ISOLATION, IDENTIFICATION AND SYNTHESIS OF ATTRACTANTS FOR THE RUSTY GRAIN BEETLE, *CRYPTOLESTES FERRUGINEUS* (COLEOPTERA:CUCUJIDAE).

by

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"Isolation, Identification and Synthesis of Attractants for the Rusty Grain Beetle, Cryptolestes ferrugineus (Coleoptera:Cucujidae)"

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ABSTRACT

Volatile compounds which are attractive for the rusty grain beetle, Cryptolestes ferrugineus, have been shown to be derived from fungus-infected wheat, the beetles themselves and beetle-produced frass. Volatiles from male beetles and from frass produced by male beetles elicit the response of both sexes and thus males produce a true aggregation pheromone. Only females respond to the odor of females.

Porapak Q has been found to be an efficient adsorbent in which to trap these attractive compounds. Soxhlet extraction of the odor laden Porapak Q with pentane removed all of the attractants. Fractionation, by preparative gas-liquid chromatography, of pentane extracts of Porapak Q-entrapped volatiles from beetle-produced frass coupled with comparative bioassays have shown that aggregation is induced by a pheromone complex of two to four compounds. Two of these compounds have the compositions $C_{12}H_{20}O_2$ and $C_{14}H_{24}O_2$. Spectral data indicate that these two attractants are probably esters or lactones. The third attractant, the major component of the pheromone, has been identified as 10-hydroxy-4,8-dimethyl,4-E,8-E,decadienoic acid lactone(I). A seven-step synthesis starting from geraniol was successfully conducted to yield I in 0.17% overall yield.

To the unions at Simon Fraser University.

"Keep the faith ..."

Tim Williams, 1980

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

The rusty grain beetle, Cryptolestes ferrugineus (Stephens) (Coleoptera:Cucujidae), is a major insect pest of stored grain in Canada[1]. It is the most serious pest of stored wheat in the prairie provinces, devouring the protein containing wheat germ[2]. It causes heating in wheat[3] and is often found in association with wheat-damaging fungus[4].

C. ferrugineus was the most frequently found insect in a survey of farm-stored grain[5] and is often found in grain elevators, box cars and cargo ships[2]. It is often found in grain exported to Great Britain from Canada[6] and does much damage to stored grain in Great Britain[7]. Because of the damage it causes and because of stipulations in the Canada Grain Act that grain be free from insect infestations, the rusty grain beetle is a major concern.

Traps currently used to detect C. ferrugineus in stored grains and in box cars rely on passive encounters of the beetles with the traps[8]. In terminal grain elevators, the vacuum sample delivery system used for detection kills 70% of the beetles before they can be observed[9]. Since other stored grain beetles utilize pheromones[10], the possibility exists that pheromones could be used to detect C. ferrugineus.

C. ferrugineus is known to be associated with fungus-infected grain. Of twenty-three species of fungi which were isolated from stored wheat and tested as feeding media and oviposition sites for the rusty grain beetle, Loschiavo and Sinha[4] showed that the best fungi for feeding and oviposition were Nigrospora sphaerica, Mucor sphaerosporus, Hormodendrum

cladosporioides and Curvularia tetramera[4]. Dolinski and Loschiavo[11], while studying the effect of fungi on the locomotory behavior of the rusty grain beetle, showed that C. ferrugineus was attracted to Penicillium corymbiferum, Scopulariopsis brevicaulis and Fusarium sp., all of which occur in stored wheat[4].

If pheromones or fungal attractants were available as pure compounds for C. ferrugineus, more effective detection and survey traps might be developed and pheromone-based control programs[12] might be feasible. The objective of this research was to isolate, identify and synthesize attractants for the rusty grain beetle.

II. AERATION AND EXTRACTION PROCEDURES AND RESULTS

A. Procedures

i. Introduction:

Attractants for insects may be obtained from several sources including the insects themselves, host feeding material, associated microflora and insect frass. Examples of insect derived attractants abound[13]. Attractants derived from host feeding material have been reported for Carpophilus spp. (Coleoptera: Nitidulidae) which are attracted to volatile compounds present in ripening figs[14]. Onion volatiles have been shown to attract and stimulate oviposition for the onion maggot, Hylemya antigua (Meigen) (Diptera:Anthomyiidae)[15,16].

Insect frass is a common source of insect attractants. Frass (a mixture of boring dust and insect feces) from several bark and timber beetles, among them ambrosia beetles, Trypodendron lineatum and Gnathotrichus sulcatus (Coleoptera:Scolytidae), have been shown to contain species specific pheromones[17,18].

All of these possible sources as well as fungus-infected wheat were examined as attractant sources for the rusty grain beetle.

ii. Experimental insects and frass production:

C. ferrugineus cultures were maintained on whole or cracked wheat and wheat germ in 3.8 ℓ glass jars at 27-30 C and 80 ± 10% relative humidity (R.H.). The lower temperature and humidity levels were beneficial for increased frass production since fungal growth was reduced; however, fewer larvae occurred under these conditions. Beetles for experiments were separated from the larger diet components with a No. 25 sieve and were collected

as they walked away from the sieved tailings. Under examination through a microscope these tailings contained approximately 50% fecal pellets on a volume basis. The remainder was made up of boring dust, chaff and larvae skins. This frass was stored in glass containers until needed.

Numbers of beetles in large samples were estimated by volume (1,000 beetles/mℓ) or by weight (2.9×10^{-4} g/beetle). If insects of either sex were required, cold-immobilized beetles were sexed by the presence of the males' ventro-lateral, mandibular tooth[19].

iii. Fungus-infected grains:

Fungus-infected grain was derived from two sources. Preliminary studies were conducted with wheat infected with unidentified fungi of several species. These samples were obtained directly from field infestations of C. ferrugineus.

In subsequent work, grain infected with a single species of fungi was used. Samples of pure identified fungi (Aspergillus flavus and Penicillium corymbiferum) on agar were obtained from Dr. J. Mills†. Initially, each fungal species to be used was scraped from the agar with a sterile wire loop and transferred to a 2 ℓ Erlenmeyer flask containing ~1,000 g of autoclaved Buckerfield's Hard Red Spring Canada No. 1 wheat (20% moisture content). The flasks of grain were allowed to stand at 29 ± 1°C and 70 ± 5% relative humidity and shaken daily for two weeks. Later, to increase the amount of fungus-infected grain, one

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† Agriculture Canada Research Station, Winnipeg, Manitoba.

gallon glass containers, each holding 3,000 g of wheat, were used.

iv. Bioassay development:

Once an odor source has been obtained, volatiles from the odor source must be tested for biological activity, usually by measuring the behavioral responses of the insects to the volatiles.

Initial studies[11] tested the locomotory behavior of C. ferrugineus through a column of wheat to fungal odors. This method was labor-intensive and time consuming (bioassays take 48 h) so a simple, more rapid bioassay method was developed based on an open arena, airflow olfactometer[20]. This apparatus was maintained at 20 ± 2 C and variable relative humidity in a room reserved for olfactory experiments. An aquarium pump forced air at 0.5-1 ℓ/min through tygon tubing into a 125 mℓ Erlenmeyer flask containing the odor source. The air passed out of the flask through glass tubing which fed the air horizontally over a paper arena surface. The paper was changed for each new stimulus.

The olfactometer was later modified to use a compressed air cylinder instead of the aquarium pump. This avoided recycling the room air through the apparatus. The tygon tubing was replaced with copper, brass and glass tubing. The Erlenmeyer flask was retained for bulk odor sources, but to ensure more uniform stimulus levels, extract stimuli were delivered for each replicate through disposable 5 cm long, 11 mm O.D. glass tubes containing a rolled, unwaxed filter paper impregnated with the extract. The paper arena was replaced by a 7.5 X 7.5 cm glass

plate which was changed for each new stimulus and washed with acetone prior to re-use. The air outlet was 1 mm from the arena surface.

Bioassay beetles were held in 25 mℓ glass vials and released in replicate groups of 10-50, 4 cm downwind of the air outlet. Responding beetles walked upwind. They were considered to be responders only if within two minutes they entered a 1 X 0.5 cm rectangle in front of the air outlet and were headed upwind. After use the beetles were returned to their 25 mℓ glass holding vial, which was placed at the end of a rotation of at least seven other vials. There was no evidence that this procedure resulted in variation in response through time.

v. Preliminary bioassay results:

Following the development of the arena airflow olfactometer, the various odor sources were tested for attractiveness for the rusty grain beetle. The experiments were performed as outlined in Table I. The first two utilized beetles as stimulus sources. The last one assessed the attractiveness of frass. The bioassays for all the experiments were completed within separate 1-4 hour sessions. All data for each experiment were pooled and compared with χ^2 tests in which all values were compared in all possible pairs. Since fungus-infected grain was already known to be attractive for *C. ferrugineus*[4], it was not bioassayed at this time.

Experiments 1 and 2 were conducted to determine if the odors of *C. ferrugineus* populations of different sizes and sexes were attractive to beetles of either or both sexes. In the first experiment the odor of 5,000 rusty grain beetles of mixed sexes attracted beetles of both sexes. In Experiment 2, there were

Table I. Response of C. ferrugineus of mixed age in laboratory bioassays to air passed over beetle populations and over frass from beetle cultures.

Experiment No.	Odor Source	Responding Beetles			
		Sex	No. replicates	No/ replicate	% response*
1	Air passed over approximately 5000 48-96 hr. old beetles	♂	10	20	58.0[b]
		♀	10	20	53.0[b]
	Air control, 0.5 ℓ/min.	♂	10	20	20.5[a]
		♀	10	20	25.5[a]
2	Air passed over 48-96 hr. old beetles:				
	500 beetles	mixed	10	20	15.5[a]
	1100 beetles	mixed	10	20	36.5[b]
	2200 beetles	mixed	10	20	50.0[c]
	Air control, 0.5 ℓ/min.	mixed	10	20	10.5[a]
3	Air passed over 12 g of frass	mixed	10	20	50.0[b]
	Air control, 0.5 ℓ/min.	mixed	10	20	6.5[a]

* Percentages within an experiment followed by same letter are not significantly different, χ^2 test, $P < 0.05$.

significant responses to 2,200 and 1,100 beetles, but not to 500 beetles. These results present strong evidence that C. ferrugineus utilizes an aggregation pheromone. Aggregation pheromones are chemicals produced by either males or females which cause other members of the same species to aggregate in a particular area[21]. For C. ferrugineus, as well as other Coleoptera, such pheromones may have arisen as mechanisms which cause aggregation at a suitable food source. This aggregation pheromone may act in conjunction with fungal volatiles to promote population build-up in grain suitable for habitation.

Experiment 2 shows that the minimum number of stimulus beetles necessary for a significant response is relatively high. This may be because the beetles produce very small quantities of pheromone or because of design problems in the olfactometer.

Results from Experiment 3 suggest that the pheromone exists in the rusty grain beetle frass and may be produced in the alimentary canal and/or the Malpighian tubules[20].

vi. Methods to capture and extract attractants:

The demonstration that odor sources were attractive to the rusty grain beetle was followed by preparation of chemical extracts containing the attractants. The two main methods of collecting attractants from solid biological material are vapour entrapment and solvent extraction.

Both of these methods were examined as outlined in Tables II-IV. Experiments using the vapour entrapment method, involved entrapment of volatiles from an odor source into Porapak Q (ethylvinylbenzene-divinylbenzene copolymer, 50/80 mesh, Applied Science Laboratories, Inc.). Porapak Q has been shown to collect

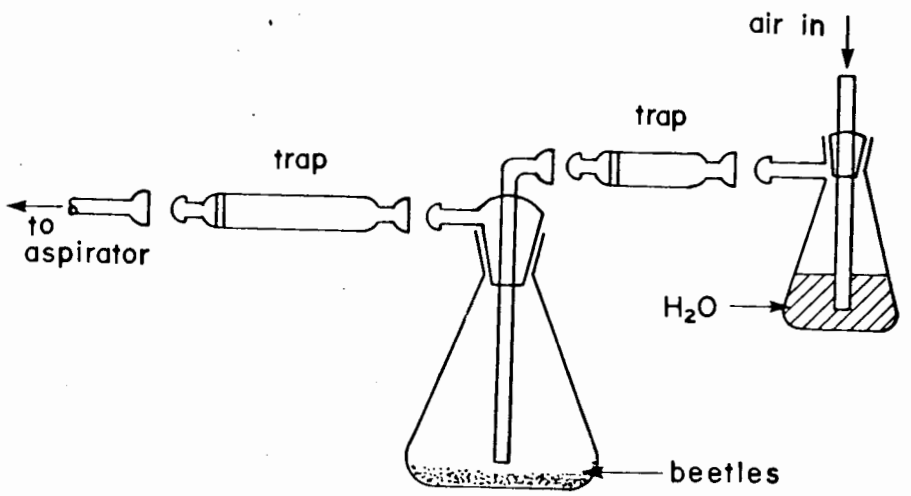
volatiles efficiently[22]. Prior to an experiment, Porapak Q was conditioned by extraction with anhydrous, reagent-grade ether in a Soxhlet extractor for a minimum of 15 hours and, after evaporation of residual ether, was stored in glass-stoppered bottles in the dark. For the capture of beetle-produced volatiles, large numbers of beetles were placed in a 2.8 ℓ sterilized glass culture flask (Figure 1a). Two S-19 ground glass joints were fitted to the top of the flask. From one of these, serving as an air inlet, 10 mm O.D. glass tubing extended to within 2 cm of the bottom of the flask. A 55/44 ground glass joint near the top of the flask permitted access. Porapak Q-filled glass tube traps (2.4 cm O.D. X 20 cm) were fitted with S-19 female and male ground joints on the top and bottom, respectively. A coarse, sintered glass disk was sealed inside each tube at the bottom. These tubes held approximately 26 g of Porapak Q. A smaller Porapak Q-filled trap (2.4 cm O.D. X 12 cm) was attached to the flask inlet and served as an air scrubber. To avoid Porapak contamination, stopcock grease was not applied to ground glass joints, which sealed firmly if kept clean. Air was drawn with a water aspirator at a rate of 1-2 ℓ/min (measured at the inlet with a Brooks Flowmeter), through water (to prevent desiccation of the beetles), through the scrubber, over the beetles and finally through the larger trap. Collection of beetle released volatiles usually involved 3-5 aerations, each lasting 2-4 days. The beetles were allowed to feed for at least two days between aerations.

To capture frass, wheat or wheat germ volatiles, the odor source was ground with a mortar and pestle and placed in a glass

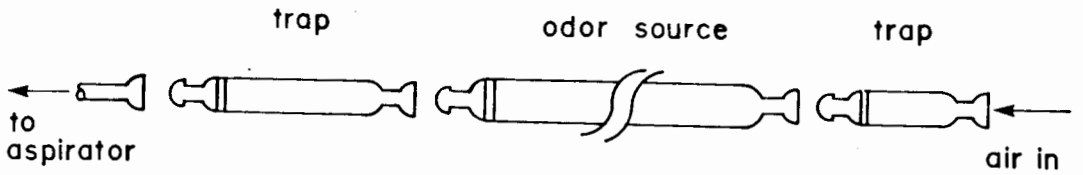
Figure 1

Aeration apparatus used for Porapak Q trapping of beetle produced volatiles (a) and frass, wheat or wheat germ volatiles (b).

(a)


air in
trap
trap
to
aspirator
H_2O
beetles

(b)


trap
odor source
trap
to
aspirator
air in

tube (3.5 cm O.D. X 45 cm) fitted with S-19 female and male ground joints on each end (Figure 1b). A coarse sintered glass disk was sealed inside the tube at the end (female joint). Near the tube inlet a 24/40 joint permitted access. This tube held approximately 160 g of odor source. A Porapak Q-filled scrubber was attached to the inlet end and a larger Porapak Q trap was connected to the other end. Air was drawn through the system with a water aspirator at approximately 1 ℓ/min for 7 to 14 days.

To capture volatiles from fungus-infected wheat, the infected wheat was placed in one to four sterilized glass chambers (approximately 3,000 g of wheat per chamber). The two-piece, cylindrical chambers (15.5 cm I.D. X 27 cm) were fitted with a 1.5 cm wide ground glass flange about 9 cm from the top. The two pieces were held together by two plastic rings that rested on the flange and were drawn together by four screws. The top and bottom of the chamber were fitted with centered S-19 female and male spherical ground joints. A Porapak-filled scrubber was attached to the top of the chamber. The chambers were supported in a stand so that the large Porapak Q trap could be connected vertically to the bottom of the chamber. Air was drawn through the chamber by a suction pump connected to the bottom of the trap at ~2 ℓ/min. In experiments where more than one chamber of the same fungus-infected grain was used, the chambers were connected in series using glass connectors.

Pentane (Caledon Laboratories Ltd.) fractionally distilled through a 30 cm glass Dufton column was used to extract odor laden Porapak Q in a Soxhlet extractor for at least 20 hours.

The extract obtained was concentrated to about 5 mℓ by distillation of the pentane through a Dufton column. The concentrate was transferred to a graduated cylinder and the volume was brought up to 10 mℓ. This solution was then placed in glass vials with Teflon-lined caps and stored in the dark until used.

In experiments where attractants were captured using solvent extraction techniques, the odor source was extracted in a Soxhlet extractor with purified pentane for at least 20 hours. The pentane extract was processed as previously described.

Experiment 11 (Table IV) was undertaken to determine if attractive compounds from Aspergillus flavus infected wheat could be obtained by steam distillation. In this experiment, 1,800 mℓ of distilled water was added to 1,300 g of Aspergillus flavus infected wheat in a 5 ℓ flask. This mixture was distilled under reduced pressure (using a water aspirator) with the first 500 mℓ of distillate being collected in a 1 ℓ flask cooled in an acetone/dry ice bath. This distillate was extracted with three 75 mℓ portions of purified pentane. After being washed with 100 mℓ of saturated NaCl solution, dried ($MgSO_4$) and filtered, this pentane extract was processed as previously described.

B. Results

i. Attractive extracts:

Table II shows the results of experiments designed to obtain pentane extracts of attractants from C. ferrugineus which are attractive to the beetles. Simple pentane extraction of the beetles (Experiment 4) did not lead to an attractive extract. This may indicate that the beetles do not store the pheromone and, instead, release it shortly after it is produced.

Table II. Response of *C. ferrugineus* of mixed age and sexes in laboratory bioassays to pentane extracts of beetles and to pentane extracts of beetle produced volatiles.

Experiment No.	Stimulus Description	Responding Beetles		
		No. replicates	No./ replicate	% response*
4	Pentane extract of beetles (Soxhlet extraction, 2 X 10^4 beetles for 20 hr).			
	50 b equiv.†	4	25	25.0[a]
	50 b equiv.	4	25	18.0[a]
	Pentane control 0.025 mℓ.	4	25	16.0[a]
5	Pentane extract of Porapak Q-trapped volatiles produced by approx. 20,000 beetles for 120 hr.			
	24,000 bhr‡	5	20	54.8[c]
	2,400 bhr	5	20	33.3[b]
	240 bhr	5	20	22.4[ab]
	24 bhr	5	20	13.1[a]
	Pentane control 0.1 mℓ.	5	20	18.3[a]

Table II (Cont'd)

Experiment No.	Stimulus Description	Responding Beetles		
		No. replicates	No./ replicate	% response*
6	Pentane extract of Porapak Q-trapped volatiles produced by 60,000 beetles for 240 h‡			
	34,000 bhr‡‡	4	50	32.5[c]
	3,400 bhr	4	50	43.5[d]
	340 bhr	4	50	35.0[cd]
	34 bhr	4	50	33.5[b]
	3.4 bhr	4	50	17.0[ab]
	0.34 bhr	4	50	17.4[ab]
	Pentane control 0.025 mℓ.	4	50	14.5[a]

* Percentages within an experiment followed by same letter not significantly different, χ^2 test, $P < 0.05$.

† One beetle equivalent (b equiv.) = the amount present in 1 beetle.

‡ One beetle hour (bhr) = the volatiles from one beetle for 1 hour.

‡‡ Starting populations and aeration durations (in parentheses): 66,000 (48 hr.), 58,000 (96 hr.), 54,000 (49 hr.), 50,000 (44 hr.). Two day feeding period allowed between aerations.

Alternatively, the concentration of pheromone in this extract may be very low and may be below the detection limit for the beetles. Whatever the reasons for the non-attractiveness, this method of obtaining attractive extracts is clearly not satisfactory considering >20,000 beetles were sacrificed in this experiment.

Experiments 5 and 6 (Table II) showed that attractive volatiles from air passed over large beetle populations could be captured on Porapak Q. The threshold stimulus concentration required to produce a response greater than the control (pentane) was 2,400 beetle hours (bhr) in Experiment 5, but only 34 bhr in Experiment 6. This difference may be because beetles in Experiment 5 were aerated for five days and were not allowed two day feeding periods between shorter aerations as in Experiment 6. Beetles denied access to food probably produce very little frass after the first one or two days.

Table III assesses the attractiveness of pentane extracts of frass and Porapak Q-trapped frass volatiles. Both of these frass-derived stimuli were significantly attractive to C. ferrugineus at most concentrations. The threshold concentration of 0.67 gram hours (ghr) for the Porapak Q extract in Experiment 8 indicates that relatively little frass is required to elicit a positive response. The response to the most concentrated stimulus of 67.2 ghr was significantly lower than to the pentane control, indicating arrestment or possibly repellency. Arrestment to pheromones at high concentrations has been found in other Coleoptera[10].

Experiment 9 (Table IV) was conducted to compare the

Table III. Response of C. ferrugineus of mixed age and sexes in laboratory bioassays to pentane extracts of frass and to pentane extracts of frass volatiles trapped in Porapak Q.

Experiment No.	Stimulus Description	Responding Beetles		
		No. replicates	No./ replicate	% response*
7	Pentane extracted frass (Soxhlet extraction 120 g for 20 hr.)			
	1.2 g equiv.†	5	20	64.6^{d}
	0.12 g equiv.	5	20	35.4^{c}
	0.012 g equiv.	5	20	26.3^{bc}
	0.0012 g equiv.	5	20	15.0^{a}
	Pentane control, 0.1 mℓ.	5	20	17.0^{ab}
8	Pentane extract of Porapak Q-trapped volatiles from 160 g of frass for 168 hr.			
	67.2 ghr‡	8	25	2.5^{a}
	13.4 ghr	4	25	28.0^{de}
	6.7 ghr	8	25	41.0^{fg}
	1.3 ghr	4	25	46.0^{g}
	0.67 ghr	8	25	32.0^{ef}
	0.07 ghr	4	25	20.0^{cd}
	Pentane control, 0.025 mℓ.	8	25	13.5^{bc}

* Percentages within an experiment followed by same letter not significantly different, χ^2 test, $P < 0.05$.

† One gram equivalent (g equiv.) = the amount present in 1 g of frass.

‡ One gram hour (ghr) = the volatiles from 1 g of material in 1 hour.

attractiveness of pentane extracts of Porapak Q-captured volatiles from C. ferrugineus produced frass, wheat and wheat germ. The pentane extract of Porapak Q-trapped wheat germ volatiles was not significantly more attractive than the pentane control. However, the responses to two extracts of wheat volatiles (1.75 ghr and 0.02 ghr) were significantly higher than the control. The Porapak Q-trapped frass volatiles (at 14.2 ghr) were significantly more attractive than those from other sources. The signficant responses to low concentrations of Porapak Q-entrapped wheat volatiles were not much greater than to the pentane control and may represent experimental artifacts. Alternatively, there may be a wheat-produced attractant. The lack of response to very high stimulus concentrations of both wheat and frass volatiles indicates an arrestment response. Such a response to high concentrations of aggregation attractants may serve to retain beetles in a suitable habitat in nature.

As with frass and beetle derived attractants, Porapak Q proved to be a good adsorbent in which to capture fungus-infected wheat volatiles (Table V). The response of C. ferrugineus in Experiment 11 to the pentane extract of odor-impregnated Porapak Q (at 4,500 ghr) was significantly higher than to the pentane control. The fungus in this case was of unknown identity and probably consisted of more than one species. In Experiment 12 the attractiveness of a pentane extract of Porapak Q-entrapped volatiles produced by Penicillium corymbiferum-infected wheat was assessed. The stimulus concentration needed to elicit a response of C. ferrugineus in this experiment was quite high (58,000 ghr). The reason that such a high concentration is needed is not known.

Table IV. Results of Experiment 9. Response of C. ferrugineus of mixed age and sexes in laboratory bioassays to pentane extracts of Porapak Q-trapped volatiles of frass, wheat and wheat germ.

Stimulus Description	Responding Beetles		
	No. replicates	No./replicate	% response*
Pentane extract of Porapak Q-trapped volatiles from 158 g frass for 354 hr.			
141.6 ghr	4	25	2.0[a]
14.2 ghr	4	25	51.0[g]
Pentane extract of Porapak Q-trapped volatiles from 240 g wheat† for 292 hr.			
175.2 ghr	4	25	5.0[ab]
17.5 ghr	4	25	9.0[bc]
1.75 ghr	4	25	23.0[ef]
0.18 ghr	4	25	17.5[de]
0.02 ghr	4	25	27.0[ef]
0.002 ghr	4	25	12.0[bcd]
Pentane extract of Porapak Q-trapped volatiles from 123 g wheat germ‡ for 340 hr.			
104.5 ghr	4	25	9.0 [bc]
10.5 ghr	4	25	14.0 [cde]
1.1 ghr	4	25	17.0 [cde]
0.1 ghr	4	25	14.0 [cde]
0.01 ghr	4	25	17.0 [cde]
Pentane control, 0.025 mℓ.	12	25	13.3 [cd]

Table IV (Cont'd)

* Percentages followed by same letter not significantly different, χ^2 test, $P < 0.05$.

† Buckerfield's Hard Red Spring Canada No. 1.

‡ Maple Leaf Mills, Lethbridge, Alberta.

Table V. Response of *C. ferrugineus* of mixed age and sexes in laboratory bioassays to pentane extracts of Porapak Q-trapped volatiles of fungus-infected wheat and whole wheat and to a pentane extract of steam distilled fungus-infected wheat.

Experiment No.	Stimulus Description	Responding Beetles		
		No. replicates	No./ replicate	% response*
10	Pentane extract of Porapak Q-trapped volatiles from 3,800 g whole wheat for 180 hr.			
	6,840 ghr	13	10	12.3[a]
	Pentane control, 0.1 mℓ.	13	10	6.9[a]
11	Pentane extract of Porapak Q-trapped volatiles from 2,500 g fungus-infected wheat† for 180 hr.			
	4,500 ghr	13	10	50.0[b]
	Pentane control, 0.1 mℓ.	13	10	6.9[a]
12	Pentane extract of Porapak Q-trapped volatiles from 13,000 g *Penicillium corymbiferum*† infected wheat for 445 hr.			
	58,000 ghr	5	20	32.6[c]
	5,800 ghr	5	20	26.5[bc]
	580 ghr	5	20	19.6[b]
	58.0 ghr	5	20	9.9[a]
	Pentane control, 0.1 mℓ.	5	20	19.6[b]

Table V (Cont'd)

Experiment No.	Stimulus Description	Responding Beetles		
		No. replicates	No./ replicate	% response*
13	Pentane extract of steam distilled Aspergilles flavus‡ infected wheat. 18.0 g equiv.	20	10	20.0[a]
	Pentane control, 0.1 mℓ.	20	10	11.0[a]

* Percentages within an experiment followed by same letter not significantly different, χ^2 test, $P < 0.05$.

† Mixed fungi of unknown identity.

‡ Penicillium corymbiferum, Aspergilles flavus from Dr. J.T. Mills, Agriculture Canada, Winnipeg, Manitoba.

In earlier experiments[11], P. corymbiferum was found to be the most attractive of various fungus species tested. Perhaps a particular strain or age of fungus is required for maximum attractant production.

Experiment 13 assessed the response of C. ferrugineus to a pentane extract of steam distilled Aspergillus flavus-infected wheat. This source did not produce extracts of significant attractiveness in comparison to a pentane control. This may indicate that either this species of fungus does not produce attractants or that steam distillation may not be a good method of obtaining an attractive extract.

ii. The role of the male:

Table VI summarizes the results of experiments undertaken to determine if male and/or female C. ferrugineus produce and respond to beetle produced attractants. Odors from sexed beetles and frass from sexed beetles were used as stimuli to test the responses of sexed C. ferrugineus. In these experiments (Experiments 14-18), both male and female rusty grain beetles significantly responded to air passed over male beetles, pentane extracts of Porapak Q-entrapped volatiles produced by male beetles and to pentane extracts of Porapak Q-entrapped volatiles from male beetle frass. Female beetles responded to volatiles from female beetles or female beetle frass in every case. Male beetles only responded to females in Experiment 17, where the stimulus was a pentane extract of Porapak Q-entrapped volatiles from female beetle frass. This response, although statistically significant, is not very high in comparison to the control. Results from these experiments present strong evidence that

Table VI. Response of C. ferrugineus in laboratory bioassays to air passed over sexed beetles and to pentane extracts of Porapak Q-trapped volatiles of sexed beetles and of the frass produced by sexed beetles. Responding beetles 2-4 weeks old.

Experiment No.	Stimulus Description	Responding Beetles			
		sex	No. replicates	No./ replicate	% response*
14	Air passed over approximately 2,000 3-20 day old female beetles.	♂	5	20	19.0[a]
		♀	5	20	35.0[b]
	Air passed over approximately 2,000 3-20 day old male beetles.	♂	5	20	31.0[b]
		♀	5	20	36.0[b]
	Air control, 1 ℓ/min.	♂	5	20	10.0[a]
		♀	5	20	19.0[a]
15	Pentane extract of Porapak Q-trapped volatiles produced by approximately 2,000 female beetles for 299 hr.†				
	7,150 bh	♂	4	25	8.0[a]
		♀	4	25	43.0[d]
	715 bh	♂	4	25	16.0[abc]
		♀	4	25	17.0[abc]
	360 bh	♂	4	25	12.0[ab]
		♀	4	25	12.0[ab]
	Pentane control, 0.025 mℓ	♂	4	25	25.0[c]
		♀	4	25	23.0[c]

Table VI (Cont'd)

Experiment No.	Stimulus Description	Responding Beetles			
		sex	No. replicates	No./ replicate	% response*
16	Pentane extract of Porapak Q-trapped volatiles produced by approximately 1,500 male beetles for 345 hr.‡				
	5980 bh	♂	4	25	28.0[b]
		♀	4	25	20.0[a]
	598 bh	♂	4	25	24.0[a]
		♀	4	25	40.0[bc]
	300 bh	♂	4	25	43.0[c]
		♀	4	25	38.0[bc]
	Pentane control, 0.025 mℓ.	♂	4	25	25.0[a]
		♀	4	25	23.0[a]
17	Pentane extract of Porapak Q-trapped volatiles from 6.4 g female beetle frass for 166 hr.				
	13.2 ghr	♂	4	25	11.0[a]
		♀	4	25	18.0[abc]
	1.32 ghr	♂	4	25	24.0[bcd]
		♀	4	25	34.0[d]
	Pentane control, 0.025 mℓ.	♂	4	25	9.0[a]
		♀	4	25	16.0[ab]

Table VI (Cont'd)

Experiment No.	Stimulus Description	Responding Beetles			
		sex	No. replicates	No./ replicate	% response*
18	Pentane extract of Porapak Q-trapped volatiles from 3.6 g male beetle frass for 166 hr.				
	14.9 ghr	♂	4	25	9.0[a]
		♀	4	25	7.0[a]
	1.49 ghr	♂	4	25	33.0[c]
		♀	4	25	40.0[c]
	Pentane control, 0.025 mℓ.	♂	4	25	9.0[ab]
		♀	4	25	16.0[b]

* Percentages within an experiment followed by same letter not significantly different, χ^2 test, $P < 0.05$.

