NATURAL PRODUCTS FROM *ALOE*

By

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CHAPTER I

INTRODUCTION

Since the earliest days of recorded history, man has made use of an *Aloe* plant (1). There are even several references to the plant in the Bible (2), although it may have been a different species from the one with which we are working, for it was used as a perfume. The species used in this study is *Aloe barbadensis* Mill. (Family *Liliaceae*) which has an objectionable odor.

The Chinese were among the earliest people who recognized the *Aloe* for its medicinal properties. They used it as a medicine for reducing fevers in children and for treating eczema and burns and as an antihelminthic agent (3).

Today, there is a widespread use of *Aloe barbadensis* Mill. (or commonly called *Aloe vera*) in folk medicine. It has been estimated that annual sales in this country alone range into the millions of dollars (4). Various claims have been made for the curative properties of *Aloe* including peptic ulcers, headaches, constipation, and burns. One use of the plant that seems to persist down through the history of folk medicine is in the treatment of burns. So widespread is its folk use in the treatment of burns that many housewives grow it as a kitchen plant just to treat minor household burns and cuts. A United States Patent has been issued covering a preparation made for its use in the treatment of burns (5).

It is this use in the treatment of burns that attracted our attention to this plant. Much progress has been made within the area of burn treatment within the last ten years. For example, the burn wound death rate at the Institute of Surgical Research at Fort Sam Houston, Texas, one of the foremost burn treatment centers of the world, has been reduced from 47.2% before 1964 to 11.3% afterwards in patients less than 15 years old and from 31.8% to 25.8% in patients older than fifteen (6). This dramatic improvement was made possible through the use of the drug "Sulfamylon" (either the hydrochloric acid salt or the acetic acid salt of α-amino-*p*-toluenesulfonamide). One of the intriguing questions that continues to drive us forward in the field of the medical sciences is "Can we still do better?" Indeed, can we lower the death rate still further, can we make the patient more comfortable, and/or can we minimize the disability caused by his wound?

The *Aloe* is not without its critics, however. Dr. Harold F. Hamit, who was Chief of the Surgical Branch of the U. S. Army Research and Development Command between 1957 and 1960, studied the effects of various preparations made from *Aloe vera* upon the burn wound. This study showed that no benefit was derived from any of these products (7).

Does the *Aloe* really contain a compound of medicinal value which has not yet been isolated, or, in the case of burns, does its juice merely form a protective film over the wound? It is the purpose of the research reported in this thesis to describe the isolation and identification of a compound or compounds which occur naturally in the *Aloe*, employing modern analytical biochemical

techniques such as thin-layer chromatography, gas - liquid chromatography, and mass spectrometry. Whether or not this compound will prove to possess physiological activity is a question to be resolved only after much testing and further research - a new drug is not and cannot be developed overnight.

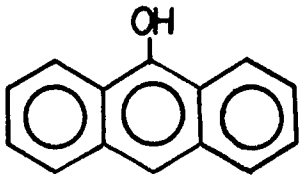
CHAPTER II

HISTORICAL REVIEW

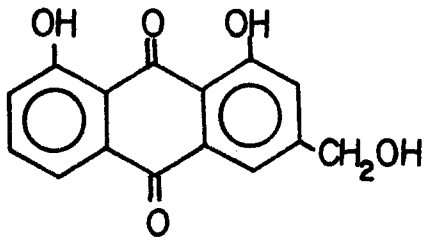
Over a hundred different species of Aloe are known and confusion has developed with respect to naming correctly some of these plants; in addition, folk usage has added to the confusion. For example, the plant used in this research is identified as Aloe barbadensis Miller, but is also known as Aloe vera and Curacao Aloe (3). The literature review will be limited to this particular species.

Because there was some question as to the true chemical composition of Aloe in the older literature, Mary, Christensen, and Beal (8) reinvestigated the Aloe using paper chromatography and identified the components by comparing their R_f values with those of known compounds as well as by studying their absorption curves. In this study, anthranol (I), aloe-emodin (II), and chrysophanic acid (chrysophanol) (III) were found.

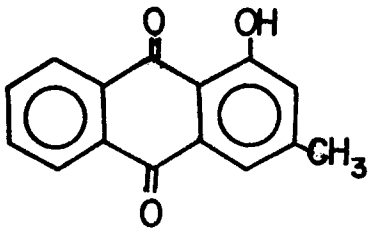
When Awe and Kuemell (7) collected the juice from fresh Aloe leaves under a nitrogen atmosphere, they found no aloe-emodin, but instead found aloin (barbaloin) (IV). They concluded that aloin had been converted to aloe-emodin by earlier investigators during their analyses. After the paper chromatograms which they used in their studies were sprayed with potassium hydroxide, these authors found p-coumaric acid (V) also. Haynes and Holdsworth (10) discovered aloesin (VI) after chromatographic analysis of a commercial sample of Aloe barbadensis.



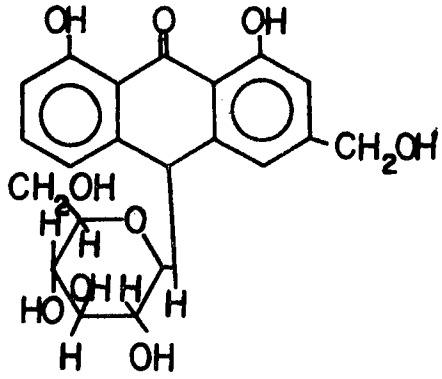
anthranol
I



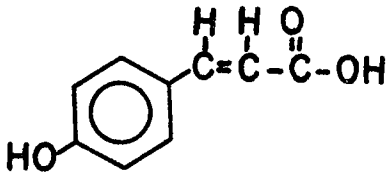
aloe-emodin
II



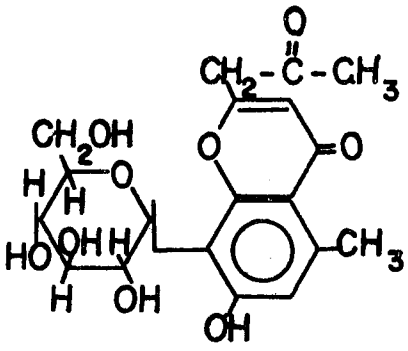
chrysophanic acid
(chrysophanol)
III



aloin
(barbaloin)
IV



p-coumaric acid
V



aloesin
VI

Figure 1. Structural formulas of compounds isolated from Aloe barbadensis

Gottshall et al. (11) found that aloin inhibited the growth of Mycobacterium tuberculosis while Döff (12) found that either aqueous or alcoholic extracts of the aloe leaf, aloin, or aloe-emodin had tuberculostatic activity, but that the most active compound in this respect was p-coumaric acid. Fly and Kiem (13) found that Aloe leaf, either as a whole or as a part, had no bacteriostatic action against Staphylococcus aureus and Escherichia coli. On the other hand, however, Lorenzetti et al. (14) found that either Aloe juice or juice which had been preserved by heating at 80°C for 15 minutes and then lyophilizing did indeed show bacteriostatic activity against Staphylococcus aureus 209, Streptococcus pyogenes, Corynebacterium xerose, and Salmonella paratyphi.

Farkas (5) studied the gel derived from Aloe leaves as a topical medication for the treatment of open wounds and burns. He found it to consist of a polyuronide composed of a polyose containing glucose and mannose and of hexuronic acids such as glucuronic acid, mannuronic acid, and galacturonic acid. As this polyuronide occurs in nature, Farkas pointed out that its molecular weight may range up to about 275,000. Segal, Taylor, and Eoff (15) reported the isolation and partial purification of a polysaccharide from the mucilage of Aloe barbadensis. Upon hydrolysis, this polysaccharide was found to contain chiefly mannose and glucose and trace amounts of arabinose, galactose, and xylose; no uronic acids could be found in the hydrolysate.

Gjerstad (16), employing infrared spectroscopy, found evidence for hydroxy, amino, ether and carboxyl groups, aromatic rings, and peptide linkages in the juice of Aloe barbadensis. The original

juice contained 0.013 percent protein and the lyophilized juice 2.5 percent protein. Analysis by cryodesiccation showed Aloe juice to contain 99.52% water. The amino acids which he found in hydrolyzed lyophilized Aloe juice are shown in Table I. Hydroxyproline, histidine, and cystine were found also in the juice as free amino acids.

Aloin and aloe-emodin also are effective as laxatives (17). Large quantities of Aloe vera are used in cosmetics (18, 19). Aloe vera gel preparations are sold commercially to improve the flavor of various foods (20).

Thus as in the case of most folk medicines, only a few of the physiologically active constituents of Aloe barbadensis have been determined, and the knowledge of the medicine has been handed down from generation to generation only because it worked - the reason why it works remains unknown.

TABLE I

AMINO ACIDS PRESENT IN THE HYDROLYZED JUICE OF *ALOE BARBADENSIS* AS FOUND BY GJERSTAD (16)

Amino Acid	Mole/mg. of protein
Lysine	0.35
Histidine	0.15
Arginine	0.23
Hydroxyproline	a
Aspartic acid	0.75
Threonine	0.34
Serine	0.44
Glutamic acid	0.92
Proline	0.38
Glycine	0.71
Alanine	0.67
Valine	0.47
½ Cystine	b
Methionine	b
Isoleucine	0.30
Leucine	0.53
Tyrosine	0.18
Phenylalanine	0.21

a Present, but not determined quantitatively.

b Most of this amino acid was destroyed during hydrolysis.

CHAPTER III

EXPERIMENTAL METHODS AND MATERIALS

Aloe Barbadensis Samples

Several *Aloe barbadensis* plants were obtained from local residents of Stillwater, Oklahoma, who had been growing them as house plants. These were young potted plants and were less than six inches tall.

Other *Aloe barbadensis* plants and leaves were obtained from Hilltop Gardens, Lyford, Texas, which is located near the Rio Grande River in the southern part of the state. These plants had been field grown and included both young and mature plants.

The plants were collected in October and were transported to Stillwater, Oklahoma, by automobile. Soon after arrival the plants were potted in clay pots or five-gallon metal cans using a 1:1:1 mixture of soil, peat moss, and perlite. The plants were allowed to grow under moderately dry conditions in a greenhouse where the temperature was maintained above 70° F at all times. As shoots grew from the *Aloe barbadensis* plants, the plants were transplanted to other pots as often as necessary to prevent crowding. Day to day care was provided by the greenhouse staff.

In order to positively identify this species of Aloe, a specimen in bloom was sent to the Plant Science Research Division

