THE PHYSICOCHEMICAL INVESTIGATION OF TERMITICIDAL COMPONENTS FROM THE LOCAL TREES, *JUNIPERUS PROCERA* AND *CROTON MEGALOCARPUS.*

By

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DECLARATION

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

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DEDICATION

Dedicated to my parents, my brothers and sisters and my grandparents.

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I extend my sincere gratitude to my parents and members of my family for their support, encouragement and patience not only during the course but throughout my academic life. I feel greatly indebted to all my friends and colleagues and members of staff, Chemistry Dept. who made my stay in the University of Nairobi a joy.

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ABSTRACT.

For many years various methods have been employed to control termite and prevent damage by termites on buildings, trees and other plants. Termiticides of various kinds have been used but there has been a general concern worldwide about the environmental problems associated with these insecticides. One alternative for termite control was the use of wood extract from trees and herbaceous plants.

Juniperus procera Hochat. (Family- *Cupressaceae*), an indigenous tree in Africa normally referred to as African pencil cedar tree and known for its durable wood, contained variable amounts of volatile oils from the extract of its heartwood. The dried wood was cut into pieces and then sawed into fine sawdust which was extracted by dry (destructive) distillation of wood method. In this case, the sawdust was heated in a large round bottomed flask into sample vials at various temperatures. These were stored in a refrigerator.

Termites were collected from colonies identified around the Chiromo area in Nairobi by excavating the nest and removing the termites together with the mould fungus and also by trapping. In trapping, a PVC pipe filled with damp soil and maize cobs was set near a nest for several days and the foraging termites collected into containers. The most prevalent species of termites in the area was *Odontotermes Stercorivorus*. Another species also used was *Odontotermes*

mautanus.

The crude extract was bioassayed against the subterranean termite species, *Odontotermes Stercorivorus* and *Odontotermes mautanus*. Various formulations (different percentages of the crude extract in distilled water) were made and their relative toxicity to the termites observed. The extract proved to be effective to both species It was apparent that the level of toxicity increased with increased concentration of the extract with the undiluted extract having the highest level of toxicity.

A tree used for comparison purposes, *Croton Megalocarpus* (family - *Euphorbiaceae*) was less toxic to the termites. TLC analysis revealed that there were ten major components in the *Juniperus procera* tree extract while the *croton* tree extract had five major components. This was confirmed by gas chromatography. The components were isolated by column chromatography and analyzed using Infrared, ultra violet and mass spectroscopy techniques. The extract contained more than 70% water which was removed by partitioning and using anhydrous sodium Sulphate. Acids were removed by sodium bicarbonate. The extract which was about 30% of the sawdust by weight was found to contain alcoholic and phenolic compounds together with acids. The compound cedrol, 17 a tertiary tricyclic alcohol was found to be in the highest proportion in the extract. Other compounds isolated were cedrene and other closely related compounds. The fraction containing the compound cedrol proved to be

effective on the termites. Previous work done by Clarence and co-workers had shown this compound to contain termiticidal properties. It had also been found to constitute a large proportion of cedar wood oil.

The use of these naturally occurring tree extracts would provide cheap and environmentally clean alternatives in controlling termites. This can be used as a part of the integrated pest management (IPM) while more research is done on the components (active ingredients) of the extract. The use of the extracts of such tree like the Cedar is a field open for more research and holds an important future in the control of pests.

CHAPTER ONE

1.0. INTRODUCTION

Over the years there has been great damage on wood and wood products due to termite attack. This has led to the selection of wood for building based on its durability and resistance to termite attack which is the most important cause of wood deterioration.

During the last four decades, numerous studies of the resistance of wood to termite attack have been carried out. These studies have covered various wood types from many parts of the world. Consequently, there has been a tremendous advancement in the production of pesticides to counteract effects of termites on wood but these pesticides have had their own toxic effects felt throughout the world in recent years [1].

Termites seldom injure or kill live trees, but can be very destructive to buildings, telephone posts, fences and other wood products. They also attack agricultural crops. These cellulose feeding pests prefer older plants or young plants that are sickly or temporarily not thriving and flaccid. More indirectly termites are harmful to growing plants by depriving them of mulches and vegetable residues before they can be broken down to be available to the plants [2]. There are many areas where mulching has been

demonstrated as a highly desirable practice but impracticable due to the termites attack on the mulching material almost as soon as it is put down. It appears that the termites activity is greatest in those soils which would benefit most from liberal additions of plant residues, both as manure and as mulch. Perhaps this is one reason for the necessity for such large quantities of farm yard manure and composite being used to produce significant improvements in yield and in soil texture in many parts of the tropics.

Other crops attacked by termites include sugar-cane. In this case appreciable damage is spasmodic in occurrence and concern for the resulting loss of crops depends to a greater extent on the development of the sugar industry in a particular area. Termite damage to seedling cotton by the subterranean nesting species is common on farms in Africa and Asia. In the Gezira area of Sudan, where cotton is grown under irrigation, the tap roots of young plants are subject to attack by *microtermes thoracatis* [1].

Wheat, rice and groundnuts are among other agricultural crops attacked by termites. A number of termites feed almost exclusively on grasses of various kinds while others include dried grass in their diet at the appropriate season. To some extent they compete with domestic animals for the available grazing. The influence of termites on tropical forestry is threefold; the destruction of seedlings and sapling required for afforestation, the reduction in yield of timber from forests where termites are active in mature trees and the

effect of termites on natural vegetation of woodlands Termites attack in nurseries and young plantations is usually more evident with exotic than with native trees and especially when growing conditions are marginal. Eucalyptus has so far been the main sufferer since it has been tried out in so many different countries to provide shelter, building poles and firewood [2].

Termite attack on field and tree crops and on forestry tree, especially in the semi-arid and sub-humid tropics, causes significant yield losses and in a major draw back on reafforestation. Since the 1940's control of this pest in agriculture and forestry has relied almost entirely on persistent organochlorine insecticides. Prior to the use of cyclodienes, highly toxic chemicals such as paris green and arsenates were used [3].

Increasing concern over damage to human health and the environment has now resulted in the banning or severe restriction of organochlorines in many countries. Research to develop alternative chemical control methods has centred on newer, less persistent insecticides, controlled release formulations of non-persistent insecticides and baiting techniques. Only controlled release formulations have so far shown significant potential in the field but they are not yet widely registered and their current high cost would be prohibitive for small scale rural farmers in developing countries.

1.1. IDENTIFICATION AND DISTRIBUTION OF TERMITES

Termites belong to the order of insects known as isoptera. They are social insects with well developed caste: These system consisting of primary reproductions, supplementary reproductives, workers and soldiers. There are four groups of termites based on their habits, namely:- (a) dampwood termites, (b) subterranean termites, (c) drywood termites, (d) powderpost termites [4].

(a) Dampwood termites attack moist wood and do not require contact with ground but require high moisture content wood. They are found in damaged poles, rotting logs, pilings and buildings.

(b) Subterranean termites belong to the family of *Rhinotermitidae.* They require constant supply of moisture. They are located in the ground. To feed above the ground they must construct covered passage ways called earthened tubes or sheltered tubes from the nest to the feeding sites. They feed on the spring wood and leave harder summer wood in ribbons.

(c) **Drywood termites** belong to the family of *kalotermitidae.* These are extremely serious pests in the tropics. They do not need contact with the ground but fly directly and enter untreated wood.

(d) Powderpost termites usually attack drywood. They do not have a ground contact and can reduce the wood to a powder. The common species of this group is *cryptermes brevis*. They cause severe damage to floors, woodwork furniture and small wooden objects [4].

1.2. Control

For many years, various methods have been employed to control termites and prevent damage by termites on buildings, trees and other plants. One of the most effective methods has been the use of termiticides of various kinds. In past years termiticides have been expected to provide 100 per cent control for several decades. However, the standard for termite control in the future may be less stringent, 7 to 10 years at 80 to 100 percent effectiveness [5].

For many years organochlorines have been used as termiticides and have been very effective but there is a general concern worldwide about the environmental problems that some pesticides, particularly DDT and other organohalide insecticides are causing.

Environmental persistent organochlorines have been used for seed dressings, planting holes or furrow treatment but currently they have been banned. While there are alternative methods for termite control in buildings, there are no proven alternative methods of termite control in agriculture and forestry [6].

One of the alternatives for controlling termites is the use of wood extracts from trees or herbaceous plants. Systematic of natural insect repellents/poisons by using extracts for wood treatment represents the most direct approach to ecologically acceptable wood protection. Another approach involves the use of structural information thus derived through the determination of structure-activity relationships to synthesize active structural analogues. A combination of the two approaches may allow the use of inactive, herbaceous plant extracts as raw materials for the synthesis of active compounds using simple synthetic procedures.

Locally available parts of plants, plant extracts and other substances have frequently been claimed to be effective in termite control although they have received little vigorous assessment in the field [3].

Woodash heaped around the base of the trunk has been recorded as preventing termite infestation of coffee bushes [7] and is said to repel them from date palms. It is said also to be effective in protecting tree seedlings if mixed into forestry nursery beds or applied as a layer below polyethylene planting tubes [8] and to protect stored yams, wooden posts and stacks of hay and maize straws. Use of woodash is a common practice which demands proper evaluation.

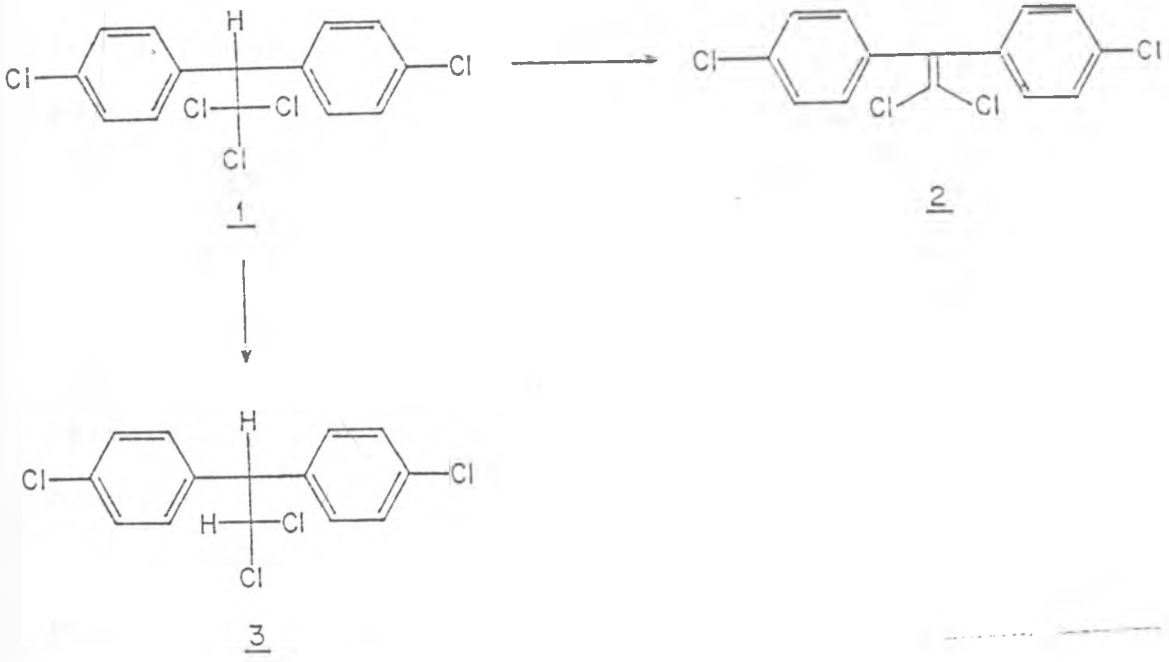
Nigerian farmers bury dead animals or fish viscera to reduce termite attack but the rationale behind this is not

clear. In India, water containing decomposed fish, tobacco and salt, or the washings from a bear skin were reputed to keep termites from mango trees [9]. Kerosene, diesel and crude oil have been recommended to prevent attack on timber and on tree bark [10]. Non-chemical control of termites is in its infancy but is a field ripe for rigorous evaluation. Current research on other insecticides and formulations may provide cheap and locally available alternatives for small farmers/foresters. Appropriate cultural methods, combined with the harnessing of resistance and the minimal use of modern or plant based insecticides and formulations in an integrated approach will provide the best answer.

Although organochlorine pesticides are very effective in pest control both in agriculture and forestry, the non-biodegradability of some of these pesticides have rendered them very undesirable for use to the extent of being banned. A good example is DDT, 1 which was until recently considered one of the most effective pesticides in the market. It later proved to be highly toxic to both land and marine life as it accumulates along the food chain. Due to the chlorine atoms present in this pesticide molecule,its toxicity is high. The chlorophenyl group doesn't break up even as the molecule breaks to other analogues i.e DDE, 2 and DDD, 3 (scheme I). Another pesticide which is also highly effective is chlorpyrifos but has been proved to be toxic to animals and marine life for example, shrimps, fish, rats etc. It has an

acute oral in rabbits of 2000 Mg/Kg and 32 Mg/Kg in chicks [12]. Other chlorinated and organophosphorus pesticides include aldrin (chlorinated hydrocarbon) chlordane (chlorinated),

SCHEME 1



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isofenphos (organophosphate), pirimiphos-ethyl (pyrimidine phosphate) and permerithrin [13]. These compounds, like DDT are not biodegradable.

Approximately 2.5 million tons of pesticides are applied each year throughout the world with a purchase price of $20 billion. Human pesticide poisoning and illnesses are clearly the highest price paid for pesticide use. A recent WHO and UNEP report (WHO/UNEP 1988) estimated that there are 1 million human pesticide poisonings each year worldwide leading to approximately 20,000 deaths [14].

A higher proportion of the pesticide poisonings and deaths occur, according to the report, in developing countries where there are inadequate occupational and other safety standards besides insufficient enforcement,poor labelling of pesticides, illiteracy, inadequate protective clothing and washing facilities. According to a report by David Pimentel and co-workers [14], a recently banned pesticide used for plant pathogen control, dibromochloropropane (DBCP), caused testicular dysfunction in animals and was linked with infertility among human workers exposed to it. The escalating prices of pesticides are preventing adequate control measures to be taken by farmers against pests especially in the developing countries.

In Kenya, which is largely an agricultural country, the role of trees, wood and wood products cannot be over-emphasised. Currently, our wood/timber production as a raw material is 3,500 million M^3 per year with fuel consuming 23 million M^3, construction, 37,000 M^3 while paper consumes 45,000 M^3 [15]. Thus there is great need to look for ways to protect this product from pests particularly termites.

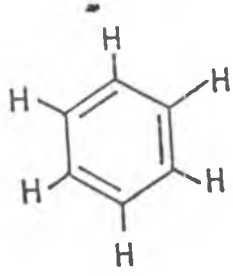
Research should be directed towards an integrated pest management programme (IPM) in which emphasis and acknowledgment of cultural and local pest management should be made [15].

Over the years many methods and chemicals have been used to protect wood and its products from termite attack.

Some of the earliest methods included the use of coal tar and wood tar. Coal tar is a by-product of gas manufacture and consists among other compounds, benzene, 4, toluene, 5 naphthalene, 6 and phenols. Wood-tar was obtained by the distillation of wood and was a by-product in the manufacture of pyroligneous acid, wood charcoal and in the distillation of pines after the extraction of the turpentine. The tar is chiefly useful in the application on stored products [16] .

For a number of years chemical toxicant had been accepted as the standard control measure for termites in many countries. However, for some time now considerable effort has been devoted to development of alternative procedures [17].

Non-chemical control of termites in agriculture and forestry is attracting renewed interest following increasing restrictions on the use of persistent



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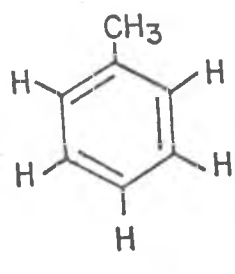

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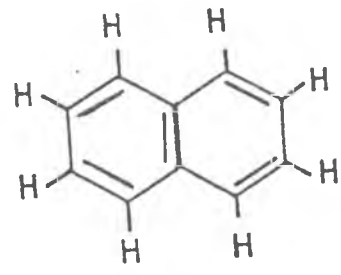

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organochlorines insecticides. These controls involve methods which, without the use of commercial pesticides, attempt to prevent termite access to the plants, reduce termite numbers

in the vicinity of the plants or reduce susceptibility of the plants themselves. Biological control by predators or pathogens is unlikely to be successful due to the termites' social structure and behaviourial responses to infected individuals.

The use of 'natural' insecticides from locally available plant products may be effective in some cases but, as they are not subject to the same rigorous safety and environmental evaluation as commercial pesticides, their use cannot be sanctioned unconditionally [18]. Despite this fact, naturally occurring anti-termite compounds are unlikely to be as toxic and environmentally persistent as the organochlorine pesticides.

Control measures differ depending on the type of termites involved. Conventionally, the plant damage by subterranean termites has been checked by persistent insecticidal barriers in the soil around roots, preventing termite access to the plant. Mound-building termites can be controlled to a limited extent by destroying or poisoning the mound and the queen. Dry-wood termites, although considerably less important, have proved extremely difficult to control chemically but a combination of cultural and chemical control may be effective against them in some instances [19].

Termites are eaten by a wide range of vertebrate and invertebrate predators whose natural influence on termite

numbers and population dynamics is partially understood in a few cases. This phenomenon is utilized in biological control which aims to manipulate other organisms (predators and pathogens/ parasites) with a view to reducing pests to economically acceptable levels [20].

In a research carried by Wu, H.J. and others, the termite species *coptotermes formosanus* and *Reticulitermes speratus*, which are major pests of structural timbers in Taiwan were tested for susceptibility to the entomogenous nematode, *Steinernema feltiae Filipfev.* This nematode proved to be a potential microbial pesticide against the termites invading the host via the mouth. All castes were susceptible to this vermin except the host eggs [21].

When the queen is removed and the nest destroyed there is substantial reduction of the activity of the mound-building termites. However, if nymphs are present at the time of de-queening replacement reproductive may develop. Thus this is not altogether a very effective method of control [22]. Nevertheless the reduction in foregoing activity may be for a long enough time to allow plants and young trees to become established or offer short term protection to crops, but only in the rare cases where mound-building species are the only serious termite pests.

There are many methods that have been in application over the years in many countries for the control of termites. Several researchers over the years have recommended an emulsion of kerosene and fish oil soap

applied to a layer of sand or ashes under pots in horticultural nurseries.

In recognition of the economic importance of over 300 species of termites as pests of agriculture, forestry and buildings, a research group was formed in 1950 to study their biology and taxonomy in relation to the problems of developing countries [23].

During the research it was found that crop losses due to termite attack in Sudan savanna were very high. This was due to removal of outer shell layers of pods by termites consequently increasing fungal infection of kernels and this produced diseased plants. Maize, wheat and other cereals were also mainly attacked by *microtermes* and *odontotermes* but yield losses did not exceed 10% except where irrigation failed.

Ridge treatment at planting time with aldrin at 1kg a.i. (1Kg active ingredient) per hectare prevented damage and reduced termite population from 267 M^{-2} to 12 M^{-2}, giving yield increases of 22%. Application of the same amount (1kg active ingredient) per hectare of pirimiphos-ethyl and chloropyrifos had less effect on the termite populations [23].

The structural diversity of the known anti-termite compound is extreme, ranging from flavonoids to sesquiterpene alcohols and ketones. All of the compounds, however contain oxygen and most of them have a hydroxyl and/or a carbonyl group. Since these are only a few

naturally occurring compounds known of any single structural type, comprehensive analysis of data for structure/action relationships would be difficult. Some structure/action information is available from reports of bioassays of minor variations of active natural products [1].

Several extracts have been tested on various families of termites. Laboratory studies have identified a number of plants containing material toxic to termites. Most research has concentrated on chemicals in timber which confer resistance to attack. Many timbers contain chemicals or complex mixtures of chemicals that repel or kill termites or interfere with their gut fauna [24], but these chemicals are difficult to extract, consequently they are not very effective pesticides in their own right this is particularly because most toxic chemicals in timbers are avoided by termites except in no-choice tests [25]. However, waste sawdust or wood chips from trees containing repellent chemicals may provide some protection if incorporated into soil.

Herbaceous plants or the leaves and fruits of trees are more likely to be effective; they are easily crushed and usually can be used without complex extraction procedures. Laboratory experiments have found numerous materials repellant or toxic to termites in such plant material.

Residues after oil such as neem or castor had been extracted were found to be effective. It is not yet known whether this is as a result of toxic effects or effects of

mulch on soil physical and chemical properties which in turn affects plant vigour and susceptibility to attack [26]. Water extracts of plants have been mixed with irrigation water, sprayed on to plants or mixed with the soil to protect trees and crops.

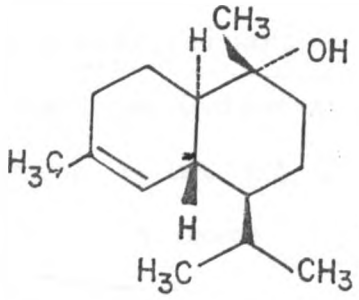
In Kenya, the leaf mulch of the plant *cassia siamea* of the *Leguminosae* family is reported to protect tree nurseries from termite attack. Other tests have shown leaf mulch of *Leucaena Leucocephala* of the same family to offer similar protection to tree nurseries [27].

Two trees, *Azadirachta Indica* (neem) and *Melia azedarach* of the family *meliaceae*, when applied as leaf mulch and leaf/ berry/ extract respectively offer considerable protection to tree nurseries in Kenya [27].

The compounds of the extractives from cypress pine, *Callitries columellaris* were shown to be toxic to the subterranean termites *coptotermes acinaciformis* and *Nasutitermes exitiosus* and repellent to *Mastotermes Darwiniensis* [28]. This prompted J.R.J. French in 1983 to carry out bioassays of extracts from Scaly ash (*Ganophyllum falcatum* Bl) against the subterranean termite *coptotermes acinaciformis* froggatt [29]. In this research the timber (*G. falcatum*) was extracted using cold methanol and the solubles separated from the insolubles. It was extracted as wood shavings and bioassays carried against 200 termites of the species *c. acinaciformis* comprising nymphs, soldiers and workers. The results indicated that limited amounts of

extracts from *G. falcatum* could be suitably bioassayed against the subterranean termite (*C. acinaformis*) with the techniques developed during this investigations.