† Starting populations and aeration durations (in parentheses): 2,580 (70 hr.), 2,090 (72 hr.), 1,850 (68.5 hr.), 1,290 (88.5 hr.). Two day feeding period between aerations.

‡ Starting populations and aeration durations (in parentheses): 1,820 (64.5 hr.), 1,560 (71.5 hr.), 1,400 (69.0 hr.), 1,220 (71.5), 950 (70.5 hr.). Two day feeding period between aerations.

C. ferrugineus utilizes an aggregation pheromone produced by the males.

iii. Summary:

Experiments outlined in Tables II-VI have demonstrated that Porapak Q is an effective adsorbent in which to capture C. ferrugineus produced attractants and attractants produced by fungus-infected wheat.

Solvent extraction techniques are not as good as the vapour entrapment method of capturing attractants. In the case of C. ferrugineus, solvent extraction was unsuccessful in producing an attractive extract for the rusty grain beetle. In the case of C. ferrugineus produced frass, solvent extraction did lead to an attractive extract. However, it did not appear to produce an extract any more attractive to C. ferrugineus than that produced by Porapak Q entrapment of volatiles from frass. Indeed, solvent extraction leads to more complicated extracts due to extraction of waxes, fats and other non-volatile compounds from the source. Extracts obtained from solvent extraction of frass may provide quantitative estimates of the amount of components present in the frass at the moment of extraction, but they give little information about relative amounts of the attractive components present in the vapour phase. For these reasons, solvent extraction of odor sources was not used for the remainder of the attractant isolation project.

Like solvent extraction, pentane extracts of Porapak Q-entrapped volatiles of frass do not give much information about the relative amounts of attractive component present in the vapour phase in nature. This method is useful, however, in that it gives

clean extracts of the volatiles present in an odor source uncontaminated by non-volatile lipids. This is extremely useful in the initial phases of an attractant isolation project. Entrapment of frass and male C. ferrugineus volatiles with Porapak Q was judged to be a good method to isolate and quantitate the attractants detected and this method was used for isolation work.

III. ISOLATION OF C. FERRUGINEUS PRODUCED ATTRACTANTS

A. Analytical GLC

Pentane extracts of Porapak Q-entrapped volatiles were analyzed directly by gas-liquid chromatography (GLC) using conditions in Table VII. Columns coated with OV-101 were found to be unsatisfactory since two of the main components of the pentane extracts eluted with almost the same retention times. Capillary columns were found to be very useful since good separation and reproducible retention data were obtained. Packed columns (Table VII) were used for most of the analytical studies since these columns were later used for preparative separations.

Typical chromatograms of pentane extracts of Porapak Q-entrapped volatiles from rusty grain beetle produced frass are illustrated in Figures 2 and 3. The extracts from which these chromatograms were obtained were from aerations of different samples of frass conducted more than eighteen months apart. In Figure 2, the extract was analyzed on a packed column, whereas in Figure 3 a capillary column was used. Each chromatogram shows the presence of at least thirty compounds. The compounds labelled A, B and C in these figures were present in most aerations and were shown, by retention data, to also be present in extracts of C. ferrugineus produced volatiles.

The relative amount of each compound in an extract was not determined because, as these figures illustrate, the composition of extracts varied. This variation was not due to isolation technique but to actual differences in volatile content of the frass samples (see Section III C). Samples of rusty grain beetle frass obtained for the more than twenty aerations which were done

Table VII. Experimental conditions for analytical gas-liquid chromatography

	Column									
Instrument*	Type	Liquid Phase	Solid Support	Length (m)	Diameter (mm)	Construction Material	Detector Temp. (°C)	Injection Temp. (°C)	He Flow Rate	Typical Oven Temp.
Varian 1200	packed	5% Carbowax 20M	Chromosorb G 70/80 mesh	3.0	3.2 (O.D.)	stainless steel	250	240	60 mℓ/min†	60° to 180°C @ 4°C/min
	packed	5% OV-101	Chromosorb G 70/80 mesh	3.0	3.2 (O.D.)	stainless steel	250	240	60 mℓ/min†	80° to 200°C @ 4°C/min
	packed	5% SP-1000	Chromosorb G 70/80 mesh	3.0	3.2 (O.D.)	stainless steel	250	240	60 mℓ/min†	160°C
Varian 2100	packed	5% Carbowax 20M	Chromosorb G 70/80 mesh	1.8	6.4 (O.D.)	glass	270	260	40 mℓ/min†	160°C
	capillary	SP-1000		33	0.66 (I.D.)	glass	270	260	20 cm/sec‡	135°C
	capillary	OV-101		38	0.27 (I.D.)	glass	270	260	20 cm/sec‡	150°C
Hewlett-Packard 5830A	capillary	SP-1000		38	0.66 (I.D.)	glass	275	260	30 cm/sec‡	180° to 210°C @ 4°C/min

* All instruments were equipped with flame-ionization detectors (FID).

† Measured at room temperature.

‡ Measured at 100°C.

Figure 2

Gas-liquid chromatogram of a pentane extract of Porapak Q-entrapped volatiles from C. ferrugineus produced frass.

Chromatogram run on a 3 m X 3.2 mm O.D. stainless steel column filled with 5% Carbowax 20 M on Chromosorb G, 70/80 mesh.

Oven temperature programmed from 60° to 150°C at 4°/min.

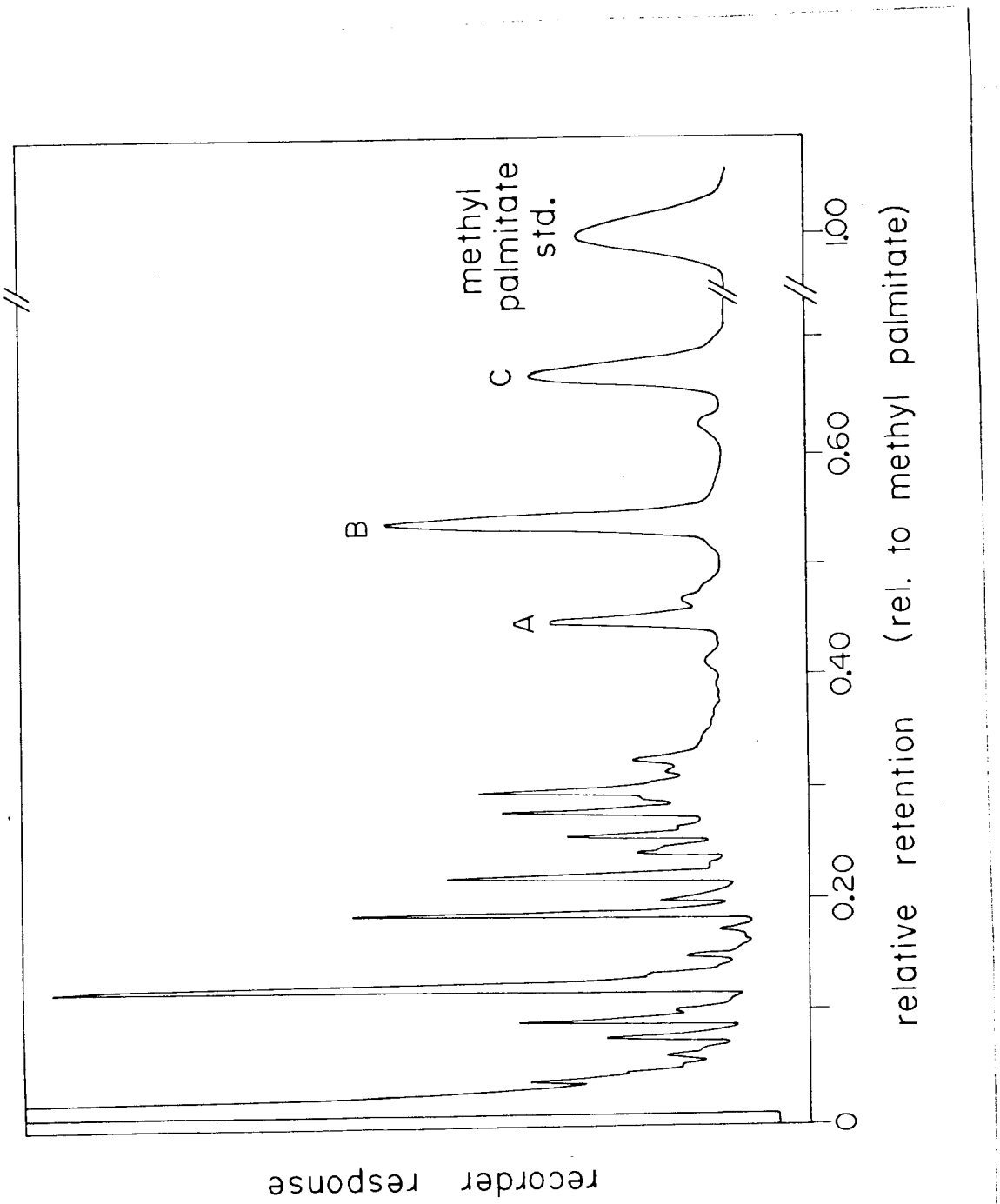
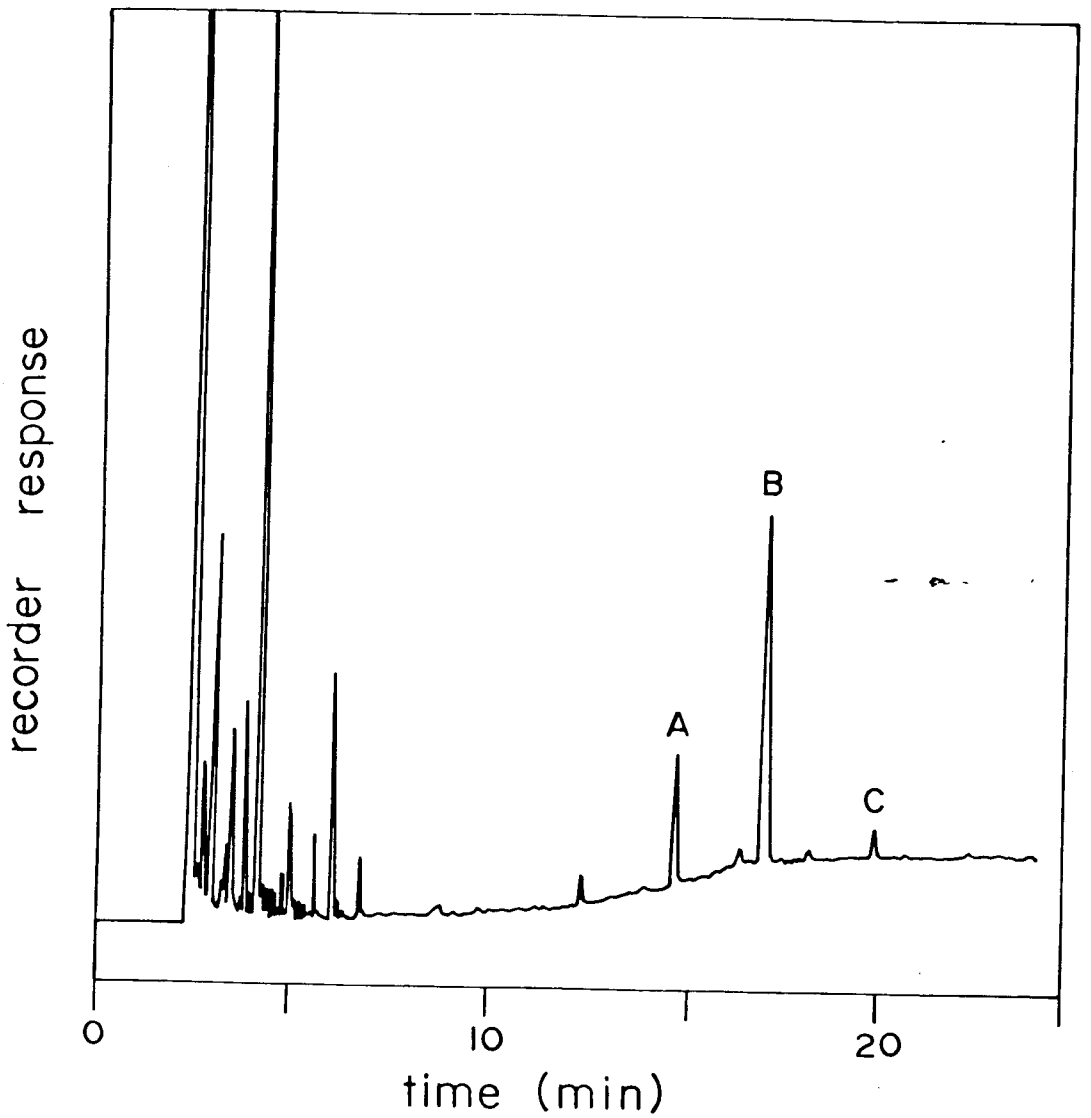

recorder response
0
0.20
0.40
0.60
1.00
relative retention (rel. to methyl palmitate)
A
B
C
methyl palmitate std.

Figure 3

Gas-liquid chromatogram of a pentane extract of Porapak Q-entrapped volatiles from C. ferrugineus produced frass.

Chromatogram run on a 38 m X 0.66 mm I.D. glass column coated with SP-1000.

Oven temperature programmed from 180° to 210°C at 4°/min.


recorder response
A
B
C
0
10
20
time (min)

differed considerably in appearance. Some were dark in colour and lumpy in texture while others were light and fluffy. This may be because of different moisture and/or fungal contents. Thus, it was not expected that these different frass samples would have the same relative quantity of volatile components. Indeed, an extract of a sample of frass taken directly from a field infestation of C. ferrugineus in Manitoba did not contain any of the compounds labelled A, B and C (Figures 2 and 3). These three compounds usually comprised 20% to 40% of the volatiles in the extracts.

Chromatograms of pentane extracts of Porapak Q-entrapped volatiles from populations of male and female C. ferrugineus are illustrated in Figure 4. There was a great difference in the composition of these extracts. Compounds A, B and C appear to be produced only by male C. ferrugineus. Since the bioassay of these extracts (Section II B ii) indicated that males produced the pheromone, these three compounds may be the attractive compounds.

B. Preparative GLC

i. Experimental:

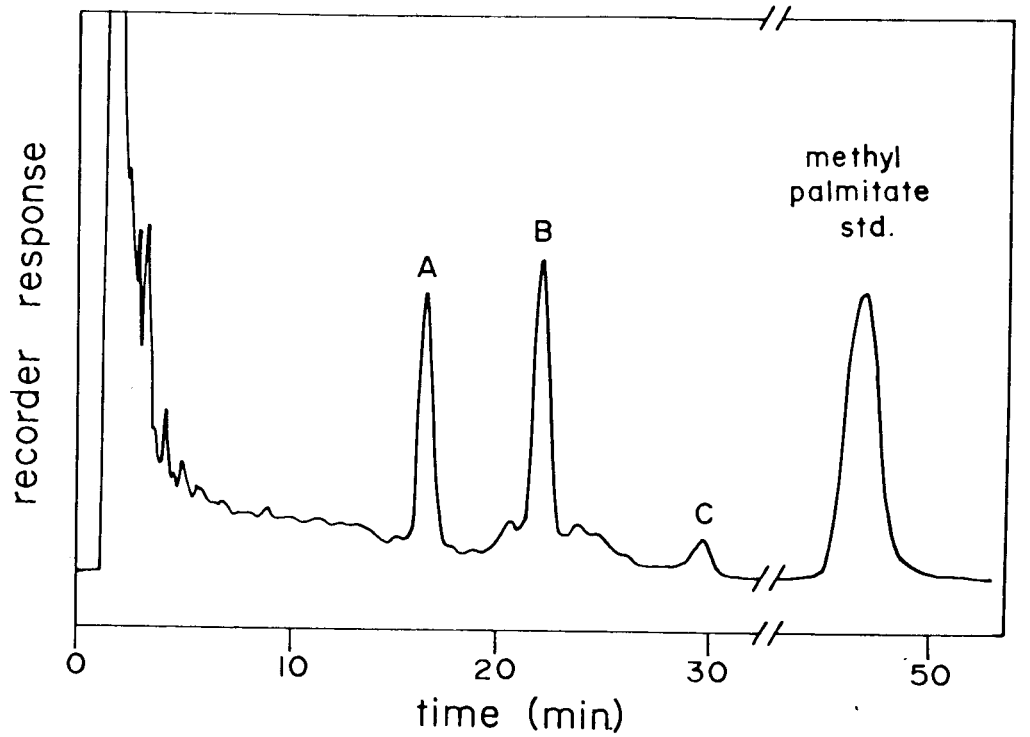
To determine which of the compounds present in a pentane extract of Porapak Q-entrapped volatiles from C. ferrugineus produced frass was attractive for C. ferrugineus, it was necessary to separate these compounds and bioassay them individually.

Initial micro-preparative gas-liquid chromatography was conducted using a Varian Aerograph 2100 gas chromatograph (GC). Column effluent was split between a 150 mm X 4.0 mm O.D. X 1.2 mm I.D. cone-tipped glass tube which extended vertically through a

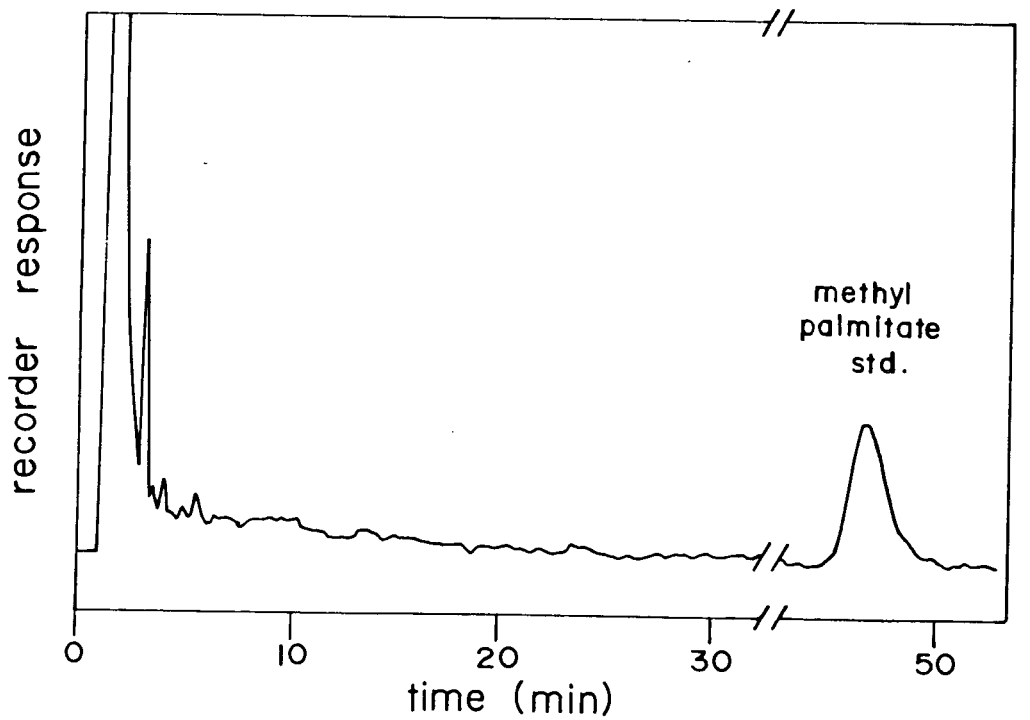
Figure 4

Gas-liquid chromatograms of pentane extracts of Porapak Q-entrapped volatiles produced by C. ferrugineus males (a) and females (b).

(a)


recorder response
A
B
C
methyl
palmitate
std.
0
10
20
30
50
time (min)

(b)


recorder response
methyl
palmitate
std.
0
10
20
30
50
time (min)

hole in the oven wall and a 140 mm X 4.0 mm O.D. glass tube which extended into the detector (Figure 5). These tubes were joined at a female S-13 joint which attached to the end of the column. By measuring the flow of carrier gas through both ends of this splitter, it was determined that ~4% of the column effluent went to the detector. A 23 cm X 4 mm O.D. X 2 mm I.D. glass tube which had nine 1.5 cm diameter spirals in its center was mounted vertically on top of the cone-end of the splitter. A flare on the bottom of this collecting tube ensured a snug fit. The collecting tube was centered in a 50 mℓ plastic cone-tipped centrifuge tube which had a 6 mm hole drilled in the bottom to allow connection of the collecting tube to the splitter. A small glass wool plug was positioned at the bottom and then this plastic jacket was filled with dry ice. When not in use, these collection tubes were sealed with a rubber serum stopper at each end. After collection was completed, each collection tube was washed into a 1/2 dram screw cap vial with several small portions of pentane. The vial was then scaled with a Teflon-lined screw cap until needed. When a sample was needed without solvent, a straight 200 mm X 4.0 mm O.D. X 2.0 mm I.D. glass tube was substituted for the spiral collector. After collection, this tube was flame sealed until the sample was needed.

Efficiency of collection was tested using β-ionone as a standard. A 1 mg sample of β-ionone in 20 µℓ pentane was injected into the GC and collected in the collector previously described. After collection was complete, the sample was washed with pentane from the tube into a 5 mℓ volumetric flask containing a known amount of a second standard (methyl palmitate). The volume

Figure 5

Effluent splitter and fraction collector for micro-preparative gas-liquid chromatography using a Varian 2100 instrument.

A. 50 mℓ plastic cone-tipped centrifuge tube filled with dry-ice. 6 mm hole in bottom.

B. Glass collector. 23 cm X 4 mm O.D. X 2 mm I.D. six to nine 1.5 cm diameter spirals.

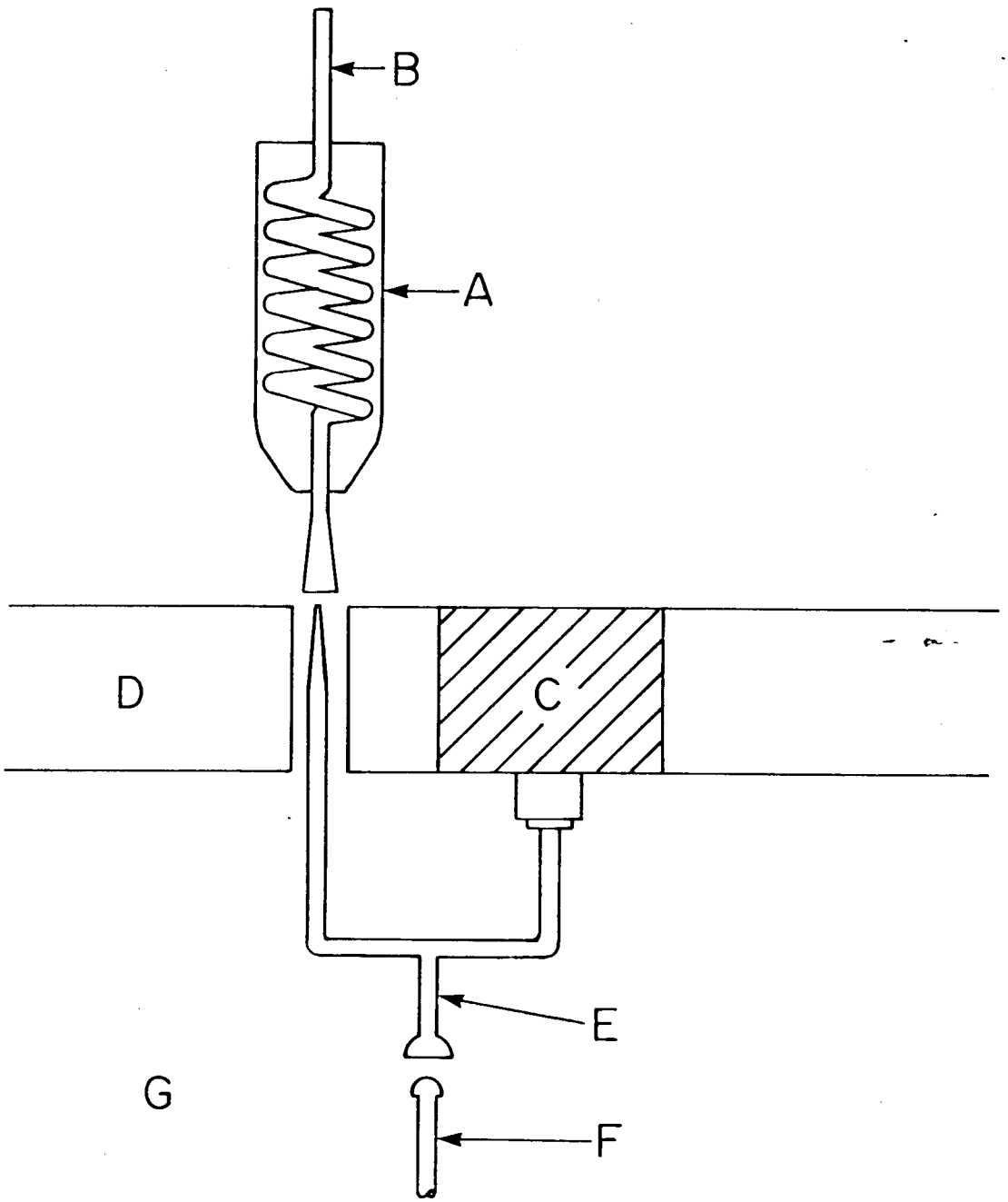
C. Detector block

D. Oven wall insulation

E. Glass splitter
   0.5 mm I.D. into detector
   1.2 mm I.D. into collector
   16 cm overall length
   S13 female joint at bottom

F. 4 mm O.D. glass column
   S13 male joint at splitter end

G. G.L.C. oven


B
A
D
C
E
G
F

in the flask was brought up to the mark. GLC analysis of this solution showed that 84% of the β-ionone had been recovered.

Later, a micro-preparative gas-liquid chromatography system was built upon a modified Varian Aerograph 1200 GC. The devices added to the basic instrument were an effluent splitter and a thermal gradient collector[23] (Figure 6). The column effluent was split between a stainless steel (s.s.) capillary detector insert (A, 100 mm X 3.2 mm O.D. X 0.25 mm I.D.) and a stainless steel tube (B, 150 mm X 1.6 mm O.D. X 1.0 mm I.D.). The s.s. tubing was silver soldered into the center of a 1/8" Swagelok s.s. union which had one end removed (C). A 1/16" zero-dead volume fitting which had been silver soldered into the cut end of the union joined the detector insert to the assembly. A stainless steel hypodermic needle (E, 18 gauge) was joined with heat-shrink Teflon tubing (D) to the other end of the tubing. By measuring the flow of carrier gas through both ends of this splitter, it was determined that ~9% of the column effluent went to the detector. This amount was decreased to <3% when a short section (80 mm) of 0.12 mm O.D. s.s. wire was inserted into the detector insert.

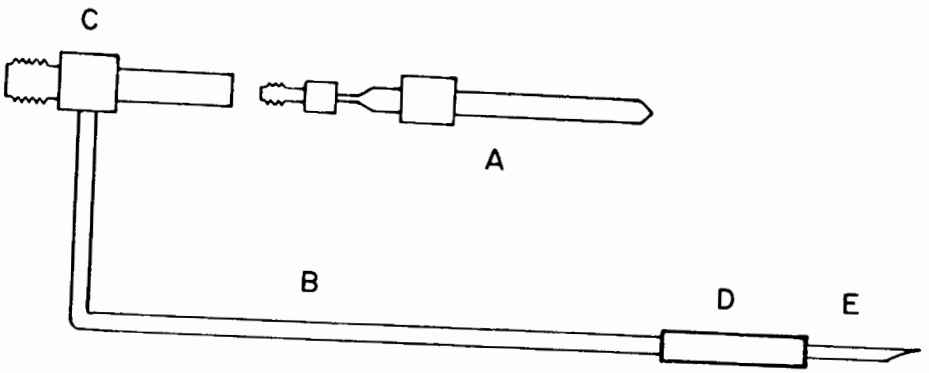
The thermal gradient collector was a modification of that devised by Brownlee and Silverstein[23]. This device (Figure 6) consisted of two aluminum blocks, one a 40 mm cube (F) which contained a 100-watt cartridge heater (G), the other (H) a 40 mm cube that had two 125 mm X 19 mm aluminum rods which extended down into a dry-ice/acetone cooling bath. During collections, the heating block was maintained at 100°C. Both blocks were mounted on a section of insulating board (I) which was supported rigidly in a horizontal position by a bracket fastened

Figure 6

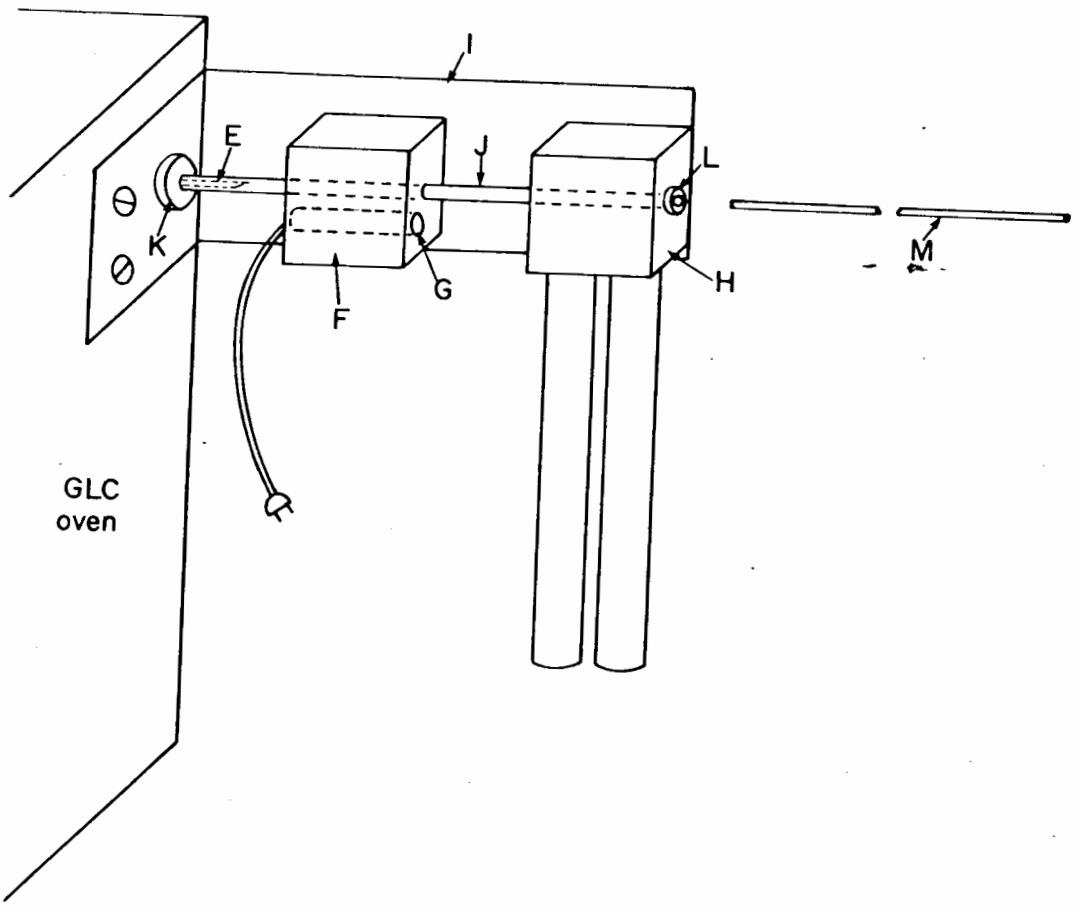
Effluent splitter (a) and thermal gradient collector (b) for micro-preparative gas-liquid chromatography using a Varian 1200 instrument.

