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I. STUDIES OF VOLATILE COMPOUNDS FROM ANTS

II. DEGRADATION STUDIES AND STRUCTURE PROOF OF *CIS*, *CIS*-NEPETALACTONE

By

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Bachelor of Science

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I. STUDIES OF VOLATILE COMPOUNDS FROM ANTS
II. DEGRADATION STUDIES AND STRUCTURE PROOF OF *CIS*, *CIS*-NEPETALACTONE

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PART I

STUDIES OF VOLATILE COMPOUNDS FROM ANTS

CHAPTER I

INTRODUCTION AND HISTORICAL

The first half of this dissertation reports the results of attempts to determine the structure and stereochemistry of the volatile compounds obtained from several species of ants. The ants for this project were collected or reared by members of the Department of Entomology at Oklahoma State University. After collection, the ants were killed by freezing, and steam distilled to obtain these compounds. Some of those identified were tested on ant colonies to determine the behavioral effects they elicited.

It has been known for over three centuries that insects secrete chemical compounds. In the seventeenth century, Samuel Fischer distilled wood ants to obtain formic acid. Numerous references have cited the influence of chemicals on insect behavior. However, because of the difficulty in obtaining these chemicals in quantity, most of them have not been identified. Entomologists have concentrated on the analysis of more easily observed phenomena.

Formic acid accounts for almost 20 per cent of the body weight of some formicine ants and is a small, familiar molecule. On the other hand, iridomyrmecin, the active principle of the poison of <u>Iridomyrmex humilis</u> (Mayr), is present to the extent of about 3.5 μg per ant and was an unknown molecule until identified from this source.[1] These small ants also produce other volatile compounds in concentrations that are orders of magnitude less than that of iridomyrmecin. The advent of the common use of nuclear magnetic resonance (nmr) and infrared spectrometry, mass spectrometry and various chromatographic techniques has facilitated the identification of minute amounts of compounds and given impetus to these studies.

Ants are a particularly good choice for experiments concerning the nature of chemical communication among insects. They have a highly developed social existence and belong to an insect order which is one of the most highly evolved in structure and behavior. Within this order their superfamily has attained maximum development of the structures used for producing and ejecting chemicals.[2] The accumulated evidence has led to the view that "most social behavior in ants is mediated by chemical releasers which are discharged at appropriate times from exocrine glands and cause stereotyped responses."[3]

It seems clear that chemical systems provide the major means of communication and defense in ants and in many other animals. Two general types of compounds are distinguished--venoms and repellents, compounds used for attack and defense; and pheromones, compounds used for communication within the species. The deciphering of these phenomena which has occurred to the present has indicated that both tend to be complex.

Roth and Eisner have reviewed the chemical defenses of arthropods.[4] They listed 31 known defense substances of which 1 is an anhydride, 3 are carboxylic acids, 9 are aldehydes, 1 is a furan, 3 are hydrocarbons, 2 are ketones, 1 is a lactone, 8 are quinones, and the remaining 3 are inorganic compounds. The defensive secretions produced by most species are a mixture of 2 or more compounds. For example, the secretion in the anal glands of Tapinoma nigerrimum (Nylander) is a mixture of 6-methyl-5-hepten-2-one, 2-methyl-4-heptanone and iridodial. The insecticidal activity is due to the ketones. The iridodial has no insecticidal properties. In air, it polymerizes to a viscous yellow substance that increases the potency of the secretion by adhering to the predator and retarding the loss of toxic volatile compounds by evaporation.[4]

Wilson[5] has distinguished 9 types of responses elicited by pheromones. They are alarm, simple attraction, recruitment, grooming, exchange of oral and anal liquid, exchange of solid food particles, facilitation, recognition of nest mates and castes and caste determination. Changes in quantity of a single pheromone may lead to a variety of effects.[6] Examples are known of pheromones which acquire additional or different meanings when presented in combination.[5] 2-Heptanone has a number of functions in Iridomyrmex pruinosus (Roger). It is primarily an alarm pheromone and attractant but apparently is also sprayed on predators for defensive purposes.[7] Blum theorizes that this alarm pheromone may have been employed originally as a defensive secretion and its use for alarm is a secondary adaptation which has been developed in order to function in a social environment. Insects are able to distinguish between geometric isomers and optical isomers.[6]

Compounds associated with nest odor and sexual attraction are present in much smaller amounts in ants than are defense chemicals. It is always possible that 1 per cent of an impurity in an isolated compound may be responsible for the behavioral effect. Up to the present, studies of ant behavior have usually been based on the analysis of response to the whole secretion of a gland. With precise identification of the molecules in the secretion, it will be possible to determine whether a single component is responsible for a behavioral effect or whether there are several, acting successively or at once.

The identification and testing of pheromones that has occurred in recent years has permitted the classification of several new biological phenomena. As well as having scientific interest, an increase in knowledge about compounds secreted by insects may have considerable economic importance. Sex pheromones have already proved to be valuable in the control of certain insects. Trail substances could conceivably serve a similar purpose. Defense substances are by nature repellents and their study could produce new ideas about safe and specific control of insect populations.

Recent reviews[2,8,9] have summarized the compounds presently identified in ants. Since these reviews, several additional developments have occurred in this field. These are summarized in Table I.

Using mass spectrometry and gas chromatography, Pavan[10] has demonstrated the presence of dimethyldisulfide and dimethyltrisulfide in the mandibular glands of the African ponerine ant Palothyreus tarsatus (Fabr.). These compounds have nauseous odors and are used to repel intruders. This is the first example of production of polysulfides by animals.

Bernardi and workers[11] have examined in greater detail the ether extract of the ant Lasius fuliginosus Latr. from which Pavan had already isolated dendrolasin. They identified eleven additional compounds including carbonyl compounds, hydrocarbons and a furan. The authors make no attempt to identify the glandular source of these compounds or to elucidate their behavioral significance.

Law et al[12] have made a study of the influence of caste on the production of volatile chemicals among some myrmecine and formicine ants. *Pheidole fallax* Mayr soldiers produce a compound tentatively identified as skatole not produced by minor workers. Male ants of the species *Lasius neoniger* Emery, *Lasius alienus* (Förster) and *Acanthomyops claviger* (Roger) were collected prior to mating flights. These ants produced a mixture of terpene-like compounds in their mandibular glands from which only 2,6-dimethyl-5-hepten-1-ol and citronellol could be positively identified along with an indole base. The authors speculate that the indole compound might be an odor fixitive for the mixture. Workers of these species produce a different mixture of compounds.

Cavill[13] has studied the contents of the Dufour's gland in *Myrmecia gulosa* (F.). The major components present are *cis*-heptadec-8-one pentadecane, and heptadecane. At least 9 other hydrocarbons are present in minor amounts. The same constituents were present in the Dufour's gland of 10 female reproductives prior to the nuptial flight as were present in workers. These hydrocarbons are not ejected with the venom when the ant stings. It has been speculated that they may function as a sting lubricant. Two ponerine ants, a myrmicine species and 2 formicine species also produced hydrocarbons in the Dufour's gland. Another myrmecine ant, *Aphaenogaster longiceps* (F. Sm.), has only α-farnesene in its Dufour's gland.[14] This was the first sesquiterpene found in ants.

TABLE I

COMPOUNDS RECENTLY IDENTIFIED IN ANTS

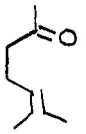
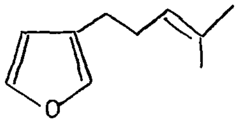
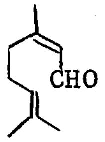
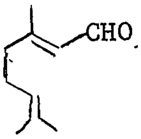
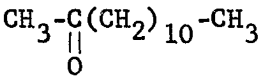
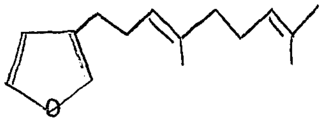
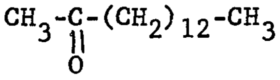
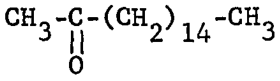
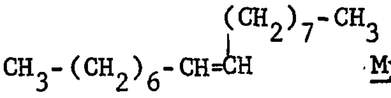
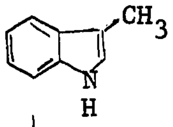
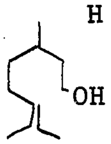
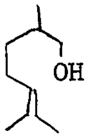
	Compound		Source	Reference
1.	Dimethyldisulfide	$CH_3-(S)_2CH_3$	*Paltothyreus tarsatus*	10
2.	Dimethyltrisulfide	$CH_3-(S)_3CH_3$	*Paltothyreus tarsatus*	10
3.	n-undecane	$CH_3-(CH_2)_9CH_3$	*Lasius fuliginosus*	11
4.	6-Me-5-hepten-2-one		*Lasius fuliginosus*	11
5.	perillin		*Lasius fuliginosus*	11
6.	n-tridecane	$CH_3(-CH_2)_{11}-CH_3$	*Lasius fuliginosus*	11
7.	cis-citral		*Lasius fuliginosus*	11
8.	n-pentadecane	$CH_3-(CH_2)_{13}-CH_3$	*Lasius fuliginosus* *Myrmecia gulosa*	11 13
9.	*trans*-citral		*Lasius fuliginosus*	11
10.	2-tridecanone	 $CH_3-\underset{\underset{O}{\Vert}}{C}(CH_2)_{10}-CH_3$	*Lasius fuliginosus*	11
11.	dendrolasin		*Lasius fuliginosus*	11

TABLE I (Continued)

Compound	Structure	Source	Reference
12. 2-pentadecanone	 $CH_3-\underset{\underset{O}{\Vert}}{C}-(CH_2)_{12}-CH_3$	<u>Lasius fuliginosus</u>	11
13. 2-heptadecanone	 $CH_3-\underset{\underset{O}{\Vert}}{C}-(CH_2)_{14}-CH_3$	<u>Lasius fuliginosus</u>	11
14. farnesal		<u>Lasius fuliginosus</u>	11
15. α-farnesene		<u>Aphaenogaster longiceps</u>	14
16. heptadecane	$CH_3-(CH_2)_{15}CH_3$	<u>Myrmecia gulosa</u>	13
17. <u>cis</u>-heptadec-8-ene	 $CH_3-(CH_2)_6-CH=\overset{\overset{(CH_2)_7-CH_3}{\vert}}{C}H$	<u>Myrmecia gulosa</u>	13
18. skatole		<u>Pheidole fallax</u>	12
19. citronellol		<u>Acanthomyops claviger</u>	12
20. 2,6-dimethyl-5-hepten-1-ol		<u>Acanthomyops claviger</u>	12

CHAPTER II

IDENTIFICATION OF 4-METHYL-3-HEPTANONE FROM POGONYMYRMEX ANTS[15]

Wilson first reported the nature of response to alarm pheromones after studies of Pogonomyrmex badius (Latreille) in 1958.[6]

> When workers are disturbed by alien mechanical or chemical stimuli they show the following characteristic alarm response. Low intensity: rate of locomotion increases, with the ant moving in wide loops and circles; the head and antennae are periodically lifted high and the gaster may be periodically lowered. High intensity: rate of locomotion increases still more, with the ant tending to move in tighter circling patterns; the head and antennae are periodically raised a moderate distance but not as high as during lower intensity alarm....

He observed that an excited worker caused a wave of alarm behavior among other workers as it encountered them. The workers discharged a volatile odorous substance when excited and Wilson assumed this was a chemical releaser of the observed behavior. Dissection revealed that the substance was in the reservoir of the mandibular glands of the head. Workers that were exposed to this unknown compound several times frequently began to show digging behavior as well as that described above.

A variety of volatile chemicals were capable of inducing behavior patterns similar to those released by the mandibular gland chemical. When groups of workers were allowed to come into direct contact with small amounts of formic acid, ethylamine, n-butyric acid, or n-caproic acid absorbed on small pieces of filter paper, they responded immediately with alarm behavior and in time with digging behavior.

Wilson[16] further defined the nature of alarm substances in tests on several dolichoderine species. Colonies of Tapinoma sessile (Say), Monacis bispinosa (Olivier), and Liometopum occidentale Emery produce volatile alarm substances. Squashes of anal glands of each species produce about the same intensity of alarm in workers of the other two species as in its own. These results can be interpreted as indicating that a common component exists in each of the ants or else that the secretions differ but the reactions are not species-specific. The ketones, 6-methyl-5-hepten-2-one and 2-methyl-4-heptanone which had been previously identified in Tapinoma nigerrimum caused intense alarm behavior for T. sessile.

Blum et al have reported on the alarm releaser of Iridomyrmex pruinosus, 2-heptanone.[7] They tested 49 ketones for relative effectiveness in eliciting alarm behavior and found that those containing 6 to 10 carbon atoms are most potent. The compounds were placed on a piece of filter paper near the nest opening and the number of ants attracted was the gague of effectiveness. Maximum activity was found in the straight chain C7 to C9 ketones. The position of the carbonyl has an influence on activity and in the C6 to C10 series, all ketones with a carbonyl group on the second carbon possess high potency.

RESULTS AND DISCUSSION

The ketone 4-methyl-3-heptanone is the mandibular gland alarm substance in Pogonomyrmex barbatus (F. Smith). It is also present in P. badius and was undoubtedly the alarm chemical whose effects Wilson observed. This pheromone is also present in P. californicus (Buckley), *P. desertorum* Wheeler, *P. occidentalis* (Cresson) and *P. rugosus* Emery.

The gas chromatogram of the ether extract of compounds obtained from steam distillation of *P. barbatus* showed a number of peaks (Figure 1). Oxidation of the crude extract with CrO_3 caused compound A-2 to disappear from the chromatogram while reduction of the extract with $LiAlH_4$ caused compound A-1 to disappear but not A-2. The major component (subsequently shown to be 4-methyl-3-heptanone) gave a 2,4-dinitrophenylphenylhydrazone when the effluent from the gas chromatograph was bubbled into 2,4-dinitrophenylhydrazine reagent. Attempts to identify the compound by purification of this derivative using thin layer chromatography and determination of melting point were unsuccessful.

Collection of larger amounts of ants allowed the isolation of A-1 using preparative gas chromatography. The nmr spectrum strongly suggested its identity. There is absorption for three protons α to a carbonyl group consisting of a quartet with an underlying multiplet centered at 2.43 δ (Figure 2). This is characteristic of an ethyl ketone with branching on the opposite side of the carbonyl group. The absorption integrated for sixteen protons. 4-Methyl-3-heptanone is the only 8-carbon ketone to fit this spectrum well.

The identification was confirmed by use of mass spectrometry followed by comparison with the spectra of an authentic sample. The major peaks in the mass spectrum of 4-methyl-3-heptanone are shown in Table II. The molecular ion occurs at m/e 128 and characteristic peaks at m/e 57 (loss of the larger alkyl group by α-cleavage), 86 (β-cleavage with rearrangement, and 71 and 29 (α-cleavage on either side of the carbonyl group with the positive charge remaining with the alkyl group).[17]

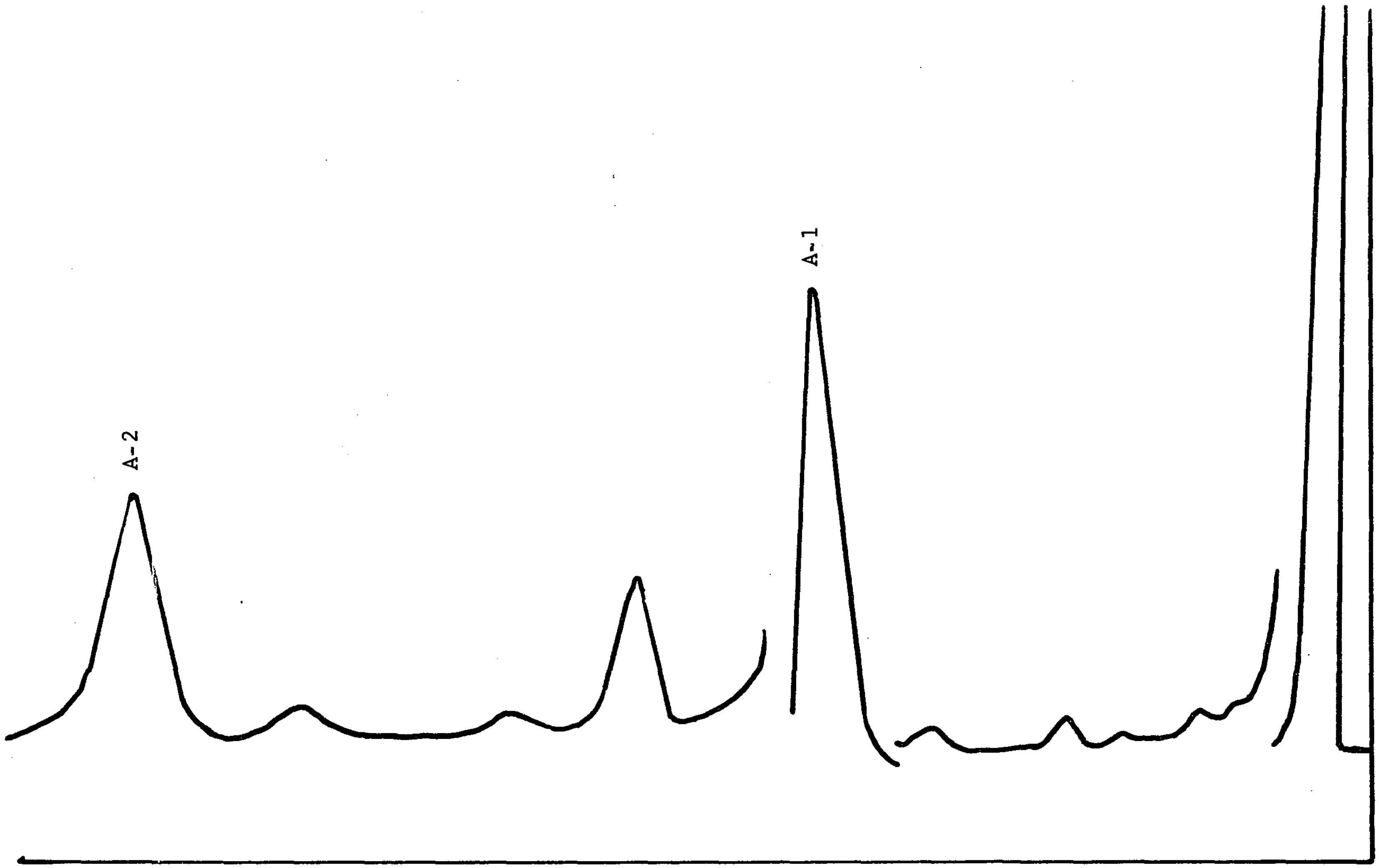


Figure 1. Volatile Compounds from <u>Pogonomyrmex barbatus</u>

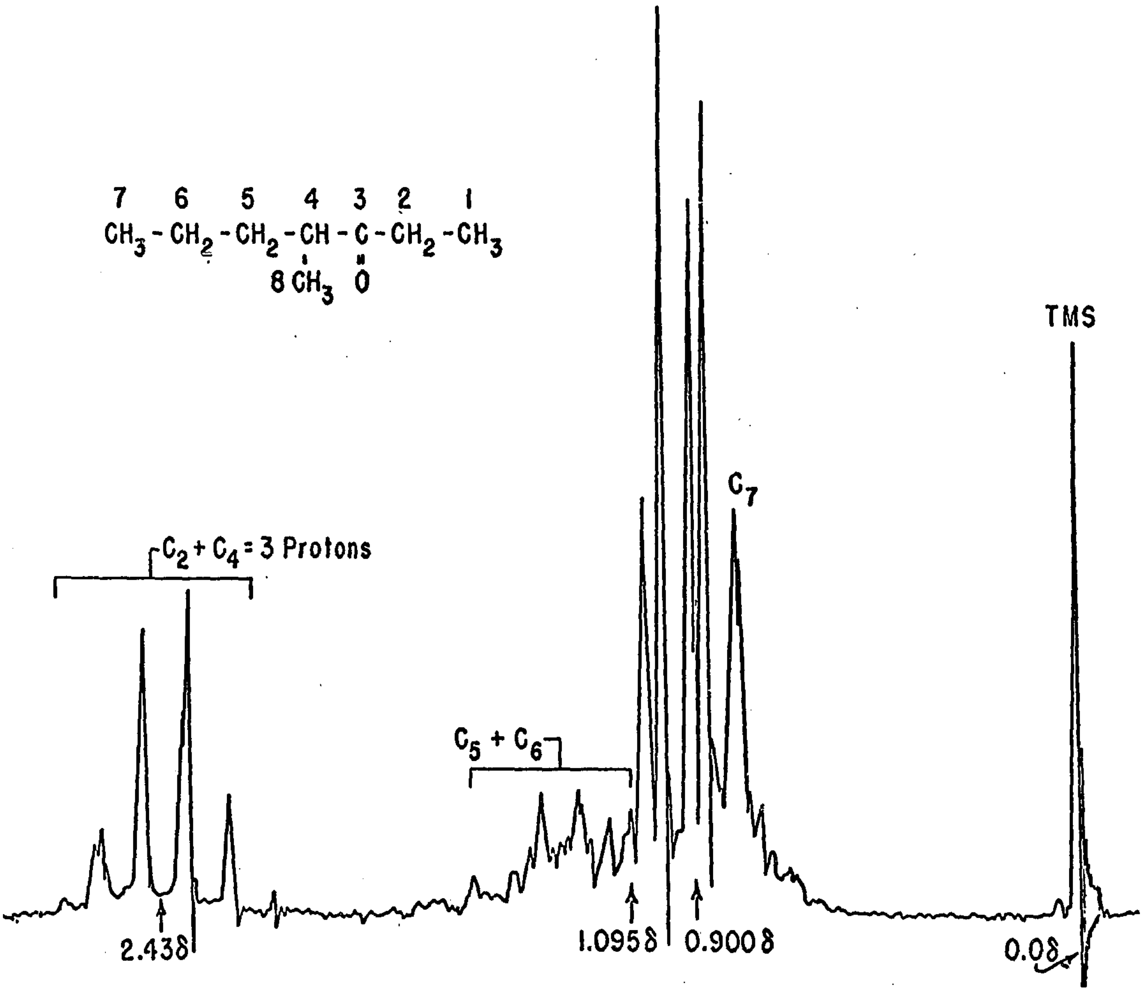


Figure 2. Nmr Spectrum of 4-Methyl-3-heptanone

This ketone releases alarm behavior in *P. barbatus* similar to that observed by Wilson in *P. badius*. The ants were at first attracted to a drop of the ketone allowed to evaporate in the nest. As it evaporated, they became alarmed and appeared to release the alarm substance themselves. Twenty nl of 4-methyl-3-heptanone, evaporated in a 5-ml syringe and injected into a 3-inch diameter, covered, petri dish nest, produced alarm behavior in the ants for 15 to 20 seconds. They quickened their movements, periodically raised their heads high above their bodies and opened their mandibles. Occasionally, they bit and stung debris in the nest. With injections of one to 5 nl, the alarm behavior intensified, and often lasted for more than a minute.

P. barbatus workers attacked crushed heads of other workers of the same colony. Crushing the abdomen or thorax of an ant had no effect on the other ants. When a small drop of 4-methyl-3-heptanone was placed on a live, active ant in a colony, the ant was attacked even more readily than the carcass of one with a crushed head. Ants which passed within 3 or 4 cm of the treated ant immediately chased and attacked it, with sometimes as many as 3 or 4 trying to bite and sting it at the same time. Often those ants which had contacted the treated ant, and therefore had acquired the odor of the chemical, were also attacked. Workers attacked objects such as wads of cotton previously treated with the alarm pheromone also. After exposure of a few minutes to this ketone, ants were observed carrying pebbles in their mandibles and infrequently making digging movements with their legs.

The presence of this ketone in the other *Pogonomyrmex* ants listed, and its location in the mandibular glands of *P. barbatus* were demonstrated by pyrolysis experiments. The glands, isolated by dissection were placed in the closed pyrolysis loop of a valved gas chromatograph. When the compounds were volatilized by heating the loop to 200°, and diverted into the column, a peak corresponding to 4-methyl-3-heptanone was obtained (Figure 3). A peak indistinguishable from this may also be obtained from a single head of *P. californicus*, *P. desertorum*, *P. occidentalis*, *P. rugosus*, or *P. badius*.

Using a combination gas chromatograph-mass spectrometer, mass spectra have been obtained of certain of the peaks from extracts of *P. barbatus*, *P. californicus*, and *P. rugosus*. Some minor constituents of these extracts have been tentatively identified from these spectra. The identifications have been strengthened by comparison of retention times with authentic samples. Compound A-2 of *P. Barbatus* is 4-methyl-3-heptanol, the alcohol corresponding to the alarm pheromone. *P. californicus* and *P. rugosus* have compounds with parent mass peak at m/e 170, base peak at m/e 57 and a splitting pattern that suggests these ants produce n-dodecane. *P. californicus* also has a compound of molecular weight 184 which seems to be n-tridecane. The close relationship between *Pogonomyrmex* ants suggests that other ants of this genus will also contain some of these compounds. It is becoming increasingly apparent that the occurrence of hydrocarbons in ants may be quite widespread.

