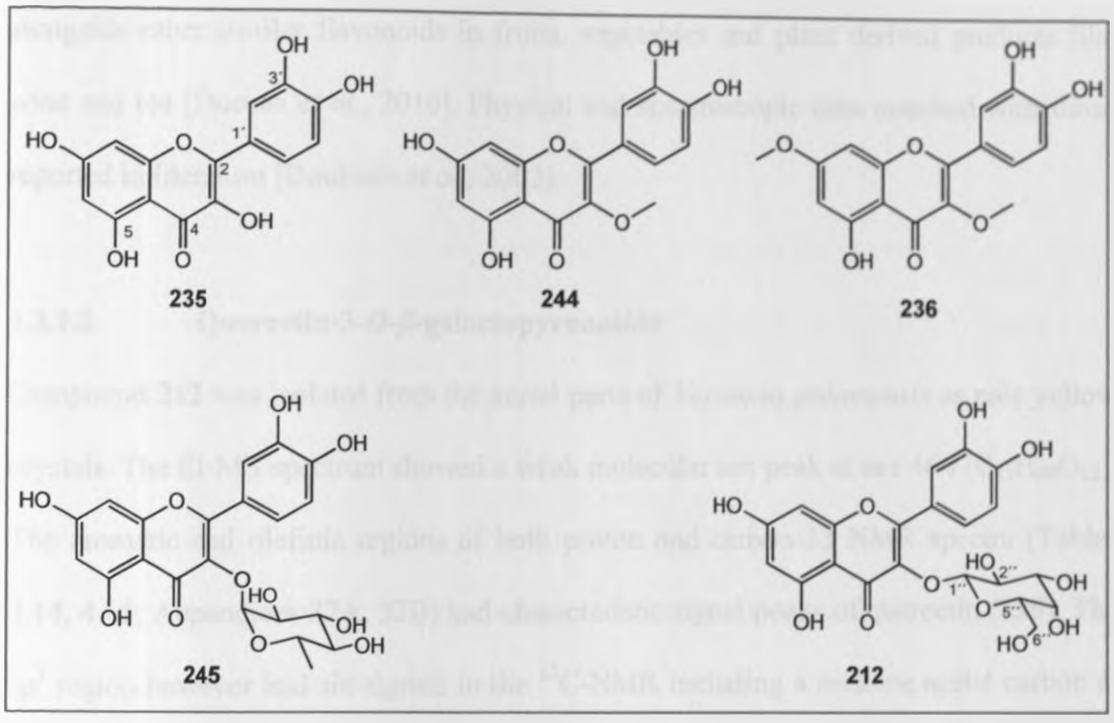


Figure 4.27: Flavanols isolated from *V.galamensis* ssp *nairobensis*

Two of the compounds, quercetin (**235**) and quercetin 3,7-dimethylether (**236**), were also isolated from *Sphaeranthus bullatus* and have been discussed above in sections 4.2.9 and 4.2.10 respectively.

4.3.1.1 Quercetin 3-methyl ether

Compound **244** was isolated as yellow crystals from the aerial parts of *vernonia galamensis*. Its EI-MS molecular ion at *m/z* 316 (Appendix 31C) corresponded to molecular formula $C_{16}H_{12}O_7$. The ^1H and ^{13}C-NMR data (Tables 4.14 and 4.15, Appendices 31A and 31B) closely correlated to those of quercetin (**235**) except for additional signals of a methoxyl group (δ_C 59.1, δ_H 3.77 *s*). The methoxy proton signal showed HMBC correlation with C-3 (δ_C 138.1) consistent to its placement at C-3.

The compound was therefore identified as quercetin-3-methyl ether, mostly found alongside other similar flavonoids in fruits, vegetables and plant derived products like wine and tea [Duenas *et al.*, 2010]. Physical and spectroscopic data matched with those reported in literature [Bouktaib *et al.*, 2002].

4.3.1.2 Quercetin-3-*O*-*β*-galactopyranoside

Compound **212** was isolated from the aerial parts of *Vernonia galamensis* as pale yellow crystals. The EI-MS spectrum showed a weak molecular ion peak at *m/z* 464 ($C_{21}H_{20}O_{12}$). The aromatic and olefinic regions of both proton and carbon-13 NMR spectra (Tables 4.14, 4.15; Appendices 32A, 32B) had characteristic signal peaks of quercetin (**235**). The sp^3 region however had six signals in the ^{13}C-NMR including a methine acetal carbon at δ_C 101.8, four other oxy-methine carbons (δ_C 68.0, 71.2, 73.2, 75.9) and a hydroxylmethylene carbon at δ_C 60.2. It was evident that there was a cyclic hexose sugar unit. The anomeric proton was observed at δ_H 5.39, with a coupling constant of 7.6 Hz implying that the sugar unit had *β*-linkage to the quercetin aglycone.

The identity of the sugar was established through analysis of coupling constants, showing *axial* coupling for the anomeric proton (H-1"), *axial-axial* coupling for H-2", *axial-equitorial* for H-3" and *equatorial-axial* for H-4". The H-4" proton was therefore in equatorial posision, making it a *β-D*-galactopyranosyl. The anomeric proton showed HMBC correlation with C-3 of quercetin aglycone (δ_C 133.5) implying that the *β-D*-galactopyranosyl was attached at C-3. The compound was therefore identified as quercetin-3-*O*-β-galactopyranoside. The NMR data were in agreement with those reported for the same compound from the flowers of *Erica sinerea* [Bennini *et al.*, 1992].

The compound had also been isolated before from the aerial parts of *Venonia galamensis* ssp *nairobensis* [Miserez *et al.*, 1996].

4.3.1.3 Quercetin 3-*O*-*α*-rhamnoside

Compound **245** was isolated from the aerial parts of *Vernonia galamensis* as pale yellow powder. The aromatic and olefinic regions of both proton and carbon-13 NMR were similar to those of quercetin (**235**). The sp^3 region displayed signals for sugar moiety; the acetal methine signals (δ_C 101.8, δ_H 5.26 *d* J = 1.2 Hz), four other oxygen-attached methine carbons (δ_C 70.1, 70.2, 70.4, 70.6) and signals for a methyl (δ_C 17.5, δ_H 0.82 *d* J = 6.0 Hz). The connectivity of a sugar unit was followed by COSY, coupling constants and HMBC to establish a rhamnosyl moiety.

The linkage between the sugar moiety and the quercetin aglycone was established to be at C-3 of the quercetin from the HMBC correlation between the anomeric proton and C-3 (δ_C 134.2) of the quercetin aglycone. The linkage was established to be *α*-linkage from the coupling constant of the anomeric proton which was 1.2 Hz. NMR data correlated to those reported for quercetin 3-*O*-*α*-rhamnoside (quercitrin) from leaves of *Myrsine seguinii* [Zhong *et al.*, 1997].

4.3.2 Lignan and benzenoid

One lignan and one benzenoid were isolated from the aerial parts of *V. galamensis* spp *nairobensis*. The lignan was identified as syringaresinol (**246**) where as the benzenoid was syringic acid (**247**).

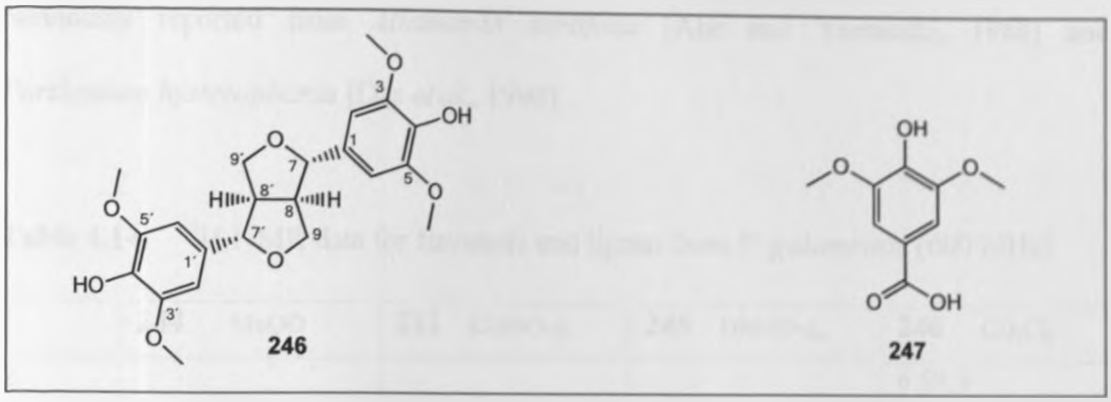


Figure 4.28: Lignan and benzoic acid from *Vernonia galamensis*

4.3.2.1 Syringaresinal

Compound **246** was isolated as yellowish oil. The EI-MS molecular ion peak was observed at *m/z* 418 ($C_{22}H_{26}O_8$) though the ^{13}C-NMR (Appendix 34B) had only eight signals, this suggested presence of symmetry within the molecule as observed amoung symmetrically substituted lignans [Abe and Yamauchi, 1988]. NMR data (Tables 4.14, 1.15; Appendices 34A, 34B) showed a 3,5-dimethoxy-4-hydroxypheny from the aromatic signals (δ_C 103.1, 132.9, 134.6, 147.5; δ_H 6.5 br*s*) and methoxy signal (δ_C 56.7; δ_H 3.87 *s*). There was also an oxy-methylene (-OCH_2-, δ_C 72.2; δ_H 4.25 *dd* J = 9.0, 7.2 Hz; 3.88 *dd* J = 9.0, 3.8 Hz) and two methines, one attached to oxygen (δ_C 86.4; δ_H 4.69 *d* J = 4.2 Hz) and the other attached to carbons (δ_C 54.9; δ_H 3.07 *ddd*, J = 7.2, 4.4, 3.8 Hz) characteristic of furanoid rings of lignans [Das *et al.*, 1998].

These NMR data resembled those obtained for furanoid lignans having 3,5-dimethoxyphenyls [Abe and Yamauchi, 1988]. HMBC correlations and coupling constants confirmed the compound to be the lignan furanoid called syringaresinal

previously reported from *Allamanda neriifolia* [Abe and Yamauchi, 1988] and *Parthenium hysterophorus* [Das *et al.*, 1998].

Table 4.14: ^{1}H-NMR data for flavanols and lignan from *V. galamensis* (600 MHz)

	244 MeOD	**212** DMSO-d_6	**245** DMSO-d_6	**246** CD_2Cl_2
2				6.59, *s*
6	6.18, *d* (1.8)	6.21, *d* (2.0)	6.21, *d* (1.8)	6.59, *s*
7				4.69, *d* (4.2)
8	6.38, *d* (1.8)	6.41, *d* (2.0)	6.40, *d* (1.8)	3.07, *ddd* (7.2, 4.4, 3.8)
9				4.25, *dd* (9.0, 7.2) 3.88, *dd* (9.0, 3.8)
2′	7.62, *d* (1.8)	7.53, *d* (2.0)	7.30, *d* (2.4)	6.59, s
5′	6.89, *d* (8.4)	6.82, *d* (8.4)	6.87, *d* (8.4)	
6′	7.52, *dd* (8.4, 1.8)	7.68, *dd* (8.4, 2.0)	7.26, *dd* (8.4, 2.4)	6.59, *s*
7′				4.69, *d* (4.2)
8′				3.07, *ddd* (7.2, 4.4, 3.8)
9′				4.25, *dd* (9.0, 7.2) 3.88, *dd* (9.0, 3.8)
1″		5.39, *d* (7.6)	5.26, *d* (1.2)	
2″		3.58, *t* (8.7, 8.4)	3.98, br *s*	
3″		3.38, *dd* (9.5, 3.3)	3.51, *dd* (9.0, 3.0)	
4″		3.66, *d* (2.9)	3.15, *dd* (9.6, 9.0)	
5″		3.34, *t* (6.0)	3.21, *m*	
6″		3.46, *dd* (10.3, 6.0) 3.30, *dd* (10.3, 6.0)	0.82, *d* (6.0)	
5-OH		12.64, *s*	12.67, *s*	
3-OMe	3.77, *s*			3,87, *s*
5-OMe				3,87, *s*
3′-OMe				3,87, *s*
5′-OMe				3,87, *s*

Table 4.15: ^{13}C-NMR data for flavanols and lignan from *V. galamensis* ssp *nairobensis* (150 MHz)

	244 MeOD	**212** DMSO-d_6	**245** DMSO-d_6	**246** CD_2Cl_2
1				132.9
2	256.6	156.3	167.4	103.1
3	138.1	133.5	134.2	147.5
4	178.6	177.5	177.8	134.6
5	161.7	161.3	161.3	147.5
6	98.3	98.8	98.7	103.1
7	164.5	164.3	164.3	86.4
8	93.3	93.6	93.7	54.9
9	158.8	156.4	156.5	72.2
10	104.4	103.9	104.1	
1′	121.5	121.1	120.7	132.9
2′	115.0	116.0	115.7	103.1
3′	145.1	144.9	145.2	147.5
4′	148.1	148.5	148.5	134.6
5′	115.0	115.2	115.5	147.5
6′	120.9	122.1	121.2	103.1
7′				86.4
8′				54.9
9′				72.2
1″		101.8	101.8	
2″		71.2	70.1	
3″		73.2	70.4	
4″		68.0	71.2	
5″		75.9	70.6	
6″		60.2	17.5	
3-OMe	59.1			56.7
5-OMe				56.7
3′-OMe				56.7
5′-OMe				56.7

4.3.2.2 Syringic acid

Compound **247** (M^+ 198, $C_9H_{10}O_5$) was isolated from the aerial parts of *Vernonia galamensis* as white powder. Its ^{1}H-NMR (Appendix 35A) had only two signals, a singlet for aromatic proton at δ_H 7.33 and a singlet for methoxy protons at δ_H 3.89. The ^{13}C-NMR (Appendix 35B) had six signals, a carboxylic acid carbonyl at δ_C 167.6, four aromatic carbon signals at δ_C 148.3, 141.5, 121.4 and 108.0 as well as a signal for methoxy carbon at 56.6.

The aromatic proton was showing HMBC correlation with its carbon of attachment implying existence of symmetrically identical carbon atom two or three bonds away from the proton. The presence of carboxylic acid carbonyl signal alongside four aromatic carbon signals suggested a symmetrical benzoic acid derivative. The position of the aromatic protons on the benzoic acid skeleton was established to be C-2 and C-6 following the HMBC correlation between the aromatic protons signal and the carbonyl C-1; δ_C 167.6) meeting the symmetrical requirements. The methoxyl groups, which were two to fit the symmetry, were at C-3 and C-5 following the HMBC correlation between the methoxyl protons signal and C-3, C-5 (δ_C 141.3) The carbon at C-4 (δ_C 148.3) was bearing a hydroxyl group. The compound was therefore identified to be 4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid), an antioxidant constituent of soybean (*Glycine max*) [Porter et al., 1986].

4.4 Secondary metabolites isolated from *Microglossa pyrifolia*

4.4.1 Clerodane diterpenoids

From the aerial parts of *Microglossa pyrifolia*, six compounds were isolated. Two were rearranged clerodane diterpenoids, 8-acetoxyisochiliolide lactone (**248**) and epoxyisochiliolide lactone (**157**).

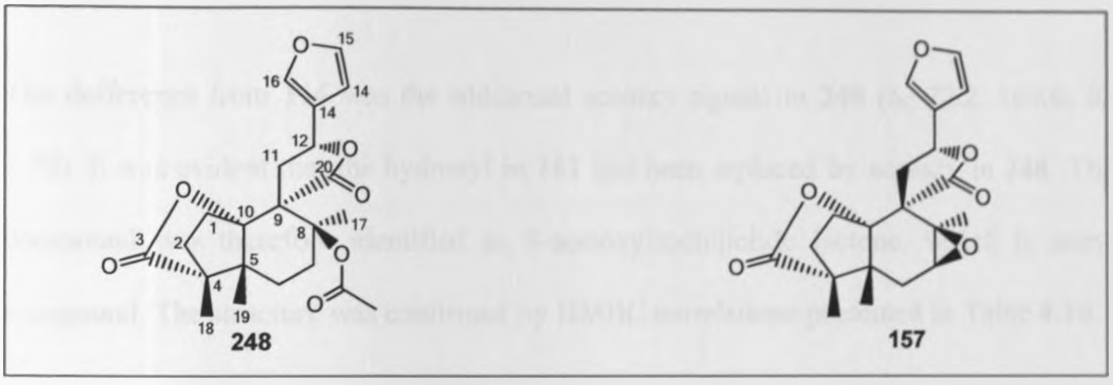


Figure 4.29: Rearranged clerodane diterpenoids from *Microglossa pyrifolia*

4.4.1.1 8-Acetoxyisochiliolide lactone

Compound **248** was isolated from the CH_2Cl_2 extract of *Microglossa pyrifolia* as white crystals. Its molecular formula was deduced to be $C_{22}H_{26}O_7$ from the HR-EIMS molecular ion peak at 402.1700 (calc for 402.1679). Its DBE of 10 was accounted by two olefinic double bonds (four olefinic carbons at δ_C 108.5, 126.0, 140.0 and 144.5), three ester carbonyls (δ_C 169.6, 172.5 and 177.5) and the remaining five was presumed to be for five rings.

The four olefinic carbons made a furanyl ring, whose protons appered at δ_H 6.45 (*d*, 1.5 Hz), 7.45 (*d*, 1.5 Hz) and 7.49 (*s*). Two of the ester carbonyls (δ_C 172.5 and 177.5) were part of two lactone rings where as the one at δ_C 169.6 was an acetoxy carbonyl as it had

HMBC correlation with a methyl singlet at δ_H 1.79. Three more methyl singlets were observed, two of which being vicinal methyls at δ_H 1.08 and 1.13 and the third methyl at δ_H 1.93. These NMR data resembled those reported for the pentacyclic isochiliolide lactone diterpenoid, 8-hydroxyisochiliolide lactone (**161**) reported from *M. pyrrhopappa* [Zdero *et al.*, 1990].

The defference from **116** was the additional acetoxy signals in **248** (δ_C 22.2, 169.6; δ_H 1.79). It was evident that the hydroxyl in **161** had been replaced by acetoxy in **248**. The compound was therefore identified as 8-acetoxyisochiliolide lactone, which is anew compound. The structure was confirmed by HMBC correlations presented in Table 4.16.

4.4.1.2 7,8-Epoxyisocholiolide lactone

Compound **157** was isolated as white crystals from the dichloromethane extract of the aerial parts of *Microglossa pyrifolia*. The ^{13}C-NMR (Table 4.16, Appendix 37B) had twenty signals implying that it was a diterpenoid with twenty carbons. Among the carbons were four olefinic carbons (δ_C 144.8, 140.8, 124.8, 108.5) for a furanyl ring (δ_H 7.58 *d* J = 1.2 Hz, 7.51 br*s*, 6.51 br*s*), two lactone carbonyls at δ_C 172.2, 178.1 and three methyls at δ_C 9.7, 17.4 and 20.8, suggesting a clerodane diterpenoid of isochiliolide lactone skeleton [Zdero *et al.*, 1990]. The adjacent oxygen bearing carbons at position 7 and 8 (δ_C 62.8, 60.8) were deduced to be forming an epoxide from the EIMS molecular ion at *m/z* 360 (Appendix 37C) and absence of OH stretch frequencies in the IR spectrum. The spectroscopic data correlated to the data reported for 7,8-epoxyisocholiolide lactone isolate from *M. pyrrhopappa* [Zdero *et al.*, 1990] confirming that it was the same compound.

Table 4.16: ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data for isochiliolide lactones **248** and **157** (CD_2Cl_2)

	248			**157**	
	^{13}C	^{1}H	HMBC	^{13}C	^{1}H
1	28.8	2.23, *ddd* (13.6, 10.5, 3.8) 1.84, *ddd* (13.6, 9.2, 4.2)	C-2 C-2, C-5	28.8	1.90, *m* 1.72, *m*
2	29.4	1.94, *m* 1.71, *ddd* (13.0, 9.2, 3.8)	C-1 C-1	28.5	1.90, *m* 2.26, *m*
3	177.5			178.4	
4	54.4			55.6	
5	51.2			50.0	
6	26.1	1.44, *td* (14.0, 3.2) 1.39, *dt* (14.0, 3.8)	C-5, C-7	28.4	1.93, br *d* (18.0) 1.87, *m*
7	31.5	2.54, *dt* (14.0, 3.6) 1.75, *m*	C-5, C-6, C-17	62.6	3.35, br *s*
8	86.0			60.8	
9	54.4			52.0	
10	93.3			91.0	
11	34.4	2.69, *dd* (13.8, 8.2) 2.61, *dd* (13.8, 8.8)	C-8, C-9, C-10, C-12, C-13 C-9, C-10, C-12, C-13, C-20	37.6	2.63, *dd* (14.4, 7.8) 2.67, *dd* (14.4, 9.4)
12	70.7	5.37, *dd* (8.8, 8.2)		72.1	5.40, *dd* (9.4, 7.8)
13	126.0			124.5	
14	108.5	6.45, *d* (1.5)	C-13, C-15, C-16	108.5	6.51, br *s*
15	144.5	7.47, *t* (1.5)	C-13, C-16	144.8	7.51, *d* (1.2)
16	140.0	7.49, *s*	C-14, C-15	140.8	7.58, br *s*
17	21.2	1.93, *s*	C-7, C-8, C-9	20.8	1.52, *s*
18	9.4	1.08, *s*	C-2, C-3, C-4, C-5	9.7	1.12, *s*
19	16.9	1.13, *s*	C-4, C-5, C-6, C-10	17.4	1.13, *s*
20	172.5			172.2	
OAc- CO	169.6				
OAc- Me	22.2	1.79, *s*	C-OAc CO		

4.4.2 Steroids

Two steroids were isolated from the aerial parts of *Microglossa pyrifolia*, stigmasterol (**66**) and *β*-sitosterol (**249**).

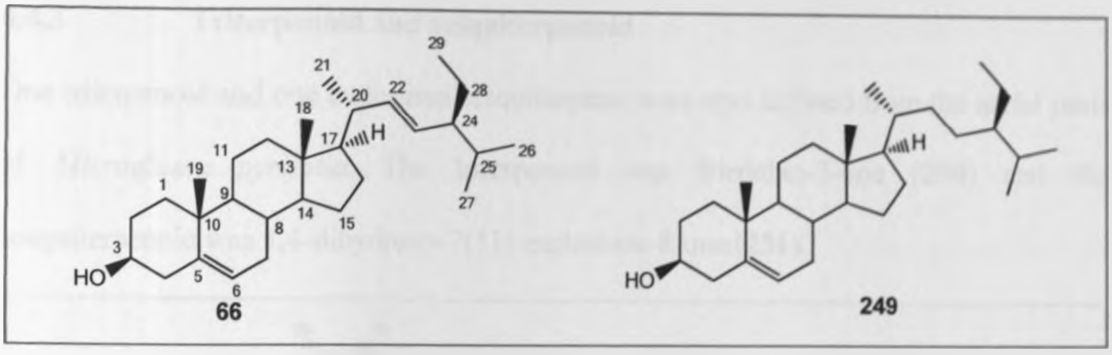


Figure 4.30: Steroids from *Microglossa pyrifolia*

4.4.2.1 Stigmasterol

Compound **66** was isolated as clear white crystals. The ^{13}C-NMR (Table 4.17, Appendix 38B) had twenty nine signals for a steroid, among them four olefinic carbons (δ_C 141.3 *s*, 121.8 *d*, 138.8 *d*, 129.6 *d*), the rest were sp^3 carbons, one (δ_C 72.0 *d*) being attached to oxygen. The DEPT experiment showed that the compound has six methyl groups, nine methylenes, eleven methines and three quaternary carbons. All spectroscopic data matched well with those reported for stigmasterol [Blunt and Stothers, 1977], a steroid that is wide spread in plants.

5.4.2.2 *β*-Sitosterol

Compound **249** was isolated as clear white crystals. The ^{13}C-NMR (Table 4.17, Appendix 39B) had twenty nine signals for a steroid, among them two olefinic carbons (δ_C 141.4 *s*, 121.8 *d*), the rest were sp^3 carbons, one (δ_C 72.1 *d*) being attached to oxygen. The DEPT experiment showed that the compound has six methyl groups, eleven methylenes, nine

methines and three quaternary carbons. NMR data matched well with those reported for β-sitosterol [Blunt and Stothers, 1977], a steroid that is wide spread in plants.

4.4.3 Triterpenoid and sesquiterpenoid

One triterpenoid and one eudesman sesquiterpene were also isolated from the aerial parts of *Microglossa pyrifolia*. The triterpenoid was friedelan-3-one (**250**) and the sesquiterpenoid was 1,4-dihydroxy-7(11)-eudesmen-8-one (**251**).

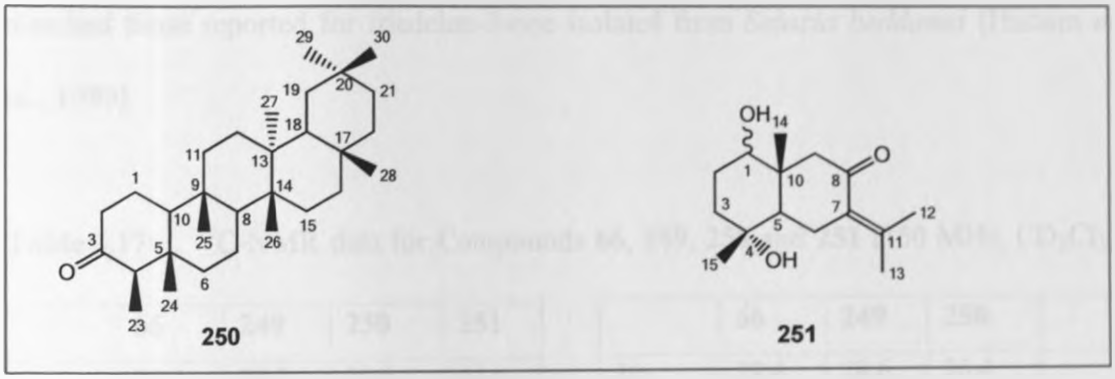


Figure 4.31: Triterpenoid and sesquiterpenoid from *Microglossa pyrifolia*

5.4.3.1 Friedelan-3-one

Compound **250** was isolated as white crystals from *n*-hexane extract of aerial parts of *Microglossa pyrifolia*. The ^{13}C-NMR (Appendix 40B, Table 4.17) had thirty carbon signals of a triterpene, all of them being sp^3 carbons except for a carbonyl carbon at δ_C 212.9. DEPT experiment showed seven quaternary carbons, four methines, eleven methylenes and eight methyls. Of these, the only carbon attached to an electronegative atom was the carbonyl carbon, as deduced from chemical shift analysis. The molecular

formula was therefore deduced to be $C_{30}H_{50}O$ (M^+ 426) with DBE of six, implying that it was a pentacyclic triterpene with one carbonyl.

The ^{1}H-NMR indicated that of the eight methyl groups, seven were singlets and one was a doublet with coupling constant of 6.6 Hz, showing HMBC correlation with the carbonyl. This implied that the triterpene was a 6-6-6-6-6 pentacyclic triterpene of friedelane skeleton with carbonyl at position three. Physical and spectroscopic data matched those reported for friedelan-3-one isolated from *Salacia beddomei* [Hisham *et al.*, 1995].

Table 4.17: ^{13}C-NMR data for Compounds **66**, **249**, **250** and **251** (150 MHz, CD_2Cl_2)

	66	**249**	**250**	**251**
1	37.7	37.7	22.6	61.6
2	32.1	32.1	41.9	23.3
3	72.0	72.1	212.9	36.1
4	42.7	42.7	58.4	60.2
5	141.3	141.4	42.4	64.2
6	121.8	121.8	41.6	29.6
7	32.3	32.3	18.6	134.6
8	32.3	32.3	53.4	207.4
9	50.6	50.6	37.8	54.9
10	36.9	36.9	59.7	58.5
11	21.5	21.5	35.9	138.2
12	40.1	40.2	30.9	21.0
13	42.6	42.7	40.1	23.2
14	57.3	57.2	38.7	17.6
15	24.7	24.7	33.2	15.8

	66	**249**	**250**	
16	29.4	28.6	36.4	
17	56.4	56.5	30.3	
18	12.2	12.0	43.2	
19	19.6	19.6	35.6	
20	40.9	36.5	28.4	
21	21.4	19.0	32.7	
22	138.8	34.3	39.6	
23	129.6	26.4	7.0	
24	51.7	46.2	14.8	
25	32.3	29.6	18.1	
26	19.2	19.2	20.4	
27	21.3	20.0	18.9	
28	25.8	23.4	32.3	
29	12.5	12.2	31.9	
30			35.1	

Table 4.18: ^{1}H-NMR data for Compounds **66**, **249** and **250** (600 MHz, CD_2Cl_2)

	66	**249**	**250**
1	1.08, *dd* (13.2, 4.8) 1.84, *dt* (13.2, 3.6)	1.07, *m* 1.84, *dt* (13.4, 3.6)	1.67, *ddd* (24.8, 12.6, 6.4) 1.96, *ddd* (12.6, 6.4, 3.0)
2	1.78, *m* 1.49, *m*	1.79, *m* 1.50, *m*	2.31, *m*
3	3.46, *m*	3.45, s	
4	2.25, *ddd* (13.2, 4.8, 2.4) 2.19, *tm* (13.2)	2.25, *ddd* (13.0, 5.0, 2.2) 2.20, *tm* (13.0)	2.24, *q* (6.6)
6	5.34, *d* (5.4)	5.34, *t* (2.9, 2.3)	1.74, *dt* (12.6, 3.0) 1.29, *m*
7	1.97, *m* 1.97, *m*	1.94-2.02, *m*	1.41, *m* 1.49, *m*
8	1.47, *m*	1.47, *m*	1.41, *m*
9	0.93, *m*	0.94, *m*	
10			1.55, *m*
11	1.53, *m* 1.53, *m*	1.51, *m*	1.46, *m* 1.29, *m*
12	1.97, *m* 1.17, *m*	2.00, *m* 1.17, *m*	1.35, *m*
14	1.02, *m*	1.01, *m*	
15	1.56, *m* 1.06, *m*	1.58, *m* 1.08, *m*	1.47, *m* 1.27, *m*
16	1.72, *m* 1.27, *m*	1.85, *m* 1.27, *m*	1.57, *m* 1.36, *m*
17	1.16, *m*	1.14, *m*	
18	0.71, *s*	0.69, *s*	1.57, *m*
19	1.01, *s*	1.00, *s*	1.22, *dd* (12.8, 5.8) 1.39, *m*
20	2.05, *m*	1.38, *m*	
21	1.03, *d* (6.6)	0.93, *d* (6.6)	1.52, *m*; 1.32, *m*
22	5.17, *dd* (15.0, 8.4)	1.35, *m* 1.03, *m*	1.52, *m* 0.93, *m*
23	5.03, *dd* (15.0, 8.4)	1.18, *m*	0.83, *d* (6.6)
24	1.55, *m*	0.95, *m*	0.78, *s*
25	1.47, *m*	1.67, *m*	0.87, *s*
26	0.80, *d* (6.6)	0.82, *d* (6.9)	1.01, *s*
27	0.85, *d* (6.6)	0.84, *d* (6.9)	1.06, *s*
28	1.43, *m*; 1.17, *m*	1.27, *m*	1.18, *s*
29	0.81, *t* (7.2)	0.85, *t* (7.4)	1.00, *s*
30			0.95, *s*

4.4.3.2 1,4-Dihydroxy-7(11)-eudesmen-8-one

Compound **251** was isolated from the aerial parts of *Microglossa pyrifolia* as pale yellow oil. The ^{13}C-NMR (Appendix 41B, Table 5.17) had fifteen signals of a sesquiterpene with a carbonyl at δ_C 207.4, two quaternary olefinic carbons at δ_C 134.6 and 138.2 as well as twelve sp^3 carbons. The HMBC spectrum showed that the olefinic carbon at δ_C 138.2 was attached to two methyl groups of δ_H 1.78 and 1.85 (δ_C 21.0 and 23.2 respectively). Two more methyl groups were observed at δ_H 1.10 and 1.40, both being singlets implying that they are attached to quaternary carbons. With the aid of coupling constants, COSY, HSQC and HMBC the compound was established to be an eudesman sesquiterpene derivative namely 1,4-dihydroxy-7(11)-eudesmen-8-one.

CHAPTER FIVE

RESULTS AND DISCUSSIONS FOR ANTI-PLASMODIAL, ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITIES

5.1 Antiplasmodial activity of compounds

As a follow up to the antiplsmodial activities shown by the extracts of the investigated plants (Table 1.1), *in-vitro* antiplasmodial tests were done for the compounds isolated from the plants. The tests aimed at identifying active components which contributed to the observed activities of crude extracts. The compounds tested are the abietane diterpenoids from *C. eriophyllum*, isochiliolide lactone diterpenoids from *M. pyrifolia*, carvotacetone and *p*-cymene derivatives from *S. bullatus* and flavanols from *S. bullatus* and *V.galamensis*. Two *P. falciparum* strains were used, Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant). Activities were observed for three abietane diterpenoids and two carvotacetone derivatives.

The abietane diterpenoids with antiplasmodial activities were taxodione (**222**), 6-hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**) where as the carvotacetone derivatives were 3,5,7-trihydroxycarvotacetone (**85**), 3-acetoxy-5,7-dihydroxycarvotacetone (**106**), 3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**) and 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**). Cytotoxicities of the compound were measured simultaneously, and the only compounds found with cytotoxicity were 6-hydroxysalvinolone (**226**) and 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**). These results are summarized in Table 5.1 and Figure 5.1.

Table 5.1: *In-vitro* antiplasmodial activity and cytoxicity

Compound	*In-vitro* antiplasmodial activity (IC_{50})		Cytotoxicity (IC_{50})
	D6 (µg/ml)	W2 (µg/ml)	(µg/ml)
Royleanone (**21**)	NA	NA	NC
Taxodione (**222**)	1.2	1.2	NC
Ferruginol (**224**)	NA	NA	NC
11-hydroxy-8,11,13-abietatriene 12-*O*-*β*-xylopyranoside (**225**)	NA	NA	NC
6-Hydroxysalvinolone (**226**)	1.8	2.5	4.5
6,16-Dihydroxysalvinolone (**227**)	3.0	4.8	NC
uncinatone (**19**)	NA	NA	NC
Bongardol (**231**)	NA	NA	NC
3,5,7-Trihydroxycarvotacetone (**85**)	3.4	2.8	NC
3-acetoxy-5,7-dihydroxycarvotacetone (**106**)	0.6	0.7	
3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**)	0.8	0.9	
3-Acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**).	1.4	2.0	2.8
3-*O*-*β*-Glucopyranoside (**233**)	NA	NA	NC
Zetaroside B (**93**)	NA	NA	NC
Quercetin-3,7-dimethylether (**236**)	NA	NA	NC
Penduletin (**237**),	NA	NA	NC
Crysoplenol D (**238**)	NA	NA	NC
Quercetin 3-*O*-*β*-*D*-galactoside (**212**)	NA	NA	NC
Quercetin 3-*O*-*α*-rhamnoside (**245**)	NA	NA	NC
8-Acetoxyisochiliolide lactone (**248**)	NA	NA	NC
Epoxyisochiliolide lactone (**157**)	NA	NA	NC
Stigmasterol (**66**)	NA	NA	NC
Friedelan-3-one (**250**)	NA	NA	NC
Chloroquine (standard)	<0.026	0.14	NC

NA = Not Active; NC = Not Cytotoxic (up to the maximum dose tested 5.0 µg/ml).

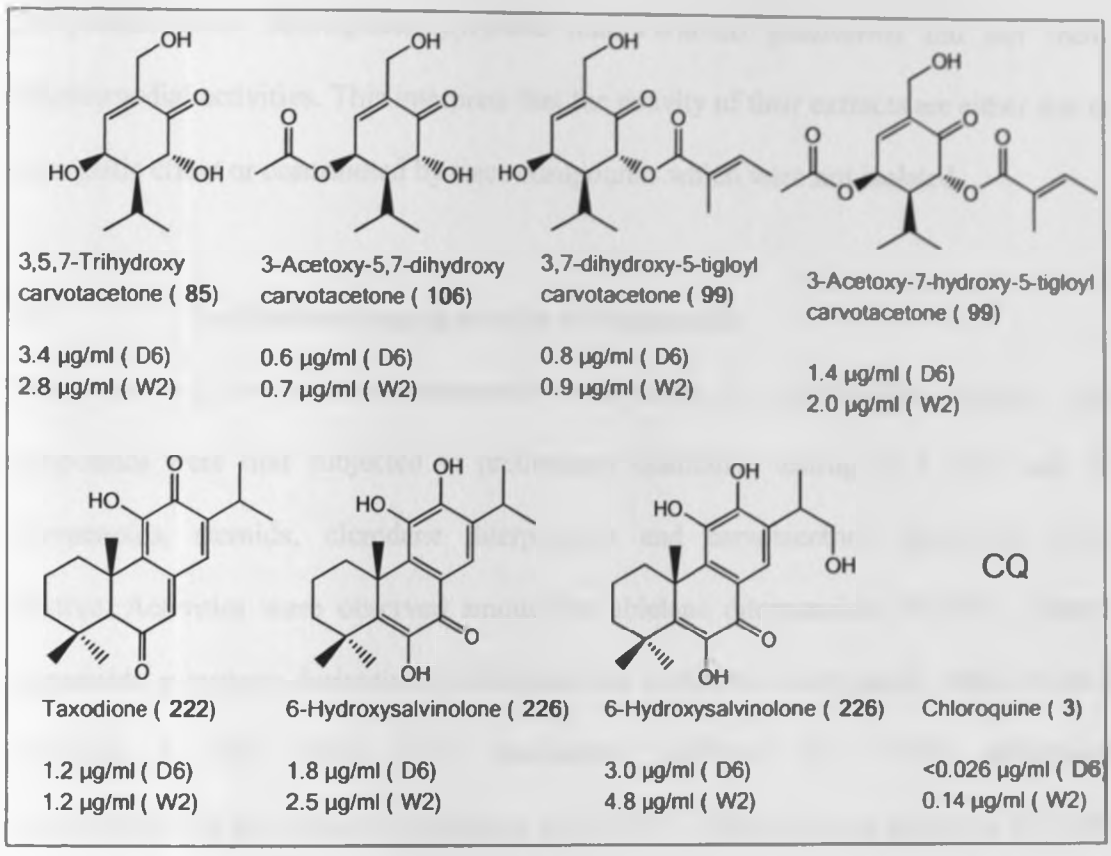


Figure 5.1: Compounds with *in vitro* antiplasmodial activities and their IC_{50}'s (µg/ml)

Compounds found active are all highly oxygenated, with hydroxyl, carbonyl and ester groups. Literature shows that these groups enhance lipophilicity of compounds hence making transportation of these compounds from site of application to site of action easier [Lacks and Pruner, 1989], and that the hydroxyls enhance binding to the receptor site and act as inhibitors of parasitic enzymes, therefore contributing to bioactivities [Collins *et al.*, 2005]. However, other structural properties have role in bioactivities, as there were no proper correlations between the number of these groups in the molecule and activity of the compound, and some compounds with these groups were inactive.

Compounds from *Microglossa pyrifolia* and *Vernonia galamensis* did not show antiplasmodial activities. This interprets that the activity of their extracts are either due to synergistic effect or contributed by trace compounds which were not isolated.

5.2 Radical scavenging activity of compounds

Thirty-eight of the isolated compounds were tested for anti-oxidant activity. The compounds were first subjected to preliminary qualitative testing on a TLC, and all triterpenoids, steroids, clerodane diterpenoids and carvotacetone derivatives were inactive. Activities were observed among the abietane diterpenoids, flavanols, phenyl propanoids, *p*-cymene derivatives and benzenoids in general. Compounds which showed activities on TLC assays were quantitavely analysed by UV-VIS absorption measurements on the compound solutions with DPPH. The results are presented in Table 5.2, 5.3, and 5.4 as well as Figure 5.2.

Table 5.2: Radical scavenging activity of flavonoids against DPPH

Compound	IC_{50}	
	µg/ml	µM
Quercetin (standard)	6.0 ± 0.2	19.9
Quercetin 3,7-dimethylether (**236**)	6.5 ± 0.4	19.7
Quercetin 3-methylether (**244**)	6.6 ± 0.3	20.9
Quercetin 3-*O*-*β*-galactopyranoside (**212**)	9.3 ± 0.4	20.0
Crysoplenol D (**238**)	10.4 ± 0.5	28.9
Quercetin 3-*O*-*α*-rhamnopyranoside (**245**)	12.9 ± 0.4	28.8
Penduletin (**237**)	> 100	-
4-Hydroxylonchocarpin (**239**)	> 100	-

Table 5.3: Radical scavenging activity of abietane diterpenoids against DPPH

Compound	IC_{50}	
	µg/ml	µM
Nellionol (**228**)	10.2 ± 0.5	29.3
6-Hydroxysalvinolone (**226**)	17.7 ± 0.4	53.6
6,16-Dihydroxysalvinolone (**227**)	20.5 ± 0.5	59.2
Taxodione (**222**)	31.5 ± 0.8	100 .0
Ferruginol (**224**)	76.3 ± 3.6	267
Sugiol (**9**)	> 100	-
Royleanone (**21**)	> 100	-
11-Hydroxy-7,9,13-abietatrien-12-one (**223**)	> 100	-
11-hydroxy-8,11,13-abietatriene *12-O-β*-xylopyranoside (**225**)	> 100	-
Uncinatone (**19**)	NT	NT
3-Oxoroyleanone (**220**)	NT	NT

Table 5.4: Radical scavenging activity of other phenolics against DPPH

Compound	IC_{50}	
	µg/ml	µM
Caffeic acid (**240**)	2.6 ± 0.3	14.4
Syringic acid (**247**)	8.3 ± 0.4	41.9
Coniferaldehyde (**241**)	14.1 ± 0.6	79.2
Syringaresinol (**246**)	21.0 ± 0.5	50.2
Zetroside B (**93**)	28.9 ± 0.6	88.1
Tyrosyloctacosanoate (**231**)	> 100	-
Thymol *3-O-β*-glucopyranoside (**233**)	> 100	-

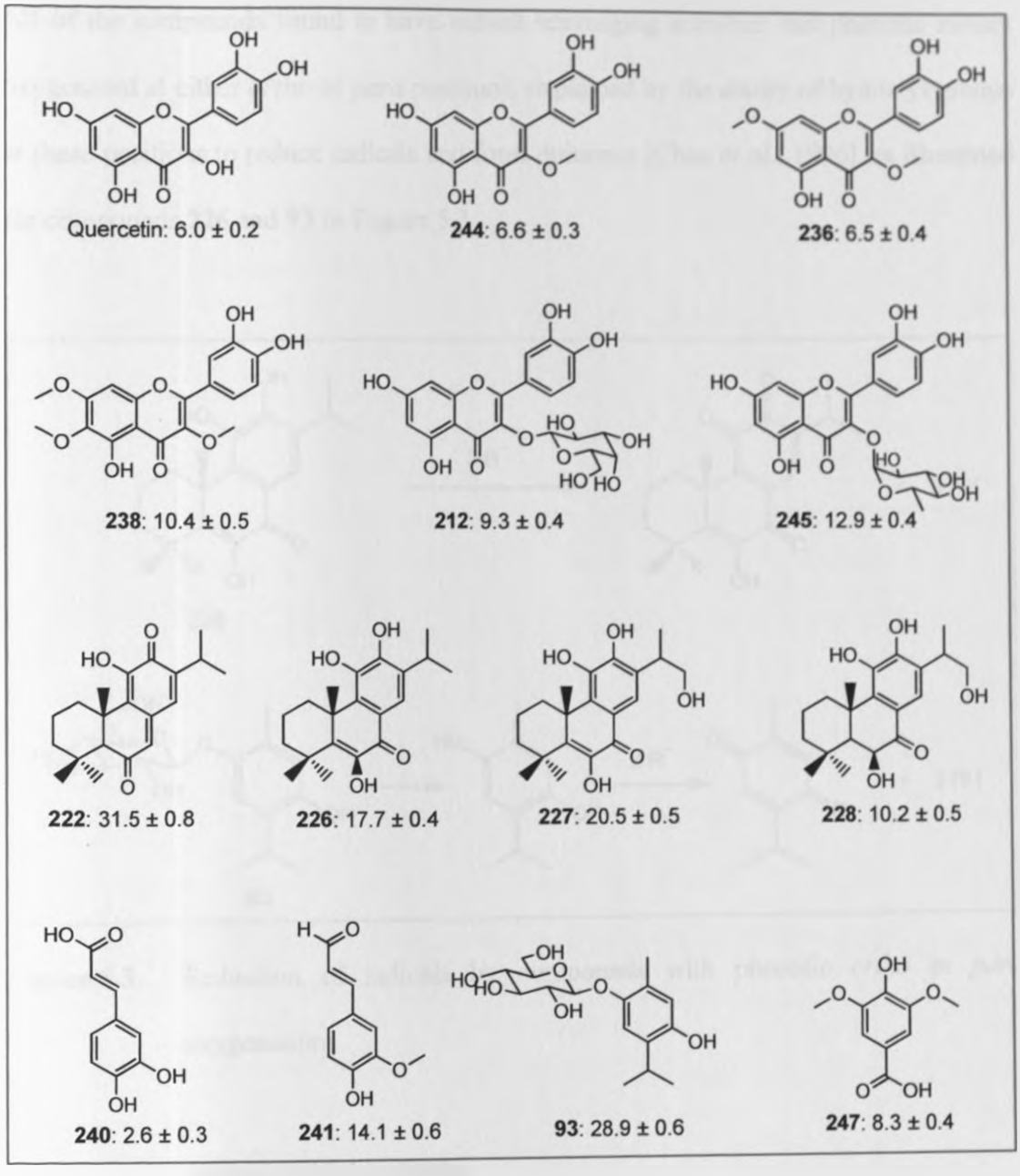


Figure 5.2: Compounds with radical scavenging activities and their IC_{50}'s (μg/ml)

The most active compound was caffeic acid (**240**), an established anti-oxidant found in coffee beverages [Andueza *et al.*, 2009]. Its radical scavenging activity of 2.6 μg/ml (14.4 μ/ml) was more than that of the standard used, quercetin (IC_{50} 6.0 μg/ml; 19.9 μM).

All of the compounds found to have radical scavenging activities had phenolic moiety oxygenated at either *ortho* or *para* positions, explained by the ability of hydroxyl groups at these positions to reduce radicals and form quinones [Chen *et al.*, 1996], as illustrated for compounds **226** and **93** in Fugure 5.3.

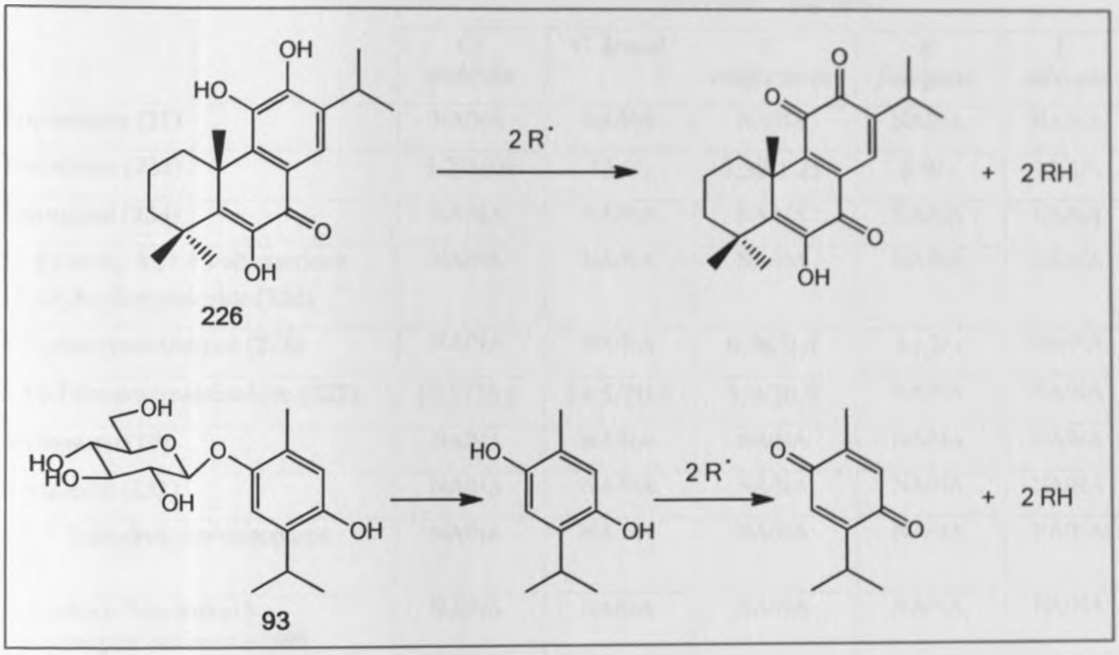


Figure 5.3: Reduction of radicals by compounds with phenolic *ortho* or *para* oxygenation.

5.3 Antimicrobial activities

The isolated compounds were tested for antifungal and antibacterial activities, using fungi *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Cryptococcus neoformans*, *Aspergillus fumigatus* as well as the bacteria *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRS), *Escherichia coli*, *Pseudomonas aeruginosa* and *Mycobacterium intracellulare*. The results are summarized in Table 5.5 for antifungal activities and Table 5.6 for antibacterial activities. Taxodione (**222**), 6-

hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**) showed antifungal activity, the best activity observed with taxodione (**222**) on *Cryptococcus neoformans* (IC_{50} 0.58 µg/ml).

Table 5.5: Antifungal activities

Compound	IC_{50}/MIC (µg/ml)				
	C. glabrata	*C. krusei*	*C. neoformans*	*A. fumigatus*	*C. albicans*
Royleanone (**21**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Taxodione (**222**)	5.2/10.0	12.0/-	0.58/1.25	8.9/-	12.5/-
Ferruginol (**224**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
11-hydroxy-8,11,13-abietatriene 12-*O*-*β*-xylopyranoside (**225**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
6-Hydroxysalvinolone (**226**)	NA/NA	NA/NA	0.96/2.5	11.2/-	NA/NA
6,16-Dihydroxysalvinolone (**227**)	14.9/20.0	14.5/20.0	5.9/20.0	NA/NA	NA/NA
uncinatone (**19**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Bongardol (**231**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
3,5,7-Trihydroxycarvotacetone (**85**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
3-Acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**).	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
3-*O*-*β*-Glucopyranoside (**233**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Zetaroside B (**93**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Quercetin-3,7-dimethylether (**236**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Penduletin (**237**),	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Crysoplenol D (**238**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Quercetin 3-*O*-*β*-*D*-galactoside (**212**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Quercetin 3-*O*-*α*-rhamnoside (**245**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
8-Acetoxyisochiliolide lactone (**248**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Epoxyisochiliolide lactone (**157**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Stigmasterol (**66**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Friedelan-3-one (**250**)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
Amphotericin B (standard)	0.31/0.65	0.95/1.25	0.44/1.25	1.29/2.50	0.43/1.25

NA =Not Active

Antibacterial activities were exhibited by taxodione (**222**), Ferruginol (**224**), 6-hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**), the best activity being of ferruginol (**224**) on methicillin-resistant *Staphylococcus aureus* (MRS) (IC_{50} 0.96 μg/ml).

Table 5.6: Antibacterial activities

Compound	IC_{50}/MIC (μg/ml)				
	S. aureus	*MRS*	*E. coli*	*P. aureginosa*	*M. intracellulare*
Royleanone (**21**)	NA	NA	NA	NA	NA
Taxodione (**222**)	1.35/5	1.47/2.5	NA	NA	11.9/-
Ferruginol (**224**)	1.33/2.5	0.96/2.5	NA	NA	14.5/-
11-hydroxy-8,11,13-abietatriene 12-*O*-*β*-xylopyranoside (**225**)	NA	NA	NA	NA	NA
6-Hydroxysalvinolone (**226**)	1.75/5	1.56/2.5	NA	NA	NA
6,16-Dihydroxysalvinolone (**227**)	6.8/20	8.44/20	NA	NA	NA
uncinatone (**19**)	NA	NA	NA	NA	NA
Bongardol (**231**)	NA	NA	NA	NA	NA
3,5,7-Trihydroxycarvotacetone (**85**)	NA	NA	NA	NA	NA
3-Acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**).	NA	NA	NA	NA	NA
3-*O*-*β*-Glucopyranoside (**233**)	NA	NA	NA	NA	NA
Zetaroside B (**93**)	NA	NA	NA	NA	NA
Quercetin-3,7-dimethylether (**236**)	NA	NA	NA	NA	NA
Penduletin (**237**),	NA	NA	NA	NA	NA
Crysoplenol D (**238**)	NA	NA	NA	NA	NA
Quercetin 3-*O*-*β*-*D*-galactoside (**212**)	NA	NA	NA	NA	NA
Quercetin 3-*O*-*α*-rhamnoside (**245**)	NA	NA	NA	NA	NA
8-Acetoxyisochiliolide lactone (**248**)	NA	NA	NA	NA	NA
Epoxyisochiliolide lactone (**157**)	NA	NA	NA	NA	NA
Stigmasterol (**66**)	NA	NA	NA	NA	NA
Friedelan-3-one (**250**)	NA	NA	NA	NA	NA
Ciprofloxacin (standard)	0.1/0.25	0.08/0.25	0.004/0.008	0.06/0.25	0.3/1.00

NA = Not Active

5.4 Antileishmanial activities

Antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes. Taxodione (**222**) and uncinatone (**19**) exhibited strong antileishmanial activity (IC_{50} 0.08 and 0.20 µg/ml respectively). Results for antileishmanial activity are summarized in Table 5.7.

Table 5.7: Antileishmanial activities

Compound	Activity against *L. donovani* (µg/ml)	
	IC_{50}	IC_{90}
3,5,7-trihydroxycarvotacetone (**85**)	17.0	>40
3-acetoxy-5,7-dihydroxycarvotacetone (**106**)	0.7	1.4
3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**)	3.0	6.9
3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**)	0.7	1.3
Royleanone (**21**)	NA	NA
Taxodione (**222**)	0.08	0.21
Ferruginol (**224**)	4.0	13.0
11-hydroxy-8,11,13-abietatriene 12-*O*-*β*-xylopyranoside (**225**)	NA	NA
6-Hydroxysalvinolone (**226**)	3.2	6.5
6,16-Dihydroxysalvinolone (**227**)	3.2	6.5
uncinatone (**19**)	0.2	0.9
Bongardol (**231**)	NA	NA
8-Acetoxyisochiliolide lactone (**248**)	NA	NA
Epoxyisochiliolide lactone (**157**)	38	NA
Pentamidine (standard)	1.4	6
Amphotericin B (standard)	0.13	0.3

NA = Not Active

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this research, four Kenyan plants used traditionally to cure malaria were investigated. The plants are *Clerodendrum eriophyllum* (Verbenaceae), *Sphaeranthus bullatus* (Asteraceae), *Microglossa pyrifolia* (Asteraceae) and *Vernonia galamensis* (Asteraceae). The investigation involved isolation and identification of secondary metabolites, *in-vitro* antiplasmodial testing, antimicrobial testing and antioxidant testing of the isolated compounds. A total of forty-four compounds were isolated, out of which two were new.

Clerodendrum eriophyllum gave fifteen compounds. One was a new compound (12-hydroxy-8,12-abietadiene-3,11,14-trione, **220**) and eleven compounds were new to the genus, being 6,7-dehydroroyleanone (**221**), taxodione (**222**), 11-hydroxy-7,9(11),13-abietatrien-12-one (**223**), ferruginol (**224**), 11-hydroxy-8,11,13-abietatriene *12-O-β*-xylopyranoside (**225**), 6-hydroxysalvinolone (**226**), 6,16-dihydroxysalvinolone (**227**), nellionol (**228**), betulinic acid (**229**), *β*-amyrin octacosanoate (**230**) and bongardol (**231**). The plant had not been investigated before this study, the study therefore helped in understanding the chemistry of the plant, revealing that the plant contained abietane diterpenoids as its major class of compounds.

Sphaeranthus bullatus gave seventeen compounds. Twelve compounds were new to the species and genus, being 5-*O-β*-glucopyranosylcarvotacetone (**232**), thymol 3-*O-β*-glucopyranoside (**233**), zetaroside A (**234**), quercetin (**235**), quercetin-3,7-dimethylether

(**236**), penduletin (**237**), crysoplenol D (**238**), 4-hydroxylonchocarpin (**239**), caffeic acid (**240**), coniferaldehyde (**241**), dammara-20,24-dienylacetate (**242**) and boehmery acetate (**243**). Compound **106** (3-acetoxy-5,7-dihydroxycarvotacetone) was new to the species but known to the genus.

Vernonia galamensis ssp *nairobensis* afforded seven known compounds, four of them being new to the species and genus, namely quercetin 3,7-dimethylether (**236**), quercetin 3-*O*-*β*-*D*-galactoside (**212**), syringaresinol (**246**) and syringic acid (**247**). Quercetin 3-methylether (**244**) is new to the species but known to the genus. *Microglossa pyrifolia* gave six compounds, 8-acetoxyisochiliolide lactone (**248**) being new compound and 1,4-dihydroxy-7(11)-eudesmen-8-one (**251**) new to the genus.

In-vitro anti-plasmodial testing showed that abietane diterpenoids from *C. eriophyllum* and carvotacetone derivatives from *S. bullatus* were potential anti-malarial agents, as they were the classes with active compounds. The abietane diterpenoids with antiplasmodial activities were taxodione (**222**) (IC_{50} = 1.2 μg/ml against both D2 and W6 strains), 6-hydroxysalvinolone (**226**) (IC_{50} = 1.8 μg/ml and 2.5 μg/ml against D2 and W6 strains respectively) and 6,16-dihydroxysalvinolone (**227**) (IC_{50} = 3.0 μg/ml and 4.8 μg/ml against D2 and W6 strains respectively), where as the carvotacetone derivatives were 3,5,7-trihydroxycarvotacetone (**85**) (IC_{50} = 3.4 μg/ml and 2.8 μg/ml against D2 and W6 strains respectively) and 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**) (IC_{50} = 1.4 μg/ml and 2.0 μg/ml against D2 and W6 strains respectively).

Anti-oxidant testing revealed that phenolic compounds with oxygenations in *ortho* or *para* positions were active. These were some of the flavonoids, some of the abietane ditepernoids, phenyl propanoids, *p*-cymeme derivatives, the lignan syringaresinol (**246**) and the benzenoid syringic acid (**247**). The most active anti-oxidant was caffeic acid (**240**), with IC_{50} of 2.6 ± 0.3 μg/ml (14.4 μM) as compared to 6.0 ± 0.2 μg/ml (19.9 μM) of quercetin, the standard used.

Taxodione (**222**) and 6-hydroxysalvinolone (**226**) exhibited potent antifungal activity (IC_{50} 0.58 and 0.96 μg/ml respectively) against *C. neoformans* where as taxodione (**222**) and uncinatone (**19**) exhibited potent antileishmanial activity (IC_{50} 0.08 and 0.20 μg/ml respectively) against *L. donovani*. Taxodione was a more potent antileishmanial compound, being active than of amphotericin B, the standard used.

The observed antiplasmodial activities for extracts and compounds from the investigated plants justifies the traditional use of the plants as cure for malaria. However, compounds from *Microglossa pyrifolia* and *Vernonia galamensis* did not show antiplasmodial activities. This interprets that the activity of their extracts are either due to synegytic effect or contributed by trace compounds which were not isolated.

Three abietane diterpenoids displayed both antiplasmodial and antioxidant activities. These are taxodione (**222**) 6-hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**). This gives a revelation for a possibility of having an anti-malarial which is also an

anti-oxidant, and hence giving a possibility of fighing malaria while minimizing the chances of developing complications from oxidative stress.

6.2 Recommandations

The compounds which displayed potent antiplasmodial and antioxidant activities, taxodione (**222**) 6-hydroxysalvinolone (**226**) and 6,16-dihydroxysalvinolone (**227**) should be tested for *in-vivo* activities and investigated further for possibilities of developing them to anti-malarial drugs.

Other parts of *Clerodendrum eriophyllum* as well as other species of the genus should be investigated for active compounds, since the roots of *Clerodendrum eriophyllum* gave more active compounds than the other plants investigated in this study.

Taxodione (**222**) should therefore be investigated for *in-vivo* activity and possibility of being developed to an antileishmanial drug, since it showed potent antileishmanial activity, being active than amphotericin B, a commercial drug.

REFERENCES

Abe F., Yamauchi T. (1988) 9α-hydroxypinoresinol, 9α-hydroxymedioresinol and related lignans from *Allamanda neriifolia*. *Phytochemistry* **27**, 575-577

Ahmed, A. A., Jakupovic J. (1990). Sesqui- and monoterpenes from *Jasonia montana*. *Phytochemistry* **29**, 3658 – 3661.

Ahmed A. A., Mahmoud A. A. (1997). Carvotacetone derivatives from the Egyptian plant *Sphaeranthus suaveolens*. *Phytochemistry* **45**, 533-535

Akah P. A., Ekekwe R. K. (1995). Ethnopharmacology of some *Asteraceae* family used in Nigerian traditional medicine. *Fitoterapia* **66**, 351-355

Akendengue B. (1992). Medicinal plants used by the Fang traditional healers in Equatorial Guinea. *J. Ethnopharmacol* **37**, 165-173

Akihisa T., Matsubara Y., Ghosh P., Thakur S., Tamura T., Matsumoto T. (1989). Sterols of some *Clerodendrum* species (Verbenaceae): Occurrence of the epimers of 24-α and 24-β- 24-ethylsterols lacking a delta 25-Bond. *Steroids* **53**, 625-638

Alfatafta A. A., Abu Zarga M. H., Sabri S. S., Freyer A. J., Shamma M. (1989). An investigation of *Bongardia chrysogonum*. *Journal of Natural Products* **52**, 818-821

Ali M. S., Saleem M., Akhtar F., Jahangir M., Parvez M., Ahmad V. U. (1999). Three *p*-cymene derivatives from *Zataria multiflora*. *Phytochemistry* **52**, 685-688

Ameho C. K., Chen C. Y. O., Smith D., Sánchez-Moreno C, Milbury P. E., Blumberg J. B. (2008). Antioxidant activity and metabolite profile of quercetin in vitamin-E-depleted rats. *Journal of Nutritional Biochemistry* **19**, 467-474

Amico A. (1977). Medicinal plants of Southern Zambesia. *Fitoterapia* **48**, 101-139

Andueza S., Manzocco L., Paz de Pena M., Cid C., Nicoli C. (2009). Caffeic acid decomposition products: Anti-oxidants or pro-oxidants? *Food Research International* **42**, 51-55

Ayanoglu E., Ulubelen A., Clark W. D., Brown G. K., Kerr R. R., Mabry T. J. (1981). Myricetin and quercetin methyl ethers from *Haplopappus integerrimus* var. *punctatus*. *Phytochemistry* **20**, 1715-1717

Bahorun T., Gressier B., Trotin F., Brunet C., Dine T., Luycks M., Vasseur J., Cazin J., Pinkas M. (1996). Oxygen species scavenging activity of phenolic extracts from Hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimittel-Forschung / Drug Resarch* **46**, 1086-1089

Balunas M.J., Kinghorn A. D. (2005). Drug discovery from medicinal plants. *Life Sciences* **78**, 431-441

Barua A. S., Chowdhury A., Adityachaudhury N. (1989). Chemical investigations of *Clerodendron fragrans* and *Clerodendron siphonanthus*. *Indian Journal of Chemistry* **28B**, 878-879

Bashwira S., Hootele C. (1988). Myricoidine and dihydromyricoidine, two new macrocyclic spermidine alkaloids from *Clerodendrum myricoides*. *Tetrtahedron* **44**, 4521-4526

Baye T., Kebede H., Belete K. (2001). Agronomic evaluation of *Vernonia galamensis* germplasm collected from Eastern Ethiopia. *Industrial Crops and Products* **14**, 179–190

Beentje H. K. (1994). Kenyan trees, shrubs and lianas. National Museums of Kenya. Nairobi, Kenya

Bennini B., Chulia A.J., Kaouadji M., Thomasson F. (1992). Flavonoid glycosides from *Erica cinerea*. *Phytochemistry* **31**, 2483-2486

Bhandary M. J., Chandrashekar K. R., Kaveriappa K. M. (1995). Medical ethnobotany of the Siddis of Uttara Kannada District, Karnataka, India. *Journal of Ethnopharmacology* **47**, 149-158

Bilia, A.R. (2006). Non-nitrogenous plant-derivered constituents with antiplasmodial activity. *Natural Product communications* **1**, 1181-1204

Blunt J. W., Stothers J. B. (1977). ^{13}C nmr spectra of steroids: A survey and commentary. *Organic Magnetic Resonance* **9**, 439-464

Borenfreund E., Babich H., Martin-Alguacil N. (1990). Rapid chemosensitivity assay with human normal and tumor cells *in vitro*: In vitro cellular & developmental biology. *Journal of the Tissue Culture Association* **26**, 1030-1034

Bouktaib M., Lebrun S., Atmani A., Rolando C. (2002). Hemisynthesis of all the *O*-monomethylated analogues of quercetin including the major metabolites, through selective protection of phenolic functions. *Tetrahedron* **58**, 10001-10009

Chakravarty A. K. (1994). Unambiguous assignment of 13C chemical shifts of some hopane and migrated hopane derivatives by 2D NMR. *Tetrahedron* **50**, 2865-2876

Chaturvedi G. N., Subramaniyam P. N., Tiwari S. K., Singh K. P. (1984). Experimental and clinical studies of diabetes mellitus evaluating the efficacy of an indigenous oral hypoglycemic drugarani (*Clrodendron phlomidis*). *Ancient Science Life* **3**, 216-224

Chawla, A. S., Kaith, B. S., Handa, S. S., Kulshreshtha, D. K., Srimal, R. C. (1990). Chemical investigation and anti-inflammatory activity of *Pluchea lanceolata* roots. *Indian Journal of Chemistry* **29B**, 918 - 922

Chen Z. Y., Chan P. T., Ho K. Y., Fung K. P., Wang J. (1996). Antioxidant activity of natural flavonoids governed by number and location of their hydroxyl groups. *Chemistry and Physics of Lipids* **79**, 157-163.

Cheng H-H., Wang H-K., Ito J., Bastow K. F., Tachibana Y., Nakanishi Y., Xu Z., Luo T-Y., Lee K-H. (2001). Cytotoxic pheophorbide-related compounds from *Clerodendrum calamitosum* and *C. cyrtophyllum*. *Journal of Natural Products* **64**, 915-919

Collins E. D., Bishop J. E., Bula C. M., Acevedo A., Okamura W. H., Norman A. W. (2005). Effect of 25-hydroxyl group orientation on biological activity and binding to the 1α,25-dihydroxy vitamin D3 receptor. *Journal of Steroid Biochemistry & Molecular Biology* **94**, 279–288

Corbett R. E., Young H., Wilson R. S. (1964). Extractives from the bark of *Olearia paniculata*. *Australian Journal of Chemistry* **17**, 712-714

Csehi J., Palinko I. (2004). Hydrogen bonding interactions in E- or Z-2-phenyl-3-(X'-pyridyl)propenoic acid (X = 2, 3 or 4) assemblies – a molecular modeling study. *Journal of Molecular Modeling* **10**, 151-154

Das B., Venkataiah B., Kashinatham A. (1998). Syringaresinol from *Parthenium hysterophorus*. *Fitoterapia* **70**, 101-102

Dellar J. E., Core M. D., Waterman P. G. (1996). Antimicrobial abietane diterpenoids from *Plectranthus elegans*. *Phytochemistry* **41**, 735-738

Dong X. P., Qiao R. X., Guo L., Xie J., Liu B. (1999). The chemical constituents of *Clerodendrum bungei* Steud. *Tianran Chanwu Yanjiu Yu Kaifa* **11**, 8-10

Dorsaz A., Marston A., Stoeckli-Evans H., Msonthi J.D., Hostettmann, K. (1985) "Uncinatone, a New Antifungal Hydroquinone Diterpenoid from *Clerodendrum uncinatum*." *Helv. Chim. Acta* **68**, 1605-1610

Duenas M., González-Manzano S., González-Paramás A., Santos-Buelga C. (2010). Antioxidant evaluation of *O*-methylated metabolites of catechin, epicatechin and quercetin. *Journal of Pharmaceutical and Biomedical Analysis* **51**, 443–449

Edwards O. E., Feniak G., Los M. (1962). Diterpenoid quinones of *Inula royleana D.C. Canadian Journal of Chemistry* **40**, 1540-1546.

El-Shamy A. M., El-Shabrawy A. R. O., El-Fiki N. (1996). Phytochemical study of *Clerodendron inerme* L. growing in Egypt. *Zagazig Journal of Pharmaceutical Sciences* **5**, 49-53

Erasto P., Grierson D. S., Afolayan A. J. (2006). Bioactive sesquiterpene lactones from *leaves of Vernonia amygdalina. Journal of Ethnopharmacology* **106**. 117-120.

Fan T. P., Min Z. D., Iinuma M. (1999a). Two novel diterpenoids from *Clerodendrum bungei. Chemical and Pharmaceutical Bulletin* **47**, 1797-1798

Fan T. P., Min Z., Song G., Iinuma M., Tanaka T. (1999b). Abietane diterpenoids from *Clerodendrum mandarinorum. Phytochemistry* **51**, 1005-1008

Ferrari B., Tomi F., Casanova J. (2005). Terpenes and acetylene derivatives from the roots of *Santolina corsica* (Astraceae). *Biochemical Systematics and Ecology* **33**, 445-449.