A. capillary detector insert, 100 mm X 3.2 mm O.D. X 0.25 mm I.D.
B. stainless steel tubing, 150 mm X 1.6 mm O.D. X 1.0 mm I.D.
C. Swagelok stainless steel union silver soldered to a 1/16" zero-dead volume stainless steel fitting
D. heat-shrink Teflon tubing
E. stainless steel hypodermic needle, 18 gauge
F. heating block, 4 cm aluminum cube
G. cartridge heater, 100 watts
H. cooling block, 4 cm aluminum cube with two 125 mm X 19 mm aluminum rods extending from bottom
I. bracket-insulating assembly
J. stainless-steel tubing, 150 mm X 3.2 mm O.D. X 2.5 mm I.D.
K. Teflon rod, 3.2 mm hole in center
L. Teflon rod, 3.2 mm hole in center
M. glass capillary tubing, 200 mm X 1.9 mm O.D. X 1.4 mm I.D.

(a)



(b)



with two screws to the side of the GC. A stainless steel tube (J, 150 mm X 3.2 mm O.D. X 2.5 mm I.D.) ran horizontally through the center of these aluminum blocks. This tube slipped into a Teflon sleeve (K) on the GC exit port in the oven wall. Provision was made for vertical and lateral alignment of the tube with the GC exit port by adjustments on the holding bracket (I). A gas tight seal was made by butting the steel tube up against a rubber septum in the Teflon sleeve. The stainless steel hypodermic needle from the end of the splitter extended horizontally through the septum into the stainless steel tube, thus connecting the splitter and collector. At the other end of the stainless steel tube, preventing frost accumulation, a short length of Teflon rod (L) with a 3.2 mm hole in the center was mounted.

Collection was made by slipping a glass tube (M, 200 mm X 1.9 mm O.D. X 1.4 mm I.D.) through the stainless steel tube and over the hypodermic needle. These glass capillary tubes were interchanged very quickly and were easily sealed for use later. Samples to be bioassayed were washed from the capillary tubes with pentane into 1/2 dram screw cap glass vials and sealed with Teflon-lined caps until used.

Samples for fractionation were prepared by first concentrating a known volume (1 to 2 mℓ) of a pentane extract to ~40 µℓ using a stream of dry nitrogen. This sample was first drawn up into a 100 mℓ gas-tight Hamilton syringe containing 10 µℓ pentane and 10 µℓ air, then injected into the GC. Fractions were collected, at appropriate time intervals, as indicated by recorder response. The purity of each fraction was checked by reinjection.

ii. Results:

Initial experiments were conducted to determine which of the compounds present in a pentane extract of frass volatiles were attractive for C. ferrugineus. In the first fractionation experiment (Figure 7a, Table VIII), a pentane extract of frass volatiles was separated into three portions: a fast eluting portion (fraction i), a slow eluting portion (fraction iii) and the portion eluting with moderate speed (fraction ii). These fractions were collected on the glass spirals illustrated in Figure 5. Bioassay results (Table IX) show that only the slow eluting fraction iii (23.4 to 46.5 minutes retention time) was attractive for C. ferrugineus. This fraction contained three major compounds (quantitative) as well as more than twenty minor components. The three compounds, A, B and C, which made up most of fraction iii had previously been shown to co-occur in volatiles from both male C. ferrugineus and from C. ferrugineus produced frass (Section III A).

Experiment 21 (Figure 7b, Table VIII) was conducted to further fractionate the portion of the pentane extract of frass volatiles which was attractive for C. ferrugineus (fraction iii). Unlike the previous experiments, the GLC was run isothermally to reduce contamination of collected fractions by bleed from the Carbowax column.

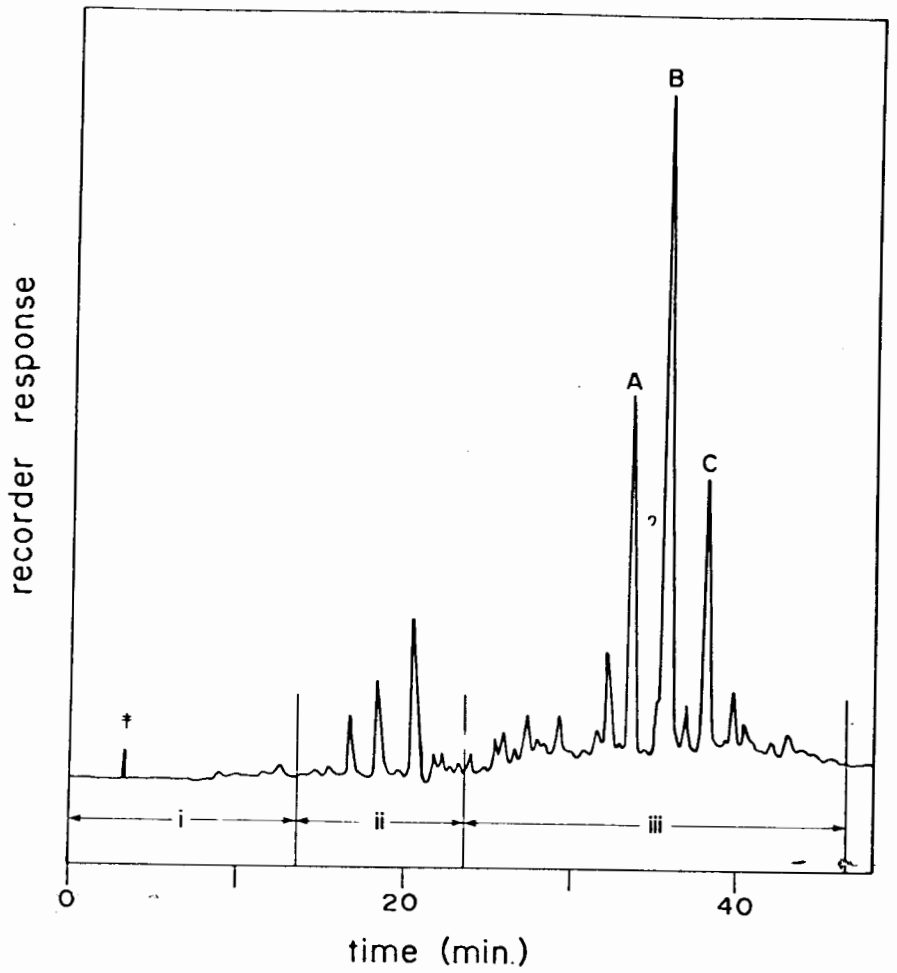
Four fractions were collected. The first fraction, v, consisted of everything eluting before compound A. The other three fractions, vi, vii and viii, were made up of mainly compounds A, B and C, respectively, with one or two minor components in each fraction. Bioassay results (Table IX) showed that fractions vi,

Figure 7

Gas-liquid chromatograms of concentrated pentane extracts of Porapak Q-entrapped volatiles from C. ferrugineus produced frass. Fractions collected for bioassays in Experiment 19(a) and in Experiment 21(b) are indicated by letters i-viii. Experimental conditions are listed in Table VIII.

† The detector flame was blown out at this point by the solvent and had to be re-lit.

(a)


recorder response
B
A
C
†
i
ii
iii
0
20
40
time (min.)

(b)

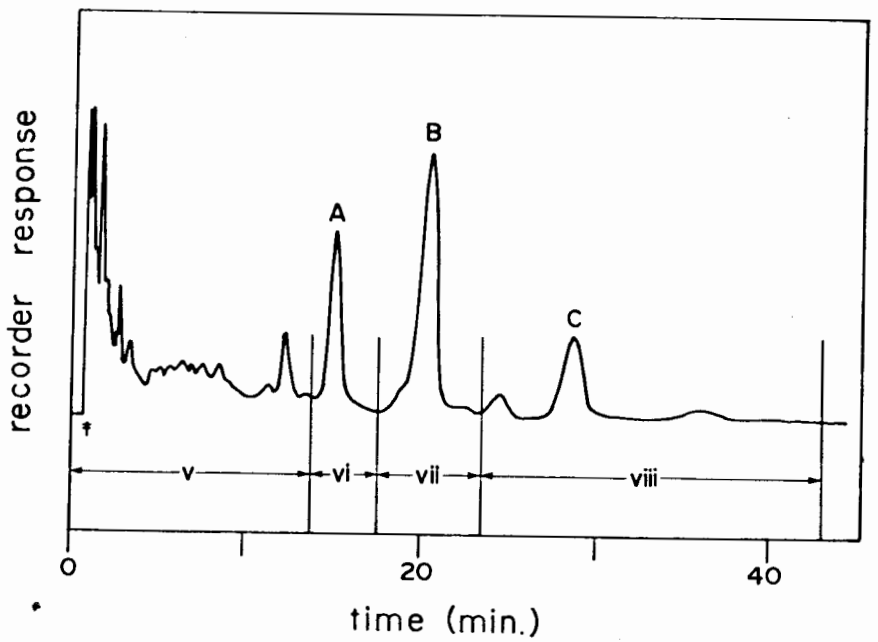

recorder response
B
A
C
†
v
vi
vii
viii
0
20
40
time (min.)

TABLE VIII. Experimental conditions for micro-preparative gas-liquid chromatography using a Varian Aerograph 2100. 1.8 m X 6.4 mm O.D. X 2.2 mm I.D. glass column packed with 5% Carbowax 20M on Chromosorb G 70/80 mesh. Detector temperature 270°C. Injector temperature 260°C. 40 mℓ/min. carrier gas. Samples concentrated to 40 µℓ.

Experiment No.	Original Volume of Extract (mℓ)	Fraction No.	GLC Oven Temp (°C)	Retention time (min)
19	2	i	60-200 @ 4°/min	0 - 13.6
	2	ii	60-200 @ 4°/min	13.6 - 23.4
	2	iii	60-200 @ 4°/min	23.4 - 46.5
20	1	iv	60-200 @ 4°/min	0 - 46.5
21	2	v	140	0 - 13.8
	2	vi	140	13.8 - 17.3
	2	vii	140	17.3 - 23.5
	2	viii	140	23.5 - 43.0
22	1	ix	140	0 - 43.0

Table IX. Response of C. ferrugineus of mixed age and sexes to preparative gas-liquid chromatography fractions of pentane extracts of Porapak Q-entrapped volatiles from beetle frass.

Experiment No.	Fraction No.	Concentration (ghr)*	% Response†
19	i	200	25.0 a
		20	27.0 a
		2.0	29.0 a
		0 (pentane control)	23.0 a
	ii	200	27.0 a
		20	20.0 a
		2.0	22.0 a
		0 (pentane control)	18.0 a
	iii	200	46.0 b
		20	79.0 d
		2.0	63.0 c
		0.2	54.0 bc
		0.02	29.0 a
		0 (pentane control)	19.0 a
20	iv	200	60.0 c
		20	54.0 c
		2.0	48.0 bc
		0.2	38.0 ab
		0.02	27.0 a
		0 (pentane control)	30.0 a

Table IX (Cont'd)

Experiment No.	Fraction No.	Concentration (ghr)*	% Response†
21	v	200	26.0 a
		20	27.0 a
		2.0	17.0 a
		0 (pentane control)	18.0 a
	vi	200	40.0 c
		20	37.0 bc
		2.0	25.0 ab
		0 (pentane control)	13.0 a
	vii	200	28.0 ab
		20	43.0 c
		2.0	34.0 bc
		0 (pentane control)	19.0 a
	viii	200	51.0 c
		20	35.0 b
		2.0	21.0 a
		0 (pentane control)	18.0 a
22	ix	200	45.0 b
		20	51.0 b
		2.0	30.0 a
		0 (pentane control)	22.0 a

* one gram-hour (ghr) = volatiles collected from one gram of frass in one hour.

† 4 replicates of 25 beetles.

Percentages in each fraction followed by same letter not significantly different, χ^2 test, $P < 0.05$.

vii and viii were all individually attractive for C. ferrugineus.

To determine which component of each biologically active fraction was attractive for C. ferrugineus, it was necessary to isolate each compound. To do this, better separation of the compounds was required. This was achieved using the Varian 1200 instrument and collector previously described.

In Experiment 23, a pentane extract of Porapak Q-entrapped volatiles from rusty grain beetle frass was separated into nine fractions (Figure 8). The first fraction, fraction x, consisted of everything from the first compounds eluting through the column to the compounds eluting immediately before the compounds which had previously been shown to be attractive (Table IX; fractions vi, vii and viii). This fraction was made up of at least thirty compounds. The remaining eight fractions contained only one compound, except for fraction xiii, which had two components.

Bioassay results showed that fractions xii, xiii, xiv, xvi and xviii were attractive for C. ferrugineus (Table XI). GLC analysis of fraction xiii, however, showed the presence of a trace amount of fraction xii. Thus, it was not certain that fraction xiii was attractive. This experiment indicated that there were four or perhaps five compounds present in the pentane extract of frass volatiles which were attractive for C. ferrugineus. It is interesting to note that three of these attractive compounds (A, B and C) are produced solely by male beetles (Section III A; Figure 4). This supports the suggestion given earlier (Section II) that the male beetle produces a true aggregation pheromone. It has yet to be determined, however, which sex produces the other one or two attractants.

Figure 8

Gas-liquid chromatogram of a concentrated pentane extract of Porapak Q-entrapped volatiles from C. ferrugineus produced frass. Fractions collected in Experiment 23 for bioassays are indicated by the letters x-xviii. Experimental conditions are listed in Table X.

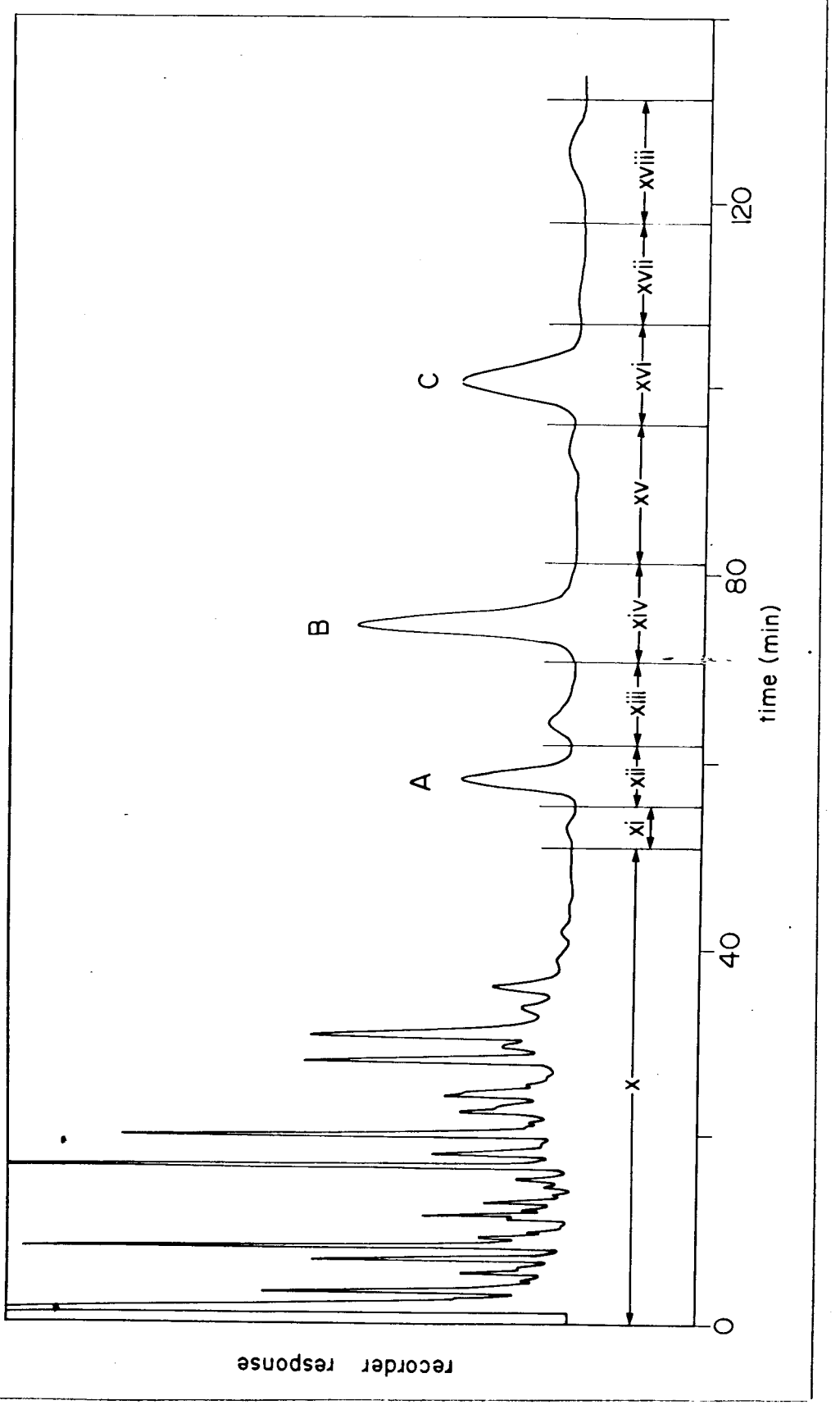

recorder response
0
40
80
120
time (min)
A
B
C
x
xi
xii
xiii
xiv
xv
xvi
xvii
xviii

Table X. Experimental conditions for micro-preparative gas-liquid chromatography using a Varian Aerograph 1200. 3.0 m X 3.2 mm O.D. stainless steel column packed with 5% Carbowax 20M on Chromosorb G 70/80 mesh. Detector temperature 250°C. Injector temperature 240°C. 40 mℓ/min He carrier gas. Samples concentrated from 1 mℓ to 40 µℓ. Oven program 60°-140°C at 4°C/min.

Experiment No.	Fraction No.	Retention time (min)
23	x	0 - 50.7
	xi	50.7 - 55.5
	xii	55.5 - 61.5
	xiii	61.5 - 70.5
	xiv	70.5 - 81.0
	xv	81.0 - 95.7
	xvi	95.7 - 106.2
	xvii	106.2 - 117.0
	xviii	117.0 - 130.2
24	xix	0 - 130.2

TABLE XI. Response of C. ferrugineus of mixed age and sexes to preparative gas-liquid chromatography fractions of pentane extracts of Porapak Q-entrapped volatiles from beetle frass. Experiments 23 and 24.

Experiment No.	Fraction No.	Concentration (ghr)*	% Response†
23	x	120	16.0 a
		12	20.0 a
		1.2	16.0 a
		0 (pentane control)	12.0 a
	xi	120	32.0 a
		12	35.0 a
		1.2	28.0 a
		0 (pentane control)	24.0 a
	xii	120	30.0 b
		12	35.0 bc
		1.2	46.0 c
		0 (pentane control)	13.0 a
	xiii	120	46.0 b
		12	49.0 b
		1.2	44.0 b
		0 (pentane control)	11.0 a
	xiv	120	52.0 b
		12	13.0 a
		1.2	16.0 a
		0 (pentane control)	17.0 a
	xv	120	22.0 a
		12	18.0 a
		1.2	23.0 a
		0 (pentane control)	15.0 a

TABLE XI (Cont'd)

Experiment No.	Fraction No.	Concentration (ghr)*	% Response†
	xvi	120	38.0 b
		12	15.0 a
		1.2	19.0 a
		0 (pentane control)	15.0 a
	xvii	120	17.0 a
		12	20.0 a
		1.2	19.0 a
		0 (pentane control)	11.0 a
	xviii	120	50.0 b
		12	12.0 a
		1.2	20.0 a
		0 (pentane control)	17.0 a
24	xix	120	58.0 b
		12	54.0 b
		1.2	51.0 b
		0 (pentane control)	12.0 a

* one gram hour (ghr) = volatiles collected from one gram of frass in one hour.

† 4 replicates of 25 beetles.
Percentages in each fraction following by same letter not significantly different, χ^2 test, $P < 0.05$

C. Isolation technique refinements

Before embarking on a large scale isolation of the attractive compounds in the pentane extracts, it was necessary to check the efficiency of the isolation technique used. The aeration, extraction and concentration procedures had to be evaluated.

Since the one to two week time period used in frass aerations was purely arbitrary, experiments were conducted to determine if any volatile material was still present in the frass at the end of the aeration. This was done by replacing the Porapak Q trap on the aeration apparatus with a fresh trap after two weeks and continuing the aeration for another two weeks. The Porapak Q in the second trap was then processed as previously described and the resulting pentane extract was analyzed by GLC.

This analysis showed that in aerations where the frass had not been pulverized, a trace of the attractive compounds was present. However, in aerations where the frass had been ground with a mortar and pestle, no attractive compounds could be detected. In subsequent aerations, the frass was ground.

To test if any compounds during an aeration were breaking through the Porapak Q trap, a second Porapak Q trap was connected in series to the first trap. After two weeks the aeration was stopped and the traps processed. GLC analysis of a pentane extract of volatiles captured in the second trap showed the presence of at least fifteen compounds. However, none of the attractive compounds could be detected.

To check efficiency of Porapak Q extraction, Porapak Q, which had been extracted with pentane for twenty-four hours, was then extracted with diethyl ether for twenty-four hours. No

attractive compounds were detected by GLC analysis of the ether extract concentrate.

As a final check of the efficiency of the isolation procedure, the pentane distilled through the Dufton column during the concentration of the pentane extract was analyzed by GLC. Again, no attractive compounds were detected.

D. Summary

Analytical GLC showed that there were large quantitative differences in composition of pentane extracts of Porapak Q-entrapped volatiles from C. ferrugineus frass from batch to batch. Qualitatively, however, the same three compounds (A, B and C) made up a large percentage of the volatiles present in each extract. These three compounds were shown to be produced by male C. ferrugineus.

An effluent splitter and a thermal gradient collector were constructed for preparative GLC. Fractionation of pentane extracts of Porapak Q-entrapped volatiles from C. ferrugineus frass followed by comparative bioassays showed the presence of four or five compounds which are attractive for C. ferrugineus. Three of these attractive compounds were produced only by male C. ferrugineus.

Experiments showed that Porapak Q efficiently trapped the attractive compounds. Soxhlet extraction of the odor laden Porapak Q with pentane removed all of the attractive compounds from the Porapak Q. Distillation of the pentane through a Dufton column concentrated the extracts without loss of the attractants.

IV. ANALYSIS OF ATTRACTANTS FOR C. FERRUGINEUS

A. Experimental

i. Quantitative GLC

Quantitative analyses were performed on a Hewlett-Packard 5830A GC and a Varian 2100 GC equipped with a Spectra-Physics Minigrator. Columns and conditions used were the same as those used previously for analytical GLC (Table VII).

ii. Mass spectrometry

Unit resolution mass spectra were obtained on a Hitachi RMU-6E mass spectrometer interfaced to a Varian 1400 GC and a System Industries 150 computer. Ionization voltage was 80 ev. Typically, a 3.6 m X 6 mm O.D. X 2 mm I.D. glass column packed with 5% Carbowax 20M on Gas Chrom Q, 100-200 mesh was used for separation of compounds.

High resolution mass spectra (HRMS) were obtained by Dr. G. Eigendorf[†] using a DS-50 mass spectrometer.

Chemical ionization mass spectra (CIMS) were obtained by Prof. W.A. Ayre[‡] on a MS12. NH_3 was used as the ionizing gas. Samples for HRMS and CIMS were isolated by preparative GLC and sealed in glass capillaries until used.

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† Department of Chemistry, University of British Columbia, Vancouver, B.C.

‡ Department of Chemistry, University of Alberta, Edmonton, Alberta.

iii. Infrared spectroscopy

Infrared spectra were obtained on a Beckman IR 4230 spectrophotometer by Mr. N. Dominelli†. Samples were run neat on AgCl plates.

iv. Microhydrogenation

A method for determining the carbon skeleton of microgram quantities of organic compounds by hydrogenolysis has been devised by Beroza[24]. The method involves passing a sample in a stream of hydrogen through a heated tube packed with catalyst. The hydrogenation products are swept into a gas chromatograph and separated. Identification of the products is made by matching retention times with those of known hydrocarbons or by analysis by mass spectrometry.

A modified version of Beroza's catalyst assembly is shown in Figure 9b. This assembly consisted of a stainless steel cylinder (A, 25 cm X 1.3 cm O.D. X 0.5 cm I.D.) screwed into the injection port of a gas chromatograph by means of a fitting D. This fitting consisted of a bolt, nut and stainless steel tubing (10 cm X 1.6 mm O.D. X 1.0 mm I.D.) silver soldered together. This assembly was jacketed by a glass tube (F, 25 cm X 1.6 cm O.D. X 1.4 cm I.D.). A length of stainless steel tubing (B, 180 cm X 1.6 mm O.D. X 1.0 mm I.D.), one end of which was silver soldered to the stainless steel cylinder, was wound around the glass tube. The entire assembly was wrapped with asbestos paper,

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† Health Protection Branch, Health and Welfare Canada, Vancouver, B.C.

Figure 9

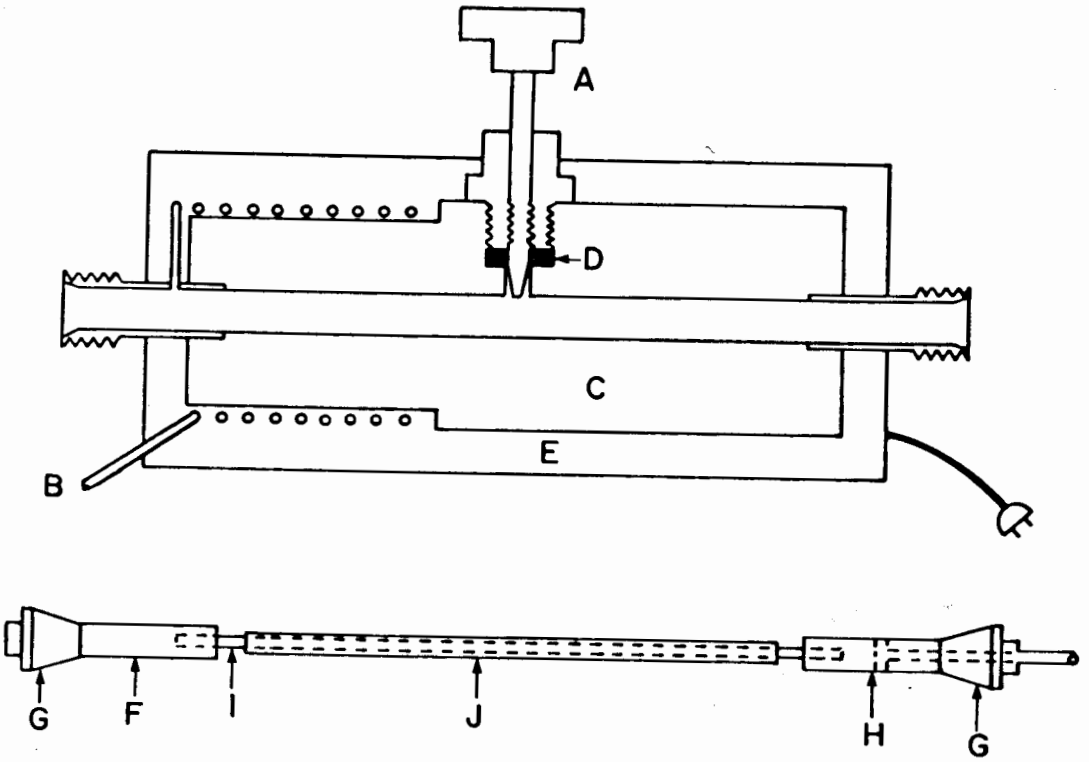
(a) Glass capillary breaker

A. stem and bonnet from Whitey valve SS-4RS6
B. stainless steel tubing, 1.2 m X 1.6 mm O.D. X 1.0 mm I.D.
C. stainless steel cylinder, 15 cm X 5 cm O.D. X 0.6 cm I.D.
D. Teflon washer
E. asbestos insulation over nichrome resistance wire
F. stainless steel rod with a 10 mm X 2.2 mm hole in center
G. 1/4" stainless steel Swagelok furrel
H. stainless steel rod with a 10 mm X 2.2 mm hole at one end and silver soldered to 1.6 mm O.D. X 1.0 mm I.D. stainless steel tubing at other end.
I. glass capillary tubing, 158 mm X 1.9 mm O.D. X 1.4 mm I.D.
J. stainless steel tubing, 135 mm X 3.2 mm O.D. X 2.5 mm I.D.

