COMPARISON OF SUBSTITUTED PHENOLS OCCURRING IN THE TICKS *IXODES SCAPULARIS* AND *DERMACENTOR VARIBILIS*

By

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ABBREVIATIONS

A	Area
A.	*Amblyomma*
Aa	*Amblyomma americanum*
Am	*Amblyomma maculatum*
AMU	Atomic Mass Unit
Att.	Attached
bp	Boiling Point
#C	Number of Carbons
°C	Degrees Centigrade
C'AMP	Cyclic Adenosine Monophosphate
cm.	Centimeter
CRC	Chemical Rubber Company
D.	*Dermacentor*
DCP	Dichlorophenol
2,6-DCP	2,6-Dichlorophenol
DDT	Dichlorodiphenyltrichloroethane
Dv	*Dermacentor variabilis*
EOG	Electroolfactograph
F	Female
°F	Degrees Farenheit
g.	Gram
GC/MS	Gas Chromatograph/Mass Spectrograph
GLC	Gas Liquid Chromatography

h	Peak Height
H.	*Haemophysalis*
I.	*Ixodes*
ID	Inner Diameter
Is	*Ixodes scapularis*
M	Male
M	Methyl
m.	Meter
m	Meta
M/e	Mass Per Unit Charge
Min.	Minute
ml.	Milliliter
mm.	Millimeter
mm^3	Cubed Millimeter
MP	Melting Point
N	Total Number of Data Points
n	Normal
ng.	Nanogram
No.	Number
o	Ortho
p	Para
pK	Negative Log of Acidity Constant
psi	Pounds Per Square Inch
R.	*Rhipicephalus*
Ref.	Literature Reference
RI	Relative Intensity
s	Standard Deviation

Σ	Summation
t	t -Value From Student T Table
ul	Microliter
um	Micrometer
U.S.	United States
W_b	Width at Peak Base
$W_{h1/2}$	Width at One Half Peak Height
x_n	Individual Data Point
$\bar{x}$	Population Average
*	Uncharacterized
4-M-2,6-DtBp	4-Methyl-2,6-di-tert-butylphenol

CHAPTER I

INTRODUCTION

Sight and sound communication, although uniquely effective in their own way, are used by relatively few animals. By far the more utilized form of communication involves the sense of smell, or more precisely, chemical communication. Higher animals use chemical communication in conjunction with sight and sound but for many of the lower species, it is the only available method of communication. Chemicals that carry a message between individuals characteristically are volatile, have at least one electron withdrawing or accepting group, and are emitted in very low concentrations. In addition, these chemicals are classified as to the individuals involved and the behavioral response elicited by the odorants.

Semiochemicals

Communication chemicals in general are called semiochemicals: chemicals that mediate interactions between organisms. Semiochemicals are further classified into groups according to the relationship of the interacting organisms. Intraspecific communication chemicals are called pheromones: chemicals that mediate interactions between organisms of the same species. Interspecific communication chemicals are called allelochemicals: chemicals that deliver behavioral messages between members of different species.

Allelochemicals. Allelochemicals are divided into groups depending on whether the sender or receiver or both are the beneficiary of the resulting interaction. A karimone is a chemical which elicits a response from another organism that is beneficial to the receiver of

the signal. An allomone is a chemical which elicits a response beneficial to the sender, and a synome is a chemical which elicits a response that is beneficial to both the sender and receiver. Allelochemicals can be used to control the population of a pest if the behavioral response to the signal can be made to limit proliferation of the pest.

Pheromones. Pheromones are divided into groups depending on the type of behavior elicited by the chemical signal. A sex pheromone is a chemical that stimulates a copulatory response from the opposite sex of the same species. Sex pheromones are the best studied of all the pheromones because of their potential for nontoxic pest population control. Alarm pheromones are chemicals that alert other members of a community to danger. Aggregation (Assembly) pheromones are chemicals that attract others of the same species to the source of the signal. These pheromones are produced by organisms that benefit from having a group of their same species with them. Antiaggregation pheromones are chemicals that repel others of the same species and are produced by organisms that benefit from solitude (Evans 1985, Silverstein 1981).

Pheromones and Pest Control

As indicated previously, semiochemicals can be used to artificially alter behavioral patterns of organisms. This effect makes pheromones a particularly attractive alternative to pesticides in insect control for not only economic reasons but toxicity and public health reasons as well. Many of the chemical pesticides used in agriculture today are quickly becoming useless due to the resistance developed by insects to these chemicals. It is common practice to cycle pesticide use to prevent the development of pesticide immunity. Another factor which is causing considerable concern recently is the toxicity of pesticides to humans and the injestion of these compounds with agricultural products. The classical example of this is DDT. In the past, DDT levels have been found in the mammary glands of human mothers as well as in the milk which they produce. This problem led to the

discontinuance of the use of DDT in the United States, but many other countries still use it.

Modern Pesticides vs. Pheromones. Modern pesticides are not as ubiquitous as DDT but still are just as toxic. The advent of organo phosphate pesticides such as Malathion and Fenthion and carbamate pesticides such as Methomyl has reduced the amount of pesticide intake in humans but waste materials from the production of these pesticides are very toxic and difficult to dispose of safely. Carbamate and organo phosphate pesticides are general cholinesterase inhibitors and work quite well on human nerve tissue but insects are much more susceptible to these toxins.

Pheromone Development for Pest Control. Development of pheromones for use in pest control could significantly reduce the use of chemical pesticides or even eliminate them in some cases. Pheromones have three qualities that make them attractive in pest control situations. First, they are entirely specific, so a single type of pest can be targeted and dealt with without the threat of eliminating beneficial or nondestructive insects. Second, nearly all pheromones are nontoxic, and their use would be in such small quantities that the ones that may be toxic are rendered harmless. Third, insects are incapable of becoming resistant to pheromones since they are naturally produced and used by the insects.

Pest Control Methods. Four proposed methods of integrated pest control using pheromones are described. Pheromones can be used in trapping insects for population monitoring and survey. Traps may be put out in the fields and routinely monitored. When the number of insects trapped is high enough, pesticides may be applied. This system would reduce the amount of pesticide needed and thereby reduce costs and environmental damage. Pheromones can be used to lure insects into circumscribed areas where they are treated with insecticides or pathogens. Pheromones can be used in mass trapping of insects for population suppression and, finally, pheromones can be used to

disrupt mating by saturation of an area with sex pheromone (Silverstein 1981, Evans 1985, Zif *et al.* 1981).

The key importance of these pest control measures is their safety, low expense, and the control of insect population rather than the annihilation of insects. Integrated pest control with pheromones is not a quick, easy method like routine spraying of pesticide, but ultimately the method is safer and less costly.

The Importance of Tick Control

Considerable investigation is necessary to impliment a pheromone pest control system. The first requirement is the behavior of a particular insect must be thoroughly understood. The second step is to identify the chemical identity of the message, and third, the chemical compounds and a delivery system must be established. This investigation concerns the second of these requirements, the identity of the chemical message stimulating the copulatory behavior of the Black-legged Tick, *Ixodes scapularis*. The Black-legged tick is considered here in comparison to the American Dog tick, *Dermacentor variabilis* because of the similarities and differences between the two ticks and because the sex pheromone of *D. variabilis*, 2,6-Dichlorophenol, has been extensively studied.

The control of *I. scapularis* is important because they serve as vectors for human pathogens. *I. scapularis* is implicated as a minor vector in the transmission of rocky mountain spotted fever, caused by *Rickettsia rickettsii* (Burgdorfer 1975), erythema chronicum migrans and Lyme arthritis, caused by *Borrelia burgdorferi* (Steere *et al.* 1978, Wallis *et al.* 1978), and babesiosis caused by *Babesia microti* (Oliveira 1979). All of these afflictions have epidemic potential and have been epidemiologically described. Thus, the potential for control of *I. scapularis* has some real and immediate value in human health and welfare.

CHAPTER II

REVIEW OF LITERATURE

Pheromones of the Ticks

Chemical communication in ticks and other closely related species has been extensively characterized and four major groups appear to exist. These pheromones are divided into groups according to the behavioral response elicited by the receiver of the stimulus.

Alarm Pheromones

Alarm pheromones are reported only from *Astigmata* (soft-bodied mites). When one of the mites is injured, the pheromone excites dispersal of others of the same species. The pheromones are the terpenoids, neryl formate, and citral (Sonenshine 1985).

Assembly Pheromones

Assembly pheromones are the most widespread of all pheromones and are reported to be interspecific in character. The response of the tick to the pheromone is cessation of ambulatory movement and congregation of numerous individuals in direct contact with each other (Sonenshine 1985). Assembly pheromones are water soluble, non heat labile, and are deposited on the tick's natural substratum. There are twelve species of soft tick, including *Argas* and *Ornithodoros*, and four species of hard ticks reported to have assembly pheromones. Aqueous solutions from the ticks have been shown to elicit behavior like an assembly pheromone but no specific components of the extract have been identified (Sonenshine, Silverstein, Rachev 1982).

Aggregation-Attachment Pheromones

Aggregation-attachment pheromones are reported only in feeding males in a few species of metastriate ticks of the genus *Amblyomma*. These pheromones regulate attachment to the host body sites where a male of the same species is already feeding (Sonenshine 1985). Aggregation-attachment pheromones are reported to be species specific. Production of these pheromones by the male occurs only after a period of feeding. The pheromone will persist at the site of feeding for two to three weeks after the male has detatched. The chemical structure and class of the aggregation-attachment pheromones is unknown.

Sex Pheromones

Sex pheromones used by ticks are generally substituted phenols or terpenes. The pheromones tend to be sex specific in that only females produce them in large quantities and only males respond. They do not, however, seem to be the stimulus to copulate but serve only to attract. Tick sex pheromones fall into three groups, arrestant, contact, and attractant pheromones.

Arrestant Sex Pheromones. Arrestant sex pheromones occur only in the mite families *Phytoseiidae* and *Tetranychidae*. The pheromones farnesol, nerodiol, geraniol, or citranellol (all terpenes) cause cessation of mobility and a "guarding" pose in males. The reception of these pheromones by the male has been called into question by new evidence suggesting that male and female mites meet by chance (Sonenshine 1971).

Attractant Sex Pheromones. Attractant sex pheromones are chemicals that attract sexually competent male ticks to a feeding female tick. True attractant sex pheromones are known only in the metastriate forms of the ixodid ticks. The females will generally produce pheromone only after feeding on the host for a few days and the pheromone only

attracts male ticks which are feeding or have fed. The pheromone stimulates male ticks to detatch and become excited (Sonenshine 1986).

Contact Sex Pheromones. Contact sex pheromones, sometimes called aphrodesiac pheromones, are only effective when the male has contacted and mounted the female and they stimulate copulation. Two examples have been reported. *Ornithodoros* females deposit a contact pheromone on their body surface in the coxal fluid. This chemical along with physical mounting of the female by the male stimulates copulation. In *Dermacentor* ticks males are attracted to 2,6-dichlorophenol but copulate only with conspecific females. This specific mating resulting from a nonspecific pheromone indicates the possible presence of a contact pheromone (Sonenshine 1982).

Sex Pheromone of *Dermacentor variabilis*

The most studied attractant sex pheromone in ticks is the compound 2,6–dichlorophenol (DCP) which has been found in several hard tick species including *Dermacentor variabilis, D. andersoni, Amblyomma americanum, A. maculatum,* and *Rhipicephalus sanguineus.* The nonspecific nature of 2,6-DCP seems to indicate that it is not the only factor controlling mating in the species that respond to it; however, different concentrations of 2,6-DCP occurring in different tick species could prevent interspecies mating (Khalil *et al.* 1983). Additional factors such as genital incompatability, female rejection of transspecific males and even a genital contact pheromone which is species specific may occur (Barker 1987).

Sonenshine *et al.* (1985) report demonstration of the presence of a genital sex pheromone in the anterior reproductive tract of female *D. variabilis.* Khalil *et al.* (1983) report, however, that the genital pheromone is apparently absent in mated females since mating attempts are very rarely seen between a male and a repleat female. Through methanol extraction and fractionation, a sex pheromone of two or more components was demonstrated to stimulate copulation in males using neutered females impregnated with the

extract. Electrophysiology was used to demonstrate that the males could indeed detect the material. The glands producing the pheromone were identified using histochemistry but the identity of the chemical stimulant has not been established.

Occurrence of the Sex Pheromone

The occurrence of 2,6-DCP in relation to age and feeding in *D. variabilis* was reported by Sonenshine *et al.* (1984). This pheromone is present only in the adult form of the tick. 2,6-DCP did not appear in females until 12 to 18 days post emergence. The amount of the pheromone per tick ranged from 0.5 to 1.5 ng per tick with the highest concentration occurring in females 158 days old. 2,6-DCP was detected in males that were fed for nine days.

Activity of Pheromones

The effective range of tick pheromones is very short with detection occuring at 2 cm. for 2,6-DCP and up to 5 cm. for *Amblyomma* aggregation-attachment pheromone. Diffusion studies have indicated that about 10% of the pheromone with a molecular weight of 100 to 300 travels 1 cm. from the source with 10,000 to 100,000 molecules occuring 2 to 3 cm. away from the source. At 10 cm. less than 1 molecule per mm^3 is present (Sonenshine *et al.* 1982). Thus with 10 ng. of pheromone extractable from the female tick, 1 ng. of 2,6-DCP is distributed in a circle of radius 1 cm. with the tick in the center.

Pheromones have been reported from several species of both hard and soft ticks. The hard ticks generally have sex pheromones while the soft ticks generally have assembly pheromones. The assembly pheromone in the soft ticks could perform the same function as the sex pheromone in hard ticks in that it brings the males and females together. Tables I and II list some of the reported tick pheromones and the ticks which use them.

TABLE I

SEX PHEROMONES OF THE VARIOUS TICKS

Tick	Pheromone	Ref.
Dermacentor andersoni	2,6-DCP, Gonopore Pheromone*	36,40
D. variabilis	2,6-DCP, Gonopore Pheromone*	36,40
D. parumapertus	2,6-DCP	24
H. anatolicum excavatum	2,6-DCP	24
Amblyomma americanum	2,6-DCP, 3-chlorophenol, 4-chloro-phenol, 2,3-DCP, 2,4-DCP, 2,5-DCP	24,3,18
D. albipictus	2,6-DCP	4
H. leporispalustris	2,6-DCP	4
A. maculatum	2,6-DCP	18
Rhipicephalus sanguineus	2,6-DCP	37
Boophilus microplus	Uncharacterized phenols	37
R. appendiculatus	Phenol, p-cresol mixture	37
R. puchellus	Phenol, p-cresol mixture	37
Ixodes ricinus	Nonphenolic,water soluble*	37

* = Uncharacterized
DCP= Dichlorophenol

TABLE II

ASSEMBLY PHEROMONES OF THE VARIOUS TICKS

Tick	Pheromone	Ref.
Argas persicus	Water soluble, uncharacterized	22
Amblyomma variegatum	o-Nitrophenol, Methyl salicylate, Pelargonic acid $2X1X8x10^{-6}$g/tick	29,32
Ixodes holocyclus	Uncharacterized	45
Aponomma concolor	Uncharacterized	45
Ornithodoros moubata	Uncharacterized	23

Sex Pheromone Production and Reception

The pheromone 2,6-DCP is reported to originate from a dermal gland associated with the fovea dorsalis called the foveal gland. A foveal gland is reported in at least six species of ixodidea and is the only pheromone producing gland identified at this time. The foveal gland is reported to be absent in *Ixodes scapularis* (Barker 1987). Foveal glands have been specifically described in *Dermacentor variabilis, D. andersoni, Amblyomma americanum,* and *A. maculatum* (Axtell & LeFurgey 1979, Layton & Sonenshine 1975).

The Foveal Gland

The foveal gland occurs on the dorsal body surface in the form of two discrete clusters of numerous pores called the foveae dorsalis. The gland is a complex of three distinct parts, the foveal pores, ducts, and secretory lobes. The tubes of the foveal pores enlarge below the fovea where they join the ducts. The ducts are microvilli lined tubes which are filled with secretory droplets when the gland is producing pheromone. The secretory lobes consist of several large cells filled with secretory droplets (Sonenshine 1985).

Sonenshine *et al.* (1981) report that the phenolic pheromone 2,6-DCP is dissolved in lipid droplets in the foveal glands of *D. variabilis* and *D. andersoni*. The droplets consist primarily of neutral lipids of the triglyceride and cholesterol ester types. The percentage of short chain saturated fatty acids is lower in the gland lipids than in the cuticular lipids. Storage of 2,6-DCP in neutral lipids masks the toxicity of the compound until it is transported out of the tick. Also, the release of the pheromone is much slower and consistant from the oil droplet than if the pheromone was just secreted onto the cuticle. It has been suggested that the components of cuticular lipids, since they are species specific, may act as a pheromone so it is possible that the components of the oil droplets from the foveal gland also perform this function (Bloomquist & Dillwith 1985).

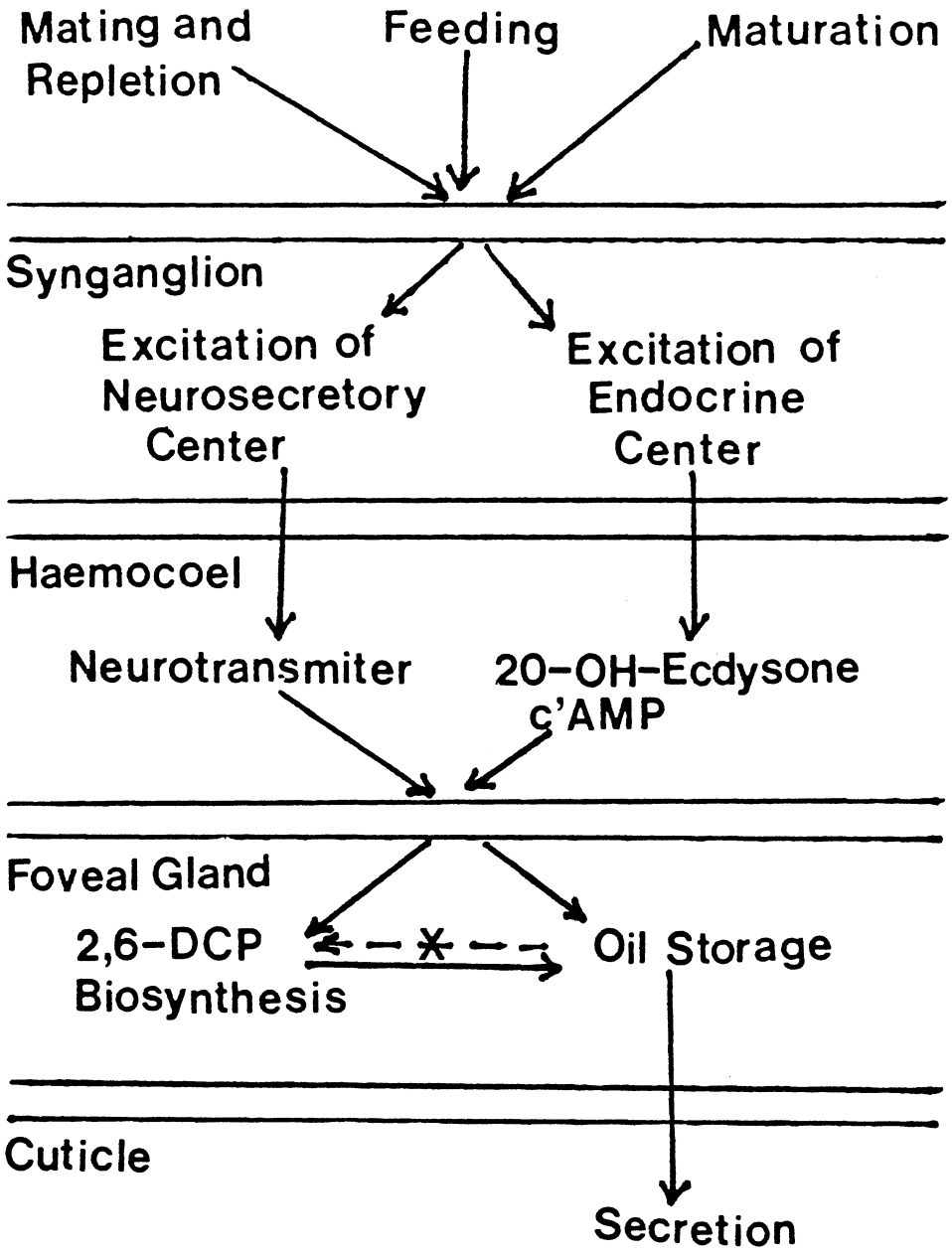
Sex pheromone production in ticks is reported to be under both neurosecretory and hormonal control. Neurosecretion of catacholamines are reported to stimulate 2,6-DCP production in *D. variabilis* since application of the monoamine inhibitors reserpine, alpha methyl-m-tyrosine methyl ester hydrochloride, or pilocarpine to females prior to feeding results in the loss of sexual attractiveness. Application of dopamine leads to elevated levels of 2,6-DCP in females (Sonenshine *et al.* 1985). Ecdysteroids and 20-hydroxy ecdysone specifically as well as juvenile hormone have been shown to stimulate 2,6-DCP production. Exogenous 20-hydroxy ecdysone administered to nymphs results in increased 2,6-DCP levels in the emerging females (Dees *et al.* 1984).

Sonenshine (1985) has proposed a hypothetical neurophysiological mechanism for control of the foveal gland production of 2,6-DCP (Figure 1). Neurosecretory or endocrine activity, stimulated by mating, feeding, or maturation, stimulates 2,6-DCP synthesis and storage in oil droplets in the secretory cells of the foveal gland.

Pheromone Receptors

Pheromone receptors are chemoreceptor sensilla that occur on three types of appendages of the tick: tarsus 1, the terminal segment of the palps, and the cheliceral digits. The one receptor that is unique to the ticks, the Haller's organ, is located on tarsus 1 and has chemoreceptors sensitive to 2,6-DCP (Sonenshine 1985). The general structure of the Haller's organ is the same on all ticks but specific shapes and sizes vary among the species (Homsher 1975). The Haller's organ is situated on the dorsal side of tarsus 1 and consists of an anterior trough and a posterior capsule. The anterior trough contains six sensillae. These sensillae are specific for perception of phenolic compounds and only one of the sensellia responds specifically to 2,6-DCP (Haggart & Davis 1981). Sonenshine *et al.* (1984) report evidence of a role for the cheliceral digits in perception of the genital sex pheromone of *D. variabilis* during mating. The cheliceral digits of the tick serve primarily

Figure 1. Diagram of Neurosecretory and Endocrine Control of the Foveal Gland (Sonenshine 1985)


Mating and Repletion
Feeding
Maturation
Synganglion
Excitation of Neurosecretory Center
Excitation of Endocrine Center
Haemocoel
Neurotransmiter
20-OH-Ecdysone
c'AMP
Foveal Gland
2,6-DCP Biosynthesis
Oil Storage
Cuticle
Secretion

for formation and transfer of the spermatophore during copulation. The inner cheliceral digits have sensilla which can detect the genital pheromone of the female.

Chemoreception

Chemoreception in any animal has been the subject of considerable research but remains a little understood phenomenon. Experiments with radiolabeled odorants indicate that the odorant is adsorbed onto the sensillium surface. The odorant then, somehow, whether through diffusion, transport, or some other process, comes in contact with the dendrite of a neuron (Boeckh 1985). It has been demonstrated by Mankin & Mayer (1983) with the use of a model olfactory system that a single odorant molecule can stimulate an action potential in a single neuron, but the action potentials of several neurons control the behavioral response of the individual so much more than a single odorant and a receptor are involved in chemical control of insect behavior.

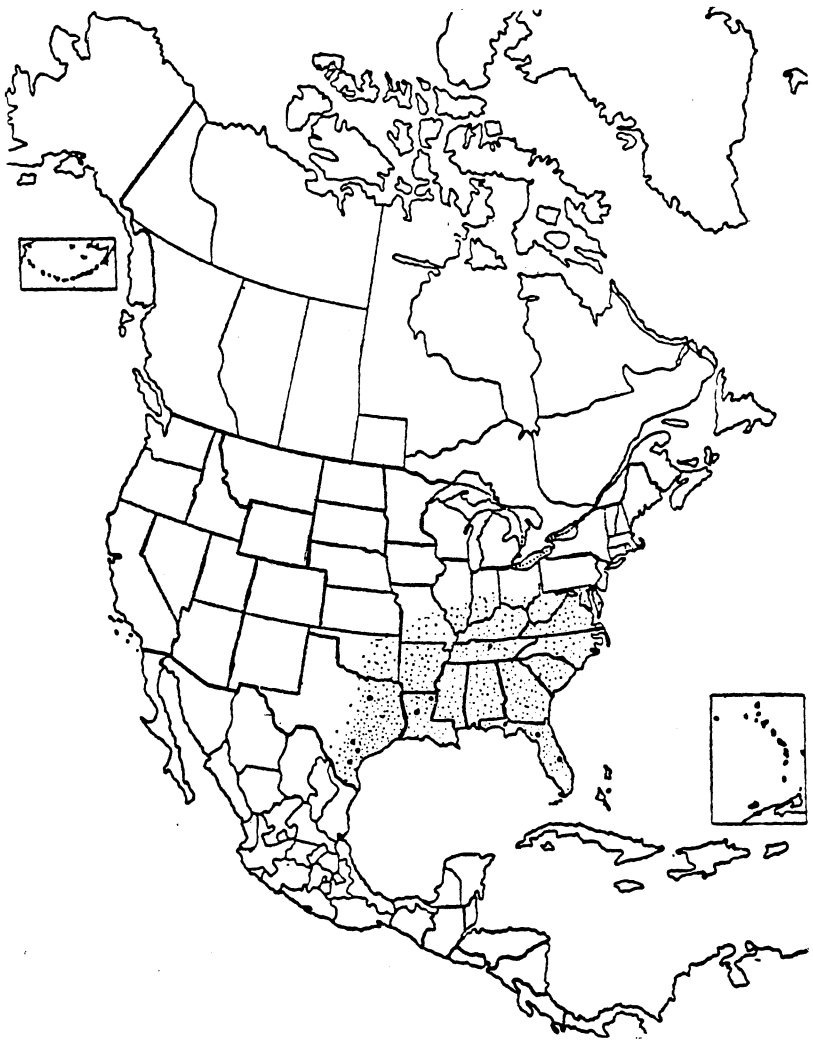
Life History of *Ixodes scapularis* and *Dermacentor variabilis*

Ixodes scapularis

Ixodes scapularis (Black-legged Tick) is found in wooded areas of eastern United States. The tick has been collected along the east coast of the U.S. from Maryland to Florida and in the central part of the U. S. from Indiana south to Texas. The tick has been reported in Mexico and in the Central American country of Costa Rica (See Figure 2).

