PHYTOCHEMICAL INVESTIGATION OF RESINS FROM KENYAN *COMMIPHORA HOLTZIANA*

BY

ROSE CHITEVA

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL CHEMISTRY OF THE UNIVERSITY OF NAIROBI

2010

THIS IS MY ORIGINAL WORK AND HAS NEVER BEEN PRESENTED FOR A DEGREE IN ANY UNIVERSITY

....

CHITEVA, ROSE

I56/7132/2006

THIS THESIS HAS BEEN SUBMITTED FOR EXAMINATION WITH OUR APPROVAL AS UNIVERSITY SUPERVISORS

....

PROF. ABIY YENESEW
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

DR. JOHN WANJOHI
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

....

DR. BEN CHIKAMAI

KENYA FORESTRY RESEARCH INSTITUTE (KEFRI)

DEDICATION

To my dear mother who ailed the entire period of my study, I thank God for sustaining your life.

ACKNOWLEDGEMENTS

First and foremost, to GOD for giving me the gift of life.

I would like to express my sincere gratitude to my supervisors, Prof. Abiy Yenesew, Dr. John Wanjohi and Dr. Ben Chikamai for their guidance, support and encouragement through out my MSc. Research.

To the Director Kenya Forestry Research Institute, (KEFRI) for allowing me time off Duty to undertake this study. To the National Program Co-ordinator, Acacia Operations, Mr. Meshack Muga through the Drylands program for funding the field Work.

To Mr. Norman Gachathi, Kenya Forest Research Institute (KEFRI) taxonomist for the identification of *Commiphora holtziana* trees in the field. To Kenya forest Service (KFS) staff both in Isiolo and Wajir, especially, District Forest Officer (DFO) Wajir, Ms. Ambia Osman for providing transport and staff to the field. To the International Center for Insect Physiology and Ecology (ICIPE) staff, Dr. W. Lwande, Mr J. Bwire, Dr. W. Torto and Mr. Wanyama for making it possible to run GC-MS analysis on my samples.

To Dr. E. Matu of Kenya Medical Research Institute (KEMRI), Dr. B.K. Amugune and Mr. Mugo of the Department of Pharmaceutical Chemistry, University of Nairobi, for their assistance in doing antimicrobial assay.

To the KEFRI- Karura Laboratory technologists namely Messer's Norman Wachira, Moses Lukibisi and Reuben Shanda for their assistance in one way or another. To Mr. Milton Manguro of KEFRI- Karura for his assistance in photocopying the thesis.

My sincere thanks go to Mr. Hannington Twinomuhwezi, PHD student in Natural Products Chemistry, University of Nairobi/Makerere University, for his support and guidance in purification of compounds.

Finally, to my parents Mr. and Mrs. Chiteva, brother Dickson and family, dear friends Pastor Charles of Uganda, Prof. Shamala of Colorado, USA and Jimmy for their perseverance, Love, encouragement and support both emotionally and financially during this period of study.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

APPENDICES

LIST OF ABBREVIATIONS

ALNAP	African Laboratory for Natural Products
CIFOR	Center for International Forestry Research
d	Doublet
dd	Doublet of a doublet
DCM	Dichloromethane ($CHCl_2$)
DEPT	Distortionless Enhancement by Polarization Transfer
DFO	District Forest Officer
EIMS	Electron Ionization Mass Spectroscopy
EtOAc	Ethyl acetate
GC – MS	Gas Chromatogram, Mass spectroscopy
ICIPE	International Center for Insect Physiology and Ecology
KEFRI	Kenya Forestry Research Institute
KEMRI	Kenya Medical Research Institute
KFS	Kenya Forest Service
m	Multiplet (multiplicity)
$[M]^+$	Molecular ion
MHz	Mega Hertz
MSD	Mass Spectrometer Detector
m/z	Mass to charge ratio

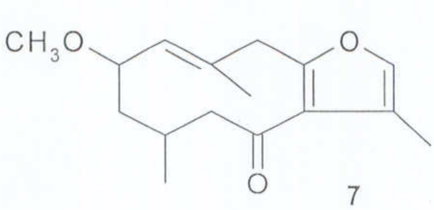
N/A	Not applicable
NMR	Nuclear Magnetic Resonance
PhD	Doctor of Philosophy
s	Singlet
ssp	Sub- species
UoN	University of Nairobi

ABSTRACT

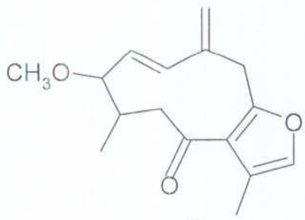
Commiphora holtziana gum resins were extracted by steam distillation to separate essential oil. The residue was successively extracted with methanol, acetone, ethyl acetate, dichloromethane and finally with hexane. A combination of chromatographic separation techniques on hexane extract of the Wajir sample of *Commiphora holtziana* led to the isolation and characterization of a new compound, 11–hydroxy-γ-muurolene **(39).** In addition, two known compounds, (1E)-2-methoxy-8,12-epoxygermacra-1(10),7,11-triene-6-one **(7)** and (1E)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one **(8)** were also characterized. A total of 14 compounds were identified by the comparison of the mass spectra with data available in the GC – MS library.

In the antimicrobial assay, the crude extract from Wajir showed an appreciable activity against three Gram positive bacteria tested namely, *Bacillus pumilis*, *Bacillus subtilis* and *Staphylococcus aureus.* Activity was also noted in the crude dichloromethane extracts from both populations which were active against the three Gram positive bacteria, the Gram negative bacteria *Escherichia coli* and the fungus *Sacharomyces cerevisiae*. The hexane extracts from both populations showed activity against the three Gram positive bacteria. In addition the hexane extract from Wajir population showed activity against the fungus *Sacharomyces cerevisiae.* The acetone extract from Wajir population showed activity against the Gram positive bacteria.

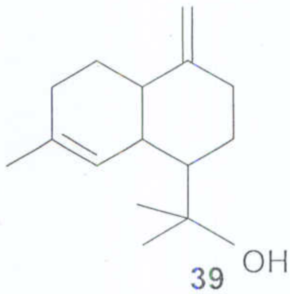
The pure compounds **7**, **8** and **39**, were also tested but no activity against the micro-organisms used were observed. The wound healing traditional use of the plant is probably related to the antibacterial and antifungal activity observed in this study.



7



8



39

CHAPTER ONE

INTRODUCTION

1.1 General Background

Resins are derivatized hydrocarbon secretions of many plants valued for their uses as incense, varnishes, adhesives and also for preparation of perfume. Fossilized resins are the source of amber. The resin produced by most plants is a viscous liquid, typically composed of volatile oils, with lesser components of dissolved non-volatile solids which make resin thick and sticky. The most common terpenes in resin are the monocyclic and bicyclic terpenes, and to a lesser extend the tricyclic terpenes. Some resins also contain a high proportion of resin acids which are protectants [Langenheim, 2003].

Among the resin producing plants is the genus *Commiphora* which produces *oleo*–gum resins. These comprise basically, essential oils, resin acids (alcohol soluble) and water soluble gums. Resins obtained from *Commiphora* species especially from *C. myrrha* are highly valued commercially. *C. myrrha* resin is composed of between 3 to 8% essential oil, 25 to 40% alcohol soluble resins and 30 to 60% water soluble gum [Tucker, 1986]. *C. holtziana* and a few other *Commiphora* species are sometimes used as adulterators of *C. myrrha* [Dekebo *et al.*, 2002a].

Kenya is among the few countries endowed with *Commiphora* trees. Isolation, identification and characterization of monoterpenes and sesquiterpenes of several *Commiphora* species has been reported [Provan *et al.,* 1987]. Many groups have studied the phytochemistry of the genus *Commiphora* and found dammarane triterpenes [Dekebo *et al.,* 2002a; Waterman and Ampoto, 1985], ferulates [Zhu *et al.*, 2001], furanosesquiterpenes [Brieskorn and Noble, 1983; Manguro *et al.*, 1996; Maradufu, 1982; Ubillas *et al.,* 1999], masumbinane derivatives [Provan *et al.,* 1992], steroids [Bajaj and Dev, 1982], lanosterols [Provan and Waterman, 1988], sesquiterpenes [Anderson *et al.,*1982; Dolara *et al.*, 2000], oxygenated alkanes [McDowell *et al.*, 1988], guggulsterones [Swaminathan *et al.*, 1987], guggutetrols [Kumar and Dev, 1987], and lignans [Provan and Waterman, 1985]. *Commiphora holtziana* is locally used by the Borana community of Northern Kenya as a tick repellant and for wound healing [Hanus *et al.,* 2005]. In this project the isolation, characterization and antimicrobial studies was conducted on *Commiphora holtziana.*

1.2 Justification of the Research

Commiphora holtziana is valued highly for its medicinal properties by the local community where it is found, and commercially in the Far-East in preparation of herbal medicines. Generation of knowledge of its chemistry, and its biological activity represent a rewarding task, as this plant has a vast untapped source with an enormous potential for developing useful products. These include tick repellants and anti–microbial agents building on their indigenous and local knowledge.

1.3 OBJECTIVES

1.3.1 General Objective

To establish the chemical profile and antimicrobial activities of the resin components of *Commiphora holtziana*.

1.3.2 Specific Objectives

(i) To separate and quantify the three components of *Commiphora holtziana,* namely: essential oils, resins and gum.

(ii) To isolate and characterize the major components of the *Commiphora holtziana* resins.

(iii) To establish the similarities and differences in the chemical profiles of resins collected from Isiolo and Wajir Districts.

(iv) To determine the *in vitro* antimicrobial activity of *Commiphora holtziana* extracts.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of Burseraceae family

Burseraceae is a moderately sized family of 17-18 genera and about 540 species of flowering plants. The actual numbers differ according to the time period in which a given source is written describing this family. The Burseraceae is also known as the Torchwood family, the frankincense and myrrh family, or simply the incense tree family. The family includes both trees and shrubs, and is native to tropical regions of Africa, Asia and the Americas [Judd *et al.,* 2008].

Some trees and shrubs of the Burseraceae are characterized by resins (having triterpenoids) and ethereal oils [Cronquist, 1981] that are present within the plant tissue from the vertical resin canals and ducts in the bark to the leaf veins. The outer bark often peels off in flakes, scrolls, strips or sheets, usually translucent, transmitting light or bluish green under the bark [Judd *et al,*. 2008]. Leaves are spirally arranged, usually without stipules, impair pinnate, 1–3 foliate or occasionally simple, rarely bi–pinnate in America [Gillet, 1991]. There are three known genera in Burseraceae, *Canarium, Boswellia* and *Commiphora.* These members are classified as shown in Figure 1 below.

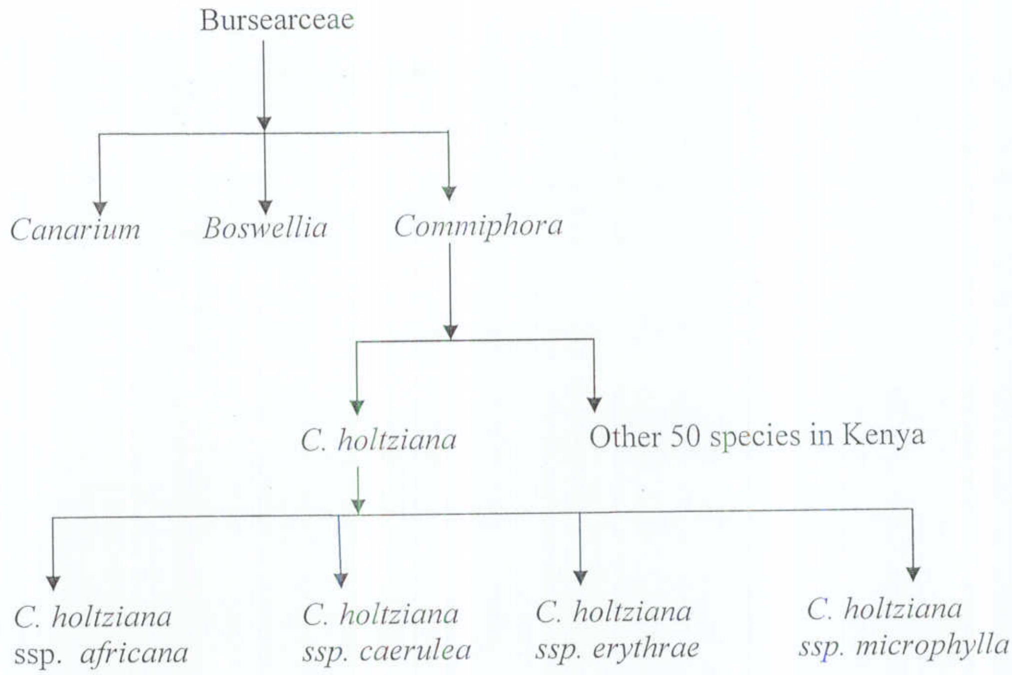


Figure 1: Taxonomy of *Commiphora* species [Gillet, 1991].

2.1.1 The genus *Commiphora*

The genus *Commiphora* (Burseraceae family) comprises over 150 species, most of which are native and confined to Eastern Africa: Kenya, Uganda, Tanzania, Ethiopia and Somalia [Beentje, 1994; Gillet, 1991]. There are however few species occurring in Arabia and India [Vollesen, 1989]. There are about fifty species distributed in the *Acacia* – *Commiphora* woodlands in Kenya [Brieskorn and Noble, 1980; 1983]. However only a few of these species are known to produce gum resins of commercial importance.

C. myrrha is economically the most important species as a source of gum resins; while, gum resins obtained from various other species of *Commiphora* including *C. holtziana* (Table 1) are used as substitutes of myrrh and the practice is widespread [Tucker, 1986]. This has complicated the chemical knowledge of myrrh, because most previous chemical studies reported on this gum resin [Brieskorn *et al.*, 1980] were based on commercial material, and not on a product obtained from properly identified trees [Maradufu, 1982].

The characteristic essential oil of myrrh with its unique scent and composition can be obtained only from *C. myrrha.* Resins of other species may seem similar in appearance to the true myrrh but chemical profiles may be totally different from myrrh. For instance *C. erlangeriana* does not yield any essential oil, lacks the characteristic sesquiterpenes of *myrrh,* contains instead the highly interesting podophyllotoxin (non – alkaloid toxin) type lignans (Dekebo *et al.,* 2002b), which exhibit cytotoxic and cytostatic activities [Habtemariam, 2003]. The full botanical account of the more than 50 *Commiphora* species that occur in Eastern Africa has been documented [Vollesen, 1989].

Myrrh and Opopanax have been used throughout history as incense and in perfumery. The two are hardened, resinous exudates obtained from trees of certain *Commiphora* species of the Burseraceae family. *Myrrh* and Opopanax oils are occasionally used as flavoring agents. Somalia, Ethiopia and Kenya are by far the largest producers of the two gum resins, while the Republic of China is the largest market for the two resins, mainly for use in traditional medicine [Chikamai and Odera, 2002].

Table 1: Some Resin-producing *Commiphora* species

Commiphora spp.	Local names	Distribution	Uses	References
C. africana (A. Rich) Engl. syn *C. pilosa*	Hamess (Borana)	Ethiopia, Sudan, Nigeria	Fragrance ingredient, termite repellent	Tucker [1986]
C. confusa Vollesen	Tichacho (Borana)	N. Kenya	-	Manguro *et al.* [2003]
C. erythraea Engl.	Haggr-ad (Somali)	Somalia, Kenya, Ethiopia, and S. Arabia	Fragrance	Brieskorn & Noble [1980 & 1983]
C. gileadensis (Forssk.) Engl.	Dakellah (Somali)	Arabia, also Djibouti, Ethiopia, Kenya, Somalia, Sudan	"more valuable than frankincense"	Chaudary and Al Jowaid [1999]
C. habessinica (Berg) Engl. syn. *C. madagascariensis* Jacq.	Jalanga (Borana)	Kenya	-	Wild [1963]
C. holtiziana ssp. *holtziana* syn. *C. caerulea* Burtt.	Hagar (Somali)	Kenya	Fragrance, tick repellent	Gachathi [1997]
C. kataf (Forssk.) Engl.	-	Kenya to Saudi Arabia, Yemen	Fragrance	Brieskorn & Noble [1980 & 1983]
C. merkeri Engl.	Ol-dimitil (Maasai)	South & East Africa	Gum-resin is used as anti-inflaming, anti-septic etc.	Fourier & Synckers [1989]

Table 1 cont'd

C. myrrha (Nees) Engl. or *C. myrrha* Engl. var. *molmol*	Mol mol (Somali)	Yemen, Saudi Arabia; also Somalia, Ethiopia & Kenya	Resin used for treatment of colds & fevers, to treat haemorrhoids & toothache.	Grieve [1995]
C. pseudopaoli JB Gillet syn. *C. paolii* Chiov.	Lailipai (Samburu)		Tick repellent.	Gachathi [1997]
C. schimperi (Berg.) Engl. syn. *C. buraensis*	Laisamis (Samburu)	Kenya		Hyde & Wursten [2009]
C. tenuis K. Vollesen	Angule (Borana)	Ethiopia	Veterinary: wound healing uses	Asres *et al.*, [1998]

Dekebo *et al.*, [2002a] has indicated that myrrh is frequently adulterated with gums of *C. sphaerocarpa* and other *Commiphora* spp., adding to the picture of widespread *Commiphora* commodity adulteration.

