ESSENTIAL OILS OF SOME PLANTS OF THE FAMILY CAPPARIDACEAE AS REPELLENTS FOR THE BROWN EAR TICK, *RHIPICEPHALUS APPENDICULATUS* AND THE MAIZE WEEVIL, *SITOPHILUS ZEAMAIS*.

BY

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A Thesis submitted in partial fulfillment for the Degree of Masters of Science of the University of Nairobi.

1995

DECLARATION

This Thesis is my original work and has not been presented for a degree in any University

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DEDICATION

This thesis is dedicated to the entire Ndakala family, whose understanding, patience and prayers during the course of this research project gave me the motivation to pursue it to the hilt.

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A B S T R A C T.

This thesis describes a chemical and bioactivity study of the essential oils of five tropical plant species of the family capparidaceae, namely *Boscia angustifolia* A. Rich var. *angustifolia*, *Boscia mossambicensis* Klotzsch, *Cadaba farinosa* Forsk, *Gynandropsis gynandra*(L.) Brig., and *Thylachium africanum* Lour. The essential oils were isolated by hydrodistillation of the aerial parts using a modified clavenger apparatus. Oils of various colours and essences were obtained.

The constituents of the essential oils were examined by routine temperature programmed GC and then subjected to GC/MS analysis utilizing the electron impact ionization technique. The examination of the mass spectra adduced, coupled with the co-chromatography of the essential oil with the authentic compounds of closely matching mass spectra, was the basis of the identification process. Thirty one compounds were identified in all the essential oils. The majority of these compounds were common to all these essential oils. These compounds were mainly monoterpenoids, aliphatic alcohols, aliphatic ketones, aliphatic and aromatic aldehydes, a few sesquiterpenoids, one diterpene alcohol, an aromatic ester, a thiazole, an isothiocyanate and an aromatic nitrile.

Repellency bioactivity studies of the essential oils and their constituents were performed on the brown ear tick, *Rhipicephalus appendiculatus* and also against the maize weevil, *Sitophilus*

zeamais. In the case of the brown ear tick, the repellency activity was based upon the Tick climbing method developed at ICIPE. The results revealed that the essential oils of *B. mossambicensis*, *T. africanum*, *G. gynandra* and *B. angustifolia* were highly repellent at the 0.1 dose level, while that of *B. mossambicensis* proved effective at the 0.01 dose level as well. The components that showed enhanced activity at the 0.1 as well as 0.01 dose level were *m*-cymene, nonanal, α-terpineol, ß-cyclocitral, nerol, *trans* geraniol, carvacrol, α-cedrene, α-ionone, *trans* geranylacetone and nerolidol, while benzaldehyde, phenylacetaldehyde, ß-ocimene, linalool, phenylacetonitrile and methyl salicylate were highly active at the 0.1 dose level but not as much at the 0.01 level. α-Terpineol, ß-cyclocitral and nerolidol proved active even at the 0.001 level with nerolidol emerging more potent. On the other hand, use was made of the Y-tube olfactometer method(Hassanali et al., 1990) to study the repellency activity against the maize weevil, *Sitophilus zeamais*. The essential oils of *B. angustifolia*, *B. mossambicensis*, *C. farinosa*, *G. gynandra* and *T. africanum* were effective at the 0.1 dose level with remarkable results being produced by the essential oils of *B. angustifolia*, *B. mossambicensis*, *C. farinosa* and *T. africanum*, which continued being active at the 0.01 dose level. For the components of the essential oils which were bioassayed, the noteworthy ones were *trans* 2-methylcyclopentanol, *cis* 3-hexen-1-ol, *trans* 2-hexen-1-ol, anisole, benzaldehyde, phenylacetaldehyde, 1,8-cineole, ß-ocimene, linalool, phenylacetonitrile, α-terpineol, ß-cyclocitral, α-cedrene, α-ionone and ß-caryophyllene which were active at the 0.1, 0.01 and 0.001

dose levels. It is interesting to note that *trans* 2-methylcyclopentanol, anisole, 1,8-cineole, linalool and phenylacetonitrile were observed to elicit activity even to the 0.0001 dose level.

CHAPTER 1

I N T R O D U C T I O N

1.0 General Considerations

Post-harvest losses of grain to stored-product pests remains among the most serious problems faced by small scale farmers throughout the tropics, accounting for substantial losses of grain in less developed countries (Cimmyt, 1970/1). On the other hand the development of the livestock industry, widely regarded as an essential factor in the growth and expansion of the traditional agro-based economy of the subsaharan region, is being hampered by the haematophagous activities and the disease transmission capabilities of ticks, making them the most serious parasites of livestock in this region (Williams and Gonzales, 1968; Wharton and Roulston, 1970; Lombardo, 1975). So to sustain the growth of the agro-based economies of the countries of this region, it's imperative that economically feasible and effective control measures be implemented to arrest the looming threat in this region by these pests.

The use of pesticides has been the most effective method of protection against these pests (Mandava, 1985; Pimentel et al., 1978; Dipeolu et al., 1992; Mathawson, 1984), but there has arisen a number of drawbacks to their use. Most crucial among these

drawbacks is that most of these pesticides are non selective, toxic, synthetic chemicals which also exert residue effects. Their indiscriminate use has led to serious social and environmental repercussions (Saxena, 1989; Barnes, 1976). In addition there is the incessant development of resistance to these pesticides (Saxena, 1989; Van Beek and De Groot, 1986; Busrine, 1976; Whitnall et al., 1952). One reason for the development of this resistance is the continuous application of these pesticides. This allows for the selection of pests with resistant alleles (Georghiou and Taylor, 1977a). It is therefore essential that supplementary methods be developed and implemented to control these pests. The Integrated pest management program (IPM) has been conceived to meet this challenge. This concept is based on the recognition that no single approach to pest control offers a universal solution, but maintains that the best defence could be provided by a fusion of various tactics into practices based on sound ecological principles. The objective of the concept is to integrate all suitable techniques and information into the most economical and ecologically acceptable system, either to reduce and maintain the pest population below damaging levels or to manipulate them in such a manner as to prevent their causing damage (McDowell, 1984). Methods of control that have attracted renewed interest are the use of sex pheromones, attractants and repellents.

It's noteworthy that there are naturally occurring compounds that have been found to exhibit repellency and attractancy properties. Such compounds have been identified from both plant and animal sources. In plants the compounds are produced either for

host plant-insect interaction or for protection from predation by pests. In animals they serve either to attract mate or as alarm-defence compounds. In general plants provide the richest and most diverse source of bioactive organic chemical compounds. These are produced in response to predation by a variety of organisms throughout the evolutionary time. The degree of predation is particularly intense in the tropical climate, where plants, in contrast to their temperate counterparts have evolved more efficient and varied chemical defense mechanisms for survival. Tropical flora thus provide a rich source of natural chemical compounds which exhibit useful activity against insect pests and disease organisms. These compounds have advantages as repellents, attractants and pesticides because the are renewable, more selective and less resistant to biological factors (Swain, 1977; Alkofahi et al., 1989; Farnsworth and Morris, 1976; Farnsworth and Bingel, 1977).

There is a potential in the isolation of natural repellents in plant odors notably in essential oils and in exudates of glandular trichomes of certain plants. These are known to either aid in natural selection by acting as attractants for certain insects to pollen, to function biologically by repelling predators or to be a defence mechanism against fungal attack (Pridham, 1966; Simonsen, 1953; Encyclopedia Britannica, 1991). It is therefore essential these attributes of tropical plants be explored and exploited with a view of isolating potent natural repellents to be used in pest control.

This thesis therefore explores the constituents of Essential

oils of some plants of the family capparidaceae and their repellency activities against the Brown ear tick, *Rhipicephalus appendiculatus* Neumann, and the Maize weevil, *Sitophilus zeamais* Motschulsky.

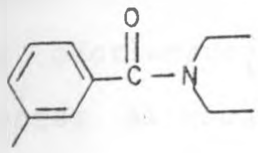
1.1.0 Repellents

A Repellent is a chemical or mixture of chemicals that, acting in the vapor phase or otherwise, causes the organism to behave in ways which result in its movement away from the source of the matter or prevents an organism from reaching a target it would otherwise be attracted to (Dethier et al., 1960; Browne, 1977). In contrast to this precise behavioral definition, workers involved in the development of repellent substances use the term "repellent" to describe a chemical that elicits a combination of behavioral responses whose net result is simply the prevention of biting by an organism. This may accrue from the fact that topical repellents were used for protection against biting organisms and the pathogens that they transmit (Davis, 1986).

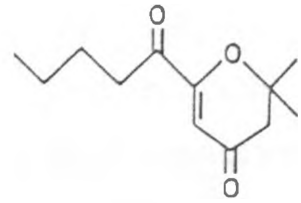
Currently, the most critical evidence suggest that repellents act directly on the chemosensory systems (Kilgore and Doult, 1976). The practical problem of repellency being essentially a behavioral one, a candidate compound must first possess inherent repellency, that is, be capable of stimulating some sensory system other than that that mediates attraction. Secondly, since the response of the organism depends upon which sensory system has been stimulated and which reflex arcs are placed in operation, the repellent must act

upon a system which has some influence on locomotion or feeding. The nature of response elicited by a repellent also depends upon a variety of intrinsic biological factors such as age and state of nutrition, the concentration of the repellent and which sensory systems are being acted upon simultaneously by other stimuli (Dethier, 1956).

Repellents may be divided into two classes depending on their mode of affording protection (Sankaria and Brown, 1951). Those materials, such as N,N-diethyl-*m*-toluamide (deet), which are sufficiently volatile to keep an organism at a distance are designated vapor or olfactory repellents. The majority of synthetic chemicals found to be effective are repellent in the vapor phase. Those that are slightly volatile, such as indalone, that the insect must approach and touch before being repelled are designated contact or gustatory repellents. Such repellents act upon specialized chemoreceptors not normally sensitive to vapors. Such receptors, located on the mouth parts and tarsi are concerned with monitoring some aspect of feeding. One would expect that repellent compounds acting on these receptors would prevent feeding (Dethier, 1956; Dethier and Chadwick, 1950; Frings, 1946; Frings and Frings, 1949; Kilgore and Doult, 1967). Since these compounds are usually detectable by other animals, including man, one of the most important criterion for an economically feasible chemical is that it be repellent to insects while still be acceptable to the animal species to whose protection it is intended (Kilgore and Doult, 1967).



Deet



Indalone

There are two types of repellent action, that is, a time dependent and a time independent variable. A material is considered to exhibit intrinsic repellency if a known amount or concentration of material demonstrates some degree of repellency independent of time. Such repellency is measured by employing an olfactometer or by testing the repellency of the surface to which a candidate agent has been applied immediately after application. In the evaluation of effective repellency, the repellency response is measured as a function of time. The effective repellency of a material may be a function of intrinsic repellency, but the latter can not be a function of the former. In the actual development of a repellent, it is the effective repellency which is of concern in the final analysis. Intrinsic repellency is of prime consideration in determining what chemical and physical factors are responsible for repellent activity (Garson and Winnike, 1968).

1.1.1 Mode of Action of Repellents: Postulations

Chemical repellents are used for protection against pathogen transmitting organisms such as mosquitoes, ticks and fleas.

N,N-Diethyl-*m*-toluamide (deet) was the first synthetic

repellent that was found broadly effective against a wide range of biting pests (Gilbert et al., 1955, 1957). It is noted for its essential odorlessness, water-like texture and long lasting effectiveness. Although deet is a very good repellent (Beuscher et al ,1983), its adverse reactions are known (Anon., 1981). It causes a burning sensation in eyes, cuts and membranous areas and will damage some plastics and synthetic fibres (James and Harwood, 1969). Consequently, investigators have been seeking new more effective repellents and new formulations of repellent compounds though without much success. One important reason for the lack of success is that we do not know how repellents act on the target organism. We know that repellents are detected by the peripheral chemosensory system and that somehow we are not bitten or atleast not as often when we use them. However, we do not understand how repellents interact with an organism to modify the behavior (Davis, 1985).

Various theories have been proposed. Information obtained from sensory physiological studies suggest that there could be more than one sensory-behavioral mechanism by which insect repellents reduce the biting activity of female mosquitoes. Repellents may act by interfering with the perception of host attractant signals (Davis and Sokolove, 1976), by exciting a receptor for a competing behavior (Davis, 1976), by switching a sensory message from attraction to repulsion (Kost et al.,1971), by activating several different receptor systems so that the repellent in effect "jams" meaningful sensory information (Davis, 1985) and/or by exciting a repellent (noxious substance) receptor (Lacher, 1971; Davis and

Rebert, 1972).

For the deet molecule it is postulated that in the vapor state it would have access to the membranes of the neurons in the chemosensilla and to the membranes of a great many body cells via pores in the cuticle and the tracheal system, respectively. In host location, a behavior chiefly mediated by olfactory cues, interaction of deet molecule with lipids perturbs the organization of the dendritic membranes in such a way that the normal responses to attractants are altered. Sufficient quantity of deet would alter significantly the sensory pattern, largely composed of responses from all the olfactory neurons, that impinges on the central nervous system (CNS). Consequently, the CNS would not elicit the muscular contractions needed to make the appropriate turning responses that would keep the mosquito within the stream of host-related attractants. Deet, having entered the body through the spiracles, may indirectly interfere with non-olfactory mediated behaviors by interaction with membranes of a large number of cells, initially affecting respiration. Other physiological processes would be affected that would eventually cause the observed behavioral changes. This indirect action on the nervous system would not be important in deets interference with the host location because the proposed alteration of the sensory pattern mechanism would occur more quickly (McIver, 1981).

1.1.2 Natural Repellents

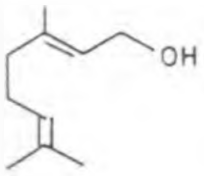
These are mainly natural compounds that are inherent in

Essential oils, exudates of glandular trichomes or hairs of certain plants. They may also be components of insect defense secretions. These mainly consist of terpenoids, quinones, phenolics etc. (Levin, 1973).

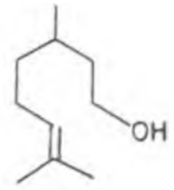
Essential oils are known to possess biological activities for pest control coupled to their perfumery values (Singh and Agarwal, 1988). The oil of citronella, which is extracted from *Andropogon nardus* (L.) contains geraniol as the primary component, with lesser amounts of citronellol, citronellal, borneol and other terpenes. Citronellol and the corresponding aldehyde, citronellal, were considered the principle mosquito repellents of the oil (Shambaugh et al., 1967; Painter, 1967). The essential oils of *Ocimum suave* Willd., leaves and *Eugenia caryophyllata* Thumb., cloves are repellent to the Maize weevil, *Sitophilus zeamais* Motsch. (Hassanali et al., 1990). Among the constituents tested for repellency activity, eugenol is reported more active than the commercial insect repellent, deet, followed by methyleugenol and isoeugenol. The essential oil of tansy (*Tanacetum vulgare*) is repellent to the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Panasiuk, 1984). The repellent components being α-terpinene, thujone, dihydrocarvone and carvone. The essential oil of *Artemisia vulgaris* is also reported to be repellent against the female mosquito, *Aedes aegypti* (Huang et al., 1985). The active component is 1,8-cineole. On the other hand, the naturally occurring aldehyde, *trans* 2-nonenal isolated from the carrot root oil (Buttery et al., 1968), is an insect repellent as well as an insecticide. It repels and kills the larvae of the parasitic, and

thus adapted insect, *Psila rosae* (F.), the carrot fly (Guerin and Ryan, 1980) and repels 98% at the 50 ppm, the German cockroach, *Periplaneta americana* (Scriven and Meloan, 1984). It is also reported effective against a broad range of 11 insects and 3 acarine pests (Taha, 1987). The essential oils of spearmint, mint and eucalyptus are reported repellent to the German cockroach (Inazuka, 1982), while menthol which emanates from trichomes of many plants of the mint family is strongly repellent to the silkworm (Levin, 1973). Oils of *Backhausia myrtifolia*, *Malaleuca bracteata* and *Ziera smithi* repel mosquitoes, march flies and sand flies (Harley, 1967; Harley and Thorsteinson, 1967).

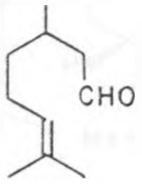
Limonene and myrcene are repellents to the western pine beetles feeding upon *Pinus pondrosa* while linalool, from the foliage of *Mentha crispa*, *Citrus bergamia* and *Cymbopogon citratus* repels the aphid, *Cavariella aegopodii* (Champman et al., 1981). Nepetalactone and metabilactone, cyclopentane monoterpenes, identified from *Nepeta cataria* and *Actinia polygama* respectively are known to repel insects. Nepetalactone shows enhanced potency (Eisner, 1970). Azadirachtin, a triterpenoid derived from neem tree, *Azadirachta indica*, is repellent to the red flour beetle, *Trogoderma granarium* Everts and the lesser grain borer, *Rhyzopertha dominica* (Jilani and Malik, 1973).



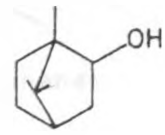
Geraniol



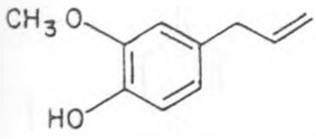
Citronellol



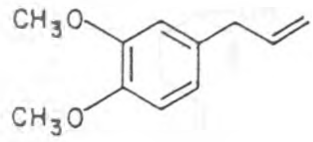
Citronellal



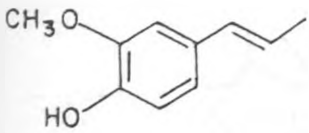
Borneol



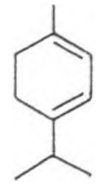
Eugenol



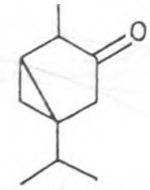
Methyl eugenol



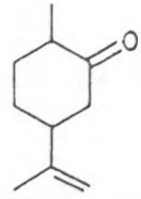
Isoeugenol



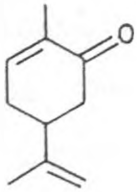
α – Terpinene



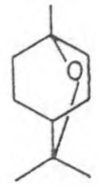
Thujone



Dihydrocarvone

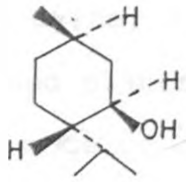


Carvone

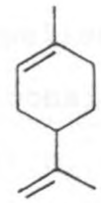


1,8-Cineole

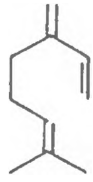
trans 2 — Nonenal



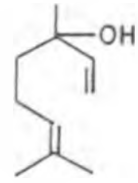
Menthol



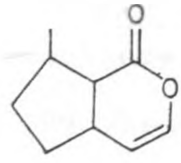
Limonene



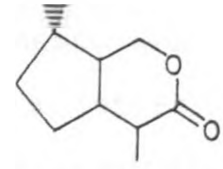
Myrcene



Linalool



Nepetalactone



Metabilactone

1.1.3 The Future for Repellent Application in Pest Control

The use of repellents is due to increase due to the increasing general awareness by the public of the dangers and problems in the indiscriminate use of potent synthetic insecticides. The persistence, the resistance problem, the damage to the environment and the threat to human health by some of these synthetic insecticides are consequences that are leading to a reappraisal of

naturally occurring compounds as substitutes and the evaluation of improved methods of pest management. Biologically active compounds of food plants are environmentally more acceptable and less hazardous than others to humans (Shaaya et al., 1991; Ryan et al., 1978; Ryan and Byrne, 1988). Natural repellents especially those derived from food plants are attracting considerable attention as an alternative mode of affording protection.

Due to the conservationist nature of the present generation, repellents might as well be the method of choice in pest management because the comfort and welfare of the subjects, to whose protection it's intended, is achieved while ecosystems are left relatively undisturbed (Egler, 1964; Rivnay, 1964; Tisdale, 1963). This is in stark contrast to the insecticides which aim at a total eradication of the pest population and therefore considerably disturbs the ecosystems. This target by insecticides further aggravates the resistance problem because the pests with resistant alleles survive the application hence stand a better chance of breeding with another pest of its kind with resistant alleles thus developing a progeny of resistant pests (De Pury, 1973).

The present generation has a high regard for cosmetics and perfumery. These products are either directly derived from essential oils due to their odoriferous nature or they are industrial replicates of the sweet smelling principles in them. Considering the fact that some of these principles of essential oils show remarkable biological activity, then it is evident essential oils tend to meet the present requirement for a more cosmetically acceptable and long-lasting all-purpose repellent for

clothing impregnation (Gilbert, 1966; Kilgore and Doult, 1967).

There is the possibility of an expanded application of repellents in Integrated pest management programmes of noxious insects. Repellents might be used more widely for temporary control in limited areas where the use of an insecticide is neither economical or practical (Prakash et al., 1990), or they might be applied to some natural breeding grounds to direct insects to other areas for control by insecticides or chemosterillants (Kilgore and Doult, 1967).

1.1.4 Why Screen Plants for Repellents

There is good reason to believe that plants have survived largely because of defensive mechanisms that have evolved in time in relation to attacks by various pathogens such as viruses, bacteria and protozoa. This is particularly so for tropical plants that are confronted with much harsher conditions of survival, such as unfavorable climate and soil conditions , than their temperate counterparts. This necessarily leads to efficient built-in defense mechanisms. It is presumably for this reason that tropical flora offer a rich and intriguing source for isolating natural products possessing attractive entomological or medicinal properties (Kubo and Nakanishi, 1977; Rice, 1983; Feeny, 1975).

As Repellents, plant odors have shown a great potential. These odors can be isolated either as exudates from glandular trichomes of certain plants but most commonly as essential oils (Levin, 1973).

1.2.0 What Are Essential Oils

These are odoriferous principles of plant origin, which are mainly present in the leaves, blossoms, fruits, twigs, roots and occasionally in the wood (Simonsen, 1953; McGraw-Hill, 1987). The oils are stored as micro-droplets in glands of plants. After diffusing through the walls of the glands, the droplets spread over the surface of the plant before evaporating and filling the air with perfume. The most odoriferous plants are found in the tropics where the solar energy is greatest. Younger plants produce more oil than the older ones, but the latter produce oil which is more resinous and darker because of the continuing evaporation of the lighter fractions of the oil (Encyclopaedia Brittanica, 1991).

Commercially, essential oils are used as (i) odorants in cosmetics, perfumes, soaps, detergents and other industrial products ranging from animal feeds to insecticides, (ii) as flavors in bakery goods, confections and soft drinks, and (iii) pharmaceuticals in dental products and a wide, but diminishing, group of medicines (Encyclopaedia Brittanica, 1991).

1.2.1 The Role of Essential Oils in Plants

The odoriferous principles of leaves and flowers probably aid in natural selection by acting as attractants for certain insects to pollen (Simonsen, 1953; Encyclopaedia Brittanica, 1991).

Terpenes are by far the most dominant constituents of essential oils. Their number in plants exceeds that in any other

group of natural products. It would therefore be unrealistic to each individual member to have a specific biological function. However, terpenoid products of plant origin have been associated with adaptive significance particularly regarding their role in pest and pathogen resistance and insect deterrence or attraction (Bridges, 1987; Ishikawa et al., 1987; Harborne, 1989). With their penetrating odor and taste, the monoterpenes present in other parts of the plant , other than in the leaves or flowers, almost function biologically by repelling predators, while sesquiterpenes may provide a defence mechanism against fungal attack (Pridham, 1966).

1.2.2 Isolation of Essential Oils

The initial step in the isolation of essential oils involves crushing or grinding the plant material to reduce the particle size and to rupture some of the cellwalls of the oil-bearing glands.

Diverse methods have been used in the isolation of essential oils. Steam distillation is the most common method of production. Three different methods of steam distillation are practised. In the simplest method, a vessel containing water and the chopped or crushed material is heated by a direct flame and water vapor and the volatile oil is recovered by a water-cooled condenser or if in small quantities the oil is extracted in a light solvent such as petroleum ether or hexane (Langenau, 1948). This method is being replaced by a process in which the plant material is suspended on a grid above the water level and steam from a second vessel is introduced under the grid. The volatiles are condensed and the oil

is separated. In the third process, the vessel containing the plant material on a grid is heated to prevent condensation of the steam so that dry distillation is attained (Encyclopaedia Brittanica, 1991).