Ixodes scapularis is a prostriate tick with three life stages: larvae, nymph, and adult. The adult female is a dark brown color with a black scutum and black legs and has no detectable foveal gland (Barker 1987). The female ranges in size from 2.5 by 1.25 mm. to 3 by 1.5 mm. unengorged. When engorged, the female is a grey to dark blue color with black legs. The engorged female is about 10 by 7 by 5.5 mm. The male of the species is about 2.3 by 1.25 mm. and has a black to dark brown color.

Figure 2. Distribution of the Black-legged Tick, *Ixodes scapularis*, in North and Central America (Hooker, Bishop and Wood 1912)



The adult *I. scapularis* will mate when not on the host, but no development occurs in the female until she engorges. Mating is more predominant on a host. The present day domestic host is most commonly cattle and dogs. The specific mating behavior is not reported in the literature but personal observations indicate that the females attach to the host and feed for a short time before the males locate them and mate.

Mating occurs when the male places his cheliceral digits into the female's genital pore and transfers the spermatophore. Apparently, the female can store the sperm for some time in a viable form since roughly 6% of the females mate before being fed on the sheep. Mating off the host is probably an artifact of the research conditions since the males and females are kept in an artificially high and crowded population. After mating, the female will feed to repleation and drop off of the host.

Oviposition occurs 15 to 25 days after the female drops off the host. The number of eggs deposited depends on the extent of engorgement of the female. The larger the blood meal, the larger the egg mass. Oviposition occurs over a 1 to 3 day period in which around 3000 eggs are produced. The eggs are light brown, shiny, smooth and about 0.445 by 0.386 mm. In oviposition, the female lays on her back and the egg mass is protruded out over the head. The female dies after oviposition. Eggs incubated at 61°F hatch in 72 days to produce larvae (Hooker, Bishop, and Wood 1912).

Unengorged larvae are dark brown and range in size from 0.616 by 0.371 mm. to 0.746 by 0.474 mm. Larvae which hatch in the laboratory live for up to 2.5 months but undoubtedly can live considerably longer before attatching to a small animal to feed. Larvae feed on small mammals of the field such as mice and rats. In the laboratory, larvae feed on a host for 3 to 9 days to become engorged. Engorged larvae are a dark bluish grey to black color and 1.28 by 0.76 mm. to 1.4 by 0.9 mm. in size. The larvae fall off the host and molt in 23 to 31 days to become nymphs.

Unengorged nymphs are a dark, smoky brown color with capitulum and shield nearly black and are 1.5 by 0.75 mm. in size. The nymphs can live up to 2 months in the

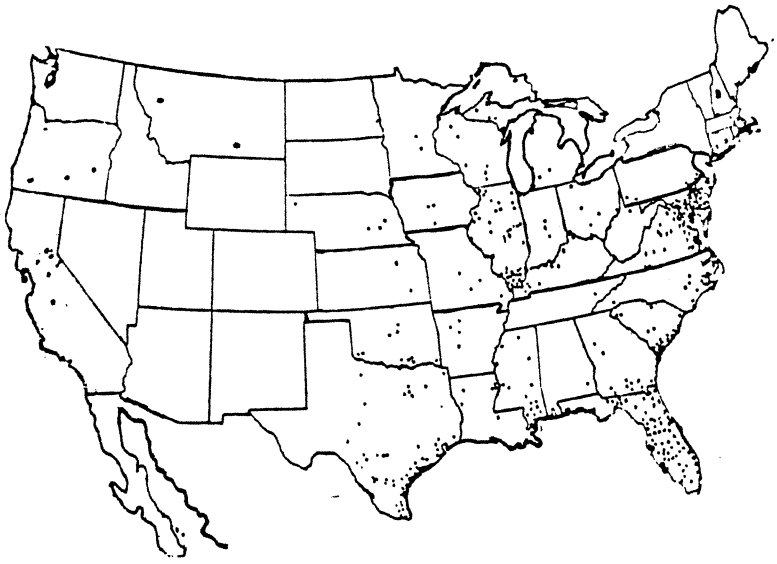
laboratory before attatching to a host and feeding. Their field hosts are the same as for the larvae. Nymphs engorge in 3 to 8 days and drop. Engorged nymphs are 2.3 by 1.5 by 1.25 mm. to 2.7 by 1.5 by 1.25 mm. in size and are a dark bluish grey color. After dropping from the host, nymphs molt in 25 days to become adults (Hooker, Bishop, and Wood 1912, Harris, 1957). A total of 141 to 165 days are required to produce a new generation of adults under optimal conditions. The time of generation, however, can vary greatly depending on the availability of hosts and mates. The adult *I. scapularis* can live for up to 5 months without feeding if the atmospheric conditions are favorable. Thus, the adults can survive a considerable time for a suitable host to become available.

Dermacentor variabilis

Dermacentor variabilis (American Dog Tick) is distributed primarily from Texas to Minnesota east to the East Coast of the U. S. Some specimens have been collected on the West Coast but the entire Central U. S. from Texas to Minnesota west to the Sierra Madres is sparsely populated with the tick. No report is made of the tick in Mexico, Central America or Canada though they may occur (See Figure 3).

More literature concerning *Dermacentor variabilis* pheromones and life cycle is available than for *Ixodes scapularis*. Not only is *D. variabilis* a more common tick, but, early in the study of spotted fever, this species was targeted as a vector of the disease. *D. variabilis* is a metastriate tick (commonly called hard tick) and has three life stages: adult, larvae, and nymph. The unengorged female is a red brown color with white zigzag markings on its dorsal side and is about 4.8 by 2.5 mm. in size. The adult male is the same red brown color as the female, but the white pattern is continous over its back giving it a bronze appearance and is 4.5 by 2.5 mm. in size (Smith, Cole, Gouck 1946). Adult *D. variabilis* are reported to live up to 1032 days in outdoor conditions before feeding, but in laboratory conditions only live up to 911 days.

Figure 3. Distribution of the American Dog Tick, *Dermacentor variabilis*, in North America (Smith, Cole, and Gouck 1946)



Both the male and female embed and feed, but there is only a small change in size in the male compared to the female. The engorged female is 13 by 10 mm and is a light tan color. There is no mating of *D. variabilis* until both the male and female have fed on the host for a short time. The males will detach and search for the female. Once the male finds a female it will mate and feed more near the female. The male may or may not detach and find another female.

After mating the females will engorge to repleation and drop from the host and roam to find a protected environment before oviposition. Oviposition can last 14 to 32 days producing 4000 to 6500 eggs. The female dies after oviposition. The egg is a yellowish-brown ellipsoidal sphere laid in a mass and covered in a viscous secretion. Incubation of the eggs is 27 to 57 days in which the eggs will hatch to produce larvae.

Larvae have no spiracles and only three pairs of legs. Unfed larvae are yellow and about 0.6 mm long. Engorged larvae are slate grey or black and are about1.5 mm. long. After feeding for 3 to 13 days, the larvae drop from the host and molt in 6 to 49 days to become nymphs.

Nymphs have four pairs of legs, are a yellowish-brown color when unfed, and are about 1.5 mm. long. Nymphs attach to a host and feed 3 to 11 days until engorged. Engorged nymphs are a slate grey and about 4 mm. long. Engorged nymphs molt in 24 to 291 days to produce adults (Smith, Cole, Gouck 1946, Campbell, Harris 1979).

The length of the period required to produce the next generation of adults is highly dependent on temperature and relative humidity and can range from 77 to 453 days. The variable and long generation period of the tick is a major obstacle when studying only the adults of the species.

Tick Feeding Behavior

Both tick development and mating are generally dependent on parasitic feeding on a host, most of its behavior is related to feeding. Waladde and Rice (1982) report tick

feeding behavior as a culmination of nine main events: appetence (seeking host), engagement, exploration (searching host for feeding spot), penetration, attachment, engorgement, detachment, and disengagement. The sequence is found in all ticks and slight variations in the feeding behavior may differentiate the species.

One major variation in feeding behavior is the secretion of a cement for attachment in the hard ticks and the absence of cement in the soft ticks. The short mouth parts occurring in hard ticks requires that a cement, which is secreted by accessory glands of the salivary gland, be produced to help the tick attach to the host. The soft ticks have relatively long mouth parts that can securely hold the tick on the host. *D. variabilis* is a hard tick that secretes cement, but *I. scapularis*, though it has many of the characteristics of the hard tick, has long mouth parts and produces no cement (Barker 1987).

The size of the blood meal ingested by a tick on feeding varies from species to species depending on the host on which the ticks are feeding. Koch and Sauer (1984) report that *D. variabilis* and *I. scapularis* both feed on dogs and that dogs are acceptable hosts for these ticks. The amount of blood ingested by *D. variabilis* and *I. scapularis* respectively was 1.45 ml. and 0.51 ml. when allowed to feed to repleation. The amount of blood ingested by *D. variabilis* on sheep was comparable to that ingested on the dog, so at least for *D. variabilis*, sheep are also a good host as they presumably are for *I. scapularis*. The quantity of blood ingested by a pregnant female will directly affect the size of the egg mass that is produced, so selection of a good host upon which to mate and feed is important.

Many hosts, when fed on by a large population of hard ticks, develop resistance and cross-resistance to the ticks. McTier *et al.* (1981) report that guinea pigs develop resistance to *D. andersoni*, *D. variabilis*, *A. americanum*, and *I. scapularis* when tick larvae in large numbers feed on them. Resistance to the ticks was determined by measuring reduced survival of the larvae after repeated infestations.

The two Dermacentor species cause considerable cross-resistance with each other but cross-resistance between the Dermacentor and the other two species was seen only in reduction of engorged weight rather than decrease in survival. Researchers report fluid filled vesicles on the guinea pigs ears when ticks were feeding indicating a T-cell type response to the allergens. Since *D. variabilis* and *I. scapularis* show little cross-resistance in guinea pigs, then they will probably show little in sheep (Speculation).

Pheromone Study

Pheromone research has evolved quickly in the last decade, primarily due to the increase in sensitivity of analytical methods during that time. Many of the early pheromone identifications involved extraction of tens of thousands of insects but today, with the best equipment available, the pheromone can be chemically characterized from ten insects in some species.

Much of the behavioral work with tick pheromones is done by placing ticks in a petri dish with lines dividing it into sections (Leahy *et al.* 1973). This work will indicate the type of behavior that can be elicited by chemical communication. Leahy ascertained that an aggregation pheromone existed in the soft tick *Argas persicus* using this method. Unmated females were allowed to contact a filter paper disk for several hours and deposit the aggregation pheromone on the disk. This disk was then used in an assay along with other unimpregnated disks to show that fed virgin females were attracted to the impregnated disk.

Once the presence of the pheromone is demonstrated, the identity of the pheromone can be ascertained. First, a suitable solvent is found by extracting ticks with different solvents and determining the response of the ticks to the extract (Norval 1979). The bioassay can be done in two ways. First, a Y-shaped olfactometer can be employed or the petri dish assay (Leahy *et al.* 1973) can be used. The correct solvent is the one which produces the most attractive extract.

A specialized bioassay which provides information about detection of pheromones but not the behavior elicited by the pheromone is the electroolfactograph (EOG). The EOG measures the membrane potential change when a chemoreceptor neuron reaches action potential. Schneider (1963) suggests that the EOG is essentially the sum of many olfactory receptor potentials recorded more or less at the same time. The EOG is an oscilloscope with two microfine electrodes, one on each side of the membrane. When an odorant stimulates the olfactory neuron to action potential, the neuron depolarizes and the depolarization is recorded as a peak of potential on the EOG. Recently, the EOG has been refined and upgraded to a system that can identify specific chemoreceptors (Borst 1984). It must be emphasized again that the EOG gives no information about the behavioral response to the odorant.

The same general method can be used to find the optimum extraction method. In most cases, for sex pheromone extraction, a freezing and thawing procedure in hexane or pentane is used (Sonenshine *et al.* 1976). The ticks are sealed in a tube with solvent and subjected to sonication, freezing in liquid nitrogen, and thawing cycles. Other methods like homogenization of the ticks (Berger 1972) have been used but this drastic method creates a considerably more complex mixture for analysis.

Analysis of the extract is performed with gas chromatography with either a flame ionization detector, electron capture detector or mass spectrograph detector. By far the best method of detection is the mass spectrograph but it is not always available in a system integrated with a gas chromatograph. Gas chromatography, however, using standards of known pheromones and possibly even a retention index such as Kovats Indicies can prove quite useful without mass spectrometry. The best inlet system for trace analysis is on column injection since none of the sample escapes the column as in split injection. The best columns for pheromone research are fused silica capillary columns since they give the best resolution of similar components of a mixture.

A specialized system of analysis for pheromone research is the gas chromatograph coupled with the EOG. In this system, the outlet of the gas chromatograph is split, one portion going to the detector and the other directed onto the receptor of the tick. The receptor is monitored using the EOG. This system indicates the peaks coming from the chromatography column for which the tick has receptors. The behavior elicited by the peak must be analysed in a separate system. Many chemical analysis methods not mentioned here are used in insect pheromone study. These methods are important but not necessarily applicable to tick research (Young, Silverstein 1975).

CHAPTER III

MATERIALS AND METHODS

Tick Feeding and Rearing

Dermacentor variabilis and *Ixodes scapularis* larvae and nymphs were raised on rabbits. The rabbit's ears were covered with nylon hose cut from the legs of No Nonsense Sheer Pantyhose. One end of the hose was secured around the rabbit's chin with velcro straps and the loose hose at the base of the ear was taped to the fur. The larvae or nymphs were poured from a bottle or carton into the ears of the rabbit, and the open end was twisted and taped shut. The rabbits were placed in confining cages 15 cm. by 15 cm. by 25 cm. and the cages were placed in fiberglass boxes. The fiberglass boxes had masking tape around the top with the adhesive exposed to prevent escape of the ticks.

After a day, the hose headgear was removed from the rabbit and most of the larvae or nymphs were attatched to the ears and feeding. The ticks were allowed to feed to repletion and drop off of the rabbits. Ticks were collected from the bottom of the boxes twice a day and placed in one cup paper ice cream cartons. The rabbits were fed sparsley on lettuce and carrots to reduce the amount of urine produced. Most of the ticks detatched from the rabbit in seven days. The rabbits were cycled so that they were used as hosts no more than twice a month and after three uses they were culled from the colony.

The engorged larvae and nymphs were stored in a non-airtight plastic humidity chambers with glass lids. The humidity of the chambers was maintaned by placing pans of saturated aqueous potassium sulfate in the bottom of the boxes. Saturated aqueous potassium sulfate ($KHSO_4$) gives a relative humidity of 86% at a temperature of 20°C

(CRC 1982-1983). The tick storage room was maintained at a temperature of 20°C. The nymphs and larvae were stored under the above described conditions until they molted, which was around 5 weeks for both nymphs and larvae of both *D. variabilis* and *I. scapularis*.

The emergent adults of *I. scapularis* would not feed for four weeks after emergence but the *D. variabilis* adults would feed almost immediately after emergence. The adult ticks were fed on wether and ewe Dorset and Ramboullet sheep. The sheep were kept inside at a temperature of around 75°F during the times ticks were feeding.

To prepare the sheep as hosts for the ticks the sheep were placed in stantions in the large animal room of the Efaw Entomology Laboratory and sheared using a Sunbeam-Stewart Model EW 311A Shearmaster animal clipper leaving about 3 mm. of wool on the skin. The sheep were washed and dried to remove dirt and excess oil from the remaining wool and skin. Any wounds occasionally inflicted with the shears were treated with Furall topical antibiotic (Furazolidone).

Eight, nine or ten circular feeding cells around 10 cm. in diameter, arranged in rows along the sheeps back, were trimmed in the wool down to skin using an Oster Model A2 small animal clipper. A ring of 3-M Scotchgrip 4799 Industrial Adhesive about 2.5 cm. wide was applied with a tongue depressor around the cells. Adhesive was applied to a 2.5 cm. strip around the inside of a 25 cm. length of Soma Med 6 in. wide tubular orthopedic stockingette and each was allowed to dry to tackiness. The stockingette was then put on around the cells and closed off on the other end with two rubber bands.

Adult ticks were separated by sex and forty to sixty female ticks were placed in each cell. The ticks had attached and were feeding usually in 24 hours, but during the winter, when temperatures fell in the sheep room to the 40 to 50°F range, the ticks either attached late and did not engorge completely or did not attach at all and died.

Ticks destined for pheromone extraction were allowed to feed for seven days at which time the cells were removed and the ticks were collected by pulling them from the

skin with forceps. The forcibly removed ticks were transported in vials to an adjoining lab and were placed in hexane filled 4 dram vials and stored at approximately -20°C until sufficient numbers had been collected for processing.

Breeding was done in the same size cells. However, twenty females and twenty males were placed in them. All ticks, with exception of male *I. scapularis* attatched within 24 hours and pregnant repleat females were dropping loose in the cells in 9 to 11 days. The last of the pregnant females were generally pulled from the sheep after 15 days. The pregnant females were placed one to a vial for *D. variabilis* and two to a vial for *I. scapularis* in a humidity chamber to await oviposition and hatching of the larvae.

Behavioral Studies

The behavioral response to 2,6-DCP was observed using a variation of the method devised by Leahy *et al.* (1973) using a 15 cm. plastic disposable petri dish. The dish was placed on a heat table set at 39°C. (103°F) which is the skin temperature of the sheep inside a feeding cell measured with a YSI model 47 scanning thermometer. Two 5 mm. diameter disks of Whatman No. 1 filter paper were placed in the dish. One of the disks was impregnated with a dilution of chemical or extract while the other contained only hexane solvent. The solvent was allowed to evaporate from the disks for 5 min. and then they were placed in the dish. Ten partially fed male *D. variabilis* or unfed *I. scapularis* were placed in the dish and the number of contacts made by the ticks on each disk was counted in a thirty minute time period.

A simple method for determining the effectiveness of the attractant is to use the number of contacts on the disk impregnated with solvent only as a background. The number of contacts on the blank disk is the number of random contacts that would be made on the other disk if it had no attractant, or for that matter, repellant on it. This finding can be used to graph the effectiveness of a compound for use as a pest control method. If the graph stays at zero, the compound is ineffective either way. If the results

are positive, it is an attractant, if negative, a repellant. This is not a statistically accurate method but it will serve the purposes of this research.

Purification of the Phenol Fraction from Ticks

Extraction I

Initial purification of phenolics from the ticks was fashioned after that reported by Sonenshine *et al.* (1976). The ticks were placed in hexane and stored in the freezer at –20°C for several days until around 700 to 800 ticks were collected. The hexane extract was collected from the ticks and two further washings were collected at 10 min. intervals. These extracts were combined and around 100 ml. of hexane were used.

Extraction I essentially removed all compounds soluble in nonpolar solvents that exist on the surface of and in the cuticle of the tick (See Table III) as well as the fecal material and some internal compounds. This fraction contains waxes, fats, and hydrocarbons occurring in the cuticular lipids plus any phenols that may be produced from the dermal glands. The waxes and hydrocarbons have a considerably higher boiling point than phenols (See Tables IVa, IVb) and are considerably more nonpolar. The hydrocarbons and wax esters elute at a higher temperature in the gas chromatograph than the low molecular weight phenols so the waxes can build up at the head of the column and plug it. Therefore extraction II is necessary to remove the phenols from the waxes.

Extraction II

Extraction II is designed to separate the phenols from the hydrocarbon type compounds. The extraction relies on the pK of the phenols being much lower than that of the hydrocarbons and much higher than that of the fatty acids (See Table V). The pK of phenols can be the basis of separation in an acid-base extraction. The hexane solvent from extraction I was vigorously extracted with 10 ml. of 1 normal sodium hydroxide (NaOH)

TABLE III

COMPOUNDS COMMONLY FOUND IN CUTICULAR LIPIDS

Compound	Structure
n-alkanes	$CH_3\text{-}(CH_2)_x\text{-}CH_3$
n-alkenes	$CH_3\text{-}(CH_2)_x\text{-}CH{=}CH\text{-}(CH_2)_y\text{-}CH_3$
2-methylalkanes	$CH_3\text{-}\underset{\displaystyle CH_3}{CH}\text{-}(CH_2)_x\text{-}CH_3$
3-methyl alkanes	$CH_3CH_2\underset{\displaystyle CH_3}{CH}\text{-}(CH_2)_y\text{-}CH_3$
Internally branched monomethylalkanes	$CH_3(CH_2)_x\text{-}\underset{\displaystyle CH_3}{CH}\text{-}(CH_2)_yCH_3$
Dimethyl alkanes	$CH_3(CH_2)_x\text{-}\underset{\displaystyle CH_3}{CH}\text{-}(CH_2)_y\text{-}\underset{\displaystyle CH_3}{CH}\text{-}(CH_2)_zCH_3$
Trimethyl alkanes	$CH_3(CH_2)_y\text{-}[\underset{\displaystyle CH_3}{CH}\text{-}(CH_2)_y]_3\text{-}(CH_2)_zCH_3$
Primary alcohol wax ester	$CH_3(CH_2)_x\text{-}O\text{-}\overset{\displaystyle O}{C}\text{-}(CH_2)_y\text{-}CH_3$
Secondary alcohol wax ester	$CH_3(CH_2)_x\text{-}\overset{\displaystyle O}{C}\text{-}O\text{-}\underset{\displaystyle (CH_2)_yCH_3}{\overset{\displaystyle H}{C}}\text{-}(CH_2)_zCH_3$
Epoxide	$CH_3(CH_2)_x\text{-}\underset{\displaystyle O}{CH}\text{-}CH\text{-}(CH_2)_yCH_3$
Ketone	$CH_3(CH_2)_x\text{-}\overset{\displaystyle O}{C}\text{-}(CH_2)_yCH_3$
Secondary Alcohol	$CH_3(CH_2)_x\text{-}\underset{\displaystyle OH}{CH}\text{-}(CH_2)_yCH_3$
Primary Alcohol	$CH_3(CH_2)_xCH_2\text{-}OH$
Free Fatty Acids	$CH_3(CH_2)_x\text{-}\overset{\displaystyle O}{C}\text{-}OH$

(Blomquist and Dillwith 1985)

TABLE IVa

PHYSICAL CONSTANTS OF SOME LONG CHAIN COMPOUNDS

Name	Source[a]	#C[c]	MP(°C)	BP(°C)
Capric Acid	1	10	31.5	270
Myristic Acid	1	14	58.5	250.5_{100}[b]
Palmitic Acid	1	16	63-64	215_{15}
Stearic Acid	1	18	69-70	383
Decane	2	10	-29.7	174.1
Hexadecane	2	16	18.2	287
Eicosane	2	20	36.8	343
Docosane	2	22	44.4	368.6
Octacosane	2	28	64.5	449.7
Docosanol	2	22	71	180_{22}
Triacontanol	2	30	88	------
Bees Wax	1	26-72	62-65	------

[a]Sources: 1 = Merk Index 10th Ed., 2 = CRC 63rd

[b]Subscript indicates mm Hg pressure

[c]Number of Carbons

TABLE IVb

PHYSICAL CONSTANTS OF SOME SUBSTITUTED PHENOLS

Name	Source[a]	#C	MP(°C)	BP(°C)
2,6-DCP	1	6	67	219-20
2,5-DCP	1	6	58	211
2,4-DCP	1	6	45	210
2,3-DCP	1	6	57	-------
3,5-DCP	1	6	68	233-4
3,4-DCP	1	6	68	253
o-Cresol	2	7	30	191-2
m-Cresol	2	7	11-12	202
p-Cresol	2	7	35.5	201.8
o-Nitrophenol	2	6	44-5	214-16
p-Nitrophenol	2	6	113-14	-------
Methyl salicylate	2		-8.6	220-24
Salicylaldehyde	2		-7	196-7
Salicylic acid	2		157-9	211
Phenol	2	6	43	182
4-Methyl-2,6-di-t-butylphenol	2	15	70	-------

[a]Sources: 1 CRC 43rd. Ed.
2 CRC 63rd. Ed.

three times using a separatory funnel. The aqueous NaOH solution containing the phenols was then acidified with concentrated hydrochloric acid (HCl) to pH 2.0.