Subsequent to our discovery of 4-methyl-3-heptanone in *P. barbatus*, Moser et al[18] found this pheromone along with 2-heptanone in the mandibular glands of *Atta Texana* (Buckley). They estimated that 4-methyl-3-heptanone released detection and attraction responses at concentrations of 2.66×10^{7} molecules/ml and alarm at 2.66×10^{8} molecules/ml. 2-Heptanone was less effective by a factor of 1000. 4-Methyl-3-heptanone

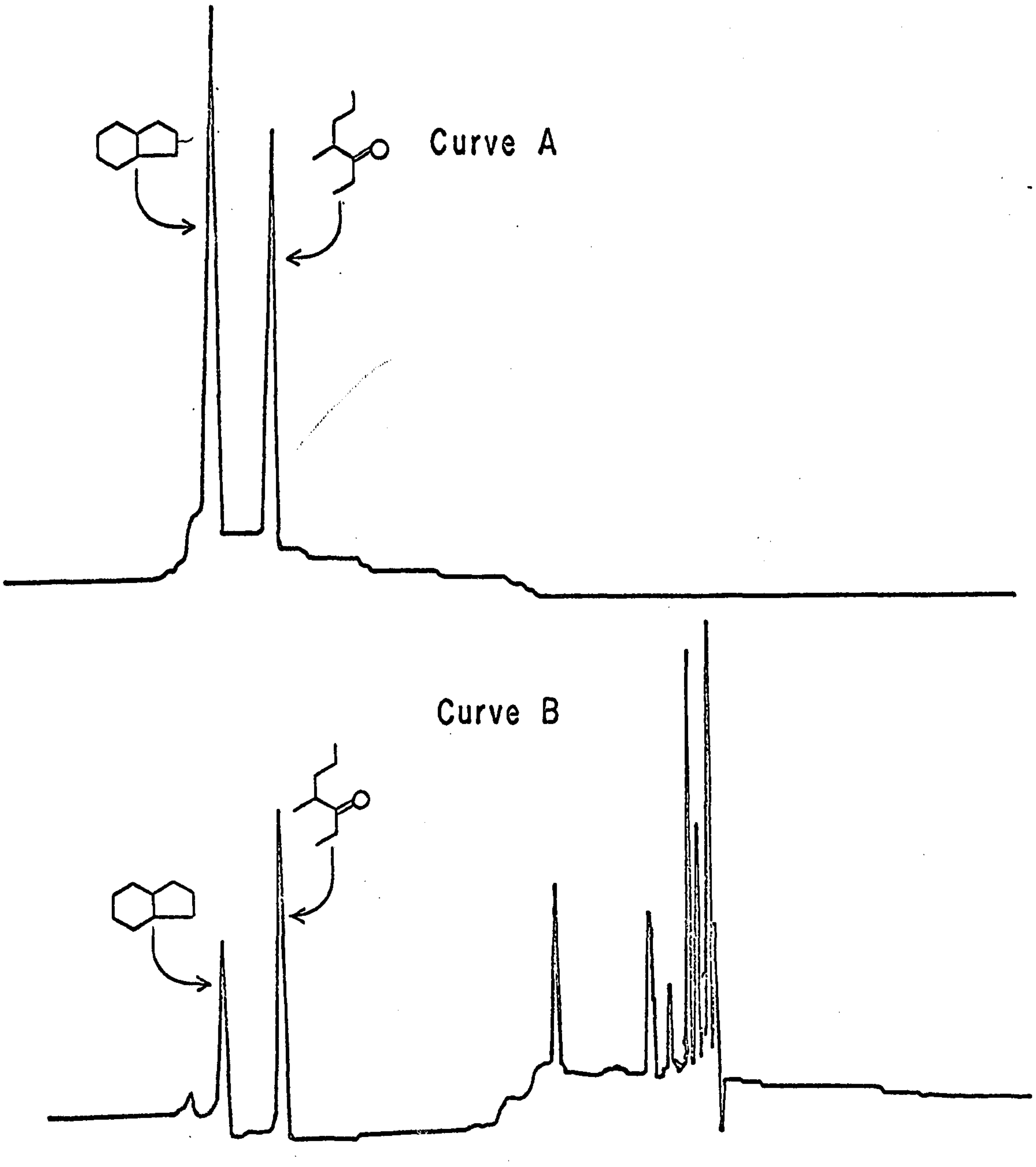


Figure 3. Enrichment of Standard 4-Methyl-3-heptanone With Pyrolysate of P. barbatus

along with other ketones and alcohols was tested in our laboratories by Ken Vick and some of his results are included for completeness.

The method of testing was that of Moser. Saturated vapor containing the compound to be tested was introduced into an air stream flowing through the nest from a syringe. From the vapor pressure of the compound tested and the dilution volume involved, the approximate concentration of compound reaching the ants could be estimated. A test was considered positive when unoccupied workers lifted their heads with antennae waving about. This behavior was followed by attempts to climb the sides of the test canister.

P. barbatus responded with alarm behavior to concentrations of 4-methyl-3-heptanone on the order of 9 x 10^{13} molecules/ml. This agrees well with a value of 4.47 x 10^{13} molecules/ml calculated for *P. badius* by a different method.[19] Other C8 ketones tested gave response levels from 10^{15} to 10^{17} molecules/ml with only 6-methyl-5-hepten-2-one giving rise to response on the same level as 4-methyl-3-heptanone. Ketones with greater or less than 8 carbon atoms gave higher response levels ranging up to 2 x 10^{18} for 3-methyl-2-butanone. Neither 4-methyl-3-heptanol nor other alcohols tested gave rise to alarm behavior at reasonable concentration levels.

In connection with this testing, a number of C8 straight chain and methyl branched ketones were synthesized or purchased. It was apparent from the literature and work that had been done in our laboratories that many ants contain C8 ketones. To facilitate identification in new ant extracts, the mass spectra of these ketones were obtained. Table II reports the major peaks in the mass spectra of 13 C8 ketones and indicates the origin of some of the fragments. Most of these spectra have not been published.

The spectra are much as predicted and agree well with published spectra.[17,20] The cleavage of the 5 methyl ketones is quite characteristic. In all cases, the 2 largest peaks are due to α-cleavage of the largest alkyl group and β-cleavage with rearrangement. The β+1 peak is prominent in all spectra and 1 of the 4 major peaks in 2 cases. The spectra of 6-methyl-2-heptanone, 5-methyl-2-heptanone and 2-octanone are so similar that differentiation of these compounds would be impossible on the basis of mass spectra alone.

β-Cleavage is less pronounced for the ethyl ketones. In 3 of the 4 spectra the base peak occurs at m/e 57. The base peak of 3-octanone is at m/e 43 as in published spectra. In the cases that β-cleavage can occur in both directions (3-methyl-4-heptanone, 2-methyl-4-heptanone, and 4-octanone), the double rearrangement peak is important.[16] The largest even-numbered peak for 4-octanone and 2-methyl-4-heptanone is at m/e 58 and 72 is comparable in size with the peak at m/e 100 in the spectrum of 3-methyl-4-heptanone.

EXPERIMENTAL

Gas chromatographs used in these analyses included a Beckman GC-2A chromatograph equipped with a thermal conductivity detector and an Instruments, Inc. model 393 valved gas chromatograph fitted with a flame ionization detector. The columns used were a 0.5 in. x 10 ft copper column packed with 25% Carbowax on 60/80 mesh acid washed Chromsorb W and a 0.02 in. x 450 ft capillary column coated with Monsanto OS-138 (a six-ring polyphenyl ether, bis[*m*-(*m*-phenoxy-phenoxy)phenyl]-ether). The nmr spectrum was obtained with a Varian HR-60 spectrometer,

TABLE II

MASS SPECTRA OF C8 KETONES

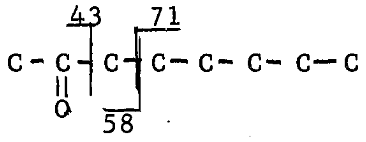
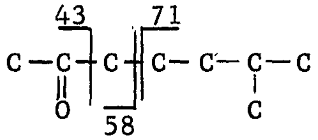
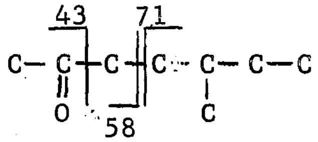
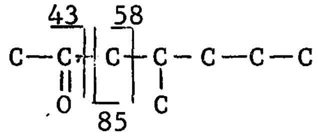
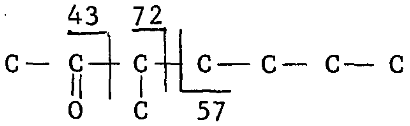
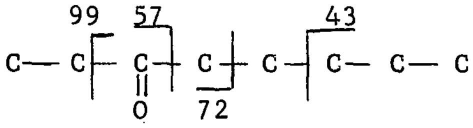
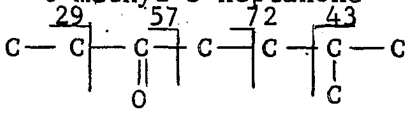
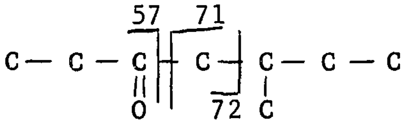
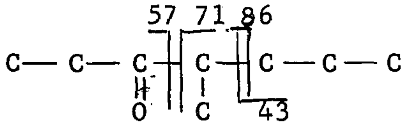
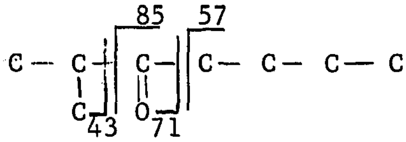
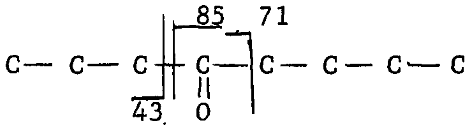
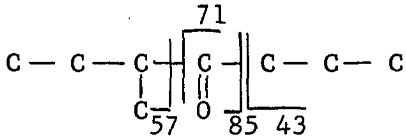
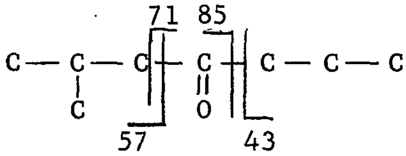
Compound	Major Peaks
2-octanone 	43, 58, 71, 59, 41, 128[a] 88 21 19 13 9.2[b]
6-methyl-2-heptanone 	43, 58, 71, 95, 70, 128[a] 70 15 11 10 3.0[b]
5-methyl-2-heptanone 	43, 58, 71, 41, 70, 128[a] 78 15 14 11 3.5[b]
4-methyl-2-heptanone 	43, 58, 59, 85, 41, 128[a] 77 34 26 12 5.0[b]
3-methyl-2-heptanone 	43, 72, 41, 57, 85, 128[a] 80 18 18 16 3.0[b]
3-octanone 	43, 57, 72, 99, 29, 128[a] 95 64 54 52 49[b]
6-methyl-3-heptanone 	57, 43, 72, 29, 81, 128[a] 76 61 43 38 12[b]

TABLE II (Continued)

Compound	Major Peaks
5-methyl-3-heptanone	57, 43, 71, 72, 29, 128[a]
	87 67 64 55 15[b]
4-methyl-3-heptanone	57, 71, 43, 86, 29, 128[a]
	74 71 49 38 2.5[b]
2-methyl-3-heptanone	57, 85, 43, 41, 71, 128[a]
	86 61 35 35 26[b]
4-octanone	71, 43, 85, 58, 41, 128[a]
	88 78 60 42 25[b]
3-methyl-4-heptanone	71, 57, 43, 41, 85, 128[a]
	70 58 21 15 15[b]
2-methyl-4-heptanone	57, 43, 85, 71, 41, 128[a]
	81 81 79 38 26[b]

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[a] Fragments appear in the sequence: base peak, 4 remaining largest peaks and parent mass peak.

[b] Per cent ionization of base peak.

using tetramethyl silane as an internal standard (δ=0). Mass spectra were obtained on a prototype of the LKB-9000 Mass Spectrometer-Gas Chromatograph[21] using the gas chromatographic inlet. The columns used on this instrument were a 0.02 in. x 50 ft capillary column coated with Apiezon L and a 5/32 in. x 10 ft glass column packed with 6% LAC 728 on 100/120 mesh acid washed Chromsorb W. The background was subtracted from all spectra. The mass spectrometer was operated at an electron energy of 70eV, accelerator voltage of 3.5KV and an ion source temperature of 290°.

6-Methyl-3-heptanone, 2-methyl-4-heptanone and 6-methyl-2-heptanone were purchased from Chemical Samples Co., and 2-octanone, 3-octanone, 4-octanone, and 5-methyl-3-heptanone from Aldrich Chemicals. 3-Methyl-2-heptanone was obtained from K & K Laboratories. The alcohols corresponding to 3-methyl-4-heptanone, and 2-methyl-3-heptanone were obtained from Columbia Chemicals and 4-methyl-3-heptanol from Aldrich. These alcohols were oxidized to the corresponding ketones using standard techniques.[22] The interpretation of the mass spectra of these compounds provided the only evidence of their identities. Since gas chromatography was used to introduce the compounds to the mass spectrometer, minor impurities should not be a factor.

Steam Distillation of P. barbatus Ants.--Ants were collected in the vicinity of Stillwater, Oklahoma, by trapping them in glass jars buried in the vicinity of the nest. For the extract from which 4-methyl-3-heptanone was isolated, 700 g were collected, killed by freezing and stored frozen until used. The ants were homogenized in a Waring Blendor and steam distilled until volatile compounds ceased to distill. After saturation with salt, the distillate was extracted with ether, dried with $MgSO_4$ and concentrated to 2 ml by distillation through an efficient column. The ether used for extraction was USP grade and contained small amounts of alcohol and ethyl acetate. 4-Methyl-3-heptanone was collected from the gas chromatograph using a 5/32 in x 12 in. coiled glass trap fitted with a connector of latex rubber and cooled in an ice bath. About 150 μl were obtained from the extract of 700 g of ants described above.

Ant Collection and Identification.--*P. rugosus* was supplied by Dr. D. E. Bryan, Tucson, Arizona, and *P. badius* by Dr. E. O. Wilson. The *P. californicus* ants were from a commercial ant farm and were donated by Miss Ann Eisenbraun. *P. occidentalis* and *P. desortorum* were collected in Oklahoma by Ken Vick. Identifications of these species and *P. Barbatus* were confirmed by Dr. Jerry Young of Oklahoma State University, and by Dr. A. C. Cole.

Chromic Acid Oxidation of P. barbatus Extract.--A 100-μl sample of concentrated extract of the steam distillate from ants was placed in a small test tube containing 1 ml of ether. One ml of oxidizing agent was added dropwise and the mixture was shaken for 10 minutes. (Reagent: 20 g of $Na_2Cr_2O_7{:}2H_2O$ and 15 ml of 96% H_2SO_4 diluted to 100 ml with H_2O).[22] The layers were separated with a pipette and the ether layer was washed with a saturated Na_2CO_3 solution, filtered through anhydrous $MgSO_4$ in a sintered glass funnel, and concentrated by distillation.

Lithium Aluminum Hydride Reduction of P. barbatus Extract.--An excess of $LiAlH_4$ (about 0.2 g) was placed in a small two-necked flask containing 3 ml of dry ether and fitted with a reflux condenser and a dropping funnel. While stirring, 100 μl of ant extract dissolved in a small amount of ether was added dropwise. The reactants were refluxed for 2 hours, and water was added to decompose excess reducing agent. The reaction mixture was filtered and the layers of the filtrate separated with a pipette. The ether layer was dried over $MgSO_4$, filtered, concentrated, and studied by gas chromatography.

Ant pyrolyses.--The mandibular glands of 5 *P. barbatus* dissected by Vick were placed in the pyrolysis loop of the valved gas chromatograph. The polyphenyl ether column described was used at 100° and a He pressure of 20 psi. The loop was heated to 200°, 10 nl of *cis*-hydrindan was injected as an internal standard, and the loop contents were diverted into the column. Under these conditions, 4-methyl-3-heptanone appeared in about 20 minutes and the standard in about 25 minutes. The identity was confirmed by comparison of retention times and enrichment of a standard sample of 4-methyl-3-heptanone injected into the loop with the hydrindan standard (Figure 3). The presence of 4-methyl-3-heptanone in all the *Pogonomyrmex* species studied was confirmed in this way. One mandibular gland or ant head was sufficient for these experiments.

Preparation of 2,4-dinitrophenylhydrazone.--The reagent consisted of 1 g of 2,4-dinitrophenylhydrazine dissolved in 20 ml of H_2SO_4 and diluted with 80 ml of water. The effluent of 4-Methyl-3-heptanone from the gas chromatograph was bubbled into a small amount of reagent. The precipitate formed was dissolved in ether and purified by chromatography on a thin layer plate coated with silica gel G using benzene as a developing solvent. After 3 purifications, the compound had a melting point of 118-126°.

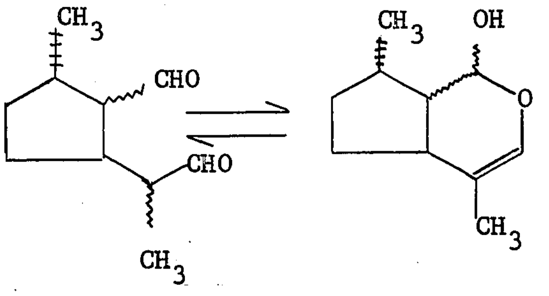
CHAPTER III

THE OCCURRENCE OF METHYLCYCLOPENTANE MONOTERPENOIDS IN THREE DOLICHODERINE SPECIES[23]

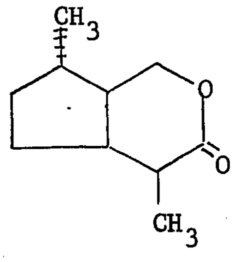
The investigation of chemicals in ants was given a decided push by Pavan when he discovered the methylcyclopentane monoterpenoid, iridomyrmecin in the dolichoderine ant, Iridomyrmex humilis and proved its structure.[24,25] This lactone was the second of a new class of natural products to have its structure elucidated and interest was heightened by the fact that it possessed both antibiotic and insecticidal activity. The extraction of four species of Iridomyrmex ants by Cavill and workers led to the discovery of several terpenoids related to iridomyrmecin.[26] A lactone from I. nitidus (Mayr) was subsequently proven to be isoiridomyrmecin, an epimer of iridomyrmecin.[27] I. detectus (F. Smith) and I. conifer (Forel) contained iridodial whose structure was established.[28] These and the related compounds dolichodial and isodihydronepetalactone have been found in several Australian Dolichoderus and Iridomyrmex species[29,30] (Figure 4). All are assumed to function as defense substances.

Several authors have proposed that the lactones result biosynthetically from iridodial.[2,31] However, none of them have yet been found with iridodial in a natural system.

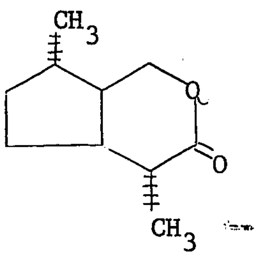
The structure and absolute configuration of the methylcyclopentane monoterpenoids have been derived by chemical correlation with nepetalic



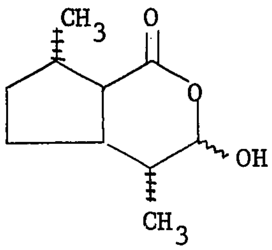
Iridodial



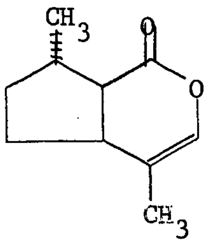
Iridomyrmecin



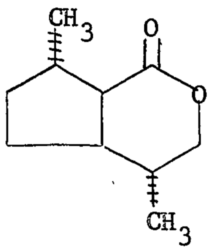
Isoiridomyrmecin



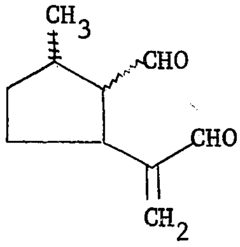
Nepetalic Acid



Nepetalactone



Isodihydro-
nepetalactone



Dolichodial

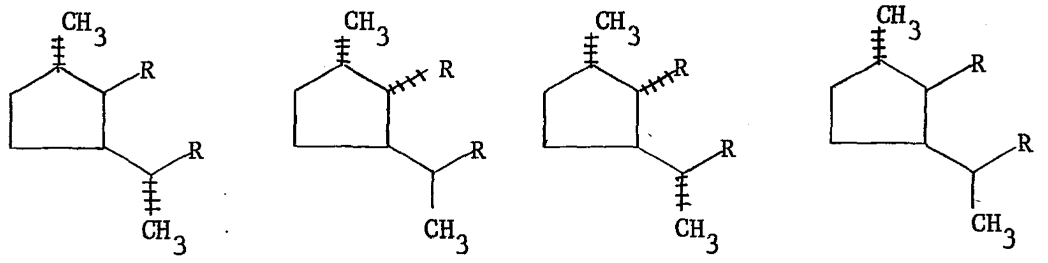
Figure 4. Methylcyclopentane Monoterpenoids
Related to Iridodial

acid and nepetalactone and the nepetalinic acids.[32,33,34] The chemical correlation has been confirmed by X-ray crystallography of iridomyrmecin and isoiridomyrmecin.[35] There are four isomers of iridodial because of possible epimerization at the carbon α to each carbonyl group. Iridodial isolated from I. detectus was reported to consist mainly of the two cis, trans isomers α and δ (Figure 5) by comparison with synthetic iridodial[36] and conversion to the α- and δ-nepetalinic acids.[28] Synthetic iridodial of the δ stereochemistry but of opposite configuration gave a mixture of all four isomers with the α isomer predominating upon heating with acetic acid or long standing.[36]

RESULTS AND DISCUSSION

Chromatographic and mass spectrometric studies of the extracts of volatile chemicals from three dolichoderine species, I. pruinosus analis (E. Andre´), Tapinoma sessile and Conomyrma pyramicus pyramicus (Roger) permitted the tentative identification of several compounds. The chromatographs of these extracts are shown in Figures 6-8 and all the compounds from which good mass spectra were obtained were numbered. The major peaks from the mass spectra of these compounds are recorded in Table III.

Iridomyrmex pruinosus produces 2-heptanone (B-1) as previously reported by Blum.[7] Compound C-2 of the T. sessile extract was shown to be 6-methyl-5-hepten-2-one. Thus, Wilson's observation[16] that T. sessile is alarmed by this compound from the anal glands of T. nigerrimum is not surprising. Compound C-3 of parent mass 124 was present in considerable quantities in T. sessile. Reduction of the whole ant extract with $LiAlH_4$ gives a new compound of mass 126 that corresponds



Nepetalinic acid,					
	$R = CO_2H$	α	β	γ	δ
	mp	85°	111°	115°	117°
Iridodial,	$R = CHO$	α	β	γ	δ
Diols,	$R = CH_2OH$	α	β	γ	δ

