# NEW NATURALLY OCCURRING COMPOUNDS FROM PEANUTS (*ARACHIS HYPOGAEA* L.)

By

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NEW NATURALLY OCCURRING COMPOUNDS FROM

PEANUTS (ARACHIS HYPOGAEA L.)

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# CHAPTER I

## INTRODUCTION

Little work has been done on basic nitrogen containing compounds, particularly alkaloids, in the fruit and vines of Arachis hypogaea L. (Family Papilionaceae), more commonly known as the peanut plant.

The research described herein was initiated as a result of a preliminary finding by C. Young of a Dragendorff positive reaction of an extract of raw peanuts (1). The Dragendorff reagent is usually considered a specific test for alkaloids, however, certain other nitrogen containing compounds, and in some cases, neutral compounds, also give positive tests (2).

This thesis outlines the systematic analysis of basic and neutral compounds obtained by methanol extraction and steam distillation, respectively, from the fruit and aerial vegetative parts of Arachis hypogaea L.

# CHAPTER II

# LITERATURE REVIEW

## Compounds Isolated from Peanuts

In 1904 Mooser (3) found what he believed was an alkaloid in Arachis hypogaea. He deduced the empirical formula to be $C_5H_{13}N_2O$ (molecular weight 117) and determined the melting points of several of its salts. Fifty-seven years later, Moll (4) showed that the supposed alkaloid was actually an impure preparation of choline.

In more recent years, carbonyls isolated as the 2,4-dinitrophenylhydrazones (5) and pyrazines (6, 7) have been isolated from the roasted peanut. Most of the compounds appeared to be formed or released during roasting. Sugars and amino acids in the raw peanut have been postulated to be the precursors of the pyrazines formed (8).

Pattee et al. (9) examined the volatile components of raw peanuts and found ten compounds: pentane, methyl formate, octane, acetaldehyde, 2-butanone, acetone, methanol, ethanol, pentanal and haxanal.

Lefort et al. (10) found that extraction of peanut oil with methanol gave an extract that on subsequent distillation revealed n-hexanol. A direct steam distillation of the oil gave predominantly 2,4-decadienal.

Lee et al. (11) isolated and identified N-methylhydroxy-proline from the alcohol soluble portion of defatted peanut flour.

Moudgal et al. (12) have shown that a glycoside isolated from the red skin covering peanuts is goitrogenic not only because it interferes with the uptake of inorganic iodide but also because it interferes with substitution of iodine into the tyrosine molecule.

A comparison of those plants closely related botanically to the peanut reveals a diversity of volatile and basic, nitrogen containing compounds, including alkaloids. Table I lists classes of compounds that have been isolated from some of the members of the family Papilionaceae, order Leguminosae (13).

## Alkaloids and Other Basic Compounds in Papilionaceae

Basic, nitrogen containing compounds can be subdivided into alkaloids and non-alkaloid compounds. The term "alkaloid", which means "alkali-like", refers to those basic compounds of plant origin containing nitrogen which are usually heterocyclic. These compounds almost always have a significant pharmacological activity and a complex molecular structure. The non-alkaloid compounds would include simple, open-chain alkylamines, choline and betaine (14).

Several alkaloids have been isolated from the plant family Papilionaceae. For example, a derivative of nicotinic acid, trigonelline, was isolated from the basic fraction of a hot ethanolic extraction of pea seeds by Hammouda and Ahmed (15). Separation of trigonelline from the other extracted compounds was achieved by paper chromatography. Sparteine and lupanine have been isolated from the Scotch Broom by various groups (16, 17). Again, as in the case of peas, extraction was by ethanol and separation by paper chromatography.

TABLE I

CLASSES OF COMPOUNDS EXTRACTED FROM SEVERAL MEMBERS OF THE PAPILIONACEAE

| Common Name | Scientific Name | Classes of Compounds Extracted |
|---|---|---|
| Broad Bean | *Vicia faba* L. | Alcohols and carbonyls (18); sulfur compounds (19) |
| Calabar Bean | *Physostigma venenosum* Balf. | Alkaloids (20, 21) |
| Clover | *Trifolium pratense* L. | Glycosides (22), carbonyls (23); alcohols (23, 24) sugars (25) |
| Scotch Broom | *Cystisus scoparius* Link | Alkaloids and amines (17); sugars (25) |
| Gum Tragacanth | *Astragalus gummifer* Lab. | Alkaloids (26) |
| Liquorice Root | *Glycyrrhiza glabra* L. | Waxes and sugars (27); triterpene acids (28) |
| Peanut | *Arachis hypogaea* L. | Pyrazines (6); hydrocarbons, alcohols, carbonyls, acids and esters (9) |
| Peas | *Pisum sativum* L. | Alkaloids and sugars (15); carbonyls (23, 30); hydrocarbons (29); acetals, esters and sulfur compounds (30); alcohols (30, 31); pyrazines (32) |

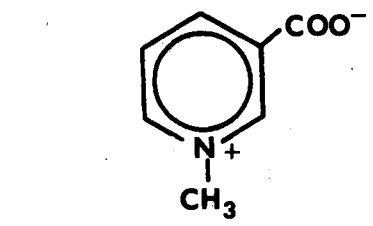
Physostigmine is present in the basic residue remaining after industrial extraction of Calabar beans (20). Robinson (21) achieved separation of physostigmine from two other alkaloids by adsorption chromatography and proposed a structure for physovenine based upon evidence from the comparison of its ultraviolet, infrared and nuclear magnetic resonance spectra with those of physostigmine. The structure of several alkaloids from the family Papilionaceae are shown in Figure 1.

It is interesting to note that Zolotnitskaya et al. (26) found that an alkaloid extracted from Gum Tragacanth had a bactericidal effect in a 1:10,000 dilution. The alkaloid was tested against Staph. aureus and E. coli. However, the structure of the compound has not been determined.

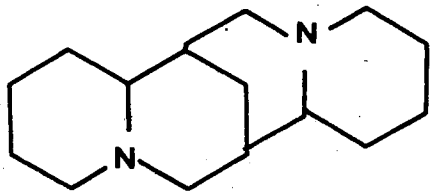
## Volatile Compounds Present in Papilionaceae

A great deal of work has been done on the volatiles contained in peas. Hall et al. (33) first investigated the volatile constituents of peas in 1950 and found evidence of primary amines, aliphatic aldehydes, methyl ketones and alcohols in the steam distillate. Murray et al. (31) isolated thirteen saturated and nine unsaturated alcohols from unblanched frozen peas. Ralls et al. (30) isolated alcohols, aldehydes and esters from the steam of a commercial pea blancher. The last two groups used gas-liquid chromatography and combined gas-liquid chromatography—mass spectrometry analysis after collection of the volatiles by either vacuum or steam distillation.

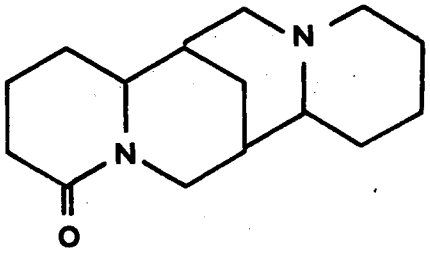
In recent years the volatiles present in clover have been of interest because they can impart an "off-flavor" in the milk of dairy



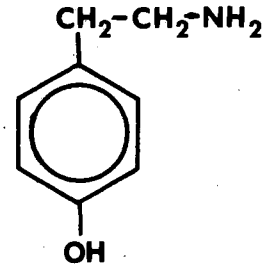

Trigonelline



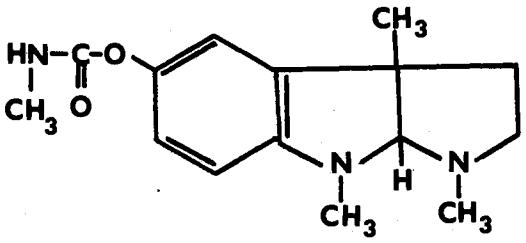

Sparteine



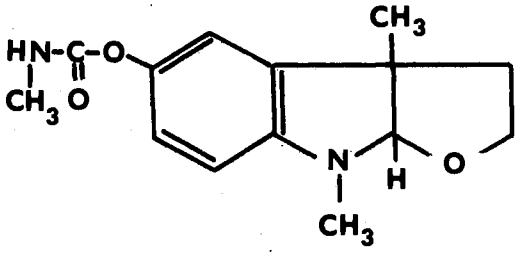

Lupanine




Tyramine




Physostigmine




Physovenine

Figure 1. Structures of Several Alkaloids and Alkaloid-Like Compounds Found in the Family Papilionaceae.

cattle. Woods and Aurand (23) were able to extract ethanal, propanal, acetone, 2-methyl propanal, butanal, butanone, 3-methylbutanal and pentanal by vacuum distillation of Ladino clover. Honkanen and Mosio (24) have also shown the presence of oct-1-en-3-ol in clover where its highest concentration (about 20 mg/kg) was found in the flowers.

# CHAPTER III

## EXPERIMENTAL METHODS

### A. Materials and Chemicals Used

#### 1. Plants

Peanut plants of the Spanish variety were grown at the Perkins Agronomy Research Station and received no insecticidal treatment. Harvesting began in early October, 1971 and ended in early November, 1971 with the first frost. All plants used were either extracted or frozen within six hours of harvesting. Only the aerial parts of the plants were used. Some of the plants used were grown in greenhouses and donated by the Department of Agronomy, Oklahoma State University.

#### 2. Nuts

One hundred pounds of Spanish peanuts, 1970 crop, were purchased from Gold Kist Peanut Growers, Durant, Oklahoma.

#### 3. Reagents

Hexyl alcohol was obtained from Eastman Organic Chemicals, Rochester, New York. $\alpha$-Terpineol, geraniol and 1-pentene-3-ol were obtained from Chemicals Procurement Laboratories, College Point, New York.

Gas chromatography packing material, Carbowax 20M, OV-61, and OV-1 were obtained either from Applied Science Laboratories, Inc., State College, Pennsylvania, or from Analabs, Inc., Hamden, Connecticul. Commercially prepared analytical and preparative silica gel GF plates were purchased from Brinkmann Instruments, Westbury, New York and Analtech, Inc., Neward, Delaware.

All other solvents and chemical reagents were analytical reagent grade unless otherwise specified.

## B. Extraction Methods

### 1. Extraction of Basic Compounds from Raw Peanuts

Methanol Extraction of Raw Peanuts. To extract the peanuts it was first necessary to remove the excess oil and the skins, which contain a red pigment (1). This helped to avoid emulsion problems further on in the extraction procedure. To make the skins easier to remove by hand, the surface oil was removed by extraction with ether for eight hours in a large Soxhlet extractor. Ten pounds of peanuts in 600 g batches were processed in this manner using 1.5 l of ether for each batch. Figure 2 outlines the procedure used.

The nuts were then coarsly ground with a manually cranked meat grinder and extracted with ether for ten hours to remove most of the remaining oil. The de-fatted peanuts were ground in a standard Number 3 Wiley Mill through a 1 mm wire screen. Six-hundred grams of the resulting fat-free peanut meal was then extracted with 1.5 l of methanol for 24 hours in a large Soxhlet extractor. Before being used for the extraction, the Soxhlet was disassembled and cleaned in an acid bath.

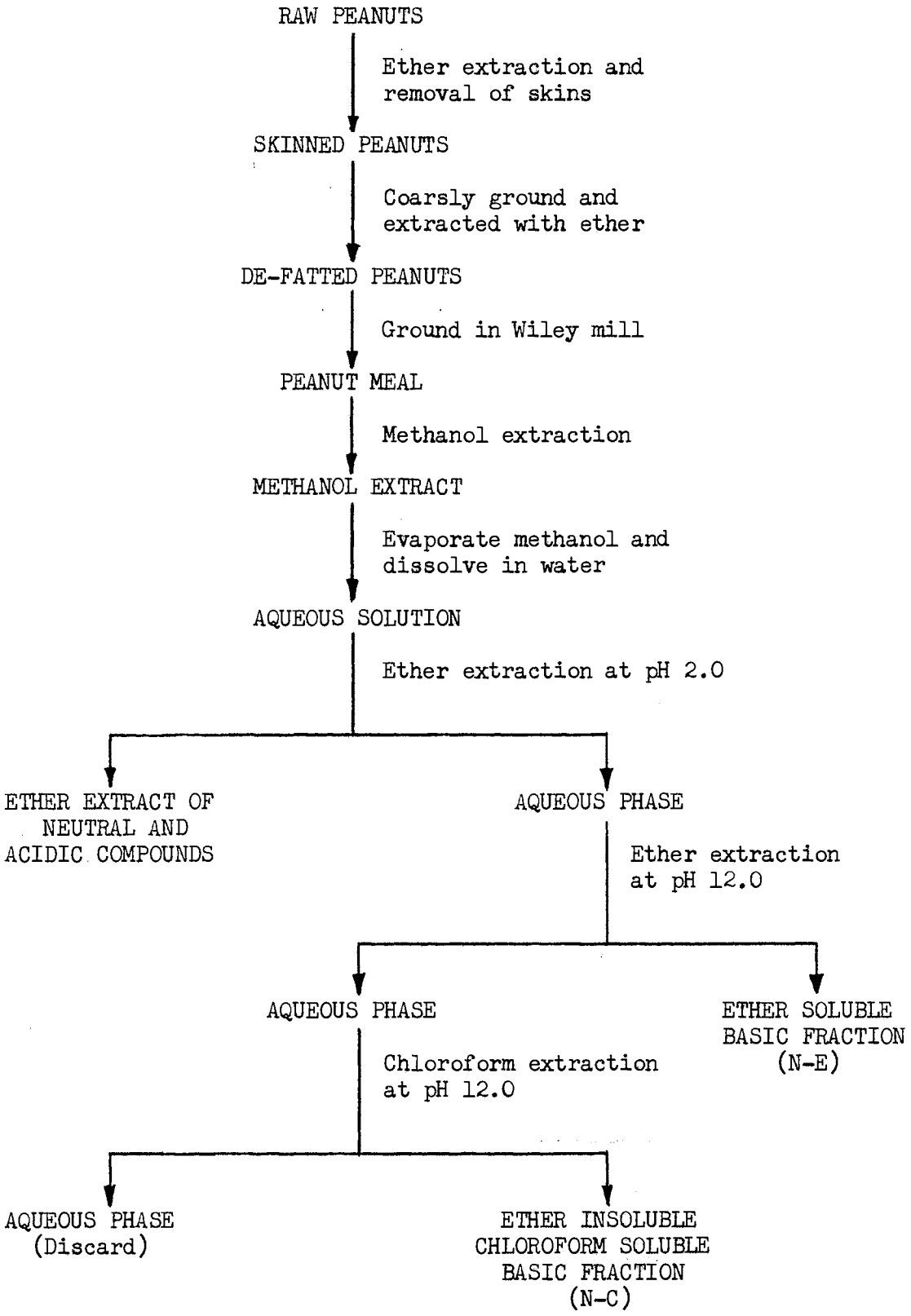



Figure 2. Flow Diagram for the Extraction of Basic Compounds from Raw Peanuts.

Separation of the Basic Fraction from the Methanol Extract. The separation method was adopted from Van Praag et al. (34) and Johnson (35). The methanol solution was reduced on a Buchler rotary evaporator to a gum-like consistancy, and redissolved in 400 ml of distilled water. The acidity was adjusted to pH 2.0 with 2N HCl. The acidified solution (about 500 ml) was placed in a one liter separatory funnel and extracted five times in succession with 100 ml of ether. The ether extracts were combined and reduced on a rotary evaporator to obtain the acid and neutral fractions which were saved for future examination. The aqueous phase was adjusted to pH 12.0 with 2N NaOH and extracted five times in succession with 100 ml of ether. The ether phases were combined and concentrated on a rotary evaporator to a volume of 0.2 ml.

2. Extraction of Basic Compounds from the Peanut Vines

Preliminary Studies. Four methods of extraction were tried on 80 g samples of the aerial parts of fresh peanut plants.

Method I - The fresh plant material was soaked in a ten percent $Ca(OH)_2$ suspension for 12 hours. The slurry was dried until brittle, crushed, and extracted with chloroform in a Soxhlet extractor (36).

Method II - The same procedure as Method I was used except ether was substituted for chloroform in the Soxhlet extraction.

Method III - The plant sample was minced in a Waring blender for two minutes with 300 ml of warm methanol, and filtered on a coarse fritted-glass Buchner funnel. The filtered plant material

was reground and filtered twice more to yield approximately 800 ml of extract and an almost colorless fiber mat.

Method IV - The same procedure as Method III was used except the plant material was extracted with a methanol: 0.5N HCl (10:1, v/v) mixture.

The organic solvent extracts from each of the four different methods were treated in the same manner as the methanol extract of raw peanuts, as shown in Figure 2, to obtain the basic fraction.

Large Scale Extraction. Method III resulted in a high yield of basic compounds and offered convenient solvent preparation, therefore it was used with a few modifications for a large scale extraction as outlined in Figure 3. A total of 10 kg (in 500 g batches) of the aerial parts of fresh peanut plants were minced in warm methanol in a large, four liter Waring blender, Model CB-2, and filtered on a coarse, fritted-glass Buchner funnel. To conserve methanol, the filtered plant material was not reground but simply washed twice with warm methanol. The resulting fiber mat still had a slight green tint.

