OVIPOSITION AND FEEDING ALLELOCHEMICALS FOR

CHILO PARTELLUS

FROM SORGHUM AND MAIZE CULTIVARS;

BIOASSAY, ISOLATION AND CHARACTERIZATION:

BY

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DECLARATION:

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

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DEDICATION:

To my father, *E. Bogita*; mother, *B. Nyarinda*; brother, *J. Nyagaka* and sisters, *Mary*, *Josephine*, *Janice*, *Zipporah* and *Ester*.

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TABLE OF CONTENTS:

PAGE:

LIST OF TABLES, GRAPHS AND FIGURES: **PAGE.**

ABSTRACT:

A study was carried out to isolate and identify the chemicals from sorghum and maize that determine the oviposition and feeding responses, respectively, of the moth and larvae of *Chilo partellus* a serious pest of these crops. Studies were carried out on a resistant and a susceptible cultivar of maize (MP 704 resistant; INBRED A susceptible) as well as sorghum (IS 1044 resistant; IS 18363 susceptible). The allelochemicals were extracted sequentially with petroleum ether, ethyl acetate and methanol. The sorghum cultivars showed quantitative rather than qualitative differences while the maize cultivars showed both qualitative and quantitative differences in respect to the concentrations of the constituent chemical compounds.

The maize feeding bioassay was carried out using impregnated cellulose acetate discs. The sorghum ovipositional bioassay was carried out using filter paper as plant substrate. The maize methanolic extracts showed greater feeding stimulation than ethyl acetate extracts and these in turn showed greater feeding stimulation than the petroleum ether extracts. The susceptible cultivar extracts elicited more feeding stimulation than those of the resistant cultivars. The sorghum petroleum ether extracts showed significant *C. partellus* ovipositional inhibition unlike the ethyl acetate and methanol extracts which showed very weak ovipositional activity or none at all. The pet-ether extract of the resistant cultivar showed the greatest oviposition inhibition.

Chemical study of the extracts revealed a variety of compounds on the leaf whorls. A series of ***n*-alkanes** with carbon chain C_{10} -C_{18} were common in all the maize and sorghum cultivars. These were however found to have no observable effect on *Chilo partellus* oviposition. The compound, **methyl 11,14,17-eicosatrienoate,** isolated from the sorghum

resistant cultivar petroleum ether extract showed significant ovipositional inhibition. This compound was in large amounts in the resistant cultivar. Three compounds were isolated from the maize resistant cultivar ethyl acetate extract; **4-hydroxybenzoic acid, 4-ethoxybenzoic acid** and **4,5,6,7-tetrahydro-2-1H-isoindole-1,3(2H)-dione.** 4-Hydroxybenzoic acid was in large amounts in the susceptible cultivar compared with the resistant cultivar and is a known *C. partellus* feeding stimulant. 4-Ethoxybenzoic acid, also in larger amounts in the susceptible cultivar compared to the resistant, has not been tested independently for bioactivity. 4,5,6,7-Tetrahydro-2-1H-isoindole-1, 3(2H)-dione was found in large amounts in the resistant cultivar. It has not been tested independently for bioactivity although a study of the H.P.L.C profiles of the cultivars suggests that this compound, along with another that was difficult to isolate, could be responsible for the observed reduced *C. partellus* feeding stimulation in the resistant cultivar by acting as antifeedants.

Structural elucidation of each of these compounds was performed on the basis of their spectroscopic data. The range of compounds isolated and characterized indicates that there may be a vast reservoir of yet untapped allelochemicals in food-crops which could be useful in the breeding of pest resistant cultivars of the principle food-crops maize and sorghum.

CHAPTER 1 :

INTRODUCTION:

1.0 General considerations:

In the tropics, maize and sorghum are some of the principle food crops. They are also used as fodder, fuel and building material. Generally grain yields in farmers fields in Africa and India are low. One of the several factors held responsible for the low yields is losses to the insect pests (Seshu, 1982). The stem borer, *Chilo partellus*, is one of the most widespread and serious maize and sorghum pests in the Indian continent, East, Central and Southern Africa (Young and Teete, 1977; Van Hamburg, 1980; Alighali, 1985). The damage caused to the grains by this pest ranges between 24-36% in maize and 15-45% in sorghum (Seshu Reddy and Sum, 1991; Seshu, 1988 and Alighali, 1986).

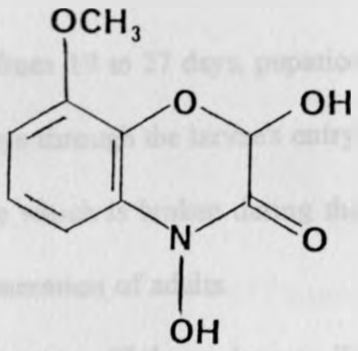
To date, a series of maize and sorghum cultivars produced by national and international breeding programs have been evaluated for resistance and varieties have been identified with different levels of preference for oviposition, feeding and establishment of *Chilo partellus* larvae (Jotwani, 1981; Seshu, R.K.V. 1985; Singh and Raina *et al.*, 1989). The degree of susceptibility of the cultivar depends on the successful larval establishment, amount of feeding and development (Alighali and Saxena, 1988).

Host plant resistance is a complex phenomenon involving multiple factors and plays a major role in the control of pest population in crops. One of the factors that plays a vital

role in the location and colonization of crops by pests is the presence or absence of secondary plant metabolites. The production of secondary plant metabolites has been shown to be mainly responsible for the resistance in different crops, for example in rice (Sogawa and Pathak, 1970), sorghum (Woodhead,1983; Haskin and Gorz,1985; Torto *et al.*,1990) and in wheat (Niemeyer, 1989). Factors which may be of major importance in relation to one pest or disease may be secondary or not relevant in another.

It has been suggested that the resistance to *Chilo partellus* by the host plant may be due to a combination of chemical compounds thus, green leave alcohols, aldehydes, terpenes, etc. that are found on the leave surface wax (Ampofo and Nyangiri, 1986; Bernays et al, 1983; Woodhead and Janeja, 1987). Indeed, 2,4-dihydroxy-8-methoxy-1,4-benzoxazin-3-one (DIMBOA), (I), found in young maize leaves, is regarded as one of the secondary plant compounds responsible for maize resistance to *Ostrinia nobilalis*, (Robinson *et al.*, 1978) though it does not appear to be a factor in the response of *Chilo partellus* to maize.

It is now well documented that the different behavioral responses shown by an insect, which lead to the selection of and successful establishment on its host plant, are influenced in part, and often predominantly, by allelochemicals. It is, therefore, important and essential to investigate the allelochemical bases for the different colonization of a pest insect on different host cultivars, to provide a better understanding of the underlying principles. Work relating to the *C. partellus* feeding allelochemicals was initiated at the International Centre of Insect Physiology and Ecology (I.C.I.P.E) in 1985 (Torto *et al.* 1990). This research sought to study *C. partellus* oviposition and feeding allelochemicals in sorghum and maize, respectively.



(I)

1.1.0 The pest *Chilo Partellus*, (Swinhoe)

(Pyralidae: Lepidoptera)

1.1.1 Description and Biology:

The *Chilo partellus* moth is medium sized and straw coloured. A female lays nearly 500 eggs in masses of 10-80 on the undersurface of a leaf often near the midrib. The eggs are flattish and oval and tend to overlap like fish scales. The eggs hatch in 4-5 days.

Chilo partellus moths have the greater part of their activity during the night. They have nocturnal habits. Emergence of male and female moths peaks 2 to 3 and 6 to 9 hours after onset of night, respectively. Maximum mating takes place during the first night after emergence, declining markedly during the successive nights. Mating commences after mid-night reaching a peak between 5 am and 7 am and then declines. Oviposition is maximum during the first night after mating and declines on successive nights. The most suitable period

for oviposition is between 4 pm and midnight (Ramachandran and Saxena, 1991; Kumar and Saxena, 1985).

The larval period lasts from 19 to 27 days, pupation takes place inside the stem and the larvae emerge in 7 to 10 days through the larvae's entry holes. During the dry season the instar larvae enter into diapause which is broken during the rainy season and pupation takes place giving rise to the first generation of adults.

Forty one species of the genus *Chilo* are known. Twenty five of these infest cereals and eighteen species occur in Africa (Beevor, *et al.*, 1990). Essentially it is a pest in hot lowland area and is seldom found above an altitude of 1500 meters (Hill , 1983).

1.1.2 Plant infestation:

The main hosts of *Chilo partellus* larvae are maize, sorghum, sugar-cane, rice, millet and wheat. The larvae may attack various parts of the crop at various stages of the plant growth; however, the larvae prefer feeding on the young plant rather than older ones (Sigh and Raina, 1989; Teete *et al.*, 1983).

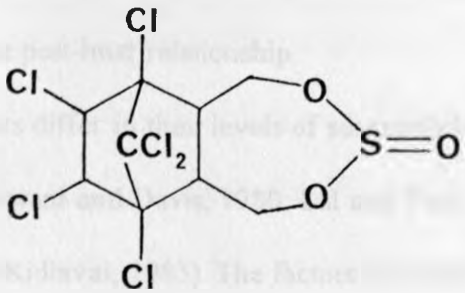
In the field, the female moth of *Chilo partellus* lays eggs mostly on the leave blades of maize and sorghum. The newly emerged larvae, after wondering around, finally enter into the whorls of young maize and sorghum plants. Once in the whorls, they feed on leaves for a while and cause a characteristic pattern of small holes and scarification of leaf epidermis. Later on, they feed on the growing stem of the young plants resulting in dead hearts. After third moult, they bore into the stem and start tunnelling. The fully mature larvae pupate inside the plant tissue. Depending on the prevailing temperature and relative humidity the incubation, larval and pupal period vary considerably from 2-9, 15- 41 and 2-12 days, respectively. A

complete generation takes 4-7 weeks. In Kenya oviposition by *Chilo partellus* starts 2-3 weeks after germination and peaks 2-3 weeks latter (Alighali, 1985).

1.1.3 Control of the pest:

Several parasites and predators are known to suppress pest density; however, there use in *Chilo partellus* control has not been fully successful. Ploughing up and destroying the stubble after harvesting is strongly recommended. Early planting with a high seed rate and removal of affected plants is advantageous (Seshu Reddy, 1981). Chemical control with Endosulfan (II) or Carboryl (III) is fairly effective but is not environmental friendly.

Chilo species are particularly difficult to control largely because of the cryptic and nocturnal habits of the adult moths and the protection offered by the stem or cob of the host crop to the developing stages (Sigh and Raina, 1989). Insecticidal measures have proved difficult to apply effectively. In the case of sugar cane, high crop density makes access for ground spaying almost impossible, while in rice cultivation insecticide application is detrimental to beneficial insects and can cause serious pollution of water ways and fish (Beevor *et al.* ,1990). Other alternative control measures that would overcome the drawbacks of insecticides are therefore required.



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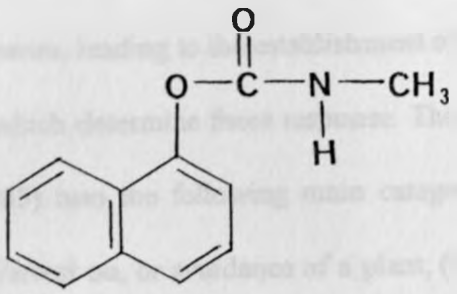


(III)

The use of sex pheromones is being tried as one of the environmental friendly measures of chilo control (Beevor, *et al.*,1990; *Ibid.* 1977; and Unnithan and Saxena 1991). Feeding and oviposition deterrents would be desirable when environmental factors are considered; moreover, feeding and oviposition deterrents and stimulants from plants or crops provide knowledge about the interrelationship between insect pests and plants. This in turn gives possibilities for developing other methods of crop protection by breeding crops of insect resistant varieties.

1.2.0 The Host - Pest relationship:

The survival and development of a pest, and consequently, the damage on the plant (crop) is directly related to the interaction between the insect pest and the host plant; thus the expression of cultivar resistance and/or insect preference for feeding, oviposition and orientation are typical of the pest-host relationship.

Various plant cultivars differ in their levels of susceptibility or resistance to the insect pest (Jotwani et al, 1987; Jotwani and Davis, 1980; Lal and Pant, 1980; Jotwani, 1981; Singh *et al.*, 1983; Dabrowski and Kidiavai, 1983). The factors that determine the level of resistance

/ susceptibility have been arranged into two broad categories by Saxena (1969 *ibid.* 1985): (1) the insect colonizing responses, leading to the establishment of it's population on the plant and (2) the plant characters which determine these response. The colonizing responses were distinguished by Saxena (1985) into the following main categories; (1) orientation of the insect determining it's arrival/arrest on, or avoidance of a plant, (2) feeding, (3) utilization of ingested food determining the insect's nutrition, (4) Development of the larvae, (5) egg production (fecundity) in the adult and (6) oviposition.

The lower the insects response in each of these categories to a cultivar, the greater will be the plant's resistance and thus, the less the insect's preference for that cultivar. Ideally these lead to reducing the insect's three major behavioral responses:- oviposition orientation and/or feeding.

1.2.1 Factors that influence oviposition and feeding of *chilo partellus*.

There are possibly many factors that influence the oviposition and feeding of *Chilo partellus* and other plant pests on the maize and sorghum cultivars, however, these can be categorized into two factors, namely;

(a) Bio-physical properties of the host plant:

Ampofo (1985) reported that the choice of oviposition site is probably influenced by the leaf surface trichomes, thus the lower leaf surface and the mid-rib concavity (the smooth areas of the plant) were noted to be preferred for oviposition with up to 92% preference for 3-4 weeks old maize plants. Ampofo suggested that tactile stimuli due to increased trichome density on the leaf surface, probably combined with other factors may be an important factor on *Chilo partellus* selection of oviposition site and orientation on the host plant. Kumar and

Saxena (1985) reported that the *Chilo partellus* moth prefers glass, polythene sheet, wax paper then filter paper in this order for the ovipositing site, while Raina (1982) reported that sorghum sootfly (*Atherigona soccata*) prefer green colour to white and a surface with ridges to a smooth substrate for oviposition.

(b) Bio-chemical properties of the host plant:

Kira *et al.* (1968) noted considerable increase in *Ostrina nubilalis* (hubner) egg production when moths were provided drinking water while Derridj and Fiala (1983) observed a direct relationship between the concentration of soluble sugars in the foliage of different maize hybrids and the number of *O. nubilalis* egg masses laid on them. Differences in glucose and/or fructose concentrations were implicated in the selection of site by *O. nubilalis.* Ampofo (1985) observed that plant exudates from ICZ2-CM maize lines significantly increased oviposition above that of distilled water while exudates from INBRED A and especially ICZ1-CM depressed oviposition. The exudates were observed to be of different chemical composition which therefore affected *Chilo partellus* oviposition and longevity differently. It has been shown that feeding by third instar larvae of *Chilo partellus* on sorghum bicolor is mediated by several surface and tissue chemicals (Torto *et al.*, 1990, *ibid.* 1991).

Considering the feeding response , for example, insect feeding involves various behavioral responses which can be divided into four distinct steps (Dethier, 1966; Munakata, 1977; Schoonhoven 1982; Miller and Sticker, 1984); (1) host plant recognition and orientation, comprising locomotion which brings the insect to it's food and cessation of locomotion on arrival, also termed as arrest; (2) initiation of feeding (biting, probing or sucking); (3) continuation of feeding and (4) termination of feeding as a result of satiation.

It has been suggested that the acceptance or rejection of a plant as food by an insect is determined by a number of factors which include the intensity of olfactory and gustatory

feeding stimulants, the intensity of repellents and deterrents, the metabolic state of the insect and learning acquired as a result of previous feeding experience (Dethier, 1982).

Electrophysiological studies at the sensory level show that chemoreceptors are the means by which the insect detects plant chemicals and is able to differentiate between a stimulant and a deterrent compound thus insects are said to respond to a summation of inputs (gestalt) from various chemoreceptor which perceive complex mixtures of compounds present in their food (Schoonhoven and Jermy, 1977; Dethier, 1980; Stadler, 1982).

1.2.2 Host plant resistance:

The seriousness of the damage caused by *Chilo partellus* larvae and other insect pests on host plants and their effect on grain or crop yield has prompted research concentrated on screening experiments with a view to identifying and developing resistant varieties. Efforts to identify sources of resistance to *Chilo partellus* and to incorporate the resistance into agronomically acceptable maize and sorghum cultivars are in progress in many research institutions.

Four factors known to affect the expression and stability of resistance of a plant cultivar are genetic, environmental, biochemical and morphological factors (Sigh, 1978, Ampofo, and Nyangiri, 1986).

It is the biochemical factors, however that are of interest to this research. Every green plant is inherently resistant to some phytophagous insect. This is to a certain extent attributed to the chemicals present in the plant. Therefore plants and especially resistant cultivars may develop defence chemistry to enable them to survive in a given habitat.

The chemical resistant factor in a plant (allelochemicals) to a large extent affect the behavioral and metabolic processes of the insect pest. Several chemicals have been identified which are essential or prohibitive to the development of the insect pest (Smith *et al.*,1979; Sagawa and Pathak, ; Torto *et al.*, 1990; Woodhead and Bernays, 1987). The improper balance of these allelochemicals in a cultivar can change the susceptibility level of the cultivar (Auclair *et al.*, 1957; Maxwel *et al.*, 1976; Maltais, 1951; Ampofo and Nyangiri, 1986).

1.2.3 Mechanisms of host plant resistance:

While it is clear that certain plant cultivars possess specific morphological and chemical characteristics which can be advantageously used for insect control, the mechanisms of resistance are generally complex and possibly interrelated (Jotwani, 1981; Painter, 1951).

Observed mechanisms include;

(i) Antibiosis; affecting insect establishment, survival, development and egg production on host plants.

(ii) Preference/non-preference for oviposition, feeding and shelter (Painter, 1958) also referred to as antoxenosis by Kogen and Ortman (1978).

(iii) Tolerance in plants involving repair regeneration of their damaged tissues (Kalode and Pant, 1966; Jatwani, 1981).

1.3.0 Allelochemicals:

Plant allelochemicals are secondary plant products that have a significant effect to the organism or plant of a species different from its source. Allelochemicals could be subdivided into four classes according to the effect on the receiving and the emitting species, where the allelochemical is the substance produced or acquired by the plant that when it contacts another species in the natural context thereby evokes in the receiver the response shown (see table 1.0 below).

Table 1.0 Classes of allelochemicals.

Allelochemical	Evoked physiological reaction
i) Kairomone	adaptively favourable to the receiver but not to the emitter.
ii) Allomone	adaptively favourable to the emitter but not to the receiver.
iii) Synomone	adaptively favourable to both the emitter and the receiver.
iv) Apneumone	adaptively favourable to the receiving organism but detrimental to an organism of another species that may be found in or on the non living material.

1.3.1 The chemistry of allelochemicals:

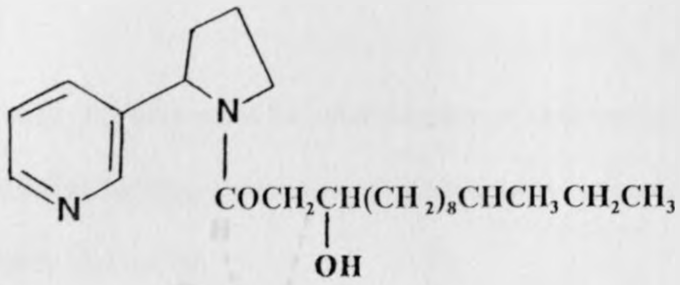
Allelochemicals belong to a few major classes of chemical compounds. These include terpenoids, phenolic compounds, phenylpropane derivatives, flavonoids, long chain cyanides,

alkaloids, purines and steroids. However, simple chemical compounds such as alkanes, alcohols, aldehydes and fatty acids have also been reported to show activity as allelochemicals. Examples of reported allelochemicals are listed in table 1.1 below.

Table 1.1 Examples of reported allelochemicals.

Allelochemical and chemical class:	Source and reported activity:	Author/reference
Phenolic compounds;		
p-Hydroxybenzoic acids	S. bicolor; feeding stimulant for *C. partellus.*	*Torto et al.* 1990.
p-Hydroxybenzaldehyde	S . bicolor; *C. partellus* feeding stimulant, *l. nubilalis* feeding inhibitor.	Torto *et al.*, 1990; Woodhead, 1982.
Phenolic acids	S.bicolor as non-deterrent esters but released as acids deterring *l. migratoria* feeding.	Woodhead and Bernays 1978.
Alkaloids and nitrogen compounds;	*N. rapenda* plant; toxic to tobacco horn worm (*manducasexta*).	Huesing and Jones 1987.
N-acyl analog of nicotine (IV)	Laguminous pasture plant (*l.pendunculatus*) roots and foliage; feeding deterrent of grass-grab (*Cosalytra zealandica*).	Granderam and Sutherland 1986.
Non-protein analogue of phenylalanine and tyrosine *eg.* Hiptagin (V).		