Essential oils are also extracted with cold fat. This process is applied to flowers that do not yield an appreciable amount of oil by steam distillation or whose odor is changed by boiling water and steam. Volatile solvents are also used for the recovery of such essential oils (Encyclopaedia Brittanica, 1991). Such extractions remove not only most of the perfume material but also waxes, coloring matter and resinous materials. The solvents are removed and the material re-extracted with alcohol to obtain oils of higher purity (McGraw-Hill, 1987).

Comparison of these extraction techniques reveals that steam distillation of foliage for 8 hrs in a circular still is a more reproducible technique for essential oil isolation than either solvent extraction by a light solvent such as pentane and petroleum ether or even steam distillation for longer or shorter periods of time (Moore, 1980; Muzika et al., 1990).

1.2.3 Chemical Composition of Essential Oils

Terpenes are by far the most dominant constituents of essential oils. Of these terpenes, monoterpenes are the most dominant constituents. However, some monoterpenoids are toxic to plant cells, so isoprenoid synthesis is limited by end-product inhibition. Each plant has its own tolerance level, with the

younger cells being less tolerant. This may also explain why plants have so diverse constituents. Individual oils may contain appreciable quantities of non-terpene, straight chain, aromatic or heterocyclic compounds. A few essential oils also contain nitrogen and sulphur compounds (Chamberlain et al., 1991; Encyclopaedia Brittanica, 1991; McGraw-Hill, 1987).

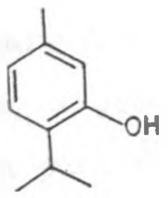
In some essential oils, one or only a few components predominate. Such are the cases for the oil of wintergreen which contains 98% of methyl salicylate and orange oil with 90% of δ-limonene. However, in most oils there is a mixture of anywhere from a few dozen to several hundred individual compounds (Encyclopaedia Brittanica, 1991).

The chemistry of the constituents of essential oils varies from plant to plant and from one geographical location to another (Anomneze and Khonje, 1981). The East indian lemon grass oil is reported to contain no myrcene, while the West indian lemon grass contains myrcene (12-20%) (Guenther, 1960). The season also influences the constitution of essential oils. The percentage of α- and ß-thujone in the essential oil of a pure clone of sage, *Salvia officinalis*, grown under cultivated conditions, increased during the season from 8% in April to more than 40% in December. At the same time the percentage of borneol decreased very sharply from 25% to 5%. The camphor percentage rose in spring from 10% to 25% and then decreased somewhat to 20% at the end of autumn (Putievsky, 1986).

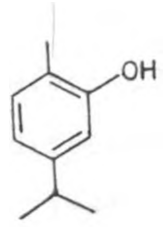
The amounts of constituents from different parts of the same plant are likely to be different. The average percentage of

methyleugenol from the whole plant, leaves, stems and flowering tops of *Cymbopogon flexuosus* Sikimensis has been reported to be 81.4, 82.4, trace and 77.6% respectively. A higher percentage of oil (1.1%) is obtained from the flowering tops than from the leaves (0.55%), though the quality of the oil is better from the leaves (Hazarika et al., 1977). Moreover, the length of storage of plants essential oils has been reported to affect the quality of the oil. The essential oil of *Cinnamon ajowan* Blume is reported to have decreased by 14% in three years. In the same period 10% of its component thymol disappeared, carvacrol decreased, while Terpinene and σ-cymol increased (Alexander and Nirmala, 1984).

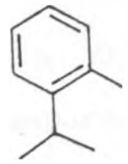
The extraction method and the duration of the extraction process has an effect on the composition of the essential oil. A comparison of the extraction methods reveals that steam distillation of foliage is a more reproducible technique than solvent extraction (Moore, 1980; Muzika et al., 1990). The composition of coniferous oils has been found to vary depending on how long the material is steam distilled. In an 8 hr steam distillation the relative amounts of oxygenated compounds and monoterpene hydrocarbons changed from equal amounts to increasing amounts of the hydrocarbons. No oxygenated compounds distilled off after about 4 hrs. The compounds distilled off in the order of their solubility in water rather than their boiling points.



Thymol



Carvacrol



σ – Cymol

1.2.4 The Chemical Analysis of Essential Oils by Gas Chromatography and Combined Gas Chromatography-Mass Spectrometry.

Gas chromatography is one of the most important tools in the analysis of essential oils. Because of the characteristic instability of terpenes i.e easy polimerization due to the presence of double bonds, and dehydration of tertiary alcohols, it is therefore necessary to determine carefully the most suitable gas chromatographic conditions to achieve good separation. Although GC is very suitable in the separation of monoterpenoids and generally sesquiterpenoids, the analysis of the latter is complicated by the lower volatility of these compounds and their occurrence in nature as complex mixtures (Heftmann, 1967).

One of the principle factors influencing separation is a suitable stationary phase. For the separation of monoterpenoids and sesquiterpenoids, a great number of different nonpolar and polar phases have been published (Heftmann, 1967). The most frequently recommended nonpolar phases are Squalen and Silicone elastomers. More polar phases, polyethylene and polypropylene glycol as carbowax are also common. It is however difficult to make the best choice because no phase is universal and even the working conditions will affect the separation.

Due to the instability of terpenes, their decomposition products formed in the preheater zone of the column may cause some difficulties (Golab and Layne, 1962). So there is the need to use lower column temperatures and consequently increase the detector sensitivity and thus reduce in effect the amount of the sample required in the analysis. The incorporation of the flame ionization detector, which is sensitive to organic compounds, thus constituted an important step forward in the qualitative analysis of terpenoids.

For the analysis of complex mixtures of terpenes, chromatography in a capillary column is very advantageous. However some of the components present in a natural essential oil in very low quantities may escape notice while the individual components can not be obtained preparatively. This method is not applicable to the identification of unknown compounds, and even the identification of the known compounds may be difficult because authentic samples of terpenic compounds are very difficult to obtain. For less complex essential oils whose chromatographic

separation can easily be achieved, the combined use of preparative gas chromatography, combined Gas chromatography-mass spectrometry, Infrared spectroscopy(Ir) and Nuclear magnetic resonance spectroscopy(Nmr) can lead to the identification of new compounds (Heftmann, 1967).

The sensitivity of the mass spectrometer and the resolving power of the combination Gas chromatography-mass spectrometer (GC-MS) has made mass spectrometry a useful tool in the elucidation of the structure of diminishingly small concentrations of compounds isolated in essential oils. Such compounds are particularly sensitive to GC-MS analysis because of their inherent volatility (Regnier, 1972). Terpenoids usually lack strong fragmentation directing groups and only in favourable cases may complete structure assignment be made by mass spectra alone. But coupled with co-chromatography, identification can be achieved. When run under the same conditions the mass spectra of terpene isomers, although generally very similar, frequently exhibit small but significant differences in the relative intensities of some peaks (Waller, 1972). Such differences can be used in the identification of the compounds.

1.3.0 The Brown Ear Tick, *Rhipicephalus appendiculatus* Neumann (Acarina: ixodidae)

The Brown ear tick, *Rhipicephalus appendiculatus*, whose preferred feeding site is the host's ear, is the chief vector of the heamoprotozoan *Theileria parva*, the causative agent of east

coast fever (ECF) in cattle and nairobi sheep disease (Nairovirus), both of which are veterinary problems of considerable magnitude and hence a major constraint to livestock production and development in Africa (FAO, 1984; Varma et al., 1974).

This important tick occurs from sea-level to about 2300 m altitude in shaded woodland or shrubby grassland with atleast 400 mm annual rainfall from southern Sudan and eastern Zaire to Kenya and South Africa (Gaafar et al., 1985).

Although there has been a belief that ECF is caused by *Theileria parva*, the biological complexity of this organism led to the recent introduction of a trinomial classification which recognizes *T. parva parva*, *T. parva lawrencei* and *T. parva bovis*. The first agent causes virulent ECF, while the second and third agents cause corridor disease and Zimbabwean malignant theileriosis respectively (Uilenberg, 1981). *R. appendiculatus* also transmits related theileria species such as *T. taurotragi*, *Ehrlichia bovis*, the agent of bovine ehrlichiosis, and *Rickettsia conori*, the agent of human tick typhus. Heavy infestation by Nairovirus causes severe toxaemia, which reduces the immunological competence and resistance to other infections and may lead to death. This is also marked by severe damage to the hosts ears, tail and udders (FAO, 1984; Gaafar et al., 1985).

1.3.1 Control of Ticks

Global socio-economic conditions have enabled the evolution of integrated tick control practices in which natural predators,

genetic resistance in cattle, organic acarides and extract from local plants play their respective roles.

Birds have provided partial tick control even before the parasite was recognized as a problem. Cattle egrets, *Bubulcus ibis*, as the constant daytime companion of the cattle, picks the arthropods from the vegetation disturbed by the walking cattle and also picks from the body of the host itself (Mansingh and Hammond, 1978).

Chemical control is the most widely practised. This at the moment is accomplished by the use of synthetic or natural acaricides / tickicides, applied in dips, dust, sprays or released from plastic bags and collars (Dipeolu et al., 1992, Mathawson, 1984). Ticks may also be controlled off the host by applying acaricides to the environment (FAO, 1984).

Many synthetic acaricides have so far been used to control ticks. The first widely used acaricide was arsenic. As arsenic trioxide (As_2O_3), it is water soluble. This has however been replaced by other acaricides amidst complaints of progressive ineffectiveness due to presumed/suspected development of resistance in the ticks (Rawlins and Mansingh, 1977a,b). Toxaphene (Barnard et al., 1983) and lindane, chlorinated hydrocarbons, are generally very stable. They provide a good kill on cattle and long periods of protection. Dioxathion (bercotex) (Wharton et al., 1969; Tatchell et al., 1969), coumaphos (asuntol) and diazino (Rawlins and Mansingh, 1977a), organophosphates, are acaricides which are generally highly effective at low concentrations, stable and safe when used directly and give adequate residual protection.

Carbamates, such as carbaryl; tick detachment agents, such as amitraz; synthetic pyrethroids such as permethrin, cypermethrin and decamethrin. clenpyrin and also chloromethiuron are also in use (FAO, 1984).

Repellents such as benzyl benzoate, dimethyl phthalate (DMP) and N, N-diethyl toluamide (deet) are also effective in the control of ticks (Hardani, 1971).

1.3.2 The Potential of Natural Products in Tick control

Due to the decreasing effectiveness of acaricides occasioned by the development of resistant strains of ticks (Solomon, 1983) coupled with the environmental pollution by acaricides, increasing attention is being focussed on natural products so as to exploit its virtues in tick control.

Although there has been some advances in the use of natural products in the control of insect pests, very little has been done to study the effects of these botanicals on ticks. However, there are some indications of the potential of natural products as tick repellents and tickicides. Essential oils of two shrubs, *Gynandropsis gynandra* (Capparidaceae) and a Labiate species have been found to exhibit repellent activity against adult *Rhipicephalus appendiculatus* (Moreka et al., 1991). Further studies showed that all the stages of *R. appendiculatus* and *Amblyomma variegatum* are repelled by the same plant. Some ticks which were continuously exposed to the leaves of the plant died, while surviving ones were found to be weak and inactive.(Malonza et al.,

1992). Some pasture plants have also been identified. Those of the genus stylosanthes secrete a sticky material from their glandular trichomes that trap and immobilize larvae of *Boophilus microplus* and prevent them from ascending the stems. The plants found most effective were *S. viscosa*, *S. scabra* and *S. guianensis* (Sutherst and Wilson, 1986; Sutherst et al., 1988). The Black sage, *Cordia curassiva*, (Smith, 1974) and the Guinea hen weed, *Petivaria alliacea* L. are used in tick control. Both the aqueous and organic solvent extracts of the weeds were reported highly toxic to engorged *B. microplus* adults when sprayed under the potters tower.

Some natural compounds have been isolated and reported promising. A tickicidal compound, 12a-hydroxy rotenone, has been isolated from the hexane extract of the root of the plant, *Neorautanenia mitis* A.Rich., which is known for its poisonous nature as a fish poison, insecticide and a remedy for syphilis in central Africa (Puyvelde et al., 1987). On the other hand some concoctions have been effective. A mixture of ground tobacco leaves (solanaceae) and magadi soda was found an effective tickicide against all stages of *R. appendiculatus*. This mixture containing Nicotine as the active principle prevented the completion of all the feeding phases of the tick, suppressed the oviposition capacity of engorged ticks and drastically reduced the hatchability of the eggs. Larvae and nymphs are killed within 2-3 days of application in invitro studies (Dipeolu et al., 1991).

1.4.0 The Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: curculionidae)

The maize weevil, *Sitophilus zeamais* Motsch., is a primary and most destructive pest of maize, rice and other grains, and their processed products. It is more cosmopolitan in distribution from tropical areas to temperate zones (Maeshima et al., 1985; De Pury, 1973; Tipping et al., 1987) and therefore causes serious economic losses of grain throughout the world (Phillips et al., 1985).

Infestation of produce typically starts in the field and is latter carried to the stores. This is especially so if the moisture content of the stored grain is high, atleast 12.5%. Damage is caused by the larvae which bores a thin tunnel from the surface towards the inside of the grain kernel (Hill and Waller, 1990; De Pury, 1973).

The adult maize weevil are brownish-black with the head prolonged into a typical snout or rostrum. The tropical species fly readily. They mate and lay eggs very rapidly and increase their numbers very quickly (Hill and Waller, 1990; De Pury, 1973).

1.4.1 Control of Storage Pests

Tropical climates offer storage pests optimal temperature and humidity conditions for their development and reproduction. This is why these pests are a serious problem to the growth of the agro-based economies of the tropical countries. In the northern latitudes, the spring and winter temperatures are below the threshold required for insect development and a combination of storage hygiene and fumigation in the summer months is sufficient for pest control (De Lima, 1987). It is therefore imperative that

control measures be placed into effect to save the grim picture in the tropics.

The control methods in practice can be classified as hygienic, physical, chemical or biological (Taylor, 1976). The most promising of the physical methods is the manipulation of temperature both cooling and heating (Evans, 1981) and the storage of grain in controlled atmospheres using either carbon dioxide or nitrogen (Shejbal, 1980; Ripp, 1984; Banks, 1981). Chemical methods must however form the spearhead of attack against pests because the alternatives, biological and physical control methods are ineffective, of limited use or even very expensive (Howe, 1978).

The types of pesticides used are as diverse as the species of stored-product pests are numerous. Malathion, pirimiphos-methyl, chlorpyrifos-methyl, methacrifos, deltamethrin, fenvalerate, bioremethrin, carbaryl, lindane, aluminium phosphide, methyl bromide, ethylene bromide and iodofenphos have been in use (Anon., 1978,1980; Morah, 1980; Hindmarsh et al., 1978; Golob, 1984; La Hue, 1976; Arthur et al., 1990; Arthur, 1992; Samson and Parker, 1989; Samson et al., 1989).

Pirimiphos-methyl is very suitable in minimizing insect damage by *Sitophilus zeamais* (La Hue, 1975; Golob, 1984; Ivbijaro, 1981). Phosphine is the major fumigant used for the disinfestation of cereal grains (Giles, 1984) particularly in the tropical and subtropical regions where the warm conditions enhance its effectiveness (Taylor and Halliday, 1986). While for the contact insecticides, malathion , a post-harvest residual grain protectant, gives satisfactory control of the major pest species of coleoptera

(Winks and Bailey, 1965). The most effective and cheap insecticide for the protection of stored maize cobs is lindane (Kockum, 1958,1965; Davis, 1960; Forsyth, 1962; Adeyemi, 1967).

1.4.2 Natural Products in Storage Pest Control

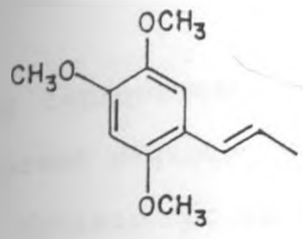
Stored product losses resulting from storage pests have been prevented predominantly through the use of pesticides. The development of resistance to widely used pesticides (Haliscak and Beeman, 1983; Horton, 1984) and concerns over chemical residues in foods dictates that alternative safe and biodegradable chemicals be investigated. This necessity has led to concerted international efforts at developing new sources from the vast store of chemical substances in plants. In a storage ecosystem, the use of plant products have many advantages over synthetic pesticides. Above all is their relative safety (Pereira and Wohlgemuth, 1982)

Essential oils of some plants and some of their components have been found effective against some stored product pests. The essential oils of Oregano, Basil, Syrian marjoram and Thyme were reported active against *Oryzaephilus surinamensis*. Sage oil was active against *Sitophilus oryzae* while the essential oils of Anise and peppermint were active against *Triboleum castaneum*. Among the components of the essential oils identified, linalool, α-terpineol and carvacrol were found most active against *O. surinamensis* while 1,8-cineole was active against *Tribolium castaneum* (Shaaya et al., 1991). The essential oil of *Dennetia tripelata* G. Baker, which grows as a shrub in southern Nigeria, is reported very effective in

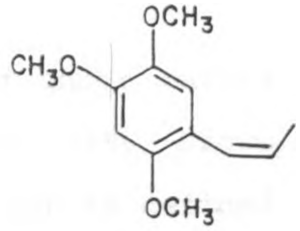
protecting cow peas and maize against *Callosobruchus maculatus* F. and Sitophilus zeamais Motsch., respectively (Osisiogu and Agbakwuru, 1978).

The insect repellent and antifeedant properties of neem, *Azadirachta indica* A. Juss., are well known. Neem leaves and cake are repellent against insect pests of stored wheat (Pruthi, 1937). Neem seed powder (1-2%) mixed with wheat grain provided protection for insect pests for 9-12 months (Jotwani and Sircar, 1965). The water and ethanol extracts of the leaves and seeds of neem repell the red flour beetle, *Tribolium castaneum* Herbst, the kharpa beetle, *Trogoderma granarium* Everst, and the lesser grain borer, *Rhyzopertha dominica* (F.) (Jilani and Malik, 1973). The insect repellent and antifeedant action of neem is attributed to the triterpenoid, azadirachtin and other compounds (Butterworth and Morgan, 1968; Zanna et al., 1975; Nakanishi, 1975). Azadirachtin also has insect growth regulating activity and is reported to have no mutagenic effect (Rembold et al., 1981; Jacobson, 1981).

The rhizomes of tumeric, *Curcuma longa* (L.) have also been used as an insect repellent (Screenivasamurthy and Krishnamurthy, 1981; Jilani, 1985). Tumeric contains pungent, odoriferous oils and oleoresins. The insect repellent constituents of tumeric are tumerone and *ar*-tumerone (Su et al., 1982). Tumeric powder repelled the granary weevil, *Sitophilus granarius* (L.) and *R. dominica*, whereas its petroleum ether extract was repellent against *Tribolium castaneum* (Jilani and Su, 1983).



α — Asarone



β — Asarone

The extract of sweet flag, *Acorus calamus* (L.) rhyzomes is toxic to the rice weevil, *Sitophilus oryzae*, *Tribolium* castaneum (Paul et al., 1965) and the long-headed flour beetle, *Letheticus oryzae*. Sweet flag oil completely stopped the development of *R. dominica*, *S. oryzae* and the anguomois grain moth, *Sitotroga cereallela* (oliver) in stored wheat (Jilani and Haq, 1984). The active component in sweet flag is asarone (Baxter et al., 1960).

1.5 Pest Resistance to Pesticides

Living organisms can carry on with their vital functions with little or no impairment in the presence of a chemical upto some level of concentration or amount. This level depends upon the species, the chemical, the method of exposure and the criterion of effect, but with these fixed it becomes a measure of the important natural resistance or tolerance of the species (Hoskins and Gordon, 1956).

Tolerance is determined by a great variety of factors such as the permeability of the integument which is a function of its anatomical structure and thickness, the pattern of behavior which affects the degree of contact with the toxicant and biochemical reactions into which the absorbed toxicant enters. Tolerance may differ greatly among various species but does not vary very much

among representative species living under natural condition in different regions (Hoskins and Gordon, 1956; Brown, 1950).

Resistance, on the other hand, can be defined at two levels. At the level of the individual insect "resistance" refers to the biochemical mechanism(s) or the phenotypic expression of one or more genes that improve, however slightly, the ability of the individual to survive and reproduce in the presence of the pesticides (Sawicki and Denholm, 1984). At the population level resistance can denote a change in response to a pesticide, through selection, such that the selected population has individuals with an upper tolerance limit greater than that of the preceding generation (Champ, 1986) or a control failure when the recommended dose nolonger gives adequate control of pests (Sawicki and Denholm, 1984). The practical problem of resistance, that is the reduction in the effectiveness of the pesticide to control a pest population in the field, is of paramount importance, and usually resistance development is well advanced by the time control failure is noticed (Kay and Collins, 1987).

The development of pesticide resistance is an evolutionary process depending on selecting for resistant individuals within the population by applying pesticides. Factors involved in the selection process can be classified as genetic (the frequency of the resistant alleles), biological (the generation time and migration), and operational (the pesticide and its mode of application) (Georghiou and Taylor, 1977a,b). When produce is sprayed those pests carrying the resistant genes survive. When the survivors breed, a large number of their offspring will have this

gene, escape the next spray, and the process will be repeated. After several generations there will exist a population, all resistant to the pesticide (De Pury, 1973). It is therefore inevitable that resistance will develop to a pesticide provided that the alleles conferring resistance are present and selection pressure is applied by using the pesticide.

In places where pesticides from all the major groups have been applied as a routine, and at relatively frequent intervals, coupled with the movement of pests in trade has left us a legacy of resistance that has become an international problem. A recent survey of resistance in the field in the major pests of stored grain (Champ, 1986; De Lima, 1987) revealed that DDT, lindane and Malathion resistance occurs in strains of the rice weevil, *Sitophilus oryzae* (L.), the maize weevil, *S. zeamais* Motsch., the red flour beetle, *Tribolium castaneum* (Herbst) and the saw toothed grain beetle, *Oryzaephilus surinamensis* (L.). lindane and malathion resistance also occur in the merchant grain beetle, O. mercator (Fauvel) and the lesser grain borer, *Rhyzopertha dominica* (F.). Strains of *S. oryzae* and *T. castaneum* are also resistant to dieldrin, pyrethrins, various pyrethroids, carbaryl and a range of organophosphorus insecticides (Kay and Collins, 1987). Of more significance in resistance is the recent development of resistance of the more resistant stages of various storage pests particularly the eggs and the pupae to phosphine (Dyte et al., 1983, De Lima, 1987), the fumigant most commonly used for the control of stored grain pests. Phosphine is easy to use and effective. However, it's often applied ineffectively. This has led to repeated underdosing

which allows some insects to survive hence select for resistance.

Resistance of ticks to acaricides is also known to occur. *Boophilus microplus*, a 1-host tick, is reported to have developed resistance against efficacy of BHC (Whitnall et al., 1952; Hitchcock, 1953), toxaphene and dieldrin (Norris and Stone, 1956; Whitehead, 1965), triazapentadiene, amitraz, the thiourea, chloromethiuron and the thiazine, cymiazole (FAO, 1984).

It is therefore essential that pesticide use be managed to minimize the development of resistance with its adverse effects on pest control programmes. Selection pressure should be reduced by employing the full range of techniques available in Integrated pest management (IPM), including the carefully managed choice of pesticides and their modes of application (Metcalf, 1983; Georghiou, 1983).

1.6.0 Capparidaceae (Capparaceae)

Capparidaceae is a medium sized family of tropical and subtropical plants that are well represented in Africa and form a conspicuous element of the dry country flora and are virtually confined to these regions (Kuchar, 1981; Ellefers and Dewolf, 1964).