Ganapaty S., Rao D. V. (1989). Constituents of *Clerodendrum neriifolium. Fitoterapia* **60**. 381

Gao J., Han G. (1997). Cytotoxic abietane diterprnoids from *Caryopteris incana. Phytochemistry* **44**, 759-761.

Geissbühler Y., Chaki P., Emidi B., Govella N. J., Shirima R., Mayagaya V., Mtasiwa D., Mshinda H., Fillinger U., Lindsay S. W., Kannady K., De Castro M. C., Tanner M., Killeen Gerry F. (2007). Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malaria Journal* **6**,126-129.

Gessler M. C., Msuya D. E., Nkunya M. H. H., Mwasumbi L. B., Schar A., Heinrich M., Tanner M. (1995). Traditional healers in Tanzania: the treatment of malaria with plant remedies. *Journal of Ethnopharmacology* **48**, 131- 144

Gessler M. C., Nkunyah M. H. H., Mwasumbi L. B., Heinrich M., Tanner M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica* **56**, 65-77

Gunatilaka A. A. L., Dhanabaladinogham B., Paredes L., Jakupovic J., Bohlmann F., Adikaram N. K. B. (1987). Microglossic acid, an alicyclic diterpene and other constituents of *Microglossa zeylanica*. *Phytochemistry* **26**, 2408-2409

Gurib-Fakim A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine* **27**, 1 – 93

Gutierrez A., Rodriguez I. M., Del Rio J. C. (2006). Chemical characterization of lignan and lipids fractions in industrial hemp bast fibres used for manufacturing high quality paper pulps. *Journal of Agricutural and Food Chemistry* **54**, 2138-2144

Han L., Huang X., Dahse H., Moellmann U., Grabley S., Lin W., Satler I. (2008). New abietane diterpenoids from the mangrove *Avicennia marina*. *Planta Medica* **74**, 432-437

Hazekamp A., Verpoorte R., Panthong A. (2001). Isolation of a bronchodilator flavonoid from the Thai medicinal plant *Clerodendrum petasites*. *Journal of Ethnopharmacology* **78**, 45-49

He L., Chen Y. Z., Ding L. S., Li P. G. (1997). Chemical constituent of *Clerodendron bungei*. *Chung Ts'ao Yao* **28**, 395-396

Hensch M., Ruedi P., Eugster C. H. (1975). Horminon, toxochnon und weitere royleanone aus zwei abessinischen *Plectranthus* spezies. *Helvetica Chimica Acta* **58**, 1921-1934

Hetzel, M. W., Iteba N., Makemba A., Mshana C., Lengeler C., Obrist B., Schulze A., Nathan R., Dillip A, Alba S., Mayumana I., Khatib R. A., Njau J. D., Mshinda H. (2007). Understanding and improving access to prompt and effective malaria treatment and care in rural Tanzania: the ACCESS Programme. *Malaria Journal* **6**, 83 – 97

Hirasawa Y., Izawa E., Matsuno Y., Kahawara N., Goda Y., Morita H. (2007). Taxodistines A and B, abietane-type diterpenes from *Taxodium distichum*. *Bioorganic & Medicinal Chemistry Letters* **17**, 5868-5871

Hisham A., Jaya Kumar G., Fujimoto Y., Hara N. (1995). Salacianone and salacianol, two triterpenes from Salacia beddomei. *Phytochemistry* **40**, 1227 - 1231

Horie T., Ohtsumu Y., Shibata K., Yamashita K., Tsukayama M., Kawamura Y. (1998). ^{13}C-NMR spectral assignment of the A-ring of polyoxygenated flavones. *Phytochemistry* **47**, 865-874

Hou W., Hsu F., Lee M. (2002). Yam (*Dioscorea batatas*) tuber mucilage exhibited anti-oxidant activities in vitro. *Planta Medica* **68**, 1072-1076

Ikram M. (1981). A review on the medicinal plants. *Hamdard* **24**, 102-129

Jacke G., Rimpler H. (1983). Distribution of iridoid glycosides in *Clerodendrum* species. *Phytochemistry* **22**, 1729-1734

Jakupovic J., Grenz M., Bohlmann F., Mungai G.M. (1990). Carvotacetone derivatives and eudesman-12,6β-olides from *Sphaeranthus* species. *Phytochemistry* **29**, 1213-1217

Jay M., Voirin, B. (1976) Les flavonoides de deux especes du genre *Chrysosplenium*. *Phytochemistry*, **15**, 517-519

Johns T., Kokwaro J. O., Kimanani E. K. (1990). Herbal remedies of the Luo of Siaya district, Kenya: Establishing quantitative criteria for consensus. *Economic Botany* **44**, 369-381.

Joshi K. C., Singh P., Mehra A. (1979). Chemical investigation of the roots of different *Clerodendron* species. *Planta Medica* **37**, 64-66

Kabouche K., Kabouche Z., Ozturk M., Kolak U., Topcu G. (2007). Antioxidant abietane diterpenoids from *Salvia barrelieri*. *Food Chemistry* **102**, 1281-1287

Kanchanapoom T., Kasaia R., Chumsric P., Hiragad Y., Yamasaki K. (2001). Megastigmane and iridoid glucosides from *Clerodendrum inerme*. *Phytochemistry* **58**, 333-336

Kamau, E. M. (2006). Roll Back Malaria and the new partnership for Africa's development: Is there potential for synergistic collaboration in partnership? *African Journal of Health Sciences* **13**, 22-27.

Kanchanapoom T., Kasai R. J., Chumsri P., Hiraga Y., Yamasaki K. (2001). Megastigmane and iridoid glucosides from *Clerodendrum inerme*. *Phytochemistry* **58**, 333-336

Kang D. G., Lee Y. S., Kim H. J., Lee Y. M., Lee H. S. (2003). Angiotensin converting enzyme inhibitory phenylpropanoid glycosides from *Clerodendron trichotomum*. *Journal of Ethnopharmacology* **89**, 151-154

Kaur K., Jain M., Kaur T., Jain R. (2009). Antimalarials from nature. *Bioorganic & Medicinal Chemistry* **17**, 3229–3256

Kawazu, S., Komaki-Yasuda, K., Oku, H., Kano, S., (2008). Peroxiredixins in malaria parasites: Parasitologic aspect. *Parasitology International* **57**, 1-7

Khan M. A., Singh V. K. (1996). A folklore survey of some plants of Bhopal district forest Madhya Pradesh India described as antidiabetics. *Fitoterapia* **67**, 416-421

Kigondu E. V. M., Rukunga G. M., Keriko J. M., Tonui W. K., Gathirwa J. W., Kirira P. G., Irungu B., Ingonga J. M., Ndiege I. O. (2009). Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. *Journal of Ethnopharmacology* **123**, 504-509.

Kirira P.G., Rukunga G.M., Wanyonyi A.W., Muregi F.M., Gathirwa J.W., Muthaura C.N., Omar S.A., Tolo F., Mungai G.M., Ndiege I.O. (2006). Anti-plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi Districts of Kenya. *Journal of Ethnopharmacology* **106**, 403–407.

Koch A., Tamez J., Pezzuto J., Soejarto D. (2005). Evaluation of plants used for antimalarial treatment by the Maasai of Kenya. *Journal of Ethnopharmacology* **101**, 95–99.

Kohler I., Janett Siems K., Kraft C., Siems K., Abbiw D., Bienzle U., Aiche E. (2002). Herbal remedies traditionally used against malaria in Ghana: Bioassay guided fractionation of *Microglossa pyrifolia* (Asteraceae). *Zeitschrift fur Naturforschung C* **57c** 1022-1027.

Kokwaro, J.O., (1976) in "Medicinal Plants of East Africa." East Africa literature bureau. Nairobi, Kenya.

Kondo Y., Ikenoue T., Takemoto T. (1963). Structure of xanthoperol. *Chemical and Pharmaceutical Bulletin* **11** 678-680.

Kuiate J. R., Zollo P. H. A., Nguefa E. H., Bessiere J. M., Lamaty G., Menut C. (1999). Composition of the essential oils from the leaves of *Microglossa pyrifolia* (Lam.) O.Kuntze and *Helichrysum odoratissimum* (L.) Less. growing in Cameroon. *Flavour and Fragrance Journal* **14**, 82-84

Kvist L. P., Christensen S. B., Rasmussen H. B., Mejia K., Gonzalez A. (2006). Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. *Journal of Ethnopharmacology* **106**, 390–402

Laks P.E., Pruner M.S. (1989). Flavanoid biocides, structure/activity relations of flavanoid phytoalexins anologues. *Phytochemistry* **28**, 87-91.

Lal S. D., Lata K. (1980). Plants used by the Bhat community for Regulating fertility. *Economic Botany* **34**, 273-275.

Leong Y., Harrison L. J. (1999). (20*R*, 23*E*)-Eupha-8,23-diene-3β,25-diol from *Tripelum cymosum*. *Phytochemistry* **50**, 849-857.

Lin Y. L., Kuo Y. H., Chen Y. L. (1989a). Three new clerodane-type diterpenoids, clerodinin C, clerodinin D and clerodiol, from *Clerodendron brachyanthum* Schauer. *Heterocycles* **29**, 1489-1495

Lin Y. L., Kuo Y. H., Chen Y. L. (1989b). Two new clerodane-type diterpenoids, clerodinins A and B, from *Clerodendron brachyanthum* Schauer. *Chemical and Pharmaceutical Bulletin* **37**, 2191-2193

Lin Y. L., Kuo Y. H. (1992). A new glycoside, brachynoside, isolated from *Clerodendron Brachyanthum* Schauer. *Chemical and Pharmaceutical Bulletin* **40**, 1928-1929

Lumbu S., Hootele C. (1993). Buchnerine and paramethoxycinamoylbuchnerine, two new macrocyclic alkaloids from *Clerodendrum buchnery*. *Journal of Natural Products* **56**, 1418-1420.

Luyengi L., Lee I. S., Mar W., Fong H. H. S., Pezzuto J. M., Kinghorn A. D. (1994). Rotenoids and chalcones from Mundelea sericea that inhibit phorbol ester-induced ornithine decarboxylase activity. *Phytochemistry* **36**, 1523-1526.

Machumi F., Samoylenko V., Yenesew A., Derese S., Midiwo J. O., Wiggers F. T., Jacob M. R., Tekwani B. L., Khan S. I., Walker L. A., Muhammad I. (2010). Antimicrobial and Antiparasitic Abietane Diterpenoids from the Roots of *Clerodendrum eriophyllum*. *Natural Product Communications* **5**, 853-858

Mahato S. B., Sen S. (1997). Advances in triterpenoid research, 1990-1994. *Phytochemistry* **44**, 1185-1236.

Makler M. T., Ries J. M., Williams J. A., Bancroft J. E., Piper R. C., Gibbins B. L, Hinriches D. J. (1993). Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. *American Journal of Tropical Medicine Hygiene* **48**, 739-741

Manandhar N. P. (1993). Herbal remedies of Surkhet District, Nepal. *Fitoterapia* **64**, 266-272

Masuda T., Yonemori S., Oyama Y., Takeda Y., Tanaka T., Andoh T., Shinohara A., Nakata M. (1999). Evaluation of the antioxidant activity of environmental plants: Activity of the leaf extracts from seashore plants. *Journal of Agricutural and Food Chemistry* **47**, 1749-1754

Matsunaga S., Tanaka R., Akagi M. (1988). Triterpenoids from *Euphorbia maculata*: *Phytochemistry* **27**, 535-537

Meyer E. F., Swanson S. M., Williams J. A. (2000). Molecular modeling and drug design." *Pharmacology & Therapeutics* **85**. 113-121

Mikus J., Steverding D. (2000). A simple colorimetric method to screen drug cytotoxicity against Leishmania by using the dye Alamar Blue. *Parasitology International* **48**. 265-269.

Miserez F., Potterat O., Marston A., Mungai G. M., Hostettmann K. (1996). Flavonol glycosides from *Vernonia galamensis* ssp. *nairobiensis*. *Phytochemistry* **43**. 283-286

Mungai G. M., Ndiege I. O. (2008). The *in vitro* anti-plasmodial and *in vivo* anti-malarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya. *Journal of Ethnopharmacology* **115**. 223–231.

Muregi F. W., Chhabra S. C., Njagi, E. N. M., Lang'at-Thoruwa C. C., Njue W. N., Orago A. S. S., Omar S. A., Ndiege I. O. (2003). In vitro antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *Journal of Ethnopharmacology* **84**. 235-239

Murugesan T., Saravanan K. S., Lakshmi S., Ramya G., Thenmozhi K. (2001). Evaluation of psychopharmacological effects of *Clerodendrum phlomidis* Linn. extract. *Phytomedicine* **8**. 472-476

Muthaura C. N., Rukunga, G. M., Chhabra S. C., Mungai G. M., Njagi E. N. M. (2007a). Traditional antimalarial phytothelapy remedies used by the Kwale community of the Kenyan Coast. *Journal of Ethnopharmacology* **114**, 377-386

Muthaura C. N., Rukunga G. M., Chhabra S. C., Omar S. A., Guantai A. N., Gathirwa J. W., Tolo F. M., Mwitari P. G., Keter L. K., Kirira P. G., Kimani C. W., Mungai G. M., Njagi E. N. M. (2007b). Antimalarial activity of some plants traditionally usedin treatment of malaria in Kwale district of Kenya. *Journal of Ethnopharmacology* **112**, 545-551

Muthaura C. N., Rukunga G. M., Chhabra S. C., Omar S. A., Guantai A. N., Gathirwa J. W., Tolo F. M., Mwitari P. G., Keter L. K., Kirira P. G., Kimani C. W., Mungai G. M., Njagi E. N. M. (2007c). Antimalarial activity of some plants traditionally used in Meru district of Kenya. *Phytotherapy Research*, **21**, 260-267.

Mwangi J. W., Achola K. J., Laurent R., Lwande W., Hassanali A. (1995). Essential oil constituents of *Sphaeranthus cyathuloides* O.Hoffm. *Journal of Essential Oil Research* **7**, 177-178

Nagaraju N., Rao K. N. (1990). A survey of plant crude drugs of Rayalaseema, Andhra Pradesh, India. *Journal of Ethnopharmacology* **29**, 137-158

Narayanan N., Thirugnanasambantham P., Viswanathan S., Vijayasekaran V., Sukumar E. (1999). Antinociceptive, anti-Inflammatory and antipyretic effects of ethanol extract of *Clerodendron serratum* roots in Experimental animals. *Journal of Ethnopharmacology* **65**, 237-241

Nisbet L.J., Mooret M. (1997). Will natural products remain an important source of drug research for the future? *Current Opinion in Biotechnology* **8**, 708-712

Nishida R., Fukami H., Miyata T., Takeda M. (1989). Clerodendrins: Feeding stimulants for the adult turnip sawfly, *Athalia rosae ruficornis*, from *Clerodendron trichotomum* (Verbenaceae). *Agriculture and Biological Chemistry* **53**, 1641-1645

Nkuo-Akenji, T.K., Menang, O.N. (2005). Prevelance of falciparum malaria together with acute diarrhoea in children residing in a malaria endemic zone. *Africa Journal of Traditional, Complementary and Alternative Medicine* **12**, 26-30.

Odugbemi, T.O., Akinsulire, O.R., Aibinu, I.E., Fabeku, P.O. (2007) Medicinal plants useful for malaria therapy in Okeigbo Ondo state, Southwest Nigeria. *Africa Journal of Traditional, Complementary and Alternative Medicine* **4**, 191-198.

Onayade O. A., Scheffer J. J. C., Schripsema J., Der VanGen A. (1990). 6-Hydroxycarvotanacetone and other constituents of the essential oil of *Laggera alata* (D. Don) Sch. Bip. ex Oliv. *Flavour and Fragrance Journal* **5**, 165-172

Osbourn A.E., Lanzotti V. (2009) in "Plant derived natural products; synthesis, function and application." Springer Dordrecht Heidelbeg London New York, pp 3.

Pandey R., Verma R. K., Singh S. C., Gupta M. M. (2003) 4α-methyl-24β -ethyl-5α-cholesta-14,25-dien-3β-ol and 24β -ethylcholesta-5, 9(11), 22e-trien-3β -ol, sterols from *Clerodendrum inerme*. *Phytochemistry* **63**, 415-420

Panthong D., Kanjanapothi T., Taesotikul T., Wongcomea V. (2003). Anti-inflammatory and antipyretic properties of *Clerodendrum petasites* S. Moorea. *Journal of Ethnopharmacology* **85**, 151-156

Pei S-J. (1985). Preliminary study of ethnobotany in Xishuang Banna, People's Republic of China. *Journal of Ethnopharmacology* **13**, 121-137

Perdue Jr. R. E., Tsichritzis F., Jakupovic J. (1993). Prevernocistifolides from *Vernonia galamensis*. *Phytochemistry* **34**, 1075-1077.

Phillipson, J. D. (2007). Phytochemistry and pharmacognosy. *Phytochemistry* **68**, 2960–2972

Pieta, P. (2000). Flavanoids as antioxidants. *Journal of Natural Products* **63**, 1035-1042.

Porter P. M., Banwart W. L., Hassett J. J. (1986). Phenolic acids and flavonoids in soybean roots and leaf extracts. *Environmental and Experimental Botany* **26**, 65-73.

Pujar P. P., Sawaikar D. D., Rojatkar S. R., Nagasampagi B. A. (2000). Eudesmanoids from *Sphaeranthus indicus*. *Fitoterapia* **71**, pp. 264-268

Raha P., Banerjee H., Das A. K. (1989). Occurrence of three 5-hydroxyflavones in *Cleodendron scandens* and *Clerodendron inermi* Linn. *Indian Journal of Chemistry* **28B**. 874

Rani S., Ahamed N., Rajaram S., Saluja R., Thenmozhi S., Murugesan T. (1999). Anti-diarrhoeal evaluation of *Clerodendrum phlomidis* Linn. leaf extract in rats. *Journal of Ethnopharmacology* **68**, 315-319

Rao C. B., Rao T. N., Vijayakumar E. K. S. (1979) Chemical examination of *Premna latifolia*; Isolation and characterization of some new Diterpenes. *Indian Journal of Chemistry Section B* **18**, 513-24

Rao R. R., Jamir N. S. (1982) Ethnobotanical studies in Nagaland. I. medicinal plants. *Economic Botany* **36**, 176-181

Ravindranath N., Ramesh C., Kishore K., Murty U. S. N., Das B. (2003). Clerodendrone, a novel hydroquinone diterpenoid from *Clerodendrum indicum*. *Journal of Chemical Research(S).* **2003**, 440-441

Rojatkar S. R., Nagasampagi A. (1992). 7-Hydroxyeudesmanolides from *Sphaeranthus indicus*. *Phytochemistry* **31**, 3270-3271

Rucker G., Kehrubau, S., Sakulas H., Lawong B., Goeltenboth F. (1992). Acetylated aurone glycosides from *Microglossa pyrifolia*. *Planta Medica* **60**, 288-289.

Rugemalila, J.B., Wanga, C.L., Kilama W.L. (2006). Sixth Africa malaria day in 2006: how far have we come after Abuja declaration? *Malaria Journal* **12**, 102-106

Sachs, J., Malaney, P. (2002). The economic and social burden of Malaria. *Nature* **415**. 680-685.

Saidu, K., Onah, J., Orisadipe, AS., Olusola, A., Wambebe, C., Gamaniel, K., (2000). Antiplasmodial, analgesic, and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina selegalensis*. *Journal of Ethnopharmacology* **71**, 275-280.

Samuelsson G., Farah M. H., Claeson P., Hagos M., Thulin M., Hedberg O., Warfa A. M., Hassan A. O., Elmi A. H., Abdurahman, A. D., Elmi A. S., Abdi Y. A., Alin M. H. (1992). Inventory of plants used in traditional medicine in Somalia II; Plants of the families Combretaceae to Labiatae. *Journal of Ethnopharmacology* **37**, 47-70

Sanogo R., Germano M. P., De Tommas N., Pizza C., Aquino R. (1998). Vernoniosides and an androstane glycoside from *Vernonia kotschyana*. *Phytochemistry* **47**, 73-78

Schmidt T. J., Hildebrand M. R., Willuhn J. (2003). New dihydrobenzofurans and triterpenoids from roots of *microglossa pyrifolia*. *Planta Medica* **69**, 258-264.

Seth K. K., Pandey V. B., Dasgupta B. (1982). Flavonoids of *Clerodendron phlomidis* flowers. *Pharmazie* **37**, 74-75

Sharma M. P., Ahmad J., Hussain A., Khan S. (1992). Folklore medicinal plants of Mewat (Gurgaon District), Haryana, India. *International Journal of Pharmacognosy* **30**, (2) 135-137

Shekhani M. S., Shah P. M., Khan K. M., Atta-Ur-Rahman (1991). New eudesmanolides from *Sphaeranthus indicus*. *Journal of Natural Products* **54**, 882-885

Shekhani M. S., Shah P. M., Yasmin A., Siddiqui R., Perveen S., Mohammed K., Shahana K., Kazmi U., Atta-Ur-Rahman (1990). An immunostimulant sesquiterpene glycoside from Sphaeranthus indicus. *Phytochemistry* **29**, 2573-2576

Shen C. C., Chang Y. C., Ho L. K. (1993). Nuclear Magnetic Resonance studies of 5,7-dihydroxyflavonoids. *Phytochemistry* **34**, 843-845.

Shrivastava N., Patel T. (2007). *Clerodendrum* and Heathcare: An Overview. *Medicinal and Aromatic Plant Science and Biotechnology* **1**, 142-150

Siddiqui S., Hafeez F., Begum S., Siddiqui B.S. (1988). Oleanderol, a new pentacyclic triterpene from the leaves of *Nerium oleander*. *Journal of Natural Products* **51**, 229-233.

Sikarwar R. L. S., Kaushik J. P. (1993). Folk medicines of the Morena District, Madhya Pradesh, India. *International Journal of Pharmacognosy* **31** 283-287

Singh R., Prakash L. (1983). Chemical examination of stem of *Clerodendron inerme* (L.) Gaertn. (Verbenaceae). *Pharmazie* **38**, 565-566

Singh V. P., Sharma S. K., Khan V. S. (1980). Medicinal plants from Ujain district Madhya Pradesh part II. *Indian Drugs and Pharmaceutical Industry* **5**, 7-12

Samoylenko V., Jacob M. R., Khan S. I., Zhao J., Tekwani B. L., Midiwo J. O., Walker L. A., Muhammad I. (2009). Antimicrobial, Antiparasitic and Cytotoxic Spermine Alkaloids from *Albizia schimperiana*. *Natural product communications* **4**, 791-796

Stenzel E., Rimpler H., Hunkler D. (1986). Iridoid glucosides from *Clerodendrum incisum*. *Phytochemistry* **25**, 2557-2561

Sy L., Brown G. D. (1999). Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*. *Phytochemistry* **50**, 781-785.

Taoubi K., Fauvel M. T., Gleye J., Fouraste I. (1992). Caffeic acid heteroside esters from Verbenaceae. *Bulletin de Liaison Groupe Polyphenols* **16**, 174-177

Taoufiq, Z., Pino, P., Dugas, N., Conti, M., Tefit, M., Mazier, D., Vouldoukis, I. (2006). Transient supplimentation of superoxide dismutase protects endothelial cells against *Plasmodium falciparum*-induced oxidative stress. *Molecular & Biochemical Palasitology* **150**, 166-173.

Tene M., Tane P., Sondengam B. L., Connolly J. D. (2005). Clerodane diterpenoids from *Microglossa angolensis*. *Tetrahedron* **61**, 2655-2658

Tian J., Sun H. D. (1995). Chemical constituents from *Clerodendrum japonicum*. *Yunnan Zhiwu Yanjiu* **17**, 103-108

Tian J., Sun H. D. (1999). Chemical constituents of *Clerodendrum indicum*. *Tianran Chanwu Yanjiu Yu Kaifa* **11**, 1-5

Tian, J., Zhao, Q.S., Zhang, H.J., Lin, Z.W., Sun, H.D. (1997). New cleroindicins from *Clerodendrum indicum*. *Journal of Natural products* **60**. 766-769.

Tian X. D., Min Z. D., Xie N., Lei Y., Tian Z. Y., Zheng Q. T., Xu R. N., Tanaka T., Iinuma M., Mizuno M. (1993). Abietane diterpenes from *Clerodendron cyrtophyllum*. *Chemical and Pharmaceutical Bulletin* **41**, 1415-1417

Tonui W. K. (2006). Situational Analysis of *Leishmania*ses Research in Kenya. *African Journal of Health Sciences* **13**, 7-21.

Topcu G., Ulubelen A. (1996). Abietane and Rearranged Abietane Diterpenes from *Salvia montbretii*. *Journal of Natural Products* **59**. 734 -737

Ulubelen A., Oksüz S., Kolak U., Birman H., Voelter W. (2000). Cardioactive terpenoids and a new rearranged diterpene from *Salvia syriaca*. *Planta Medica* **66**. 627-629

Vera R., Smadja J., Conan J. Y. (1990). Preliminary assay of some plants with alkaloids from Reunion Island. *Planta Medica Phytother.* **24**. 50-65

Vigneron J. P. (1978). Antiappetant substances of natural origin. *Annales de Zoologie Ecologie Animale* **10**, 663-694

Watt J. M., Breyer-Brandwijk M. G. (1962). In "The medicinal and poisonous plants of Southern and Eastern Africa." 2nd Ed, E. + S. Livingstone Ltd., London.

World Report (2007). Malaria returns to Kenyan highlands as temperature rise *www.thelancet.com* **370**, 925-926.

Yadava R. N., Kumar S. (1999). A novel isoflavone glycoside from the leaves of *Sphaeranthus indicus*. Fitoterapia **70**. 127-129

Yang G. Z., Li Y. F., Yu X., Mei Z. N. (2007). Terpenoids and flavonoids from *Laggera pterodonta*. *Acta Pharmaceutica sinica*, **42**, 511-515

Yang H., Hou A-J., Mei S-X., Sun H-D., Che C-T. (2002). Constituents of *Clerodendrum bungei*. *Journal of Asian Natural Products Research* **4**, 165- 169

Yang H., Jiang B., Na Z., Guo Y. P., Sun H. D. (2000a). Two new iridoid glucosides from *Clerodendrum serratum*. *Chinese Chemical Letters* **11**, 231-234

Yang H., Wang J., Hou A. J., Guo Y. P., Lin Z. W., Sun H. D. (2000b). New steroids from *Clerodendrum colebrookianum*. *Fitoterapia* **71**, 641-648

Zdero C., Ahmed A. A., Bohlmann F., Mungai G. M. (1990a). Diterpenes and sesquiterpene xylosides from East African *Conyza* species. *Phytochemistry* **29**, 3167-3172

Zdero C., Bohlmann F., Mungai G.M., (1990b). Rearranged clerodanes and other diterpenes from *Microglossa pyrrhopappa*. *Phytochemistry* **29**, 3233-3241.

Zdero C., Bohlmann F., Mungai G.M. (1990c). A glaucolide-type sesquiterpene lactone from *Vernonia galamensis* ssp. *Nairobensis*. *Phytochemistry* **29**, 3668-3669.

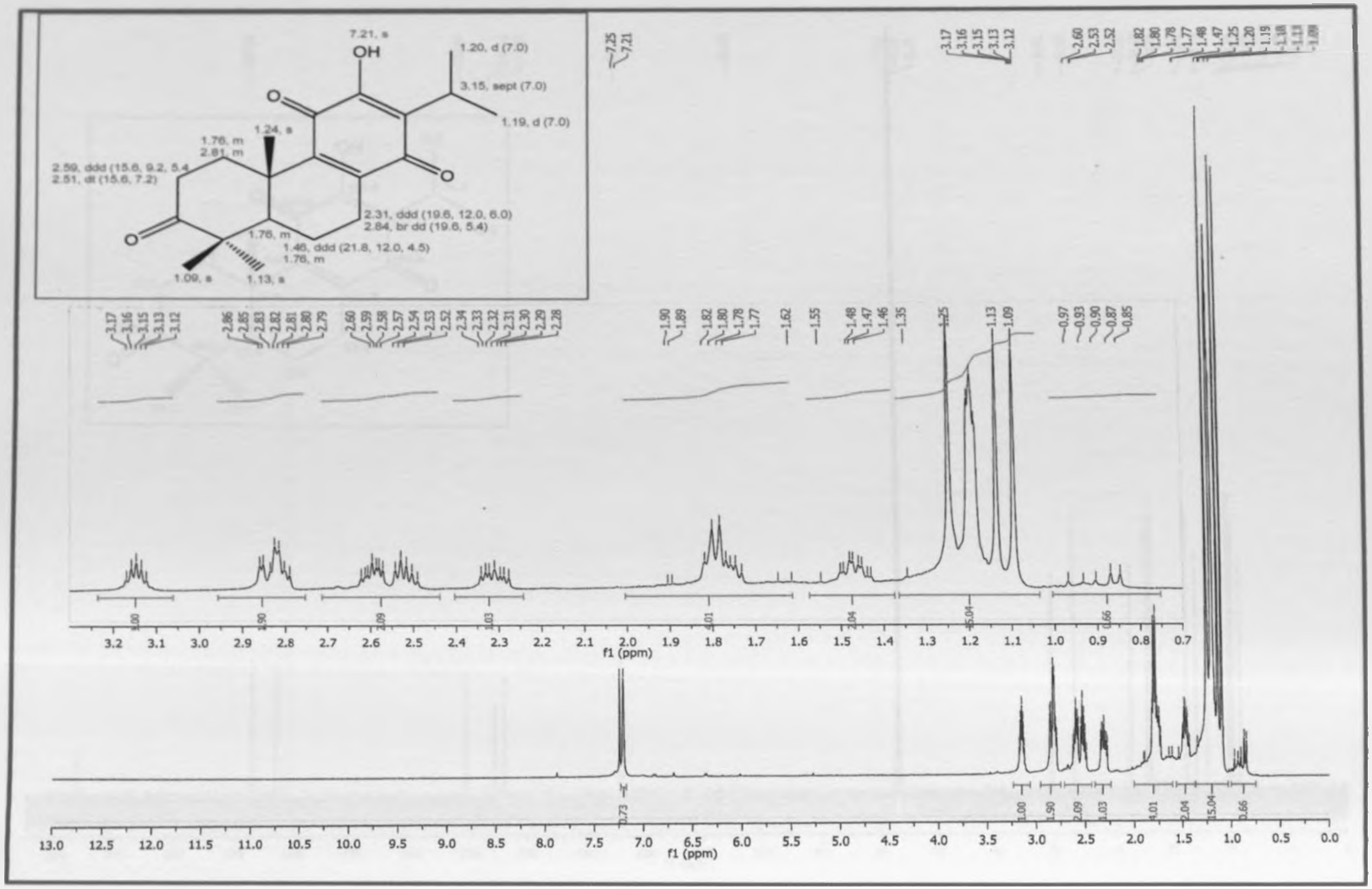
Zdero C., Bohlmann F., Mungai G. M. (1991). Carvotacetone derivatives and other constituents from representatives of the *Sphaeranthus* group. *Phytochemistry* **30**, 3297-3303

Zhong X.N., Otsuka H., Ide T., Hiraka E., Takushi A., Takeda Y. (1997). Three flavanol glycosides from leaves of *Myrsine seguinii*. *Phytochemistry* **46**, 943-947.

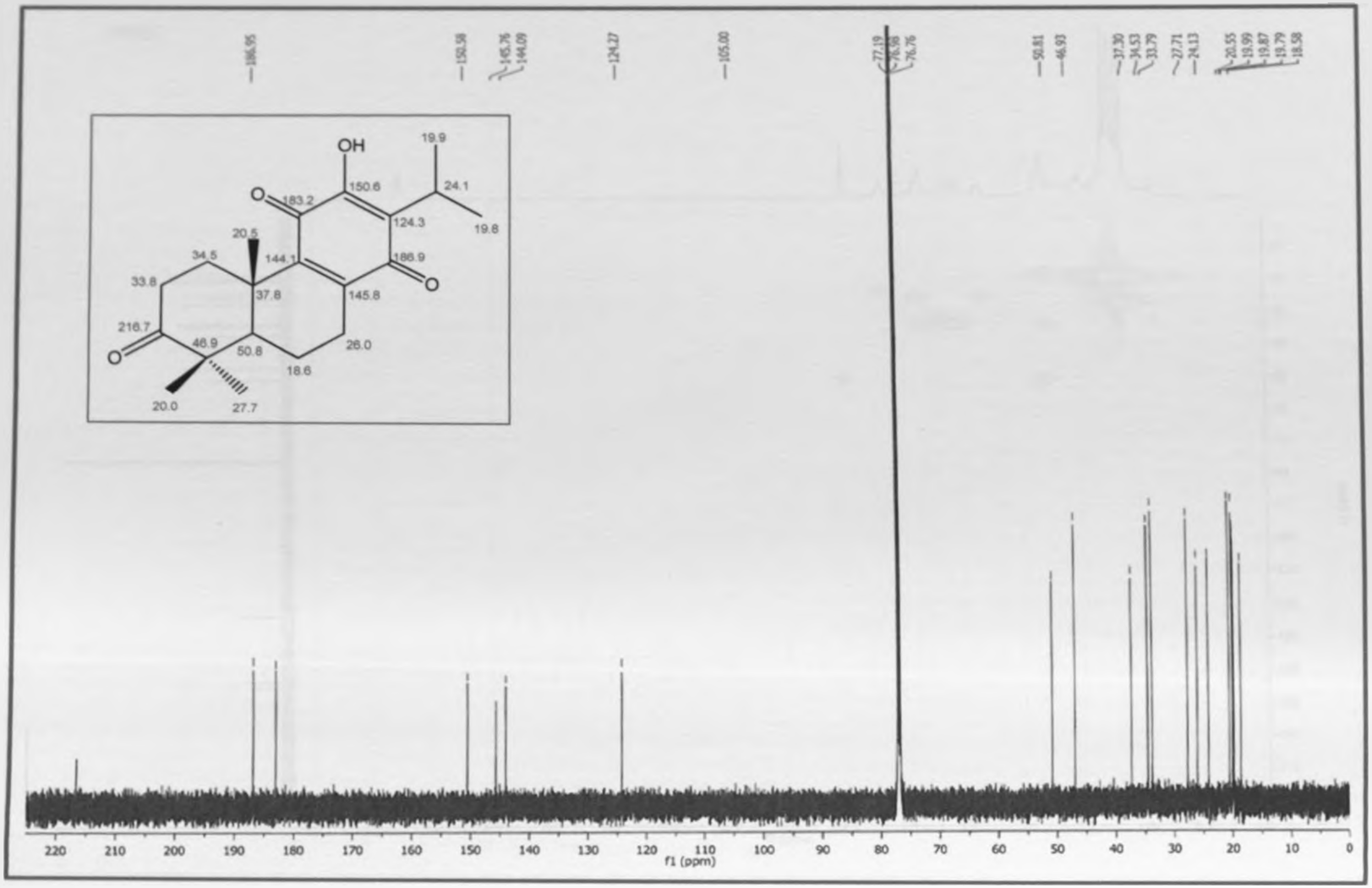
Zhou P., Pang Z., Hso H. Q. (1982). Studies on chemical constituents of *Clerodendron bungei*. *Zhiwu Xaebao* **24**, 564-567

Zhu M., Phillipson J. D., Greengrass P. M., Borwery N. G. (1996). Chemical and biological investigation of the root bark of *Clerodendrum mandarinorum*. *Planta Medica* **62**, 393-396

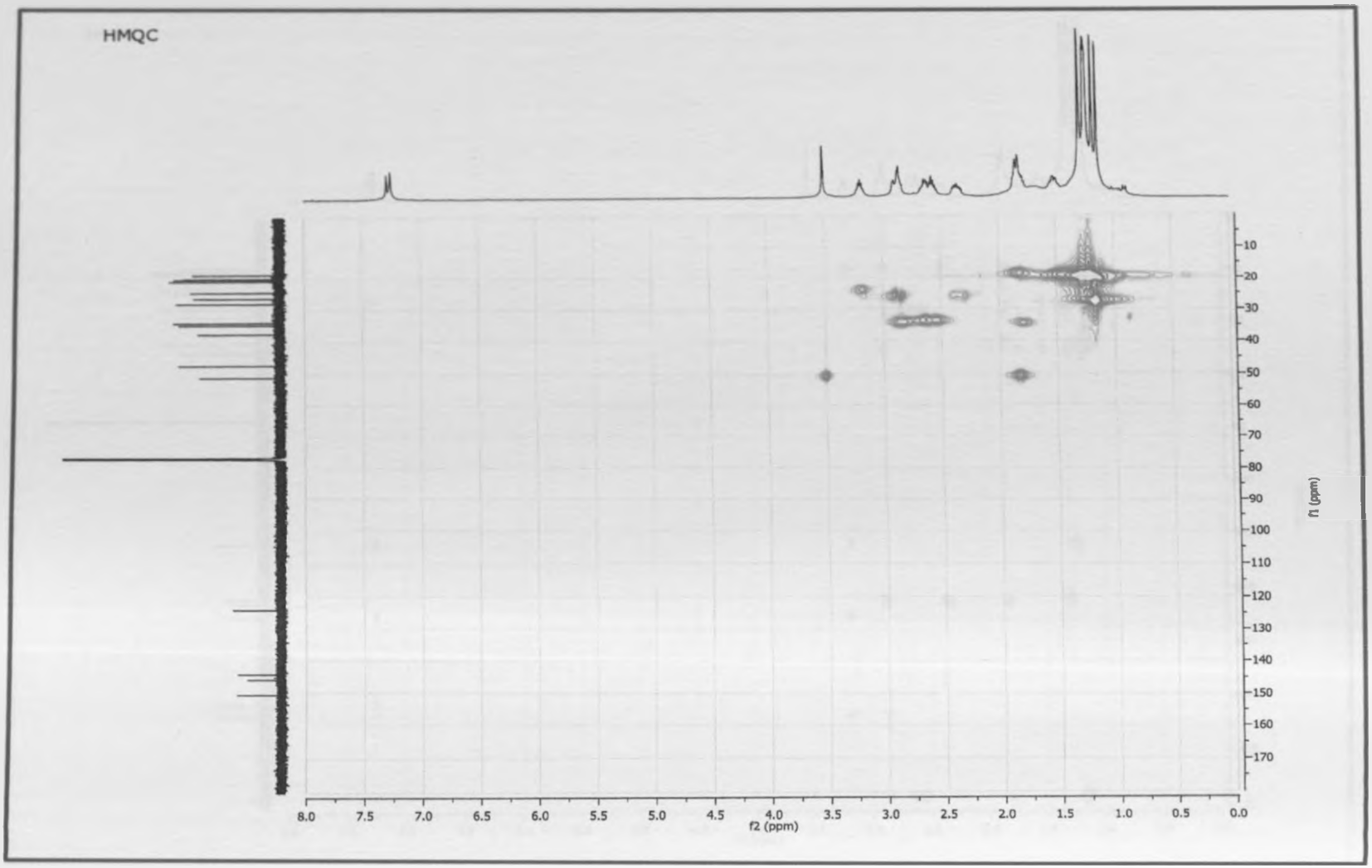
Appendix 1A: ^{1}H-NMR spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (**220**), $CDCl_3$, 500 MHz



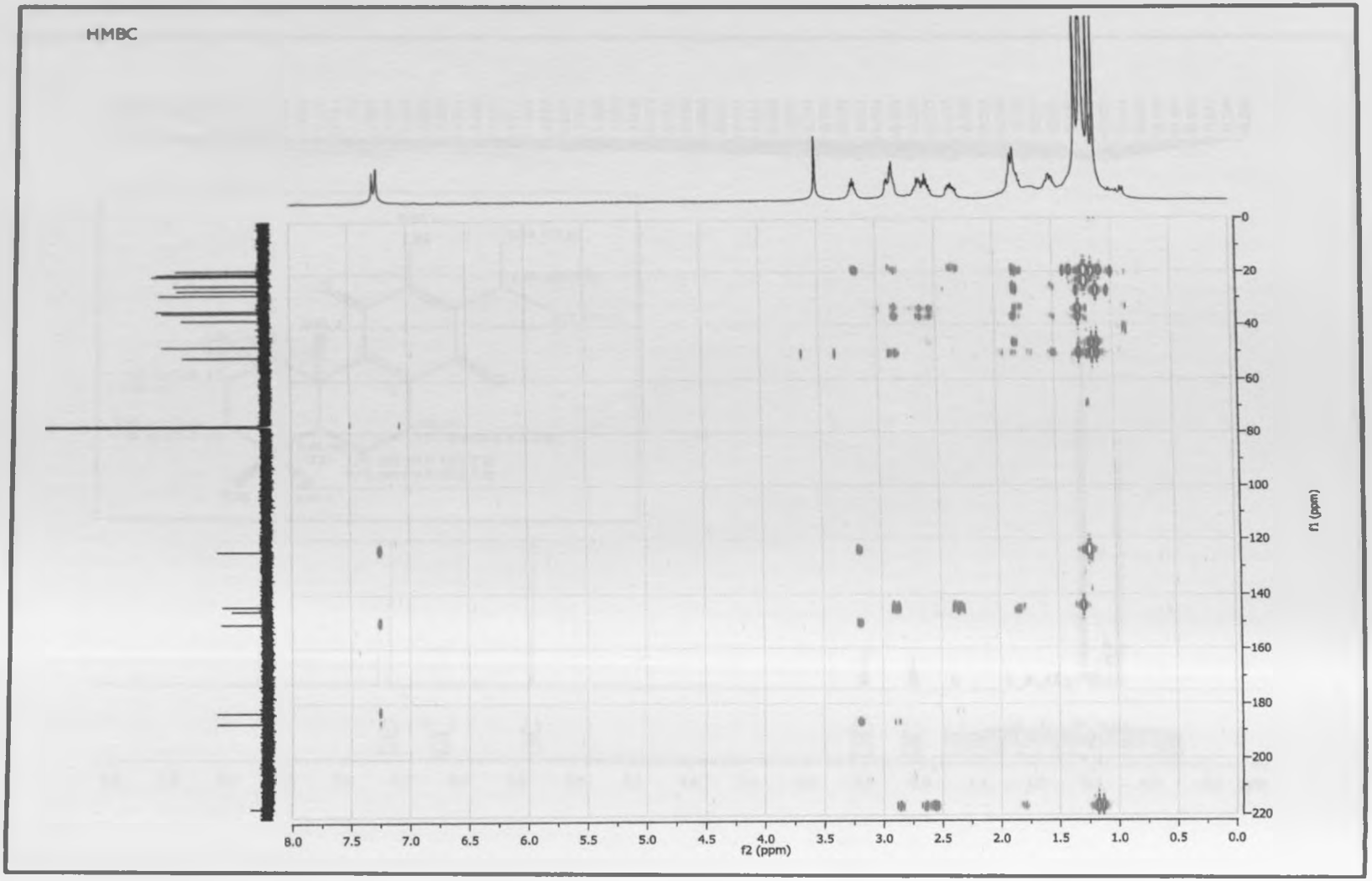
Appendix 1B: ^{13}C-NMR spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (**220**), $CDCl_3$, 125 MHz



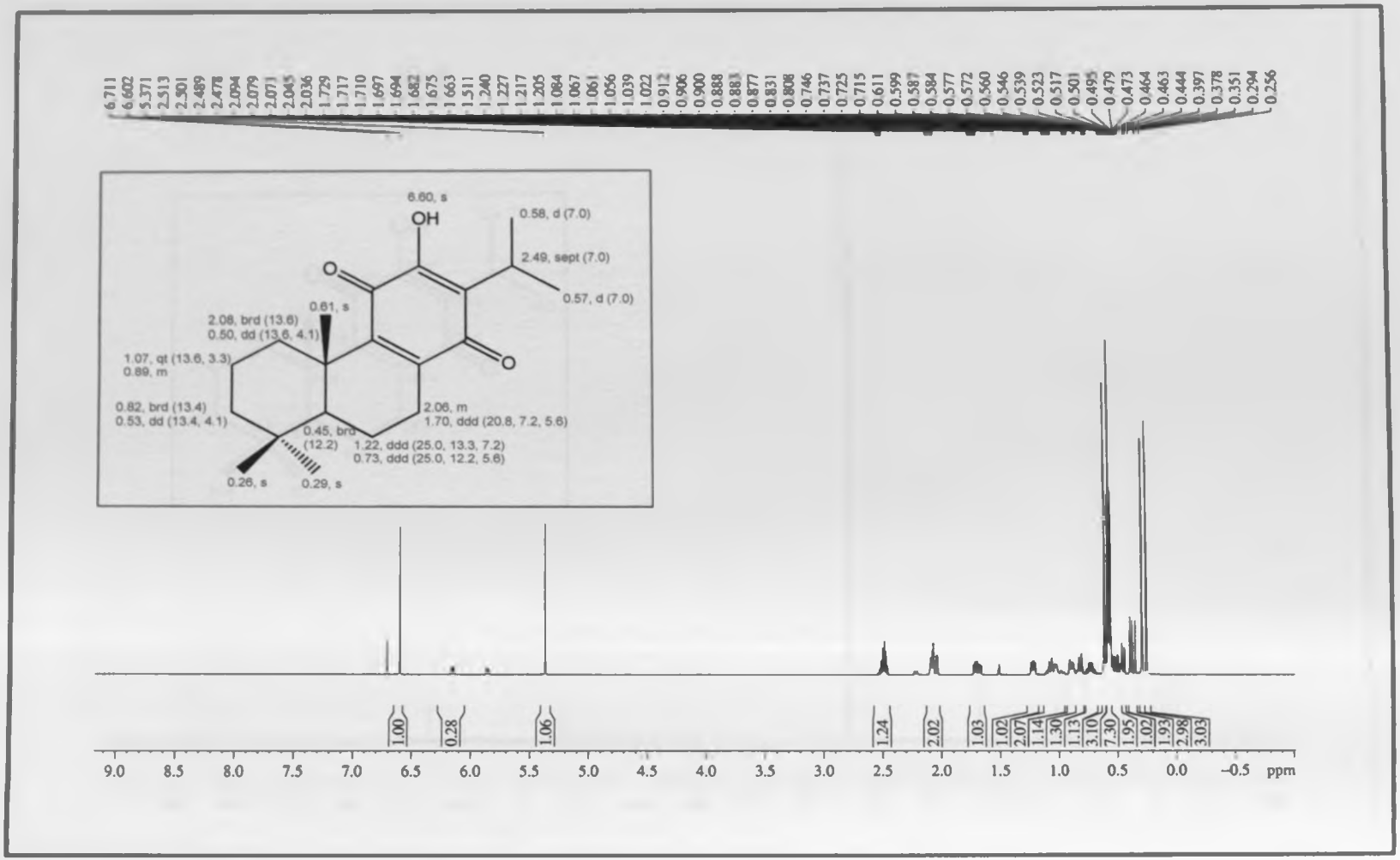
Appendix 1C: HMQC spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (**220**), $CDCl_3$, 500 MHz



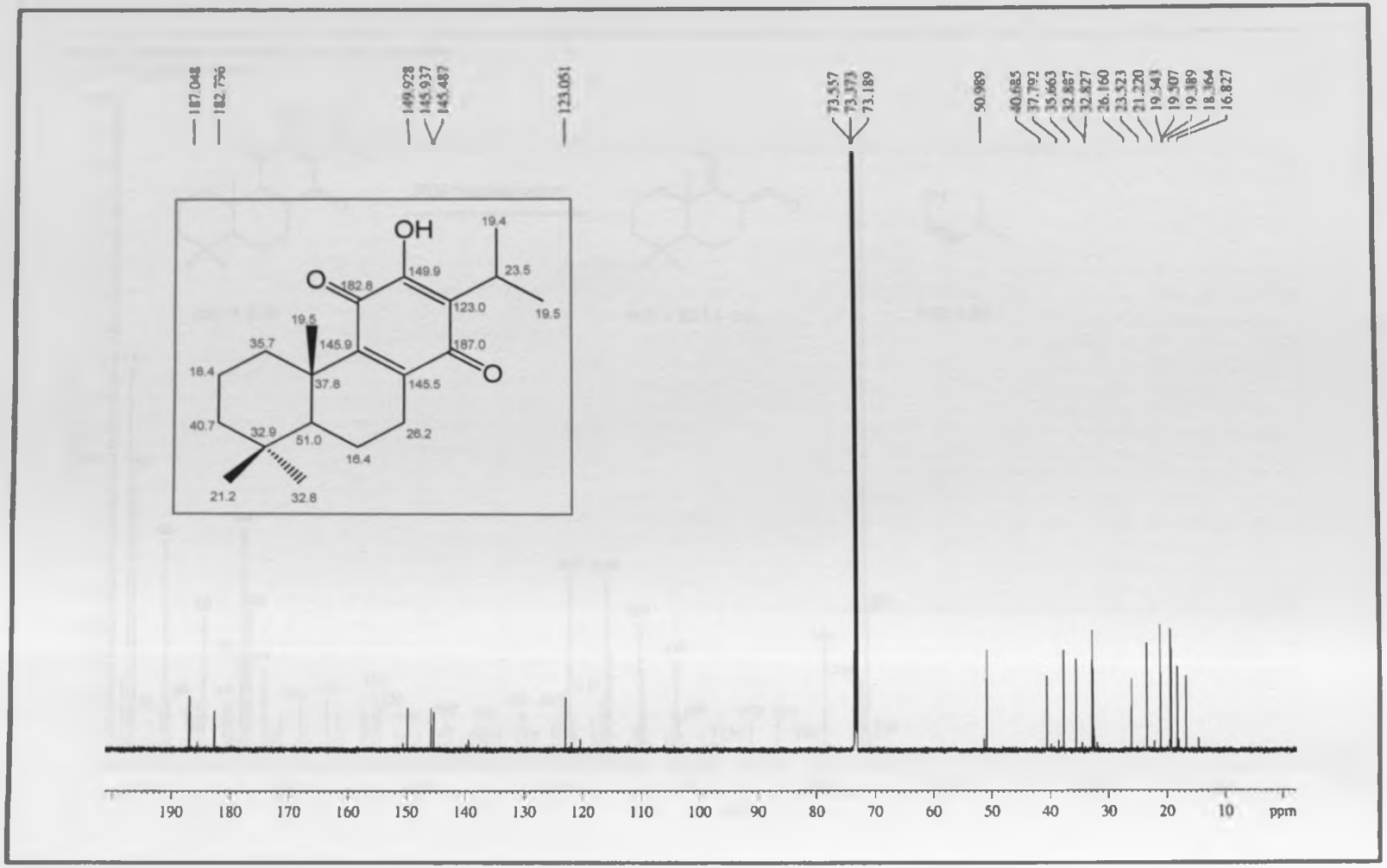
Appendix 1D: HMBC spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (**220**), $CDCl_3$, 500 MHz



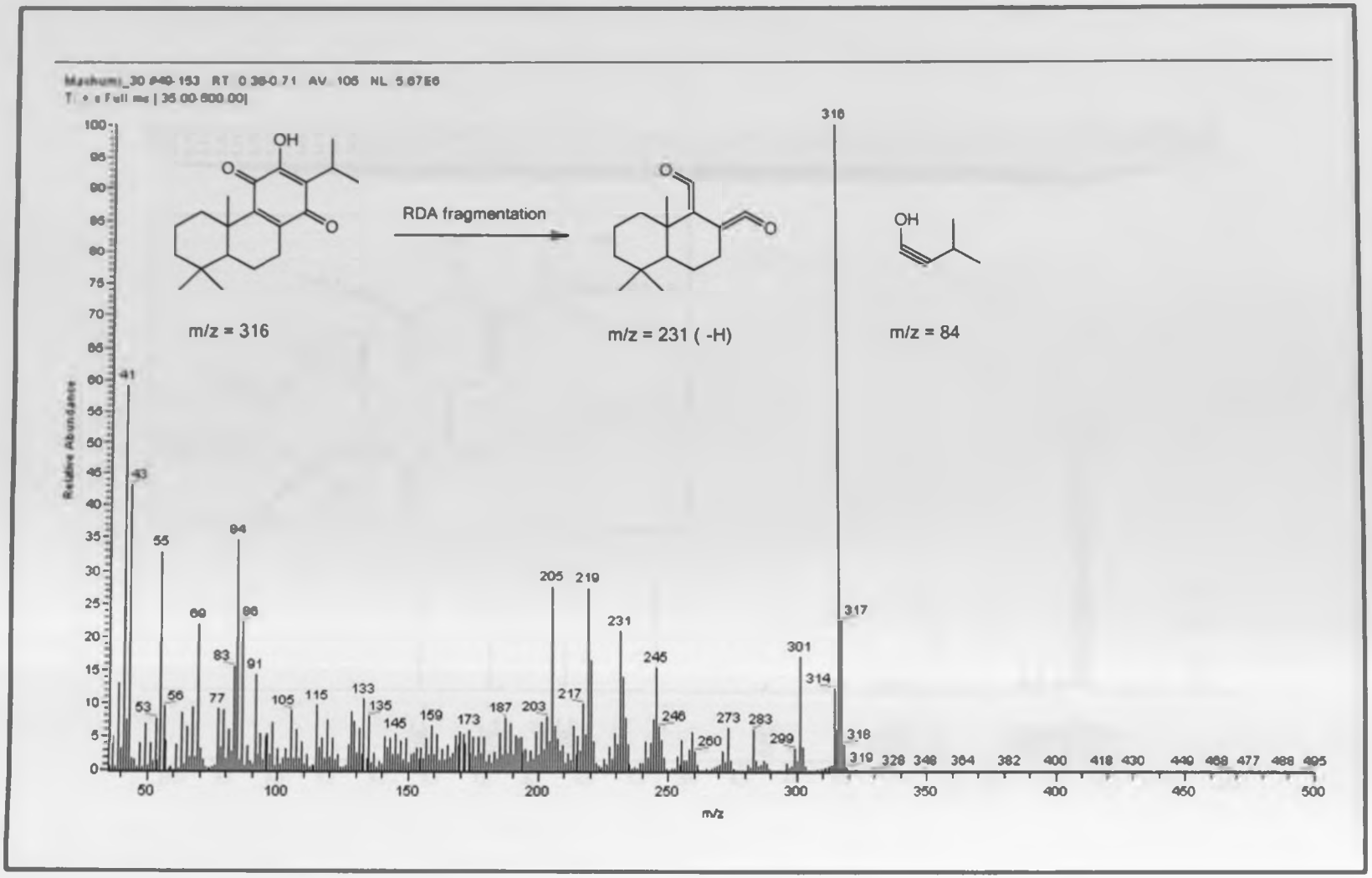
Appendix 2A: ^{1}H-NMR spectrum of royleanone (**21**), $C_2Cl_4D_2$, 600 MHz



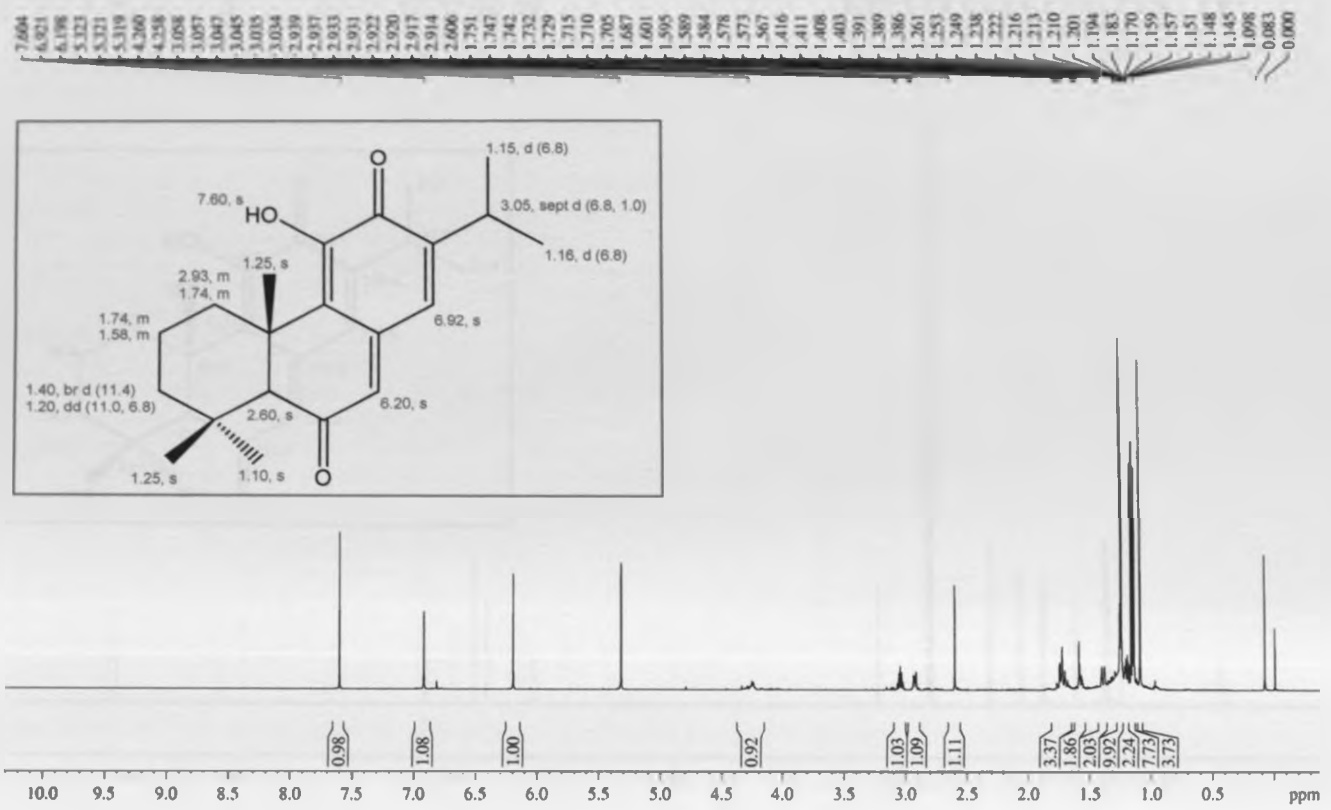
Appendix 2B: ^{13}C-NMR spectrum of royleanone (**21**), $C_2Cl_4D_2$, 150 MHz



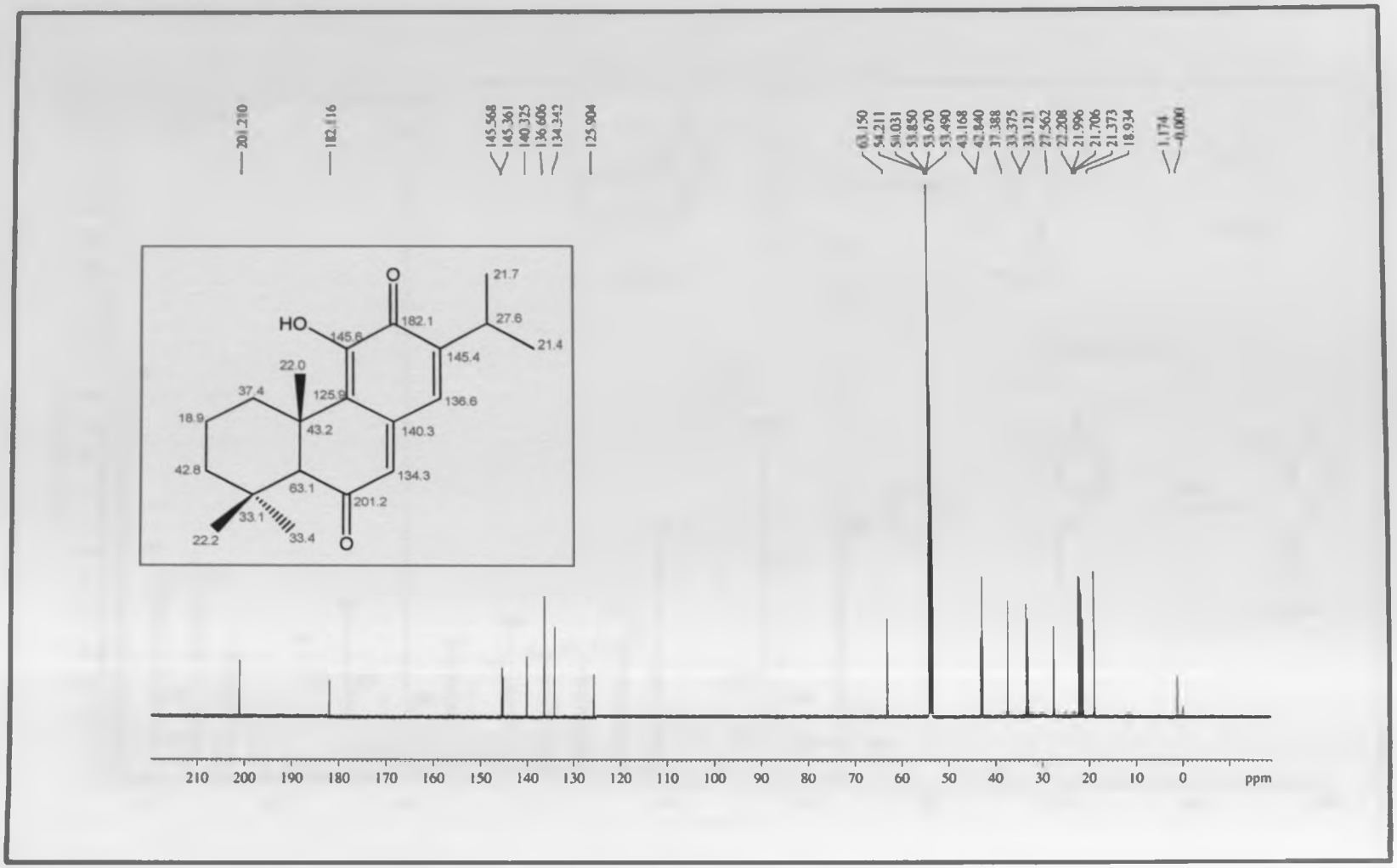
Appendix 2C: EI-MS spectrum of royleanone (**21**)



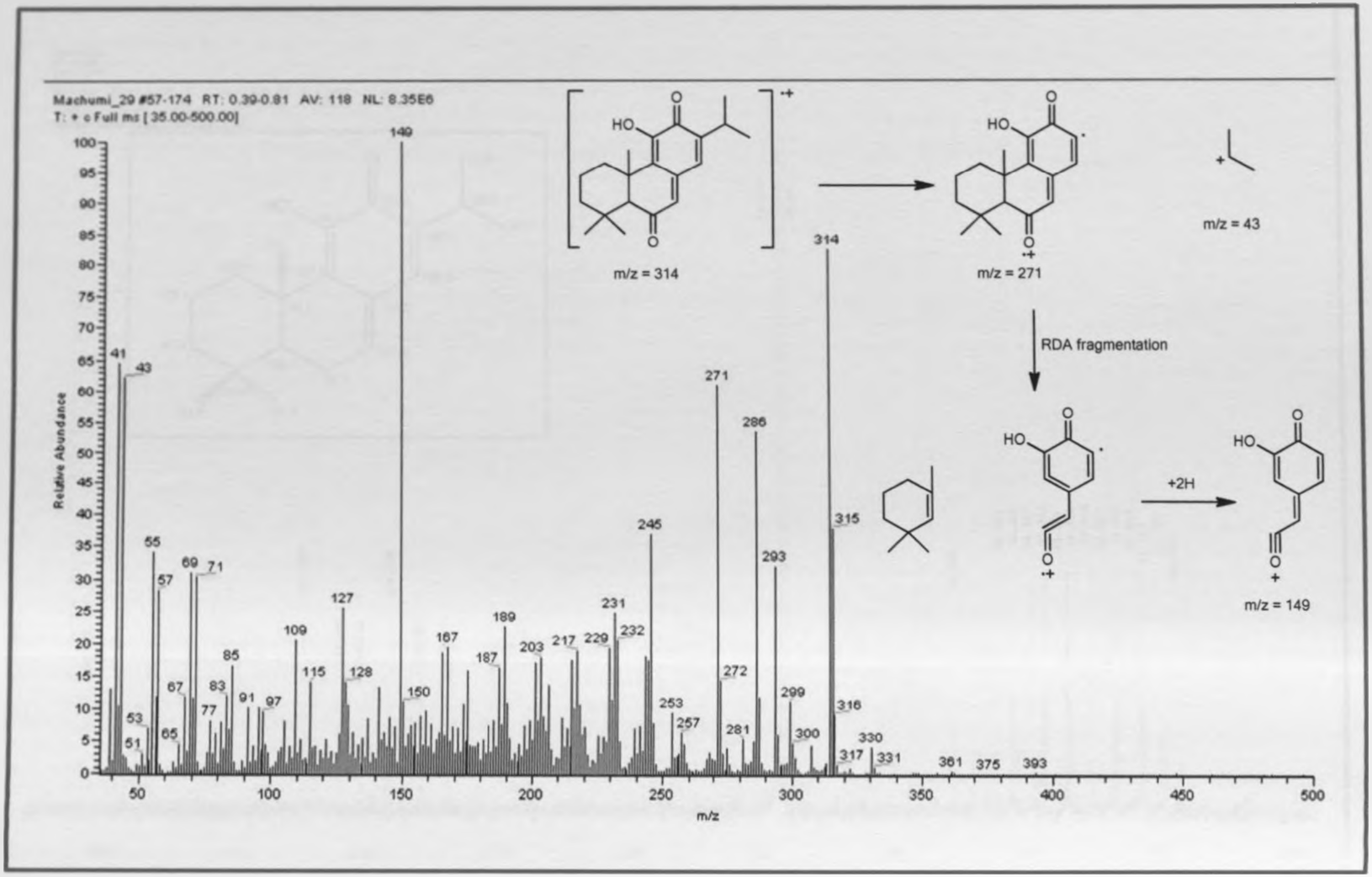
Appendix 3A: ^{1}H-NMR spectrum of taxodione (**222**), CD_2Cl_2, 600 MHz



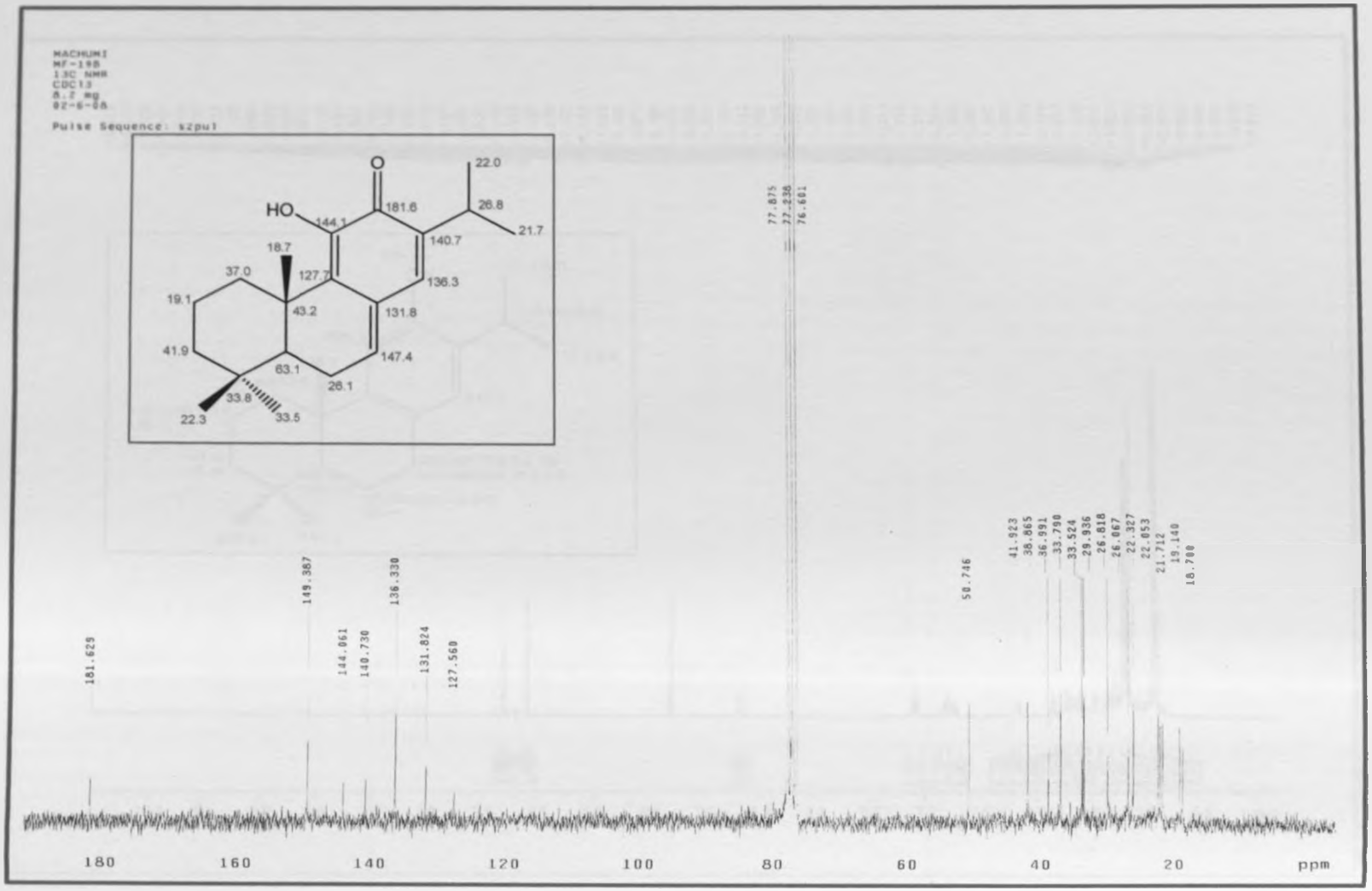
Appendix 3B: ^{13}C-NMR spectrum of taxodione (**222**), CD_2Cl_2, 150 MHz