The separation of the basic extract from the acid and neutral compounds was essentially the same as shown in Figure 2. The methanol extract, totaling 28 l, was evaporated in four liter beakers on a steam table until only a moist green residue remained. However, since the residue was more soluble in a low polarity solvent than water, most of the residue was dissolved with ether. The remaining gum was washed out with distilled water. The resulting 2 l mixture of green colored ether and water was divided into two portions, each portion being processed separately in a 2 l separatory funnel. The mixture was extracted five times in succession with 250 ml of 0.01N HCl. The acid extracts were

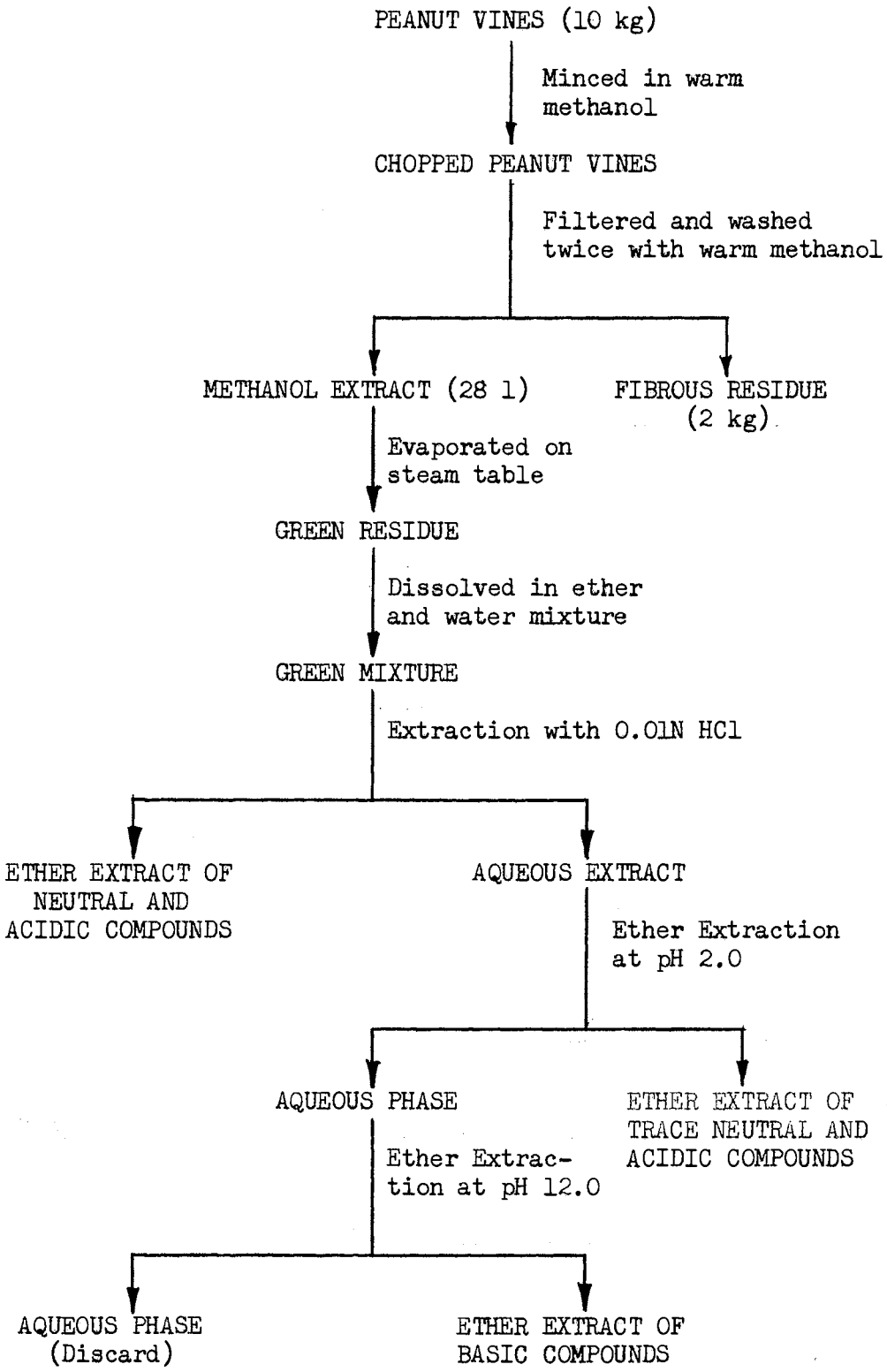



Figure 3. Flow Diagram for the Extraction of Basic Compounds from Peanut Vines

combined, the acidity adjusted to pH 2.0 with 2N HCl, and extracted five times in succession with 200 ml of ether. This was done to remove green coloration due to traces of acidic and neutral compounds. The aqueous solution was adjusted to pH 12.0 with 2N NaOH and extracted five times in succession with 200 ml of ether to yield the basic fraction which was reduced to one ml on a rotary evaporator, transferred to a vial, dried under nitrogen and weighed (376 mg).

3. Steam Distillation of Neutral Compounds from the Vine

The procedure used is essentially the same as that of H. Auda et al. (37) and is shown in Figure 4. Fifteen-hundred grams of the aerial parts of peanut plants were chopped in distilled water in a large Waring blender. The resulting seven liters of homogenate was steam distilled in a 12 l flask for five hours and three liters of distillate were collected. The distillate was saturated with NaCl and extracted five times in succession with 500 ml of ether. The combined ether extract was dried over anhydrous $Na_2SO_4$ and reduced to 100 ml on a Buchler rotary evaporator, then to 5 ml under nitrogen and transferred to a vial.

## C. Analytical Methods

1. Thin Layer Chromatography (TLC)

Concentrated neutral or basic extracts were subjected to analysis by both analytical and preparative thin layer chromatography using plates coated with silica gel GF. The main solvents used were benzene: ethyl acetate: diethyl amine (10:1:1, v/v/v) and hexane: acetone: ethanol (40:10:4, v/v/v). Plates were examined in ultra-violet light at

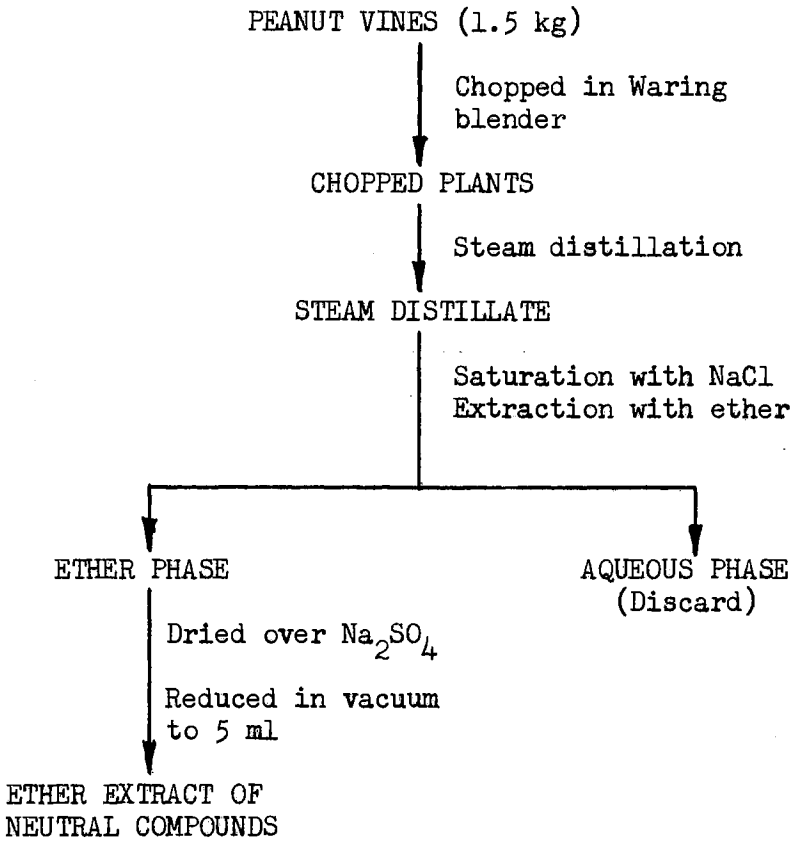



Figure 4. Flow Diagram for the Extraction of Neutral Compounds from Peanut Vines

both 366 nm and 254 nm, then sprayed with Dragendorff's reagent, according to Munier (38).

The spots of interest were scraped from plates which had not been sprayed, eluted with either chloroform or ether and concentrated under nitrogen for further study.

## 2. Gas-Liquid Chromatography (GLC)

Analytical GLC. Gas-liquid chromatography was performed on a modified Barber-Colman Model 5000 gas chromatograph equipped with a hydrogen flame ionization detector (40). The column packings used were 1% OV-61 on 80-100 mesh Gas Chrom Q or 15% Carbowax 20M, on 60-80 mesh Gas Chrom Q. Both columns were 1/4 inch by 11 foot silanized glass.

Preparative GLC. Preparative GLC analyses were performed on a Varian Aerograph Autoprep 711. The column packings used were 4% OV-1 on 80-100 mesh Chromosorb W in a 3/8 inch by 16 foot aluminum column and 15% Carbowax 20M on 60-80 mesh Anakrom ABS in a silanized 3/8 inch by 16 foot glass column. Injections of 20 to 50 $\mu$l were made, depending on the resolution required and the particular sample being used. Those peaks of interest were collected in anhydrous ether either at room temperature or in an isopropanol-dry ice slush (-72°C), depending on the volatility.

## 3. Mass Spectrometry (MS)

Low Resolution MS. Low resolution mass spectra (LRMS) were obtained on a prototype of the LKB-9000 combination gas chromatograph-mass spectrometer as described by Waller (40). The mass spectra were

obtained either by direct probe or as the compounds were eluted from the gas chromatograph using either the 1% OV-61 or 15% Carbowax 20M column. The following conditions were used: ionization voltage of 20 or 70ev, 3.5 kV accelerating voltage, 65 μA trap current, 1.7 kV electron multiplier voltage, ion source temperature of 250°C, separator temperature of 225°C, helium flow rate of 20 to 35 ml per minute. The column temperature was usually programed, with the parameters being adjusted according to the particular column used. A recording of the total ionization current (TIC) from the collector slit in the analyzer tube served as the gas chromatographic tracing. The vertical slash marks along the tracings indicate where mass spectra were taken. The heights of the spectra peaks were measured manually, then keypunched into an IBM 360/65 computer which was used to drive a Cal Comp Model 565 Plotter which plotted the spectra (41).

High Resolution MS. High resolution mass spectra (HRMS) were obtained on CEC 21-110 B mass spectrometers courtesy of Continental Oil Company, Ponca City, Oklahoma and Massachusetts Institute of Technology, Cambridge, Massachusetts. The samples were introduced by direct probe and the spectra recorded on a photoplate. Perfluoro-kerosene spectra were superimposed on the sample spectra to provide known mass standards.

The distances from mass standard peaks to sample peaks for the Continental Oil Company data were determined on a Nikon comparator and by measurement of a microdensitometer recording of the photoplate. The equation used to calculate the exact mass was:

$$M_x = \left[\frac{d_x - d_2}{d_2 - d_1}\left(\sqrt{M_2} - \sqrt{M_1}\right) + \sqrt{M_1}\right]^2 \qquad \text{(Equation 1)}$$

$M_x$ = Unknown mass (normally located between $M_1$ and $M_2$)

$M_1$ = Low $\underline{m/e}$ calibration peak

$M_2$ = High $\underline{m/e}$ calibration peak

(M is in grams/mole and e is the charge, usually one.)

$d_x$, $d_1$, $d_2$ = distances in arbitrary units of the respective masses $M_x$, $M_1$ and $M_2$ from any convenient reference point.

Equation 1 may be derived from Equation 2, the fundamental expression for the selection of a particular ion by changes in the intensity of the magnetic field or acceleration potential:

$$\frac{m}{e} = k \frac{H^2 r^2}{V} \qquad \text{(Equation 2)}$$

where m/e = the mass to charge ratio of an ion.

k = a constant.

H = intensity of the magnetic field in gauss.

r = radius of curvature of trajectory in centimeters.

V = acceleration potential in volts.

## 4. Infrared Spectroscopy

Infrared spectra (IR) were obtained on a Perkin-Elmer 457 Grating Infrared Spectrophotometer using a medium scan speed (15 minutes to scan from 2.5 $\mu$ to 40 $\mu$ ) with samples of 2 $\mu$l to 5 $\mu$l in a Wilks Mini-Cell equiped with AgCl windows. Standards were run either in a Mini-Cell or in a disposable AgCl cell made by Research and Industrial Instruments Co., London, England. A calibration peak at 5.138 $\mu$ from polystyrene was superimposed on several spectra to check the accurary of the instrument.

## 5. Nuclear Magnetic Resonance Spectrometry

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian XL-100 MHz spectrometer equipped with a Varian C-1024 time averaging computer. The operational conditions were as follows: sweep width 1000 Hz, sweep time 250 seconds, RF field 63 dB and spectrum amplitude 63. The solvent used was deuterated chloroform and the internal standard was trimethylsilane.

# CHAPTER IV

# RESULTS AND DISCUSSION

## A. Basic Compounds Extracted From Raw Peanuts and Peanut Vines

### Evaluation of Extraction Methods

A preliminary study of different extraction methods for peanut plants was undertaken because the plant is easier to extract than the peanuts, which have a high oil content. The four different methods of extraction are described in Chapter III and the total yield of the basic fraction for each method is shown in Table II. The yields of Methods I and II were low compared to Methods III and IV. Method I gave emulsion problems during the separation of the basic compounds from the neutral and acidic compounds. Even though the plant material was thoroughly extracted, the basic compounds represented only 0.006% of the fresh weight. Method III was chosen for the large scale extraction because it gave a relatively high yield of basic compounds and did not require a special solvent mixture for extraction as did Method IV.

To ensure reproducibility of the compounds extracted, four independent extractions were made of field-grown peanut vines that were harvested in Fall, 1971. A fifth extraction of greenhouse-grown peanut vines was made in June, 1972. Two extractions of defatted peanut

meal were made, as described in Chapter III, to obtain the extracts N-E and N-C.

TABLE II

TOTAL YIELD OF BASIC EXTRACT FOR DIFFERENT METHODS OF EXTRACTION OF PEANUT PLANTS

| Method | Solvent | Weight of Basic Extract (mg/80g fresh weight) | Percent (w/w) of fresh weight |
|---|---|---|---|
| I | $CHCl_3$ | 1.98 | .0025 |
| II | $(Et)_2O$ | 3.08 | .0039 |
| III | MeOH | 4.45 | .0056 |
| IV | MeOH: 0.5N HCl (10:1, v/v) | 4.89 | .0061 |

Thin Layer Chromatography

The basic extracts obtained as described in Chapter III were subjected to analytical TLC on 5 by 20 cm silica gel GF plates using two different solvent systems. Figure 5 shows those spots which appeared after spraying with Dragendorff's reagent as well as those visible when viewed under ultraviolet light of 254 nm. Two major and one minor Dragendorff positive reactions were observed. Using Plate II in Figure 5 as an example, the darkest spot (Compound I), originating from the basic fraction of the methanol plant extract, had and $R_f$ of 0.33 while the other spot (Compound II), originating from the chloro-

**Figure 5.** Tracing of the Thin-Layer Chromatographic Separation of the Basic Extracts Obtained from Raw Peanuts and Peanut Vines.

Plate I was developed in hexane:acetone: ethanol (40:10:4, v/v/v)
Plate II was developed in benzene: ethyl acetate: diethylamine (10:1:1, v/v/v)
A--Basic fraction of the methanol extract of peanut vines
B--Chloroform soluble, ether insoluble basic fraction of raw peanut extract (N-C)
C--Ether soluble basic fraction of raw peanut extract (N-E)

Spots were detected by Dragendorff's reagent (⊘) or by viewing in ultraviolet light at 254 nm and 366 nm (○).