Allelochemical and chemical class:	Source and reported activity:	Author /reference
Tannins	American deciduous tree defence against insect feeding.	Barbosa and Krischcha 1987.
Terpenoids		
Momordicine II (VI)	Cucurbit (*M. charantia*) reduces feeding of red pumpkin beetle (*Aulaephora foveccollis)*	Chandravadana 1987.
Tenulin (VII)	Bitter weed (*H. amarum*) Antifeedant and reduces growth and development of European corn borer *O. nubilalis*	Broughtton *et al.* 1986
Flavonoids		
Luteolin-7-O-(6-O- melanylglucoside) (VIII)	Carrot leaf; oviposition stimulant to swallowtail moth *(P. polyxenes*).	Feeny 1988.
Hydrocarbons	S. bicolor; cultivar resistance to stem borers.	Woodhead and Janeja 1987.
32-carbon chain hydrocarbon.	Rice cultivar IR-22 Resistance to Brown plant hopper *(N. lugens)*	Woodhead and Padgham 1988.
Short chain hydro-carbons C_{10} to C_{18}.	1). S. bicolor; feeding deterrents for *L. migratoria*	Woodhead 1983.
Alcohols, aldehydes and fatty acids.	2). Rice; resistance to *N. lugens.*	Woodhead and Padgham 1988.



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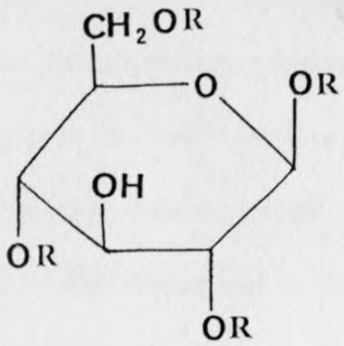
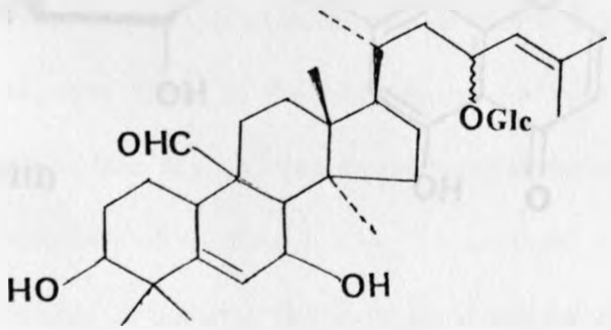


R = $COCH_2CH_2NO_2$

(V)



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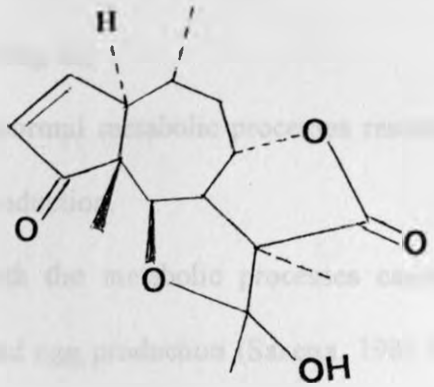

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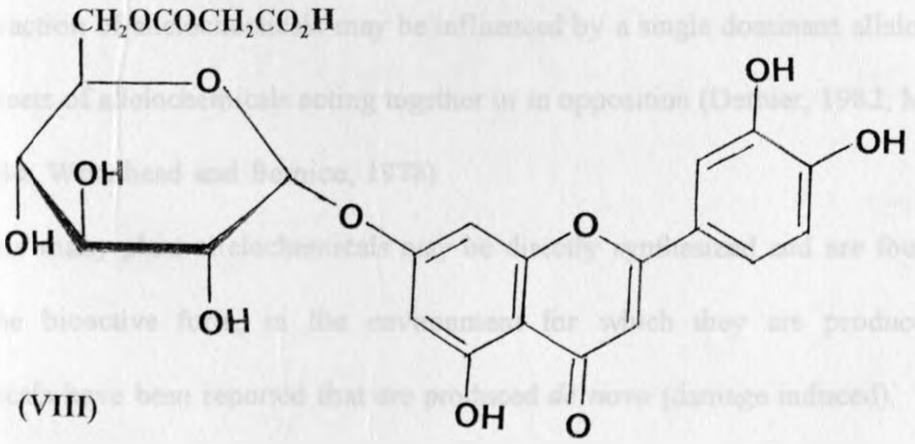


(VIII)

1.3.2 Bioactivity of allelochemicals:

Plant allelochemicals can determine the establishment of an insect species on the plant by determining its orientation, feeding and/or oviposition behaviour towards the plant and/or influencing the metabolism serving as;

a) Nutrients promoting normal metabolic processes resulting in the insects survival, development and egg production.

b) toxins interfering with the metabolic processes causing failure of the insect's survival, development and egg production (Saxena, 1985 *ibid.* 1987).

The allelochemical may provide olfactory stimuli perceivable at a distance from the plant or contact stimuli perceivable after the insect's arrival on the plant. The orientation of the insect can be determined by the olfactory stimuli whereas its feeding and oviposition responses can be determined by the contact and to some extent by the olfactory stimuli (Dethier, 1947; Thorseinson, 1966; Norris and Kogen, 1980; Pathak and Dole, 1983).

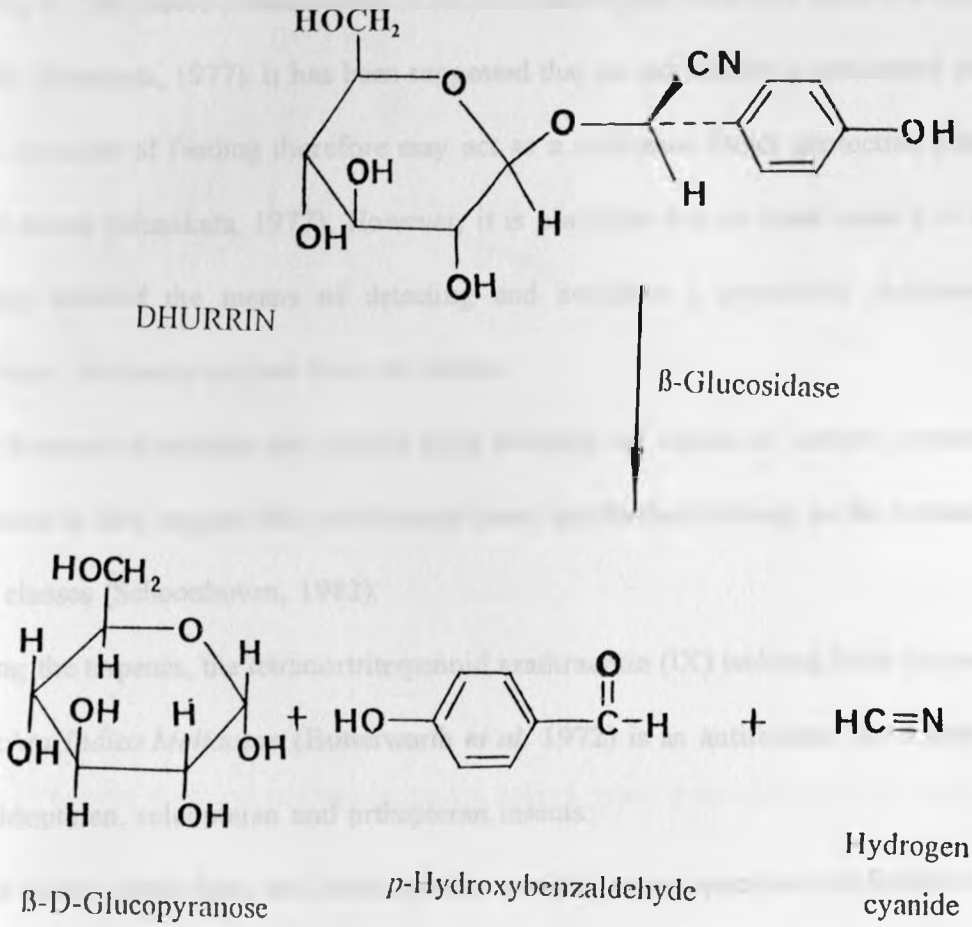
The action of allelochemicals may be influenced by a single dominant allelochemical or different sets of allelochemicals acting together or in opposition (Dethier, 1982; Miller and Sticker, 1984; Woodhead and Bernice, 1978).

While many plant allelochemicals may be directly synthesized and are found in the plant in the bioactive form, in the environment for which they are produced, some allelochemicals have been reported that are produced *de novo* (damage induced).

In the resistance of sorghum bicolor, for example, to attack by *L. migratoria*, the relative unpalatability of seedling is the result of several deterrent chemicals which are produced only at the time of feeding. This is true of HCN, stored as the glucoside Dhurrin, and the phenolic acids, stored as esters. As a result of damage to the plant tissues these

substances are brought into contact with enzymes to produce the bioactive compounds though the substrate themselves are not bioactive (Woodhead and Bernice, 1978)

The enzymatic hydrolysis of Dhurrin to *p*-hydroxybenzaldehyde and HCN is stoichiometric as outlined below.



In maize, damage induced DIMBOA production protects maize against stalk borers (Cooper Driver and Wain, 1976; Bernice *et al.*, 1977).

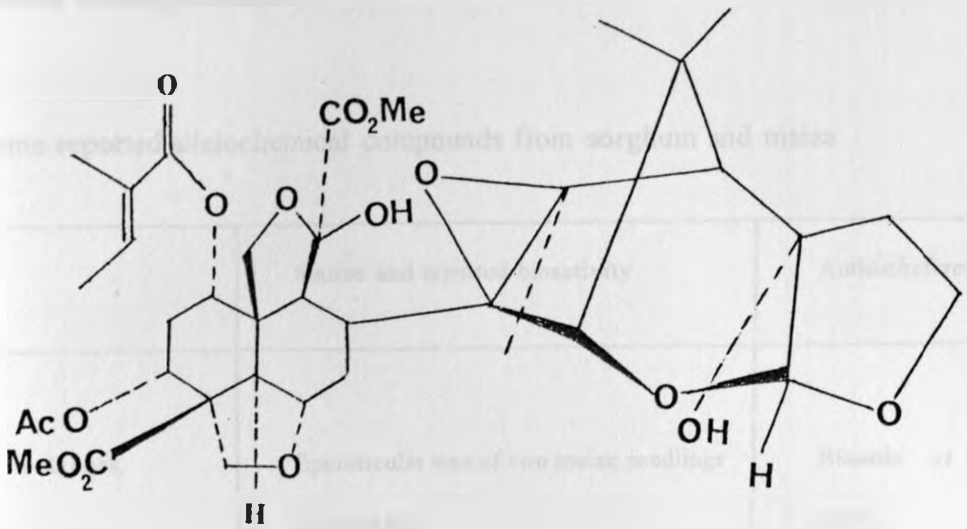
1.3.3 Insect feeding deterrents:

Plant chemicals which possess feeding deterrent activities against insects are known as antifeedants (Dethier, *et al.* 1966). They prevent or decrease the feeding of the insect without killing it. The insect remains near or on the treated plant and dies from starvation (Dethier, 1980; Munakata, 1977). It has been suggested that an antifeedant is concerned with deterring the initiation of feeding therefore may act as a resistance factor protecting plants against insect attack (Munakata, 1977). However, it is plausible that in some cases it is the insect that has evolved the means of detecting and avoiding a potentially deleterious compound. Many deterrents are also toxic to insects.

Plant deterrent chemicals are derived from virtually all classes of natural products. However studies to date suggest that most potent insect antifeedants belong to the terpenoid and alkaloid classes (Schoonhoven, 1982).

Among the terpenes, the tetranortriterpenoid azadirachtin (IX) isolated from the neem tree *Azadirachta Indica Meliaceae* (Butterworth *et al.* 1972) is an antifeedant for a host of different lepidopteran, coleopteran and orthopteran insects.

While many antifeedants are insect species specific, broad spectrum antifeedants are also known; apart from azadirachtin, the mustard glycoside sinigrin, the carbohydrate phlorizin, the flavonoid glycoside rutin, several quinones, napthoquinones and some ellagitaninnins have been found to be broad spectrum insect antifeedants (Schoonhoven, 1972; Bernays and Chapman, 1977; Hedin *et al.*, 1977; Norris, 1986; Jones and Klocke 1987).



(IX)

1.4.0 Previous work on maize, sorghum, insect pests, oviposition and feeding:

Extensive research has been done on sorghum and maize regarding their biology, cultivation and breeding for pest resistance on host plant-pest relationship as reflected by the references quoted; however, not much has been done on the biochemical aspects of the plants in relation to their insect pests, especially on the chemicals possibly involved the oviposition and feeding of *Chilo partellus*. Outlined below is some of the previous work.

1.4.1 Previous work on maize and sorghum:

Tabulated below (table 1.2) are some of the compounds and their reported activities that have been isolated from maize and sorghum but may not be necessarily involved in *C. partellus* oviposition and/or feeding bioactivity.

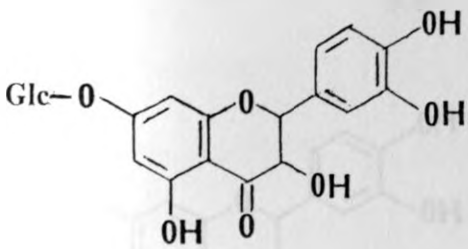
Table 1.2. Some reported allelochemical compounds from sorghum and maize.

Compound:	Source and reported bioactivity	Author/reference
C_{27},C_{29} and C_{31} n-alkanes, dotriacontanal, *n*-octacosanal, *n*-tricotanal, *n*-dotriacotanol, *n*-octaosol, *n*-tricontanol, several esterified primary alcohols and fatty acids.	Epicuticular wax of two maize seedlings gl2 and GL (no bioassay done)	Bianchi *et al.* 1975.
(z)-Hexenyl acetate, (z)-hexenol, cyclosalirene (sesquiterpene), x-ylangene,	Corn leaf volatile (no bioassay done)	Buttery and Lousa 1984.
2-heptanone, 4-hepten-2-one, and caryophyllene.	Corn leaf volatile (no bioassay done)	Buttery and Lousa 1984.

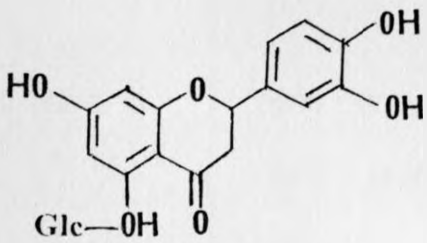
Compound:	Source and reported bioactivity	Author/reference
Toluene, hexanal, (*z*)-3-hexen-1-ol, *m*-xylene, *o*-xylene, (*z*)-3- hexen-1-ol acetate, nonanal and decanal.	S. bicolor seedlings (no bioassay done)	Lwande and Bentley 1987.
(+)-Taxifolin-7-o-B-glucoside,(Xa), eriodictyol-5-*o*-ß-glucoside (Xb), 4,2',4',6',-tetra-hydroxychalcone-4-o-ß-glucoside (Xc), 4-procyanidins (XIa - XId),	Sorghum grains	Rene *et al* 1986.
Flavan-4-ols	Sorghum grains, leaf, and tissue (of resistant cultivar to grain mold)	Ramamurthi *et al*. 1986.
ß-Caryophllene, longifolene, bazzanene, cyclosativene and x-ylangene (sesquiterpenes).	Volatile of cone roots (no bioassay done)	Buttery and Loisa, 1985.

Most of the above compounds vary in concentration with the cultivar of the plant and with the plant maturation (Buttery and Louisa, 1984; Woodhead, 1982). While most of the above compounds have not been tested for bioactivity, some of them could be playing a role in the insect pest behaviour.

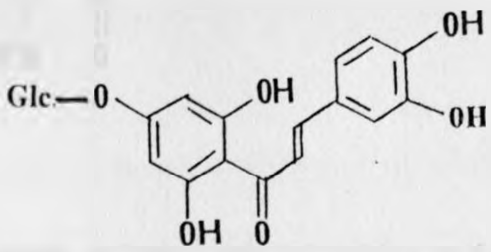
stu Xa - X1d



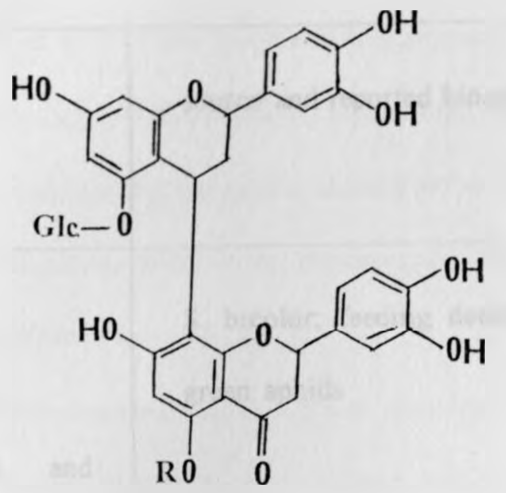
(Xa)



(Xb)

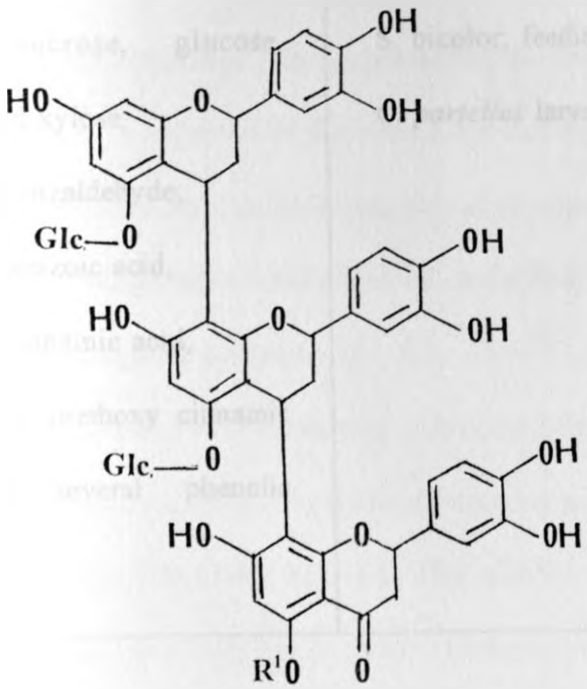


(Xc)



(XIa), R = H.

(XIb), R = Glucose.



(XIc), R^1 = H.

(XId), R^1 = Glucose.

1.4.2 Previous work on insect pest oviposition and feeding:

Besides the compounds reported elsewhere in this thesis, the following are some of the compounds that have been reported to have allelochemical effects on insect pest oviposition and/or feeding:

Table 1.3 Some sorghum feeding/oviposition allelochemicals.

Compound	source and reported bioactivity	Author and reference
Dhurrin, *p*-Hydroxybenzaldehyde, *p*-hydroxybenzoic acid, and procyanidin (unidentified)	S. bicolor; feeding deterrents to green aphids	Dreyer *et al.* 1980.
Sugars; sucrose, glucose, fructose and xylose, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, *p*-hydroxycinnamic acid, *p*-hydroxy-*m*-methoxy cinnamic acid and several phenolic analogs.	S. bicolor; feeding stimulants to *C. partellus* larvae.	Torto *et al.* 1990 *ibid.* 1991.

Raina (1981) reported oviposition deterrence of sorghum shootfly (*Atherigonia soccata*) by what he associated to an unidentified deterrent pheromone in the water soluble glue with which females attach their eggs to the leaves. Under low population density conditions, the females lay one egg per sorghum plant, hence repeated oviposition is deterred by the suggested pheromone.

Unnithan *et al.* (1987) and Saxena (1990) reported that acetone extracts of resistant, susceptible and wild sorghum elicited high ovipositional response to shootfly on maize, a non host plant, therefore suggesting a potential method for reducing the population of the pest by diverting oviposition to non host plants. However, the oviposition stimulants in the extract have not yet been identified.

Kumar (1986) reported that the maize plants infested with *Chilo partellus* larvae elicited more oviposition than the uninfested plants. Ampofo (1985) and Ampofo and Nyangiri (1986) reported differing oviposition among maize genotypes with INBRED A eliciting maximum oviposition. They suggested that certain conditioning stimuli were central to larvae acceptance or rejection of the plant on which eggs were laid. Their analysis indicated that different chemical characteristics played a certain role in larvae acceptance of the plant. Kumar (1985) made similar observations on the ovipositional responses to the susceptible and resistant sorghum cultivars.