This family comprises of 30 genera and 650 species. There are 11 genera represented by 92 species in east Africa. 10 genera are found in Kenya and are represented by 54 species (Elleffers and Dewolf, 1964; Kuchar,1981). They are mostly large shrubs, very rarely herbaceous plants with a firm hairy or leathery leaves that

can also feel slightly fleshy (Kuchar, 1981).

Some seed oils of boscia, capparis and cleome genera contain sulphur compounds. They are irritating and usually cause reddening and even vesication on the skin, and also act as carminatives in small doses or as emetics in large doses (Kokwaro, 1976). The isothiocyanates, in particular, produce allergic contact dermatitis (Mitchell, 1974).

Many capparids indicate soil calcium and few indicate saline soils (Elffers et al, 1964; Kuchar, 1981). Many capparids are in large part not palatable or atleast not relished by animals and many species are avoided entirely (Kuchar, 1981).

1.6.1 *Boscia angustifolia* A. Rich. var. *angustifolia*

This member of capparidaceae is a small evergreen tree upto 10 m tall with a spreading rounded crown and smooth grey or silvery bark. The leaves are alternate and the fruits spheroid or slightly ellipsoid. The leaves are always completely glabrous beneath (Elffers et al., 1964).

The fruit is edible but somewhat bitter while the seeds are eaten when cooked. The fruit is crushed in water and given as a purgative. It is also used for colds and snake bites (Kuchar, 1981). The leaves are repellent to *Locusta migratoria* (Bernays and Chapman, 1977).

This species habits deciduous woodland and bushland, and grassland with scattered trees (Elffers et al., 1964).

1.6.2 *Boscia mossambicensis* Klotzsch

This member of capparaceae is a dense, evergreen shrub or tree, upto 10 m tall, often with a rather flat crown. The leaves alternate and are clearly spaced. The twigs are glabrous. The fruits are spheroid, smoothly verrulose, glabrous and rather shiny (Elffers et al., 1964).

The fruit is edible while the leaves are commonly chewed for sore throat. The leaves are also used for cleaning milk gourds to eliminate bad odor (Kuchar, 1981).

This plant habits deciduous woodland, bushland and thicket, and grassland with scattered trees (Elffers et al., 1964).

1.6.3 *Cadaba farinosa* Forsk

This is a slender, much branched and rather twiggy and tangled shrub with arching branches upto 5 m tall. The leaf blade is elliptic to roundly elliptic. The fruits are narrowly cylindrical, obscurely torulose and farinaceous. Seeds are surrounded by a red or orange membrane or flex (Ellfers et al., 1964).

All parts of the plant are used medicinally for coughs and colds, chicken pox, anthrax, uterine obstructions, anthelmintic especially roundworm, stimulant and as an antidote for poisoning. A decoction of the leaves is drunk for the treatment of gonorrhea. The root is reportedly poisonous (Kokwaro, 1976; Kuchar, 1981).

This plant habits deciduous bushland, grassland with scattered

trees, semi-desert shrub and riverine vegetation (Elffers et al., 1964).

1.6.4 *Thylachium africanum* Lour

This capper is a shrub or small tree, rarely a dwarf shrub with tuberous roots. The stems are branched or several slender unbranched stems arise from thickened rootstock. The leaves are glabrous and the fruits ellipsoid (Elffers et al., 1964).

Concoctions of this plant have been used as a cure for pain in the body. The bark is bitter to taste and the roots are used for snake bite treatment (Kokwaro, 1976).

This plant habits deciduous woodland, bushland and thicket, grassland with scattered trees, riverine forest and old cultivated areas (Elffers et al., 1964).

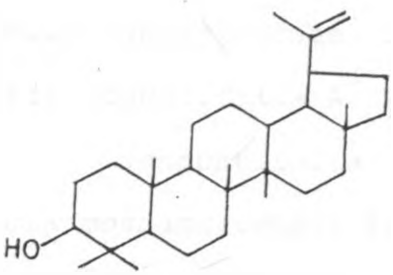
1.6.5 *Gynandropsis gynandra* (L.) Brig

This is an annual herb, branched and rather stout, upto 1 m tall. It's a flowering plant commonly found in the tropical and subtropical climate on waste, disturbed or cultivated grounds, roadsides, perhaps wild on rocky shallow soil in deciduous bushland (Grainage and Ahmed, 1988; Elffers et al., 1964). The stem is thickly glandular, more rarely varying to glabrous. The leaves are petiolate, while the seeds are dark brown, finely longitudinally straite with low or slightly cristate transverse ridges (Elffers et al., 1964).

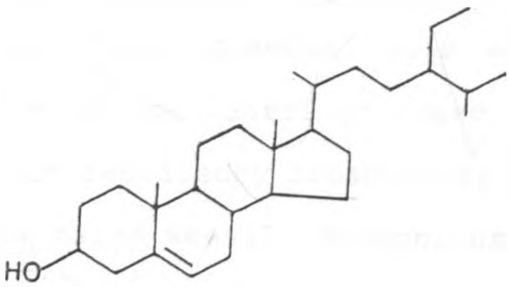
The leaves are used as foods for humans. As a vegetable, it is reported to have a high leaf protein (Imbamba, 1973). The leaves yield lupeol, ß-sitosterol and kaempherol (Ramiah and Nair, 1984). The leaves, seeds and oil are medicinal. The seed is reported to have phenolic components (Jain and Gupta, 1985). The oil has been used as an insect repellent against headlice, *Pediculus humanus capitis* and as a general vermicide in hairdressing (Jacobson, 1975; McIndoo, 1945; Mitchell and Brandwijk, 1962). The oil exhibits repellent activity against *Rhipicephalus appendiculatus*. The highest repellency of 90.9% being obtained at a dose of 0.1 ml per 25 cm long metal rod (Moreka et al., 1991). The leaves of the plant also repel all stages of *R. appendiculatus* and *Amblyomma variegatum*. Ticks which were continuously exposed to the leaves of the plant died while the surviving ones were weak and inactive. The effect is more pronounced on nymphs than adults (Malonza et al., 1992). The seeds and oil are antinematode (Usher, 1973). The whole plant is a fish poison, alkaloids being the active principles (Chopra and Badhar, 1940; Quisumbing, 1947; Anon., 1960; Smolenski et al., 1975). The alcoholic extract of this plant is reported to exert maximal anti-inflamatory activity in carrageenin induced inflamation, to reduce cotton pellet granuloma and to inhibit phospholipase A_2 activity in rats (Kumar and Sadique, 1987), while the petroleum ether extract at only 2% concentration gave 100% mortality to insect pests of cruciferous painted bug, *Bagrada cruciferanum* (Verma and Pandey, 1981).

Traditionally the plant has been used in treatment of rheumatism, headache, epileptic fits, stomachache, conjunctivitis,

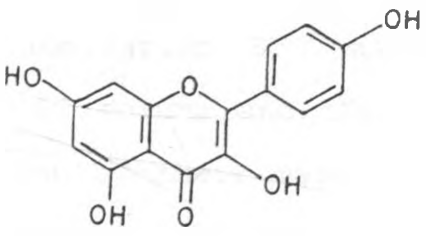
stiffneck, scurvy, earaches and severe infection of threadworms (Mitchell and Brandwijk, 1962; Kokwaro, 1976).



Lupeol



β – Sitosterol



Kaempherol

1.7 Aim of the Project

The aim of the project was four-fold:-

1) To isolate the essential oils from leaves of five tropical plants of the family capparidaceae, namely:

a) *Boscia angustifolia* A. Rich. var. *angustifolia*

b) *Boscia mossambicensis* Klotzsch.

c) *Cadaba farinosa* Forsk.

d) *Gynandropsis gynandra* (L.) Brig.

e) *Thylachium africanum* Lour.

The isolation of these essential oils was to be carried out by hydrodistillation of the leaves of these plants.

2) To carry out repellency bioactivity studies of the essential oils against the maize weevil, *Sitophilus zeamais* Motsch., by the Y-tube olfactometer method (Hassanali et al., 1990) and also against the brown ear tick, *Rhipicephalus appendiculatus* Neumann by the Tick climbing method.

3) To characterize the components of the essential oils by their electron impact ionization (EI^+) mass spectral data, their order of elution on the GC column and their GC relative retention time values, comparison of their mass spectra and GC retention times with those of authentic samples and finally co-injection with these authentic samples.

4) To carry out the repellency bioassay of the identified compounds in the essential oils against the maize weevil as well as the brown ear tick using the same methods as in 2 above.

CHAPTER 2.

RESULTS AND DISCUSSION.

2.1: Extraction of the Essential Oils.

The essential oils of the aerial parts of the five tropical plants of the family capparidaceae namely *Boscia angustifolia* A. Rich var. *angustifolia*, *Boscia mossambicensis* Klotzsch, *Cadaba farinosa* Forsk, *Gynandropsis gynandra*(L.) Brig and *Thylachium africanum* Lour were isolated by hydrodistillation using a modified clavenger apparatus as described under section 3.5. This process yielded oils of various essences and percentage yields(fresh weight basis) as shown in Table 1.

It is evident from Table 1 that *G. gynandra* yielded more essential oil, followed by *B. angustifolia* then *T. africanum*. This could be attributed to the fact that *Gynandropsis gynandra* is an aromatic shrub whose aroma normally characterizes its habitat. *G. gynandra* is observed to spring out rapidly during the rains. Plant cells of such plants are known to produce essential oil rapidly as opposed to trees which grow rather slowly and hence do not accumulate as much essential oil as its evaporation rate is not counteracted by an enhanced production rate. This may account for

the rather low yield of oil from the rest of the plants most of which are trees and furthermore were collected during the dry season. The prevalent harsh conditions

Table 1: The percentage yields and the colours of the essential oils isolated.

Plant species	Colour of the essential oil	Percentage yield
Boscia angustifolia A. Rich var. *angustifolia*	Yellow	0.18
Boscia mossambicensis Klotzsch	Orange	0.05
Cadaba farinosa Forsk	Pale Yellow	0.08
Gynandropsis gynandra (L.) Brig	Yellow	0.26
Thylachium africanum Lour	Yellow	0.12

may have led to considerable volatilization of the leaf essential oils and hence the low essential oil yield. Lack of water during the same period may have limited plant cell biochemistry or even determined the biochemical pathway and therefore may have had its own toll not only to the essential oil production but also its composition. *Boscia angustifolia* has rather shiny leaves. This may attest to its higher oil content as compared to the other plants that were collected under the same conditions. All in all, the above results are in agreement with the expectation that shrubs on

account of their aromaticity, produce more essential oil than trees (Panasiuk, 1984).

3.2: The Biological Activity of the Essential Oils.

The repellency bioactivity of the essential oils was carried out on the brown ear tick, *Rhipicephalus appendiculatus*, and the maize weevil, *Sitophilus zeamais*, using the Tick climbing repellency method and the Y-tube olfactometer method, respectively. These methods are previously described in section 3.8.1 and 3.8.2, respectively. The results of the repellencies of the essential oils utilizing these respective repellency bioassay methods compared with those elicited by the commercial arthropod repellent N, N-diethyl-*m*-toluamide(deet) are as stipulated in Tables 2 and 3 for each appropriate method and corresponding test organism.

In the Tick climbing repellency bioassay against the brown ear tick, *Rhipicephalus appendiculatus*, the most effective essential oil at the highest dose level (0.1) was that of Boscia mossambicensis which had a percentage repellency of 100. This was closely followed by the essential oils of *Gynandropsis gynandra* (98.9%), *Thylachium africanum* (96.2%), *Boscia angustifolia* (89.1%) and finally the essential oil of *Cadaba farinosa* (73.2%). It is noteworthy to indicate that at this dose level (0.1), all the essential oils except that of *C. farinosa* were either more repellent or closely comparable to that of the commercial arthropod repellent N, N-diethyl toluamide (Deet) which showed a percentage repellency of 84. At the 0.01 dose level only the essential oils

Table 2: The mean percentage repellencies(± s.e) of the essential oils and Deet against the brown ear tick, *Rhipicephalus appendiculatus*, using the Tick climbing method.

	Concentration(μl/μl)			
Essential oil of	0.1	0.01	0.001	0.0001
Boscia angustifolia A. Rich var. *angustifolia*	89.1±2.3	35.7±3.8	–	–
Boscia mossambicensis Klotzsch	100.0±0.0	97.8±1.0	31.3±3.0	–
Cadaba farinosa Forsk	73.2±3.2	31.5±3.0	–	–
Gynandropsis gynandra (L.) Brig	98.9±0.0	89.9±0.0	70.5±3.6	50.5±0.0
Thylachium africanum Lour	96.2±1.4	38.9±2.8	–	–
Deet	84.0±3.9	82.8±3.6	75.6±4.5	70.5±3.6

of *B. mossambicensis* and *G. gynandra* proved more effective than Deet whereas at the subsequent dose levels, the percentage repellencies of all the essential oils fell well below those of Deet, whose repellency is rather stable with dilution.

These essential oils also proved effective against the Maize weevil, *Sitophilus zeamais*, in Y-tube olfactometer repellency bioassay. The essential oils which proved more potent in their respective orders of efficacy were those of *Boscia mossambicensis* (92.8%), *Gynandropsis gynandra* (86.1%), *Thylachium africanum* (85.4%), *Boscia angustifolia* (82.9%) and then the essential oil of

Cadaba farinosa (74.3%). All the essential oils were more effective at all the dose levels tested against the maize weevil than the commercial arthropod repellent, Deet.

Table 3: Mean percentage repellencies (± s.e) of the essential oils and Deet against the maize weevil, *Sitophilus zeamais*, using the Y-tube olfactometer method.

	Concentration(μl/μl)			
Essential oil of	0.1	0.01	0.001	0.0001
Boscia angustifolia A.Rich var. *angustifolia*.	82.9±1.7	71.5±3.2	57.6±1.9	_
Boscia mossambicensis Klotzsch	92.8±1.5	87.6±3.8	74.0±3.9	68.8±2.5
Cadaba farinosa Forsk	74.3±2.0	64.2±6.6	62.8±1.2	44.9±6.7
Gynandropsis gynandra (L.) Brig	86.1±3.9	49.7±2.1	39.5±0.5	_
Thylachium africanum Lour	85.4±1.4	80.5±1.2	69.9±3.9	59.4±3.9
Deet	52.2±0.3	40.5±0.2	33.8±0.2	25.6±0.1

Comparing the repellency results obtained against the brown ear tick with those against the maize weevil one notes the fact that these essential oils seem in general terms more effective against the maize weevil as compared to the brown ear tick. This may be due to the rather close relationship of the maize weevil to plants and hence plant odors as compared to the brown ear tick

which is mostly associated to animals rather than plants. The brown ear tick only uses plants to expose itself to a vantage position awaiting to mount on its host so doesn't damage plant tissues to warrant specific development of defence odours against this livestock pest.

It is therefore evident from the results produced that the essential oils investigated in the present analysis have components that are effective repellents of both the brown ear tick and the maize weevil. Therefore these plant products could provide some level of protection against these species. This kind of protection is normally taken advantage of in the practice of intercropping especially so with the active food plants as happens to be for the vegetable, *Gynandropsis gynandra*, which was investigated in this analysis. This interplanting of crop plants with plants that are known to possess insect repelling properties has attracted renewed interest in Integrated pest management experiments (Latheaf and Irwin, 1980; Theunissen and Denouden, 1980; Panasiuk, 1984). Never the less, it's dangerous to assume that this is always to man's advantage (Latheaf and Irwin, 1980).

2.3: Chemical Analysis of the Essential Oils to Characterize the Biologically Active Components.

The chromatographic separation of the components of the essential oils was achieved by routine temperature programmed Gas chromatography using a crosslinked methyl silicone column. This

chromatographic separation afforded the GC profiles shown in figures 1-5 for each individual essential oil.

The chemical identification of the components of the essential oils was achieved by GC-MS and computerized mass spectral matching of the adduced spectra with those published for the pure compounds. The identity was further accertained by the GC-cochromatography of the essential oils with commercially obtained authentic samples of the tentatively identified compounds. As a consequence of these analyses 31 different compounds were identified as constituents of all five of the essential oils investigated. The mass spectra of the compounds identified are shown in figures 6-36, whereas their chemical identity and respective chemical structures are as stipulated in Table 4 and Scheme 1, respectively. Table 4 also depicts the peak numbers of these compounds corresponding directly with those on the GC profiles as well as those in Scheme 1. Quoted also are the retention times of these compounds as produced under the instrumental conditions previously described in section 3.1 and the corresponding essential oils in which these compounds were identified and their various percentage compositions in the respective essential oils.

Figure 1: The GC profile of the essential oil of *Boscia angustifolia* A.Rich var.*angustifolia*. Column: 50m x 0.32mm x 0.17μm crosslinked methyl silicone. Temperature program: 45°c(5mins.) to 180°c(0mins.) at 5°c/min, then to 280°c(15mins.) at 20°c/min.

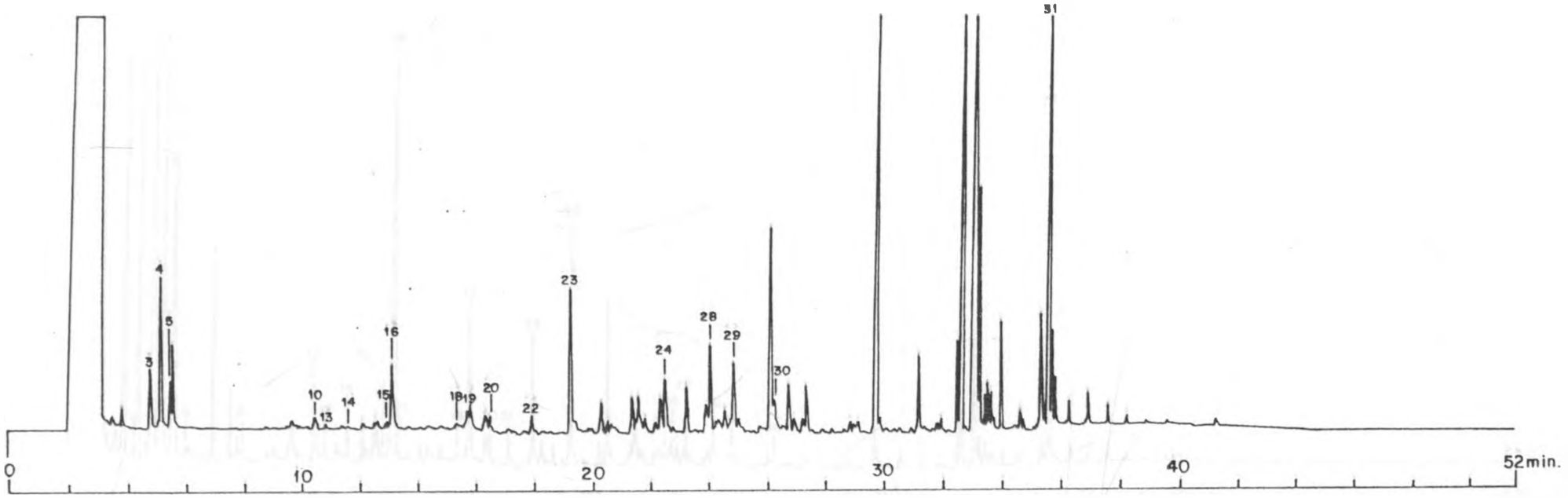


Figure 2: The GC profile of the essential oil of *Boscia mossambicensis* Klotzsch. Column: 50m x 0.32mm x 0.17μm crosslinked methyl silicone. Temperature program: 45°c(5min.) to 180°c(0min.) at 5°c/min then to 280°c(15mins.) at 20°c/min.

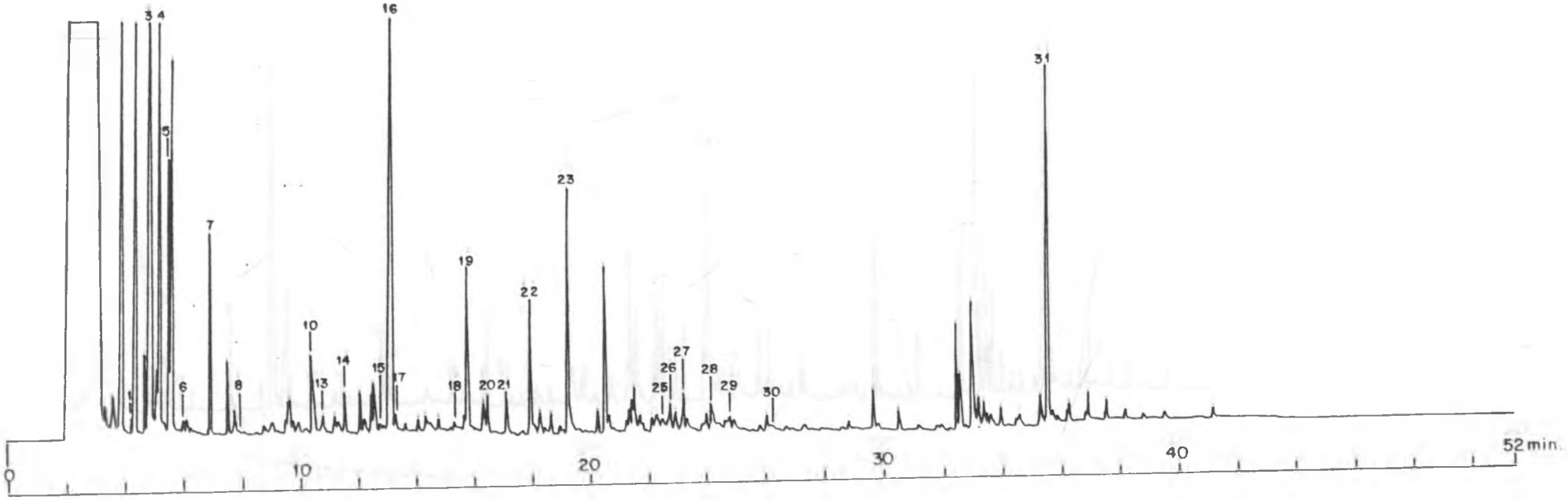


Figure 3: The GC profile of the essential oil of *Cadaba farinosa* Forsk.Column: 50m x 0.32mm x 0.17μm crosslinked methyl silicone. Temperature program: 45°c(5min.) to 180°c(0min.) at 5°c/min, then to 280°c(15mins.) at 20°c/min.

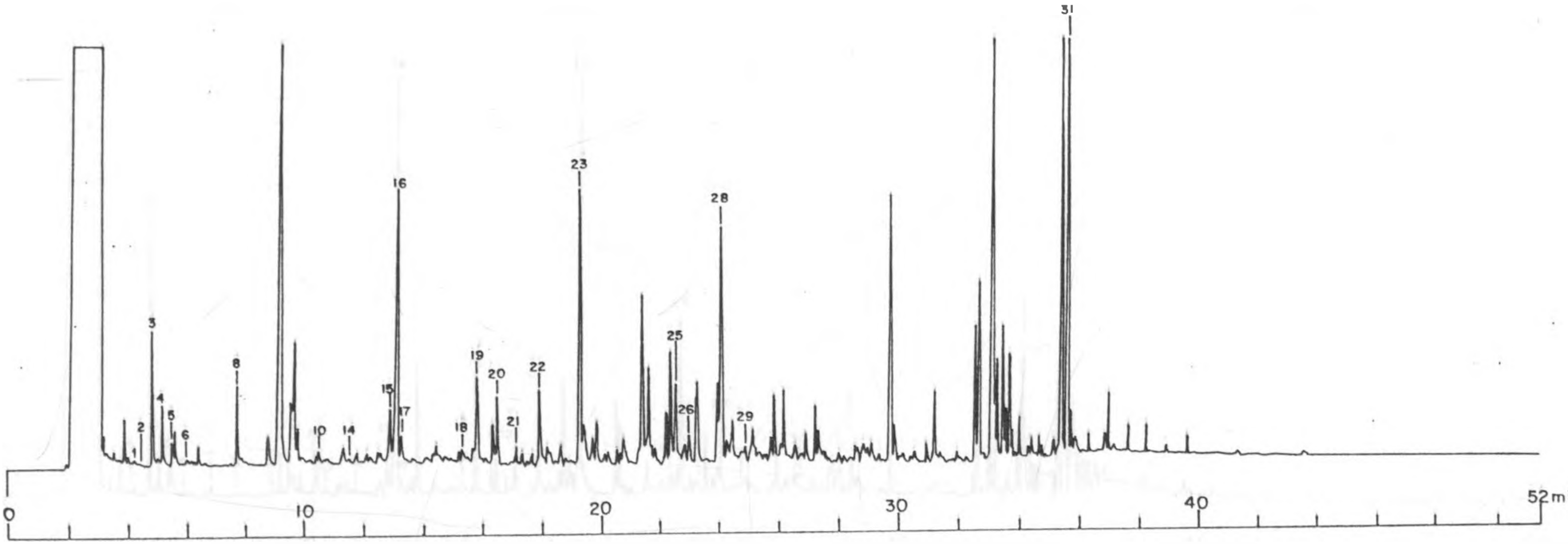


Figure 4: The GC profile of the essential oil of *Gynandropsis gynandra* (L.) Brig. Column: 50m x 0.32mm x 0.17μm crosslinked methyl silicone. Temperature program: 45°c(5min.) to 180°c(0min.) at 5°c/min, then to 280°c(15mins.) at 20°c/min.