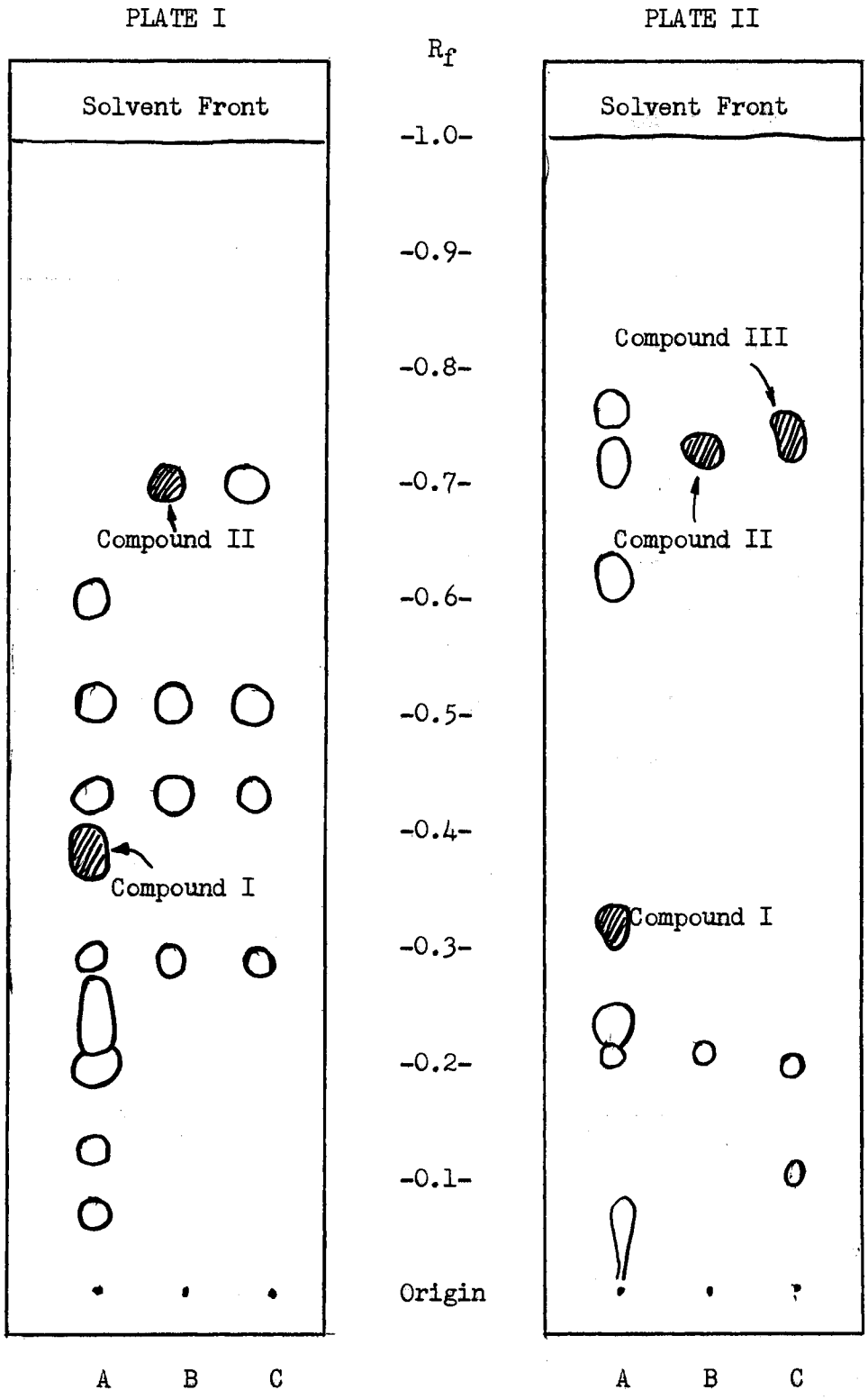


PLATE I
PLATE II
$R_f$
Solvent Front
Solvent Front
-1.0-
-0.9-
-0.8-
-0.7-
-0.6-
-0.5-
-0.4-
-0.3-
-0.2-
-0.1-
Origin
Compound III
Compound II
Compound II
Compound I
Compound I
A B C
A B C

form soluble, ether insoluble basic fraction of raw peanut extract (N-C), had an $R_f$ of 0.72. The minor Dragendorff positive spot (Compound III) also had an $R_f$ of 0.72 and was from the ether soluble basic fraction of raw peanut extract (N-E). No perceptible Dragendorff positive reaction was detected for Compound III on Plate I. All three Dragendorff positive spots quenched ultraviolet light at 254 nm before the plate was sprayed. When the developed plate was sprayed with 2, 4 dinitrophenylhydrazine solution instead of Dragendorff's reagent, yellow and orange colorations developed for Compounds I and II, respectively, indicating the presence of the carbonyl functional group. No ninhydrin positive reaction was detected for any of the compounds isolated.

The three compounds were located by their ultraviolet quenching on a plate that had not been sprayed, scraped off, eluted with chloroform, and subjected to mass spectral analysis using the direct probe. Figures 6, 7 and 8 show typical spectra of Compounds I, II and III that have molecular weights of 206, 390 and 355 respectively.

Attempts to isolate the darkest Dragendorff positive spot (Compound I) by preparative TLC met with limited success. The basic fraction of the methanol extraction was streaked on a 20 cm by 20 cm, 1000 $\mu$ silica gel HF preparative plate and developed in the benzene: ethyl acetate: diethylamine solvent system. The resolution was much lower than for the analytical plate, resulting in the bands overlapping each other. The Dragendorff positive band (Band I, $R_f$ 0.33) was also visually weak. Gas chromatographic analysis of Band I revealed that only partial separation had been achieved, resulting in a purity of about 70% for Compound I. Mass spectral analysis of the major component in Band I gave a spectrum identical to Compound I.

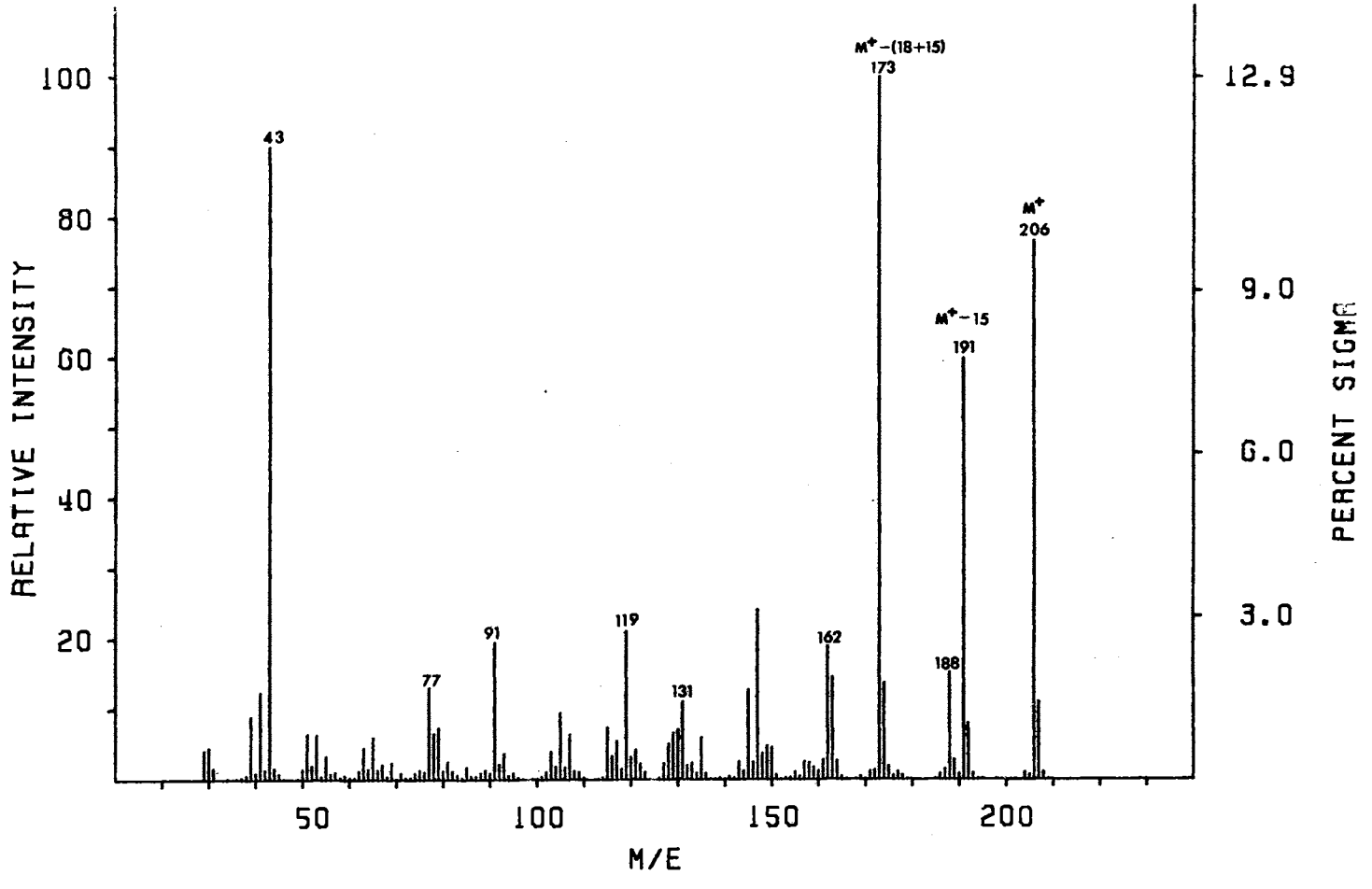



Figure 6. Mass Spectrum of Compound I.

MS 2694 2-4

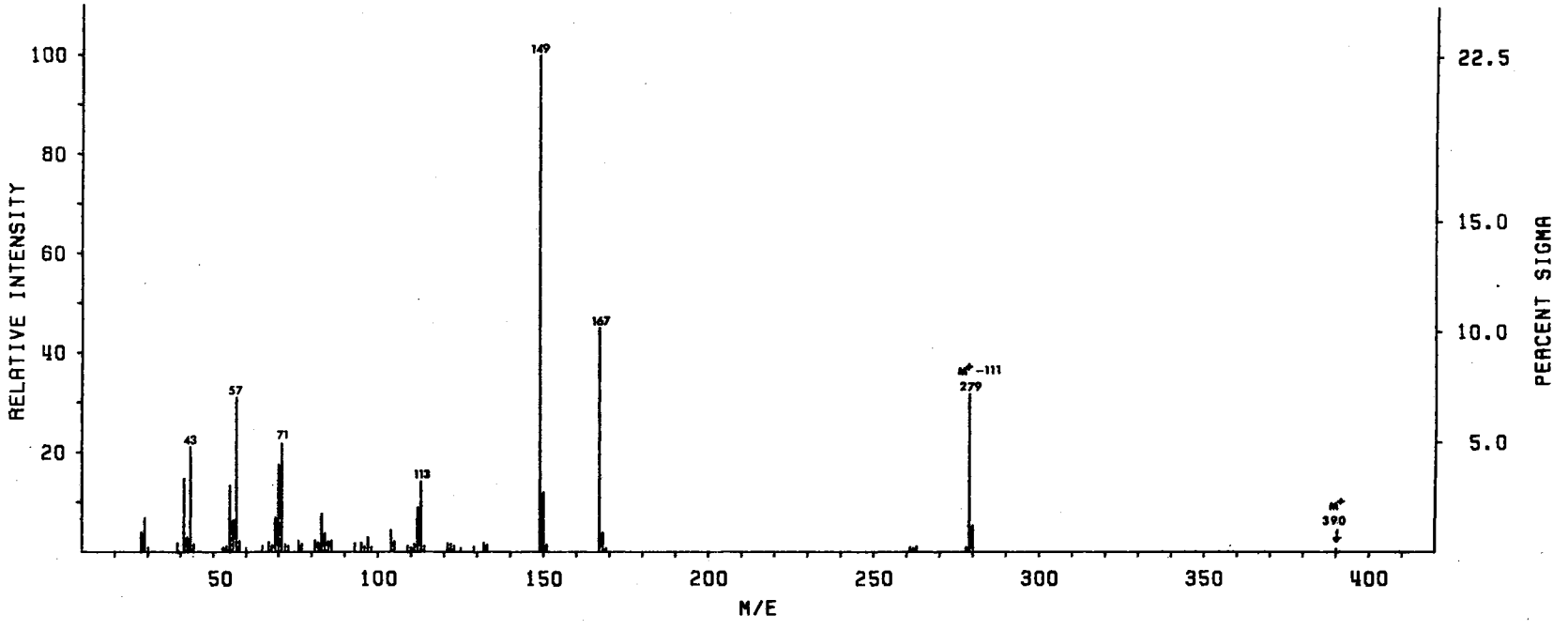



Figure 7. Mass Spectrum of Compound II.

MS 2892 3-4

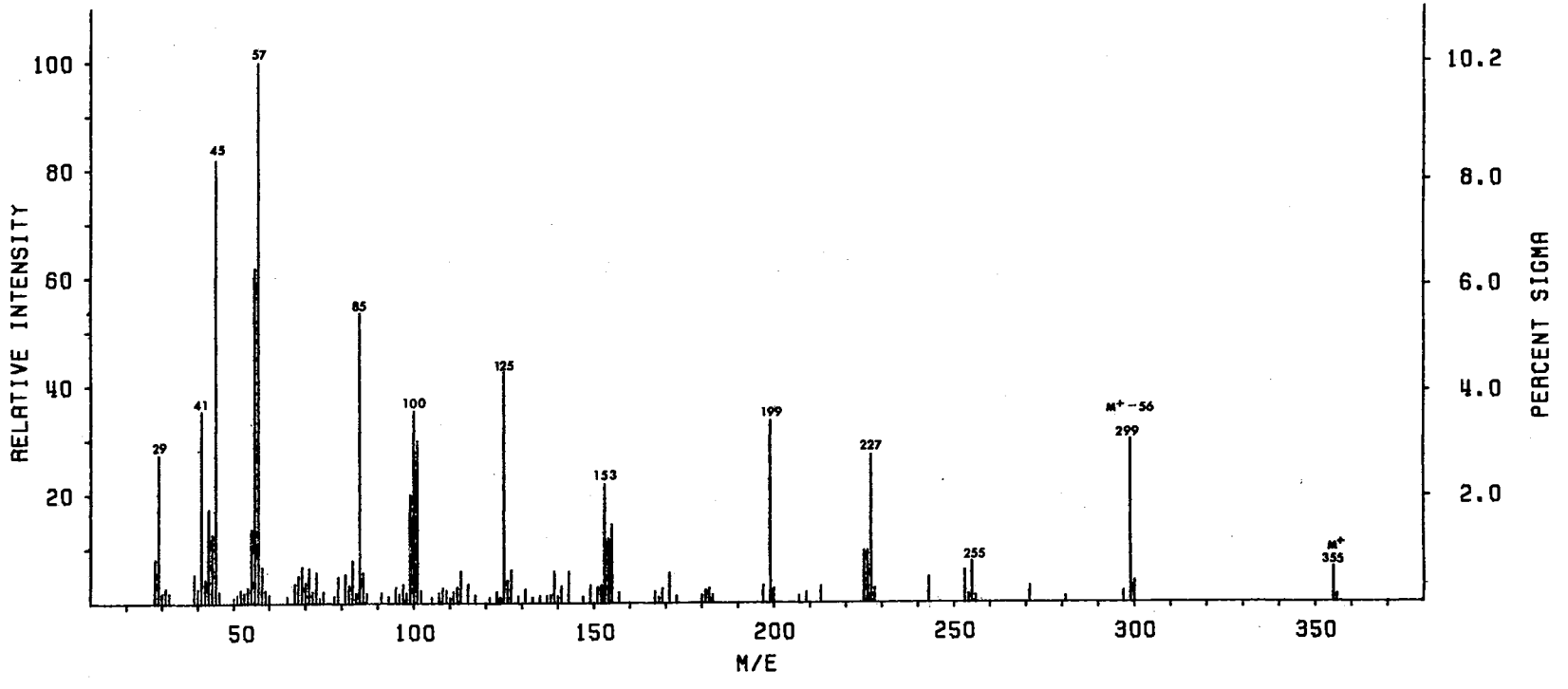



Figure 8. Mass Spectrum of Compound III.

MS 2728 6-8

Gas-Liquid Chromatography--Mass Spectrometric Analysis

Mass spectra were taken of the basic compounds present in the five methanol extracts and the N-E and N-C extracts as they were eluted from the 1% OV-61 column. In Figure 9, Peak 10 had a mass spectrum identical to Compound I, which comprised 25 to 50% of the methanol extract depending upon the maturity of the extracted plant. Although complete time studies were not performed, there was a greater concentration of Compound I in the more mature plants. Another large peak (Peak 28), one of the last to come off the column, had a molecular ion at m/e 355, corresponding to Compound III. In Figure 10, Peak 15, which comprised 65% of the total extract of N-E, had a mass spectrum identical to Compound III. Compounds I and II were also present but to a much lesser degree. As shown in Figure 11, Peak 33 comprised at least 80% of the total extract of N-C and had a mass spectrum identical to Compound II.

Table III summarizes the distribution and provides a preliminary estimate of the amount of compounds extracted from raw peanut meal and peanut vines. Compound I was not found in the raw peanut extracts (N-E and N-C).

The ten most intense ions for Compound I had m/e values of 173, 43, 206, 191, 147, 119, 91, 162, 188 and 163 with relative intensities of 100, 90, 77, 60, 24, 21, 20, 19, 15 and 14% respectively. In some spectra m/e 43 was the base peak with m/e 173 at 70%. The relative intensities between m/e 173, 191 and 206 did not change. The ten most intense ions for Compound II had m/e values of 149, 167, 279, 57, 71, 43, 70, 41, 113 and 55 with relative intensities of 100, 45, 32, 31, 22, 21, 18, 15, 14 and 13% respectively. A peak at m/e 279 (M-111) is the first large peak below the molecular ion. A loss of a large part

Figure 9. Total Ion Current Recording of the Basic Fraction of the Methanol Extract of Peanut Vines.