Saxena (1987) employing techniques specially developed to test the role of cultivars in oviposition reported remarkable differences in oviposition of the susceptible, IS 18363, and the resistant, IS 1044, cultivars. The number of eggs laid as well as the ovipositional preference were high for IS 18363, while the number of eggs as well as the ovipositional preference were low and medium respectively for IS 1044. Other workers (I.C.I.P.E annual report 1988) show that *n*-hexane and/or acetone extracts of susceptible and resistant cultivars of sorghum (IS 18363, IS 1044) exhibit deferent levels of oviposition stimulation and/or inhibition.

1.5 Objectives of the study:

The review of the feeding and ovipositional allelochemical work on the spotted borer *C. partellus* shows that no detailed and methodical study has been carried out to identify the chemicals from sorghum and maize which affect the oviposition and feeding, respectively, of the moth and larvae of this insect; Besides, the literature available on this subject suggests that not much work has been undertaken to demonstrate whether or not there are any allelochemical bases for oviposition and feeding preferences shown by the moths and larvae to different sorghum and maize cultivars .

The objectives of the study therefore are;

I). Isolation of allelochemicals of maize and sorghum through sequential solvent extraction of the leaves followed by fractionation of the extracts using chromatographic techniques and their structural elucidation using spectroscopic methods.

II). Carrying out feeding and oviposition bioactivity assays of the crude extracts and where possible of the isolated compounds against the stalk borer *C. partellus* moths and larvae.

Ultimately this study would enhance the knowledge on *C. partellus* oviposition, feeding and establishment on the host plants sorghum and maize. This would therefore lead to better methods of control of the pest; methods that would be environmental-friendly and both technologically and socio-economically feasible for the small scale resource poor farmer.

CHAPTER 2:

2.0. RESULTS AND DISCUSSION:

The results of this work are outlined below. The differences between the resistant cultivars and the susceptible cultivars of both maize and sorghum are clearly depicted. Consequently an attempt is made to use the observed cultivar chemical differences to explain the observed biological behaviour of the *Chilo partellus* pest on the host crops. As reflected by the quotation below, this results serve to demonstrate the important linkage between chemistry and biology in the natural world.

"Much of life can be understood in rational terms if expressed in the language of chemistry. It is an international language, a language for all time, a language that explains where We have come from, what We are and where the physical world will allow us to go. Chemical language has great aesthetic beauty and links the physical sciences to the biological sciences. Unfortunately, the full use of this language to understand life processes is hindered by a gulf that separates chemistry from biology. The gulf is not nearly so wide as the one between humanities and sciences. Yet chemistry and biology are two distinct cultures and the rift between them is serious, generally unappreciated and counterproductive....." (*Nobel Laureate Arthur Kornberg on Chemistry and Biology*).

The yields of the extracts from the cultivar whorls and the oviposition and feeding bioactivity of the crude extracts and some of the isolated and characterized compounds are reported. The characterized compounds include a series of *n*-alkanes of carbon chain length C_{10} to C_{18}, three phenolic compounds, one methyl-ester and one heterocyclic compound.

2.1 Yields of extracts from the sorghum and maize whorls:

Table 2.1 Yields of the extracts.

Cultivar	Plant material		Weight of extract			Total Yield
	Fresh wt gms	Dry wt. gms	Pet Ether mgs.	EtAc. mgs.	MeOH. mgs.	mgs.
	Sorghum season I (28th June 1992):					
IS1044	443.01	49.1	422.03	451.63	2217.64	3091.3
IS18520	551.03	56.1	233.1	613.79	3023.52	3870.41
IS18363	482.83	9.3	279.24	385.18	2694.64	3359.06
	Sorghum season II (8th September 1993):					
IS1044	750.23	63.2	795.42	756.42	3714.27	5266.11
IS18520	459.68	46.8	489.83	869.10	4281.83	5640.76
IS18363	357.47	36.5	471.05	693.50	4853.61	6018.16
	Maize season I (June 1991):					
MP 704	674	71.22	214.6	136.0	4895.2	5245.8
INBRED A	800	80.36	382.0	219.9	15000.0	15601.9
	Maize season II (28th June 1992):					
MP 704	335.97	35.5	214.64	354.39	4286.25	4855.28
INBRED A	722.72	72.6	318.71	563.31	8058.77	8940.79
	Maize season III (8th September 1993):					
MP 704	579.19	61.2	765,80	1124.6	10206.55	12096.95
INBRED A	706.79	71.0	707.94	922.01	9894.41	11524.36

Table 2.2 Average yield in mgs per gm of dry weight plant material (mg/g).

Cultivar	Pet-ether	Ethyl acetate	methanol	total
Sorghum.				
IS 1044	10.6	10.59	51.97	73.14
IS 18520	7.34	14.76	72.70	94.76
IS 18363	6.82	13.41	93.82	116.51
Maize.				
MP 704	7.19	10.09	118.75	136.03
INBRED A	6.37	7.83	145.67	159.87

The yields of the petroleum ether (P.E.), ethyl acetate (EtAc.) and methanol (MeOH) extracts were dependent on the cultivar, the season of planting and on the age at harvesting time (tables 2.1 & 2.2). In general the susceptible cultivars of both sorghum and maize gave higher yields of the total extracts compared to the resistant cultivars. This was generally true of both the ethyl acetate and methanol extracts for sorghum and methanol extract for maize, however for sorghum there was a remarkably higher yield of the petroleum ether extract from the resistant (IS 1044) cultivar compared to the susceptible (IS 18363), while for maize there was higher yield of the ethyl acetate extract from the resistant cultivar (MP 704) compared to the susceptible (INBRED A).

2.2 Sorghum; oviposition bioassay:

The relative suitability of the test extracts and samples was compared on the basis of the ovipositional preference (O.P) for the cultivar (extract) relative to the non-plant substrate (blank). The O.P. represented the percentage as 100(T-B)/(T+B), T and B being the number of eggs laid on the treated half disc and the blank half disc, respectively. Positive O.P values reflect attraction and/or contact stimulation while negative values reflect, avoidance and/or contact inhibition of the oviposition by the cultivar extract or sample. O.P values close to zero reflect lack of involvement of the plant extract or sample in oviposition.

Table 2.3 Percentage oviposition preference. (O.P ± Std Error)

Sample/Dose (µg/cm²)	10	20	40	80	160	320
IS 1044 P.E.	13.35	-22.32	-35.4	-31.31	-31.78	
	±11.88	±7.59	±10.22	±7.77	±11.14	
IS 18363 P.E.	16.31	-11.59	-5.82	-27.1	-29.04	
	±13.23	±15.17	±15.76	±10.39	±14.78	
IS 18520 P.E.	18.04	-18.81	-24.68	-30.02	-29.00	
	±12.94	±12.46	±17.85	±12.92	±12.12	
IS 1044 EtAc.		20.98	-8.51	1.42	-0.95	
		±11.82	±14.49	±16.55	±19.5	

Sample/Dose (µg/cm²)	10	20	40	80	160	320
IS 18363 EtAc.		16.96	21.81	-15.56		
		±13.86	±10.88	±13.87		
IS 1044 MeOH		-7.74	7.78	9.43	14.4	
		±11.57	±14.72	±8.58	±14.18	
IS 1044 P.E:-						
Remixed Fxn		-11.96	-16.85	-21.76		
		±10.21	±10.78	±12.02		
Fxn. I			-7.61	-2.72	9.66	29.71
			±18.61	±8.48	±14.57	±15.18
Fxn II.			18.0	22.07		
			±9.74	±12.55		
Fxn III.		-20.56	-24.56	-27.40		
		±13.31	±11.13	±11.31		
p-Hydroxy-	0.39	-1.34	16.97	8.06	-0.14	
benzaldehyde	±12.43	±9.57	±11.58	±12.25	±14.00	
p-Hydroxybenzoic acid	-3.83	-2.00	16.97	-4.32	6.03	
	±12.00	±11.98	±12.99	±9.48	±10.06	

Table 2.4 Percentage insects laying more than 1.5 times as many eggs on the blank as on the treated surface.

Sample/Dose (µg/cm²)	10	20	40	80	160	320
IS 1044 P.E.	50	45	60	75	75	
IS 18363 P.E.	16.7	42.86	35.7	66.6	68.75	
IS 18520 P.E.		33.3	50	58	58	
IS 1044 EtAc.	33.3	16.7	41.67	33.33	41.67	
IS 18363 EtAc.		14	21.43	41.67		
IS 1044 MeOH		33	30.77	23.1	25	
IS 1044 P.E:-						
Remixed Fxn		53.3	42.86	66.67		
Fxn. I			41.67	37.5	27.8	33
Fxn II.			25	25		
Fxn III.		50	55.56	60.14		
p-Hydroxy- benzaldehyde	33.3	33.3	22.2	27.27	333	
p-Hydroxybenzoic acid	30.77	35.71	27.78	47.37	27.78	

Table 2.5 Average number of eggs laid on the treated compared to the blank surfaces.

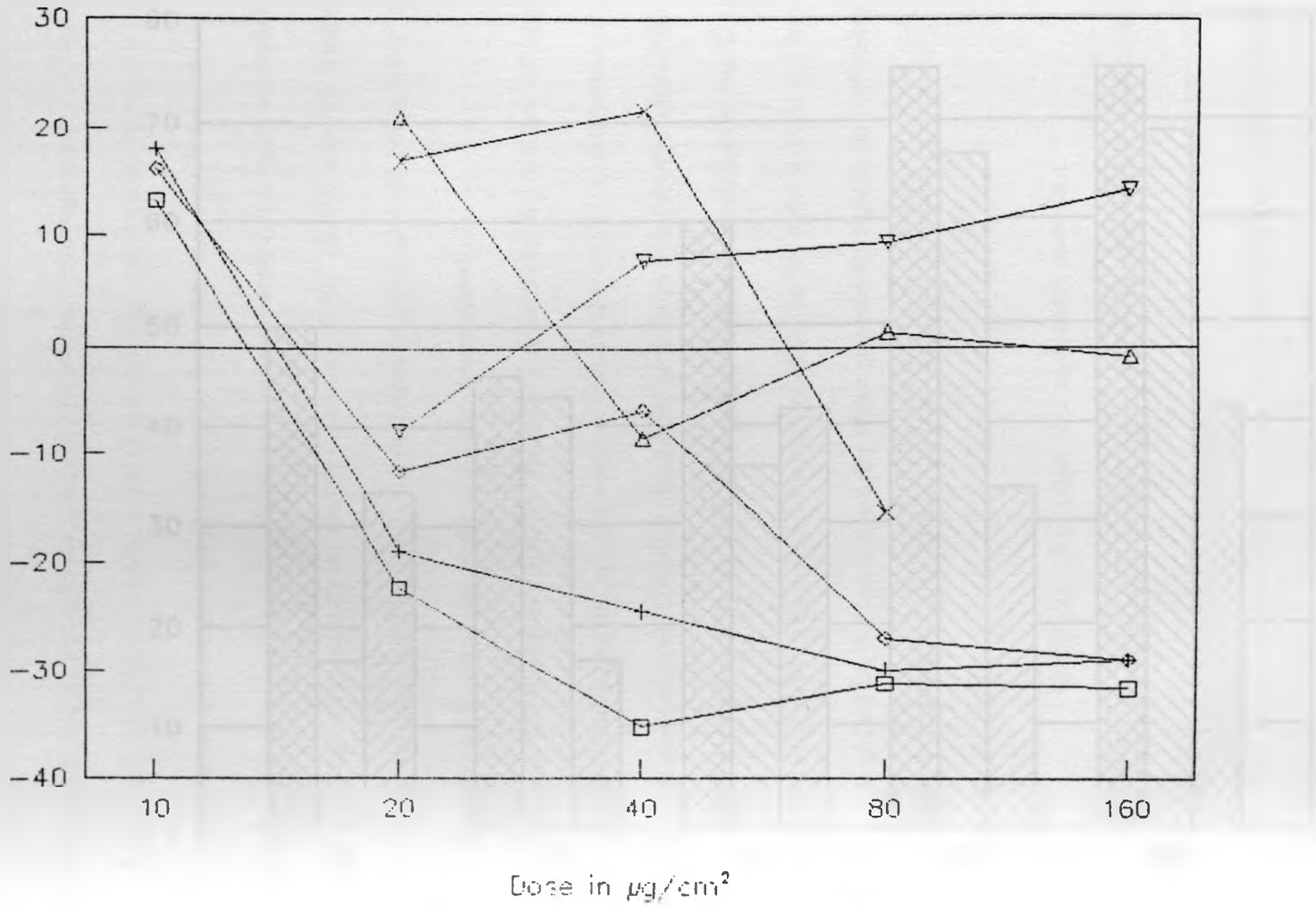
Sample/Dose (µg/cm²)	10	20	40	80	160	320
IS 1044 P.E.	101	105	76	91	83	
	±16.7	±15.5	±15.9	±13.6	±12.4	
Blank.	134	138	147	160	174	
	±12.21	±30.3	±18.1	±17.1	±25.9	
IS 18363 P.E.	120	127	85	106	77	
	±21.0	±22.2	±17.2	±17.1	±16.5	
Blank.	113	184	122	186	137	
	±25.4	±27.4	±27.4	±20.3	±19.9	
IS 18520 P.E.		97	77	87	77	
		±20.4	±19.1	±17.8	±11.0	
Blank.		133	144	158	154	
		±17.9	±28.8	±20.0	±21.4	
IS 1044 EtAc.	146	209	139	144	95	
	±20.0	±27.8	±26.9	±25.4	±24.8	
Blank	127	126	174	156	134	
	±25.6	±19.2	±23.5	±29.6	±27.6	
IS 18363 EtAc.		163	161	114		
		±23.3	±24.9	±24.8		
Blank		102	98	123		
		±15.6	±16.9	±17.3		

Sample/Dose (µg/cm²)	10	20	40	80	160	320
IS 1044 MeOH		120	141	195	174	
		±19.7	±19.7	±22.6	±24.0	
Blank		147	120	141	111	
		±21.1	±18.3	±16.4	±17.8	
IS 1044 P.E:-						
Remixed Fxn		139	122	124		
		±20.0	±19.1	±20.4		
Blank.		186	158	181		
		±24.4	±20.3	±23.0		
Fxn I			105	133	115	132
			±25.1	±14.0	±17.9	±19.2
Blank.			93	141	92	119
			±23.8	±13.7	±18.8	±27.1
Fxn II.			194	162		
			±26.6	±20.4		
Blank.			149	110		
			±32.4	±22.6		
Fxn III.		79	78	84		
		±15.0	±11.7	±14.9		
Blank		108	107	150		
		±18.8	±11.7	±18.5		

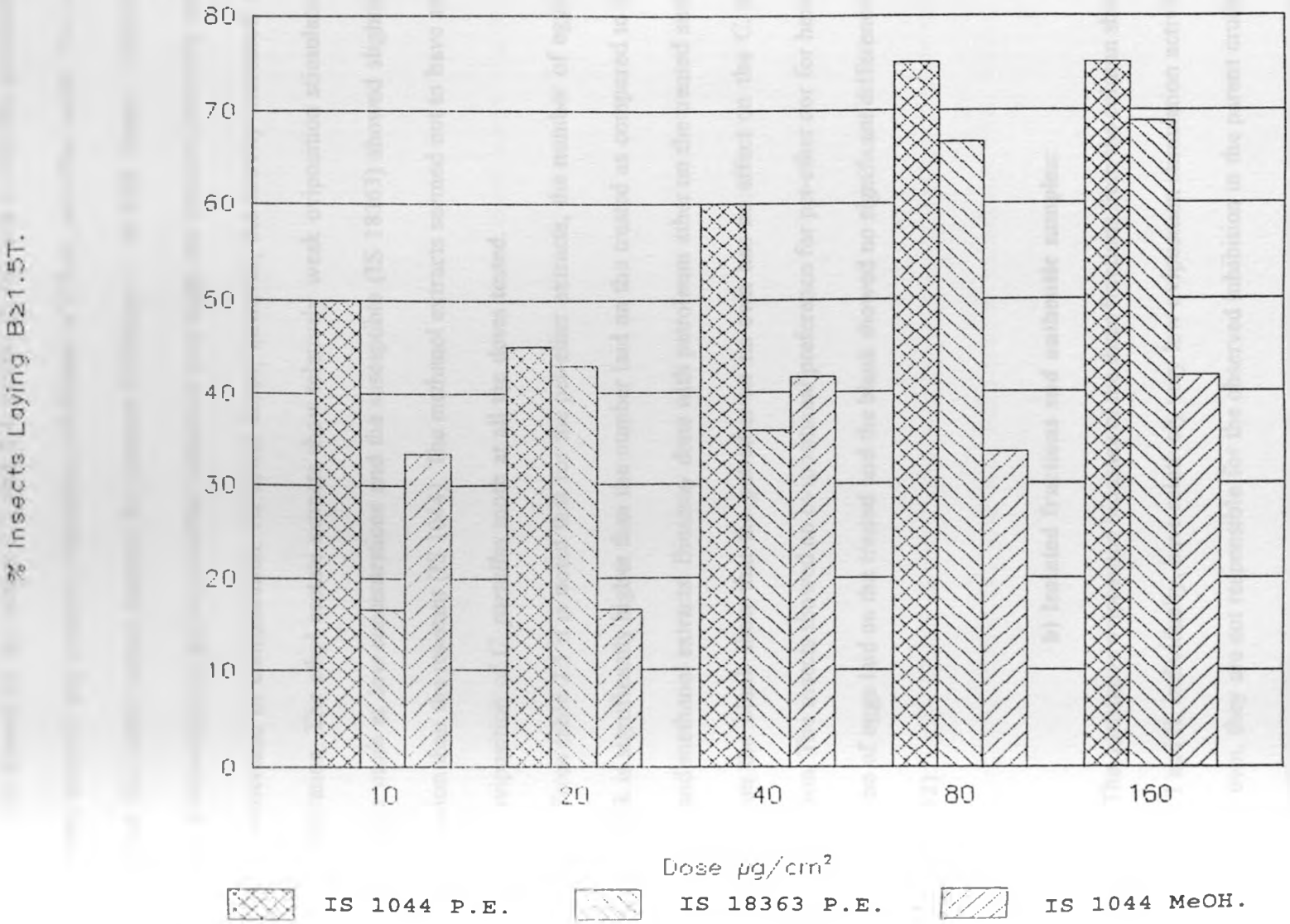
Sample/Dose (µg/cm²)	10	20	40	80	160	320
p-Hydroxy-	122	130	134	159	151	
benzaldehyde	±16.0	±17.4	±18.0	±22.7	±23.5	
Blank.	148	125	103	139	134	
	±23.1	±15.0	±18.9	±20.0	±22.4	
p-Hydroxybenzoic acid	148	152	144	116	119	
	±23.5	±21.5	±22.1	±14.9	±14.7	
Blank.	150	149	114	130	121	
	±19.4	±19.0	±23.8	±17.7	±16.7	

These oviposition bioassay results are summarized in the graphs 2.1a and 2.1b below.

Graph 2.1a Cultivar Oviposition Response (sorghum).



Graph 2.1b % Insects with O.P. ≤ -20.



a) bioassay of the crude extracts:

As shown on the tables 2.3 and 2.4, graphs (2.1a) and (2.1b) the petroleum ether extracts showed the greatest oviposition inhibition of *Chilo partellus* moth. The resistant cultivar (IS 1044) extract showed the greatest inhibition. At high doses ($80\mu l/cm^2$ and higher) the activity of all the pet-ether extracts is very high and remains relatively unaffected by the increase in concentration. At lower doses, however, the activity increased with the concentration. The ethyl acetate extracts show relatively weak oviposition stimulation of *C. partellus* moth at low concentrations and the susceptible (IS 18363) showed slightly higher stimulation than the resistant (IS 1044). The methanol extracts seemed not to have any effect on the oviposition of *C. partellus* moth at all the doses tested.