Figure 5. Stereochemical Correlation of Iridodials With the Nepetalinic Acids

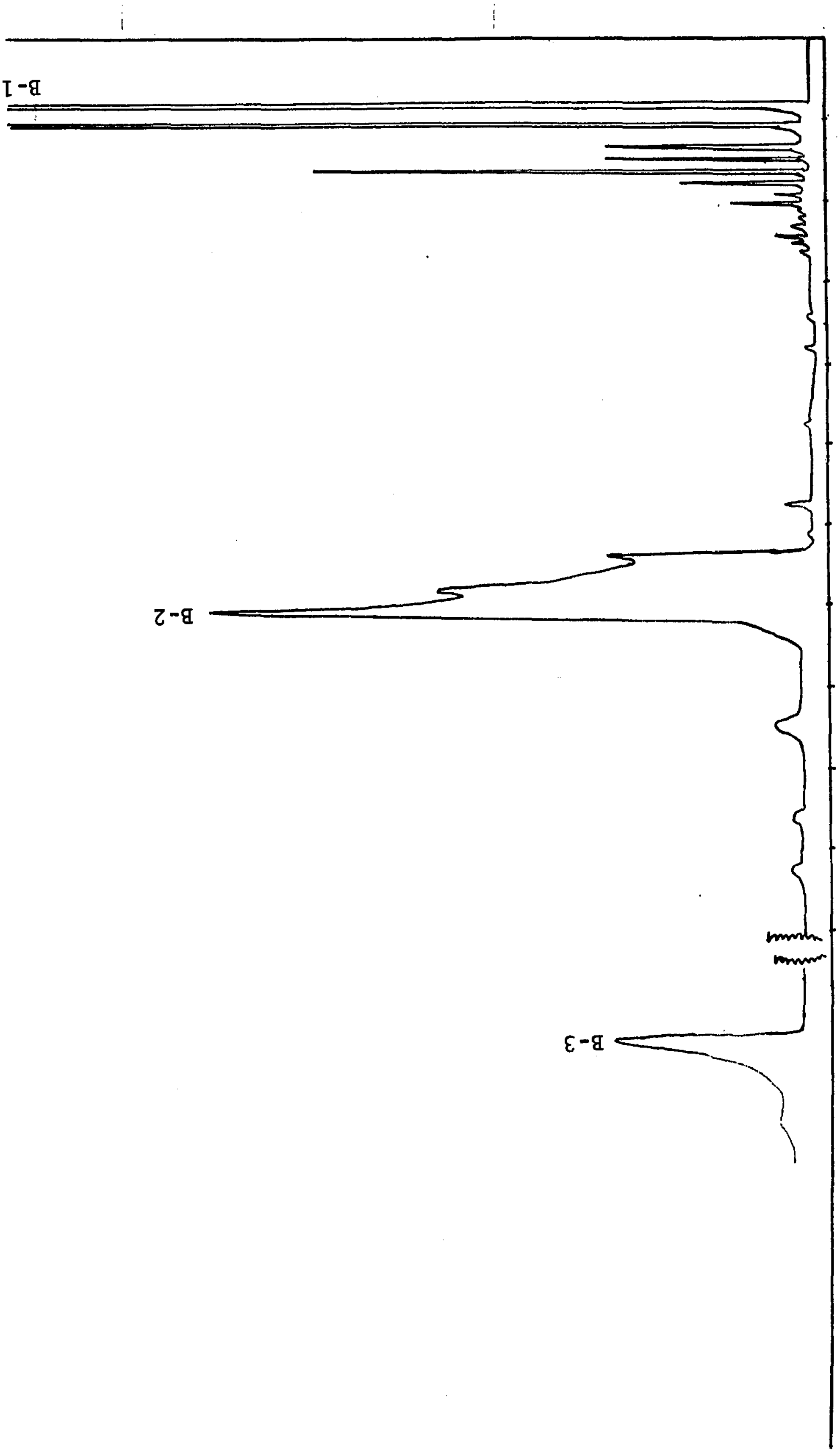


Figure 6. Volatile Compounds from *Iridomyrmex pruinosus*

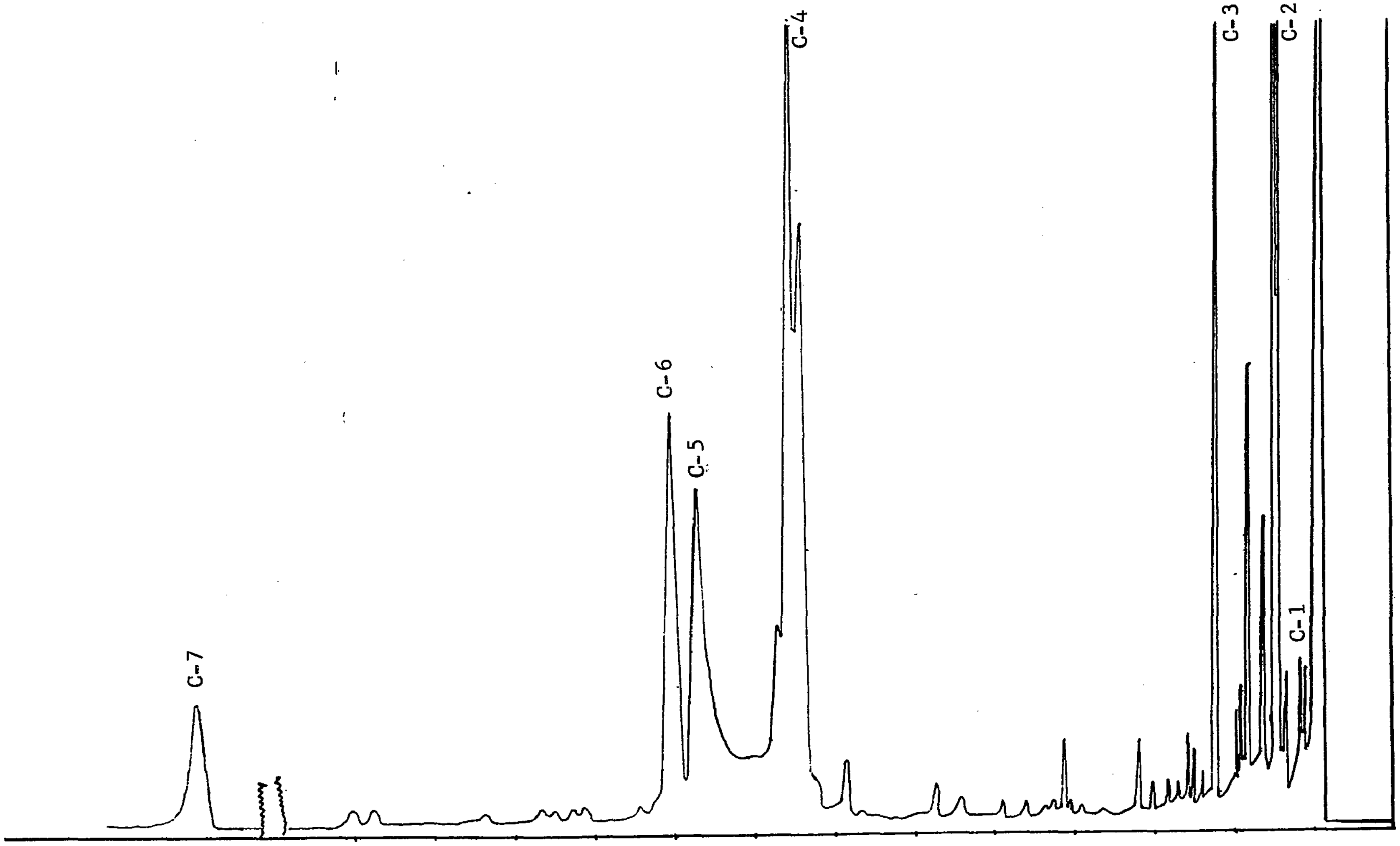


Figure 7. Volatile Compounds from *Tapinoma sessile*

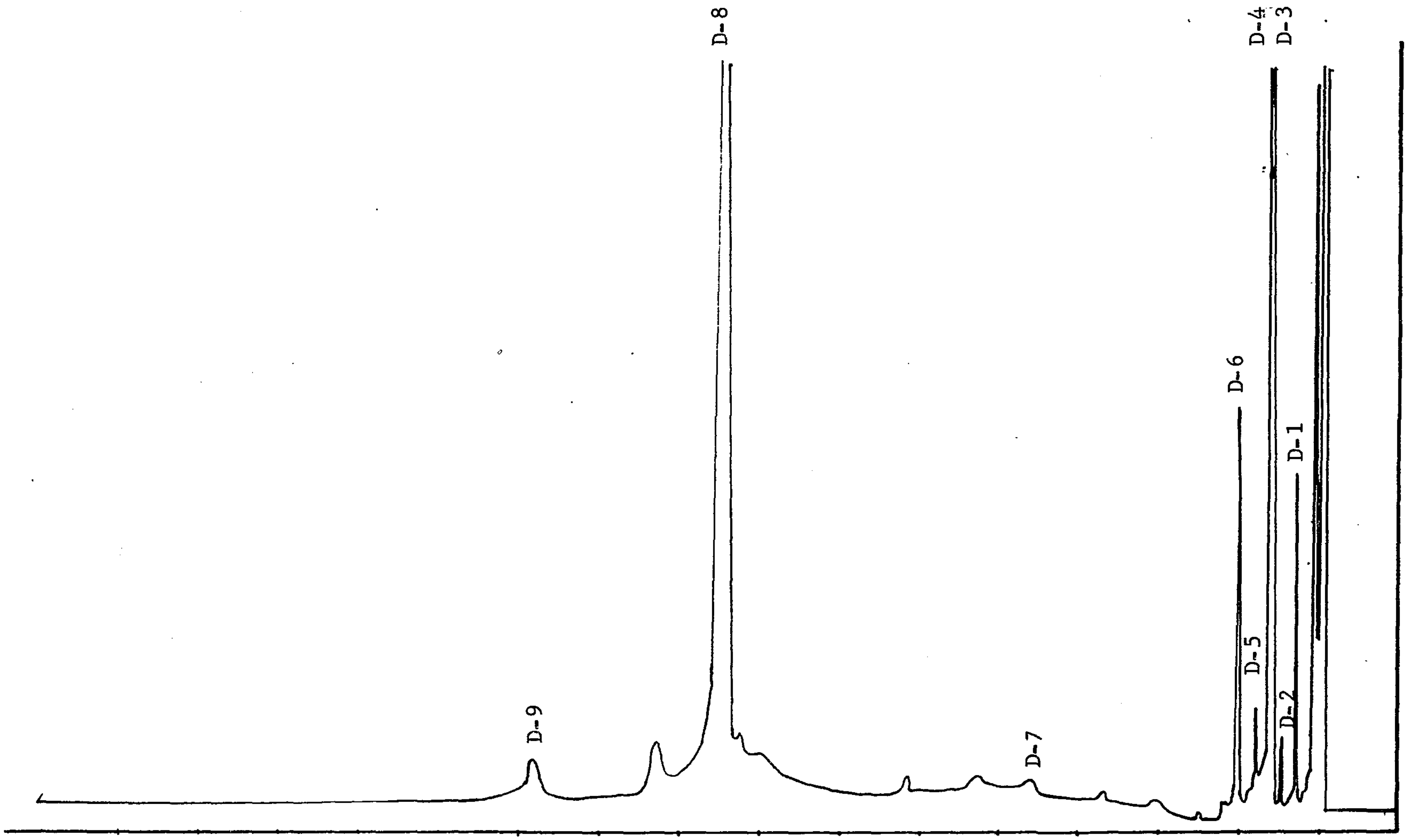


Figure 8. Volatile Compounds from <u>Conomyrma pyramicus pyramicus</u>

TABLE III

MASS SPECTRA OBTAINED FROM STUDIES ON DOLICHODERINE ANTS

B-1[a]		B-2[a]		B-3[a]		C-1[a]	
2-heptanone		Iridodial		Iridomyrmeçin		4-me-2-pentanone	
114	13%	168	7.8%	168	5.7%	100	12%
58	100	81	100	95	100	43	100
43	55	110	62	109	57	58	25
59	24	109	53	81	39	57	14
71	24	58	50	82	30	41	11
99	5.8	95	37	68	29	85	11

C-2[a]		C-3[a]		C-4[a]		C-5[b]	
6-me-5-hepten-2-one		Unknown		Iridodial		Unknown	
126	11%	124	54%	168	--	190	44%
43	100	68	100	81	100%	175	100
41	56	67	80	41	91	122	36%
69	39	96	37	67	77	161	22%
55	37	81	30	58	75	162	22
108	37	39	29	55	68	176	13

C-6[b]		C-7[a]		C-8[a]		D-1[b]	
Unknown		Isoiridomyrmecin		C-3 reduced		Unknown	
168	25%	168	19%	126	4.9	98	100%
147	100	95	100	93	100	69	37
132	83	67	90	108	59	41	24
95	76	81	82	67	55	83	23
109	55	41	56	41	50	55	16
82	35	109	55	69	50	43	14

D-2[b]		D-3[b]		D-4[a]		D-5[b]	
Unknown		6-me-5-hepten-2-one		6-me-5-hepten-2-ol		Unknown	
106	100%	126	33%	128	14%	124	85%
43	70	108	100	95	100	68	100
71	70	43	44	41	91	96	65
86	67	69	39	110	57	81	49
57	67	111	38	69	45	95	38
72	52	71	25	71	36	82	37

TABLE III (Continued)

D-6[b] Unknown		D-7[b] Unknown		D-8[b] Iridodial		D-9[a] Unknown	
148	13%	154	3%	168	12%	236	3%
69	100	111	100	81	100	81	100
58	89	84	52	111	96	41	82
111	77	93	52	109	84	111	74
85	66	43	45	58	71	55	68
146	21	81	34	135	57	67	61
				110	55		

Silanized α-iridodiol[a]

316	xx	75	55%
226	13%	81	55
73	100	103	45
143	62	147	42

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[a] Spectrum taken at 70 eV

[b] Spectrum taken at 20 eV

with C-3 in size relative to the size of other peaks in the gas chromatogram (mass spectrum C-8). The identity of this compound was never established, however. Compound C-1 was identified from its mass spectral fragmentation pattern to be either 4-methyl-2-pentanone or 2-hexanone (parent mass m/e 100, base peak m/e 43, large peak at m/e 58). However comparison of retention times and mass spectra with authentic compounds still proved inconclusive.

C. pyramicus also contained 6-methyl-5-hepten-2-one (D-3). Blum reported that the major volatile constituent in these ants was 2-heptanone.[37] This compound could not be detected in our extracts of *C. pyramicus* collected near Stillwater. *C. pyramicus bicolor* W. M. Wheeler, collected near Tucson, Arizona, also contained 6-methyl-5-hepten-2-one and not 2-heptanone. *C. pyramicus* ants which were collected near Alexandria, Louisiana contained 2-heptanone. Coloration, markings and the place of collection lead to the belief that the Oklahoma ants are *C. pyramicus pyramicus* (Roger) and the Louisiana ants are *C. pyramicus flavopectus* M. R. Smith.

A compound in the extract of *C. pyramicus* which is not separated from 6-methyl-5-hepten-2-one in Figure 8 gave a mass spectrum quite similar to 6-methyl-5-hepten-2-ol (D-4). Compound D-1 has a parent mass of 98. Its splitting pattern resembles that of an α, β-unsaturated 6 carbon aldehyde or of a C7 α olefin. The combination gas chromatographic-mass spectrometric analysis of the extracts from these three ants indicated the presence of more than one isomer of iridodial in each ant (B-2, C-4, D-8). It was not possible to separate the four dialdehydes completely using gas chromatography. Consequently they were identified by conversion to silyl ethers of iridodiols and gas

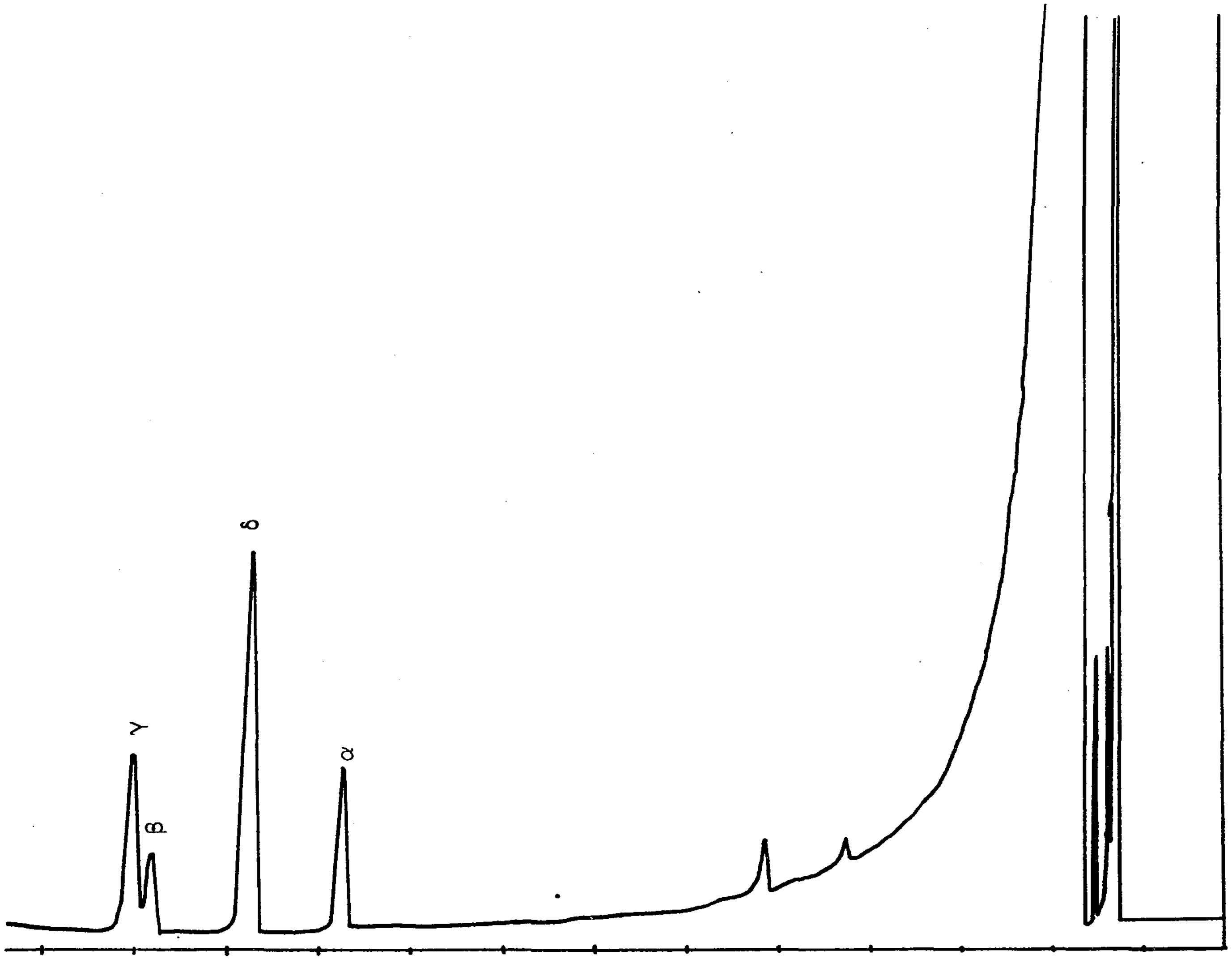

Figure 9. Gas Chromatogram of Standard Iridodiols

chromatographic comparison with the silyl ethers of nepetadiol standards. The standards were obtained by silylation of lithium aluminum hydride reduction products of nepetalinic acids remaining from earlier studies.[38,39]

To obtain iridodiols from ants, about 200 were crushed in ether, filtered and the extract reduced immediately with $LiAlH_4$. The ether was evaporated and the diols silanized and chromatographed immediately. Figure 9 shows a chromatogram of the four diols. The identity of the isomers present in the ants was confirmed by comparison of retention times and enrichment of reduced ant extracts with standard iridodiols to determine which peaks increased in size.

To further demonstrate the presence of iridodial in these ants, the mass spectra of the peaks presumed to be silanized diols from the ant extracts were obtained, compared with the mass spectra of the standard silanized diols obtained from nepetadiols and found to be identical. The mass spectra of all four isomers of these compounds are similar and only that of standard α-iridodiol silyl ether has been included in Table III. No unidentified compounds which could give iridodiol were present in these ant extracts nor was iridodiol itself present before reduction. No new compounds appeared in the chromatograms when the extracts were treated with diazomethane showing the absence of acidic products which might be reduced to iridodiols.

Each of the species contained over 80% of a single iridodial isomer. (This allows for reduction of the iridolactones discussed in the next paragraph). This isomer was different in each case and at least a trace of each isomer was present in all three species of ants (Table IV). It is doubtful that extensive epimerization occurred

TABLE IV

GAS CHROMATOGRAPHY ANALYSIS OF THE SILYL ETHERS OF THE IRIDODIOLS

	Retention times of silyl ethers (min)	*I. pruinosum* (%)	*C. pyramica* (%)	*T. sessile* (%)
α-Iridodial	50	A	Trace	B
β-Iridodial	60	A	B	A
γ-Iridodial	61	A	A	A
δ-Iridodial	55	B	Trace	A

Column and conditions: 0.02-in. x 450-ft, lined with a film of Monsanto OS-138 (a six-ring polyphenyl ether, bis [*m*-(*m*-phenoxyphenoxy) phenyl]ether) at 120° C, 30 psi helium.

A: Minor peak, less than 12%.

B: Major peak, greater than 80%.

during isolation. The extracts were immediately reduced. Iridodiol has been reported to be stable to distillation without loss of configuration.[36] Reduction itself would not be expected to epimerize the dialdehydes.[40] The isomer ratio did not change significantly if the extract was allowed to stand for 24 hours before reduction.

By comparison of gas chromatographic retention times, enrichment experiments and mass spectrometry data, it was found that iridomyrmecin (B-3) is present in _I_. _pruinosus_ and isoiridomyrmecin (C-7) in _T_. _sessile_. In each case the iridolactone possesses the stereochemistry of the predominant iridodial isomer in that ant, as would be expected if the proposed biosynthetic routes are correct. Iridolactone of the β configuration could not be detected in _C_. _pyramicus_.

_C_. _pyramicus_ _flavopectus_ produced iridodial of the α configuration instead of β iridodial which was the major isomer in _C_. _pyramicus_ _pyramicus_. Apparently variation in isomers of ant compounds can occur even between two very closely related species. Several samples of each of the three species studied were tested over the course of two years and the results were essentially identical.

It is not clear what is the significance of the production of different isomers of a defense chemical, iridodial, by ants of the same subfamily. Isoiridomyrmecin and iridomyrmecin have been reported to have different biological activities.[35] It was assumed that these differences were related to the overall shape of the molecule and not to the epimeric relationship. Presumably the various lactol structures which may result from cyclization of the epimeric iridodials could provide a variety of conformations. At this time, there are no studies available which clearly define this point. It seems likely that these isomer variations are of biosynthetic rather than of entomological significance.

EXPERIMENTAL

Gas chromatography of the ant extracts was performed with the Instruments, Inc. valved gas chromatograph fitted with the 0.02 in. x 450 ft polyphenyl ether column described in the previous chapter. The column conditions were 150°, 35 psi He. The silanized iridodiols were separated using the same system with column conditions of 120° and 30 psi He.

Mass spectra were obtained on the LKB prototype described earlier. The ant extracts were run using an Apiezon L capillary column (0.02 in. x 50 ft) at 100° and a flow of 15 ml/min. The accelerator voltage was 3.5 KV and the ion source temperature 290°. The spectra were taken using electron energies of both 20 and 70 eV. The iridodiol silyl ethers were introduced into the mass spectrometer through the polyphenyl ether capillary column held at 115° and a flow rate of 15 ml/min. The spectra were taken at 70 eV electron energy and a source temperature of 310°. The background was subtracted from all spectra.

Tapinoma sessile, *C. pyramicus pyramicus* and *I. pruinosus analis*. were collected by K. Vick in the vicinity of Stillwater, Oklahoma. Several samples were collected over a period of two years and gas chromatography of the extracts indicated all were essentially identical. The extracts used to obtain the mass spectra shown in Table III were obtained from 119 g of *T. sessile*, 5.6 g of *C. pyramicus* and 42 mg of *I. pruinosus*. This is a reasonable estimate of the relative amounts of these ants that were available for study. *C. pyramicus bicolor* were collected in the vicinity of Tucson, Arizona by Mrs. D. E. Bryan, and C. *pyramicus flavopectus* by K. Vick near Alexandria, Louisiana. Identification of species was by K. Vick, Dr. W. A. Drew and Dr. J. Young. Ant extracts were obtained in a manner identical to that described for P. *barbatus*.

Identifications presented as definite in this chapter were confirmed by comparison of retention times and mass spectra with authentic samples of the compounds involved. The presence of 6-methyl-5-hepten-2-one and absence of 2-heptanone in C. *pyramicus pyramicus* and the converse in C. *pyramicus flavopectus* were demonstrated by pyrolysis experiments identical to those used to prove the presence of 4-methyl-3-heptanone in *Pogonomyrmex* species. Five ants were sufficient for a pyrolysis run.

Reduction of Ant Extracts.--Using a glass rod, about 200 ants were crushed in a small beaker of ether. Without drying, this extract was immediately decanted dropwise into a small flask fitted with a reflux condenser and containing a pea-sized lump of $LiAlH_4$ dissolved in 5 ml of ether. The reaction mixture was refluxed for 2 hours and the excess hydride decomposed with water. The solution was acidified, extracted with ether and the extract dried over $MgSO_4$. The ether was then evaporated and the residue used to prepare silyl ethers.

Preparation of Silyl Ethers.--To an ant extract of this size in a small vial, was added 0.1 ml of pyridine followed by 0.01 ml of hexamethyldisilazane and 0.003 ml of trimethylsilyl chloride.[41] The extracts were allowed to stand for 5 minutes then chromatographed on the polyphenyl ether capillary column.

Preparation of Standard Iridodiols.--To an excess of $LiAlH_4$

dissolved in 10 ml of ether was added 100 mg of nepetalinic acid. After reduction and decomposition of excess hydride, the aqueous layer was extracted before acidification to obtain the iridodiol. The aqueous layer was then acidified and extracted again with ether to obtain any lactones formed during the reduction.

CHAPTER IV

VOLATILE COMPOUNDS PRESENT IN NINE ADDITIONAL SPECIES

In addition to the ants already mentioned, extracts of volatile compounds have been obtained and chromatographed under standard conditions from nine additional ant species. Seven of these species were also studied using mass spectrometry. Several compounds have been tentatively identified in these extracts but for a variety of reasons, usually difficulty in obtaining the ants or difficulty in identifying the compounds whose mass spectra have been obtained, these ants have not been studied exhaustively. The data obtained from these ants are presented in this chapter.

RESULTS AND DISCUSSION

Eciton nigrescens (Cresson) and Eciton opacithorax Emery (Figures 10-11).--These are the only species studied from the Dorylinae subfamily. The only mass spectrum obtained from E. opacithorax that could be interpreted was that of compound K-1 (Figure 11, Table V). It has a parent mass ion at m/e 114 and a splitting pattern that corresponds well with that of standard 2-heptanone. E. nigrescens was available in larger amounts and could be studied in greater detail. Compound F-2 also seems to be 2-heptanone. The fragmentation of F-3 indicates that it is a saturated C-11 hydrocarbon, probably n-undecane. F-10 and F-11 have parent ions at odd mass numbers. Their spectra are closely related to the published mass spectra of indole and skatole respectively. Compound F-10 has a retention time identical with that of indole. A peak corresponding to F-11 appears in the *E. opacithorax* extract (K-2).

Novomessor cockerelli (E. Andre') (Figure 12).--This ant contains a number of saturated hydrocarbons in its abdomen. From mass spectrometry it is concluded that *Novomessor* produces all the straight-chain hydrocarbons from C_{11} to C_{19}. In addition, branched chain C_{18} and C_{16} hydrocarbons are present along with a number of compounds whose parent mass peak could not be determined. This ant does not possess a sting as does other ants in which hydrocarbons have been found and the function of these hydrocarbons is uncertain.

Pheidole dentata Mayr and Apaenogaster texana Emery (Figures 13-15).--Chromatograms are presented for both major and minor *Pheidole* workers. Apparently both castes contain largely the same compounds. The only spectra from *Pheidole* which could be interpreted were those of compounds G-3, G-4, G-10 and G-12. These are probably n-pentadecane, n-hexadecane, n-octadecadecane and n-nonadecane respectively. *Aphaenogaster* also contains pentadecane.

Crematogaster lineolata punctulata Emery and Crematogaster laeviuscula Mayr (Figures 16-17).--*C. lineolata* proved an interesting species although only two compounds were tentatively identified from the extract. They are 3-octanone (I-1) and 3-decanone (I-3). The extract contains a number of compounds of molecular weights 248, 246, and 274 with base peaks at m/e 94 which are obviously closely related. These compounds could not be identified from the information at hand, however, and *C. lineolata* could not be obtained in the quantities necessary to study these compounds in greater detail. No identifications were possible from the spectra obtained from C. laeviuscula.

Correlations Between Species.--The gas chromatographic tracings of extracts of Camponotus pennsylvanicus (DeGeer), and Monomorium minimum (Buckley) under standard conditions are shown in Figures 18 and 19. No mass spectral studies were made of these ants. Camponotus contained formic acid as would be expected for a formicine ant.

In several cases, similar compounds seem to appear in several species. The most obvious example of this is the presence of straight-chain hydrocarbons in several species. Some of the compounds which have not been identified also appear in more than one species. Compounds G-5 and G-8 of Pheidole dentata, J-3 of Crematogaster laeviuscula, H-2 of Aphaenogaster texana, and F-9 of Eciton nigrescens all have an apparent molecular weight of 220 with the base peak at m/e 205. Although neither the mass spectra nor the retention times of these compounds are identical they are similar enough to suggest a close relationship.

Compounds G-9 of P. dentata, H-3 of A. texana and D-9 of C. pyramicus all have a molecular weight of 236 with large peaks at m/e 67 and 81. C. laeviuscula also has a compound of this molecular weight, but since this spectrum was taken at 20 eV, direct comparison is difficult. Three of the four species that contain the "220 compound" mentioned above have a compound of parent mass 236 closely following this compound in the gas chromatogram. Compounds G-1 of P. dentata, F-5 of E. nigrescens and D-6 (20 eV) of C. pyramica may also be related, having parent masses of 148 with large fragments at m/e 146.

With the use of the gas chromatography-mass spectrometer combination, identification of compounds from ants is no longer an almost impossible task. However, this knowledge has a limited utility unless combined with knowledge of the source of these chemicals in the ants and the behavioral function of the compounds. Studies of this kind may be valuable in differentiating between closely related species and in discovering new relationships between species not so closely related.

EXPERIMENTAL

Mass spectra were taken on the instrument described for earlier experiments.

N. cockerelli, A. texana, E. nigrescens, and P. dentata spectra were taken using the 0.02 in. x 450 ft polyphenyl ether capillary column at a temperature of about 140° and a flow rate of 15 ml/min. The electron energy was 70 eV, the accelerator voltage 3.5 KV, and the ion source was at a temperature of 310°.

E. opacithorax and C. laeviuscula spectra were taken at an electron energy of 20 eV with a source temperature of 290° using the 0.02 in. x 50 ft Apiezon L capillary column.

Spectra for C. lineolata were taken at 70 eV, 3.5 KV and source temperature of 290°, using a glass column (5/32 in. x 10 ft.) packed with 6% LAC 728 on acid washed Chromsorb W 100/120 mesh. The flow rate was 15 ml/min and the temperature was programed from 120 to 150°. The background noise was subtracted from all spectra.

More than one sample was collected for each of these species over several years time. One of the larger samples is described below for each variety to give an idea of the quantity of ants involved in studies of that species. No major differences between samples were noted. Ants were identified by K. Vick and Drs. J. Young and W. A. Drew.

Unless stated differently, they were collected by K. Vick near Stillwater, Oklahoma. Ants were killed by freezing and stored frozen until workup. The treatment was the same as that described earlier--homogenization, steam distillation, extraction and concentration.

Aphaenogaster texana: 60 mg of ants collected in April, 1965.

Camponotus pennsylvanicus: 2.0 g of ants collected in the spring of 1966. These ants smelled strongly of formic acid.

Crematogaster laeviuscula: 100 g of ants collected by D. McGurk and K. Vick near Sulphur, Oklahoma, June 1967.

Crematogaster lineolata punctulata: 28 g of ants collected from autumn 1965 to April 1966.

Eciton nigrescens: 16 g from a laboratory colony.

