A PHYTOCHEMICAL INVESTIGATION ON

BOTHRIOCHLOA INTERMEDIA

AND RELATED STUDIES

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A PHYTOCHEMICAL INVESTIGATION OF

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TO MY PARENTS

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GLOSSARY OF ABBREVIATIONS

Anal.	elemental analysis
BP	boiling point
C	Celsius
cm^{-1}	wave number (IR)
col	column
d	doublet
g	gram
GC	gas chromatography
GC:R_t	retention time as measured by gas chromatography
hr	hour
Hz	Hertz (cycles per second)
IR	infrared spectroscopy
J	coupling constant (NMR)
Kg	kilogram
l	liter
m	multiplet (NMR)
M^+	molecular ion in mass spectrum
m/e	mass to charge ratio
min	minute
mg	milligram
ml	milliliter
MP	melting point

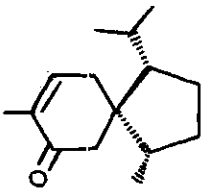
NMR	nuclear magnetic resonance spectroscopy
p.	page
ppm	parts per million
q	quartet (NMR)
R_t	retention time
s	singlet (NMR)
sec	seconds
t	triplet (NMR)
TMS	tetramethylsilane

SUMMARY

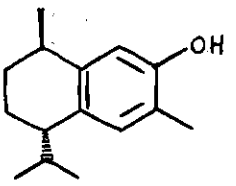
The purpose of this research was threefold. First, several secondary plant metabolites were identified in the hexane extract of Peltophorum inerme (Roxb.) Naves. Since Peltophorum inerme showed confirmed preliminary anti-tumor activity, extracts were sent to the National Institute of Health for further testing. Second, several secondary plant metabolites in the steam volatile oil of the hybrid 56*627-1, prepared from two forms of Bothriochloa intermedia (R. Br.) A. Camus were identified. Third, a gas chromatographic study was made of the steam volatile oils from some accessions, hybrids, and strains of Bothriochloa intermedia for the purpose of determining palatability to cattle, insect antifeedant properties, and aiding taxonomic identification.

A total of eight long straight-chain alkanes were identified in the hexane extract of Peltophorum inerme. Four of the compounds, n-heptacosane (n-$C_{27}H_{56}$), n-nonacosane (n-$C_{29}H_{60}$), n-hentriacontane (n-$C_{31}H_{64}$), and n-tritriacontane (n-$C_{33}H_{68}$) were isolated by preparative gas chromatography and identified by mass spectral analysis. The other four compounds, n-octacosane (n-$C_{28}H_{58}$), n-triacontane (n-$C_{30}H_{62}$), n-dotriacontane (n-$C_{32}H_{66}$), and n-tetratriacontane (n-$C_{34}H_{70}$), were not isolated; however, they were identified by mixed injections, analytical gas chromatography, with known samples of these four compounds.

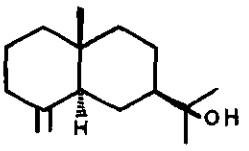
Five compounds, acorenone-B (7), kessane (1), elemol (2), β-eudesmol (6-A), and 7-hydroxycalamene (9), were isolated from the steam volatile oil of the hybrid grass 56x627-1. Conclusions have been drawn as to the biogenetic origin of these five compounds based on their co-occurrence. Gas chromatographic studies of the steam volatile oils of the various grasses of Bothriochloa intermedia in this research are only the initial stages of a long range study of the grasses' palatability to cattle and insect antifeedant properties and at this point it appears a high acorenane-B (7) content is related to low palatability to cattle and high insect resistance. Studies on chemical taxonomy as related to Bothriochloa intermedia are still inconclusive due to Bothriochloa intermedia's genetic aggressiveness.



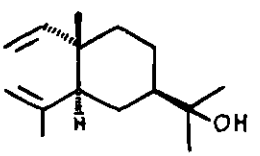
acorenone-B (7)



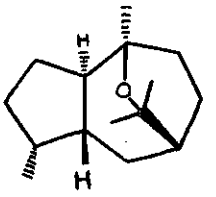
7-hydroxycalamenene (9)



β-eudesmol (6-A)



elemol (2)



kessane (1)

Figure 1. Sesquiterpenes Isolated from the Hybrid Grass 56x627-1

CHAPTER I

INTRODUCTION

The purpose of this research was threefold. First, several secondary plant metabolites were identified in the hexane extract of Peltophorum inerme (Roxb.) Naves. Since Peltophorum inerme showed confirmed preliminary anti-tumor activity, extracts were sent to the National Institute of Health for further testing. Second, several secondary plant metabolites in the steam volatile oil of the hybrid grass 56x627-1, prepared from two forms of Bothriochloa intermedia (R. Br.) A. Camus were identified. Third, a gas chromatographic study was made of the steam volatile oils from some accessions, hybrids, and strains of Bothriochloa intermedia for the ultimate purpose of determining palatability to cattle, insect antifeedant properties, and aiding taxonomic identification.

Peltophorum inerme is a medium-sized to large evergreen tree 30-65 feet in height and 1.5 feet or more in trunk diameter, with spreading branches and dense foliage. Common names for Peltophorum inerme are yellow flamboyant and yellow poinciana. The tree is native in Ceylon, southern India, Malaya, East Indies, Philippines, and northern Australia; however, it is not native in Puerto Rico, where it was collected by the U.S. Department of Agriculture for this research. Peltophorum inerme is grown in Puerto Rico as an ornamental shade tree because of its yellow flowers and reddish fruits. Flowering

occurs from spring to fall (April to September) and fruiting occurs chiefly in the winter.[1]

Peltophorum inerme extracts were screened by the National Institute of Health for anti-tumor activity. Activity was found in KB-Cell Culture. Our interest was in concentrating the KB activity by successively extracting and partitioning the extracts with various solvents and sending these to the National Institute of Health for screening. After the anti-tumor activity was concentrated in a particular extract, then the compounds responsible for this anti-tumor activity could be isolated and identified. It turned out that even though the plant showed preliminary anti-tumor activity, the various extracts of the plant showed very little or no anti-tumor activity. An outline of the extraction procedure is shown on Chart 1, p. 20.

The hexane extract (Chart 1, p. 20) of Peltophorum inerme was subjected to analytical gas chromatography (Col II, 272°C), GC trace #1 (Appendix 1, p. 73). From this trace it can be seen that there are eleven major compounds present. A total of eight of these compounds were identified. Four of the compounds, n-heptacosane (n-$C_{27}H_{56}$), n-nonacosane (n-$C_{29}H_{60}$), n-hentriacontane (n-$C_{31}H_{64}$), and n-tritriacontane (n-$C_{33}H_{68}$) were isolated by preparative gas chromatography (Col III, 278°C) and identified by mass spectral analysis. The other four compounds, n-octacosane (n-$C_{28}H_{58}$), n-triacontane (n-$C_{30}H_{62}$), n-dotriacontane (n-$C_{32}H_{66}$), and n-tetratriacontane (n-$C_{34}H_{70}$) were not isolated; however, they were identified by mixed injections, analytical gas chromatography (Col II, 272°C), with known samples of these four compounds.

Alkanes are very widely distributed in both the plant and animal kingdoms. In plants they are most abundant in the cuticle waxes which act as protective coatings on leaves and stems. The term wax refers to an ester of a higher fatty acid and a higher aliphatic alcohol, but in the present context it applies to all substances of "waxy" character isolated from the plant. The waxy coating on the leaves of plants performs at least two major functions. First, the waxy coating assists in controlling the water-balance of the plant, especially under excessively moist or dry conditions. Second, the waxy layer seems to contain substances which inhibit bacteral, fungal, and insect attack. The major constituents of plant waxes are C_{27}, C_{29}, C_{31}, and C_{33} n-alkanes. The content of odd-carbon-number alkanes is usually greater than that of even-carbon-number alkanes by a factor of more than ten.[2] The most probable mechanism for the biogenesis of alkanes in plants involves decarboxylation of the corresponding long-chain fatty acids. Horning and her co-workers have shown the possible biogenesis of both odd and even-carbon-number long chain fatty acids.[3] This biogenesis is shown below.

$$RCO\text{-}CoA + n\left[\begin{array}{l} COOH \\ | \\ CH_2CO\text{-}CoA \end{array}\right] \rightarrow R(CH_2CH_2)_nCOOH$$

If R- is methyl the acid will be even-carbon-numbered. If R- is ethyl the acid will be odd-carbon-numbered. Since the even-carbon-numbered carboxylic acids are produced in larger amounts than the odd-carbon-numbered carboxylic acids, it can be seen why the odd-carbon-numbered

alkanes (arising by decarboxylation) are produced in greater amounts than the even-carbon-numbered alkanes.

Bothriochloa intermedia is a member of the grass family (Gramineae). Common names for Bothriochloa intermedia are sundhaur, Burnett River blue grass, poverty grass, sour grass, and purple tassel grass. The grass is native in Africa, India, Pakistan, Ceylon, Assam, Burma, Malaya, Indo-China, China, Australia, and most of the Pacific islands.[4]

The Department of Agronomy at Oklahoma State University, Stillwater, Oklahoma, gathered seeds of Bothriochloa intermedia from those countries in which it is native. The seeds were then planted in Stillwater, Oklahoma, or Fort Reno, Oklahoma, for the purpose of finding new and improved grasses which would be used to feed cattle.

In this dissertation a form is defined as a distinct variety of Bothriochloa intermedia. Hybrids were prepared from different forms of Bothriochloa intermedia. Hybridizations were carried out by means of hand emasculations and pollinations. An accession is defined as a particular plot of Bothriochloa intermedia which could be a pure form, a hybrid, or a strain. A strain is defined as a blend of hybrids or accessions. The term "grass" will be used to denote any of the nine samples of Bothriochloa intermedia studied in this dissertation.

Bothriochloa intermedia was of particular interest to the agronomists at Oklahoma State University because of its winter hardiness under Oklahoma conditions. Different forms of Bothriochloa intermedia were tried in the south from Florida to California due to

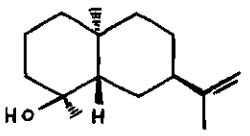
its tropical origin. It was hoped that by making hybrids from various forms of Bothriochloa intermedia the grass could be even better suited to the United States.[5] One such hybrid that was prepared was the hybrid 56x627-1, prepared from two forms of Bothriochloa intermedia, a gangetica type (male) indigenous to the Gangetic Plain of India, and an indica type (female) indigenous to Punjab, India.[5] The major part of this dissertation is concerned with the isolation and identification of the metabolites in the steam volatile oil of this hybrid. "K" strain is a blend of three accessions indigenous to the Kulu valley in India. Both "I-F" and "I-T" strains are blends of thirty hybrids, each of which contains either accession 5297 (Lahnavala, India) or accession 5410 (Punjab, India) as a parent. Accession 5297 is indigenous to Lahnavala, India. Accession 5752 is indigenous to Kedah, Malaya. Finally, accessions 8967, 8969, and 8907 are all indigenous to Turkey.[6]

Table 1 shows the compounds present in the steam volatile oils of the nine grasses. As noted in the table, acorenone-B (7), kessane (1), elemol (2), β-eudesmol (6-A), and 7-hydroxycalamenene (9) were isolated from the hybrid 56x627-1. Also intermedeol[9,10] (11) was isolated from accession 5297, neointermedeol[11] (12) was isolated from accession 5752, and acorenone-B (7) was isolated from "K" strain. The presence of any of the above compounds in any other grass was determined only by gas chromatographic retention times (GC:R_t) with mixed injections. The structures of the sesquiterpenes isolated from Bothriochloa intermedia are shown in Figure 2.

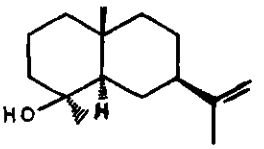
Table 1. Compounds Present in the Nine Grasses of Bothriochloa intermedia

	Acorenone-B	Intermedeol	Neointermedeol	Kessane	Elemol	β-Eudesmol	7-Hydroxycalamenene
Hybrid 54x627-1	isolation	--*	--*	isolation	isolation	isolation	isolation
"I-F" strain	GC:R_t	GC:R_t ?	--*	GC:R_t	GC:R_t	GC:R_t ?	--*
"I-T" strain	GC:R_t	GC:R_t ?	--*	GC:R_t	GC:R_t	GC:R_t ?	--*
"K" strain	isolation	--*	--*	--*	--*	--*	--*
Access. 5297	--*	isolation	--*	--*	--*	--*	--*
Access. 5752	--*	--*	isolation	--*	--*	--*	--*
Access. 8967	GC:R_t	GC:R_t ?	--*	--*	--*	--*	--*
Access. 8969	GC:R_t	GC:R_t ?	--*	--*	--*	--*	--*
Access. 8907	--*	GC:R_t	--*	--*	--*	--*	--*

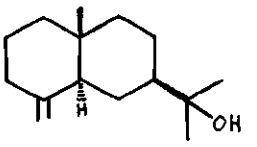
*Not determined.



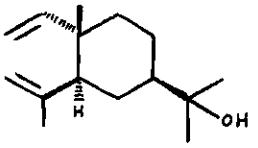
intermedeol 11



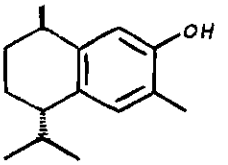
neointermedeol 12



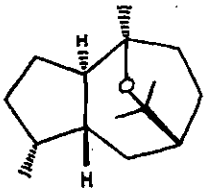
β-eudesmol 6-A



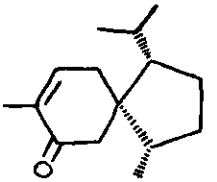
elemol 2



7-hydroxycalamenene 9



kessane 1



acorenone-B 7

Figure 2. Sesquiterpenes isolated from Bothriochloa intermedia.

It is interesting to note that the five sesquiterpenes isolated from the hybrid 56x627-1 all contain different structural types: acorenone-B (7) (acorane), kessane (1) (guaiane), elemol (2) (elemane), β-eudesmol (6-A) (eudesmane), and 7-hydroxycalamenene (9) (cadinane). Enough is known about the principle routes of biogenesis to permit the conclusion that a group of closely related compounds occurring in a single plant are all derived by secondary alteration of a common precursor.[12] In the case of these five co-occurring structural types two common precursors can be written. The common precursor for

acorenone-B (7) and 7-hydroxycalamenene (9) could be the β-bisabolyl cation (14) derived from cis,trans-farnesyl pyrophosphate (13). The common precursor for elemol (2), β-eudesmol (6-A), and kessane (1) could be different conformations of the trans,trans-1,5-cyclodecadiene cation (16) derived from trans,trans-farnesyl pyrophosphate (15). The biogenesis scheme for these five structural types is shown in Figures 3 and 4.

As noted in the first paragraph of the Introduction, a gas chromatographic study was made of the nine grasses for the purpose of determining palatability to cattle, insect antifeedant properties, and aiding taxonomic identification. Of the nine grasses studied, cattle liked the "I-F" and "I-T" strains the most and "K" strain the least. From this small sample no conclusion could yet be drawn as to the relationship between palatability to cattle and the compounds in the steam volatile oils; however, as can be see from GC tracs #'s 4, 5 and 6 (Appendix 1, p. 76, 77, and 78) "I-F" and "I-T" strains are rich in monoterpenes and sesquiterpenes whereas "K" strain contains practically 100% acorenone-B (7). A scientific study has not yet been made on the insect antifeedant properties of Bothriochloa intermedia; however, it has been observed that when the various grasses were growing near each other no foraging insects, such as grasshoppers, were observed in the "K" strain plot, whereas grasshoppers were observed in plots of the other grasses. Again, no conclusions could be drawn as to the insect resistance of "K" strain; however, as noted before, the steam volatile oil of this grass contains practically 100% acorenone-B (7). Finally, it may be impossible to relate the composition of the

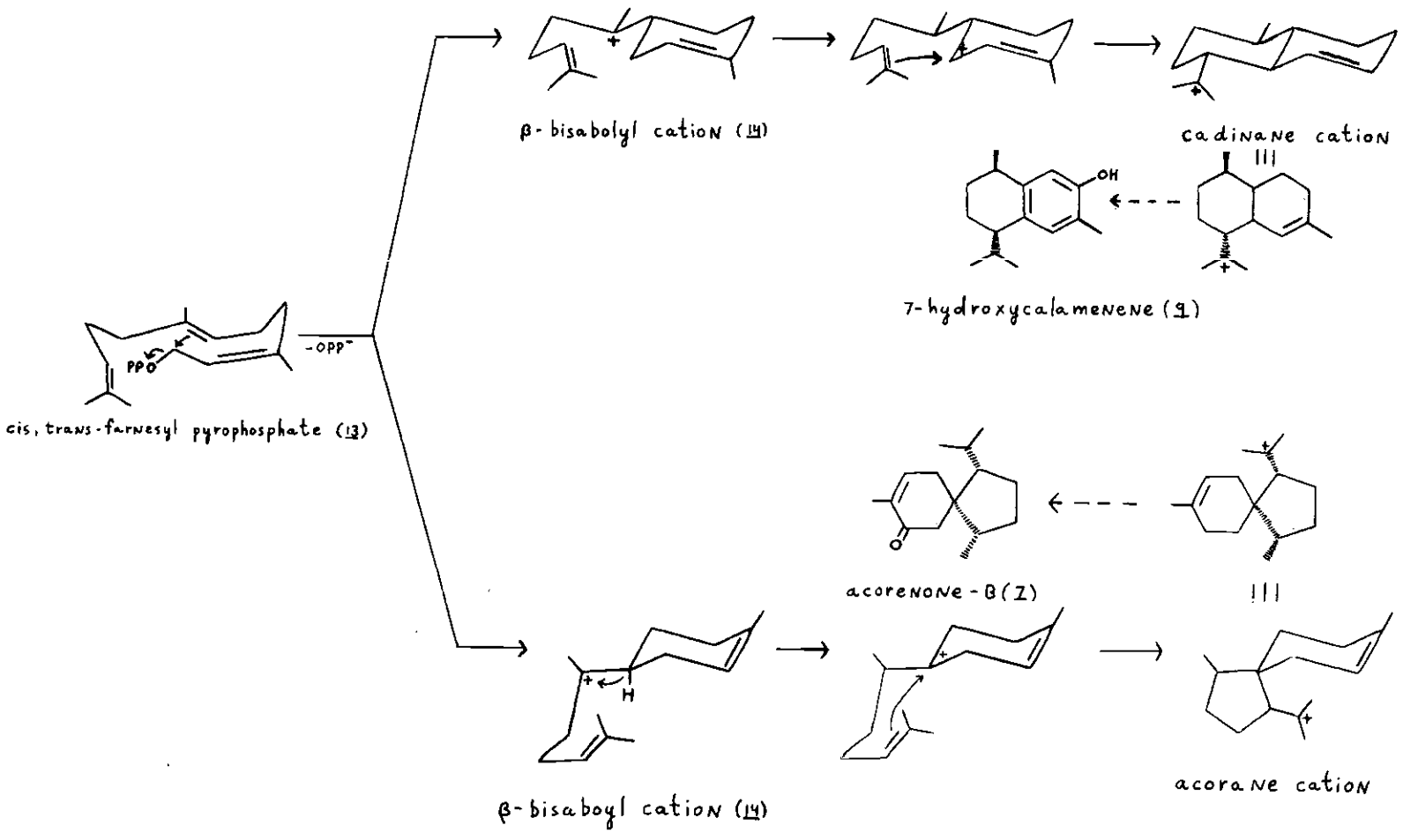


Figure 3. Biogenetic Scheme to Acorenone-B and 7-Hydroxycalamenene (9).

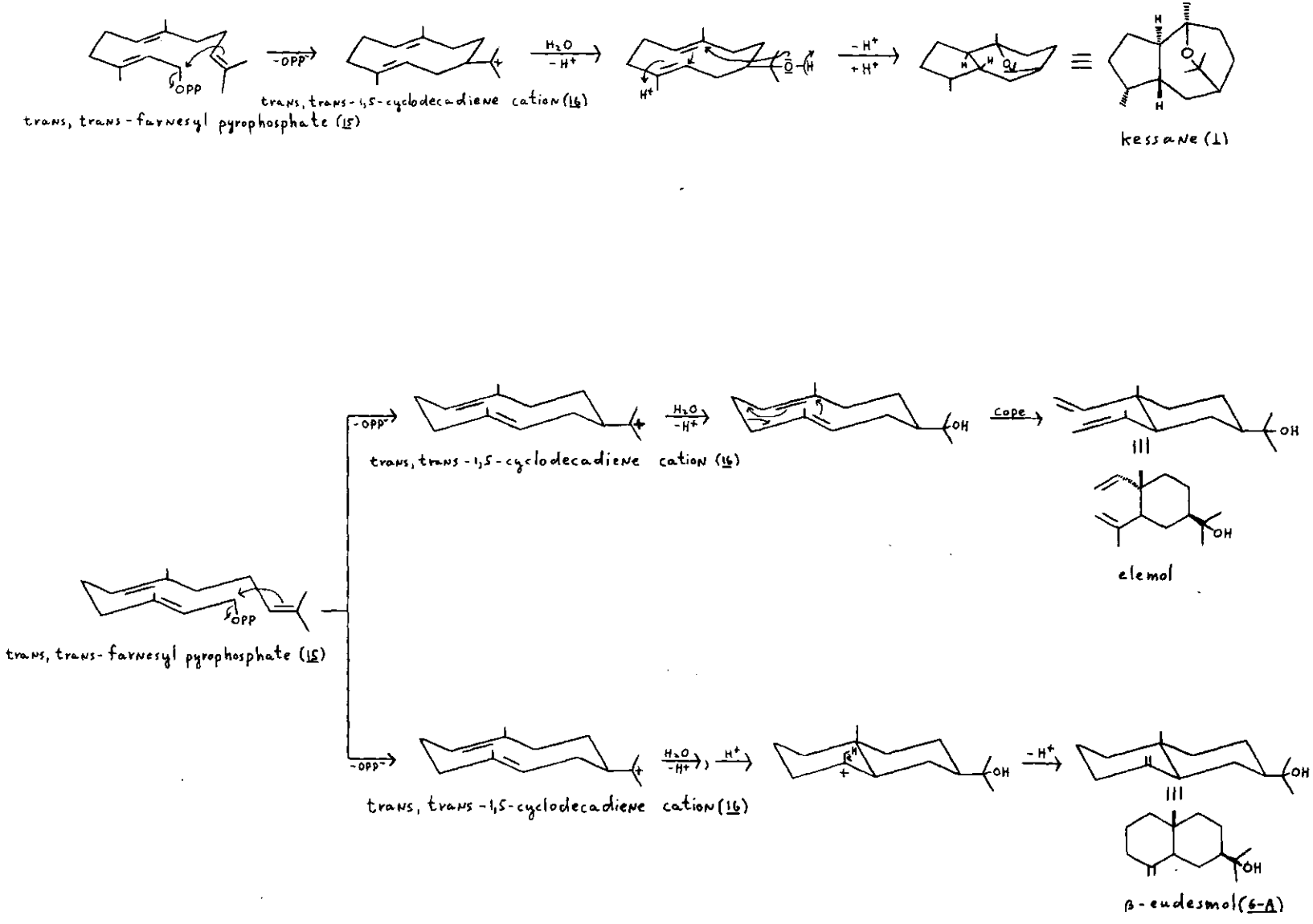


Figure 4. Biogenetic Scheme to B-Endesmol (6-A), Elemol (2), and Kessane (1).

steam volatile oils of the various grasses of Bothriochloa intermedia to taxonomic identification. The difficulty in relating steam volatile oil composition to taxonomic identification lies in Bothriochloa intermedia's genetic aggressiveness. Harland[13] has stated: "It gradually became apparent that Bothriochloa intermedia itself is a hodgepodge of germ plasm assembled from at least five species belonging to three genera. Every accession of Bothriochloa intermedia in our collection seems to be some sort of cross, backcross, or introgression product of one kind or another. We have over two hundred collections of the species from Cape Town to Kenya to Pakistan, India, Burma, Malaya, Taiwan, the Philippines, Indonesia, New Guinea, and Australia, and we are not sure that any of these represent a really original Bothriochloa intermedia. In the course of assimilating germ plasm from various related species, Bothriochloa intermedia seems to have genetically consumed its own ancestral form."