Studies carried out by Clarence McDaniel in 1989 on the major termiticidal components of the heartwood of port-orford cedar, *Charmaecyparis lawsoniana*, revealed that the extracts were toxic to termites [30]. Isolation and characterization of compounds in various solvents used for extraction showed that the compound α-cadinol, 7 was present in the highest percentage along with other compounds such as α-terpineol, 8, T-cadinol, 9 and T-murrolol, 10. The bioassays on the subterranean termites *R. flavipes* and *R. Virginicus* Banks and the formosan subterranean termite *Coptotermes formosanus* shiraki proved the effectiveness of the extracts on the termites. These were sesquiterpene



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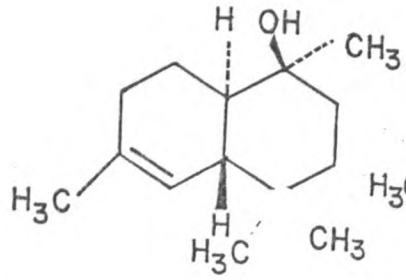

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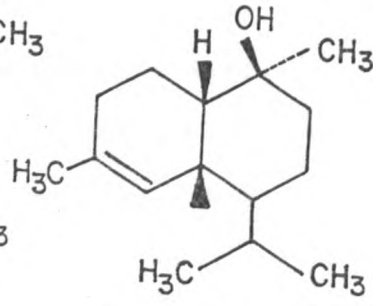

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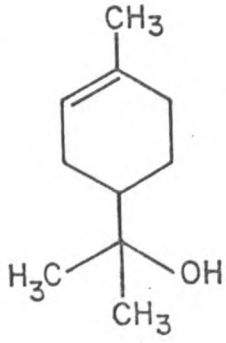

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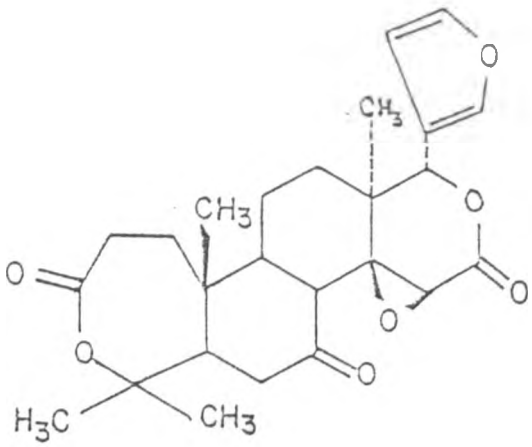
alcohols and were found to exhibit some toxicity to *R. flavipes* species of termites.

Recently, a sutra placed in a wooded pagoda in Japan about 1200 years ago was discovered in a wall preserved state without damage by insects or micro organisms. Experiments showed that extracts of *Phelladendron amuranse* had been added during its making. Analysis showed that the methanol extract of *p. amuranse* back had strong anti-feedant activity against *Reticulitermes speratus*, a serious termite pest of paper and wood [31].

The extract was sequently partitioned with hexane, chloroform, ethylacetate and water and the activities observed in both chloroform and water fractions. The active ingredient isolated from the chloroform fraction were obacunone, 11 and Kihadanin A, while those from the water fraction were berbarine chloride, and palmatine iodide, 12. The palmatine iodide appears as orange-yellow needles with a melting point of 246°c and found as an alkaloid in *Jateorhiza palmata* columba and a large number of other species [31].

The natural resistance of timber to damage caused by *Psammotermes hybostoma* and fungi was tested on thirty seven tree species grown in Sudan. Resistance was evaluated in terms of weight loss. Lignin and phenolic compounds were thought to confer some resistance to both termites and fungal damage. In this study by Abushama and Abdel, extracts



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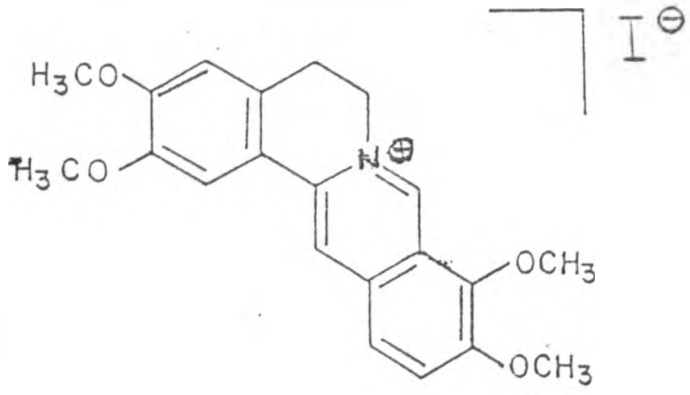

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from highly resistant timbers were tested for effectiveness using susceptible species [32].

In similar studies, a non-choice bioassay indicated that neem oil deterred feeding in *reticulitermes speratus*. A methanol extract of the oil was 4-fold more active than the whole oil. Eleven main Limonoids, accounting for 81.5% of the activity were purified from the active chromatographic fractions of the methanol extracts.
No acute toxicity was found, although *R. speratus* that were given large doses tended to die faster than unfed ones [33].

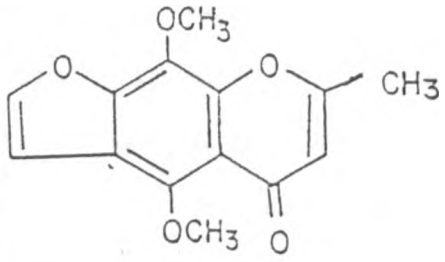
Evaluation of various parts of the paw-paw tree, Asimina triloba *Annonaceae* as a commercial source of the pesticidal annonaceous acetogenins has been done in the U.S.A. Various parts of *Asimina tribola* were extracted and partitioned to concentrate a mixture of acetogenins into a standardised pesticidal extract. A bioassay with *Artimina salina* showed that the extract was toxic. Small twigs yielded the most potential extracts with a lethal concentration necessary to exhibit a 50% mortality rate of 0.04 ppm. (LC_{50} = 0.04 ppm.) while the leaves gave the poorest activities [34].

It was concluded that by pollanding the entire twigs and small branches of *Asimina tribola*, it could be processed to produce a potent acetogenin mixture. Scientists undertaking the research concluded that the biomass could be made available in quantities needed for commercialization of the pesticidal product [34].

There is no literature of any tests done using this extract on termites. Another study done on isolates from the root bark of the plant *celastrus angulatus*, showed that it contained an insecticidal sesquiterpene polyestan, which had strong insecticidal and anti-feedant effects against *Heliothis armigera*, *Aphis gossypii*, *Pieris rapae* and *Brevicoryre bressiceae* [35].

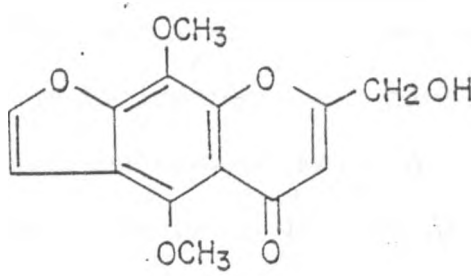
Several secondary plant metabolites belonging to different classes viz terpenoids, alkaloids, coumarin, phenylpropanes are known to be feeding deterrents to a number of insects. The presence of such feeding deterrents in plants may be of a ecological importance in protecting them against herbivorous insects. Reports on recent tests done with extracts from the plant *Pimpinella monoica* Dalz. (*umbelliferae*) show that the plant contains anti-feedants that cause significant feeding inhibition to *Spodoptera literal* F. larvae, a tobacco catapillar [36]. Some of the compounds isolated were khellin, 13, Ammiol, 14 and Isopimpinellin, 15. The plant proved to contain furochromones and could serve as an alternative practical source of these compounds.

Previously, *Ammi visnaga* a plant of Egyptian origin has been the major natural source of furochromones. Amongst furochromones, visnagin, 16, showed maximum feeding inhibition, although its methoxylated derivative (Khellin) caused two fold reduction in bioactivity. During isolation, most of these furochromones were obtained through



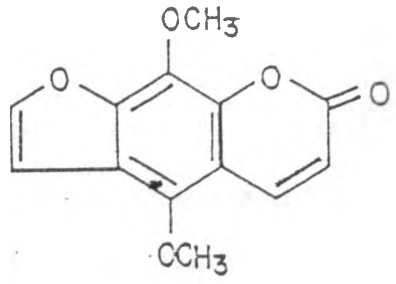
13

This compound exhibited a 55% inhibition on feeding at 330PPM.



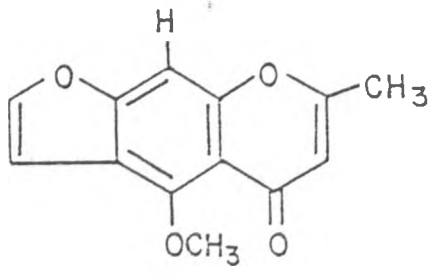
14

Showed similar inhibition characteristics to Khellin.



15

Effective concentration was 5PPM (EC_{50}: 5PPM) to inhibit 50% feeding.



16

The Cpd. had a EC_{50} of 69.2PPM.

preparative thin layer chromatography. The compounds, khellin, visnagin, isopimpinellin and visamminol, were extracted using n-hexane and then chromatographed using a silica gel column and the solvents n-hexane, chloroform and ethylacetate used for elution respectively [36].

Richardson and co-workers have recently reported the identification and preparation of antisectan dienols from *diptenocarpus kerii* tree resin [37].

The two uncharacterised sesquiterpenes were synthesised from α-gurjunene, a novel process involving meta-chloroperoxybenzoic acid. Oxidation of α-gurjunene produced the sesquiterpene in one step. Bioassays with termites demonstrated that one of the sesquiterpenes was more toxic than the other, resulting in a 50% mortality in seven days when offered to *Neotermes* (*Isoptera*) [37].

1.3 WOOD CHEMISTRY

Wood is composed of about 50% carbon, 6% hydrogen and 44% oxygen and minor amounts of nitrogen. The chief compound in wood are cellulose, hemicellulose, lignin and extractives. (1) **Cellulose** which is a linear polysaccharide built up from anhydroglucose units which are connected with each other by 1 4-**β** -glucosidic linkages. (2) The **hemicelluloses** which comprise all non-cellulosic polysaccharides and related compounds e.g. uronic acids and their derivatives. (3) **Lignin** which contains a basic

skeleton of four or more substituted phenylpropane units per molecule.

(4) **Extractives** which consists of a large number of organic compounds which can be extracted with organic solvents and also partially with water, and which do not belong to the hemicellulose group [38].

These extractives are the ones of most interest since they contain most of the termicidal compounds of a tree. Most woods contain aliphatic, aromatic and alicyclic compounds; hydrocarbons; alcohols; esters; acid-esters; and phenolic compounds. Also found may be sterols, tannins, essential oil resins, dye stuffs, lignans, proteins, waxes and some alkaloids. With regard to the chemical structure of the oleoresin it can be said that volatile oil and the resin acids belong to the terpenoids which are derived from the hydrocarbons known as terpenes. They are cyclic and acyclic in character [39] and include phenols, Condensed and hydrolysable tannins.

Hydrolysable tannins are esters of a sugar, mostly glucose with one or several polyphenolic carboxylic acids. The **condensed tannins** are built up of monomers of the catechin type. They are found chiefly in the bark but sometimes also in the wood.

Other extractives include quinones, fixed oils, ketones aldehydes, terpenes, cyclitols and colouring matter. These extractives are found within the cell cavities and adhering

to the walls of the wood. Some solvents used to remove these compounds include, ether, benzene, alcohol, acetone, gasoline, water or steam.

Extraneous components of the various woods serve to characterise a wood i.e. durability (resistance to insects and fungal attack), its colour, odour, taste, inflammability, toxicity etc. All these characteristics are often correlated with the presence or absence of these non-cellular components. Investigations show that 1% of a dihydric phenol (Pinosylvin) prevents the successful sulfite pulping of pine heartwood. It also stops decay in an old pine stumps. A satin wood lumber *Chloroxylon swietenia* is reported to cause dermatitis to people working on it [39].

A compound group referred to as Thujaplicins are responsible for the western red cedar wood durability but these Thujaplicins also cause corrosion of metal equipment during the alkaline pulping operation. In one Phillipine wood, Ginjo, which is notoriously corrosive, Schorgen showed the presence of 0.2% of a mixture of acetic and formic acids. Most of the essential oils, oil turpentine, etc of tropical woods, resins tannins and colouring matter derived from wood have found numerous industrial uses all over the world [39].

1.3.1 VOLATILE OILS

These consist of essential and ethereal oils. Most of the important oils are by turpentining operations and steam

distillation of wood. Usually these oils are liquids at ordinary temperatures. They are optically active and their optical rotation usually ranges from +55° to - 122°. Their specific gravity ranges from 0.683 to over 1.0. Pine terpentines (terpene hydrocarbons), consistently fall in the narrow range of 0.850 to 0.875. An oil may contain several compounds e.g. (a) Hydrocarbons (i) [Terpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$) and diterpenes ($C_{20}H_{32}$)] (ii) Other cyclic terpenes and sesquiterpenes; (b) Acids and phenols; (c) Alcohols of terpenes and sequiterpenes and other alcohols; (d) Esters; (e) Ethers; (f) Aldehydes and ketones; (g) Oxides and Lactones [39].

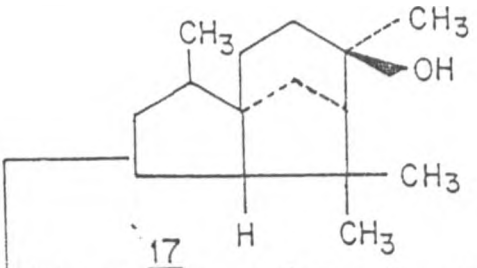
1.3.2 OLEORESINS

These are viscous exudate of the non-volatile solids (Rosin) and the liquid essential oil secreted by the resin forming cells of the sapwood when the tree is wounded by scarifying or boring. Gum terpentines refer to the distillates of the above oleoresins.

1.3.3 WOOD TERPENTINES

This group comprises wood oils which are distillates of steam distillation of the heartwood.

Volatile oil of the heartwood (wood turpentine) from *Juniperus procera Hochat.*(Abyssinian juniper) include 2% oil, 38% cedrol, 17. Another tree of the same family of

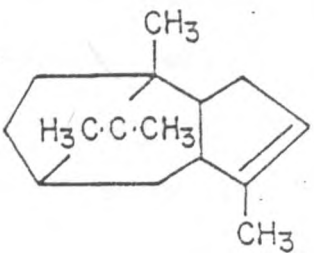


Tertiary tricyclic alcohol.
α-cedrol

17

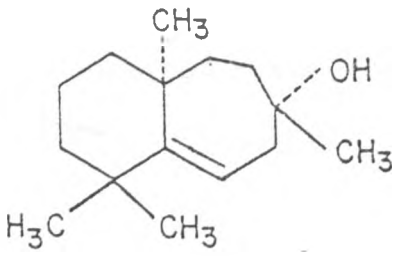
This is an anti-termite compound found in Juniperus procera, Juniperus virginiana trees etc.

readily dehydrates
to α-Cedrene



An Olefin

18



19

Cupressaceae that is *juniperus virginiana* L., eastern red-cedar contains wood oil (turpentine) whose content is 2.5 to 4.5% oil, cedrene, 18, cedrol, 17, widdrol, 19 cedrenol and pseudo cedrol.

White cedar oil from *Thuja accidentalis* L. Northern white-cedar contains 37.2% cedrol, with a yield of 1 to 1.5% from small branches. While *chamaecyparis lawsoniana* parl (port orford cedar) contains 45.7% d-α-pinene, 26% d-borneol, 21%, d-cadinene, 3.9%- L. cadinol, 3.2% d-limonene and aliphatic acid esters [39].

Juniperus excelsa M.B. (Grecian juniper) has α-pinene, cedrene and cedrol. Cedrol is a tertiary tricyclic alcohol ($C_{15}H_{26}O$), which frequently separates from cedar-wood oils in the form of silky white needles. Dehydration to the corresponding olefin, cedrene takes place readily.

1.3.4 OTHER TERMITICIDAL COMPOUNDS

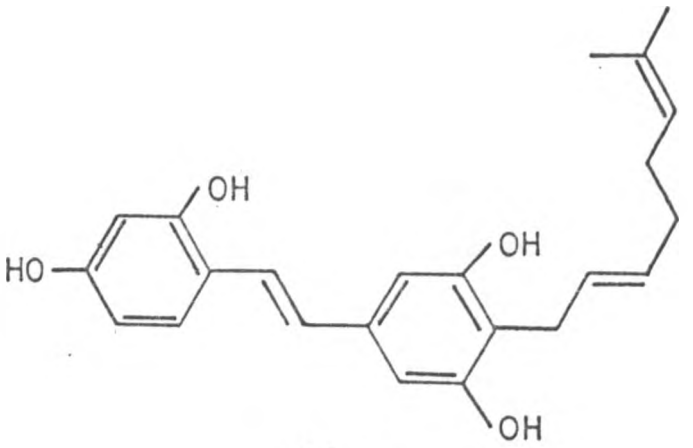
Throughout the tropics, certain timbers have local reputation for resistance to termite attack. Such timbers are usually indigenous, but some are imported as in the case of mangrove which has for centuries been carried from East Africa to the countries around the Persian Gulf.

Specific chemical substances are present in some woods in sufficient quantities to drive away termites. In other woods the amount present is only sufficient to repel the termite after it has eaten a minute particle.

Once again it is the heartwood that usually has an effective concentration of repellent substance. Extracts of resistant heartwoods have been used to impregnate palatable softwoods and so render them termite repellent for long periods. These extracts have been analysed, and in some cases it has been proved possible to synthesis likely constituents in quantity in order to test them individually [40]. Work done by Wolcott around 1958 revealed that chlorophorin, 20, which occurs in the timber iroko (*chlorophora excels*) is effective as a one-half per cent solution. Pinosylvin, 21 and its derivative, Pinosylvin methyl ether, 22, from *Pinus sylvestris*, was shown to be similarly effective at a dilution of one-hundredth of one per cent. In view of the lack of resistance to termite attack shown by *pinus sylvestris* timber, one can only assume that pinosylvin is present in exceedingly small amount in the tree [41].

Tectoquinone, 23 from Teak (*Tectona grandis*) was considered by Wolcott to be the active agent in this resistant timber, and experiments with it showed that a 0.5 per cent solution protected flamboyant wood for over nine years against dry wood termites.

Other termiticidal compounds include, anthraquinone, nathoquinone from the family of plant referred to as *Leguminoseae*, stilbene and aldehyde (furfural) from the *pinaceae* family.



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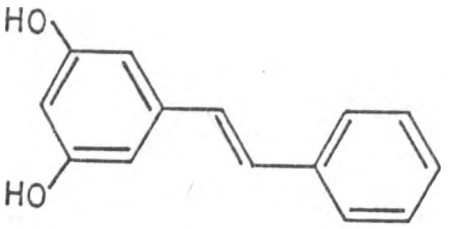

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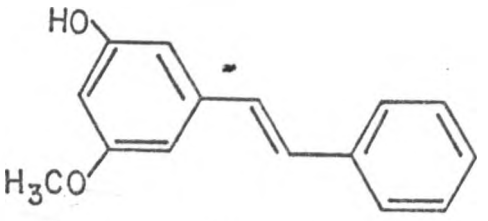

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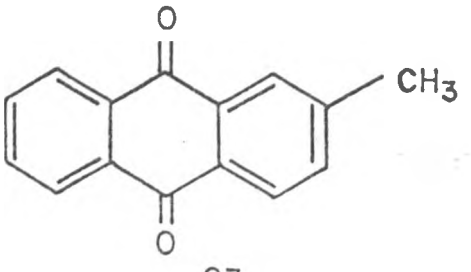
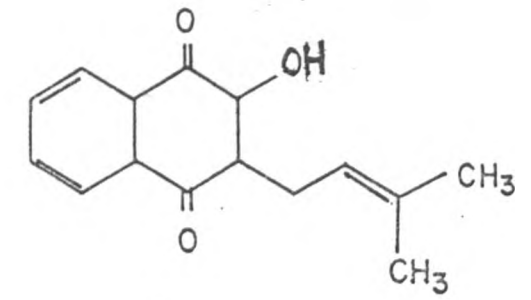


23

Cupressaceae family of plants produces sesquiterpene alcohols and unsaturated ketones. The same family of trees has 1-citronellic acid, 24, chamaecynone, 25 from *callitris columellaris* and *callitris pisifera* respectively.



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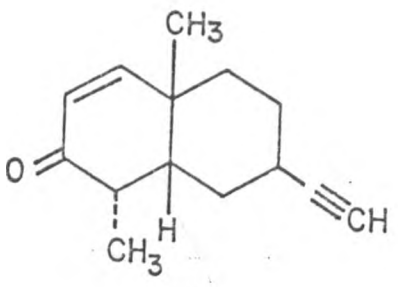

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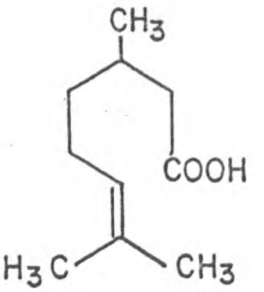
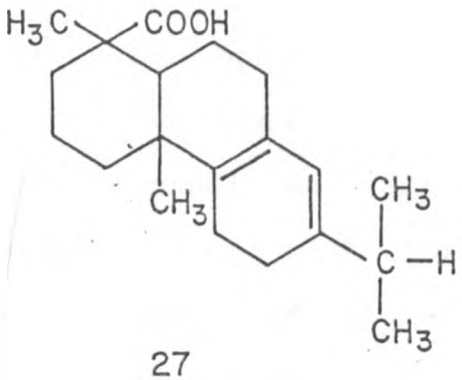
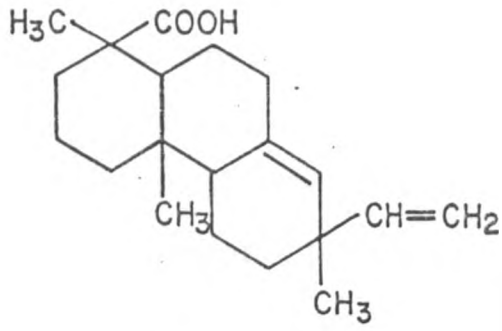


24

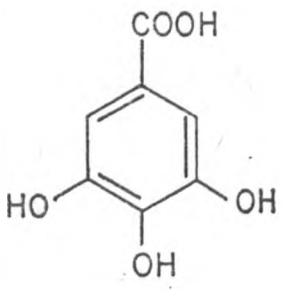
Pinus lambertiana has palmitic acid, margaric acid ($C_{16}H_{33}COOH$), stearic acid and pinocembrin. other termiticidal compounds include, Lapacol, 26, Palustric acid, 27, Dextropimaric acid, 28, Gallic acid, 29, Catechin, 30 and Conidendrin, 31.