Column - 1/4" x 11' silanized glass
Column Packing - 1% OV-61 on Gas Chrom Q
Column Temperature - Linearly programmed from 120°C to 200°C at 3°/min.
Inlet Temperature - 225°C
Carrier Gas - Helium
Flow Rate - 25 ml/min.
Detector - Mass spectrometer
Attenuation - 1

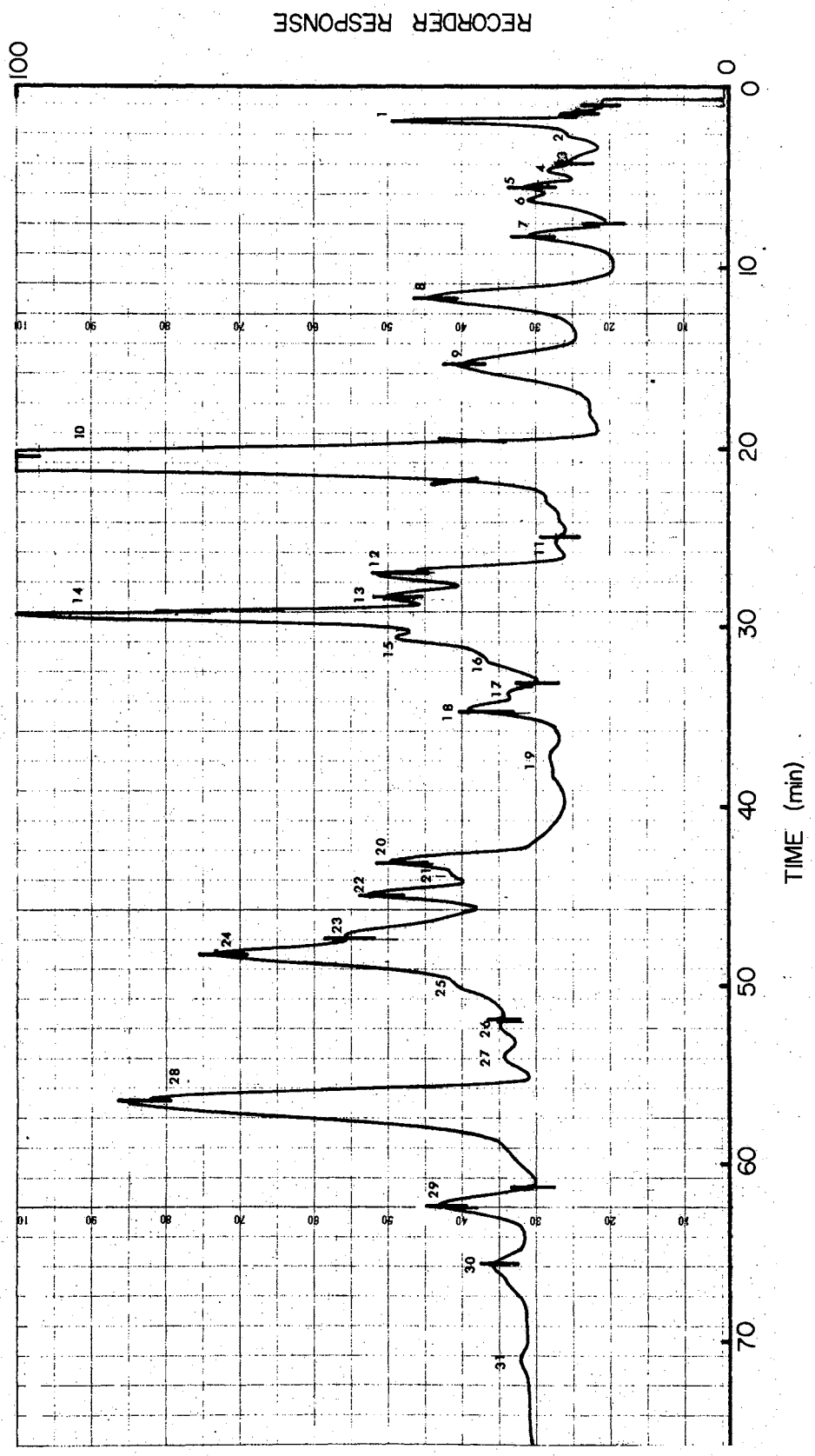

RECORDER RESPONSE
100
0
0
10
20
30
40
50
60
70
TIME (min)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

Figure 10. Total Ion Current Recording of the Basic Fraction N-E Extracted from Raw Peanuts.

Column - 1/4" x 11' silanized glass
Column Packing - 1% OV-61 on Gas Chrom Q
Column Temperature - Linearly programmed from 120°C to 200°C at 5°/min.
Inlet Temperature - 225°C
Carrier Gas - Helium
Flow Rate - 25 ml/min.
Detector - Mass spectrometer
Attenuation - 1

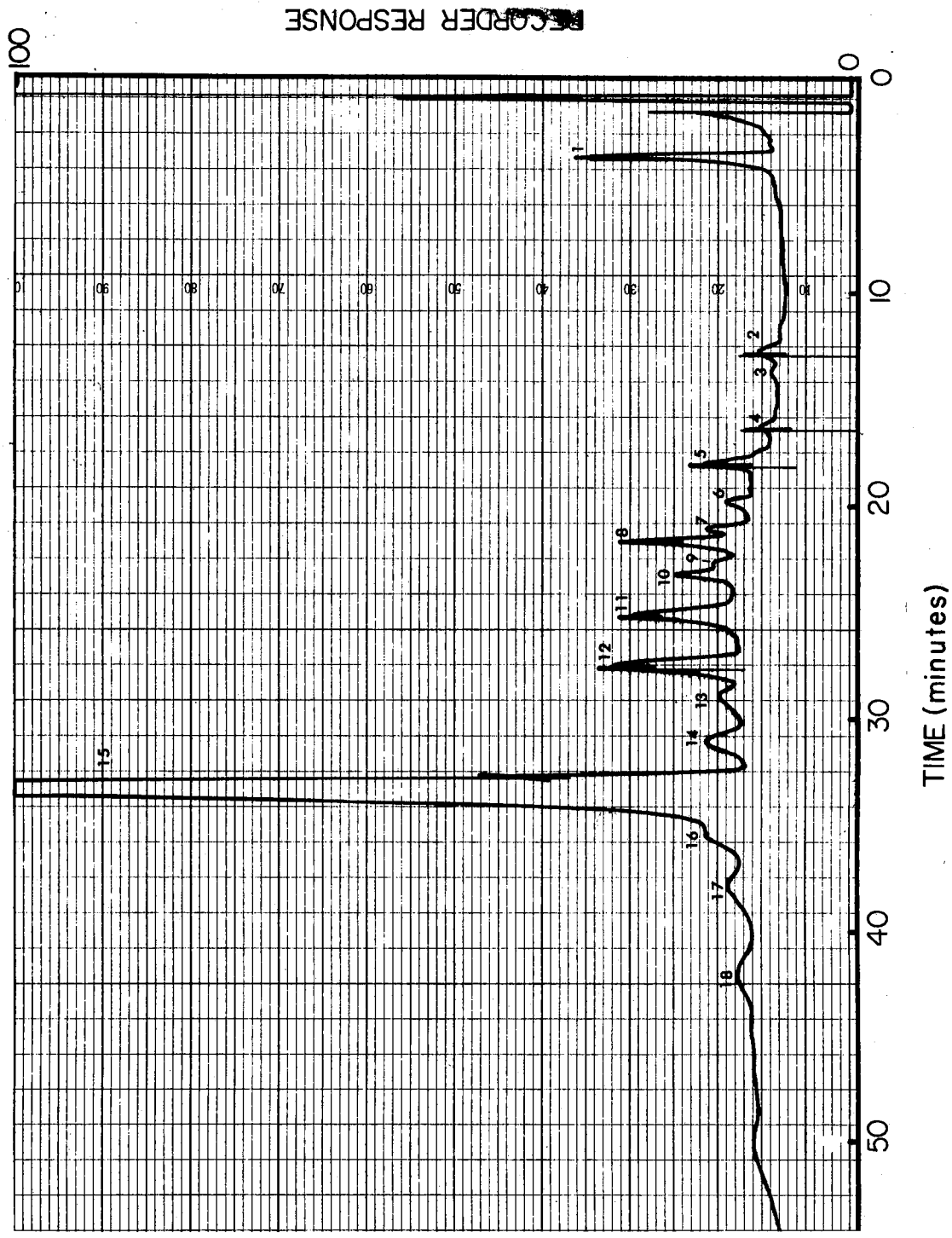
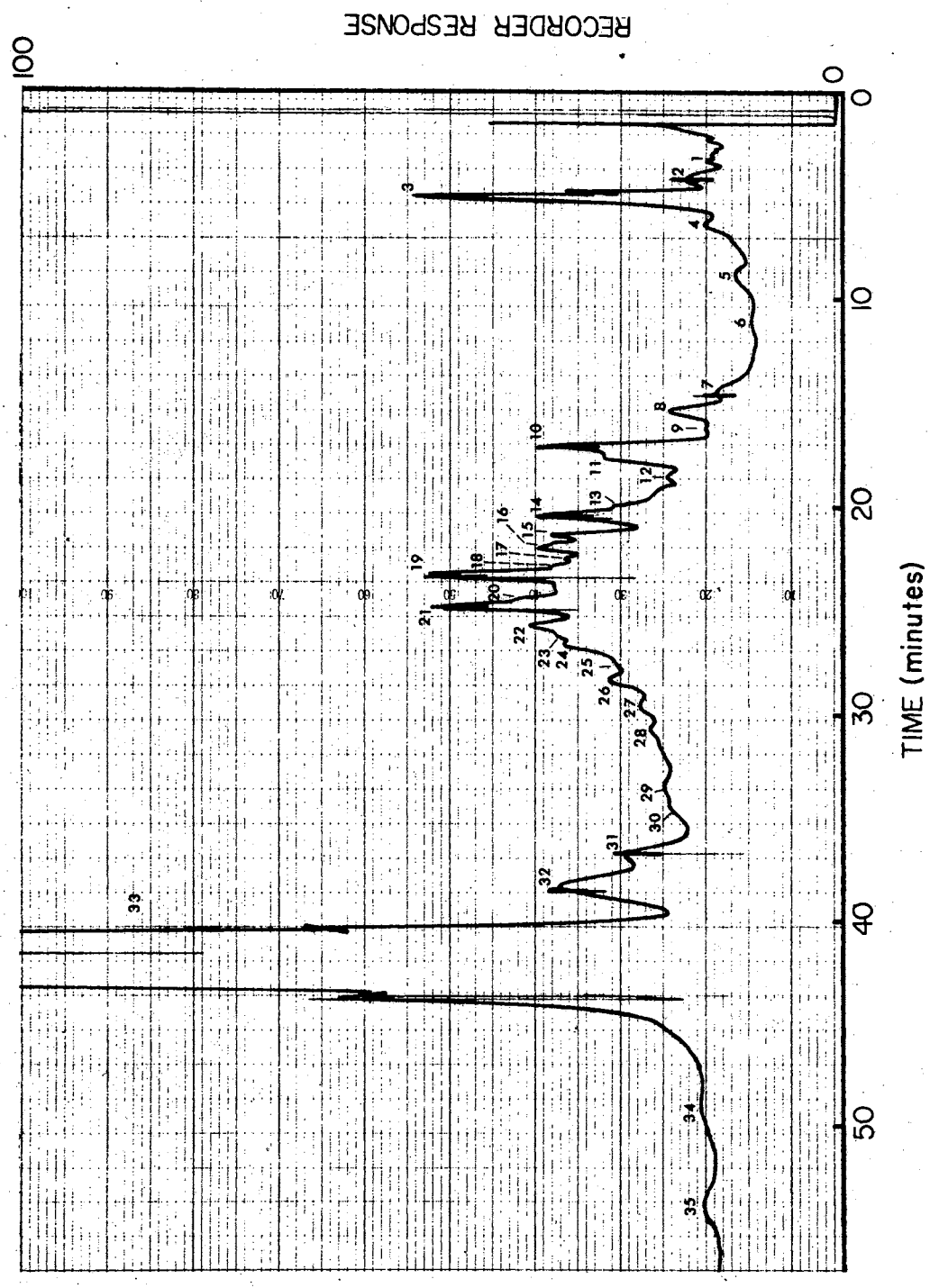

RECORDER RESPONSE
100
0
0
10
20
30
40
50
TIME (minutes)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Figure 11. Total Ion Current Recording of the Basic Fraction N-C Extracted from Raw Peanuts.

Column - 1/4" x 11' silanized glass
Column Packing - 1% OV-61 on Gas Chrom Q
Column Temperature - Linearly programmed from 120°C to 200°C at 6°/min.
Inlet Temperature - 225°C
Carrier Gas - Helium
Flow Rate - 25 ml/min.
Detector - Mass spectrometer
Attenuation - 1


RECORDER RESPONSE
100
0
0
10
20
30
40
50
TIME (minutes)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

of the molecule is typical in the mass spectra of alkaloids. A transition from the second most intense peak (m/e 167) to the base peak (m/e 149) produced a very strong metastable ion at an apparent m/e of 132.9.

TABLE III

DISTRIBUTION AND AMOUNT OF BASIC COMPOUNDS EXTRACTED FROM RAW PEANUTS AND PEANUT VINES[a]

| Compound | Molecular Weight | Raw Peanut Extract | | Methanol Extract of the Vine | Present in the Original Plant |
|---|---|---|---|---|---|
| | | N-E | N-C | | |
| | | % | % | % | % |
| I | 206 | N.F. | N.F. | 50 | .003 |
| II | 390 | * | 80 | * | -- |
| III | 355 | 65 | * | * | -- |

[a] Amounts were estimated from peak areas of GLC and TIC tracings.
* Minor component of the extract.
N.F. Not found.

The ten most intense ions for Compound III had m/e values of 57, 45, 56, 85, 125, 41, 100, 199, 101 and 299 with relative intensities of 100, 82, 62, 54, 43, 35, 35, 34, 30 and 30% respectively. The first major loss (M-56) occurred at m/e 299 and is characteristic of quinones. Compound III was the only basic compound extracted in quantity that had

an odd molecular ion (m/e 355), an indication that it contained an odd number of nitrogens, most likely one or three.

Table IV shows the analysis of the metastable peak information for all three compounds isolated.

TABLE IV

METASTABLE PEAKS IN COMPOUNDS I, II AND III

| Compound | Metastable Peak Observed | Probable Transition Denoted | Probable Neutral Fragment |
|---|---|---|---|
| I | 177.1 | m/e $206^+ \rightarrow$ m/e $191^+ + 15$ | $CH_3$ |
| | 171.6 | m/e $206^+ \rightarrow$ m/e $188^+ + 18$ | $H_2O$ |
| | 159.2 | m/e $188^+ \rightarrow$ m/e $173^+ + 15$ | $CH_3$ |
| | 156.7 | m/e $191^+ \rightarrow$ m/e $173^+ + 18$ | $H_2O$ |
| | 133.5 | m/e $162^+ \rightarrow$ m/e $147^+ + 15$ | $CH_3$ |
| | 113.1 | m/e $191^+ \rightarrow$ m/e $147^+ + 44$ | $C_2H_4O$ |
| I-TMS[a] | 248.9 | m/e $278^+ \rightarrow$ m/e $263^+ + 15$ | $CH_3$ |
| | 133.4 | m/e $162^+ \rightarrow$ m/e $147^+ + 15$ | $CH_3$ |
| II | 132.9 | m/e $167^+ \rightarrow$ m/e $149^+ + 18$ | $H_2O$ |
| | 39.1 | m/e $83^+ \rightarrow$ m/e $57^+ + 26$ | $C_2H_2$ |
| III | 72.2 | m/e $100^+ \rightarrow$ m/e $85^+ + 15$ | $CH_3$ |
| | 37.2 | m/e $85^+ \rightarrow$ m/e $56^+ + 29$ | CHO |

[a] TMS - trimethylsilyl derivative

Preparative Gas-Liquid Chromatography

In order to study Compound I in greater detail, 8 mg of the basic fraction of the methanol plant extract was separated by preparative GLC as shown in Figure 12. Fractions X, Y and Z were collected in anhydrous ether at room temperature (about 25°C) from six injections of 20 µl each. The amount injected for each gas chromatographic separation was kept low to prevent excessive loss of resolution which was lower for the OV-1 column than for the OV-61 column. An OV-61 preparative column was not used as the liquid phase was not available.

Gas-liquid chromatography and GLC-MS analysis of Fraction Y revealed a purity of 88% for Compound I and two impurieites, both of which had molecular ions of $M^+$ 190.

