IDENTIFICATION AND SYNTHESES OF MACROLIDE PHEROMONES FROM *CRYPTOLESTES FERRUGINEUS*

by

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"Identification and Synthesis of Macrolide Pheromones from

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ABSTRACT

Three compounds were isolated from the pentane extract of Porapak Q-captured frass volatiles from *Cryptolestes ferrugineus* (Stephens), a serious coleopteran (beetle) pest of stored grain, by a combination of high performance liquid chromatography and preparative gas-liquid chromatography. The structures of these compounds were revealed by spectroscopic analyses to be (Z,S)-3-dodecen-11-olide (II), (Z)-5-tetradecen-13-olide (III) and (Z,Z)-3,6-dodecadien-11-olide (IV). Compound II, which was found in frass and beetle volatiles, elicited aggregation behavior from adult beetles of both sexes in a two-choice, pitfall olfactometer. Synergism was observed for a binary mixture of II and a compound I, (E,E)-4,8-dimethyl-4,8-decadien-10-olide, which had been isolated from *C. ferrugineus* in a previous study. A mixture of compounds I and II (1:1), which were named ferrulactone I and ferrulactone II, was equivalent in attractiveness to beetle volatiles but only 88% as attractive as frass volatiles. The presence of attractive fungal volatiles was proposed to account for the additional activity in frass volatiles. Compound III was not attractive alone, or in combination, with the ferrulactones. Several other macrolides which occurred in both frass and beetle volatiles were identified by mass spectroscopy on the natural products and their hydrogenated derivatives. The compounds, (Z,Z)-5,8-tetradecadien-13-olide, 11-dodecanolide and (E)-3-dodecen-11-olide were minor constituents and were not attractive in the laboratory bioassay.

Synthesis of I, (R,S)-II, (R)-II, (S)-II and (R,S)-III were achieved by routes which were based on the intramolecular esterification of an ω-hydroxy acid. Racemic ferrulactone II and pure (S)-II were both attractive, while the (R)-enantiomer was neither attractive nor inhibitory.

Preliminary field tests were carried out in the annex bins of grain elevators, located in southern Manitoba, on traps baited with a 9:1 mixture of ferrulactones I and II. Results indicated that the pheromones may be useful for detection of *C. ferrugineus* in grain storage bins.

For Avy, for her love, patience,

and understanding.

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I would like to acknowledge the technical assistance provided by Mr. G. Owen for obtaining the mass spectra and Ms. M. Tracey for the NMR spectra.

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LIST OF ABBREVIATIONS

AcOH	acetic acid
CMR	carbon magnetic resonance
DMF	dimethylformamide
EtOAc	ethyl acetate
Eu(tfc)$_3$	tris-[3-(trifluoromethylhydroxymethylene)-d-camphorato] europium (III)
GLC	gas-liquid chromatography
HEX	hexanes
HMPA	hexamethylphosphoramide
HRMS	high resolution mass spectrometry
IR	infrared
$LiAlH_4$	lithiumaluminum hydride
MEM	methoxyethoxymethyl
PMR	proton magnetic resonance
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMS	tetramethylsilane
UV	ultraviolet

I. INTRODUCTION

1) The Rusty Grain Beetle

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is a serious pest of stored products throughout the world (Wheeler, 1921; Rilett, 1949; Howe and Lefkovitch, 1957). This beetle has been found in dried fruits from the United States (Barnes and Kaloostian, 1940), Britain and Greece (Richards and Herford, 1930) and in oilseeds from the U.S.S.R. (Belyaev *et al.*, 1932) and Canada (Hurlock, 1963). Other stored products such as spices, chillies (Richards and Herford, 1930) and cacao beans (Howe and Lefkovitch, 1957) have also been attacked by *C. ferrugineus.* However, the principal targets of the rusty grain beetle are stored grain and processed grain products. Infestations by *C. ferrugineus* in stored grain have been reported from the U.S.S.R. (Davies, 1949), North America (Bishop, 1959), Britain (Hurlock, 1963) and Australia (Banks, 1979). *C. ferrugineus* has also been found in flour mills in Poland (Shmal'Ko, 1939) and Britain (Ogijewicz, 1934).

In Canada, the rusty grain beetle was seldom detected in stored grain prior to 1942, but by 1943 the Board of Grain Commissioners declared that it was the most important pest of stored grain in Western Canada (Rilett, 1949). In 1981 *C. ferrugineus* was still the most frequently intercepted primary insect pest in stored grain. Infestations have been found in farm-stored grain (Loschiavo, 1975), prairie elevators (Liscombe and Watters, 1962), terminal elevators (J.R. Elvidge, personal communication)[1], boxcars

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[1] Canada Grain Commission, Vancouver, B.C.

(Loschiavo, 1974) and cargo ships (Monro, 1969).

Due to transportation problems caused by the Second World War, large quantities of grain were stored in temporary sheds and annexes. This practice led directly to the rapid increase of rusty grain beetle infestations (Rilett, 1949; Howe and Lefkovitch, 1957). The temporary storage facilities were not weatherproof or equipped for the turning of grain. Thus moisture was allowed to reach the grain and hasten deterioration. Also, grain stored in these facilities remained warm through the winter due to the good insulating property of grain. The combination of these factors provided an ideal environment for insect infestations. The rusty grain beetle's tolerance to low temperature (Smith, 1975) and high intrinsic rate of natural increase (Smith, 1965) also contribute to its success in infesting stored grain.

Infested wheat suffers a loss of germinating capacity since the beetle specifically consumes the protein and vitamin-rich wheat germ (Campbell and Sinha, 1976). However, the major damage to stored wheat caused by the rusty grain beetle is due to the metabolic heat generated by an infestation (Freeman, 1952; Watters, 1969). Heated grain cakes, sprouts, becomes mouldy and suffers a loss of milling and baking qualities (Anderson, 1943). Furthermore, insect infested grain is unacceptable for export and therefore requires fumigation (Hurlock, 1963). For example, 32 terminal bins and 116 boxcars were fumigated for primary insect infestations at Vancouver in 1979 (J.R. Elvidge, personal communication)[1]. The majority of these fumigations were for *C. ferrugineus*.

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[1] Canada Grain Commission, Vancouver, B.C.

The control of *C. ferrugineus* in stored grain is dependent on detection coupled with fumigation. Insect detection can be accomplished by visual inspection or Berlese funnel extraction. Since these methods rely on the chance occurrence of insects in random samples,the probability of detecting insects in lightly infested grain is not high. Traps developed to detect *C. ferrugineus* in stored grain (Loschiavo and Atkinson, 1967, 1973; Loschiavo, 1974) also rely on passive encounters of beetles with the traps. If aggregation pheromones were available for *C. ferrugineus* they could be used to optimize the efficiency of survey traps. Behavior exhibited by *C. ferrugineus* suggests that an aggregation pheromone is utilized. The build up of local populations of *C. ferrugineus* to the level necessary to initiate heating is difficult to explain, particularly in dry grain (Freeman, 1952). This aggregation behavior could be accounted for by the presence of a pheromone which is attractive to both sexes. The ability of *C. ferrugineus* to respond to chemotactic stimuli from fungus-infected wheat has been shown by Loschiavo and Sinha (1966). These observations suggest that the rusty grain beetle utilizes an aggregation pheromone to establish populations in suitable environments.

Pheromones are known for a number of stored-product coleoptera and lepidoptera (Burkholder, 1981). Some of these pheromones have been utilized as attractants in traps for monitoring stored-product insects. For example, large numbers of *Trogoderma variabile* (Ballion) have been caught with pheromone-baited traps at U.S. ports by the U.S.D.A.'s Plant Protection and Quarantine program. In storage facilities the greater sensitivity of detection that was made possible by the use of pheromone-baited traps had allowed infestations to be detected before they reached the economic threshold. For example, a population of *T. variabile* in a Milwaukee warehouse was only detected by the use of pheromone-baited traps (Barak and Burkholder, 1976). Once an infestation has been detected, prompt application of control measures such as pesticides, pathogens or insect growth regulators could prevent the infestation from spreading. The rapid control of an infestation is essential since stored food products are high-value items which represent an investment in time and energy (Brand *et al.*, 1979). Impetus for an investigation of *C. ferrugineus* pheromones was provided by the possibility that a pheromone-based monitoring system could be developed.

2) Response of the Rusty Grain Beetle to an Aggregation Pheromone: Results from Previous Studies

The results from experiments done prior to this study have provided strong evidence for the utilization of an aggregation pheromone by *C. ferrugineus* (Borden *et al.*, 1979). Beetles of mixed age and sex oriented positively to the odor of beetles, frass, pentane extracts of frass and pentane extracts of Porapak Q-captured volatiles from beetles or frass in an open arena, airflow olfactometer. Porapak Q-captured volatiles from wheat[2] and wheat germ were not significantly attractive to the beetles. In an earlier study, Loschiavo and Sinha (1966) also observed that uninfected wheat kernels were not attractive to *C. ferrugineus*, but found that fungus (*Nigrospora sphaerica*) infected wheat kernels elicited a strong aggregation

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[2] Significant responses to low concentrations of wheat volatiles were only slightly higher than the pentane control and were probably artifacts due to an insufficient number of replicates.

response in a two-choice test. These observations indicated the presence of both beetle and fungal-produced attractants for the rusty grain beetle.

Verigin (1980) has shown that compounds produced only by the male beetles were responsible for eliciting aggregation behavior from both sexes. The fractionation of pentane extracts of Porapak Q-captured frass volatiles by preparative gas-liquid chromatography (GLC) resulted in the separation of several compounds. At least four compounds were attractive to *C. ferrugineus* in the open arena olfactometer (Borden *et al.*, 1979). The major compound was isolated and identified as (E,E)-4,8-dimethyl-4,8-decadien-10-olide (I) Verigin (1980). The proposed structure was confirmed by a seven-step synthesis from geraniol which resulted in a 0.17% yield of I. The elemental compositions of two of the remaining attractants were determined to be $C_{12}H_{20}O_2$ (II) and $C_{14}H_{24}O_2$ (III) by high resolution mass spectroscopy (HRMS). Structures were not elucidated for II or III in the previous study.

A comparison of the activities of synthetic I (27% response in the arena olfactometer) and the pentane extract of Porapak Q-captured frass volatiles (38%) revealed that I was not the only pheromone for *C. ferrugineus*. One or more additional components must be present to enhance the activity of I.

3) Current Objectives

The major objectives of this proposed research were to: 1) isolate and identify all of the aggregation pheromone components for the rusty grain beetle, 2) synthesize the pheromones and determine the most attractive mixture and, 3) field test the synthetic pheromone in the trap developed by Loschiavo and Atkinson (1973). An improved synthesis of I was also required to provide material for field tests.

II. EXPERIMENTAL METHODS

4) Insect Rearing

C. ferrugineus cultures were maintained on a mixture of Canada No. 2 Red Spring wheat and wheat germ (95:5, w/w) in 3.8 L glass jars. Since the beetles were sensitive to dessication, the wheat was adjusted to a moisture content of 18% by the addition of water followed by a 30 min autoclaving period. Cultures were inoculated with 1000 beetles (0.29 g) and incubated in the dark at 30 ± 1^{o} and 80±10% relative humidity. New adults emerged approximately 30 days after inoculation.

Beetles that were required for aerations or bioassays were separated from the wheat with a coarse sieve and collected by aspiration. Frass was obtained by sifting the finer components of spent media through a U.S. standard 250 μm sieve. The collected frass was stored in glass jars at -30^{o} until required.

5) Capture and Extraction of Beetle and Frass Volatiles

Borden *et al.* (1979) and Verigin (1980) have shown that attractive volatiles from beetles and frass could be trapped onto Porapak Q and recovered by pentane extraction. Therefore, these methods were employed in this study to obtain attractive extracts from beetles and frass.

Beetle volatiles were captured on Porapak Q in a set-up which was essentially the same as the one described by Verigin (1980). Air, drawn by an aspirator (1-2 L/min), was bubbled through water and then passed through an air scrubber packed with activated charcoal (50-80 mesh, Fisher). The moistened, purified air was then passed over beetles held in a 2 L erlenmeyer flask and through a glass trap (20 cm x 2.4 cm O.D., S-19 male and female joints and a coarse sintered glass disk at one end) packed with Porapak Q (26 g, 50-60 mesh, Applied Science Laboratories). The Porapak Q was conditioned before use by Soxhlet extraction (20 h) with ether. A typical aeration of mixed-sex beetles was carried out at 23° according to the following schedule: 8.26×10^4 beetles (48 h), 8.21×10^4 beetles (48 h), 7.63×10^4 beetles (48 h) and 6.95×10^4 beetles (48 h) for a total of 1.49×10^7 beetle-hours (bh). After each 48 h aeration period the dead beetles were removed, and the survivors allowed to feed in wheat (48 h) since it was observed that production of volatiles decreased when the beetles were starved.

Frass volatiles were captured on Porapak Q by the method used for beetle volatiles. A glass tube (45 cm x 4.8 cm O.D., fitted with a 24/40 joint at the inlet and a coarse sintered glass disk and male S-19 joint at the outlet) packed with frass was fitted between a charcoal air scrubber and a Porapak Q trap. Air was drawn through the system by aspiration (1-2 L/min) at an ambient temperature of 23°. A water bubbler was not required for frass aerations. After 48 h the frass was removed from the aeration assembly and ground in a ceramic ball mill (Norton, Chemical Process Products Division) for 20 min. The frass was then repacked and aerated for 288 h. A typical frass aeration (427 g aerated for 336 h) yielded 1.43×10^5 gram-hours (gh) of volatiles, based on the weight of the frass recovered after aeration.

It is important to note that gh and bh are only approximate measures of the quantities of compounds contained in the captured volatiles. The absolute quantities of compounds present in the captured volatiles depend on factors such as their release rates from beetles and frass. Since the release rates of compounds decrease with increased duration of aeration, it is clear that bh and gh cannot be quantitative units in a linear sense. However, since frass and beetle aerations were always performed under similar conditions these units (gh and bh) were sufficiently accurate for our purposes.

The Porapak Q-captured volatiles were recovered by Soxhlet extraction (20 h) with pentane (Caledon Laboratories), which had been distilled through a 30 cm Dufton column. The pentane extracts of frass and beetle volatiles were concentrated to about 6-7 mL by removal of the pentane (30 cm Dufton column). Stock solutions of the volatiles were prepared by transferring the concentrated extracts to 10 mL volumetric flasks and diluting to the mark with distilled pentane. The stock solutions were stored in 4 dram, teflon-lined, screw cap vials at -30° until required. Concentrated extracts of frass volatiles (58.0 gh/μL), required for pheromone isolation, were prepared by combining the pentane extracts of three frass aerations prior to concentration.

6) Bioassay Procedures

The open arena, airflow olfactometer bioassay which was developed to test the response of *C. ferrugineus* to volatile stimuli (Borden *et al.*, 1979), was considered too time consuming to perform for large numbers of stimuli. Furthermore, the response of the beetles to the most attractive stimulus did not exceed 80%. Therefore, a bioassay was required which would yield higher responses and was more convienent to perform. A two-choice, pitfall olfactometer, used to assess the olfactory response of *Oryzaephilus* spp. (Pierce *et al.*, 1981) appeared to satisfy these requirements. Ninety tests could be set up within 2 h, and responses greater than 90% were observed for attractive stimuli. The bioassay apparatus consisted of a plastic petri dish roughened on the inside bottom with sandpaper and drilled with two holes (1.4 cm diameter) 6.5 cm apart. A glass vial (4.8 cm x 1.9 cm) was held under each hole by vial stoppers which had their centers cut out. The stimulus and solvent control were deposited on 1.27 cm filter paper disks and placed in the glass vials after the solvent had evaporated. Fifteen beetles were released into the bioassay dish which was then covered with a lid and left in darkness for 2 h . The numbers of beetles in the stimulus and control vials were recorded. Prior to each bioassay, test beetles (mixed sex, 1-3 months of age) were held in 25 mL glass vials in the dark at 23° for 18 h without food. All bioassays were performed between 0900 h and 1300 h in an environmental chamber maintained at 23° . Dilutions of a test stimulus were assayed in a single session and each stimulus was replicated six or ten times.

The raw data were analyzed using the _t_-test and the results were expressed as the mean percent response of the total number of beetles per treatment. Threshold is defined as the concentration of pentane extract at which significant ($P<0.01$) positive response was first detected. All of the bioassay data reported in this study were obtained from the two-choice, pitfall bioassay.

7) Isolation of Pheromones

i) Analytical and Preparative GLC

Analytical separations of extracts containing frass or beetle volatiles were carried out with a Hewlett-Packard model 5830A gas chromatograph equipped with a 18835B capillary inlet system. Samples (1-5 μL) were introduced by splitless injection onto a 33 m x 0.66 mm I.D. glass open-tubular column coated with SP-1000 (column A). The following temperature program (A) was used for most analyses: initial oven temperature 70° for

2 min, program rate 4^{o} /min, final temperature 200^{o} for 20 min (He flow 30 cm/s).

The quantitation of pheromone components in extracts of beetle and frass volatiles was accomplished using geranyl acetate as the internal standard. The relative weight response (RWR) of I to geranyl acetate was determined to be 0.86 by the injection of a solution containing known quantities of pure synthetic I and geranyl acetate. Compounds II-VIII, which eluted in the same region of the chromatogram as I, were assumed to have the same RWR. The concentrations of compounds were reported in parts per million (ppm, µg/g of frass or beetles).

Preparative GLC separations were carried out with a Varian model 1200 gas chromatograph that was equipped with a 10:1 effluent splitter, flame ionization detector and thermal gradient collector (Brownlee and Silverstein, 1968). The thermal gradient collector has been described in detail by Verigin (1980). Purification of pheromones was achieved on a 3.0 m x 3.2 mm I.D. stainless steel column packed with 10% SP-1000 on 100/120 mesh Chromosorb W (column B). Samples were eluted with temperature program A (He flow 60 mL/min) and collected in 20 cm x 1.6 mm O.D. glass tubes. The collected samples were either washed into half-dram glass vials with pentane for bioassay, or sealed with deuterated chloroform ($CDCl_3$) for nuclear magnetic resonance (NMR) experiments. The purity of collected samples was determined by analysis on column A.

ii) High-Performance Liquid Chromatography (HPLC)

The fractionation of beetle volatiles for bioassay was carried out by

HPLC on a Varian LC5000, equipped with a series 634 UV/VISIBLE Spectrophotometer. A pentane extract (10 μL, 1.5 x 10^4 bh) of Porapak Q-captured beetle volatiles was loaded onto a Micropak SI-10 column (30 cm x 4 mm I.D.) which was equilibrated with 0.1% isopropyl alcohol (IPA, Omnisolv BDH Chemicals)/hexane (HPLC grade, Caledon Laboratories). The column was eluted with a gradient of IPA/hexane (0.1% to 2.0% at 10 min, 10% at 30 min) at a flow rate of 2 mL/min. Effluent was monitored at 213 nm and fractions (∿2 mL) were collected to separate individual components.

A pentane extract (30 μL, 5.12 x 10^4 gh) of frass volatiles was fractionated by HPLC via the procedure described for beetle volatiles. Thirty-nine 2 mL fractions were collected into 2 dram screw-cap vials for bioassay.

Large scale fractionations of frass volatiles, for pheromone isolation, were carried out on a Whatman Magnum 9 Partisil 10/50 column. Samples were concentrated to a volume of 100 μL and loaded onto the column which was equilibrated with 0.2% IPA/hexane. The column was eluted with a gradient of IPA/hexane (0.2% from 0 to 8 min, 1.0% at 15 min, 2.0% at 25 min, 10% at 35 min) at 4 mL/min. Effluent was monitored at 213 nm.

iii) Isolation of (Z,S)-3-Dodecen-11-olide

The isolation of II from a pentane extract of frass volatiles involved a combination of HPLC and preparative GLC. Compound II eluted between 56-61 ml when 4.04 x 10^5 gh of frass volatiles were fractionated on the Magnum 9 column under the conditions described previously. The HPLC fraction, which contained 87% II (column A, temperature program A), was concentrated to 40 μL under a stream of nitrogen and subjected to preparative GLC on column B (program A). Approximately 750 µg of II (>99.7% pure) was collected between 28.3 and 29.4 min.

iv) Isolation of (Z)-5-tetradecen-13-olide and (Z,Z)-3,6-dodecadien-11-olide

Compounds III and IV were isolated from *C. ferrugineus* frass volatiles by the procedure used for the isolation of II. Component III was collected between 48 mL and 55 mL from the Magnum 9 column. Approximately 200 µg of III was obtained from 4.04 x 10^5 gh of frass volatiles by preparative GLC on column B (elution time 34.2-36.2 min). Component IV (∿100 µg) was obtained pure after HPLC (elution volume 66.0-70.5 mL) followed by preparative GLC (elution time 31.2-32.0 min).

8) Identification of Pheromones

i) Spectroscopic Methods

The infra-red (IR) spectra of II and III were obtained with a Beckman IR 4230 instrument on neat samples (AgCl plates). The spectra were run by Mr. N. Dominelli (Health Protection Branch, Health and Welfare Canada, Vancouver, B.C.) on samples prepared by Mr. V. Verigin (1980).

Unit resolution mass spectra were obtained on a Hewlett-Packard 5985B GC/MS/DS equipped with a fused silica open-tubular column (30 m x 0.32 mm I.D.) coated with Carbowax 20M (column C). Chemical ionization (CI) and high resolution (HR) mass spectra of compounds II and III were obtained by Verigin (1980).

The proton magnetic resonance (PMR) spectra of II, III and IV, in $CDCl_3$ (99.8%-d, Merck, Sharp and Dohme), were recorded with a Bruker 400 MHz instrument by M. Tracey (Chemistry Department, Simon Fraser University and Chemistry Department, University of British Columbia). Chemical shifts were calculated from $CHCl_3$ ($\delta_{TMS}=\delta_{CHCl_3}+7.27$ ppm). Lanthanide induced shift experiments on II were performed by the addition of aliquots of a $CDCl_3$ solution of tris-[3-(trifluoromethylhydroxymethylene)-d-camphorato], europium (III) ($Eu(tfc)_3$, Aldrich Chemical Company) to a sample of II. The determination of the enantiomeric purity of synthetic (R)-II was carried out with $Eu(tfc)_3$ in CCl_4 instead of $CDCl_3$.