H3C
COOH
CH3
CH3
C—H
CH3

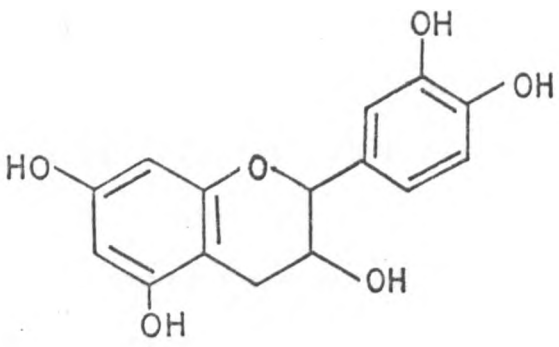
27


H3C
COOH
CH3
CH=CH2
CH3

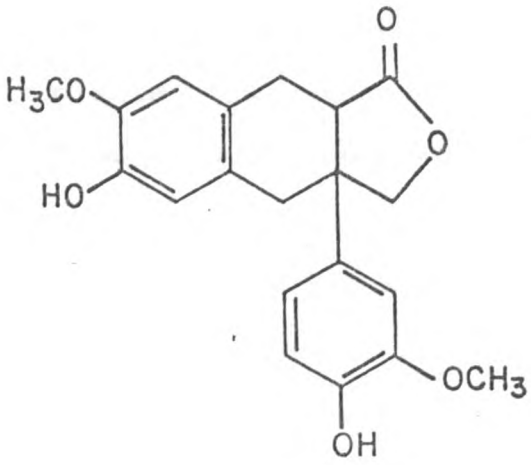
28


COOH
HO
OH
OH

29


OH
OH
HO
O
OH
OH

30


O
H3CO
O
HO
OCH3
OH

31

Most plant based insecticides break down rapidly in the soil and would not present long term environmental problems. Research into the use of plant extracts for termite control so far suggests that they may have considerable potential in providing a cheap locally available method of control

The use of waste sawdust which is a by-product in almost all timber processing industries and carpentry workshops, and the use of cheap extraction procedures is one way of achieving this goal. Moreover, trapping of wasteful gaseous products during charcoal preparation would be another way of reaching the above objective [41].

Termite resistant crop species are often already known and should be promoted if other social and economic constraints allow. There is however need for development and identification of varieties resistant to termites. More is known of resistance of tree species but insufficient acknowledgement of local and regional differences has meant that much of the literature is of little specific value. Use of resistant plants requires no development or implementation of new techniques once the appropriate species/variety is identified. This seems especially suitable in developing countries where other methods are difficult to justify economically. Although very little research work has been done on pesticides, particularly on naturally occurring anti-termites in Africa, work previously done or on-going research has given encouraging results.

The trend to look for alternatives to chemical pesticides has been brought about by the prohibitive prices of the pesticides and erratic supply especially in developing countries and accumulation (non-biodegradability) of some pesticides. For example, in Nigeria the use of pesticides for control of insects pests and disease of major food and cash crops in the field and in store has increased during the last 5-10 years but due to the problems sited above majority of farmers with small-scale holdings have been adopting alternative methods of control [42]. Some involve use of Neem extracts, vegetable oil ashes, heat and soke treatment. Also included are scopa solutions, mulches and organic soil amendments [42].

In Kenya not much has been done in this field but unpublished reports indicate that there is on-going research to test alternative methods and their efficacy in controlling insect pests particularly termites which are the most serious pests of wood and wood products.

Reports from the workshop proceedings of 1992 on termite research and control indicates that there is a current joint programme between Kenya Agricultural Research Institute (KARI) and Danish Technological Institute (DTI) to initiate a regional approach for termite control in East and Central Africa [43]. Some of the goals of the 3 year-programme initiated in 1991 are: (i) compilation of information on the extent of damage caused by termites in Agriculture, forestry and the building industry, (ii)

development of an integrated pest management strategy (IPM) for damaging termites, (iii) investigation of the possibilities of utilizing locally occurring natural enemies for control of termites with the emphasis on fungal pathogens and (iv) evaluation of the possible use of the new insecticides within the integrated pest management programme.

Some of the trials being carried out in this project involve the use of the neem tree extracts. Mostly it is the seeds that have been used. Unpublished reports indicated that the seeds of the neem tree are dried and then crushed into powder. This powder is then applied to trees in the field and their durability as compared to controls is observed [44].

Although previous work has been done elsewhere in this field, it was apparent that the pest problems in the country needed to be addressed with a view to acquire cheap control methods which could be incorporated in the integrated pest management (IPM) programme. The species of trees and termites investigated in this work were different from those used in any other work. The high costs of pesticides, including termiticides, prompted us to try and look for a control which was easily and cheaply available. Therefore, the method (destructive distillation of wood) was used. It is a cheap, simple and easy approach. Moreover the volatiles can be collected even from charcoal burning processes.

OBJECTIVES OF THE RESEARCH

1. To carry out a preliminary study on termite related problems.
2. To explore naturally occurring materials or plants that would provide by-products capable of repelling and/or killing termites.
3. Work with appropriate extracts from partial destructive distillation of wood that according to preliminary studies seem promising as anti-termites.
4. Use analytical procedures to study the most effective extracts and active ingredients.
5. Develop formulations to base on material extracts in order to produce effective termiticides.
6. Use termites from various sites (termite nests) to test the efficacy of the new environmentally clean product.

CHAPTER 2

EXPERIMENTAL SECTION

2.0. General

The first extraction material was obtained from the pencil cedar tree referred to as *Juniperus procera* Hochat. It is of the family *Cupressaceae* and is indigenous in the Kenya. It is found in the Kenyan highlands,between 1800-2950m; and distributed around the Rift valley. Its heartwood is best known for its durability especially when used as fencing posts [45].

The second tree extract was obtained from *croton megalocarpus* of the Euphorbia family. This tree is common around Nairobi and in dry upland evergreen or semi-deciduous forests and used as a boundary marker. The wood is perishable and splits badly, and although a good firewood, the smoke irritates the eyes [45].

Most solvents used are laboratory reagents. These include petroleum ether, dichloromethane, methanol and hexane. Other analytical grade reagents used include anhydrous sodium sulphate, dichloromethane, activated charcoal, carbon tetrachloride and bromine.

Some reagents were prepared in the laboratory for analysis of the extracts. Chromatographic studies were carried out using both commercial pre-coated silica plates

and plates made by spreading a silica gel slurry onto glass plates, dried and oven activated.

2.0.1 Material collection and preparation

A portion of the tree trunk that had previously been felled and cut into pieces, was dried and chopped into small sizes. The drying period was about four weeks. The pieces were then cut into smaller pieces of about 10 cm each across the length and then subdivided across the width. The resulting pieces were then stored in paper bags.

These pieces of wood were later taken to the wood workshop for grinding. A file pre-cleaned with acetone was used to file the pieces into a fine dust of wood. A radial arm saw was used to make sawdust from the longer pieces of wood.

This sawdust was then stored in polyethylene bags each containing about 400 g in weight of the dust. The bags were then stored in a refrigerator at about 4°C awaiting extraction. This was to avoid any evaporation of the volatile components in the sawdust and to prevent any possible reactions with the atmosphere e.g. oxidation etc.

2.0.2 Extraction

Apparatus: 1L Round bottomed flask, a condenser, receiver adaptor, thermometer, heating mantel, ice bath, thermometer jacket and sample vials.

Procedure: sawdust was and put into the 1 litre round bottomed flask (pyrex). The apparatus were then set up for distillation. The dry sawdust was gradually heated and the temperature monitored. The distillate (volatile oil) was collected over ice at 0°C in sample vials, corked, weighed and stored in a refrigerator. The total weight of sawdust extracted was 600.60 g and the total weight of the crude extract obtained was 182.03 g. The crude extract was the extract got from the process before any further purification or separation was done.

2.1.0 BIOASSAY OF THE CRUDE EXTRACT ON TERMITES

After the extraction of the crude sample from the tree, the samples were stored in a refrigerator awaiting tests on termites and other analysis. Preliminary survey done showed that the most prevalent termite species around the Chiromo area were *odontotermes stercorivorus* and *odontotermes mautanus* [46]. The species *O. stercorivorus* was used for bioassays since its mounds were easy to find and numerous and easy to dig out.

2.1.1 Collection of termites

The first lot of termites was collected on 31st January 1994 at the National Museum of Kenya, a compound adjacent to the Nairobi river. The mound of this species do not protrude above the ground and therefore was located by identifying the mouths of the tunnels leading to the nest.

The nest was dug by first making a small trench around the nest and digging progressively deeper from the edges of the nest towards the nest centre. There was need to dig deep into the nest (to the queen's chamber) since the termites, especially the workers retreated, very deep into the nest as the digging progressed. The first encounter with a substantial number of termites showed a very high proportion of the soldiers as compared to the workers. This was due to the fact that the soldiers are always left behind during an attack to guard the nest as the members of the other castes hide in their chambers.

After one and half meter, the main chamber was reached, which contained eggs and the fungus. This is where most of the workers were found. This chamber was collected together with nymphs, workers and several soldiers. The termites collected this way, together with the fungus and nest soil, could survive for at least a week. The basin was not covered to prevent mould from growing on the damp soil.

The caste of workers was the one of most interest for bioassay since these are the ones that forage for food and

bring to the other castes for consumption. Several soldiers, however, were used in each test.

2.1.1.0 Bioassay - principle

This was a no-choice bioassay. The main objective was to observe the effect of the crude extract after being consumed orally or/and through contact by the termites. The mortality rate was to be noted for termites exposed to the extract and compared with the mortality rate of those un-exposed to the extract.

2.1.1.1 Preparation of extract solutions

The crude extract was diluted to several different concentrations using distilled de-ionized water in 50 mls volumetric flasks. The percentage concentrations made were, 100 (undiluted extract), 20, 10, 2, 0.4, 0.2 and 0.0 (distilled, de-ionised water).

The extract was measured and transferred into the volumetric flasks using 10 mls pipettes and then diluted to the mark using the de-ionized, distilled water. The tests were carried out in triplicates and seven different concentrations were used including the control which was the distilled, de-ionized water.

2.1.1.2 Procedure of bioassay

A wooden box measuring 1 m x ½ m x ½ m was obtained and painted black and sealed to prevent as much light as

possible from penetrating into it. This was then placed conveniently in the laboratory such that no contact with water would be made whatsoever.

Sterilized plastic petri dishes (disposable) each measuring 90 mm, diameter and 15 mm high were used. In each petri dish a pad-paper (filter paper), 70 mm diameter (Whatmann No. 1) was placed. In total twenty one petri-dishes were used in this first test.

For each concentration, 0.1 ml treatment solution (extract) was pipetted and applied evenly onto the pad-papers in triplicates and then 0.5 ml of water were applied on each treated pad to moisten the pad papers. This was done to maintain humid conditions ideal for termite survival.

20 termites (18 healthy workers and 2 soldiers) were placed into each petri-dish containing the impregnated (treated) pad-papers. The termites had previously been sorted out and the health workers kept aside with several soldiers. The petri-dishes were then arranged randomly in the box and the box closed.

The petri-dish covers were loosely fitting so some air was allowed to circulate inside. All these procedures were followed to create a termite-nest atmosphere inside the petri-dishes so that termite mortality due to other factors other than the extract toxicity was reduced to a minimum.

The number of dead termites was determined at different intervals for one day and the results recorded. At each stage the dead termites were removed from the petri-dishes.

2.2.0. BIOASSAY 2

In this case the procedure for treatment was the same as in the previous test and the species of termite used was the same that is, *odontotermes stercorivorus*. The volume of treatment solution applied on each filter paper was reduced to 0.3 ml to test for the most effective optimum concentration of the extract. Due to the time involved in locating and digging out a termite nest and the amount of work required, a different method of obtaining the termites was employed. This involved the use of a trap.

2.2.1. Trapping

To make the termite trap, a pvc pipe 14 cm in diameter and meter in length was obtained. A plastic cooking fat container (½ kg) was cleaned and fixed at one end of the pvc pipe to seal it. This seal was achieved by pressing the narrower bottom side of the container into the end of the pvc pipe after smearing a thin coat of pvc pipe cement onto the inner walls of the pipe and the outer part of the plastic container at the ends to be sealed. The seal was left to dry for more than eight hours. The pvc pipe cement used was 'TANGIT' which is a petroleum mixture.

Soil was put into the pvc pipe and enough water added to make the soil damp. Dry maize cobs and small twigs were stuck into the soil and more soil and water added. The process was repeated successively until the pipe was filled up. A mound of the termite species, *odontotermes*

stercorivorus was identified adjacent to the Nairobi river and the pipe and its contents inverted up side down at the mouth of the termites nest and left for three days, as shown in plate A.

After this period the trap was removed from the site and the contents emptied into a basin. The approximate number of termites trapped this way was six hundred. Most the termites trapped were workers since their caste is responsible for looking for food, They were healthy and well fed.

The previously prepared samples were used with fresh filter papers and new petri-dishes. Twenty termites (two soldiers and 18 workers) were put into the impregnated (treated) pad papers as in the former case and the same procedure followed for 24 hours.

PLATE A: A termite trap (a PVC pipe sealed at one end) inverted at the mouth of a termite nest.

Dead termites were removed from the petri-dishes at each stage during observation.

There was no cause to think that the mode of trapping the termites influenced the mortality rate as far as only healthy workers and soldiers were used in the tests.

2.2.2 Bioassay 3

The test was carried out in the same way as in the previous cases but in this case the species of the termites was *odontotermes mautanus*. The nest was dug out as in the first case in the chiromo campus compound. Since the mortality rate was not very high as in the first test, the period of examination was increased by twelve hours.

2.3.0 Croton megalocarpus tree extract

2.3.1 Dry distillation

The procedure followed was similar to the one described for the first tree and the extract was stored under the same conditions.

2.3.2 Soxhlet extraction

A medium sized soxhlet extractor was used and a piece of cloth was used to hold the sawdust during extraction. The cloth had been cleaned and soxhlet extracted with dichloromethane for one hour. A solvent system of n-hexane and dichloromethane in the ratio of 1:2 was used. 300 mls of the solvent mixture were used to extract 100g of the

sawdust. Siphoning was done after every 1½ hours for a period of 18 hours. The extract obtained was concentrated using a rotary evaporator and stored in a refrigerator. A 1L round bottomed flask (pyrex) was used. The apparatus was set up and the solvent put into the flask. After the soxhlet extractor had been set up the sample was put inside and the condenser set up. The heating mantel was regulated at 70°C.

2.4.0 T.L.C. ANALYSIS

The crude extract was first dissolved in dichloromethane before being spotted onto precoated TLC plates that had been cut into convenient sizes for the analysis. Trials tests were done to obtain the best solvent system for development. A mixture of hexane and dichloromethane in the ratio of 1:1 was observed as giving the best results and adopted as the developing solvent system throughout the analysis.

After development the plates were air dried and then sprayed with anisaldehyde-sulfuric acid reagent as the locating agent. The plates were then left to dry and then the developed plates were placed in an oven at 100°C and heated for one and half minutes. the spots of different compounds appeared as multi-coloured spots on the plates, the colours ranged from yellow, green and red. The other identification technique employed was putting the plates in an iodine tank. The spots appeared as yellow but it was not

as effective as the anisaldehyde reagent identification method. The R_f values were recorded.

2.4.1 Preparation of Anisaldehyde reagent.

Concentrated sulfuric acid (8 ml) were added to 0.5 ml of anisaldehyde (p-methoxybenzaldehyde). This mixture was added to a mixture of 85 ml methanol and 10 ml glacial acetic acid under cooling with ice. The reagent prepared was a spraying solution and could last for more than two weeks.

2.5.0 FRACTIONAL DISTILLATION

The crude extract was put in a 250 ml round bottomed flask and a fractionating column connected to it. The extract was then heated using a heating mantel gradually, collecting the distillate at various temperatures. This method failed to separate any pure components but separated out some water from the extract. Partitioning using dichloromethane was done to get the aqueous layer (AqP) out before the columns were run.

2.6.0 COLUMNS

In this case, several columns were run to try and separate the various components in the extracts. A trial column was first run using a burette of diameter 1 cm (pyrex). The most effective solvent system was dichloromethane/Hexane (1:1) and then washing with methanol. Before the exercise, all the solvents were distilled and the silica gel activated overnight in an oven at 150°C.

2.6.1.0 Column packing: COLUMN A

The sample was concentrated to about 2 mls using a rotary evaporator. Activated silica gel was weighed after the sample was weighed. The ratio of the silica gel to the sample used was 20:1.

A glass column, 2.5 cm in diameter and 1m in length was cleaned and dried. Glass wool that had previously been soxhlet extracted using dichloromethane was made into a ball and dropped to the bottom of the column (end with a tap). A little acid washed sand was added and then the column filled a third way with hexane.

Silica gel (90g) were made into a slurry in a beaker using hexane and poured into the column with occasional tapping of the column to ensure even packing of the silica gel. Then the sample, 2.5g was introduced onto the gel using a 10 cm^3 pipette. The sample was sucked into the pipette and then closing the upper end of the pipette, it was lowered until the end just touched the surface of the packed silica gel. The extract was then released slowly and formed a uniform layer. Glass wool was then put to cover the layer. The column was then run using 200 mls of Hexane followed by 200 mls of 20% dichloromethane in Hexane, 100 ml of 50% dichloromethane in Hexane, 100 ml dichloromethane and then washed with 100 ml of methanol.

The rate of collection was 2 CM^3 MIN^{-1} and 700 ml run for five hours. The analysis was carried out and indicated that further separation was necessary. Micro columns were run.

2.6.1.1. Micro columns: COLUMN B

The column (pyrex) of diameter 1 cm was hexane packed and the amount of silica gel used was 20 g. The fractions in solvent were concentrated to 1.3 ml using the rotary evaporator. The rate of collection of fractions was 1.0 ml per minute.

The column was then run using the solvent proportions as shown in table 2.1 and the fractions collected. Another column run, utilized a column of diameter 2 cm and was Hexane packed. 30 g of silica gel were used and the following proportions of solvents used to run it as shown in Table 2.2

Table 2.1: Volumes of solvent systems for eluting column A.

Total volume of solvent added (ml)	Volume of Hexane (ml)	Volume of Dichloromethane (ml)	Volume of methanol (ml)
40	40	0	0
40	32	8	0
20	10	10	0
30	12	18	0
20	6	14	0
20	0	20	0
20	0	0	20

Table 2.2: Volumes of solvent systems used to elute column B.

Total volume of solvent added (ml)	Volume of Hexane(ml)	Volume of Dichloromethane (ml)	Volume of methanol (ml)
100	100	0	0
200	160	40	0
200	120	80	0
100	50	50	0
100	30	70	0
100	0	100	0
100	0	0	100

2.7.0 CHEMICAL TESTS

2.7.1.0 Brady's test (2,4-dinitrophenylhydrazine test)

2.7.1.1 Preparation of 2,4-dinitrophenylhydrazine reagent.

2,4-Dinitrophenylhydrazine (3 g) was dissolved in 15 ml of concentred sulfuric acid. This solution was then added with stirring to a solution of water (20 ml) and 70 ml of 95% ethanol. The resulting solution was shaken thoroughly, left to stand for a short time and then filtered.

2.7.1.2. Reactions

For each of the fractions, 2 drops were added to 2 ml of ethanol in a test tube and then 3 ml of the Brady's reagent added. it was then shaken vigorously and observed for any precipitate. The precipitates are due to dinitrophenylhydrazones which are insoluble solids formed

from a reaction of the reagent and most aldehydes and ketones.

2.7.2.0 Bromine water

A solution of the samples was transferred into the test tube and to each solution bromine water was added drop by drop and observations made as to whether decolorisation occurred.

2.7.3.0 Fehlings reagent test

2.7.3.1 Preparation of fehlings reagent

Solution A: approximately 17.5 g of copper sulphate crystals, $CUSO_4.5H_2O$, were dissolved in distilled water and diluted to 250 cm^3. The solution formed a part of fehlings reagent.

Solution B: 87.50 g of Rochelle salt (potassium sodium tartrate) and 35 g of solid sodium hydroxide were dissolved in distilled water and diluted to 250 cm^3.

To use these solutions (fehlings reagents), a mixture of the two was made by mixing equal volumes of solution A and B. This was done only during the experiment otherwise the solution were stored separately [47].

2.7.3.2 Reactions

A few drops of the test sample solution were put into a test tube and some drops of fehlings reagent added, the mixture was gradually heated with shaking until boiling

Table 2.2: Volumes of solvent systems used to elute column B.

Total volume of solvent added (ml)	Volume of Hexane(ml)	Volume of Dichloromethane (ml)	Volume of methanol (ml)
100	100	0	0
200	160	40	0
200	120	80	0
100	50	50	0
100	30	70	0
100	0	100	0
100	0	0	100

2.7.0 CHEMICAL TESTS

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For each of the fractions, 2 drops were added to 2 ml of ethanol in a test tube and then 3 ml of the Brady s reagent added. it was then shaken vigorously and observed for any precipitate. The precipitates are due to dinitrophenylhydrazones which are insoluble solids formed

point. Colour changes or any deposits were observed and recorded.

2.8.0.0 GAS CHROMATOGRAPHY AND SPECTROSCOPY

2.8.1.0 INSTRUMENTATION

2.8.1.1 Gas Chromatography

This technique was employed for the separation of components of the crude extracts and for the determination of purity of the isolated components from the extract.

A PERKIN ELMER 8500 gas chromatography at the Chemistry Department, University of Nairobi, was used.

In this technique, an inert gas such as nitrogen or argon is used as the mobile phase. Nitrogen was employed as the mobile phase in this work. The stationary phase used in this type of work usually consists of an inert solid impregnated with a non-volatile organic liquid. The column is installed in a heated cabinet and a stream of gas is passed through it at a constant rate. A solution of the extract was injected into the hot column at the end nearest the gas inlet. The extract vaporises and is carried through the column by the gas at different rates [47]. The rates are influenced by different partition-coefficients between the liquid stationary phase and the mobile phase of the different components in the extract.

Reasonably good flow rate is achieved with a two stage pressure regulator at the cylinder head in conjunction with a good needle valve. If the column used presents a

substantial impendence to flow, this will also help to maintain constancy of flow rate. The pressure at the exit of the apparatus is usually allowed to be atmospheric.

Columns: columns for analytical GC studies are either capillary columns (small capillary tubing packed only with a stationary phase) or large-diameter columns packed with a stationary phase dispersed oven an inert support (e.g. pulverised firebrick) [48].

In this analysis work, fused silica capillary columns were used. Specifically, an SE-30 column and a CARBOWAX 20 M columns were employed during the GC analysis and GC-MASS SPEC analysis, respectively. The SE-30 column (capillary) is composed of 100% dimethyl polysiloxane (gum) and is non-polar. It is best for phenols, hydrocarbons, amines, sulfur compounds, pesticides and PCBs among others. The carbowax capillary column comprises of polyethylene glycol and is polar in nature. It is used mainly in the analysis of free acids, alcohols, ethers, essential oils, glycols and solvents. The upper limit temperatures for SE-30 and carbowax columns are 325°C and 220°C, respectively.