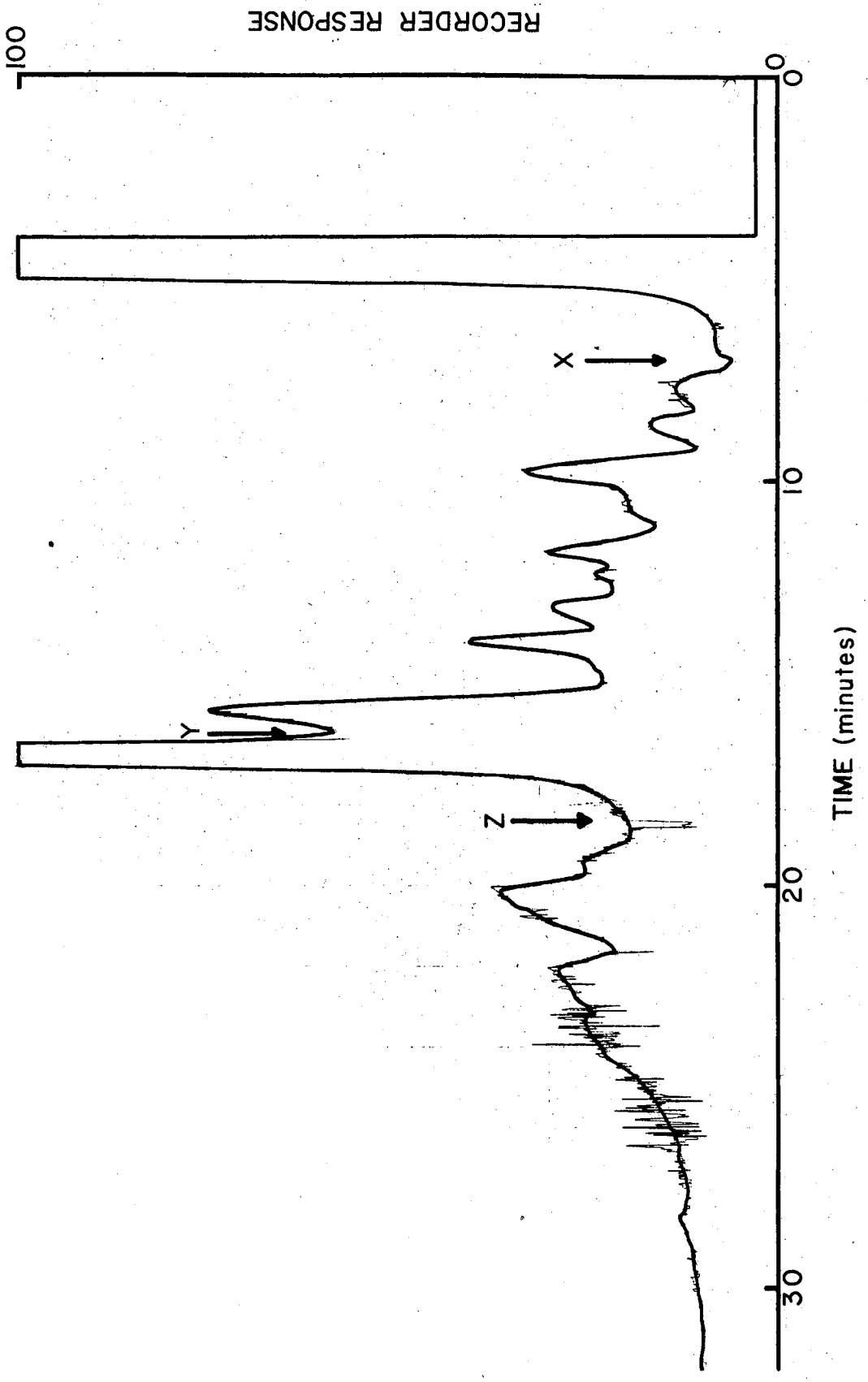
Structural Stuidies on Compound I

The infrared spectrum of neat 88% pure Compound I is shown in Figure 13. Silverstein and Bassler (42) indicated that the broad peak at 3.0 µ was typical of an R-OH group, while the multiple absorption band in the 3.3 µ to 3.5 µ region corresponded to $-CH_2-$ stretching. The peak at 5.8 µ indicated the presence of the $R_2C=O$ group, a result that supported the positive 2,4-dinitrophenylhydrazine reaction on the TLC plate. A very strong and informative peak occurred at 4.6 µ . Only certain groups, most of which contain a triple bond, will cause such an absorption. The possible groups are nitrile, cyanate, isocyanate, thiocyanate, isothiocyanate, acetylenic and carbodiimide (43). The thiocyanate group fits the sharpness, width and position of the peak best; however, examination of the intensities of the M+1 and M+2 peaks of the mass spectra indicated that sulfur was not present. Another

Figure 12. Preparative Gas-Liquid Chromatographic Recording of the Methanol Extract of Peanut Vines.

Column - 3/8" x 16' aluminum
Column Packing - 4% OV-1 on Chromosorb W
Column Temperature - Increased from 220°C to 260°C after 20 min.
Inlet Temperature - 280°C
Carrier Gas - Nitrogen
Flow Rate - 120 ml/min.
Detector - Hydrogen flame ionization
Attenuation - 8

Arrows indicate points where separate fractions were collected.


RECORDER RESPONSE
100
0
0
10
20
30
TIME (minutes)
X
Y
Z

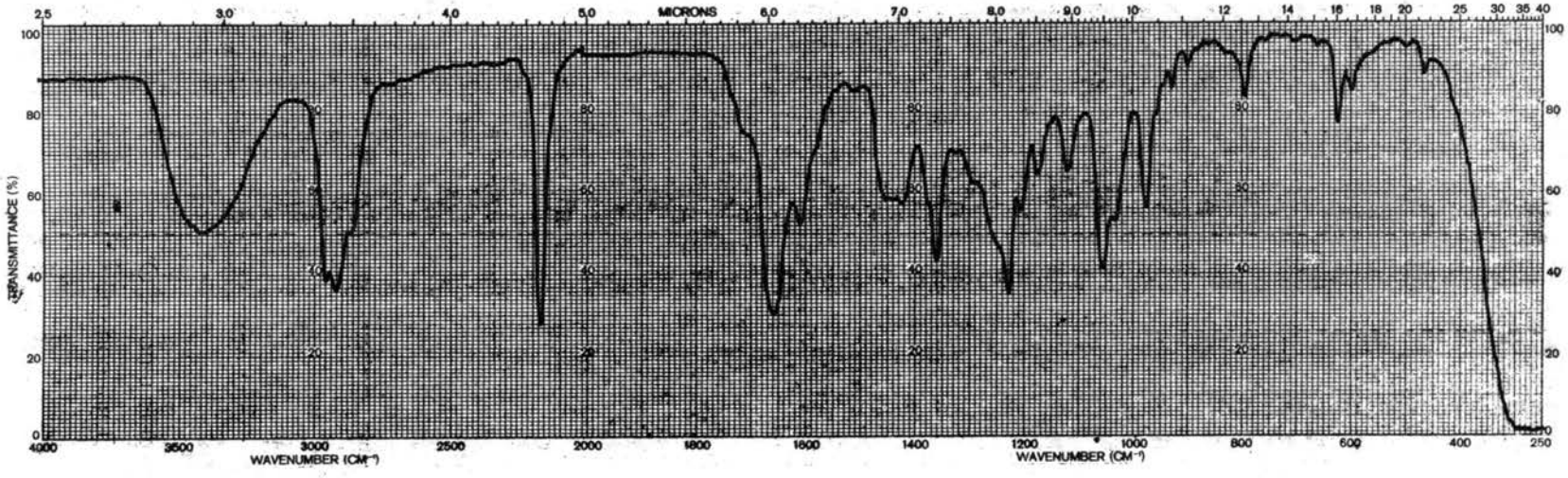



Figure 13. Infrared Spectrum of Compound I.

strong possibility is an N-substituted nitrile of an amine. The N-substitution shifts the usual absorption of the nitrile group from 4.4$\mu$ to 4.6$\mu$. All of the possible groups, with the exception of the acetylenic group, contain nitrogen, therefore the compound probably contains two or four nitrogens since the molecular weight (206) is even. Lack of absorption peaks in the 5.0$\mu$ to 5.8$\mu$ region apparently eliminated the possibility of the compound being aromatic.

Since infrared analysis had revealed the presence of a hydroxyl group in Compound I, a trimethylsilyl (TMS) derivative was prepared and Figure 14 shows its mass spectrum. The molecular weight was increased by 72 mass units to $M^+$ 278 indicating that only one TMS group was added. Therefore, the compound had only one free hydroxyl group. Ions at m/e 263 ($M^+$-15; $CH_3$), m/e 235 ($M^+$-43; $CH_3Si$), m/e 207 ($M^+$-71; $C_3H_7Si$) and m/e 188 ($M^+$-90; $C_3H_{10}OSi$) gave proof that the derivative had been made. The two prominent metastable peaks and the transitions that they correspond to are listed in Table IV.

Calculations of the exact mass from high resolution mass spectra (courtesy of Continental Oil Company, Ponca City, Oklahoma) using Equation 1 in Chapter III gave an average value of 206.1249 $\pm$ 10 mmu. The computer print-out from Massachusetts Institute of Technology gave a molecular weight of 206.1263 $\pm$ 3.2 mmu. The possible empirical formulas for Compound I are listed in Table V. Earlier mass spectral and infrared studies had shown the presence of at least one nitrogen atom and at least two oxygen atoms. Therefore, the only empirical formula that fits the requirements is $C_8H_{18}N_2O_4$.

The nuclear magnetic resonance spectrum of 2 mg of 70% pure Compound I is shown in Figure 15. Other than a peak at $\delta$ 7.25 due to the

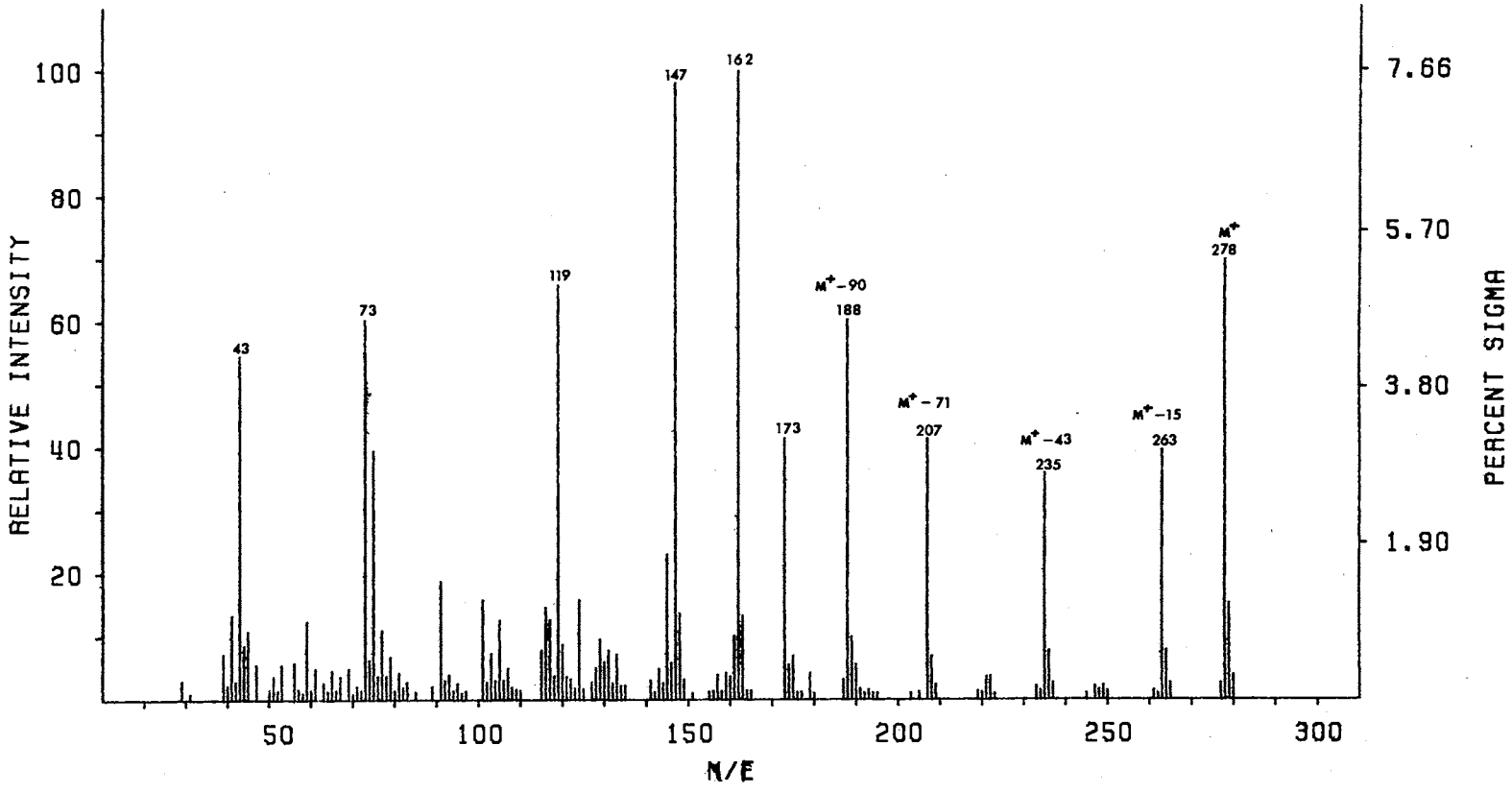



Figure 14. Mass Spectrum of the Trimethylsilyl Derivative of Compound I. MS 2885 4-5

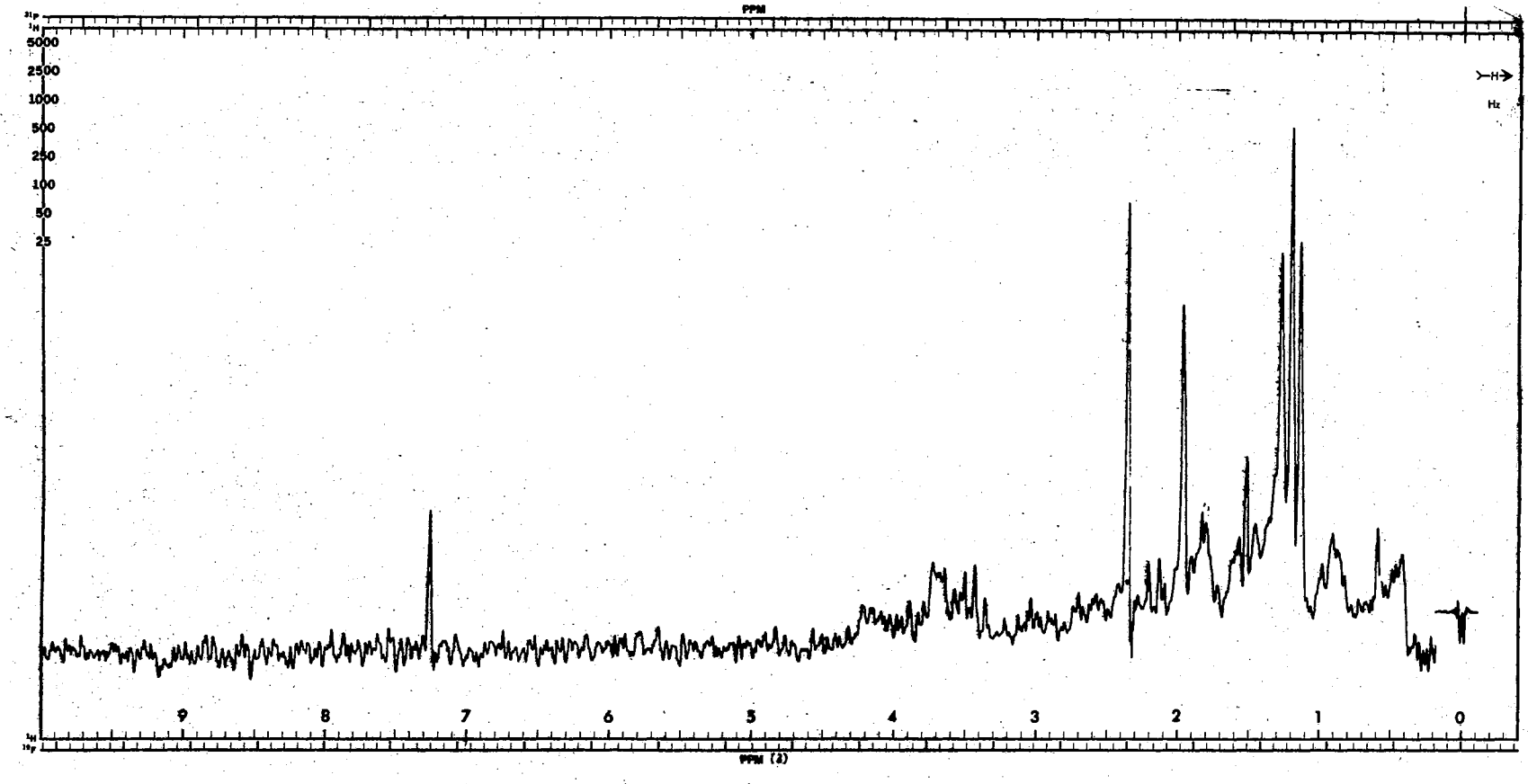



Figure 15. Nuclear Magnetic Resonance Spectrum of Compound I in Deuterated Chloroform.

deuterated chloroform, there were no peaks above $\delta$ 5.0; a result which indicated that the compound is not aromatic. The weak triplet at $\delta$3.68 may be due to the $\alpha$ proton of the $R_2CH-OH$ group (41). The strong triplet at $\delta$1.20 is probably due to a terminal methyl group. Two strong singlets appeared at $\delta$1.96 and $\delta$2.34. Time averaging of the signals was attempted without success as the NMR spectrometer would not stay locked on the internal standard for the required time of 12 hours.

TABLE V

POSSIBLE EMPIRICAL FORMULAS FOR COMPOUND I

| Molecular Weight (44) | Formula | Comments |
|---|---|---|
| 206.1181 | $C_{12}H_{16}NO_2$ | Mass too low. |
| 206.1253 | $C_6H_{16}N_5O_3$ | No evidence for 3 oxygen atoms. |
| 206.1265 | $C_8H_{18}N_2O_4$ | Most likely based on experimental results. |
| 206.1280 | $C_9H_{14}N_6$ | No oxygen atoms. |
| 206.1293 | $C_{11}H_{16}N_3O$ | Only one oxygen atom. |
| 206.1307 | $C_{13}H_{18}O$ | No nitrogen atoms. |
| 206.1419 | $C_{12}H_{18}N_2O$ | Mass too high. |

The ultraviolet absorption spectrum of 70% pure Compound I is shown in Figure 16. The maximum occurred at 284 nm with an extinction coefficient of about 7000. A compound with conjugated double bonds is one possibility.