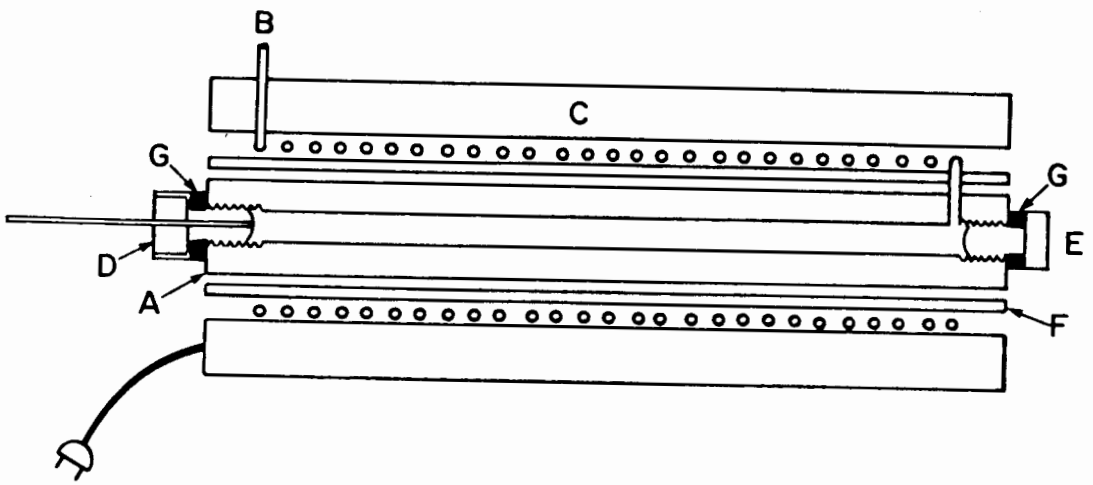
(b) Catalyst assembly

A. stainless steel cylinder, 25 cm X 1.3 cm O.D. X 0.5 cm I.D.
B. stainless steel tubing, 180 cm X 1.6 mm O.D. X 1.0 mm I.D.
C. asbestos insulation over nichrome resistance wire.
D. fitting connecting gas chromatograph injection port with catalyst assembly (bolt, nut and stainless steel tube silver soldered together).
E. bolt
F. glass tube, 25 cm X 1.6 cm O.D. X 1.4 cm I.D.
G. silicon septa with 0.5 cm diameter holes in the center.

(a)



(b)



then nichrome resistance wire (600 cm, 1.6 Ω/ft) and finally asbestos tape. The ends of the wire were connected to a variable transformer. The temperature of the assembly was determined by a thermocouple, placed between the glass tube (F) and the catalyst tube (A). Bolt E allowed addition or removal of catalyst. Silicon septa, which had a 0.5 cm hole cut in their centers, were used to ensure a leak tight connection between the injection port fitting (D) and the cylinder and the bolt (E) and the cylinder.

The catalyst, 1% palladium (by weight as the metal), was prepared by the method of Beroza and Sarmiento[25]. 178 mg $PdCl_2$ (Alfa) was dissolved in 100 mℓ 5% aqueous acetic acid. Gas-Chrom P (60-80 mesh, 9.9 g, Applied Science) was added and the solvent was removed on a rotary evaporator. After drying in vacuo over night, the catalyst was added to a solution containing 80 mg NaOH. The solvent was removed and the product was again dried in vacuo. The dried powder was then packed into the catalyst assembly and hydrogen gas was slowly passed through the tube (20 mℓ/min). The assembly was not attached to the gas chromatograph and the hydrogen was vented into a fume hood. The catalyst was activated by heating at ~125°C for 30 minutes, at 200°C for 30 minutes and at the operating temperature for 30 minutes.

To introduce neat samples directly from a sealed capillary collector tube to the catalyst assembly, a glass capillary breaker was used[23], Figure 9a. A glass capillary tube (I, 158 mm X 1.9 mm O.D. X 1.4 mm I.D.) was held axially in a stainless steel cylinder (C, 150 cm X 5 cm O.D. X 0.6 cm I.D.) by means of fixtures (F and H) on each end. The center of the capillary tube was sheathed in a 135 mm length of 3.2 mm O.D., 2.5 mm I.D.

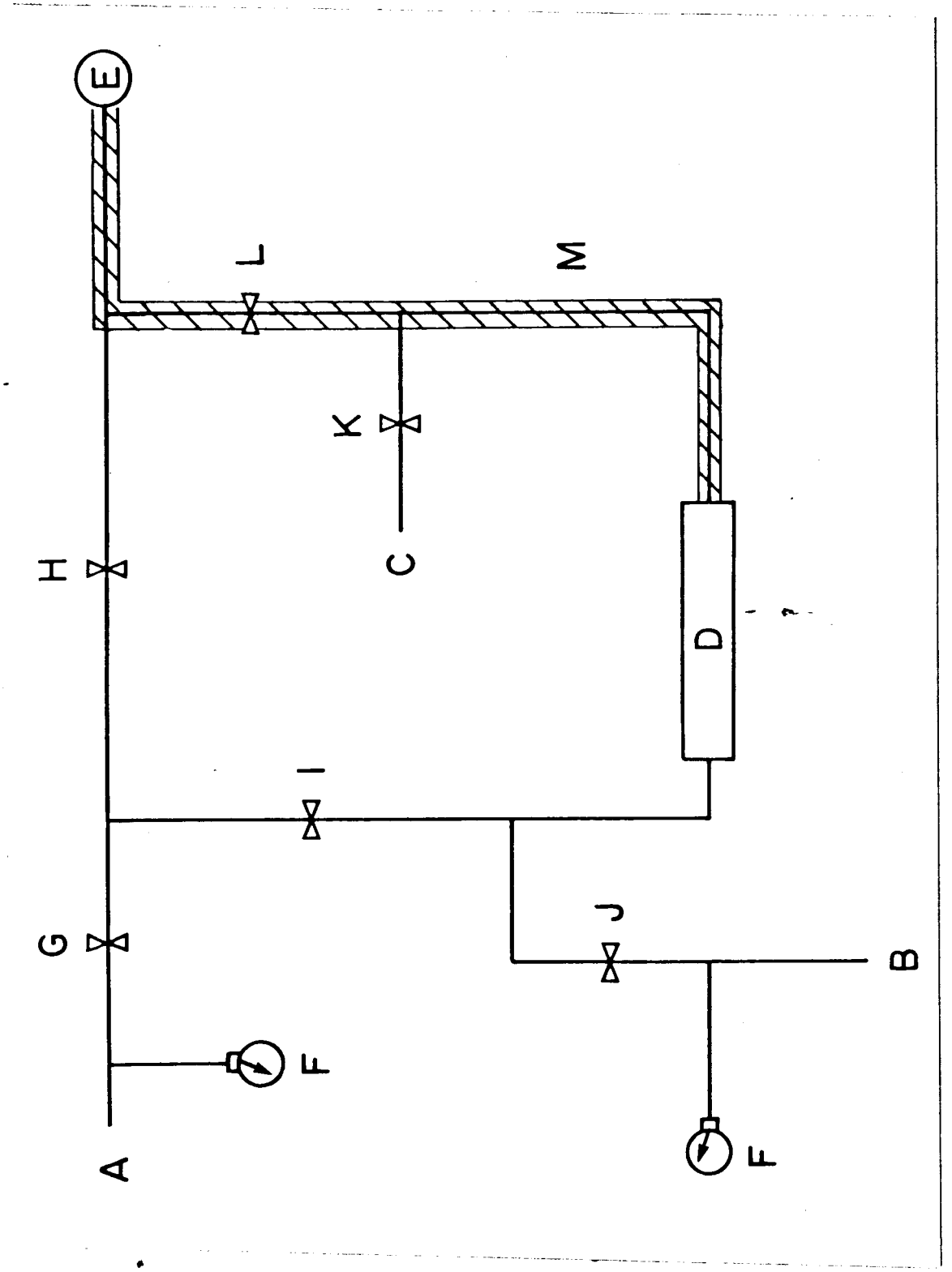
stainless steel tubing (J). The stem and bonnet from a Whitey valve (A) were mounted in a threaded hole in the cylinder. When the valve was turned, the stem pushed against the center of the stainless steel tube and caused the glass capillary to break at both ends. To ensure a clean break, each end of the capillary tube was scored with a diamond-tipped pencil. A 1.2 m X 1.6 mm O.D. X 1.0 mm I.D. stainless steel tube (B), coiled around one end of the cylinder, served as a carrier gas preheater. The cylinder and carrier gas were heated by asbestos insulated nichrome wire. Temperature was measured by a thermocouple.

To begin operation (Figure 10), all valves except the catalyst assembly valve (H) and the flow control valve (G) were closed. Thus, the gas chromatograph was isolated from the capillary breaker. The catalyst assembly was maintained at approximately 210°C. The glass capillary tube containing the neat sample was loaded into the capillary breaker (D). The capillary breaker, sample line and interface valve (L) were heated to approximately 200°C. The hydrogen purge valve (J) and valve K were opened. The hydrogen gas, after purging the sample chamber of volatile contaminants, was vented to the fume hood. After 15 minutes, valve K was closed. When the pressure in the capillary breaker reached that of the rest of the system (38 psi), as indicated by the back pressure valve (F), the purge valve (J) was closed. The interface valve (L) was opened and hydrogen gas was diverted through the capillary breaker by closing the catalyst assembly valve (H) and opening the capillary breaker valve (I). The glass capillary was then broken. The sample was swept into the assembly and the hydrogenolysis products entered

Figure 10

Schematic diagram of gas supply manifold for use with glass capillary breaker and catalyst assembly.

A. H_2 supply
B. purge supply
C. vent
D. capillary breaker
E. catalyst assembly
F. back pressure gauge
G. flow control valve, Nupro B4MG
H. catalyst assembly valve, Whitey 1KS4
I. capillary breaker valve, Whitey 1KS4
J. purge valve, Whitey 1KS4
K. purge bleed valve, Whitey 1KS4
L. interface valve, Whitey S.S.-3NBS4
M. heating tape


E
L
M
K
C
H
D
I
G
J
B
F
F
A

directly onto the chromatographic column. Hydrogen, the carrier gas, was maintained at a flow of 20 mℓ/min. Identification of the products was made by matching retention times with those of known hydrocarbons or by mass spectrometry.

Analysis by gas-liquid chromatography was carried out on a 3.0 m X 3.2 mm O.D. X 2.5 mm I.D. stainless steel column packed with 5% OV-101 on Chromosorb G 70-80 mesh fitted into a Varian 1200 gas chromatograph. Analysis conditions have been previously described (Table VII).

For analysis by mass spectroscopy, the hydrogenolysis assembly was fitted to the injection port of the gas chromatograph-mass spectrometry system previously described.

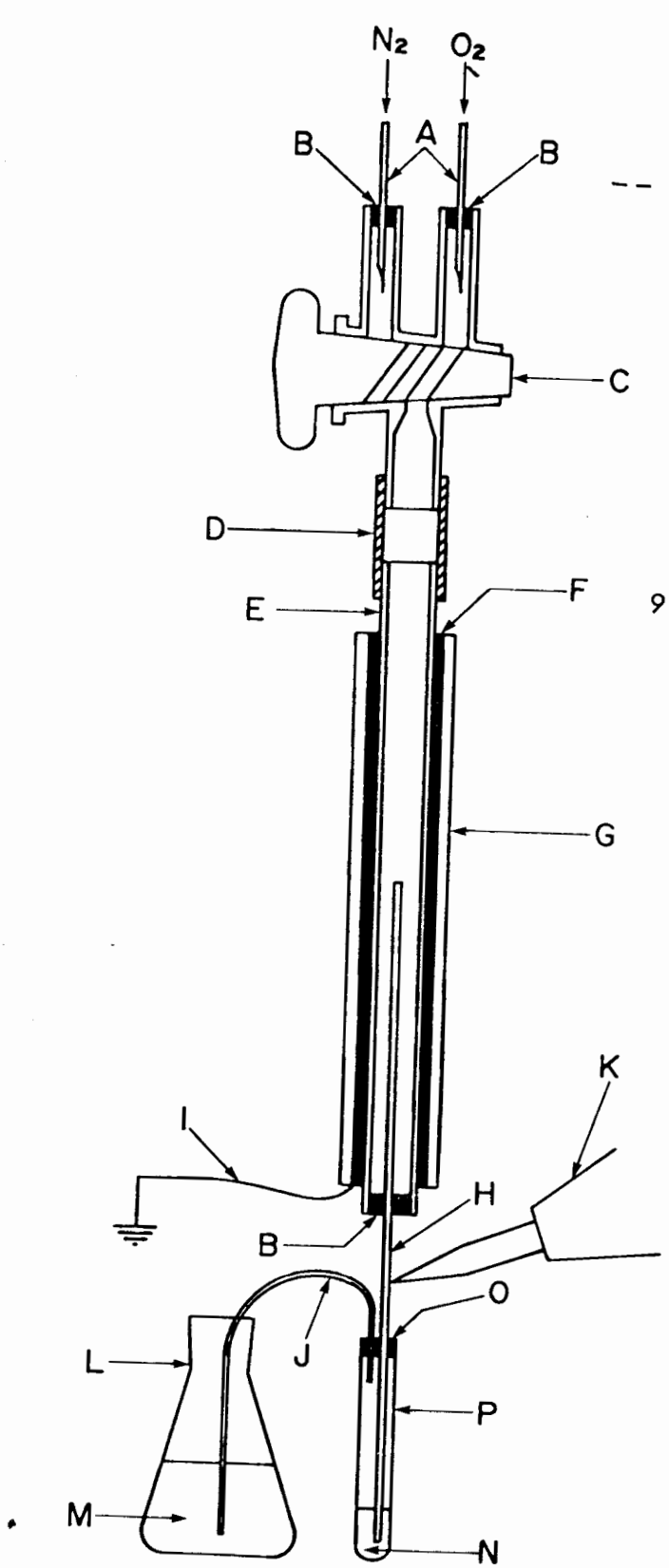
v. Micro-ozonolysis

A small ozonizer, for the analysis of microgram amounts of material, has been devised by Beroza[26]. A micro-ozonizer based on Beroza's design is shown in Figure 11. A sheet of aluminum foil (F) was folded over a length of copper wire (I, 20 gauge) and then wrapped several times around glass tubing (E, 10 cm X 8 mm O.D. X 6 mm I.D.) to within 1 cm of the ends. The foil was held on the tubing with rubber tubing (G, 8 cm). A stainless steel needle (H, 20 gauge, 14 cm) was pushed through a silicon septum (B) at one end of the glass tube. To the other end of the glass tube, a 3-way glass stopcock (C) was attached by means of a rubber sleeve (D). Nitrogen or oxygen was supplied through 1.6 mm O.D. teflon tubing connected to stainless steel needles (A, 20 gauge) which were pushed through silicon septa into the entry tubes on the stopcock. The reaction tube (P) was a 5 mm X 50 mm culture tube stoppered with a 5 mm rubber septum (O). The

Figure 11

Micro-ozonizer

A. stainless steel needles, 20 gauge
B. silicon septa
C. 3-way glass stopcock
D. rubber tubing
E. glass tubing, 10 cm X 8 mm O.D. X 6 mm I.D.
F. aluminum foil
G. rubber tubing
H. stainless steel needle, 20 gauge, 14 cm
I. grounded copper wire
J. Teflon tubing, 1.6 mm O.D.
K. Tesla coil
L. 10 mℓ Erlenmeyer flask
M. indicating solution, 4 mℓ
N. solution of compound, 100 μℓ
O. serum septum, 5 mm
P. culture tube, 5 mm X 50 mm


N2
O2
A
B
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P

needle stock (H) was pushed through the septum to the bottom of the tube. Teflon tubing (J, 1.6 mm O.D.) pushed through the septum conducted the gases emerging from the tube into 4 mℓ of indicating solution (M) contained in a 10 mℓ Erlenmeyer flask (L). A dry ice-methanol bath was used to cool the reaction tube.

To conduct an experiment, approximately 40 μg of compound in 100 μℓ of pentane (Caledon, distilled) were placed in the reaction tube (P). Oxygen (Matheson Extra Dry) was flushed through the system at 20 mℓ/min with the inner end of the needle stock tube (H) just above the septum (B). The solution was cooled in a dry ice-methanol bath. After about one minute, when the system was purged of air, the needle stock tube was pushed about halfway into the glass tube. The gas emerging from the reaction tube was bubbled, through Teflon tubing (J), into 4 mℓ of a solution of 5% KI (Allied Chemicals, reagent grade) and 0.5% soluble starch (Allied Chemicals, reagent grade) in 5% aqueous H_2SO_4 contained in the Erlenmeyer flask (L). Ozone was generated by touching the needle stock tubing with a Tesla coil (K). When a blue colour was seen in the indicating solution, showing the presence of excess ozone, the Tesla coil was removed. Usually, only 20 to 30 seconds was required for ozone generation. The three-way stopcock (C) was then turned and the solution was purged with nitrogen (Linde). The cold bath was removed, the septum (O) was slipped off the reaction tube and about 0.5 mg of $P\phi_3$ (Matheson, recrystallized from diethyl ether) was dropped into the solution which was then analysed by GC-MS.

vi. NMR

Chloroform-d (minimum 99.8% deuterated, Merck, Sharp and

Dohme), $CDCl_3$; maintained over 4Å molecular sieves, was used as solvent for all NMR experiments. Tris (6,6,7,7,8,8,8-heptafluoro-perdeutero-2,2-dimethyl-3,5-octanedionato)europiumIII, $Eu(fod)_3$-$\underline{d}_{30}$ (Alfa), dried *in vacuo* for 24 hours and then stored in a dessicator, was used for shift reagent studies.

Samples for proton NMR analysis were collected by preparative GLC in 20 cm X 1.9 mm O.D. X 1.4 mm I.D. glass capillary tubes. One end of the tube was flame sealed and 50 µℓ $CDCl_3$ was added using a syringe. Mixing was achieved by agitating the solution with a Vibrograver (Burgess Vibrocrafters, Inc.). The open end of the tube was sealed with a small plug cut from a silicon septum. For shift reagent experiments, aliquots of a solution of $Eu(fod)_3$-$\underline{d}_{30}$ in $CDCl_3$ were added with a syringe to the capillary tube.

Proton NMR spectra were obtained on a Varian XL 100 Fourier Transform spectrometer. The capillary tube containing a sample solution was mounted coaxially in a thin-wall (5 mm O.D.) NMR tube (Willmad Glass Co., Inc). Samples were run with either external ^{19}F lock or internal ^{2}H lock.

^{1}H-noise-decoupled natural-abundance ^{13}C NMR and decoupled proton NMR were performed at 20 MHz and 80 MHz, respectively, by Dr. J.N. Shoolery†.

B. Results

i. Quantitative GLC

An estimate of the amount of attractants present in a pentane extract of Porapak Q-entrapped volatiles was made by GLC.

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† Dr. J.N. Shoolery, Varian Associates, Applications Laboratory, Palo Alto, California.

In Figure 3, a typical chromatogram of a pentane extract, compounds A, B and C comprise approximately 9%, 22% and 2%, respectively, of the volatiles present. The other two compounds which may also be attractants are present in a fraction of a percent. This analysis is based on the assumption that these compounds have similar detector responses. In Figure 7, a chromatogram of a different pentane extract, compounds A and B make up about the same percentages as above, but the amount of compound C increases to 10%. Although there was considerable variance in the relative amounts of the attractive compounds present in an extract, compound B was always present in the largest amount, approximately 200 µg per aeration of 160 g frass.

ii. Identification of rusty grain beetle attractant B

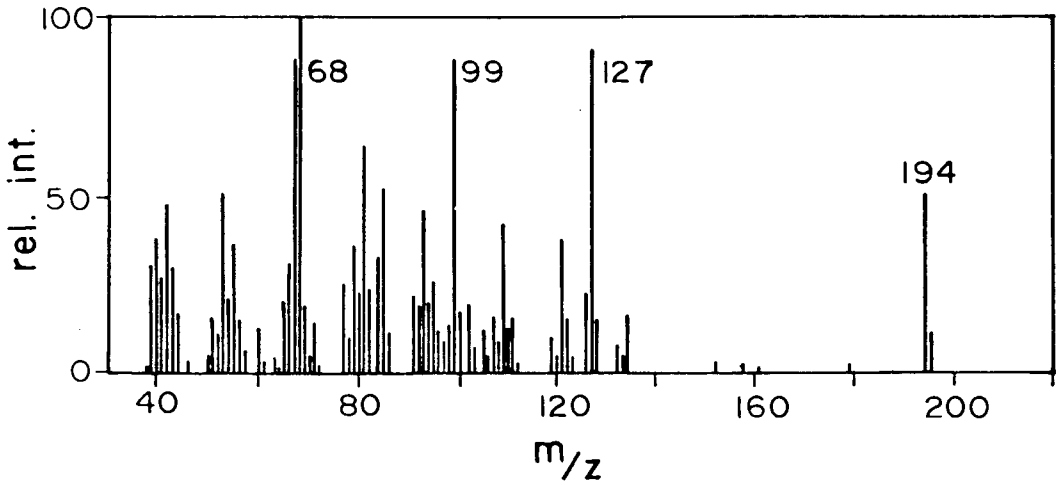
The electron impact mass spectra (Figure 12a and Table XII) suggested the molecular weight of compound B was 194.1291, corresponding to a molecular composition of $C_{12}H_{18}O_2$ (194.1307). Chemical ionization mass spectrometric analysis (Figure 12b) confirmed that m/z 194 was indeed the molecular ion peak. This molecular formula corresponds to a compound with 4 sites of unsaturation. The infrared spectrum (Figure 13) showed that one of these sites of unsaturation could be assigned to a carbonyl (ν 1730 cm^{-1}) of an ester or a small ring ketone. The former was suggested by an intense absorption at 1132 cm^{-1} (C-O stretch of an ester). The IR spectrum is devoid of hydroxyl and aromatic absorption, thus excluding the possibility of these groups occurring in compound B.

The mass spectrum of the micro-ozonolysis product of compound B (Figure 14) exhibited fragment peaks typical of an

Figure 12

Electron impact (a) and chemical ionization (b, NH_3 reagent gas) mass spectra of rusty grain beetle attractant B.

(a)


100
50
0
rel. int.
68
99
127
194
40
80
120
160
200
m/z

(b)

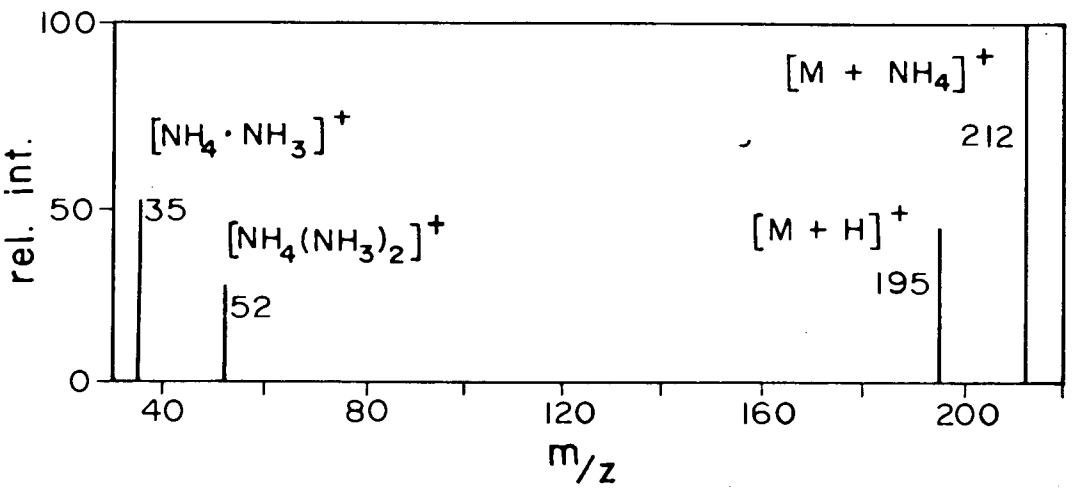

100
50
0
rel. int.
[NH4 · NH3]+
35
[NH4(NH3)2]+
52
[M + NH4]+
212
[M + H]+
195
40
80
120
160
200
m/z

TABLE XII. Significant peaks in the High Resolution Mass Spectrum of rusty grain beetle attractant B.

Nominal m/z	Measured Mass	Calculated Mass	Composition
43	43.0212	43.0184	$C_2H_3O^+$
43	43.0574	43.0548	$C_3H_7^+$
53	53.0396	53.0391	$C_4H_5^+$
67	67.0542	67.0548	$C_5H_7^+$
68	68.0618	68.0626	$C_5H_8^+$
81	81.0707	81.0704	$C_6H_9^+$
85	85.0653	85.0653	$C_5H_9O^+$
85	85.1013	85.1017	$C_6H_{13}^+$
93	93.0692	93.0704	$C_7H_9^+$
99	99.0445	99.0446	$C_5H_7O_2^+$
109	109.0659	109.0653	$C_7H_9O^+$
109	109.1023	109.1017	$C_8H_{13}^+$
121	121.1027	121.1017	$C_9H_{13}^+$
127	127.0754	127.0759	$C_7H_{11}O_2^+$
194	194.1291	194.1307	$C_{12}H_{18}O_2^+$

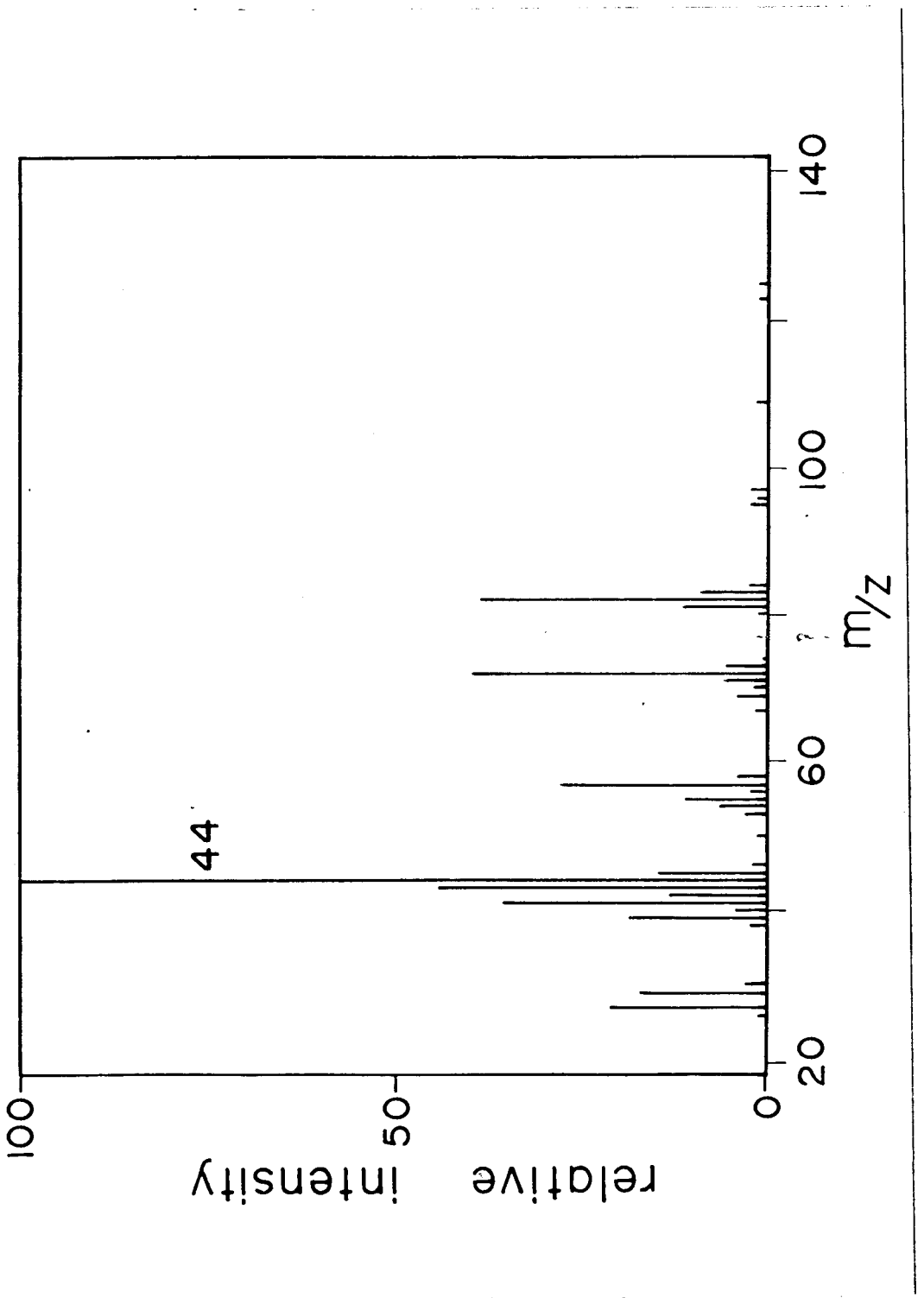
Figure 13

Infrared spectrum of rusty grain beetle attractant B. Neat on a AgCl plate.