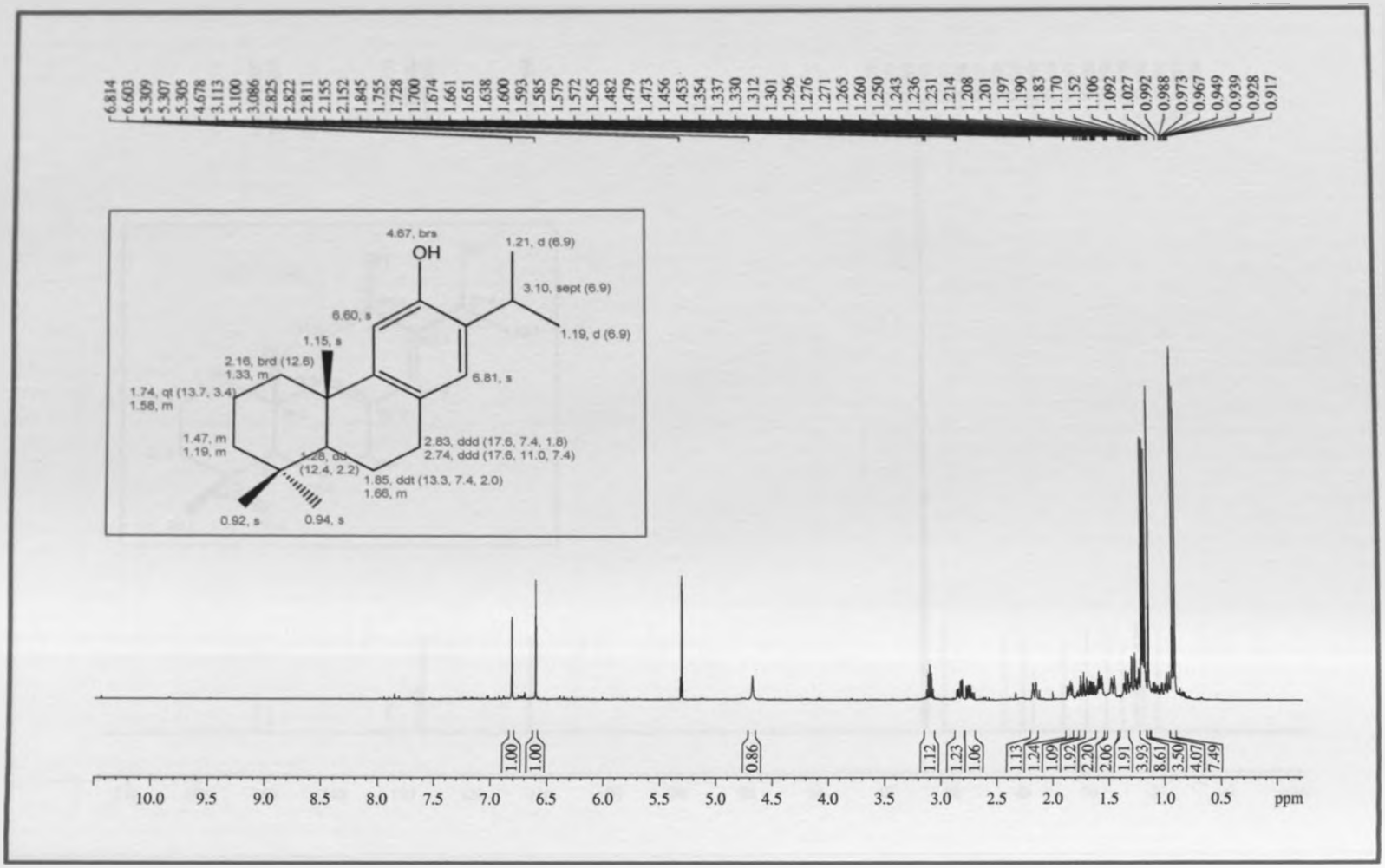
Appendix 3C: EI-MS spectrum of taxodione (**222**)



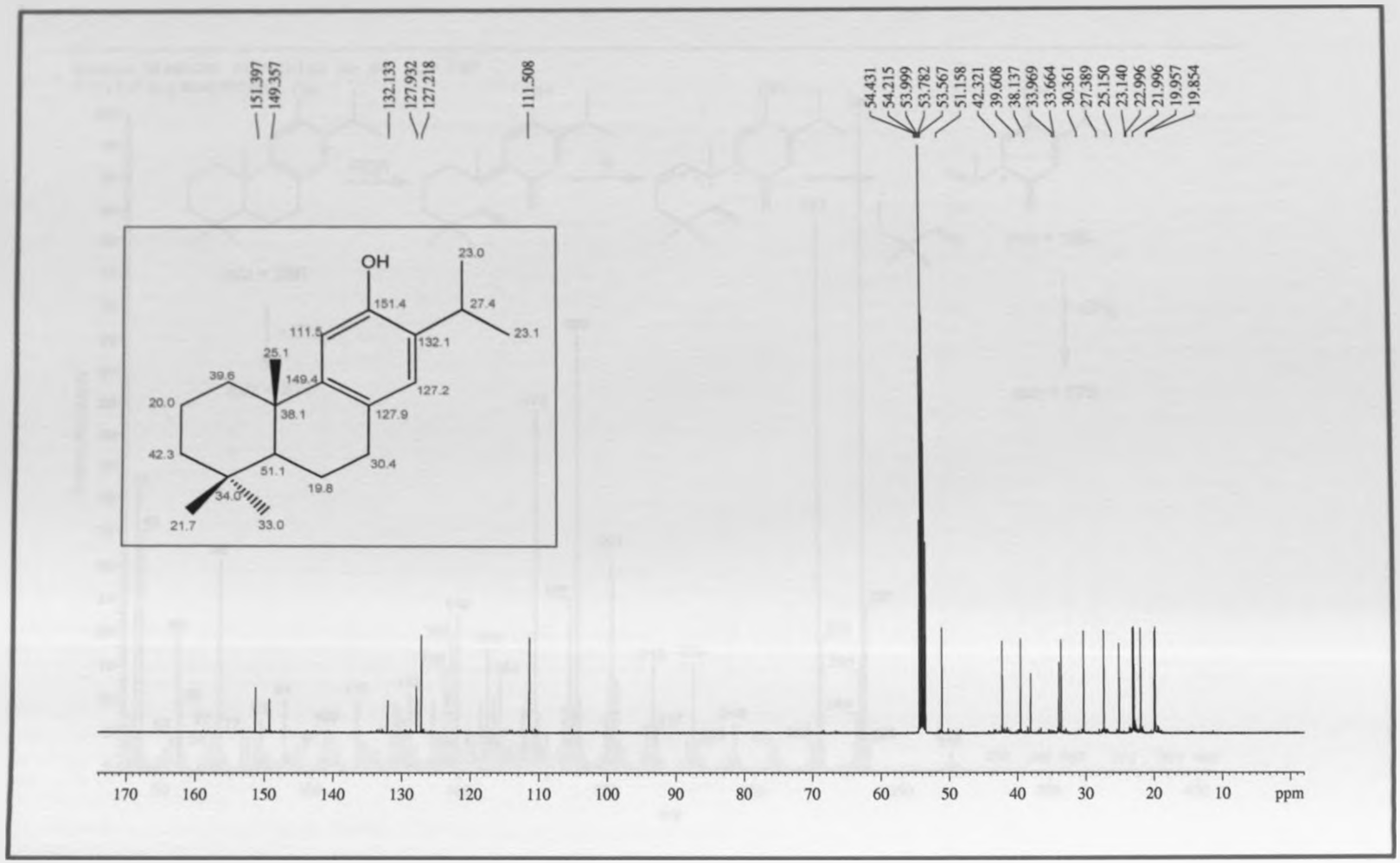
Appendix 4A: ^{13}C-NMR spectrum of 11-Hydroxy-7,9(11),13-abietatrien-12-one (**223**) $CDCl_3$, 50 MHz



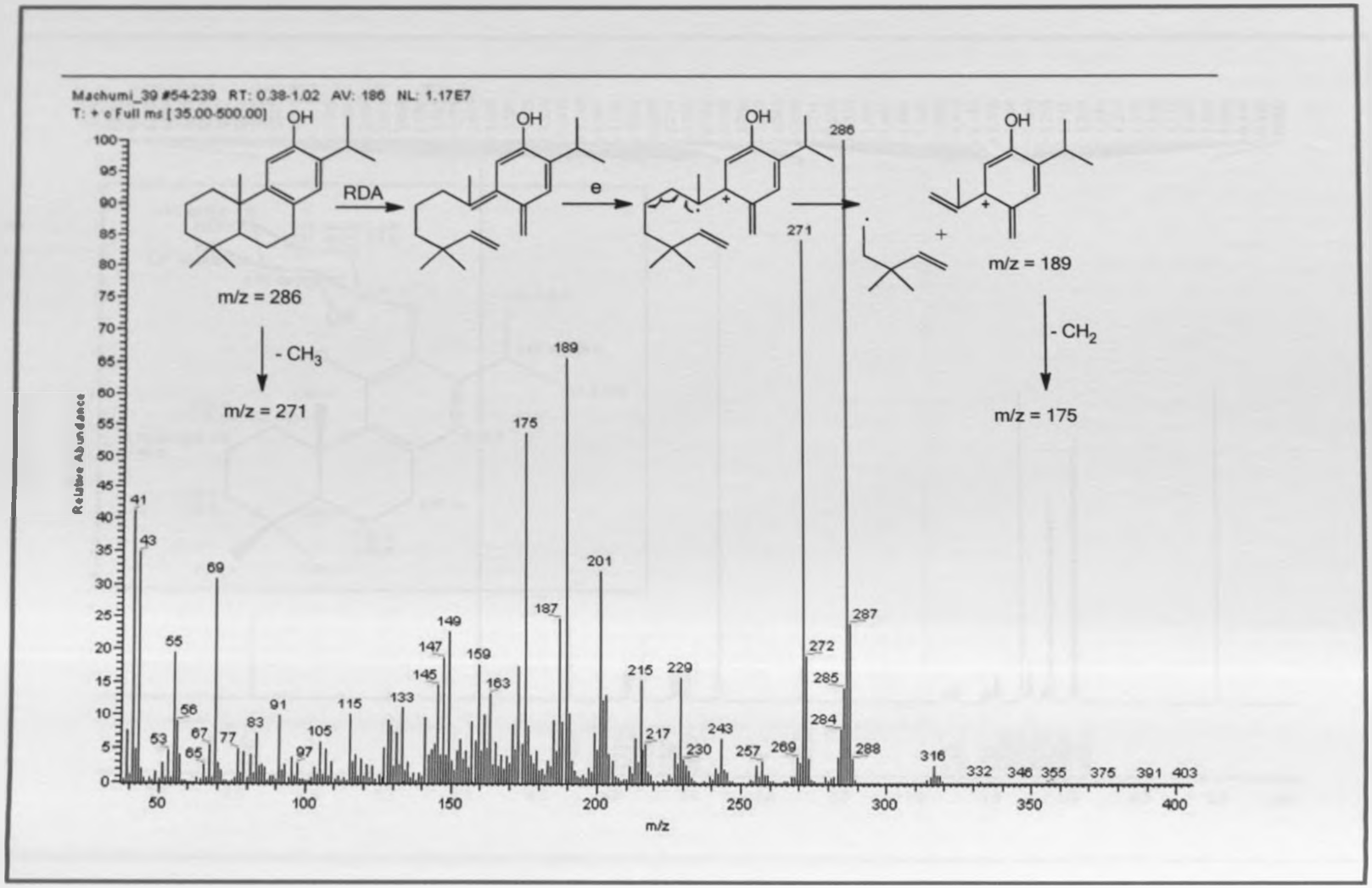
Appendix 5A: ^{1}H-NMR spectrum of ferruginol (**224**), CD_2Cl_2, 600 MHz



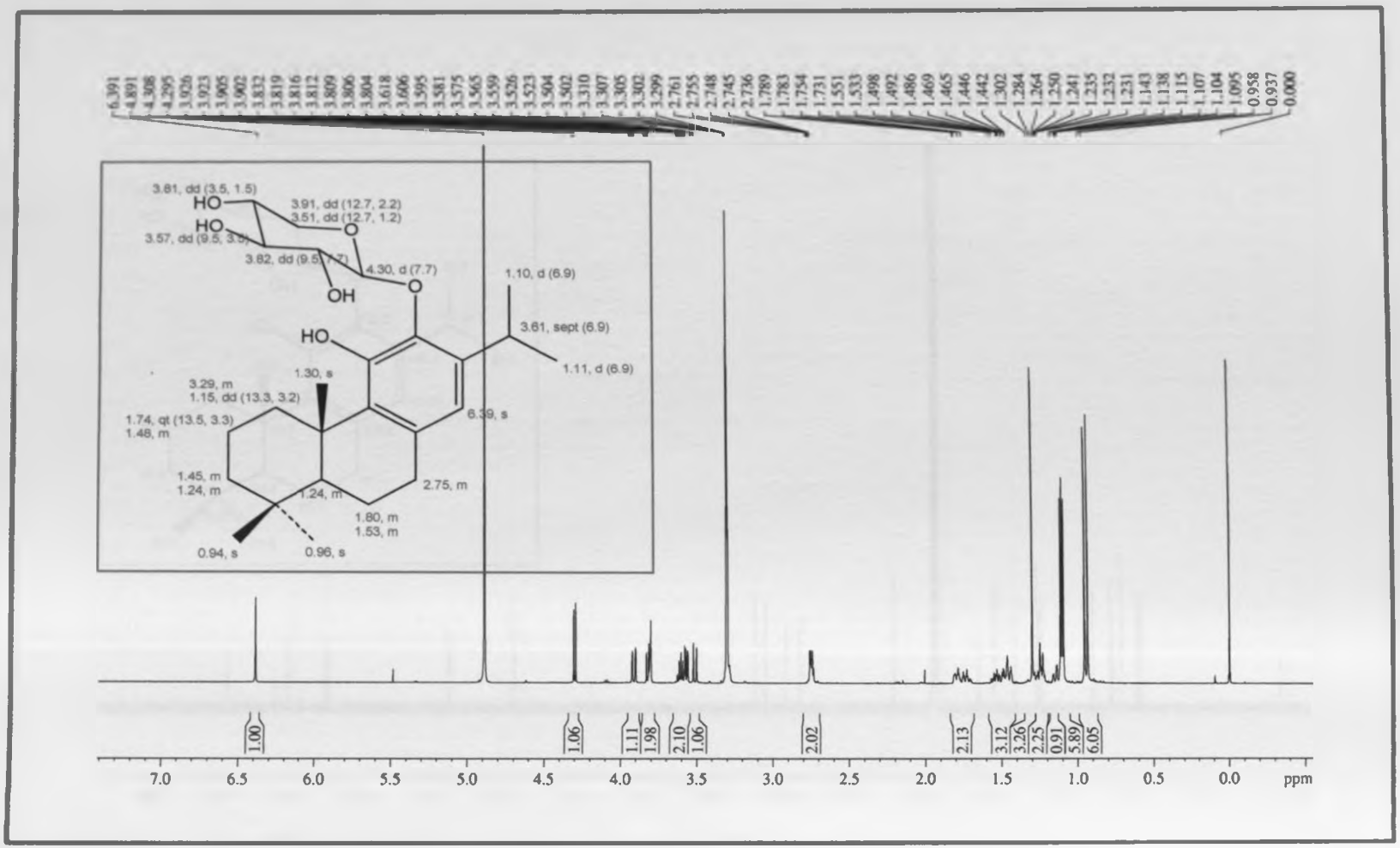
Appendix 5B: ^{13}C-NMR spectrum of ferruginol (**224**), CD_2Cl_2, 150 MHz



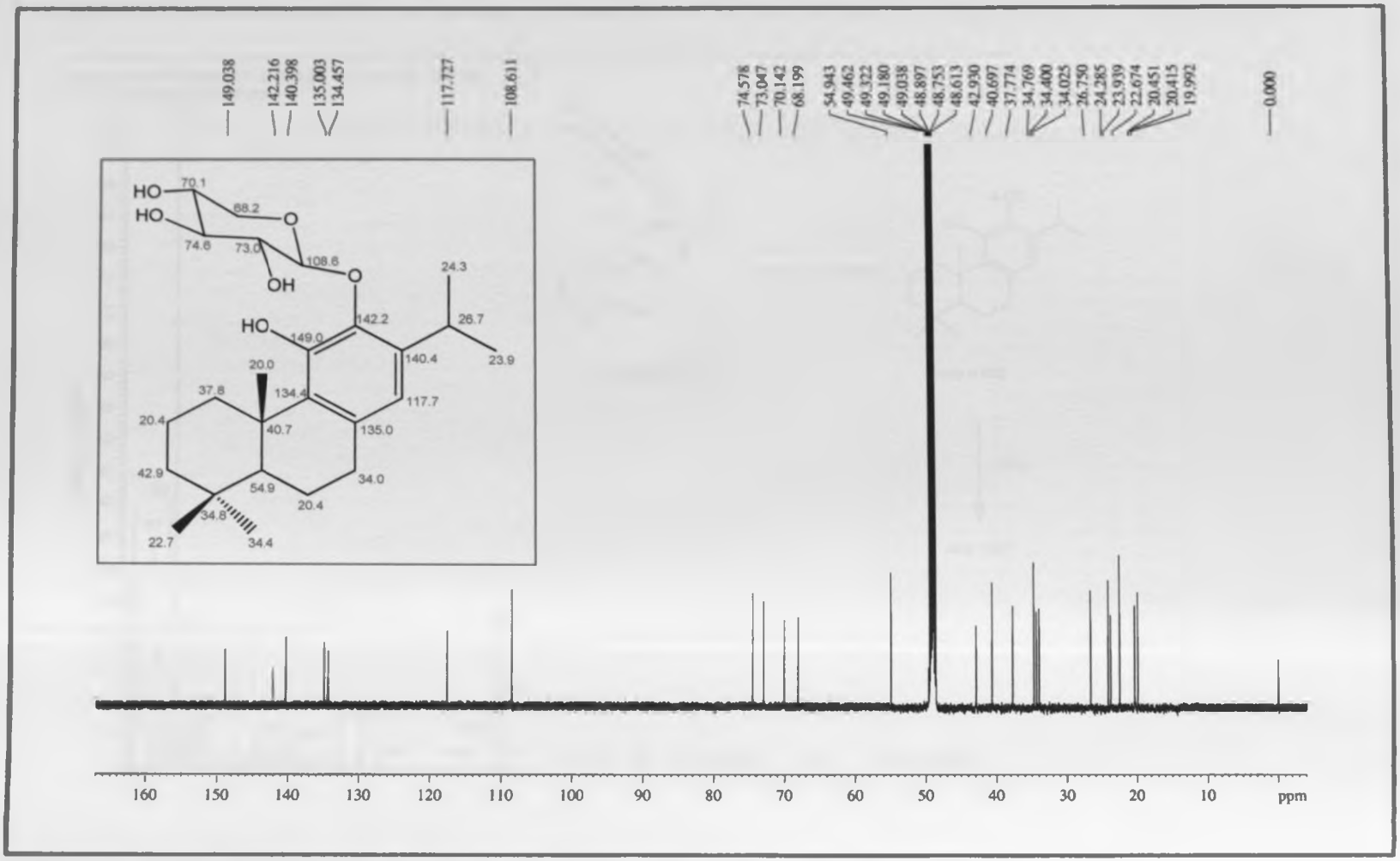
Appendix 5C: EI-MS spectrum of ferruginol (**224**)



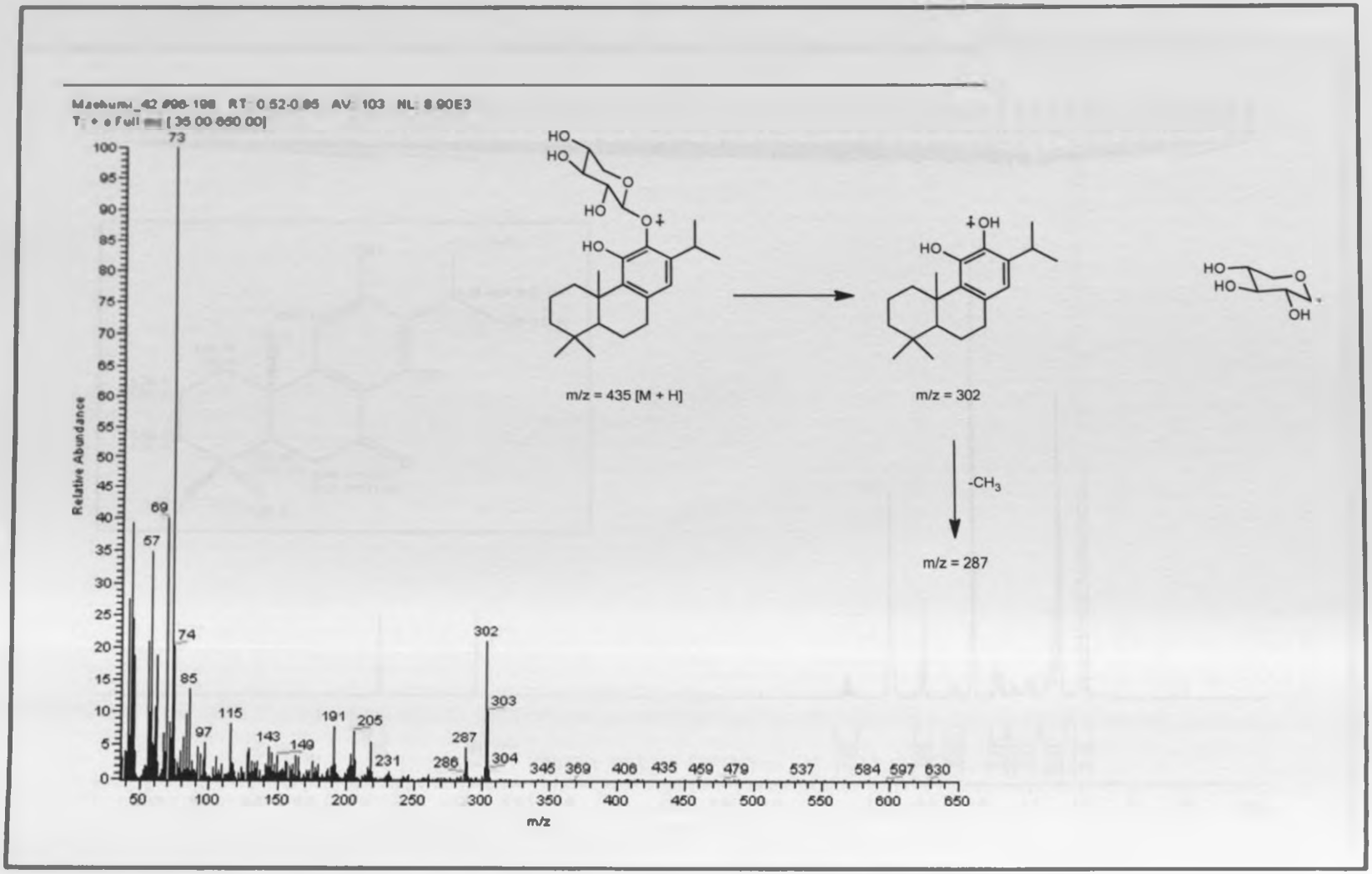
Appendix 6A: ^{1}H-NMR spectrum of 11-hydroxy-8,11,13-abietatriene *12-O-β*-xylopyranoside (**225**) MeOD, 600 MHz



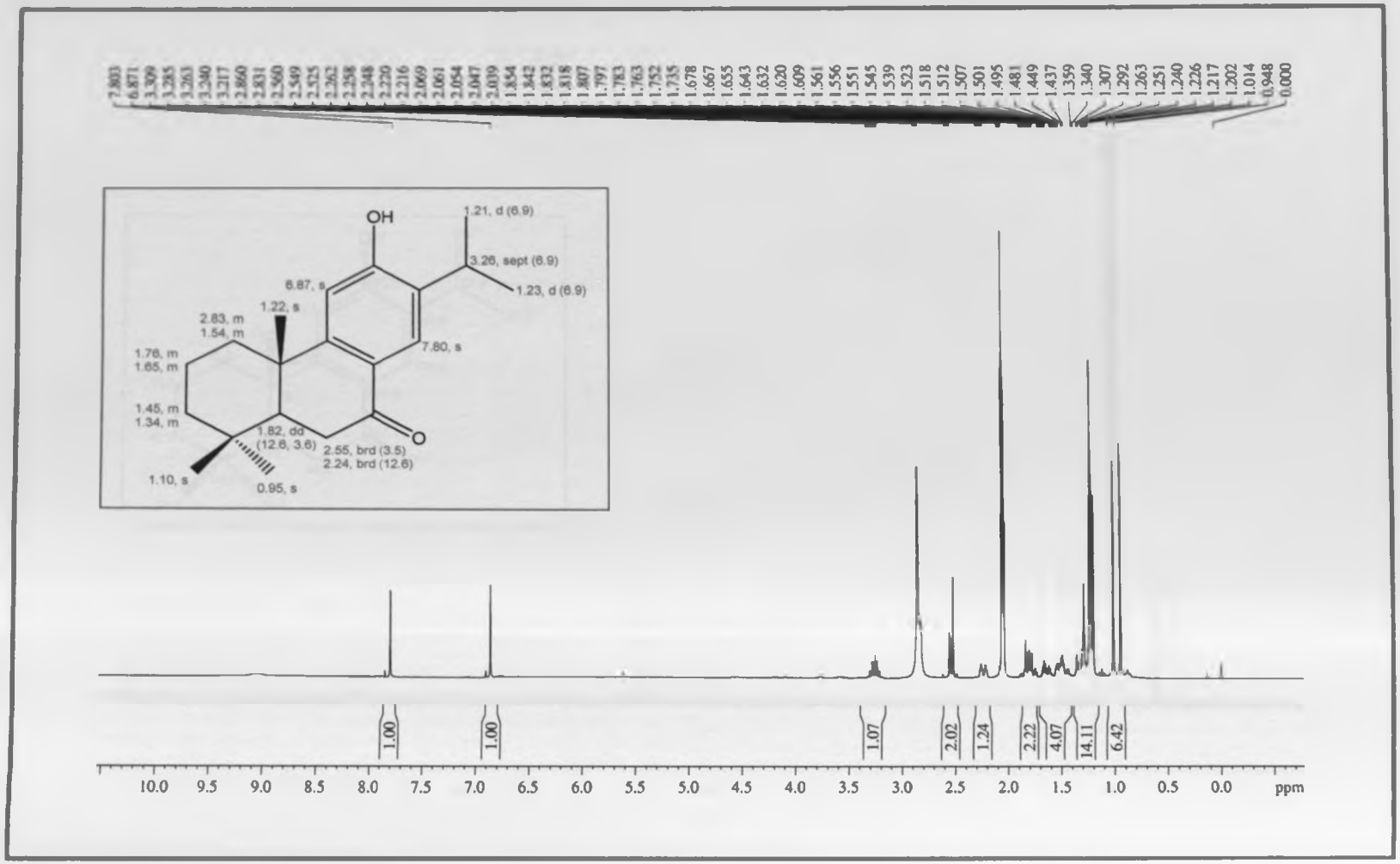
Appendix 6B: ^{13}C-NMR spectrum of 11-hydroxy-8,11,13-abietatriene *12-O-β*-xylopyranoside (**225**) MeOD, 150 MHz



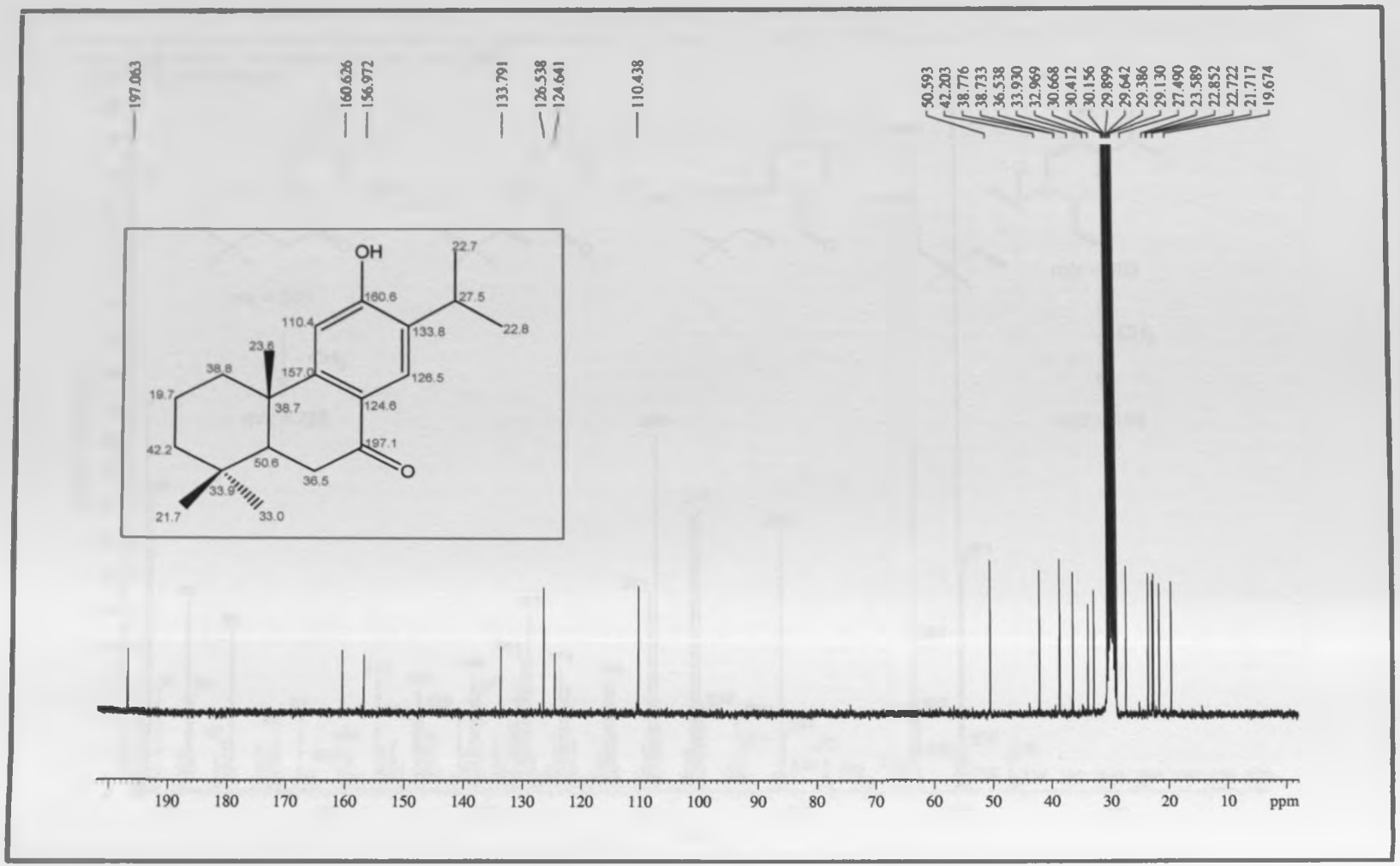
Appendix 6C: EI-MS spectrum of 11-hydroxy-8,11,13-abietatriene *12-O-β*-xylopyranoside (**225**)



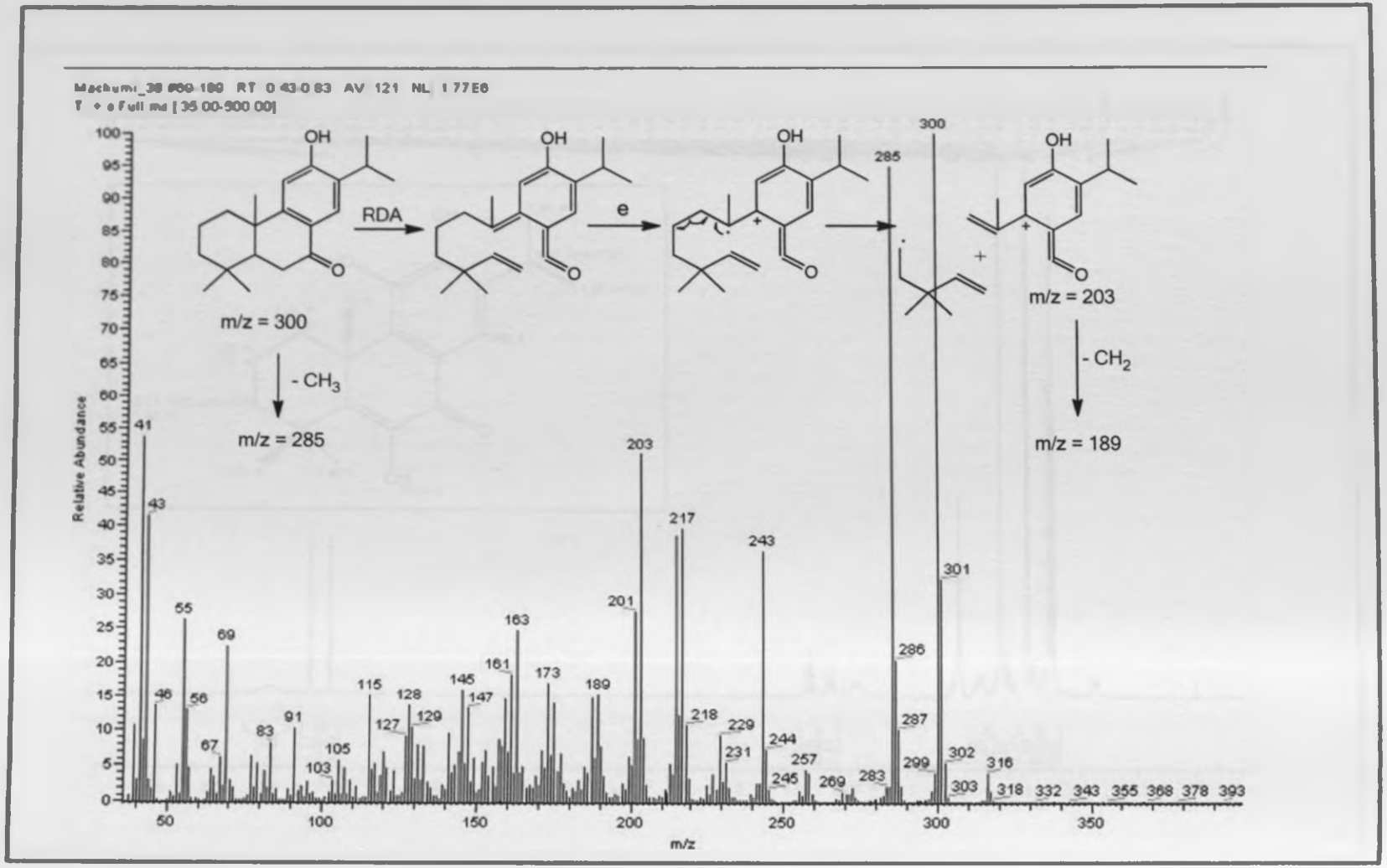
Appendix 7A: ^{1}H-NMR spectrum of sugiol (**9**), Acetone-d_6, 600 MHz



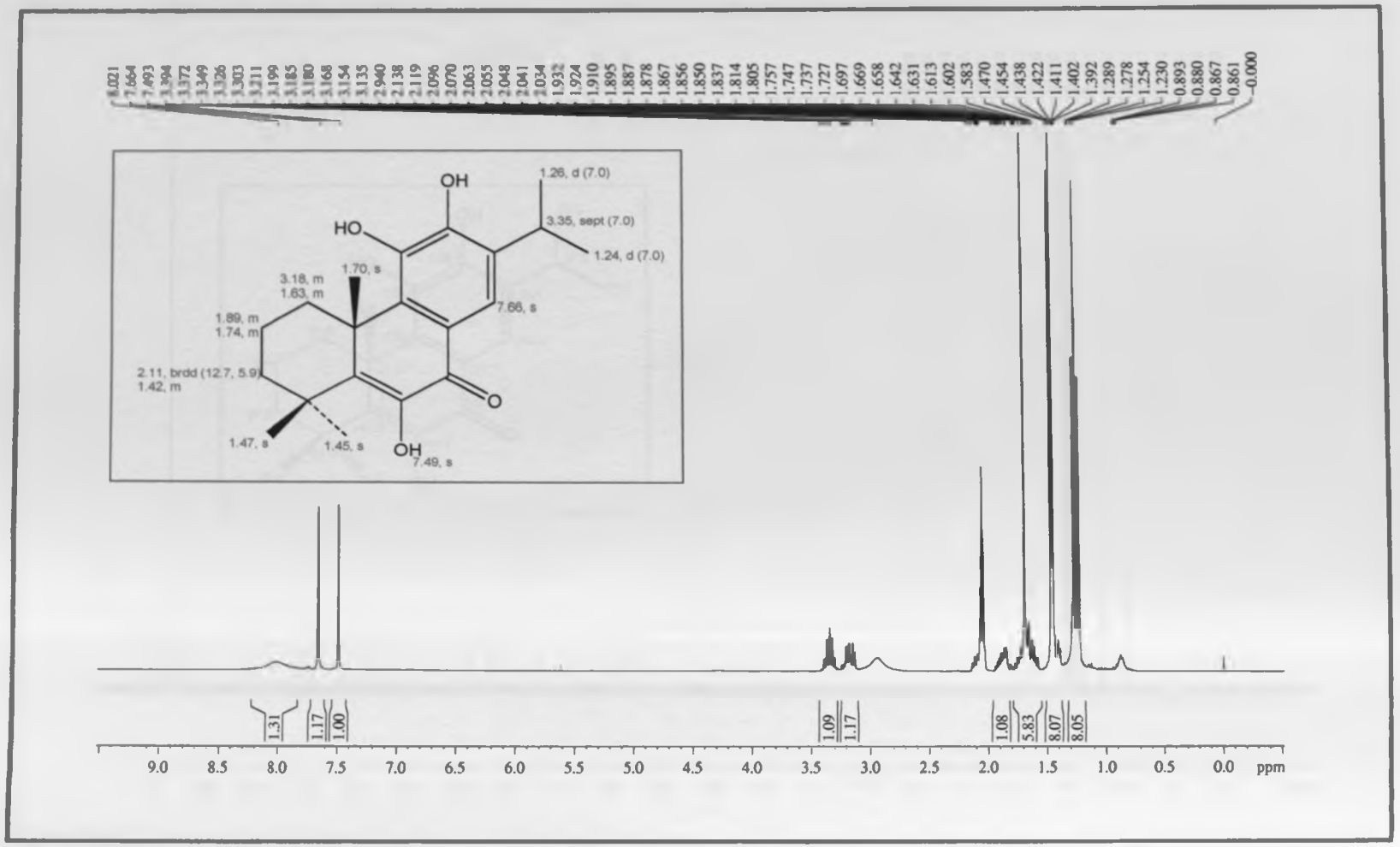
Appendix 7B: ^{13}C-NMR spectrum of sugiol (**9**), Acetone-d_6, 150 MHz



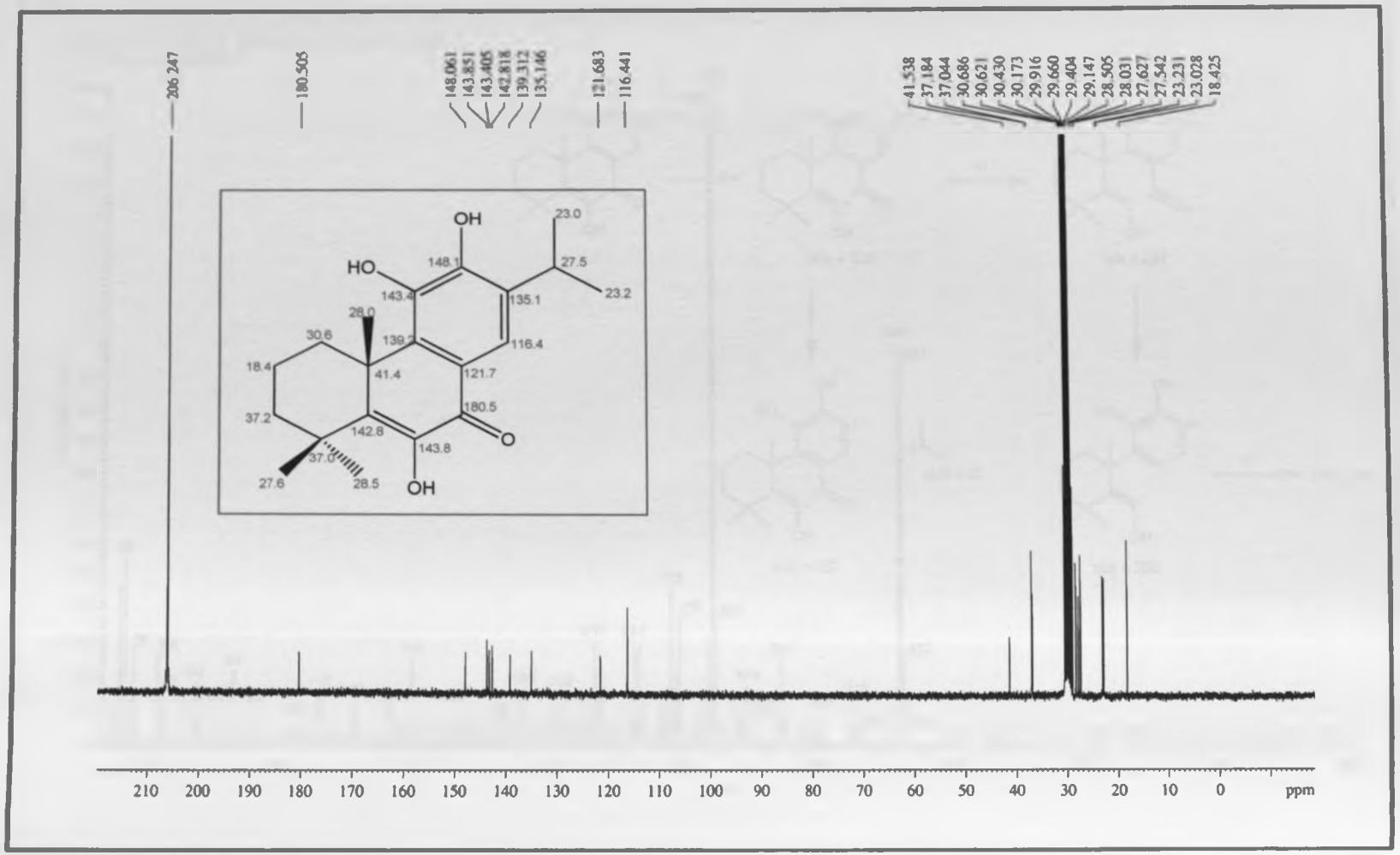
Appendix 7C: EI-MS spectrum of sugiol (**9**)



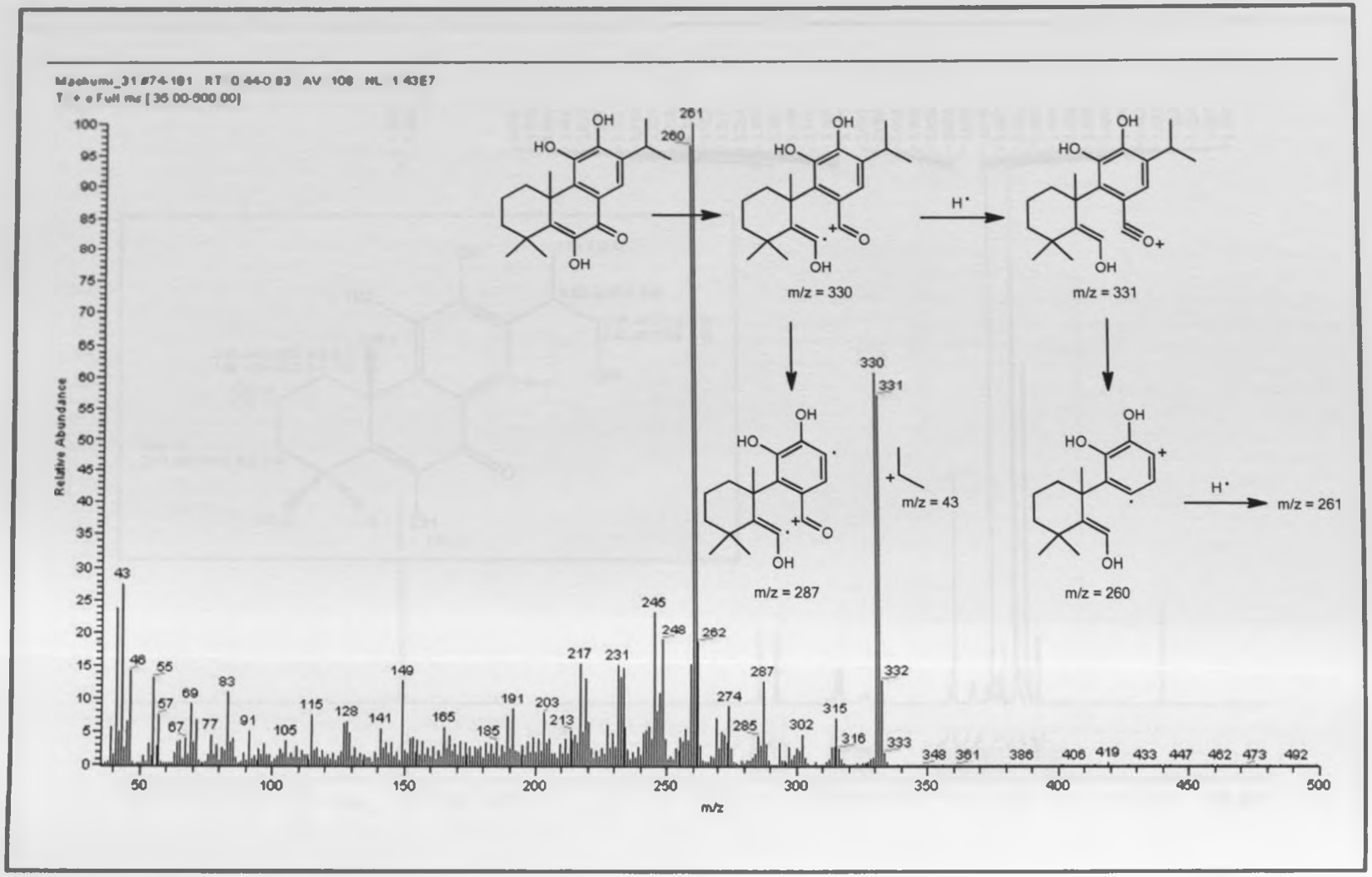
Appendix 8A: ^{1}H-NMR spectrum of 6-hydroxysalvinolone (**226**), Acetone-d_6, 300 MHz



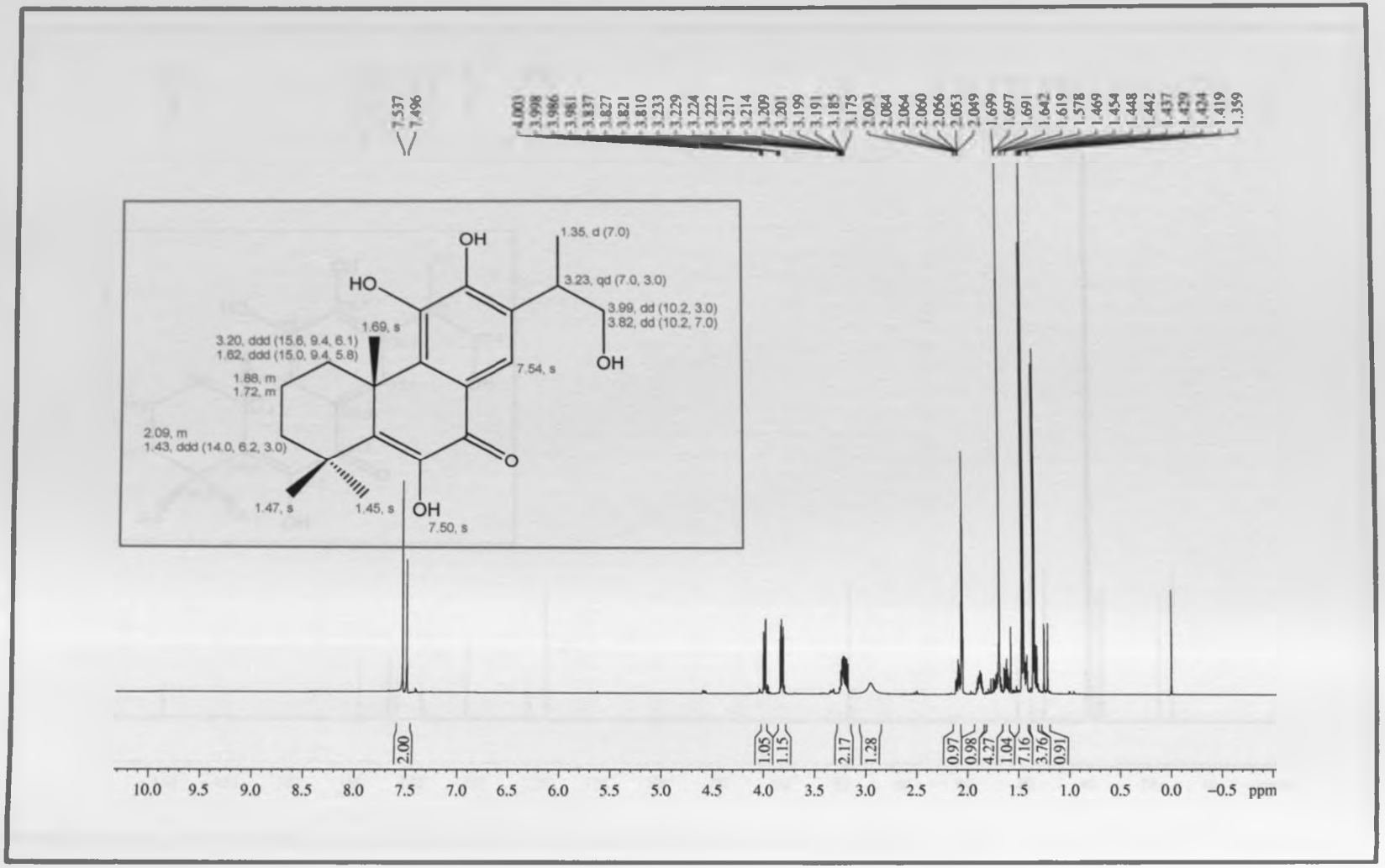
Appendix 8B: ^{13}C-NMR spectrum of 6-hydroxysalvinolone (**226**), Acetone-d_6, 75 MHz



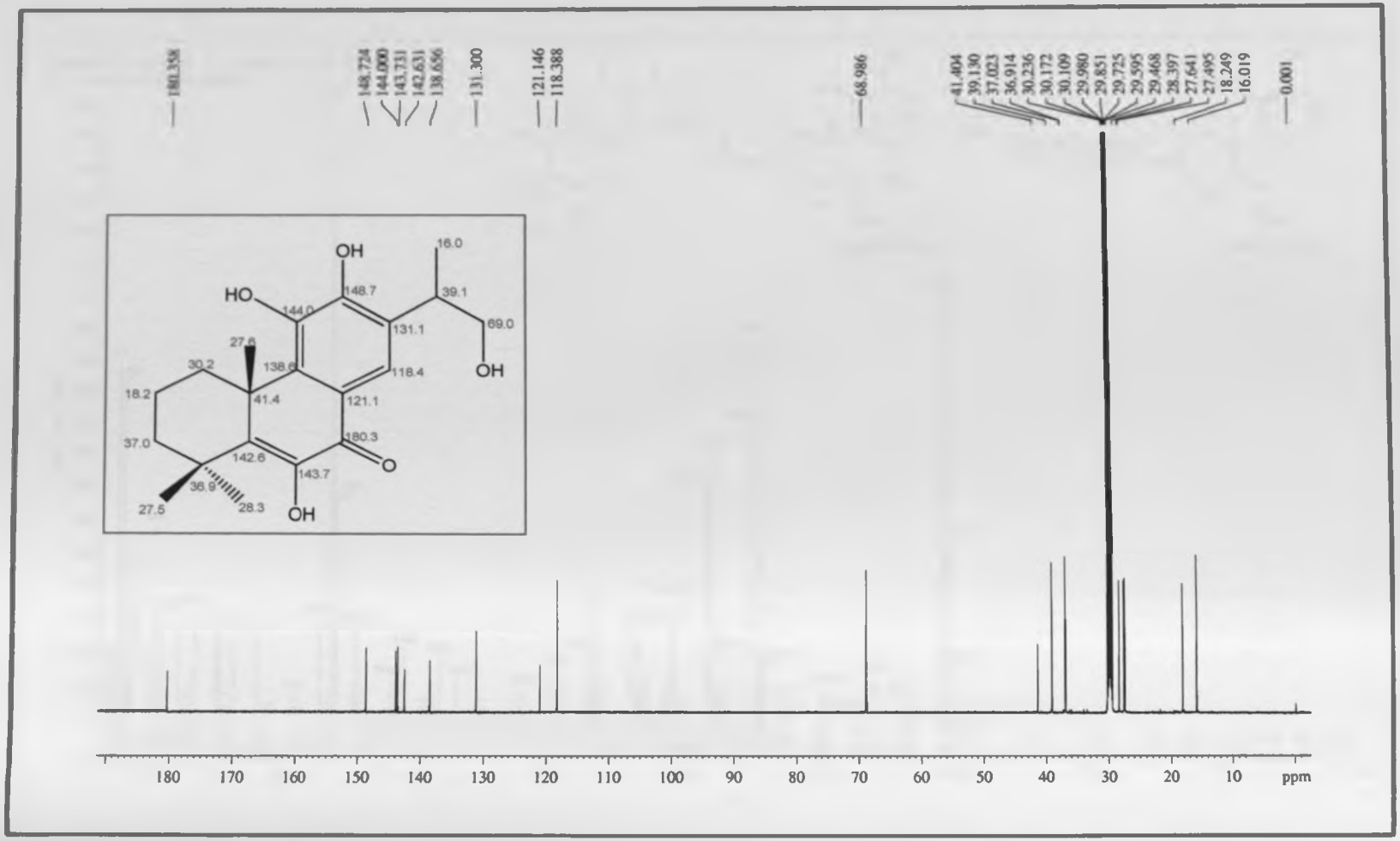
Appendix 8C: EI-MS spectrum of 6-hydroxysalvinolone (**226**)



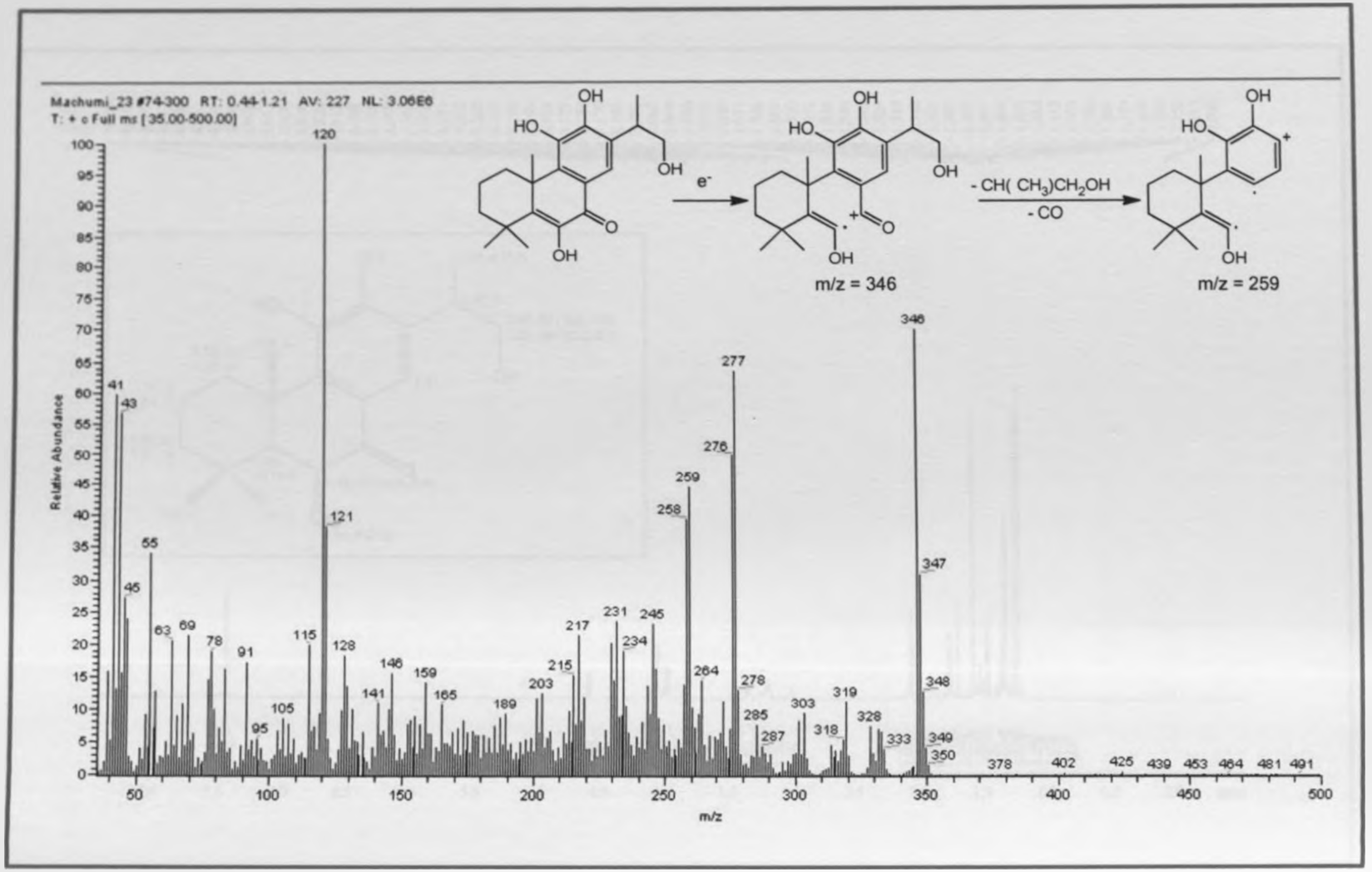
Appendix 9A: ^{1}H-NMR spectrum of 6,16-dihydroxysalvinolone (**227**), Acetone-d_6, 600 MHz



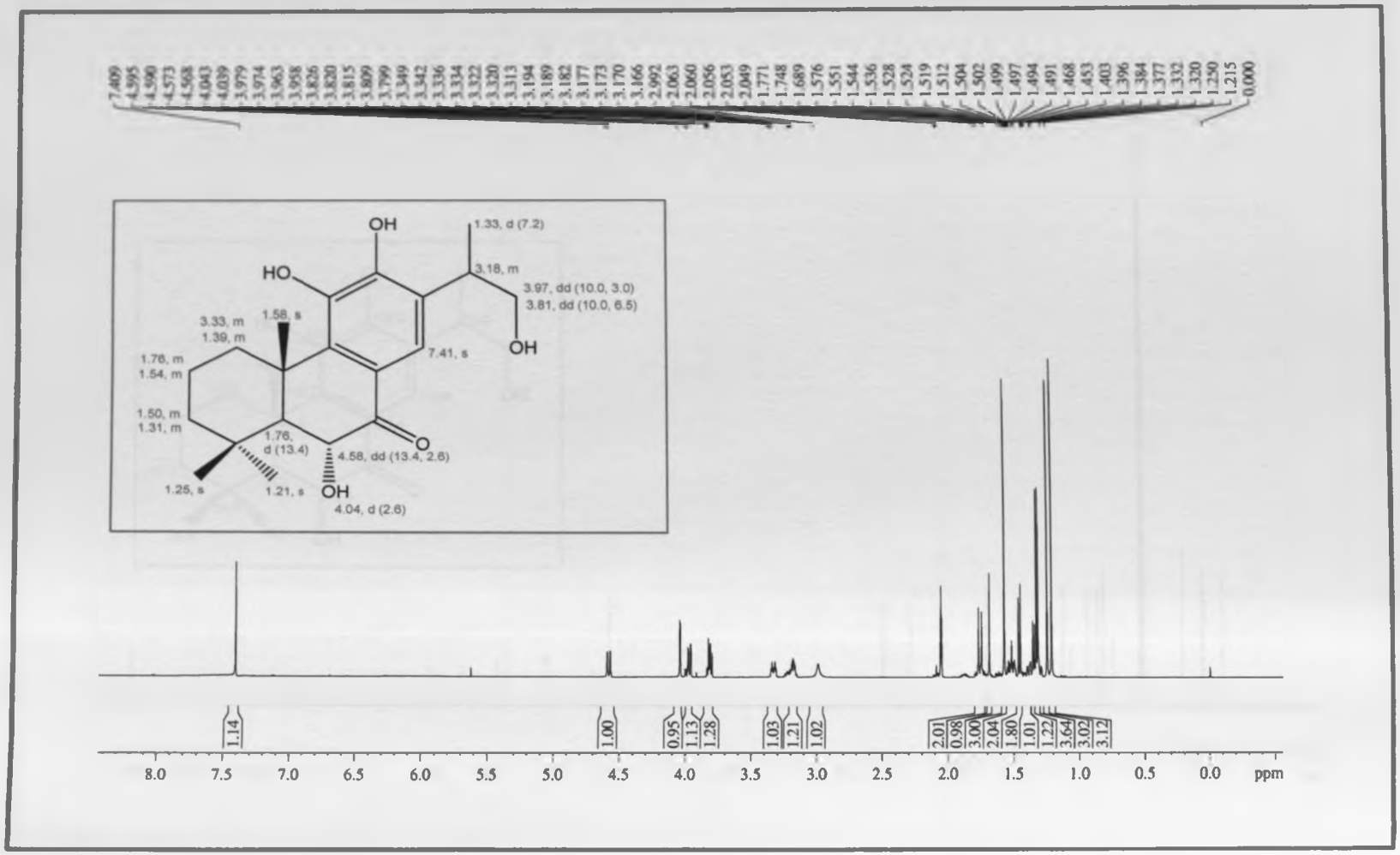
Appendix 9B: ^{13}C-NMR spectrum of 6,16-dihydroxysalvinolone (**227**), Acetone-d_6, 150 MHz



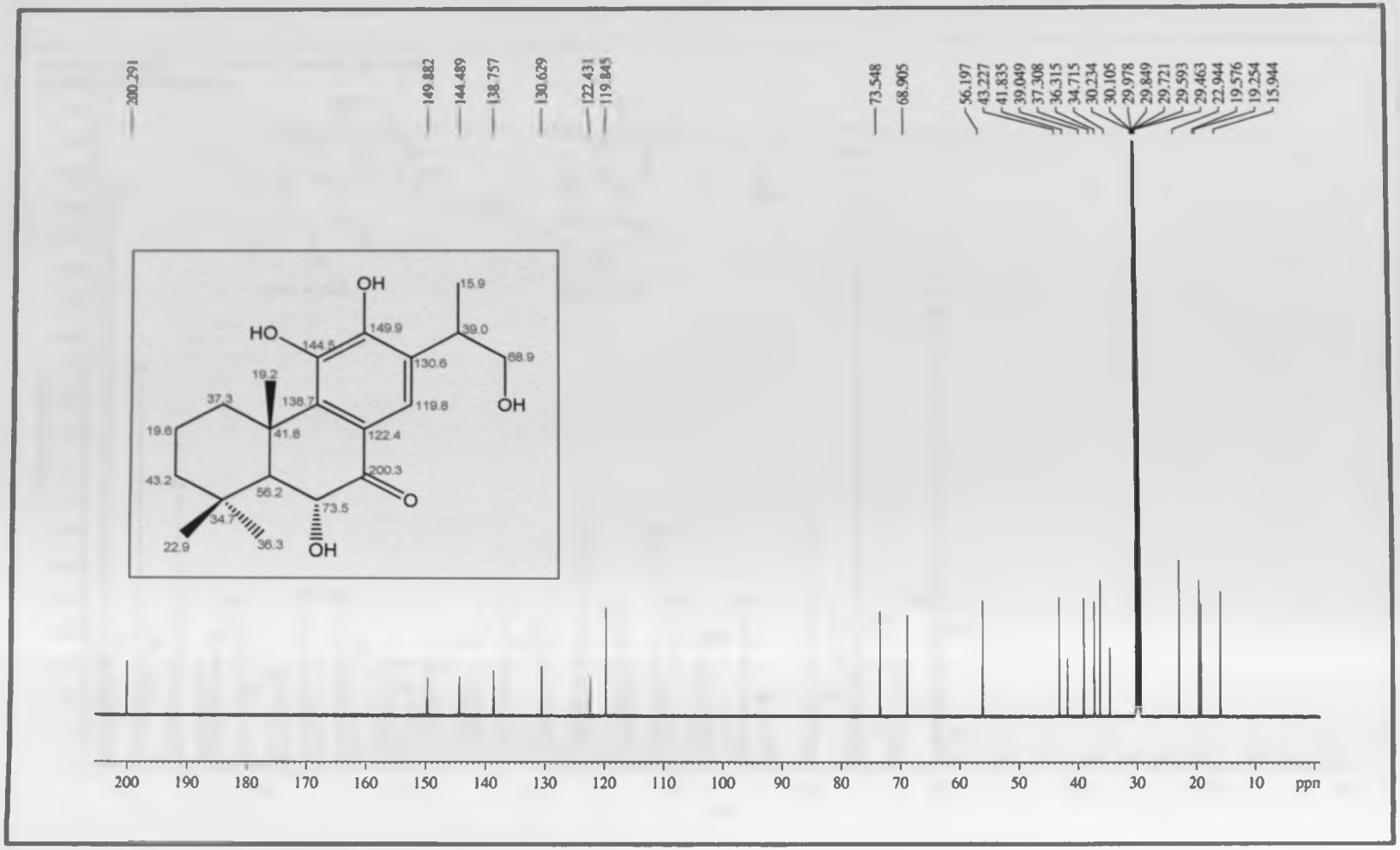
Appendix 9C: EI-MS spectrum of 6,16-dihydroxysalvinolone (**227**)



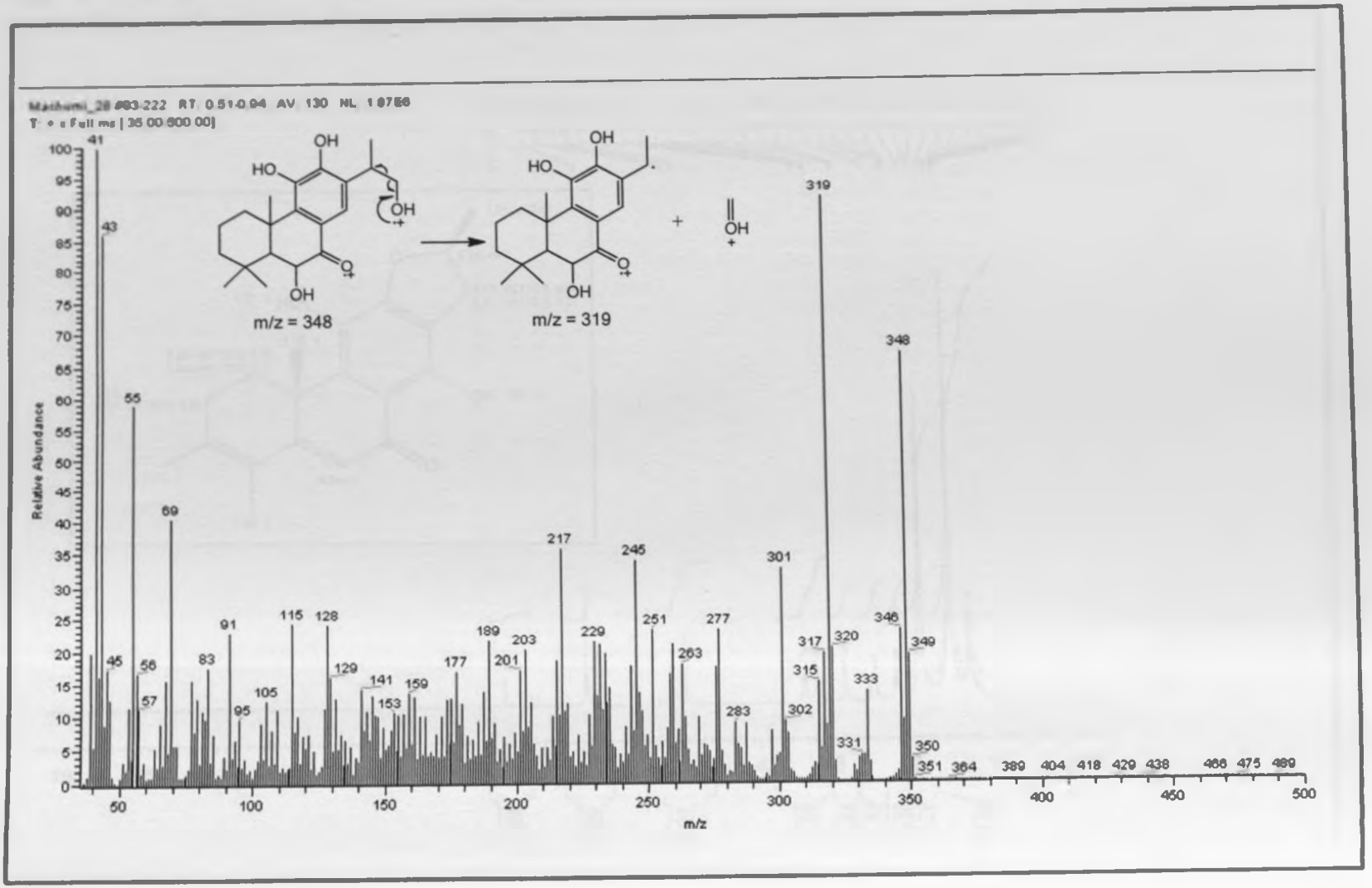
Appendix 10A: ^{1}H-NMR spectrum of nellionol (**228**), Acetone-d_6, 600 MHz