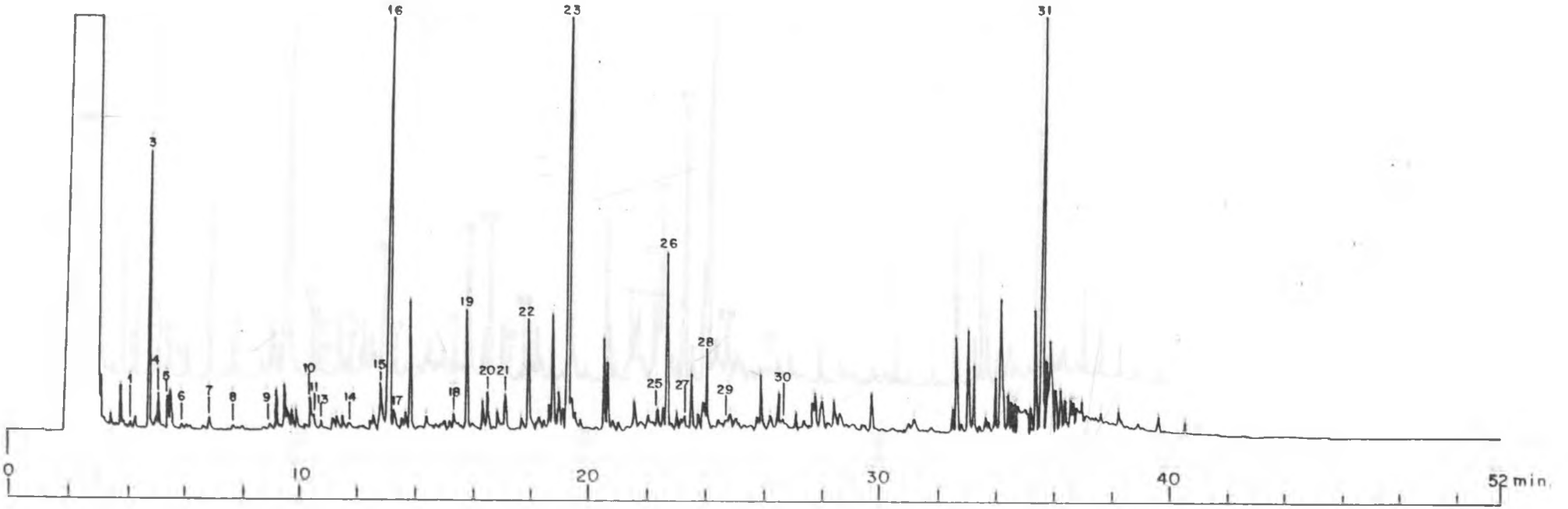


Table 4: The identity of the compounds, their retention times(Rt) and their respective percentage compositions in the essential oils.

Peak #	Compound identity	Rt(min.)	Essential oil of				
			Boscia angust.	Boscia mossam.	Cadaba farino.	Gynand. gynand.	Thylach africa.
1	Methyl isothiocyanate	4.97	–	3.30%	–	0.11%	1.41%
2	2-Hexanol	5.38	–	–	trace	–	–
3	*trans* 2-Methyl-cyclopentanol	5.52	0.43%	30.72%	3.79%	4.01%	10.04%
4	*cis* 3-Hexen-1-ol	5.90	1.39%	3.78%	1.53%	0.52%	0.44%
5	*trans* 2-Hexen-1-ol	6.27	0.42%	1.94%	0.32%	0.52%	0.21%
6	Heptan-2-one	6.49	–	trace	0.20%	0.14%	trace
7	Anisole	7.64	–	–	–	0.21%	2.16%
8	Benzaldehyde	8.47	–	–	2.13%	0.21%	trace
9	2,4,5-Trimethyl-thiazole	9.82	–	–	–	0.19%	–

Table 4 cont'd

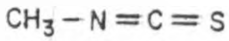
Peak #	Compound identity	Rt(min.)	Essential oil of				
			Boscia angust.	Boscia mossam.	Cadaba farino.	Gynand. gynand.	Thylach africa.
19	α-Terpineol	17.13	0.55%	2.38%	1.36%	1.85%	0.36%
20	ß-Cyclocitral	17.87	0.26%	0.22%	0.81%	0.48%	1.20%
21	Nerol	18.47	_	0.74%	0.71%	0.55%	trace
22	*trans* Geraniol	19.27	0.33%	2.61%	1.07%	1.66%	trace
23	Carvacrol	20.61	2.73%	4.37%	3.22%	15.40%	3.24%
24	α-Cedrene	23.96	1.18%	_	_	_	_
25	α-Ionone	23.96	_	trace	1.24%	0.60%	0.90%
26	ß-Caryophyllene	24.15	_	_	0.31%	2.55%	3.08%
27	*trans* Geranylacetone	24.71	_	0.93%	_	0.20%	3.26%
28	ß-Ionone	25.71	1.16%	0.61%	2.35%	1.33%	5.21%

Table 4 cont'd

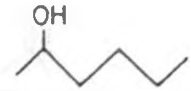
Peak #	Compound identity	Rt(min.)	Essential oil of				
			Boscia angust.	Boscia mossam.	Cadaba farino.	Gynand. gynand.	Thylach africa.
29	Tridecanal	26.32	0.94%	trace	trace	trace	1.12%
30	Nerolidol	27.61	0.64%	trace	_	trace	trace
31	*trans* Phytol	36.76	35.77%	7.58%	5.42%	12.92%	8.53%

Boscia angust.= *Boscia angustifolia* A. Rich. var. *angustifolia*
Boscia mossam.= *Boscia mossambicensis* Klotzsch
Cadaba farino.= *Cadaba farinosa* Forsk
Gynand. gynand.= *Gynandropsis gynandra* (L.) Brig
Thylach. africa.= *Thylachium africanum* Lour
Rt = Retention time.

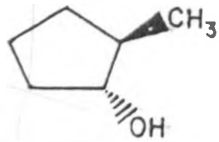
Scheme 1: The chemical structures of the compounds isolated in the essential oils.



(1) Methyl isothiocyanate



(2) 2 – Hexanol

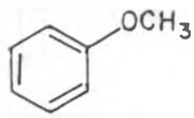


(3) trans 2 – Methylcyclopentanol

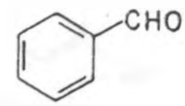
(4) cis 3 – Hexen – 1 – ol

(5) trans 2 – Hexen – 1 – ol

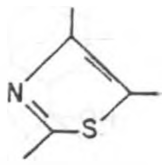
(6) Heptan - 2 - one



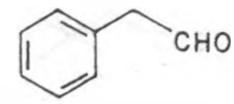
(7) Anisole



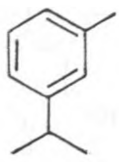
(8) Benzaldehyde



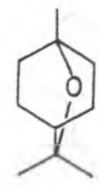
(9) 2, 4, 5-Trimethylthiazole



(10) Phenylacetaldehyde



(11) m – Cymene

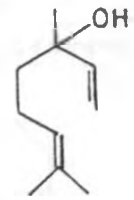


(12) 1 , 8 – Cineole

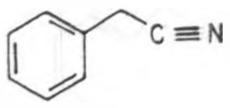
(13) d – Limonene

(14) β – Ocimene

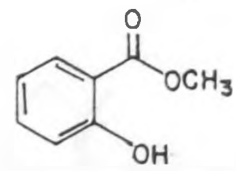
(15) Nonanal



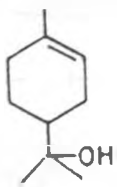
(16) Linalool



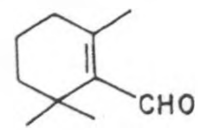
(17) Phenylacetonitrile



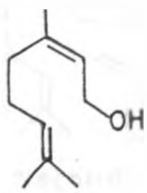
(18) Methyl Salicylate



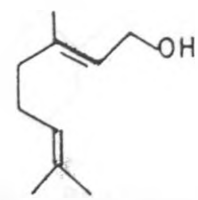
(19) α – Terpineol



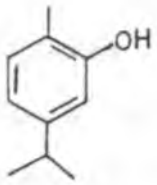
(20) β – Cyclocitral



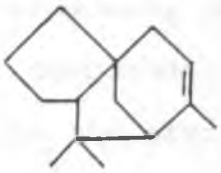
(21) Nerol



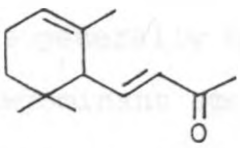
(22) trans Geraniol



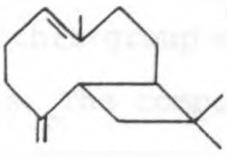
(23) Carvacrol



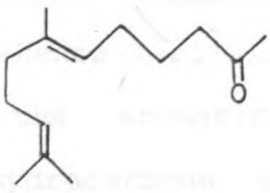
(24) α — Cedrene



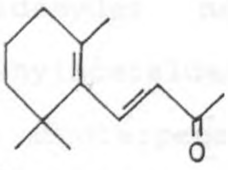
(25) α — Ionone



(26) β — Caryophyllene

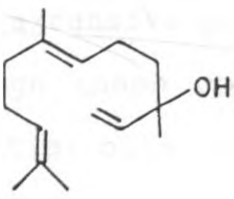


(27) trans Geranylacetone

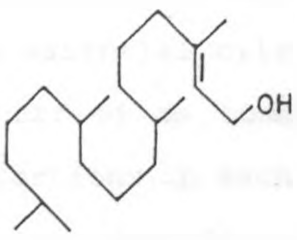


(28) β — Ionone

(29) Tridecanal



(30) Nerolidol



(31) trans Phytol

A close examination of the GC profiles of the essential oils shown in Figures 3-7 and their corresponding peak identities reveals that a large portion of the compounds identified and possibly those unidentified, judging from the similarity of their mass spectra, are common to most of these species investigated. Most of the compounds that were identified were terpenes. This confirms the generally held finding that this group of compounds is the most predominant among essential oils. The compounds that were identified to be common to all these essential oils were the aliphatic alcohols, *trans* 2-methylcyclopentanol, *cis* 3-hexen-1-ol and *trans* 2-hexen-1-ol; the aliphatic aldehydes, nonanal and tridecanal; the aromatic aldehyde, phenylacetaldehyde; the monoterpene hydrocarbons, ß-ocimene and the monoterpene alcohols, linalool, α-terpineol, *trans* geraniol and carvacrol. Also identified in all the essential oils was the monoterpene aldehyde, ß-cyclocitral; the monoterpene ketone analogue, ß-ionone and finally the diterpene alcohol, *trans* phytol, which was apparently present in extensive portions in all the essential oils.

Although these compounds were identified as common to all these essential oils, their relative proportions in each essential oil was so different, occasionally varying so significantly to the extent of some essential oils possessing compounds unique to their particular selves. This may lend credence to the presence of 2-hexanol in the essential oil of *C. farinosa*, 2,4,5-trimethylthiazole in the essential oil of *G. gynandra*, 1,8-cineole in the essential oil of *T. africanum* and α-cedrene in the essential

oil of *B. angustifolia* var. *angustifolia* and not in any that other studied in this analysis.

The essential oils were predominated by *trans* 2-methylcyclopentanol, linalool, carvacrol and *trans* phytol. *trans* 2-Methylcyclopentanol is highest in the essential oil of *B. mossambicensis*(30.72%) followed by the essential oil of *T. africanum*(10.04%), while linalool was present in comparable amounts in the essential oils of *B. mossambicensis*(8.80%) and *G. gynandra*(7.18%) and was detected in equally good amounts in the essential oil of *C. farinosa*(4.27%). On the otherhand carvacrol was predominant in the essential oil of *G. gynandra*(15.40%), and was present in comparable amounts in the essential oils of *B. mossambicensis*(4.37%), *T. africanum*(3.24%), *C. farinosa*(3.22%) and *B. angustifolia*(2.73%). Prominent among all the essential oils was the diterpene alcohol, *trans* phytol which was present in enhanced amounts in the essential oil of *B. angustifolia*(35.77%) and in appreciable propositions in the essential oils of *G. gynandra*(12.92%), *T. africanum*(8.53%), *B. mossambicensis*(7.58%) and *C. farinosa*(5.42%).

Most of the compounds identified have previously been isolated in essential oils of other plant species and families. Some of these components serve an intricate biogenetic network. The network of whose some of its products were also identified as components of these very same essential oils. One such striking component that was identified in some of the essential oils was methyl isothiocyanate. This was identified to be present in the essential oils of *B. mossambicensis*, *G. gynandra* and *T. africanum*. Previous

investigations revealed this to be a product of enzymic hydrolysis of the glucoside, glucocapparin, which is present in the seeds and roots of *G. gynandra* and *T. africanum* respectively (Kjaer and Thomsen, 1963). It has also been identified in the headspace volatiles of the shrub, *Boscia senegalensis*, whose fruits and leaves have been used traditionally in Senegal to control cereal pests. The biological activity of this shrub is attributed to the liberation of methyl isothiocyanate from the glucosinolate precursor, glucocapparin(Seck et al., 1993). The other constituent of the essential oils with an allied biogenesis to that of methyl isothiocyanate is phenylacetonitrile. This was identified in the essential oils of *B. mossambicensis*, *C. farinosa* and *G. gynandra*. phenylacetonitrile has previously been identified in the essential oils of *Inula racemosa*(Bokadia et al., 1986) and Peony flowers, *Peony albiflora*(Kumar and Motto, 1986). It is also present in the volatile components of citrus fruit blossoms as well as in some mango cultivars(Attaway et al., 1966b; MacLeod and Nirmala, 1984). Phenylacetonitrile is a product of the autolysis of benzyl glucosinolate. This process is generally accompanied with the production of benzyl isothiocyanate, but some reports point to the failure to detect the latter in essential oils in which phenylacetonitrile was positively identified(Bokadia et al., 1986; Cole, 1975; Gil and MacLeod, 1980; MacLeod et al., 1981). In the present analysis there was no conclusive evidence of the presence of benzyl isothiocyanate in any of the essential oils investigated.

The unsaturated leaf alcohols, *trans* 2-hexen-1-ol and *cis* 3-hexen-1-ol have been reported as volatile components of numerous

plant species belonging to a variety of plant species(Gildemeister and Hoffmann, 1960, 1963; Straten, 1977). They have been isolated in the essential oils from the foliage of coast redwood, *Sequoia sempervirens* and in the volatile components of some mango cultivars (Gregonis et al., 1968; MacLeod and Nirmala, 1984). In this investigation, these alcohols were identified in all the essential oils investigated. These unsaturated alcohols are formed by the oxidative degradation of plant lipids. However the ratio between the several products of the biosynthesis-components varies in and over different plant species. Within the same plant species, the proportions are modified seasonally (Hatanaka et al., 1976) as caused by the expressions and/or the shifts in expressions of the several enzymes involved, owing to the plant aging and injury (Buttery et al., 1971; Kazeniac and Hall, 1970; Sayo and Takeo, 1975).

The plant monoterpene alcohol, linalool is an ubiquitous component of essential oils and headspace volatiles. This has been isolated in citrus leaf oils (Attaway et al., 1966a), in wood oleoresins of *Pinus edulis* and *P. monophylla* (Snajberk and Zavarin, 1975) and in the essential oils of *Mikania micrantha*, *M. amara*, *M. banisteriae* and *M. congesta* (Nicollier and Thompson, 1981; Da Silva et al., 1984). In the present investigation, linalool was identified in all the essential oils analyzed. This compound has been proposed as a precursor in the biogenesis of δ-limonene and ß-ocimene (Attaway et al., 1966a). It is therefore interesting to note that in almost all the essential oils in which linalool was present, save for the essential oil of *C. farinosa*, these compounds

ß-ocimene and δ-limonene were subsequently identified as components. In the essential oil of *C. farinosa*, linalool and traces of ß-ocimene were present but there was no evidence of the presence of δ-limonene. δ-limonene and ß-ocimene in combination with linalool have previously been identified as volatile components of some mango cultivars (MacLeod and Nirmala, 1984), whereas ß-ocimene and linalool without any mention of δ-limonene are reported as components of citrus leaf oils (Attaway et al., 1966a).

The other important monoterpene alcohol that was identified in all the essential oils investigated is α-terpineol. This is biosynthesized from geraniol. These two compounds share a common biosynthetic route to δ-limonene (Nicholas, 1967; Stork and Burgstahler, 1955). It is therefore noteworthy to observe that α-terpineol and *trans* geraniol were present in all the essential oils. Particularly captivating is the striking relationship that seems to exist between the proportions of these two components in each of the essential oils investigated. The trend that emanates is that of these two components assuming a comparable proportion in the individual essential oils. This may further enhance the proposition of a common biosynthetic route. These two components also occur in the wood oleoresins of *Pinus edulis* and *P. monophylla* (Snajberic and Zavarin, 1975).

The other terpene alcohols, carvacrol, nerolidol and *trans* phytol also occur frequently in essential oils. Carvacrol occurs in the essential oil of spanish origanum, *Coridothymus capitatus* (Sendra and Cunat, 1980), while the sesquiterpene alcohol,

nerolidol occurs in the volatiles isolated from the floral parts of citrus plants (Attaway et al., 1966b). *trans* phytol has been isolated from the hot hexane extract of the red algae, *Cracilaria andersoniana*.(Sims and Pettus, 1976). Whereas carvacrol was present in good proportions in all the essential oils studied, nerolidol was only isolated in near to trace amounts and was absent in the essential oil of *C. farinosa*. However the diterpene alcohol, *trans* phytol was the most abundant single component in practically all the essential oils. Phytol is unusual among the terpenes in that it has a definite role in plant biochemistry as part of the chlorophyll molecule and also in vitamins E and K. So phytol biogenesis may be closely linked to chlorophyll, from which it is derived by hydrolysis (Sims and Pettus, 1986; Nicholas, 1967).

Although the majority of the compounds that were identified were terpenes, there were also compounds identified which though closely resemble terpenes by their partial isoprenoid unit linkage, didn't possess the complete isoprenoid chain linkage that is characteristic of terpenes. These could have been derived from their terpene congeners by biodegradative processes. However other compounds identified didn't seem to have any linkage to the isoprenoid units that characterize terpenes. Clearly such compounds probably could not have been derived from terpenes. A notable example of the former group of compounds are the ionones as α- and ß-ionone which were identified to be present in all the essential oils except for the essential oil of *B. angustifolia* in which α-ionone was absent. ß-Ionone is a key intermediate in the biosynthesis of vitamin A. Previous investigations reveal it's

present in the essential oil of *Boronia megastigma* Nees, the water melon, *Citrullus vulgaris* Schrad fruit as well as in the volatiles of the musk melon fruit (Budavari et al., 1987; Kemp et al., 1973; Kemp, 1975). On the other hand α- and β-ionone have both been isolated in the essential oil of *Inula racemosa* (Bokadia et al., 1986). A striking example of compounds which were not related to the terpenes is in the thiazole, 2,4,5-trimethylthiazole which was only isolated in the essential oil of *G. gynandra*.

Apart from the terpenes, the other prevalent group of compounds were the aliphatic and aromatic aldehydes. This group of compounds seem another common feature of the constituents of essential oils. Particularly prevalent among the essential oils studied were phenyl acetaldehyde, nonanal and tridecanal, while the other aldehyde benzaldehyde was not as prevalent. The structural similarity of phenylacetaldehyde and phenyl- acetonitrile may point to a common biosynthetic pathway. The former may be derived from the latter by partial hydrolysis and therefore may be linked to the degradation of plant carbohydrates. Benzaldehyde and phenylacetaldehyde are regular components of essential oils. These have been isolated jointly in the essential oil of the giant cordgrass, *Spartina cynosuroides* and in the volatiles of the peony flowers, *Peony albiflora* (Kumar and Motto, 1976, Mody et al., 1974). Nonanal is also a common constituent of plant essential oils and also their volatiles. It has been isolated in cassava, rice, and *cannabis sativa*, while in combination with benzaldehyde are present in the air space volatiles of the cotton plant(Hedin et al., 1975). It is also a constituent of the essential oils of the

water melon, *Citrullus vulgaris* schrad fruit and *Sarracenia flava* (Hedin, 1975; Kemp, 1975). Tridecanal, on the other hand, is rather rare having been isolated in the essential oils of *Mikania amara*, *M. banisteriae* and *M. congesta*(Da Silva et al., 1984).

2.4: The Biological Activity of the Constituents of the Essential Oils.

The repellency bioactivity of the constituents identified in the essential oils was carried out using the same procedure as was done for the essential oils and as outlined in section 3.8.1 and 3.8.2 for the brown ear tick, *Rhipicephalus appendiculatus*, and the maize weevil, *Sitophilus zeamais*, respectively. Table 5 and 6 shows the percentage repellencies of these constituents under the same dose levels as was done for the essential oils. These repellencies are compared against those of the commercial arthropod repellent N, N-diethyl toluamide, Deet.

The constituents of the essential oils that emerged particularly active against the brown ear tick, *R. appendiculatus* at both the 0.1 and 0.01 dose levels were *m*-cymene, nonanal, α-terpineol, ß-cyclocitral, nerol, *trans* geraniol, carvacrol, α-cedrene, α-ionone, *trans* geranylacetone and nerolidol. At these two dose levels these components were highly active compared to the commercial arthropod repellent, Deet. However there were some constituents which were highly active at the 0.1 dose level and yet only mildly active, if at all, at the 0.01 dose level. Such were the compounds in benzaldehyde, phenyl- acetaldehyde, ß-ocimene,

Table 5: Mean percentage repellencies (± s.e) of the compounds isolated from the essential oils of *Boscia angustifolia*, *Boscia mossambicensis*, *Cadaba farinosa*, *Gynandropsis gynandra* and *Thylachium africanum* and N,N-diethyl toluamide(Deet) to *Rhipicephalus appendiculatus*.

	Concentration (µl/µl)			
Compound	0.1	0.01	0.001	0.0001
2-Hexanol	45.9±3.2	–	–	–
trans 2-Methyl-cyclopentanol	30.8±3.1	–	–	–
cis 3-Hexen-1-ol	15.9±4.3	–	–	–
trans 2-Hexen-1-ol	42.5±4.7	–	–	–
Heptan-2-one	45.1±4.0	–	–	–
Anisole	14.8±5.1	–	–	–
Benzaldehyde	82.8±2.8	45.0±3.4	–	–
2,4,5-Trimethyl-thiazole	45.3±2.1	–	–	–
Phenylacetaldehyde	96.8±0.9	38.6±3.7	–	–

Table 5 cont'd...