Detectors: detectors mostly used are flame ionization (FID), thermal conductivity (TCD) and electron capture (ECD).

In this analysis, work was done using a flame ionization detector (FID). This consists of a hydrogen flame burning at a jet in a supply of filtrated air. The jet acts as one electrode and a small wire ring or gauze above the

flame as the other electrode. Changes in the ionization current due to components in the gas stream are detected and fed to an amplifier [49]. The flame was ignited with a glowing tip of a heated built-in spark coil.

2.8.1.2 Ultraviolet/Visible (UV/VIS) Spectrometer

The instrument used was PYE UNICAM SP8-150 UV//VIS-Spectrophotometer. This was available in the Chemistry Department, University of Nairobi.

This instrument consists of five sections (a) radiation-source, (b) monochromator, (c) sample area, (d) detector with amplifier and (e) read out.
The radiation source is a hydrogen discharge tube, but it can be replaced by a tungsten filament lamp. The monochromatic light that emerge from the exit slit is pulsed by a chopper and split into sample and reference beams.

The UV spectra of the compounds are determined either in solution or in the vapour form. The cells used vary in length but in this work, 1 cm path length cells were used. The absorption in the UV/Vis region of the spectrum is dependent on the electronic structure of the molecule especially for conjugated systems. The spectrum is a plot of the wavelength or frequency of absorption versus the absorption intensity (transmittance or absorbance). The ultra-violet region is commonly divided for practical reasons into the near ultraviolet regions (200-350 nm) and far or vacuum ultraviolet region. Only molecules containing

multiple bonds (with a few exceptions) give rise to sufficiently stable excited states to absorb in the near UV region. Thus saturated hydrocarbons, alcohols and ethers are transparent in this region [48].

The basis of this technique is the Beer-Lambert law which states that the proportion of light absorbed by a transparent medium is proportional to the number of absorbing molecules in the path length [50].

$$Log_{10}[I_o/I] = acl$$

where Io = Intensity of the incident light,

I = intensity of the transmitted light,

a = absorptivity,

c = concentration of solute,

l = cell (path) length (cm), [50].

2.8.1.3 Infrared spectrometer

This technique was employed to identify the functional groups in the isolated components. An IR spectrometer was available in the Chemistry Department; a PYE UNICAM SP 300. This instrument was a double beam, null balanced. Like the UV spectrophotometer, it contains the five principle sections Radiation source, sampling area, photometer, monochromator and detector.

The radiation is produced by electrically heating a source usually a nernst filament or a globar to 100-1800°C. The filament is fabricated from a binder and oxides of zicornium, Thorium and Cerium.

Reference and sample beams enter the sampling area and pass through the reference cell and sampling cell respectively, thus the sample beam is absorbed by the sample, the attenuator is driven into the reference beam until its intensity matches that of the sample beam. The amplified off-null signal of the detector is used to position the optical attenuator so that the radiation from the reference and sample beam are kept at equal intensity. the movement of the attenuator is recorded by the recording chart pen [50].

Infra-red radiation refers mainly to that wavelength between 1μ and 100μ. The limited portion of infra-red radiation between 2.5μ and 15μ (4000 cm^{-1}-660 cm^{-1}) is of greatest practical use.

Band intensities are expressed either as transmittance, T (ratio of radiant power transmitted by sample to radiant power incident on sample) or as absorbance, A (the logarithm to base ten of the reciprocal of the transmittance, $A = \log_{10} 1/T$) [49].

2.8.1.4 Gas chromatography-Mass spectrometer (GC/MS)

This technique was employed with a view to characterise the components isolated from the crude extract. A HEWLETT-PACKARD, VG 12-250 GC/MASS SPEC model of the instrument was available at the international centre for insect physiology and ecology (ICIPE).

The functions of this instrument can be divided into two phases, (i) separation and (ii) identification. Biologically active natural products and environmentally objectionable organic pollutants comprises two categories of materials of rapidly growing interest. The subject material of the investigation is often obtained at very low concentrations in a highly complex mixture of other organic compounds. Even after the application of multi-stage extractions and other clean up procedures, including preparative scale chromatographic methods, a biologically active substance, observed only in bioassay, may not appear visibly nearer to the desired condition of isolation [51].

The coupling of the separation power of the gas chromatography to the analytical sensitivity and specificity of the mass spectrometer is made possible by the fact that the physical characteristics of the systems are so well matched. A data acquisition and speed processing computer is also coupled to the system. The combined apparatus is capable of showing the presence of a large number of compounds in a short time and printing out their spectrum.

In the search of a biochemical not previously identified, bioassay requires the collection of fraction that can be correlated with the GC/MASS data files. Secondly, in the search for a material whose mass spectrum exists in the computer's library collection, identification of a newly acquired spectrum by comparison will probably be accomplished quickly [51]. However, if the chemical has not been found before, its spectrum will not be in the library. If the structure is not too complicated or too different from known ones, it may be derived from the mass spectrum alone.

Packed gas chromatographic columns were initially connected to the ion sources of the mass spectrometers by means of stream-splitting valves that admitted as much of the gas stream as could be tolerated and vented the remainder to the atmosphere. This led to a loss of a large proportion of the sample. Means were sought for the enrichment of the input stream through the selective elimination of the carrier gas. This is accomplished by (a) enrichment by effusion through fine pores or a narrow slit, (b) preferential diffusion of carrier gas or of sample through semipermeable membrane and, (c) fractionation of gases in an expanding jet stream.

A separator is not needed for capillary column GC. The pumping speed of a good system is adequate to handle the entire effluent stream and the capillary column is connected directly to the ion source.

For this work, a carbowax 20 M capillary column was employed, thus it was connected directly to the ion source. The most important process in the mass spectrometry is the displacement of one electron from the molecule (M) to form the molecular ion (M^+, a radical cation).

$M + e^- = M^+ + 2e^-$ [50].

The ionization does not change significantly the mass of the particle and allows particle acceleration by means of an applied potential. The accelerated ions pass into a magnetic field and acquire a circular path, the radius of curvature of which is proportional to the mass to-charge ration (m/e) of the particle. Since the magnitude of the charge (e) is normally 1, the magnitude of m/e is a measure of mass.

Other fragmented ions may be of the form.

$M^+ \quad F_1^+ + F_2\cdot$

The molecular ion of a pure sample can often be identified as the largest peak in the cluster of peaks of highest m/e values.

Mass spectrometry is basically a means of analyzing mixtures of positive ions. In the magnetic analyzer the ions, after their formation are passed through a slit and then subjected to a potential of several thousand volts, yielding an essentially mono energetic ion beam [49].

2.8.2.0 GC AND SPECTROSCOPIC ANALYSES

2.8.2.1 Gas Chromatography

(1) Crude extract: The extracted and partitioned material was shaken three times with 5 per cent (W/V) $NaHCO_3$ to remove organic acids. The extract material had previously been partitioned using hexane and dichloromethane respectively.

The extract was dried over anhydrous sodium sulphate (Na_2SO_4) and then concentrated using a rotary evaporator. The extract was then monitored by gas chromatography utilising the instrument previously mentioned.

The detector used was flame ionization detector (FID) while the column employed was a fused silica capillary column of the type: SE-30 (100% dimethylpolysiloxane (Gum), length (30 M), an internal diameter of 0.2 µM and film thickness of 0.3 µM. Nitrogen gas was used as the carrier gas at a flow rate of 10 ml/min. The temperature parameters employed were: Initial oven temp: 50°C

Ramp rate: 5°C/min

Final oven temp: 225°C (hold 5 min)

Injection port: 175°C

Detector: 250°C

The ramp rate was later increased to 10°C/min while injection port temp. was increased to 275°C and detector temperature to 350°C. The isolated components from the column chromatography were also analysed using this technique.

2.8.2.2 GC/MS spectrometry

Fraction monitoring and component identification were also performed by employing gas chromatography-mass spectrometry (GC/MASS LAB 12-250 quadruple Gas chromatography-mass spectrometer with a HEWLETT PACKARD HP-5790 SERIES Gas Chromatography and Data system.

The injection was splitless and the carrier gas was helium (He) with a flow rate of 10 ml/min. The GC column used was a capillary column of the type: CARBOWAX 20 M (consisting of polyethylene glycol) with a length of 50M, an internal diameter of 0.2 µM and a film thickness of 0.2µM. The oven temperature program utilised was,

initial temp: 60°C (hold 8 min),

ramp rate: 5°C/min,

final temp.: 220°C (Hold 20 min),

run time: 60 min (1 hr).

The ion-source parameters were,

mode: Electron ionization (EI),

source temperature: 180°C,

electron energy: 70 ev (electron volts).

The GC/ms data files from the computer provided mass spectra libraries for compounds to be compared with the newly run spectra. In this technique, very pure solvents were used. The isolated components were also analysed the same way.

2.8.2.3 UV/VIS Spectroscopy

The test samples dissolved in triply distilled dichloromethane and in ethanol were analysed and λ_{max} recorded for each sample. Scanning was done within wavelength range of 750 to 190 nm.

The samples were also analysed using a BECKMAN DU-50 UV spectrophotometer at the International Centre for Insect Physiology and Ecology (ICIPE).

2.8.2.4 Infra-red spectroscopy

Some samples were run as neat since they were oils. A thin film was spread evenly on the I-R sodium chloride windows and then the windows pressed together while turning the windows. They were then mounted in the spectrometer in a holder and the spectrum run.

Other samples were minute traces in solutions so a solution cell was used. The solvent was first evaporated and then spectroscopic grade Carbon tetrachloride, CCl_4 was used to dissolve the sample. The solution was introduced into the inclined cell through a hypodermic syringe. The cell was wiped off any spilling solution and then the cell mounted in the spectrometer and spectrum run.

Due to the small quantities of samples, nujol mull technique proved to be very unsuccessful. The peaks obtained were mostly due to the nujol mull and very few peaks could be distinctly be identified as arising from the sample.

The pellet technique was also employed. The sample was introduced into a mortar and ground and a little spectroscopic grade KBr introduced and ground too. The mixture was later put into a die and then connected to a pump to eliminate occluded air from the pellet. The pellet was then pressed to pressure of 10 tons/sq in. and the clear transparent disc was mounted onto a holder and the spectrum obtained.

CHAPTER 3

RESULTS AND DISCUSSION

3.0.0. EXTRACTION

3.0.1. DISTILLATION

Crude extract (182.03 g) was obtained from the dry distillation of the sawdust of *Juniperus procera* (African pencil cedar) tree. The weight of sawdust prior to the dry distillation was 600 g.

The crude sample which was about 30.31% of the starting material was collected in vials at different temperatures as shown in Table 3.1 below.

Table 3.1: Extraction from *Juniperus procera* tree

Vial Nos.	Collection temp. range (°C)	Collection time (Hr)	Weight of extract (g)	Extract description
1	90-110	6	119.30	An aqueous Yellow solution
2	111-160	5	62.73	Mixture: red- brown oily layer and traces of yellow solution

After partitioning and fractional distilling, it was observed that a large proportion of the extract was water (about 77.5% v/v). The other part consisted of yellow and reddish-brown oily layers which were soluble in organic solvents e.g dichloromethane. The acqueous layer contained

predominantly water with traces of the extract. So the two layers were mixed together.

3.1. THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS.

The TLC analyses of the various isolates and crude extract from *Juniperus procera* and *Croton megalocarpus* trees showed that there were about ten distinct components in the *Juniperus procera* extract with retardation factor (R_f) values of 0.93, 0.86, 0.84, 0.81, 0.71, 0.66, 0.64, 0.57, 0.31 and 0.20. The developing solvent system used in all the TLC analyses was n-hexane mixed with dichloromethane (50:50 v/v).

The TLC analysis of the soxhlet extracted sample of the *J.procera* tree revealed that it contained seven compounds with R_f values of 0.91, 0.83, 0.64, 0.54, 0.25, 0.16, and 0.11.

This shows that the soxhlet extraction yielded fewer compounds than dry distillation of wood. Since the sawdust was prepared in a similar manner for both extraction techniques, the results suggest that dry distillation of wood was a more effective technique for the extraction of the wood components.

This extraction method was preferred since it was cheaper and tended to destroy the entire wood organic matrix which ultimately released the trapped volatile wood components. This clearly shows that the components obtained from the sawdust depended on the extraction method used.

On the other hand, the *Croton megalocarpus* crude extract yielded fewer components (seven) than that of *J.procera*. These had R_f values of 0.72, 0.58, 0.50, 0.47, 0.33, 0.24 and 0.15. This indicates that for a particular extraction procedure, the components obtained, depend on the species of the plant under investigation.

3.2. COLUMN CHROMATOGRAPHY

The plant extracts were isolated using column chromatography. Several portions of the extracts were run through a hexane packed column and eluted with hexane, dichloromethane and methanol respectively. Three visible bands (yellow, orange, red) were observed. several fractions, F_1, F_2, F_3, F_4, and F_5, were sequentially collected and spotted on TLC plates. The R_f values obtained were 0.69, 0.67, 0.95, 0.71 and 0.91, respectively.

3.3.0 BIOASSAY

3.3.1 Termites:

Preliminary surveys done indicated that the most prevalent termite species around the area (Chiromo) were *Odontotermes stercorivorus* and *Odontotermes mautanus* [46]. They destroy trees, posts and pasture. The species *Odontotermes stercorivorus* was collected for bioassay. The nest was located at an area within the National Museums of Kenya complex, adjacent to the Nairobi river. The termites

species are of the family - *termitidae*, sub-family - *macrotermitinae*, genus - *odontotermes*.

3.3.2 Preparation of extract solutions:

The crude extract was diluted with distilled water to different concentrations using volumetric flask (50 mls). The concentrations made are as shown in table 3.2.

Table 3.2: Various concentrations of *Juniperus procera* tree crude extract.

Volume of crude extract in 50mls of solution (mls)	Concentration (v/v).
Undiluted crude extract	100%
10	20%
5	10%
1	2%
0.2	0.4%
0.1	0.2%
0.0 (Control)	0.0% (Pure distilled water).

The toxic effects of the various concentrations of the *Juniperus procera* tree crude extract on the termites are presented in Table 3.3. After the first hour all the termites (workers) on the highest loading of the crude extract (100%) were dead. More than 50% of the workers on the 20% loading of crude extract were dead after the same

duration. The mortality rate was observed to decrease proportionally with decrease in concentration of the crude extract (table 3.3).

After a 24 hour duration the workers on the 100%, 20% and 10% loadings were all dead, while about 60% mortality rate was observed for termites on the 2% loading. The lowest loading of 0.2% crude extract registered a mortality rate of about 30% after the same duration, 24hr, while the control had negligible mortality. All the tests were done in triplicates and the mean mortality taken for each concentration at each given time.

Table 3.3: Effect of various concentrations of *juniperus procera* extract on the termite species *O.stercorivorus.*

% V/V	Number of dead termites at a given time (Hours).										
	1	3	5	7	9	11	13	15	17	21	24
100	20	20	20	20	20	20	20	20	20	20	20
20	14	17	19	19	20	20	20	20	20	20	20
10	8	11	12	14	16	17	18	18	18	20	20
2	3	5	5	6	6	7	8	9	10	11	12
0.4	1	2	3	5	5	6	6	7	8	9	9
0.2	0	0	1	1	2	3	3	4	4	5	6
0.0	0	0	0	0	0	0	0	0	0	1	2

The experiments clearly indicated that the crude extract sufficiently affects the termites to warrant further investigation. Although the crude extract in its undiluted

form was very effective on the termites, considerations of cost-effectiveness and optimum applications showed that a 10% crude extract loading was most suitable. From the observations, it was evident that the lethal concentration needed to kill 50% of the termites (i.e., LC_{50}) after a duration of 24 hours was a concentration of 2% of the crude extract. A close observation of the termites after the introduction of the test sample revealed that they were dying from contact and from the asphyxiating vapours of the extract. Some termites collapsed immediately after contact with the solutions of the higher loadings (concentrations) of the extract, while others withdrew to the sides of the Petri-dishes. Some later became weak and staggered around the Petri-dish in a daze. This was due to the asphyxiating effect of the extract. A graph of the number of the dead termites versus time in hours (Fig. 3.1) showed a linear relationship. The relatively large gradient of the graphs upto around 10 hours after application of test sample shows how the effect of the extract reduced with prolonged time but there was consistency in the mortality trends of the termites in all the different concentrations of the extract employed in the experiment. The control showed negligible mortality as expected rendering the observed mortality in the other loadings, acceptable within experimental errors.

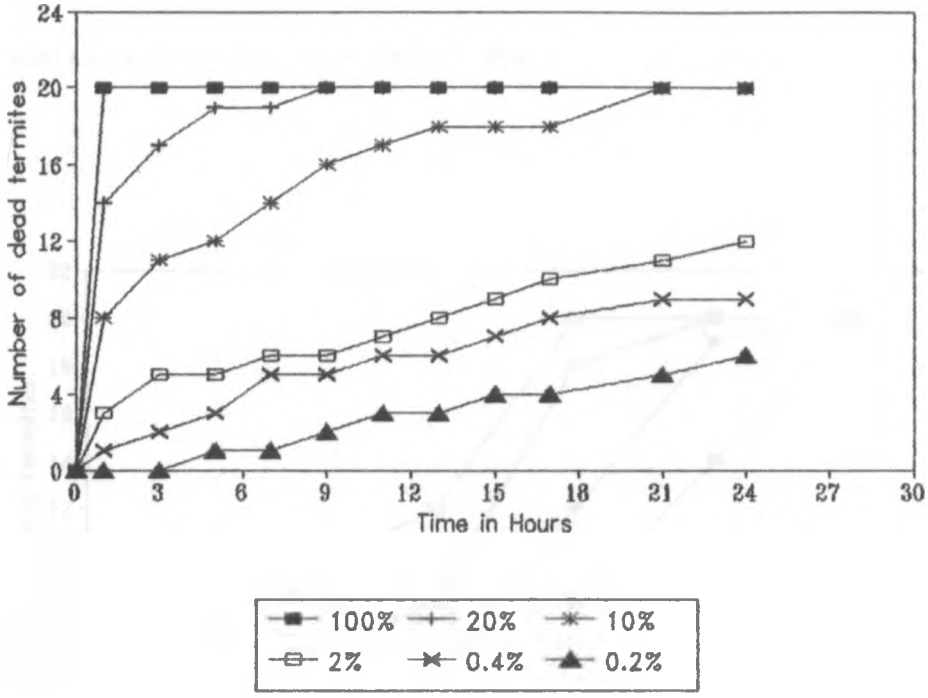


Fig.3.1: Dependence of termite (*O. stercorivorus*) mortality with exposure time for the *juniperus procera* extract

Within the duration of observation, various time lapses were taken and a plot of the Logarithm of percentage concentration against the average number of the dead termites drawn (Fig. 3.2).

From the graph, it was evident that the mortality rate of termites increased with increased concentration of the extract.

A second bioassay was carried out on the termites; The volume of loading for each concentration was reduced

from 0.5 ml to 0.3 ml. This was to compare the mortality and determine the most suitable concentration of the extract for application to the termites.

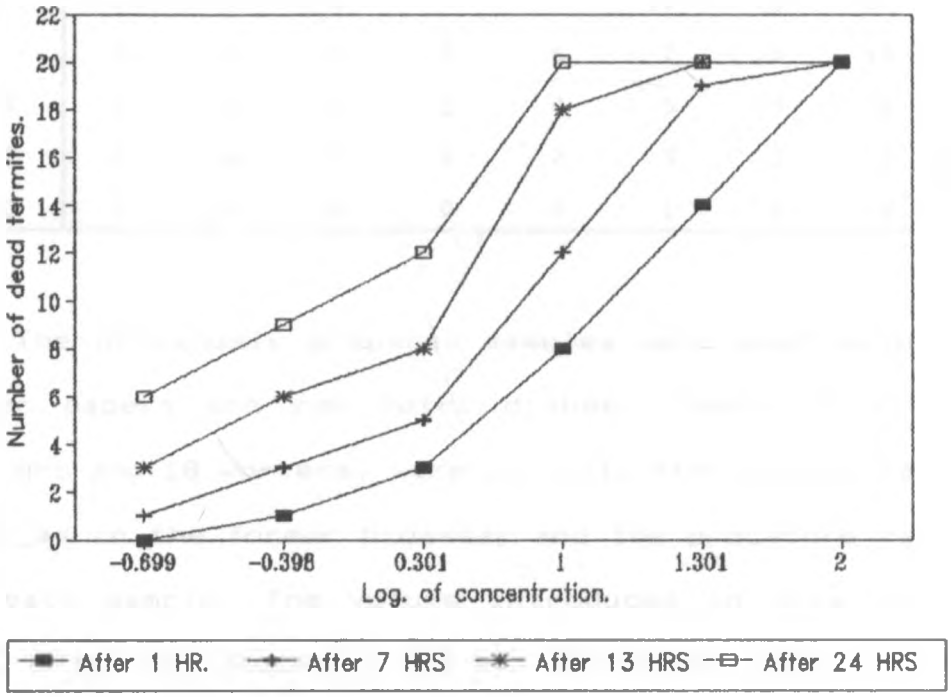


Fig.3.2: Dependence of termite (*O. stercorivorus*) mortality on the concentration of the extract (*J. procera* tree) at various times.

Due to the time involved in locating and digging out a termite nest and the amount of work required, a different method of obtaining the termites was employed. This involved a termites trap made from a PVC pipe sealed at one end (see PLATE A) under the section of methodology.

Table 3.4: Toxicity of *Juniperus procera* crude extract on the termite species *O.stercorivorus.*

% V/V	Number of dead termites at a given time (Hours).							
	1	3	5	7	9	15	20	24
100	8	14	14	16	19	20	20	20
20	6	8	9	11	11	15	18	20
10	5	5	6	7	7	10	15	17
2	3	3	4	5	6	7	10	14
0.4	1	1	3	3	4	5	5	6
0.2	0	0	1	2	2	3	3	4
0.0	0	0	0	0	1	1	1	2

The previously prepared samples were used with fresh filter papers and new Petri dishes. Twenty termites (2 soldiers and 18 workers) were put onto the impregnated pad-paper as in the former bioassay and the procedure repeated for each sample. The volume introduced in this case per Petri dish was reduced to 0.3 ml. There was reduction in the rate of mortality of the termites which could be reasonably attributed to the smaller volume of extract applied (Table 3.4).

This reduction though significant, indicates that for a certain percentage concentration, a lesser volume (say 0.3 ml) may be prefered rather than applying a large volume leading to waste of the extract. This is evident from a comparison of mortalities in Table 3.3 and 3.4. A graph of average number of dead termites against time, Fig. 3.3 gave the same trends as in bioassay 1 but the general gradients of the plots were reduced.

