ETHNOBOTANICAL USES, PHYTOCHEMICAL ANALYSIS, BIOACTIVITY AND MOSQUITO REPELLENCY OF *CYPERUS ARTICULATUS L.* FROM THE FAMILY *CYPERACEAE* FROM THARAKA MERU

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DECLARATION

THIS IS MY ORIGINAL WORK AND HAS NEVER BEEN SUBMITTED FOR AWARD OF ANY DEGREE IN ANY UNIVERSITY.

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DEDICATION

This work is dedicated to my husband, David Kiogora, my son Newton Murithi, my parents, brothers and sisters.

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ABBREVIATIONS AND ACRONYMS

CC	Column Chromatography
Cfu	colony forming units
DCM	Dichloromethane
DMF	Dimethylformade
EI	Electron Ionization
GC-MS	Gas Chromatography Mass Spectrometry
MIC	Minimum Inhibition Concentration
MS	Mass Spectrometry
NIST	National Institute of Science and Technology
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
UV	Ultra Violet
UV-VIS	Ultra Violet-Visible Spectroscopy
WHO	World Health Organization
PACN	Pan African Chemistry Network
RISE	Regional Initiative in Science and Education
AFFNET	African Natural Products Training Network
JKUAT	Jomo Kenyatta University of Agriculture and Technology

ABSTRACT

An ethno botanical survey of *C. articulatus L* (Ndago) was carried out in Tharaka-Meru district, Kenya from 8th to 10th October 2010. This study was aimed at determining traditional uses and procedures used by Traditional medicine practitioners (TMPs) to prepare concoctions of *C. articulatus L* for treatment of various ailments. It commenced with a reconnaissance survey where the researchers met with the TMPs and were briefed on the ethno-botanical uses of *C. articulatus L*. At the meeting a one day workshop was arranged and a date was fixed. The workshop was attended by thirty traditional medicine practitioners and five scientists from the University of Nairobi. During the workshop the TMPs were issued with questionnaires from which data was collected and analyzed. The data collected from the questionnaires indicated that all the TMPs were using *C. articulatus L* for treatment of typhoid fever, stomachache, headaches, blisters, wounds, skin rush, abdominal pains; cough, as perfume, as a mouth freshener and insect repellent. The extinction of the plant was established. The results of this survey were documented for further reference. The root tubers of *C. articulatus L* were subjected to extraction with the following solvent systems; 100% CH_2Cl_2, 1:1CH_2Cl_2/CH_3OH, 5% H_2O/CH_3OH. The resultant crude extract of 100% CH_2Cl_2 was subjected to a combination of chromatographic techniques including column chromatography (CC) and preparative thin layer chromatography(PTLC) for the isolation of compounds. Spectroscopic analysis including UV and GC- MS were done to determine the structures of the compounds. A total of 59 compounds were identified, of which 48 (82.76%) were terpenes. Amongst the terpenes were 27 sesquiterpenes (45.76%), 20 monoterpenes (33.90%) 1 triterpene (1.69%), and 11 non-terpenes (18.64 %). The major sesquiterpene identified in this essential oil was α cubenene.

This made it unique amongst all the other oils extracted from *C. articulatus L* from other parts of the world (Nigeria, Brazil, Japan, Taiwan, Thailand, Hawaii, and Philippines). The 100% CH_2Cl_2 crude extract was subjected to anti-bacterial tests against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Salmonella typhi* bacterial strains. The inhibition zones were taken and averaged and the extracts showed activity for the three bacterial strains. The undiluted crude extract of 100% *C.articulatus L* showed inhibition against the growth of the micro-organisms tested. The 100% CH_2Cl_2 crude extract was repellent against the mosquito *Aedes egyptii*. The above results supported the claims by the traditional medicine practitioners that *C.articulatus L* could treat skin lashes and repel the mosquitoes Aedes egyptii.

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Absence of modernized socio-economic and public healthcare systems reinforces reliance of rural and lower-income urban populations on the use of traditional medicinal-herbs and plants as complementary aids to routine pharmaceutical market products (Muhammad *et al.*, 2011). Plants and other organisms are the most efficient tools for synthesis, capable of making a diversity of secondary metabolite organic molecules (natural products) that have complex structures with a variety of physical, chemical, and biological properties. Some of these products are complex and may not be easily synthesized in the laboratory or are easily accessible from nature. For example, the anticancer drug (vincristine**1** and vinblastine **2**) extracted from periwinkle, *Catharantus roseus,* are among the most widely used natural products in the pharmaceutical industry. These products have not been challenged by any new synthetic substitutes and almost half of the available cancer chemotherapeutic drugs are of plant origin (Kinghorn *et al.*, 1999). Similarly, 25% of all anti-malarial drugs are endemic to plants, in particular the commonly known quinine which was obtained from *Cinchona species* from South America. The currently recommended artemesinin isolated from *Artemisia annua.* Today, there is a worldwide emphasis and research on herbal drugs, where majority of the studies are based on plants that have been traditionally used or claimed to have potential therapeutic properties(WHO 2003). Presently, about 200 plant-derived chemical compounds are being used as drugs or as agents for improving human health (Farnsworth *et al.*, 1985). It was estimated that the booming trade in

herbal medicine will have reached approximately US$500 billion by the year 2000 World Health Organization (WHO 2003) and governments in most developing countries are campaigning for the promotion and integration of herbal remedies in healthcare, as supplementary contribution to modern medical facilities especially in rural areas where medical care is too expensive for some people. Except for few anti-malarial drugs, all the other commonly used anti-malarial molecules are based upon plant-derived compounds (Geoffrey, 1996). Africa has a rich tradition of plant use, an immense range of climates, cultures and species and has the human and natural resources to become an even greater producer of natural plant products. The pharmaceutical potential of African medicinal plants are immense (Rukangira., 2001). In the traditional set-up, there have been a number of concoctions of plant origin for the bacterial infection. Many people in developing countries especially in the sub-Saharan Africa depend on crude extracts from plants for treatment of diseases such as malaria, cancer, allergies and AIDS (Abram *et al.*, 1990). Kenya is endowed with vast resources of medicinal and aromatic plants of which *C. articulatus L* is one. Natural treatments and alternative medicine can serve to complement more traditional therapies though some alternative approaches have questions regarding their efficacy and safety. The main aim of this research was to study the phytochemistry of rhizomes of *C. articulatus* L (Ndago) growing in Meru- Kenya. *C. articulatus L* has been used in the ethno-medicine of the Ameru for various purposes such as treatment of cough, fever, malaria, fungal infection, arthritis and abdominal pains during menstrual periods, surface skin infections, boils and wounds. It has also been used as a mosquito repellent, perfume and as a mouth freshener. Most people from the larger Meru community have used Ndago

in one way or another. It was popularly known in the Ameru tradition as a wonder drug. The popular perfume of Ndago was used on special occasions in the Ameru community , for example it was given to the newly circumcised young men and women of their age who were being prepared for marriage to apply shortly before the commencement of their popular dance for lovers (Bandago), meaning a sweet lover who has the sweet scent (smell) of Ndago. Repellency is known to play an important role in preventing the vector borne diseases by reducing man-vector contact. Synthetic chemicals and insecticides used for control of vectors are causing irreversible damage to the eco-system, as some of them are non-degradable in nature. Some repellents of synthetic origin may cause skin irritation and affect the dermis. Majority of commercial repellents are prepared by using chemicals that have been reported to be unsafe for public use. Due to unpleasant smell, oily feeling to some users and potential toxicity some people prefer to use natural insect repellent products. Repellents of plant origin do not pose hazards of toxicity to human and domestic animals and are easily biodegradable. Natural products are safe for human when compared to that of synthetic compound (Das *et al.*, 2003)

1.2 Problem statement

Tharaka district in Kenya is situated in the semi arid parts of the larger Meru region. By the time of this study there was not a single tarmac road in the whole district and medical services were not easily accessible. The nearest well equipped public hospital is Meru general hospital situated in Meru central district in Meru county .The other hospitals are Nkubu Consolata and Chogoria PCEA mission hospitals that are situated

in neighboring districts and are too expensive for most poor people of Tharaka district. Traditional medicines practitioners (TMPs) were the most accessible and affordable, as they even accepted payment in kind (chicken, goats and cows). *C. articulatus L* is one of the most widely used medicinal plant by the TMPs for the treatment of malaria, fever, typhoid, cough, common cold, flu, pneumonia, abdominal pains, fungal infection, skin rush , wounds, as mouth freshener and as a perfume.

1.3 Justification

Several plants are traditionally used in Tharaka for treatment of typhoid, abdominal pains, stomachache, common cold, flu, fever, malaria, skin rush, wounds, blisters, swollen breasts and fungal infections. *C. articulatus L* is one of these most popular plants used traditionally by Ameru in Tharaka and the neighboring Tigania, Igembe, Chogoria, Mwimbi, Chuka and Iment communities of Meru for the treatment of the above named diseases, it is also used as mouth freshener, perfume and as mosquito repellent hence the need to carry out a research on it to establish the scientific basis of its popular use.

1.4 Objectives

1.4.1 General objective

To document the ethno-botanical uses, characterize the components, determine the antimicrobial and mosquito repellency potency of *C.articulatus* L from Tharaka Kenya.

1.4.2 Specific objectives

1. To conduct an ethno botanical survey of *C. articulatus L* in Tharaka Meru.
2. To extract root tubers of *C. articulatus L* using different organic solvents systems.

3. To determine the components of the extract using chromatographic and spectroscopic methods.

4. To carry out anti-bacterial assays on the extract.

5. To carry out mosquito repellency test on the extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical information

The following ethno-botanical information was used to classify *C. articulatus* L from Tharaka.

Kingdom *Plantae* – Plants

Subkingdom *Tracheobionta* – Vascular plants

Super division *Spermatophyta* – Seed plants

Division *Magnoliophyta* – Flowering plants

Class *Liliopsida* –Monocotyledons

Subclass *Commelinidae*

Order *Cyperales*

Family *Cyperaceae* – Sedge family

Genus *Cyperus L.* – flatsedge

Species *Cyperus articulatus L.* – jointed flatsedge

2.1.1 Family Cyperaceae (Sedge Family)

The plants in the family *Cyperaceae* grow in wet areas along rivers, ponds or swamps. Their stems are usually solid and three-angled (those edges - you may have to slice it toward the base to really see it). The leaves, when present, are slender but with a substantial stem clasping basal sheath with fused edges. The flowers (or florets in this case) are clustered in spikelets. There are usually 3 stamens, and 2 to 4 feathery stigmas on the pistil. The family *Cyperaceae* includes approximately 36 genera and about 128 species of *Cyperus (*Neville *et al.*, 1968).

2.1.1.1 *Cyperus rotundus*

C. rotundus is a traditional medicinal plant appearing among Indian, Chinese and Japanese natural drugs used against involuntary muscle contraction and stomach disorders. The rhizome oils of this plant from different countries show compositional differences, suggesting the existence of phytochemical varieties. The essential oil as well as solvent extracts of the rhizomes of *C. rotundus* have been subjected to numerous studies resulting in the isolation of many terpenoids. Essential oils do not crystallize (Mesmin *et al.*, 2001). In Amazonian region *C. prolixus* and *C. rotundus* were cultivated mostly in home gardens for medicinal purposes and aromatization of dish washers. They have been widely utilized for various medicinal purposes, including birth control and induction of labor and in hallucinogenic preparations (Maria *et al.*, 2008). In South Africa *Cyperus rotundus* is a multipurpose plant used in traditional medicine to treat stomach ailments, wounds, boils and blisters. A number of pharmacological and biological activities including anti-candida, anti-inflammatory, anti-diabetic, anti-diarrhea, anti-mutagenic, anti-microbial, anti-bacterial, anti-oxidant, anti-pyretic and analgesic activities have been reported for this plant. Previous phytochemical studies on *C. rotundus* revealed the presence of alkaloids, flavonoids, tannins, starch and many sesquiterpenoids. The observed compositional difference between *C. rotundus* found in South Africa and the rest of the world could be due to climactic and environmental conditions, chemo types, nutritional status of the plants, and other factors, which can influence essential oil composition (Oladipupo *et al.*, 2009). Essential oil from the tubers of *Cyperus rotundus*, obtained by steam distillation by Kilani *et al*, was analyzed

by GC and GC/MS and a total of 33 compounds were identified, the oil was characterized by its high content of sesquiterpenes with cyperene (30.9%) being major. The antibacterial activity of oil from tubers of *Cyperus rotundus*, showed more important activity against the bacteria *Staphylococcus aureus* (Kilani *et al.*, 2005).

2.1.1.2 *Cyperus articulatus L*

C. articulatus L was described in 1753 by Carl Linnaeus. The name is considered as validly published (Brickell, 2003). *C. articulatus L* is a type of reed-like tropical grass, used in Meru for medicinal purposes as earlier mentioned. It is an aromatic herbaceous species of grass with short rhizomes, thin and resistant roots. It grows in damp, marshy and flooded areas along the rivers and streams (where it can help to control soil erosion). It can attain a height of 6 feet as shown in figure 2.2.

Figure 2.1 *Cyperus articulatus L*

It grows in clumps from dividing rhizomes which are about 1cm in diameter some times in a series of two or three, connected by an underground stem (Rain Tree Nutrition,

2006). *C. articulatus L* often occurs in almost pure stands in tropical and warm temperate localities that provide permanent water. It is distinguished by its robust, leafless culms. (Gordon *et al.*, 2006) The tall green stems are fibrous, round, and hollow at the base with jointed flat edge. Its blackish red tubers are 1 to 3 cm long. This is as shown in figure 2.3.

Figure 2.2 Dried tubers of *C.articulatus L*

C. articulatus L has an aroma similar to lavender and the aromatic properties are used in folklore medicine to cause a feeling of warmth in the body which aids in the treatment of digestive disorders and its sedative effects (Rain tree Nutrition, 2006).

2.1.1.2.3 Cultivation

C. articulatus L prefers a sunny to half shady site. It grows best in loamy wet soil (Brickell, 2003).

2.1.1.2.4 Geographical Distribution of *C. articulatus L*

C. articulatus L is native to Asia, Africa, Texas, the South East of the US, Florida, Mexico, Central America and S. America (Brickell, 2003). A number of plants species that are found in wetland areas are important economic resources for women in Swaziland. C. *articulatus L* and *Schoenoplectus corymbosus* plants are used for making food mats, sleeping mats, bags, and baskets, hence provide economic livelihood to many women (Edje, 2006). *Cyperacea* grown in Egypt have been investigated for their flavanoids (Habershy *et al.*, 1989). Although native to the Amazon, *C. articulatus* L (piri-piri) can be found in many other tropical areas and countries. It occurs alongside the Nile River in Africa just as it grows alongside the Amazon River in South America (Taylor, 2001).

2.2 Ethno-Medicinal Uses of *C. articulatus L*

Ethnopharmacology and natural product drug discovery remains a significant hope in improving the poor livelihoods of rural communities (Nanyingi *et al.*, 2008). Over many years plants have been used for drugs and as fragrance materials. The chemical characterization of rhizomes of *C. articulatus L.* shows the presence of flavonoids, saponins, triterpenes, sesquiterpenes and ketones. As some of the diseases treated with *C. articulatus L.* (migraines, headaches and according to a personal communication also epilepsy) concern the nervous system, some pharmacological work has been done to define its interaction with this system. Decoction of rhizomes of *C. articulatus L.* possesses depressant activity in the central nervous system (Ngo *et al.*, 2001). *C. articulatus L* (Piri-piri) has a long history of use in herbal medicine systems in South

America. It is a very common remedy for treating nausea, vomiting, stomach-aches and intestinal gas throughout the continent. In Peru, piri-piri is considered as an abortifacient, anticonvulsant and anti-epileptic and treats stomach-ache (Taylor, 2001). The crude drug prepared from the rhizomes of this plant has been used in traditional medicine as contraceptive (Helena *et al.*, 2006). It is used for diarrhea, dysentery, digestive disorders and intestinal infections, intestinal worms, epilepsy, to stop bleeding (internally and externally) and to heal wounds. In Africa, piri-piri is used for malaria, toothaches, headaches, diarrhea, indigestion and coughs (Taylor, 2006). *C. articulatus L* is popularly known as priprioca in Pará State (Brazil). Priprioca has aroused scientific and economic interest because of the pleasant aroma of the volatile oil obtained from the plant rhizome. This species has great importance in the local pharmacopoeia of Brazil. It is mainly used as a contraceptive, a painkiller, and in the treatment of diarrhea. The volatile part of the priprioca extract (the essential oil obtained by hydro-distillation) mainly consists of α-pinene, β-pinene, limonene, myrtenol, α-copaene, and caryophyllene oxide (Lucinewton *et al.*, 2008). In Brazil *C. articulatus L* is cultivated and commercialized by small holders for direct market sale, and as a raw material for the perfumery industries. Anticonvulsant, sedative, antibacterial, and activity on epilepsy were reported from this plant (Maria *et al.*, 2008). Extracts from rhizomes of *Cyperus articulatus L.* (*Cyperaceae*) used in Africa and Amazonia has been used for many different ailments including; digestive disorders, menstrual irregularity and has been used for its sedative properties and anticonvulsant properties in the treatment of epilepsy (Bum *et al.*, 2004). Rhizomes of *C. articulatus L.* pocesses anticonvulsant properties in animals and this explains its use as a traditional medicine for epilepsy in

Africa (Ngo., 2001). In Cameroon qualitative chemical characterization of the total extract showed that *C. articulatus L* contains flavonoids, saponins, polyphenols, tannins, terpenes and sugars. The total extract of the rhizome of *C. articulatus L* did not appear to possess either an aesthetic or paralyzing effects. In contrast, spontaneous motor activity is significantly reduced by the extract. However, *C. articulatus L* does not seem to have muscle relaxant effects. When associated with sodium thiopental or diazepam, the extract facilitates sleep induction, and increases the total sleep time without any concomitant analgesic effects (Vincent *et al.,* 2000). *C. articulatus L* has been used traditionally for the treatment of pain, cough, flu, common cold, fever, malaria and typhoid. Intensive research on the plant in relation to a number of ailments has been carried out in Brazil, S Africa, Cameroon, Nigeria and Peru. Many of its biological actions are attributed to various sesquiterpenes called cyperones which are also found in other *Cyperus* plants in the family. Nyasse *et al (1988)* have isolated the sesquiterpenoic ketones, mandassidione, mustakone, corymbolone and the alcohol corymbolol from Cameroonian grown *C. articulatus L*. Earlier work on the essential oils from the Canadian grown *C. articulatus L* has led to the isolation and characterization of a bicyclic ketone, cyperotundone (Nyasse *et al.*, 1988). Two of these compounds called cyperotundone and α-cyperone, have been reported to posses anti-malarial activities, as well as the ability to inhibit nitric oxide synthesis and *prostaglandin synthetase* inhibitor. Aspirin and ibuprofen are *prostaglandin synthetase* inhibitors (Taylor 2006). Corymbolone is a sesquiterpenoid keto-alcohol first isolated in 1985, in South America from the rhizomes of *Cyperus corymbosus Rottboll*. Some years later, corymbolone was isolated in Cameroon, from *C. articulatus L* along with

another sesquiterpene. Two sesquiterpenes, corymbolone and mustakone, isolated from the chloroform extract of the rhizomes of *C. articulatus L*, exhibited significant anti-plasmodial properties. Mustakone was approximately ten times more active than corymbolone against *Plasmodium falciparum* (Rukunga *et al.*, 2008). In 2009 ant-malaria activity of a water and methanol extract of the same plant was reported by Rukunga and Muthaura et al 2011. *C. articulatus L* has several uses in many parts of the world. Table 2.1 gives a brief summary of its uses around the world and table 2.2 shows compounds reported from the plant from various regions of the world.

Table 2.1 Documented ethno medicinal use of *C.articulatus L* from various parts of the world

Part/location	**Documented ethno medicinal use**
Rhizome / Africa	Used for toothaches, headaches, diarrhea, indigestion, and coughs
Rhizome / Brazil	Used as an antivenin for snakebite
Rhizome / Ecuador	Mix ground rhizome with water for fever, and influenza,
Rhizome / Guyana	Used for stomachache.
Rhizome/ Peru	Used for snakebite: the fresh raw rhizome is chewed fresh and the juice swallowed, then the pulp is put onto the bite after it has bled .Used as a contraceptive. Used as a hair tonic and for baldness and as a hemostat. Juice taken as a nerve tonic; in cases of Stress, nervous and mental disorders. Juice is taken for malignant tumors and throat cancer Juice is taken as an abortifacien Used for dysentery and other severe intestinal infections digestive disorders Used as a rheumatic pain reliever. Used for healing wounds.
Rhizome / USA	Used to control nausea, stomach pain, and gas. Used for headaches, epilepsy, blood in the urine, menstrual irregularity, breast pain, and vaginal discharge. Used for vomiting (2 ml fluid extract). Used as aromatic tonic, and Anthelmintic. (Called andrue), Considered

	Gently stimulating, warming, diffusive,used as a gastric tonic. Used to soothe the nervous system and increase skin blood circulation, As an anti-emetic and carminative; for digestive disorders, and intestinal gas
Leaves/Guinea	Used as a cerebral anti-malarial., Used for wounds and hemorrhages

Adopted from Leslie Taylor 2006

2.3 Essential oils

For several centuries terpenes are known to be components of essential oils (fragrant oils) from leaves, flowers and fruits of plants. Example are α Pinene, β Pinene Caryophyllene oxide, Mertenol, thymol and eucalyptol (Zhang 2005). Essential oils are highly concentrated volatile, aromatic essences of aromatic plants and they have been known since ancient times to possess biological activity. They have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons and oxygenated compounds which are highly odoriferous (Rios & Recio, 2005). All essential oils are principally composed of a class of organic compounds built of *"isoprene units."* An isoprene unit is a set of five connected carbon atoms with eight hydrogens attached. Molecules built of isoprene units are all classified as *"terpenes"* (Dewick, 2002). Terpenes contained in essential oils are compounds with tiny molecular structures and very small molecular weights (Smith *et al.*, 2001). Monoterpenes, with sesquiterpenes, are the main constituents of essential oils. While a few, such as camphor, occur in a near pure form, most occur as complex mixtures, often of isomers difficult to separate (Solomons, 1996).

2.3.1 Composition

Literature information is scanty on the chemical composition of the oil from *C. articulatus L* from East Africa and Kenya. However, research works on the oil composition of other *Cyperus* species have been reported (Nureni *et al.*, 2006). The chemical composition of the volatile oils of *Cyperus rotundus* has been extensively studied and four chemo types from different parts of Asia have been reported. The H-type from Japan was found to contain α-cyperone (36.6%), β-selinene (18.5%), cyperol (7.4%) and caryophyllene (6.2%) as the main constituents. The M-type from China, Hong Kong, Japan, Taiwan and Vietnam had α-cyperone (30.7%), cyperotundone (19.4%), β-selinene (17.8%), cyperene (7.2%) and cyperol (5.6%) as the main constituents. The O-type from Japan, Taiwan, Thailand, Hawaii and the Philippines was characterized by cyperene (30.8%), cyperotundone (13.1%) and β-elemene (5.2%). In addition, the Hawaiian O-type had cyperotundone (25.0%) and cyperene (20.7%) as the major compounds. Finally, the K-type, also from Hawaii, was dominated by cyperene (28.7%), cyperotundone (8.8%), patchoulenyl acetate (8.0%) and sugeonyl acetate (6.9%) (Oladipupo *et al.*, 2009).

2.3.2 Source and Isolation

Almost all odoriferous plants contain essential oils. Depending on the type of the plant, various parts of the plant may be used for isolation of essential oils, e.g. fruits, seeds, buds and flowers, leaves, and stems, roots, bark or wood. The raw material from which essential oils are manufactured may be fresh, partially dehydrated or dried, but flower

oils must be fresh. Many methods are used for the isolation of essential oils. These include;

Distillation-It is the most important method in obtaining essential oils from plants.

Merceration & enfleurage- This is used for obtaining oils from flowers and yields highly fragrant oils.

Solvent Extraction- This technique is used in order to increase yield of oil, or to extract products that cannot be obtained by any other processes, this was the method that was used in this study.

Extraction by cold pressing expression- This is applied only for Citrus oils (Mesmin et al., 2000).

2.3.3 Chemical Analysis

Many methods have been used for studying the chemical composition of essential oil, these include; IR, UV, NMR spectroscopies and gas chromatography. Chemical analysis of essential oils is generally performed using GC (quantitative analysis) and GC/MS (qualitative analysis). Gas chromatography has three main advantages over other analytical methods, it is very rapid, it has very high separation capacity and also very great sensitivity. These may be the reasons for its great preference in essential oils analysis. Identification of the main components is carried out by the comparison of both the GC retention times and MS data against those of the reference standards (with known source). Analytical conditions and procedures used should carefully be described. These include; apparatus of oil analysis (make and model number of the equipment), column type and dimensions, carrier gas flow rate, the temperature

programming conditions including injection temperature, detector and column temperatures, in addition to mass spectra (electronic impact). Sometimes identification by GC/MS must be confirmed by retention indices on two columns of different polarity but at a different temperature. Data should thus include essential oils optical rotation, density and refraction index. On the other hand, compounds which are not easily separated by GC, and molecules structurally similar like stereo-isomeric compounds of essential oils are analyzed by 13C NMR (Lahlou., 2004).

2.3.3.1 GC/MS Technique

When a beam of electrons (70eV) from the heated filament in the GC-MS collides with sample molecules it produces a positively charged ion which is the molecular ion. The molecular ion peak in this case was [M-H] +. The produced molecular ion undergoes a series of fragmentation reactions to produce other smaller fragments both positive and negative. Most fragmentations occurs soon after the molecule is ionized ($<10^{-6}$ s) in the ion source. The positively charged ions are repelled by the magnet and pushed forward towards the Mass Spectrometer (MS) which in this case was used as the detector. These fragmentation reactions yield 'finger print' (mass spectrum) that are detected by the MS. Only positive fragments are analyzed by the mass spectrometer therefore the spectrum shown in the appendices for compounds one to fifty nine consisted of only positive species. The fragmentation is as a result of unimolecular reactions and is solely due to the internal energy of the ions. This internal energy is imparted during the ionization step. The ionization energies of most organic molecules are known to be in the range of 7-10eV while the Ionizing electrons has 70eV and imparts 5-8eV of internal energy to the molecular ion as it is being formed. The internal energy imparted

to the ion during formation is more than enough to break bonds (2-4eV) and this causes further fragmentation. This fragmentation is through real chemical reactions with defined mechanisms, they are not random. Fragmentation reactions are fairly fast because ions are present in the ion source region for just a few microseconds (10-6s).

2.3.4 Uses of essential oils in medicine and modern civilization

The use of essential oils is potentially in the pharmacological field and in food preservation technology for its antimicrobial effects (Ciani et al., 2000). Essential oils are commercially important as the basis of natural perfumes and also of spices and used for flavoring purposes in the food industry (Okigbo *et al.*, 2009). Terpenoids which are components of essential oils display a wide range of biological activities against cancer, malaria, inflammation, and a variety of infectious diseases (Zhang *et al,* 2005). Many essential oils such as eucalyptus oil and peppermint oil are used as additives in pharmaceutical preparations. They are used not only for their flavor and fragrant properties but also for their biological activity (Mesmin *et al.*, 2000). The essential oil of *Thymus vulgaris* (*Lamiaceae*) is the natural source of Thymol a monocyclic phenolic compound ($C_{10}H_{14}O$). It is widely used in medicine for its antimicrobial and antiseptic action against oral bacteria and wound healing properties. Previous investigations have reported its antioxidant properties (Archan *et al.*, 2009). Thymol has been reported as anti-cancer agent, but its anti-cancer mechanism has not yet been fully elucidated (Dipanwita *et al.*, 2011).The structure of Thymol is as shown in Figure 2.4 below.

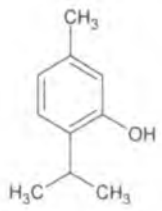


Figure 2.3 Thymol

In Austria Thymol isolated from *C.articulatus L* is said to be an important additive in cosmetic products (e.g. mouthwash, bath essences, etc.), several traditionally used medicines, and also a main ingredient in different spices and herbs (e.g. thyme, oregano, savory, etc.) which results in the fact that these products are daily consumed in considerable amounts (Thalhamer *et al.,* 2011). In 2004, Brazil was the 10th largest essential oil importer ($42 million) in the world. Simultaneously, the country is the world's fourth leading essential oil exporter (US$98 million). The domestic cosmetic and perfumery industry fragments as follows: hair care 25%, fragrance 13%, oral care 10%, bath 10%, skin care 9% and deodorant 9%. The country's growing sales rate in cosmetics (17%) has outstripped everyone (including China), except for Argentina. The market share of the Brazilian cosmetics segment breaks down into: 69% personal care, 18% cosmetics and 13% perfumes. Brazil has already commercialized a number of traditional raw materials for the fragrance industry. Some interesting emerging essential oils of the region include Priprioca (*C. articulatus L*) (6^{th} international congress on perfumery and natural raw materials Grasse-France).

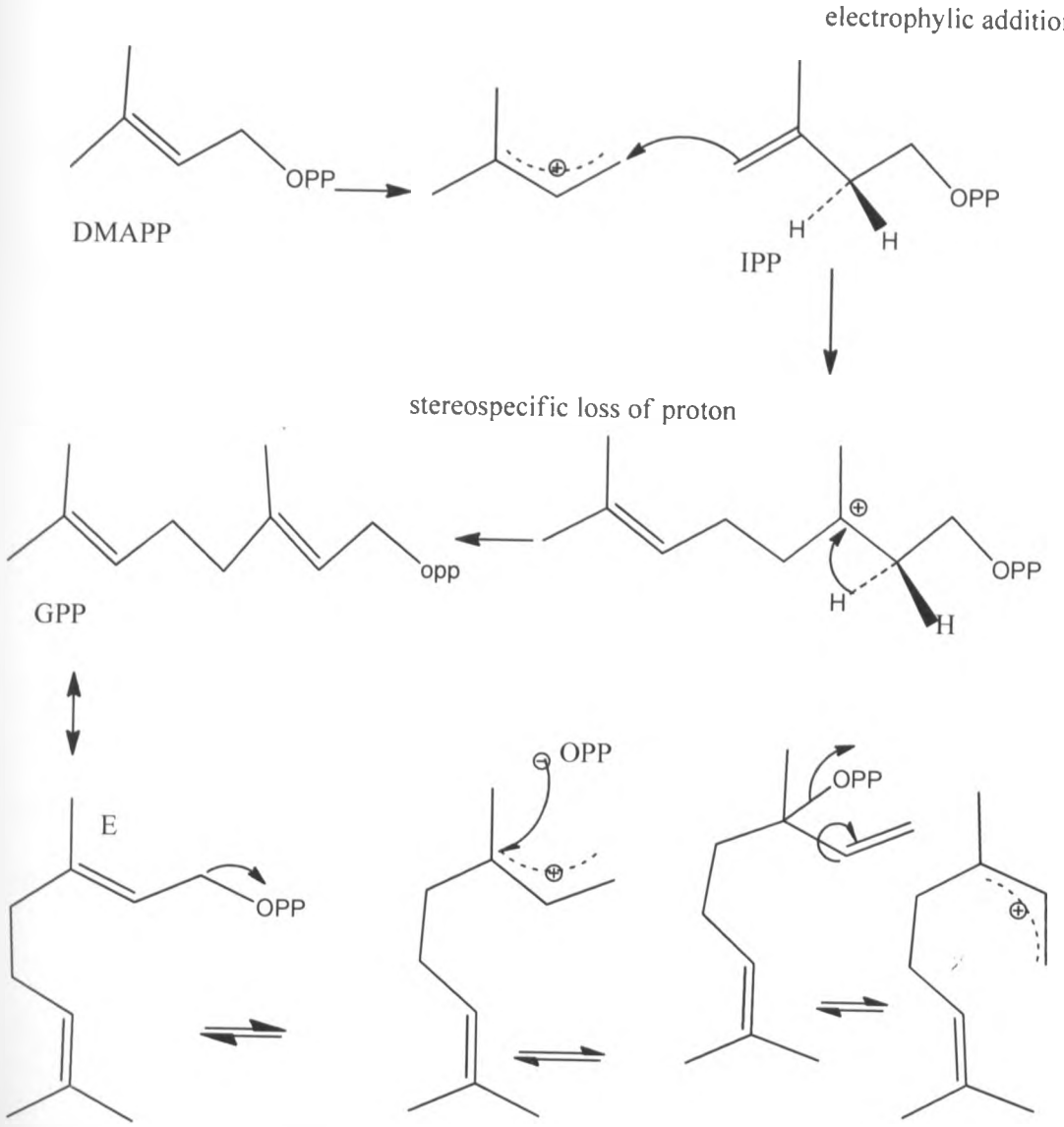
2.4 Terpenes as constituents of essential oils

Terpenes are small organic hydrocarbon molecules, they may be cyclic or acyclic, saturated or unsaturated (Smith *et al.*, 2001). Terpenoids, also referred to as terpenes,

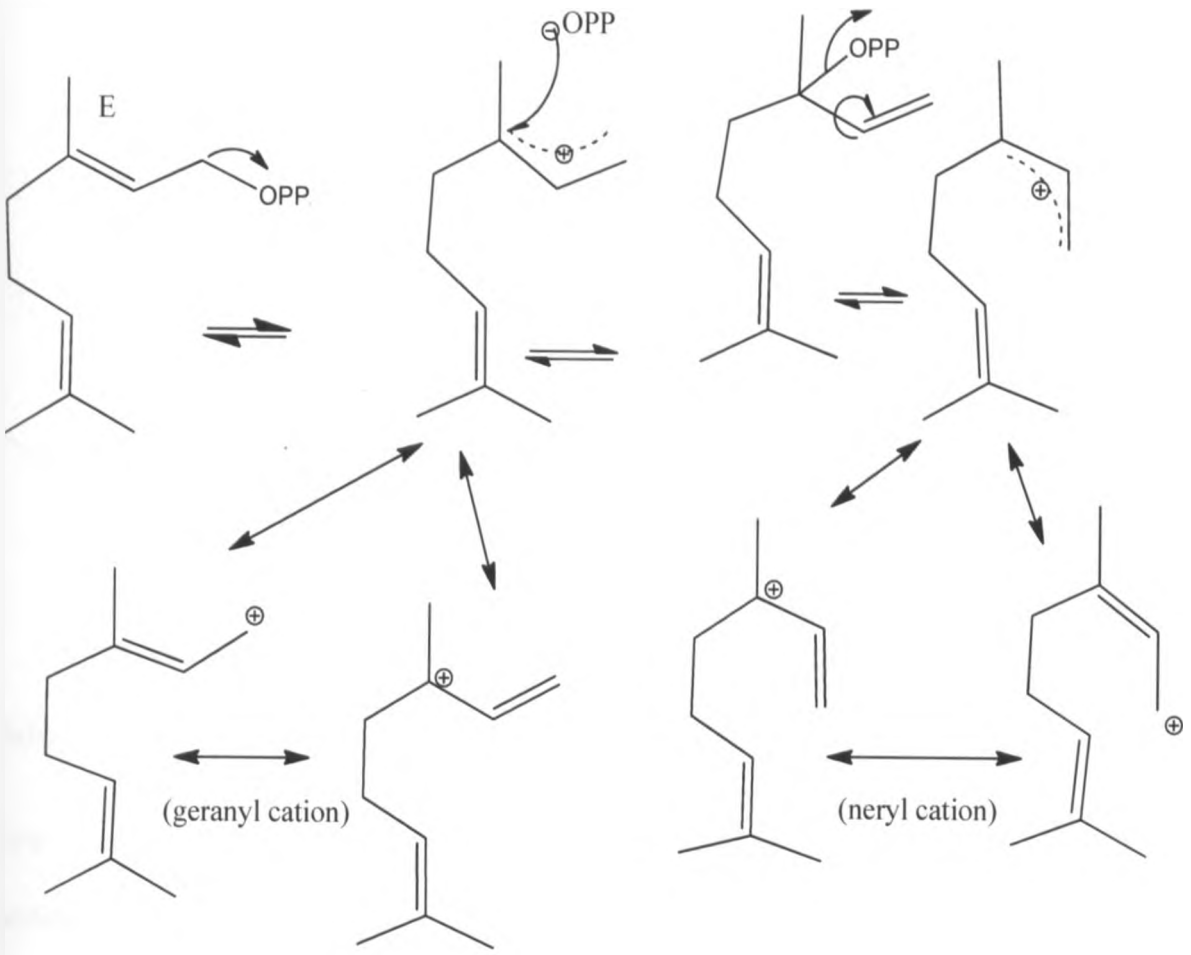
are the largest group of natural compounds. Many terpenes have biological activities and are used for the treatment of human diseases. The worldwide sales of terpene-based pharmaceuticals in 2002 were approximately US $12 billion. Among these pharmaceuticals, the anticancer drug taxol and the ant-malarial drug artimesinin are two of the most renowned terpene-based drugs. Based on the number of the building blocks, terpenoids are commonly classified as monoterpenes C_{10}, sesquiterpenes C_{15}, diterpenes C_{20}, sesterterpenes C_{25}, triterpenes C_{30} ,Carotenoids C_{40}, and polyisoprinoids $C_{\geq 40}$ (Zhang *et al*,. 2005). C_{10} and C_{15} terpenes are the chief constituents of essential oils. Terpenes are what make essential oils unique in the world of natural substances. The terpenes are structurally diverse and widely distributed. Most terpenes have been isolated from plants.

2.4.1 Biosynthesis of terpenes

The 5 carbon building blocks of all terpenoids are isopentenyl phyrophosphate (IPP) and dimethylally diphosphate (DMAPP). The complete sequence of the formation of the IPP from the five carbon unit has not yet been elucidated. DMAPP may be derived from IPP or may be produced independently (not yet clarified). Combination of DMAPP and IPP via the enzyme prenyl transferase yields geranyl diphosphate (GPP). Linalyl PP and neryl PP are isomers of geranyl PP which are likely to be formed from GPP by ionization to the allylic cation. These three compounds give rise to a range of monoterpenes (Dewick 2002). Scheme 2.1 gives a proposed mechanism for biosynthesis of terpenes.



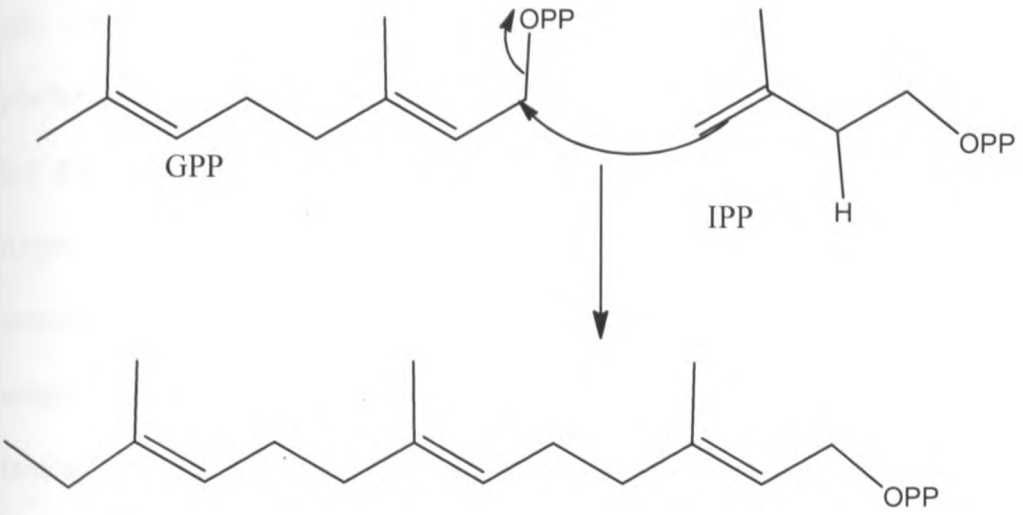
Scheme 2.1a Mechanism for Biosynthesis of Terpenes



C10 Compunds

Scheme 2.1b Biosynthesis continued

Addition of a further C_5 IPP unit to GPP leads to farnesyl diphosphate (FPP) which is the sesquiterpene precursor.



FPP

C-15 Compounds
(sesquiterpenes)

Scheme 2. 2 Sesquiterpene (C_{15} Compounds)

FPP gives rise to linear and cyclic sesquiterpenes. The increased chain length and additional double bond leads to possible cyclization and a huge range of mono, bi- and tri-cyclic sesquiterpene structures can result. In some cases the tartially diphosphate nerolidyl PP has been implicated as a more immediate precursor than farnesyl PP. (Dewick. 2002).

2.4.2. Monoterpenes C10

The monoterpenes are isolated by either distillation or extraction and find considerable industrial use in flavors and perfumes. Combination of DMAPP and IPP via enzyme prenyl transferase yields geranyl diphosphate (GPP) (Scheme 2.1). This produces geranyl PP (GPP). Linaryl PP and neryl PP are isomers of geranyl PP. These three by modest changes give rise to a range of monoterpenes found as components of volatile

oils used in flavouring and perfumery like camphor, α-pinene, α-phellandrene, β-phellandrene and β-pinene .(Dewick, 2002).

2.4 .3 Sesquiterpenes C_{15}

Approximately 5000 sesquiterpenes have been reported. Most appear to be derived from mevalonic acid. Sesquiterpenes are found in most plants and many fungi accumulate sesquiterpenes .The biosynthesis is not as well worked out as for monoterpenes, but farnesyl pyrophosphate appears to be the intermediate in the biosynthesis of almost all other sesquiterpenes. FPP-synthetase forms *E*-farnesyl pyrophosphate or diphosphate. An ionization mechanism is involved. Geranyl-OPP is an intermediate, but may exist only in combination with the enzyme (Stan forth, 2006).

2.4.4 Functions and utilities of sesquiterpenes

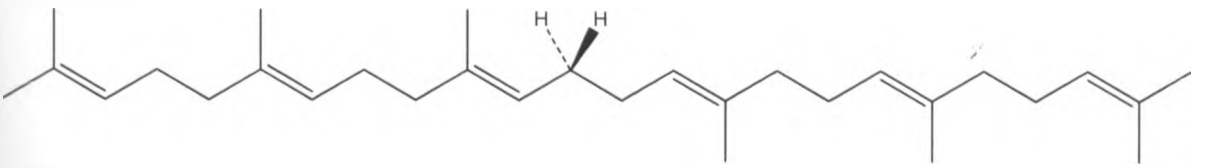
Scientists are interested in unearthing the reason why plants, insects, and fungi produce sesquiterpenoids as secondary metabolites. Some sesquiterpenes play a role as pheromones (chemicals secreted by animals that influence the behavior and development of other members of the same species) this means that they are responsible for communication between individuals of the same species. Often they serve as attractants, thus facilitating mating, or in the case of social insects as guides to food sources. Sesquiterpenes are also secreted as defense substances to fight possible predators. They have unpleasant odor and sometimes they are toxic. Others are known as juvenile hormone or they have growth inhibitory or growth-regulatory activity (Mesmin *et al.*, 2000).

2.4.5. Diterpenes C_{20}

The diterpenes represent a large group of terpenoids with a wide range of biological activities, isolated from a variety of organisms. One of the simplest and most important acyclic diterpenes is phytol, a reduced form of geranylgeraniol. This terpenoid is perhaps the most studied of those found in aquatic environments, and it is a side chain of chlorophyll. Phytol isolated from *Lucas volkensii* exhibits significant ant tuberculosis activity (Zhang *et al.*,2005).