This research has raised a number of questions which will make interesting research projects. These possible projects are discussed in Chapter V of this dissertation. The Gramineae contain over seven thousand species, yet remain relatively untouched in the area of natural product isolation and identification. The Gramineae are rich in compounds with interesting and diverse structures as demonstrated by the structures of the compounds in one of its members, Bothriochloa intermedia.

All of the compounds isolated in this research from Peltophorum inerme and Bothriochloa intermedia can be classified as "secondary plant metabolites". "Primary plant metabolites" can be distinguished

from "secondary plant metabolites" in two ways. First, the "primary plant metabolites" such as the low molecular weight carboxylic acids of the Krebs cycle, the twenty or so amino acids that make up the majority of the proteins, the common fats and lipids, and the common sugars and sugar derivatives are universal in their distribution. The "secondary plant metabolites", such as alkaloids, steroids, terpenes, and phenolic compounds are more restricted in distribution and more characteristic of specific botanical sources. Second, the "primary plant metabolites" have a clearly recognized function in the metabolic activities of the plant, whereas, many of the "secondary plant metabolites" do not.

Until a few years ago these "secondary plant metabolites" were considered to be only waste materials from primary metabolic processes. They are now believed to be responsible for allelochemic[14,15] interactions, which are interactions involving chemicals by which organisms of one species affect the growth, health, behavior, or population biology of organisms of another species.[16] Coevolution is the evolutionary interactions between genetically isolated groups of organisms.[17] Two types of interactions will be briefly discussed. One type of interaction is the suppression of growth of some plants by chemicals released from another plant. This phenomenon is known as allelopathy.[14,15,18] The other type of interaction is the defense, through the use of chemicals, which some plants have developed for their protection from herbivores.

One example of allelopathy involves soft chaparral in the hills near Santa Barbara, California. When soft chaparral invades grasslands

on some soils during dry periods it has been noted that the invading shrub patches are surrounded by belts of bare soil one to two meters wide and devoid of herbs and wider belts in which growth of the grassland plants is reduced. The cause of these bare belts is believed to be due to the volatile terpenes (camphor, cineole, etc.) which give the shrub community its characteristic fragrance. The terpenes are absorbed from the air onto soil particles in amounts effective in inhibiting germination and growth of herb seedlings.[19-22] This is just one example of allelopathic phenomena. Allelopathic effects have been reported for agricultural and wild species from rain-forest trees[10] to desert shrubs.[24,25] There are also a number of pathways by which the toxic "secondary plant metabolites" find their way into the soil. Some allelopathic materials are relased by rainwash, fog drip, volatilization from leaves, excretion or exudation from roots, and decay of the plant.[26] Thus, it can be seen that allelopathy is widespread and allelopathic materials are released by a variety of routes.

The second type of interaction, that between plants and herbivores, will now be briefly discussed. According to coevolutionary theory, plants are under powerful evolutionary pressures by herbivores, and defend themselves by metabolizing compounds, "secondary plant metabolites", which are not palatable to the herbivores. The herbivores, in turn, circumvent the toxic chemicals by detoxication or excretion and thus continue the evolutionary pressure on the plants.[27] Thus, a reciprocal process continues in a stepwise fashion. Fraenkel[28] was the first to propose that "secondary plant metabolites" were produced by plants as a means of protecting themselves against herbivores.

Over two hundred plant species have shown some insect antifeedant behavior; however, very few of these compounds have been tested under controlled conditions.[29] Two alkaloids that are insect antifeedants are tomatin and nicotine which repel the Colorado potatoe beetle, Leptinotarsa decemlineata.[30] Two terpenes that are insect antifeedants are shiromodiol diacetate and shiromodiol monoacetate.[31,32] These are just a few of the many "secondary plant metabolites" which repel herbivores.

There are thousands of "secondary plant metabolites" which have been discovered and many thousands more which remain to be discovered. These compounds, with their wide structural variety, cannot be simply waste products of primary plant metabolism. Levin[33] has said that this multitude ostensibly is the consequence of (1) selection for novel products which confer pathogen and herbivore resistance; (2) selection for distinctive chemical profiles, (3) the numerous avenues by which selection for effective and distinctive chemical profiles may be successful. He concludes that the diversity of secondary compounds is an evolutionary product, indefinite and indeterminate, and subject to self-augmentation through time.

Fraenkel[34] has pointed out that the majority of biochemists and organic chemists has been totally unaware of and disinterested in the reasons for the existance of "secondary plant metabolites". Due predominantly to the recent work of entomologists and botanists, we are developing a clearer understanding of the vital role played by these so-called "secondary plant metabolites".

CHAPTER II

INSTRUMENTATION AND EQUIPMENT

Melting points were determined on either a Thomas-Hoover capillary melting point apparatus or a Thomas-Kofler micro hot stage Model 651 and are uncorrected. Microanalyses were performed by Atlantic Microlabs, Atlanta, Georgia. Solvents were removed in vacuo on a Büchler Instruments rotary evaporator at water aspirator pressure.

Infrared spectra were recorded using a Perkin-Elmer 237B spectrophotometer with solids in the form of potassium bromide pellets or in carbon tetrachloride or chloroform solution and liquids in the form of thin films between sodium chloride plates or in carbon tetrachloride or chloroform solution. The band at 1601 cm^{-1} of polystyrene film was used as a reference. Nuclear magnetic resonance spectra were obtained in deuterochloroform solutions containing tetramethylsilane as an internal standard. All 60 MHz ^{1}H-NMR's were obtained using Varian Associates A-60D or T-60A spectrometers, while 100 MHz ^{1}H-NMR's and ^{13}C-NMR's were done on a JEOL PFT-100 Fourier transform spectrometer. Mass spectra were obtained using either an Hitachi Perkin-Elmer Model RMU-7L or a Varian Model M-66 mass spectrometer.

Gas chromatographic traces were obtained with either a Hewlett-Packard Model 402 gas chromatograph or an F and M Model 400 gas chromatograph using flame ionization detectors. Preparative gas chromatography was performed on an Aerograph Autoprep Model A-700 gas

chromatograph with a thermal conductivity detector. All columns were glass and are listed below.

Most physical separations were accomplished by the use of medium pressure high performance chromatography equipment. The pump that was used was an FMI Model RP-SY. A prepacked EM Reagents silica gel 60 size C column was used with the medium pressure high performance chromatography equipment. See Figure 5. Some samples were purified by high pressure high performance chromatography equipment. The pump that was used was a Milton Roy Model 396 Instrument minipump. The column that was used was a prepacked Partisil M9 10/25 by Whatman. See Figure 6. Spinning band distillations were carried out using a Nester/Faust annular teflon spinning band distillation column (24 x 0.5 inches).

Column Number	Liquid Phase	Support	Column Size
I	5% SE-30	80/100 Chromosorb W	5'9" x 1/4"
II	3% OV-17	100/120 Gas-Chrom Q	5'7" x 1/4"
III	5% SE-30	80/100 Chromosorb W	4'9" x 3/8"
IV	3% OV-17	100/120 Gas-Chrom Q	4'9" x 3/8"

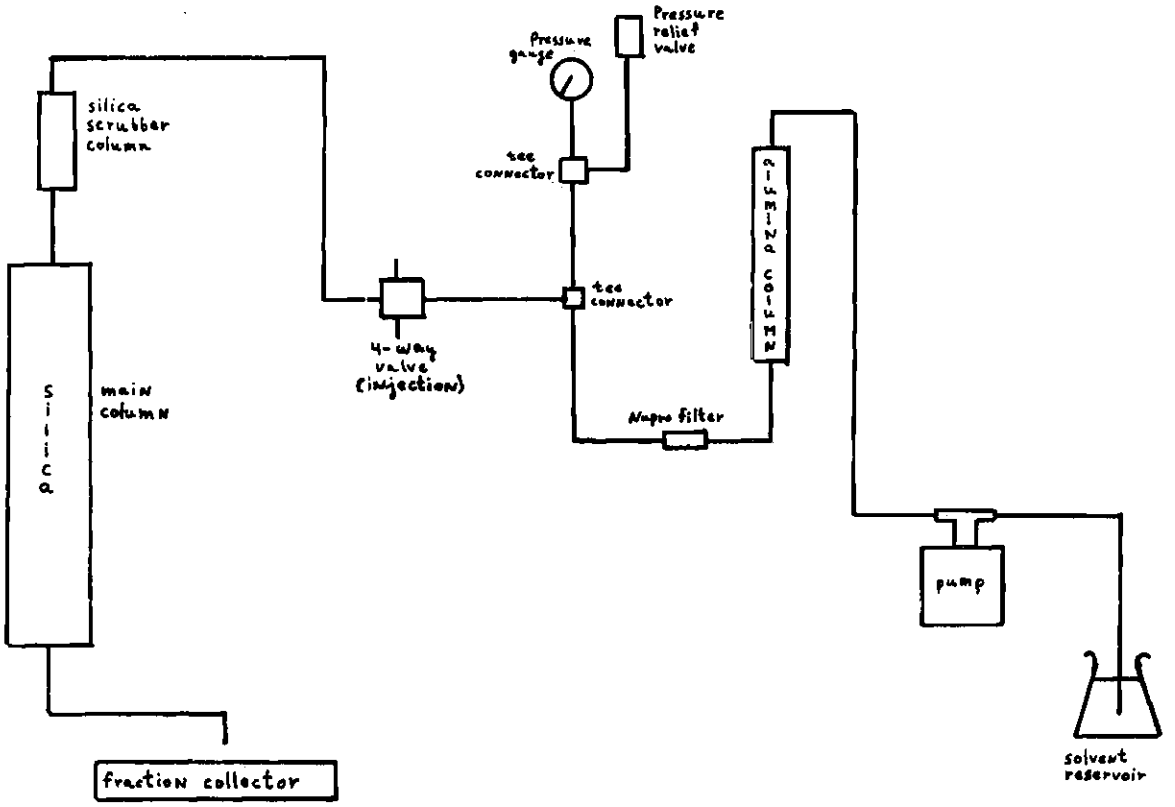


Figure 5. Medium Pressure Chromatography Setup.

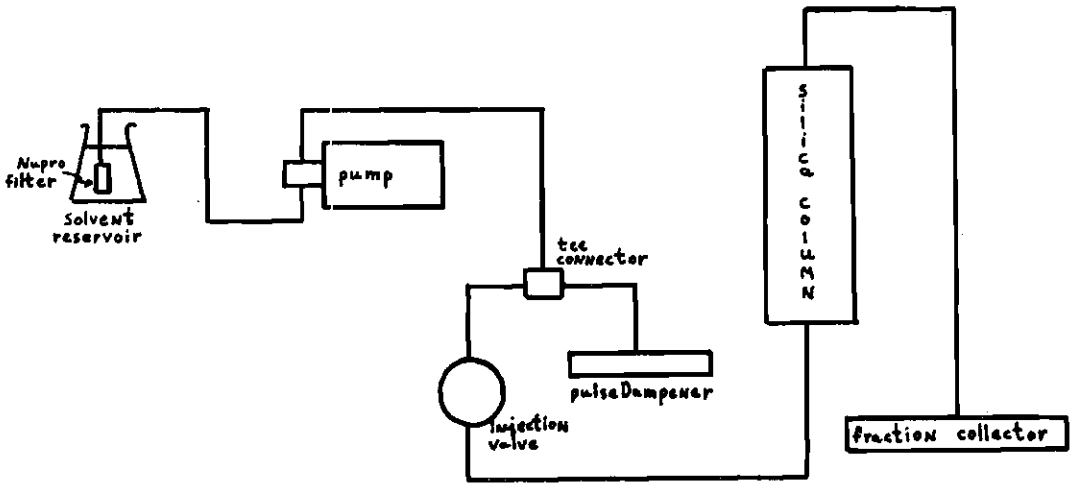


Figure 6. High Pressure Chromatography Setup.

CHAPTER III

EXPERIMENTAL

Collection of Plant Material

Peltophorum inerme was collected in Puerto Rico by the U.S. Department of Agriculture in April, 1969. The stems and leaves were dried, and then shipped in cardboard containers.

Bothriochloa intermedia was grown at Stillwater or Fort Reno, Oklahoma, by the Agronomy Department at Oklahoma State University. The grasses were either dried and shipped in bales or shipped green in cardboard containers.

Extraction of Peltophorum inerme

Peltophorum inerme leaves (2.37 Kg) were ground in a Waring blender containing hexane. The ground-up leaves, suspended in hexane, were placed in a large Soxhlet extractor and continuously extracted with hexane (9 ℓ) until the hexane in the siphon tube was colorless (five days). The hexane was removed by a rotary evaporator yielding a dark green solid (34.22 g). The hexane extract was subjected to analytical gas chromatography (Col II, 272°C), GC trace #1 (Appendix 1, p.73). The plant material was then continuously extracted with 95% ethanol (9 ℓ) until the ethanol in the siphon tube was colorless (five days). The ethanol was removed by a rotary evaporator yielding a dark green gum (212.96 g). The solid plant material was then discarded. The ethanol extract was partitioned between chloroform and water (1:1)

until the fresh chloroform from the continuous extractor was colorless (four days). The chloroform was removed by a rotary evaporator yielding a dark green solid (61.23 g). The water was removed by a rotary evaporator yielding a brown solid (92.19 g). Samples of the hexane extract, ethanol extract, chloroform extract, and aqueous extract were sent to the National Institute of Health for testing. See Chart 1 for an outline of the above procedure. Table 2 contains the cancer screen data.

Isolation of n-Heptacosane ($n\text{-}C_{27}H_{56}$)

n-Heptacosane ($n\text{-}C_{27}H_{56}$) was isolated from the hexane extract of Peltophorum inerme. The hexane extract (11.3 g), which was green in color, was washed with diethyl ether. The mother liquor, which was green in color, was decanted yielding a white solid (1.4 g). This ether insoluble white solid was then subjected to preparative gas chromatography (Col III, 278°C). The first peak to appear, R_t - 2.9 min, was collected. This sample was then subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 2.4 min, GC trace #1 (Appendix 1, p.73). This component had the same spectral and physical properties as n-heptacosane ($n\text{-}C_{27}H_{56}$).[35,36]

GC: R_t - 2.4 min (Col II, 272°C)

MP: 58-60°C lit. 59.1°C [35,36]

Mass Spectrum: M^+ = 380 (2%), 57 (100%)

Chart 1. Extraction Procedure for Peltophorum inerme

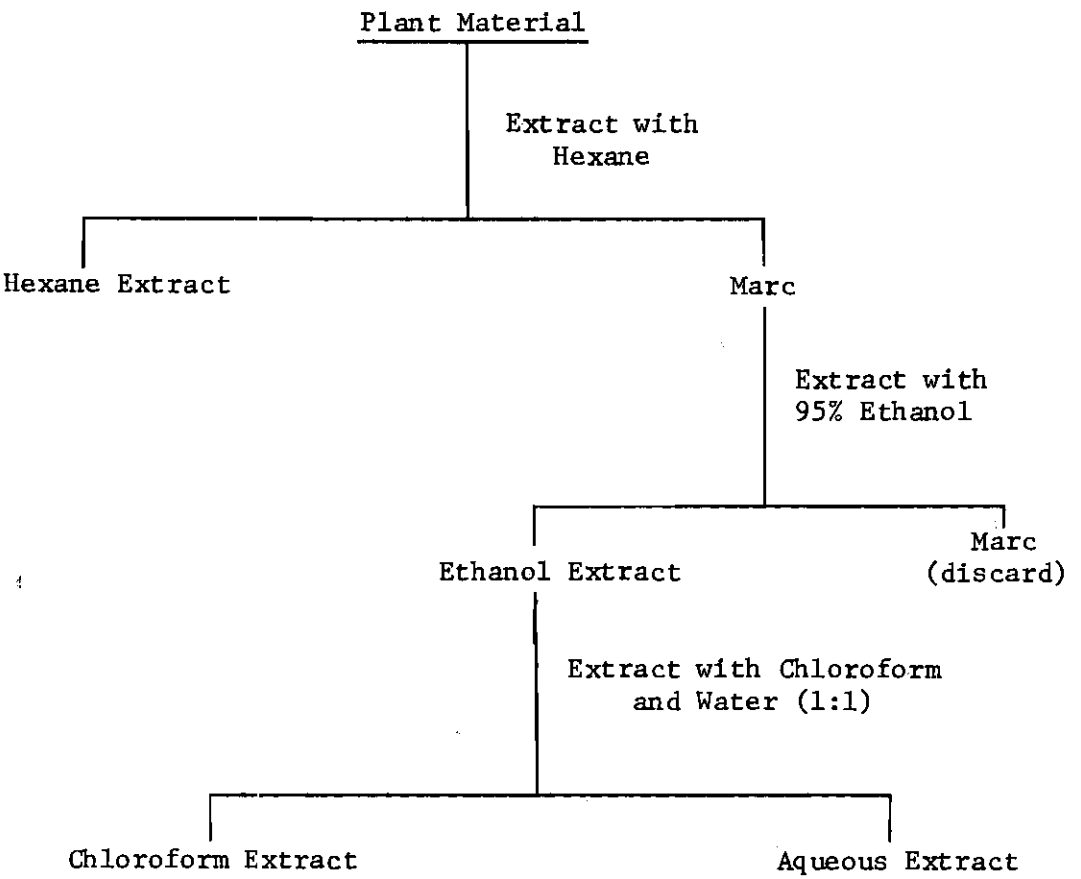


Table 2. National Cancer Institute Screen Data on the *Peltophorum inerme* Extracts

Extract	GIT No.	CCNSC No.	Tumor
Hexane	F004	B636170-F004	KB with slope -0.00 ED50 2.0
Chloroform	F005	B636170-F005	KB with slope -0.00 ED50 2.0
Water	F006	B636170-F006	KB with slope -0.00 ED50 2.0
$CHCl_3$:MeOH (2:1) soluble	F007	B636170-F007	KB with slope -0.00 ED50 2.0
$CHCl_3$:MeOH (2:1) insoluble	F008	B636170-F008	KB with slope -0.00 ED50 2.0

The interpretation of the screening data can be found in "Instruction 14, Screening Data Summary Interpretation", Drug Research and Development, Division of Cancer Treatment, NCI, Bethesda, MD 20014.

The T/C parameters were either mean or median survival time, except for host KB (Cell Culture). The host in all other cases was mouse.

Isolation of n-Nonacosane ($n\text{-}C_{29}H_{60}$)

n-Nonacosane ($n\text{-}C_{29}H_{60}$) was isolated from the hexane extract of Peltophorum inerme. The hexane extract (11.3 g), which was green in color, was washed with diethyl ether. The mother liquor, which was green in color, was decanted yielding a white solid (1.4 g). This ether insoluble white solid was then subjected to preparative gas chromatography (Col III, 278°C). The fourth peak to appear, R_t - 8.6 min, was collected. This sample was then subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 4.1 min, GC trace #1 (Appendix 1, p.73). This component had the same spectral and physical properties as n-nonacosane ($n\text{-}C_{29}H_{60}$).[35,37]

GC: R_t - 4.1 min (Col II, 272°C)

MP: 62-64°C lit. 63.7°C [35,37]

Mass Spectrum: M^+ = 408 (1%), 57 (100%)

Isolation of n-Hentriacontane ($n\text{-}C_{31}H_{64}$)

n-Hentriacontane ($n\text{-}C_{31}H_{64}$) was isolated from the hexane extract of Peltophorum inerme. The hexane extract (11.3 g), which was green in color, was washed with diethyl ether. The mother liquor, which was green in color, was decanted yielding a white solid (1.4 g). This ether insoluble white solid was then subjected to preparative gas chromatography (Col III, 278°C). The sixth peak to appear, R_t - 15.2 min, was collected. This sample was then subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 7.0 min, GC trace #1 (Appendix 1, p.73). This component had the same spectral and physical properties as n-hentriacontane ($n\text{-}C_{31}H_{64}$).[35,38]

GC: R_t - 7.0 min (Col II, 272°C)

MP: 68°C lit. 67.9°C [35,38]

Mass Spectrum: M^+ = 436 (2%), 57 (100%)

Isolation of n-Tritriacontane ($n\text{-}C_{33}H_{68}$)

n-Tritriacontane ($n\text{-}C_{33}H_{68}$) was isolated from the hexane extract of Peltophorum inerme. The hexane extract (11.3 g), which was green in color, was washed with diethyl ether. The mother liquor, which was green in color, was decanted yielding a white solid (1.4 g). This ether insoluble white solid was then subjected to preparative gas chromatography (Col III, 278°C). The eighth peak to appear R_t - 24.9 min, was collected. This sample was then subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 12.0 min, GC trace #1 (Appendix 1, p. 73). This component had the same spectral and physical properties as n-tritriacontane ($n\text{-}C_{33}H_{68}$).[35]

GC: R_t - 12.0 min (Col II, 272°C)

MP: 71°C lit. 72°C [35]

Mass Spectrum: M^+ = 464 (7%), 57 (100%)

Column Chromatographies of the Hexane Extract of Peltophorum inerme

During the course of the research involving the isolation of long straight-chain hydrocarbons from the hexane extract of Peltophorum inerme many column chromatographies were done in an attempt to isolate pure compounds. None of these column chromatographies was successful in that no pure compounds were ever isolated in this manner; however, many fractions were obtained which were rich in mixtures of two to four compounds. This was helpful in that some of

these mixtures were later used to isolate pure compounds by preparative gas chromatography. Several of these chromatographys will now be discussed.

Alumina (activity III)

The hexane extract of Peltophorum inerme (1.0 g) was chromatographed on 112.5 g of alumina (acid-washed, Merck, activity III) packed in hexane. The following elution scheme was used.

Fraction	Solvent	Volume (ml)	Weight (g)
1	Hexane	30	--
2	Hexane	40	0.1149
3	Hexane	35	0.1007
4	Hexane	30	0.0052
5	Hexane	40	0.0081
6	Hexane	30	0.0061
7	Benzene-Hexane (1:9)	40	0.0082
8	Benzene-Hexane (1:9)	40	0.0102
9	Benzene-Hexane (1:9)	35	0.0158
10	Benzene-Hexane (1:3)	40	0.0108
11	Benzene-Hexane (1:3)	40	0.0046
12	Benzene-Hexane (1:3)	40	0.0037
13	Benzene-Hexane (1:1)	35	0.0044
14	Benzene-Hexane (1:1)	40	0.0071
15	Benzene-Hexane (1:1)	40	0.0288
16	Benzene-Hexane (3:1)	40	0.0278
17	Benzene-Hexane (3:1)	40	0.0067

Fraction	Solvent	Volume (ml)	Weight (g)
18	Benzene-Hexane (3:1)	40	0.0059
19	Benzene	40	0.0045
20	Benzene	40	0.0364
21	Benzene-Chloroform (1:1)	40	0.0625
22	Benzene-Chloroform (1:1)	40	0.0233
23	Benzene-Chloroform (1:1)	40	0.0089
24	Chloroform	40	0.0060
25	Chloroform	40	0.011
26	Methanol-Chloroform (1:9)	40	0.0113
27	Methanol-Chloroform (1:9)	40	0.0200
28	Methanol-Chloroform (1:9)	30	0.0568
29	Methanol-Chloroform (1:1)	40	0.0179
30	Methanol-Chloroform (1:1)	40	0.0200
31	Methanol	40	0.1007
			0.6477
			65% recovery

Fractions 2, 4, 6, 8, 10, 13, 15, 18, 22, 25, and 26 were subjected to analytical gas chromatography (Col II, 272°C). All were mixtures.