2.1.1.1 *Commiphora holtziana*

Commiphora holtziana is a spiny tree up to 6m tall with a well defined trunk. The outer bark is white to yellow, peeling in large papery flakes (Figure 1) from the bluish green under bark. The exudates are faintly scented, forming gum resins commonly known as *hagarsu* in Borana. The leaves are greyish–green, 3 foliate or occasionally 5 foliate on long shoots. In Kenya, *C. holtziana* is widely distributed in the dry lands, particularly in Northern Kenya, in *Acacia - Commiphora* bush land, on well drained red sandy soils, 20 – 1100m with rainfall 220 – 630mm annually [Gachathi, 2005]. Oleo-gum resin (Figure 2) from *hagar* (local name for *C. holtziana*) is collected from plant exudations caused by insects or animal damage.

Figure 2: *Commiphora holtziana* flaky bark

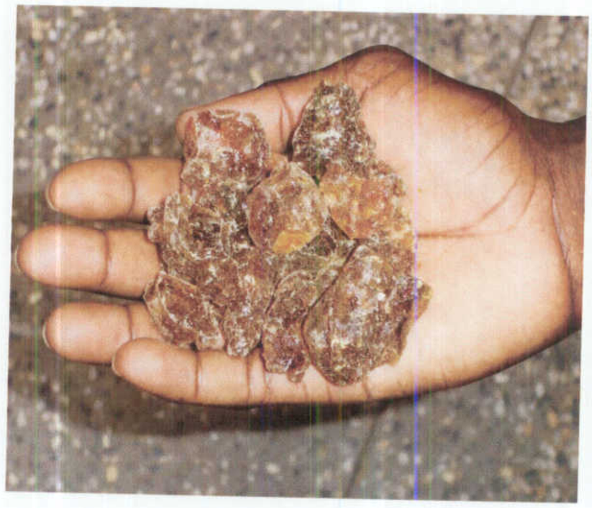


Figure 3: *C. holtziana* oleo-gum resin

Commiphora holtziana has four known sub – species; *C. africana* which is taller*, C. erythrae, C. caerulae and C. microphylla* which has smaller leaves and fruits than the *C. holtziana*

2.1.1.1.1 *Commiphora africana*

C. africana is a spiny tree up to 10m tall: trunk cylindrical, usually beset from near the base with horizontal spiny branches; bark peeling in shiny reddish brown or grey scrolls; slash mottled red granular; exudates milky, slightly scented, producing hard gummy resin. Leaves never completely glabrous; all leaflets crenate-serrate, the laterals sometimes especially on long shoots much shorter than terminals. The most widespread variety is found in *Acacia - Commiphora* bush land between 5 – 1780m above sea level with rainfall of between 250 – 800mm annually [Bentje,1994].

2.1.1.1.2 *Commiphora caerulea*

Deciduous tree. Bark smooth, distinctly pale bluish, peeling in papery translucent straw-coloured strips; young branches greyish, velvety, not spine-tipped. Leaves clustered, imparipinnate with 2-4 pairs of leaflets, more rarely 3-foliolate, both types together on the same tree; leaflets oblong-elliptic, up to 8 × 4.5 cm, greyish velvety below, hairless or with a few scattered hairs on the veins and midrib above; margin scalloped or finely toothed [Bentje,1994].

2.1.1.1.3 *Commiphora microphylla*

Tree 3 – 4m. Petiole less than 12mm long. Lateral leaflets up to 8 by 6mm, terminally up to 10 by 7mm. Both male and female flowers solitary. Found in open *Acacia – Commiphora* bushland, 60 – 270m above sea level with a rainfall of 220 – 350mm annually [Bentje,1994].

2.2 Gums and Resins

The terms "gum" and "resin" are used interchangeably in everyday language: both terms refer to something that is sticky, smooth and elastic. However, specialists differentiate between the two terms based on numerous characteristics as seen in Table 2.

Table 2: Differences between gums and resins according to Nair [2007]

Gums	Resins
1. Water soluble 2. Exude from disintegration of cell wall 3. Edible 4. Not fragrant 5. Does not burn	1. Insoluble in water 2. Produced spontaneously or as a result of injury to the plant 3. Not edible 4. Aromatic 5. Flammable

The role of gums and resins in plants is to protect them against insect, fungal and other infestation, or to seal the tissue in incidents of physical damage [Nair, 2007].

2.2.1 Uses of Gums and Resins

The resins from African Burseraceae are important items of commerce, in preparation of glue, medicines and perfumes. The chemical components of the resins of many plants of the Burseraceae family have not yet been identified [Hanus *et al.*, 2005], though these resins have been used extensively throughout history [Hanus *et al.*, 2005]. The Dhofaris in the southern part of Oman use *Commiphora* plants to disinfect wounds and also as anthelmintic and hair shampoo [Miller and Moris, 1988].

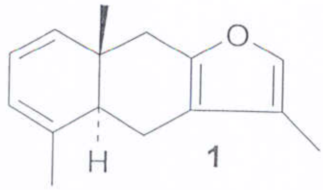
Table 3: Uses of Resins

Type of Resin	Examples/source	Uses
1. Hard and transparent	Dammars	Varnishes and cement
2. Soft and odoriferous	Turpentine	Therapeutic and incense
3. Gum resins with essential oils	Myrrh	Therapeutic and incense
4. Rosin (Resin without volatiles)	*Pinus* species	Adhesive and vanishes

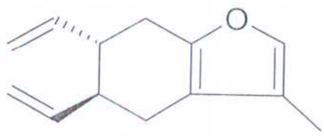
Gums are produced by members of a large number of families but commercial exploitation is restricted to a few species of Leguminosae, Sterculiaceae and Combretaceae. Gum is also extracted from seeds, seaweeds, micro-organisms and *Aloe barbadensis* (*Aloe* gum), wood chips of *Larix occidentalis* (stractan), seed coats or barns of corn, wheat, oats, barley, rice and soyabean (Hemi-cellulose). Resins, on the other hand, occur in a wide range of plants. They are formed in the specialized structures called ducts [Nair, 2007].

2.2.1.1 Uses of some Resins of the *Commiphora* species

Uses of *Commiphora* species are many. For example, it was reported that two compounds of myrrh, furanoeudesma-1,3-diene **(1)** and curzarene **(2),** are responsible for the pain relieving (analgesic) properties of myrrh in traditional therapies [Archaelogy, 1996]. The anti-inflammatory, antipyretic, antihistaminic [Tariq *et al.,* 1985], antigastric and cytoprotective [Al–Harbi *et al.,* 1997], anti-tumour [Queshi *et al.,* 1993] properties are among the few other activities of *Commiphora myrrha.*



1



2

Commiphora myrrha is an economically and ecologically important plant species found mainly in the horn of Africa particularly in Ethiopia, Somalia and Kenya and to some extent in the Arabian peninsula. The tree yields the aromatic gum or resin known as myrrh which is broadly defined as "a product of several species of *Commiphora*" [Blumenthal *et al.,* 2000]. This is however erroneous and misleading as the so called other species yield resins which are chemically different and lacking the active principles for which myrrh is so well known for. In fact mixing myrrh with resins from other *Commiphora* species leads to the perennial problem of adulteration. This problem has been clarified by the works of Dekebo *et al.,* [2002a].

Myrrh was used since several millennia as medicine as well as for ceremonial and religious purposes. In many cultures in Europe, Asia and Africa, myrrh has enjoyed various traditional and industrial uses and applications. A recent study conducted in Saudi Arabia on the prevalence and pattern of use of alternative medicine, based on interviews of 1408 individuals, revealed the most frequently used medicines were honey (40%), black seed (39%) and myrrh (35%) [Al-Faris *et al.,* 2008]. The traditional medicinal use of myrrh extends to several countries where it has been used as a cleansing and purifying agent since ancient times [Al-Faris *et al.,* 2008].

2.2.1.2 Historical and Traditional Uses of *Myrrh*

Myrrh is one of the oldest medicines in the world. It has been mentioned in Egyptian medical texts since 2,800 BC, and is one of many herbs mentioned in the Ebers Papyrus, which documents over eight hundred medicinal recipes. The Egyptians consumed large amounts of myrrh, both in temple rituals and embalming; it was also burned in temples of Babylon, Greece, India, Rome and China. It is one of the ingredients of the famous magic-inducing incense, Kyphi, and the ointment Metopian, used for treating infections and wounds. In Chinese medicine, the use of myrrh was recorded as early as 600 BC during the Tang Dynasty, where it was used in a similar manner. Like frankincense, myrrh was an important trade item for more than a thousand years [Davis, 1999].

Traditionally, myrrh was used for as many diverse purposes as frankincense. It was a primary ingredient in incenses and holy oils used to inspire prayer, deepen meditation, and revitalize the spirit. It was used to fumigate the body to promote cleanliness and stimulate immunity, and continues to have an important role in cosmetics and perfumery. It has also been used to treat cattle and camels, and burned to repel snakes [Essential science, 2007].

2.2.1.3 Therapeutic Uses of Myrrh

Like frankincense, myrrh resin is a predominant part of the tree's immune system. Many of the therapeutic functions of myrrh are therefore similar to frankincense. A comparison of the two reveals that myrrh is more astringent, antiseptic, disinfectant, bitter, and tonic; while frankincense is more anti-inflammatory, blood vitalizing, and mentally uplifting. The two are often combined. Like frankincense, myrrh has a long history of use for a wide range of conditions, with virtually no toxicity [Lawless, 1995).

The Eclectic physician Dr. Ellingwood describes the therapeutic properties of *myrrh* as follows: "This agent has always been highly esteemed as a stimulant, although its influence is more of a local than a general character [Davis, 1999]. It exercises the characteristic influence of most of the stimulants upon the excretions and secretions, acting as a diaphoretic, expectorant, sialagogue, and to a certain extent emenagogue. As a most active general stimulant in ulcerative, engorged, flabby and atonic conditions of the mucous membranes of the mouth and throat this agent acts promptly. It stimulates the capillary circulation, restores tone and normal secretion and causes the healing of ulcerations. In its influence upon the digestive apparatus myrrh is direct in its action. It quickly increases the power of the digestive function, stimulating the peptic glands to extreme action. It increases the appetite and promotes the absorption and assimilation of nutrition. It is given in atonic dyspepsia in the absence of inflammatory action, especially if there is excessive mucous discharge from the bowels" [Davis, 1999].

Below is a brief list of the most important therapeutic applications of myrrh, which is by no means complete; like frankincense, its uses are so numerous that it can also be described as a panacea [Davis, 1999].

2.2.1.3.1 Mouth and Throat

Myrrh is a specific and highly effective antiseptic astringent for inflammations of the mouth, throat, and gums. It is a common ingredient of herbal toothpowders and mouthwashes, and is widely used through India and the Middle East for oral and dental problems. The German Commission has approved myrrh for treating mouth inflammation. Its list of indications includes mouth sores and ulcers, gingivitis, irritation from dentures, soreness and looseness of teeth and gums, gum disease, tooth decay, and bad breath. Myrrh is also very effective for infectious and inflammatory conditions of the throat, including strep throat, tonsillitis, and pharyngitis. For these various symptoms, tincture of myrrh can be diluted and used as a mouthwash and gargle, or applied directly to sores. It is frequently combined with Echinacea and/or golden seal for these purposes [Dolara *et al.,* 2000].

2.21.3.2 Digestion

In the digestive tract myrrh acts as a stimulant, carminative, tonic and chologogue. Its bitter principles stimulate the appetite and the flow of digestive juices, improving digestion and absorption. It both relaxes and invigorates the stomach, calming spasms, relieving gas, and combating fatigue associated with weak digestion. Its antibacterial and antifungal powers help reduce *candida* and other pathogenic factors in the gut. Myrrh has pronounced anti-parasitic properties. By improving digestion myrrh clears toxins from the digestive tract and acts as a general detoxifying and anti-inflammatory remedy, thereby treating the root causes of arthritis, rheumatism, and gout. It can be combined with *Aloe vera* for treatment of both the symptoms and causes of constipation [Davis, 1999].

2.2.1.3.3 Respiratory System

Myrh is a stimulant, expectorant, and decongestant with antibacterial properties. It is helpful for relieving bronchitis, asthma, and colds. In Ayurvedic terms, it dries kapha (mucous secretions), reduces pitta (antibiotic), and stimulates prana (opens breathing). In Chinese terms, it is a stimulant of Wei Chi (respiratory immune enhancing). It can be a specific remedy for chronic sinusitis. It can be used in carrier oil as a chest rub [Kiringe, 2006].

2.2.1.3.4 Skin

Myrrh is an astringent antiseptic that is beneficial for acne, rashes, and inflammatory skin problems. The tincture, powder, or essential oil of myrrh can be applied directly to ulcerated sores, wounds, and abrasions. It can be made into salves for treating hemorrhoids and bed sores. For boils it can be taken as a blood cleanser while also being applied externally. It is an excellent addition to the medicine cabinet of those who live in tropical places such as Hawaii, where staph infections can be easily acquired from coral cuts or walking on beaches [Dolara *et al.*, 2000].

2.2.1.3.5 Wounds and Bruising

Myrrh is similar to frankincense in its wound-healing and blood-vitalizing properties, and the two are often combined in liniments. *Myrrh* is also given for pain and without the resin (*C. mol*) it's used in abscesses [Lans *et al.*, 2006]. *Commiphora caudata* is also used in the treatment of mouth ulcers and wound healing [Ganesan *et al.*, 2002].

2.2.1.3.6 Antimicrobial and Immune Stimulant

Myrrh is both an antimicrobial agent and a direct stimulant of white blood cell production. It increases resistance to infection, and is one of the most effective of all known disinfectants from the plant kingdom. It is a rejuvenating tonic, and is reputed to enhance of the intellect [Dolara *et al.*, 2000].

A sampling of studies published on PubMed concerning myrrh derived from different species of *Commiphora* reveals that the resin reduces cholesterol and triglycerides; that it is a promising non-hepatotoxic anti-helminthic for schistosomiasis; that it is highly effective (100 per cent cure rate) on fascioliasis parasite without remarkable side effects; that its triterpene myrrhanol A is a more potent anti-inflammatory than hydrocortisone; that it possesses smooth muscle-relaxing properties; that its sesquiterpene fractions had antibacterial and antifungal activity against pathogenic strains of *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*; and that its extract has strong efficacy as an insecticide against the cotton leaf worm [Barakat *et al*,. 2005].

A sesquiterpenoid isolated from myrrh is highly effective against drug-resistant tumor cells found in the breast and prostate, without toxicity to healthy cells [Siegel, 2002].

Commiphora africana has a fragrant gum that is chewed, used as arrow heads on arrow shafts for play by new initiates during circumcision. The species is also used as live hedge [Ng'ethe *et al.*, 1999]. It is also used in open wounds, as a skin moisturizer and for killing jiggers [Kiringe, 2006].

The oleo – gum resin of *Commiphora mukul* is a versatile indigenous drug claimed by the Indian system of medicine to be highly efficacious in the treatment of rheumatism, obesity, neurological disorders, ills of syphilitic nature, scrofulous affections, urinary disorders and a few skin disorders. It is also used in the treatment of swollen gums, chronic tonsillitis and ulcerated throat. Recent pharmacological investigations on crude drug, its different fractions and pure constituents have revealed significant anti – inflammatory, anti-rheumatic [Atal *et al.,* 1975]; [Batchelder *et al.,* 2002].

2.2.1.4 Phytochemistry of *Commiphora* species

The gum of *Commiphora* species contains polysacharides and proteins, while the organic solvent extract is composed of steroids, sterols and terpenes [Hanus *et al.,* 2005]. Natural gums (gums obtained from plants) are hydrophilic carbohydrate polymers of high molecular weights, generally composed of monosaccharide units joined by glucocidic bonds [Davison, 1980]. They are generally insoluble in oils or organic solvents such as hydrocarbons, ether, or alcohols, but are either water soluble or absorb water and swell - up or disperse in cold water to give a viscous solution or jelly [Davison, 1980].

Resins are a complex mixture of polyterpenes containing various functional groups as a result of oxidation. Resins are soluble in organic solvents, but do not have affinity for water. The resins that are less soluble in organic solvents, can be made to dissolve by a process known as 'running' or sweating. When the resins contain essential oils, they are called oleoresins or soft resins. Gum resins are a combination of resins and true gums with a characteristic mixture of both. Certain gum resins contain small amount of essential oil. They are called oleo-gum resins. Small quantities of resins exude on the surface of the trunk due to injury by wind, fire, lightening or wound caused by animals. However, for commercial purpose tapping is necessary. Sometimes the natural exudation is so copious that the resins become buried and fossilized in the soil around the trunk. Vast deposits of resin may be found where the original forest has disappeared. Amber is an example of fossil resins [Davison, 1980]. Tree resins are composed of terpenes, diterpenoids and triterpenoids being the most common [Hanus *et al.,* 2005].

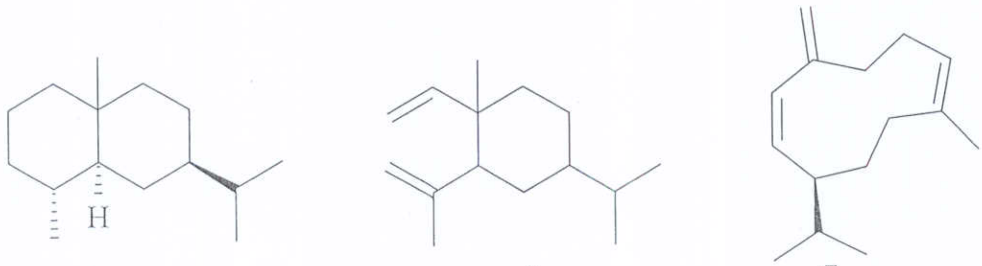
2.2.1.4.1 Terpenes

Terpenes are naturally occurring organic compounds formed through the combination of two or more C_5 units. Terpenoids can be thought of as modified terpenes, wherein methyl has been shifted or removed, or oxygen atoms added. Just like terpenes, the terpenoids can be classified according to the number of isoprene units used as seen in table 4.