2.4.6 Triterpenes C_{30}

Triterpenes are not formed by an extension of IPP instead two molecules of farnesyl PP are joined tail to tail to yield the hydrocarbon Squalene (figure 2.5). Squalene is a precursor for triterpenes and steroids. Several seed oils are quite rich sources of squalene, e.g. *Amaranthus cruentus* (Dewick, 2002).



Scheme 2.3 Squalene

2.4.7 Tetraterpenes C_{40}

The tetraterpenes are represented by only one group of compounds, carotenoids. These compounds play a role in photosynthesis but are also found in non-photosynthetic plant tissues, in fungi and bacteria. Formation of a tetraterpene involves coupling of

geranylgeranyl diphosphate (GGPP) in a sequence similar to that of squalene and triterpenes (Dewick, 2002).

CHAPTER THREE

METHODOLOGY

3.1 Ethno Botanical Survey of *C. articulatus L* from Tharaka Meru

Ethno botanical studies involve field explorations of indigenous medicinal knowledge and biodiversity. An ethno botanical survey was carried out in Tharaka-Meru district during the month of October 2010. Tharaka is found in the larger Meru region in the former Eastern province. During the time of this study the area was experiencing a drought that had affected most parts of Kenya and Tharaka being a semi-arid area was as dry as can be seen in figure 3.1 below.

Figure 3.1 Dry vegetation

Position of Tharaka district in the map of Kenya is shown in figure 3.2.

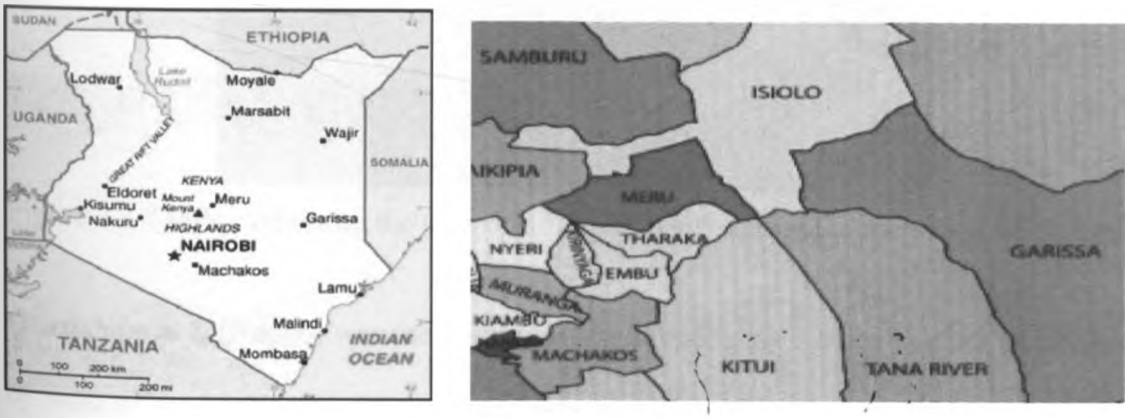


Figure 3.2 Map of Kenya and Tharaka district.

3.1.1 Reconnaissance survey

A two day reconnaissance survey was carried out in Tharaka Meru where a total of 7 leaders of the Tharaka Traditional Medicine Practitioners (TMPs) from Ciokariga, Mukothima, Marimanti and Gatunga divisions met with the researchers at the Marimanti Methodist Guest House. Each of them gave a brief history on their traditional medicinal practice in general and displayed the plants they use for treatment of various diseases as shown in figure 3.3. In the afternoon a brief field visit was made and TMPs explained the various plants found in the field as shown in figure 3.4. After this *C. articulatus L* was selected for detailed study.

Figure 3.3 TMPs explaining the uses of different plants during the afternoon field visit

C. articulatus L (Ndago) was the plant most mentioned as a medicinal plant being used by most of the TMPs and great concern was raised on its being an endangered species. Each of them claimed to have used it. After the meeting it was agreed that a one day workshop be conducted at the same place with more TMPS from the four divisions of

Tharaka (Marimanti, Ciokariga, Mukothima and Gatunga) who practice traditional medicine and use *Cyperus articulatus L.* It was also agreed that a man (Mutonga) from neighboring Mitunguu (Iment South be invited) as he was the one who supplies the Tharaka people with *C. articulatus L* from Iment South (neighboring district) during the dry season when it was scarce in Tharaka. The TMPs were asked to bring *C. articulatus L* samples during the workshop.

3.1.2 Ethno-Botanical Survey Workshop

The workshop was conducted by five scientists (one student and four University of Nairobi lecturers). A semi-structured questionnaire was administered to the registered traditional herbal medicine practitioners (TMP). Procedures involved in the making of the medicinal preparation from *C. articulatus L* were sought. These included procedures used in the treatment of stomachache, abdominal pain, menstrual period pains, typhoid, fever, malaria, common cold, cough, flu, running nose, swollen breasts, baby skin rush, blisters, fungal infection, wound. Other ethno medicinal uses that were studied included; mosquito repellency, mouth freshener and perfume. The sample questionnaire that was used is shown in Appendix 1. Data was sought regarding the availability of *C. articulatus L* , methods of preparation and formulation, dosage, treatment and outcomes, recurrence as well as patient satisfaction. Data obtained from the questionnaires was analyzed using the appropriate methods (descriptive statistics) and the plant collected for taxonomic identification.

3.2 Plant Material Collection

Fresh root tubers (rhizomes) of *C. articulatus L* were collected from Ciokariga, Marimanti, Gatunga, Mukothima and Mitunguu in October 2010 and were identified at the School of Biological Sciences, University of Nairobi Herbarium. Due to the ongoing drought during the time of study the plant material collection covered a vast region and the plant collection exercise took longer than anticipated. As can be seen from the Figure 3.4 below the plants were dry and tilling the hard ground was not easy. More field assistants were to be hired and strict supervision done to ensure the right plants were being harvested.

Figure 3.4 TMPs digging up tubers of *C.articulatus L*

3.3 Plant Extraction

The root tubers of *C. articulatus L* were washed dried under shade and ground using a Wiley mill from the school of Biological Sciences, University of Nairobi. The powder was weighed and yielded 6 kg and 2 kg were preserved in a refrigerator for further

analysis. The remaining 4 kg were subjected to serial extraction using dichloromethane (CH_2Cl_2) , 1:1 dichloromethane/methanol (CH_2Cl_2/ CH_3OH) and finally 5% water in methanol. Each extract was concentrated using a rotatory evaporator at temperatures of 40°C to 60°C with an aspirator vacuum. The 5% water /methanol extract after concentration was freeze dried at the School of Biological Science University of Nairobi for 24 hours to ensure the water was removed. The dry powder was tightly corked and kept in the fridge awaiting further analysis. The crude extracts for each of the others were combined, tightly corked and stored in the refrigerator to wait for further processing. The quantities yielded were 100% dichloromethane extract 100 grams, 1:1 methanol/dichloromethane 60 grams, and 5% water in methanol yielded 36 grams. The three extracts were brown in color, oily and all with a pleasant odour.

3.4 Laboratory Analysis

3.4.1 General

Merck Silica gel 60 (0.063 -0.200 mm) was used for column chromatography (CC) as the stationery phase.; PTLC on Merck Silica gel 60 $PF_{254+366}$, coated on glass plates (20 by 20 cm) to make 1.0 mm layers; Analytical TLC was carried out using aluminum base plates (0.25 mm) coated with silica gel ($60F_{254}$, Merck) and spots visualized under UV lamp 254-366 nm, followed by spraying with 1% vanillin in H_2SO_4 spray reagent. EI-MS spectra were recorded on Agilent Gas chromatography/ mass spectrometer (GC-MS) .

3.4.2 Column Chromatography

Fifty grams of the DCM extract was subjected to column chromatography on silica gel using varying ratios of ethyl acetate / hexane. A total of 108 fractions were collected and analyzed using analytical thin layer chromatography (TLC) and the ones found similar combined. Further purification was carried out using column chromatography on silica gel and preparative thin layer chromatography (PTLC). After combining they were renamed A-P. The separation of compounds was monitored using analytical thin layer chromatography using aluminium coated factory made plates. Fraction C was weighed and gave three grams and was subjected to further separation as follows. A column of fifty grams of silica gel was packed with 2% ethyl acetate in hexane. The three grams were mixed with 5 ml of the 2% ethyl acetate in hexane and charged on the column. The column was eluted with varying ratios of n-hexane/ethyl acetate (in order of increasing polarity). A total of forty nine fractions were collected and concentrated using a rotary evaporator with similar fractions being combined on the basis of TLC analysis. Fraction 37 which weighed 100.58 mg was mounted on 6 silica gel precoated PTLC plates and developed twice in 10% ethyl acetate/hexane. It produced C_1 which when viewed on UV $_{254\text{-}366nm}$ revealed one sport, these were later sprayed with vanillin .and also exposed to iodine , the single sports revealed numerous overlapping sports, that were too difficult to isolate by column chromatography or by preparative thin layer chromatography (PTLC). Sephadix was also used. The process of isolation of compounds was rigorous and difficult and did not yield any pure compounds. These methods (CC and PTLC) didn't work for the isolation of these compounds.

3.4.3 GC-MS

Two microlitres of the crude extract of *C.articulatus L* 100% DCM was injected (introduced) in the ion source. This crude extract of *C.ariculatus* was run on an Agilent Technologies 7890A GC system connected to an Agilent Technologies 5975C Inert XL EI/CI MSD mass spectrometer with a triple axes detector. An HP-SMS column with a length of 30 m and i.d of 0.25 nm was used with a film thickness of 0.25 microns and a split ratio of 50:1. The oven temperatures were as follows; Starting temperature was at 50 degrees and was held for 3 minutes then ramped up at 10 degrees per minute up to 250 degrees, and held at 250 degrees for 2 minutes. The injection temperature was 250 degrees and the detector temperature was 230 degrees. Helium was used as the carrier gas. A heated filament that produced a beam of electron (70eV) was used to bombard the sample. The retention times were as shown in the table in Appendix 2. The diagram on figure 3.5 shows the GC-MS set up that was used.

Figure 3.5 GC-MS set up

3.5 Identification of the Compounds Using GC-MS

Gas chromatography is certainly a very rapid method of separation, since no preliminary operations are required. It is also a method of choice when only a very small quantity of oil is available. Constituents of the oil of *C. articulatus L* were identified by comparing the experimental gas chromatogram shown in Appendix 2, retention indices shown in Appendix 3 and MS spectra of the compounds shown in Appendices 4 to 62 with corresponding reference data (Adams, 1995). For this report the National Institute of Science and Technology (NIST) library was used. The percentage compositions of the oils were computed in each case from GC peak areas shown in Appendix 3. The components of the oils were identified by matching their retention indices shown in Appendix 2 and mass spectra indicated in Appendices 4 to 62 with those standards of NIST library mass spectra data base of the GC-MS system from Surrey University Library in the UK. Appendices 4 to 62 gives the spectra produced during the fragmentation of the compound and below each spectrum is the spectrum that was searched from National Institute of Science and Technology library. These spectra were used to produce the structures of the compounds and it is from them that the fragmentation patterns of the compounds were proposed.

3.6 Antibacterial Activity Assays

Bacterial clinical isolates *Salmonella typhi*, *Streptococcus pneumoniae* and *Staphylococcus aureus* were obtained from the department of Medical Microbiology School of Medicine, University of Nairobi. The strains were isolated 3 times on nutrient agar plates, identified and confirmed using the standard bacteriological methods at the

Biotechnology Laboratory, Department of Veterinary Anatomy and Physiology, University of Nairobi, they were maintained on nutrient agar slopes at 4 °C. The cultures were spread on nutrient agar plate band incubated aerobically at 37 ^{0}C overnight (18-24 hrs) before use. Using plate dilution method the cultures were adjusted to yield 1:10 colony forming units per ml (cfu/ml). The MIC was taken as the lowest concentration that inhibited the growth of organisms after incubation and the results were recorded in Table 4.4.

3.6.1 Disc Impregnation

A steady air current was used to dry the plant extract for 24 hours. A volume of 0.5 ml of 20% dimethyl-sulphate (DMSO) was used to reconstitute the extract. Sterile discs made from Whitman filter paper No. 1 were soaked in 100 µl of the extract to an approximate concentration of 20 mg /ml per disc. Discs soaked in 20% dimethyl sulphate (DMSO) and sterile saline acted as control. The impregnated discs were sterilized under ultra violet rays (UV) for 1hour.

3.6.2 Seeding of Nutrient Agar Plates

Inoculums of 0.5 ml from each bacteria species were introduced onto the dry, sterile surface of the nutrient agar (Muller Hinton agar 9OXOID) . The seeding rate was $1.0*10^{5}$ cfu/ml and a sterile glass spreader was used to make an evenly distributed culture. The plates were left to dry for 2 hours.

3.6.3 Bacterial Susceptibility Testing

Sterile impregnated discs of known concentrations were carefully placed on the labeled seeded plates in duplicates. The plates were allowed to stand for 1 hr to allow diffusion to take place, and then they were incubated aerobically at 37 ^{0}C and examined for zones

of inhibition after 24 hrs. Discs impregnated with 20% dimethyl sulphate (DMSO) and normal saline were examined as control. The experiment was repeated 5 times. The crude extract of 100% CH_2Cl_2 was screened for antibacterial activities against three different strains of bacteria and the plates used were as shown in the results section. The inhibition diameters were measured after 24 hours of introducing the extract to the colonies in the Petri dishes. Different inhibition diameters were recorded for each of the three experiments and an average reading was calculated and recorded on Table 4.3.

3.6.4 Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration (MIC) was determined by Microtiter Dilution Method using microtiter plates. Six hundred milligrams (600 mg) of the extract was dissolved in 1 ml N, N usually dimethylsulphate (DMSO) and made into a final volume of 3 ml using nutrient broth (200 mg/ ml). Serial dilution of the 100% CH_2Cl_2 extract resulted into six concentrations. Racks carrying the dilution test tubes were gently shaken to mix the content. The negative and positive controls contained only the nutrient broth. A bacteria suspension containing 1.5 x 10^6 colony forming units/ml (cfus) of the test organism was added to each test tube except for the negative control. The plates were incubated at 37 °C for 24 hours and observed for turbidity. The lowest concentration of each extract that showed no sign of turbidity indicating growth inhibition was recorded as the minimum inhibition concentration in mg/ml in Table 4.4.

3.7 Mosquito Repellent Test

This test was carried out using laboratory reared *Aedes egyptii* mosquitoes. The test was carried out in a dark room at 25 °C using human baits. *Aedes egyptii* adult mosquitoes

were raised in netted cages at 25 -30 °C from a larval colony. The mosquitoes were fed for five days on exposed skin of live rabbit ears. One hundred of these adult mosquitoes were starved for 48 hours and placed in a cage. Human bait was used for the evaluation. Before each test, the readiness of the mosquitoes to bite was confirmed by having subject insert an untreated forearm into the test cage. Once subject observed five mosquito landings on the untreated arm, the arm was withdrawn from the cage and *Cyperus articulatus L* crude extract applied as the repellent being tested (Faradin *et al.*, 2002). The *C. articulatus L* extract was applied thinly using cotton wool on the bare forearm from elbow to the fingers and placed in the mosquito cage. Exposure time was 5 min, 15min, 30min and 60min .This experiment was carried out in a semi darkened room. The number of bites and landings were counted for each exposure period and recorded in Table 4.5.

3.8 Structure Elucidation.

The structures of the compounds were determined using spectroscopic methods. The chromatogram from the GC and spectra obtained from GC-MS were as shown in Appendices 4 to 59. These spectra were compared with those of the National institute of science and technology (NIST) library of Surrey University in the UK and from these the structures shown on table 4.2 were proposed. The retention times were as shown in the table in Appendix 2.

CHAPTER FOUR

RESULTS AND DISCUSIONS

4.1 Ethno Botanical Survey in Tharaka

The information from the questionnaire were analyzed and the following were the findings, From the information gathered from the TMPs it was clear that they were using *C. articulatus L* for treatment of diseases recorded in Table 4.1. Judging from the responses in the questionnaires and from the little amount of *C.articulatus L* the herbalists brought on that day it was clear that *C. articulatus L* was becoming scarce in Tharaka. This is because many people from the neighboring districts which had better growing conditions (loamy soil and shady wet conditions) for the plant had uprooted it. Due to scarcity of *C.articulatus L* some people had started using it sparingly. They understood all its uses and most of what they were mentioning is supported by literature from other parts of the world, Peru, Brazil, S. Africa, Cameroon and Nigeria. The plant is becoming scarce in Tharaka and very hard to find at times especially during the dry season because Tharaka is a dry semi arid area. From the information gathered from the questionnaires most people agreed that it was becoming less and less with time

4.2 Ethno-Medicinal Uses and preparation methods for concoctions of *C. articulatus L* from Tharaka

The Tharaka people use *C. articulatus L* in several ways. A summary of the uses mentioned by the traditional medicine practitioners during the workshop in October 2010 were summarized in the Table 4.1.

Table 4.1 Uses of *C.articulatus L* from Tharaka Meru

Diseases treated/use	Method of preparation/ dosage/usage
Cough	Tubers washed chewed 2 tubers 3 times /day
Blocked nose	Washed ground powder applied around the nose 3 times /day
Common cold	Washed ground and applied around the nose, or taken 1 spoonful 3 times/day
Running nose	Tubers cut into pieces boiled taken 1 cup 3times/day
Flu	Tubers washed and chewed or into powder applied around the nose
Fever	Washed ground mixed with water and taken 1 cup 3times/day
Malaria	Washed ground into powder taken1tablespoon 3 times /day or tubers cut into pieces boiled in water taken 1 cup 3 times/day
Pneumonia	Whole plant cut into pieces boiled, taken 1 cup 3-times a day.
Rheumatism	Tubers washed boiled and taken 1 cup 3times/day
Wounds	Tubers washed ground into powder applied on wounds twice /day
Blisters	Washed ground into powder applied on Blisters
Fungal infection	Washed ground into powder, applied on affected Areas (between toes)
Baby skin rush	Tubers washed ground into powder applied as baby powder or all over the body (affected area of the skin)
Typhoid	Tubers washed ground mixed with water taken 1 cup 3times/day
Swollen breasts	Tubers washed ground into powder applied on swollen breasts twice/day
Stomachache	Tubers washed and chewed 2-3 tubers 3 times a day
Abdominal pains	Tubers washed and chewed 2-3 tubers 3times/day
Menstrual period pains	Tubers washed and chewed2 tubers 3 times/day
Goat cough	Whole plant cut into pieces mixed with water, boiled and given to goats
Mosquito repellency	Tubers ground into pounder mixed with water and sprinkled

	around the houses and on the floor shortly before dusk.
Perfume	Powder ground and applied around the armpits, neck and around the waist, sometimes in the olden days mixed with chalk or red earth and applied on the hair.
Mouth freshener	Tubers washed peeled and chewed

4.3. Characterization of essential oils of *C.articulatus L*

4.3.1 Column Chromatography

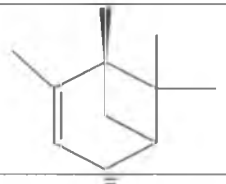
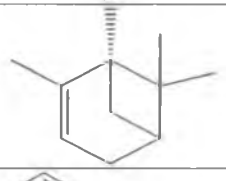
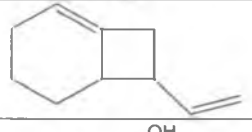
The TLC of the crude extract of 100%CH_2Cl_2 revealed UV $_{254\ -\ 366nm}$ active spots. Chromatographic separation of the crude extract did not yield any pure compounds, however characterization using GC-MS resulted to terpenoids with a large number of mono terpenes (twenty) which are generally known to be difficult to isolate as pure compounds as mentioned in the literature review. The student leant that Column chromatography does not always work.

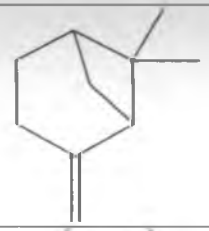
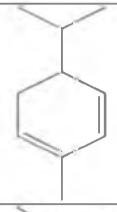
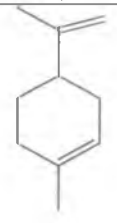
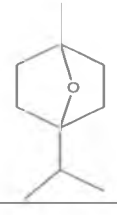
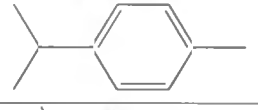
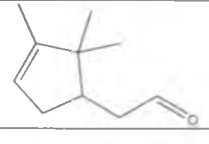
4.3.2.1 Chemical Analysis of the Compounds from *C. articulatus L* from Tharaka

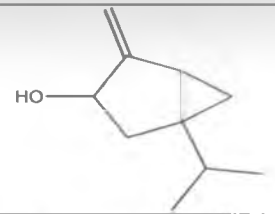
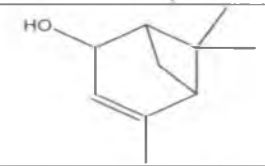
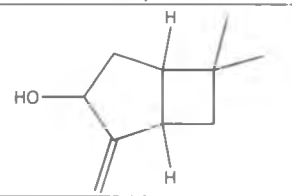
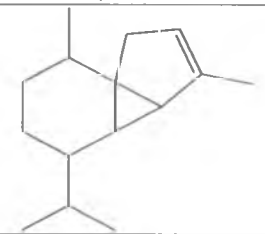
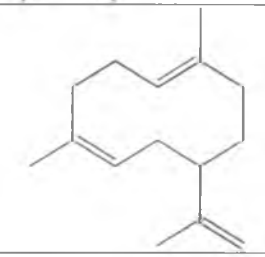
GC chromatogram shown in appendix 2 was first produced. And from each of the peaks shown in the GC chromatogram the spectra for each compound were produced. The retention indices and the spectra shown in the appendices section were used to identify the structures of the compounds. From the 100% dichloro methane extract of *C. articulatus L* from Tharaka fifty nine compounds were detected. Terpenes accounted for the highest number of compounds analyzed with forty eight in number (81.36 %). From the peak areas of these 59 compounds the quantities of each compound was computed. There were twenty seven sesquiterpenes (45.76%), twenty monoterpenes (33.90%) one triterpene (1.69%) and eleven other compounds (18.64%). The most abundant terpene was found to be the sesquiterpene α-cubenene. Table 4.2 gives the GC order number, compound name, molecular formula, structure, retention time, and relative peak areas of

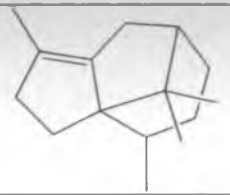
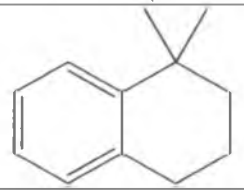
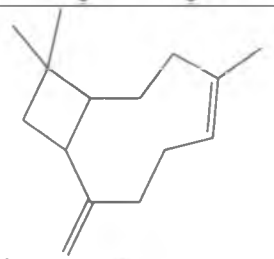
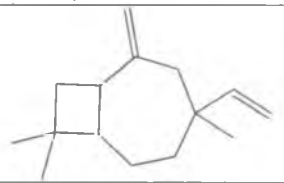
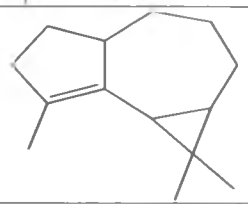
the compounds. The relative peak area percentages were used to compute the quantities for the compounds. As in the essential oils analyzed by Nureni *et al* 2006 in Nigeria the essential oil from Tharaka had terpenes as the major constituents accounting for a total of 48 out of the 59 compounds. Both the crude extracts and all the fractions from *C. articulatus L.* were sweet smelling. This was attributed to the high number of terpenes in the essential oils. Table 4.2 gives a summary of comparative analyses of these compounds. The fragmentation pattern in this table compared to those in the NIST library and suggested these structures. It is from the details in this table and the spectra that the fragmentation pattern of compound thirty three was proposed by the author.

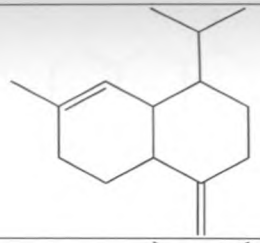
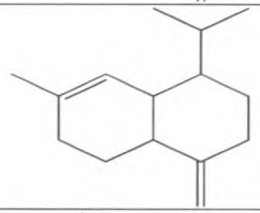
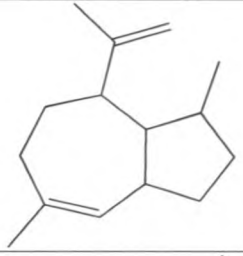
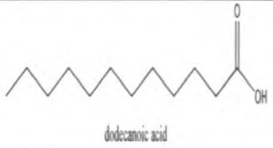
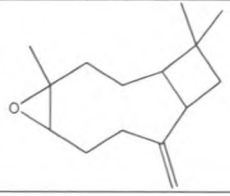
Table 4.2 Retention Time, Molecular Formula and Corresponding Peak Area % of Maximum for compounds from C. articulatus L from Tharaka

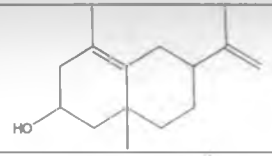
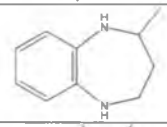
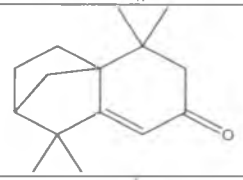
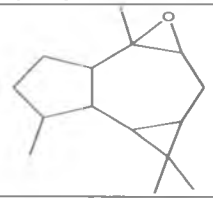
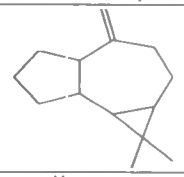
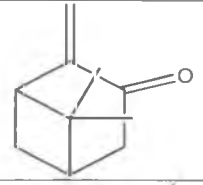
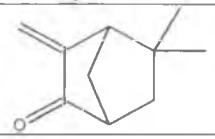
GC-Order No.	Compound Name	Molecular formula	Molecular Structure	Retention Time	Relative peak area % of maximum
1	1*R*-α pinene	$C_{10}H_{16}$		6.16	1.16
2	1*S*-α pinene	$C_{10}H_{16}$		6.59	0.57
3	7 vinyl-Bicyclo[4.2.0]oct-1-ene	$C_{10}H_{14}$		6.57	2.90
4	Bicyclo[3.1.0] hex-3-ene-2-ol,2methyl 1(1methyl ethyl),(5,alpha)	$C_{10}H_{14}O$		6.71	1.55

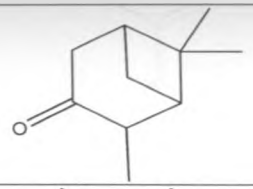
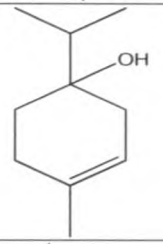
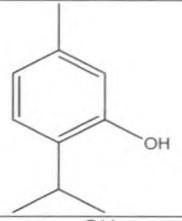
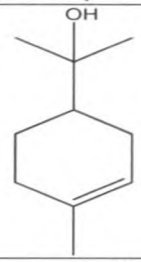
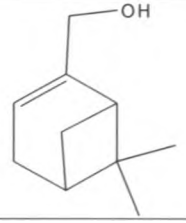
5	Beta pinene	$C_{10}H_{16}$		7.01	3.99
6	alpha phellandrene	$C_{10}H_{15}$		7.53	0.66
7	D-Limonene	$C_{10}H_{15}$		9.98	1.39
8	Eucalyptol	$C_{10}H_{18}O$		8.03	0.52
9	Benzene,1-methyl-4(1-methylethyl)(cymol, paracymene, β cymene).	$C_{10}H_{14}$		7.90	1.39
10	3cyclopentene-1-acetaldehyde 2,2,3` trimethyl. (α campol).	$C_{10}H_{16}O$		7.68	1.11

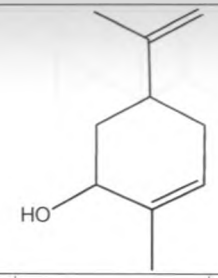
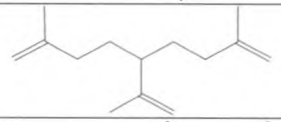
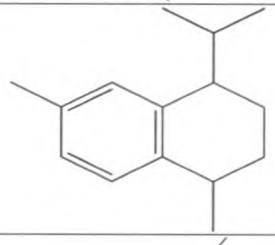
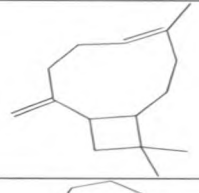
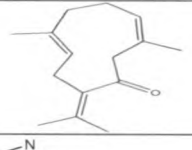
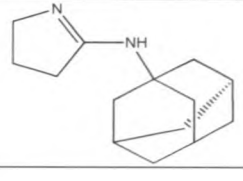
11	Bicyclo[3.1.0]hexane-3-ol,4methylene,-1-(1methyl)1s-(1alpha 3 beta ,5alpha).(sabinol).	$C_{10}H_{16}O$		9.92	18.25
12	Bicylo(3.1.1)hept-3-en-2-ol4,6,6 trimethy (lS -(alph,.2beta,5alpha). ((s) cis verbenol)	$C_{10}H_{14}O$		10.01	13.13
13	Bicyclo[3.2.0]-3-ol,2methylene,6,6,dimethyl	$C_{10}H_{16}O$		10.18	0.78
14	Alpha cubenene	$C_{15}H_{24}$		13.50	77.89
15	8 Isopropyl-1,5-dimethyl cyclodica -1,5-diene	$C_{15}H_{24}$		13.68	2.54

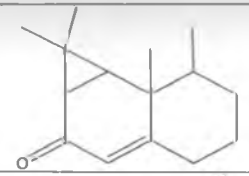
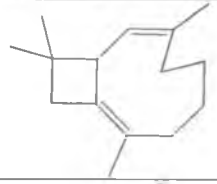
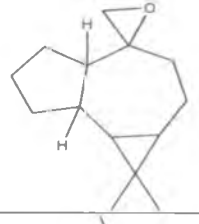
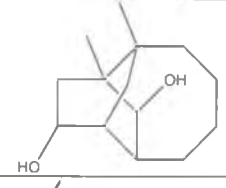
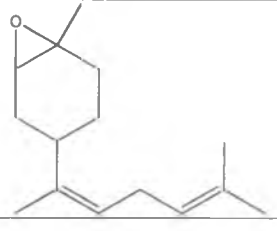
16	3H-3a,7methano2,4,5,6,7,8 hexahydro-1,4,9,9tetramethyl-(3a-alpha,4beta,7alpha (cyperana)	$C_{15}H_{24}$		13.85	77.89
17	Naphthalene 1,2,3,4 tetrahydro-1,1,6 trimethyl (lonene)	$C_{13}H_{18}$		13.89	100
18	Caryophylene	$C_{15}H_{24}$		14.09	5.75
19	Bicyclo[5.2.0]nonane,2methylene4,8, 8 trimethyl,4,vinyl	$C_{15}H_{24}$		14.08	1.87
20	Azulene1,2,3,4,5,6,7,8,octahydro-1-4-dimethyl-7-[1alpha,7,alpha] (Quaiene)	$C_{15}H_{24}$		14.30	6.90

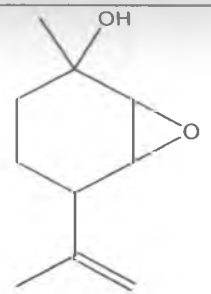
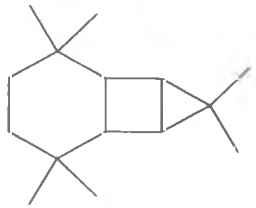
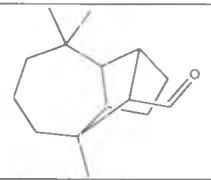
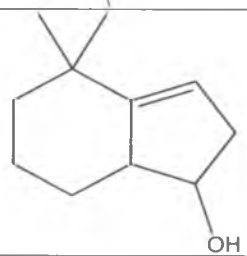
21	1H-cycloprop(e)azulene,1a,2,3,4 ,4a,5,6 ,7b octahydro-1,1,4,7tetramethyl(1aR (1a ,alpha,4alpha,4abeta,7a,alpha] (α gurjunene).	$C_{15}H_{24}$		14.44	1.97
22	Naphthalene1,2,3,4,4a,5,6,8,octahydro7methyl-4methylene-1-(1-methyl)-(1alpha,4a,alpha,8a,alpha) (muurolene).	$C_{15}H_{24}$		14.444	2.76
23	Azulene1.2.3.5.6.7.8,8aoctahydro7,methyl-4methylene-(1-methyl)-(1alpha,4a,alpha,8a,alpha) (bulnesene)	$C_{15}H_{24}$		15.18	16.41
24	Dodecanoic acid	$C_{12}H_{24}O$	 dodecanoic acid	15.78	7.15
25	Caryophylene oxide	$C_{15}H_{24}O$		16.19	51.63

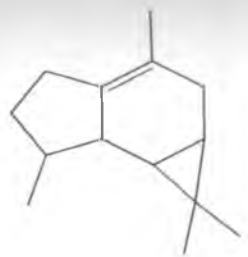
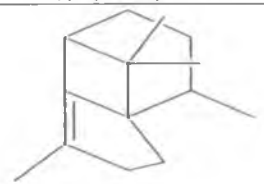
26	6-isopropyl-4.8a-dimethy8al-1,2,3,5,6,7,8 , octahydro naphthalene-2-0	$C_{15}H_{22}O$		16.49	19.99
27	1H1,5Benzodiazepine2,3,4,5tetrahydro-2-methyl	$C_{10}H_{14}N_2$		16.56	14.99
28	1,2,3,4,5,6 hexahydro 1,1,5,5,tetramethyl-2,4a-methanonaphthalene-7(4a,H)-one	$C_{15}H_{22}O$		16.65	24.34
29	Isoaromandrene epoxide	$C_{15}H_{24}O$		16.74	6.64
30	1H-cycloprop[e]azulene,decahydro-1,1,7trimethyl-4-methylene	$C_{15}H_{24}$		16.99	12.34
31	2(10)-pinen-3-one	$C_{10}H_{14}O$		10,31	6.77
32	Bicyclo(2.2.1)heptan-3-one6,6dimethyl-2-methylene	$C_{10}H_{14}O$		10.31	6.77

33	Bicyclo(3,1,1)hepta-3-one,2,6,6 trimethyl. (pinocamphone, 3 pinanone ,trans 3 pinanone)	$C_{10}H_{16}O$		10.49	1.16
34	3cyclohexane-1-o l- 4-methyl1-(1-methyl)-R	$C_{10}H_{17}0$		10.53	1.16
35	Thymol	$C_{10}H_{14}O$		10.63	2.22
36	3cyclohexene-1-methano,alpha,alpha,4 trimethyl	$C_{10}H_{18}O$		10.74	1.70
37	Myrtenol	$C_{10}H_{16}O$		10.84	18.32

38	2-cyclohexane-1-ol,2methyl-5-(1methyl ethyl	$C_{10}H_{16}O$		11.16	2.30
39	1,8Nonadiene,2,7dimethyl-5-(1methylethyl)	$C_{14}H_{24}$		12.72	3.69
40	Naphthalene1,2,3,4tetrahydro-1,6dimethyl-4-(1-methylethyl)-(1s-cis). (calamenene)	$C_{15}H_{22}$		13.31	7.20
41	Bicyclo(7.2.0)undec-4-ene 4,11,11trimethyl(-8-metylone (β caryophyllene)	$C_{15}H_{24}$		17.09	12.38
42	3,7,cyclodec-1-one 3,7 dimethyl-10-(-1-methylethylidene(-EE) (germacron)	$C_{15}H_{22}O$		17.42	17.20
43	1Pyrroline-2-amine,N-(1 adamantyl)	$C_{14}N_2H_{19}$		17.50	30.87

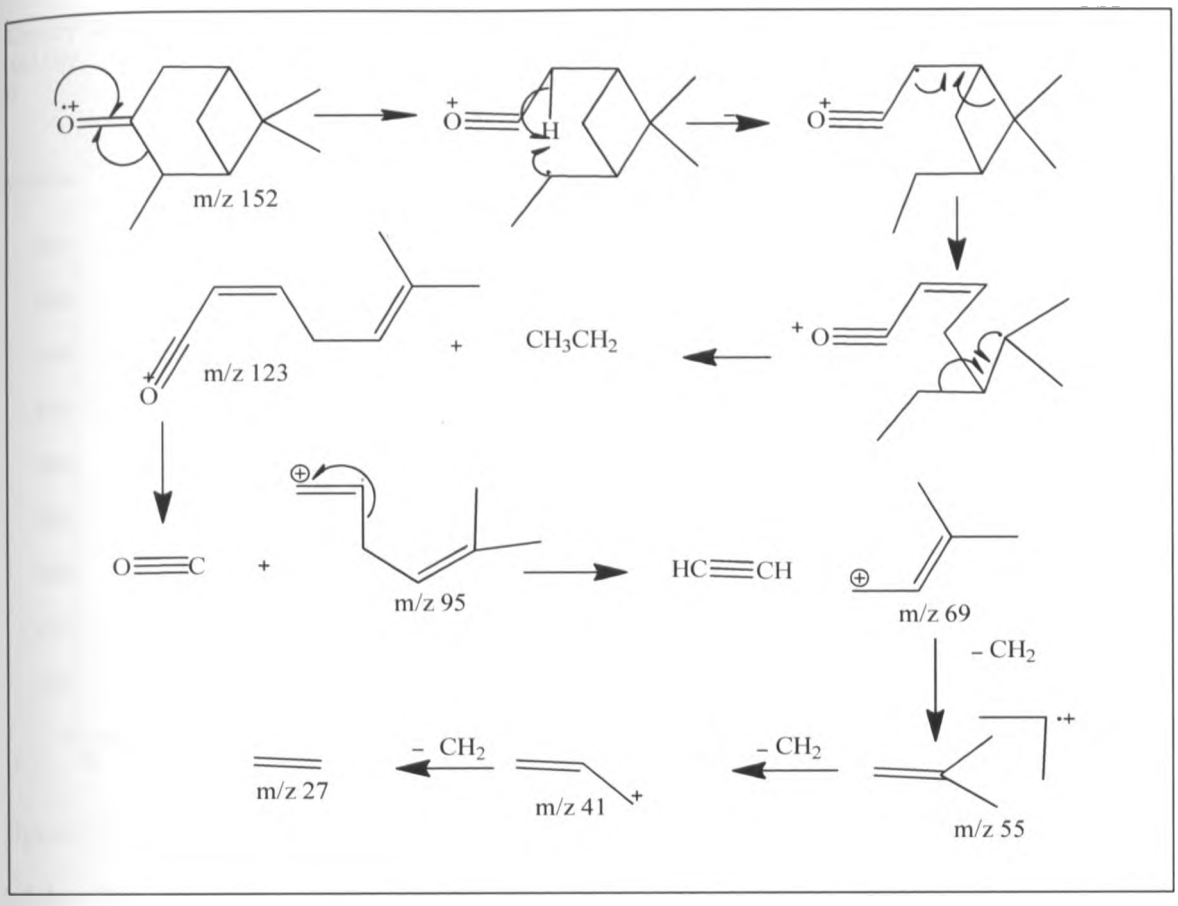
44	2H-Cycloprop[a]naphthalene-2-one ,1,1a,4 ,5,6, 7, 7a,7b octahydro-1,1,7,7a,tetramethyl (1a,alpha, 7 alpha,7a, alpha,7b,alpha).(aristolene)	$C_{15}H_{22}O$		17.61	22.70
45	Caryophylene(11)	$C_{15}H_{24}$		18.11	36.31
46	. Alloaromandrene oxide-(1)	$C_{15}H_{22}O$		18.51	37.67
47	Culmorine	$C_{15}H_{24}O_2$		18.95	32.18
48	Ciz-2-alpha Bisobolene epoxide	$C_{15}H_{24}O$		19.03	42.16

49	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	$C_{10}H_{15}O_2$		19.23	38.44
50	Tricyclo[4.3.0.0](7,9)nonane 2,2,5,5,8,8hexamethyl-alpha.6beta,7alpha,9alpha. 2,2,5,5,8,8-Hexamethyl-tricyclo[4.3.0.0*7,9*]nonane	$C_{15}H_{26}$		19.39	22.65
51	Longifolenaldehide	$C_{15}H_{24}O$		19.68	36.72
52	1H-Inden-1 ol-2,4,5,6,7,7a hexahydro-4,4,7a-trimethyl	$C_{12}H_{20}O$		20.76	8.95

53	53).1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b,octahydro,-1,1,4,7,tetramethyl,9,1aR(1a,alpha,7a, beta,7,alpha	$C_{15}H_{24}$		23.78	3.58
54	54). Methyl4,6-decadienyl ether	$C_{11}H_{18}O$	$(CH_3)_2$-O-$(CH)_5(CH)_4$	25.33	8.96
55	55). Dodecanoic acid, tetradecyl ester	$C_{26}H_{52}O_2$	$(CH_3)_2(CH_2)_{23}$CHO-O	26.80	7.76
56	56). Dodecanoic acid, hexadecyl ester	$C_{28}H_{58}O_2$	$(CH_3)_2(CH_2)_{26}$COO	28.21	18.15
57	57). Succinic acid, heptyl tridec-2-ynl ester	$C_{21}H_{41}O_4$	$CH_3)_2(CH_2)_{15}CH_2)_2$COCH OCOO	28.92	2.06
58	58). Dodecanoic acid ,octadecyl ester	$C_{31}H_{62}O_2$	$CH_3)_2(CH_2)_{28}$COO	29.76	4.02
59	59). Longipinocarveol,trans	$C_{15}H_{22}O$		28.62	1.27

4.3.2.2 Proposed Fragmentation Pattern for Compound 33

Compound 36 was chosen to illustrate the fragmentation pattern for terpenes. The spectrum in figure 4.1was used. It is from this spectrum that the fragmentation pattern for compound 33 was proposed by the author. Compound 33 contains the hetero atom oxygen which has two lone pairs of electrons. An electron was knocked from the lone pair of electrons by the beam of electrons that bombarded the sample at the ionization chamber. This created the molecular ion shown in the first step in Scheme 4.1 with a mass to charge ratio (*m/z*) of 152. The bond adjacent to the hetero atom formed a triple bond with oxygen at C-1. The remaining electron forms a radical at C-6. Through a hydride shift the radical shifted to C-2 and using this radical the bond adjacent to C-3 broke to form a double bond between C-2 and C-3. The radical shifted to C-4. This coupled with an electron from the bond adjacent to C-6 and cleaved the bond. This gave rise to an ethylene and another positive ion (6-methylheptan-1 ol) with an *m/z* 123. Carbon monoxide was then lost to form another positive ion with *m/z* 95. Then an ethylyne was lost to produce another positive ion of *m/z* 69 and eventually loss of three consecutive methyls to produce positive fragments of *m/z* 55, *m/z* 41 and m/z 27 consecutively. This fragmentation pattern is illustrated stepwise in Scheme 4.1.