Compound	Concentration (µl/µl)			
	0.1	0.01	0.001	0.0001
m-Cymene	100.0±0.0	98.4±0.8	26.2±3.6	–
1,8-Cineole	44.7±3.3	–	–	–
δ-Limonene	27.2±2.6	–	–	–
ß-Ocimene	93.6±1.5	40.8±2.5	–	–
Nonanal	100.0±0.0	98.0±0.8	19.7±4.3	–
Linalool	94.9±2.2	35.8±2.7	–	–
Phenylacetonitrile	100.0±0.0	33.2±4.4	–	–
Methyl salicylate	99.4±0.4	19.0±4.5	–	–
α-Terpineol	89.9±0.0	89.0±0.0	68.2±3.7	37.4±2.9
ß-Cyclocitral	100.0±0.0	98.9±0.5	54.3±3.8	–
Nerol	100.0±0.0	100.0±0.0	44.1±2.1	–

Table 5 cont'd...

	Concentration (µl/µl)			
Compound	0.1	0.01	0.001	0.0001
trans Geraniol	100.0±0.0	97.6±0.9	43.5±3.3	_
Carvacrol	89.9±0.0	77.0±4.9	26.6±2.8	_
α-Cedrene	98.7±0.6	79.4±2.6	26.3±4.1	_
α-Ionone	100.0±0.0	97.9±1.1	34.9±3.3	_
ß-Caryophyllene	8.3±6.5	_	_	_
trans Geranylacetone	100.0±0.0	97.9±0.8	35.8±2.6	_
ß-Ionone	48.7±7.1	_	_	_
Tridecanal	34.7±4.7	_	_	_
Nerolidol	100.0±0.0	100.0±0.0	98.3±0.7	30.2±2.1
trans Phytol	48.4±7.1	_	_	_
Deet	84.0±3.9	82.0±3.6	75.6±4.5	70.5±3.6

Table 6: Mean percentage repellencies (± s.e) of the compounds isolated from the essential oils of *Boscia angustifolia*, *Boscia mossambicensis*, *Cadaba farinosa*, *Gynandropsis gynandra* and *Thylachium africanum* and N,N-diethyl toluamide(Deet) to *Sitophilus zeamais*.

	Concentration (µl/µl)			
Compound	0.1	0.01	0.001	0.0001
2-Hexanol	94.8±1.9	60.7±4.0	42.6±4.5	–
trans 2-Methyl-cyclopentanol	93.1±3.6	86.2±5.3	53.7±8.3	63.3±5.9
cis 3-Hexen-1-ol	100.0±0.0	84.1±1.7	63.1±6.4	46.2±7.8
trans 2-Hexen-1-ol	100.0±0.0	96.7±1.9	70.7±3.3	49.3±4.7
Heptan-2-one	69.6±3.8	82.4±7.2	38.5±1.5	–
Anisole	95.5±1.5	87.2±2.1	83.6±2.7	65.3±5.0
Benzaldehyde	98.3±1.7	62.1±4.3	50.2±3.6	–
2,4,5-Trimethyl-thiazole	86.1±6.2	49.2±5.8	–	–
Phenylacetaldehyde	87.5±1.8	87.2±4.6	53.3±6.1	–

Table 6 cont'd...

Compound	Concentration (µl/µl)			
	0.1	0.01	0.001	0.0001
m-Cymene	72.9±11.6	55.3±9.3	–	–
1,8-Cineole	82.5±3.9	73.7±1.4	75.8±5.7	54.4±3.1
δ-Limonene	70.6±0.9	59.1±4.9	–	–
ß-Ocimene	93.9±3.0	88.1±4.1	68.7±10.5	49.7±5.2
Nonanal	98.5±1.6	90.1±4.9	42.1±5.2	–
Linalool	87.6±5.9	84.3±2.6	79.5±2.6	59.9±7.6
Phenylacetonitrile	93.6±4.5	87.3±2.1	89.5±4.9	75.5±3.6
Methyl salicylate	67.1±4.6	38.4±11.9	–	–
α-Terpineol	67.6±3.2	60.3±0.5	52.4±1.2	48.6±0.2
ß-Cyclocitral	78.2±2.6	84.6±5.5	81.5±3.8	40.7±6.5
Nerol	65.3±10.9	43.6±3.5	–	–

Table 6 cont'd...

Compound	Concentration (µl/µl)			
	0.1	0.01	0.001	0.0001
trans Geraniol	69.2±7.6	55.4±6.6	_	_
Carvacrol	72.0±5.2	61.6±2.0	22.2±0.8	_
α-Cedrene	90.7±1.9	88.7±4.1	73.4±3.6	49.9±5.0
α-Ionone	83.3±4.0	72.4±4.3	62.2±6.1	_
ß-Caryophyllene	81 4±2.1	90.2±4.4	69.6±8.9	49.9±4.1
trans Geranylacetone	21.0±12.9	_	_	_
ß-Ionone	22.7±4.6	_	_	_
Tridecanal	60.6±5.9	28.4±7.1	_	_
Nerolidol	66.7±10.8	60.0±6.3	_	_
trans Phytol	38.2±0.1	_	_	_
Deet	52.2±0.3	40.5±0.2	33.8±0.2	25.6±0.1

linalool, phenylacetonitrile and methyl salicylate. The activity of these compounds was also higher than that of Deet at the 0.1 level but feeble to the selfsame at the 0.01 dose level. Prominent among the repellencies of these constituents of essential oils are those of α-terpineol, ß-cyclocitral and nerolidol. These components show enhanced repellency to the 0.001 dose level with Nerolidol emerging rather more potent. In this bioassay, one of the constituents identified, methyl isothiocyanate was not tested on account of its high toxicity in space.

There are some characteristic trends that seem to emanate from the results of this study. For instance, the biological activity of the constituents which have low molecular weight and therefore very volatile, seem to elicit feeble repellency activity. This could be because the ready volatility decreases their strength with time hence don't persist long enough to keep away *R. appendiculatus* over the entire period of the bioassay. However as the molecular weight increases the activity also seems to increase although with some exceptions. Some of the very high molecular weight compounds are also not as active. Since most repellents act on the olfactory senses and therefore have to be perceived in the vapour phase, a good repellent would be that which produces enough vapour to keep away the test species and last long enough to continue eliciting this response. So the activity of these constituents at this level may be a function of the balance between their volatilities and their persistence in time.

A close examination of the active constituents of the

essential oils reveals that they are mostly monoterpene alcohols, few monoterpene hydrocarbons, aliphatic and aromatic aldehydes and some aliphatic ketones. In general there is the prevalence of the oxygen atom either as a hydroxylic group or as a carbonyl. This agrees well with the generalization drawn by Dethier (1956) and DAVIS (1985) as to the prevalent functionality of repellent compounds. The prevalence of oxygen as the rather polar hydroxylic or carbonyl form may point to some form of electrostatic interaction of these compounds to the active centre of the olfactory receptor. What looks rather astounding are the results of the repellencies of α- and ß-ionone. α-Ionone emerges very active and yet the immediate positional isomer, ß-ionone is only mildly active although the only difference in their structures lies in the shifting of the double bond a single bond position away. However the characteristic aroma of these two compounds are unique to each one. α-Ionone evokes a stronger burning sensation to the nostrils while ß-ionone has only a mild effect. So what finally comes out of these results point to the repellency being a function of the molecular weight and hence volatility, the persistence of the compound in space and finally the functionality of the molecule with the alcohols and carbonyl compounds making an upstart.

The components of the essential oils which may be responsible for their repellencies to the brown ear tick may therefore be phenylacetaldehyde, nonanal, ß-ocimene, ß-cyclocitral, nerol, *trans* geraniol, carvacrol, α-cedrene, α-ionone, *trans* geranylacetone, linalool, methyl salicylate and then nerolidol. However there may be other active components which were not identified in this

analysis and therefore not tested.

In the case of the maize weevil, *S. zeamais*, the constituents of the essential oils that proved active at the 0.1, 0.01 and 0.001 dose level were *trans* 2-methylcyclopentanol, *cis* 3-nexen-1-ol, *trans* 2-hexen-1-ol, anisole, benzaldehyde, phenyl- acetaldehyde, α-terpineol, ß-cyclocitral, α-cedrene, α-ionone and ß-caryophyllene. All these components were more active at these dose levels than Deet. It is noteworthy to observe that *trans* 2-methylcyclopentanol, anisole, 1,8-cineole, linalool and phenyl- acetonitrile were still highly active to the 0.0001 dose level. In the course of the bioassay methyl salicylate was observed to exhibit some knockdown properties to the maize at the 0.1 dose level.

In general, most of the compounds identified were active repellents of *S. zeamais* as compared to *R. appendiculatus*. Although those components that repelled *S. zeamais* are almost well distributed throughout the molecular weight ranges covered, it's important to note that the lower molecular weight compounds were slightly more regularly active than the higher molecular weight ones. What surfaces from the results is that the low molecular weight oxygenated compounds mostly as alcohols were highly active. This is in total contrast to the results obtained for *R. appendiculatus*, which indicate the relative potency of the medium molecular weight range oxygenated compounds as compared to the low molecular weight ones.

Among the components of the essential oils that were found active against the maize weevil and the brown ear tick, nerolidol is already documented a feeding deterrent for the gypsy moth larvae

Doskotch et al., 1980) while linalool is repellent to the aphid, *Cavariella aegopodi*, the German cockroach, *Blatella germanica* and the female mosquitoe, *Aedes aegypti* (Inazuka, 1983; Chapman et al., 1981; Hwang et al., 1985). Linalool is also a feeding deterrent of the oligophagous locust, *Locusta migratoria* (Bernays and Chapman, 1977) and is toxic to such stored product pests as *Zabrotes subfaciatus* (Bohem), *Acanthoscelides obtectus* (Say), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Oryzaephilus surinamensis* (Shaaya et al., 1985; Weaver et al., 1991). Linalool has particularly attracted a lot of interest in research pertaining to pest control on the basis of some of the virtues it exhibits. It is reported to be an olfactory cue in the seeking of host plants by numerous phytophagous invertebrates and acts as a reversible competitive inhibitor of the enzyme, acetylcholinestrase (Ryan and Byrne, 1988). It's also effective against all life stages of the cat flea, *Ctenocephalides felis* Bouche (Hink et al., 1988). Another component of essential oils that enjoys as wide a reputation as an insect repellent is the oxygeneted monoterpene, 1,8-cineole. This in the present investigation was mainly active against the maize weevil, *S. zeamais*. It is reportedly repellent to the American cockroach (Scriven and meloan, 1984), the colorado potatoe beetles (Schearer, 1984), the female mosquitoe, *Aedes aegypti*, and a host of other insect species (Klocke et al., 1987). Geraniol and limonene just as linalool are deterrent to *Locusta migratoria* (Bernays and Chapman, 1977), while limonene alone is repellent to the female mosquitoe, *Aedes aegypti* (Huang et al., 1985). Nerol, which was repellent to the brown ear tick, is reported an alarm

substance of the stingless bee, *Trigona fulviventris* (Johnson and Wiemer, 1982) and to be repellent to the German cockroach, *Blatella germanica* (L.) (Inazuka, 1983), while α-terpineol and Carvacrol are toxic to *Oryzaephilus surinamensis* (Shaaya et al., 1985). Also reported active have been the leaf alcohols, *trans* 2-hexen-1-ol and *cis* 3-hexen-1-ol. These in this analysis were effective repellents of only the maize weevil, while elsewhere they are reported to significantly reduce the aphid population on tomato leaves (Hildebrand et al., 1993).

Whereas the above review may not be exhaustive, it however adduces some evidence to the effect that constituents of essential oils exhibit a wide range of bioactivity to insect species and more interestingly so to those insect species associated to those particular plant species whose essential oil constituents are under consideration. This may point therefore to these constituents serving a particular role to the plant species in which they are isolated and hence be contrary to the had been widely held view that these were simply waste products of the plant biochemical processes. Indeed evidence keeps building up emphasizing that these essential oil and by extension their constituents may serve either a defensive role as repellents to insect predators or as attractants to insect pollinators. This could therefore be taken advantage of, on account of their relatively low toxicity to mammals and their amenability to biodegradation processes, and be utilized in the control of some notorious insect species just as evidence point to the potency of some essential oil constituents the likes of linalool.

Figure 6: The mass spectra of methyl isothiocyanate.

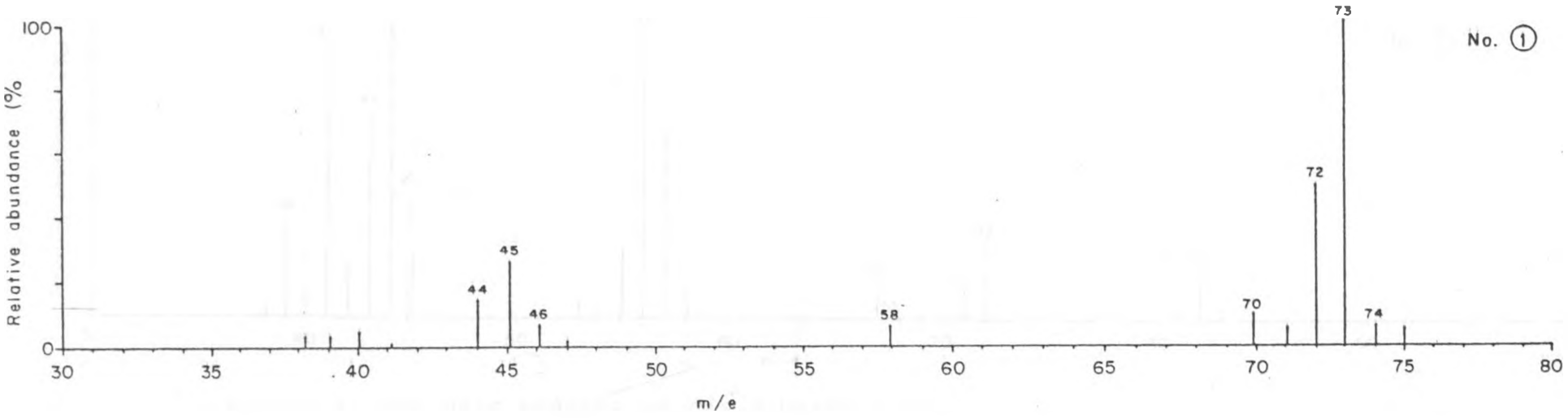


Figure 7: The mass spectra of 2-hexanol.

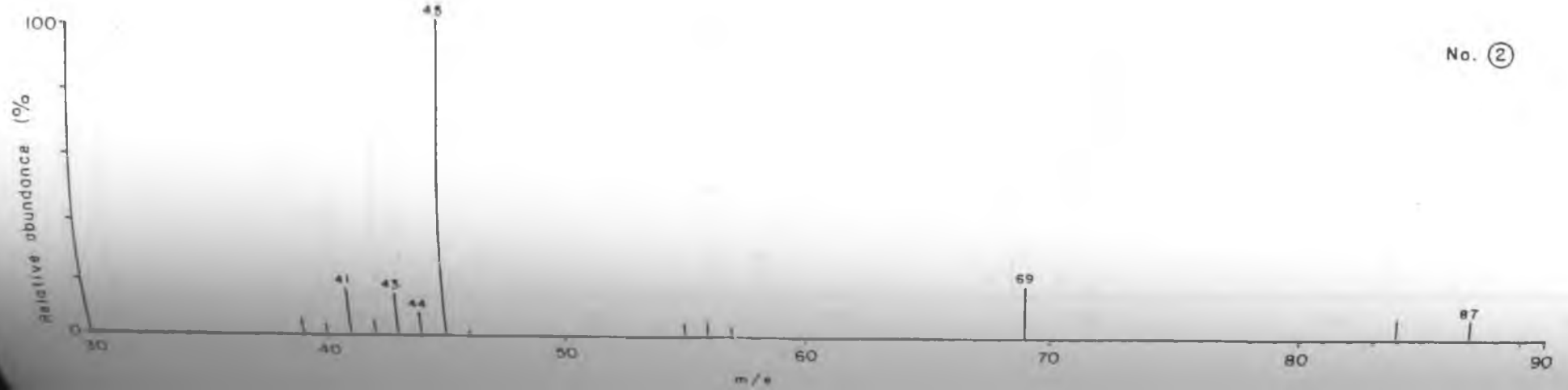


Figure 8: The mass spectra of *trans* 2-methylcyclopentanol.

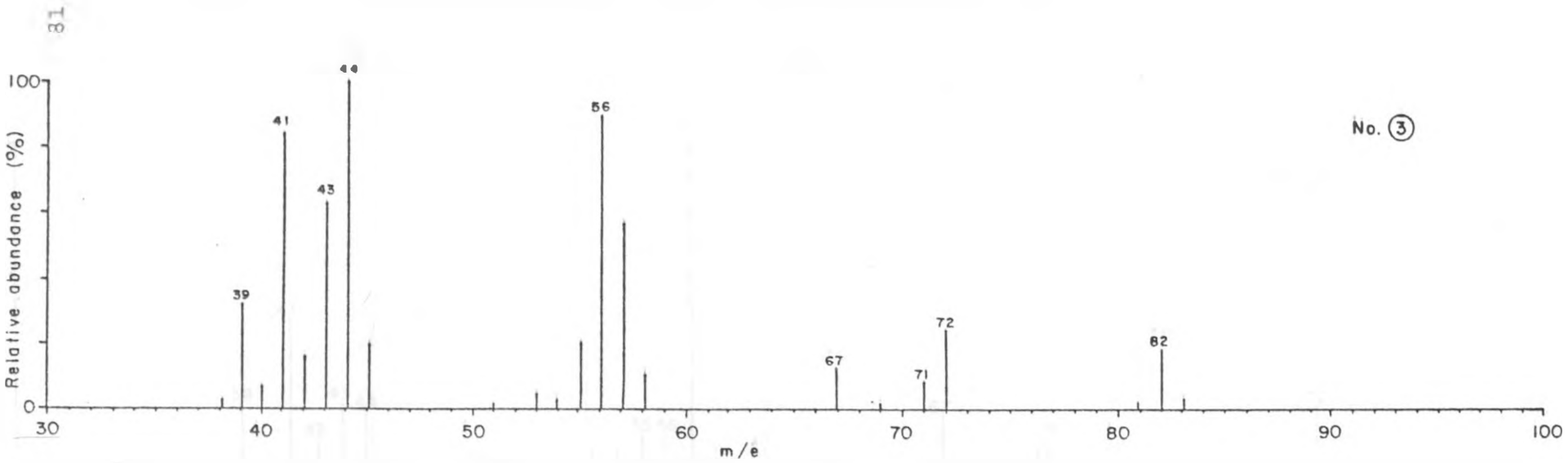


Figure 9: The mass spectra of *cis* 3-hexen-1-ol.

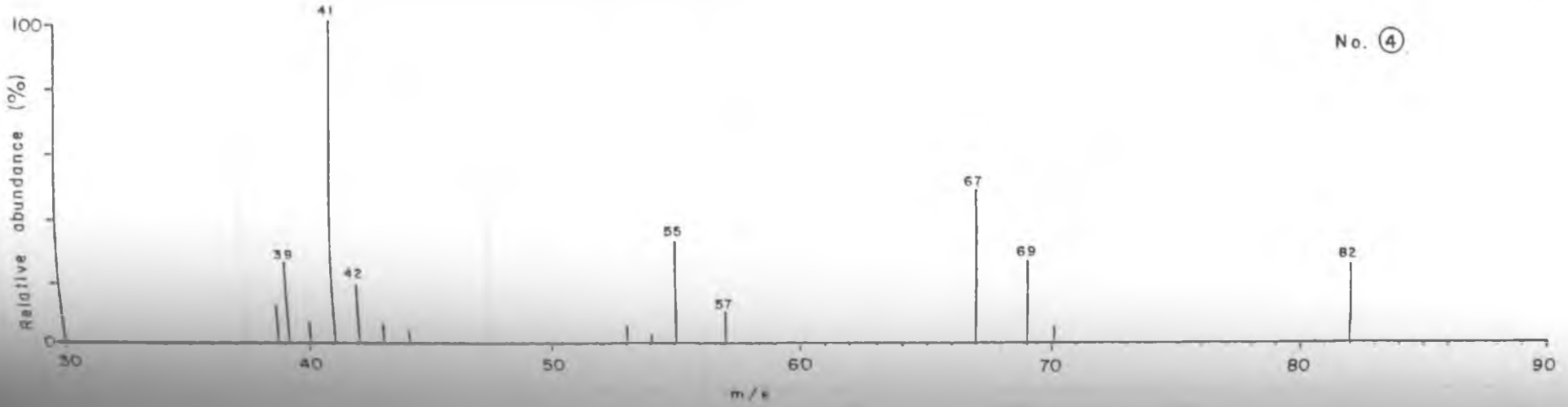


Figure 10: The mass spectra of *trans* 2-hexen-1-ol

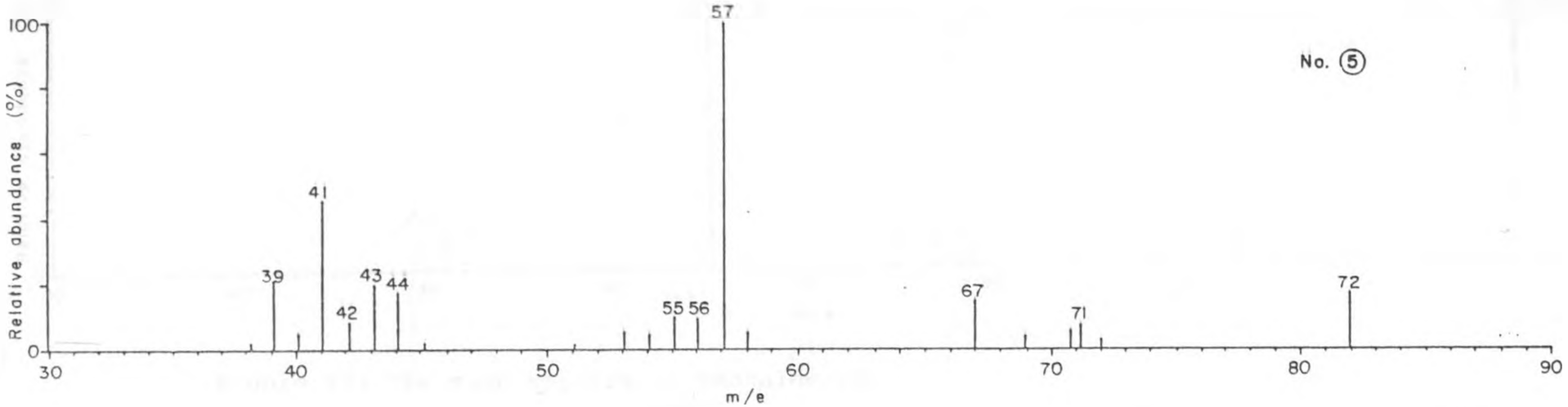


Figure 11: The mass spectra of heptan-2-one.

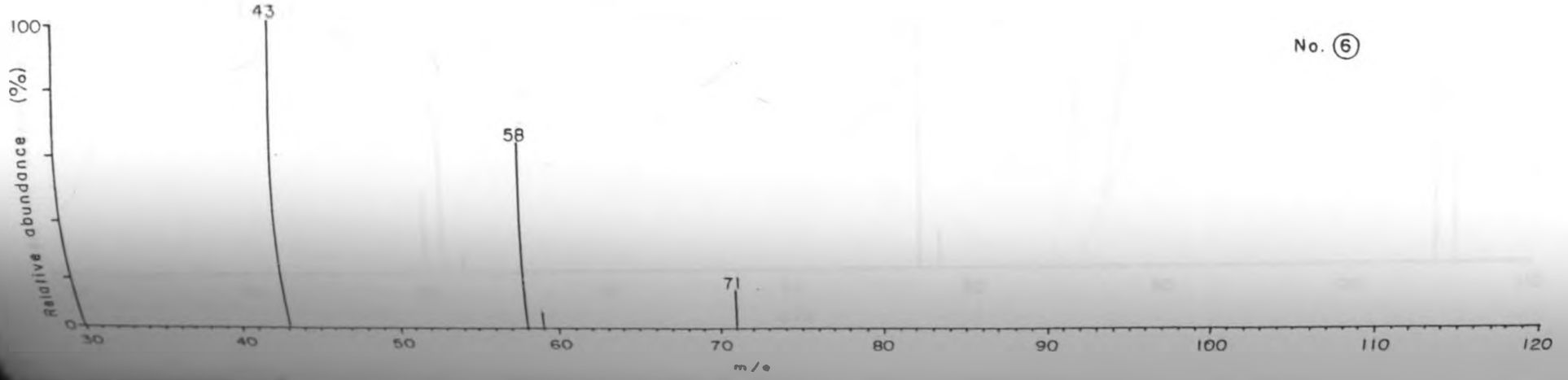


Figure 12: The mass spectra of anisole.