TABLE V

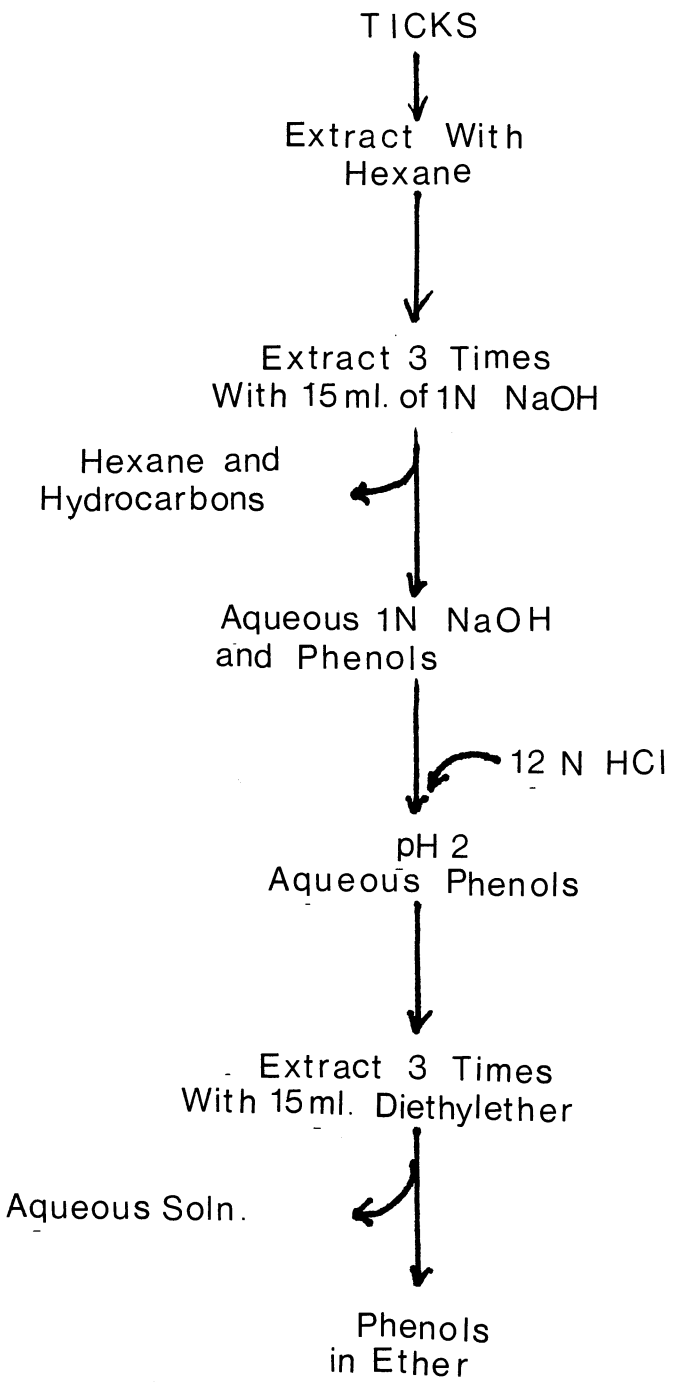
ACIDITY CONSTANTS OF SUBSTITUTED PHENOLS

Name	Acidity Const.*,[a]
Phenol	1.3×10^{-10}
o-Cresol	6.3×10^{-11}
m-Cresol	9.8×10^{-11}
p-Cresol	6.7×10^{-11}
o-Nitrophenol	6.8×10^{-8}
p-Nitrophenol	7×10^{-8}
m-Chlorophenol	1.6×10^{-9}
p-Chlorophenol	6.3×10^{-10}
o-Chlorophenol	7.7×10^{-9}

* In Water at 25°C.
[a] Source: T.W.G. Solomons

The acidified aqueous solution was then extracted three times with 20 ml. of diethyl ether. The ether containing the phenols was then dried with sodium sulfate. This dried ether was evaporated to dryness using a nitrogen gas stream and a water bath. The residues were dissolved in 1.0 ml of hexane and transferred to a vial. The hexane was partially evaporated with nitrogen gas to concentrate the sample (See Figure 4).

Figure 4. Diagram of Extraction Method


TICKS
Extract With Hexane
Extract 3 Times With 15ml. of 1N NaOH
Hexane and Hydrocarbons
Aqueous 1N NaOH and Phenols
12 N HCl
pH 2 Aqueous Phenols
Extract 3 Times With 15ml. Diethylether
Aqueous Soln.
Phenols in Ether

Chromatographic and Mass Spectral Analysis

Chromatographic Analysis

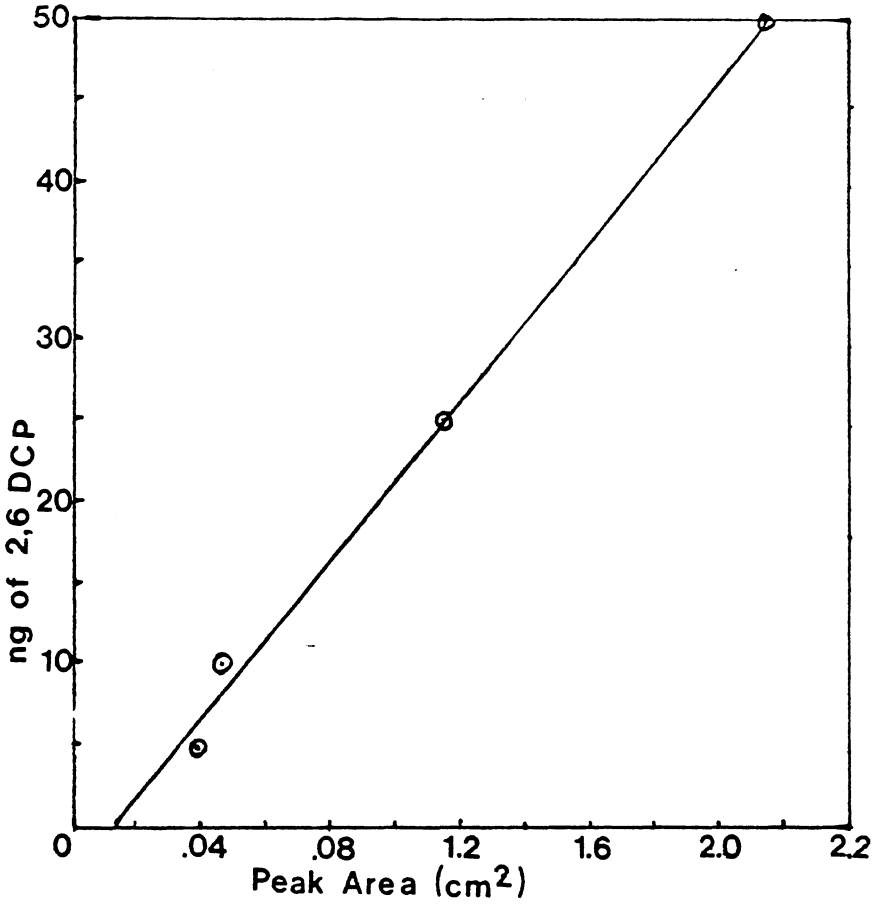
Chromatographic analysis was performed on a Tracor 560 gas chromatograph fitted with a J & W Scientific on column injector. The column was a 30 m. by 0.53 mm. ID DB–1 bonded phase 0.25 um. film, fused silica capillary column from All Tech Associates and the drive gas was helium with a column head pressure of 11 psi. Detection was with a flame ionization detector and an electron capture detector.

Standardization was with a series of substituted phenols (See Table IVb) and the limit of detection of 1 to 5 ng/ul. was determined with 2,6-DCP (See Figure 5). Internal standards were analyzed with selected samples by adding 50 ng/ul of 2,6-DCP to a portion of the sample. A sample of 1 ul. of tick extract was injected on column at an initial oven temperature of 75°C. The temperature of the oven was increased 3°C per minute to 200°C.

Mass Spectral Analysis

Mass spectral data was performed using a Kratos MS25Q mass spectrometer integrated with a Carlo Erba gas chromatograph. Chromatography was performed with a DB-1 column, splitless injection, and a temperature program from 75-250°C at 8°C per minute. Additional analysis was performed using a LKB-2091 capillary gas chromatograph/mass spectrometer/data analysis system. The capillary column was a DB-5 30 m. by 0.25 mm. bonded phase column with 0.1 um. film thickness from J&W Scientific Co. The column exit was connected directly to the ion source of the mass spectrometer. Each sample was evaporated to dryness and dissolved in 10 ul. of hexane. A sample of 1 ul. of this was injected with a 1:4 split at an initial oven temperature of 70°C. The temperature was raised at 4°C per minute to 150°C and then raised at 15°C per minute to 200°C. Mass scans were recorded at a rate of 1.402134 seconds per scan. The

Figure 5. 2,6-Dichlorophenol Concentration Verses Peak Area by GLC


50
40
30
20
10
0
ng of 2,6 DCP
.04
.08
1.2
1.6
2.0
2.2
Peak Area (cm2)

detection limit was 2 ng. entering the detector so a total of 10 ng. of any one component per 1 ul. of sample was required.

Statistical Methods

The population mean of the retention time data was approximated using the average (x) which is defined:

$$x = \frac{(x_n)}{N}$$

where x_n is the individual data point, and N is the total number of data points. The population variance of the same data was approximated using the standard deviation(s) which is defined:

$$s = \frac{(x_i - x)^{1/2}}{N - 1}$$

where x is the average, x_i is the individual data point, and N is the number of data points.

If the standard deviation(s) was larger than 10% of the average retention time, the individual data points were tested for validity using the Q-test. The Q-test was performed as follows:

1. Determine the range of the data (high value - low value).
2. Find the difference between the outlying data point and its nearest neighbor.
3. Divide the difference in outlier and neighbor by the range to find the quotient Q.
4. Compare the calculated Q value to that on a Q-table with the correct number of data points.

If $Q_{calculated}$ is greater than Q_{table} then the outlying data point is considered invalid and can be cropped from the data.

After testing the data, a 90% confidence interval was established for each retention time using the Student T table and the equation:

$$x \pm \frac{ts}{n}$$

where x is the average, t is the value from the Student T table, s is the standard deviation, and N is the total number of data points. This confidence interval is used to try to correlate the peaks from different samples and to provide a tentative identification of peaks.

CHAPTER IV

RESULTS AND DISCUSSION

Tick Rearing and Feeding

In this research, a total of 209 cells were put on sheep with 129 containing *I. scapularis* females for extraction, 46 containing *D. variabilis* females for the same purpose, and 29 cells were used for breeding and other purposes. Thirteen sheep were used for the 209 cells (See Table VI). To avoid resistance of the sheep to the ticks, they were all used only three times except for one, Sheep 19, which was used four times.

The 46 cells containing *D. variabilis* had a total of 1855 females put in them with 1400 feeding, 258 either feeding and disengaging or not feeding, and 197 dying. On a basis of all cells together, 89.4% of the ticks lived and 75.5% were feeding when they were removed. *D. variabilis* females had access to a host for an average of 6.09 days (See Appendix A). It was noted that the ticks fed more readily during the summer months when the temperature and humidity inside the sheep room were more constant. *D. variabilis* produces a cement to help them attach to a host. This cement caused considerable damage to the sheep's skin when the ticks were forcibly removed because the skin came off with the tick in spots of 1 to 3 mm. diameter.

The 129 cells containing *I. scapularis* had a total of 6182 females placed in them with 5099 of them feeding. No live females were found not feeding at collection time unless they were repleat, pregnant females. Of the 5099 fed females 4982 of them were extracted. On a basis of all cells together, 82.5% of the ticks lived through feeding with 80.6% being used for extraction. *I. scapularis* females had access to a host for an average of 8.35 days (See Appendix B). Most of the *I. scapularis* engorged more fully during the

TABLE VI

SHEEP UTILIZATION

Sheep	Sex	Tick	No. Cells	No. Ticks	Date In	Date Out
13	F	Is	62	40	11/1	11/7
		Dv	8	320	2/9	2/21
		Dv	6	240	3/30	4/4
14	F	Dv	7	280	1/16	1/30
		Is	10	600	4/13	4/18
15	F	Is	8	320	1/16	1/23
16	F	Is	8	320	1/20	1/31
17	F	Is	8	320	1/26	1/31
18	F	Is, Am, Aa	8	320	11/20	11/26
		Dv	8	320	2/23	2/28
		Is, Dv	8	320	3/30	4/4
		Is	8	320	11/1	11/7
19	F	Dv, Am	9	335	2/10	2/21
		Is	9	360	3/30	4/4
		Is	10	600	4/7	4/13
20	F	Is	8	320	11/20	11/26
		Is	10	600	4/23	4/29
51	M	Dv, Is	7	195	10/17	10/24
		Is	10	600	4/13	4/18
59	F	Dv, Is	8	300	10/17	10/24
		Dv	7	280	-------	-------
61	M	Dv, Is	8	447	10/3	10/15
		Is, Am, Aa	8	330	12/4	12/12
84	F	Dv, Is	6	350	9/23	10/3
		Is, Am, Aa	8	330	12/4	12/12

Is - *Ixodes scapularis*
Dv - *Dermacentor variabilis*
F - Female
Aa - *Amblyomma americanum*
Am - *Amblyomma maculatum*
M - Male

fall and spring than during the winter. The long mouth parts of *I. scapularis* caused a hematoma 3 to 5 mm. in diameter on feeding but little damage when they were forcibly removed.

Rate of Occurrence of Pregnant Female Ticks

When separating *I. scapularis* females from males, some of the females were found mating with the males. This occurrence indicates that a sex pheromone or assembly pheromone of I. scapularis is produced even when they are not feeding. Development of eggs does not occur until the female feeds on the host so the female can store the male's sperm in a viable form for some time. Of 47 *I. scapularis* cells surveyed with 1742 female ticks in them, 116 of the females were pregnant from mating off the host. On a basis of all 47 cells together, 6.7% of the females were found to be pregnant after an average of 6.9 days of feeding. Thus, in any sample of *I. scapularis* ticks used for extraction, 6.7% of them were pregnant (See Appendix C).

Host Resistance

Host resistance was observed in the form of skin lesions, reduced engorgement, and reduced survival of the ticks fed on sheep and in the form of granulomas forming in the skin on the rabbit's ears. Little, if any, cross-resistance was observed between *D. variabilis* and I. scapularis on either the sheep or the rabbits. Sheep 59 was very resistant to *D. variabilis* but was a good host for *I. scapularis* (See Appendices A and B). Sheep 59 on 10/17/86 had 40 *I. scapularis* put in cells 1 through 4 with 37 to 39 surviving. Cells 5 through 8 on 59 at the same time had 35 *D. variabilis* put in them with 20 to 23 surviving. Also, on 1/13/87, sheep 59 was mistakenly used for a mating sheep for *D. variabilis* and in three days none of the ticks had fed.

Attraction of Ticks to 2,6-DCP

Solutions of 2,6-DCP in hexane were prepared containing 50, 40, 30, 20, 10, and 2 ng. of 2,6-DCP per 5 ul. of solution. These solutions were used to place 50, 40, 30, 20, 10, and 2 ng. of 2,6-DCP on 5 mm. Whatman No. 1 filter paper disks. The disks were placed in 15 cm. plastic petri dishes maintaned at 90 to 95°F on a heat table. The relative humidity was 48% and the behavior of each set of 10 male ticks was observed for 30 minutes. Each new test was done with males that had not been previously used. The concentrations were too high to elicit responses from the ticks so further dilutions were done.

Unfed *D. variabilis* males were not attracted to 2,6-DCP concentrations of 0.25, 0.20, 0.15, 0.10, 0.05, and 0.01 ng in paper disks but fed males were attracted. Fed males responded to disks with 0.25, 0.20, and 0.15 ng. of 2,6-DCP by coming in contact with the disks and trying to crawl beneath them like they would if they were trying to mount a female for mating. On some occasions, several males would mob the disk and all try to crawl beneath it.

The male *I. scapularis* could not be induced to feed on a sheep in this study so unfed males were tested. It was apparent that unfed males respond to some signal from a female that is also unfed since male and female *I. scapularis* were observed mating off the host. The tests of unfed male *I. scapularis* ticks showed no positive response to any of the 2,6-DCP concentrations tested. The concentrations may have been too high or too low, but it is evident that *I. scapularis* males are not attracted to the same sex attractant system used by *D. variabilis*.

Chromatographic Analysis

Efficiency of Extraction II described in materials and methods was established using known amounts of 2,6-DCP in hexane. The extraction was performed and the 2,6-DCP occurring in the final sample was determined. A percentage of 72.3 was established as the

amount of the initial 2,6-DCP that was transferred across Extraction I. This means that roughly 30% more ticks are necessary for extraction than if the sample were used without extraction to meet the limit of detection.

Samples of fed female ticks were collected over a period of time. A total of 3 *D. variabilis* and 5 *I. scapularis* samples were prepared with a known number of ticks in each (Table VII). The samples were prepared as described in materials and methods except for one variation. Samples Is 6 and Is 7 had 5 mg. of 2,6-Dibromophenol in them for monitoring the efficiency of the extraction.

TABLE VII

SAMPLE PREPARATIONS

Sample	Tick	Bottle No.	No. of Ticks
Dv1	*D. variabilis*	1	271
Dv2	*D. variabilis*	4	930
Dv3	*D. variabilis*	6	257
----	*I. scapularis*	2	698
Is1	*I. scapularis*	3	1073
Is2	*I. scapularis*	4	576
Is3	*I. scapularis*	5	971
Is6	*I. scapularis*	6	888
Is7	*I. scapularis*	7	533
----	*I. scapularis*	8	243

Retention times were measured in centimeters from the start of the solvent peak. Chromatograms were recorded at a linear rate of 1.0 cm./sec. so translation to time is quite simple. Area beneath the curve for individual peaks was calculated using the equation:

$$A = h\, W_{h1/2}$$

with A being area, h being peak height, and $W_{h1/2}$ being the peak width at one half the height. If the peak went to the top of the chromatogram, the area was impossible to determine. Percentage of each component in the sample was impossible to find since some residual unsaturated hydrocarbons and fatty acids remained in the sample.

Substituted phenol reference compounds which have been reported to be attractive to ticks were analysed for retention time data. These compounds include phenol, three isomers of methyl phenol (cresol), two isomers of nitrophenol, 2,6-dibromophenol, six isomers of dichlorophenol, salicylaldehyde, methyl salicylate, and 4-mehtyl-2,6-di-tert-butylphenol (See Table VIII). Substituted phenols gave sharp nearly symmetrical peaks except for the halogenated phenols which all produced a tailing peak indicating a resistance to mass transfer into the mobile phase.

Each reported retention time is accompanied by its 90% confidence interval. Most of the confidence intervals on the retention times of the references are small since there were no complex mixtures involved. The confidence intervals for retention times of compounds found in the tick extracts were considerably higher (See Tables IX and X). Different factors produced these variations in retention times. First, the mechanism of the on column injector prevented establishment of a start point, requiring that retention times be measured from the emergence of the solvent peak. Second, the time from the initial placement of the sample on the column to the time at which the temperature program was started varied slightly for each injection. Finally, the long chain carbon compounds tended to impair the efficiency of the column and affect the resolution of the column.

The chromatograms obtained from the samples are complex. The best chromatogram from *D. variabilis* produced 130 peaks, 49 of which were major peaks. *I. scapularis* produced 105 peaks of which 29 were major peaks. The earlier peaks are probably produced by phenolic compounds while many of the later peaks are probably produced by the waxes and fatty acids that were not excluded from the sample during extraction II.

TABLE VIII

RETENTION TIME OF SUBSTITUTED PHENOLS

Compound	T_R*(Min. from Solvent Peak)
Phenol	3.85 ± 0.05
o-Cresol	5.35 ± 0.09
m-Cresol	6.05 ± 0.05
p-Cresol	5.98 ± 0.14
o-Nitrophenol	7.40 ± 0.14
p-Nitrophenol	6.85 ± 0.98
Salicylaldehyde	4.97 ± 0.06
Methylsalicylate	9.65 ± 0.14
4-Methyl-2,6-di-tert-butylphenol	22.13 ± 0.02
2,3-Dichlorophenol	9.05 ± 0.09
2,4-Dichlorophenol	8.83 ± 0.05
2,5-Dichlorophenol	8.60 ± 0.00
2,6-Dichlorophenol	10.20 ± 0.02
3,4-Dichlorophenol	18.88 ± 0.61
3,5-Dichlorophenol	18.03 ± 0.33
2,6-Dibromophenol	9.90 ± 0.09

* Retention times were determined on a Tracor 560 Gas Chromatograph with a DB-1 column and a temperature program from 75-150°C at 3°C per minute.

TABLE IX

RETENTION TIMES OF PEAKS FROM *I. SCAPULARIS* EXTRACTS

Peak	Is3 T_R (Min.)	Is6 T_R (Min.)	Is7 T_R (Min.)	Range of T_R* (Min.)	Tentative Identification
A	4.00	4.20	4.15	4.12 ± 0.09	
B	4.70	4.90	----	4.80 ± 0.19	
C	6.00	6.20	----	6.10 ± 0.19	m-Cresol
D	6.25	6.40	6.50	6.38 ± 0.12	
E	9.70	9.95	10.00	9.88 ± 0.15	m-Salicylate
F	11.20	11.40	11.50	11.37 ± 0.14	
G	13.45	13.55	13.65	13.55 ± 0.09	
H	13.80	13.90	14.00	13.90 ± 0.09	
I	18.25	18.30	18.50	18.35 ± 0.12	
J	19.50	19.50	19.70	19.57 ± 0.11	
K	20.45	20.40	20.65		
		20.45		20.49 ± 0.08	
L	21.10	20.75	21.40		
		21.10		21.40 ± 0.21	
M	21.80	22.50	22.60	22.30 ± 0.42	4-M-2,6-D-t-BP
N	22.40	22.50	22.80	22.57 ± 0.20	
O	23.30	22.60	----	22.95 ± 0.67	
P	26.60	26.50	26.80	26.60 ± 0.14	
Q	29.00	28.90	29.20	29.03 ± 0.14	
R	29.95	29.60	29.90	29.82 ± 0.18	
S	30.60	30.45	30.20	30.42 ± 0.19	
T	34.20	34.20	34.40	34.27 ± 0.11	
U	35.50	35.40	35.55	35.48 ± 0.08	
	35.60	35.45	35.55	35.53 ± 0.08	
V	36.50	36.35	36.60	36.48 ± 0.12	

* Average of the chromatograms shown. There were eleven chromatograms but only the best resolved chromatograms were used. Chromatography was performed on a Tracor 560 GC with a DB-1 column and a temperature program from 75 to 200°C at 3°C per minute.

TABLE X

RETENTION TIMES OF PEAKS FROM *D. VARIABILIS* EXTRACTS

Peak	Dv3 T_R* (Min.)	Dv3' T_R* (Min.)	Dv3" T_R* (Min.)	Range of T_R (Min.)	Tentative
A'	4.20	4.20	4.20	4.17 ± 0.06	
B'	4.90	4.90	4.80	4.87 ± 0.06	Salicylaldehyde
C'	6.20	6.15	6.05	6.13 ± 0.08	p-Cresol
D'	6.45	6.40	6.30	6.38 ± 0.08	
E'	6.80	6.80	6.70	6.78 ± 0.06	
F'	6.90	6.85	6.80	6.85 ± 0.05	p-Nitrophenol
G'	------	9.80	10.10	9.95 ± 0.28	2,6-Dibromophenol
H'	11.40	11.25	11.30	11.32 ± 0.08	
I'	13.60	13.40	13.40	13.47 ± 0.11	
J'	18.35	18.05	18.15	18.18 ± 0.14	
K'	19.60	19.20	19.30	10.67 ± 0.20	
L'	21.00	20.50	20.55	20.68 ± 0.26	
M'	21.60	20.80	20.95	21.12 ± 0.41	
N'	22.40	22.05	22.10	22.18 ± 0.18	4-M-2,6-D-t-BP
O'	------	26.20	26.30	26.25 ± 0.09	
P'	------	28.60	28.70	28.65 ± 0.09	
Q'	------	29.30	29.40	29.35 ± 0.09	
R'	------	29.60	29.75	29.68 ± 0.15	
	------	29.70	29.90	29.80 ± 0.19	
S'	------	30.10	30.20	30.15 ± 0.09	
	------	30.30	30.50	30.40 ± 0.19	
T'	------	35.00	35.10	35.05 ± 0.09	
	------	35.10	35.20	35.15 ± 0.09	
U'	------	36.05	36.20	36.13 ± 0.15	
V'	------	36.80	36.90	36.85 ± 0.09	
W'	------	38.50	38.60	38.55 ± 0.09	
X'	------	40.00	40.15	40.88 ± 0.15	

* All three chromatograms are of sample DV3. DV3 is Figure 8, DV3' is Figure 9, and DV3" is Figure 10. Chromatography was performed on a Tracor 560 GC with a DB-1 column and a temperature program from 75-200°C at 3°C per minute.

Many of the peaks occurring in the *I. scapularis* samples were in the retention time range of the substituted phenols (3 to 23 minutes). The later peaks were generally associated with a slight increase in the baseline due to the temperature program. Many of the peaks were concentrated enough to reach the top of the chromatogram preventing calculation of relative percentages of each fraction. A sample concentrated enough to show the small peaks well had enough waxes and other material to impair resolution and efficiency of the column.

The retention times of the peaks from three *I. scapularis* samples run at least three times each were averaged with a 90% confidence interval calculated for each. Of the many peaks on the chromatogram (See Figure 6), 22 were selected for retention time analysis. Table IX shows the peak designations, retention times and tentative identifications for each of the 22 major peaks. Peak number and retention time are given on the best chromatogram of each of the three samples and then an average is given for the peaks with a 90% confidence interval.

The letter designation in Table IX is derived from a qualitative comparison of the chromatograms of the different samples. The confidence interval is an indication of the effectiveness of the qualitative comparison. Tentative identification of peaks C as m-cresol, E as methyl salicylate, and M as 4-methyl-2,6-di-tert-butylphenol were determined using retention times. The chromatograms from which the data was obtained are shown in Figures 6, 7, and 8.

The *D. variabilis* extracts, like the *I. scapularis* extracts, produced a complex chromatogram, and since the peaks again reached the top of the chromatogram, relative percentages of fractions was impossible to calculate. Table X shows a letter designation with a (') indicating a qualitative comparison of peaks from different chromatograms. The retention times, average retention times, and a 90% confidence interval. A tentative identification of the peaks was made using retention times from Table VII. Peak B' is tentatively identified as salicylaldehyde, C' as p-cresol, F' as p-nitrophenol, G' as 2,6-

Figure 6. Gas Chromatogram of *I. scapularis* Extract Is3. Chromatography was performed on a Tracor 560 Gas Chromatograph with a DB-1 column at a temperature program from 75 to 200° C. at 3° C. per minute.