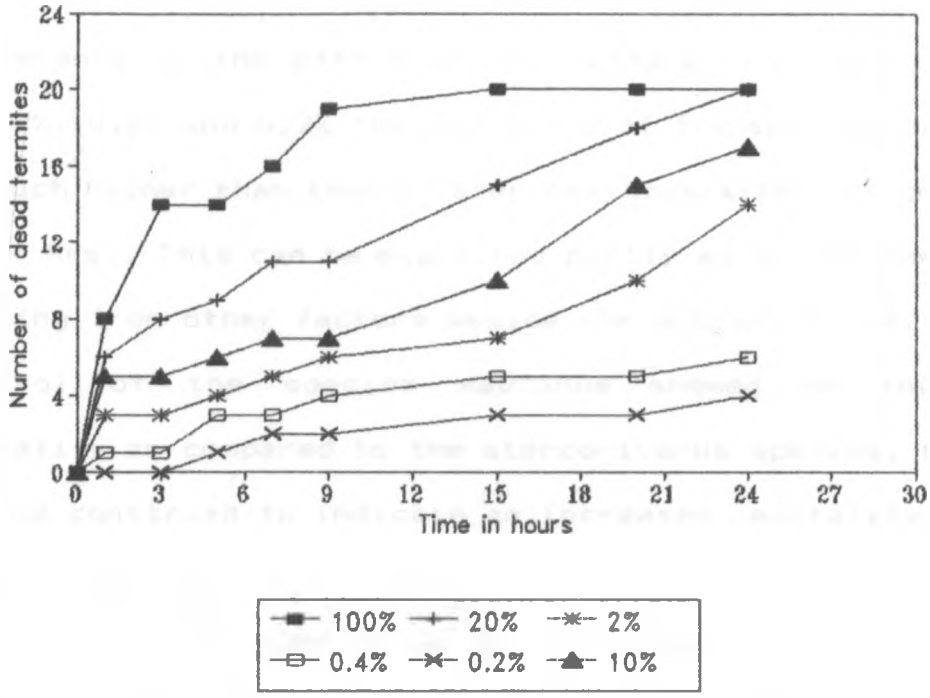


Fig.3.3: Change of termite (*O. stercorivorus*) mortality with exposure time. (*Juniperus procera* extract, volume per petri dish = 0.3 ml.).

From this second bioassay it seems that a 10% loading of the extract is still the most suitable one for applying on the *Odontotermes stercorivous* termites species.

In a third bioassay, a different species of termites was used and the same crude extract tested on the species to compare the toxicity of the extract on the two species of termites. The species, *Odontotermes mautanus* was collected and exposed to the extract in the same way as the former species (Table 3.5).

A comparison of the mortality rate in Table 3.4 with those in Table 3.5 reveals that there is no significant difference in the effect on the different species. Though for 2%, 0.4% and 0.2% the mortality of the species *mautanus* is much higher than that of stercorivorus after the duration of 24 hrs., This can be explained partly as due to mortality arising from other factors beside the extract toxicity. The control of the species *mautanus* showed an increased mortality as compared to the stercorivorus species, thus it can be construed to indicate an increased mortality in the other loadings.

These two species were collected within the same location and since they are from the same family and genus, it's not surprising that they responded almost the same way to that same extract.

Table 3.5: Toxicity of *juniperus procera* crude extract on the termite species *O.mautanus*.

% V/V	Number of dead termites at a given time (Hours).									
	1	3	5	7	9	10	15	18	24	36
100	12	13	14	16	19	19	20	20	20	20
20	6	7	8	10	12	13	17	19	20	20
10	3	3	4	6	9	10	13	15	18	20
2	0	1	2	3	5	6	8	10	14	20
0.4	0	0	3	3	3	4	6	7	10	18
0.2	0	0	1	1	2	2	4	5	8	14
0.0	0	0	0	1	1	1	1	2	3	5

A second tree extract from the tree, *Croton megalocarpus* was bioassayed against the *Odontotermes stercorivorus* termite species and the effect compared with that of the *Juniperus procera* extract. The dilutions made were like in the previous case.

There was a marked reduction in the mortality of the termites and the duration of observation was extended to 48 hrs. The plots of the average number of dead termites against time in hours and of concentration against average number of dead termites showed that same general trend as that of the *Juniperus extract* (Figure 3.4 and 3.5). The reduction in mortality in the case of the *croton* tree extract (see Table 3.7) can be envisaged to mean that the toxic components affecting the termites were in reduced amounts in this species of tree as compared to the *Juniperus* tree.

A comparison in the mortality rates of the two tree species after a 24 hour duration (Figure 3.6) shows that there is a marked difference. The *Juniperus procera* extract is much more effective on the termites than that of *croton megalocarpus* tree. This tree is more susceptible to termites than *Juniperus procera* tree thus it is possible that some of these components that are toxic to termites are the ones that prevent termites attack on the trees.

Table 3.6: Concentration of the *Croton-megalocarpus* crude extract and its effect on termite mortality

Percentage Concentration of crude extract by Vol.	logarithm of Percentage concentration Log_{10}[Conc.]	Total No. of dead termites after 10 hours
100	2	14
50	1.699	9
20	1.301	7
10	1.000	2
2	0.3010	1
0.4	-0.398	0
0.2	-0.699	0

Table 3.7: Toxicity of *Croton megalocarpus* crude extract on the termite species *O.stercorivorus*

% V/V	Number of dead termites at a given time (Hours).											
	2	4	8	10	18	24	28	32	36	40	44	48
100	8	12	14	14	18	19	20	20	20	20	20	20
50	4	5	7	9	14	18	19	19	20	20	20	20
20	3	4	5	7	8	11	13	13	16	18	19	20
10	1	1	2	2	6	7	7	9	10	12	14	19
2	0	0	1	3	3	4	5	6	6	8	9	10
0.4	0	0	0	0	1	2	3	4	4	4	6	7
0.2	0	0	0	0	0	1	2	3	3	4	4	5
0	0	0	0	0	0	0	0	1	1	1	1	1

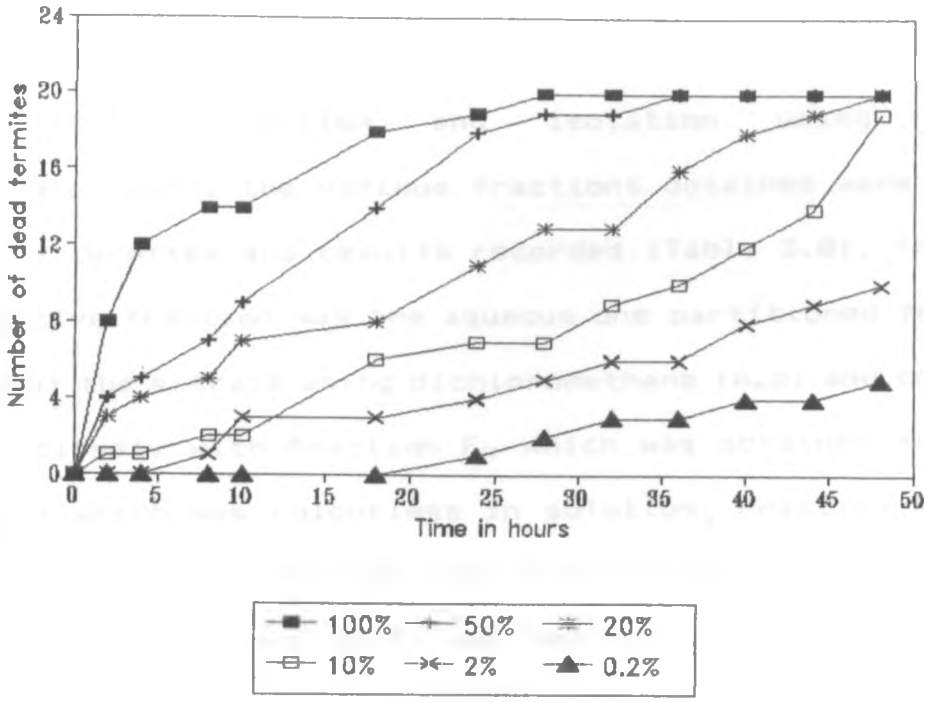


Fig.3.4: Dependence of termite (*O. stercorivorus*) mortality on exposure time. (*C. megalocarpus* tree).

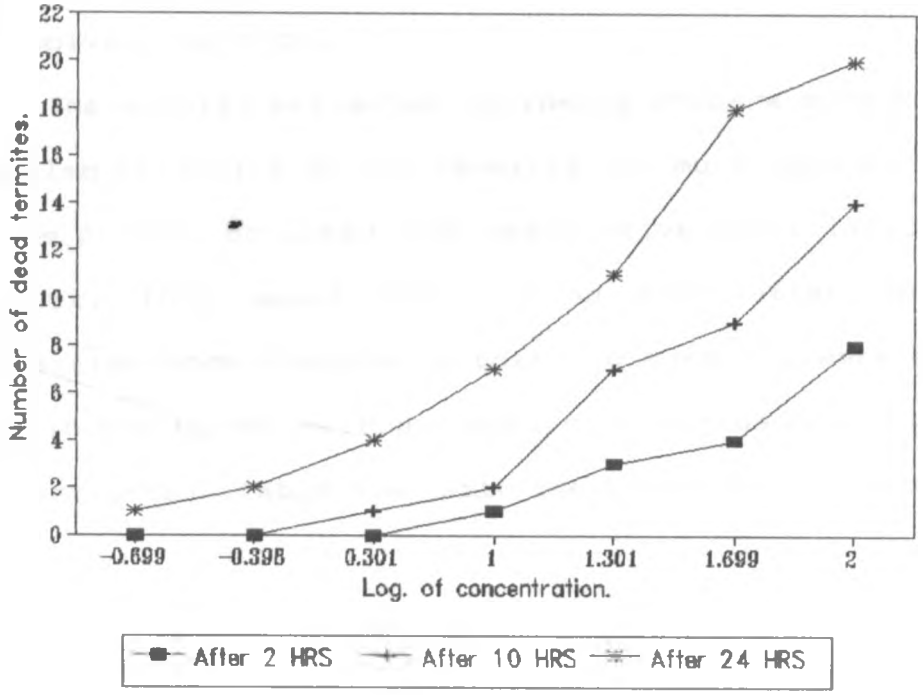


Fig.3.5: Change in termite (*O. stercorivorus*) mortality with increase in concentration for the *croton megalocarpus* tree extract.

After extraction and isolation using column chromatography, the various fractions obtained were tested on the termites and results recorded (Table 3.8). The most effective fraction was the aqueous one partitioned from the rest of the extract using dichloromethane (A_qp) and compares very closely with fraction F_3 which was obtained from the column which was colourless in solution. Fraction F_4 also had an effect on the termites almost equal to fraction F_3 and A_qP. This fraction F_4 was reddish-brown in colour and contained at least two compounds with very close R_f values. The solvent control used in this case was dichloromethane (DMSC) since some of the fractions were insoluble in water. The fraction F_5 was also a colourless solution that didn't show any significant effect on the termites as compared to the solvent control.

The soxhlet extracted *Juniperus procera* product (JPSE) was also effective on the termites but much less as compared to the product obtained from destructive distillation of the sawdust. This means that during the latter method of extraction more components toxic to termites were obtained than in the former method (compare *Juniperus procera* soxhlet extract(JPSE) (table 3.8) with the 100% extract (table 3.3).

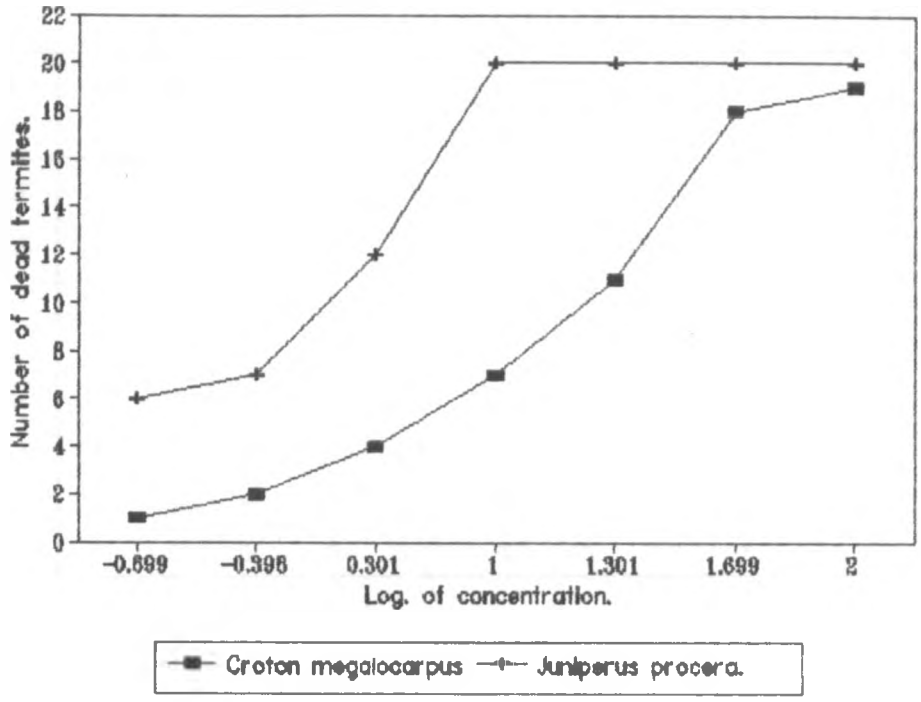


Fig.3.6: Dependence of mortality rate on extract concentration for the two trees, *J.procera* and *C.megalocarpus*. (After 10 hours).

Table 3.8: Toxicity of the *Juniperus* isolated fractions and soxhlet extract (JPSE) on *O.stercorivorus* termites.

Sample label	Number of dead termites after a given time (Hours).											
	2	4	8	10	18	24	28	32	36	40	44	48
DCMSC	0	0	0	1	2	3	3	3	3	3	3	5
JPSE	1	2	2	3	6	9	11	15	17	18	19	20
F_1	0	0	0	1	3	4	5	7	7	9	9	9
F_2	0	0	2	3	5	7	10	14	18	18	19	19
F_3	1	2	4	6	12	13	14	15	18	19	20	20
A_GP	1	3	4	7	14	15	16	16	19	19	20	20
F_4	3	1	4	6	8	10	14	16	17	18	20	20
F_5	0	0	0	0	3	3	3	4	5	5	5	6

3.4.0 GAS CHROMATOGRAPHY/MASS SPEC. ANALYSES

For the GC/MS analysis solution systems were prepared from the extract and each solution run separately and water eliminated from the extract. This was achieved by solvent partitioning using solvents compatible with the GC/MS column and detector system. The crude extract was initially separated into the aqueous layer and the oily layer using a separatory funnel. The oily layer, which floated on the aqueous layer, was extracted with dichloromethane (system 1). Two layers were produced, the dichloromethane layer and a layer containing the rest of the extract. This latter layer was now extracted with hexane (system 2). The aqueous layer was also extracted in a similar manner and again two layers were obtained, the hexane portion (system 3) and the

other layer containing the rest of the extract. This other layer was now extracted with dichloromethane (system 4).

These extract systems were analyzed by GC/MS spectrometer. The gas chromatogram of system 1, labelled as A_2-oil gave a major peak with a scan number 727 (figure 3.7) and a retention time of 23.2 minutes (the GC/MS used was a Hewlett Parkcard model VG 12- 250). Other minor peaks appeared as impurities.

The mass spectrum of the peak at scan number 727 (figure 3.8) showed the largest mass ion peak at a m/e value of 204. The base peak (most abundant) had a mass (m/e) of 119 and an equally large peak of mass 93. The most likely compounds that would give this spectrum would be, cedrol, 17 and ylangene, 32 with the molecular formulae $C_{15}H_{26}O$ (Mwt.222) and $C_{15}H_{24}$ (Mwt. 204), respectively. For cedrol, the peak at mass 204 would be as a result of a loss of a water molecule from the molecular ion of mass 222. In this case, the ion with a m/e value of 204 would not be the molecular ion. A further loss of $C_6H_{13}\cdot$ fragment gives the peak at mass 119. The fragment, C_9H_{11} gives the peak at mass 93. For ylangene, the molecular ion would be at mass (m/e) 204 and then a similar fragmentation like the one above would follow. Further analysis, after isolation of the fractions, confirmed the presence of cedrol.

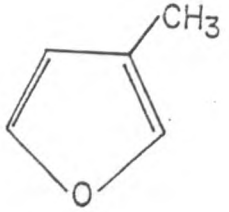
The hexane extracted oily portion, system 2 contained the same components as system 1 but in negligible quantities thus it was concluded that most of the components in the

oily layer were insoluble in hexane. The chromatogram of the system 3 which was labelled A_1-Q_{HEX}, Fig. 3.9 gave several peaks. There were more than twelve components but the major peaks were eight in number. The most prominent peak with a scan number of 573 had a retention time of 24.3 minutes while the other peaks with scan numbers 425, 447, 573, 625, 705, 999, 1125 and 1192 had retention times of 15.6, 16.8, 21.8, 23.0, 30.55, 33.8 and 35.54 minutes, respectively.

The mass spectrum of this system 3 (A_1-Q_{HEX}) was run and the spectra of the main components analysed to determine their identities. The spectrum of component with scan number 447 gave a base peak of mass 39 and a peak arising from the molecular ion with a mass of 82. The fragment of mass 39 was due to $C_3H_3^+$. The molecular ion (mass 82) loses the CH_3 group to give the peak of mass 67. This showed it to be most likely 3-methylfuran C_5H_6O, 34.

The largest peak, with a scan number of 573, had a mass spec. with major fragments of masses 39, 67 and 96 among other smaller peaks (figure 3.11). The molecular ion was of mass 96 which on loss of a $C_2H_4\cdot$ fragment gives the peak of mass 67. This further fragments to give the peak of mass 39. The most likely compound was 2H-pyran-2-one, $C_5H_4O_2$, 33 with a molecular weight of 96.

Another component with a significant peak was the one with a scan number 999. Its mass spectrum (fig. 3.12) gave a parent ion of mass 124 and a base peak of mass 109. Other fragments had masses of 81, 65, 53, 39 etc.



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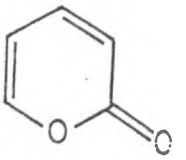

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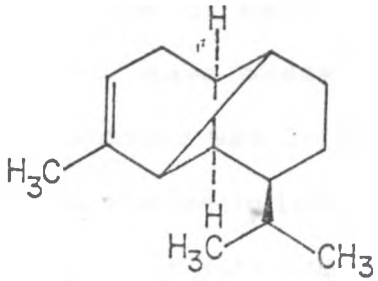

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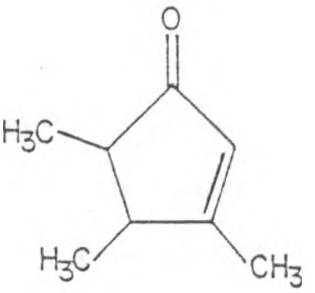
A loss of a $-CH_3$ from the molecular ion of mass 124 gives the peak of mass 109. Fragmentation of a $C_2H_3O\cdot$ fragment from the molecular ion gives the mass 81. The likely structure corresponding to this spectrum is that of the compound, 3,4,5-trimethyl-2-cyclopenten-1-one, 36. The peak with scan number 1125, fig 3.13 gave a mass spectrum with a molecular ion of mass 108 and a base peak of mass 94. Other fragments gave masses of 79, 77, 39, 50 and 62. The most likely compound was 2- methylphenol, 35. The $-CH_3$ group fragments from the methylphenol to give the fragment with a mass of 94. Loss of -OH group gives a peak at mass 79. The phenyl group (C_6H_5) gives rise to the peak at mass 77. The aqueous dichloromethane extracted portion (system 4) contained essentially the same compounds as the hexane extracted portion (system 3), Fig. 3.10 but the peaks obtained were much smaller than those of system 3.

A gas chromatogram of the hexane/dichloromethane soxhlet extracted *Juniperus procera* sample (note this method is different from that of dry distillation of wood) revealed more than ten compounds but only four major ones with retention times 1.75, 8.30, 10.73 and 10.58 mins, (Fig. 3.14) using a Perkin Elmer 8500 GC model.

A look at the chromatograms obtained from the destructive distillation extract of the same tree and the soxhlet extracted sample shows that many of the components were obtained in both methods thus it is more appropriate to use the former method of extraction since it avoids the use



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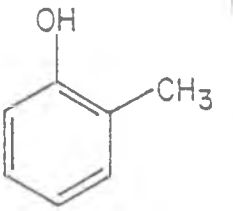

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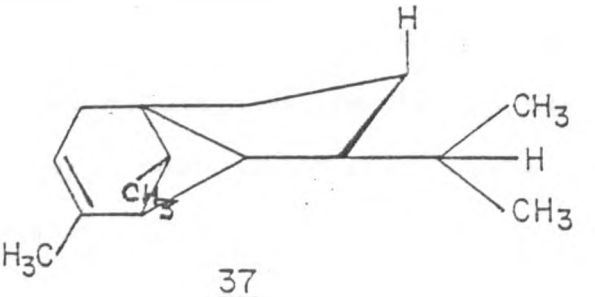

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of the expensive organic solvents and gives components toxic to termites. Further GC/MS analysis was carried out after isolation of the components besides other tests to confirm the presence of the detected compounds.

3.4.1 Analyses of the isolated components.

The dry distillation extracts of *Juniperus procera* were introduced into a column A packed with silica gel. Overall, five fractions were isolated.

The isolated components from the column were analyzed using the GC and GC/MASS spectrometer respectively. Several components detected earlier in the crude extract were isolated and analysed again by GC/MS besides other analyses. Not all of the components detected in the crude extract were isolated. A fraction, F_1, showed a single peak with a retention time of 7.98 minutes on the Perkin Elmer 8500 GC (Fig. 3.15). It gave a peak with scan number 733 and a retention time of about 24.50 minutes (under a different GC program) on the Hewlett-Parkcard VG 12-250 GC/MASS instrument (Fig. 3.16). A mass spectrum of the compound at peak number 733 gave a base peak of 119 and fragments at 41, 55, 69, 93, 105, 161 and 204 (Fig. 3.17).

The peak at mass (m/e) 204 is as a result of the loss of an -OH fragment from molecular ion at 222, a further loss of four - CH_3 groups attached to the rings and two after ring opening, gives a peak at 119 due to the fragment, $C_9H_{11}^+$. Other fragments are obtained easily as the ring breaks up.

This can easily be deduced from the structure of cedrol, 17. Thus, the fraction F_1 contained cedrol which had earlier been detected in the crude extract.