Eciton opacithorax: 1 g collected in April 1965 near Morrison, Oklahoma by Dr. J. Young.

Monomorium minimum: 20 mg collected in March, 1965.

Novomessor cockerelli: 100 g of ants collected by D. McGurk and K. Vick near Tucson, Arizona, November 1966.

Pheidole dentata: 5.1 g of major workers collected in May, 1966; 4.7 g of minor workers collected at the same time.

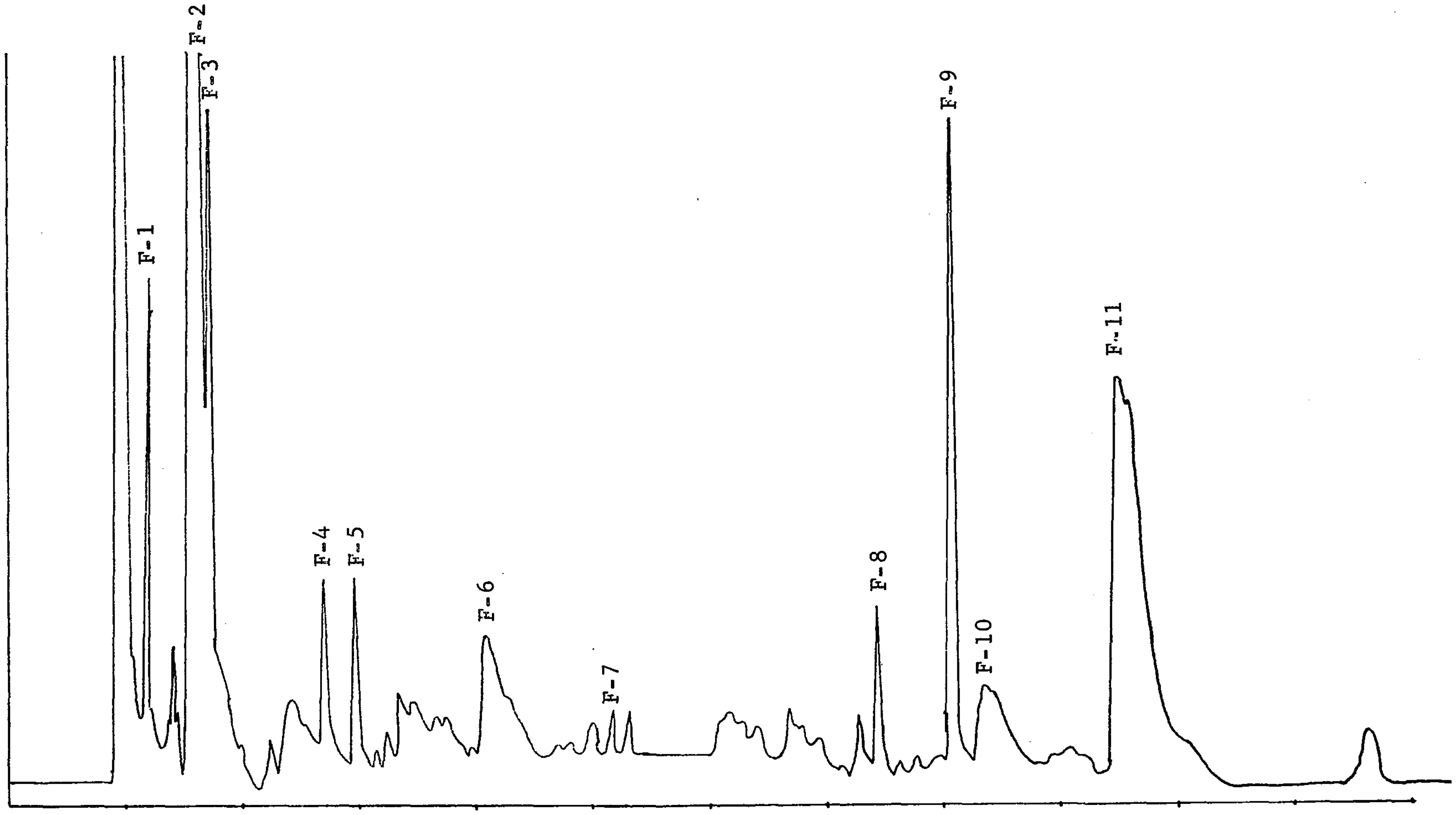

Figure 10. Volatile Compounds from Eciton nigrescens

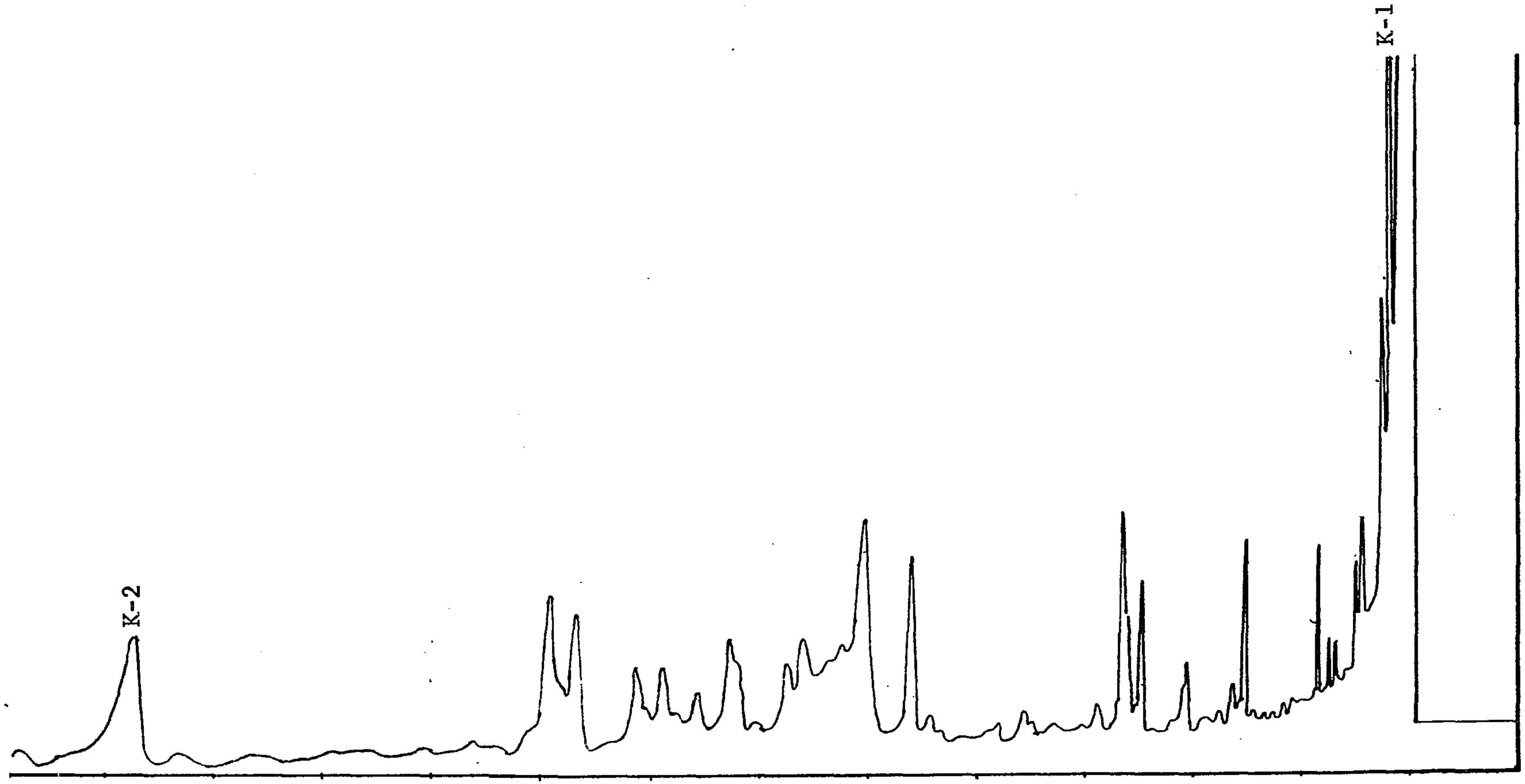

Figure 11. Volatile Compounds from Eciton opacithorax

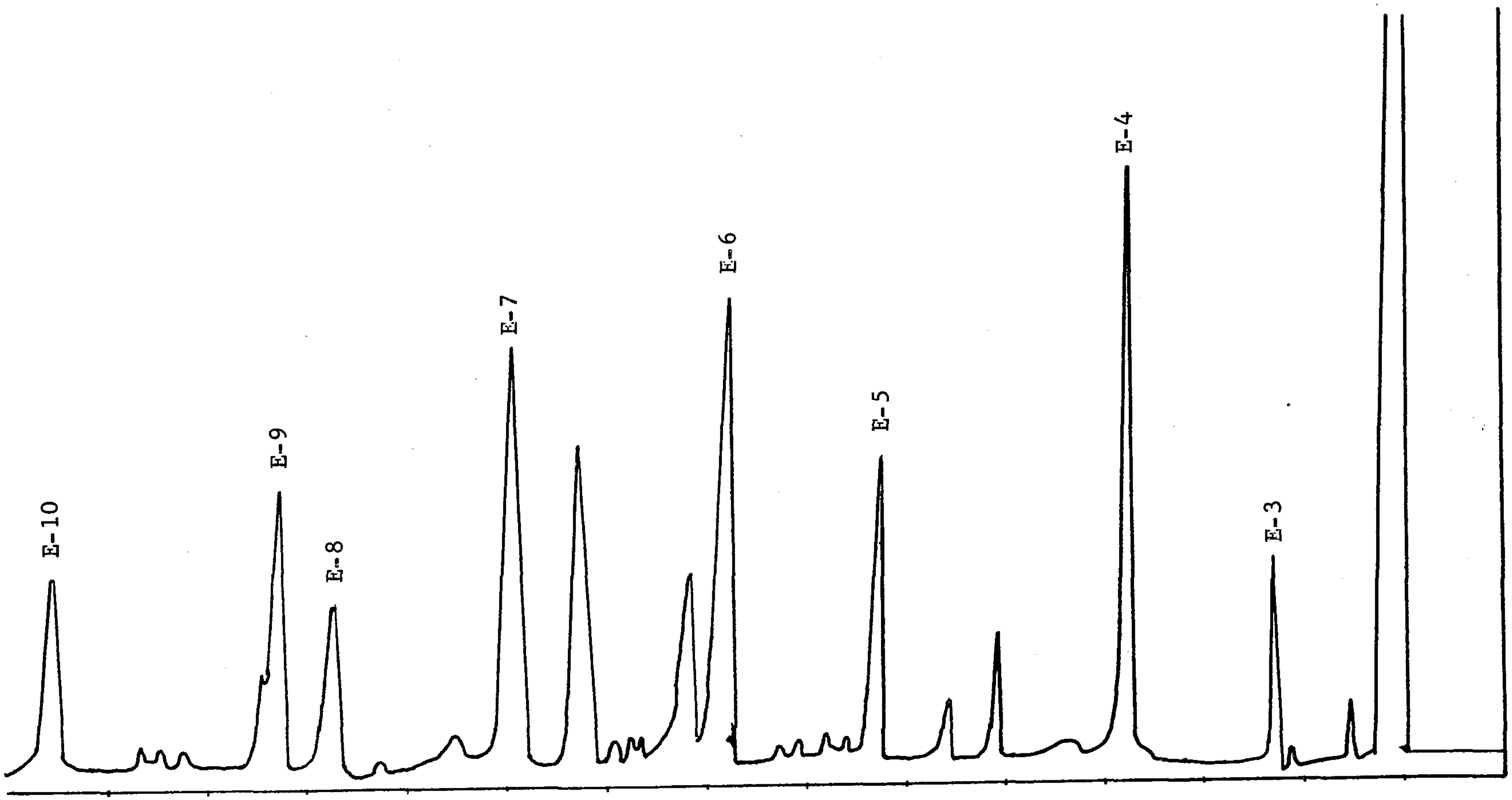

Figure 12. Volatile Compounds from <u>Novomessor cockerelli</u>

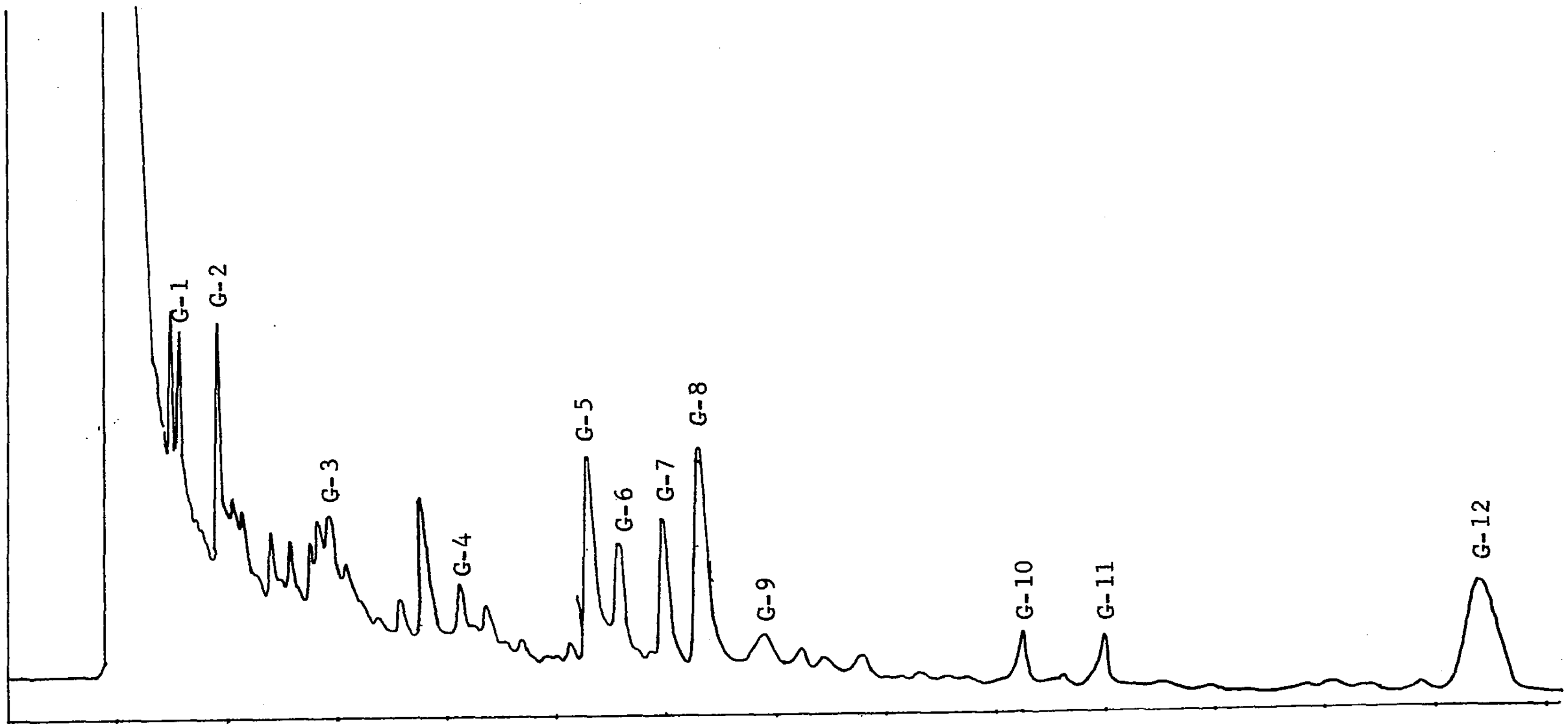

Figure 13. Volatile Compounds from *Pheidole dentata* minor workers

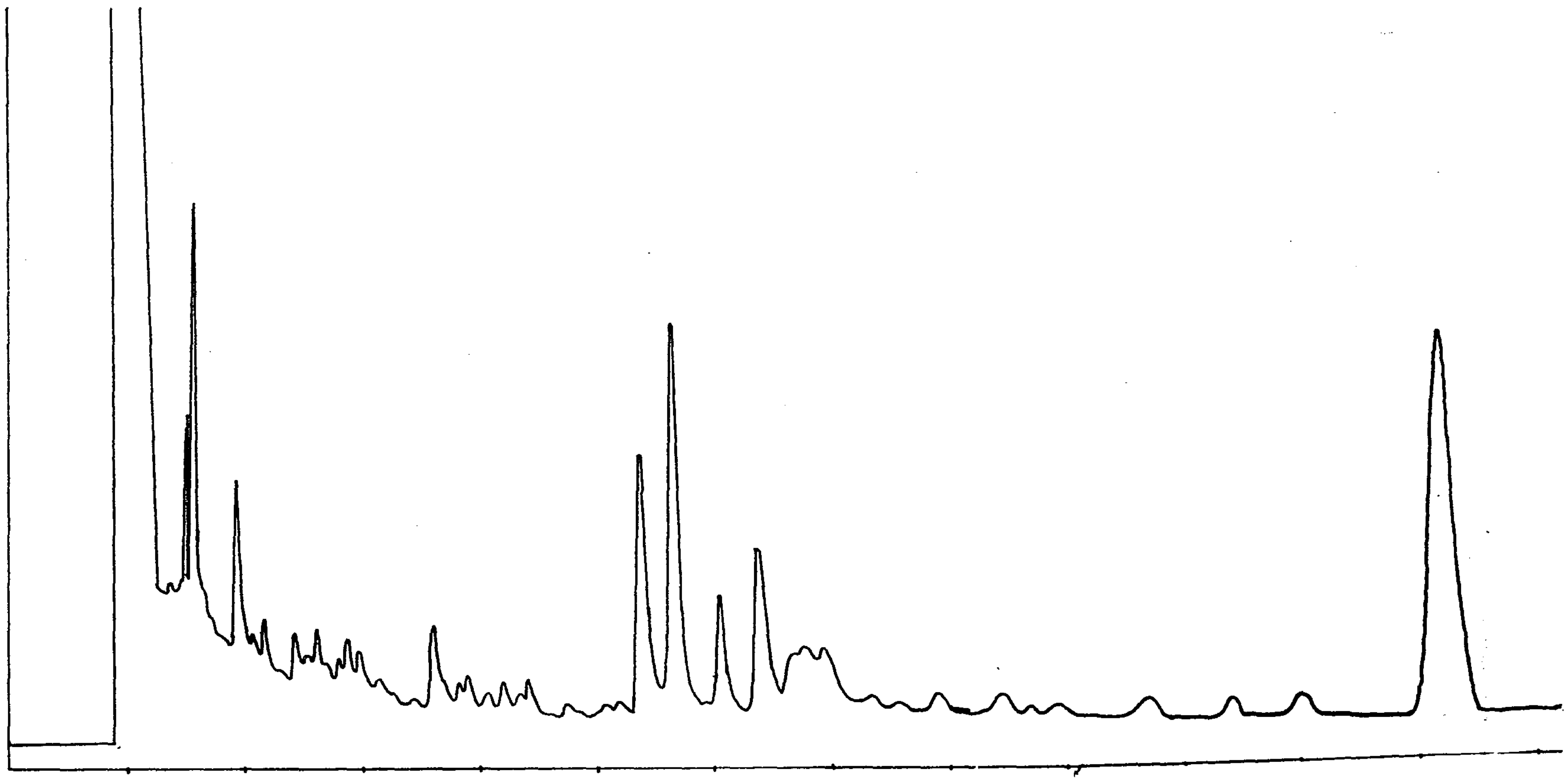

Figure 14. Volatile Compounds from *Pheidole dentata* Major Workers

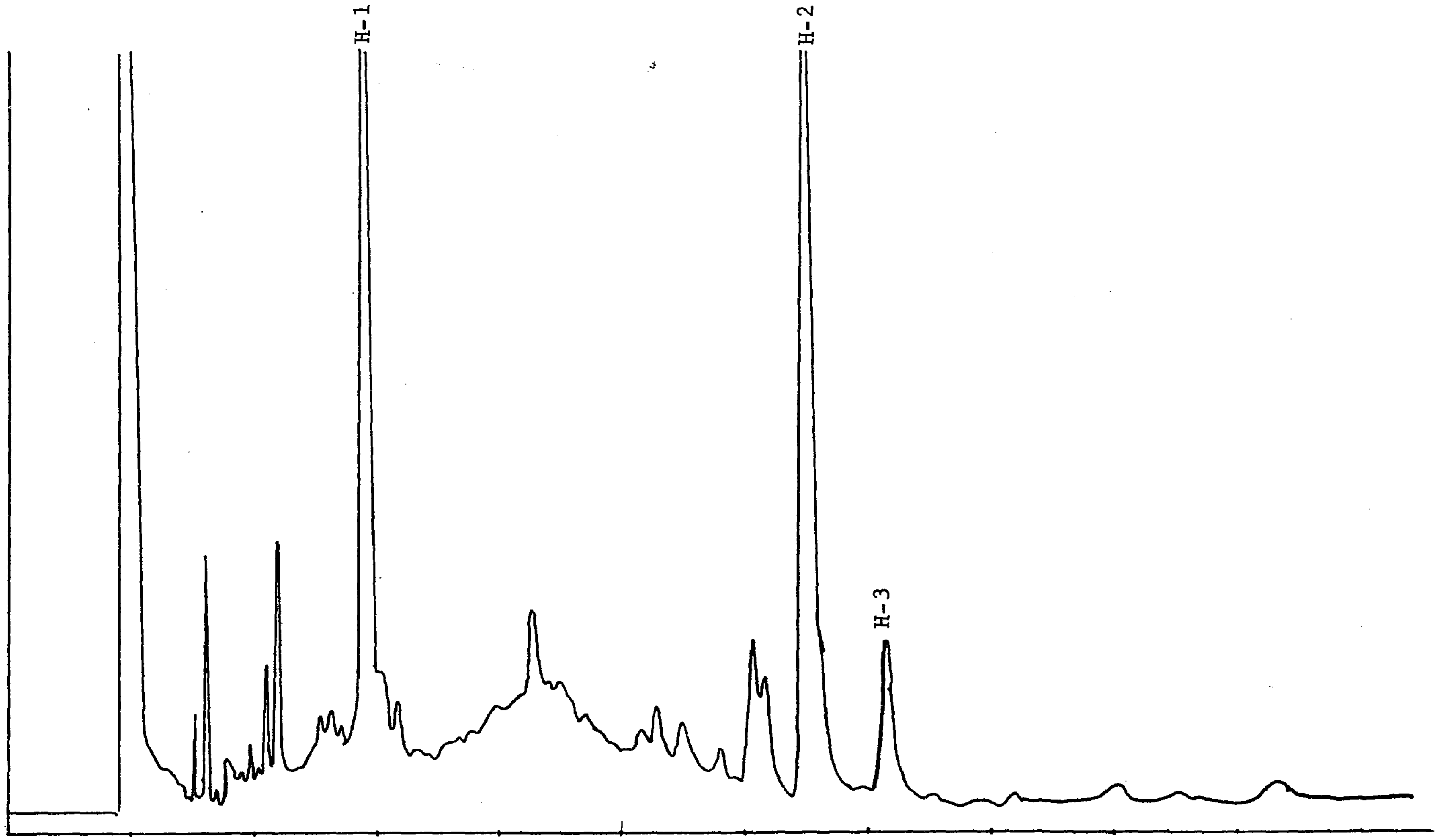

Figure 15. Volatile Compounds from <u>Aphaenogaster texana</u>

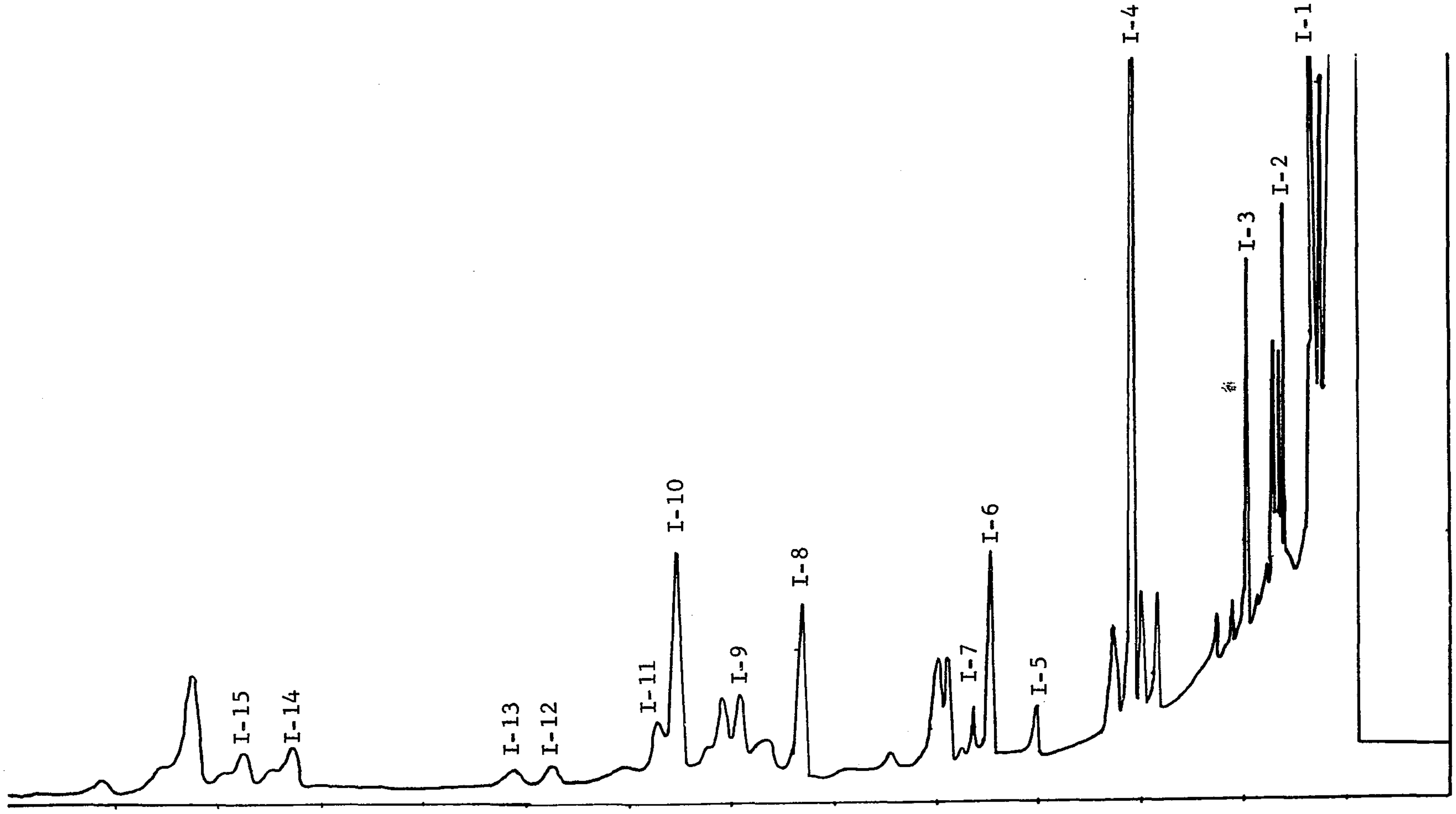

Figure 16. Volatile Compounds from *Cremotogaster lineolata punctulala*

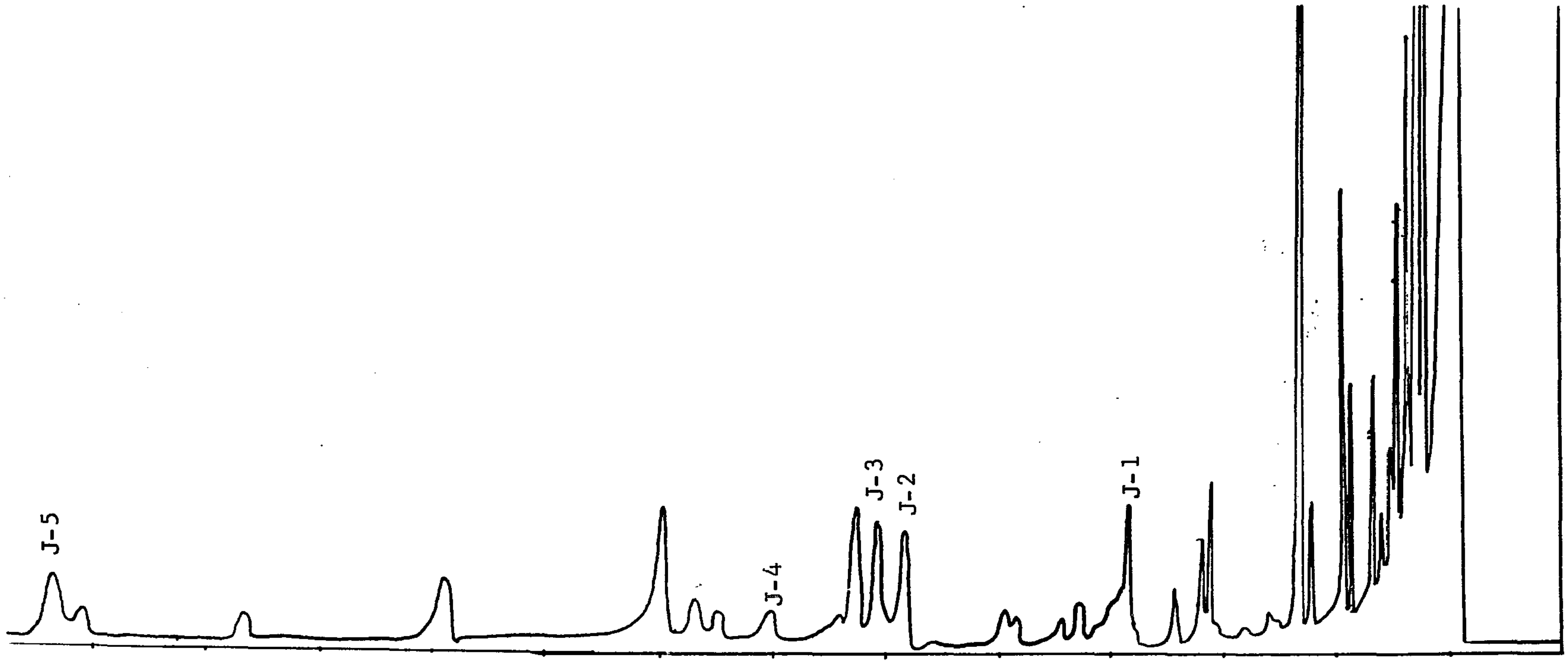

Figure 17. Volatile Compounds from <u>Cremotogaster laeviuscula</u>

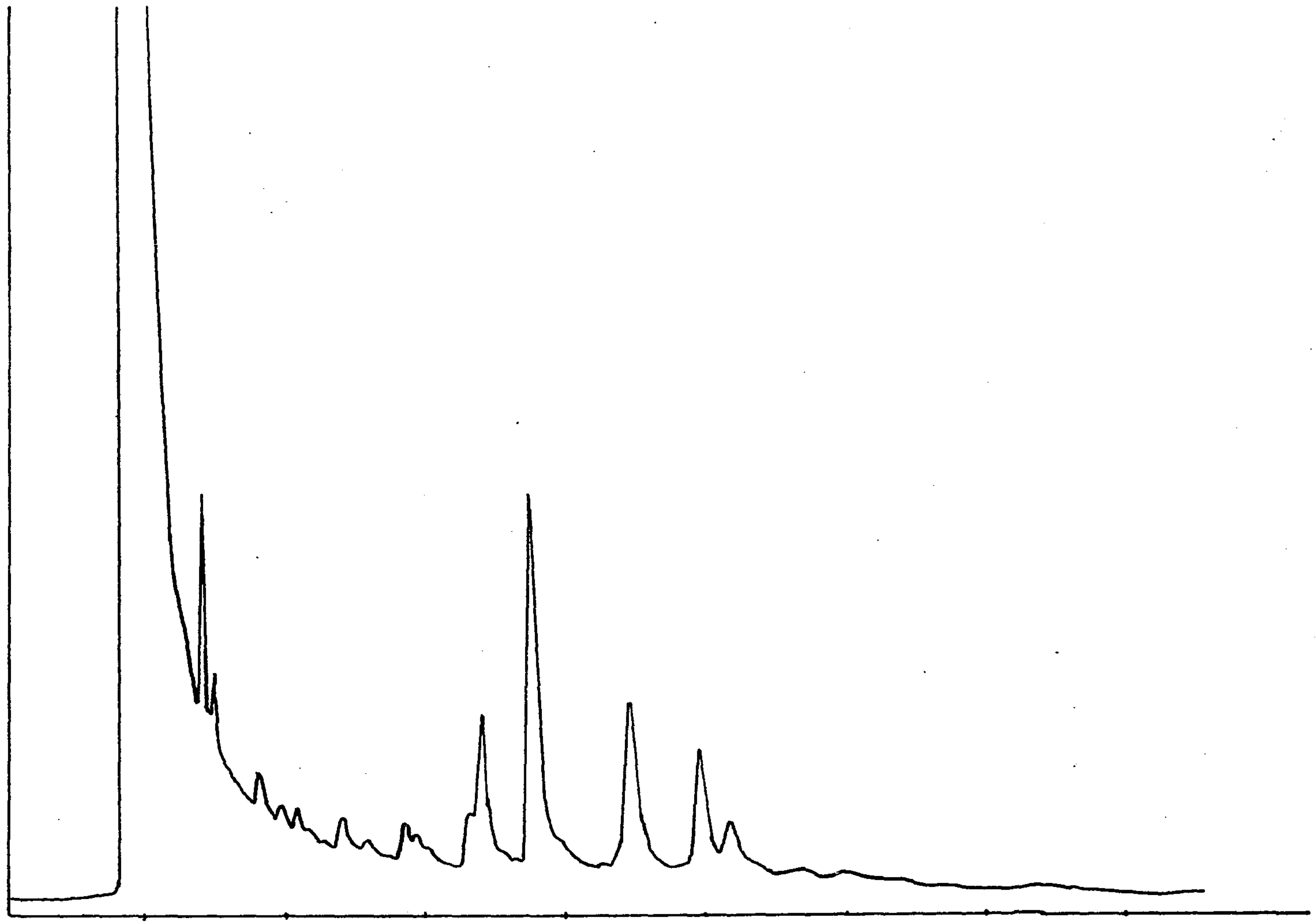

Figure 18. Volatile Compounds from <u>Camponotus</u> <u>pennsylvanicus</u>

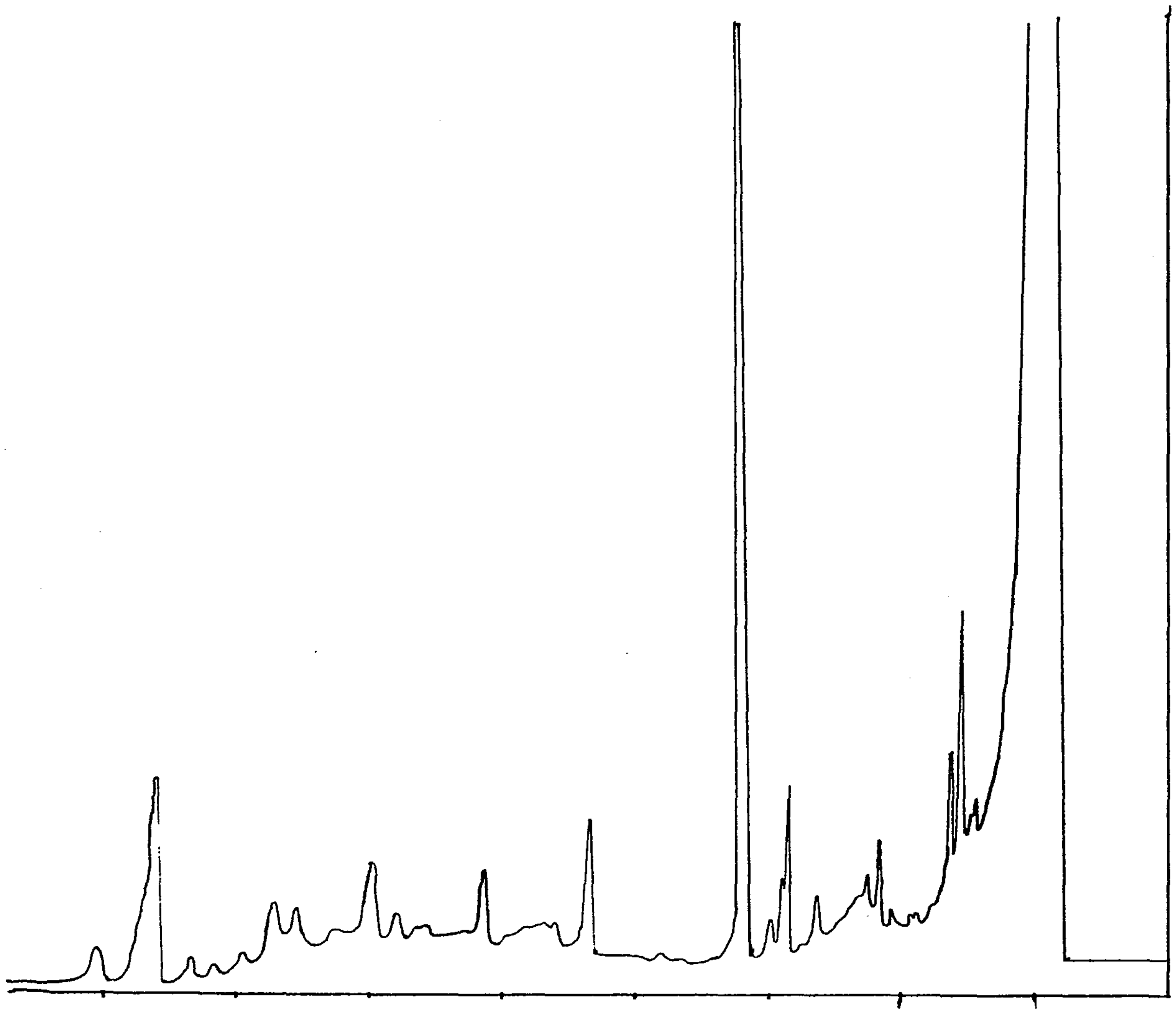

Figure 19. Volatile Compounds from *Monomorium minimum*

TABLE V

MASS SPECTRA FROM ANT STUDIES

Eciton nigrescens

F-1		F-2		F-3		F-4	
Unknown		2-heptanone		n-undecane		Unknown	
142[a]	76%	114	5.3%	156	xx	142	100%
91	100	43	100	57	100	47	67
92	68	58	61	43	100	50	60
94	36	71	15	71	100	62	48
127	31	27	13	41	100	127	43
62	25	41	13	85	xx	45	38

F-5		F-6		F-7		F-8	
Unknown		Unknown		Unknown		Unknown	
146	100%	136	90%	142	69%	136	4.5%
148	67	135	100	45	100	93	100
111	39	42	57	41	99	41	99
75	32	39	38	59	89	55	63
74	22	56	28	47	73	69	62
50	21	108	24	74	55	107	46

						K-1	
F-9		F-10		F-11		*Eciton opacithorax*	
Unknown		Indole		skatole		2-heptanone[b]	
220	25%	117	100%	131	56%	114	15%
205	100	90	49	130	100	58	100
117	29	116	38	77	17	43	55
57	23	89	27	103	13	59	37
145	17	51	18	65	10	71	32
206	16	39	14	51	9	99	10

TABLE V (Continued)

Pheidole dentata

G-1 Unknown		G-2 Unknown		G-3 n-pentadecane		G-4 n-hexadecane	
148	32%	120	20%	212	2.2%	226	3.8%
41	100	91	100	57	100	57	100
57	82	41	38	43	90	43	78
55	67	43	37	71	58	71	74
56	61	57	30	85	44	85	42
146	40	92	26	41	36	41	37

G-5 Unknown		G-6 Unknown		G-7 Unknown		G-8 Unknown	
220	28%	198	5.2%	188	22%	220	24%
205	100	58	100	173	100	205	100
161	45	43	67	41	30	57	56
57	43	71	31	158	21	41	26
41	37	41	25	163	15	43	30
203	34	57	19	149	15	71	24

G-9 Unknown		G-10 n-octadecane		G-11 Unknown		G-12 n-nonadecane	
236	12%	254	x	228	20%	268	xx
67	100	57	100	229	16	57	100
81	89	43	74	43	100	43	69
41	78	71	67	41	78	71	55
93	60	85	50	60	45	85	47
95	53	41	38	39	38	41	22

Aphaenogaster texana

H-1 n-pentadecane		H-2 Unknown		H-3 Unknown	
212	2.8%	220	24%	236	22%
57	100	205	100	67	100
43	78	57	34	41	76
71	65	41	18	81	65
85	38	206	17	82	64
41	37	145	16	55	50
				95	50

TABLE V (Continued)

Novomessor cockerelli

E-1 n-undecane[b]		E-2 n-dodecane[b]		E-3 n-tridecane		E-4 n-tetradecane	
156	14%	170	20%	184	6%	198	7%
57	100	57	100	57	100	57	100
71	59	71	90	43	75	43	84
85	48	85	68	71	56	71	80
43	28	43	25	41	28	85	39
99	14	99	22	85	17	41	28

E-5 n-pentadecane		E-6 n-hexadecane		E-7 n-heptadecane	
212	3.5%	226	5%	240	3%
57	100	57	100	57	100
43	65	43	76	43	72
71	55	71	66	71	61
85	38	85	45	85	42
41	25	41	26	41	24

E-8 Unknown		E-9 n-octadecane		E-10 n-nonadecane	
254	3%	254	4%	268	2%
57	100	57	100	57	100
71	83	71	69	43	85
43	53	43	61	71	80
85	48	85	37	85	58
41	19	41	23	41	40
127	19				

Crematogaster lineolata

I-1 3-octanone		I-2 Unknown		I-3 3-decanone		I-4 Unknown	
128	12%	140	0.9%	156	3.4%	140	0.8%
43	100	43	100	57	100	69	100
57	92	69	68	72	70	84	52
72	62	99	53	43	37	41	28
29	53	41	44	29	34	125	10
99	50	55	31	127	27	39	9

TABLE V (Continued)

I-5 Unknown		I-6 Unknown		I-7 Unknown		I-8 Unknown	
166	2.0%	164	15%	164	14%	210	3.3%
57	100	41	100	41	100	41	100
43	100	55	88	55	73	55	92
41	70	57	66	67	52	69	89
82	66	56	64	69	41	81	81
55	63	81	63	68	37	67	67
		67	63				

I-9 Unknown		I-10 Unknown		I-11 Unknown		I-12 Unknown	
248	26%	248	24%	248	28%	246	35%
94	100	94	100	94	100	94	100
81	66	45	71	107	90	107	83