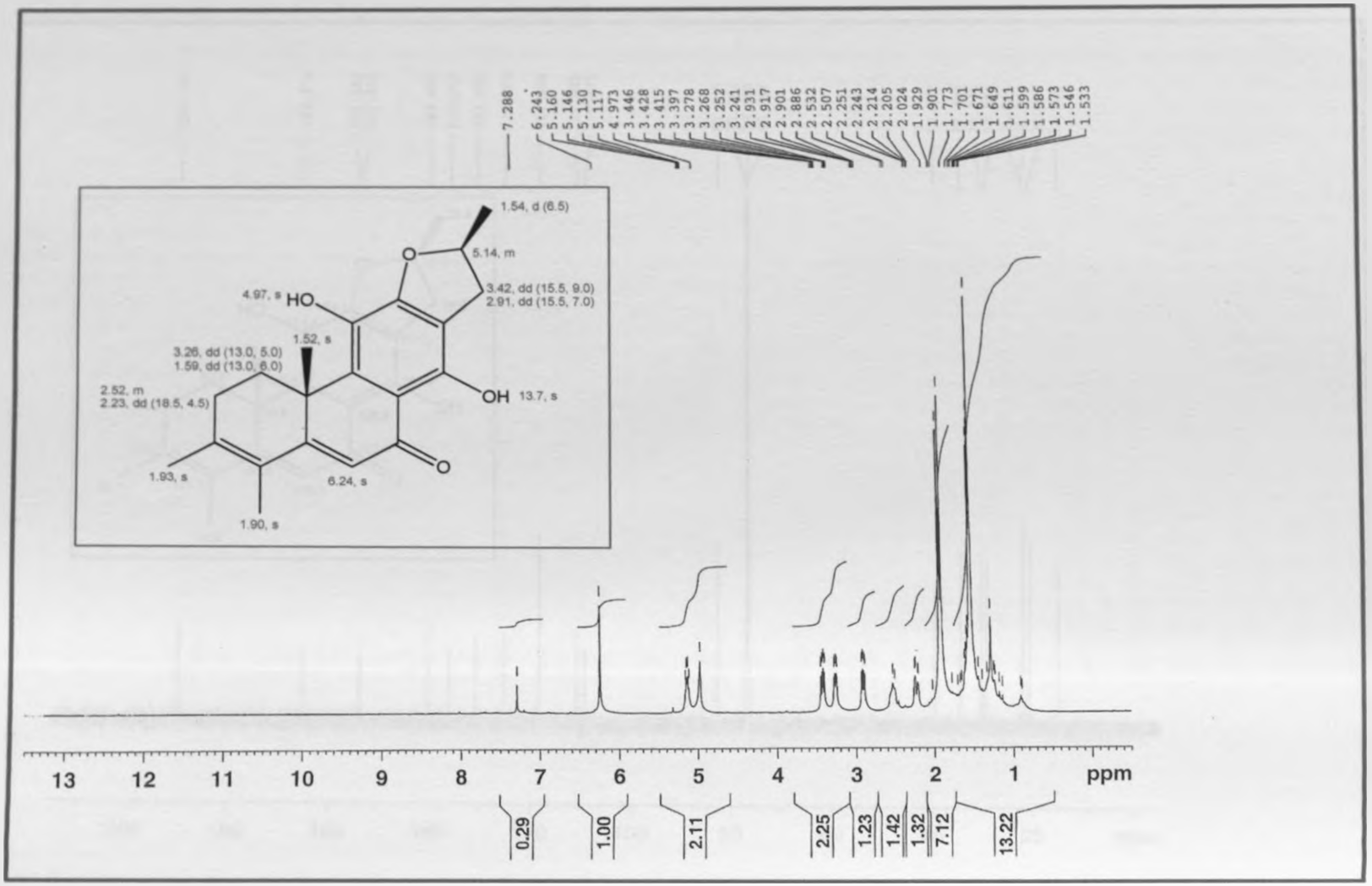
Appendix 10B: ^{13}C-NMR spectrum of nellionol (**228**), Acetone-d_6, 150 MHz



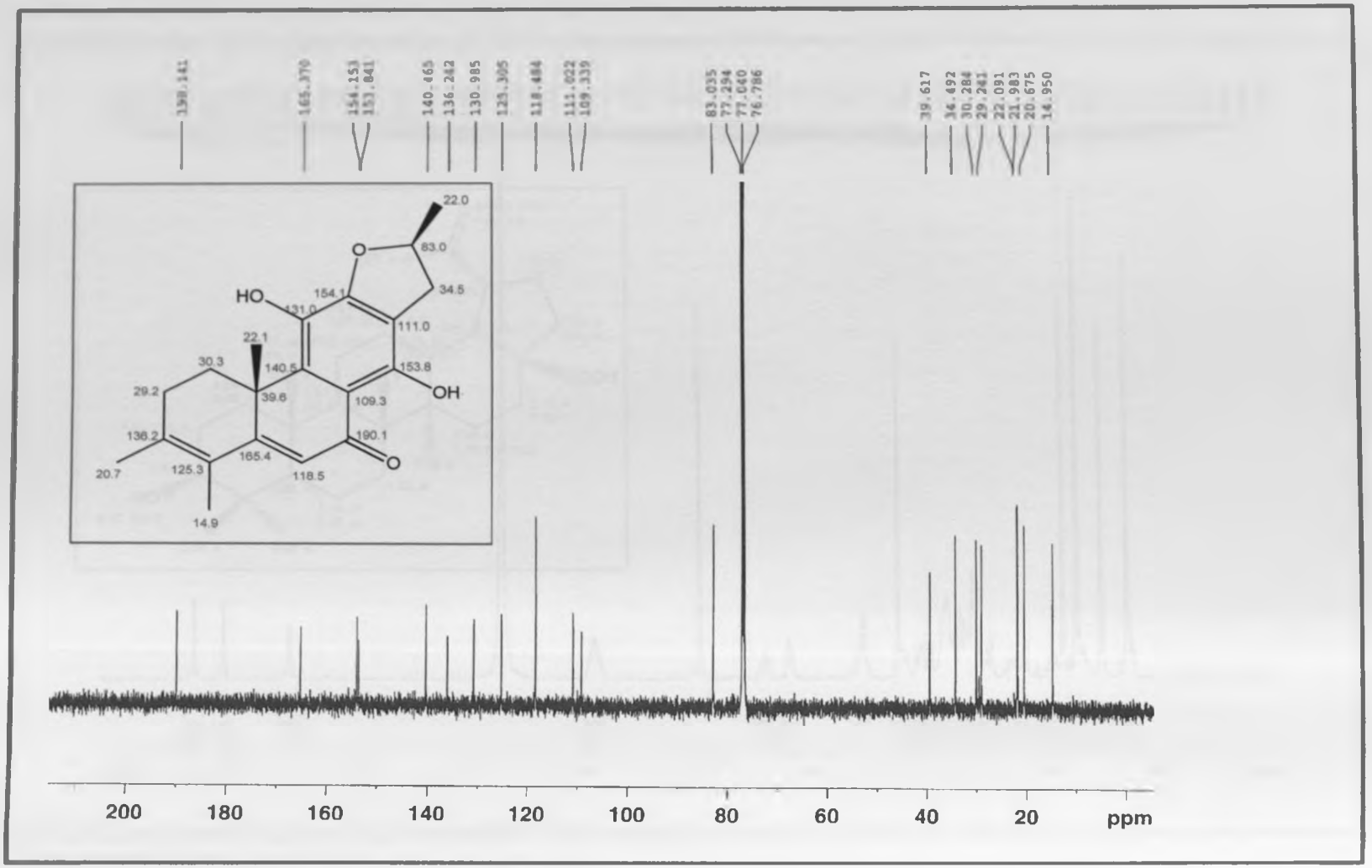
Appendix 10C: EI-MS spectrum of nellionol (**228**)



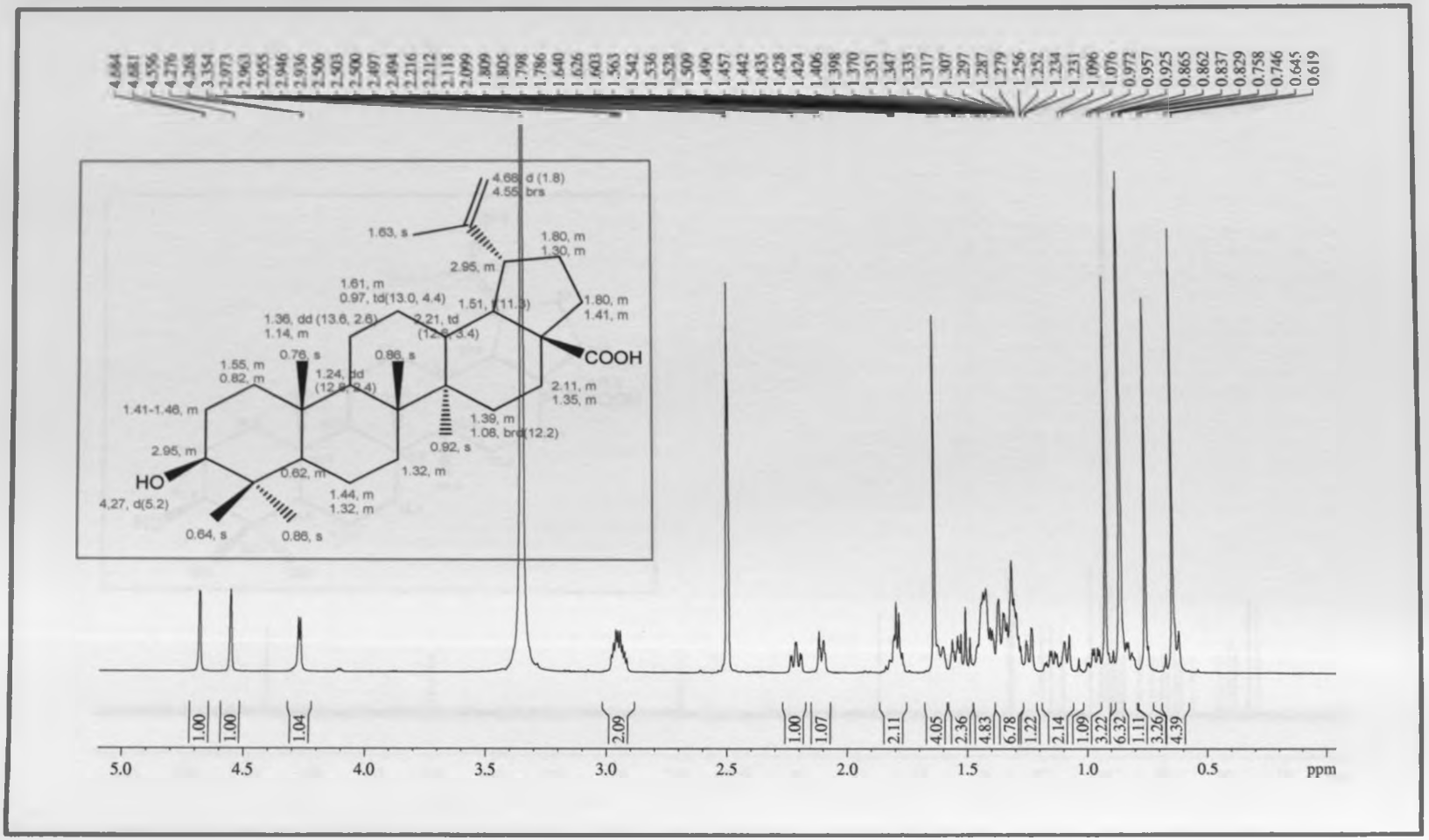
Appendix 11A: ^{1}H-NMR spectrum of uncinatone (**19**), $CDCl_3$, 500 MHz



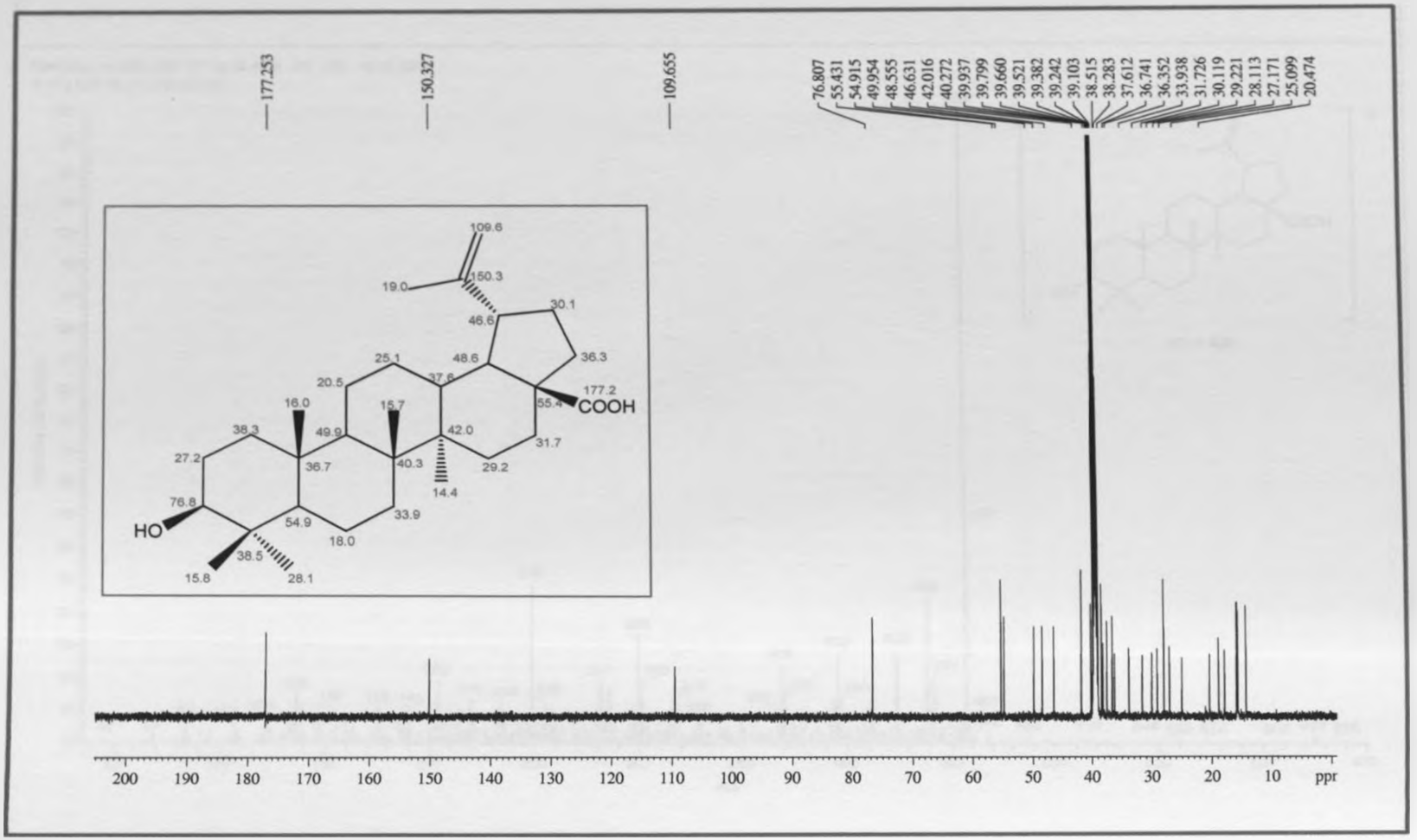
Appendix 11B: ^{13}C-NMR spectrum of uncinatone (**19**), $CDCl_3$, 125 MHz



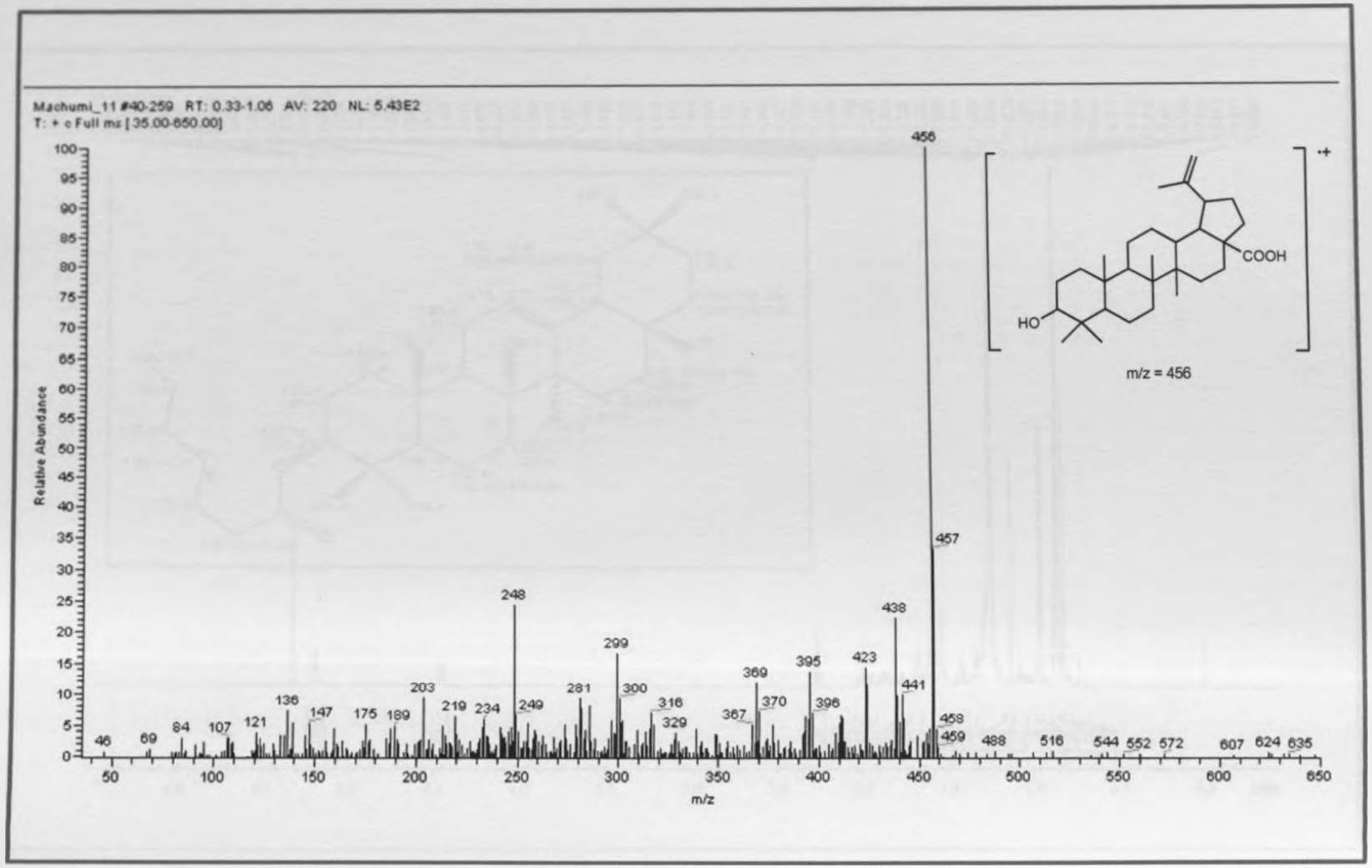
Appendix 12A: ^{1}H-NMR spectrum of betulinic acid (**229**), DMSO, 600 MHz



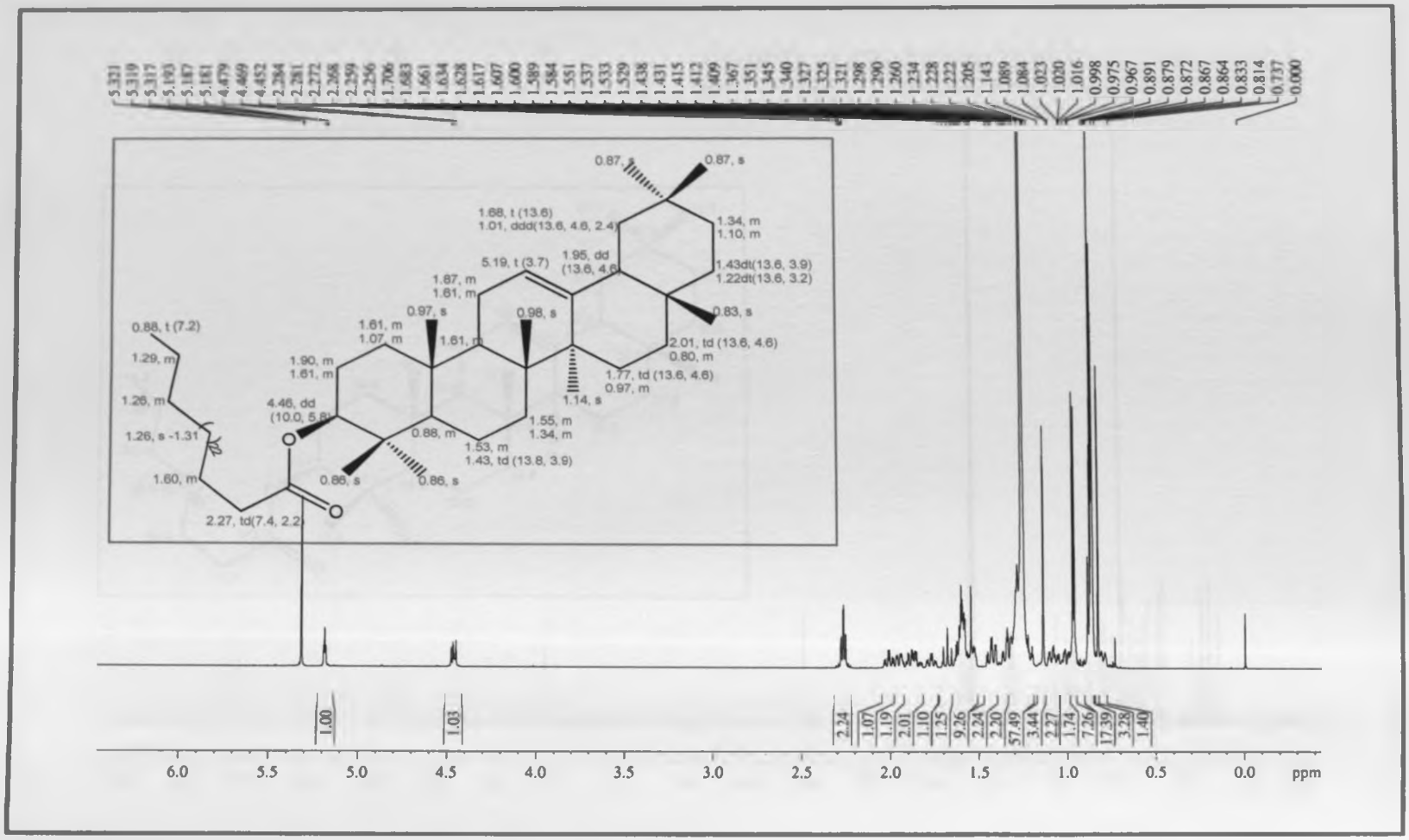
Appendix 12B: ^{13}C-NMR spectrum of betulinic acid (**229**), DMSO, 150 MHz



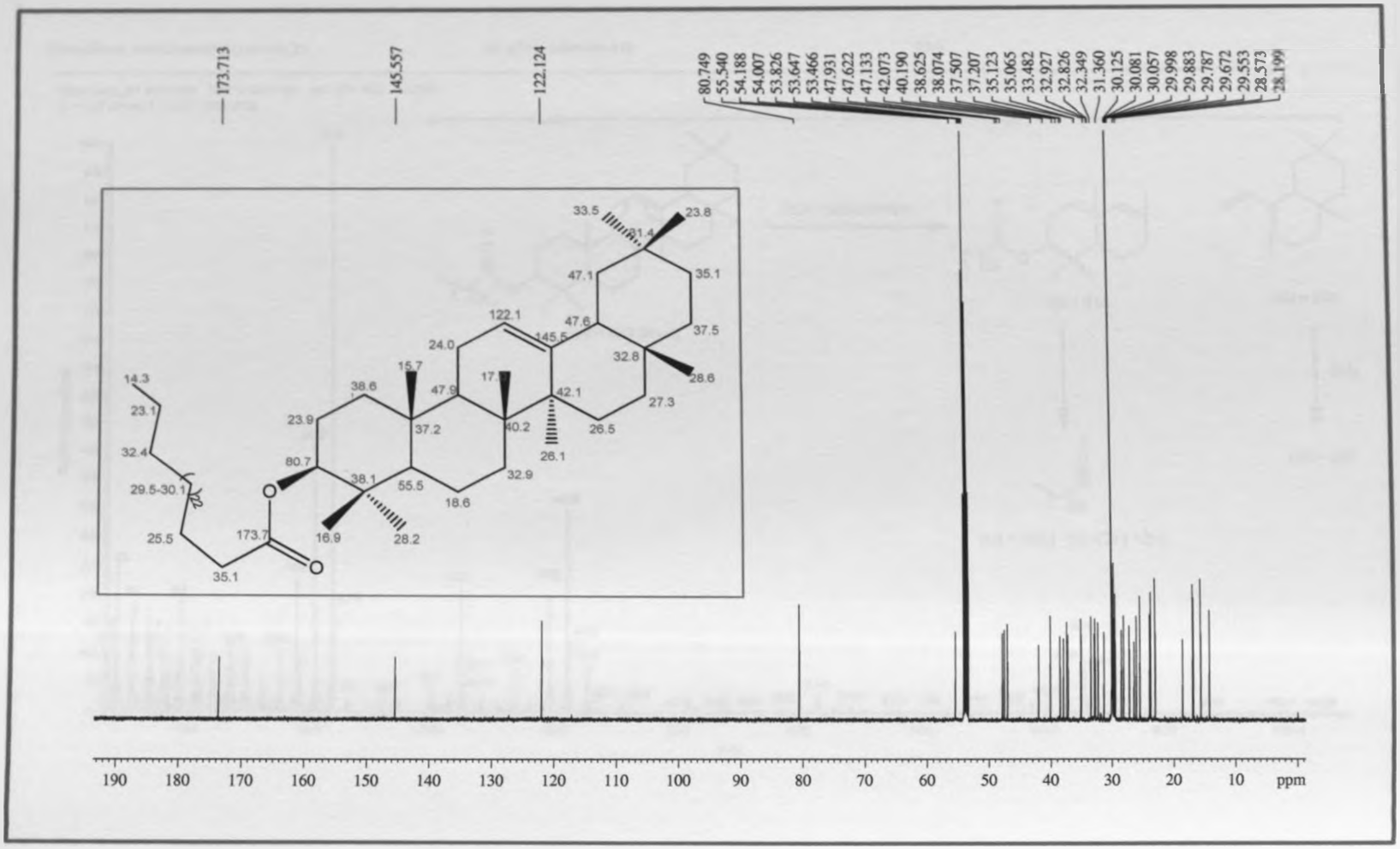
Appendix 12C: EI-MS spectrum of betulinic acid (**229**)



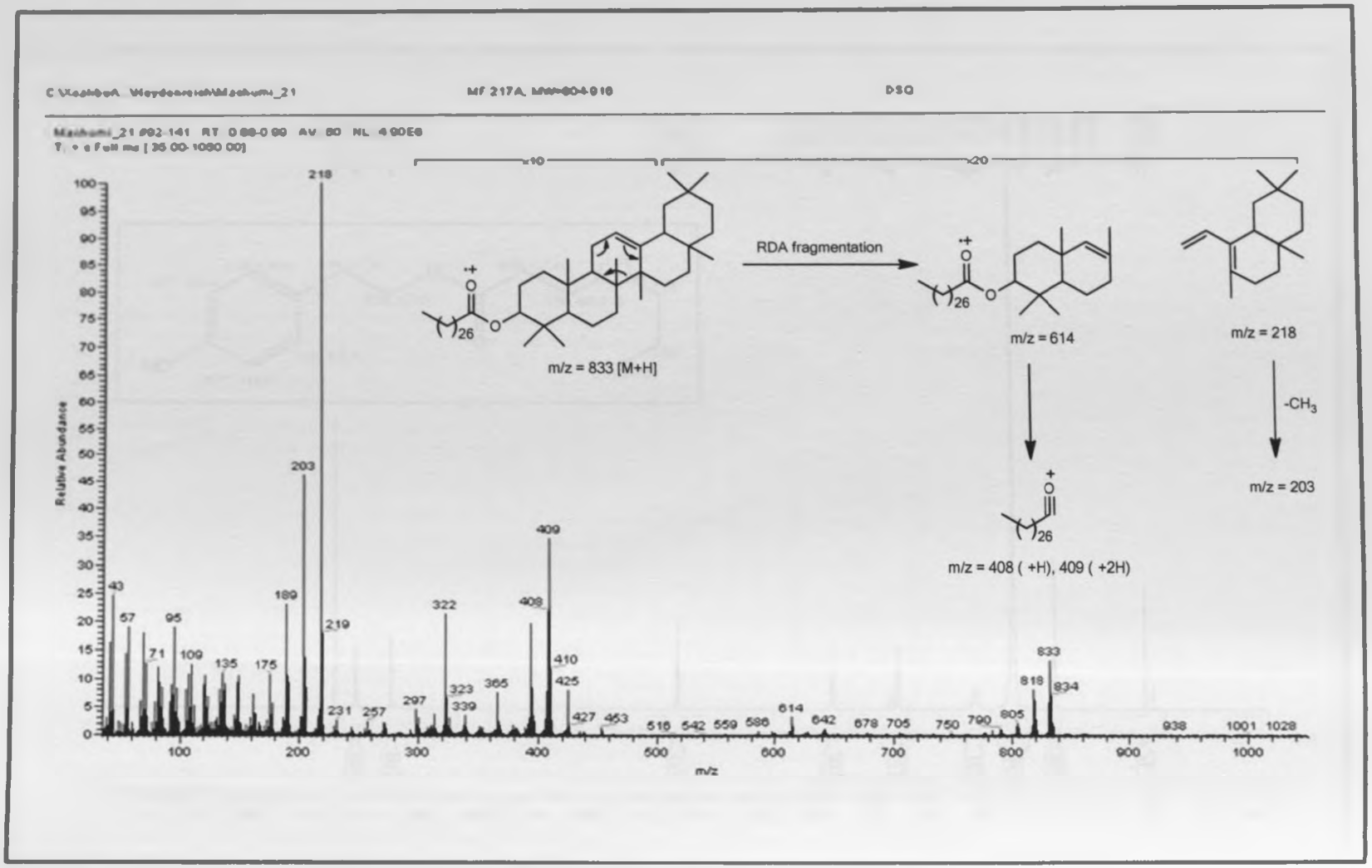
Appendix 13A: ^{1}H-NMR spectrum of β-amyrin octacosanoate (**230**), CD_2Cl_2, 600 MHz



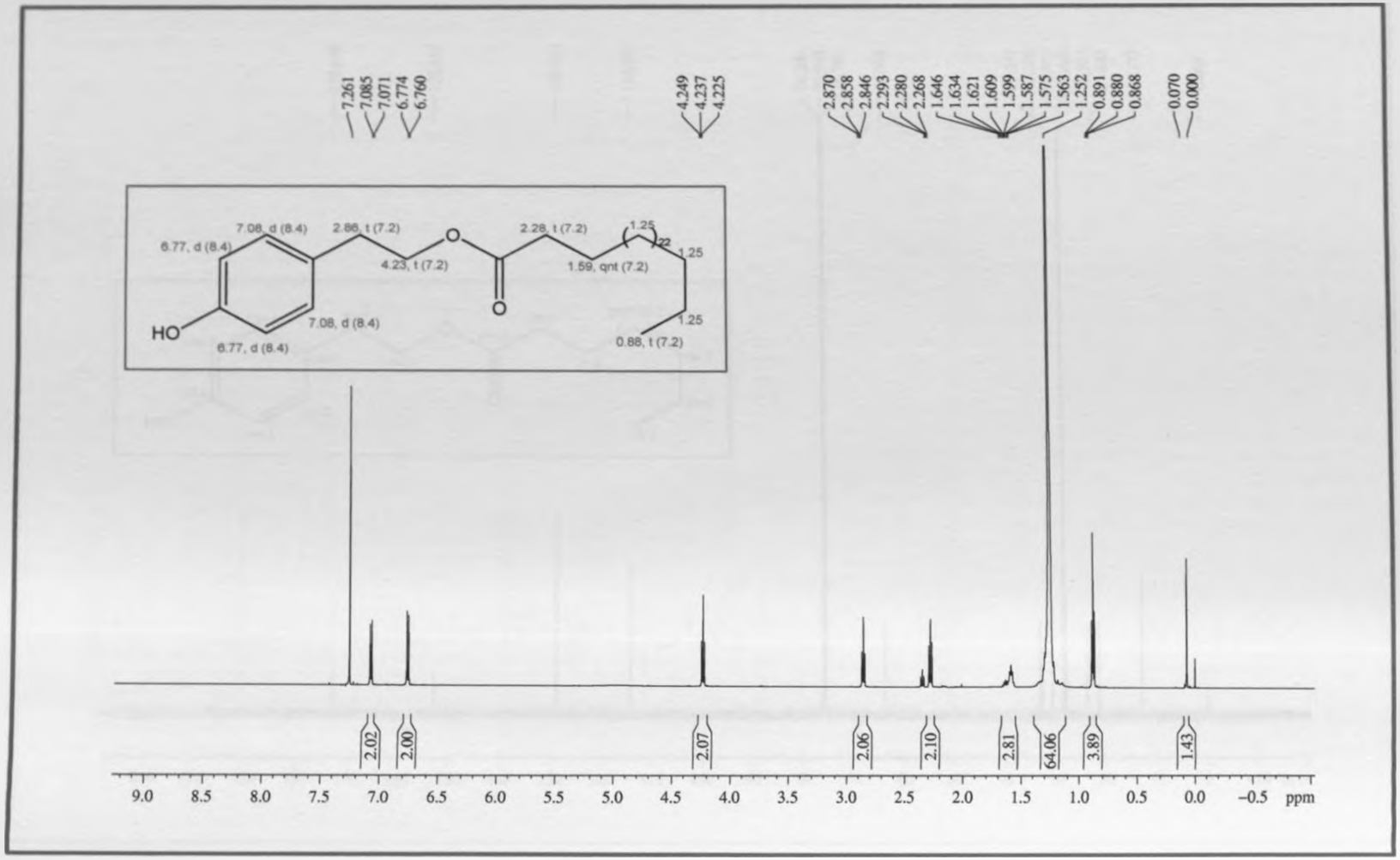
Appendix 13B: ^{13}C-NMR spectrum of β-amyrin octacosanoate (**230**), CD_2Cl_2, 600 MHz



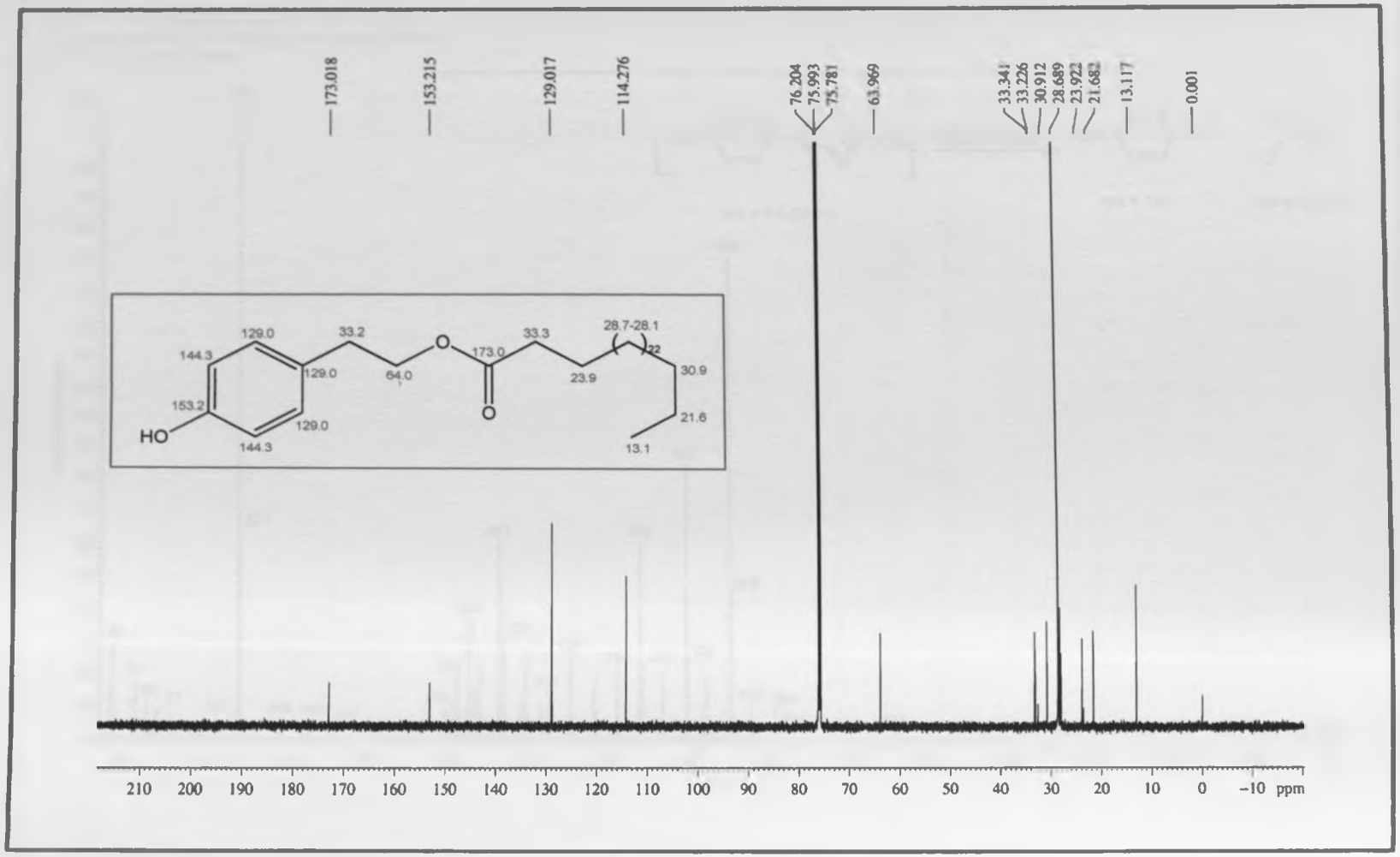
Appendix 13C: EI-MS spectrum of β-amyrin octacosanoate (**230**)



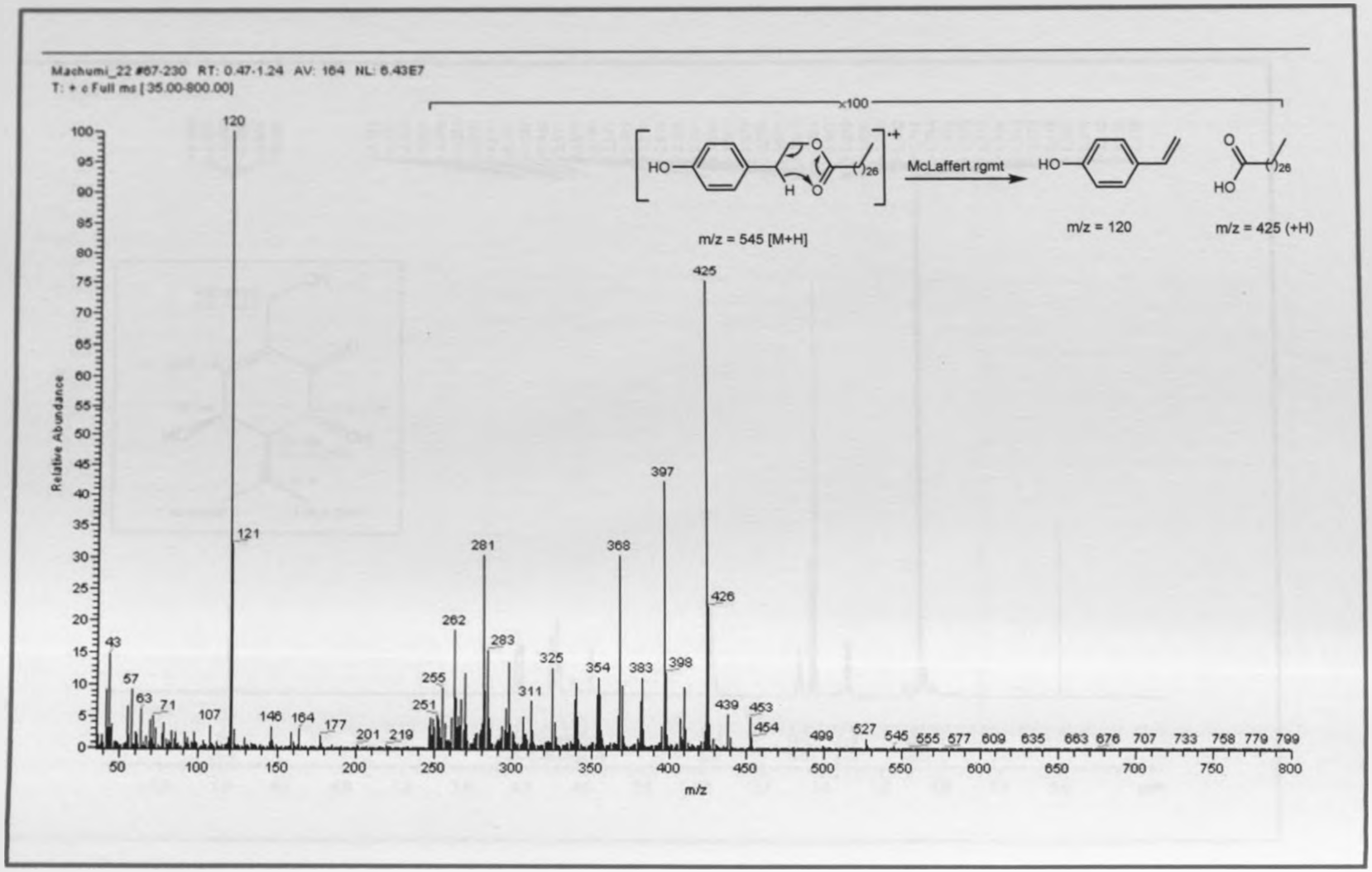
Appendix 14A: ^{1}H-NMR spectrum of Bongardol (**231**), $CDCl_3$, 600 MHz



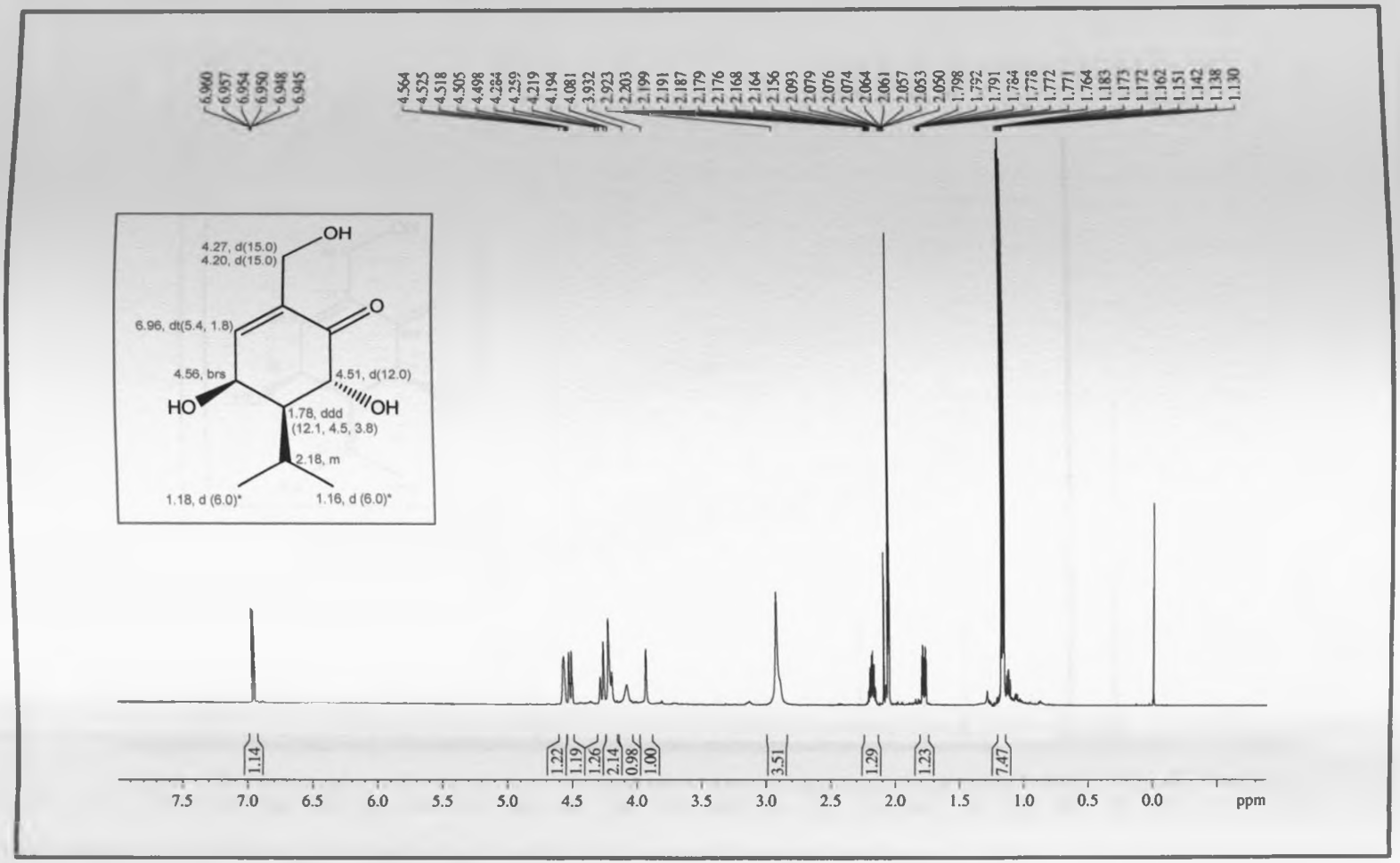
Appendix 14B: ^{13}C-NMR spectrum of Bongardol (**231**), $CDCl_3$, 150 MHz



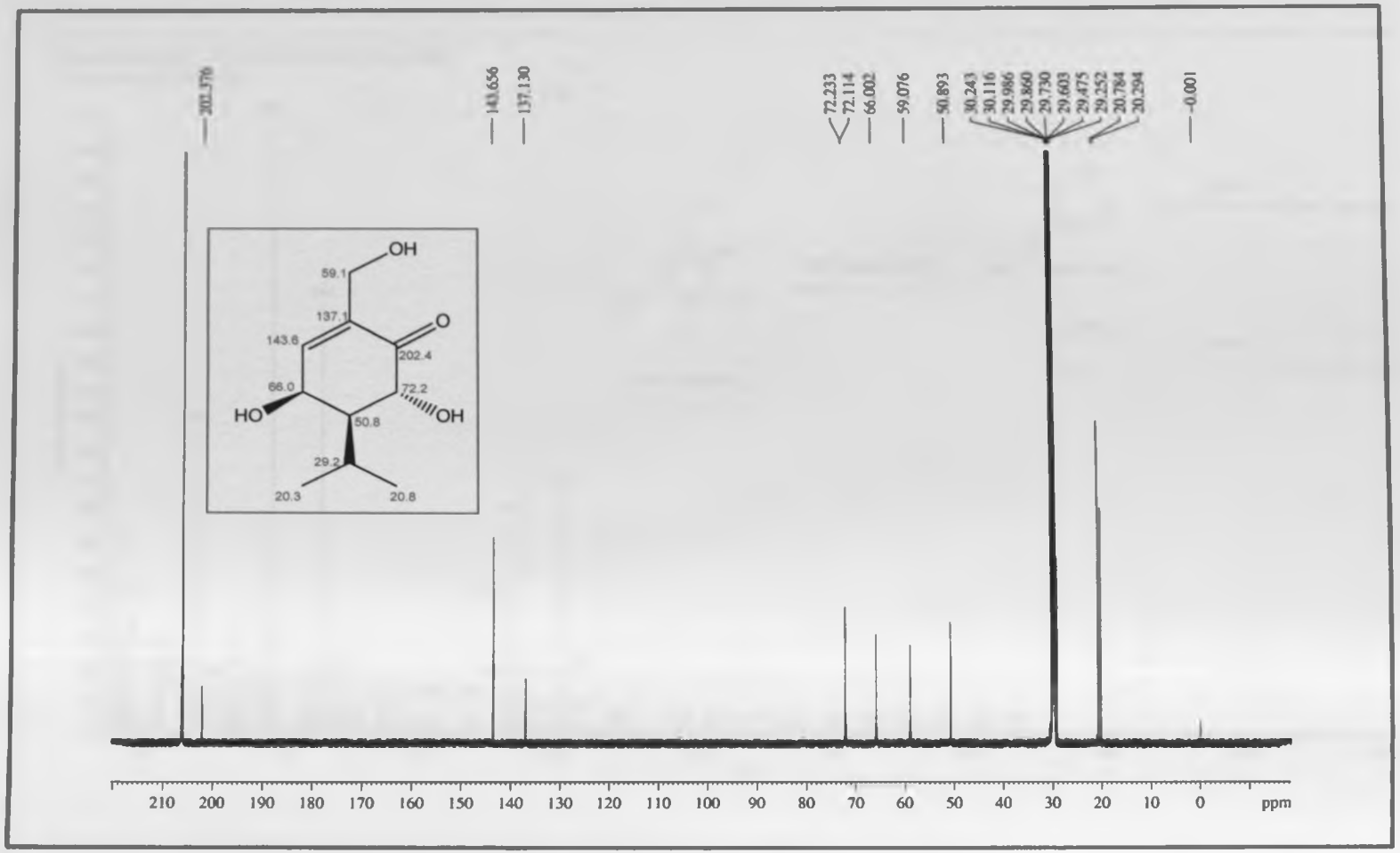
Appendix 14C: EI-MS spectrum of Bongardol (**231**)



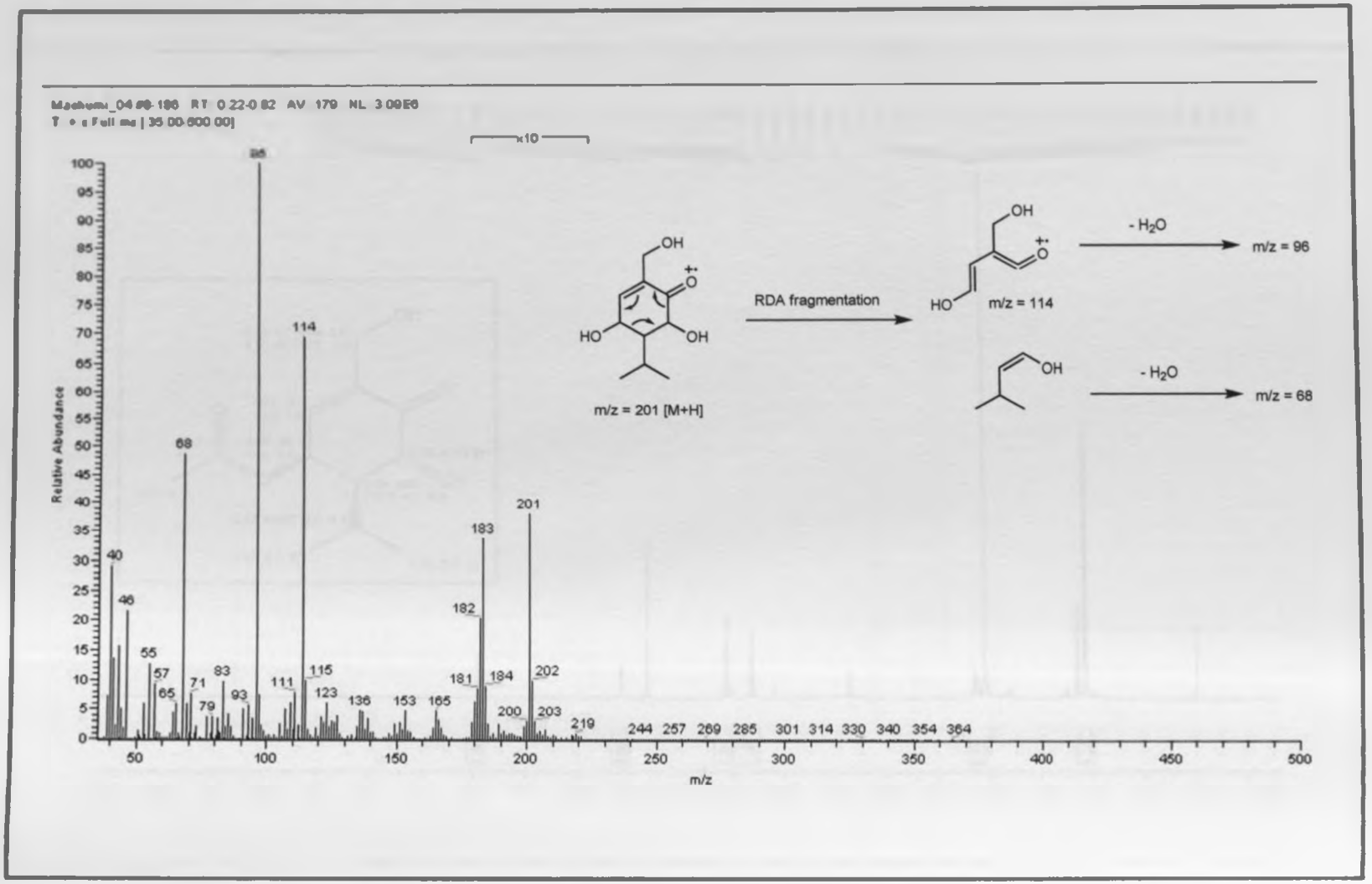
Appendix 15A: ^{1}H-NMR spectrum of 3,5,7-trihydroxycarvotacetone (**85**), Acetone d_6, 600 MHz



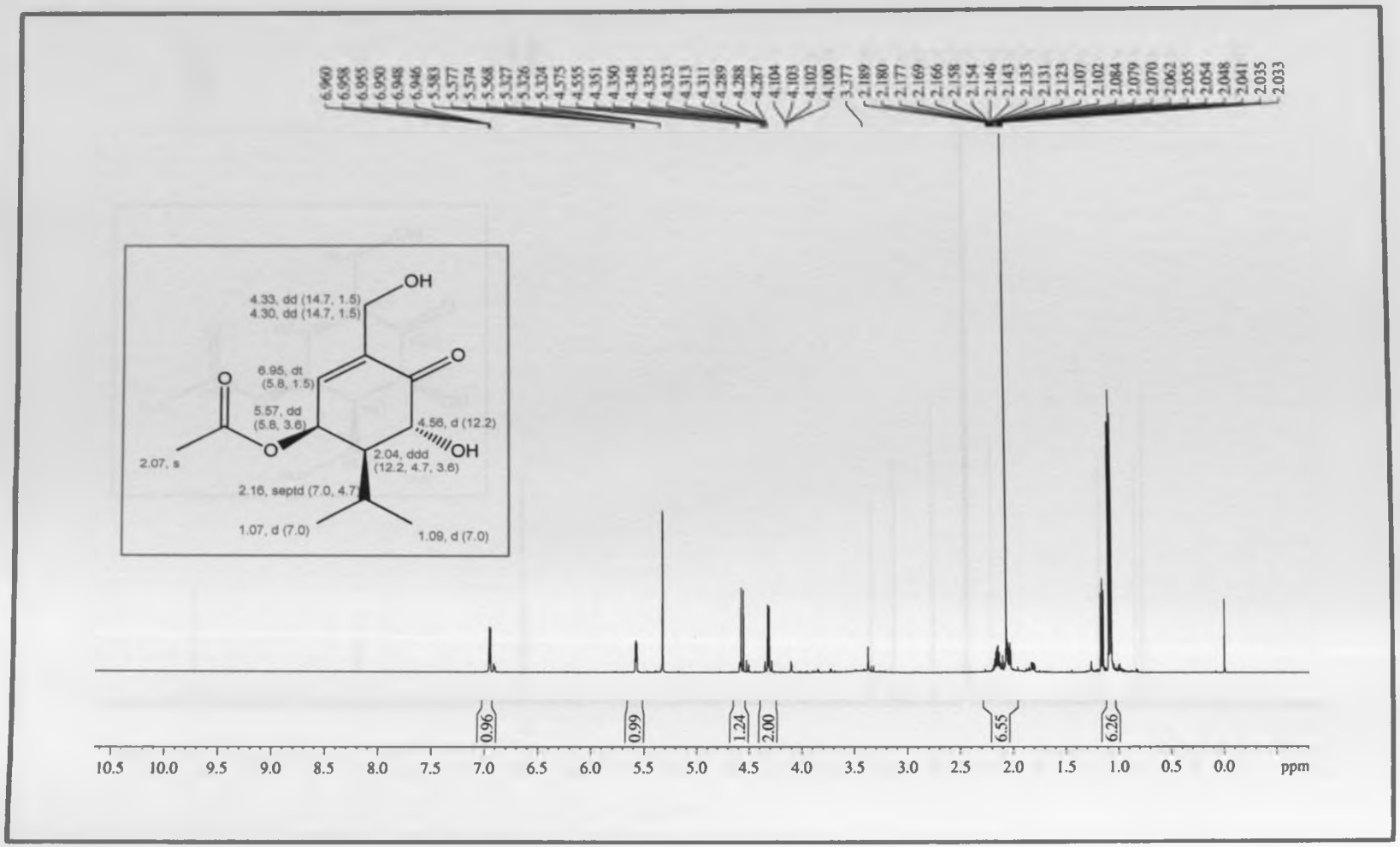
Appendix 15B: ^{13}C-NMR spectrum of 3,5,7-trihydroxycarvotacetone (**85**), Acetone d_6, 150 MHz



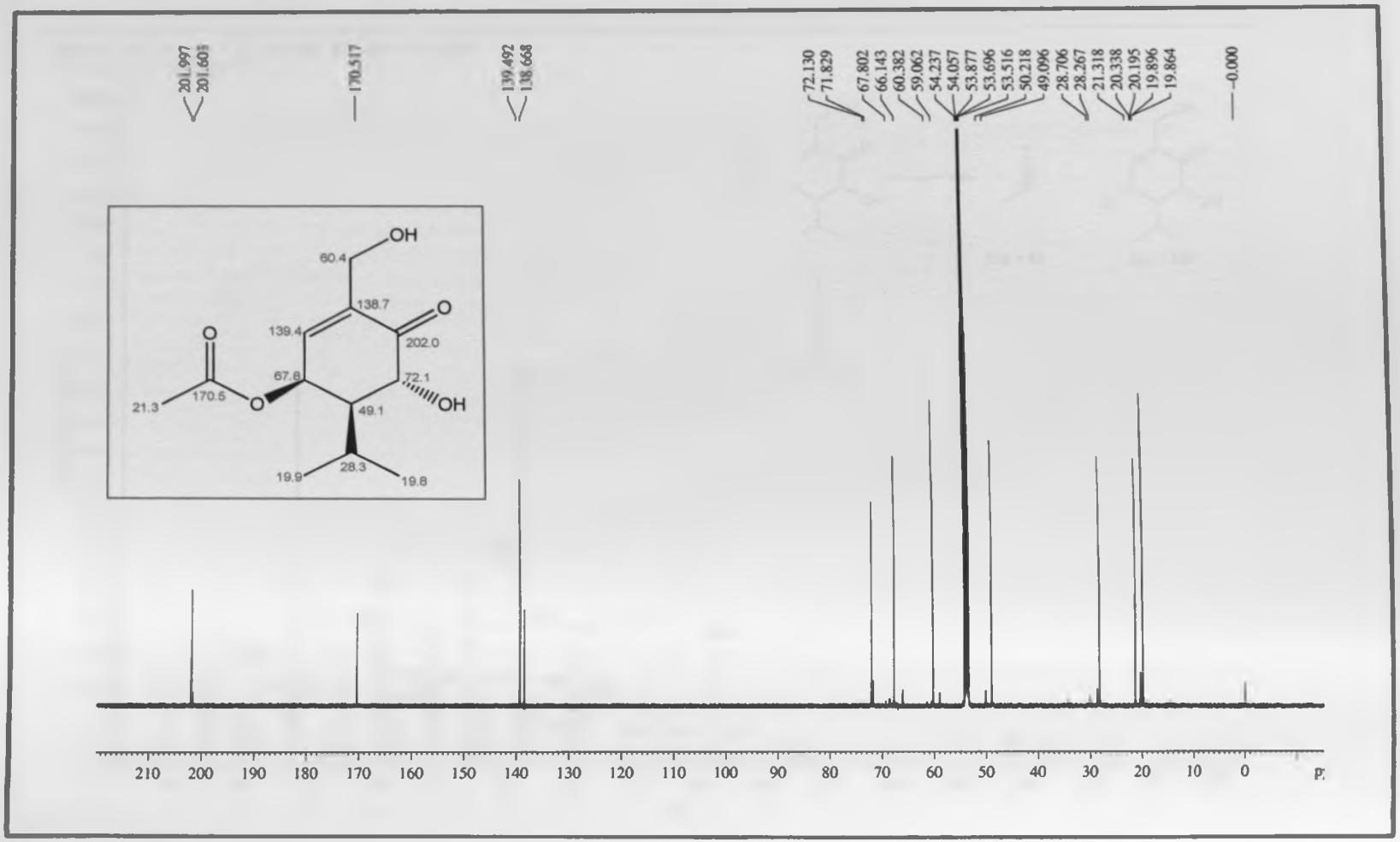
Appendix 15C: EI-MS spectrum of 3,5,7-trihydroxycarvotacetone (**85**)



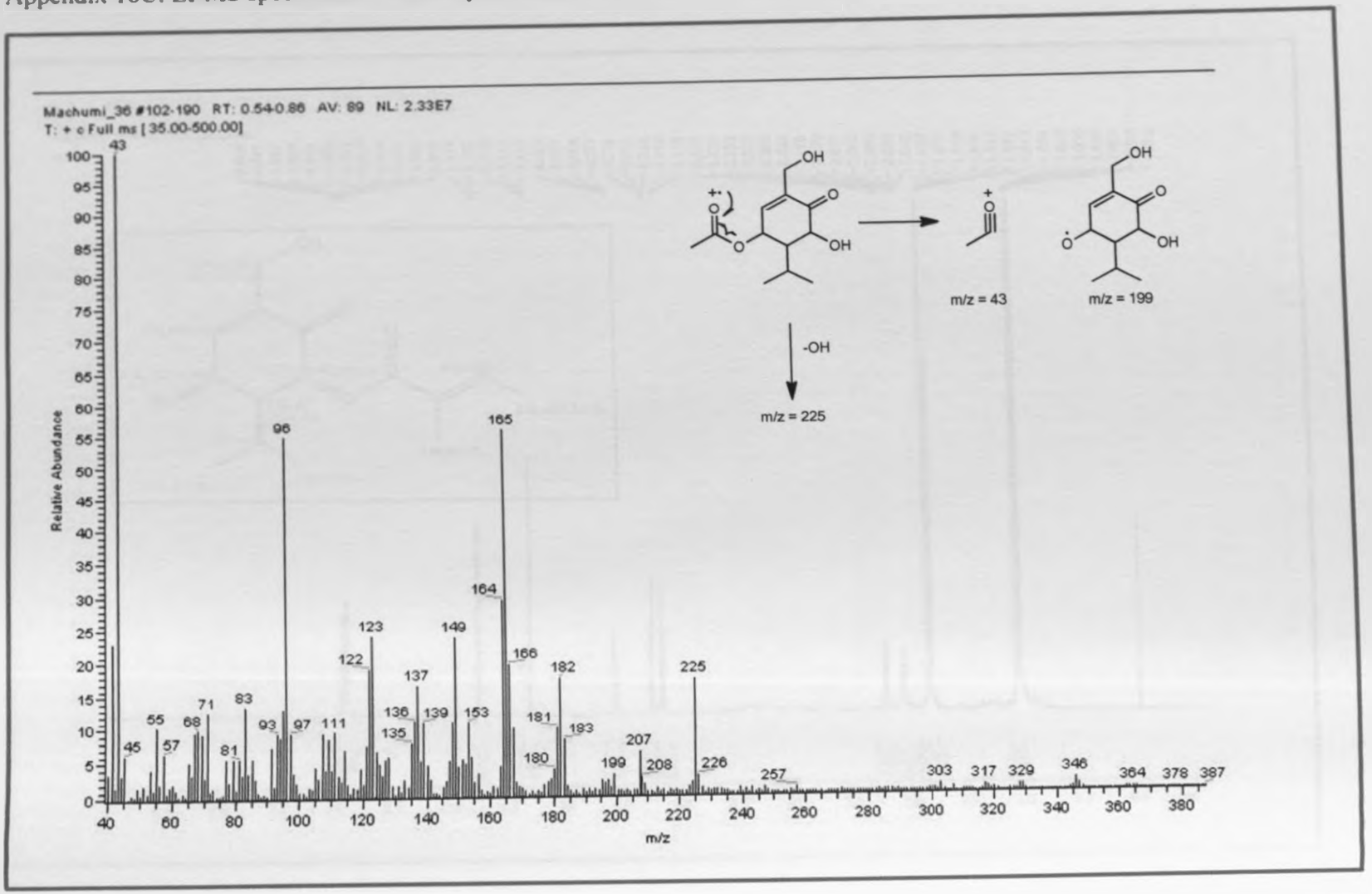
Appendix 16A: ^{1}H-NMR spectrum of 3-acetoxy-5,7-dihydroxycarvotacetone (**106**). CD_2Cl_2, 600 MHz



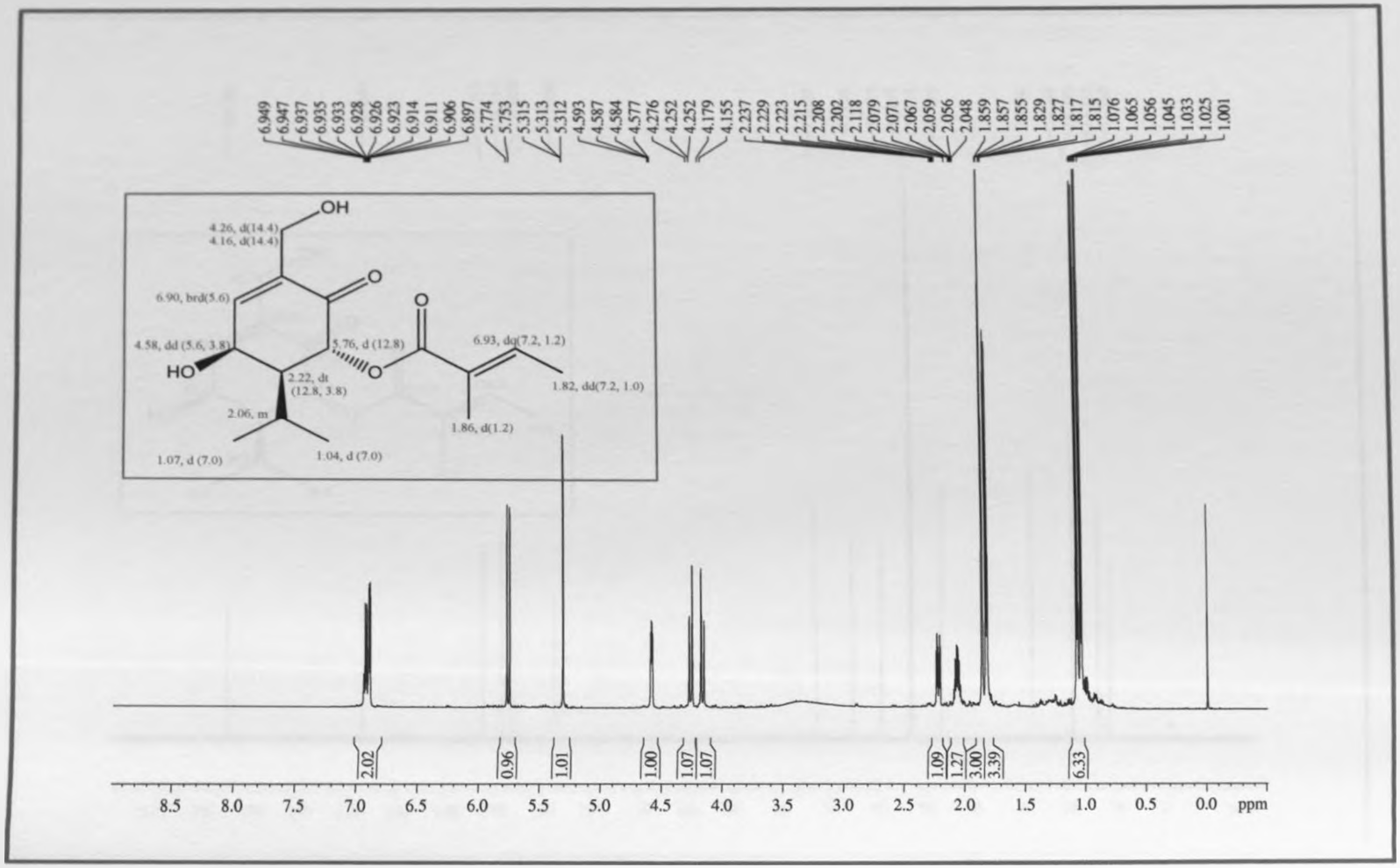
Appendix 16B: ^{13}C-NMR spectrum of 3-acetoxy-5,7-dihydroxycarvotacetone (**106**), CD_2Cl_2, 150 MHz