Alumina (activity II)

The hexane extract of Peltophorum inerme (0.0233 g) was chromatographed on 1.2 g of alumina (acid-washed, Merck, activity II) packed in hexane. The following elution scheme was used:

Fraction	Solvent	Volume (ml)	Weight (g)
1	Hexane	5	0.0082
2	Hexane-Benzene (4:1)	5	0.0020
3	Hexane-Benzene (4:1)	5	0.0004
4	Benzene	5	0.0037
5	Benzene	5	0.0007
6	Benzene-Chloroform (1:1)	5	0.0003
7	Benzene-Chloroform (1:1)	5	0.0002
8	Chloroform	5	0.0007
9	Chloroform	5	0.0007
10	Chloroform-Methanol (9:1)	5	0.0003
11	Methanol	5	0.0002
			0.0174
			75% recovery

Fractions 1, 2, 4, and 7 were subjected to analytical gas chromatography (Col II, 272°C). All were mixtures. Since most of the material came off in the first four fractions it was decided that activity I alumina should be used next.

Alumina (activity I)

The hexane extract of Peltophorum inerme (0.0234 g) was chromatographed on 1.2 g of alumina (acid-washed, Merck, activity II) packed in hexane. The following elution scheme was used:

Fraction	Solvent	Volume (ml)	Weight (g)
1	Hexane	5	0.0034
2	Hexane-Benzene (4:1)	5	0.0005
3	Hexane-Benzene (4:1)	5	0.0004
4	Benzene	5	0.0002
5	Benzene	5	0.0006
6	Benzene-Chloroform (1:1)	5	0.0002
7	Benzene-Chloroform (1:1)	5	0.0098
8	Chloroform	5	0.0019
9	Chloroform	5	0.0006
10	Chloroform-Methanol (9:1)	5	0.0015
11	Methanol	5	--
			0.0189
			81% recovery

Fractions 1, 7, 8, and 9 were subjected to analytical gas chromatography (Col II, 272°C). All were mixtures.

Silica Gel

The hexane extract of Peltophorum inerme (0.0200 g) was chromatographed on 1.1 g of silica gel (100-200 mesh) packed in hexane. The following elution scheme was used:

Fraction	Solvent	Volume (ml)	Weight (g)
1	Hexane	5	--
2	Hexane-Benzene (4:1)	5	0.0033
3	Hexane-Benzene (4:1)	5	0.0027
4	Benzene	5	--
5	Benzene	5	--
6	Benzene-Chloroform (1:1)	5	0.0016
7	Benzene-Chloroform (1:1)	5	0.0011
8	Chloroform	5	0.0023
9	Chloroform	5	0.0021
10	Chloroform-Methanol (9:1)	5	0.0018
11	Methanol	5	--
			0.0149
			75% recovery

Fractions 1, 4, 5, 6, 8, and 9 were subjected to analytical gas chromatography (Col II, 272°C). All were mixtures.

These column chromatographies were not the only ones carried out. The hexane extract of Peltophorum inerme (1.0 g) was chromatographed on 109.1 g of alumina (acid-washed, Merck, activity IV) packed in hexane. The elution scheme was similar to the ones previously described. Even though 48 fractions were taken no pure compounds were isolated. Still other chromatographies were done in which the enriched fractions from one chromatography were rechromatographed. None of these chromatographies yielded pure compounds.

After trying these column chromatographies it was decided that column chromatography was not the way to separate the mixture of long straight-chain hydrocarbons found in the hexane extract of Peltophorum inerme. Since the hydrocarbons could be separated by analytical gas chromatography it was decided to use preparative gas chromatography to isolate the compounds. This method proved successful, although the yields were poor.

Extraction of Bothriochloa intermedia - "K" Strain

Bothriochloa intermedia, "K" strain, (2.0 Kg) was placed on a large Soxhlet extractor and continuously extracted with 95% ethanol (9 ℓ) until the solvent in the siphon tube was colorless (four days). The ethanol was removed by a rotary evaporator yielding a dark green gum (162.0 g). The ethanol extract was partitioned between chloroform and water (1:1) until the chloroform in the continuous extractor was colorless (four days). The chloroform was removed by a rotary evaporator yielding a dark green solid (36.2 g). The water was removed by a rotary evaporator yielding a brown solid (80.4 g). The aqueous extract was partitioned between hexane and methanol-water (9:1) by stirring overnight. The hexane was removed by a rotary evaporatory yielding a brown solid (17.1 g). The methanol-water was removed by a rotary evaporator yielding a brown solid (14.1 g). Samples of the ethanol extract, chloroform extract, aqueous extract, hexane extract, and methanol-water extract were sent to the National Institute of Health for testing. See Chart 2 for an outline of the above procedure. Table 3 contains the cancer screen data.

Chart 2. Extraction Procedure for Bothriochloa intermedia

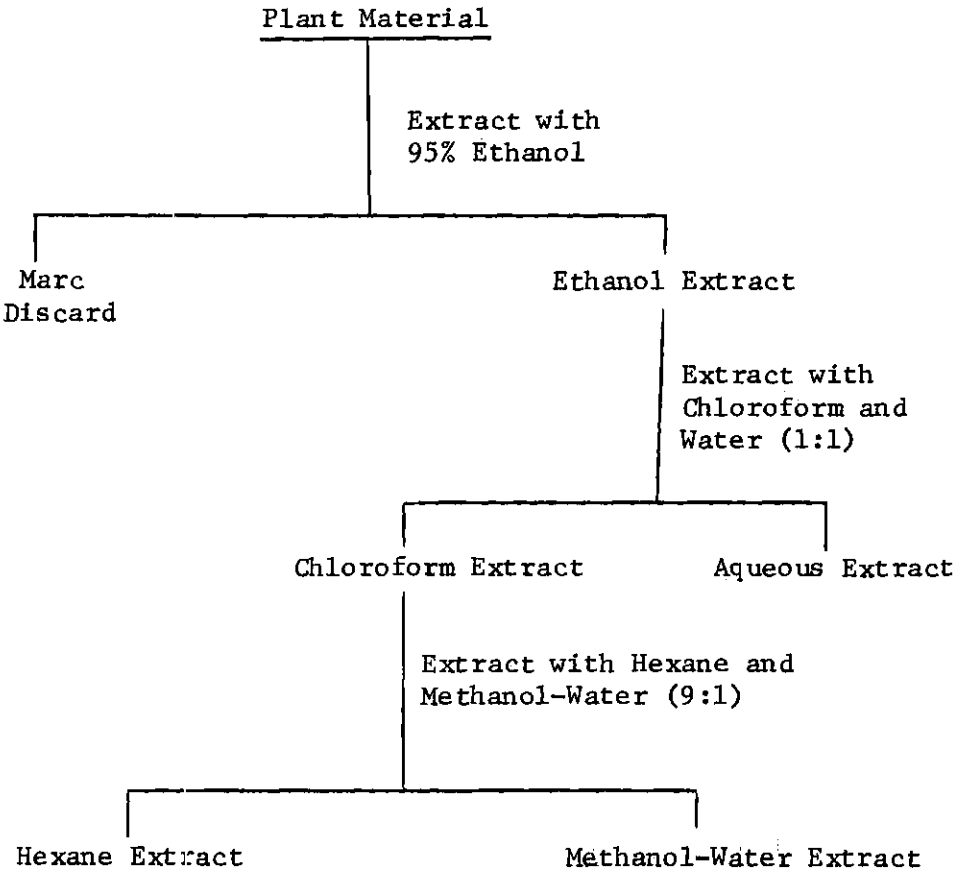


Table 3. National Cancer Institute Screen Data on Some Bothriochloa intermedia Extracts

Extract	GIT No.	CCNSC No.	Tumor	Dose (mg/Kg)	Toxicity (survivors)	%TC
"K" strain EtOH	JB-III-59-1	B837103	PS	400	616	100
			PS	200	616	98
			PS	100	616	99
				KB w slope - 0.25 ED50 1.0 x 10		
"K" strain $CHCl_3$	JB-III-59-2	B837104	PS	400	616	110
			PS	200	616	108
			PS	100	616	104
				KB w slope - 0.30 ED50 1.0 x 10		
"K" strain H_2O	JB-III-59-3	B837105	PS	400	616	100
			PS	200	616	101
			PS	100	616	105
				KB w slope - 0.00 ED50 1.0 x 0		
"K" strain hexane	JB-III-61-1	B837106	PS	400	616	108
			PS	200	616	97
			PS	100	616	107
				KB w slope - 0.00 ED50 1.0 x 0		
"K" strain $MeOH-H_2O$	JB-III-61-2	B837107	PS	400	616	106
			PS	200	616	126
			PS	100	616	118
				KB w slope - 0.53 ED50 5.9 x 10		

continued...

Table 3. (continued)

Extract	GIT No.	CCNSC No.	Tumor	Dose (mg/Kg)	Toxicity (survivors)	%TC
"I-F" strain	JB-III-60-1	B837108	PS	400	616	100
			PS	200	616	110
EtOH			PS	100	616	92
				KB w slope - 0.00 ED50 1.0 x 10		

Extraction of Bothriochloa intermedia - "I-F" Strain

Bothriochloa intermedia, "I-F" strain, (1.54 Kg) was placed in a large Soxhlet extractor and continuously extracted with 95% ethanol (9 ℓ) until the solvent in the siphon tube was colorless (four days). The ethanol was removed on a rotary evaporator yielding a dark green gum (223.8 g). A sample of the ethanol extract was sent to the National Institute of Health for testing. Table 3 contains the cancer screen data.

Isolation of the Steam Volatile Oil of the Hybrid Grass (56x627-1)[8]

The hybrid 56x627-1 was prepared from two forms of Bothriochloa intermedia (5410 x 5400). The chopped dry grass was steam distilled in a 12 liter round-bottom flask half-filled with grass. The steam distillation was continued until there was no oil in the condensate, which usually required six to ten liters of condensate. The condensate was continuously extracted with ether. The etheral solution was dried ($MgSO_4$) and the ether removed by distillation at 40° with a Vigreux column to give 0.2% oil based on dry plant. In this manner a total of 120 g of oil was collected. The oil was then subjected to analytical gas chromatography (Col I, 152°C), GC trace #2 (Appendix 1, p. 74), and (Col I, 184°C), GC trace #3 (Appendix 1, p. 75). On both GC traces the peaks are labeled along with their corresponding retention times. This oil will later be referred to as 627 grass oil.

Isolation of Kessane (1)

Kessane (1) was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of *Bethriochloa intermedia* (5410 x 5400). Kessane (1) was isolated in pure form by the use of medium pressure high performance chromatography. The 627 grass oil (3.0 g) was chromatographed on a silica gel 60, size C, pre-packed column. Kessane (1) (0.56 g) was isolated in pure form from a 1:1 hexane-benzene fraction. Purity was determined by analytical gas chromatography (Col I, 152°C). Mixed injections were used to determine that Kessane corresponds to peak #1 on both GC traces #2 and #3 (Appendix 1, p. 74 and 75). This compound had the same physical and spectral properties as Kessane (1).[39,40] Spectra (^{1}H-NMR and IR) were kindly supplied by Yoshikoshi.

GC: R_t - 10.0 min (Col I, 152°C) and R_t - 3.6 min (Col I, 184°C)

BP: 85°C/0.2 mm Hg [47] lit. b.p. 110~112°C/6 mm Hg [39,40]

I.R.: ν_{max}^{film} 2950, 2879, 1450, 1375, 1360, 1250, 1230, 1095, 985, 975 cm^{-1}

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.791 (3H,d, J = 6.6 cps), 1.110 (3H,s), 1.248 (6H,s), 1.675 (14H,m)

^{13}C-NMR (25 MHz, δ, $CDCl_3$): 74.5, 73.6 (ether carbons), 50.1, 41.4, 35.7, 34.7, 33.2, 32.8, 32.1, 31.0, 29.6, 28.2, 24.2, 18.4

MS: M^+ = 222 (6%), 126 (100%)

Anal: 81.34% C, 11.97% H (Calcd. for $C_{15}H_{26}O$: 81.02% C, 11.79% H)

Isolation of Elemol (2)

Elemol (2) was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). Elemol (2) was isolated in pure form by the use of column chromatography and preparative gas chromatography. The 627 grass oil (2.0 g) was chromatographed on silica gel 60 (230-400 mesh) (127.0 g). Only compounds which corresponded to peaks #2 (R_t - 10.6 min) and #6-A and 6-B (R_t - 16.7 min) on GC trace #2 (Appendix 1, p. 74) were present in the benzene fraction. This fraction was then subjected to preparative gas chromatography (Col III, 148°C). The compound corresponding to the first peak to appear was collected. The sample (a white solid) was then subjected to analytical gas chromatography (Col I, 152°C). Only one peak was present (R_t - 10.6 min). Mixed injections were used to determine that elemol (2) corresponds to peak #2 on GC trace #2 (Appendix 1, p. 74). This compound had the same physical and spectral properties as elemol (2).[41,42]

GC: R_t - 10.6 min (Col I, 152°C)

MP: 52-53°C lit. 52-53°C [41]

IR: ν_{max}^{film} 3300, 3070, 2960, 2930, 2850, 1630, 1465, 1430, 1370, 910, 880 cm^{-1}

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.986 (3H,s), 1.204 (6H,s), 1.717 (3H,s), 4.603-4.975 (4H,m), 5.819 (1H,q,J=10,8 cps)

MS: M^+ - 18 = 204 (7%), 59 (100%)

Anal: 81.10% C, 11.83% H (Calcd. for $C_{15}H_{26}O$: 81.02% C, 11.79% H)

Isolation of β-Eudesmol (6-A)

β-Eudesmol (6-A) was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). β-Eudesmol was isolated in pure form by the use of a spinning band distillation, medium pressure high performance chromatography, and high pressure high performance chromatography. The 627 grass oil (54.4 g) was distilled using an annular teflon spinning band column. The reflux ratio was adjusted to approximately 10:1. After the distillation, the material remaining in the still pot was subjected to analytical gas chromatography (Col I, 152°C). Only compounds which corresponded to two peaks were present, a small peak with R_t - 16.7 min and a large peak with R_t - 19.3 min. The still pot material (3.0 g) was chromatographed on a silica gel 60, size C, pre-packed column. β-Eudesmol (6-A) (14 mg) was isolated from one of the chloroform fractions. The β-eudesmol (6-A) that was isolated needed to be purified, and this was accomplished by chromatographing the impure β-eudesmol (6-A) (14 mg) on a partisil, M9 10/25, pre-packed column using chloroform as the elutant. Approximately 10 mg of pure white crystalline β-eudesmol (6-A) was recovered. Purity was determined by analytical gas chromatography (Col I, 152°C). Mixed injections were used to determine that β-eudesmol (6-A) corresponds to peak #6-A on both GC traces #2 and #3 (Appendix 1, p. 74 and 75). This compound had the same physical and spectral properties as β-eudesmol (6-A).[43,44]

GC: R_t - 16.7 min (Col I, 152°C) and R_t - 5.5 min (Col I, 184°C)

MP: 69°C lit. 68-69°C [43]

IR: $\nu_{max}^{CCl_4}$ 3400, 3075, 2925, 1642, 1450, 1380, 940, 920, 890, 845 cm^{-1}

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.701 (3H,s), 1.604 (6H,s), 4.438 (1H,s), 4.708 (1H,s)

MS: M^+ = 222 (10%), 149 (100%)

Anal: 81.05% C, 11.95% H (Calcd. for $C_{15}H_{26}O$: 81.02% C, 11.79% H)

Isolation of Acorenone-B (7)

Acorenone-B (7) was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). Acorenone-B was isolated in pure form by the use of medium pressure high performance chromatography. The 627 grass oil (3.0 g) was chromatographed on a silica gel 60, size C, pre-packed column. Acorenone-B (7) (1.2 g) was isolated in pure form from a 3:1 benzene-chloroform fraction. Purity was determined by analytical gas chromatography (Col I, 152°C). Mixed injections were used to determine that acorenone-B (7) corresponds to peak #7 on both GC traces #2 and #3 (Appendix 1, p. 74 and 75). This compound had the same physical and spectral properties as that of a known sample of acorenone-B (7).[7,8]

GC: R_t - 19.3 min (Col I, 152°C) and R_t - 6.3 min (Col I, 184°C)

BP: 68°C/0.01 mm [47]

IR: ν_{max}^{film} 1670 cm^{-1}

^{1}H-NMR (60 MHz, δ, $CDCl_3$): 0.77 (3H,d,J=6 cps), 0.87 (3H,d,J=6 cps), 0.95 (3H,d,J=6 cps), 1.75 (3H,m), 2.2 (2H,m), 2.1, 2.4, 2.6, 2.9 (2H, doublet of doublets, J = 17 cps), 6.7 (1H,m)

^{13}C-NMR (25 MHz, δ, $CDCl_3$): 199.2 (C=O), 143.5 (=C $\begin{matrix}R\\H\end{matrix}$), 134.7 (=$C(R)_2$), 49.2 ($c(R)_4$), 56.7, 48.1, 45.8, 29.7, 28.9, 25.8, 25.1, 24.0, 21.1, 16.9, 15.3

MS: M^+ = 220 (82.8%), 135 (100%)

Anal: 81.53% C, 10.96% H (Calcd. for $C_{15}H_{24}O$: 81.76% C, 10.98% H)

Isolation of 7-Hydroxycalamenene (9)

7-Hydroxycalamenene (9) was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). 7-Hydroxycalamenene (9) was isolated in pure form by the use of medium pressure high performance chromatography. The 627 grass oil (3.0 g) was chromatographed on a silica gel 60, size C, prepacked column. 7-Hydroxycalamenene (9) and a compound corresponding to peak #8 on GC trace #3 (Appendix 1, p. 75) were isolated from a 1:1 hexane-benzene fraction. This mixture was then subjected to preparative gas chromatography (Col III, 170°C). The compound corresponding to the second peak to appear was collected. The sample (an oil) was then subjected to analytical gas chromatography (Col I, 184°C). Only one peak was present (R_t - 9.8 min). Mixed injections were used to determine that 7-hydroxycalamenene (9) corresponds to peak #9 on GC trace #3 (Appendix 1, p. 75). This compound had the same physical and spectral properties as 7-hydroxycalamenene (9).[45] Spectra (^{1}H-NMR and IR) were kindly supplied by Rowe.

GC: R_t - 9.8 min (Col I, 184°C)

BP: Oil

IR: ν_{max}^{film} 3350, 2950, 1620, 1500, 1460, 1260, 880 cm^{-1}

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.757 (3H, doublet, J=6.59 cps), 1.03 (3H, doublet, J= 7.0 cps), 1.22 (3H, doublet, J=7.0 cps), 2.203 (3H, singlet), 6.639 (1H, singlet), 6.880 (1H, singlet)

MS: M^+ = 218 (12%), 175 (100%)

Exact Mass: 218.16707 (-1.46) (20 ppm) ($C_{15}H_{22}O$)

Isolation of 627-A

Compound 627-A [GC traces #2 and #3 (Appendix 1, p. 74 and 75)] was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). Compound 627-A was isolated in pure form by the use of a spinning band distillation, medium pressure high performance chromatography, and preparative gas chromatography. The 627 grass oil (54.4 g) was distilled using an annular teflon spinning band column. The reflux ratio was adjusted to approximately 10:1. After the distillation, fraction #11 was subjected to analytical gas chromatography (Col I, 152°C). Among the compounds present was one with R_t - 7.3 min. Fraction #11 (1.15 g) was chromatographed on a silica gel 60, size C, prepacked column. Compound 627-A was isolated in impure form from the 1:1 benzene-chloroform fraction. Compound 626-A was then subjected to preparative gas chromatography (Col III, 148°C). The compound corresponding to the first peak to appear was collected. The sample (an oil) was then subjected to analytical gas chromatography (Col I, 152°C). Only one peak was present (R_t - 7.3 min). Mixed injections were used to determine that compound 627-A corresponds to peak "A" on GC trace #2 (Appendix 1, p. 74).

GC: R_t - 7.3 min (Col I, 152°C)

BP: Oil

IR: $\nu_{max}^{CCl_4}$ 3350, 2940, 2860, 1725, 1460, 1376, 1175, 1050 (plate V, p. 90)

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.880 (2H,t,J=6.35 Hz), 1.273 (10H,s), 1.562 (2H,s), 2.058 (2H,d,J=6.35 Hz), 2.327 (2H,q,J=6.35 Hz), 3.643 (2H,t,J=6.35 Hz), 5.295-5.633 (2H,m) (plate VI, p. 90)

MS: $M^+ - 18 = 166$ (4%), 68 (100%) (plate VII, p. 90)

Exact Mass: 166.1691 18 ppm ($C_{12}H_{22}$)

Isolation of 627-3

Compound 627-3 [GC traces #2 and #3 (Appendix 1, p. 74 and 75)] was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). Compound 627-3 was isolated in pure form by the use of a spinning band distillation, medium pressure high performance chromatography, and preparative gas chromatography. The 627 grass oil (54.5 g) was distilled using an annular teflon spinning band distillation column. The reflux ratio was adjusted to approximately 10:1. After the distillation, fraction #11 was subjected to analytical gas chromatography (Col I, 152°C). Among the compounds present was one with R_t - 12.4 min. Fraction #11 (1.15 g) was chromatographed on a silica gel 60, size C, pre-packed column. Compound 627-3 was isolated in impure form from the 3:1 hexane-benzene fraction. Compound 627-3 was then subjected to preparative gas chromatography (Col III, 148°C). The compound corresponding to the first peak to appear was collected. The sample (an oil) was then subjected to analytical gas chromatography (Col I, 152°C). Only one peak was present (R_t - 12.4 min). Mixed injections were used to determine that compound 627-3 corresponded to peak #3 on GC trace #2 (Appendix 1, p. 74).

GC: R_t - 12.4 min (Col I, 152°C) and R_t - 4.4 min (Col I, 184°C)

BP: Oil

IR: ν_{film}^{max} 2950, 2925, 2850, 1740, 1460, 1375, 1170, 1110, 975 cm^{-1} (plate VII, p. 91)

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.826-0.969 (m), 1.288 (s), 1.369-1.572 (m), 1.997-2.146 (m), 2.308 (t,J=7.57 Hz), 4.512 (d,J=5.38 Hz), 5.402-5.925 (m) (plate IX, p. 92)

MS: M^+ = 226 (3%), 57 (100%) (plate X, p. 92)

Anal: 75.99% C, 12.02% H (Calcd. for $C_{14}H_{26}O_2$: 74.29% C, 11.58% H)

Isolation of 627-6-B

Compound 627-6-B [GC traces #2 and #3 (Appendix 1, p. 74 and 75)] was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). Compound 627-6-B was isolated in pure form by the use of column chromatography and preparative gas chromatography. The 627 grass oil (2.0 g) was chromatographed on silica gel 60 (230-400 mesh) (127.0 g). Only compounds which corresponded to peaks #1 (R_t - 10 min) and #6-A and 6-B (R_t - 16.7 min) on GC trace #2 (Appendix 1, p. 74) were present in the 1:1 hexane-benzene fraction. This fraction was then subjected to preparative gas chromatography (Col III, 148°C). The compound corresponding to the second peak to appear was collected. The sample (a white solid) was then subjected to analytical gas chromatography (Col I, 152°C). Only one peak was present (R_t - 16.7 min). Mixed injections were used to determine that compound 627-6-B corresponds to peak #6-B on GC trace #2 (Appendix 1, p. 74).

GC: R_t - 16.7 min (Col I, 152°C)

MP: 68°C

IR: ν_{max}^{film} 2940, 2850, 2800, 1680, 1630, 1445, 1365, 880 cm^{-1} (plate XI, p. 93)

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.959 (d,J=3.41 Hz), 1.256 (s), 1.602-1.715 (m), 2.257-2.735 (m), 4.84 (d,J=3.41 Hz), 6.546 (t,J=8.175 Hz), 8.977 (1H,s) (plate ZII, p. 93)

MS: M^+ = 222 (2%), 59 (100%) (plate XII, p. 94)

Exact Mass: 222.19628 (2.08) (20 ppm) ($C_{15}H_{26}O$)

Isolation of 627-8

Compound 627-8 [GC traces #2 and #3 (Appendix 1, p. 74 and 75)] was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). Compound 627-8 was isolated in pure form by the use of medium pressure high performance chromatography and preparative gas chromatography. The 627 grass oil (3.0 g) was chromatographed on a silica gel 60, size C, pre-packed column. 7-Hydroxycalamenene (9) and a compound corresponding to peak #8 on GC trace #2 (Appendix 1, p. 74) were isolated from a 1:1 hexane-benzene fraction. This mixture was then subjected to preparative gas chromatography (Col III, 170°C). The compound corresponding to the first peak to appear was collected. The sample (an oil) was then subjected to analytical gas chromatography (Col I, 184°C). Only one peak was present (R_t - 6.9 min). Mixed injections were used to determine that compound 627-8 corresponded to peak #8 on GC traces #2 and #3 (Appendix 1, p. 74 and 75).