From table 2.5 it is noted that for the pet-ether extracts, the number of eggs laid on the blank is significantly higher than the number laid on the treated as compared to the ethyl acetate and methanol extracts. Bioassay done with petroleum ether on the treated surface and hexane on the blank show that the pet-ether on its own has no effect on the *C. partellus* oviposition. The insects showed no ovipositional preference for pet-ether nor for hexane. The average no of eggs laid on the treated and the blank showed no significant difference (T; 143 & B; 132).

b) Isolated fractions and authentic samples:

The qualitative oviposition bioassay of the three fractions from the column showed that fraction I and II, individually, have relatively very low oviposition stimulation activity. Thus, on their own, they are not responsible for the observed inhibition in the parent crude extract. Fraction III, however, showed relatively high oviposition inhibition though not as high as the parent crude extract (Tables 2.3, 2.4 and 2.5). Comparing the activity of the crude extract, the

column fraction III and the remixed fractions, it was noted that the bioactivity of the remixed fractions was lower than that of the crude extract and that of fraction III, however, higher than that of the ethyl acetate and methanol crude extracts.

p-Hydroxybenzaldehyde and *p*-hydroxybenzoic acid which have been isolated from the sorghum ethyl acetate extract (Torto *et al.* 1990) were assayed and they showed relatively weak oviposition stimulation at low doses but decreased in activity at higher doses.

2.3 Compounds identified from IS 1044 pet-ether extract:

Fraction I from the column was analyzed by GC-MS and identification was confirmed by co-injection in the GC with the authentic samples. A series of n-alkanes of C_{10} to C_{18} were characterized as outlined in table 2.6 below.

As would be expected, the GC profile (fig. 2.2a) shows the characteristic arrangement of the peaks at regular intervals which decrease slightly from one peak to the next one. The observed interval represents the CH_2 difference between successive alkanes.

The concentrations of the *n*-alkanes were notedly varying with the season of planting/harvesting. During season (I), pentadecane, hexadecane and heptadecane were most abundant while in season (II) dodecane, tridecane and tetradecane were most abundant (fig.2.2a).

Table 2.6 n-alkanes characterized from the pet-ether extract.

n-alkanes	GC Rt. min.	% comp. Season I	% comp. Season II	MASS-SPECT. m/e of M+ ion	% M+ abd.
Decane	9.17	0.233	2.046	142	10
Undecane	11.272	0.961	1.355	156	10
Dodecane	13.085	0.496	3.517	170	8
Tridecane	14.69	1.604	2.570	184	9
Tetradecane	16.156	4.621	4.391	198	8
Pentadecane	17.532	14.836	1.028	212	8
Hexadecane	18.823	34.46	0.239	226	8
Heptadecane	19.876	18.516	0.190	240	7
Octadecane	20.769	1.928	0.099	254	6

As observed from the mass spectra (fig. 2.2b table2.6 & table 2.7) the molecular ion peak (M+) of the *n*-alkanes is always present but decreases in intensity with the *n*-alkane chain length. The fragmentation pattern is characterized by clusters of peaks, and the corresponding peaks of each cluster are 14 (CH_2) mass units apart. The largest peak in each cluster represents a C_nH_{2n+1} fragment. This is accompanied by C_nH_{2n} and C_nH_{2n-1} fragments. The most abundant fragments are at C_3 and C_4 and the fragment abundance decreases in a smooth curve down to M-C_2H_5. The M-CH_3 peak is characteristically very weak or missing.

Table 2.7 Summary of the mass spectra fragmentation pattern of the *n*-alkanes and the relative abundance of the fragments.

n-alkane chain		C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}
Fragment (+).	m/e									
$CH_3(CH_2)_2$	43	98	96	80	80	80	78	84	76	74
$CH_3(CH_2)_3$	57	100	100	100	100	100	100	100	100	100
$CH_3(CH_2)_4$	71	21	23	58	61	62	70	83	75	70
$CH_3(CH_2)_5$	85	15	15	38	40	44	44	58	50	51
$CH_3(CH_2)_6$	99	5	4	8	11	12	16	20	19	19
$CH_3(CH_2)_7$	113	4	2	4	4	5	6	12	11	10
$CH_3(CH_2)_8$	127		1	2	2	3	3	4	4	6
$CH_3(CH_2)_9$	141			1	1	3	3	3	3	4
$CH_3(CH_2)_{10}$	155				1	2	2	3	3	3
$CH_3(CH_2)_{11}$	169					1	2	2	2	2
$CH_3(CH_2)_{12}$	183						1	2	2	2
$CH_3(CH_2)_{13}$	197							1	1	2
$CH_3(CH_2)_{14}$	211								1	1
$CH_3(CH_2)_{15}$	225									1

As observed and expected, n-alkanes containing more than eight carbon atoms show fairly similar spectra; hence identification depends on the molecular ion peak (Silverstein *et al.* 1981).

The presence of branched alkanes is ruled out from the observed spectra because although the spectra of branched chain hydrocarbons are grossly similar to those of the straight chain compounds, the smooth curve of the decreasing intensities is broken by preferred fragmentation at each branch.

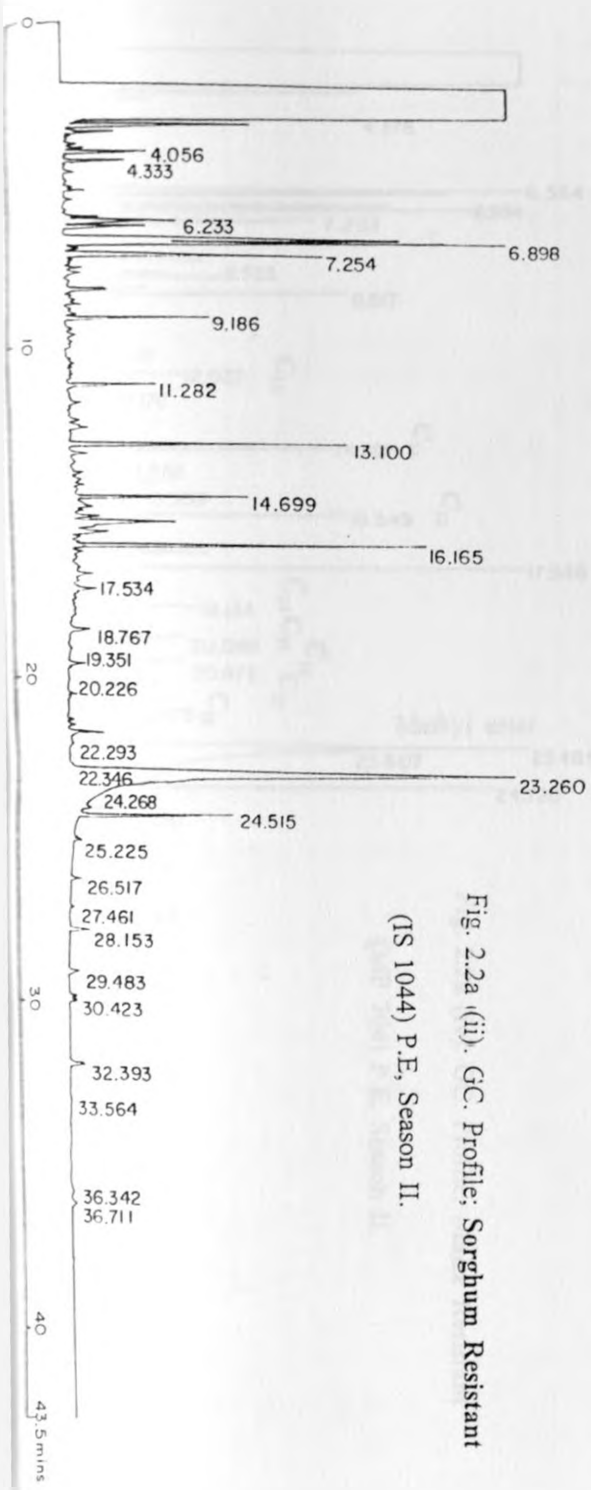


Fig. 2.2a (i). GC. Profile; **Sorghum Resistant** (IS 1044) P.E Season I.

Methyl ester.

Fig. 2.2a (ii). GC. Profile; **Sorghum Resistant** (IS 1044) P.E, Season II.

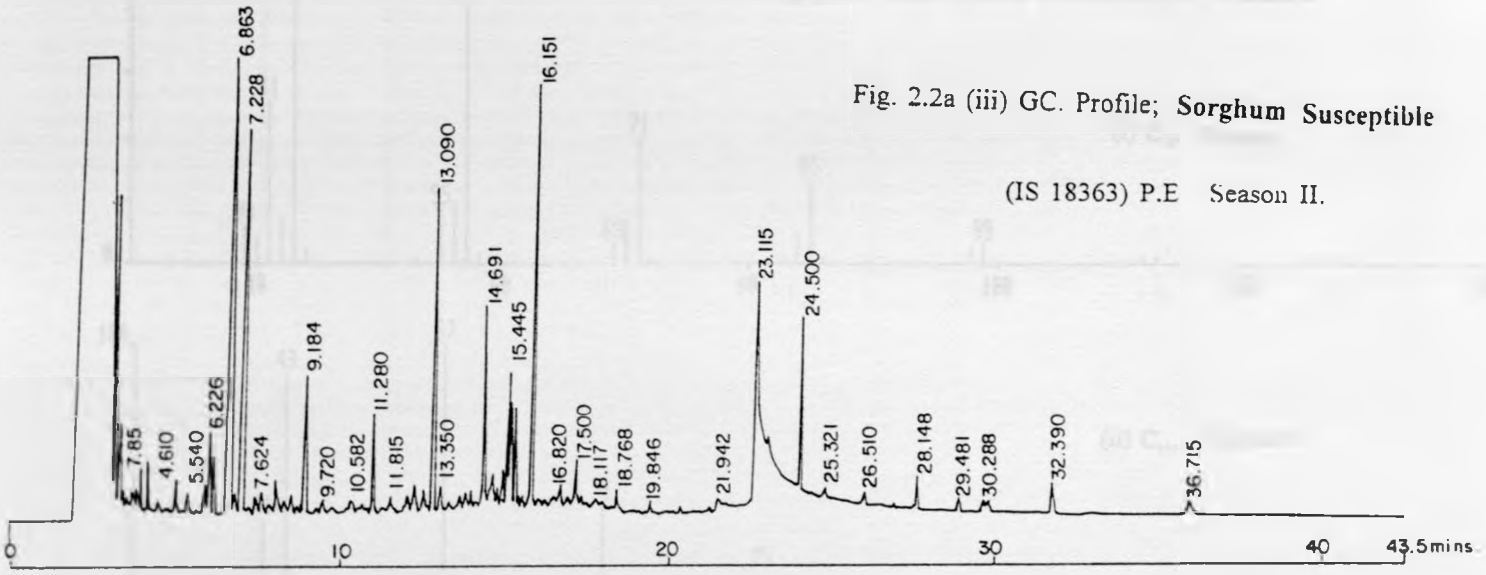


Fig. 2.2a (iii) GC. Profile; **Sorghum Susceptible**

(IS 18363) P.E Season II.

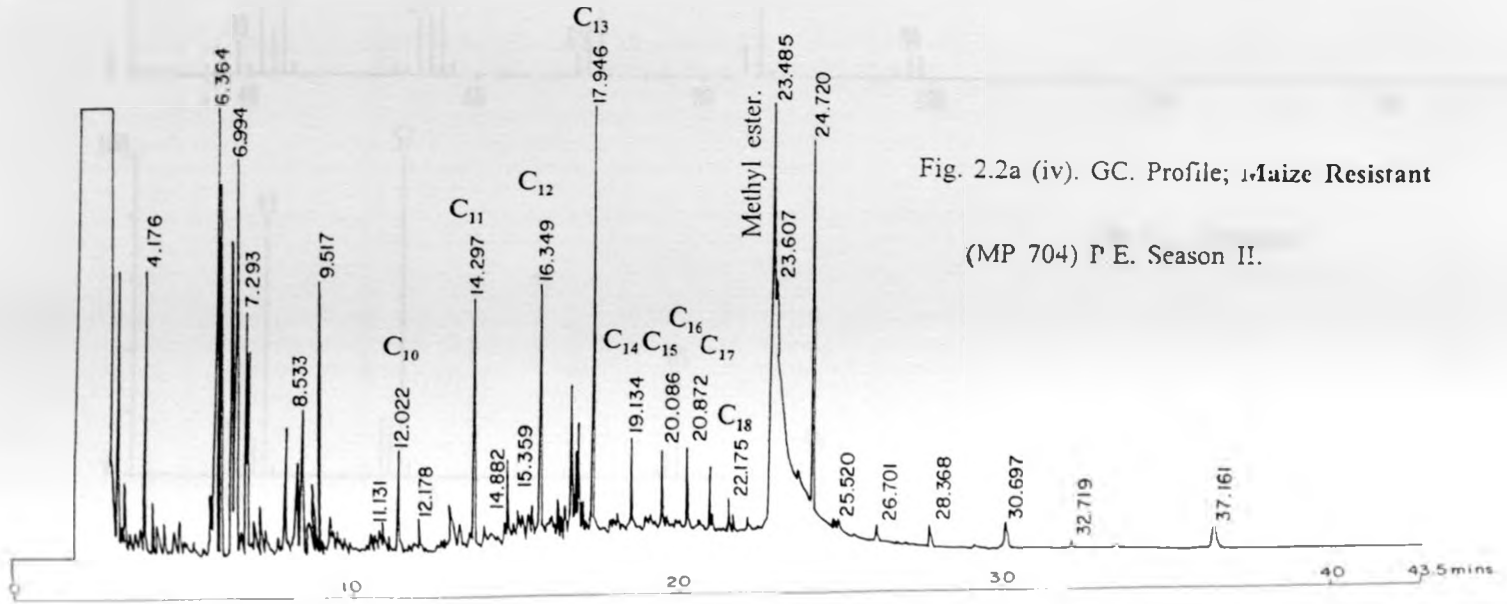


Fig. 2.2a (iv). GC. Profile; **Maize Resistant**

(MP 704) P.E. Season II.

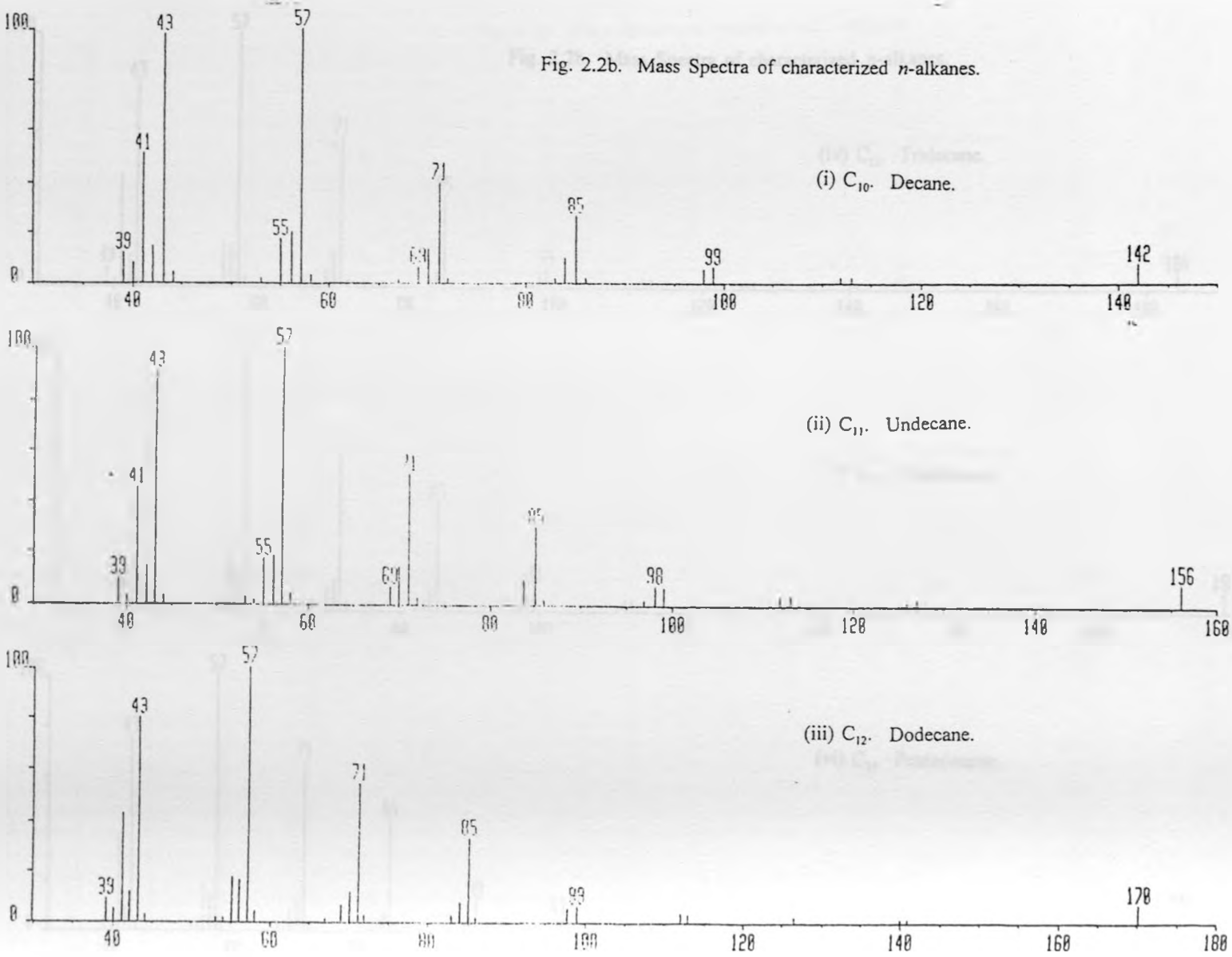


Fig. 2.2b. Mass Spectra of characterized *n*-alkanes.

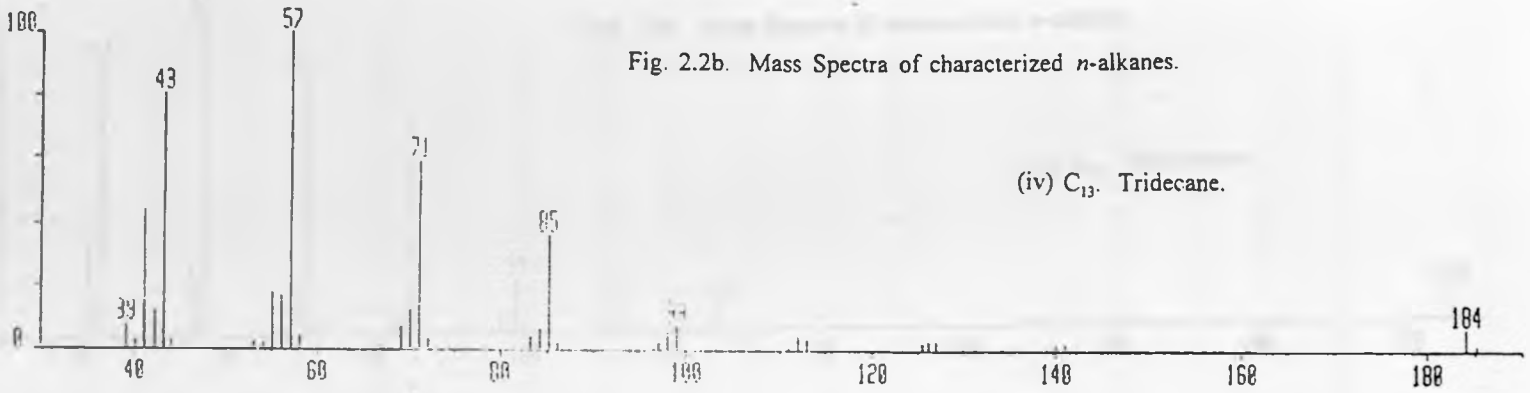
(i) C_{10}. Decane.

(ii) C_{11}. Undecane.

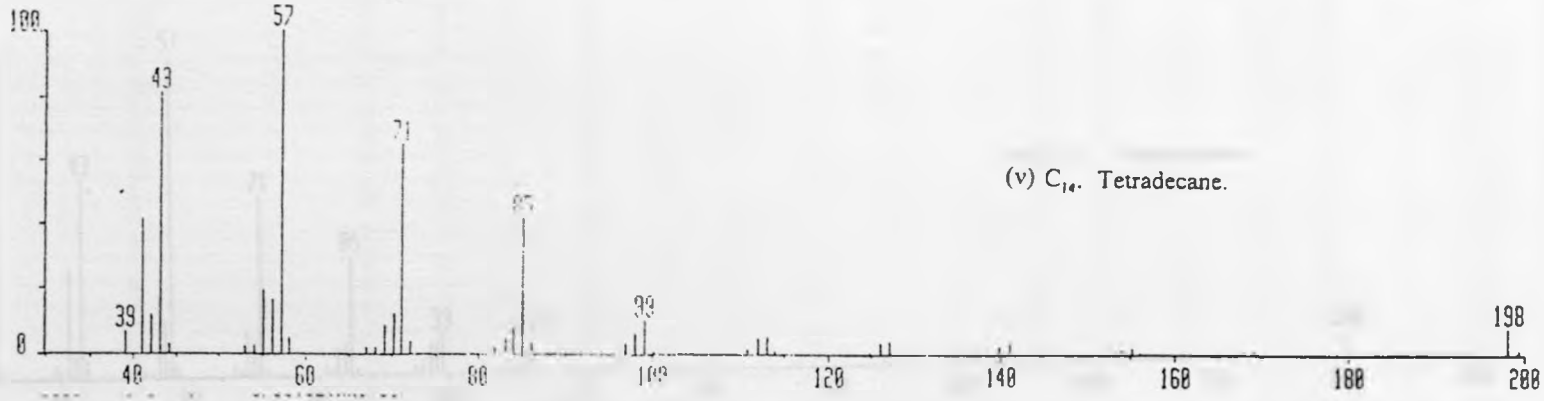
(iii) C_{12}. Dodecane.