Table 4: Classification of terpenoids [Newman, 2006]

Terpenoids	No. of C_5 units	No. of Carbon atoms	Occurence
Monoterpenoids	2	10	Essential oil
Sesquiterpenoids	3	15	Resin
Diterpenoids	4	20	Resin
Triterpenoids	6	30	Resin or saponin
Tetraterpenoids	8	40	Carotenoid

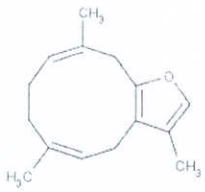
Resin samples collected from Kenya and attributed to *Commiphora myrrha* and *Commiphora holtziana* have been examined. These have yielded a wide range of sesquiterpenes notably furanosesquiterpenes based on eudesmane **(3)**, elemane **(4)** and germacrene **(5)** [Provan *et al.*, 1987].



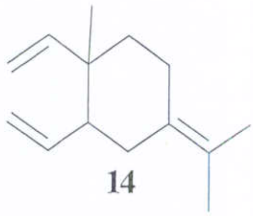
3 4 5

Table 5: Major sesquiterpenes identified from *Commiphora myrrha* and *C. holtziana*

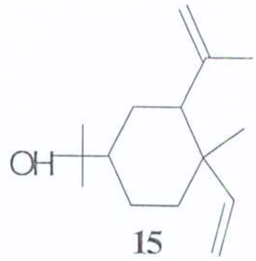
Components	***Commiphora myrrha***	***Commiphora holtziana***
Furanoeudesma-1,3-diene **(1)**	+	+
Isofuranogermacrene **(2)**	+	-
-(2*R*)-methyl-5*S*-acetoxy-4*R*-furanogermacr-1(10)Z-en-6-one **(6)**	-	+
1(10)E,2R,4R)-2-methoxy-8,12-epoxygermacra-1(10),7,11-triene-6-one **(7)**	-	+
(1E)-3-methoxy-8,12-epoxygermacra -1,7(8),10(15),11-tetraen-6-one **(8)**	-	+
Furanodiene **(9)**	+	-
2 - Methoxyfuranodiene **(10)**	+	-
4,5-dihydrofuranodiene-6-one **(11)**	+	+
Lindestrene **(12)**	+	+
β-Eemene **(13)**	+	-
γ-Elemene **(14)**	+	+
δ-Elemene **(15)**	-	+
Elemol **(16)**	-	+
Curzerenone **(17)**	+	+
α-Cubebene **(18)**	+	-
β-Bourbonene **(19)**	-	+



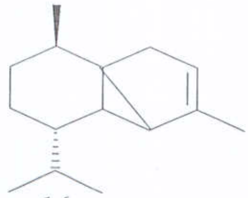
9



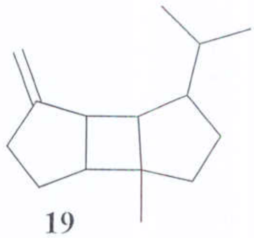
14



15



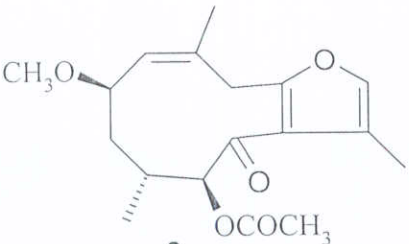
16



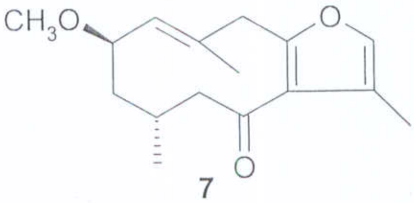
19

From table 5 it is possible to tell if a *C. myrrha* sample is adulterated with *C. holtziana* by checking out for the presence of compounds **6, 7, 8, 15, 16** and **19**. From the work done by Dekebo *et al.*, [2002a], it is interesting to note that the chemical profiles of resins from other *Commiphora* species, *C. holtziana* included, are quite distinct. Thin layer chromatography can be used as a tool to distinguish true myrrh from its common adulterants. Based on the presence or absence of isofuranogermacrene, furanodiene and furanodesma- 1, 3- diene, it is possible to detect adulteration of *C. myrrh* [Maradufu *et al.,* 1988].

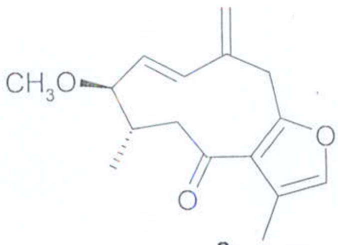
The resins from African Burseraceae are important items of commerce. The compounds of these resins are not yet well identified. Three known – *rel*-(2*R*)-methyl-5*S*-acetoxy-4*R*-methyl-furanogermacr-1(10)Z-en-6-one **(6),** (1E)-3-methoxy-8,12-epoxygermacra-1,7,10,11-tetraen-6-one **(8)** and (1(10)E,2R,4R)-2-methoxy-8,12-epoxygermacra-1(10),7,11-triene-6-one **(7)** have been isolated and identified from the ethanolic extract of a resinous exudates (a commercial sample) of *Commiphora holtziana* from Kenya [Hanus *et al.*, 2005].



6

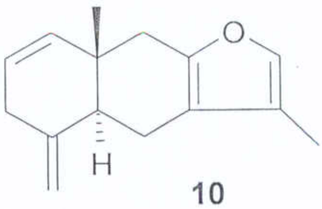


7

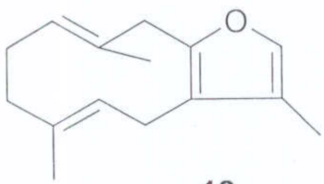


8

Gum resins of *Commiphora myrrha* (Nees) Engler are important commercial products (fragrant oil) in Kenya, Ethiopia and Somalia. Hexane soluble viscous oil is responsible for the odor of the gum. With the help of HPLC, the oil is separated into pure compounds which are then identified using GC/MS as the known furanosesquiterpenoids isofuranogermacrene/isogermafurene) **(2),** lindestrene **(12)**, furanoeudesma-1, 3-diene **(1)** and furanodiene (=isofuranodiene) **(10)** [Hanus *et al.,* 2005].

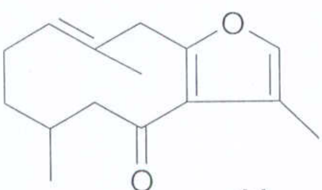


10



12

Myrrh consists of water soluble gum, alcohol soluble resins and volatile oil. It's characteristic odor is derived from furanosesquiterpenes like furanodesma-1, 3-diene **(1)** and 4,5-dihydrofuranodiene-6-one **(11)** [Hanus *et al.,* 2005].



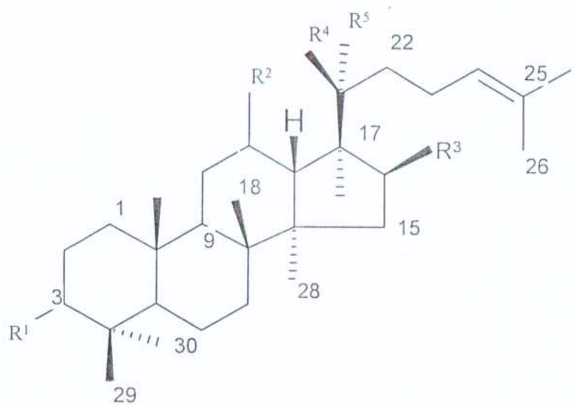
11

Fractionation of steam distilled residue of *Commiphora confusa* resin yielded (Table 6) four novel dammarane triterpenes **(20 – 24)** along with several known compounds **(25 – 28).** Their structure were established on the basis of extensive spectroscopic and chemical studies [Manguro *et al.*,2003].

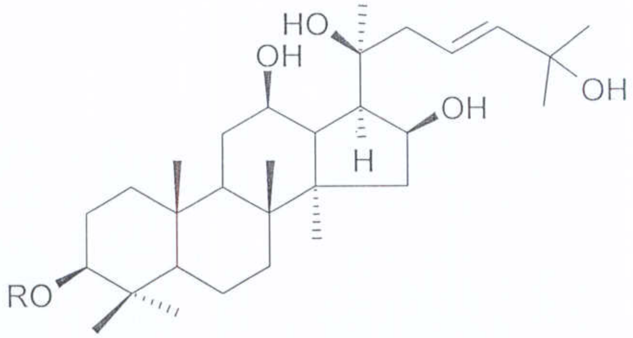
Table 6: Triterpenes of *Commiphora confusa*

Name	**Source (Plant part)**	**Reference**
(20*S*)-3β-acetoxy-12*β*,16-trihydroxydammar-24-ene **(20)** (20*S*)12*β*,16*β*-25,25-tetrahydroxydammar-23-ene **(21)**	Resin	Ahmad *et al.*, [1985]
3β-acetoxydammar-16*β*-hydroxydammarane-24-ene **(22)**	"	Brieskorn & Noble. [1983]
(20*R*)-3*β*,16*β* -trihydroxydammar-24-ene **(23)**	"	Brieskorn & Noble. [1983]
(20*S*)-3*β*-Acetoxy-12*β*,16*β*-25 tetrahydroxydammar-23-ene **(24)**	"	Fattorusso *et al.*, [1985] Provan & Waterman [1986]
(20*S*)-3*β*,12*β*,16*β*,25 tetrahydroxydammar-23-ene **(25)**	"	Fattorusso *et al.*, [1985] Provan & Waterman [1986]
3β-amyrinacetate,2-methoxyfuranodinone,2-acetoxyfuranodienone,(20R)-3*β*-acetoxy-	"	Fattorusso *et al.*, [1985] Provan & Waterman [1986]

16β-dihydroxydammar-24-ene (**26**)		
3β-hydroxydammar-24-ene (**27**)	"	Fattorusso *et al*., [1985] Provan & Waterman [1986]
3β-acetoxydammar-24-ene (**28**)	"	Fattorusso *et al*., [1985] Provan & Waterman [1986]

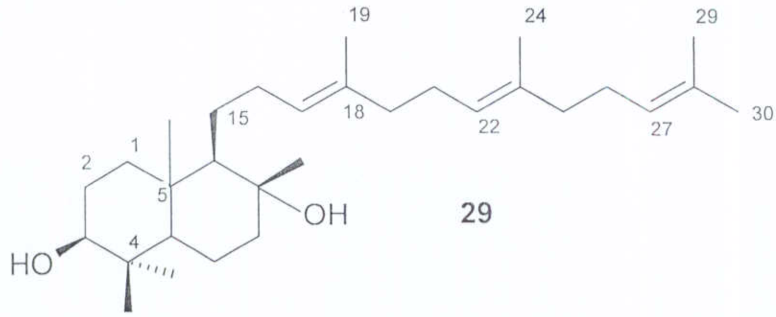


	R[1]	R[2]	R[3]	R[4]	R[5]
20	OAc	OH	OH	OH	Me
21	O-glu	OH	OH	OH	Me
24	OAc	H	OH	H	OH
25	OH	H	H	H	Me
26	OAc	H	H	H	Me
27	OAc	H	OH	H	Me
28	OH	H	OH	H	OH

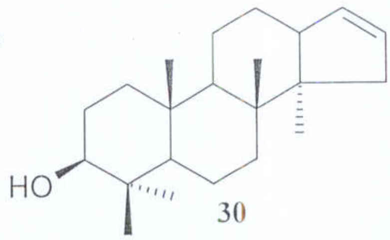


	R
22	Ac
23	H

From the methanolic extract from gulggul–gum resin, the resin of *Comiphora* (Balsamodendron) mukul, three new polypodane–type triterpenes, myrrhanol B **(29)**, dammarane-type triterpene, epimansumbinol **(30)** among others were isolated [Hanus *et al.*, 2005].

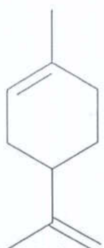


29

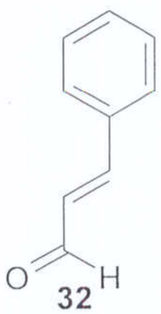


30

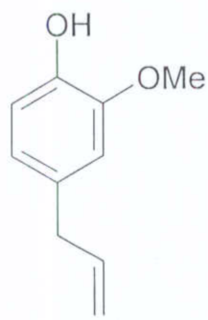
In the phytochemical studies of myrrh, the monoterpene derivatives limonene **(31)**, Cinnamic aldehyde **(32)** and m–cresol **(33)** have been identified [Hanus *et al.*, 2005].



31



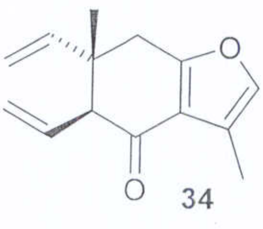
32



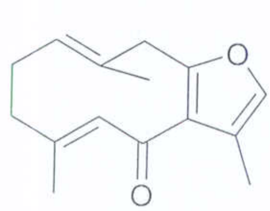
33

The composition of true myrrh, derived from *C. myrrha* was compared [Hanus *et al*., 2005] with some of its adulterants (*C. sphaerocarpa* Chiov., *C. holtziana* Engl. and *C. kataf* (Forssk. Engl.). The petrol ether extract of *C. myrrha* gave, after chromatography over silica gel, six compounds. One of them, (1E)-8,12-epoxygermacra-1,7(8),10(15),11-tetra-6-one **(8),** a furanosesquiterpene [Hanus *et al*., 2005]. *C. holtziana* also gave six compounds that were not reported in the *C. myrrh*. These compounds were – *rel*-(2*R*)-methyl-5*S*-acetoxy-4*R*-furanogermacr-1(10)-Z-en-6-one **(6),** (1(10)E,2R,4R)-2methoxy-8,12-epoxygermacra-1(10),7,11-triene-6-one **(8)**, (1E)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one **(8),** elemol **(16),** δ- elemene **(15)** and β-bourbonene **(19).**

A petrol extract of resin of *C. sphaerocarpa* was chromatographed over silica gel eluting with petrol/EtOAc mixtures of increasing polarities to afford curzerenone **(34),** furanodienone **(35)**, 3-methoxy-8,12-epoxy-germacra-1,7,10,11-tetraene-6-one **(8)** and (10)E,2R,4R)-2-methoxy-8,12-epoxygermacra-1(10),7,11-triene-6-one **(7)** [Dekebo *et al*., 2002b].



34



35

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 The components of *Commiphora holtziana*

The components of *C. holtziana* essential oils, organic solvent soluble resins and water soluble gums were obtained through extraction and the yield compared with the literature (Table 7).

Table 7: Quantification of *C. holtziana* components from Wajir and Isiolo populations

COMPONENT	YIELD OBTAINED (%)		LITERATURE YIELD (%) [Tucker, 1986]
	Wajir	Isiolo	
Essential oil	9.2	9.1	2 – 10
Solvent soluble resin	41.0	44.0	25 – 40
Water soluble gum	40.2	39.8	30 – 60

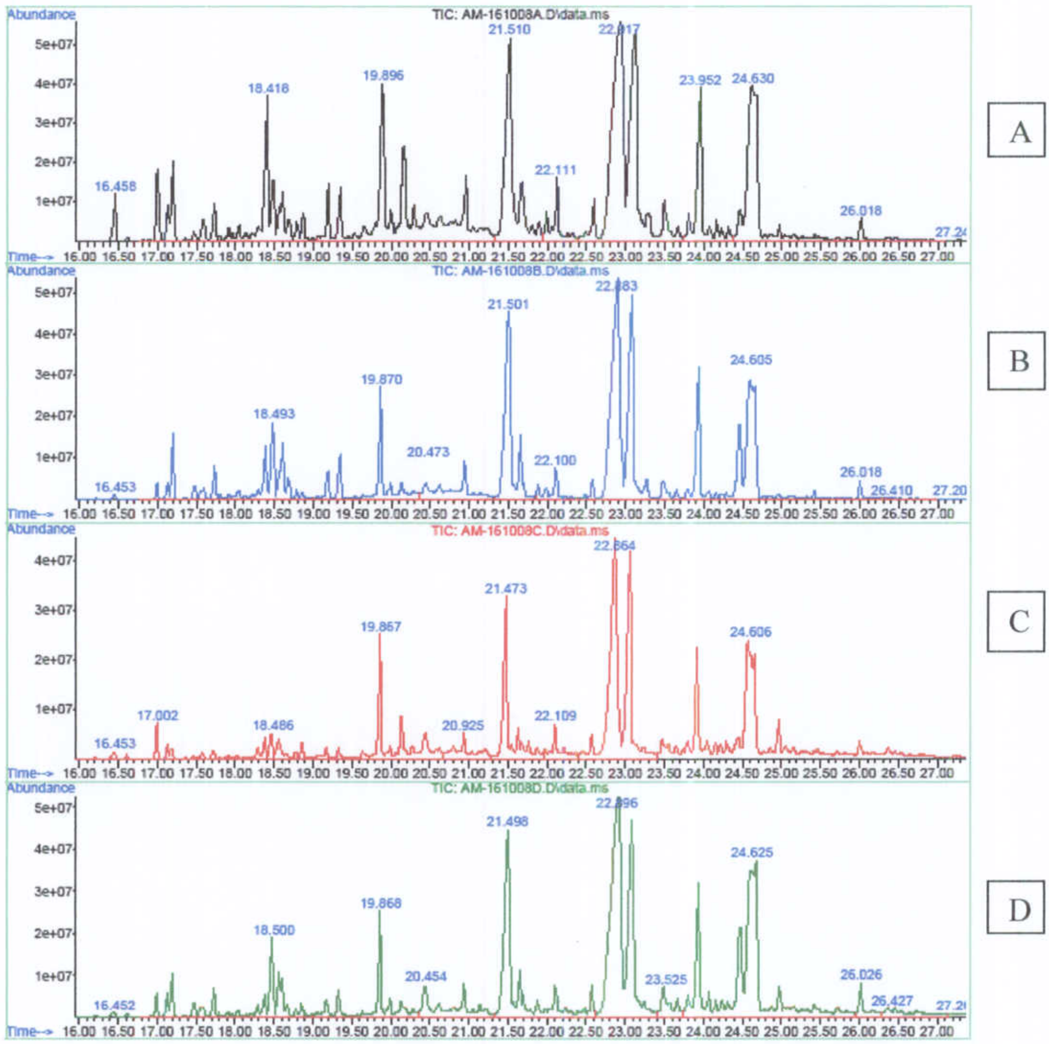
The yield of essential oil and the water soluble gum obtained from *C. holtziana* were within range according to Tucker [1986], but the solvent soluble resin was above the range reported in literature (Table 7).