GC: R_t - 21.7 min (Col I, 152°C) and R_t - 6.9 min (Col I, 184°C)

BP: Oil

IR: ν_{max}^{film} 2945, 2860, 1710, 1470, 1380, 1090, 840, 825 cm^{-1} (plate XIV, p. 94)

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.781 (d,J=6.11 Hz), 0.896 (d,J=6.59 Hz), 1.017 (d,J=6.59 Hz), 1.160-1.256 (m), 1.371 (s), 1.548 (s), 1.160-1.256 (m), 2.038-2.200 (m), 3.194-3.435 (m) (plate XV, p. 95)

MS: M^+ = 236 (2%), 43 (100%) (plate XVI, p. 95)

Exact Mass: 236.16252 (-6.02) (40 ppm) ($C_{18}H_{20}$)

Isolation of 627-10

Compound 627-10 [GC trace #3 (Appendix 1, p. 75)] was isolated from the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). Compound 627-10 was isolated in pure form by the use of a spinning band distillation, medium pressure high performance chromatography and preparative gas chromatography. The 627 grass oil (54.5 g) was distilled using an annular teflon spinning band column. The reflux ratio was adjusted to approximately 10:1. After the distillation, 3 g of the material remaining in the still pot was chromatographed on a silica gel 60, size C, pre-packed column. Compound 627-10 was isolated from one of the chloroform fractions. The 627-10 that was isolated needed to be purified and this was accomplished by preparative gas chromatography (Col IV, 260°C). The compound corresponding to the third peak to appear was collected. The sample (an oil) was then subjected to analytical gas chromatography (Col I, 184°C). Only one peak was present (R_t - 15.9 min). Mixed injections were used to determine that compound 627-10 corresponds to peak #10 on GC trace #3 (Appendix 1, p. 75).

GC: R_t - 15.9 min (Col I, 184°C)

BP: Oil

IR: ν_{max}^{film} 2955, 2870, 1740, 1590, 1575, 1460, 1280, 1205, 1150, 1080, 740 cm^{-1} (plate XVII, p. 96)

^{1}H-NMR (100 MHz, δ, $CDCl_3$): 0.937-1.025 (m), 1.582-1.720 (m), 4.144-4.372 (m), 4.831 (s), 7.512-7.606 (m), 7.738-7.834 (m) (plate XVIII, p. 96)

MS: $M^+ - 17 = 263$ (93%), 149 (100%) (plate XIX, p. 97)

Anal: 64.42% C, 7.26% H (Calcd. for $C_{15}H_{20}O_5$: 64.27% C, 7.19% H)

Isolation of the Steam Volatile Oil of Bothriochloa intermedia - "K" Strain

Procedure I

Bothriochloa intermedia - "K" Strain (350 g) was steam distilled in a 12 liter round-bottom flask. The steam distillation continued until there was no oil in the condensate. The condensate was extracted with ether. The etheral solution was dried ($MgSO_4$) and the ether removed by a rotary evaporator at about 30°C to yield a brown oil (0.7 g). The oil was then subjected to analytical gas chromatography (Col I, 152°C), GC trace #6 (Appendix 1, p. 78).

Procedure II

Bothriochloa intermedia - "K" Strain (2.0 Kg) was placed in a large Soxhlet extractor and continuously extracted with 95% ethanol (9 ℓ) until the solvent in the siphon tube was colorless (four days). The ethanol was removed by a rotary evaporator; there remained a dark green gum (162.0 g). The ethanol extract (162.0 g) was steam distilled and continuously extracted with ether for eight days. The etheral solution was dried ($MgSO_4$) and the ether removed by a rotary evaporator at about

30°C to yield a brown oil (7.96 g). The oil was then subjected to analytical gas chromatography (Col I, 152°C). The GC trace was exactly the same as GC trace #6 (Appendix 1, p. 78).

Isolation of the Steam Volatile Oil of Bothriochloa intermedia - "I-F" Strain

Bothriochloa intermedia - "I-F" Strain (1.5 Kg) was placed in a large Soxhlet extractor and continuously extracted with 95% ethanol (9 ℓ) until the solvent in the siphon tube was colorless after four days. The ethanol was removed on a rotary evaporator; there remained a dark green solid (223.8 g). The ethanol extract (223.8 g) was steam distilled and continuously extracted with ether for about 18 hours. The etheral solution was dried ($MgSO_4$) and the ether removed by a rotary evaporator at about 30°C to yield a yellow oil (3.16 g). The oil was then subjected to analytical gas chromatography (Col I, 152°C), GC trace #4 (Appendix 1, p. 76).

Isolation of the Steam Volatile Oil of Bothriochloa intermedia - "I-T" Strain

Bothriochloa intermedia - "I-T" Strain (341 g) was steam distilled in a 12 liter round-bottom flask. The steam distillation continued until there was no oil in the condensate. The condensate was extracted with ether. The etheral solution was dried ($MgSO_4$) and the ether removed by a rotary evaporator at about 30°C to yield a yellow oil (0.7 g). The oil was then subjected to analytical gas chromatography (Col I, 152°C), GC trace #5 (Appendix 1, p. 77).

Isolation of the Steam Volatile Oil of Bothriochloa intermedia - Accession 5297

Bothriochloa intermedia (accession 5297) was chopped and steam distilled in a 12 liter round-bottom flask. The steam distillation continued until there was no oil in the condensate. The condensate was continuously extracted with ether. The etheral solution was dried ($MgSO_4$) and the ether removed by distillation at 40°C with a Vigreux column to give 0.1% oil based on dry plant. The oil was then subjected to analytical gas chromatography (Col I, 152°C), GC trace #7 (Appendix 1, p. 79).

Isolation of the Steam Volatile Oil of Bothriochloa intermedia - Accession 5752

Bothriochloa intermedia (accession 5752) was chopped and steam distilled in a 12 liter round-bottom flask. The steam distillation continued until there was no oil in the condensate. The condensate was continuously extracted with ether. The etheral solution was dried ($MgSO_4$) and the ether removed by distillation at 40°C with a Vigreux column to give 0.2% oil based on dry plant. The oil was then subjected to analytical gas chromatography (Col I, 152°C), GC trace #8 (Appendix 1, p. 80).

Isolation of the Steam Volatile Oils of Bothriochloa intermedia - Accessions (8967, 8969, and 8907)

The dry grasses were each ground-up in a Waring blender with water. The ground-up grasses were steam distilled with mechanical stirring and continuously extracted with ether for about four days.

The etheral solutions were dried ($MgSO_4$) and the ether removed by distillation at 40°C with a Vigreux column to give about 0.5% oil based on each dry plant. Each oil was subjected to analytical gas chromatography (Col I, 152°C).

Accession 8967 GC trace #9 (Appendix 1, p. 81)

Accession 8969 GC trace #10 (Appendix 1, p. 82)

Accession 8907 GC trace #11 (Appendix 1, p. 83)

CHAPTER IV

DISCUSSION OF RESULTS

As stated in the Introduction, the purpose of this research was threefold. First, several secondary plant metabolites were identified in the hexane fraction of Peltophorum inerme. Since Peltophorum inerme showed confirmed preliminary anti-tumor activity, extracts were sent to the National Institute of Health for further testing. Second, several secondary plant metabolites in the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400) were identified. Third, a gas chromatographic study was made of some accessions, hybrids, and strains of Bothriochloa intermedia as an aid in determining palatability to cattle and taxonomic identification.

Extraction of Peltophorum inerme and the Isolation of Several Long Straight-Chain Hydrocarbons

Extracts of Peltophorum inerme were screened by the National Institute of Health for anti-tumor activity. Activity was found in KB-Cell Culture. Our interest was in concentrating the KB activity by successively extracting the plant with various solvents and sending the extracts to the National Institute of Health for testing. After the anti-tumor activity was concentrated in a particular extract, then the compound responsible for this anti-tumor activity could be isolated and identified. It turned out that even though the plant showed preliminary anti-tumor activity, the various extracts of the plant showed very little or no anti-tumor activity.

The plant material was collected in Puerto Rico by the U.S. Department of Agriculture in April, 1969. The leaves (2.37 Kg) were separated from the stems and ground in a Waring blender containing hexane. The extraction procedure is that shown on Chart 1, p. 20.

The ground-up leaves were placed in a large Soxhlet extractor and continuously extracted with about nine liters of hexane until the hexane in the siphon tube was colorless. The hexane extraction procedure took five days. Removal of the hexane from the hexane solution produced 34.22 g of hexane soluble extract.

The plant material remaining in the Soxhlet extractor after the hexane extraction was extracted with about nine liters of 95% ethanol until the ethanol in the siphon tube was colorless. The ethanol extraction procedure took five days. Removal of the ethanol from the ethanol solution produced 212.96 g of ethanol soluble extract.

The ethanol soluble extract was partitioned between chloroform and water (1:1) in a large chloroform extractor. The extraction was continued until the fresh chloroform extract was colorless. The chloroform and water (1:1) partitioning took four days. Removal of the chloroform from the chloroform solution produced 61.23 g of chloroform soluble extract. Removal of the water from the water solution produced 92.19 g of water soluble extract. Samples of the hexane soluble extract, ethanol soluble extract, chloroform soluble extract, and water soluble extract were sent to the National Institute of Health for testing. Very little or no anti-tumor activity was found in each extract (see Table 2).

The hexane extract was subjected to analytical gas chromatography (Col II, 272°C), GC trace #1 (Appendix 1, p. 73). From this GC trace it can be seen that there are eleven major compounds present. Column chromatography of the hexane extract on alumina - activity I, II, III, IV, and silica gel resulted in some separation; however, a pure compound could never be isolated in this manner. The isolation of four of the compounds in the hexane extract was accomplished by preparative gas chromatography. Four other compounds in the hexane extract were identified by mixed injections of known compounds with the hexane extract. The hexane extract was washed with diethyl ether to give a white waxy solid. This white waxy solid had the same GC trace as that of the green hexane extract. Preparative gas chromatography was conducted on this white waxy solid instead of the green hexane extract, which was a gum, because it was felt it would do less damage to the prep column.

The ether insoluble white solid was subjected to preparative gas chromatography (Col III, 278°C). The compound corresponding to the first peak to appear was collected. This sample was then subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 2.4 min. This peak corresponds to the peak labeled n-$C_{27}H_{56}$ in GC trace #1 (Appendix 1, p. 73) which was proven by mixed injections. The IR and ^{1}H-NMR of this compound, which had a melting point of 58-60°C, indicated it was a hydrocarbon; however, the structure was determined from its mass spectrum (Appendix 2, p. 85) which showed a M^+ = 380 (2%), and had a pattern characteristic for straight chain hydrocarbons, i.e. the gradual increase from $(M-15)^+$ to m/e 57 in

abundance of C_nH_{2n+1} fragments.[46] The compound was thus identified as n-heptacosane (n-$C_{27}H_{56}$) whose melting point was reported as 59.1°C.[35,36]

The compound corresponding to the fourth peak to appear was collected when the ether insoluble white solid was subjected to preparative gas chromatography (Col III, 278°C). This sample was then subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 4.1 min. This peak corresponds to the peak labeled n-$C_{29}H_{60}$ in GC trace #1 (Appendix 1, p. 73) which was proven by mixed injections. The IR and ^{1}H-NMR of this compound, which had a melting point of 62-64°C, indicated it was a hydrocarbon; however, the structure was determined from its mass spectrum, (Appendix 2, p. 86) which showed a M^+ = 408 (1%), and had a pattern characteristic for straight chain hydrocarbons.[46] The compound was thus identified as n-nonacosane (n-$C_{29}H_{60}$) whose melting point was reported as 63.7°C.[35,37]

The compound corresponding to the sixth peak to appear was collected with the ether insoluble white solid was subjected to preparative gas chromatography (Col III, 278°C). This sample was then subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 7.0 min. This peak corresponds to the peak labeled n-$C_{31}H_{64}$ in GC trace #1 (Appendix 1, p. 73) which was proven by mixed injections. The IR and ^{1}H-NMR of this compound, which had a melting point of 68°C, indicated it was a hydrocarbon; however, the structure was determined from its mass spectrum (Appendix 2, p. 87) which showed M^+ = 436 (2%), and had a pattern characteristic for straight chain hydrocarbons.[46] The compound was thus identified as n-hentriacontane (n-$C_{31}H_{64}$) whose reported melting point was 67.9°C.[35,38]

The compound corresponding to the eighth peak to appear was collected when the ether insoluble white solid was subjected to preparative gas chromatography (Col III, 278°C). This sample was subjected to analytical gas chromatography (Col II, 272°C). Only one peak was present, R_t - 12.0 min. This peak corresponds to the peak labeled n-$C_{33}H_{68}$ in GC trace #1 (Appendix 1, p. 73) which was proven by mixed injections. The IR and ^{1}H-NMR of this compound, which had a melting point of 71°C, indicated it was a hydrocarbon; however, the structure was determined from its mass spectrum (Appendix 2, p. 88) which showed M^+ = 464 (7%) and had a pattern characteristic for straight chain hydrocarbons.[46] The compound was thus identified as n-tritriacontane (n-$C_{33}H_{68}$) whose melting point was reported as 72°C.[35]

Four other straight chain hydrocarbons were found in the hexane extract by mixed injections of the extract with known samples of n-octacosane (n-$C_{28}H_{58}$), R_t - 3.1 min; n-triacontane (n-$C_{30}H_{62}$), R_t - 5.4 min; n-dotriacontane (n-$C_{32}H_{66}$), R_t - 9.4 min; and n-tetratriacontane (n-$C_{34}H_{70}$), R_t - 16.0 min (Col II, 272°C). The analysis of the hexane extract is summarized in GC trace #1 (Appendix 1, p. 73).

Alkanes are very widely distributed in both the plant and animal kingdoms. In plants they are most abundant in the cuticle waxes which act as protective coatings on leaves and stems.[2] The alkanes in plants are formed by the decarboxylation of the corresponding long-chain fatty acids. Since the even-carbon-numbered carboxylic acids are produced in larger amounts than the odd-carbon-numbered carboxylic acids, it can be seen why the odd-carbon-numbered alkanes are produced in greater amounts than the even-carbon-numbered alkanes.[3]

Extraction of Bothriochloa intermedia "K" and "I-F" Strains

The purpose for extracting Bothriochloa intermedia with various solvents and sending the extracts to the National Institute of Health for testing was to determine whether or not this particular strain possessed anti-tumor activity. It turned out that the methanol-water (9:1) extract of "K" strain did possess anti-tumor activity. The extraction procedure is that shown on Chart 2, p. 30.

Bothriochloa intermedia "K" and "I-F" strains were grown by the Agronomy Department at Oklahoma State University either at Stillwater, Oklahoma, or Fort Reno, Oklahoma, in 1976. The grasses were harvested, dried, baled, and shipped in burlap bags. The extraction procedure began with grinding up the grass, "K" strain, (2.0 Kg) in a Waring blender using 95% ethanol as a solvent. The ground-up grass was then placed in a large Soxhlet extractor and continuously extracted with about nine liters of 95% ethanol until the ethanol in the siphon tube was colorless. The ethanol extraction procedure took four days. Removal of the ethanol from the ethanol solution produced 162.0 g of ethanol soluble extract.

The ethanol soluble extract was extracted with chloroform and water by partitioning it between chloroform and water (1:1) in a large chloroform extractor. The extraction was continued until the chloroform layer was colorless. The chloroform and water (1:1) partitioning took four days. Removal of the chloroform from the chloroform solution produced 36.2 g of chloroform soluble extract. Removal of the water from the water solution produced 80.4 g of water soluble extract.

The water soluble extract was extracted with hexane and methanol-water (9:1) by partitioning it between hexane and methanol-water (1:1)

in a large erlenmeyer flask and stirring overnight. Removal of the hexane from the hexane solution produced 17.1 g of hexane soluble extract. Removal of the methanol-water from the methanol-water solution produced 14.1 g of methanol-water soluble extract. Samples of the ethanol soluble extract, chloroform soluble extract, hexane soluble extract, and methanol-water (9:1) soluble extract were sent to the National Institute of Health for testing. Only the methanol-water (9:1) soluble extract showed anti-tumor activity. See Chart 2, p. 30 for an outline of the above procedure.

Bothriochloa intermedia - "I-F" strain (1.54 Kg) was ground up in a Waring blender using 95% ethanol as a solvent. The ground-up grass was then placed in a large Soxhlet extractor and continuously extracted with about nine liters of 95% ethanol until the ethanol in the siphon tube was colorless. The ethanol extraction procedure took four days. Removal of the ethanol from the ethanol solution produced 223.8 g of ethanol soluble extract. A sample of the ethanol soluble extract was sent to the National Institute of Health for testing. This extract did not exhibit any anti-tumor activity.

Isolation and Identification of Kessane (1), Elemol (2), β-Eudesmol (6-A), Acorenone-B (7), and 7-Hydroxycalamenene (9) from 627 Grass Oil

Until this work, only one compound, acorenone-B (7),[7] was known to be present in the steam volatile oil of the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia (5410 x 5400). This oil will be referred to simply as 627 grass oil.

Kessane (1) was isolated by using medium pressure high performance chromatography. The chromatography was carried out on a silica gel 60, size C, pre-packed column. From 3.0 g of 627 grass oil 0.56 g of pure kessane (1) was obtained from a (1:1) hexane-benzene fraction. This sample was subjected to analytical gas chromatography (Col I, 152°C). Only one peak was present with a retention time of 10.0 min. Mixed injections were used to determine that kessane (1) corresponds to peak #1 in both GC traces #2 and #3 (Appendix 1, p. 74 and 75). That this oil was kessane (1) was determined by comparing its spectral properties with the spectra obtained from kessane (1) which were kindly supplied by Yoshikoshi.[39,40] Both the IR's and ^{1}H-NMR's were completely identical. A new piece of data (^{13}C-NMR) was obtained on kessane which is in the experimental section of this dissertation, p. 34.

Elemol (2) was isolated by using a combination of column chromatography and preparative gas chromatography. The chromatography was carried out on 127.0 g of silica gel 60 (230-400 mesh). From 2.0 g of 627 grass oil a mixture was obtained from the benzene fraction which consisted of compounds corresponding to peaks #2 and #6-A and 6-B on GC trace #2 (Appendix 1, p. 74). This mixture was then subjected to preparative gas chromatography (Col III, 148°C). A white solid with a melting point of 52-53°C was obtained when the compound corresponding to the first peak to appear was collected. This sample was subjected to analytical gas chromatography (Col I, 152°C). Only one peak was present with a retention time of 10.6 min. Mixed injections were used to determine that elemol (2) corresponds to peak #2 on both GC traces #2 and #3 (Appendix 1, p. 74 and 75). That this solid was elemol (2) [41]

was determined by comparing its spectral properties with the literature's spectral properties of elemol (2).[41,42] Both the IR's and ^{1}H-NMR's were completely identical.

β-Eudesmol (6-A) was isolated by carrying out a spinning band distillation on 627 grass oil, chromatographing 3.0 g of the material remaining in the still pot by the use of medium pressure high performance equipment, and purifying one of the fractions by using high pressure high performance chromatography. An annular teflon spinning band column was used to distill 54.4 g of 627 grass oil. A silica gel 60, size C, pre-packed column was used to chromatograph 3.0 g of the material remaining in the still pot. From one of the chloroform fractions, 14 mg of approximately 95% pure β-eudesmol (6-A) was isolated. Further purification was accomplished by chromatographing the β-eudesmol on a partisil, M9, 10/25, pre-packed column using chloroform as the eluent. In this manner 10 mg of pure white β-eudesmol (6-A) with a melting point of 69°C was obtained. This material had a retention time of 16.7 min when subjected to analytical gas chromatography (Col I, 152°C). Mixed injections were used to determine that β-eudesmol (6-A) corresponded to peak #6-A on both GC traces #2 and #3 (Appendix 1, p. 74 and 75). That this solid was β-eudesmol (6-A) was determined by comparing its spectral properties with the spectra from a known sample of β-eudesmol (6-A) which was kindly supplied by Pinder. Both the IR's and ^{1}H-NMR's were completely identical.[43,44]

Acorenone-B (7) was isolated and its structure determined by Zalkow and McClure.[7,8] During the course of this work it was isolated by the use of medium pressure high performance chromatography. The

chromatography was carried out on a silica gel 60, size C, pre-packed column. From 3.0 g of 627 grass oil, 1.2 g of acorenone-B (7) was obtained from a (3:1) benzene-chloroform fraction. This sample was subjected to analytical gas chromatography (Col I, 152°C). Only one peak was present with a retention time of 19.3 min. Mixed injections were used to determine that acorenone-B (7) corresponds to peak #7 on both GC traces #2 and #3 (Appendix 1, p. 74 and 75). That this oil was acorenone-B (7) was determined by comparing its spectral data with the spectra obtained from a known sample of acorenone-B (7). Both the IR's and ^{1}H-NMR's were completely identical. A new piece of data (^{13}C-NMR) was obtained on acorenone-B which is in the experimental section of this dissertation, p. 37.

7-Hydroxycalamenene (9) was isolated by a combination of medium pressure high performance chromatography and preparative gas chromatography. The chromatography was carried out on a silica gel 60, size C, pre-packed column. From 3.0 g of 627 grass oil, and oil was obtained from a (1:1) hexane-benzene fraction, which when subjected to analytical gas chromatography (Col I, 184°C) showed two peaks. When this oil was subjected to preparative gas chromatography (Col III, 170°C), and the compound corresponding to the second peak to appear was collected, a pure oil was obtained as shown by analytical gas chromatography (Col I, 184°C). This oil had a retention time of 9.8 min. Mixed injections were used to determine that 7-hydroxycalamenene (9) corresponded to peak #9 on GC trace #3 (Appendix 1, p. 75). That this oil was 7-hydroxycalamenene (9) was determined by comparing its spectral

properties with the spectra obtained from 7-hydroxycalamene (9) which were kindly supplied by Rowe.[45]

Isolation of Five Compounds Which Were Not Identified From 627 Grass Oil

Along with the five compounds which were isolated and identified from 627 grass oil were five other compounds which were isolated; however, their structures have not been determined. The reason the structures were not determined is due to the fact that they were all isolated in pure form by preparative gas chromatography in amounts of between 5 and 10 mg. This small amount of material was enough to obtain an IR, ^{1}H-NMR (100 MHz), mass spectrum, and an exact mass or analysis, but not enough to obtain a crystal for x-ray analysis. What follows will be a discussion of the isolation procedures and any structural features evident from the data that has been collected. These five compounds are called 627-A, 627-3, 627-6-B, 627-8, 627-10, and are labeled A, 3, 6-B, 8, and 10 respectively, on GC traces #2 and #3 (Appendix 1, p. 74 and 75). All spectra for these compounds are in Appendix 3, p. 89.

Compound 627-A was isolated by carrying out a spinning band distillation on 627 grass oil, chromatographying one of the fractions by the use of medium pressure high performance equipment, and subjecting one of the fractions to preparative gas chromatography. An annular teflon spinning band column was used to distill 54.4 g of 627 grass oil. A silica gel 60, size C, pre-packed column was used to chromatograph 1.15 g of a fraction which contained among other compounds one

which had a retention time of 7.3 min when subjected to analytical gas chromatography (Col I, 152°C). From the (1:1) benzene-chloroform fraction an oil was obtained which was rich in compound 627-A. The oil was then subjected to preparative gas chromatography (Col III, 148°C). The compound corresponding to the first peak to appear was collected. When this compound was subjected to analytical gas chromatography (Col I, 152°C) only one peak was present (R_t - 7.3 min). Mixed injections were used to determine that this oil was the compound responsible for the peak labeled (A) in GC trace #1 (Appendix 1, p. 74). The IR, ^{1}H-NMR (100 MHz), and mass spectra of this compound are shown in Appendix 3, p. 89. The exact mass was 166.1691 from which a molecular formula of $C_{12}H_{22}$ was calculated; however, since the IR spectrum indicates the presence of an alcohol (3350 cm^{-1}), the molecular formula is probably $C_{12}H_{24}O$, and the last peak in the mass spectrum is due to the loss of water (M^+-18). If the molecular formula is $C_{12}H_{24}O$ then the molecule contains one unit of unsaturation. The ^{1}H-NMR spectrum indicates vinyl protons (δ = 5.22-5.63) which means that the one unit of unsaturation is a double bond and no rings are present.