Carbon 13 magnetic resonance (CMR) spectra for II and III were recorded on a Varian XL-100 spectrometer equipped with a microprobe. All NMR spectra of isolated pheromones were obtained on samples sealed in 1.6 mm O.D. glass tubes which contained approximately 30 μL of $CDCl_3$. For PMR, the capillary tubes were positioned inside a 5 mm tube with Teflon vortex plugs which had been drilled through the Center.

ii) Microscale Hydrogenations

The hydrogenation of small quantities of isolated pheromones (∿25 μg) was carried out in half-dram glass vials equipped with a Teflon-lined septum. A hexane solution of the sample was stirred for one hour under hydrogen with a trace of 10% palladium on carbon.

9) Syntheses of Pheromones

i) General Procedures

The IR spectra of synthetic intermediates and products were determined on a Perkin-Elmer 599B spectrophotometer. PMR spectra were determined on a

Varian EM-360 (60 MHz) or XL-100 (100 MHz) instrument. Chemical shifts are reported in δ units, parts per million (ppm) downfield from tetramethylsilane (TMS). Splitting patterns are described as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and b, broad. Coupling constants are reported in Hertz (Hz). CMR spectra were determined on the XL-100 instrument. Mass spectra (electron impact, EI or chemical ionization, CI) were obtained on direct probe or GLC-MS samples at 70 eV on a HP 5985B mass spectrometer. High resolution mass spectra were obtained on a DS-50 instrument at U.B.C. Boiling points are uncorrected. Microanalyses were performed by Mr. M. Yang (Department of Biological Sciences, Simon Fraser University) on a Perkin-Elmer Model 240 elemental analyzer. Optical rotations were measured with a Rudolph polarimeter (Model 70) on samples contained in a 1 dm x 2 mm I.D. cell (Zeiss, Germany). Concentrations (C) are reported as g/100 mL of solvent.

Thin layer (0.25 mm) and preparative layer (20 cm x 20 cm x 0.75 mm) plates were made from silica gel 60 GF_{254} (E. Merck, Darmstadt). All column chromatography was performed on kieselgel 60 (40-63 μm, E. Merck, Darmstadt) as described by Still *et al.* (1978). Columns were eluted with mixtures of ethyl acetate and hexanes with compositions reported as volume percent. Chromatography solvents (ethyl acetate, EtOAc and hexanes, HEX) were distilled before use.

All reactions requiring anhydrous conditions were run in flame-dried glassware under a positive pressure of dry nitrogen or argon. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride ($LiAlH_4$), dimethylformamide (DMF), benzene and toluene were distilled from calcium hydride prior to use.

Dry HMPA was stored over activated molecular sieves (Linde 13x, Alfa Products). Anhydrous ether was stored over sodium wire.

A standard procedure was adopted for product isolation. Unless otherwise noted, the organic phase derived from product extraction was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure on a rotary evaporator.

Product purity was usually determined by TLC and/or GLC in the solvent or column conditions (12 m x 0.2 mm I.D. fused-silica column coated with OV-101 (column C) or 31 m x 0.25 mm I.D. open-tubular column coated with SP-2100 (column D), HP 5880A gas chromatograph) reported. Spectroscopic analyses and combustion analyses were also used to verify the purity of isolated compounds.

ii) Attempted Synthesis of I via Intramolecular Alkylation (Fig. 16)

a) Preparation of 3,7-Dimethyl-2-E,6-E-octadienylphenylthioacetate (1)

Phenylthioacetyl chloride (34.1 g, 183 mmol), prepared from phenylthioacetic acid (Aldrich Chem. Co., Mooradian *et al.*, 1949), was added dropwise over 1 h to an ice-cold solution of geraniol (25 g, 162 mmol, Eastman) and pyridine (17 m) in 25 ml of dichloromethane (CH_2Cl_2). The reaction mixture was stirred for 2 h and then poured into 150 mL of ice-cold 0.1 N HCl. The CH_2Cl_2 extract (3 x 100 mL) of the aqueous mixture was washed successively with 0.1 N HCl (2 x 100 mL), saturated aqueous sodium bicarbonate ($NaHCO_3$) and water. Product isolation resulted in a dark yellow liquid which was chromatographed on 275 g (30 cm x 4.5 cm I.D. column) of silica gel.

Elution with 10% EtOAc in HEX gave 49.0 g (99.3%) of 1. The light yellow liquid was analyzed by TLC (50% EtOAc in HEX) and found to be homogeneous: PMR (60 MHz, $CDCl_3$) δ1.56 (3H, CH_3, s), 1.63 (6H, gem-dimethyl, s), 2.03 (4H, C_4, C_5, m), 3.58 (2H, $-O-CH_2$, s), 4.58 (2H, C_1, d, J=7.2 Hz), 4.85-5.60 (2H, C_2, C_6, m), 7.1-7.5 (5H, phenyl, m); mass spectrum, m/z (relative intensity) 304 (0.4), 168 (28), 136 (27), 123 (61), 121 (24), 93 (63), 81 (45), 69 (100).

Analysis calculated for $C_{18}H_{24}O_2S$: C, 71.01; H 7.95. Found: C, 70.60; H 8.07.

b) Preparation of 3,7-Dimethyl-8-hydroxy-2-E,6-E-octadienylphenylthioacetate (2)

Selenium dioxide (11.5 g, 103 mmol, Alfa) was added to a solution of 1 (42.0 g, 138 mmol) and pyridine (25 mL) in 350 mL of 95% ethanol and refluxed for 4.5 h . The reaction mixture was filtered through a sintered glass funnel, and the filtrate concentrated by rotary evaporation to give a viscous, red-brown crude product. The crude product was then dissolved in THF (100 mL), acidified (pH 3) with HCl, cooled in an ice-bath and treated with 3.3 g (53 mmol) of sodium cyanoborohydride (Aldrich). The ice-bath was removed after 1 h and the reaction mixture stirred at 23° for 18 h . Workup of this reaction was carried out by pouring the reaction mixture into water (200 mL), extracting the aqueous mixture with diethyl ether (3 x 100 mL) and washing the ether extract with saturated aqueous sodium chloride (brine). Product isolation yielded 48.5 g of a light yellow liquid. The crude reduced product was divided into eight portions and chromatographed on 180 g

(20 cm x 4.5 cm I.D.) of silica gel. Regeneration of the silica gel by successive washings with EtOAc (500 mL) and the elution solvent (50% EtOAc in HEX, 500 ml) enabled each column to be used twice before the silica gel was discarded. The fractions (20 mL) containing the alcohol (2) were pooled to give 17.07 g (45.9%) based on recovered 1 of a light yellow liquid which gave one spot (R_f 0.48, 50% EtOAc in HEX) when analyzed by TLC : PMR (60 MHz, $CDCl_3$) δ 1.62 (6H, CH_3-C_3, CH_3-C_7, s), 2.08 (4H, C_4, C_5, m), 3.60 (2H,-CO-CH_2, s), 3.95 (2H, C_8, s), 4.60 (2H, C_1, d, J=7.2 Hz), 5.1-5.5 (2H, C_2, C_6, m), 7.1-7.5 (5H, phenyl, m); IR (film) 3400 (OH); mass spectrum, m/z (relative intensity) 303 (2.3), 197 (6.9), 169 (7.6), 153 (18.8), 151 (13.6), 137 (29), 135 (100), 125 (17.5), 123 (27), 111 (56), 107 (25).

Analysis calculated for $C_{18}H_{24}O_3S$: C, 67.47; H, 7.55. Found: C, 67.46; H, 7.59.

c) Preparation of 3,7-Dimethyl-8-bromo-2-E,6-E-octadienylphenylthioacetate(3)

Because 3 was unstable its preparation was carried out in small batches immediately before it was required. To an anhydrous ether (50 mL) solution of carbon tetrabromide (CBr_4, MC&B, 2.05 g, 6.24 mmol) and 2 (1.0 g, 3.12 mmol) was added triphenylphosphine (Ph_3P, Aldrich, 1.64 g, 6.24 mmol) at 23° . The reaction was stirred for 20 h and then filtered through a sintered glass funnel to remove the white precipitate of triphenylphosphine oxide. The residue, which remained after removal of the ether *in vacuo*, was chromatographed on 160 g (18 cm x 4.5 cm I.D.) of silica gel. Elution with 25% EtOAc in HEX afforded 1.19 g (99.8%) of 3. Analysis of the product on TLC

(25% EtOAc in HEX) revealed one spot (R_f 0.45) : PMR (60 MHz, $CDCl_3$) δ 1.67 (3H, CH_3-C_3, s), 1.72 (3H, CH_3-C_7, s), 3.61 (2H,-CO-CH_2, s), 3.94 (2H, C_8, s), 4.60 (2H, C_1, d, 7.2 Hz), 5.1-5.7 (2H, C_2, C_6, m), 7.1-7.75 (5H, phenyl, m); mass spectrum (CI, CH_4), m/z (relative intensity) 413 (1.3), 411 (1.7), 385 (1.3), 383 (1.5), 303 (19), 217 (35), 215 (35), 169 (23), 151 (13), 135 (100), 123 (45), 107 (9), 93 (14), 81 (30); HRMS calculated for $C_{18}H_{23}O_2SBr$: m/z384.1455/382.1475. Found: m/z 384.0589/382.0608.

d) Preparation of (*E*,*E*)-2-phenylthio-4,8-dimethyl-4,8-decadien-10-olide

A three-necked 100 ml flask fitted with a condenser and a thermometer was flushed with dry nitrogen and charged with sodium hydride (35 mg of a 57% mineral oil dispersion, 0.83 mmol, Alfa) and 40 mL of dry HMPA. To the stirred suspension maintained at 55° was added 3 (240 mg, 0.63 mmol) in 5 mL of dry THF over 30 min. The reaction was then heated to 93° (bath temperature) for 23 h after which it was poured into water (80 mL), extracted with ether (3 x 50 mL) and washed with brine (2 x 50 mL). The residue left after product isolation was applied onto a single preparative TLC plate and eluted with 25% EtOAc in HEX. A band at R_f 0.54 (UV visualization, 254 nm) was scraped into a sintered glass funnel and eluted with ether to give 70.2 mg (37%) of a colorless liquid (4), which was homogeneous by TLC analysis : PMR (60 MHz, $CDCl_3$) δ 1.66 (3H, CH_3-C_4 or CH_3-C_8, s), 1.67 (3H, CH_3-C_4 or CH_3-C_8, s), 2.0-2.6 (6H, C_3, C_6, C_7, m), 3.92 (1H, C_2, dd, J=4.5 Hz, 12 Hz), 4.41 (1H, C_{10a}, dd, J=9.6 Hz, 11 Hz), 4.76 (1H, C_{10b}, dd, J=6 Hz, 11 Hz), 4.92 (1H, C_5, bdd, J=12 Hz, 2.5 Hz), 5.61 (1H, C_9, bt, J=6 Hz, 9.6 Hz), 7.36 (5H, phenyl, m); mass spectrum, m/z (relative intensity)

302 (14), 234 (26), 150 (14), 135 (22), 123 (17), 121 (21), 110 (19), 109 (14), 107 (18), 105 (16), 95 (11), 93 (29), 91 (19), 81 (100), 80 (54), 79 (48), 77 (25), 67 (17), 65 (16), 55 (16), 53 (20), 41 (17).

The result obtained in this reaction was not reproducible despite numerous attempts. The major product isolated from these unsuccessful attempts had an R_f (0.54, 25% EtOAc in HEX) which was identical to that of the phenylthiolactone (4). This product was identified as 3,7-dimethyl-2-E,5-E,7-octatrienyl-phenylthioacetate (28) : PMR (60 MHz, $CDCl_3$) δ 1.66 (3H, CH_3-C_3, s), 1.82 (3H, CH_3-C_7, s), 2.77 (2H, C_4, bd, J=6.5 Hz), 3.58 (2H,-CO-CH_2, s), 4.60 (2H, C_1, d, J=7.0 Hz), 4.87 (2H, exo-methylene, bs), 5.28 (1H, C_2, t, J=7.0 Hz), 5.60 (1H, C_5, dd, J=6.5 Hz, 15 Hz), 6.13 (1H, C_6, d, J=15 Hz); mass spectrum, m/z (relative intensity) 302 (0.9), 218 (3), 168 (12), 134 (100), 123 (100), 119 (60), 107 (32), 105 (29), 93 (63), 91 (51), 81 (27), 79 (47), 77 (48), 69 (20), 65 (22), 55 (50), 45 (41), 41 (36); high resolution mass spectrum, m/z 302.1343 ($C_{18}H_{22}O_2S$); Calculated, 302.2214.

e) Preparation of I via the Desulfurization of 4

Raney nickel (450 mg, W-2, Mozengo, 1955) was added to a solution of 4 (45 mg, 0.15 mmol) in 9 ml of absolute ethanol. The suspension was stirred at room temperature and analyzed directly by GLC (column A and C). Analysis of a sample (column C, 210° isothermal) removed after 1 h revealed that the reaction was 45% completed based on the consumption of 4. Heating the reaction mixture to reflux did not increase the yield of product. The addition of a second portion of Raney nickel (450 mg) followed by 20 min of reflux resulted in the complete disappearance of both starting material (4) and product (I). Presumably, the organic material was irreversibly adsorbed onto the catalyst. Therefore no product was recovered from this reaction. However, an aliquot removed from the reaction prior to the second addition of Raney nickel was analyzed by GLC(column A) and found to contain material which co-eluted with authentic I. No trace of isomeric products were detected. Due to a lack of the starting material (4), further work on the desulfurization of the phenylthiolactone was not carried out.

iii) Synthesis of I by Intramolecular Esterification (via Diethyl Malonyl Derivative) (Fig. 17)

a) Preparation of 8-Acetoxy-2,6-dimethyl-2-E,6-E-octadien-1-ol (5)

The oxidation of geranyl acetate was carried out according to the procedure described by Umbreit and Sharpless (1977). Tert-butyl hydroperoxide (28 mL 90% solution, 242 mmol, MC&B) was added to a suspension of selenium dioxide (7.1, 64 mmol) in dichloromethane (75 mL) and stirred for 30 min at 10°. Geranyl acetate (25 g, 128 mmol; bp. 73-74°C (0.65 mm), prepared by the treatment of geraniol with acetic anhydride and pyridine) was added all at once to the selenium dioxide solution and stirred for 3 h (10°). Benzene (50 mL) was added to the reaction mixture and the dichloromethane removed by rotary evaporation. The benzene solution was diluted with ether (100 mL) and washed with 10% aqueous potassium hydroxide (4 x 50 mL) and brine (2 x 50 mL). The residue which remained after product isolation contained aldehyde and was therefore subjected to reduction with sodium borohydride (1.9 g, 50 mmol; in 25 mL abs. ethanol for 10 min). This reaction was diluted with water (50 mL), extracted with ether (3 x 50 mL), and washed with brine (2 x 50 mL). Product isolation yielded a light yellow liquid which was subjected to vacuum distillation through a Vigreux column: yield 15.4 g; bp. 126-135° (0.9 mm). The distilled product contained the alcohol (5) (R_f 0.32; 50% EtOAc in HEX) and a contaminant (∿20%, R_f 0.09) which was identified as 2,6-dimethyl-8-hydroxy-2-E,6-E-octadien-1-ol : PMR (60 MHz, $CDCl_3$) δ 1.63 (6H, C_2, C_6, s), 2.10 (4H, C_4, C_5, m), 3.98 (2H, C_1, s), 4.14 (2H, C_8, d, J=7.0 Hz), 5.43 (2H, C_3, C_7, bt, J=7.0 Hz). Separation of 5 from the contaminant was achieved by chromatography on silica gel (200 g; 18 cm x 5 cm I.D. column). The sample was eluted with 50% EtOAc in HEX and 250 mL fractions were collected. Fractions 2-5 were combined to give 12.0 g (45%) of the pure alcohol (5) : PMR (60 MHz, $CDCl_3$) δ 1.64 (3H, CH_3-C_6, s), 1.66 (3H, CH_3-C_2, s), 2.00 (3H,-CO-CH_3, s), 2.05 (5H, C_4, C_5, OH, m), 3.94 (2H, C_1, s), 4.54 (2H, C_8, d, J=7.2 Hz), 5.30 (2H, C_3, C_7, bt, J= 7.2 Hz); mass spectrum (EI), m/z (relative intensity) 134 (13), 121 (5), 119 (7), 93 (12), 84 (48), 68 (60), 67 (34), 55 (13), 43 (100), 41 (25); CI (iso-butane) 287 (3), 269 (1), 213 (15), 195 (12), 153 (18), 135 (100), 107 (10), 95 (8), 93 (12). Analysis calculated for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50. Found: C, 68.10; H, 9.44.

b) Preparation of 8-Acetoxy-2,6-dimethyl-2-E,6-E-octadienyl bromide (6)

Hooz and Gilani's (1968) procedure for the bromination of alcohols was used for the preparation of 6. To an ice-cold solution of 5 (14.2 g, 67 mmol) and carbon tetrabromide (44.2 g, 133 mmol) in anhydrous ether (250 mL) was added 35.0 g (133 mmol) of triphenylphosphine. The reaction mixture was then stirred at 4° for 24 h, after which it was filtered through a sintered glass funnel. The residue obtained after removal of the ether was diluted with 100 mL of ice-cold hexanes which precipitated the remainder of the triphenylphosphine oxide. Filtration of the hexane solution followed by evaporation of the filtrate left the crude product which was chromatographed on 350 g (30 cm x 5 cm I.D. column) of silica gel (10% EtOAc in HEX; 20 mL fractions). Fractions 28-60 were combined to give 17.4 g (94.5%) of 6 which was homogeneous by TLC analysis (R_f 0.59, 50% EtOAc in HEX) : PMR (60 MHz, $CDCl_3$) δ 1.68 (3H, CH_3-C_6, s), 1.72 (3H, CH_3-C_2, s), 2.02 (3H,-CO-CH_3, s), 2.10 (4H, C_4, C_5, m), 3.94 (2H, C_1, s), 4.55 (2H, C_8, d, J=7.2 Hz), 5.15-5.70 (2H, C_3, C_7, m); mass spectrum (CI, isobutane), m/z (relative intensity) 217 (48), 215 (50), 195 (19), 135 (100).

Analysis calculated for $C_{12}H_{19}O_2Br$: C, 52.38; H, 6.96. Found: C, 52.53; H, 6.97.

c) Preparation of 10-Acetoxy-2-carboethoxy-4,8-dimethyl-4-E, 8-E-ethyl decadienoate (7)

A three-necked 500 mL flask fitted with a thermometer and a dropping funnel was flushed with nitrogen and charged with sodium hydride (2.34 g of a 60% mineral oil dispersion, 59 mmol). The sodium hydride dispersion was washed free of mineral oil with dry hexanes and suspended in 180 mL of dry DMF. Diethyl malonate (9.4 g, 59 mmol) was added over 20 min, and the resultant mixture was stirred for 1 h . Bromide (6) (14.6 g, 53 mmol) was added over 15 min to the solution of sodio-diethyl malonate which was then heated to 60^{o} for 4.5 h followed by 12 h at 23^{o} . Workup of the reaction was carried out by dilution of the reaction mixture with water (250 mL), extraction with ether (3 x 150 mL) and washing with brine (3 x 100 mL). Product isolation gave a yellow liquid which was chromatographed on 350 g (30 cm x 5 cm I.D.) of silica gel (25% EtOAc in HEX, 20 mL fractions). Fractions 31-60 were combined and concentrated to give 16.3 g (87%) of product. Analysis by GLC on column C (200^{o}) revealed two major products: 7 (77%) and diethyl malonate (11%). Since the diester (7) was subjected to decarbalkoxylation without further purification, a small sample was purified by preparative TLC (R_f 0.27, 30% EtOAc in HEX) for analysis : PMR (60 MHz, $CDCl_3$) δ 1.24 (6H, CH_3-CH_2O, t, J=7.0 Hz), 161 (3H, CH_3-C_8, s), 1.68 (3H, CH_3-C_4, s), 2.01 (3H,-CO-CH_3, s; 4H, C_6, C_7, m), 2.57 (2H, C_3, d, J=7.6 Hz), 3.51 (1H, C_2, t, J=7.6 Hz), 4.17 (4H, CH_3-CH_2-O, q, J=7.0 Hz), 4.57 (2H, C_{10}, d, J=7.2 Hz), 5.0-5.5 (2H, C_5, C_9, m); mass spectrum (CI, CH_4), m/z (relative intensity) 295 (100), 249 (23), 227 (34), 135 (30).

Analysis calculated for $C_{19}H_{30}O_6$: C, 64.38; H, 8.53. Found: C, 64.22; H, 8.64.

d) Preparation of 10-Acetoxy-4,8-dimethyl-4-E,8-E-ethyl decadienoate (8)

The decarbalkoxylation of 7 was carried out according to the procedure of Krapcho and Lovey (1973). To a solution of partially purified 7 (15.5 g, ∿42 mmol based on 77% purity) in DMSO (100 mL) was added 3.3 g (57 mmol) of sodium chloride and 2 mL of water. The mixture was heated to 160°C for 7 h and then stirred for 12 h at 23° . Analysis of an aliquot by GLC (column C, 210°) revealed that all of the starting material had been consumed. The reaction mixture was poured in water (150 mL), extracted with ether (3 x 100 mL), and washed with brine (2 x 100 mL). Product isolation yielded 11.5 g of a yellow liquid which was chromatographed on 300 g (28 cm x 5 cm I.D. column) of silica gel (20% EtOAc in HEX). The product recovered after chromatography (8.3 g, 58% yield from 6) was 91% pure by GLC (column C, 210°) : PMR (60 MHz, $CDCl_3$) δ 1.25 (3H, $C\underline{H}_3CH_2$-O, t, J=7.2 Hz), 1.62 (3H, CH_3-C_8, s), 1.71 (3H, CH_3-C_4, s), 2.04 (3H,-CO-CH_3, s; 4H, C_6, C_7, m), 2.34 (4H, C_2, C_3, s), 4.13 (2H, CH_3-$C\underline{H}_2$-O, q, J=7.2 Hz), 4.59 (2H, C_{10}, d, J=7.0 Hz), 4.95-5.50 (2H, C_5, C_9, m); mass spectrum (CI, CH_4), m/z (relative intensity) 283 (8), 223 (100), 177 (7), 135 (31).