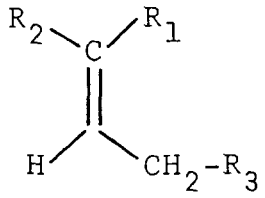
50
25
0
transmission (%)
4000
3000
2000
1600
1200
800
wavenumber (cm⁻¹)

Figure 14

Mass spectrum of micro-ozonolysis product of rusty grain beetle attractant B.


relative intensity
100
50
0
20
60
100
140
m/z
44

aldehyde[27]. The peaks at m/z 29 and 44 are typical fragment ions of aliphatic aldehydes and are usually due to the species CHO^+ and $CH_2{=}\overset{+\cdot}{C}HOH$. Because the ozonolysis product appears to be an aldehyde, compound B must contain a vinyl hydrogen. This information leads to the partial structure of



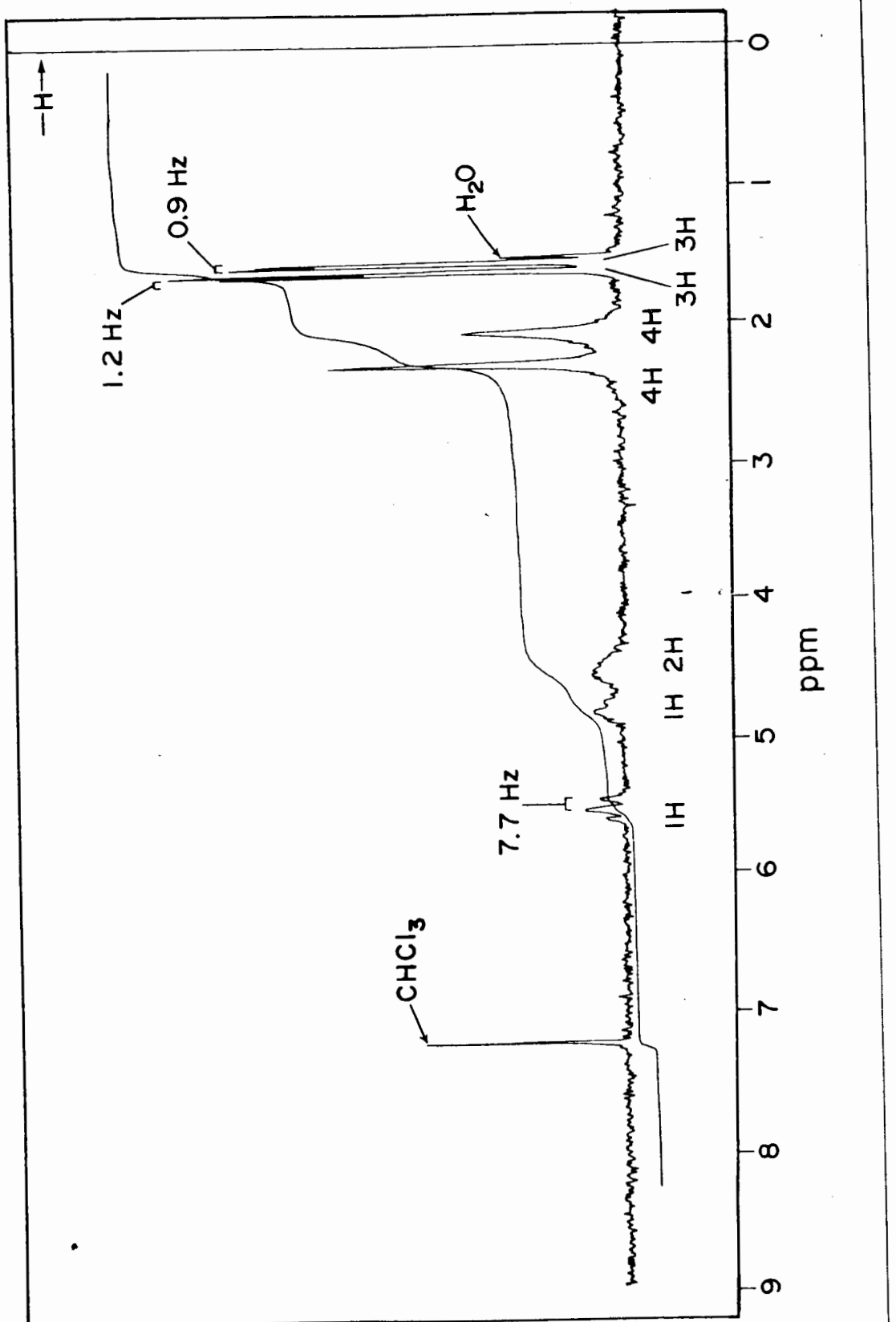
for compound B.

Microhydrogenation of compound B did not yield any GLC detectable products. Because of the destructive nature of the technique and because of a shortage of compound B, only a small amount of compound B was committed to establishing the correct experimental conditions. Unfortunately, this amount was not sufficient. Experimental conditions had previously been determined for the hydrogenation of several model compounds. Geraniol, upon hydrogenation gave a product indentified by GC-MS as 2,6-dimethylheptane. Hydrogenation of dodecene gave dodecane, the expected product. Hydrogenation of compound B under the experimental conditions determined for the model compounds did not, however, yield any products in sufficient amount for identification.

The most revealing structural information about compound B was derived from its ^1H NMR (Figure 15). The spectrum showed two-three hydrogen doublets (δ1.57, J = 0.9 Hz and δ1.64, J = 1.2 Hz), two-four hydrogen signals (δ2.09 and δ2.32), a two hydrogen multiplet (δ4.56), a one hydrogen multiplet (δ = 4.82) and a one

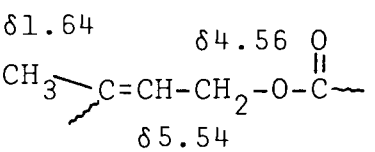
Figure 15

100 MHz ^{1}H NMR spectrum of rusty grain beetle attractant B. 200 µg in 50 µℓ $CDCl_3$. 128 scans, 5 second delay.

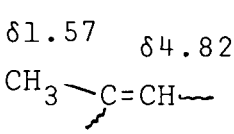

—H—
1.2 Hz
0.9 Hz
H_2O
3H 3H
4H 4H
1H 2H
1H
7.7 Hz
$CHCl_3$
0
1
2
3
4
5
6
7
8
9
ppm

hydrogen triplet (δ5.51, J = 7.7 Hz). The effect of progressive addition of shift reagent, $Eu(fod)_3$-d_{30}, on the ^1H NMR spectrum is shown in Figure 16. The peaks at δ2.32, δ4.56 and δ5.54 are shifted downfield the greatest (~1.1 ppm) indicating that they are close to nucleophilic sites. The other signals are shifted less (0.3 ppm) indicating that they are farther removed from these sites.

The signal at δ4.56 is assignable to a methylene group between an oxygen of an ester and a vinyl group. Irradiation at δ4.56 caused the triplet structure at δ5.54 to collapse. Conversely, the broad signal at δ4.56 collapsed when the sample was irradiated at δ5.54. Irradiation at δ5.54 also caused the vinyl methyl signal at δ1.64 to change but had no effect on the other vinyl methyl group at δ1.57. This indicates that these methyl groups are on different double bonds and leads to the following partial structure:



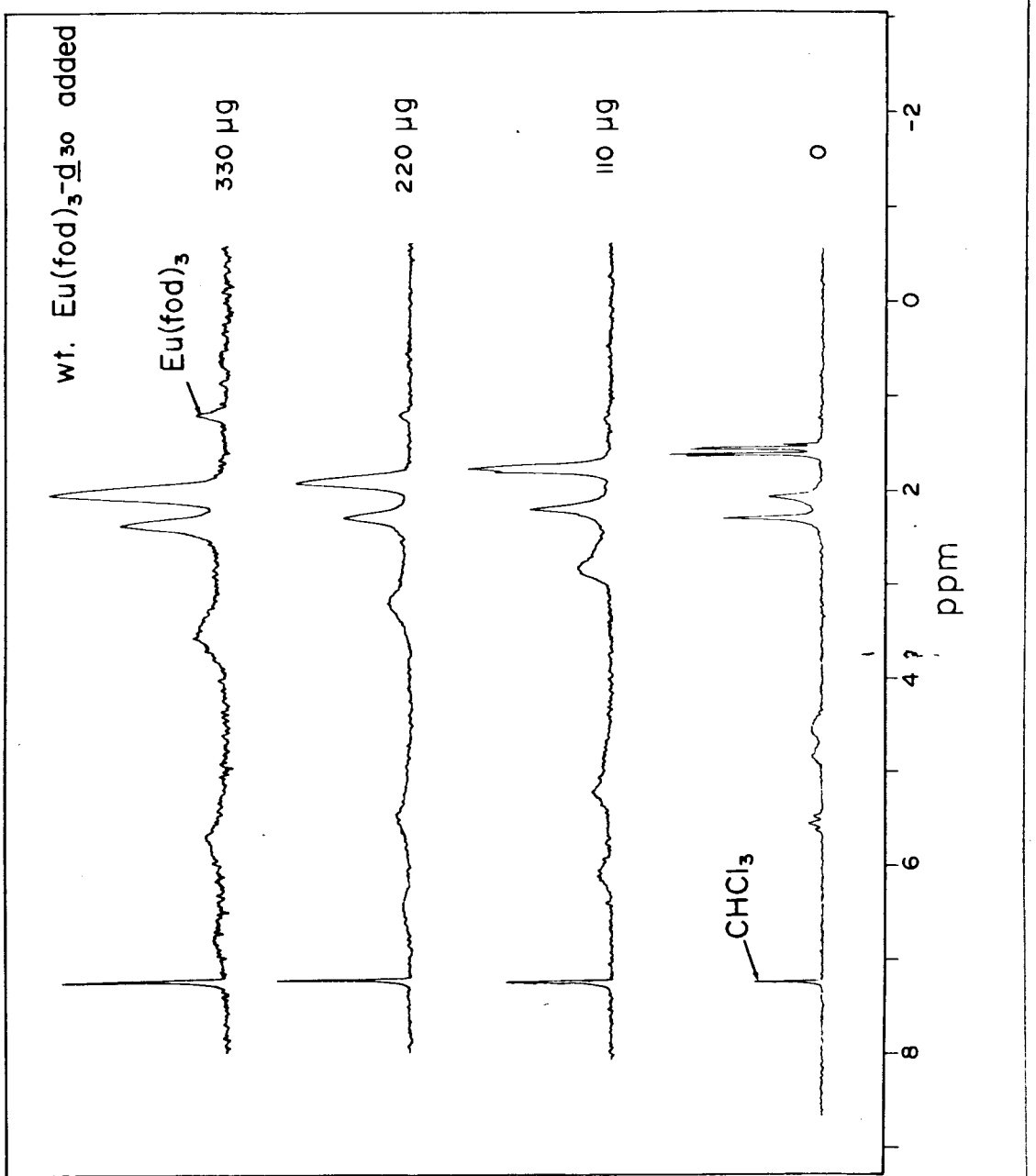
The signal at δ4.82 can be assigned to the vinyl hydrogen. Irradiation at δ4.82 caused the methyl group at δ1.57 to change, indicating the partial structure:

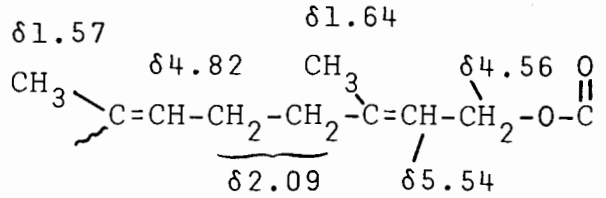


The four hydrogen signal at δ2.09 has the chemical shift expected for hydrogens on carbons between two vinyl groups. When this signal was irradiated, the only signal affected was that at δ4.82. This allows extension of the partial structure to:

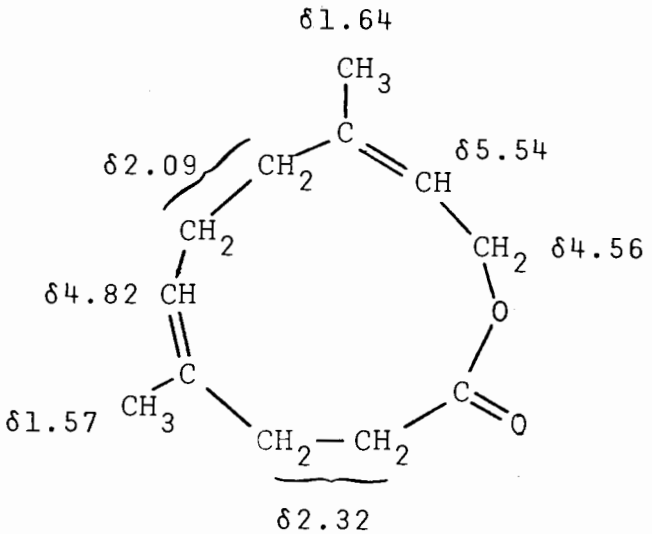
Figure 16

The effect of progressive addition of $Eu(fod)_3$-$\underline{d}_{30}$ on the 100 MHz ^1H NMR spectrum of rusty grain beetle attractant B. 200 µg attractant B. 128 scans, 5 second delay.

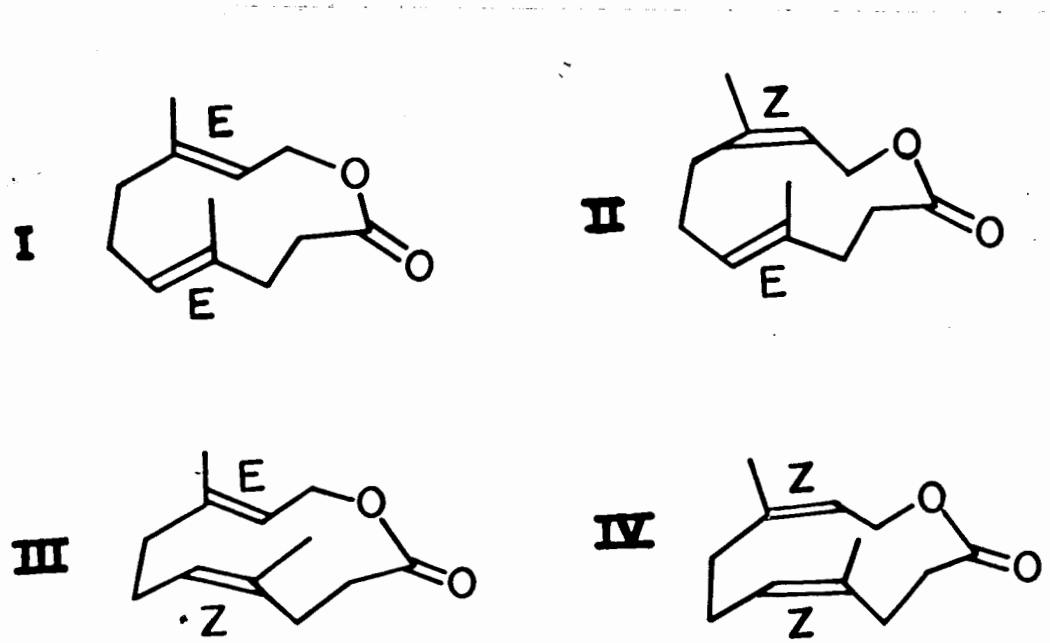

wt. Eu(fod)3-d30 added
Eu(fod)3
330 µg
220 µg
110 µg
0
CHCl3
8
6
4
2
0
-2
ppm



The remaining four hydrogens (δ2.32) must then be on carbons connecting the chain, as in:



This compound may exist in one of four isomeric forms:



These structures possess terpenoid carbon skeletons and it is possible to envision B as being derived from oxidation of farnesol at the terminal bond followed by cyclization of the resulting hydroxy-carboxylic acid. Since the most prevalent natural isomer of farnesol is the E-E-isomer, one would expect the E-E-isomer of B (I) to be the most likely to occur.

Although in theory it is possible to assign the E/Z geometry of the double bonds of B from the ^{13}C chemical shifts of the vinyl methyls in the proton noise decoupled spectrum, the small amount available precluded this (Figure 17). Indeed, complete assignment of the resonances in the proton noise decoupled ^{13}C NMR spectrum of attractant B was not possible because of impurities in the sample. The ^{1}H NMR spectrum of the ^{13}C spectral sample showed significant impurities resulting in signals at δ1.2 (doublet, J = 8 Hz) and δ3.0 (multiplet). Furthermore, the ^{13}C NMR experiment was conducted in California (Varian Associates) two months after the sample was prepared.

By comparison with ^{13}C NMR assignments of model compounds (Figure 18) the ^{13}C NMR spectrum of compound B is consistent with the structures postulated. The resonance for the carbonyl carbon must be either 164.9 ppm or 160.5 ppm. The other of these resonances must be due to an impurity. The resonances for the two disubstituted vinyl carbons, typically at 130 to 145 ppm, are not visible above noise. There are two vinyl carbon resonances at 126.6 ppm and 122.5 ppm which are consistent with resonances from singly protonated vinyl carbons in trisubstituted double bonds in terpenoid chains. The resonance at 61.2 ppm is easily assigned to the alkyl carbon bearing the ester oxygen.

Figure 17

^{13}C NMR spectrum of rusty grain beetle attractant B. 200 µg in 7 µℓ $CDCl_3$. 314,000 transients.

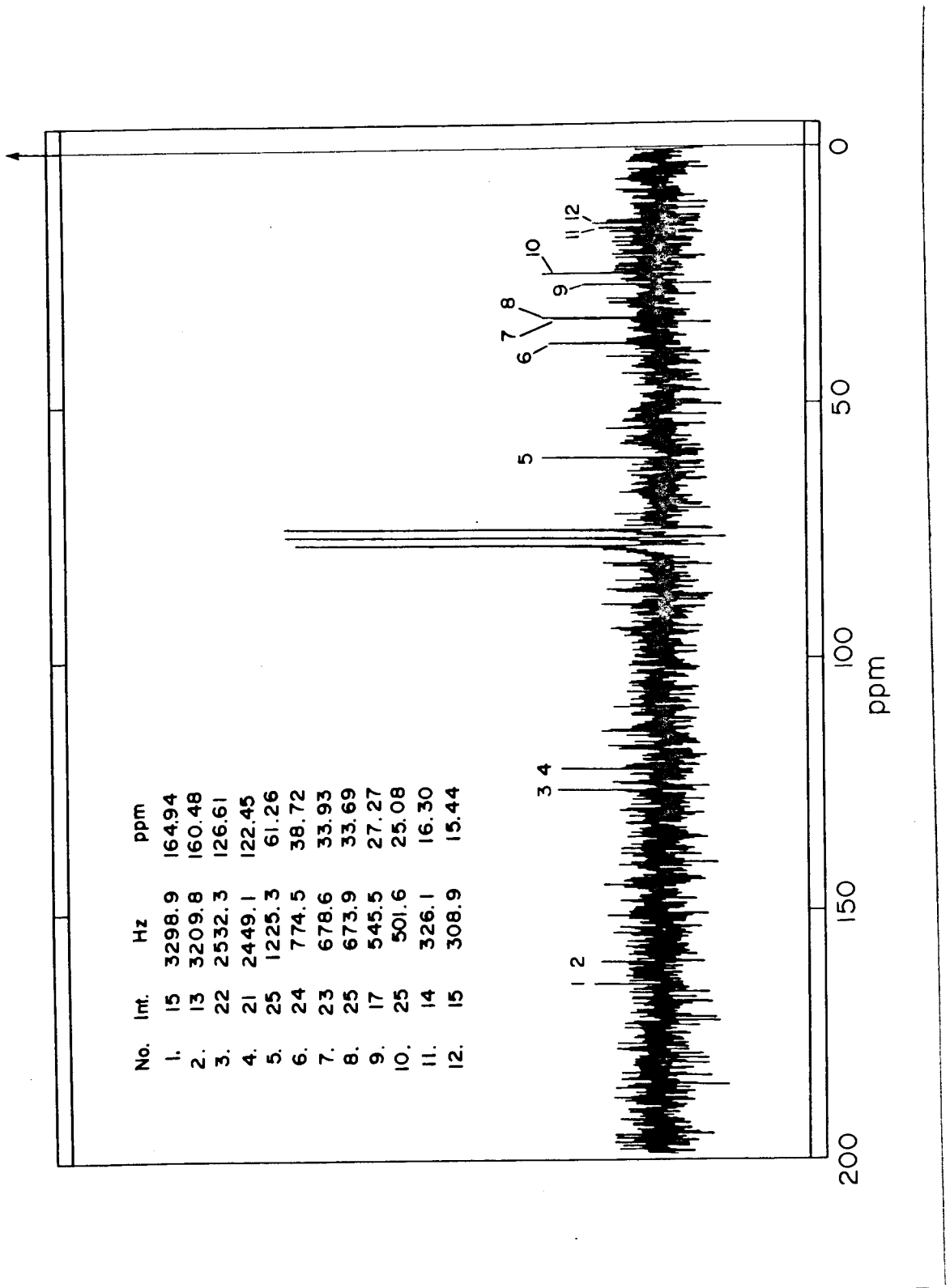
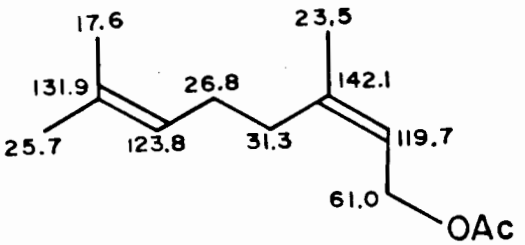
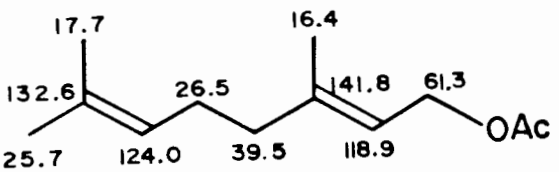

No. Int. Hz ppm
1. 15 3298.9 164.94
2. 13 3209.8 160.48
3. 22 2532.3 126.61
4. 21 2449.1 122.45
5. 25 1225.3 61.26
6. 24 774.5 38.72
7. 23 678.6 33.93
8. 25 673.9 33.69
9. 17 545.5 27.27
10. 25 501.6 25.08
11. 14 326.1 16.30
12. 15 308.9 15.44
1 2
3 4
5
6 7 8
9
10
11 12
0
50
100
150
200
ppm

Figure 18

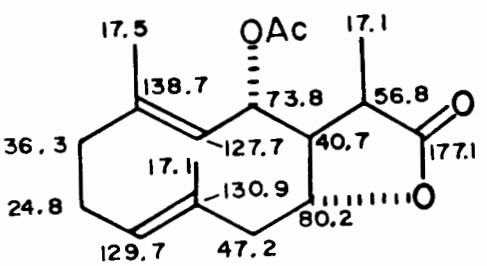
^{13}C NMR chemical shift data of selected compounds. Shifts are in ppm relative to TMS.



neryl acetate[28]

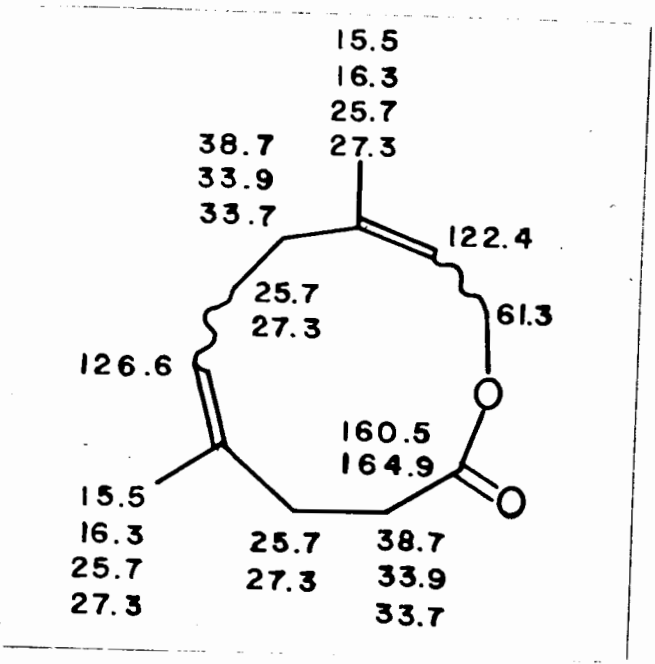


geranyl acetate[28]

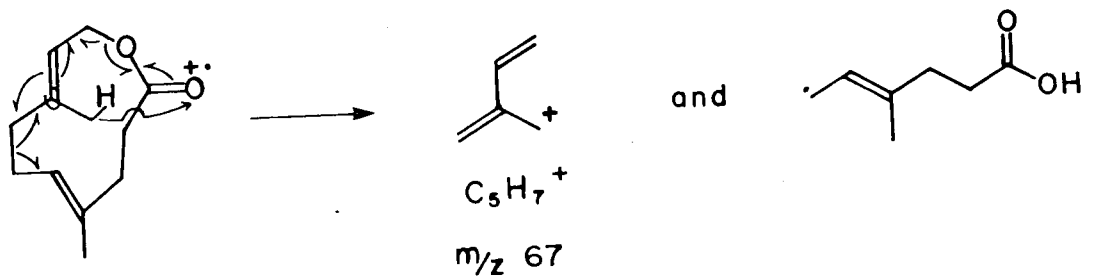


dihydrolaurenobiolide[38]

The signals at 38.7, 33.9, 33.7, 27.3 and 25.1 are suggestive of methylene carbons in a polyisoprene chain. The signals at 27.3 and 25.1 ppm, however, could be due to vinyl methyls where the geometry of the double bond is Z. The resonances at 16.3 and 15.4 ppm are consistent with vinyl methyls where the geometry of the double bond is E. Since there are only six carbons not assigned, clearly one of these resonances from 38.7 to 15.4 ppm is either noise or from an impurity. At least one of the double bonds must be in the E conformation.



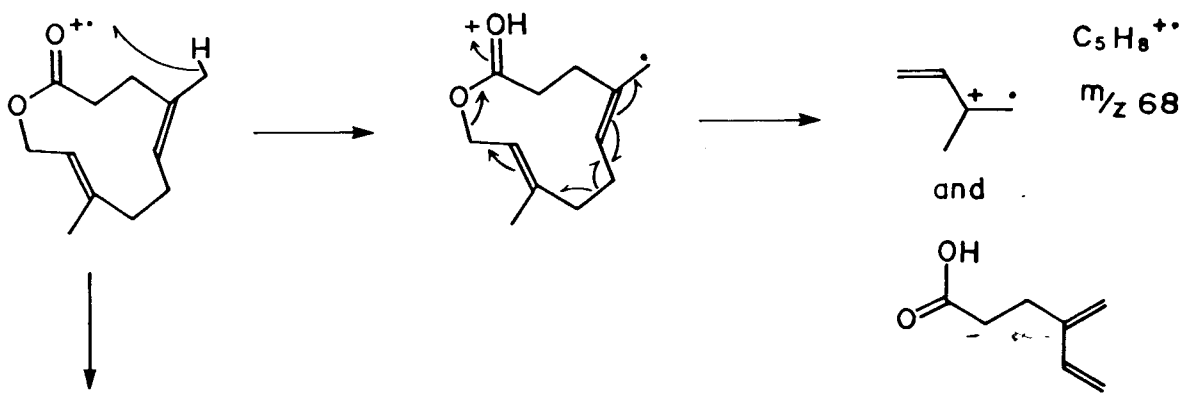
The mass spectral fragmentation pattern (Figure 12, Table XII) can also be interpreted in terms of the proposed structures. The fragmentation pattern for the E,E, structure (I) is given as follows:



$C_5H_7^+$

m/z 67

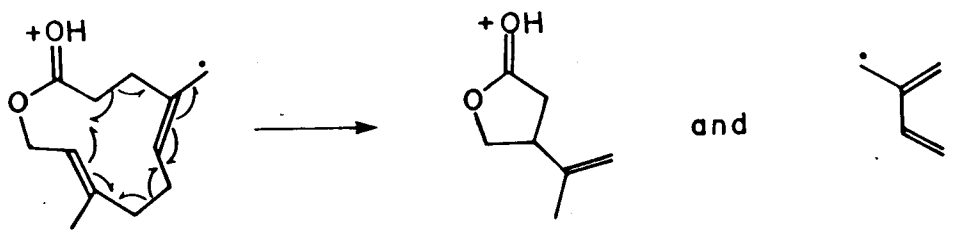
and



$C_5H_8^{+\cdot}$

m/z 68

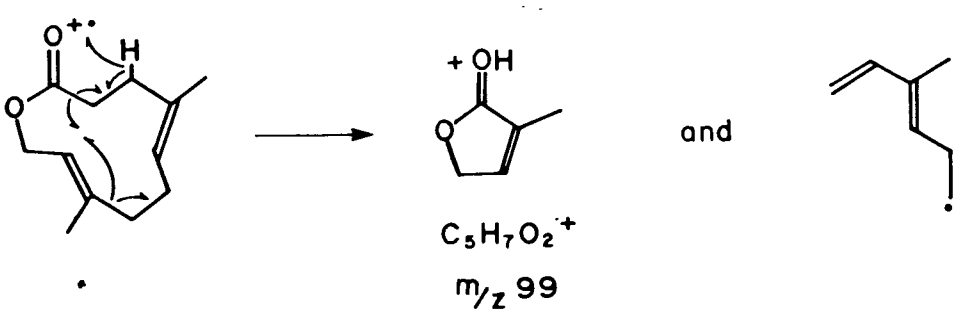
and



$C_7H_{11}O_2^+$

m/z 127

and



$C_5H_7O_2^+$

m/z 99

and

iii. Identification of rusty grain beetle attractants A and C

The electron impact mass spectra (Figure 19a, Table XIII) suggested that the molecular weight of compound A was 196.1449, which corresponds to a molecular composition of $C_{12}H_{20}O_2$ (196.1463). Chemical ionization mass spectrometric analysis (Figure 19b) confirmed this assumption. The infrared spectrum (Figure 20) showed that one of the three sites of unsaturation in this compound can be assigned to a carbonyl (ν = 1730 cm^{-1}) of an ester or lactone. The IR spectrum is quite simple and is devoid of hydroxyl and aromatic absorption, thus excluding the possibility of these groups occurring in compound A. The high resolution mass spectrum indicates that there are no major fragments containing any oxygen atoms. This is unusual for a compound containing a carbonyl function and indicates branching or unsaturation γ or δ to the carbonyl carbon.

Compound C has a molecular weight, determined by mass spectrometry (Figure 21, Table XIV), of 224.1779. This corresponds to a molecular composition of $C_{14}H_{24}O_2$ (224.1776). This molecular formula corresponds to a compound with three sites of unsaturation. The infrared spectrum (Figure 22) shows that one of these sites can be assigned to a carbonyl (ν = 1725 cm^{-1}) of an ester or lactone. The infrared spectrum is quite similar to that of compound A. Since the composition of compound A differs from the composition of compound C by C_2H_4, these compounds may be homologs.