3.2 Identification of the Major Compounds of *Commiphora holtziana*

3.2.1 Identification of components of *Commiphora holtziana* extracts

The hexane and dichloromethane extracts of *C. holtziana* collected from both Wajir and Isiolo populations were analyzed by GC–MS (Figure 4) and a total of 14 major compounds detected (Table 8). In the Wajir population, 7 compounds were detected from both the hexane extract and dichloromethane extract, 4 appeared only in the hexane extract and 3 only in the DCM extract. While from the Isiolo population a total of 7 compounds were detected with three being detected from the hexane extract, four from the dichloromethane extract and three of which were common to both solvents used (Table 8 and 9). Compounds **7**, **8**, **36** - **40** were identified using the GC – MS library as seen in tables 8 and figure 4.

```
File       :C:\msdchem\1\DATA\AM-161008A.D
Operator   :
Acquired   : 16 Oct 2008  16:49      using AcqMethod VOLATILES-35 TO 280.M
Instrument :    ICIPE MSD
Sample Name: HEX Extr. 3.163g
Misc Info  : Essential Oil HEX Extract 3.163g
Vial Number: 1
```



A-Dichloromethane extract and B- hexane extract from Isiolo collection
C- Dichloromethane extract and D- hexane extract from Wajir collection

Figure 4: GC tracings of *Commiphora holtziana* from Wajir and Isiolo collections

Table 8: Composition of extracts of the *C. holtziana* crude gum resin collected from Wajir (W) and Isiolo (I). (GC – MS)

			Concentration (%)					
Peak No.	Rt.	Compound per population	Hexane extract (W)	CH_2Cl_2 extract(W)	Hexane extract (I)	CH_2Cl_2 extract (I)	Total in Wajir	Total in Isiolo
1	16.45	CH-1	0.68	0.32	0.18	0.14	1.00	0.32
2	17.00	CH-2 (α–Copaene, **36**)	1.29	0.79	-	0.84	2.08	0.84
3	18.50	CH-3 (γ-muurolene, **37**)	3.57	0.27	-	0.17	3.84	0.17
4	19.35	CH-4	1.08	-	1.48	-	1.08	1.48
5	19.90	CH-5 (Cis -α-Bergamotene, **38**)	0.55	4.02	3.30	2.42	4.57	5.72
6	**20.18**	**CH-6 (11-hydoxy-γ-muurolene, 39)**	3.24	0.80	-	-	4.04	-
7	20.45	CH-7	-	1.83	-	1.90	1.83	1.90
8	20.92	CH-8	-	1.12	-	-	1.12	-
9	21.50	CH-9	8.93	-	12.01	-	8.93	12.01
10	22.12	CH-10	1.42	0.65	2.36	0.81	2.07	3.17
11	22.90	CH-11 (Compound **7**)	10.58	-	19.97	-	10.58	19.97
12	23.96	CH-12 (Compound **8**)	4.17	-	4.68	4.24	4.17	8.92
13	24.61	CH-13(β – elemene, **40**)	-	12.88	10.83	-	12.88	10.83
14	26.02	CH-14	0.45	1.54	-	1.11	1.89	1.11

7. 2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one.

8. (1E)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one.

CH-1 = *Commiphora holtziana* compound 1

Rt = Retention time (mins)

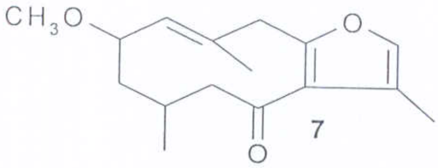
" - " = Not detected

Table 9: Summary of the presence of *C. holtziana* compounds per population

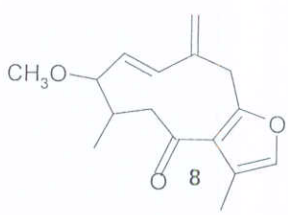
Peak No.	Compound per population respectively	CH_2Cl_2 extract (W)	CH_2Cl_2 extract (I)	Hexane extract (W)	Hexane extract (I)
1,5,10	CH-1, **38**, CH-8	√	√	√	√
2,3,14	**36, 37**, CH-12	√	√	√	X
4,9,11	CH-4, CH-7, **8**	X	X	√	√
6	**39**	√	X	√	X
7	CH-6	√	√	X	X
8	CH-8	√	X	X	X
12	7	X	√	√	√
13	**40**	√	X	X	√

Peaks 1, 5(α-Bergamotene) and 10 were detected from all solvents. Peaks 2 , 3 (γ-muurolene) and 14 were detected in all solvents except in the hexane extract from Isiolo. From the hexane extracts of both populations, peaks 4, 9 and 11 (2-methoxy-8,12-epoxygermacra-1(10),7,10,11-trien-6-one were detected, whereas peak 6 (11-hydoxy-γ-muurolene), the new compound, was detected from CH_2Cl_2 and hexane extracts of the Wajir population and not in the Isiolo population. Peak 7 appeared only in CH_2Cl_2 extracts from both populations whereas peak 8 only appeared in CH_2Cl_2 extract of the Wajir population. No compound appeared only in Isiolo population.

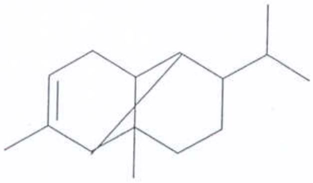
KEY

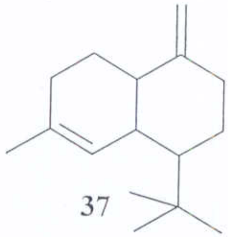


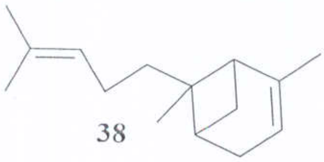
7

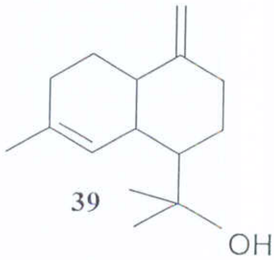


8

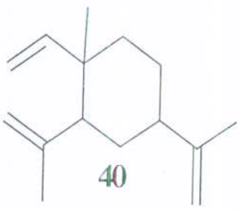

36


37

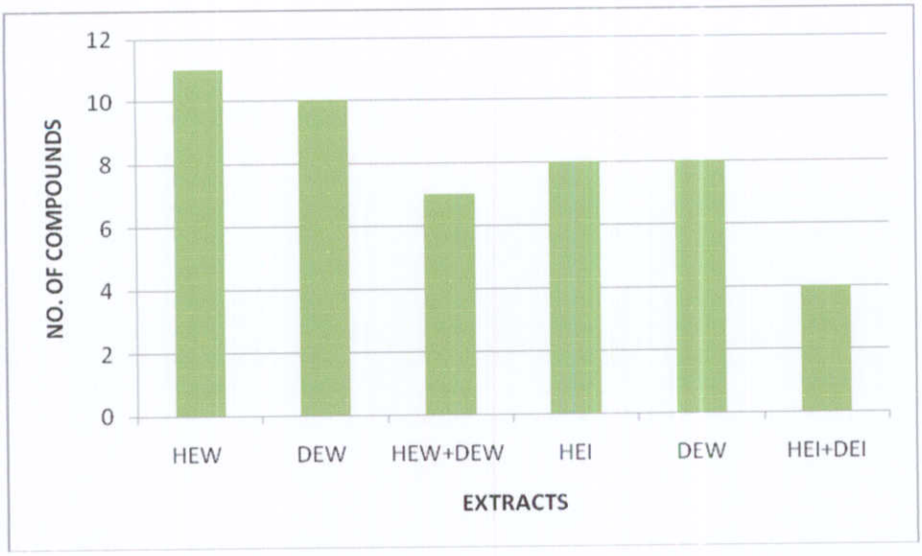

38



39


40

A further illustration of the differences in the number of compounds detected from the two populations is shown in figure 3. Here the number of compounds detected from each solvent extraction per population is illustrated. In Wajir sample, the hexane extract showed the presence of 11 compounds while eight were detected from the hexane extract of Isiolo population.



HEW – Hexane extract from the Wajir population
DEW – Dichloromethane extract from the Wajir population
HEI-Hexane extract from Isiolo population
DEI- Dichloromethane extract from Isiolo population

Figure 5: Compounds with a concentration ≥ 1 % detected per solvent

Out of the 14 major compounds detected by GC from hexane and dichloromethane extracts of the *C. holtziana* in the two populations, only 7 natural compounds could be identified from the GC-MS library.

Peak 6 (Table 8) corresponds to 11-hydroxy – γ - muurolene **(39)** with a molecular mass of 220.35 ($C_{15}H_{24}0$) and has not been identified from *Commiphora holtziana* earlier. This compound was eluted at retention time 20.18 and was found only in the Wajir population. This compound **(39)** is a 11-hydroxy derivative of γ-muurolene **(37)**, a compound which has been identified in the same extract having a retention time of 18.50 and a molecular mass of 204 (See appendix 4).

Peak 11 in table 8 corresponds to (1E)-2-methoxy-8,12-epoxygermacra-1,(10),7,11-trien-6-one **(7)** with a molecular mass of 262.3 ($C_{16}H_{22}O_3$) and has been reported from this plant earlier [Dekebo *et al*., 2000]. It was eluted at retention time 22.90 minutes and was found in the hexane extracts from both Wajir and Isiolo populations. It had concentration of 10.58% and 19.97% respectively.

Peak 12 in table 8 corresponds to (1E)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one **(8)** with a molecular mass of 259.3 ($C_{16}H_{19}O_3$), and has been identified from this plant by Dekebo *et al.*, [2002a]. It was eluted at retention time 23.96 and was found in all extracts except DCM extract of the Wajir population. It had an average concentration of 4.36%.

3.2.2 Characterization of Isolated Compounds

The hexane extract of *Commiphora holtziana* resins was subjected to column chromatography on Silica gel and eluted with hexane containing increasing amounts of ethyl acetate (3%, 6% and 50% yielding three compounds. The dichloromethane extract was subjected to the same treatment and purified to yield one pure compound. This appeared to be a major compound as it appeared in several fractions.

Compound **39** (16.1mg) was isolated as colorless oil, retention time 20.18 in hexane extract. EIMS showed a $[M]^+$ peak at m/z 220 corresponding to molecular formula $C_{15}H_{24}O$. ^{13}C NMR spectrum also showed the presence of 15 non – equivalent carbon atoms, in agreement with the MS data. These data as well as chemotaxonomic considerations suggested that this compound could be a sesquiterpene derivative.

The ^{13}C/DEPT spectrum (Table 10) showed fifteen carbon atoms, four methine (-CH), five methylenes

($-CH_2$), three methyl ($-CH_3$) and the remaining three were quaternary carbon atoms.

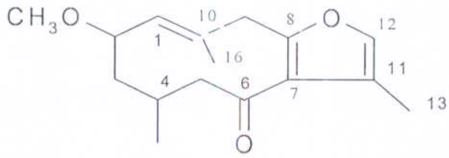
The ^1H (Table 10) and ^{13}C NMR further showed that compound **39** is a sesquiterpene with close similarity to γ-muurolene **(37)**. Thus the presence of two oleifinic groups at C-3 (δ_H 5.55 *br s* for H-4; δ_C 121.6 for C-4; 149.9 for C-3) and C-9(15) (δ_H 4.75 and 4.70 for CH_2-15, δ_C 154.1 for C-9; 106.7 for C-15) was evident from NMR.

37 **39**

Comparison of ^1H and ^{13}C NMR data of compound **(39)** with those of γ-muurolene **(37)** showed that the signals for the decaline moiety were in close agreement showing that compound **(39)** has the same skeleton. The principal difference is the presence of an oxygenated quartenary sp^3 carbon (δ_C 80.9) for C-11 instead of a methine and hence this new compound was characterized as 11-hydroxy-γ-muurolene. (See appendices 1A – 1D).

Compound 7 (9.1 mg) was isolated as a yellow oil, retention time 22.90 minutes, from hexane extract of the Wajir population. EIMS showed a $[M]^+$ peak at m/z 262.1 corresponding to molecular formula $C_{16}H_{22}O_3$. ^{13}C NMR also showed the presence of 16 non – equivalent carbon atoms in agreement with the MS data. These data suggested that this compound could be a sesquiterpene [Dekebo *et al.*, 2000]. The ^{13}C/DEPT showed four methine (-CH), three methylene ($-CH_2$), three methyl ($-CH_3$), one methoxy (OMe) and the remaining five were quaternary carbon atoms.

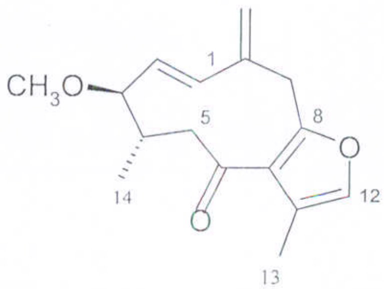
The ^{1}H and ^{13}C NMR spectra (Table 11 and 12) further showed that this compound is of sesquiterpene skeleton thus has three olefinic groups at C-10 (δ_H 5.18, *br s* for H – 1; δ_C 133.1 for C–1; 133.6 for C – 10) and at C – 11 (δ_H 6.99, s for H – 12; δ_C 126.2 for C – 11; 137.7 for C – 12).



7

Comparison of the ^{1}H (Table 11) and ^{13}C (Table 12) NMR data of compound 7 with a compound identified in Dekebo *et al.,* [2002a] showed that the signals were in agreement indicating this is the same compound. This compound was therefore identified as (1(10)E-2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one, (See appendices 2A – 2C). The complete ^{1}H NMR and ^{13}C NMR data are shown in tables 11 and 12 respectively.

Compound **8** (62.7 mg) was isolated as a yellow oil, retention time 23.96 minutes from hexane extract. EIMS showed a $[M]^{\cdot+}$ peak at m/s 259.3. In ^{13}C NMR spectrum, the presence of sixteen non – equivalent carbon atoms was observed. These data Dekebo *et al.,* [2002a] suggested that this compound could also be a sesquiterpene derivative.



8

The ^{13}C/DEPT showed sixteen carbons, five methine (-CH), three methylene ($-CH_2$), three methyl ($-CH_3$) and the remaining five were quaternary carbon atoms.

The ^{1}H and ^{13}C NMR spectra further showed that this compound is a sesquiterpene similar to that identified in Dekebo *et al.*, [2002a] with a furan ring located at C-7 and C-8 (Table 11 and 12)

Comparison of the ^{13}C (Table 12) NMR data of compound **(8)** with a compound reported in Dekebo *et al.*[2002a] showed that the signals were in agreement indicating that compound **(8)** is *(1E)*-3-methoxy-8,12-epoxygermacra-1,7,10,11-tetraen-6-one.