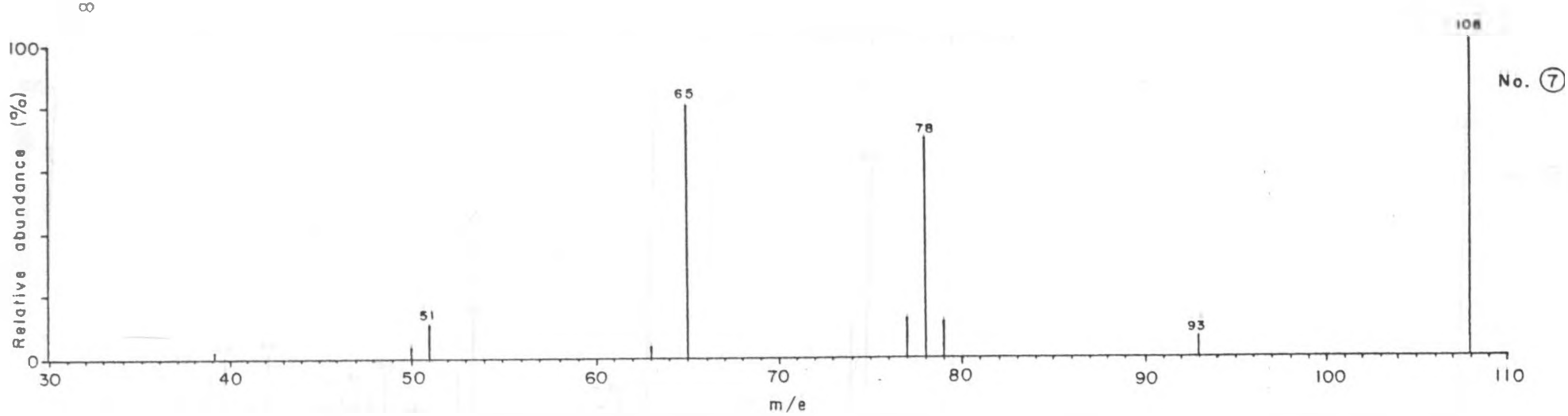


Figure 13: The mass spectra of benzaldehyde.

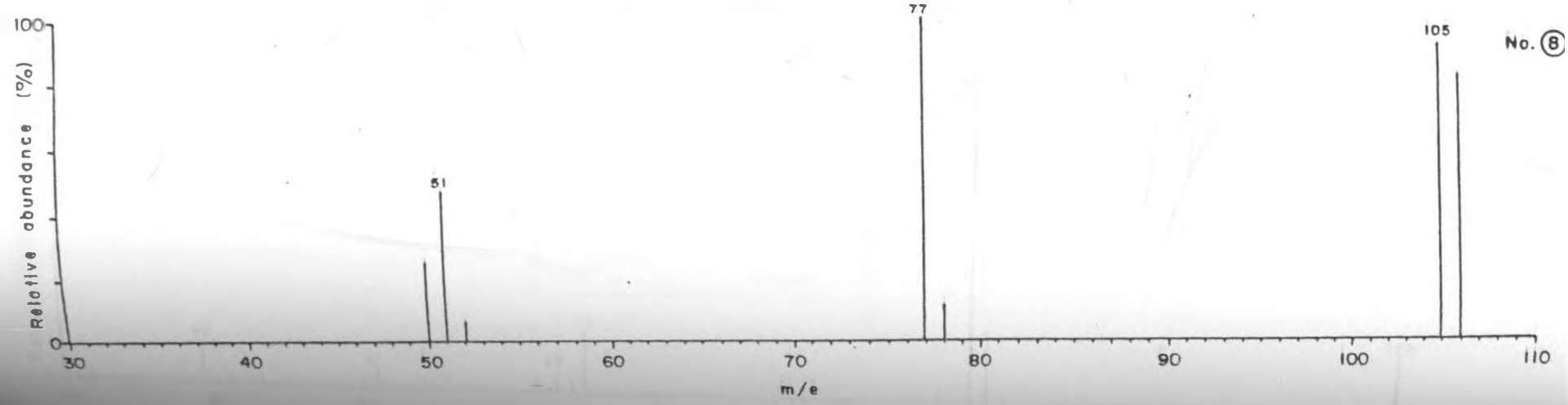


Figure 14: The mass spectra of 2,4,5 trimethylthiazole.

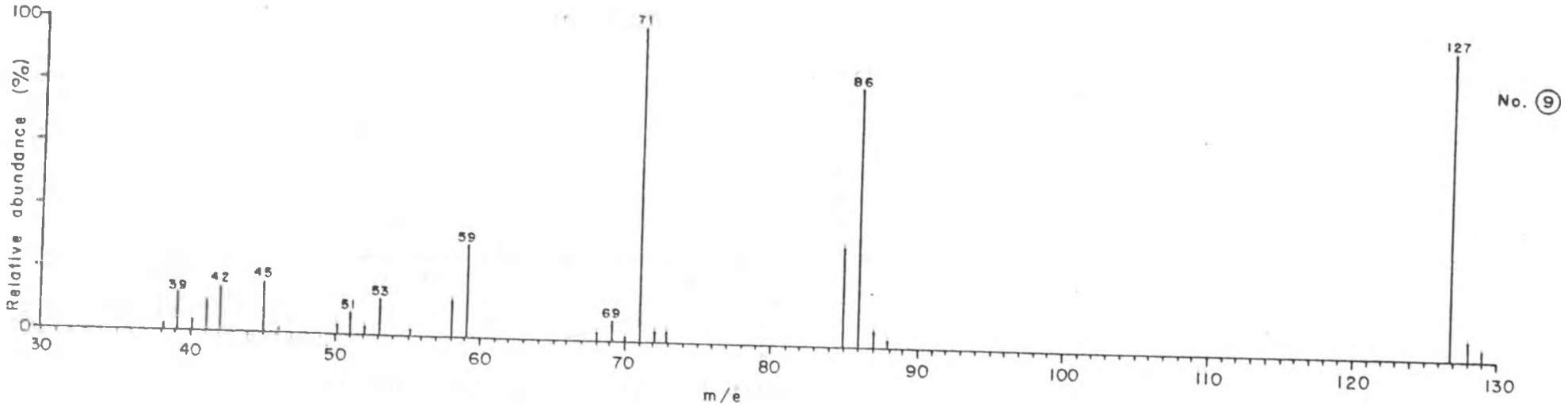


Figure 15: The mass spectra of phenylacetaldehyde.

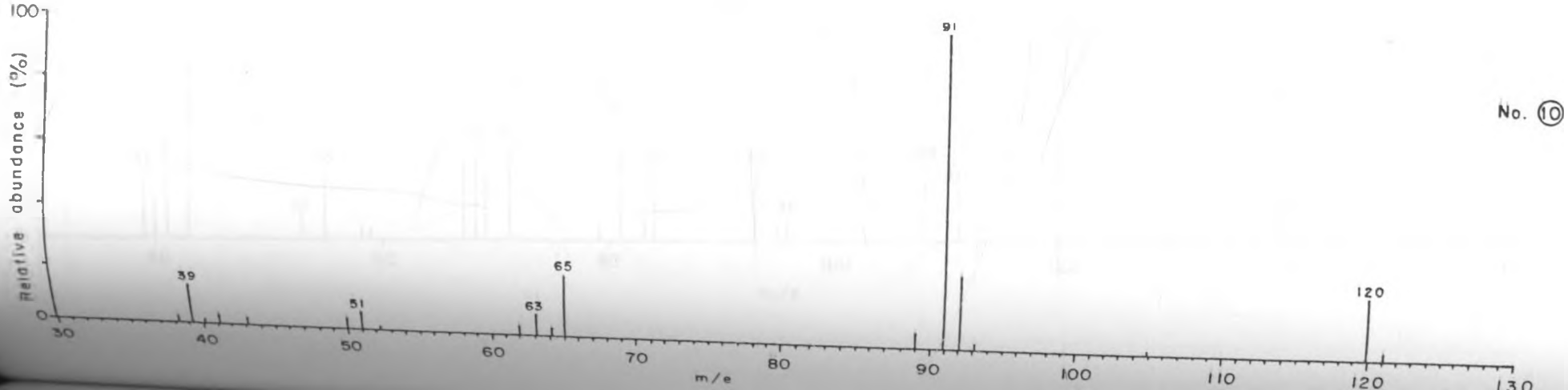


Figure 16: The mass spectra of *m*-cymene.

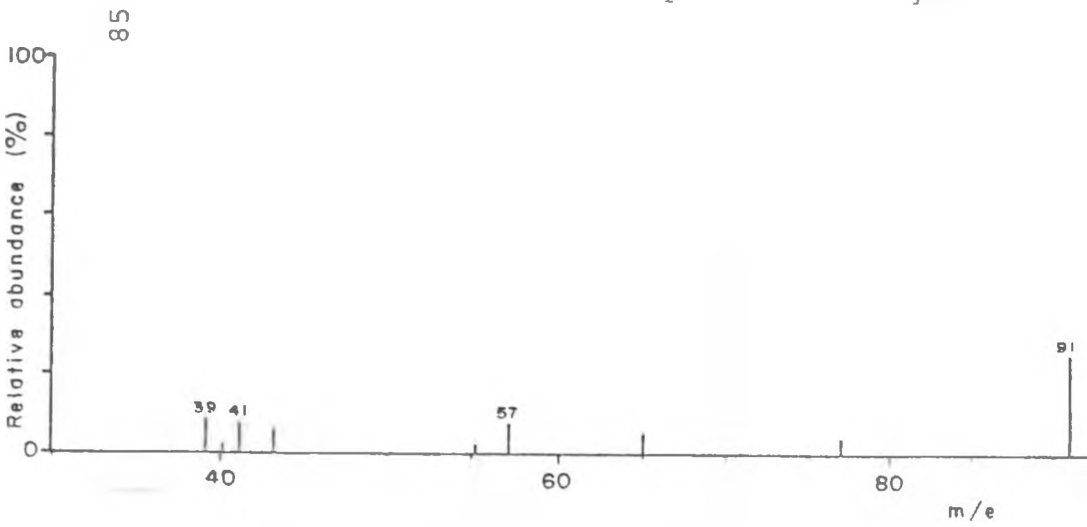
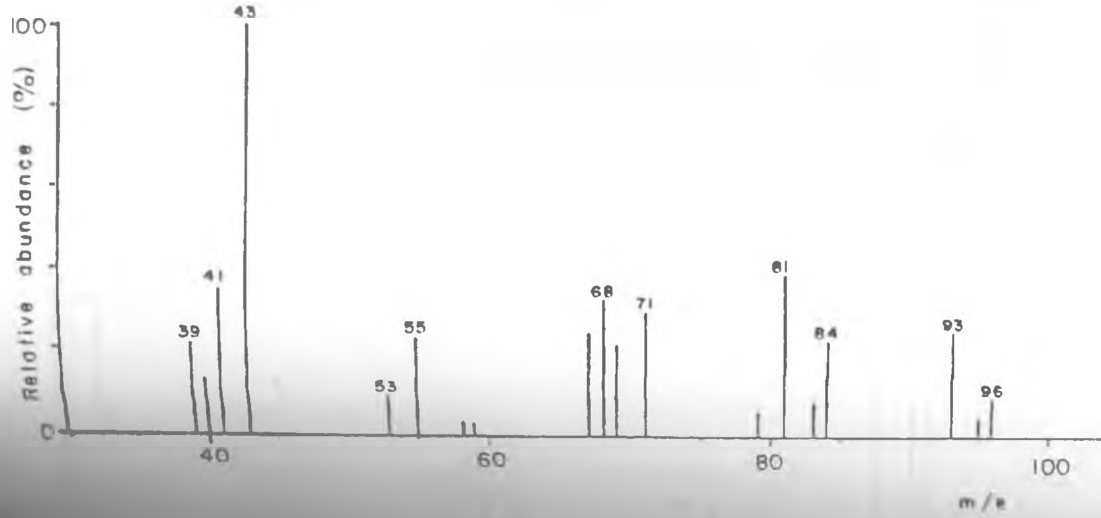
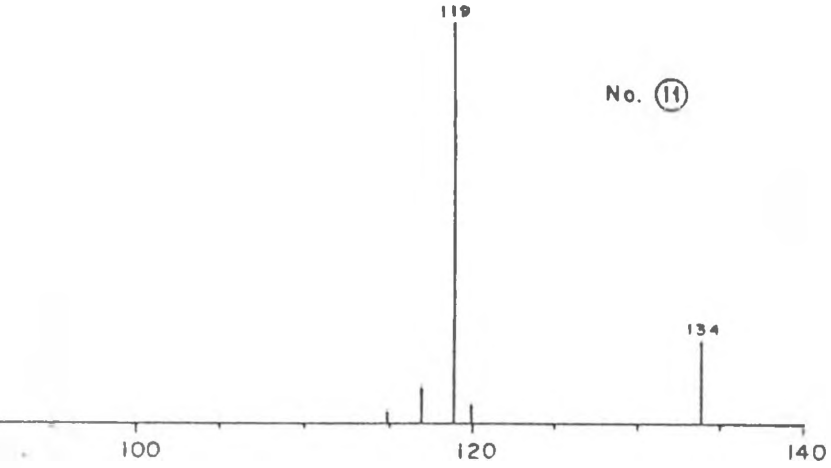


Figure 17: The mass spectra of 1,8-cineole.




119
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134
100
120
140

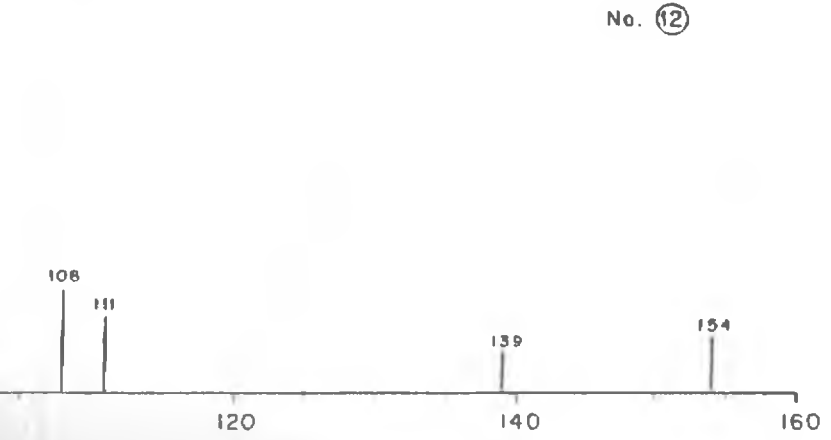

No. (12)
108
111
139
154
120
140
160

Figure 18: The mass spectra of δ-limonene.

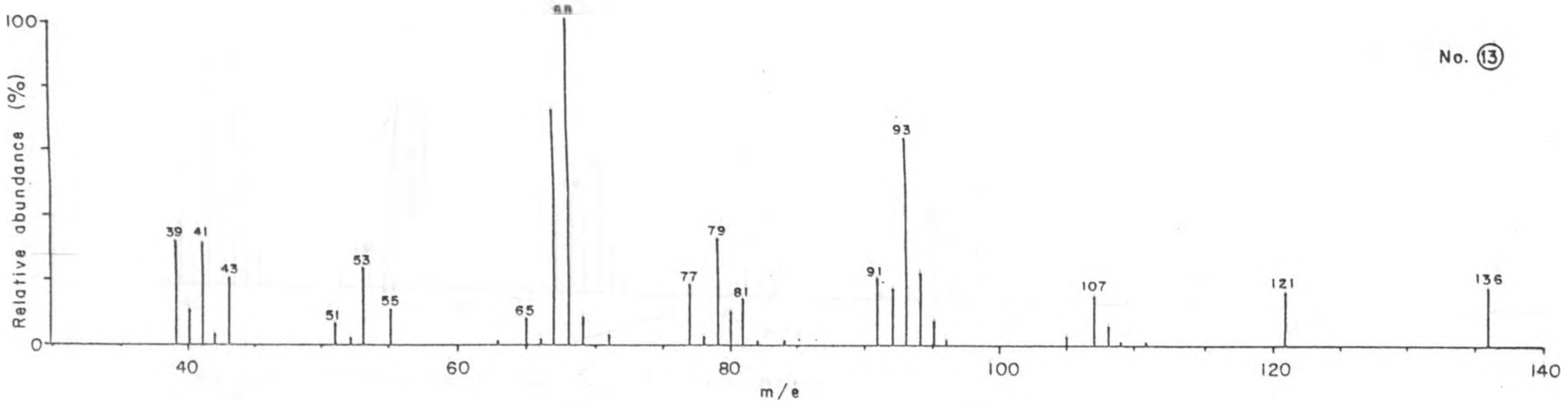


Figure 19: The mass spectra of ß-ocimene.

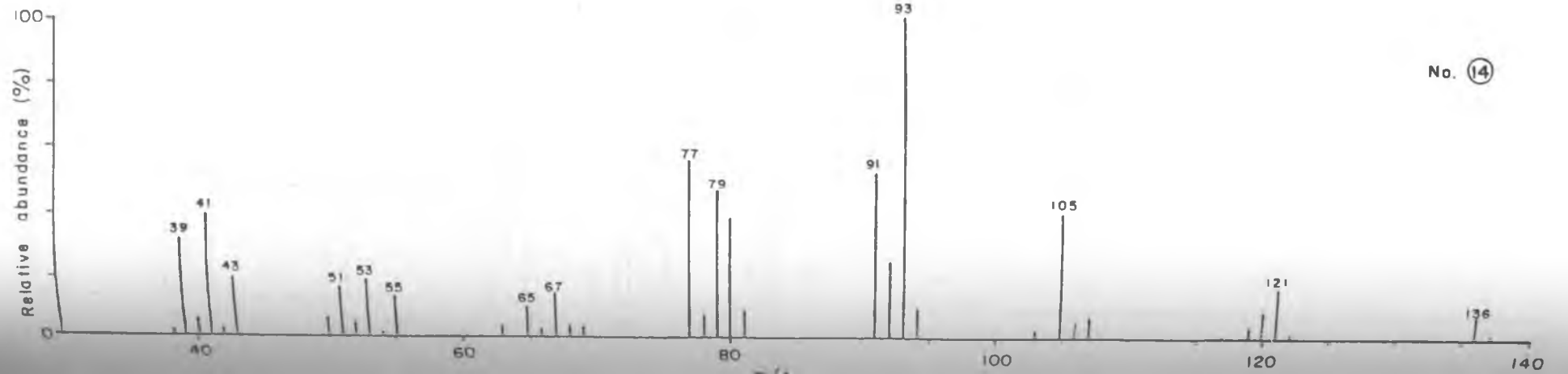


Figure 20: The mass spectra of nonanal.

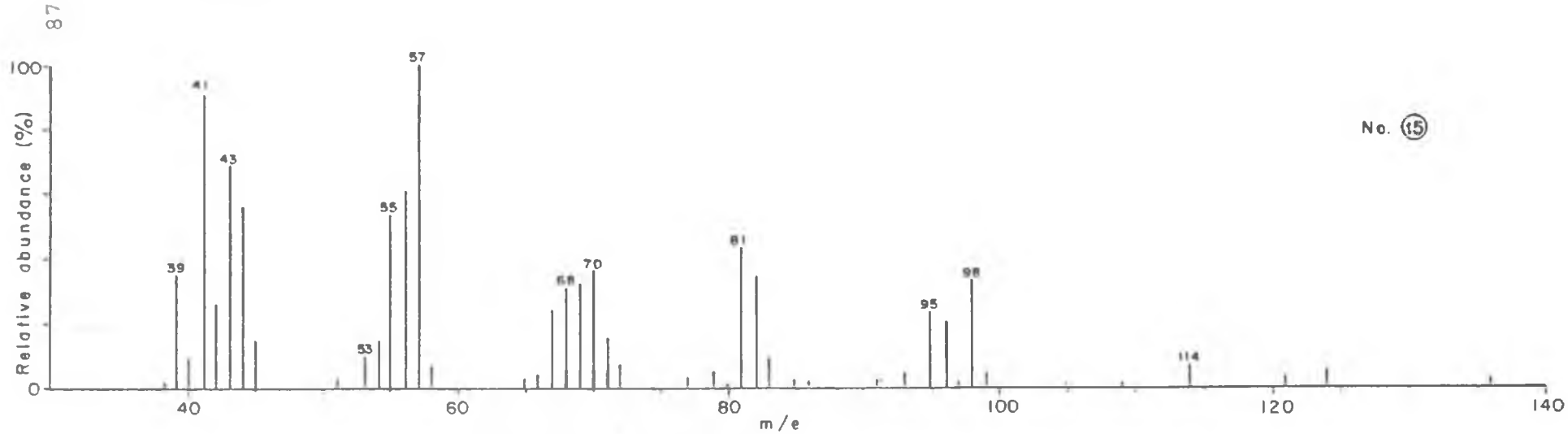


Figure 21: The mass spectra of linalool.

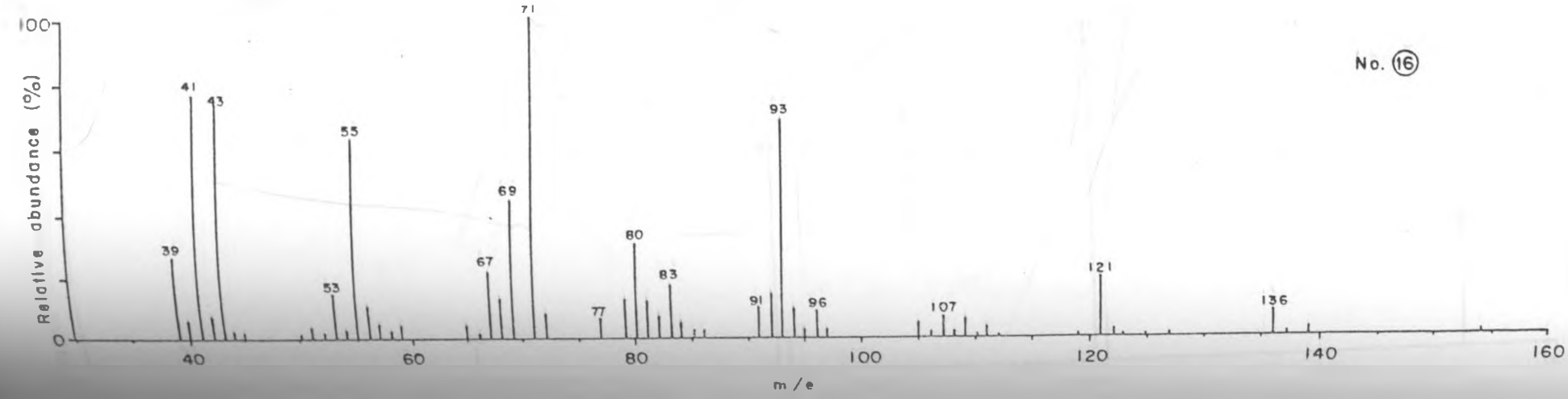


Figure 22: The mass spectra of phenylacetonitrile.