Analysis calculated for $C_{16}H_{26}O_4$: C, 68.05; H, 9.28. Found: C, 67.75; H, 9.50.

e) Preparation of 4,8-Dimethyl-10-hydroxy-4-E,8-E-decadienoic acid (9)

To a solution of 8 (7.3 g, 26 mmol) in 50 mL of dioxane was added 4.1 g of sodium hydroxide in 15 mL of water. The mixture was refluxed for 2.5 h after which it was diluted with water (100 mL), acidified to pH 2 with concentrated HCl, extracted with ether (4 x 50 mL) and washed with brine (2 x 50 mL). Product isolation yielded a viscous oil (4.2 g) which was chromatographed on 280 g (25 cm x 5 cm I.D. column) of silica gel (EtOAc : HEX : acetic acid, 49.5 : 49.5 : 1) to give 3.98 g (73%) of 9. Analysis by TLC (solvent system used for column chromatography) revealed a major spot at R_f 0.25 (9) and minor impurities at R_f 0.19 and 0.39. PMR of 9 (60 MHz, $CDCl_3$) δ 1.61 (6H, CH_3-C_4, CH_3-C_8, s), 2.07 (4H, C_6, C_7, m), 2.37 (4H, C_2, C_3, m), 4.16 (2H, C_{10}, d, J=7.0 Hz), 5.12 (1H, C_5, m), 5.36 (1H, C_9, bt, J=7.0 Hz), 7.20 (2H, OH, COOH, bs); IR (neat film) 3400, 1710 cm^{-1}; mass spectrum (EI), m/z (relative intensity) 194 (8), 181 (5), 127 (43), 121 (29), 117 (12), 109 (51), 99 (30), 97 (12), 95 (10), 94 (10), 93 (33), 85 (93), 84 (24), 83 (15), 81 (100), 68 (55), 67 (51), 41 (27).

Analysis calculated for $C_{12}H_{20}O_3$: C, 67.89; H, 9.49. Found: C, 68.15, H, 9.59.

f) Preparation of I via the Intramolecular Esterification of 9

A three-necked 500 mL flask fitted with a low temperature thermometer and a reflux condenser was flushed with nitrogen and charged with 302 mg (1.43 mmol) of 9, 856 mg (2.14 mmol) of 2.2'-bis-(4-t-butyl-N-isopropyl) imidazolyl disulfide (prepared by Dr. A.C. Oehlschlager according to the procedure of Corey and Brunelle (1976))and 20 mL of dry toluene. Triphenylphosphine (561 mg, 2.14 mmol) was added to the orange mixture (-68°) which immediately turned yellow and formed a white precipitate. The mixture was allowed to reach -10° , stirred for 2.5 h and then diluted with 300 mL of cold toluene (-35°). The cloudy, yellow reaction mixture was allowed to reach room temperature (23°) and then heated to reflux for 2 h . Samples removed from the reaction (filtered through neutral alumina and eluted with dichloromethane) and analyzed by GLC (column C, 170°) revealed that the 2 h of reflux did not increase the yield of I. Most of the toluene was removed by distillation (10 mm) through a Vigreux column and the remainder (∿5 mL) was removed *in vacuo*. Chromatography of the residue on 150 g of silica gel (28 cm x 3.5 cm I.D. column; 7.5% EtOAc in HEX) gave 5.2 mg (1.9%) of a product at R_f 0.35 (7.5% EtOAc in HEX) and 102 mg (37%) of I. Analysis of I by TLC (R_f 0.50, 7.5% EtOAc in HEX) and GLC (column C, 210°) revealed a purity of greater than 99% : PMR (60 MHz, $CDCl_3$) δ 1.57 (3H, CH_3-C_4, d, J=0.9 Hz), 1.64 (3H, CH_3-C_8, d, J=1.2 Hz), 2.09 (4H, C_6, C_7, bs), 2.32 (4H, C_2, C_3, s), 4.56 (2H, C_{10}, bd, J=7.0 Hz), 4.82 (1H, C_5, m), 5.54 (1H, C_9, dt, $J_{C_{10}-C_9}$=7.0 Hz, $J_{C_7-C_9}$=1.5 Hz); CMR (25.2 MHz, $CDCl_3$) δ 14.7, 15.3, 24.9, 33.5, 38.5, 61.5, 122.3, 126.4, 132.8, 142.8, 174.2; mass spectrum (EI), m/z (relative intensity) 194 (1.8), 127 (31), 121 (5), 109 (8), 99 (37), 93 (7), 85 (11), 81 (20), 68 (100), 67 (62), 53 (15), 41 (17).

Analysis calculated for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.48; H, 9.47.

Spectroscopic analysis of the crystalline minor product (R_f 0.35) showed that it was the diolide of I : PMR (60 MHz, $CDCl_3$) δ 1.60 (6H, CH_3-C_4, s), 1.67 (6H, CH_3-C_8, s), 2.05 (8H, C_6, C_7, m), 2.33 (8H, C_2, C_3, bs), 4.57 (4H, C_{10}, d, J=7.0 Hz), 4.8-5.3 (4H, C_5, C_9, m); mass spectrum (EI), m/z (relative intensity), 195 (26), 194 (43), 193 (29), 177 (10), 175 (13), 127 (85), 121 (52), 109 (26), 99 (42), 93 (77), 81 (76), 68 (100), 67 (98), 55 (20), 53 (18).

iv) Synthesis of Racemic (Z)-3-Dodecen-11-olide (R, S-II) (Fig. 18)

a) Preparation of 1-Tert-butyldimethylsiloxy-3-butyne (10)

To an ice-cold solution of 3-butyn-1-ol (35 g, 0.5 mol, Farchan Labs) and imidazole (85 g, 1.25 mol, Sigma) in 150 mL of dry DMF was added 90.3 g (0.6 mol) of t-butyl dimethylchlorosilane (Silar Labs). The ice-bath was removed after 30 min and the reaction stirred for 4 h at 23°. The reaction mixture was then poured into water (300 mL), extracted with pentane (4 x 150 m) and washed with brine (3 x 100 mL). Product isolation yielded a light yellow liquid which was distilled through a Vigreux column (10 mm) to give 90.2 g (98%) of 10 : bp 82-89° (10-15 mm). Analysis by GLC on column C (130°) revealed a purity of 95%. PMR (60 MHz, $CDCl_3$) δ 0.12 (6H, CH_3-Si, s), 0.92 (9H, t-butyl-Si,s), 1.96 (1H, C_4, t, J=3.0 Hz), 2.42 (2H, C_2, dt, J_{1-2}=7.2 Hz, J_{4-2}=3.0 Hz), 3.75 (2H, C_1, t, J=7.2 Hz). HRMS calculated for $C_9H_{17}O_1Si$ ($M-CH_3$): m/z 169.2135. Found: m/z 169.1046.

b) Preparation of 1-Chloro-5-iodopentane (11)

The dihalo-alkane (11) was prepared from 1,5-pentanediol by the procedure of Taylor and Strong (1950). To an ice-cold solution of 1,5-pentanediol (104.2 g, 1 mole, Aldrich) in 25 mL of pyridine was added 219 mL (3 mole) of thionyl chloride (dropwise over 2 h). The yellow reaction mixture was then stirred for 18 h at 23° . Ice was cautiously added to the mixture until no further reaction was observed. The organic layer was separated in a separatory funnel, washed with concentrated sulfuric acid (2 x 15 mL), dilute aqueous sodium bicarbonate (4 x 100 mL) and water (4 x 100 mL). The crude product was then diluted with pentane (300 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield 137.4 g (97.4%) of 1,5-dichloropentane: PMR (60 MHz $CDCl_3$) δ 1.63-2.03 (6H, C_2, C_3, C_4, bm), 3.45-377 (4H, C_1, C_5, t, J=6.0 Hz).

The 1,5-dichloropentane (50 g, 0.35 mol) was then dissolved in 650 mL of acetone which contained 53.2 g (0.35 mol) of sodium iodide. The resultant mixture was stirred vigorously and refluxed for 24 h . Filtration of the reaction mixture through a Büchner funnel removed the sodium chloride that was formed. The filtrate was concentrated *in vacuo*, dissolved into pentane (200 mL) and washed with aqueous sodium thiosulfate (2 x 100 mL) and water (2 x 100 mL). Product isolation gave a light yellow crude product (77 g) which was distilled through a Vigreux column: bp 64-66° (0.3 mm) lit. 104-105° (14 mm) (Taylor and Strong, 1950);yield 36.0 g (87%) of 1-chloro-5-iodopentane (98% pure by GLC, column C; 100° -140° at 10°/min); PMR (60 MHz, $CDCl_3$) δ 1.45-2.15 (6H, C_2, C_3, C_4, bm), 3.17 (2H, CH_2-I, t, J=6.4 Hz), 3.53 (2H, CH_2-Cl, t, J=6.0 Hz).

c) Preparation of 1-Tert-butyldimethylsiloxy-9-chloro-3-nonyne (12)

A three-necked 250 mL flask fitted with a dropping funnel and a thermometer was flushed with nitrogen and charged with 10 (18.4 g, 100 mmol) in 50 mL of dry THF. n-Butyllithium (66 mL, 1.6 M, 105 mmol) was added dropwise over 20 min to the vigorously stirred, ice-cold solution of 10. After n-Butyllithium addition, 23.3 g (100 mmol) of 11 (and 40 mL of dry THF) was added all at once. Dry HMPA (50 mL) was then added dropwise into the mixture resulting in a slightly exothermic reaction. The ice-bath was removed and the reaction was stirred at 23° for 30 min. Workup was carried out by dilution of the reaction mixture with water (250 mL), and extraction with ether (4 x 75 mL) followed by a brine wash (2 x 150 mL). The crude products from this reaction, and a second reaction performed under identical conditions (on 165 mmol of 10), were combined for distillation through a Vigreux column: bp. 95°-112° (0.05 mm); 47.5 g (62.2%), purity of 76.4% based on GLC analysis, column C, 120°-250° at 10°/min. This product was suitable for iodination without further purification. Therefore, a small sample was purified by careful distillation through a Vigreux column (bp. 110°-118°, 0.2 mm) for analysis: GLC analysis on column C (120°-250° at 10°/min) revealed a purity of 93%; PMR (60 MHz, $CDCl_3$) δ 0.12 (6H, CH_3-Si, s), 0.92 (9H, t-butyl-Si, s), 1.34-194 (6H, C_6, C_7, C_8, bm), 2.0-2.63 (4H, C_2, C_5, bm), 3.56 (2H, C_9, t, J=6.0 Hz), 3.72 (2H, C_1, t, J=7.0 Hz); mass spectrum (EI), m/z (relative intensity) 273 (0.1), 233 (3), 231 (9), 123 (69), 105 (16), 95 (57), 93 (100), 89 (43), 79 (87), 75 (40), 73 (81), 67 (20), 59 (16), 55 (28).

Analysis calculated for $C_{15}H_{29}OSiCl$: C, 62.36; H, 10.12. Found: C, 62.64; H, 10.01.

d) Preparation of 1-Tert-butyldimethylsiloxy-9-iodo-3-nonyne (13)

To 7.9 g (27 mmol) of 12 was added 100 mL of acetone containing 8.2 g (55 mmol) of sodium iodide. The resultant solution was refluxed for 65 h and then concentrated *in vacuo*, diluted with water (150 mL) and extracted with ether (3 x 75 mL). The ether extract was washed with aqueous sodium thiosulfate (2 x 75 mL) and brine (2 x 75 mL). Distillation of the isolated product through a Vigreux column gave 10.0 g (96%) of 13, purity of 94% by GLC (column C, 120^{o}-250^{o} at 10^{o}/min): bp. 120^{o}-122^{o} (0.1 mm), PMR (60 MHz, $CDCl_3$) δ 0.12 (6H, CH_3-Si, s), 0.92 (9H, t-butyl-Si, s), 1.27-2.07 (6H, C_6, C_7, C_8, bm), 2.07-2.64 (4H, C_2, C_5, bm), 3.25 (2H, C_9, t, J=6.8 Hz), 3.74 (2H, C_1, t, J=6.8Hz).

e) Preparation of 1-Tert-butyldimethylsiloxy-11-hydroxy-3-dodecyne (14)

The three-necked 100 mL flask fitted with a dropping funnel and a reflux condensor was flushed with argon and charged with magnesium powder (830 mg, 35 mmol, Mallinckrodt) and 13 (8.7 g, 23 mmol) in 40 mL of dry THF. Grignard formation was initiated by the addition of ethylbromide (0.1 mL, ∿1.3 mmol), and maintained by refluxing the reaction mixture for 3 h . The formation of the Grignard reagent was monitored by GLC (column C; 160^{o}-250^{o} at 10^{o}/min): aliquots taken from the reaction were quenched into saturated aqueous ammonium chloride, extracted with ether, dried over anhydrous magnesium sulfate and injected into column C. After the iodide (13) was completely consumed, 2.2 g (11.5 mmol) of cuprous iodide (BDH) was added to the mixture, which was cooled in an ice-bath, and stirred for 30 min. Propylene oxide (2.7 g, 46 mmol, Aldrich) diluted with 10 mL of dry THF was added dropwise to the black reaction mixture. The reaction was stirred for 30 min at 23° and then quenched with saturated aqueous ammonium chloride (100 mL). The ether extract (3 x 75 mL) of the aqueous mixture was washed with brine (3 x 75 mL). Product isolation gave a crude product which was purified by chromatography on 180 g (20 cm x 4.5 cm I.D. column) of silica gel (20% EtOAc in HEX): 3.3 g (46%) of 14 (>99% pure by GLC on column C; 180°-250° at 10°/min); PMR (60 MHz, $CDCl_3$) δ 0.08 (6H, CH_3-Si, s), 0.89 (9H, t-butyl-Si, s), 1.19 (3H, C_{12}, d, J=6.0 Hz), 1.36 (10H, C_6-C_{10}, bs), 1.77 (1H, -OH, s), 2.00-2.57 (4H, C_2, C_5, bm), 3.70 (3H, C_1, t, J=7.0 Hz), 3.50-3.90 (1H, C_{11}, bm); mass spectrum (EI), m/z (relative intensity) 255 (1.2), 237 (1.6), 121 (18), 107 (25), 105 (50), 95 (23), 93 (33), 89 (27), 81 (30), 79 (24), 75 (100), 73 (44), 67 (20), 59 (10), 55 (19), 45 (15).

Analysis calculated for $C_{18}H_{36}O_2Si$:C,69.15; H, 11.61. Found: C, 69.41; H, 11.77.

f) Preparation of 1,11-dihydroxy-3-dodecyne (15)

To a solution of 14 (2.9 g, 9.4 mmol) in the THF (15 mL) was added 19 mL of tetra-n-butylammonium fluoride (19 mmole, 1M solution in THF, Aldrich). The reaction was stirred for 1 h at 23° then diluted with 75 mL of water. The aqueous mixture was extracted with ether (3 x 50 mL) and the ether extract washed with brine (2 x 50 mL). Product isolation yielded 1.86 g (100%) of the diol (15) which was analyzed by TLC (25% EtOAc in HEX). A small amount of one impurity (R_f 0.37) was detected above the diol (R_f 0.29). No further purification was carried out on 15 : PMR (60 MHz, $CDCl_3$) δ 1.14 (3H, C_{12}, d, J=6.0 Hz), 1.34 (10H, C_6-C_{10}, bs), 1.94-2.57 (4H, C_2, C_5, bm), 2.85 (2H, OH, bs), 3.63 (2H, C_1, t, J=6.3 Hz), 3.38-3.83 (1H, C_{11}, bm). HRMS calculated for $C_{12}H_{22}O_2$: m/z 198.1614. Found: m/z 198.1624.

g) Preparation of 11-hydroxy-3-dodecynoic acid(16)

The inverse addition oxidation procedure developed by Holland and Gilman (1974) was used for the oxidation of 15. To a vigorously stirred, ice-cold solution of chromic trioxide(5.4 g, 54 mmol, MC&B) in 50 mL of 6N sulfuric acid was added dropwise over 3 h , 1.76 g (8.9 mmol) of 15 in 50 mL of acetone. The reaction was kept in an ice-bath for 30 min and then warmed to 23° before workup. Water (200 mL) was added to the reaction mixture which was then extracted with ether (4 x 75 mL). The ether extract was washed with brine (3 x 50 mL). Product isolation yielded crude 11-keto-3-dodecynoic acid which was purified by crystallization from 10% ether in HEX (mp 49-51°). Analysis of the methyl ester of the crystalline 11-keto-3-dodecynoic acid(prepared by treatment of the keto-acid with an excess of ethereal diazomethane) by GLC on column C (180°-250° at 10°/min) revealed that it was 90% pure: yield 1.28 g (68%); PMR (60 MHz, $CDCl_3$)

δ 1.08-1.77 (8H, C_6-C_9, bm), 2.10 (3H, C_{12}, s), 1.95-2.60 (4H, C_5, C_{10}, bm), 3.27 (2H, C_2, t, J=2.2 Hz), 10.3 (1H, COOH, bs).

The keto-acid was converted quantitatively to 16 by reduction with sodium borohydride. To an ice-cold solution of the keto-acid (676 mg, 3.2 mmol) in 10 mL of absolute ethanol was added 244 mg (6.4 mmol) of sodium borohydride. After 5 min the reaction was warmed to 23°, diluted with cold 5% HCl (30 mL) and extracted with ether. Product isolation yielded 680 mg (99%) of 16 which was 97% pure by GLC analysis (of methylester) on column C (180°-250° at 10°/min): PMR (60 MHz, $CDCl_3$) δ 1.17 (3H, C_{12}, d, J=6.0 Hz), 1.35 (10H, C_6-C_{10}, bs), 2.0-2.4 (2H, C_5, bm), 3.26 (2H, C_2, t, J=2.2 Hz), 3.6-4.0 (1H, C_{11}, bm), 6.77 (2H, COOH, OH, bs). HRMS calculated for $C_{12}H_{18}O_3$: m/z 210.1251. Found: m/z 210.1261.

h) Preparation of 11-hydroxy-3-Z-dodecenoic acid (17)

Partial reduction of the triple bond in 16 was accomplished with P-2 nickel catalyst (Brown and Ahuja, 1973). To a vigorously stirred mixture of nickel acetate (1g, MC&B) and absolute ethanol (25 mL, saturated with hydrogen) was added 5 mL of the filtrate from a solution of sodium borohydride (500 mg) in ethanol (12 mL) and 2N aqueous sodium hydroxide (0.63 mL). After hydrogen evolution had ceased, the black suspension was treated with 0.7 mL of ethylene diamine. The alkyne (16, 643 mg, 3.0 mmol) was then added to the P-2 nickel preparation and stirred for 1 h at 23°. The reaction was diluted with brine (100 mL), acidified with 5% HCl and extracted with ether (4 x 50 mL). HRMS calculated for $C_{12}H_{20}O_3$: m/z 212.1407. Found: m/z 212.1403.

After the ether extract was washed with brine (2 x 50 mL), product isolation gave 620 mg (96%) of 17 which was 96% pure by GLC analysis of the methylester on column C (180^o-250^o at 10^o/min): PMR (60 MHz, $CDCl_3$) δ 1.20 (3H, C_{12}, d, J=6.0 Hz), 1.34 (10H, C_6-C_{10}, bs), 1.87-2.20 (2H, C_5, bm), 3.12 (2H, C_2, d, J=5.4 Hz), 3.5-4.0 (1H, C_{11}, bm), 5.4-5.7 (2H, C_3, C_4, m), 7.15 (2H, COOH, OH, bs). HRMS calculated for $C_{12}H_{22}O_3$: m/z 214.1563. Found: m/z 214.1577.

i) Preparation of (3Z, 11RS)-3-Dodecen-11-olide ((R,S)-II)

Triphenylphosphine (196 mg, 0.75 mmol) was added to a solution of 2,2'-dipyridyl disulfide (165 mg, 0.75 mmol, Aldrich) and 17 (80 mg, 0.37 mmol) in 4 mL of dry acetonitrite and stirred for 1.5 h at 23^o under argon. The yellow solution was then diluted with 26 mL of dry xylenes and added dropwise over 4 h to a refluxing solution of silver perchlorate (387 mg, 1.9 mmol, Alfa) in xylenes (50 mL, under nitrogen). The reaction was refluxed for 6 h after the addition of the thioester and then filtered through a sintered glass funnel to remove the brown precipitate that had been formed. The filtrate was diluted with 160 mL of hexane and forced through 80 g (20 cm x 3 cm I.D. column) of silica gel which had been packed with hexanes. The column was washed free of xylenes with hexanes (200 mL) and then eluted with 5% EtOAc in HEX. Fractions which contained the lactone (R,S-II) were combined to give 61 mg of a material which was 78% pure by GLC analysis on column C (150^o-250^o at 10^o/min). Kugelrohr distillation of the product recovered from chromatography yielded 22 mg (30%) of (R,S)-II: bp. 60-70^o (0.1 mm), 97% pure by GLC analysis; spectroscopic data obtained for the synthetic product were identical to those obtained for the isolated natural product (see section III-12-i). GLC analysis of the distilled material on column A (program A) revealed the presence of the E isomer and 11-dodecanolide in a ratio of 376:18:1, Z:11-dodecanolide:E.