Fig. 2.2b. Mass Spectra of characterized *n*-alkanes.

(iv) C_{13}. Tridecane.



(v) C_{14}. Tetradecane.



(vi) C_{15}. Pentadecane.

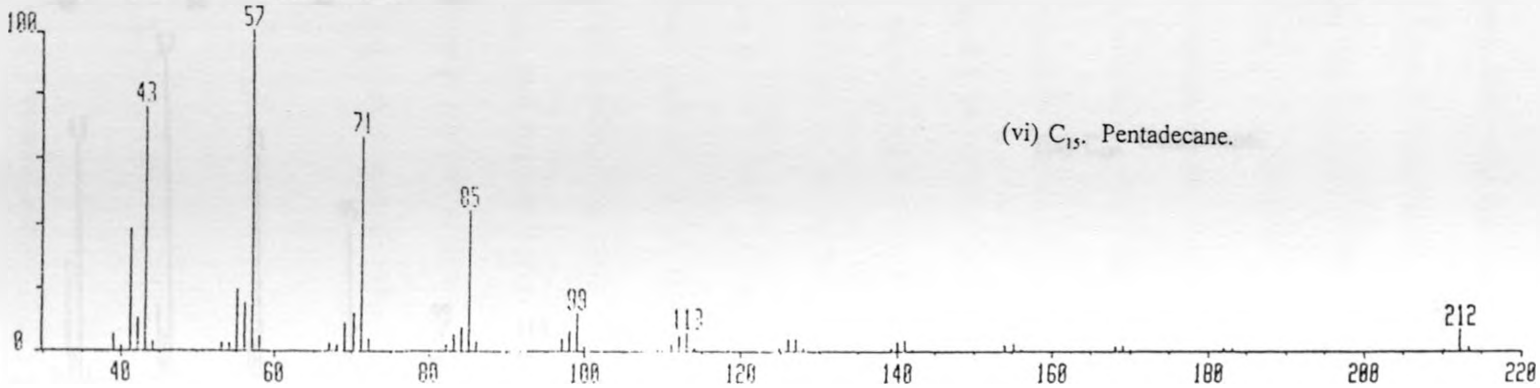
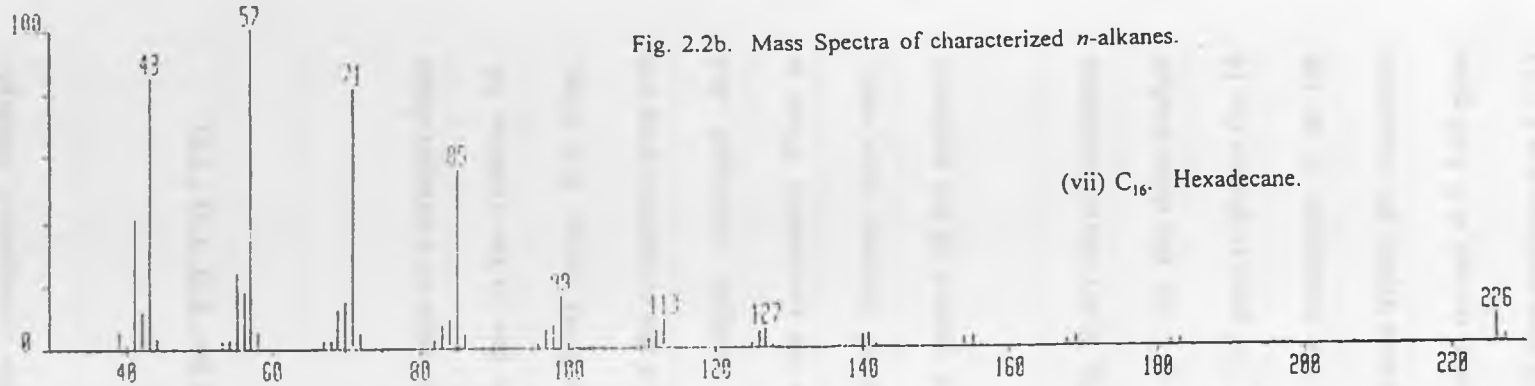
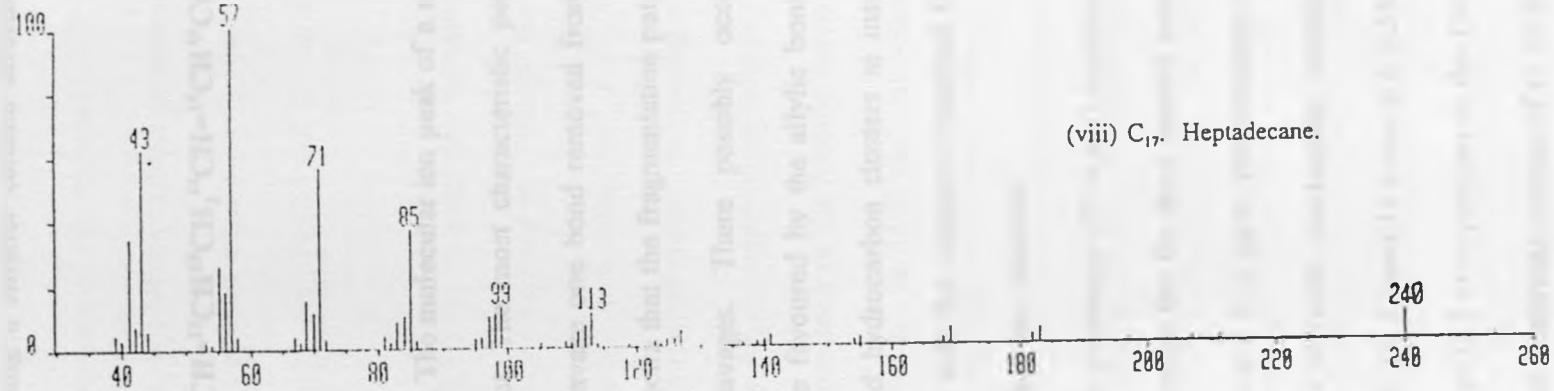


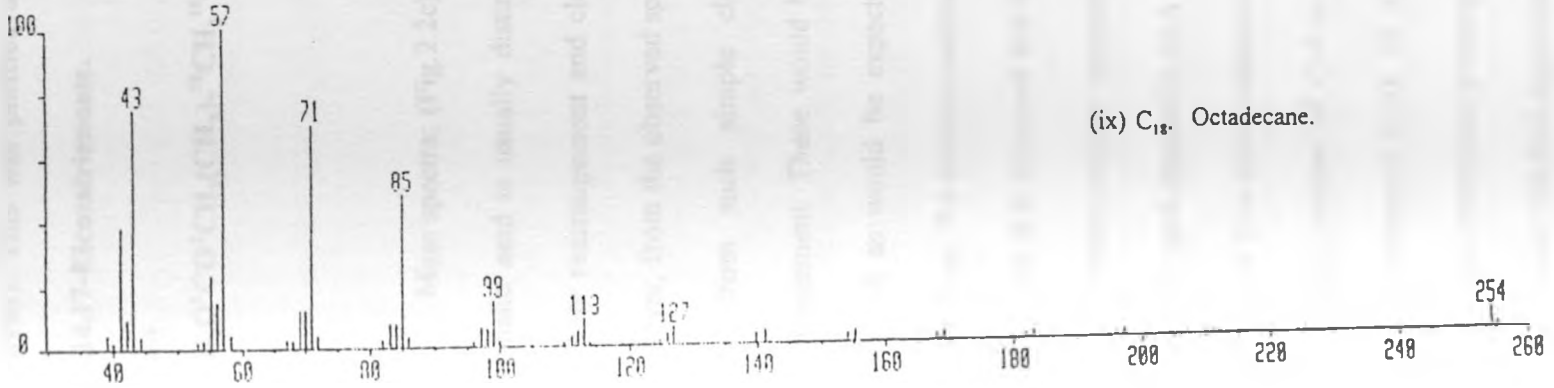
Fig. 2.2b. Mass Spectra of characterized *n*-alkanes.



(vii) C_{16}. Hexadecane.



(viii) C_{17}. Heptadecane.



(ix) C_{18}. Octadecane.

Fraction (III) from the column was predominantly one compound (over 90% by GC. analysis). This was purified using a smaller column affording the compound; **Methyl-11,14,17-Eicosatrienoate.**

structure

$^{1}CH_3O^{1}CO^{2}CH_2(CH_2)_7{}^{10}CH_2{}^{11}CH={}^{12}CH^{13}CH_2{}^{14}CH={}^{15}CH^{16}CH_2{}^{17}CH={}^{18}CH^{19}CH_2{}^{20}CH_3$

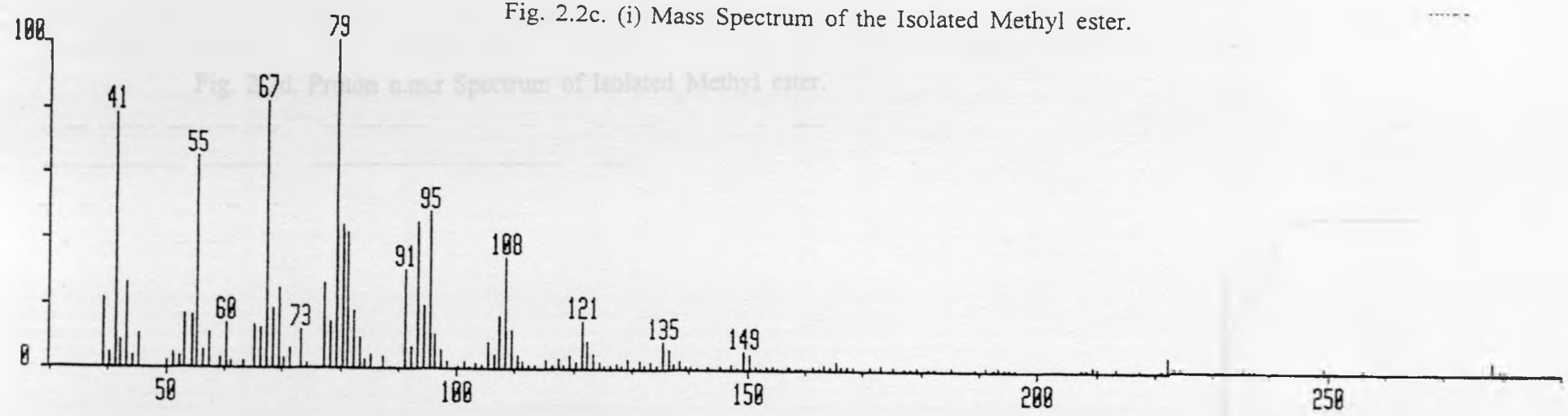
Mass spectra; (Fig.2.2c). The molecular ion peak of a methyl ester of a straight chain aliphatic acid is usually distinct. The most characteristic peak is due to the familiar *Mc Lafferty* rearrangement and cleavage one bond removed from the **C=O** group. It is clear, however, from the observed spectra that the fragmentation pattern of the compound does not arise from such simple cleavages. There possibly occurs multiple cleavages and rearrangement. These would be favoured by the allylic bonds in the compound. There is observed as would be expected hydrocarbon clusters at intervals of fourteen mass units. Comparing the sample spectra with the computer matched library spectra of the authentic compound it is observed that they are identical.

Proton nuclear magnetic resonance (^{1}H n.m.r) spectrum (Fig. 2.2d) shows resonance at δ 0.90 ppm appearing as a triplet for the three methyl protons of *C-20*, the three methyl protons of *C-a* show resonance at δ 1.30 ppm, the resonance at δ 1.35 ppm is due to the 14 methylene protons of *C-3 to C-9* (with overlapping coupling). The resonance of the six olefinic protons of *C-11, 12, 14, 15, 17 and 18* occur at δ 5.35 ppm as a triplet, the resonance of the two methylene protons of *C-2* at α-position to the C=O group occurs at δ 2.80 ppm as a tetralet. The four equivalent methylene protons of *C-13 & C-16* give resonance at δ 2.35 ppm as a triplet while the remaining four methylene protons of *C-10 and C-19* show resonance at δ 2.15 ppm as overlapping tetralet and pentatet.

^{13}C n.m.r spectrum (fig. 2.2e) was interpreted by comparing with published ^{13}C n.m.r spectral data (Silverstein, *et al.* 1981) and the assignment was as follows; the ester carbonyl carbon was confirmed by the presence of the resonance at δ 179.01 ppm . The presence of one methyl carbon δ 14.206 ppm, the olefinic carbons (methine) δ 127.212, 127.835, 128.333, 130.265 and 132.009 ppm; thus at least three olefinic bonds. The methylene carbons at δ 33.953, 29.592, 29.094, 27.256, 25.636, 24.731 and 20.588 ppm; at least seven methylene carbons.

Infra-red spectrum (fig. 2.2f) run by preparing the sample as a film cast, confirms the presence of the ester C=O by the strong band at 1710 cm^{-1} corresponding to C=O stretching. The olefinic C-H stretching appears as a weak band at 3224 cm^{-1}. The most characteristic vibrational mode of olefins are the out of plane C-H bending vibration between 1000 cm^{-1} and 650 cm^{-1} these bands are usually the strongest in the spectra of olefins. Therefore the strong band at 725 cm^{-1} confirms the presence of the olefinic bonds. The C=C stretching expected to occur in the region of 1680 cm^{-1} to 1630 cm^{-1} are usually weak and often absent in symmetrical disubstituted or tetrasubstituted trans olefins. This is true of this compound. Olefins which lack symmetry absorb more strongly than trans olefins and internal double bonds generally absorb more weakly than terminal double bonds because of pseudosymmetry. The band at 1260 cm^{-1} corresponds to the asymmetric stretching of the C-O-C bonds and is accompanied with the symmetric stretching at 1075 cm^{-1}. The bands at 2926 cm^{-1} and 2853 cm^{-1} correspond to the asymmetric and symmetric methylene C-H stretching. The weak bands at 1410 cm^{-1} and 1460 cm^{-1} correspond to the methyl C-H symmetric and asymmetric bending. The symmetric band is stronger than the asymmetric band as expected. (Silverstein *et al.* 1981).

Fig. 2.2c. (i) Mass Spectrum of the Isolated Methyl ester.



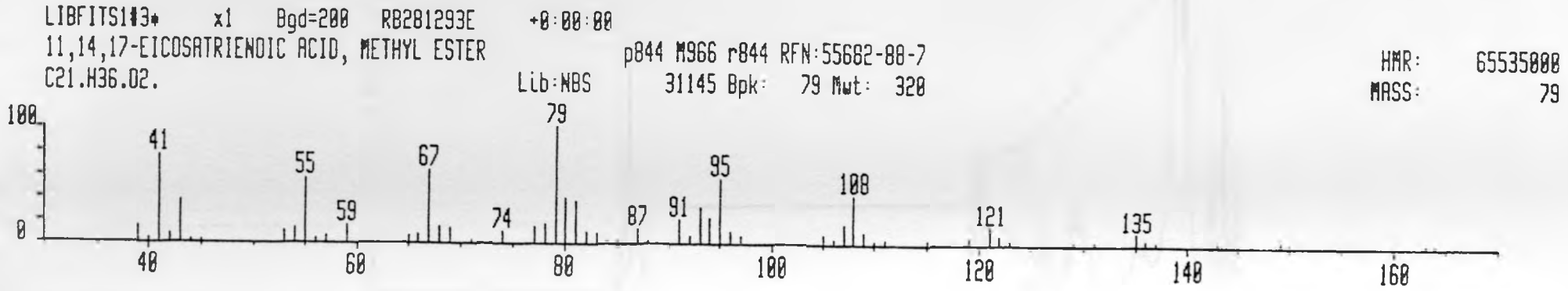
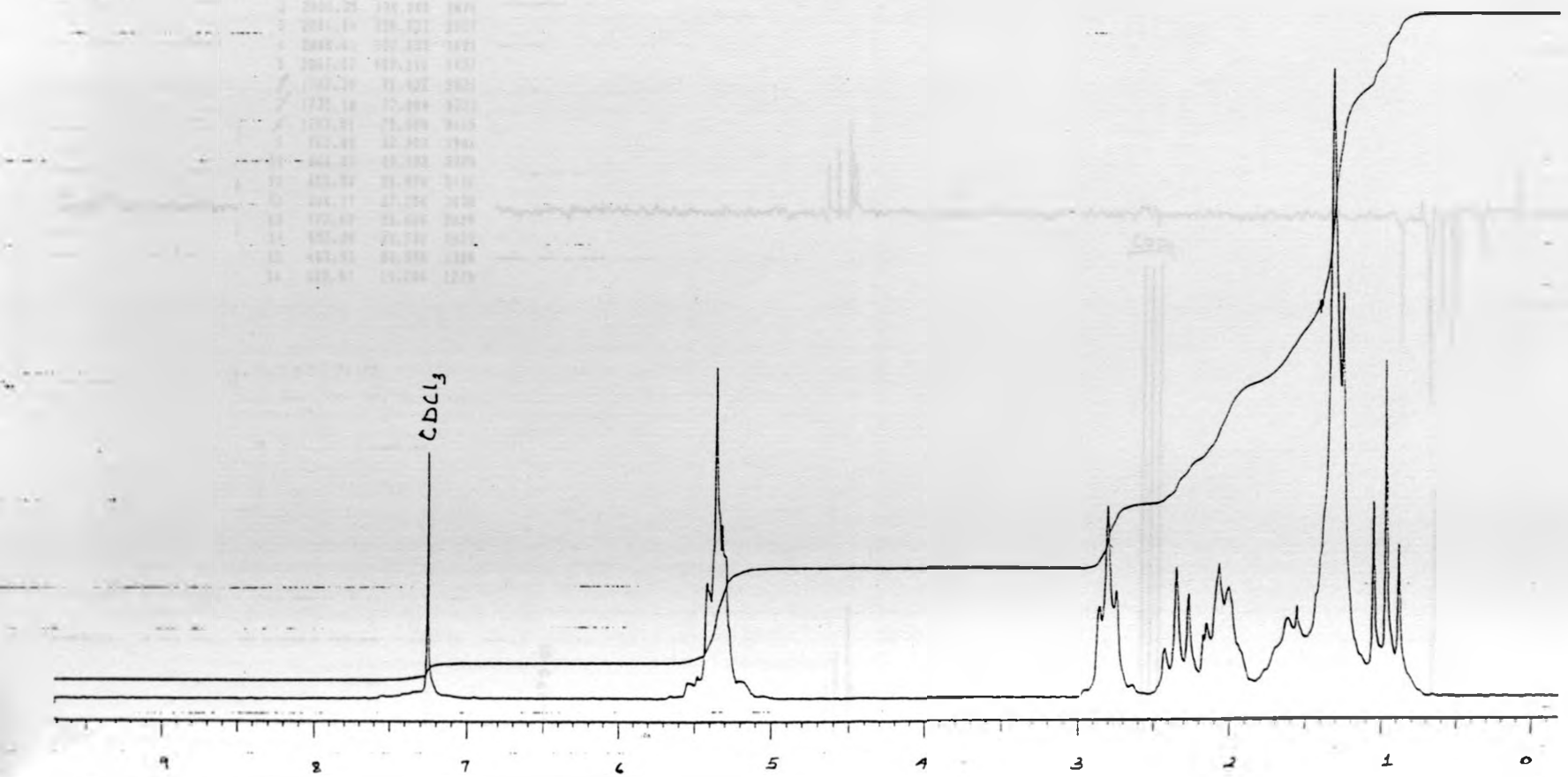


Fig. 2.2c. (ii) Mass Spectrum of the Authetic sample (Library).

Fig. 2.2d. Proton n.m.r Spectrum of Isolated Methyl ester.