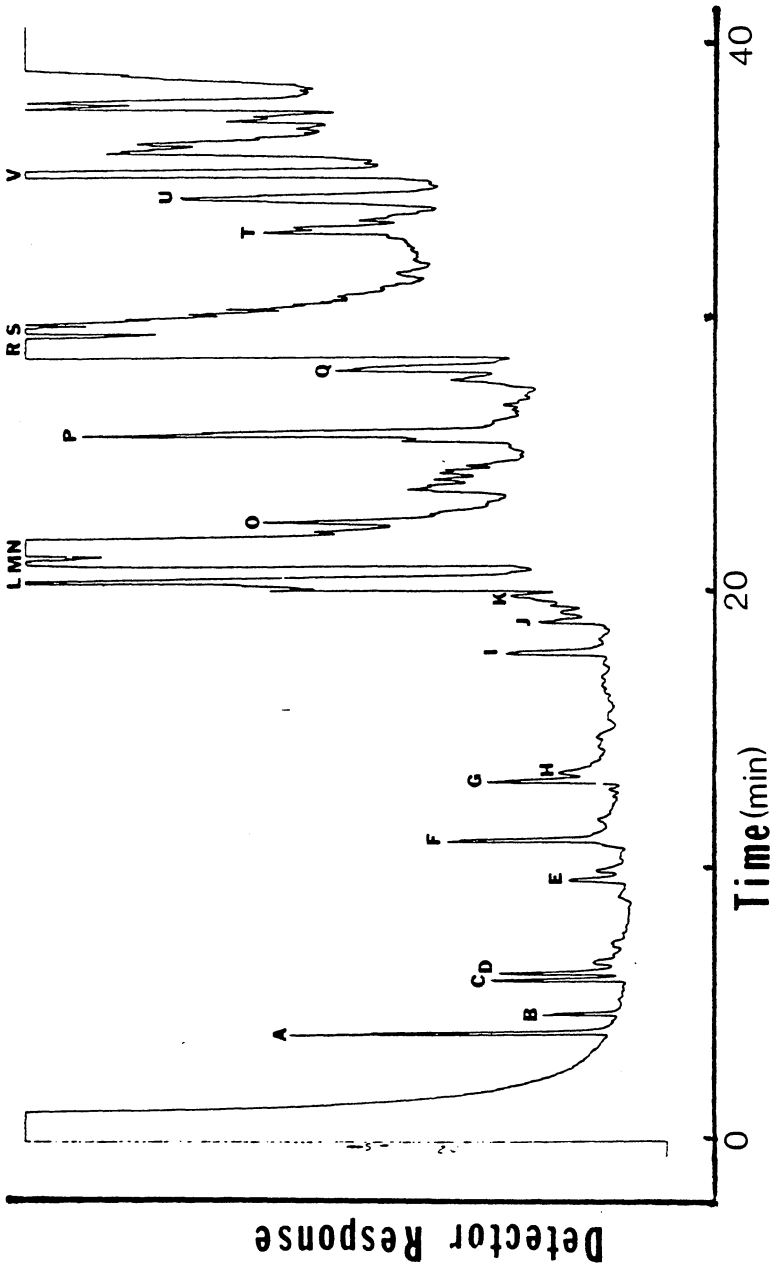

Detector Response
Time(min)
0
20
40
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V

Figure 7. Gas Chromatogram of *I. scapularis* Sample Is6. Chromatography was performed on a Tracor 560 Gas Chromatograph with a DB-1 column at a temperature program from 75 to 200° C. at 3° C. per minute.

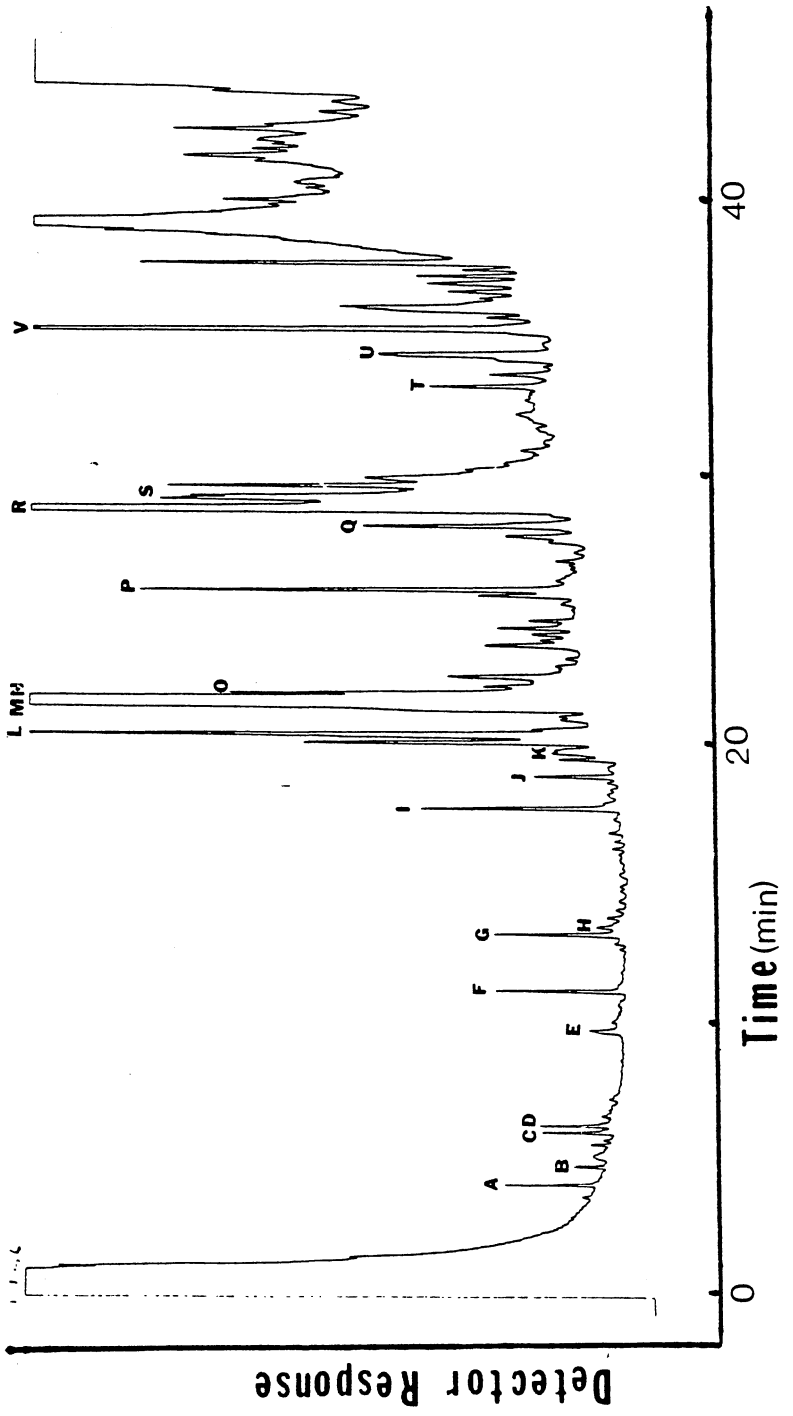
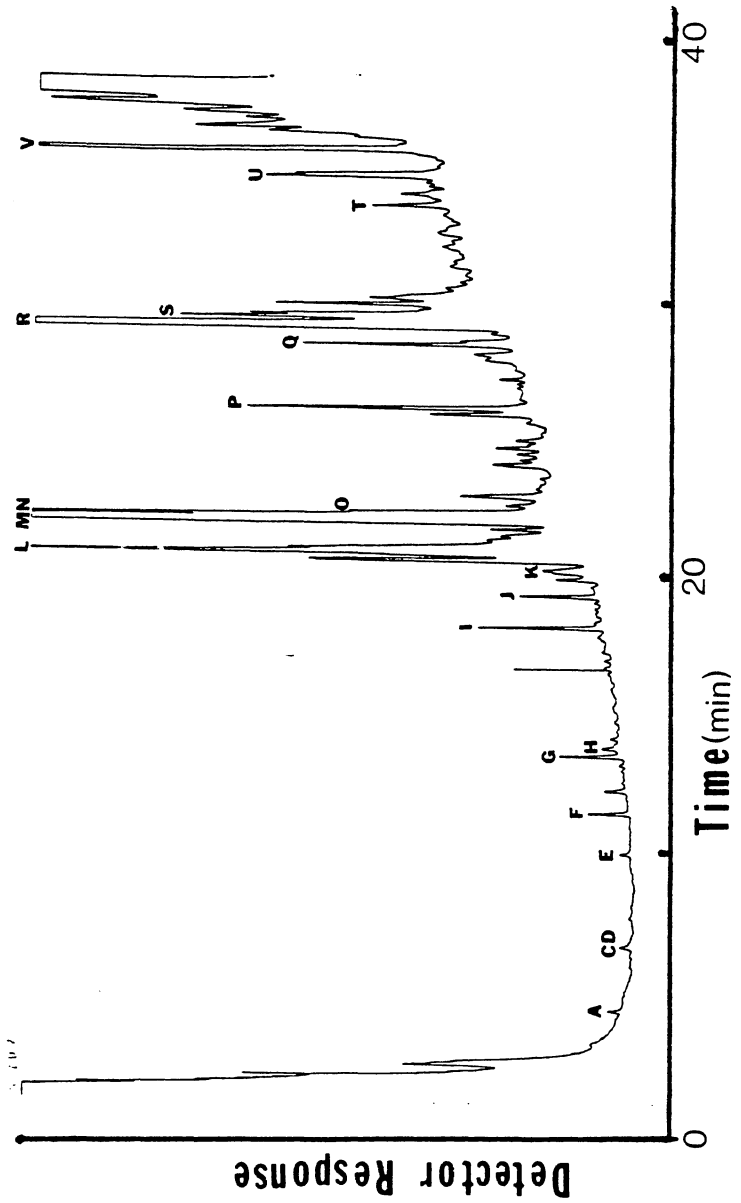

Detector Response
Time (min)
0
20
40
A
B
CD
E
F
G
H
I
J
K
L MN
O
P
Q
R
S
T
U
V

Figure 8. Gas Chromatogram of *I. scapularis* Sample Is7. Chromatography was performed on a Tracor 560 Gas Chromatograph with a DB-1 column at a temperature program from 75 to 200° C. at 3° C. per minute.


Detector Response
Time(min)
0
20
40
A
CD
E
F
G
H
I
J
K
L MN
O
P
Q
R
S
T
U
V

dibromophenol, and M' as 4-methyl-2,6-di-tert-butylphenol. The chromatograms from which the data was obtained are shown in Figures 9, 10, and 11.

An internal standard of 2,6-dichlorophenol was put in the *D. variabilis* sample and it was analysed in the chromatograph shown in Figure 11. When compared to the chromatogram of the *D. variabilis* sample with no added 2,6-dichlorophenol, shown in figure X, it is apparent that no 2,6-dichlorophenol occurred in this sample.

Comparing chromatograms from *I. scapularis* and *D. variabilis* shows a tentative correlation of 15 major peaks between the two species, only one of which correlates in the tentative identification of the peaks. Peak M from *I. scapularis* and peak N' from *D. variabilis* both correlate between the species and are both tentatively identified as 4-methyl-2,6-di-tert-butylphenol (See Table XI). This high correlation indicates a relationship between the ticks in the material they produce in their cuticles and bodies.

4-methyl-2,6-di-tert-butylphenol is a member of a group of isomers collectively known as butylated hydroxytoluene (BHT). BHT is commonly used as a preservative and antioxidant. Dry cereals, gasoline, paper products, and many other common products have BHT as an additive to preserve them. BHT is considered not to be a natural product because of the rarity of the tert-butyl functional group in biomolecules.

Mass Spectral Analysis

Gas chromatography integrated with mass spectrometry (GC/MS) provides a means of identifying the specific chemical compound emerging from the chromatograph. The mass spectrum of each compound is essentially a fingerprint that can identify the compound when compared to a reference spectrum. Some general information about the structure of the compound can be gained from the spectrum without actually identifying the compound so a general class of reference compounds can be selected for identification.

Mass spectral data from the tick extracts is presented generally in the same way as data is tabulated in Eight Peak Index of Mass Spectra (1974) with the masses of the peaks

Figure 9. Gas Chromatogram of *D. variabilis* Sample Dv3. Chromatography was performed on a Tracor 560 Gas Chromatograph with a DB-1 column at a temperature program from 75 to 200° C. at 3° C. per minute.

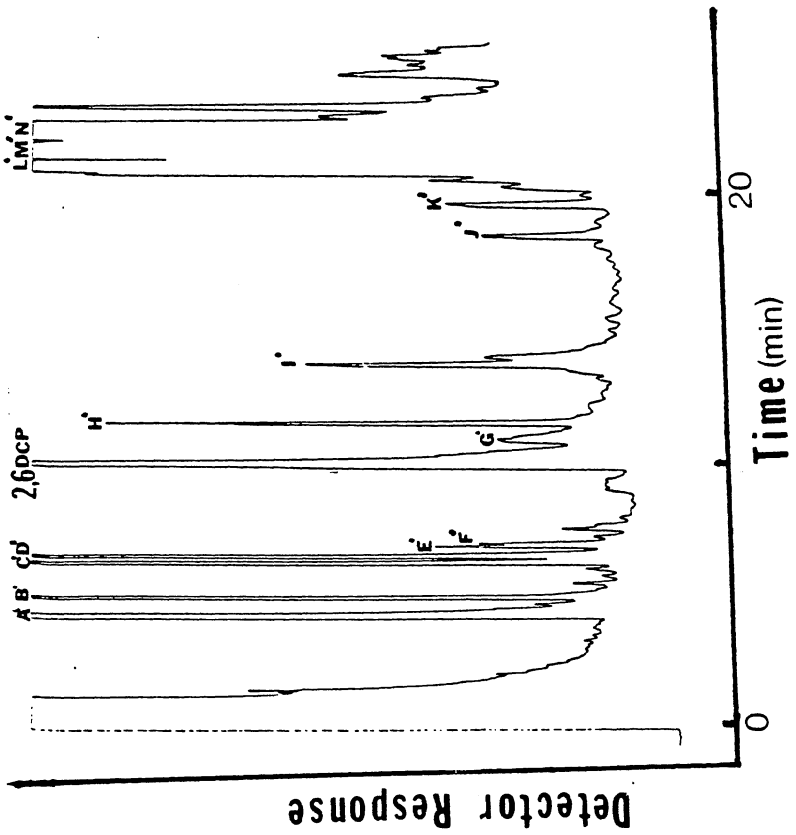

Detector Response
Time (min)
0
20
A' B' C' D'
E' F'
2,6DCP
G'
H'
I'
J'
K'
L' M' N'

Figure 10. Gas Chromatogram of *D. variabilis* Sample Dv3'. Chromatography was performed on a Tracor 560 Gas Chromatograph with a DB-1 column at a temperature program from 75 to 200° C. at 3° C. per minute.

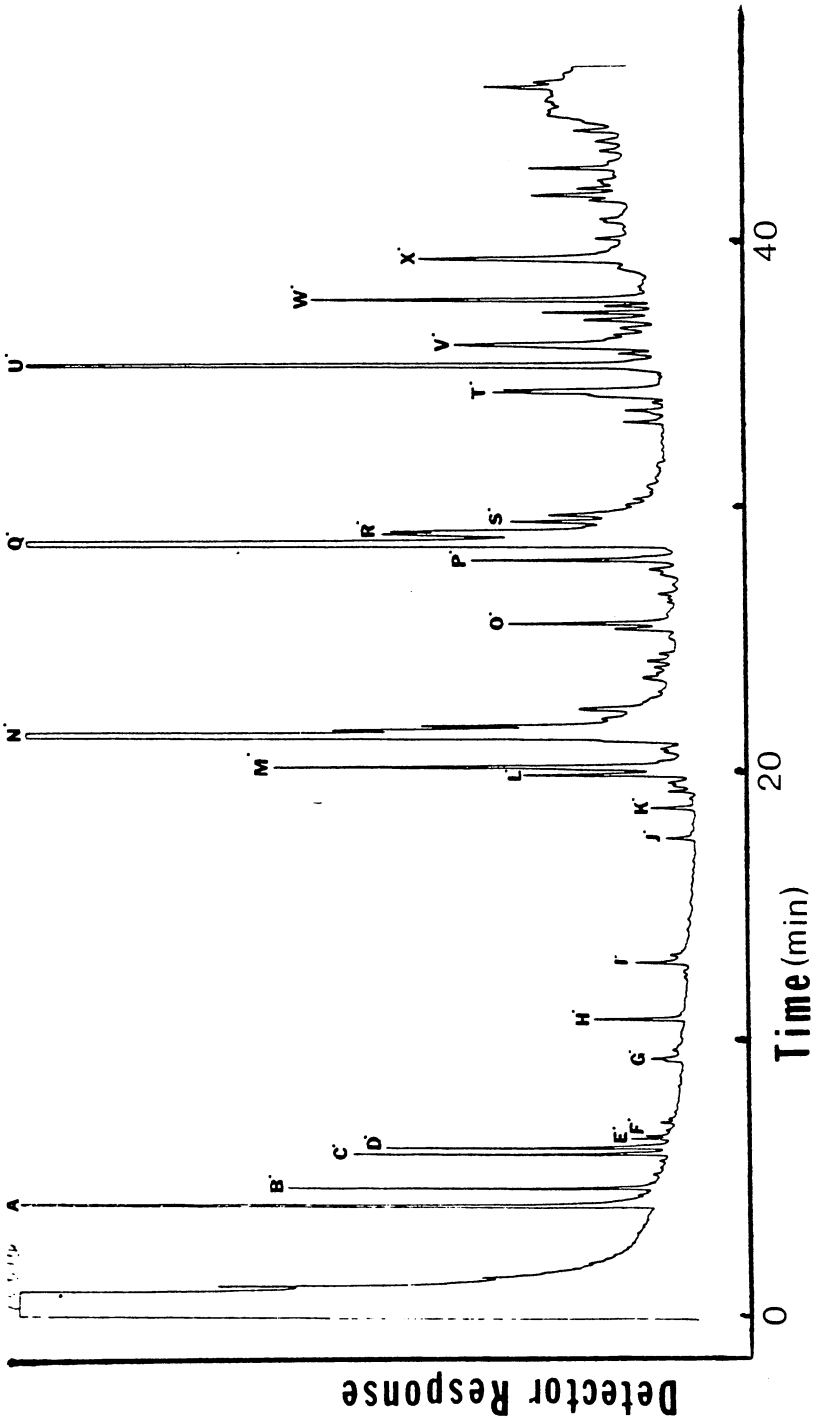

Detector Response
Time (min)
0
20
40
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X

Figure 11. Gas Chromatogram of *D. variabilis* Sample Dv3". Chromatography was performed on a Tracor Gas Chromatograph with a DB-1 column at a temperature program from 75 to 200° C. at 3° per minute.

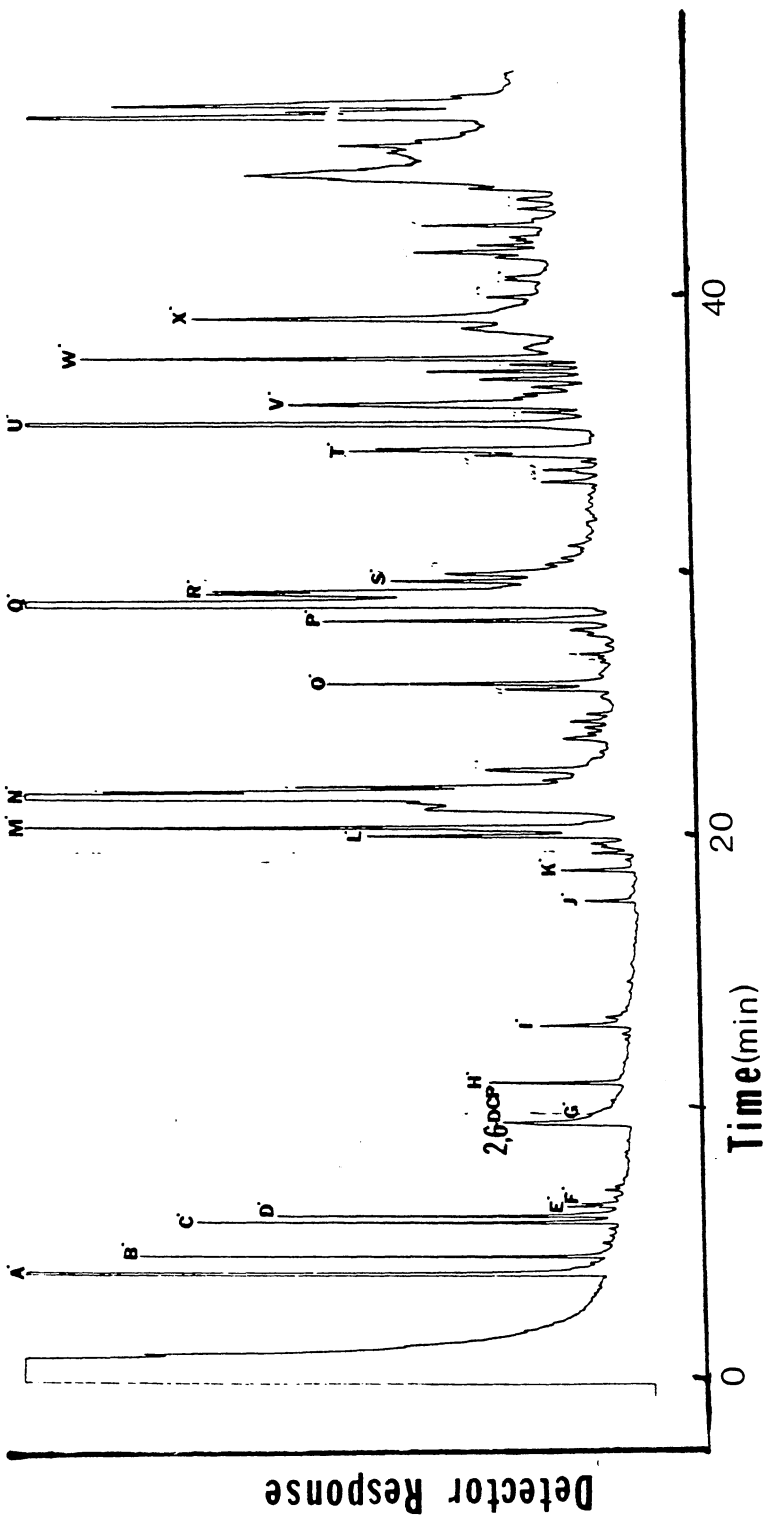

Detector Response
Time(min)
0
20
40
A
B
C
D
E
F
2,6DCP
H
G
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X

TABLE XI

PEAK CORRELATION BETWEEN *I. SCAPULARIS* AND *D. VARIABILIS* EXTRACTS USING THEIR RETENTION TIMES

Is Peak	T_R* (Min.)	Dv Peak	T_R* (Min.)	Is Tent. Ident.	Dv Tent. Ident.
A	4.12 ± 0.09	A'	4.17 ± 0.06		
B	4.80 ± 0.19	B'	4.87 ± 0.06		Salicylaldehyde
C	6.10 ± 0.19	C'	6.13 ± 0.08	m-Cresol	p-Cresol
D	6.38 ± 0.12	D'	6.38 ± 0.08		
E	9.88 ± 0.15	G'	9.85 ± 0.28	M-salicylate	2,6-DBP
F	11.37 ± 0.14	H'	11.32 ± 0.08		
G	13.55 ± 0.09	I'	13.47 ± 0.11		
I	18.35 ± 0.12	J'	18.18 ± 0.14		
J	19.70 ± 0.11	K'	19.67 ± 0.20		
K	20.49 ± 0.08	L'	20.68 ± 0.26		
L	21.09 ± 0.21	M'	21.12 ± 0.41		
M	22.30 ± 0.42	N'	22.18 ± 0.18	4-M-2,6-D-t-BP	4-M-2,6-D-t-BP
Q	29.20 ± 0.14	Q'	29.35 ± 0.09		
R	29.82 ± 0.18	R'	29.68 ± 0.15		
			29.80 ± 0.19		
S	30.42 ± 0.19	S'	30.15 ± 0.19		
			30.40 ± 0.19		
V	36.48 ± 0.12	U'	36.13 ± 0.15		

* Retention times are from Tables IX and X.

reported with the relative intensity. Spectral data from the substituted phenol references presented in table 14 are from the above reference. Some of the reference compound data were gained from GC/MS analysis.

Figure 12 shows the gas chromatogram of *I. scapularis* sample Is3 and Table XII shows the mass spectral data from the numbered peaks in Figure 12. The mass spectrums can be found in Appendix D. The mass spectral data of the substituted phenol references appears in Tables XIV and XV and the mass spectrums of the dichlorophenol isomers are in Appendix E. From these tables it is apparent that only one of the major peaks of the chromatogram corresponds to any of these compounds. Peak 2 has the same mass spectrum of 4-methyl-2,6-di-tert-butylphenol and a comparable retention time as well. Retention time of peak 2 is 14.10 while that of 4-methyl-2,6-di-tert-butylphenol is 14.08.

Figure 13 shows the gas chromatogram of *D. variabilis* sample Dv3 and Table XIII shows the mass spectral data of the numbered peaks. Comparison with data in Tables XIV and XV shows that only peak 2 corresponds to 4-methyl-2,6-di-tert-butylphenol. The retention time of peak 2 is 13.54 and that of 4-methyl-2,6-di-tert-butylphenol is 14.10. None of the other peaks correspond to any of the substituted phenol references.

A comparison of the mass spectral data from *I. scapularis* and *D. variabilis* samples shows that *I. scapularis* peaks 1, 2, 3, 4, 5, and 7 correspond to *D. variabilis* peaks 1, 2, 3, 4, 5, and 6, respectively, but no chemical identification of the peaks is available except for peak 2 of both samples.

Many of the mass spectra from the tick samples are related since the masses 29, 41, 43, 57, and 69 occur commonly in most of them. Many of the mass spectra show a pattern of mass peaks separated by 14 mass units characteristic of hydrocarbons (CH_2 = 14 AMU). The three most common mass peaks in substituted phenols, 39, 63, and 65, occur in relatively few of the spectra from the tick samples. The mass of tert-butyl (57AMU) occurs quite often in tick samples of both species.