107	53	107	53	79	28	81	24
95	41	43	39	95	17	79	24
82	33	55	38	81	15	77	24

I-13 Unknown		I-14 Unknown		I-15 Unknown		J-1 Unknown[b]	
246	28%	274	11%	274	22%	236	12%
94	100	94	100	94	100	220	64
107	77	81	23	147	96	205	100
81	18	55	19	107	63	180	96
77	18	120	14	120	63	165	50
95	13	107	58	81	32	221	42
						57	37

Crematogaster laeviuscula

J-2 Unknown[b]		J-3 Unknown[b]		J-4 Unknown[b]		J-5 Unknown[b]	
220	100%	220	65%	250	17%	234	50%
177	75	205	100	193	100	219	100
205	34	206	20	194	13	220	15
163	28	221	12	57	11	57	11
192	24	177	3	97	10	97	10
135	22	57	4	149	8	71	9

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[a] Order of peaks is: parent mass base peak, and the largest 4 remaining fragments.

[b] Spectrum taken at 20 eV., all others taken at 70 eV.

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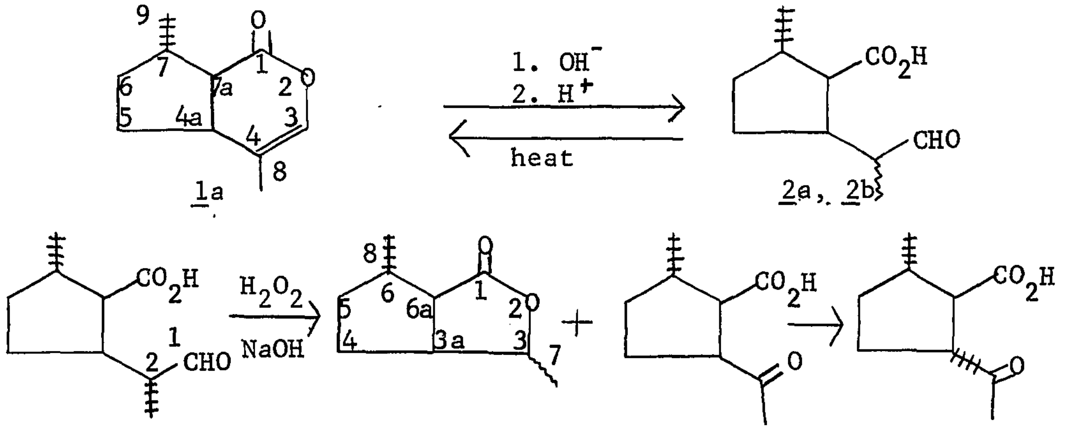
PART II

DEGRADATION STUDIES AND STRUCTURE PROOF OF *CIS*, *CIS*-NEPETALACTONE

CHAPTER I

INTRODUCTION AND HISTORICAL

The study of the compounds present in oil of catnip began at the University of Wisconsin. Nepetalic acid (2a) was isolated after hydrolysis of nepetalactone (1a).[1] This molecule was then degraded in alkaline hydrogen peroxide to give several products.

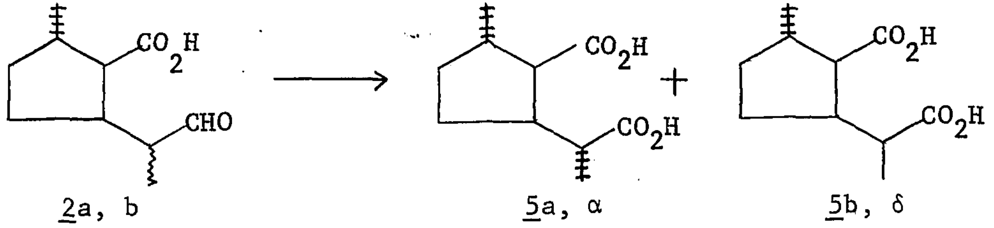


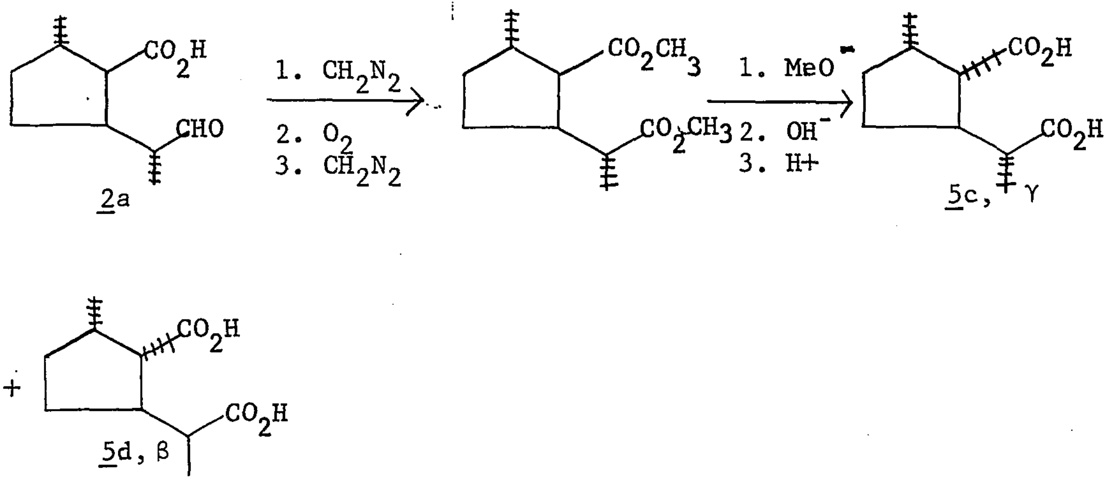
1a 2a, 2b

2a 3a, 3b 4a 4b

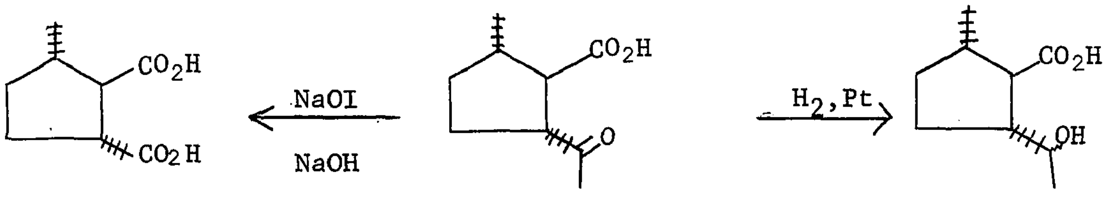
The structure and stereochemistry of these compounds were elucidated by McElvain and Eisenbraun.[2] The absolute configuration at C_7 was established by conversion of *trans*, *trans*-nepetonic acid (4b) into (-)-(S)-methylglutaric acid. The relative stereochemistry of nepetalactone at C_{4a} and C_{7a} was inferred from studies of the nepetalinic acids and the absolute structure at C_2 of nepetalic acid and the corresponding position of the nepetalinic acids from studies of the hydrogenation of bulnesol.[3,4]

Oxidation of nepetalic acid (2a, b) with chromic acid gives two epimeric nepetalinic acids designated as the α and δ isomers (5a, b). Esterification of crystalline nepetalic acid (2a) with diazomethane followed by oxidation with molecular oxygen gives the half ester of nepetalinic acid (5a) which after epimerization and saponification gives a mixture of the original acid and a new acid, 5c, designated as the isomer. The diester of 5a could be epimerized to give after saponification a mixture of 3 acids, 5a, 5c, and 5d, the remaining possible compound in this series. The groups at C_{4a} and C_{7a} must be oriented *trans* to one another in nepetalactone or it would be impossible to obtain 3 isomers from α-dimethyl nepetalinate (5a).



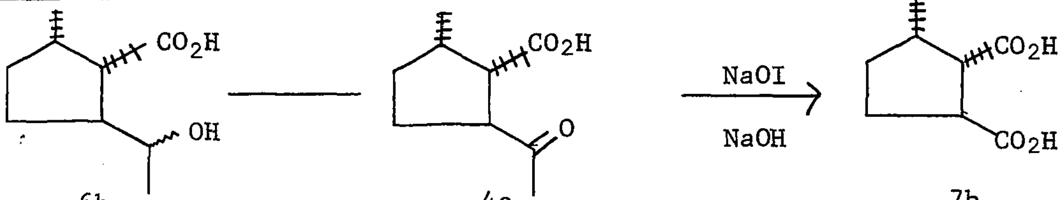


Ozonization of nepetalactone and decomposition of the ozonide in $NaBH_4$ gives the epimeric nepetolactones (3a, b).[5] The stereochemistry of *trans*, *trans*-nepetonic acid (4b) was demonstrated by reduction to a nepetolic acid (6a) which did not cyclize to a lactone and by conversion to trans, trans-nepetic acid (7a) of known structure.[6]

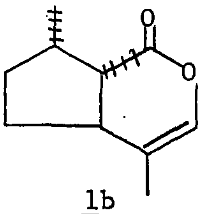


7a 4b 6a

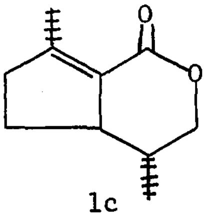
The presence of more than one nepetalactone isomer in Nepeta cataria oil was first demonstrated by Bates et al.[5] Nepetalinic acid (5a) could be removed from oxidation mixtures of nepetalactone from oil of catnip by formation of its insoluble barium salt. From the difficulty in then inducing nepetalinic acid (5b) to crystallize, it was hypothesized that a mixture of compounds were present. A nepetolic acid (6b) different from 6a was isolated from the acid fraction obtained by ozonization of the nepetalactone mixture and decomposition of the ozonide with $NaBH_4$. This hydroxy acid could be oxidized to the new trans, cis-nepetonic acid (4c) which could be degraded to the known trans, cis-nepetic acid (7b). From these results, the existence of trans, cis-nepetalactone (1b) (epinepetalactone) was inferred.



6b 4c 7b



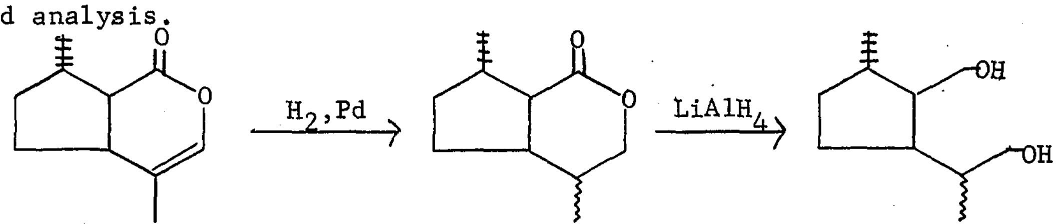
1b



1c

Sakan et al[7] isolated 1b from commercial *Nepeta cataria* oil using column chromatography and showed that it was converted into nepetalactone by heating with K_2CO_3 in xylene. These workers also isolated a third lactone from the leaves and galls of *Actinidia polygama* of molecular weight 166 ($C_{10}H_{14}O_2$) which they called neonepetalactone (1c).