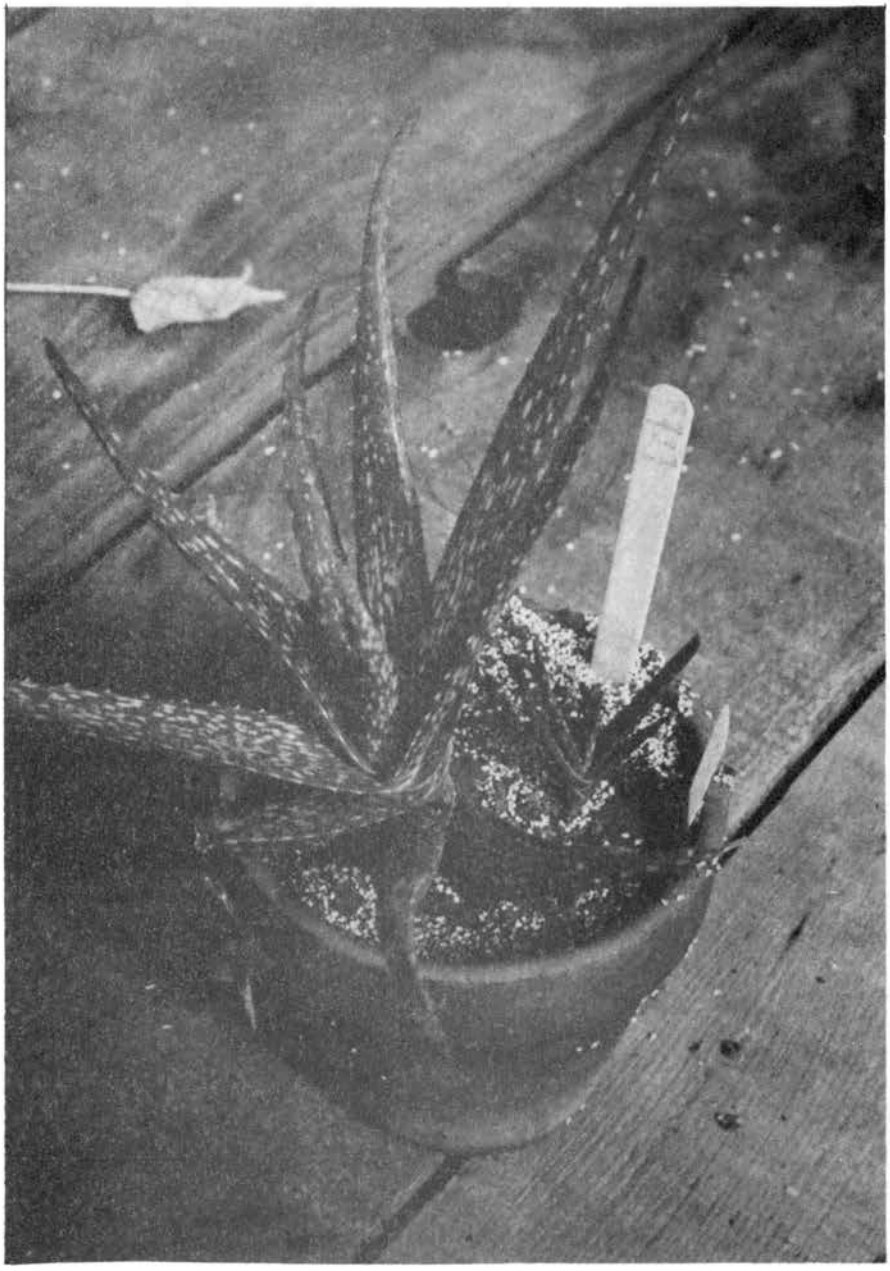


Figure 2. Young Aloe barbadensis plants

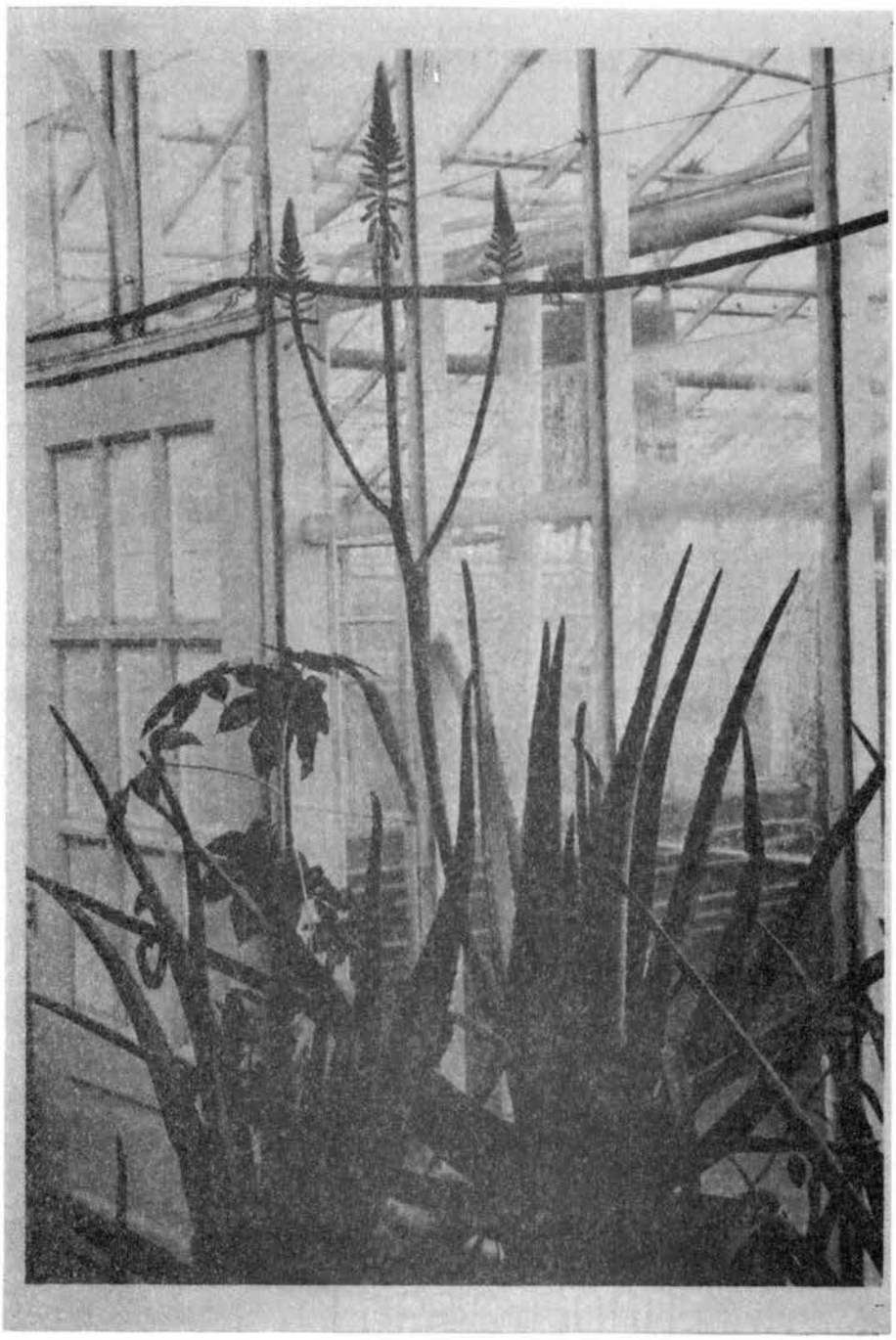


Figure 3. Mature Aloe barbadensis plants in flower

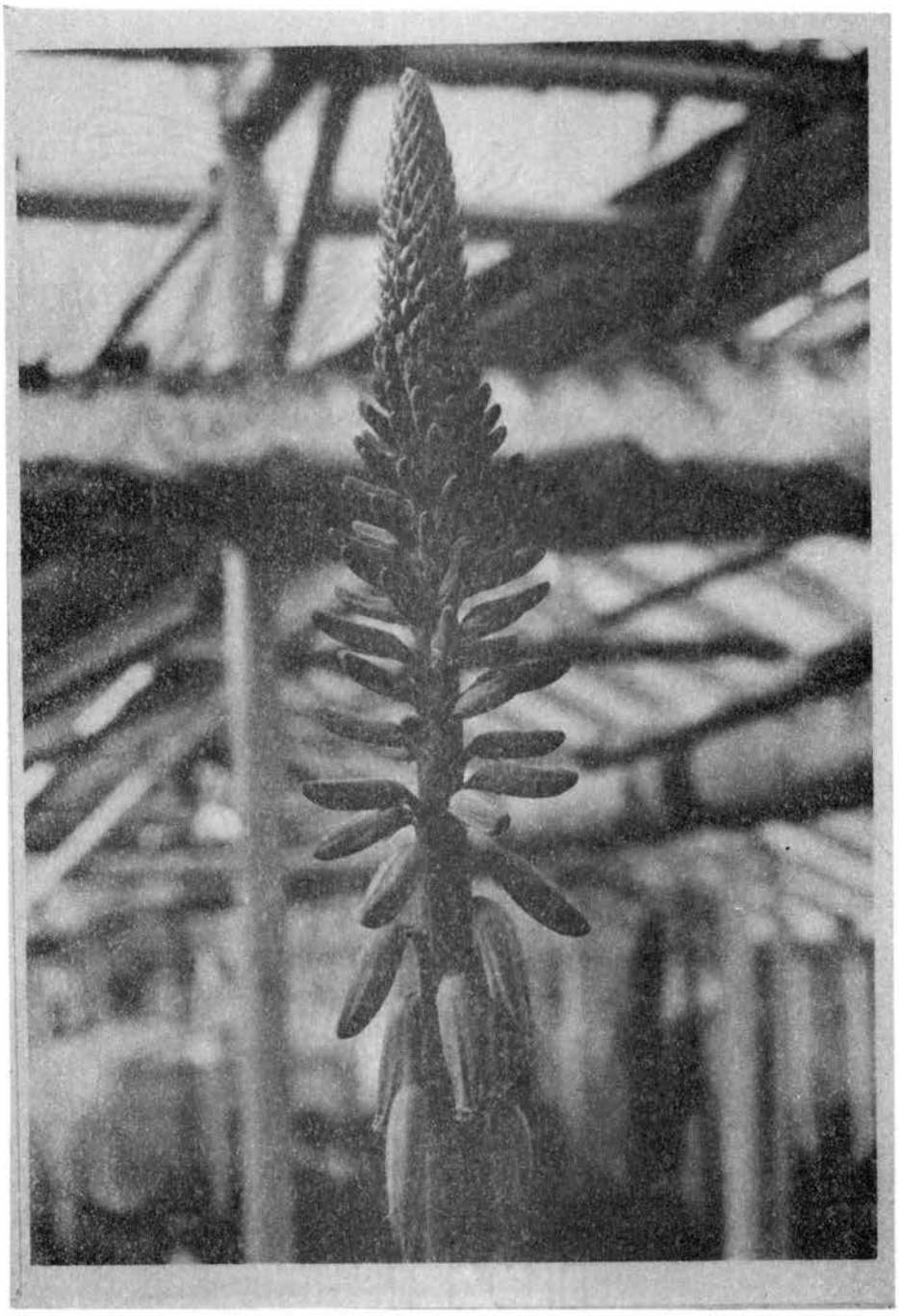


Figure 4. Detailed view of the upper part of the flowering stalk

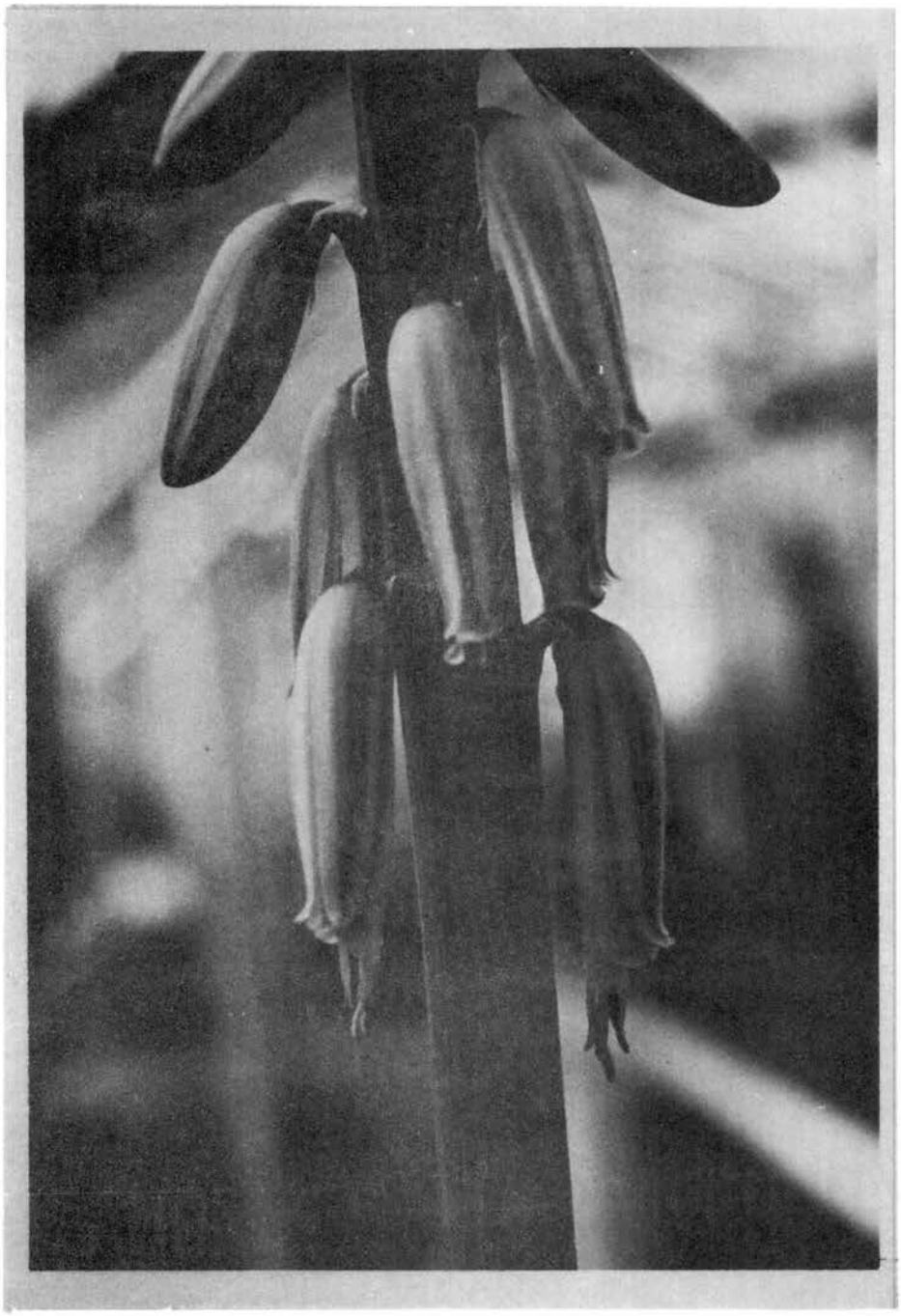


Figure 5. Close-up photograph of the Aloe barbadensis flowers which were greenish yellow in color

of the United States Department of Agriculture, Beltsville, Maryland, where it was identified as Aloe barbadensis Miller (synonymous with Aloe vera).

Apparatus and Reagents

Gas - Liquid Chromatography

All gas - liquid chromatographic analyses were performed on a modified Barber - Colman Model 5000 gas chromatograph equipped with a hydrogen-flame detector (21). The hydrogen was supplied by a Milton Roy Model E-150A hydrogen generator. Helium was used as the carrier gas. The column used was a 10-foot silanized glass column (¼ inch O.D.) and was packed with 20% Apiezon L on Anakrom ABS, 60 - 80 mesh.

Thin-Layer Chromatography

Commercially prepared analytical and preparative Silica Gel GF thin-layer plates were used which were obtained from Brinkmann Instruments, Westbury, New York, or from Analtech, Newark, Delaware. Two different solvent systems were used. One was chloroform:methanol (5:1, v/v) and the other was hexane:acetone:ethanol (40:10:4, v/v/v).

Mass Spectrometry

Mass spectrometry was performed on a prototype of the LKB Model 9000 combination gas chromatograph - mass spectrometer as described by Waller (21). This instrument was built in the laboratories of Dr. Ragnar Ryhage at the Karolinska Institutet, Stockholm, Sweden.

The same gas chromatography column was used in this instrument as is described above. The peak heights of the collected spectra were measured manually. These data were introduced into an IBM 360/65 computer which plotted them on a Cal Comp Model 565 plotter.

Miscellaneous Equipment

A VirTis Model 23 homogenizer and a Waring Blender Model CB-2 were used to prepare the Aloe samples for analysis. The volume of solvent where indicated was reduced with a Buchler Instrument rotary evaporator. A Chromato-Vue ultraviolet view box with a short UV lamp (254 nm) and a long UV lamp (366 nm) was used for determining the spots which fluoresce or absorb in UV on the thin-layer plates. All residues were lyophilized on a VirTis lyophilizer.

Reagents

All solvents and reagents were of a reagent grade and were used as received without further purification. The nitrogen which was used for evaporations was Linde dry grade.

Methods

Isolation of Compounds

Two general methods were used for the isolation of compounds:

Solvent Extraction. A procedural scheme is shown in Figures 6 and 7 . Since there are reports that severed Aloe leaves rapidly lose their medicinal properties (3,12), it was decided not to dry the plant material before extraction. Therefore, freshly collected

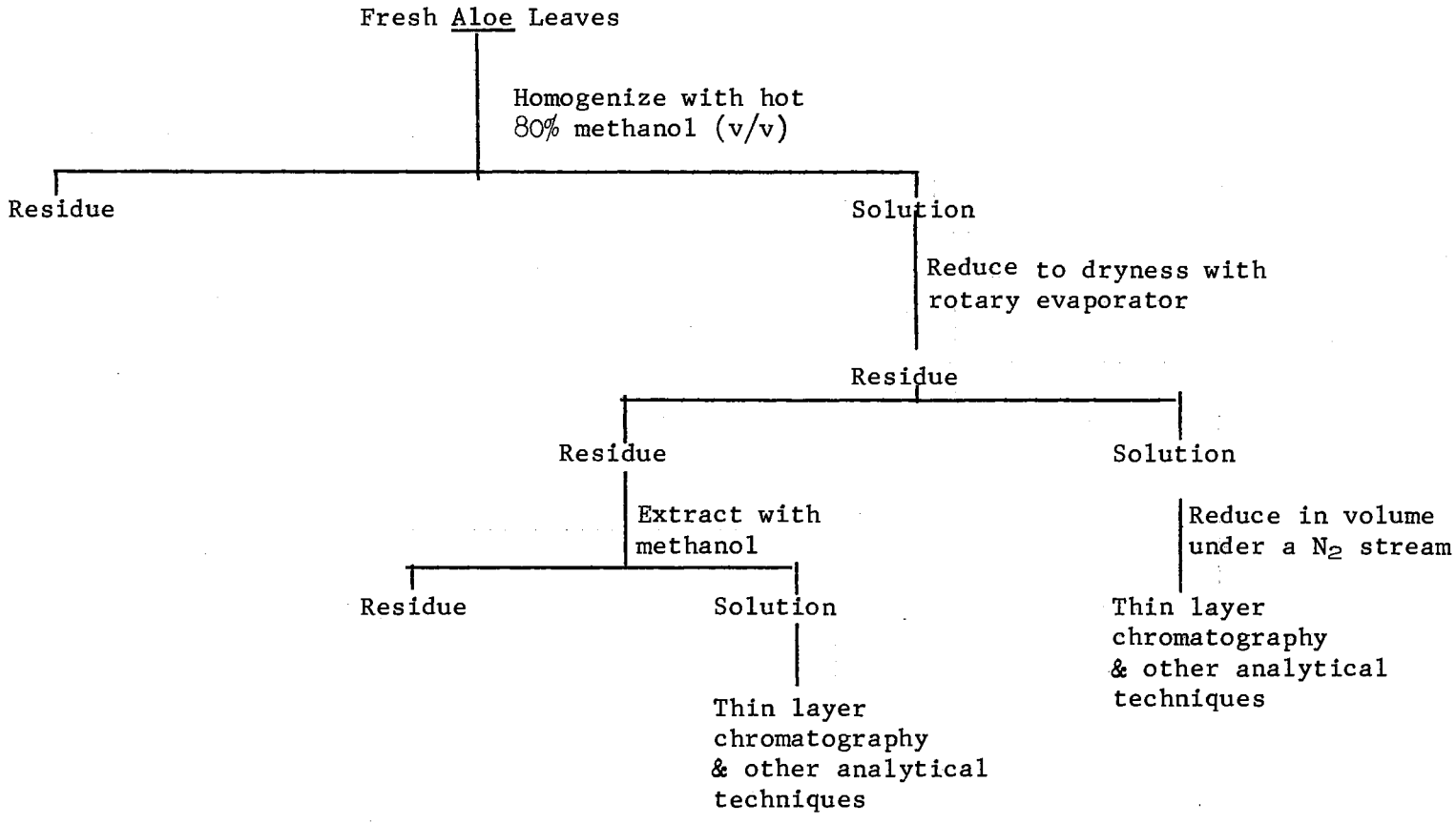


Figure 6. Scheme for solvent extraction of whole fresh Aloe leaves using a hot 80% methanol solution