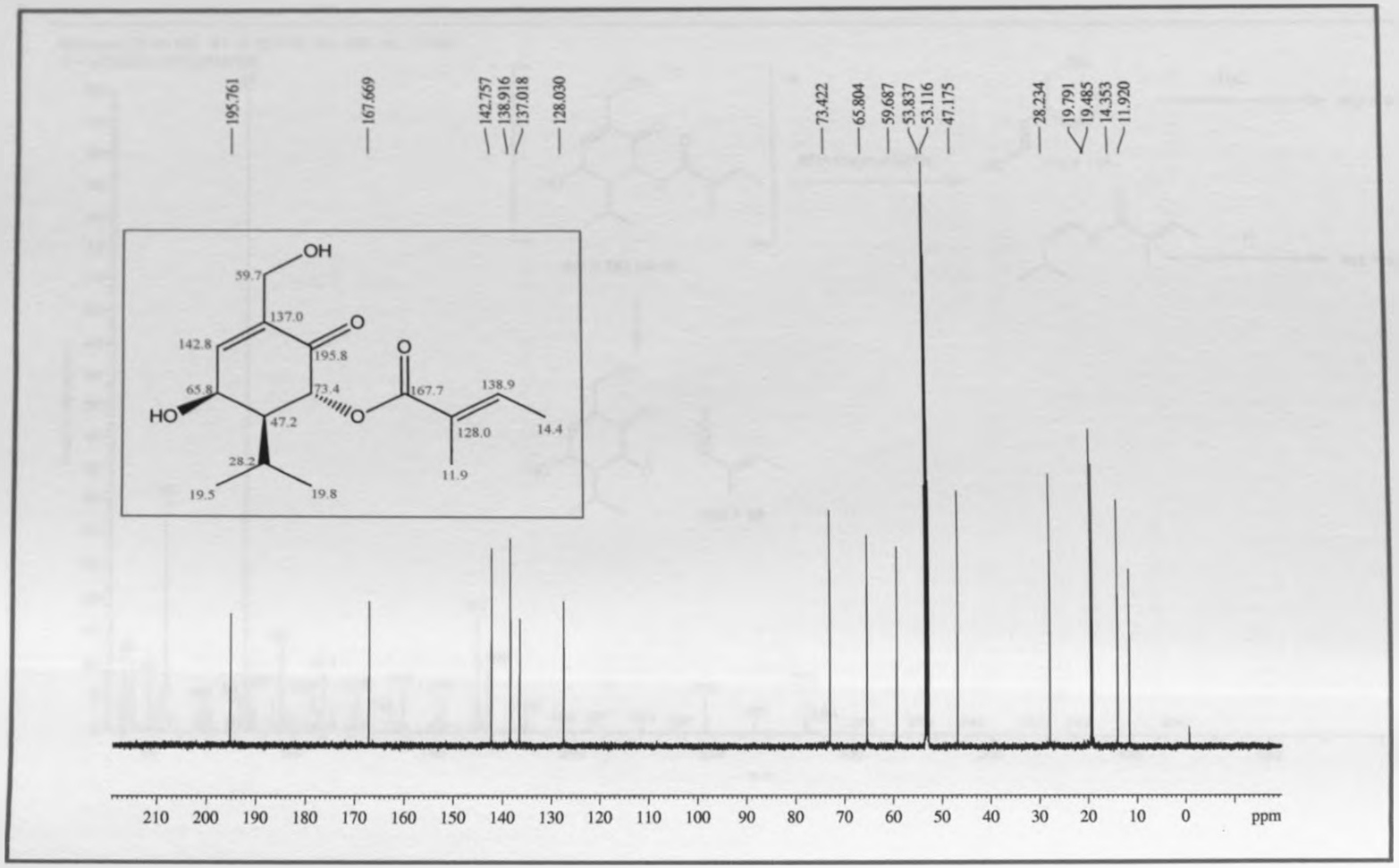
Appendix 16C: EI-MS spectrum of 3-acetoxy-5,7-dihydroxycarvotacetone (**106**)



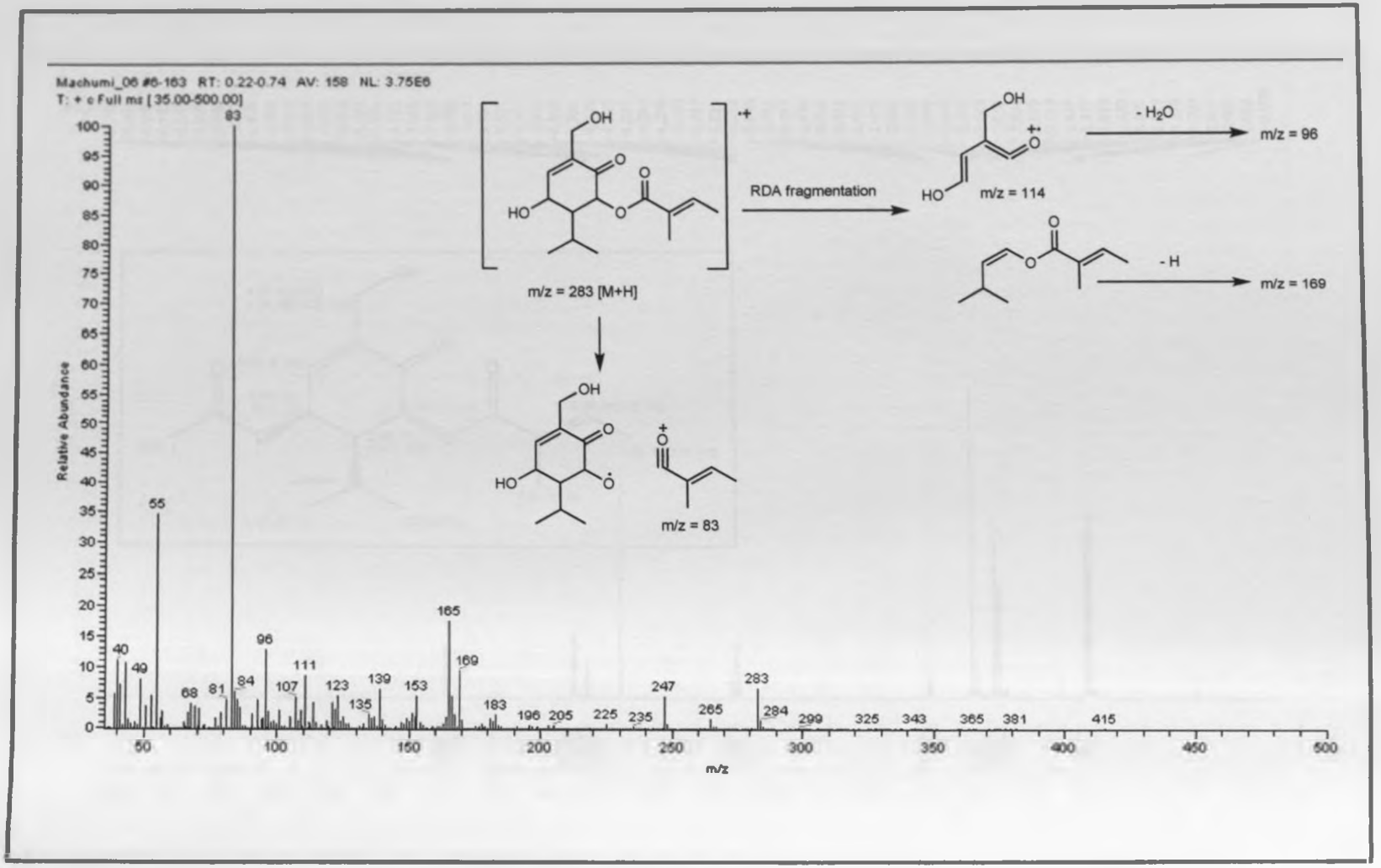
Appendix 17A: ^{1}H-NMR spectrum of 3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**), CD_2Cl_2, 600 MHz



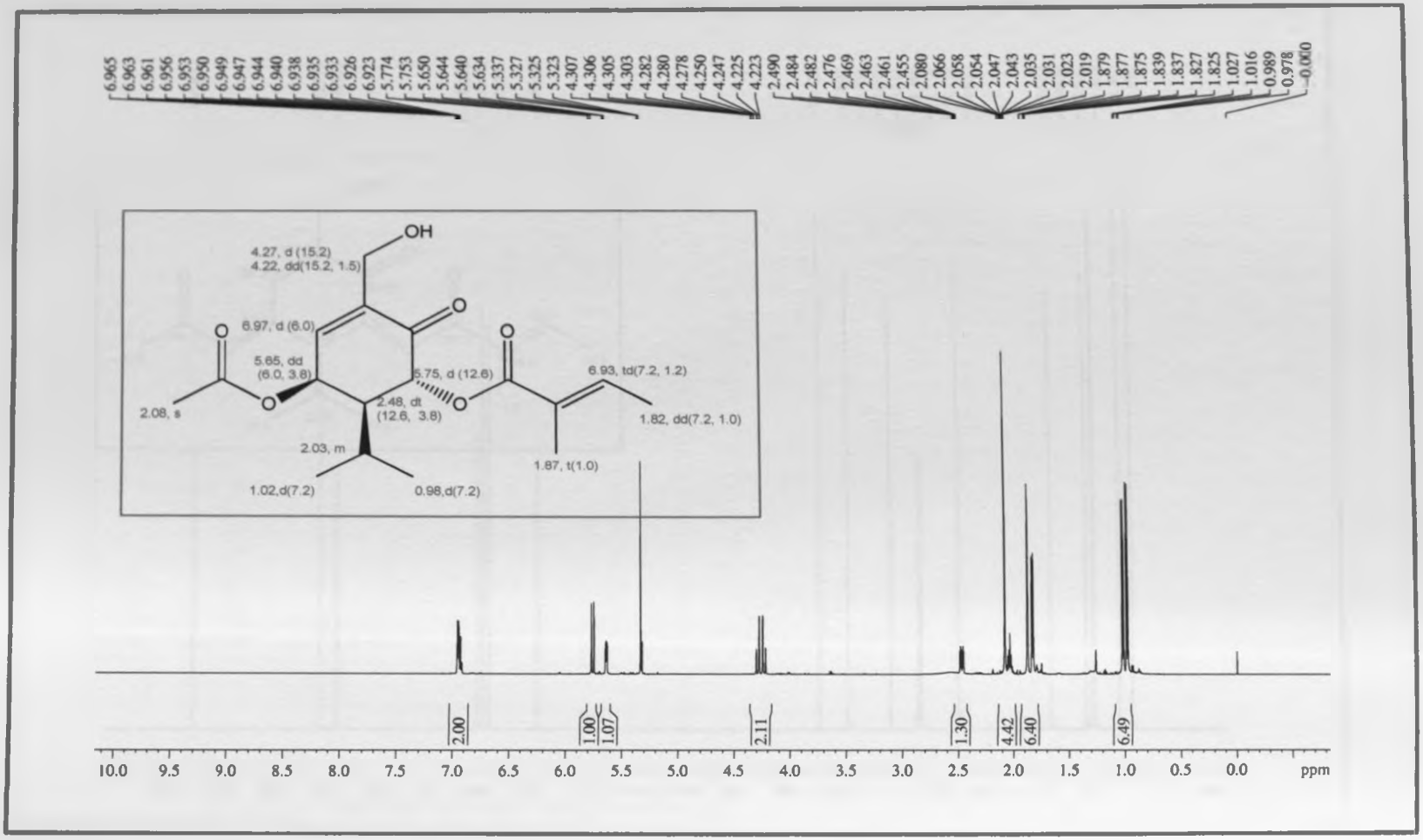
Appendix 17B: ^{13}C-NMR spectrum of 3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**), CD_2Cl_2, 150 MHz



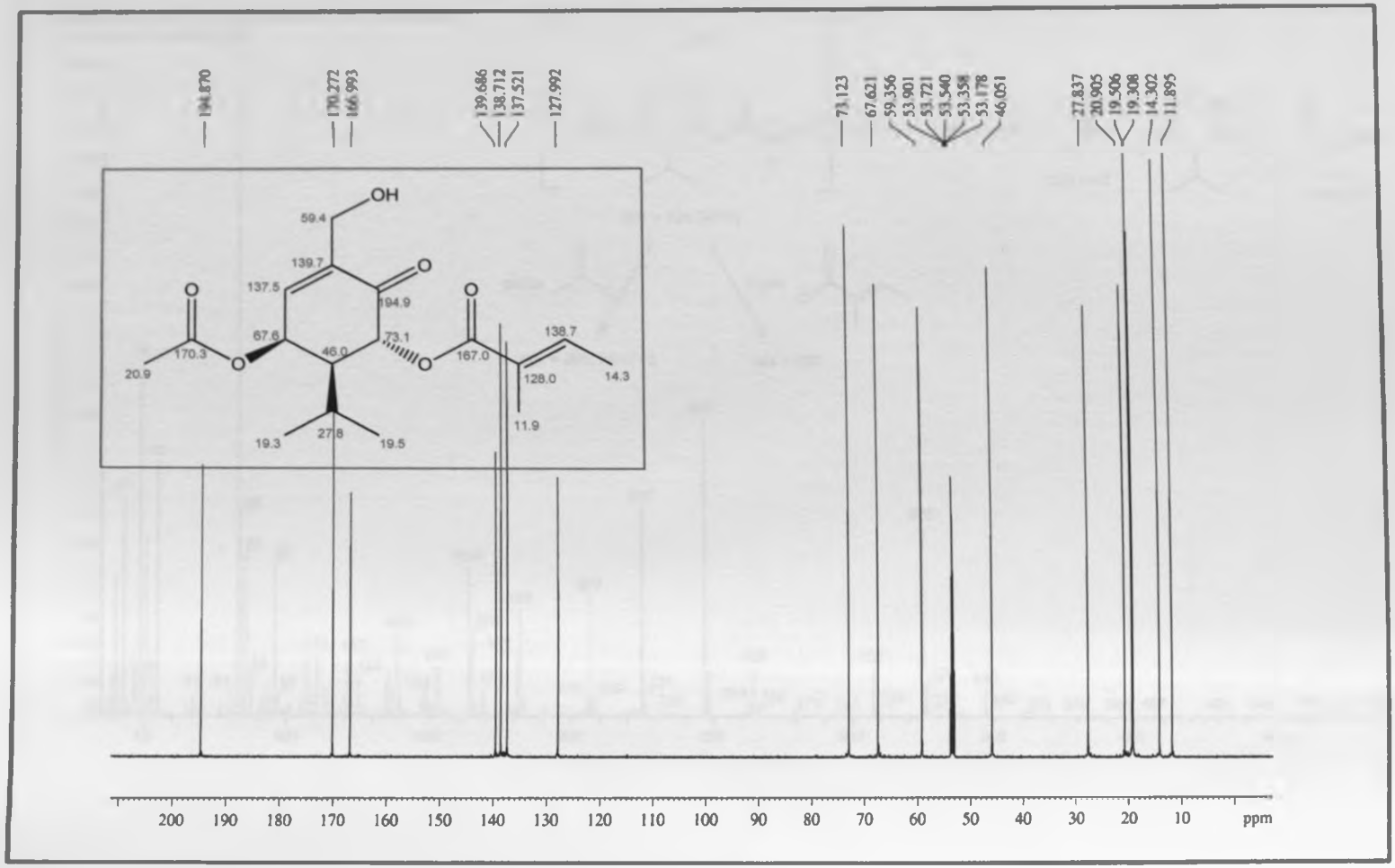
Appendix 17C: EI-MS spectrum of 3,7-dihydroxy-5-tigloyloxycarvotacetone (**88**)



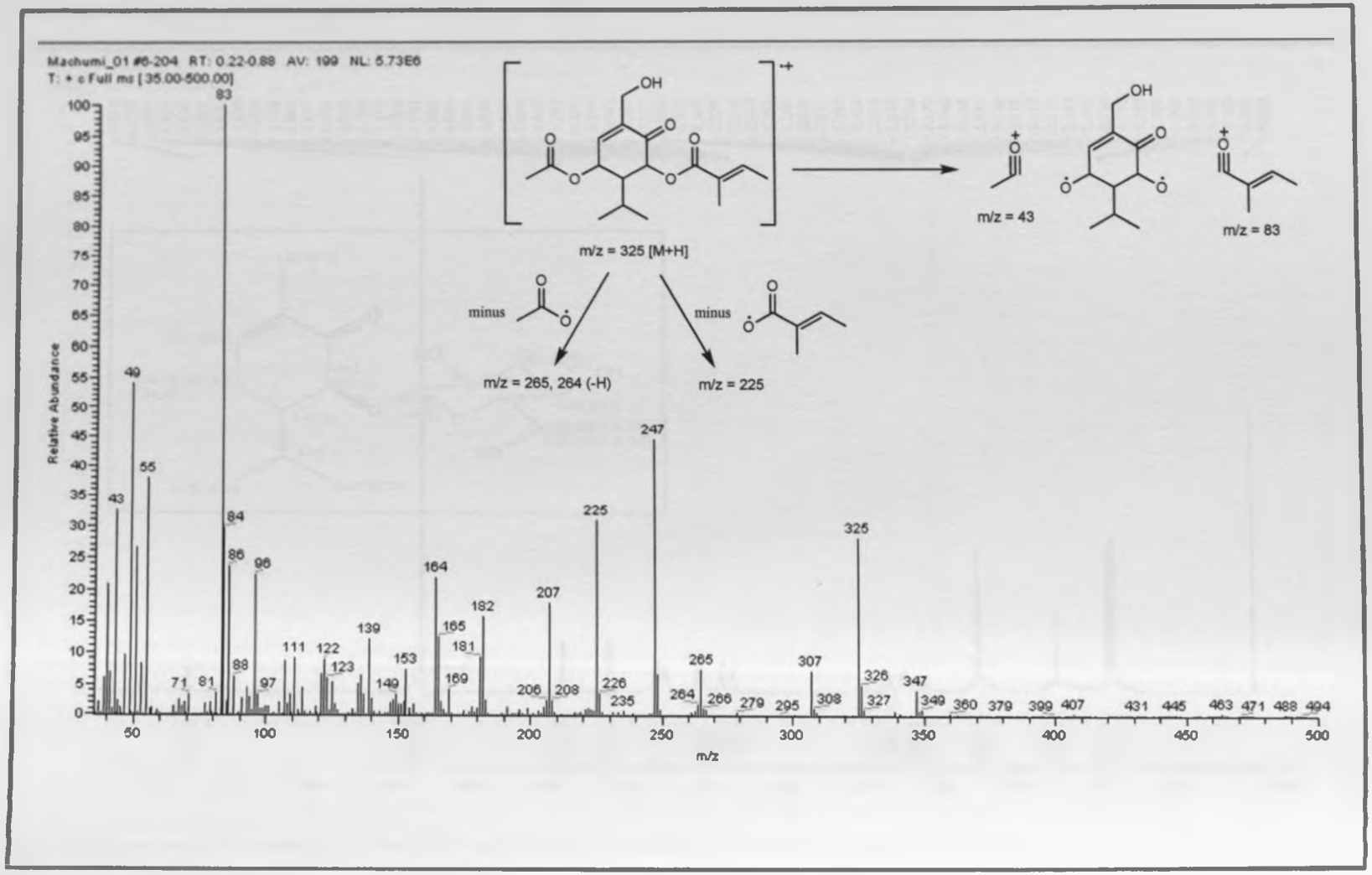
Appendix 18A: ^{1}H-NMR spectrum of 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**), CD_2Cl_2, 600 MHz



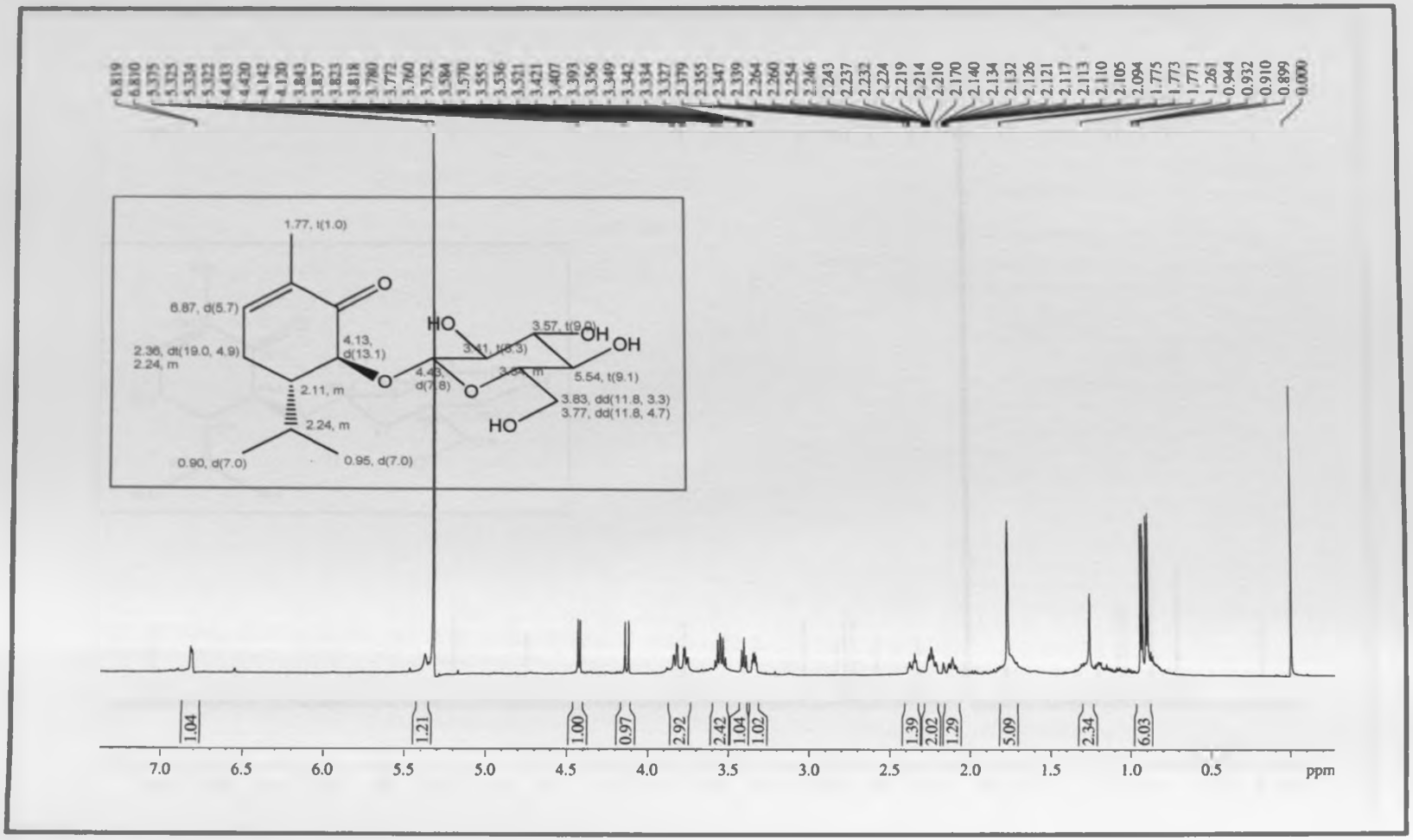
Appendix 18B: ^{13}C-NMR spectrum of 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**), CD_2Cl_2, 150 MHz



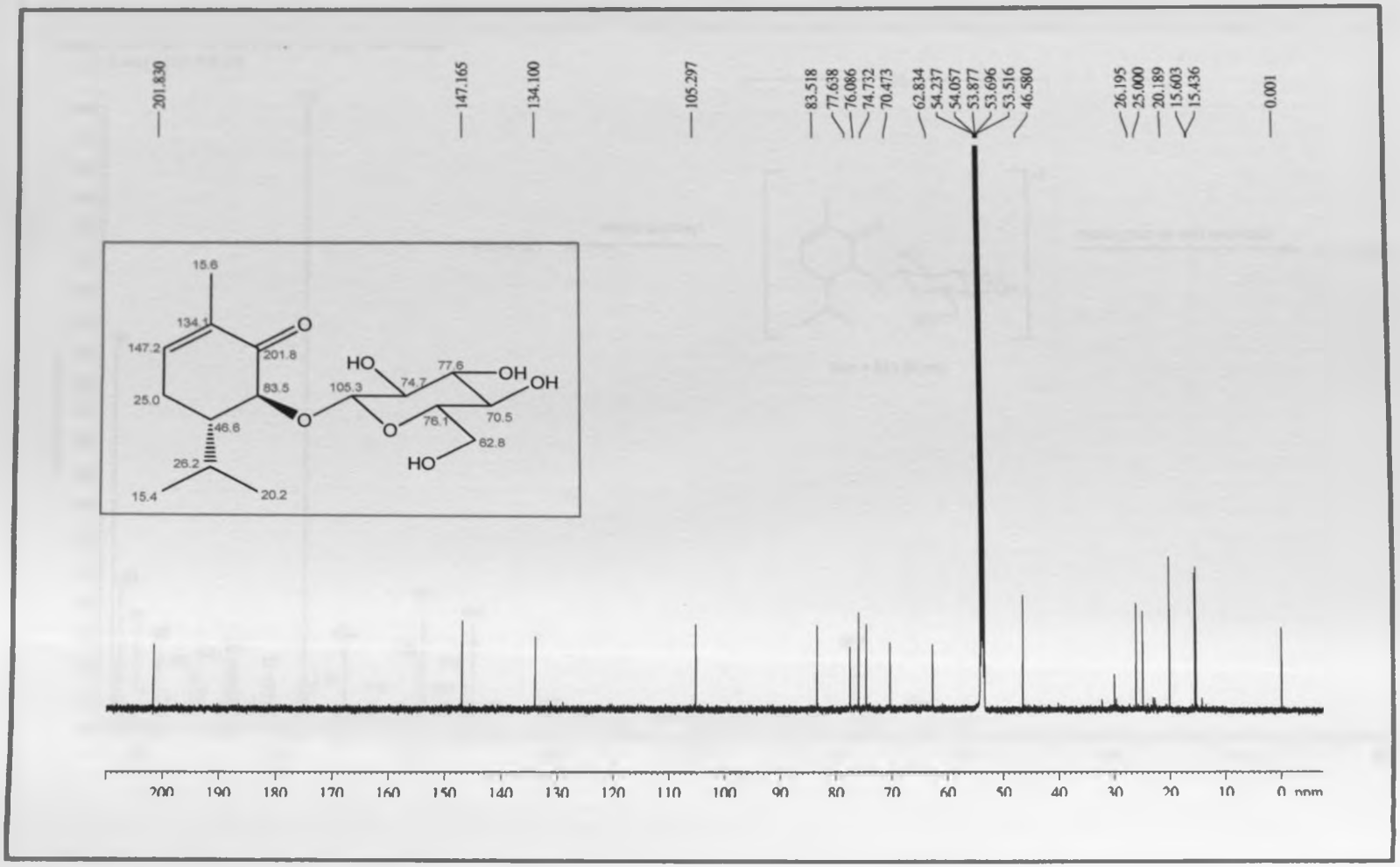
Appendix 18C: EI-MS spectrum of 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (**99**)



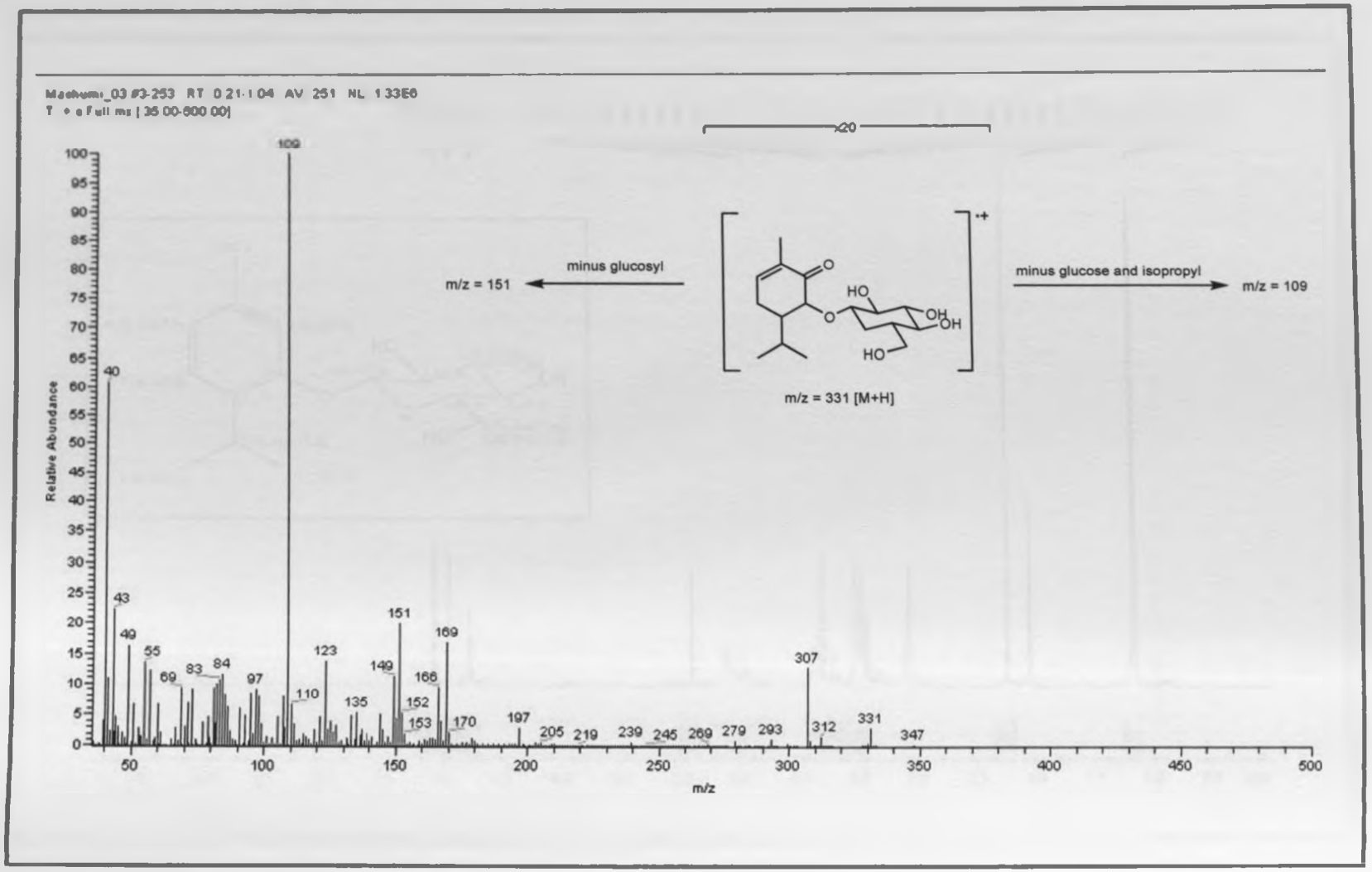
Appendix 19A: ^{1}H-NMR spectrum of 5-*O*-*β*-glucopyranosylcarvotacetone (**232**), CD_2Cl_2, 600 MHz



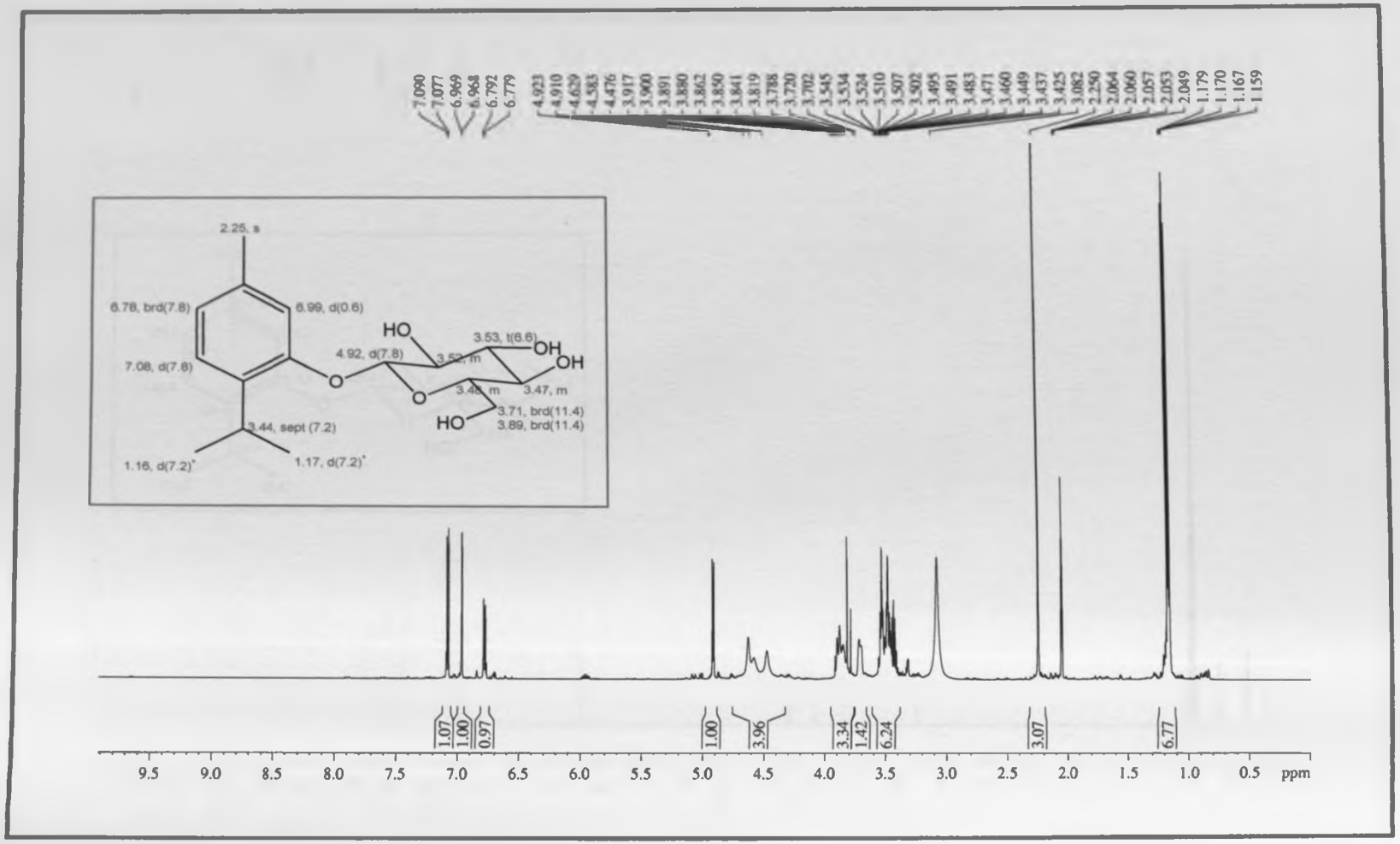
Appendix 19B: ^{13}C-NMR spectrum of 5-*O*-*β*-glucopyranosylcarvotacetone (**232**), CD_2Cl_2, 150 MHz



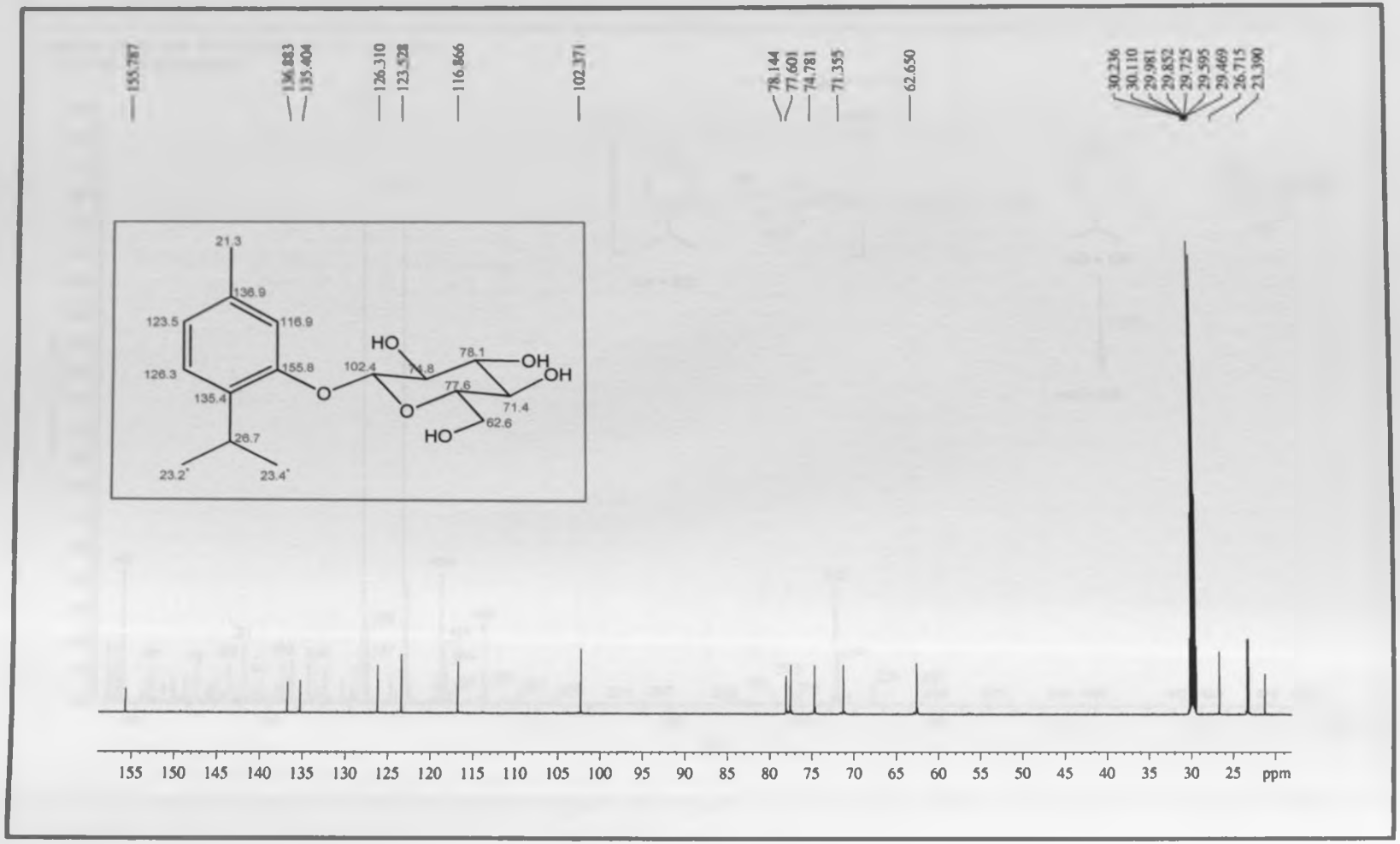
Appendix 19C: EI-MS spectrum of 5-*O*-*β*-glucopyranosylcarvotacetone (**232**)



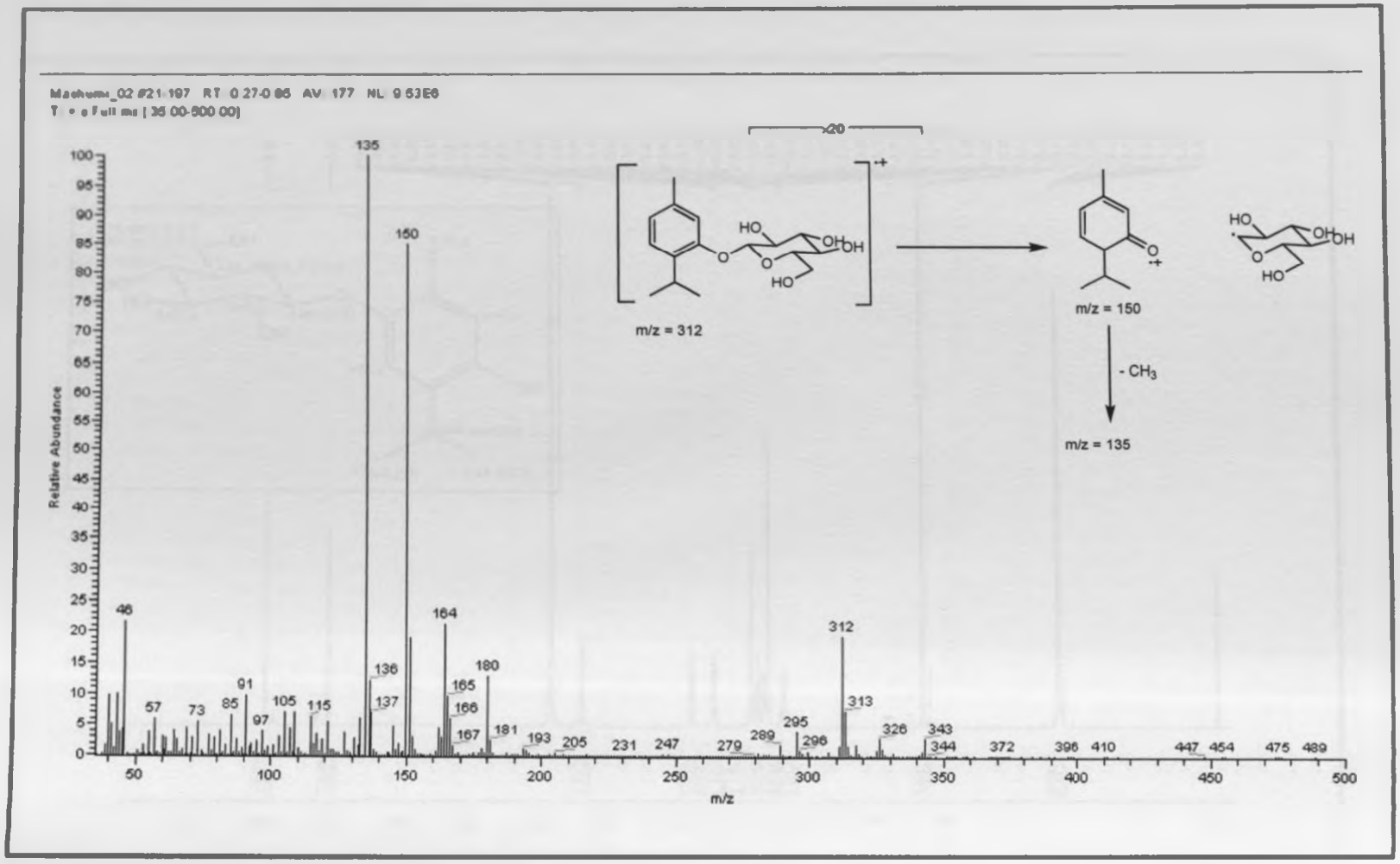
Appendix 20A: ^{1}H-NMR spectrum of thymol 3-*O*-*β*-glucopyranoside (**233**), Acetone d_6, 600 MHz



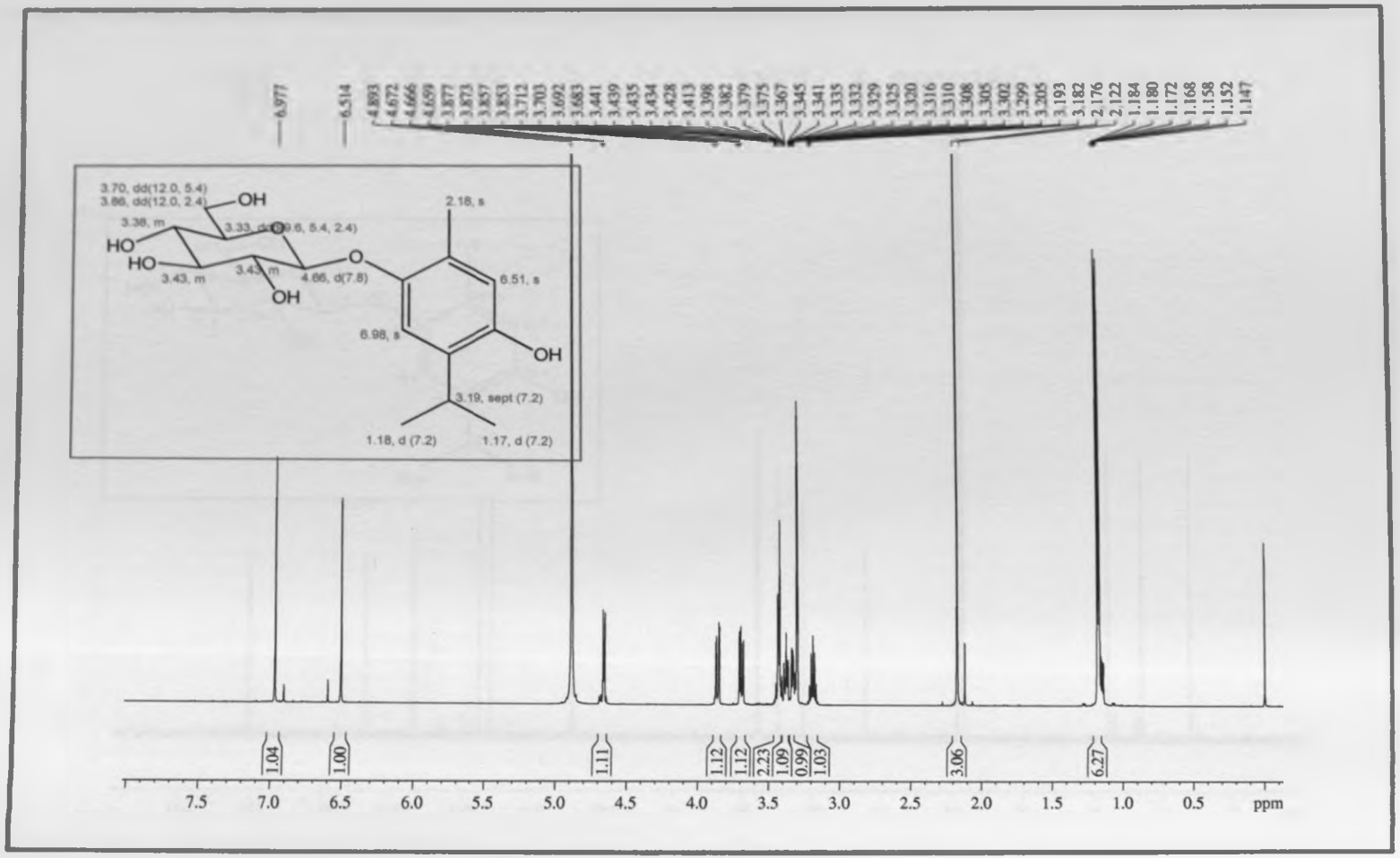
Appendix 20B: ^{13}C-NMR spectrum of thymol 3-*O*-*β*-glucopyranoside (**233**), Acetone d_6, 150 MHz



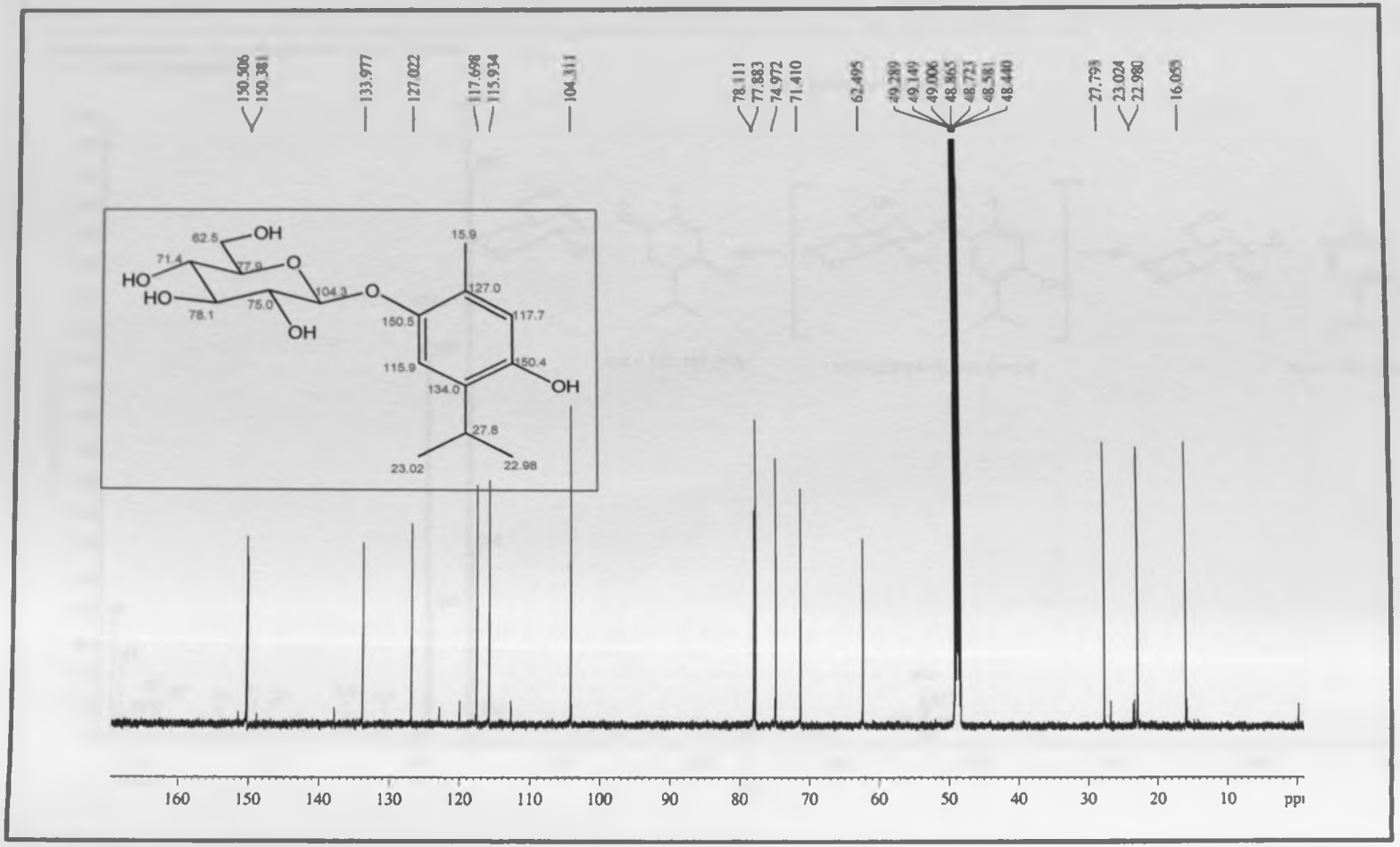
Appendix 20C: EI-MS spectrum of thymol 3-*O*-*β*-glucopyranoside (**233**)



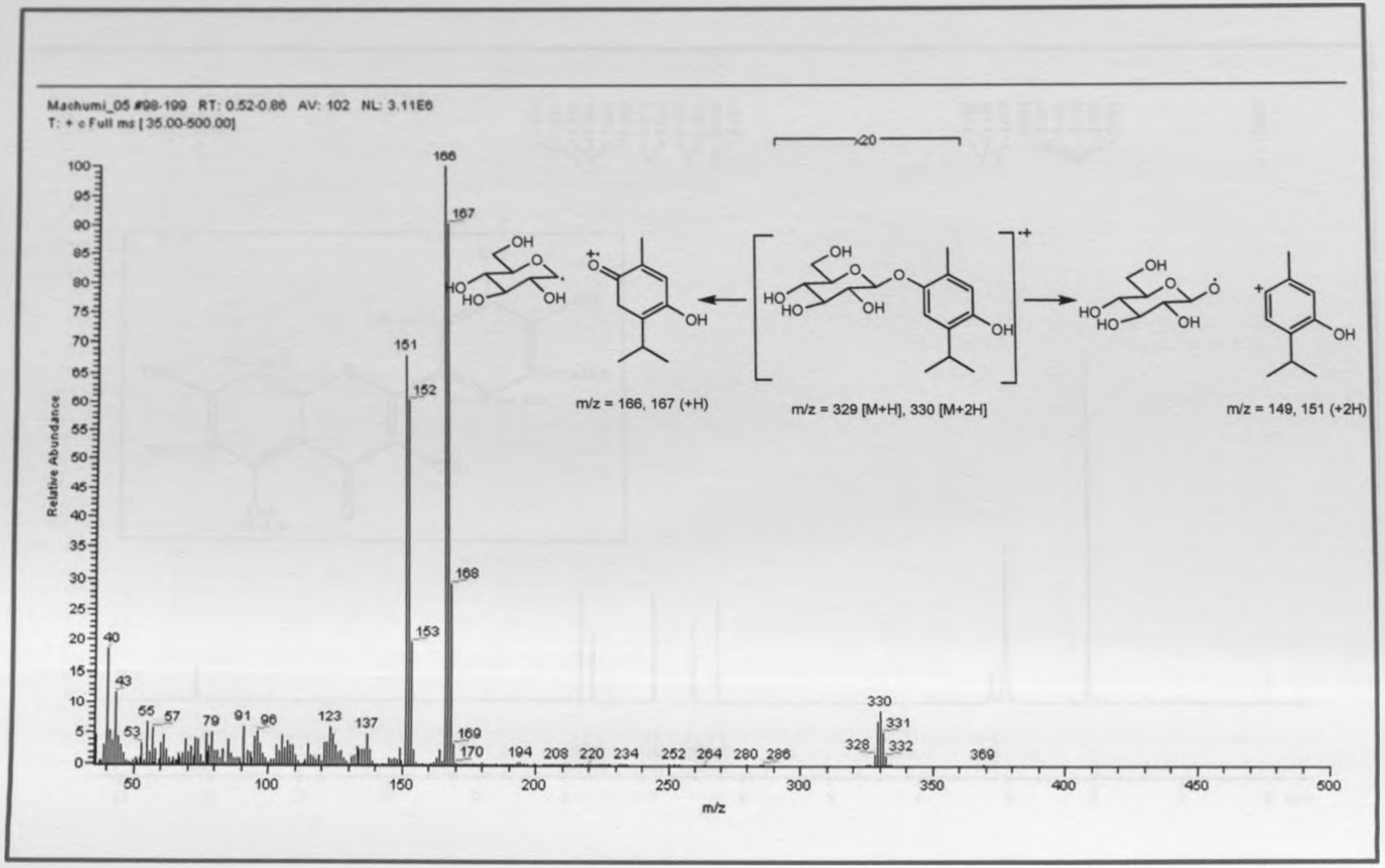
Appendix 21A: ^{1}H-NMR spectrum of zetaroside B (**93**), MeOD, 600 MHz



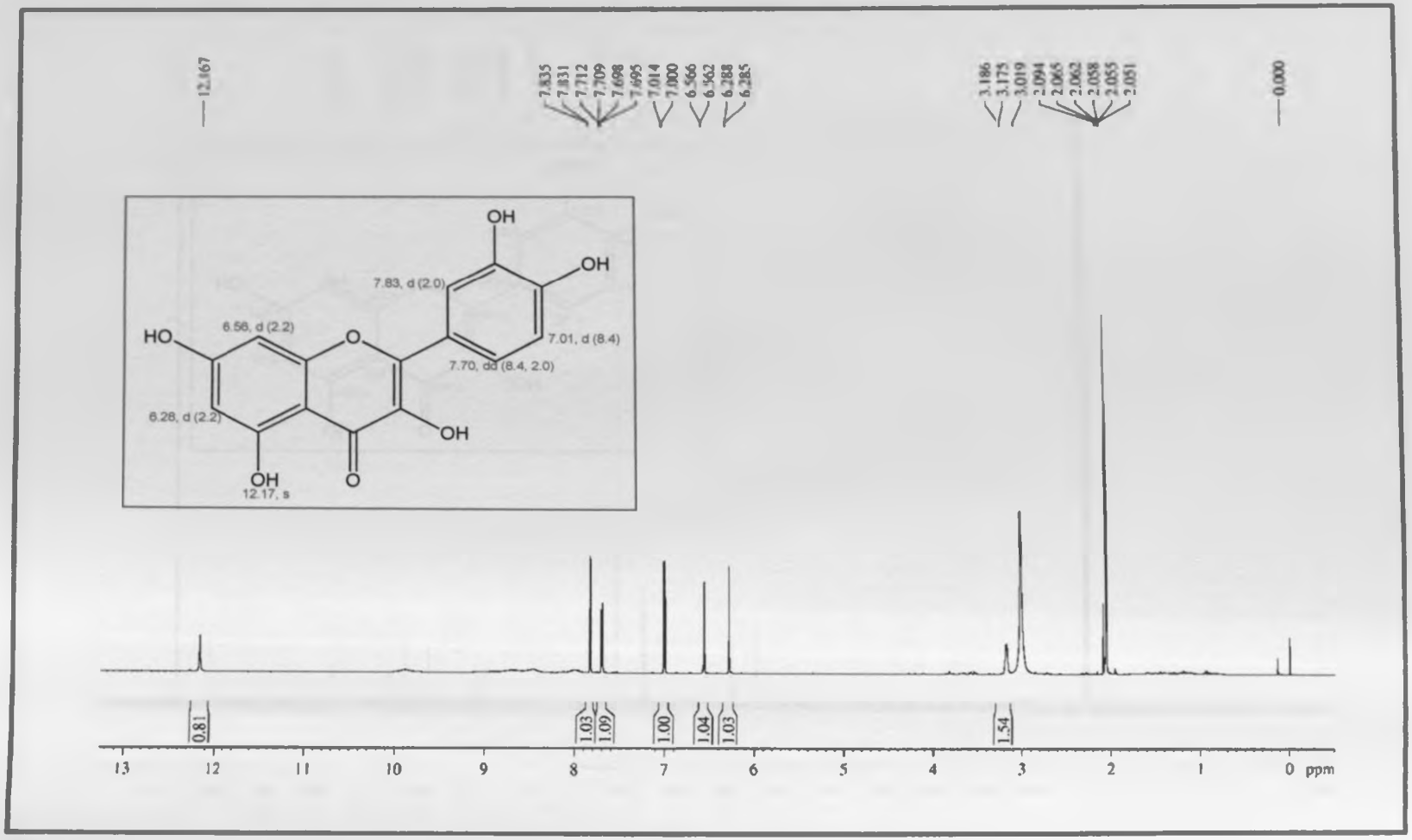
Appendix 21B: ^{13}C-NMR spectrum of zetaroside B (**93**), MeOD, 150 MHz



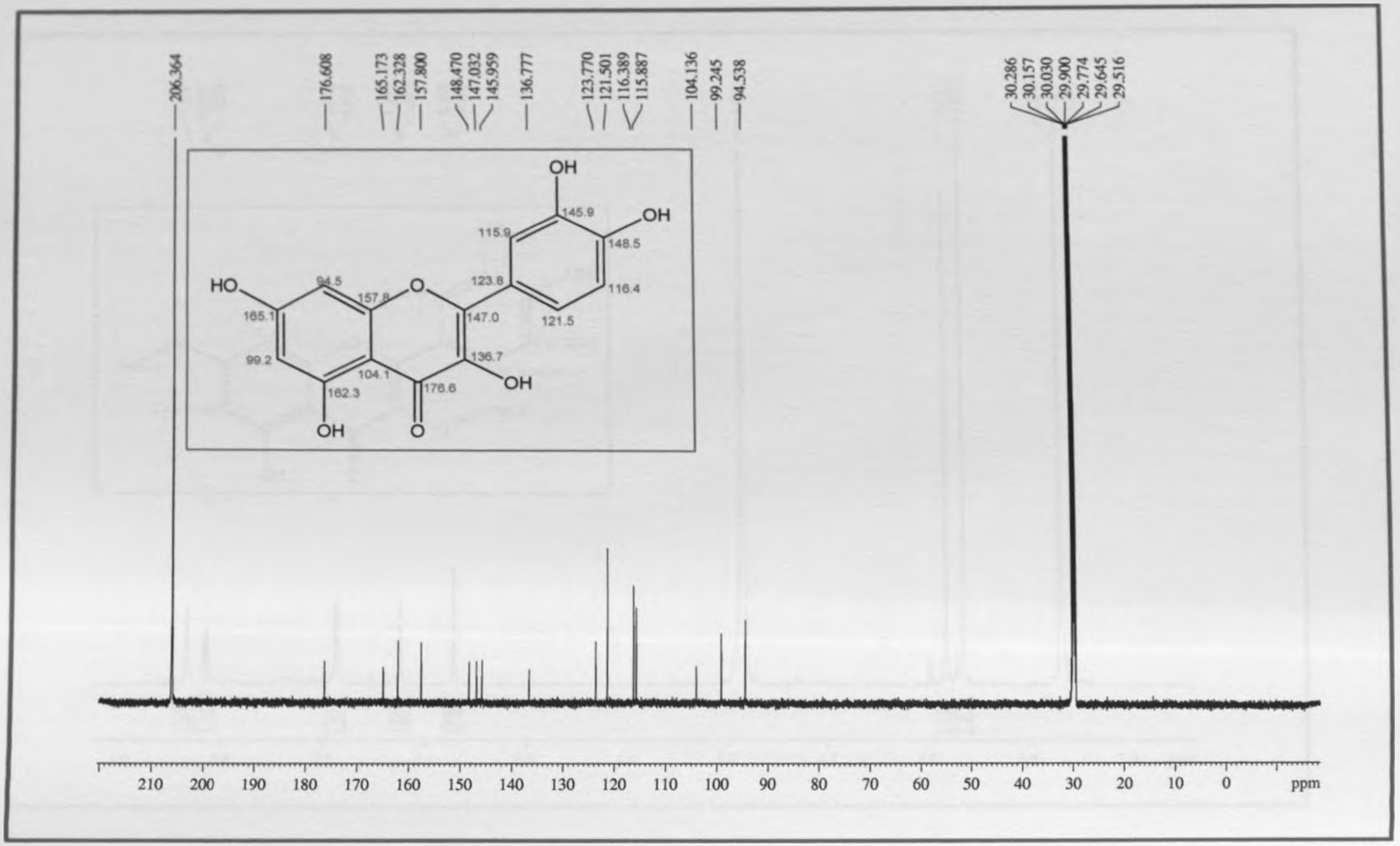
Appendix 21C: EI-MS spectrum of zetaroside B (**93**)



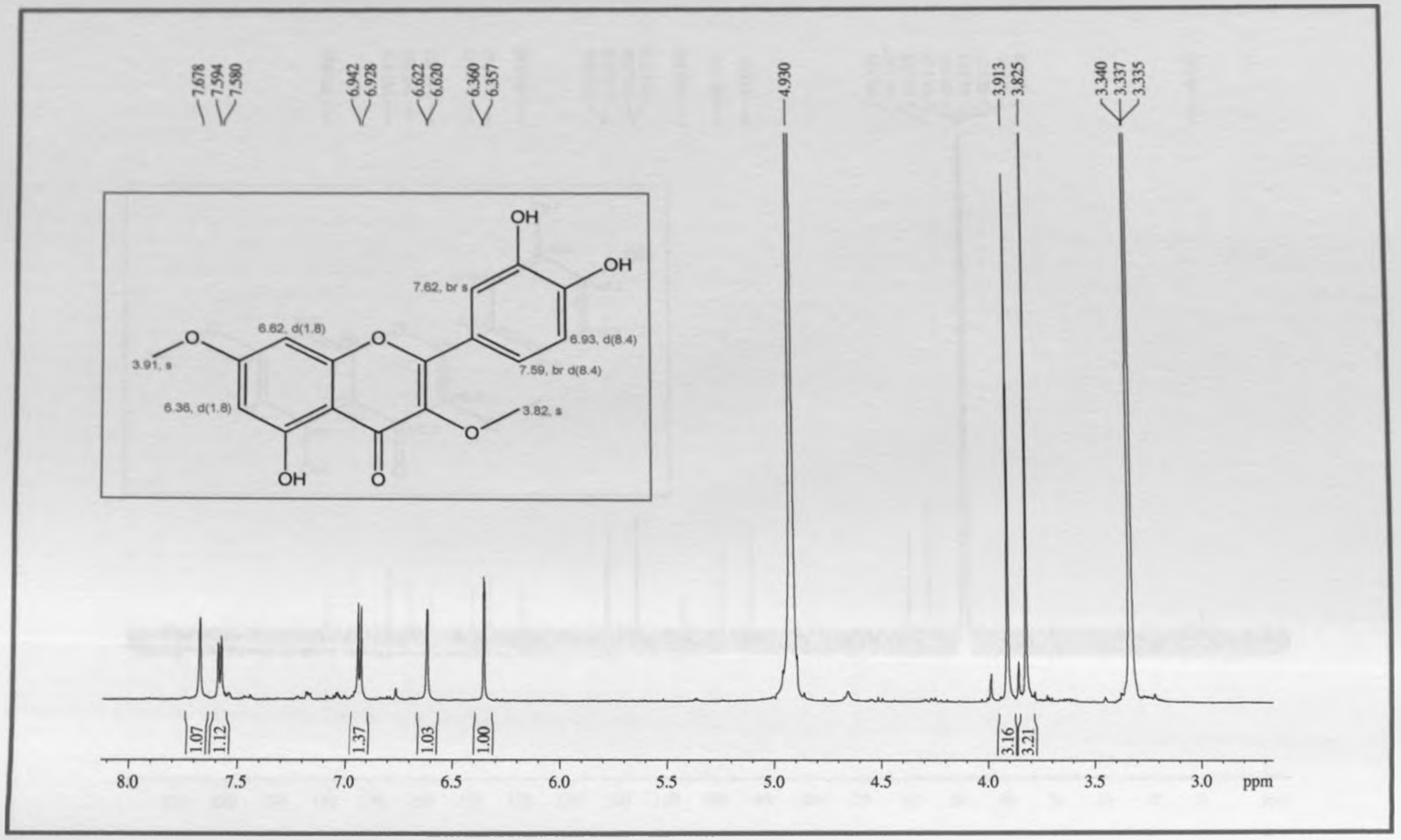
Appendix 22A: ^{1}H-NMR spectrum of quercetin (**235**), Acetone d_6, 600 MHz



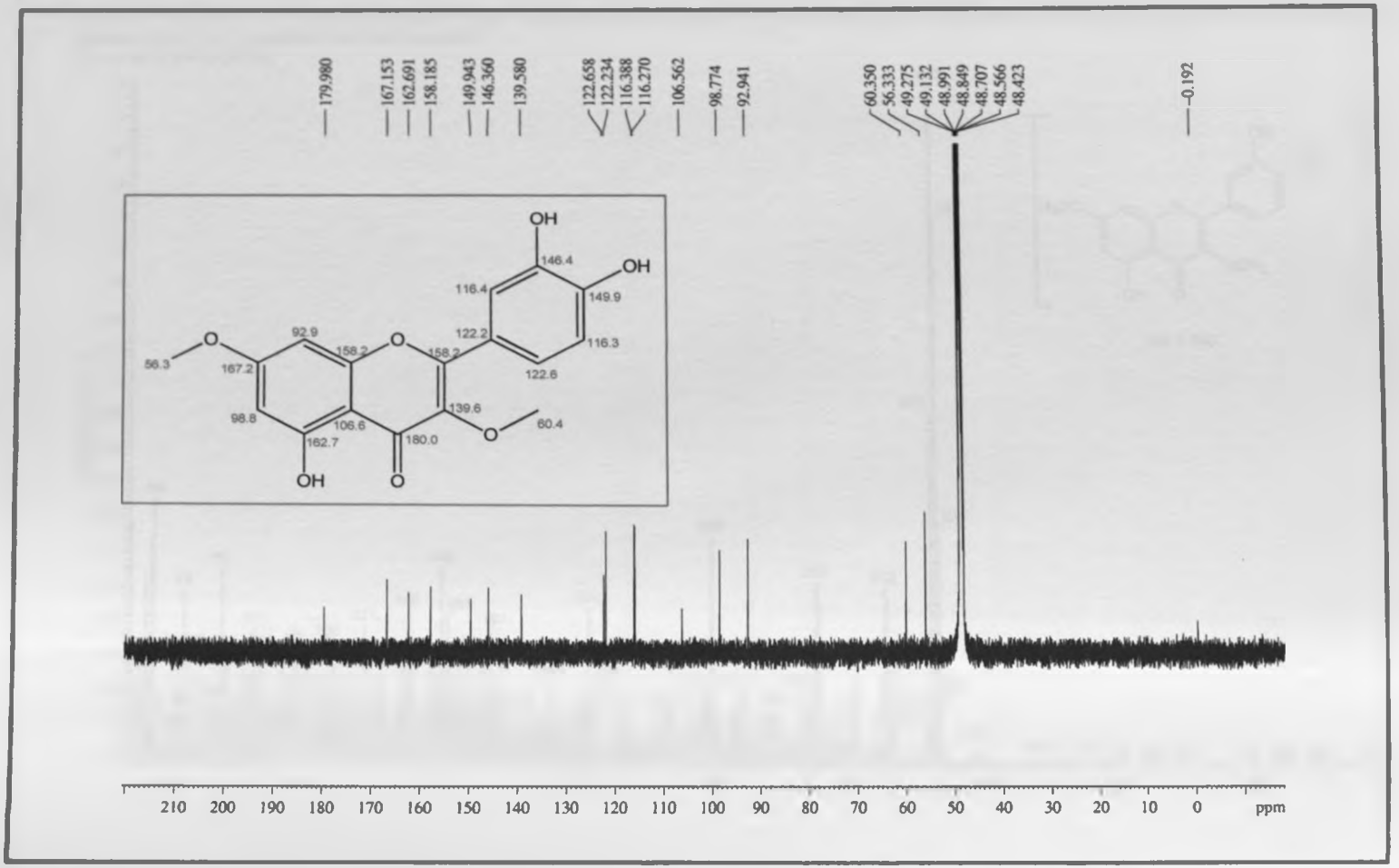
Appendix 22B: ^{13}C-NMR spectrum of quercetin (**235**), Acetone d_6, 150 MHz