C. SUMMARY

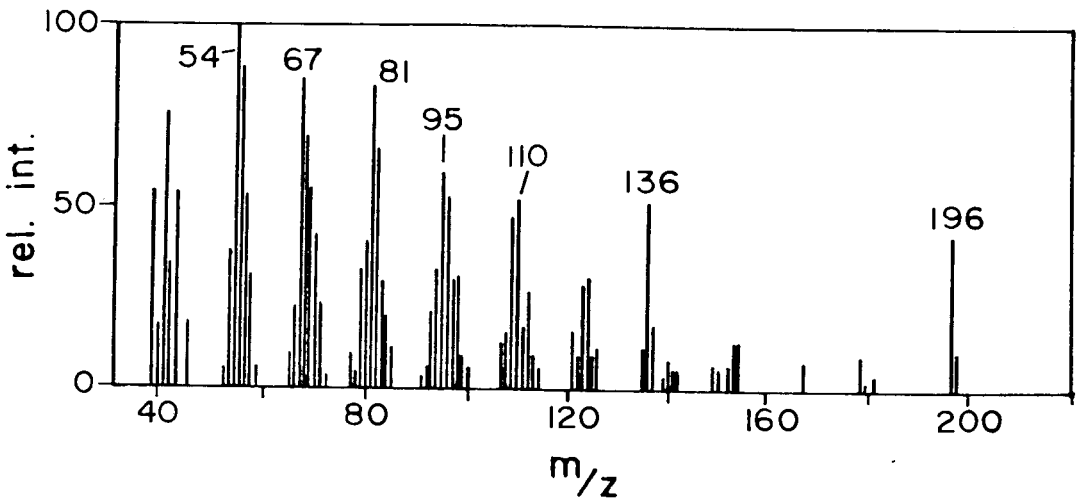
Three attractants for the rusty grain beetle, compounds A,

A

Figure 19

Electron impact (a) and chemical ionization (b, NH_3 reagent gas) of rusty grain beetle attractant A.

(a)


100
50
0
rel. int.
54
67
81
95
110
136
196
40
80
120
160
200
m/z

(b)

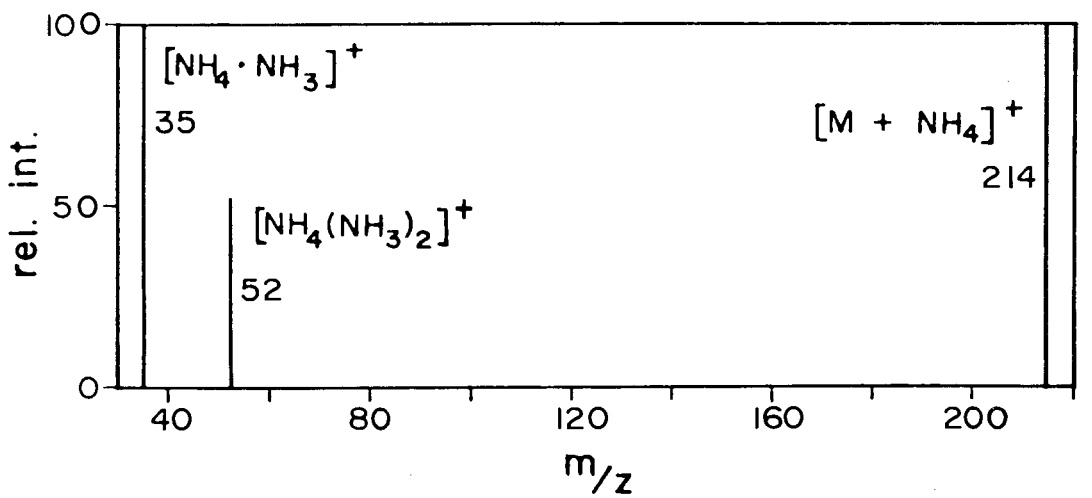

100
50
0
rel. int.
[NH4 · NH3]+
35
[NH4(NH3)2]+
52
[M + NH4]+
214
40
80
120
160
200
m/z

TABLE XIII. Significant peaks in the High Resolution Mass Spectrum of rusty grain beetle attractant A.

Nominal m/z	Measured Mass	Calculated Mass	Composition
39	39.0277	39.0235	$C_3H_3^+$
41	41.0429	41.0391	$C_3H_5^+$
43	43.0217	43.0184	$C_2H_3O^+$
43	43.0581	43.0548	$C_3H_7^+$
54	54.0484	54.0469	$C_4H_6^+$
55	55.0563	55.0548	$C_4H_7^+$
56	56.0640	56.0626	$C_4H_8^+$
67	67.0556	67.0548	$C_5H_7^+$
68	68.0628	68.0626	$C_5H_8^+$
69	69.0705	69.0704	$C_5H_9^+$
81	81.0683	81.0704	$C_6H_9^+$
82	82.0760	82.0782	$C_6H_{10}^+$
95	95.0850	95.0861	$C_7H_{11}^+$
96	96.0923	96.0939	$C_7H_{12}^+$
109	109.0652	109.0653	$C_7H_9O^+$
109	109.0998	109.1017	$C_8H_{13}^+$
110	110.1078	110.1095	$C_8H_{14}^+$
124	124.0919	124.0888	$C_8H_{12}O^+$
124	124.1253	124.1252	$C_9H_{16}^+$
136	136.0882	136.0888	$C_9H_{12}O^+$
136	136.1261	136.1251	$C_{10}H_{16}^+$
167	167.1067	167.1092	$C_{10}H_{15}O_2^+$
178	178.1353	178.1358	$C_{12}H_{18}O^+$
196	196.1449	196.1463	$C_{12}H_{20}O_2^+$

Figure 20

Infrared spectrum of rusty grain beetle attractant A. Neat on a AgCl plate.

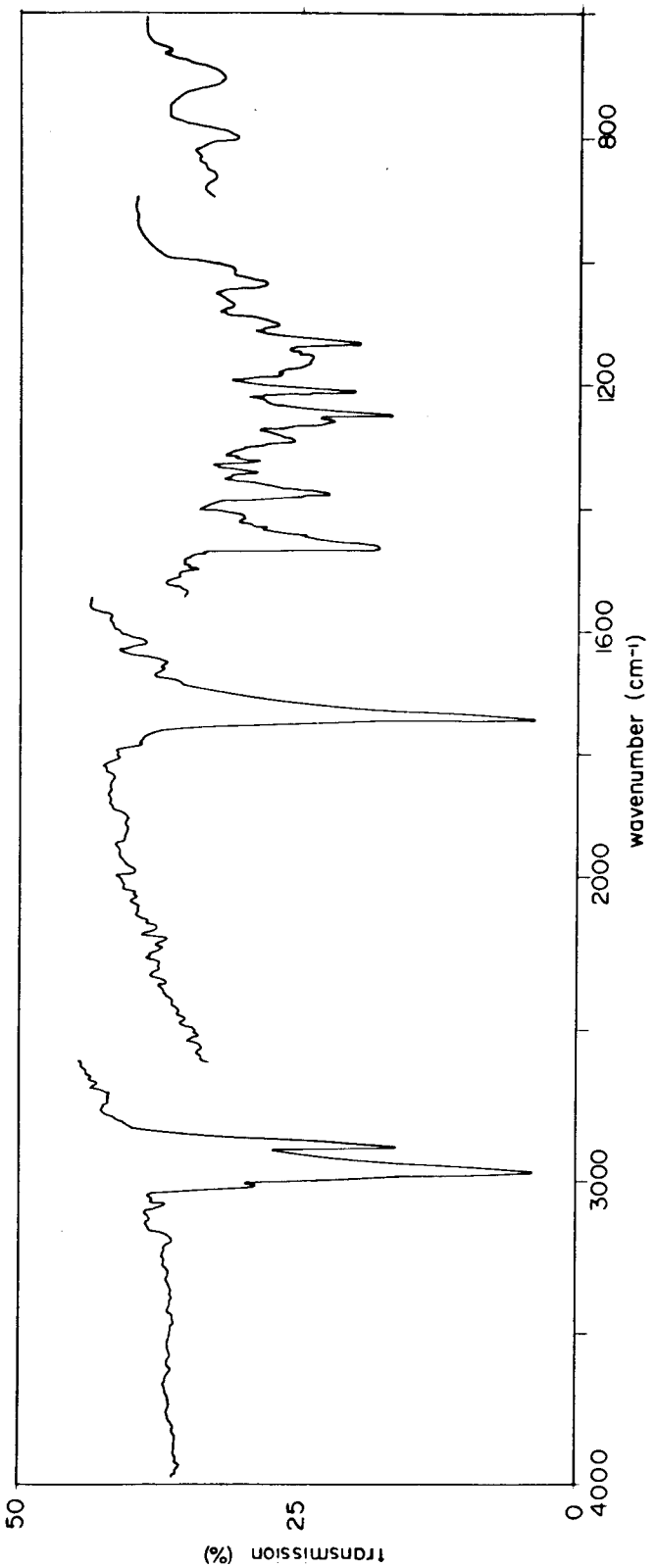
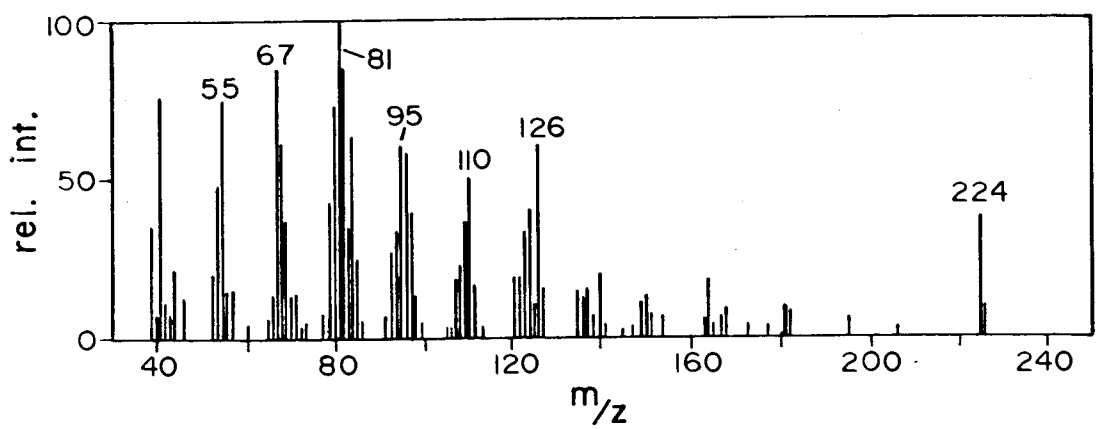

50
25
0
transmission (%)
4000
3000
2000
1600
1200
800
wavenumber (cm⁻¹)

Figure 21

Electron impact (a) and chemical ionization (b, NH_3 reagent gas) of rusty grain beetle attractant C.

(a)


100
50
0
rel. int.
55
67
81
95
110
126
224
40
80
120
160
200
240
m/z

(b)

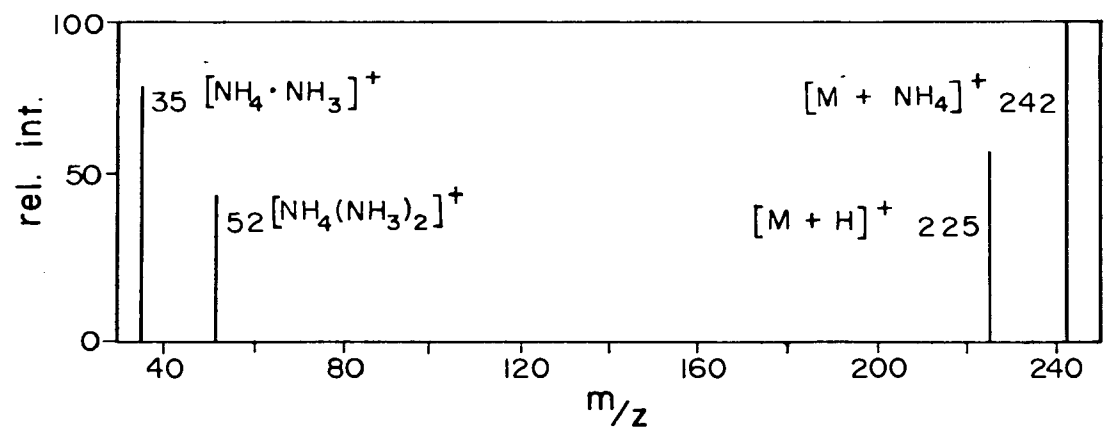
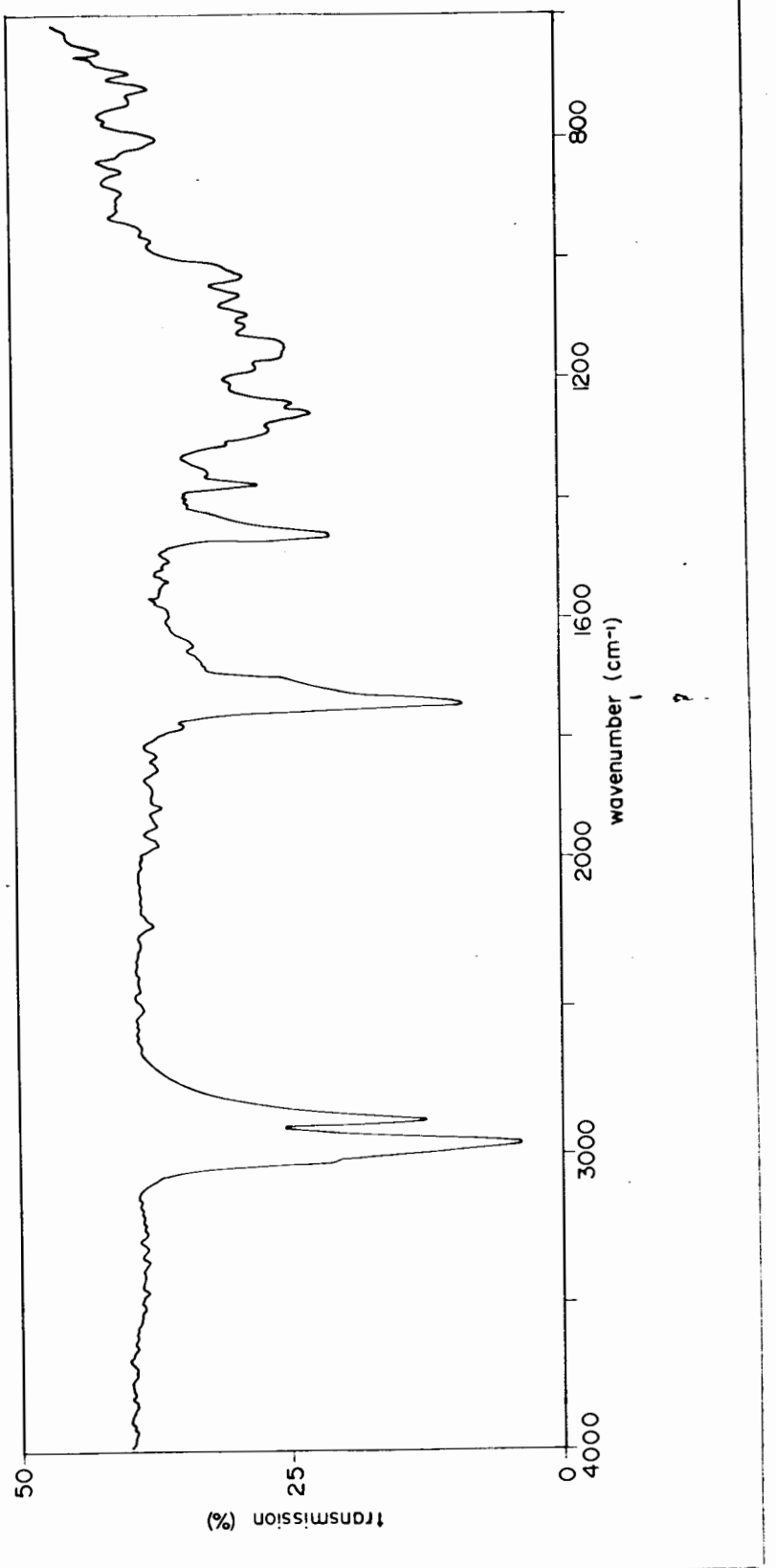

100
50
0
rel. int.
35 [NH4 · NH3]+
52 [NH4(NH3)2]+
[M + NH4]+ 242
[M + H]+ 225
40
80
120
160
200
240
m/z

TABLE XIV. Significant peaks in the High Resolution Mass Spectrum of rusty grain beetle attractant C.

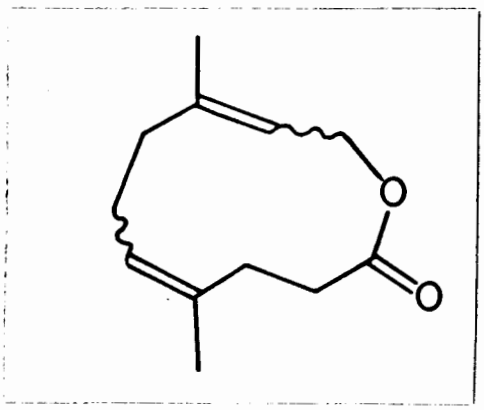
Nominal m/z	Measured Mass	Calculated Mass	Composition
67	67.0547	67.0548	$C_5H_7^+$
68	68.0607	67.0626	$C_5H_8^+$
80	80.0630	80.0626	$C_6H_8^+$
81	81.0709	81.0704	$C_6H_9^+$
82	82.0782	82.0782	$C_6H_{10}^+$
84	84.0558	84.0575	$C_5H_8O^+$
95	95.0857	95.0861	$C_7H_{11}^+$
96	96.0936	96.0939	$C_7H_{12}^+$
110	110.1096	110.1095	$C_8H_{14}^+$
126	126.0682	126.0681	$C_7H_{10}O_2^+$
140	140.0829	140.0837	$C_8H_{12}O_2^+$
164	164.1556	164.1565	$C_{12}H_{20}^+$
224	224.1779	224.1776	$C_{14}H_{24}O_2^+$

Figure 22

Infrared spectrum of rusty grain beetle attractant C. Neat on a AgCl plate.


transmission (%)
50
25
0
4000
3000
2000
1600
1200
800
wavenumber (cm⁻¹)

B and C, have been shown to have molecular compositions of $C_{12}H_{20}O_2$, $C_{12}H_{18}O_2$ and $C_{14}H_{24}O_2$, respectively. Each of these compounds has a strong infrared absorption at approximately 1730 cm^{-1} corresponding to that of an ester or lactone. Compound B which has a composition of $C_{12}H_{18}O_2$, has been identified as an 11-membered, dimethyl substituted, unsaturated lactone:



The geometry of the double bonds is uncertain.

V. SYNTHESIS OF ATTRACTANT B

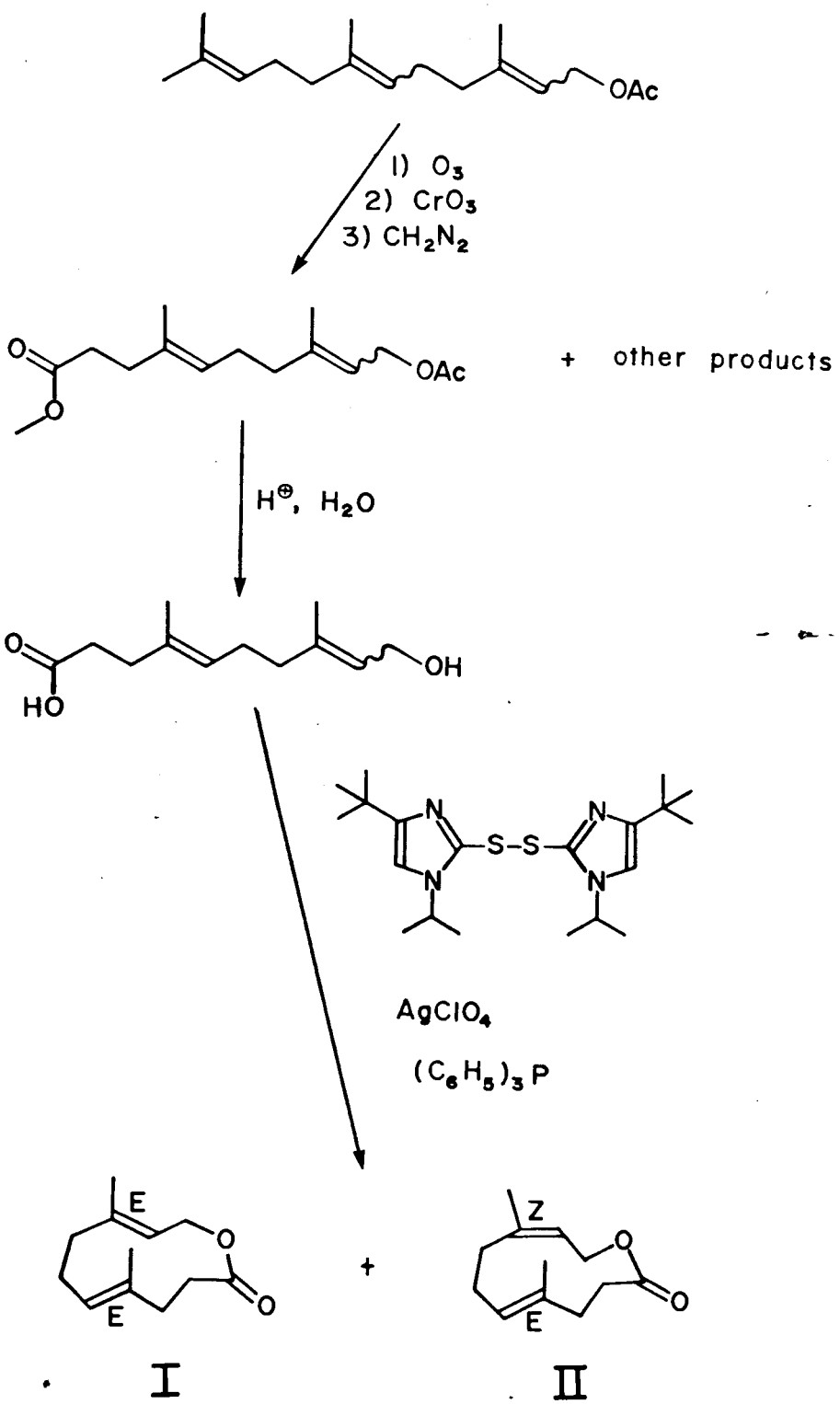
A. Synthetic approaches

Since the geometry of the double bonds of attractant B was not known, synthesis of all four possible isomers were considered. Geraniol, nerol, farnesol and isomers were thought to be good starting materials because they possessed structures which were similar to attractant B. Syntheses starting from geraniol or nerol would involve chain extension followed by cyclization. This cyclization would involve a C-C or C-O bond formation, depending on which end of the molecule was chain extended. A synthesis starting from farnesol or a similar molecule would involve oxidation of a C-C double bond followed by cyclization.

In early 1979, Dr. A.C. Oehlschlager[29] conducted a synthesis of attractant B from a mixture of farnesol acetate, E,E, E,Z and Z,E isomers. These isomers were subjected first to ozonolysis then to oxidation by Jones reagent and finally to esterification using diazomethane (Figure 23). The acetoxy esters formed were separated from the other reaction products using TLC. During this purification the Z,E isomer was separated from the other two. The products were identified by ^{13}C NMR, ^{1}H NMR and MS. After hydrolysis, the E,E and E,Z isomers were cyclized using the method of Corey, et al.[30] to yield two major inseparable products in low overall yield. GLC analysis showed that the faster eluting compound had a retention time identical to that of attractant B. Bioassay results showed that this mixture of products was attractive for C. ferrugineus. This indicated that attractant B was either isomer I or isomer II. The double bond δ to the ester

Figure 23

Oehlschlager[29] synthesis of attractant B.


OAc
1) O_3
2) CrO_3
3) CH_2N_2
OAc
+ other products
$H^{\oplus}$, H_2O
OH
HO
N
S-S
N
N
N
$AgClO_4$
$(C_6H_5)_3P$
E
O
O
E
+
Z
O
O
E
I
II

oxygen must be in the E configuration. The geometry of the other double bond, β to the ester oxygen, was still unknown.

Since the GLC data indicated that the attractive compound was the isomer eluting first through the carbowax column and since nerol eluted through the same column before geraniol, it was assumed that attractant B had an E,Z configuration (II). With this in mind, two syntheses starting from nerol were attempted.

B. Syntheses from nerol

i. Seven-step synthesis

The first synthetic scheme attempted is shown in Figure 24. Nerol was first coupled to ethyl malonyl chloride[31]. The isopropylidene terminus of the coupling product, 3, was stereospecifically oxidized with SeO_2[32] to yield the E aldehyde, 4. This aldehyde was reduced[33] to the allylic alcohol, 5, using $NaBH_4$ which was in turn converted to the allylic acetate, 6. Cyclization of the sodium salt, 7, was attempted by activating the vinyl acetate end of the molecule through the formation of a π-allyl palladium complex[34,35]. This cyclization attempt proved unsuccessful.

a. experimental

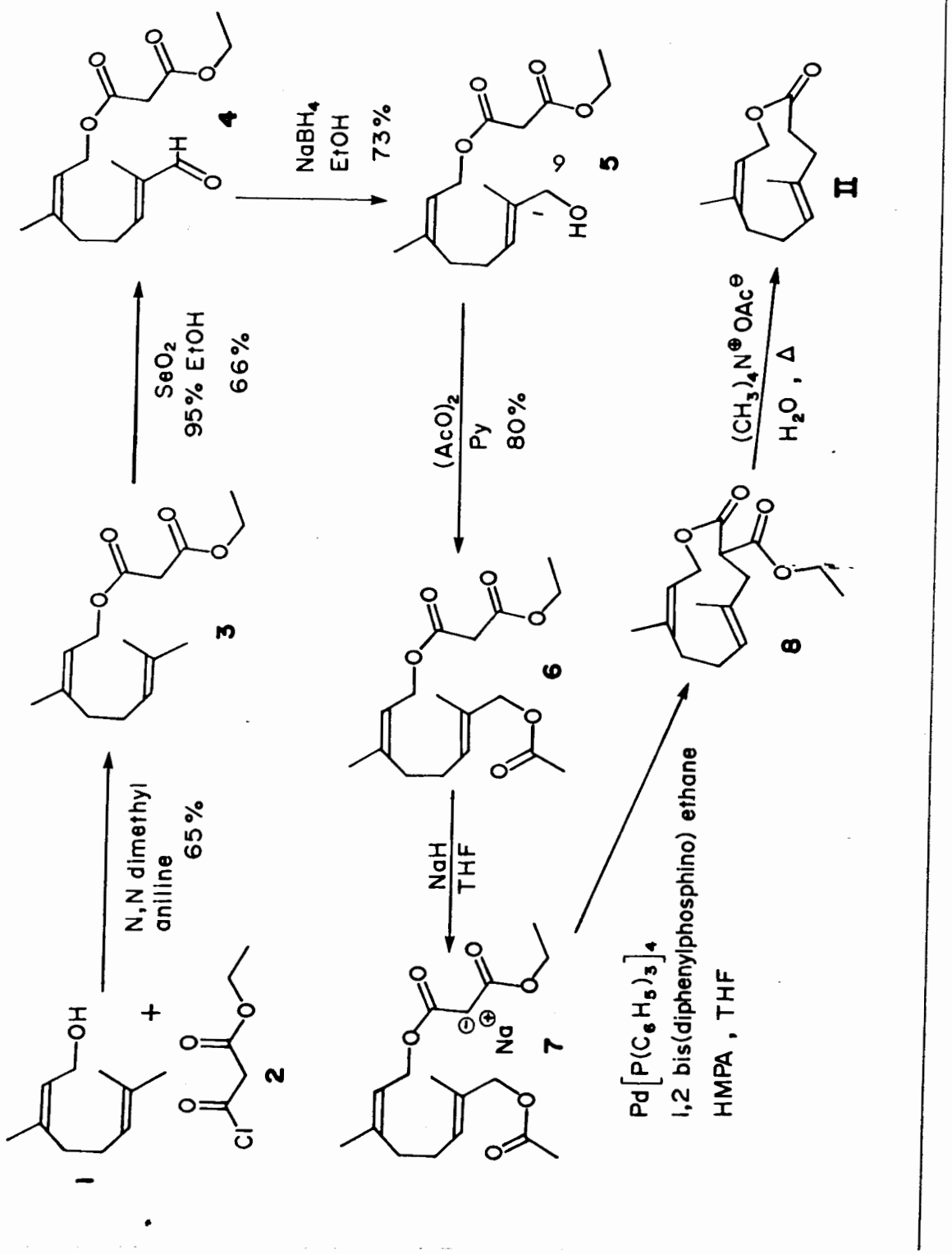
NMR spectra were recorded with a Varian A60 instrument. Chemical shifts are reported in parts per million relative to TMS.

Ethyl malonyl chloride (2)

In a 2-liter three-necked flask equipped with a mechanical stirrer, reflux condenser and 500 mℓ pressure equalizing dropping funnel were placed 200 g (1.25 moles) of diethyl malonate

Figure 24

First attempted synthesis of compound II. Yields are for crude products.


1
2
N,N dimethyl aniline
65%
3
SeO2
95% EtOH
66%
4
NaBH4
EtOH
73%
5
9
HO
(AcO)2
Py
80%
6
NaH
THF
7
Na
Pd[P(C6H5)3]4
1,2 bis(diphenylphosphino) ethane
HMPA , THF
8
(CH3)4N⊕OAc⊖
H2O , Δ
II

(Fisher, purified grade, dried over $MgSO_4$) and 700 mℓ of absolute ethanol (Fisher). To this was added, with stirring over a period of one hour, a solution of 70 g (1.25 moles) KOH (American Scientific, reagent) in 500 mℓ of absolute ethanol. A white precipitate formed. After stirring for three hours and standing overnight the precipitate was filtered off. Concentration of the mother liquid to about 250 mℓ yielded more crystals. This monopotassium salt of ethyl hydrogen malonate was dried overnight under vacuum to yield 157 g (74%).

To a chilled solution of the potassium salt (95 g, 0.56 mole) in 60 mℓ of distilled water (immersed in an ice-bath) 55 mℓ of concentrated HCl (1.78 g) were slowly added with stirring. After 30 minutes, the mixture was filtered with suction and the precipitate of potassium chloride was washed with 75 mℓ of ether. The aqueous layer was separated and extracted with ether (3 X 50 mℓ). The combined ether extracts were dried ($MgSO_4$) and evaporated to yield 51.5 g (70%) of ethyl hydrogen malonate.

In a 500 mℓ two-necked round-bottomed flask equipped with a dropping funnel and reflux condenser were placed 100 g (0.50 mole) phthaloyl chloride (Eastman, freshly distilled). Ethyl hydrogen malonate (50 g, 0.38 mole) was placed in the dropping funnel, the flask immersed in an oil bath heated to ~105°C and the ethyl hydrogen malonate was added with stirring over thirty minutes. Stirring and heating were continued for two hours. The mixture was then distilled at 15 mm Hg (80°C) to yield 31 g (65%) of ethyl malonyl chloride.