Regnier et al[8] investigated oil of catnip in greater detail. Both 1a and 1b were isolated from *Nepeta cataria* oil by preparative gas chromatography. These were converted to the corresponding dimethyl nepetalinates and compared with authentic compounds through gas chromatographic studies. As a second proof of identity, these compounds were converted into nepetadiols (8a, b) and the silyl ethers of these compounds were compared chromatographically with silyl ethers of nepetadiols prepared from standard nepetalinic acids.(cf iridodial isomer proof, Part I, this dissertation). It was shown that epimerization of epinepetalactone and nepetalactone did not occur during their isolation and analysis.



1a

8a, b

Regnier et al also made comparative studies of the composition of the oil from three *Nepeta* species, *N. cataria*, *N. mussini*, and *N. citriodora*.[9] They found that *N. cataria* and *N. citriodora* produce mainly nepetalactone (1a) with small amounts of epinepetalactone (1b). *Nepeta mussini* was found to produce mainly 1b with minor amounts of 1a present. This last result was demonstrated using only the nepetadiol approach described above. A minor amount of a third nepetalactone isomer was detected in N. cataria and N. citriodora using gas chromatography-mass spectrometry. It was suggested that this isomer was neonepetalactone (1c).

McElvain and Eisenbraun had reported[2] that reaction of crystalline nepetalic acid (2a) with basic hydrogen peroxide gave about a 65% yield of trans, trans-nepetonic acid (4b) and approximately 7% of the nepetolactones (3a, b). If nepetalactone which was regenerated by pyrolysis of nepetalic acid at 250° was opened again in base and then reacted with H_2O_2, these yields changed to about 40% and 25% respectively. It was speculated that these differences resulted from epimerization during the pyrolysis, but it is difficult to explain how epimerization to epinepetalactone (1b) could be responsible for a higher yield of nepetolactones.

The mechanism of formation of these products was discussed by Regnier.[10] The formation of nepetolactone can be rationalized by a Baeyer-Villiger type reaction with attack of the $O\text{-}O\text{-}H^-$ anion at the carbonyl carbon.[11] Nepetonic acid formation was explained with recourse to attack of hydroperoxide anion at the position α to the carbonyl group in strong base, referring to the work of Doering[12] and Elkik[13] on the autoxidation of ketones in basic media. These mechanisms are shown in Figure 1. The formation of the hydroperoxide by attack of hydroperoxide anion at a negative center represents a mechanistic improbability, however. Because of the known ease of decomposition of basic hydrogen peroxide to oxygen, it would be well to consider the possibility that molecular oxygen is involved in this reaction.

Studies were undertaken to investigate further the mechanism of hydrogen peroxide degradation of nepetalic acid and to study further

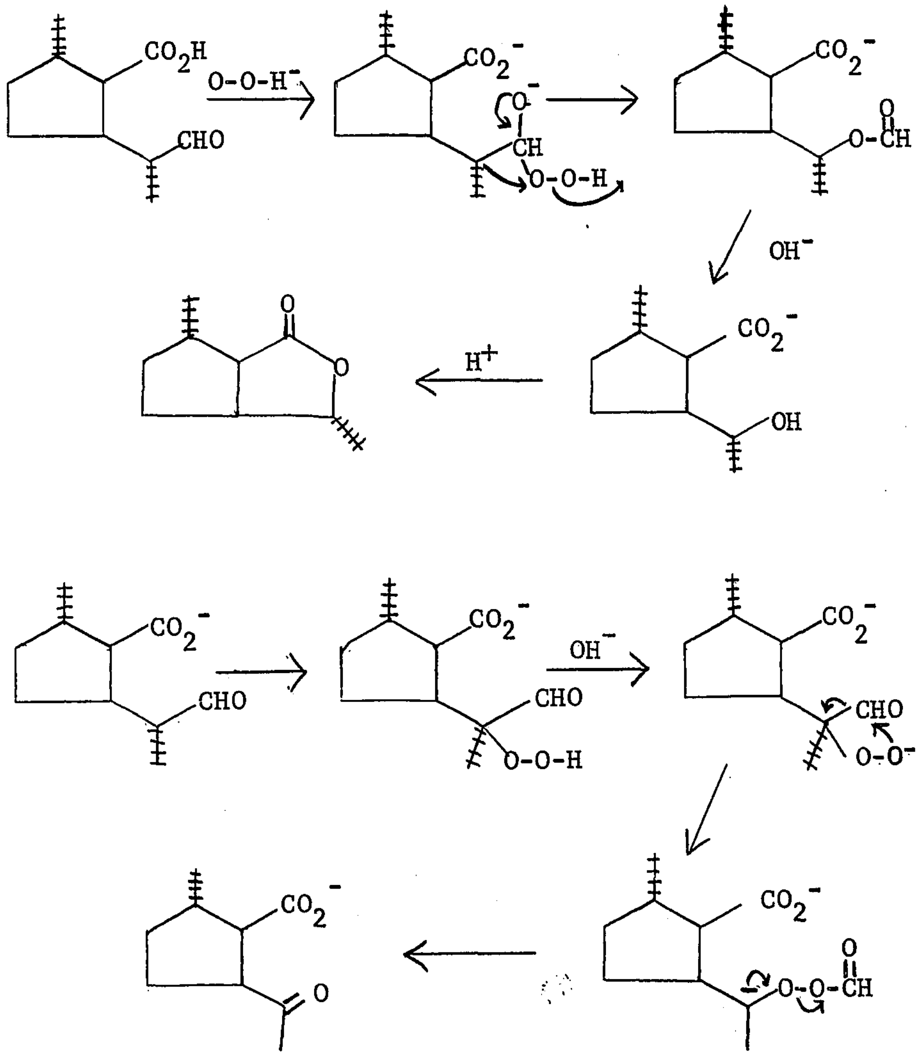
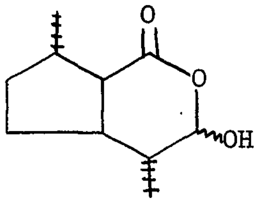


Figure 1. Literature Mechanisms for Formation of Nepetolactones and Nepetonic Acids

the stereochemistry of the reactants and products. It was planned to investigate the possible effects of the stereochemistry at C_2 of nepetalic acid on the course of this reaction as well as the possible role of cyclic lactol and pseudo acid forms 9 and 10. It was also planned to study the degradation of "epinepetalactone" obtained from Nepeta mussini oil in greater detail.



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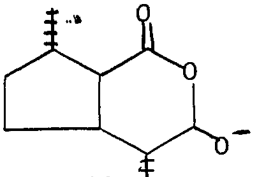

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CHAPTER II

RESULTS AND DISCUSSION

Nepeta cataria which was grown by the Botany Department of Oklahoma State University was harvested and steam distilled to obtain about 186 g of catnip oil that was used for the studies described in this dissertation. Gas chromatography-mass spectrometry showed only two nepetalactone isomers in this oil in the ratio of 91/9. These were assumed to be nepetalactone (1a) and epinepetalactone (1b) respectively. It was attempted to duplicate the results of Eisenbraun[2] for degradation of nepetalactone in basic hydrogen peroxide.

Best yields were obtained using approximately his procedure on a smaller scale. Typically, reactions were run on about 1.5 g quantities of starting material. This was opened in an excess of 10% NaOH, extracted to remove neutrals and then reacted with excess hydrogen peroxide while allowing the pot temperature to rise to about 60°. The reaction was stirred magnetically for 3 hours. Usual per cent yields from this reaction were about 30% of nepetonic acids and 20% of nepetolactones. Starting from crystalline nepetalic acid, about 40% and 15% respectively of these products were obtained. Yields were determined using gas chromatography. The products formed from the epinepetalactone contaminent are included in these figures.

The yields of this reaction were studied holding the reaction temperature at 0°, 25°, and 50°. The base concentration was varied from the minimum amount to maintain solution to a considerable excess and the influence of peroxide concentration was investigated. None of these variations improved the yield of this reaction and often lower yields were obtained. Timed studies indicated that the reaction was complete after 3 hours under the conditions used and further reaction tended to lower the yield due to degradation of nepetonic acids.

Perhaps the smaller scale of reaction is responsible for the disparity in percent yields from those obtained previously. Not enough reactions were run to be certain that the differences in starting from crystalline nepetalic acid and nepetalic acid from nepetalactone opened _in situ_ were significant. In several cases, yields were practically identical for the two cases. Nepetalic acid obtained from nepetalactone was pyrolyzed to regenerate nepetalactone. No new compounds of as much as 5% of the nepetalactone concentration were detected after pyrolysis.

The gas chromatogram of the esterified products of a typical reaction is shown in Figure 2. Identification of these compounds was established by comparison of retention times and enrichment experiments using authentic samples of these compounds and by mass spectrometry studies. Compound A-6 had an identical retention time with _trans_, _trans_-methyl nepetonate (_4_b). Mass spectra indicated that compounds A-7 and A-8 were also methyl nepetonates (Table I). When the reaction was run for a long time or when the base concentration was increased, the amount of A-8 relative to A-6 and A-7 was greatly reduced. It was assumed on this basis that A-8 was _cis_, _trans_-methyl nepetonate (_4_a), and A-7 was the nepetonate produced from the epinepetalactone contaminant, _trans_, _cis_-methyl nepetonate (4c). A-7 was not present if crystalline nepetalic acid was used as starting material.

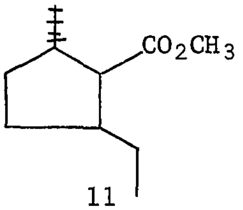
Figure 2. Degradation Products of <u>cis</u>, <u>trans</u>-Nepetalactone

Comparison of mass spectra (Table II) and retention times with authentic samples indicated that A-9 and A-10 were the epimeric nepetolactones (3a, b). When the acid fraction was separated from the lactone fraction by bicarbonate wash before esterification, these compounds appeared in the lactone fraction. Compounds A-11 and A-12 have molecular weights of 228 and correspond in retention times to those of α and δ-dimethylnepetalinate respectively. The mass spectrum for A-12 is shown in Table III.

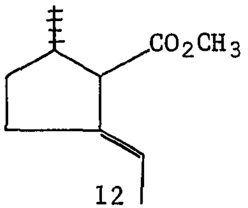
The Baeyer-Villiger reaction of nepetalic acid using peroxytrifluoroacetic acid gave about equal amounts of nepetolactones and nepetalinic acids. When the nepetolactones (3a,b) were opened in base and treated with excess hydrogen peroxide for 2 days, no nepetonic acid was formed. An increase of reaction temperature (during the peroxide degradation) favored the formation of nepetolactones relative to nepetonic acids. Evidently these products are formed by different mechanisms.

When molecular oxygen was bubbled into a basic solution of nepetalic acid, the major product was <u>trans</u>, <u>trans</u>-nepetonic acid rather than the expected nepetalinic acids formed by aldehyde autoxidation. After two days reaction at room temperature, about 60% of the starting material was reacted. A possible mechanism for nepetonate formation would be attack of the anion formed at the α position of the aldehyde of nepetalic acid by oxygen. However, a radical reaction seems more likely.

It has been previously reported that aldehydes can give hydrocarbons when oxidized by molecular oxygen in basic solution.[15-17] The mass spectra of unknown compounds A-1 and A-4 are included in Table III. These compounds are acids of molecular weights 156 and 154 respectively and the congruence of major peaks with those from the fragmentation of other compounds studied, suggests they contain the methylcyclopentane nucleus. They are not present in the starting material. These spectra compare reasonably with what would be expected for compounds such as 11 and 12.



11



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When untreated tank nitrogen was bubbled through the reaction mixture, the yield of products A-1 and A-4 increased greatly with a corresponding decrease in the yields of nepetonic acids and nepetolactones. If the gaseous products from the reaction were swept into a trap containing PdCl$_2$-phosphomolybdic acid reagent, a color change was observed indicative of the evolution of CO. On the basis of this evidence, it seems likely that formation of nepetonic acids involves a radical mechanism such as the one shown in Figure 3. Hydroperoxides are known to decompose to carbonyl compounds in both acidic and basic solution.[18]

About 38 g of *Nepeta mussini* oil was obtained by steam distillation of plants grown by the Botany Department at Oklahoma State University. Gas chromatography-mass spectrometry indicated the presence of 2 nepetalactone isomers in the ratio of 91/9. Regnier[9] has stated that this plant produces epinepetalactone (*trans*, *cis*-nepetalactone, 1b) in major amounts. Degradation studies of *N. mussini* oil indicated that the major nepetalactone isomer in these samples is *cis*, *cis*-nepetalactone (1d), a molecule not previously reported and that *cis*, *trans*-

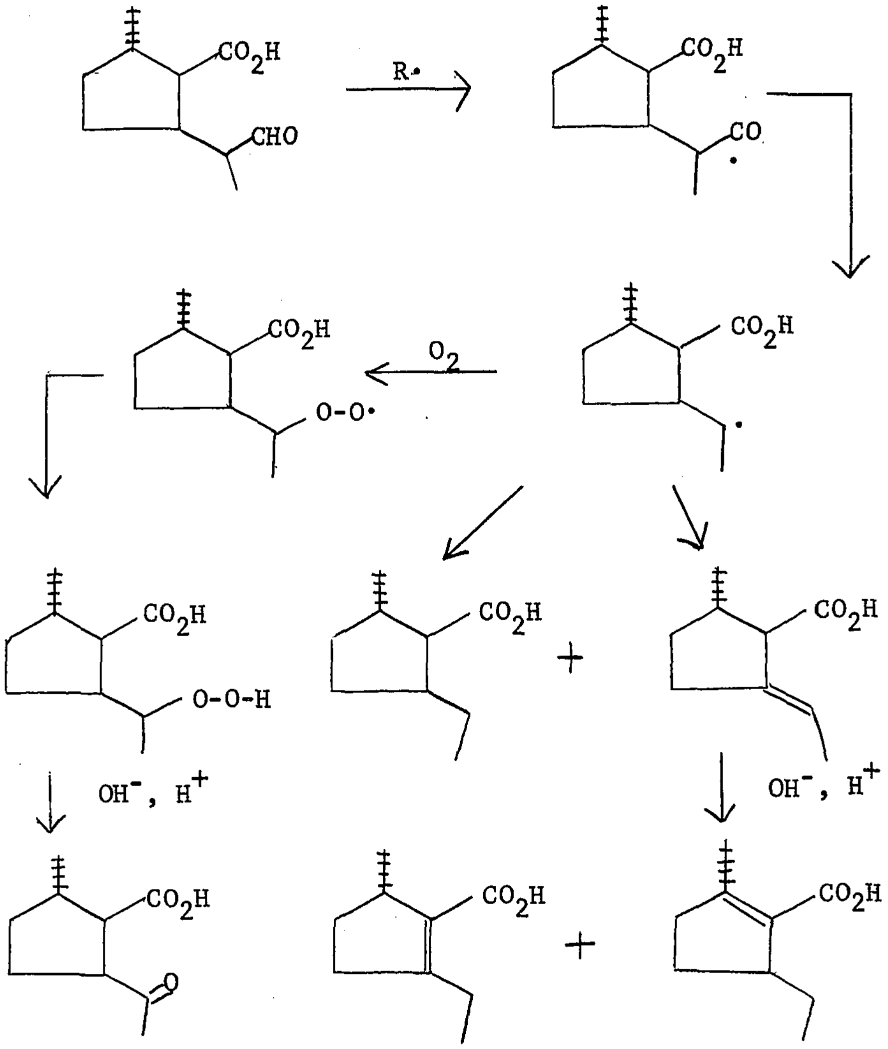
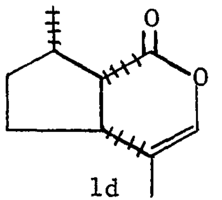
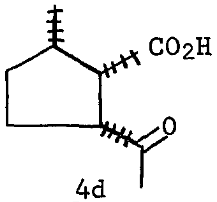


Figure 3. Proposed Radical Mechanism for Formation of Nepetonic Acids

nepetalactone (1a) is formed in minor amounts.



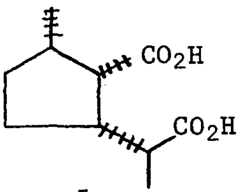
1d



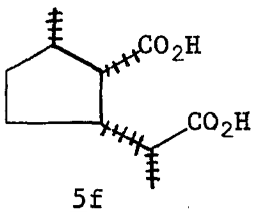
4d

Degradation of the nepetalactones from *N. mussini* in alkaline hydogen peroxide gave rise to the product mixtures shown in Figures 4 and 5. Mass spectral evidence indicated that compounds B-1, B-2, B-3, and B-4 were all methyl nepetonates (Table I). B-1, B-2, and B-3 had the same retention times as *trans*, *trans*-, *trans*, *cis*-, and *cis*, *trans*-methyl nepetonates respectively from degradation mixtures of *N. cataria* nepetalactones. Permutation of the two centers involved leaves only *cis*, *cis*-methyl nepetonate (4a) remaining of the 4 possible isomers of this series of compounds. It is difficult to explain how this thermodynamically unstable compound could be formed from either *trans*, *cis*- or *cis*, *trans*-nepetalactone in basic solution. The amount of B-4 in the reaction mixture is reduced if the reaction time is prolonged.

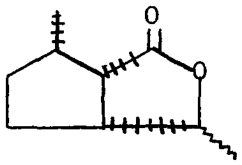
Compounds B-6 and B-7 correspond in retention time with that of α- and δ-dimethyl nepetalinate (5a, b). Mass spectrometry indicates that B-5 and B-8 are also dimethylnepetalinates, presumably from 5e and 5f. The spectrum of B-5 is presented in Table III.



5e



5f



3c, 3d

The neutral products are shown in Figure 5 (Table II). Mass

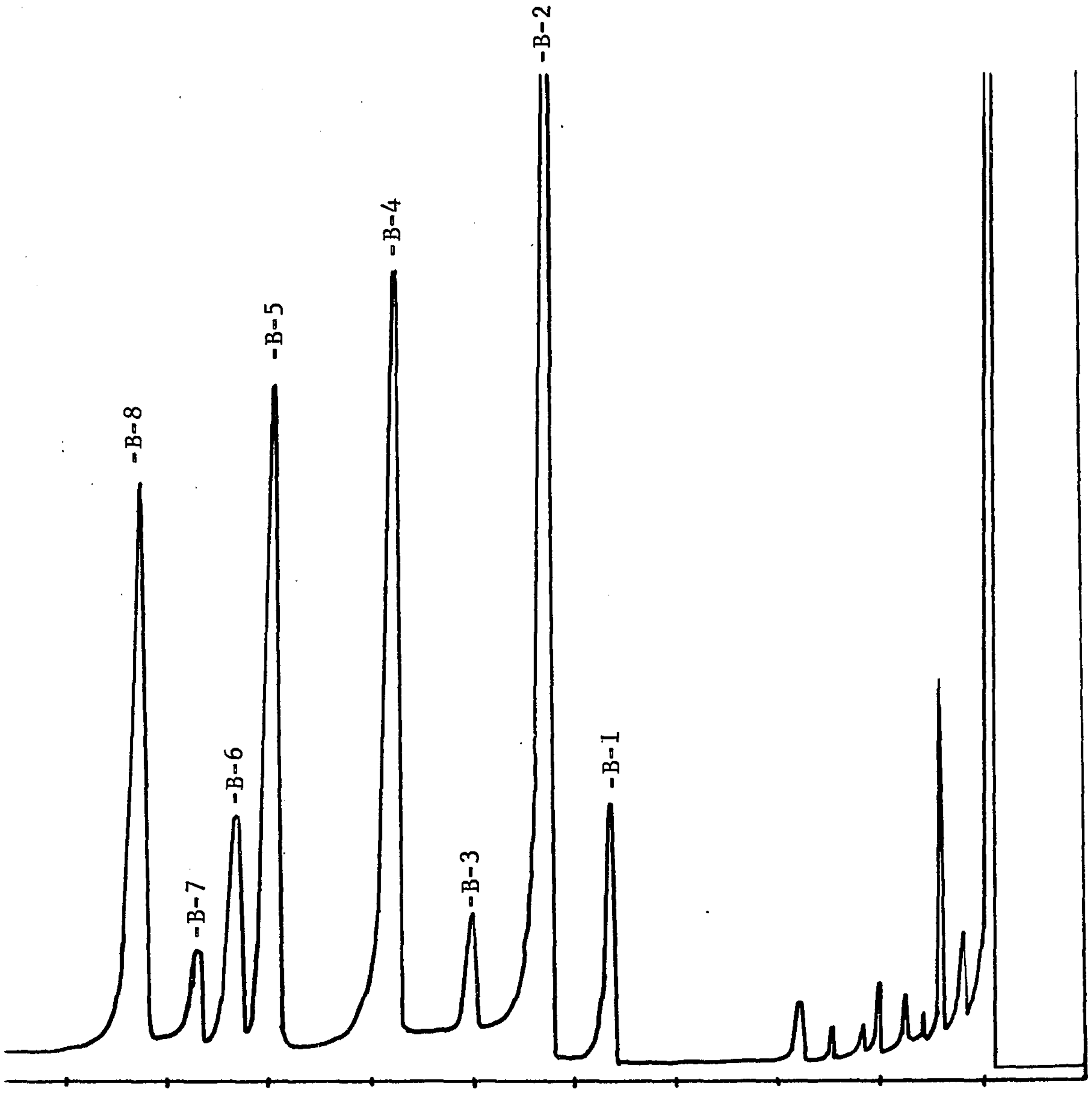


Figure 4. Acidic Products from Degradation of <u>cis</u>, <u>cis</u>-Nepetalactone

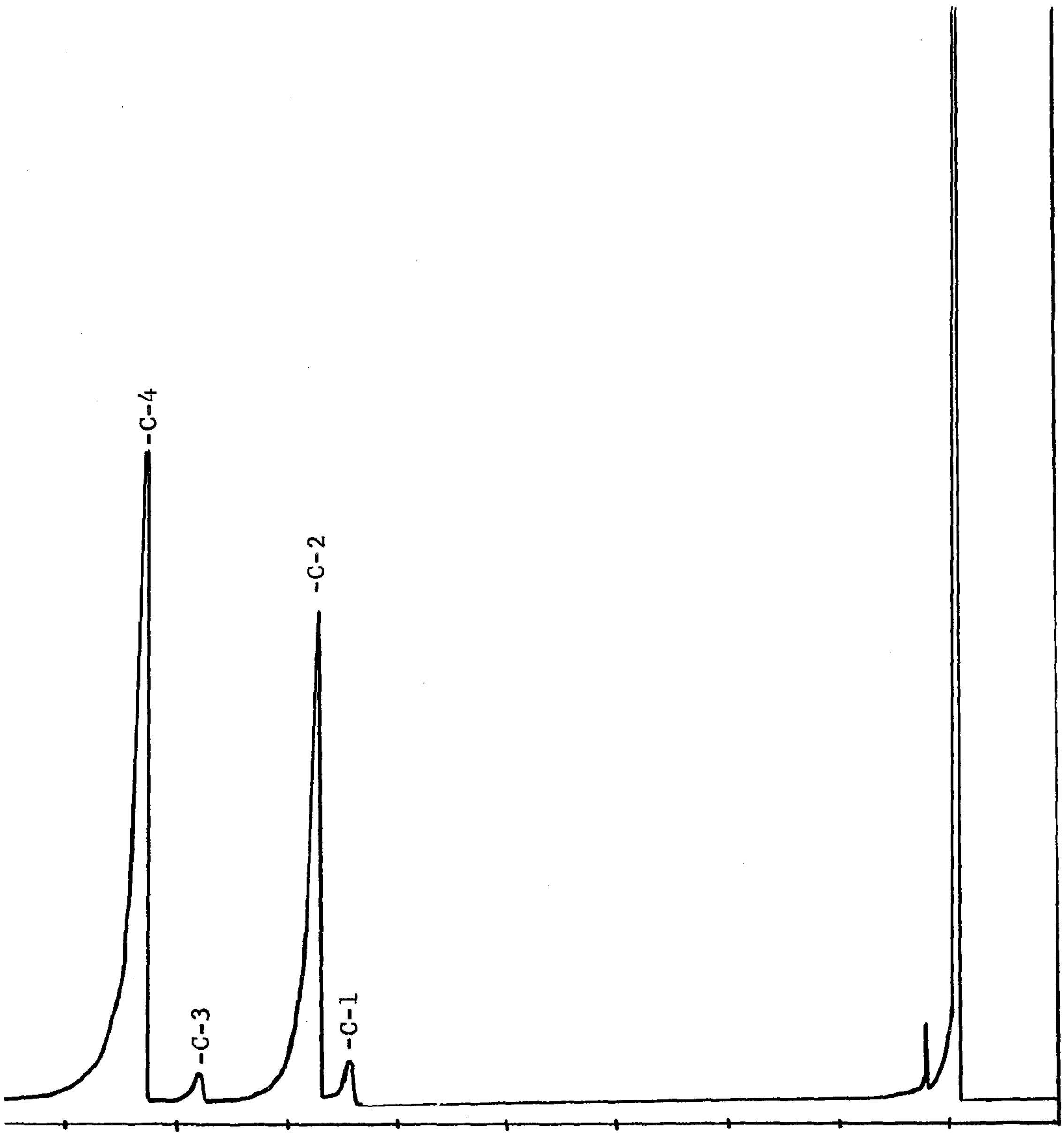


Figure 5. Neutral Products from Degradation of <u>cis</u>, <u>cis</u>-Nepetalactone

spectra indicate that all 4 products are nepetolactones. Compounds C-1 and C-3 correspond to the cis, trans-nepetolactones (3a, b) from nepetalactone. The only other possible nepetolactones in this series are the cis, cis-nepetolactones (3c, d). Again, it is difficult to provide a mechanism for conversion of trans, cis- or cis, trans-nepetalactone to these products. Per cent yields were not studied extensively for degradation of these lactones but seemed to be approximately 20% nepetonic acid formation and 18% nepetolactone formation.

The nmr spectra of cis, trans-nepetalactone (1a) and cis, cis-nepetalactone (1d) in CCl_4 are shown in Figures 6 and 7. The C_9 methyl protons absorb at 1.17δ (1.22), the C_8 methyl protons at 1.62δ (1.66) and the C_3 proton at 6.10δ (6.25). Literature values in $CDCl_3$ solution are in parentheses.[19] Cis, cis-nepetalactone (1d) obtained from N. mussini oil by preparative gas chromatography absorbed at .97δ (C_9), 1.61δ (C_8) and 6.21δ (C_3) in CCl_4 solution. The literature value[19] given for the C_9 methyl protons of epinepetalactone (trans, cis-nepetalactone, 1b) purified from Nepeta cataria oil in $CDCl_3$ is 1.12δ , indicating that different compounds are probably involved.