HRMS calculated for $C_{12}H_{20}O$:m/z 196.1458. Found: m/z 196.1463.

v) Synthesis of (S) and (R)-Z-3-Dodecen-11-olide (Fig. 19)

a) Preparation of (S)-1-Tert-butyldimethylsiloxy-11-hydroxy-3-dodecyne((S)-18) and (R)-18

The Grignard reagent of 13 (4.0 g, 10.5 mmol) was coupled with (S)-(-)-propylene oxide (1.2 g, 21 mmol; $[\alpha]_D^{24}$ -12.8°, neat, prepared by the Method of Seuring and Seebach, 1977), via the procedure described for the preparation of 14, to give 1.65 g (42%) of (S)-18 (α_{Obs}^{22} +4.0°, neat). Analysis by GLC (column C, 180°-250° at 10°/min) revealed a purity of 81%. The PMR spectrum (60 MHz, $CDCl_3$) of (S)-18 was identical to that of 14.

(R)-18 was prepared from 19.0 g (50 mmol) of 13 and 3.5 g (60 mmol) of (R)-(+)-propylene oxide ($[\alpha]_D^{24}$ + 13.9° (neat), prepared by the method of Johnston and Slessor, 1979) following the procedure described above. After workup and column chromatography, 4.9 g (31%) of (R)-(-)-18 was obtained: α_{Obs}^{24} -3.0°, neat, purity of 88% determined by GLC on column C (180°-250° at 10°/min; PMR spectrum of (R)-(-)-18 (60 MHz, $CDCl_3$) was identical to the spectrum of 14.

b) Preparation of (S)-(+)-11-β-Methoxyethoxymethyl-3-dodecyn-1-ol ((S)-(+)-19) and (R)-(-)-19

To an ice-cold mixture of (S)-(+)-19 (1.2 g, 3.8 mmol) and diisopropylethylamine (0.74 g, 5.7 mmol) in dry dichloromethane (4 mL), was added 0.71 g (5.7 mmol) of β-methoxyethoxymethyl chloride (MEM chloride, Aldrich). After 30 min the reaction was warmed to 23° and stirred for 5 h. The reaction was then diluted with water (15 mL), extracted with dichloromethane (3 x 25 mL) and washed with brine (2 x 25 mL). Product isolation yielded 1.60 g of the MEM ether which was 92% pure by GLC analysis on column C (180°-250° at 10°/min): PMR (60 MHz, $CDCl_3$) δ 0.08 (6H, CH_3-Si, s), 0.90 (9H, t-butyl-Si, s), 1.16 (3H, C_{12}, d, J=6.4 Hz), 1.28-1.60 (10H, C_6-C_{10}, m), 1.96-2.57 (4H, C_2, C_5, bm), 3.42 (3H, CH_3-O, s), 3.53-3.83 (7H, O-CH_2CH_2-O, C_1, C_{11}, bm), 4.72 (2H, O-CH_2-O, s). The MEM ether was stirred for 5 h in a mixture of AcOH:THF:water (3:1:1, v/v/v) at 23°C. Water (50 mL) was then added to the reaction, and the resultant aqueous mixture was extracted with ether (3 x 50 mL) and washed with brine (2 x 50 mL). Chromatography of the isolated product on silica gel (60 g, 15 cm x 3 cm I.D. column; 75% EtOAc in HEX) gave 0.78 g (72% from (S)-(+)-18) of (S)-(+)-19 (98% pure by GLC, column C, 180°-250° at 10°/min): α^{22}_{Obs} +10.8°, neat; PMR (60 MHz, $CDCl_3$) δ 1.14 (3H, C_{12}, d, J=6.0 Hz), 1.32 (10H, C_6-C_{10}, bs), 1.89-2.64 (5H, C_2, C_5, -OH, bm), 3.34 (3H, CH_3-O, s), 3.47-3.83 (7H, -OCH_2CH_2O-, C_1, C_{11}, bm), 4.69 (2H, O-CH_2-O, s).

Analysis calculated for $C_{16}H_{30}O_4$: C, 67.08; H, 10.56. Found: C, 67.34; H, 10.56.

(R)-(-)-19 was prepared from 4.5 g (14.4 mmol) of (R)-(-)-18 by the procedure described above except that the tert-butyl-dimethyl silyl ether was cleaved with tetra-n-butyl ammonium fluoride instead of the AcOH:THF: water mixture (section II-iv-f). After chromatography, 2.54 g (62% yield from (R)-(-)-18) of (R)-(-)-19 were recovered (99% pure by GLC, column D, 180°-250° at 10°/min): α^{22}_{Obs} -10.7°, neat; PMR spectrum of (R)-(-)-19 was identical to the spectrum of (S)-(+)-19.

c) Preparation of (S)-11-β-Methoxyethoxymethyl-3-dodecynoic acid ((S)-20) and (R)-20

To an ice-cold solution of chromium trioxide (1.1 g, 11 mmol) in 2N sulfuric acid (15 mL) was added dropwise 0.78 g (2.7 mmol) of (S)-(+)-19 dissolved in 30 mL of acetone. After the addition was completed (2 h), the reaction was warmed to 23° and stirred for 3 h . The acetone was removed *in vacuo* leaving a residue which was diluted with water (50 mL) and extracted with ether (3 x 50 mL). The ether extract was washed with brine (3 x 50 mL) and then subjected to product isolation which gave 0.72 g of crude product. Analysis of the crude product by GLC (column C, 180°-250° at 10°/min) after treatment with diazomethane revealed a purity of 50%. This material was utilized for the next step without further purification.

The (R) enantiomer of 20 was prepared from 2.12 g (7.41 mmol) of (R)-(-)-19. To a solution of chromium trioxide (2.97 g, 30 mmol) in 2N sulfuric acid (42 mL) maintained at 10° , was added (R)-(-)-19 (in 85 mL acetone) over 30 min. The reaction was then stirred for 3 h at 23° and worked up by the method described above. Partial purification of the crude product was achieved by chromatography on 170 g (15 cm x 5 cm I.D. column) of silica gel (EtOAc:HEX:AcOH, 49.5:49.5:1.0) : yield, 0.87 g (39%) of (R)-20, purity of 77% by GLC analysis of methyl ester (column D, 180°-250° at 10°/min); PMR (60 MHz, $CDCl_3$) δ 1.14 (3H, C_{12}, d, J=6.0 Hz), 1.36 (10H, C_6-C_{10}, bs), 1.95-2.60 (2H, C_5, bm), 3.27 (2H, C_2, t, J=1.8 Hz), 3.38 (3H, CH_3-O, s), 3.48-388 (5H, OCH_2CH_2O, C_{11}, m), 4.73 (2H, OCH_2O, s), 10.25 (1H, COOH, bs).

d) Preparation of (S)-11-hydroxy-3-dodecynoic acid ((S)-16) and (R)-16

To an ice-cold solution of (S)-20 (section II-iv-c) in 35 mL of dry dichloromethane (under nitrogen) was added 13.5 g (60 mmol) of anhydrous zinc bromide (dried under vacuum at 100° for 24 h). The resultant suspension was stirred for 2 h after which it was filtered through a sintered glass funnel, evaporated to dryness, dissolved in ether (50 mL), and washed with brine (2 x 50 mL). Chromatography of the crude product on 60 g (15 cm x 3 cm I.D. column) of silica gel (EtOAc:HEX:AcOH, 49.5:49.5:1, v/v/v) gave 71.6 mg (12.5% from (S)-(+)-19) of (S)-16 which was 84% pure by GLC analysis of the methyl ester (column C, 180°-250° at 10°/min). The PMR spectrum (60 MHz, $CDCl_3$) of (S)-16 was identical to the spectrum for racemic 16 (section II-iv-g).

A modification of the procedure described above was used for the cleavage of the MEM ether in (R)-20. To a solution of (R)-20 (720 mg, ∿1.8 mmol) in 20 mL of dry dichloromethane was added 10.7 g (47 mmol) of anhydrous zinc bromide. The suspension was shaken vigorously for exactly 5 min at 23° and then diluted with 50 mL of brine. The aqueous mixture was acidified (ph 2) with 2N sulfuric acid, extracted with dichloromethane (3 x 40 mL) and washed with brine (2 x 50 mL). Product isolation gave a crude product which was purified by chromatography on 90 g (23 cm x 3 cm I.D. column) of silica gel: yield, 190 mg of (R)-16 (49%); purity of 80% by GLC analysis of methyl ester on column D, 180°-250° at 10°/min); PMR spectrum (60 MHz, $CDCl_3$) was identical to spectrum obtained for racemic 16.

e) Preparation of (S)-17 and (R)-17

The (S) enantiomer of 16 (71.6 mg, 0.34 mmol) was hydrogenated with P-2 nickel catalyst by the procedure described in section II-iv-h. A yield of 83% was obtained for the crude product ((S)-17 which was 90% pure by a GLC analysis of the methyl ester (column C, 180°-250° at 10°/min).

(R)-17 (153 mg, 84% pure by GLC analysis) was prepared in 84% yield by the same procedure.

f) Preparation of (S)-(+)-II and (R)-(-)-II

The lactonization of (S)-17 and (R)-17 was carried out by the procedure described for (R,S)-II (section II-iv-i). Lactonization of 60.3 mg (0.28 mmol) of (S)-17 gave 15.6 mg (28%) of (S)-II after chromatography and Kugelrohr distillation. This material also contained the E isomer and 11-dodecanolide in the ratio that was observed for (R,S)-II: $[\alpha]_D^{22.5}$ =+70.5° (c=0.96, $CHCl_3$); spectroscopic data for (S)-II was identical to that obtained for (R,S)-II.

The lactonization of 128 mg (0.6 mmol) of (R)-17 gave 43 mg (37%) of (R)-II after chromatography and distillation. The ratios of the E isomer and 11-dodecanolide to (R)-II were identical to those observed for (S)-II and (R,S)-II : $[\alpha]_D^{22.5} = -78^o$ (C=16.4, $CHCl_3$).

vi) Synthesis of (5Z,13RS)-5-Tetradecen-13-olide ((R,S)-III)

a) Preparation of 1-Tert-butyldimethylsiloxy-5-hexyne (21)

To an ice-cold solution of 5-hexyn-1-ol (9.8 g, 0.1 mol, Farchan Labs) and imidazole (17 g, 0.25 mol) in dry DMF (50 mL) was added 18.1 g (0.12 mol) of t-butyldimethylsilyl chloride. After the chloride was added, the reaction was warmed to 23^o and stirred for 1.5 h . The reaction was then diluted with brine (100 mL), extracted with ether (3 x 100 mL) and washed with brine (2 x 100 mL). Distillation of the crude product through a Vigreux column gave 20.7 g (98%) of 21: bp. 39^o-41^o (0.15 mm); 99% pure by GLC analysis on column C, 120^o ; PMR (60 MHz, $CDCl_3$) δ 0.08 (6H, CH_3-Si, s), 0.91 (9H, t-butyl-Si, s), 1.50-1.80 (4H, C_2, C_3, m), 1.97 (1H, C_6, t, J=2.8 Hz), 2.26 (2H, C_4 m), 3.71 (2H, C_1, m). Analysis calculated for $C_{12}H_{24}OSi$: C, 67.86; H, 11.39. Found: C, 67.95; H, 11.49.

b) Preparation of 1-Tert-butyldimethylsiloxy-11-chloro-5-undecyne (22)

n-Butyllithium (23 mL, 2.2M, 50.6 mmol) was added dropwise over 30 min to an ice-cold solution of 21 (10.6 g, 50 mmol) in 25 mL of dry THF (under argon). The lithium acetylide of 21 was then transferred to a dropping funnel and added over 1 h to a solution of 11 in a mixture of THF and HMPA (25 m and 10 mL) maintained at -50^o . After the acetylide addition was complete, the reaction was warmed to 23° and stirred for 3 h . Workup was carried out by dilution of the reaction with water (100 mL), extraction with ether (3 x 75 mL) and washing with brine (2 x 100 mL). Vacuum distillation of the crude product through a Vigreux column gave 13.2 g (70%) of 22 (96% pure by GLC analysis on column C, 120°-200° at 10°/min): bp. 101-118°C (0.05 mm); PMR (60 MHz, $CDCl_3$) δ 0.08 (6H, CH_3-Si, s), 0.90 (9H, t-butyl-Si, s), 1.34-1.94 (10H, C_2, C_3, C_8-C_{10}, bm), 2.15 (4H, C_4, C_7m), 3.53 (2H, C_{11}, t, J=6.0 Hz), 3.62 (2H, C_1, t, J=6.0 Hz); mass spectrum (EI) m/z (relative intensity) 259 (2.3), 149 (13), 125 (7), 123 (13), 121 (10), 107 (42), 95 (49), 93 (100), 91 (32), 81 (89), 79 (59), 77 (24), 75 (55), 73 (38), 67 (68), 55 (30). Analysis calculated for $C_{17}H_{33}OSiCl$: C, 64.41; H, 10.49. Found: C, 64.09; H, 10.61.

c) Preparation of 1-Tert-butyldimethylsiloxy-13-hydroxy-5-tetradecyne (23)

A 100 mL three-necked flask fitted with a dropping funnel and reflux condensor was flushed with nitrogen and charged with 1.4 g (58 mmol) of magnesium turnings (Fisher) and 30 mL of dry THF. The chloride (22; 9.5 g, 30 mmol) was added to the magnesium turnings followed by three drops of n-butyl bromide to initiate Grignard formation. After 10 h of reflux, Grignard formation was complete. The reaction was cooled to 23° , charged with 2.9 g (15 mmol) of cuprous iodide and stirred for 15 min. Propylene oxide (3.5 g, 60 mmol), diluted into 5 mL of dry THF, was added dropwise over 20 min to the black reaction mixture which was maintained at 0°C. The reaction was then warmed to 23° and stirred for 1 h before being quenched with saturated aqueous ammonium chloride (75 mL). The aqueous mixture was extracted with ether (3 x 75 mL) and the ether extract washed with brine (2 x 75 mL). Product isolation yielded 10.8 g of crude product which was chromatographed on 180 g (20 cm x 4.5 cm I.D. column) of silica gel (22.5% EtOAc in HEX): yield, 4.8 g (47%) of 23; GLC analysis on column C (140°-240° at 10°/min) revealed a purity of 98%; PMR (60 MHz, $CDCl_3$) δ 0.08 (6H, CH_3-Si, s), 0.92 (9H, t-butyl-Si, s), 1.20 (3H, C_{14}, d, J=6.0 Hz), 1.34-1.70 (15H, C_2, C_3, C_8-C_{12}, OH, bm), 2.18 (4H, C_4, C_7, m), 3.65 (2H, C_1, t, J=5.5 Hz), 3.47-3.95 (1H, C_{13}, bm); mass spectrum (EI) m/z (relative intensity) 283 (1.5), 281 (3.0), 189 (3.0), 145 (5), 135 (5), 121 (8), 109 (18), 107 (17), 105 (15), 95 (27), 93 (21), 81 (28), 79 (22), 75 (100), 73 (26), 55 (18)

HRMS calculated for $C_{20}H_{40}O_2Si$: m/z 340.3878. Found: m/z 340.2785.

d) Preparation of 13-β-Methoxyethoxymethyl-5-tetradecyn-1-ol (24)

The MEM ether of 23 was prepared from 4.4 g (13 mmol) of 23 by the method described for the preparation of (S)-19 (section II-v-b). Cleavage of the silyl ether was performed directly on the crude product with 26 mL (26 mmol) of tetra-n-butylammonium fluoride (section II-v-b). After chromatography on 225 g (20 cm x 5 cm I.D. column) of silica gel (75% EtOAc in HEX), 3.8 g (94% from 23) of 24 was recovered (98.5% pure by GLC analysis on column C, 180°-250° at 10°/min): PMR (60 MHz, $CDCl_3$) δ 1.15 (3H, C_{14}, d, J=6.0 Hz), 1.27-1.80 (14H, C_2, C_3, C_8-C_{12}, bm), 1.94-2.30 (5H, C_4, C_7, OH, bm), 3.36 (3H, CH_3O, s), 3.62 (7H, OCH_2CH_2O, C_1, C_{13}, m), 4.72 (2H, OCH_2O, s). Analysis calculated for $C_{18}H_{34}O_4$: C, 68.31; H, 11.47. Found: C, 68.19; H, 11.29.

e) Preparation of 13-hydroxy-5-tetradecynoic acid (25)

To an ice-cold solution of chromium trioxide (2.8 g, 28 mmol) in 30 mL of 2N sulfuric acid was added 2.2 g (7.0 mmol) of 24 in 70 m of acetone (over 3 h). The reaction was maintained at 0° and stirred for 7 h before workup. The residue left after removal of the acetone (rotary evaporation) was dissolved in ether (75 mL) and washed with 100 mL of water. The aqueous layer was extracted with ether (2 x 75 mL) and the ether extracts combined and extracted with 2N aqueous sodium hydroxide (3 x 25 mL). The sodium hydroxide extract was then acidified (pH 2) with concentrated HCl, extracted with ether (3 x 75 mL), and the ether extracts were washed with brine (2 x 75 mL). The crude product (1.2 g, MEM-acid) was subjected to MEM ether cleavage without further purification.

To an ice-cold solution of the crude MEM-acid (993 mg, 3.0 mmol) in 50 mL of dry dichloromethane was added 16.8 g (75 mmol) of anhydrous zinc bromide. After 3 h of vigorous stirring, the reaction mixture was filtered through a sintered glass funnel and the filtrate was evaporated to dryness. The residue was dissolved in ether (50 mL) and then extracted with 2N aqueous sodium hydroxide (3 x 25 mL). Acidification (pH 2, c. HCl) of the sodium hydroxide extract followed by ether extraction (3 x 50 mL) and a brine wash (2 x 50 mL) gave the crude product. Product isolation yielded 551 mg (76%) of 25. Analysis of the methyl ester of crude 25 by GLC on column C (180°-250° at 10°/min) revealed a purity of 76%. The major impurity was the starting material (MEM-acid, 10.8%). PMR spectrum (60 MHz, $CDCl_3$) of the crude 25: δ 1.22 (3H, C_{14}, d, J=6.0 Hz), 1.39 (12H, C_3, C_8-C_{12}, bs), 1.67-2.70 (6H, C_2, C_4, C_7, bm), 3.70 (1H, C_{13}, bm), 7.95 (2H, OH, COOH, bs), signals at 3.36, 3.6, and 4.7 are due to the unreacted MEM-acid (∼11%).

f) Preparation of 13-Hydroxy-5-Z-tetradecenoic acid (26)

The hydrogenation of 25 (710 mg, 2.9 mmol) with P-2 nickel catalyst was carried out via the procedure described for the preparation of 17 (section II-iv-h). After workup and product isolation, 508 mg (72%) of crude 26 was obtained: the product was 72% pure by GLC analysis of the methyl ester of 26 on column C (180°-250° at 10°/min); PMR (60 MHz, $CDCl_3$) δ 1.19 (3H, C_{14}, d, J-6.0 Hz), 1.33 (12H, C_3, C_8-C_{12}, bs), 1.60-2.57 (6H, C_2, C_4, C_7, bm), 3.70 (1H, C_{13}, m), 5.35 (2H, C_5, C_6, m), 8.34 (2H, OH, COOH, bs). The crude hydroxy-acid (26) was subjected to lactonization without purification.

HRMS calculated for $C_{14}H_{26}O_3$: m/z 242.1875. Found: m/z 242.1880.

g) Preparation of (5Z,13RS)-5-Tetradecen-13-olide ((R,S)-III)

The unpurified hydroxy-acid (26; 508 mg, ∼1.5 mmol) was lactonized by the procedure described in section II-iii-f. The residue left after evaporation of the toluene was chromatographed on 160 g (23 cm x 3 cm I.D. column) of silica gel (6% EtOAc in HEX). The product recovered after chromatography was distilled (Kugelrohr) to give 32 mg (9.5%) of (R,S)-III: bp. 120°-130° (0.1 mm); 98% pure by GLC analysis on column C (150°-250° at 10°/min), analysis on column A (program A) also revealed a purity of 98% and did not show any E isomer or 13-tetradecanolide. The chromatographic and spectroscopic parameters for synthetic (R,S)-III were identical to those obtained for the natural isolate.

HRMS calculated for $C_{14}H_{24}O_2$: m/z 224.1776. Found: 224.1779.

10) Field Test of Synthetic Pheromones

A field test of the synthetic pheromones, I and (R,S)-II, was conducted in grain storage facilities located in southern Manitoba in collaboration with Dr. S.R. Loschiavo (Agriculture Canada, Winnipeg Research Station). The tests were carried out between September 16 and October 5, 1981.

i) Pheromone Formulation and Release Rate Determination

A mixture which contained I and (R,S)-II (9:1, w/w) was used as the bait since it was determined to be the most attractive combination in the laboratory bioassay (section III-13). Racemic II (13.8 mg) was thoroughly mixed with 124 mg of I to give 138 mg of the pheromone bait. Analysis of the bait by GLC (column A, program A) showed the presence of I, (R,S)-II, 11-dodecanolide and the E isomer of (R,S)-II in the ratio of 3615:377:18:1. The bait was loaded into a hollow polyester fibre (100 cm x 0.20 mm I.D., Conrel Division, Albany International Co., Columbus, Ohio) by the application of suction via a syringe (the fibre was inserted through a rubber septum which was fitted onto the syringe), and sealed into 3 cm sections with a warm soldering iron. The loaded fibre was then cut at the sealed portions with a razor blade to give pheromone-filled fibres (3 cm long, ∼1 mg pheromone) sealed at both ends. These fibres were stored in a Teflon-lined screw-cap vial until required.

The release rate of the pheromone from the fibres was determined by opening one end of each fibre with a sharp razor blade (to avoid deformation of the circular opening) and measuring the meniscus drop at 24 h intervals. The release rate was only determined for pure I at 20^o and 30^o.

ii) Trap Design and Testing Procedure

The trap (Fig. 1) used in the field test was designed by Loschiavo and Atkinson (1973). Pheromone-loaded fibres (one or two) were taped to the end of a plastic drinking straw (∿14 cm), placed in the insect collecting vial and inserted into the trap so that the fibres were visible through the perforated brass screen. Baited traps and unbaited control traps were placed at various positions and depths (1 ft (30 cm), 5 ft (152 cm) and 8 ft (244 cm) in the annex bin of a grain elevator (Manitoba Pool, Elm Creek, Manitoba). Trap locations are shown in Fig. 14. After one week the traps were recovered and the number of beetles determined by counting or by weight (3450 rusty grain beetles/g).