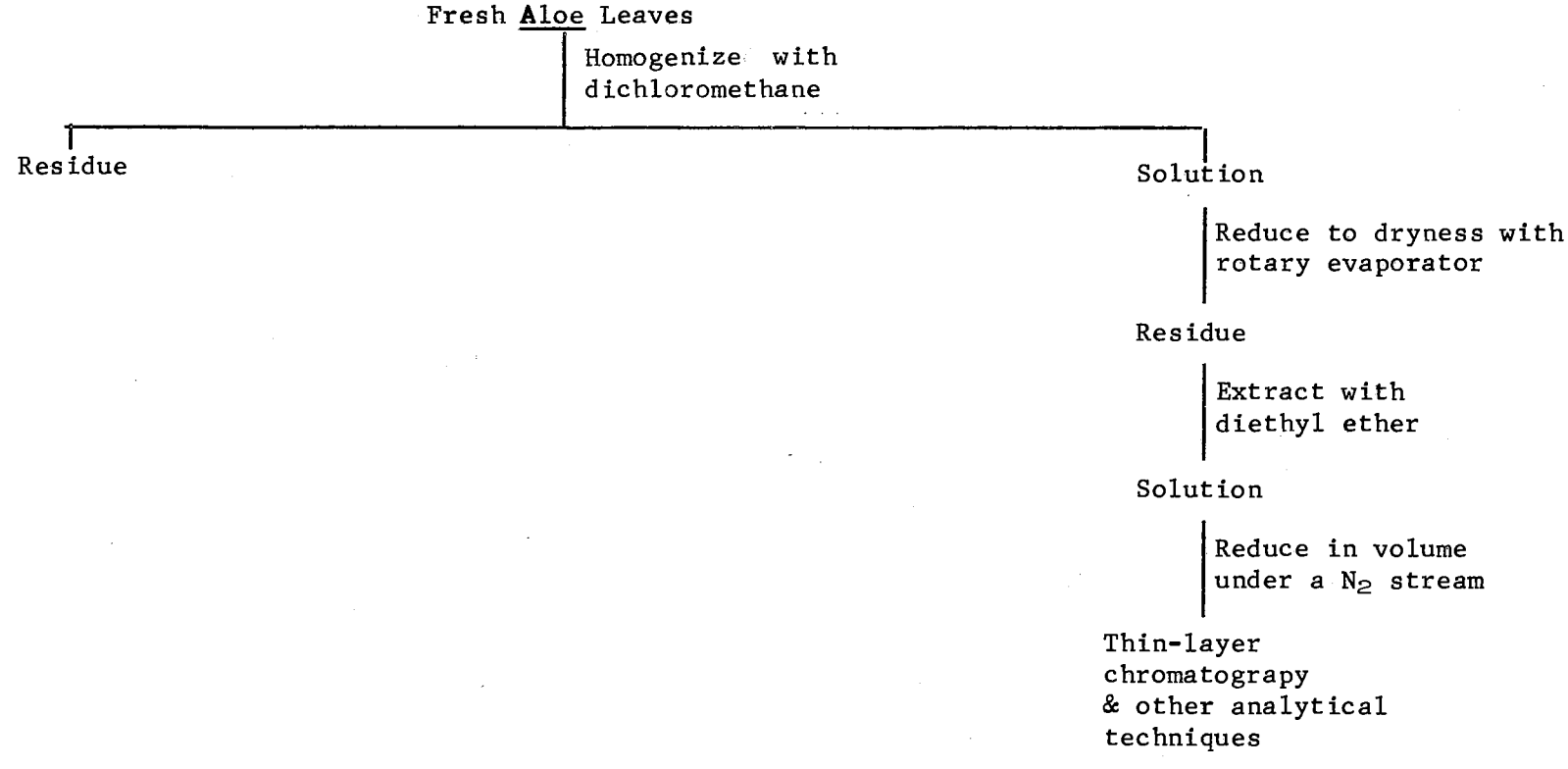


Figure 7. Scheme for solvent extraction of whole fresh Aloe leaves using dichloromethane

leaves from Aloe barbadensis which had been obtained locally were homogenized with either dichloromethane or boiling 80% methanol using a volume equal to ten times the weight of the plant material. The solution was removed from the plant residue by vacuum filtration using a water aspirator and a sintered-glass Buchner funnel of coarse porosity. The plant residue was reintroduced into the homogenizing flask, and the above process was repeated four times.

The solvent was removed from the solution obtained from the extraction process by vacuum distillation using a Buchler rotary evaporator. Diethyl ether was used to extract the residue which remained in the flask. When dichloromethane was used as the original extraction solvent, the residue was completely soluble in diethyl ether, but when 80% methanol was used, part of the residue did not dissolve in diethyl ether. The part that did not dissolve in diethyl ether was dissolved in methanol. The volume of the diethyl ether was reduced under a stream of dry nitrogen.

Steam Distillation. A procedural scheme is shown in Figure 8. Leaves and mature plants which had been brought from Texas were used. Before the leaves were steam distilled, the tough outer portion consisting of the cuticle, epidermis, and mesophyll was cut away leaving a colorless, almost transparent, gel beneath (see Figure 9). If the leaves were kept for more than three or four days, they began to turn dark and translucent; when the leaves began to develop these spots, they were immediately discarded. Both the green outer portion and the colorless inner part as well as the "stalk" (that portion of the plant above the ground remaining after the leaves had

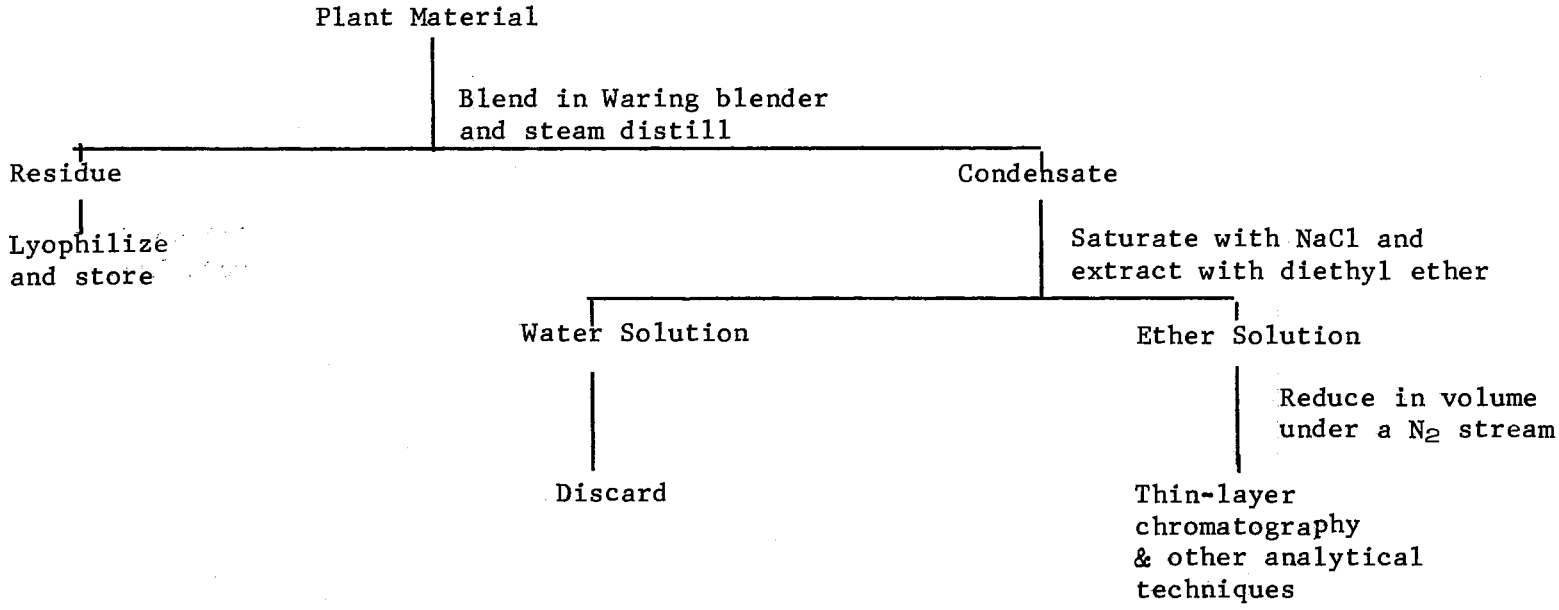


Figure 8. Scheme for steam distillation of various parts of Aloe barbadensis

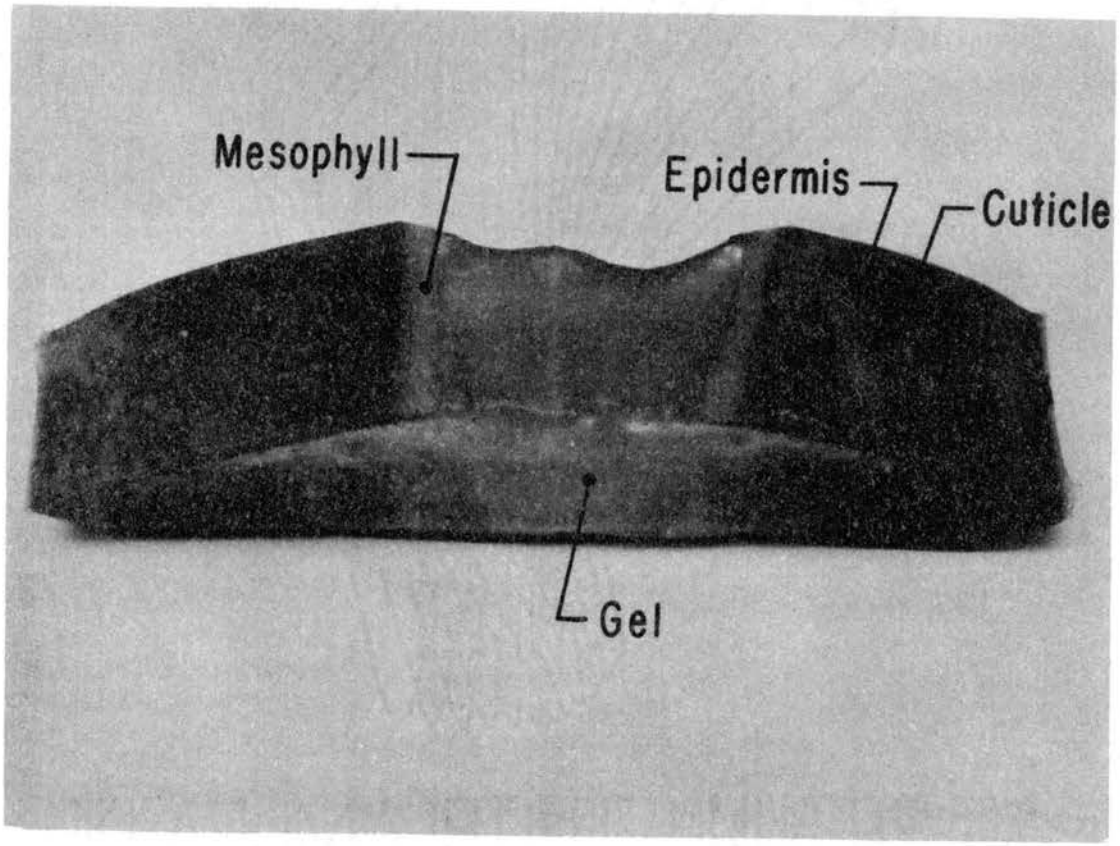


Figure 9. Photograph of a 1-inch section of an Aloe leaf which has been partly cut to show the various layers. The leaf was originally 24 inches long and approximately 4 inches wide at the base

been removed) and the roots were steam distilled, each separately. The distillation was allowed to proceed as long as the condensate had a definite odor; this required approximately five hours.

The condensate from the steam distillation was saturated with sodium chloride after which it was extracted with diethyl ether. The diethyl ether phase was dried with anhydrous sodium sulfate and was reduced in volume using a stream of dry nitrogen.

Separation of the Isolated Compounds

The plant extract from either solvent extraction or steam distillation was spotted on analytical chromatographic thin-layer plates. In the case of extract obtained from solvent extraction, the chloroform:methanol system was used while the hexane:acetone: ethanol system was used with the extract obtained from the steam distillation. Following chromatography, the retention factors (R_f) of the spots that could be seen under visible light and those that could be seen under ultraviolet (UV) light were measured. In order to identify areas in which alkaloids may be present, the analytical plates were sprayed with Dragendorf's reagent (22), and the R_f of the positive spots was determined.

To separate larger quantities of material for further identification, plant extract was streaked on preparative thin-layer plates. After determining the areas of interest from the R_f values, the bands were scraped off the glass plate and were eluted with diethyl ether. The volume of the diethyl ether was reduced under a stream of dry nitrogen.

Identification of the Compounds

This concentrated ether extract from the thin-layer plates then was subjected to analysis by gas chromatography - mass spectrometry. The mass spectra obtained were compared with tables of published data (23, 24, 25, 26). Following a tentative identification based upon matched spectra, a sample of the pure suspected compound was obtained and chromatographed on a gas - liquid chromatograph. The retention times of the pure compound and of the unknown were compared. Following this comparison, the unknown and the known were co-chromatographed. Mass spectra of the unknown and of the known were obtained under identical conditions and were compared. If all of these comparisons were positive, then the known and the unknown were assumed to be identical.

Sugar Determination

A sample of the transparent gel-like portion of the Aloe leaf which had been freed of all the green material was lyophilized and then submitted to Dr. B. G. Hudson's laboratory for a sugar analysis. The lyophilized sample was hydrolyzed in 2N H_2SO_4 for four hours at 100°C. The hexoseamines were separated from the neutral sugars on a Dowex H column and the neutral sugars were determined by the method of Lee, McKelvy, and Lang (27).

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Investigation

Solvent Extraction

Two solvents of different polarities were used for the extraction of Aloe leaves. A methanol:water solution (80:20, v/v) was chosen as a solvent to extract the more polar compounds from the whole leaves and dichloromethane was chosen to extract the less polar compounds.

