MASS SPECTRAL EXAMINATION OF THE EXUDATES OF ERECT GLANDULAR PLANT HAIRS (*MEDICAGO SCUTELLATA* AND *MEDICAGO SATIVA* L. *SUBSP. PRAEFALCATA*)

by

Donna Carol Triebe

B. S., Kansas State University

Manhattan, Kansas, 1978

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1981

Approved by:

Clayton E. Melvin

Major Professor

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF FIGURES

page

LIST OF FIGURES (continued)

page

LIST OF FIGURES (continued)

page

LIST OF FIGURES (continued)

page

LIST OF TABLES

INTRODUCTION

The alfalfa weevil [Hypera postica (Cyllenhaul)] is one of the more serious pests attacking alfalfa (Medicago sativa L.) in the Midwest. In Kansas, where over one million acres of alfalfa are grown, weevil infestation reached its peak in 1974. In that year, at least 95% of the acreage was infested. More than 824,000 acres were treated with insecticides in 1974, while only 220,000 were treated in 1973 and 49,000 in 1972.[1] The excessively cold winters over the past few years have reduced the weevil infestion, but if a few warm winters in a row occur then it is anticipated that excessive weevil damage will again occur.

Certain annual Medicago species are resistant to the weevil both in the larval stage[2,3,7] and the adult stage.[4,5,6] Studies have been performed on both diploid species (containing two sets of chromosomes) and tetraploid species (containing four sets of chromosomes). Results show the tetraploids as being more resistant than the diploids to the weevil in the larval stage[2] and the diploids more resistant than the tetraploids to the weevil in the adult stage.[2] The alfalfas cultivated in Kansas are the perennial M. sativa (the annual species are not grown in Kansas). The annual species of particular interest are diploid Medicago disciformis DC and tetraploid Medicago scutellata (L.) Mill as many preliminary studies have been performed on these species. The surfaces of these plants, the leaves, stems, and floral parts (except the petals), are covered with many capitate erect glandular hairs. These hairs secrete an exudate which is believed to either immobilize the weevil on the plant, causing death to the weevil, or perhaps emit a substance toxic to the weevil.[2,3,7]

The purpose of this project is to provide chemical identification of the exudates from the erect glandular hairs of M. scutellata and M. sativa L. subsp. praefalcata. This will help to determine if alfalfa weevil mortality is due to toxic components of the exudate or to total immobilization. Previous, unpublished studies have been conducted with M. disciformis exudate. Researchers performing these studies proposed the primary component of the exudate to be a long chain aliphatic hydrocarbon with ester and/or keto functions.[8] M. scutellata was chosen over M. disciformis for the project described in this thesis because of its superior vigor.

Alfalfa in the United States has shown little resistance to the alfalfa weevil and does not contain erect glandular hairs on the plant parts as do the annual Medicago species. However, this characteristic has been bred into the tetraploid perennial M. sativa L. subsp. praefalcata. Plants selected from this population were resistant to the alfalfa weevil larva in the greenhouse.[3]

Studies have been performed on the glandular secretory systems of both the annual and perennial Medicago species.[9,10] Small procumbent glands (non-erect hairs) composed of a few cells and erect glands (erect hairs) composed of numerous head cells and multicellular stalks were found on both the annuals and perennials. Erect glands outnumbered the procumbent glands on the annual species observed. Both the procumbent and erect glands exhibited secretory activity but not in equal amounts. The procumbent glands secrete only a small amount of exudate, which becomes hard and dry. These procumbent glands are not known to provide resistance to the insect predators of alfalfa. The erect glands produce plentiful secretions, apparently intermittently during the life of the plant. Sudan staining indicates that lipids are present as the main components.[10] It has been shown that high resistance to

pests of alfalfa can be attributed to the erect hairs.

Scanning electron micrographs show the density of the erect glands of M. scutellata (Figure 1) and M. sativa L. subsp. praefalcata (Figure 5). Alfalfa weevil larvae appear to be immobilized by the erect glandular hairs on the vegetative parts of M. scutellata (Figures 1-3). A weevil larva, apparently confused by the "forest" of hairs (Figure 1), raises up to see over the hairs (Figure 2) and gets caught in the sticky glands of M. scutellata (Figure 3). Secretion blisters are seen on the head of an erect glandular hair (Figure 1A). The secretion blisters have broken on the heads of the hairs and exudate is oozing down the stalks (Figures 1B and 2A). Only a few procumbent glands are seen among the erect glands of M. scutellata (Figure 3A). The exudate from erect glands of M. sativa L. subsp. praefalcata appears to immobilize spider mites (Figures 4 and 5). Blotches of exudate are covering the erect glands of M. sativa L. subsp. praefalcata (Figure 5). Secretion blisters are also seen on the heads of these hairs (Figures 4 and 5A). Exudate oozes down the stalk of an erect glandular hair (Figure 5B).

Exudate was collected from M. scutellata and M. sativa L. subsp. praefalcata plants which had been raised in the greenhouse. Collection was made to a much lesser extent on the latter plant because it was not as robust as M. scutellata and did not secrete as much exudate. The samples were then taken to the laboratory and prepared for chromatographic analysis.

FIGURE 1

Scanning electron micrograph (x70) of an alfalfa weevil larva attempting to climb up the stem of Medicago scutellata (Courtesy of the Departments of Agronomy and Entomology, Kansas State University)

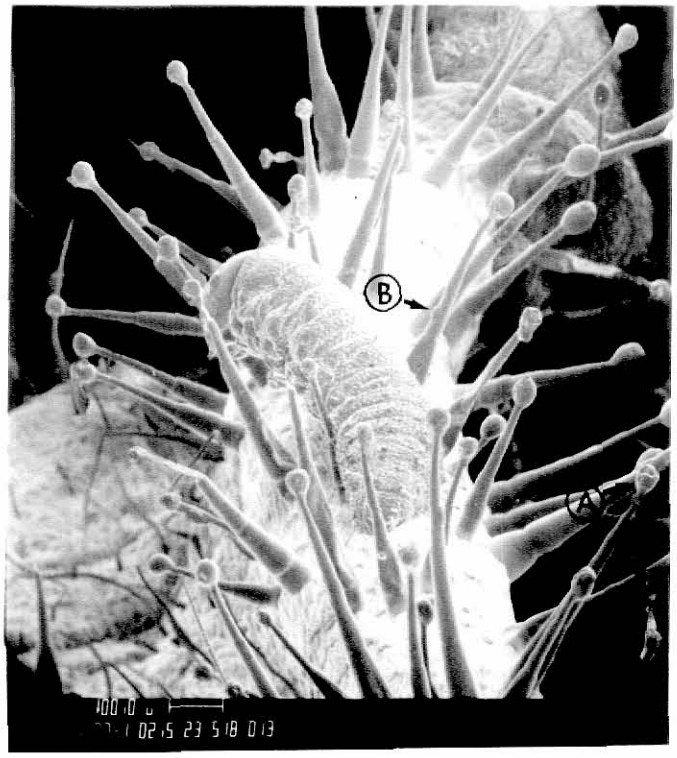

B
A

FIGURE 2

Scanning electron micrograph (x100) of an alfalfa weevil larva raising itself to see over the erect glandular hairs of Medicago scutellata

(Courtesy of the Departments of Agronomy and Entomology, Kansas State University)

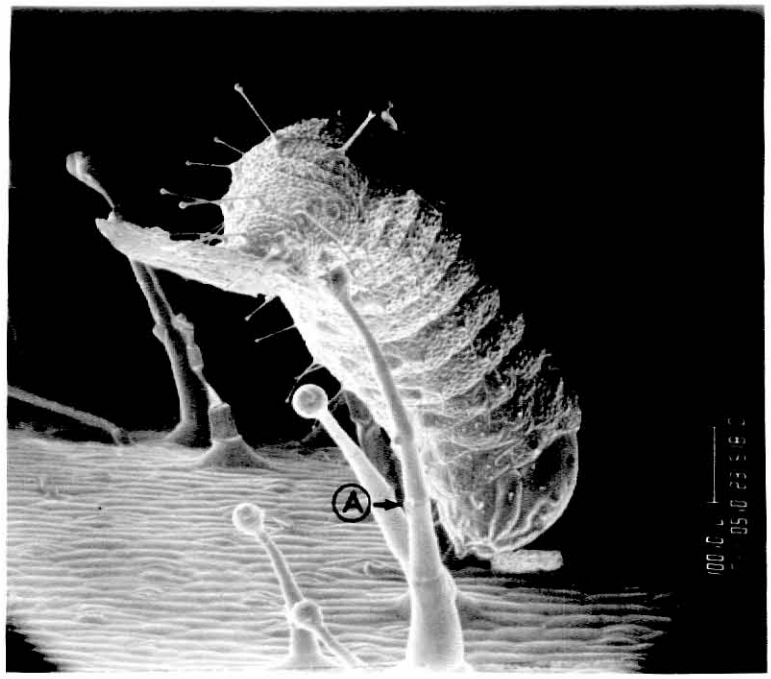

A

FIGURE 3

Scanning electron micrograph (x100) of an alfalfa weevil larva caught in the erect glandular hairs of Medicago scutellata (Courtesy of the Departments of Agronomy and Entomology, Kansas State University)

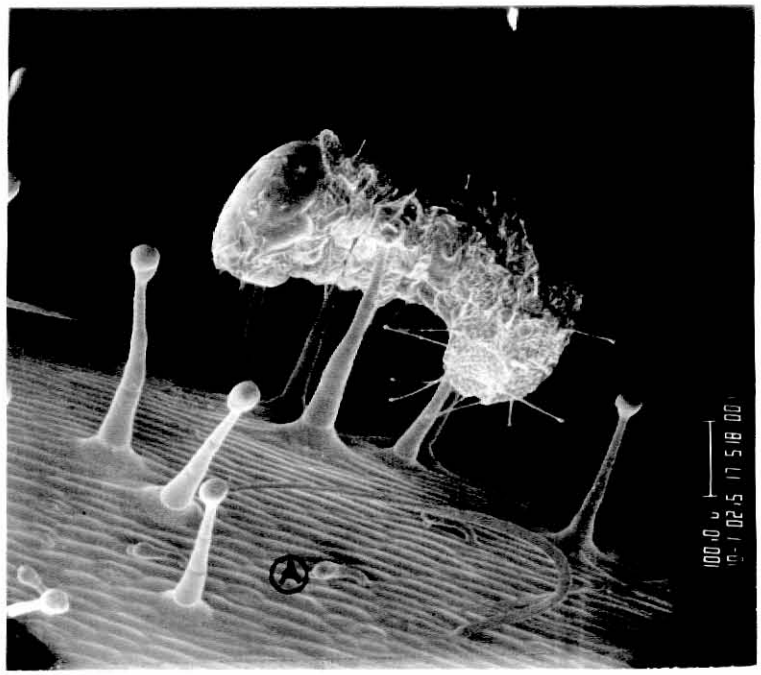

100.0 µm
10-1 02.5 17 5.18 00

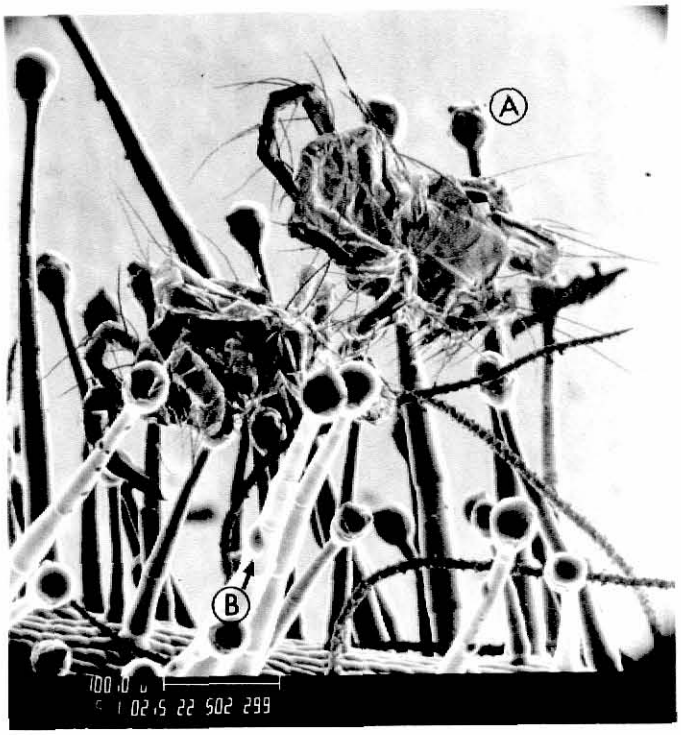
FIGURE 4

Scanning electron micrograph (x150) of a spider mite "glued" to an erect glandular hair of Medicago sativa L. subsp. praefalcata (Courtesy of the Departments of Agronomy and Entomology, Kansas State University)


100.0 µ
15-1 02:5 21 605 005

FIGURE 5

Scanning electron micrograph (x150) of two spider mites caught in the erect glandular hairs of *Medicago sativa* L. *subsp. praefalcata* (Courtesy of the Departments of Agronomy and Entomology, Kansas State University)


A
B

EXPERIMENTAL

Chemicals

All chemicals used were of reagent grade unless otherwise specified.

The glass rod used in sample collection was etched with hydrofluoric acid, lot number 45417, obtained from J.T. Baker Chemical Company.

Chemicals used in sample preparation were chloroform and benzene, both obtained from the Fisher Scientific Company. Nitrogen gas was passed through Drierite obtained from the W. A. Hammond Drierite Company.

Standards for GC analysis were: ethyl oleate, lot number A5E, obtained from the Eastman Kodak Company and 4-tert-butylphenol, lot number 5411CE, obtained from the Aldrich Chemical Company, Inc.

Insecticides used were: Omite 30W obtained from Uniroyal, White Fly Spray obtained from the Patterson Chemical Company, Pentac WP obtained from the Hooker Chemicals and Plastics Corporation, and Cygon 2-e obtained from the Black Leaf Products Company.

Instruments

The following gas chromatographs were used in the laboratory: Tracor model 560 with a flame ionization detector (FID), Hewlett-Packard model 700 with a FID, and a Bendix model 2200 with a FID.

The following gas chromatography/mass spectrometry systems were used: Finnegan model 4000 system with an Encos data system located at the Midwest

Research Institute in Kansas City, Missouri and an AEI MS5076 mass spectrometer with a Perkin-Elmer Sigma 2 gas chromatograph and a Kratos data system at the Midwest Center for Mass Spectrometry, Lincoln, Nebraska. The Finnegan 4000 mass spectrometer is a quadrupole unit while the AEI MS5076 is a magnetic sector instrument.

Gas Chromatography Column Packings

The following GC column packings were used:

3% OV-101, lot number 8169, obtained from Applied Science Laboratories, Inc., on 80/100 Chromosorb W AWDMCS, lot number 863, obtained from the Johns-Manville Sales Corporation

3% OV-17, lot number 8240, obtained from Applied Science Laboratories, Inc., on 80/100 Chromosorb W AWDMCS, lot number 863, obtained from the Johns-Manville Sales Corporation

3% OV-225, lot number 8250, obtained from Applied Science Laboratories, Inc., on 80/100 Chromosorb W HP, lot number 331, obtained from Analabs, Inc.

3% OV-1 on 80/100 Supelcoport obtained from Supelco, Inc.

5% SE-30 obtained from Wilkens Instrument and Research, Inc., on 80/100 Chromosorb W AWDMCS, lot number 863, obtained from the Johns-Manville Sales Corporation

3% Dexsil 300GC, lot number 1125, obtained from the Dexsil Chemical Corporation, on 80/100 Chromosorb W HP, lot number 85, obtained from the Johns-Mansville Sales Corporation

3% SP-2250 on 100/120 Supelcoport, lot number GA1973, obtained from Supelco, Inc.

Raising the Plants

Medicago disciformis and Medicago scutellata seeds were obtained from the Department of Agronomy. The surfaces of the seeds were scratched with sand paper until they were no longer shiny and then placed in a germination chamber. The humidity in the chamber was kept very high with the temperature between 70 and 80°F. The sand paper treatment helps the seeds absorb moisture.

Three days later, many of the seeds had roots 5-6 mm long and were ready to plant. In preparation for the planting, peat pots were partially filled with soil which was pressed down to form a uniform surface. The germinating seeds were then placed on the soil (one seed to a peat pot) and were sprinkled with Nitragin, a black powdery substance consisting of bacteria in ground peat moss. The plant then obtains its nitrogen from the bacteria. The seeds were then covered with enough sand to prevent them from being washed away when watered. Sand is used to prevent diseases that develop under moist conditions. The sand does not hold moisture but dries out, so there is no moisture to support disease organisms. The seeds were watered and placed in the growth chamber. The chamber was continuously illuminated and the temperature was kept between 68 and 72°F.

Optimum Procedure for Sample Collection, Preparation, and Analysis

In 26 days, the plants were placed in the greenhouse where the collection of the plant exudate could begin. It was discovered that M. scutellata, being a more robust plant than than M. disciformis, seemed to produce more

exudate and the collection was easier with this plant. Studies of the Medicago annual species were performed only with M. scutellata. Collections were also made on M. sativa L. subsp. praefalcata, a perennial. This plant was not nearly as robust as M. scutellata and was harder to collect from. For this reason, more emphasis was placed on the chemical identification of M. scutellata exudate and the small amount of M. sativa exudate collected was used to see if some components were the same in the two exudate samples.

The following is the optimum procedure found for the collection of the exudate, the preparation of the sample, and the GC analysis of the exudate sample:

Collection of the exudate was best on sunny days, between 11:30 a.m. and 1:00 p.m., when the temperature of the greenhouse was 85 to 95°F and the exudate was the most fluid. The younger plants gave off more exudate than the older ones and the petiol or leaf stem was the best part of the plant for collection since the hairs are the most dense there. The exudate adhered more readily to a rough surface than a smooth one so the surface of a glass rod was etched with concentrated hydrofluoric (HF) acid by immersing the rod in HF for about one minute. The etched glass rod was rolled in a spiral fashion across the stem of the plant until approximately 6 cm of the rod was covered with exudate. The rod was then immersed and stirred in a 9.5 cm tall vial of $CHCl_3$. The cap of the vial was lined with teflon to prevent contaminants from entering the sample. This process was repeated (roll rod along stem and immerse and stir it in $CHCl_3$) many times on several different plants until 45 minutes to one hour had elapsed from when collection had begun.

The sample contained in $CHCl_3$ was condensed to about 2 ml (by running a gentle stream of N_2 over the vial) and then filtered with a Waters Associates' Sample Clarification Kit, with a filter for organics, pore size 0.5 micrometers. The filtering syringe was then rinsed several times with $CHCl_3$, the washings collected and added to the filtered sample, and the total sample condensed to approximately 0.5 ml with N_2. Due to the small concentration of exudate in each sample, it is best to combine several of these samples that have been collected, condensed, and filtered in the manner described above. The combined sample was condensed to 1 ml or less and stored in a 1 to 2 ml capacity Wheaton microproduct vial with a teflon cap liner.

The best gas chromatographic results were obtained with the following conditions:

Column: stainless steel, 1/8 inch O.D. by 6 foot length, packed with 3% OV-101 on 80/100 Chromosorb W AWDMCS

The column should have been conditioned for at least 8 hours before use with these specifications:

carrier gas (N_2) flow: approximately 25 ml/min.

injection port temperature: 350°C

temperature program: 50°C hold 15 min.

50 to 325°C at 5°/min.

325°C hold for 8 hours or more

Carrier gas: N_2 at 30 ml/min.

Injection port temperature: 325°C

Use a high temperature, low bleed septum.

Detector: Flame ionization

Detector temperature: 350°C

Temperature program: 150°C hold 2 min.

150 to 300°C at 10°/min.

300°C hold 3 min.

Sample size: 1 to 2 microliters, depending on sample concentration

Detector attenuation will vary with sample concentration.

Gas Chromatography Columns

Several gas chromatography (GC) columns were used for the separation of the exudate components. All of the columns were made from 1/8 inch O.D., 6 foot lengths of stainless steel, except the OV-1 column used at the Midwest Research Institute. It was a glass column, 1/4 inch O.D., and 6 feet in length. The OV-101 column of 3% loading on Chromosorb W AWDMCS gave the best resolution of the exudate components with this temperature program:

150°C hold 2 min.

150-300°C at 10°/min.

300°C hold 3 min.

The other columns were tried but were not used for GC/MS work for the following reasons:

A column of 3% OV-225 on Chromosorb W HP was used and programmed to 225°C. Only a few peaks on a hump were shown on the GC chromatogram. The maximum temperature of the OV-225 column is only 250° which is not a high enough temperature to elute all of the components from the column. The column may also be too polar.

A loading of 3% SE-30 on Chromosorb W AWDMCS was also used but programmed to 300°C. Several broad peaks on a hump were shown on the GC chromatogram. Because of its poor resolution, this column was not used further.

A column containing 3% OV-17 on Chromosorb W AWDMCS gave a fairly good separation of the components when programmed to 300°C. Many peaks were present on the GC chromatogram, most on a hump. However, the OV-101 column seemed to give slightly better response and separation with the same samples.

The 3% SP-2250 on 100/120 Supelcoport column gave a chromatogram of only seven peaks, all on a hump, as the column was programmed to 300°C. The resolving power of this column was not sufficient for the intended research.

A Dexsil 300GC column of 3% loading on Chromosorb W AWDMCS was used because of its stability at high temperatures. The column was programmed to 350°C but it appeared that no components came off the column above 300°. The chromatogram produced was similar to that of OV-101 (again a hump was present) but the peaks did not appear to be as well resolved as those of the OV-101 chromatogram.

Insecticides

Four different insecticides had been applied to the *M. scutellata* plants in the greenhouse. Because of this application, these insecticides may be present in the exudate sample. In order to eliminate peaks from these insecticides that may appear on the gas chromatogram of the exudate sample, GC studies were done. Available information about each insecticide is listed below along with the results of the GC studies.

The following GC program was used with each column and each sample:

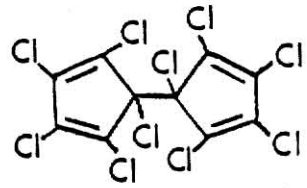
150°C hold 2 min.

150-300°C at 10°/min.

300°C hold 3 min.

Pentac WP Miticide

active ingredient: Bis(pentachloro-2,4-Cyclopentadien-1-yl)



$C_{10}Cl_{10}$

Pentac is an acaricide expressing long residual activity and therefore will be present on the plant for some time after application. It is a solid at room temperature, with the consistency of a powder and light brown in color. Particles of Pentac could stick to the glass rod when it is applied to the plant during sample collection.

GC Studies with Pentac

Several particles of the Pentac powder were placed in a small vial containing 1 ml of $CHCl_3$. The vial was shaken to aid the powder in going into solution. However, the particles did not appear to dissolve. The sample was filtered to prepare for injection onto the GC column.

Three different columns of these stationary phases were used: OV-101, Dexsil 300GC, and SP-2250. On injection of the Pentac sample, no peaks but the solvent peak appeared for any of the three columns. This result leads to one of the following conclusions:

1) Pentac is slightly soluble in $CHCl_3$ but does not give a response with a

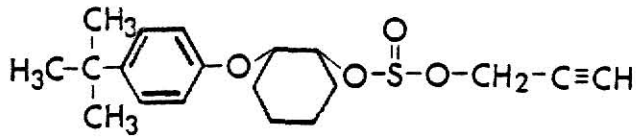
Flame Ionization Detector (FID). If this is the case, Pentac should show up in the mass spectral data.

2) Pentac is not soluble in $CHCl_3$ and will not be present in the exudate sample unless it is soluble in the exudate itself.

Mass spectral data does not indicate that Pentac or any of its fragments are present in the exudate sample. Data taken of the exudate sample at the Midwest Center for Mass Spectrometry (MCMS) in Lincoln, Nebraska (Figures 7-52) show no chlorinated compounds. The data taken at the Midwest Research Institute (MRI) in Kansas City, Missouri (Figures 54-67) do show two chlorinated compounds, but their structures are not similar to the structure of Pentac. The mass spectrum for Pentac was found in the literature and no spectra obtained from the exudate sample were similar to the Pentac spectrum.[21]

Omite

active ingredient: 2-(p-tert-butylphenoxy)cyclohexyl-2-propynyl sulfite



$C_{19}H_{26}O_4S$

Omite expresses residual activity. It is a white, powdery solid at room temperature. If Omite is present on the plant at the time of sample collection, some particles could stick to the glass rod as it is being applied to the plant.

GC Studies with Omite

Several particles of the Omite powder were placed in a small vial containing 1 ml $CHCl_3$. The vial was shaken so the Omite could be dissolved in

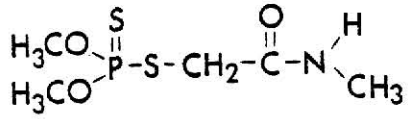
the $CHCl_3$. It appeared that some of the Omite may have gone into solution. The sample was filtered in preparation for GC analysis.

The injection of the Omite sample onto the OV-101 column produced two significant peaks. These peaks are most likely due to decomposition products of Omite. It appears that Omite is soluble in $CHCl_3$ to a certain extent and will enter the sample when Omite is collected with the exudate from the plant.

The M. scutellata exudate sample was spiked with Omite in $CHCl_3$ and injected onto the OV-101 column to determine which peaks of the gas chromatogram should be attributed to the Omite. Results showed that one low boiling component of the exudate sample is indeed a decomposition product of Omite. The higher boiling decomposition product did not seem to enhance any of the exudate sample peaks. Possibly the compound could be hidden in the "hump" of the M. scutellata spectrum and is not a major component.

Cygon

active ingredient: Dimethoate (O,O-dimethyl-S-(N-methylcarbamoylmethyl) phosphoradithioate)



$C_5H_{12}NO_3PS$

Cygon is a systemic and contact insecticide.[20] It is a very strong-smelling liquid at room temperature. It is almost colorless but has a yellow tint.

GC Studies with Cygon

One or two drops of Cygon were placed in a small vial containing 1 ml of $CHCl_3$. The vial was shaken to mix the two solutions. The Cygon appeared to dissolve readily in the $CHCl_3$.

When the Cygon sample was injected onto the OV-101 column, many peaks due to low boiling compounds were exhibited on the chromatogram. Cygon most likely decomposes in the $CHCl_3$ or on the GC column, and the decomposition products are very volatile.

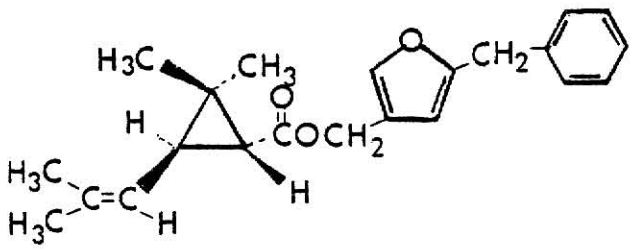
The M. scutellata exudate sample was spiked with the Cygon sample and injected onto the OV-101 column to determine which peaks of the gas chromatogram are due to Cygon. Results showed that possibly one peak may be due to Cygon. This is a very small peak near the beginning of the chromatogram. Therefore, Cygon definitely does not contribute to a major component in the M. scutellata exudate sample.

White Fly Spray (Bioresmethrin)

active ingredients: (5-benzyl-3-furyl)methyl-2,2-dimethyl-3-(2-methylpropenyl) cyclopropanecarboxylate

related compounds

aromatic petroleum hydrocarbons



$C_{22}H_{26}O_3$

Bioresmethrin is a potent synthetic pyrethroid insecticide. It shows rapid biodegradability and little or no persisting residue.[20] It is a strong-smelling liquid at room temperature, red-brown in color.

GC Studies with White Fly Spray

A few drops of Bioresmethrin were placed in a small vial containing 1 ml of $CHCl_3$. The vial was then shaken to mix the solutions. The Bioresmethrin seemed to go readily into solution.

When the Bioresmethrin sample was injected onto the OV-101 column, several peaks due to low boiling compounds and one large peak due to a less volatile compound were shown on the gas chromatogram. Only the higher boiling component received further consideration as it was in much greater concentration than the more volatile components.

The M. scutellata exudate sample was spiked with the Bioresmethrin sample. It did appear that one of the peaks, at retention time approximately 9.5 minutes, was enhanced. By the time this spiked injection was made, several injections of M. scutellata exudate sample had been made. The separating ability of the column appeared to degrade after each injection of the M. scutellata exudate sample, so resolution was not good for the spiked injection. For this reason, the peak at 9.5 minutes retention time may have been due to Bioresmethrin and another compound which were not resolved. According to the mass chromatogram of the M. scutellata exudate sample, the Bioresmethrin does not show up at 9.5 minutes. However, it may be present but masked by column bleed peaks that appear at that retention time.

RESULTS

Specifications for GC/MS

Mass spectra were taken at the Midwest Research Institute (MRI) at Kansas City, Missouri according to the following specifications:

Column: glass, 1/4 inch O.D. by 6 foot length, packed with 3% OV-1 on 80/100 Supelcoport

Carrier gas: He at 30 ml/min.

Solvent: benzene

Injection port temperature: 325°C

Temperature program: 150°C hold 5 min.
150 to 300°C at 15°/min.
300°C hold 10 min.

Sample: exudate of *M. scutellata* in benzene

Mass spectra were taken at the Midwest Center for Mass Spectrometry (MCMS) at Lincoln, Nebraska according to the following specifications:

Column: stainless steel, 1/8 inch O.D. by 6 foot length, packed with 3% OV-101 on 80/100 Chromosorb W AWDMCS

Carrier gas: He at 30 ml/min.

Solvent: $CHCl_3$

Injection port temperature: 325°C

Temperature program: 150°C hold 2 min.
150 to 300°C at 10°/min.
300°C hold 3 min.

Samples: exudate of *M. scutellata* in $CHCl_3$
exudate of *M. sativa* L. *subsp. praefalcata* in $CHCl_3$

Though different specifications were used at MRI than at MCMS, similar spectra should have been obtained for the following reasons:

1) Both column packings consisted of methyl silicon and should have given nearly identical results.

2) The carrier gas flow rates and injection port temperatures were the same.

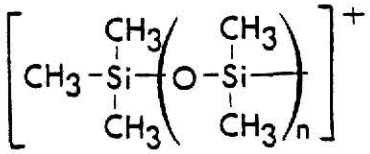
3) The temperature programs were only slightly different and so the retention times for the same compound should be very close.

4) The solvents were different but they both are very low boiling and should not interfere with the other components of the exudate.

5) The exudate samples collected were both from M. scutellata. An additional sample was collected from M. sativa L. subsp. praefalcata and run at MCMS.

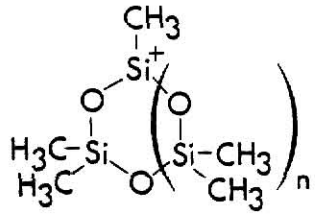
Column Bleed

Due to the high temperatures to which the column is subjected, column bleed will enter the mass spectrometer. This fact must be considered in the interpretation of the mass spectra. The following are column bleed fragments of the liquid phase OV-1 along with their masses.[22]



n = 0, 1, 2, 3, 4, 5, 6

m/e=73,147,221,295,369,443,517



n = 0, 1, 2, 3, 4, 5, 6

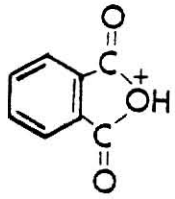
m/e=133,207,281,355,429,503,577

OV-1 is 100% methyl silicon as is OV-101, so these column bleed peaks will be the same for OV-101.

Phthalates

Some phthalic acid esters (phthalates) are present in the exudate samples, according to the following mass spectra. The presence of phthalates is indicative of contamination from plastics. Though great care was taken to keep the sample free from such contaminants, phthalates may have been picked up during sample preparation. The phthalates could also have been present due to a dirty syringe or from the septum. The latter two possibilities are more likely than contracting phthalates from sample preparation because of the four phthalates that are present in the first GC/MS set of data from the M. scutellata exudate sample taken at the MCMS in Lincoln, Neb. (Figures 7-29), only one showed up in the second set of data (Figures 31-52).

A very large peak (sometimes the base peak) at m/e 149 is a strong indication that a phthalate is present. The m/e 149 peak is due to the fragment:[13]



Explanation of 100% Intensities for MCMS Spectra

The mass intensity report on each spectrum taken at MCMS in Lincoln, Neb. (Figures 7-52) gives an indication as to how weak or strong that particular spectrum is. The 100% intensity is equated to a certain number of counts which may be different for each spectrum. Any signal below 50 counts may be due to noise. To determine what percentage intensity 50 counts is, a simple calculation can be performed. For example, in Figure 31, 100% intensity is 69 counts.

$$69 \text{ counts}/100\% = 50 \text{ counts}/n$$

$$n = 72.5\%$$

Therefore, any signal below 72.5% is most likely due to noise and cannot be interpretated.

Components Containing Base m/e 131

Several of the mass spectra of the exudate samples contain a very significant peak at m/e 131; sometimes it is the base peak. Not enough

information is contained in the spectra for interpretation. In the mass chromatogram of Medicago scutellata exudate, many of the peaks are on top of a hump, which starts at about eight minutes retention time and continues throughout the rest of the spectrum. The components which have a significant m/e 131 peak elute after the hump appears. Also, components with large m/e 131 peaks are contained in the background (hump). This background is subtracted out of all the peaks on the hump. It was proposed that these unexplainable peaks might be due to 1) bleed from the column or septum due to high temperatures or 2) insecticides.

To check for septum and column bleed, a new septum (high temperature, low bleed) was punctured and the column was programmed as for an exudate sample injection. The resulting chromatogram baseline was fairly flat, no hump appeared, indicating minimal septum and column bleed. An injection of exudate sample was then made. After all the components had eluted, the column was taken to the initial temperature and programmed to 300°C again. The resulting chromatogram baseline was still fairly flat.

Referring to the Insecticides section, it was found that two insecticides, Omite and Bioresmethrin, gave peaks at retention times close to a component which has a base peak at m/e 131 when injected in $CHCl_3$ onto the GC column. However, injections of these insecticides in $CHCl_3$ did not produce the hump observed in the M. scutellata exudate sample chromatogram. Also, injection of an exudate sample from plants not exposed to insecticides gave a chromatogram very similar to that of an exudate sample with insecticides, except the early eluting Omite peak was missing. The components which have a base peak at m/e 131 were present in the no-insecticide chromatogram along

with the hump. Therefore, these peaks do not appear to be due to insecticides.

It appears that something in the exudate sample may be reacting with the column packing. After the column has been conditioned overnight, the first injection of the exudate sample produces good resolution of the peaks on the hump and the hump is not large. However, on the second injection of the sample, resolution of peaks on the hump is poor and the hump becomes large. The column must then be conditioned again for at least eight hours to obtain adequate resolution of the exudate sample components. The column must also be repacked at least once a month. All of the columns used for the separation of the exudate sample components gave chromatograms which had a large hump. Each coating used in packing the columns contains silicon. The compounds whose spectra have a large peak at m/e 131 could contain silicon as the peaks at m/e 132 and m/e 133 are of the abundances indicating silicon. It is possible that some component(s) is(are) reacting with the silicon coating of the column and is(are) causing it to bleed off, gradually destroying the column.

Interpretation of Mass Spectra

from MCMS, Lincoln, Nebraska

of Medicago scutellata (first set of data)

Peak 1 of Figure 6

The mass spectrometer is vented for the first two minutes to rid the system of the early boiling solvent. Perhaps this peak is due to a small amount of solvent, $CHCl_3$, detected by the mass spectrometer. The retention time of this peak (0:25) is very close to that of $CHCl_3$ injected onto the OV-101 column with this temperature program. No mass spectrum was taken of this peak.

Figure 7 (Peak 2 of Figure 6)

This spectrum is very weak and therefore does not contain enough useful information for a thorough interpretation. Both an alkane pattern at m/e 57, 71, and 85 and an alkene pattern at m/e 55, 69, and 83 are present. Because of the low intensity of this spectrum, the peak at m/e 149 is not significant. It is most likely due to noise and not to true signal. Since insecticides such as Cygon, Omite, and Bioresmethrin that were used on the plants give low boiling peaks upon their injection in $CHCl_3$, this peak may be due to some insecticide fragments.

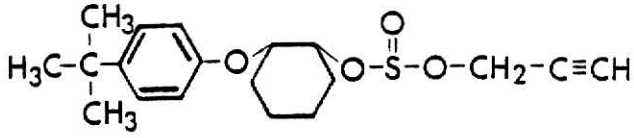
Figure 8 (Peak 3 of Figure 6)

This spectrum is also very weak. Only a few observations can be made about the compound producing this spectrum. An alkane pattern is present at m/e 57, 71, and 85 and an alkene pattern also, at m/e 55, 69, 83, and 97. The large peak at m/e 88 suggests an oxygen containing compound. The spectrum is much too weak to interpret fully.

Figure 9 (Peak 4 of Figure 6)

From earlier GC work (see Insecticides section), it has been shown that this peak arises from a decomposition product of the insecticide Omite.

Omite has the following structure:



From the mass spectrum, the compound that this peak is attributed to contains the p-tert-butylphenol group.

1) From m/e 150 to m/e 135 there appears to be a significant loss of CH_3, most likely from more than one CH_3 group. The base peak arises from the fragment $(CH_3)_2CC_6H_5OH$.

2) There appears to be a further loss of two CH_3 groups and the C to which these CH_3 groups were attached picks up 2 H's to form $CH_2C_6H_5OH$ and the peak at m/e 107.

3) The peak at m/e 91 arises from $CH_2C_6H_5$.

4) The peak at m/e 77 arises from C_6H_5.

5) The peak at m/e 57 indicates the t-butyl group on the phenol.

However, the compound is not p-tert-butylphenol, but one of higher molecular weight containing p-tert-butylphenol as a group. This was shown by injecting a standard of p-tert-butylphenol in $CHCl_3$ onto the OV-101 column. The retention time of p-tert-butylphenol was only approximately two minutes while the early boiling Omite component had a retention time of approximately six minutes.

Figure 10 (Peak 5 of Figure 6)

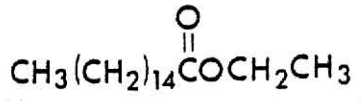
The base peak of this compound is m/e 149, which suggests a phthalate. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component in the exudate.

Figure 11 (Peak 6 of Figure 6)

The large peak at m/e 149 in this spectrum suggests that a phthalate is present. However, when comparison of this spectrum is made with that of Peak 7 of Figure 30 (Figure 35) whose retention time (7:25) is very close to the retention time of Peak 6 of Figure 6 (7:32), these spectra are very similar. The only significant differences are that Figure 35 does not contain peaks at m/e 149 and m/e 251, while this spectrum (Figure 11) does contain these peaks. A further comparison of Figure 11 with Figure 10 indicates that these peaks are common to both at m/e 149 and m/e 251 (of significant intensity) and those peaks at m/e 76, 93, 125, 150, and 167 (at lower intensities). Those peaks that have been cited as being common in Figures 10 and 11 are not common in

Figures 11 and 35. Therefore, it is proposed that Peak 6 of Figure 6 contains some of the phthalate which eluted immediately before this peak. When this phthalate spectrum is subtracted from Figure 11, a spectrum arises which is nearly identical to Figure 35.

Proposed structure:



1) The parent peak is taken to be at m/e 284.

2) The large peak at m/e 239 indicates a loss of CH_3CH_2O (mass 45). Esters where the predominant portion is the acid portion tend to first lose R'-O (where the ester is RCO_2R').[13]

3) The peak at m/e 157 indicates a further loss of $(CH_2)_4CO$ from the molecule.

4) The peak at m/e 143 shows a further loss of CH_2, and from m/e 143 to m/e 115, a loss of CH_2CH_2.

5) The significant peak at m/e 101 arises from the fragment $CH_2CH_2CO_2CH_2CH_3$.

6) The base peak at m/e 88 indicates $CH_2C(OH)OCH_2CH_3$. A significant peak at m/e 88 is common for ethyl esters.[14]

Figure 12 (Peak 7 of Figure 6)

The base peak at m/e 149 indicates a phthalate (see Phthalates section). An alkene pattern is apparent at m/e 55, 69, 83, and 97, but the spectrum is too weak to determine if another compound is present besides the phthalate, as in Figure 11.

Figure 13 (Peak 8 of Figure 6)

Proposed structure:

$$CH_3(CH_2)_7CH=CH(CH_2)_7\overset{O}{\overset{\|}{C}}OCH_2CH_3$$

1) The significant peak at m/e 310 is taken as the parent peak.

2) The large peak at m/e 264 indicates a loss of CH_3CH_2OH. Esters where the predominant portion is the acid portion tend to first lose R'-O (where the ester is RCO_2R').[13]

3) The peak at m/e 222 indicates a further loss of CH_2CO from the molecule. After losing R'-O, esters where the predominate portion is the acid, then lose RCO or CH_2CO.[13]

4) The significant peak at m/e 123 indicates a loss of C_7H_{15}, which breaks the molecule to $CH_3(CH_2)_7CH=CH$.

5) A loss of CH then creates a peak at m/e 110.

6) The large peak at m/e 88 is possibly due to the fragment $CH_2C(OH)OCH_2CH_3$.

7) The significant peak at m/e 101 arises from the fragment $CH_2CH_2CO_2CH_2CH_3$.

8) The pattern of peaks at m/e 55, 69, 83, 97, etc. indicates an alkene. It is very difficult to place the double bond. The basis for placing the double bond at the C_9 position of the acid is:

a) the loss of 99 (C_7H_{15}) from m/e 222 to m/e 123 and then the loss of 13 (CH) from m/e 123 to m/e 110 and

b) this spectrum matches very well with that of ethyl oleate.[18]

9) The M. scutellata exudate sample was spiked with ethyl oleate and Peak 8 of Figure 6 was enhanced. Therefore, this peak is positively identified as being ethyl oleate.

Figure 14 (Peak 9 of Figure 6)

1) The parent peak is taken to be at m/e 342.

2) The loss of 15 from m/e 342 to m/e 327 indicates a loss of CH_3.

3) An alkane pattern is present at m/e 57, 71, 85, and 99 and an alkene pattern at m/e 55, 69, 83, and 97.

No further interpretation could be done on this spectrum. See the special section on the components whose spectra contain m/e 131 base peaks for further postulations.

Figure 15 (Peak 10 of Figure 6)

This spectrum is very similar to Figure 14. This similarity may arise from the fact that Peaks 9 and 10 of Figure 6 are not well resolved. Therefore, Peak 10 could contain some background from Peak 9.

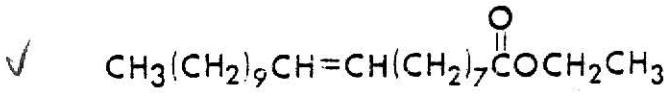
1) The parent peak is taken to be at m/e 342.

2) The loss of 15 from m/e 342 to m/e 327 indicates a loss of CH_3.

See the special section for components with spectra containing base peaks at m/e 131 for further discussion.

Figure 16 (Peak 11 of Figure 6)

Proposed structure:



$$CH_3(CH_2)_9CH{=}CH(CH_2)_7\overset{\overset{O}{\|}}{C}OCH_2CH_3$$

1) The peak at m/e 338 is taken as the parent peak.

2) A loss of CH_3CH_2O gives a peak at m/e 292. This indicates an ester whose

predominate portion is the acid.

3) The peak at m/e 250 then indicates a loss of CH_2CO.

4) The peak at m/e 88 is due to the fragment $CH_2C(OH)OCH_2CH_3$.

5) The peak at m/e 101 is due to the fragment $CH_2CH_2CO_2CH_2CH_3$.

6) An alkene pattern is present at m/e 55, 69, 83, 97, etc., indicative of an alkyl double bond. The position of the double bond is not as apparent as in the ethyl oleate spectrum--no reference spectrum was available. Possibly the unusual loss of 24 from m/e 135 to m/e 111 could indicate the double bond. This would position it at C_9 of the acid. Since this spectrum also is very similar to the spectrum of ethyl oleate, only 28 units longer, the double bond is tentatively assigned to the C_9 position of the acid.

Figure 17 (Peak 12 of Figure 6)

The base peak of m/e 149 in this spectrum indicates that the compound represented here is most likely a phthalate. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component in the exudate.

Figure 18 (Peak 13 of Figure 6)

A phthalate is probably present as indicated by the large peak at m/e 149. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component in the exudate. There is a strong alkene pattern present at m/e 55, 69, 83, etc. After m/e 149, the peaks are all fairly small and in no particular pattern. Since the spectrum

is fairly weak and therefore contains noise spikes, the high molecular weight peaks are not interpretable. The phthalate could be masking another compound which has an alkene function. However, there is not enough information to be obtained from this spectrum to determine if there is another compound present in addition to the phthalate.

Figure 19 (Peak 14 of Figure 6)

The peak giving this spectrum is not well separated from Peak 15 of Figure 6, and is in fact the shoulder of Peak 15. Therefore, this spectrum probably contained some of the Peak 15 spectrum (Figure 20).

1) The parent peak is taken to be at m/e 396.

2) The loss of 15 from m/e 396 to m/e 381 indicates a loss of CH_3.

See the special section on components whose specta contain base peaks at m/e 131 for further discussion.

Figure 20 (Peak 15 of Figure 6)

1) The parent peak is taken to be at m/e 398.

2) The loss of 15 from m/e 398 to m/e 383 indicates a loss of CH_3.

This spectrum is very intense, but aside from the base peak at m/e 131, the other peaks of any significance are all less than 10% intensity. The spectrum is therefore difficult to interpret. The special section on components containing base m/e 131 peaks in their spectra provides further discussion.

Figure 21 (Peak 16 of Figure 6)

Proposed structure: straight chain alkyne or alkyl diene of C_{26}

1) The parent peak is hard to locate. It could possibly be at m/e 366 because a loss of 15 (CH_3) is seen from m/e 366 to m/e 351. However, this molecular weight does not make sense for the alkyne or alkyl diene.

2) The peak at m/e 57 indicates the alkane fragment C_4H_9, while the base peak at m/e 69 indicates the alkene fragment C_5H_9, and the peak at m/e 81 indicates the alkyne or alkyl diene fragment C_6H_9. The two unsaturations pattern continues at m/e 95, 109, 123, 137, ..., 333.

3) Since the large peaks of the spectrum occur at the low molecular weights and the peak intensities tail off in the high molecular weights, the compound appears to be straight chained. If the compound is a straight chain alkyne or alkyl diene, the parent peak may not be apparent.

4) The peak at m/e 100 is significant but hard to explain. It may be due to $C_6H_{12}O$, but it is difficult to fit this fragment into the rest of the spectrum.

5) Column bleed peaks are present at m/e 73, 143, 207, 221, 281, 295, 355, 429, and 503.

Figure 22 (Peak 17 of Figure 6)

Proposed structure: straight chain alkane of length C_{28} or greater

Peaks 16 and 17 of Figure 6 are not well resolved and therefore each spectrum for the individual peaks may not be of only one compound. Both spectra (Figures 21 and 22) have posed problems in interpretation and so no definite structures will be assigned with the data available.

A strong alkane pattern is shown in this spectrum (Peak 17 of Figure 6, retention time 14:38) as in Figure 23 (Peak 18 of Figure 6, retention time 14:51) and Figure 48 (Peak 20 of Figure 30, retention time 14:51). The compound giving rise to this spectrum could be a straight chain alkane of high molecular weight (larger than C_{28}) whose parent peak is not detected. If so, the peaks at m/e 149 and m/e 292 are due to impurities. This compound doesn't appear to be a branched alkane because 292 and 149 are not correct mass units for alkane or alkene fragments. The peaks at m/e 429 and m/e 503 are column bleed peaks.

Figure 23 (Peak 18 of Figure 6)

Proposed structure: straight chain alkane or

branched alkane of length C_{36} or greater

1) A very strong alkane pattern is present at m/e 57, 71, 85, etc.

2) Column bleed peaks are present in significant intensities at m/e 207, 281, 355, 429, and 503.

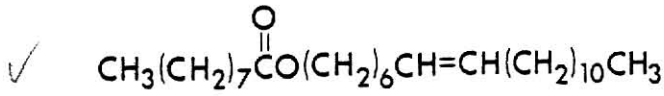
3) Since the peak giving this spectrum is not well resolved and is not symmetric in shape, there is a possibility that more than one compound is being fragmented in this spectrum. Therefore, comparison is made with Figure 48, the spectrum of a peak (Peak 20 of Figure 30) having the same retention time as this peak. Peak 20 of Figure 30 is well resolved and well shaped. Its spectrum seems to be that of a straight chain alkane. Therefore, it is likely that this spectrum is also that of a straight chain alkane with the peak at m/e 408 arising from an impurity.

4) If the peak at m/e 408 is not an impurity, it could arise from the fragment $CH_3(CH_2)_2CH(CH_2)_{24}CH_3$. This would indicate that the compound is a branched

alkane whose parent peak is not detected in the spectrum.

Figure 24 (Peak 19 of Figure 6)

Proposed structure:



1) The large peak at m/e 422 is taken to be the parent peak.

2) The significant peak at m/e 264 indicates a loss of $CH_3(CH_2)_7CO_2H$. Esters where the predominant portion is the alcohol lose a molecule of acid in the same way alcohols lose water.[17]

3) The large peak at m/e 158 is from the acid $CH_3(CH_2)_7CO_2H$.

4) An alkyl pattern is present all through the spectrum indicating a long chain alkane function, no branching is likely.

5) The peaks at m/e 111 and m/e 113 are of the same intensity and indicate a double bond at the C_7 position of the alcohol. However, the double bond tends to migrate during mass fragmentation and so its location cannot be definitely determined.

Figure 25 (Peak 20 of Figure 6)

Proposed structure: straight chain alkane of length C_{36} or greater

1) The alkane pattern of peaks at m/e 57, 71, 85, etc. continues visibly until the peak at m/e 393 and then the pattern tails off.

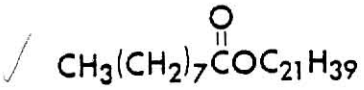
2) In the spectra of many high molecular weight alkanes, the parent peak is not visible as is possibly the case with this spectrum.[17]

3) The large peak at m/e 281 and smaller peaks at m/e 355, 429, and 503 are

due to column bleed.

Figure 26 (Peak 21 of Figure 6)

Proposed structure:



(no branching)

1) The parent peak is taken to be at m/e 448.

2) The peak at m/e 291 could arise from the loss of $CH_3(CH_2)_7CO_2$, indicating an ester whose predominant portion is the alcohol.

3) A loss of 24 is seen from m/e 291 to m/e 267. This could possibly be due to loss of C≡C, but it is highly unlikely.

4) The peak at m/e 158 is due to the acid $CH_3(CH_2)_7CO_2H$.

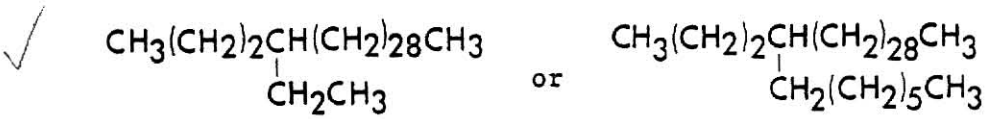
5) A strong alkyl pattern is present at m/e 57, 71, 85, etc.

6) Column bleed peaks of significant intensity are present at m/e 280, 354, 428, and 502.

7) It appears that the alkyl fragment (after $C_8H_{17}CO_2$ is lost) of the molecule has a mass of 291, indicating $C_{21}H_{39}$. This formula suggests two unsaturations in the form of a triple bond or two double bonds. However, a strong alkane pattern is shown in the spectrum. Aside from the loss of 24 from m/e 291 to m/e 267 the spectrum does not give any further indication of the position(s) of the unsaturations.

Figure 27 (Peak 22 of Figure 6)

Proposed structure:



1) There are two possibilities for the parent peak of this spectrum, m/e 492 or m/e 562.

2) A loss of C_2H_4 (mass 28) or C_7H_{14} (mass 98) results in the significant peak at m/e 464. This indicates branching in the alkane.

3) A strong alkane pattern is present throughout the spectrum at m/e 57, 71, 85, etc.

4) The significant intensity of the peak at m/e 281 is due to column bleed. The peaks at m/e 355, 429, and 503 are also due to column bleed.

5) The large peak at m/e 57 indicates possible branching at the C_4 position.

Figure 28 (Peak 23 of Figure 6)

Proposed structure: ester or straight chain alkane of length C_{33} or greater

1) The parent peak is not apparent in this spectrum.

2) A strong alkane pattern is present throughout the spectrum at m/e 57, 71, 85, ..., 323.

3) The large peak at m/e 479 could be due to a number of things:

a) The compound represented by this spectrum could be an ester where the $CO(CH_2)_n$ group is lost first, leaving $CH_3(CH_2)_{31}CH_2O$, and giving rise to the peak at m/e 479. The parent peak may not be discernable due to the weak spectrum.

b) The compound represented here may be an ester whose alkyl portion is

lost first, leaving $CH_3(CH_2)_{30}CO_2$, giving a peak at m/e 479. Again the parent peak is not detected.

c) The peak at m/e 479 could be due to an oxygen containing fragment, but not in the form of a double bond (C=O).

d) The peak at m/e 479 could be due to an impurity or an artifact, and if that is the case, this compound is most likely a straight chain alkane of length C_{33} or greater.

Figure 29 (Peak 24 of Figure 6)

This spectrum is very weak. Much of the spectrum is due only to noise and column bleed. Column bleed peaks are present at m/e 147, 207, 281, and 503. This is to be expected as this is the last peak of the GC/MS run.