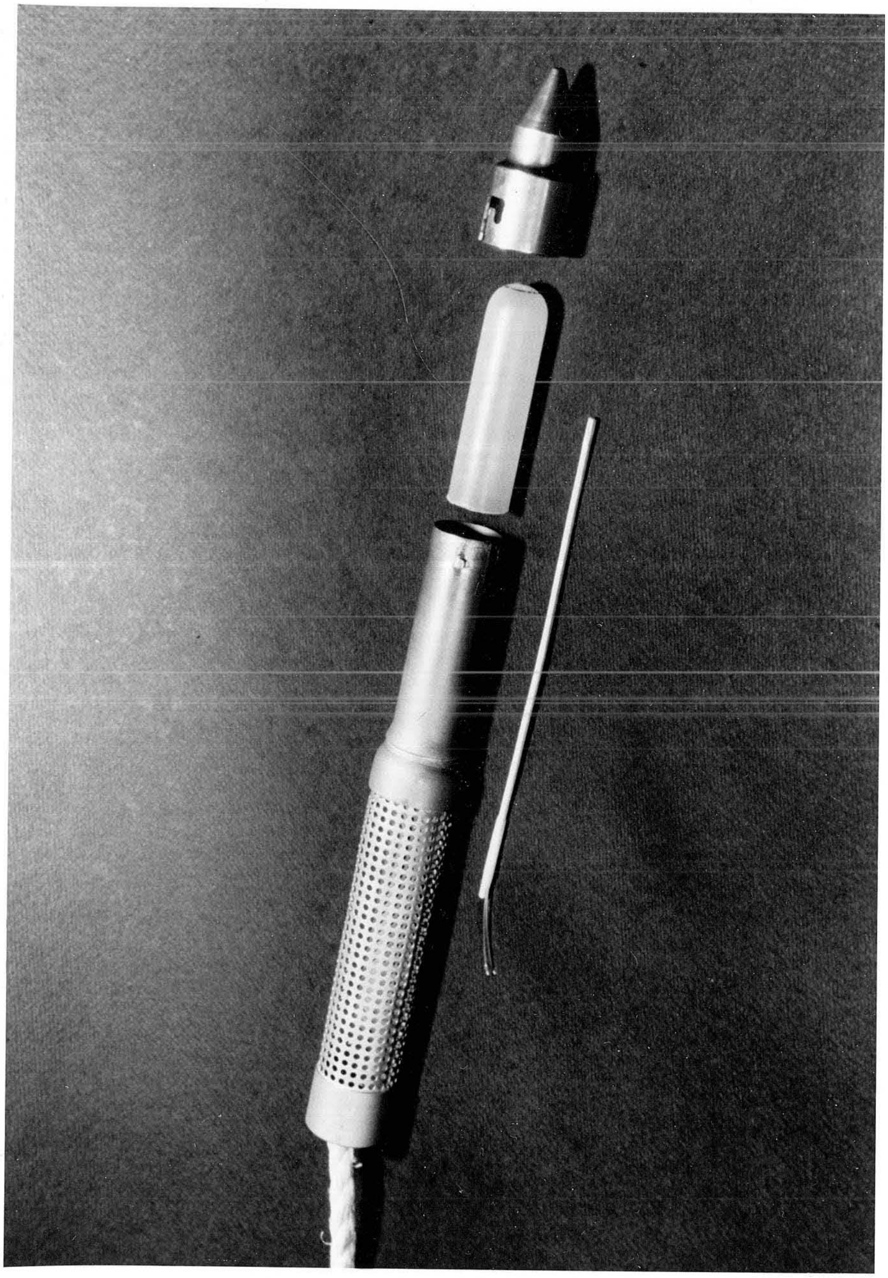

Figure 1. Rusty grain beetle trap and Conrel fibres loaded with pheromone bait

III. RESULTS AND DISCUSSION

11) Response of *C. ferrugineus* to Attractive Stimuli in the Two-Choice, Pitfall Olfactometer

Results from previous experiments (Verigin, 1980) in which preparative GLC fractionations of frass volatiles were monitored by arena olfactometer bioassays, indicated that two or possibly three of the male-produced compounds remained to be identified as pheromones for the rusty grain beetle. However, because the fractionations and analyses were performed on packed columns, it is possible that the activity in a fraction was due to an inseparable minor component instead of the major component. Since the male-produced compounds all have similar retention times, the cross-contamination of adjacent fractions was a significant possibility. A second problem which hindered the identification of this pheromone was due to the arena olfactometer bioassay. In addition to the drawbacks mentioned in section II-6, the arena olfactometer assay also suffered from variability of response to experimental and control stimuli. Therefore, a quantitative assay which would yield reproducible responses to a given stimulus was required. The two-choice, pitfall olfactometer developed by Dr. A. Pierce (Pierce *et al.*, 1981) satisfied these requirements in that reproducible responses of greater that 95% were obtained for attractive stimuli. Because of the two problems discussed above, the repetition of some of Verigin's experiments was required. The response of *C. ferrugineus* to frass and beetle volatiles, and fractions thereof, was monitored by the two-choice, pitfall olfactometer, and the results obtained were used in conjunction with Verigin's results to identify which compounds were the rusty grain beetle pheromones.

i) Beetle Volatiles

The GLC analysis (column A, program A) of beetle volatiles resulted in a chromatogram which was virtually devoid of peaks except for the region between 28 min and 38 min (Fig. 2). Peaks I-IV were detected in male beetle volatiles only (Verigin, 1980). The trace components, V, VII and VIII were not detected in Verigin's experiment; however, this was probably due to the higher detection limit for the packed column which was used in his analysis. Quantitation by the internal standard method revealed the presence of I-V in the ratio of: 11.7:13.9:1.8:1.5:0.2 ppm (w/w/w/w/w). Compounds VII and VIII were also present in small quantities (<0.5 ppm).

The response of *C. ferrugineus* (mixed sexes, 1-3 months) to beetle volatiles in the two-choice, pitfall olfactometer is shown in Figure 3 (bottom). The threshold stimulus (1000 bh) was much higher than the previous value (34 bh) determined by Borden *et al.* (1979). This difference in the threshold stimulus may be caused by the different olfactometer assays which were used.

The separation of beetle volatiles by HPLC (section II-7-ii) gave fractions in which the major compounds, I and II, were completely separated. Fraction 8 (16.8-18.5 mL) only contained II, uncontaminated by any of the other compounds found in the beetle volatiles, while fraction 9 (18.5-20.0 mL) contained I and IV in the ratio of 9.3:1. The responses of *C. ferrugineus* to the separate and recombined HPLC fractions were compared to beetle volatiles in Figure 4. While fraction 8 was inactive and fraction 9 was only moderately attractive the combined mixture of 8 and 9 (I:II:IV, 9.5:7:1) was as attractive as the unfractionated beetle volatiles. Compound

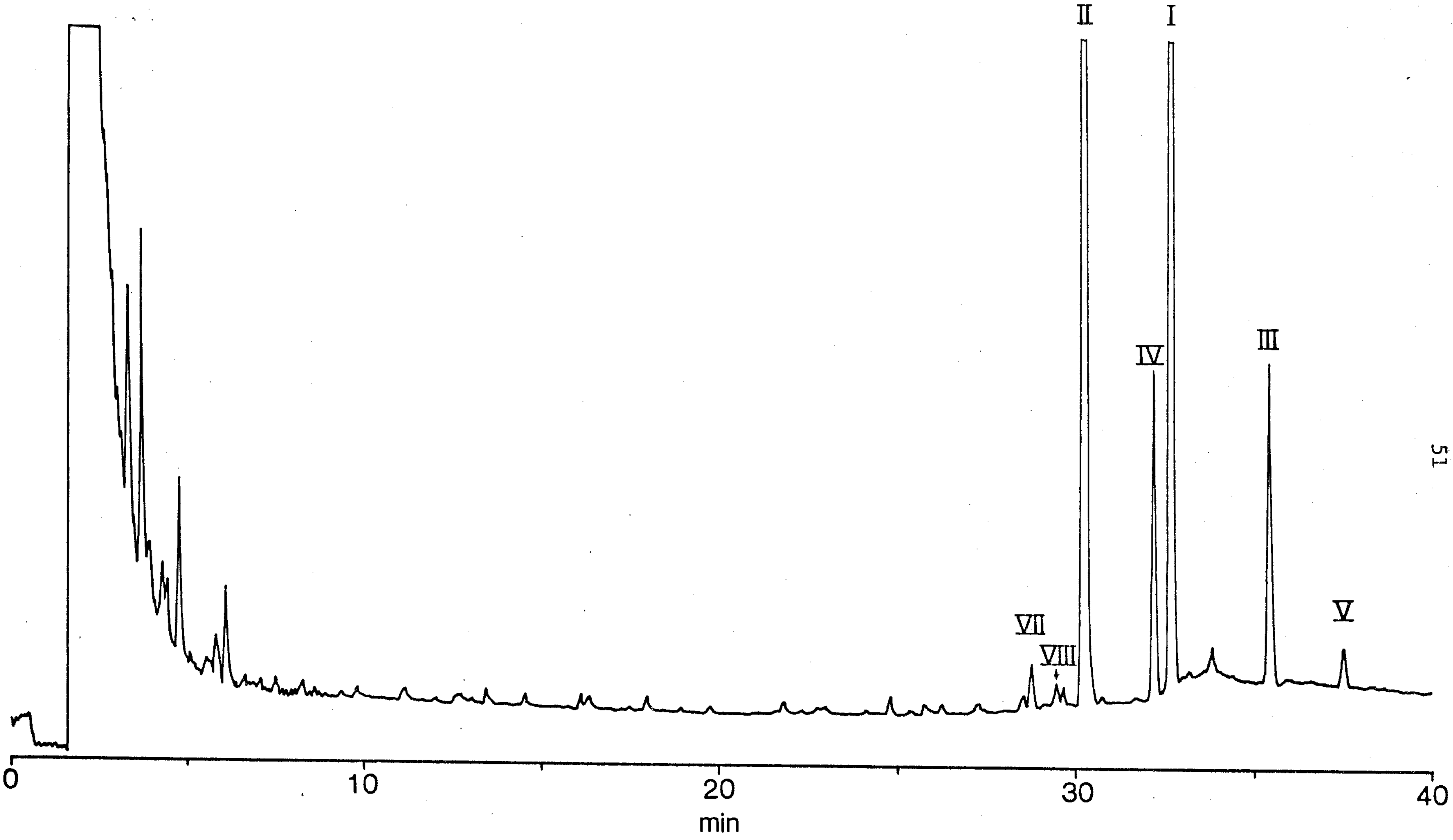

Figure 2. Gas-liquid chromatogram of the pentane extract of Porapak Q-captured beetle volatiles (column A, program A)

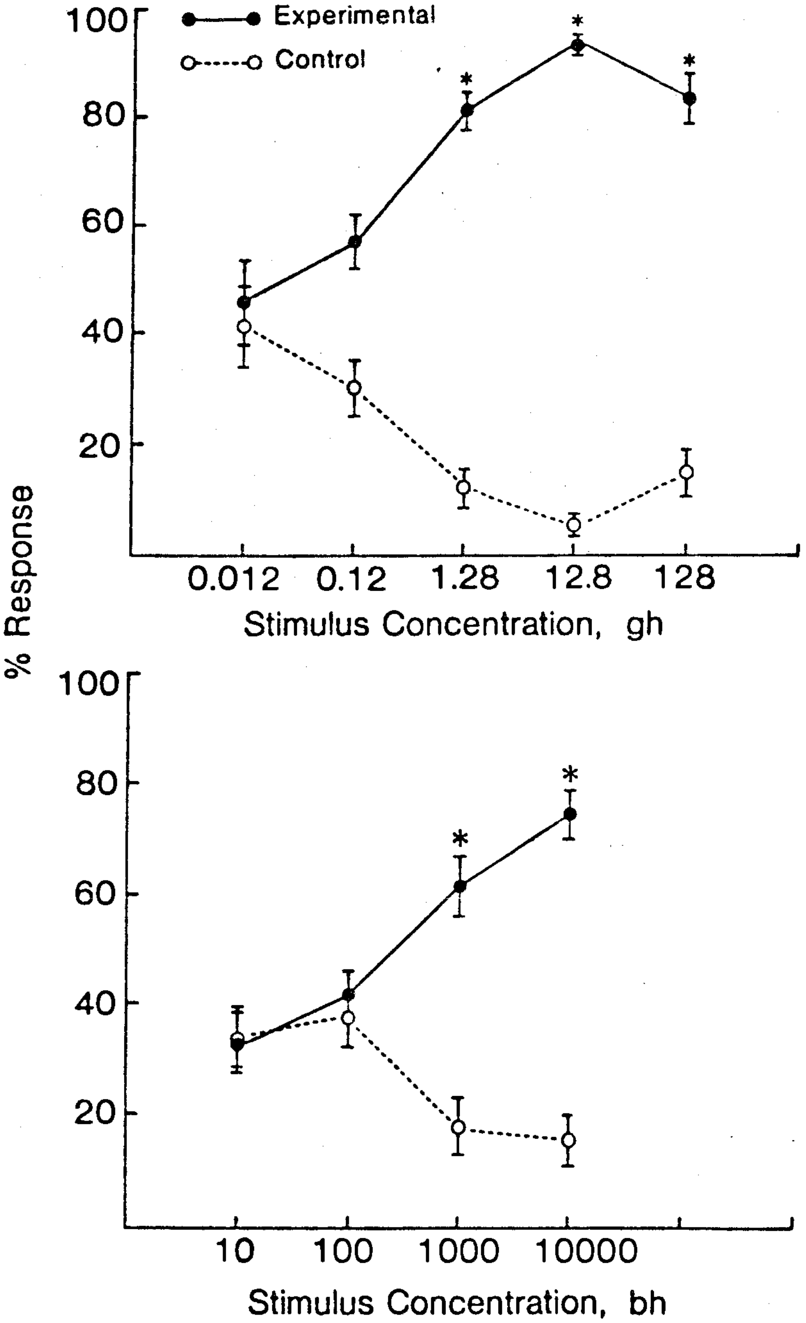

Figure 3. Response of *C. ferrugineus* to Porapak Q-captured volatiles of frass (top) and beetles (bottom) in the two-choice, pitfall bioassay. A significant response (t-test), to experimental stimulus is indicated by *P<0.01. N=6 replicates

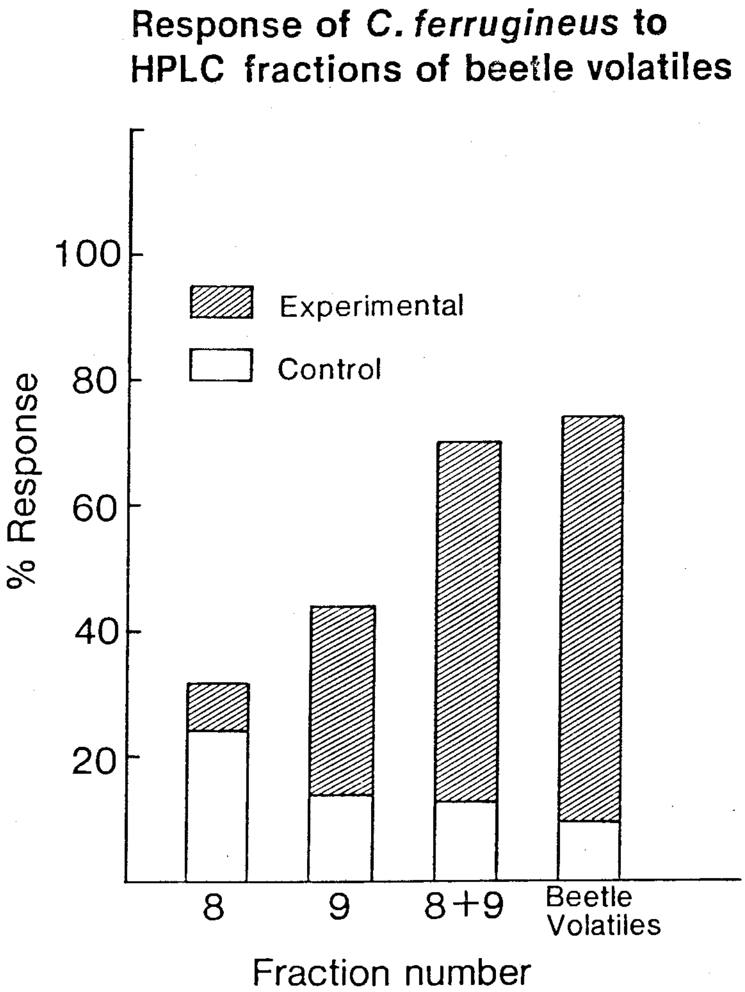

Figure 4. Response of *C. ferrugineus* to HPLC fractions of beetle volatiles in the two-choice, pitfall bioassay. Fraction 9, Fraction 8 + 9 and beetle volatiles significantly different from controls, t-test, P<0.001; Fraction 8, not significant. N=10 replicates

III was not added to the mixture and was not required for the recovery of total activity. This experiment indicated that the rusty grain beetle pheromone is composed of two or perhaps three synergistic components.

ii) Frass Volatiles

A GLC analysis of frass volatiles revealed the presence of the beetle-produced compounds (I-V, VII, VIII), and many other compounds which eluted earlier in the chromatogram (Fig. 5). We noted the presence of compound VI, which was not detected in beetle volatiles. The concentrations of I-V in frass volatiles were 3.0:1.8:0.17:0.22:0.02 ppm (w/w/w/w/w).

The response of *C. ferrugineus* to frass volatiles is shown in Figure 3 (top). The threshold stimulus of 1.28 gh is comparable to the 0.67 gh threshold stimulus observed by Borden *et al.* (1979). A direct comparison can be made between beetle and frass volatiles since 1.28 gh of frass volatiles contains approximately the same quantity of I and II as 1000 bh of beetle volatiles. While the threshold for activity is approximately the same for both stimuli, frass volatiles are more attractive. The source of this increased attractiveness may be fungal volatiles derived from the fungi which are visibly present in *C. ferrugineus* cultures. Dolinski and Loschiavo (1973) have shown that *C. ferrugineus* responded positively to fungus-infected wheat kernels in a laboratory bioassay. The presence of a fungal attractant has also been implicated for another stored-product insect, *Oryzaephilus mercator* (Pierce *et al.*, 1981). The decrease in response observed at the highest concentration of frass probably indicates sensory adaptation (Seabrook, 1977; Pierce *et al.*, 1981).

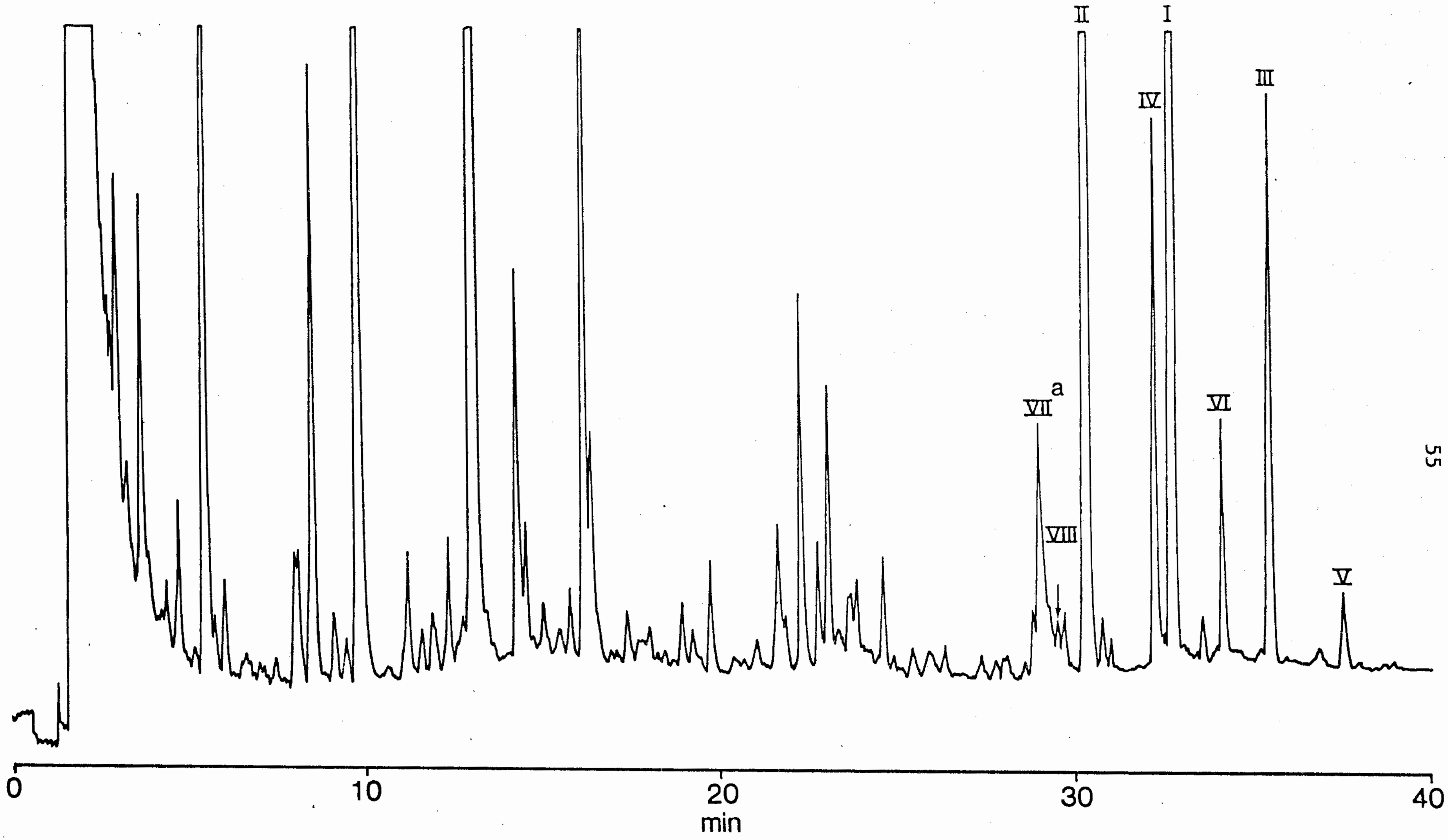

Figure 5. Gas-liquid chromatogram of the pentane extract of Porapak Q-captured frass volatiles (column A, program A). [a]Compound VII coeluted with a peak at 28.8 min

The fractionation of frass volatiles by HPLC was carried out in an attempt to locate the remaining biological activity. The response of *C. ferrugineus* to the 39 fractions that were obtained is shown in Figure 6. While fractions which contained II (9 and 10) appeared to be slightly attractive, the fraction which contained I (12) was not attractive. Some fractions (32-34) even seemed to be repulsive. However, the most important feature of Figure 6 was that none of the individual fractions were significantly attractive. These results supported the previous observation (section III-11-i) that suggested the requirement for two or more synergists for maximum activity. Fractions were recombined in an attempt to reconstitute total activity. The response of *C. ferrugineus* to the recombined fractions is shown in Table 1. Attention was focused on the fractions which contained I-IV, since they were in attractive fractions that were isolated from beetle volatiles. Note that the fractions containing I (12) and II (9,10) are individually attractive, but that the fraction containing III was not. The addition of III (fraction 7) to fractions which contained I or II did not result in any increase in activity. However, the recombination of fractions 9, 10 and 12 yielded a mixture which was significantly more attractive than the individual fractions. This result supports the previous observation (section III-11-i) that mixtures which contain I, II and perhaps IV are synergistic. No increase or decrease in activity was observed when III was added to the mixture of I, II and IV. Therefore, III does not possess any pheromonal activity for *C. ferrugineus*. This result is contrary to Verigin's data which indicated that III was attractive to *C. ferrugineus* in the arena olfactometer bioassay.