Methanol Extraction. The green-colored residue which remained after evaporation of the methanol was extracted with diethyl ether. This residue was only slightly soluble in ether. The material that remained after this extraction was then further extracted with undiluted methanol. A tan residue was left which became dark green in color within an hour upon exposure to air. This dark green solid was soluble in water, but insoluble in diethyl ether, dichloromethane, methanol, acetone, glacial acetic acid, or diethyl amine. When stored in a tightly stoppered container, this tan residue remained unchanged in color.

Following thin-layer chromatography of the methanolic extract in chloroform:methanol (5:1) three major spots could be seen on the thin-layer plate when viewed at 366 nm; an orange spot with an R_f

of 70, a bright blue spot with an R_f of 57, and another orange spot with an R_f of 23. Other minor spots were observed. When sprayed with Dragendorf's reagent, material that had remained at the origin became blue upon continued exposure to light; however, no true Dragendorf positive spots were seen.

Dichloromethane Extraction. Essentially all of the green-colored residue which remained after evaporation of the dichloromethane was dissolved in diethyl ether. When this ether was submitted to thin-layer chromatography a number of spots, the more predominant of which are indicated in Figure 10, were seen when the plate was observed under 366 nm light. This extract contained no positive Dragendorf material.

Film Production of Dichloromethane. The plant material that had been extracted with dichloromethane was dried at room temperature for one week prior to storage. At the end of this time, it was observed that a thin transparent, colorless, and flexible film had been formed on the glass container around the residue. An insufficient amount of film was formed for a qualitative or quantitative study.

Steam Distillation

Before steam distillation, the plant was separated into its various components, i.e., the leaves, the stalk, the flower, and the roots. The leaves were further dissected so that the inner gel was separated from the outer layers.

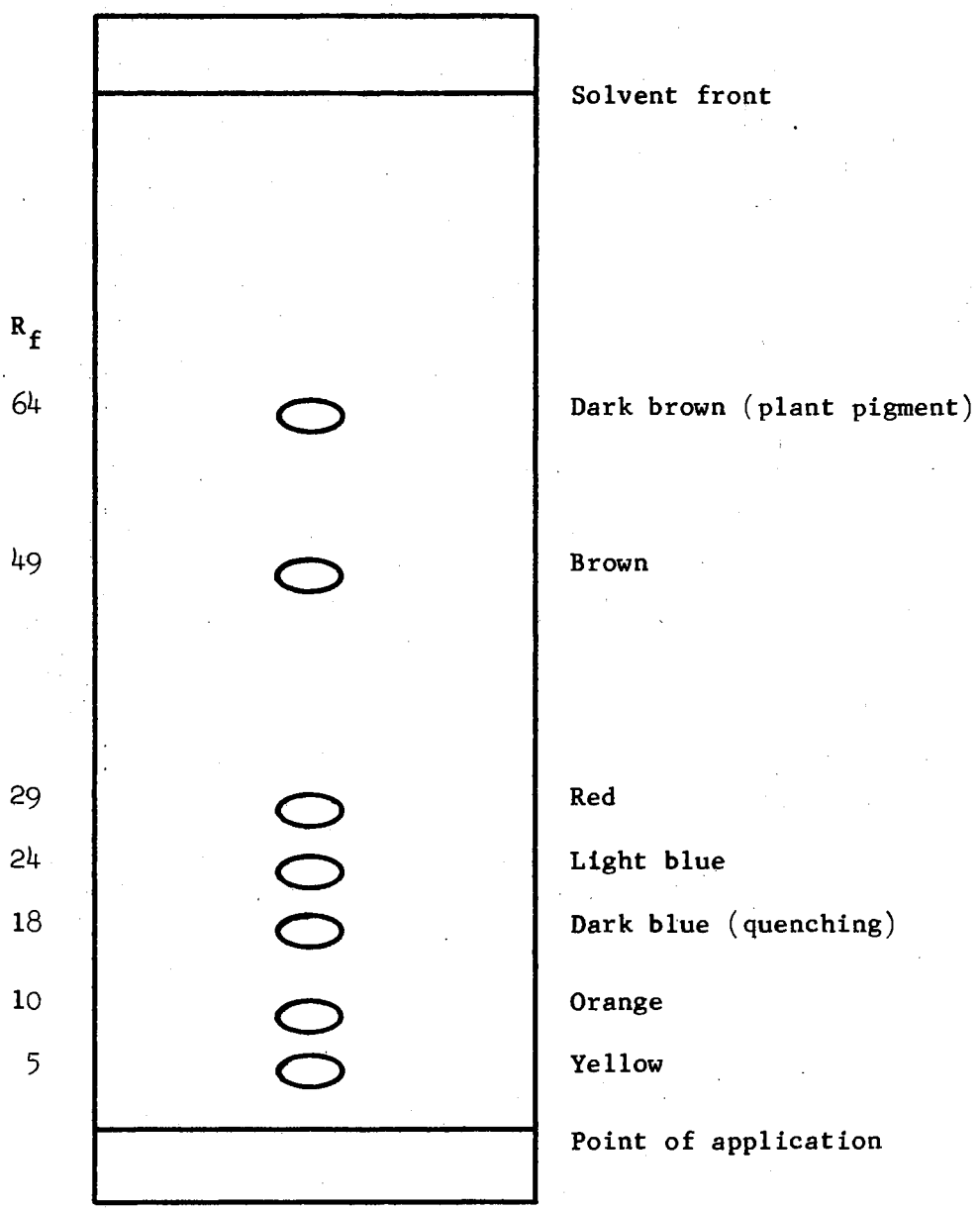


Figure 10. Thin-layer chromatogram developed in chloroform: methanol (5:1) of a dichloromethane extract of Aloe barbadensis leaves as viewed at 366 nm

As the blended gel was heated during the distillation, it changed from nearly colorless to a very deep pink. The color change was not due to a change in pH, for the pH remained at 5.0 during the distillation. Following the acid distillation, the gel was made basic with 10% aqueous sodium hydroxide and distillation was continued. The gel became very dark in color and appeared to be partly digested by the base, while the distillate had an odor similar to that of burnt wood.

After the condensate had been saturated with sodium chloride and had been extracted with diethyl ether an attempt was made to determine if all of the organic compounds had been removed. Since the solution was saturated with salt, it could not be frozen and therefore could not be lyophilized. Therefore, the water was removed under vacuum in a rotary evaporator at as low a temperature as possible. Following the removal of water, the salt which remained was submitted for mass spectrometry using the direct probe. If any organic compounds remained, a spectrum would be observed. Unfortunately, the salt still contained so much water that this test could not be satisfactorily performed.

A comparison of the spots resulting from thin-layer chromatography of the volatiles from the gel of the inner part of the Aloe leaves, the outer layers of the leaves, the stalk, and the roots as seen under visible light and 366 nm light and after spraying with Dragendorf's reagent is seen in Figure 11. Such a comparison, however, has three shortcomings: (1) it was extremely difficult even to estimate whether or not the same amount of material was being applied to the thin-layer plate since the amount of the original

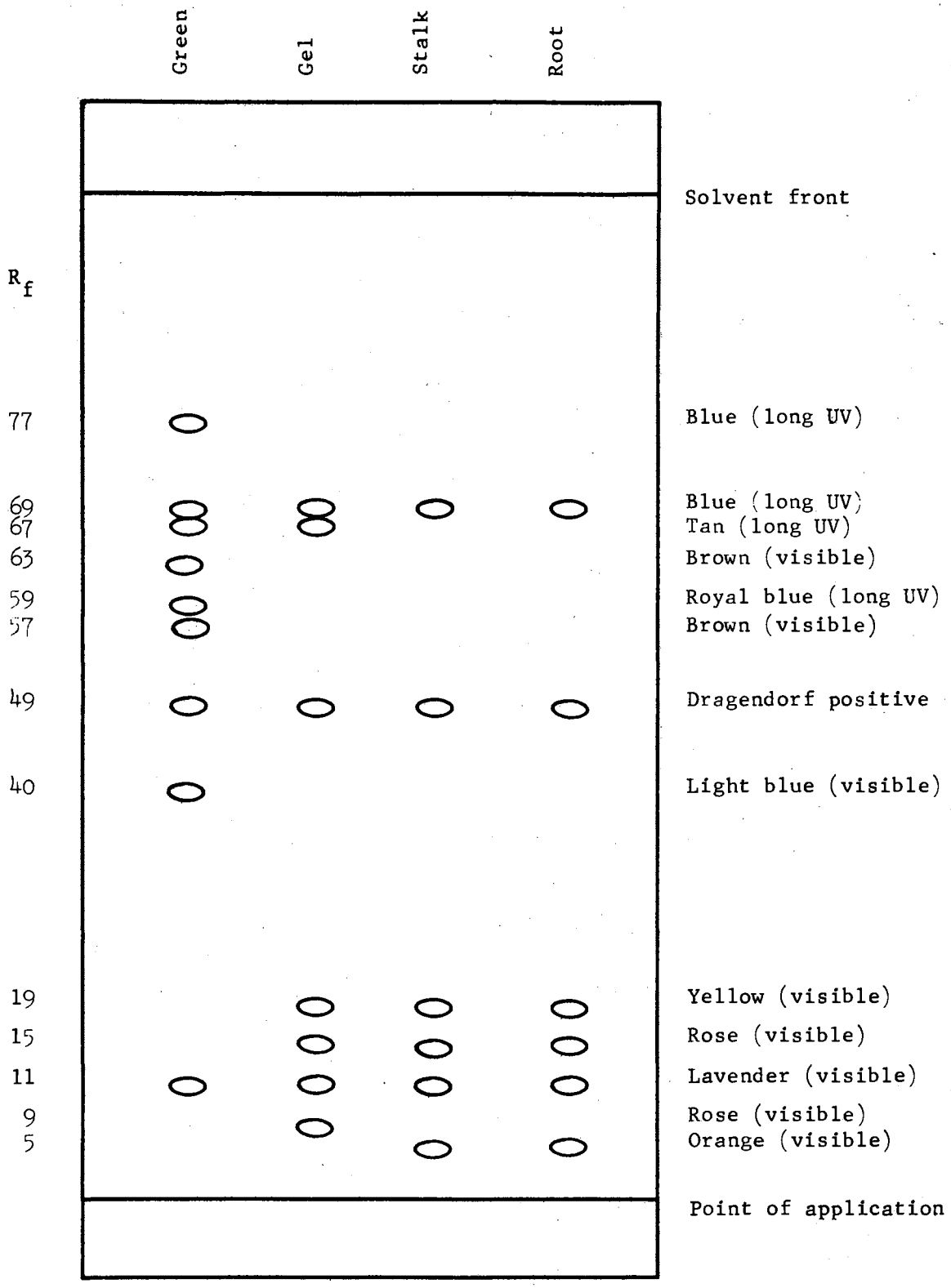


Figure 11. A comparison of the steam volatiles from the inner leaf gel, the outer leaf layers ("green" above), the stem, and the roots of Aloe barbadensis as seen on a thin-layer chromatogram developed in hexane:acetone:ethanol (40:10:4)

plant extracted was too large for the scales available and therefore could not be weighed accurately; (2) some of the gel from the inner part of the leaf remained on the outer portion as the leaf was dissected; and (3) near the top of the plant, it was hard to determine where the leaves began and the stalk ended.

Gas Chromatography - Mass Spectrometry

Following the steam distillation of the various parts of the Aloe plant, the compounds which had been distilled were extracted from the condensate with diethyl ether. The ether solution was concentrated and this concentrate was analyzed by gas chromatography - mass spectrometry.

Outer Layers of the Leaves

When the diethyl ether extract containing the steam volatile compounds was subjected to thin-layer chromatography (TLC), a positive Dragendorf spot at R_f 49 was observed. Another aliquot of this extract was subjected to thin-layer chromatography using preparative plates and the area which contained this compound(s) was scraped off the plate and extracted with diethyl ether. This TLC extract was reduced in volume and subjected to gas - liquid chromatography, and the results are shown in Figure 12.

When the TLC extract was analyzed by gas chromatography - mass spectrometry, the peak labeled A was found to represent a compound having a retention time of 10½ minutes at 208° C and appeared to have a molecular weight of 220 and a base peak at m/e 205. Peaks which occurred at ½ m/e values are 63½, 64½,

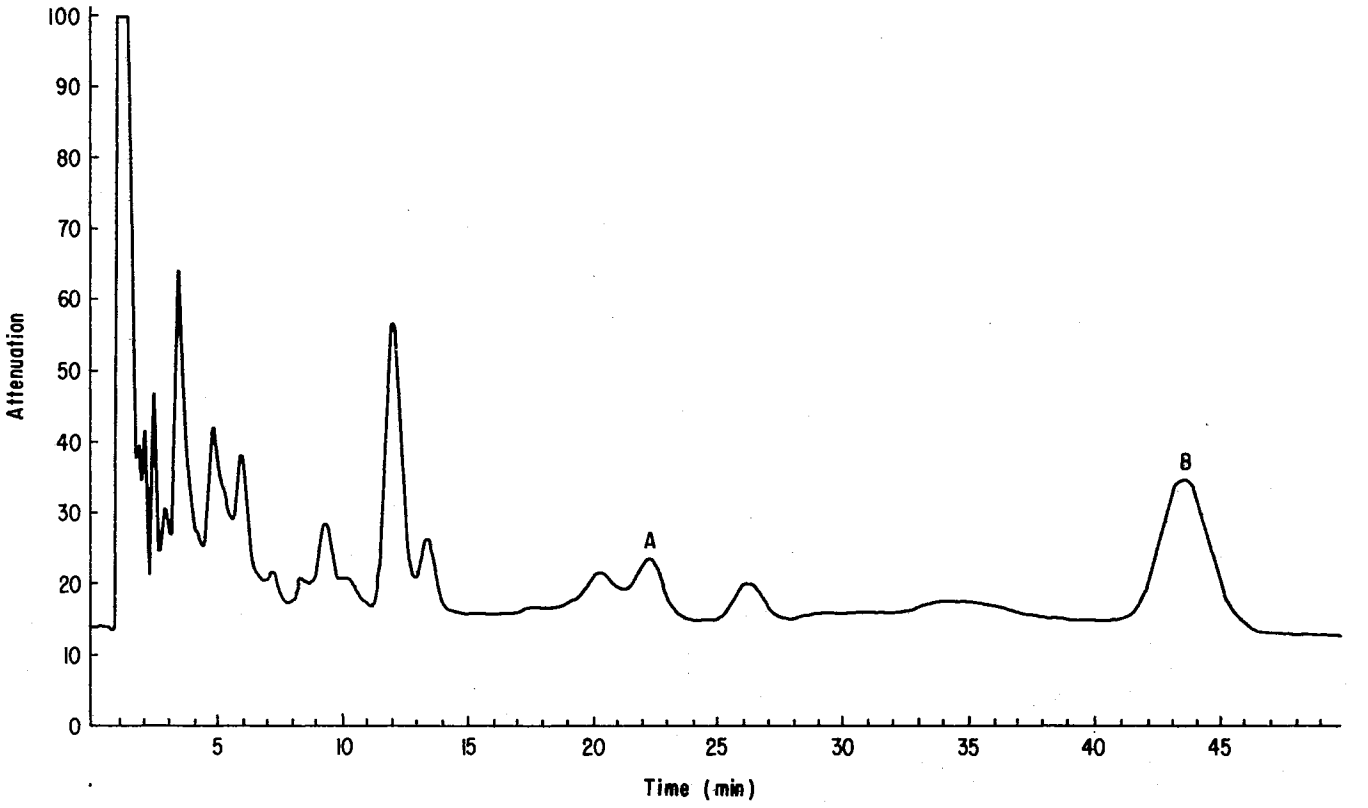


Figure 12. Gas - liquid chromatogram of the extract from the area (R_f 49) of a thin layer chromatogram which yielded a Dragendorf positive spot.