The nepetalic acids obtained from N. cataria and N. mussini oil were converted to nepetalinic acid half esters by esterification with diazomethane and air oxidation. These products were then esterified a second time and chromatographed. The major products from the experiment with N. cataria oil were α and δ-dimethyl nepetalinate (5a, b) with small amounts of β- and γ-dimethyl nepetalinate present (5c, d). From N. mussini oil, small amounts of α- and δ-dimethyl nepetalinates were obtained along with two additional compounds, presumably 5e and

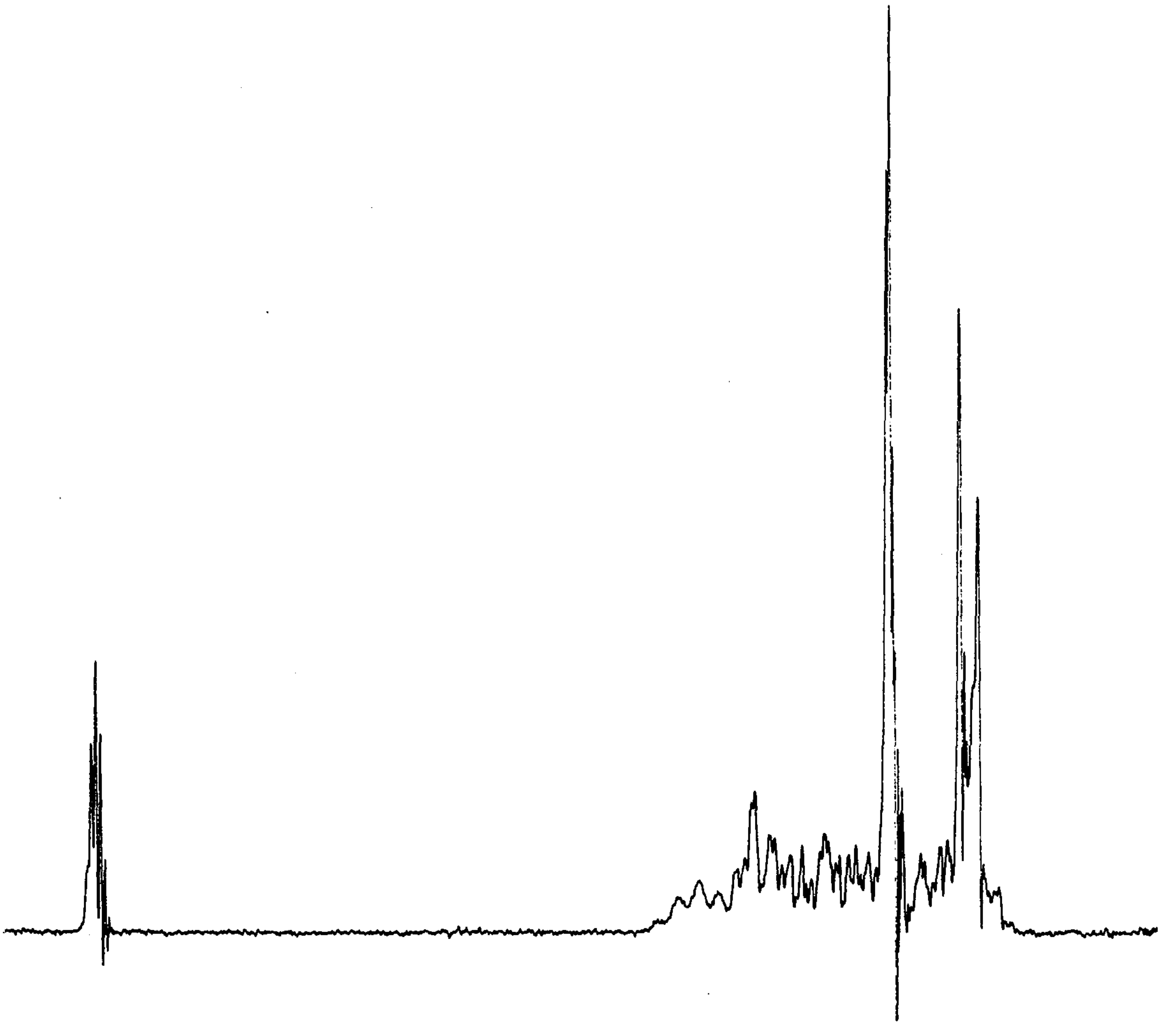


Figure 6. Nmr Spectrum of cis, trans-Nepetalactone, 1a

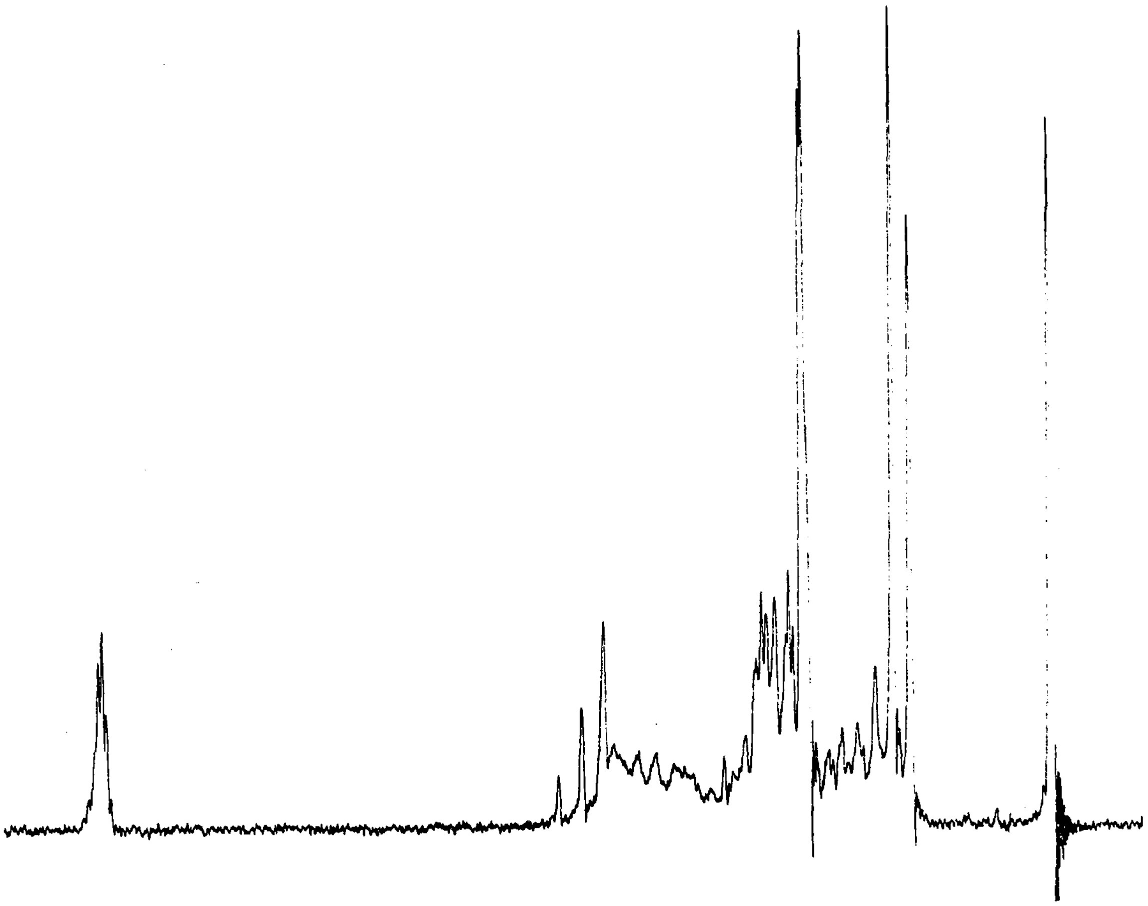
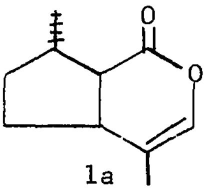
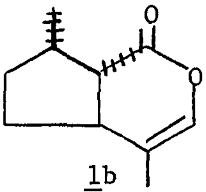


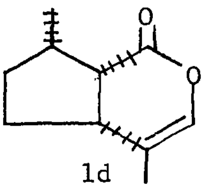
Figure 7. Nmr Spectrum of cis, cis-Nepetalactone, 1d

5f in major amounts. The products of this experiment are shown in Figure 8 for comparison with the standard dimethyl nepetalinates.

From these experiments, it seems likely that nepetalactone from *N. cataria* consists mainly of *cis*, *trans*-nepetalactone (1a) with small amounts of *trans*, *cis*-nepetalactone (1b) present. *N. mussini* produces *cis*, *cis*-nepetalactone (1d) contaminated with a small amount of *cis*, *trans*-nepetalactone (1a). This problem was complicated by the fact that it was not possible to separate 1b and 1d though several chromatographic systems were tried. There are several possible reasons for the difference between the results of studies of *N. mussini* reported in the literature and those reported here. Most obvious is the possibility that the plants were different. However, the nepetadiol structure proof does not seem to be as convincing as others. The major component present after the formation of silyl ethers is pyridine which gives a long trailing peak on many chromatographic systems and could distort the retention times of compounds appearing after it. Secondly, the stereochemistry at C_{4a} could be affected by the hydrogenation. Finally, it is possible that the *trans*, *cis*-nepetadiols have a similar retention time to that of the *cis*, *cis*-nepetadiols.


1a


1b


1d

Several of the original experiments in the structure proof of products in the *cis*, *trans*-nepetalactone series were repeated for *cis*, *cis*-nepetalactone. For comparison and practice, they were first

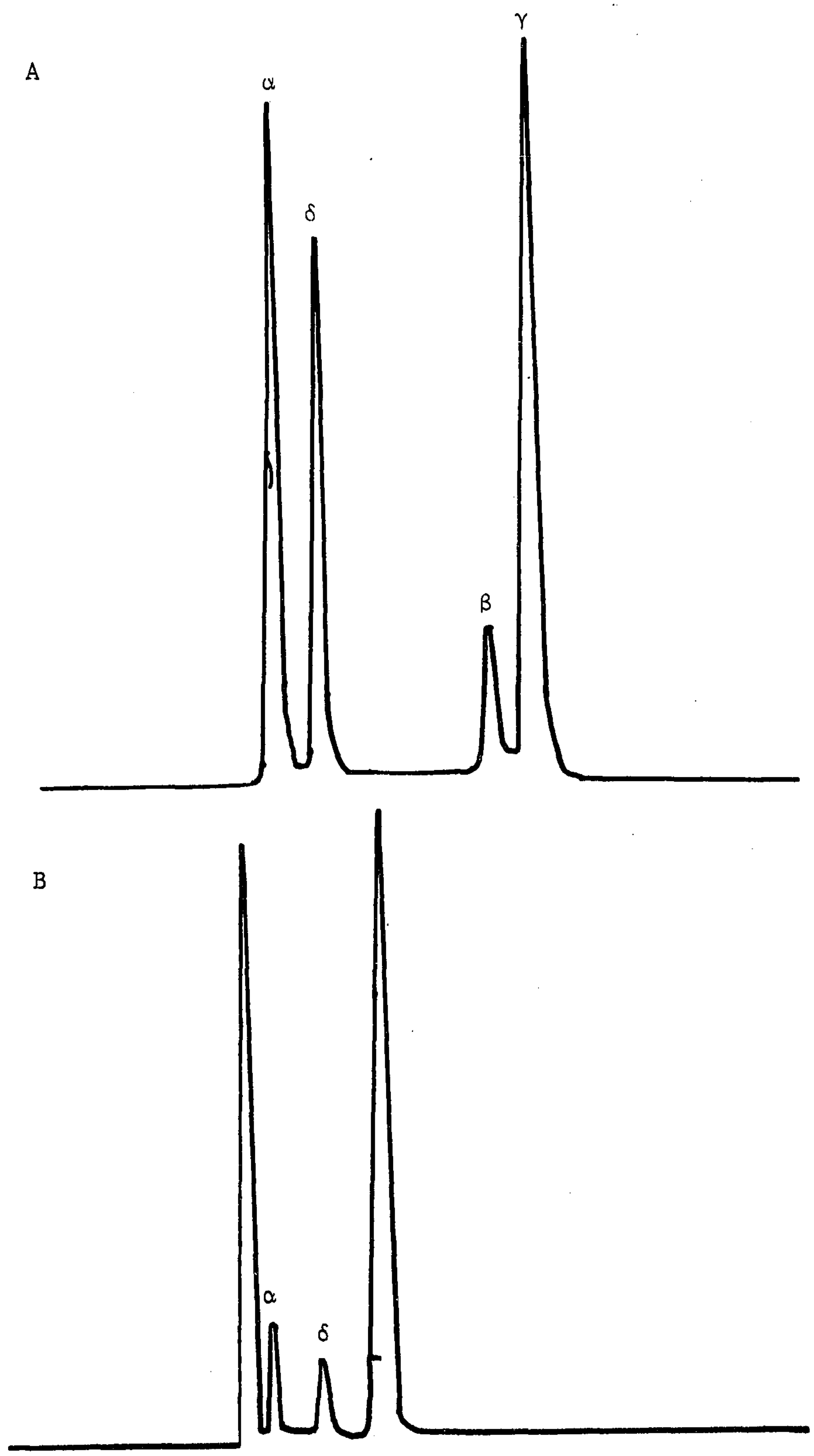


Figure 8. Comparison of Standard Dimethyl Nepetalinates With Dimethyl Nepetalinates Prepared from *Nepeta Mussini* Oil

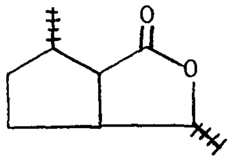
repeated for nepetalactone (1a). Nepetalactone (1a), obtained from *N. cataria* oil by preparative gas chromatography, was ozonized in methanol with pyridine added.[5] Decomposition of the ozonide with $NaBH_4$ gave the two *cis*, *trans*-nepetolactones. No other compounds were formed in yields as large as 10% of these products. This experiment was repeated using *cis*, *cis*-nepetalactone from *N. mussini*. Again the major products appeared in the lactone fraction and not the acid fraction and corresponded in retention time to the *cis*, *cis*-nepetolactones from degradation studies.

Nepetalactone was ozonized in CH_2Cl_2 with no pyridine present and the ozonide decomposed with zinc dust. A single compound whose retention time corresponded with that of *cis*, *trans*-methyl nepetonate (4a) represented about 90% of the products in the esterified extract. Heating the acid obtained from ozonization of 1a for 1 hour in 10% NaOH converted this product to a compound which when esterified had a retention time identical with *trans*, *trans*-methyl nepetonate (4b). The *cis*, *trans*-nepetonic acid was converted to nepetic acids by an iodoform reaction. About equal amounts of the *cis*, *trans* (7c) and *trans*, *trans* (7a) isomers were obtained. An iodoform reaction on *trans*, *trans*-nepetonic (4b) acid gave no *cis*, *trans*-nepetic acid (7c).

This experiment was repeated starting with purified *cis*, *cis*-nepetalactone (1d) from *N. mussini*. After ozonization, a compound was obtained which when esterified had a retention time identical to that of *cis*, *cis*-methyl nepetonate (4d) and could be epimerized to *trans*, *cis*-nepetonic acid (4c). An iodoform reaction on the ozonization product (*cis*, *cis*-nepetonic acid) gave *trans*, *cis*- (7a) and *cis*, *cis*-

nepetic (7d) acids in a ratio of 60/40. Because several extraneous compounds were formed in the iodoform reaction, the dimethyl nepetates (13) were epimerized in NaOMe/MeOH to further confirm their identity. After 1 hour, the *cis*, *cis*-dimethyl nepetate (13d) had disappeared from the mixture and a peak corresponding to the *trans*, *trans* isomer (13a) had appeared. After 4 hours, this was the major compound in the mixture. These transformations are summarized for the *cis*, *cis*-nepetalactone series in Figure 9. A mass spectrum of *cis*, *cis*-dimethyl nepetate (13d) was obtained from the above mixture (Table III) and corresponds well with a spectrum of the standard.

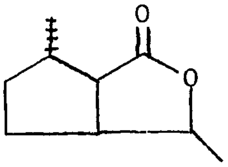
The nmr spectra of the nepetolactones are shown in Figures 10-13. On the basis of these spectra, it is possible to assign the following structures to these compounds:



C-1 3a

3R-*cis*, *trans*-nepetolactone

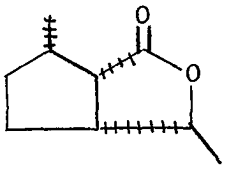
C_8	CH_3	1.21δ
C_7	CH_3	1.44δ
C_3	H	4.19δ



C-2 3b

3S-*cis*, *trans*-nepetolactone

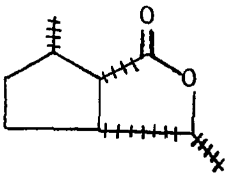
C_8	CH_3	1.12δ
C_7	CH_3	1.32δ
C_3	H	4.75δ