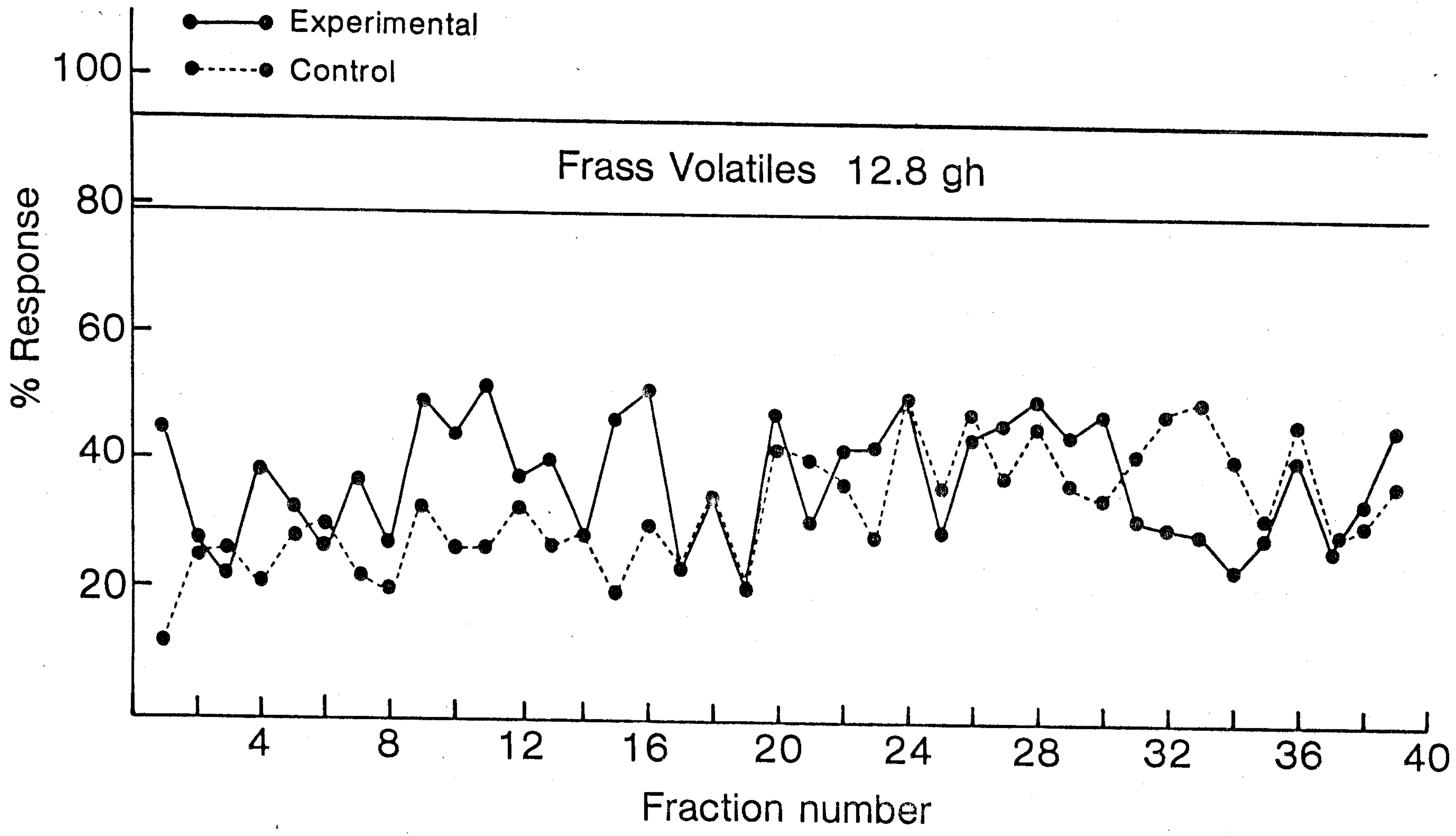

Figure 6. Response of *C. ferrugineus* to HPLC fractions of frass volatiles in the two-choice, pitfall bioassay. N=6 replicates

Table 1. Response of *C. ferrugineus* to individual and recombined HPLC fractions of frass volatiles in the two-choice, pitfall, olfactometer. N=6 replicates.

Experimental[b] Stimulus	Stimulus Concentration, gh	% Response[a] ($\bar{X}$ ± S.E.)	
		Experimental Stimulus	Blank Control
Fraction 7	128	45.6 ± 4.2	32.2 ± 4.2 NS
	12.8	30.0 ± 6.3	38.9 ± 6.3 NS
III:unknowns	1.28	27.8 ± 3.0	35.6 ± 3.0 NS
16:1	0.128	26.7 ± 5.4	36.7 ± 5.4 NS
Fraction 9	128	74.4 ± 3.2	25.6 ± 3.2**
	12.8	61.1 ± 5.4	18.9 ± 5.4*
II:VII:unknowns	1.28	57.8 ± 6.6	24.4 ± 6.6 NS
70: 1:5	0.128	26.7 ± 5.2	36.7 ± 5.2 NS
Fraction 10	128	84.4 ± 3.4	15.6 ± 3.4***
	12.8	64.4 ± 2.8	30.0 ± 2.8**
II:VII:unknowns	1.28	54.4 ± 6.6	37.8 ± 6.6 NS
80: 1:6	0.128	51.1 ± 6.3	20.0 ± 6.3 NS
Fraction 12	128	68.9 ± 6.7	25.6 ± 6.7*
	12.8	77.8 ± 2.5	11.1 ± 2.5***
I:IV:unknowns	1.28	58.9 ± 3.1	18.9 ± 3.1**
14:1:7	0.128	32.2 ± 4.7	37.8 ± 4.7 NS
Fractions 7 and 12	128	63.3 ± 3.7	17.1 ± 3.7**
	12.8	72.2 ± 3.7	22.2 ± 3.7**
I:III:IV:unknowns	1.28	55.6 ± 6.7	23.3 ± 6.7 NS
17: 1:1.3:7	0.128	37.8 ± 4.5	23.3 ± 4.5 NS

Table 1. (cont'd)

Experimental[b] Stimulus	Stimulus Concentration, gh	% Response[a] ($\bar{X}$ ± S.E.) Experimental Stimulus	% Response[a] ($\bar{X}$ ± S.E.) Blank Control
Fraction 7, 9 and 10	128	72.2 ± 3.7	21.1 ± 3.7**
	12.8	73.3 ± 4.6	12.2 ± 4.6**
II:III:unknowns	1.28	47.8 ± 6.2	27.8 ± 6.2 NS
16:1.3:1	0.128	38.9 ± 4.6	33.3 ± 4.6 NS
Fraction 9, 10 and 12	128	88.9 ± 3.7	10.0 ± 3.7***
	12.8	82.2 ± 1.9	14.4 ± 1.9**
I:II:IV:unknowns	1.28	68.9 ± 3.5	26.7 ± 3.5**
13:9: 1:6	0.128	36.7 ± 8.7	32.2 ± 8.7 NS
Fraction 7, 9, 10 and 12	128	74.4 ± 3.8	17.8 ± 3.8**
	12.8	85.6 ± 3.9	13.3 ± 3.9***
I:II:III:IV:unknowns	1.28	67.8 ± 5.5	25.6 ± 5.5*
17:12:1:1.3:5:5	0.128	27.8 ± 6.6	24.4 ± 6.6 NS
Frass volatiles	128	82.7 ± 4.0	14.7 ± 4.0***
	12.8	93.3 ± 2.0	5.6 ± 2.0***
I:II:III:IV:unknowns	1.28	81.1 ± 3.4	12.2 ± 3.4***
16:11:1:1.3:38	0.128	56.7 ± 5.1	30.0 ± 5.1 NS

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[a] Significant response (t-test) to experimental stimulus was indicated by: ***P<0.001, **P<0.01, *P<0.05, NS=not significant.

[b] The ratios of compounds reported for the individual and recombined fractions of frass volatiles were calculated from the counts detected for each compound by GLC. The presence of compounds which eluted before the beetle-produced compounds was indicated by : unknowns.

A comparison of the responses of *C. ferrugineus* to the mixture of fractions 9, 10 and 12 and unfractionated frass volatiles showed that the mixture (fractions 9, 10 and 12) was only 88% as attractive as frass volatiles. Other HPLC fractions of frass volatiles were added to the mixture of fractions 9, 10 and 12 in an attempt to locate the remaining activity. However, none of the fractions tested enhanced the activity of fractions 9, 10 and 12. Indeed the recombination of all 39 fractions produced a mixture which was no more attractive than fractions 9, 10 and 12. Apparently, 12% of the activity in frass volatiles was lost during HPLC fractionation. A selective loss of the more volatile components could have occurred during concentration of the pentane extract under a nitrogen stream. Another possibility is that a loss of activity could have occurred during passage of the frass volatiles through the silica column. It is clear from these experiments however, that two or three compounds (I, II and IV) are responsible for all of the activity in beetle volatiles and 88% of the activity in frass volatiles.

iii) Pure Synthetic I and Pure Isolated II

The response of *C. ferrugineus* to pure I and II was tested in the two-choice olfactometer to determine whether IV was required for maximum pheromone activity (Table 2). Synthetic I (>99% pure by GLC analysis (splitless injection) in columns A and C) was prepared by the route described in section II-9-iii. Compound II was isolated from frass volatiles by the procedure described in section II-7-iii. Analysis of isolated II by GLC (column A, splitless injection) revealed the absence of other male beetle-produced compounds, and a purity of greater than 99%. The mixture of I and

Table 2. Response of *C. ferrugineus* to pure synthetic I and pure isolated II, individually and in combination, in the two-choice, pitfall, olfactometer.

Experimental Stimulus	Stimulus Concentration ng	No. of Replicates	% Response[a] ($\bar{X}$ ± S.E.)	
			Experimental Stimulus	Blank Control
Synthetic I	200	6	53.3 ± 1.3	35.6 ± 1.3**
	20	6	53.3 ± 4.3	34.4 ± 4.3 NS
	2.0	6	50.0 ± 5.3	40.0 ± 5.3 NS
	0.2	6	33.3 ± 6.6	54.4 ± 6.6 NS
Isolated II	200	6	65.6 ± 6.3	26.7 ± 6.3*
	20	6	41.1 ± 6.4	34.4 ± 6.4 NS
	2.0	6	47.8 ± 5.0	35.6 ± 5.0 NS
	0.2	6	42.2 ± 6.7	38.9 ± 6.7 NS
I and II (1:1, w/w)	400	10	70.7 ± 3.2	20.0 ± 3.2***
	40	10	60.7 ± 3.7	40.0 ± 3.7**
	4.0	10	50.0 ± 3.2	40.0 ± 3.2 NS
	0.4	10	51.3 ± 4.1	43.3 ± 4.1 NS

[a] Significant response (*t*-test) to experimental stimulus was indicated by: ***$P<0.001$, **$P<0.01$, *$P<0.05$, NS=not significant.

II was significantly more attractive than the individual pure compounds, and was equal in activity to the mixture of fractions 8 and 9 (Fig. 4) and beetle volatiles (Fig. 3, bottom; 10^4 bh contains approximately 200 ng, each of I and II). The results shown in Table 2 confirmed that all of the activity in beetle volatiles was accounted for by a mixture of I and II and that compound IV was not needed. Frass volatiles obviously contained one or more compounds which added to the activity of I and II. Note that while pure II (Table 2) elicited a response which was the same as that obtained for fraction 9 and 10 (Table 1, 12.8gh ∿200 ng of II), the response observed for fraction 12 (Table 1) was higher than the response elicited by pure I. This observation suggested that the unknowns in fraction 12 were responsible for some attraction. Since host volatiles (wheat and wheat germ) were not significantly attractive (Borden *et al.*, 1979), the additional activity in frass volatiles was probably due to fungal volatiles.

iv) Summary of Bioassay Results

The HPLC fractionation of beetle and frass volatiles coupled with quantitative bioassays confirmed Verigin's observation that II was required for pheromone activity. Compound III, however, was shown to be inactive either alone or in combination with I and II. The deletion of IV from a mixture of I, II and IV did not result in a decrease in activity. Therefore, IV does not appear to play a role in the *C.ferrugineus* pheromone, which is composed of two synergistic components (I and II). The possible presence of fungal attractants in frass volatiles was not pursued any further in this project.

12) Identification of Pheromones and Related Compounds Isolated from *C. ferrugineus* Frass Volatiles

Although bioassay data had indicated that only compound II remained to be identified as a *C. ferrugineus* pheromone, all of the unknowns (II-VIII) were subjected to structure elucidation. One reason for the identification of all the unknowns was to see whether they were isomers of I and II, or structurally related compounds that could have an interspecific communication function. Species differentiation by the use of different geometric isomers of pheromones, is known for a couple of coleopterous insects. The black carpet beetle, *Attagenus megatoma*, uses E,Z-3,5-tetradecadienoic acid as a sex-attractant, while the Z,Z- isomer is used by *A. elongatulus* (Fukui *et al.*, 1977). Males of five *Trogoderma* spp. are attracted by E and Z-14-methyl-8-hexadecenal.*T. variabile* and *T. inclusum* release only the Z- isomer while *T. glabrum* releases the E- isomer. A 9:1 mixture of Z:E is produced by female *T. granarium* (Levinson and Levinson, 1979).

Frass volatiles were chosen as the starting material for pheromone isolation even though the concentrations of compoundsII-VIII were higher in beetle volatiles on a weight basis (i.e. μg pheromone/g of frass or beetles). The reasons for this choice were: 1) frass could be accumulated for large scale aerations, and 2) the aeration of frass did not require the time consuming feeding periods required during beetle aerations.

The proposed structures for compounds II-VIII, which are all macrolides except for VI which is a γ-lactone, are shown in figure 7 along with the structure of I which was identified by Verigin (1980).

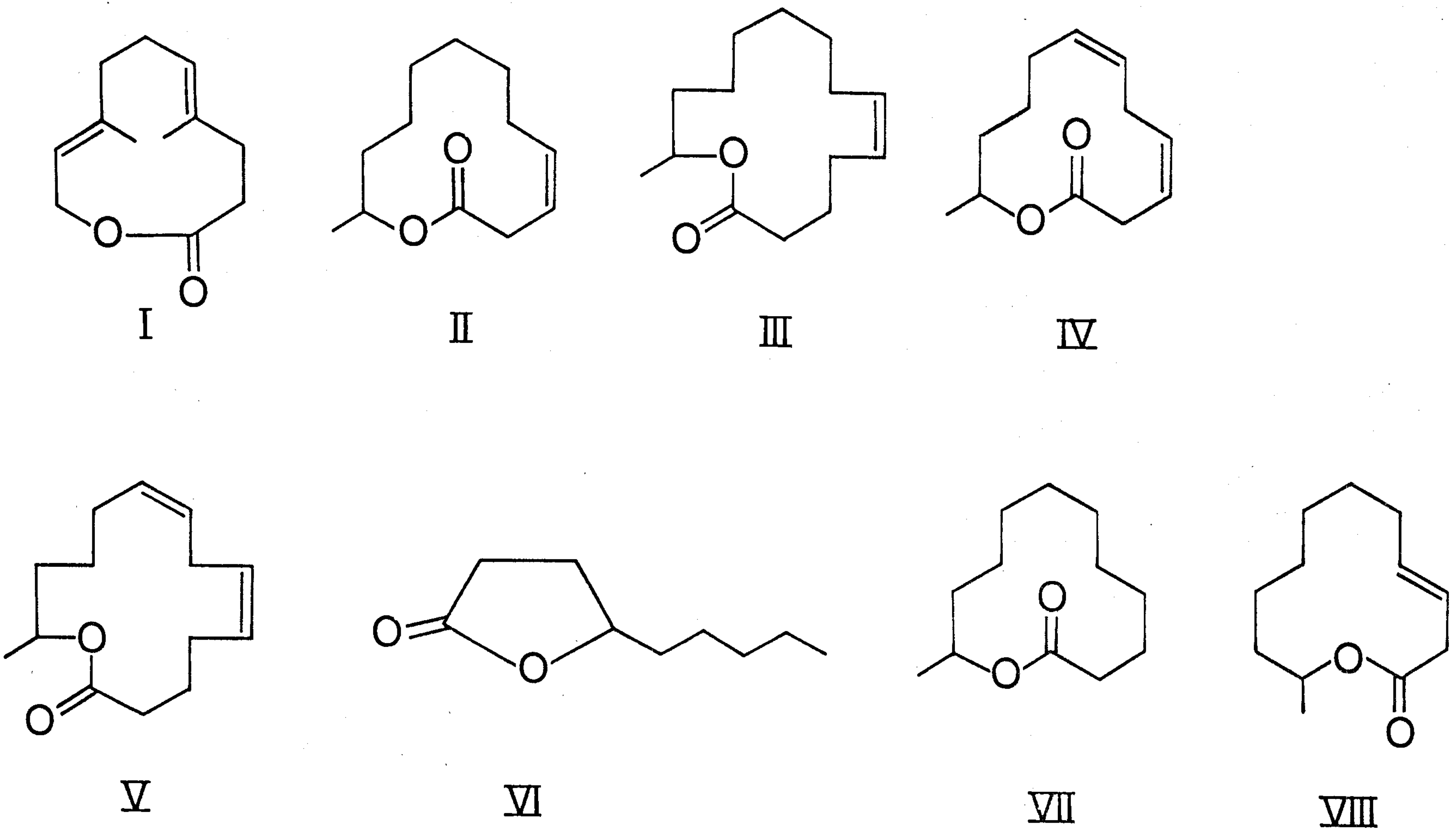


Figure 7. Structures of macrolides isolated from *C. ferrugineus* frass volatiles

i) Identification of II

The highest peak in the unit resolution mass spectrum of II (Fig. 8) was observed at m/z 196. A prominent peak at m/z 214 ($M^+ + NH_4$) in the chemical ionization mass spectrum (NH_3 reagent gas) suggested that the m/z 196 ion was the molecular ion. The high resolution mass spectrum revealed an exact mass of 196.1449 which indicated a molecular formula of $C_{12}H_{20}O_2$ (three sites of unsaturation). Although the molecular weights of I and II only differed by two mass units, the electron impact mass spectra were quite different. A small peak at m/z 181 (M-15) in the mass spectrum of II was indicative of a methyl branch in the molecule. Groups of peaks separated by fourteen mass units (m/z 123, 109, 95, 81, 67) suggested the presence of an unsaturated hydrocarbon chain. The IR spectrum contained a strong absorption at 1730 cm^{-1} (C=O stretch), and was devoid of a hydroxyl absorption which indicated that both oxygens were in an ester or lactone function. The presence of an even mass fragment ion at m/z 136 (M-60) in the mass spectrum also indicated an ester function since the loss of 60 could be the elimination of acetic acid.