65½, 67½, 73½, 80½, 81½, 94½, 95½, and 102½; peaks were observed also at 57½ and 58½ in other experiments. In addition, a metastable ion occurred at m/e 191; this confirmed the m/e 220 ⟶ m/e 205 transition. The ten most intense peaks together with these ½ m/e peaks correspond to those published for 2,6-di-tertiary-butyl-4-methylphenol (BHT) (25). A sample known to be BHT was obtained and analyzed in an identical manner. A comparison of the spectrum of the BHT standard and the spectrum of the unknown is shown in Figure 13; not shown are the peaks that occur at ½ m/e values which also corresponded with each other.

The peak labeled B was found to represent a compound having a retention time of 22½ minutes at 208° C. This compound was missed in earlier studies run at a lower temperature (150° C) because of its long retention time (approximately 120 minutes). The base peak is also the parent peak (M^+ 169). Peaks which occurred at ½ m/e values are 69½, 70½, 71½, 82½, 83½, and 84½; peaks were also observed at 57½ and 58½ in other experiments. The eight most intense peaks corresponded to those published for diphenylamine (23). A sample known to be diphenylamine was obtained and analyzed in an identical manner. A comparison of the spectra of the diphenylamine standard and of the unknown is shown in Figure 14; in addition, the peaks that occurred at ½ m/e values which are not shown also correspond. The proposed partial fragmentation is shown in Figure 15.

As added proof, a small amount of BHT was added to the extract. Figure 16 shows that peak A was increased. Similar results are shown in Figure 16 with peak B when a small amount of diphenylamine

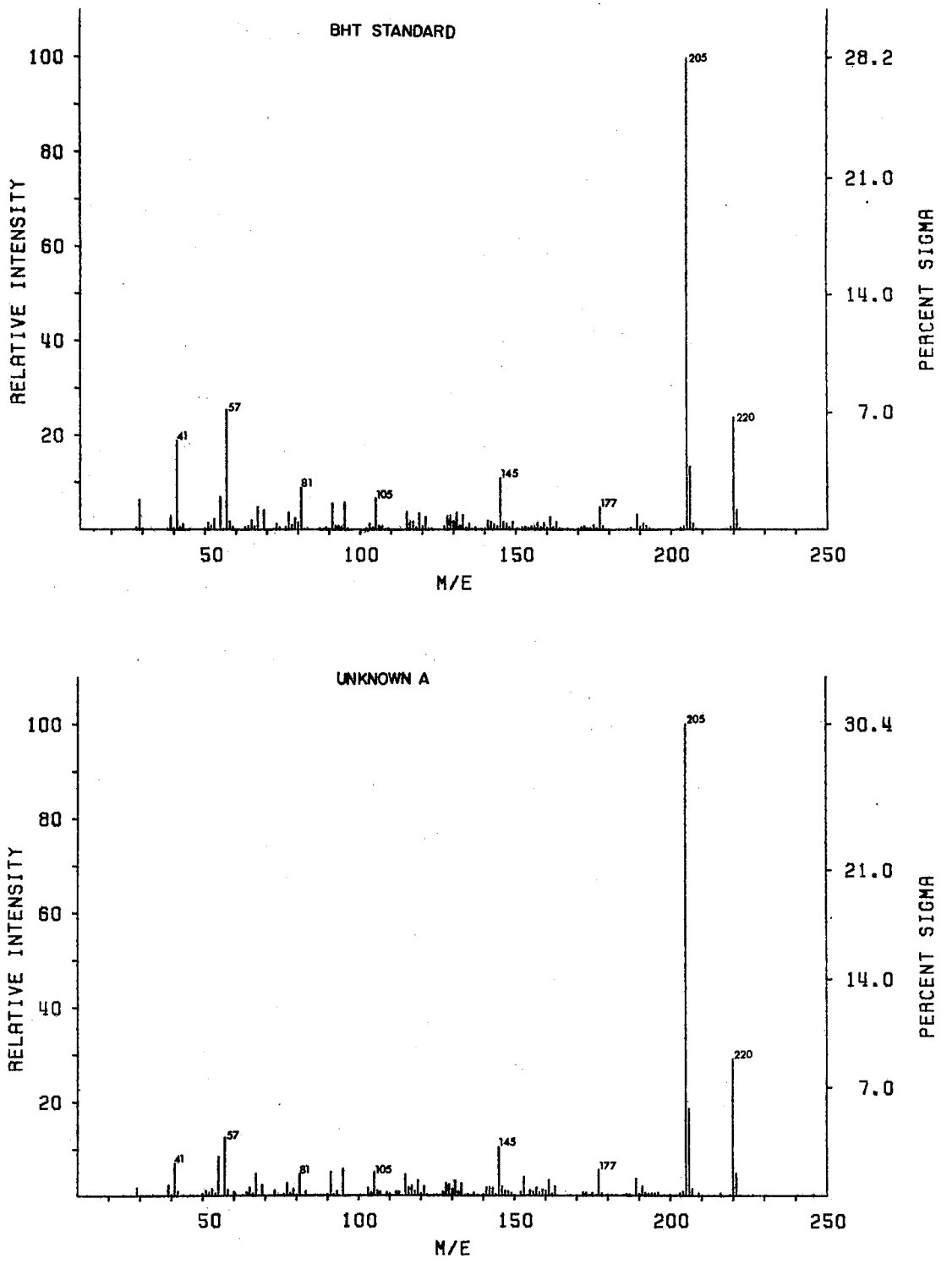


Figure 13. Mass spectra of 2,6-di-tertiary-butyl-4-methylphenol (BHT) and unknown A.

MS 2921 5-4 : MS 2846 7-5

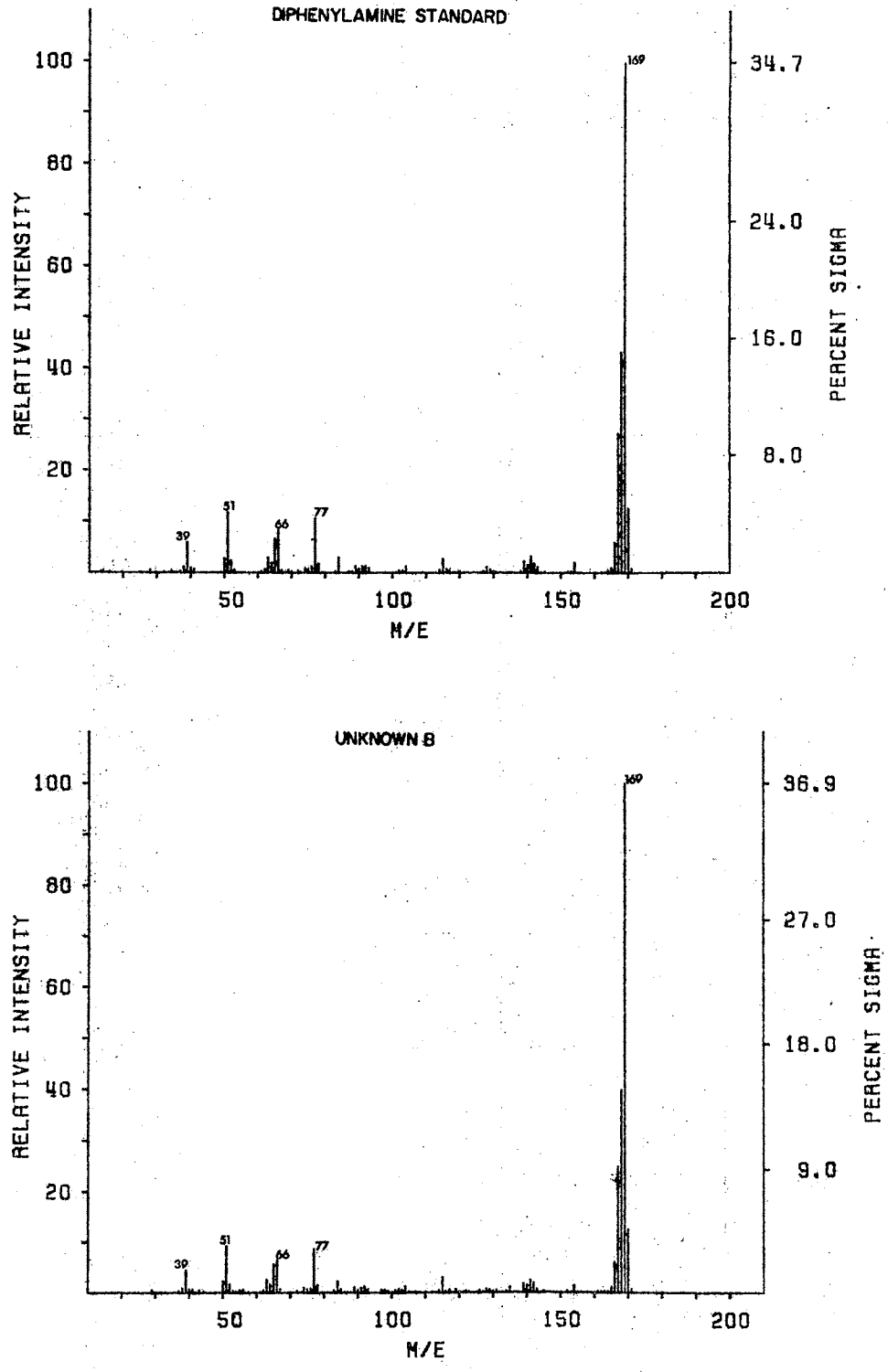


Figure 14. Mass spectra of diphenylamine and unknown B

MS 2922 4-3 : MS 2846 11-13

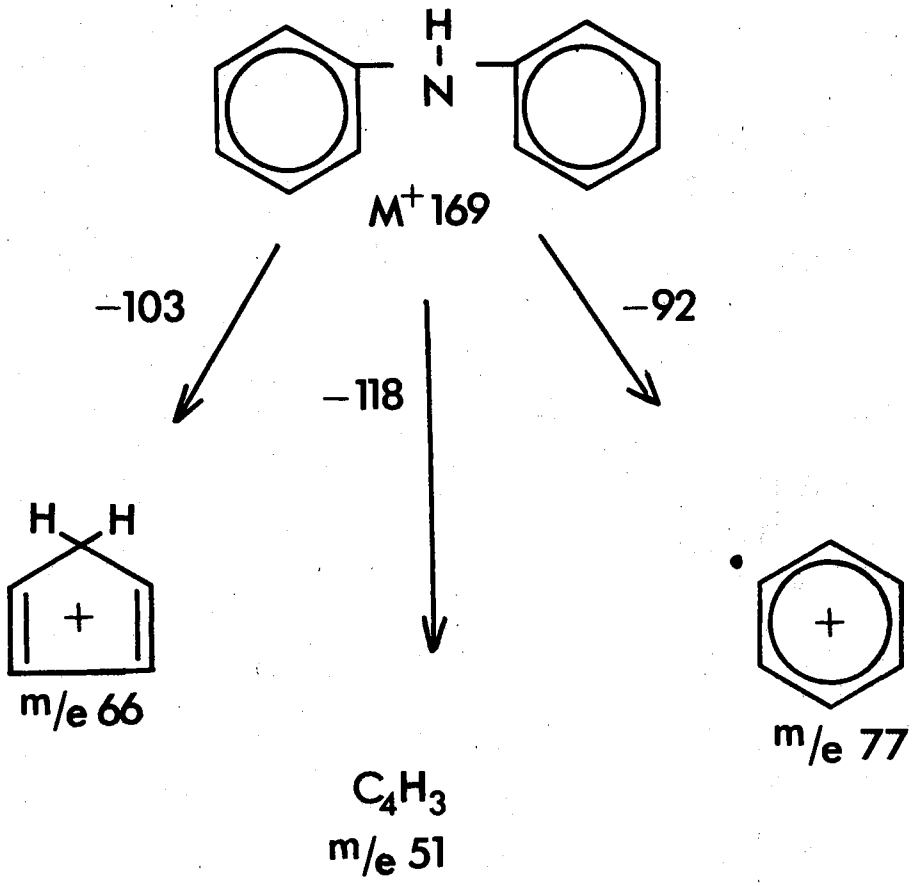


Figure 15. Proposed partial fragmentation of diphenylamine

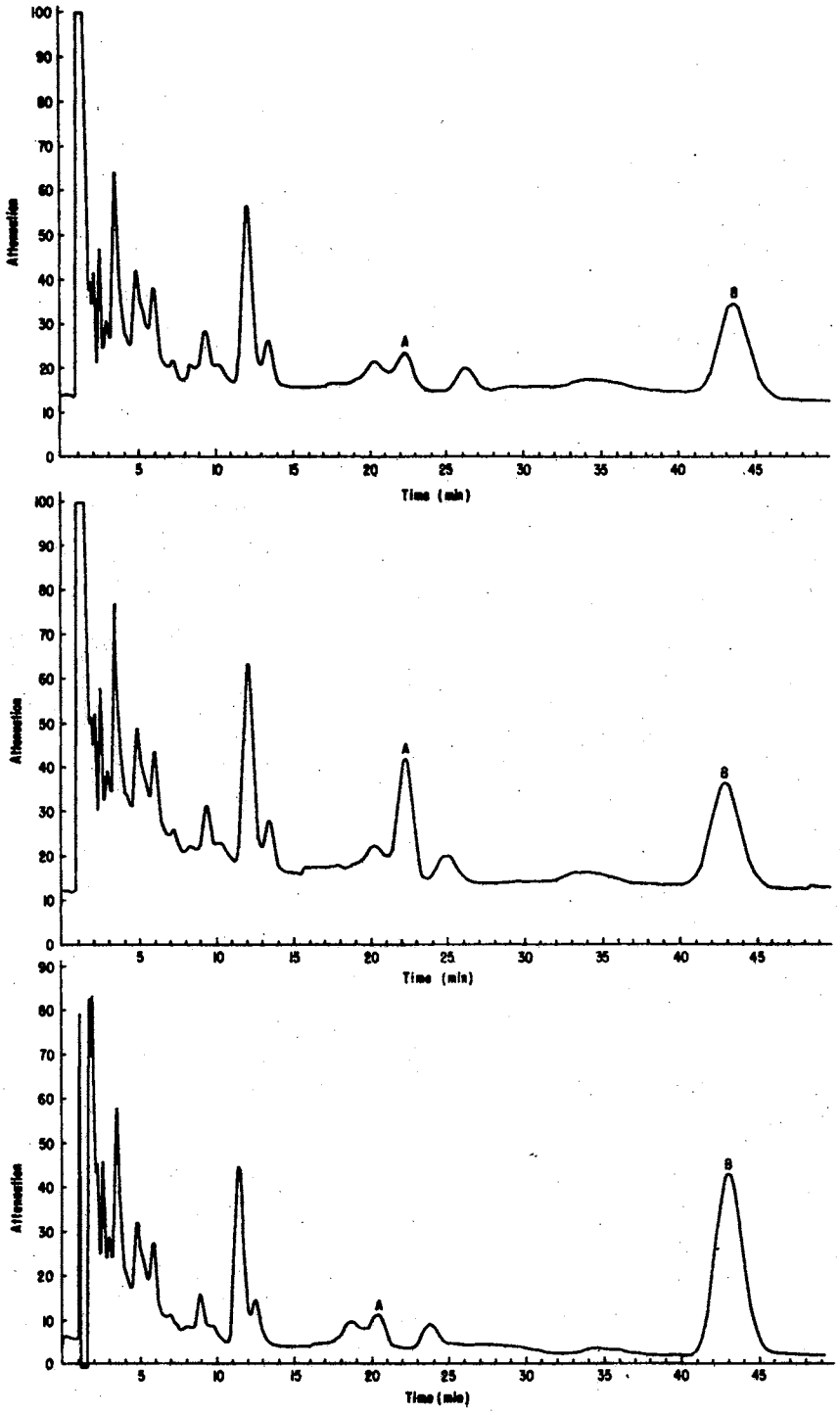


Figure 16. Gas - liquid chromatogram of an Aloe leaf extract, (top) with added BHT, (center), and with added diphenylamine (bottom)