TABLE XII

MASS SPECTRAL DATA FROM *I. SCAPULARIS* SAMPLE IS3[a]
(SEE FIGURE XII FOR CHROMATOGRAM)

Peak	T_R	Mol. Ion	Mass Spectral Data (M/e)								
1	13.31	205	57	43	71	85	29	99	113	154	127
		RI	100	77	46	34	23	14	8	7	3
2	14.10	220	205	57	41	220	29	145	177	105	91
		RI	100	59	59	38	34	31	25	23	21
3	15.49	127	57	43	71	29	85	97	113		
		RI	100	96	44	40	24	7	5		
4	17.08	168	55	43	69	83	29	97	111	127	140
		RI	100	73	67	61	50	50	28	16	9
5	20.02	238	57	43	69	83	111	97	29	125	82
		RI	100	84	63	38	36	30	21	13	8
6	21.06	205	43	57	117	29	69	83	97	108	205
		RI	100	65	45	41	34	28	21	6	4
7	25.32	264	41	55	69	29	83	97	111	264	
		RI	100	96	55	40	37	28	13	4	
8	27.33	211	57	41	70	29	83	112	100	211	
		RI	100	88	70	46	29	24	20	5	
9	33.59	259	129	57	41	70	29	112	83	147	101
		RI	100	90	65	50	34	33	30	21	13

[a]Kratos MS25Q Mass Spectrometer with Carlo Erba Gas Chromatograph

Figure 12. Gas Chromatogram of *I. scapularis* Sample Is3. Chromatography was performed on a Carlo Erba GC with a Kratos MS25Q mass spectrometer as a detector. Temperature program was from 75-250° C. at 8° C. per minute on a DB1 column.

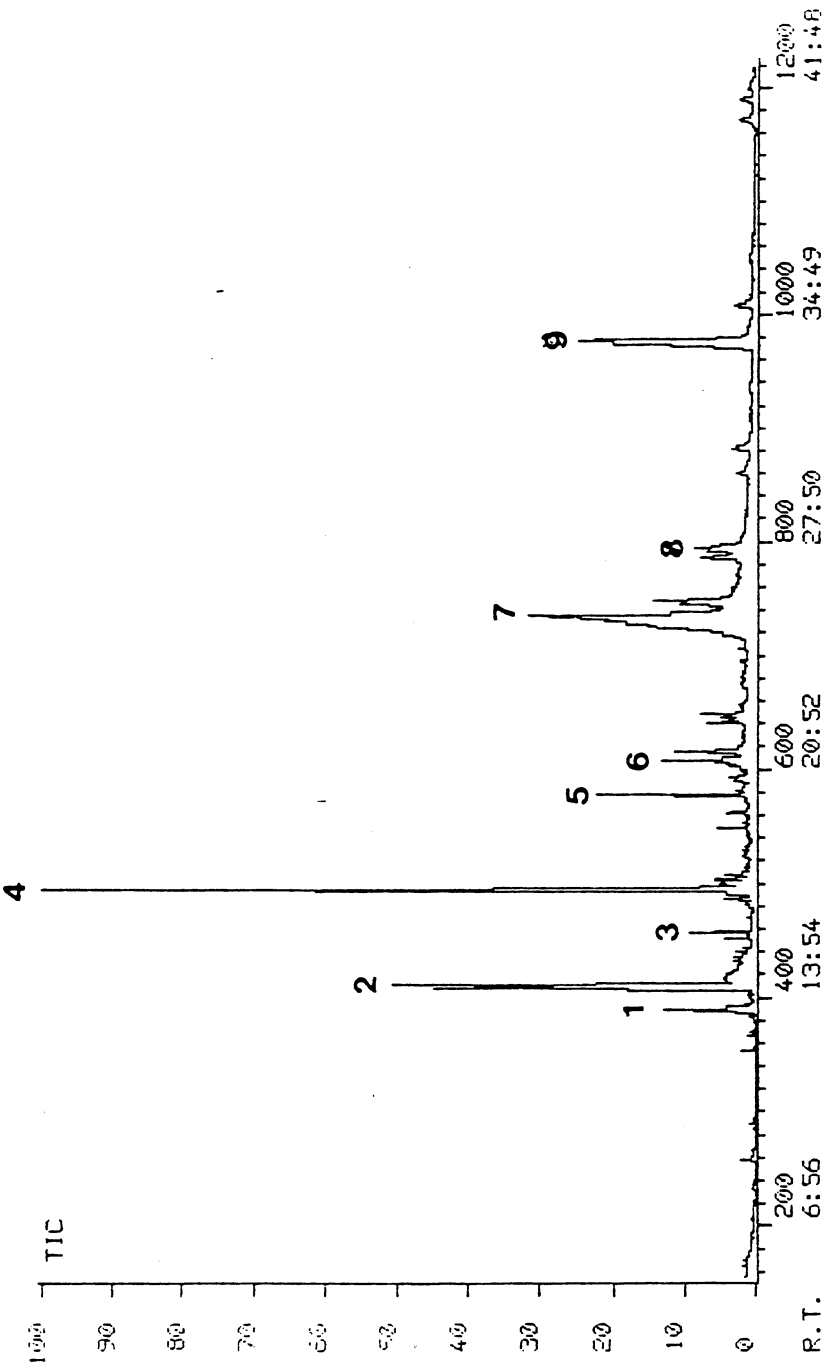

TIC
R.T.
6:56
13:54
20:52
27:50
34:49
41:48
1
2
3
4
5
6
7
8
9

TABLE XIII

MASS SPECTRAL DATA OF PEAKS FROM *D. VARIABILIS* SAMPLE DV3[a]
(SEE FIGURE XIII FOR CHROMATOGRAM)

Peak	T_R	Mol. Ion	Mass Spectral Data (M/e)									
1	13.12	154	57	43	71	29	99	113	154	127		
		RI	100	92	54	24	15	8	7	4		
2	13.54	220	205	57	41	220	145	177	29	105	69	91
		RI	100	46	47	45	30	27	21	20	19	19
3	15.30	197	57	43	71	85	29	99	113	127		
		RI	100	84	54	32	29	10	6	4		
4	16.47	168	55	43	69	83	29	97	111	127	140	168
		RI	100	75	68	63	59	50	28	17	10	5
5	20.44	205	43	57	29	117	69	83	97			
		RI	100	61	38	39	33	27	19			
6	24.55	264	41	55	69	29	83	97	111			
		RI	100	81	53	43	36	26	13			
7	26.59	247	57	43	69	85	111	29	125	168		
		RI	100	88	53	33	30	20	10	7		

[a]Kratos MS25Q Mass Spectrometer with Carlo Erba Gas Chromatograph

Figure 13. Chromatogram of *D. variabilis* Sample Dv3. Chromatography was performed on a Carlo Erba GC with a DB1 column. The detector was a Kratos MS25Q mass spectrometer. A temperature program from75-250° C. at 8° C. per minute was used.

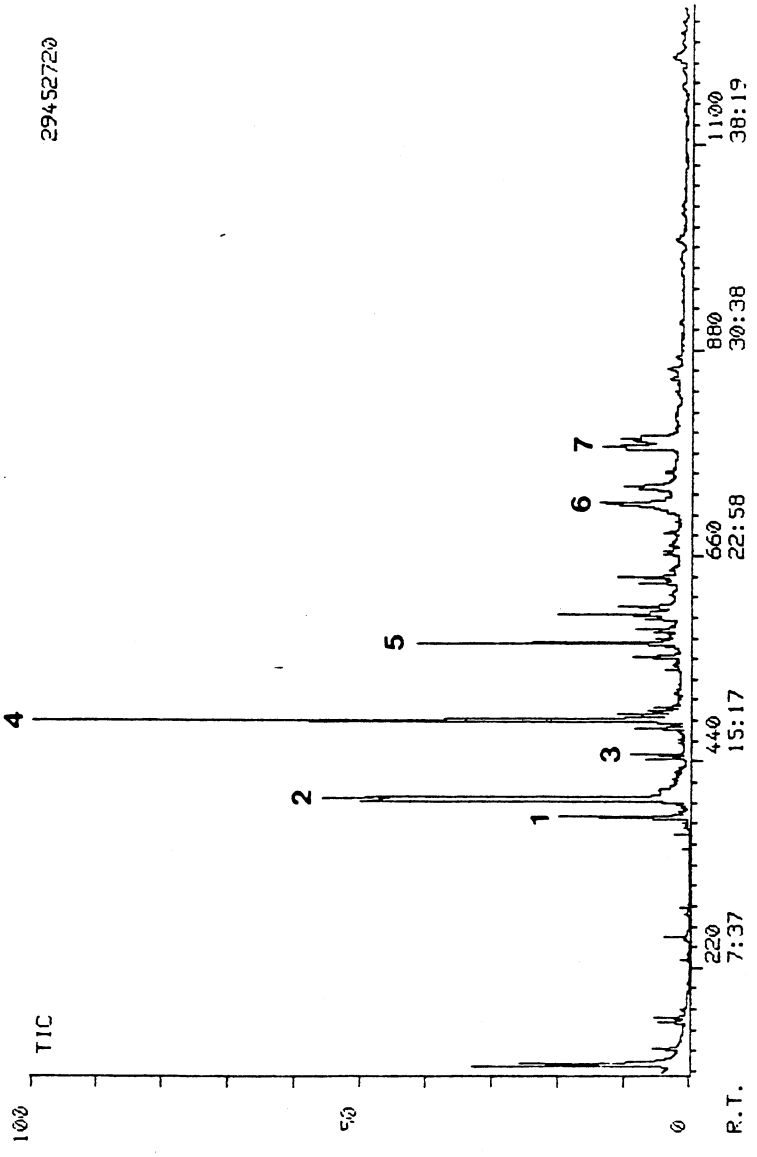

TIC
2945720
100
50
0
R.T.
220
7:37
440
15:17
660
22:58
880
30:38
1100
38:19
1
2
3
4
5
6
7

TABLE XIV

MASS SPECTRAL DATA FROM REFERENCE PHENOLS[a]

Compound	Mol. Ion	Mass Spectral Data							
Phenol	94	94	66	39	65	40	38	63	55
	RI	100	83	60	48	27	21	15	14
o-Cresol	108	108	107	77	29	51	39	90	50
	RI	100	75	32	32	26	26	23	16
m-Cresol	108	108	107	39	79	77	51	53	38
	RI	100	80	33	30	27	16	14	12
p-Cresol	108	107	108	77	28	79	53	91	51
	RI	100	90	18	15	14	8	8	8
o-Nitrophenol	139	139	65	64	63	53	93	81	38
	RI	100	36	22	22	13	13	11	9
p-Nitrophenol	139	65	139	109	39	28	81	93	63
	RI	100	90	70	66	46	33	30	30
M-salicylate	152	120	92	152	39	121	65	15	63
	RI	100	58	41	35	32	26	24	17

[a]Eight Peak Index of Mass Spectra (1974)

TABLE XV

MASS SPECTRAL DATA FROM DICHLOROPHENOLS[a] AND 4-METHYL-2,6-DI-TERT-BUTYLPHENOL[b]

Compound	Mol. Ion	Mass Spectral Data (M/e)										
3,4-DCP	162	160	63	164	98	62	99	73	61	100	37	126
	RI	100	91	68	47	23	23	19	19	17	19	13
2,5-DCP	162	63	162	164	98	99	62	61	73	53	37	38
	RI	100	96	79	44	35	30	23	23	20	18	16
2,6-DCP	162	162	63	164	98	126	62	73	61	99	166	100
	RI	100	99	74	28	25	22	21	18	15	15	14
3,4-DCP	162	162	99	164	63	73	62	101	61	98	37	50
	RI	100	63	60	46	25	25	19	19	17	14	13
3,5-DCP	162	162	164	99	63	62	73	101	98	61	127	
	RI	100	68	55	34	29	25	23	18	14	14	
4-M-2,6-DtBp	220	57	205	41	29	220	145	105	91	67	177	81
	RI	100	80	63	37	19	16	15	15	13	13	12

[a]LKB 2091 GC/MS/DS

[b]Kratos MS25Q Mass Spectrograph with Carlo Erba Gas Chromatograph

In general, the mass spectral data from the two tick samples are quite similar. Many of the spectra are of hydrocarbon components of the ticks cuticular lipid while others are quite obviously of more complex molecules.

CHAPTER V

CONCLUSIONS

1. Sheep are an adequate host for feeding adult *I. scapularis* and *D. variabilis* ticks however, they do develop immune reactions to the ticks.
2. *D. variabilis* are attracted to the compound 2,6-dichlorophenol. *I. scapularis* are not attracted to the same concentrations of 2,6-dichlorophenol that *D. variabilis* are attracted to.
3. Neither *D. variabilis* nor *I. scapularis* produce enough 2,6-dichlorophenol or any of the other substituted phenol attractants to be detected using the extraction and analysis methods described in this study.
4. Both *I. scapularis* and *D. variabilis* contain a relatively high concentration of 4–methyl-2,6-di-tert-butylphenol (BHT) when raised and fed with the materials used in this study.
5. Several other major components occuring in the phenol fraction were molecules containing hydrocarbon components and none of them appeared to be phenolic compounds.

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APPENDICES

APPENDIX A

DERMACENTOR VARIABILIS FEEDING DATA

APPENDIX A

DERMACENTOR VARIABILIS FEEDING DATA

No.	Sheep	Cell	No. Ticks On	No. Ticks Off	No. Ticks Not Att.	Days	Sample No.	No. Ticks In Sample	Date On	Date Off
1	84	1	25	--	--	16	1		9/17	10/3
2	"	3	50	--	--	"		30	"	"
3	61	1	50	41	--	7	1	41	10/3	10/10
4	"	3	"	34	--	12	"	34	10/3	10/15
5	"	4	"	31	--	"	"	31	"	"
6	"	6	"	47	--	7	"	47	10/3	10/10
7	59	5	35	20	--	7	1	20	10/17	10/24
8	"	6	"	22	--	"	"	22	"	"
9	"	7	"	22	--	"	"	23	"	"
10	"	8	"	23	--	"	"	23	"	"
11	13	1	40	29	8	5	4	37	2/9	2/14
12	"	2	"	23	15	"	"	38	"	"
13	"	3	"	16	22	"	"	38	"	"
14	"	4	"	26	13	"	"	39	"	"
15	"	5	"	21	16	"	"	37	"	"
16	"	6	"	28	13	"	"	41	"	"
17	"	7	"	16	21	"	"	37	"	"
18	"	8	"	22	12	"	"	34	"	"
19	19	1	40	37	2	5	4	39	2/16	2/21
20	"	2	"	29	11	"	"	40	"	"
21	"	3	"	35	4	"	"	39	"	"
22	"	4	"	30	10	"	"	40	"	"
23	"	5	"	33	6	"	"	39	"	"
24	"	6	"	37	3	"	"	40	"	"
25	"	7	"	29	11	"	"	40	"	"
26	"	8	"	38	7	"	"	45	"	"
27	18	1	40	38	2	5	4	40	2/23	2/28
28	"	2	"	32	6	"	"	38	"	"
29	"	3	"	38	0	"	"	38	"	"
30	"	4	"	32	5	"	"	37	"	"
31	"	5	"	39	0	"	"	39	"	"
32	"	6	"	38	0	"	"	38	"	"
33	"	7	"	38	1	"	"	39	"	"
34	"	8	"	36	2	"	"	38	"	"
35	18	1	40	38	0	5	6	38	3/30	4/4
36	"	2	"	36	0	"	"	36	"	"
37	"	3	"	40	0	"	"	40	"	"
38	"	5	"	36	3	"	"	39	"	"
39	"	6	"	35	2	"	"	37	"	"
40	"	7	"	36	2	"	"	38	"	"
41	13	1	40	29	6	5	6	35	3/30	4/4
42	"	3	"	27	10	"	"	37	"	"
43	"	4	"	21	19	"	"	40	"	"
44	"	5	"	33	7	"	"	40	"	"

No.	Sheep	Cell	No. Ticks On	No. Ticks Off	No. Ticks Not Att.	Days	Sample No.	No. Ticks In Sample	Date On	Date Off
45	"	7	"	32	9	"	"	41	"	"
46	"	8	"	26	10	"	"	36	"	"
Total			1855	1400			278	1658		

APPENDIX B

IXODES SCAPULARIS FEEDING DATA

APPENDIX B

IXODES SCAPULARIS FEEDING DATA

No.	Sheep	Cell	No. Ticks On	No. Ticks Off	Days	No. Preg.	Sample No.	No. in Sample	Date On	Date Off
1	84	2	25	-	16	-	2		9/17	10/3
2	"	7	50	-	"	-	"	42	"	"
3	61	5	"	41	7	7	2	34	10/3	10/10
4	"	7	47	36	9	3	"	33	10/8	10/15
5	"	8	50	30	"	4	"	26	10/6	10/15
6	59	1	40	38	7	1	2	37	10/17	10/24
7	"	2	"	37	"	4	"	33	"	"
8	"	3	"	37	"	3	"	34	"	"
9	"	4	"	39	"	3	"	36	"	"
10	13	6	40	27	6	1	3	26	11/1	11/7
11	"	7	"	29	"	1	"	38	"	
12	19	1	40	41	6	4	3	37	"	"
13	"	2	"	39	"	3	"	36	"	"
14	"	3	"	39	"	3	"	36	"	"
15	"	4	"	40	"	1	"	39	"	"
16	"	5	"	38	"	2	"	36	"	"
17	"	6	"	41	"	4	"	37	"	"
18	"	7	"	39	"	3	"	36	"	"
19	"	8	"	40	"	2	"	38	"	"
20	18	3	40	36	6	-	3	36	11/20	11/26
21	"	4	"	32	"	-	"	32	"	"
22	"	5	"	35	"	-	"	35	"	"
23	"	6	"	40	"	-	"	40	"	"
24	"	7	"	40	"	-	"	40	"	"
25	"	8	"	40	"	-	"	40	"	"
26	20	1	40	37	6	-	3	36	11/20	11/26
27	"	2	"	36	"	-	"	36	"	"
28	"	3	"	37	"	-	"	37	"	"
29	"	4	"	38	"	-	"	38	"	"
30	"	5	"	37	"	-	"	37	"	"
31	"	6	"	37	"	-	"	37	"	"
32	"	7	"	35	"	-	"	35	"	"
33	"	8	"	39	"	-	"	39	"	"
34	84	3	40	27	8	1	3	26	12/4	12/12
35	"	4	"	27	"	1	"	26	"	"
36	"	5	"	29	"	1	"	28	"	"
37	"	6	"	28	"	0	"	28	"	"
38	"	7	"	29	"	2	"	27	"	"
39	"	8	"	32	"	4	"	28	"	"
40	61	3	40	33	8	0	3	33	12/4	12/12
41	"	4	"	16	"	1	2	15	"	"
42	"	5	"	33	"	6	"	27	"	"
43	"	6	"	36	"	"	"	30	"	"
44	"	7	"	29	"	9	"	20	"	"

No.	Sheep	Cell	No. Ticks On	No. Ticks Off	Days	No. Preg.	Sample No.	No. in Sample	Date On	Date Off
45	"	8	"	37	"	3	"	34	"	"
46	15	1	40	41	7	2	2	39	1/16	1/23
47	"	2	"	36	"	0	"	36	"	"
48	"	3	"	40	"	2	"	38	"	"
49	"	4	"	48	"	3	"	45	"	"
50	"	5	"	38	"	"	"	35	"	"
51	"	6	"	36	"	2	"	34	"	"
52	"	7	"	33	"	"	"	31	"	"
53	"	8	"	40	"	1	"	39	"	"
54	16	1	40	37	5	-	4	37	1/26	1/31
55	"	2	"	39	"	-	"	39	"	"
56	"	3	"	37	"	-	"	37	"	"
57	"	4	"	34	"	-	"	34	"	"
58	"	5	"	39	"	-	"	39	"	"
59	"	6	"	33	"	-	"	33	"	"
60	"	7	"	33	"	-	"	33	"	"
61	"	8	"	36	"	-	"	36	"	"
62	17	1	40	39	5	-	4	39	1/20	1/31
63	"	2	"	37	"	-	"	37	"	"
64	"	3	"	39	"	-	"	39	"	"
65	"	4	"	37	"	-	"	37	"	"
66	"	5	"	44	"	-	"	44	"	"
67	"	6	"	55	"	-	"	55	"	"
68	"	7	"	37	"	-	"	37	"	"
69	19	1	40	31	5	-	5	31	3/30	4/4
70	"	2	"	28	"	-	"	28	"	"
71	"	3	"	29	"	-	"	29	"	"
72	"	4	"	41	"	-	"	41	"	"
73	"	5	"	31	"	-	"	31	"	"
74	"	6	"	49	"	-	"	49	"	"
75	"	7	"	26	"	-	"	26	"	"
76	"	8	"	34	"	-	"	34	"	"
77	"	9	"	39	"	-	"	39	"	"
78	18	4	40	17	5	-	5	17	3/30	4/4
79	"	8	"	56	"	-	"	56	"	"
80	19	1	60	57	6	-	5	57	4/7	4/13
81	"	2	"	61	"	-	"	61	"	"
82	"	3	"	44	"	-	"	44	"	"
83	"	4	"	20	"	-	"	20	"	"
84	"	5	"	38	"	-	"	38	"	"
85	"	6	"	43	"	-	"	43	"	"
86	"	7	"	30	"	-	"	30	"	"
87	"	8	"	39	"	-	"	39	"	"
88	"	9	"	22	"	-	"	22	"	"
89	"	10	"	44	"	-	"	44	"	"
90	51	1	60	47	5	-	6	47	4/14	4/18
91	"	2	"	48	"	-	"	48	"	"
92	"	3	"	49	"	-	"	49	"	"

No.	Sheep	Cell	No. Ticks On	No. Ticks Off	Days	No. Preg.	Sample No.	No. in Sample	Date On	Date Off
93	"	4	"	53	"	-	"	53	"	"
94	"	5	"	48	"	-	"	48	"	"
95	"	6	"	48	"	-	"	48	"	"
96	"	7	"	44	"	-	"	44	"	"
97	"	8	"	57	"	-	"	57	"	"
98	"	9	"	44	"	-	"	44	"	"
99	"	10	"	53	"	-	"	53	"	"
100	14	1	60	37	6	-	5	37	4/13	4/18
101	"	2	"	55	"	-	"	55	"	"
102	"	3	"	54	"	-	"	54	"	"
103	"	4	"	46	"	-	"	46	"	"
104	"	5	"	45	"	-	6	45	"	"
105	"	6	"	52	"	-	"	52	"	"
106	"	7	"	67	"	-	"	67	"	"
107	"	8	"	34	"	-	"	34	"	"
108	"	9	"	54	"	-	"	54	"	"
109	"	10	"	44	"	-	"	44	"	"
110	69	1	60	34	6	0	6	34	4/18	4/24
111	"	2	"	25	"	0	"	25	"	"
112	"	3	"	42	"	0	"	42	"	"
113	"	4	"	45	"	5	7	40	"	"
114	"	5	"	34	"	0	"	34	"	"
115	"	6	"	52	"	4	"	48	"	"
116	"	7	"	56	"	6	"	50	"	"
117	"	8	"	44	"	2	"	42	"	"
118	"	9	"	58	"	0	"	58	"	"
119	"	10	"	53	"	1	"	52	"	"
120	20	1	60	52	6	-	8	52	4/23	4/29
121	"	2	"	49	"	-	"	49	"	"
122	"	3	"	50	"	-	"	50	"	"
123	"	4	"	50	"	-	"	50	"	"
124	"	5	"	42	"	-	"	42	"	"
125	"	6	"	25	"	-	7	25	"	"
126	"	7	"	54	"	-	"	54	"	"
127	"	8	"	44	"	-	"	44	"	"
128	"	9	"	50	"	-	"	50	"	"
129	"	10	"	36	"	-	"	36	"	"
Total			6182	5099	1077			4982		

APPENDIX C

PREGNANCY RATE IN FEMALE *IXODES SCAPULARIS*

APPENDIX C

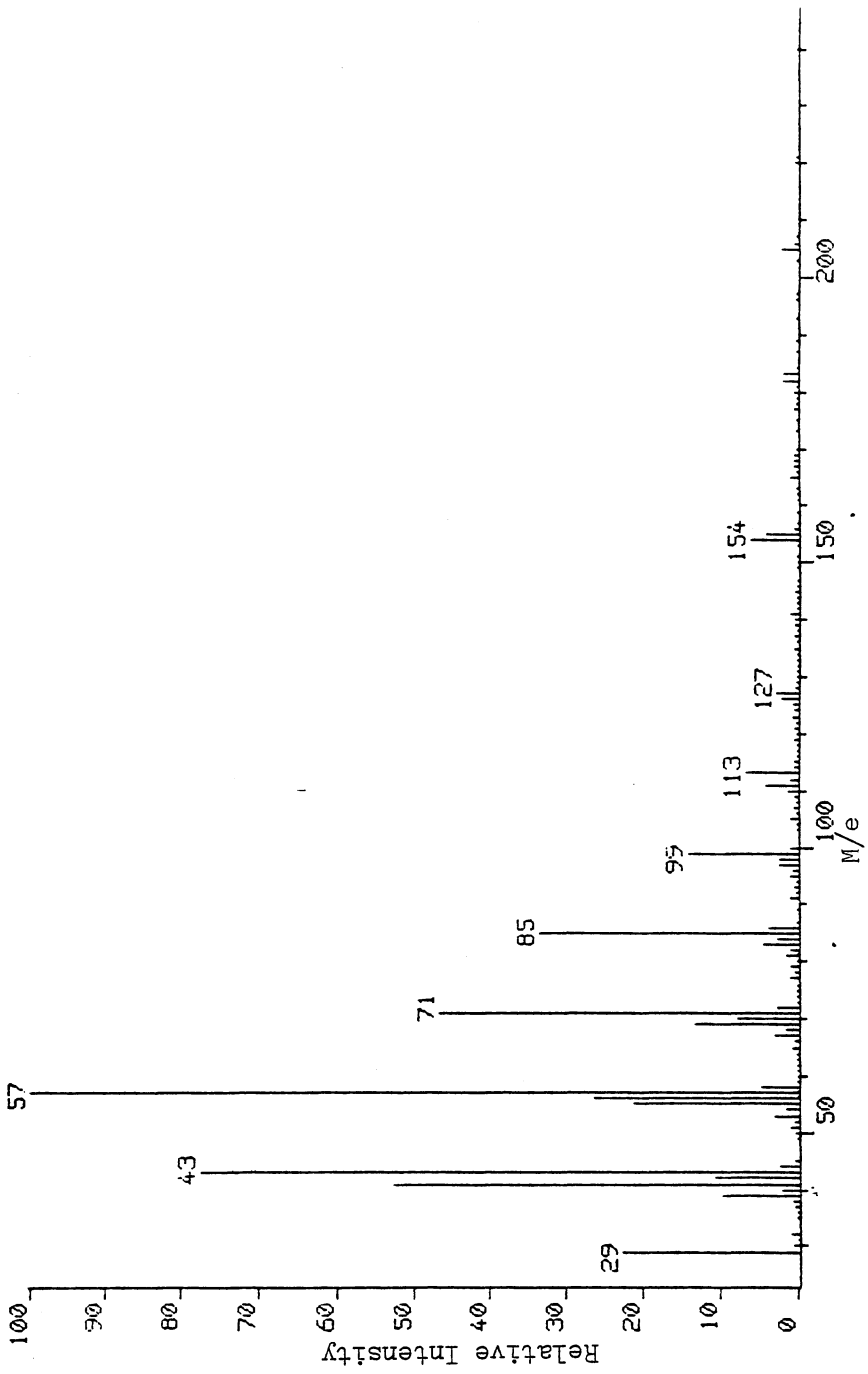
PREGNANCY RATE IN FEMALE *IXODES SCAPULARIS*