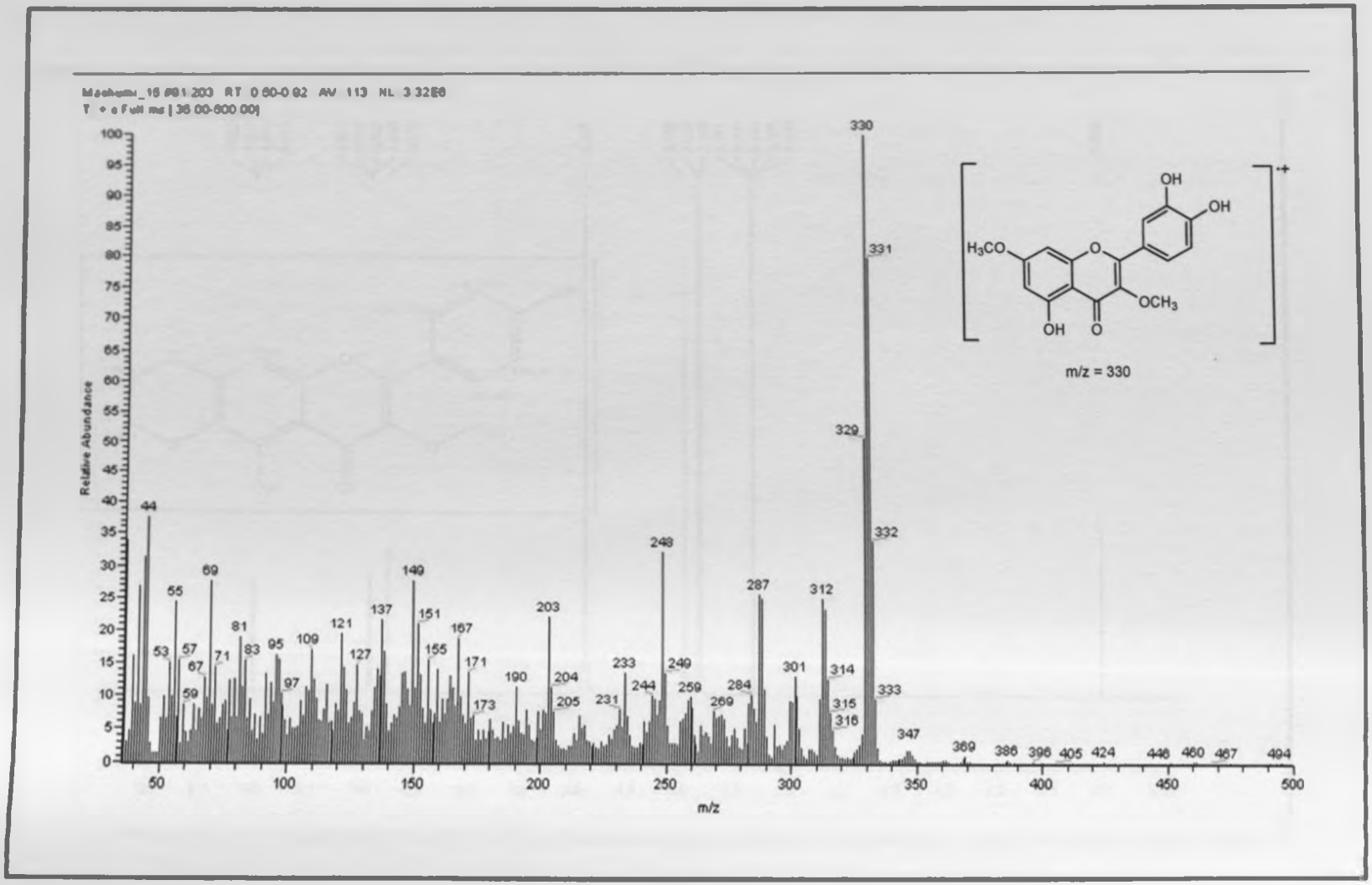
Appendix 23A: ^{1}H-NMR spectrum of quercetin-3,7-dimethylether (**236**), MeOD, 600 MHz



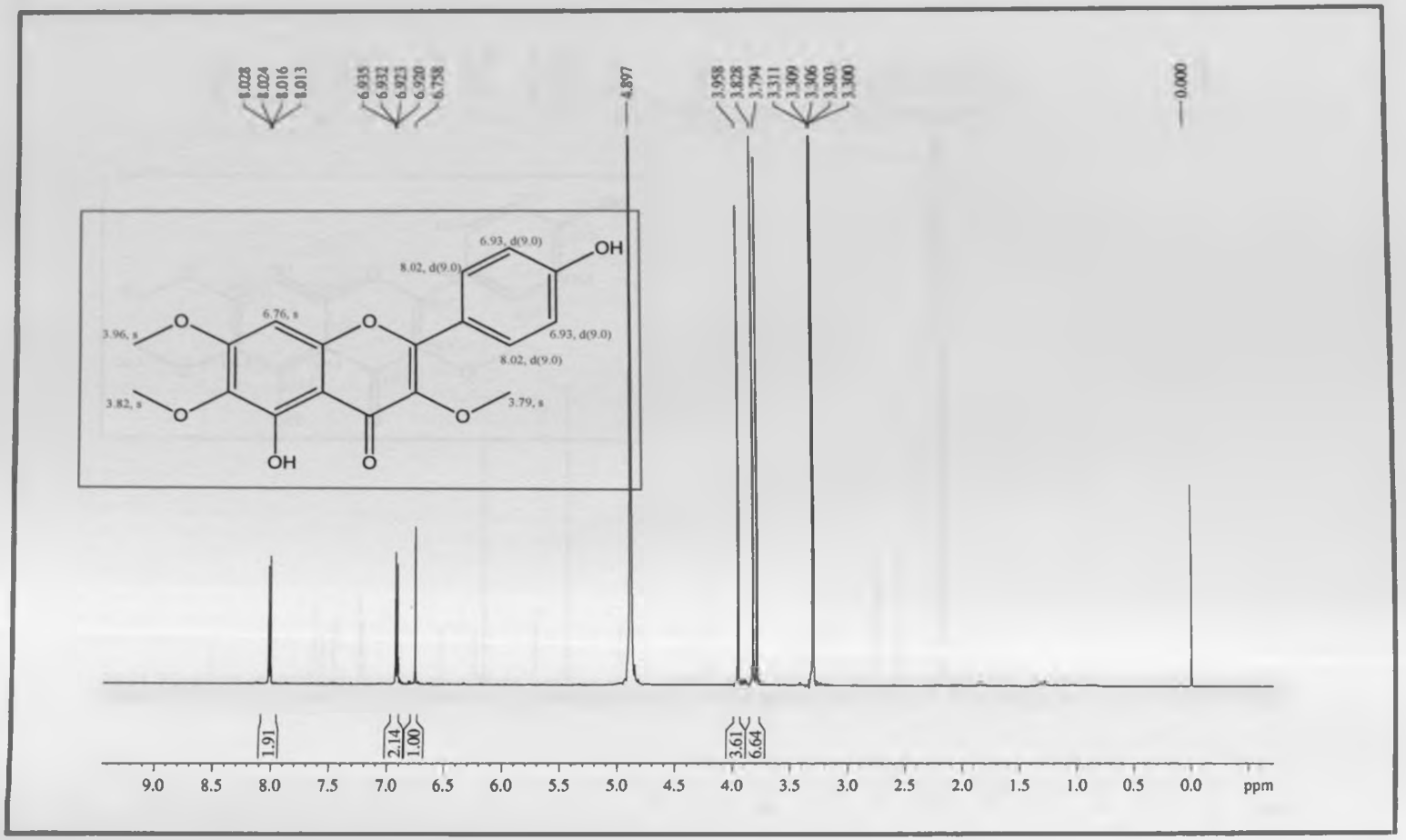
Appendix 23B: ^{13}C-NMR spectrum of quercetin-3,7-dimethylether (**236**), MeOD, 150 MHz



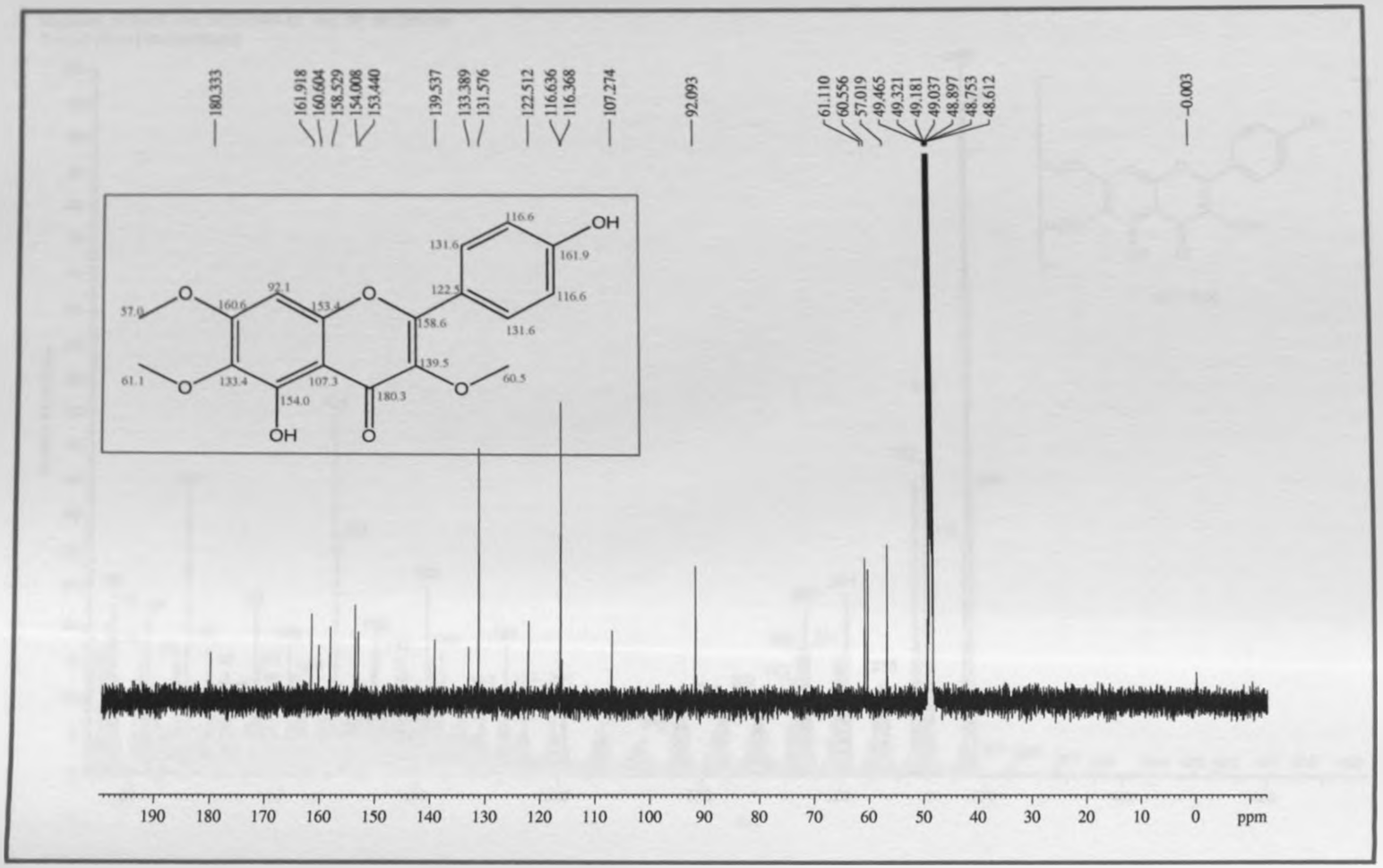
Appendix 23C: EI-MS spectrum of quercetin-3,7-dimethylether (**236**)



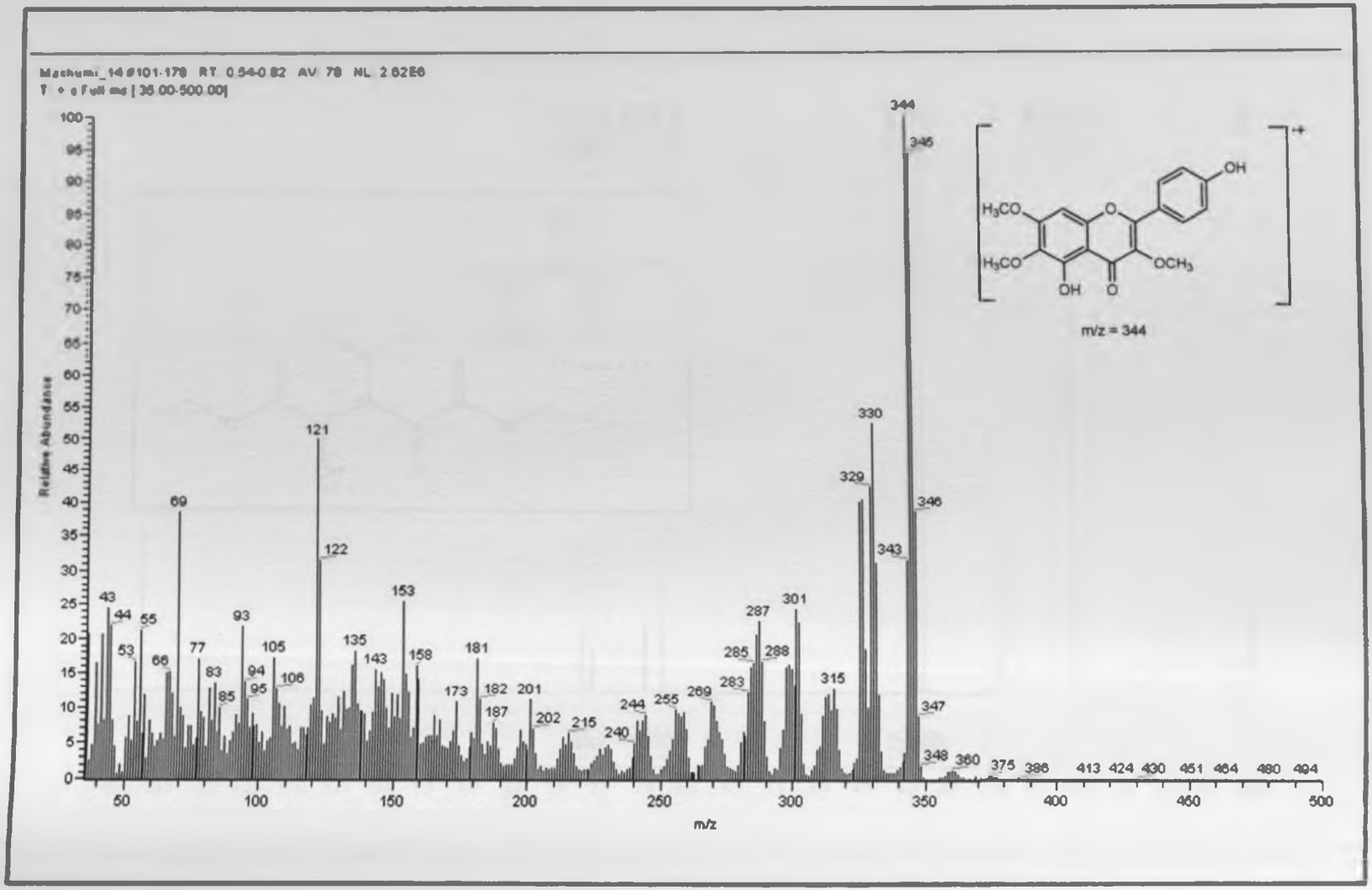
Appendix 24A: ^{1}H-NMR spectrum of penduletin (**237**). MeOD. 600 MHz



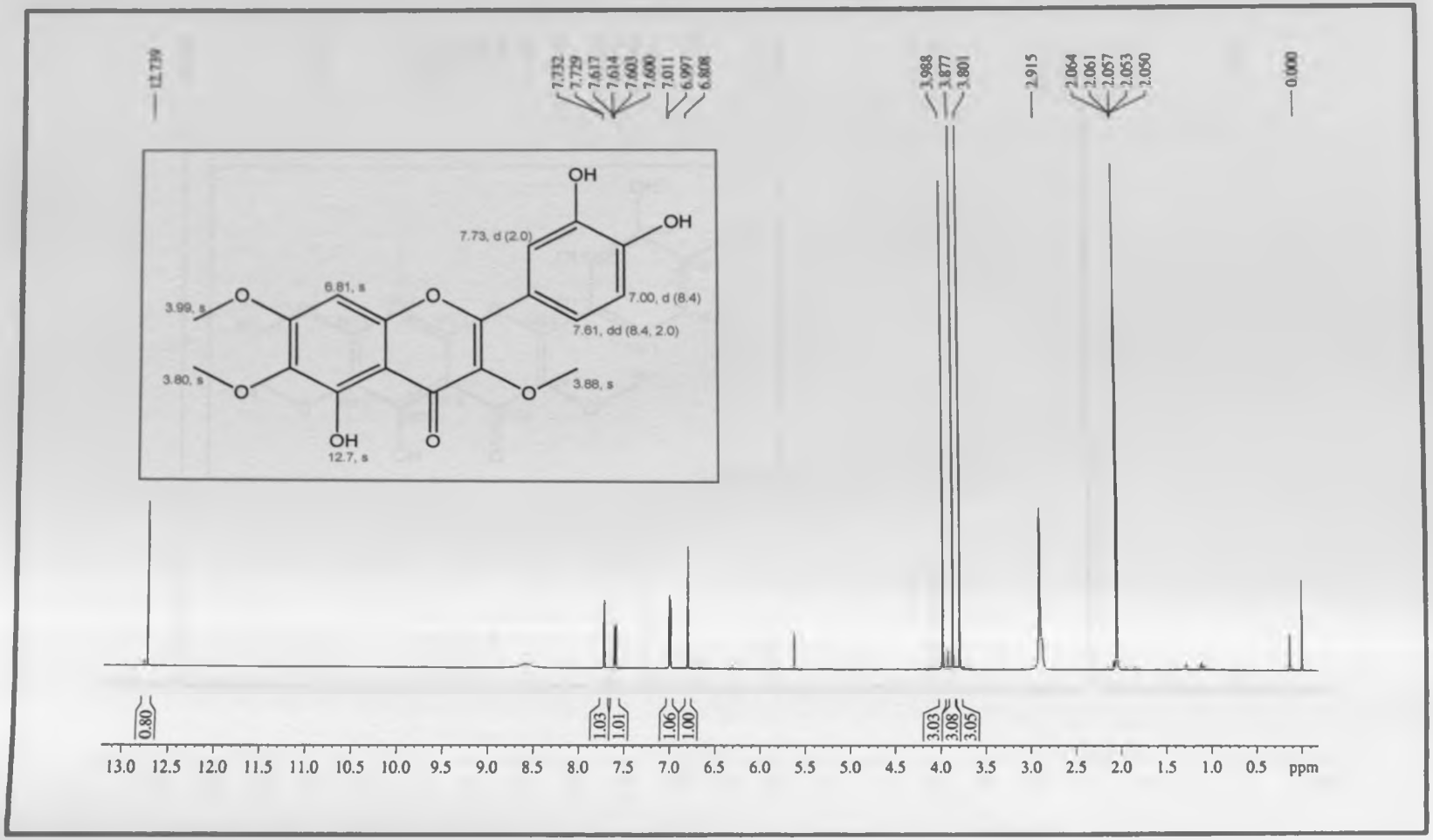
Appendix 24B: ^{13}C-NMR spectrum of penduletin (**237**), MeOD, 150 MHz



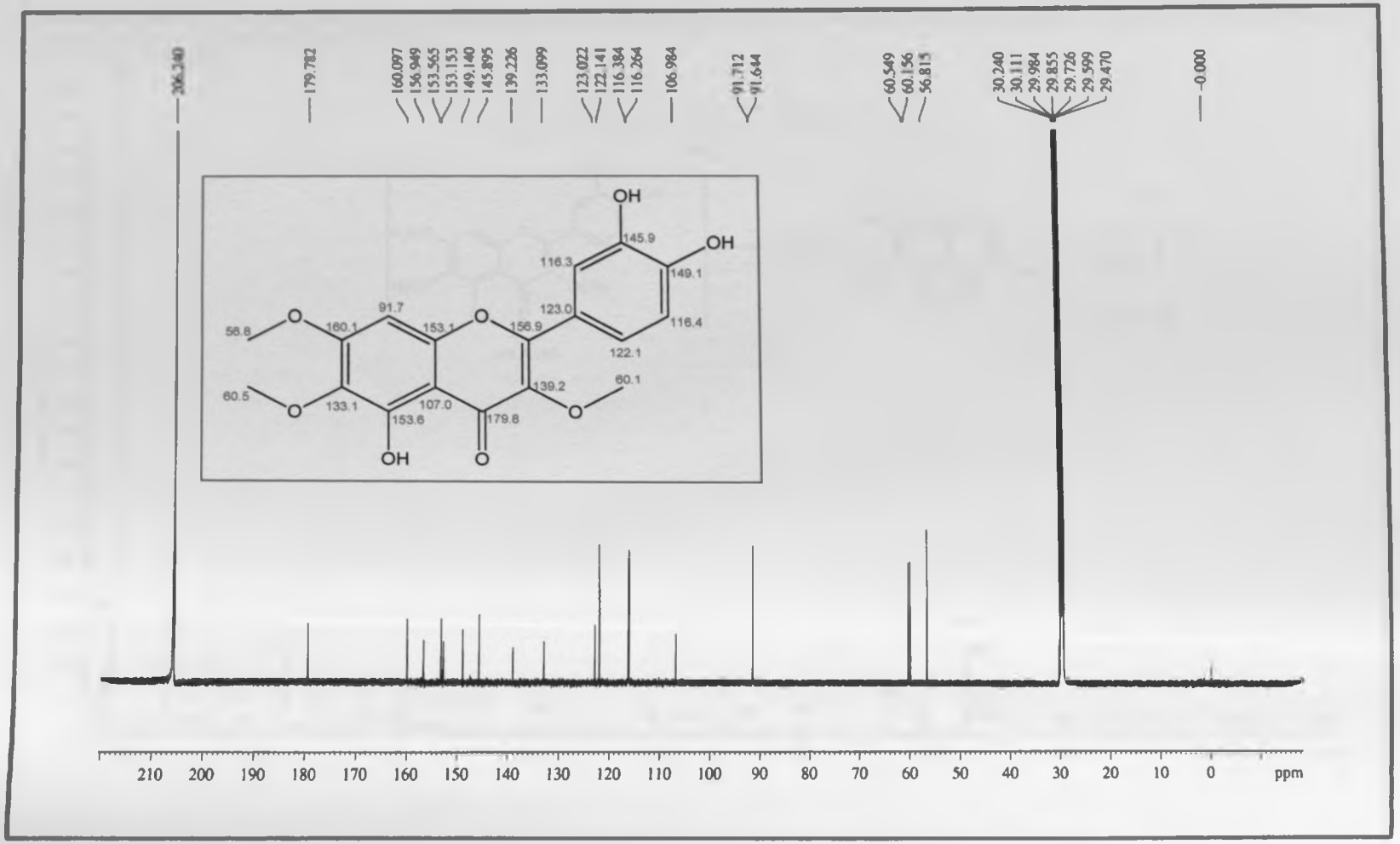
Appendix 24C: EI-MS spectrum of penduletin (**237**)



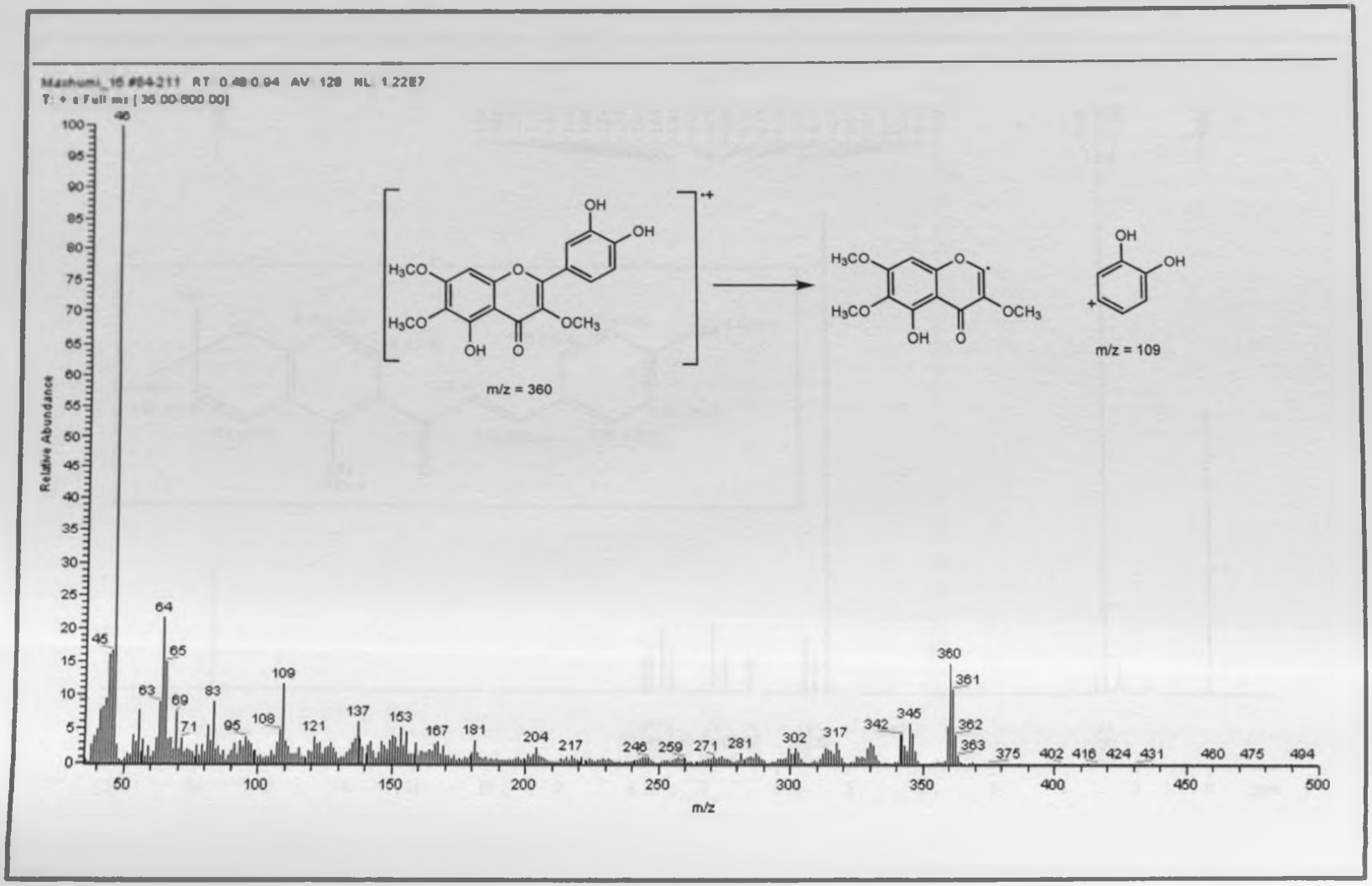
Appendix 25A: ^{1}H-NMR spectrum of crysoplenol D (**238**), Acetone d_6, 600 MHz



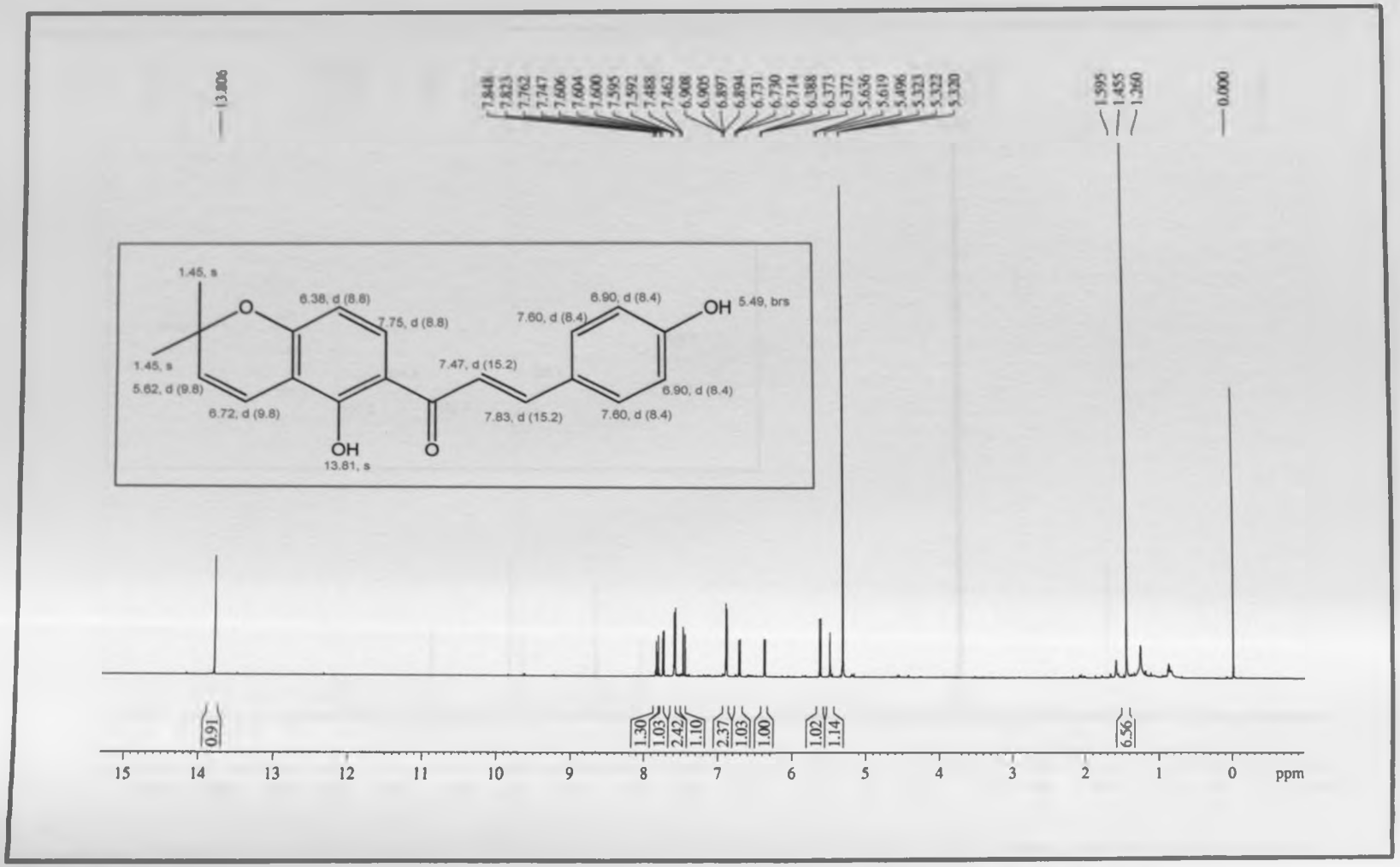
Appendix 25B: ^{13}C-NMR spectrum of crysoplenol D (**238**), Acetone d_6, 150 MHz



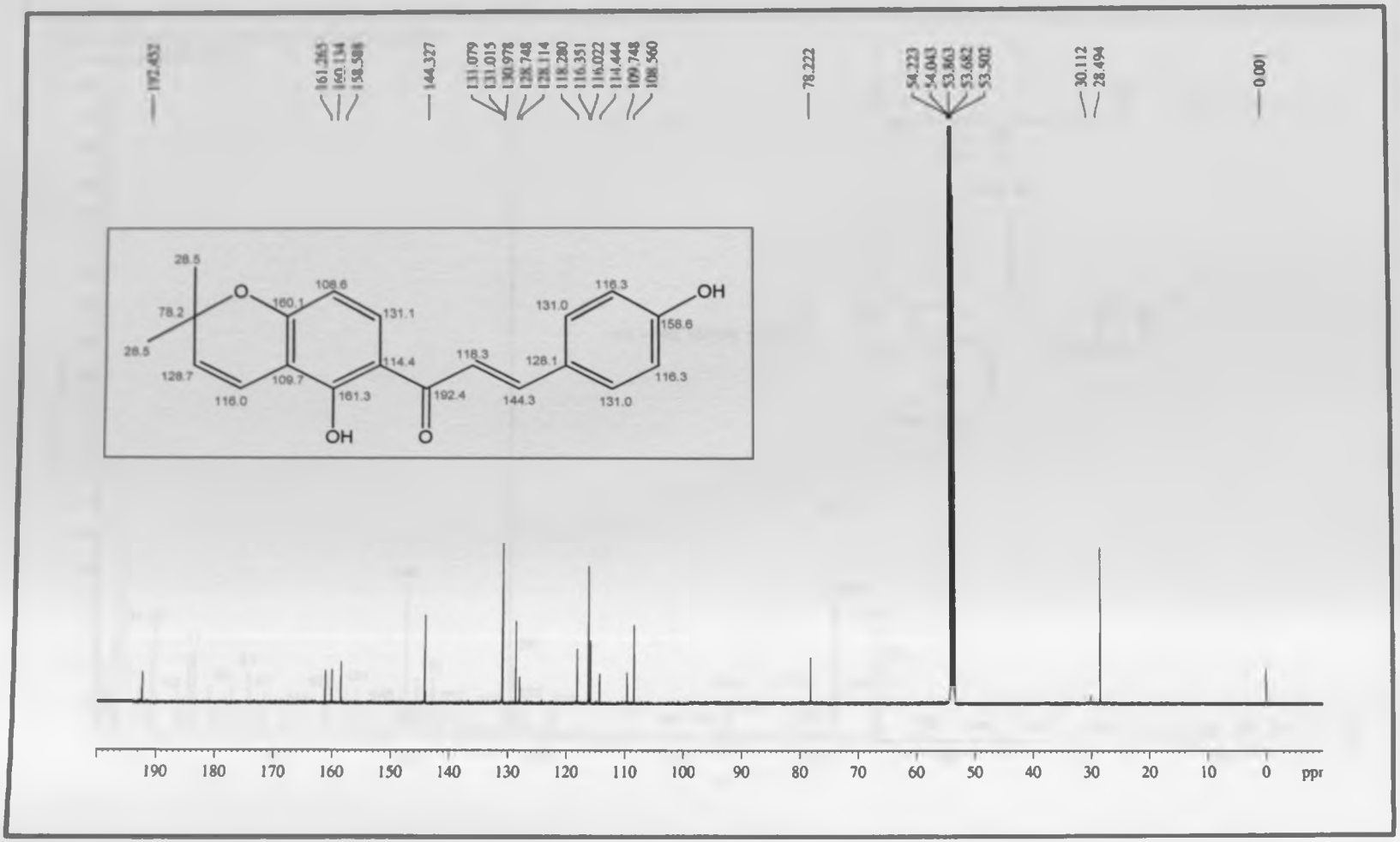
Appendix 25C: EI-MS spectrum of crysoplenol D (**238**)



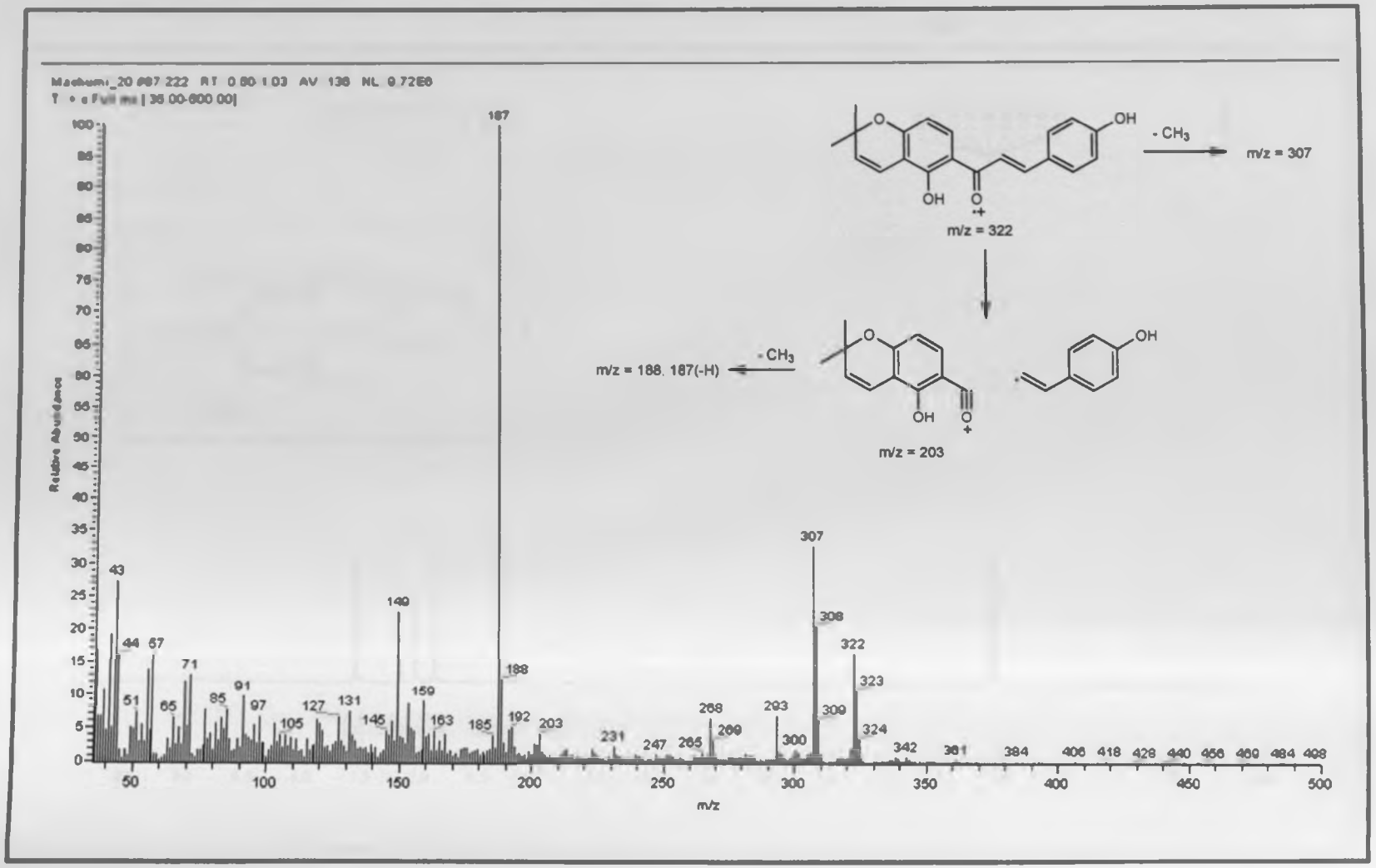
Appendix 26A: ^{1}H-NMR spectrum of 4-hydroxylonchocarpin (**239**), CD_2Cl_2, 600 MHz



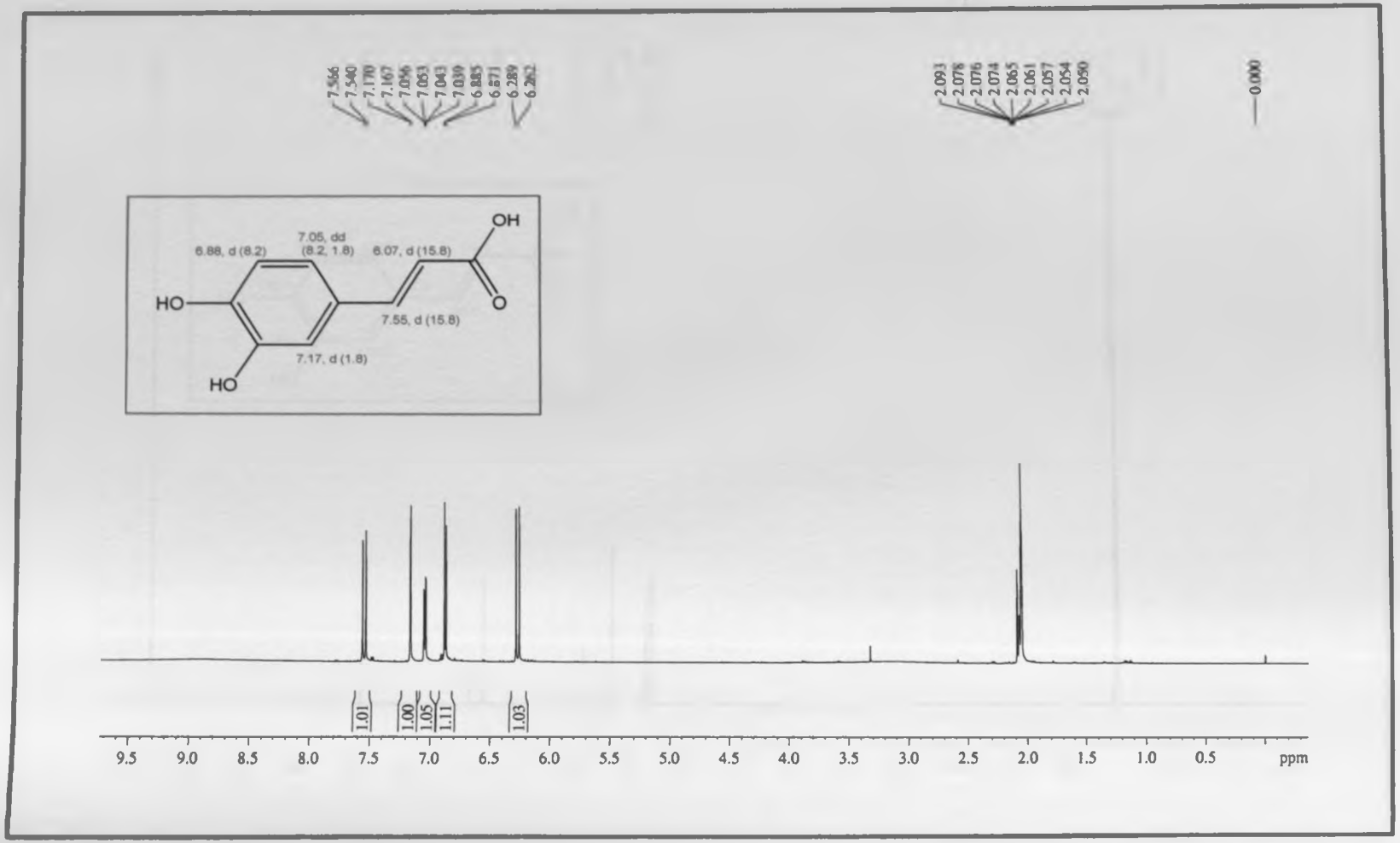
Appendix 26B: ^{13}C-NMR spectrum of 4-hydroxylonchocarpin (**239**), CD_2Cl_2, 150 MHz



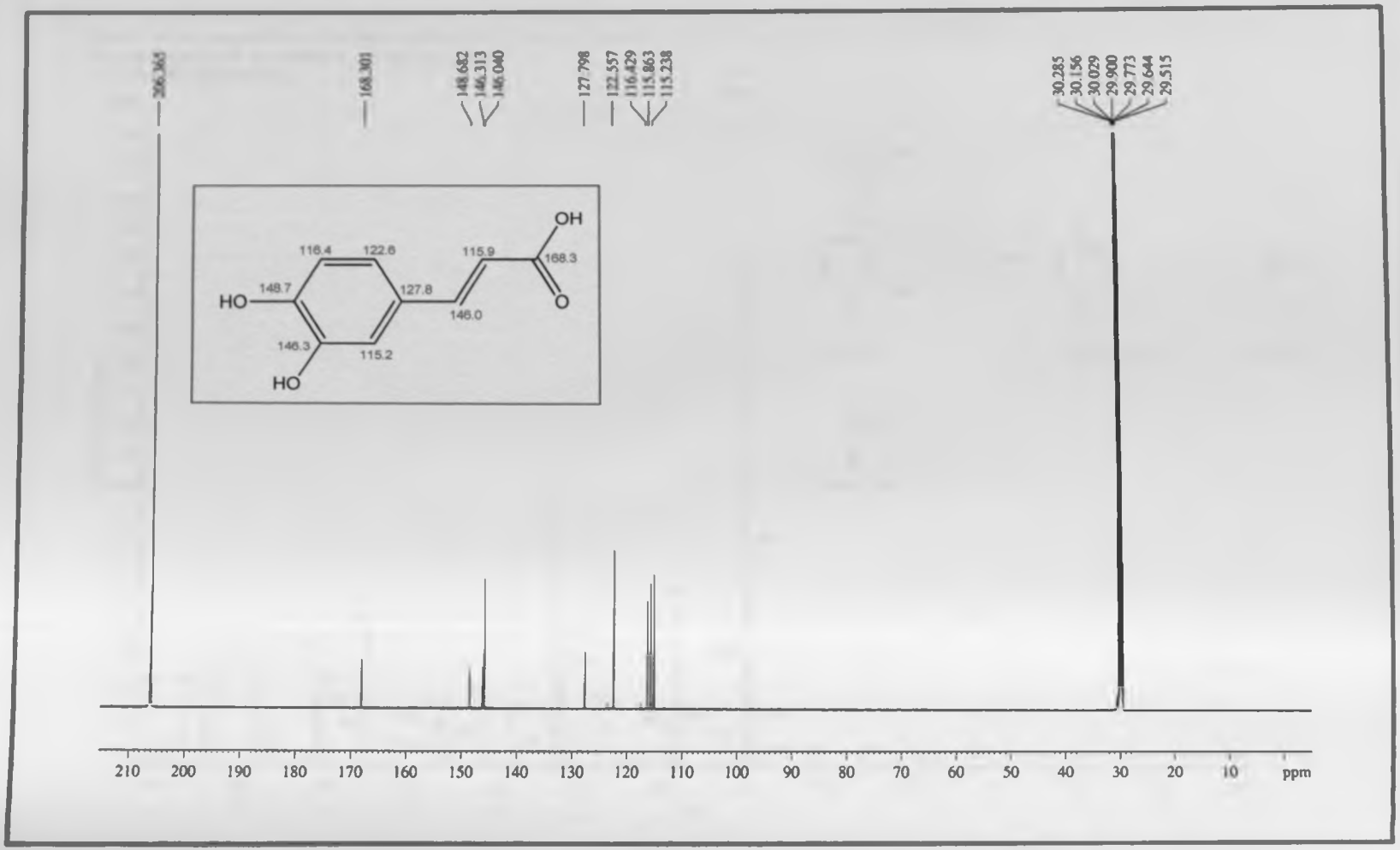
Appendix 26C: EI-MS spectrum of 4-hydroxylonchocarpin (**239**)



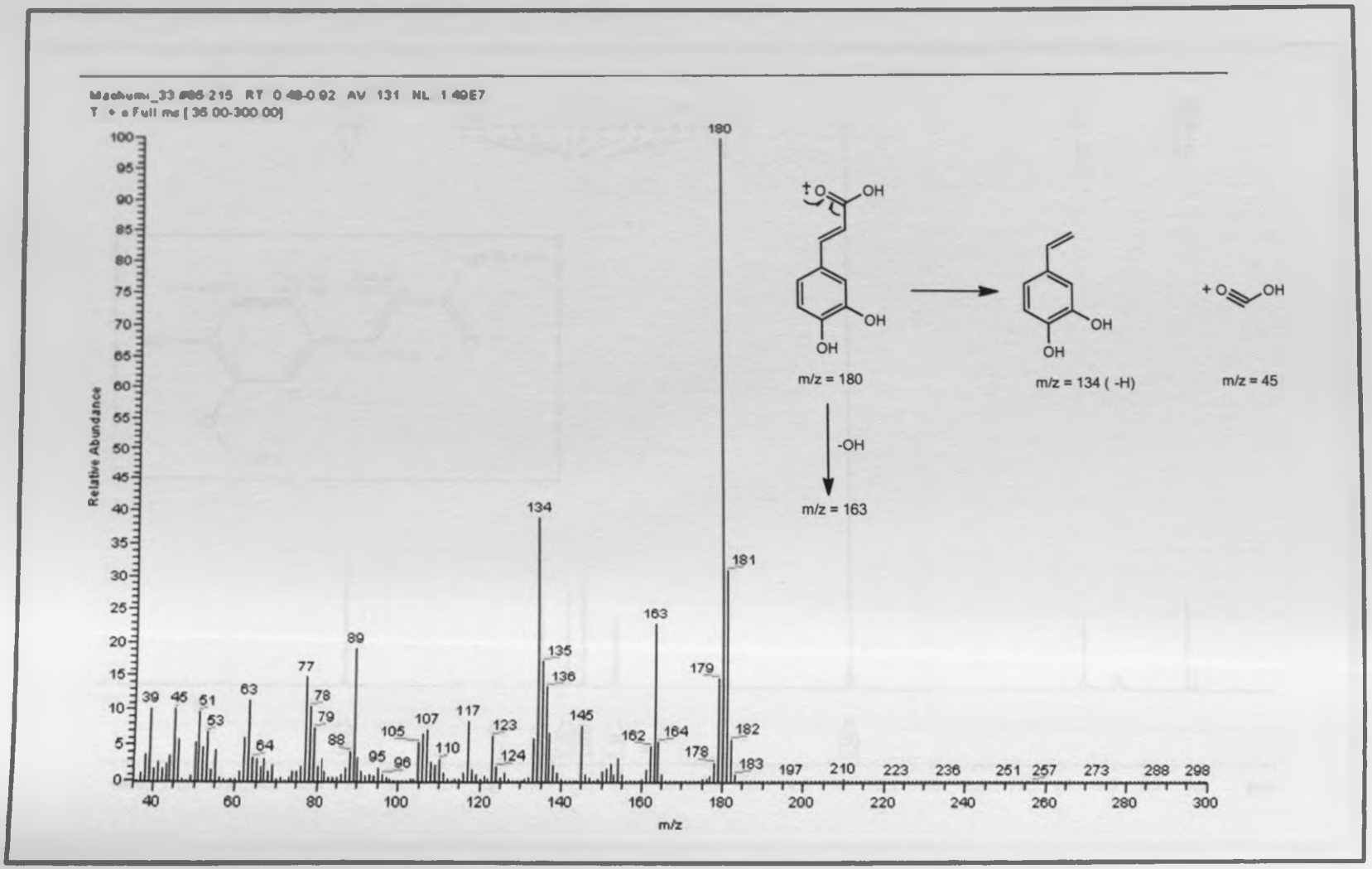
Appendix 27A: ^{1}H-NMR spectrum of caffeic acid (**240**), Acetone d_6, 600 MHz



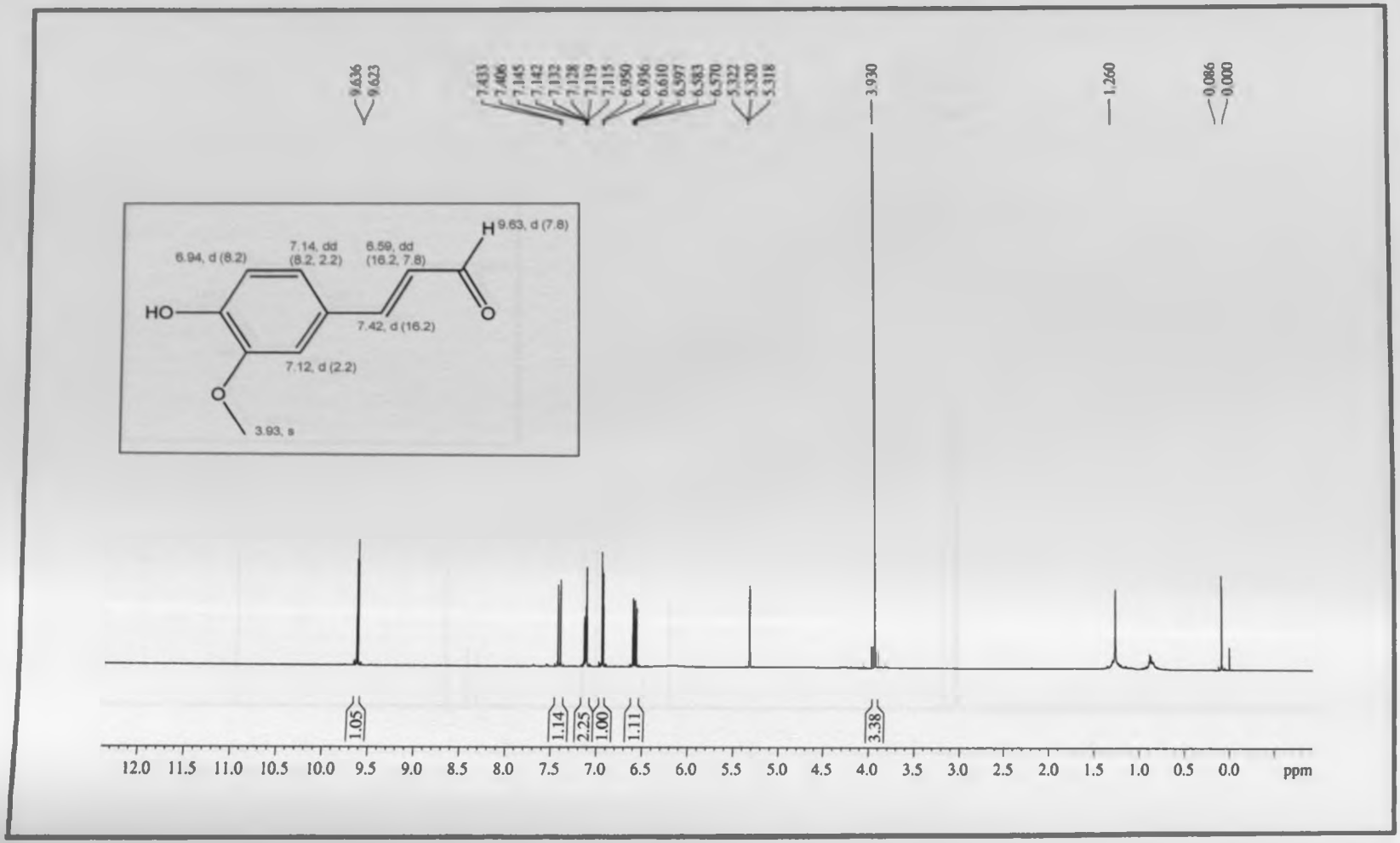
Appendix 27B: ^{13}C-NMR spectrum of caffeic acid (**240**), Acetone d_6, 150 MHz



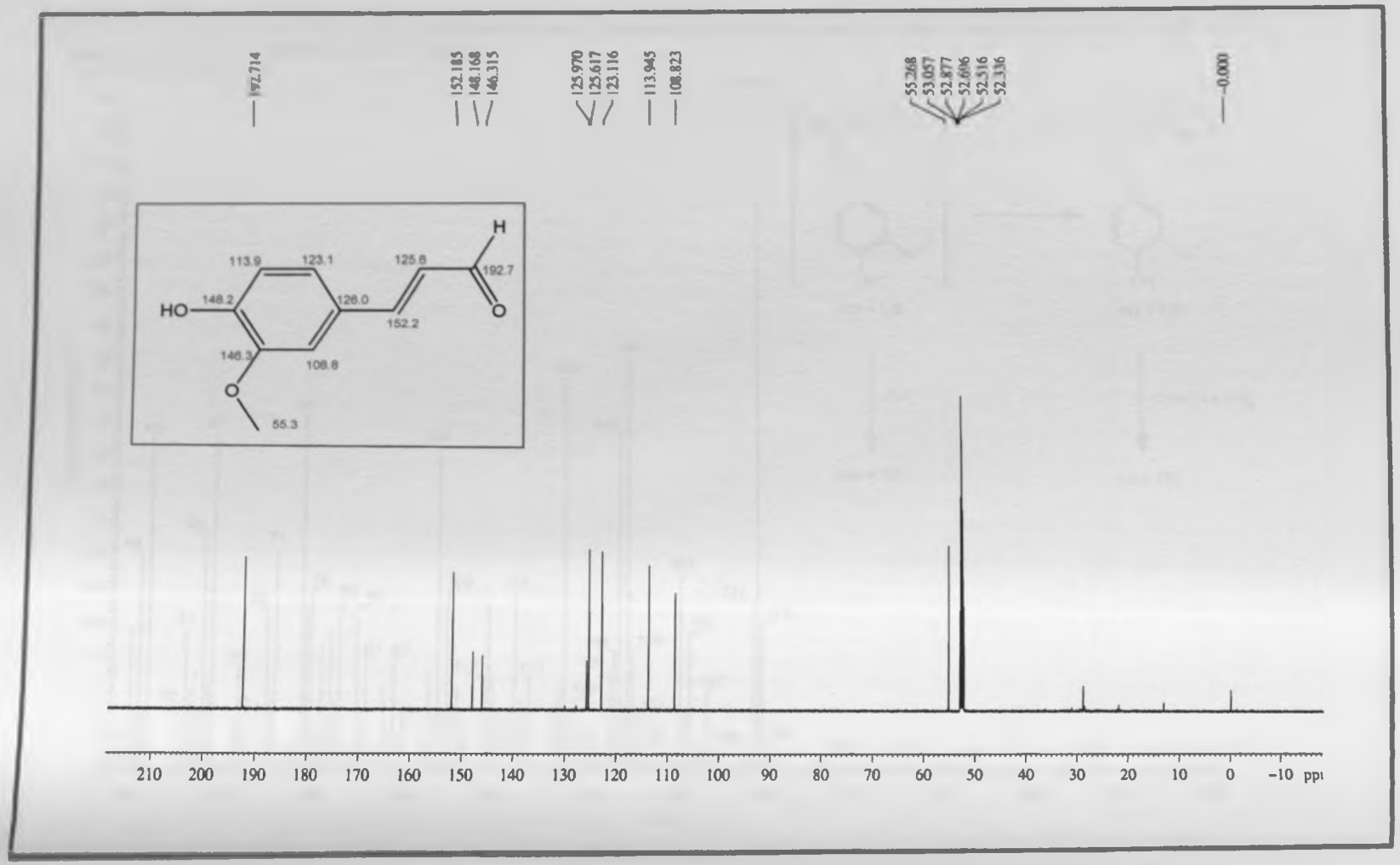
Appendix 27C: EI-MS spectrum of caffeic acid (**240**)



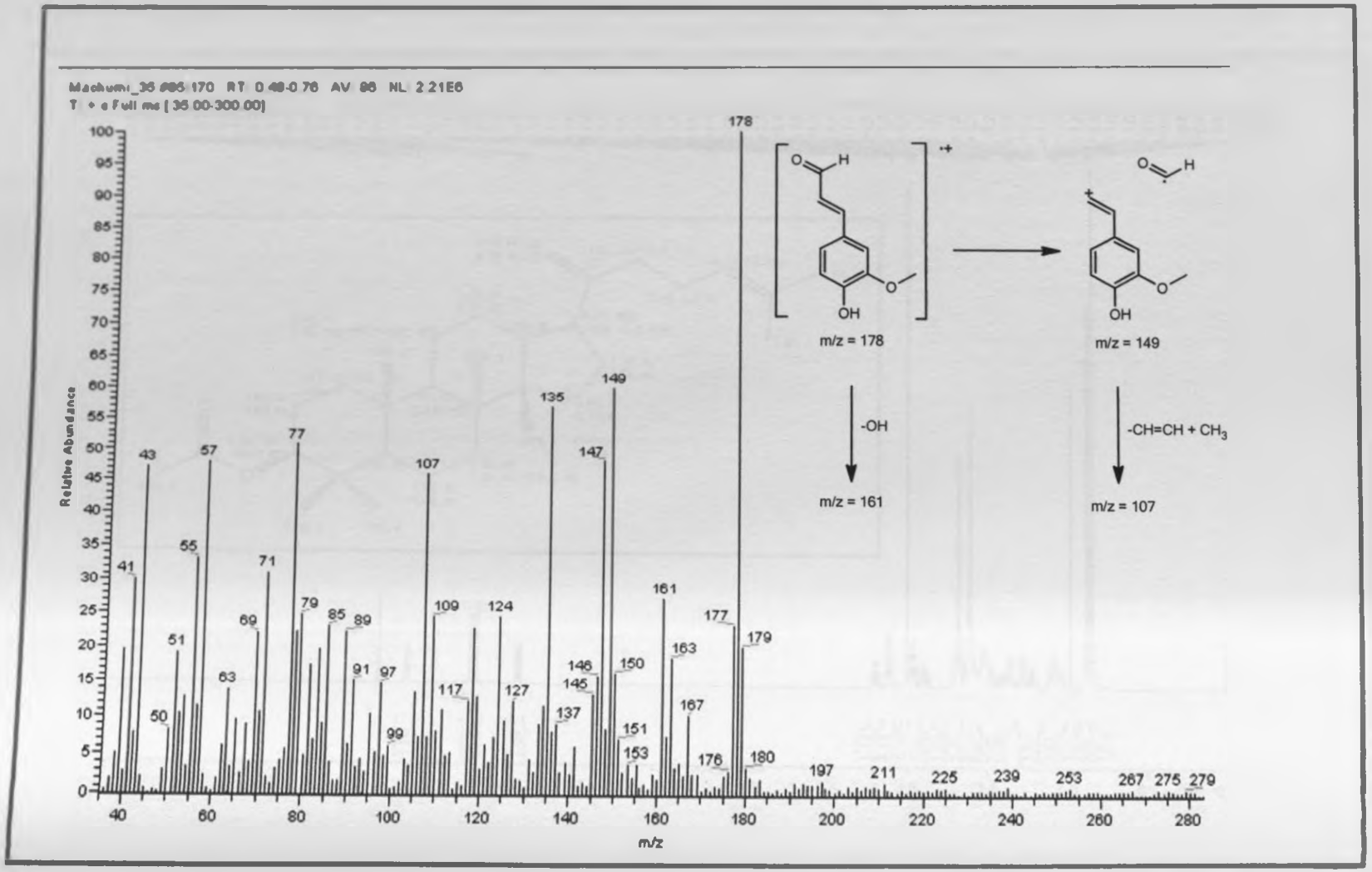
Appendix 28A: ^{1}H-NMR spectrum of coniferaldehyde (**241**), CD_2Cl_2, 600 MHz



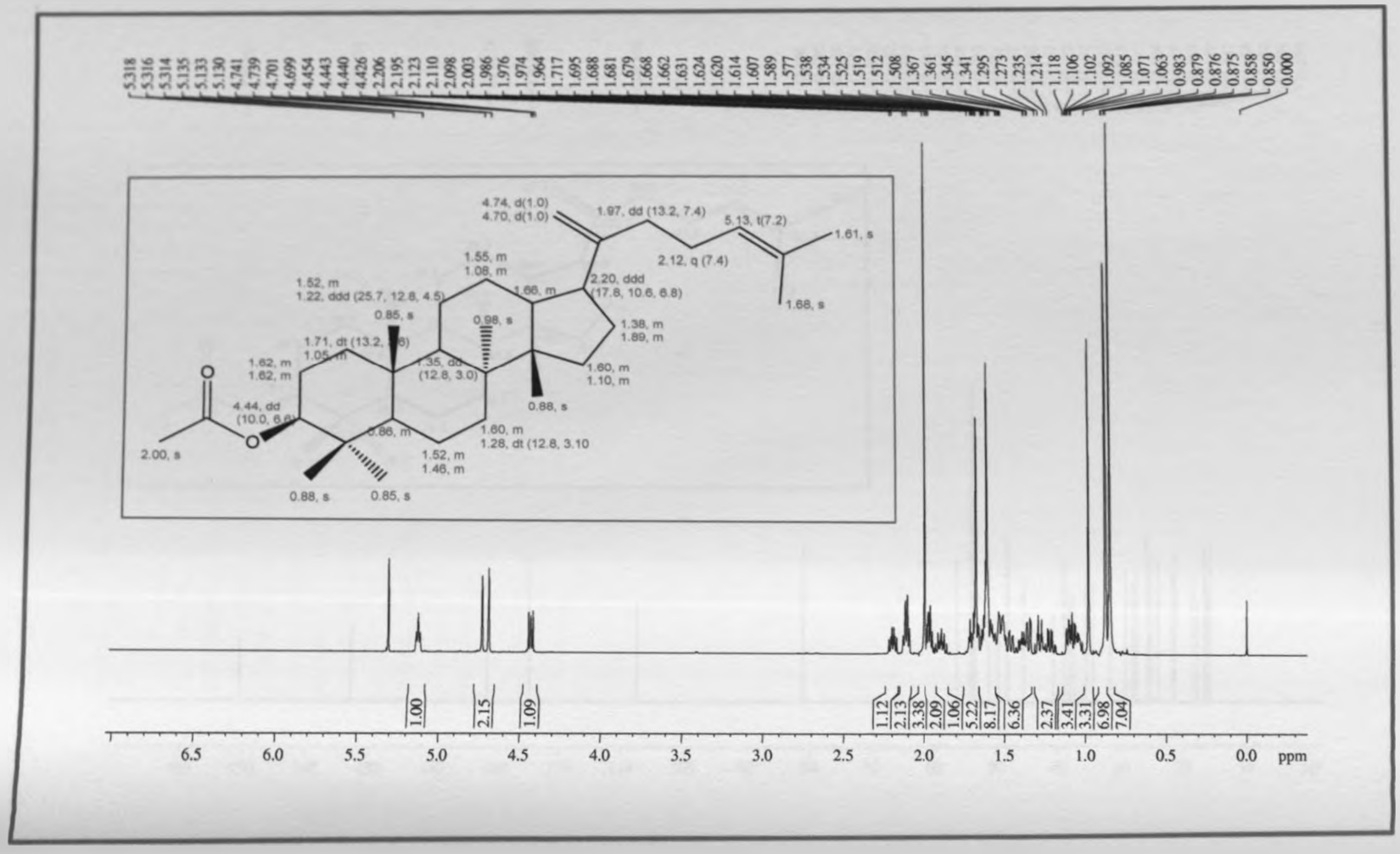
Appendix 28B: ^{13}C-NMR spectrum of coniferaldehyde (**241**), CD_2Cl_2, 150 MHz



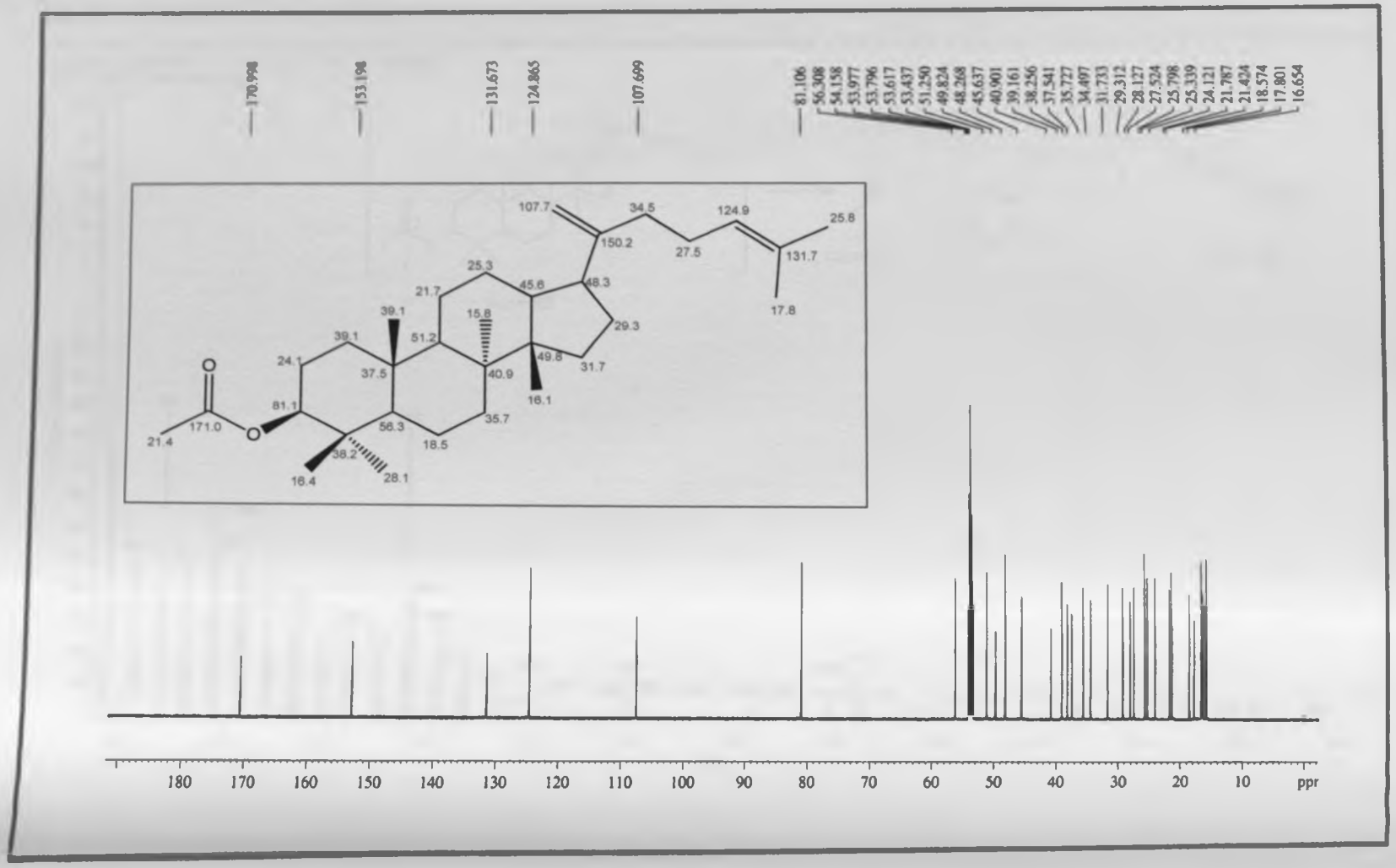
Appendix 28C: EI-MS spectrum of coniferaldehyde (**241**)