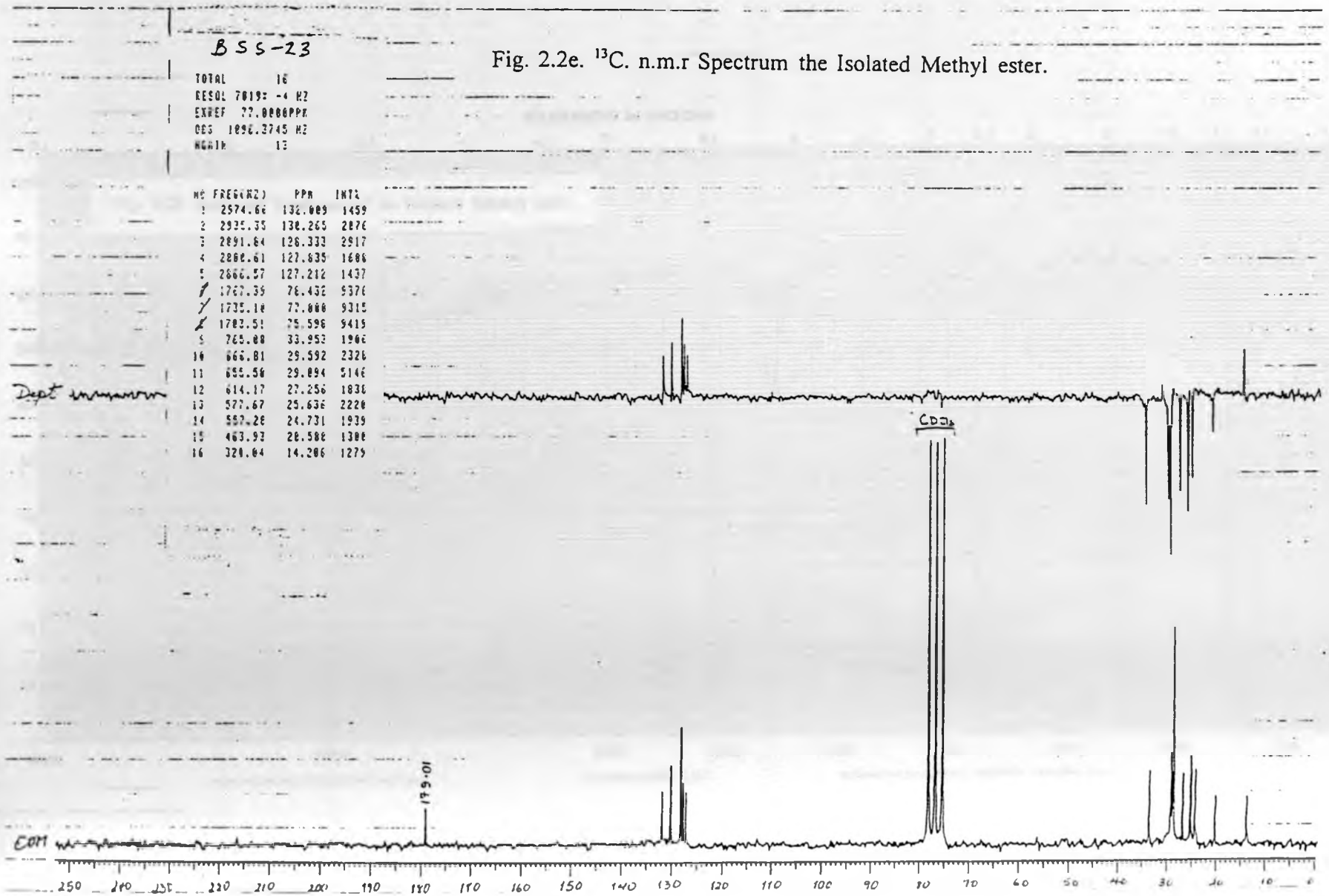


Fig. 2.2e. ^{13}C. n.m.r Spectrum the Isolated Methyl ester.

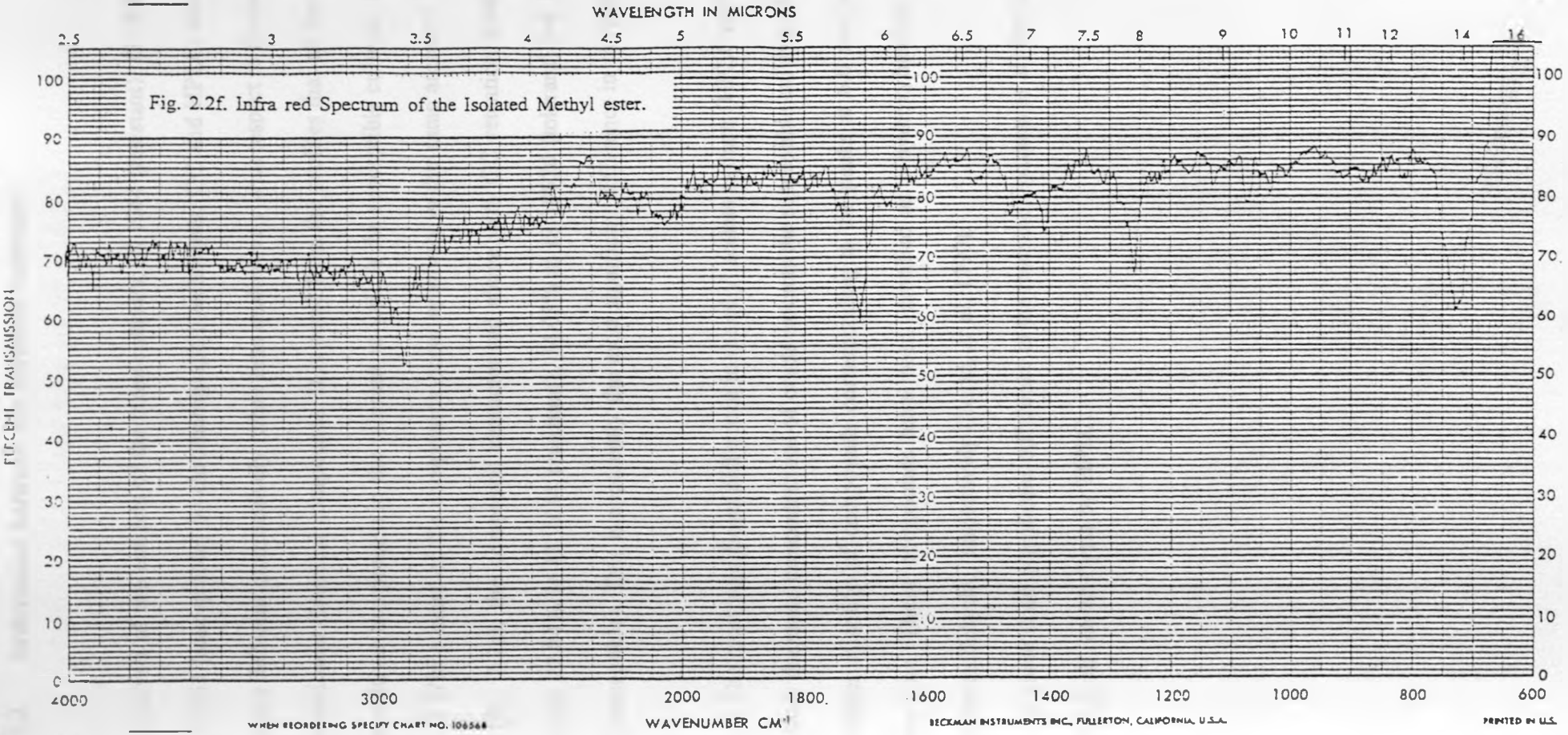


Fig. 2.2f. Infra red Spectrum of the Isolated Methyl ester.

2.4 Differences between the sorghum cultivars:

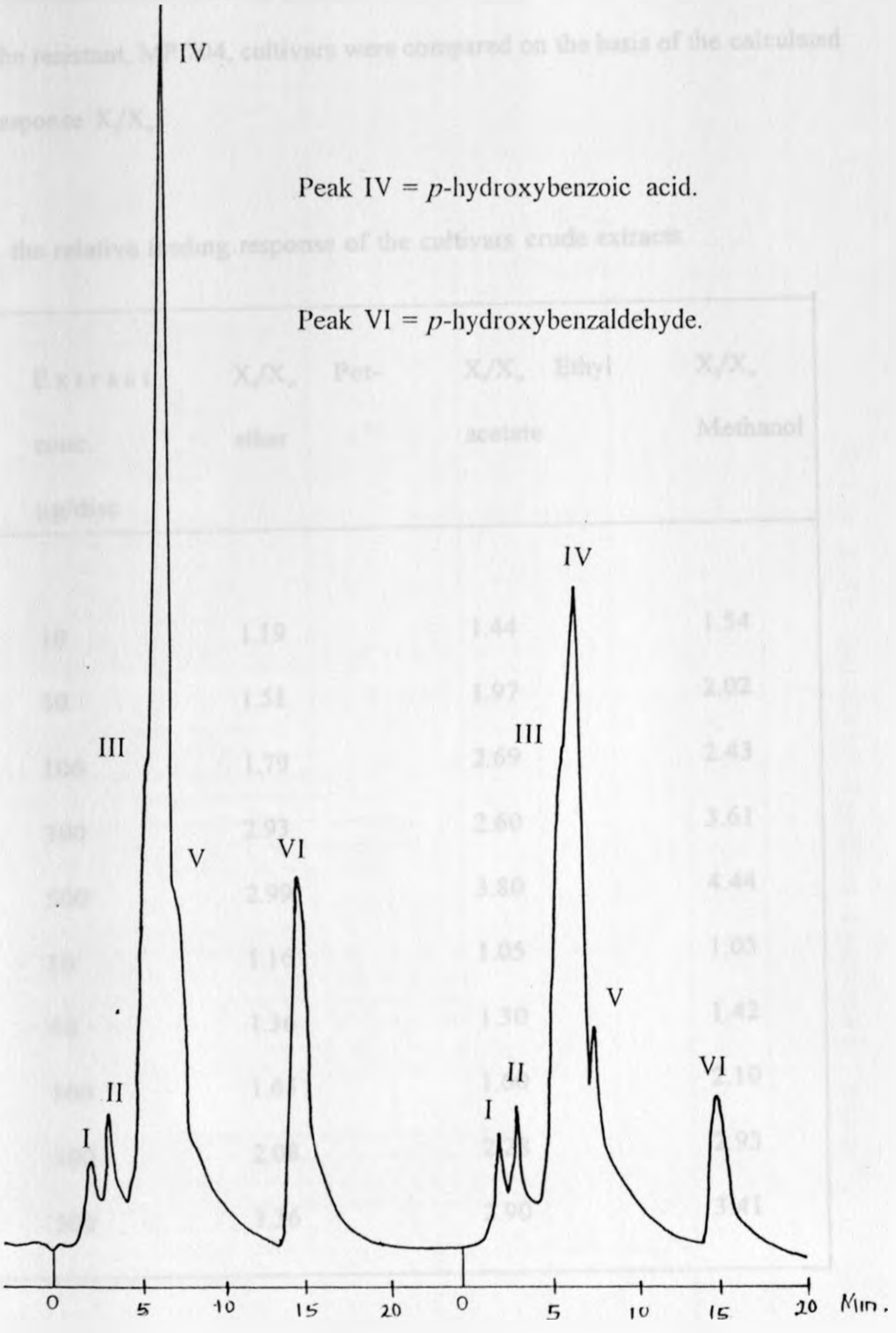
Analysis of the extracts of the three cultivars; IS 1044 (resistant), IS 18520 (tolerant) and IS 18363 (susceptible) by chromatographic techniques, GC and HPLC, showed that the cultivars are different quantitatively rather than qualitatively with respect to the concentrations of the constituent chemical compounds. It is noted from the profiles that all the peaks in the resistant cultivar are present in the tolerant as well as the susceptible cultivar; however,

1) While most of the compounds occur relatively in the same amounts from the GC profile (fig. 2.2a), the compound characterized above with GC retention time 23.115 min occurs with 22.84% in IS 1044 (resistant), 14.03% in IS 18520 (tolerant) but 12.99% in IS 18363 (susceptible). This is apparently the most significant difference in the petroleum ether extract.

2) The most significant difference in the ethyl acetate extracts (HPLC profiles fig.2.3a) is that from the same concentrations of crude extracts the susceptible cultivar (IS 18363) has much higher quantity of compounds corresponding to the peaks at 6.0 min and 15.0 min retention time. These compounds have been identified as *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde, respectively (Torto, *et al.* 1990).

3) There is no significant difference in the methanol extracts as all the cultivars show only one peak on analysis by HPLC.

Fig. 2.3a. HPLC Profile of the sorghum cultivars.



IS 18363 (Susceptible) - EtAc. IS 1044 (Resistant) - EtAc.

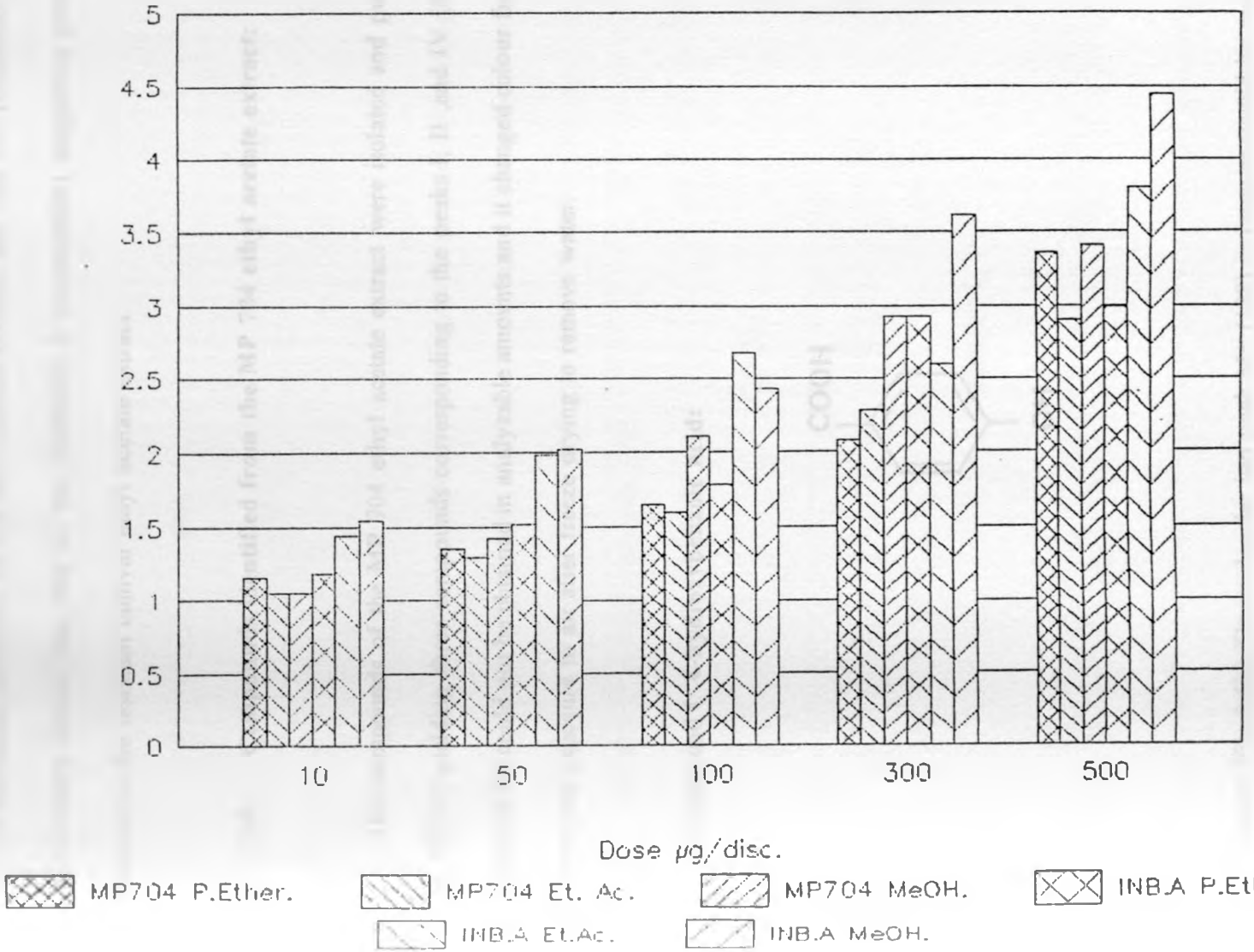
2.5 Maize; feeding bioassay:

The *Chilo partellus* larvae feeding response on the crude extracts of the susceptible, INBRED A, and the resistant, MP 704, cultivars were compared on the basis of the calculated relative feeding response X_t/X_o.

Table 2.8. the relative feeding response of the cultivars crude extracts.

Cultivar.	Extract conc. µg/disc	X_t/X_o Pet-ether	X_t/X_o Ethyl acetate	X_t/X_o Methanol
Inbred A	10	1.19	1.44	1.54
	50	1.51	1.97	2.02
	100	1.79	2.69	2.43
	300	2.93	2.60	3.61
	500	2.99	3.80	4.44
MP 704	10	1.16	1.05	1.05
	50	1.36	1.30	1.42
	100	1.65	1.60	2.10
	300	2.08	2.28	2.93
	500	3.36	2.90	3.41

Graph 2.4a Cultivar feeding response (Maize).



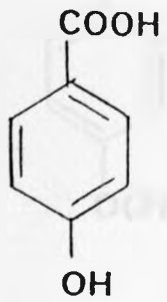
In general the methanol extracts were most stimulating followed by the ethyl acetate extracts and lastly petroleum ether extracts. Extracts derived from the susceptible cultivar were more stimulatory than those derived from the resistant cultivars.

In particular, however, for the ethyl acetate extracts, the MP 704 (resistant) response is significantly lower than that of the INBRED A (susceptible) suggesting presence of allomones in the resistant cultivar ethyl acetate extract.

2.6 Compounds identified from the MP 704 ethyl acetate extract:

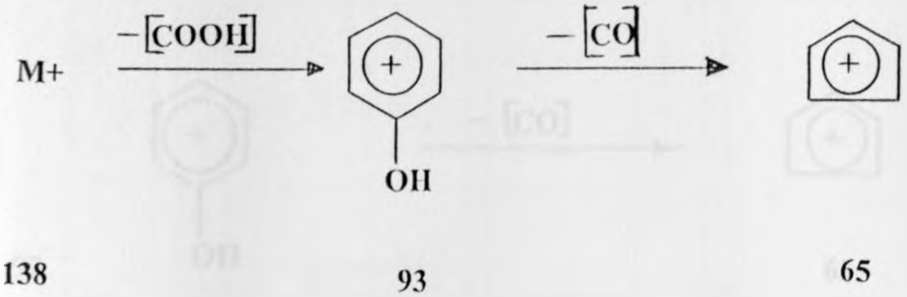
The compounds of the MP 704 ethyl acetate extract were isolated and purified by Prep. HPLC yielding three compounds corresponding to the peaks I, II ,and IV (fig. 2.5a). Compound III could not be obtained in analyzable amounts and it changed colour from white to brown on exposure to air after freeze drying to remove water.

Compound I: **4-hydroxybenzoic acid:**



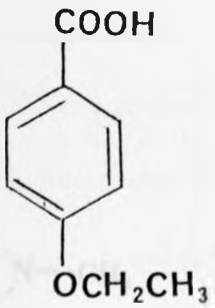
Mass spectra (Fig.2.5b) The molecular ion peak M+ (138) is large as would be expected of aromatic acids and phenols (Silverstein *et al.* 1981). The other diagnostic peaks that are prominent are M+ - OH (121) and M+ - COOH (93). The peak M+ - (COOH+CO) ie 65 is

predominant and is formed as a result of fragmentation by lose of COOH group followed by rearrangement and loss of CO characteristic of phenols.