Table 10: ^{1}H-NMR (200 MHz, $CDCl_3$) and ^{13}C-NMR (50 MHz, $CDCl_3$) data of compound **39**

No.	**39** δ_H (int, mult, j in Hz)	**39** δ_C
1		30.2
2		37.3
3	5.55 (1H, *br s*)	149.9
4		121.6
5		37.7
6		55.1
7		24.3
8		40.4
9		154.1
10		47.5
11		80.9
12	0.99/0.97 (3H, *s*)	21.5
13	0.97/0.99 (3H, *s*)	21.7
14	1.23 (3H, *s*)	25.0
15	4.75 (1H, *br s*), 4.70 (1H, *s*)	106.7

Table 11: ^{1}H-NMR data of compounds 7, 8 and their references (200 MHz, $CDCl_3$)

No.	7 δ_H (int, mult, *j* in Hz)	Literature value for compound 7 [Dekebo *et al.*,2000]	8 (int, mult, *j* in Hz)	Literature value for compound 8 [Dekebo *et al.*, 2002a]
1	5.18 (1H, *br s*)	5.18 (1H, *br d*)	5.95 (1H, d, 16.4)	5.92 (1H, d, 16.4)
2	3.99 (1H, td, 8.8, 2.6)	3.98 (1H, dt, 8.8,2.2)	5.38 (1H, *dd*,16.4, 9.4)	5.30 (1H, *dd*,16.5, 9.5)
3	1.71 (1H, *m*), 1.96 (1H, *m)*	1.71 (1H, *m*), 1.96 (1H, *m*)	3.05 (1H, *t*, 9.2) 2.6 (1H, *m*)	3.05 (1H, *t*, 9.5)
4	2.40 (1H, *m*)	2.40 (1H, *m*)	2.32 (2H, *m*)	
5	2.50 (2H, *m*)	2.50 (2H, *m*)		
6				
7				
8			3.73(1H, *d*, 14.6), 3.35 (1H, *d*, 14.6)	3.73 (1H, *d*, 14.6), 3.35 (1H, *d*, 14.6)
9	3.20 (1H, *m*), 3.53 (1H, *m*)	3.20 (1H, *m*), 3.53 (1H, *m*)		
10				
11				
12	6.99 (1H, *br s*)	6.99 (1H, *br s*)	7.27, (1H, *br s*)	6.91 (1H, *br s*)
13	1.88 (3H, *br s*)	1.88 (3H, *br s*)	1.98 (3H, *d*, 1.2)	1.96 (3H, *d*, 1.2)
14	1.10 (3H, *d*, 7.0)	1.10 (3H, *d*, 7.0)	1.14 (3H, *d*, 6.4)	1.14 (3H, *d*, 6.0)
15	1.78 (3H, *d*, 1.1)	1.78 (3H, *d*, 1.1)	5.18(1H, *br s*) 4.89 (1H, *br s*)	5.18 (1H, *br s*) 4.89 (1H, *br s*)
16	4.75 (1H, br s), 4.70 (1H br s)	4.75 (1H, br s), 4.70 (1H br s)		
OMe	3.24 (3H, *s*)	3.24 (3H, *s*)	3.24 (3H, *s*)	3.24 (3H, *s*)

Table 12: ^{13}C-NMR data (δ) of **7** and **8** (50 MHz, $CDCl_3$) and their references

Position	7	Literature value for compound 7 [Dekebo *et al.*, 2002a]	8	Literature value for compound **8** [Dekebo *et al.*, 2002a]
1	133.7	133.1	135.1	135.0
2	74.8	74.6	132.3	132.2
3	37.1	36.9	88.4	88.3
4	25.5	25.3	38.2	38.0
5	50.7	50.5	48.5	48.4
6	203.1	202.8	204.1	203.8
7	120.0	119.7	117.9	117.7
8	151.7	151.5	151.5	151.4
9	38.2	38.3	33.9	33.8
10	133.6	133.4	142.7	142.5
11	126.2	126.0	128.8	128.6
12	137.7	137.4	138.0	137.9
13	8.5	8.3	9.4	9.2
14	22.1	21.9	19.1	18.9
15	18.4	18.1	115.9	115.7
OMe	55.8	55.5	56.8	56.7

3.3 Antimicrobial Activity of *Commiphora holtziana*

The crude extracts from Isiolo and wajir collections were tested for antimicrobial and antifungal activities against different organisms (Table 13). The crude methanolic extract of *Commiphora holtziana* was active against all Gram - positive bacteria, *Bacillus pumilis*, *Bacillus subtilis* and *Staphylococcus aureus* with inhibition zones of 9.5, 9.2 and 10.7 mm respectively for the Wajir methanol extract at 5mg/well. Similar results were reported by Musa [2008] where the methanolic extract of *C. kerstingii* Engl. inhibited the growth of several bacteria with the highest inhibition zone of 30 mm at 5 mg/well against *S. aureus*.

This results show that *Commiphora holtziana* is also effective in suppressing the growth of *S. aureus* and other bacteria.

Both dichloromethane crude extracts of *Commiphora holtziana* collected from Wajir and Isiolo were active against the Gram negative bacteria (*Escherichia coli*) with the Isiolo population sample giving an inhibition zone of 10.3 mm while the Wajir population sample gave 9.6 mm at 5 mg/well. Methanol and hexane extracts from both populations were inactive against *E. coli* . There was also no activity in the acetone extract from Wajir population.

Both dichloromethane extracts were active against the fungus *Sacharomyces cerevisiae* with the sample from the Isiolo population giving highest inhibition zone of 11.4 mm while that from Wajir population gave an inhibition zone of 10.6 mm. The hexane extract from the Wajir population was also active against *Sacharomyces cerevisiae*. The same fungi was resistant to methanol extracts, the hexane extract from Isiolo population and acetone extract from the Wajir population.

The pure compounds, 11-hydroxyl-γ-muurolene **(39)**, (1(10)*E*, 2*R**,4*R**)-2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one **(7)** and (1E)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one **(8)** were also tested for activity at a concentration of 5mg/well in Dimethyl Sulfoxide (DMSO) against *Candida albicans* and *Aspergillus clavatus*. However no inhibition was observed.

Table 13: Antimicrobial activities of *Commiphora holtziana* crude extracts

Zone of inhibition (mm)									
Crude extracts (5 mg/ml)								Antimicrobial agents	
Organism	DEI	MEI	Hexane Extract(I)	DCM Extract(W)	Acetone Extract(W)	Hexane Extract(W)	Methanol Extract(W)	NYS (0.30mg/ml)	GTN (0.32mg/ml)
Bacillus pumilus	7.85	8.38	7.84	8.60	8.31	8.25	9.46	**N/A**	**24.30**
Bacillus subtilis	8.54	8.58	8.07	8.75	8.64	8.54	9.16	**N/A**	**24.38**
Escherichia coli	10.32	-	-	9.58	-	-	-	**N/A**	**10.60**
Staphylococcus aureus	8.66	8.56	8.37	9.31	8.83	8.36	10.66	**N/A**	**25.00**
Sacharomyces Cerevisiae	11.36	-	-	10.62	-	10.54	-	**12.79**	N/A

DEI – Dichloromethane extract from Isiolo
DCM – Dichloromethane
MEI – Methanol extract from Isiolo
NYS- Nystatin (For Fungi)
GTN- Gentamycin (For bacteria)
N/A – Not applicable

3.4 Conclusions and Recommendations

3.4.1 Conclusions

1) Three components of *C.holtziana* namely essential oils, resins and gums were separated and quantified.

2) Three compounds, namely 11-hydroxyl-γ-muurolene **(39)**, (1(10)*E*, 2*R**,4*R**)-2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one **(7)** and (1*E*)-3-methoxy-8,12-epoxygermacra -1,7(8),10(15),11-tetraen-6-one **(8)** were isolated and characterized from this plant of which compound **39** appears to be new.

3) Comparative study by GC – MS analysis of two populations of *Commiphora holtziana* shows appearance of similar compounds in both populations with a variation only in their abundance. Using this method the identified compounds were (1(10)*E*, 2*R**,4*R**)-2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one **(7)**, (1E)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one **(8)**, α-copaene **(36)**, γ-muurolene **(37)**, cis - α- bergamotene **(38)**, 11-hydoxy-γ-muurolene **(39)** and β-elemene **(40)**.

4) The crude extract of *Commiphora holtziana* showed some anti-microbial activity, probably suggesting why the plant is used traditionally for wound healing.

3.4.2 Recommendations

Most of the peaks detected by GC – MS in this study have not been structurally identified. These compounds should be isolated and characterized.

It is recommended to isolate and test all the constituents of *Commiphora holtziana* for antimicrobial activities. In addition, toxicity assay is required to determine the safety level of the plant extract since it is used in the healing of wounds.

Comparative analysis of different populations of this plant should be done to establish the chemical profile.

CHAPTER FOUR

EXPERIMENTAL

4.1 General

Column chromatography was carried out using Merck Silica gel 60 (70 – 230 mesh) and Sephadex LH–20. Analytical TLC was done using Merck pre–coated 60 PF_{254}. Preparative thin layer chromatography (PTLC) was done on silica gel (Merck). Chromatographic zones were detected under UV (254, 366 nm) light and/or by exposing to iodine vapor in some cases.

GC-MS analyses was performed on an Agilent HP 7890A GC system using a fused Silica capillary column (30 m x 0.25mm i.d., thickness 0.25μm, DB-5), fitted with an on – column injector, which was directly coupled to a magnetic sector 5975C triple axis MSD, (Thermo-Finnigan MAT95 XP, Bremen, Germany). Ionization was by electron impact (70eV, source temperature 285°C). The oven temperature was maintained at 35°C for 10 min, and then programmed at 10°C/min to 280°C which was maintained for 10.5 minutes. The carrier gas was helium, with a flow rate of 1.2ml/min. The total run time was 40 minutes. Tentative identifications were given by the GC–MS library

The NMR spectra were recorded on a Varian – Mercury 200 instrument. The chemical shifts were measured in ppm (δ) values relative to the internal standard tetramethylsilane (TMS). The operating frequencies for ^{1}H was 200 MHz and 50 MHz for ^{13}C.

4.2 Plant material

The *Commiphora holtziana* gum resin was collected from Isiolo and Wajir districts in September 2007. They are among the dry areas of Kenya where the species is abundantly found. The plants were collected and identified with the help of a KEFRI taxonomist. Samples were picked (Figure 6) by hand and packed in clear poly-ethene paper bags and coded.

Figure 6: Collecting Oleo - gum resins

4.3. Sample Preparation

The oleo-gum resins were cleaned manually by removing the grass and any other foreign bodies stuck to it. The samples were then dried under room temperature until they were dry enough to be ground using pestle and mortar. They were then placed in a plastic bag and sealed.

4.4 Extraction and Isolation of Compounds

4.4.1 Separation of the Gum Resin Components

To separate and quantify the oleo-gum resin, 400g of the sample were first steam distilled and oil collected using an essential oil extractor. The volume of the oil was determined by reading from the graduated collector. The remaining sample was then extracted first with methanol, followed by acetone then dichloromethane, and finally with n-hexane. Removal of solvent from each extract gave four crude extracts. The reverse order of extraction completely eliminates the essential oils. Weights of each extract were noted and eventually summed up as total extract by solvents. The remaining gum was weighed and all parameters recorded as a percentage of the original resin.

4.4.2 Extraction and Isolation

For isolation of compounds, 400g of the Wajir sample was extracted first with hexane, followed by dichloromethane, acetone and finally with methanol. The acetone and methanol extracts were obtained in small quantities and only antimicrobial test was done on these extracts. This methodology was done reverse to that in section 4.4.1, starting with the less polar solvent hexane to the most polar, methanol, in order to capture all components of the resin including monoterpenes which are easily removed in the steam distillation to obtain essential oils. These four extracts were used for further analysis as described below.

4.4.2.1 Chromatographic Isolation of Compounds from Hexane Extract of *C. holtziana* Resins

The hexane extract (24 g) was subjected to column chromatography with column size (4.4 cm x 20 cm) on Silica gel (200 g) eluting with hexane containing increasing amounts of ethyl acetate. The fraction eluted with 10% ethyl acetate in hexane (1.3 g) was subjected to further column chromatography Silica gel (1.8 cm x 20 cm) eluting with hexane containing increasing amounts of ethyl acetate and then Sephadex LH-20 (eluting with CH_2Cl_2 /MeOH; 1:1) and PTLC (eluent, n-hexane/acetone, 10:0.5) on Silica gel to give compounds **39** (102.8 mg), 7 (334.6 mg) and **8** (131.9 mg).

4.4.2.2 Extraction and Isolation of Compounds from Dichloromethane Extract of *C. holtziana* Resins

The dichloromethane extract (21 g) was chromatographed as above. The fractions from the elution with 100% hexane gave a mixture which was separated by PTLC (eluent, n-hexane/acetone, 10:0.5) to give compound **8** (132.1 mg).

4.5 Physical and Spectroscopic Data for the Isolated Compounds

11- Hydroxy- γ-muurolene (39)

Colorless oil, ^{1}H ($CDCl_3$, 200 MHz): 5.55 *br s* (H–4), 4.75, 4.70 *s* (H–15), 1.23 *s* (H–14), 0.99 *s* (H–13), 0.97 *s* (H–12), ^{13}C (**$CDCl_3$, 50 MHz**): 154.1 (C-9), 149.9 (C-3), 121.6 (C-4), 106.7 (C-15), 80.9 (C-11), 55.1 (C-6), 47.5 (C-10), 40.4 (C-8), 37.3 (C-2), 37.7 (C-5), 30.2

(C-1), 25.0 (C-14), 24.3 (C-7), 21.7 (C-13), 21.5 (12). **EIMS** (70ev): 220 (20) $[M]^+$, 205 (27) $[M-Me]^+$, 202 (41) $[M-H20]^+$,187 (38), 177 (31), 122 (18), 159 (91), 147 (45), 119 (100), 105 (39), 91 (55), 79 (20), 43 (24).

(1E)-2-Methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one (7)

Yellow oil, ^{1}H **($CDCl_3$, 200 MHz):** 6.99 *br s* (H–12), 5.18 *br s* (H–1), 4.75 ,4.70 *br s* (H-16), 3.99 t*d* (8.8, 2.6) (H–2), 3.53 *m* (H–9), 3.24 (OMe–2), 3.20, 2.50 *m* (H–5), 2.40 *m* (H–4), 1.88 *br s* (H–13), 1.96, 1.71, *m* (H–3), 1.78 *d* (1.1) (H–15), 1.10 *d* (7.0) (H–14), ^{13}C **($CDCl_3$, 50 MHz):** 203.1 (C-6), 151.7 (C-8), 137.7 (C-12), 133.6 (C-10), 133.7 (C-1), 126.2 (C-11), 120.0 (C-7), 74.8 (C-2), 55.8 (OMe), 50.7 (C-5), 38.2 (C-9), 37.1 (C-3), 25.5 (C-4), 22.1 (C-14), 18.4 (C-15), 8.5 (C-13). **EIMS** (70ev): 262 (21.4) $[M]^+$, 247 (14.4), 230 (19.8), 173 (100), 162 (69), 145 (58.6), 41 (15.8).

(1E)-3-Methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one (8)

Yellow oil, ^{1}H **($CDCl_3$, 200 MHz):** 3.24 (OMe–3), 5.94 *d* (6.4) (H–1), 5.38 *dd* (16.3, 9.4) (H–2)), 3.05 *t* (9.2) (H – 3), 2.6 *m* (H– 4), 2.32 *m* (H–5), 3.73 *d* (14.6) (H–9), 7.27 *s* (H–12), 1.98 *d* (1.2) (H–13), 1.14 *d* (6.4) (H–14), 4.89, 5.17 *br s* (H–15). ^{13}C **(50 MHz):** 204.1 (C-6), 151.5 (C-8), 142.5 (C-10), 138.0 (C-12), 135.1 (C-1), 132.2 (C-2), 128.7 C-11), 117.7 (C-7), 115.9 (C-15), 88.4 (C-3), 56.8 (OMe), 48.5 (C-5), 38.1 (C-4), 33.9 (C-9), 19.1 (C-14), 9.4 (C-13). **EIMS (70ev):** 260 (33) $[M]^+$, 245 (1), 228 (5), 213 (17), 175 (100), 159 (18), 43 (2).

4.6 Antimicrobial Activity of *Commiphora holtziana*

4.6.1 Test Organisms

The Gram-negative bacteria *Escherichia coli* and the Gram-positive bacteria *Bacillus pumilus* and *Bacillus subtilis* were used. These are clinical isolates obtained from Kenyatta Hospital. The fungal isolates *Sacharomyces cerevisiae* and the Gram positive bacteria *Staphylococcus aureus* were obtained from the Pharmaceutical Chemistry Department of the University of Nairobi. All the bacteria strains were suspended in water and incubated at 121°C for 15 minutes and cooled to about 50°C. Mueller Hinton agar (MHA) and Tryptone soya agar (Liofilchem, Scozia, Italy) were used in the test for antibacterial activity, while sabourauds dextrose agar (Oxoid, Basingstoke, United, Kingdom) was used in the test for antifungal activity [Musa, 2008].

Incubation conditions for the fungi was 48 hours under 6% O, 10% CO at 37°C, then followed by anaerobic conditions for 48 – 72 hours.

4.6.2 Determination of Antimicrobial Activity

4.6.2.1 Crude Samples

Antimicrobial activity of the extracts was evaluated by the agar diffusion assay method. Plates were inoculated with microbes and poured into sterile Petri – dishes: 20ml for the bacteria and 15ml for the fungi. This was further cooled until they solidified. With the use of a cork borer, 6 wells of 6.95mm diameter and 2cm apart were punctured in the culture media using sterile borers for the first four samples plus a blank and a standard; and five in the second Petri – dish for the last three samples.

50μl of each crude extract was introduced into the wells as a DMSO solution and left to stand for 1 hour for diffusion to take place. Incubation for bacteria was done at 37^0C and 25^0C for fungi all for 18 hours. Gentamycin (0.32 mg/ml) was used as the standard for antibacterial activity while nystatin (0.30 mg/ml) for fungi. After incubation, antimicrobial activity was determined by measurement of the width of the zones of inhibition using electronic vernier calipers.

4.6.2.2 Pure Compounds

Isolated pure compounds were divided into two portions. One to be used for testing against *Candida,* a yeast fungus, and the other to be used against *Aspergillus* which is a filamentous fungus. Paper discs were soaked in a solution of the compounds dissolved in Dimethyl sulfoxide (DMSO). Micro-organisms were then introduced on a prepared agar and spread evenly. The soaked discs were then put on the marked positions of the agar using forceps. Incubation was then done at 37°C for 24 hours for *Candida albicans* and at 25°C for a minimum of seven days for the *Aspergillus clavitus.*

REFERENCES

Ahmad, V. U., Ali, A., Baqai, F. T. and Zafar F. N. (1985). Dammarane triterpenes from the resins of *Commiphora confusa*. *Phytochemistry* **24**: 1035-1037.

Al-Faris, E. A., Al-Rowais, N., Mohamed, A. G., Al-Rukban, M. O., Al-Kurdi, A., Balla, A., M. A., Al-Harby, S. and Sheikh, A. (2008). Prevalence and Pattern of Alternative Medicine Use: The Results of a Household Survey. Ann. *Saudi Med.* **28**: 4-10.

Al-Harbi, M. M., Qureshi, S., Raza, M., Ahmed, M. M., Afzal, M. and Shah, A. (1997). Gastric anti-ulcer and cytoprotective effect of Commiphora molmol in rats. *J Ethnopharmacol* **55**: 141-150.

Anderson, M., Bergendorff, O., Shan, R., Zygmont, P., and Sterner, O. (192). Minor components with smooth muscle relaxing properties from scented myrrh (*Commiphora guidotti*). *Planta medica* **63**:251-254.

Archaelogy (1996). News brief – medicinal myrrh **49**:3-5. Archaelogical institute of America.