The fraction F_2 contained the same component as fraction F_1 but in a much higher quantity as evident from the gas chromatogram of the fraction (Fig. 3.18), which contains a very prominent sharp peak at a retention time of 8.18 minutes which, within experimental error correlates very well with that of fraction F_1 i.e 7.98 minutes with a difference of 0.2 minutes. The GC/MASS Spec. chromatogram gives the peak with scan number 732 (Fig. 3.19) which again is the same as that of fraction F_1 at 733. The mass spectrum (Fig. 3.20) gave a base peak of 119 and other fragments at 161, 204, 105, 93, 77, 69 and 41 among others which was the case for fraction F_1.

This spectrum turns out to be that of the compound cedrol which is confirmed from the IR spectrum of the fraction which shows a peak (broad) between 3520-3440 cm^{-1} which indicates the presence of a hydroxyl group which is hydrogen bonded. The sharp peak at 2929 cm^{-1} is the C-H stretching frequency of the $-CH_3$ group. The band around 1300 cm^{-1} is due to C-O stretching. Asymmetric bending of the group $-C-CH_3$ is given by the band between 1500-1460 cm^{-1} while the symmetric bend is given by the band at around 1350 cm^{-1}. The band between 1260-1240 cm^{-1} is due to C-O stretching in a Cyclic molecule. The UV spectrum didn't yield any significant peak. This leads to the conclusion

that the fraction F_2 contained the compound cedrol.

The isolated fraction F_3 from column A contained essentially one component with a retention time of 9.59 minutes (Fig. 3.22). This component appeared as a single strong peak at scan number 757 in the VG 12- 250 GC/MS spectrometer with a retention time of about 24.2 minutes (Fig. 3.23). The mass spectrum of this peak (Figure 3.24) showed major fragments of masses 204, 161. 119, 105, 93, 77, 69, 55 and 41 with the base peak being at mass 119. The largest mass ion had a m/e of 204. The mass spectrum closely resembled that of cedrol. This compound labelled F_3, decolorised Bromine water but didn't react with Brady's reagent or Fehling's reagent (tests for carbonyl groups). This indicated unsaturation in the molecule which is absent in cedrol. Moreover, failure to give positive Brady's and Fehling's tests suggest absence of a carbonyl group.

The molecular weight of the component was found, from the molecular ion to be 204 g/mol. From the mass spectrum it was evident that the molecule was an alkene, cedrene which possesses a double bond. The compound cedrene is also found in the cedar oil together with cedrol [39]. This was confirmed from the IR spectrum of the compound which showed a peak beyond 3000 cm^{-1} suggesting unsaturation. The peak at around 2929-2960 cm^{-1} is due to C-H stretching frequency while the peak at 1600 cm^{-1} is due to Carbon double stretching frequency. The peaks between 1400-1500 cm^{-1} suggest presence of CH_3 groups whose asymmetric bending

frequencies lie in the region. There is also a peak at 1370 cm^{-1} which is due to symmetric bending mode of CH_3 groups. The bends between 1300 and 750 cm^{-1} suggest that the compound is not a straight chain but rather has rings. The UV spectrum
(Fig. 3.25) gave an absorption maxima at 228 nm which suggests unsaturation [48]. The molecule, cedrene (see 18) mwt. 204, occurs in cedar-wood and in many other oils.

The column B was similar to column A but the eluting solvent volumes were different. This was with a view to try and separate further the fractions from column A which had not fully separated. Unseparated components were detected by spotting on TLC plates. Fraction F_4 from column B, gave a very prominent peak at 12.89 mins on the Perkin-Elmer 8500 GC (Fig. 3.26) and the same peak appeared as peak number 1343 on the VG 12-250 GC/MASS spec. model (Fig. 3.27) with a retention time of about 40 minutes. The GC/MS was programmed to run for one hour while the Perkin-elmer 8500 GC was run for 18 minutes. The mass spectrum of this component was very similar to that of component F_1 but with an extra peak at 248. This suggests a probable derivative of cedrol compound or a derivative of the compound copaene, 37, of mwt. 204 g/mol, mostly likely a carboxylic acid derivative with the additional $-CO_2H$ group to make the molecular weight 248 (the molecular ion has a mass of 248), Fig. 3.28.

From this investigation, it was evident that the water

content in the extract was high (around 77.5% v/v). Of the isolated components, the major ones were cedrol, cedrene and probably a derivative of the compound copaene, 37. From the UV spectrum and mass spectra of the extracts, it was apparent that besides these compounds, other probable components included phenolic and alcoholic simpler molecules. Some of these included methanol which is almost always produced during the distillation of wood. Methylphenol and 2H-pyranone were compounds detected by the mass Spectrometer technique. Some of these compounds could be by-products as a result of decomposition during the extraction which involved heating. These compounds could account for the high toxicity of the crude extract as compared to the isolated fractions.

From the gas chromatogram peak areas it could be estimated that cedrol was about 10% of the total components in the extract while cedrene was about 6%.

Fraction F_5 contained a peak with a retention time of 8.08 mins. (Perkin-Elmer 8500 GC) and a mass Spectrum which compared very well with cedrol/cedrene compound standard . This indicated that the fraction also contained predominantly the compound cedrol or cedrene.

Due to the presence of the ion with am/e value of 204 in most of the mass spectra, it was probable that cedrene was present in substantial amounts. This could be explained in terms of dehydration of cedrol to the corresponding alkene, cedrene. This would have taken place during

extraction which involved heating. Besides cedrene is also found in the cedar tree species.

The partitioned aqueous layer portion (A_qP) showed a toxicity level almost equal to that of fraction F_3. The gas chromatogram of this fraction (A_qP) revealed that it contained several compounds including those found in fractions F_2, F_1 and F_3 although in lesser amounts. This could explain its enhanced toxicity to the termites (*O. Stercorivorus*). The extract was also suspected to contain carboxylic acids (pH less than 5) which were removed using 5% sodium bicarbonate ($NaHCO_3$) before analysis was carried out. The water was removed using anhydrous sodium sulphate (Na_2SO_4) to dry the samples before analysis.

The detection of the compound cedrol in high proportion in the isolated components of the extract was in agreement with previous work done which showed cedrol to make up to 30% of cedar wood oil [40]. Small quantities of this same compound extracted using solvents by Clarence A. and co-workers proved to be toxic to termites [30].

About 70% of the crude extract was water with some components dissolved in it most likely -OH containing species. This portion after partitioning was effectively bioassayed against the termites (portion A_qP). It contained some of the more polar compounds from the extract. Its high toxicity level showed that it contained the compounds cedrol and cedrene (table 3.7), though in small quantities.

4.0. CONCLUSION AND RECOMMENDATIONS

This work started from a very basic level and due to the numerous unknown components extracted, the determination of the individual compounds was difficult. The isolation was on the basis of trial and error since there were no laid down isolation procedures for this extraction method before this work was started.

The results show that the extract from the *Juniperus procera* was successfully bioassayed against the termites species, *Odontotermes stercorivorus* and *O. Mautanus*. The 100% crude extract killed the termites almost instantly indicating a very high potency level. A 10% solution of the extract (10% extract and 90% water) was to be an optimum concentration for application to the termites. This killed half the termites (10) in 5 hours and achieved 100% mortality (20 termites) within 21 hours. The control tree *croton megalocarpus* had fewer components in its extract (Fig. 3.29) which showed a substantial degree of toxicity although to a lesser degree than the *J. procera*. This indicates that there could be a direct relationship between the natural resistance to termite attack of a wood and the toxic components in the wood extract, since the former tree is more resistant to termite attack than the latter. Isolation and spectroscopic analysis together with previous analytical data showed the presence of compounds like cedrol and cedrene about 10% and 6% respectively which according to previous work had been found to be toxic to termites [30].

The product of the wood distillation contained the active ingredients and cedar wood oil which is also useful for other Industrial purposes. It is evident that naturally occurring pesticides are currently under-utilized but could offer a remedy to some of the pest problems without causing harm to the environment.

There is thus need to investigate more of the plants for pesticides especially from plants that show a marked resistance to the pests of interest. The extract, if produced in large scale and other residual components removed could make a formidable formulation against termites. The crude extract could be made into formulations and sprayed directly to the termites.

Although investigations of termiticidal properties of plants have been carried out elsewhere, very little has been done in our country. Various local trees and plants are potential sources of termiticides. This, coupled with the high costs of pesticides, prompted us to carry out this work. Although there might be some enviromental impact of the large scale production of the extract, the major source of the extract would be sawdust from the millers and other timber processes.

4.1. RECOMMENDATIONS:

The work carried out indicated that the extract was a potential pesticide against termites. There is thus need for further investigation in the field with the extract which

could include other species of termites in addition to the ones already used. A comparison with available termiticides in the market could be made.

Complete isolation, purification and characterization using NMR spectroscopy could be carried out on the components and the efficacy of the pure compounds determined. Synthesis of the most effective compound could be carried out using alternative procedures to produce this compound or its analogues.

With a view to maximizing the use of the extract, other types of insects (pests) should be used during tests for toxicity. Other parts of the tree e.g. seeds, leaves and bark should be extracted to determine the termiticidal components present.

Treatment could be carried out on pieces of wood from the more susceptible species of wood e.g. *Eucalyptus Salignas* using the extract and their durability observed as compared to untreated ones. The period of activity and persistence after application should be observed.

The by-product of the extraction (charcoal dust) can be pressed into small cubes and used as fuel.

SPECTRA AND CHROMATOGRAMS

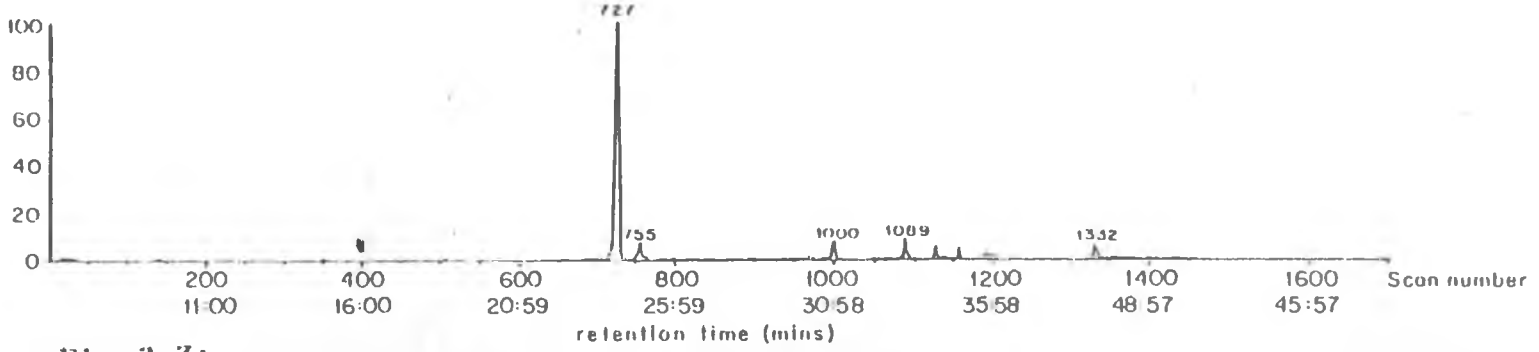


Fig. 3.7:- Chromatogram of the dichloromethane soluble oily layer of the crude extract (A_2-OIL).

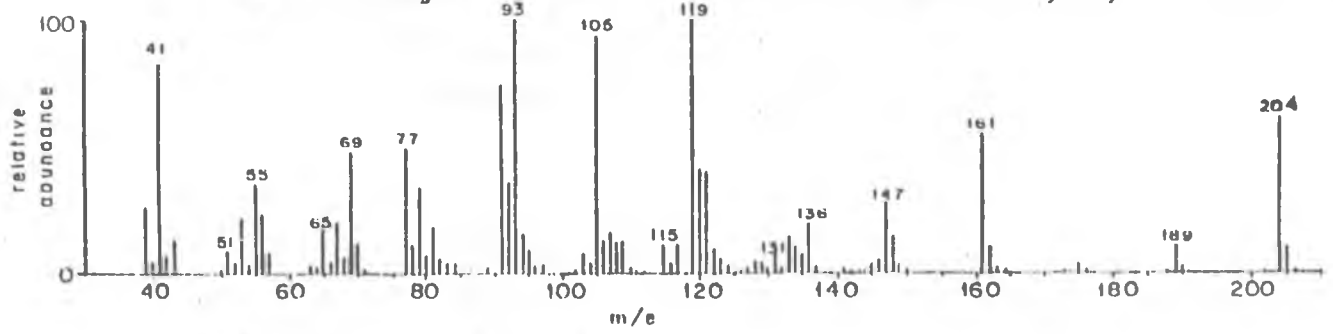


Fig. 3.8:- Mass spectrum of the largest peak of the sample (A_2 - OIL), peak no. 727.

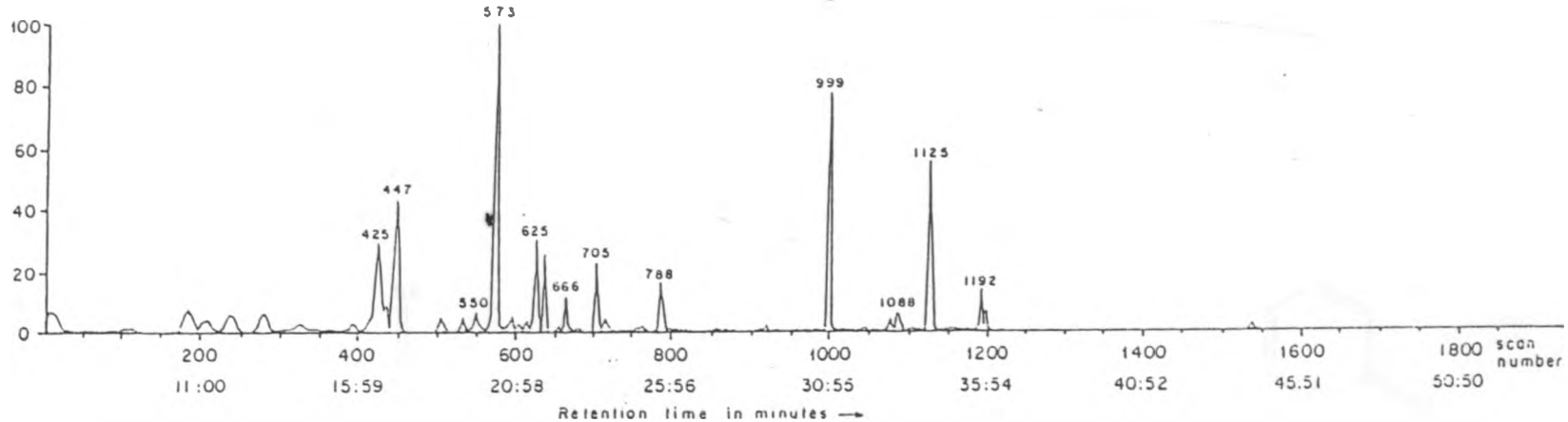


Fig.3.9:. Chromatogram of the crude Hexane soluble extract portion (A_1-Q_{HEX}) using GC/mass spectrometry technique .

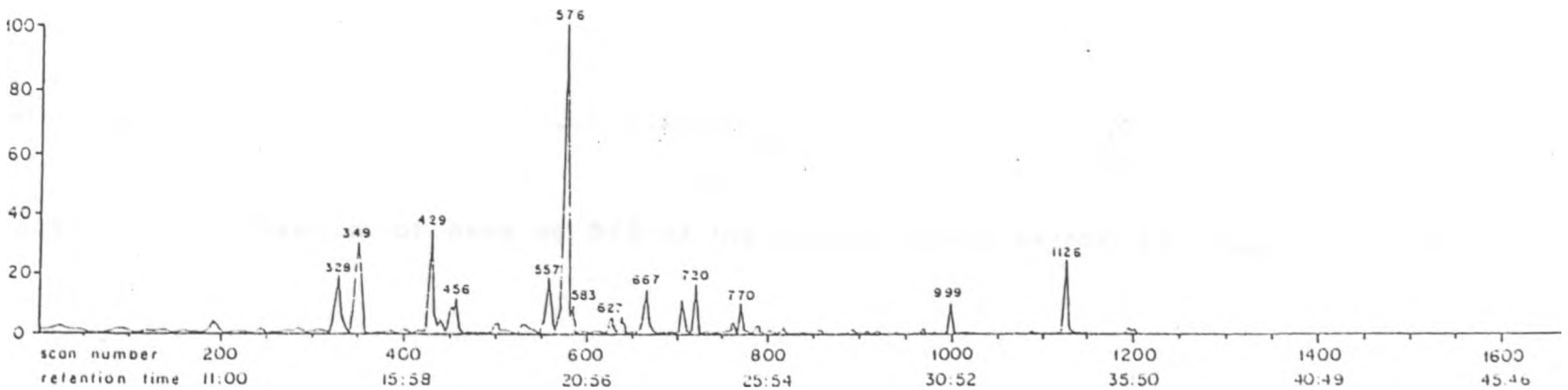
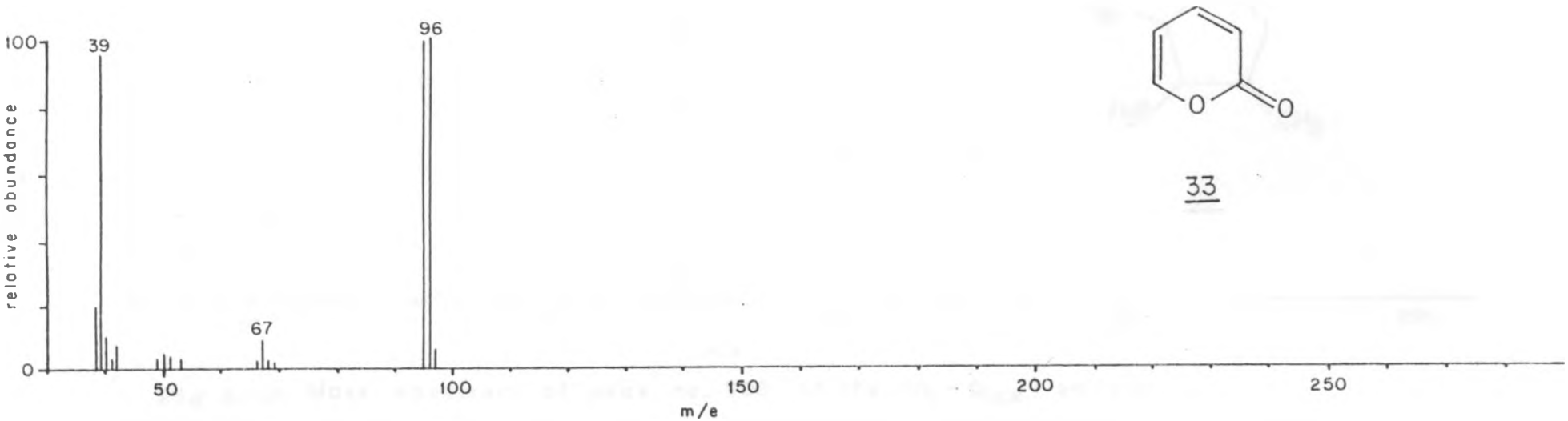


Fig.3.10: Chromatogram of the Dichloromethane soluble portion of the aqueous layer of the crude extract .



33

Fig.3.11: Mass spectrum of peak no. 573 of the Hexane soluble extract ($A_1 - Q_{HEX}$) with the base peak at 96.

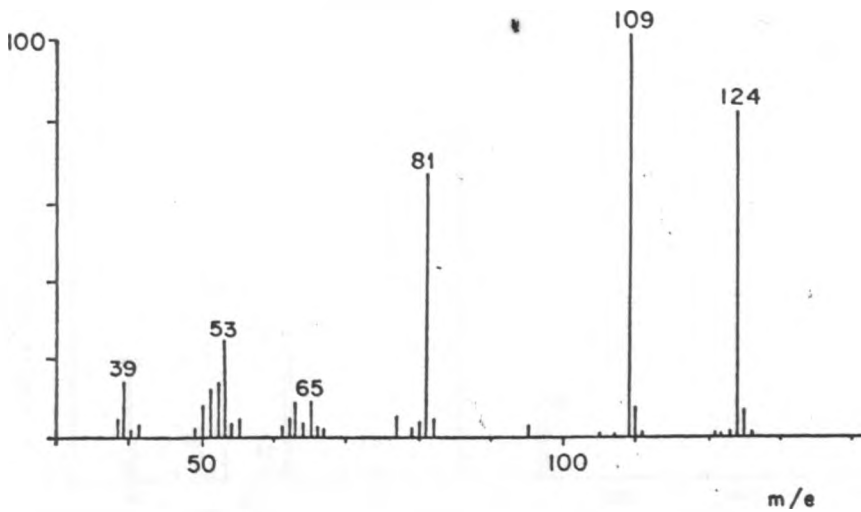
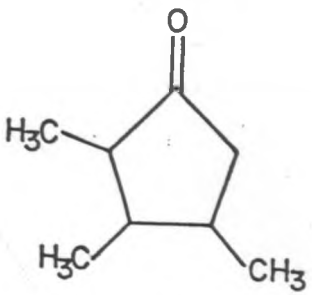


Fig.3.12: Mass spectrum of peak no. 999



36

150 200 250

of the $(A_1 - Q_{HEX})$ sample.