Ethyl neryl malonate (3)

In a two-necked 250 mℓ flask equipped with a reflux

condenser and a dropping funnel were placed 12.4 g (0.080 mole) nerol (MCB, freshly distilled), 9.8 g (0.080 mole) N,N-dimethyl aniline (Fisher reagent, dried over KOH) and 30 mℓ anhydrous ether. The stirred solution was heated to reflux, the heat removed and 13.4 g (0.080 mole) of ethyl malonyl chloride in 20 mℓ of anhydrous ether slowly added. The mixture continued to reflux without external heat and a solid (N,N-dimethylaniline hydrochloride) crystallized. After the reaction subsided, the mixture was refluxed for 3 hours and allowed to stand overnight. 20 mℓ water was added and the two phases were separated. The aqueous phase was extracted with ether and the combined ether extracts were washed with 10% sulfuric acid until no cloudiness was observed in the washings when made alkaline. The ether extract was then dried ($MgSO_4$) and evaporated to yield an oil. This oil was fractionated (0.2 mm Hg, 120°C) to yield 14.0 g (65%) product: ^{1}H NMR, δ 1.25 (t, J = 7 Hz, 3H, -O-CH_2-$\underline{CH_3}$). 1.58 (s, 3H, $\underline{CH_3}$-olefin), 1.65 (s, 6H, $(\underline{CH_3})_2$- olefin), 2.08 (m, 4H, -$\underline{CH_2}$-$\underline{CH_2}$-), 3.28 (s, 2H, -CO-$\underline{CH_2}$-CO-), 4.12 (q, J = 7 Hz, 2H, -O-$\underline{CH_2}$-CH_3), 4.60 (d, J = 7 Hz, 2H, olefin-$\underline{CH_2}$-O-), 5.06 (m, 1H, $(CH_3)_2$C=$\underline{CH}$-), 5.34 (t, J = 7 Hz, 1H, $\rangle$C=$\underline{CH}$-CH_2-O-).

Ethyl (3,7-dimethyl,2-Z,6-E,8-al,octadienyl) malonate (4)

3.5 g (32 mmoles) selenium dioxide (Alfa, 99.4%) were added to a solution of ethyl neryl malonate (4.5 g, 16 mmoles) in 240 mℓ of 95% EtOH in a 500 mℓ round bottom flask. The mixture was heated under reflux for 2-1/2hours. The mixture was then filtered into 200 mℓ of water and extracted with ether. The ether extracts were washed with sodium bicarbonate solution, dried ($MgSO_4$) and evaporated to yield 3.1 g (66%) of crude product:

^{1}H NMR, δ 1.28 (t, J = 7 Hz, 3H, -O-CH_2-CH_3), 1.74 (s, 6H, CH_3-olefin), 2.10-2.60 (m, 4H, -CH_2-CH_2-), 3.35 (s, 2H, -CO-CH_2-CO-), 4.20 (q, J = 7 Hz, 2H, -O-CH_2-CH_3), 4.65 (d, J = 7 Hz, 2H, olefin-CH_2-O-), 5.40 (t, J = 7 Hz, 1H, >C=CH-CH_2-O-), 6.45 (t, J = 7 Hz, 1H, >C=CH-CH_2-CH_2-), 9.38 (s, 1H, aldehyde H).

Ethyl (8-hydroxy-3,7-dimethyl,2-Z,6-E,octadienyl) malonate (5)

3.0 g (0.11 mole) of the crude andehyde, 4, were placed in a 100 mℓ round-bottom flask containing 30 mℓ absolute ethanol. After the solution was cooled to 0°C in an ice-bath, 0.107 g (0.0028 mole) of $NaBH_4$ (MCB) was slowly added. After 5 minutes the reaction mixture was poured into water and stirred for one half hour. The solution was then extracted with ether (3 X 50 mℓ). The ether extracts were dried ($MgSO_4$) and evaporated to yield 2.2 g (73%) of crude product, 5: ^{1}H NMR δ 1.28 (t, J = 7 Hz, 3H, -O-CH_2-CH_3), 1.66 (s, 3H, CH_3-olefin), 1.73 (s, 3H, CH_3-olefin), 2.15 (m, 4H, -CH_2-CH_2-), 3.0 (s, 1H, D_2O exchangeable, -OH), 3.37 (s, 2H, -CO-CH_2-CO-), 3.97 (s, 2H, -CH_2-OH), 4.20 (q, J = 7 Hz, 2H, -O-CH_2-CH_3), 4.67 (d, J = 7 Hz, 2H, olefin-CH_2-O-), 5.38 (t, J = 7 Hz, 2H, vinyl H).

Ethyl (8-acetoxy-3,7-dimethyl,2-Z,6-E,octadienyl) malonate (6)

2.2 g (7.7 mmoles) of the allylic alcohol, 5, were dissolved in a solution of 10 g pyridine (Fisher) and 10 g acetic anhydride (American Chemical, reagent). The solution was stirred overnight at room temperature. The mixture was then poured into 50 mℓ water and extracted with ether (3 X 50 mℓ). The ether extracts were washed with water several times, with

0.3N HCl, with 2% $NaHCO_3$ until CO_2 evolution ceased and finally with water. The extract was then dried ($MgSO_4$) and evaporated to yield 2.0 g (80%) of crude product, 6: ^1H NMR, δ 1.28 (t, J ▫ 7 Hz, 3H, -O-CH_2-CH_3), 1.67 (s, 3H, CH_3-olefin), 1.73 (s, 3H, CH_3-olefin), 2.04 (s, 3H, -CO-CH_3), 2.15 (m, 4H, -CH_2-CH_2-), 3.36 (s, 2H, -CO-CH_2-CO-), 4.18 (q, J = 7 Hz, 2H, -O-CH_2-CH_3), 4.46 (s, 2H, -CH_2-O-acetate), 4.63 (d, J = 7 Hz, 2H, >C=CH-CH_2-O-), 5.35 (t, J = 7 Hz, 2H, vinyl H).

Tetrakis(triphenylphosphine)palladium(0)[36]

1.77 g (0.010 mole) of $PdCl_2$ (Alfa), 13.1 g (0.050 mole) of $P(C_6H_5)_3$ (MCB) were mixed with 120 mℓ dimethylsulfoxide (Fisher) in a 250 mℓ two-necked round-bottomed flask. A rubber septum and a nitrogen line were connected to the outlets. The mixture was heated under a N_2 atmosphere until complete solution occurred (~150°C). The bath was taken away and 2.0 g (0.040 mole) of hydrazine hydrate (Mallinckrodt) were added with a hypodermic syringe. The solution was cooled to room temperature and filtered. The precipitate was washed with ethanol (2 X 20 mℓ) and ether (2 X 20 mℓ) and recrystallized from benzene and hexane to yield 5.7 g (50%) of the yellow crystalline product.

Cyclization of ethyl (8-acetoxy-3,7-dimethyl,2-Z,6-E-octadienyl) malonate (6)

To 40 mℓ THF (Fisher, distilled from $LiAlH_4$) containing 1.5 g (4.6 mmoles) of 6, 0.23 g of a 57% oil dispersion (5.5 mmoles) of NaH (Ventron) was slowly added. The resulting red solution was filtered into a dropping funnel.

To 50 mℓ of a refluxing mixture of HMPA and THF (1/1, V/V) containing 1.06 g (0.92 mmoles) of tetrakis(triphenylphosphine)-

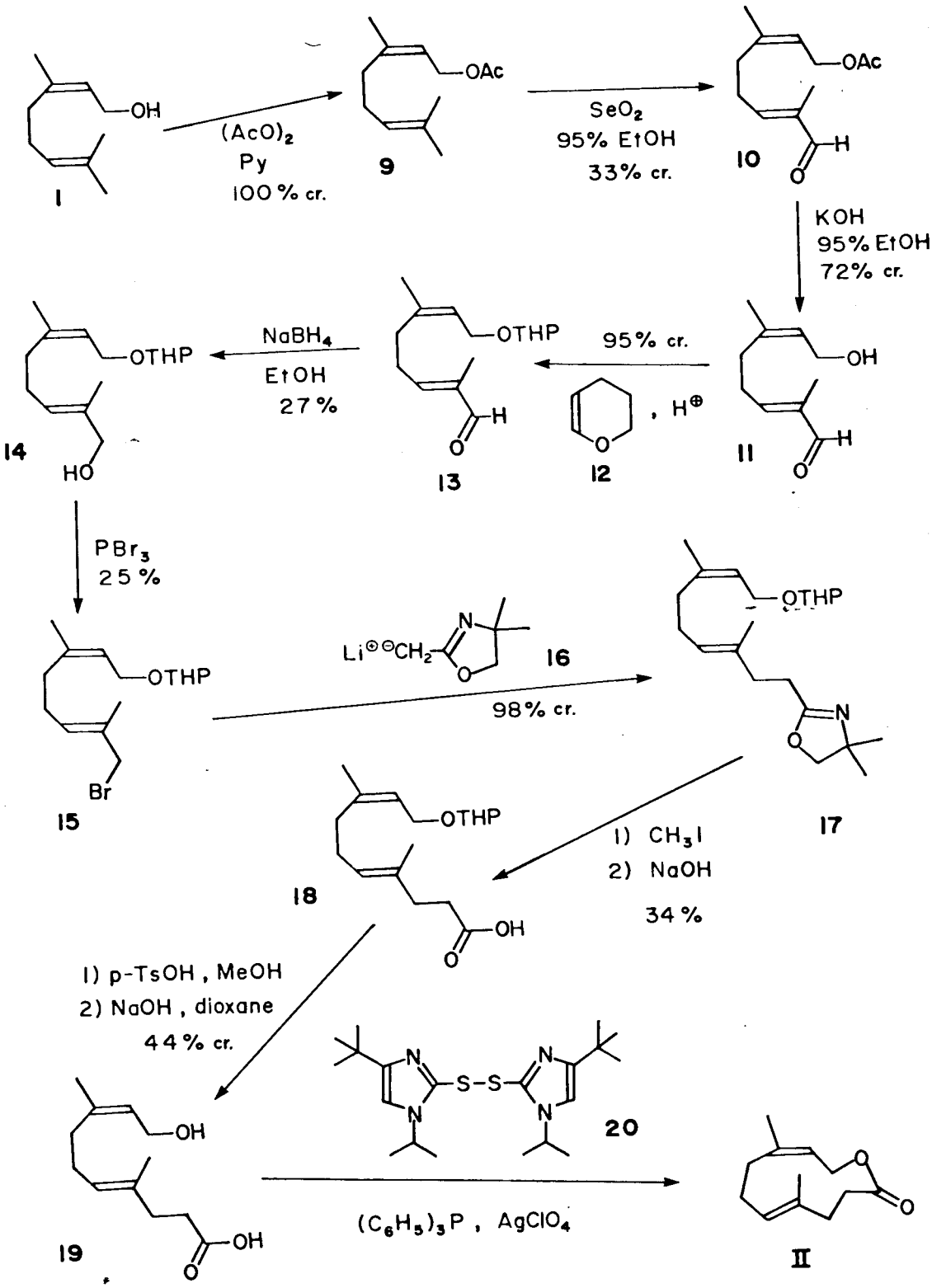
palladium(0) and 0.40 g (0.92 mmole) of 1,2-bis(diphenylphosphine)ethane (Alfa Inorganic) the sodium salt of 6, prepared above, was added slowly over a period of 4 hours. The mixture was left to reflux overnight and was then partitioned between saturated aqueous ammonium chloride and ether. The ether extracts were dried ($MgSO_4$) and evaporated to yield an oil. GLC and GLC-MS analysis showed the presence of at least 5 major compounds including nerol. ^1H NMR analysis indicated that no cyclized product was present.

ii. Ten step synthesis

A second synthetic scheme, which did not depend on Pd catalysts, was designed (Figure 25). Neryl acetate, 9, was oxidized by SeO_2 to yield the E aldehyde, 10. The acetate group was cleaved in alcoholic KOH and replaced with a tetrahydropyranyl (THP) protecting group[31], 13. The aldehyde was then reduced to the alcohol, 14, with $NaBH_4$ which was converted to the bromide[31], 15, using PBr_3. This bromide was alkylated with the lithium salt of 2,4,4-trimethyl-2-oxazoline[37], 16, to yield 17. The methyl iodide salt of this alkylated ozazoline was hydrolyzed in base to yield the carboxylic acid[31], 18. Removal of the THP group[33] yielded the hydroxy acid, 19. Cyclization of this hydroxy acid was achieved by simultaneously activating both hydroxyl and carboxyl functions with 2,2'-bis-(4-t-butyl-N-isopropyl)imidazolyl disulfide (BID)[30,39], 20. This resulted in the formation of a carbon-oxygen bond to give II. This long synthesis resulted in the formation of only a small amount of product suspected to be II. Complete analysis of intermediates and product was not possible. GLC analysis, however, showed that II was not attractant

Figure 25

Synthesis of compound II. Percentages followed by the letters "cr" indicate yields on the crude products.


OH
1
(AcO)2
Py
100 % cr.
OAc
9
SeO2
95% EtOH
33% cr.
OAc
H
O
10
KOH
95% EtOH
72% cr.
OH
H
O
11
95% cr.
, H⊕
O
12
OTHP
H
O
13
NaBH4
EtOH
27 %
OTHP
HO
14
PBr3
25 %
OTHP
Br
15
Li⊕⊖CH2
N
O
16
98% cr.
OTHP
N
O
17
1) CH3I
2) NaOH
34 %
OTHP
OH
O
18
1) p-TsOH , MeOH
2) NaOH , dioxane
44 % cr.
OH
OH
O
19
N
S-S
N
N
N
20
(C6H5)3P , AgClO4
O
O
II

B. The initial assumption that attractant B possessed the E,Z configuration was incorrect. Attractant B must be in the E,E configuration (compound I).

a. experimental

NMR spectra were recorded with a Varian A60 instrument. Chemical shifts are reported in parts per million relative to TMS.

Neryl acetate (9)

15 g (0.10 mole) of nerol (MCB, freshly distilled) were mixed with 25 g acetic anhydride (American Chemical, reagent) and 25 g pyridene (Fisher) in a 100 mℓ round-bottomed flask and stirred overnight at room temperature. The mixture was diluted with 50 mℓ water and extracted with ether (3 X 50 mℓ). The combined ether extracts were washed with water, with 0.3N HCl, with 2% $NaHCO_3$ (until CO_2 evolution ceased) and finally with water. The extracts were dried ($MgSO_4$) and evaporated to yield 20 g (100%) crude product: ^1H NMR, δ 1.62 (s, 3H, $\underline{CH_3}$-olefin), 1.70 (s, 6H, $\underline{CH_3}$-olefin), 2.02 (s, 3H, $\underline{CH_3}$-CO-), 2.10 (m, 4H, -$\underline{CH_2}$-$\underline{CH_2}$-), 4.57 (d, J = 7 Hz, 2H, -$\underline{CH_2}$-O-), 5.10 (m, 1H, $(CH_3)_2C$=$\underline{CH}$-), 5.38 (t, J = 7 Hz, 1H, $\rangle$C=$\underline{CH}$-CH_2-O-).

8-Acetoxy-2,6-dimethyl,2-E,6-Z,octadienol (10)

45 g (0.408 mole) selenium dioxide (Alfa, 99.4%) was added to a solution of 40 g (0.204 mole) of neryl acetate in 400 mℓ 95% ethanol in a 1,000 mℓ flask. The mixture was heated under reflux for 2 hours. The reaction mixture was then poured into 1 liter of water and extracted with ether. The ether was evaporated and the resulting red oil was extracted with pentane (3 X 200 mℓ). The pentane extract was dried ($MgSO_4$) and

evaporated to yield 14 g (33%) of crude aldehyde: ^{1}H δ 1.76 (s, 6H, $\underline{CH_3}$-olefin), 2.02 (s, 3H, $\underline{CH_3}$-CO-), 2.20-2.60 (m, 4H, -$\underline{CH_2}$-$\underline{CH_2}$-), 4.56 (d, 2H, -$\underline{CH_2}$-O-), 5.42 (t, J = 7 Hz, 1H, >C=$\underline{CH}$-CH_2-O-), 6.50 (t, J = 7 Hz, 1H, >C=$\underline{CH}$-CH_2-CH_2-), 9.38 (s, 1H, aldehyde $\underline{H}$).

8-Hydroxy-2,6-dimethyl-2-E,6-Z,octadienal (11)

14 g (0.067 mole) of the aldehyde, 10, were added to a 200 mℓ round-bottom flask containing 50 mℓ of 95% ethanol. To this mixture, which was cooled to -10°C in an ice/salt-bath, were added over 45 minutes 4 g (0.071 mole) of KOH (American Scientific, reagent) in 50 mℓ of 95% ethanol. The mixture was stirred for an additional 30 minutes then was neutralized with dilute HCl. The mixture was diluted with 200 mℓ of water and then extracted with ether (3 X 100 mℓ). The ether extracts were washed with water, dried ($MgSO_4$) and evaporated to give 8.1 g (72%) of the crude product, 11: ^{1}H NMR, δ 1.74 (s, 6H, $\underline{CH_3}$-), 2.20-2.60 (m, 4H, -$\underline{CH_2}$-$\underline{CH_2}$-), 3.38 (s, 1H, D_2O exchangeable, -O$\underline{H}$), 4.13 (d, J = 7 Hz, 2H, -$\underline{CH_2}$-O-), 5.46 (t, J = 7 Hz, 1H, >C=$\underline{CH}$-CH_2-O-), 6.52 (t, J = 7 Hz, 1H, >C=$\underline{CH}$-CH_2-CH_2-), 9.40 (s, 1H, aldehyde $\underline{H}$).

8-Tetrahydropyranyloxy-2,6-dimethyl-2-E,6-Z,octadienal (13)

8.0 g (0.048 mole) of the alcohol prepared above, 11, and 10 g (0.12 mole) of dihydropyran (12, Aldrich, freshly distilled) were mixed in a 50 mℓ round-bottom flask. 4 drops of conc. HCl were added and the mixture was stirred overnight at room temperature. Ether was added and the mixture was shaken with 10% sodium hydroxide solution. The ether layer was dried ($MgSO_4$) and evaporated to yield 120 g (95%) of the crude product, 13: ^{1}H NMR, δ 1.40-1.80 (m, 12H, $\underline{CH_3}$-, -($\underline{CH_2}$)$_3$-CH_2-O-), 2.20-2.60 (m, 4H, olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 3.40-4.20 (m, 4H, -$\underline{CH_2}$-O-), 4.60 (s, 1H,

methine H), 5.44 (t, J = 7 Hz, 1H, >C=CH-CH_2-O-), 6.48 (t, J = 7 Hz, 1H, >C=CH-CH_2-CH_2-), 9.40 (s, 1H, aldehyde H).

8-Tetrahydropyranyloxy-2,6-dimethyl,2-E,6-Z,octadienol (14)

12.0 g (0.047 mole) of the crude andehyde, 13, were mixed with 100 mℓ ethanol in a 200 mℓ round-bottom flask. The solution was cooled in an ice-bath and 1.3 g (0.017 mole) of $NaBH_4$ (MCB) was slowly added. After stirring for 2 hours, the reaction mixture was poured into water and stirred for one half hour. The solution was then extracted with ether (3 X 100 mℓ). The ether extracts were dried ($MgSO_4$) and evaporated to yield 8.2 g of an oil. This oil was purified by column chromatography (600 g silica-gel, hexanes:ether, 3:2) to yield 3.2 g (27%) of the alcohol, 14: ^{1}H HMR, δ 1.66 (s, 9H, CH_3-, -$(CH_2)_3$-CH_2-O-), 1.72 (s, 3H, CH_3-), 2.12 (d, J = 4 Hz, 4H, olefin-CH_2-CH_2-olefin), 2.90 (s, 1H, D_2O exchangeable, -O-H), 3.30-4.20 (m, 6H, -CH_2-O-), 4.60 (s, 1H, methine H), 5.38 (t, J = 7 Hz, 2H, vinyl H).

8-Tetrahydropyranyloxy-2,6-dimethyl,2-E,6-Z,octadienyl bromide (15)

To a solution of 1.5 g (5.9 mmoles) of the alcohol, 14, in 8 mℓ ether and 8 mℓ HMPA (Aldrich) in a 50 mℓ round-bottom flask were added with stirring and ice-cooling 3.2 mℓ of 2.2 M (7.0 mmoles) n-BuLi in hexane (Alfa). 1.2 g (13 mmoles) p-TsCl (Eastman, recrystallized from pentane) were added portionwise to the stirred solution. Then 14 g (16 mmoles) LiBr (Matheson, Coleman and Bell, Anhydrous Reagent) were added and the solution was stirred overnight at room temperature. The solution was then poured into 20 mℓ water and extracted with ether (3 X 50 mℓ). The ether extract was washed with water and brine, dried ($MgSO_4$)

and concentrated *in vacuo* to yield an oil. This oil was flash chromatographed (ethyl acetate/hexanes; 6:94 v/v) to yield 0.55 g (25%) of the bromide: ^{1}H NMR δ 1.66 (m, 12H, $\underline{CH_3}$-, -($\underline{CH_2}$)$_3$-CH_2-O-), 2.13 (d, J ▫ 4 Hz, 4H, olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 3.30-4.20 (m, 6H, -$\underline{CH_2}$-O-, -$\underline{CH_2}$-Br), 4.58 (s, 1H, methine H), 5.33 (m, 2H, vinyl H).

2,4,4-Trimethyl-2-oxazoline

25.1 g (0.28 mole) of 2-amino-2-methyl-1-propanol (Sigma, freshly distilled) were added to a 100 mℓ round-bottom flask equipped with a reflux condenser. 33.8 g (0.56 mole) glacial acetic acid were slowly added. This mixture was magnetically stirred and heated until the reflux temperature dropped from a high of ~120°C to 110°C. The mixture was distilled azeotropically at 98°-130°C through a 15 cm Vigreaux column into 200 mℓ pentane. The pentane layer was separated and the water layer was extracted several times with pentane. The combined pentane extracts were dried ($MgSO_4$) and evaporated to yield an oil. This oil was distilled (75°C) to yield 22.0 g (70%) product: ^{1}H NMR δ 1.2 (s, 6H, ($\underline{CH_3}$)$_2$C), 1.90 (s, 3H, vinyl $\underline{CH_3}$), 3.85 (s, 2H, -$\underline{CH_2}$-O-).

2-(9-Tetrahydropyranyloxy-3,7-dimethyl,3-E,7-Z,nonadienyl) 4,4-dimethyl-2-oxazoline (17)

0.21 g (1.8 mmoles) of 2,4,4-trimethyl-2-oxazoline was magnetically stirred with 10 mℓ THF (American Scientific, distilled from $LiAlH_4$) at -78°C (dry ice-acetone bath) under a N_2 atmosphere. 0.91 mℓ of 2.2 M (2.0 mmoles) n-BuLi in hexane (Alfa) was slowly added with a syringe through a rubber septum and the resulting solution was stirred for 30 minutes. 0.54 g (1.7 mmoles) of the bromide, 15, was slowly added and the resulting mixture was stirred for another 30 minutes. After slowly

warming to room temperature, the solution was poured into 50 mℓ of saturated NaCl solution and extracted with ether (3 X 50 mℓ). The ether extract was dried ($MgSO_4$) and concentrated to give 0.59 g (98%) of crude product: ^{1}H NMR δ 1.23 (s, 6H, $(\underline{CH_3})_2C<$), 1.50-1.75 (m, 12H, vinyl $\underline{CH_3}$, $-(\underline{CH_2})_3-CH_2-O-$), 2.08 (d, J = 7 Hz, 4H, C-5'$\underline{CH_2}$, C-6'$\underline{CH_2}$), 2.30 (s, 4H, C-1'$\underline{CH_2}$, C-2'$\underline{CH_2}$), 3.30-4.25 (m, 6H, $-\underline{CH_2}-O-$), 4.57 (s, 1H, methine $\underline{H}$), 5.05-5.55 (m, 2H, vinyl $\underline{H}$).

10-Tetrahydropyranyloxy-4,8-dimethyl,4-E,8-Z,decadienoic acid (18)

0.594 g (1.70 mmoles) of the oxazoline prepared above was converted to the methyl iodide salt by stirring in excess methyl iodide (Matheson, Coleman and Bell) overnight at room temperature. After the volatiles were evaporated in vacuo, the crude salt was added to 5 mℓ 1N NaOH and stirred for 15 hr at room temperature. The solution was acidified to pH 2 with 10% HCl and extracted with ether (3 X 30 mℓ). The extracts were dried ($MgSO_4$) and concentrated in vacuo to leave 0.514 g of crude product. This oil was purified by preparative TLC (silica gel, ethyl acetate:hexanes, 1:1 v/v with 1% acetic acid, Rf 0.45 to 0.75) to yield 0.173 g (34%) product: ^{1}H NMR δ 1.66 (m, 12H, $\underline{CH_3}$-, $-(\underline{CH_2})_3-CH_2-O-$), 2.05 (s, 4H, olefin-$\underline{CH_2}-\underline{CH_2}$-olefin), 2.35 (s, 4H, HOOC-$\underline{CH_2}-\underline{CH_2}$-), 3.30-4.30 (m,4H, $-\underline{CH_2}-O-$), 4.63 (s, 1H, methine $\underline{H}$), 5.05-5.55 (m, 2H, vinyl $\underline{H}$), 9.10 (s, 1H, exchangeable with D_2O, -COO$\underline{H}$).

10-Hydroxy-4,8-dimethyl,4-E,8-Z,decadienoic acid (19)

0.173 g (0.580 mmole) of the acid, 18, was added to a solution of 0.0044 g (0.023 mmole) p-TsOH (Eastman) in 5 mℓ of methanol. The solution was poured into 20 mℓ of water and

extracted with ether (3 X 50 mℓ). The ether extracts were dried ($MgSO_4$) and evaporated. This reaction product was then stirred overnight with 2 mℓ 1,4-dioxane (Fisher) and 3 mℓ 4% NaOH in a 10 mℓ round bottomed flask. The solution was acidified to pH 2 with HCl, poured into 20 mℓ of water and extracted with ether (3 X 50 mℓ). The ether extracts were dried ($MgSO_4$) and evaporated to yield 0.090 g of the crude product. ^{1}H NMR analysis showed that ~60% of this product was the acid-alcohol and 40% was the methyl ester-alcohol: ^{1}H NMR δ 1.67 (m, 6H, $\underline{CH_3}$-), 2.10 (m, 4H, olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 2.36 (m, 4H, HOOC-$\underline{CH_2}$-$\underline{CH_2}$-), 3.72 (s, $\underline{CH_3}$-O-CO-), 4.12 (d, J = 7 Hz, 2H, -$\underline{CH_2}$-O-), 5.00-5.50 (m, 2H, vinyl $\underline{H}$), 6.52 (s, -O-$\underline{H}$).

10-Hydroxy-4,8-dimethyl,4-E,8-Z,decadienoic acid lactone (II)

0.090 g (~0.25 mmole) of the crude acid-alcohol prepared above, 19, 0.198 g (0.44 mmole) of 2,2'-bis-(4-t-butyl-N-isopropyl) imidazolyl disulfide (20, courtesy of Dr. A.C. Oehlschlager[29]), 0.130 g (0.44 mmole) triphenylphosphine Matheson, Coleman and Bell, recrystallized) and 0.120 g (0.44 mmole) silver perchlorate (Alfa) were dissolved in 5 mℓ toluene (Fisher) in a 50 mℓ round bottom flask. The solution was purged with nitrogen and stirred overnight at 4°C under a N_2 atmosphere. After the solution was siluted with 30 mℓ dry toluene, a reflux condenser was attached to the flask and the solution was refluxed under a nitrogen atmosphere for 5 hr. A one milliter aliquot was then filtered through silica gel and eluted with 3 mℓ CH_2Cl_2. GLC analysis of this solution showed that the product formed was not attractant B, but instead had the same retention time as the second isomer produced in the Oehlschlager[29] synthesis.

C. Synthesis from geraniol

i. Seven-step synthesis

A seven-step synthesis of attractant B starting from geroniol was undertaken (Figure 26). The alcohol was first protected by forming the tetrahydropyranyl ether[40], 22. The isopropylidene terminus of this compound was stereospecifically oxidized with SeO_2 to yield the E alcohol[41], 23. Stork's method[42] was used for the conversion of the alcohol into the corresponding bromide, 24. This bromide was alkylated with the lithium salt of 2,4,4-trimethyl-2-oxazoline, 16, to yield 25. The methyl iodide salt of this alkylated oxazoline was hydrolyzed in base to yield the carboxylic acid, 26. Removal of the protecting group[43] yielded the hydroxy acid, 27. This compound was cyclized using BID to yield I in 0.17% overall yield. The ^1H NMR spectrum, mass spectra, infrared spectrum and GLC retention time of the synthetic compound proved identical to those for the natural compound (attractant B).

Bioassay results comparing the attraction of pentane solutions of pure natural attractant B, pure synthetic attractant B and Porapak Q-entrapped volatiles from frass are shown in Table XV. The synthetic compound was attractive for C. ferrugineus proving that I is the structure of attractant B.

a. experimental

NMR spectra were recorded with Varian XL-100 and EM-360 instruments in $CDCl_3$ using tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million relative to TMS. Unit resolution and chemical ionization mass spectra were measured on a Hewlett-Packard Model 5985

Figure 26

Synthesis of rusty grain beetle attractant B (I).