Scheme 4.1: Proposed Fragmentation Pattern for Compound 33

```
Library Searched : C:\Database\NIST08.L
Quality          : 94
ID               : 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-
                   (1a.alpha.,4a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-
```

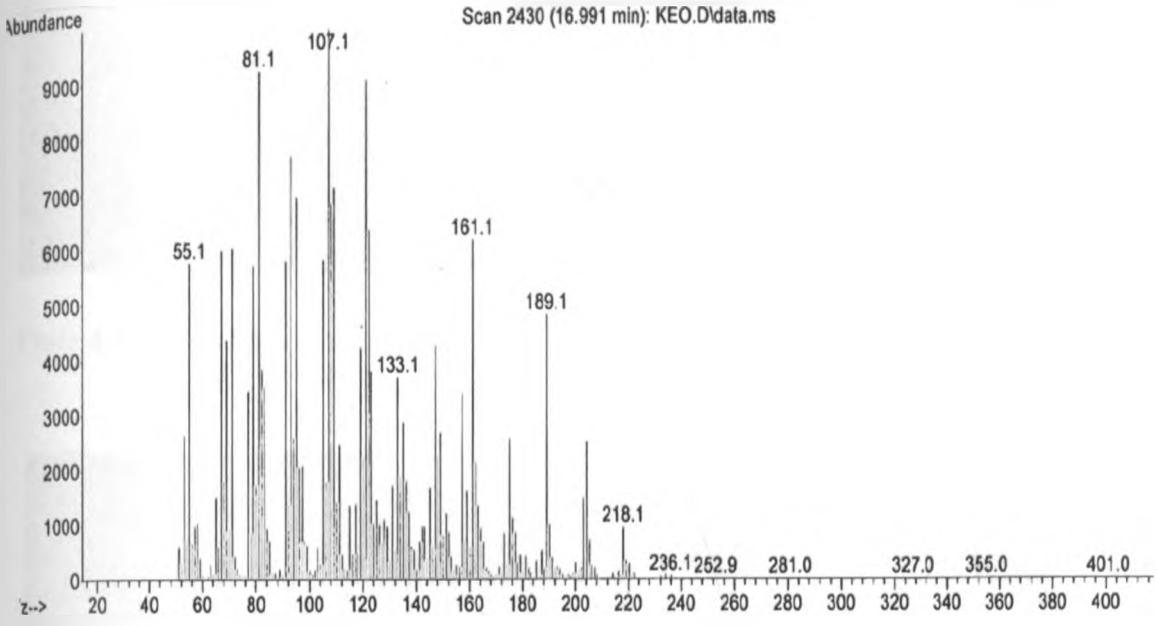


Figure 4.1 mass spectrum for compound 33

4.4 *In-vitro* Antimicrobial Activities

The extract obtained using 100% CH_2Cl_2 exhibited activity against all bactaria strains tested (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Salmonella typhi)*. This is as shown in plates 4.1 and 4.2 below. The inhibition zones for the strains tested were : *S.aureus* 12.0 mm, *S.pneumonia* 9.0 mm and *S. typhi* 8.5 mm.

Plate 4.1 ***Staphylococcus aureus***

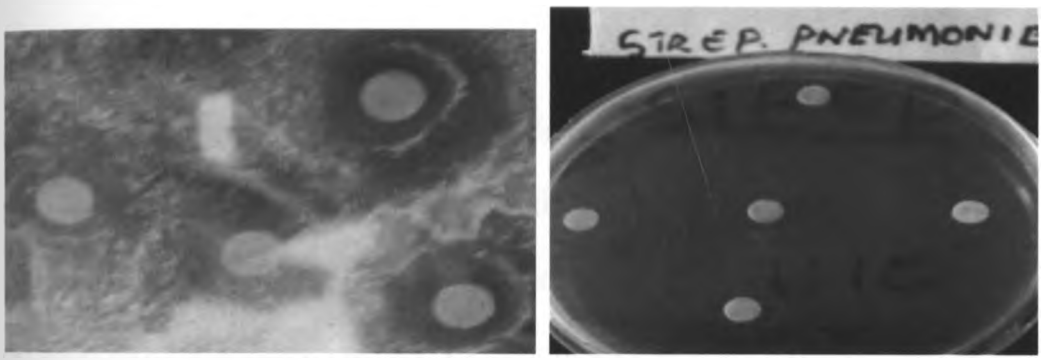


Plate 4.2 ***Streptococcus pneumonia***

Table 4.3 Anti microbial activities of the100% CH_2Cl_2 crude extract of *C.articulatus L* from Tharaka .

Bacteria strain Average inhibition diameter	*Staphylococcus aureus*	*Streptococcus Pneumonia*	*Salmonella Typhi*
Neat concentration	15 mm	12 mm	1 mm
1:5-concentration	12 mm	8.5 mm	9 mm
1:10- concentration	9 mm	8 mm	8 mm
1:100-concentration	8 mm	9 mm	7 mm

4.4.1 Minimum Inhibition Concentration

The minimum inhibition concentrations were taken and recorded. At 100 % concentration (undiluted crude extract of dichloro methane) there was no growth observed for the three bacterial strains. At 10mg/ml concentration there was no growth for Staphylococcus aureus but there was partial growth for both *S. pneumonia* and *S. typhi*. At 1mg/ml concentration there was partial growth for *Staphylococcus aureus* and full growth for *Streptococcus pneumonia* and *Salmonella typhi*. At 0.1mg/ml concentration there was full growth for all the three bacteria strains.

4.5 Mosquito Repellent Test Results

When the untreated arm was placed in the cage five landings were observed indicating the readiness of the mosquitoes to bite. When the 100% CH_2Cl_2 extract was smeared on the forearm of the experimenter and the hand immediately placed inside a darkened cage with approximately 100 adult mosquitoes that had been starved for 48 hours, the following was observed;

1. The mosquitoes were ready to bite as observed in figure 4.5a, five landings were recorded.
2. When the hand with extract was introduced into the cage all the mosquitoes flew away and not a single landing was observed.
3. When the untreated arm was re-introduced into the cage the mosquitoes landed and baits were recorded in Table 4.3 below.

Table 4.4 Number of bites at different periods of exposure

Period of exposure	5minutes	15minutes	30minutes	60minutes
Control Hand/bites	30	42	52	55
Landings	39	46	61	63
Treated Hand/bites	0	0	0	0
Landings	1	0	0	0

The percentage bites were calculated by the formula; $\frac{B}{X} \times 100$, Where B = number of bites and X =-number of landings.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The traditional medicine practitioners in Tharaka were using *C. articulatus L* for treatment of various diseases. Form the ethno-botanical survey it was evident that most people had uprooted *C. articulatus L* from their farms. *C. articulatus L* was becoming scarce as was evidenced by the long distance herbalists covered searching for the plant. The constituents of *C. articulatus L* from Tharaka had the sesquiterpene α- cubenene as the major component. The compound α- cubenene was not detected in all the other essential oils extracted from *C. articulatus L* from Nigeria, S. Africa, Brazil, Japan, Taiwan, Thailand, Hawaii and Philippines. The following compounds were found in both the essential oils of *C. articulatus L* from Tharaka and the one reported from Nigeria by Nureni *et al* 2006; Caryophyllene, Caryophyllene oxide, cymene, pinene, gurjunene, and muurolene. The following compounds were detected from the essential oils of *C. articulatus L* from Tharaka and absent in that from Nigeria; phellandrene, D-limonene, eucalyptol, mertenol, thymol, alloaromandrene oxide, culmarine, lsoaromandrene epoxide and α-cubenene. Cyperene was a compound reported from *C. articulatus* grown in Brazil, Japan, Taiwan, Thailand, Hawaii, Philippines, and *C. rotundus* from S. Africa but was missing in *C. articulatus L* from Tharaka in Kenya. *C. articulatus L* from Nigeria was reported by Nureni *et al* 2006 to have cyperotundone as its major component and this was absent in the *C. articulatus L* from Tharaka. Comparing the results of *C. articulatus L* from Tharaka with those previously reported in literature on essential oils of *C. articulatus L* and other related species it is apparent that there are many differences regarding the major constituents of the oil and its other components. This could be due to different climatic and environmental conditions in Kenya and other parts of the world as explained by Oladipupo *et al.*, 2009. This means that the Kenyan essential oil is unique. As in the previous findings the sesquiterpenes were the major components, twenty seven in number and going by the computation of relative peak area percentages given in table 4.2 they were in larger quantities than the monoterpenes which were twenty in number and in smaller quantities. This was the case in the Nigerian essential oil where the sesquiterpenes were major and the monoterpenes were the minor components. The number of compounds indentified in

Kenya was greater than that in Nigeria. Kenyan essential oil had 59 compounds, Nigerian- red tubers-37 and black tubers-47. This is could be because in Kenya the method used was solvent extraction which is known to extract more compounds than all the other methods, in Nigeria hydro-distillation was the method extraction used. Attempts to isolate the essential oils of *C. articulatus L* obtained by using 100 % CH_2Cl_2 extract from Tharaka were unsuccessful. This was attributed to the presence of a large number of number of monoterpenes which have very close retention values and therefore difficult to separate by column chromatography. Crystallization also failed as a method of compounds purification in this study because sesquiterpenes are oils at room temperature and do not usually crystallize as stated by Mesmin *et al.*, 2001. *C. articulatus L* from Tharaka had a lot of similarities with that extracted from Brazil and other parts of the world, this means that it has the potential for drugs and other products ranging from mosquito repellent, perfumes, air freshener, mouth-freshener and a range of other cosmetic products as evidenced in the results in chapter four of this report. The crude extract of *C. articulatus L* was active against the three bacterial strains: *S. pneumonia, S. aureus* and *S. typhi.* The best and most effective dose was the undiluted 100% CH_2Cl_2 extract for all the bacterial strains except for *S. aureus* that was inhibited in both undiluted 100% CH_2Cl_2 extract and the 1 mg/ml according to the records on tables 4.3 and 4.4. The extract was repellent against the mosquito *A. egyptii* with a repellency of 100% as recorded on table 4.5 which supported the TMPs claim that it was being used as an insect repellent. The use of *C. articulatus L* by the entire Meru community for treatment of typhoid, stomachache, blisters and wounds could be related to its anti-bacterial activities reported in this study. It is being reported for the first time here that *C. articulatus L* from Tharaka has activity against *S.typhi*, *S.aureus* and *S. pneumoniae.* We also report for the first time that the crude extract of *C. articulatus L* from Tharaka has repellent activity against the mosquito *Aedes egyptii.*

5.2 Recommendations

The farmers in Meru should be encouraged to plant more *C. articulatus L* as its oil composition is similar to that of France, Brazil and other countries that are using it in cosmetics and perfumery industry in hair care, fragrance, oral care, skin care, and deodorant. We here recommend that formulation as a perfume, deodorant, air freshener, mouth-freshener and a

mosquito repellent be done on this plant. It should also be examined for anti- allergenic effects because its sweet smell does not seem to affect people who are allergic to other commercial perfumes. Use of botanical derivatives in mosquito control instead of synthetic insecticides will reduce the cost and risks of environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fractions of this plant extracts for development of eco-friendly chemicals and indigenous plant base oil for protection against the bites of insects and the above mentioned uses. Other chromatographic methods example preparative gas chromatography (GC) should be tried for isolating the pure compounds and further tests be done on the pure compounds. This is because sesquiterpenes structures may serve as new prototypes, or templates for synthetic organic chemists to use in the design of potentially superior chemotherapeutic or otherwise biologically active agents. The research should also be extended to the other *Cyperus* species found in the area of study like *Cyperus rotundus* which from S. Africa is reported to have related compounds.

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APPENDICES

Appendix 1 Questionnaire used for traditional medicine practitioners

EVALUATION OF MEDICINAL PLANT PREPARATIONS USED IN THE TREATMENT OF FEVER AND PAIN

Serial number of the questionnaire .....

Name of interviewer.....Date.....

PART ONE: CONSENT

A RESEACHER'S DECLARATION

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual proprietary rights of the herbal practitioners.
2. We will at no time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretence.
3. We will be under no obligation to edit or tamper with the information provided by the respondents.
4. The information collected will be used for the described research purpose and not any undisclosed intentions.

Researchers:

Karambu Muriithi
Dr. Mbaabu Mathiu
Prof. J.O Midiwo
Prof..S.G.Kiama
Dr. J.M Wanjohi

RESPONDENTS CONSENT AGREEMENT

I .....hereby agree to participate in the study with my full consent and conscience, and declare that to the best of my knowledge the information that I have provided is true, accurate and complete.

Signature / Thumb print.....

PART TWO
A: BIODATA

Name
.....age(years).....gender.....
Location of practice.....in .....Division
Number of years of practice.....
How did you acquire your skills.....
Level of education*(none, primary, secondary, college, other.....
Contact address/telephone.....

B: MEDICINAL USE OF CYPERUS ARTICULATUS

1. Which diseases / conditions do you treat with *Cyperus articulatus* L (Ndago)

.....
.....

	Disease/condition	Plant part used	How do you prepare?	How much do you administer/ how many times in a day	Remarks
1					
2					
3					
4					
5					
6					
7					

i) Is *Cyperus articulatus L* (Ndago) readily available? .....
ii) How far (long) do you go (take) to get Ndago today compared to 10 years ago?
iii) Is Ndago cultivated or collected wildly from the forest?
(a) cultivated
(b) collected from the forest
iv) Apart from the medicinal uses what else do you use Ndago for?
.....
v) What part(s) of the plant do you use?
1. Stem
2. Roots
3. Tubers
4. Flowers

vi) How do you prepare the medicine? (detail the entire process of harvest, processing and mixing including ratios until ready for taking)
.....
.....
.....
.....
.....

vii) How long can you keep the medicine before it goes bad?
.....

viii) How does the patient take the medicine?

ix) How long does the patient take to get well? .....

x) What problems do you encounter in herbal medicine practice?
.....
.....
.....

xi) Is there any reported case of toxicity? .....

xii) What amount of medicine do you give to:
1. Adults
2. Children

xiii) Are there any differences in usage by:
1. Male
2. Female

Appendix 2.GC-MS Results for 100% DCM Extract

```
     Data Path  : C:\msdchem\1\data\Staff\M_Langat\
     Data File  : KEO.D
     Acq On     : 11 Jan 2011   11:41
     Operator   :
     Sample     : KEO
     Misc       :
     ALS Vial   : 1   Sample Multiplier: 1

     Integration Parameters: autoint1.e
     Integrator: ChemStation

     Method     : C:\msdchem\1\methods\Standard.M
     Title      :

    42   14.368   1965 1972 1977 VV 4 1010770   24859884    2.00%    0.106%
    43   14.444   1977 1985 1989 VV   1162000   24445886    1.97%    0.104%
    44   14.629   2005 2017 2025 VV   18755194  412698889   33.28%    1.752%
    45   14.706   2025 2031 2035 VV   1627781   34256020    2.76%    0.145%

    46   14.793   2035 2046 2063 VV 2 7302692   196204741   15.82%    0.833%
    47   15.046   2077 2090 2093 VV 2 1904768   47229419    3.81%    0.200%
    48   15.097   2093 2099 2103 VV 3 4046256   84307337    6.80%    0.358%
    49   15.175   2103 2113 2122 VV 2 8081650   203452896   16.41%    0.863%
    50   15.314   2122 2137 2143 PV 3 4773749   136601977   11.02%    0.580%

    51   15.377   2143 2148 2158 VV 2 4709146   118383990    9.55%    0.502%
    52   15.459   2158 2162 2165 VV 4  824152    17298124    1.40%    0.073%
    53   15.533   2165 2175 2181 VV 3 4506690   116225334    9.37%    0.493%
    54   15.647   2181 2195 2201 VV   8560572   200710901   16.19%    0.852%
    55   15.702   2201 2205 2210 VV 7  802530    21088740    1.70%    0.090%

    56   15.794   2210 2221 2228 VV 4 3004044   88610037    7.15%    0.376%
    57   15.894   2228 2238 2242 VV 2 1741287   42624073    3.44%    0.181%
    58   15.969   2242 2251 2254 VV 2 3768999   94861378    7.65%    0.403%
    59   16.047   2254 2265 2269 VV 2 13819863  383331419   30.92%    1.627%
    60   16.080   2269 2271 2274 VV   9485830   141610226   11.42%    0.601%

    61   16.128   2274 2279 2284 VV   22223946  484377748   39.06%    2.056%
    62   16.194   2284 2291 2307 VV 2 18189279  640157834   51.63%    2.717%
    63   16.314   2307 2312 2315 VV 2 1199270   23728132    1.91%    0.101%
    64   16.353   2315 2318 2321 VV 2 1212624   23120262    1.86%    0.098%
    65   16.427   2321 2331 2335 VV 4 3749262   136373720   11.00%    0.579%

    66   16.498   2335 2344 2349 VV 5 7714385   247803287   19.99%    1.052%
    67   16.561   2349 2355 2360 VV 2 7885395   185891117   14.99%    0.789%
    68   16.649   2360 2370 2383 VV 2 8117382   301799900   24.34%    1.281%
    69   16.745   2383 2387 2391 VV 4 3728710   82272006    6.64%    0.349%
    70   16.792   2391 2395 2401 VV 5 2964533   75642384    6.10%    0.321%

    71   16.840   2401 2404 2406 VV 2 1520255   25207992    2.03%    0.107%
    72   16.953   2406 2423 2427 VV 7 4203679   202504747   16.33%    0.859%
    73   17.002   2427 2432 2436 VV   6565682   153031414   12.34%    0.649%
    74   17.053   2436 2441 2444 VV 3 4693852   105604720   8.52%    0.448%
    75   17.095   2444 2448 2453 VV 2 6854606   153562368   12.38%    0.652%

    76   17.263   2453 2477 2480 VV 5 19201392  872195940   70.34%    3.702%
    77   17.325   2480 2488 2501 VV 2 29096646  1044383549  84.23%    4.433%
    78   17.426   2501 2506 2510 VV 2 9340968   213258430   17.20%    0.905%
    79   17.503   2510 2519 2522 VV 4 16099611  494387812   39.87%    2.098%
    80   17.549   2522 2528 2535 VV   23792738  655595589   52.87%    2.782%

    81   17.619   2535 2540 2545 VV   12704519  281473643   22.70%    1.195%
    82   17.684   2545 2551 2557 VV 3 9161332   269495156   21.73%    1.144%
    83   17.744   2557 2562 2565 VV 6 6039825   145210654   11.71%    0.616%
    84   17.785   2565 2569 2573 VV 2 6060337   150798330   12.16%    0.640%
    85   17.832   2573 2577 2586 VV 7 4942976   177822491   14.34%    0.755%

    86   17.950   2586 2598 2603 VV 3 6902973   251459796   20.28%    1.067%

Standard.M Mon Jul 18 09:39:46 2011

    44.297   1945 1959 1965 VV    4190889   85613301    6.90%    0.363%

Standard.M Mon Jul 18 09:39:46 2011
```

Data Path : C:\msdchem\1\data\Staff\M_Langat\
Data File : KEO.D
Acq On : 11 Jan 2011 11:41
Operator :
Sample : KEO
Misc :
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

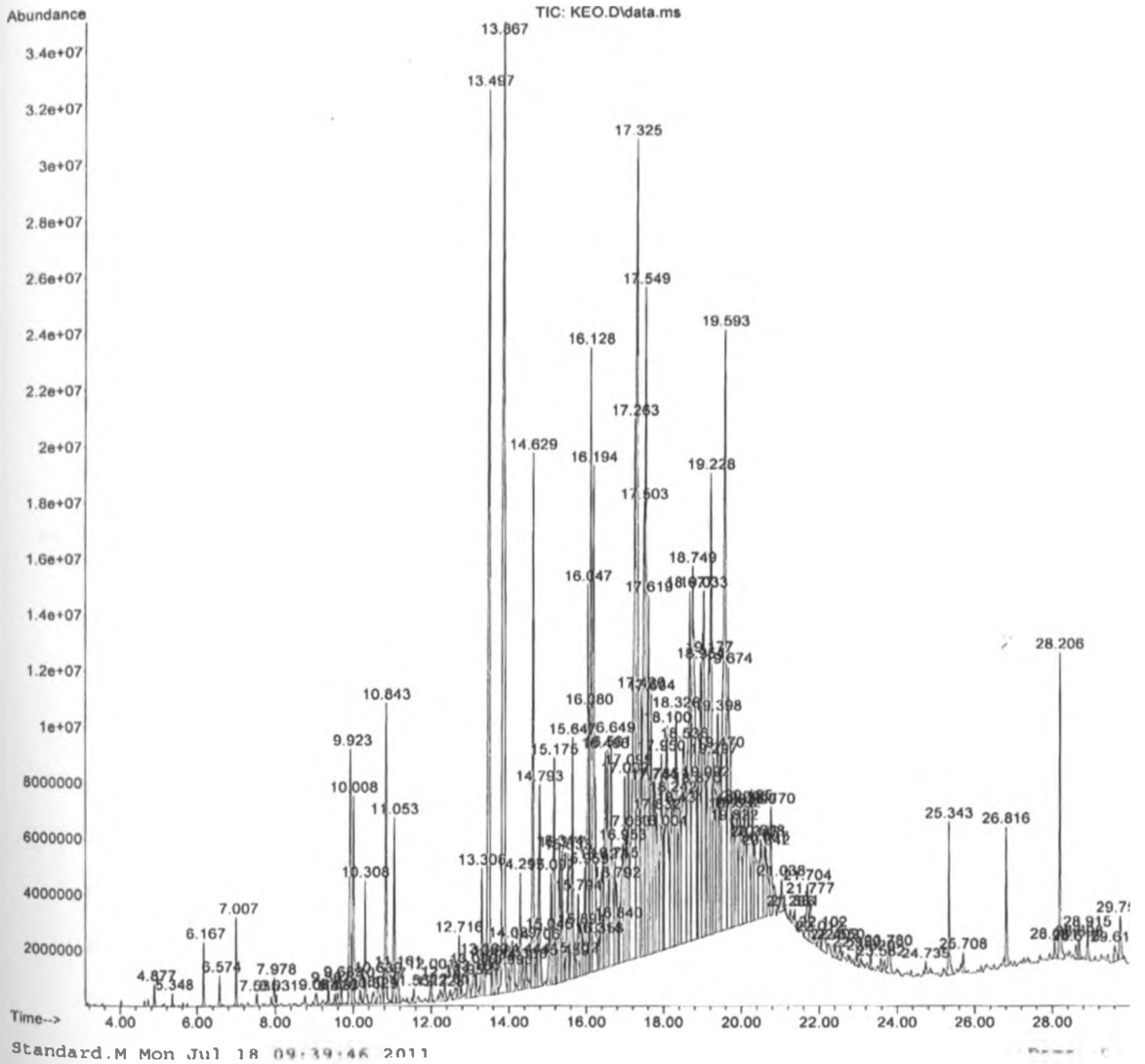
Method : C:\msdchem\1\methods\Standard.M
Title :

132	25.343	3875	3890	3915	VV		5431310	111075986	8.96%	0.471%
133	25.708	3947	3953	3962	VV		740704	16683416	1.35%	0.071%
134	26.816	4139	4147	4160	VV		4911600	96215719	7.76%	0.408%
135	28.061	4353	4365	4378	BV	4	618437	17466143	1.41%	0.074%
136	28.206	4378	4390	4409	VV		10915950	225095371	18.15%	0.955%
137	28.618	4455	4462	4470	VV	6	559191	15736951	1.27%	0.067%
138	28.698	4470	4476	4487	VV	4	729356	17709918	1.43%	0.075%
139	28.915	4506	4514	4531	BV		1102900	25531440	2.06%	0.108%
140	29.612	4620	4636	4652	BV	5	605723	25038292	2.02%	0.106%
141	29.759	4652	4661	4674	VV	6	1567452	49810212	4.02%	0.211%

Sum of corrected areas: 23561624684

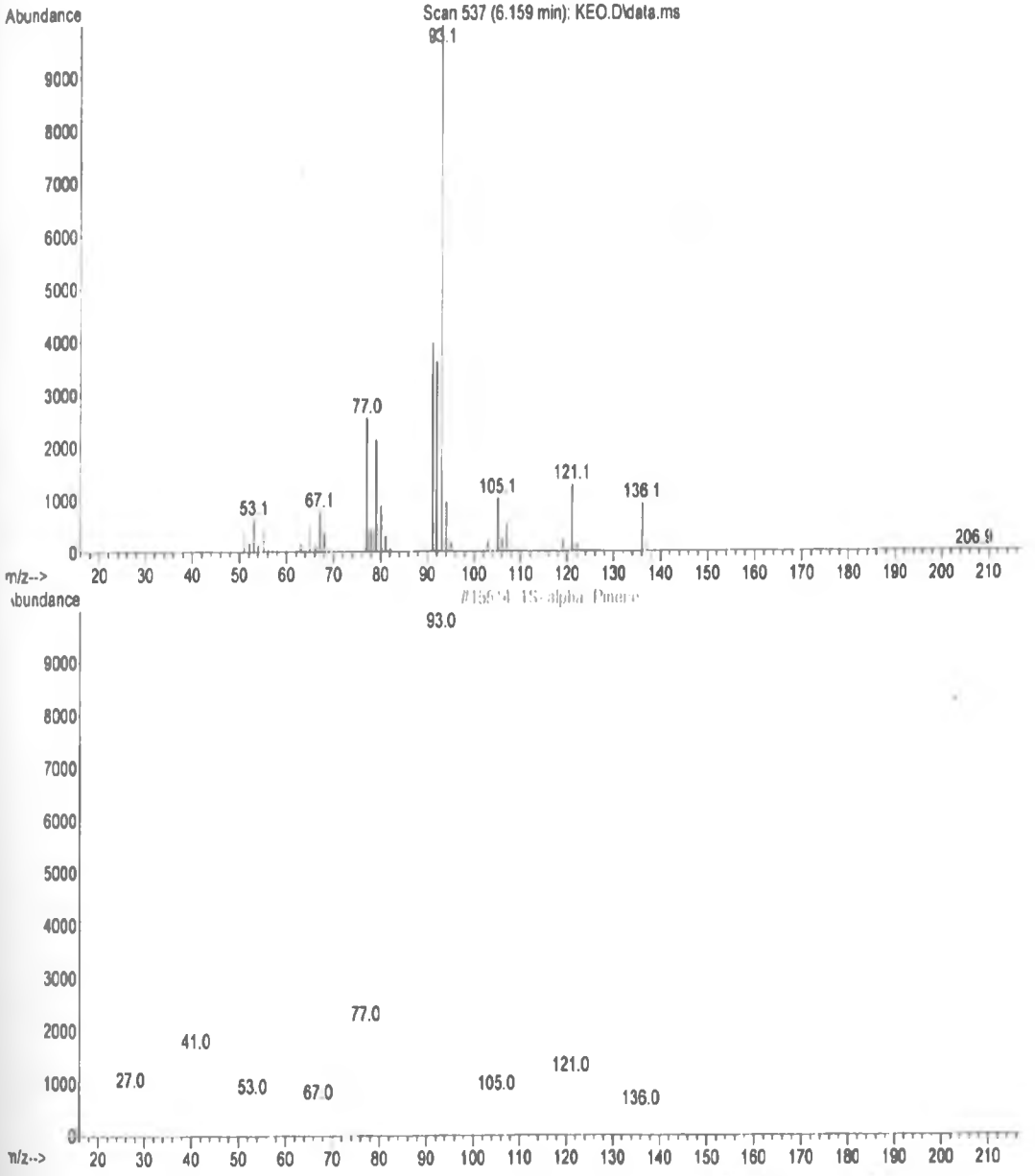
Appendix 3 Gas Chromatogram for the100% Crude Extract

```
Data Path : C:\msdchem\1\data\Staff\M_Langat\
Data File : KEO.D
Acq On    : 11 Jan 2011  11:41
Operator  :
Sample    : KEO
Misc      :
ALS Vial  : 1   Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method    : C:\msdchem\1\methods\Standard.M
Title     :
```



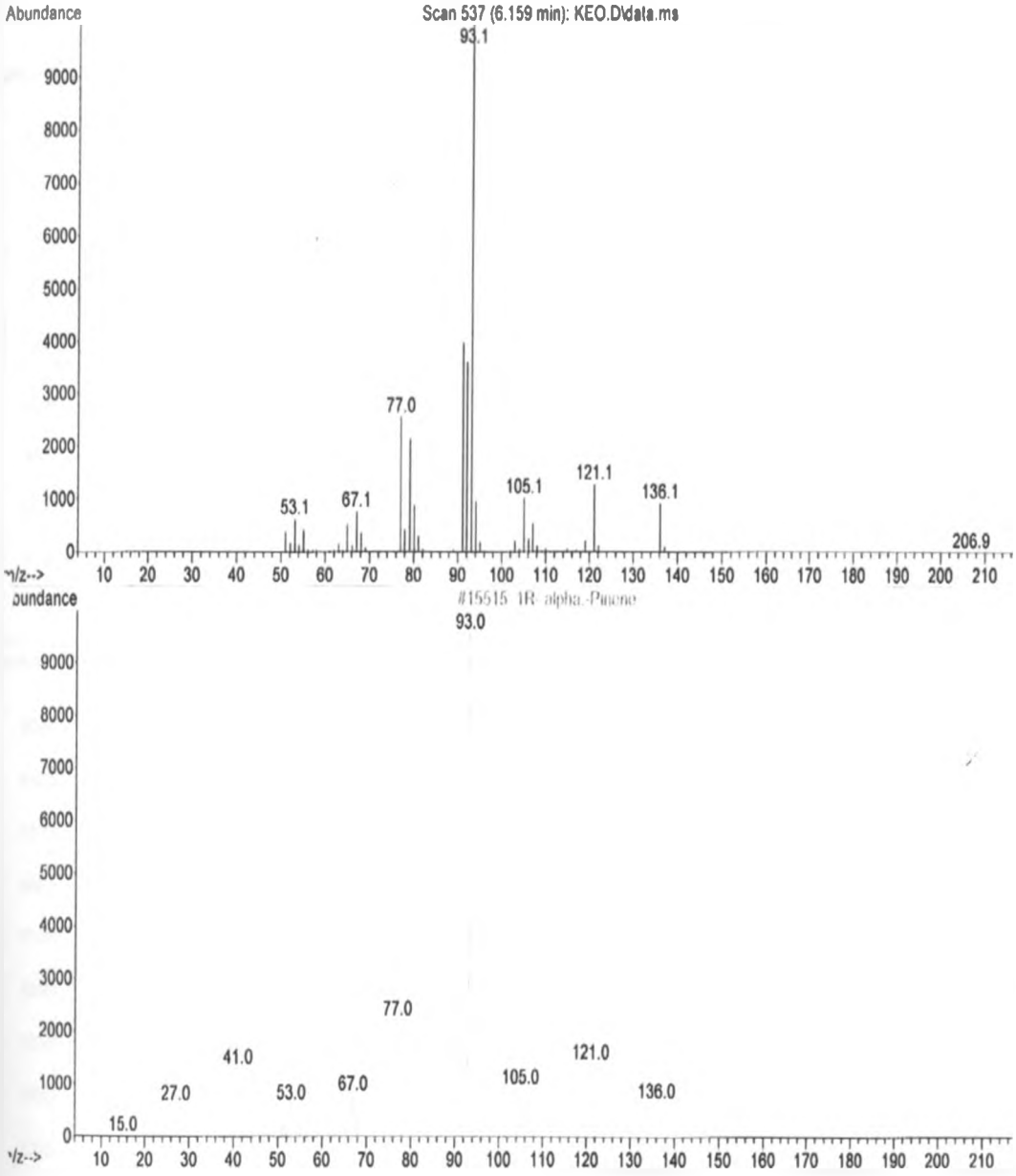
Appendix 4 Mass Spectrum for Compound 1

```
Library Searched : C:\Database\NIST08.L
Quality          : 96
ID               : 1S-.alpha.-Pinene
```



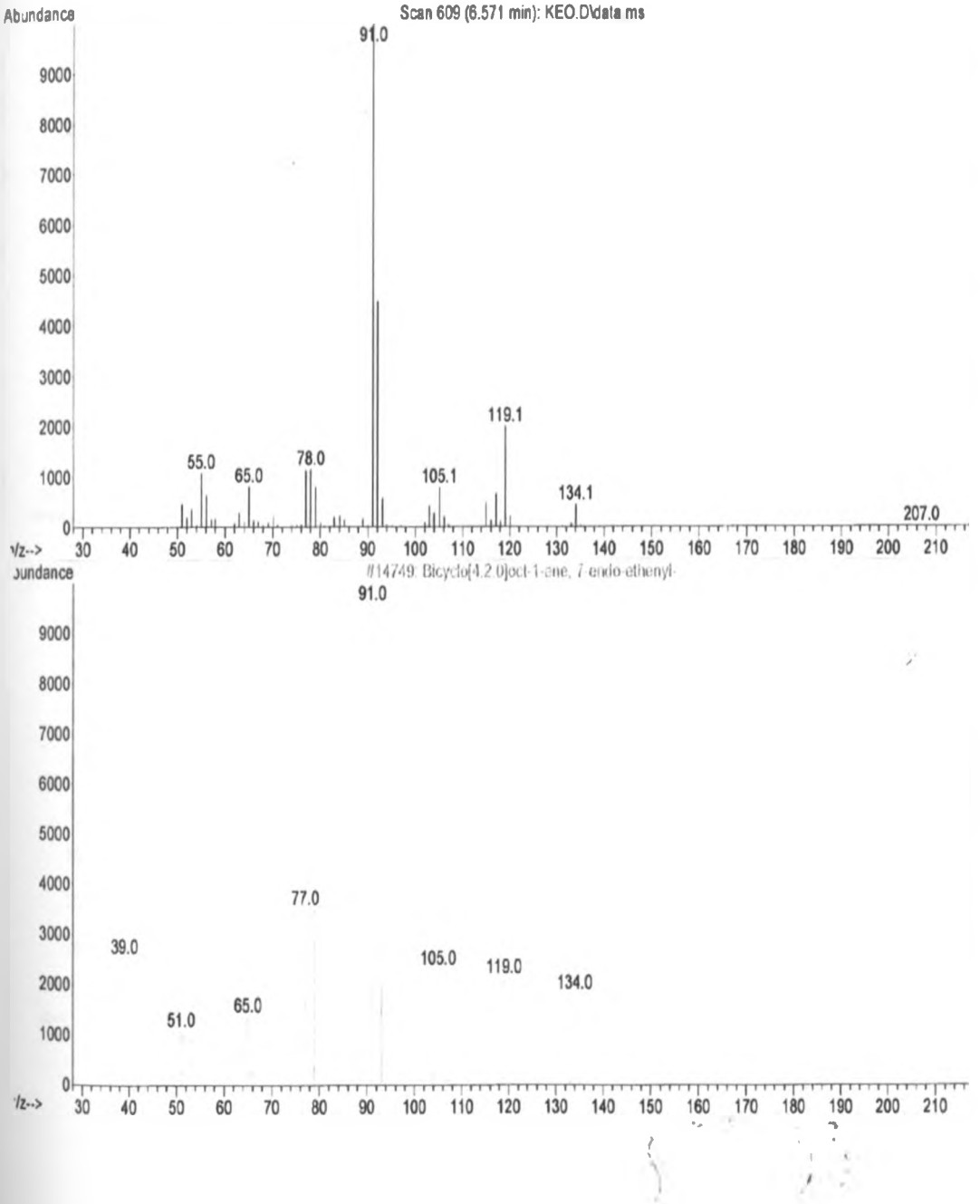
Appendix 5 Mass Spectrum for Compound 2

```
Library Searched : C:\Database\NIST08.L
Quality          : 96
ID               : 1R-.alpha.-Pinene
```



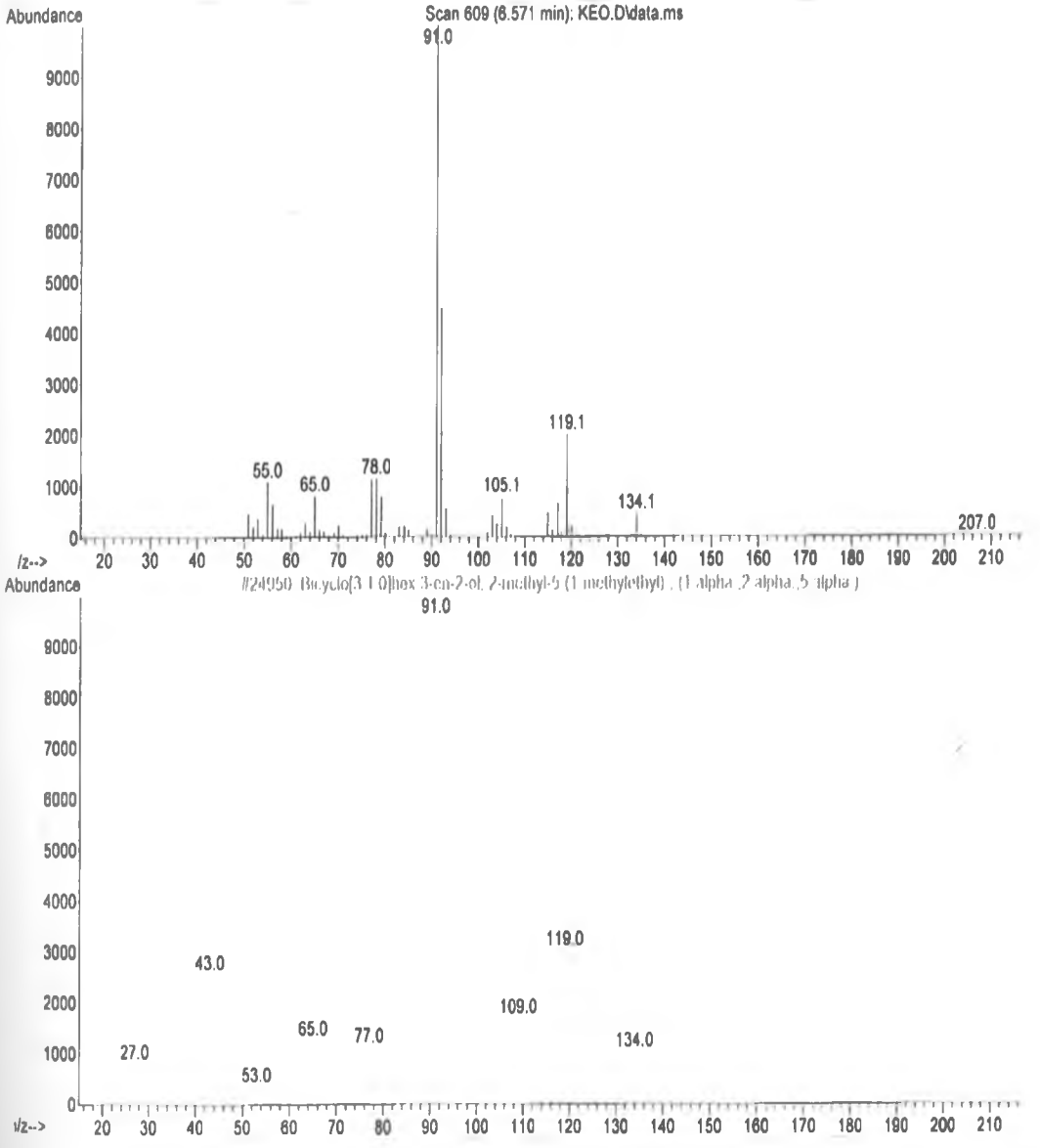
Appendix 6 Mass Spectrum for Compound 3

```
Library Searched : C:\Database\NIST08.L
Quality          : 64
ID               : Bicyclo[4.2.0]oct-1-ene, 7-endo-ethenyl-
```



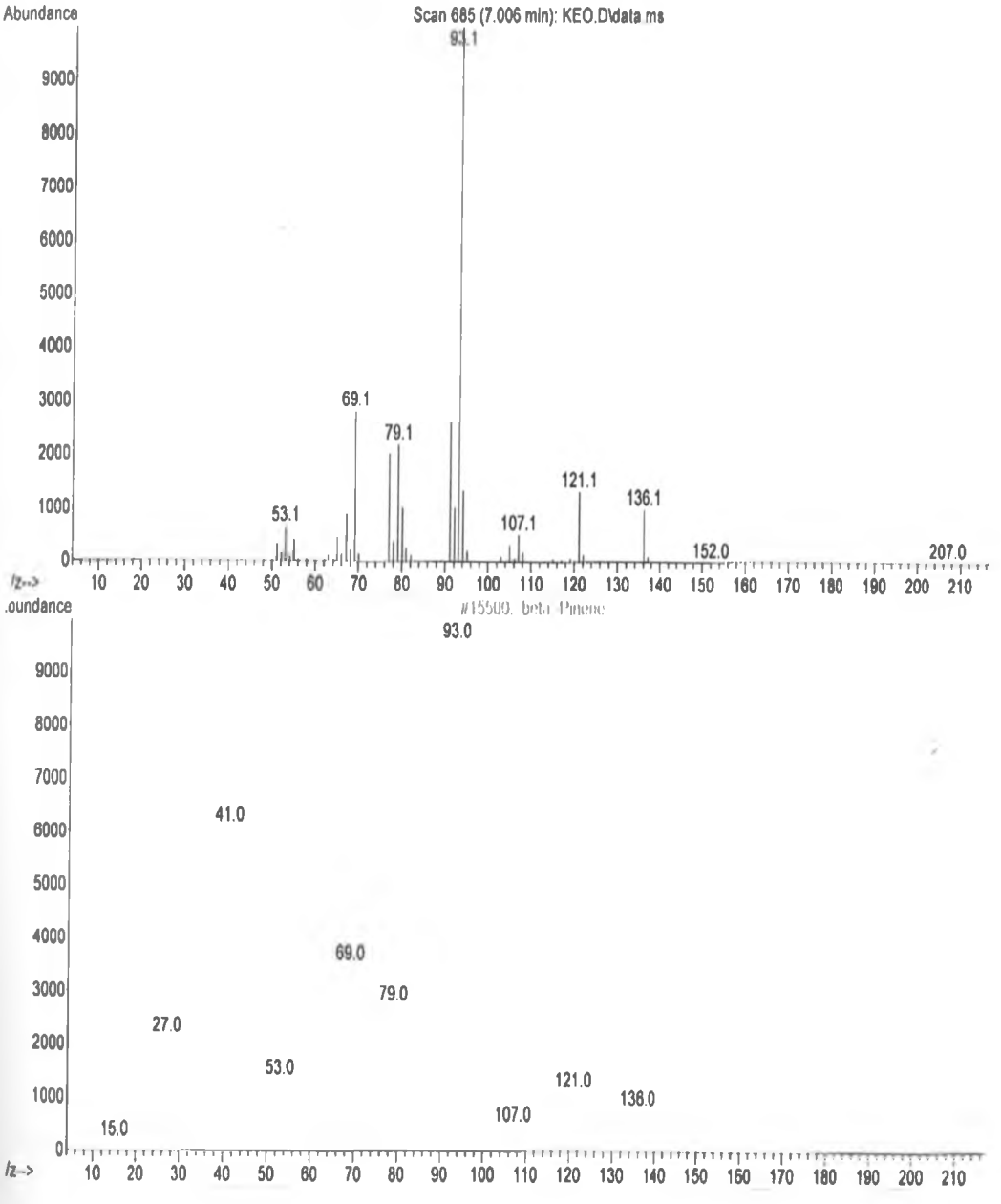
Appendix 7 Mass Spectrum for Compound 4

```
Library Searched : C:\Database\NIST08.L
Quality          : 64
ID               : Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2
                   .alpha.,5.alpha.)-
```