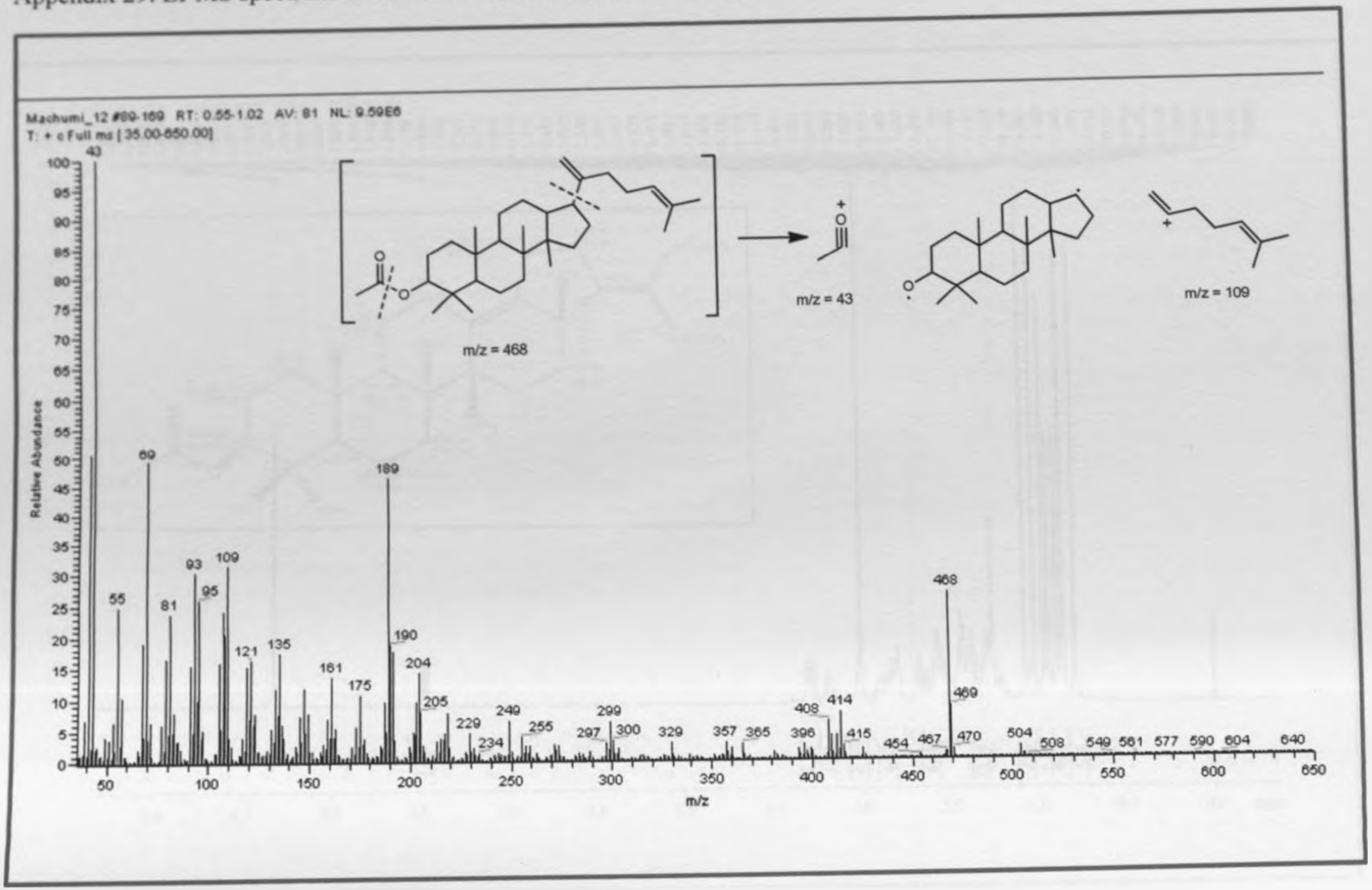
Appendix 29A: ^{1}H-NMR spectrum of dammara-20,24-dienylacetate (**242**), CD_2Cl_2, 600 MHz



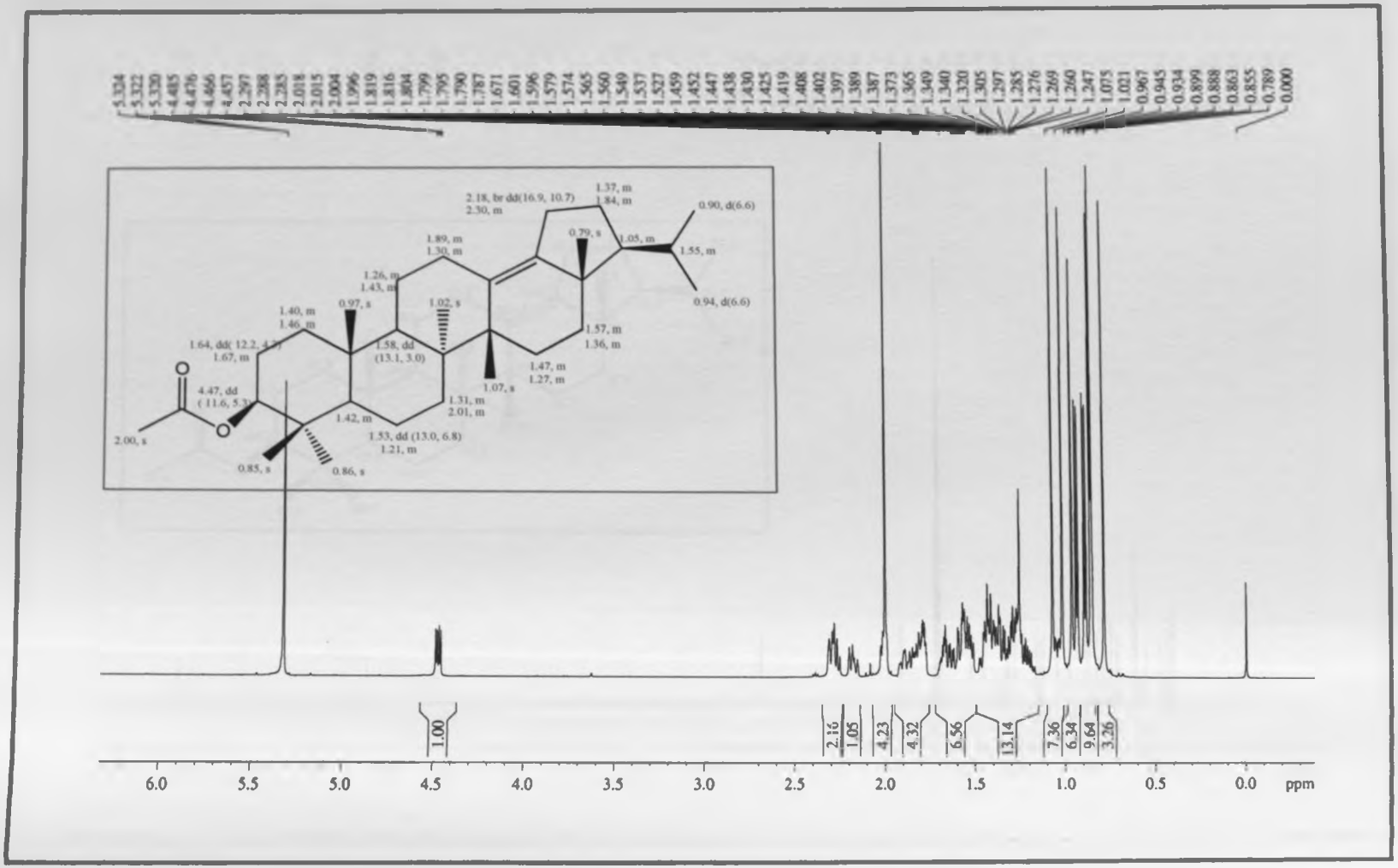
Appendix 29B: ^{13}C-NMR spectrum of dammara-20,24-dienylacetate (**242**), CD_2Cl_2, 150 MHz



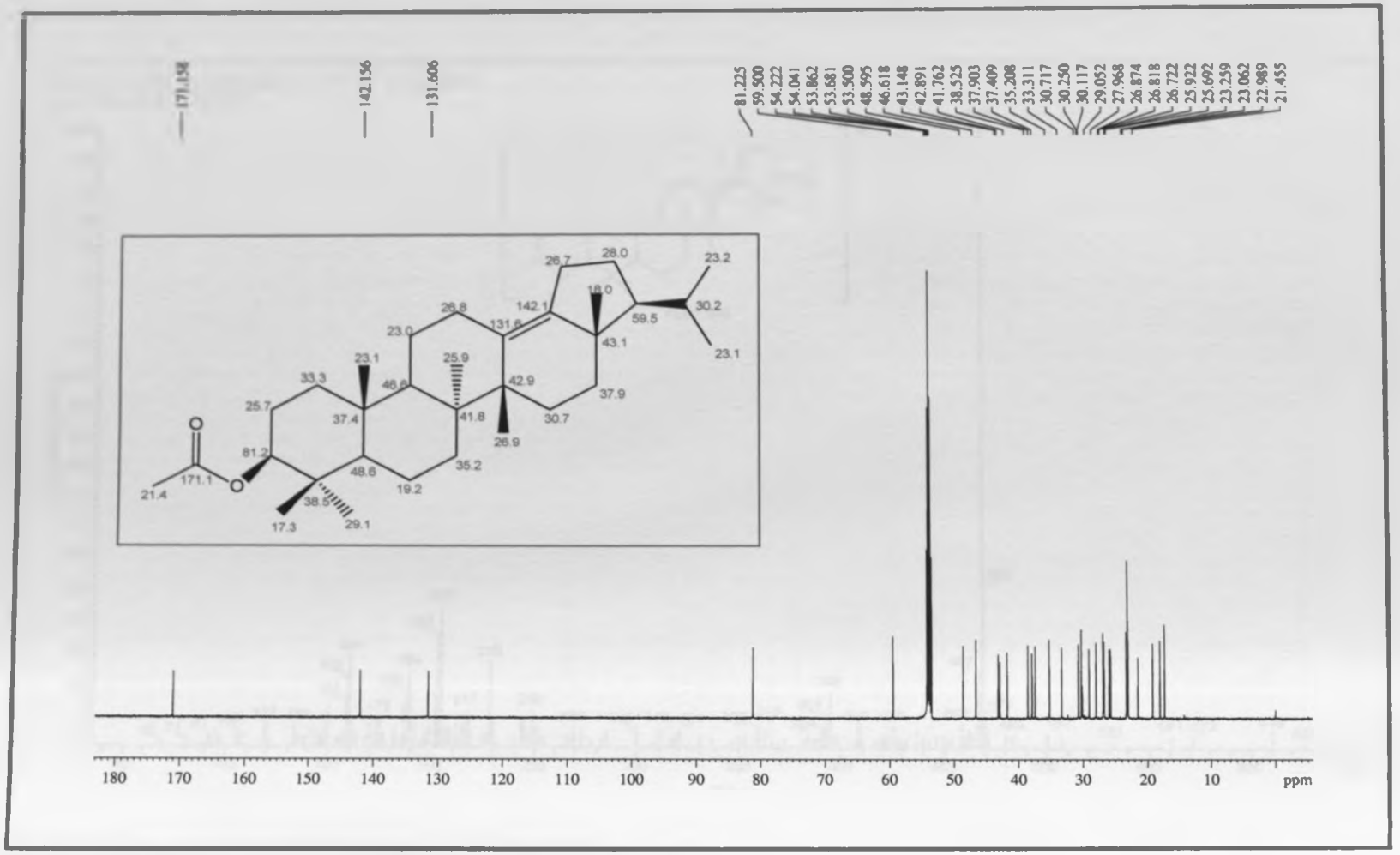
Appendix 29: EI-MS spectrum of dammara-20,24-dienylacetate (**242**)



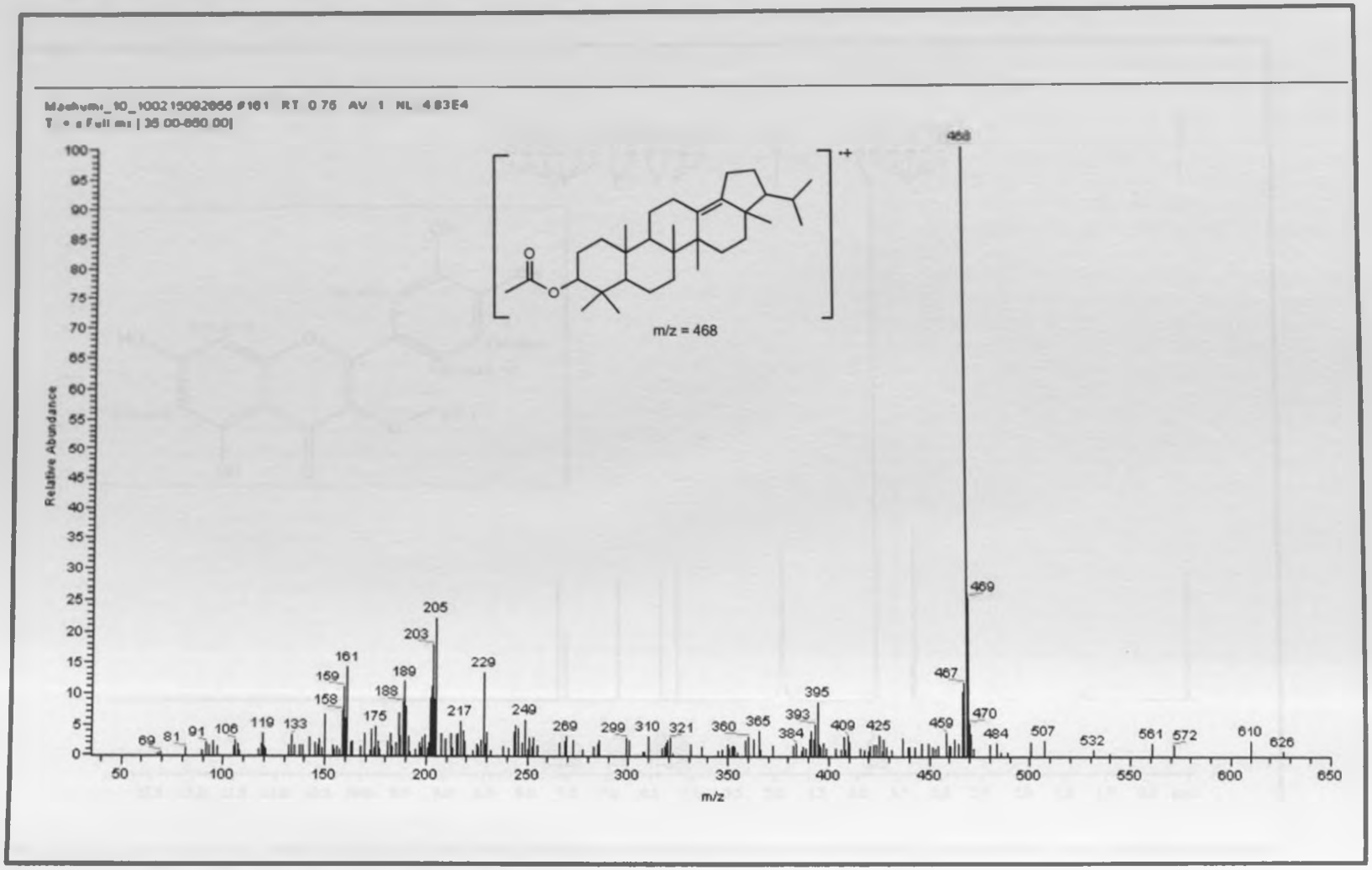
Appendix 30A: ^{1}H-NMR spectrum of boehmery acetate (**243**), CD_2Cl_2, 600 MHz



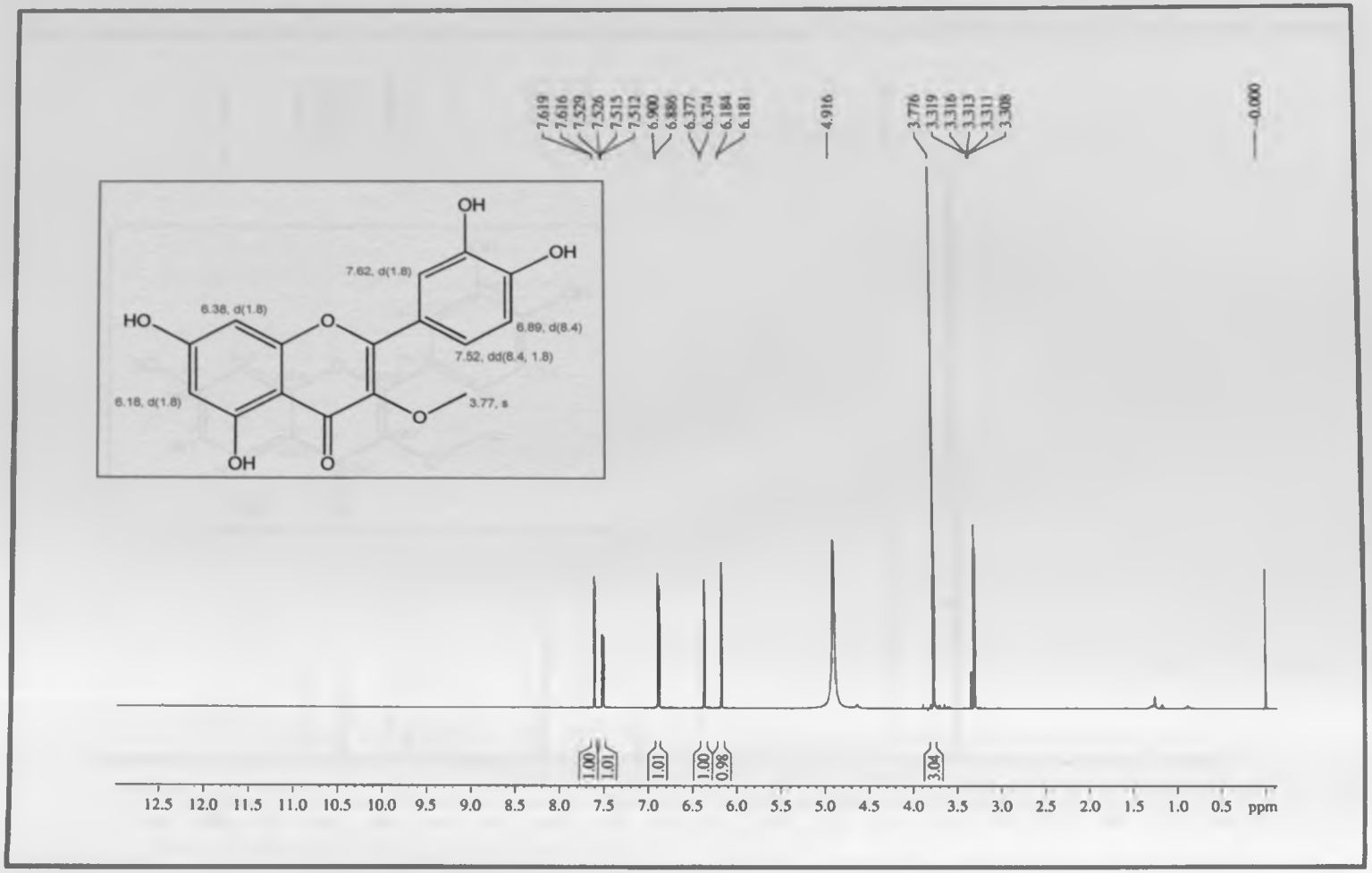
Appendix 30B: ^{13}C-NMR spectrum of boehmery acetate (**243**), CD_2Cl_2, 150 MHz



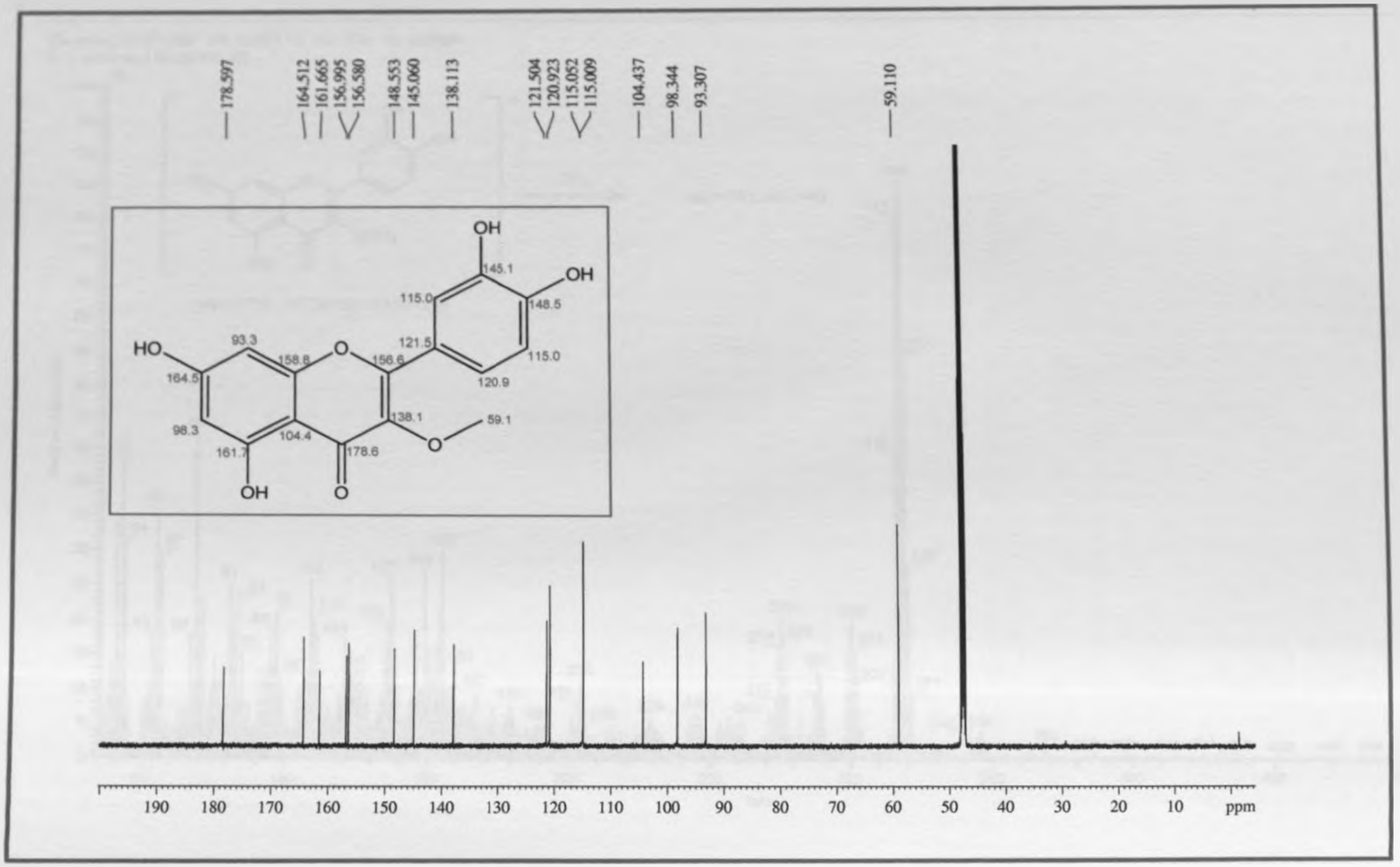
Appendix 30C: EI-MS spectrum of boehmery acetate (**243**)



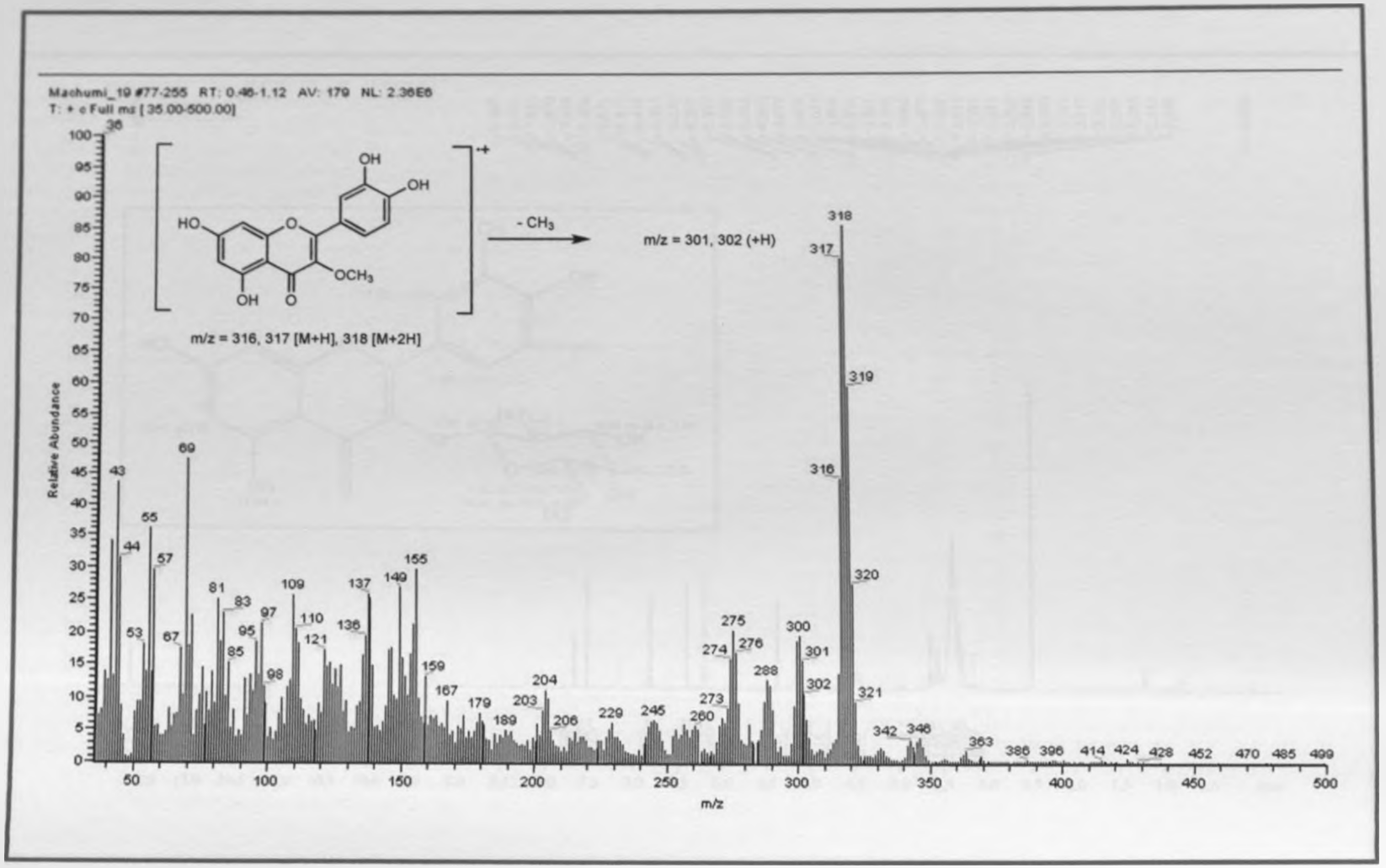
Appendix 31A: ^{1}H-NMR spectrum of quercetin 3-methylether (**244**), MeOD, 600 MHz



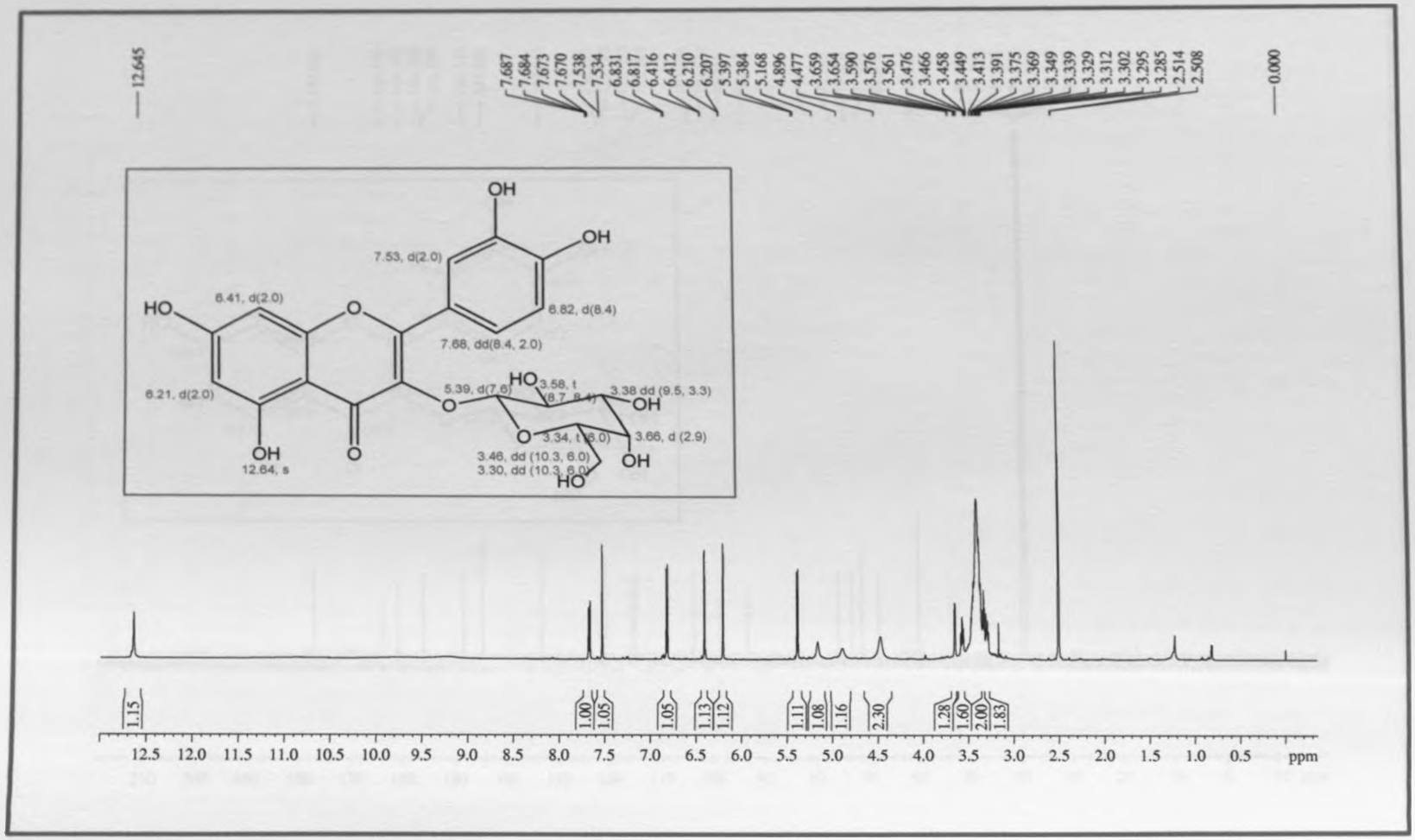
Appendix 31B: ^{13}C-NMR spectrum of quercetin 3-methylether (**244**), MeOD, 150 MHz



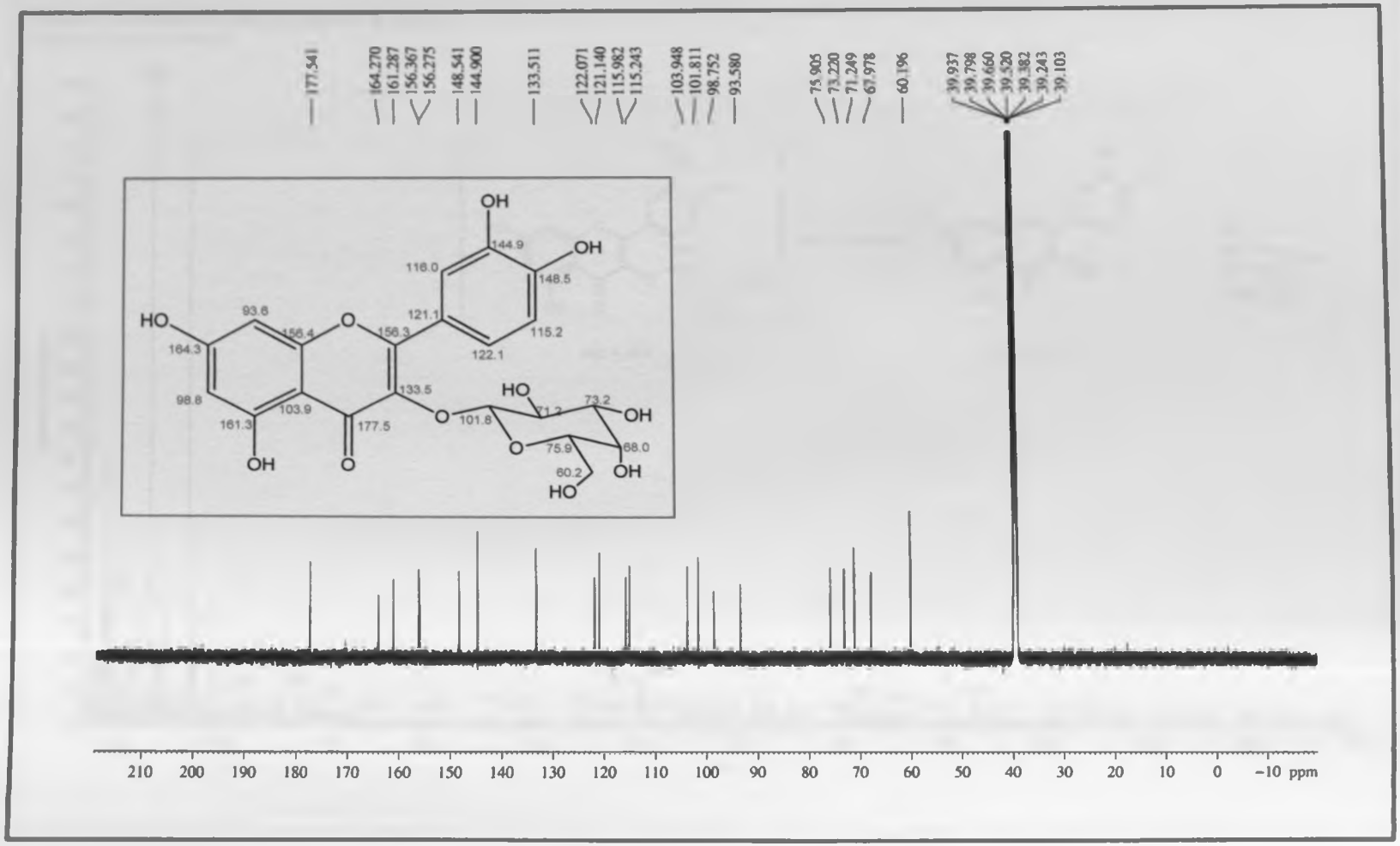
Appendix 31C: EI-MS spectrum of quercetin 3-methylether (**244**)



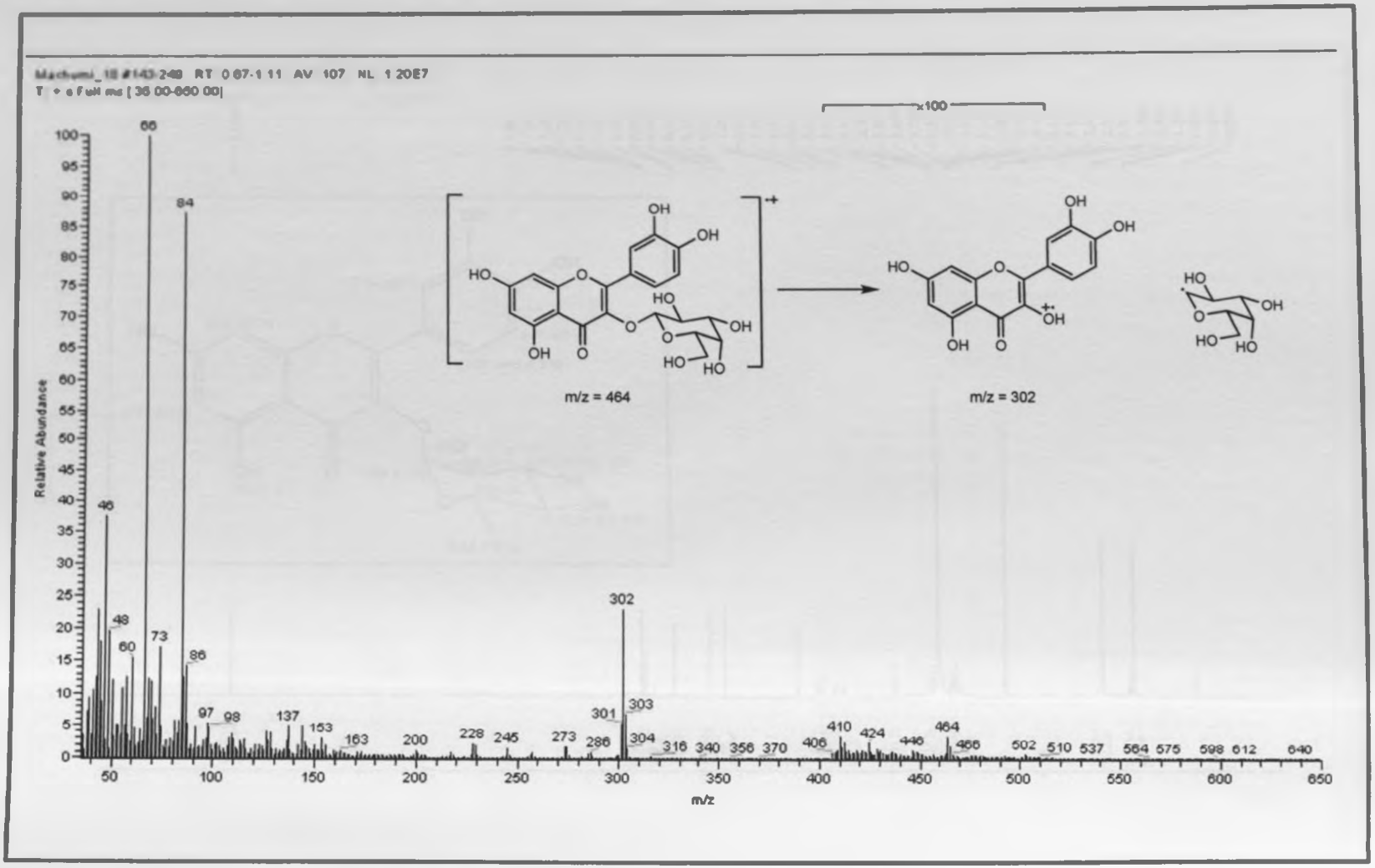
Appendix 32A: ^{1}H-NMR spectrum of quercetin 3-*O*-*β*-*D*-galactoside (**212**) DMSO d_6, 600 MHz



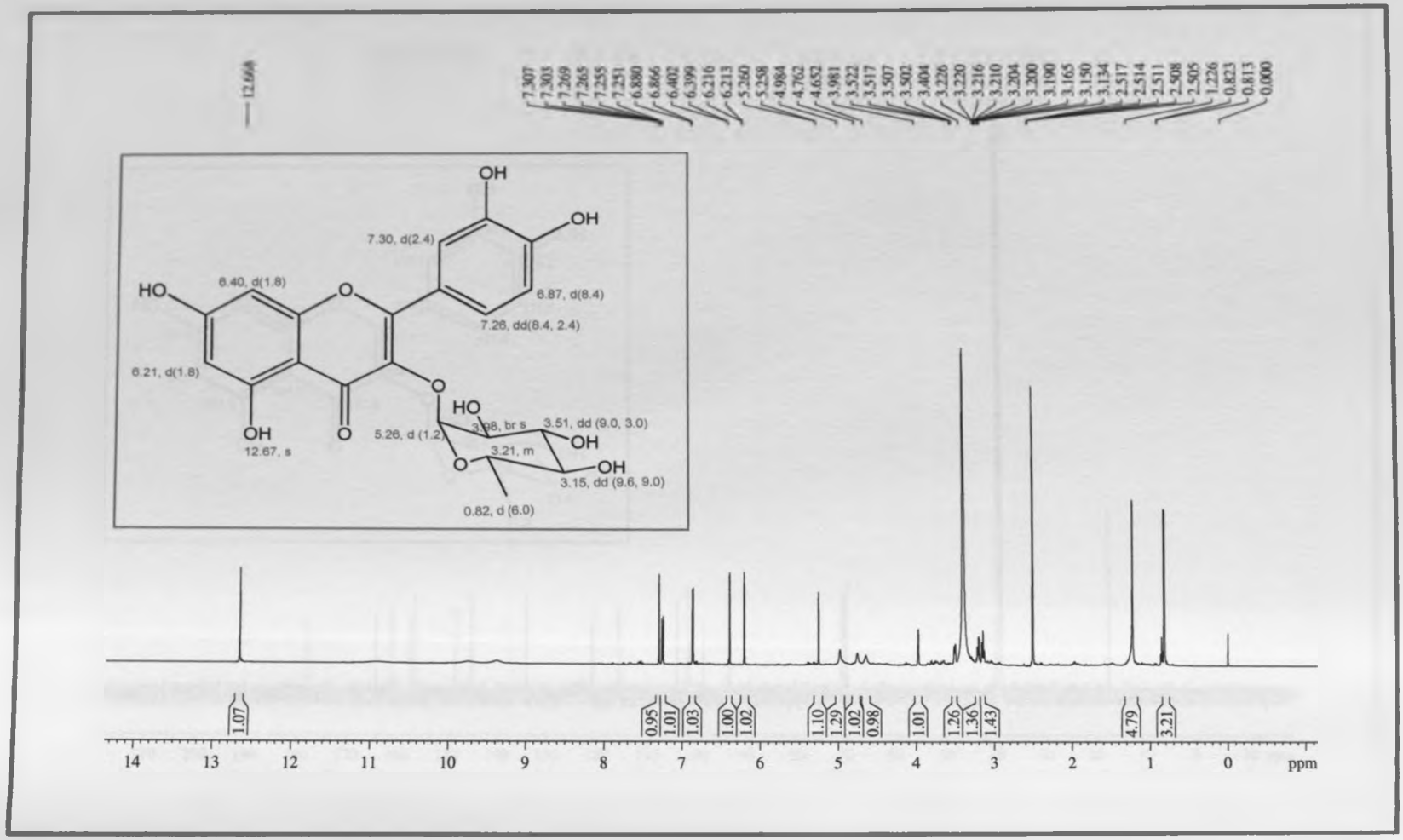
Appendix 32B: ^{13}C-NMR spectrum of quercetin 3-*O*-*β*-*D*-galactoside (**212**) DMSO d_6, 150 MHz



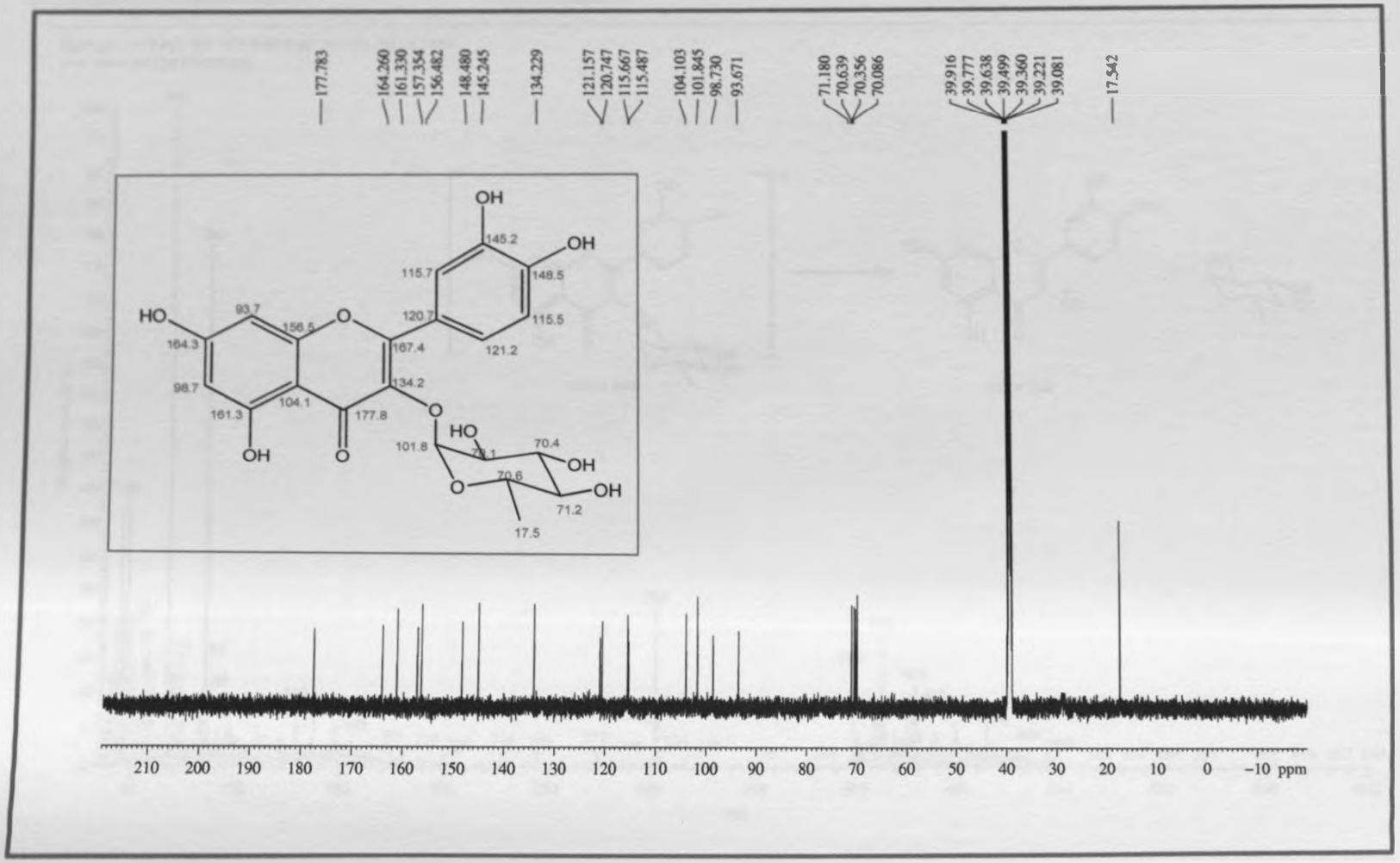
Appendix 32C: EI-MS spectrum of quercetin 3-*O*-*β*-*D*-galactoside (**212**)



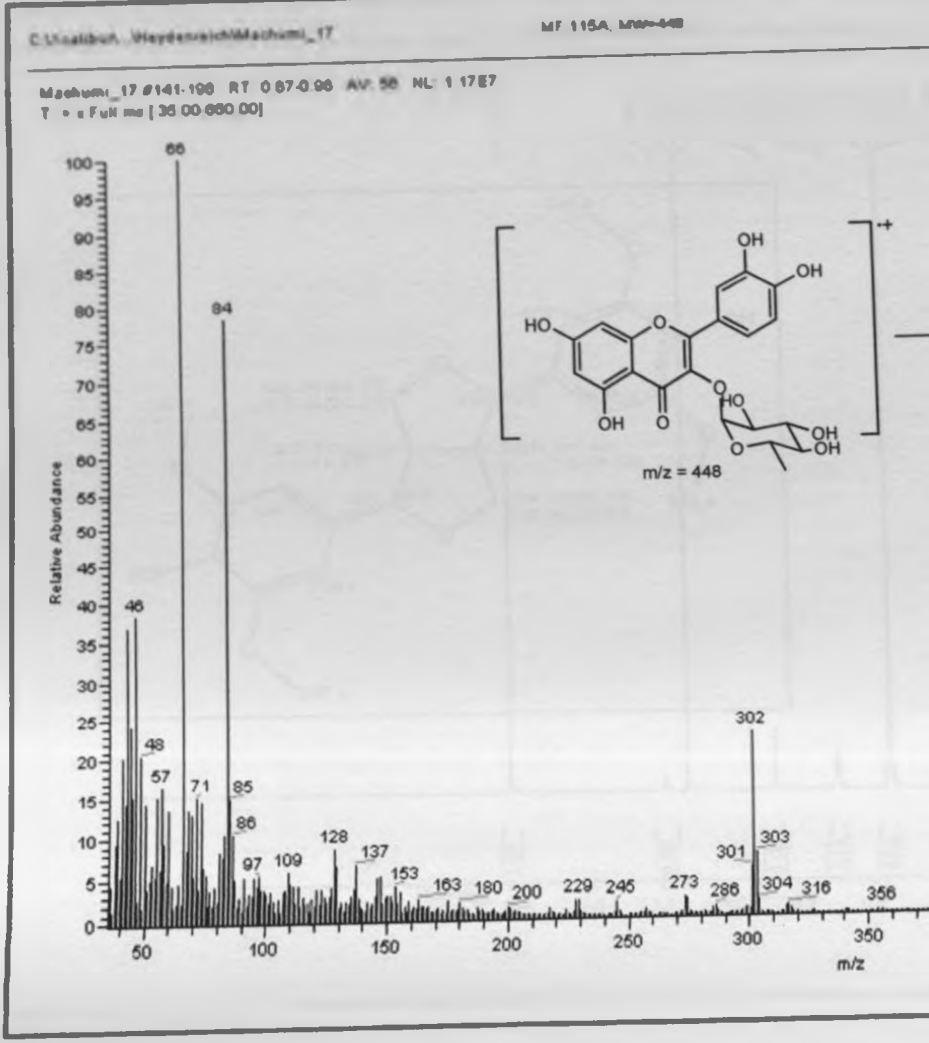
Appendix 33A: ^{1}H-NMR spectrum of quercetin 3-*O*-*α*-rhamnoside (**245**), DMSO d_6, 600 MHz

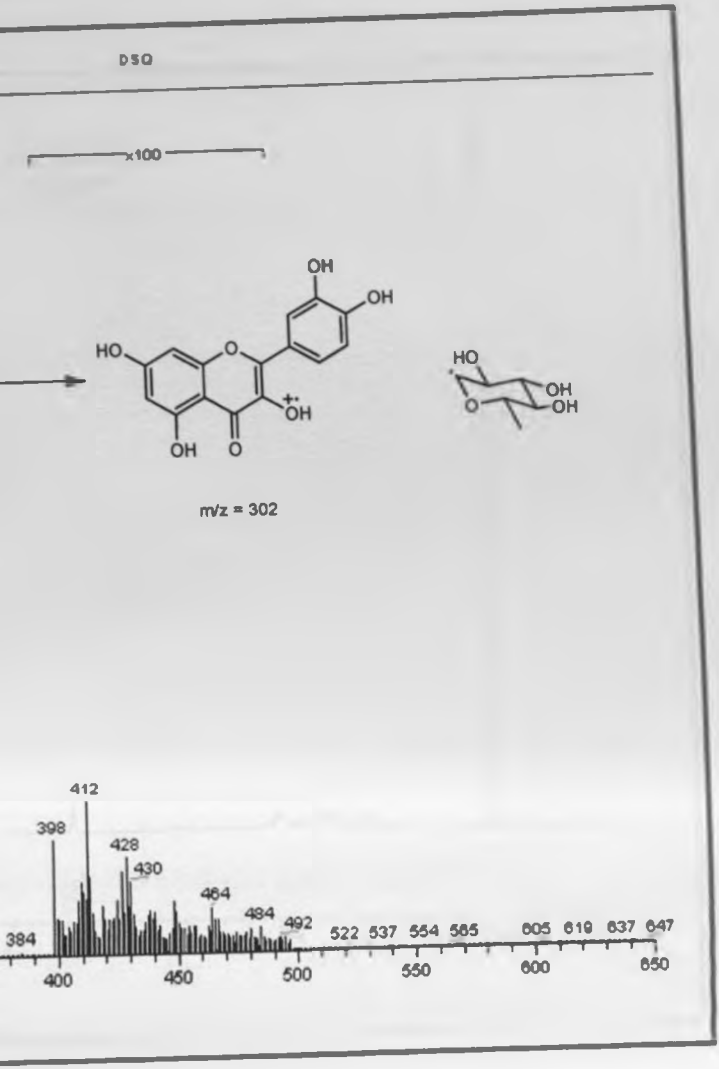


Appendix 33B: ^{13}C-NMR spectrum of quercetin 3-*O*-*α*-rhamnoside (**245**), DMSO d_6, 150 MHz

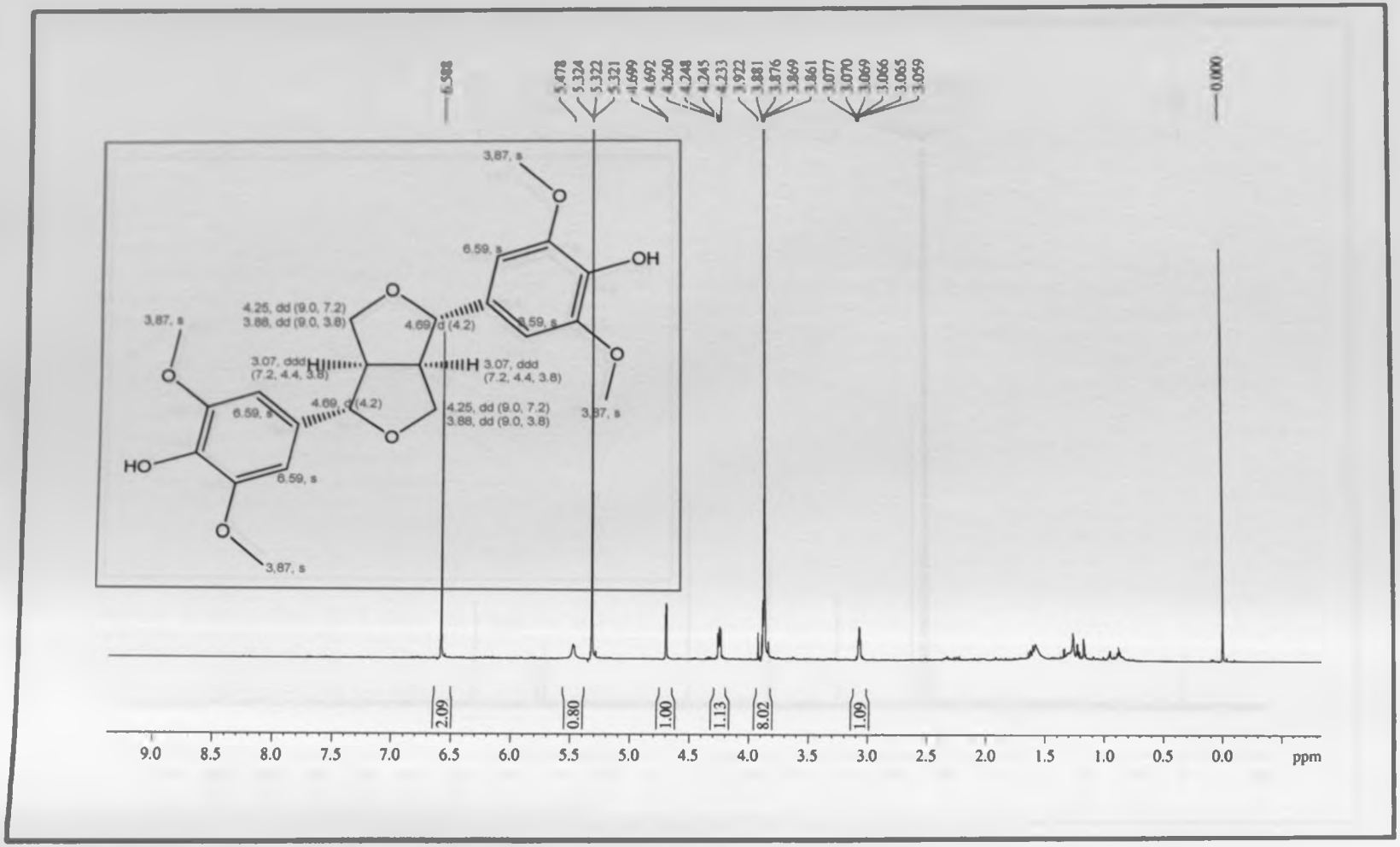


Appendix 33C: EI-MS spectrum of quercetin 3-*O*-*α*-rhamnoside (**245**)

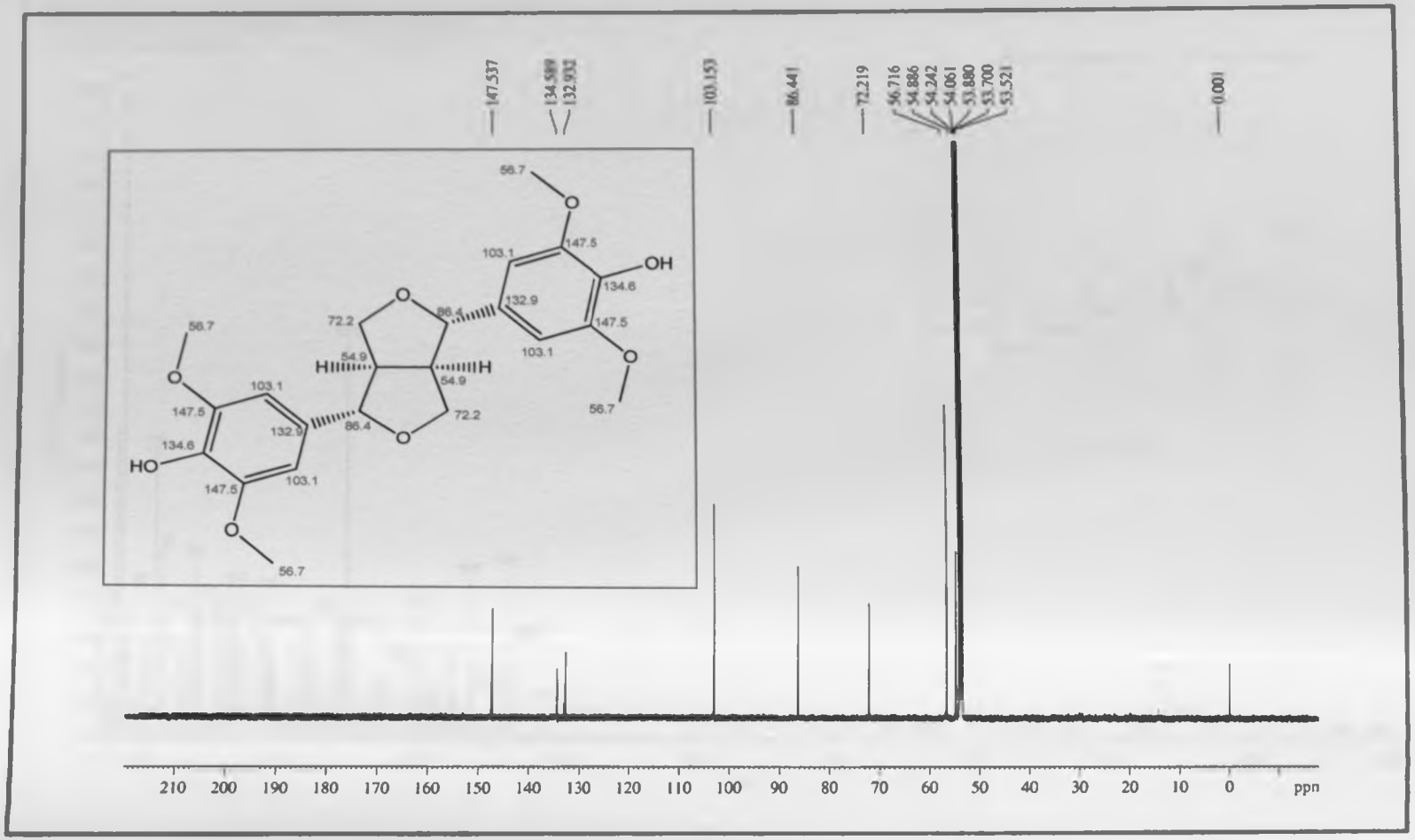



DSQ
x100
OH
OH
HO
O
+·
OH
OH
O
HO
OH
OH
O
m/z = 302
412
398
428
430
464
484
492
384
522 537 554 565
605 619 637 647
400
450
500
550
600
650

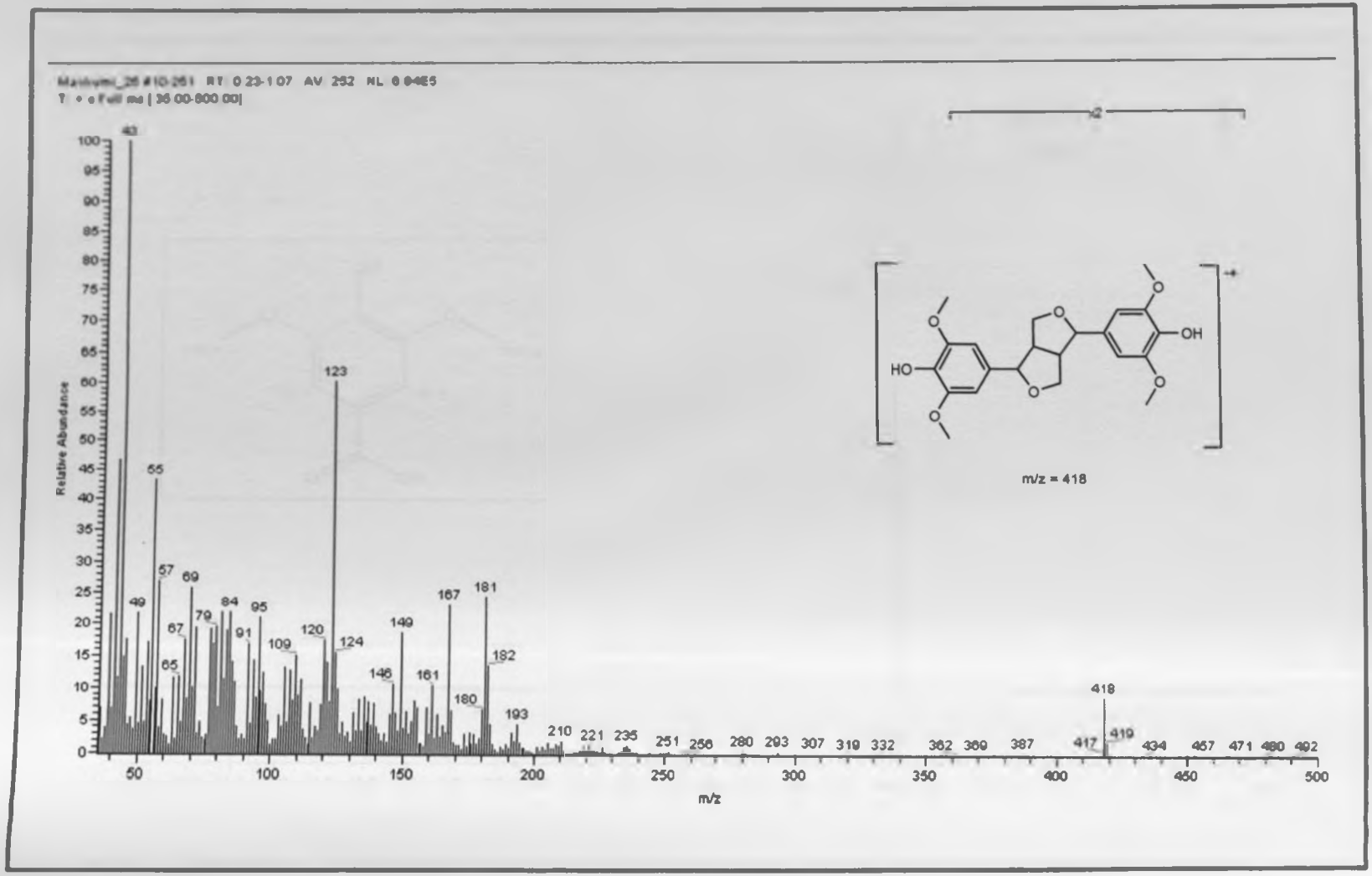
Appendix 34A: ^{1}H-NMR spectrum of syringaresinol (**246**), CD_2Cl_2, 600 MHz



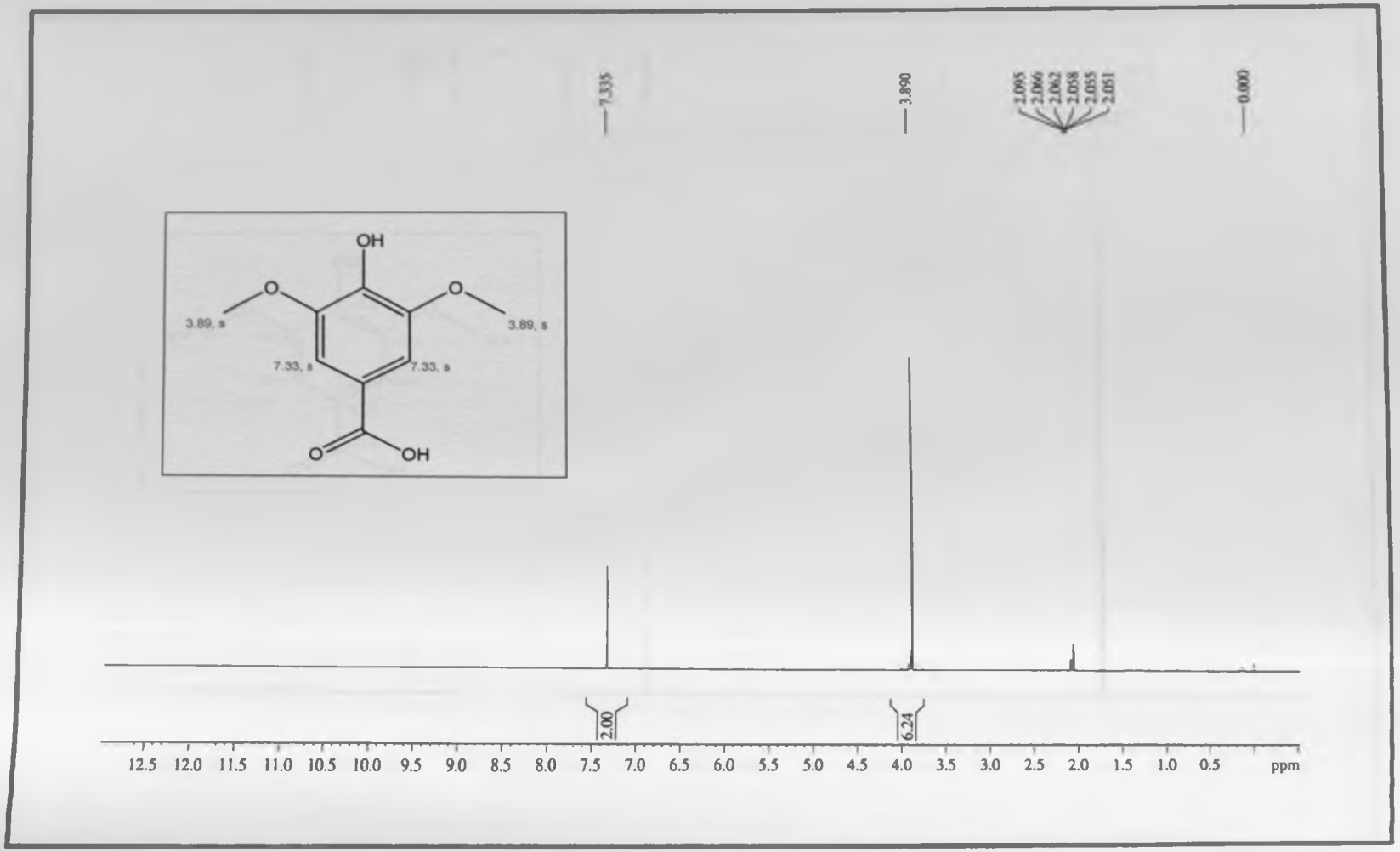
Appendix 34B: ^{13}C-NMR spectrum of syringaresinol (**246**), CD_2Cl_2, 150 MHz