Asres, K., Tei, A., Moges, G., Sporer, F. and Wink, M. (1998). Terpenoid composition of the wound induced bark exudates of *Commiphora tenuis* from Ethiopia. *Planta Medica* **64**:473-5.

Atal, K. C., Gupta, P. O. and Afaq, H. S. (1975). *Commiphora mukul*: Source of guggal in Indian systems of medicine. *Economy Botany* **29**:209-218.

Bajaj, G. A. and Dev, S. (1982). Chemistry of ayurvedic crude drugs-V: Guggul (resin from Commiphora mukul)-5 – some new steroidal components and stereochemistry of guggulsterol–1 at C-20 and C-22. *Tetrahedron letters* **38**:2949-54.

Barakat, R., Elmorshedy, H. and Fenwick, A. (2005). Efficacy in the treatment of *schistomiasis mansoni*. *American journal of tropical medicine and hygiene* **73**:365-367.

Batchelder, T., Roy, A. and Saraf, S. (2002). Ethno-medicinal approach in Biologiacl and Chemical investigation of phytochemicals as antimicrobials. Ethno-medicine for anthritis – mical Anthropology.

Beentje, H. J. (1994). Kenya Trees, shrubs and Lianas.The National Museums of Kenya, Nairobi, Kenya.

Bluementhal, M., Goldberg, A. and Brinkmann, J. (eds). (2000). Herbal medicine: Expanded Commission Monographs. Boston (MA): Integrative Medicine Communications.

Brieskorn, C. H. and Noble. (1983). Two furanoeudesmanes from the essential oil of myrrh. *Phytochemistry* **22**:187-189.

Brieskorn, C. H. and Noble, P. (1980). Drei Neu Furanogermacrene aus myrrhe. *Tetrahedron Letters* **21**: 1511 – 1514. (ALNAP Database Ref.ID: 1340).

Chaudary, A. S. and Jowaida, L. (1999).Vegetation of the Kingdom of Saudia Arabia. Pub. Ministry of Agric. and Water, Kingdom of Saudia Arabia.

Chikamai, B. and Odera, A. J. (eds). (2002). Commercial plant gums and Gum Resins in Kenya: Sources of alternative livelihood and economic development in the drylands. Executive printers, Nairobi, Kenya.

Cronquist, A. (1981). An Integrated System of Classification of Flowering Plants. Columbia University Press, New York, New York, USA.

Davis, P. (1999). *Aromatherapy an A-Z* CW Daniel, Saffron Walden, quoting *The Yearbook of Pharmacy & Transactions of the British Pharmaceutical Conference* (1907) pp. 217.

Davison, R. L. (1980). Handbook of water soluble gums and resins. McGrow Hill Book company, New York.

Dekebo, A., Dagne, E. and Sterner, O. (2002a). Furanosesquiterpenes from *C. sphaerocarpa and* related adulterants of true myrrh. *Fitoterapia* 73: 48 – 55.

Dekebo, A., Lang, M., Polborn, K., Dagne, E. and Steglich, W. (2002b). Four lignans of *Commiphora erlangeriana. Journal of Natural Products* **65**:1252-7.

Dekebo, A., Dagne, E., Hansen, K. L., Gautun, R. O. and Aasen, J, A. (2000). Crystal structure of two furanosesquiterpenes from *Commiphora sphaerocarpa. Tetrahedron letters* **41**: 9875 – 9878.

Dolara, P., Corte, B., Ghelardini, C., Monserrat, C., Aiollis, S., Luceri, F., Lodovici, M., Menichettis, S. and Romanelli, M. N. (2000). Analgesic effects of Myrrh. *Nature* **29**:379.

Essential science. (2007). Essential oils desk reference USA: Essential science publishing.

Fattorusso, E., Santacrose C. and Xaasan F.C. (1985). Characterization of archeological frankincense by gas chromatography – mass spectroscopy. *Phytochemistry* **24**:1035 – 1037.

Fourier, T. G. and Snyckers, F. O (1989). A Pentacyclic triterpene with anti–inflammatory and analgesic activity from the roots of *Commiphora merkeri*. *Journal of Natural Products* **52**:1129-31.

Gachathi, F. N. (2005). Gums and Resin species in Kenya. A paper presented to the Training of Trainers workshop at Transit Hotel, Isiolo, Kenya.20 – 25th March 2005.

Gachathi, F. N. (1997). Recent advances of classification and status of main gum producing species in the family Burseraceae.

Ganesan, S., Ramar, N. and Banumathy, N. (2002). Ethnomedicinal survey of Alagarkoil Hills (Reserved forest), Tamil Nadu, India.

Gillet, J. B. (1991). Burseracea. In: Flora of Tropical East Africa. A. A. Balkema/Rotterdam/Brookfield.

Grieve, M. (1995). Myrrh In: A modern herbal. Dover Publications, Inc. New York, USA.

Hanus, O. L., Renzanka, T., Dembitsky, M. V. and Moussaieff, A. (2005). Myrrh-*Commiphora* Chemistry. *Biomedical papers* **149:** 3–28.

Habtemariam, S. (2003). Cytotoxic and activity of erlangerins from *Commiphora erlangeriana*. *Toxicon* **41**:723-7.

Hyde, M. A. and Wursten, B. (2009). Flora of Zimbabwe: Species information *Commiphora schimperi*. http//www.zimbabwean flora.zw/species data retrieved 18th May, 2009.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F. and Donoghue, M. J. (2008). Plant Systematics: A Phylogenetic Approach 3rd ed. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

Kiringe, W. J. and Okello, M. M. (2006). A survey of traditional Health Remedies used by the Maasai of southern Kajiado district, Kenya. *Ethnobotany Research & applications journal* **4:**061 – 073.

Kumar, V. and Dev, S. (1987). Chemistry of Ayurvedic crude drugs-VII guggul (Resin from *Commiphora mukul*) – 6: Absolute stereochemistry of guggultetrols. *Tetrahedron letters* **43**:5933-5948.

Langenheim, J. (2003). Plant Resins: Chemistry, evolution, ecology and ethnobotany. Timber Press, Portland, OR.

Lans, C., Turner, N., Brauer, G. M., Lourenco, G. and Georges, K. (2006). Ethno-veterinary medicines used for horses in Trinidad and British Columbia, Canada. *Journal of Ethno-biology and Ethno-medicine* **2**:31

Lawless, J. (1995). The illustrated Encyclopaedia of essential oils. UK: Thorsons.

Manguro, A. O. L., Ugi, I. and Lemmen, P. (2003). Dammarane Triterpenes of *confusa* Resin. *Chemical & Pharmaceutical bulletin* **51**: 5483 – 5487.

Manguro, L. O. A., Mukonyi, K. W. and Githiomi, J. K. (1996). Bisabolenes and furanosesquiterpenes of Kenyan *Commiphora kua* resin. *Planta Med*ica **62**: 84-85.

Maradufu, A., and Warthen, J. D. (1988). Furanosesquiterpenes from Commiphora myrrh oil. Plant science **57**: 181 – 184.

Maradufu, A. (1982). Irritant potential of some constituents from oleo–gum resins of *Commiphora myrrha*. *Phytochemistry* **21**:677 – 680.

McDowell, W., Tlusty, A., Rott, R., BeMiller, N. J., Bohn, A. J., Meyers, W. R. and Schwarz, T. R. (1988). Inhibition of glycoprotein oligosaccharide processing in vitro and in influenza-virus-infected cell by alpha – D - mannopyranosymethyl – P-nitrophenyltriazine. *Biochemical Journal* **255**:991-998.

Miller, A.G. and Morris, M. (1988). Plants of Dhofar - The southern Region of Oman: Traditional Economic and medicinal uses. The office of the adviser for conservation of the environmemt, Diwan of Royal court, Sultanate of Oman, pp.82.

Musa, A. A. (2008). Antioxidant and Antibacterial activity of *Commiphora kerstingii* Engl. stem bark extract. *Research journal of Phytochemistry* **2**:106-111.

Nair, B. (2007). Sustainable utilization of gum resins by improved tapping technique in some species. In: Harvesting of Non – Wood Forest Products. FAO Corporate documentary repository, pp 1–15.

Newman, A. A. (2006). Chemistry of Terpenes. Pp. 155-206. Academic Press, London and New York.

Ng'ethe, R., Kariuki, A. and Opondo, C. (1999). Some experience on adaptive research input on natural resource use: the case of Gums and Resins in Mukogodo rangelands, Laikipia district, Kenya. A paper presented during a Regional Conference for Africa in Nairobi, Kenya 6-10 October 1997.

Provan, G. J., Gray, I. and Waterman, P. G. (1992). Masombinane derivatives from stem bark of *Commiphora kua. Phytochemistry* **31**:2065-2068.

Provan, G. J. and Waterman, P. G. (1988). Major triterpenes from the resins of *Commiphora incisa* and *Commiphora kua* and their potential chemotaxonomic significance. *Phytochemistry* **27**:3841-3843.

Provan, G. J., Gray, I. and Waterman, P. G. (1987). Monoterpene–rich Resins from some Kenyan Burseraceae. *Flav. Frag. J* **2**: 115 – 118. (ALNAP Database Ref. ID: 3729).

Provan, G. J. and Waterman, P. G. (1986). A hydroxylated mansumbinen – 28-oic acid from *Combretum coccineum*. *Phytochemistry* **25**: 917-922.

Provan, G. J. and Waterman, P. G. (1985). "Picropolygamain: A new lignin from *Commiphora incise Resin"*. *Planta Medica* Pp.271-272.

Queshi, S. Al-Harbi, M. M., Ahmed, M. M., Raza, M., Giangreco, A. B. and Shah, A. H. (1993). Evaluation of the genotoxic, cytotoxic, and antitumor properties of *Commiphora molmol* using normal and enrlich ascites carcinoma cell-bearing Swiss albino mice. Cancer Chemotheraphy and Pharacology **33:** 130-138.

Siegel, J. (2002). *Myrrh*. Retrieved 15:20pm, May 19, 2009 from http://foodsci:rutgers.edu/

Swaminathan, S., Bakshi, R. K. and Dev, S. (1987). Higher Isoprenoids XIX. Guggulsterones to dexamethasone. *Tetrahedron* letters **43**:3827-3838.

Tariq, M., Ageel, A.M., Al-Yahya, M.A., Mossa, J.S., Al-Said, M.S. and Parmar, N.S. (1985). Anti-inflammatory activity of *Commiphora molmol*. Agents and Actions **17**: 381-382.

Tucker, A. O. (1986). Frankincense and myrrh. *Economic Botany* **40:** 425 – 433.

Ubillas, R. P., Mendez, C. D., Lolad, S. D., Luo, J., King, S. R., Carlson, T. J. and Fort, D. M. (1999). Antihyperglycemic furanosesquiterpenes from *Commiphora myrrha*. *Planta Medica* **65**:778-779.

Vollesen K. (1989). In: Hedberg I, Edwards, (eds.) (1989). Burseraceae. Flora of Ethiopia. Addis Ababa: Addis Ababa University, Ethiopia **3**:442–478.

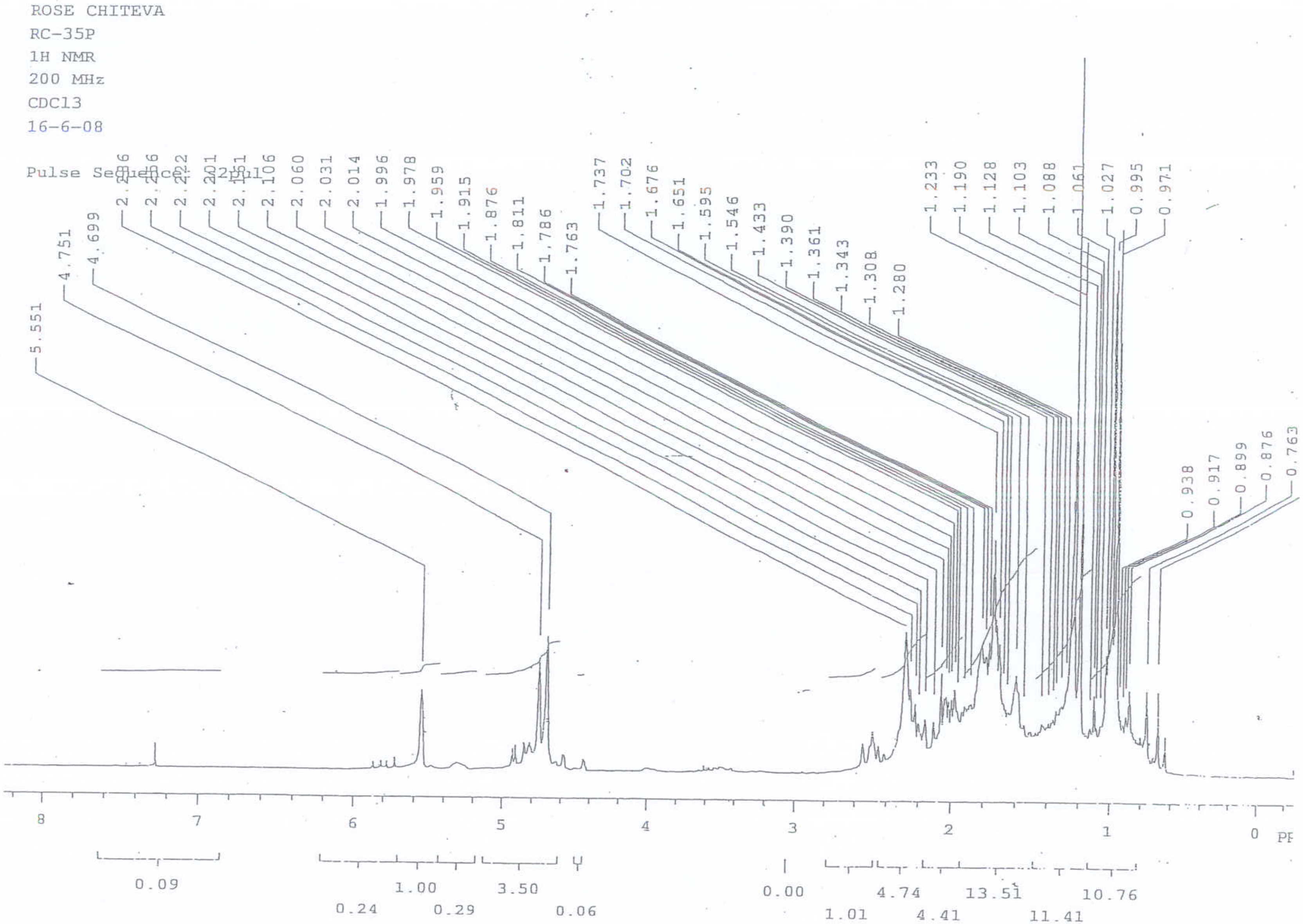
Wild, H. (1963). *Commiphora habessinica*. *Flora zambesica* **2**:263.

Zhu, N., Kikuzaki, H., Sheng, S., Sang, S., Rafi, M. M., Wang, M., Nakatani, N., Dipaola, R. S., Rosen, R. T. and Ho, C. T. (2001). Furanosesquiterpenoids of *Commiphora myrrha*. *Journal of Natural Products* **64**:1460-1462.