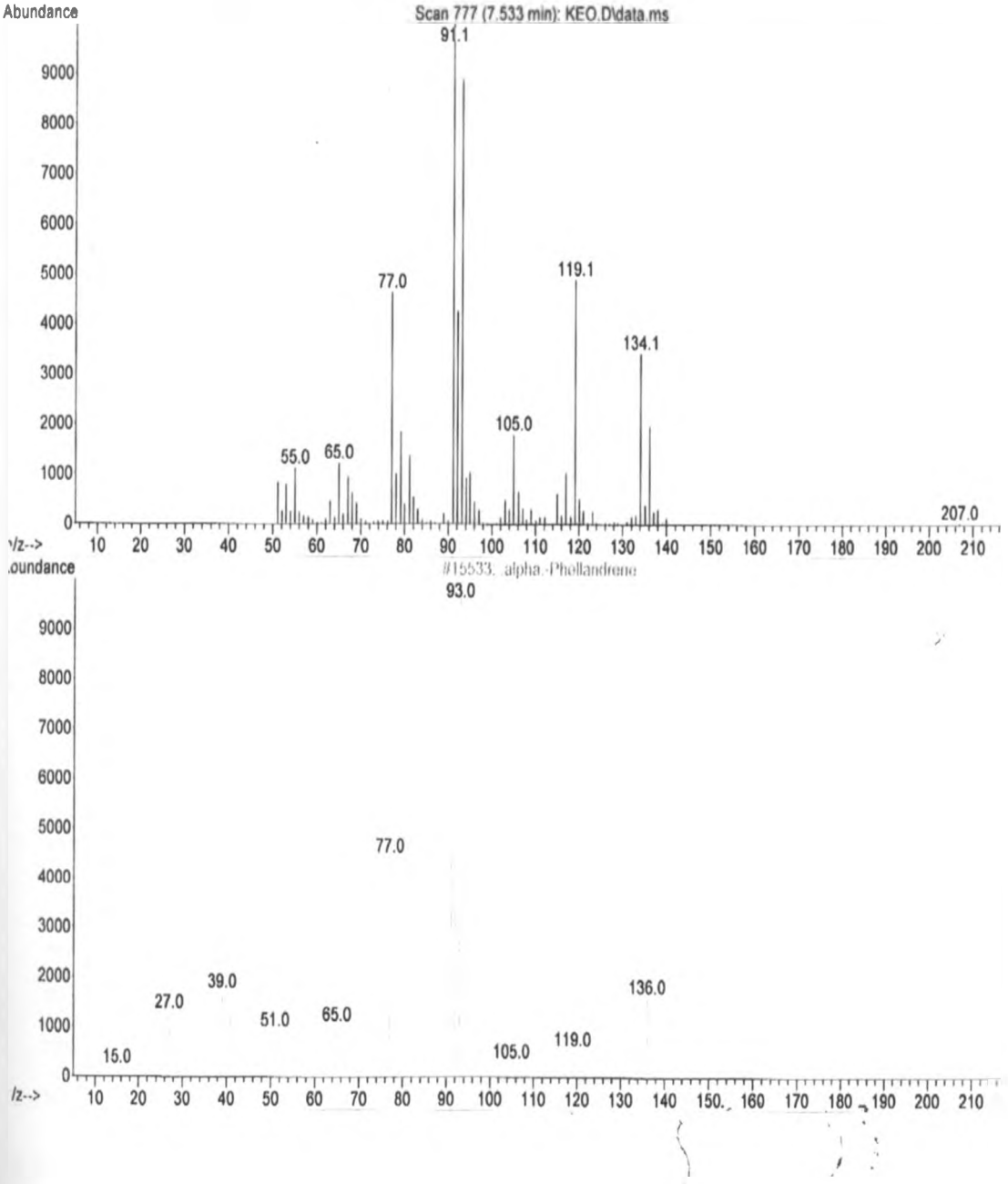
Appendix 8 Mass Spectrum for Compound 5

```
Library Searched : C:\Database\NIST08.L
Quality          : 91
ID               : .beta.-Pinene
```



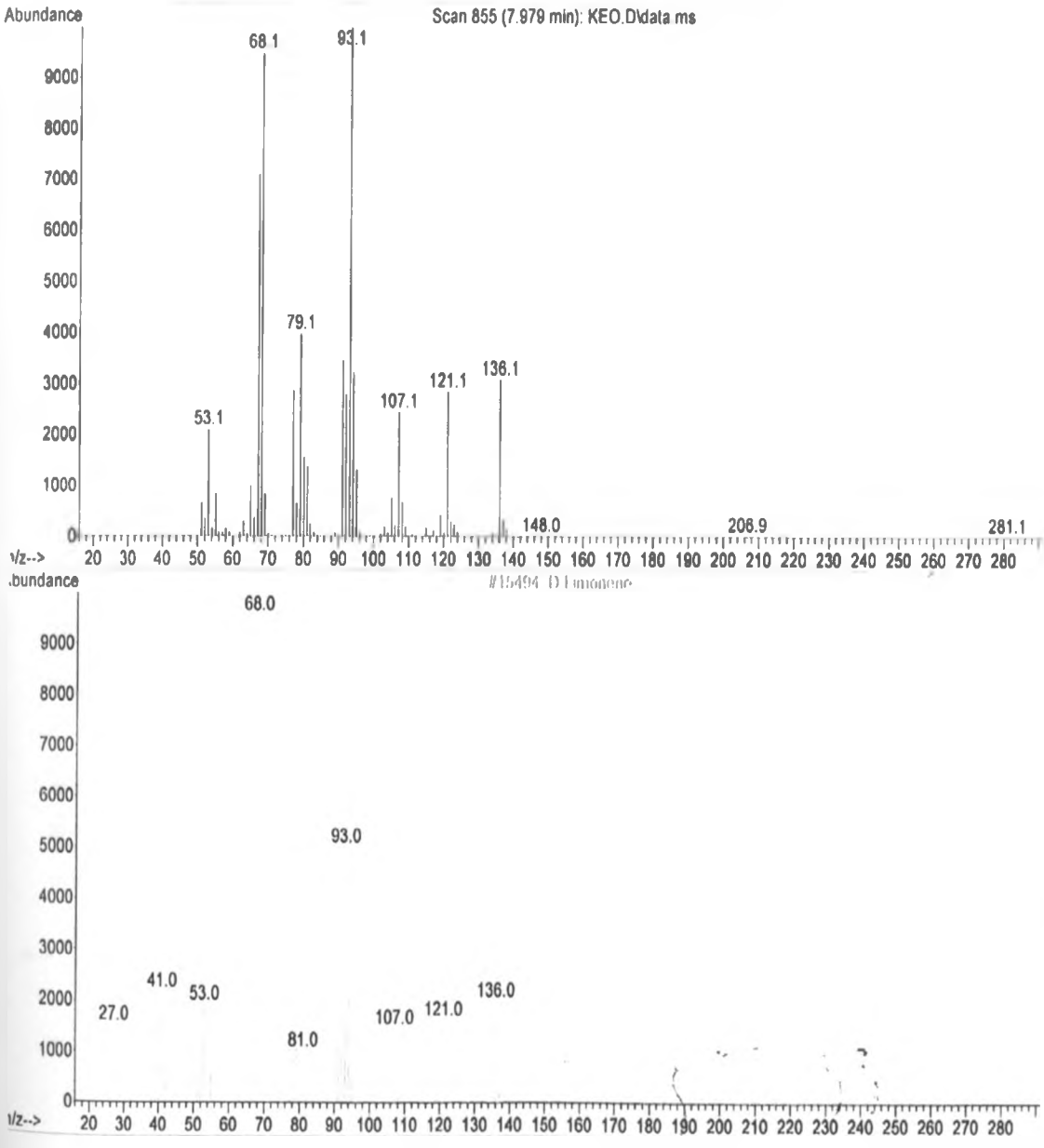
Appendix 9 Mass Spectrum for Compound 6

```
Library Searched : C:\Database\NIST08.L
Quality          : 70
ID               : .alpha.-Phellandrene
```



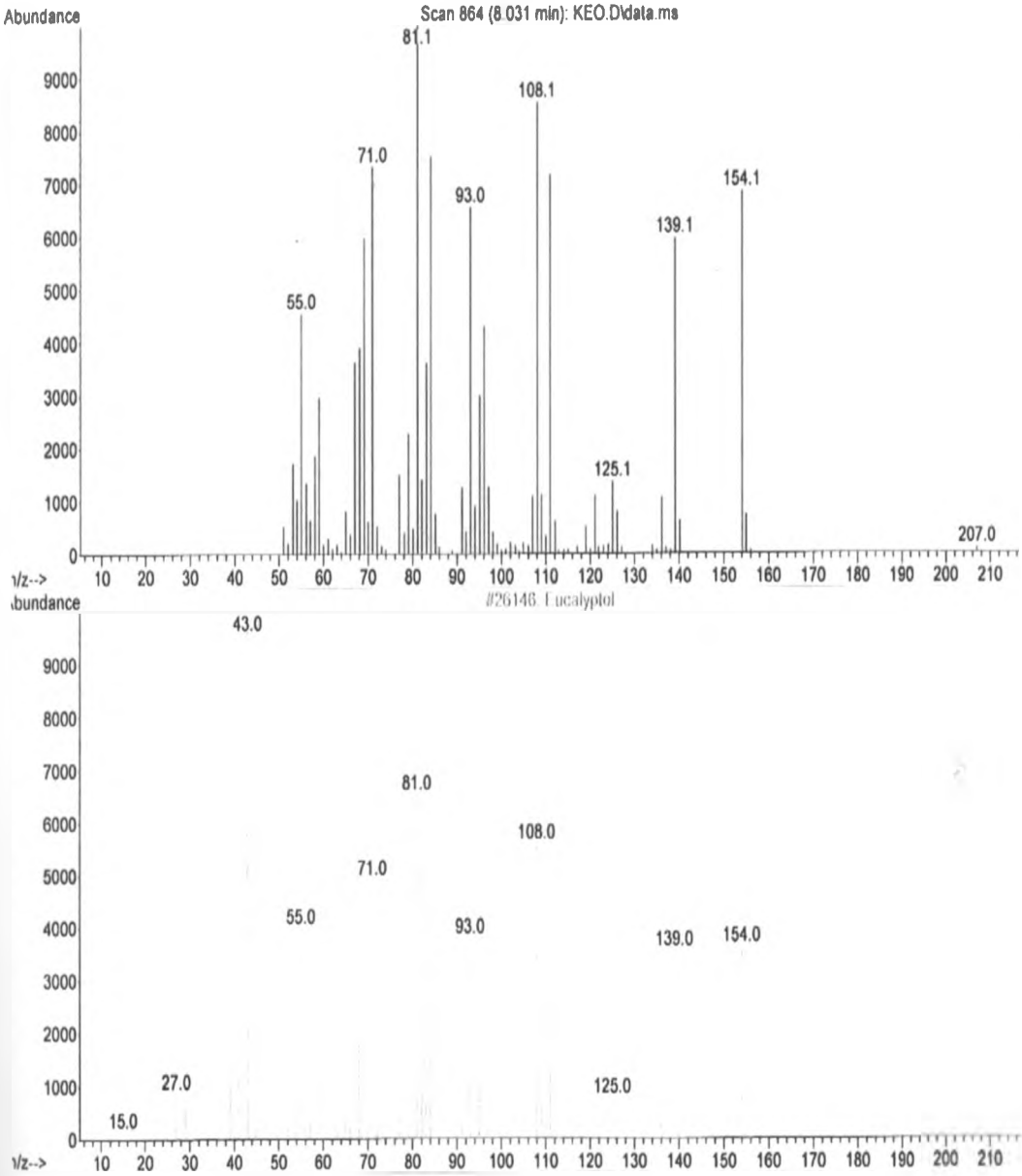
Appendix 10 Mass Spectrum for Compound7

```
Library Searched : C:\Database\NIST08.L
Quality          : 95
ID               : D-Limonene
```



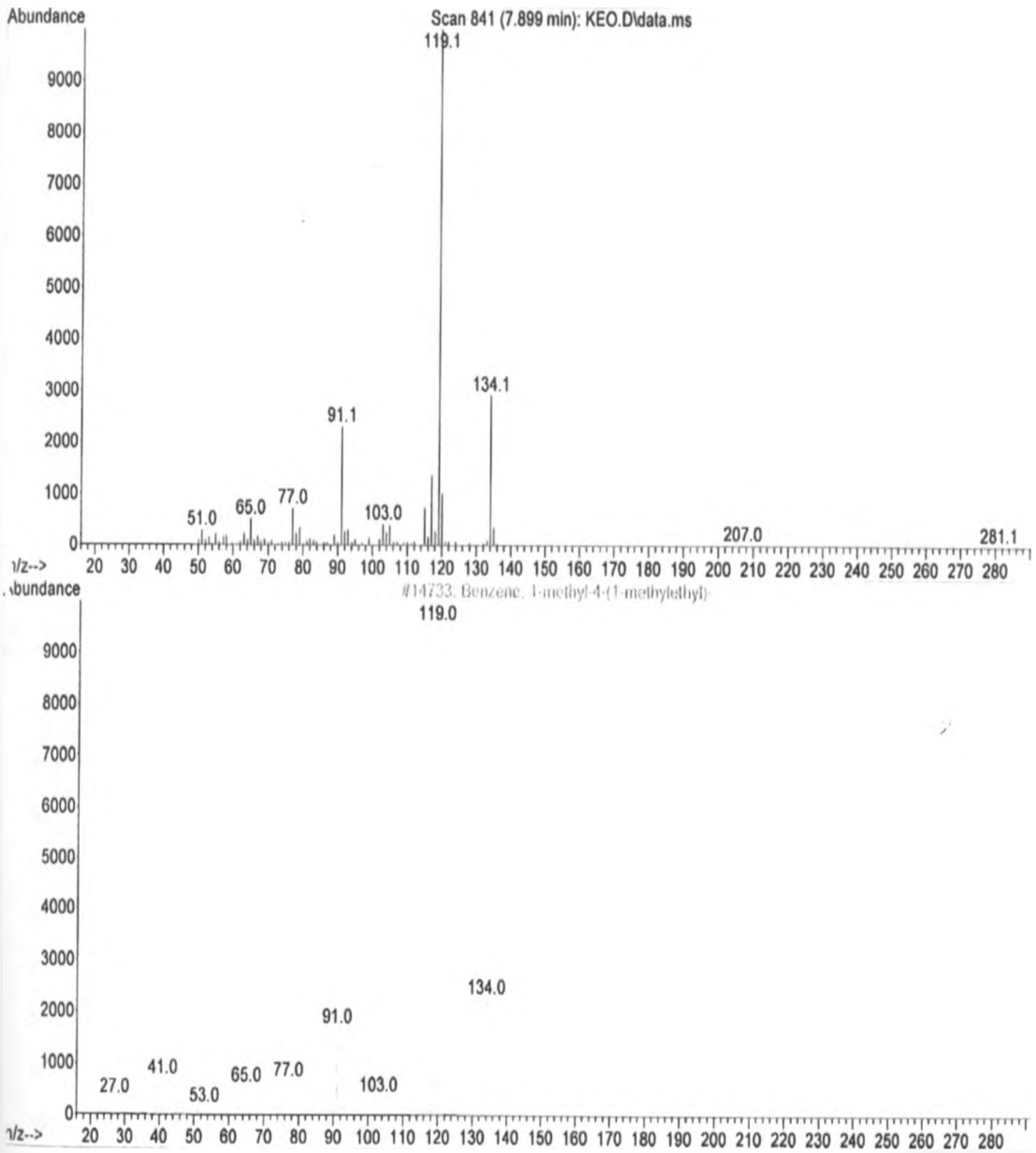
Appendix 11 Mass Spectrum for Compound 8

```
Library Searched : C:\Database\NIST08.L
Quality          : 98
ID               : Eucalyptol
```



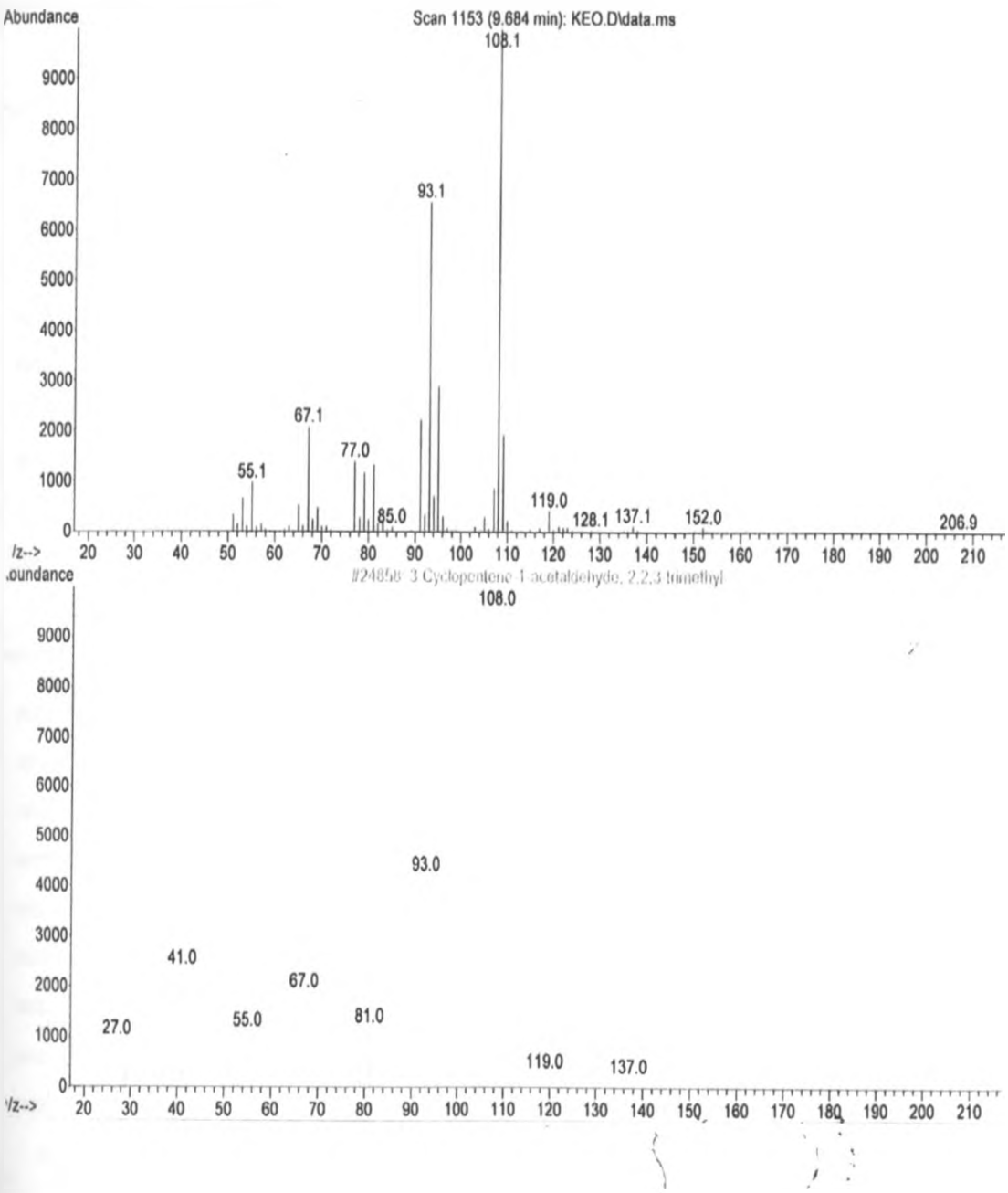
Appendix 12 Mass Spectrum for Compound 9

```
Library Searched : C:\Database\NIST08.L
Quality          : 97
ID               : Benzene, 1-methyl-4-(1-methylethyl)-
```



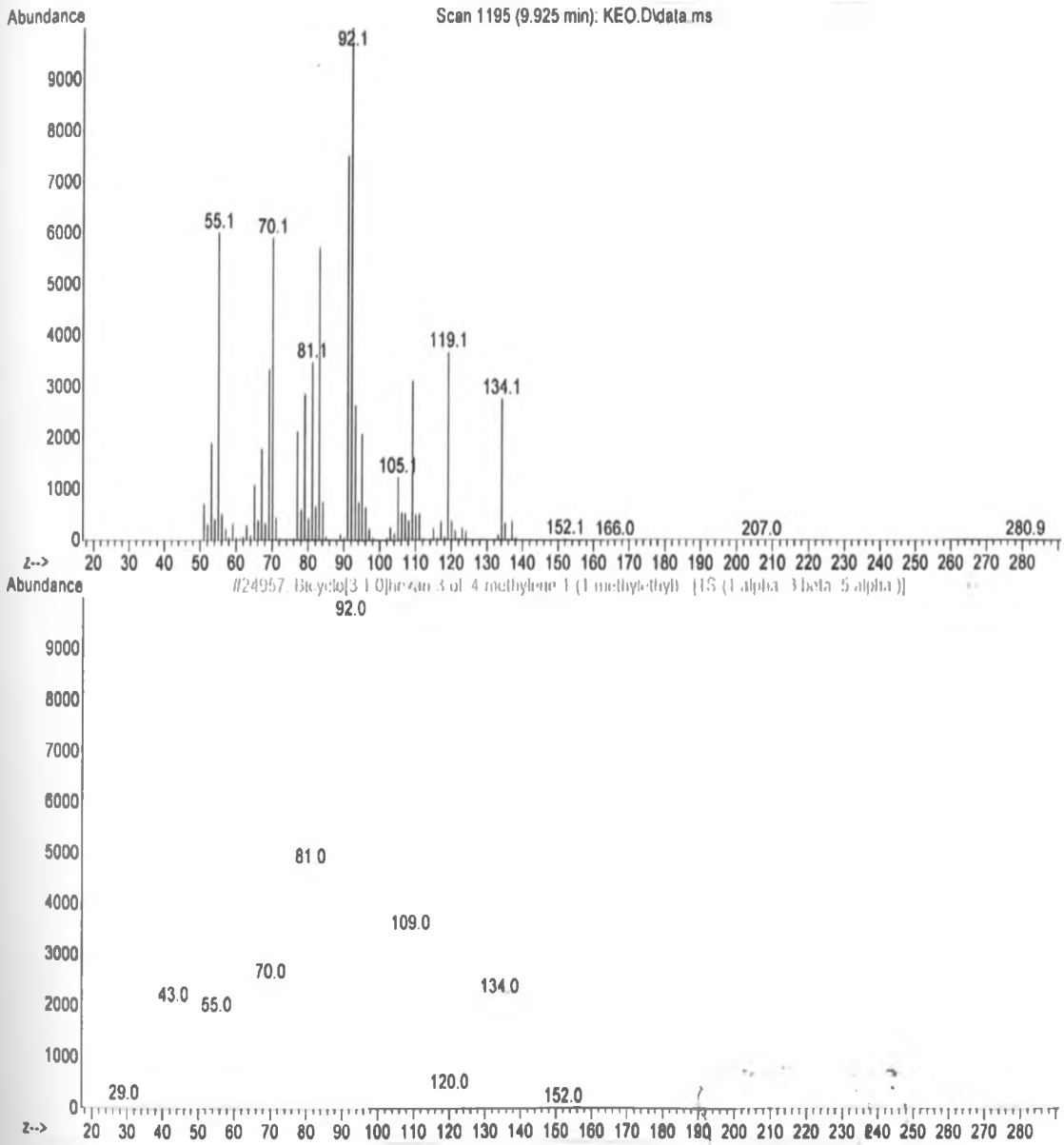
Appendix 13 Mass Spectrum for Compound 10

```
Library Searched : C:\Database\NIST08.L
Quality          : 90
ID               : 3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-
```



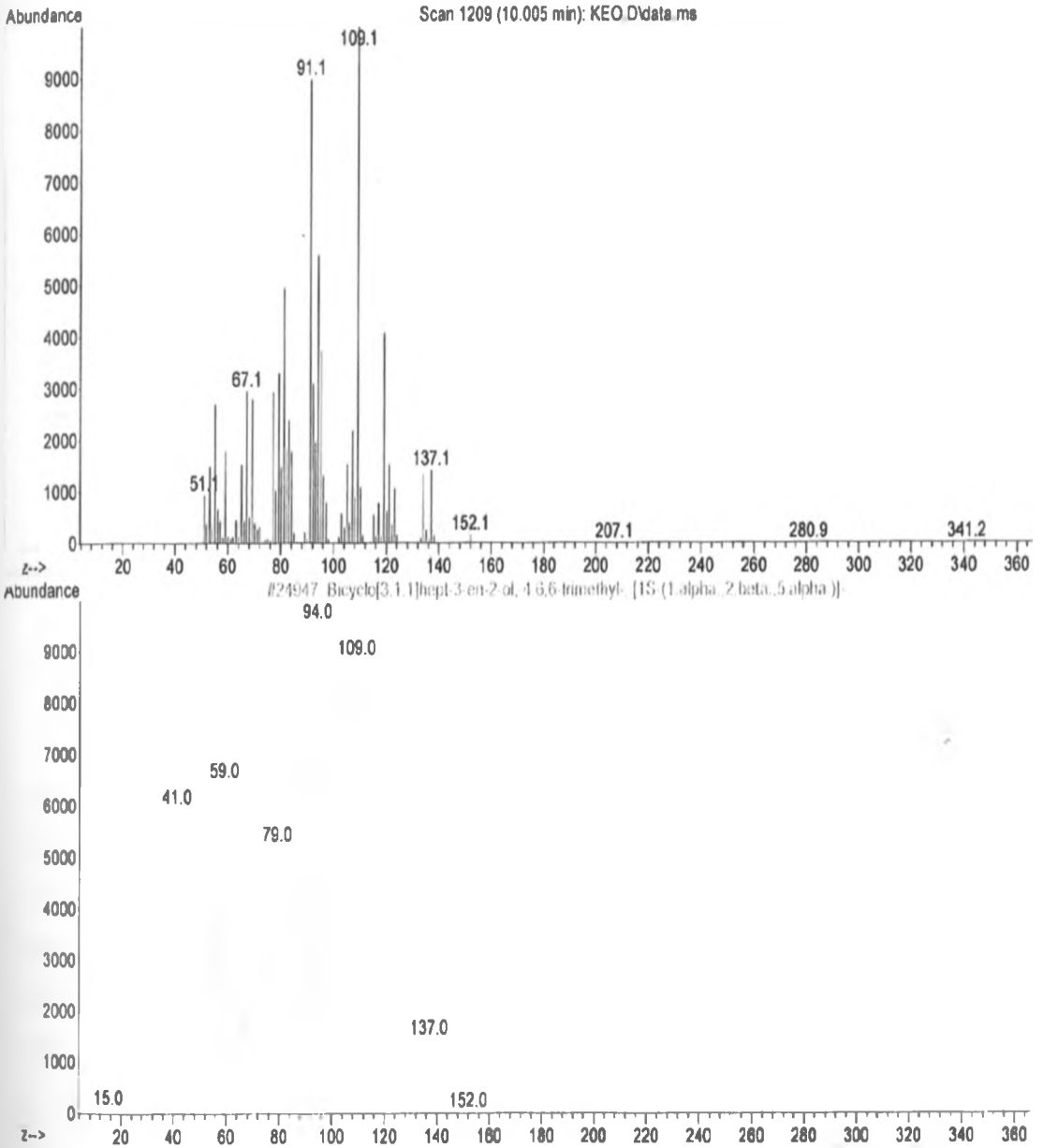
Appendix 14 Mass Spectrum for Compound 11

```
Library Searched : C:\Database\NIST08.L
Quality          : 76
ID               : Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, [1S-(1.alph
                   a.,3.beta.,5.alpha.)]-
```



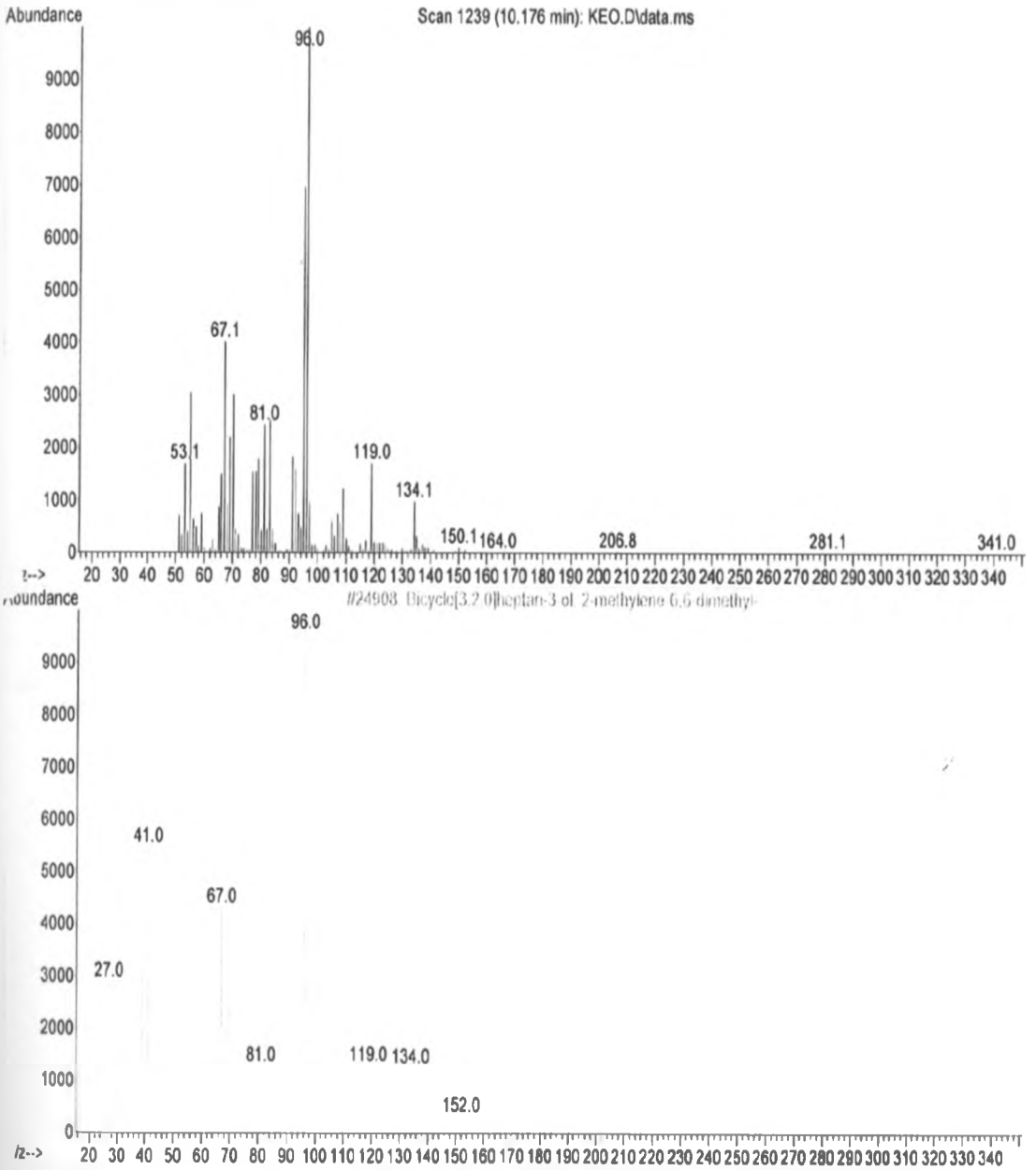
Appendix 15 Mass Spectrum for Compound 12

```
Library Searched : C:\Database\NIST08.L
Quality          : 59
ID               : Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1.alpha.,2.beta.
                   ,5.alpha.)]-
```



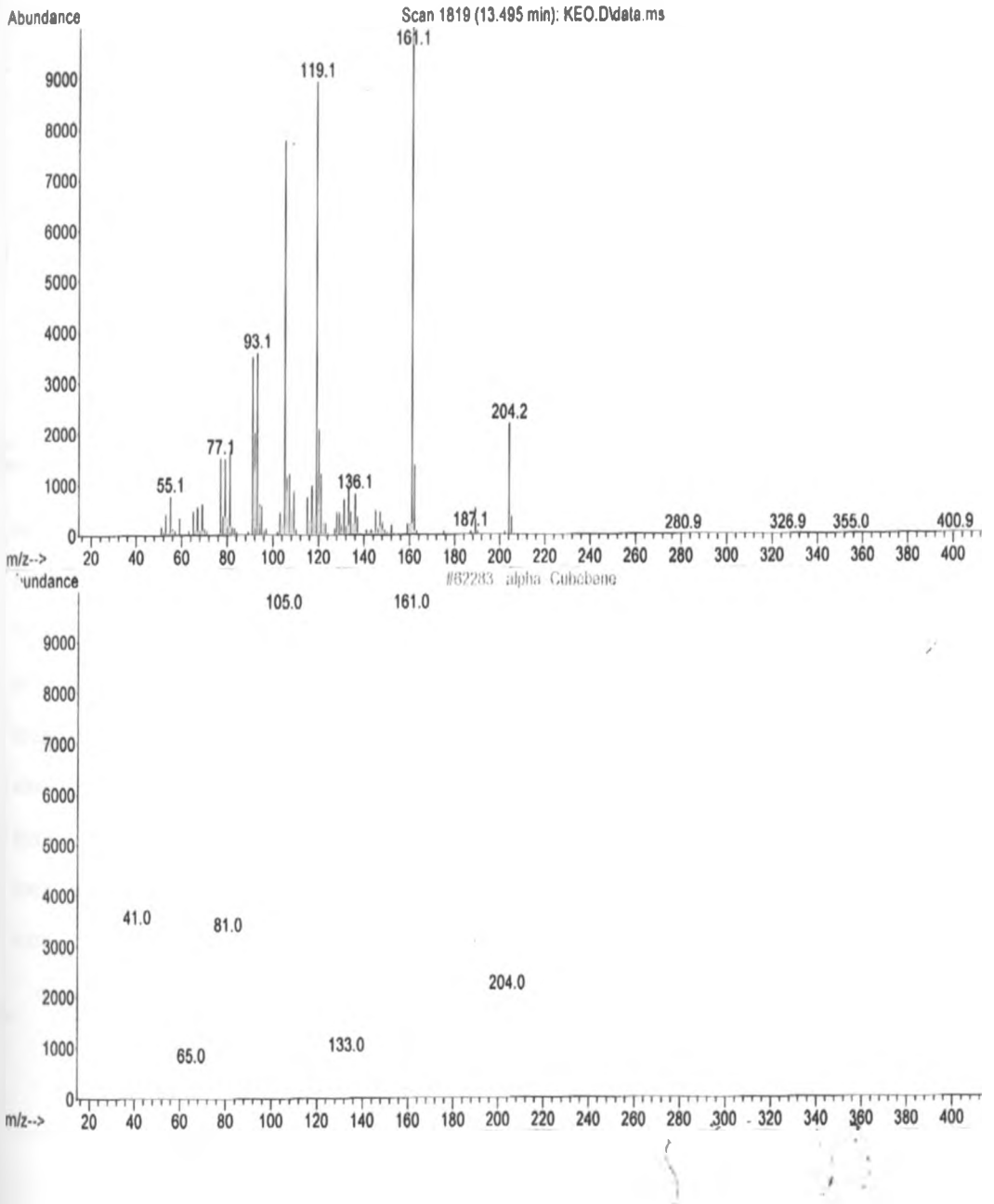
Appendix 16 Mass Spectrum for Compound 13

```
Library Searched : C:\Database\NIST08.L
Quality          : 94
ID               : Bicyclo[3.2.0]heptan-3-ol, 2-methylene-6,6-dimethyl-
```



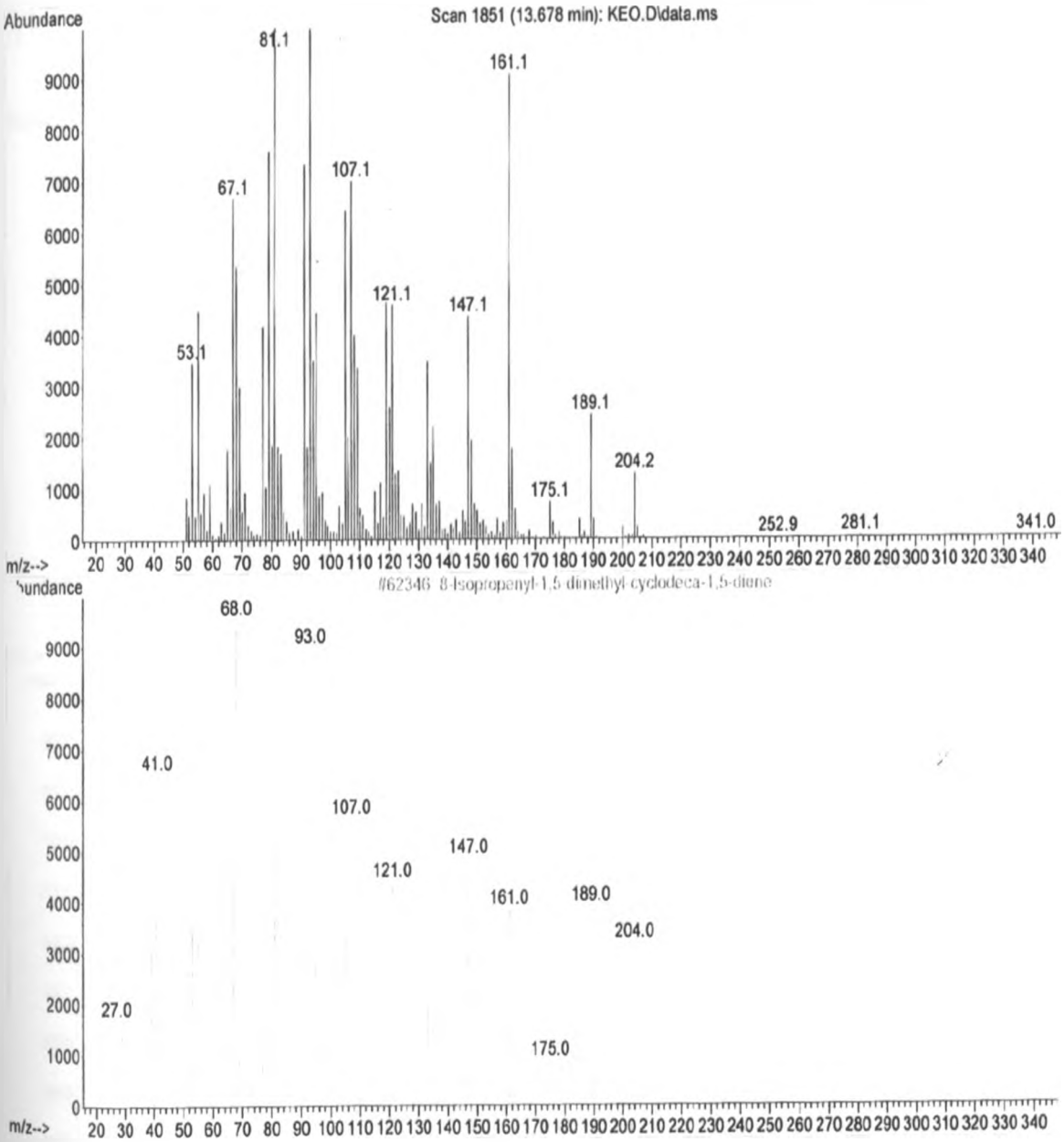
Appendix 17 Mass Spectrum for Compound 14

```
Library Searched : C:\Database\NIST08.L
Quality          : 98
ID               : .alpha.-Cubebene
```



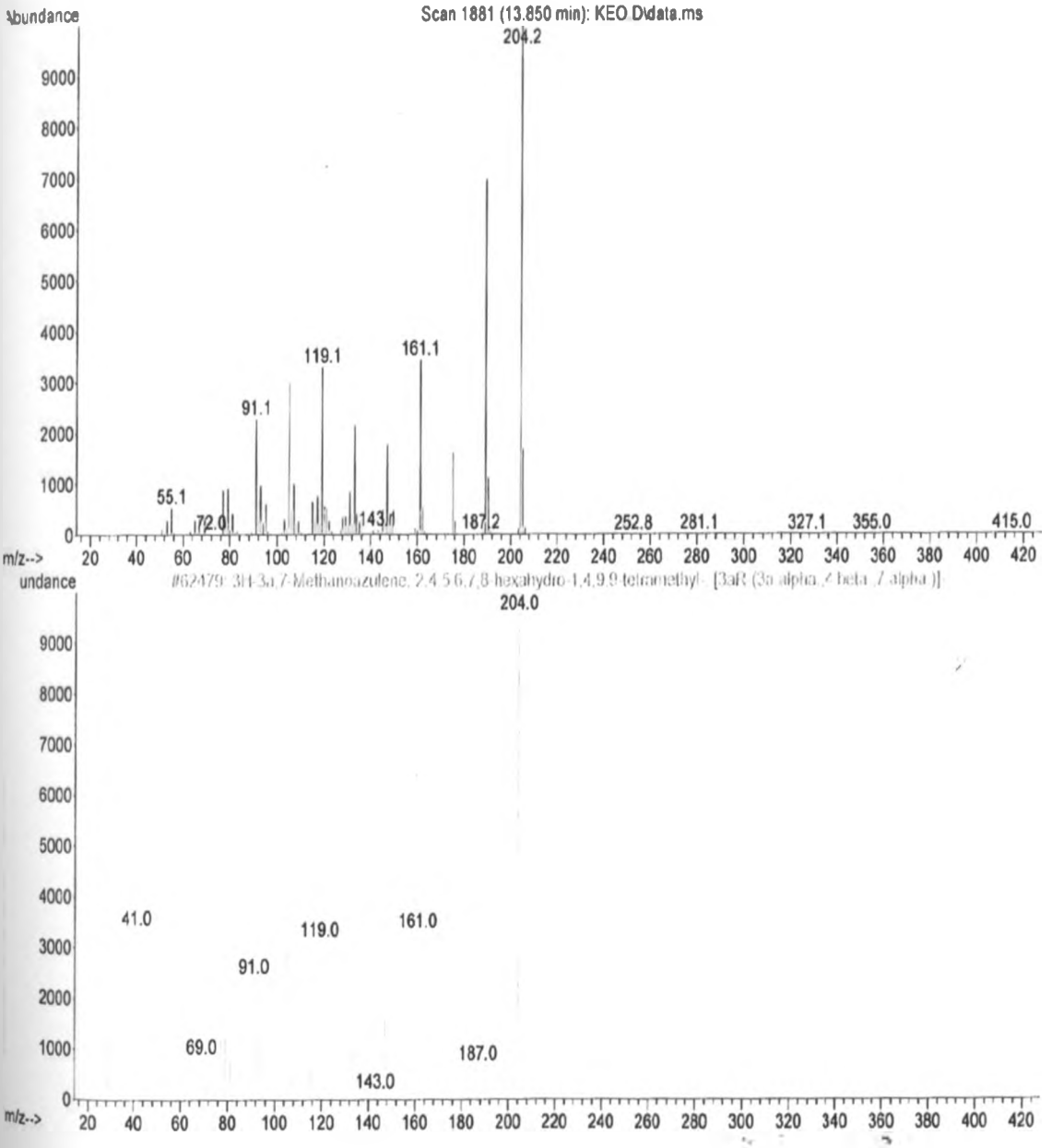
Appendix 18 Mass Spectrum for Compound 15

```
Library Searched : C:\Database\NIST08.L
Quality          : 89
ID               : 8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene
```