Compound 627-3 was isolated by carrying out a spinning band distillation on 627 grass oil, chromatographying one of the fractions by the use of medium pressure high performance equipment, and subjecting one of the fractions to preparative gas chromatography. An annular teflon spinning band column was used to distill 54.5 g of 627 grass oil. A silica gel 60, size C, pre-packed column was used to chromatograph 1.15 g of a fraction which contained among other compounds one which had a retention time of 12.4 min when subjected to analytical gas chromatography (Col I, 152°C). From the (3:1) hexane-benzene fraction

an oil was obtained which was rich in compound 627-3. The oil was then subjected to preparative gas chromatography (Col III, 148°C). The compound corresponding to the first peak to appear was collected. When this compound was subjected to analytical gas chromatography (Col I, 152°C) only one peak was present (R_t - 12.4 min). Mixed injections were used to determine that this oil was the compound responsible for the peak labeled (3) in GC trace #2 (Appendix 1, p. 74). The IR, ^{1}H-NMR (100 MHz), and mass spectra of this compound are shown in Appendix 3, p. 89 . The mass spectrum showed a M^+ = 226 (3%). Combustion analysis gave 75.99% C, 12.02% H (Calcd. for $C_{14}H_{26}O_2$: 74.29% C, 11.58% H). If the molecular formula is $C_{14}H_{26}O_2$ then there are two units of unsaturation in the molecule. Since there is a carbonyl group present (IR - 1740 cm^{-1}) and olefinic protons present (δ - 5.402-5.925) there are no rings in the molecule.

Compound 627-6-B was isolated by column chromatography and preparative gas chromatography. Silica gel 60 (230-400 mesh) (127.0 g) was used to chromatograph 2.0 g of 627 grass oil. The (1:1) hexane-benzene fraction consisted of an oil which contained two compounds, one of which had a retention time of 16.7 min (Col I, 152°C). When this oil was subjected to preparative gas chromatography (Col III, 148°C) a white solid with a melting point of 68°C was collected. When this solid was subjected to analytical gas chromatography (Col I, 152°C) only one peak was present (R_t - 16.7 min). Mixed injections were used to determine that this compound was responsible for the peak labeled 6-B on GC trace #2 (Appendix 1, p. 74). The IR, ^{1}H-NMR (100 MHz), and mass spectra of this compound are shown in Appendix 3, p. 89. The

exact mass was 222.19628, from which a molecular formula of $C_{15}H_{26}O$ was calculated. The molecular formula indicates there are three units of unsaturation. The molecule contains an α,β-unsaturated aldehyde, as determined from the IR and ^{1}H-NMR [(IR - 1680, 2700, 2800 cm^{-1}) (δ - 8.977, 6.460-6.624)]. This would account for two units of unsaturation. The molecule also contained an exocyclic double bond as determined from the IR and ^{1}H-NMR [(IR - 880 cm^{-1}) (δ - 4.841)]. Since all three units of unsaturation have been accounted for the molecule does not contain any rings.

Compound 627-8 was isolated by medium pressure high performance chromatography and preparative gas chromatography. A silica gel 60, size C, pre-packed column was used to chromatograph 3.0 g of 627 grass oil. An oil was isolated from a (1:1) hexane-benzene fraction which contained two compounds, one of which had a retention time of 21.7 min (Col I, 152°C). When this oil was subjected to preparative gas chromatography (Col III, 170°C) an oil was collected which showed only one peak (R_t - 21.7 min) according to analytical gas chromatography (Col I, 152°C). Mixed injections were used to determine that this compound was responsible for the peak labeled (8) on GC trace #2 (Appendix 1, p. 74). The IR, ^{1}H-NMR (100 MHz), and mass spectra of this compound are shown in Appendix 3, p. 89. The exact mass was 236.16252 from which a molecular formula of $C_{18}H_{20}$ was calculated with an error of -6.02 in 40 ppm. This formula is incorrect because a carbonyl group is present in the molecule (IR - 1710 cm^{-1}). The molecular formula can not be $C_{18}H_{22}O$ because a hydroxy group is not present in the molecule (there is not an IR band above 3000 cm^{-1}). From

the molecular formulas of the other known compounds in 627 grass oil the molecular formula could be $C_{15}H_{24}O_2$ (mol. wt. - 236). This would require four units of unsaturation.

Compound 627-10 was isolated by carrying out a spinning band distillation on 627 grass oil, chromatographying the material remaining in the still pot by use of medium pressure high performance equipment, and subjecting one of the fractions to preparative gas chromatography. An annular teflon spinning band column was used to distill 54.4 g of 627 grass oil. A silica gel 60, size C, pre-packed column was used to chromatograph 3.0 g of the material remaining in the still pot. From one of the chloroform fractions, an oil was isolated which was rich in compound 627-10. This oil was then subjected to preparative gas chromatography (Col IV, 260°C). An oil was isolated which when subjected to analytical gas chromatography (Col I, 184°C) showed only one peak (R_t - 15.9 min). Mixed injections were used to determine that this oil was the compound responsible for the peak labeled (10) on GC trace #3 (Appendix 1, p. 75). The IR, ^{1}H-NMR (100 MHz), and mass spectra of this compound are shown in Appendix 3, p. 89. Combustion analysis gave 64.42% C, 7.26% H (Calcd. for $C_{15}H_{20}O_5$: 64.27% C, 7.19% H). The last peak (263, 98%) present in the mass spectrum must be due to M^+-17. This mass spectrum was not run on a high resolution instrument. If the molecular formula is $C_{15}H_{20}O_5$ (mol. wt. - 280), then the molecule contains six units of unsaturation. There is present in the molecule a carbonyl group (IR - 1740 cm^{-1}), a double bond (δ - 4.14-4.37), and an aromatic ring (δ - 7.51-7.83). This accounts for all six units of unsaturation.

It was noted in the introduction that intermedeol (11) and neointermedeol (12) were isolated from the steam volatile oil of Bothriochloa intermedia, accessions 5297 and 5752 respectively.

Intermedeol (11):[9,10]

GC: R_t - 18.3 min (Col I, 152°C)

MP: 47-48°C

$[\alpha]_0^{25}$: +10.7 (C., 0.957 in EtOH)

IR: ν_{max}^{film} 3480, 1645, 890, 910 cm^{-1}

^{1}H-NMR (60 MHz), δ, $CDCl_3$): 0.90 (s,3H), 1.00 (s,3H), 1.72 (s,3H), 4.83 (s,3H)

^{13}C-NMR (25 MHz, δ, $CDCl_3$): 146.5 (=C(R_2)), 110.5 (=CH_2), 7.20 (R_2 $\overset{R}{\underset{OH}{}}$), 49.2, 43.6, 41.5, 40.5, 39.5, 35.4 (CR_4), 23.7, 22.9, 22.5, 20.3, 18.7

Anal: 81.36% C, 11.82% H (Calcd. for $C_{15}H_{26}O$: 81.01% C, 11.79% H)

Neointermedeol (12):[11]

GC: R_t - 14.4 min (Col I, 152°C)

BP: 85-87°C (0.5 mm)

$[\alpha]_0^{25}$: +7.5 (C., 2.635 in EtOH)

IR: ν_{max}^{film} 3500, 1640, 890 cm^{-1}

^{1}H-NMR (60 MHz, δ, $CDCl_3$): 1.02 (3H,s), 1.12 (3H,s), 1.72 (3H,s), 4.62 (2H,m)

Gas Chromatographic Study of Several Accessions, Hybrids, and Strains of Bothriochloa intermedia

The methods used to isolate the steam volatile oils from the various grasses of Bothriochloa intermedia and to analyze the oils by gas chromatography are described in detail in the experimental part of this dissertation. Three different methods were used to isolate the steam volatile oils from the grasses. The first method involved first extracting the grass with 95% ethanol, then steam distilling the ethanol extract by boiling the extract with water while continually extracting with ether. The second method involved directing steam into the chopped grass, after which the oil-water condensate was extracted with ether. The third method involved boiling the chopped grass with water while continually extracting with ether. The grass was mechanically stirred during the whole process. It was discovered that the GC trace of the steam volatile oil from a particular grass was independent of the method used to isolate the oil. The only difference in the above methods were the yields of oil that were obtained. The third method gave the highest yields of oil, while the yields of oil from the first and second methods were approximately equal. All of the gas chromatographic traces of the various oils that appear in Appendix 1, p. 72 were run on an F and M Model 400 gas chromatograph using a flame ionization detector. A 5'9" x 1/4" glass column packed with 5% SE-30 on 80/100 Chromosorb W was used to analyze the oil from each grass.

All of the grasses were grown by the Agronomy Department at Oklahoma State University either at Stillwater, Oklahoma, or Fort Reno, Oklahoma; however, the seeds were obtained primarily from three

countries, India, Malaya, and Turkey. In this dissertation a form will be defined as a distinct variety of Bothriochloa intermedia. Hybrids are prepared from different forms of Bothriochloa intermedia. An accession is defined as a particular plot of Bothriochloa intermedia which could be a pure form, a hybrid, or a strain. A strain is defined as a blend of hybrids or accessions. The hybrid 56x627-1 was prepared from two forms of Bothriochloa intermedia, a gangetica type (male) indigenous to the Gangetic Plain of India and an indica type (female) indigenous to Punjab, India. The hybridization was carried out by means of hand emasculation and pollination. "K" strain is a blend of three accessions indigeneous to the Kulu Valley in India. Both "I-F" and "I-T" strains are blends of thirty hybrids, each of which contains either accession 5297 (Lahnavala, India) or accession 5410 (Punjab, India) as a parent. Accession 5297 is indigeneous to Lahnavala, India. Accession 5752 is indigeneous to Kedah, Malaya. Finally, accessions 8967, 8969, and 8907 are all indigeneous to Turkey.

Table 1 shows the compounds present in the steam volatile oils of nine grasses. As noted in the table, acorenone-B (7), kessane (1), elemol (2), β-eudesmol (6-A), and 7-hydroxycalamenene (9) were isolated from the hybrid 56x627-1. Also intermedeol (11) was isolated from accession 5297, neointermedeol (12) was isolated from accession 5752, and acorenone-B (7) was isolated from "K" strain. The presence of any of the above compounds in any other grass was determined only by gas chromatographic retention times (GC:R_t) with mixed injections.

Of the nine grasses studied, cattle liked the "I-F" and "I-T" strains the most and "K" strain the least. From this small sample no

conclusion could be drawn as to the relationship between palatability to cattle and the compounds in the steam volatile oils; however, as can be seen from GC traces #'s 4, 5, and 6 (Appendix 1, p. 76, 77 and 78) "I-F" and "I-T" strains are rich in monoterpenes and sesquiterpenes, whereas "K" strain contains practically 100% acorenone-B (7). A scientific study has not been made on the insect antifeedant properties of Bothriochloa intermedia; however, it has been observed that when the various grasses were growing near each other no foraging insects, such as grasshoppers, were observed in the "K" strain plot, whereas grasshoppers were observed in plots of the other grasses. Again, no conclusions could be drawn as to the insect resistance of "K" strain; however, as noted before, the steam volatile oil of this grass contains practically 100% acorenone-B (7). Finally, studies on chemical taxonomy as related to Bothriochloa intermedia are still inconclusive due to Bothriochloa intermedia's genetic aggressiveness. The difficulty in relating steam volatile oil composition to taxonomic identification lies in Bothriochloa intermedia's genetic aggressiveness. Harland[13] has stated: "It gradually became apparent that Bothriochloa intermedia itself is a hodgepodge of germ plasm assembled from at least five species belonging to three genera. Every accession of Bothriochloa intermedia in our collection seems to be some sort of cross, backcross, or introgression product of one kind or another. We have over two hundred collections of the species from Cape Town to Kenya to Pakistan, India, Burma, Malaya, Taiwan, the Philippines, Indonesia, New Guinea, and Australia, and we are not sure that any of these represent a really original Bothriochloa intermedia. In the course of assimilating germ

plasm from various related species, Bothriochloa intermedia seems to have genetically consumed its own ancestral form."

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The hexane extract (Chart 1, p. 20) of Peltophorum inerme was subjected to analytical gas chromatography (Col II, 272°C), GC trace #1 (Appendix 1, p. 73). From this trace it can be seen that there are eleven major compounds present. A total of eight of these compounds were identified. Four of the compounds, n-heptacosane ($n\text{-}C_{27}H_{56}$), n-nonacosane ($n\text{-}C_{29}H_{60}$), n-hentriacontane ($n\text{-}C_{31}H_{64}$), and n-tritriacontane ($n\text{-}C_{33}H_{68}$) were isolated by preparative gas chromatography (Col III, 278°C) and identified by mass spectral analysis. The other four compounds, n-octacosane ($n\text{-}C_{28}H_{58}$), n-triacontane ($n\text{-}C_{30}H_{62}$), n-dotriacontane ($n\text{-}C_{32}H_{66}$), and n-tetratriacontane ($n\text{-}C_{34}H_{70}$) were not isolated; however, they were identified by mixed injections, analytical gas chromatography (Col II, 272°C), with known samples of the four compounds. Peltophorum inerme is a member of the Legume Family (Leguminosae), and as such should be rich in alkaloids. The chloroform extract (Chart 1, p. 20) should be subjected to various chromatographic separation procedures in order to isolate and identify as many secondary metabolites as possible. This work would not just be an exercise in natural product elucidation since the compounds could be sent to the National Institute of Health for anti-tumor activity testing.

A total of five compounds, acorenone-B (7), kessane (1), elemol (2), β-eudesmol (6-A), and 7-hydroxycalamenene (9), were isolated from the hybrid 56x627-1 prepared from two forms of Bothriochloa intermedia. Two other compounds, intermedeol (11) and neointermedeol (12) have been isolated from other accessions of Bothriochloa intermedia. Table 1 shows the compounds present in the steam volatile oils of all nine of the grasses of Bothriochloa intermedia that were examined in this research. The five co-occurring compounds in the hybrid 56-627-1 had five different structural types, acorane, guaiane, elemane, endesmane, and cadinane. It was shown that a possible common precursor for acorenone-B (7) and 7-hydroxycalamenene (9) could be the β-bisabolyl cation (14) derived from cis,trans-farnesyl pyrophosphate (13), while a possible common precursor for elemol (2), β-Eudesmol (6-A), and kessane (1) could be different conformations of the trans, trans-farnesyl pyrophosphate (15). Gas chromatographic studies of the steam volatile oils of the various grasses of Bothriochloa intermedia in this research are only the initial stages of a long range study of the grasses' palatability to cattle and insect antifeedant properties. It appears at this time that a high acorenone-B (7) content is related to low palatability to cattle and high insect resistance. These observations should be further investigated.

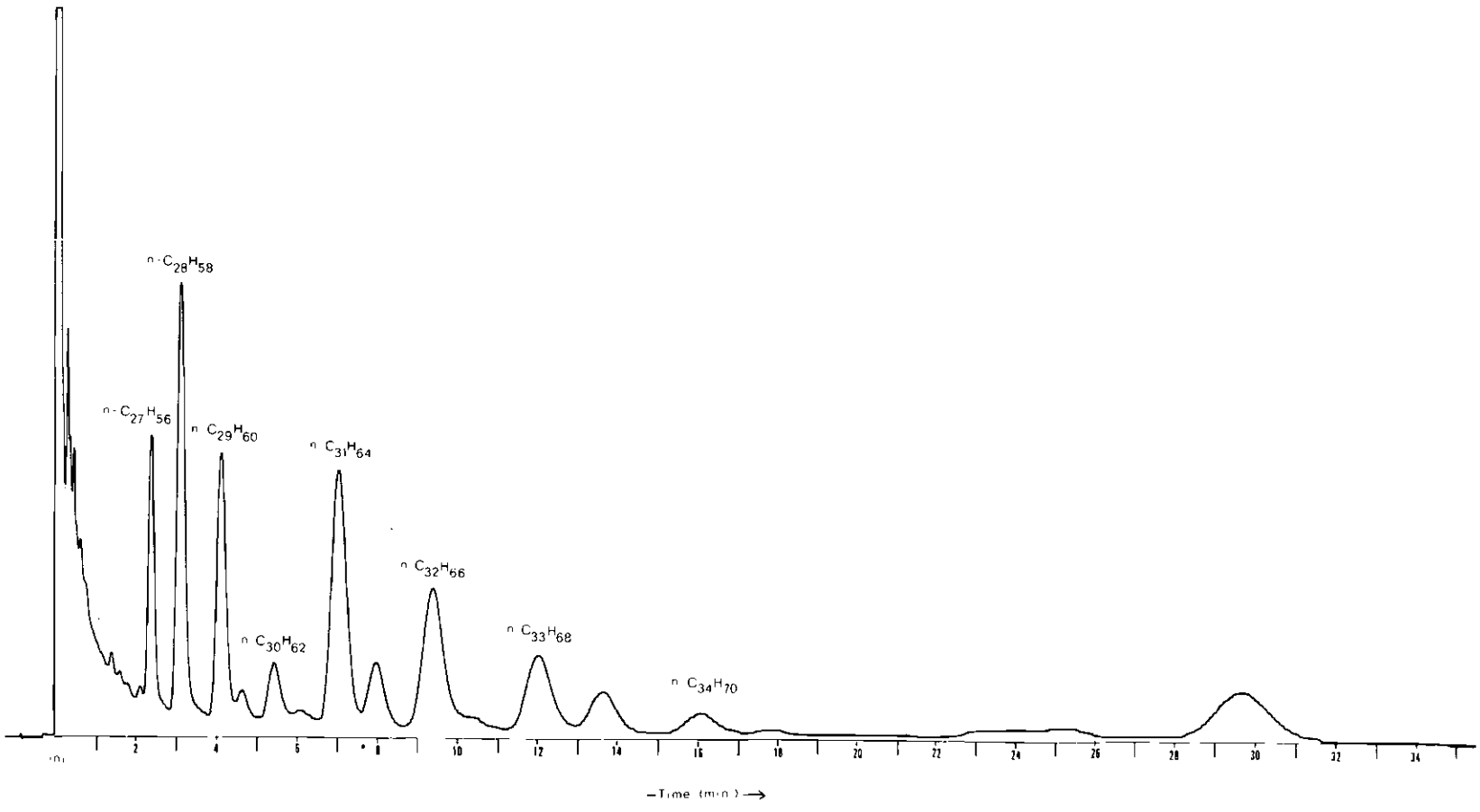
Finally, the parents of the hybrid 56x627-1 should be investigated. It is known that the female parent (accession 5410, indica type) contains intermedeol (11) and does not contain acorenone-B (7). The male parent (accession 5400, gangetica type) should be investigated to see whether or not intermedeol (11) and acorenone-B (7) are present.

The results of this investigation should be interesting since the hybrid 56x627-1 contains a large amount of acovenone-B (7) and no intermedeol (11).