FIGURE 6

Mass chromatogram of *Medicago scutellata* exudate sample for the first GC/MS run at MCMS, Lincoln, Nebraska

DS-55 CROSS SCAN REPORT, RUN: 1757A

+ TIC

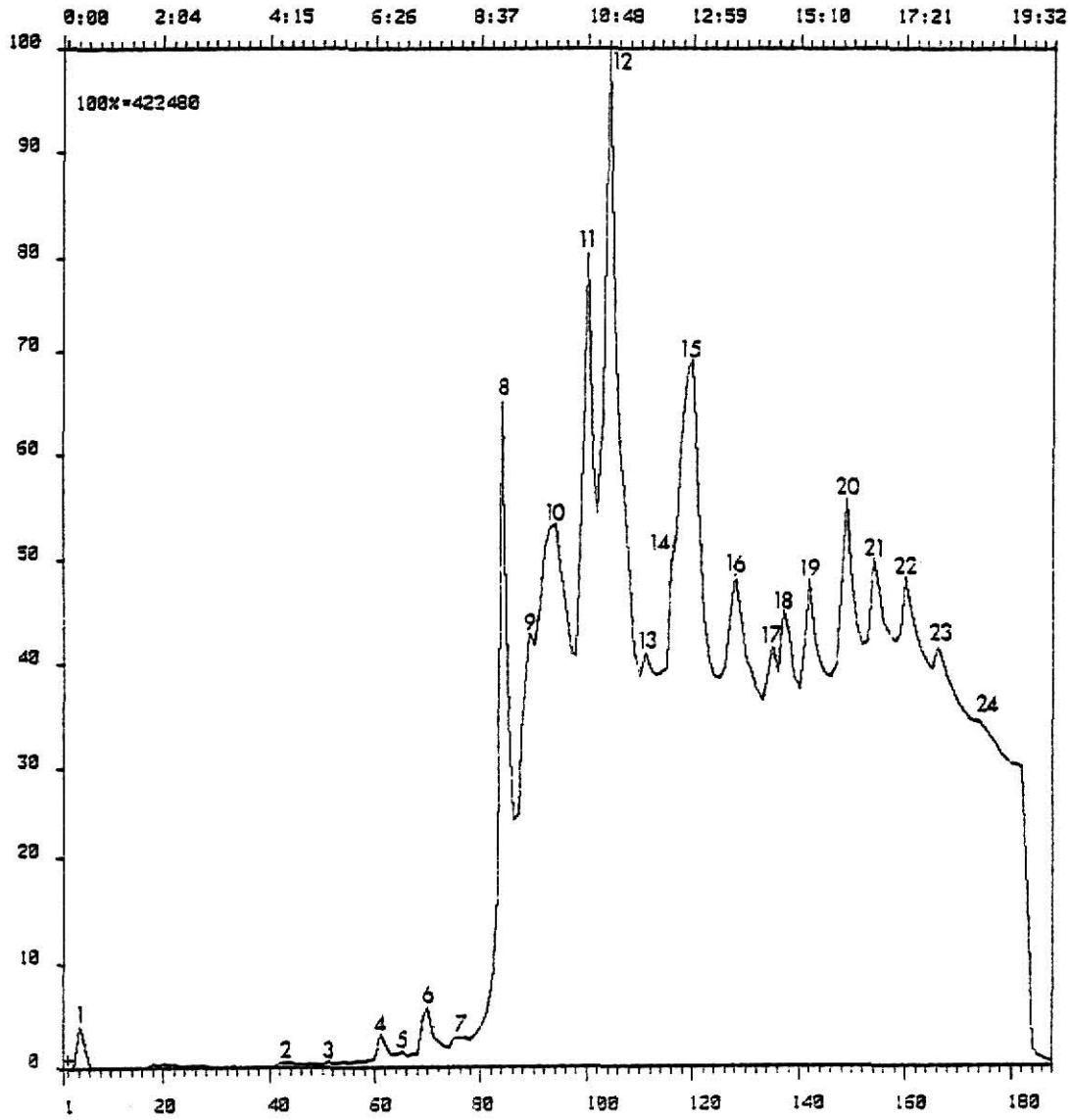


FIGURE 7

Mass spectrum of

Peak 2 of Figure 6

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
DISP8K.42 [TIC=975, 100%=162] EI

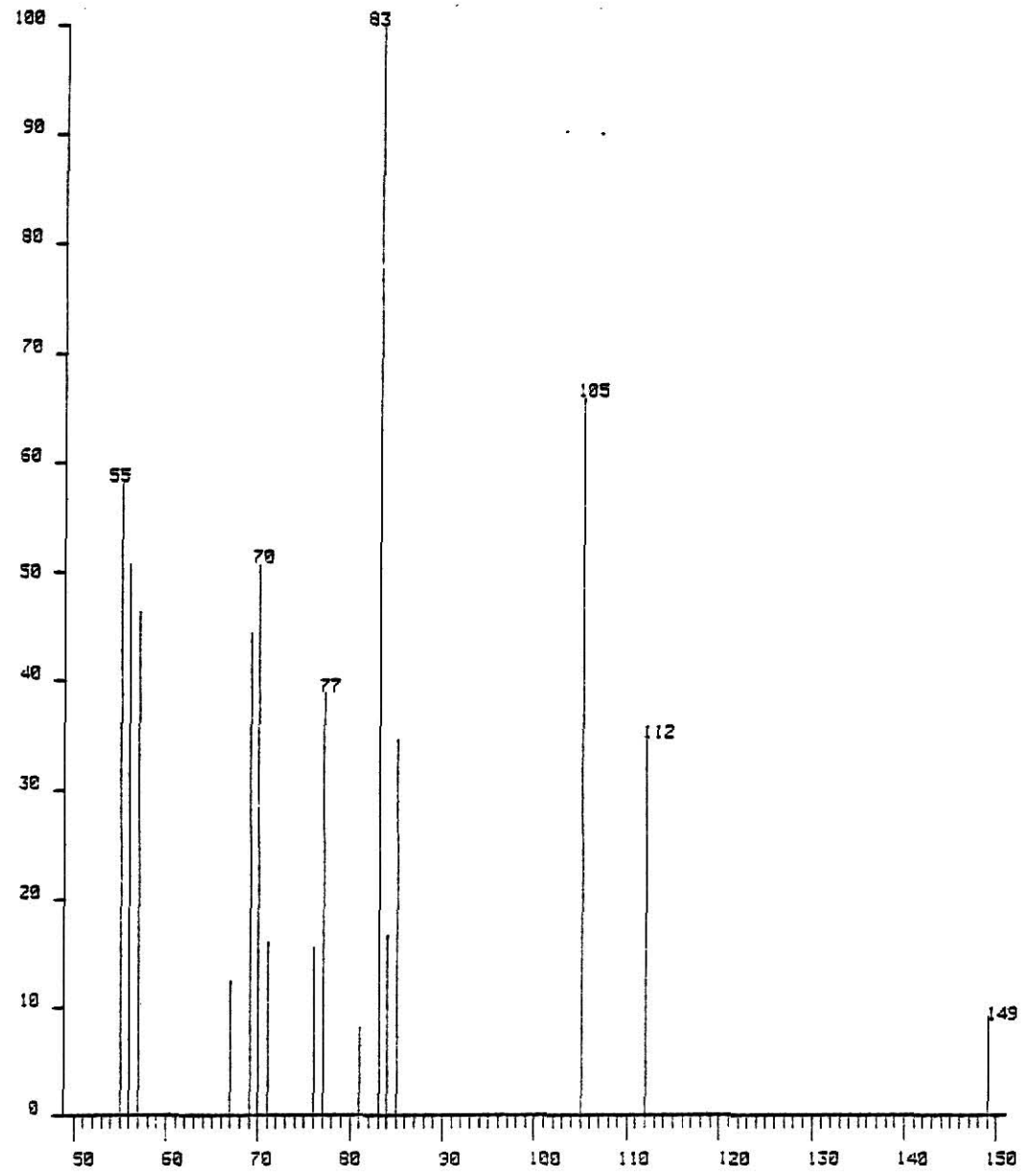


FIGURE 8

Mass spectrum of

Peak 3 of Figure 6

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
DISP8K.51 [TIC=604, 100%=74] EI

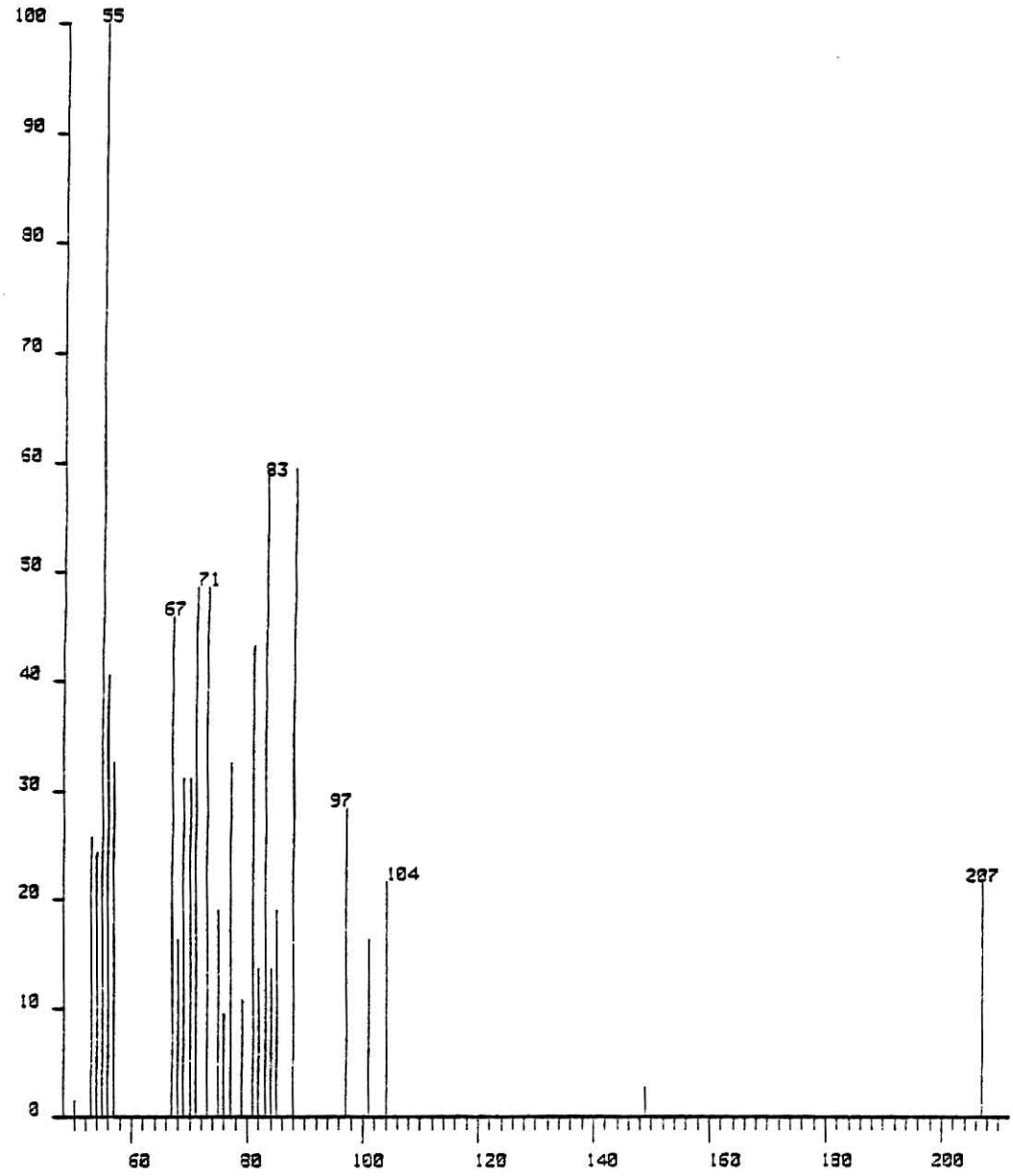


FIGURE 9

Mass spectrum of

Peak 4 of Figure 6

Proposed structure: Omite fragment

DS-55 MASS INTENSITY REPORT:
DISPBK.61 [TIC=9731, 100%=3447] EI

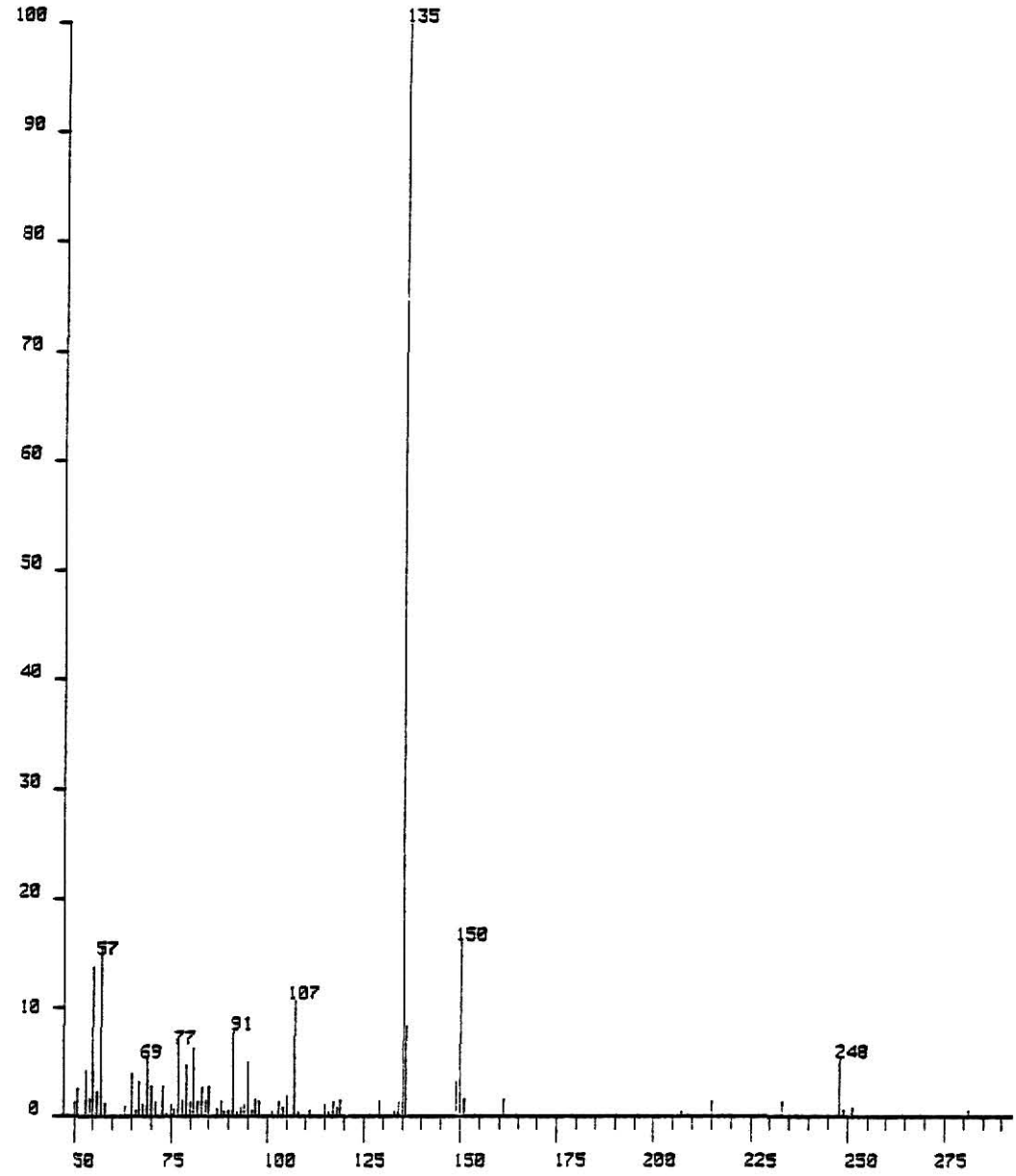


FIGURE 10

Mass spectrum of

Peak 5 of Figure 6

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:
DISP8K.65 [TIC=1406, 100%=795] EI

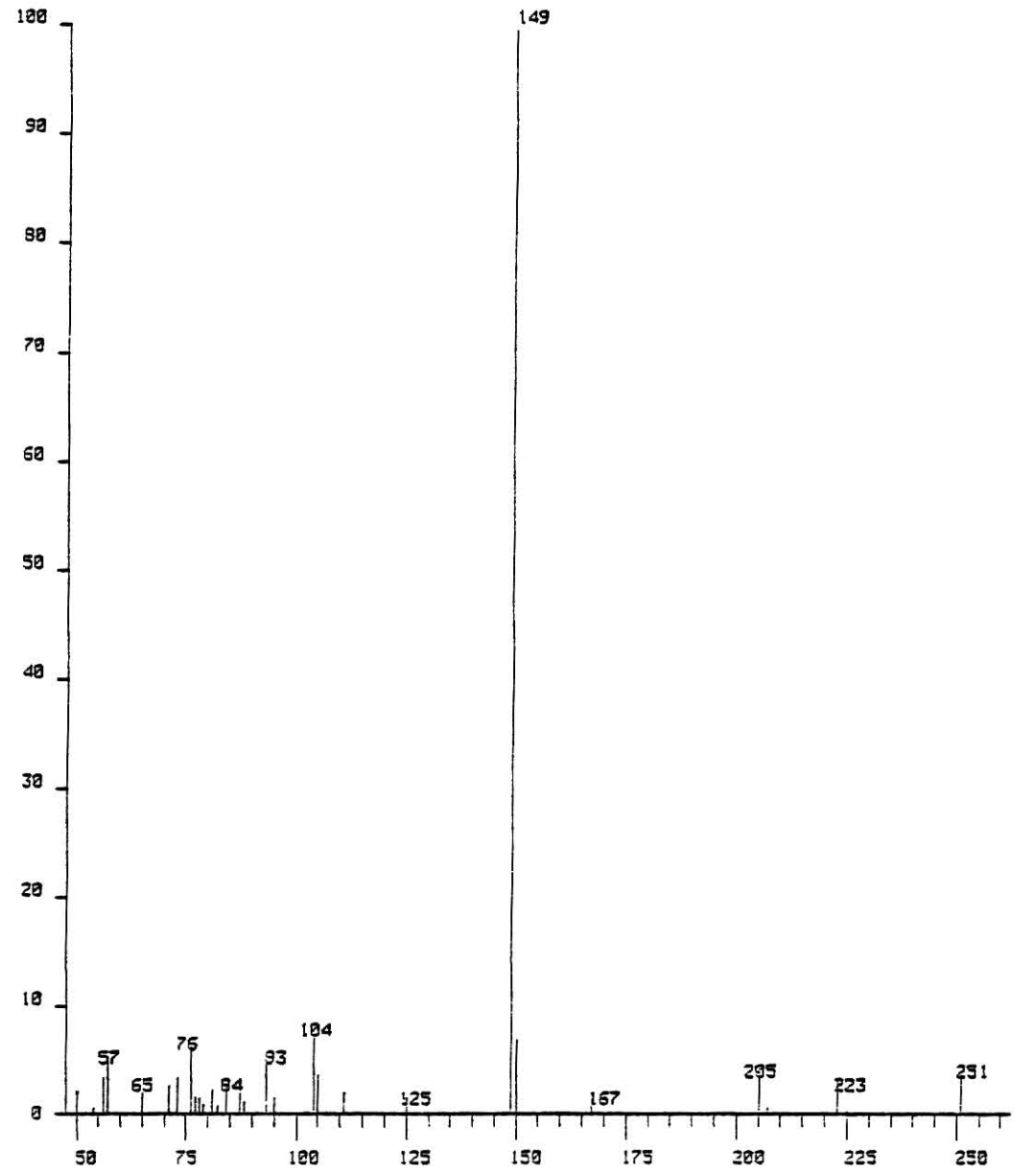
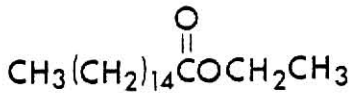


FIGURE 11

Mass spectrum of

Peak 6 of Figure 6

Proposed structure:



DS-55 MASS INTENSITY REPORT:
DISP8K.70 [TIC=15465, 100%=2275] EI

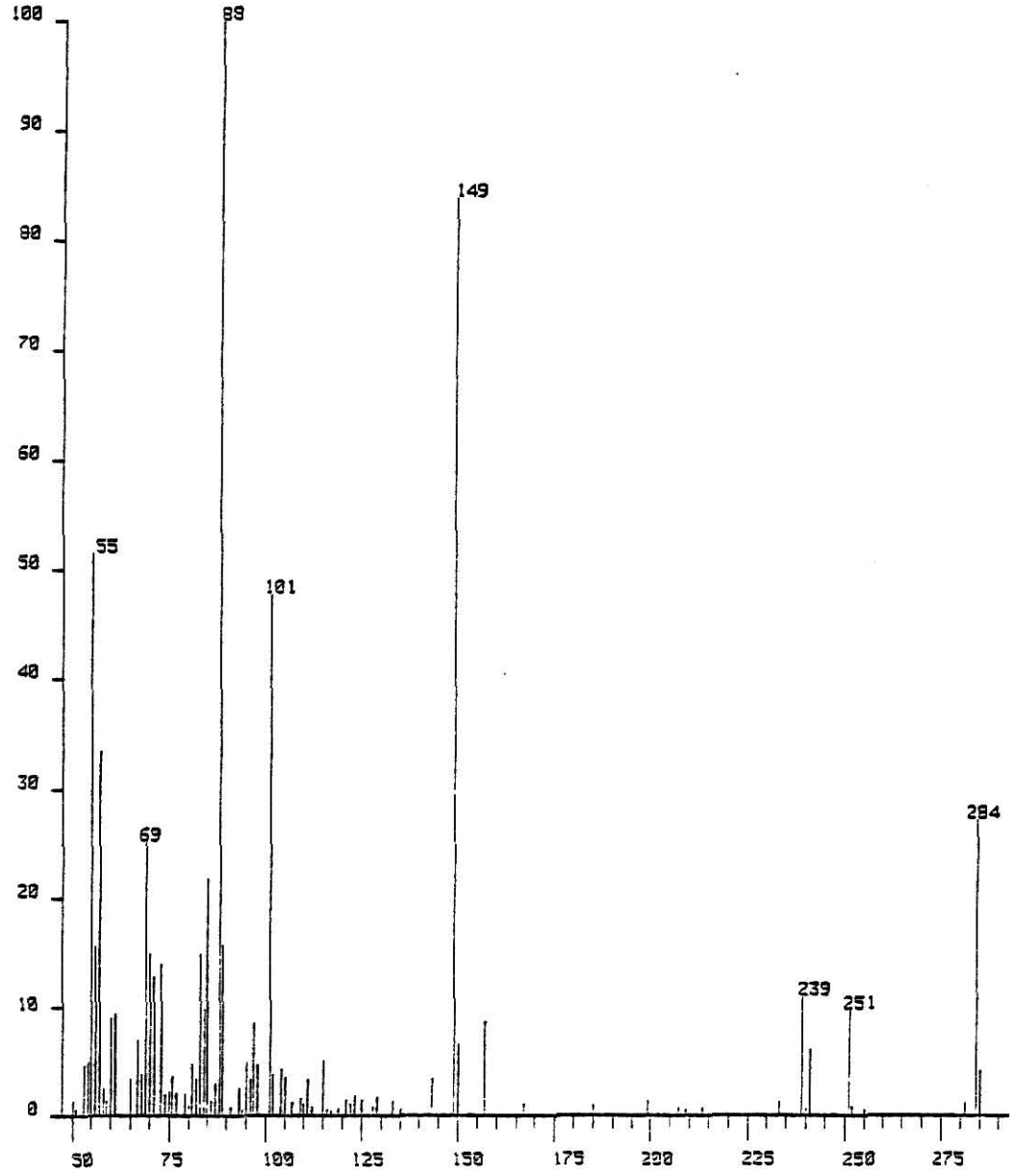


FIGURE 12

Mass spectrum of

Peak 7 of Figure 6

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:
DISPBK.76 [TIC=3496, 100%=990] EI

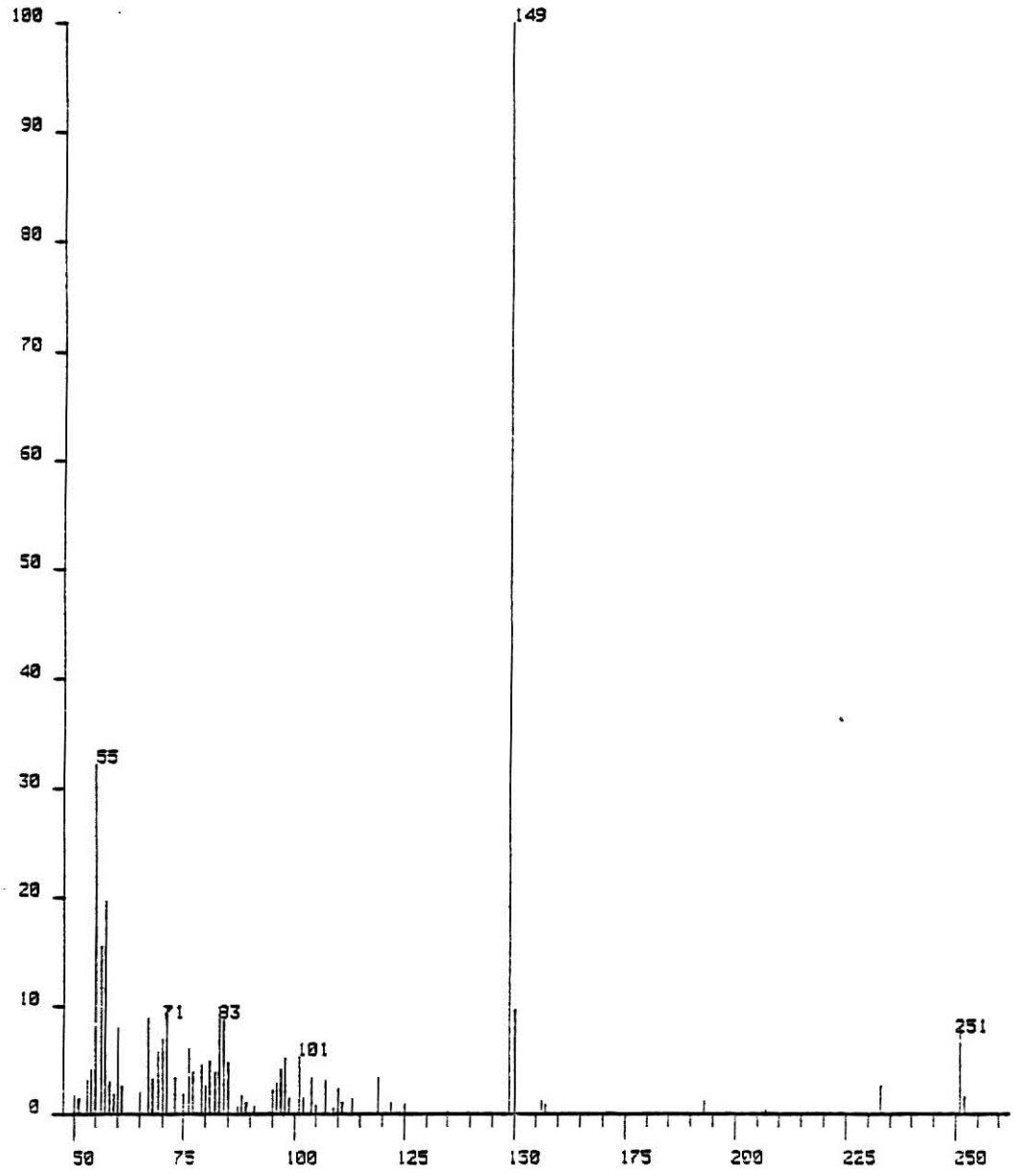
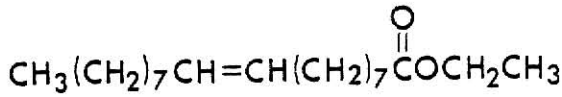


FIGURE 13

Mass spectrum of

Peak 8 of Figure 6

Proposed structure:



DS-55 MASS INTENSITY REPORT:

DISP8K.84 [TIC=156980, 100%=14025] EI

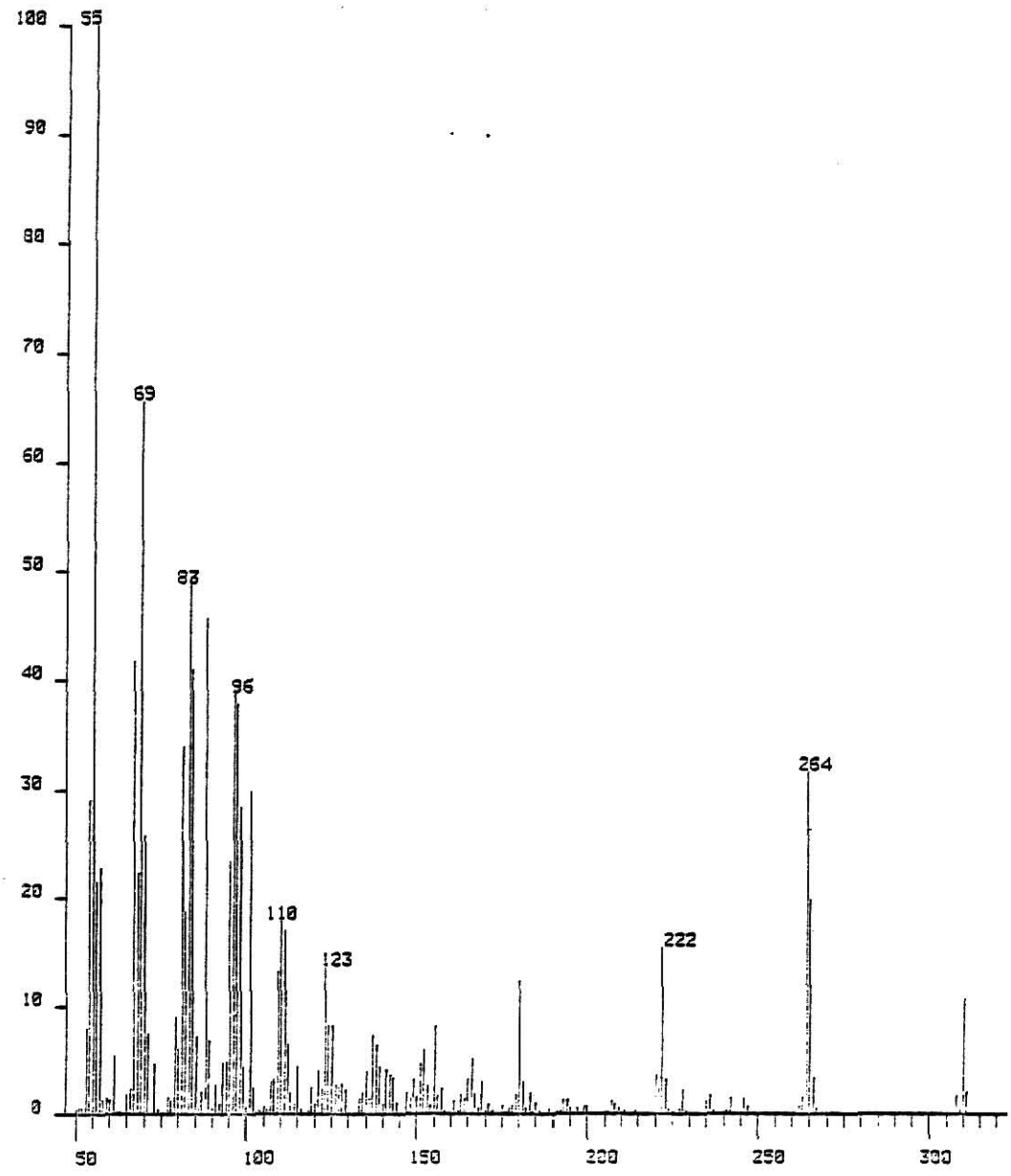


FIGURE 14

Mass spectrum of

Peak 9 of Figure 6

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
DISP8K.89 [TIC=64806, 100%=12437] EI

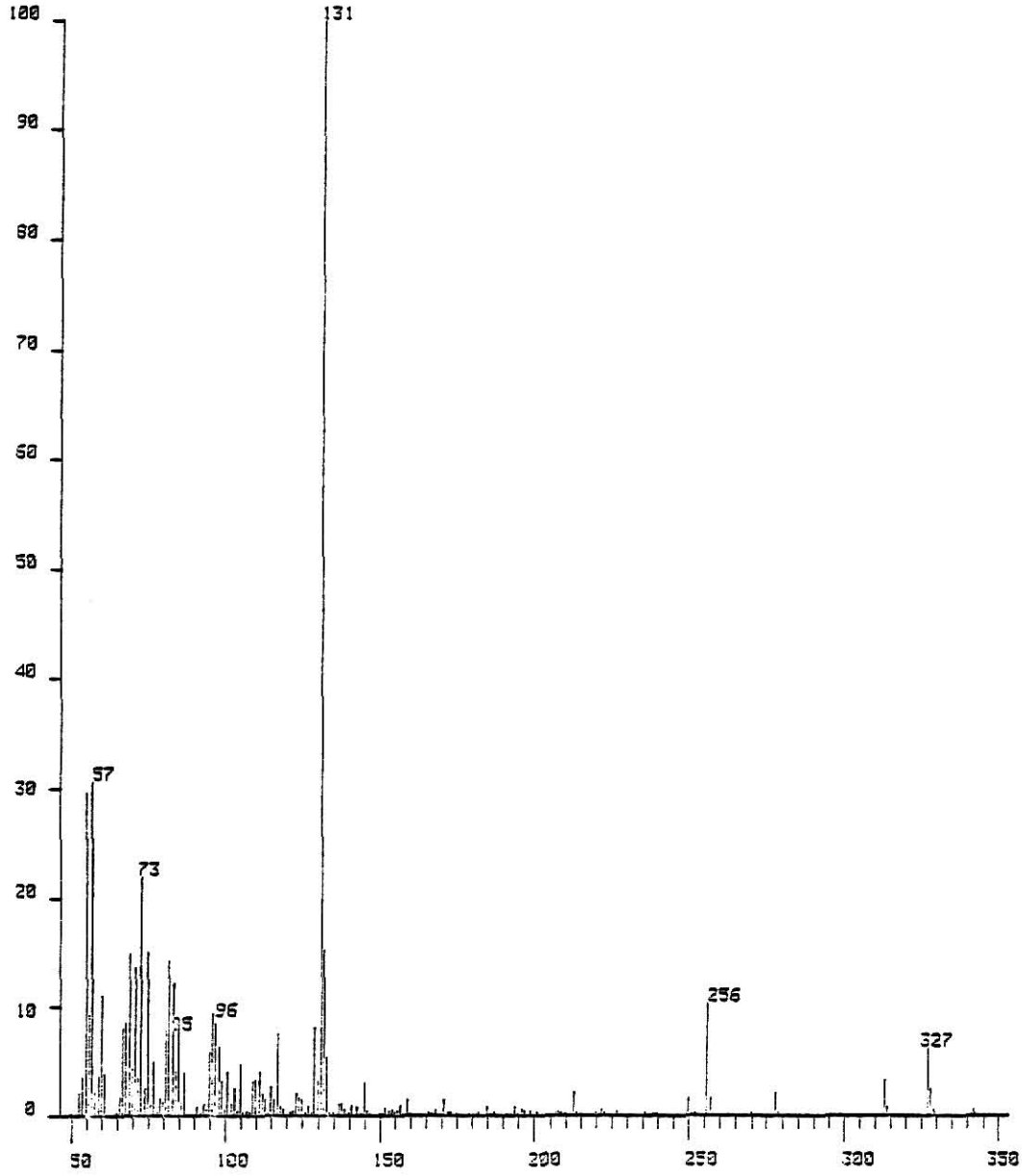


FIGURE 15

Mass spectrum of

Peak 10 of Figure 6

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
DISPBK.94 [TIC=47882. 100%=129831 EI

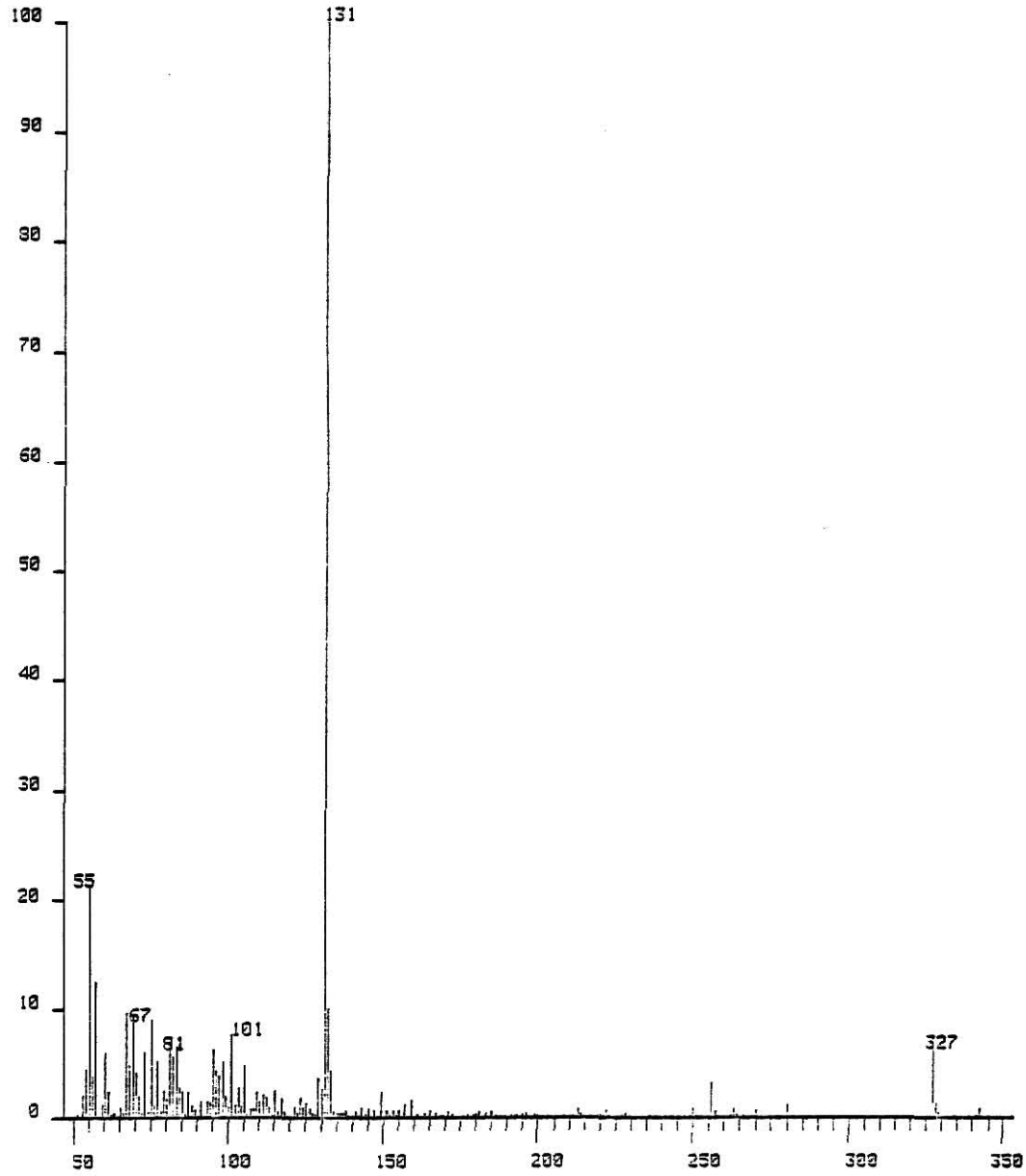
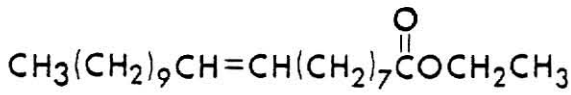


FIGURE 16

Mass spectrum of

Peak 11 of Figure 6

Proposed structure:



DS-55 MASS İNTENSITY REPORT:
DISP8K.100 [TIC=113932, 100%=10453] EI

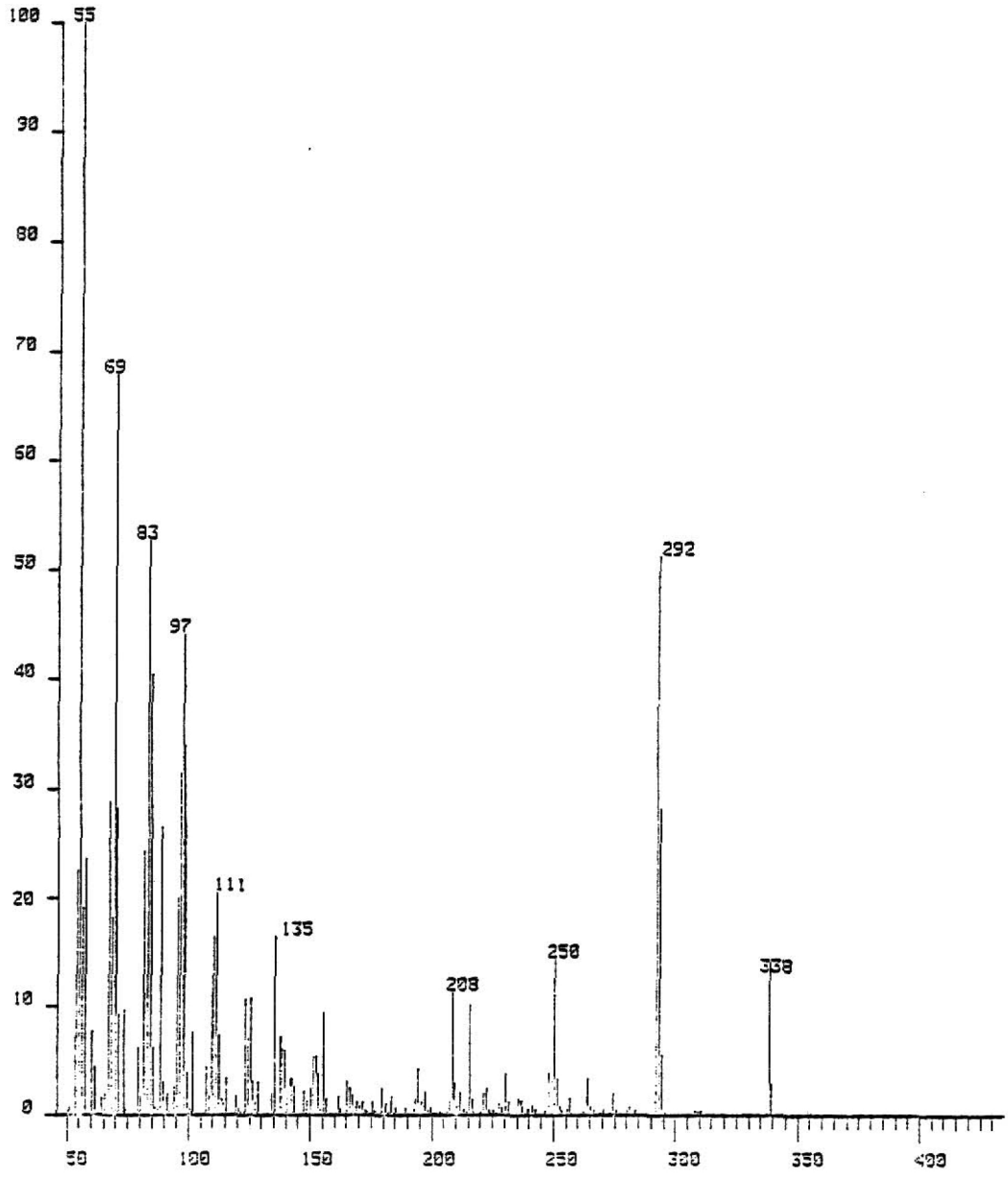


FIGURE 17

Mass spectrum of

Peak 12 of Figure 6

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:

DISPBK.104 [TIC=194116, 100%=50647] EI

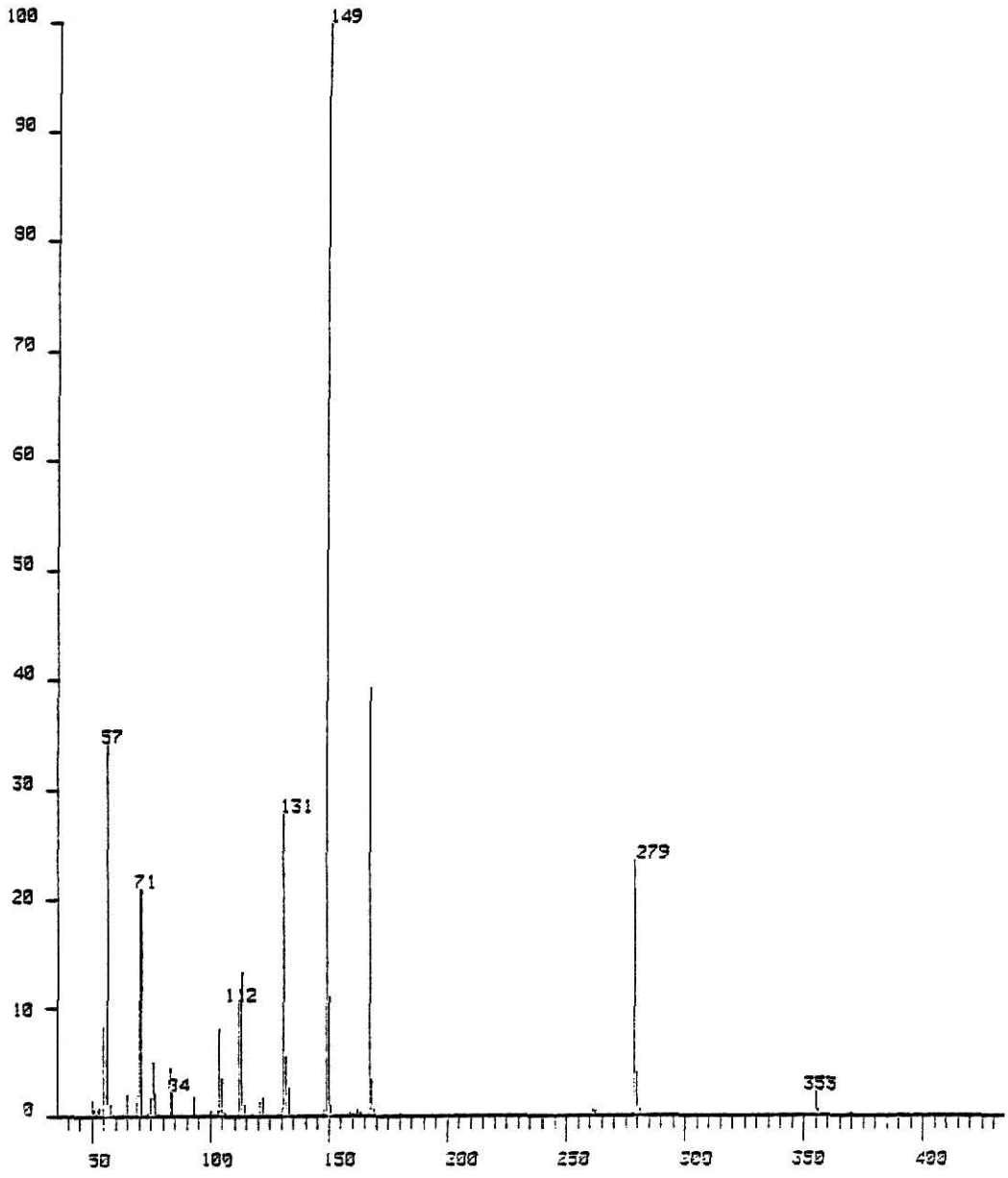


FIGURE 18

Mass spectrum of

Peak 13 of Figure 6

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:
DISPBK.111 [TIC=10940. 100%=878] EI

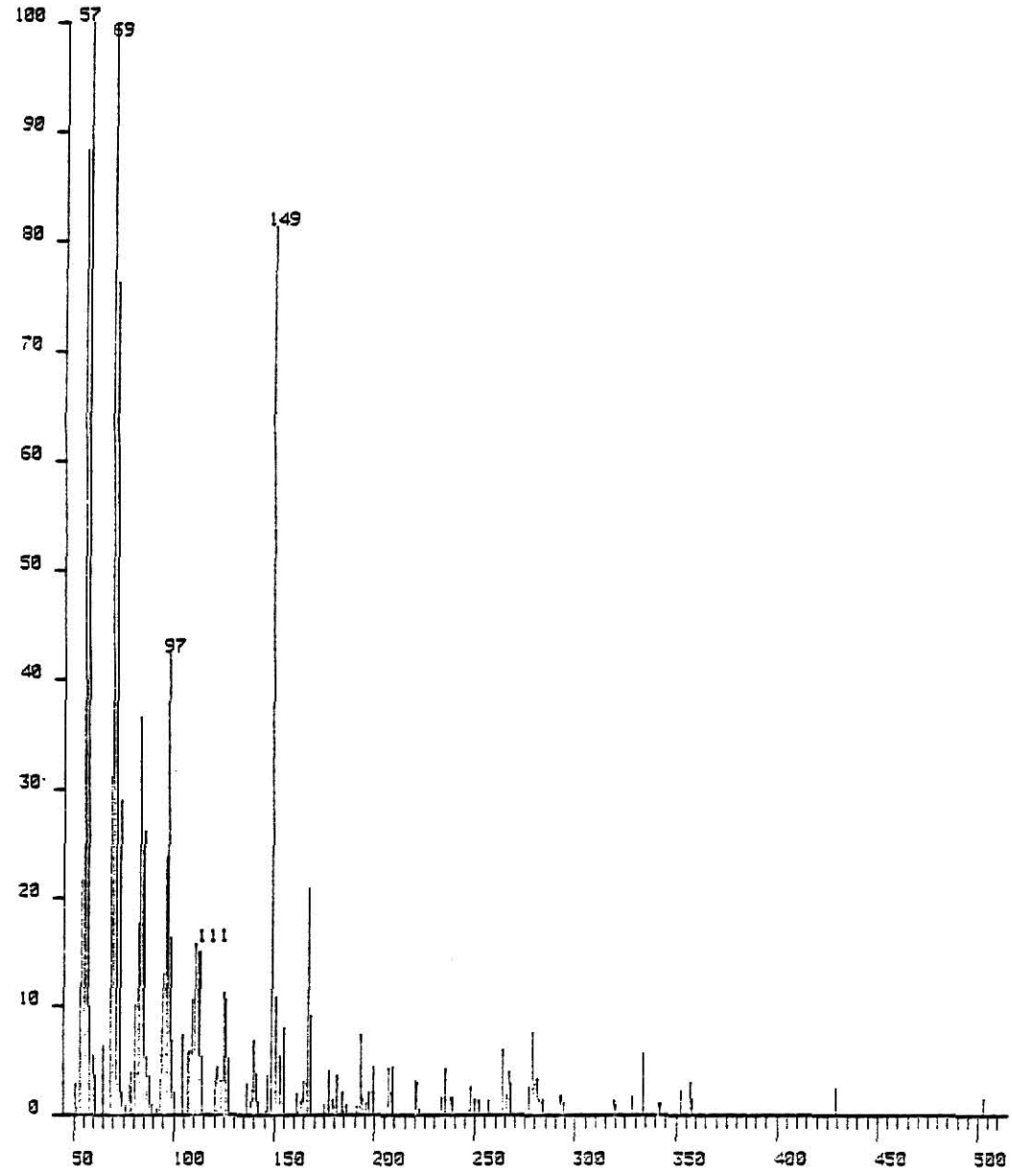


FIGURE 19

Mass spectrum of

Peak 14 of Figure 6

Proposed structure: None

DS-55 MASS INTENSITY REPORT:

DISP8K.116 (TIC=40780. 100%=36211) EI

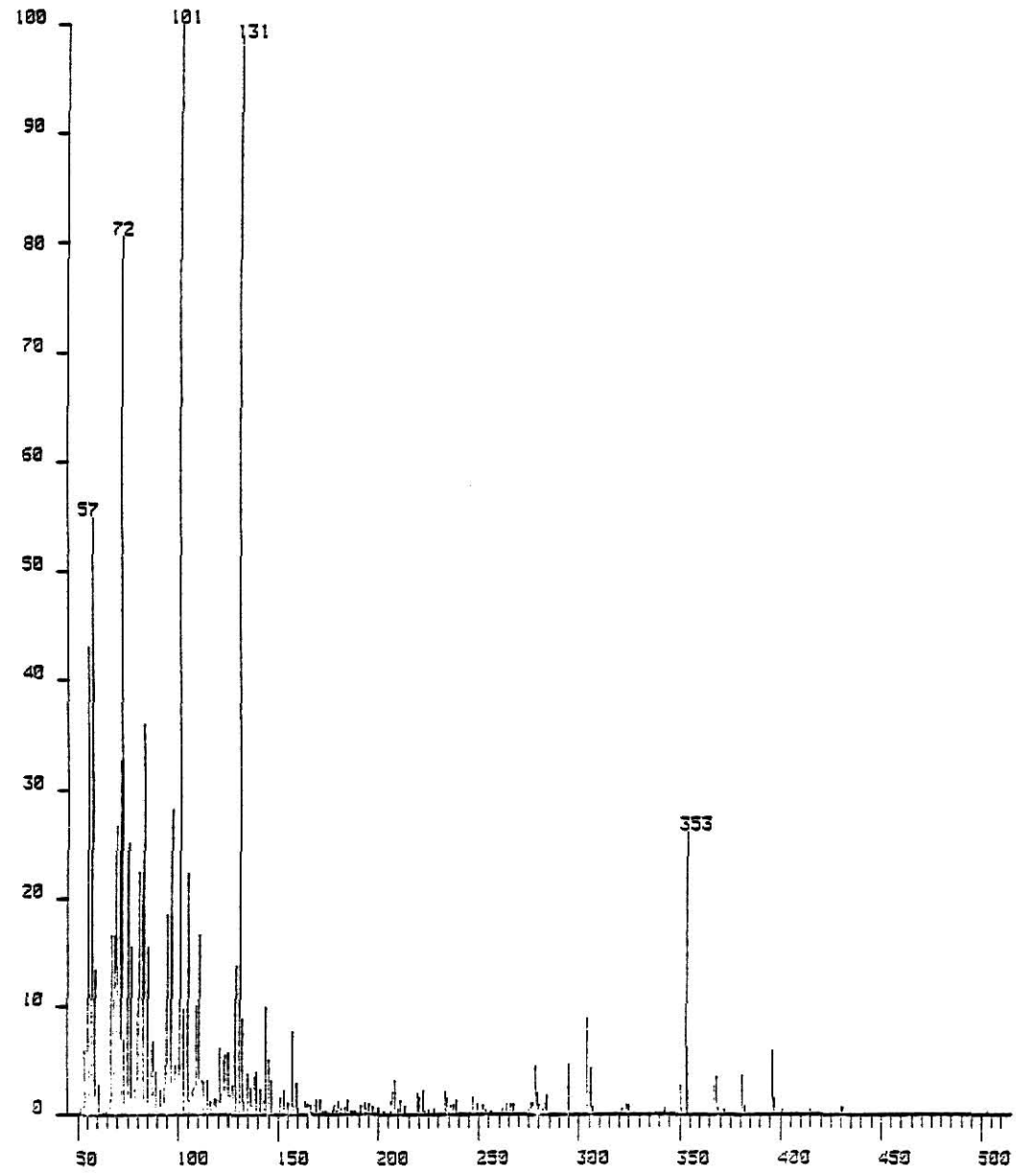


FIGURE 20

Mass spectrum of

Peak 15 of Figure 6

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
DISPBK.120 [TIC=124304, 100%=55704] EI

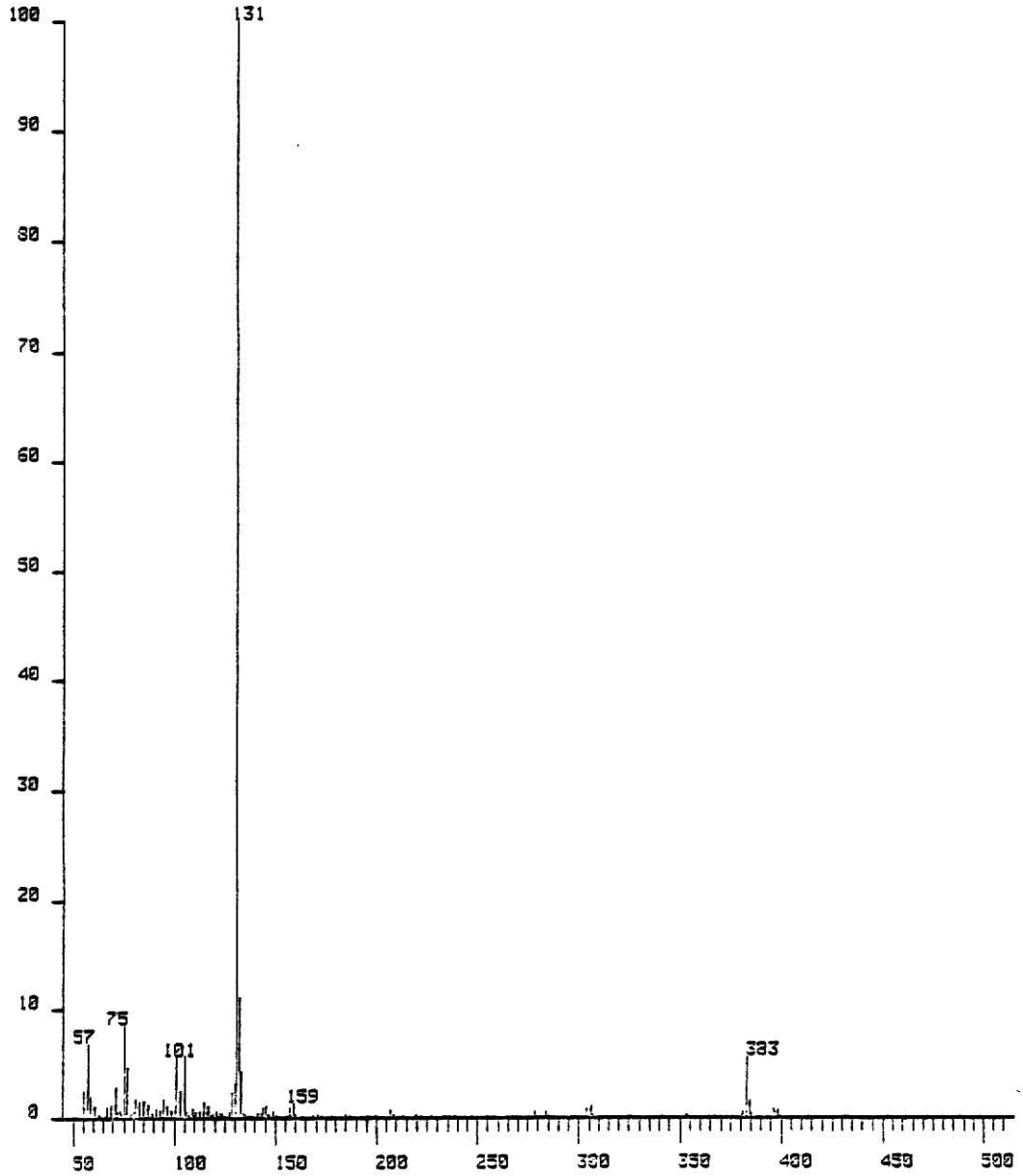


FIGURE 21

Mass spectrum of

Peak 16 of Figure 6

Proposed structure:

straight chain alkyne or alkyl diene of length C_{26}

DS-55 MASS INTENSITY REPORT:
DISP8K.128 [TIC=43619, 100%=6228] EI

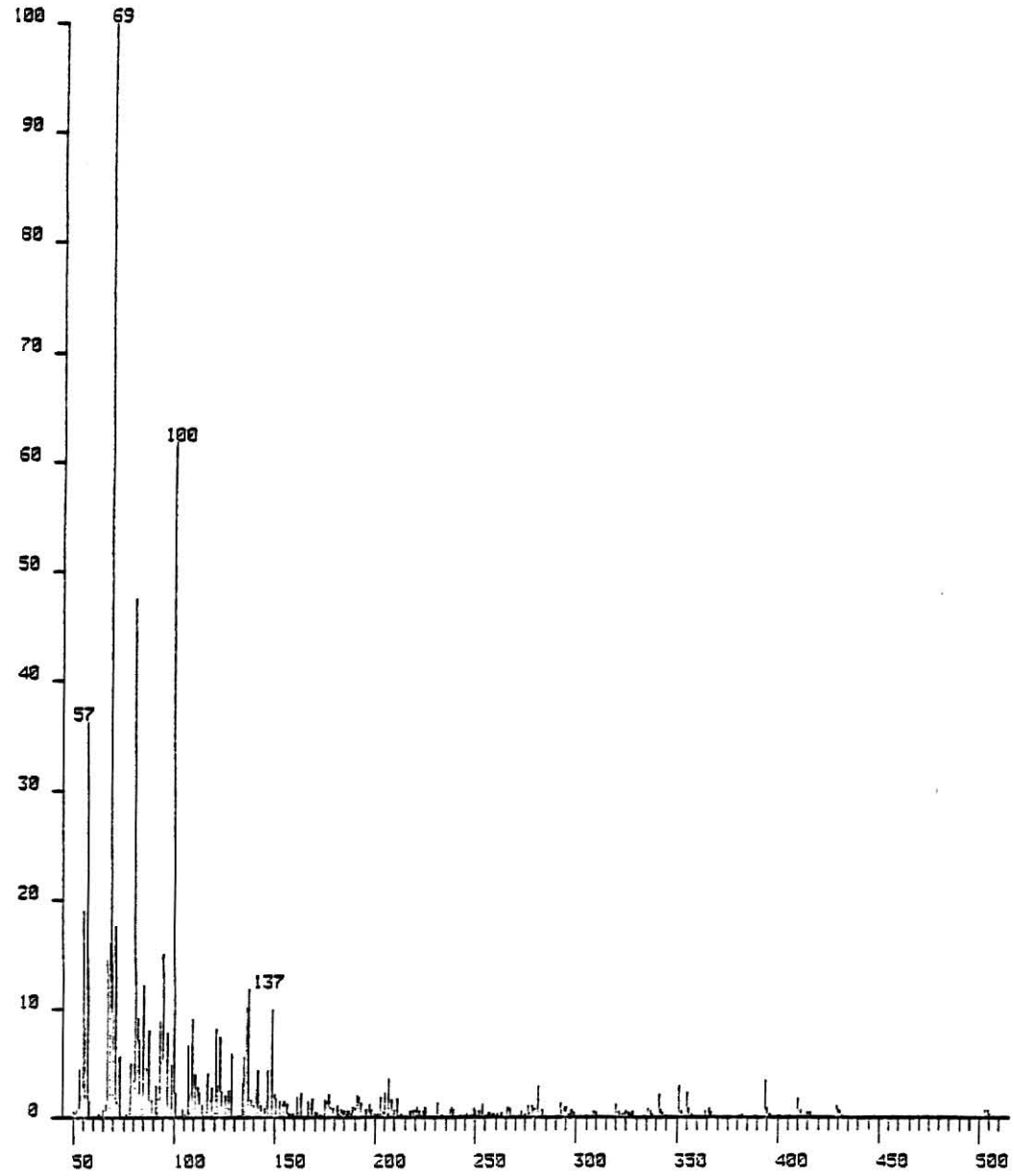


FIGURE 22

Mass spectrum of

Peak 17 of Figure 6

Proposed structure:

straight chain alkane of length at least C_{28}

DS-55 MASS INTENSITY REPORT:
DISP8K.135 [TIC=20912, 100%=3958] EI

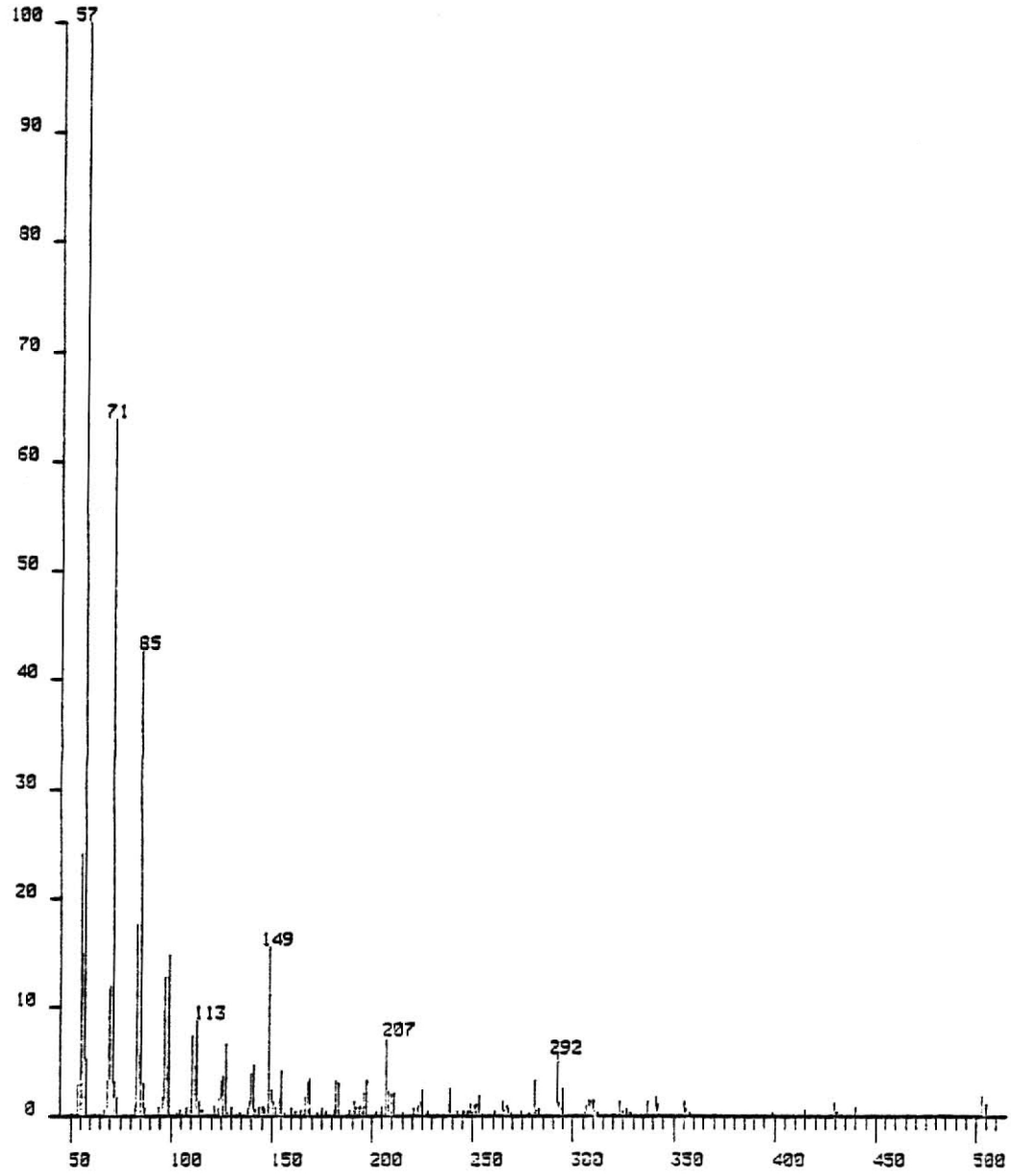


FIGURE 23

Mass spectrum of

Peak 18 of Figure 6

Proposed structure:

straight chain or branched alkane of length at least C_{36}

DS-55 MASS INTENSITY REPORT:
DISPBK.137 [TIC=33115. 100%=6430] EI

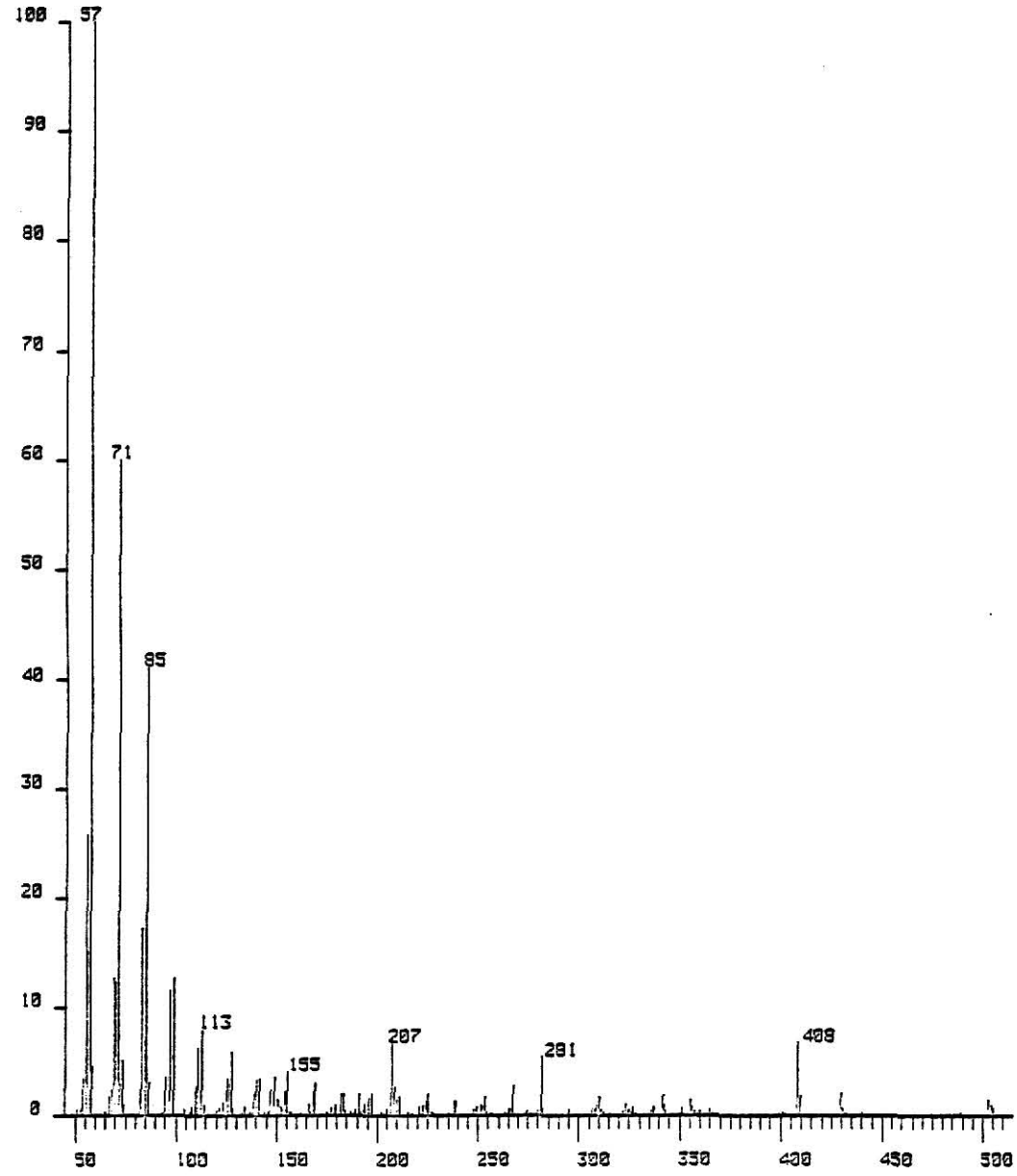
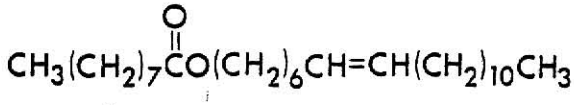