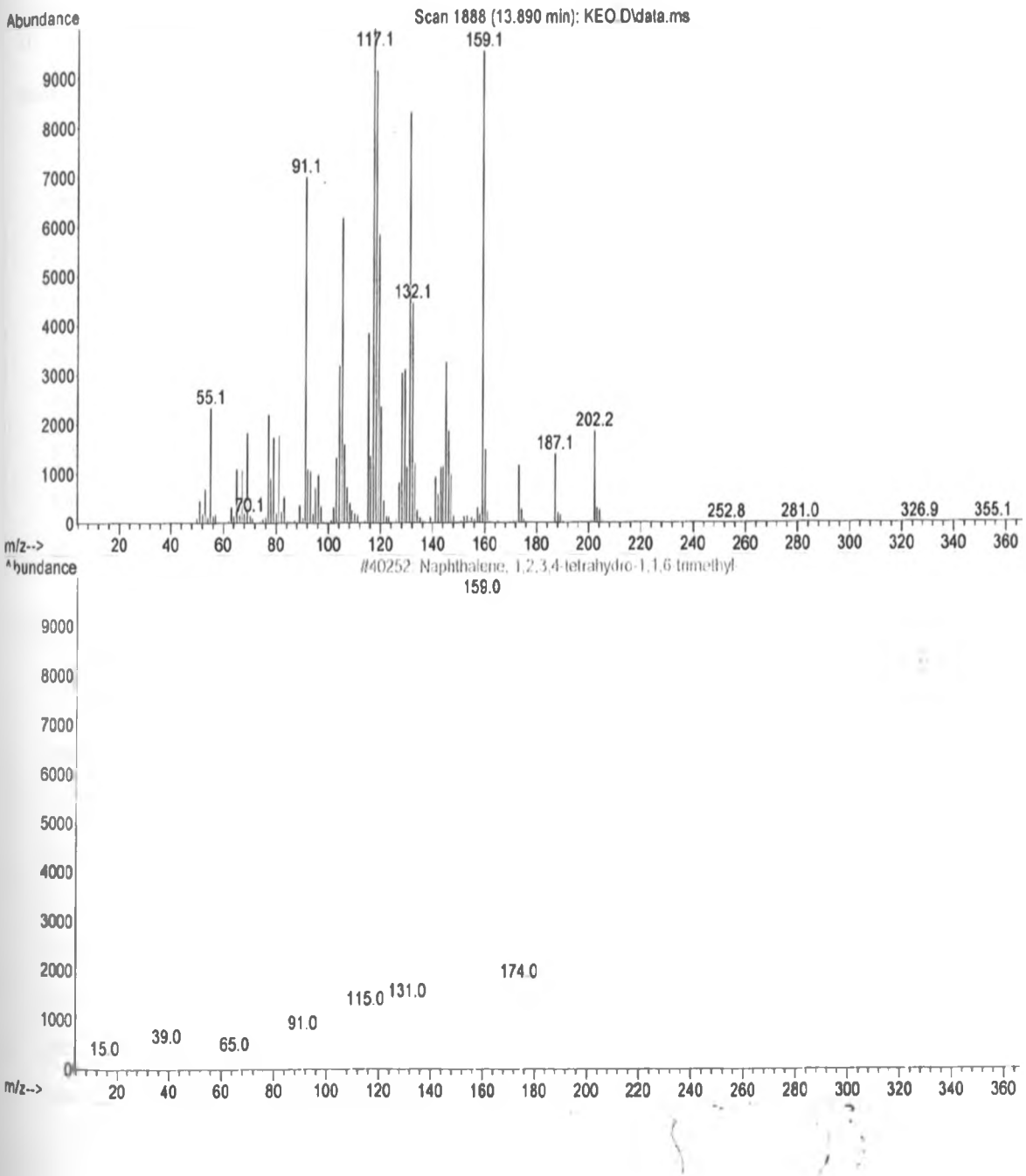
Appendix 19 Mass Spectrum for Compound 16

Library Searched : C:\Database\NIST08.L
Quality : 95
ID : 3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3a.alpha.,4.beta.,7.alpha.)]-



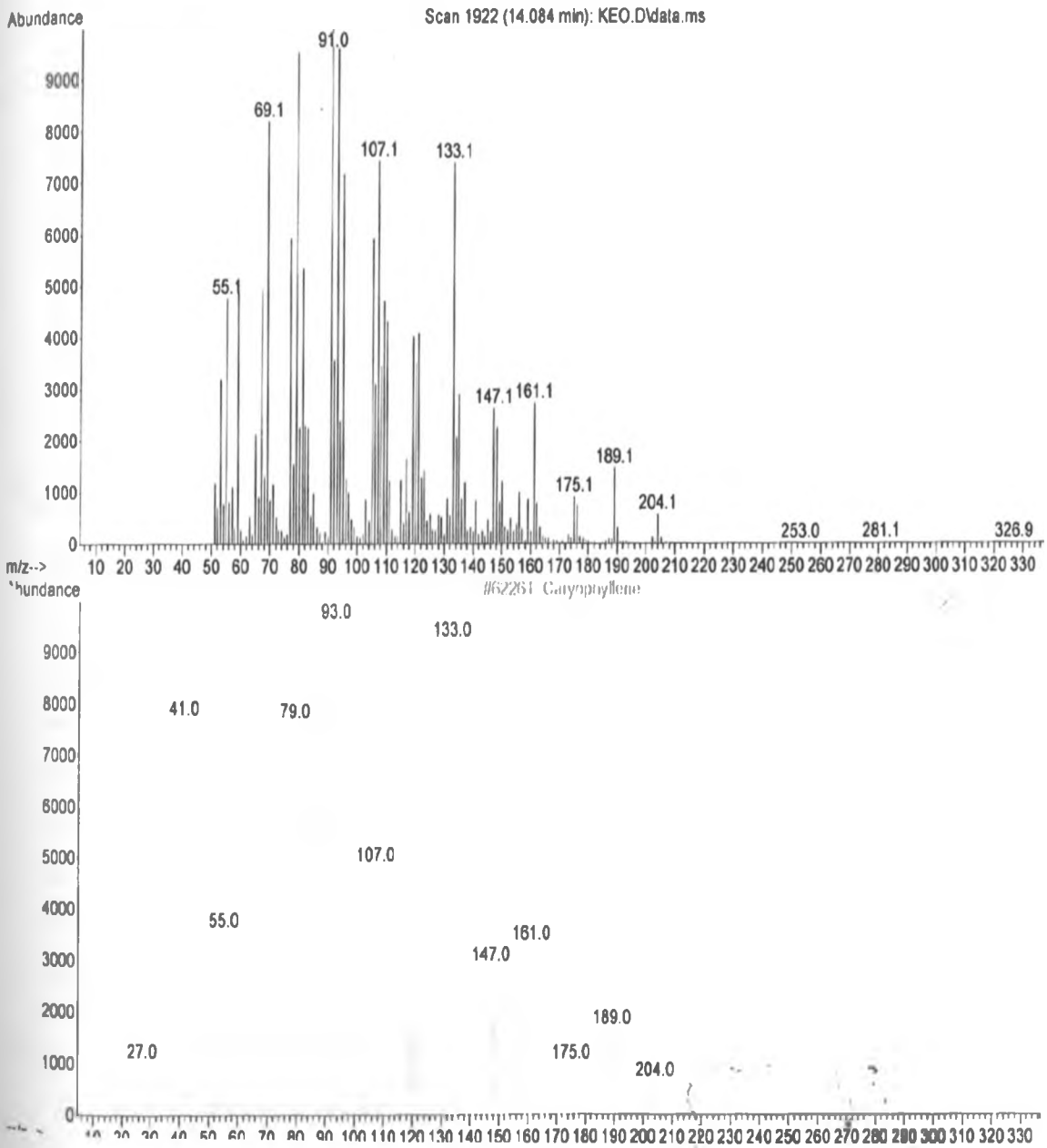
Appendix 20 Mass Spectrum for Compound 17

```
Library Searched : C:\Database\NIST08.L
Quality          : 64
ID               : Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-
```



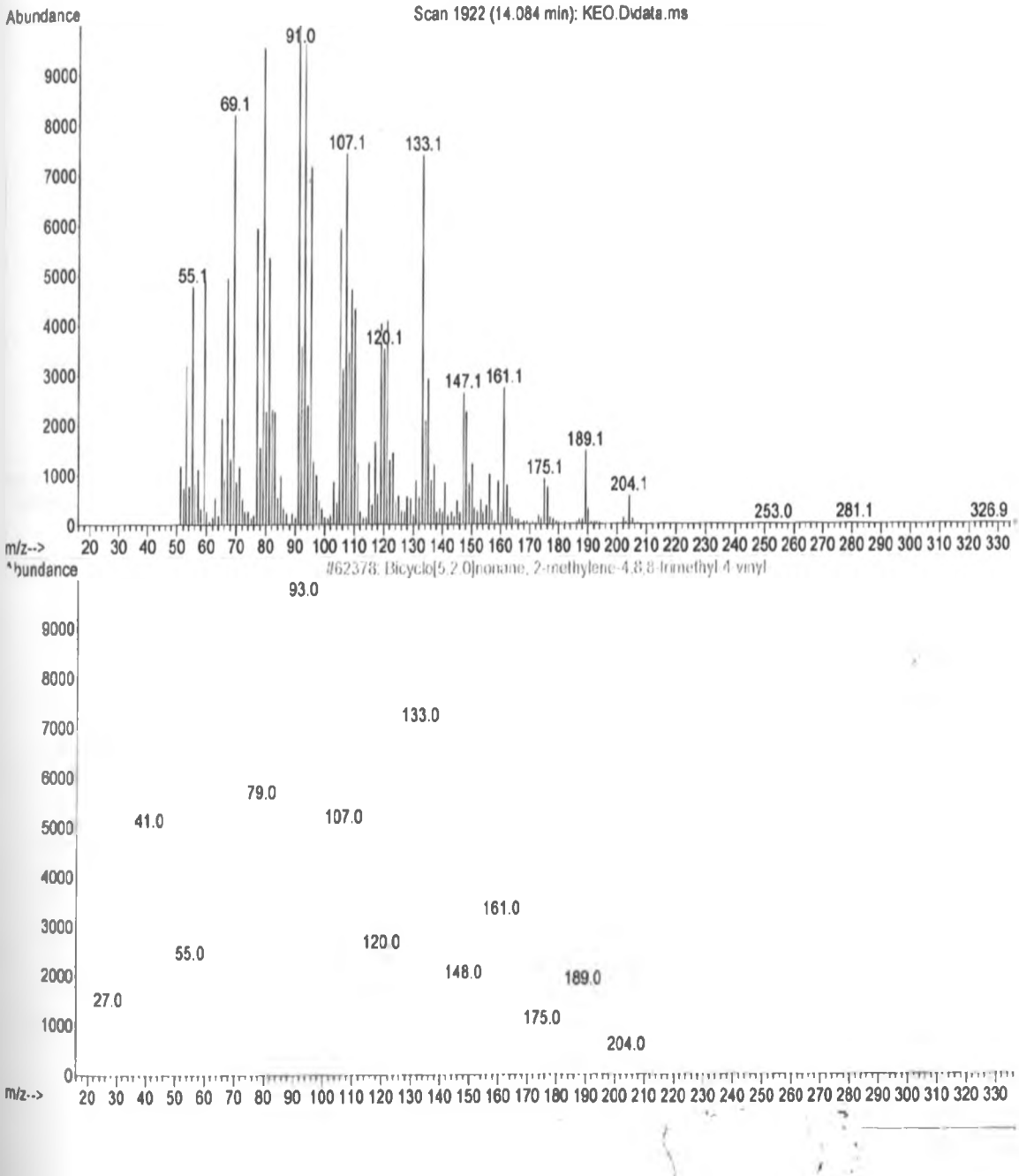
Appendix 21 Mass Spectrum for Compound 18

```
Library Searched : C:\Database\NIST08.L
Quality          : 96
ID               : Caryophyllene
```



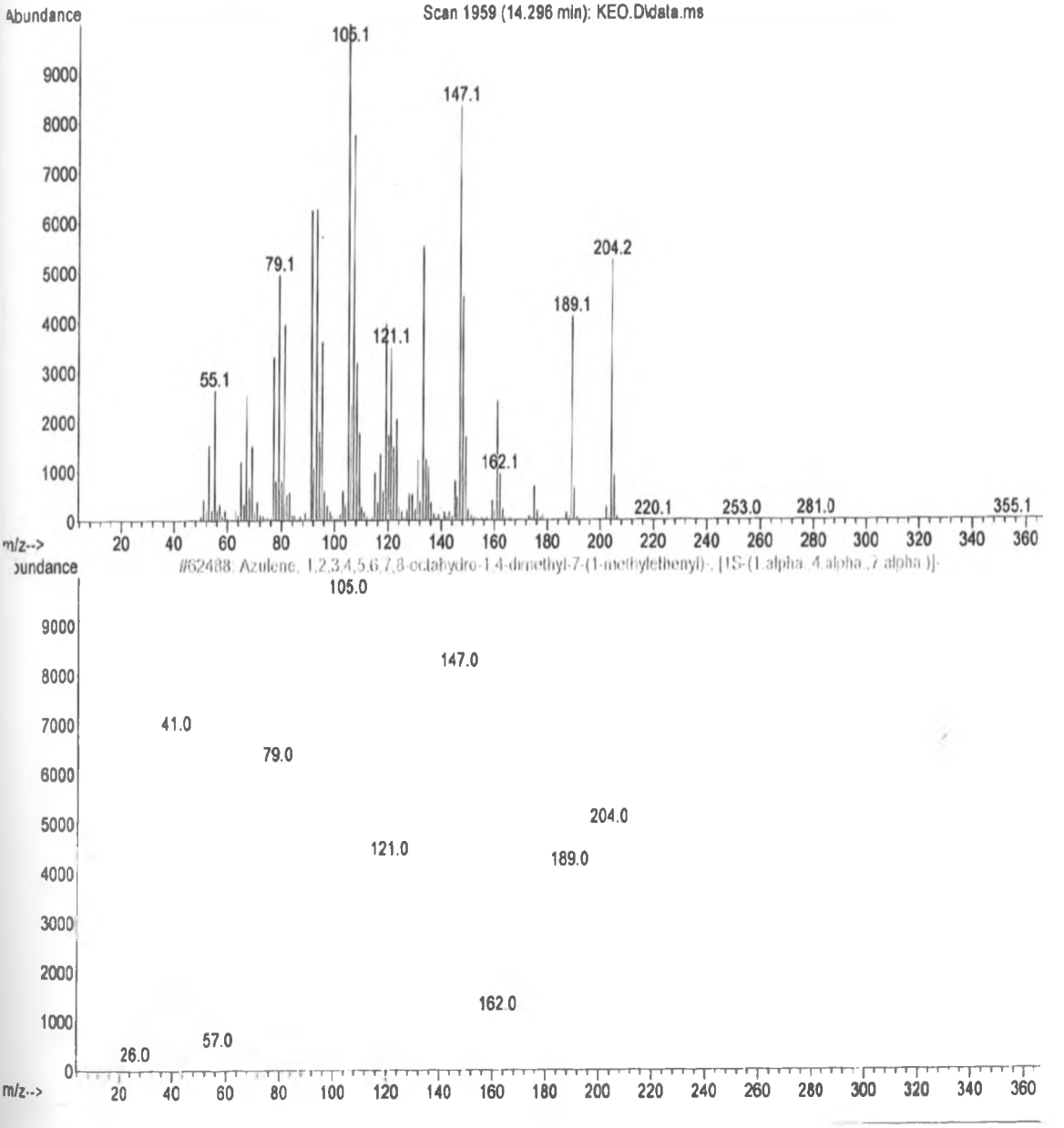
Appendix 22 Mass Spectrum for Compound 19

```
Library Searched : C:\Database\NIST08.L
Quality          : 94
ID               : Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-
```



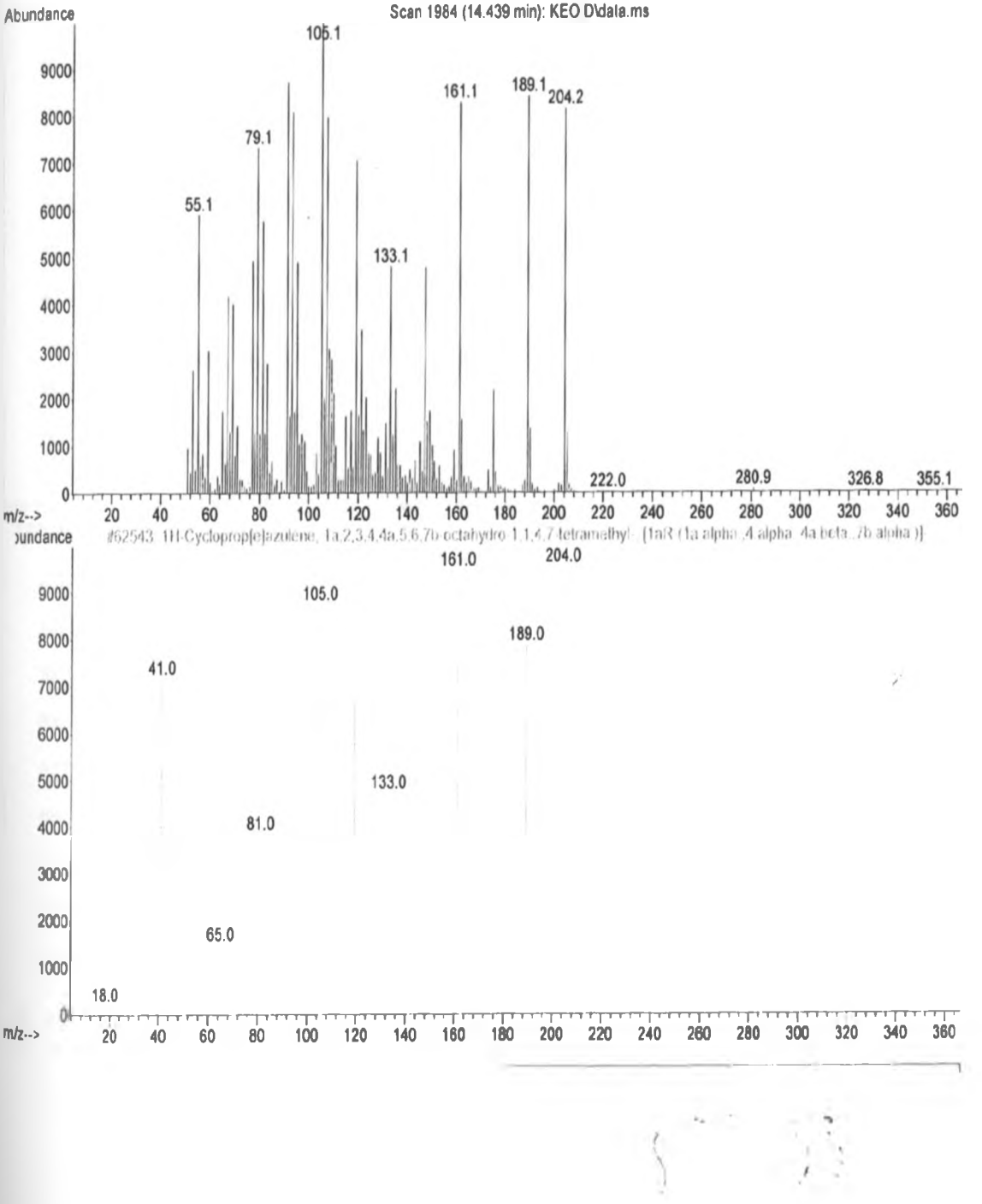
Appendix 23 Mass Spctrum for Compound 20

```
Library Searched : C:\Database\NIST08.L
Quality          : 99
ID               : Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,
                   [1S-(1.alpha.,4.alpha.,7.alpha.)]-
```



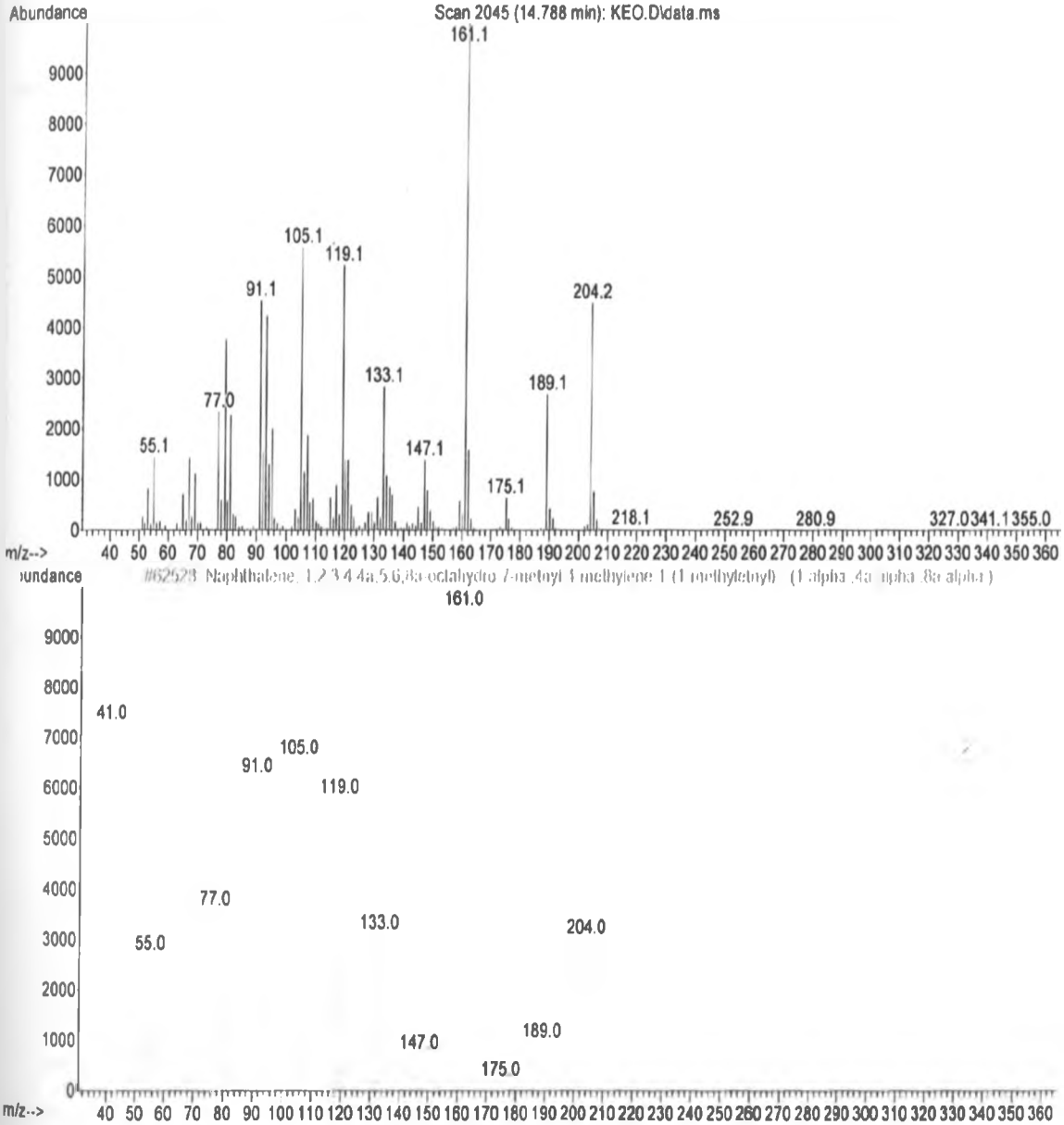
Appendix 24 Mass Spectrum for Compound 21

```
Library Searched : C:\Database\NIST08.L
Quality          : 95
ID               : 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramet
                   hyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-
```



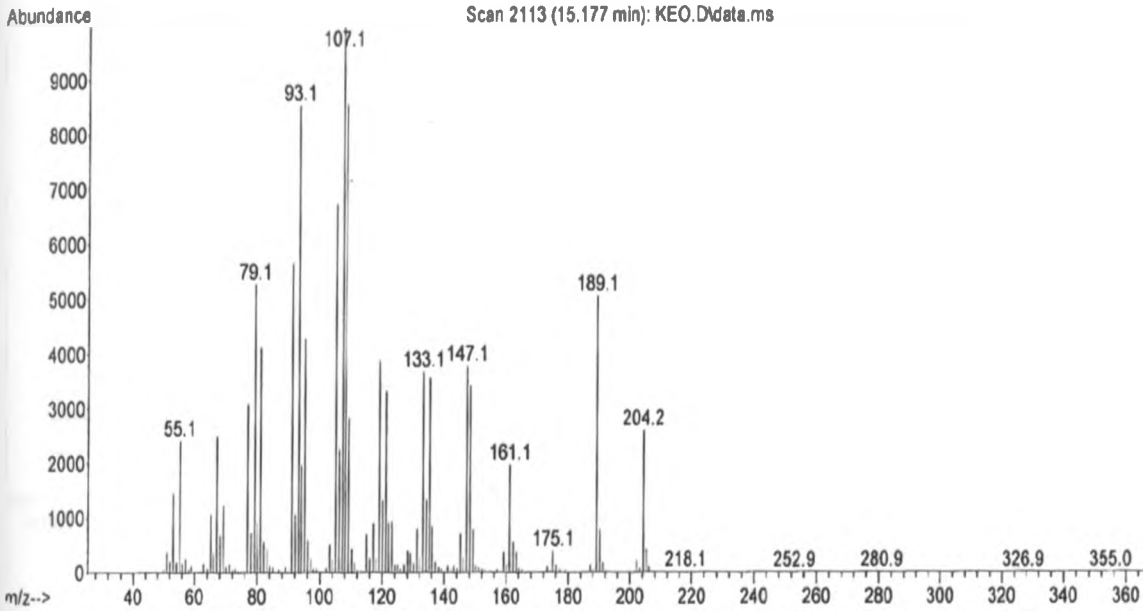
Appendix 25 Mass Spectrum for Compound 22

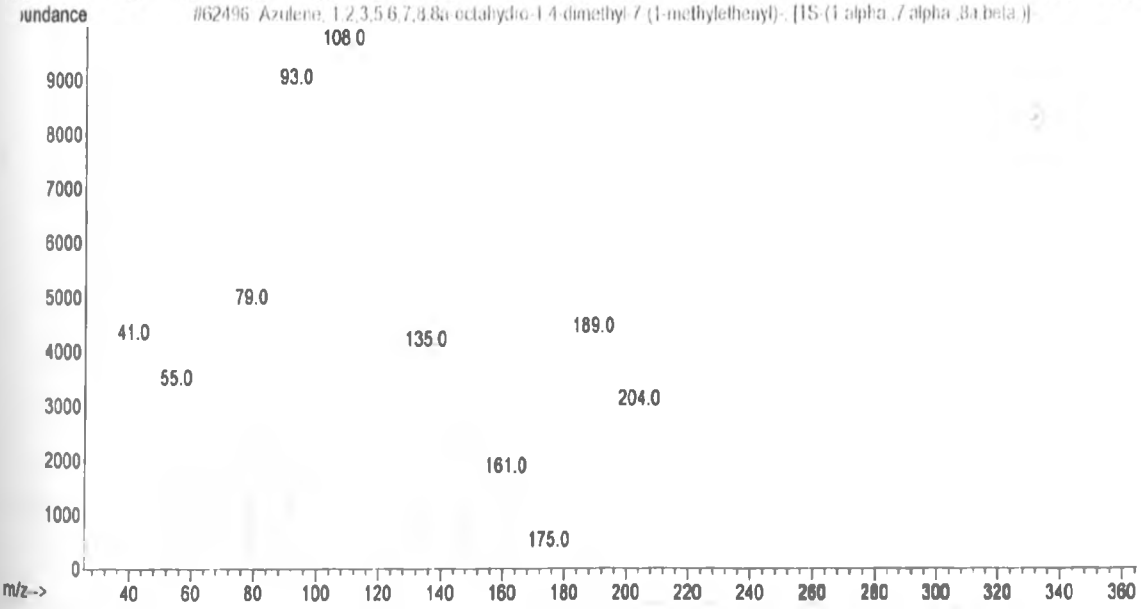
```
Library Searched : C:\Database\NIST08.L
Quality          : 98
ID               : Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-me
                   thylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-
```



Appendix 26 Mass Spectrum for Compound 23

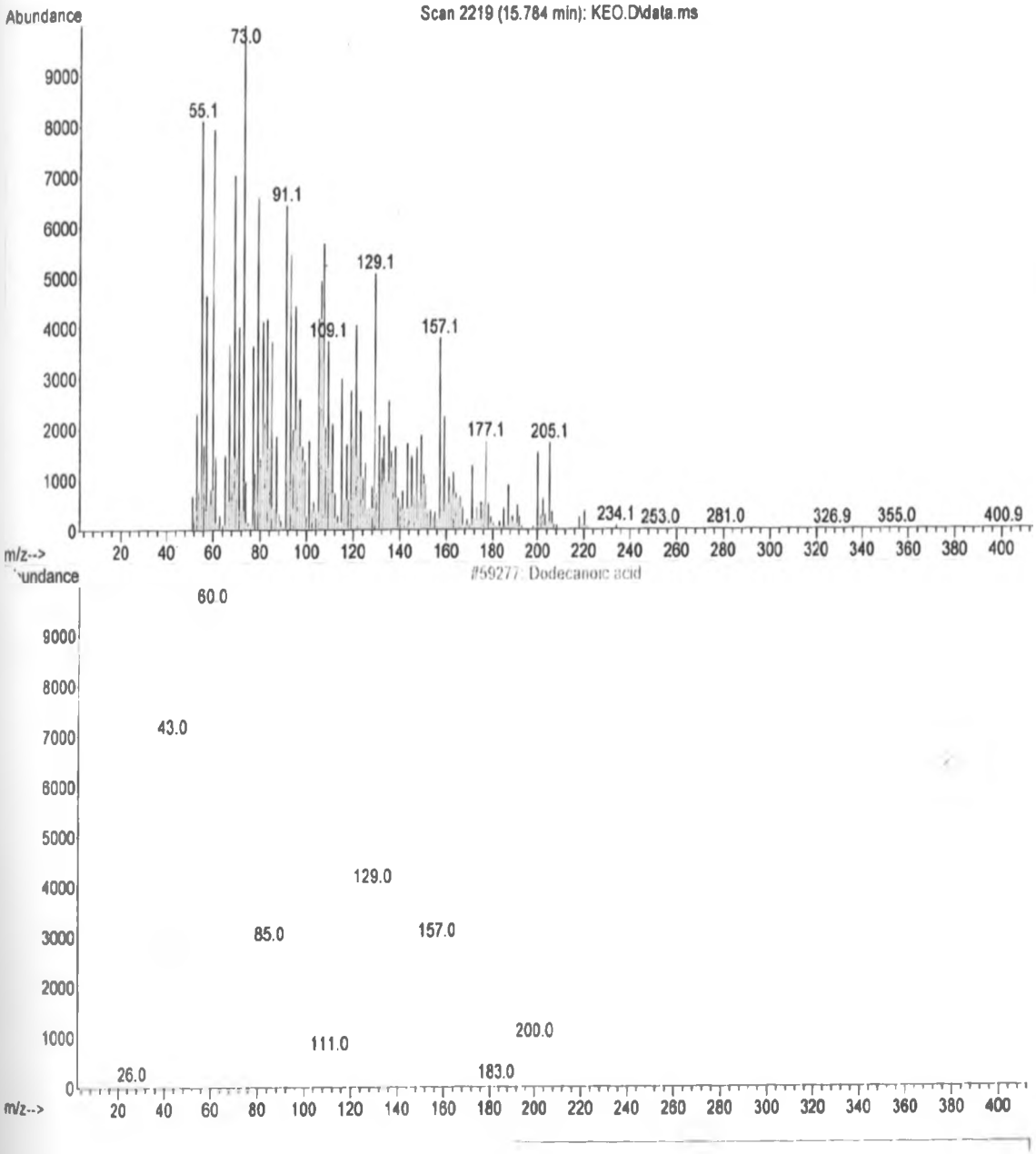
```
Library Searched : C:\Database\NIST08.L
Quality          : 99
ID               : Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-
                   , [1S-(1.alpha.,7.alpha.,8a.beta.)]-
```





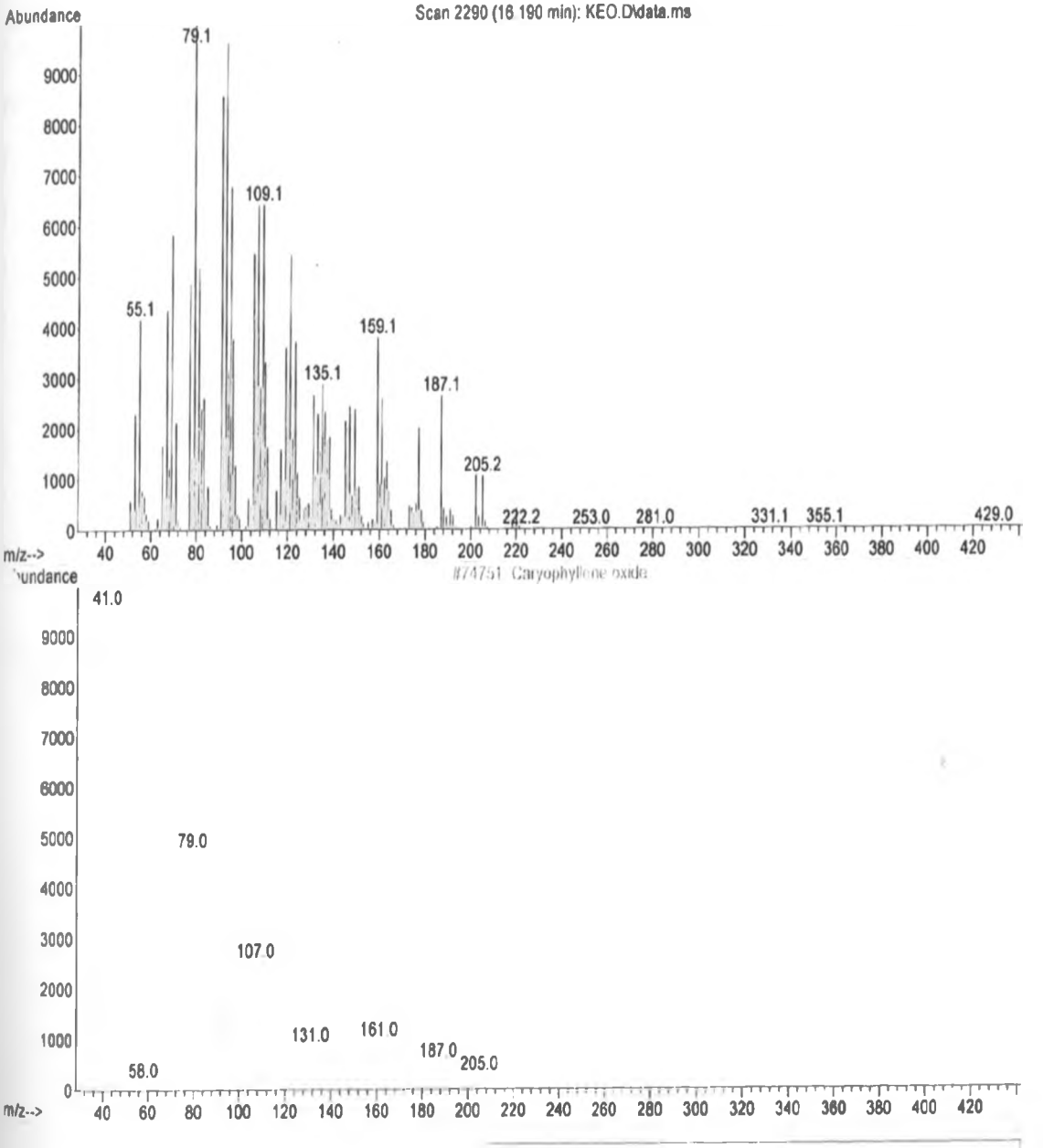
Appendix 27 Mass Spectrum for Compound 24

```
Library Searched : C:\Database\NIST08.L
Quality          : 93
ID               : Dodecanoic acid
```



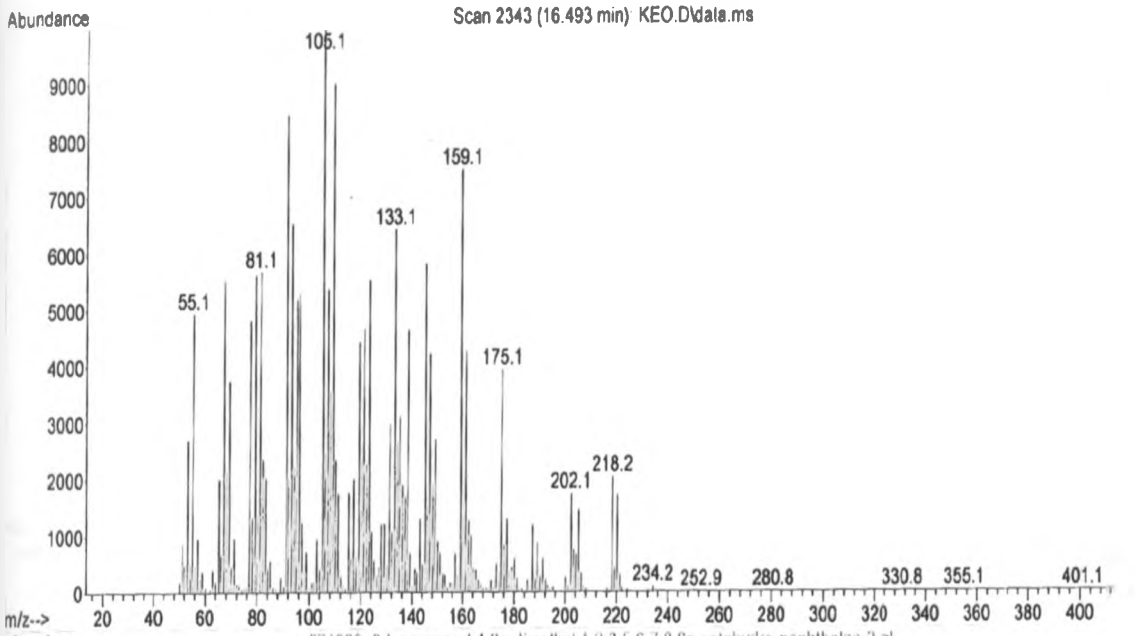
Appendix 28 Mass Spectrum for Compound 25

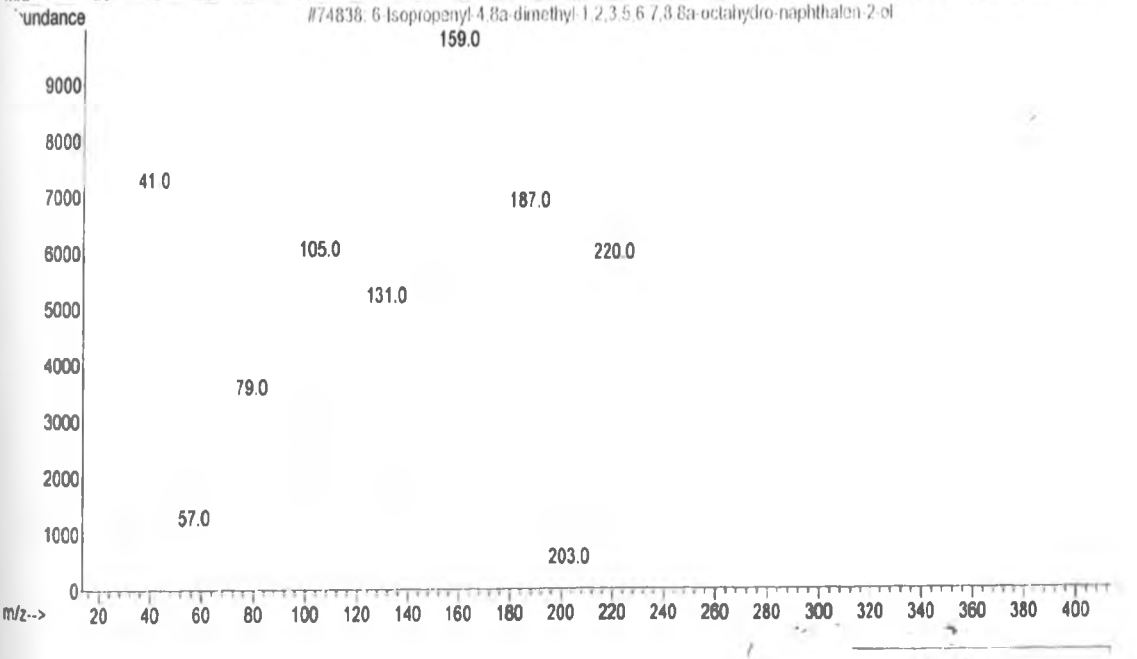
```
Library Searched : C:\Database\NIST08.L
Quality          : 92
ID               : Caryophyllene oxide
```



Appendix 29 Mass Spectrum for Compound 26

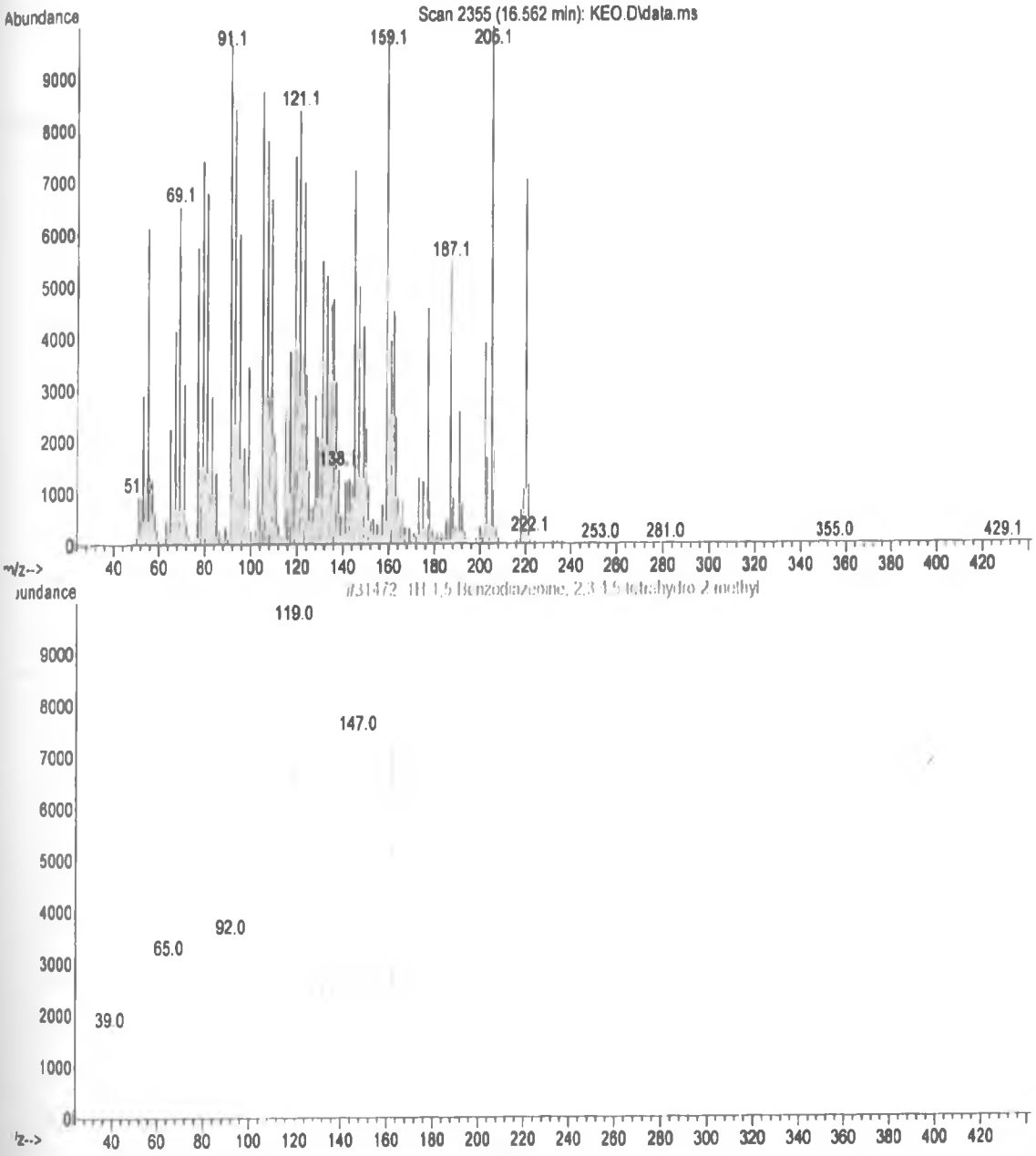
```
Library Searched : C:\Database\NIST08.L
Quality          : 59
ID               : 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-o
```