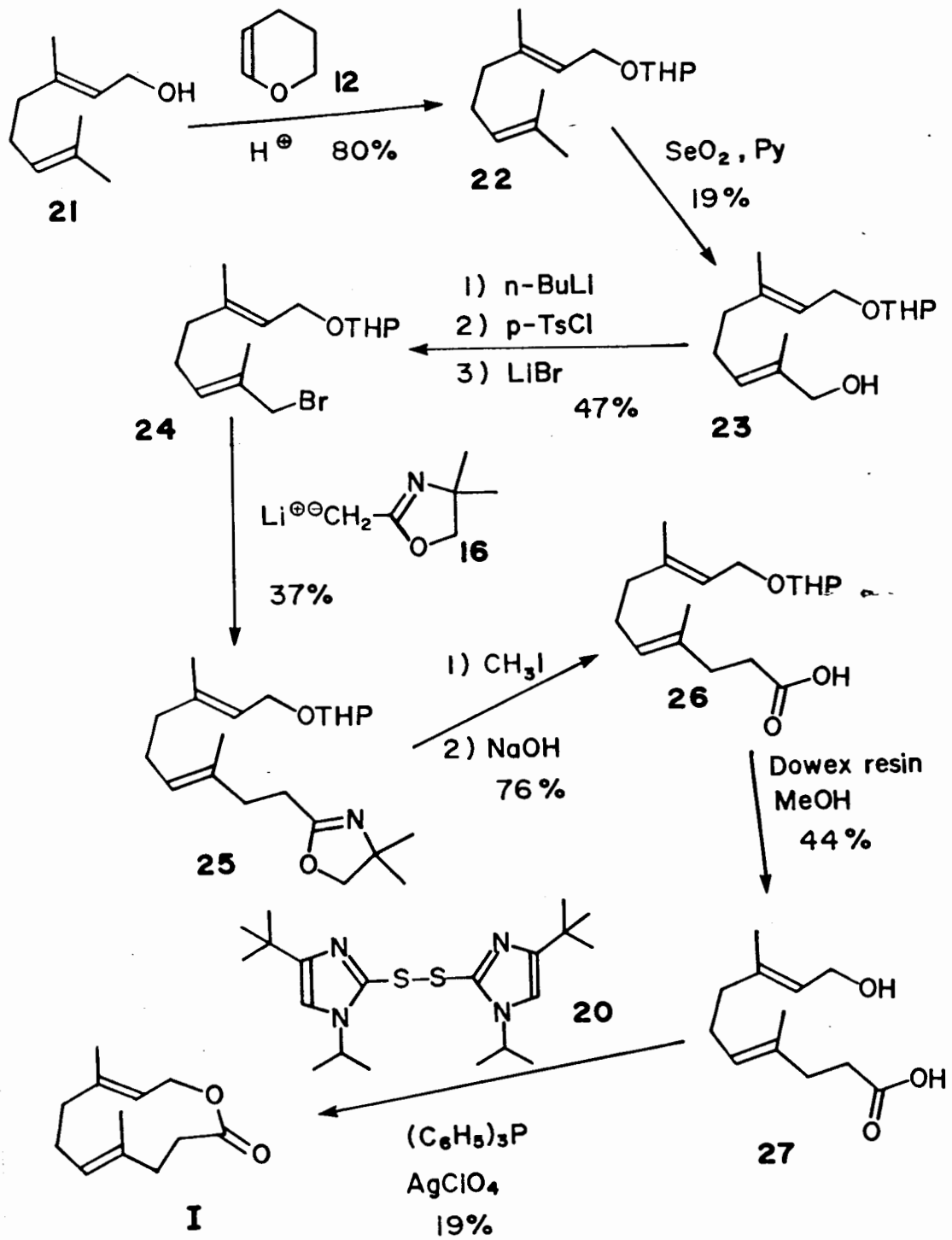

OH
12
H⊕ 80%
21
OTHP
22
SeO2, Py
19%
OTHP
OH
23
1) n-BuLi
2) p-TsCl
3) LiBr
47%
OTHP
Br
24
Li⊕⊖CH2
N
O
16
37%
OTHP
25
1) CH3I
2) NaOH
76%
OTHP
OH
O
26
Dowex resin
MeOH
44%
S-S
20
OH
OH
O
27
O
O
I
(C6H5)3P
AgClO4
19%

TABLE XV. Response of C. ferrugineus of mixed age and sexes to pentane solutions of natural and synthetic attractant B and to pentane extracts of Porapak Q-entrapped volatiles from rusty grain beetle frass. Experiment 25.

Stimuli	Concentration	% Response*
Pentane extract of Porapak Q-entrapped volatiles from 160 g beetle frass for 312 hr.	125 ghr†	6.9 a
	15 ghr	38.4 d
Natural attractant B in pentane.	125 ghr equiv‡	20.4 c
	15 ghr equiv	35.6 d
	1.2 ghr equiv	17.4 bc
Synthetic attractant B in pentane.	125 ghr equiv	9.0 ab
	15 ghr equiv	27.2 cd
	1.2 ghr equiv	18.4 bc
Pentane control, 0.025 mℓ.		8.0 a

* Percentages followed by the same letter not significantly different, χ^2, $P < 0.05$.

† One gram hour (ghr) = the volatiles from 1 g of material in 1 hr.

‡ One gram hour equivalent (ghr equiv) of a compound ▫ the amount in gram hours that this concentration of compound is equivalent to.

instrument. High resolution mass spectra were run by Dr. G. Eigendorf† using a DS-50 mass spectrometry data system. Infrared spectrum (IR) were obtained with neat samples on NaCl plates on a Perkin-Elmer 599B instrument. Chemical ionization mass spectral data and ^{13}C NMR data are reported in Table XVI and Figure 27, respectively. Flash chromatography was conducted using essentially the method of Still et al.[44].

Geranyl tetrahydropyranyl ether (22)

Geraniol (Matheson, Coleman and Bell, technical) was distilled on a 55 cm spinning band column with a reflux ratio of 10:1. The fraction distilling at 125-130°C (13-14 mm Hg) was collected. 25.0 g (0.162 mole) of geraniol and 13.7 g (0.162 mole) dihydropyran (Aldrich, freshly distilled) were mixed in a 100 mℓ round bottom flask equipped with a calcium chloride drying tube. 10 drops of concentrated HCl were added to the flask and the solution was magnetically stirred overnight. Ether (Fisher) was added and the solution was shaken with a 10% sodium hydroxide solution. The ether layer was dried ($MgSO_4$) and evaporated to yield a yellow oil. This oil was fractionated (104-105°C, 0.3 mm Hg) to yield 30.6 g (80%) of product: ^1H NMR δ 1.60 (m, 15H, $\underline{CH_3}$-, -($\underline{CH_2}$)$_3$-CH_2-O-), 2.00 (s, 4H, olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 3.2-4.2 (m, 4H, -$\underline{CH_2}$-O-), 4.57 (s, 1H, methine $\underline{H}$), 5.05 (br.s, 1H, $(CH_3)_2C{=}C\underline{H}$-), 5.30 (t, J = 7 Hz, 1H, $\rangle C{=}C\underline{H}$-$CH_2$-O-); IR 2950(s), 1450(m), 1390(m), 1210(m), 1150(m), 1035(s), 920(m), 880(m), 830(m) cm^{-1}; MS (rel. intensities) m/z 238 (M^+, 0.1%), 93 ($C_7H_9^+$,

† Department of Chemistry, University of British Columbia, Vancouver, B.C.

TABLE XVI. Selected ions in the chemical ionization mass spectra of compounds in the synthesis of attractant B. Isobutane reagent gas.

Compound	Ion	m/z	% Rel. Ab.
geraniol (21)	$[M + H - H_2O]^+$	137	100
geranyl tetrahydropyranyl ether (22)	$[M + H]^+$	239	100
8-tetrahydropyranyloxy-2,6-dimethyl-2-E,6-E,octadien-1-ol (23)	$[M + H - H_2O]^+$	237	63
	$[M + H]^+$	255	35
8-tetrahydropyranyloxy-2,6-dimethyl-2-E,6-E,octadienyl-bromide (24)	$[M + H]^+$	317	21
		319	15
2-(9-tetrahydropyranyloxy-3,7-dimethyl,3-E,7-E,nonadienyl)-4,4-dimethyl-2-oxazoline (25)	$[M + H]^+$	350	100
10-tetrahydropyranyloxy-4,8-dimethyl,4-E,8-E,decadienoic acid (26)	$[M + H - H_2O]^+$	279	100
	$[M + H]^+$	297	43

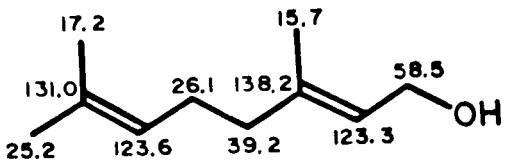
TABLE XVI (Cont'd)

Compound	Ion	m/z	% Rel. Ab.
10-hydroxy-4,8-dimethyl,4-E, 8-E,decadienoic acid (27) I	$[M + H - H_2O]^+$	195	100
	$[M + H]^+$	195	100

Figure 27

^{13}C chemical shifts of compounds in the synthesis of attractant B (I). Shifts are in ppm relative to TMS.

★ indicates ambiguous assignments.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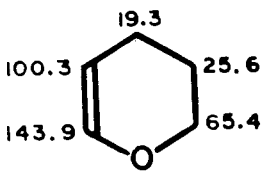
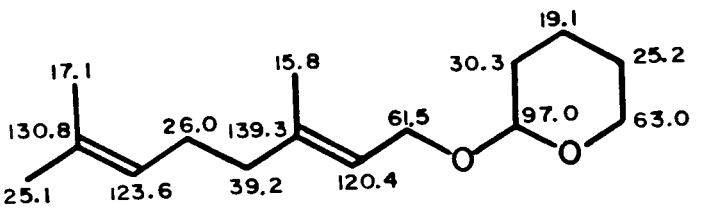
15.7
131.0
26.1
138.2
58.5
OH
25.2
123.6
39.2
123.3

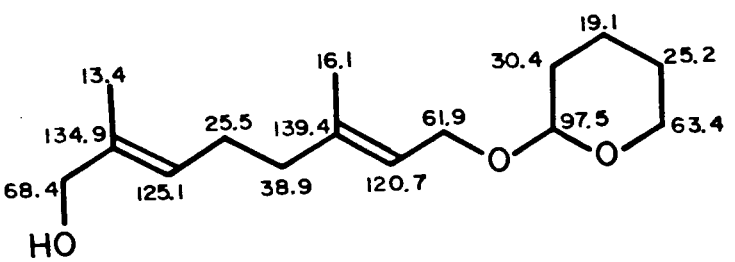
21


19.3
100.3
25.6
143.9
65.4
O

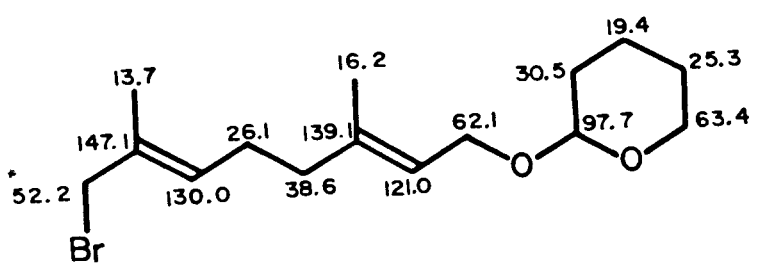
12


19.1
17.1
15.8
30.3
25.2
130.8
26.0
139.3
61.5
97.0
63.0
O
O
25.1
123.6
39.2
120.4

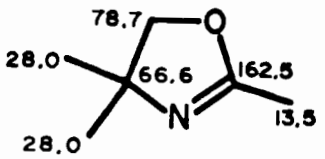
22


19.1
13.4
16.1
30.4
25.2
134.9
25.5
139.4
61.9
97.5
63.4
O
O
68.4
125.1
38.9
120.7
HO

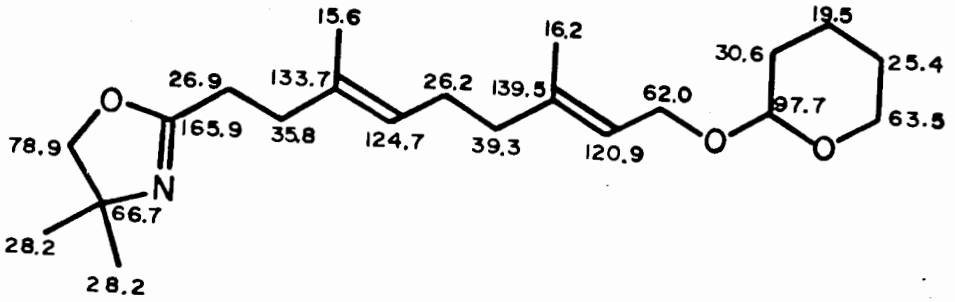
23


19.4
13.7
16.2
30.5
25.3
147.1
26.1
139.1
62.1
97.7
63.4
O
O
52.2
130.0
38.6
121.0
Br

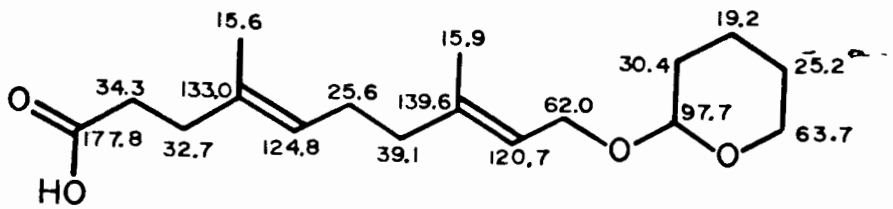
24


78.7
O
28.0
66.6
162.5
N
13.5
28.0

16


15.6
16.2
19.5
30.6
25.4
26.9
133.7
26.2
139.5
62.0
97.7
63.5
O
165.9
35.8
124.7
39.3
120.9
O
O
78.9
N
66.7
28.2
28.2

25


15.6
15.9
19.2
30.4
25.2
34.3
133.0
25.6
139.6
62.0
97.7
63.7
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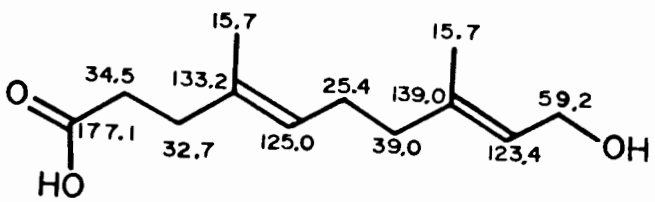
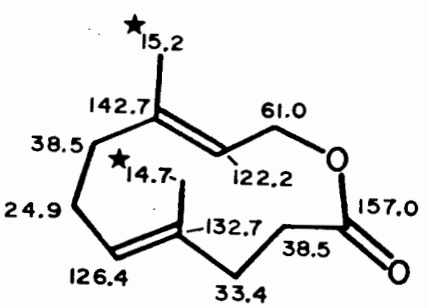
32.7
124.8
39.1
120.7
O
O
HO

26


15.7
15.7
34.5
133.2
25.4
139.0
59.2
O
177.1
32.7
125.0
39.0
123.4
OH
HO

27


15.2
142.7
61.0
38.5
14.7
122.2
O
24.9
132.7
157.0
38.5
126.4
33.4
O

I

238 (M^+, 0.1%), 93 ($C_7H_9^+$, 10.5%), 85 ($C_5H_9O^+$, 100%), 81 ($C_6H_9^+$, 14.5%), 69 ($C_5H_9^+$, 45.6%), 67 ($C_5H_7^+$, 15.7%); high resolution mass spectrum, calculated for $C_{15}H_{26}O_2$ 238.1933, found 238.1933.

8-Tetrahydropyranyloxy-2,6-dimethyl,2-E,6-E octadien-1-ol (23)

10.9 g (0.100 mole) selinium dioxide (Alfa, 99.4%) were added to a solution of 22 (28.6 g, 0.120 mole) in 350 mℓ of 95% EtOH in a 500 mℓ round bottom flask. 14.0 g (0.240 mole) pyridine (Fisher certified) were added and the mixture was heated under reflux for 4 hr. The solvent was evaporated *in vacuo* and the residue was extracted with ether (3 X 50 mℓ). The combined extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated to give an oil (16.9 g). This oil was flash chromatographed (ethyl acetate/hexanes: 2/3, v/v) to yield 6.9 g of a yellow oil. This oil was distilled (145-160°C, ~0.3 mm Hg) to yield 5.7 g (19%) of a colourless oil: ^{1}H NMR δ 1.63 (m, 12H, $\underline{CH_3}$-, -($\underline{CH_2}$)$_3$-CH_2-O-), 2.07 (s, 4H, olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 2.70 (br.s, 1H, D_2O exchangeable, -O-$\underline{H}$), 3.20-4.25 (m, 6H, -$\underline{CH_2}$-O-), 4.57 (br.s, 1H, methine $\underline{H}$), 5.30 (t, J = 7 Hz, 2H, vinyl $\underline{H}$); IR 3420(br.m), 2930(s), 1440(m), 1380(m), 1200(w), 1115(m), 1020(m), 905(m), 730(m) cm^{-1}; MS (rel. intensities) m/z 254 (M^+, 0.1%), 93 ($C_7H_9^+$, 9.5%), 85 ($C_5H_9O^+$, 100%), 81 ($C_6H_9^+$, 13.8%); high resolution mass spectrum, calculated for $C_{15}H_{26}O_3$ 254.1882, found 254.1889.

8-Tetrahydropyranyloxy-2,6-dimethyl,2-E,6-E,octadienyl bromide (24)

To a solution of 5.5 g (0.0217 mole) of the alcohol, 23, in 17 mℓ HMPA (Aldrich) in a 100 mℓ round bottom flask were added,

with stirring and ice-cooling under a N_2 atmosphere, 9.8 mℓ of 2.2 M (0.0220 mole) n-BuLi in hexane (Alfa). 4.3 g (0.050 mole) p-TsCl (Eastman, recrystallized from pentane) were added portionwise to the stirred solution. Then 5.0 g (0.058 mole) LiBr (Matheson, Coleman and Bell, Anhydrous Reagent) were added and the solution was stirred overnight at room temperature. The solution was then poured into 50 mℓ water and extracted with ether (3 X 75 mℓ). The ether extract was washed with water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo to yield 6.5 g of oil. This oil was flash chromatographed (ethyl acetate/hexanes:6:94 v/v) to yield 3.2 g (47%) of product: ^{1}H NMR δ 1.67 (m, 12H, $\underline{CH_3}$-, -$(\underline{CH_2})_3$-CH_2-O-), 2.07 (s, 4H, olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 3.20-4.25 (m, 6H, -$\underline{CH_2}$-Br, -$\underline{CH_2}$-O-), 4.60 (br.s, 1H, methine $\underline{H}$), 5.35 (m, 2H, vinyl $\underline{H}$); IR 2946(s), 1450(m), 1390(m), 1270(m), 1210(m), 1125(s), 1035(s), 920(m), 880(m), 830(m), 770(m), 700(m) cm^{-1}; MS (rel. intensities) m/z 237 ($C_{15}H_{25}O_2^+$, 0.4%), 135 ($C_{10}H_{15}^+$, 7.3%), 85 ($C_5H_9O^+$, 100%), 81 ($C_6H_9^+$, 13.5%), 67 ($C_5H_7^+$, 25.3%); high resolution mass spectrum, calculated for $C_{15}H_{25}O_2{}^{79}Br$ 316.1038, found 316.1062.

2-(9-Tetrahydropyranyloxy-3,7-dimethyl,3-E,7-E nonadienyl)-4,4-dimethyl-2-oxazoline (25)

0.80 g (7.1 mmoles) of 2,4,4-trimethyl-2-oxazoline was magnetically stirred with 10 mℓ THF (American Scientific, distilled from $LiAlH_4$) at -78°C (dry ice-acetone bath) under a N_2 atmosphere. 3.7 mℓ of 2.2 M (8.1 mmoles) n-BuLi in hexane (Alfa) were slowly added with a syringe through a rubber septum and the solution was stirred for 30 minutes. 3.0 g (6.3 mmoles) of the bromide, 24, were slowly added and the resulting mixture was stirred for

another 30 minutes. After slowly warming to room temperature, the solution was poured into 50 mℓ of saturated NaCl solution and extracted with ether (3 X 50 mℓ). The ether extract was dried ($MgSO_4$) and concentrated to give 2.1 g of crude product. This oil was flash chromatographed (ethyl acetate:hexanes, 1:1 v/v) to give 0.85 g (37%) product: ^1H NMR δ 1.25 (s, 6H, $(\underline{CH_3})_2C<$), 1.63 (br.s, 12H, vinyl $\underline{CH_3}$, $-(\underline{CH_2})_3-CH_2-O-$), 2.05 (s, 4H, C-5'$\underline{CH_2}$, C-6'$\underline{CH_2}$), 2.32 (s, 4H, C-1'$\underline{CH_2}$, C-2'$\underline{CH_2}$), 3.20-4.30 (m, 6H, $-\underline{CH_2}-O-$), 4.63 (br.s, 1H, methine $\underline{H}$), 5.00-5.50 (m, 2H, vinyl $\underline{H}$); IR 2930(s), 1668(s), 1450(m), 1390(m), 1365(m), 1200(m), 1120(m), 1025(s) cm^{-1}; MS (rel. intensities) m/z 349 (M^+, 0.8%), 264 ($C_{16}H_{26}NO_2^+$, 63.0%), 248 ($C_{17}H_{12}O_2^+$, 59.4%), 180 ($C_{11}H_{18}NO^+$, 100%), 113 ($C_6H_{11}NO^+$, 75.0%), 85 ($C_5H_9O^+$, 78.9%), 67 ($C_5H_7^+$, 40.5%); high resolution mass spectrum, calculated for $C_{21}H_{35}NO_3$ 349.2616, found 349.2612.

10-Tetrahydropyranyloxy-4,8-dimethyl,4-E,8-E,decadienoic acid (26)

0.768 g (2.20 mmole) of the oxazoline prepared above, 25, was converted to the methiodide salt by stirring in excess methyl iodide (Matheson, Coleman and Bell) overnight at room temperature. After the volatiles were evaporated in vacuo, the crude salt was added to 5 mℓ 1N NaOH and stirred for 20 hrs at room temperature. The solution was acidified to pH 2 with 10% HCl and extracted with ether (3 X 30 mℓ). The extracts were dried ($MgSO_4$) and concentrated in vacuo to leave 0.564 g of crude product. This oil was purified by preparative TLC (silica gel, ethyl acetate:hexane, 1:1 v/v with 1% acetic acid) to yield 0.498 g (76%) product: ^1H NMR δ 1.63 (br.s, 12H, $\underline{CH_3}$-, $-(\underline{CH_2})_3-CH_2-O-$), 2.05 (s, 4H,

olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 2.35 (s, 4H, HOOC-$\underline{CH_2}$-$\underline{CH_2}$-), 3.30-4.30 (m, 4H, -$\underline{CH_2}$-O-), 4.63 (br.s, 1H, methine $\underline{H}$), 4.90-5.50 (m, 2H, vinyl $\underline{H}$), 8.75 (s, 1H, D_2O exchangeable, -O-$\underline{H}$); IR 3000(br.m), 2920(s), 1700(s), 1010(m), 905(m), 730(s) cm^{-1}; MS (rel. intensities) m/z 296 (M^+, 0.1%), 154 ($C_9H_{14}O_2^+$, 12.8%), 127 ($C_7H_{11}O_2^+$, 12.8%), 101 ($C_5H_9O_2^+$, 18%), 85 ($C_5H_9O^+$, 100%), 68 ($C_5H_8^+$, 15.2%), 67 ($C_5H_7^+$, 27.6%); high resolution mass spectrum, calculated for $C_{17}H_{28}O_4$ 296.1988, found 296.1986.

10-Hydroxy-4,8-dimethyl,4-E,8-E,decadienoic acid (27)

Dowex-50W-X8 resin (Baker) was activated by washing the resin in a chromatography column with 6 M HCl. Excess acid was washed from the column with distilled water until the washings showed no reaction with silver nitrate. The resin was then washed with methanol.

0.459 g (1.55 mmoles) of the acid, 26, was added to 5 mℓ of methanol containing 0.75 g of the activated resin. The heterogeneous mixture was stirred at room temperature for 1 hr. The mixture was then filtered, added to 30 mℓ of distilled water and extracted with ether (3 X 25 mℓ). The ether layer was dried ($MgSO_4$) and evaporated to yield an oil. This oil was purified by preparative TLC (silica gel, ethyl acetate:hexanes, 1:1 v/v with 1% acetic acid) to yield 0.146 g (44%) product: ^1H NMR δ 1.62 (s, 6H, $\underline{CH_3}$-), 2.08 (s, 4H, olefin-$\underline{CH_2}$-$\underline{CH_2}$-olefin), 2.34 (s, 4H, HOOC-$\underline{CH_2}$-$\underline{CH_2}$-), 4.12 (m, 2H, -$\underline{CH_2}$-O-), 4.90-5.50 (m, 2H, vinyl $\underline{H}$), 6.18 (s, 2H, D_2O exchangeable, -O-$\underline{H}$); IR 3400(br.m), 2930(s), 1710(s), 1440(m), 1385(m), 1165(m), 995(m) cm^{-1}; MS (rel. intensities) m/z 212 (M^+, 1.4%), 194 ($C_{12}H_{18}O_2^+$, 12.3%), 109 ($C_7H_9O^+$, 32.9%), 99 ($C_5H_7O_2^+$, 32.2%), 85 ($C_5H_9O^+$, 100%),

81 ($C_6H_9^+$, 85.6%), 68 ($C_5H_8^+$, 60.7%), 67 ($C_5H_7^+$, 65.5%), 55 ($C_4H_7^+$, 43.8%), 43 ($C_2H_3O^+$, 92.7%), 41 ($C_3H_5^+$, 83.4%); high resolution mass spectrum, calculated for $C_{12}H_{20}O_3$ 212.1412, found 212.1418.

10-Hydroxy-4,8-dimethyl,4-E,8-E,decadienoic acid lactone (I)

0.092 g (0.437 mmoles) of the hydroxy acid, 27, 0.494 g (1.12 mmoles) of 2,2'-bis-(4-t-butyl-N-isopropyl) imidazolyl disulfide, 20, and 0.294 g (1.12 mmoles) triphenylphosphine (Matheson, Coleman and Bell, recrystallized) were dissolved in 6 mℓ benzene (Fisher, distilled from CaH_2) in a 50 mℓ round bottom flask. The solution was purged with nitrogen and stirred overnight at 4°C under a nitrogen atmosphere. After the solution was diluted with ~30 mℓ dry benzene, 0.232 g (1.12 mmoles) silver perchlorate (Alfa) was added. The flask was equipped with a reflux condenser and the solution was refluxed under a nitrogen atmosphere for 5 hr. The mixture was then filtered through silica gel and eluted with 50 mℓ CH_2Cl_2. After the solution was concentrated, preparative GLC yielded 16 mg (19%) product. The ^1H NMR spectrum, mass spectra, infrared spectrum and GLC retention time of the synthetic compound proved identical to those for the natural compound (attractant B). High resolution mass spectrum, calculated for $C_{12}H_{18}O_2$ 194.1306, found 194.1307.

D. Summary

Preliminary investigations[29] indicated that of the four possibilities attractant B was either isomer I or II. The double bond δ to the ester oxygen was in the E configuration. The geometry of the other bond was assumed to be in the Z configuration (II) because of GLC analysis. The first synthesis of II

from nerol was unsuccessful because the palladium catalyzed cyclization failed. A second synthesis of II from nerol, using BID as an activator for cyclization, resulted in the formation of a small amount of product. Because of the small amount of compound produced, spectral analysis was incomplete. GLC analysis, however, showed that this product was not attractant B. The initial assumption that attractant B possessed the E,Z configuration was incorrect. A seven-step synthesis of the E,E isomer was successfully conducted to yield I in 0.17% overall yield. The ^{1}H NMR spectrum, mass spectra, infrared spectrum and GLC retention time of the synthetic compound proved identical to those for the natural compound (attractant B). Bioassay results showed that the synthetic compound was attractive for C. ferrugineus, showing that compound I is attractant B.

VI. CONCLUSIONS

Porapak Q has been demonstrated to be an effective adsorbent in which to capture C. ferrugineus produced attractants and attractants produced by fungus-infected wheat. Solvent extraction techniques were shown to be not as good as the vapour entrapment method of capturing attractants for C. ferrugineus.

The construction of an effluent splitter and a thermal gradient collector lead to the isolation by preparative GLC of four compounds from a pentane extract of Porapak Q-entrapped volatiles from C. ferrugineus produced frass which were attractive for C. ferrugineus. Three of these compounds were produced only by male C. ferrugineus. This observation and the result of the bioassay of extracts of volatiles produced by sexed beetles show that C. ferrugineus utilizes an aggregation pheromone produced by the males.

The quantitative composition of extracts varied from batch to batch, however three attractants (A, B and C) were present in all extracts and comprised 20-40% of the total volatiles present. Compound B was always present in the largest amount, approximately 200 µg per aeration of 160 g frass.

Spectral analysis of these three attractants, A, B and C, showed that these compounds have molecular compositions of $C_{12}H_{20}O_2$, $C_{12}H_{18}O_2$ and $C_{14}H_{24}O_2$, respectively. Each of these compounds has a strong infrared absorption at approximately 1730 cm^{-1} corresponding to that of an ester or lactone. Compound B was identified as one of four isomers of an 11-membered dimethyl substituted unsaturated lactone.

After two unsuccessful attempts, compound B was synthesized from geraniol in 0.17% overall yield. The ^{1}H NMR spectrum, mass spectra, infrared spectrum and GLC retention time of the synthetic compound proved identical to those for the natural compound. Bioassay results showed that the synthetic compound was attractive for C. ferrugineus, proving that attractant B is 10-hydroxy-4,8-dimethyl,4-E,8-E,decadienoic acid lactone(I).

Identification of the other rusty grain beetle produced compounds which are attractive for C. ferrugineus, followed by rational syntheses and comparison with the natural compounds will complete the identification of the rusty grain beetle pheromone complex.

Once fungal attractants for C. ferrugineus have been identified and synthesized, these compounds, together with the rusty grain beetle pheromone, can be tested for synergism.

Field tests, in which synthetic compounds will be tested for their attraction to beetles in grain storage areas, will follow the development of efficient chemical synthetic pathways which will yield large amounts of synthetic chemicals of known authenticity and purity. These attractants may be incorporated into traps with effective chemical release devices and used as survey and/or control tools against C. ferrugineus.

After two unsuccessful attempts, compound B was synthesized from geraniol in 0.17% overall yield. The ^{1}H NMR spectrum, mass spectra, infrared spectrum and GLC retention time of the synthetic compound proved identical to those for the natural compound. Bioassay results showed that the synthetic compound was attractive for C. ferrugineus, proving that attractant B is 10-hydroxy-4,8-dimethyl,4-E,8-E,decadienoic acid lactone(I).

Identification of the other rusty grain beetle produced compounds which are attractive for C. ferrugineus, followed by rational syntheses and comparison with the natural compounds will complete the identification of the rusty grain beetle pheromone.

Once fungal attractants for C. ferrugineus have been identified and synthesized, these compounds together with the rusty grain beetle pheromone can be tested for synergism.

Field tests, in which synthetic compounds will be tested for their attraction to beetles in grain storage areas, will follow the development of efficient chemical synthetic pathways which will yield large amounts of synthetic chemicals of known authenticity and purity. These attractants may be incorporated into traps with effective chemical release devices and used as survey and/or control tools to be used against C. ferrugineus.

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