FIGURE 24

Mass spectrum of

Peak 19 of Figure 6

Proposed structure:



DS-55 MASS INTENSITY REPORT:
DISP8K.142 [TIC=39017, 100%=5282] EI

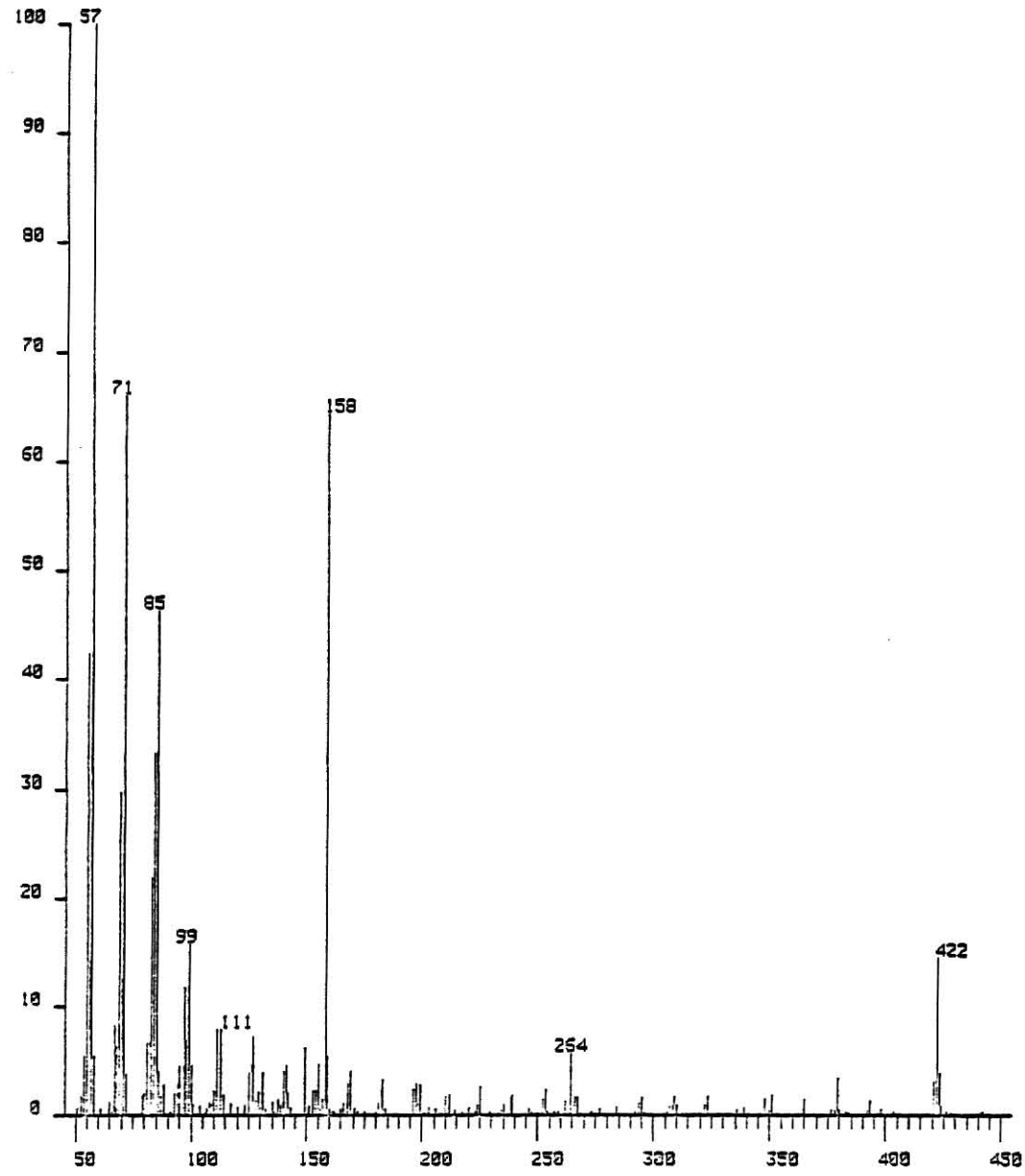


FIGURE 25

Mass spectrum of

Peak 20 of Figure 6

Proposed structure:

straight chain alkane of length at least C_{36}

DS-55 MASS INTENSITY REPORT:
DISPBK.149 [TIC=61217, 100%=13265] EI

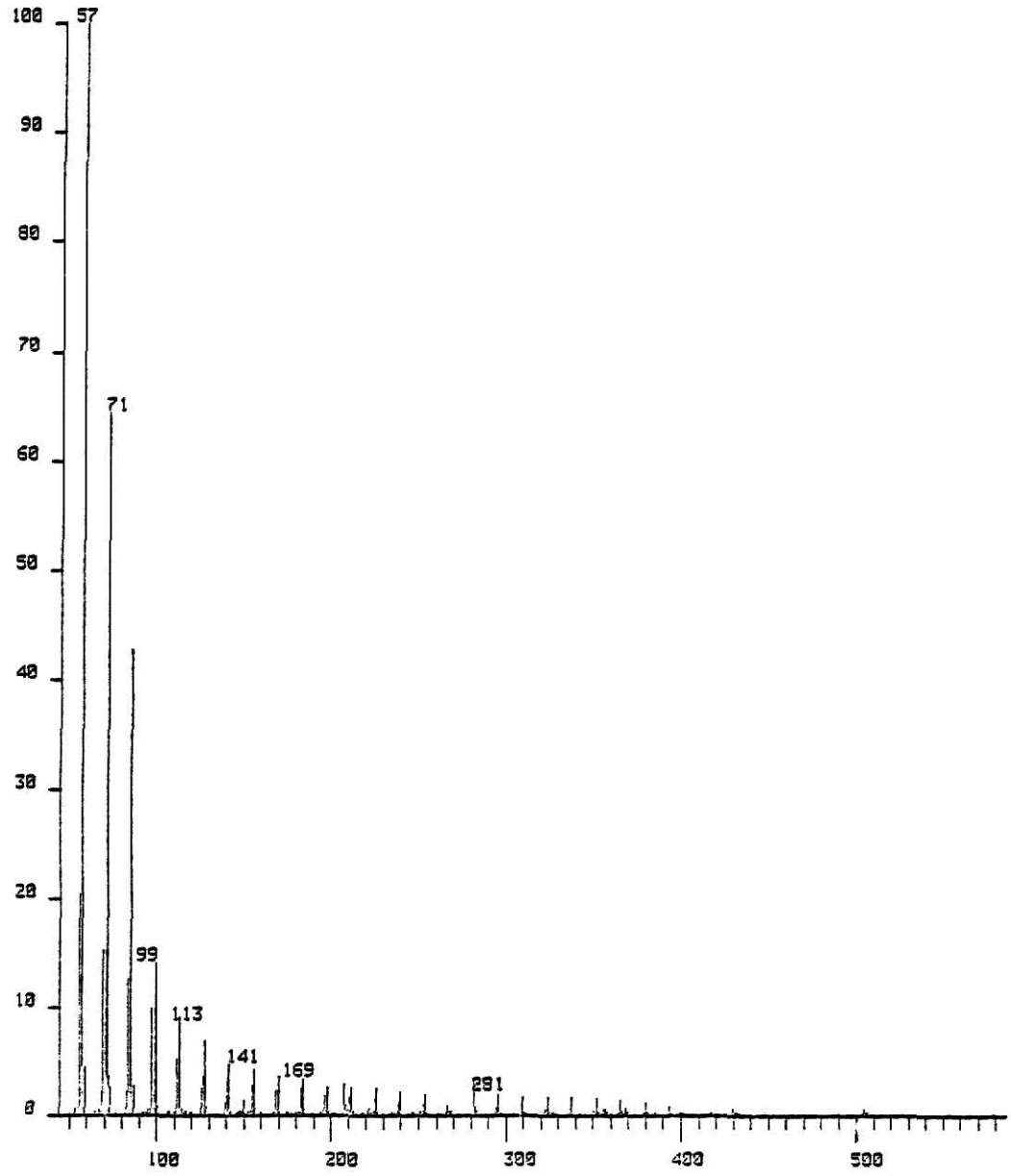
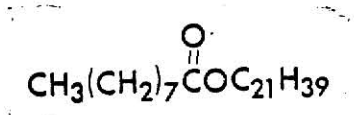


FIGURE 26

Mass spectrum of

Peak 21 of Figure 6

Proposed structure:



(no branching)

DS-55 MASS INTENSITY REPORT:
DISPBK.154 [TIC=45476, 100%=6868] EI

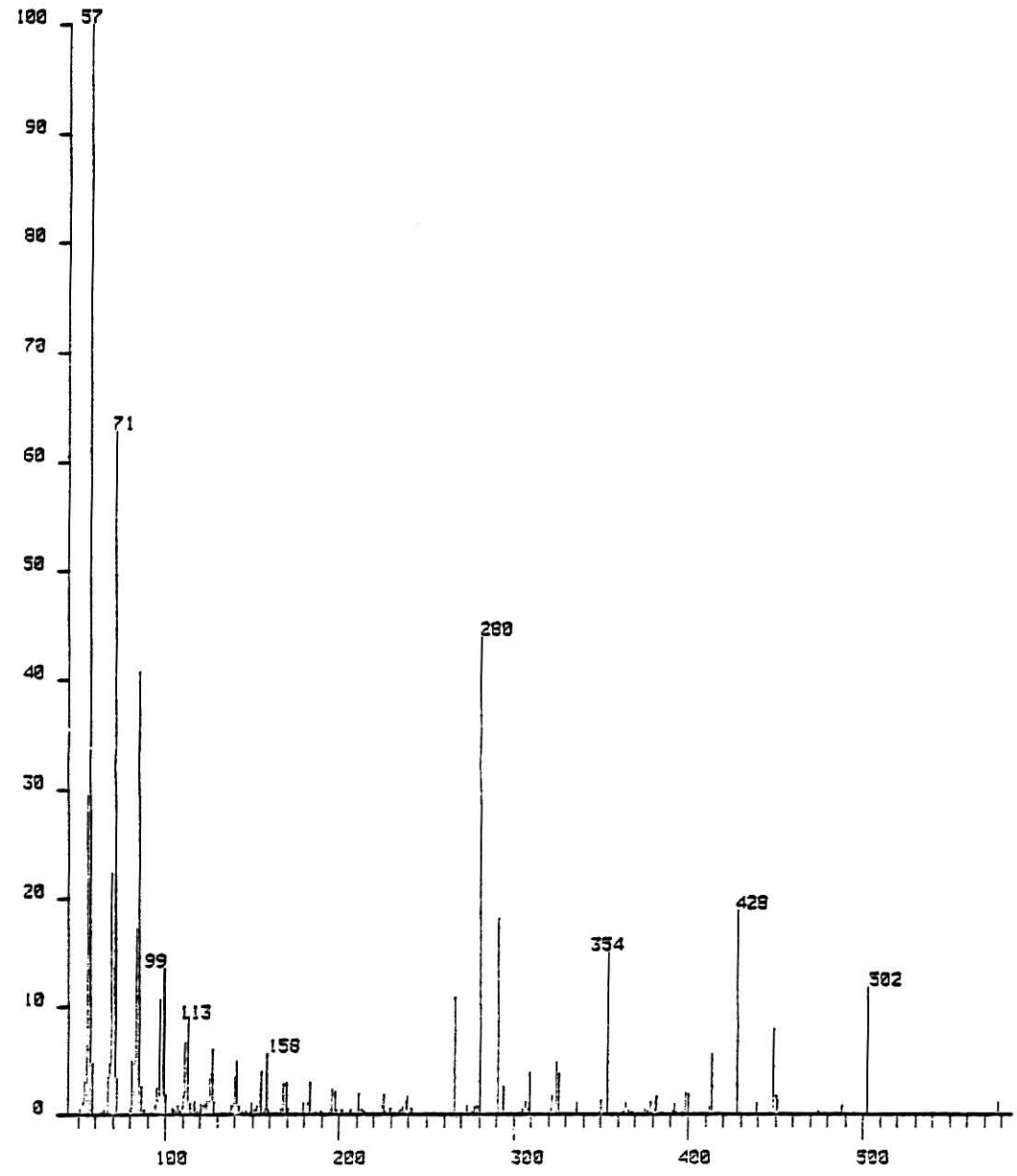
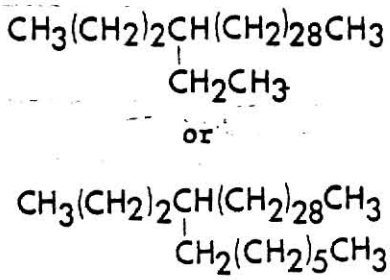


FIGURE 27

Mass spectrum of

Peak 22 of Figure 6

Proposed structure:



or

DS-55 MASS INTENSITY REPORT:
DISPBK.160 [TIC=23796, 100%=4694] EI

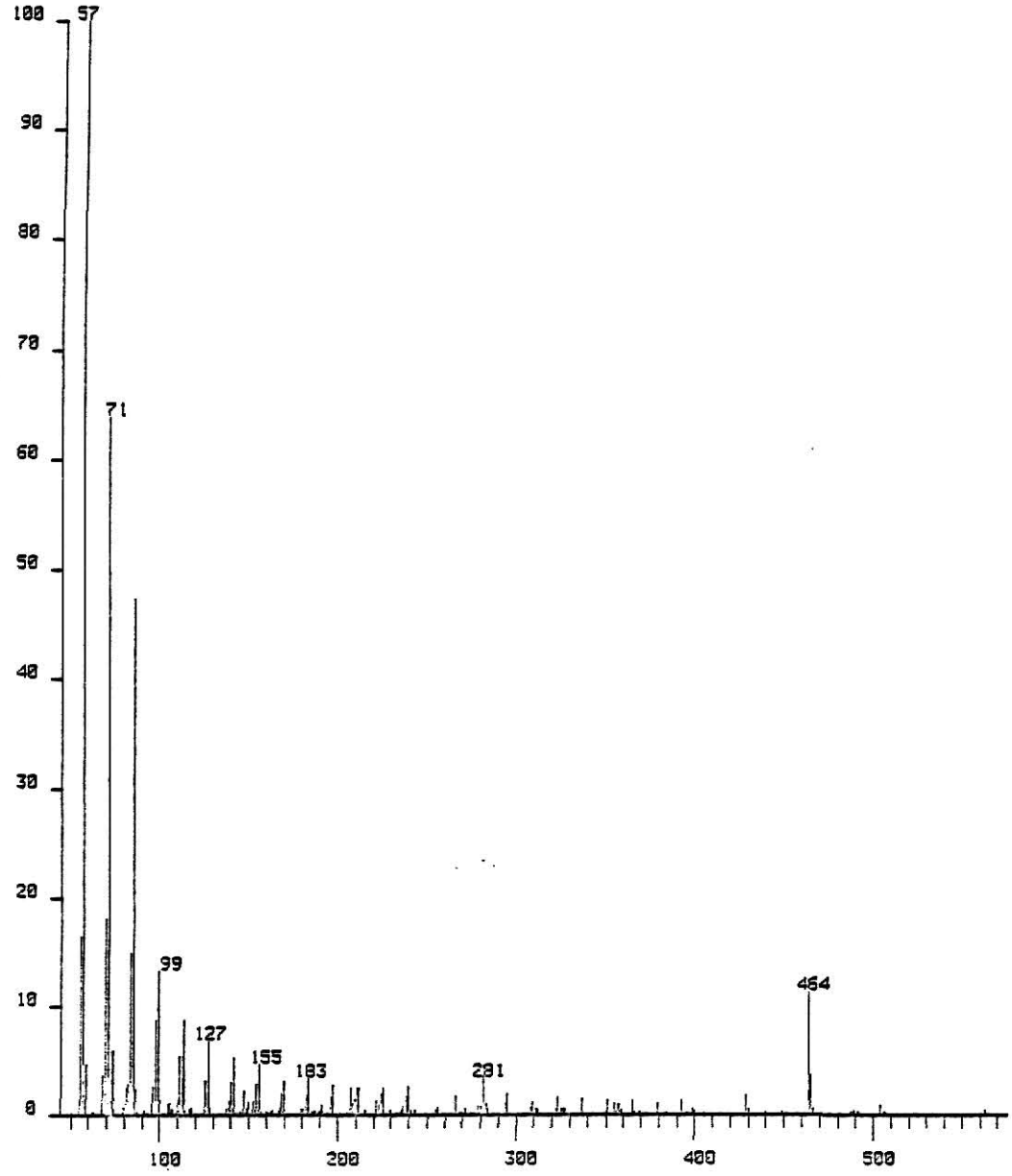


FIGURE 28

Mass spectrum of

Peak 23 of Figure 6

Proposed structure:

ester or straight chain alkane of length at least C_{33}

DS-55 MASS INTENSITY REPORT:
DISP8K.166 [TIC=7922, 100%=1952] EI

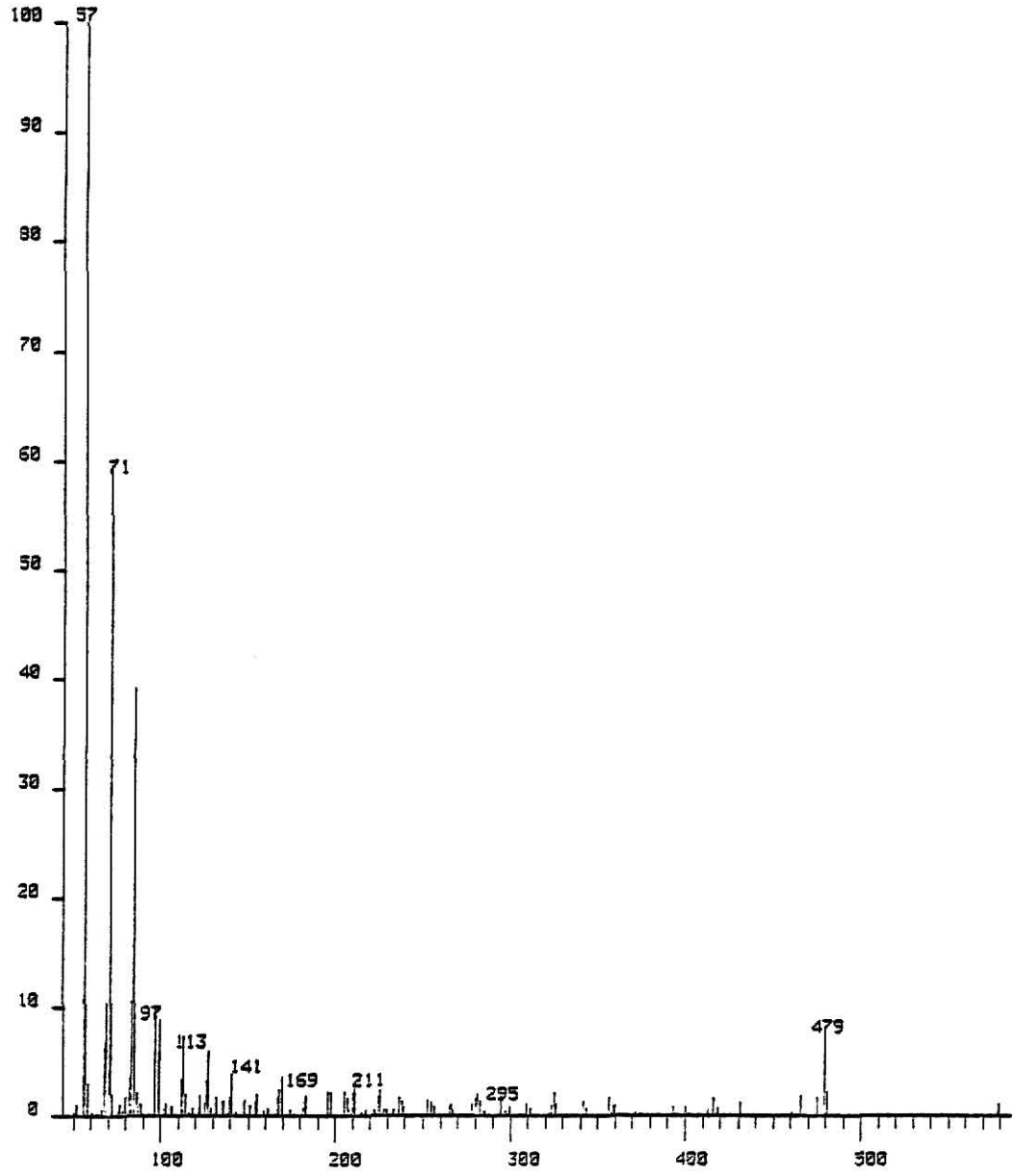


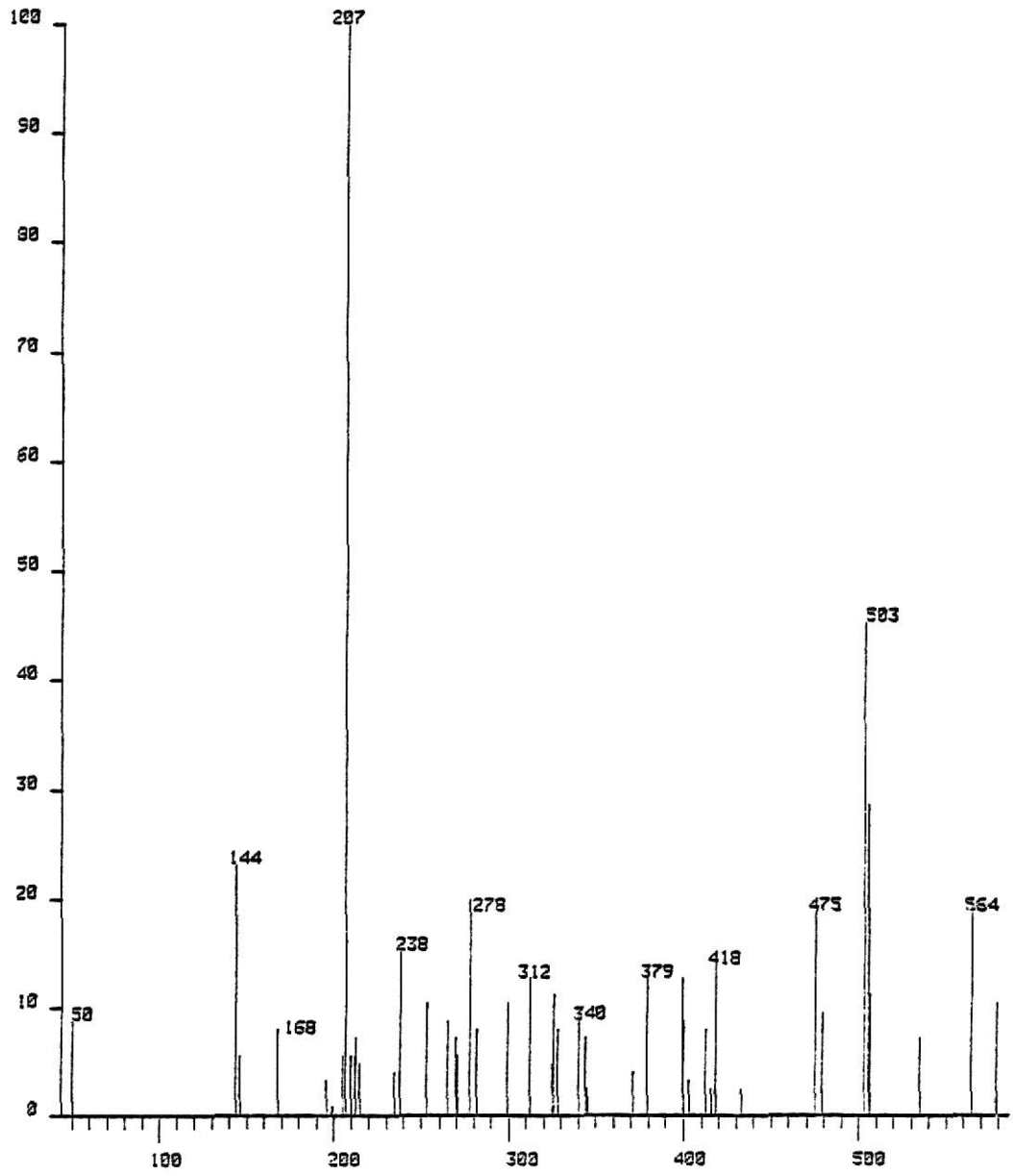
FIGURE 29

Mass spectrum of

Peak 24 of Figure 6

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
DISP8K.174 [TIC=681, 100%=126] EI



Interpretation of Mass Spectra

from MCMS, Lincoln, Nebraska

of Medicago scutellata (second set of data)

Peak 1 of Figure 30

This peak is due to the solvent ($CHCl_3$). Though the system is being vented during this time, a trace may reach the mass spectrometer. No mass spectrum was taken of this peak.

Peak 2 of Figure 30

The system is vented for the first two minutes of the GC run. This peak arises after the system is closed. No mass spectrum was taken of this peak.

Figure 31 (Peak 3 of Figure 30)

This mass spectrum is much too weak for interpretation. The peak represented here could be due to insecticide fragments, as some of the insecticides have low boiling components. This spectrum is very similar to Figure 7.

Figure 32 (Peak 4 of Figure 30)

This spectrum is also very weak. The intense peaks of this spectrum match with those intense peaks of Figure 8.

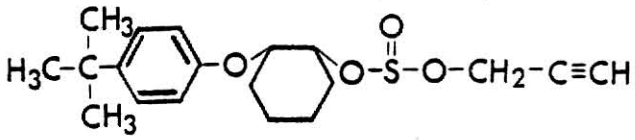
Figure 33 (Peak 5 of Figure 30)

This is another weak spectrum and does not contain sufficient information for interpretation.

Figure 34 (Peak 6 of Figure 30)

This spectrum matches well with Figure 9. From earlier GC work (see Insecticides section), it has been shown that the peak giving this spectrum arises from a decomposition product of the insecticide Omite which was applied to the plant.

Omite has the following structure:

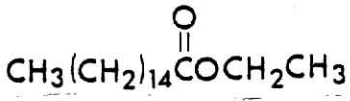


1) The peak at m/e 150 probably arises from $(CH_3)_3CC_6H_5OH$.

2) The peak at m/e 135 then arises from loss of CH_3, representing the fragment $(CH_3)_2CC_6H_5OH$. See the explanation of Figure 9 for the further breakdown of the spectrum.

Figure 35 (Peak 7 of Figure 30)

Proposed structure:



1) The parent peak is taken to be at m/e 284.

2) The peak at m/e 239 arises from loss of CH_3CH_2O.

3) The peak at m/e 157 then indicates further loss of $(CH_2)_4CO$.

4) The loss of 14 from m/e 157 to m/e 143 indicates a loss of CH_2 and the loss of 28 from m/e 143 to m/e 115 indicates a loss of CH_2CH_2.

5) The large peak at m/e 101 represents the fragment $CH_2CH_2CO_2CH_2CH_3$.

6) The base peak at m/e 88 arises from the fragment $CH_2C(OH)OCH_2CH_3$.

7) An alkane pattern at m/e 57, 71, and 85 and an alkene pattern at m/e 55, 69, 83, and 97 are present.

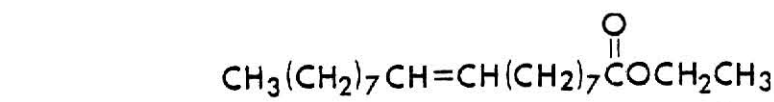
This spectrum matches well with Figure 11 except this spectrum has no phthalate impurities.

Figure 36 (Peak 8 of Figure 30)

Only a few observations about the compound represented by this spectrum can be made because the spectrum is very weak. The significant peak at m/e 207 and the small peak at m/e 281 are due to column bleed. An alkane pattern at m/e 57, 71, and 85 and an alkene pattern at m/e 55, 69, 83, 97, and 111 are present. The compound giving this spectrum most likely contains an alkene function.

Figure 37 (Peak 9 of Figure 30)

Proposed structure:



1) The parent peak is taken to be at m/e 310.

2) The large peak at m/e 264 arises from the loss of CH_3CH_2OH.

3) The peak at m/e 222 indicates the further loss of CH_2CO.

4) The peak at m/e 123 indicates a loss of C_7H_{15} which breaks the molecule to $CH_3(CH_2)_7CH{=}CH$.

5) A loss of CH then creates the peak at m/e 110.

6) The large peak at m/e 88 is due to the fragment $CH_2C(OH)OCH_2CH_3$.

7) The significant peak at m/e 101 arises from the fragment $CH_2CH_2CO_2CH_2CH_3$.

8) The alkene pattern at m/e 55, 69, 83, etc. suggests an alkyl double bond. The basis for placing the double bond at the C_9 position of the acid is:

a) the loss of 99 (C_7H_{15}) from m/e 222 to m/e 123 and then the loss of 13 (CH) from m/e 123 to m/e 110 and

b) this spectrum matches very closely with that of ethyl oleate.[18]

9) The M. scutellata exudate sample was spiked with ethyl oleate and Peak 9 of Figure 30 was enhanced. Therefore this peak is positively identified as being ethyl oleate.

Figure 38 (Peak 10 of Figure 30)

This spectrum is very similar to Figure 15. Both contain a large peak at m/e 131. However, the peak at m/e 131 is the base peak of Figure 15 while it is not in this particular spectrum. Also, the alkene pattern at m/e 55, 69, 83, etc. and the peaks at m/e 256 and m/e 327 are much more intense in this spectrum than in Figure 15.

1) The parent peak is taken to be at m/e 342.

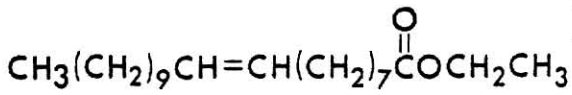
2) A significant loss of CH_3 is seen from m/e 342 to m/e 327.

3) A very strong alkene pattern is present at m/e 55, 69, 83, etc.

No structure could be assigned with the information given. See the special section concerning components whose spectra contain base peaks at m/e 131 for further discussion.

Figure 39 (Peak 11 of Figure 30)

Proposed structure:



1) The peak at m/e 338 is taken to be the parent peak.

2) The large peak at m/e 292 is due to loss of CH_3CH_2O.

3) Further loss of CH_2CO gives a peak at m/e 250.

4) The peak at m/e 88 is due to the fragment $CH_2C(OH)OCH_2CH_3$.

5) The peak at m/e 101 is due to the fragment $CH_2CH_2CO_2CH_2CH_3$.

6) An alkene pattern is present at m/e 55, 69, 83, etc. which indicates an alkyl double bond is present. Because this spectrum is very similar to that of ethyl oleate, except 28 units longer, the double bond is tentatively assigned to the C_9 position of the acid.

Figure 40 (Peak 12 of Figure 30)

1) The parent peak could possibly be at m/e 368.

2) A loss of 15 from m/e 368 to m/e 353 indicates the loss of CH_3.

3) An alkane pattern is present at m/e 57, 71, 85, etc. and an alkene pattern at m/e 55, 69, 83, etc.

No further interpretation could be done on this spectrum. See the special section about the components containing base peaks at m/e 131 in their spectra for further discussion.

Figure 41 (Peak 13 of Figure 30)

This spectrum is nearly identical to Figure 40. This could be due to the fact that the two peaks giving these spectra (Peaks 12 and 13 of Figure 30) are not well separated so the spectrum of peak 13 may contain some of the spectrum of Peak 12 (Figure 40).

1) The parent peak is taken to be at m/e 368.

2) A loss of 15 from m/e 368 to m/e 353 indicates a loss of CH_3.

3) An alkane pattern at m/e 57, 71, 85, etc. and an alkene pattern at m/e 55, 69, 83, etc. are present.

See the special section concerning components whose spectra contain base peaks at m/e 131 for further discussion.

Figure 42 (Peak 14 of Figure 30)

This spectrum (Peak 14 of Figure 30, retention time 12:00) is similar to Figure 17 (Peak 12 of Figure 6, retention time 11:14) and Figure 18 (Peak 13 of Figure 6, retention time 12:00). It was proposed that Figures 17 and 18 were spectra of phthalates. The presence of the base peak at m/e 149 of this spectrum suggests that a phthalate is represented here also. But the presence of a strong alkane pattern at m/e 57, 71, 85, etc. suggests the spectrum is

of more than one compound. Column bleed peaks are present at m/e 147, 206, 354, 430, and 503. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component of the exudate itself.

Figure 43 (Peak 15 of Figure 30)

The peak giving this spectrum is actually the shoulder of Peak 14 of Figure 30 and so its spectrum should be very similar to that of Peak 14 (Figure 42). It is somewhat similar to Figure 42 but it is without the phthalate spectral features.

1) The base peak at m/e 117 could arise from $CH_3(CH_2)_4C(OH)_2$.

2) The loss of CH_2CH_2 (28) would then give rise to the peak at m/e 89.

3) Further loss of H_2O (18) results in the peak at m/e 71.

4) The peak at m/e 55 suggests C_4H_7 is present.

5) If this compound is an ester, as the peaks at m/e 117, 89, and 71 might indicate, it looks like the ester may contain a C_6 acid. If that is the case, the predominant portion of the ester would be the alcohol. The initial loss from the parent peak would then be a molecule of acid, in this case, $CH_3(CH_2)_{14}CO_2H$, mass 116. However, there is no significant loss of 116 seen in this spectrum.

6) If this compound is an ethyl ester, losses of 46 (CH_3CH_2OH) and 42 (CH_2CO) should be seen. A loss of 46 is seen from m/e 338 to m/e 292, and a loss of 42 is seen from m/e 292 to m/e 250. However, these peaks are not of great enough intensity to be from this type, if from any type, of ester. Also, there is no logical breakdown pattern after the peak at m/e 250.

7) Column bleed peaks are present at m/e 73, 133, 147, 281, and 429.

8) No tentative structure can be assigned with the information available.

Figure 44 (Peak 16 of Figure 30)

This spectrum is almost identical to Figure 20. The peaks giving these intense spectra (Peak 16 of Figure 30 and Peak 15 of Figure 6) are very large and are therefore in significant concentration.

1) The parent peak is taken to be at m/e 398.

2) The loss of 15 from m/e 398 to m/e 383 indicates a loss of CH_3.

Aside from the base peak at m/e 131, very little helpful information is given in the spectrum, hindering interpretation. The special section about components whose spectra contain base peaks at m/e 131 provides further discussion.

Figure 45 (Peak 17 of Figure 30)

This spectrum (Peak 17 of Figure 30, retention time 13:39) is similar to Figure 66 (Peak 13 of Figure 53, retention time 15:00), especially in the lower molecular weight region. (When comparing retention times between the GC/MS runs made at MCMS and those made at MRI, note that the temperature programs were slightly different). However, the similarities are not as great in the higher molecular weight region. There appear to be many more intense high molecular weight peaks in this spectrum than in Figure 66. When the interpretation of this spectrum is made without the use of Figure 66 as a reference, these observations are made:

a) The spectrum is weak so noise peaks may interfere with the interpretation and the parent peak is therefore difficult to ascertain.

b) Column bleed peaks of significant intensities are present at m/e 73, 134, 147, 207, 281, 430, and 504.

c) The significant peaks at m/e 384 and m/e 336 are difficult to explain. The loss of 48 from m/e 384 to m/e 336 is not common and a fragment will not be proposed for mass 48.

If the portion of the mass spectrum from m/e 266 to the lower end of the spectrum is considered, interpretation can be made as for Figure 66:

1) The parent peak is taken to be at m/e 266.

2) The peak at m/e 265 arises from $C_{16}H_{29}CO_2$.

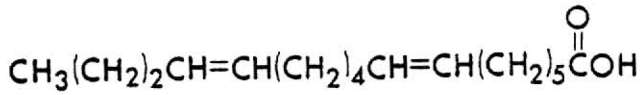
3) The peak at m/e 249 arises from the loss of OH from the molecule.

4) The peak at m/e 221 arises from the further loss of C=O.

5) The base peak at m/e 117 is due to the fragment $CH_3(CH_2)_4C(OH)_2$.

6) The two alkyl double bonds are tentatively placed at the C_7 and C_{13} positions of the acid for the reasons contained in the explanation of Figure 66.

Proposed structure:



It is proposed that Peak 17 of Figure 30 contains the above acid along with another component which causes the unexplainable higher molecular weight peaks of the spectrum.

Figure 46 (Peak 18 of Figure 30)

The peak giving this spectrum is not well separated from Peak 17 of Figure 30 and so its spectrum may contain some of the spectrum from Peak 17 (Figure 45).

1) The high molecular weight peaks are very small and it is difficult to determine the true parent peak. The parent peak may possibly be at m/e 366.

2) With the parent peak placed at m/e 366, a loss of CH_3 (15) is seen from m/e 366 to m/e 351. No other significant losses are observed until the lower molecular weights.

3) The peak at m/e 117 may be due to $CH_3(CH_2)_4C(OH)_2$.

4) The peak at m/e 100 could be due to $CH_3(CH_2)_4COH$.

5) The peak at m/e 87 is probably due to an oxygen containing fragment of the empirical formula $C_5H_{11}O$.

6) The peak at m/e 73 appears to arise from an oxygen containing fragment of the empirical formula C_4H_9O.

7) The peak at m/e 56 could be due to the fragment C_4H_8.

Because of the lack of information given in the higher molecular weight region of the spectrum, the molecule cannot be further pieced together, and so no tentative structure can be assigned.

Figure 47 (Peak 19 of Figure 30)

The peak giving this spectrum is only a small shoulder on the side of Peak 18 of Figure 30 and so part of its spectrum (Figure 46) will probably be seen here.

1) This spectrum is weak. The parent peak is difficult to determine because the true signal and noise spikes are difficult to differentiate.

2) The peak at m/e 158 may be due to $CH_3(CH_2)_7CO_2H$.

3) The peak at m/e 117 could arise from the fragment $CH_3(CH_2)_4C(OH)_2$.

4) A further loss of OH (17) leaves $CH_3(CH_2)_4COH$ and thus creates the peak at m/e 100.

5) The peak at m/e 88 could be due to the fragment $CH_3(CH_2)_4OH$ or to another fragment of the empirical formula $C_5H_{12}O$.

6) Column bleed peaks of significant intensity are present at m/e 73, 281, and 429, and smaller ones at m/e 147 and m/e 503.

Figure 48 (Peak 20 of Figure 30)

Proposed structure: straight chain alkane of length C_{36} or greater

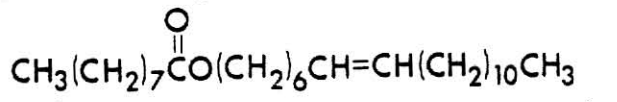
1) The parent peak is not detectable in this mass spectrum, characteristic of high molecular weight alkanes.[17]

2) The alkane pattern is seen throughout the spectrum, m/e 57, 71, 85, ..., 365.

3) No oxygen containing fragments are seen in the spectrum.

Figure 49 (Peak 21 of Figure 30)

Proposed structure:



This spectrum, in its entirety, is not easily interpreted. Much column bleed is present in these peaks: m/e 73, 147, 207, 221, 356, 430, and 504. According to the mass chromatogram of this GC/MS run (Figure 30), Peak 21 is not well formed, and from its shape it could possibly be two peaks which are not resolved. Since the separation ability of the column seems to have degraded from the first run, the hump called Peak 21 of Figure 30 could very well contain two peaks, not resolved. Therefore, because the retention time (15:22) of this peak is very close to that of Peak 19 of Figure 6 (15:25), it is proposed that Peak 21 of Figure 30 contains the ester in Peak 19 of Figure 6 plus one or more other compounds.

1) The parent peak is taken to be at m/e 422.

2) A loss of 158 ($CH_3(CH_2)_7CO_2H$) gives rise to the peak at m/e 264.

3) The base peak at m/e 158 arises from the acid fragment $CH_3(CH_2)_7CO_2H$.

4) In Figure 24 (Peak 19 of Figure 6), alkane and alkene patterns are present which do not show up strongly in this spectrum. In Figure 24, the alkane pattern dominates the alkene pattern until m/e 111 and m/e 113 where they are equal in intensity. Therefore, the double bond is tentatively positioned at C_7 in the alcohol portion.

5) The strong peaks which are not due to column bleed and are not common with Figure 24, m/e 84, 100, 293, 342, and 416, are thought to arise from another compound within the Peak 21 hump.

Figure 50 (Peak 22 of Figure 30)

Proposed structure: straight chain alkane of length C_{36} or greater

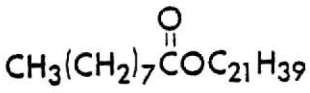
1) No parent peak is detectable, which is characteristic of a high molecular weight alkane.[17]

2) No oxygen containing peaks are seen in the spectrum.

3) An alkane pattern is present throughout the spectrum at m/e 57, 71, 85, ..., 393.

Figure 51 (Peak 23 of Figure 30)

Proposed structure:



(no branching)

1) The parent peak is taken to be at m/e 448.

2) The peak at m/e 291 could arise from the loss of $CH_3(CH_2)_7CO_2$ from the molecule.

3) A loss of 24 is seen from m/e 291 to m/e 267. This could possibly be due to the loss of C≡C, but it is highly unlikely.

4) The base peak at m/e 158 is due to the fragment $CH_3(CH_2)_7CO_2H$.

5) An alkyl pattern is seen at m/e 57, 71, 85, etc.

6) It appears that the alkyl fragment (after $C_8H_{17}CO_2$ is lost) of the molecule has a mass of 291, indicating $C_{21}H_{39}$ with two unsaturations. However, aside from the loss of 24 from m/e 291 to m/e 267, no indication is given as to the position(s) of the unsaturations.

Figure 52 (Peak 24 of Figure 30)

This spectrum is fairly weak and so noise spikes interfere with the interpretation. Column bleed peaks of significant intensities are seen at m/e 73, 207, 281, 356, 429, 503, and 578. All of the other peaks are low in intensity and could be due to noise. Because this is the last peak of this GC/MS run, the peak produced is most likely due only to column bleed.

FIGURE 30

Mass chromatogram of Medicago scutellata exudate sample for the second GC/MS run at MCMS, Lincoln, Nebraska

DS-55 CROSS SCAN REPORT. RUN: 17578

+ TIC

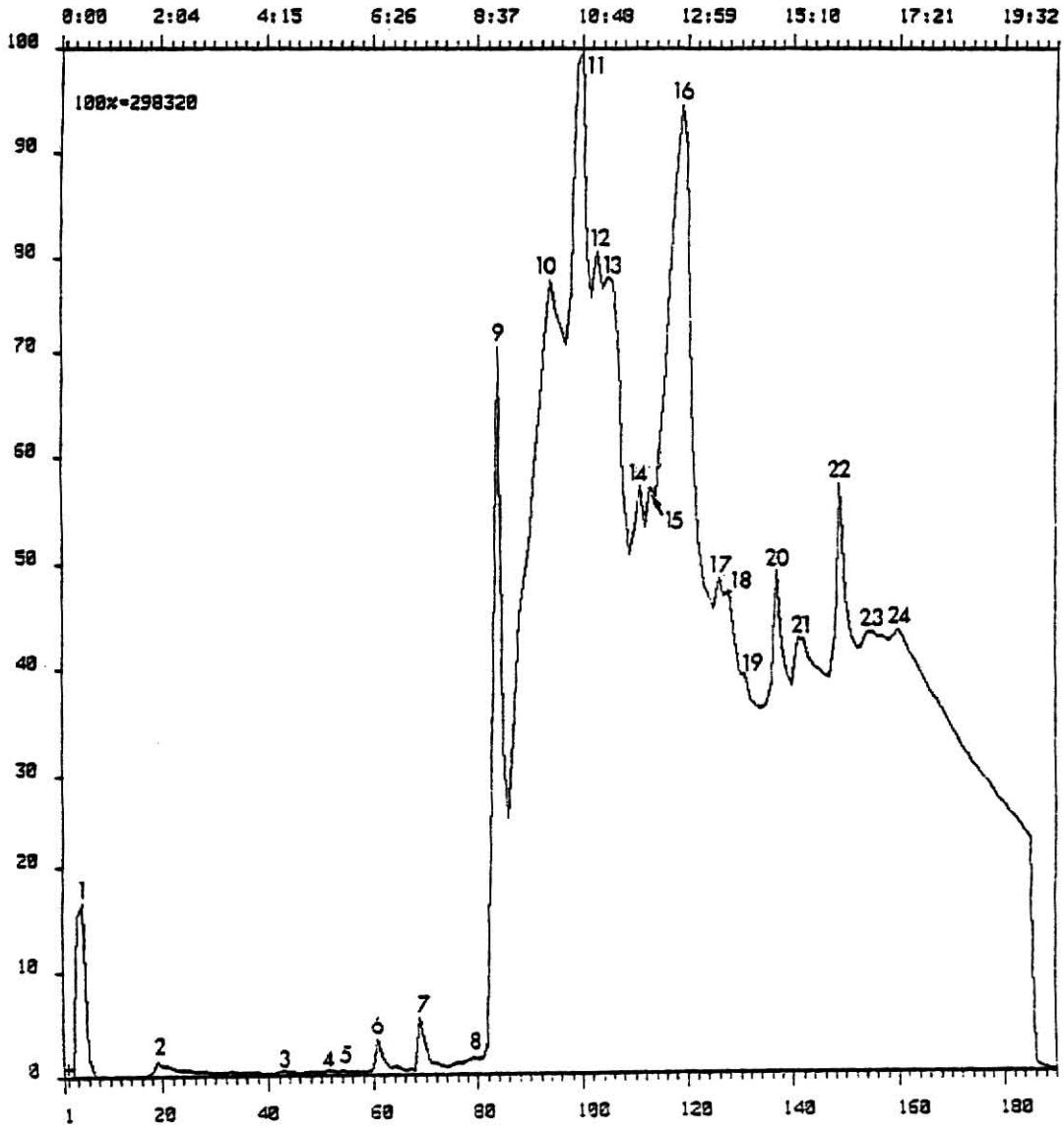


FIGURE 31

Mass spectrum of

Peak 3 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
175782.42 [TIC=384, 100%=69] EI

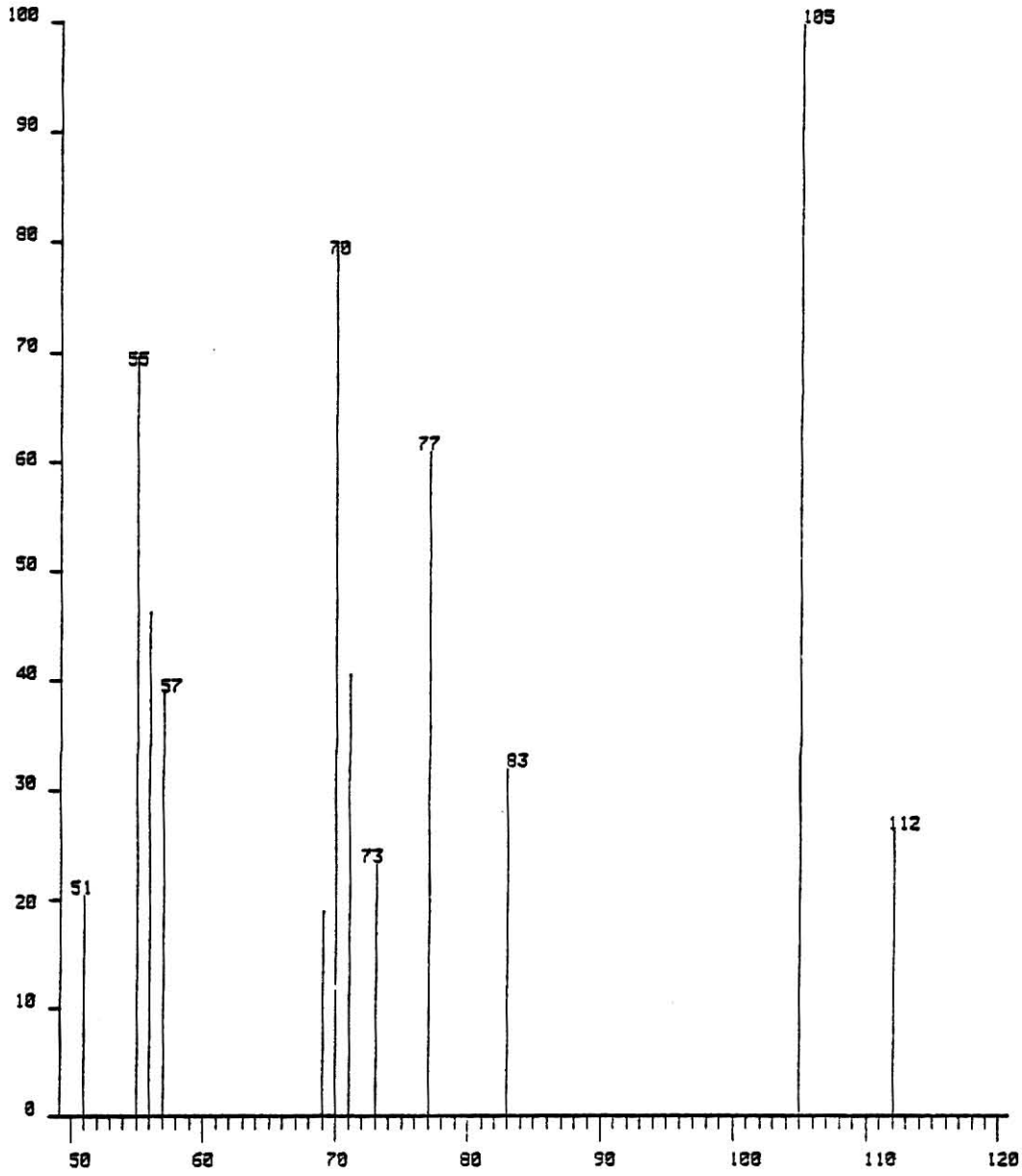


FIGURE 32

Mass spectrum of

Peak 4 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17578Z.51 CTIC=286, 100%=60] EI

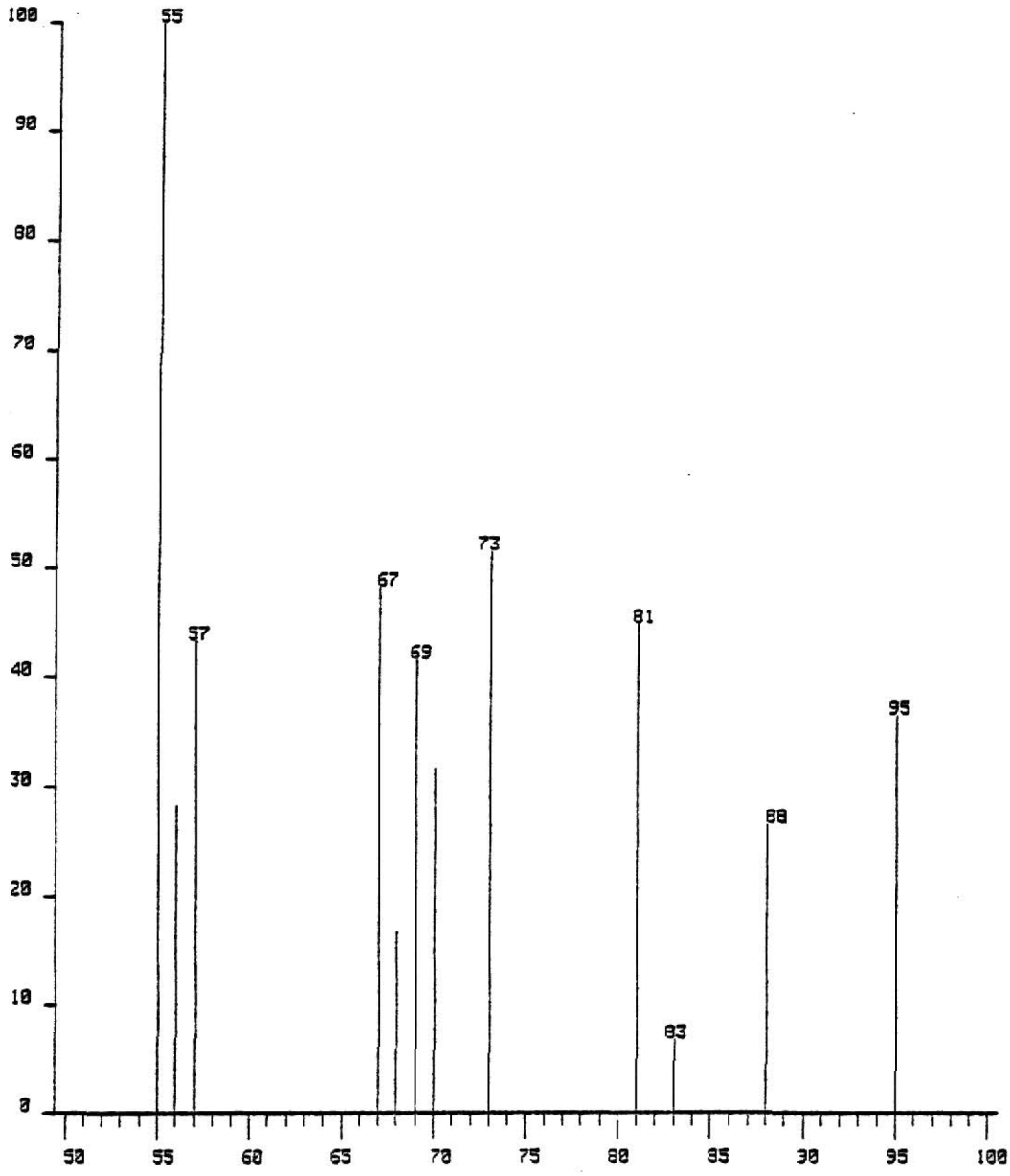


FIGURE 33

Mass spectrum of

Peak 5 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17578Z.54 [TIC=308, 100%=74] EI