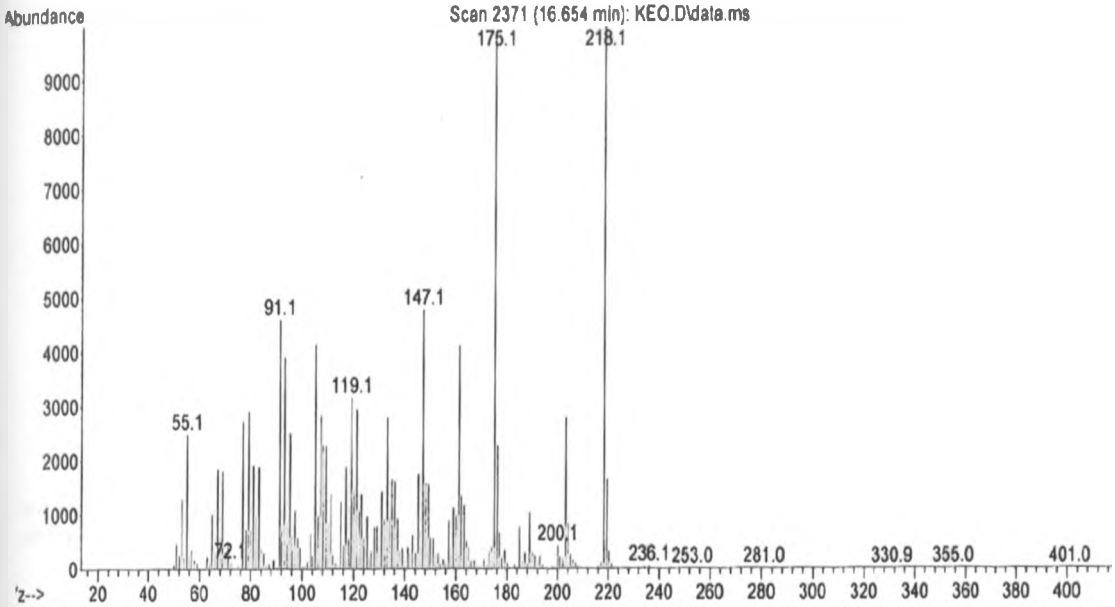
Appendix 30 Mass Spectrum for Compound 27

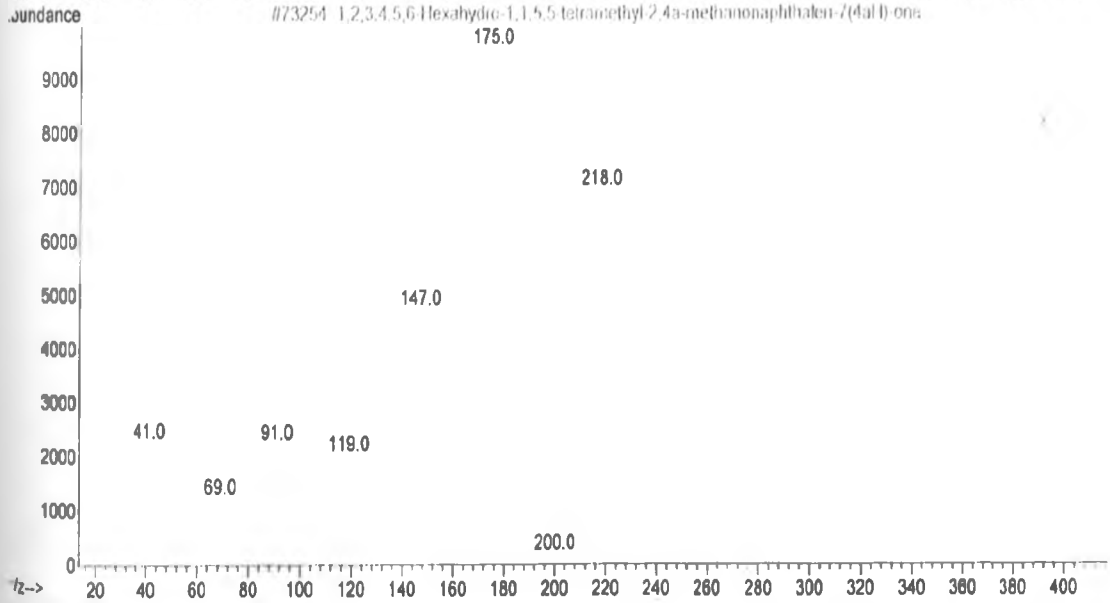
```
Library Searched : C:\Database\NIST08.L
Quality          : 56
ID               : 1H-1,5-Benzodiazepine, 2,3,4,5-tetrahydro-2-methyl-
```



Appendix 31 Mass Spectrum for Compound 28

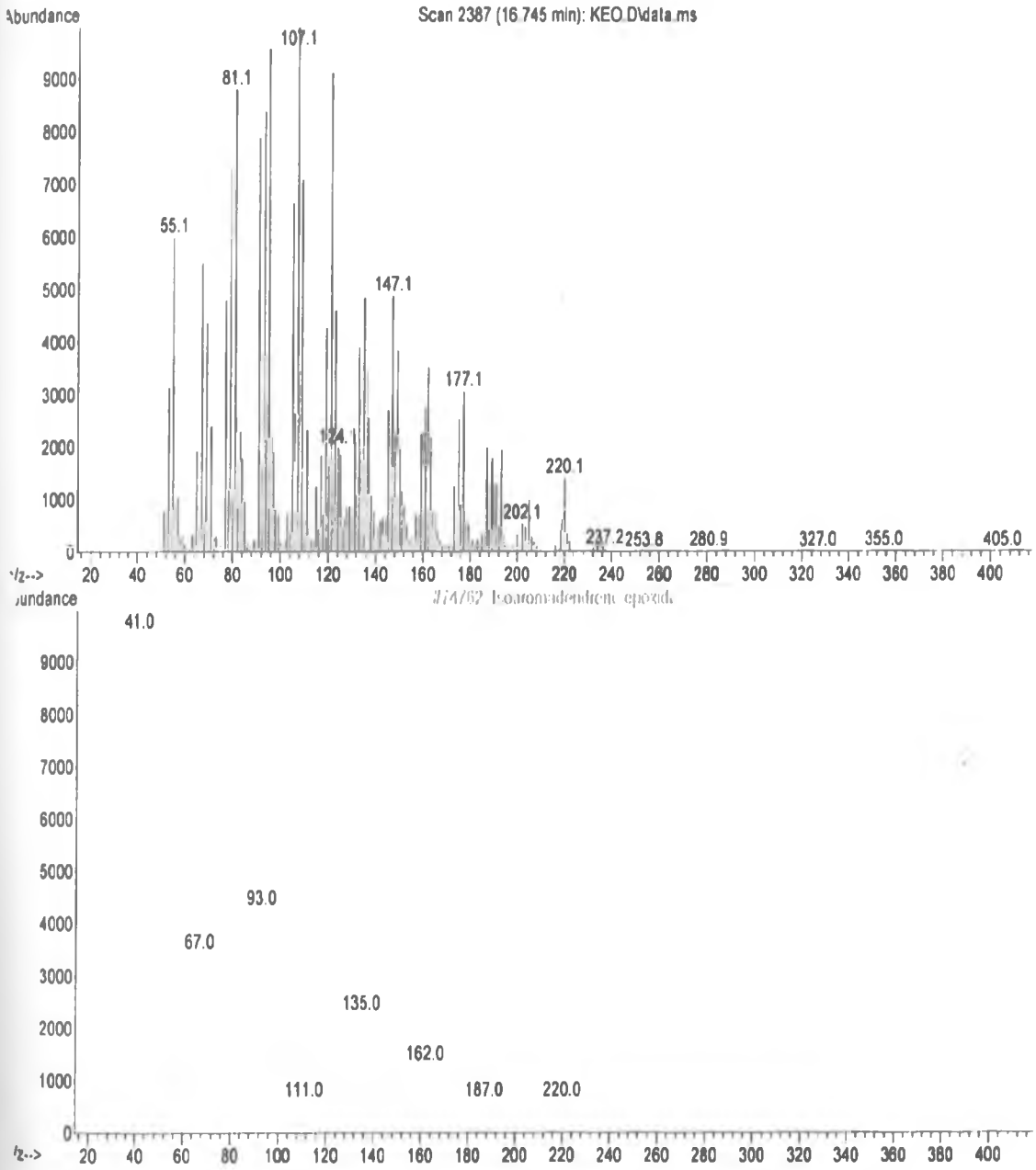
```
Library Searched : C:\Database\NIST08.L
Quality          : 90
ID               : 1,2,3,4,5,6-Hexahydro-1,1,5,5-tetramethyl-2,4a-methanonaphthalen-7(4a
                   H)-one
```





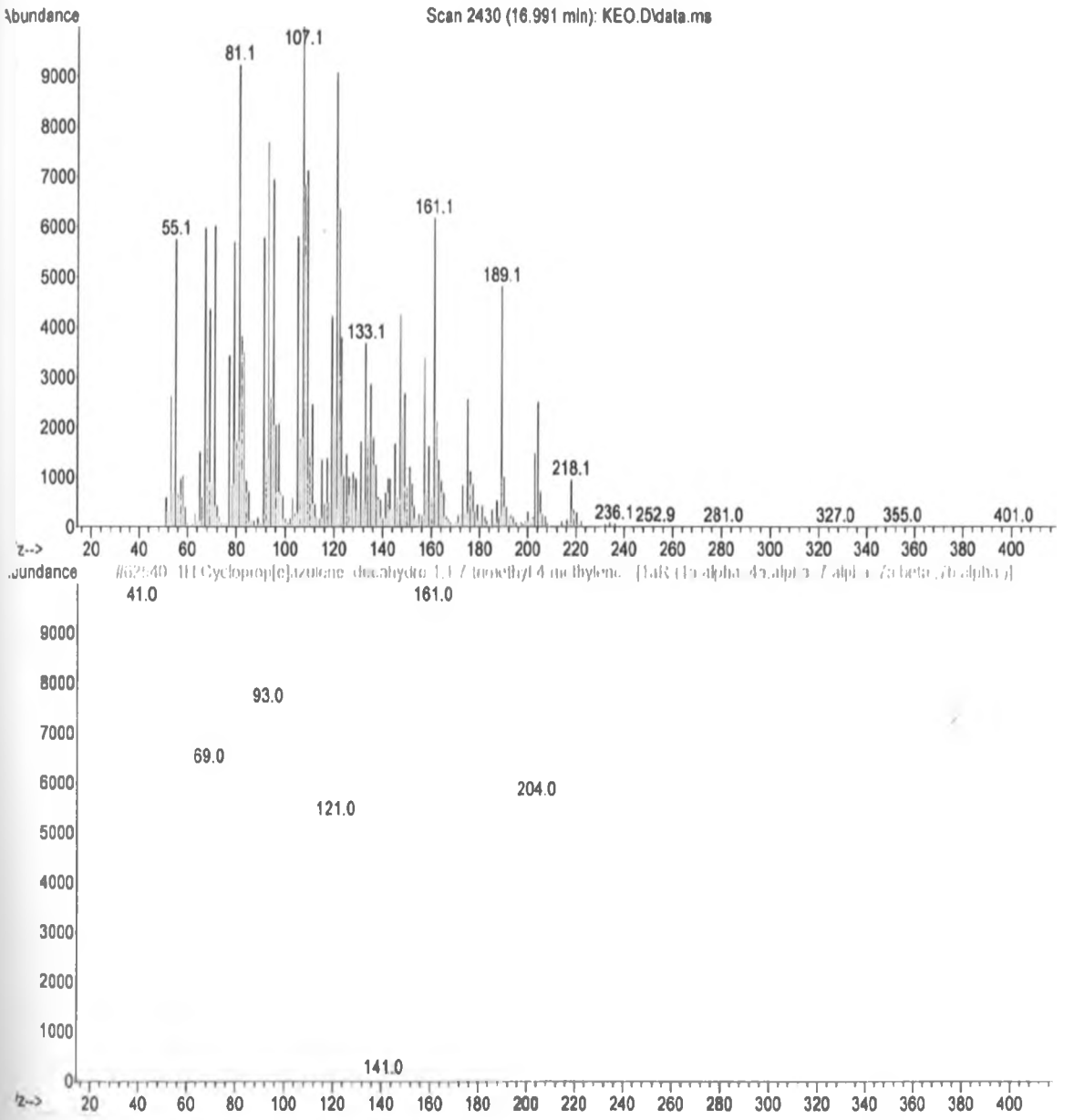
Appendix 32 Mass Spectrum for Compound 29

```
Library Searched : C:\Database\NIST08.L
Quality          : 95
ID               : Isoaromadendrene epoxide
```



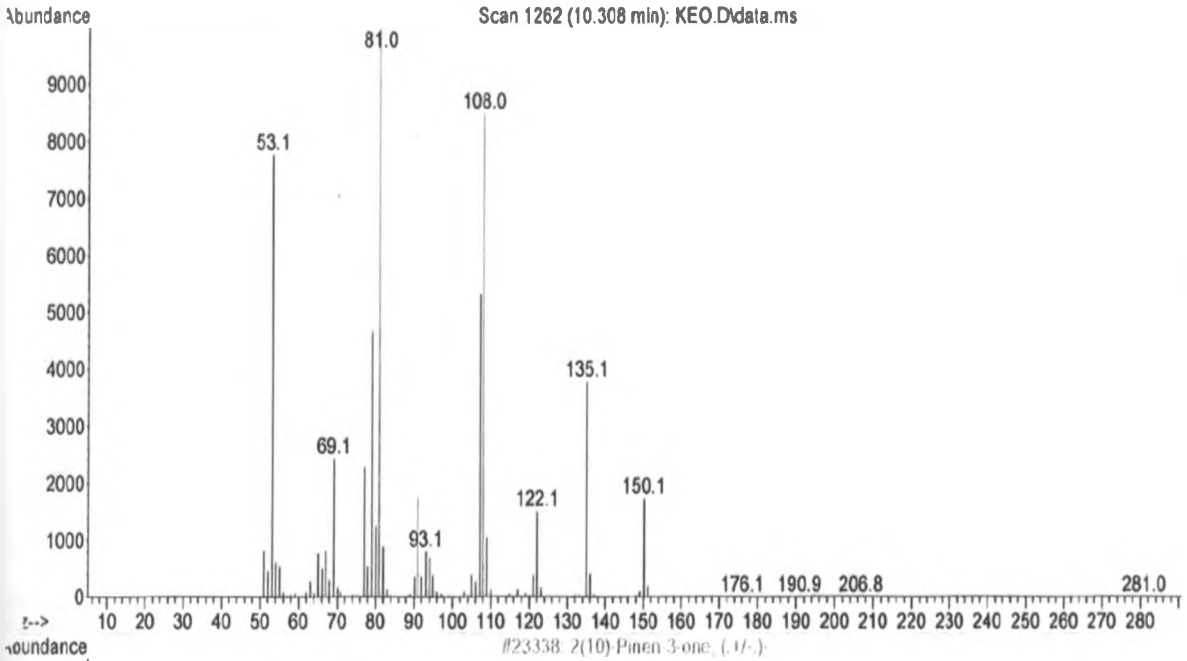
Appendix 33 Mass Spectrum for Compound 30

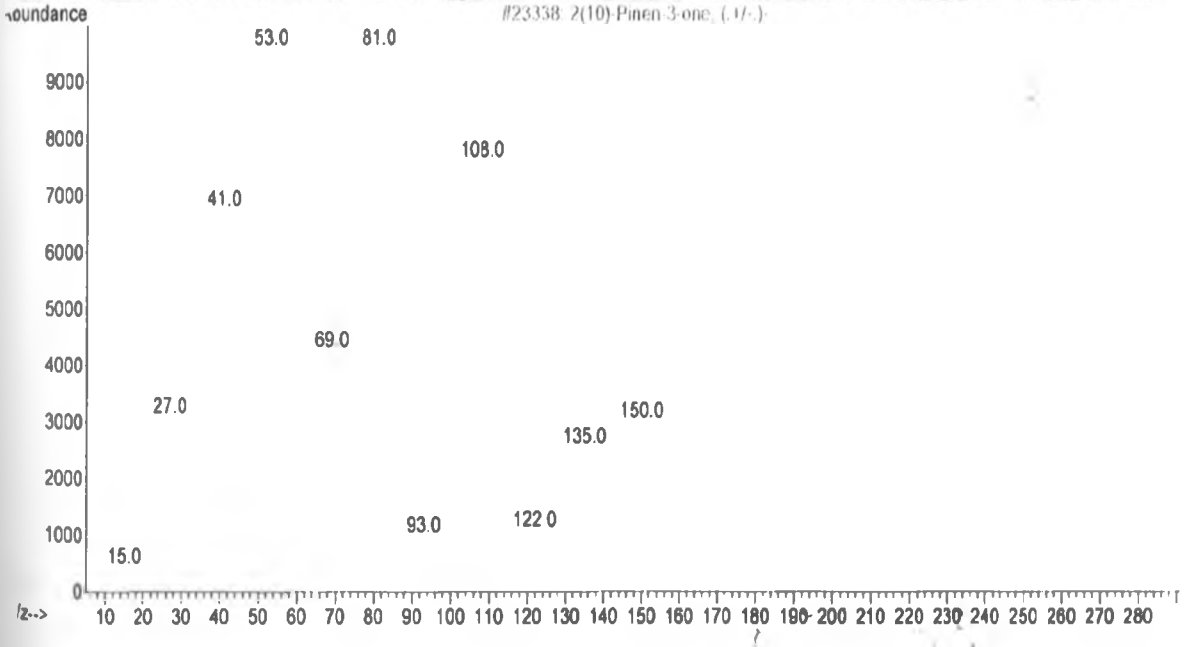
```
Library Searched : C:\Database\NIST08.L
Quality          : 94
ID               : 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-
                   (1a.alpha.,4a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-
```



Appendix 34 Mass Spectrum for Compound 31

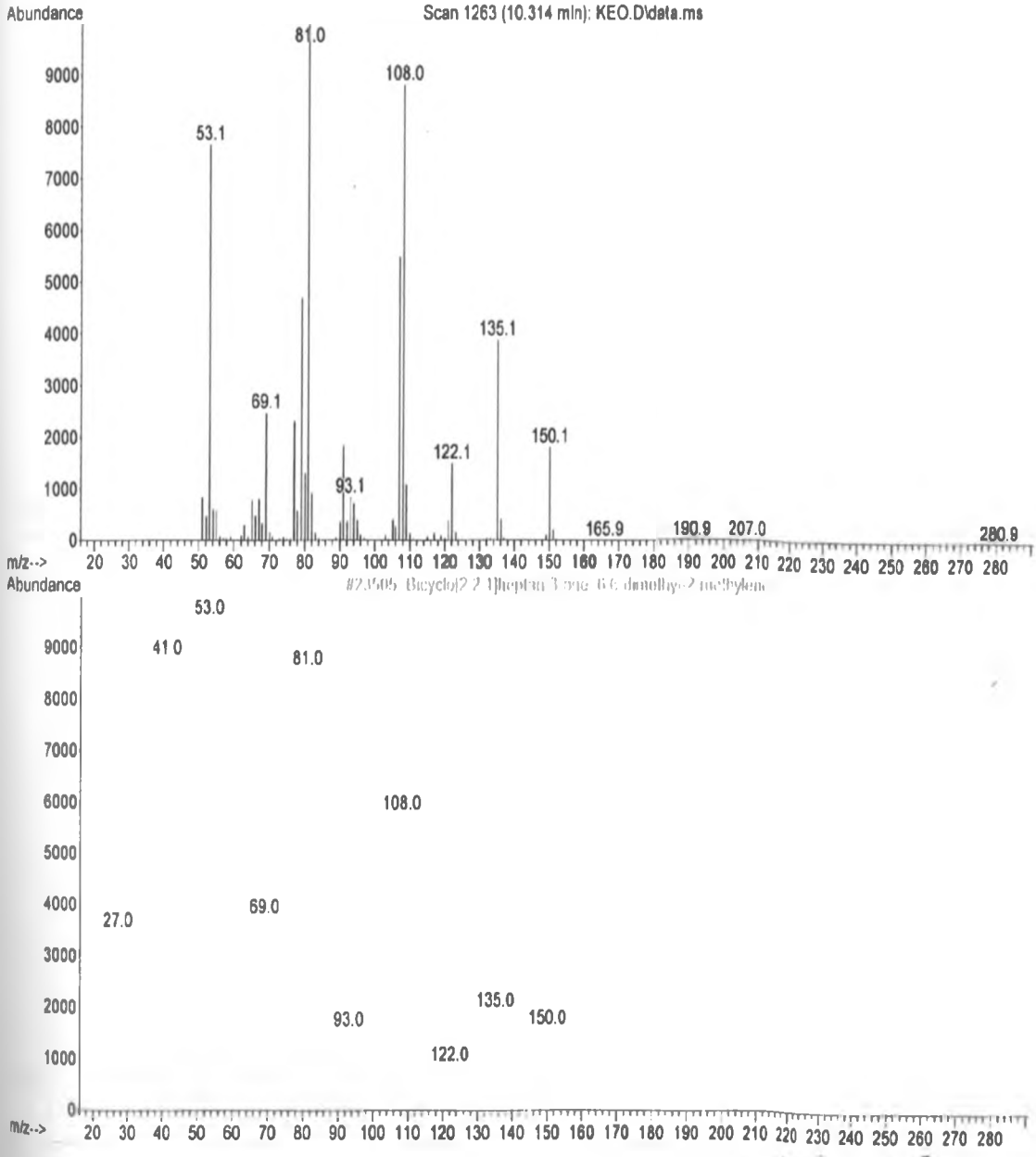
Library Searched : C:\Database\NIST08.L
Quality : 58
ID : 2(10)-Pinen-3-one, (.+/-.)-





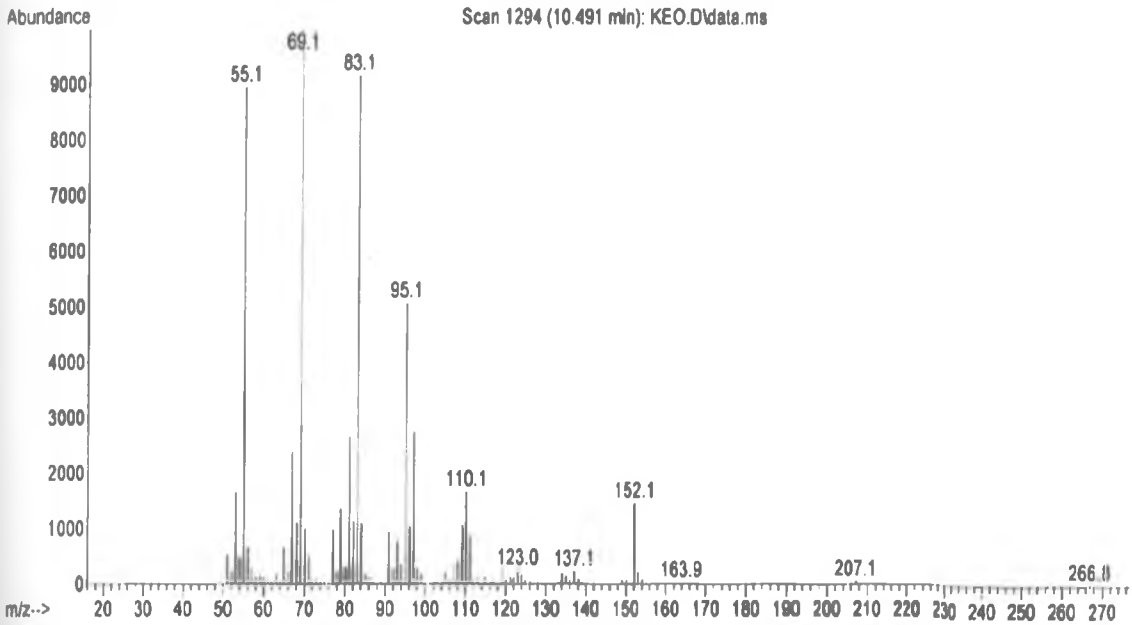
Appendix 35 Mass Spectrum for Compound 32

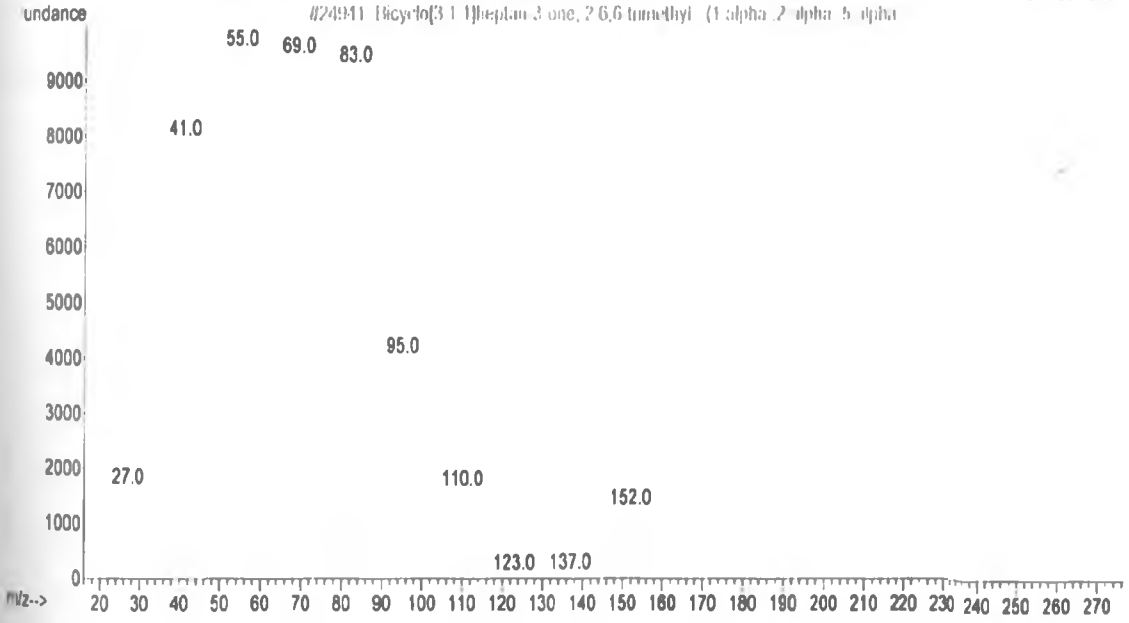
```
Library Searched : C:\Database\NIST08.L
Quality          : 78
ID               : Bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene-
```



Appendix 36 Mass Spectrum for Compound 33

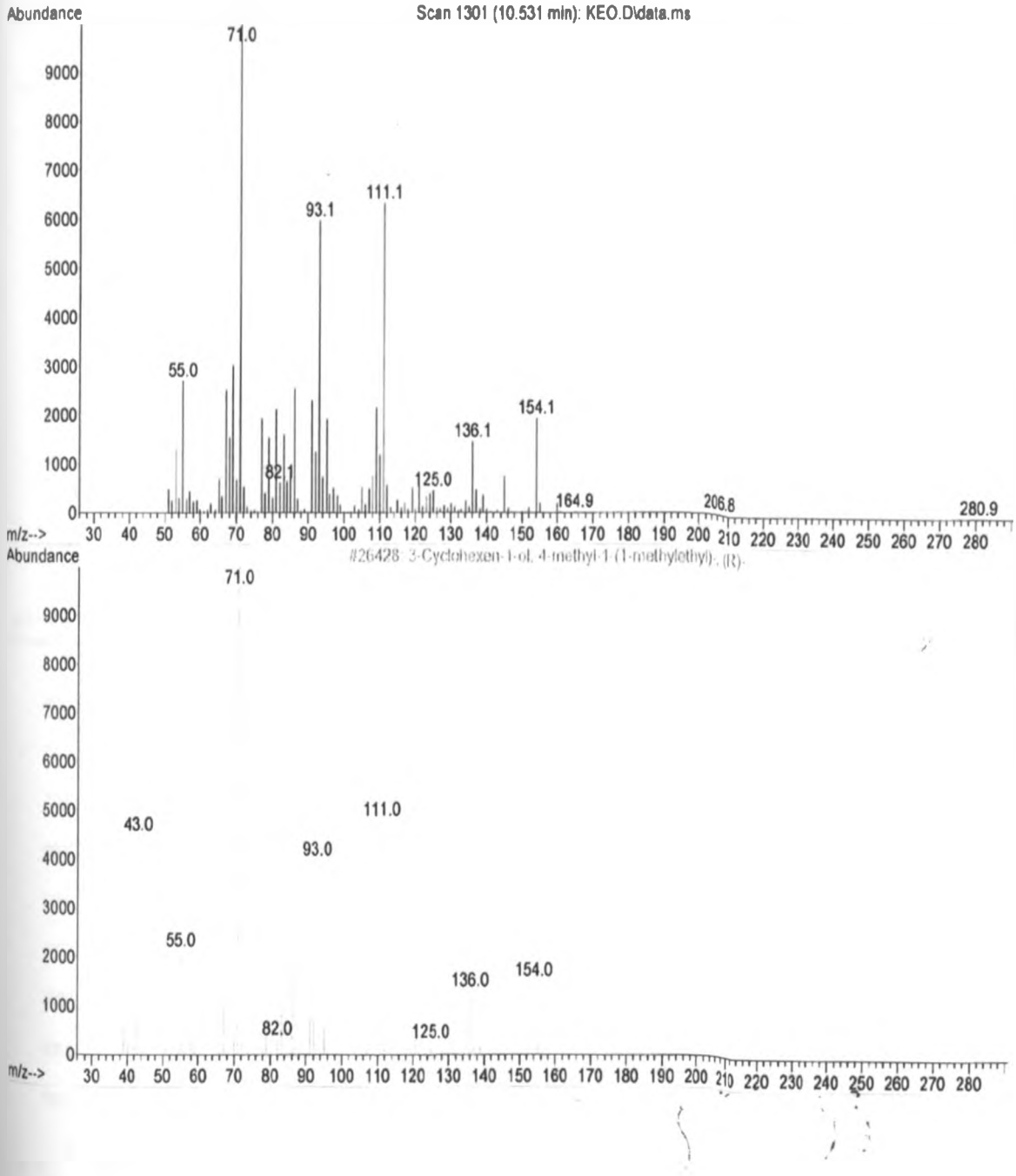
```
Library Searched : C:\Database\NIST08.L
Quality          : 96
ID               : Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.alpha.,5.al
                   pha.)-
```





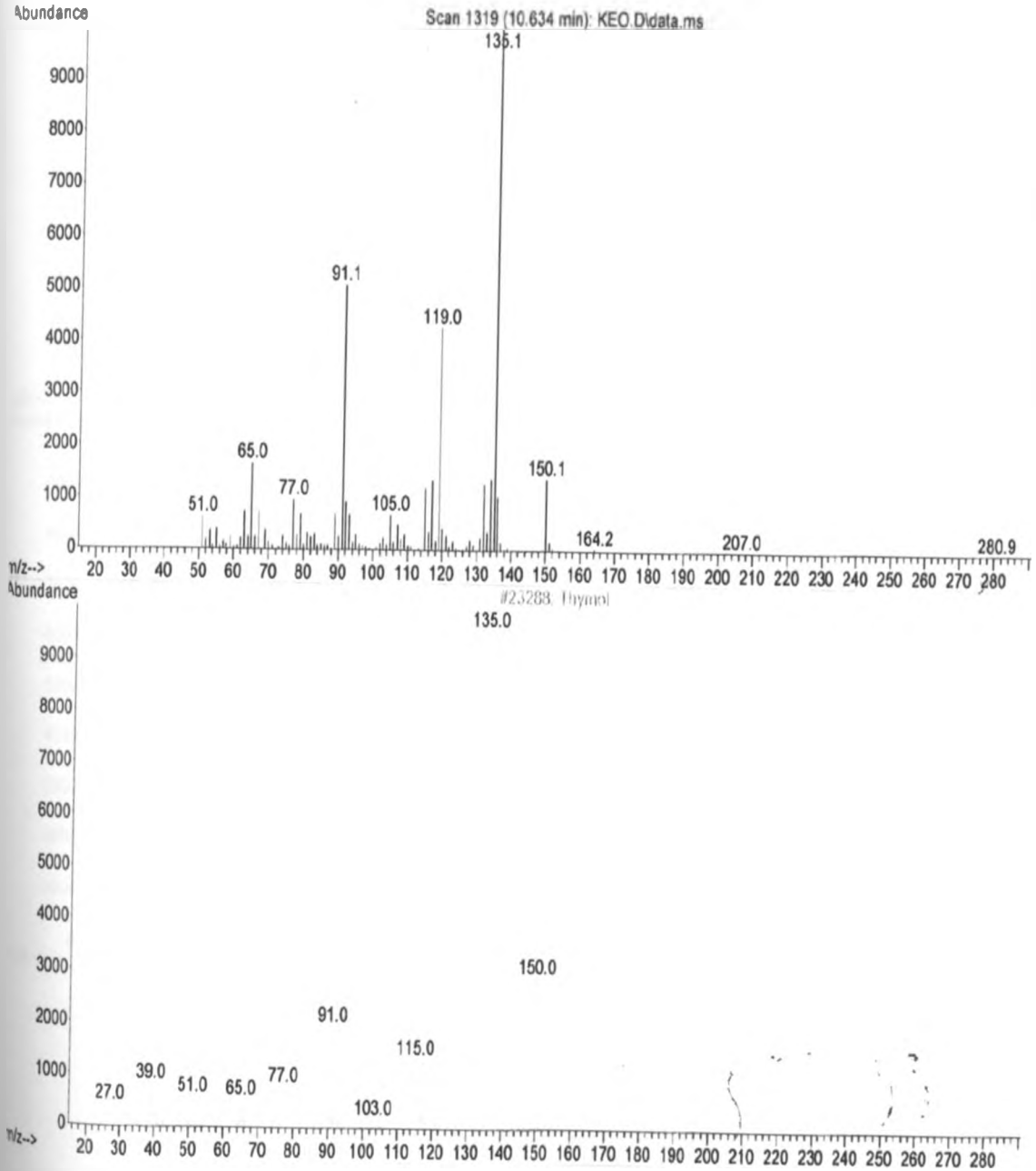
Appendix 37 Mass Spectrum for Compound 34

```
Library Searched : C:\Database\NIST08.L
Quality          : 94
ID               : 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-
```



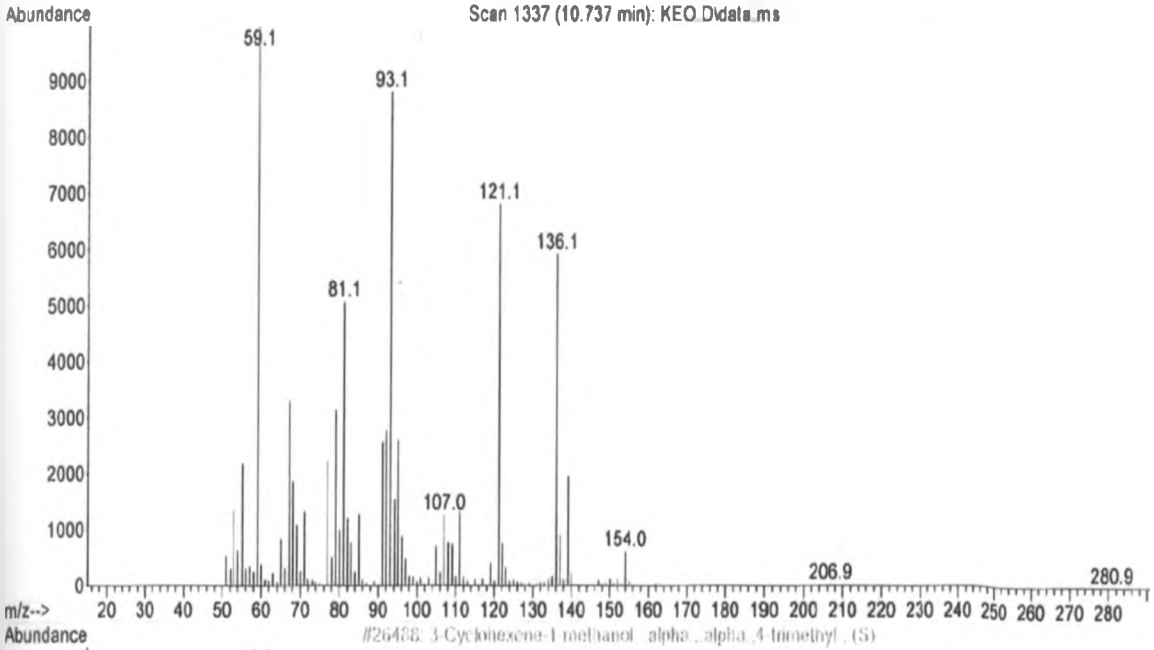
Appendix 38 Mass Spectrum for Compound 35

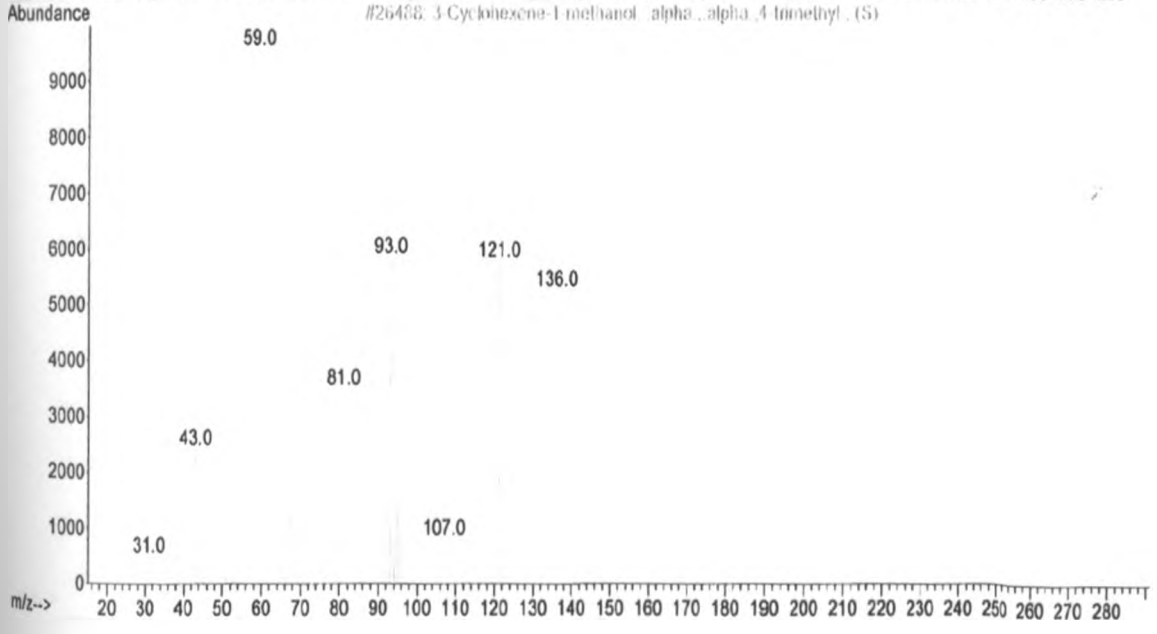
```
Library Searched : C:\Database\NIST08.L
Quality          : 64
ID               : Thymol
```



Appendix 39 Mass Spectrum for Compound 36

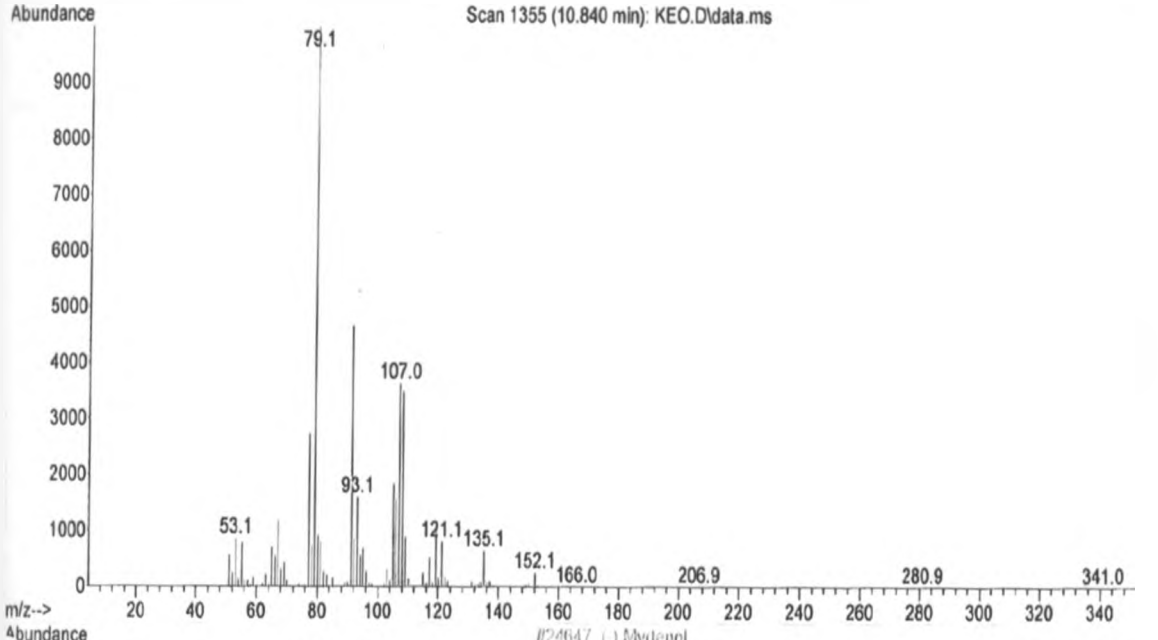
```
Library Searched : C:\Database\NIST08.L
Quality          : 86
ID               : 3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)-
```