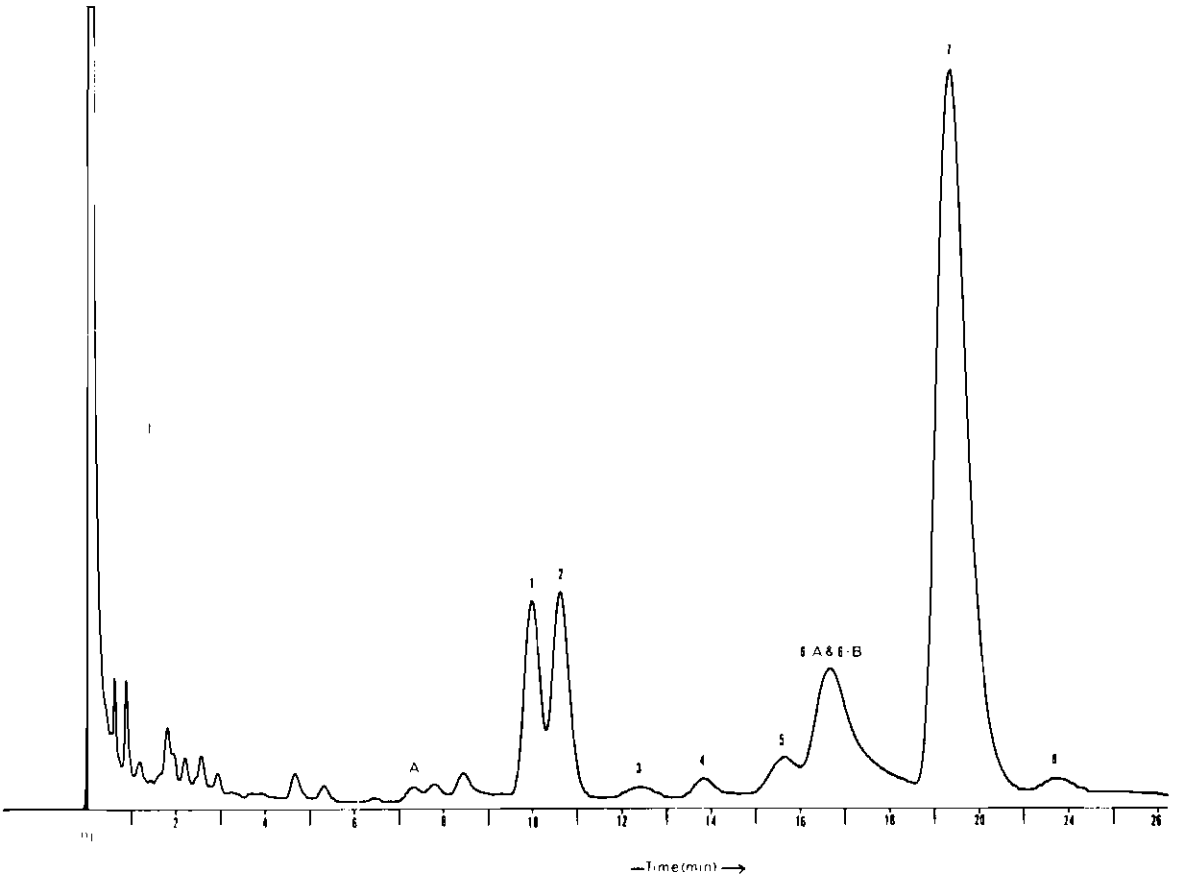
APPENDICES

APPENDIX 1

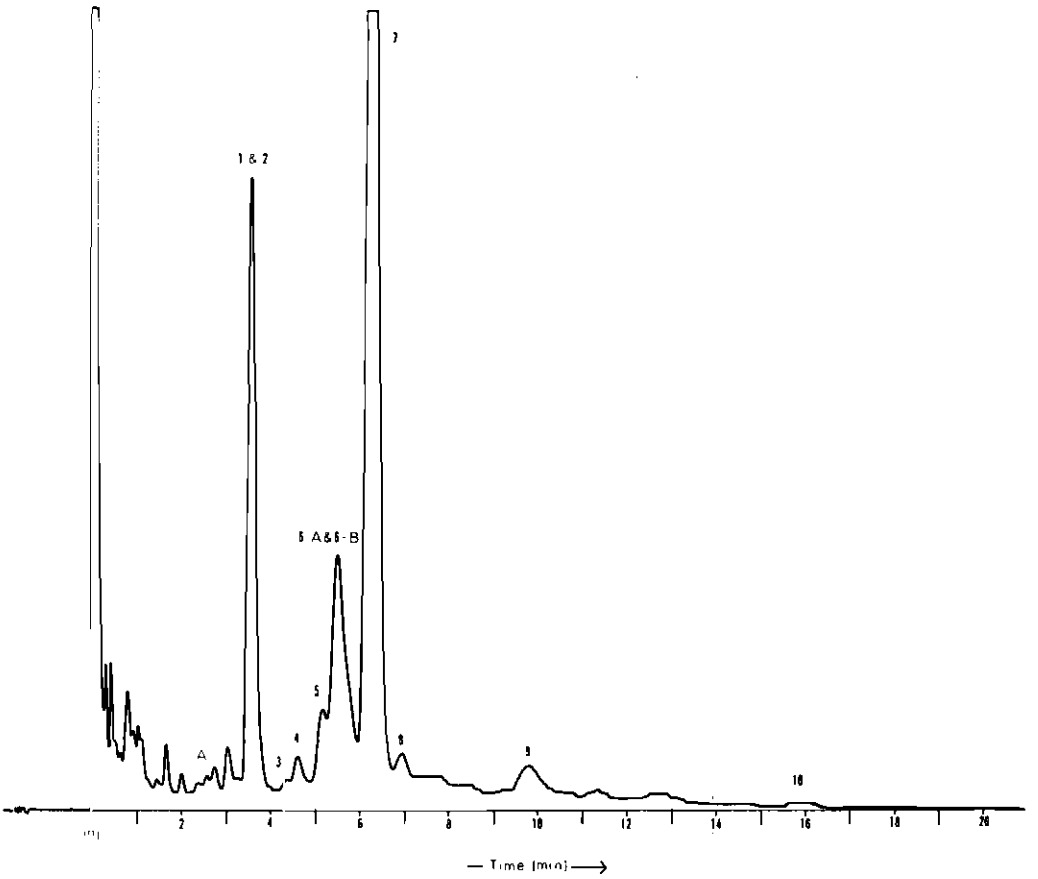
GAS CHROMATOGRAPHY TRACES



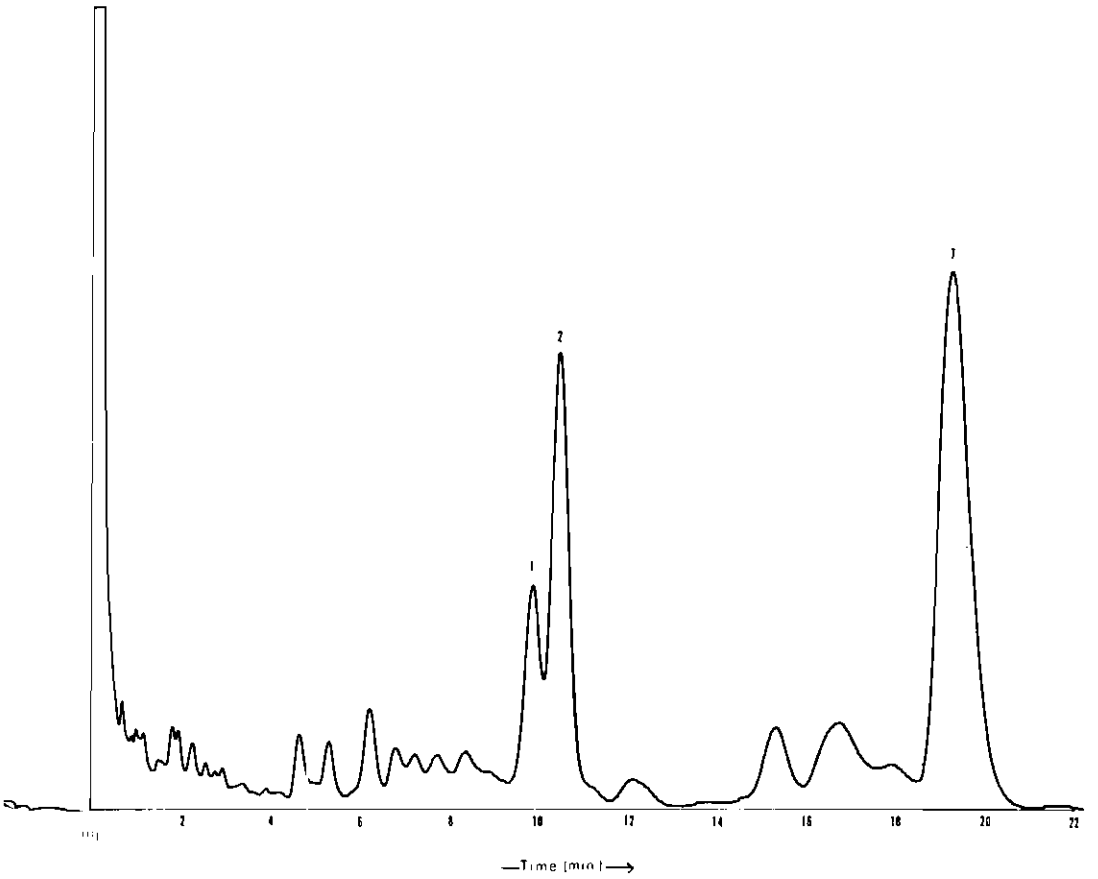
GC Trace 1. Hexane Extract of Peltophorum inerme. (Col II, 272°C)



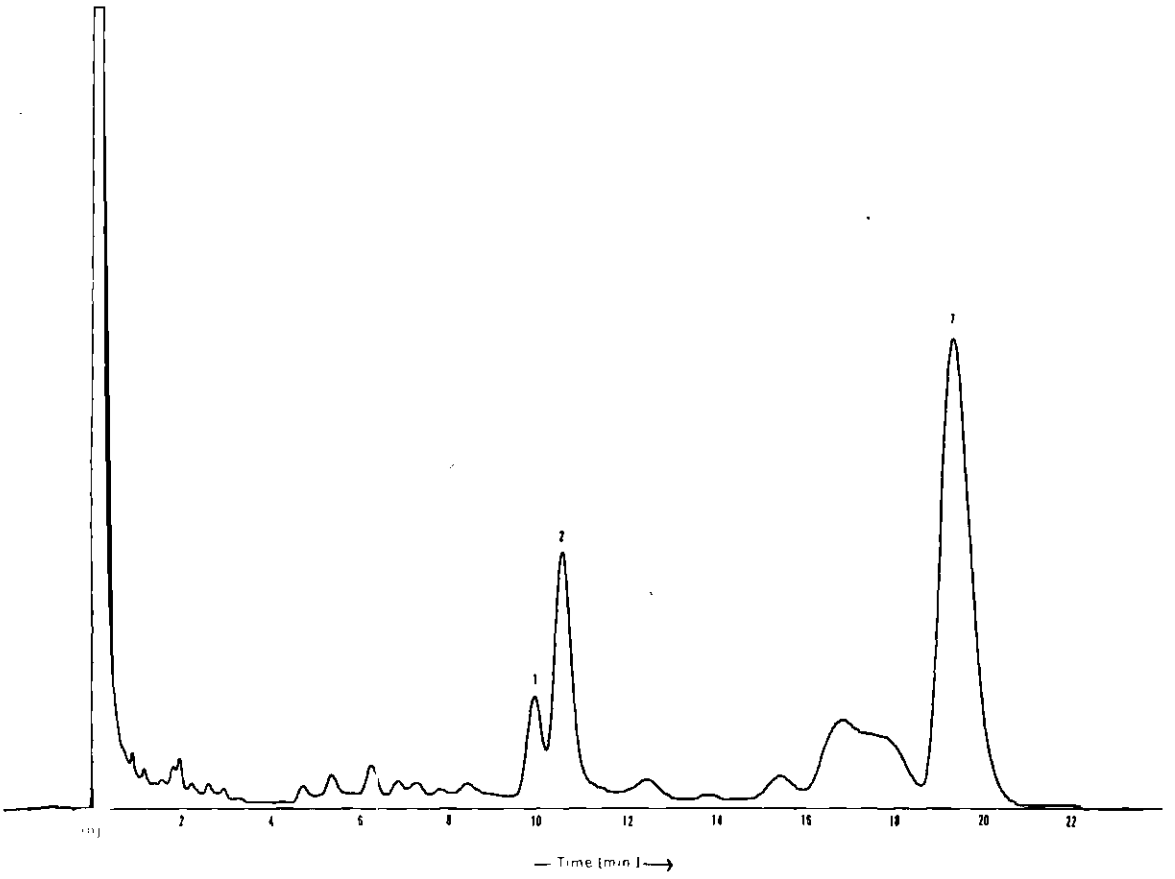
GC Trace 2. Steam Volatile Oil of Bothriochloa intermedia (Hybrid Grass 56 x 627-1) (Col I, 152°C)



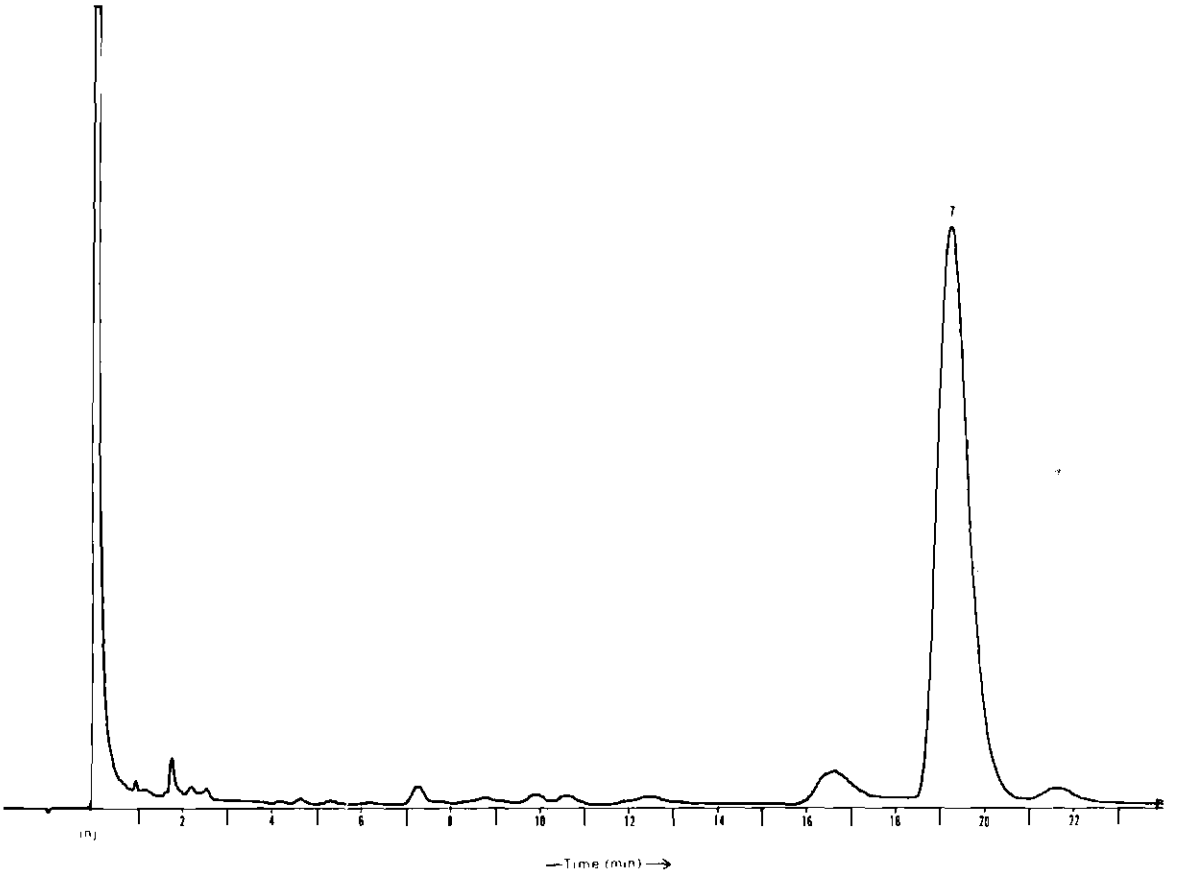
GC Trace 3. Steam Volatile Oil of Bothrichloa intermedia (Hybrid Grass 56 x 627-1) (Col I, 184°C)



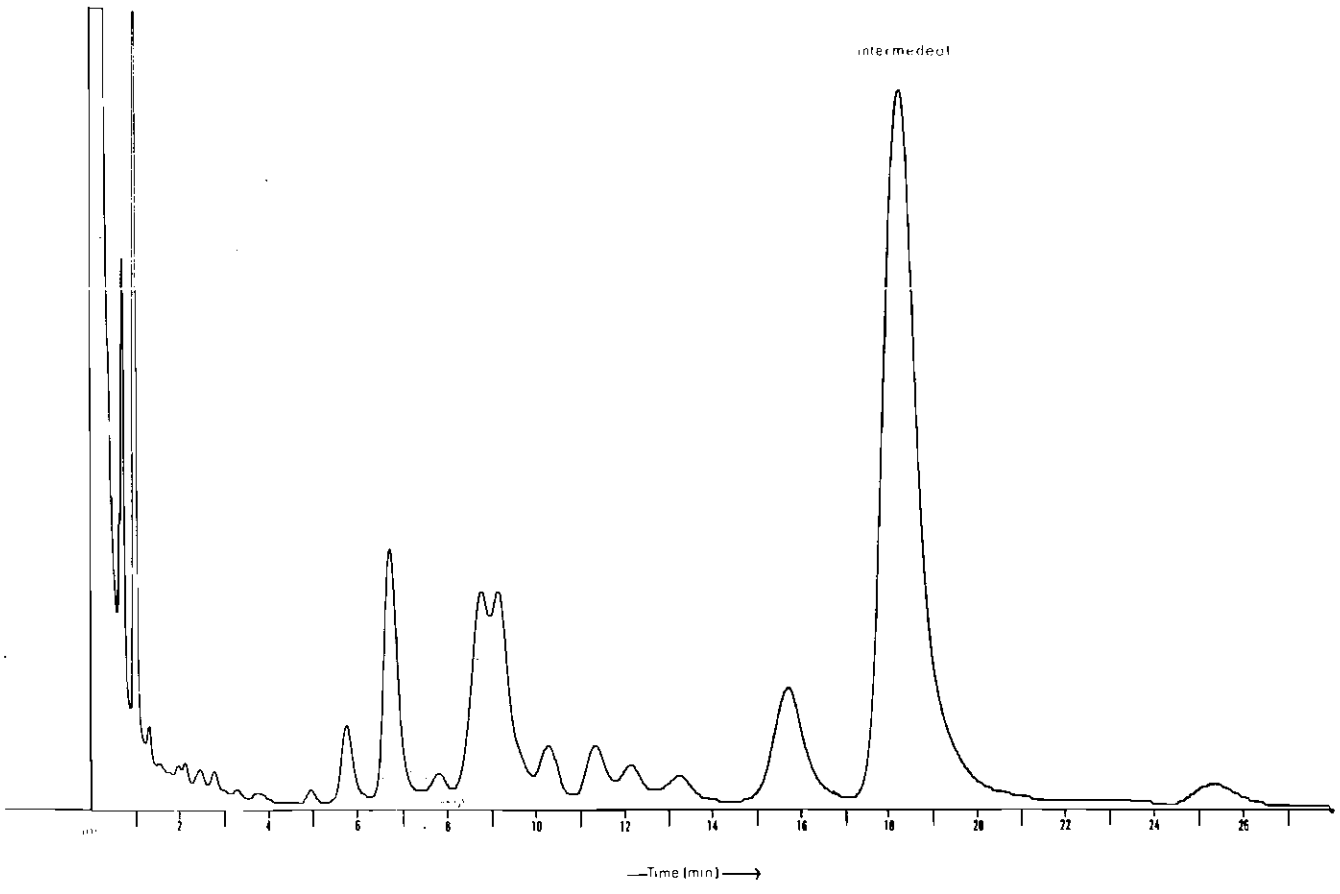
GC Trace 4. Steam Volatile Oil of Bothriochloa intermedia ("I-F" strain). (Col I, 152°C)



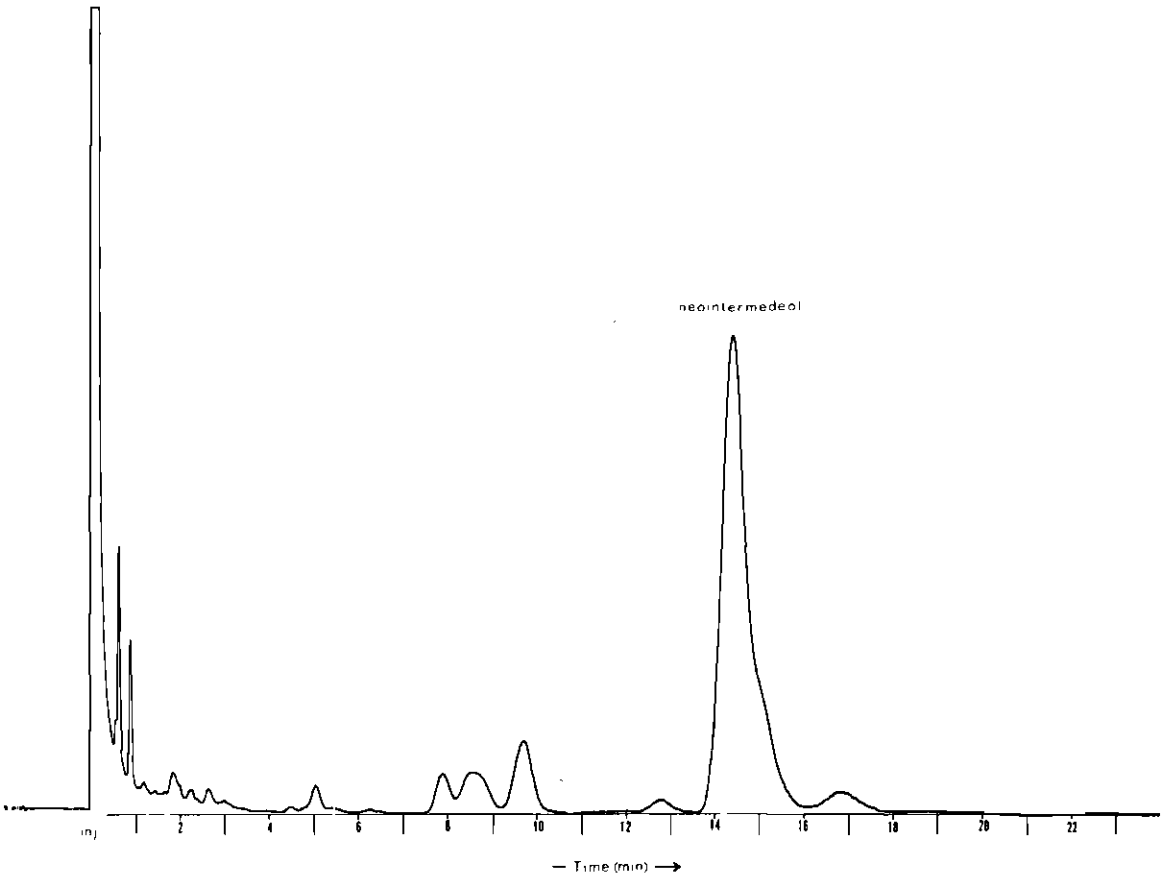
GC Trace 5. Steam Volatile Oil of Bothriochloa intermedia ("I-T" strain). (Col I, 152°C)



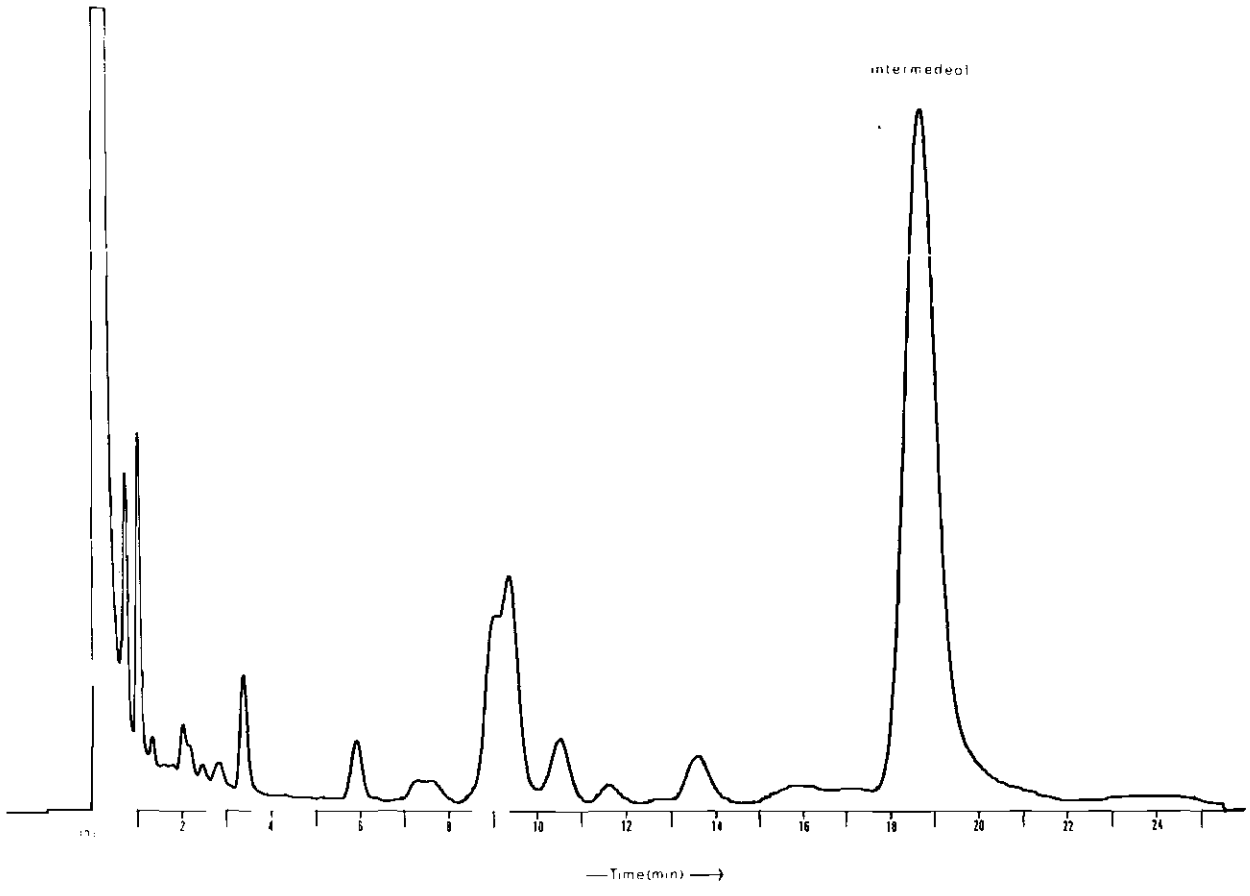
GC Trace 6. Steam Volatile Oil of Bothriochloa intermedia ("K" strain). (Col I, 152°C)