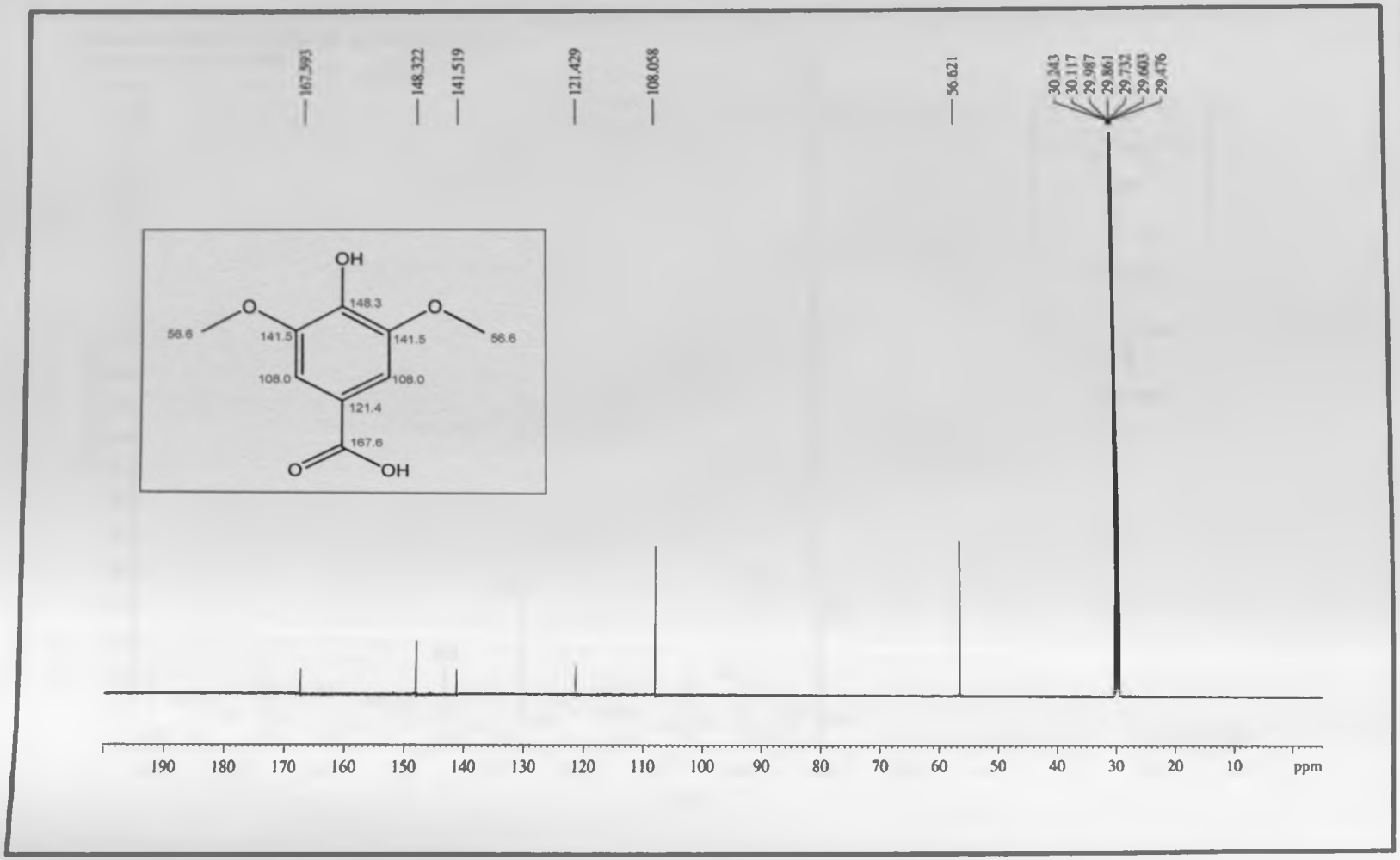
Appendix XXXIVC: EI-MS spectrum of syringaresinol (**246**)



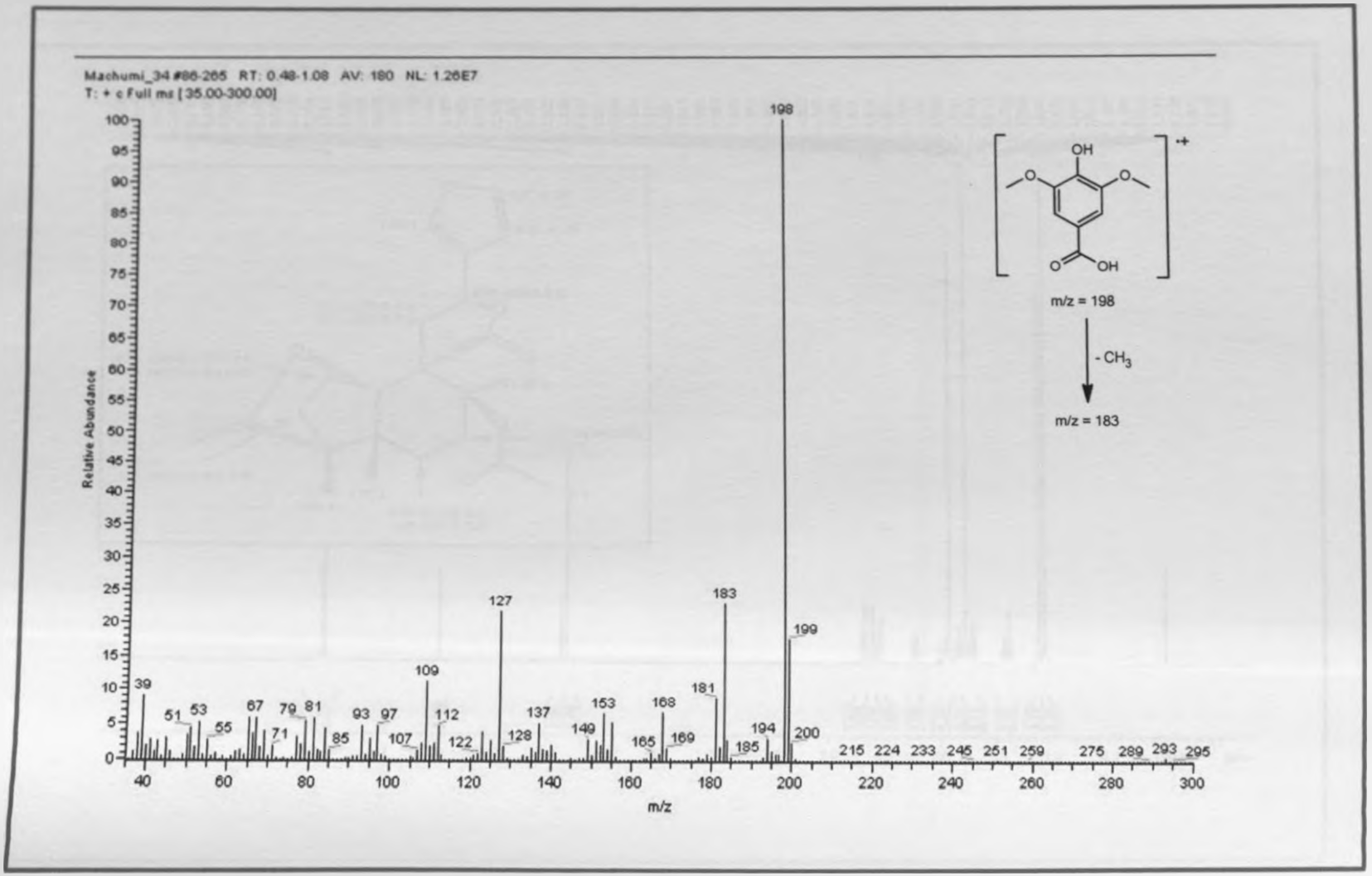
Appendix 35A: ^{1}H-NMR spectrum of syringic acid (**247**), Acetone d_6, 600 MHz



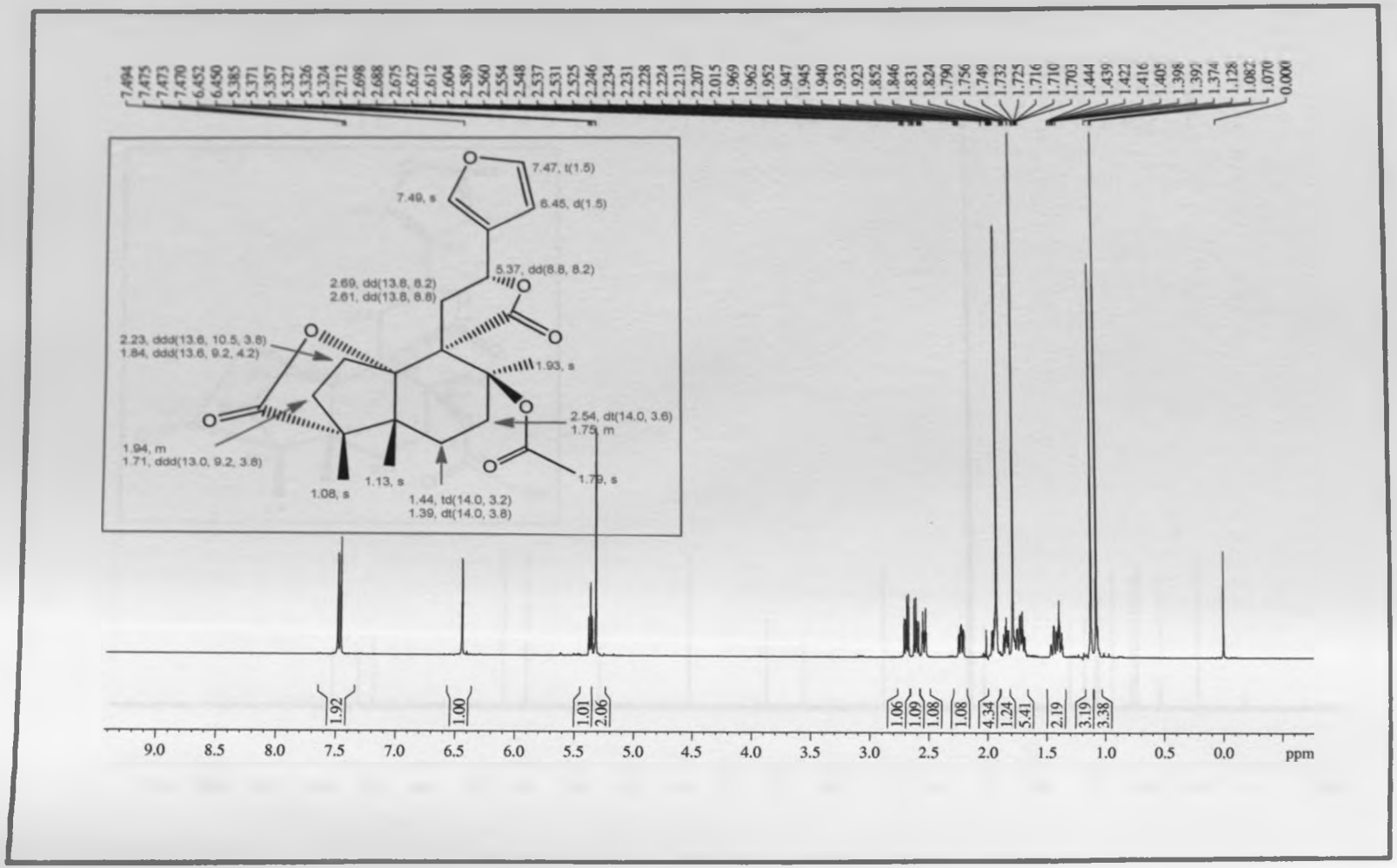
Appendix 35B: ^{13}C-NMR spectrum of syringic acid (**247**), Acetone d_6, 150 MHz



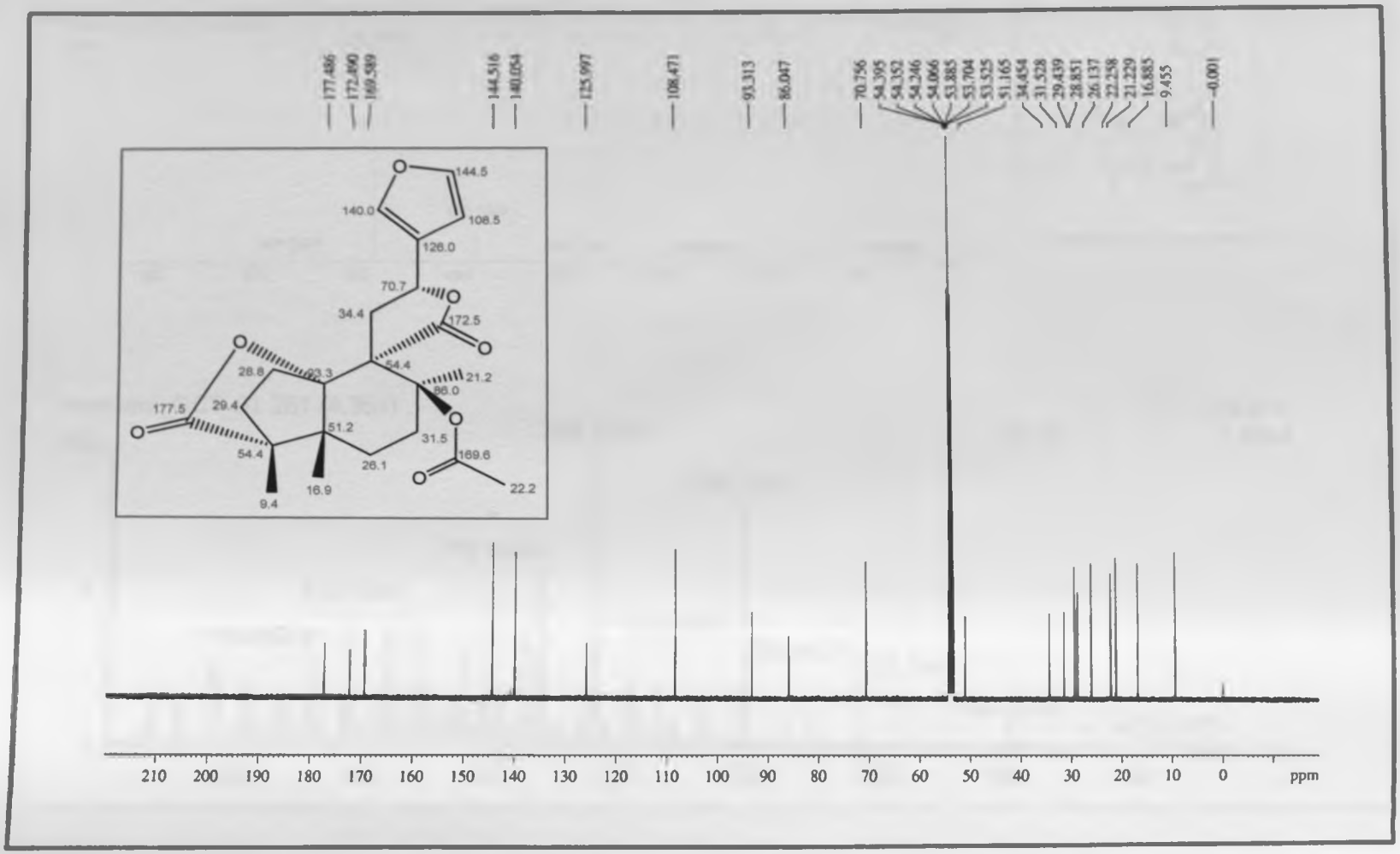
Appendix 35C: EI-MS spectrum of syringic acid (**247**)



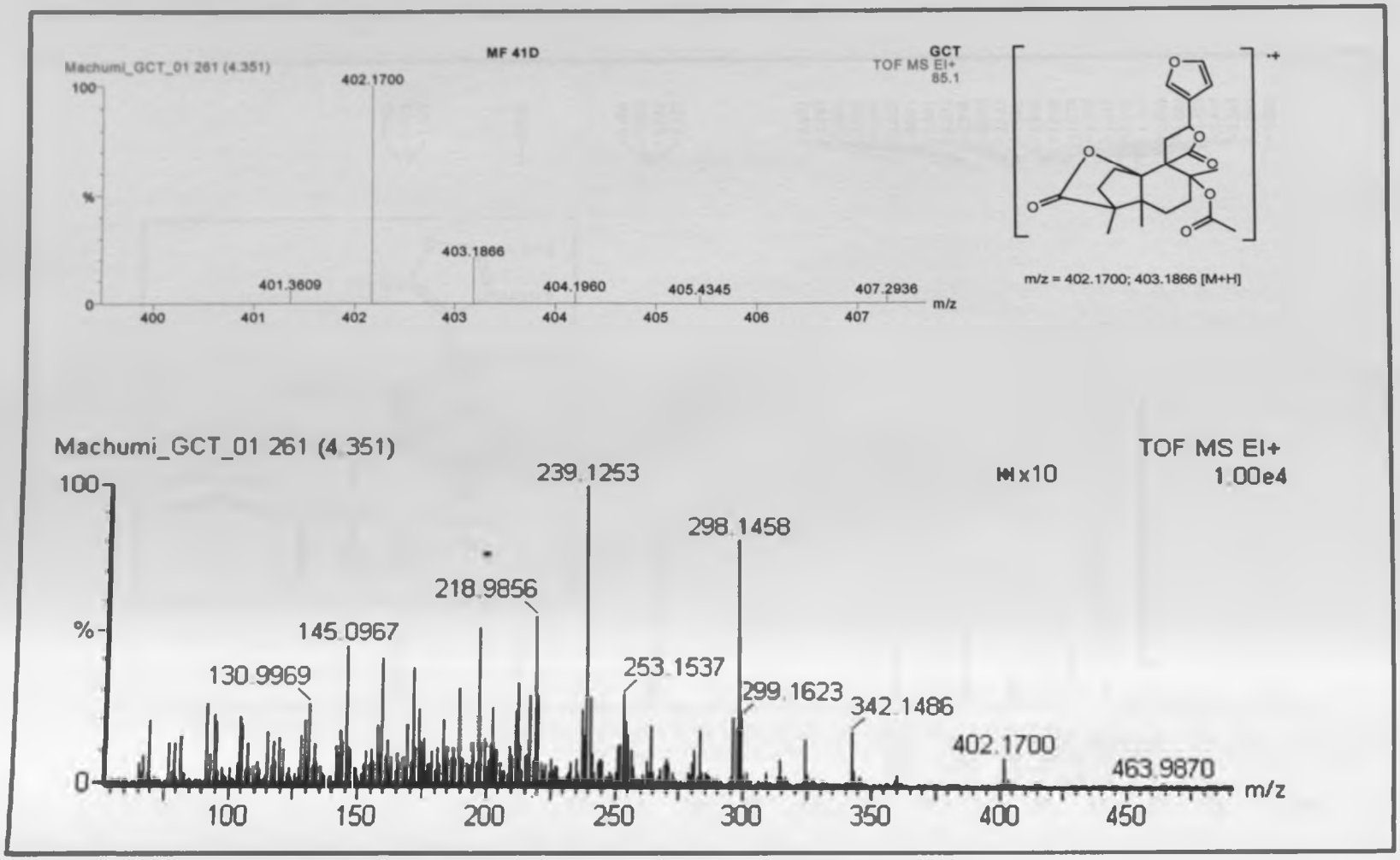
Appendix 36A: ^{1}H-NMR spectrum of 8-acetoxyisochiliolide lactone (**248**), CD_2Cl_2, 600 MHz



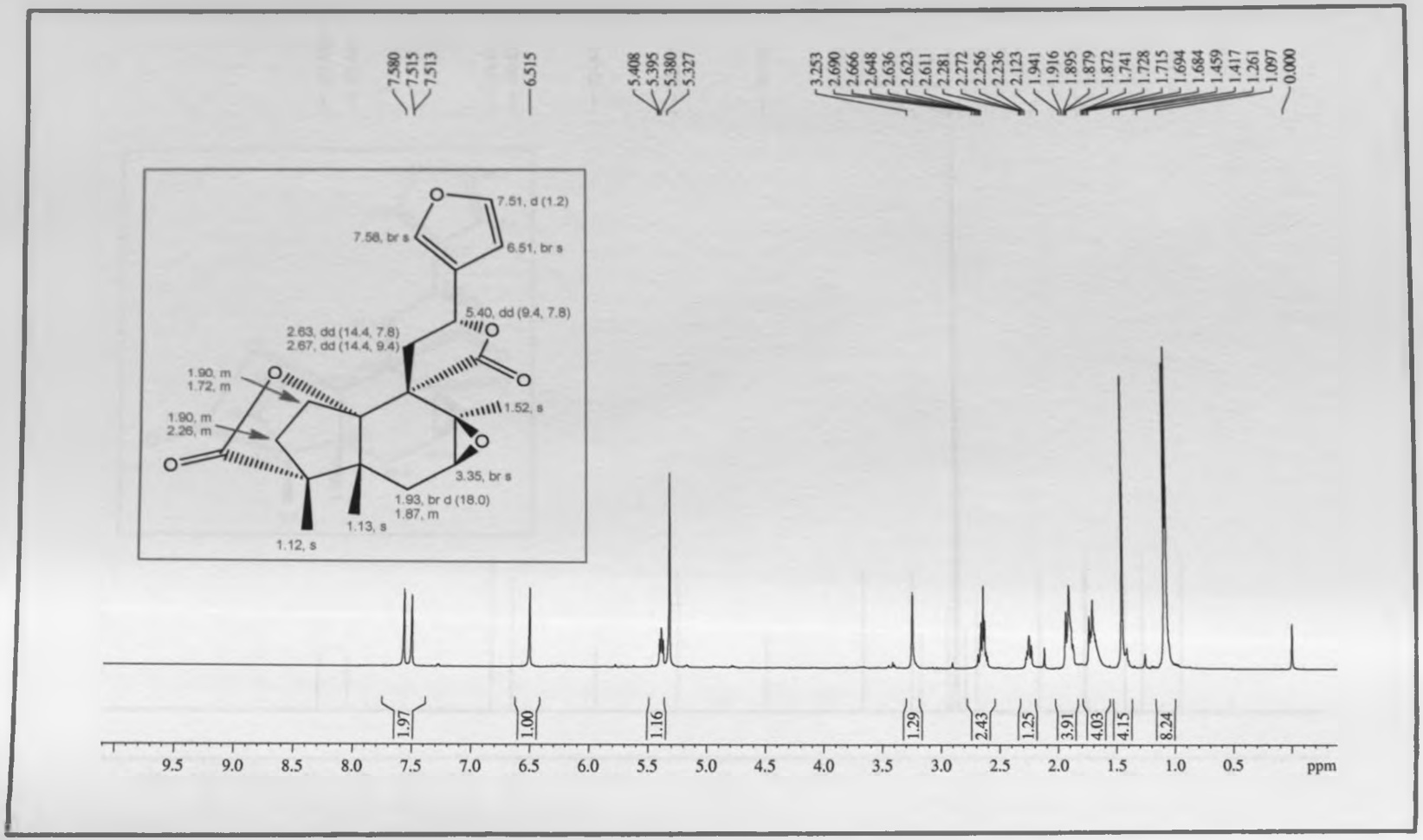
Appendix 36B: ^{13}C-NMR spectrum of 8-acetoxyisochiliolide lactone (**248**), CD_2Cl_2, 150 MHz



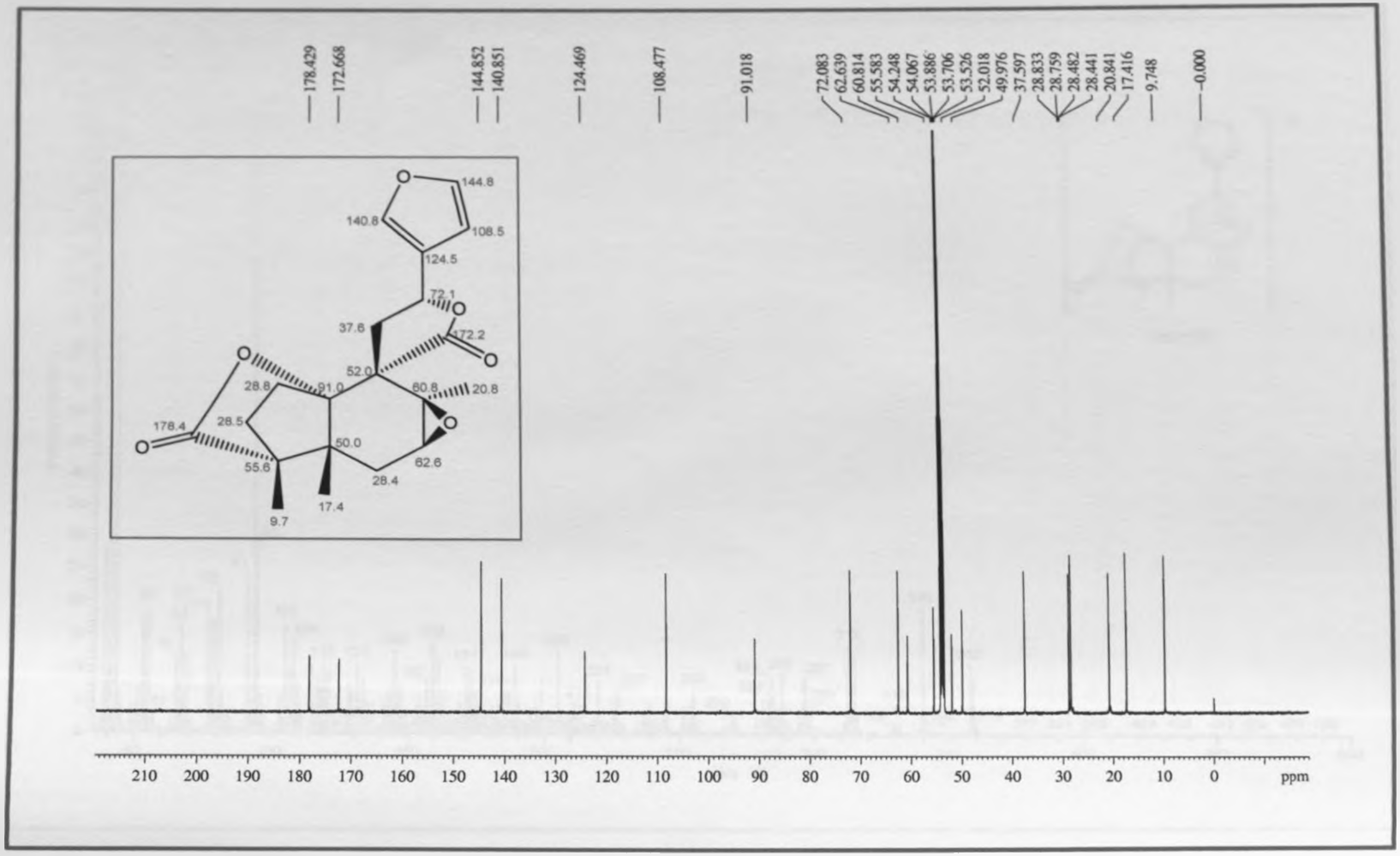
Appendix 36C: HREI-MS spectrum of 8-acetoxyisochiliolide lactone (**248**)



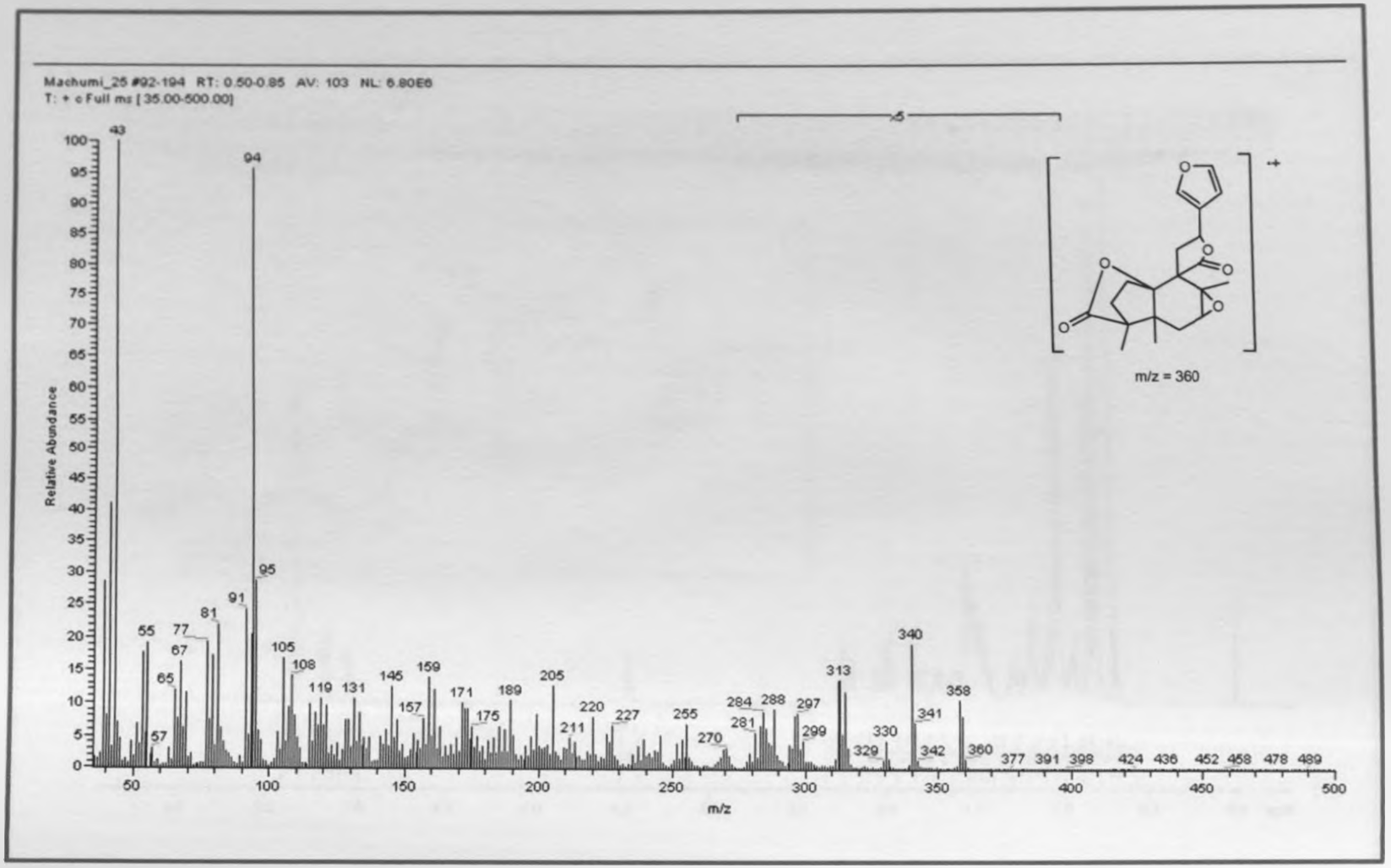
Appendix 37A: ^{1}H-NMR spectrum of epoxyisochiliolide lactone (**157**), CD_2Cl_2, 600 MHz



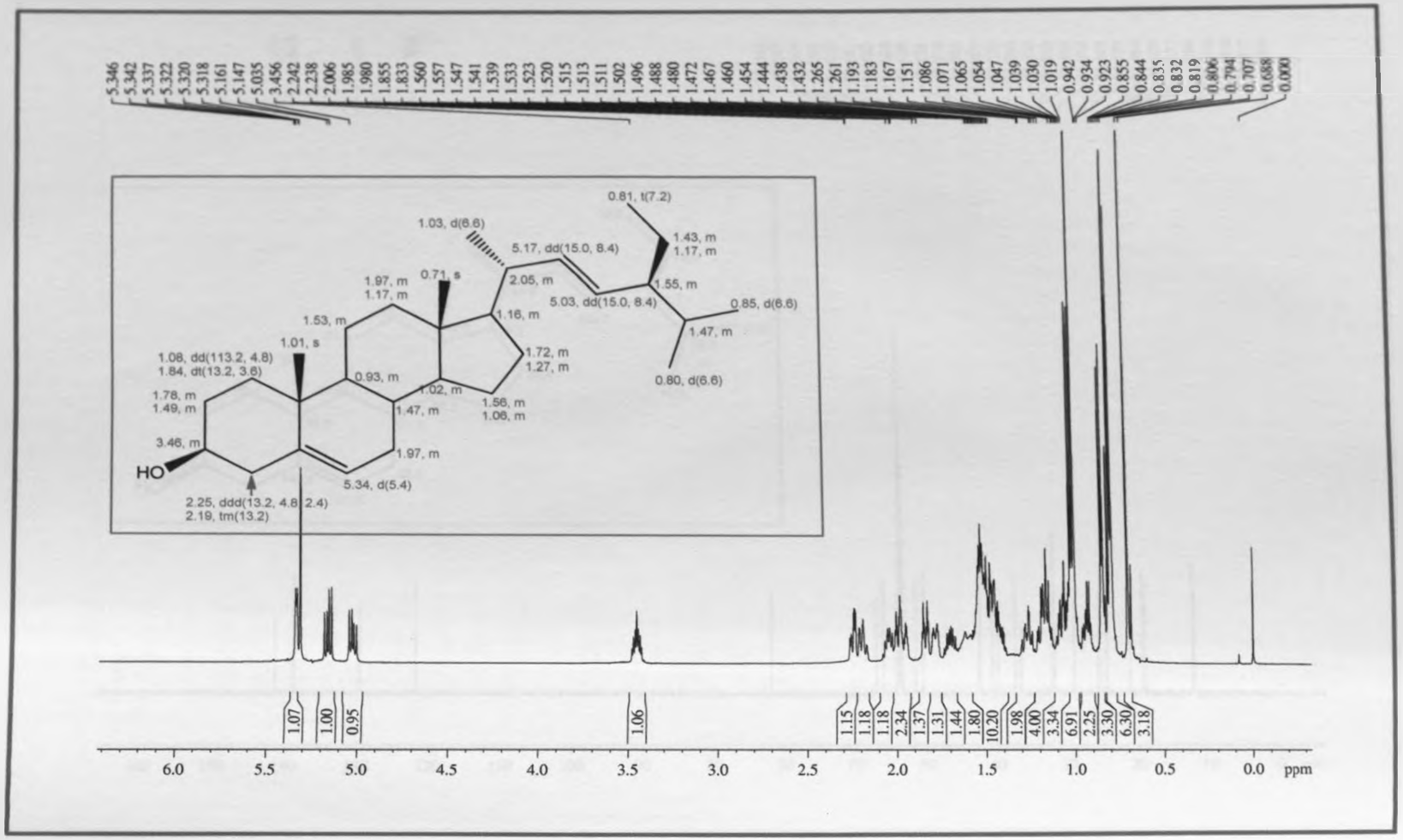
Appendix 37B: ^{13}C-NMR spectrum of epoxyisochiliolide lactone (**157**), CD_2Cl_2, 150 MHz



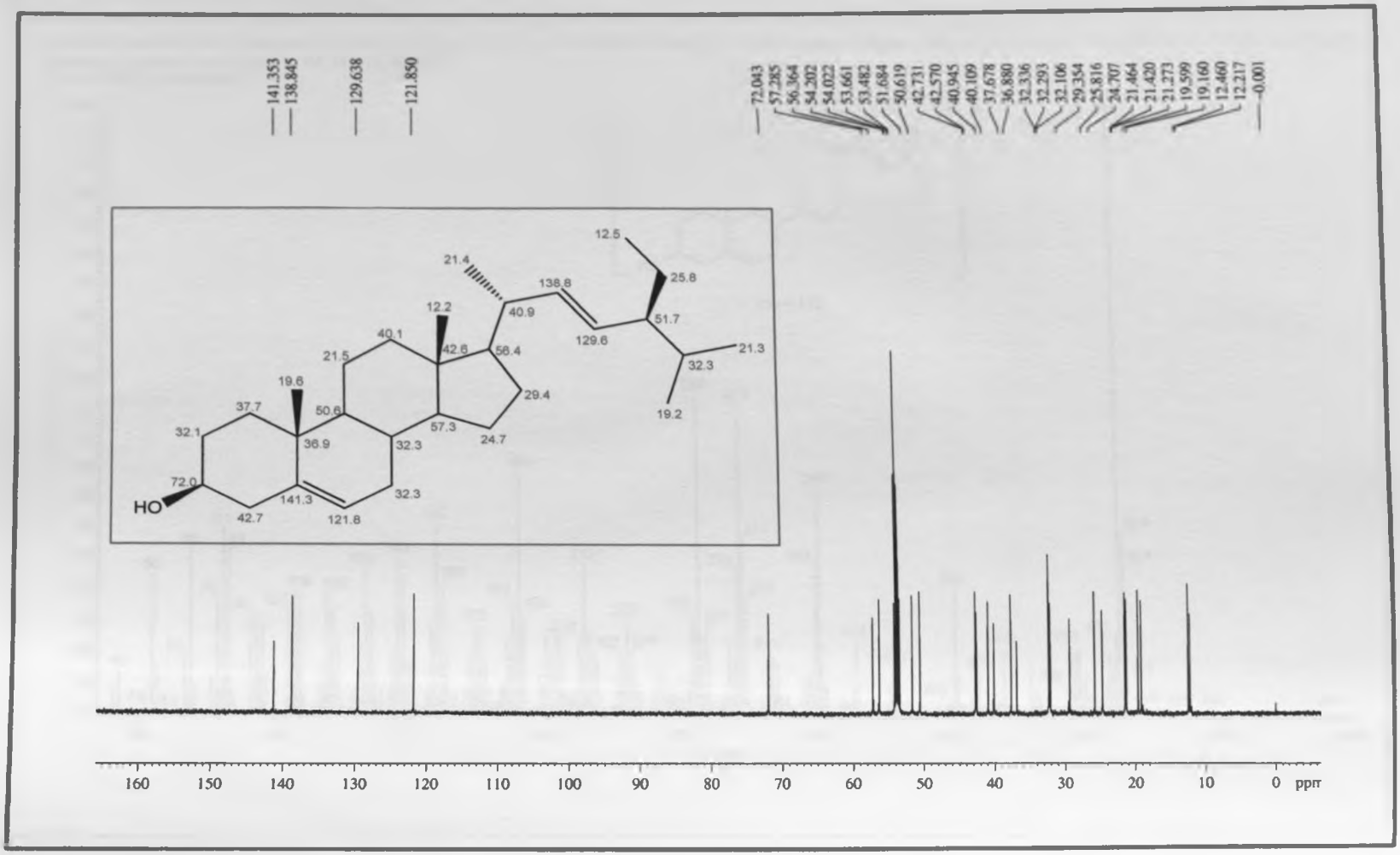
Appendix 37C: EI-MS spectrum of epoxyisochiliolide lactone (**157**)



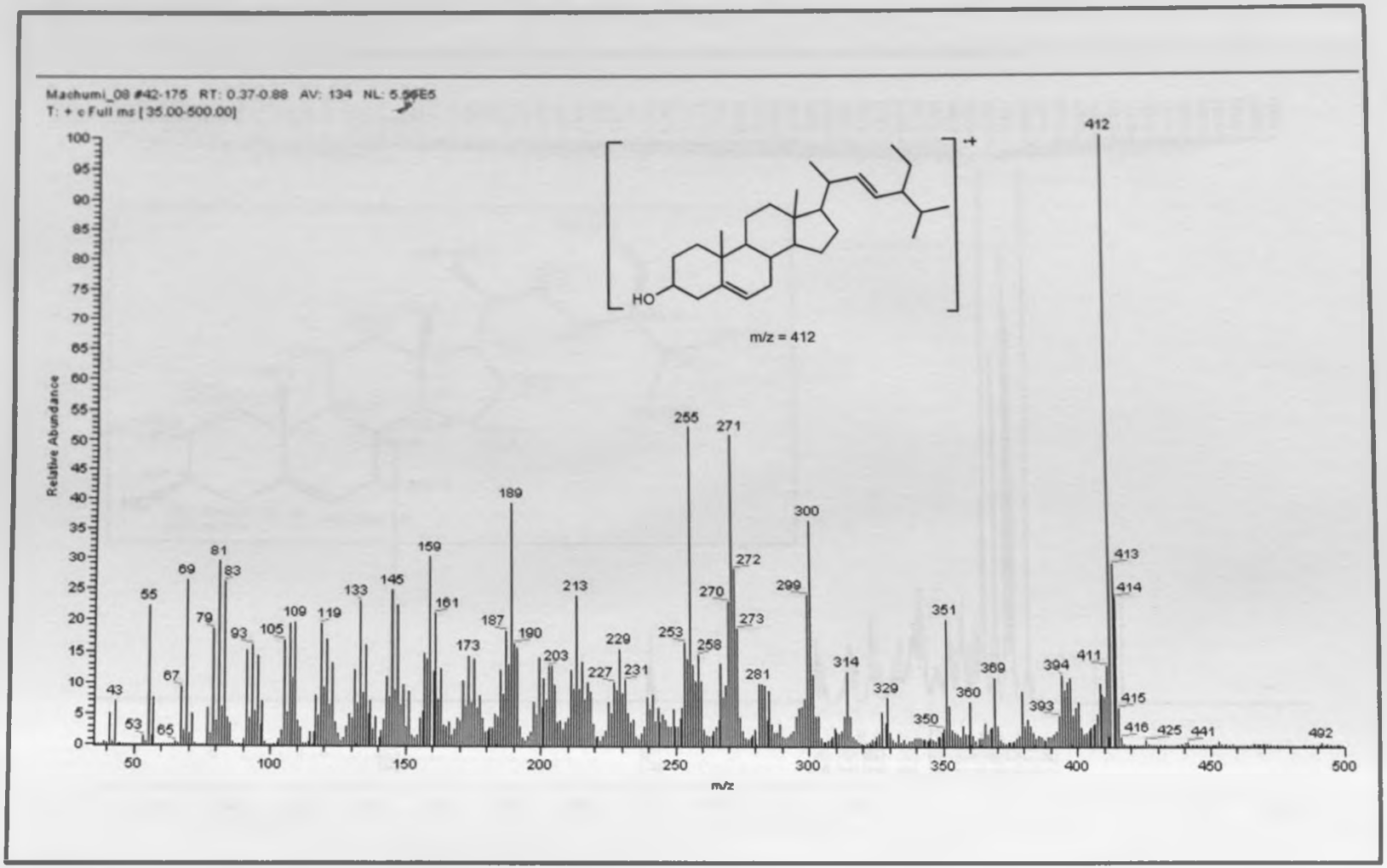
Appendix 38A: ^{1}H-NMR spectrum of stigmasterol (**66**), CD_2Cl_2, 600 MHz



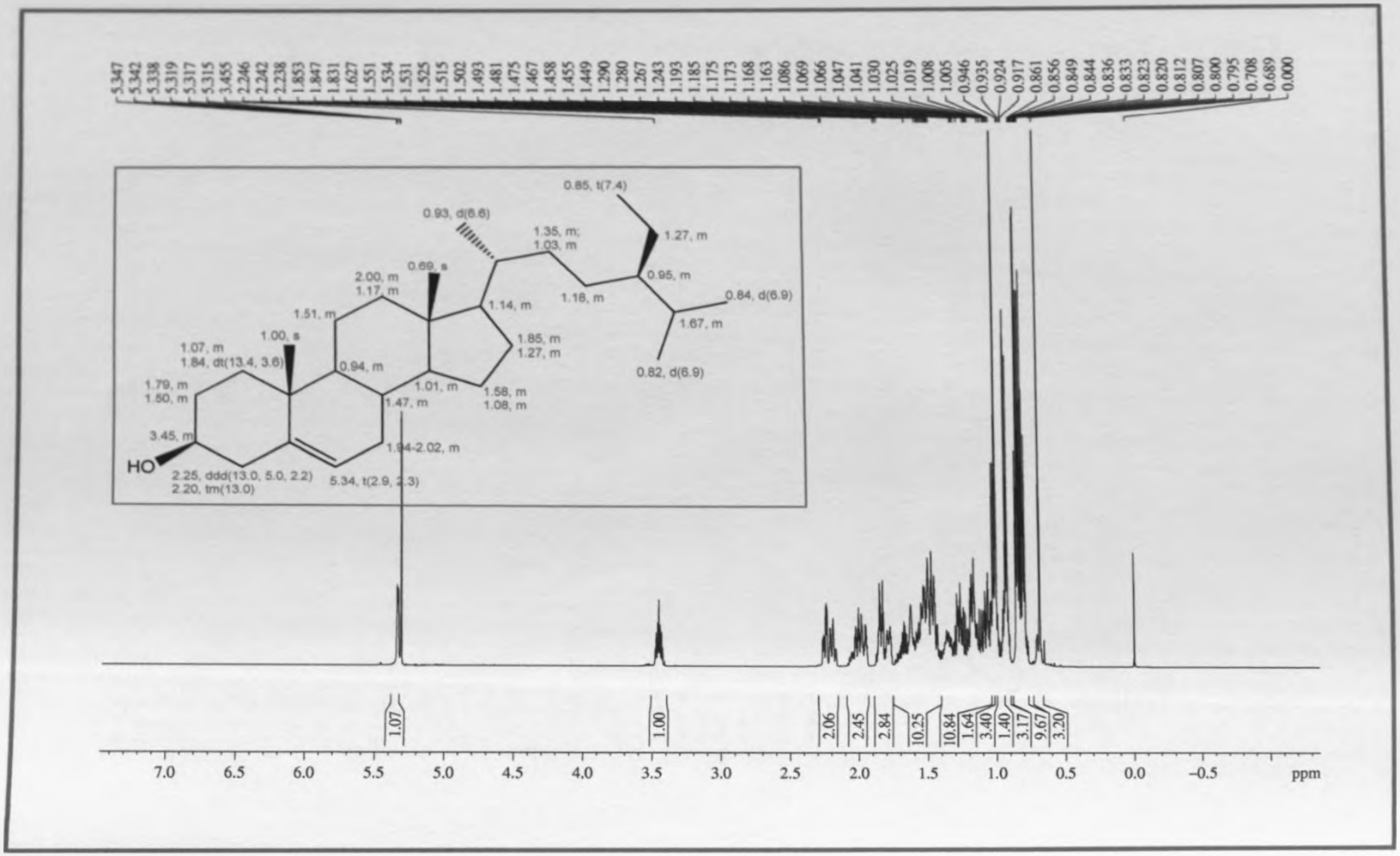
Appendix 38B: ^{13}C-NMR spectrum of stigmasterol (**66**), CD_2Cl_2, 150 MHz



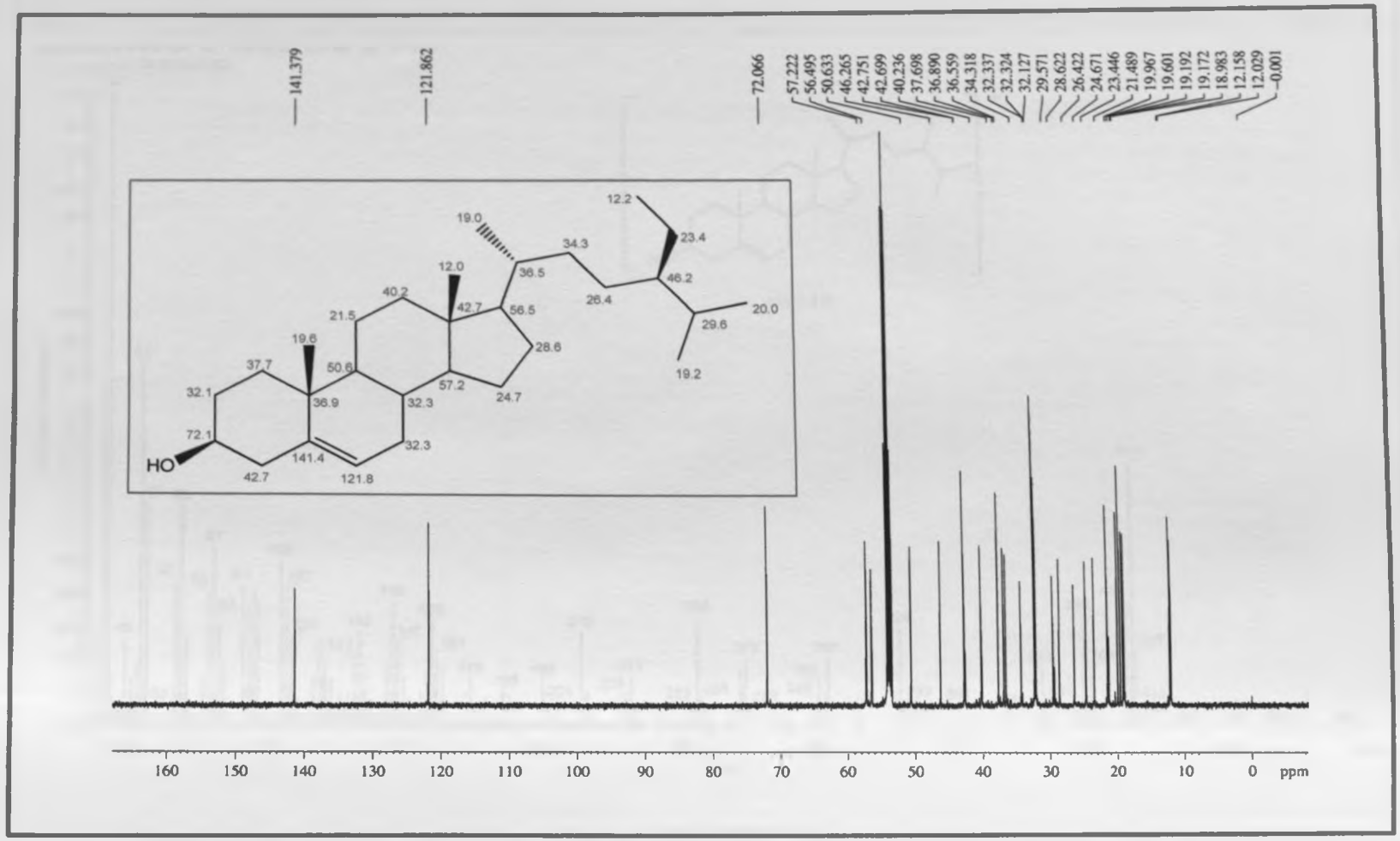
Appendix 38C: EI-MS spectrum of stigmasterol (66)



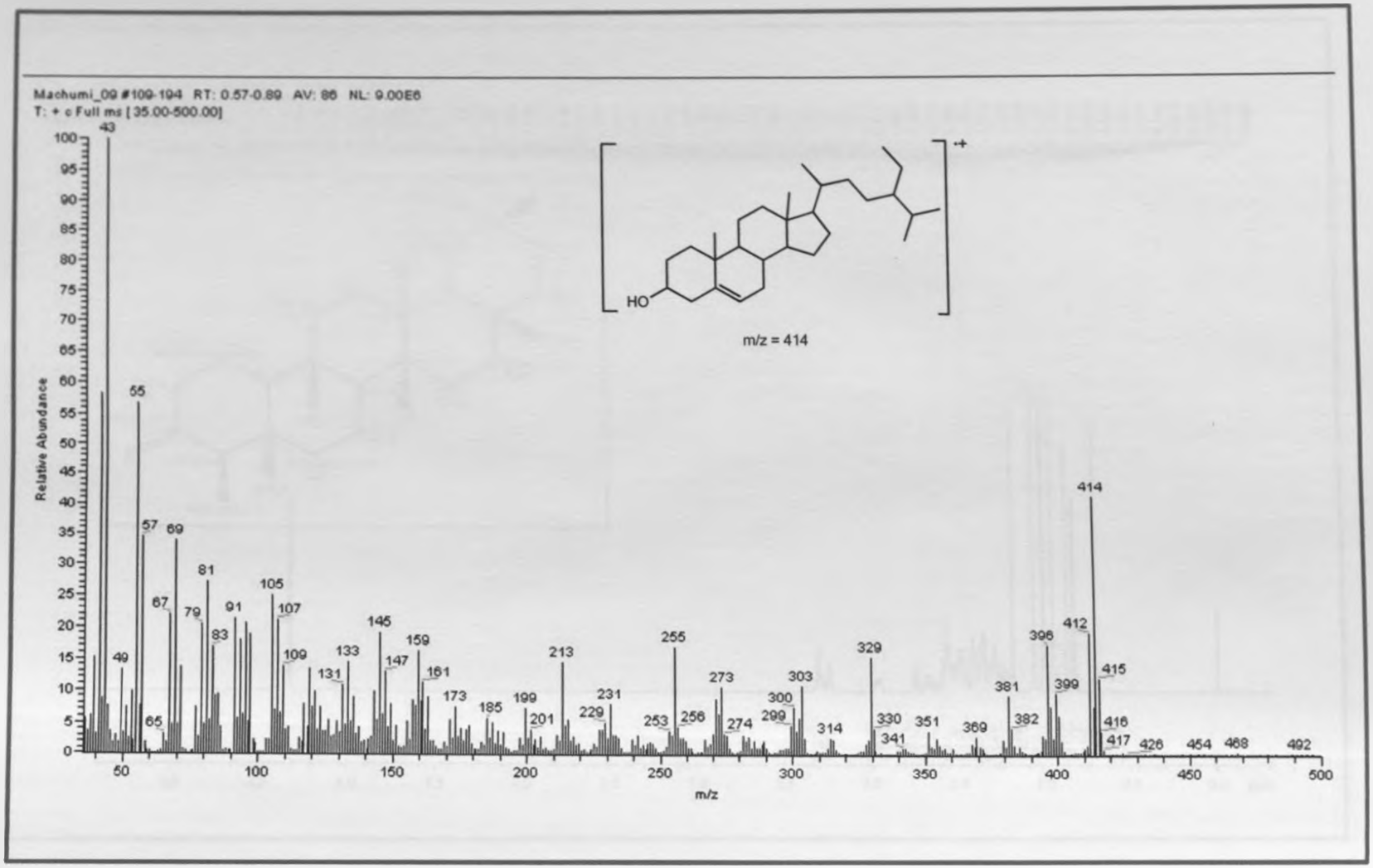
Appendix 39A: ^{1}H-NMR spectrum of β-sitosterol (**249**), CD_2Cl_2, 600 MHz



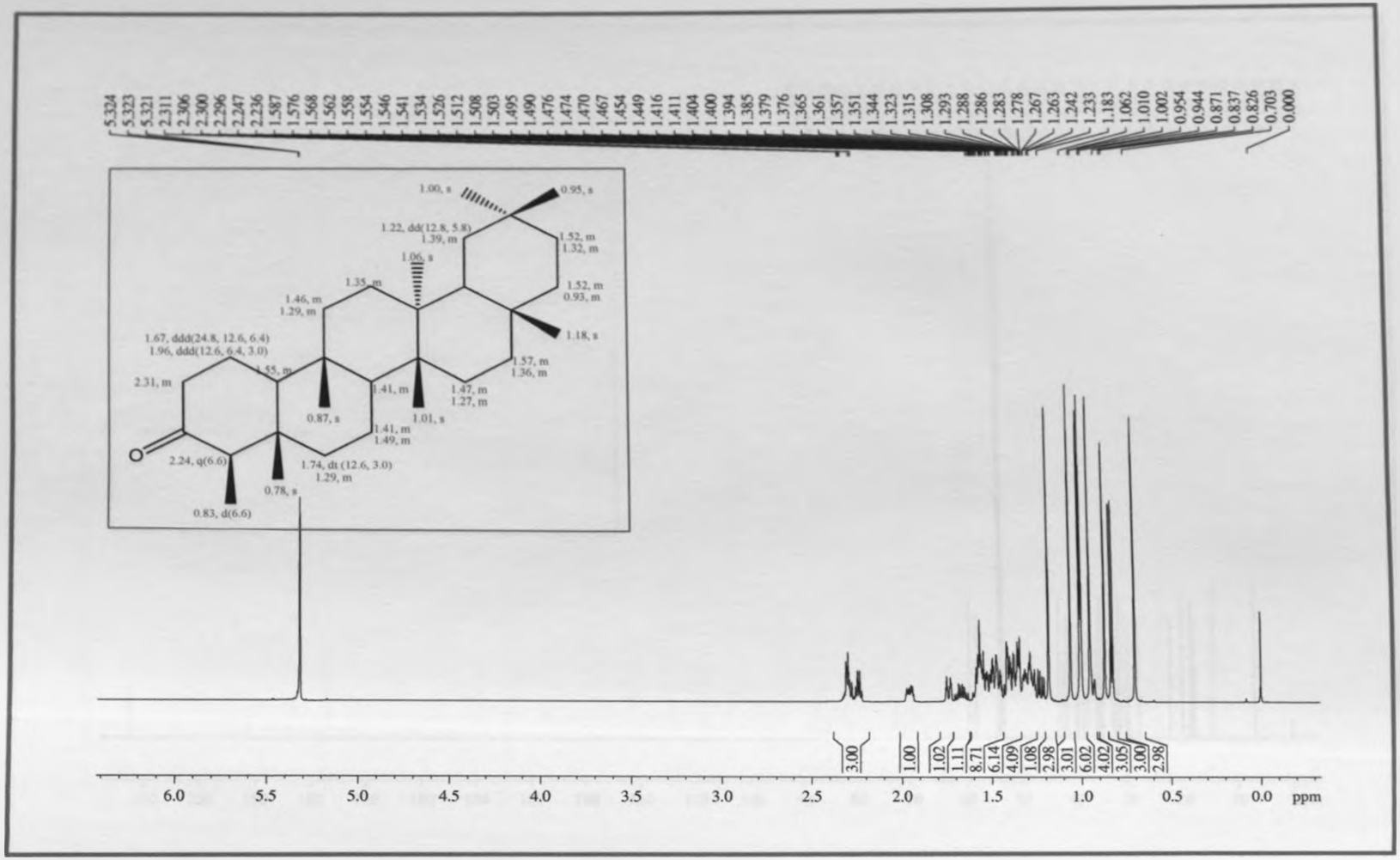
Appendix 39B: ^{13}C-NMR spectrum of β-sitosterol (**249**), CD_2Cl_2, 150 MHz



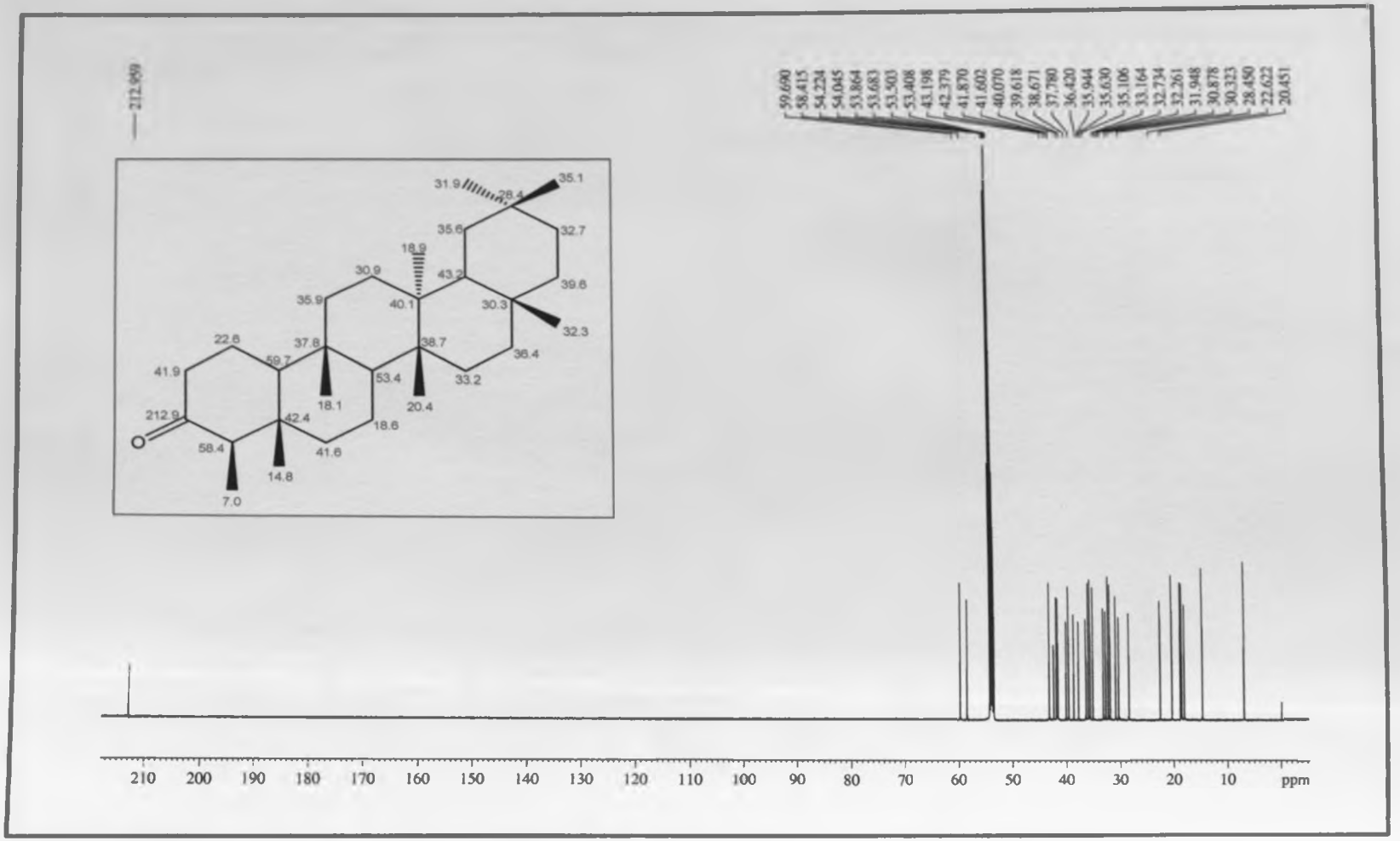
Appendix 39C: EI-MS spectrum of β-sitosterol (**249**)



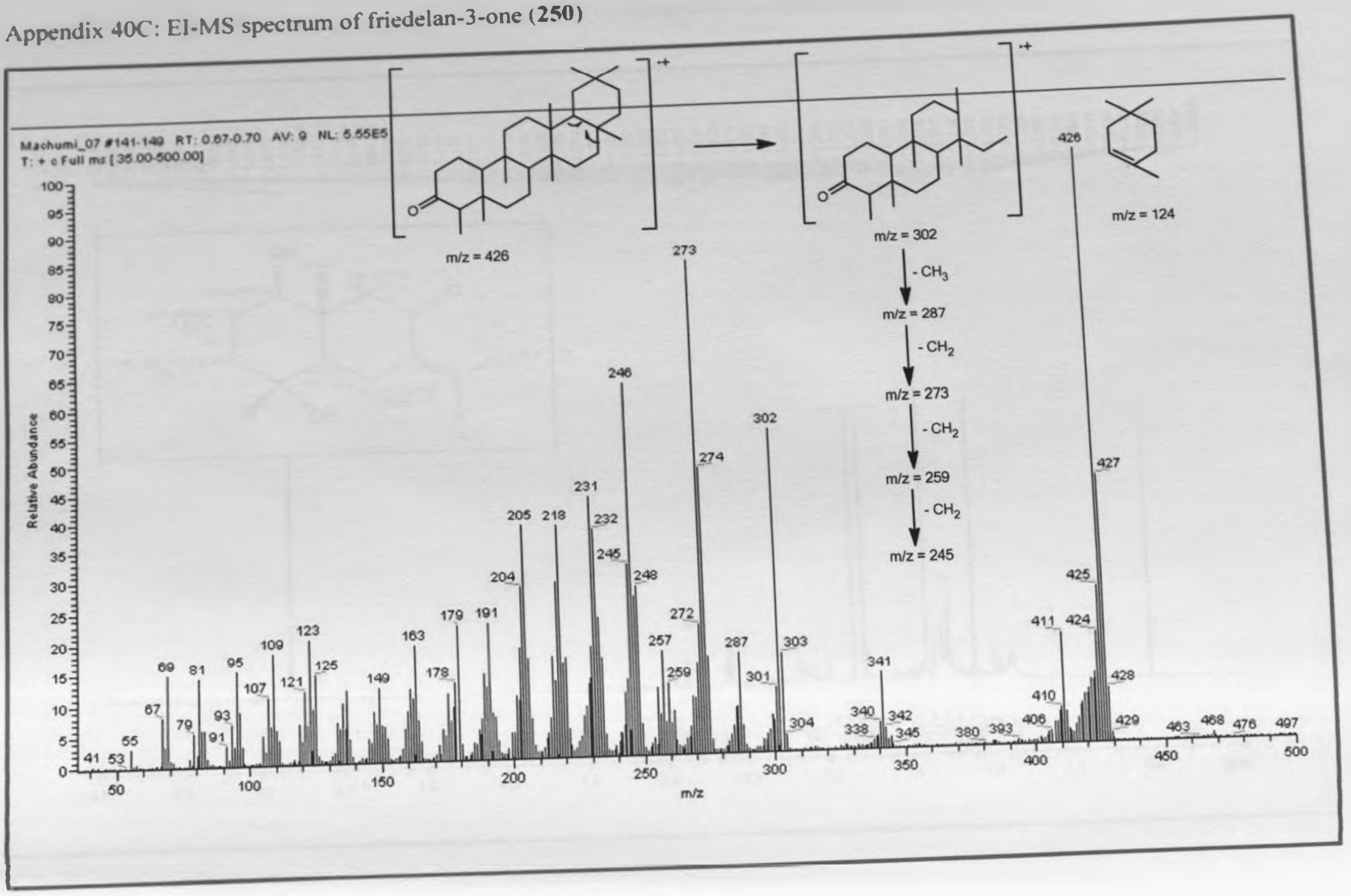
Appendix 40A: ^{1}H-NMR spectrum of friedelan-3-one (**250**), CD_2Cl_2, 600 MHz



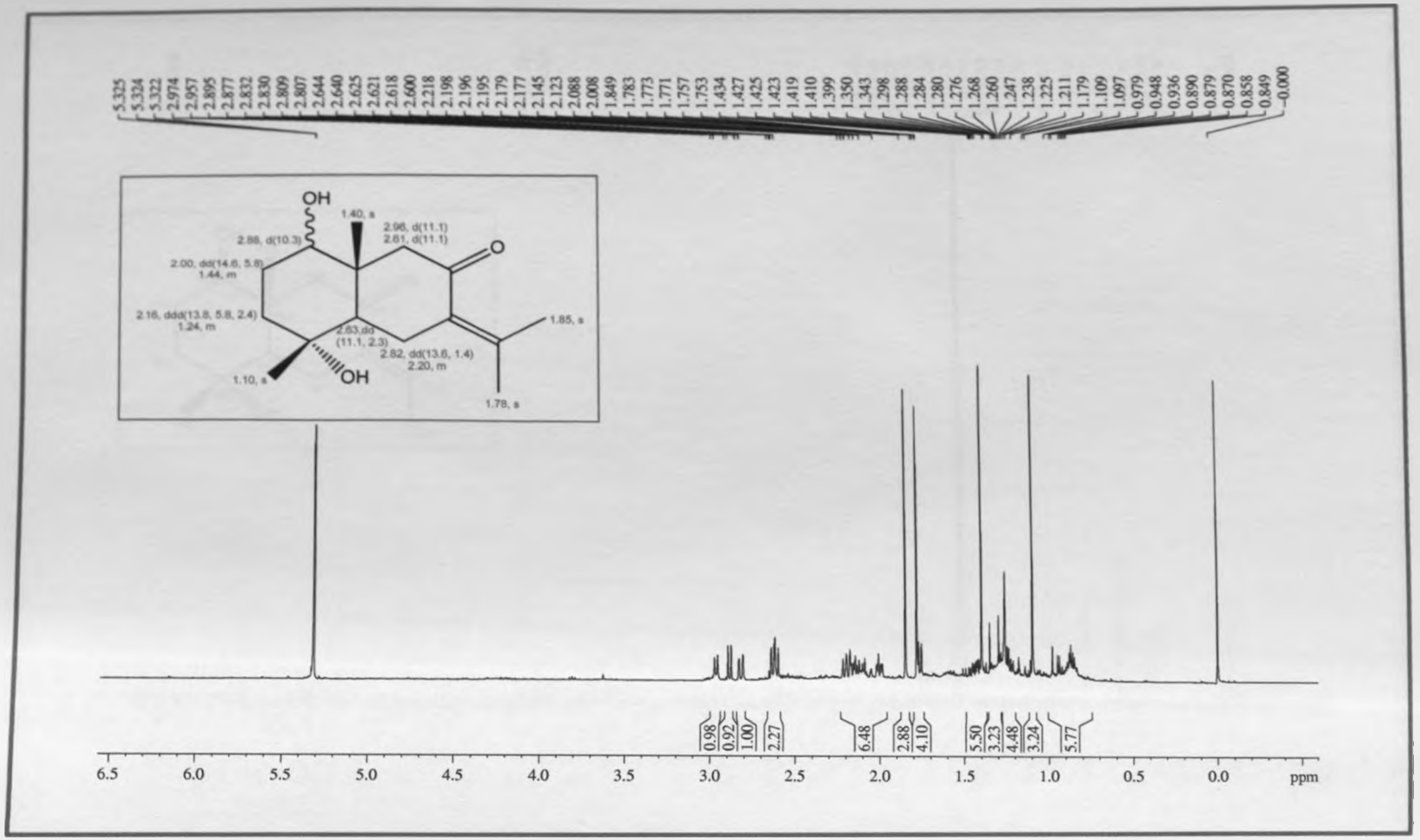
Appendix 40B: ^{13}C-NMR spectrum of friedelan-3-one (**250**), CD_2Cl_2, 150 MHz



Appendix 40C: EI-MS spectrum of friedelan-3-one (**250**)



Appendix 41A: ^{1}H-NMR spectrum of 1.4-dihydroxy-7(11)-eudesmen-8-one (**251**), CD_2Cl_2, 600 MHz



Appendix 41B: ^{13}C-NMR spectrum of 1,4-dihydroxy-7(11)-eudesmen-8-one (**251**), CD_2Cl_2, 150 MHz