Two coupled, single proton resonances in the PMR spectrum (Fig. 9) of II at δ 5.50 and δ 5.62 ppm (J=9.0 Hz) indicated the presence of a single, disubstituted double bond with Z geometry. The Z geometry was supported by the absence of a strong absorption at 970 cm^{-1} (C-H bending of E disubstituted olefins) in the IR spectrum. Since the olefin and ester functional groups accounted for only two sites of unsaturation, the presence of one ring was required. The ten methylene protons (δ 1.3-1.7 ppm), and the absence of any signal at a higher field than the methyl doublet (δ 1.26 ppm) suggested that

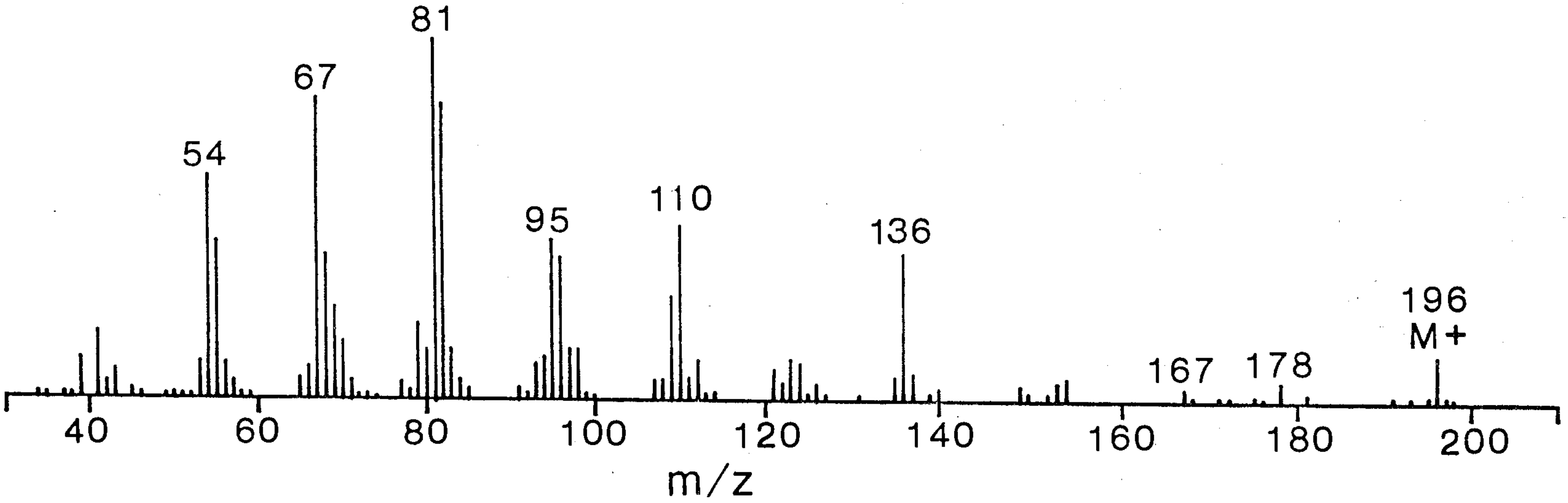


Figure 8. Unit resolution mass spectrum of II

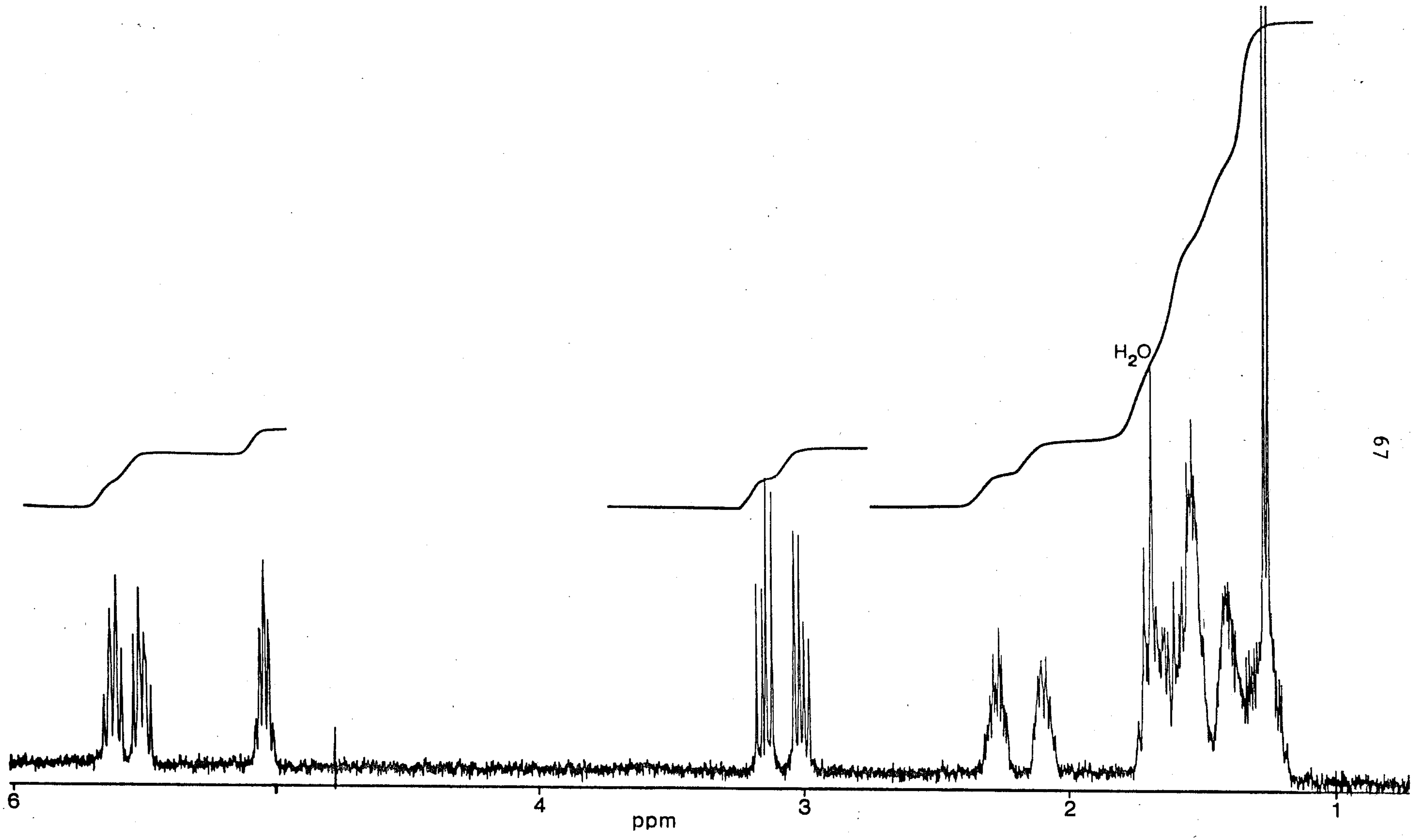


Figure 9. PMR spectrum (400 MHz) of II in CDCl$_3$

the methylenes were in a large ring rather than an open chain terminated by a methyl group. In view of the structure of I, and the data discussed above, a macrolide structure was proposed for II. A twelve-membered ring was suspected since only a single methyl doublet was observed. The chemical shifts of the methyl doublet and the signal of the methine proton (δ 5.04 ppm, J=6.6 Hz) to which it was coupled indicated that the methyl group was on the carbon directly adjacent to the oxygen atom. A number of macrolides which all have methyl groups on the carbon adjacent to the oxygen atom exhibit similar proton chemical shifts for the methyl and methine protons (Kaiser and Lamparsky, 1978). The position of the double bond was established by the presence of the two coupled resonances at δ 3.01 and 3.14 (J=14.4 Hz) which were also coupled to the vinyl proton signal at δ 5.62 ppm (J=7.7 Hz and 8.7 Hz respectively). The chemical shifts of these signals were appropriate for an allylic methylene group adjacent to an ester carbonyl. Therefore, II was considered to be (Z,S)-3-dodecen-11-olide.

The CMR spectrum of II (δ 20.2, 25.0, 25.8, 26.1, 27.8, 31.4, 34.4, 78.0, 122 and 135 ppm) also supported the proposed structure. The two signals at 122 and 135 ppm could be assigned to the vinyl carbons while the resonance at 78.0 ppm could be assigned to the carbon directly adjacent to the ester alkyl oxygen. The remaining signals could be assigned to the methyl carbon (20.2 ppm) and the seven methylene carbons. Since only six signals were unaccounted for, two of the methylene carbons resonances must be unresolved. The structure proposed for II accommodated all the spectral data. Additional confirmation of the proposed structure was obtained by hydrogenation of II, which yielded a single compound with a molecular weight of 198 and a fragmentation pattern in the mass spectrum which was identical to that of 11-dodecanolide (VII, Vesonder *et al.*, 1971).

Confirmation of the structure of II was obtained by the synthesis of (S)-II, (R)-II and the racemic modification of II. The synthetic products were chromatographically and spectroscopically identical to the isolated material. Determination of the absolute configuration of II was carried out by the addition of the chiral NMR shift reagent, $Eu(tfc)_3$, to a $CDCl_3$ solution of II. In the PMR spectra recorded on samples which contained $Eu(tfc)_3$, the methine proton signal (δ 5.04 ppm) was irradiated to simplify the methyl proton signal to a singlet. The addition of 20 mol % $Eu(tfc)_3$ to racemic II (prepared as described in section II-9-iv) resulted in two methyl singlets separated by 5.4 Hz. Broadening of the spectral lines offset the increased shift difference that was observed when more shift reagent was added. Only one methyl singlet was detected in the spectra of isolated II and synthetic (S)-II (prepared as described in section II-9-v) which were combined with $Eu(tfc)_3$ (20 mol %). The addition of racemic II to the samples of isolated II and synthetic (S)-II which contained $Eu(tfc)_3$, both resulted in the enhancement of the high-field methyl singlet. The low-field methyl singlet was increased when racemic II was added to synthetic (R)-II in the presence of 20 mol % $Eu(tfc)_3$. Therefore, the natural product appeared to be comprised of only the (S)- enantiomer. The small quantity of natural material that was isolated (<1 mg) precluded the determination of optical rotation. The trivial names ferrulactone II and ferrulactone I are proposed for compounds II and I, respectively.

ii) Identification of III

An exact mass of 224.1779 was found for the molecular ion of compound III by high resolution mass spectroscopy. This molecular weight indicated a formula of $C_{14}H_{24}O_2$ which corresponds to three sites of unsaturation. Both the IR spectrum (1725 cm^{-1}, C=O stretch of ester) and the mass spectrum (m/z, 224, 209, 154, 140, 126, 110, 95, 81, 67, 55, 41) of III, were similar to the spectra for II. The similarities suggested that III was a homolog of II.

The 400 MHz PMR spectrum of III provided sufficient information to allow deduction of its structure. Signals were observed at δ 1.12 (1H, m) 1.22 (3H, C_{14}, d, J=6.1 Hz), 1.24-1.66 (10H, C_8-C_{12}, bm), 1.72 (1H, C_7, m, 1.78-1.95 (2H, C_3, C_7, m), 2.12-2.26 (2H, C_2, C_4, m), 2.30 (1H, C_4, m), 2.41 (1H, C_2, m), 4.98 (1H, C_{13}, m), 5.30 (1H, C_6, m), 5.38 (1H, C_5, m). Many similarities between the PMR spectra of II and III were apparent. The methyl doublet (δ 1.22 ppm) was coupled to the signal for the methine proton at δ 4.98 which indicated the presence of a methyl group on a carbon adjacent to an ester oxygen. The two coupled, vinyl proton signals at δ 5.30 and 5.38 ppm (J=10.5 Hz) indicated the presence of a Z disubstituted double bond. A hydrocarbon chain in the molecule was suggested by the signals for ten methylene protons (δ 1.24-1.66 ppm). On the basis of these spectroscopic data, III was proposed to be a mono- unsaturated fourteen-membered lactone. The position of the double bond was tentatively determined by decoupling experiments. Irradiation of one of the proton signals α to the carbonyl δ 2.41 ppm) resulted in a simplification of the signal centered around δ 1.82 ppm. The proton signal at δ 1.82 ppm could thus be assigned to one of the protons β to the carbonyl. When the β proton signal was irradiated, the expected simplification of the δ 2.41 ppm signal occurred (loss of 9.0 Hz coupling) along with the simplification of a pair of allylic proton signals at δ 2.19 and 2.30 ppm. The direct coupling of the allylic proton signals and the vinyl proton signal (δ 5.38 ppm) was demonstrating by irradiation of the signal at δ 2.30 ppm. This set of decoupling experiments revealed that the double bond was δ to the carbonyl.

Catalytic hydrogenation of III yielded a single compound with a molecular weight of 226 and a mass spectral fragmentation pattern which was identical to that obtained for 13-tetradecanolide (Kaiser and Lamparsky, 1978). This result confirmed the carbon skeleton proposed for III. Confirmation of the double bond position was obtained by a total synthesis of racemic III (Z)-5-tetradecen-13-olide. (see section II-9-vi)

iii) Identification of IV

The unit resolution, electron impact mass spectrum of IV (Fig. 10) revealed a molecular ion at 194 which was identical to the molecular weight of I. However, the fragmentation patterns of I and IV were quite different. The catalytic hydrogenation of IV yielded a compound with a molecular weight of 198 and a GLC retention time identical to that of VII. Furthermore, the fragmentation patterns for VII and reduced IV were identical. These experiments showed that IV had the same carbon skeleton as II, but had one more double bond.

The 400 MHz PMR spectrum (Fig. 11) showed as expected, a signal due to a methyl group (δ 1.23 ppm, d, J=6.3 Hz) coupled to a single proton resonance

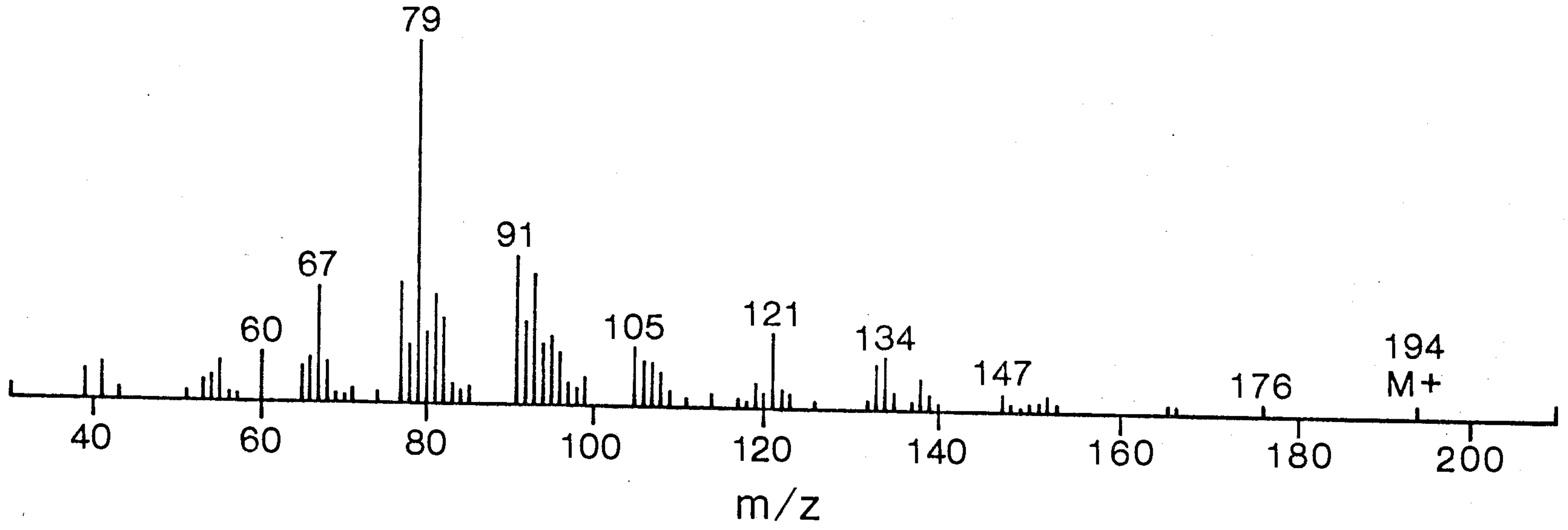


Figure 10. Electron impact mass spectrum of IV

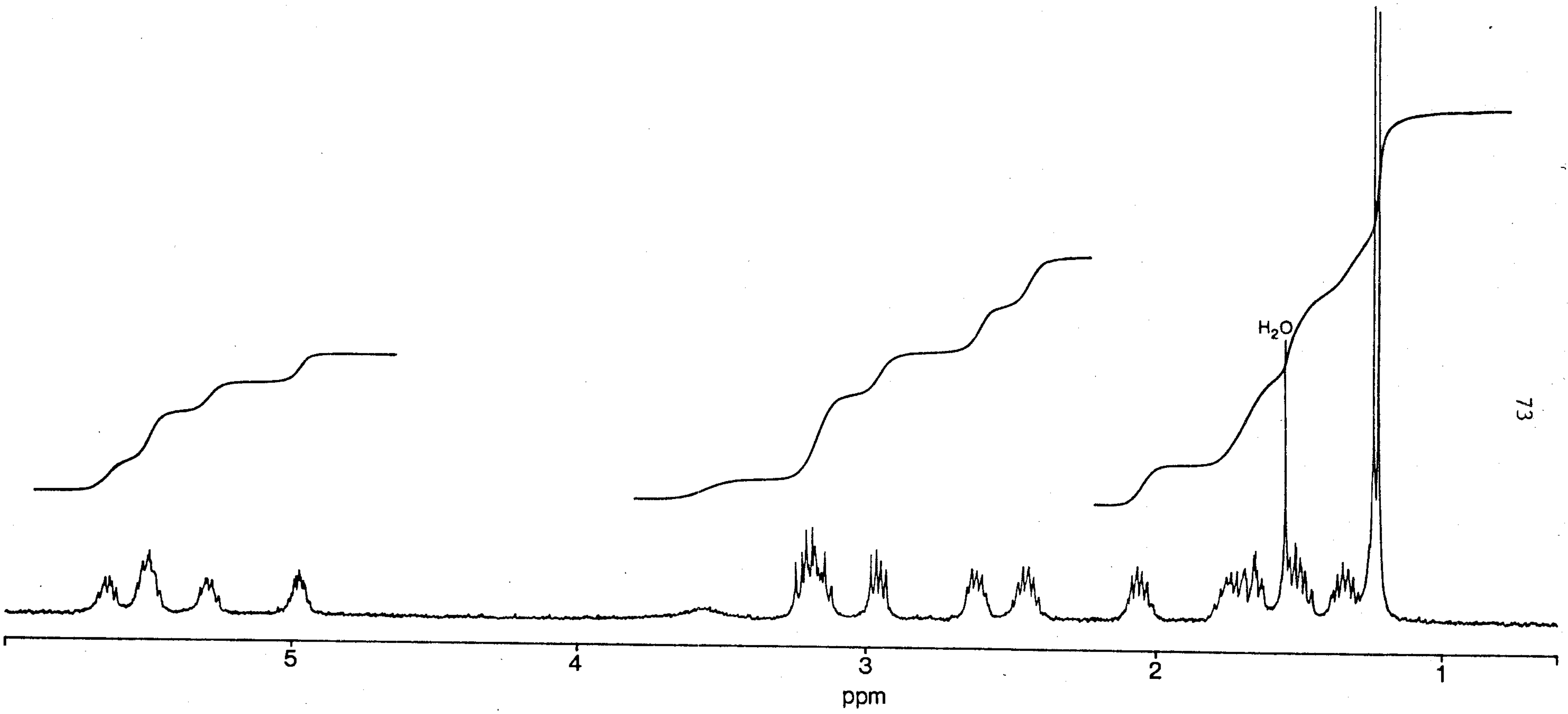


Figure 11. PMR spectrum (400 MHz) of IV in CDCl$_3$

at δ 4.96 ppm. Signals for four vinyl protons at δ 5.30 (1H, C_7, m, J=9.0 Hz), 5.50 (2H, C_3 C_6, m) and 5.64 (1H, C_4, m, J=9.0 Hz) indicated that two double bonds were present. There was no evidence for conjugation of the double bonds with each other or with the carbonyl since the chemical shifts of the vinyl protons were all higher field than 6 ppm. The coupling constants for the vinyl protons indicated a Z,Z geometry. Since only six protons were observed in the allylic proton region (δ 2.05 (1H, C_8, m), 2.45 (1H, C_8, m), 2.61 (1H, C_5, m), 2.93 (1H, C_2, m), and 3.18 (2H, C_2, C_5, m)), it was clear that IV possessed a 1,4-diene system. The position of the 1,4-diene system was established by the presence of the signals due to two coupled protons at δ 2.93 and 3.18 ppm (J=14 Hz). These signals were virtually identical in chemical shift and splitting pattern to the signals due to the methylene protons in II, which were in between the carbonyl and the double bond. Based on this information, the structure of IV was proposed to be (Z,Z)-3,6-dodecadien-11-olide.

iv) Identification of V

The electron impact mass spectrum of V (Fig. 12) closely resembled the spectrum of IV except that the molecular ion was at m/z 222. Hydrogenation of V yielded a compound with a molecular ion at 226 and a GLC retention time and fragmentation pattern identical to that of 13-tetradecanolide Therefore, V must be a homolog of IV. Since the quantity of natural material available was insufficient for PMR, the position of the double bonds were not determined. However, if the analogy between II and IV holds true for III and V, then V should be (Z,Z)-5,8-tetradecadien-13-olide.

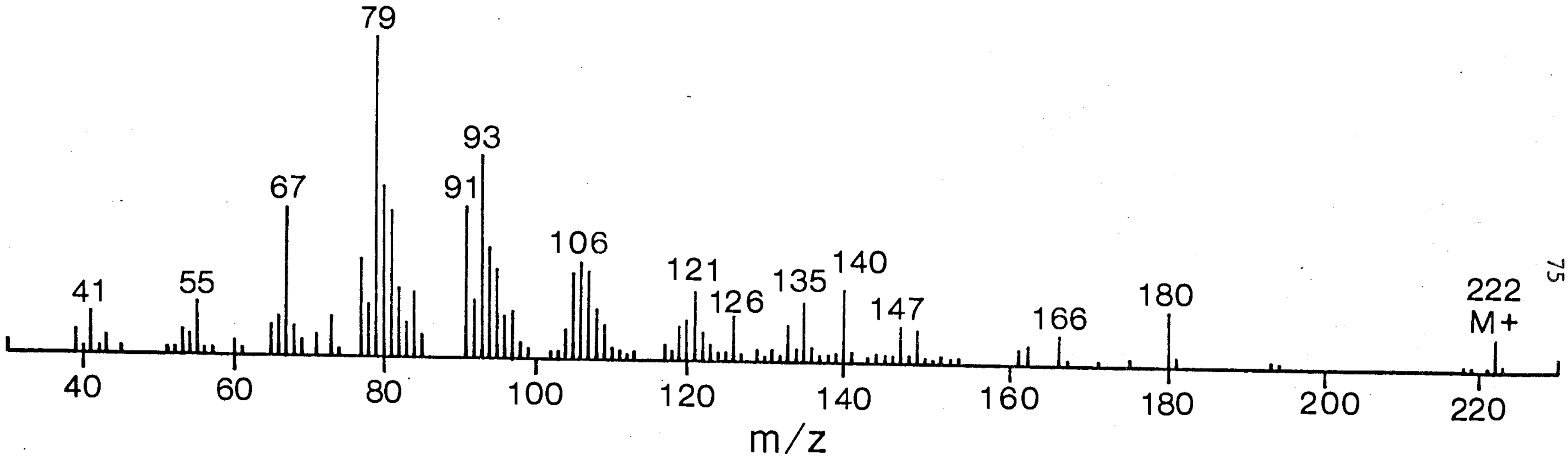


Figure 12. Electron impact mass spectrum of V

v) Identification of VI

The structure of VI was identified on the basis of its GLC retention characteristics and mass spectral fragmentation pattern. An intense peak at m/z 85 indicated that VI could be a γ-lactone. Cleavage at the alkyl side chain would result in the lactone moiety bearing almost all the charge. The spectra of VI and γ-nonalactone (Jennings and Shibamoto, 1980) were identical. Confirmation of the structure of VI was obtained by the co-elution of VI and authentic γ-nonalactone (ICN Pharmaceuticals) from column A.

vi) Identification of VII and VIII

A small quantity of VII was detected in frass and beetle volatiles by GLC-MS analysis. The natural material had a retention time and fragmentation pattern which was identical to that of authentic 11-dodecanolide.