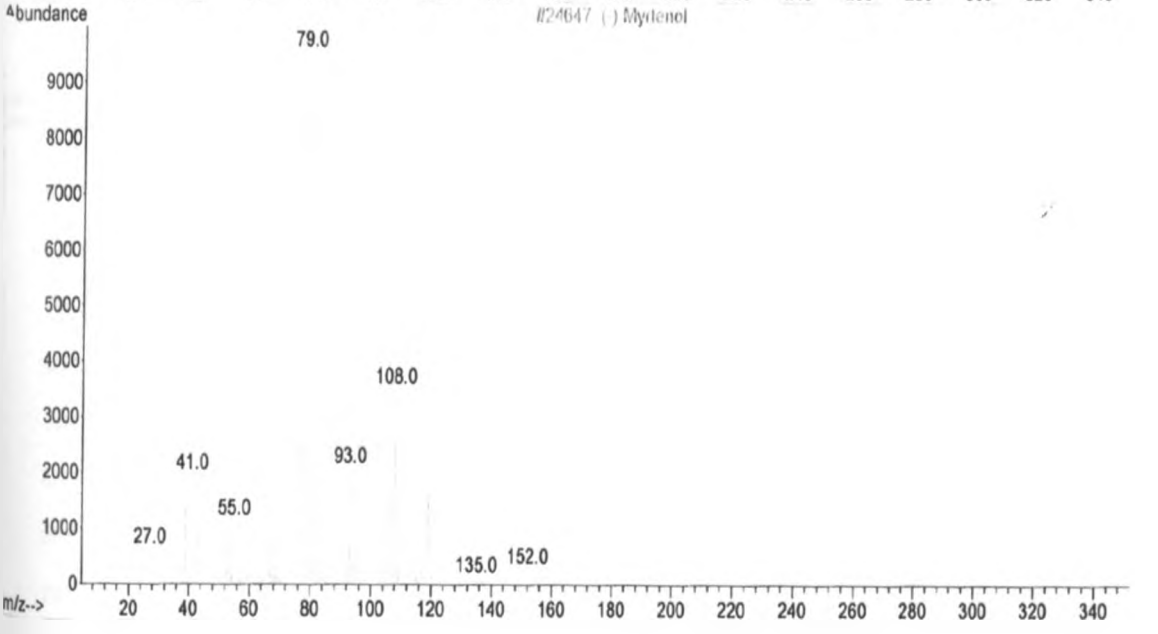




Appendix 40 Mass Spectrum for Compound 37

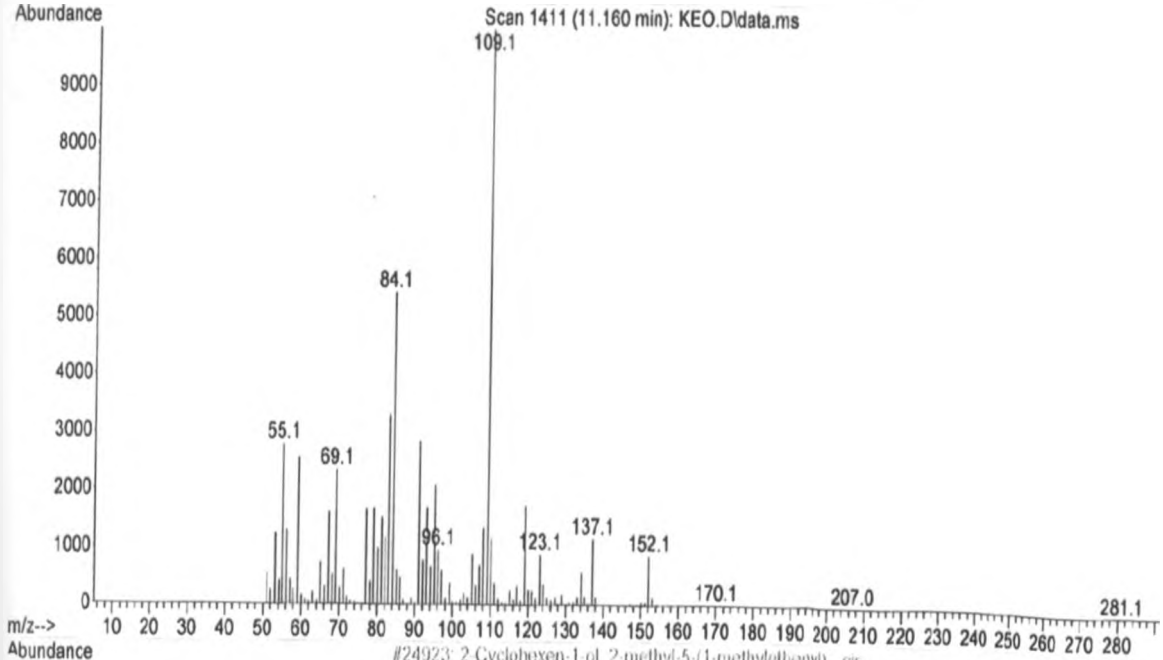
```
Library Searched : C:\Database\NIST08.L
Quality          : 81
ID               : (-)-Myrtenol
```

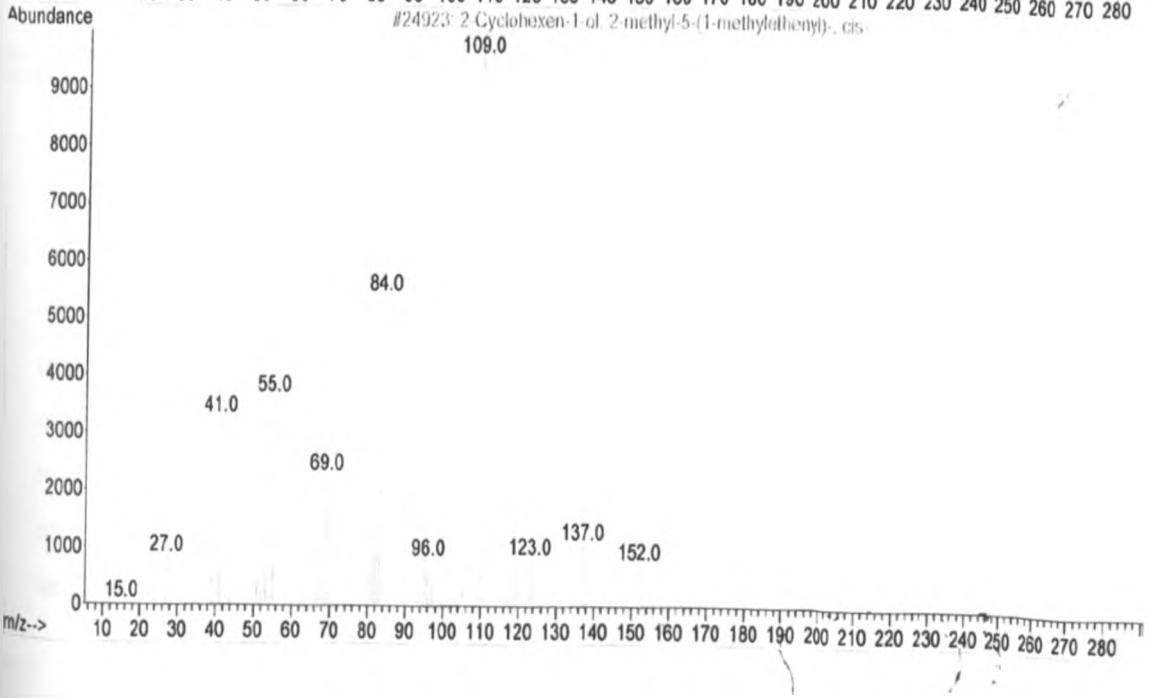




Appendix 41 Mass Spectrum for Compound 38

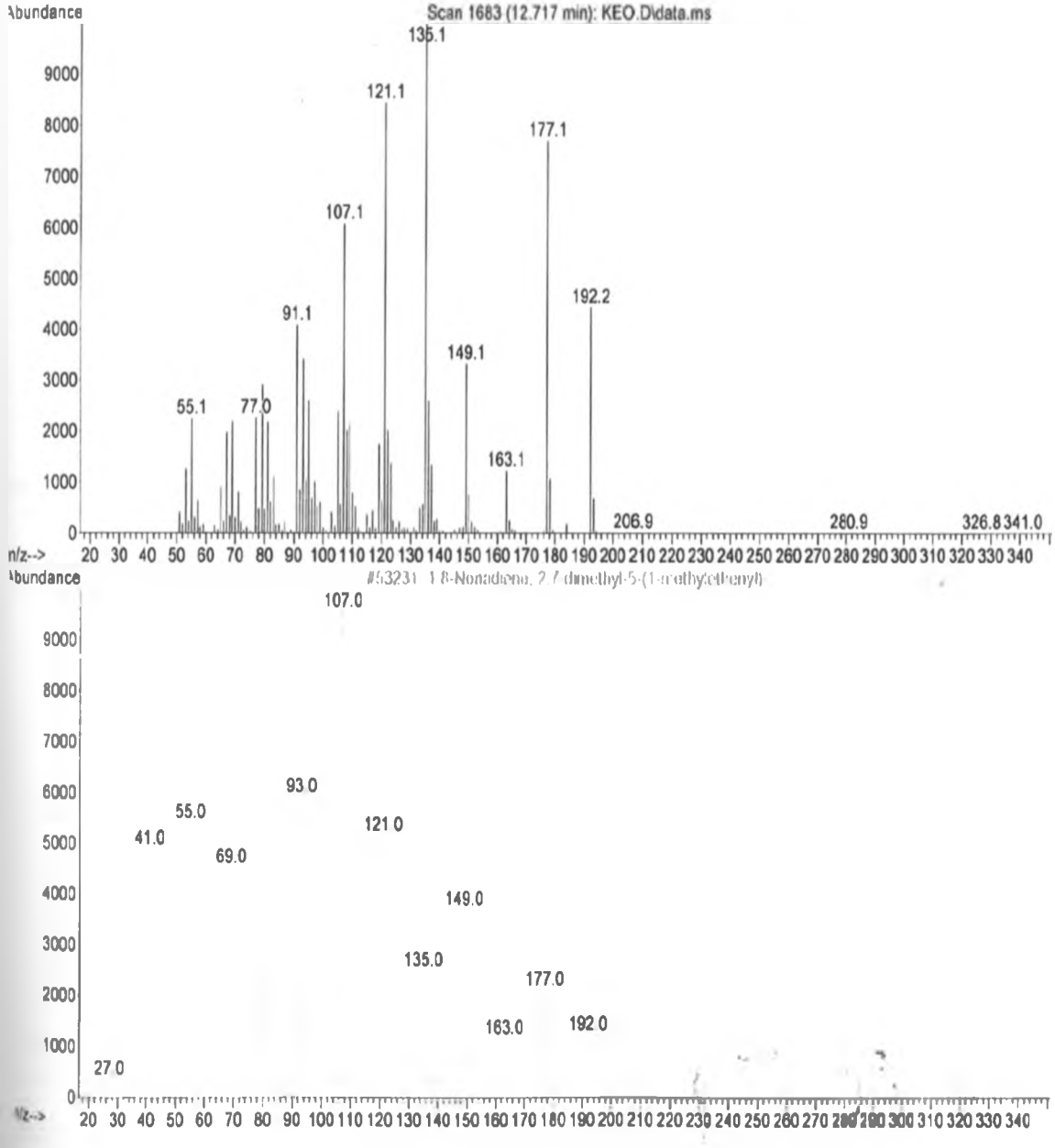
```
Library Searched : C:\Database\NIST08.L
Quality          : 98
ID               : 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-
```





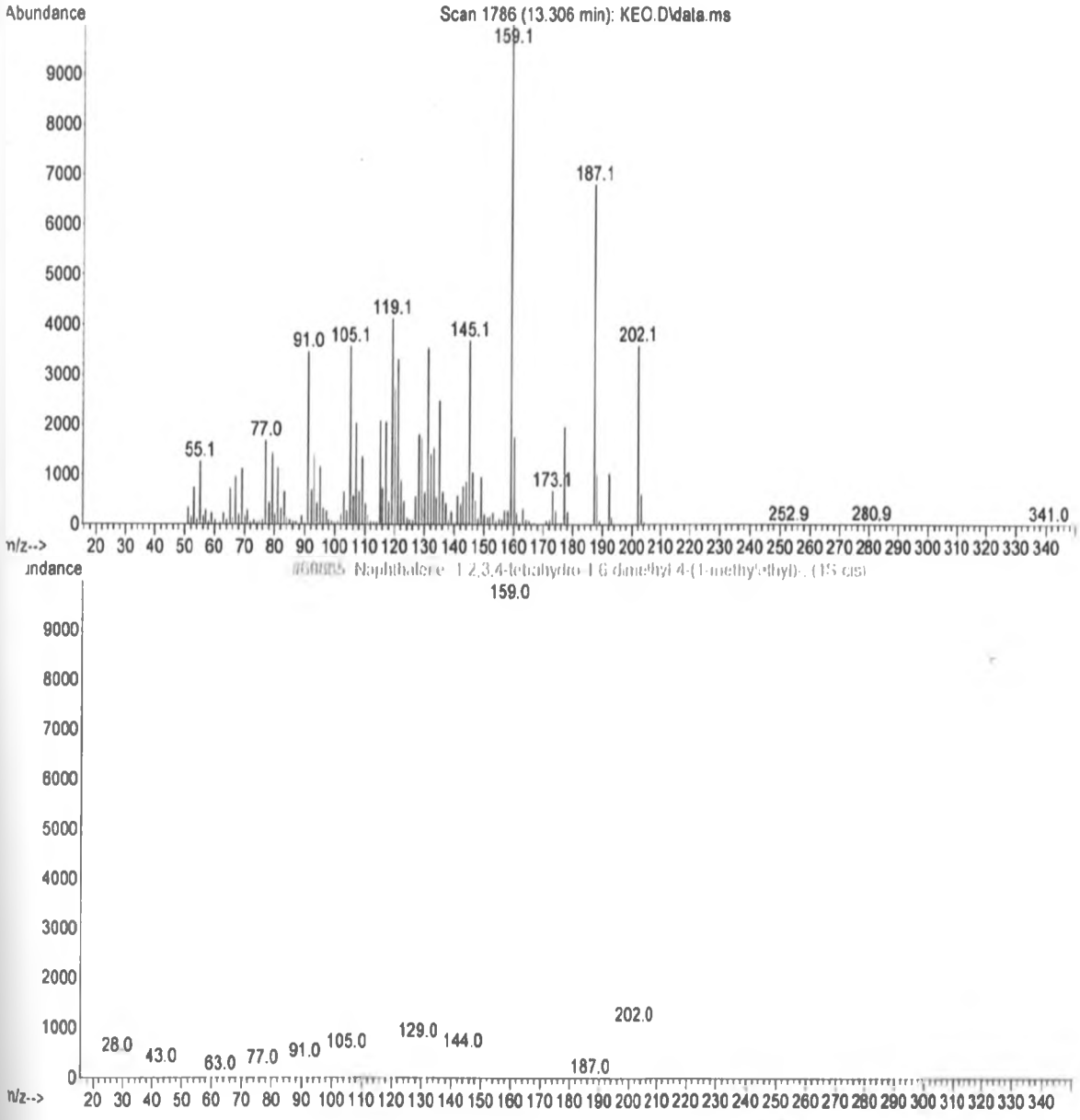
Appendix 42 Mass Spectrum for Compound 39

```
Library Searched : C:\Database\NIST08.L
Quality          : 70
ID               : 1,8-Nonadiene, 2,7-dimethyl-5-(1-methylethenyl)-
```



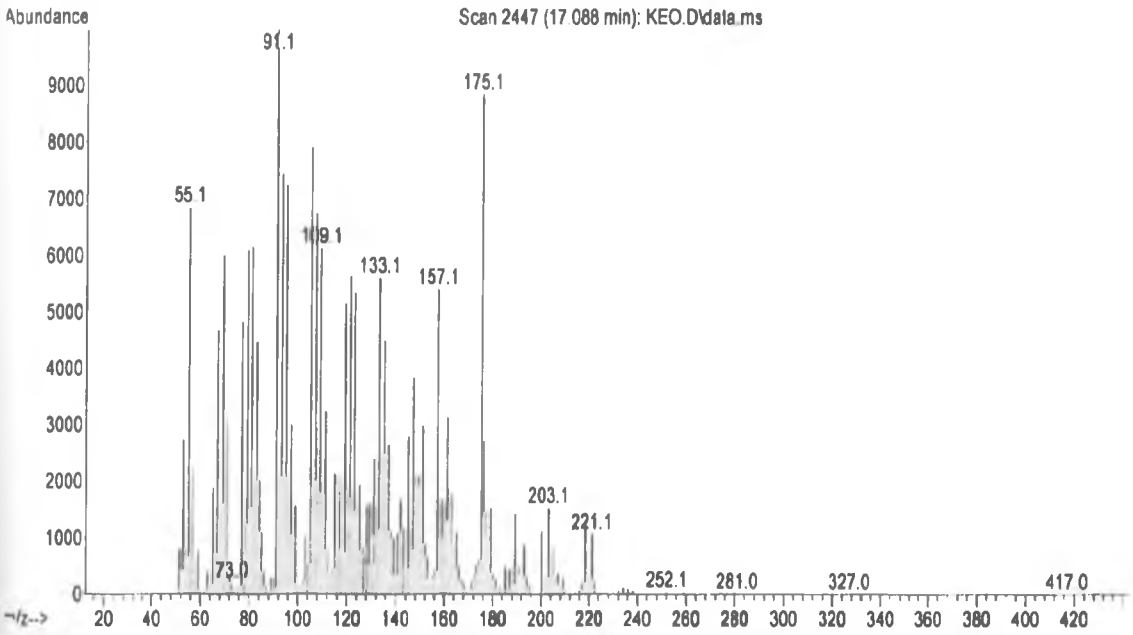
Appendix 43 Mass Spectrum for Compound 40

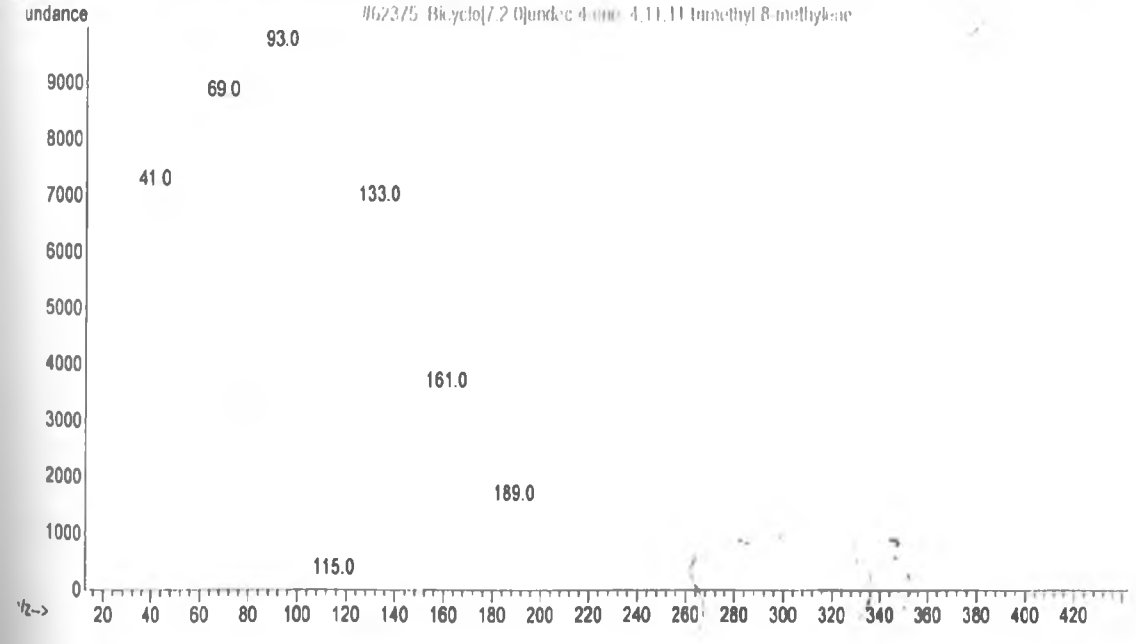
```
Library Searched : C:\Database\NIST08.L
Quality          : 93
ID               : Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-
                   cis)-
```



Appendix 44 Mass Spectrum for Compound 41

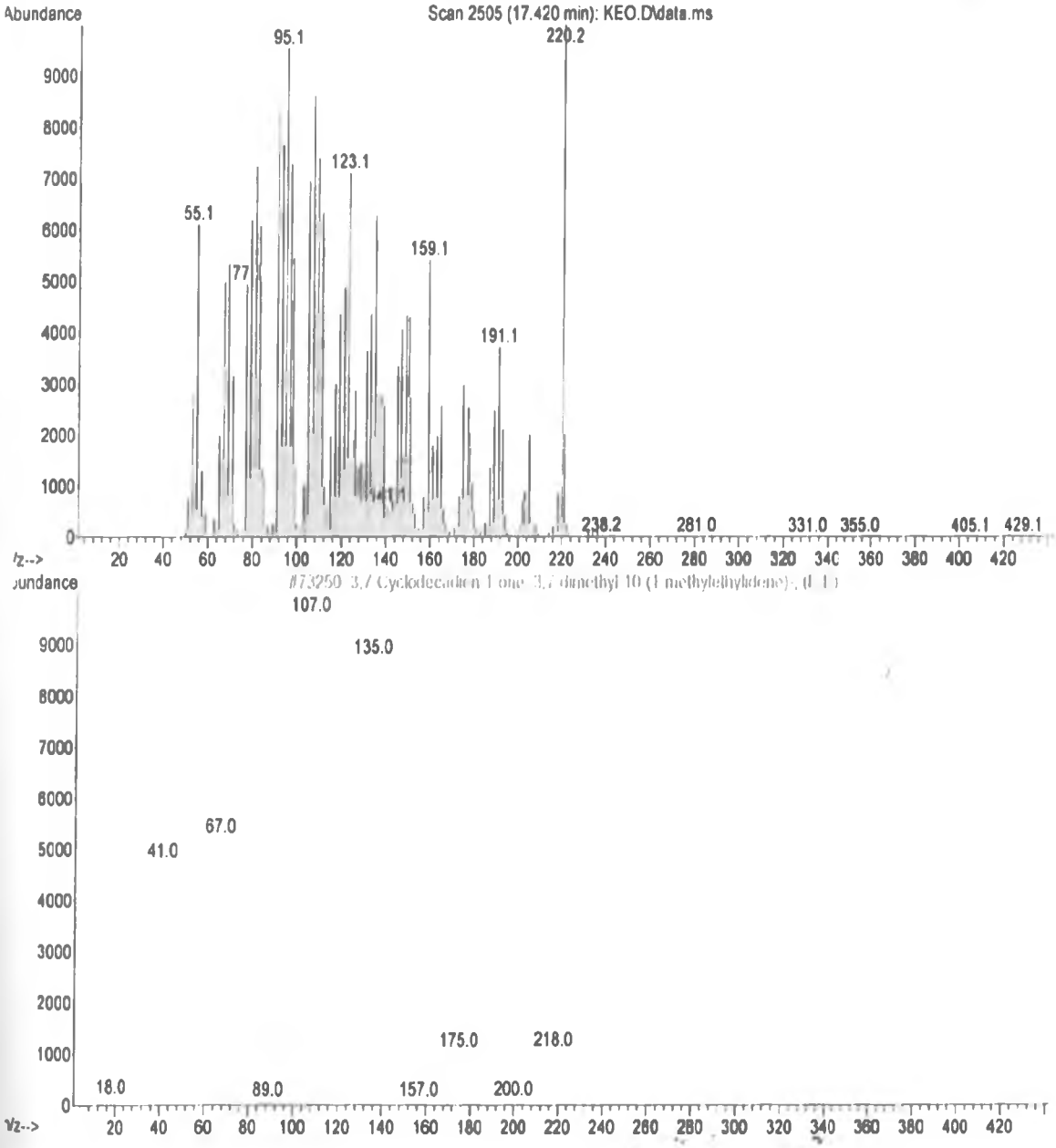
```
Library Searched : C:\Database\NIST08.L
Quality          : 90
ID               : Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-
```





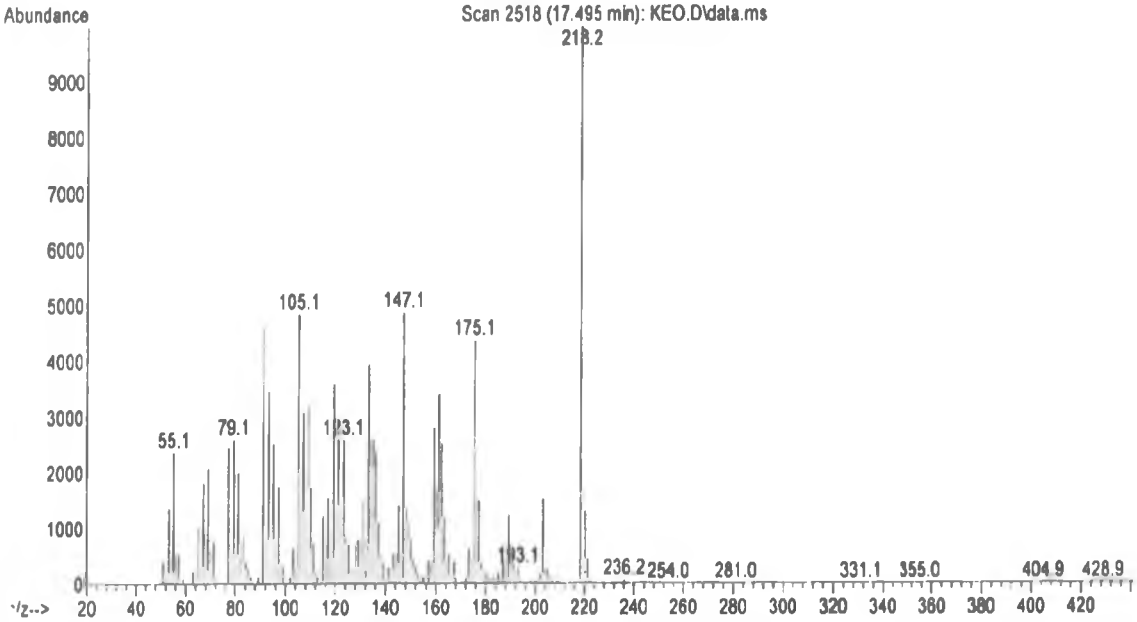
Appendix 45 Mass Spectrum for Compound 42

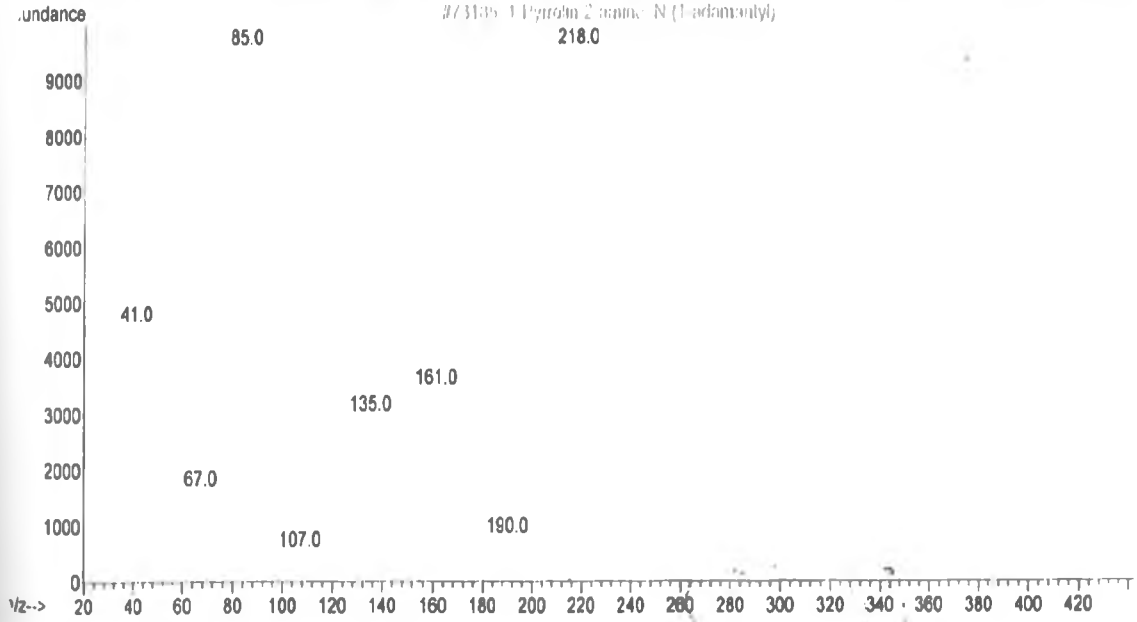
```
Library Searched : C:\Database\NIST08.L
Quality          : 53
ID               : 3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)
```



Appendix 46 Mass Spectrum for Compound 43

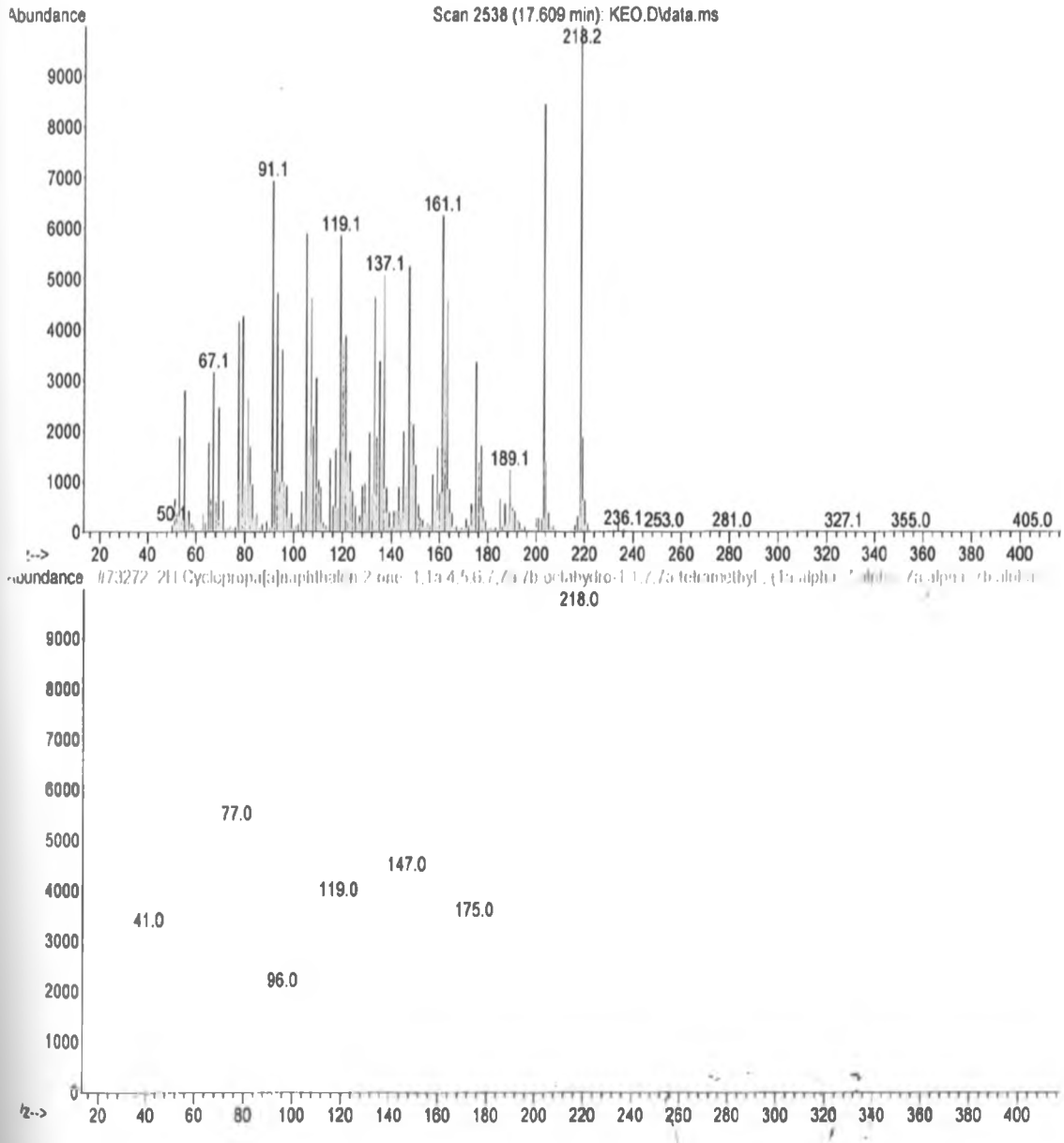
```
Library Searched : C:\Database\NIST08.L
Quality          : 86
ID               : 1-Pyrrolin-2-amine, N-(1-adamantyl)-
```





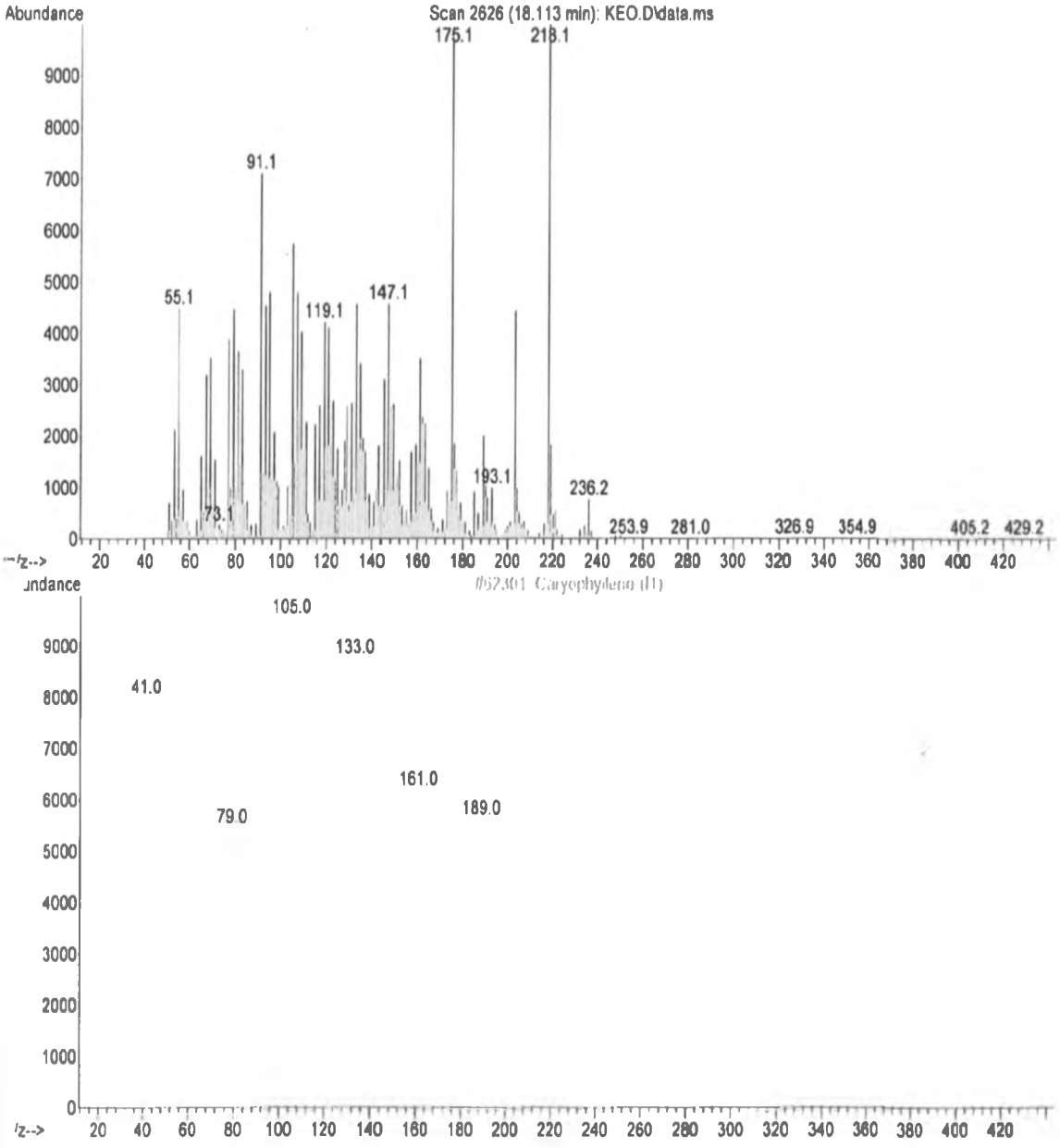
Appendix 47 Mass Spectrum for Compound 44

```
Library Searched : C:\Database\NIST08.L
Quality          : 86
ID               : 2H-Cyclopropa[a]naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,
                   7a-tetramethyl-, (1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)-
```



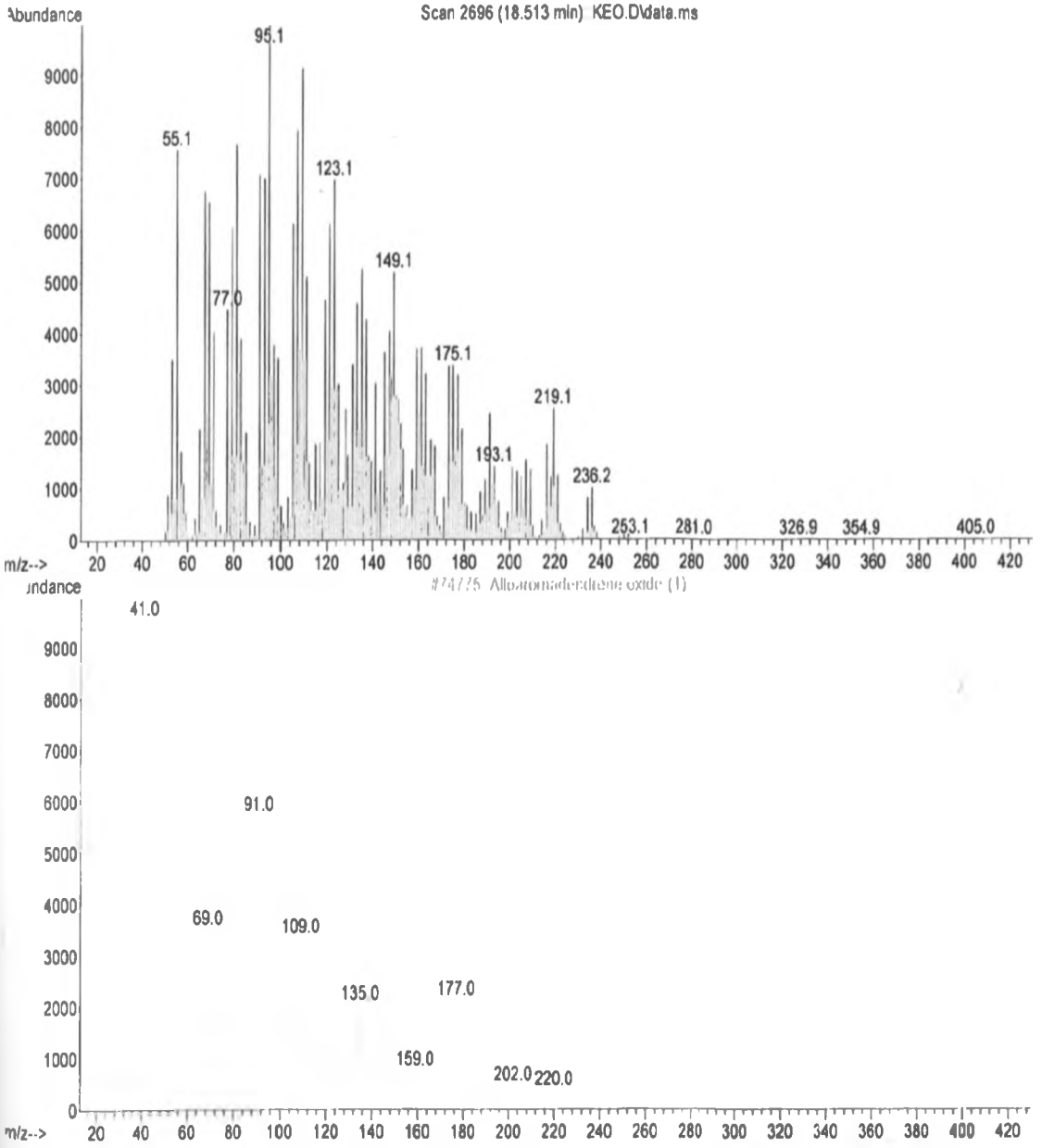
Appendix 48 Mass Spectrum for Compound 45

```
Library Searched : C:\Database\NIST08.L
Quality          : 94
ID               : Caryophyllene-(I1)
```



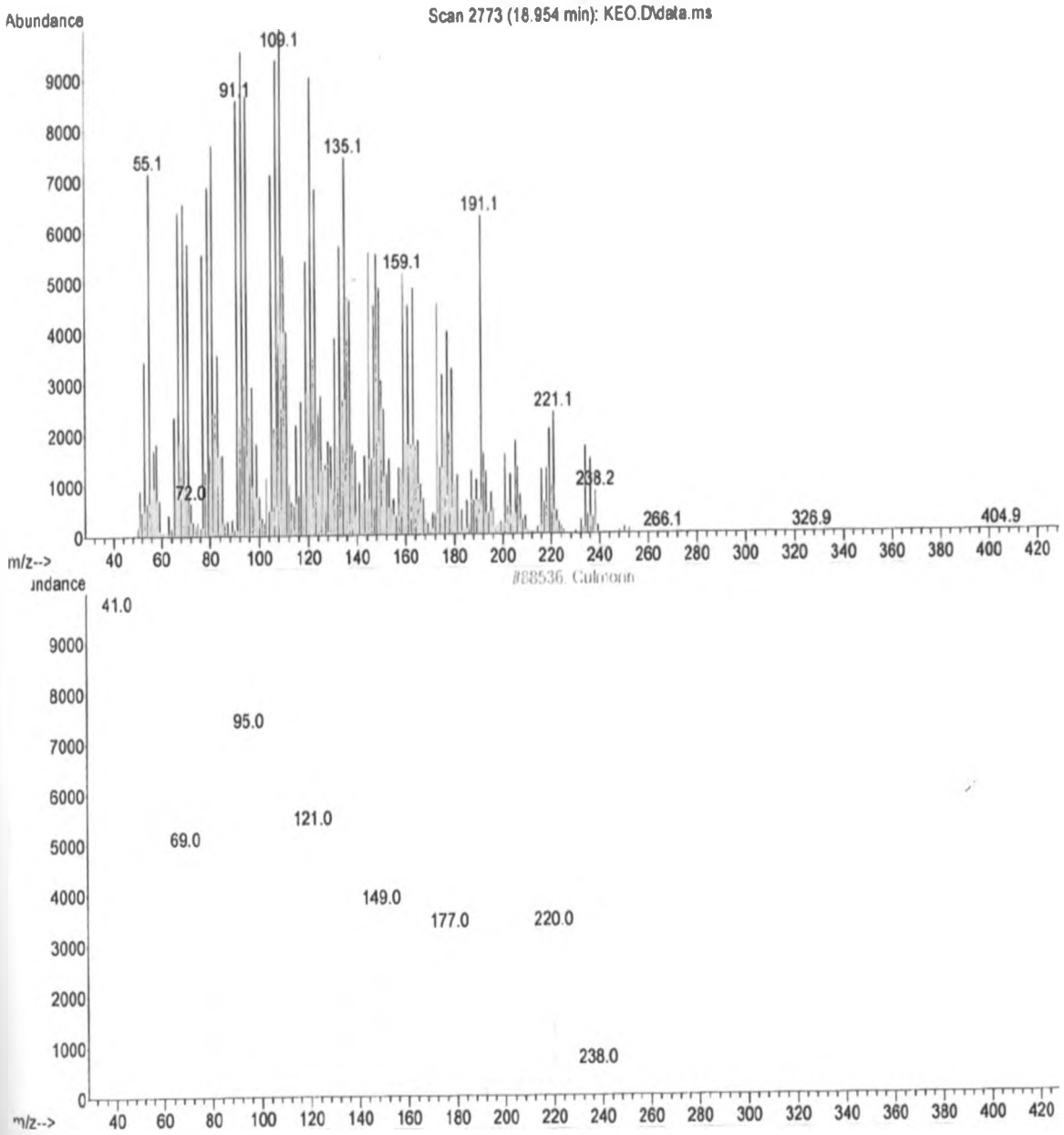
Appendix 49 Mass Spectrum for Compound 46

```
Library Searched : C:\Database\NIST08.L
Quality          : 91
ID               : Alloaromadendrene oxide-(1)
```