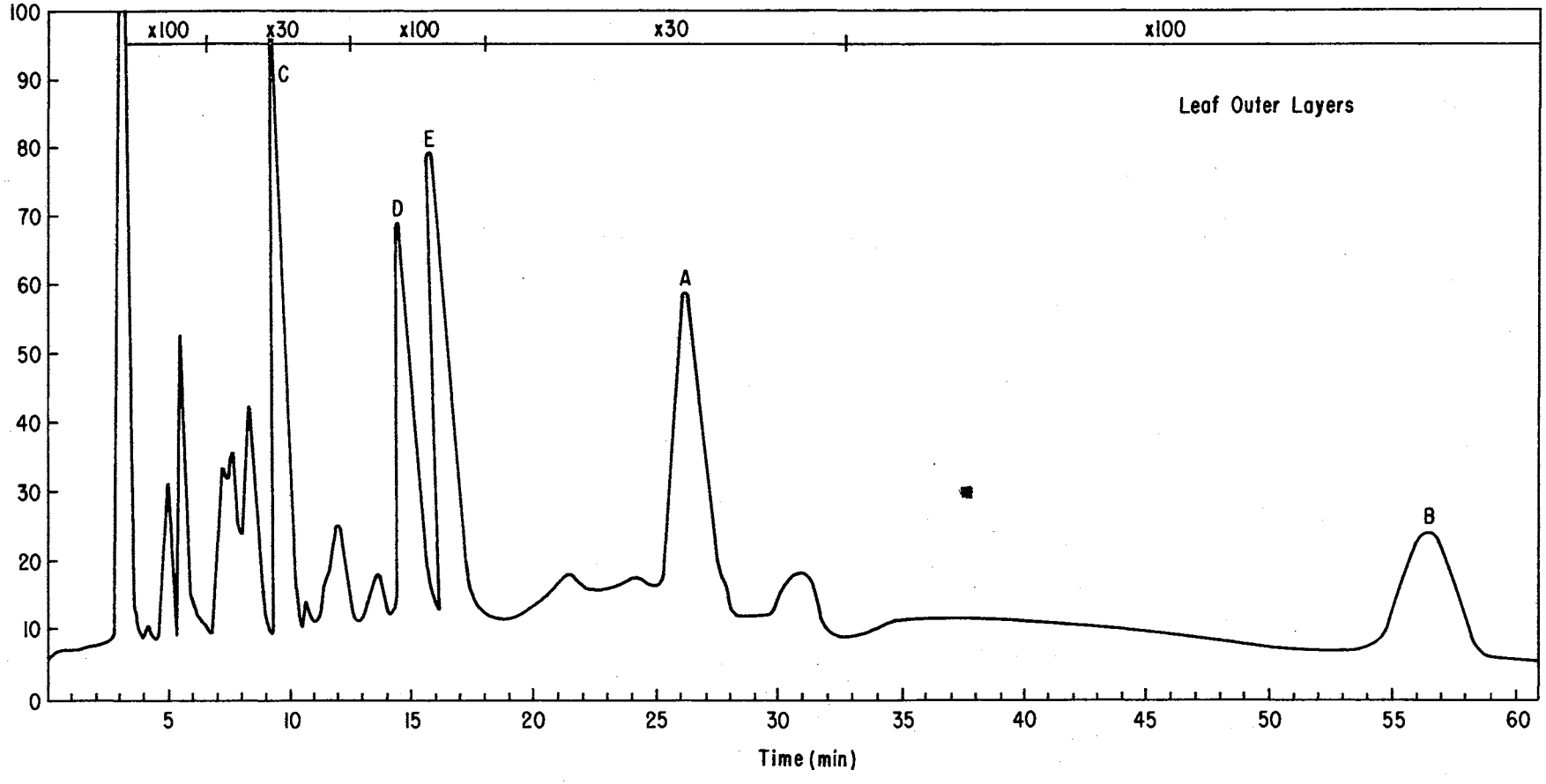


Figure 17. Gas - liquid chromatogram of an extract containing the steam volatiles from the outer part of Aloe leaves.

was added to the extract. Therefore, it is assumed that peak A represents BHT and peak B represents diphenylamine.

To rule out the possibility that the BHT and the diphenylamine were present only in the plants that had been brought from Texas, six plants which had been obtained in Stillwater, Oklahoma, and which had been grown in the greenhouse were cut up and steam distilled together. The steam volatiles were extracted from the condensate and were analyzed by gas chromatography - mass spectrometry. BHT and diphenylamine were found to be present in these plants at about the same concentration as was found in the plants of Texas origin. Identification was based on identical gas - liquid chromatographic retention times and on mass spectra.

Other major peaks which were observed in the gas - liquid chromatogram of the outer part of the Aloe leaves were those labeled in Figure 16 as C, D, and E. Analyzed by mass spectrometry, peak C which had a retention time of 6 minutes at 195° C had a molecular weight of 180 and a base peak at m/e 72 (Figure 18). The ten most intense peaks in decreasing order of m/e are 72, 44, 161, 29, 100, 132, 179, 60, 180, and 88 with relative intensities of 100, 93, 67, 60, 33, 23, 22, 21, 15 and 13, respectively. In addition, a peak which occurred at a ½ m/e is 82½ and a small metastable ion appeared at m/e 25.2. Peak D which had a retention time of 9½ minutes at 195° C had a molecular weight of 135 which was also its base peak. The ten most intense peaks in decreasing order of m/e are 135, 108, 69, 136, 63, 54, 91, 82, 137, and 45 with relative intensities of 100, 31, 9, 8, 7, 7, 6, 6, 4, and 3, respectively. A metastable ion

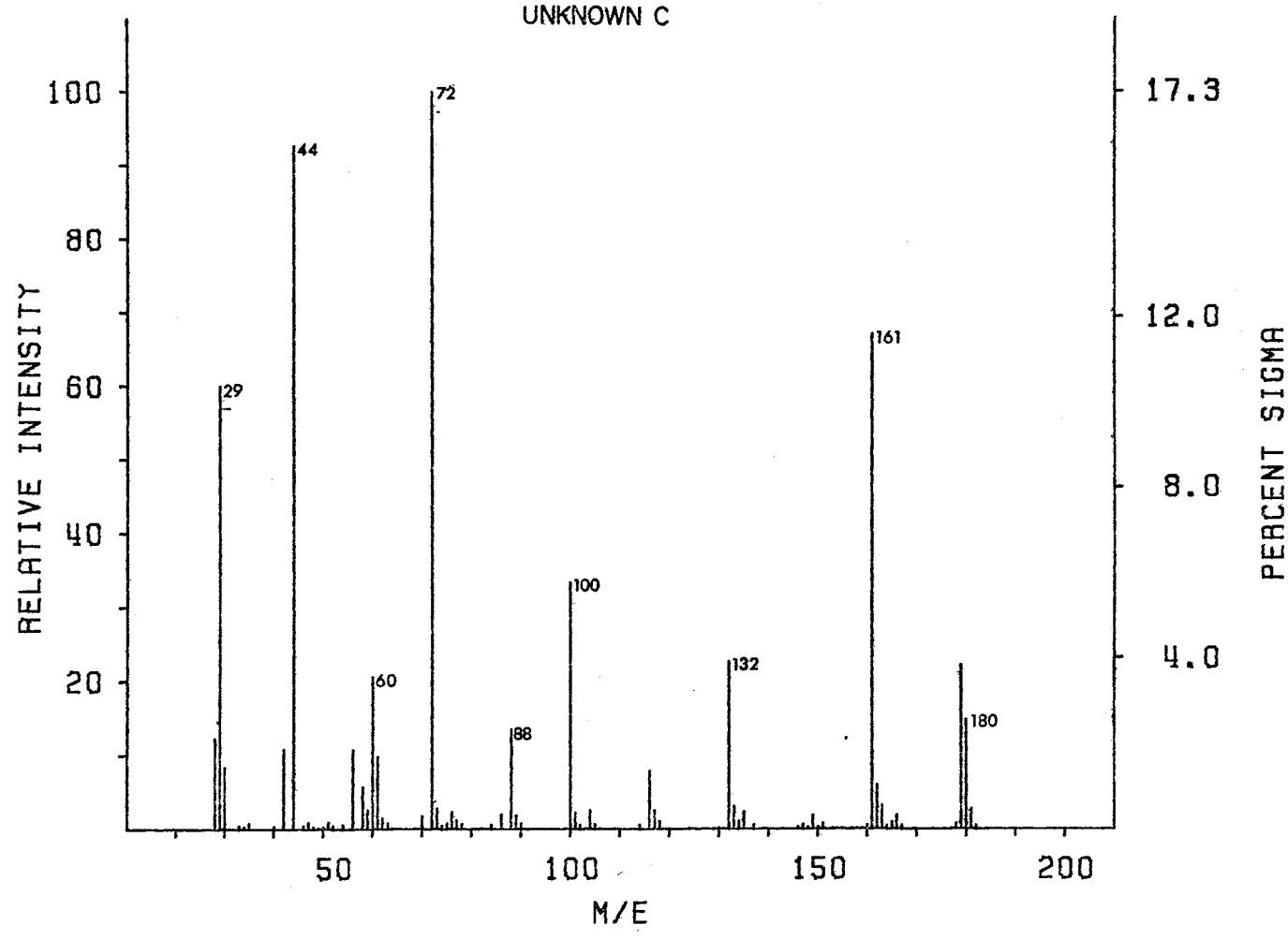


Figure 18. Mass spectrum of unknown C.

MS 2923 7-5

occurred at m/e 86.5, and peaks which occurred at ½ m/e values are 52½, 53½, 54½, and 67½. Compounds which possess a tendency to yield peaks at a ½ m/e are monoolefins, polyolefins, aromatic compounds, and heterocyclic compounds (28). Since the P+2 peak is relatively very large (52% of the P+1 peak), the presence of a sulfur atom in the molecule is to be suspected. Peak D may represent benzothiazole or a compound similar to benzothiazole. A comparison of the published spectrum of benzothiazole (26) and the spectrum of unknown D is shown in Figure 19. In addition, the published spectrum of benzothiazole lists peaks at ½ m/e as 52½, 54½, and 67½. The proposed partial fragmentation is shown in Figure 20. Benzothiazole is volatile with steam (17). A sample of benzothiazole, however, was not available so that it could be co-chromatographed with the unknown nor could its mass spectrum be obtained under conditions identical to those used for unknown spectrum. Benzothiazole has not been reported to occur naturally. Although disputed, a report has appeared that pea roots can form 4-methyl-5(2-hydroxyethyl)-thiazole from thioformamide and 4-ketopentanol (29).

Peak E which had a retention time of 10½ minutes at 195° C had a molecular weight of 101 and a base peak at m/e 57 (Figure 21). The ten most intense peaks in decreasing order of m/e are 57, 45, 56, 41, 75, 29, 100, 85, 59, and 101 with relative intensities of 100, 51, 33, 28, 27, 18, 17, 16, and 15, respectively. Metastable ions occurred at m/e 72.2 and 29.5. The odd molecular weight of this compound indicated that it contained an odd number of nitrogen atoms.

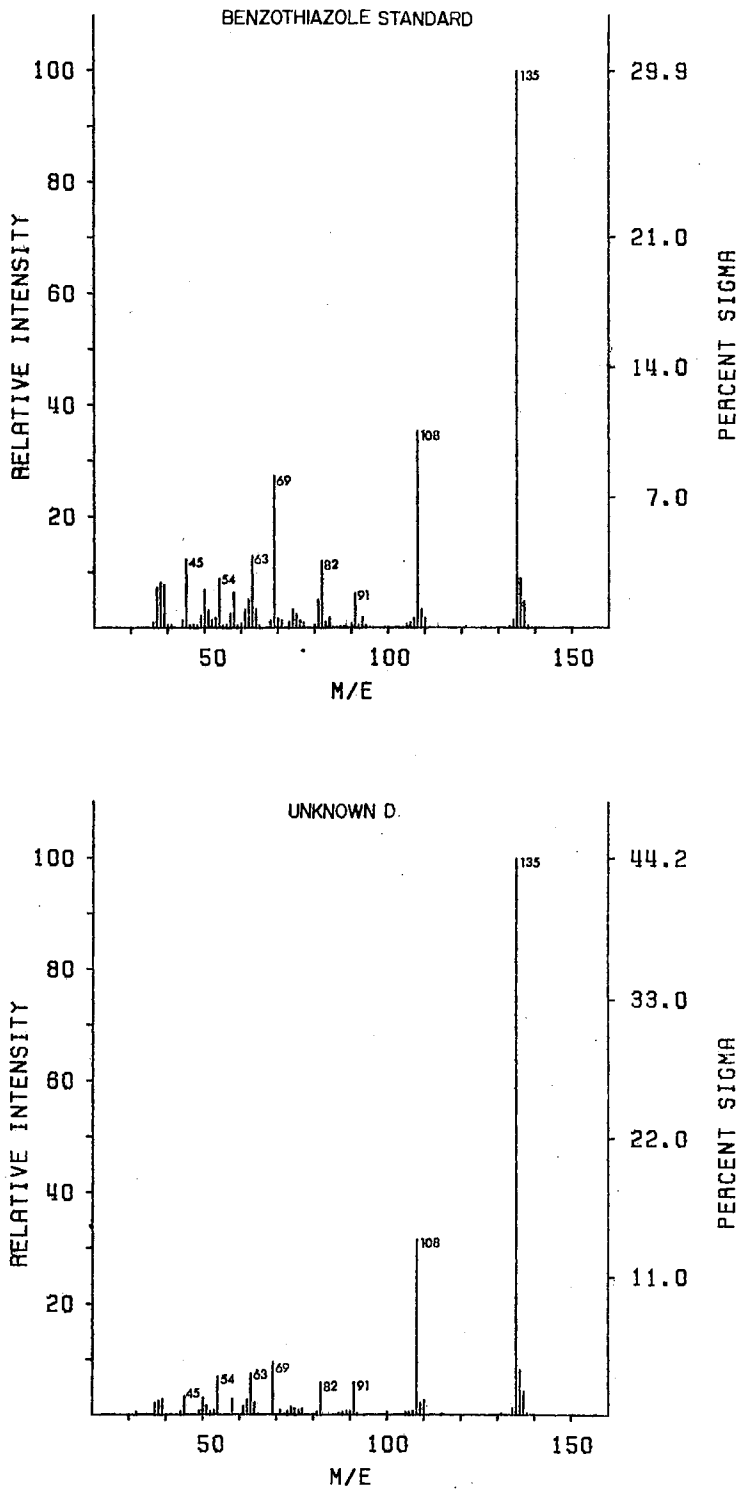


Figure 19. Comparison of mass spectra of benzothiazole and unknown D

MS 2923 11-10 (bottom)