No.	Sheep	Cell No.	No. Ticks On	No. Ticks Off	Day	No. of Pregnancies	Date On	Date Off
3	61	5	50	41	7	7	10/3	10/10
4	"	7	47	36	9	3	10/6	10/15
5	"	8	50	30	"	4	10/6	10/15
6	59	1	40	38	7	1	10/17	10/24
7	"	2	"	37	"	4	"	"
8	"	3	"	37	"	3	"	"
9	"	4	"	39	"	3	"	"
10	13	6	40	27	6	1	11/1	11/7
11	"	7	"	29	"	1	"	"
12	19	1	40	41	6	4	11/1	11/7
13	"	2	"	39	"	3	"	"
14	"	3	"	39	"	3	"	"
15	"	4	"	40	"	1	"	"
16	"	5	"	38	"	2	"	"
17	"	6	"	41	"	4	"	"
18	"	7	"	39	"	3	"	"
19	"	8	"	40	"	2	"	"
34	84	3	40	27	8	1	12/4	12/12
35	"	4	"	27	"	1	"	"
36	"	5	"	29		1	"	"
37	"	6	"	28	"	0	"	"
38	"	7	"	29	"	2	"	"
39	"	8	"	32	"	4	"	"
40	61	3	40	33	8	0	12/4	12/12
41	"	4	"	16	"	1	"	"
42	"	5	"	33	"	6	"	"
43	"	6	"	36	"	6	"	"
44	"	7	"	29	"	9	"	"
45	"	8	"	37	"	3	"	"
46	15	1	40	41	7	2	1/10	1/23
47	"	2	"	36	"	0	"	"
48	"	3	"	40	"	2	"	"
49	"	4	"	48	"	3	"	"
50	"	5	"	38	"	3	"	"
51	"	6	"	36	"	2	"	"
52	"	7	"	33	"	2	"	"
53	"	8	"	40	"	1	"	"
110	69	1	60	34	6	0	4/18	4/24
111	"	2	"	25	"	0	"	"
112	"	3	"	42	"	6	"	"
113	"	4	"	45	"	5	"	"
114	"	5	"	34	"	0	"	"
115	"	6	"	52	"	4	"	"
116	"	7	"	56	"	6	"	"
117	"	8	"	44	"	2	"	"

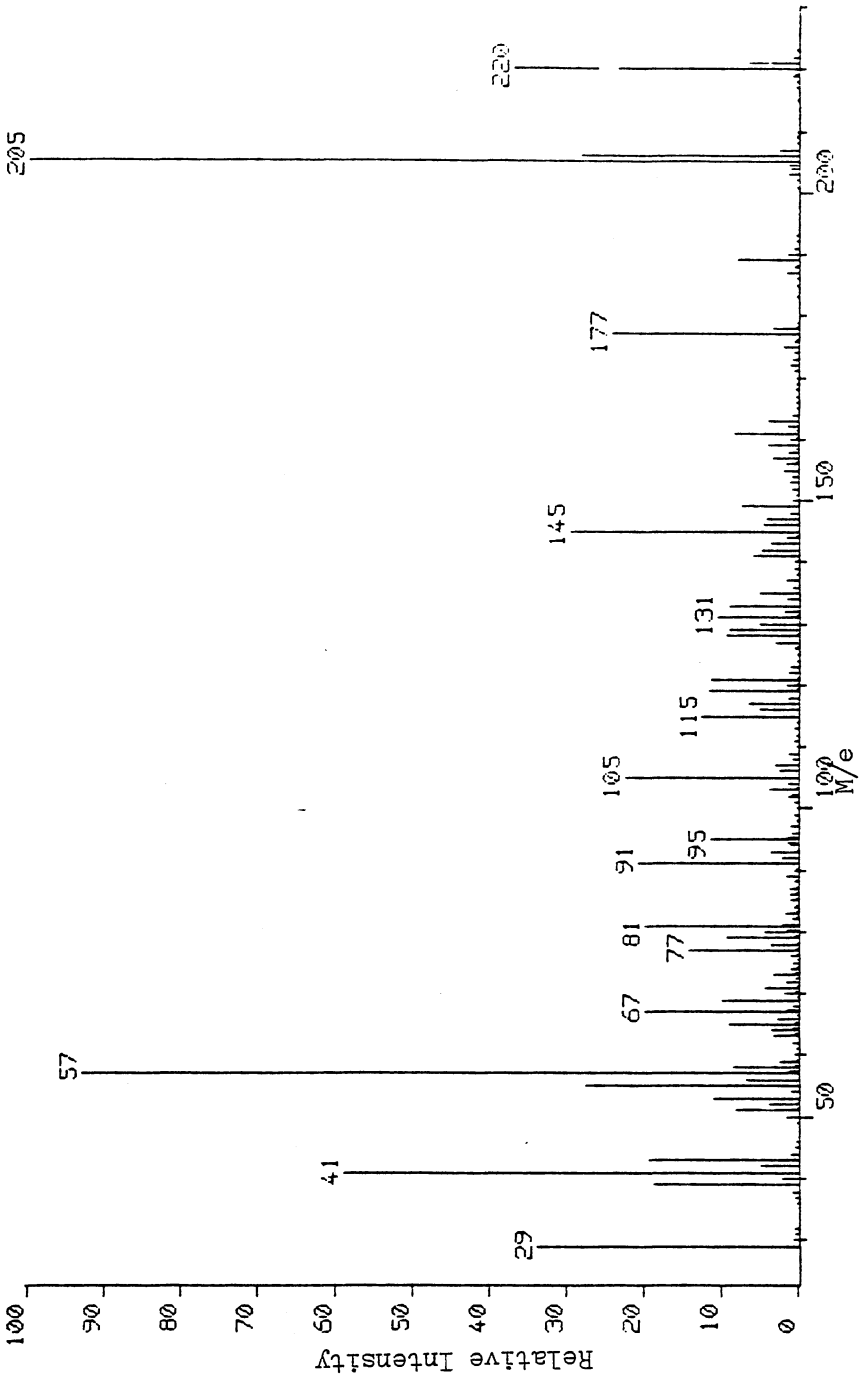
No.	Sheep	Cell No.	No. Ticks On	No. Ticks Off	Day	No. of Pregnancies	Date On	Date Off
118	"	9	"	58	"	0	"	"
119	"	10	"	53	"	1	"	"
Total:	47 Cells			1742	325	116		