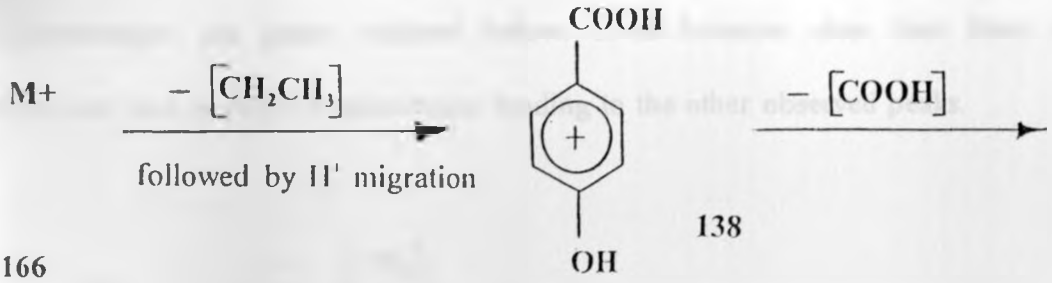


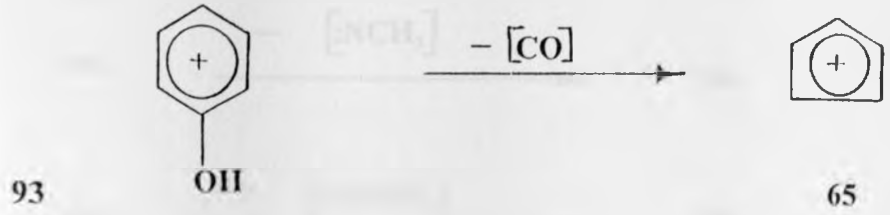
The presence and structure of this compound were confirmed by co-injection of the extract with the authentic sample in the H.P.L.C and consequent observation of enhancement.

Compound II: ***p*-Ethoxybenzoic acid.**

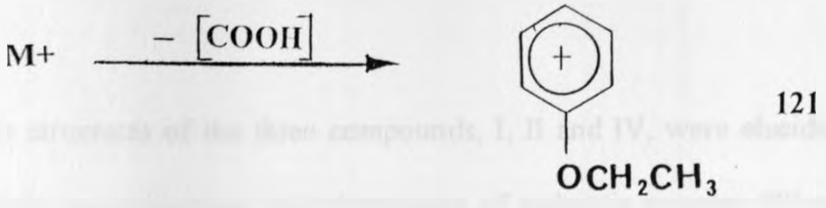


Mass spectra; The parent peak M+ (166) is large as is expected of aromatic acids. The characteristic peaks are as a result of the fragmentation pattern outlined below (Silverstein *et al.* 1981).

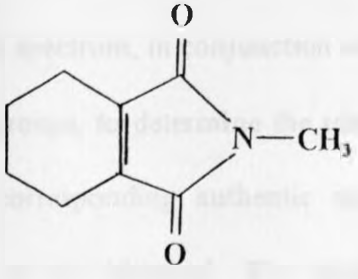




The peak at m/e 121 is obtained from the parent ion by loss of the (COOH) group.

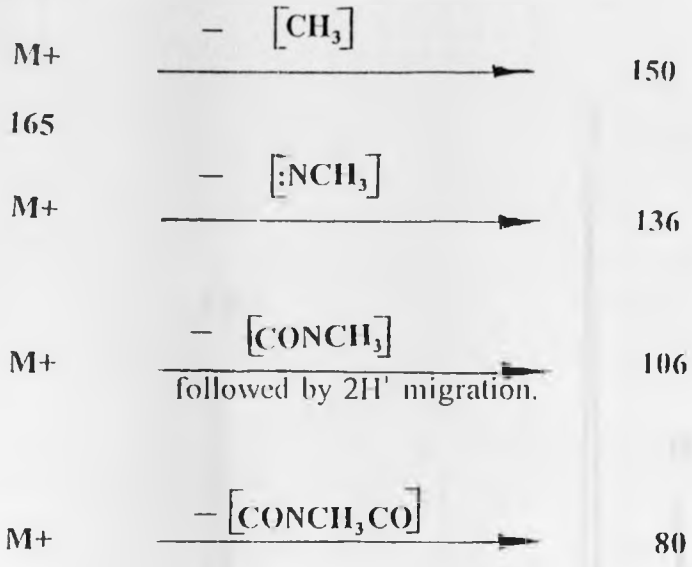


Compound IV: **4,5,6,7-Tetrahydro-2-1H-Isoindole-1,3 (2H)-dione.**



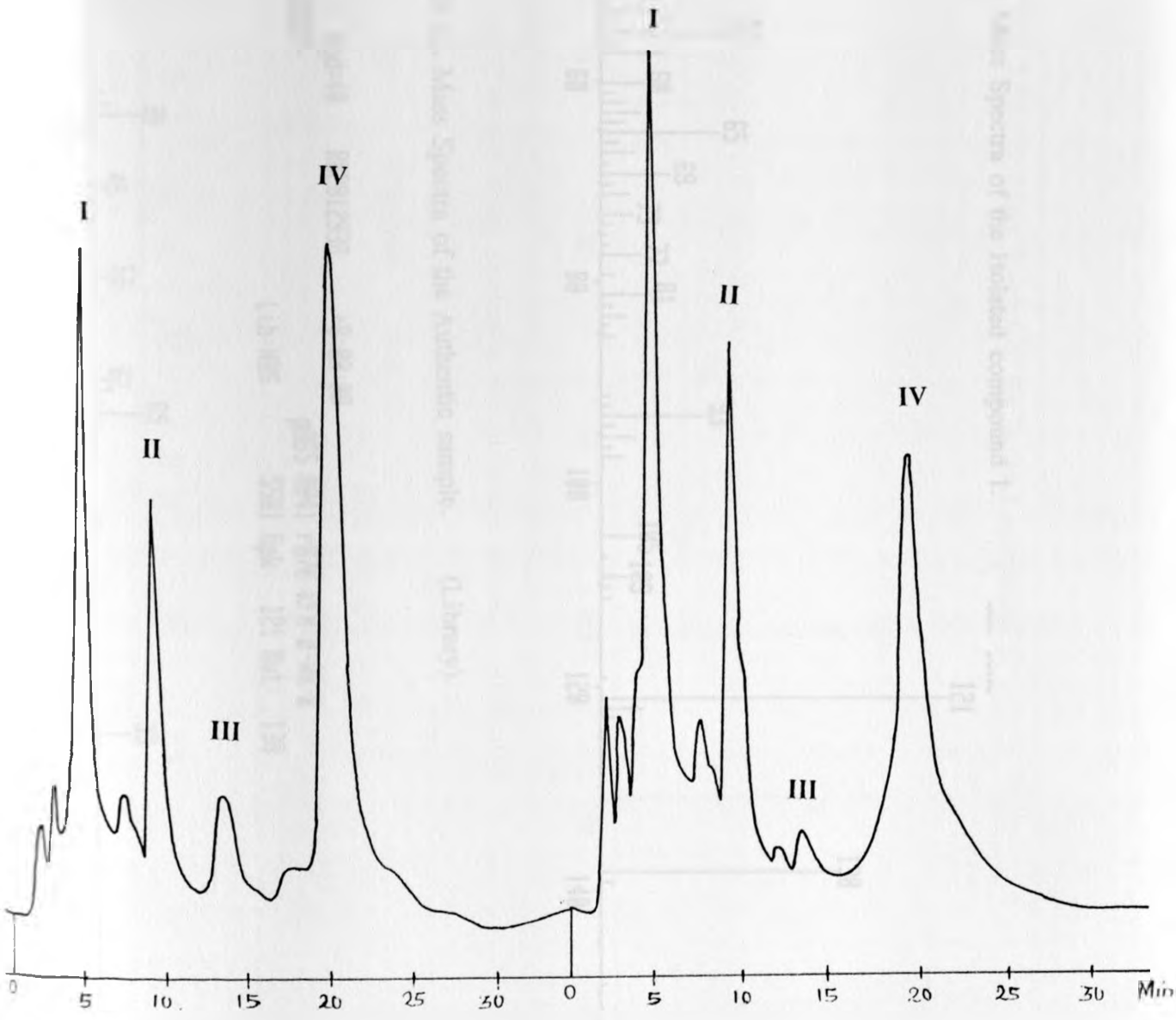
Mass spectra; The parent peak, as would be expected, is very intense M+ (165). The fragmentation pattern may not be a straight forward one, however, cleavage is expected to occur at the C-C bond adjacent to the oxygen atom and at the C-N bonds; Thus leading to

the fragmentation ion peaks outlined below. It is however clear that there occurs rearrangement and multiple fragmentation leading to the other observed peaks.



The structures of the three compounds, I, II and IV, were elucidated by comparison of the sample mass spectrum with the spectra of authentic samples (Silverstein *et al.* 1981). This was done by computerised search and matching of the sample spectrum with the computer library spectra of authentic samples. The computer gave a print out of the five best fitting spectra for further analysis. The five spectra then were manually analyzed peak by peak comparing with the sample spectrum, in conjunction with the expected fragmentation pattern of the various functional groups, to determine the identically fitting spectrum. The isolated sample spectra and the corresponding authentic sample spectra (fig.2.5bi, fig.2.5bii & fig.2.5biii) were found to be identical. The three compounds were therefore easily characterized.

Fig. 2.5a. HPLC profiles of the maize cultivars.



MP 704 (Resistant) - EtAc.

INBRED A (Susceptible) - EtAc.

Fig. 2.5b (i). Mass Spectra of the Isolated compound I.

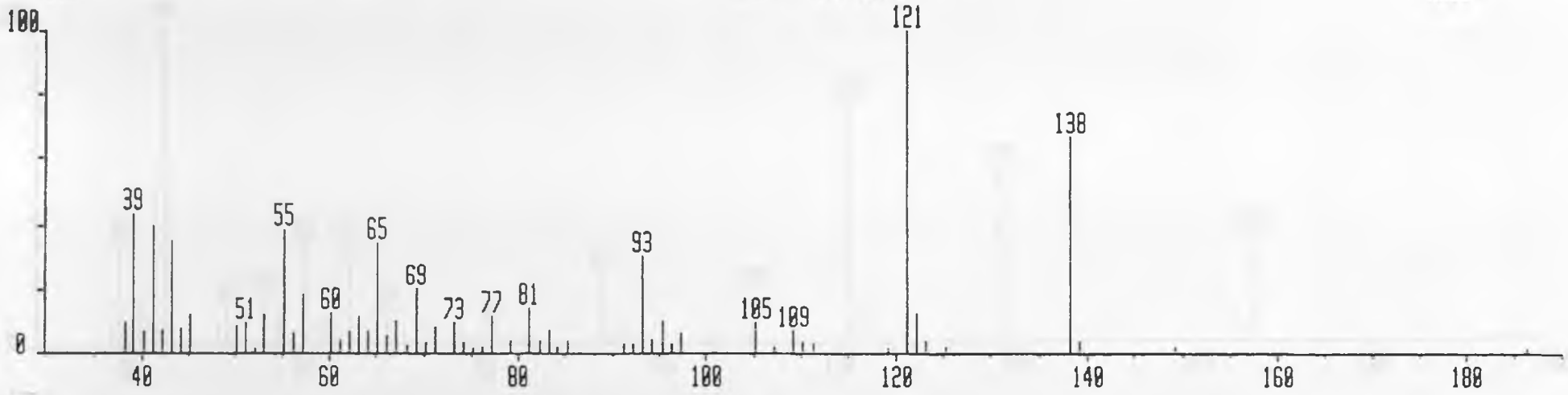


Fig. 2.5b (i). Mass Spectra of the Authentic sample. (Library).

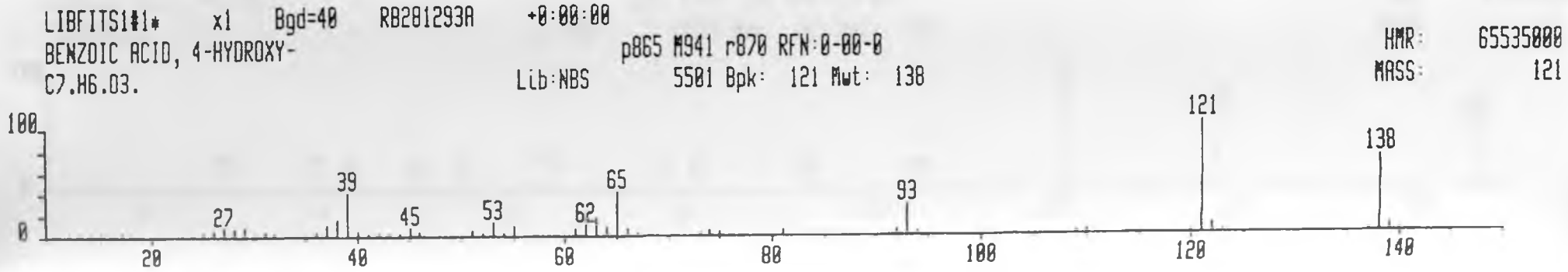


Fig. 2.5b (ii). Mass Spectra of the Isolated compound II.

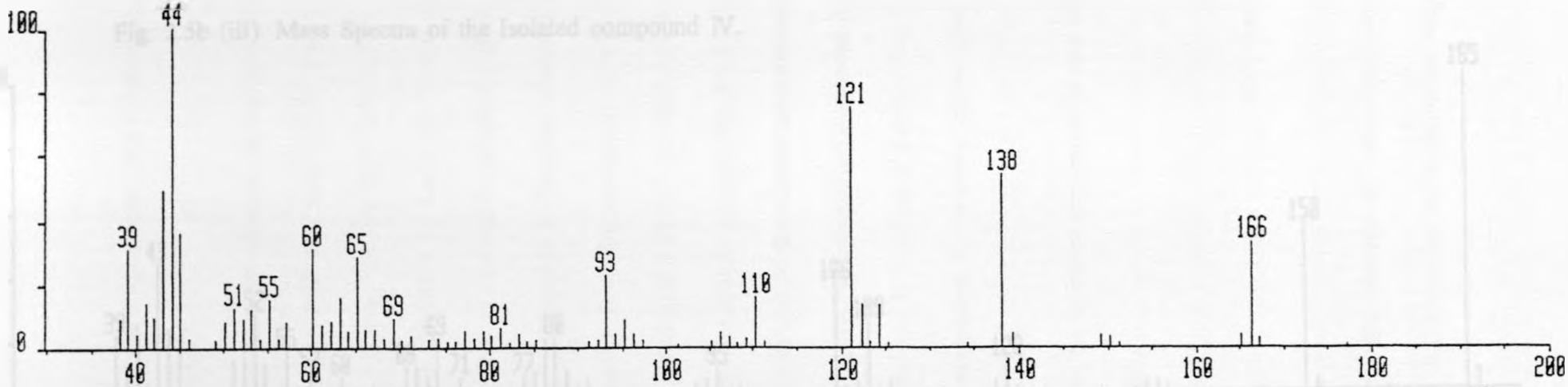


Fig. 2.5b (ii). Mass Spectra of the Authentic sample. (Library).

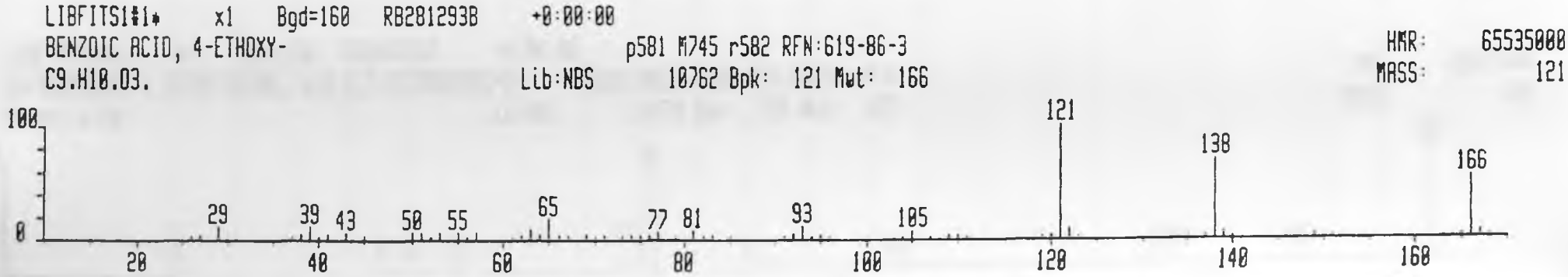


Fig. 2.5b (iii). Mass Spectra of the Isolated compound IV.

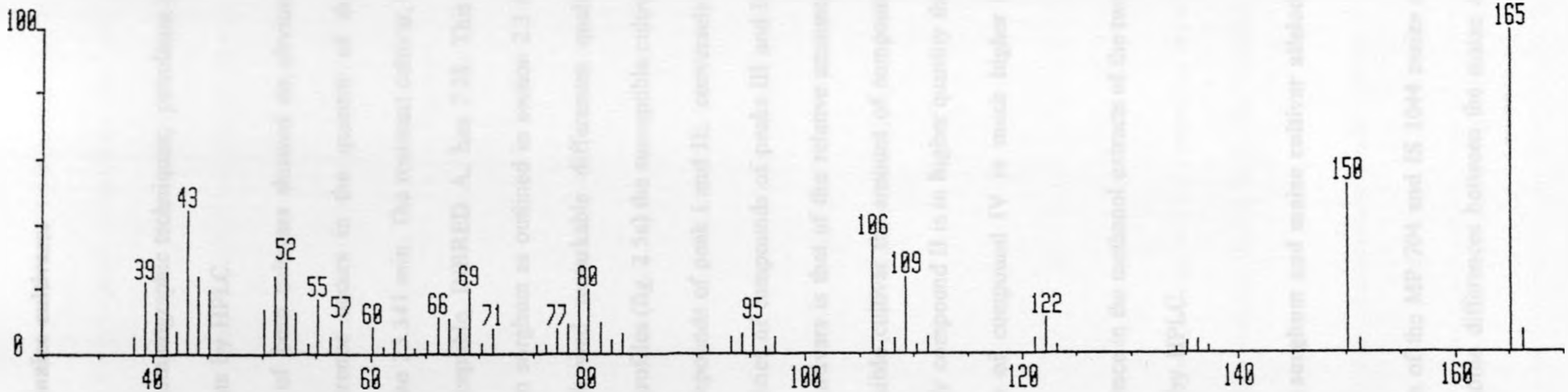
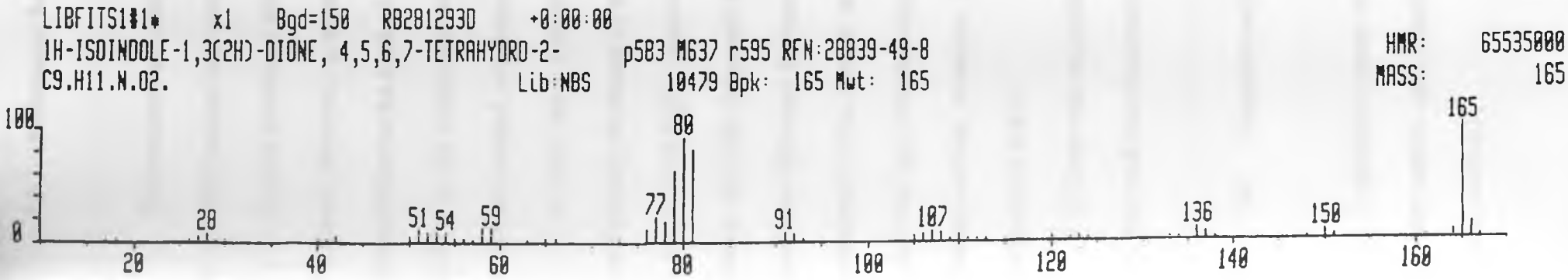


Fig. 2.5b (iii). Mass Spectra of the Authentic sample. (Library).



2.7 Differences between the maize cultivars:

The extracts were analyzed by chromatographic techniques; petroleum ether extracts by GC, ethyl acetate and methanol extracts by HPLC.

1) The petroleum ether extracts of both cultivars showed no obvious qualitative difference; however, a significant difference occurs in the quantity of the compound corresponding to the peak of retention time 23.341 min. The resistant cultivar, MP 704, has 19.27% of the compound while the susceptible, INBRED A, has 7.28. This is the same compound isolated and characterized from sorghum as outlined in section 2.3 above.

2) The ethyl acetate extracts showed remarkable differences qualitatively and quantitatively. As shown by the H.P.L.C profiles (fig. 2.5a) the susceptible cultivar, INBRED A, has a much higher quantity of the compounds of peak I and II; conversely the resistant cultivar, MP 704, has much higher quantities of compounds of peaks III and IV. The other significant difference between the two cultivars is that of the relative amounts of the four compounds in the extract. In the susceptible cultivar the amount of compound I is much higher than that of compound IV, similarly compound II is in higher quantity than IV. In the resistant cultivar, however, the quantity of compound IV is much higher than that of compounds I and II.

3) There was no significant difference in the methanol extracts of the two cultivars as only one peak was observed on analysis by HPLC.

2.8 Comparison between the sorghum and maize cultivar allelochemicals:

A qualitative study of the profiles of the MP 704 and IS 1044 maize and sorghum cultivars reveals some similarities and some differences between the maize and sorghum surface chemistry.