SPECTRA FOR COMPOUND 39

APPENDIX 1A: ^{1}H NMR SPECTRUM FOR COMPOUND 39

Solvent $CDCl_3$; SF: 200 MHZ

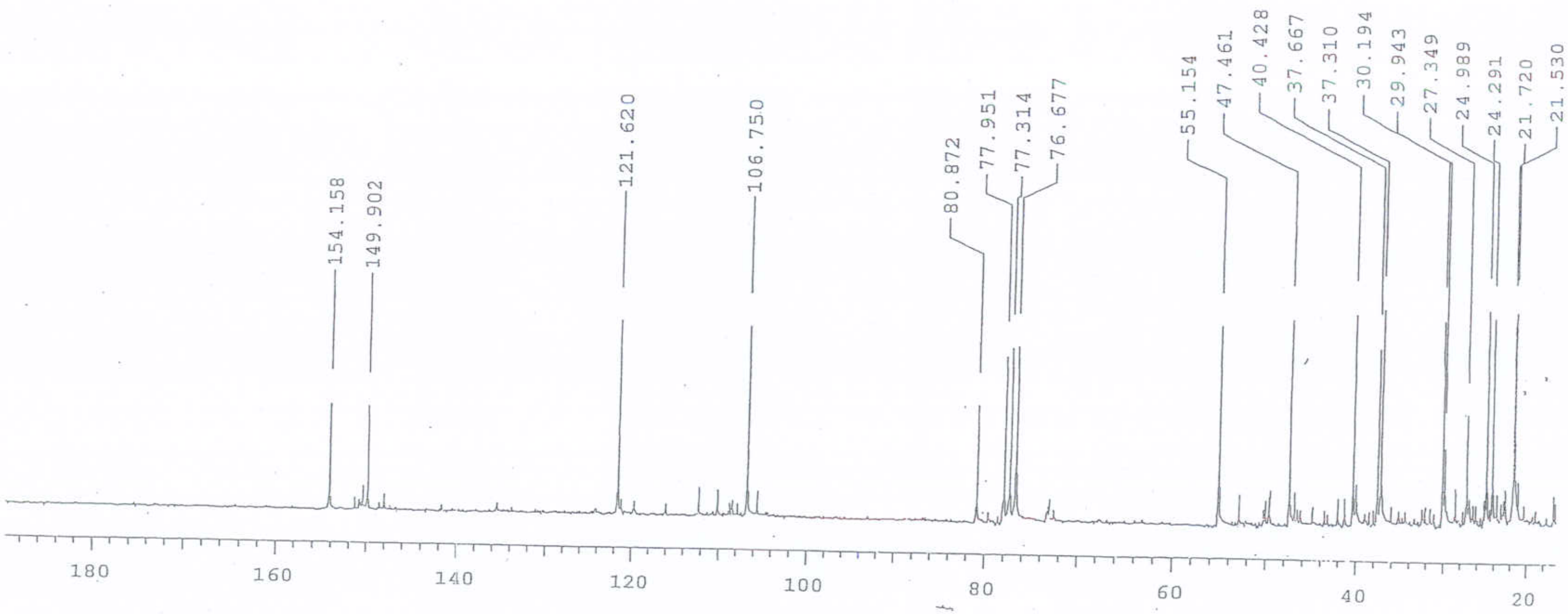


ROSE CHITEVA
RC-35P
13C NMR
50 MHz
CDCl3
17-6-08

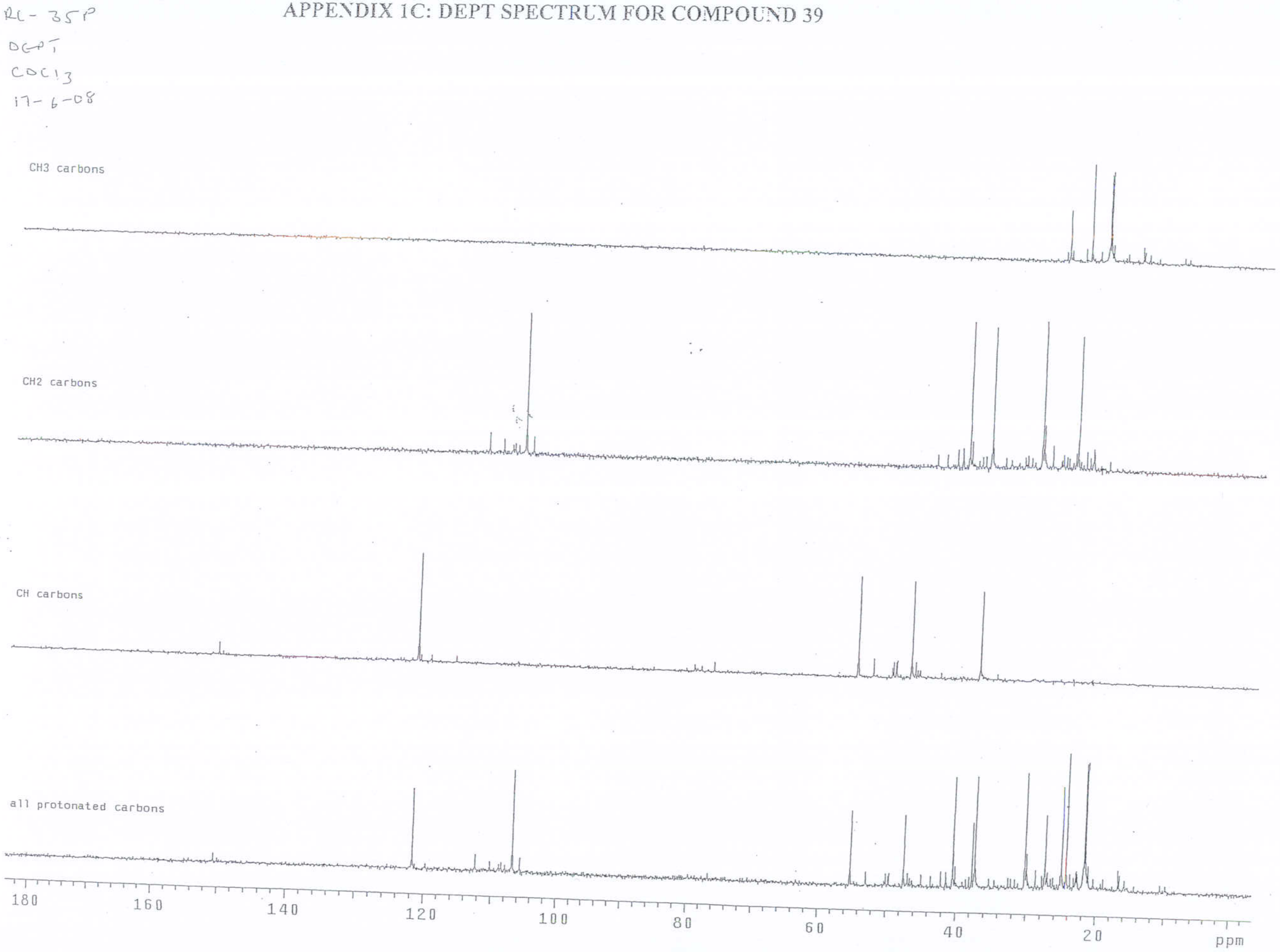
Pulse Sequence: s2pul

APPENDIX 1B: ^{13}C NMR SPECTRUM FOR COMPOUND 39

Solvent $CDCl_3$; 50 MHz.



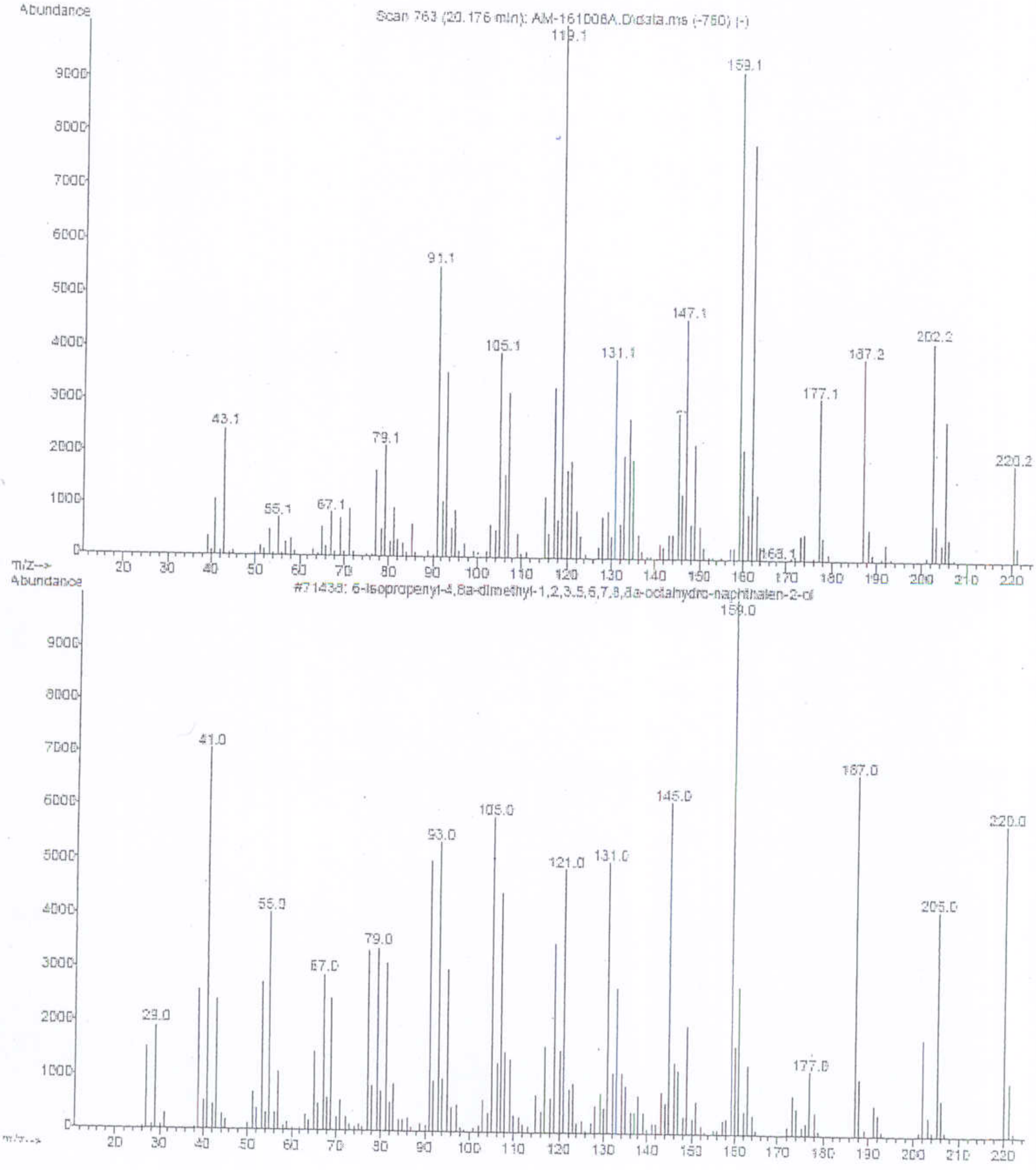
APPENDIX 1C: DEPT SPECTRUM FOR COMPOUND 39



APPENDIX 1D: GC – MS SPECTRUM FOR COMPOUND 39

7890A GC system, 5975C inert XL EI/CI MSD triple axis detector, 7683B series auto sampler

```
File        : C:\msdchem\1\DATA\AM-161008A.D
Operator    :
Acquired    : 16 Oct 2008  16:49     using AcqMethod VOLATILES-35 TO 290.M
Instrument  :    ICIPE MSD
Sample Name: HEX Extr. 3.163g
Misc Info   : Essential Oil HEX Extract 3.163g
Vial Number: 1
```



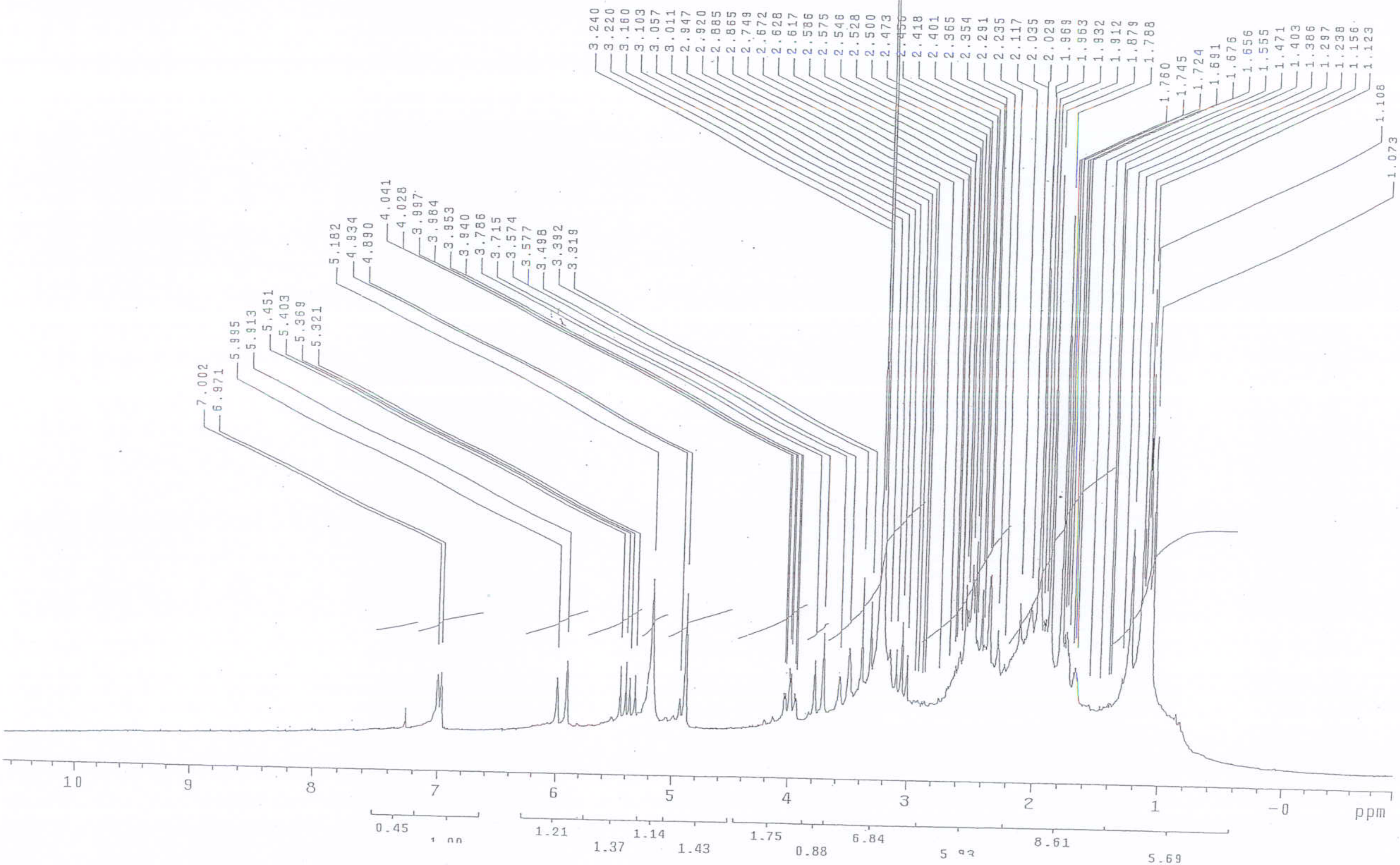
SPECTRA FOR COMPOUND 7

CHITEVA
RC-38C
1H NMR
CDCL3
26-7-08

Pulse Sequence: s2pul

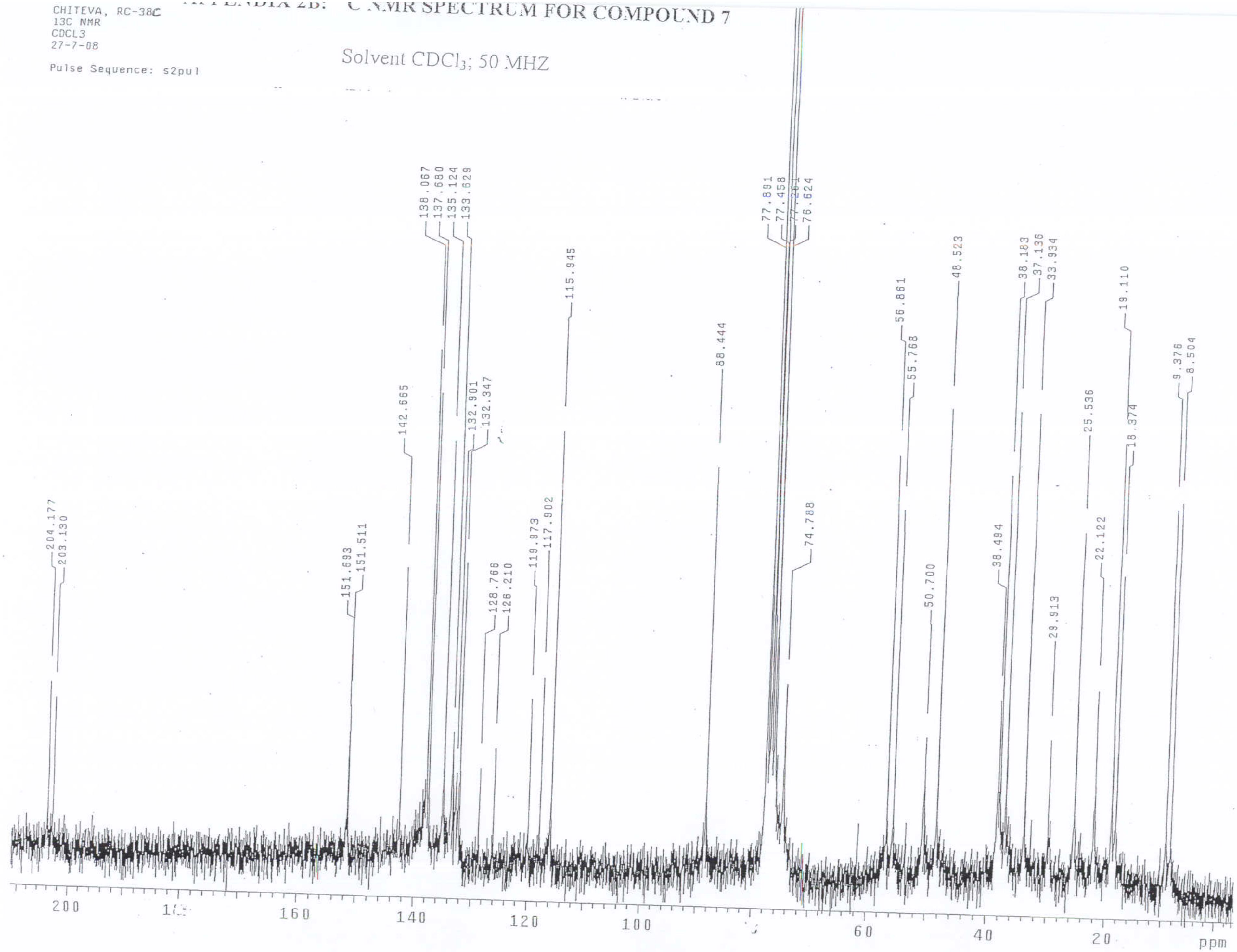
APPENDIX 2A: ^{1}H NMR SPECTRUM FOR COMPOUND 7