C-3 3c

3S-*cis*, *cis*-nepetolactone

C_8	CH_3	1.14δ
C_7	CH_3	1.35δ
C_3	H	4.04δ



C-4 3d

3R-*cis*, *cis*-nepetolactone

C_8	CH_3	1.19δ
C_7	CH_3	1.30δ
C_3	H	4.58δ

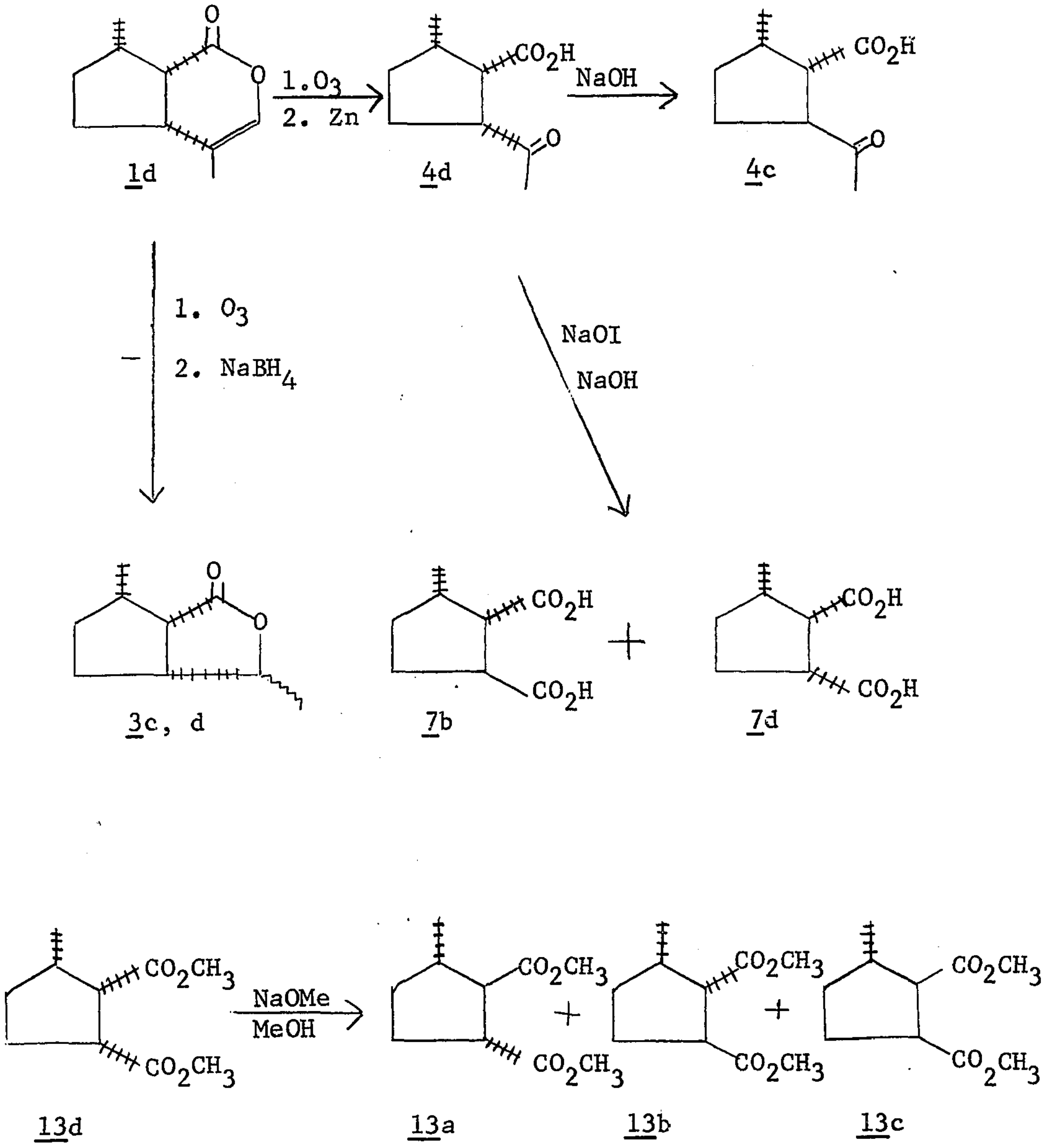


Figure 9. Further Degradation Studies of <u>cis</u>, <u>cis</u>-Nepetalactone

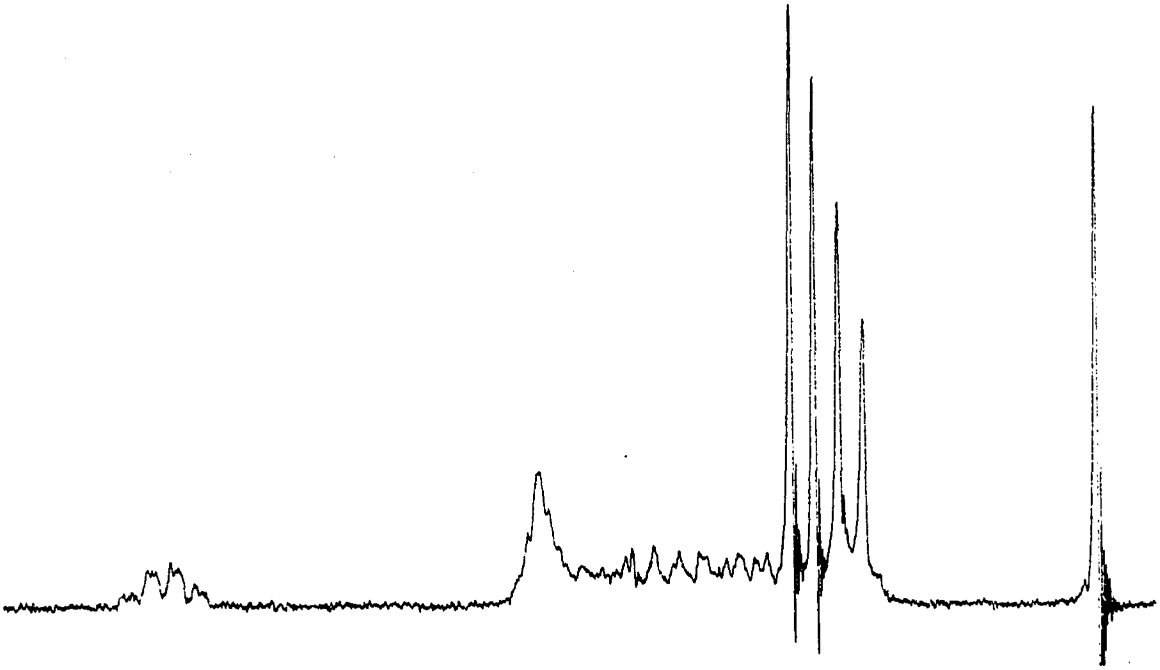


Figure 10. Nmr Spectrum of 3R-cis, trans-Nepetolactone, 3a

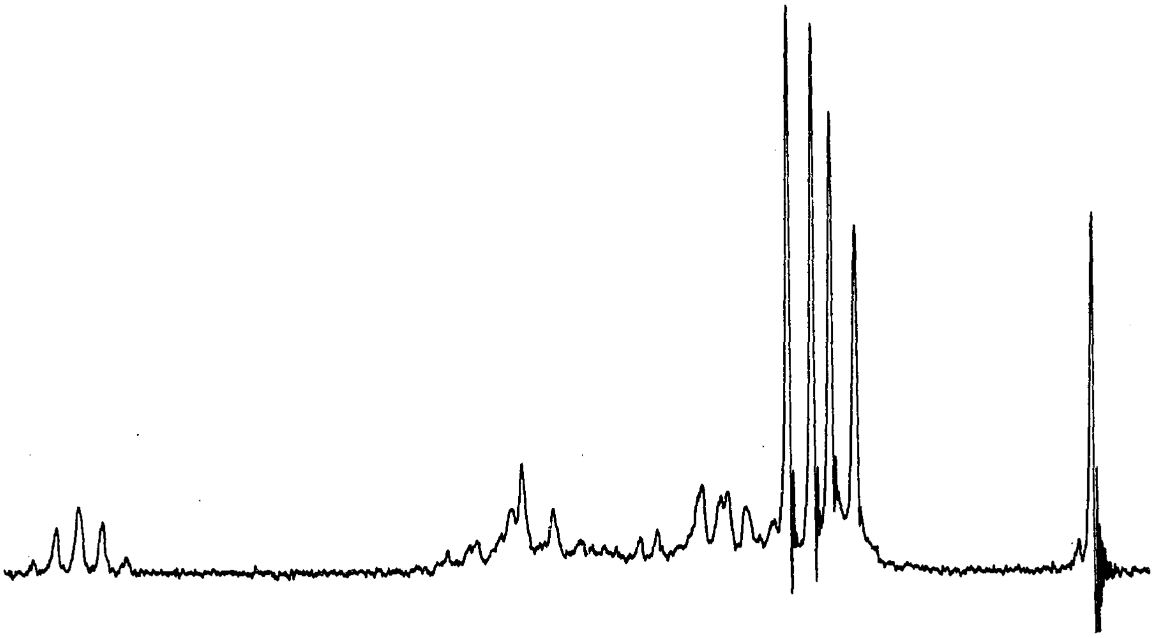


Figure 11. Nmr Spectrum of 3S-*cis*, *trans*-Nepetolactone, 3b

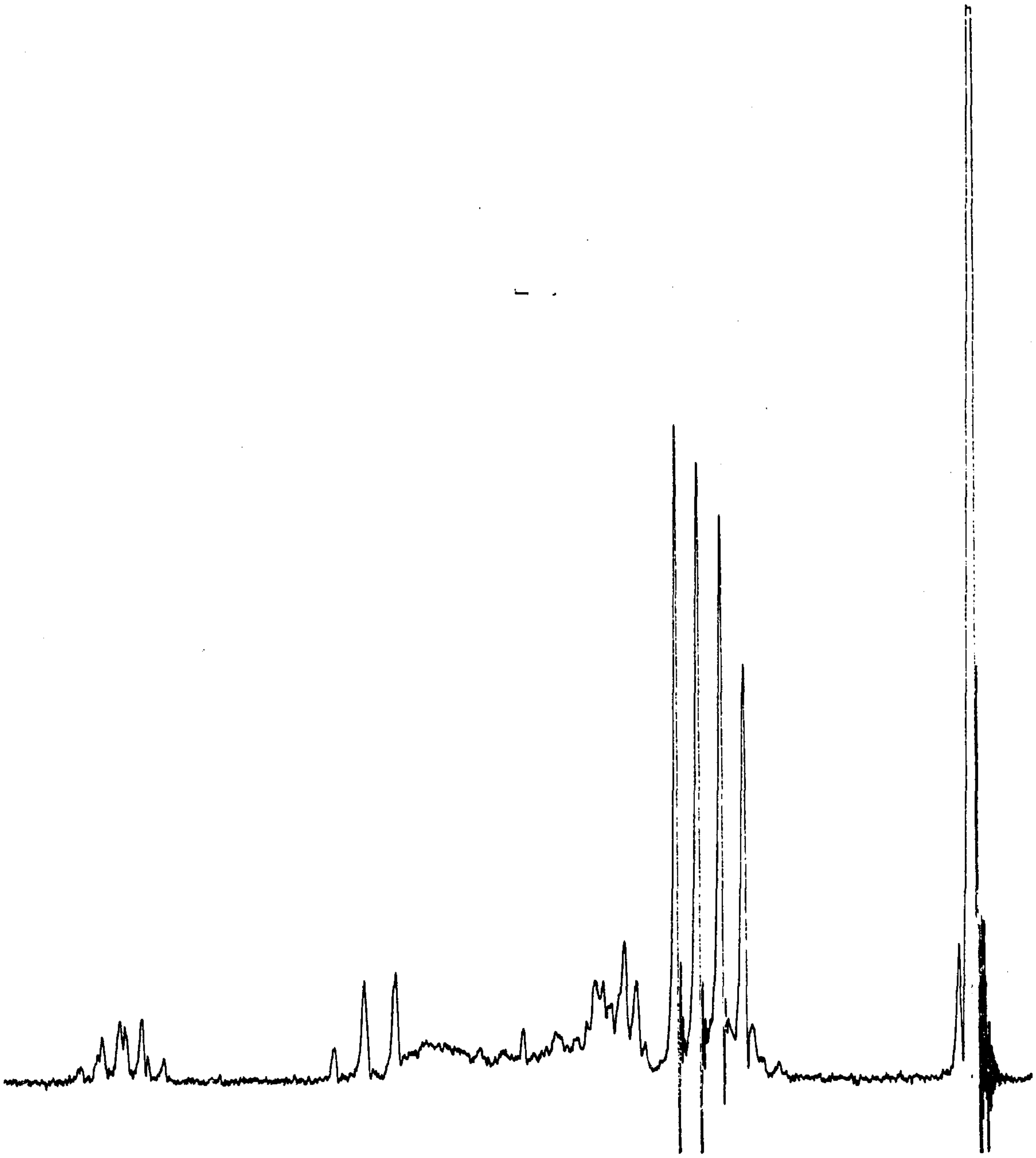


Figure 12. Nmr Spectrum of 3S-cis, cis-Nepetolactone, 3c

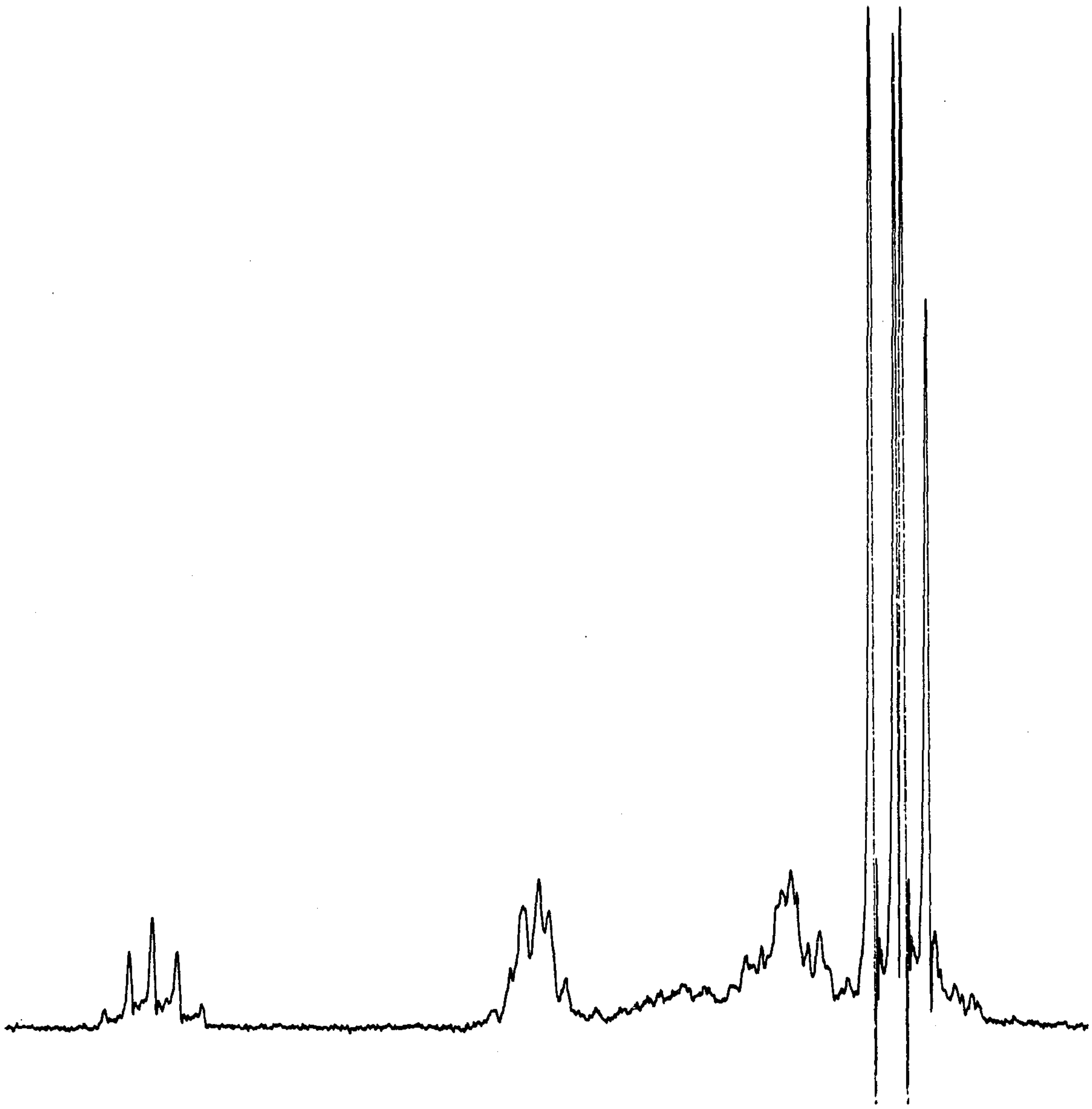


Figure 13. Nmr Spectrum of 3R-cis, cis-Nepetolactone, 3d

For compounds 3a and 3c, the C_3 proton is inside the envelope of the fused 5-membered rings. As a result of this shielding, this proton absorbs at a higher field, for these compounds, than the corresponding protons of 3b and 3d. For 3b and 3d, the C_7 methyl protons are shielded and absorb at lower δ values than the corresponding protons in 3a and 3c. The mass spectra of compounds 3a and 3c which have the more exposed C_7 methyl groups have a much larger M-15 peak than do 3b and 3d. (17%/8% and 27%/6.4% respectively).

TABLE I

MASS SPECTRA OF METHYL NEPETONATES

Mass	*trans*, *trans*	*cis*, *trans*	*trans*, *cis*	*cis*, *cis*
39	9.6	9.7	9.7	13
40	2.6	2.2	2.6	
41	15	14	13	21
42	3.0	3.0	2.6	
43	100	100	80	100
44	3.1	2.8	2.8	5.0
45	2.8	2.6	2.8	3.7
50		1.1		5.6
51	2.3	2.0	2.1	
53	7.8	7.5	7.1	13
54	1.7	2.0	1.9	
55	10	9.0	9.0	4.0
56	1.7	1.7	1.6	
59	11	9.8	9.5	12
61		1.3		
65	2.4	2.3	2.5	
66	1.9	1.9	2.0	
67	16	17	13	14
68	3.9	4.4	3.9	3.3
69	7.8	8.6	6.9	15
71	3.7	7.7	9.6	9.6
74				3.5
75		1.6		1.6
77	3.5	3.3	3.1	
79	6.6	6.2	6.4	12
80	3.6	4.7	4.6	7.7
81	97	89	100	89
82	13	16	14	12
83	4.5	7.7	7.2	4.0
84		1.5		
85		1.8	1.7	2.9
87	10	13	6.5	21
88		1.3		3.8
91		1.2	1.6	
95	2.8	3.2	2.7	2.9
97	8.5	9.5	5.8	11
98	10	6.3	4.2	9.7
99		1.9	2.0	4.3

TABLE I (Continued)

Mass	trans, trans	cis, trans	trans, cis	cis, cis
100	21	28	15	35
101	5.7	6.2	3.3	4.3
105				5.3
107	2.1	2.0	3.2	
108			1.6	
109	28	19	28	26
110	5.6	4.2	6.0	4.9
111	1.7		2.1	3.7
112				2.8
114	12	28	34	9.2
115		2.3	2.9	1.9
124	15	15	20	11
125	12	6.7	12	7.4
126			1.8	3.6
127	14	17	9.0	23
128		1.7		
129	22	16	8.5	45
130	2.2	1.3		
135				2.4
140			2.0	6.4
141	15	6.9	12	8.8
142	13	16	7.1	36
143		1.8		4.9
152	18	13	10	14
153	14	16	11	19
154	1.7	1.7		
168				6.1
169	5.2	4.1	1.6	3.9
184	5.5	3.8	3.0	3.8
185	.92	.66	.71	2.4

TABLE II

MASS SPECTRA OF NEPETOLACTONES

Mass	3R-*cis*, *trans* Nepetolactone	3S-*cis*, *trans*- Nepetolactone	3S-*cis*, *cis*- Nepetolactone	3R-*cis*, *cis*- Nepetolactone
39	15	15	27	24
40		4.2	5.9	7.5
41	20	17	35	29
42	2.9	2.2	4.0	
43	18	16	27	26
44		1.6	4.3	4.3
45		1.4	3.1	3.2
50		1.2		
51	2.6	3.1	4.9	5.3
52	3.1	1.5	16	
53	9.1	8.4	29	15
54	5.0	3.8	39	24
55	23	15	5.6	25
56		2.2		
57		1.2		
63		.76		
65	3.6	2.9	5.3	5.4
66	4.0	2.5	4.7	4.6
67	69	53	100	99
68	18	9.4	27	16
69	8.1	3.6	9.8	6.9
71		.94	5.3	5.0
77	7.4	3.6	10	7.5
78		1.1	4.9	
79	9.3	5.7	17	16
80	3.3	3.4	5.9	5.8
81	100	100	93	100
82	50	40	76	75
83	6.5	3.8	7.1	7.9
90		1.6		
91	4.6	1.6	5.9	4.7
93	13	4.0	26	11
94			40	20
95	44	30	51	45
96	4.2	2.8	5.1	5.3
97			3.1	
99		.95	70	48
100		.95	10	7.5
108			9.5	14
109	3.4	2.5	13	22

TABLE II (Continued)

Mass	3R-*cis*, *trans* Nepetolactone	3S-*cis*, *trans*- Nepetolactone	3S-*cis*, *cis*- Nepetolactone	3R-*cis*, *cis*- Nepetolactone
110	3.5	1.7	3.0	
111	4.0	.98	12	5.0
112			6.3	13
113			2.5	
125			5.9	16
126			2.4	3.5
135			2.4	
136			4.3	8.7
139	17	1.8	27	6.4
140	3.4		2.8	
153	1.3	.96		2.7
154	1.9	.41	23	16
155	.88	.18	3.3	2.6

TABLE III

ADDITIONAL MASS SPECTRA FROM DEGRADATION STUDIES

Mass	A-12 δ-Dimethyl nepetalinate	B-5 Dimethyl nepetalinate	A-1 Unknown	A-4 Unknown	cis,cis-Dimethyl nepetate
39	11	9.5	12	15	14
41	27	22	22	21	23
43	9.5	10	8.1	17	6.1
44	2.0	2.5			
45			2.9		
50			2.5		3.6
51				4.5	
52	3.5	1.7			
53	11	9.3	9.1	10	12
55	17	18	25	19	15
56	7.9	7.3			
57	8.9	7.2			
59	18	17	12	11	23
67	21	31	9.7	44	20
68		7.7			6.4
69	22	20	37	13	13
74				13	
77		4.9		12	6.4
79	8.9	7.7		19	14
80					17
81	100	100	29	23	100
82	12	20			13
83			14		
88	35	19			
91				11	
93	7.3	6.0		24	
95	6.9	8.7	8.9		
96		9.6	26		
101	15	17	100		63
107		7.1		11	
108	13	11		34	13
109	58	50	12	100	28
110	6.6	6.7		12	
111			12		6.3
113	9.5	21			89
114	7.6	3.8			12
115			62		

TABLE III (Continued)

Mass	A-12 δ-Dimethyl nepetalinate	B-5 Dimethyl nepetalinate	A-1 Unknown	A-[illegible] Unknown	*cis*,*cis*- Dimethyl nepetate
125	6.6	6.0			4.3
126					4.7
136	4.4	5.3			
137	6.2	7.6			
138			8.1		
139	7.8	9.5	6.2	28	
140					36
141	32	58	6.8		12
142		6.4			
145					47
146					4.3
153	14	12		4.4	
154		3.8			
155			1.6		
164		5.0			
168	60	44		27	18
169	12	8.1			35
170			1.6		3.7
171		14			
173		3.4			
196	8.5	12			
197	15	18			
228	x	1.1			

CHAPTER III

EXPERIMENTAL

Harvesting of N. cataria and N. mussini.--Nepeta cataria plants which were grown by the Botany Department of Oklahoma State University were harvested while in seed in September, 1967 after growing all summer. About 255 kg of plants were steam distilled. The distillate was saturated with salt, extracted 3 times with ether, and the ether extract was concentrated by distillation. This concentrate was washed with saturated $NaHCO_3$ to remove acidic impurities, dried with $MgSO_4$ and the remainder of the ether was removed to give 186 g of oil. Chromatography indicated that nepetalactone (1a) and epinepetalactone (1b) were present in a ratio of 91/9. This extract was distilled before further reaction.

N. mussini plants grown by the Oklahoma State Botany Department were harvested while in flower in October, 1967. From 38 kg of plants, 23.7 g of oil was obtained. Another batch cut in May, 1968 gave 14 g of oil from 16 kg of plants. These extracts were essentially identical. Both contained cis, cis-nepetalactone (1d) and cis, trans-nepetalactone (1a) in a ratio of 91/9. This was confirmed by gas chromatography and by identity of results from degradation experiments.

Crystallization of Nepetalic Acid.--The nepetalic acid was prepared by treating freshly distilled N. cataria oil with base, extraction to remove neutrals, acidification and a second extraction. The compound was taken up in a minimum amount of hot petroleum ether, seeded with a few crystals of standard nepetalic acid and the container was scratched with a glass rod. The container was allowed to sit in the refrigerator for a week. After this time, crystals had formed. Three recrystallizations from ether-petroleum ether gave essentially "pure" nepetalic acid. (mp 72-73^{o}). Nepetalic acid obtained from N. mussini oil could not be induced to crystallize by this procedure.

Degradation of Nepetalic Acid in Alkaline Hydrogen Peroxide.--A 1.47 g sample of freshly distilled N. cataria oil was shaken for 5 minutes in 15 ml of 10% KOH and extracted 3 times with ether to remove neutral compounds (162 mg of neutrals were removed leaving about 1.328 g of the 2 nepetalactones). This sample was placed in a 3-necked flask fitted with a reflux condenser, dropping funnel and thermometer and while stirring magnetically, 8 ml of 30% H_2O_2 were added dropwise over a period of 15 min so that the pot temperature stayed below 70^{o}. After 3 hours, the reaction mixture was acidified with 3N HCl and extracted with ether. The extract was washed with water, twice with a dilute solution of $FeSO_4$ and again with water to remove peroxides and extracted then with saturated $NaHCO_3$. The ether layer was dried with $MgSO_4$, filtered and the ether removed with a rotary evaporator. Gas chromatography indicated the presence of the epimeric cis, trans-nepetolactones (3a, b) in this fraction, in a yield of about 21%. The bicarbonate extract was acidified with dilute HCl, saturated with salt and extracted with ether. After drying of the ether and esterification with CH_2N_2, gas chromatography indicated the presence of three methyl nepetonates in the ratio of 14:1:9 with trans, trans-methyl nepetonate (4b) predominating. The yield of these nepetonic acids was about 32%.

Crystalline nepetalic acid (1.382 g, mp 71-72°, lit.[1] 73°) was reacted in a manner analogous to the above procedure except for the preliminary extraction. The yields estimated gas chromatographically were 40% of two nepetonic acids (4b, a) (ratio 25:4) and 15% for 2 nepetolactones (3a, b). In a similar experiment, 1.265 g of lactones obtained from *N. mussini* oil was reacted with 8 ml of 30% H_2O_2 in 15 ml of 10% KOH, maintaining the pot temperature at 50° during the 3 hour reaction. The approximate yields were 20% of three nepetonic acids (4c, b, d) and 19% of the two *cis*, *cis*-nepetolactones (3c, d).

Gas Chromatography.--Unless stated differently, all analyses were performed using an Instruments Incorporated Model 393 valved gas chromatograph fitted with a flame ionization detector and using the 0.02 in. x 450 ft polyphenyl ether capillary column described in the first part of this dissertation. Typical conditions for analysis of degradation mixtures from nepetalactones were a temperature of 150° and a helium pressure of 35 psig (flow of about 15 ml/min). For chromatography of the nepetalactones, the temperature was increased to 170°. Under these conditions, nepetalactone (1a) and epinepetalactone (1b) were separated by about 10 minutes.

Preparative gas chromatography of nepetalactone, *cis*, *cis*-nepetalactone, and the four nepetolactones was performed on an F and M Model 700 gas chromatograph fitted with a thermal conductivity detector, using a 0.5 in. x 13 ft. copper column packed with 25% Carbowax 20M on 80/100 mesh acid washed Gas Pack. Collections were made at 210° and a flow rate of 90 ml/min. Under these conditions, all of the above compounds could be separated from the impurities in the samples from which they were obtained by a sufficient amount to obtain them in about 99% purity from a single collection. About 70 μl of oil or reaction mixture were injected per run. Samples were collected using 5/32 in. x 15 in. coiled-glass collection tubes with a latex rubber connector to the exhaust of the chromatograph. The tubes were heated near the top with a heat gun while the lower half was cooled in an ice bath.

Per cent yields were estimated from chromatography performed on an Aerograph Model 1520 gas chromatograph, using a thermal conductivity detector, and the carbowax column and conditions described above. Peak areas were measured by a Disc Chart Integrator, Model 224-4 from Disc Instruments, Inc. Standard samples of the two cis, trans-nepetolactones, trans, trans-methyl nepetonate, and α-dimethyl nepetalinate were prepared. Multiple injections were made of the standard solution followed by multiple injections of the reaction mixture. A typical degradation mixture was diluted to 5 ml with ether in a volumetric flask and the average was taken of three, 10 μl injections. For quick analysis of later runs, yields were estimated using indene as internal standard. Relative molar responses were approximately 1.0 (indene), 1.0 (nepetolactone), 1.2 (methyl nepetonate) and 1.7 (dimethyl nepetalinate).

Baeyer-Villiger Reaction of Nepetalic Acid.--Peroxytrifluoroacetic acid was prepared by dropping 2.1 ml of trifluoroacetic anhydride into 246 μl of 90% H_2O_2 in 5 ml of CH_2Cl_2 while cooling in an ice bath. This solution was added over a period of 10 min to a suspension of 5 g of Na_2HPO_4 in 10 ml of CH_2Cl_2 and 700 mg of dissolved nepetalic acid. After 30 min the reaction was stopped, washed with $NaHCO_3$ and this wash along with the insoluble salts remaining were acidified with 3N HCl and extracted with ether. The ether was removed and the products refluxed for 1 hour in 10% NaOH. The solution was then acidified, extracted with ether and the extract dried over $MgSO_4$, esterified with CH_2N_2 and gas chromatographed. Yields were estimated chromatographically to be 30% of the cis, trans-nepetolactones (3a, b) and 30% of α- and δ-dimethyl nepetalinates (5a, b).

Reaction of Nepetolactones with Hydrogen Peroxide.--A 243 mg sample of cis, trans-nepetolactone (equal ratios of each isomer) was shaken for 5 min in 11 ml of 10% KOH. To this was added dropwise, 5 ml of 30% H_2O_2 with stirring. After 1 hour, 8 ml of the reaction mixture was removed, acidified and extracted. This extract was treated in the normal manner. The total extract was esterified and chromatographed. No change had occurred in the ratio of nepetolactone isomers and no peaks corresponding to methyl nepetonates were present. The remainder of the reaction mixture was processed after 20 hours, with the same results. The recovery of nepetolactones was estimated chromatographically to be 72%.

Reaction of Nepetalic Acid with Oxygen.--A 1 g sample of N. cataria oil was dissolved in 10 ml of 5% NaOH. After extraction of the neutral compounds, O_2 was bubbled into the solution contained in a 3-necked flask fitted with a reflux condenser. The mixture was stirred magnetically. After 21 hours an aliquot was removed, acidified and extracted with ether and the extract dried, esterified, concentrated and gas chromatographed. A peak which corresponded to trans, trans-methyl nepetonate had appeared and represented approximately 25% of the acidic products. After 48 hours, the ratio of this peak to the two methyl nepetalate peaks was about 3/2. Peaks corresponding to cis, trans- and trans, cis-methyl nepetonate were also present. The amount of dimethyl nepetalinate present was less than 5% of the amount of nepetonate. Identities were confirmed by correspondence of retention times and peak enrichment experiments.

Proof of Evolution of CO During Peroxide Degradations.--The phosphomolybdic acid-$PdCl_2$ test was used to demonstrate the evolution of CO during the degradation of nepetalactone in alkaline hydrogen peroxide. With nitrogen bubbling through the system, 1.5 g of N. cataria oil from which neutral compounds had been removed was reacted with 9 ml of 30% H_2O_2 in the usual way. The gases were swept from the reaction flask through traps filled with 30% KOH, fuming H_2SO_4, 30% KOH, and concentrated H_2SO_4 and into a trap containing 3 ml of solution one and 2 ml of solution two. (Solution one: 17 mg of $PdCl_2$ dissolved in 300 ml of water with 2 ml of HCl. Solution two: a 0.5% solution of $20MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$). After about 10 min the test solution had begun to change color from yellow-green to blue-green and after 2 hours it had acquired a deep blue color. Carbon monoxide generated by dropping H_2SO_4 into formic acid gave a similar color. When nitrogen was bubbled through a mixture of NaOH and H_2O_2 and into the test solution, no color change was observed after 16 hours.

Preparation of Nepetalinic Acids from Nepetalactones.[8]--A 100 mg sample of nepetalic acids was prepared from the crude plant oil by hydrolysis in base, extraction to remove neutrals, acidification with dilute HCl and a second ether extraction. These acids were esterified with CH_2N_2 and stirred for 48 hours in an open flask. The sample was taken up in 2 ml of 10% NaOH, extracted to remove neutrals, acidified and extracted again to obtain the methyl nepetalinates present. After a second esterification, the mixture was studied gas chromatographically.

Preparation of Nepetolactones by Ozonization of Nepetalactones.[5]--A 200 mg sample of nepetalactone (1a) of greater than 99% purity was dissolved in 15 ml of methanol, the solution cooled to -70° with a dry ice-acetone bath and 0.3 ml of pyridine added. Ozone was passed into the solution until a blue color was observed. A solution of 0.15 g of $NaBH_4$ in 1.5 ml of water was then added. After 30 min, a similar portion of $NaBH_4$ was added and the temperature was allowed to rise to 0°. Similar portions of reducing agent were added after 2,4, and 10 hours. After 10 more hours at 0°, 2 g of NaOH was added along with excess water and the solution was evaporated to remove the methanol. The solution was acidified, and extracted with ether. Gas chromatography showed the presence of only two major products, the *cis*, *trans*-nepetolactones (3a, b). A similar experiment was performed on 100 mg of purified *cis*, *cis*-nepetalactone (1d) from *N. mussini* oil to obtain the *cis*, *cis*-nepetolactones (3c, d) as the only major products.

Preparation and Epimerization of Nepetonic Acids from Nepetalactones.--An 80 mg sample of nepetalactone (1a) purified by gas chromatography and distilled using a short-path distillation apparatus was ozonized at -70° in 10 ml of CH_2Cl_2 without added pyridine. The sample took up ozone and the end point could be recognized by a permanent blue color. The ozonide was decomposed by the addition of 1 g of zinc dust and the sample was allowed to warm to room temperature. The zinc was removed by filtration and the solution extracted with saturated $NaHCO_3$. The aqueous layer was acidified and extracted to obtain cis, trans-nepetonic acid (4a) estimated chromatographically to represent about 90% of the products present. This acid was dissolved in 5 ml of 10% NaOH and the solution stirred for 1 hour. The major product obtained after acidification and extraction was trans, trans-nepetonic acid (4b).

A 70 mg sample of purified cis, cis-nepetalactone (1d) from N. mussini was treated as above. Gas chromatography before epimerization indicated that about 90% of the sample was a compound whose retention time was identical with that of the second methyl nepetonate peak from degradation of N. mussini oil with peroxide (cis, cis-methyl nepetonate, 4d). After epimerization for 1 hour in 5 ml of 10% NaOH, a new peak had become the major compound of the mixture. This corresponded to the first methyl nepetonate peak from peroxide degradation mixtures of cis, cis-nepetalactone (trans, cis-methyl nepetonate, 4c).

Preparation of Nepetic Acids.--A 40 mg sample of cis, cis-nepetonic (4d) acid was prepared from cis, cis-nepetalactone (1d) using the above procedure. A solution of 800 mg of iodine and 1.6 g of KI in 3 ml of water was heated to 50° in a small flask while stirred magnetically. To this was added the nepetonic acid in 5 ml of 10% NaOH. After 5 hours, the reaction mixture was filtered to remove iodoform, acidified with 6N HCl and treated with $NaHSO_3$ to remove excess iodine. After extraction with ether, the extract was esterified with CH_2N_2 and chromatographed. The two major peaks corresponded in retention time to trans, cis- and cis, cis-dimethyl nepetate (13b, d). The ether was removed and the sample was added to sodium methoxide solution prepared by addition of a small lump of sodium to 2 ml of anhydrous methanol.

Aliquots were withdrawn after 1, 4 and 16 hours, acidified with dilute HCl, and extracted with ether. After 16 hours, a peak which corresponded to *trans*, *trans*-dimethyl nepetate (13a) was the major compound of the mixture and no *cis*, *cis*-dimethyl nepetate (13d) was present.

Mass spectra were taken on the LKB prototype described in the first part of this thesis at an electron energy of 70 eV and an accelerator voltage of 3.5 KV. The nmr spectra were obtained with a Varian HR-60 spectrometer, using tetramethylsilane as an internal standard ($\delta = 0$).

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