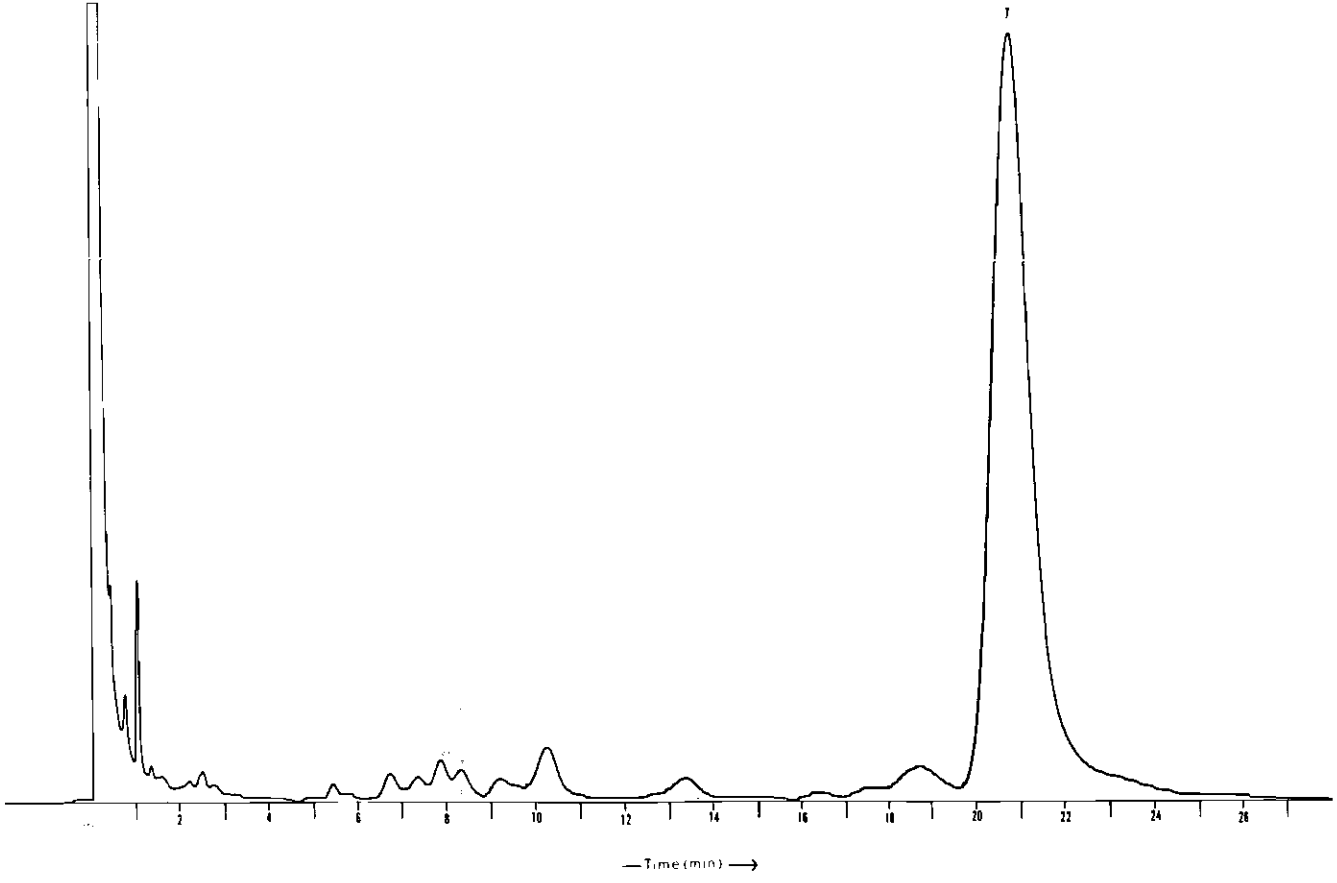
GC Trace 7. Steam Volatile Oil of Bothriochloa intermedia (Accession 5297). (Col I, 152°C)



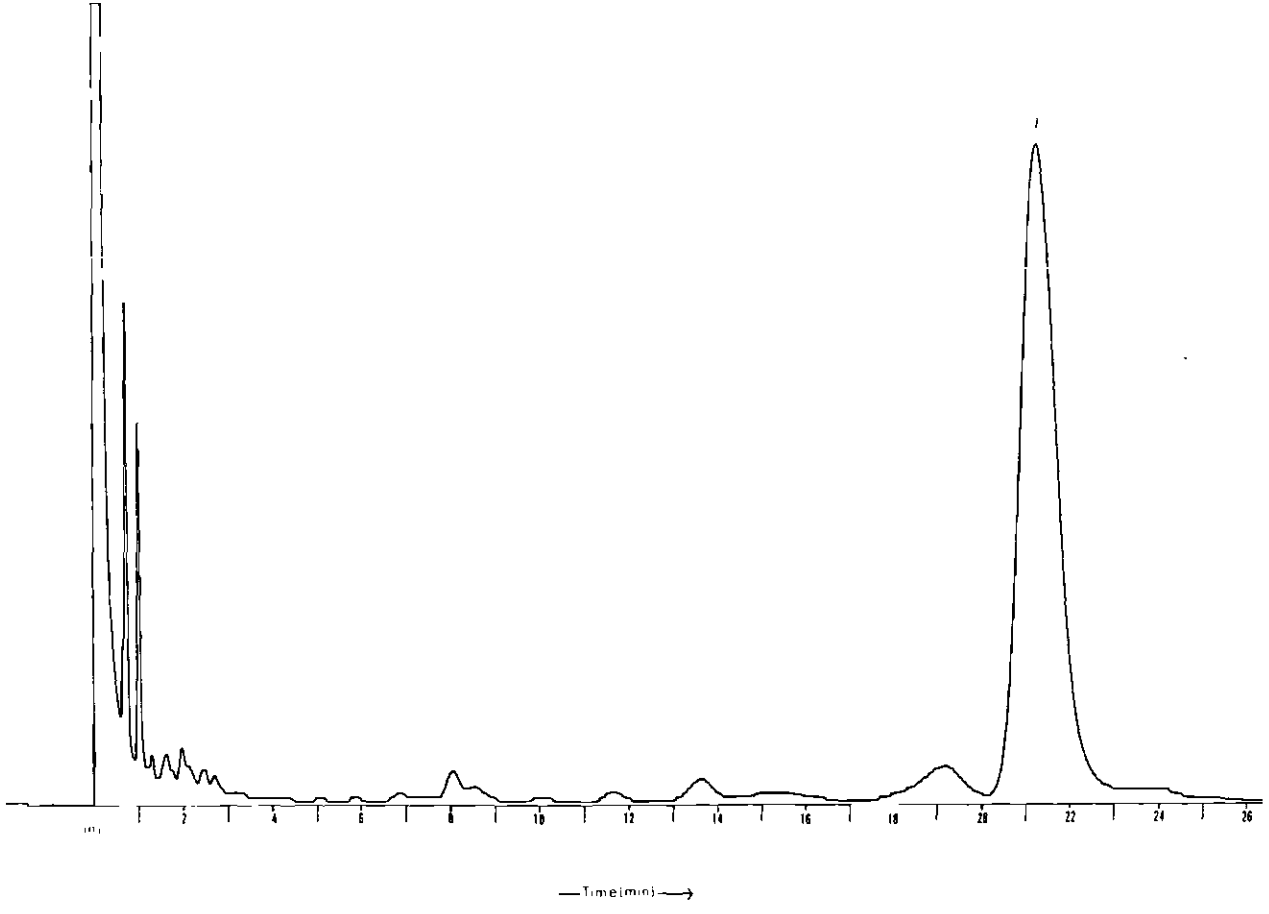
GC Trace 8. Steam Volatile Oil of Bothriochloa intermedia (Accession 5752). (Col I, 152°C)



GC Trace 9. Steam Volatile Oil of Bothriochloa intermedia (Accession 8967). (Col I, 152°C)



GC Trace 10. Steam Volatile Oil of Bothriochloa intermedia (Accession 8969). (Col I, 152°C)



GC Trace 11. Steam Volatile Oil of Bothriochloa intermedia (Accession 8907). (Col I, 152°C)

APPENDIX 2

MASS SPECTRA

OF

LONG STRAIGHT-CHAIN HYDROCARBONS

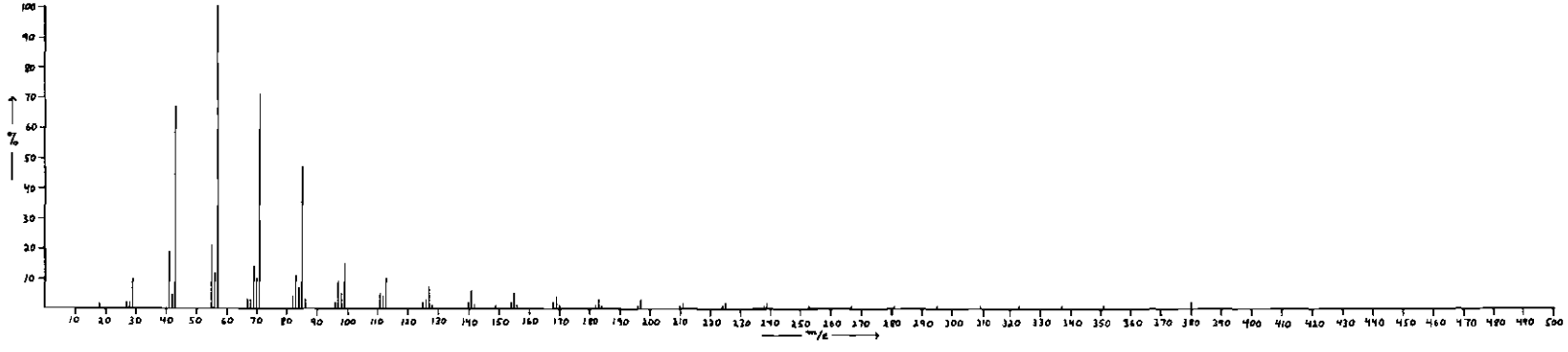


Plate I. Mass Spectrum of n=Heptacosane (n-C_{27} H_{56}).

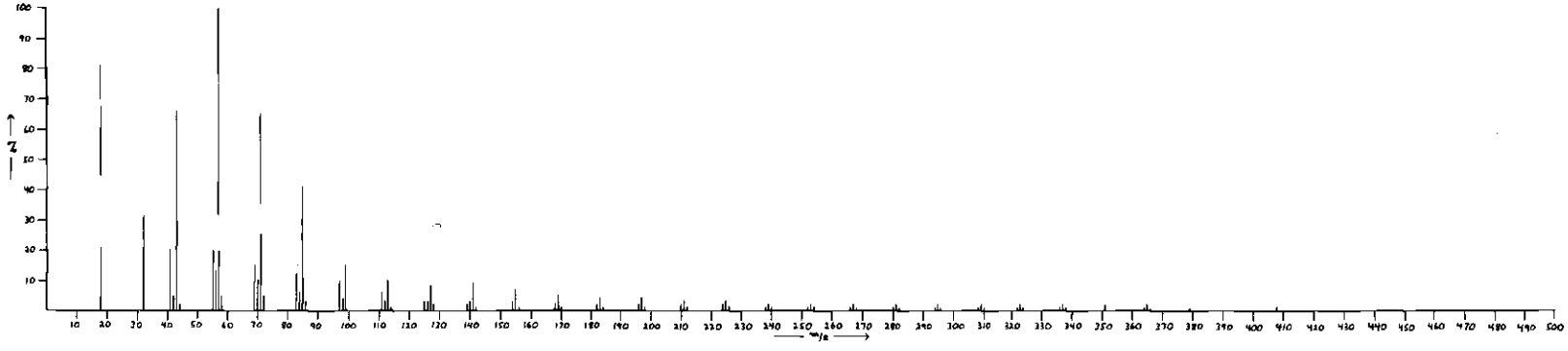


Plate II. Mass Spectrum of n-Nonacosane (n-C_{29} H_{60}).

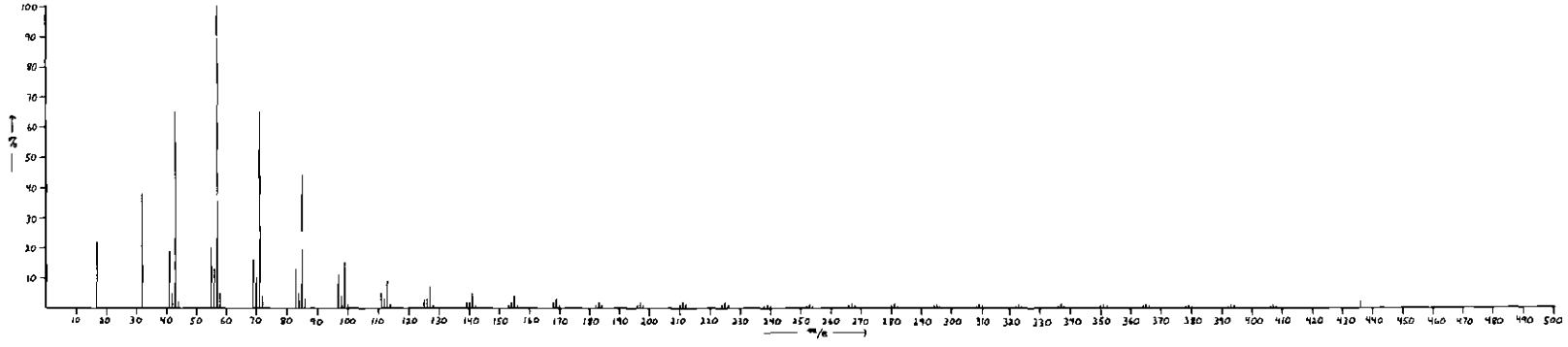


Plate III. Mass Spectrum of n-Hentriacontane (n-C_{31} H_{64}).

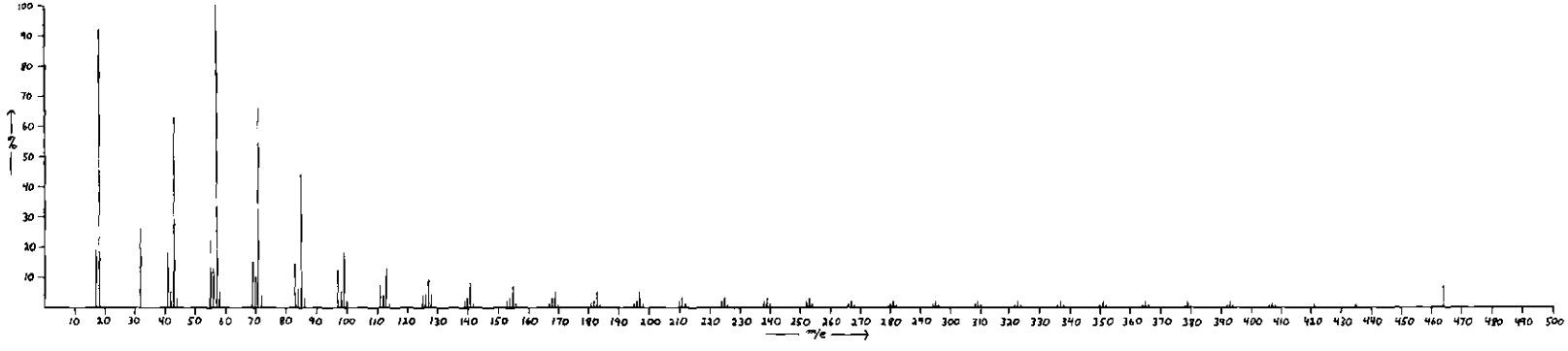


Plate IV. Mass Spectrum of n-Tritriacontane (n-C_{33} H_{68}).

APPENDIX 3

NMR, IR, AND MASS SPECTRA

OF

FIVE UNIDENTIFIED COMPOUNDS ISOLATED

FROM

627 GRASS OIL

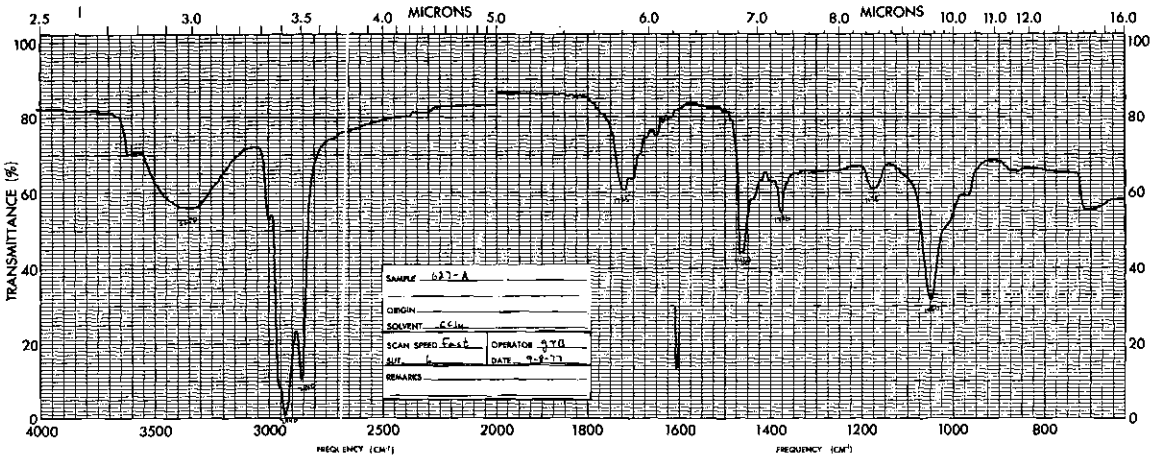


Plate V. Infrared Spectrum of Compound 627-A.

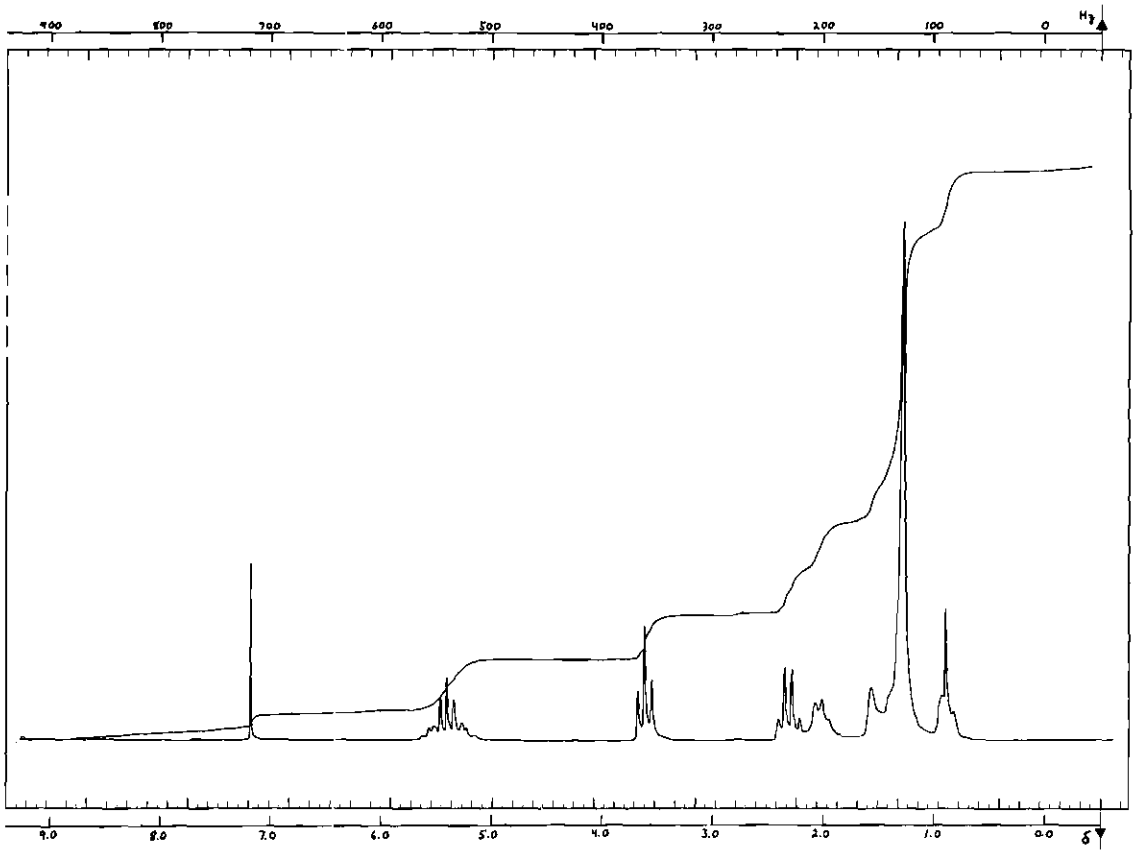


Plate VI. ^{1}H-NMR Spectrum of Compound 627-A.

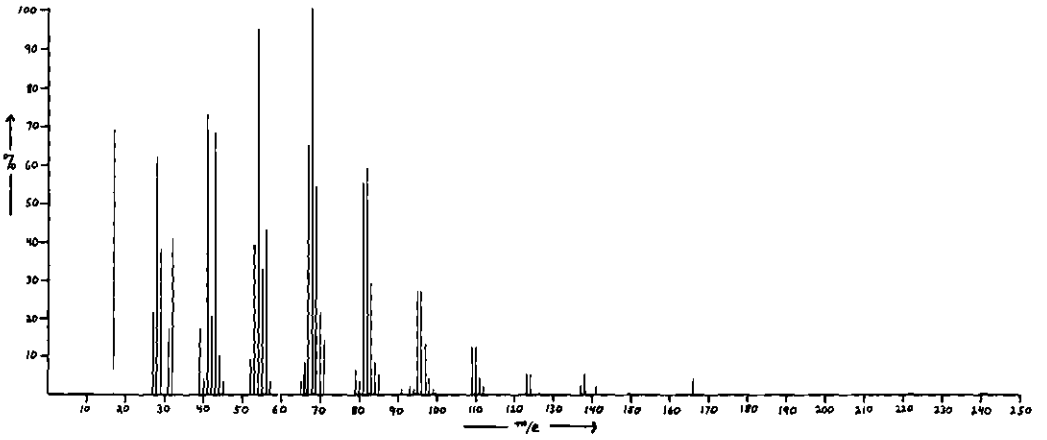


Plate VII. Mass Spectrum of Compound 627-A.

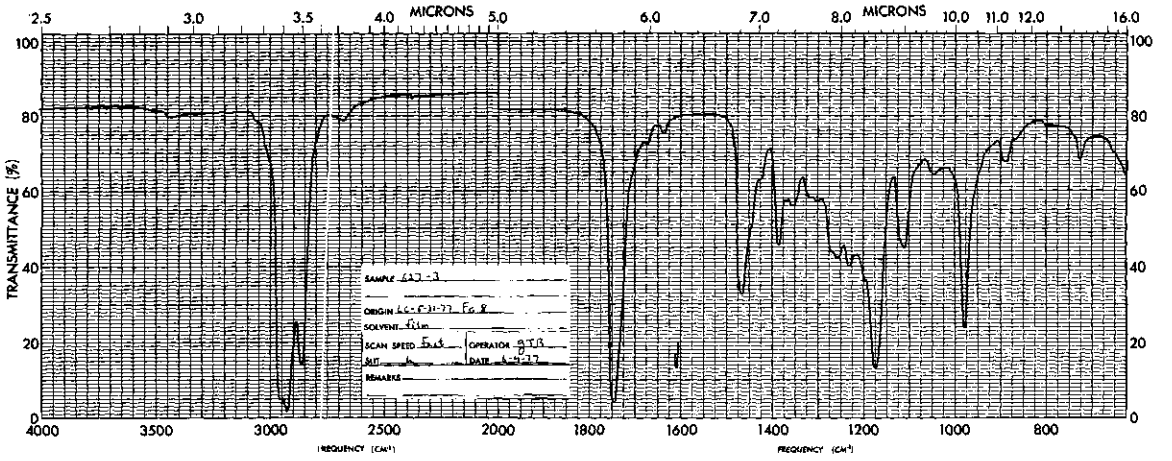


Plate VIII. Infrared Spectrum of Compound 627-3.