Future Studies

It is recommended that future studies include; first, the unequivocal identification of Compound I; second, the determination of the concentration of Compound I in peanut vines as a function of the maturity of the plant; and third, the determination of the concentration of Compound I in the flowers, leaves, stems and roots of the mature plant. A similar study could also be done for Compounds II and III. If any of the compounds gave evidence of being an alkaloid, a study of the physiological activity, if any, should be done using experimental animals.

## B. Neutral Compounds Steam Distilled from Peanut Vines

Preliminary Observations

Two separate steam distillations were made. Steam Distillation I was made in late October, 1971, using freshly collected peanut plants and Steam Distillation II was made in January, 1972, using frozen peanut plants harvested in early November, 1971. The ether extraction of the first steam distillation was much darker in color than the extract of the second distillation. This was attributed to the use of impure ether which had been stored in polyethylene wash bottles. The second steam distillation was extracted with anhydrous ether that had been

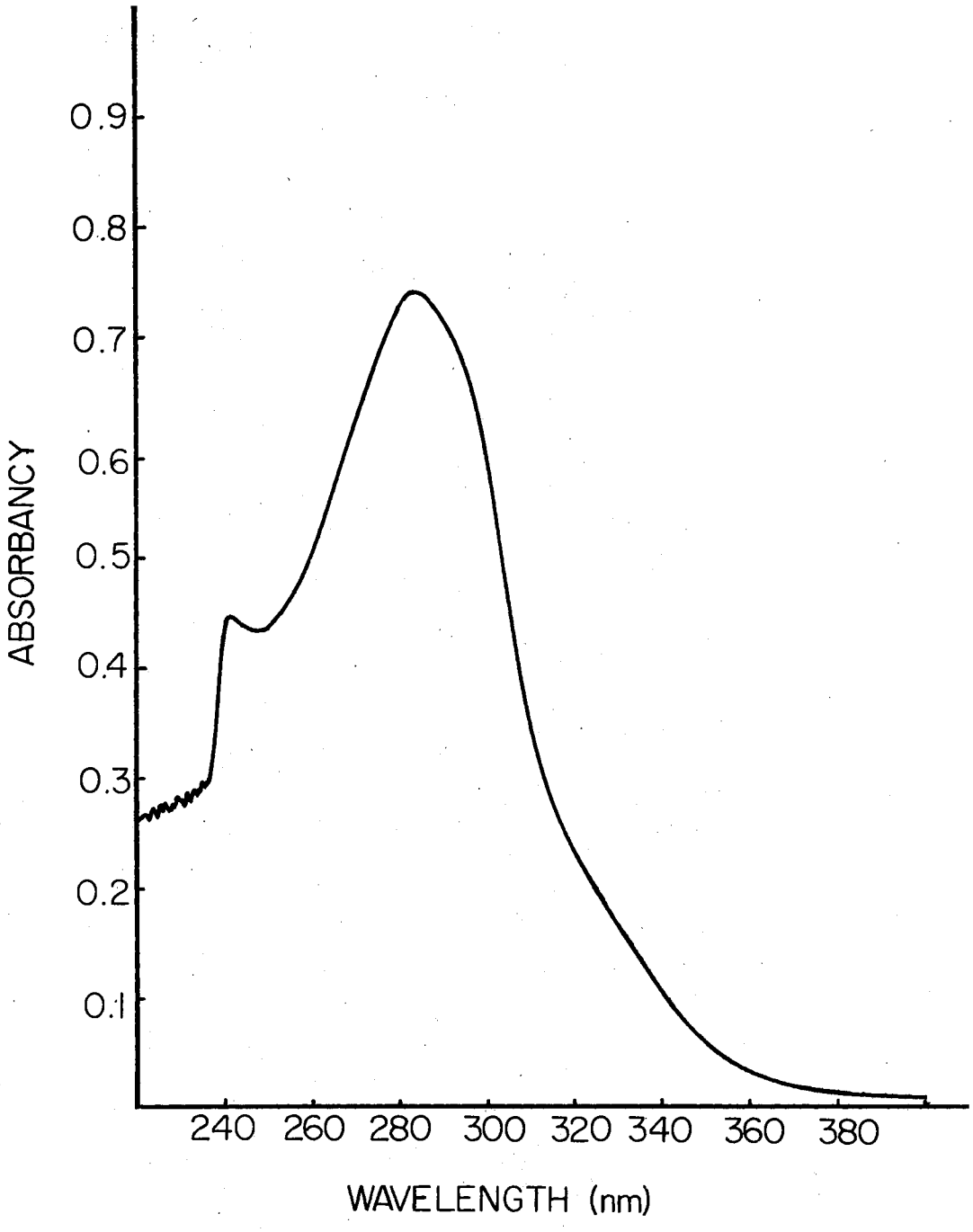



Figure 16. Ultraviolet Spectrum of Compound I in Chloroform.

stored in metal cans. Use of contaminated ether for the extraction of Steam Distillation I also resulted in false Dragendorff positive reactions when the extract was screened for possible alkaloids. The false positive reaction occurred at an $R_f$ of 0.6 on silica gel HF plates developed in benzene: ethyl acetate: diethylamine (10:1:1, v/v/v). Linalool (3,7-dimethyl-1,6-octadien-3-ol), later identified as one of the major components of the steam distillate, also had an $R_f$ of 0.6. If Dragendorff's reagent about one year old was used, linalool would give a false positive test, presumably due to the reaction of one of its double bonds.

Due to the volatility of the compounds extracted, visualization of the separated compounds on the thin layer plate by ultra-violet light at 254 and 366 nm was found to be impractical, except for linalool. The original extract had a disagreeable odor; however, after the plate had been developed, and the solvent evaporated, an odor characteristic of linalool was observed.

Gas-Liquid Chromatography

Although there was no significant difference in the gas-liquid chromatographic separations of the two distillations on the 15% Carbowax 20M column (Figure 17), the relative areas of several of the peaks changed. Table VI shows that for Steam Distillation II, the relative percentages of Peaks D, E and F decreased while Peak H increased. This was probably caused either by storage of the plants at -18°C for 10 weeks or by harvesting of the plants two weeks later than those used for Steam Distillation I.

Figure 17. Gas-Liquid Chromatographic Recordings of Steam Distillations I and II of Peanut Vines.

Column - 1/4" x 11' silanized glass
Column Packing - 15% Carbowax 20M on Gas Chrom Q
Column Temperature - Linearly programmed from 100°C to 200°C at 3°/min.
Inlet Temperature - 225°C
Carrier Gas - Helium
Flow Rate - 30 ml/min.
Detector - Hydrogen flame ionization.
Attenuation - 100

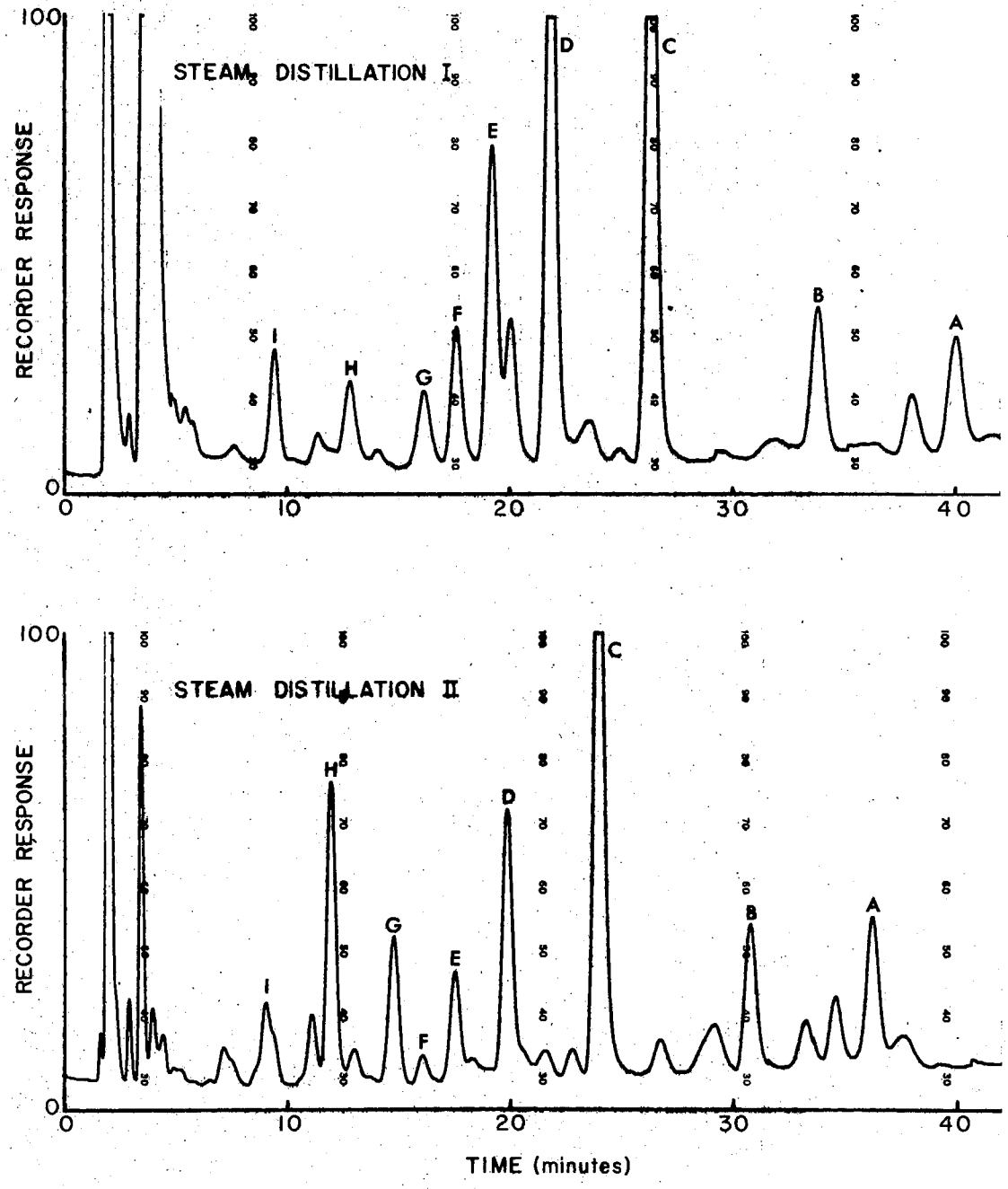

STEAM DISTILLATION I
STEAM DISTILLATION II
RECORDER RESPONSE
TIME (minutes)
100
0
10
20
30
40
A
B
C
D
E
F
G
H
I

TABLE VI

RELATIVE CONCENTRATION OF COMPOUNDS IN STEAM DISTILLATIONS I AND II*

| Peak | Percent of Total Extract Steam Distillation I | II |
|---|---|---|
| A | 6.9 | 7.1 |
| B | 7.7 | 6.6 |
| C | 34.3 | 28.5 |
| D | 28.0 | 11.0 |
| E | 15.4 | 4.5 |
| F | 5.9 | 1.1 |
| G | 4.1 | 6.1 |
| H | 4.1 | 10.5 |
| I | 4.3 | 4.2 |

* Relative concentrations were determined from the peak areas of gas-liquid chromatograms.

A procedural blank revealed only the presence of diethyl ether and ethanol when analyzed by gas chromatographic retention times. Since anhydrous ether contains 0.01% ethanol by volume, evaporation of a large volume of ether would preferentially concentrate the ethanol.

Gas-Liquid Chromatography--Mass Spectrometric Analysis

The total ion current recording from the GLC-MS analysis of Steam Distillate II is hown in Figure 18. Comparison of the mass spectra obtained with published spectra revealed that Peaks A, B, C, F and I were geraniol, α-terpineol, linalool, 1-hexanol and 1-pentene-3-ol respectively (45). Co-gas chromatographic analysis of Steam Distillate II with each of the pure standards confirmed the findings. Table VII lists the peaks in reverse order of elution from the 15% Carbowax 20M column and the retention times with respect to linalool.

TABLE VII

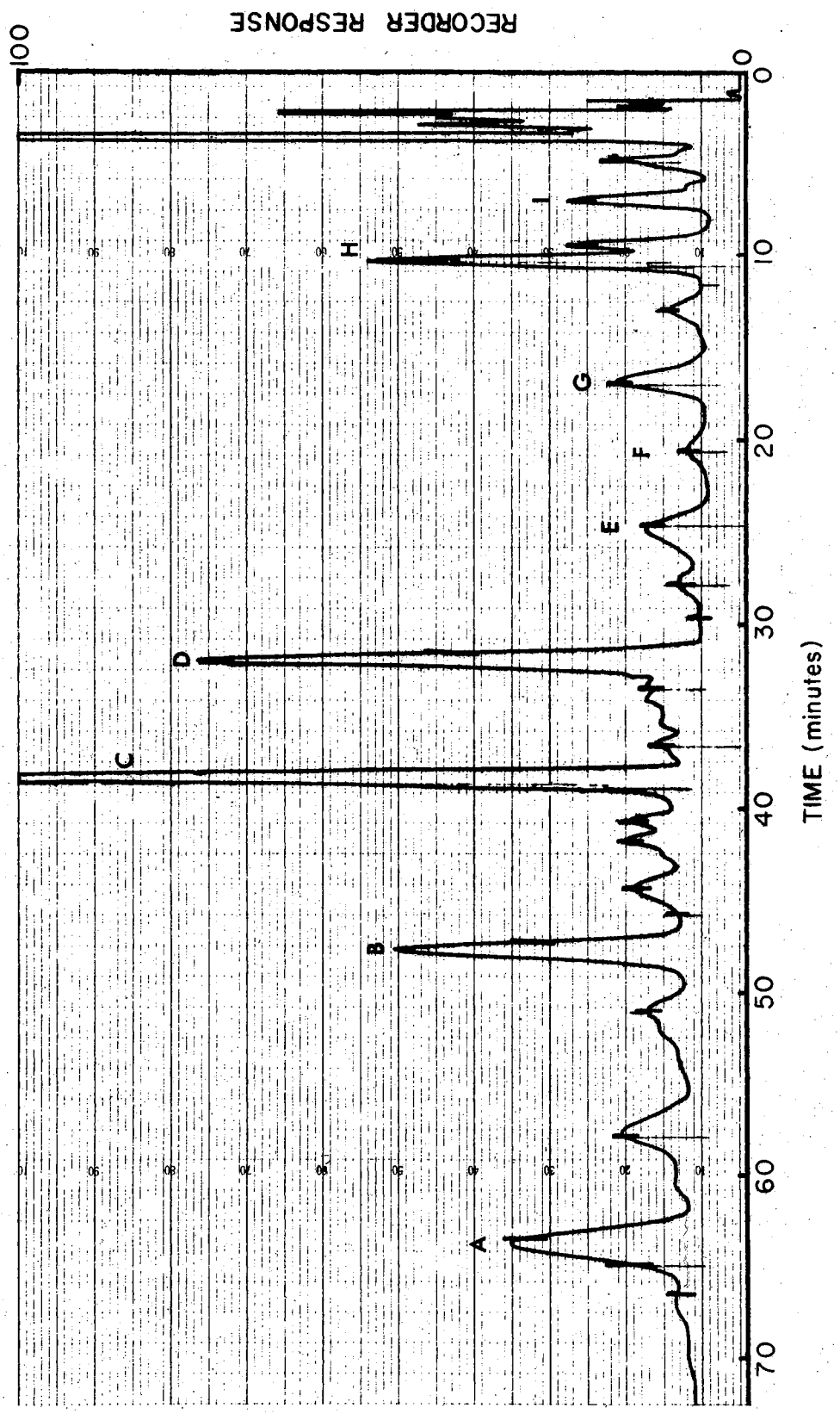
RETENTION TIMES OF COMPONENTS OF STEAM DISTILLATE II RELATIVE TO LINALOOL*

| Compound | Peak | Relative Retention Time |
|---|---|---|
| Geraniol | A | 3.02 |
| α-Terpineol | B | 1.85 |
| Linalool | C | 1.00 |
| Unknown | D | .72 |
| Unknown | E | .62 |
| 1-Hexanol | F | .54 |
| Unknown | G | .50 |
| Unknown | H | .44 |
| 1-Pentene-3-ol | I | .33 |

* Compounds were separated on a 15% Carbowax 20M column.

Figure 18. Total Ion Current Recording of Steam Distillation II.