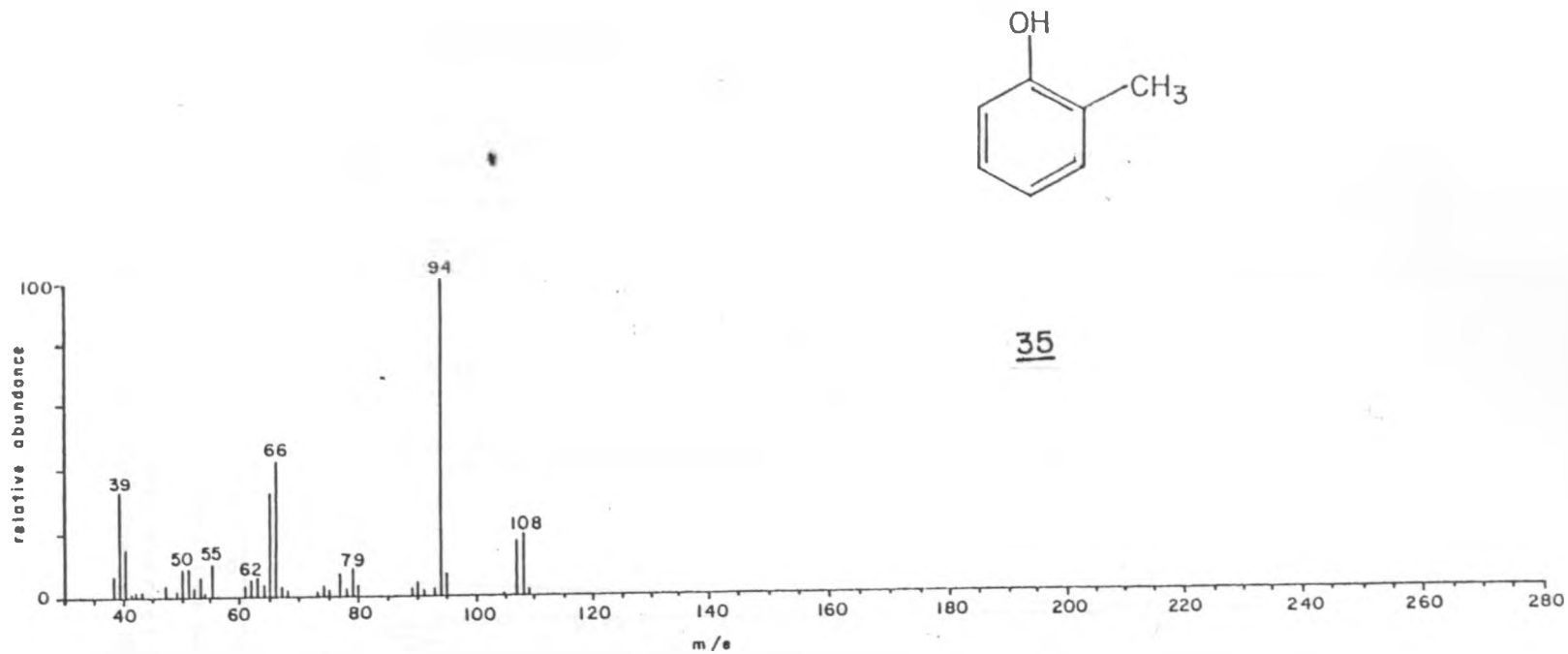


Fig. 3.13: Mass spectrum of peak no. 1125 of the ($A_1 - Q_{HEX}$) sample with base peak of 94

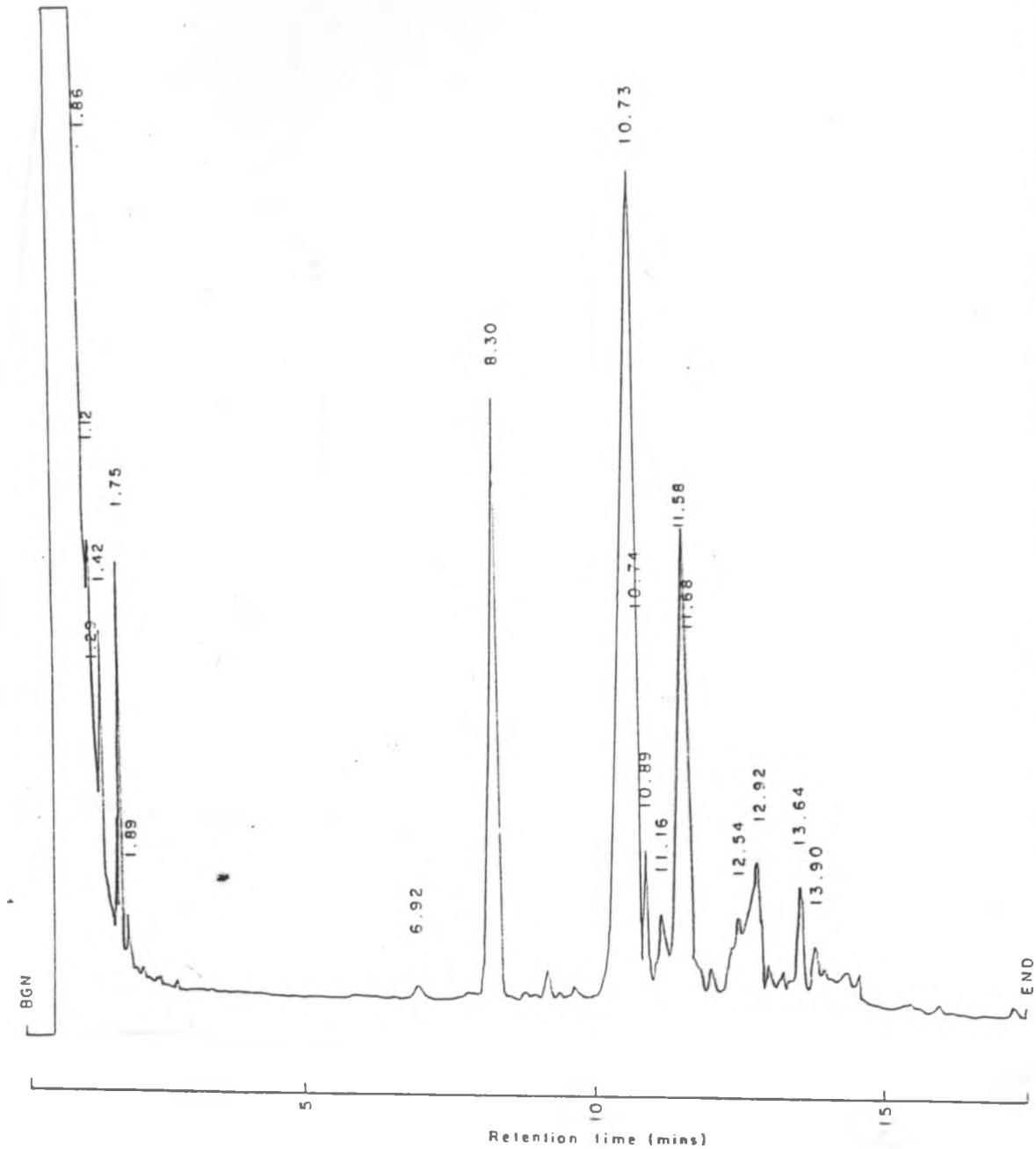


Fig.3.14: Gas chromatogram of soxhlet extract from Juniperus procera tree (solvents used : Dichloromethane and Hexane)

Fig.3.15 : Chromatogram of fraction F1

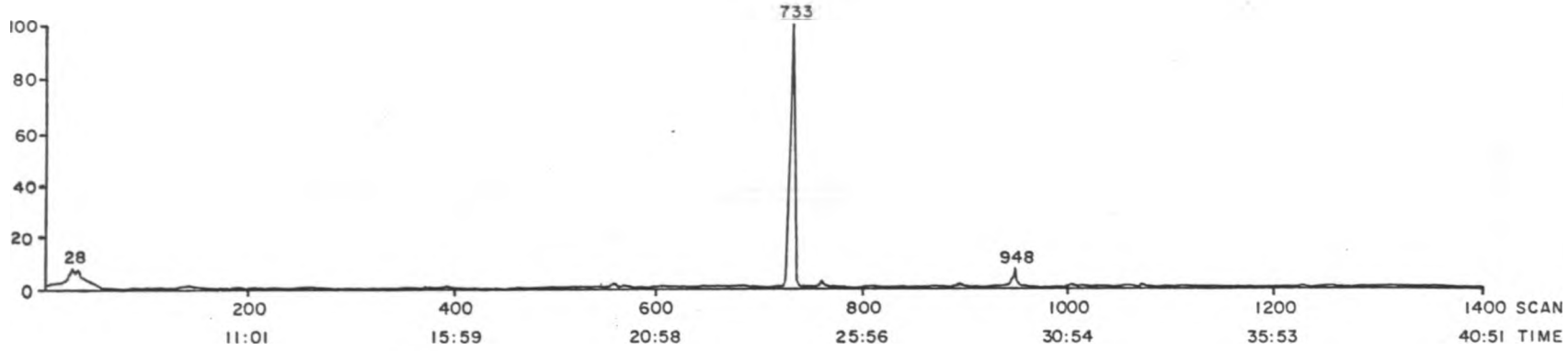


Fig.3.16: GC/MS Chromatogram of fraction F_1 showing a peak at scan number 733.

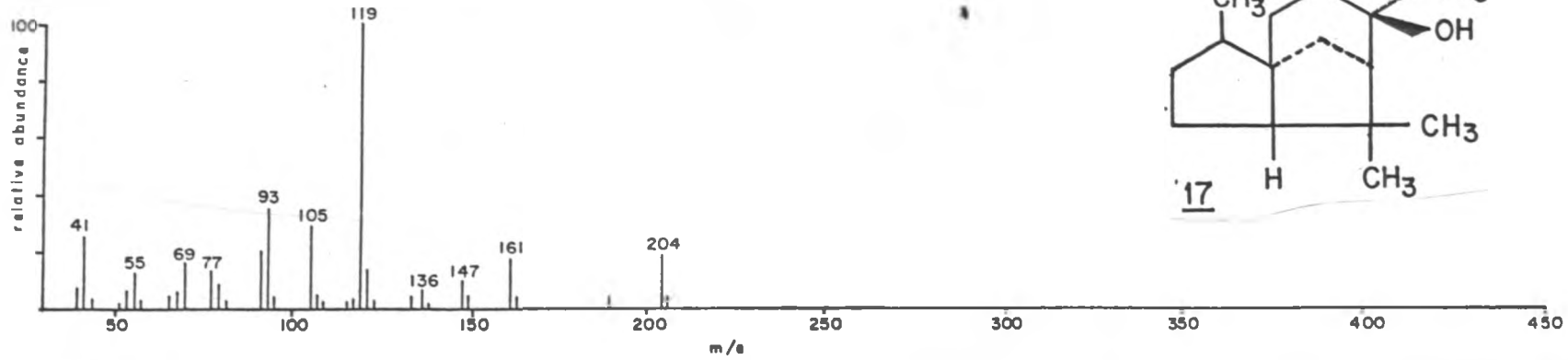


Fig.3.17: Mass spectrum of component 733 of fraction F_1 |

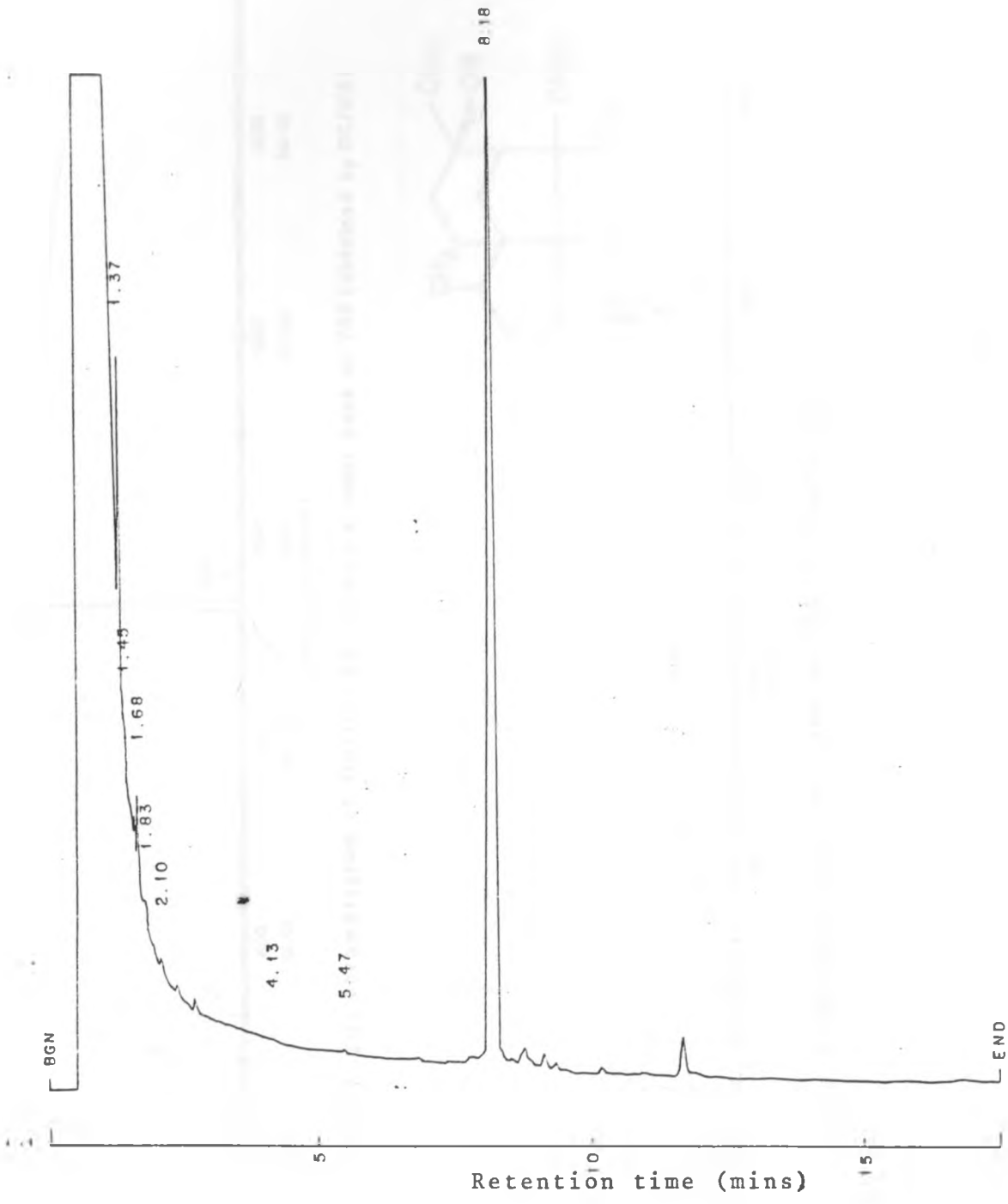


Fig.3.18: Chromatogram of fraction F_2.

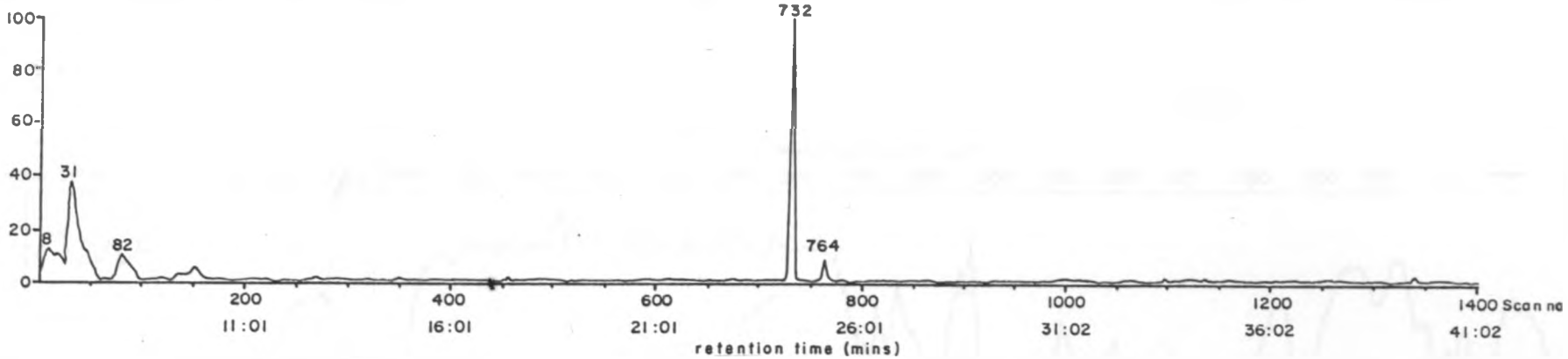


Fig.3.19: Chromatogram of fraction **F2** showing a major peak at 732 (obtained by GC/MS).

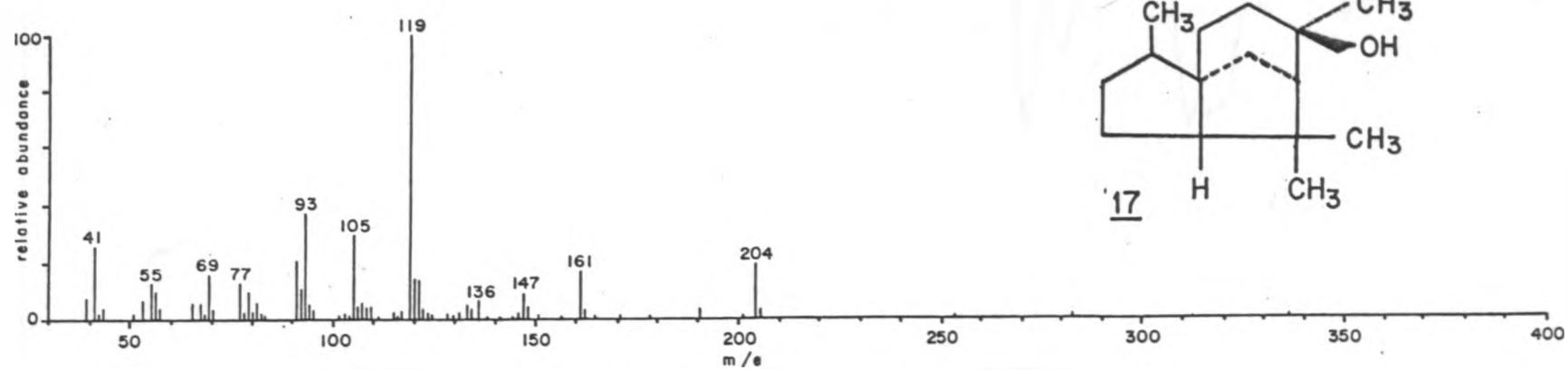


Fig.3.20: Mass spectrum of peak no. 732 of fraction **F2**

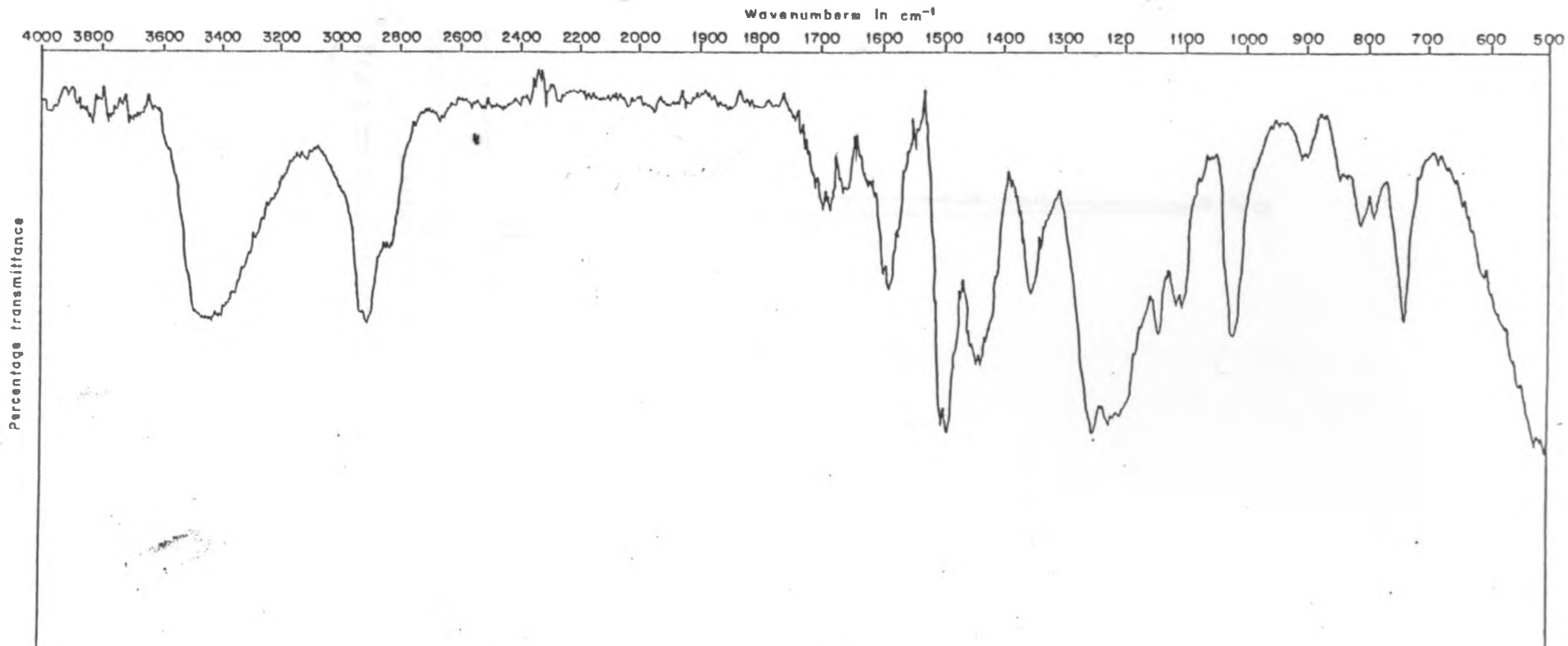


Fig.3.21: I-R Spectrum of fraction F_2 containing α-Cedrol $C_{15}H_{26}O$ Mwt 222g/mol.