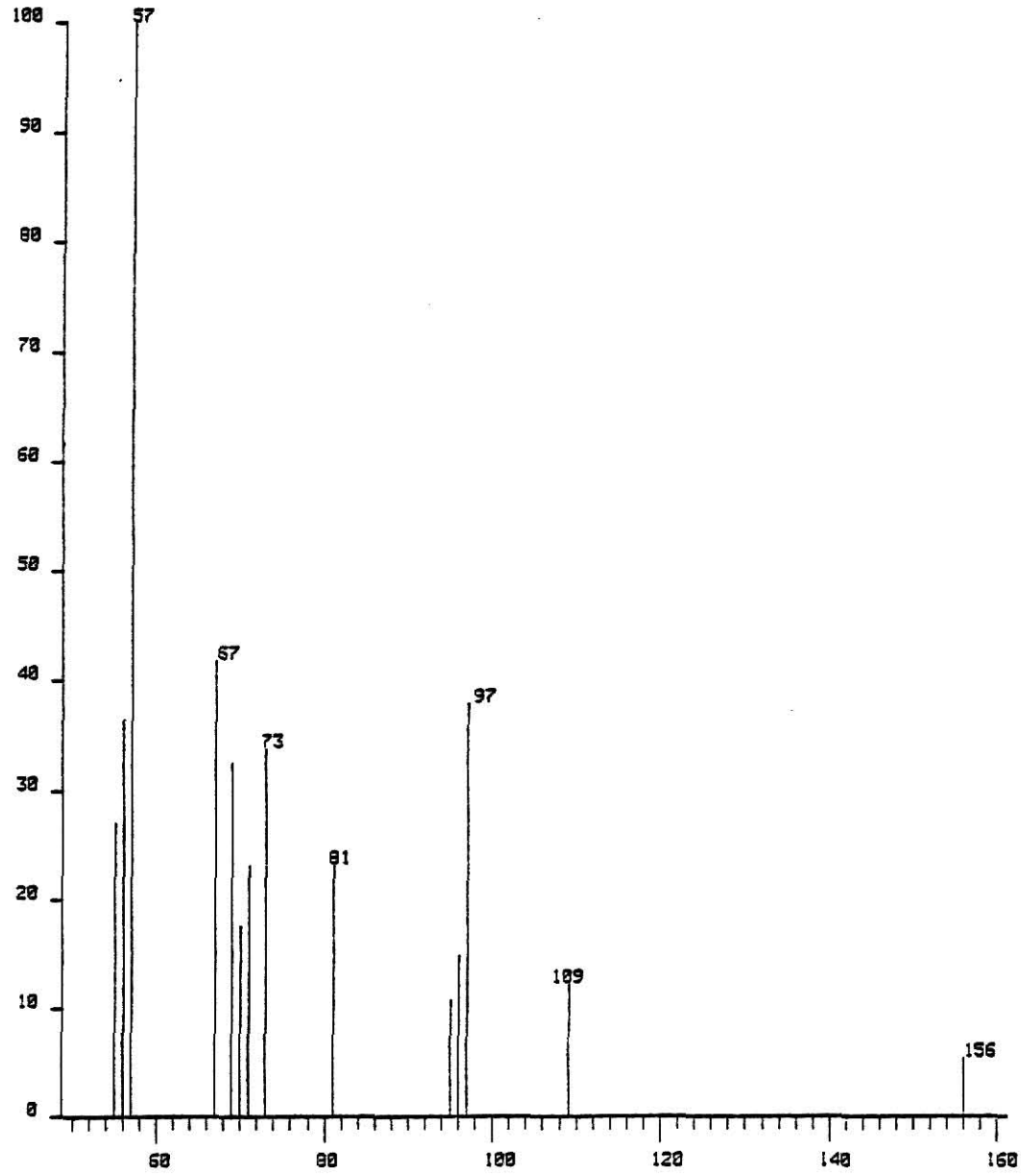


FIGURE 34

Mass spectrum of

Peak 6 of Figure 30

Proposed structure: Omite fragment

DS-55 MASS INTENSITY REPORT:
175782.61 [TIC=8073, 100%=3475] EI

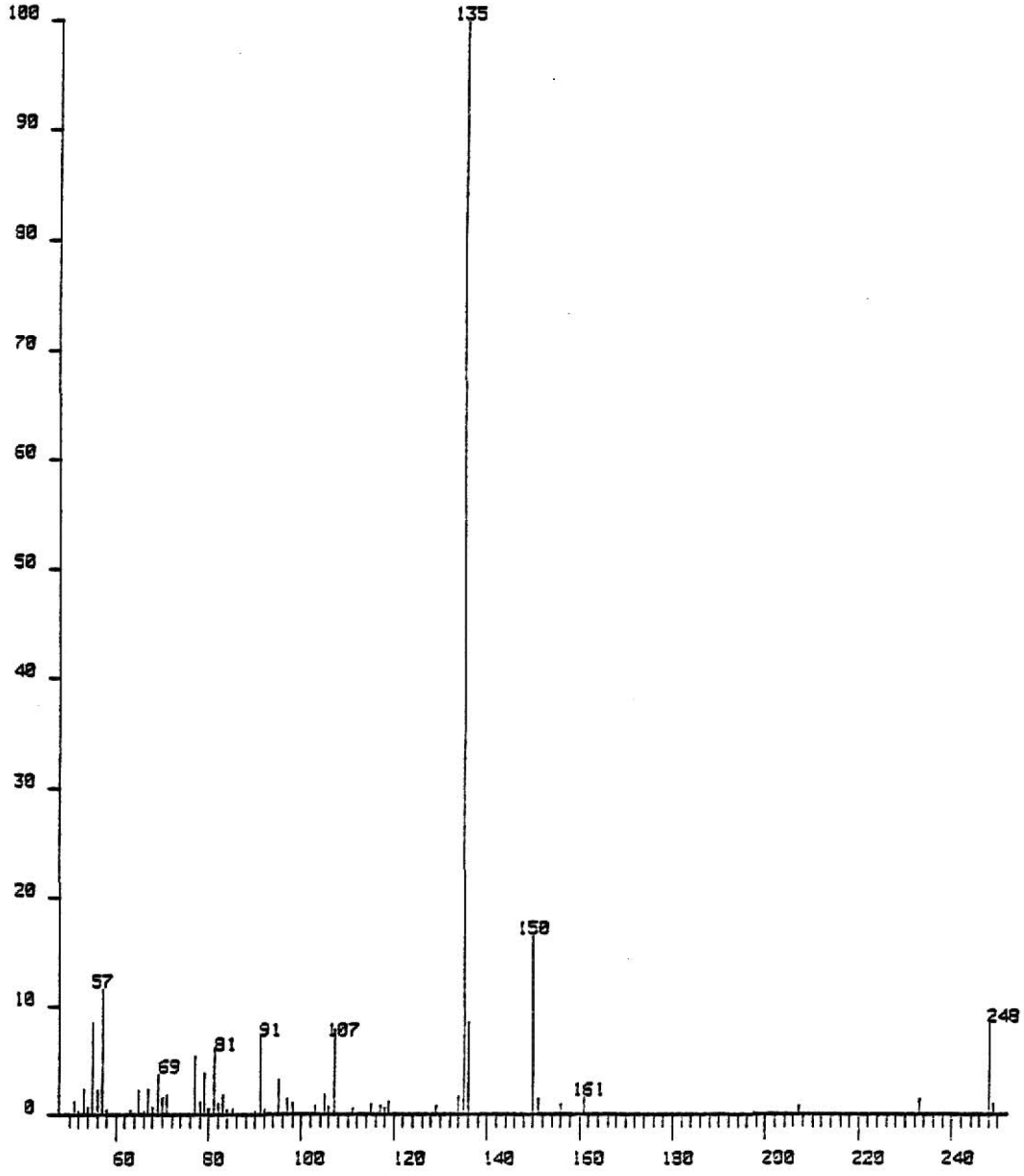
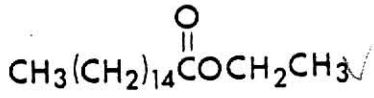


FIGURE 35

Mass spectrum of

Peak 7 of Figure 30

Proposed structure:



DS-55 MASS INTENSITY REPORT:

17578Z.69 [TIC=11229, 100%=2738] EI

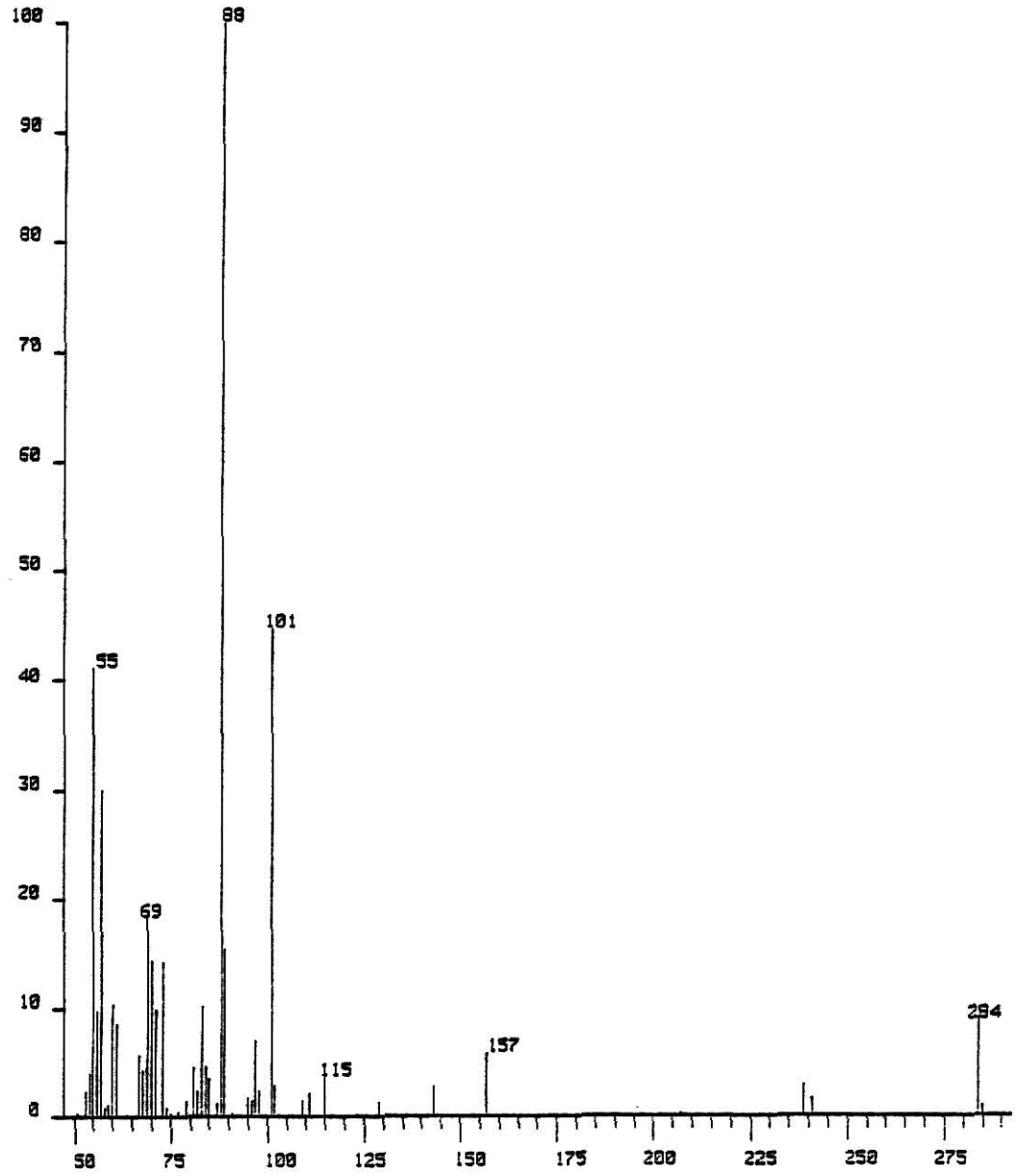


FIGURE 36

Mass spectrum of

Peak 8 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
I75782.79 [TIC=1939, 100%=213] EI

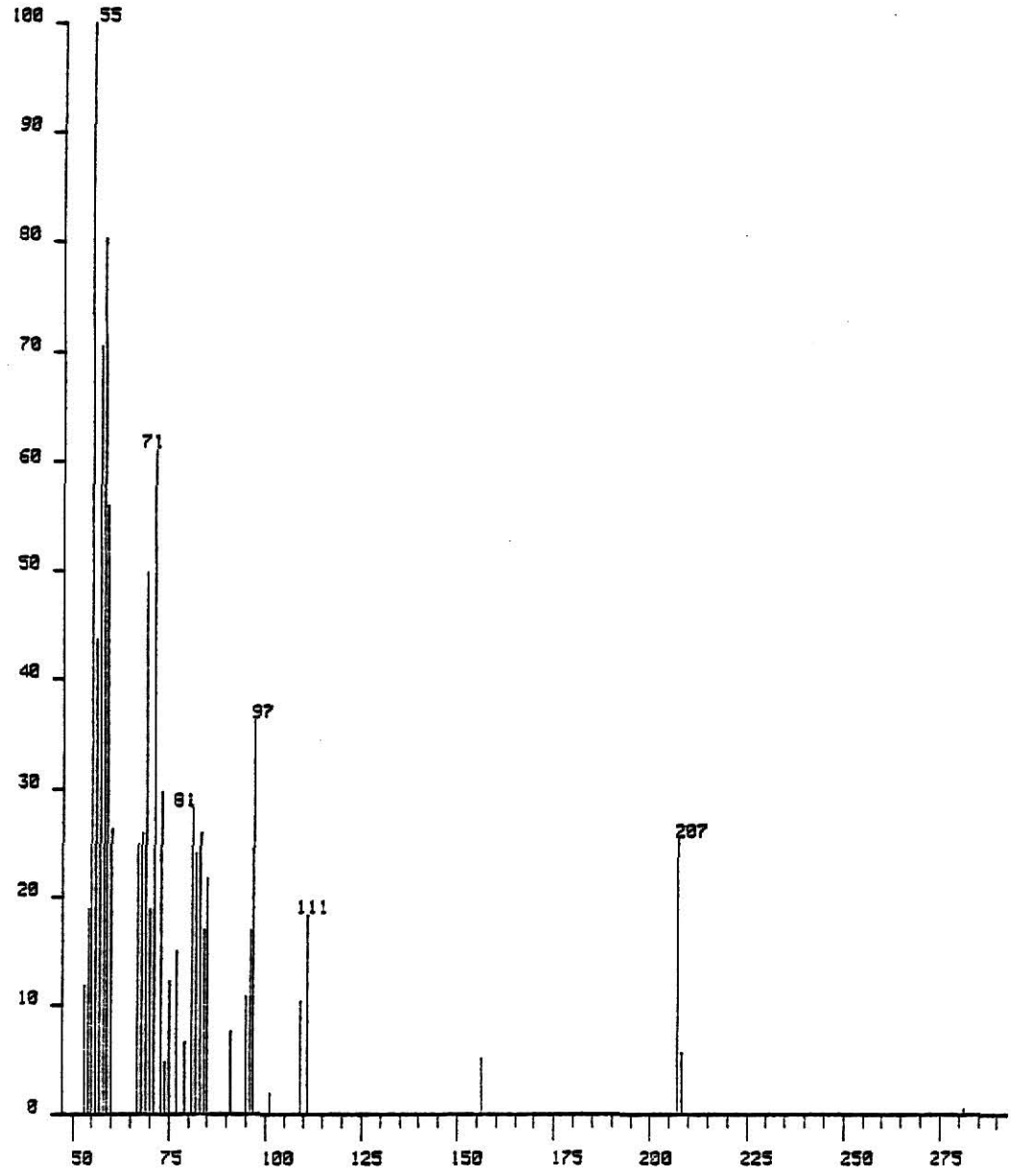
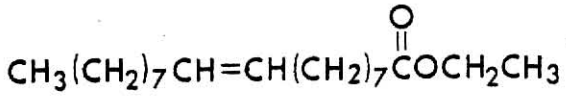


FIGURE 37

Mass Spectrum of

Peak 9 of Figure 30

Proposed structure:



DS-55 MASS INTENSITY REPORT:
175782.84 [TIC=126852, 100%=10214] EI

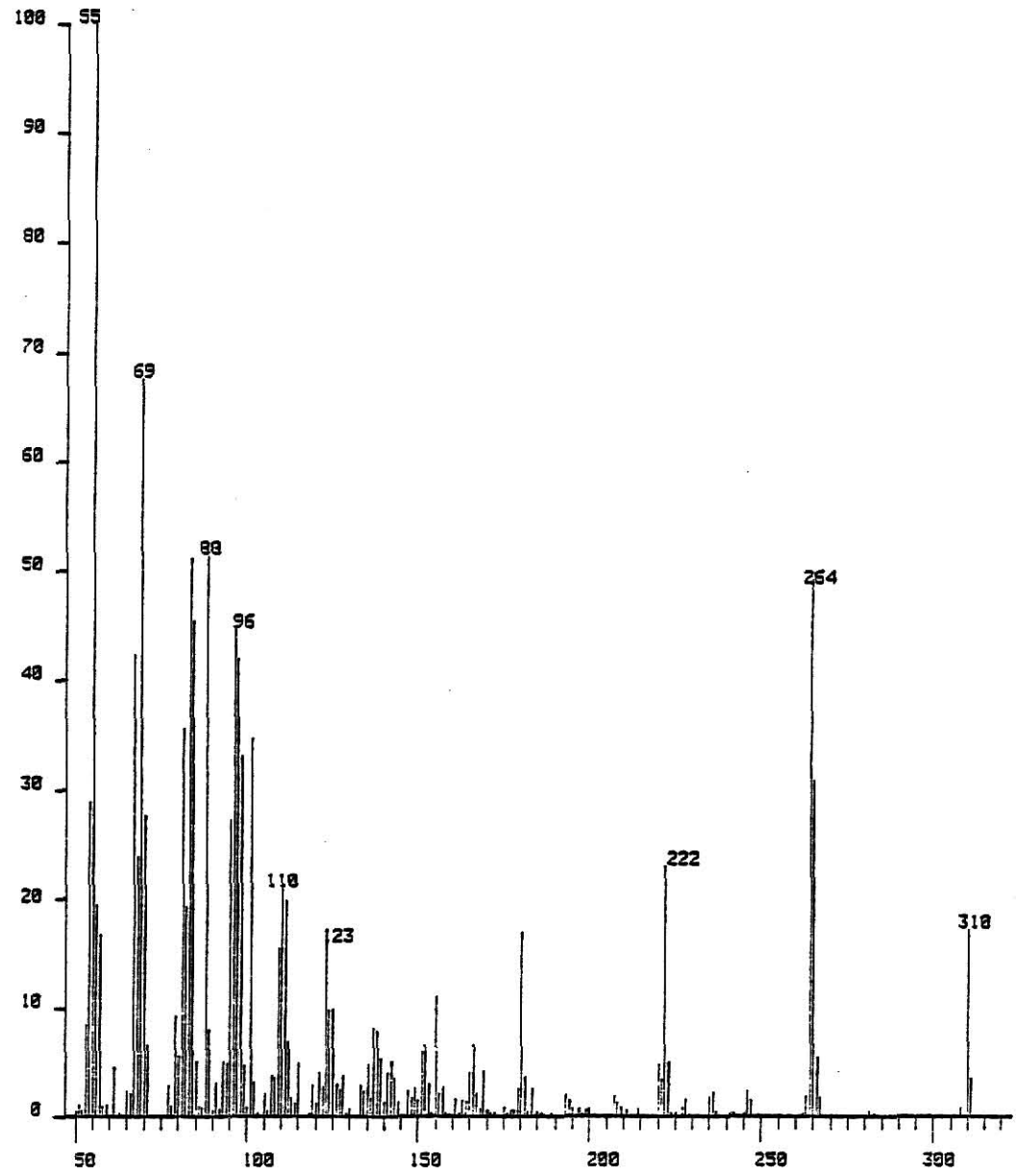


FIGURE 38

Mass spectrum of

Peak 10 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17578Z.94 [TIC=69520, 100%=6031] EI

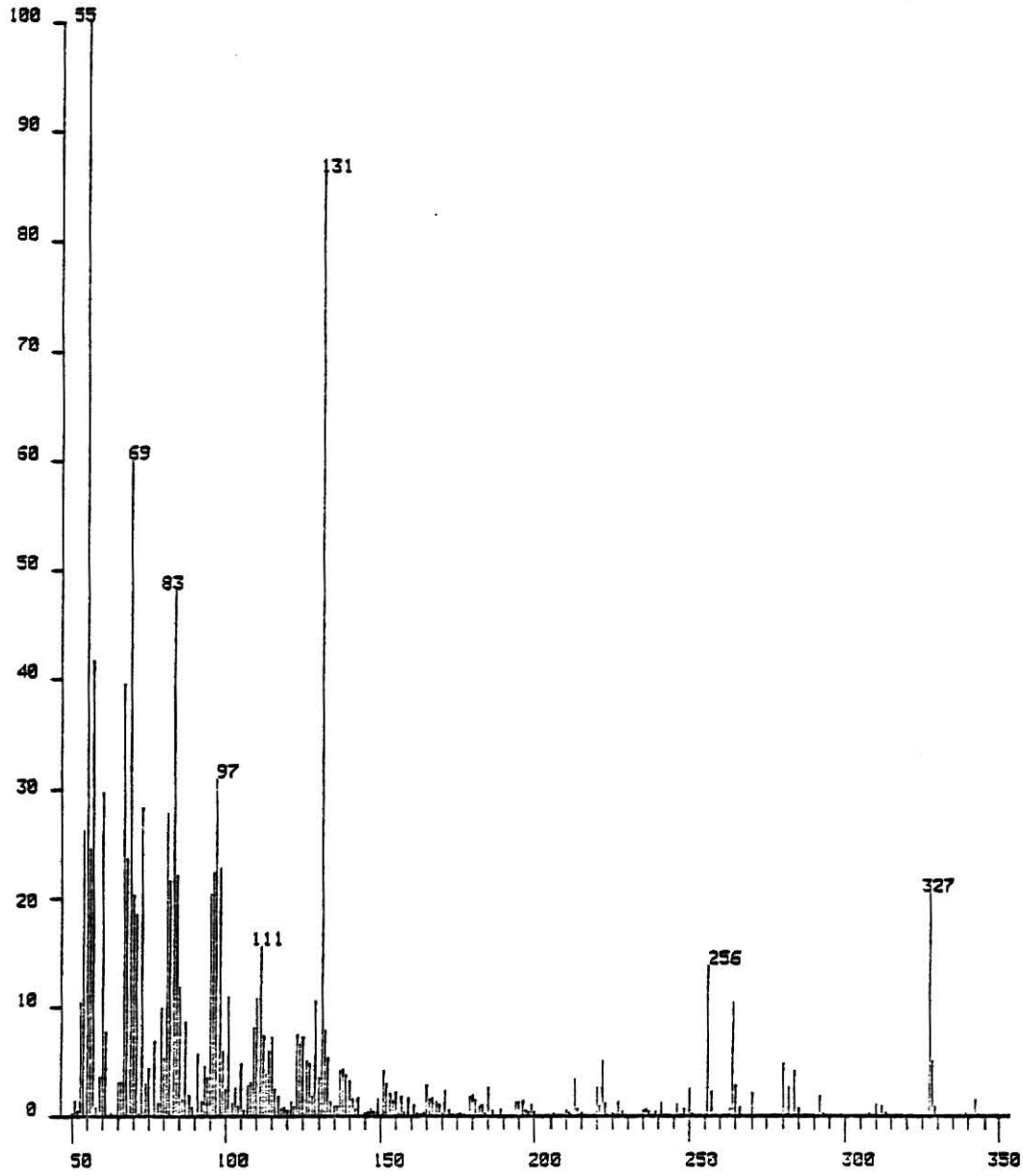
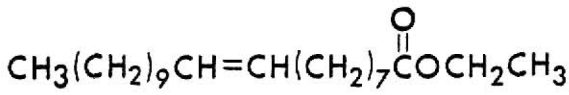


FIGURE 39

Mass spectrum of

Peak 11 of Figure 30

Proposed structure:



DS-55 MASS INTENSITY REPORT:

175782.100 [TIC=143968, 100%=11782] EI

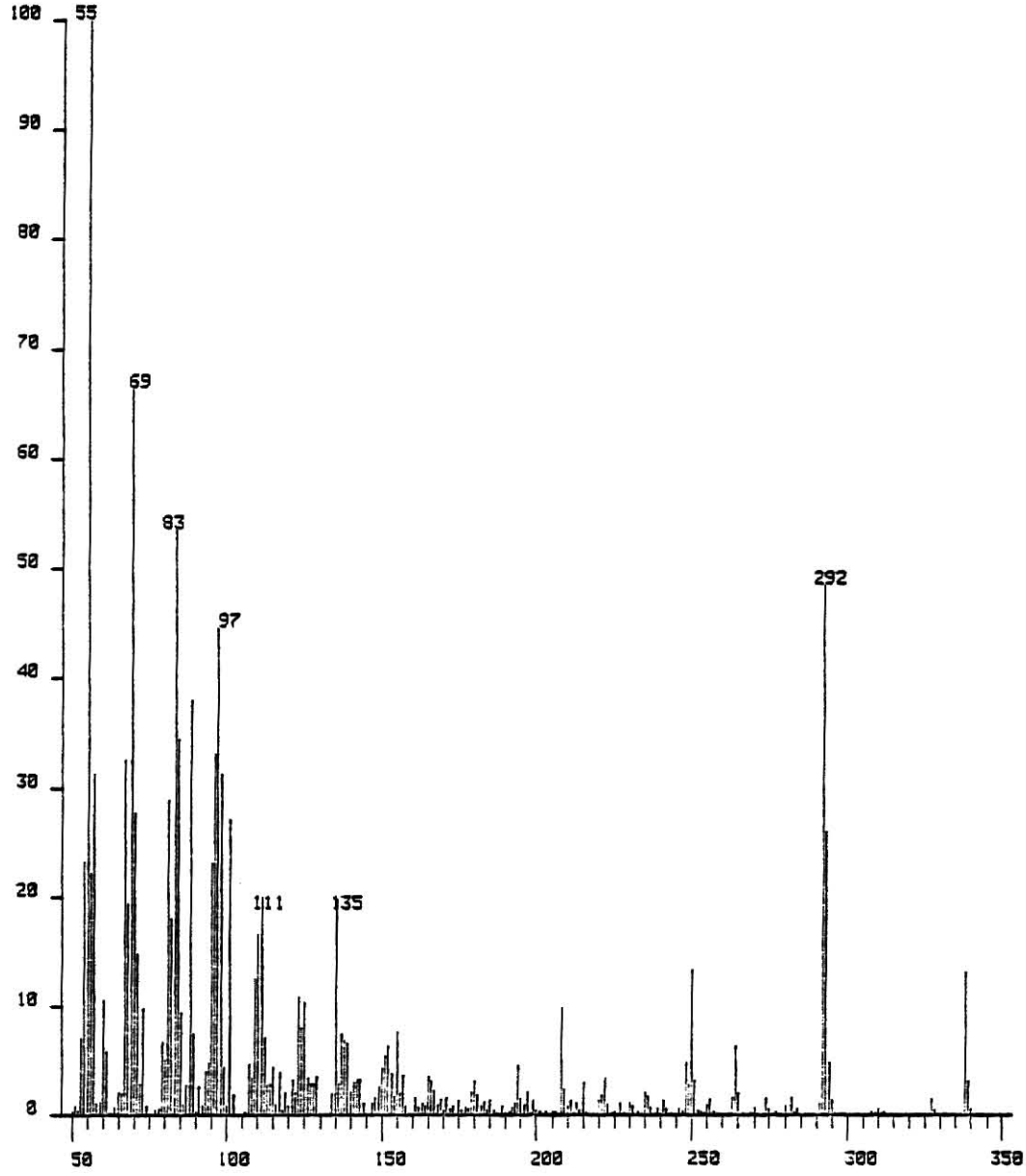


FIGURE 40

Mass spectrum of

Peak 12 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17578Z.103 [TIC=60408. 100%=16930] EI

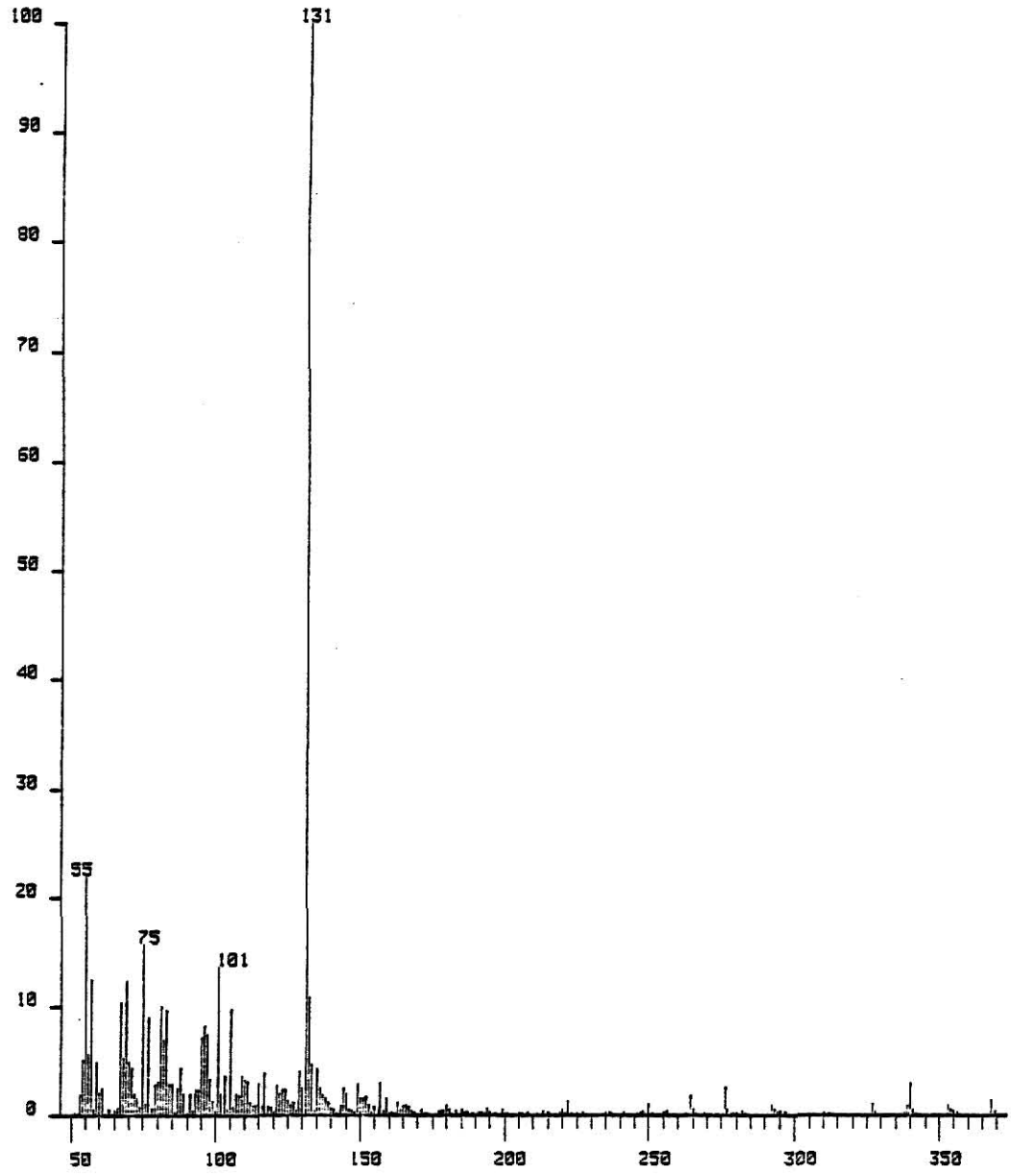


FIGURE 41

Mass spectrum of

Peak 13 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:

17578Z.105 [TIC=74708, 100%=19700] EI

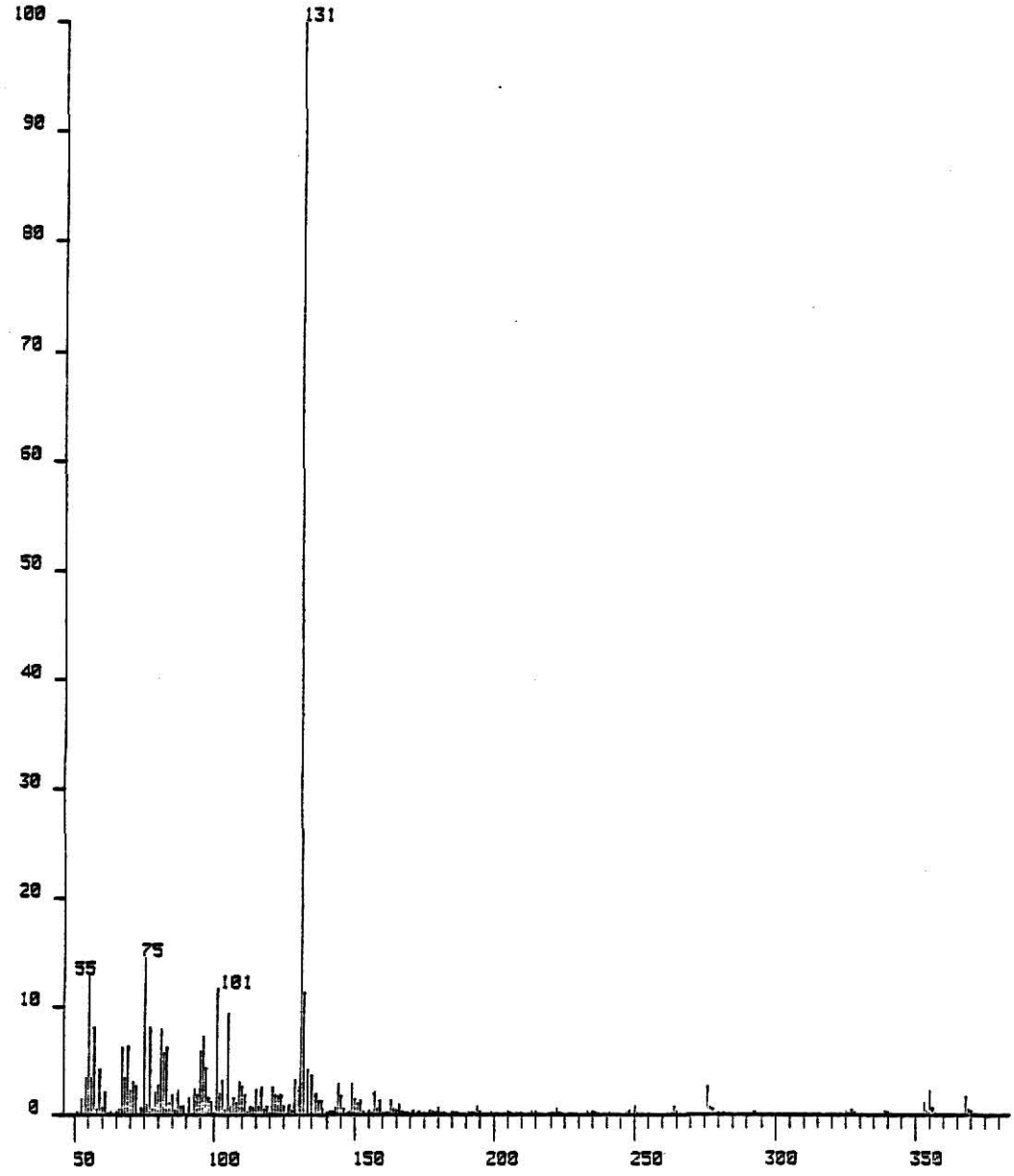


FIGURE 42

Mass spectrum of

Peak 14 of Figure 30

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:

17578.111 [TIC=12404. 100%=1273] EI

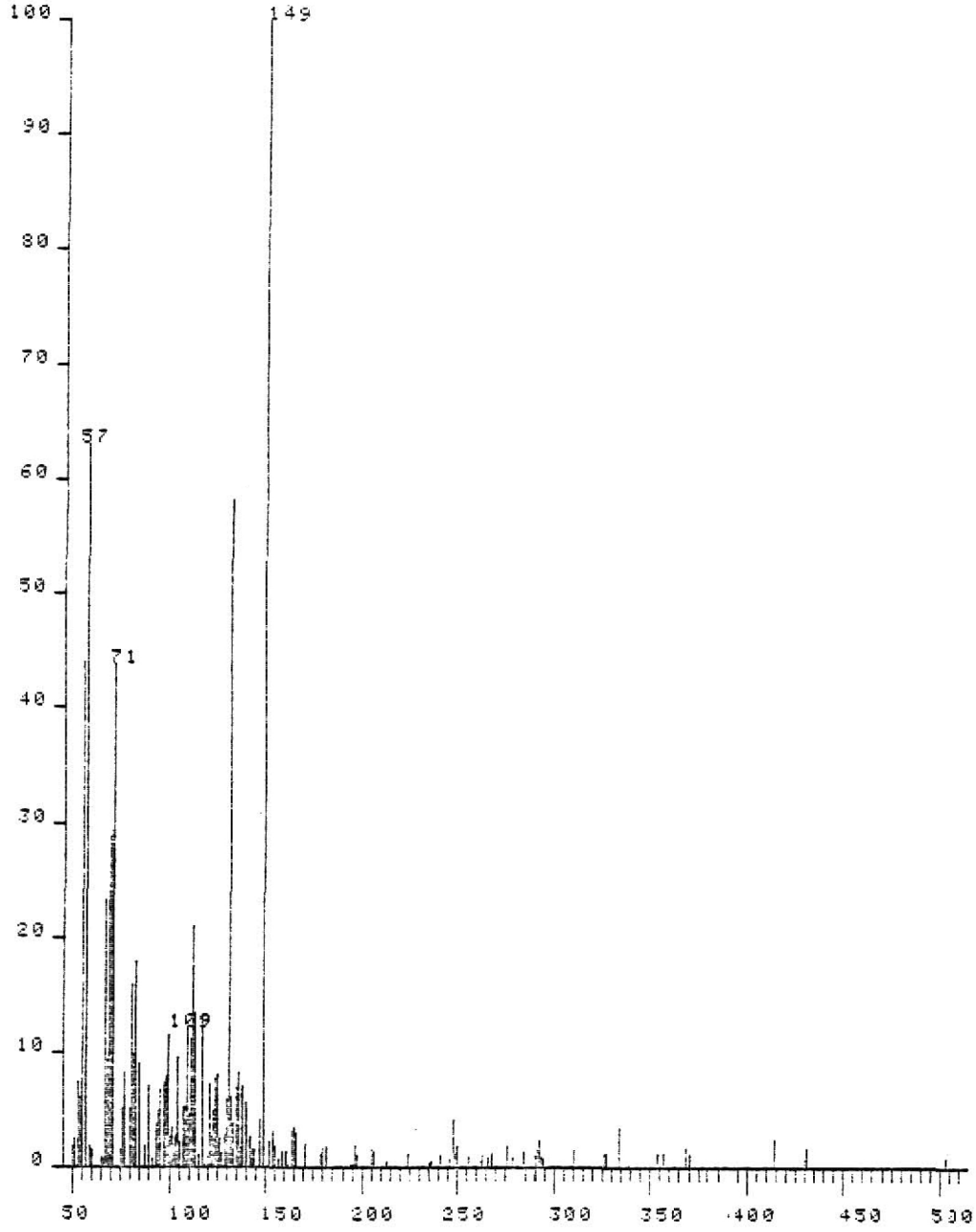


FIGURE 43

Mass spectrum of

Peak 15 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT.
17578.113 [TIC=12024, 100%=2321] EI

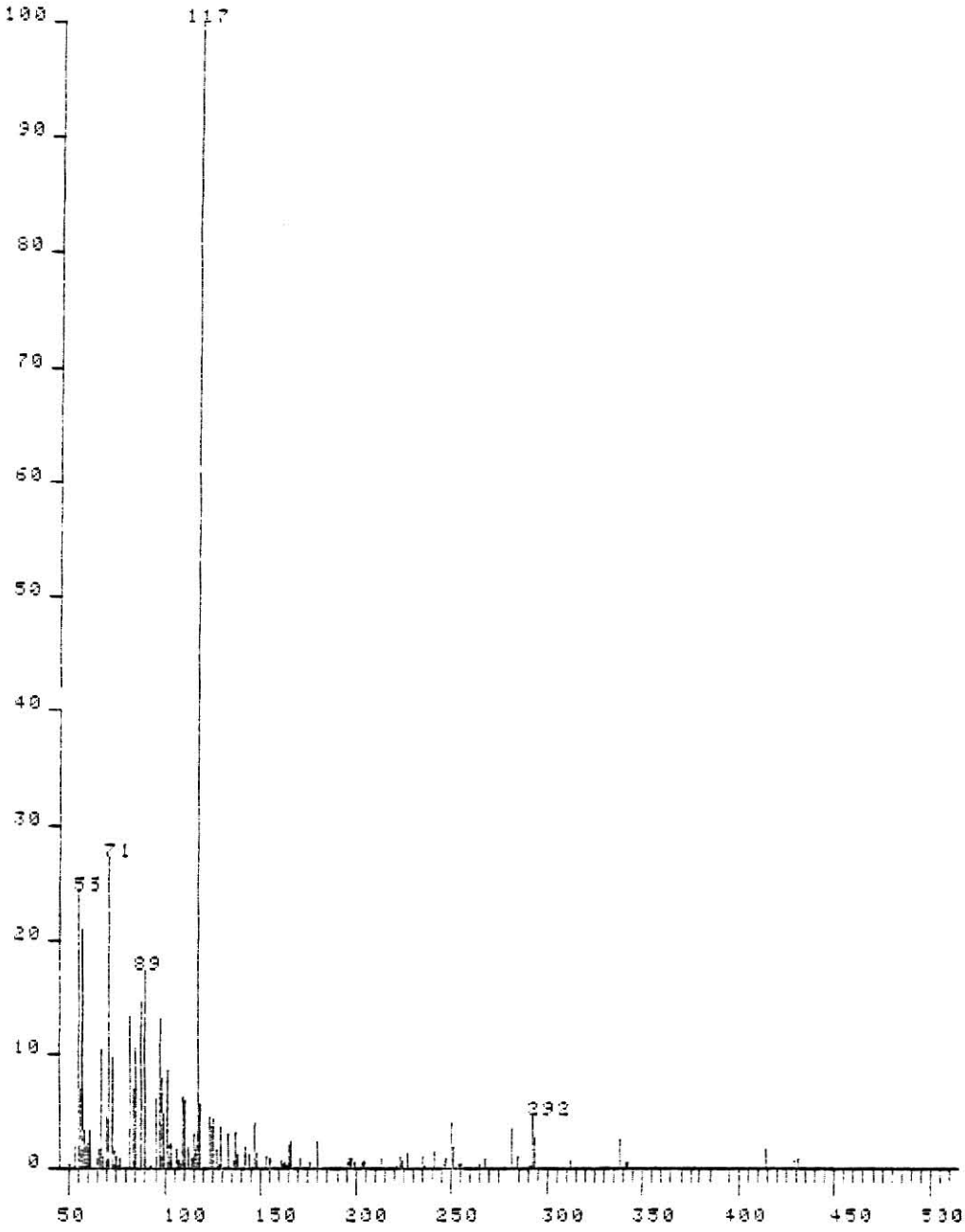


FIGURE 44

Mass spectrum of

Peak 16 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17578Z.119 [TIC=135000, 100%=53488] EI

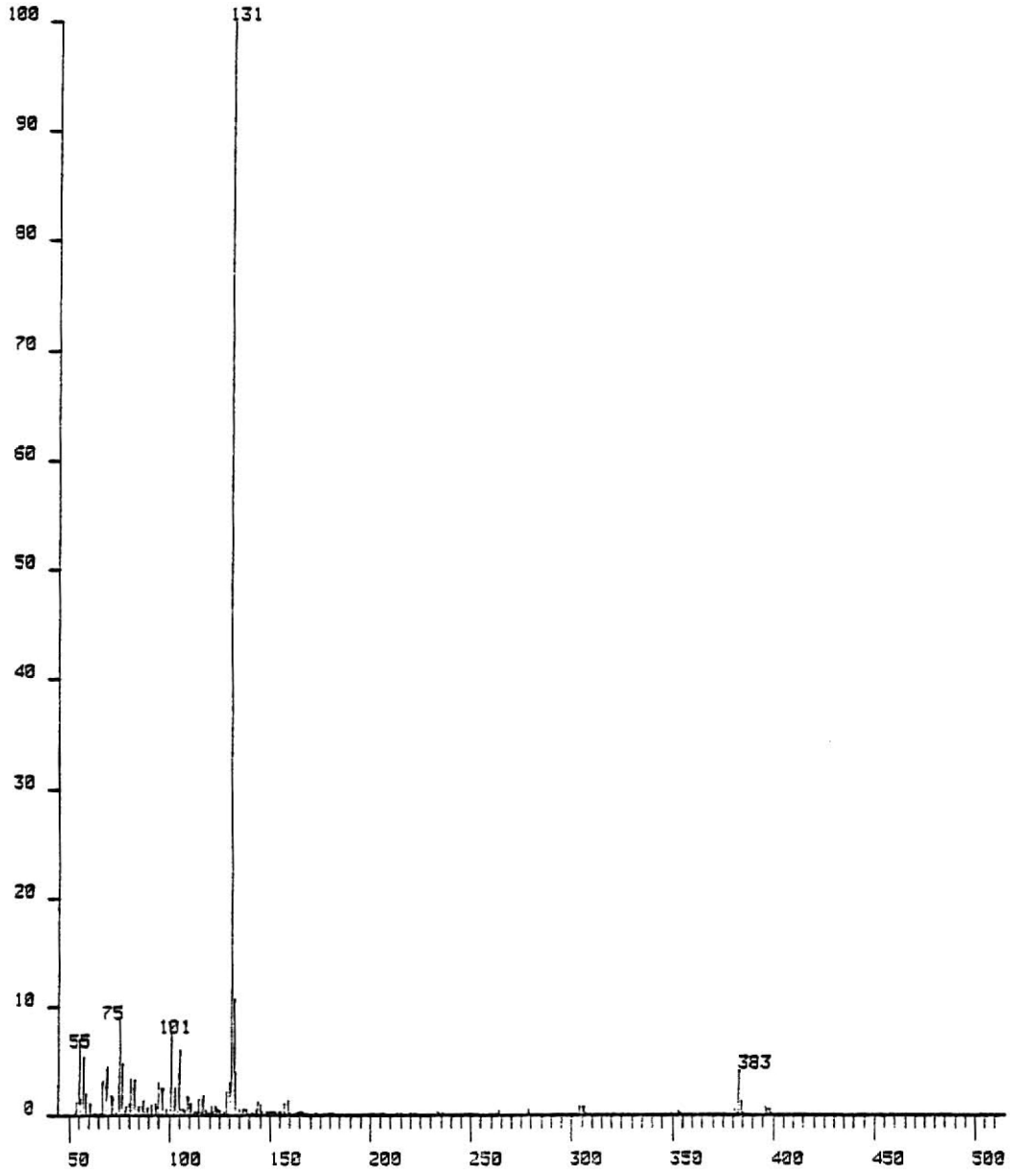
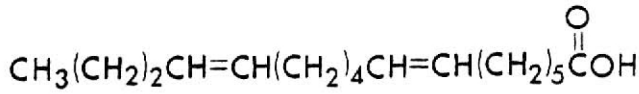


FIGURE 45

Mass spectrum of

Peak 17 of Figure 30

Proposed structure:



DS-55 MASS INTENSITY REPORT:

175782.126 [TIC=8188, 100%=725] EI

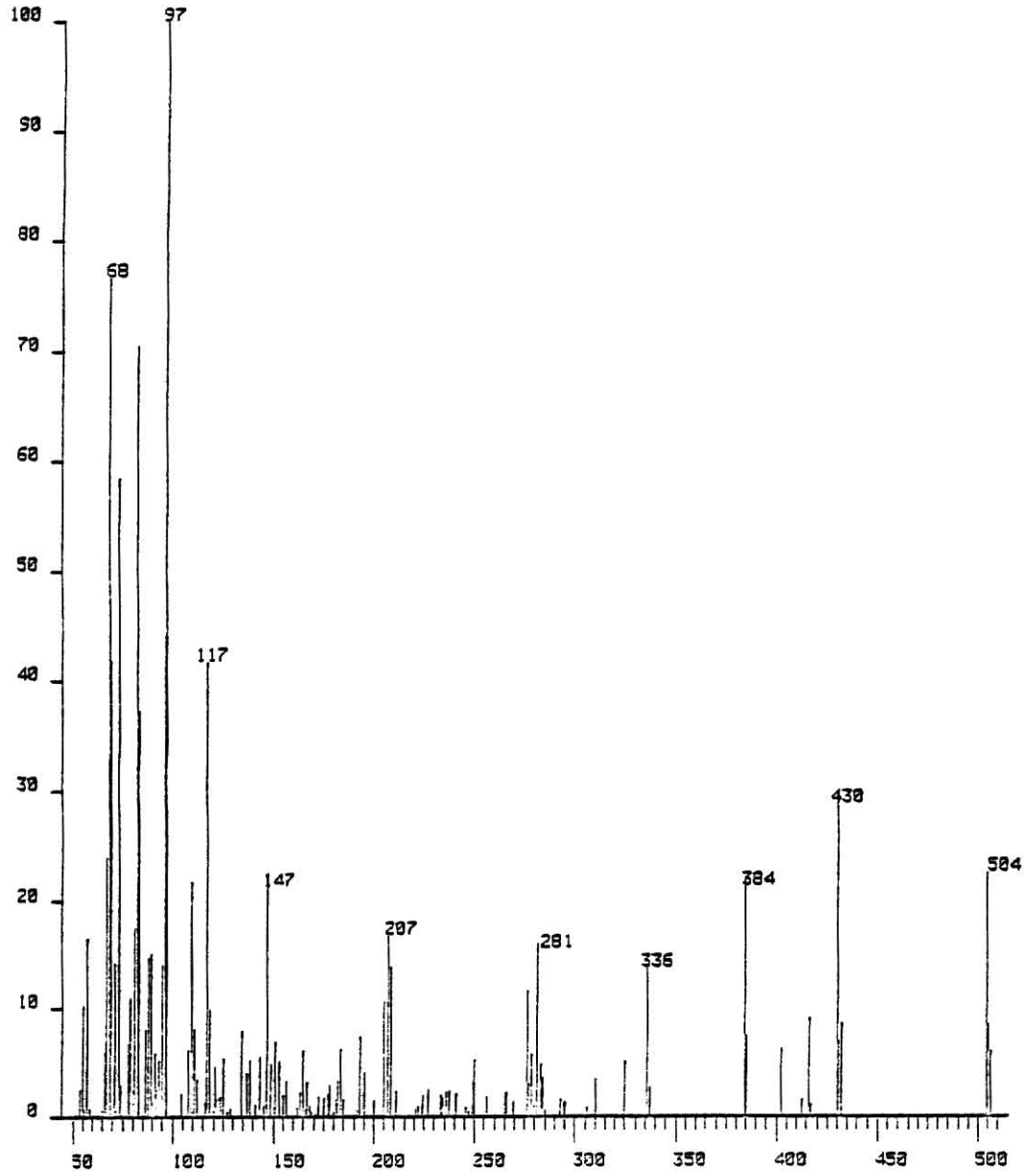


FIGURE 46

Mass spectrum of

Peak 18 of Figure 30

Proposed structure: None

DB-5S MASS INTENSITY REPORT:

17378.128 [TIC=15982, 100%=9295] EI

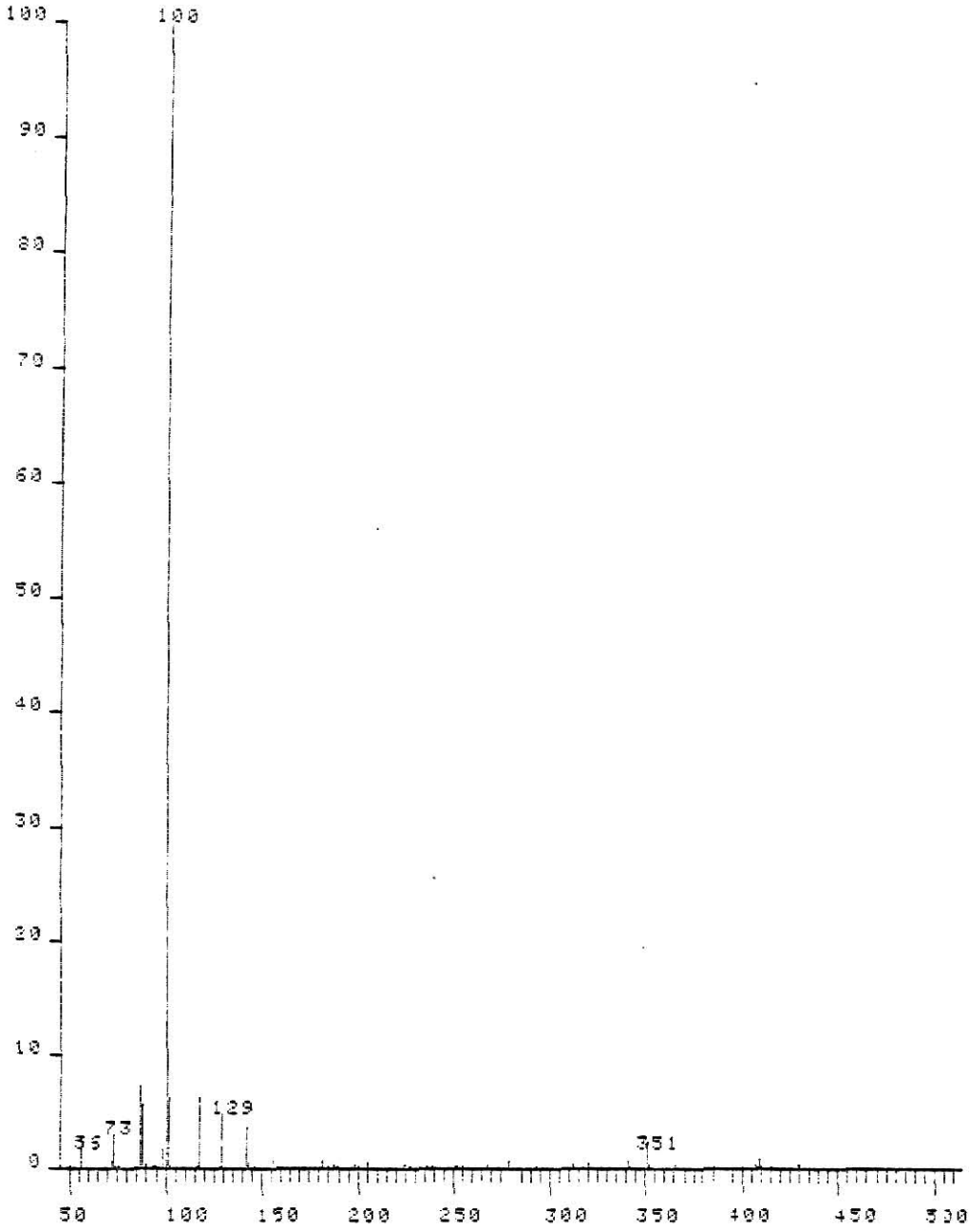


FIGURE 47

Mass spectrum of

Peak 19 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:

17578.130 [TIC=3534, 100%=750] EI

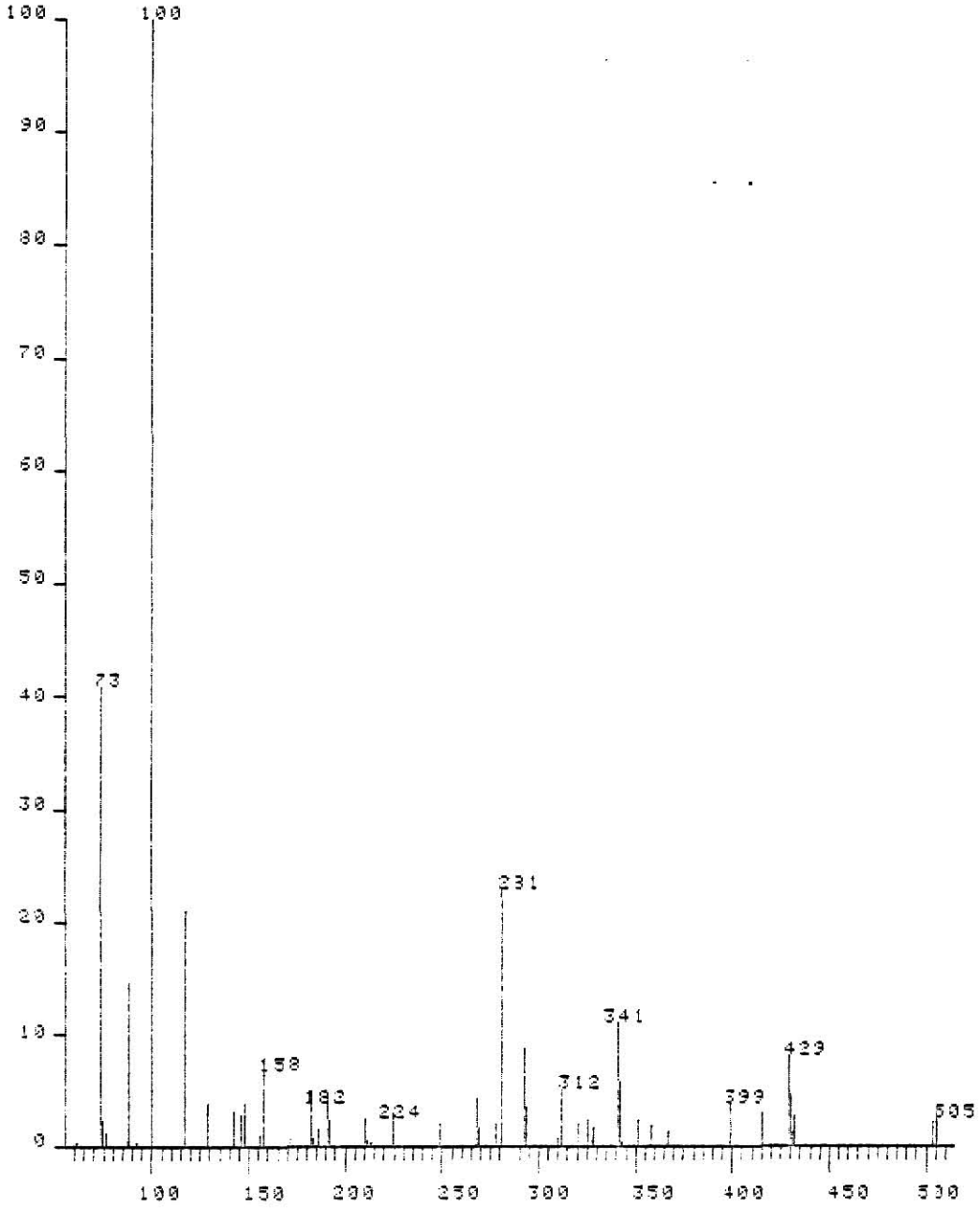


FIGURE 48

Mass spectrum of

Peak 20 of Figure 30

Proposed structure:

straight chain alkane of length at least C_{36}

DS-55 MASS INTENSITY REPORT:
17578Z.137 [TIC=29893, 100%=6620] EI

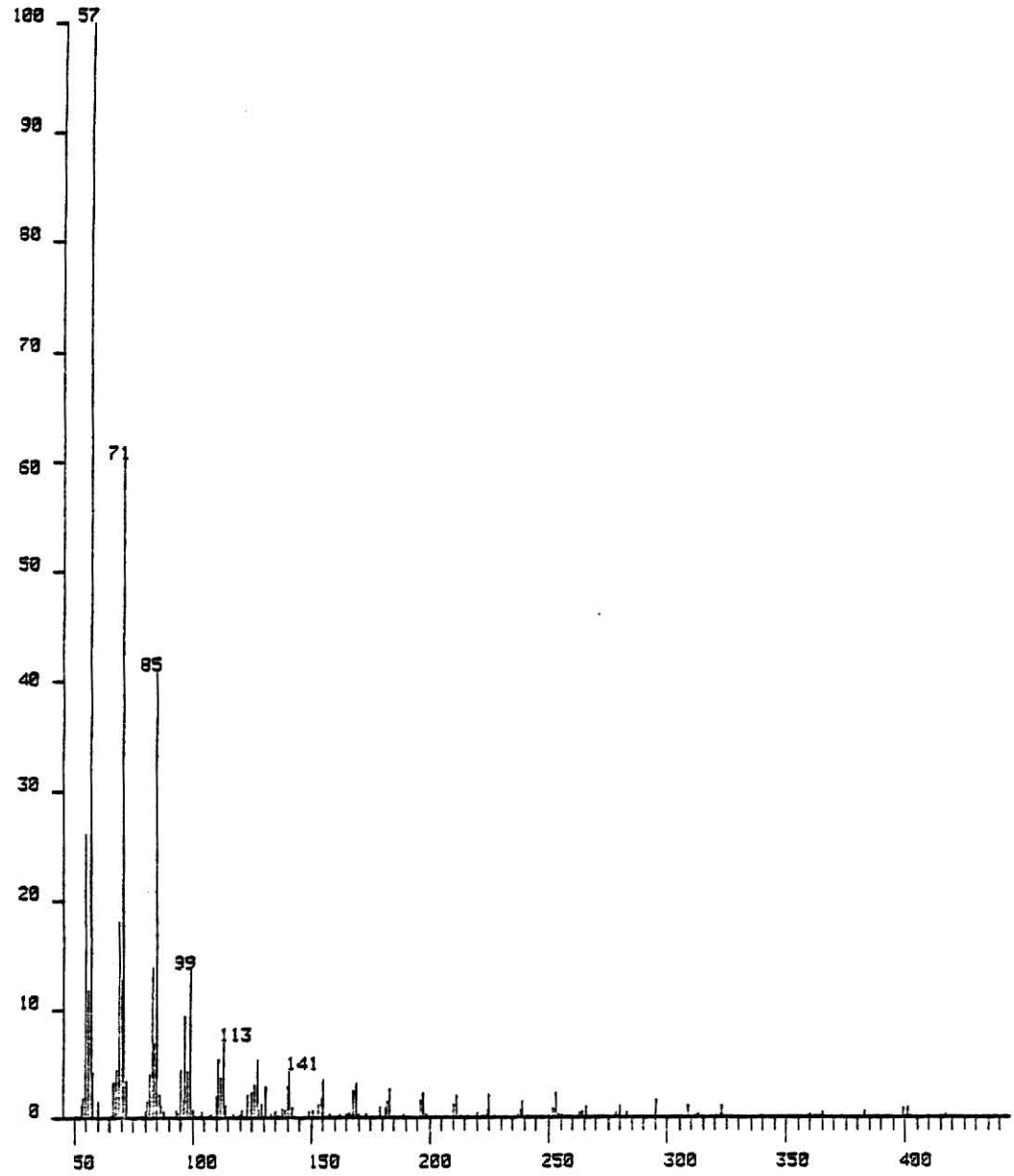
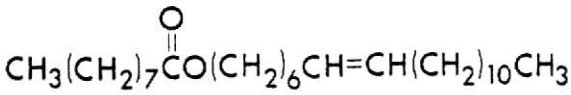