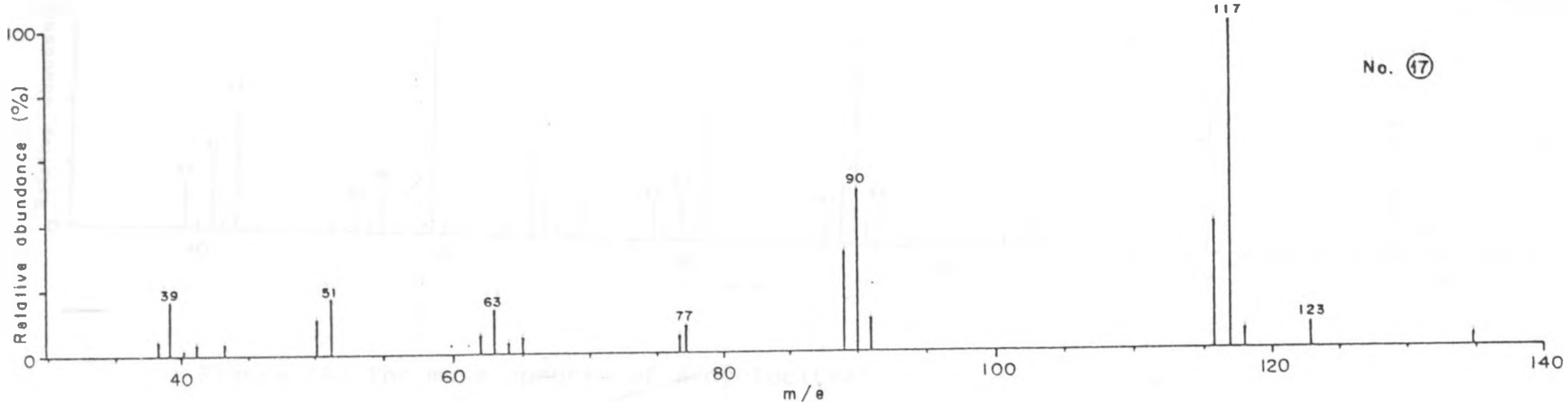


Figure 23: The mass spectra of methyl salicylate.

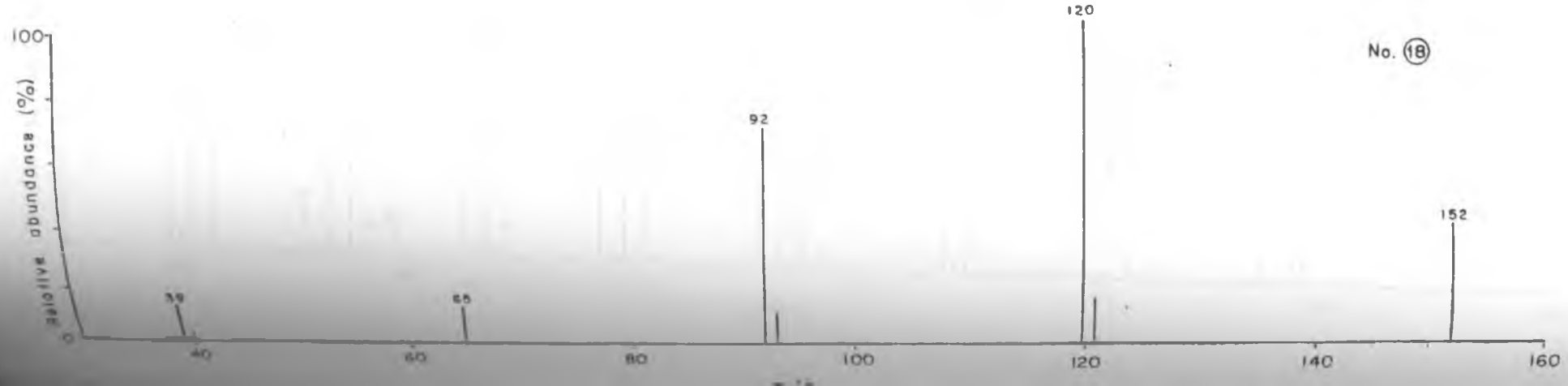


Figure 24: The mass spectra of α-terpineol.

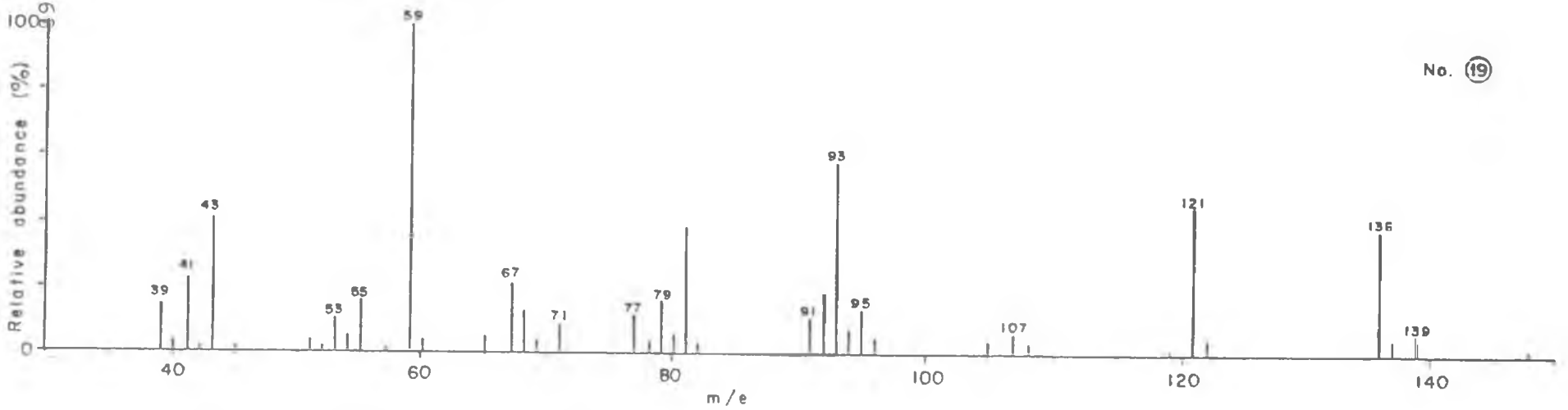


Figure 25: The mass spectra of β-cyclocitral.

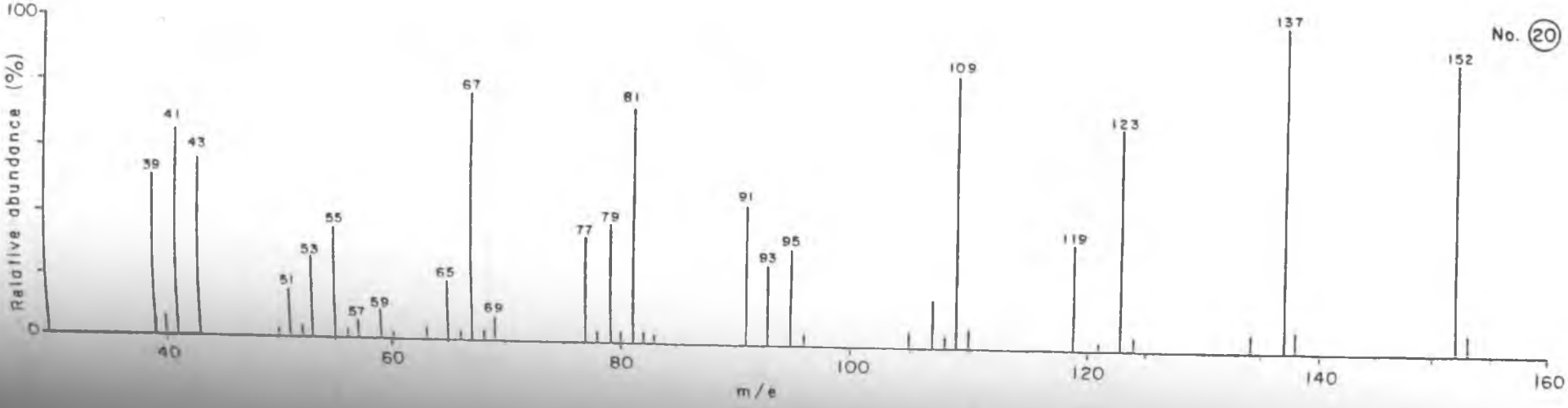


Figure 26: The mass spectra of nerol.

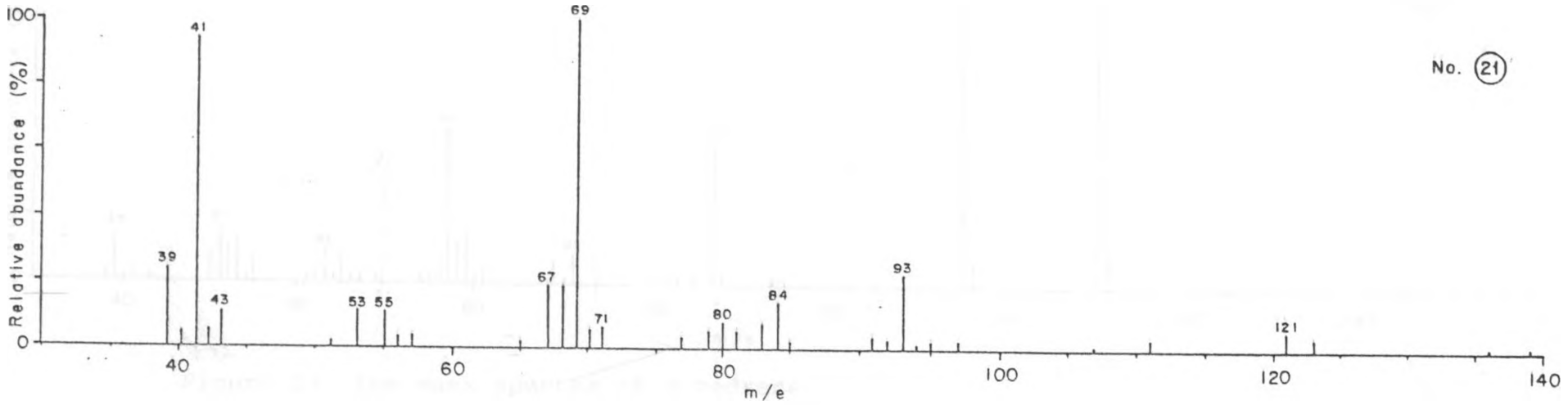


Figure 27: The mass spectra of *trans* geraniol.

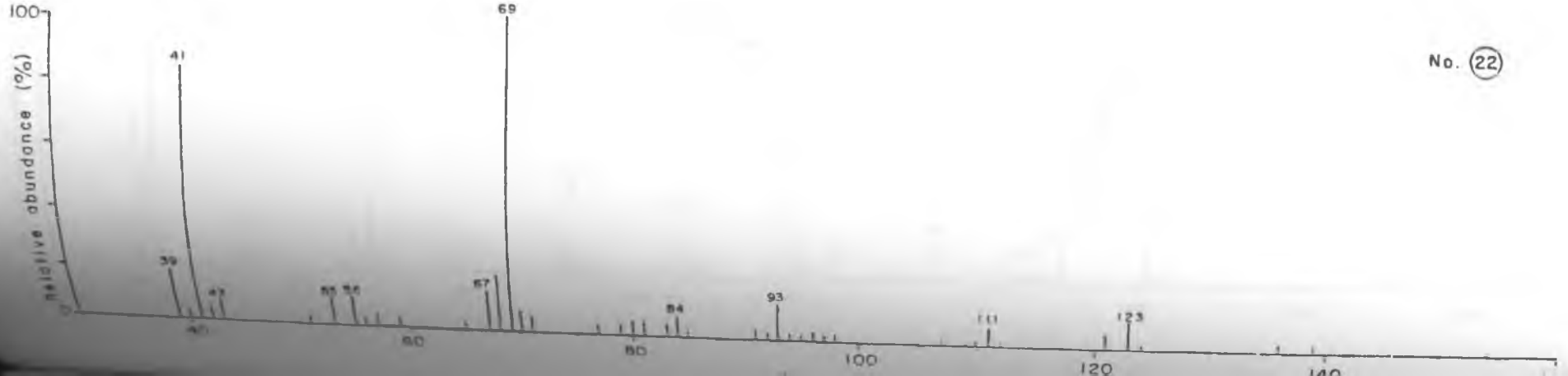


Figure 28: The mass spectra of carvacrol.

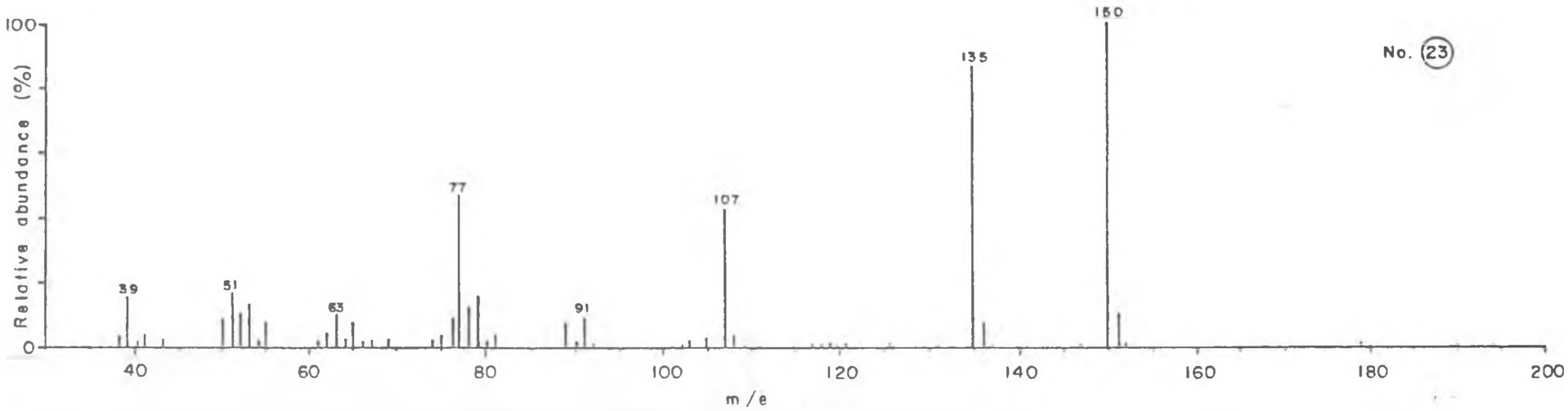


Figure 29: The mass spectra of α-cedrene.

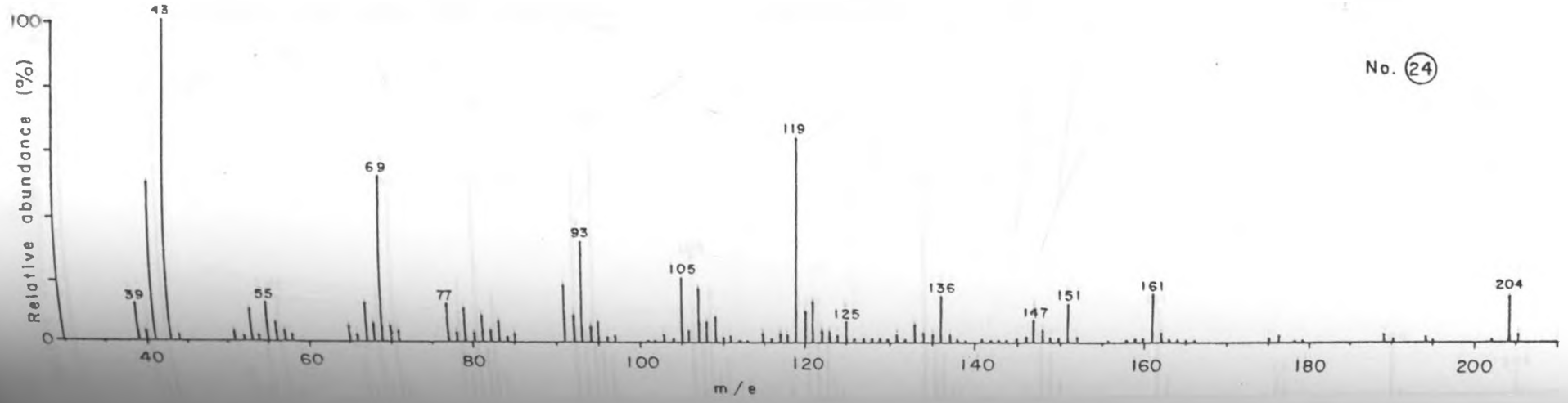


Figure 30: The mass spectra of α-ionone.

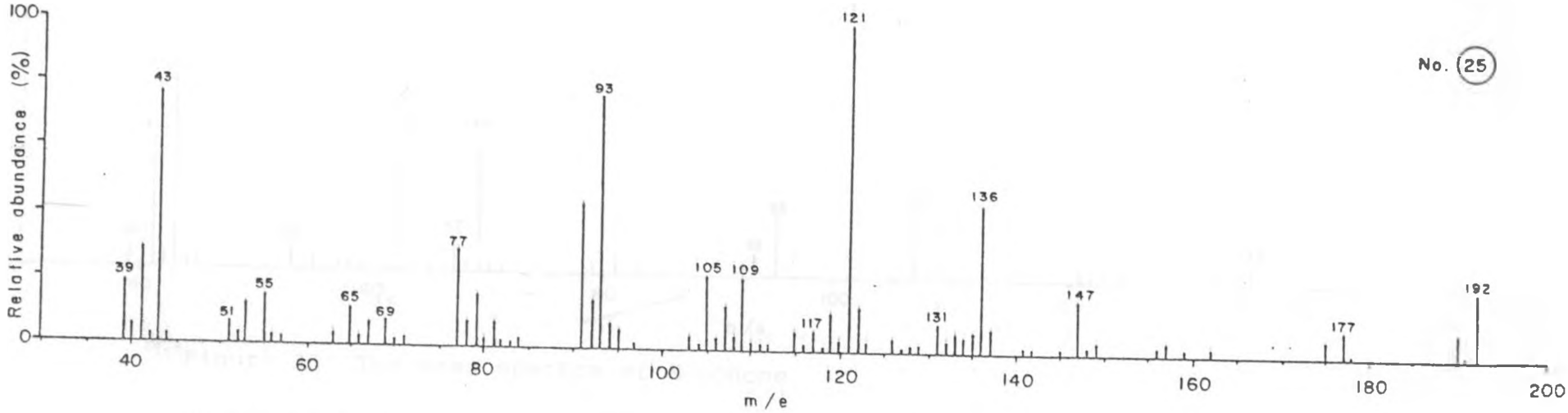


Figure 31: The mass spectra of β-caryophyllene.

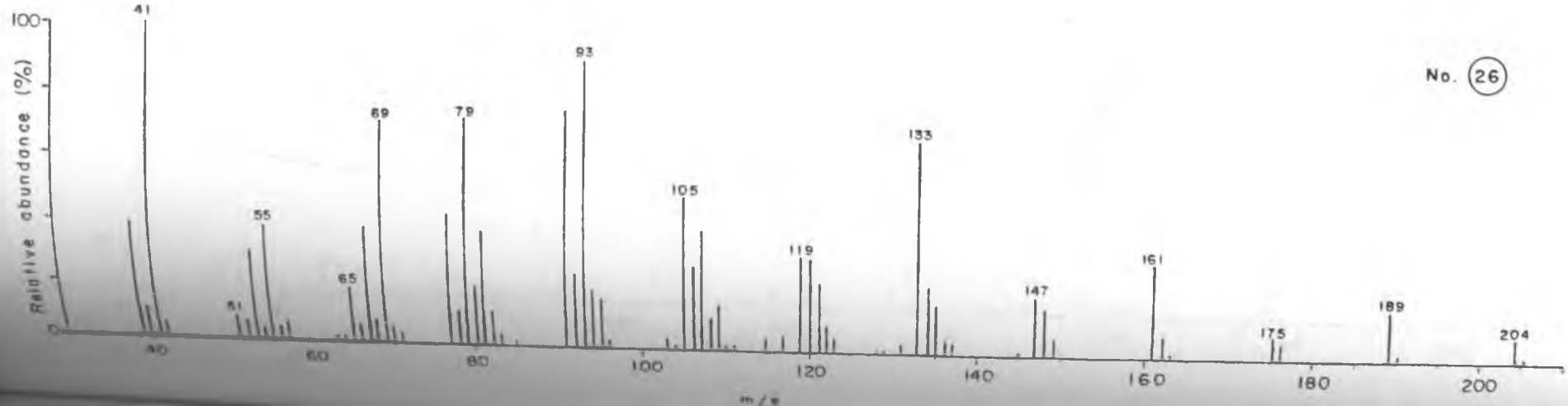


Figure 32: The mass spectra of *trans* geranylacetone.

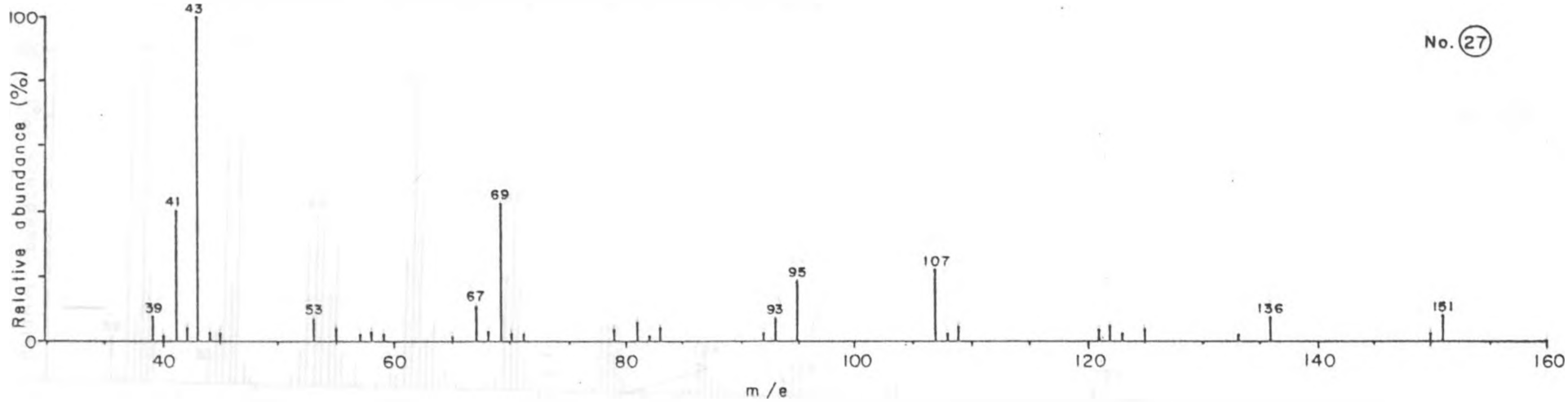


Figure 33: The mass spectra of ß-ionone.

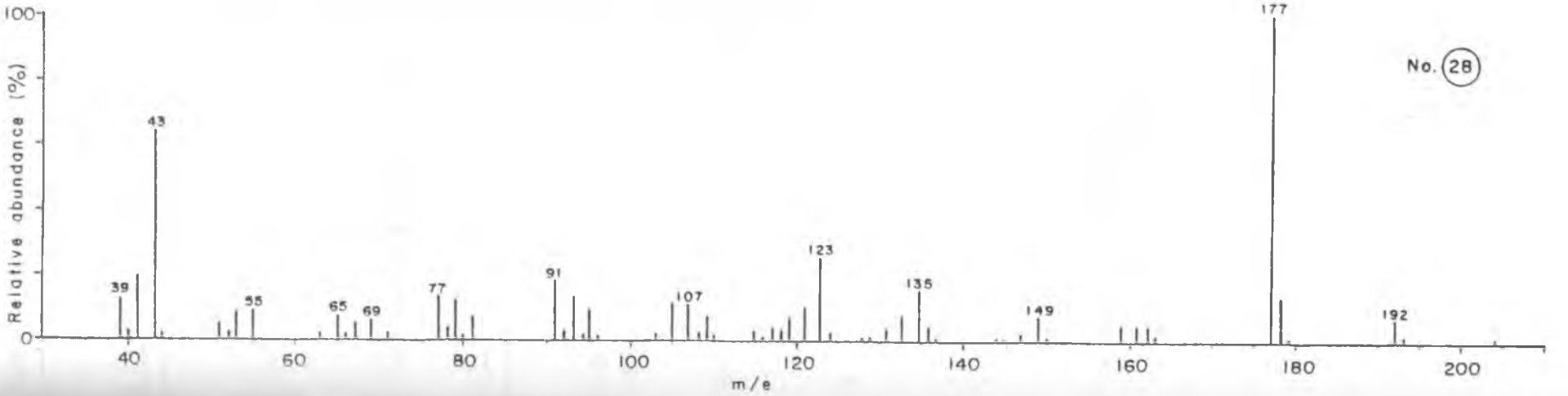


Figure 34: The mass spectra of tridecanal.