Solvent $CDCl_3$; SF: 200 MHZ



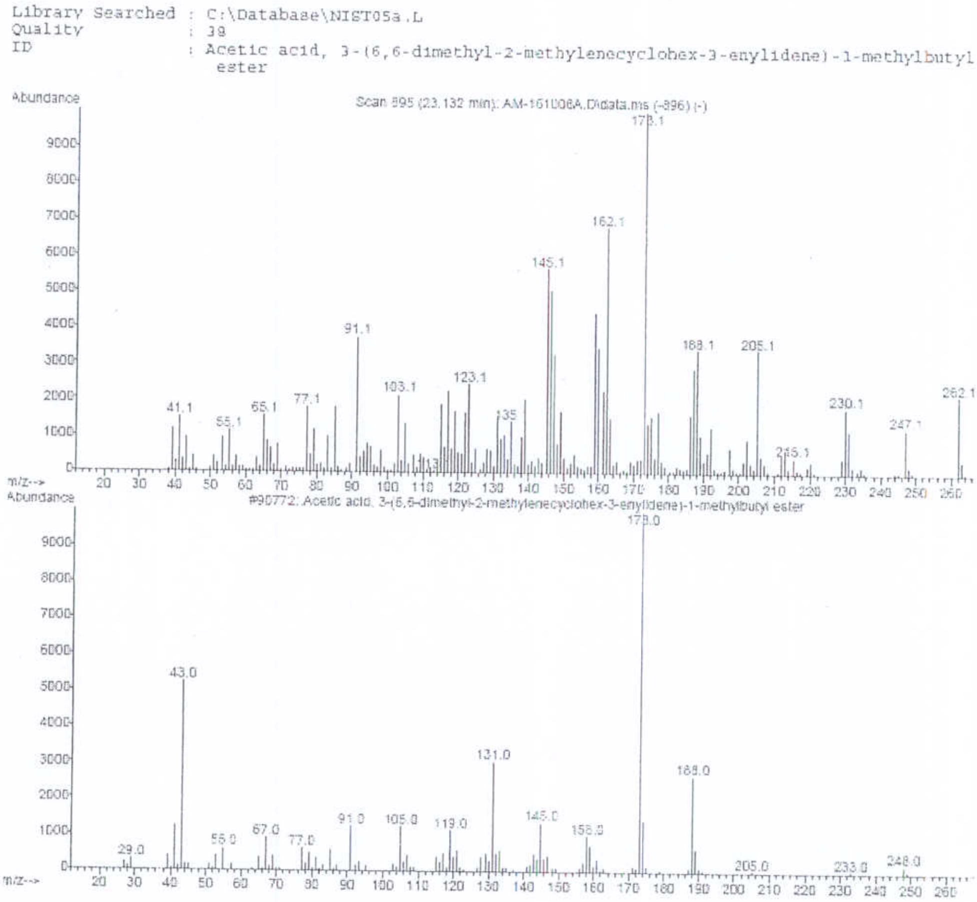
APPENDIX 2B: ^{13}C NMR SPECTRUM FOR COMPOUND 7

Solvent $CDCl_3$; 50 MHZ



APPENDIX 2C: GC- MS SPECTRUM FOR COMPOUND 7

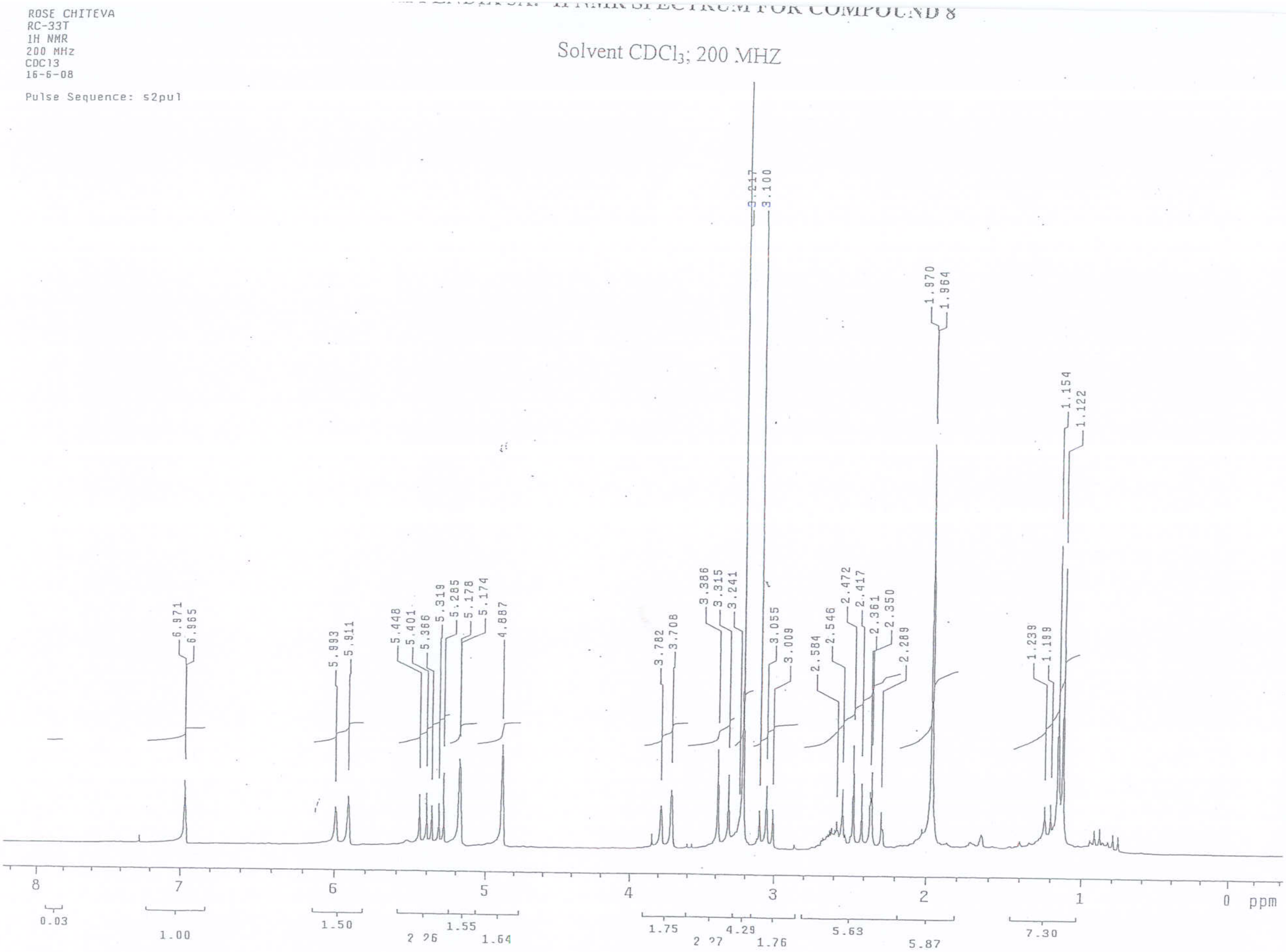
7890A GC system, 5975C inert XL EI/CL MSD triple axis detector, 7683B series auto sampler



SPECTRA FOR COMPOUND 8

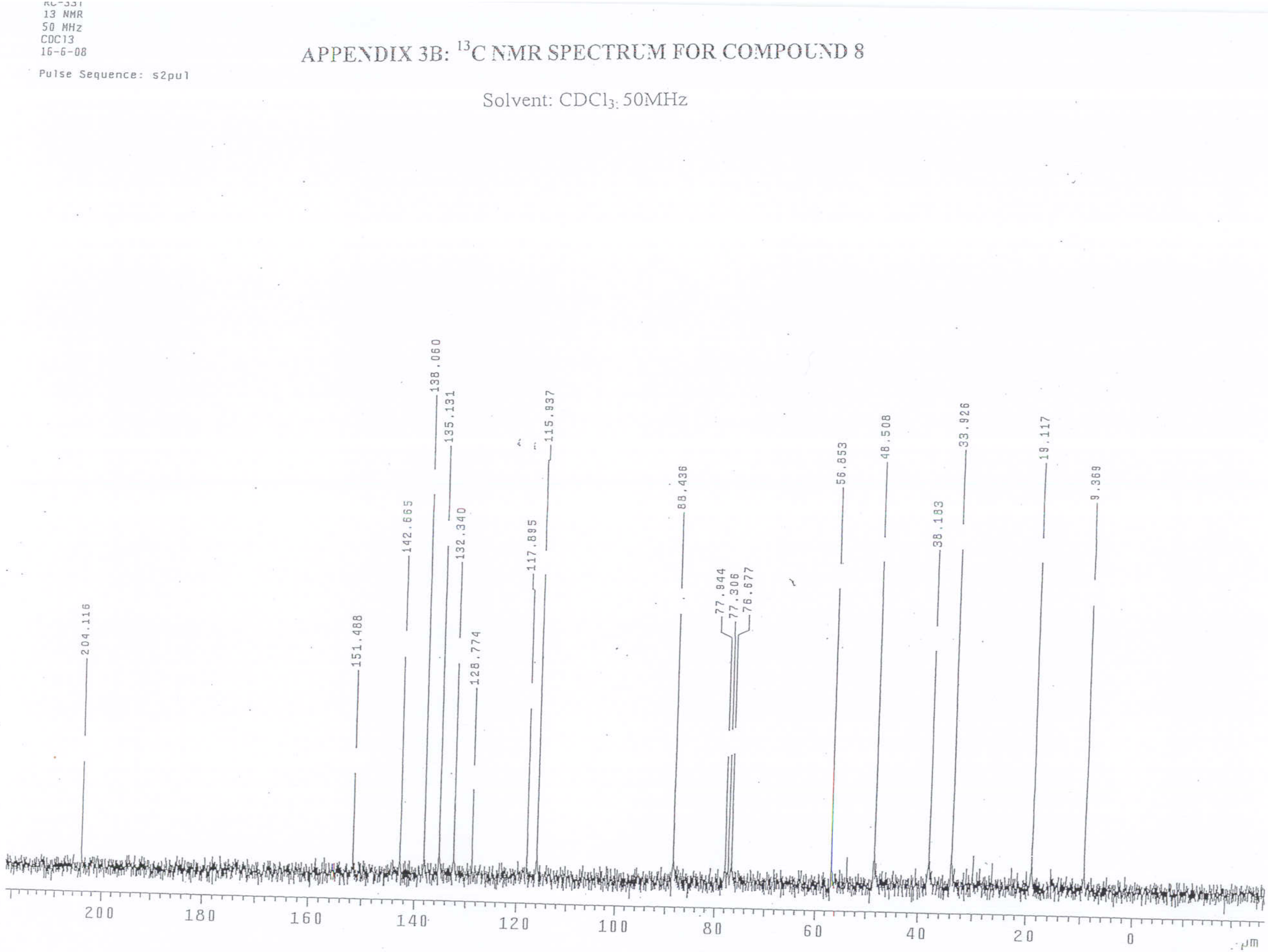
SPECTRUM FOR COMPOUND 8

Solvent $CDCl_3$; 200 MHZ



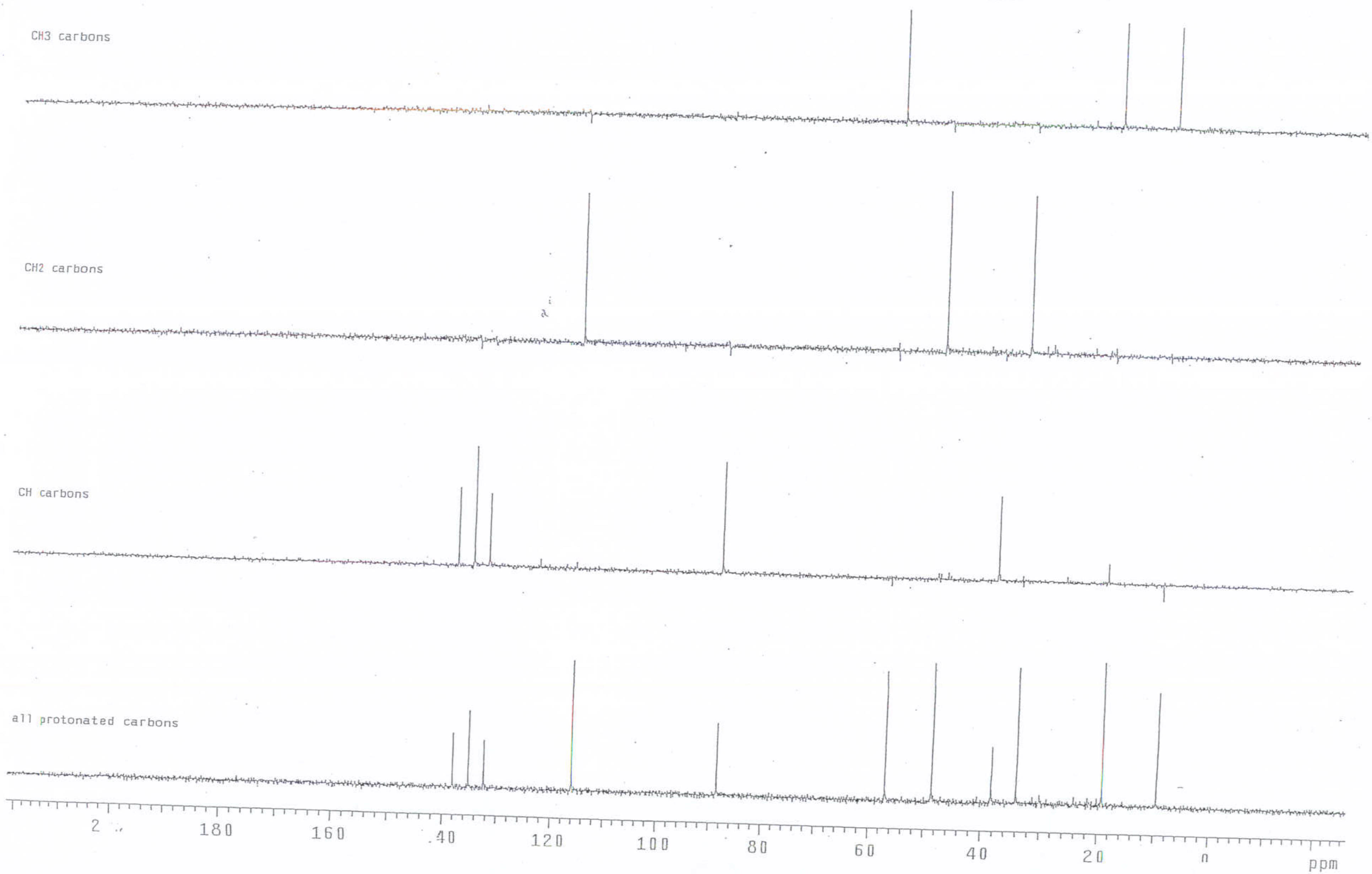
APPENDIX 3B: ^{13}C NMR SPECTRUM FOR COMPOUND 8

Solvent: $CDCl_3$; 50MHz



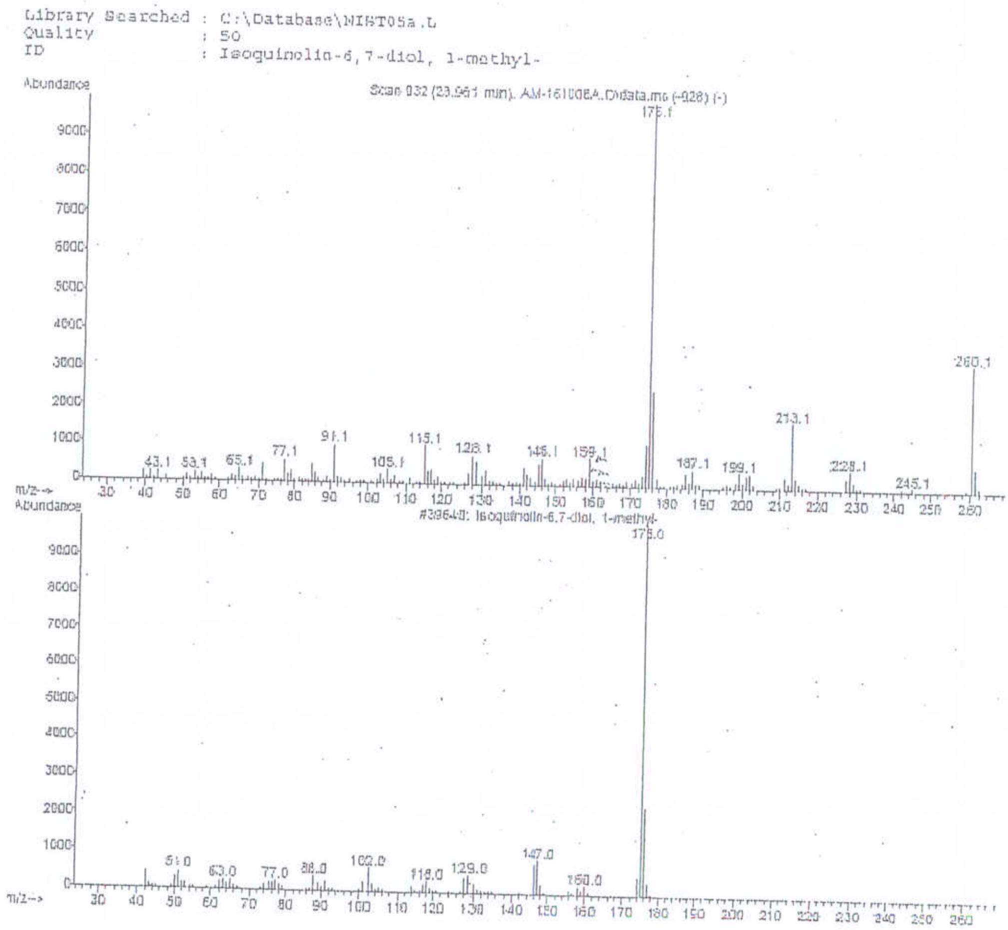
RL - 33 T
DEPT

APPENDIX 3C: DEPT SPECTRUM FOR COMPOUND 8



APPENDIX 3D: GC-MS SPECTRUM FOR COMPOUND 8

7890A GC system, 5975C inert XL EI/CL MSD triple axis detector, 7683B series auto sampler



APPENDIX 4: GC – MS SPECTRUM FOR COMPOUND 37

7890A GC system, 5975C inert XL EI/CL MSD triple axis detector, 7683B series auto sampler

```
Library Searched : C:\Database\NIST05a.L
Quality          : 97
ID               : 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-
```