APPENDIX D

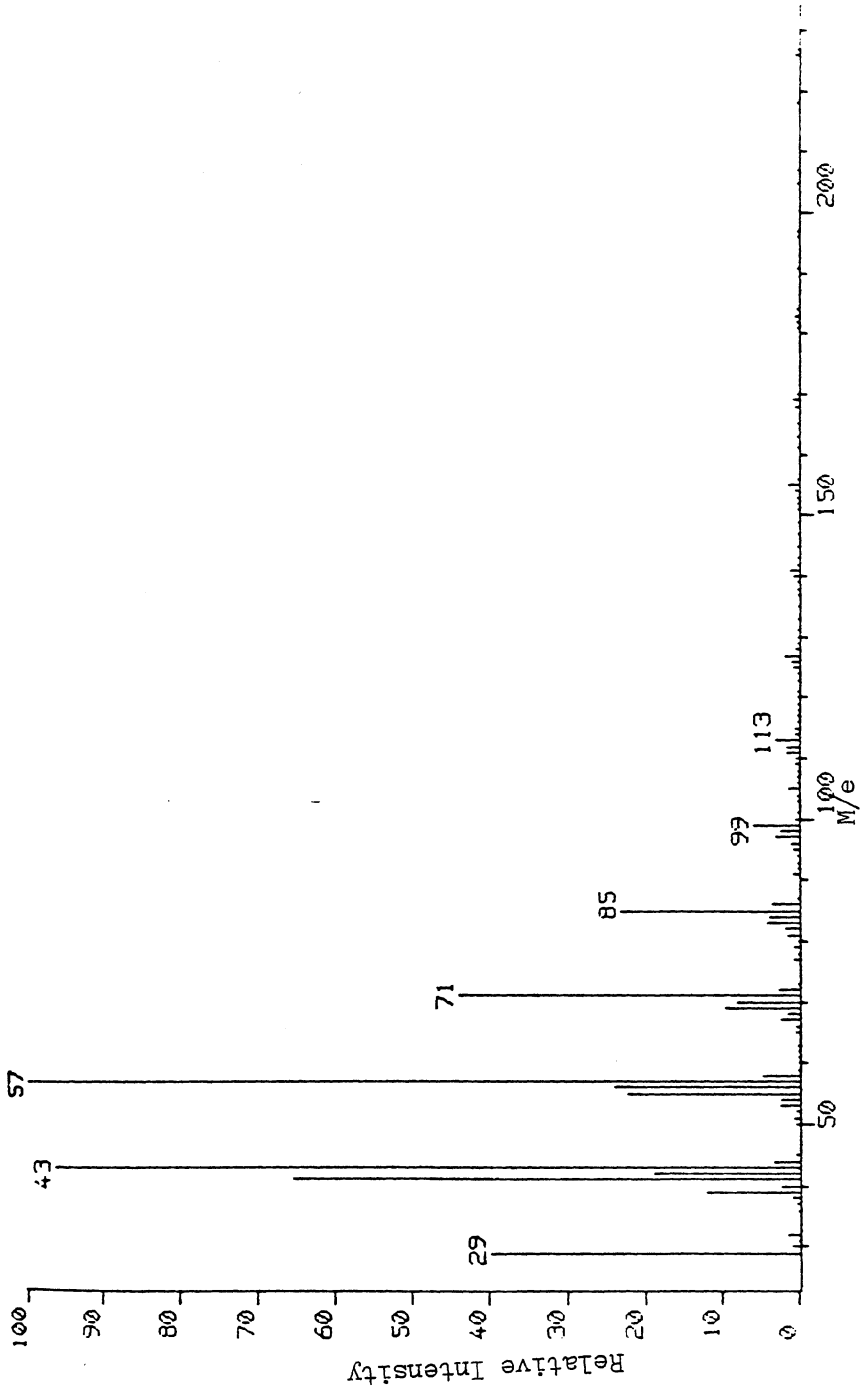
MASS SPECTRA OF EXTRACTION FRACTIONS



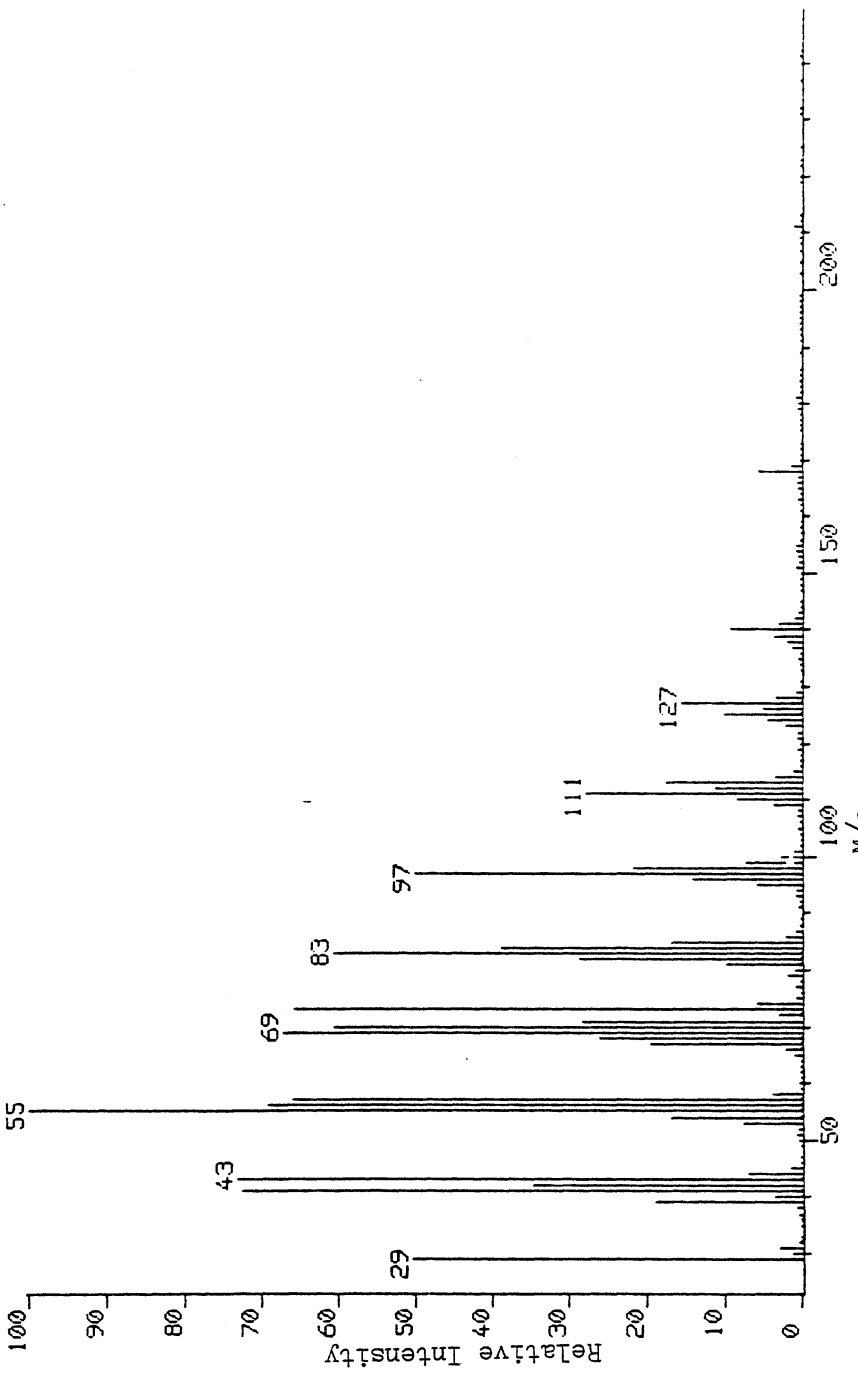
Mass Spectrum of Is3 peak 1



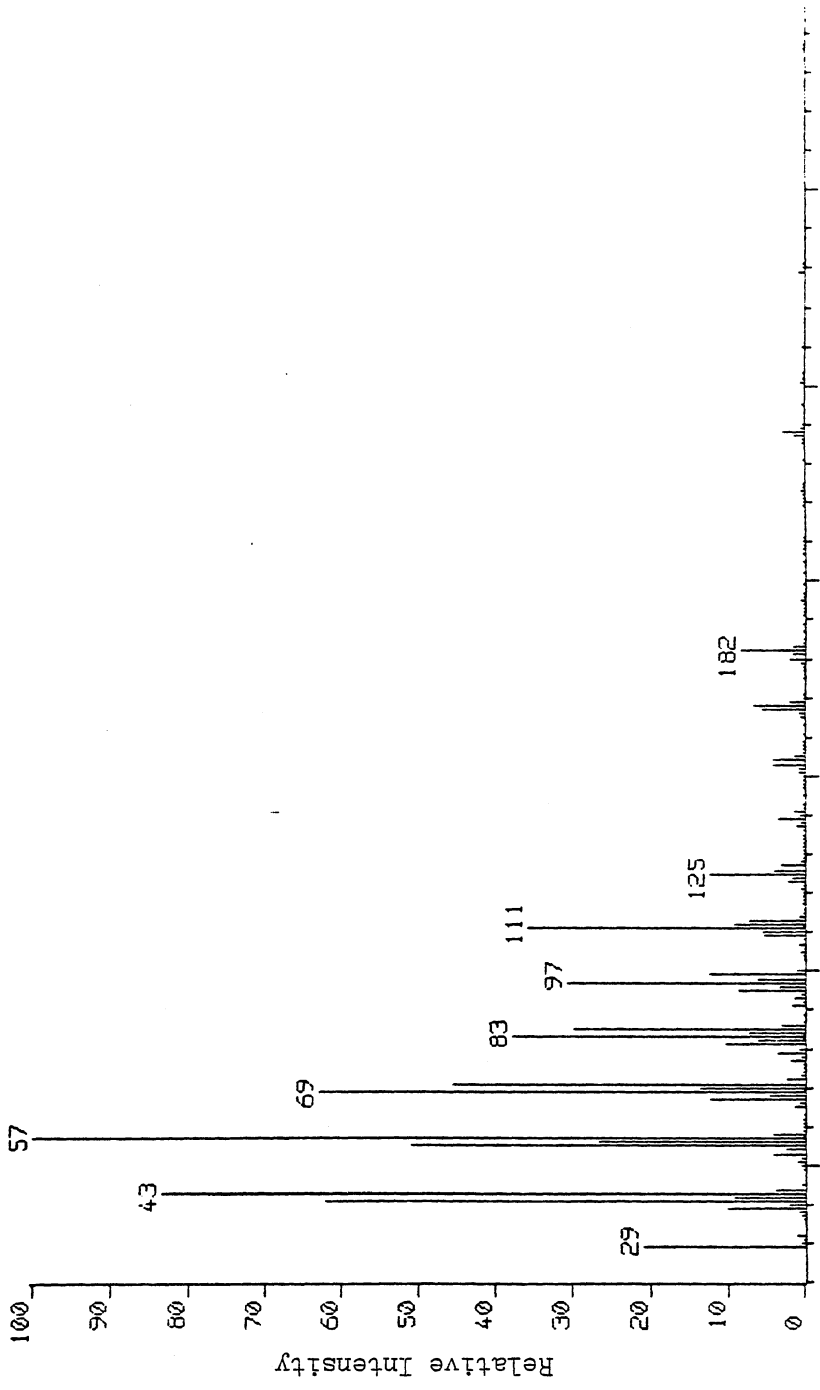
Mass Spectrum of Is3 peak 2



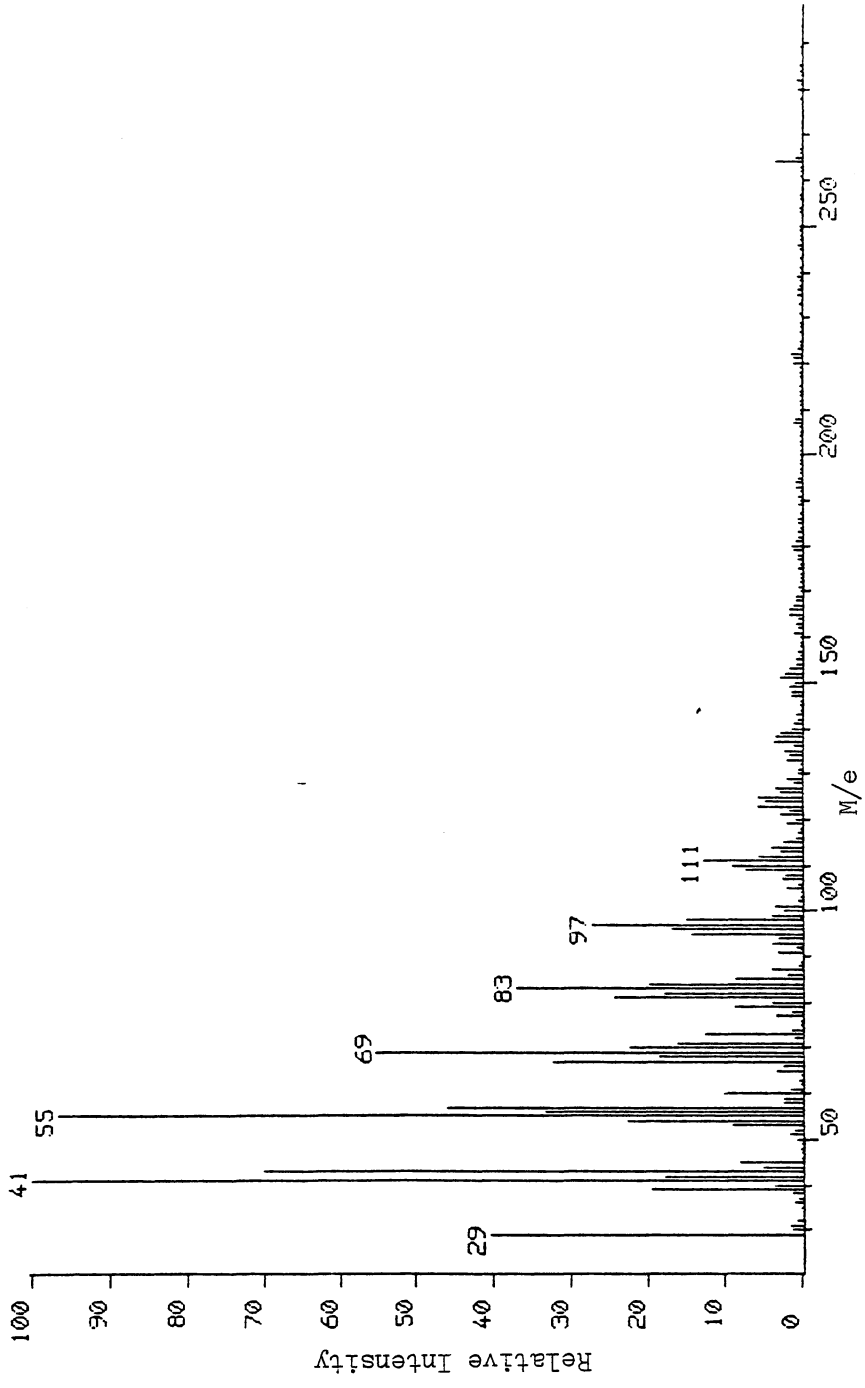
Mass Spectrum of Is3 peak 3



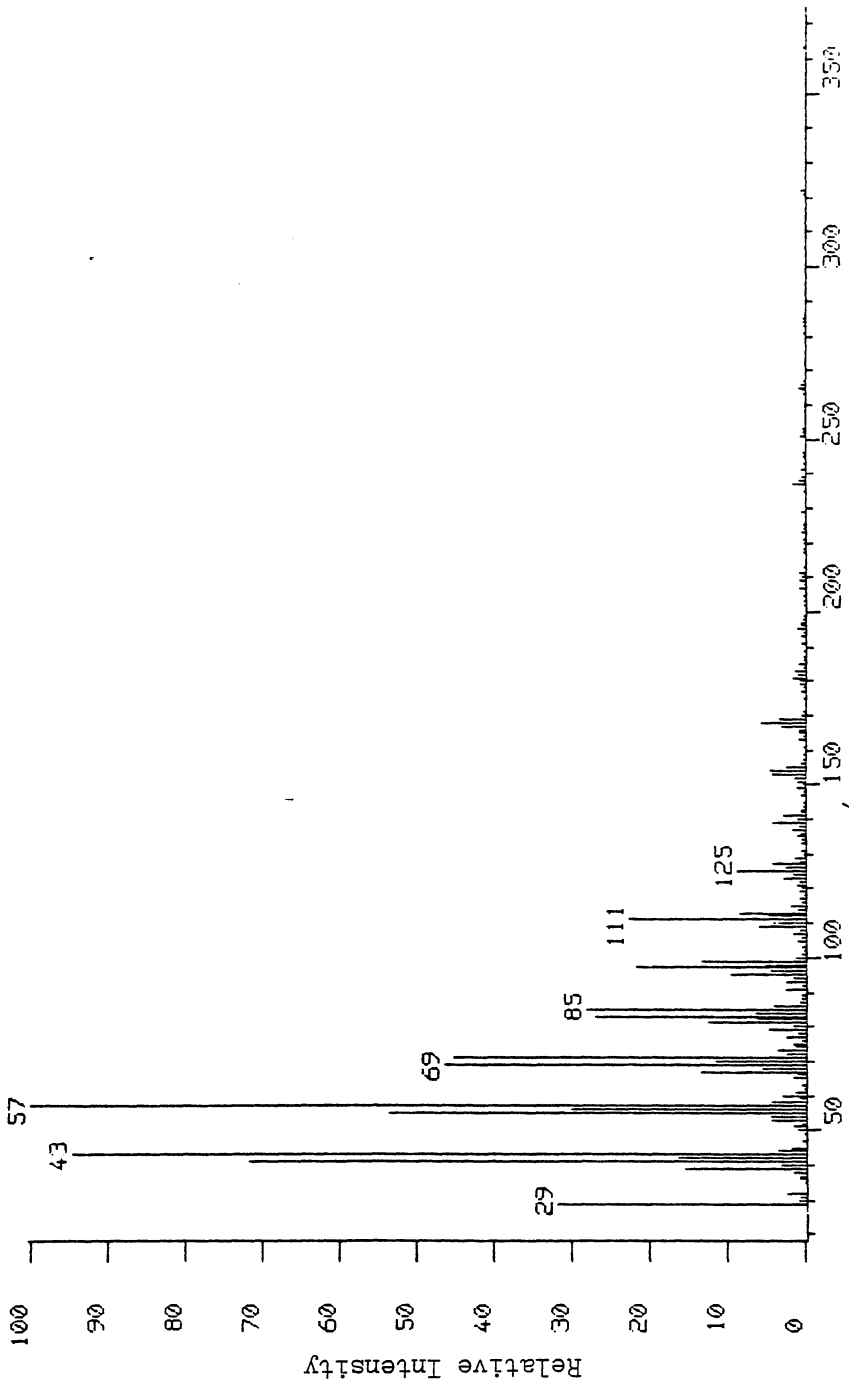
Mass Spectrum of Is3 Peak 4



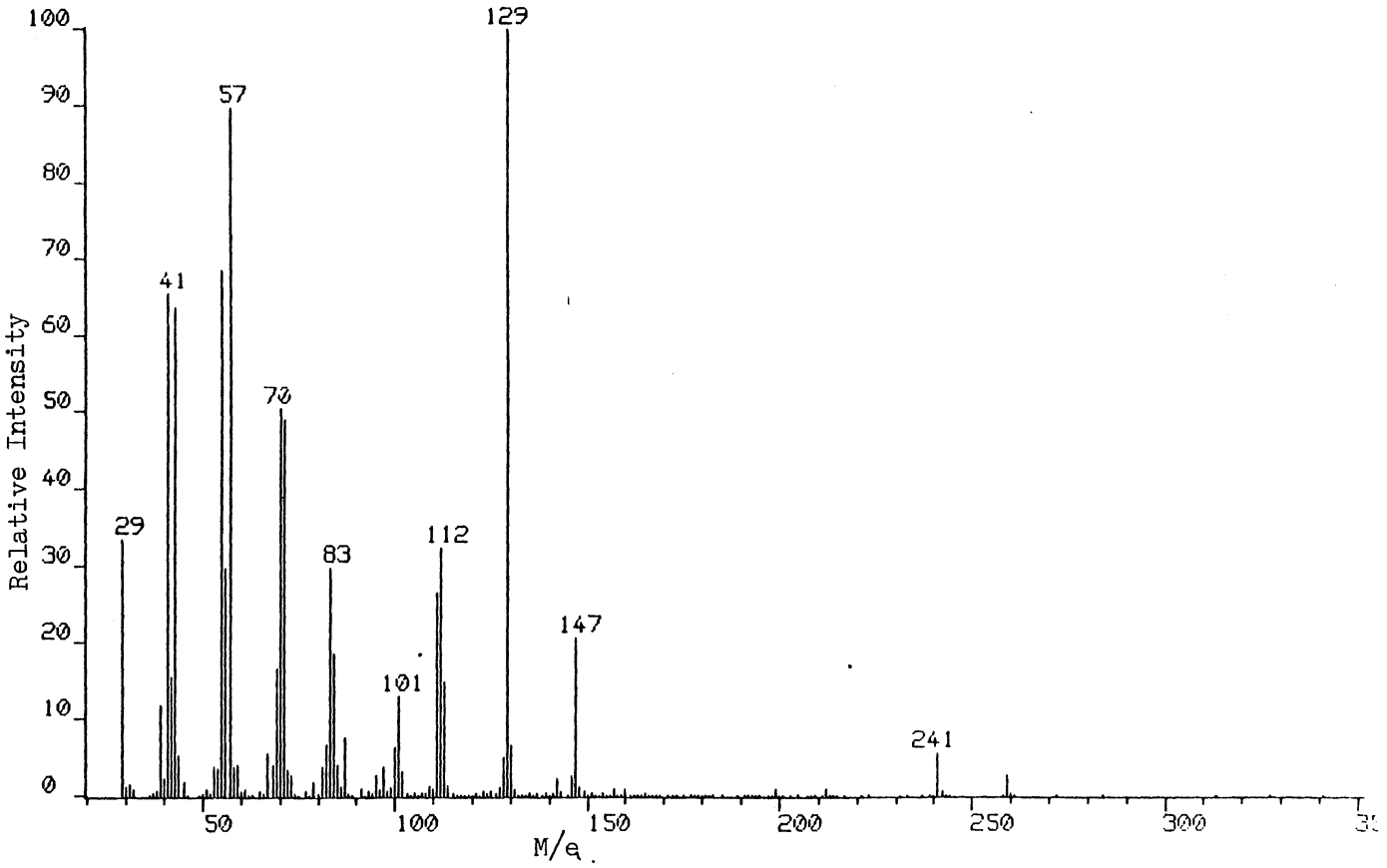
Mass Spectrum of Is3 Peak 5



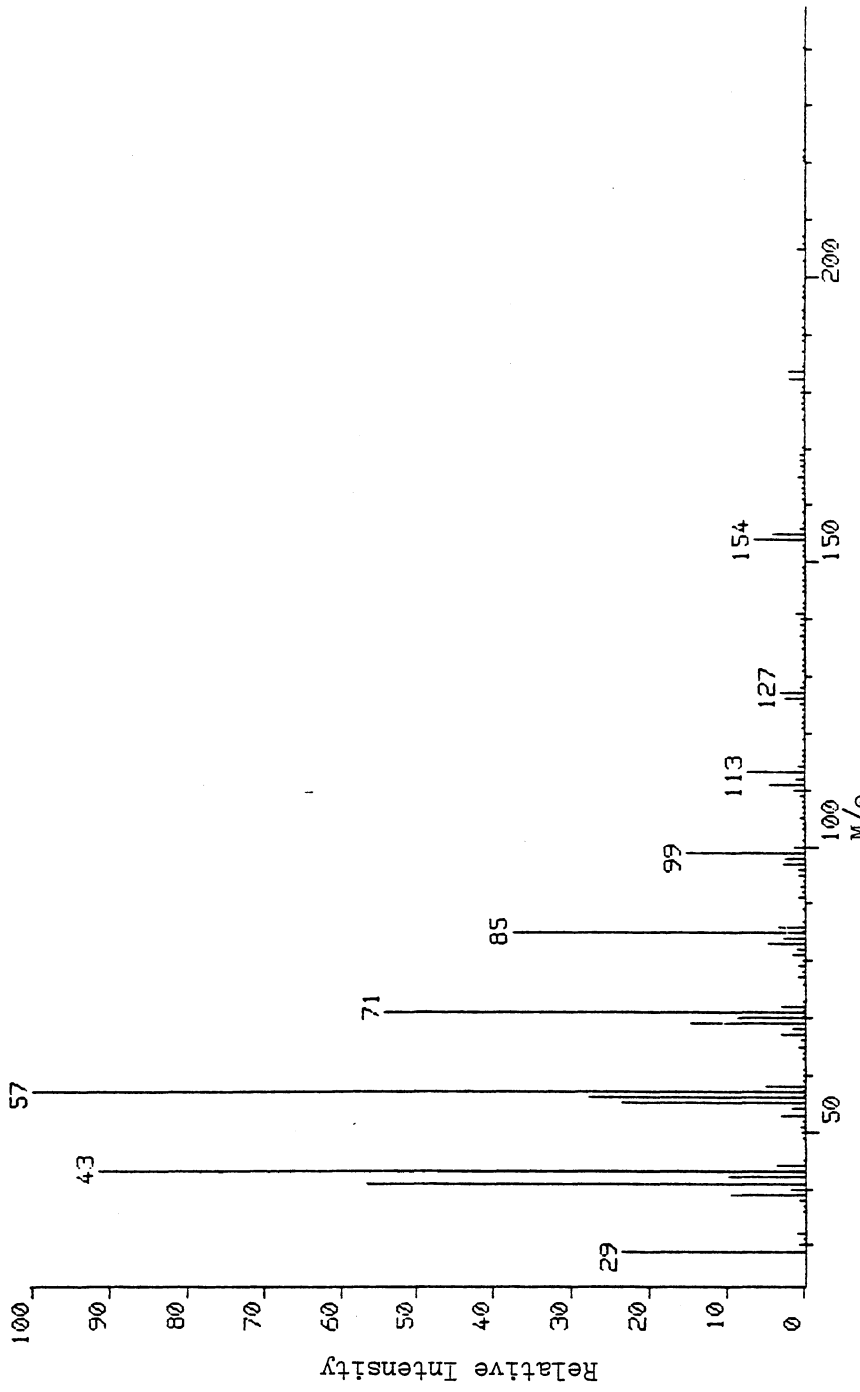
Mass Spectrum of Is3 Peak 7



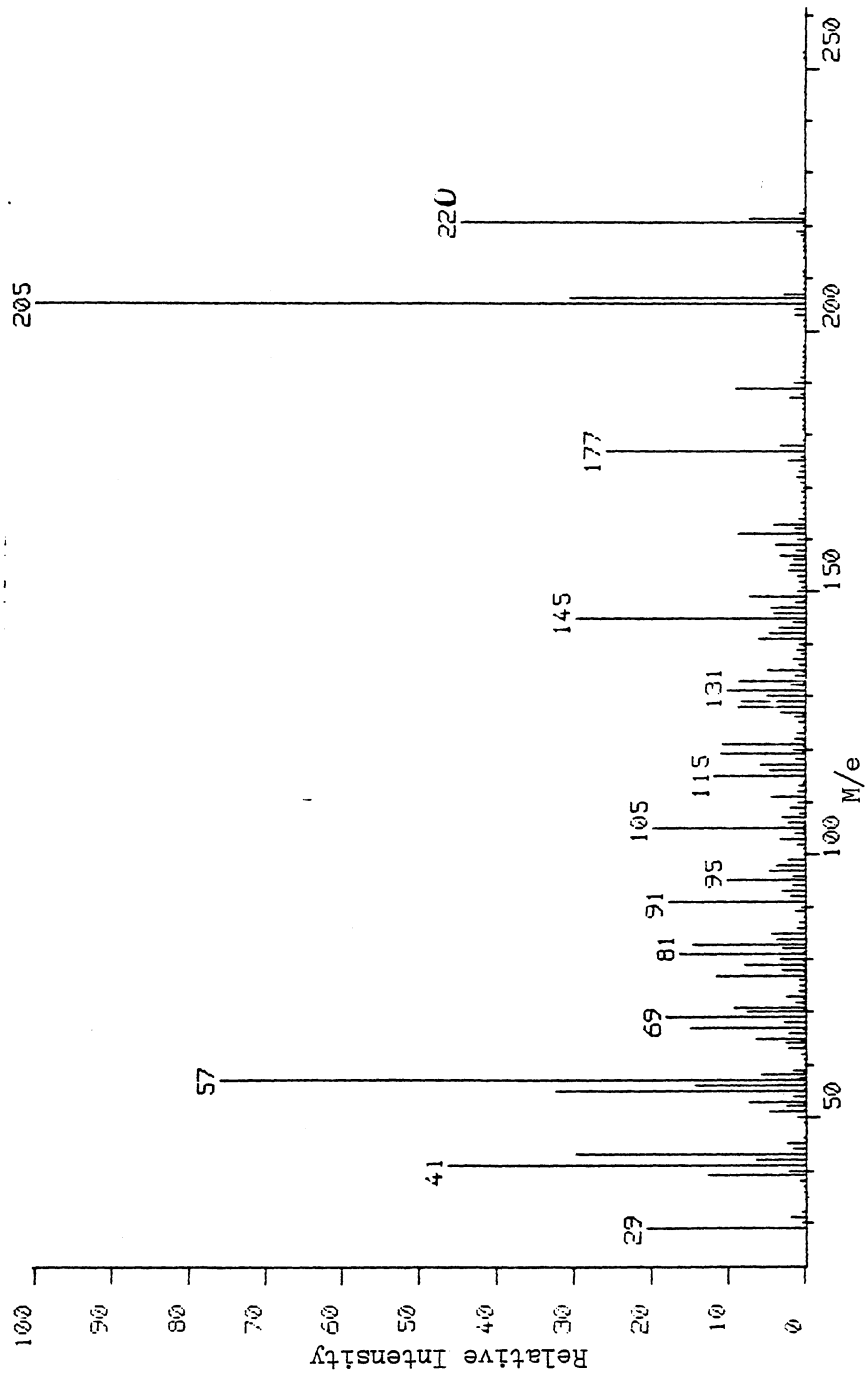
Mass Spectrum of Is3 Peak 8



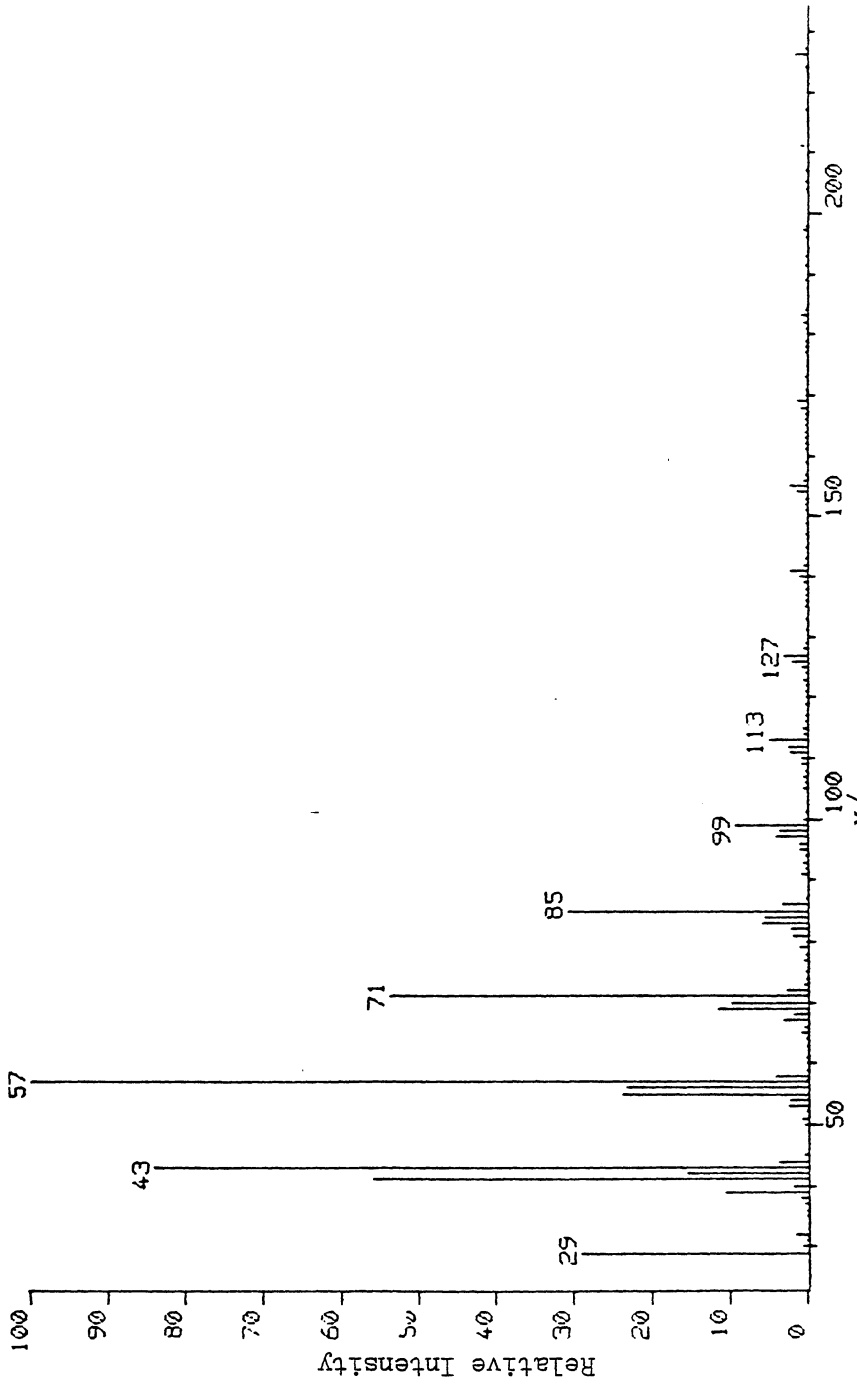
Mass Spectrum of Is3 Peak 9



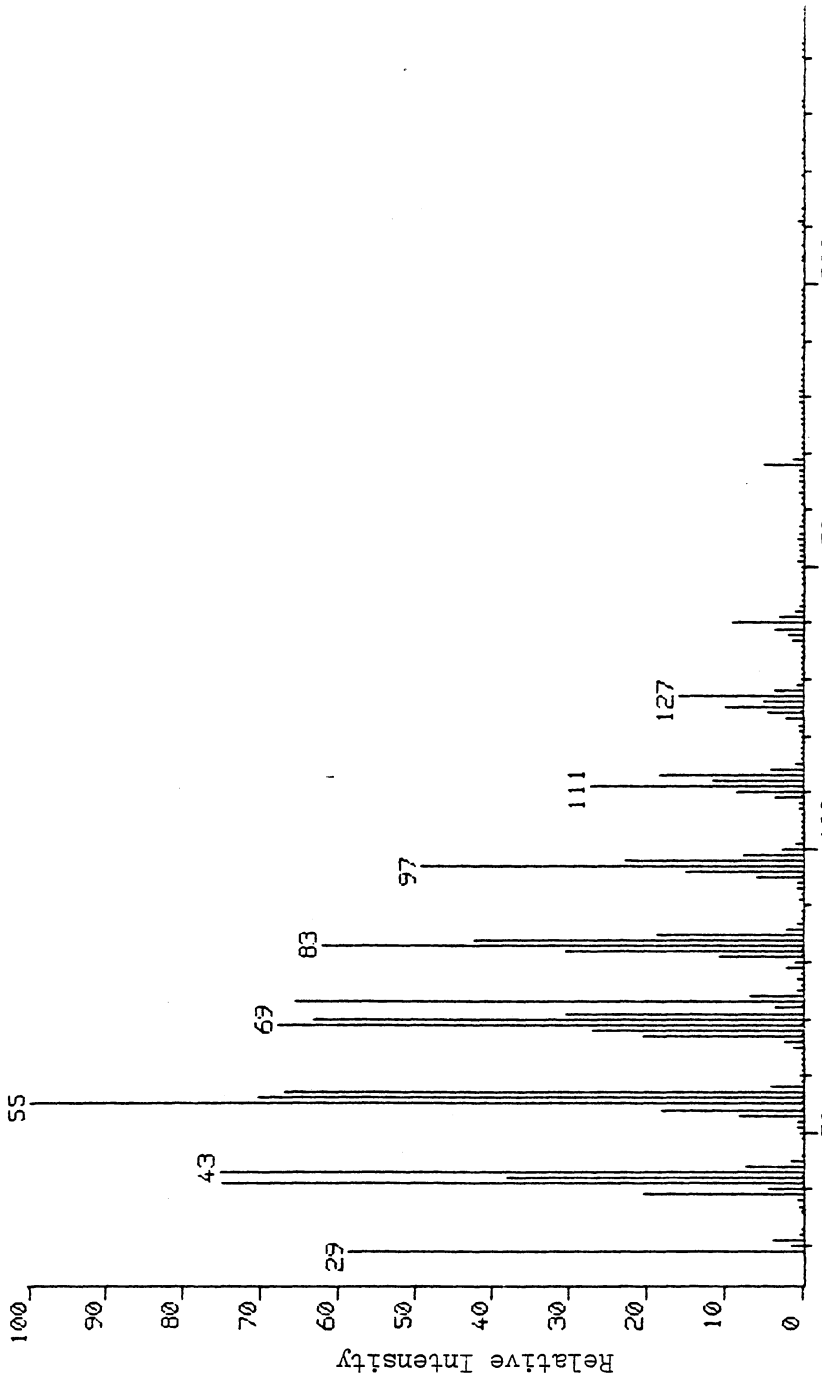
Mass Spectrum of Dv3 Peak 1



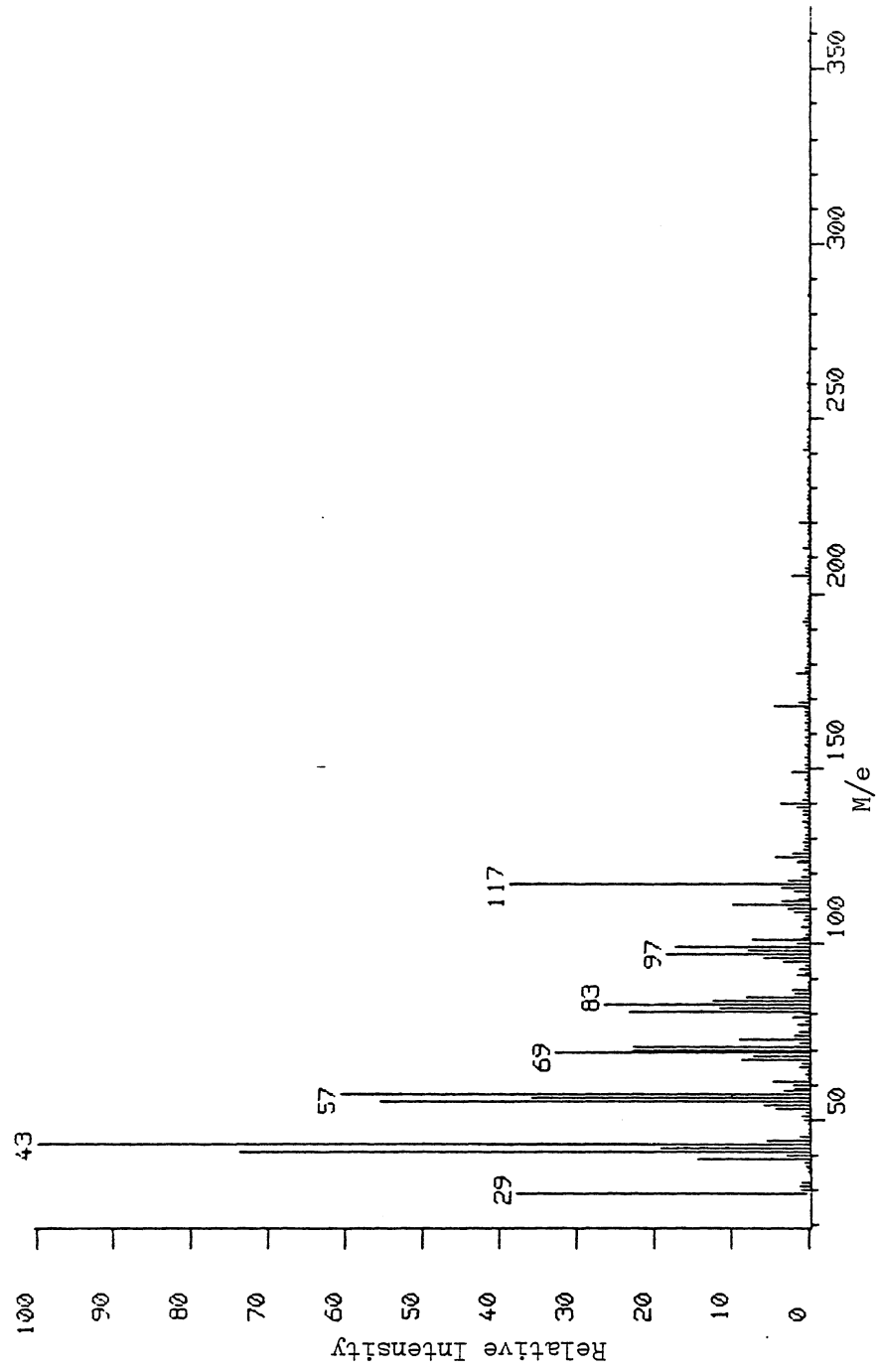
Mass Spectrum of Dv3 Peak 2



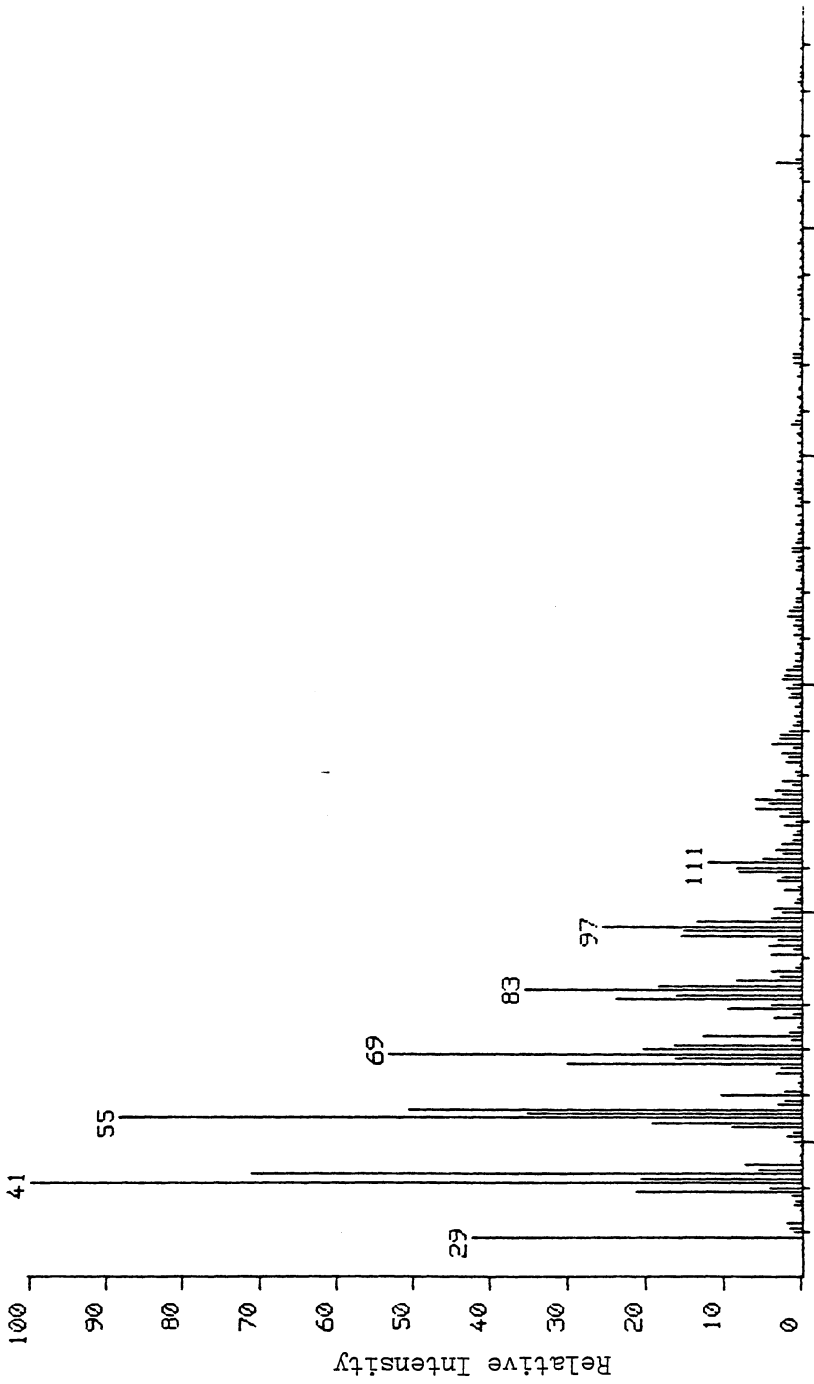
Mass Spectrum of Dv3 Peak 3



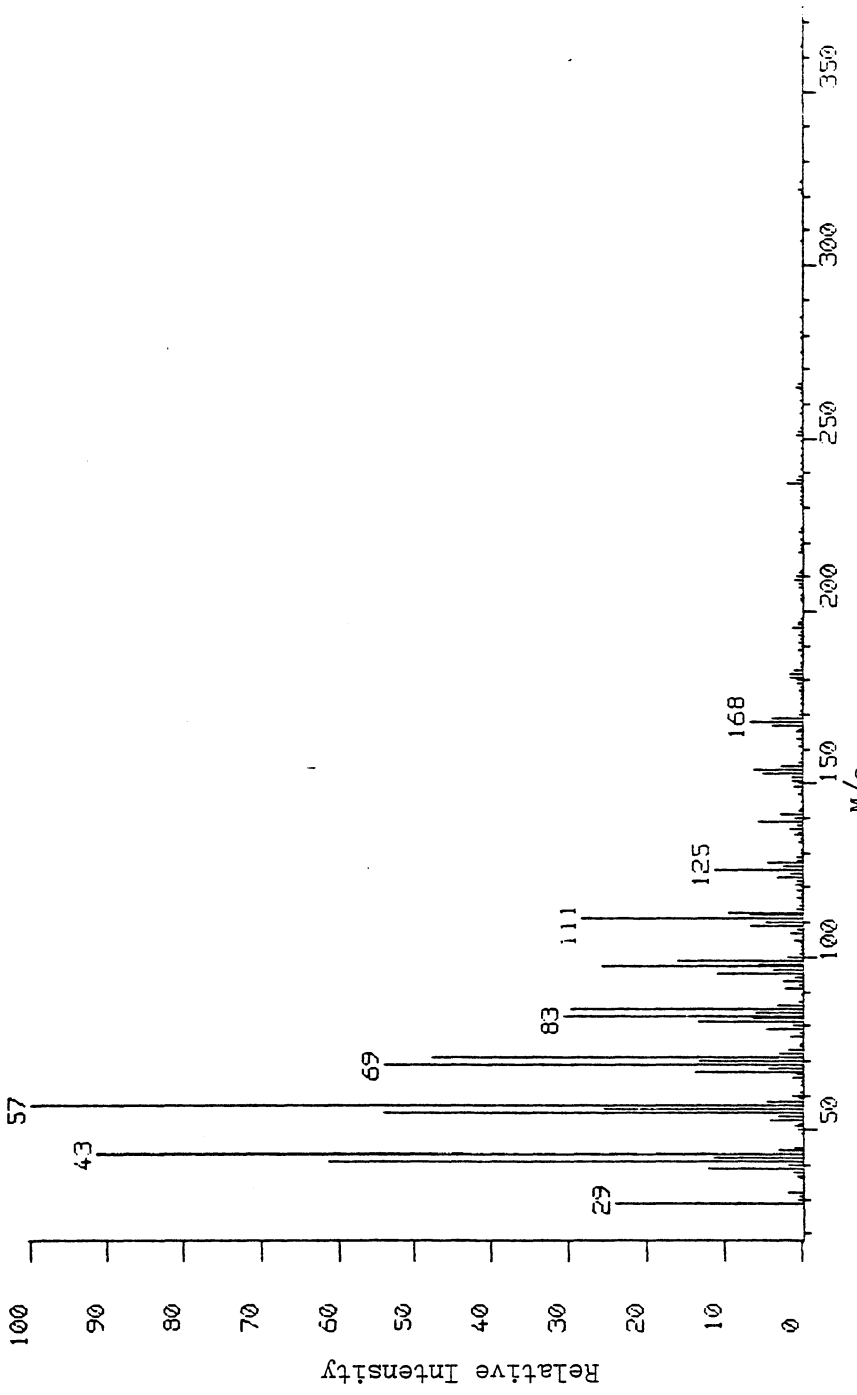
Mass Spectrum of Dv3 Peak 4