FIGURE 49

Mass spectrum of

Peak 21 of Figure 30

Proposed structure:



DS-55 MASS INTENSITY REPORT:

17578Z.141 [TIC=18133, 100%=2932] EI

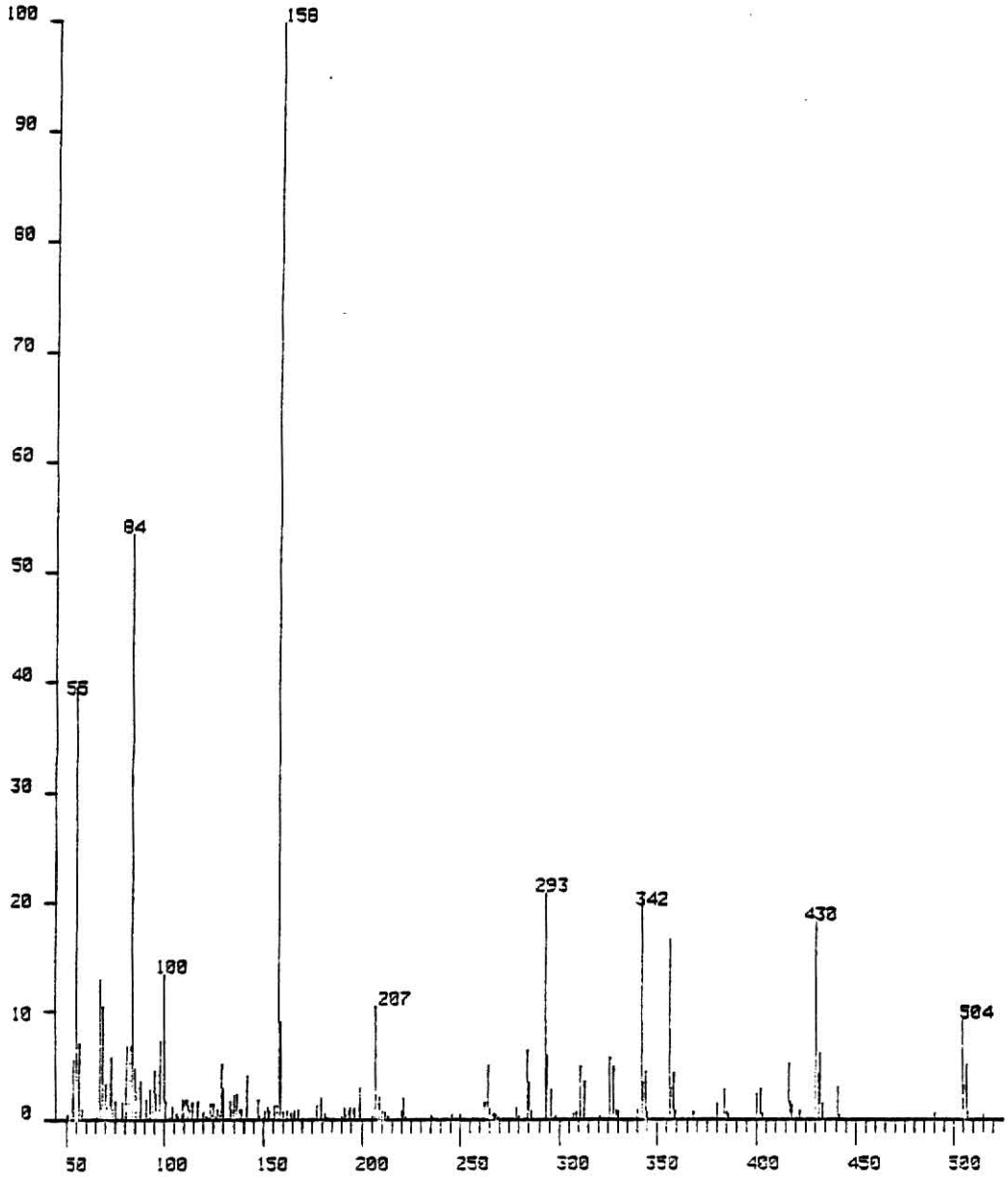


FIGURE 50

Mass spectrum of

Peak 22 of Figure 30

Proposed structure:

straight chain alkane of length at least C_{36}

DS-55 MASS INTENSITY REPORT:

175782.149 [TIC=44672, 100%=9616] EI

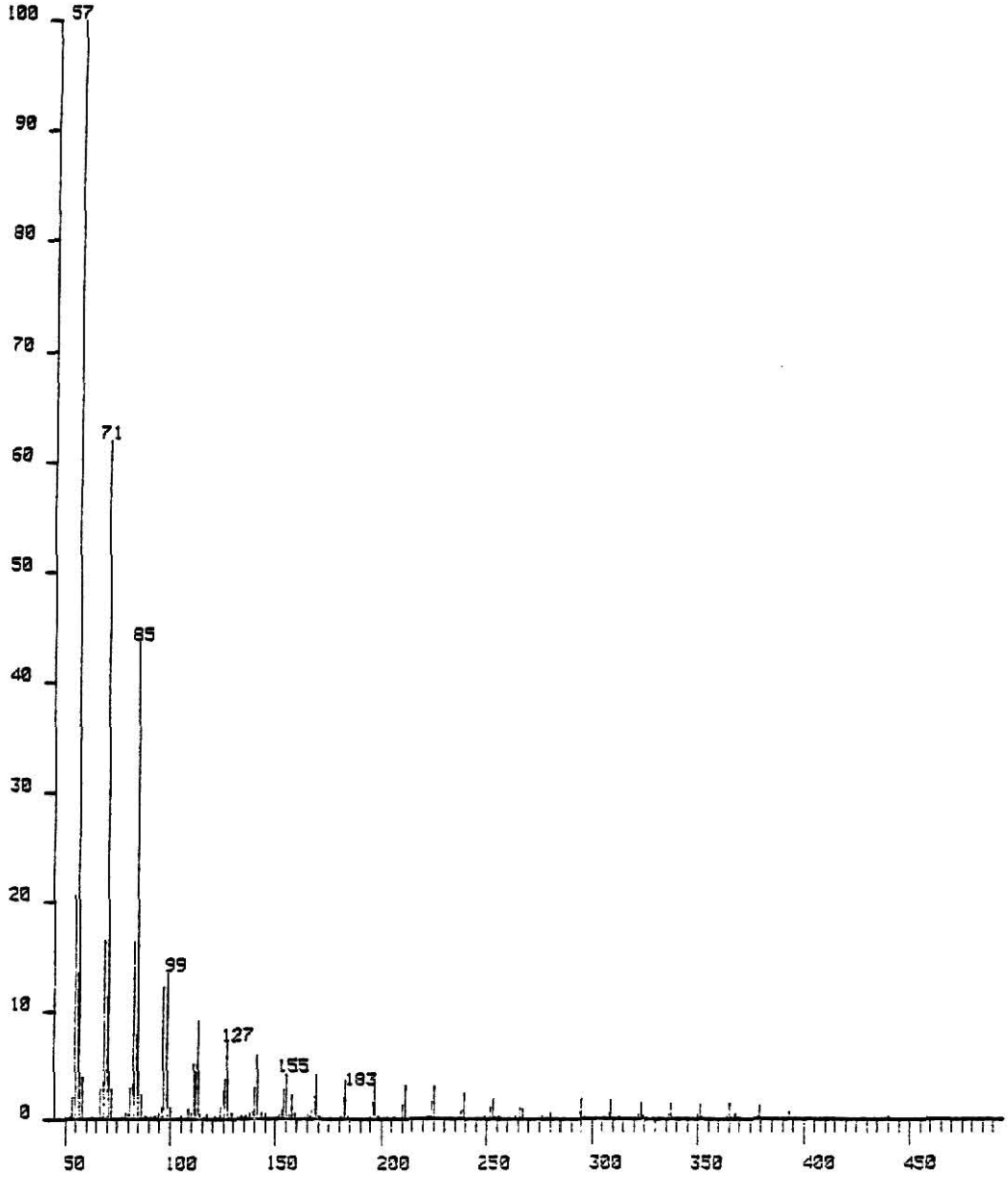
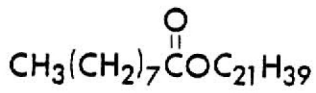


FIGURE 51

Mass Spectrum of

Peak 23 of Figure 30

Proposed structure:



(no branching)

DS-55 MASS INTENSITY REPORT:
17578.154 (TIC=7741, 100%=905) EI

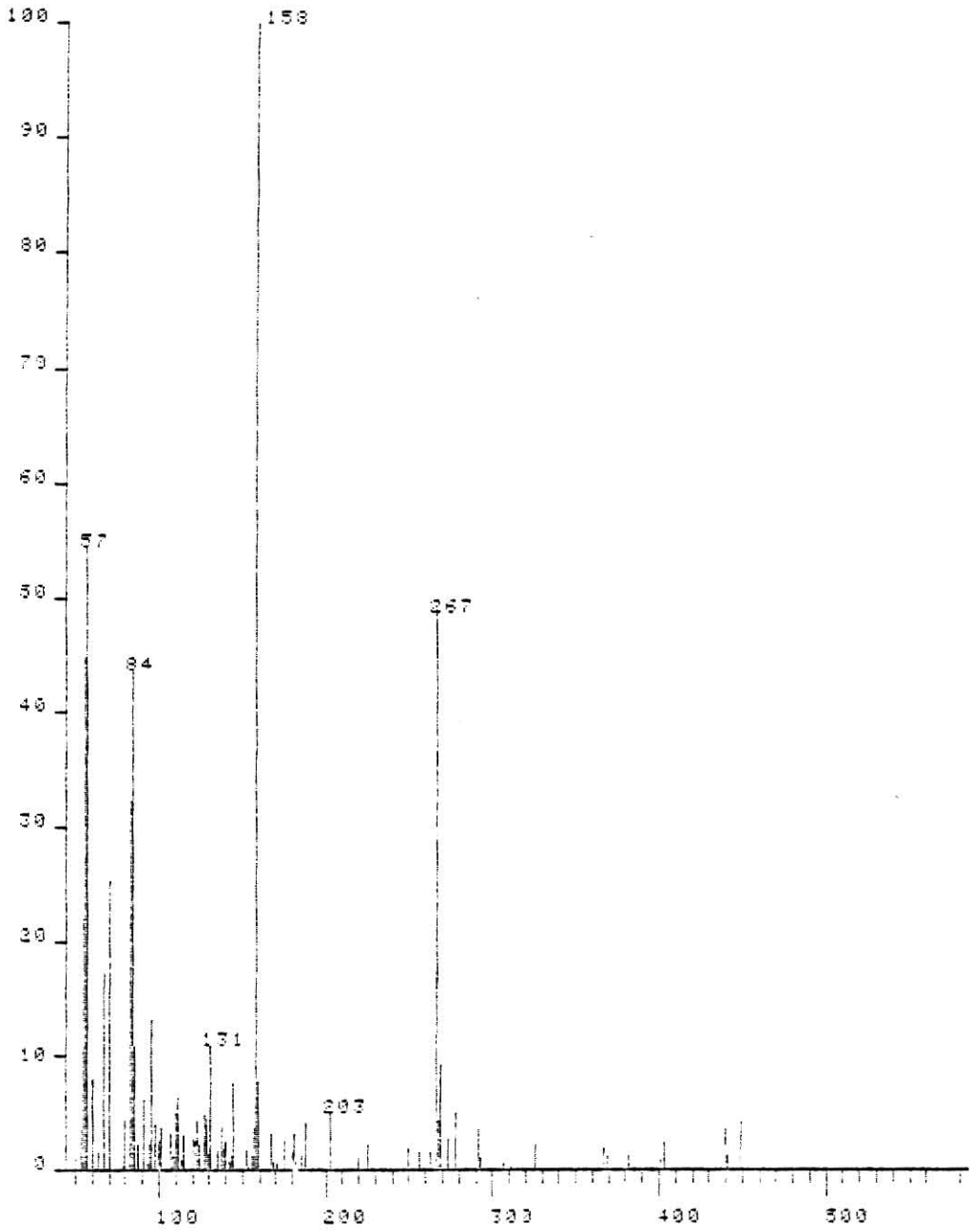


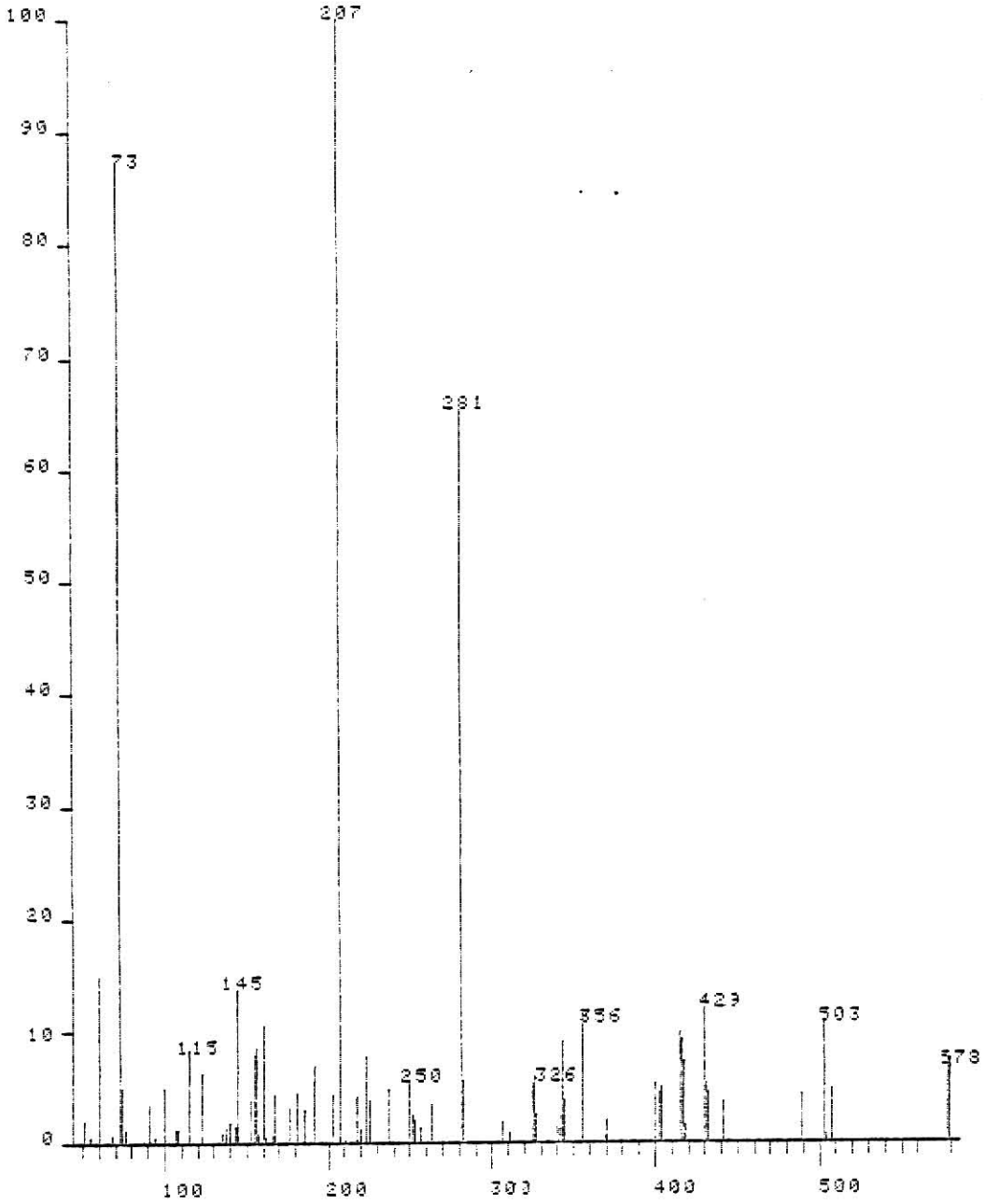
FIGURE 52

Mass spectrum of

Peak 24 of Figure 30

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17378 159 [TIC=4177, 100%=445] EI



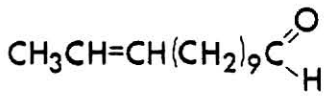
Interpretation of Mass Spectra

from MRI, Kansas City, Missouri

of Medicago scutellata

Figure 54 (Peak 1 of Figure 53)

Proposed structure:



1) The parent peak is taken to be at m/e 196. The peak at m/e 222 was not chosen because the steady fragmentation pattern which occurs throughout the spectrum stops with m/e 196. There is then a gap of 26 mass units before the signal at m/e 222.

2) The peak at m/e 179 arises from the loss of OH from the molecule.

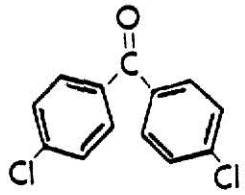
3) The peak at m/e 166 arises from the loss of HCHO from the molecule.

4) A loss of CH_2=CHOH from the molecule gives rise to the peak at m/e 152.

5) Both alkane and alkene patterns are present at m/e 57, 71, 85, etc. and m/e 55, 69, 83, etc., respectively. The alkene pattern dominates at m/e 55 and throughout the higher molecular weight region of the spectrum. For this reason, the double bond is tentatively assigned to the C_{11} position. However, the double bond most likely migrates during mass fragmentation of the molecule since it appears to be straight chained. Thus, the true position of the double bond cannot be ascertained here.

Figure 55 (Peak 2 of Figure 53)

Proposed structure:



or some isomer of this compound

1) The peak at m/e 250 is taken as the parent peak. The M+2 peak is 68% of the parent peak. The isotopic abundance of Cl^{37} is 32.7%, so this indicates two Cl's are present.

2) The loss of 35 from m/e 250 to m/e 215 indicates a loss of Cl.

3) The base peak at m/e 139 is due to the loss of C_6H_5Cl from the parent molecule, leaving COC_6H_4Cl. The m/e 141 peak is 31% of the m/e 139 peak, which indicates one Cl.

4) The peak at m/e 111 is due to the loss of C=O, leaving C_6H_4Cl.

5) The peak at m/e 75 is due to the loss of HCl, leaving C_6H_3.

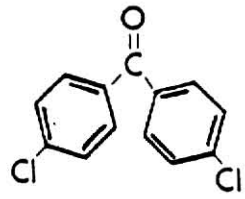
It is not likely that the compound represented by this spectrum is a component of the exudate but may be formed from the solvents used in the sample preparation. The sample was originally in $CHCl_3$ and then evaporated to dryness. The residue was then taken up in benzene. Or possibly insecticide fragments could have dissolved in the benzene to produce this compound. It is not believed that this compound is a component of the exudate because:

a) the other compounds found thus far are esters, aldehydes, alkanes, and alkenes of high molecular weights and

b) this compound has not shown up in the other two M. scutellata exudate sample GC/MS analyses.

Figure 56 (Peak 3 of Figure 53)

Proposed structure:



or some isomer of this compound

This spectrum is nearly identical to Figure 55.

1) The parent peak is taken to be at m/e 250. The M+2 peak is 63% of the parent peak. The isotopic abundance of Cl^{37} is 32.7%, so this indicates two Cl's are present.

2) The loss of 35 from m/e 250 to m/e 215 indicates the loss of Cl.

3) The base peak at m/e 139 is due to the loss of C_6H_5Cl from the parent molecule, leaving COC_6H_4Cl. The peak at m/e 141 is 31% of the m/e 139 peak indicating one Cl.

4) The peak at m/e 111 is due to the loss of C=O, leaving C_6H_4Cl.

5) The peak at m/e 75 is due to the loss of HCl, leaving C_6H_3.

The compound represented by this spectrum and the compound represented in Figure 55 are not believed to be components of the exudate for these reasons:

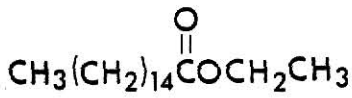
a) Thus far, the compounds which have been tentatively identified are esters, aldehydes, alkanes, and alkenes, all of high molecular weights.

b) This compound has not been seen in the GC/MS work done on the M. scutellata exudate sample at the MCMS, in Lincoln, Nebraska (Figures 7-52).

The compound could be formed from insecticide fragments or their interaction with the $CHCl_3$ and benzene used in the sample preparation.

Figure 57 (Peak 4 of Figure 53)

Proposed structure:



1) The parent peak is taken to be at m/e 284.

2) The peak at m/e 239 indicates a loss of CH_3CH_2O (mass 45). Because of the great similarities of this spectrum to Figures 11, 34, and 35 which contain small if any peaks at m/e 248, 250, 255, and 267, these peaks will not be considered significant in the interpretation of this spectrum.

3) The peak at m/e 157 indicates further loss of $CH_2(CH_2)_3CO$ from the molecule.

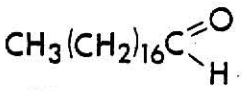
4) The peak at m/e 143 indicates a further loss of CH_2.

5) The significant peak at m/e 101 arises from the fragment $CH_2CH_2CO_2CH_2CH_3$.

6) The base peak at m/e 88 is due to $CH_2C(OH)OCH_2CH_3$.

Figure 58 (Peak 5 of Figure 53)

Proposed structure:



1) The parent peak is taken to be at m/e 268.

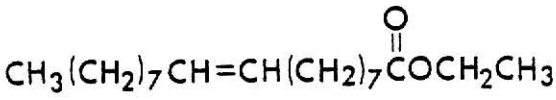
2) The peak at m/e 250 results from the loss of H_2O from the molecule.

3) The peak at m/e 224 arises from the loss of CH_2=CHOH from the molecule.

4) An alkane pattern is present at m/e 43, 57, 71, etc. and an alkene pattern is present at 41, 55, 69, etc.

Figure 59 (Peak 6 of Figure 53)

Proposed structure:



1) The parent peak is taken to be at m/e 310.

2) The peak at m/e 264 results from the loss of CH_3CH_2OH, and the peak at m/e 265 results from the loss of CH_3CH_2O.

3) The peak at m/e 222 indicates a further loss of CH_2CO.

4) The peak at m/e 123 indicates a further loss of C_7H_{15}, leaving $CH_3(CH_2)_7CH{=}CH$.

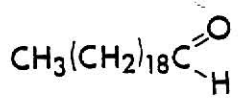
5) The further loss of CH then creates a peak at m/e 110.

6) The base peak at m/e 73 arises from $CO_2CH_2CH_3$.

This spectrum is similar to Figures 13 and 37 and the retention times of the peaks represented by these spectra (Peak 6 of Figure 53, retention time 9:36, Peak 8 of Figure 6, retention time 9:03, and Peak 9 of Figure 30, retention time 9:03) are comparable. Peaks which appear significant in this spectrum at m/e 60, 129, 157, 213, and 256 are not significant in Figures 13 and 37. These differences may be due to the different temperature programs and columns used for the sets of data collected at MCMS and MRI.

Figure 60 (Peak 7 of Figure 53)

Proposed structure:



1) The parent peak is taken to be at m/e 296.

2) A loss of H_2O gives rise to the peak at m/e 278.

3) A loss of CH_2=CHOH gives rise to the peak at m/e 252.

4) From m/e 250 to m/e 236 there is a loss of 14 (CH_2) and this loss is seen clearly all through the spectrum, m/e 236 to m/e 222, m/e 222 to m/e 208, m/e 208 to m/e 194, etc.

5) A fairly strong alkane pattern is present in m/e 43, 57, 71, etc. and an alkene pattern at m/e 41, 55, 69, etc.

Figure 61 (Peak 8 of Figure 53)

Proposed structure:

1) The parent peak is taken to be at m/e 310.

2) The peak at m/e 251 results from the loss of $CH_3CH_2CH_2O$.

3) The next significant peak at m/e 139 arises from the further loss of $(CH_2)_6CO$. After losing R'-O, esters whose predominant portion is the acid then lose RCO or CH_2CO. However, in this case it appears that only part of R is broken off, and this break indicates the tentative double bond location.

4) The base peak at m/e 59 arises from the fragment $CH_3CH_2CH_2O$.

Figure 62 (Peak 9 of Figure 53)

1) The parent peak may be at m/e 368.

2) A loss of 15 from m/e 368 to m/e 353 indicates a loss of CH_3.

3) An alkene pattern is present at m/e 55, 69, 83, etc.

No tentative structure could be made from the information available. See the special section about components whose spectra contain base peaks at m/e 131 for further discussion.

Figure 63 (Peak 10 of Figure 53)

Aside from the very intense peak at m/e 131, the other peaks of this spectrum are very weak and so make interpretation very difficult. The spectrum is similar to Figures 14, 15, 20, 40, 41, and 44 all of which contain m/e 131 base peaks. See the special section for components producing these unusual spectra for further discussion.

Figure 64 (Peak 11 of Figure 53)

The base peak at m/e 149 of this spectrum strongly suggests that a phthalate is present. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component of the exudate itself.

Figure 65 (Peak 12 of Figure 53)

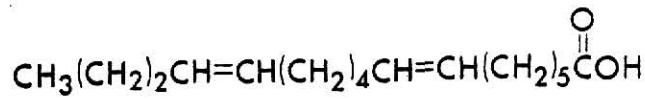
1) The parent peak is taken to be at m/e 398.

2) The loss of 15 from m/e 398 to m/e 383 indicates a loss of CH_3.

3) An alkene pattern is present at m/e 55, 69, 83, etc.

Figure 66 (Peak 13 of Figure 53)

Proposed structure:



1) The parent peak is taken to be at m/e 266.

2) The large peak at m/e 265 is due to $C_{16}H_{29}CO_2$.

3) The loss of 16 from m/e 265 to m/e 249 indicates a loss of O.

4) Further loss of CO is indicated from m/e 249 to m/e 221.

5) The base peak at m/e 117 is due to $CH_3(CH_2)_4C(OH)_2$.

6) The two alkyl double bonds are tentatively placed at the C_7 and C_{13} positions of the acid because of this reasoning:

a) The fragment of $CH_3(CH_2)_4C(OH)_2$ at m/e 117 indicates there are five saturated C's after the first acid C. This fragment of the acid is probably broken off after a double bond:

$$CH{=}CH\dagger(CH_2)_5CO_2H.$$

b) The peak at m/e 43 arises from the fragment C_3H_7. This means there are three saturated C's at the end of the acid molecule. This C_3H_7 fragment is probably broken off after a double bond:

$$CH_3(CH_2)_2\dagger CH{=}CH.$$

c) Piecing the previous two arguments together, the locations of C_7 and C_{13} are proposed for the alkyl double bonds of this acid molecule.

Figure 67 (Peak 14 of Figure 53)

Besides the base peak at m/e 135 and the very large peak at m/e 197, this spectrum does not contain much useful information. The base peak at m/e 135 is indicative of a phenol: $(CH_3)_2CC_6H_4OH$.

Figure 67 (Peak 14 of Figure 53)

Besides the base peak at m/e 135 and the very large peak at m/e 197, this spectrum does not contain much useful information. The base peak at m/e 135 is indicative of a phenol: $(CH_3)_2CC_6H_4OH$.

FIGURE 53

Mass chromatogram of *Medicago scutellata* exudate

sample taken at MRI, Kansas City, Missouri

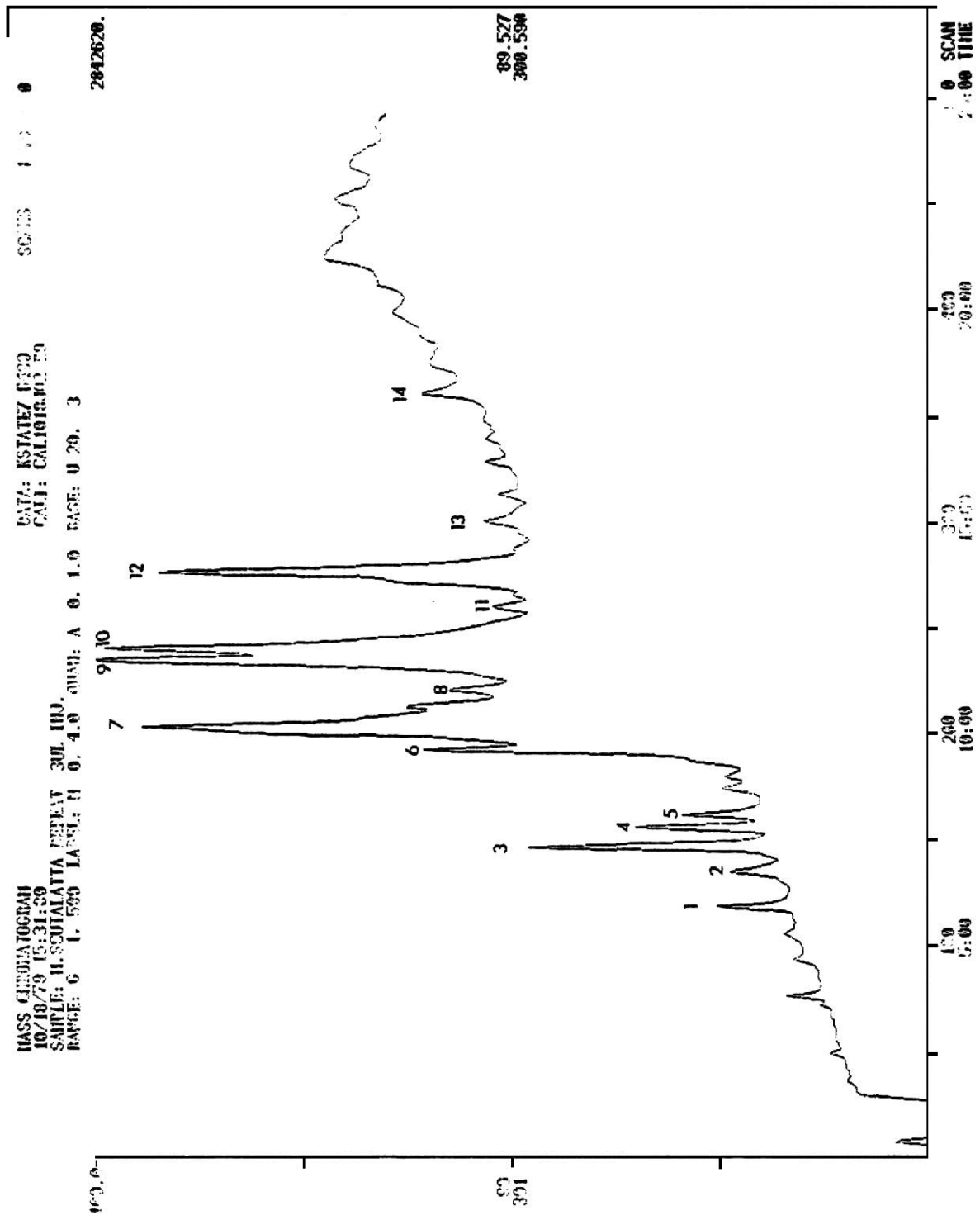
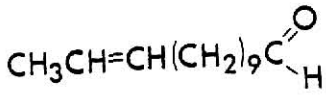

MASS CHROMATOGRAM
10/18/79 15:31:59
SAMPLE: 11.SCUTALATTA REPEAT 30L INJ.
RANGE: 6 1.599 LABEL: 11 6. 4.0 BASE: A 6. 1.0 BASE: U 29. 3
DATA: KSTATE7 0329
CAL: CAL1018.M0 59
2842620.
89.527
300.590
1
2
3
4
5
6
7
8
9
10
11
12
13
14
SCAN TIME

FIGURE 54

Mass spectrum of

Peak 1 of Figure 53

Proposed structure:



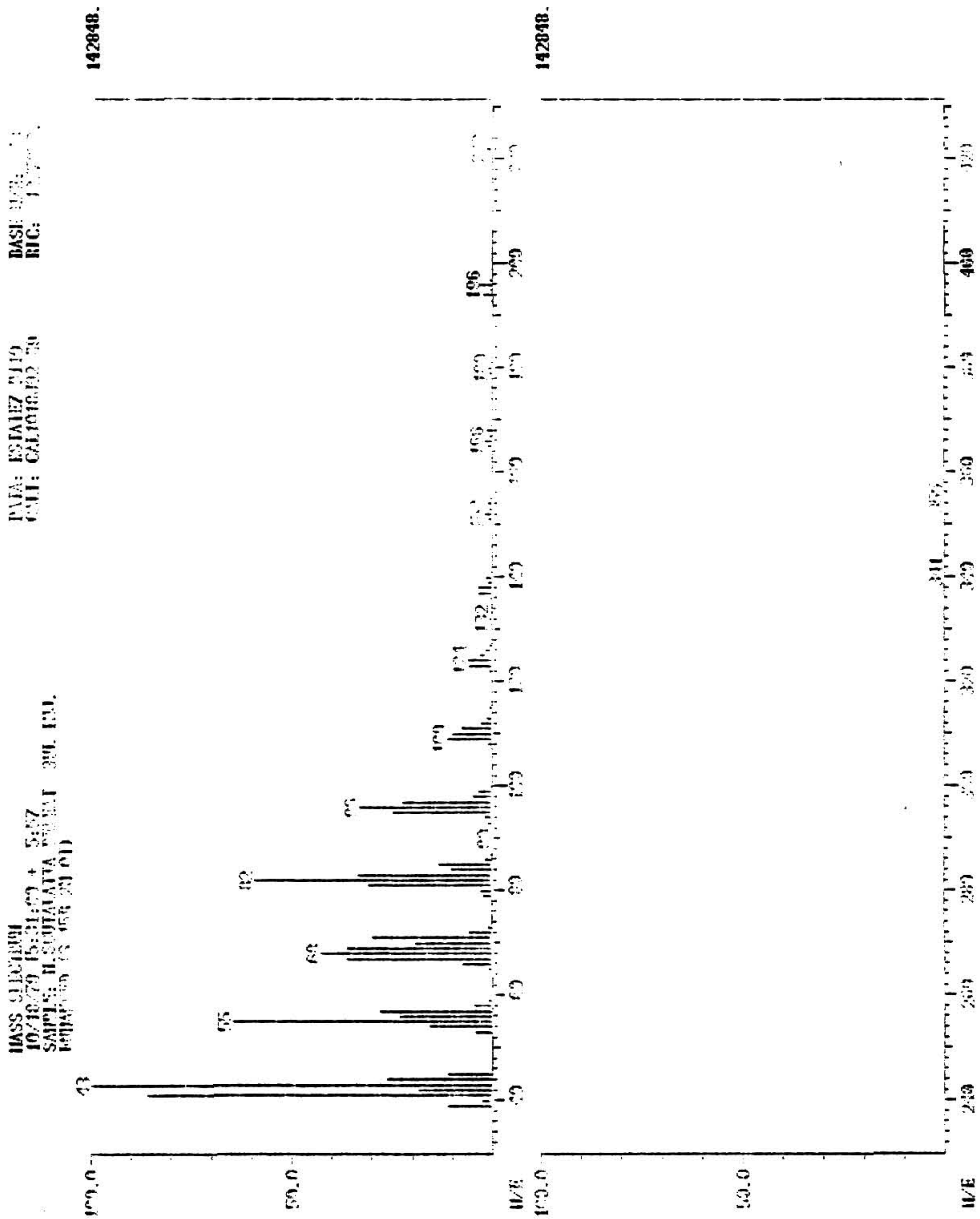
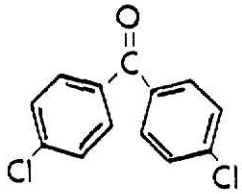

142848.
142848.
43
55
82
196
50.0
M/E
M/E
400

FIGURE 55

Mass spectrum of

Peak 2 of Figure 53

Proposed structure:



or some isomer of this compound

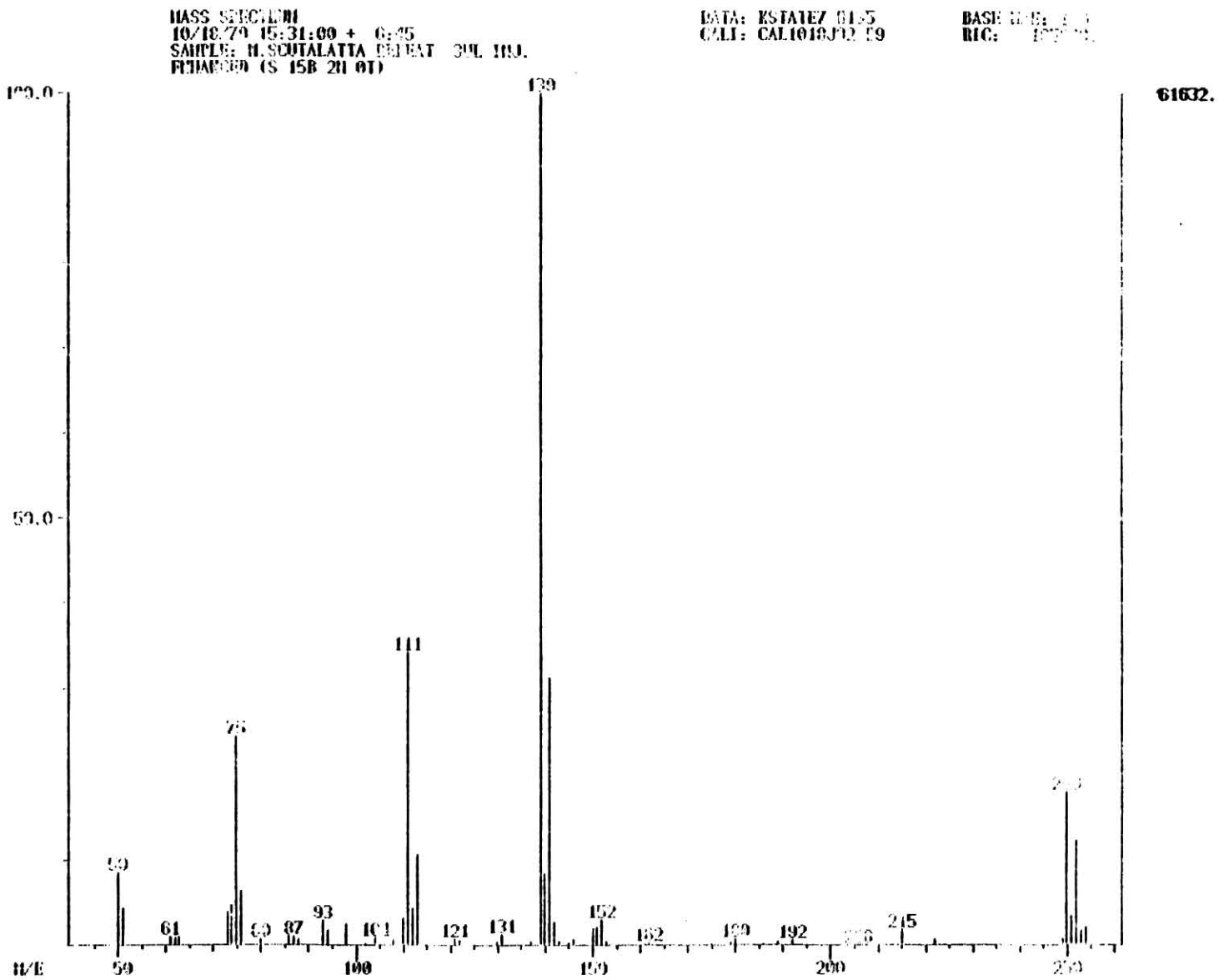
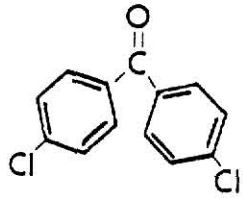

MASS SPECTRUM
10/18.79 15:31:00 + 0:45
SAMPLE: M.SCUTALATTA
DATA: KSTA1EZ 0155
CALI: CAL1010J12 09
BASE
RIC:
100.0
50.0
61632.
139
111
75
59
93
152
215
M/E
50
100
150
200
250

FIGURE 56

Mass spectrum of

Peak 3 of Figure 53

Proposed structure:



or some isomer of this compound

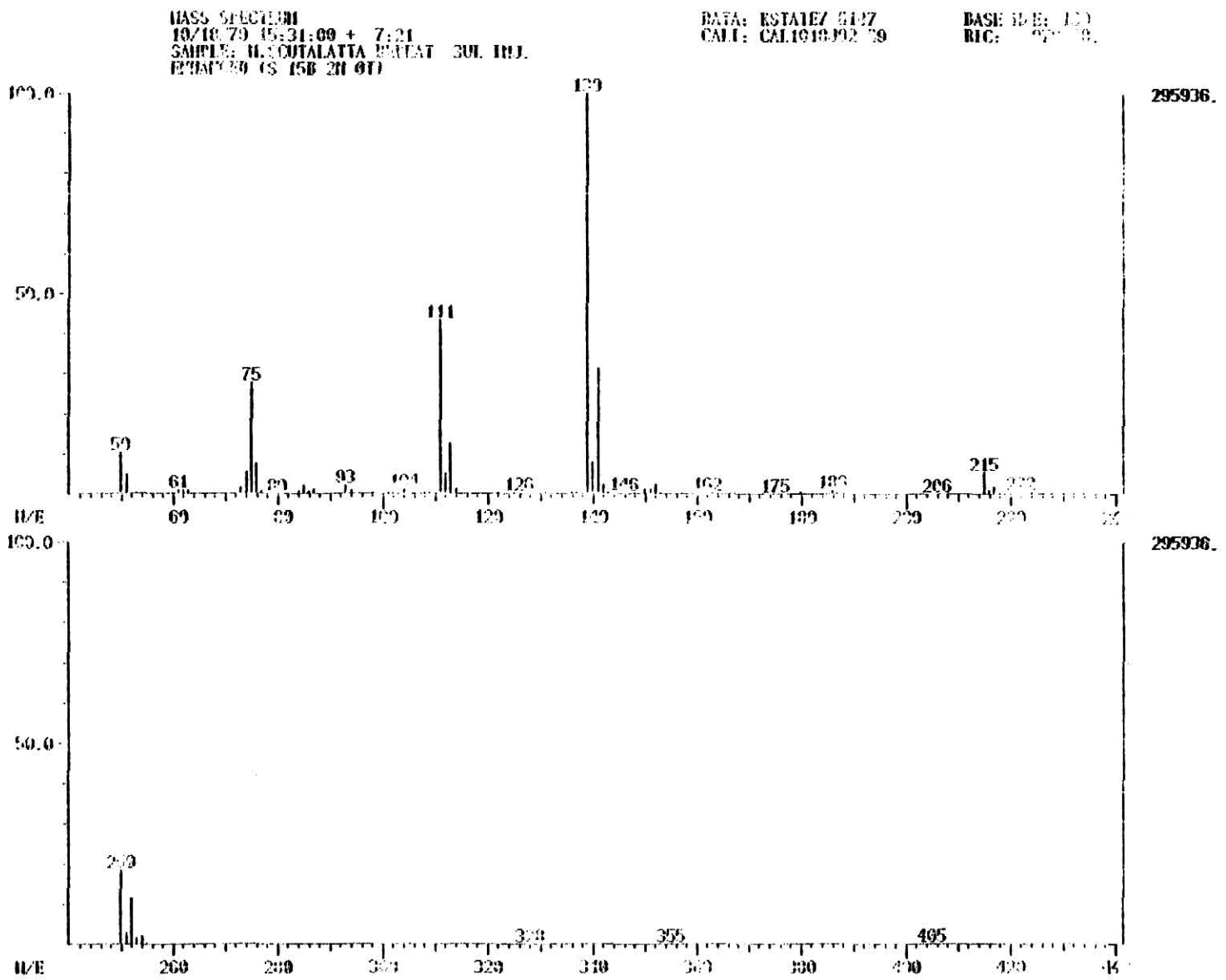
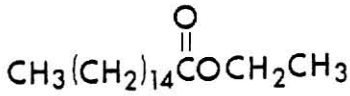

MASS SPECTRUM
10/10.79 15:31:00 + 7:21
SAMPLE: H.CUTALATTA EXTRAT 30L INJ.
DATA: ESTATE7 0127
CALI: CAL1010J02 79
100.0
50.0
295936.
75
111
129
215
M/E
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
295936.
240
328
355
405

FIGURE 57

Mass spectrum of

Peak 4 of Figure 53

Proposed structure:



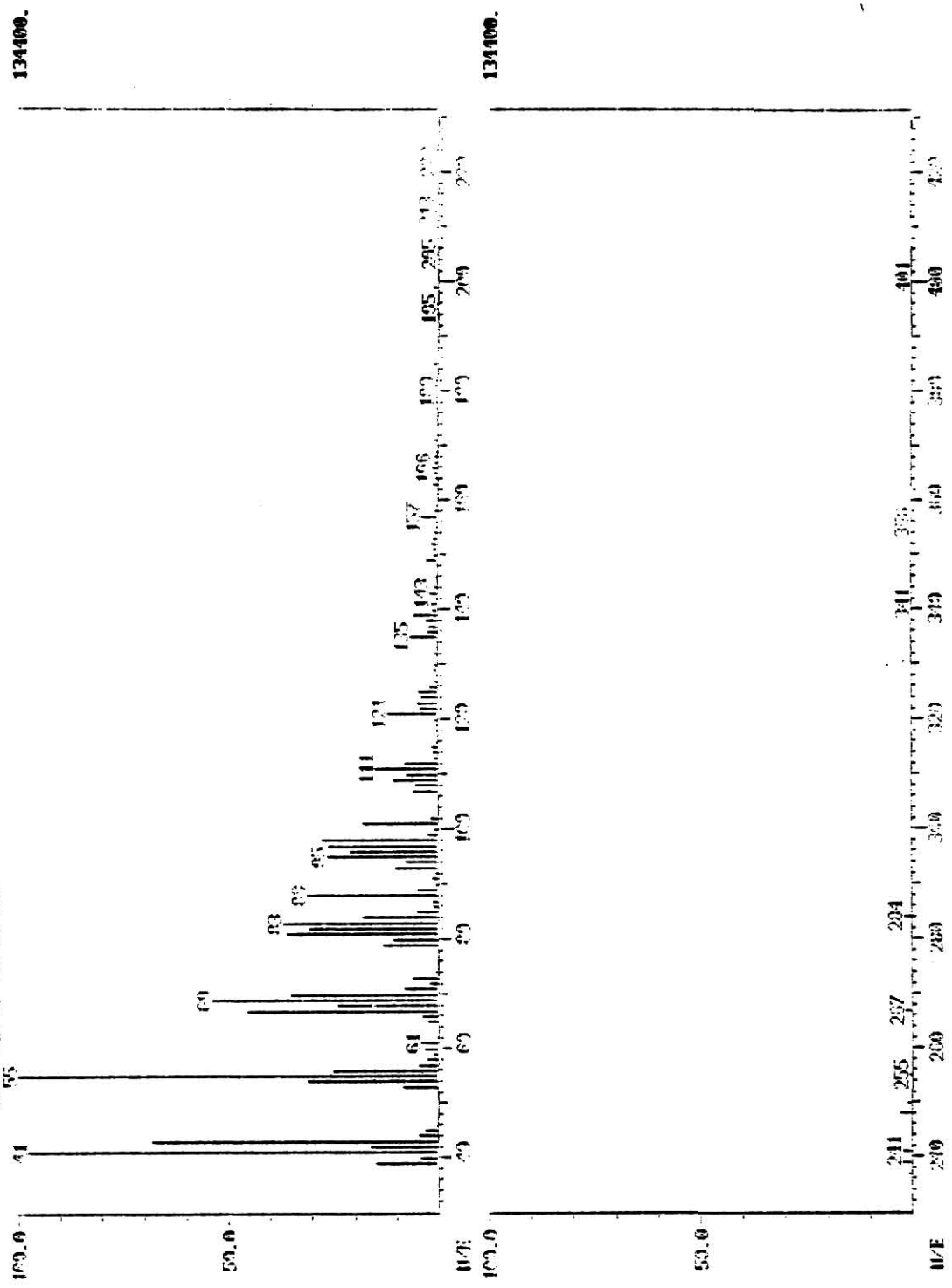
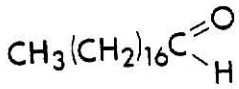

MASS SPECTRUM
DATA: ESTAIEZ 6156
CALI: CAL101802 69
BASE
RIC:
SAMPLE: H.SOUMATTA
134400.
134400.
100.0
50.0
M/E

FIGURE 58

Mass spectrum of

Peak 5 of Figure 53

Proposed structure:



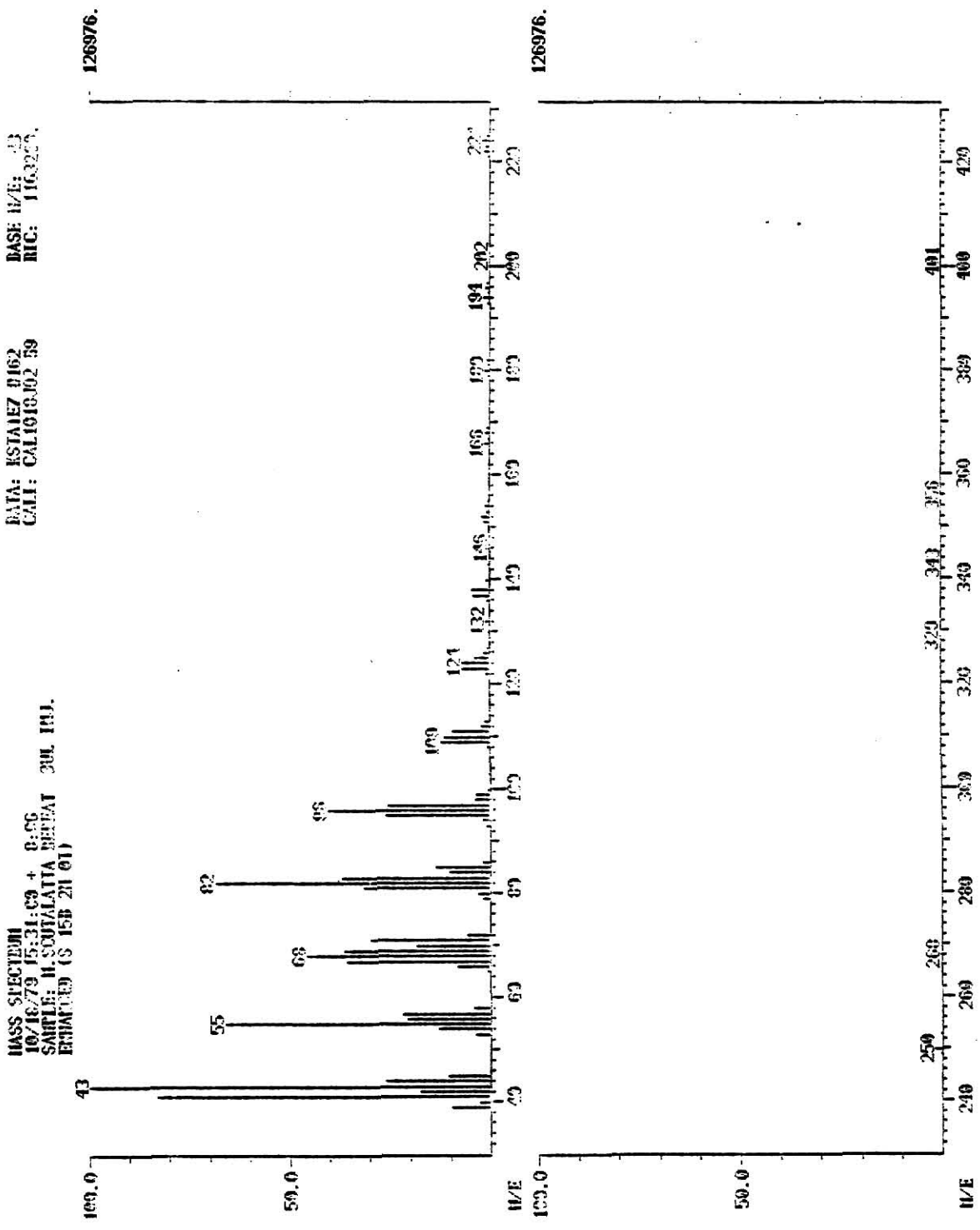
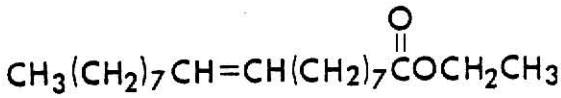

MASS SPECTRUM
10/16/79 15:31:09 + 8.56
SAMPLE: H.SCUTALATTA REPEAT 30L INJ.
ENHANCED (S 15B 21 01)
DATA: KSTA1E7 0162
CAL: CAL1018J02 59
BASE H/E: 43
RIC: 116328.
126976.
100.0
50.0
M/E
43
55
68
82
95
109
121
132
146
166
194
202
126976.
100.0
50.0
M/E

FIGURE 59

Mass spectrum of

Peak 6 of Figure 53

Proposed structure:



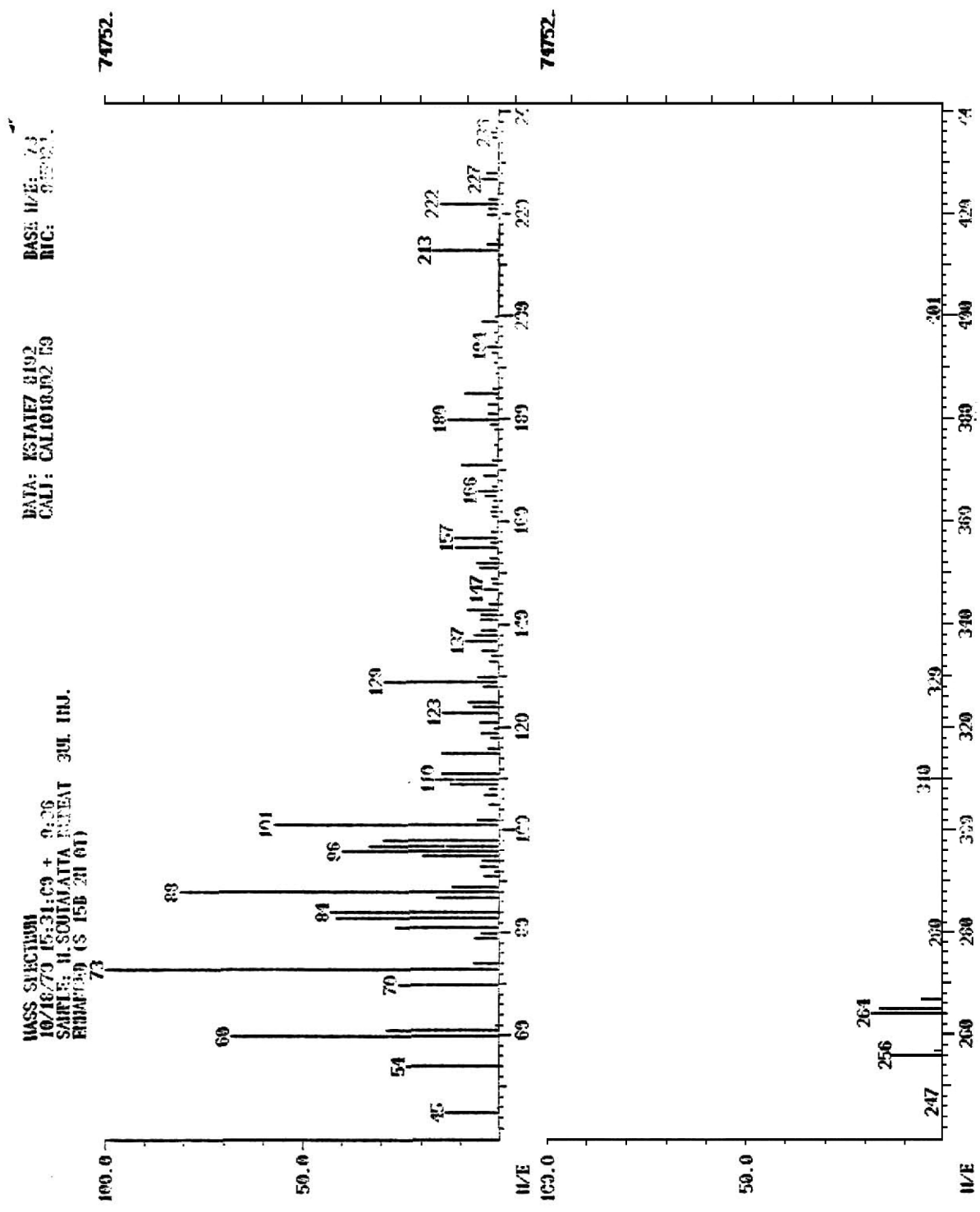
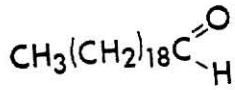

MASS SPECTRUM
10/16/73 15:31:09 + 9:36
SAMPLE: H SOUTAATTA BREAT 3UL INJ.
DATA: KSTAIE7 8192
CALI: CAL1010J02 59
BASE M/E
RIC:
74752.
74752.
100.0
50.0
M/E
100.0
50.0
M/E
45
54
69
70
73
83
84
96
101
110
123
129
137
147
157
166
189
213
222
227
247
256
264

FIGURE 60

Mass spectrum of

Peak 7 of Figure 53

Proposed structure:



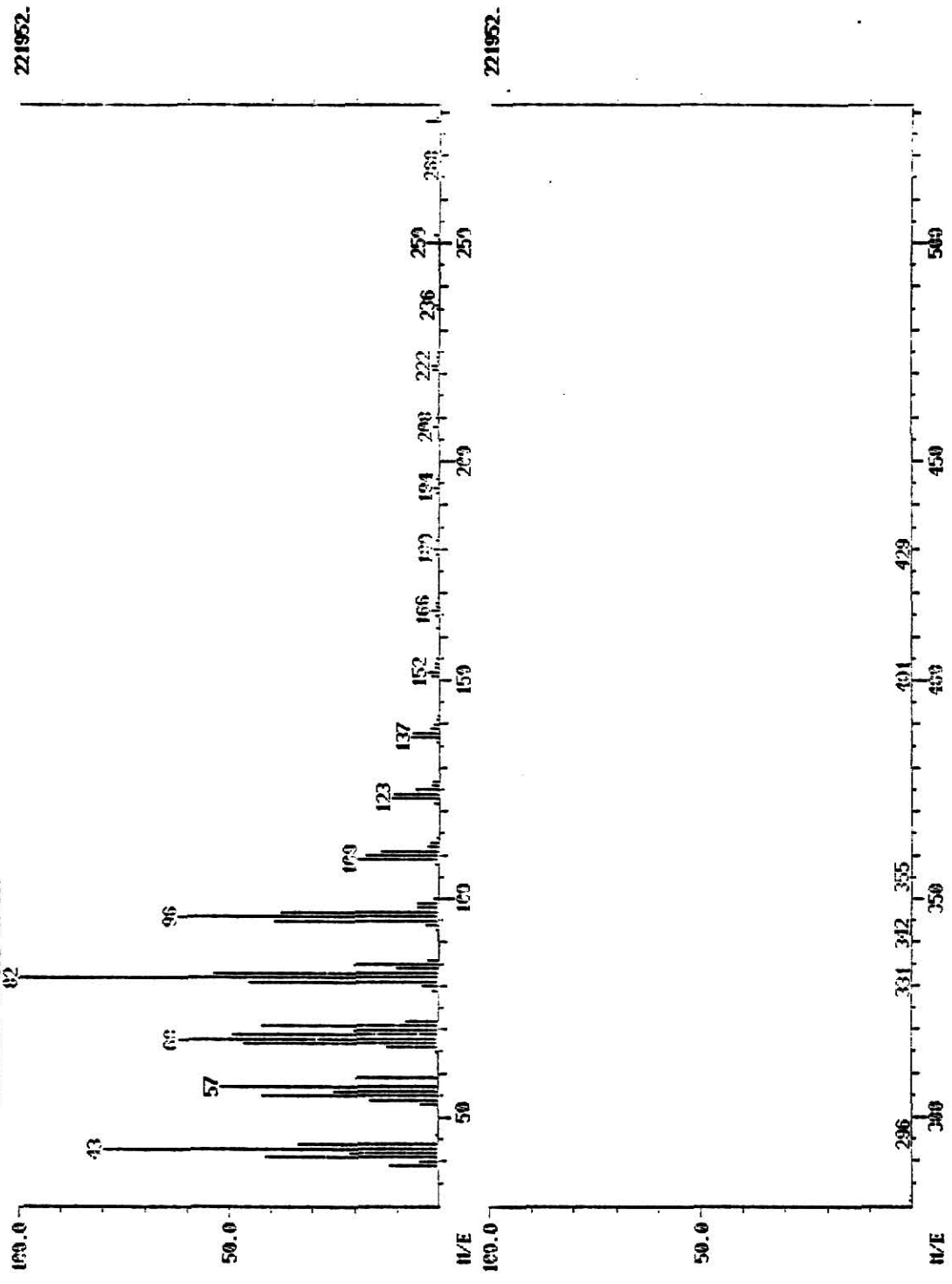
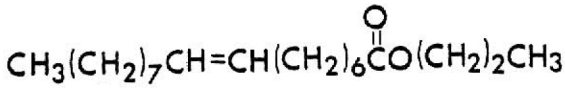

MASS SPECTRUM
10/16/79 15:31:09 + 10:12
SAMPLE: H.SUBTALATTA REPEAT 30L INJ.
EMMCOD (S 150 20 01)
DATA: KSTATE7 0271
CALI: CAL1018J02 09
BASE M/E:
RIC:
221952.
221952.
100.0
50.0
M/E
43
57
69
82
96
109
123
137
152
166
194
208
222
236
259
269
296
300
331
342
355
429
450
491
500

FIGURE 61

Mass spectrum of

Peak 8 of Figure 53

Proposed structure:



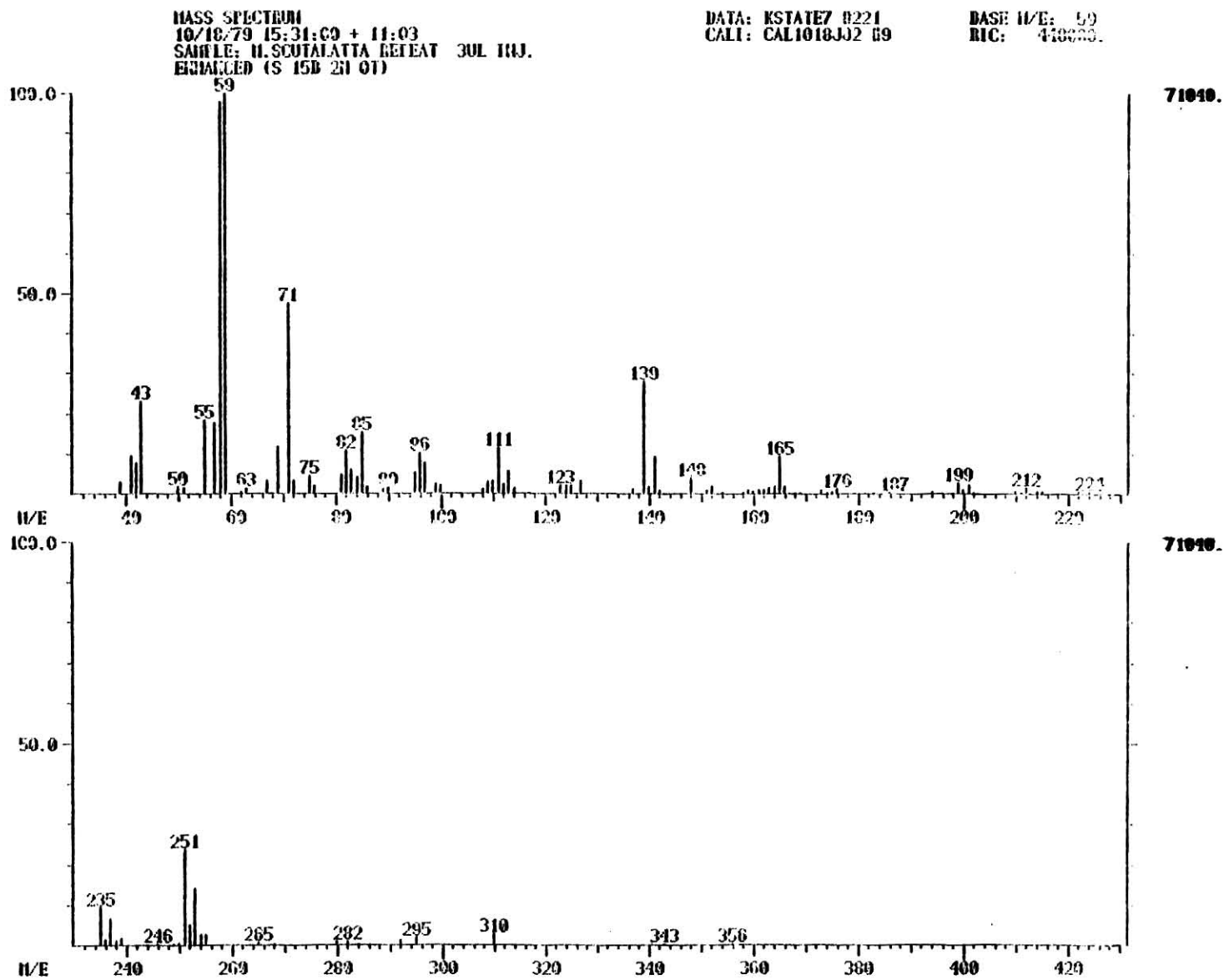

MASS SPECTRUM
10/18/79 15:31:09 + 11:03
SAMPLE: H.SCUTALATTA REPEAT 30L INJ.
ENHANCED (S 15B 25 0T)
DATA: KSTATE7 0221
CALI: CAL1018JJ2 09
BASE M/E: 59
RIC: 418033.
100.0
50.0
71010.
59
71
43
55
139
85
82
96
111
165
75
63
50
123
148
176
187
199
212
221
M/E
40
60
80
100
120
140
160
180
200
220
100.0
50.0
71010.
251
235
246
265
282
295
310
343
356
M/E
240
260
280
300
320
340
360
380
400
420

FIGURE 62

Mass spectrum of

Peak 9 of Figure 53

Proposed structure: None

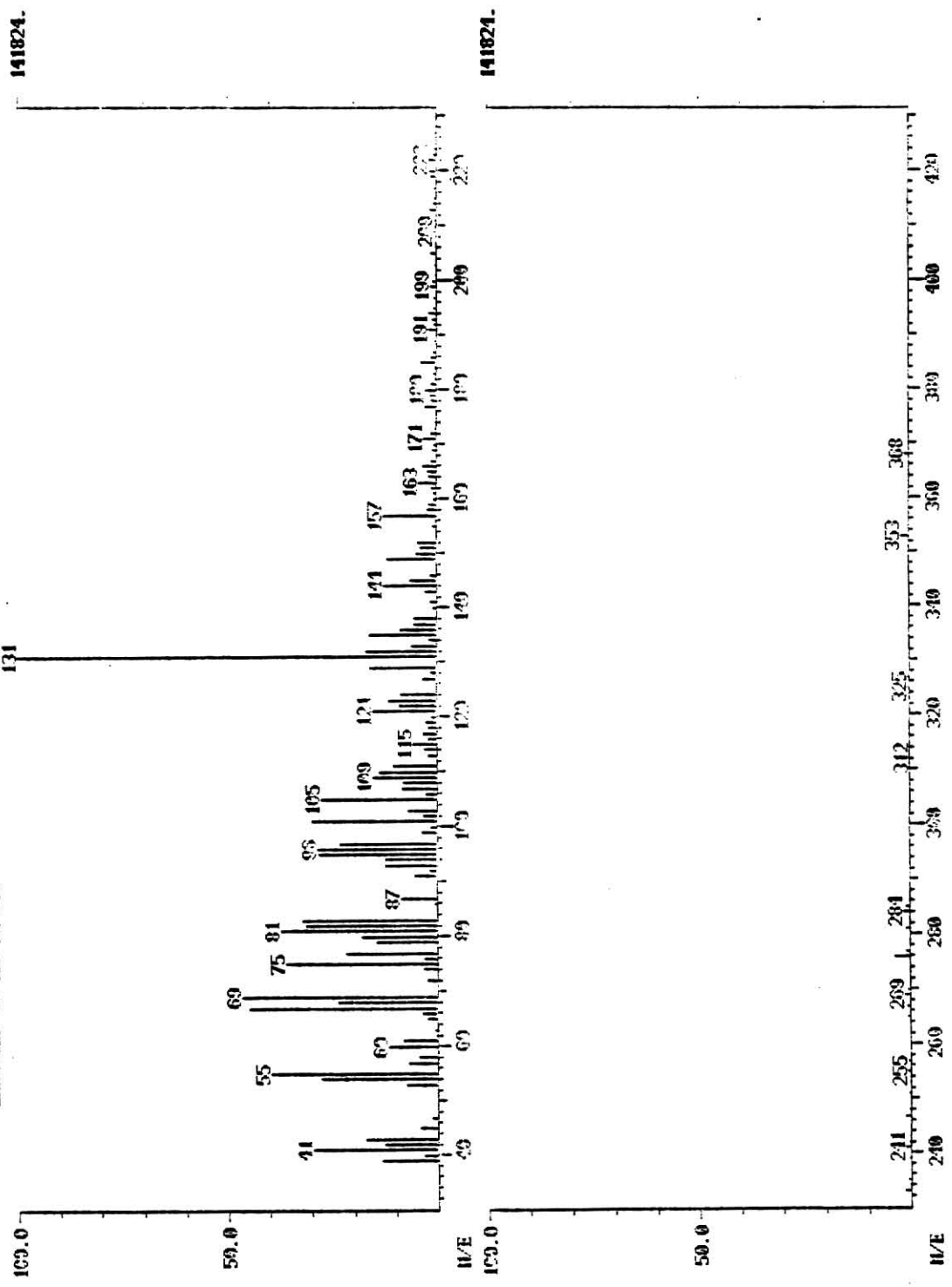

MASS SPECTRUM
10/16/79 15:31:00 + 11:30
SAMPLE: H.SCUTALATTA BEAT 30L INJ.
EDUCTED (S 15D 2H 01)
DATA: KS1A1E7 0236
CALI: CAL1616J02 59
BASE M/E: 131
RIC: 163
141824.
131
M/E
100.0
50.0
141824.
M/E
100.0
50.0

FIGURE 63

Mass spectrum of

Peak 10 of Figure 53

Proposed structure: None

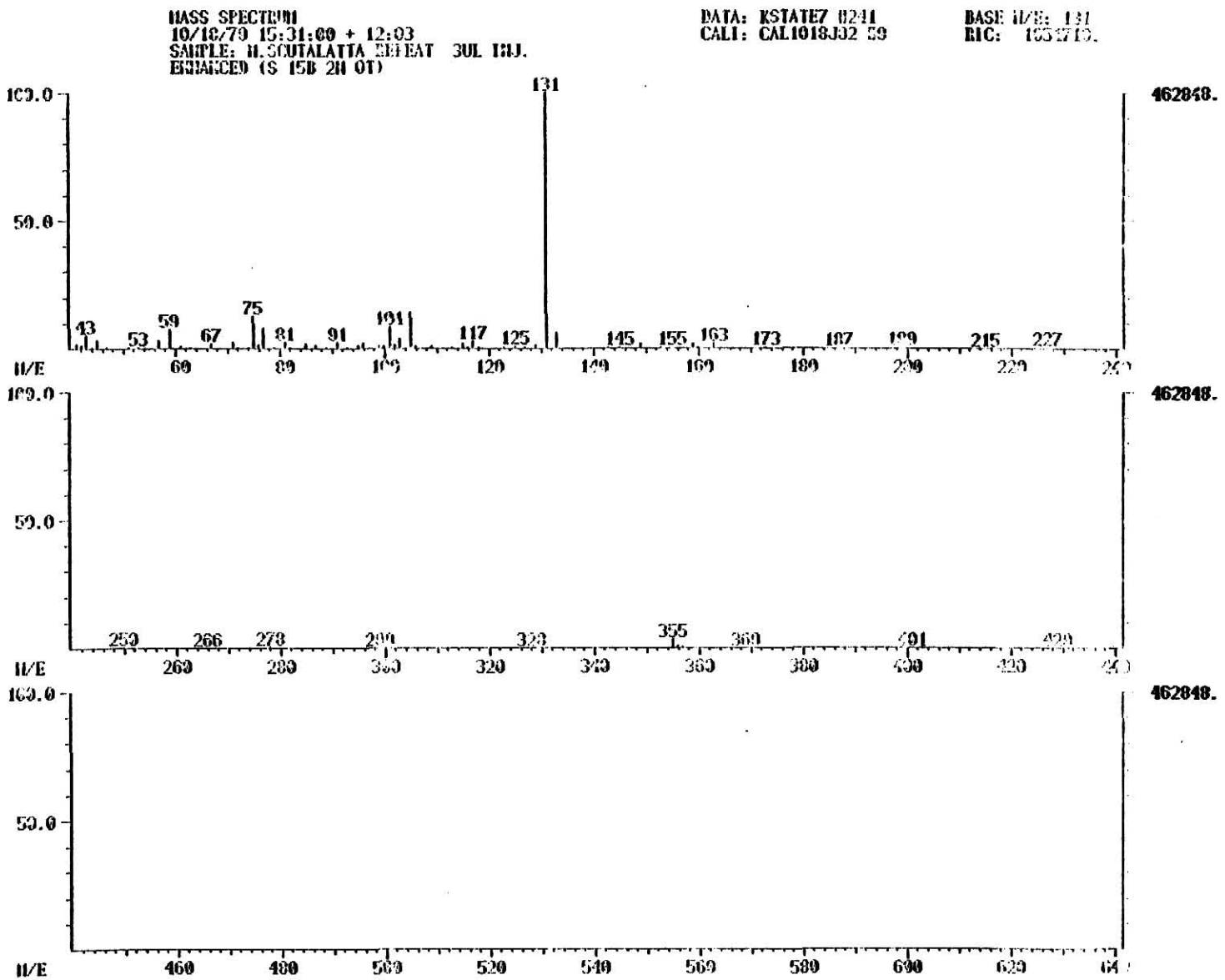

MASS SPECTRUM
10/10/79 15:31:00 + 12:03
SAMPLE: H.SCUTALATTA 2DFEAT 30L IBJ.
ENHANCED (S 15B 2H 0T)
DATA: KSTATE7 0241
CALI: CAL1018J02 59
BASE H/E: 131
RIC: 1053710.
100.0
50.0
462848.
H/E
131
75
59
43
53
67
81
91
101
117
125
145
155
163
173
187
199
215
227
250
266
278
299
323
355
391
429
460
480
500
520
540
560
580
600
620

FIGURE 64

Mass spectrum of

Peak 11 of Figure 53

Proposed structure: Phthalate

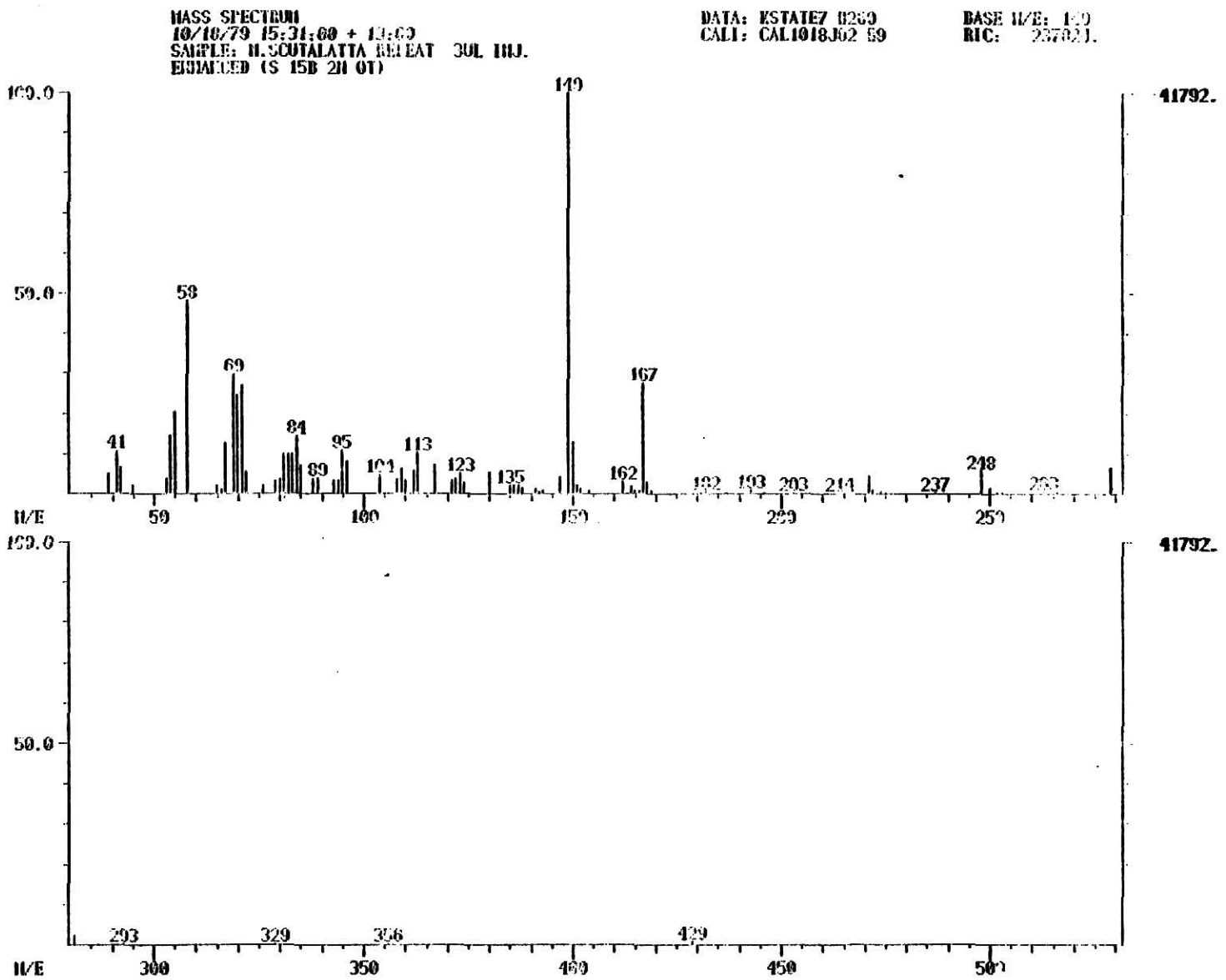

MASS SPECTRUM
10/10/79 15:31:00 + 13:00
SAMPLE: H.SCUTALATTA REHEAT 30L INJ.
ENHANCED (S 15B 2H 0T)
DATA: ESTATE7 B269
CALI: CAL1018J02 59
BASE H/E: 149
RIC: 237021.
100.0
50.0
41792.
149
58
69
167
41
84
95
113
89
101
123
135
162
182
193
203
214
237
248
H/E
50
100
150
200
250
100.0
50.0
41792.
293
329
356
429
H/E
300
350
400
450
500

FIGURE 65

Mass spectrum of

Peak 12 of Figure 53

Proposed structure: None

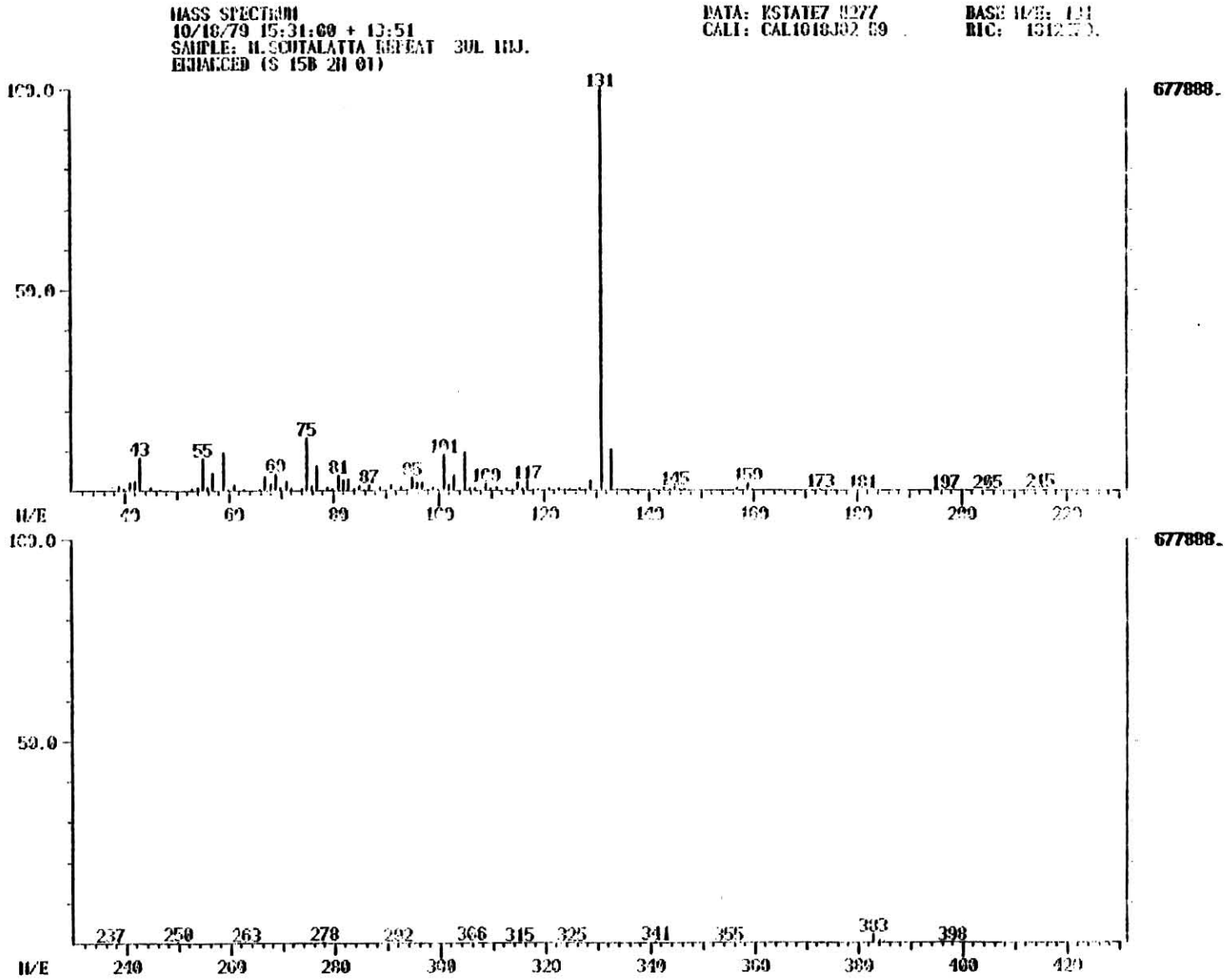
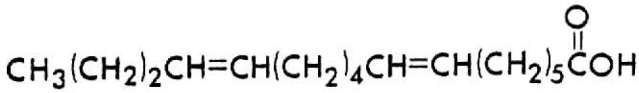

MASS SPECTRUM
10/18/79 15:31:00 + 13:51
SAMPLE: H.SCUTALATTA REPEAT 30L INJ.
ENHANCED (S 15B 2H 01)
DATA: KSTATE7 0277
CALI: CAL1018J02 89
BASE M/E: 131
RIC: 1312...
100.0
50.0
677888.
131
75
43
55
60
81
87
95
101
109
117
145
159
173
181
197
205
215
M/E
40
60
80
100
120
140
160
180
200
220
100.0
50.0
677888.
237
250
263
278
292
306
315
325
341
355
383
398
M/E
240
260
280
300
320
340
360
380
400
420

FIGURE 66

Mass spectrum of

Peak 13 of Figure 54

Proposed structure:



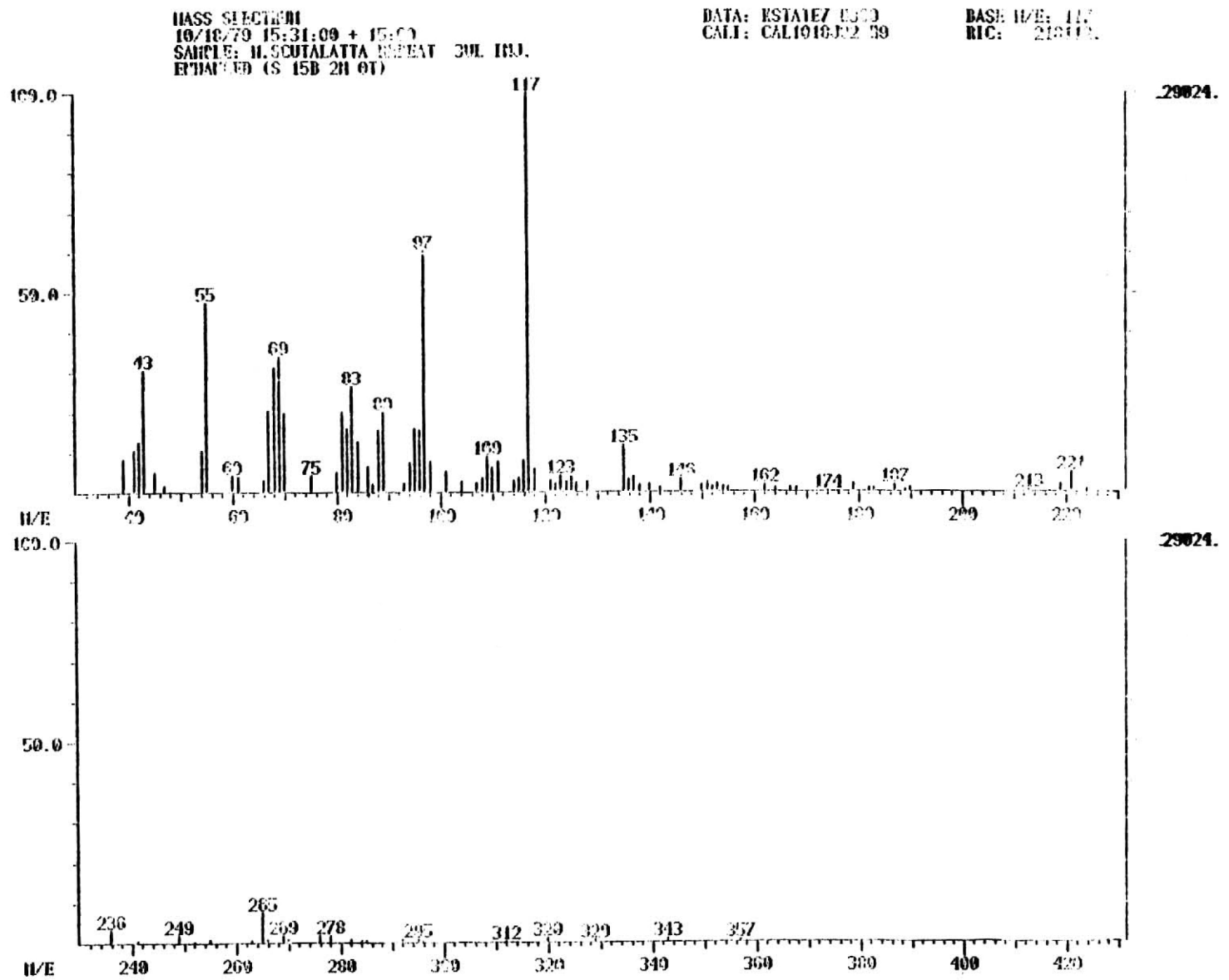
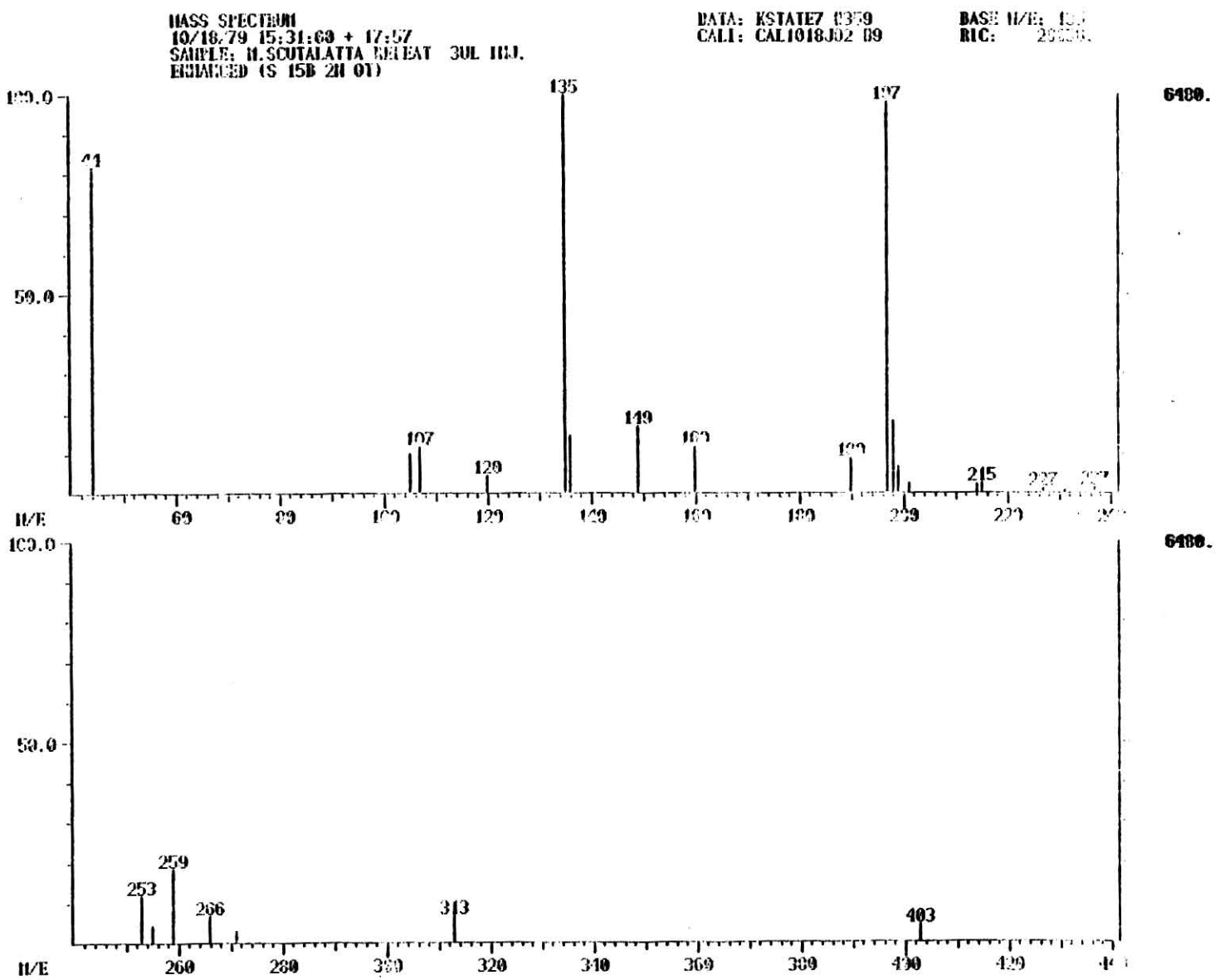

MASS SPECTRUM
10/10/79 15:31:00 + 15:00
SAMPLE: H.SCUTALATTA REPEAT 30L IBJ.
EXTRACTED (S 15B 2H 0T)
DATA: ESTATE7 0.00
CALI: CAL1010.D2 39
BASE M/E: 117
RIC: 21811.
29024.
100.0
50.0
M/E
43
55
69
83
97
117
135
169
221
29024.
100.0
50.0
M/E
236
249
265
269
278
295
312
329
339
343
357
240
260
280
300
320
340
360
380
400
420

FIGURE 67

Mass spectrum of

Peak 14 of Figure 53

Proposed structure: None


MASS SPECTRUM
10/18/79 15:31:00 + 17:57
SAMPLE: M.SCUTALATTA REPEAT 30UL INJ.
ENHANCED (S 15B 2H 01)
DATA: KSTATE7 0359
CALI: CAL1018J02 09
BASE M/E:
RIC:
100.0
50.0
M/E
6480.
41
107
120
135
149
197
215
253
259
266
313
403

Interpretation of Mass Spectra

from MCMS, Lincoln, Nebraska

of Medicago sativa L. subsp. praefalcata

Peak 1 of Figure 68

This peak is due to the solvent, $CHCl_3$. No mass spectrum was taken of this peak.

Peak 2 of Figure 68

After injection of the sample in $CHCl_3$, the mass spectral system is vented for two minutes because of the large amount of solvent eluting from the column during this time. This peak arises from the system being closed after the initial two minutes.

Figure 69 (Peak 3 of Figure 68)

The strong peak at m/e 149 indicates a possible phthalate. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component of the exudate itself. The spectrum is too weak for further interpretation.

Figure 70 (Peak 4 of Figure 68)

The signals in this spectrum could all be due to noise since the spectrum is so weak. This fact makes interpretation impossible.

Figure 71 (Peak 5 of Figure 68)

The most intense peaks of this spectrum at m/e 73, 147, 221, 281, 355, and 578 are due to column bleed. Besides those peaks, there are faint alkane and alkene patterns present. No further interpretation can be made due to the weak spectrum.

Figure 72 (Peak 6 of Figure 68)

This spectrum is much too weak for interpretation. The pattern of peaks at m/e 57, 71, and 85 indicates an alkane group.

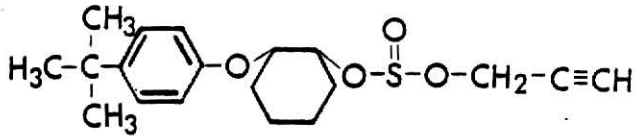
Figure 73 (Peak 7 of Figure 68)

Of the intense peaks in this spectrum, these peaks at m/e 73, 147, 207, 221, 281, 355, and 429 are due to column bleed. The information given that is not due to column bleed is not sufficient for interpretation.

Figure 74 (Peak 8 of Figure 68)

This spectrum is very similar to Figures 9 and 34. It has been shown from earlier GC work (see Insecticides section) that this peak arises from a decomposition product of the insecticide Omite. Since Omite had been applied to this plant also, it only follows that Omite should be in the exudate sample.

Omite has the following structure:



From the mass spectrum, Peak 8 of Figure 68 arises from a decomposition product that contains the p-tert-butylphenol group. Further breakdown is included in the explanation of Figure 9.

Figure 75 (Peak 9 of Figure 68)

The presence of the base peak at m/e 149 of this spectrum strongly suggests that a phthalate is present in the exudate sample. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component of the exudate itself.

Figure 76 (Peak 10 of Figure 68)

This spectrum is weak and therefore difficult to interpret. Peaks of the retention time 7:32, Peak 6 of Figure 6 and Peak 7 of Figure 30, display spectra (Figures 11 and 35, respectfully) similar to this spectrum but contain more information. Both Figures 11 and 35 (spectra from the M. scutellata exudate sample) are much more intense and show higher molecular weight fragments. In those spectra, the base peak is at m/e 88 and the next most intense peak is at m/e 101. This spectrum (Figure 76) also has a base peak at m/e 88 and an intense peak at m/e 101. Possibly a small amount of the same

compound found in the M. scutellata exudate sample is also in the M. sativa L. subsp. praefalcata exudate sample but in too small a concentration for all of the true signals to show up through the noise of the spectrum.

1) The base peak at m/e 88 could arise from the fragment $CH_2C(OH)CH_2CH_3$.

2) The intense peak at m/e 101 could be due to the fragment $CH_2CH_2CO_2CH_2CH_3$.

The peaks at m/e 88 and m/e 101 are usually fairly intense in the mass spectra of ethyl esters as seen from previous spectra.

The structure proposed by using Figures 11 and 35 (M. scutellata exudate sample spectra) as reference spectra is:

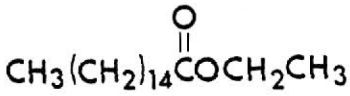


Figure 77 (Peak 11 of Figure 68)

This spectrum is weak and fragments of mass greater than 135 are not shown. From the information given, the compound represented here contains an alkane function. An alkane pattern is present at m/e 57, 71, and 85 and an alkene pattern at m/e 55, 69, 83, 97, and 111. The alkene peak at m/e 69 is equivalent in intensity to the alkane peak at m/e 71, but in the higher mass units the alkene peaks dominate. No oxygen fragments are detected.

Figure 78 (Peak 12 of Figure 68)

This is a weak spectrum which contains column bleed peaks at m/e 73, 147, 207, and 281. The peak at m/e 73 is so intense that it could also be due to a true signal in addition to the column bleed. The peak at m/e 73 could arise

from an oxygen containing fragment of the empirical formula C_4H_9O. An alkene pattern is also present at m/e 55, 69, 83, and 97.

Figure 79 (Peak 13 of Figure 68)

This spectrum is very weak and therefore noise peaks can be mistaken for true signals from the sample. Both an alkane pattern at m/e 57, 71, and 85 and an alkene pattern at m/e 55, 69, 83, 97, and 111 are present, though the alkene pattern dominates after m/e 71.

Figure 80 (Peak 14 of Figure 68)

Useful information is lacking in the molecular weight region greater than m/e 116 of this spectrum. Therefore, proposals made will be based on the information given in the lower molecular weight region.

1) The peak at m/e 116 could arise from the acid fragment $CH_3(CH_2)_4CO_2H$.

2) A dominating alkene pattern is present at m/e 83 (C_6H_{11}), m/e 97 (C_7H_{13}), and m/e 111 (C_8H_{15}).

3) The base peak at m/e 57 is due to C_4H_9 and the peak at m/e 71 is due to C_5H_{11}.

The presence of the m/e 116 peak, which could be due to hexanoic acid, indicates that the compound could be an ester which loses a molecule of acid upon mass fragmentation. The predominant portion of the ester would then be the alcohol since this type of ester tends to lose a molecule of acid initially. If this compound is a hexanoic acid ester, the alcohol portion of

the molecule contains one unsaturation. The peaks at m/e 239, 257, and 312 do not fit in with this line of reasoning. With no other peaks present in this region, no interpretation concerning these peaks will be made.

Figure 81 (Peak 15 of Figure 68)

The most intense peaks in this spectrum, besides the base peak at m/e 131, are due to column bleed: m/e 73, 147, 207, 221, 281, and 355. The parent peak cannot be detected due to the lack of useful information in the higher molecular weight region. An alkane pattern at m/e 57, 71, and 85 and an alkene pattern at m/e 55, 69, 83, and 97 are present. See the special section for components whose spectra contain base peaks at m/e 131 for further discussion.

Figure 82 (Peak 16 of Figure 68)

The presence of a base peak at m/e 149 strongly suggests a phthalate is represented here. See the Phthalates section for proposals as to the origin of the phthalate. It is most likely not a component of the exudate. The peak at m/e 207 is due to column bleed.

Figure 83 (Peak 17 of Figure 68)

This spectrum has a base peak at m/e 135 which suggests a phenol is present. The portion of the spectrum from m/e 50 to m/e 150 is very similar

to Figure 77 (Peak 8 of Figure 68). Peak 8 of Figure 68 was attributed to a decomposition product of the insecticide Omite. From earlier GC studies (see Insecticides section), it was found that when Omite in $CHCl_3$ is injected onto an OV-101 column, two significant peaks appear on the chromatogram. Peak 8 of Figure 68 was found to be the first peak due to the lower boiling decomposition product. Since some similarities of this spectrum are found with Figure 74 (Peak 8 of Figure 68), possibly this peak (Peak 17) is due to the higher boiling decomposition product of Omite. This peak may not have shown up in the M. scutellata chromatograms because it may have been there only in a small concentration and was lost in the background (hump). The peaks at m/e 73, 147, 207, 221, 281, 355, and 429 are due to column bleed.

Figure 84 (Peak 18 of Figure 68)

This spectrum is very similar to Figure 81. The parent peak cannot be determined due to the lack of information in the higher molecular weight region. The only peak in that region is at m/e 207 and is due to column bleed. An alkane pattern is present at m/e 57, 71, 85, and 99 and an alkene pattern at m/e 55, 69, 83, and 97. See the special section for components whose spectra contain base peaks at m/e 131 for further discussion.

Figure 85 (Peak 19 of Figure 68)

The base peak at m/e 149 of this spectrum gives a strong indication that the compound represented here is a phthalate, a contaminant (see Phthalates section). This spectrum resembles Figure 18 (also of a phthalate) as these

peaks are in common: m/e 57, 71, 76, 83, 95, 104, 113, 149, 167, and 279.

Figure 86 (Peak 20 of Figure 68)

This spectrum is very weak and so the only useful bit of information that can be obtained is the base peak at m/e 131. See the special section concerning components whose spectra contain base peaks at m/e 131 for further discussion.

Figure 87 (Peak 21 of Figure 68)

This spectrum is similar to the other spectra whose base peaks are at m/e 131. The parent peak cannot be determined because the only peaks in the high molecular weight region are due to column bleed: m/e 207 and m/e 281. An alkane pattern is present at m/e 57, 71, and 85 and an alkene pattern at m/e 55, 69, 83, and 97. See the special section for components whose spectra contain base peaks at m/e 131 for further discussion.

Figure 88 (Peak 22 of Figure 68)

This spectrum is weak and so contains only a small amount of useful information. Column bleed peaks are present at m/e 73, 149, 207, 281, and 429. A fairly strong alkane pattern is present at m/e 57, 71, 85, 97, and 111. The compound represented here most likely contains an alkane function.

Figure 89 (Peak 23 of Figure 68)

This spectrum is much too weak for interpretation as most of the signals are probably due to noise. The peaks at m/e 73, 147, 208, 221, 283, 355, and 403 are due to column bleed.

Figure 90 (Peak 24 of Figure 68)

This spectrum is weak and lacks useful information in the higher molecular weight region. An alkane pattern is present at m/e 55, 69, 83, and 97 and column bleed peaks appear at m/e 73, 221, and 281. The compound represented by this spectrum contains an alkene function.

Figure 91 (Peak 25 of Figure 68)

Proposed structure: straight chain alkane of length C_{30} or greater

1) A parent peak cannot be detected here due to the low intensity of the spectrum. In spectra of high molecular weight alkanes, the parent peak is almost always non-detectable, even in more intense spectra.[17]

2) A very strong alkane pattern is present at m/e 57, 71, 85, 99, and 113. After m/e 113, the true signals are so weak that noise may be masking them.

3) Column bleed peaks are present at m/e 207, 281, 355, 429, and 503.

4) This spectrum (Peak 25 of Figure 68, retention time 14:50) is very similar to Figure 48 (Peak 20 of Figure 30, retention time 14:51), though much weaker than that spectrum. Since the retention times of the peak represented here and Peak 20 of Figure 30 are so close and their spectra are similar, it is proposed that the compound represented by this spectrum is a straight chain

alkane of high molecular weight as is Peak 20 of Figure 30.

Figure 92 (Peak 26 of Figure 68)

This spectrum is very weak and the most intense peaks at m/e 73, 147, 221, 281, 355, and 429 are due to column bleed. Because this peak comes near the end of the GC/MS run, column bleed is to be expected.

Figure 93 (Peak 27 of Figure 68)

This spectrum is weak but some useful information can be obtained from it. A very strong alkane pattern at m/e 57, 71, 85, 99, and 113 and a slightly less intense alkene pattern at m/e 55, 69, 83, 97, and 111 are present. Other intense peaks at m/e 73, 133, 147, 207, 281, and 356 are due to column bleed. The compound represented here most likely contains an alkane function.

Figure 94 (Peak 28 of Figure 68)

Proposed structure: straight chain alkane of length C_{30} or greater

1) The parent peak is not detected in this spectrum, characteristic of high molecular weight alkanes.[17]

2) A very strong alkane pattern is present at m/e 57, 71, 85, ..., 337.

3) The intensity of this spectrum is fairly strong and so column bleed and other background noise do not interfere as they do in weaker spectra.

As seen from the mass chromatogram (Figure 68), the compound represented here is a major component of the M. sativa L. subsp. praefalcata exudate sample and is in much greater concentration than the other components of the sample.

This spectrum (Peak 28 of Figure 68, retention time 16:09) matches well with Figure 25 (Peak 20 of Figure 6, retention time 16:09) and Figure 50 (Peak 22 of Figure 30, retention time 16:09), both spectra proposed to be of high molecular weight straight chain alkanes.

Figure 95 (Peak 29 of Figure 68)

This spectrum is weak and lacks useful information in the high molecular weight region. Strong alkane and alkene patterns are present at m/e 57, 71, 85, 99, 113, and 127, and at m/e 55, 69, 83, 97, 111, and 125, respectfully. Because the alkene pattern dominates the alkane pattern after m/e 83, the compound represented here most likely contains an alkene function.

Figure 96 (Peak 30 of Figure 68)

Because the entire mass chromatogram of M. sativa L. subsp. praefalcata exudate sample (Figure 68) is fairly weak as the exudate is low in concentration, column bleed and noise have interfered in many of the spectra. This spectrum is no exception as peaks at m/e 73, 147, 207, 221, 281, 355, and 429 are attributed to column bleed.

Proposed structure: branched alkene or

straight chain alkene of length C_{30} or greater

1) Both an alkane pattern at m/e 57, 71, 85, etc. and an alkene pattern at m/e 55, 69, 83, etc. are present. The alkene pattern dominates at m/e 97 and in the higher molecular weight region. This indicates a double bond is present in the C_7 position of the alkene function.

2) All of the higher molecular weight peaks except possibly m/e 342 are most likely due to column bleed or noise.

a) If the peak at m/e 342 is due to a true signal, it is probably not the parent peak but may arise from branching of the alkene.

b) If the peak at m/e 342 is due to noise, then the compound represented here is probably a straight chain alkene.

FIGURE 68

Mass chromatogram of Medicago sativa L. subsp. praefalcata exudate sample taken at MCMS, Lincoln, Nebraska

DS-55 CROSS SCAN REPORT, RUN: 1757C

+ TIC

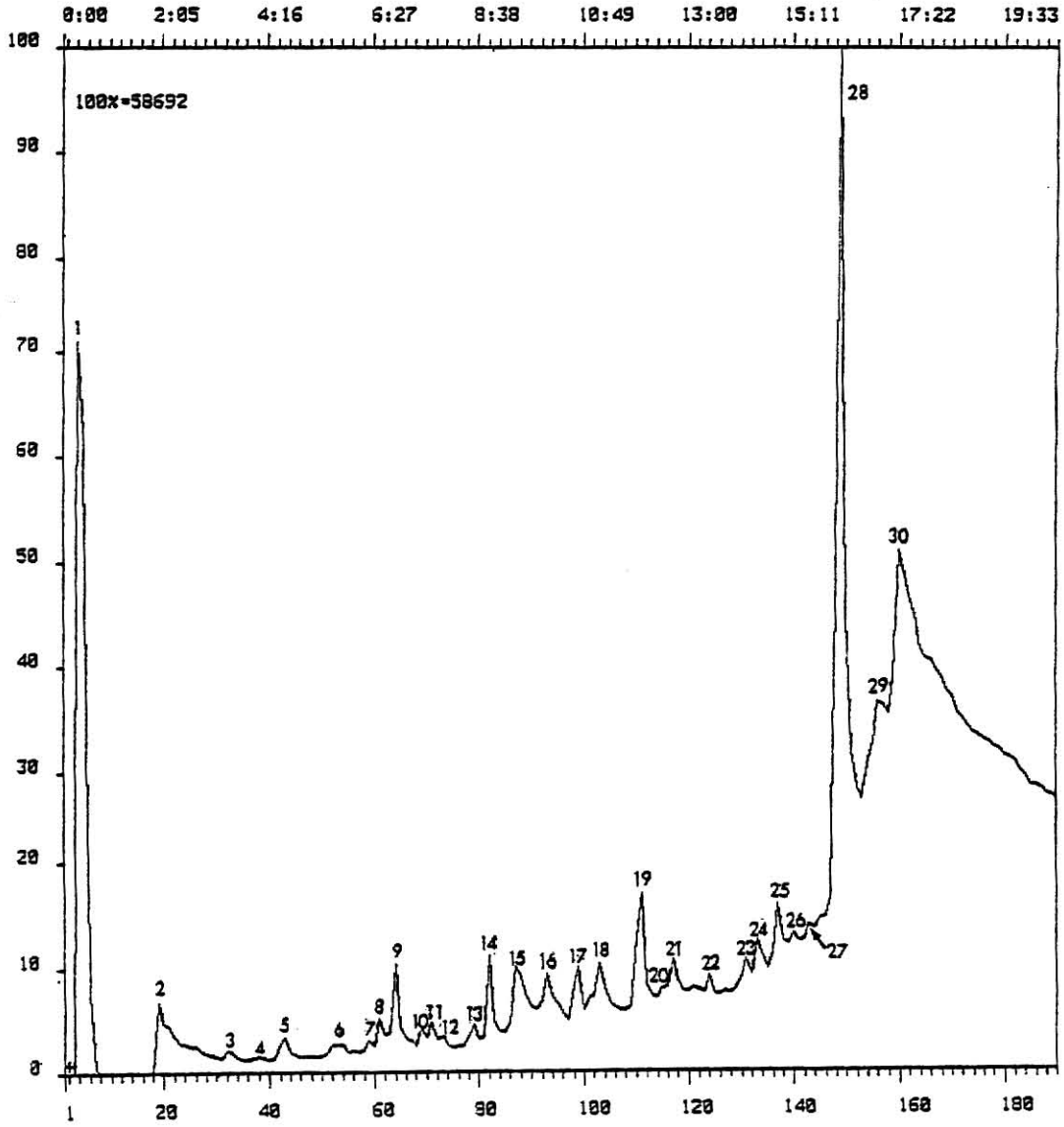


FIGURE 69

Mass spectrum of

Peak 3 of Figure 68

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:
1757CZ.32 [TIC=243, 100%=87] EI

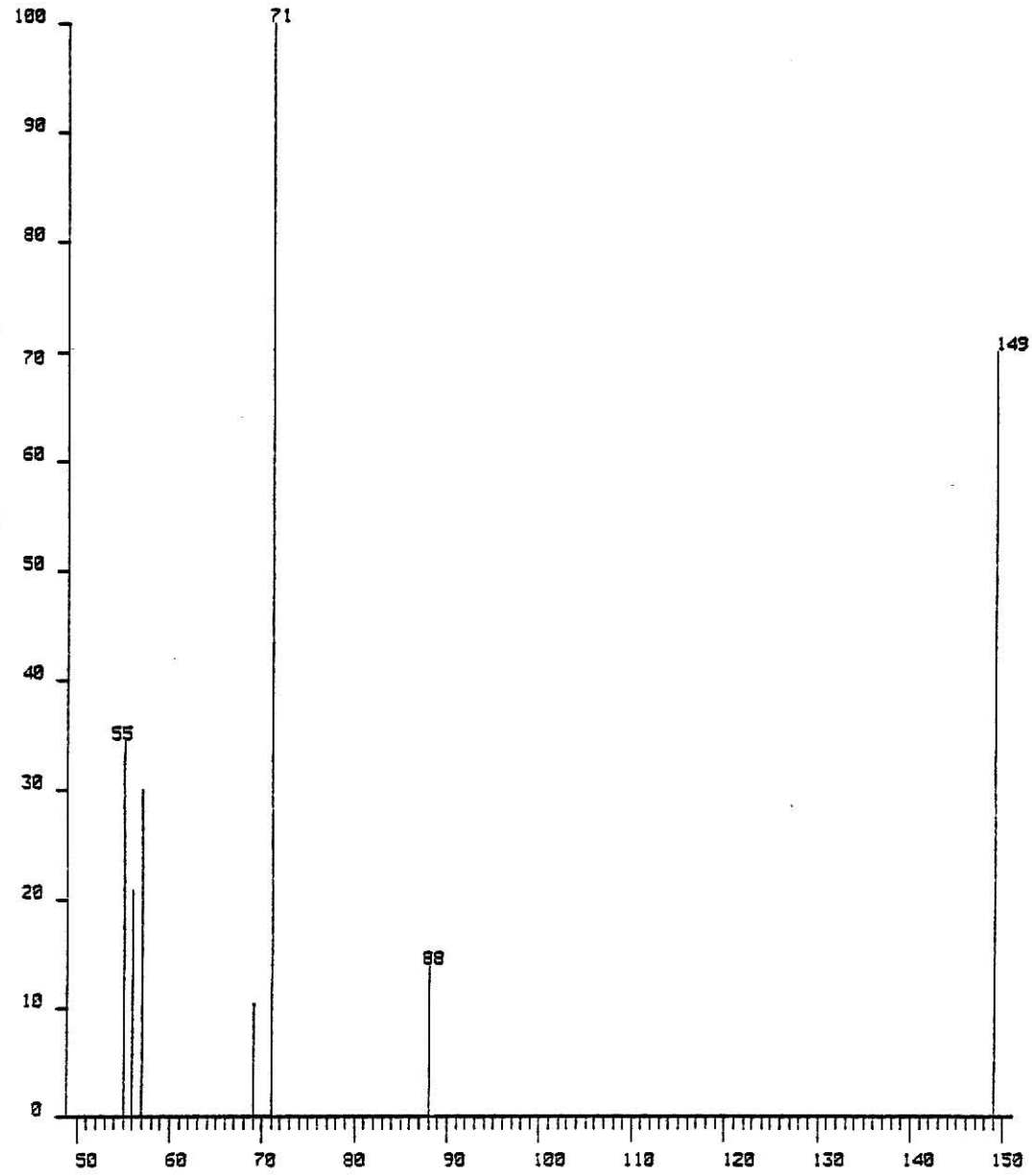


FIGURE 70

Mass spectrum of

Peak 4 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.38 [TIC=86, 100%=29] EI

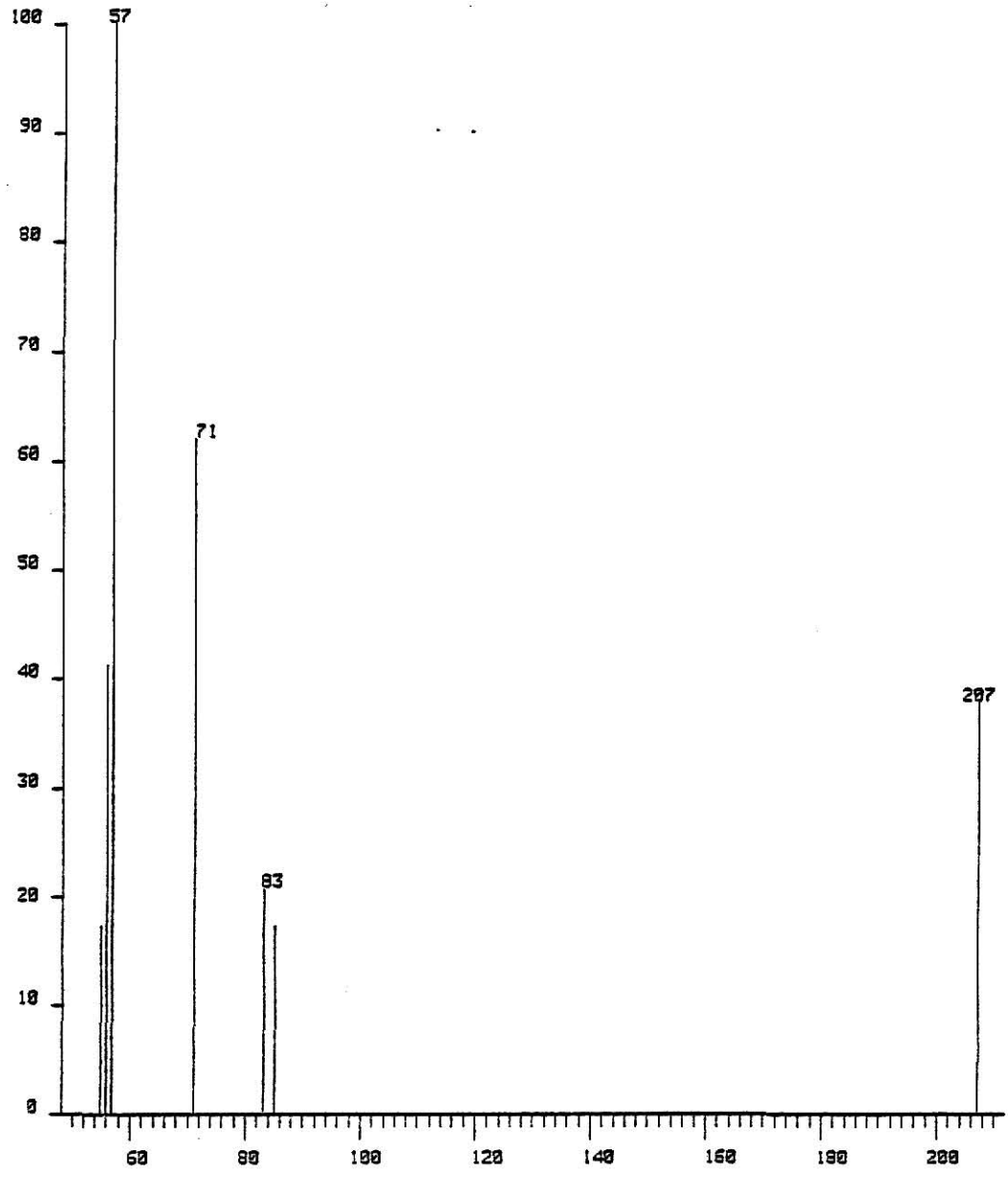


FIGURE 71

Mass spectrum of

Peak 5 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.43 [TIC=1026, 100%=353] EI