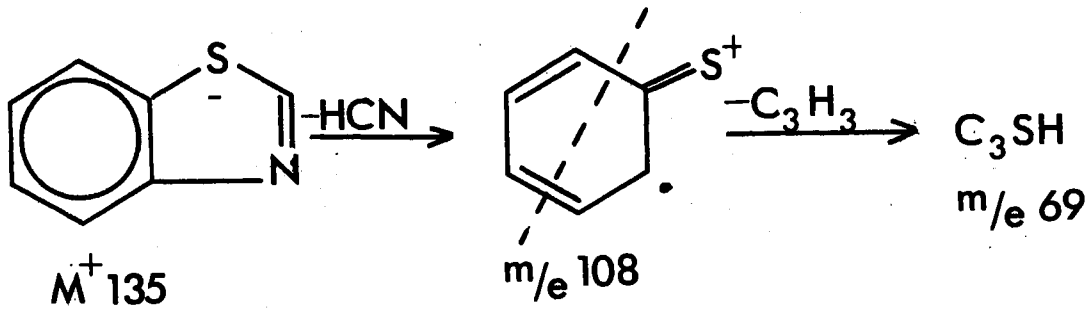


Figure 20. Proposed partial fragmentation of benzothiazole

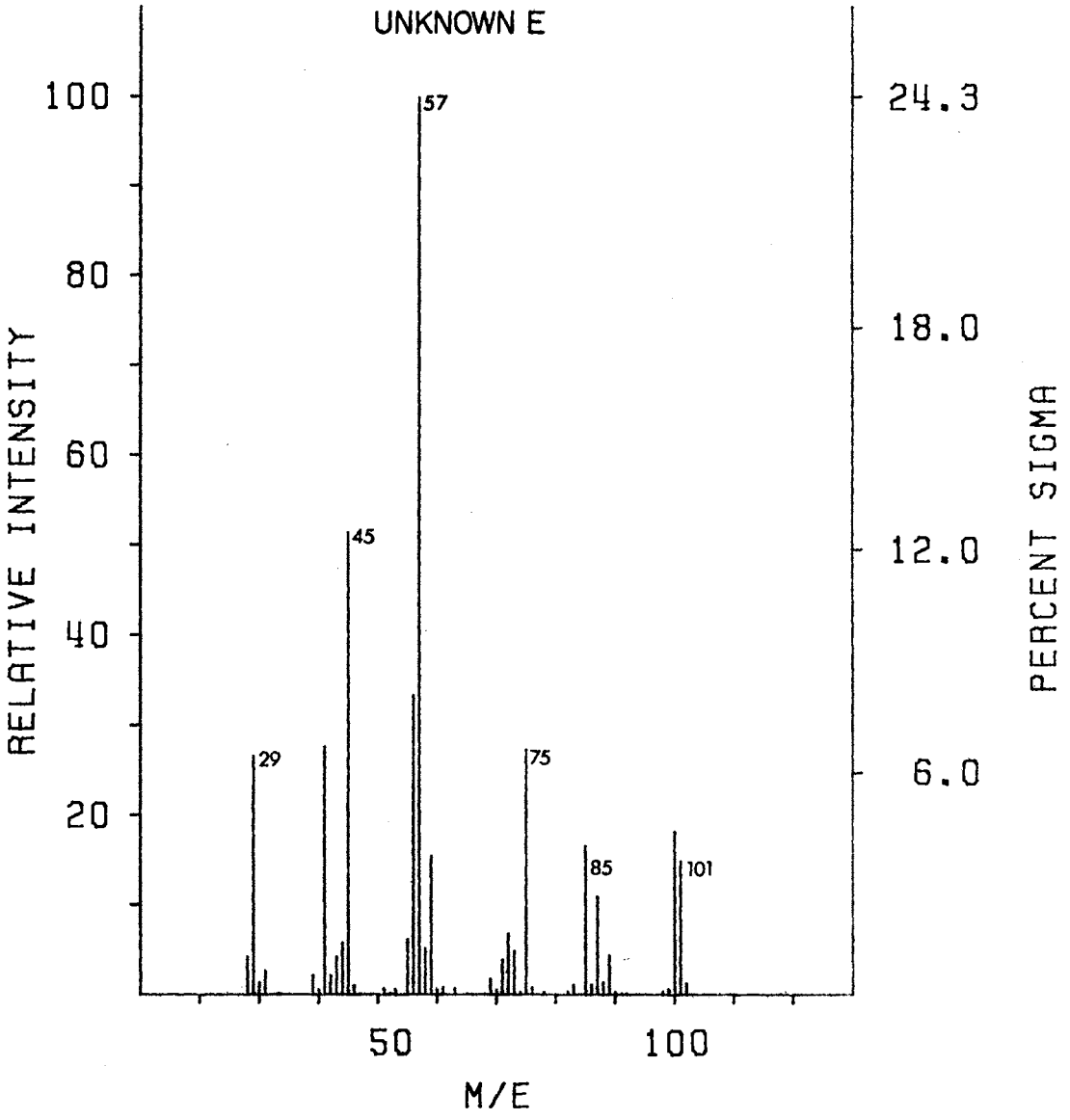


Figure 21. Mass spectrum of unknown E

MS 2923 13-12

Inner Gel of the Leaves

Acid Distillation. Although the analysis of the extract by gas chromatography - mass spectrometry was conducted at too low a temperature (150° C) and for too short a time (80 minutes) to detect the diphenylamine, BHT was detected. However, a comparison of the chromatograms of the leaf outer layers and of the gel performed later at 212° C is shown in Figure 22. As shown in the figure, peaks A (BHT), B (diphenylamine), D, and E from the leaf outer layer have corresponding peaks in the chromatogram from the gel.

Basic Distillation. A solution in diethyl ether of the volatiles which had been extracted from the condensate from the steam distillation of the Aloe gel after it had been made basic was analyzed by gas chromatography - mass spectrometry. The total ion current tracing from the analysis is shown as Figure 23.

Two major (F and G) and several minor peaks can be seen in this tracing. The compound labeled F, which had a retention time of 4½ minutes at 150 C, appeared to have a molecular weight of 126 and a base peak at m/e 54 (Figure 24). The ten most intense peaks in decreasing order are m/e 54, 126, 39, 43, 93, 55, 53, 82, 66, and 28 with relative intensities of 100, 39, 21, 18, 13, 10, 10, 7, 5, and 5, respectively. There is a less intense peak at mass 111 which indicates that the parent ion may lose a methyl group. Based upon other spectra, the peak with m/e 135 is a small amount of another unknown.

The other major peak (G) with a retention time of 7½ minutes had a molecular weight of 129 and to have its base peak at m/e 30

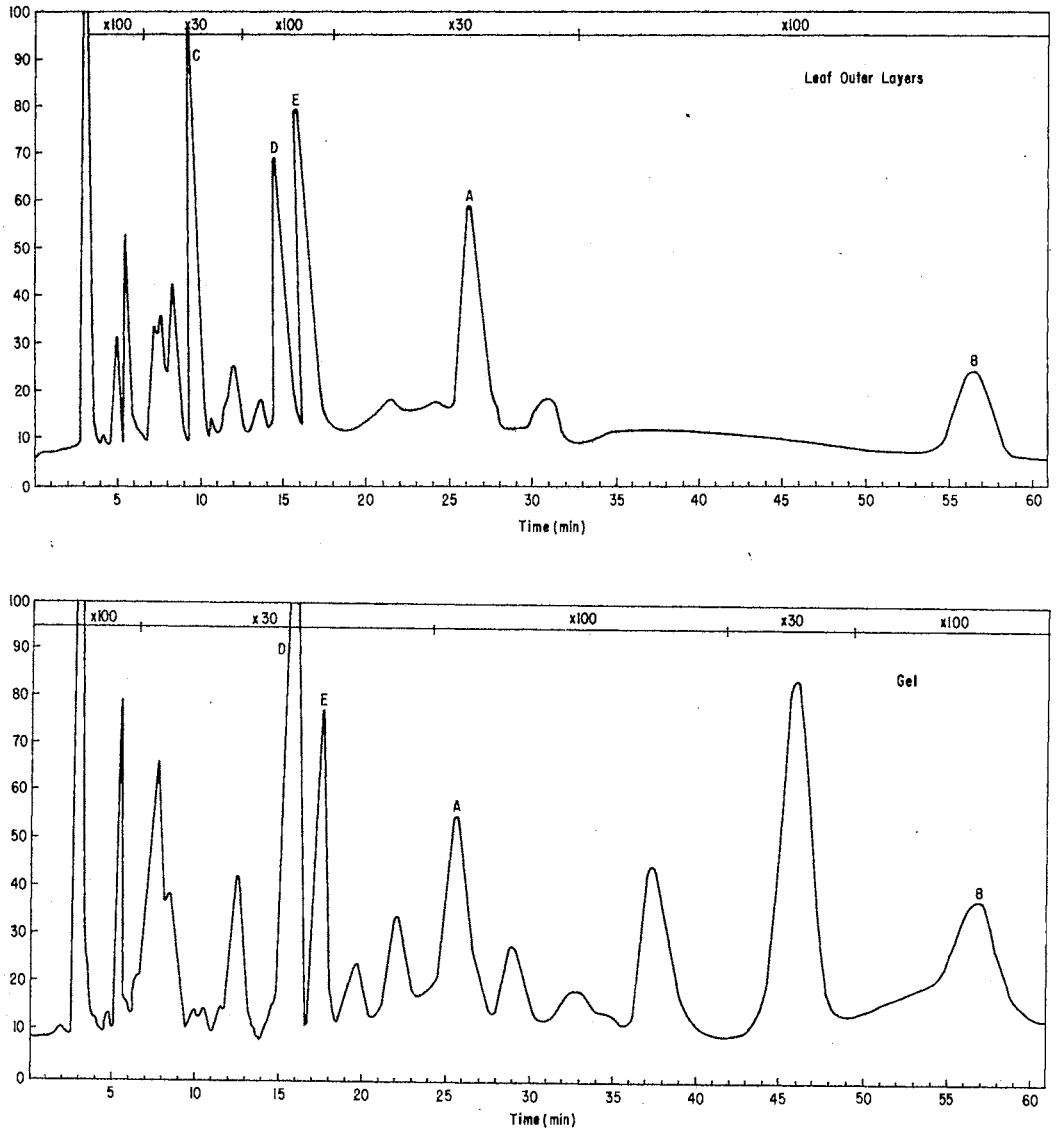


Figure 22. A comparison of the steam-distillable fractions from the Aloe leaf outer layers with the gel by gas - liquid chromatography

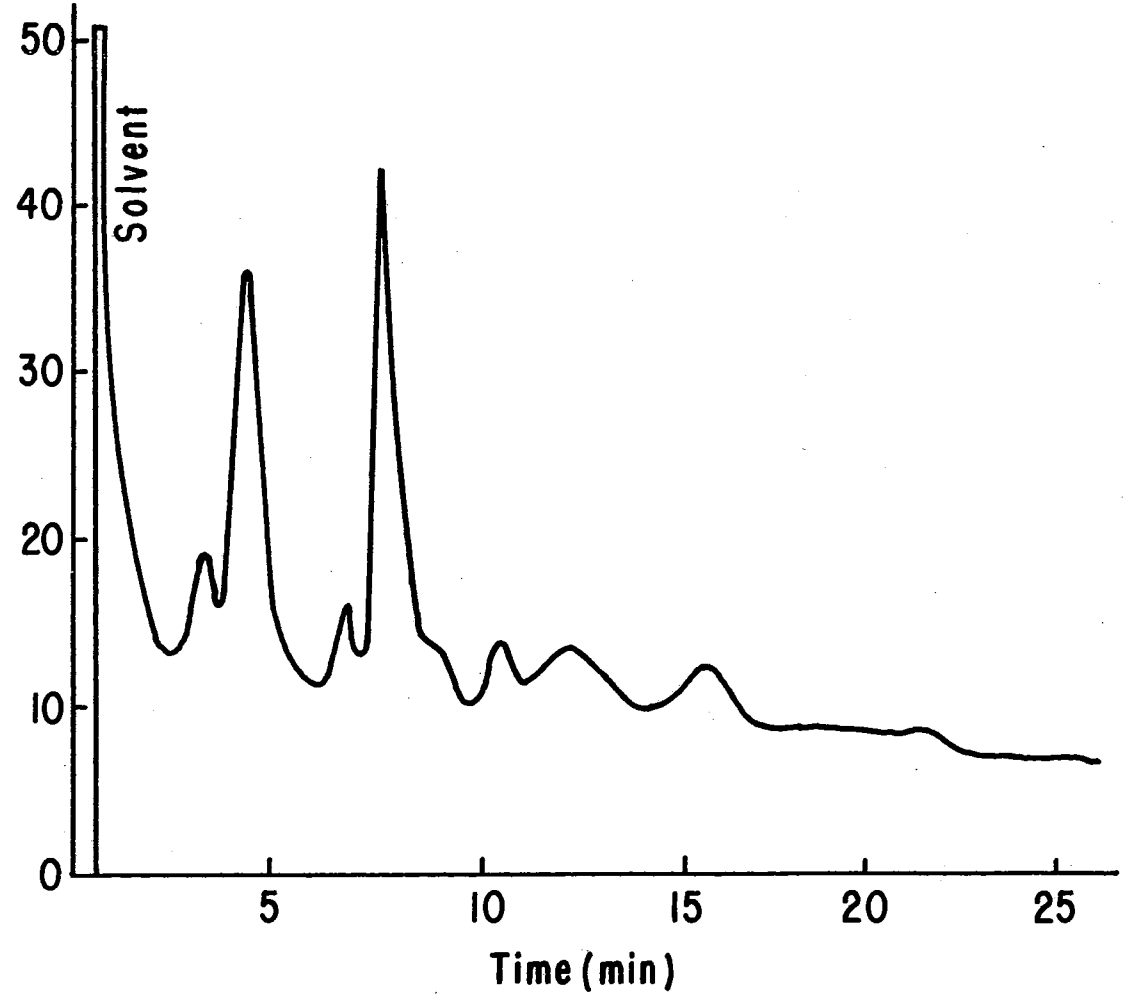


Figure 23. Total ion current tracing of a GC-MS analysis of the volatiles from the basic steam distillation of the gel from Aloe leaves