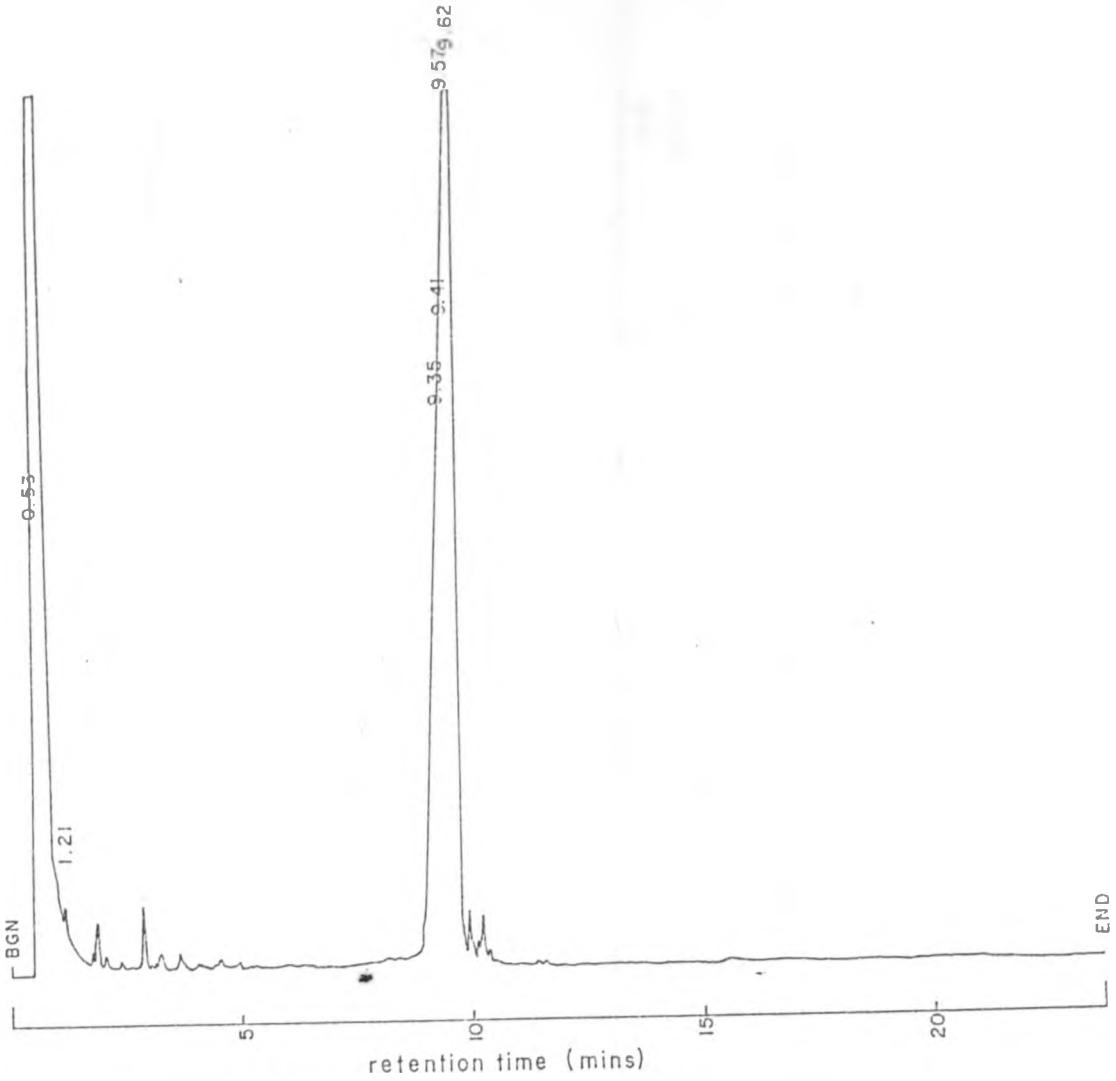


Fig.3.22: Chromatogram of fraction F_3

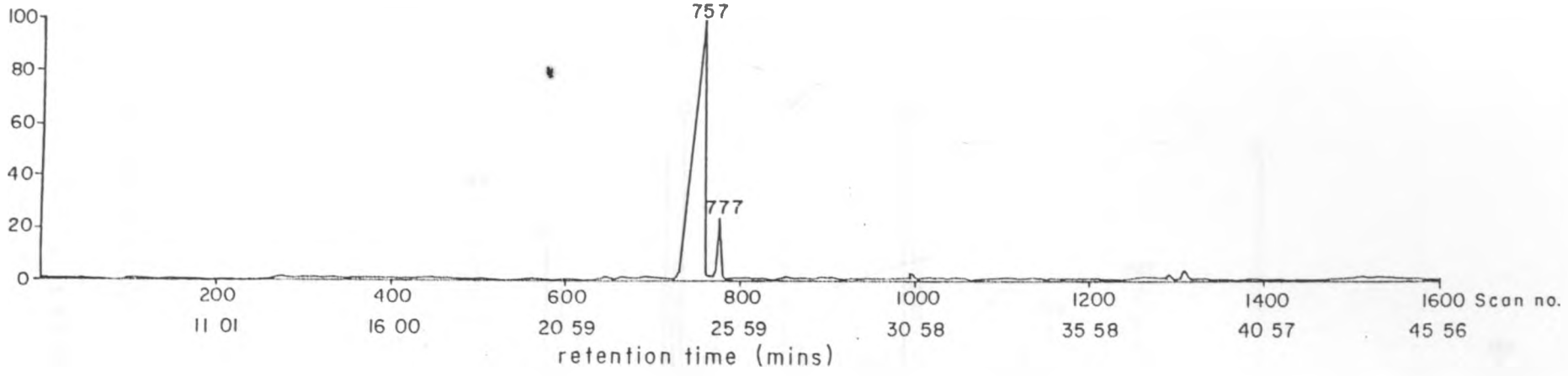


Fig.3.23: Chromatogram of sample F_3, showing a major peak number 757 .

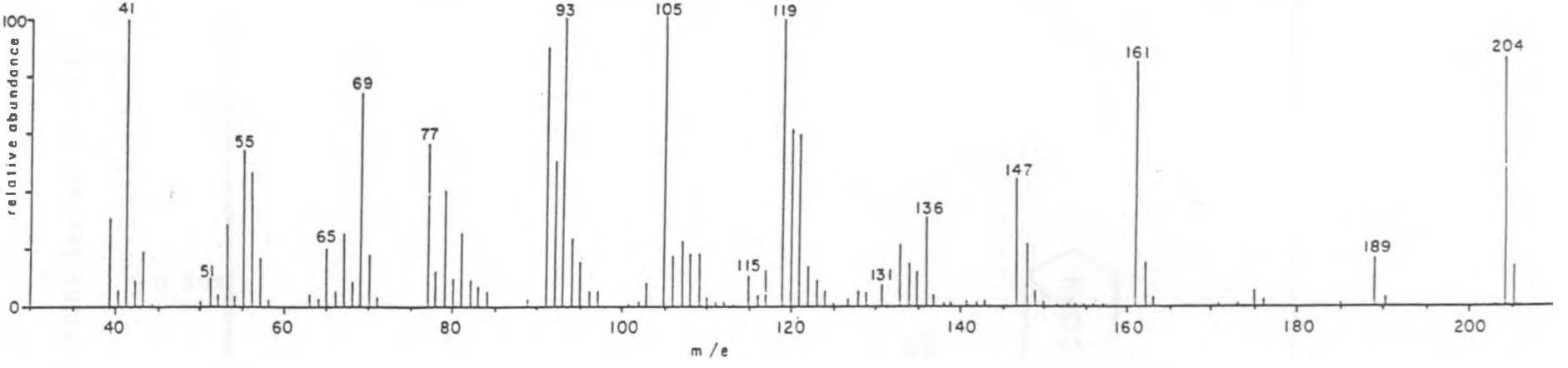


Fig.3.24: Mass spectrum of peak 757 of sample F_3 .

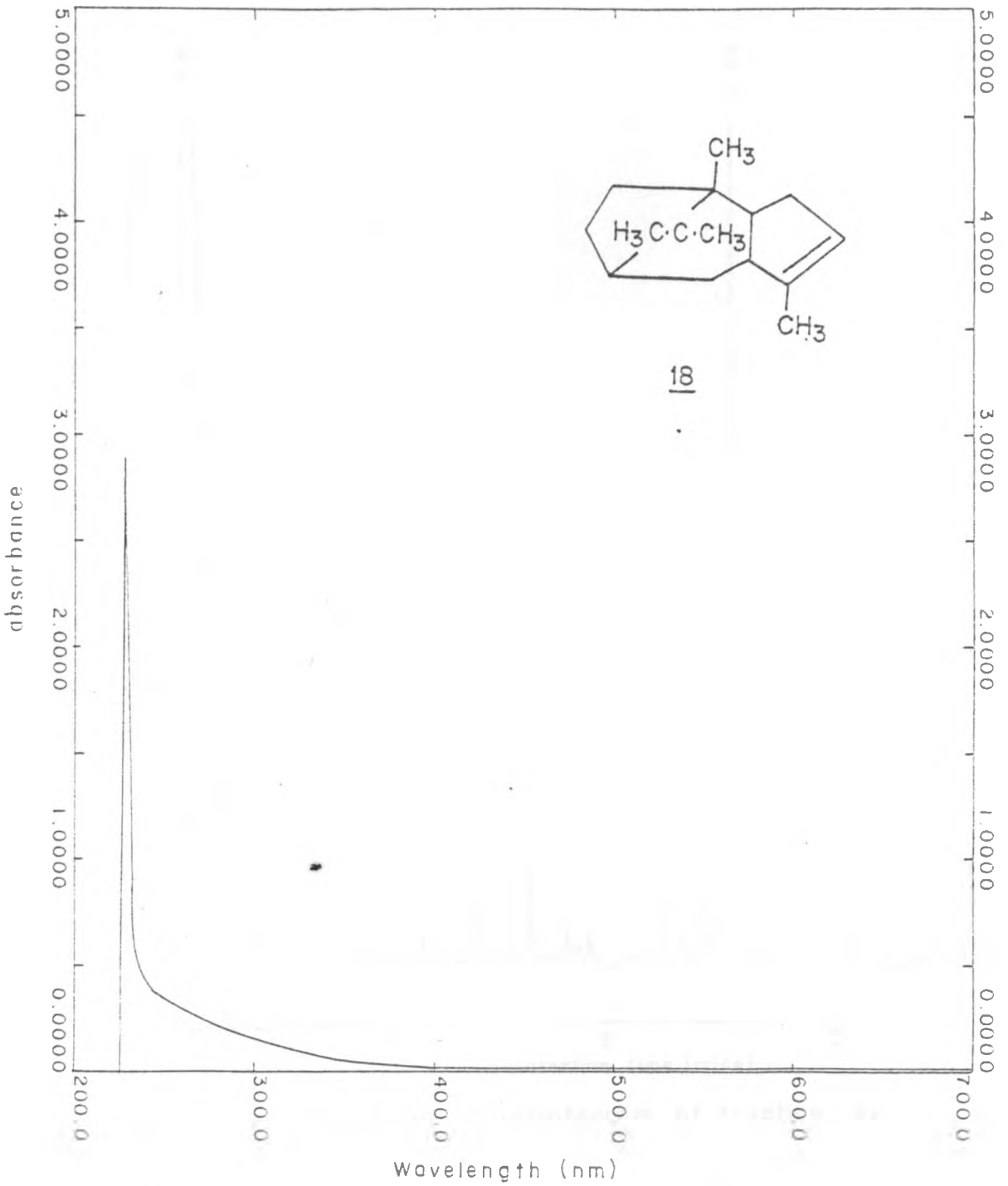


Fig.3.25: UV – Spectrum of the sample **F3** in dichloromethane

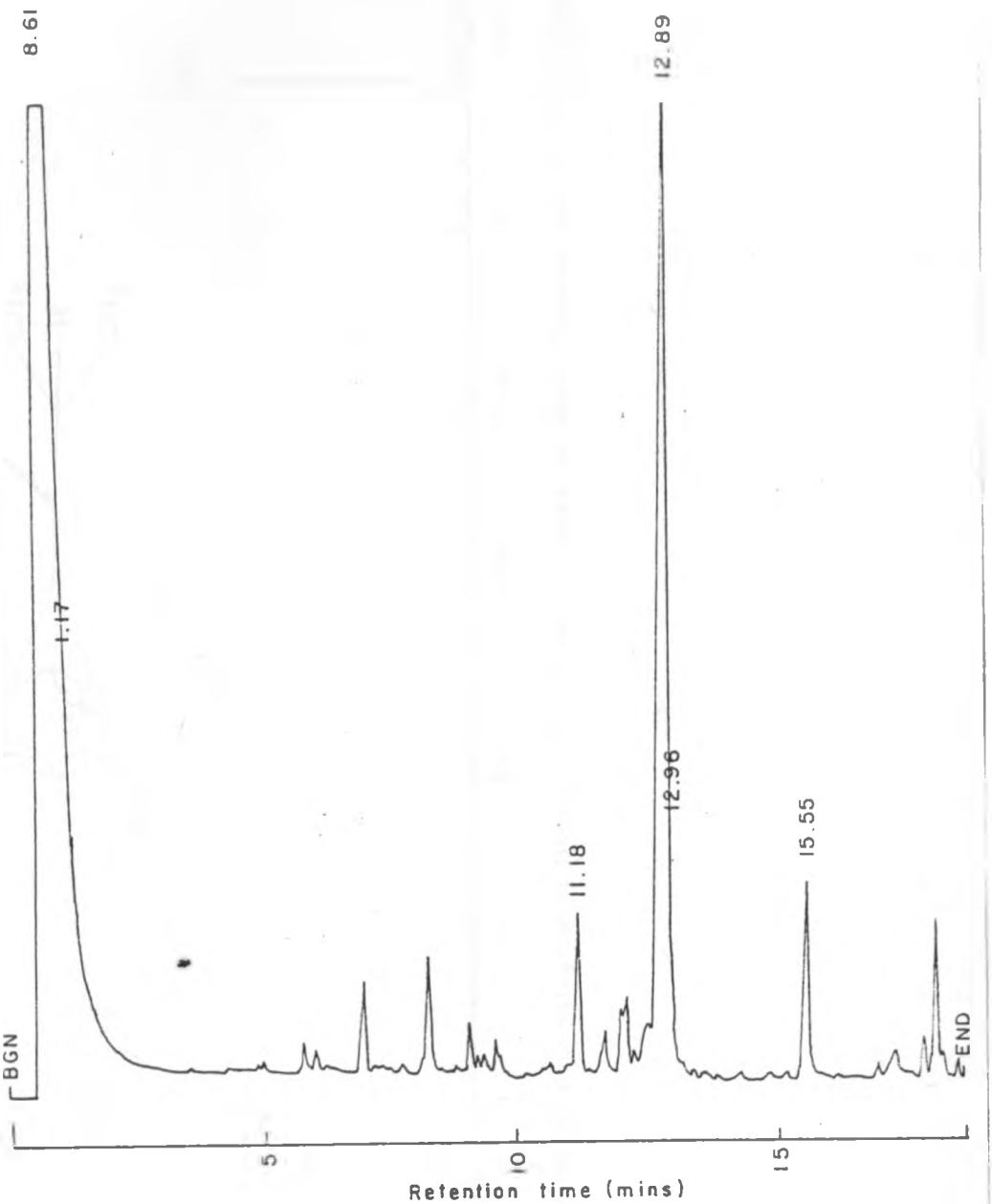


Fig.3.26 : Chromatogram of fraction F_4

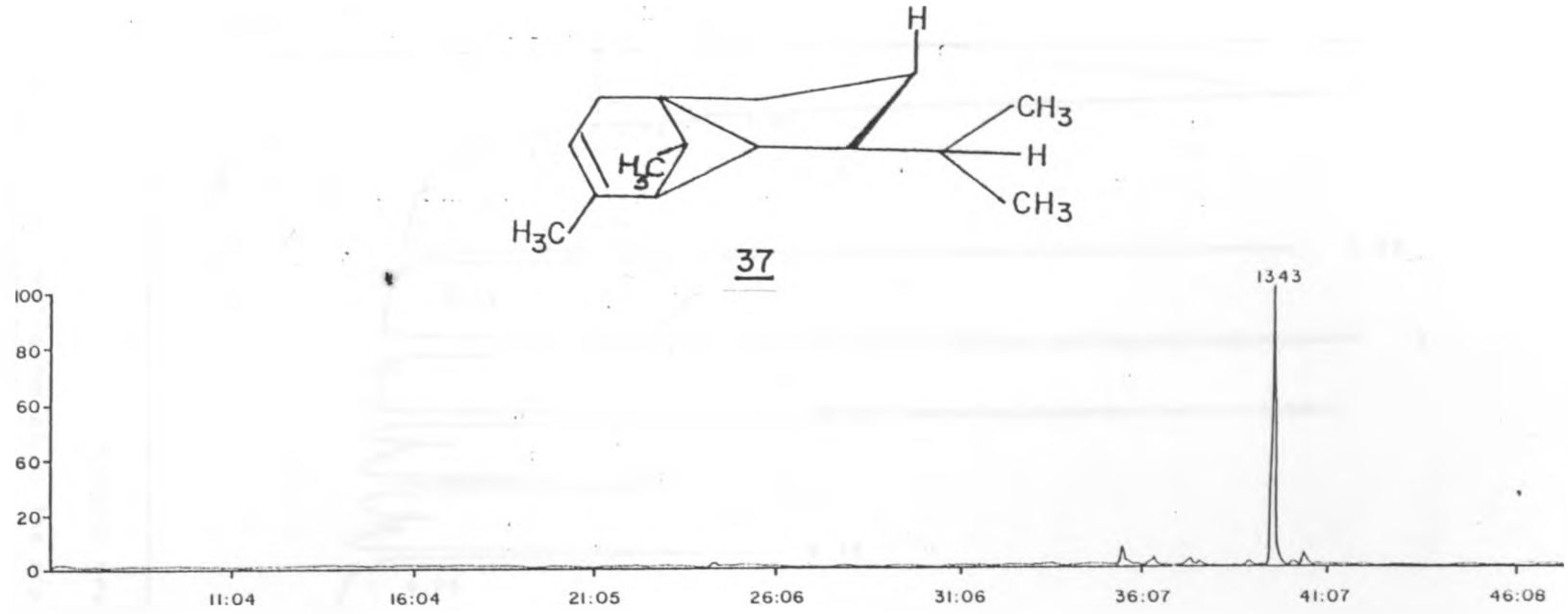


Fig. 3.27: Chromatogram of fraction F_4 showing a peak at about 40 mins, scan no. 1343 (obtained using GC/MS).

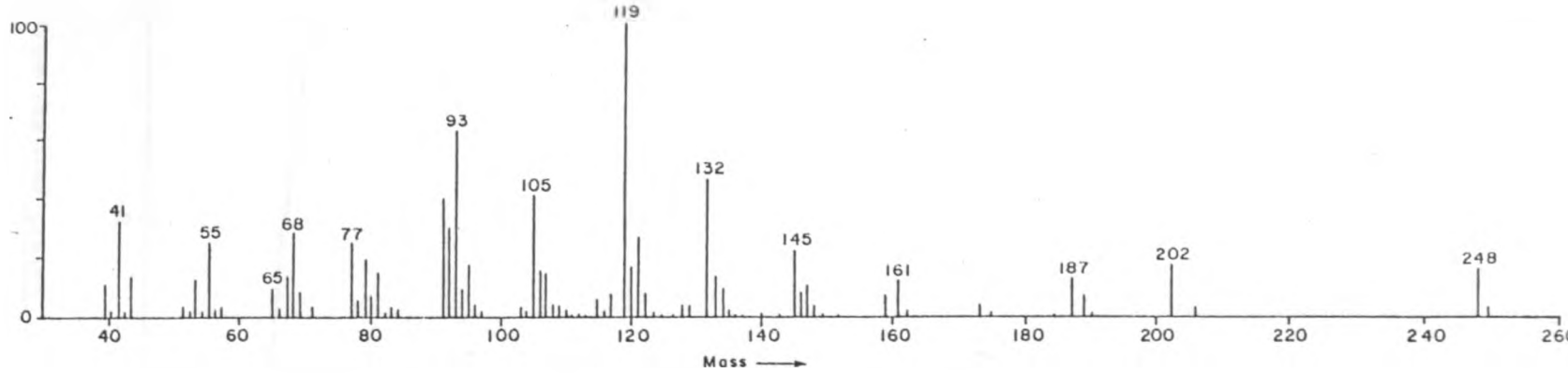


Fig. 3.28: Mass spectrum of peak no. 1343 of fraction F_4.

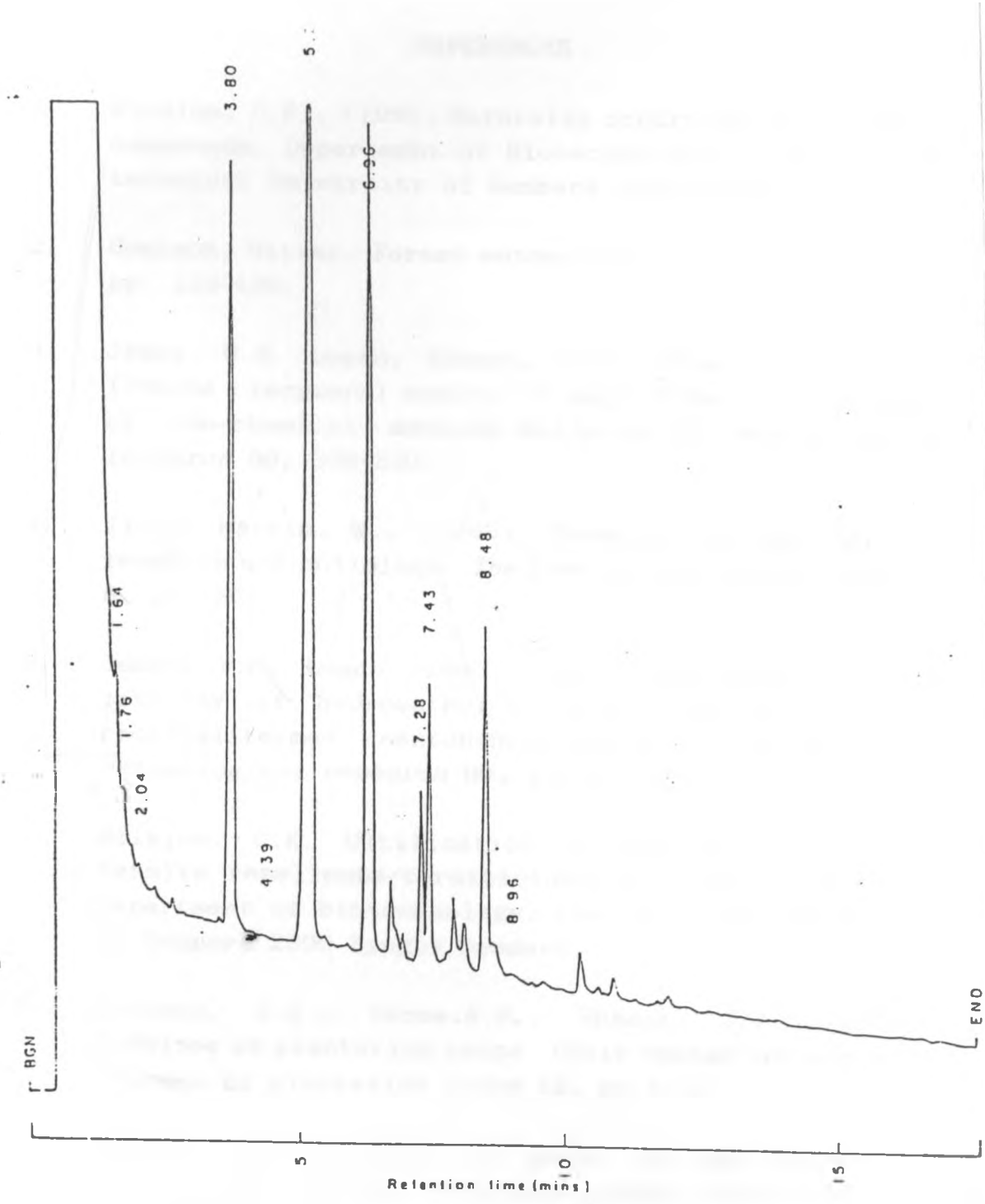


Fig.3.29: Chromatogram of the crude extract from the tree croton megalocarpus .

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