Column - 1/4" x 11' silanized glass
Column Packing - 15% Carbowax 20M on Gas Chrom Q
Column Temperature - Linearly programmed from 80°C to 130°C at 3°/min.
Inlet Temperature - 150°C
Carrier Gas - Helium
Flow Rate - 25 ml/min.
Detector - Mass spectrometer
Attenuation - 1


RECORDER RESPONSE
100
0
10
20
30
40
50
60
70
TIME (minutes)
A
B
C
D
E
F
G
H
I

Figures 19 through 23 compare the mass spectra of Peaks A, B, C, F and I from the GLC-MS analysis of the steam distillates with mass spectra of known standards. Both standards and samples were run on the same LKB 9000 mass spectrometer, as described in Chapter III. It was found necessary to run standards on the same instrument under the same conditions of ionization potential, inlet temperature and gas-chromatographic column in order to obtain reporducible spectra. Published spectra were inadequate for several of the compounds. For example, the mass spectra of geraniol as published by von Sydow (46) was obtained at 20 eV instead of the standard 70 eV, resulting in a m/e 41 peak of only 28% instead of 70%. All five compounds exhibited peaks at m/e $M^+ - 18$, indicative of alcohols, and all but hexanol had a peak at m/e $M^+ - 15$ corresponding to the loss of a methyl group. Table VIII lists the metastable ions and probable transitions associated with each of the three terpene alcohols isolated.

There were no published mass spectra similar to Peak D which had a molecular weight of 155, indicating that it contained an odd number of nitrogens, most likely one. A $M^+ - 18$ peak at m/e 137 confirmed that m/e 155 was the molecular ion.

## Preparative Gas-Liquid Chromatography

Preparative GLC analysis was made on Steam Distillate I using the 15% Carbowax 20M preparative column. As shown in Figure 24 the chromatographic elutant was divided into seven different fractions collected in 10 successive runs with 50$\mu$l of the distillate injected for each run.

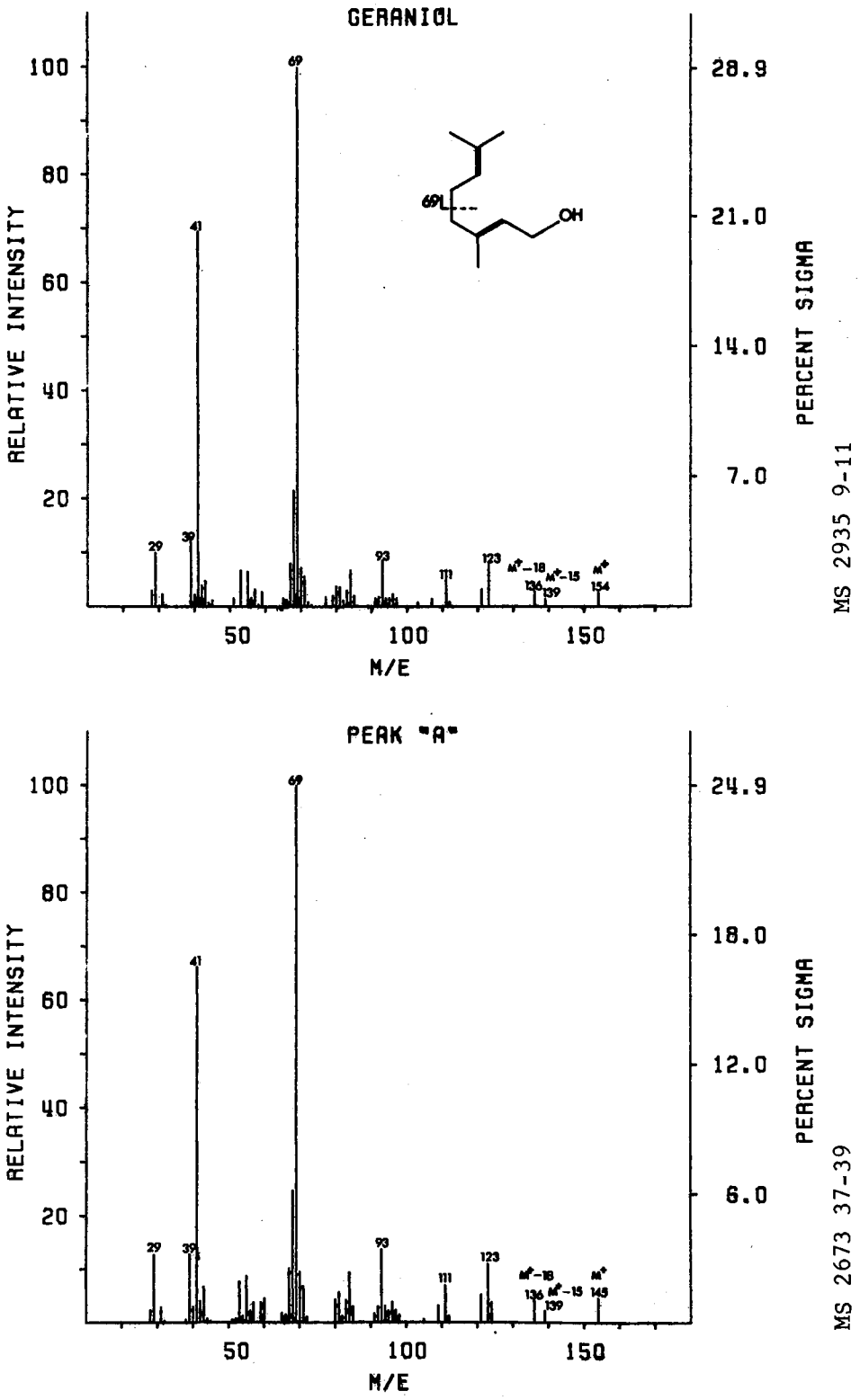



Figure 19. Mass Spectra of Geraniol and Peak A.

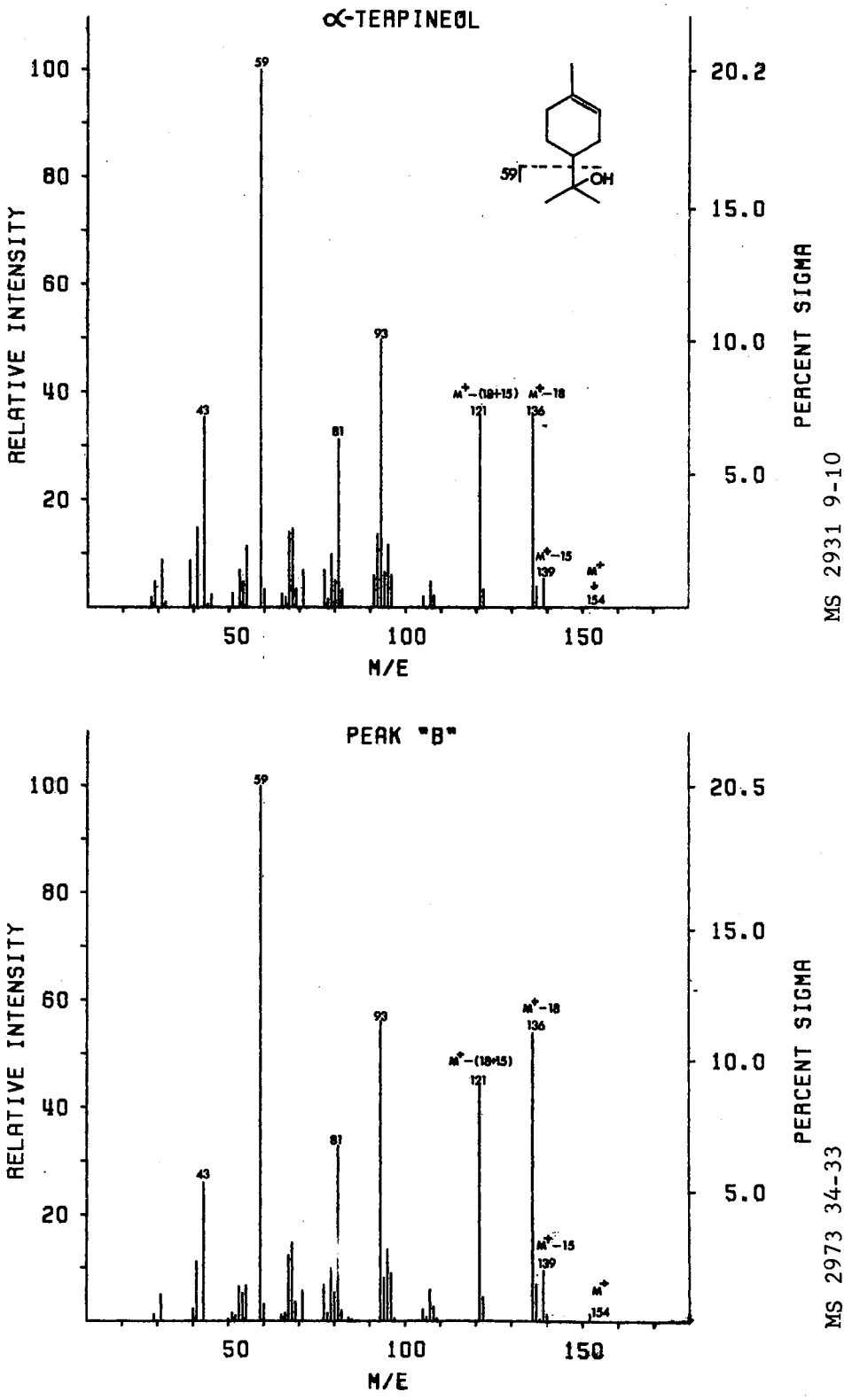



Figure 20. Mass Spectra of ∝-Terpineol and Peak B.

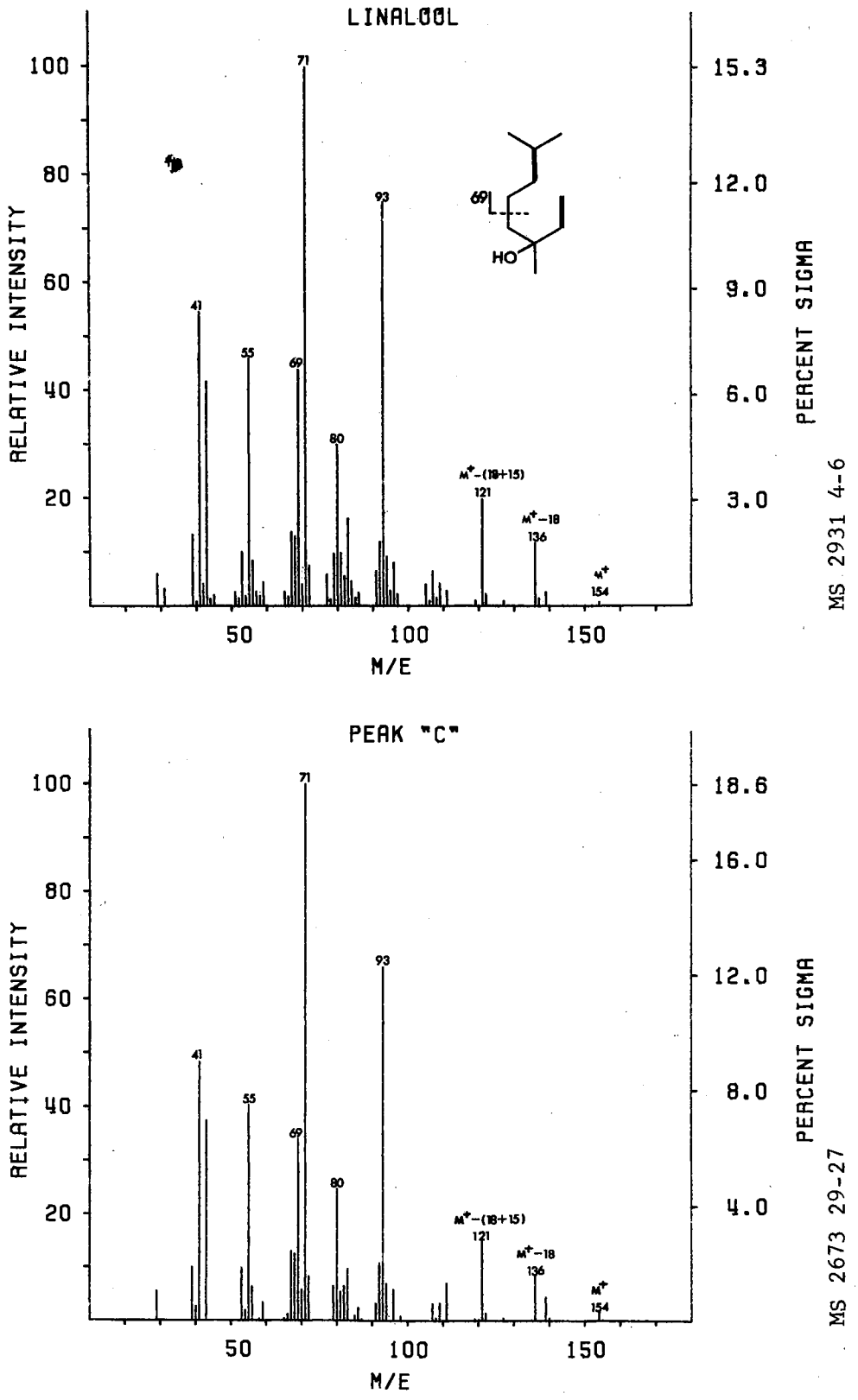



Figure 21. Mass Spectra of Linalool and Peak C.