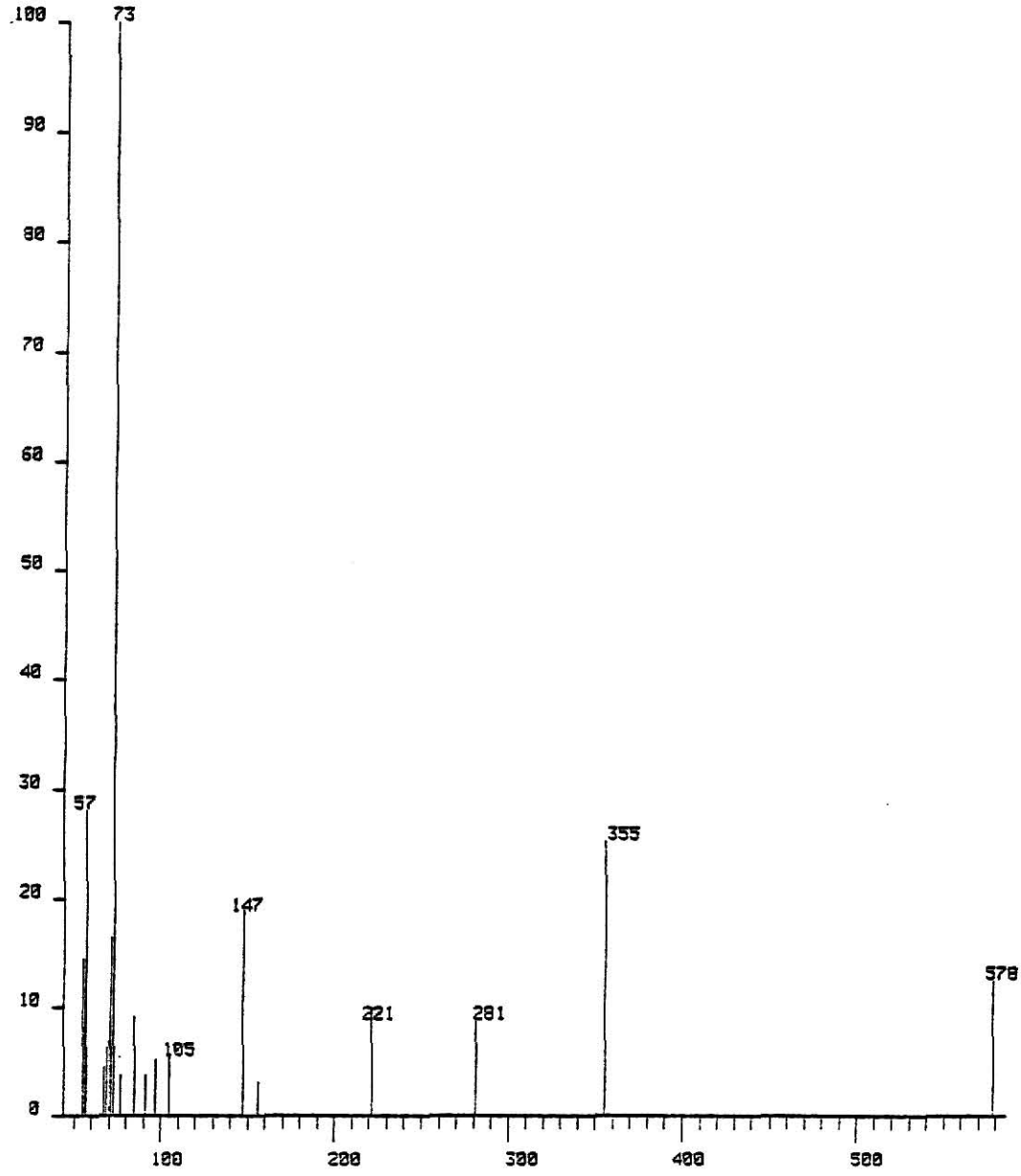


FIGURE 72

Mass spectrum of

Peak 6 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:

1757CZ.52 [TIC=291, 100%=71] EI

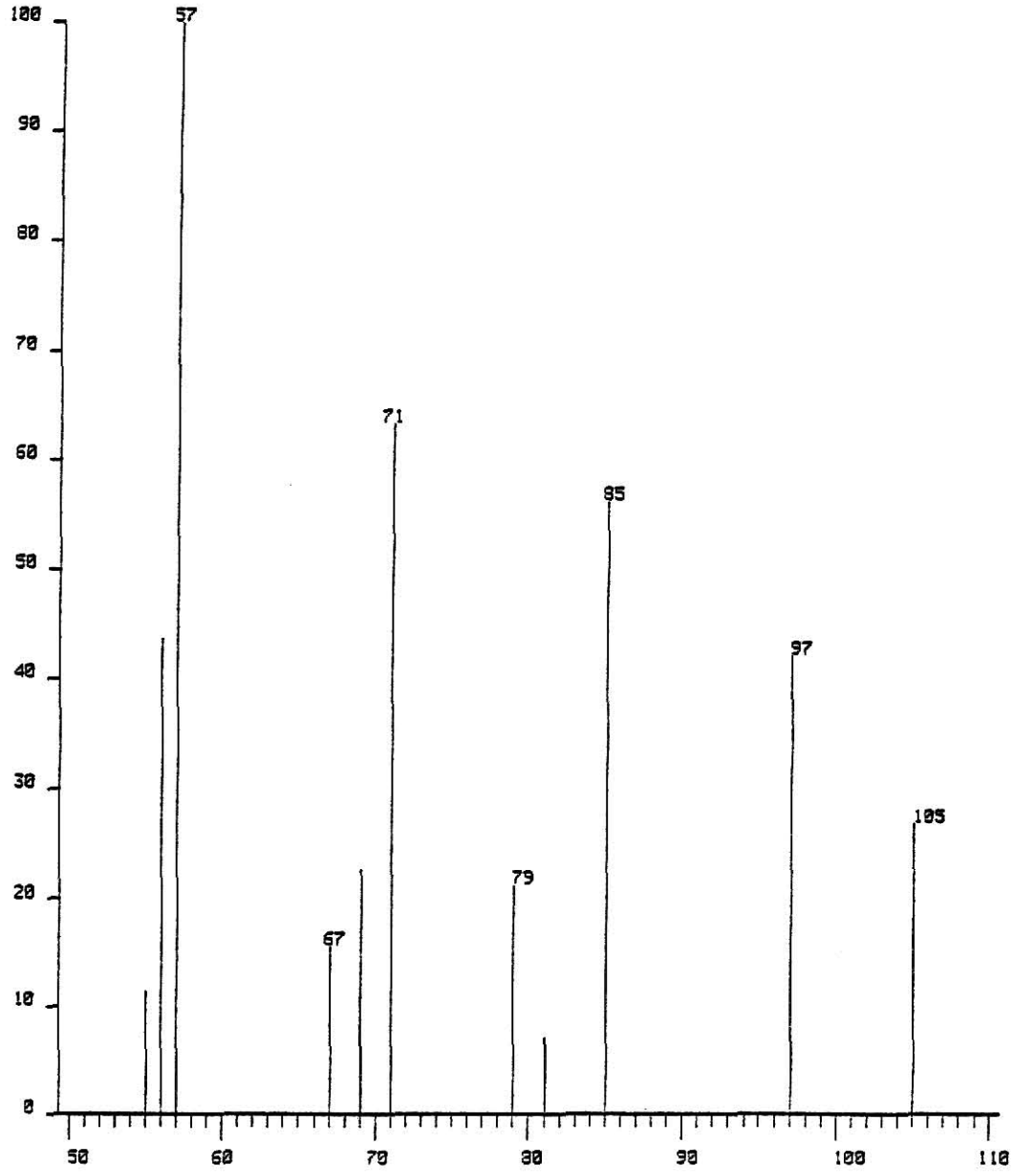


FIGURE 73

Mass spectrum of

Peak 7 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.59 [TIC=715, 100%=3571 EI

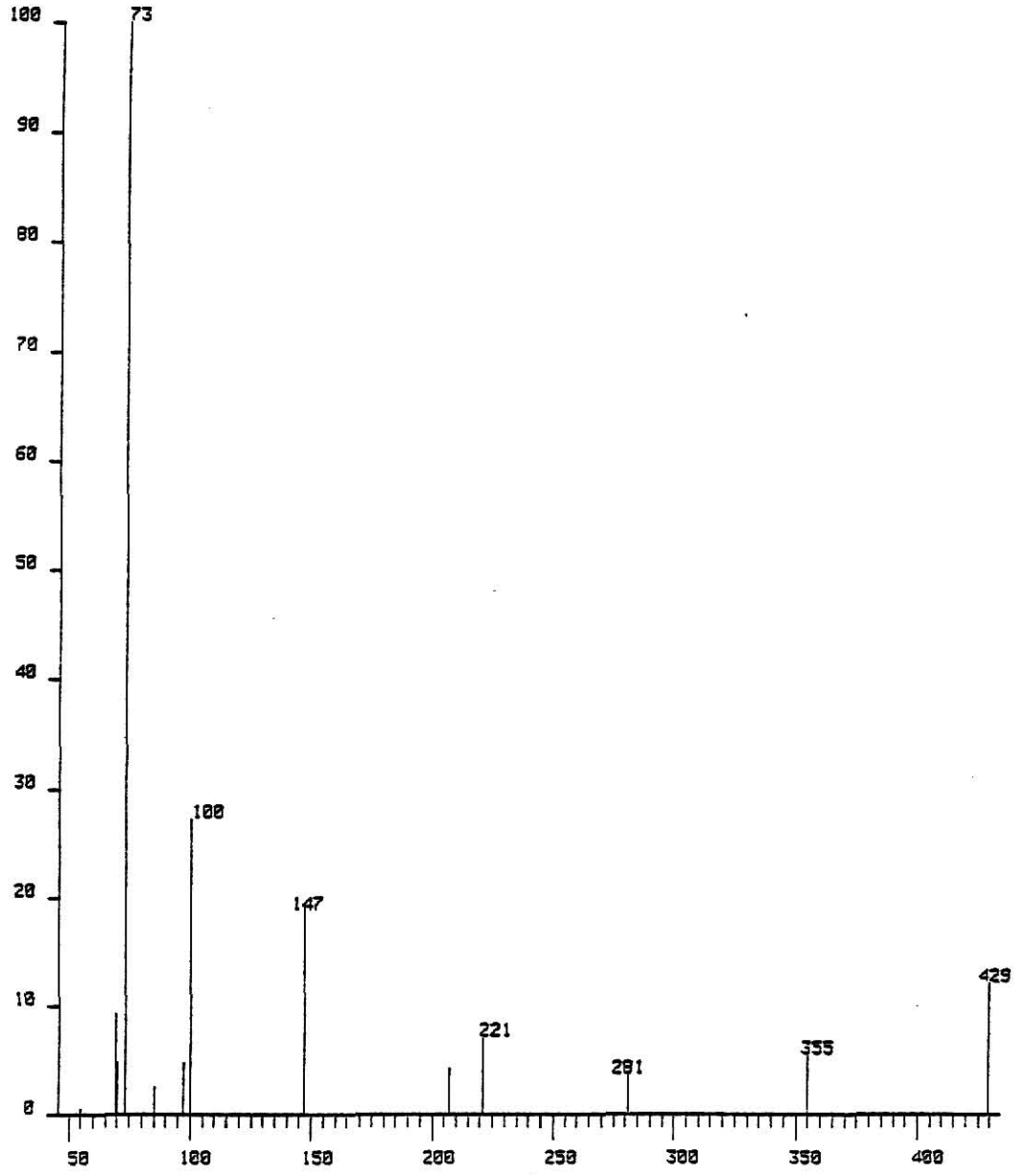


FIGURE 74

Mass spectrum of

Peak 8 of Figure 68

Proposed structure: Omite fragment

DS-55 MASS INTENSITY REPORT:
1757CZ.61 [TIC=1435, 100%=575] EI

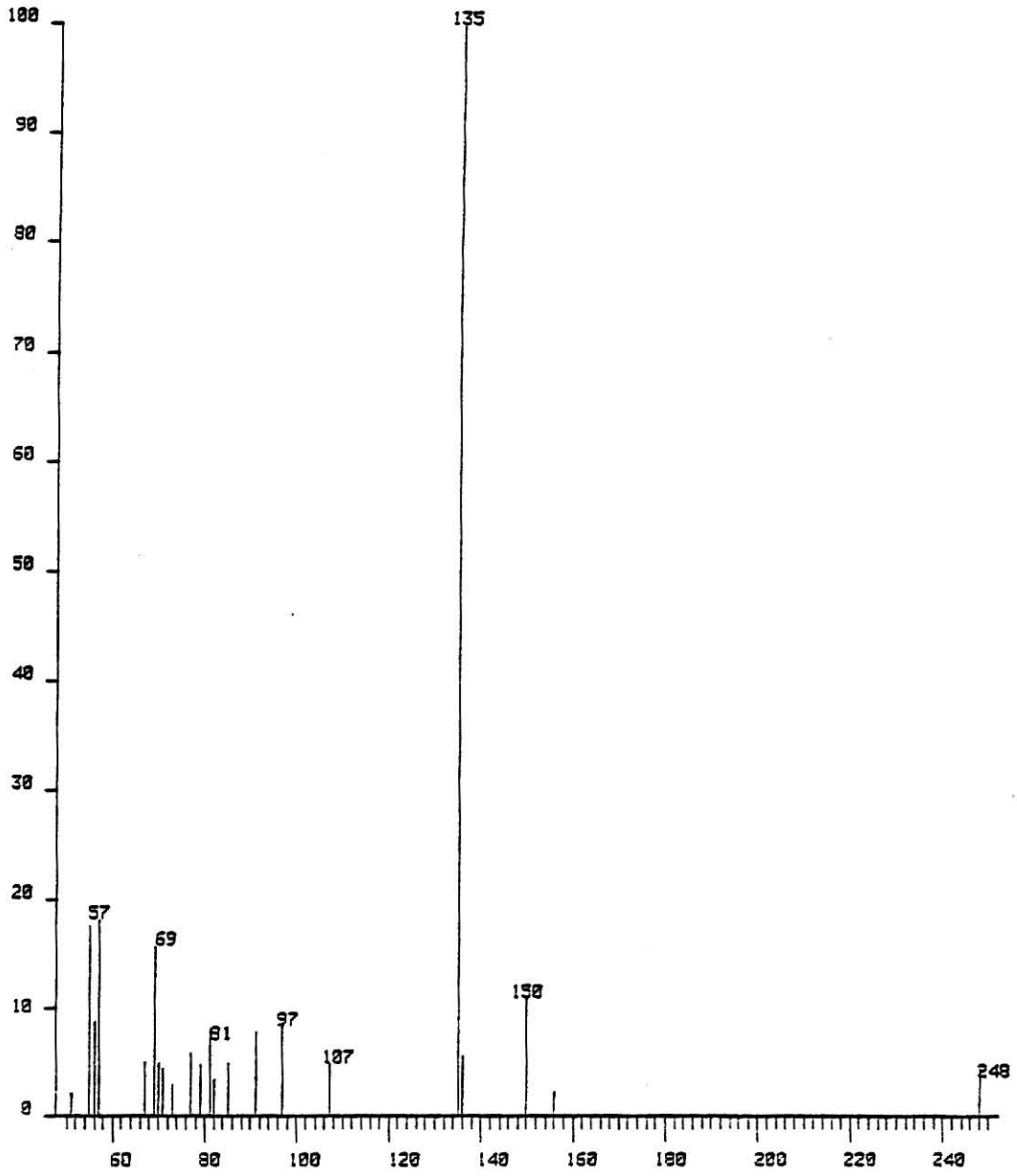


FIGURE 75

Mass spectrum of

Peak 9 of Figure 68

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:

1757CZ.64 [TIC=4345, 100%=2473] EI

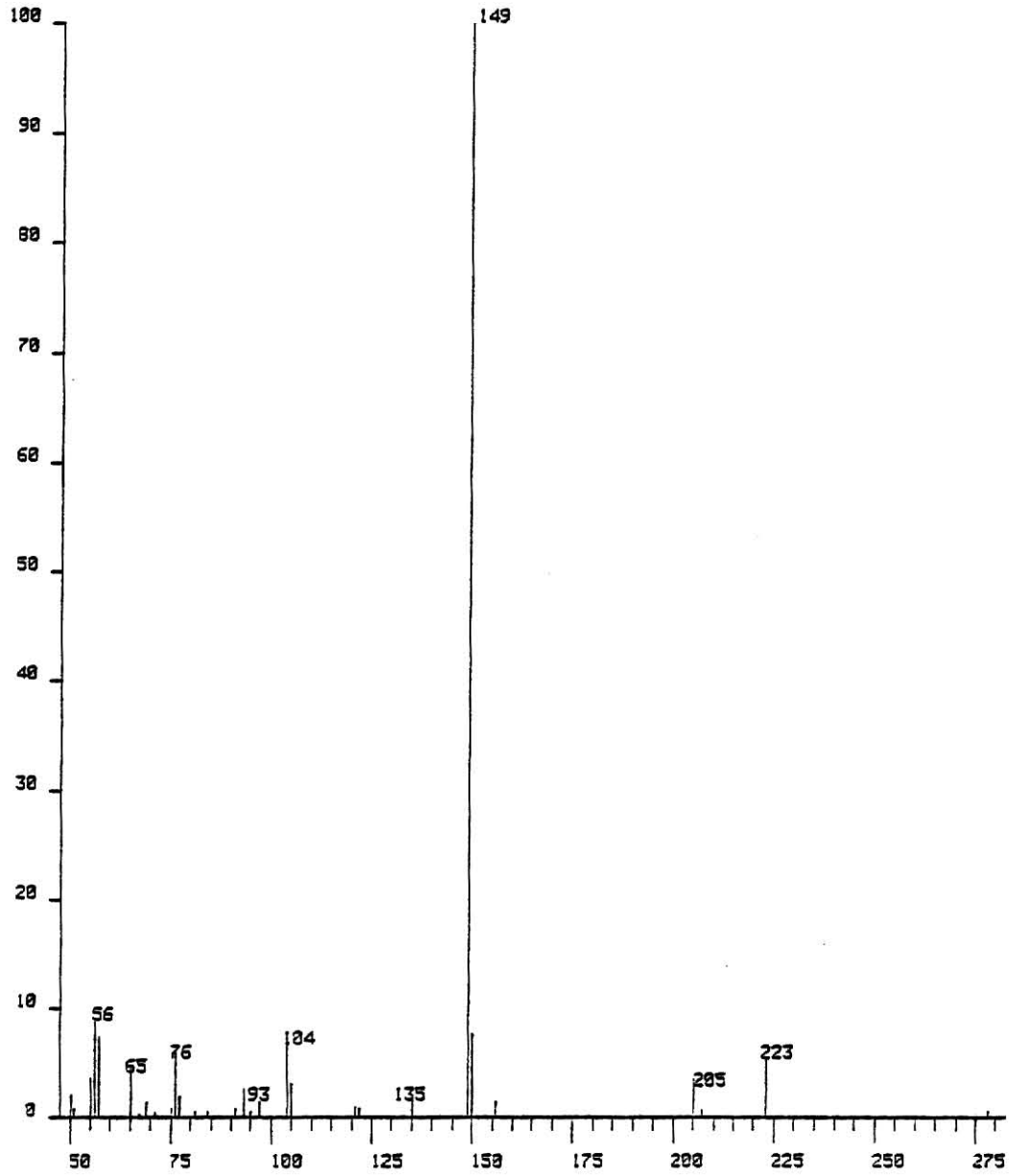
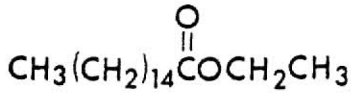


FIGURE 76

Mass spectrum of

Peak 10 of Figure 68

Proposed structure:



DS-55 MASS INTENSITY REPORT:

1757CZ.69 [TIC=703, 100%=216] EI

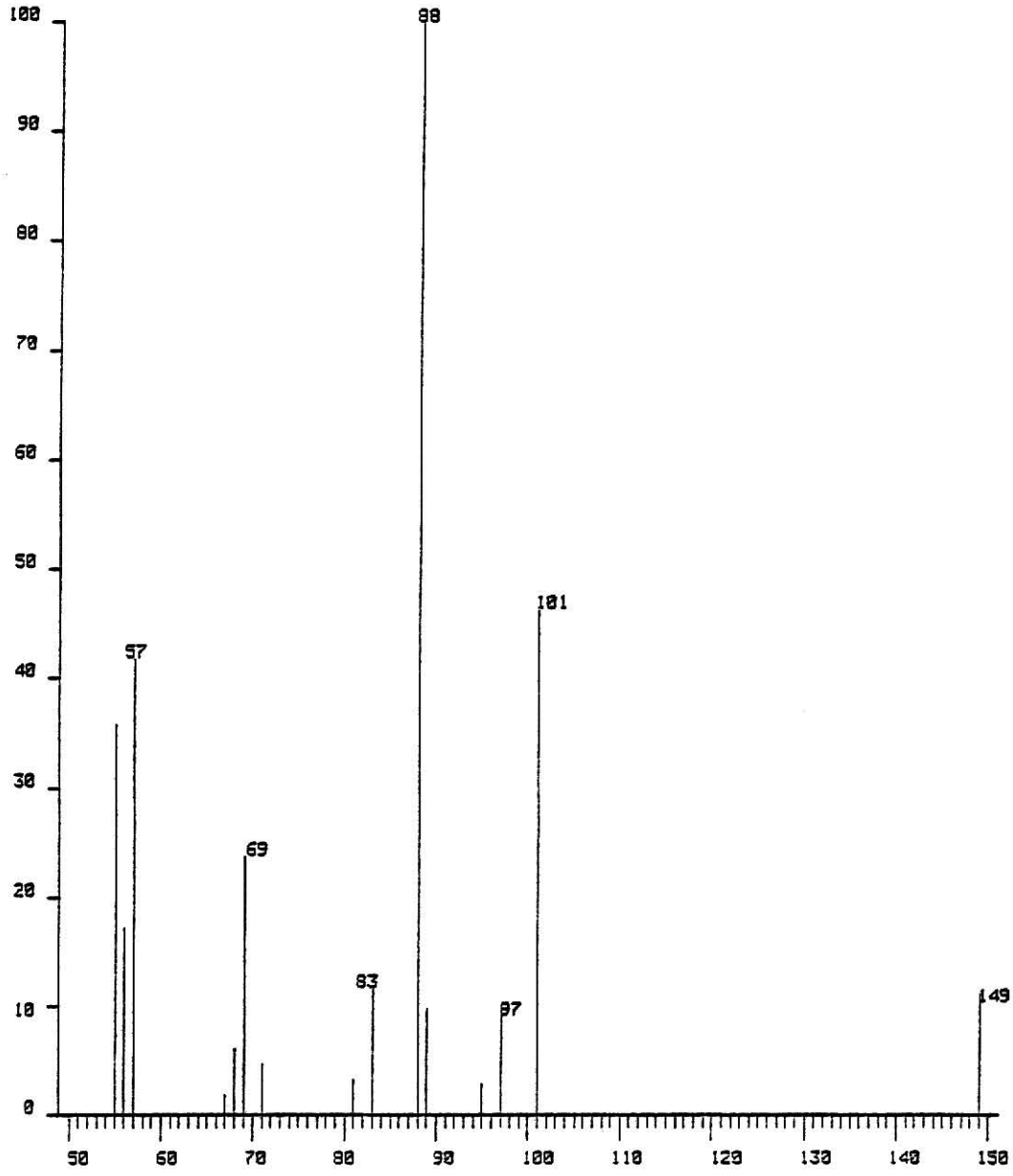


FIGURE 77

Mass spectrum of

Peak 11 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757C2.71 [TIC=927, 100%=168] EI

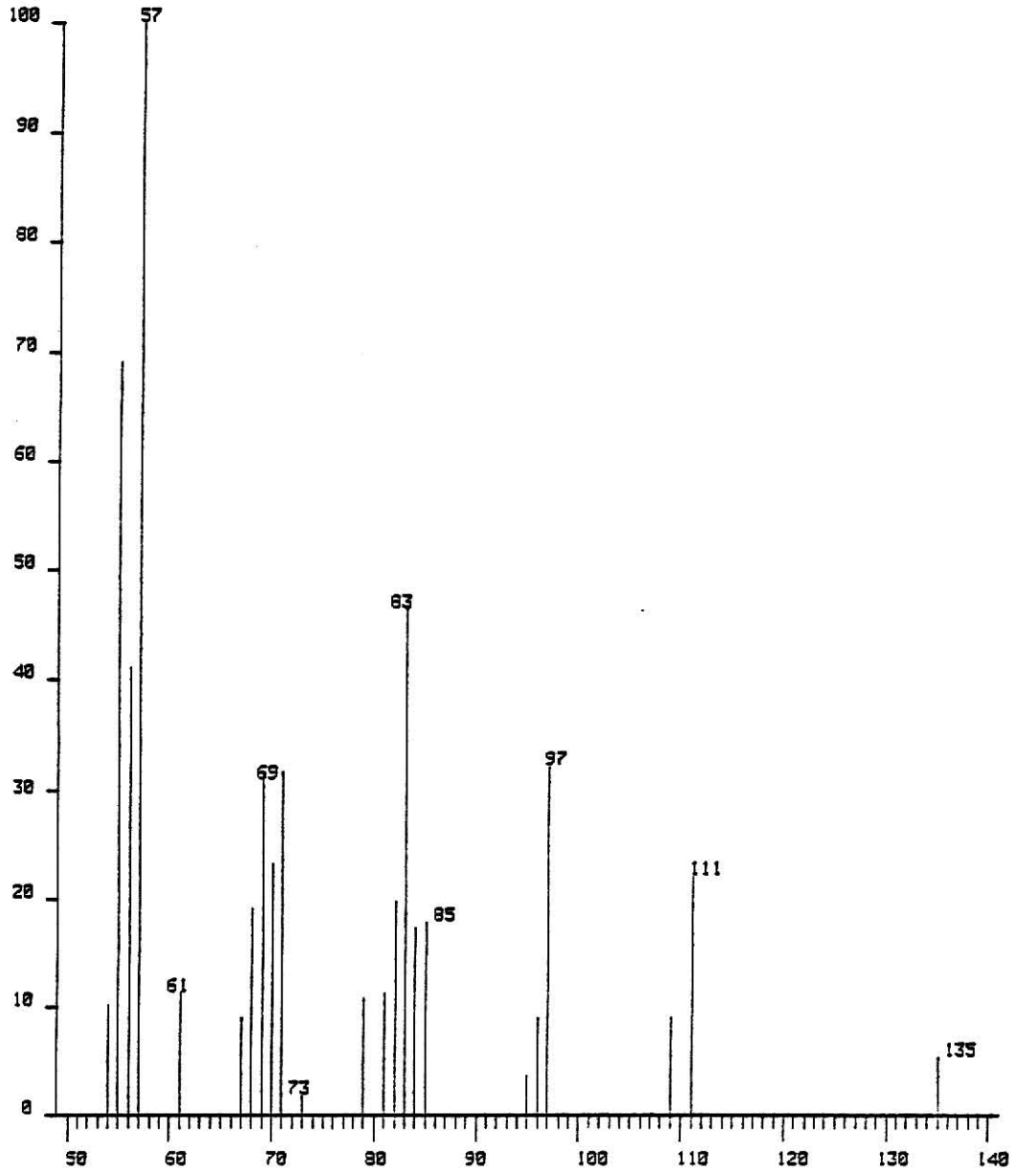


FIGURE 78

Mass spectrum of

Peak 12 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.73 [TIC=562, 100%=343] EI

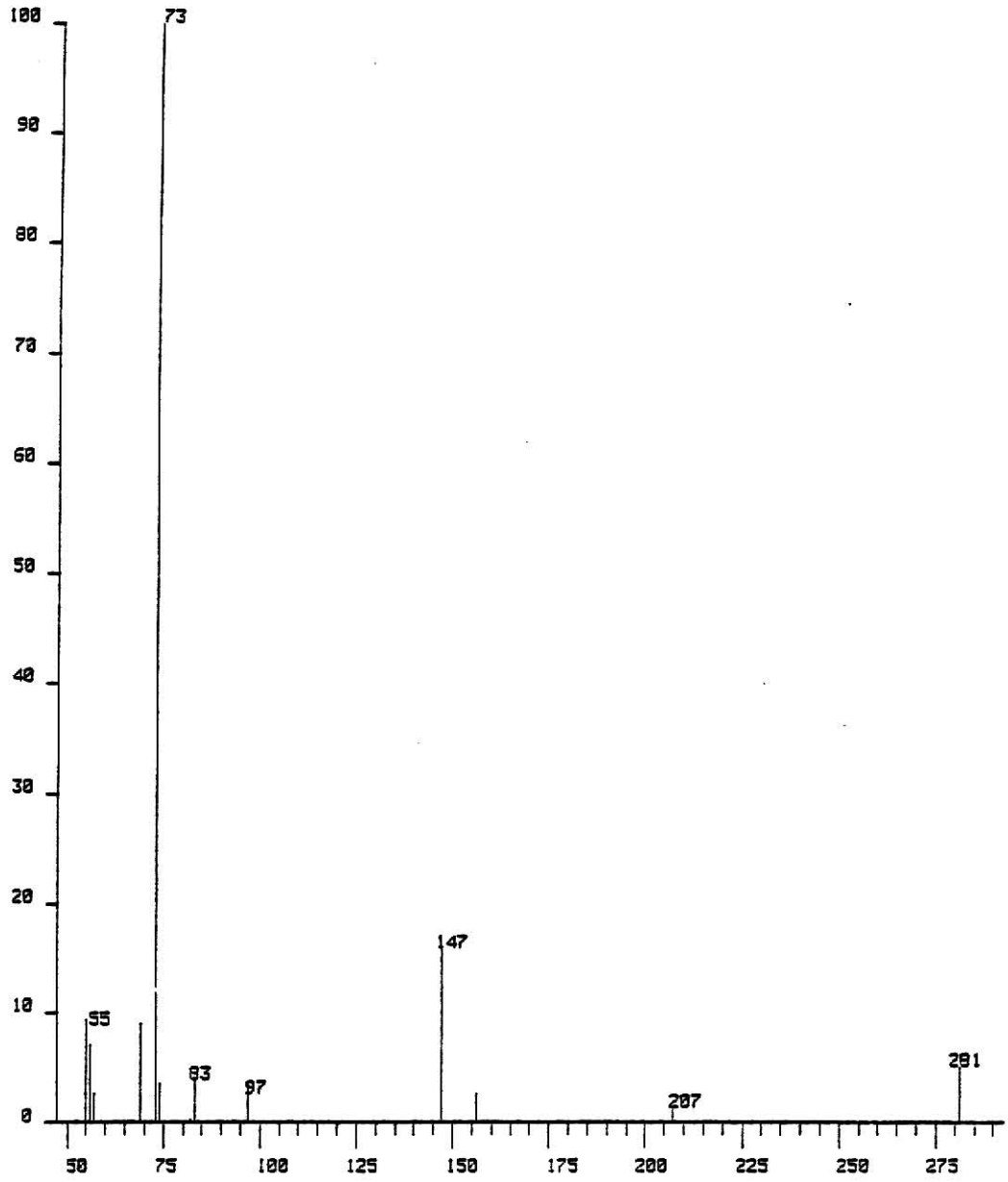


FIGURE 79

Mass spectrum of

Peak 13 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.79 [TIC=497, 100%=67] EI

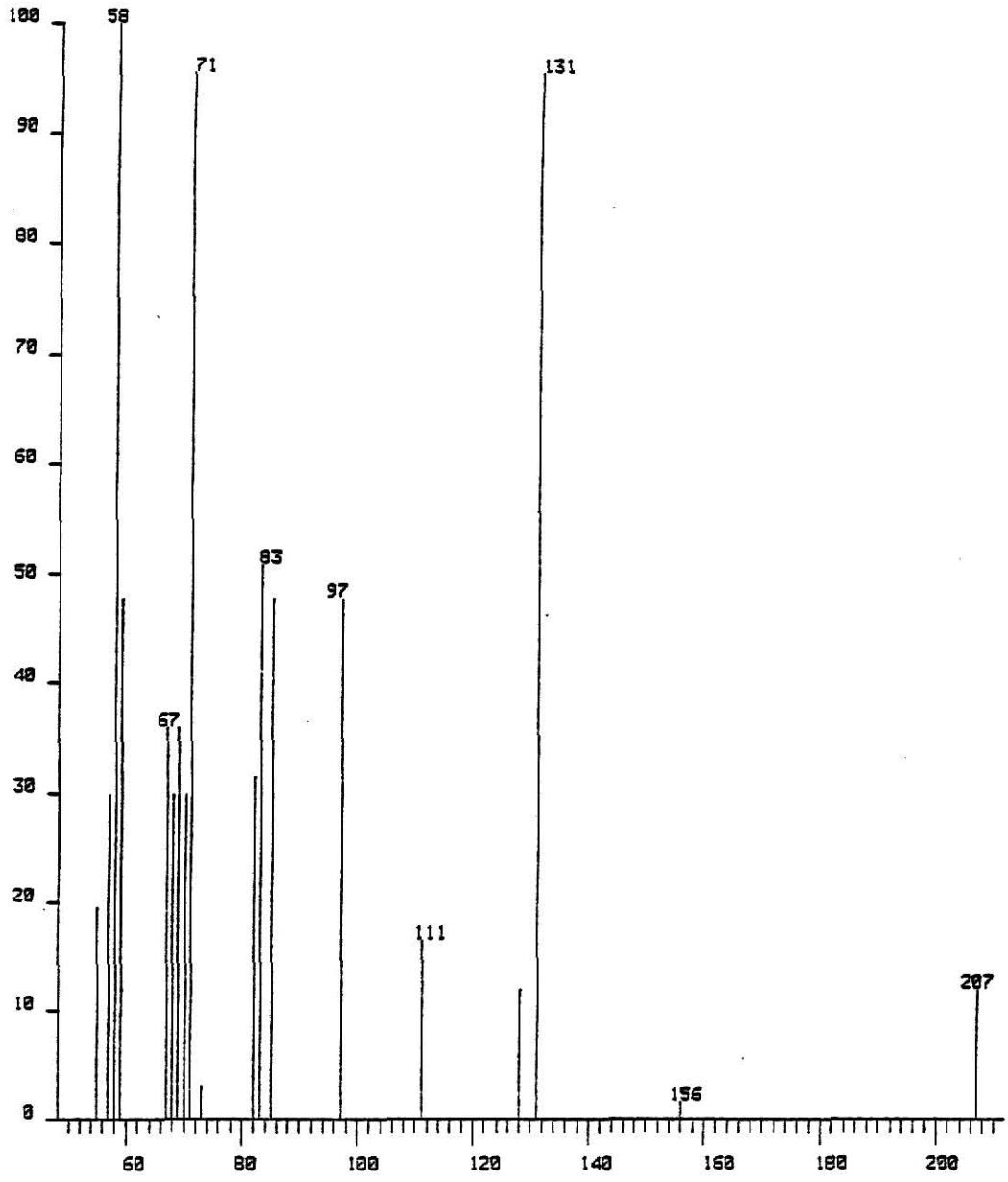


FIGURE 80

Mass spectrum of

Peak 14 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.82 [TIC=3493, 100%=862] EI

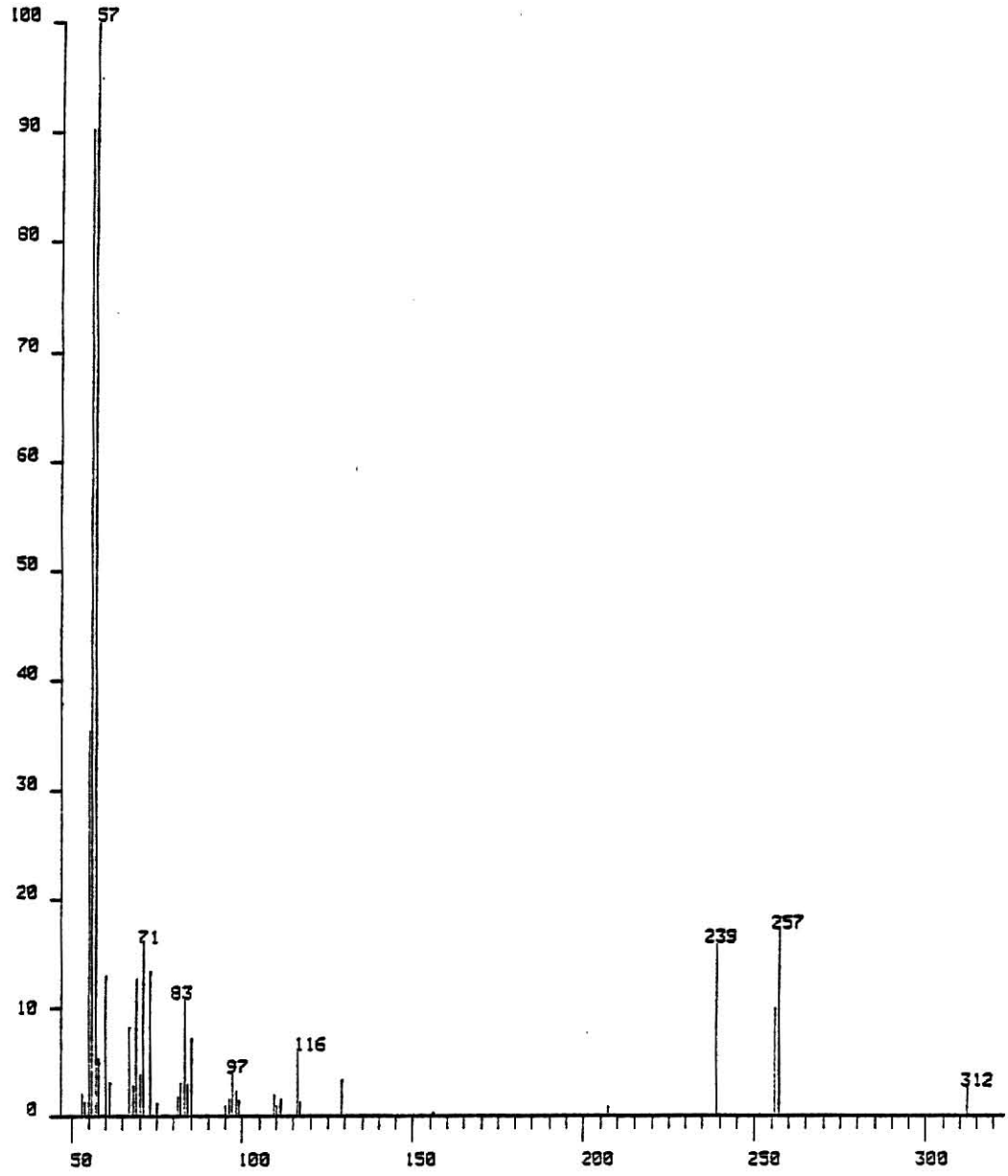


FIGURE 81

Mass spectrum of

Peak 15 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.87 [TIC=2029, 100%=596] EI

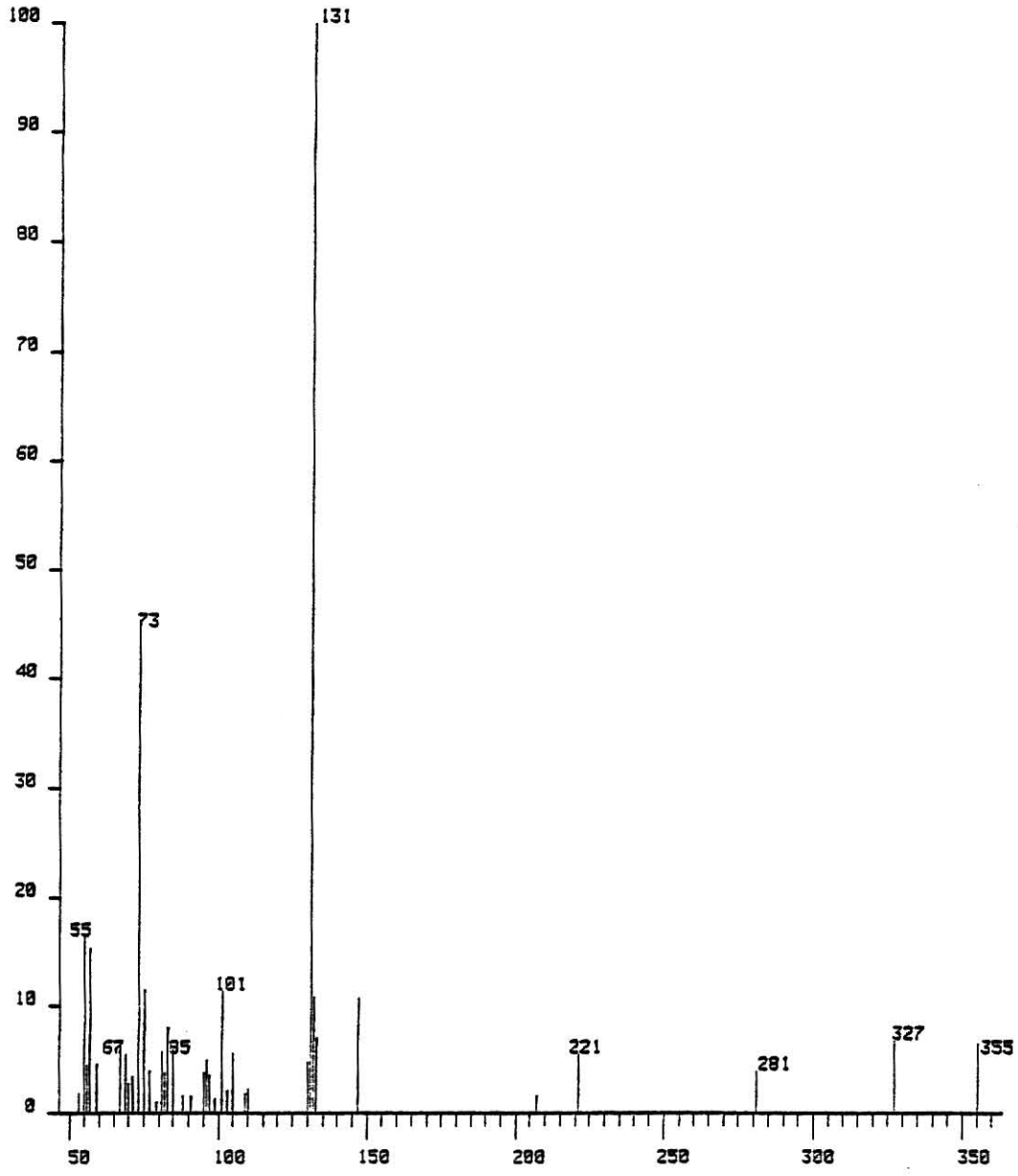


FIGURE 82

Mass spectrum of

Peak 16 of Figure 68

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:
1757CZ.93 [TIC=1799, 100%=588] EI

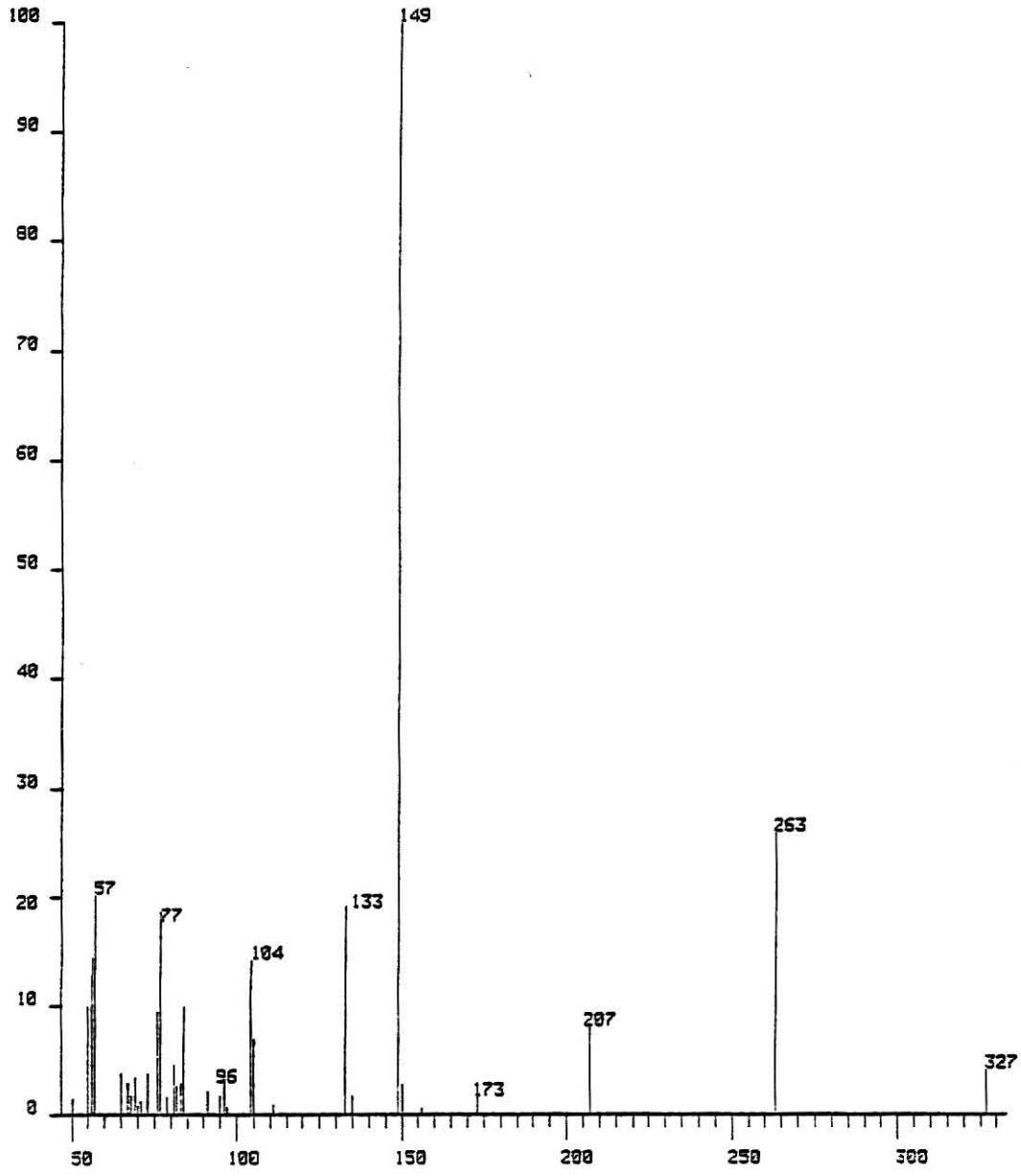


FIGURE 83

Mass spectrum of

Peak 17 of Figure 68

Proposed structure: Omite fragment

DS-55 MASS INTENSITY REPORT:

17S7CZ.99 [TIC=2351, 100%=596] EI

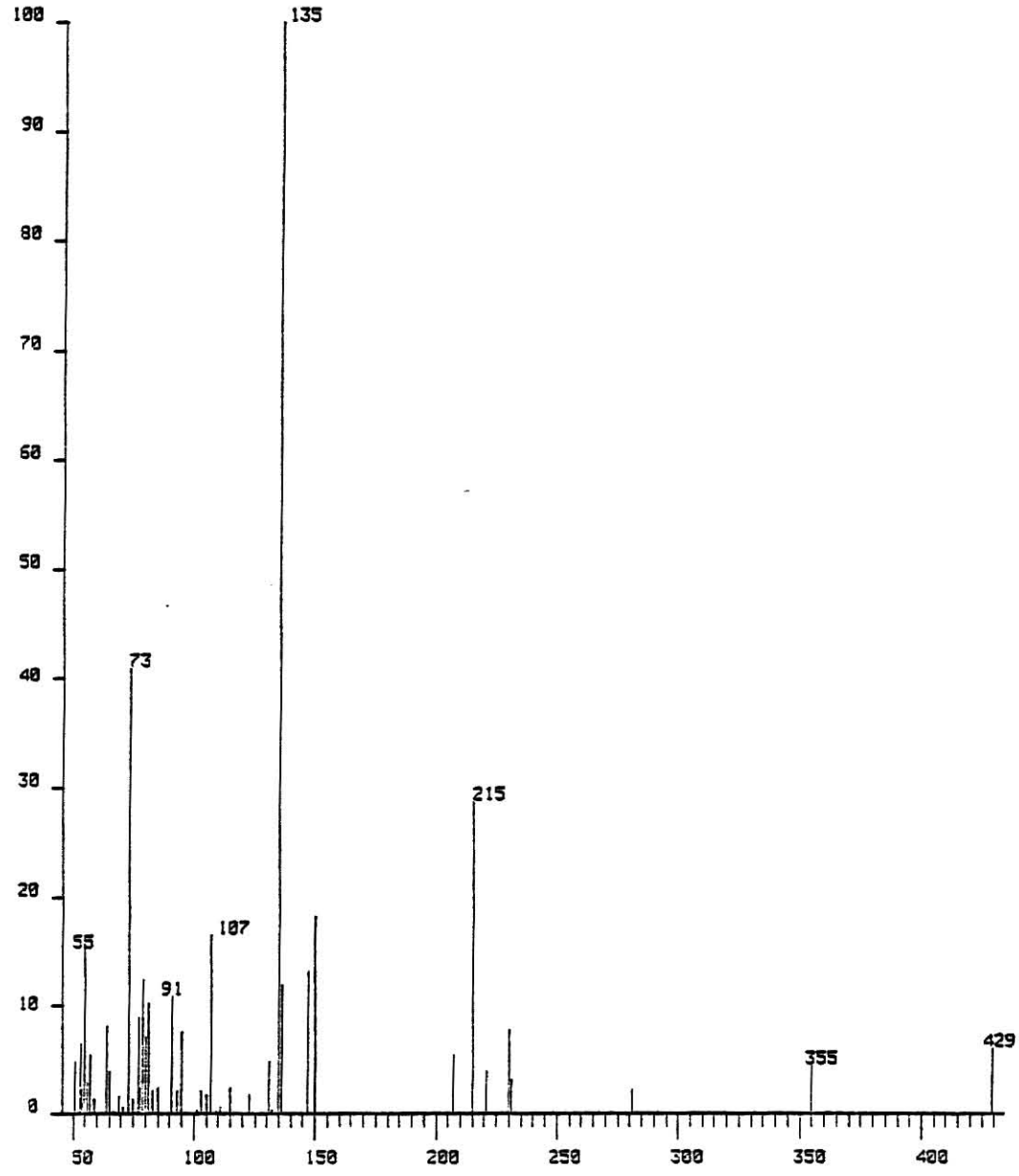


FIGURE 84

Mass spectrum of

Peak 18 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.103 [TIC=2280. 100%=771] EI

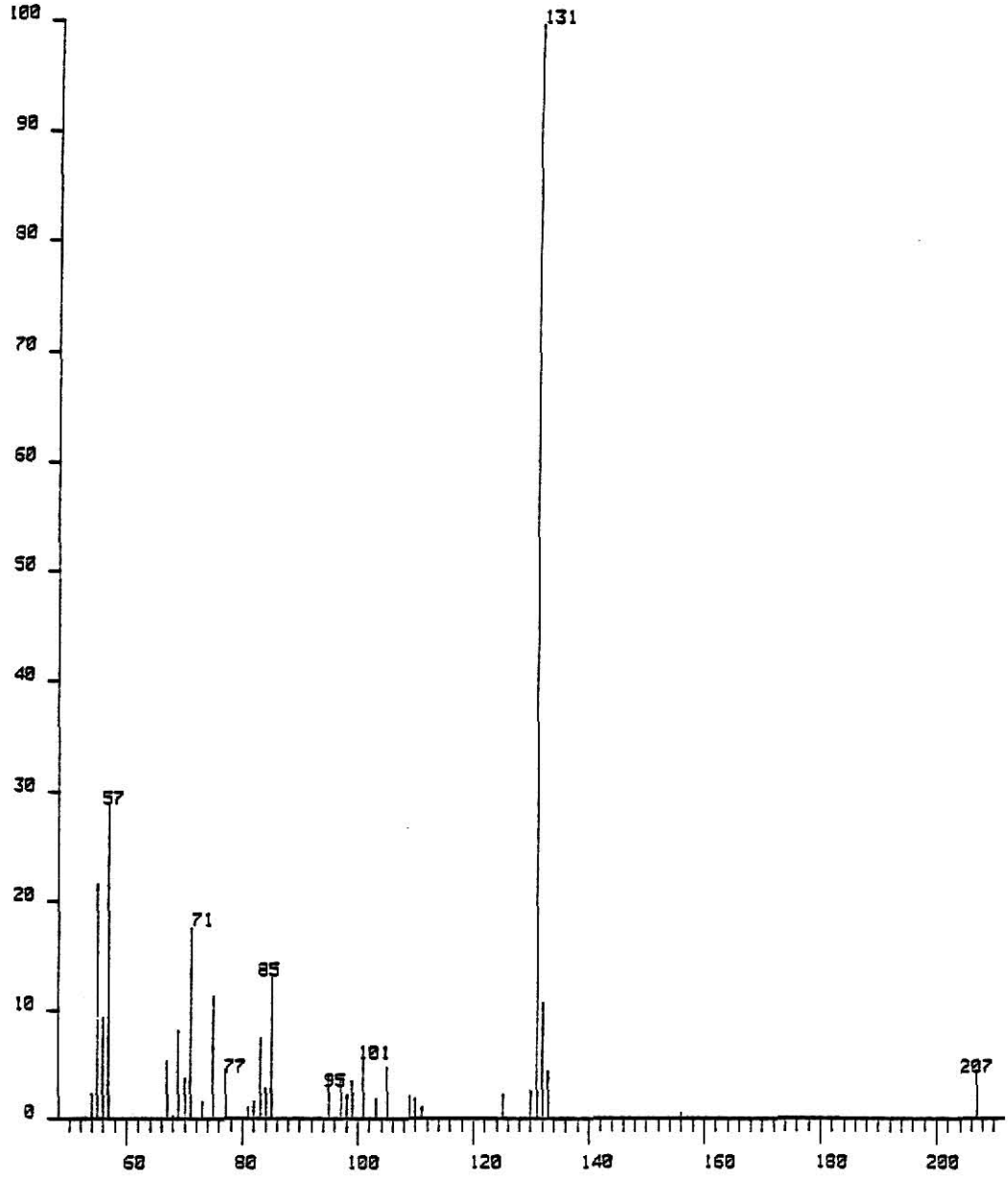


FIGURE 85

Mass spectrum of

Peak 19 of Figure 68

Proposed structure: Phthalate

DS-55 MASS INTENSITY REPORT:
1757CZ.111 [TIC=5041, 100%=1199] EI

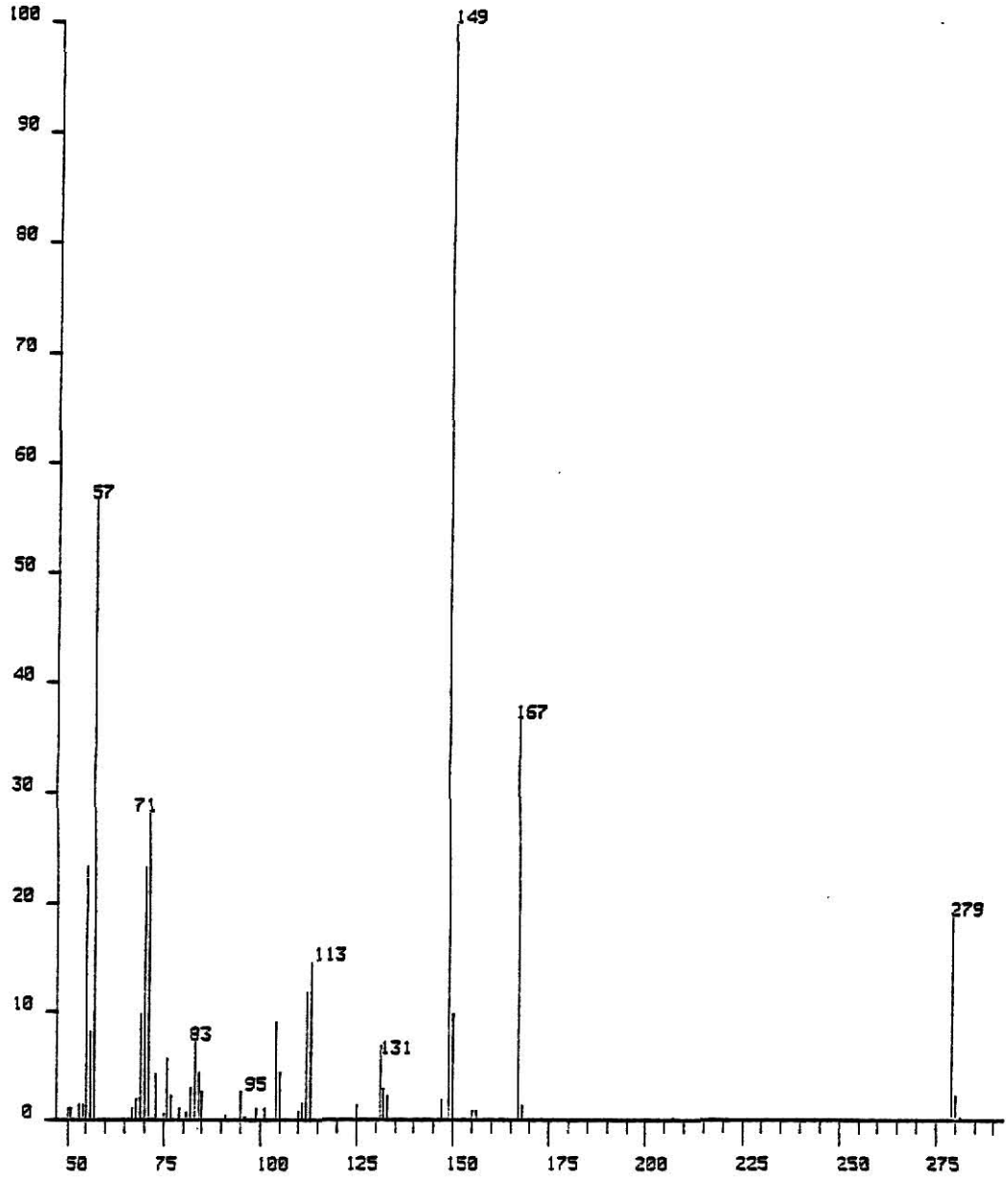


FIGURE 86

Mass spectrum of

Peak 20 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:

1757C2.115 [TIC=460, 100%=69] EI

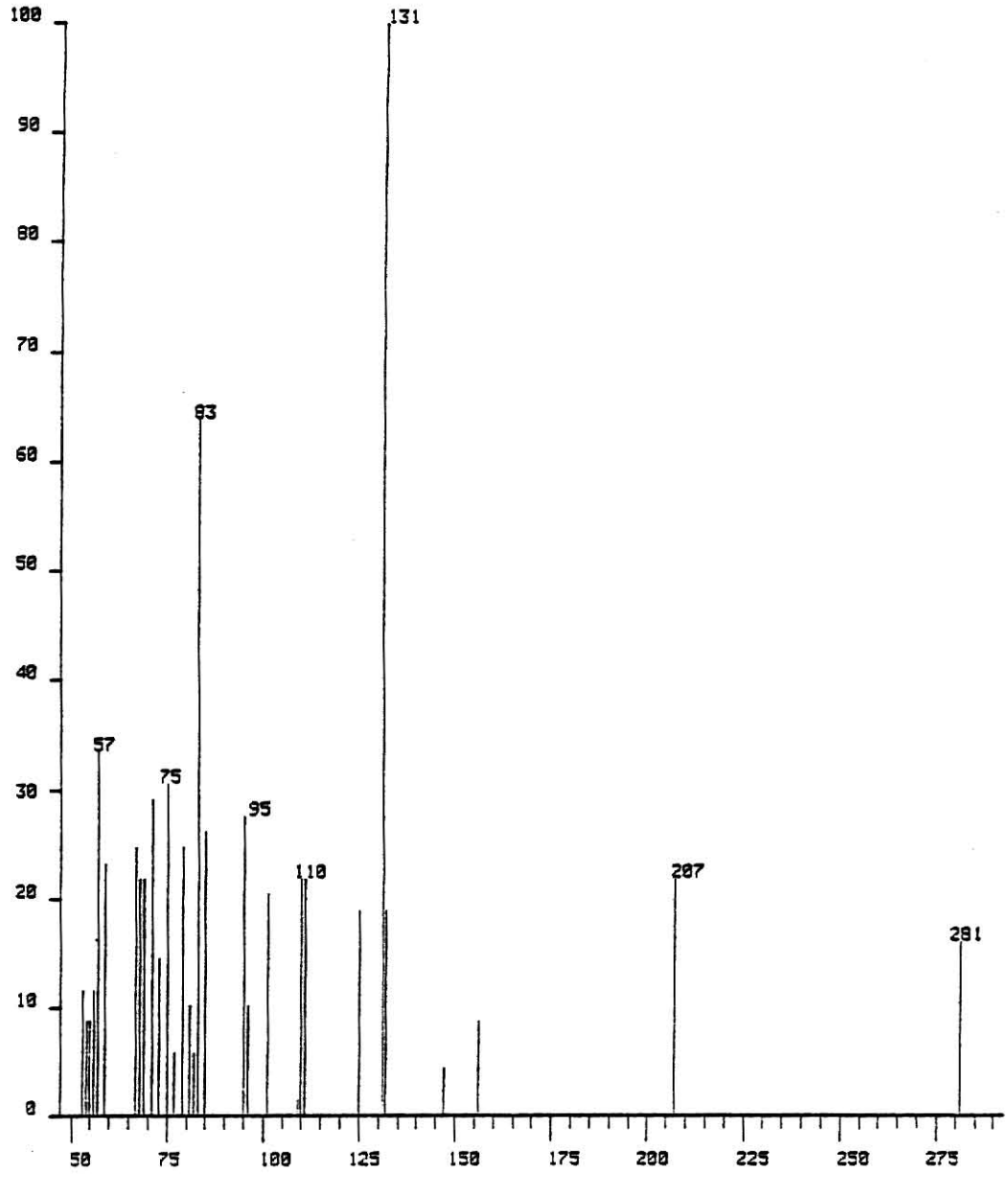


FIGURE 87

Mass spectrum of

Peak 21 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:

1757CZ.117 [TIC=1879, 100%=626] EI

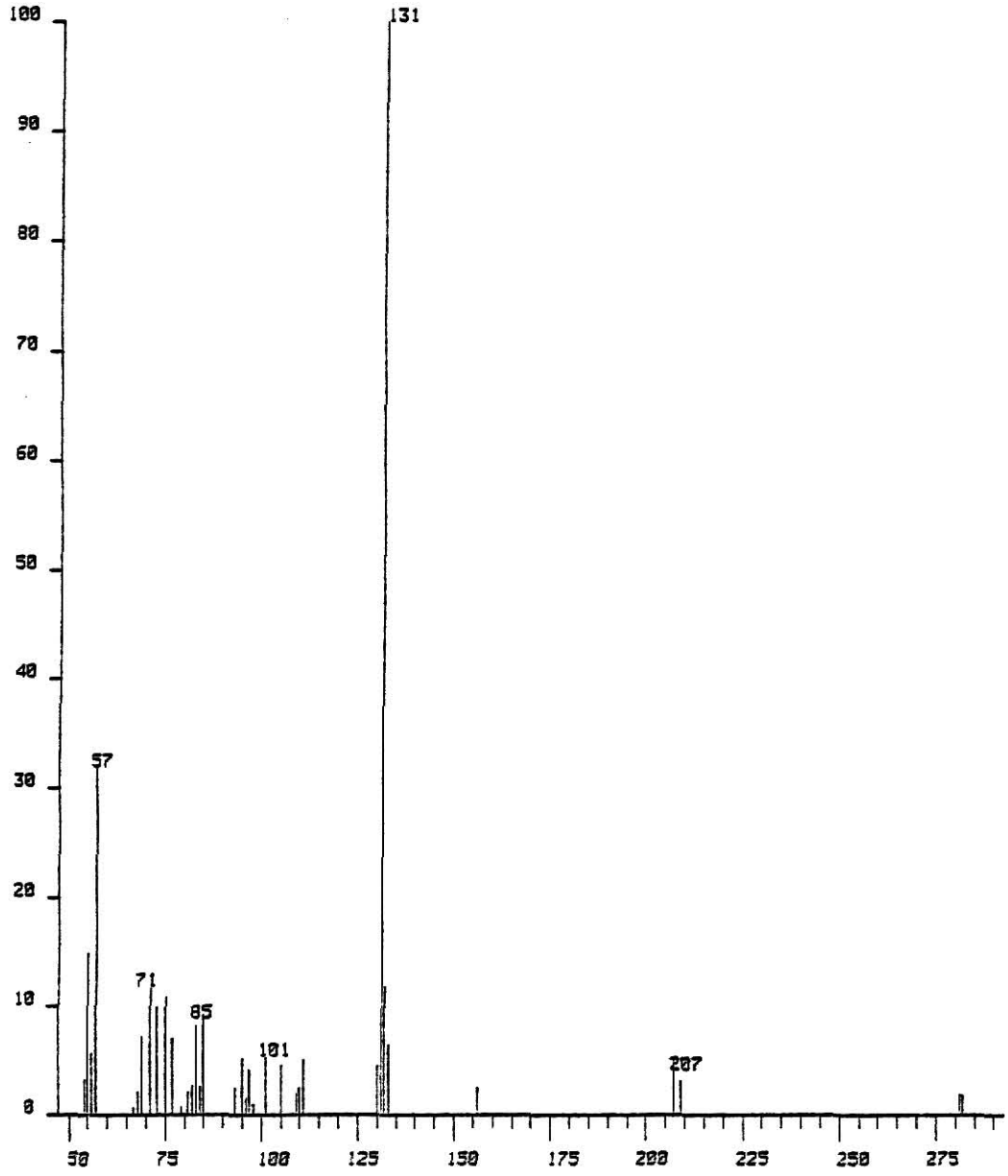


FIGURE 88

Mass spectrum of

Peak 22 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.124 [TIC=876, 100%=200] EI

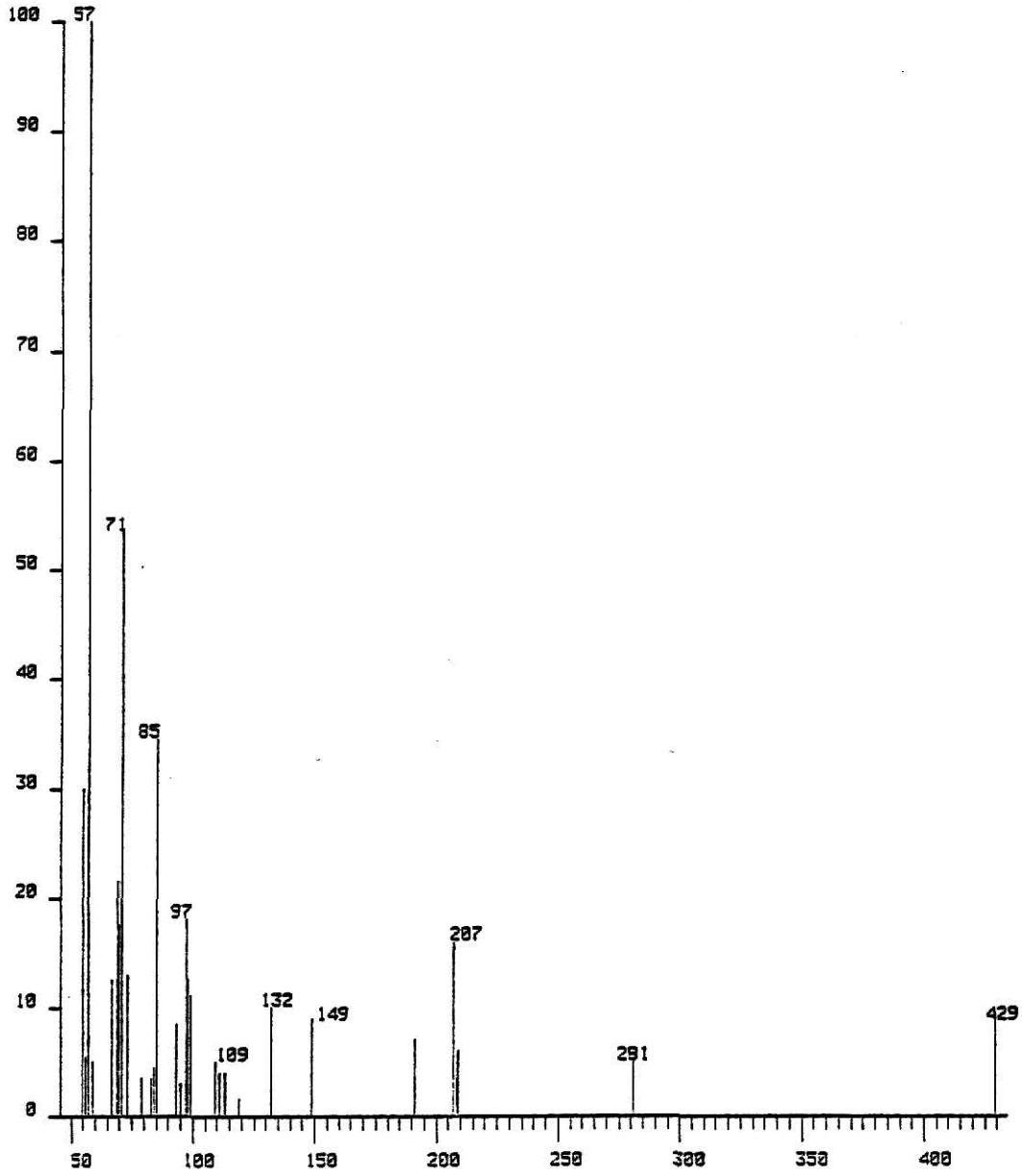


FIGURE 89

Mass spectrum of

Peak 23 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757C2.131 [TIC=747, 100%=76] EI

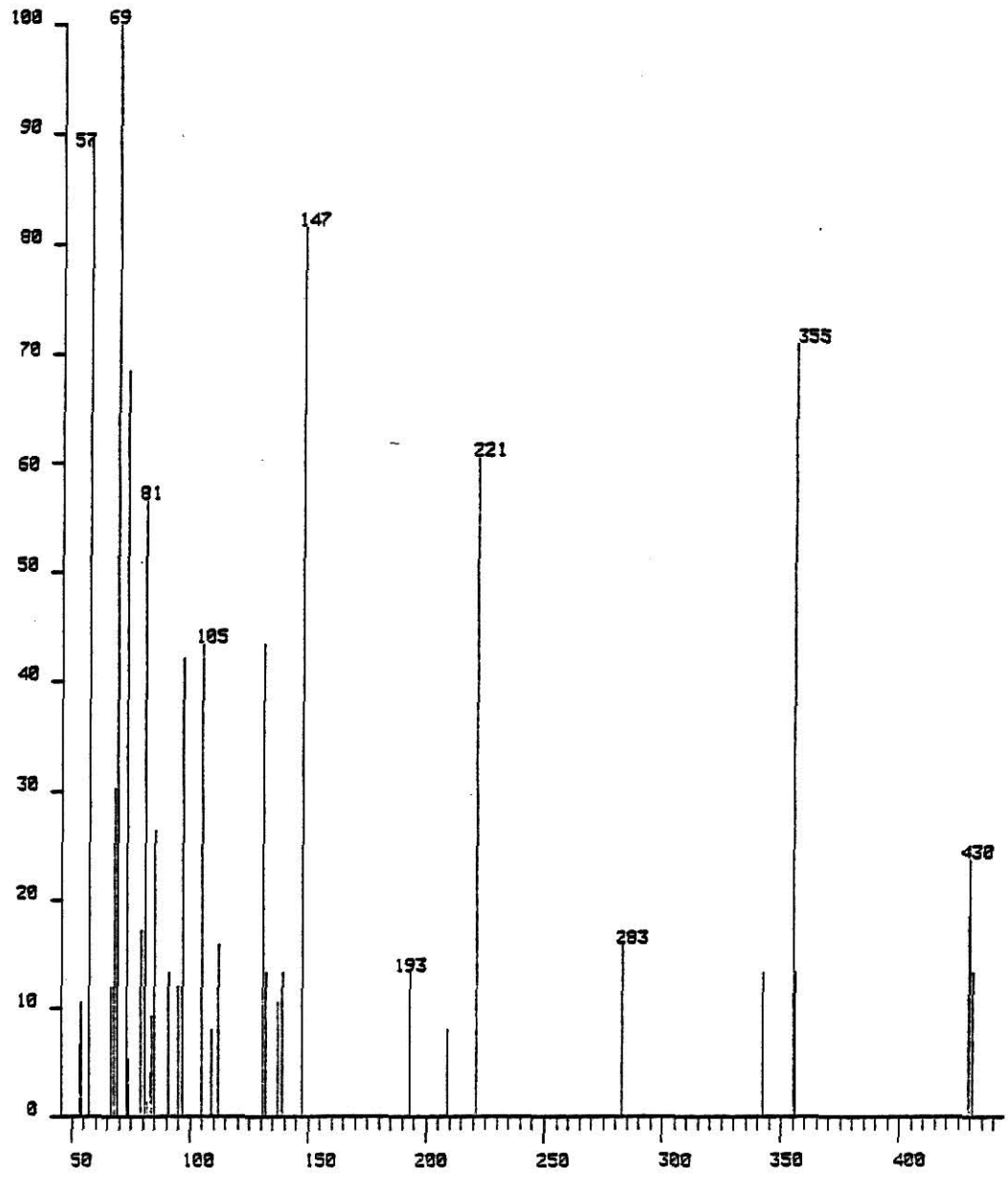


FIGURE 90

Mass spectrum of

Peak 24 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:

1757CZ.133 [TIC=1257, 100%=187] EI

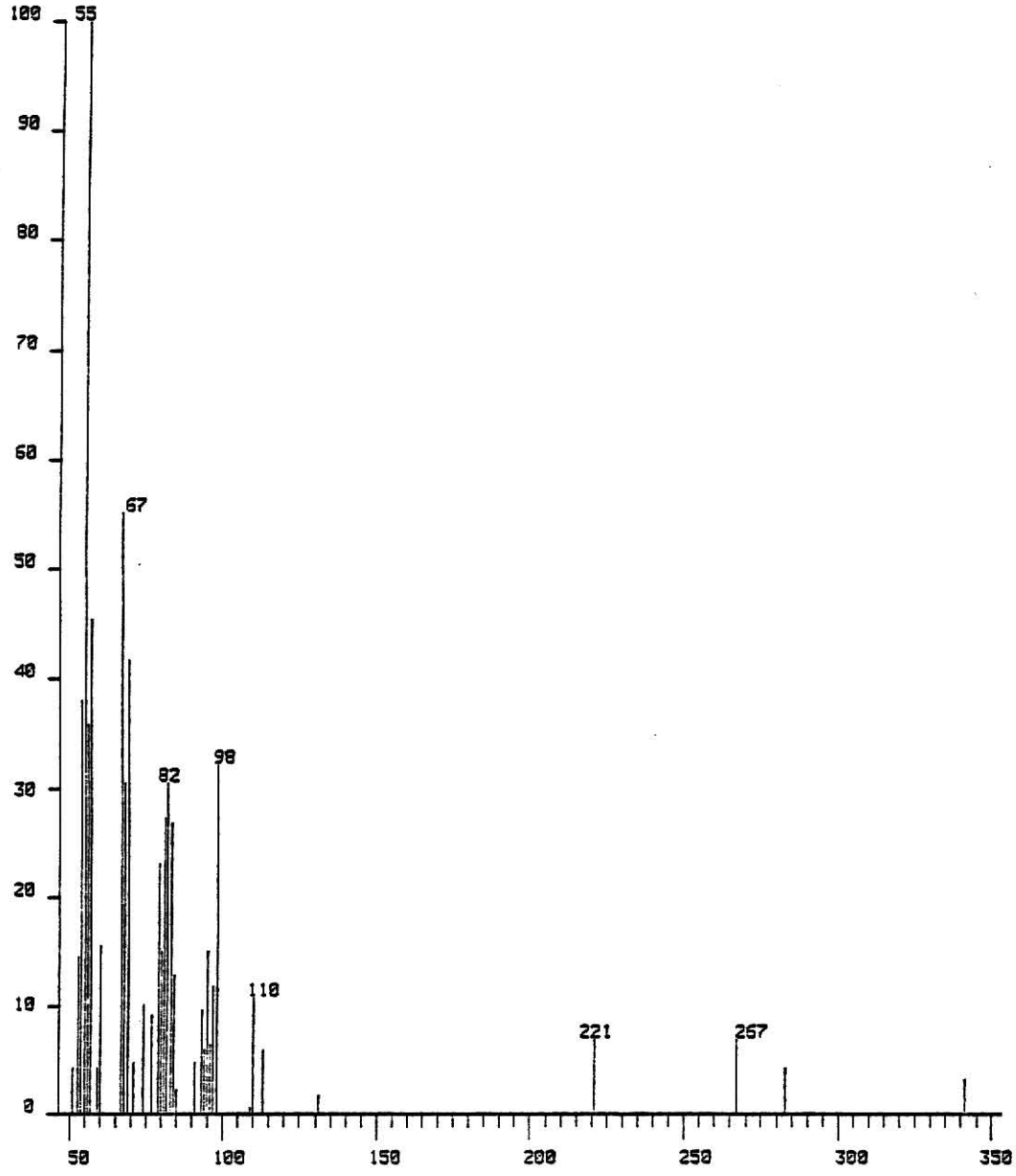


FIGURE 91

Mass spectrum of

Peak 25 of Figure 68

Proposed structure:

straight chain alkane of length at least C_{30}

DS-55 MASS INTENSITY REPORT:
1757CZ.137 [TIC=2930, 100%=714] EI

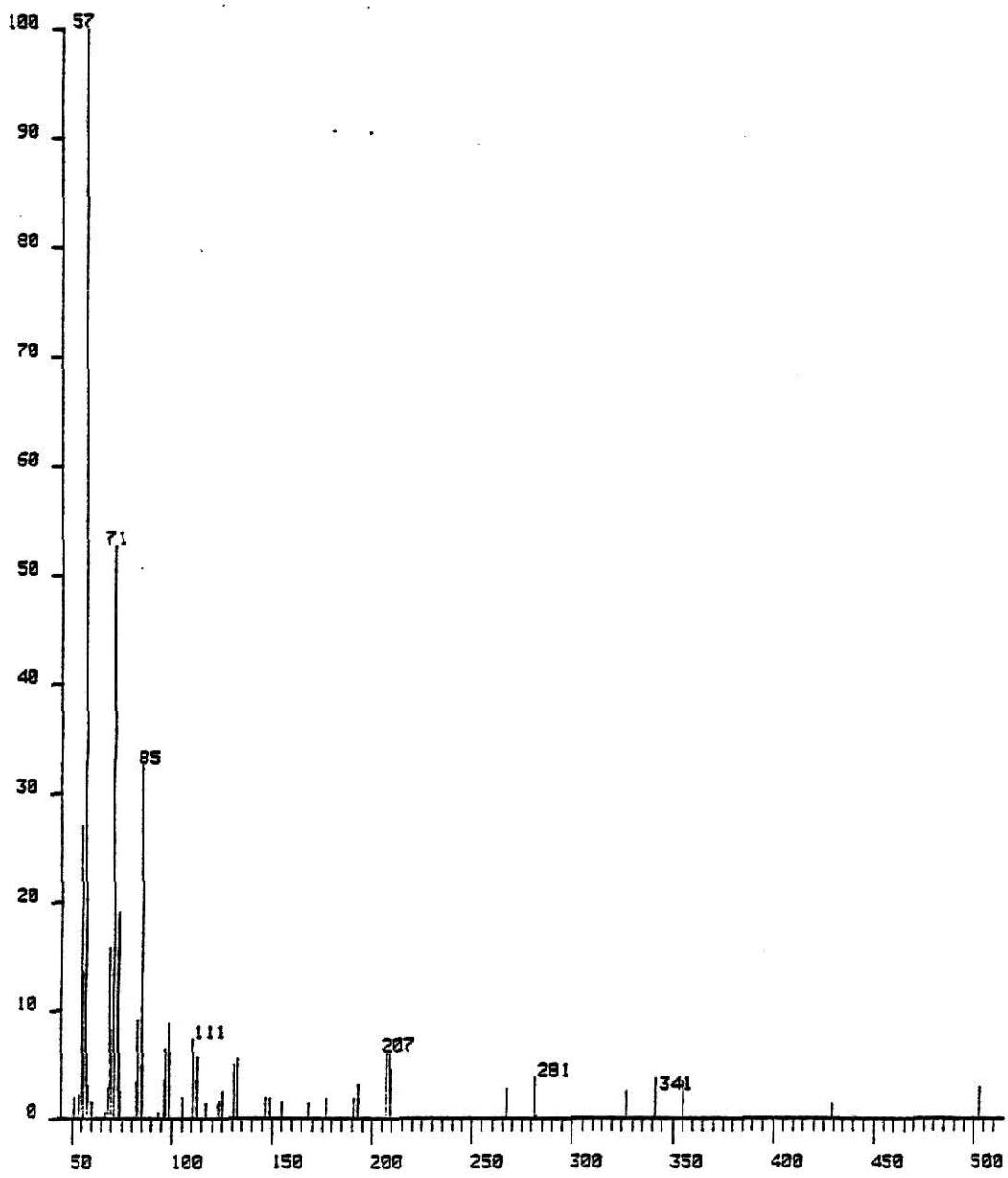


FIGURE 92

Mass spectrum of

Peak 26 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17570.140 [TIC=626, 100%=62] EI

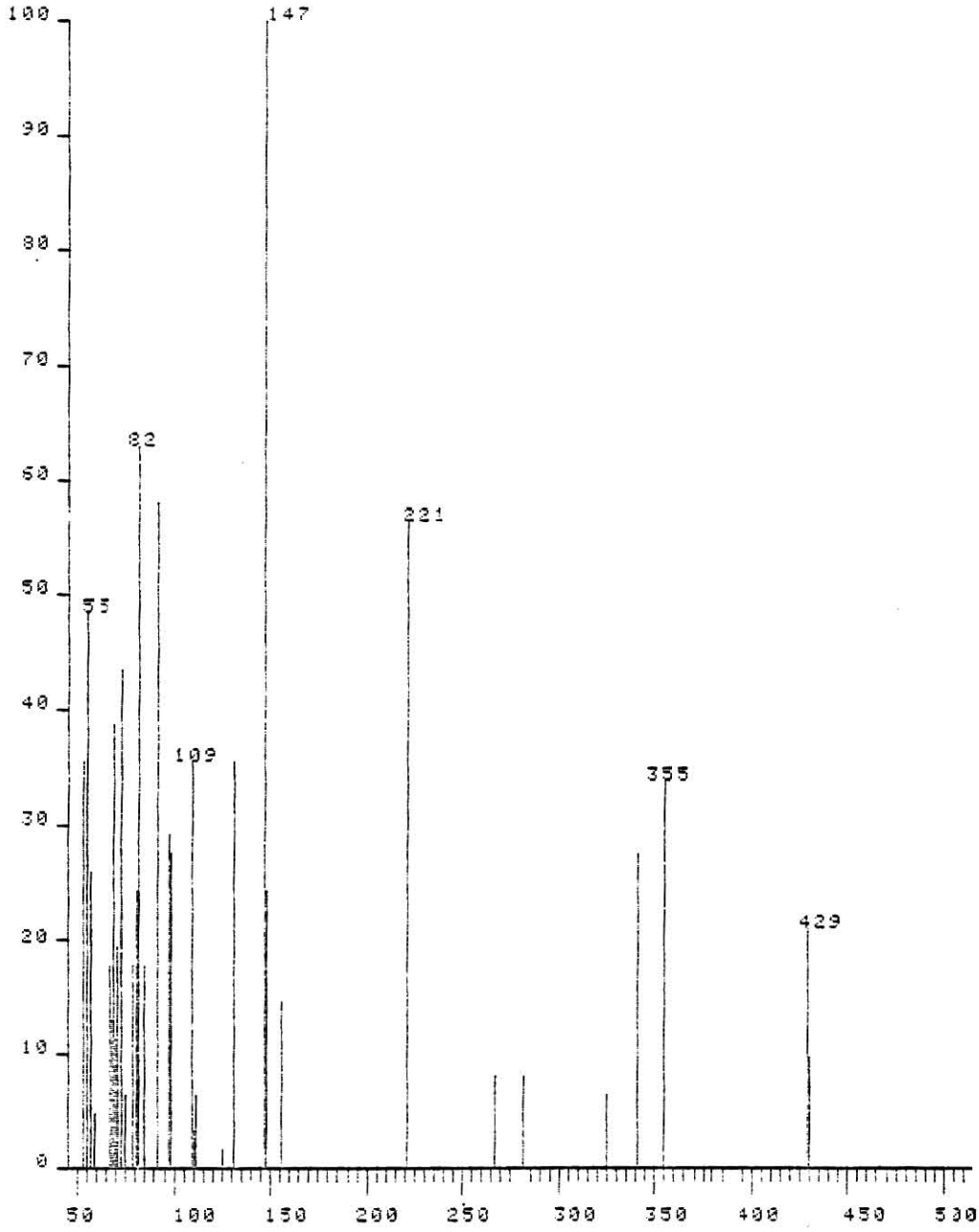


FIGURE 93

Mass spectrum of

Peak 27 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
17570.143 [TIC=875, 100%=1331 EI

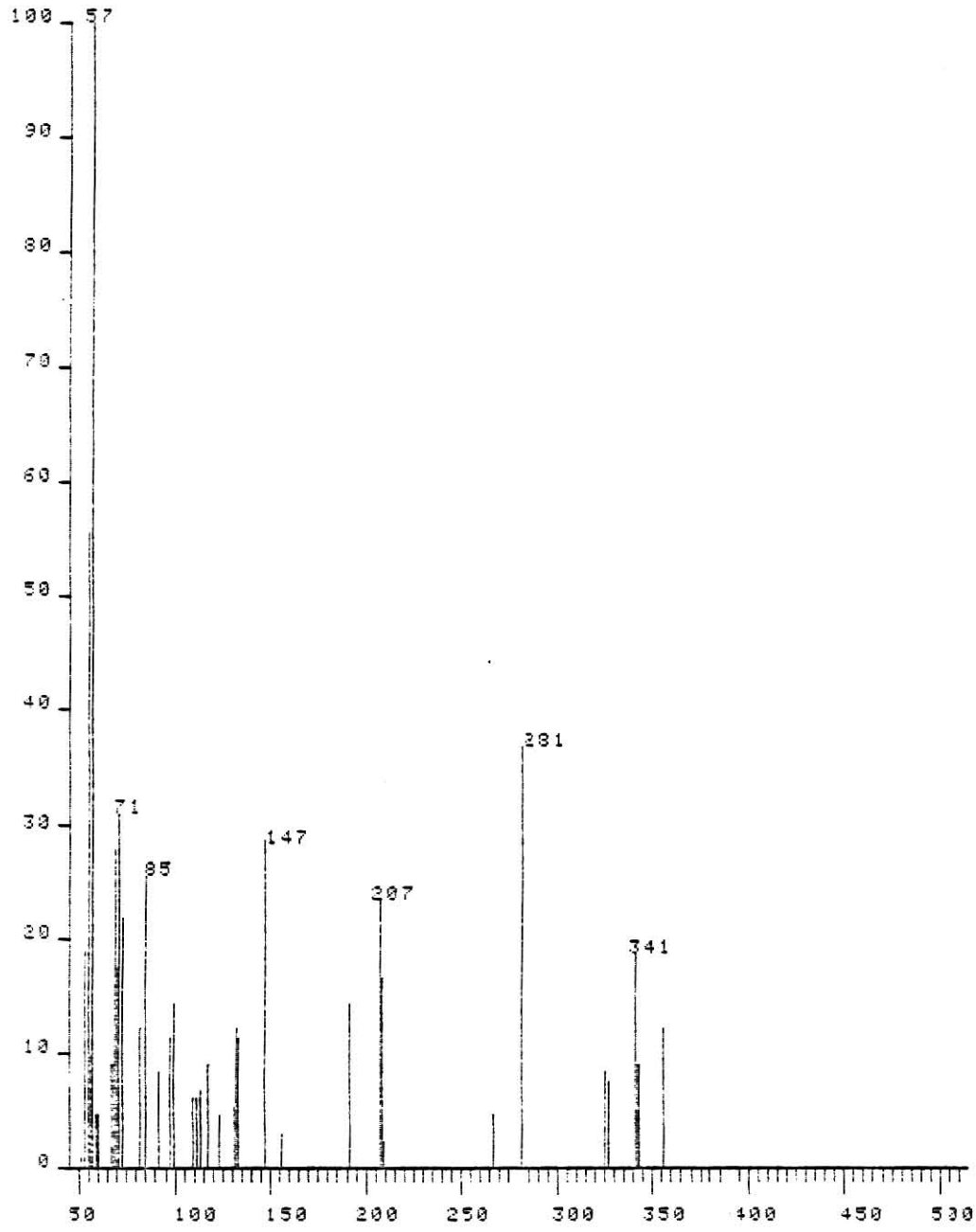


FIGURE 94

Mass spectrum of

Peak 28 of Figure 68

Proposed structure:

straight chain alkane of length at least C_{30}

DS-55 MASS INTENSITY REPORT:
1757CZ.149 CTIC=34266. 100%=7401] EI

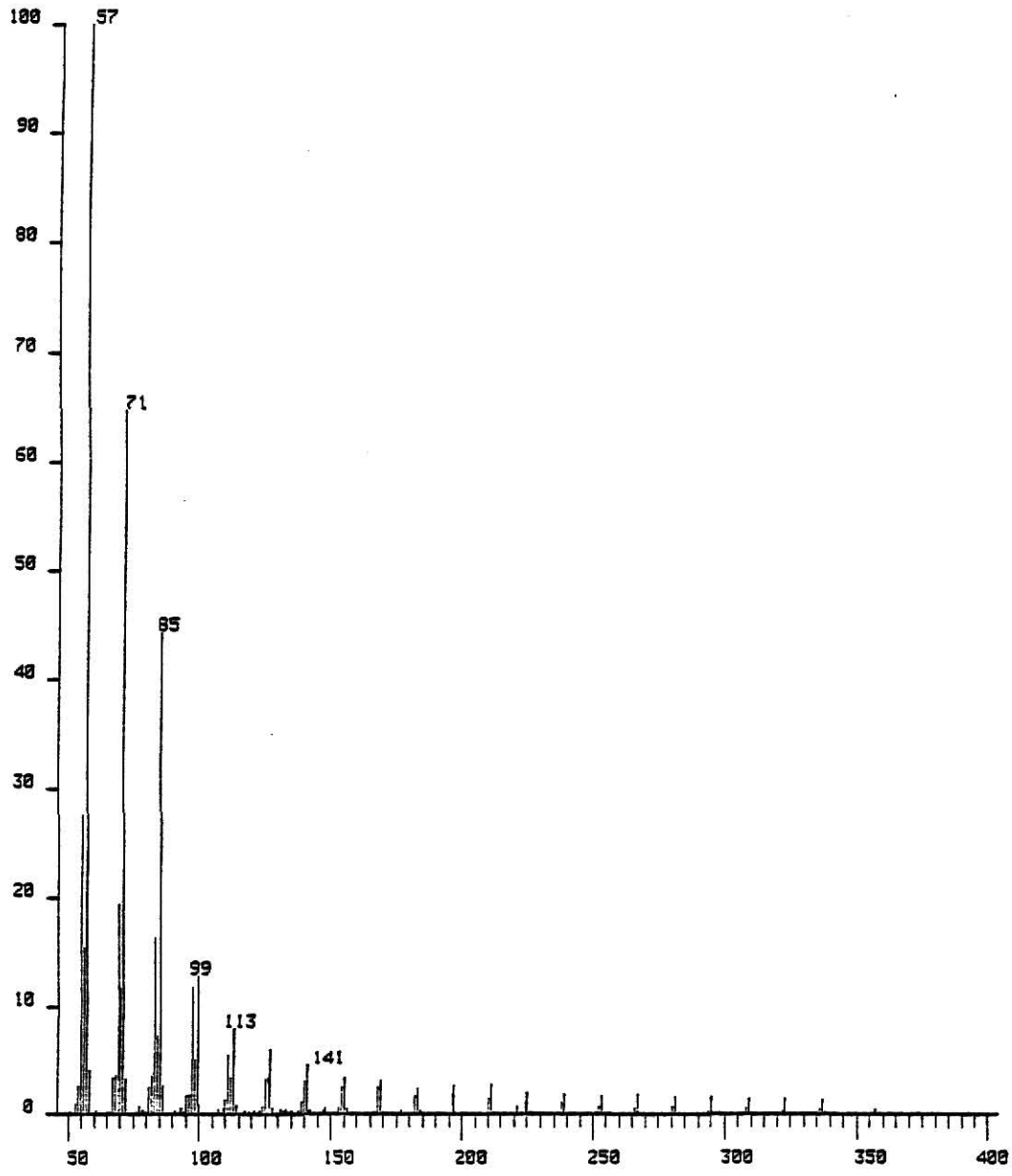


FIGURE 95

Mass spectrum of

Peak 29 of Figure 68

Proposed structure: None

DS-55 MASS INTENSITY REPORT:
1757CZ.156 [TIC=2138. 100%=356] EI

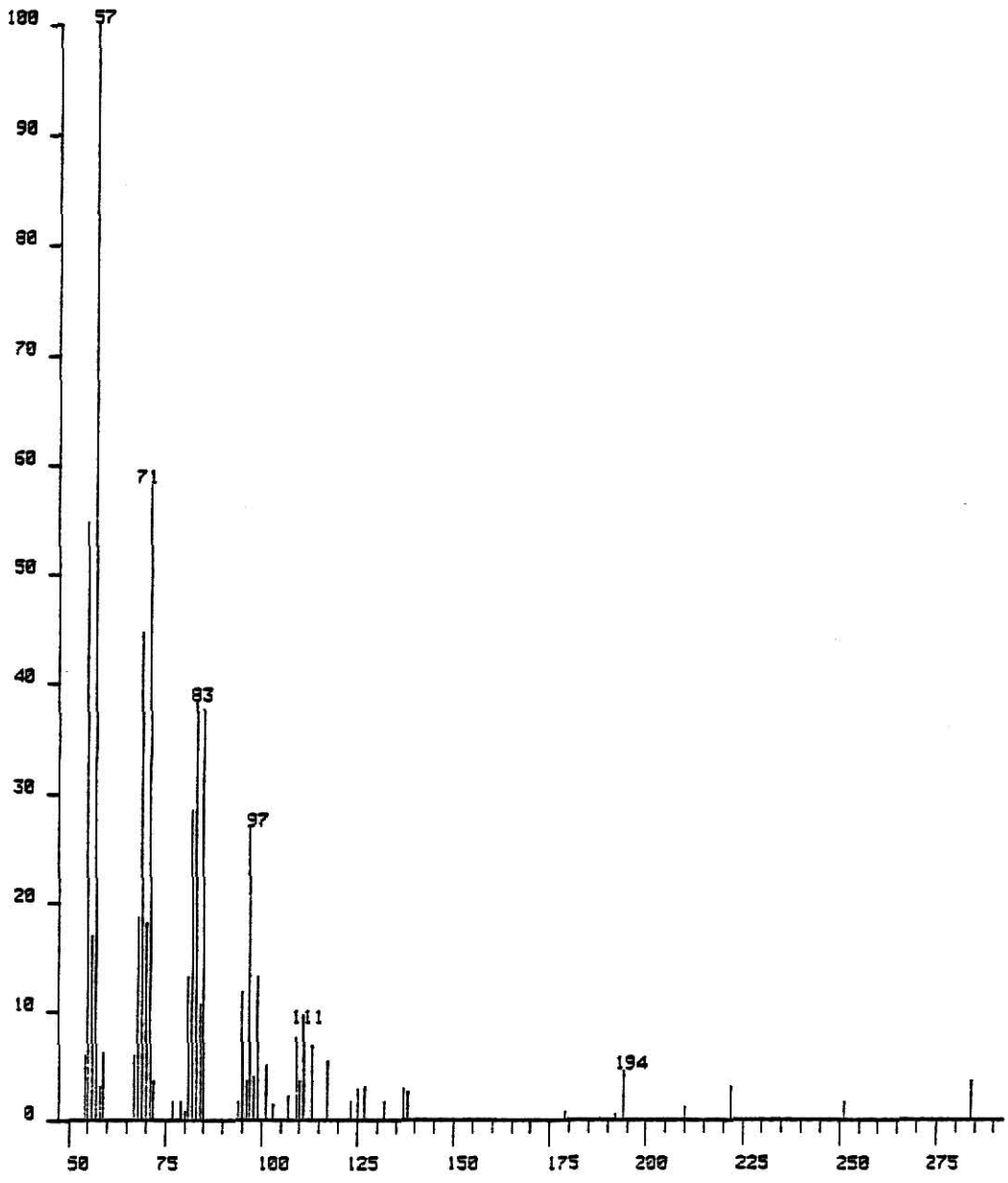


FIGURE 96

Mass spectrum of

Peak 30 of Figure 68

Proposed structure:

branched or straight chain alkene of length at least C_{30}

DS-55 MASS INTENSITY REPORT:

. 1757CZ.160 [TIC=7738, 100%=964] EI

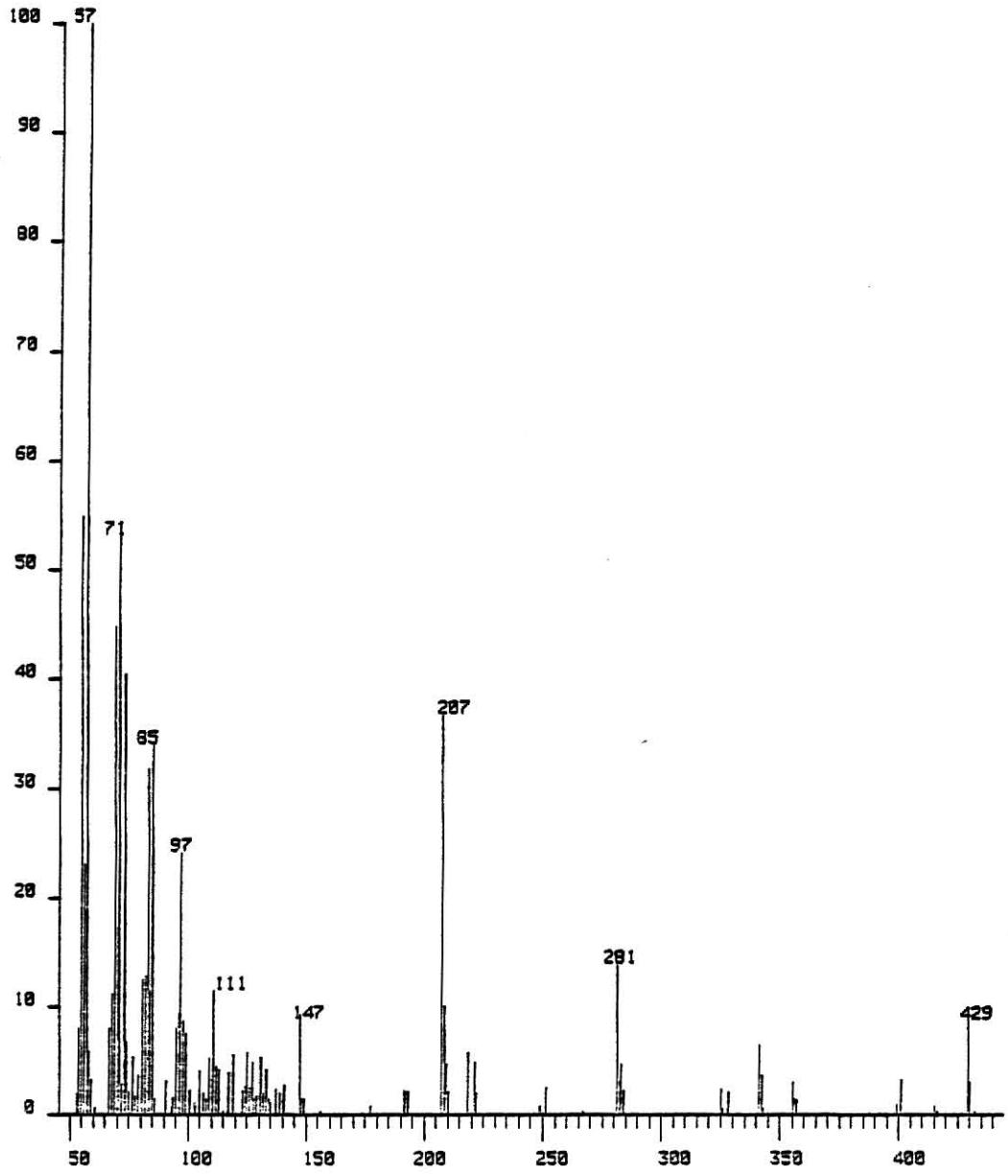


TABLE 1

Compounds Tentatively Identified for Medicago scutellata

(First data set from MCMS)

(Peaks are of Figure 6)

Peak	Spectrum	Compound
1	None	Solvent ($CHCl_3$)
2	Figure 7	Not identified
3	Figure 8	Not identified
4	Figure 9	Omite fragment
5	Figure 10	Phthalate
6	Figure 11	$CH_3(CH_2)_{14}CO_2CH_2CH_3$
7	Figure 12	Phthalate
8	Figure 13	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_2CH_3$
9	Figure 14	Not identified (base m/e 131)
10	Figure 15	Not identified (base m/e 131)
11	Figure 16	$CH_3(CH_2)_9CH{=}CH(CH_2)_7CO_2CH_2CH_3$
12	Figure 17	Phthalate
13	Figure 18	Phthalate
14	Figure 19	Not identified (large m/e 131)
15	Figure 20	Not identified (base m/e 131)
16	Figure 21	Straight chain alkyne or alkyl diene (length C_{26})
17	Figure 22	Straight chain alkane (length at least C_{28})
18	Figure 23	Straight chain or branched alkane (length at least C_{36})
19	Figure 24	$CH_3(CH_2)_7CO_2(CH_2)_6CH{=}CH(CH_2)_{10}CH_3$
20	Figure 25	Straight chain alkane (length at least C_{36})
21	Figure 26	$CH_3(CH_2)_7CO_2C_{21}H_{39}$ (no branching)
22	Figure 27	Branched alkane (length C_{35})

TABLE 1 (continued)

Peak	Spectrum	Compound
23	Figure 28	Ester or straight chain alkane (length at least C_{33})
24	Figure 29	Not identified

TABLE 2

Compounds Tentatively Identified for Medicago scutellata

(Second data set from MCMS)

(Peaks are of Figure 30)

Peak	Spectrum	Compound
1	None	Solvent ($CHCl_3$)
2	None	Peak appears after venting
3	Figure 31	Not identified
4	Figure 32	Not identified
5	Figure 33	Not identified
6	Figure 34	Omite fragment
7	Figure 35	$CH_3(CH_2)_{14}CO_2CH_2CH_3$
8	Figure 36	Not identified
9	Figure 37	$CH_3(CH_2)_7CH=CH(CH_2)_7CO_2CH_2CH_3$
10	Figure 38	Not identified (large m/e 131)
11	Figure 39	$CH_3(CH_2)_9CH=CH(CH_2)_7CO_2CH_2CH_3$
12	Figure 40	Not identified (base m/e 131)
13	Figure 41	Not identified (base m/e 131)
14	Figure 42	Phthalate
15	Figure 43	Not identified
16	Figure 44	Not identified (base m/e 131)
17	Figure 45	$CH_3(CH_2)_2CH=CH(CH_2)_4CH=CH(CH_2)_5CO_2H$
18	Figure 46	Not identified
19	Figure 47	Not identified
20	Figure 48	Straight chain alkane (length at least C_{36})
21	Figure 49	$CH_3(CH_2)_7CO_2(CH_2)_6CH=CH(CH_2)_{10}CH_3$
22	Figure 50	Straight chain alkane (length at least C_{36})

TABLE 2 (continued)

Peak	Spectrum	Compound
23	Figure 51	$CH_3(CH_2)_7CO_2C_{21}H_{39}$ (no branching)
24	Figure 52	Not identified

TABLE 3

Compounds Tentatively Identified for Medicago scutellata

(Data set from MRI)

(Peaks are of Figure 53)

Peak	Spectrum	Compound
1	Figure 54	$CH_3CH{=}CH(CH_2)_9CHO$
2	Figure 55	$ClC_6H_4COC_6H_4Cl$
3	Figure 56	$ClC_6H_4COC_6H_4Cl$
4	Figure 57	$CH_3(CH_2)_{14}CO_2CH_2CH_3$
5	Figure 58	$CH_3(CH_2)_{16}CHO$
6	Figure 59	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_2CH_3$
7	Figure 60	$CH_3(CH_2)_{18}CHO$
8	Figure 61	$CH_3(CH_2)_7CH{=}CH(CH_2)_6CO_2(CH_2)_2CH_3$
9	Figure 62	Not identified (base m/e 131)
10	Figure 63	Not identified (base m/e 131)
11	Figure 64	Phthalate
12	Figure 65	Not identified (base m/e 131)
13	Figure 66	$CH_3(CH_2)_2CH{=}CH(CH_2)_4CH{=}CH(CH_2)_5CO_2H$
14	Figure 67	Not identified

TABLE 4

Compounds Tentatively Identified for Medicago sativa L. subsp. praefalcata

(Data from MCMS)

(Peaks are of Figure 68)

Peak	Spectrum	Compound
1	None	Solvent ($CHCl_3$)
2	None	Peak appears after venting
3	Figure 69	Phthalate
4	Figure 70	Not identified
5	Figure 71	Not identified
6	Figure 72	Not identified
7	Figure 73	Not identified
8	Figure 74	Omite fragment
9	Figure 75	Phthalate
10	Figure 76	$CH_3(CH_2)_{14}CO_2CH_2CH_3$
11	Figure 77	Not identified
12	Figure 78	Not identified
13	Figure 79	Not identified
14	Figure 80	Not identified
15	Figure 81	Not identified (base m/e 131)
16	Figure 82	Phthalate
17	Figure 83	Omite fragment
18	Figure 84	Not identified (base m/e 131)
19	Figure 85	Phthalate
20	Figure 86	Not identified (base m/e 131)
21	Figure 87	Not identified (base m/e 131)
22	Figure 88	Not identified

TABLE 4 (continued)

Peak	Spectrum	Compound
23	Figure 89	Not identified
24	Figure 90	Not identified
25	Figure 91	Straight chain alkane (length at least C_{30})
26	Figure 92	Not identified
27	Figure 93	Not identified
28	Figure 94	Straight chain alkane (length at least C_{30})
29	Figure 95	Not identified
30	Figure 96	Straight chain or branched alkene (length at least C_{30})

DISCUSSION OF RESULTS

The three different sets of data from the GC/MS runs of M. scutellata exudate sample have made structural proposals easier by combining their spectral information. The data from the GC/MS runs made at MCMS in Lincoln, Nebraska especially complemented each other. Some peaks that were well separated with one set of conditions were not with the second set of conditions, and visa versa. By combining the spectral information of the peaks with similar retention times, a structure could usually be proposed, while with only one set of data, there was not enough information. Most of the tentative structures proposed for the components of the M. sativa L. subsp. praefalcata exudate sample could not have been made without the aid of the spectral information taken from the M. scutellata exudate sample.

The spectra obtained from the first GC/MS run of M. scutellata exudate sample taken at MCMS (Figures 7-29) match very well with the spectra of the second GC/MS set (Figures 31-52). However, not as many of these spectra are common to those obtained with M. scutellata exudate sample at MRI (Figures 54-67). Several proposals will be made here as to why these differences have occurred:

1) A different GC column was used at MRI than at MCMS. A 3% OV-1 on 80/100 Supelcoport, 1/4 inch O.D. by 6 foot length glass column was used at MRI while a 3% OV-101 on Chromosorb W AWDMCS, 1/8 inch by 6 foot length stainless steel column was used at MCMS. However, since both phases consist of methyl silicon, this would cause very minor differences.

2) The temperature programs were different as this program was used at MRI:

150°C hold 5 minutes

150-300C at 15°/min.

300°C hold 10 minutes

and this program was used at MCMS:

150°C hold 2 minutes

150-300°C at 10°/min.

300°C hold 3 minutes

But once again, this should cause only minor differences in the results. Retention times should be slightly different. The peaks of the MCMS chromatogram may have slightly better resolution due to the slower program rate.

3) The samples were in different solvents. The samples run at MRI were in benzene and the samples run at MCMS were in $CHCl_3$. This could account for the spectra obtained in the MRI data which appeared to be from $ClC_6H_4COC_6H_4Cl$. The benzene may have dissolved the insecticides in the exudate sample to form these isomers.

4) The samples run at MRI were collected several months before the samples run at MCMS. More Pentac was possibly contained in the MRI sample because Pentac was replaced by Omite in the greenhouse after the MRI sample was collected and before the MCMS sample was collected. Possibly the Pentac may have dissolved in the solvent (benzene) along with other insecticides to form the $ClC_6H_4COC_6H_4Cl$.

5) The fact that Omite was being applied to the M. scutellata plants when the

MCMS sample was collected and not when the MRI sample was collected may account for some differences. The peak due to Omite of retention time 6:32 (Figure 9) found in the MCMS data may be masking the aldehyde of retention time 5:57 (Figure 54) of the MRI data. Or a peak possibly due to more Omite fragments of retention time 5:27 (Figure 8) may be masking the aldehyde. However, Figure 8 is so weak in intensity that it is impossible to determine if it is indeed due to Omite fragments.

6) Many more phthalates were proposed from the MCMS data than the MRI data. The phthalate seen at retention time 6:59 (Figure 10) or the one of retention time 8:06 (Figure 12) of the MCMS data could be masking the aldehyde seen at retention time 8:06 (Figure 58) of the MRI data. There is an alkene pattern present in Figure 12, similar to the one in Figure 58.

7) There also seem to be more peaks containing base m/e 131's in their spectra in the MCMS data than in the MRI data. From the MRI data, there is an aldehyde at retention time 10:12 (Figure 60) while in the MCMS data, at retention times 9:36 and 10:08, there appear spectra with base peaks at m/e 131 (Figures 14 and 15 respectfully). If the latter two spectra are of column bleed, one of them could be masking the aldehyde. Figure 14 does have these peaks in common with Figure 60: m/e 57, 68, 71, 82, and 96. The ethyl ester proposed from the MCMS data at retention time 10:48 (Figure 16) may have been masked by the peak of the MRI data of retention time 11:48 giving a base m/e 131 peak in its spectrum (Figure 62).

8) The exudate samples used at MRI and MCMS may have varied in concentrations. Because the exudate on the glass rod is not visible to the naked eye during collection, it is impossible to determine the concentration of the exudate sample. However, this does not seem to explain the matter as some compounds proposed from the MRI data were not from the MCMS and visa versa.

The compounds proposed that were common to all three sets of M. scutellata exudate sample GC/MS data are $CH_3(CH_2)_{14}CO_2CH_2CH_3$ and $CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_2CH_3$, and $CH_3(CH_2)_2CH{=}CH(CH_2)_4CH{=}CH(CH_2)_5CO_2H$ was proposed from Figure 45 (MCMS) and Figure 66 (MRI). Only ethyl oleate was positively identified as none of the other compounds was commercially available for testing. By combining the data obtained from all three GC/MS runs of the M. scutellata exudate sample, the compounds listed in Tables 1, 2, and 3 have been tentatively identified as being components of the exudate.

The proposals made from the MCMS data are more certain than those made from the MRI data for the following reasons:

1) The MCMS data was taken more recently. Therefore, techniques in collecting the sample and preparing it for GC work were improved from when the MRI sample was collected and prepared.

2) Because the MCMS GC/MS data was taken with a slower temperature program (10°/min.) than at MRI (15°/min.) the separation of components was better at MCMS.

3) Data from two GC/MS runs of the M. scutellata exudate sample were taken at MCMS while only one set of data was taken at MRI. Therefore, the reproduced spectra from the first GC/MS run to the second at MCMS support the proposed structures made from these spectra.

The concentration of M. sativa L. subsp. praefalcata exudate in the exudate sample itself seemed to be quite low as many of the spectra of the GC/MS run (Figures 69-96) were very weak. This arises from the fact that the M. sativa L. subsp. praefalcata plant is not nearly as robust as M. scutellata and does not give off as much exudate so only a small amount of the M. sativa L. subsp. praefalcata exudate was collected. However, when the

spectra from the M. scutellata exudate sample GC/MS runs (Figures 7-67) are compared with the M. sativa L. subsp. praefalcata exudate sample spectra (Figures 69-96), these compounds are proposed as being common in the two exudate samples, as seen in Table 4: $CH_3(CH_2)_{14}CO_2CH_2CH_3$ and two straight chain alkanes of high molecular weight. One straight chain alkane appears to be present in much greater concentration than the rest of the exudate components. This exudate sample also produced spectra containing base m/e 131's. If this is due to column destruction, there does not appear to be as much of it in this GC/MS run as in the M. scutellata GC/MS runs. This may also be due to the small concentration of exudate in the sample. The exudate was actually in too small a concentration to determine if more compounds were common to the M. scutellata exudate components.

Summary

The types of compounds found in the exudate samples of Medicago scutellata and Medicago sativa L. subsp. praefalcata are straight chain esters (length C_{17} and greater), straight chain alkanes (C_{30} and greater), straight chain aldehydes (length C_{12} and greater), and a straight chain acid (C_{17}). All of these compounds are large molecules and are non-volatile. All contribute to the viscous nature of the exudate. From the results obtained, it appears that the weevil is totally immobilized by the sticky exudate and death most likely does not occur due to toxic components in the exudate.

FUTURE WORK

The major difficulty with this project right now is in determining what is causing the spectra with base peaks at m/e 131. The hump or background gives this type of spectrum. Suggested future work would include using a GC column packing that does not contain silicon. If the problem is not cured this way, it is possible that a very high molecular weight substance is breaking down, creating the spectra with base m/e 131's. A mass spectrograph could be obtained of the exudate which would indicate if a tremendously high molecular weight component is present. Also, the technique of capillary GC could be employed for better separation of the exudate components. If this is successful, capillary GC/MS could then be done.

When the hump is eliminated and better GC resolution of the exudate components is achieved, several steps can be taken to identify the compounds positively. The individual peaks could be collected by preparative GC and then NMR used for the determination of double bonds, etc. Also, mass spectrographs could be taken of the individual peaks collected. High resolution GC/MS could be run on the exudate sample (without collecting peaks) if the peaks were well resolved.

The tentatively identified compounds could be synthesized and their retention times compared or a greater search for these compounds already made could be done.

The technique of HPLC could be employed, using a refractive index detector.

REFERENCES

1. E. L. Eshbaugh, E. L. Sorensen, and W. A. Moore, "Two Ways to Fight Alfalfa Weevils", AES Keeping Up with Research, Ag. Exp. Station, Kansas State Univ., Manhattan, Kansas, 14,(1975).

2. K. J. R. Johnson, E. L. Sorensen, and E. K. Horber, Crop Sci., 20, 631(1980).

3. E. K. Horber, E. L. Sorensen, and K. J. R. Johnson, Report of the 27th Alfalfa Improvement Conference, in press.

4. K. J. R. Johnson, E. L. Sorensen, and E. K. Horber, Environ. Entomol., 9, 133(1980).

5. K. J. R. Johnson, E. L. Sorensen, and E. K. Horber, Environ. Entomol., 9, 241(1980).

6. K. J. R. Johnson, E. L. Sorensen, and E. K. Horber, Environ. Entomol., in press.

7. R. E. Shade, T. E. Thompson, and W. R. Campbell, J. Econ. Entomol., 68, 339(1975).

8. W. R. Campbell, R. Shade, and T. Thompson, unpublished results.

9. G. L. Kreitner and E. L. Sorensen, Crop Sci., 19, 380(1979).

10. G. L. Kreitner and E. L. Sorensen, Crop Sci., 19, 499(1979).

11. R. B. Othman, E. L. Sorensen, G. H. Liang, and E. K. Horber, Bot. Gazette, in press.

12. G. H. Liang and E. L. Sorensen, J. Submicro. Cytol., 10, 429(1978).

13. H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds", Holden Day, Inc., San Francisco, 1964.

14. J. H. Beynon, "Mass Spectrometry and its Applications to Organic Chemistry", Elsevier Publishing Co., New York, 1960.

15. R. J. Reed, "Applications of Mass Spectrometry to Organic Chemistry", Academic Press, New York, 1966.

16. F. W. McLafferty, "Interpretation of Mass Spectra", W. A. Benjamin, Inc., New York, 1967.

17. R. M. Silverstein, G. C. Bassler, and T. C. Morrill, "Spectrometric Identification of Organic Compounds", John Wiley and Sons, Inc., New York, 1974.

18. "Atlas of Mass Spectral Data", E. Stenhagen, et. al., eds., Vol. 3, Interscience Publishers, New York, 1969.

19. J. C. Uphof, "Plant Hairs", Gebruder Borntraeger, Berlin, 1962.

20. "The Merck Index", M. Windholz, et. al., eds., Ninth ed., Merck and Co., Inc., Rahway, 1976.

21. S. Safe and O. Hutzinger, "Mass Spectrometry of Pesticides and Pollutants", CRC Press, Inc., Cleveland, 1973.

22. "Biochemical Applications of Mass Spectrometry", G. R. Waller, ed., John Wiley and Sons, Inc., New York, 1972.

ACKNOWLEDGEMENTS

I would like to thank the Kansas State Agricultural Experiment Station, under the direction of Floyd Smith, for its financial support of this project.

I would like to acknowledge the people at the Midwest Research Institute in Kansas City, Missouri for the use of their GC/MS system and for their help with spectral interpretation.

I would also like to thank the people at the Midwest Center for Mass Spectrometry at Lincoln, Nebraska for the use of their GC/MS system and especially Dr. Frank Crow for his help with spectral interpretation.

Dr. E. L. Sorensen of the Agronomy Department was most helpful in providing the plants and space in the greenhouse for exudate collection, and in giving his advice concerning this project.

I would like to express my appreciation to my research director Professor Clifton E. Meloan for his guidance and support throughout this project.

I would like to thank my parents for their support and encouragement throughout the years. And I would especially like to thank my husband Fred for his love, patience, and support throughout this project.

VITA

The author was born in Hutchinson, Kansas on January 22, 1956. She attended Hutchinson High School in Hutchinson, Kansas, from which she graduated in May of 1974. She then attended Kansas State University and received her B. S. degree in May of 1978. The author entered the graduate school at Kansas State University in August of 1978.

The author was married to Frederick M. Triebe in March of 1978, in Hutchinson, Kansas.

MASS SPECTRAL EXAMINATION OF THE EXUDATES OF ERECT GLANDULAR PLANT HAIRS (*MEDICAGO SCUTELLATA* AND *MEDICAGO SATIVA* L. *SUBSP. PRAEFALCATA*)

by

Donna Carol Triebe

B. S., Kansas State University

Manhattan, Kansas, 1978

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1981

ABSTRACT

The alfalfa weevil [Hypera postica (Cyllenhaul)] is a serious pest attacking alfalfa (Medicago sativa L.) in Kansas and infested 95% of the acreage in 1974. Certain annual Medicago species are resistant to the weevil. The annual Medicago scutellata L. (Mill) and perennial M. sativa L. subsp. praefalcata were studied. Their plant surfaces are covered with erect glandular hairs that secrete an exudate which either immobilizes the weevil, causing death, or is toxic to it. Tentative chemical identification of the components in the exudate was made through the technique of gas chromatography/mass spectrometry.

The exudates (collected with an etched glass rod rolled along the plant stem) were dissolved in $CHCl_3$ and the samples were condensed. The M. scutellata exudate sample was separated into 24 components with the aid of a gas chromatograph and the mass spectrum of each peak was taken with the mass spectrometer. Compounds tentatively identified include straight chain esters, alkanes, aldehydes, and an acid, all of molecular weight 200 or greater. Ethyl oleate was positively identified as a component. The exudate from the perennial M. sativa L. subsp. praefalcata was also analyzed and comparisons made with M. scutellata exudate data. One ester and two alkanes were compounds found to be possibly common to both exudates.