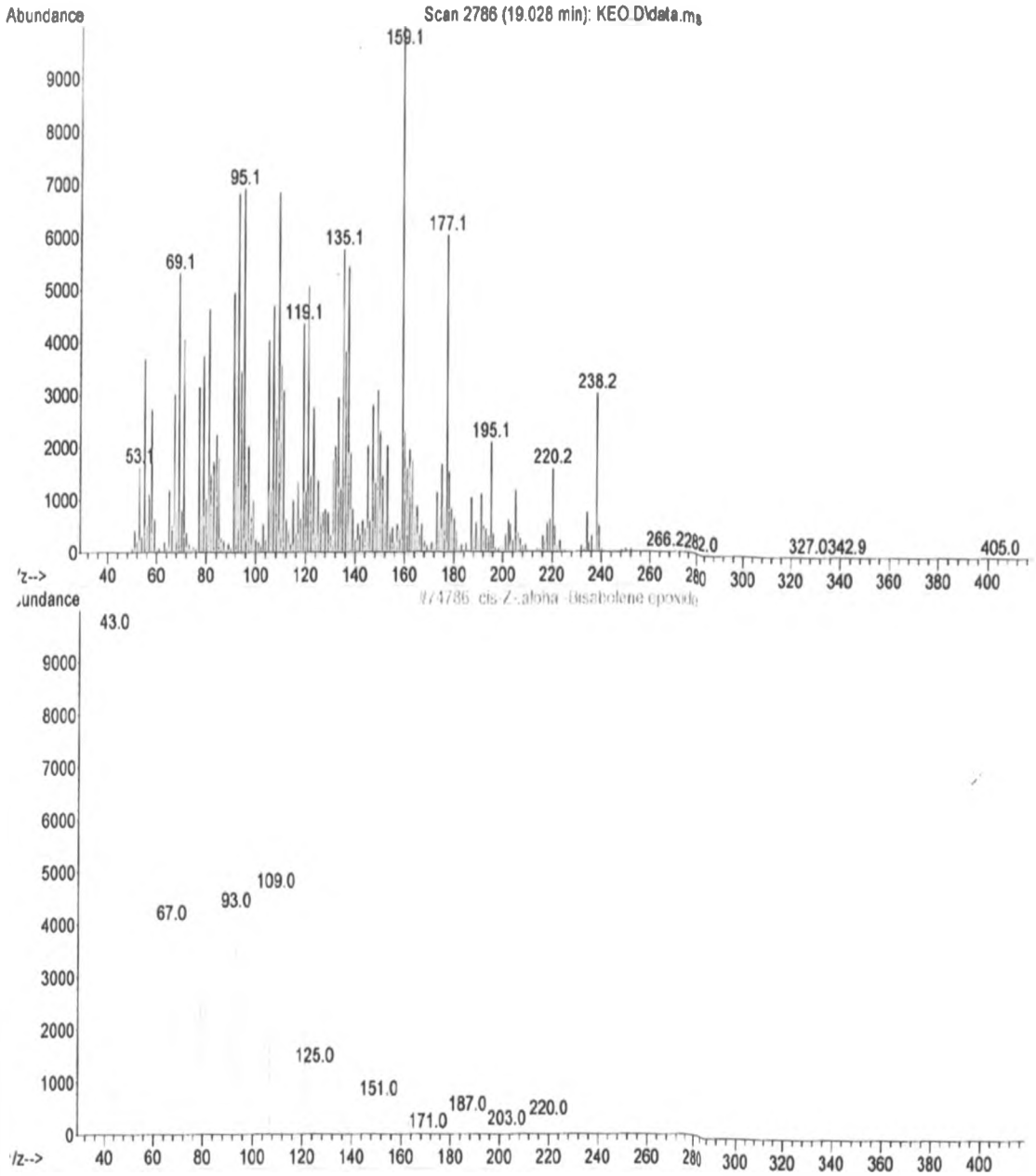
Appendix 50 Mass Spectrum for Compound 47

```
Library Searched : C:\Database\NIST08.L
Quality          : 90
ID               : Culmorin
```



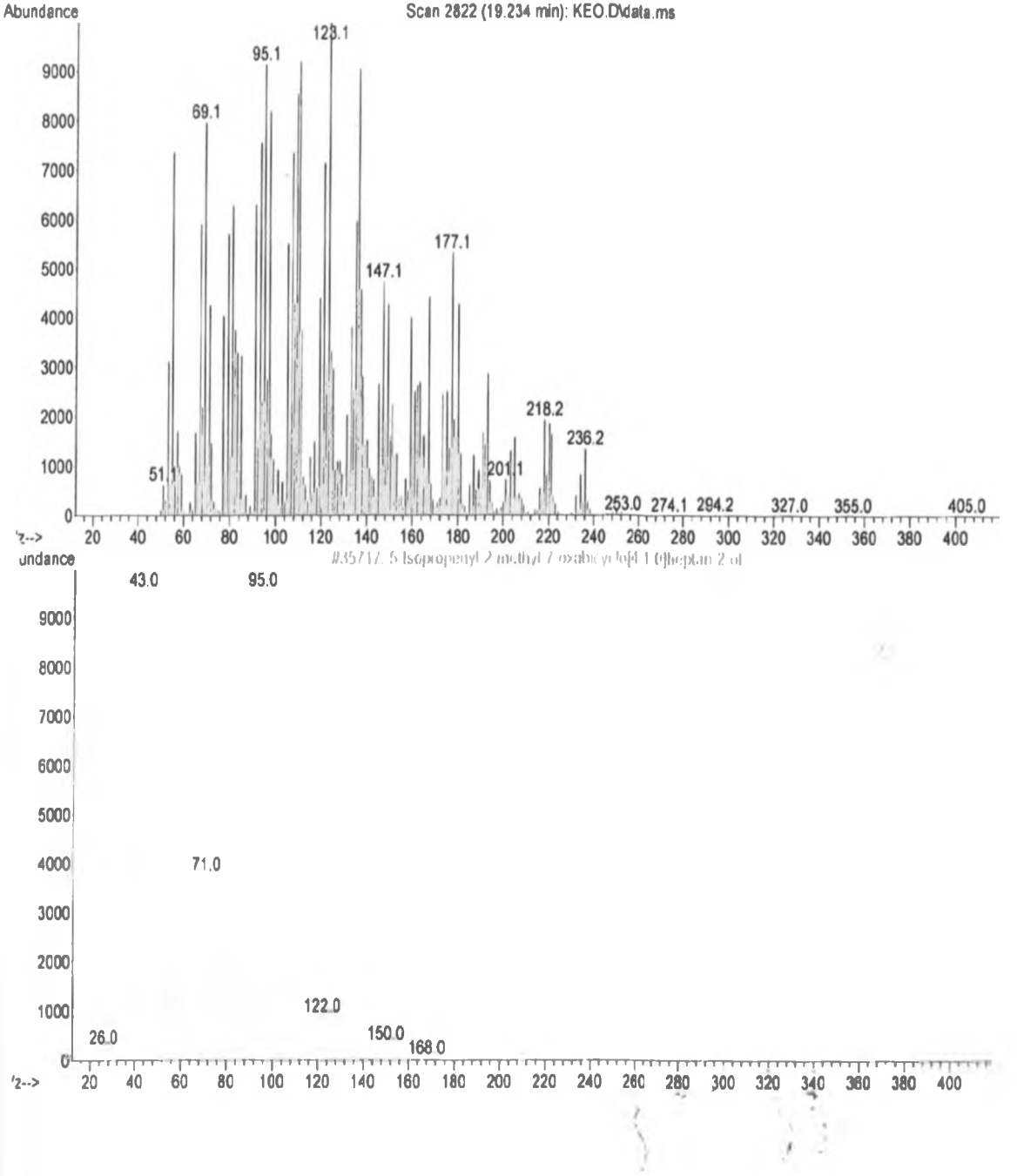
Appendix 51 Mass Spectrum for Compound 48

```
Library Searched : C:\Database\NIST08.L
Quality          : 47
ID               : cis-Z-.alpha.-Bisabolene epoxide
```



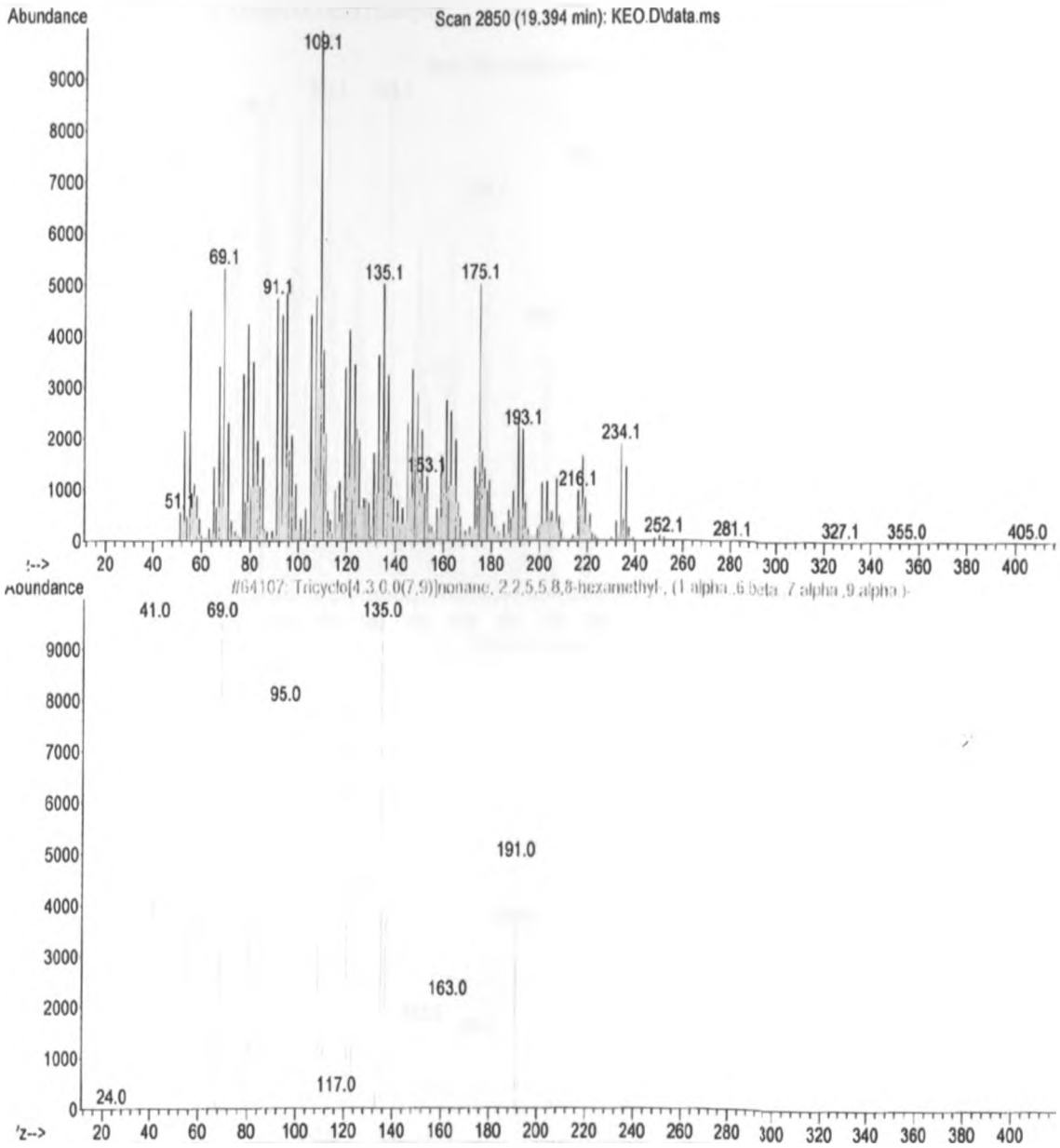
Appendix 52 Mass Spectrum for Compound 49

```
Library Searched : C:\Database\NIST08.L
Quality          : 83
ID               : 5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol
```



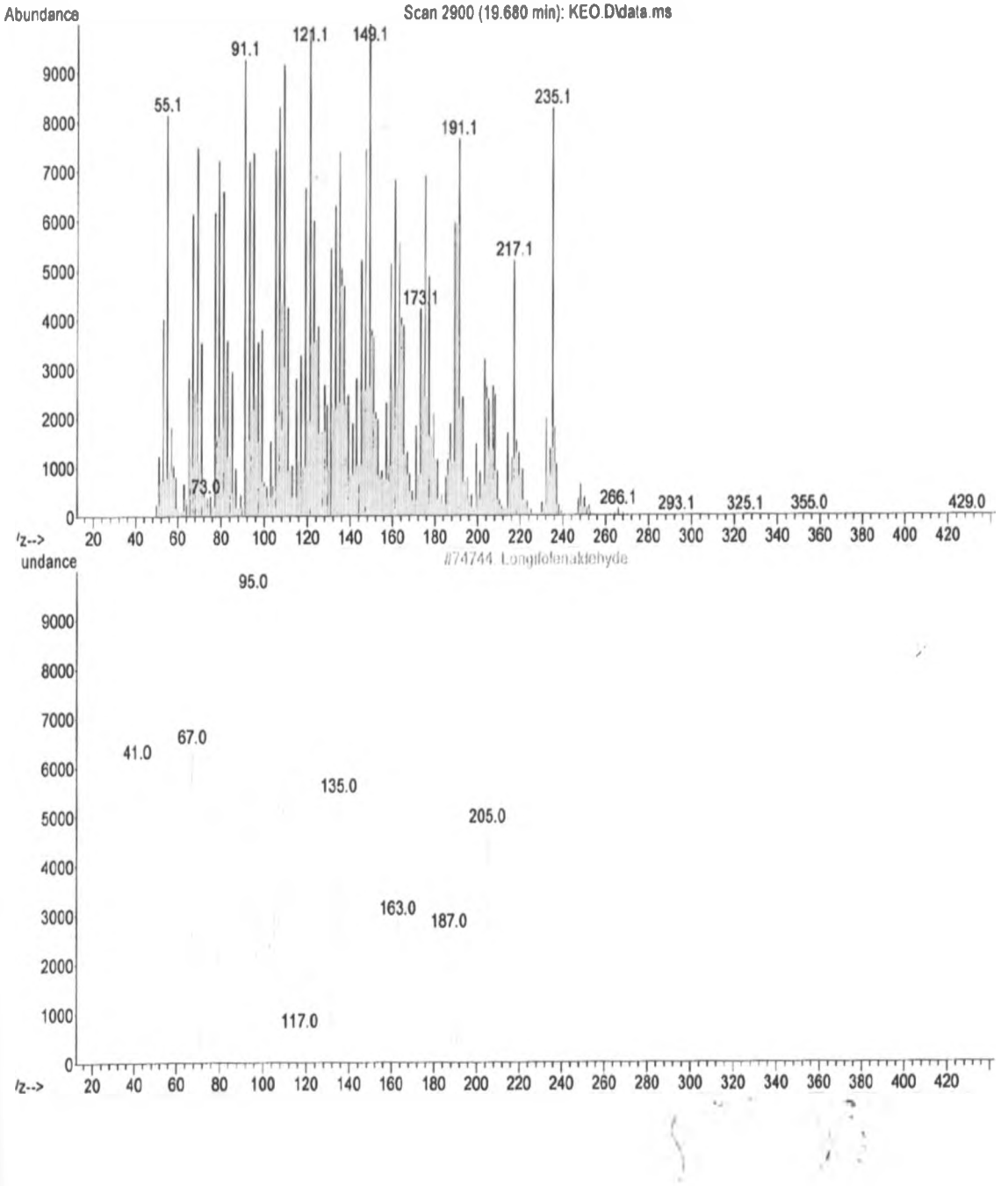
Appendix 53 Mass Spectrum for Compound 50

```
Library Searched : C:\Database\NIST08.L
Quality          : 89
ID               : Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.be
                   ta.,7.alpha.,9.alpha.)-
```



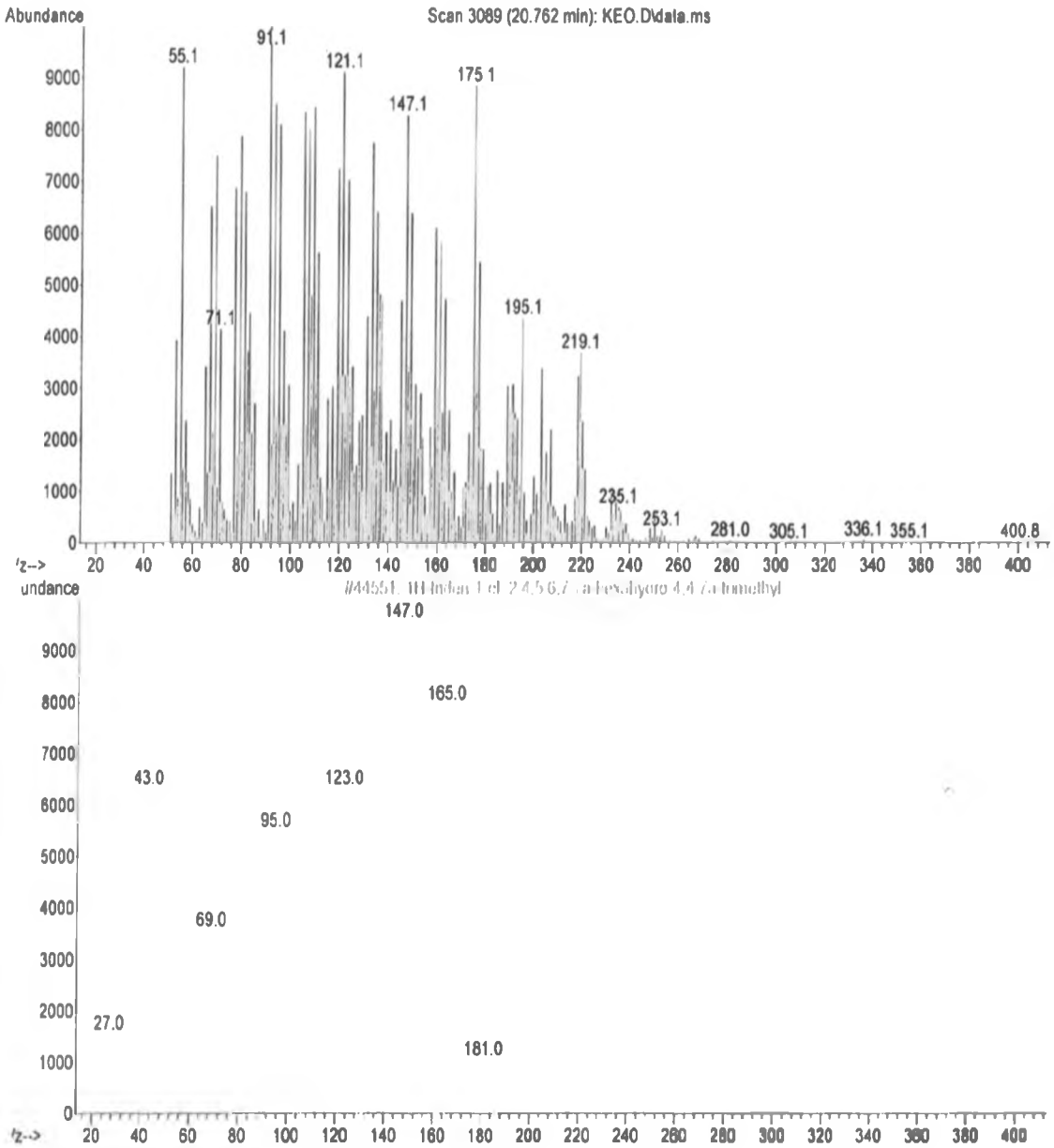
Appendix 54 Mass Spectrum for Compound 51

```
Library Searched : C:\Database\NIST08.L
Quality          : 78
ID               : Longifolenaldehyde
```



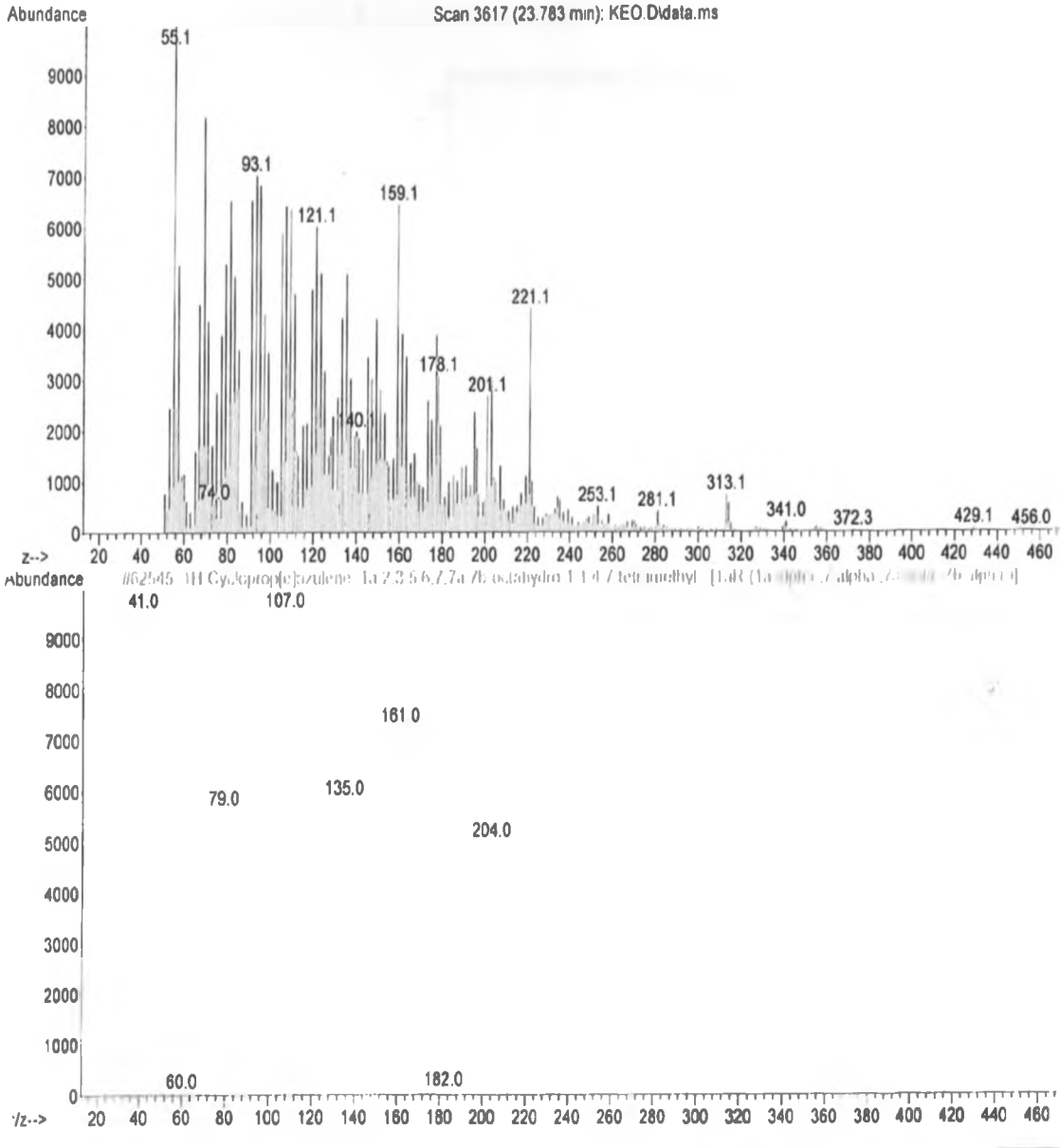
Appendix 55 Mass Spectrum for Compound 52

```
Library Searched : C:\Database\NIST08.L
Quality          : 83
ID               : 1H-Inden-1-ol, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-
```



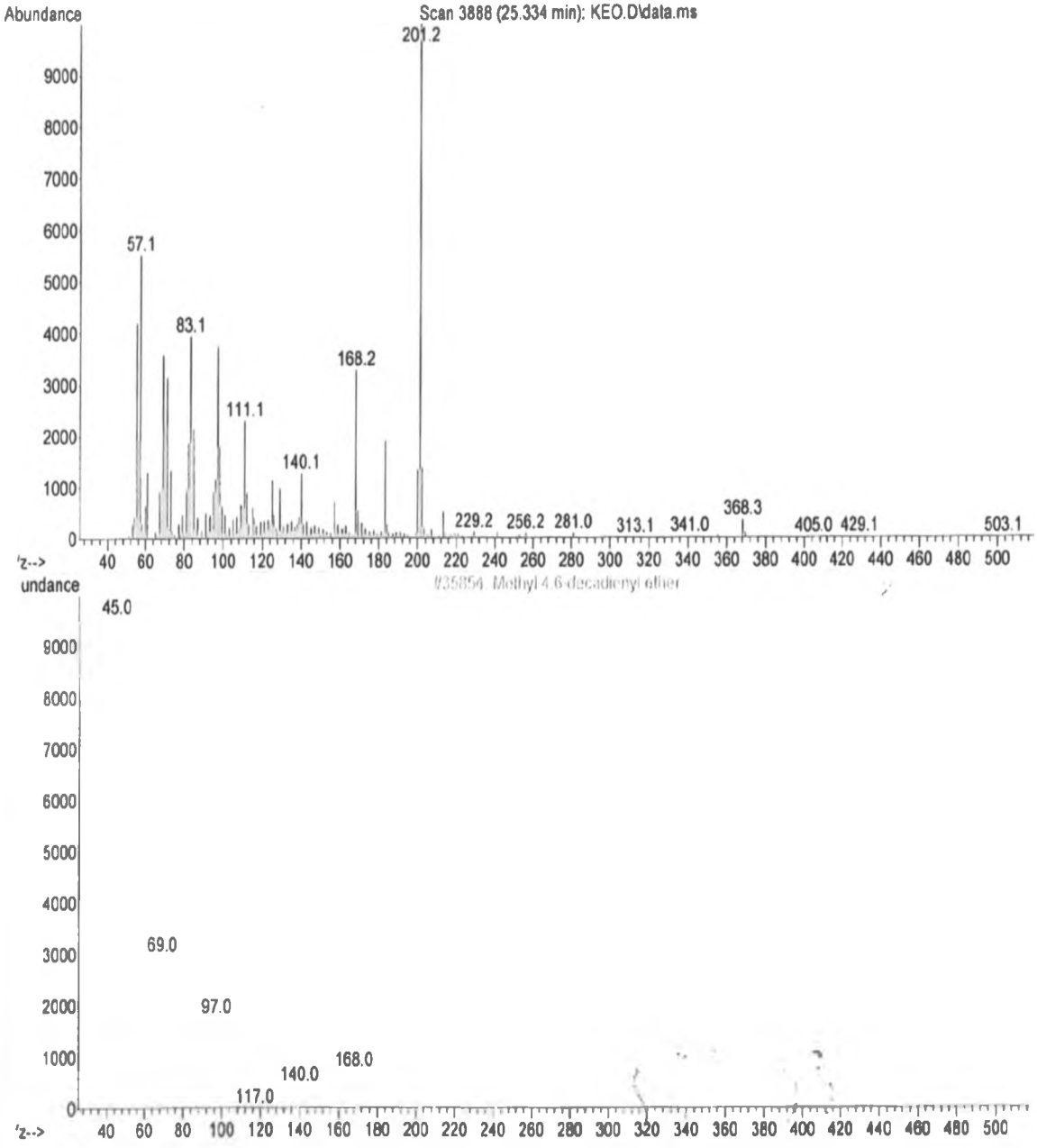
Appendix 56Mass Spectrum for Compound 53

Library Searched : C:\Database\NIST08.L
Quality : 92
ID : 1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-



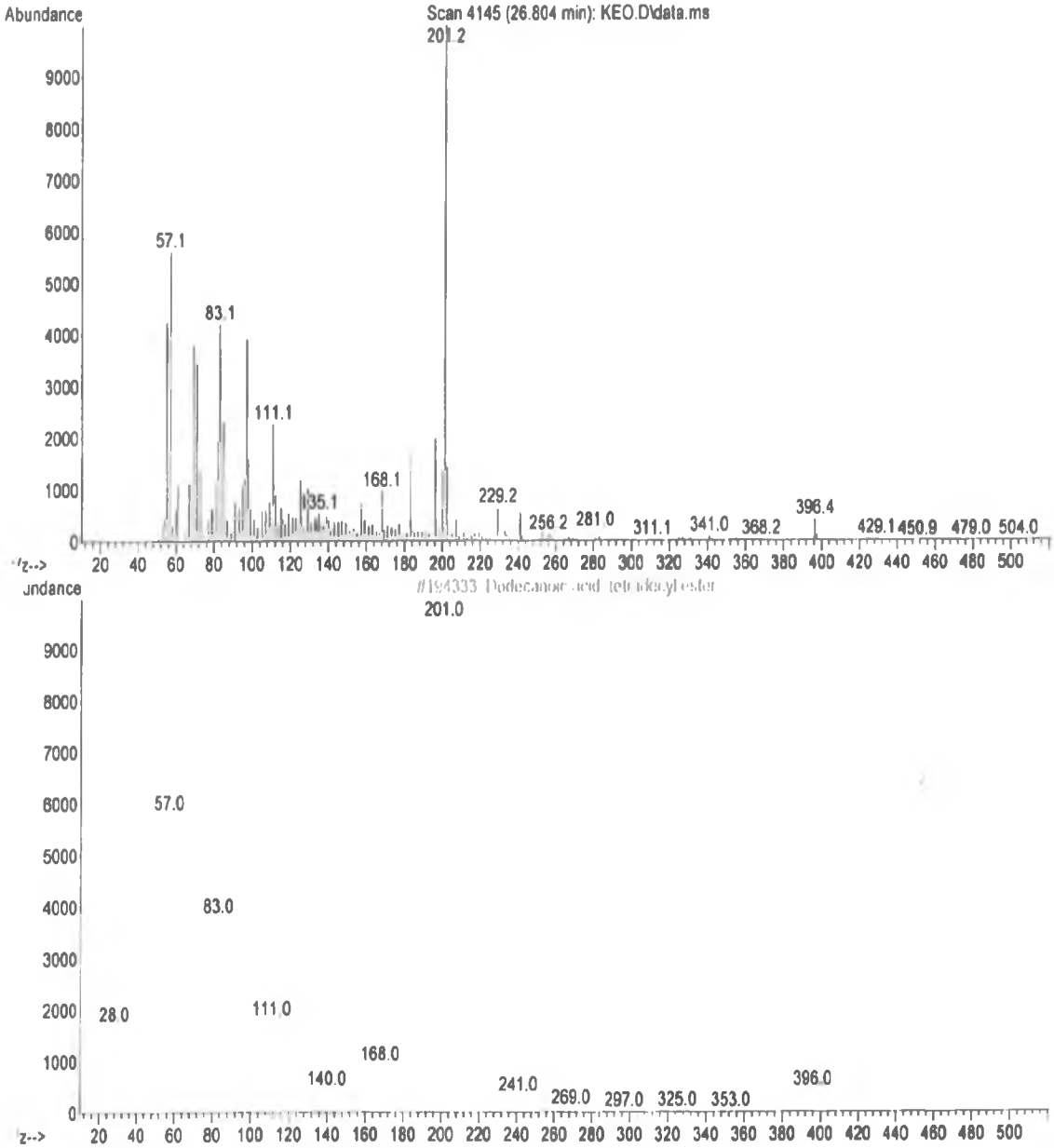
Appendix 57- Mass Spectrum for Compound 54

```
Library Searched : C:\Database\NIST08.L
Quality          : 50
ID               : Methyl 4,6-decadienyl ether
```



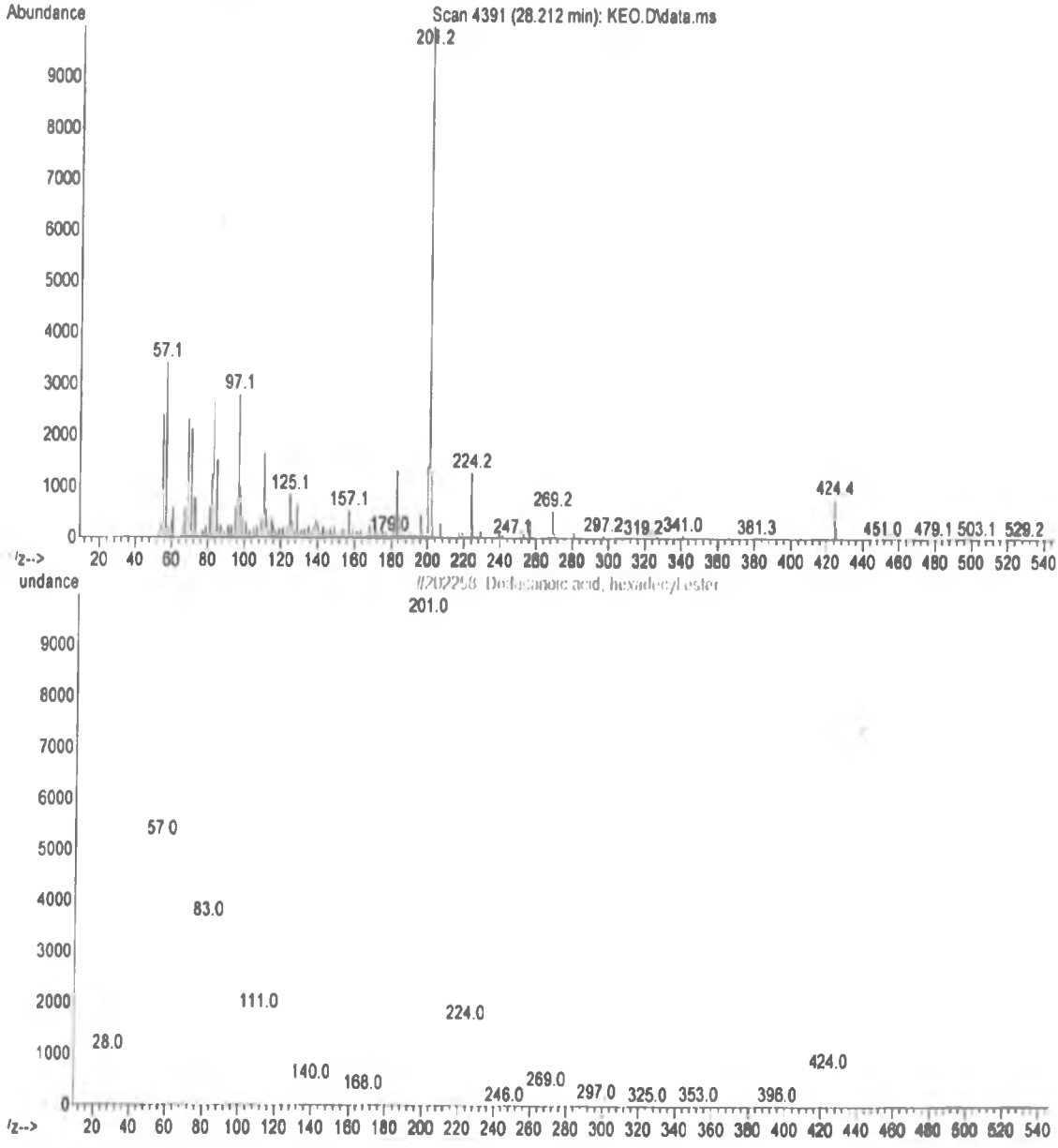
Appendix 58Mass Spectrum for Compound 55

```
Library Searched : C:\Database\NIST08.L
Quality          : 99
ID               : Dodecanoic acid, tetradecyl ester
```



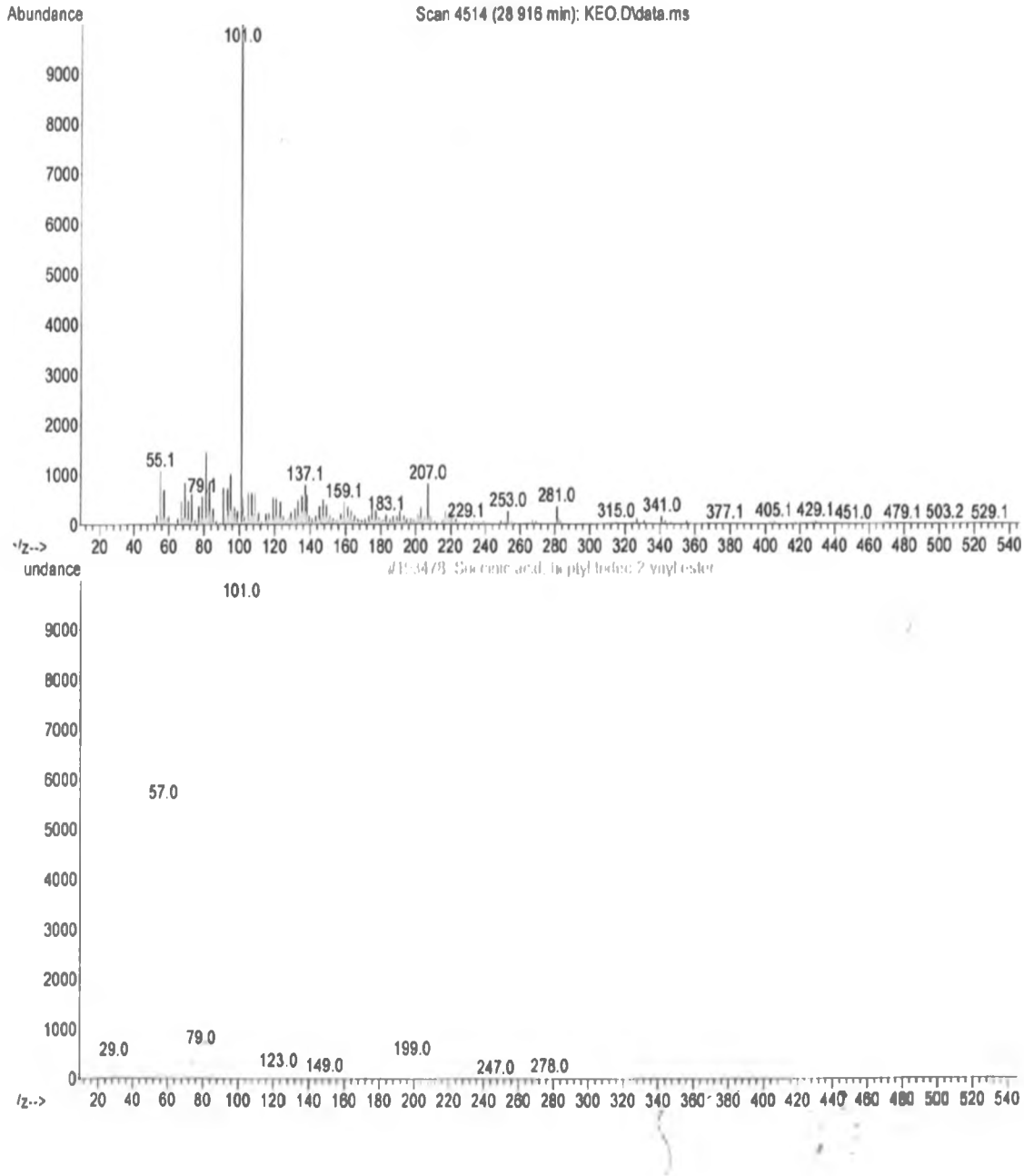
Appendix 59 Mass Spectrum for Compound 56

```
Library Searched : C:\Database\NIST08.L
Quality          : 99
ID               : Dodecanoic acid, hexadecyl ester
```



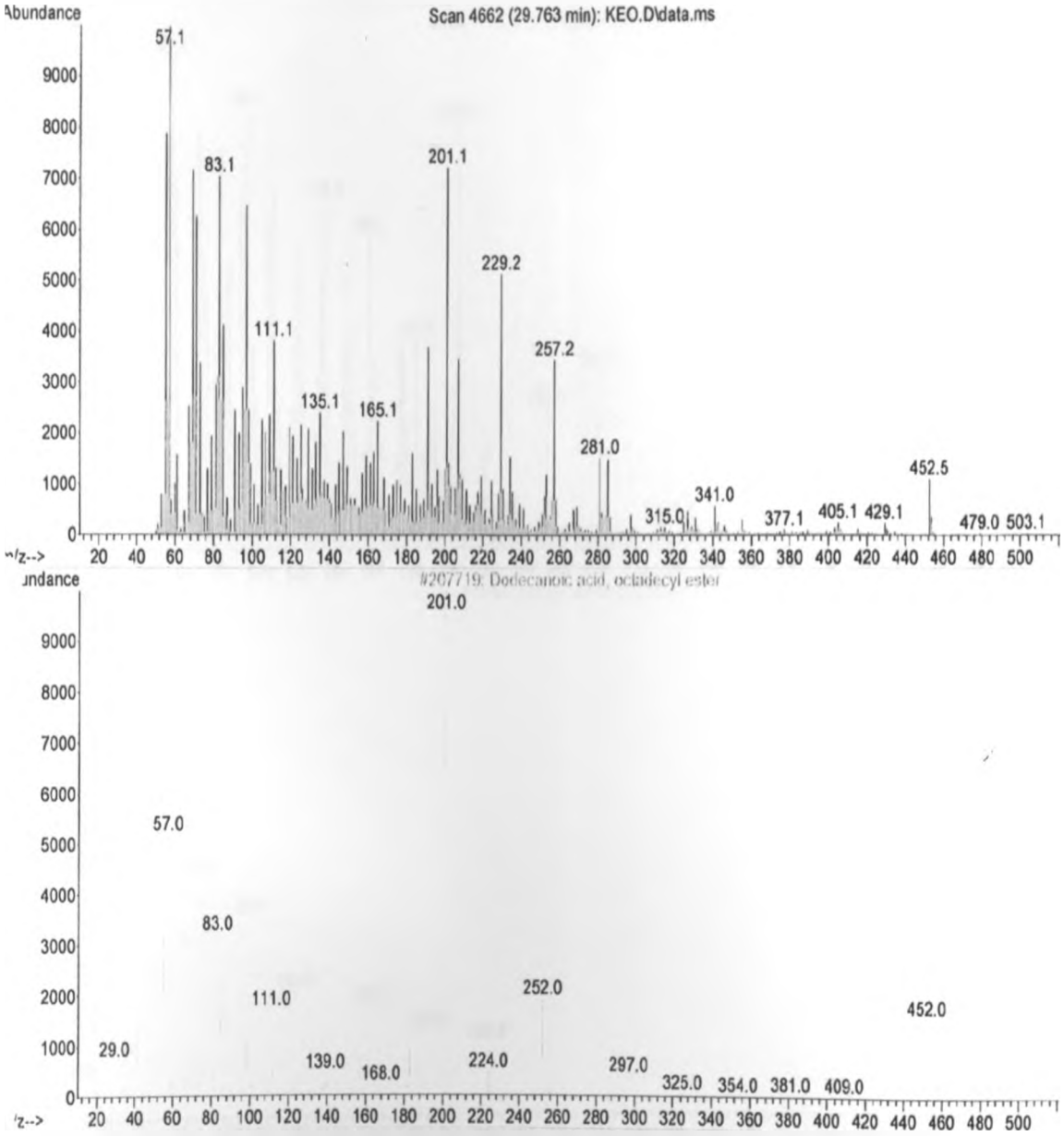
Appendix 60 Mass Spectrum for Compound 57

```
Library Searched : C:\Database\NIST08.L
Quality          : 72
ID               : Succinic acid, heptyl tridec-2-ynyl ester
```



Appendix 61 Mass Spectrum for Compound 58

```
Library Searched : C:\Database\NIST08.L
Quality          : 98
ID               : Dodecanoic acid, octadecyl ester
```



Appendix 62 Mass Spectrum for Compound 59

```
Library Searched : C:\Database\NIST08.L
Quality          : 70
ID               : Longipinocarveol, trans-
```