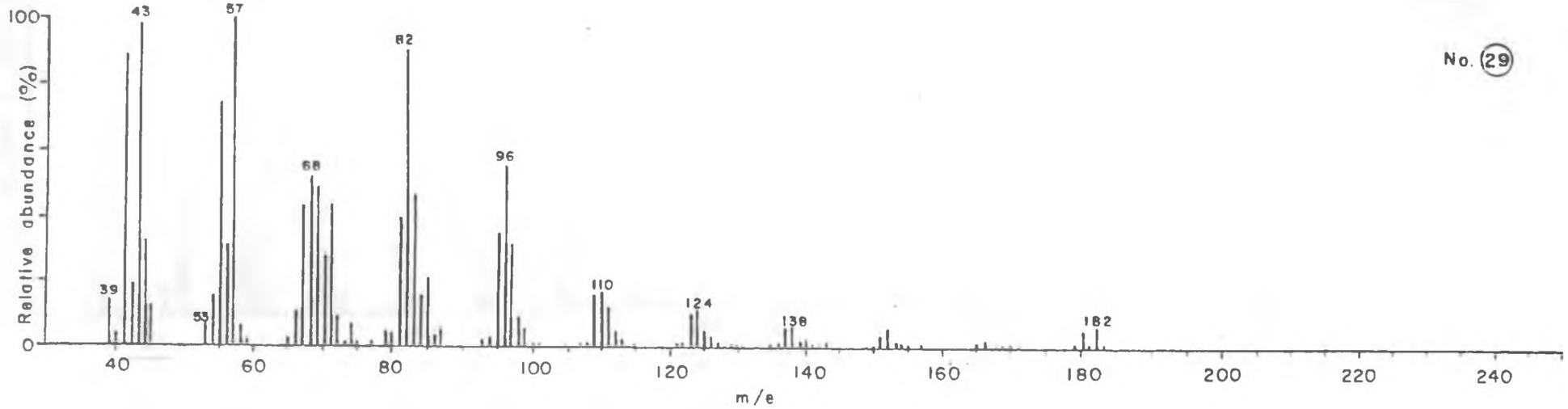


Figure 35: The mass spectra of nerolidol.

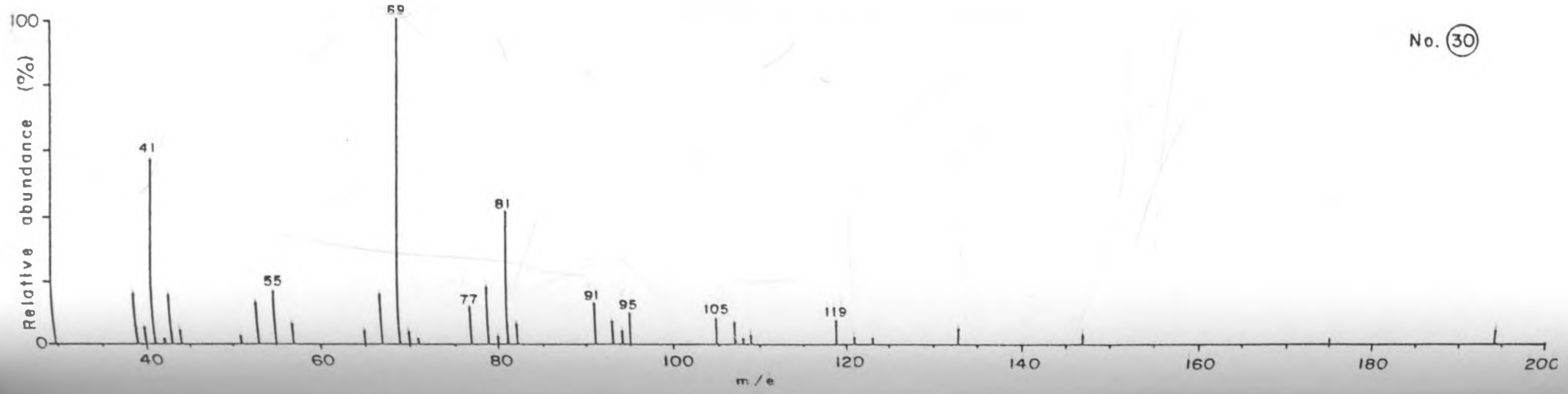
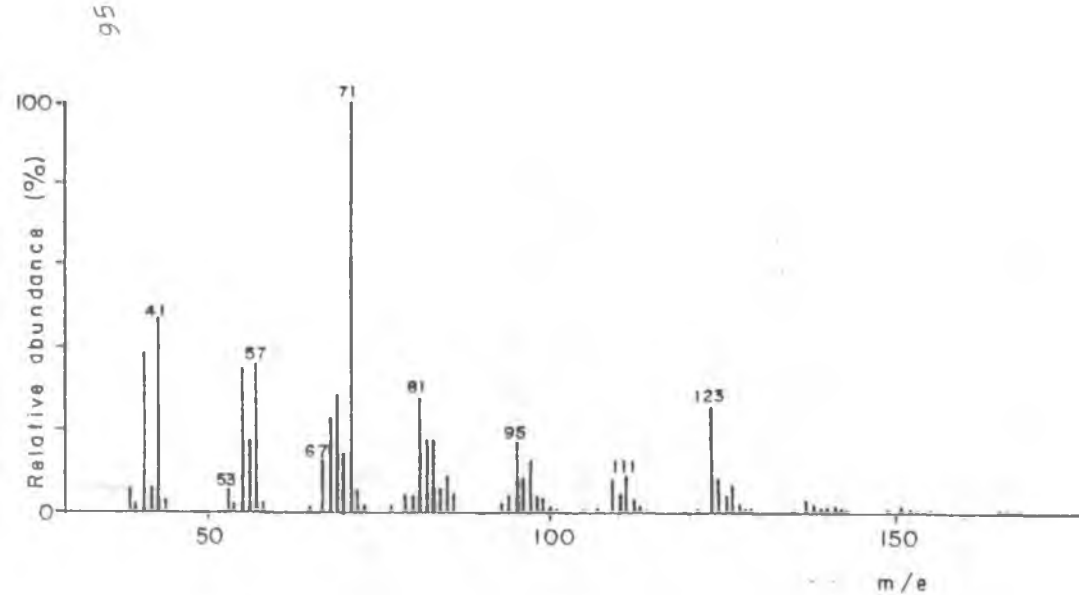


Figure 36: The mass spectra of *trans* phytol



No. (31)

CHAPTER 3

EXPERIMENTAL

3.1 Instrumentation

Gas chromatography (GC) of the essential oils was performed on a Hewlett packard model HP5890A series Gas chromatograph equipped with a split/splitless injector and a flame ionization detector (FID) both of which were maintained at 250°C. All the GC analyses were performed in the splitless mode. Chromatographic separations were achieved using a fused silica capillary column (Ultra 1 Hewlett packard(50 m x 0.32 mm ID)) coated with crosslinked methyl silicone (0.17 μm film thickness). Nitrogen (WSN) at a flow rate of 3.45 cm^3/min was the carrier gas, while hydrogen and medical air at flow rates of 45 cm^3/min and 360 cm^3/min respectively were used as fuel gas. The GC oven was temperature programmed to run from 45°C where it is maintained for 5 min then to rise at 5°C/min to 180°C then rise at 20°C/min to 280°C at which it is maintained for 15 min. The whole run takes a cumulative 52 mins. The peak areas, the percentage compositions and the retention times were calculated on a Hewlett packard model HP3393A integrator.

Gas chromatograph linked electron impact ionization mass spectrometric (GC-MS) analyses were performed on a Hewlett packard model HP5790A series Gas chromatograph coupled to a VG-Masslab 12-250 analytical organic mass spectrometer equipped with a data library system. Chromatographic separations were achieved using a

fused silica capillary column (50 m x 0.2 mm ID) coated with crosslinked methyl silicone (0.33 μm film thickness). All the GC-MS analyses were performed in the splitless mode with Helium as the carrier gas at a linear flow rate of 20 cm/sec. The oven temperature program was the same as that used during the GC analyses except for the final temperature (280°C) which was maintained for 20 mins, while the ion source and injector temperatures were 180°C and 240°C respectively.

3.2 Plant Material.

The leaves of the plants, *Boscia angustifolia* A.Rich. var. *angustifolia*, *Boscia mossambicensis* Klotzsch, *Cadaba farinosa* Forsk., and *Thylachium africanum* Lour., of the family capparidaceae were collected in Kenya at various locations along the Nairobi-Mombasa highway before and around Kibwezi in september 1992. This happened to be during a dry spell. These plants were however still leafy and green during this period. The identity of the plants was confirmed at the Nairobi university herbarium based at chiromo campus, by Mr Simon Mathenge, Botanist, University of Nairobi, Kenya. Voucher specimen were deposited at the same Herbarium. The leaves of *Gynandropsis gynandra* (L.) Brig.were obtained from a cultivated sample grown at Nairobi using seeds obtained from western Kenya, where it is grown as a common vegetable. The leaves of all these plants were separately packed in polythene bags, sealed and preserved in a deep freezer at -20°C awaiting the hydrodistillation process.

The voucher specimen numbers of the plants utilized in this analysis are as indicated in parenthesis:

a) *Boscia angustifolia* A. Rich. var. *angustifolia* {# 92/4, Ndakala & Mathenge},

b) *Boscia mossambicensis* Klotzsch.{# 92/3, Ndakala & Mathenge},

c) *Cadaba farinosa* Forsk. {# 92/1, Ndakala & Mathenge},

d) *Gynandropsis gynandra* (L.) Brig. {# 92/6, Ndakala & Mathenge} and

e) *Thylachium africanum* Lour. {# 92/7, Ndakala & Mathenge}.

3.3 Bioactivity Test Organisms.

The brown ear tick, *Rhipicephalus appendiculatus* Neumann: These were reared at the International centre for insect physiology and ecology's (ICIPE) insect mass rearing unit, Nairobi, Kenya. These are reared on the ears of rabbits according to the methods described by Bailey (1960) and Irvin and Brocklesby (1970). The ticks were collected in glass vials which were then sealed with cotton wool. These were then immersed halfway in moist sand to maintain the humidity in the tubes. This is precisely for storage purposes.

The maize weevil, *Sitophilus zeamais* Motschulsky: Maize weevils were obtained from a laboratory colony reared under ambient conditions with natural photoperiods on untreated (insecticide-free) maize obtained from small-scale holdings in Vihiga, Kenya. Weevils of variant ages and sexes were used in the bioassays.

3.4 Authentic Samples.

Authentic samples of the compounds identified in the essential oils from the plants were purchased from Aldrich chemical co., Gullingham, UK. and Sigma chemical co., Poole , UK.

3.5 Extraction of Essential Oils from the Leaves of the Plants.

The essential oils of the leaves of the plants were extracted by hydrodistillation of the leaves. This hydrodistillation process follows the same procedure, though with slight modifications, as that used by Von Rudloff (1969). The leaves were placed in a 5 litre round bottomed flask filled with 1 litre of water. This was in turn connected to a modified clavenger-type circulatory steam distillation apparatus connected to a condenser at the end of the set-up.

The oils were extracted into a layer of doubly distilled hexane (2 ml) placed in the collection arm of the modified clavenger apparatus. This hexane layer was collected and renewed every 2 hrs over a period of 8 hrs of the steam distillation process of each batch of leaves. The hexane extracts which therefore contained small amounts of water, were preserved at -20°c in the deep freezer in tightly capped glass vials. The extracts of each plant were combined in a clean beaker then dried over anhydrous Sodium sulphate (Na_2SO_4) to remove the water. The hexane solvent was carefully distilled off at low heat leaving behind the

essential oil.

3.6 Identification of the Components of the Essential Oils .

The chemical analysis utilizes conventional techniques routinely used in the separation and characterization of components of essential oils (Klocke et al., 1985; Komai and Tang, 1989). Chromatographic separations of the components of the essential oils were achieved by conventional Gas chromatography under the conditions previously described in section 3.1. These instrumental conditions and the column type were carefully chosen after they were found to produce good separations during the preliminary analyses undertaken.

The components were tentatively identified by Gas chromatography-mass spectrometry through computerized matching of the acquired electron impact ionization (EI^+) mass spectra with the stored NBS mass spectral library in the data system of the GC-MS and also by comparison with the published mass spectral data in THE WILEY/NBS REGISTRY OF MASS SPECTRAL DATA (Mc Lafferty and Stauffer,1989). The identifications were confirmed by their order of elution on the GC column, comparison of their relative retention time (Rt) values with those of authentic samples and co-injection of the essential oils with authentic samples.

3.7 Preparation of Test Samples for Bioassay.

The test samples for bioassay were prepared by diluting 100 μl of the essential oil or synthetic compound to 1000 μl of solution

in hexane. Successive dilutions of 10 times of the resultant solution were made subject to the observed repellency of the resultant dose. These subsequent dilutions were made until the dosage fell below ED_{50} (effective dose for 50% repellency).

3.8 Bioactivity Studies

3.8.1 Tick Climbing Repellency Bioassay

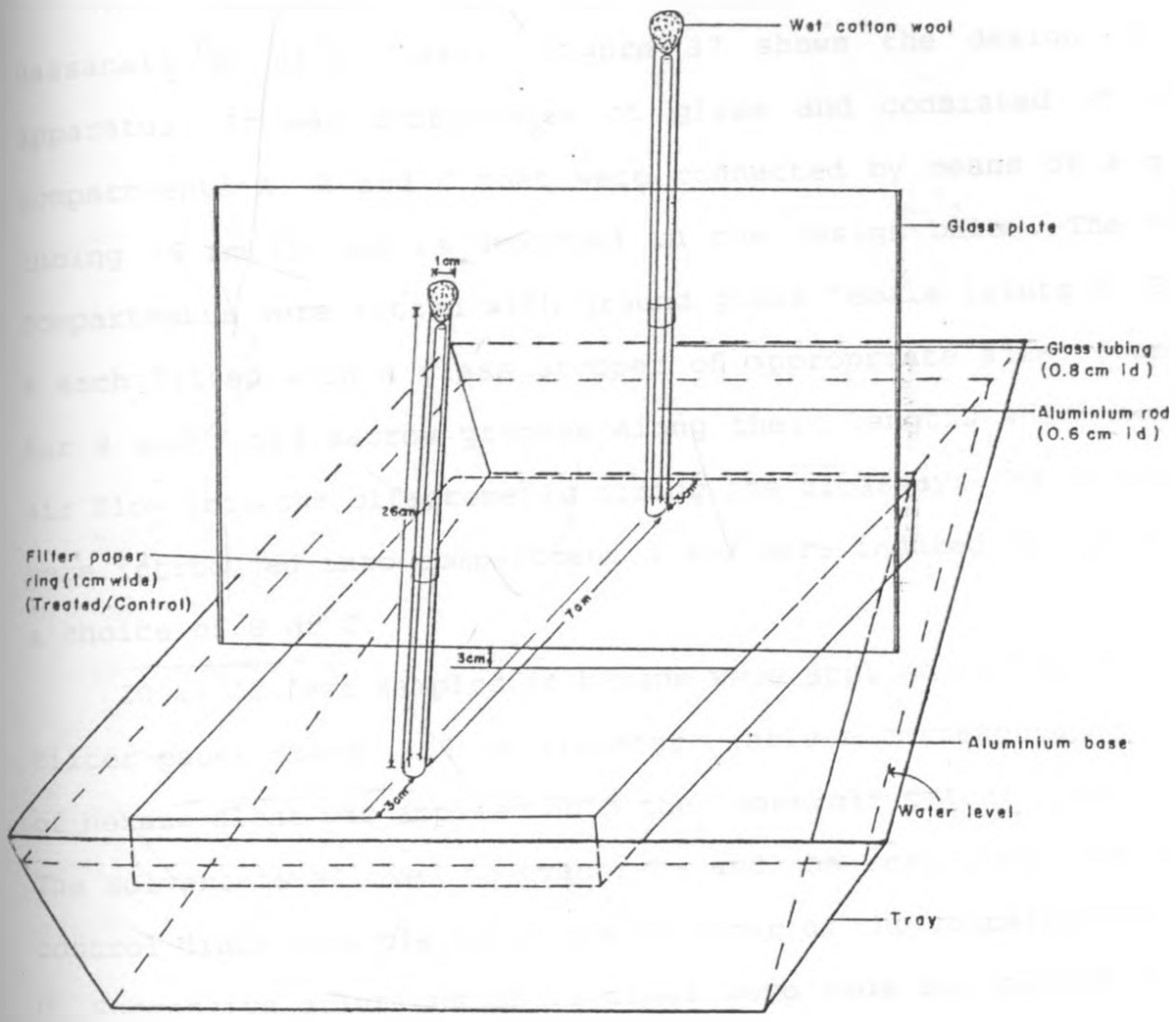
A tick climbing bioassay was used to test for *R. appendiculatus* repellency of the essential oils and the synthetic identified components. The bioassay was developed based on the observations of Browning (1976) that the unfed stages of *R. appendiculatus* climb the available vegetation to await a passing host. The longer they remain at an exposed vantage point on the vegetation, the greater their chances of locating a host. These ticks also actively avoid high temperatures and low humidity.

The design of the apparatus is illustrated in figure 36. It consisted of an aluminium base (14.0 cm wide x 14.0 cm long x 1.5 cm deep) on which were attached two aluminium rods (0.6 cm diameter x 24.0 cm long), placed 7.0 cm apart. Two glass tubes (0.8 cm diameter) were slipped over the aluminium rods and plugs of moist cotton wool were placed at the open end of the glass tubes. The moist cotton wool tended to "arrest" ticks which climbed up to the tip of the glass lined rods due to humidity effects. Strips of filter paper (1.0 cm wide) were wrapped around each glass tube, 10.0 cm from the aluminium base. The apparatus was then placed in

a metallic tray (24.0 cm x 40.0 cm) containing a pool of water such that the upper surface was above the water level. The water pool around the aluminium base serves to prevent ticks from leaving the aluminium base so most ticks climb the rods hence get in contact with the treated surfaces. A glass plate (28.0 cm x 40.0 cm) was suspended midway between the two rods such that it hanged 3.0 cm above the aluminium base. The glass plate aided in separating odors around the two stems. 100 μl of a given dose of the test material in hexane was then applied to one of the filter paper strips (treated) while an equal volume of hexane was then applied to the other filter paper (control). The bioassay assembly was uniformly illuminated with fluorescent light from above. All bioassays were conducted at 28±1°C and 75±5% relative humidity.

60 Newly moulted unfed adult *Rhipicephalus appendiculatus* ticks of mixed ages and sexes, selected and activated by moistening overnight in glass vials, are placed on the aluminium base midway between the aluminium rods. The assay was left to run for 1 hr after which the number of ticks on and above the filter paper on the control rod (N_c) and the treated rod (N_t) were counted. After each test the room was exhausted of air using a fan while the apparatus was thoroughly cleaned, rinsed with acetone then dried in the oven at 100°C. The assay for each dose of material was replicated 18 times. Percentage repellency (PR) values were computed using the formula $PR=\{(N_c-N_t)/(N_c+N_t)\} \times 100$.

Figure 37: The apparatus design for the Tick climbing method to study the repellency bioactivity against the brown ear tick (*Rhipicephalus appendiculatus*)



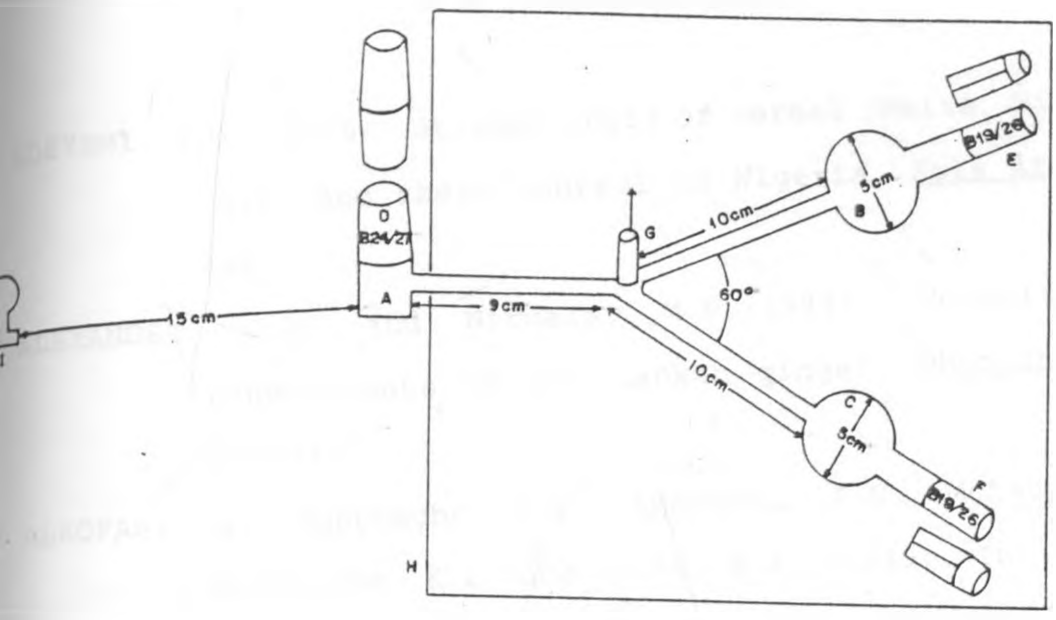
3 8.2 Y-tube Olfactometer Bioassay for Maize Weevil Repellency.

The olfactometer used in the bioassay was that developed by Hassanali et al., (1990). Figure 37 shows the design of the apparatus. It was constructed of glass and consisted of three compartments A, B and C that were connected by means of a glass tubing (6 mm ID) as is depicted in the design below. The three compartments were fitted with ground glass female joints D, E and F each fitted with a glass stopper of appropriate size. Stoppers for E and F had narrow grooves along their lengths which allowed air flow into the olfactometer during the bioassays. Maize weevils were introduced into compartment A and were induced to migrate to a choice of B or C.

20 µl of Test samples in hexane were applied to the "treated" filter paper discs (1.8 cm diameter) while a corresponding volume of hexane alone was applied onto the "control" filter paper discs. The solvent is allowed to evaporate and the resulting treated and control discs were placed in one or other of the compartments B and C. Successive dilutions of 10 times were made but subject to the tested dose being above ED_{50}.

Prior to the introduction of test materials, air suction was applied at G by means of an aspirator pump at a flow rate of 1.5 ml/min. This ensured that the olfactometer did not become saturated with the test material, which was confined to the olfactometer arm that contained the treated filter paper disc (treated arm).

Figure 38: The Y-tube olfactometer set-up for the maize weevil (*Sitophilus zeamais*) repellency bioassay.



For each assay, 60 randomly selected adult maize weevils of mixed sex and age were introduced into compartment A. Advantage is taken of the fact that the weevils are negatively phototactic by illuminating compartment A with light from a 60 watt bulb (I) placed 15 cm away and screening the rest of the olfactometer in a paper carton (H). The assay was left to run for 1 hr and then the number of weevils in the control arm (N_c) and in the treated arm (N_t) of the olfactometer were counted. After each assay, the olfactometer was thoroughly cleaned and dried at 100°C. The assay for each dose of material was replicated 4 times. Percentage repellency (PR) values were computed using the formula $\{(N_c-N_t)/(N_c+N_t)\} \times 100$.

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