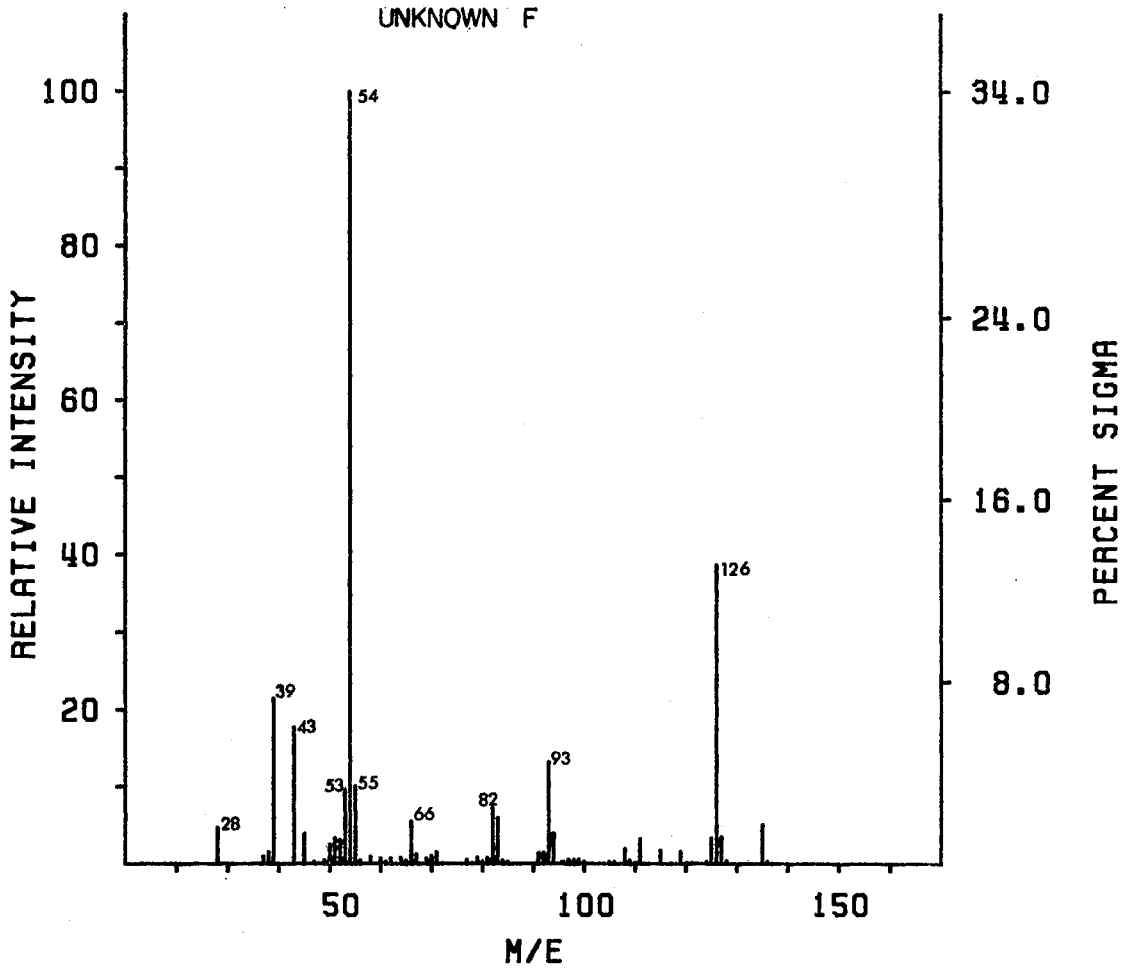


Figure 24 . Mass spectrum of unknown F

MS 2571 33-30

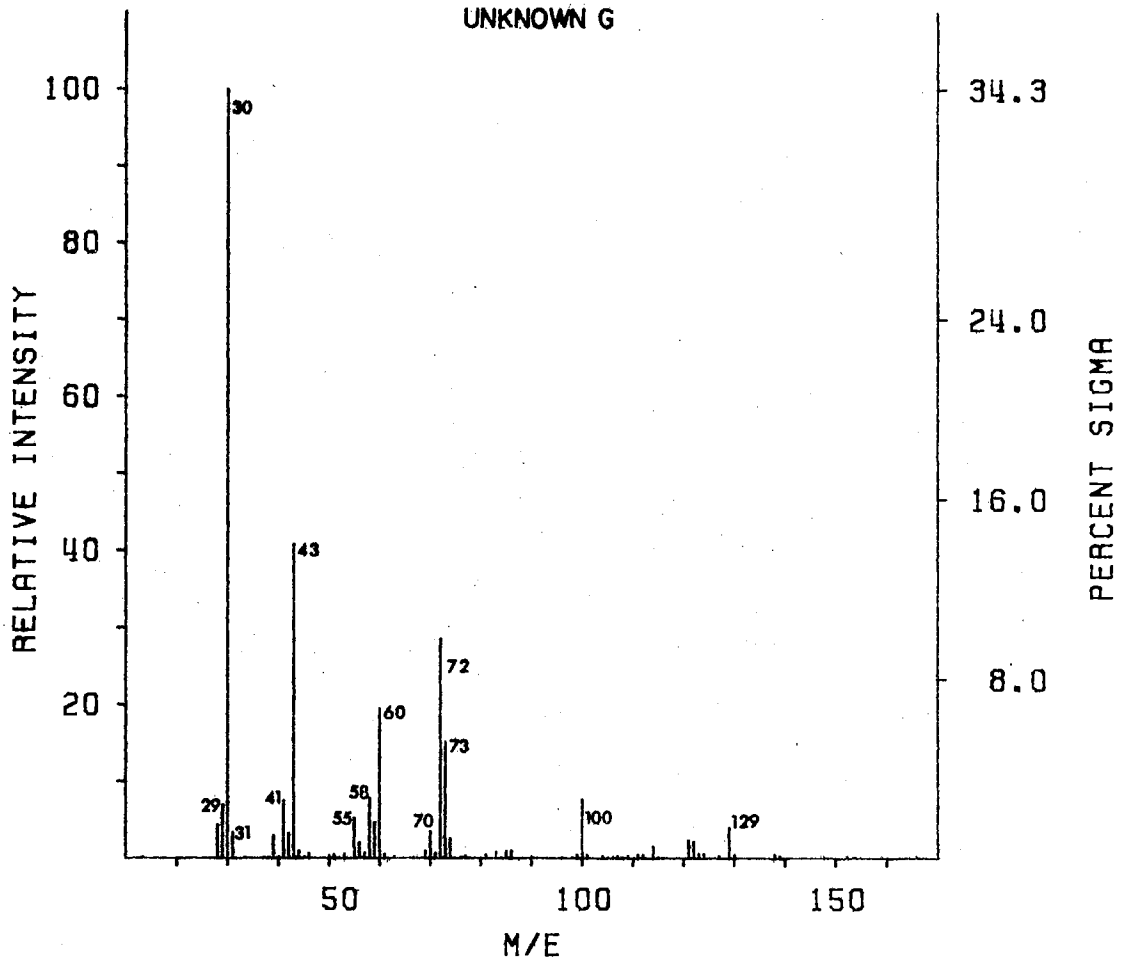


Figure 25. Mass spectrum of unknown G

MS 2571 37-34

(Figure 26). The ten most intense peaks are m/e 30, 43, 72, 60, 73, 58, 41, 100, 70, and 31 with relative intensities of 100, 41, 28, 19, 15, 8, 8, 8, 3, and 3, respectively. In addition, there appears to be a small metastable ion at m/e 77.8. The odd molecular weight of this compound indicates that it contains an odd number of nitrogen atoms. The loss of 29 mass units in the transition m/e 129 → m/e 100 may represent the loss of either an aldehyde or ethyl group.

Whether or not BHT and diphenylamine are environmental contaminants was not established although both compounds were found in plants which had been obtained in Texas where they had been field grown and in Oklahoma where they had been grown as house plants. Although BHT is a widely used preservative and diphenylamine is used in the packing of citrus products for shipment, neither compound was used as a spray near the field of Aloe in Texas (30) nor in the greenhouse.

Stalk

When the extract containing the steam volatile compounds of the stalk was subjected to preliminary analysis by gas chrpmatography - mass spectrometry, no BHT could be found although diphenylamine was present.

A very large peak was seen in the total ion current tracing of the mass spectrometer after 175 minutes at 210° C. The compound represented by this peak had a molecular weight of 299 and a base peak at m/e 57 (Figure 26). The ten most intense peaks are m/e 57, 45, 56, 85, 125, 41, 100, 29, 101, and 199 with relative intensities of

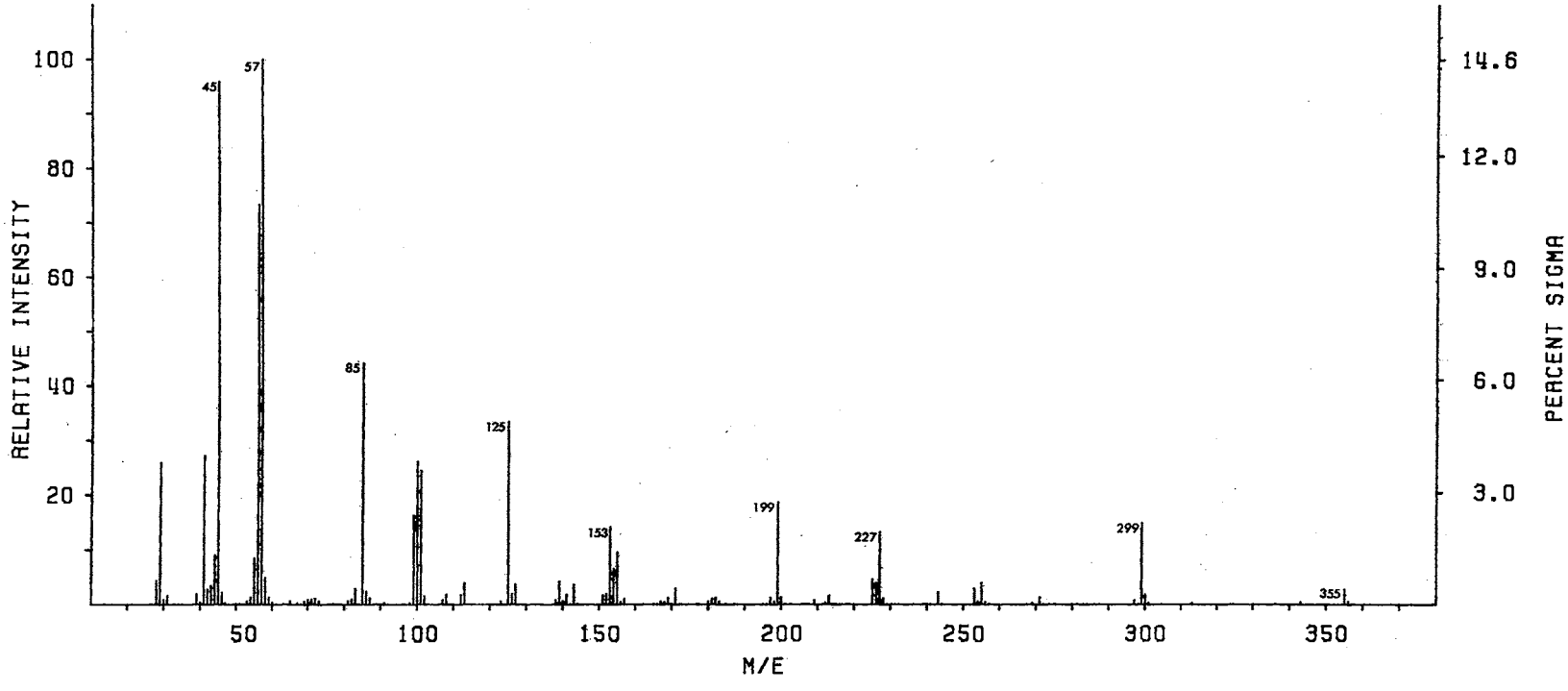


Figure 26. Mass spectrum of unknown steam-volatile compound found in Aloe stalk.

MS 2936 60-62

100, 96, 73, 44, 33, 27, 26, 26, 25, and 19, respectively.

Sugar Determination

Since a difference in the composition of a polysaccharide isolated from Aloe barbadensis has been reported by different authors (5, 15), a sugar determination was performed on the hydrolyzed lyophilized gel. As shown in Table II, Farkas reported the finding of mannose and glucose only (the relative molar ratios were not stated) while Sega, Taylor, and Eoff found mannose and glucose in a relative molar ratio of 9 - 10:1, and trace amounts of arabinose, galactose, and xylose. On the other hand, Farkas reported the finding of hexuronic acids while Segal, Taylor, and Eoff could find none.

In the present investigation, no attempt was made to isolate either a polyuronide or a particular polysaccharide before the analysis, but rather a lyophilized sample of the whole gel was subjected to the analysis. (It was found that 88% of the weight of the gel was lost during lyophilization.) If several different polysaccharides had been present, these also would have been measured using this technique. The results obtained in this laboratory are shown in Table III; the hexuronic acid content of the gel was not measured. Mannose and glucose were found in a molar ratio of 5:4, and trace amounts of xylose, rhamnose, galactose, and either arabinose or fucose were also found.

TABLE II

THE SUGAR CONTENT OF ALOE BARBADENSIS AS REPORTED BY VARIOUS AUTHORS[a]

Compound	Found by Farkas (5)	Found by Segal, Taylor and Eoff (15)	Found in this Laboratory
Arabinose	-	+	+[b]
Galactose	-	+	+
Glucose	+	+	+
Mannose	+	+	+
Rhamnose	-	-	+
Xylose	-	+	+
Hexuronic Acids	+	-	c

a The symbol + in the table indicates that the compound was found in the Aloe leaves and the symbol - indiates that it was not found.

b As determined in this laboratory, arabinose could not be distinguished from fucose.

c Hexuronic acids were not determined.

TABLE III

SUGAR CONTENT OF ALOE GEL AS DETERMINED IN THIS LABORATORY

Sugar	Sugar in whole gel N mole/g	Total sugar in lyophilized residue %
Arabinose	4.23 (a)	4.7 (a)
Galactose	3.60	4.3
Glucose	31.3	37.7
Mannose	39.4	47.5
Rhamnose	1.27	1.5
Xylose	4.44	4.4

(a) Calculated as fucose.

CHAPTER V

SUMMARY

The purpose of this study was to isolate and to identify a compound or compounds which occur in Aloe barbadensis. Qualitative analyses were performed using a combination of thin-layer chromatography, gas - liquid chromatography, and gas chromatography - mass spectrometry.

The leaves of young Aloe plants were extracted with either dichloromethane or 80% methanol in a preliminary study. This extract was analyzed by thin-layer chromatography only.

Various parts of mature Aloe plants were steam distilled. The steam-volatile compounds were extracted from the condensate and were analyzed by thin-layer chromatography, gas - liquid chromatography, and gas chromatography - mass spectrometry. The complex mixture contained more than 16 compounds. 2,6-Di-tertiary-butyl-4-methylphenol was isolated from the leaves and diphenylamine was isolated from the leaves and stalk. A compound which may be benzothiazole or which may be similar in structure to benzothiazole was also isolated from the leaves. Neither BHT, diphenylamine, nor benzothiazole previously have been reported to occur naturally.

Much work remains to be done: 1) the compound or compounds which gave a positive Dragendorf test have yet to be identified; 2) the steam-volatile compounds found in the stalk and roots

require further analyses; 3) compounds which are found in the plant but which are not steam volatile should be characterized; 4) the unknown compounds in the steam distillate from the leaves must be identified; and 5) an attempt should be made to determine which of the compounds found in Aloe barbadensis are of physiological significance.

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