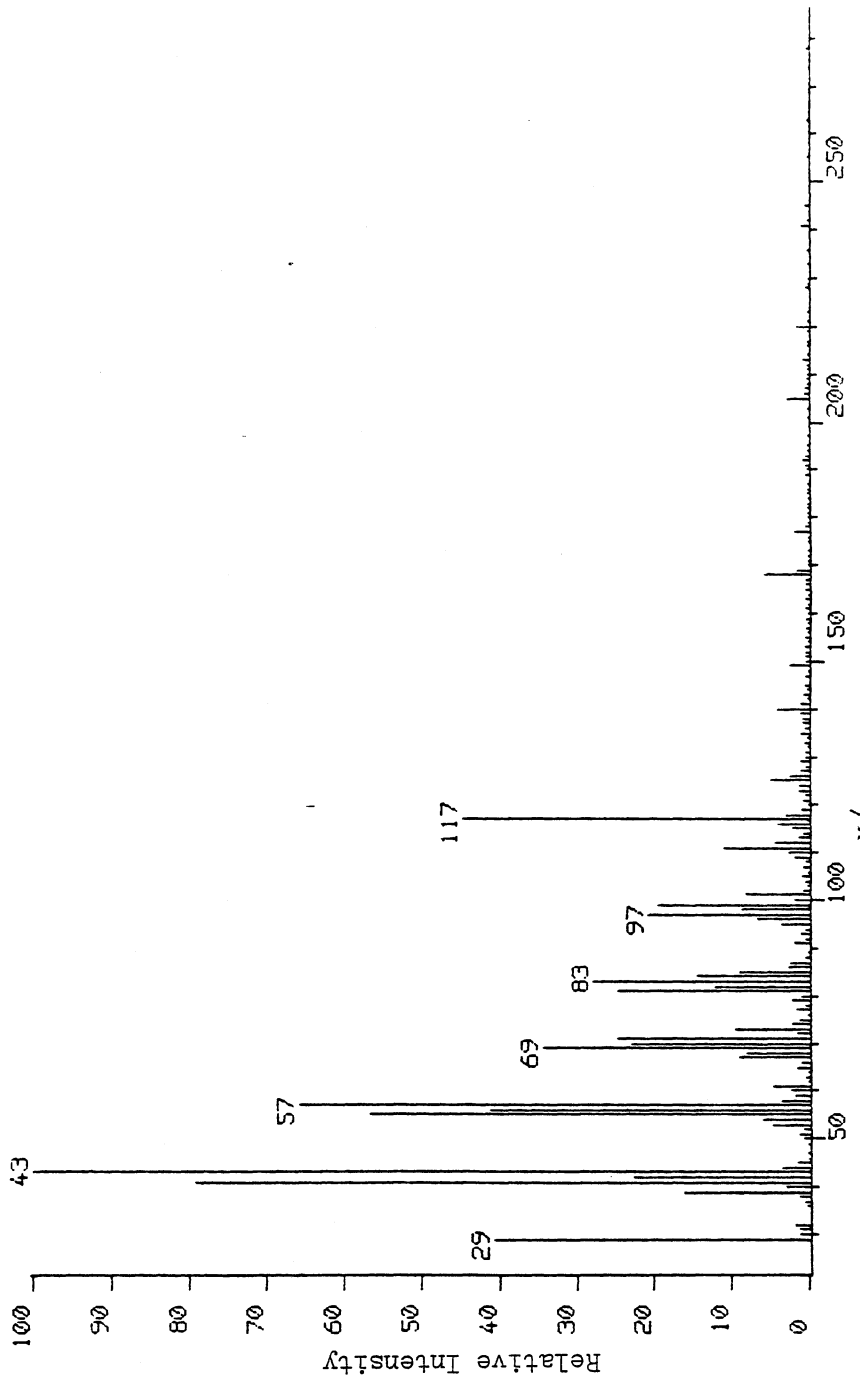
Mass Spectrum of Dv3 Peak 5



Mass Spectrum of Dv3 Peak 6



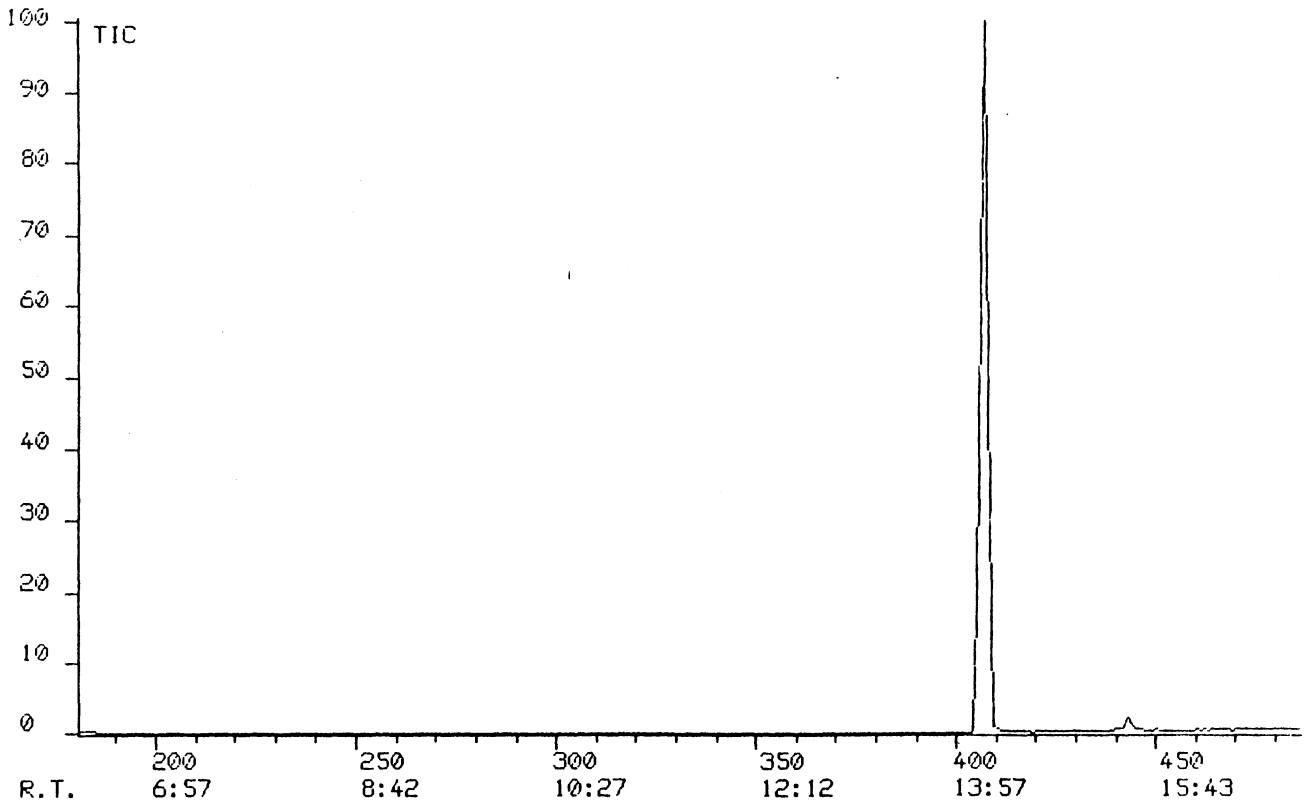
Mass Spectrum of Dv3 Peak 7



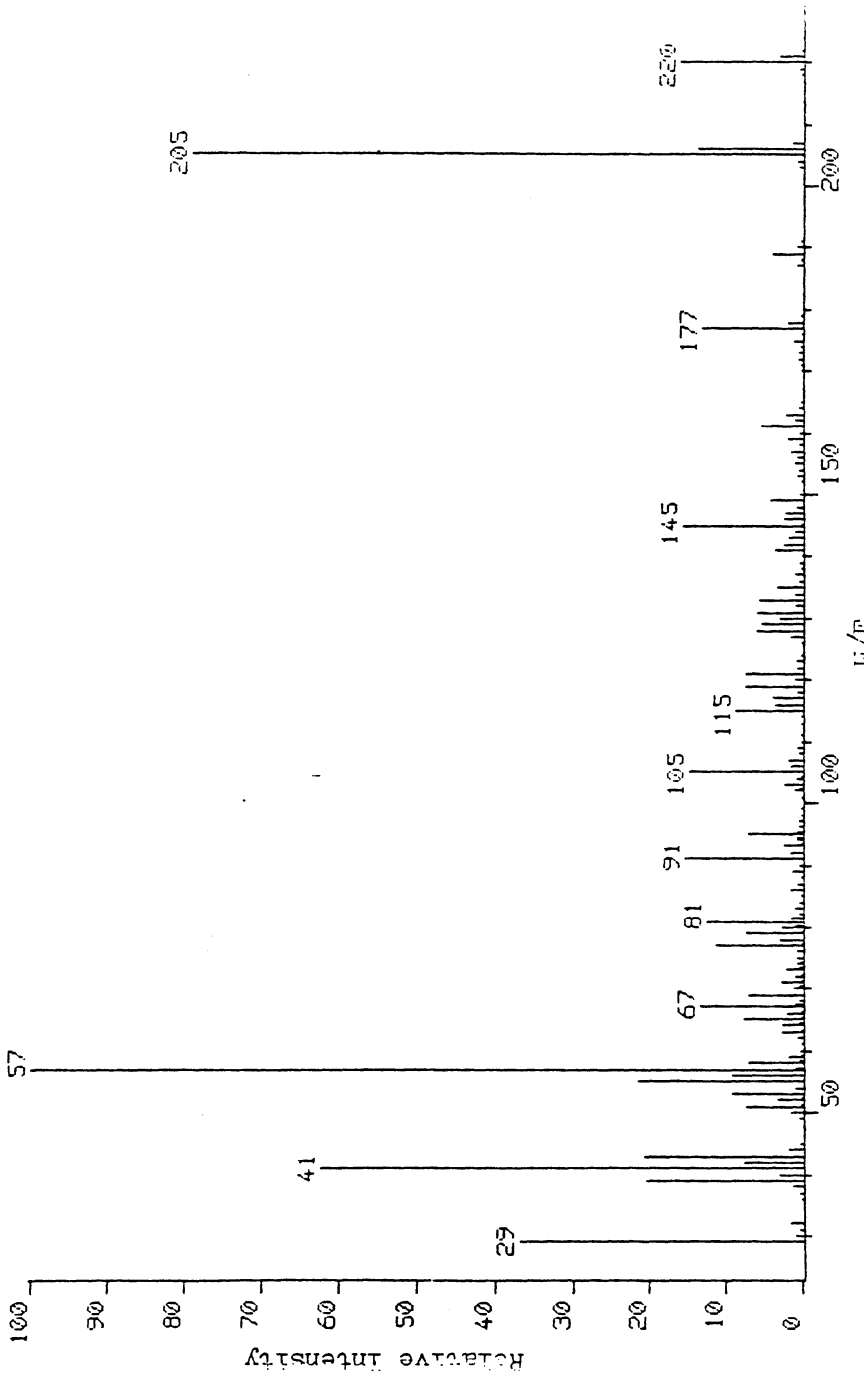
Mass Spectrum of Is3 Peak 6

APPENDIX E

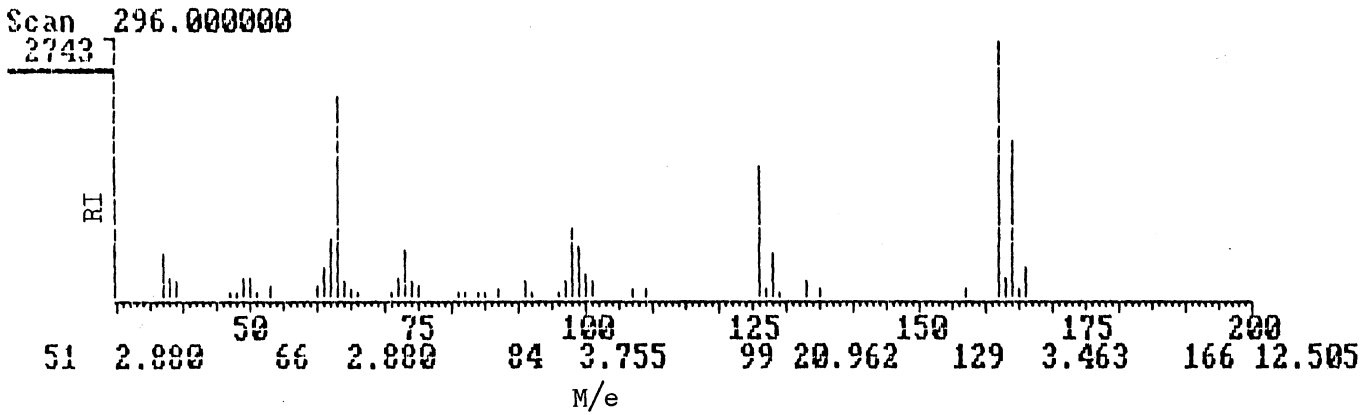
MASS SPECTRA OF REFERENCE COMPOUNDS



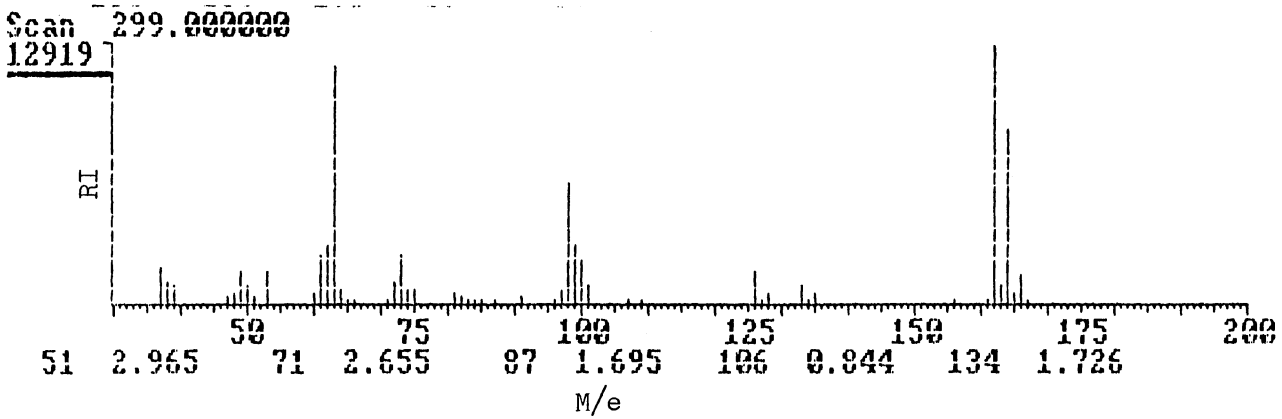
Gas Chromatogram of 4-M-2,6-tert-butylphenol



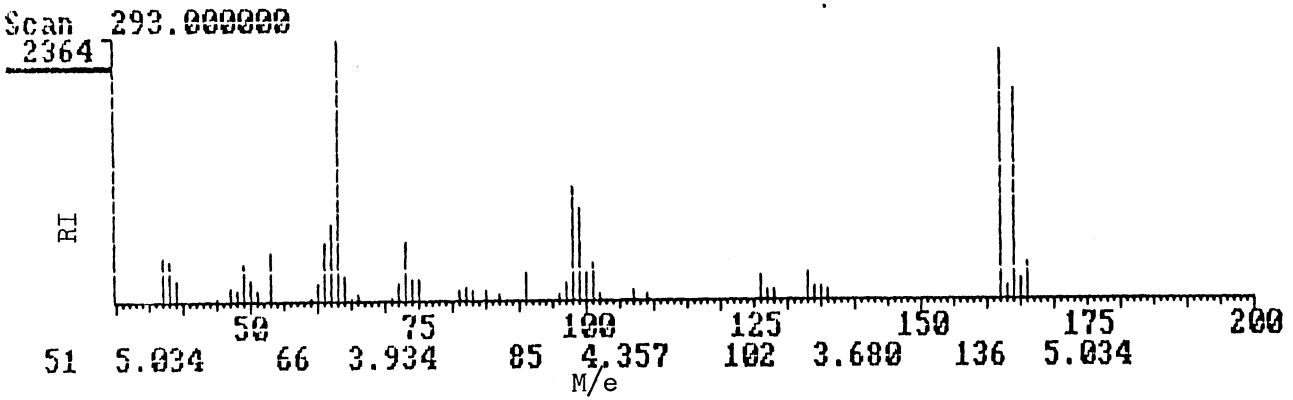
Mass Spectra of 4-M-2,6-tert-butylphenol



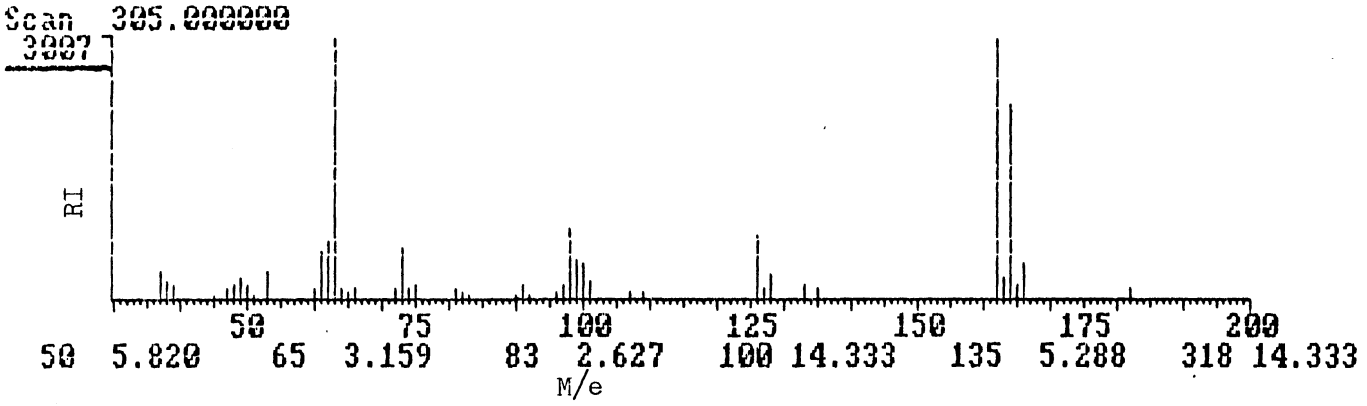
Mass Spectrum of 2,3-Dichlorophenol



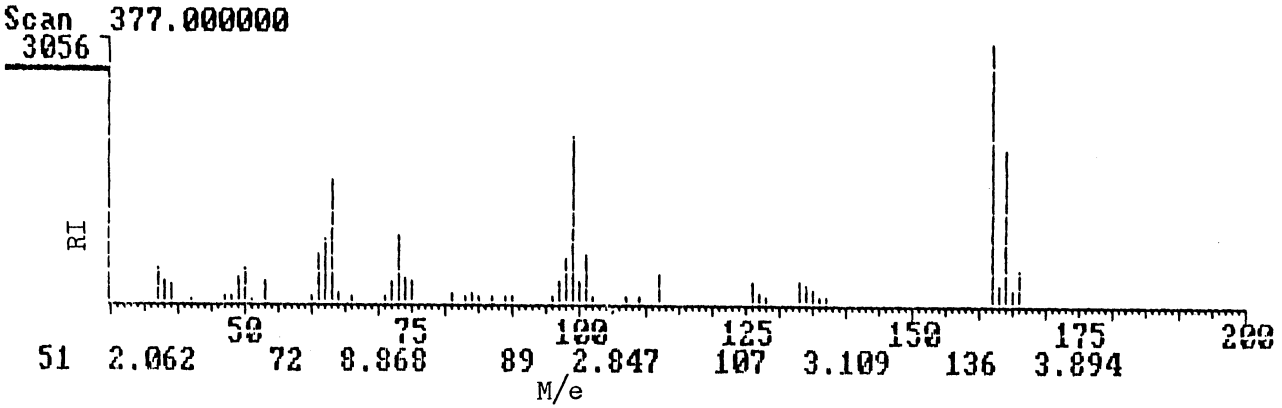
Mass Spectrum of 2,4-Dichlorophenol



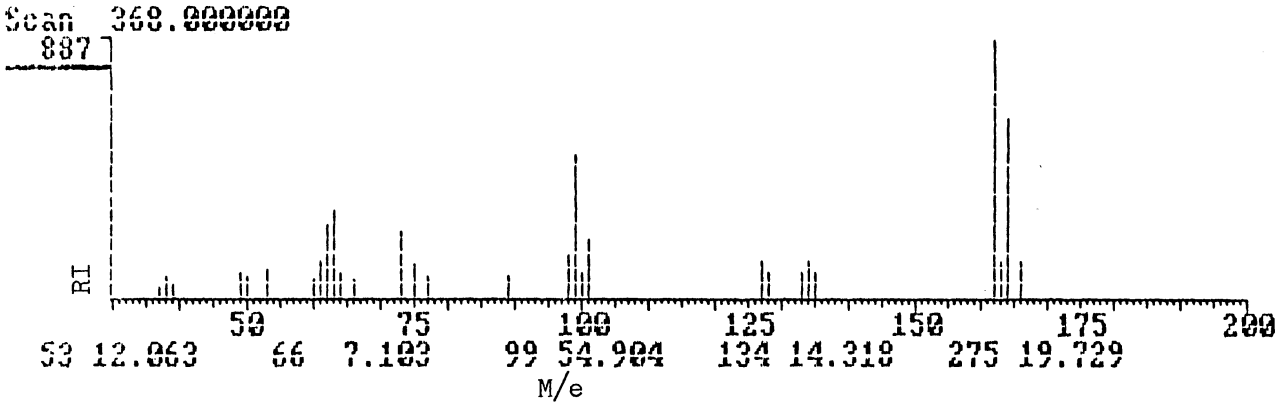
Mass Spectrum of 2,5-Dichlorophenol



Mass Spectrum of 2,6-Dichlorophenol



Mass Spectrum of 3,4-Dichlorophenol



Mass Spectrum of 3,5-Dichlorophenol

VITA

Paul Douglas Swartz

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