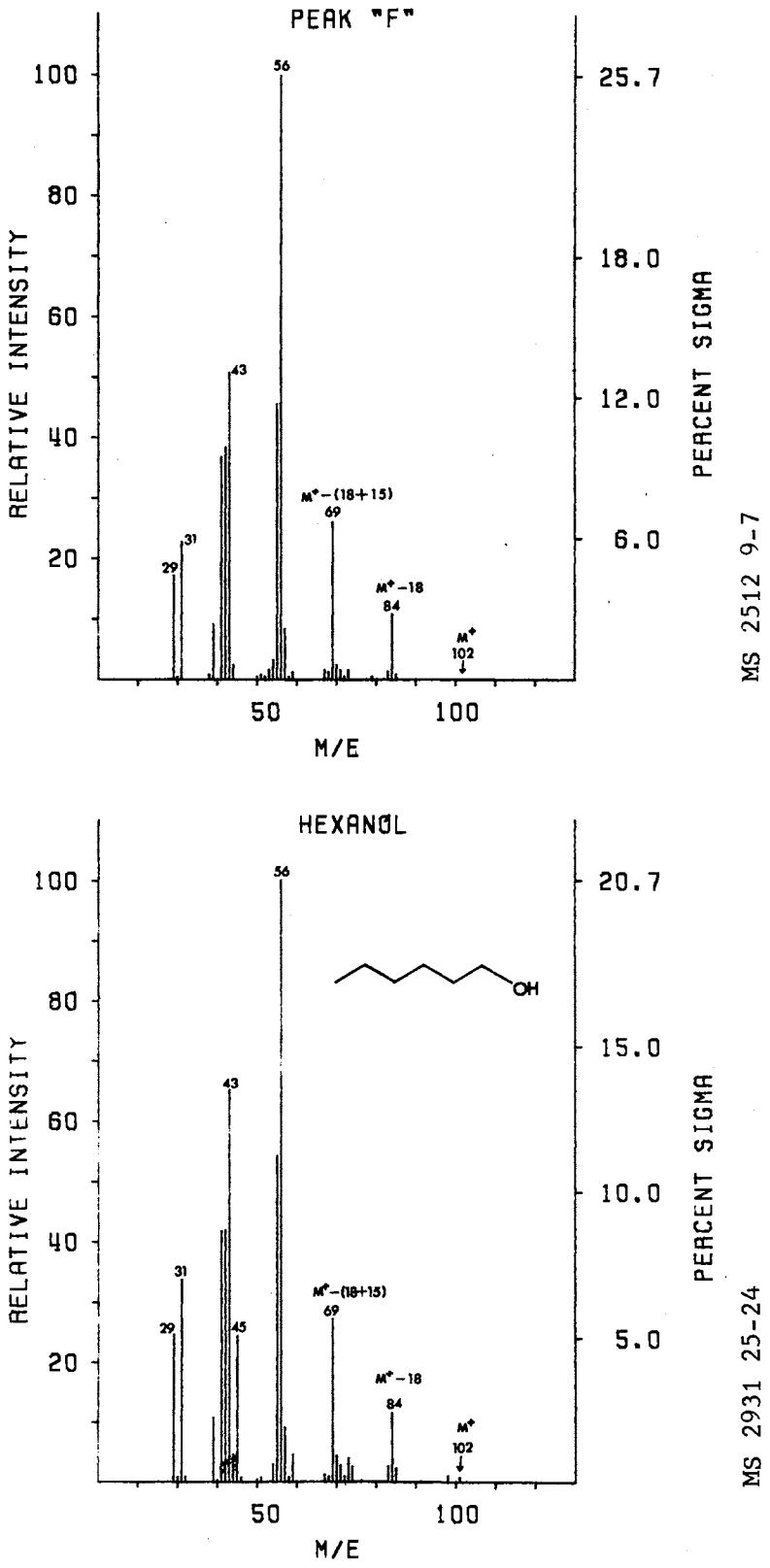



Figure 22. Mass Spectra of 1-Hexanol and Peak F

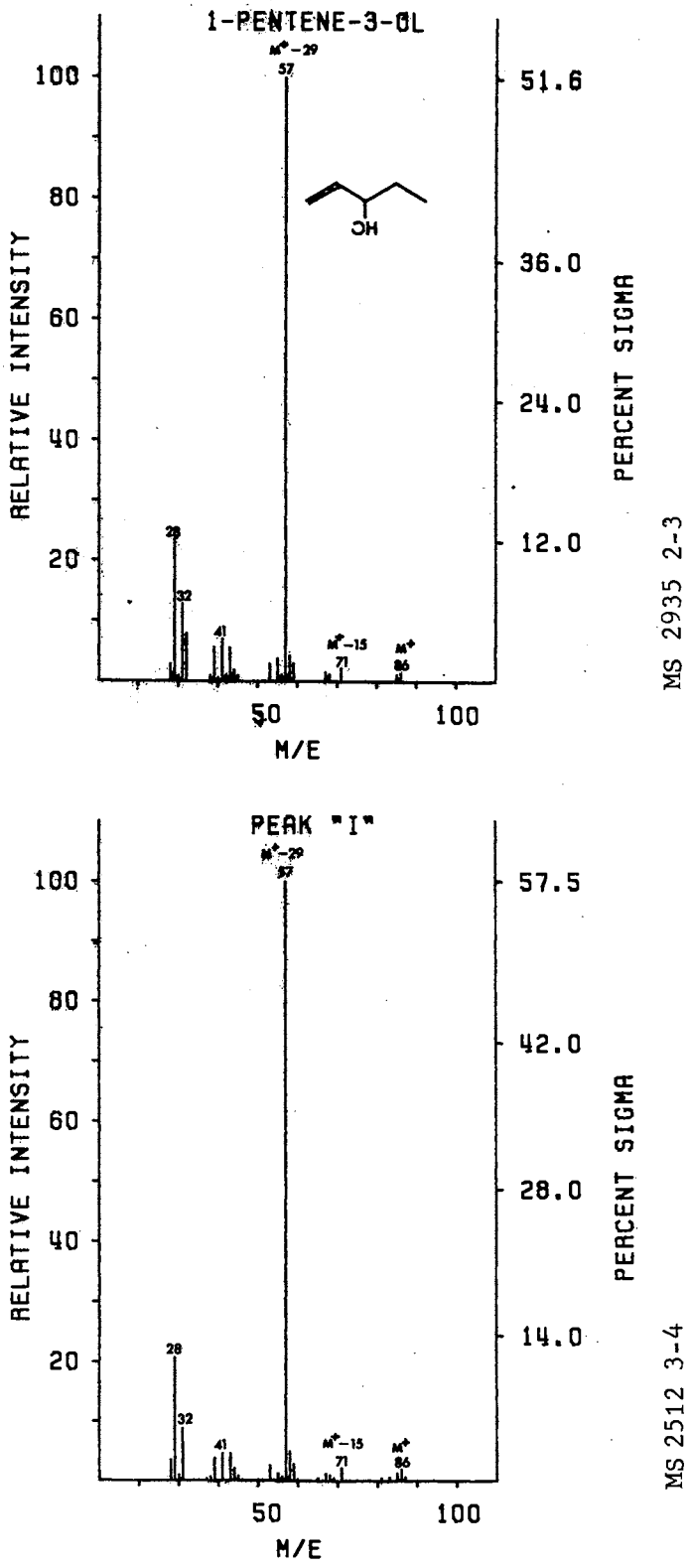



Figure 23. Mass Spectra of 1-Pentene-3-ol and Peak I.

TABLE VIII

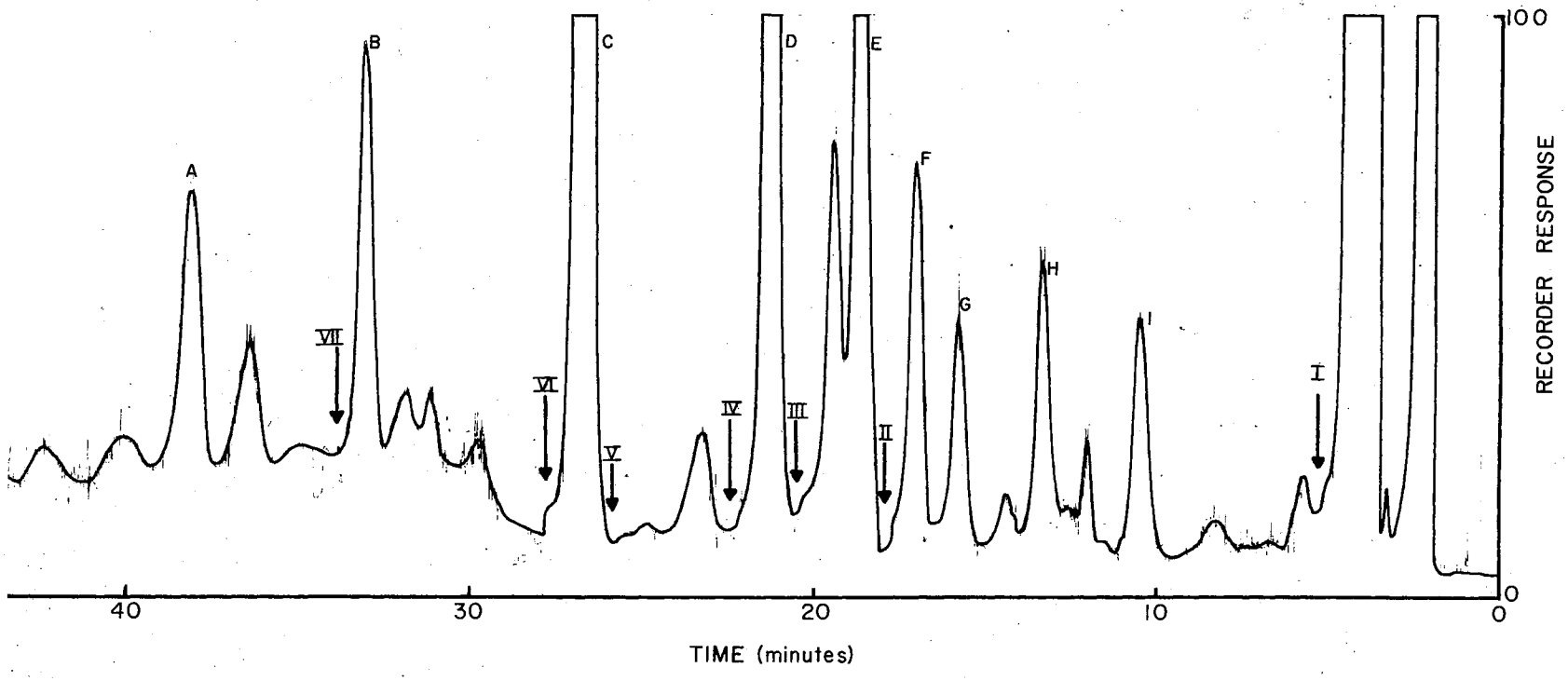
METASTABLE PEAKS IN GERANIOL, α-TERPINEOL AND LINALOOL

| Metastable Peak Observed | Probable Transition Denoted | Probable Neutral Fragment | Geraniol | α-Terpineol | Linalool |
|---|---|---|---|---|---|
| 125.5 | m/e $154^+ \rightarrow$ m/e $139^+ + 15$ | $CH_3$ | X | | |
| 107.7 | m/e $136^+ \rightarrow$ m/e $121^+ + 15$ | $CH_3$ | | X | X |
| 105.3 | m/e $139^+ \rightarrow$ m/e $121^+ + 18$ | $H_2O$ | | | |
| 89.0 | m/e $93^+ \rightarrow$ m/e $91^+ + 2$ | $H_2$ | X | X | X |
| 71.5 | m/e $121^+ \rightarrow$ m/e $93^+ + 28$ | $C_2H_4$ | | X | X |
| 63.6 | m/e $136^+ \rightarrow$ m/e $93^+ + 43$ | $C_2H_3O$ | | X | X |
| 37.3 | m/e $81^+ \rightarrow$ m/e $55^+ + 26$ | $C_2H_2$ | X | X | X |
| 37.1 | m/e $121^+ \rightarrow$ m/e $67^+ + 54$ | $C_4H_6$ | X | X | X |

Figure 24. Preparative Gas-Liquid Chromatographic Recording of Steam Distillation I.

Column - 3/8" x 16' silanized glass
Column Packing - 15% Carbowax 20M on Anakrom ABS
Column Temperature - Linearly programmed from 120°C to 200°C at 5°/min.
Inlet Temperature - 225°C
Carrier Gas - Nitrogen
Flow Rate - 120 ml/min.
Detector - Hydrogen flame ionization
Attenuation - 8

Arrows indicate points where separate fractions were collected.


A
B
C
D
E
F
G
H
I
VII
VI
V
IV
III
II
I
100
0
RECORDER RESPONSE
40
30
20
10
0
TIME (minutes)

Fraction V was found to be 98% pure for Peak C when run on the analytical OV-61 column. However, when an infrared spectrum was run, the finger-print region did not match that of a linalool standard. This was attributed to the inadvertent use of diethyl ether stored in polyethylene wash bottles. The infrared spectrum did show the presence of R-OH at 3.0 $\mu$ and $CH_2$ stretching at 3.42 $\mu$ and 3.5 $\mu$. There was no evidence for an aromatic nucleus or a carbonyl group. Fraction III (Peak D) exhibited a similar infrared spectrum, eliminating the possibility of a nitrile, isothiocyanate or similar nitrogen containing compound.

## Future Studies

It is recommended that future studies of the steam distillate of peanut vines include a positive identification of Peak D, which appears to be a nitrogen containing alcohol.

# CHAPTER V

## SUMMARY

Three compounds, I, II and III, were isolated from the basic fractions of extracts of Arachis hypogaea L. by preparative thin-layer chromatography and gas-liquid chromatography. Mass spectrometric analysis of Compounds I, II and III revealed molecular weights of 206, 390 and 355, respectively. Compound I was found only in peanut vines while Compounds II and III were isolated from raw peanuts but were also found to a smaller extent in peanut vines. Compound I, a viscous liquid at room temperature, was found to have one hydroxyl group, a carbonyl group, a prominent infrared absorption peak at 4.6 $\mu$ and an empirical formula of $C_8H_{18}N_2O_4$.

Analysis of the steam distillation of peanut vines by combined gas-liquid chromatography--mass spectrometry revealed the presence of 1-pentene-3-ol, 1-hexanol, linalool, $\alpha$-terpineol and geraniol. None have been previously identified in peanut plants. Two of the alcohols, 1-pentene-3-ol and 1-hexanol, have previously been identified in the steam distillate of peas (16). Linalool, $\alpha$-terpineol and geraniol are terpene alcohols that are common to a wide variety of plants. Preliminary evidence suggested that one of the unidentified compounds isolated was a nitrogen containing alcohol.

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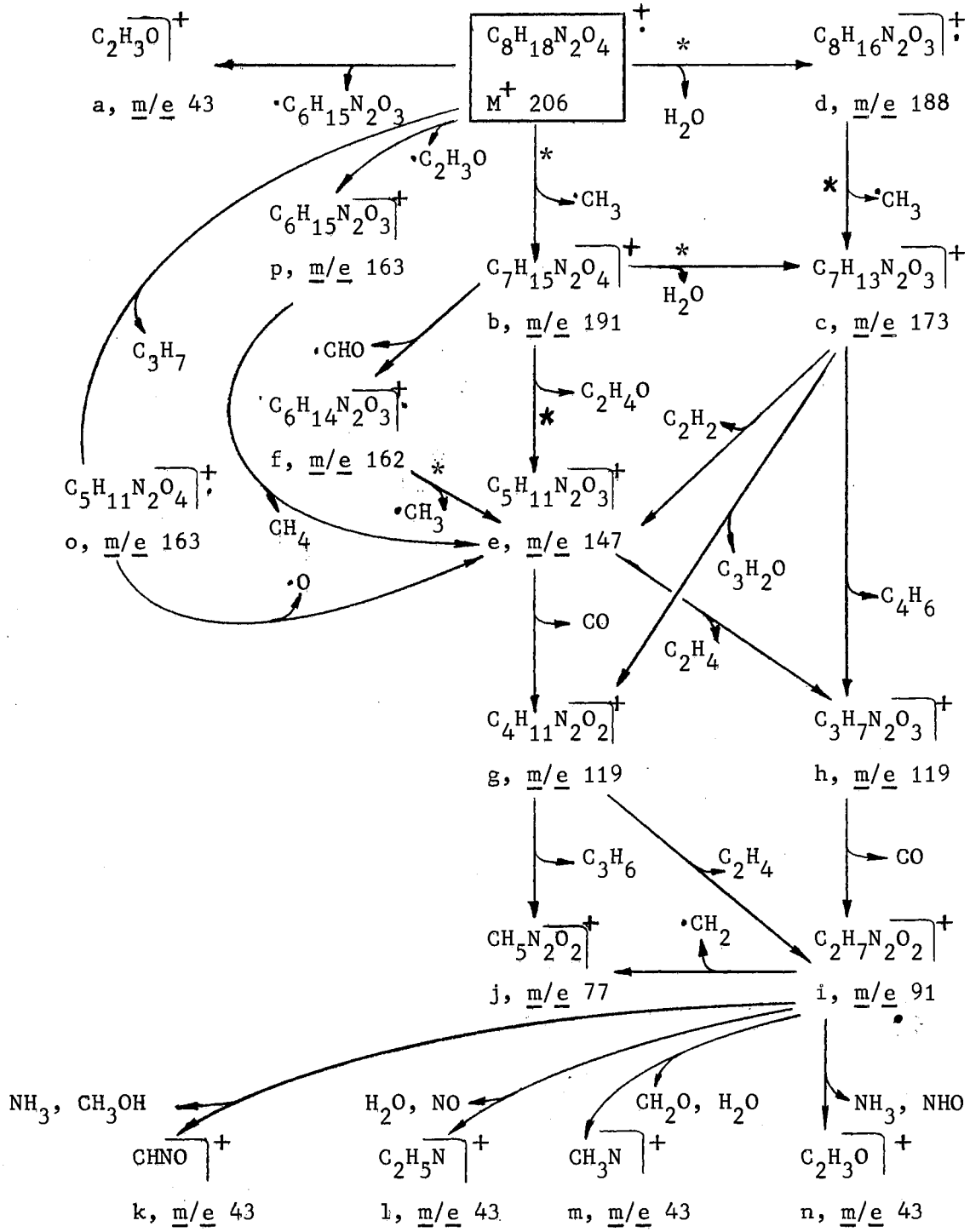
# APPENDIX

## PROPOSED PARTIAL FRAGMENTATION OF COMPOUND I

Scheme I shows the proposed partial fragmentation of Compound I as deduced from low resolution mass spectra and the Massachusetts Institute of Technology high resolution mass spectra. The molecular ion, $M^+$ 206, can undergo loss of $C_6H_{15}N_2O_3$ to yield ion a, m/e 43. Examination of low resolution mass spectra showed that m/e 43 had a relative intensity between 90 and 100%. This indicated that m/e 43 corresponds to an easily cleaved group. Examination of the high resolution mass spectra showed that m/e was actually a quartet with exact masses corresponding to $C_2H_3O$, CHNO, $C_2H_5N$ and $CH_3N_2$ in a 5:3:3:1 ratio; hence, several fragmentations are involved.

The molecular ion may also lose a methyl group to yield ion b, m/e 191 which in turn may lose water to yield ion c, m/e 173. An alternate pathway involves the loss of water from the molecular ion to form ion d, m/e 188 which can then undergo loss of a methyl group to form ion c, m/e 173. Ion b, m/e 191 may lose $C_2H_4O$ to form ion e, m/e 147 or lose CHO to form ion f, m/e 162. Ion f, m/e 162 may lose a methyl group to yield ion e, m/e 147. The formation of ions b, c, d and e have been confirmed by metastable ions as listed for Compound I in Table IV.

The molecular ion may also lose either $C_3H_7$ or $C_2H_3O$ to form ions o or p respectively, both m/e 163. Ion e, m/e 147 may be formed from




* = Transition confirmed by a metastable ion as listed in Table IV.

Scheme I. Proposed Partial Fragmentation of Compound I.

ion o, m/e 163 by loss of an oxygen radical or from ion p, m/e 163 by loss of methane.

The empirical formulae listed in Scheme I for m/e 191, 173, 163, 147 and 119 were not listed in the computer printout of the high resolution data, probably since their exact masses exceeded the error limit of 2.7 milli-mass units. The empirical formulae for these ions were obtained by subtracting the neutral fragment from their respective parent ions. The empirical formula of the cleaved neutral fragment was determined from the exact mass difference between the parent and daughter ions.

Ion c, m/e 173 may lose $C_2H_2$ to yield ion e, m/e 147. Ion c, m/e 173 may also lose either $C_3H_2O$ or $C_4H_6$ to form ions g or h respectively, both at m/e 119. Ion h is preferentially formed in a 3:1 ratio over ion g. Ion e, m/e 147 may lose either CO or $C_2H_4$ to form ions g or h respectively. In this case ion g is preferentially formed in a 2:1 ratio over ion h. Ion i, m/e 91 may be formed from ion g, m/e 119 by loss of $C_2H_4$ or from ion h, m/e 119 by loss of CO. Ion j, m/e 77 may be formed from ion g, m/e 119 by loss of $C_3H_6$ or from ion i, m/e 91 by loss of a methylene radical. Ion i, m/e 91 may lose $NH_3 + CH_3OH$, $H_2O + NO$, $CH_2O + H_2O$ or $NH_3 + NHO$ to form ions k, l, m and n respectively.

The structure of Compound I could not be determined despite the information obtained from high resolution mass spectra concerning the partial fragmentation pattern.

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