Comparison of the GC profiles (fig. 2.2a) of the pet-ether extracts showed that both maize and sorghum possess the major constituents of the non-polar compounds; the *n*-alkanes from C_{10} to C_{18}. The compounds with retention times 24.5, 25.224, 26.515, 28.158, 29.49, 30.697, 32.402 and 36.72 minutes, which have not been identified and had no significant effect on the oviposition of *C. partellus*, are common to both maize and sorghum and relatively in similar amounts. The compound of retention time 23.144 minutes, which is most likely the *C. partellus* oviposition inhibitor, occurs in relatively higher quantities in the resistant cultivars of both maize (19%) and sorghum (22%) but in lower quantities in the susceptible cultivars.

The HPLC profiles (fig. 2.2f & fig. 2.5a) show both qualitative and quantitative similarities and differences. The most remarkable similarity is the presence of *p*-hydroxybenzoic acid in relatively large amounts. The sorghum ethyl acetate extract however, is predominantly consisting of *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde while the maize extract does not seem to possess detectable amounts of the *p*-hydroxybenzaldehyde, instead it contains compounds II, III, and IV which are interestingly absent in sorghum.

2.9 DISCUSSION.

The available literature on the role of plant characters in determining resistance or susceptibility of different sorghum and maize cultivars to *Chilo partellus* is inadequate. It is the interaction of the different factors rather than any one of them that determine a cultivars resistance or susceptibility to the pest (Saxena, 1990).

These results are in agreement with observations made earlier where larval establishment was found to be high on the leaves of the maize susceptible cultivar INBRED A but low on the resistant cultivar ICZ2-CM. The adult moth oviposition was found to be

high on the leaves of sorghum susceptible cultivar IS 18363 but very low on the resistant cultivar IS 1044 (Ampofo and Nyangiri, 1986; Saxena, 1985 and Saxena, 1987).

The susceptible cultivars of both maize and sorghum give higher quantities of crude extracts than the resistant cultivars, especially so of the methanolic extracts. The methanolic extracts have been shown to consist mainly of sugars which have been shown to be *C. partellus* feeding stimulants acting synergistically with other known stimulants (Torto, *et al.* 1990). This explains the higher feeding stimulation observed in the INBRED A extracts and in the IS 18363 as was observed by Torto, *et al.* It is of importance to note that the sorghum resistant cultivar IS 1044 has higher quantities of the petroleum extractables and more so of the compound methyl 11,14,17-eicosatrienoate compared to the susceptible IS 18363. The maize resistant cultivar MP 704, on the other hand, has higher quantities of the ethyl acetate extractables and especially so of the compounds 4,5,6,7-tetrahydro-2-1H-isoindole-1,3(2H)-dione and another that has not been identified compared to the susceptible cultivar INBRED-A.

It is clear from the bioassay results that the resistant cultivars of the maize and sorghum possess chemicals that inhibit oviposition for sorghum and feeding for maize. From this study it may be concluded that the presence of the compound methyl 11,13,17 eicosatrienoate in high amounts does play a significant role in combination with the alkanes and other unidentified compounds in inhibiting the *C. partellus* oviposition on the highly resistant cultivar IS 1044. It may be concluded too from this study that the presence of the compound 4,5,6,7-tetrahydro-1,3(2H)-dione along with the unidentified compound in high amounts play a significant role in inhibiting (by acting as antifeedants) the feeding of the *C. partellus* larvae on the maize resistant cultivar MP 704. Therefore the low quantities or absence of these compounds in the susceptible cultivar INBRED A leads to high feeding stimulation due to the compound p-hydroxybenzoic acid and possibly *p*-ethoxybenzoic acid which are in high quantities in the cultivar. The combination of the various compounds in the

cultivar is clearly important for the cultivars resistance or susceptibility. This is reflected by the feeding bioassay results, the H.P.L.C profiles of the maize cultivars (fig. 2.5a) and by the fact that the various column fractions and the remixed fraction of the sorghum resistant cultivar IS 1044 petroleum extract show much weaker oviposition bioactivity compared to the crude extract.

The bioassay methods used in this study give a reflection of the real life field situation. It however must be noted that the field situation more often than not is far from the laboratory controlled situation, therefore, the improvement of the bioassay conditions so as to approach the field conditions would serve to improve the bioassay results. As observed from the oviposition bioassay there is a wide response variation between individual insects. On any one accession it is not unusual to find that 5% - 10% of the insects do not follow the general pattern. No explanation can be given for this and it is not known whether such insects would consistently be the exceptional ones.

The leaf surface has been shown to be important to locusts and grasshoppers in the selection of food plants (Blaney and Chapman, 1970; Chapman, 1977). Such insects can perceive chemicals in the wax on the leaf surface by contact chemoreceptors on the maxillary palps (Bernays, *et al.*, 1976).

Alkanes are always present in plant surface waxes, but normally the major alkanes have long chains of 27, 29, 31 or 33 carbon atoms (Eglinton and Hamilton, 1963). In sorghum, however, the dominant alkanes vary with cultivar. Bianchi, *et al.*(1978) found C_{29} and C_{31} *n*-alkanes predominantly in cultivars SD 102 and Alliance, but Wilkinson and Cummins, (1981) showed that in "bloom" leaves of cultivars Redbine 60 the most abundant n-alkanes in the surface wax were C_{35}, C_{36}, C_{37} and C_{38}, whereas in "bloomless" leaves of the same cultivar, the C_{25} alkane was in highest concentration.

In young sorghum plants of cultivar CSH 1 and IS 1082, the major alkanes are C_{27}, C_{29} and C_{31} with negligible amounts of any alkanes of shorter chain length (Atkin and

Hamilton, 1982). Woodhead, (1983) found in the cultivar 65D surface wax *n*-alkanes of C_{19} to C_{25} with C_{23}, C_{24} and C_{25} being the most abundant. Woodhead found that only the C_{19}, C_{21} and C_{23} *n*-alkanes did reduce feeding of *L. migratoria*.

It is of interest to note that in this study the *n*-alkanes found in the cultivars IS 1044, IS 18520 and IS 18363 are of much shorter carbon chain than any reported earlier, and C_{14}, C_{15} and C_{16} are the most abundant in the cultivar IS 1044. Although these *n*-alkanes do not exhibit any observable *C. partellus* oviposition bioactivity for any of the doses tested, they could be acting in combination with the methyl 11,13,17 eicosatrienoate to produce the observed oviposition inhibition in the sorghum resistant cultivar. There is need therefore for a detailed study of the effect of these short chain *n*-alkanes to the pest *C. partellus*.

Alkanes in plant surface waxes have previously been reported to be involved in food selection by *Acyrthosiphon pisum* with C_{32} serving as a feeding stimulant and by *L.migratoria* with C_{19}, C_{21} and C_{23} acting as feeding deterrents (Klingaur *et al.*,9171; Woodhead, 1983). C_{10} - C_{18} *n*-alkanes have been shown to be involved in rice cultivar IR-22 resistance to the brown plant hopper (*N. lupens*) (Woodhead and Padgam, 1988). They have also been reported to affect oviposition of Delia (*Hylemya brassicae*) (Stadler, 1978).

Most higher plant waxes have mainly C_{20}, C_{22} and C_{24} acid components of their wax esters (Martin and Juniper, 1970). Seedlings of sorghum cultivar 65D have mainly shorter-chain acid components while seedlings of cultivars CSH 1 and IS 1082 generally have longer-chain fatty acids (Atkin and Hamilton, 1982). Woodhead (1983) reported C_{12} to C_{24} esters in the leaf wax of the sorghum cultivar 65D with only traces of odd carbon chain esters.

Methyl esters of C_{10} and C_{12} fatty acids have been reported to adversely affect growth and cause mortality in bollworm (Binder *et al.*, 1979), and there are many reports of the use of free short-chain fatty acids as contact insecticides. Mortality was highest with C_{10} and C_{12} compounds in, for example, mosquitoes (Saxena and Thorsteinson, 1971) and houseflies (Quraishi, 1971).

The short-chain lauric acid (C_{12}) has been reported to stimulate *L.migratoria* feeding while its methyl ester was strongly deterrent (Woodhead, 1983).

p-Hydroxybenzoic acid, several phenolic compounds and their derivatives have been shown to be *C. partellus* feeding stimulants (Torto *et al.*, 1990 *Ibid.* 1991). Fisk (1990) found that a phenolic extract from sorghum was stimulatory to the homopteran *Peregrinus maidis*, and Baker *et al.* (1986) showed that 4-hydroxybenzaldehyde and some of its derivatives are strong feeding stimulants for the elm bark beetle *Scolytus multistratus*. On the other hand *p*-hydroxybenzaldehyde has been shown to deter feeding by the *L. migratoria* and the aphid *Schizaphis graminum* (Woodhead and Bernays, 1978; Dreyer *et al.*, 1981; Woodhead, 1982).

Not many heterocyclic compounds have been isolated from either sorghum or maize. However, the compound DIMBOA, isolated from young leaves of one resistant cultivar of maize, has been shown to be responsible for maize resistance to *O. nobilalis* and the 1,4-benzoxazin-3-one functional group seemed important for its activity (Robinson *et al.* 1978). It therefore may be of interest to establish the bioactivity of the isolated compound 4,5,6,7-tetrahydro-2-1H-Isoindole-1,3-(2H)-dione and possibly the importance of the isoindole group or the dione arrangement.

The importance of the surface of the young sorghum and maize plant may have been underestimated in previous work when the strongly deterrent nature of the seedlings was attributed to the internal cellular cynogenic glucoside, dhurrin, and phenolic acid esters (Woodhead and Bernays, 1978). The presence of deterrent chemicals in the surface of the plant is of particular interest in that it provides information to the insect resulting in rejection of the plat prier to or at palpation before the internal feeding deterrents are encountered. As proposed by Chapman (1977) there are obvious advantages to the plant in protecting itself from potential predators by advertising its unsuitability on its surface rather than keeping its defence hidden until it is damaged by being bitten.

From the point of view of a plant breeder, the potential for developing resistant varieties of a crop is probably much higher than may be immediately obvious. The number of possible resistant varieties will be a function of not only of the number of individual chemicals which may individually be increased in amount, but also of the possible combinations and mixtures of individual secondary compounds each of which may be of no obvious significance when tested alone.

CHAPTER 3

(3) EXPERIMENTAL:

3.0 General experimental procedures:

Thin layer chromatography (TLC) was done on MN polygram precoated silica gel/UV254 40x80 mm plates (0.25mm thickness). They were eluted in 10% ethyl acetate - hexane, developed with 50% aqueous sulphuric acid and subsequently heated at 120°C for 30 minutes. Column chromatography was done with (columns 15mm and 10mm diameters) normal phase silica gel (60) particle size 0.040-0.063mm (230-400-mshs ASTM). High performance liquid chromatography (HPLC) was performed on Varian 5000L.C model, equipped with MCH-5 and MCH-10 reverse phase columns. Gas chromatography (GC) profiles were obtained using a Howlett Packard model HP 5890A series gas chromatograph. GC-MS and MS spectra were done on a Howlett Packard model HP 5790A series gas chromatograph coupled to a VG Masslab 12-250 analytical organic mass spectrometer. Nuclear magnetic resonance (proton NMR and ^{13}C- NMR) were done on Perkin Elmer Spectrometer model R12B-90MHz. Infra-red spectra was done on a Beckman Infrared spectrophotometer.

The solvents used; hexane, petroleum ether, ethyl acetate, methanol, dichloromethane and acetonitrile were all of analar grade. The hexane used for GC profiles was double distilled while the methanol and acetonitrile used for HPLC were of HPLC grade (solvents were obtained from Aldrich chemical company Ltd, Gillingham UK).

3.1 Chromatography:

Column chromatography was used to isolate compounds from the IS 1044 pet-ether extract. The column was packed using hexane. The extract was dissolved in minimal hexane, introduced in the column and eluted with hexane adjusted in polarity with ethyl acetate and eventually with dichloromethane. The eluates were monitored by their GC profiles.

The ethyl acetate and methanol extracts were analyzed by HPLC. For the profiles, 5mg/ml each of the extracts were prepared in methanol, filtered through a wad of cotton wool plugged in a pasteur pipette and 10µl of each extract were injected and analyzed on the MCH-5 (Zorbex 4.6mmx25cm) reverse phase column. The column was eluted with 30% and 20% methanol - water for maize and sorghum extracts, respectively. The flow rate was 1ml per minute, the column pressures were 312 and 270 atm., respectively. The uv. detector, 240nm, was used.

The pet-ether extracts were analyzed by GC. Solution (2µl) containing 20µg/µl of the extract were used. The GC was equipped with a split/splitless injector and a flame ionization detector (FID) at 250°C. The column used was Ultra 1 crosslinked methyl silicone gum of dimension (50mx0.32mm ID; 0.17µm film thickness). White spot nitrogen (3.45cm^3/sec.) was the carrier gas. Hydrogen (45cm^3/sec.) and medical air (360cm^3/sec.) were the fuel gases. The total flow was 450cm^3/sec. All the GC analyses were done in the splitless mode with the injector temperature at 280°C. The oven temperature was programmed to stay at 45°C for 5min rise to 180°C at a rate of 10°/min and further to 280 at 20°/min The retention times, the peak areas and the percentage composition were calculated on a Howlett Packard model HP 3393A integrator.

3.2 Biological activity tests (bioassays):

Bioassay of the crude extracts, fractions and isolated compounds were done on the *Chilo partellus* larvae and moth to establish the stimulation or inhibition, if any, of feeding and oviposition by the maize and sorghum allelochemicals respectively.

(a) ***Chilo partellus*** **feeding bioassay;** The no choice feeding tests were conducted in small glass vials (23x27mm) with tight porous caps. A wad of moist cotton wool placed inside the cap maintained a high humidity. Test samples in solvents were applied topically to both sides of the cellulose acetate disks (12mm diameter, 7.902 ± 0.348 mg, Cole- parmer Co. Chicago, Illinois) and were dried in a stream of warm air (Doss and Shank, 1986). Each test disk was then dampened with 15µl of double distilled water and placed in the vial. Control disks dipped into solvent and air dried were similarly treated with double distilled water. Three third instar larvae, starved for 24 hours prior to testing, were placed into each vial. All the feeding tests were conducted for 24 hours in the dark at 65 ± 2% relative humidity and 29 ± 2°C. Test and control disks were weighed several times to a constant weight before the assay on a Mettler AT261 Delta range balance to ± 0.001mg. Each test was replicated ten times.

The relative feeding response, X_t/X_o, was calculated for each dose by dividing the mean weight of the treated disks consumed (X_t) by that of the control disks consumed (X_o).

(b) ***Chilo partellus*** **oviposition bioassay;** These were conducted in a circular chamber, (Kumar and Saxena, 1985; and Ramachandran and Saxena, 1991). The extract or compound was impregnated on a semi circular Piece of filter paper (Whitman Filter paper No. 1, 9cm. diameter) covering half the area, a similar paper without extract covering the other half and serving as the control. A single ovipositing female was released within the circular

chamber overnight and the number of egg masses and eggs laid on the treated and the control semi circles were counted. The percentage preference / non-preference was calculated by dividing the deference in the number of eggs laid on the treated and the control by the total number of eggs laid, (T-B/T+B)100, The percentage number of insects laying one and a half (1.5) times or more as many eggs on the blank/treated as on the treated/blank was also calculated. Each test dose was replicated 15-20 times.

All the oviposition tests were conducted for 12 hours in the dark (at night) at 60 ± 5% relative humidity and 29 ± 2°C.

3.3 Plant material:

Whorls of maize; cultivars MP 704 (Resistant) and INBRED A (susceptible), and sorghum; cultivars IS 1044 (resistant) IS 18520 (tolerant) and IS 18363 (susceptible) were obtained from the screen house at the Mbita Point Field Station.

3.4 Insects:

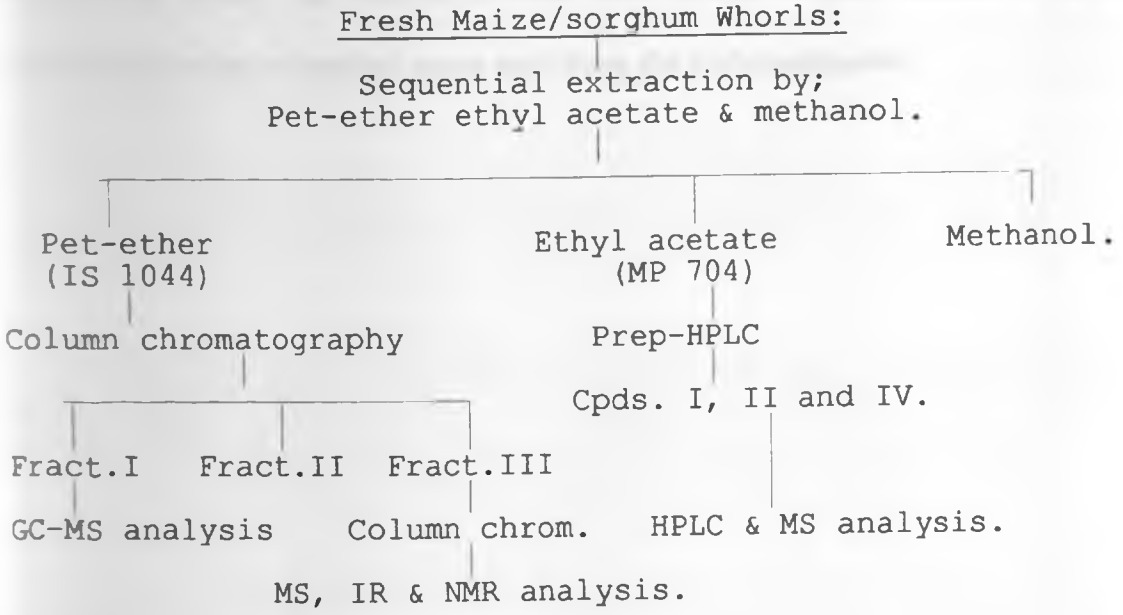
Freshly molted third instar larvae and freshly emerging pupae, of *Chilo partellus*, were obtained from stock cultures maintained on an artificial diet at the I.C.I.P.E insectary (Ochieng *et al.* 1985). Third instar larvae feed first on the leaves of the host plant before gradually boring into the stem. This stage was selected for bioassay owing to the long-term objective of elucidating the basis for the change in the larval feeding sites. The freshly emerged pupae were sexed and paired (male and female) for mating the first night after emerging and oviposition assayed the fist night after mating (Kumar and Saxena, 1985).

3.5 **Extraction:**

The plants were extracted three weeks after emergence. The whorl leaves of each of the maize and sorghum cultivars were extracted by successively soaking them in petroleum ether (20min), ethyl acetate (20 min) and methanol, 20min (for sorghum / oviposition work) and 24 hours (for maize / feeding work). The extract solutions of pet-ether and ethyl acetate were filtered and dried with anhydrous sodium sulphate then concentrated to dryness *in vacuo* at low temperature (40°C). The methanol extracts were filtered and concentrated to a small volume and freeze dried to remove residual water. The residual plant material was also freeze dried to obtain the weight of the dry plant material.

3.6 Fractionation and isolation:

The summary of the fractionation of the extracts and isolation of the constituent allelochemicals is outlined in the diagram below. Fresh maize and sorghum whorls were sequentialy extracted in petroleum ether, ethyl ecetate and methanol. The extracts were then subjected to chromatographic techniques to isolate the relevant allelochemicals.



3.6.1 Fractionation and isolation of IS 1044 pet-ether extract compounds:

The IS 1044 petroleum ether extract was subjected to column chromatography yielding three main fractions, (I, II & III). Fraction I was further analyzed by GC-MS and by co-injection with authentic standards in the GC.

Fraction III was found to be predominantly one compound by GC analysis and was further purified by column chromatography using a smaller column to obtain the pure compound for Mass Spectroscopic, Nuclear Magnetic Resonance and Infra red analysis.

3.6.2 Isolation and purification of MP 704 ethyl acetate extract compounds:

The allelochemicals of the MP 704 ethyl acetate extract were isolated and purified by preparative High Performance Liquid Chromatography (HPLC). The ethyl acetate extract (each of 100mg) was dissolved in 5ml of methanol and filtered. The sample (50µl) was injected into the HPLC Varian 5000 LC. The column used was MCH-10 reverse phase (50cm x 8mm). The solvent system was 30% methanol-water, the flow rate was 3ml/min and the detector was 240nm. The fractions obtained were reduced to small volumes *in vacuo* and freeze dried to remove residual water and obtain the allelocompounds.

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