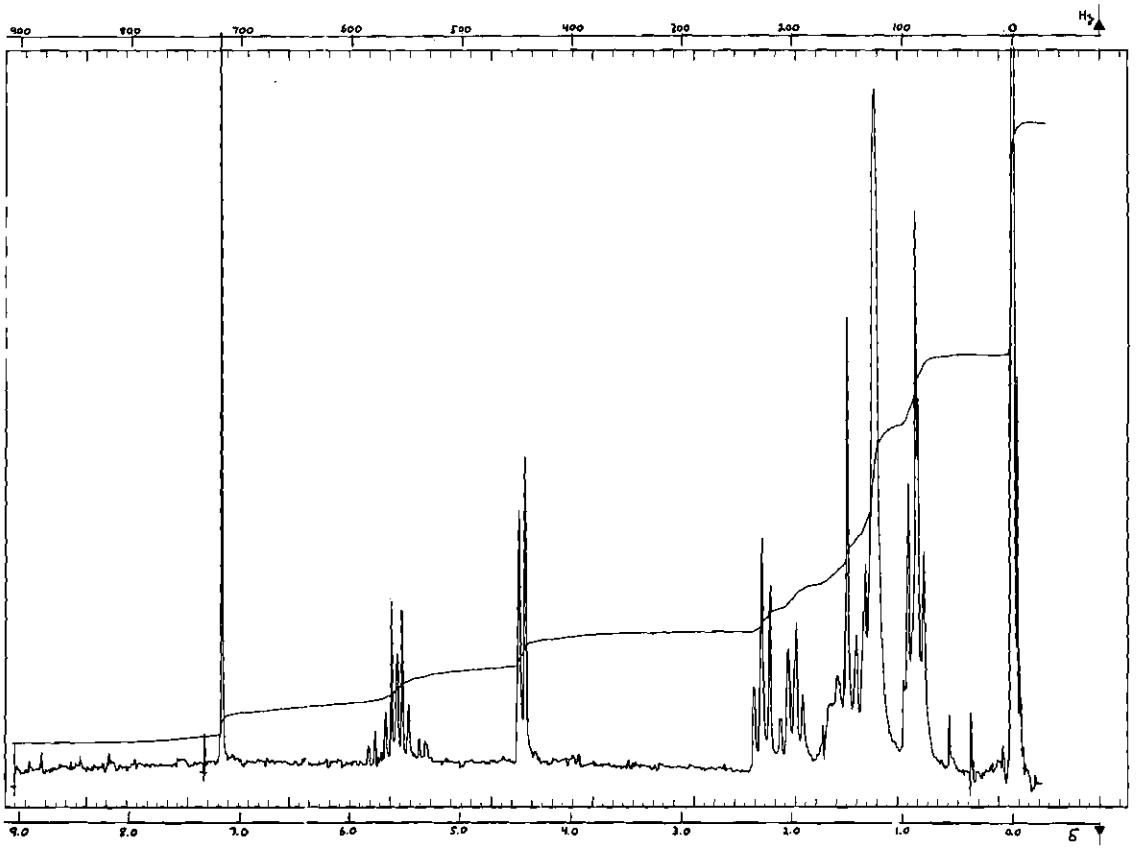


Plate IX. ^{1}H-NMR Spectrum of Compound 627-3.

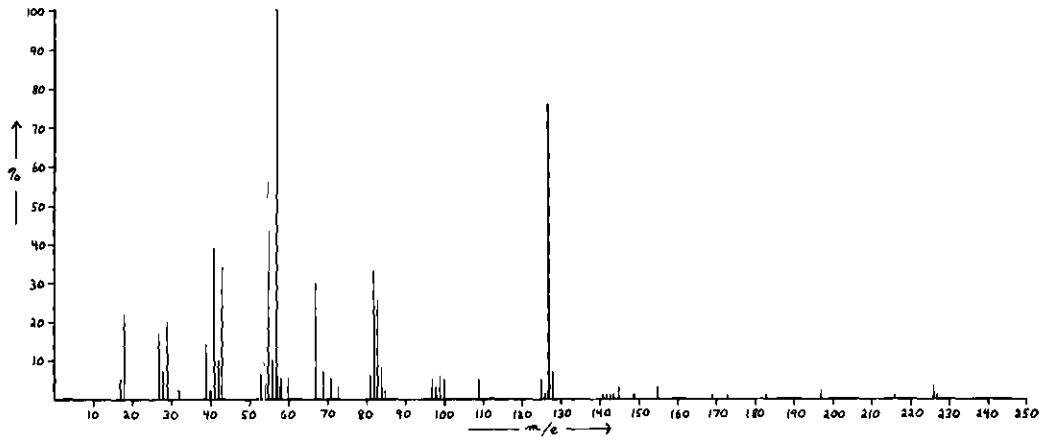


Plate X. Mass Spectrum of Compound 627-3.

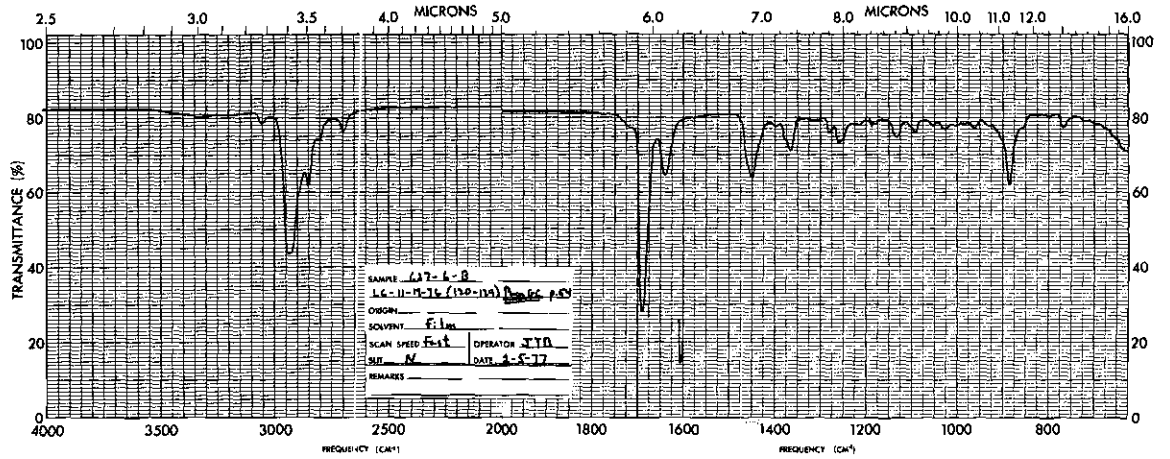


Plate XI. Infrared Spectrum of Compound 627-6-B.

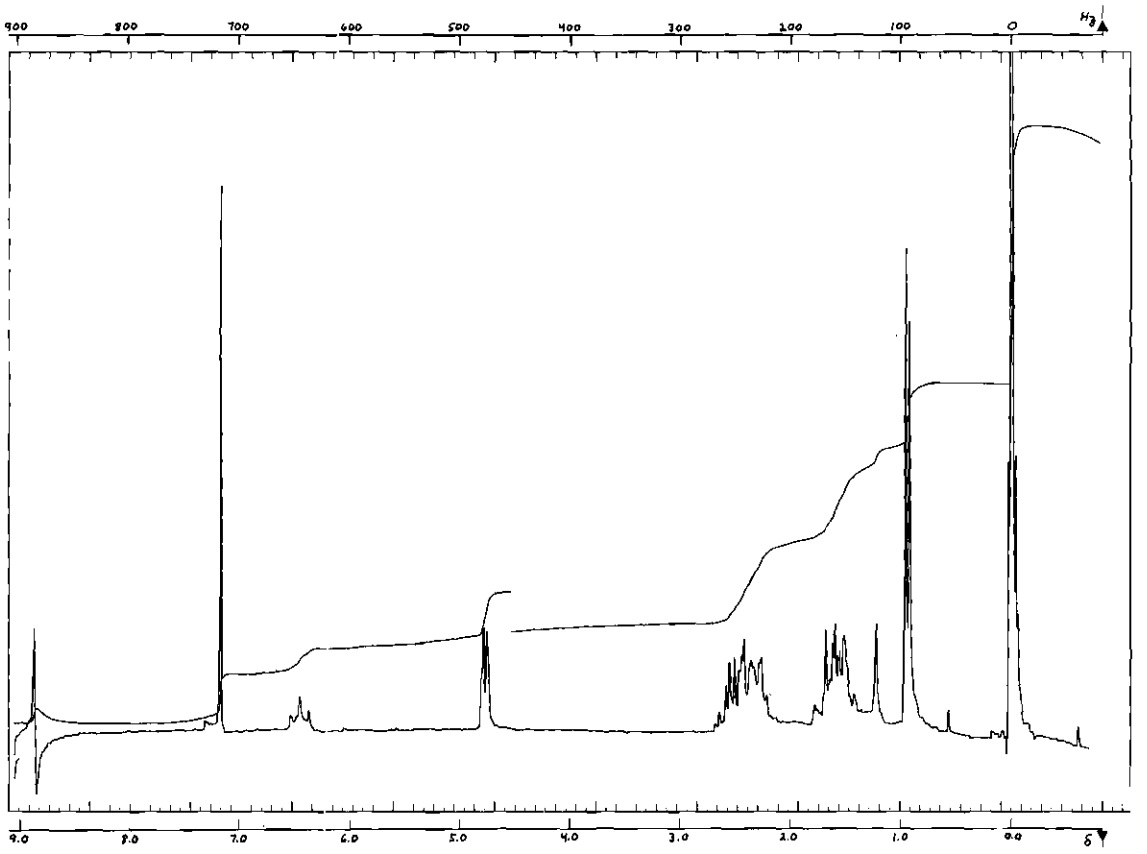


Plate XII. ^{1}H-NMR Spectrum of Compound 627-6-B.

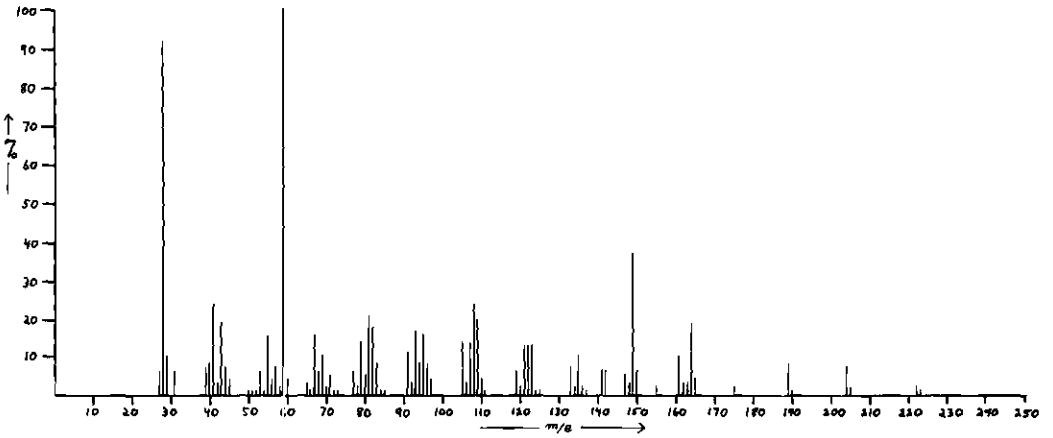


Plate XIII. Mass Spectrum of Compound 627-6-B.

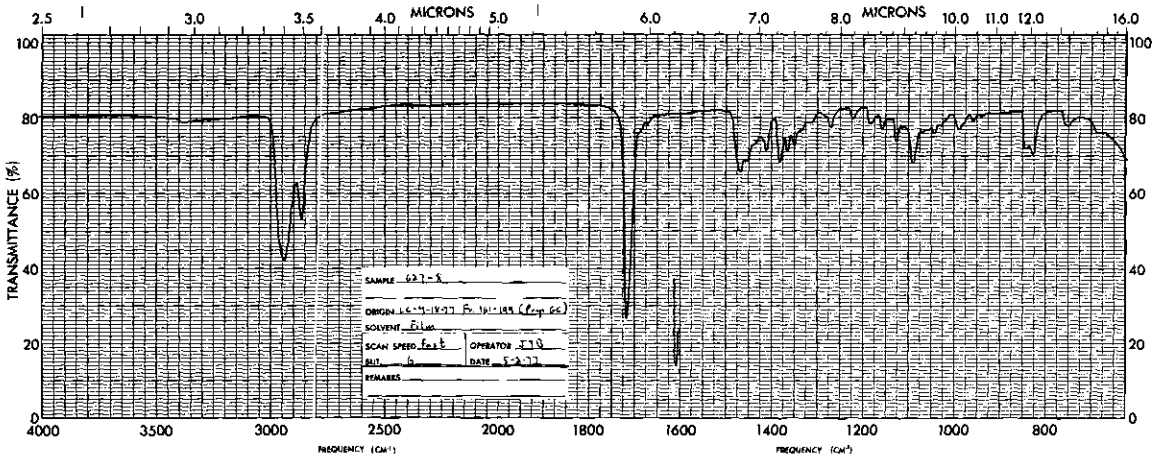


Plate XIV. Infrared Spectrum of Compound 627-8.

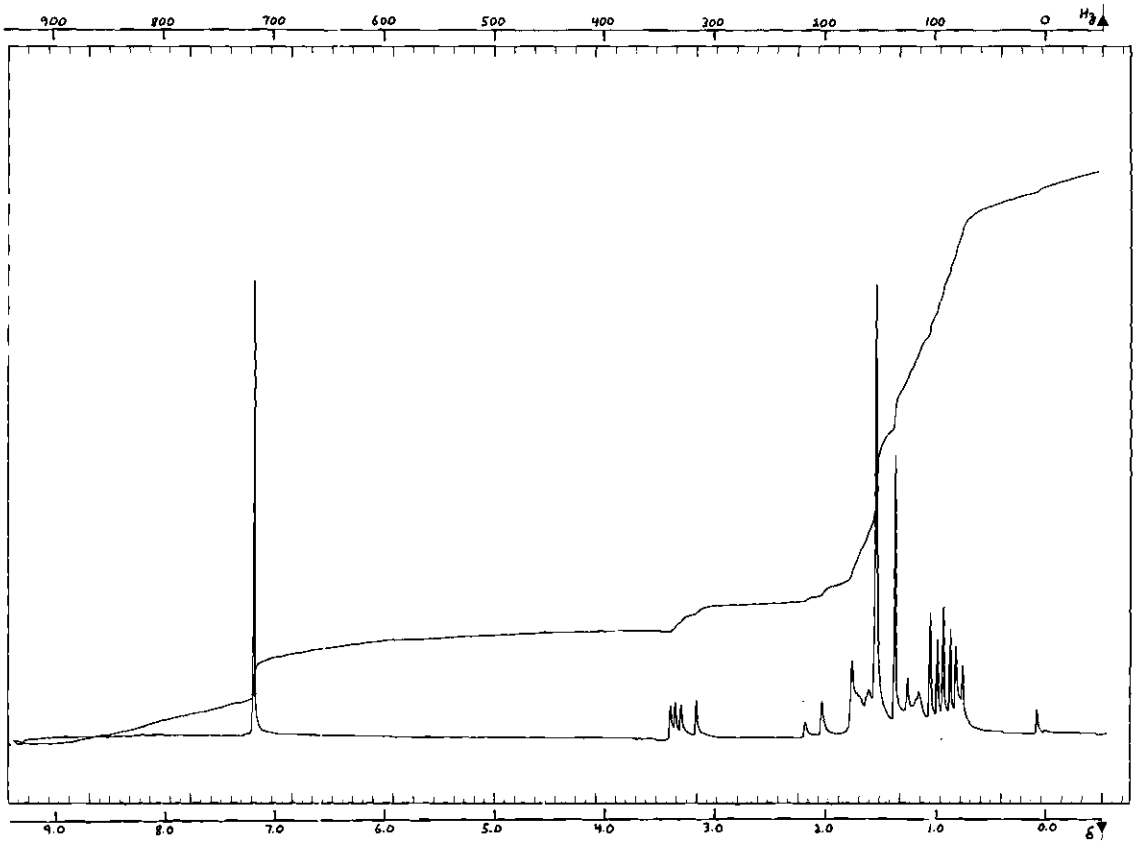


Plate XV. ^{1}H-NMR Spectrum of Compound 627-8.

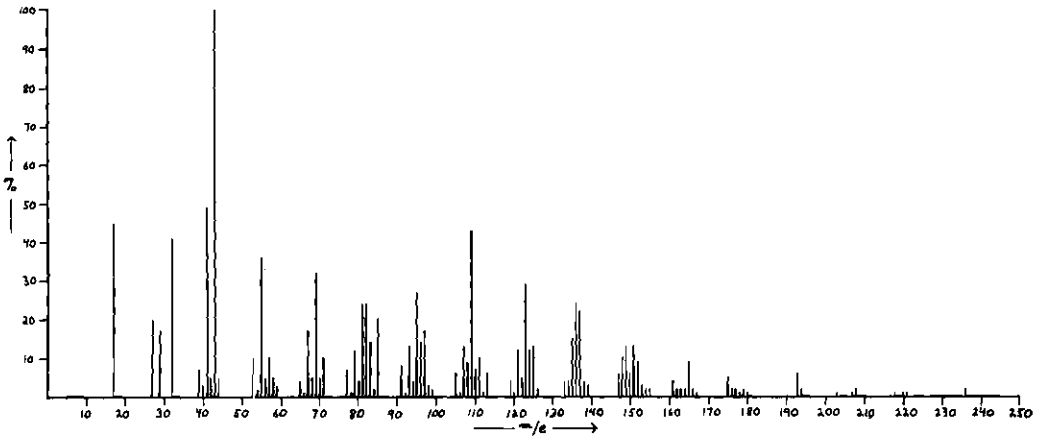


Plate XVI. Mass Spectrum of Compound 627-8.

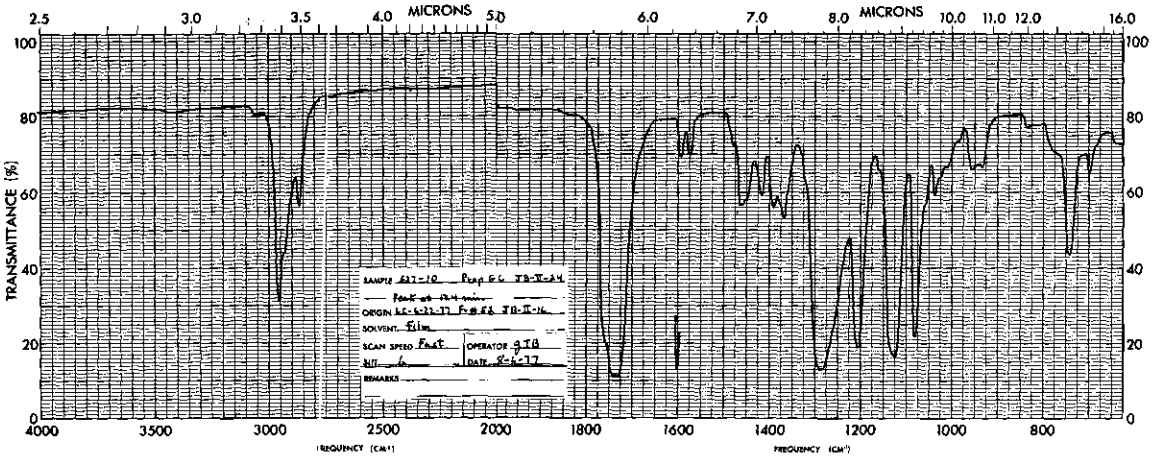


Plate XVII. Infrared Spectrum of Compound 627-10.

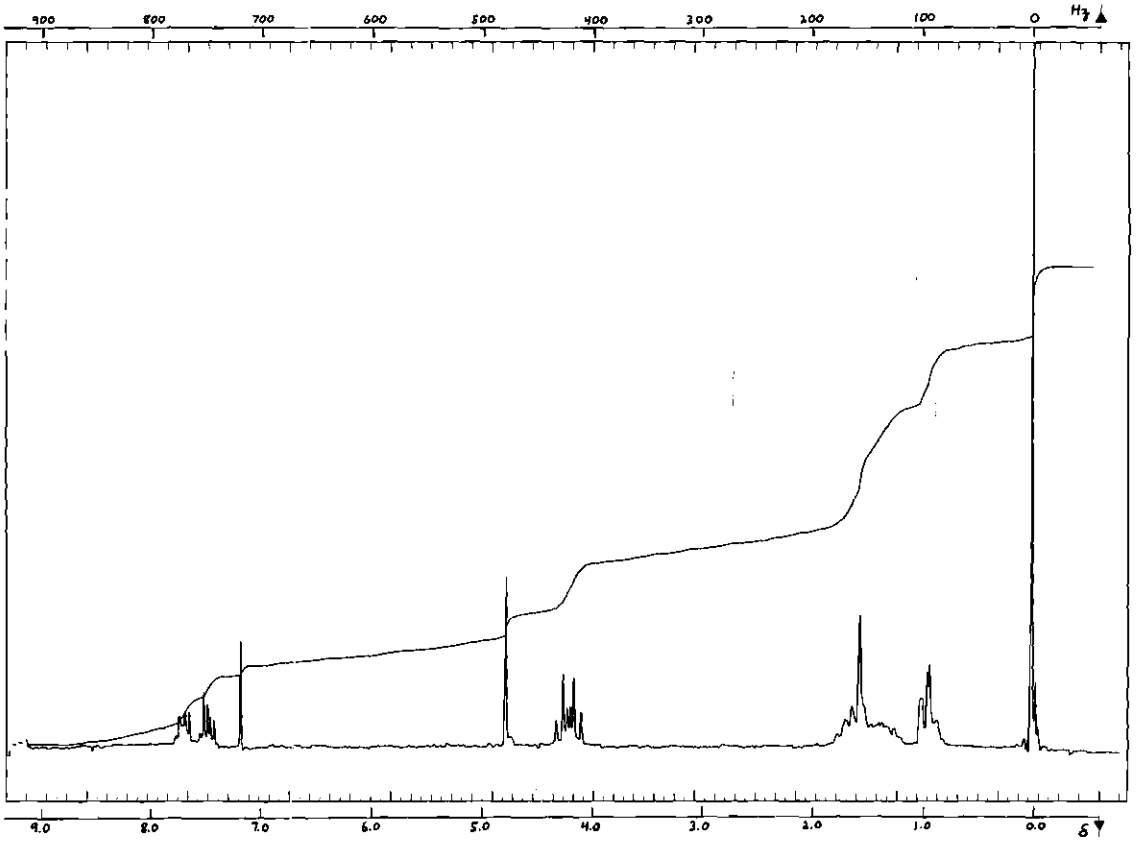


Plate XVIII. ^{1}H-NMR Spectrum of Compound 627-10.

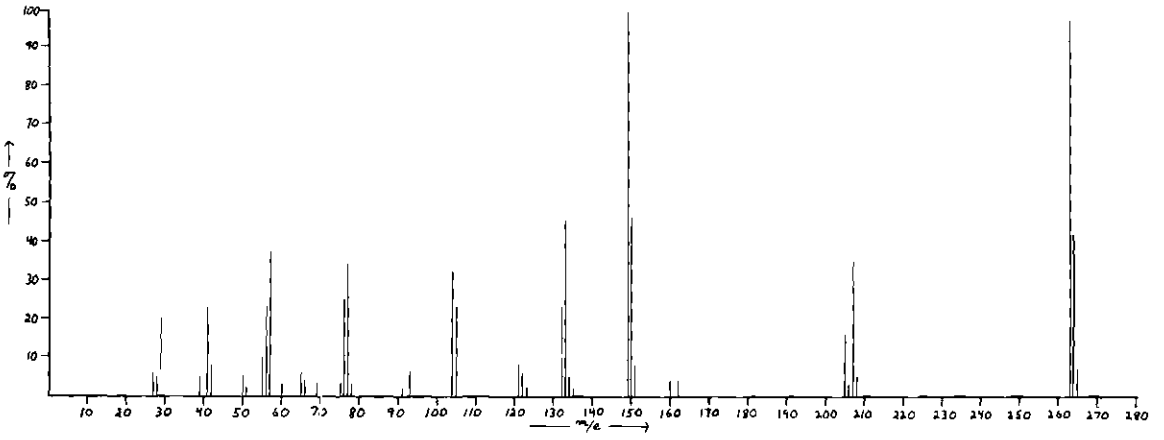


Plate XIX. Mass Spectrum of Compound 627-10.

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47. Hot Box Distillation (bulb to bulb vacuum distillation heated by hot air).

VITA

James Thomas Baxter, eldest son of Mr. and Mrs. James W. Baxter, was born April 27, 1944, in Norfolk, Virginia. He attended Hahira High School in Hahira, Georgia, and graduated in June 1962.

Mr. Baxter attended Valdosta State College in Valdosta, Georgia, where he received his Bachelor of Science in Chemistry degree in June 1966.

In September 1966, he began graduate study in the School of Chemistry at the Georgia Institute of Technology, Atlanta, Georgia, under the direction of Dr. Leon H. Zalkow.

In September 1972, he held the position of Temporary Assistant Professor at Valdosta State College. In September 1973, he taught high school at Palmetto High School in Palmetto, Georgia. In September 1974, he became an Instructor at Valdosta State College, a position he has held until the present time.

In June 1975, he married Judy Dianne Wellborn of Atlanta, Georgia.

Mr. Baxter received his Master of Science in Chemistry degree from the Georgia Institute of Technology, under the direction of Dr. Leon H. Zalkow, in March 1976.