The compound (VIII) which eluted just prior to II was proposed to be (E)-3-dodecen-11-olide based on the identity of mass spectral fragmentation patterns for the two compounds. Isomers in which the unsaturation is at different positions would be expected to have different fragmentation patterns.

13) Response of *C. ferrugineus* to Synthetic Compounds

The response of *C. ferrugineus* to the synthetic macrolides is shown in Figure 13. Synthetic I and (S)-II were individually attractive, and elicited maximum responses at a stimulus concentration of 200 ng. The responses observed for synthetic (S)-II were comparable to the responses obtained for isolated II (Table 2). The (R)- enantiomer of II was not significantly attractive over the concentration range that was tested. Since the response curves obtained for (S)-II and (R,S)-II were virtually identical, (R)-II must not be inhibitory. The slightly lower activity of (R,S)-II

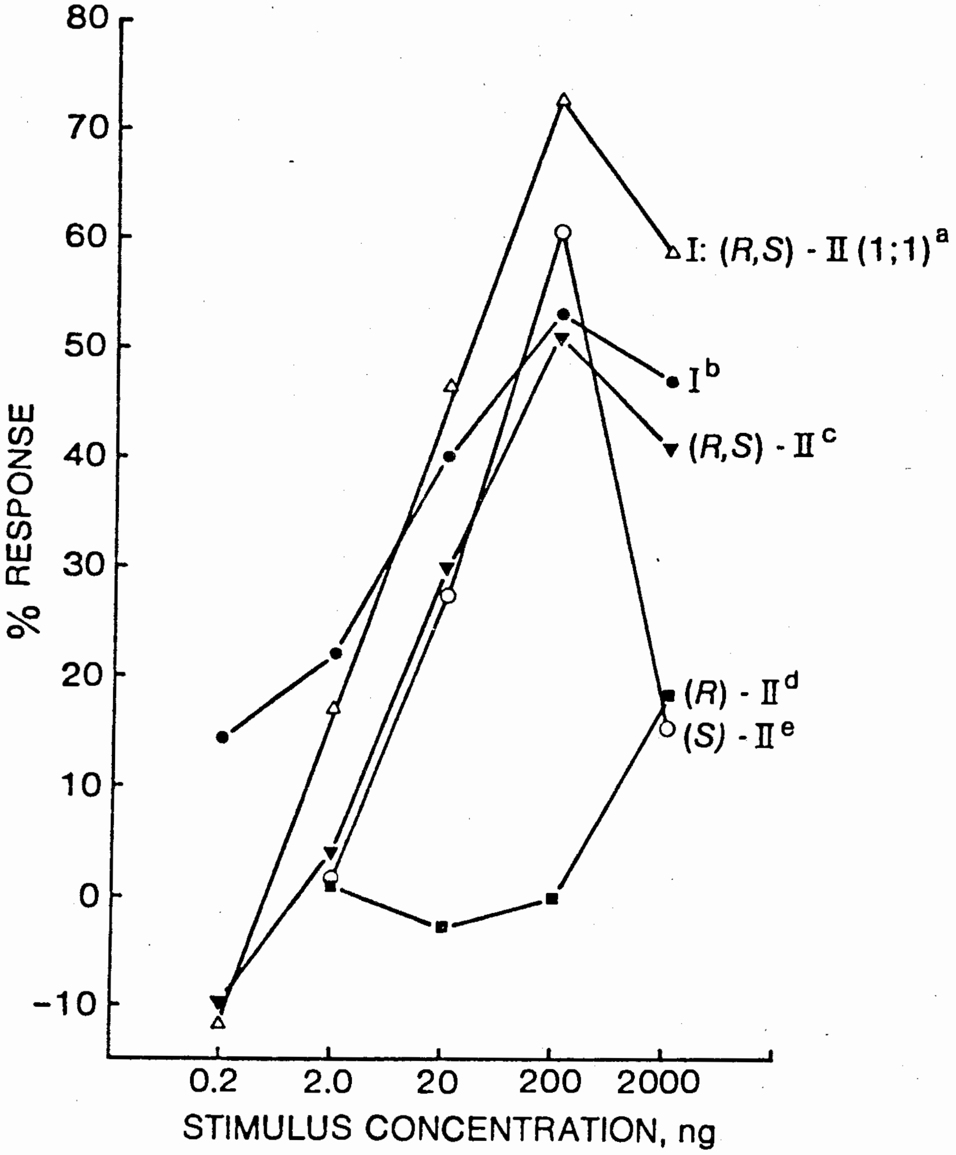


Figure 13. Response of *C. ferrugineus* to synthesized pheromones in the two-choice, pitfall bioassay. [a]$P<0.01$ at 20 ng and above. [b]$P<0.05$ at 20 ng and above. [c]$P<0.05$ at 200 ng and $P<0.01$ at 2000 ng. [d]NS at all concentrations. [e]$P<0.05$ at 20 ng and $P<0.01$ at 200 ng. Percent response calculated by (Experimental-Control/Experimental+Control) x 100. N=6 replicates. t-test used to analyse data.

compared to (S)-II could be accounted for by the fact that a sample of (R,S)-II contains only one-half the amount of (S)-II that is found in an equivalent sample of pure (S)-II. This situation, in which an insect produces only one active enantiomer and responds poorly or not at all to the opposite enantiomer, is well known (Silverstein, 1979). For example, (S)-(+)-4-methyl-3-heptanone, the alarm pheromone for *Atta cephalotes*, is approximately two-hundred times more active than the (R)-(-)- enantiomer (Riley *et al.*, 1974).

The synergistic behavior of I and II, proposed in section III-11-i, was confirmed when a significantly higher response was observed for a one to one mixture of I and (R,S)-II (Fig. 13), compared to the activities of the individual compounds. A similar response was observed for a one to one mixture of I and (S)-II (data not shown) which indicated that the (R)-enantiomer did not inhibit the activity of the binary mixture. Since (R)-II was not inhibitory, the more easily synthesized (R,S)-II could be used in field tests. The presence of small quantities of VII and VIII, produced in the hydrogenation (P-2 nickel) reaction (section II-9-iv-i), did not appear to inhibit the activities of synthetic (S)-II and (R,S)-II compared to pure isolated II.

Different ratios of I and (R,S)-II were tested in the two-choice olfactometer to determine the most attractive mixture for field tests (Table 3). Despite the fact that the ratios of I:(S)-II were 1.6:1 in frass volatiles and 0.84:1 in beetle volatiles, the most attractive mixture was a 9:1 ratio (equivalent to an 18:1 mixture of I:(S)-II. The 9:1 mixture was significantly more attractive than the 3:1 and 1:1 mixture.

Synthetic (R,S)-III (0.2-2000 ng) was tested in the two-choice olfacto-

Table 3. Response of *C. ferrugineus* to different ratios (9:1, 3:1) of synthetic I and (R,S)-II in a two-choice, pitfall, olfactometer. N=6 replicates.

		% Response[a] ($\bar{X}$ ± S.E.)	
Experimental Stimulus	Stimulus Concentration, ng	Experimental Stimulus	Blank Control
I:(R,S)-II	4500 :500	75.0 ± 4.1	17.0 ± 3.8**
	450 :50	85.1 ± 3.7	14.9 ± 2.5***
	45 : 5	80.0 ± 5.2	13.3 ± 1.6***
	4.5 : 0.5	66.3 ± 9.7	33.7 ± 8.4*
	0.45: 0.05	42.5 ± 7.0	46.0 ± 12.3 NS
I:(R,S)-II	1500 :500	77.8 ± 4.5	18.9 ± 4.8**
	150 : 50	78.7 ± 1.4	20.2 ± 2.0***
	15 : 5	59.8 ± 2.0	31.5 ± 6.4*
	1.5 : 0.5	51.1 ± 4.0	30.0 ± 4.6 NS
	0.15: 0.05	44.3 ± 3.9	27.3 ± 2.2 NS

[a] Significant response (t-test) to experimental stimulus indicated by: ***P<0.001, **P<0.01, *P<0.05, NS=not significant.

meter and found to be unattractive (data not shown), alone, or in combination with I and (R,S)-II. This result together with previous data (section III-11-ii) confirmed that III was not required in the *C. ferrugineus* pheromone. Compounds IV-VIII were not tested for pheromone activity in pure form since bioassay results indicated that they were not attractive. However, their presence may be required for communication with other *Cryptolestes* spp.

14) Results of Field Tests on I:(R,S)-II (9:1)

The results from preliminary field tests indicated that more rusty grain beetles were caught in pheromone baited traps than unbaited control traps. Figure 14 contains a three-dimensional representation of a wheat-filled annex bin marked with the locations of one fibre traps, two fibre traps, and control traps. The number of beetles caught decreased with increased depth of traps, with most beetles (10282) caught near the surface. At a depth of 152 cm, 901 beetles were found in baited and control traps while only 85 beetles were caught at 244 cm. This distribution of beetles is in rough agreement with the observation (Loschiavo, 1974) that rusty grain beetles are found preferentially in the top and bottom third of large wheat-filled containers. The baited traps caught 10.2 times as many beetles as control traps (per trap) at the surface and 19.6 times as many (per trap) at 152 cm. However, at 244 cm where the total catch was low, the number of beetles caught in control traps outnumbered those in baited traps by a factor of 1.5. Two fibre traps caught more beetles (1723/trap) than the one fibre traps (211/trap). However, if the 7300 beetles caught in the single two fibre trap are not included, then the catches are roughly equal.

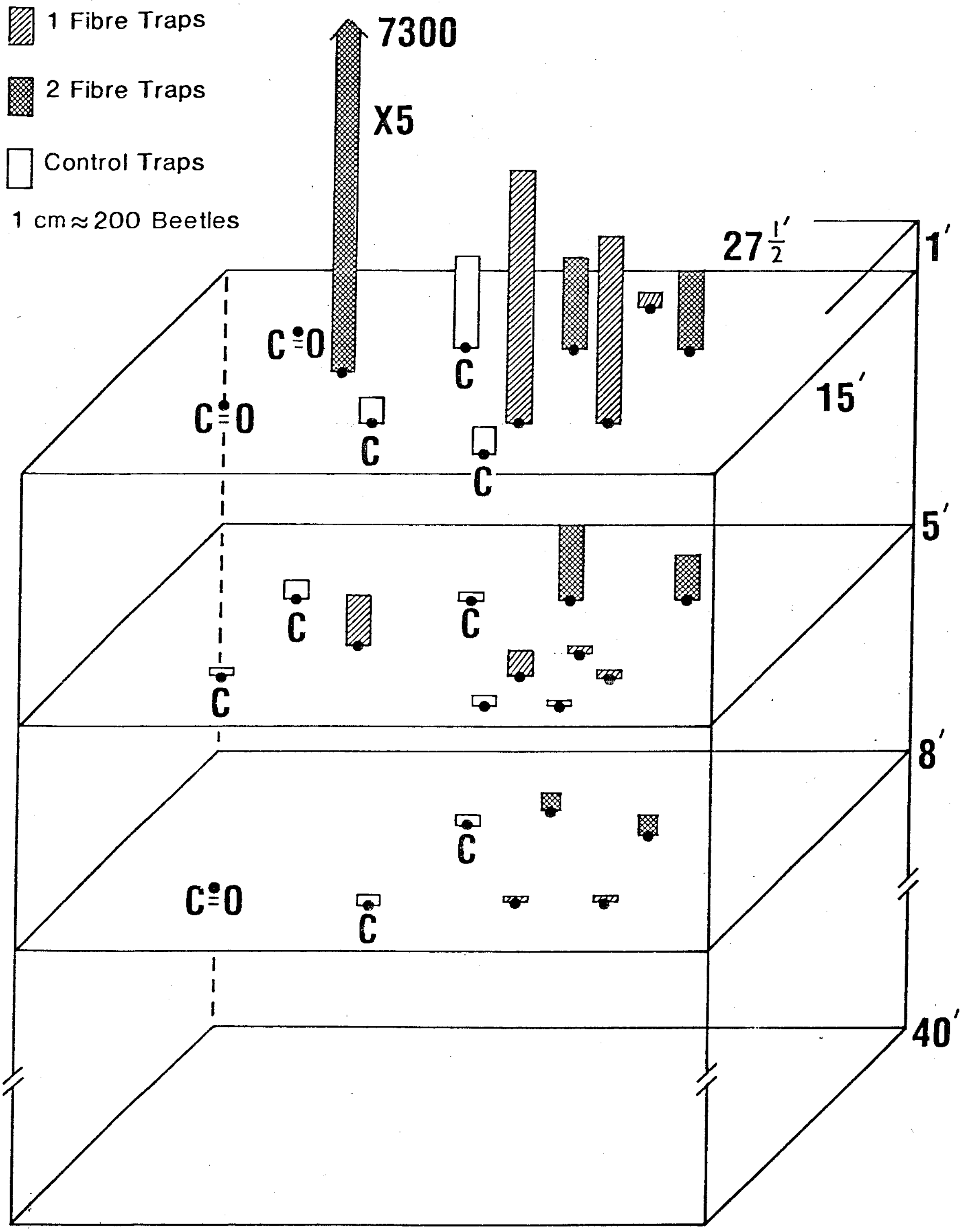


Figure 14. Trap locations and catches of rusty grain beetles in a wheat-filled annex bin, Elm Creek, Manitoba

Therefore it was not possible to conclude that the two fibre traps were more efficient than the one fibre traps. The large catch in the single two fibre trap may have been the result of a local infestation with a high population density at the trap site. It is noteworthy to point out that several control traps placed within 152 cm (horizontal distance) of the two fibre trap did not catch many insects. These results suggest that the 9:1 mixture of I and (R,S)-II may be useful as a bait for the detection of rusty grain beetles in grain-filled storage areas. Further tests are required to test the range and efficiency of the pheromone bait.

The length of time that the baited traps were left in the grain (one week) was based on the release rate that was determined for I. At 30° most of the pheromone had evaporated by the end of one week, while at 20° pheromone was still left after three weeks (Fig. 15). These release rates were determined in ventilated surroundings, and actual release rates in grain bins may differ from these experimental results.

15) Syntheses of Macrolide Pheromones

The term "macrolide" refers to medium (8-11 atoms) and large (12 or more) ring compounds which contain a lactone moiety. A large number of naturally occurring macrolides have been discovered (Keller-Schielein, 1973; Masamune *et al.*, 1977) since the isolation of the first macrolide, ambrettolide ((Z)-7-hexadecenolide), was reported by Kerschbaum (1927). Some examples of major groups of macrolides, classified according to structural type, include the polyoxo (pikromycin, erythromycin), polyene (amphoterican B, tetrin), ionophoric (nonactin, boromycin), and the lactam-containing ansa

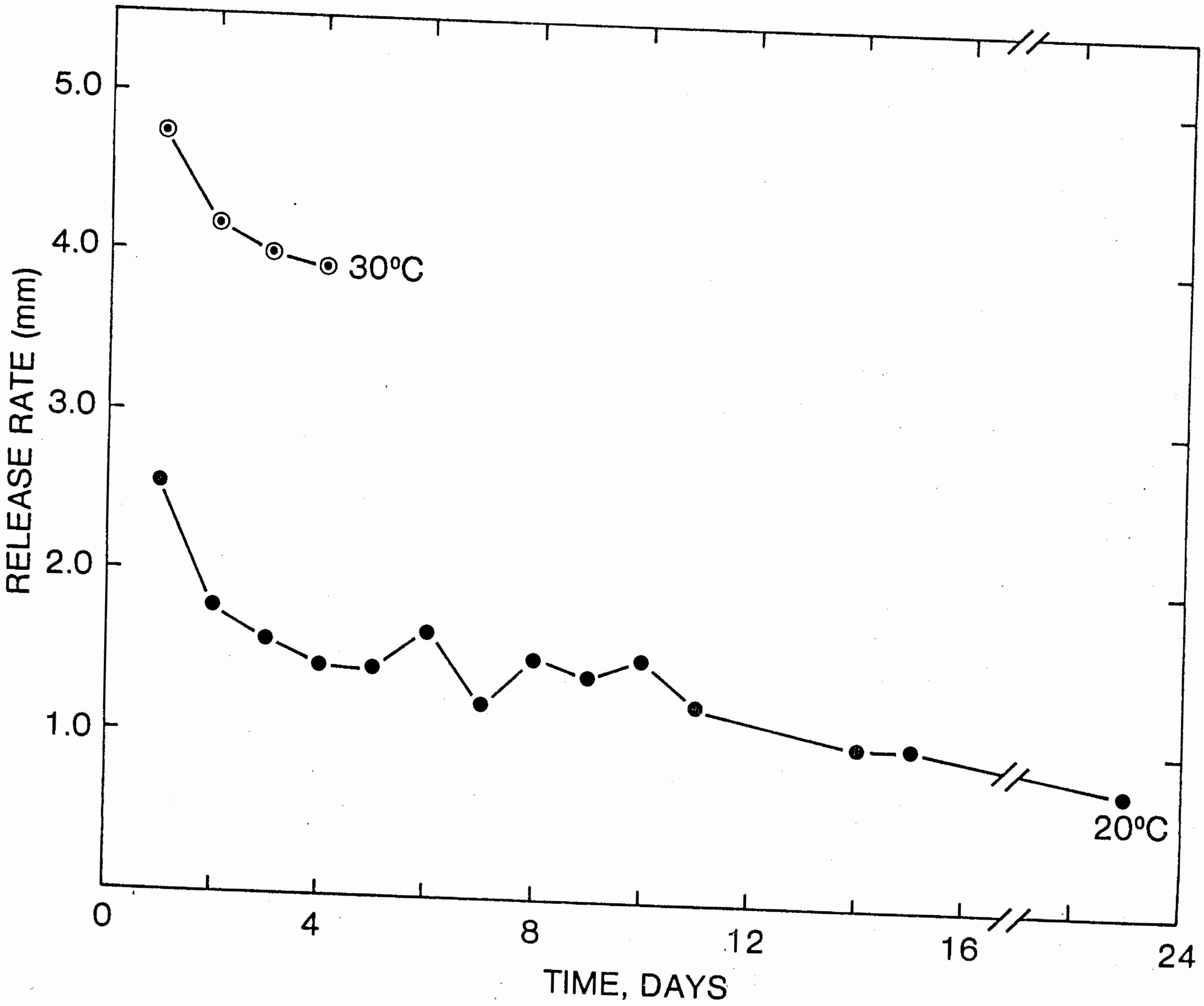


Figure 15. Release rates of I from 0.203 mm I.D. Conrel fibres at 20°C and 30°C

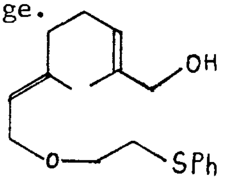
(maytansine) macrolides (Trost and Verhoeven, 1980). The structurally diverse macrolides have been isolated from a variety of organisms including plants (ambrettolide), insects (phoracantholides, Moore and Brown, 1976) fungi (diplodialides, Ishida and Wada, 1975; recifeiolide, Vesonder *et al.*, 1971) and bacteria (pikromycin). Although the existence of macrolides had been known since 1927, it was not until the discovery of the first macrolide antibiotic, pikromycin (Brockmann and Henkel, 1950), that real interest was shown in their biological function and chemical synthesis.

The two major methods that have been employed for the synthesis of macrolides are: 1) the expansion or contraction of a ring, and 2) the cyclization of a bifunctional acyclic precursor. Examples of ring expansion procedures include the Baeyer-Villiger oxidation of macrocyclic ketones (Mookherjee *et al.*, 1972; Kaiser and Lamparsky, 1978) and the Fe^{2+} ion promoted fragmentation of alkoxy hydroperoxides (Schreiber, 1980). Except for simple symmetrical ketones, the Baeyer-Villiger oxidation is not generally applicable for macrolide synthesis because of the formation of isomers and the relatively harsh conditions that are required (peracids). Although a highly regio- and stereoselective synthesis of recifeiolide was achieved by the fragmentation of an alkoxy hydroperoxide precursor, this method has not been tested on molecules with sensitive functional groups.

The cyclization of bifunctional acyclic molecules has been the most frequently employed method for the synthesis of macrolides. Examples of bifunctional molecules include: 1) ω-hydroxy-acids (Corey and Nicolaou, 1974), 2) ω-halo-acids (Kruizinga and Kellog, 1979), 3) ω-halo-phenyl-thio acetates (Takahashi *et al.*, 1981), and 5) ω-allylic acetate-benzenesulfonylacetates (Trost and Verhoeven, 1980). Ring closure can be achieved by the intramolecular displacement of a leaving group by a stabilized carbanion for bifunctional precursors of type 3, 4 and 5. However, most ring closures to date have been carried out by the intramolecular esterification of an activated ω-hydroxy-acid. This was the method chosen for the synthesis of the *C. ferrugineus* pheromones.

i) Synthesis of Ferrulactone I

Although I had been synthesized by Verigin (1980), the low yield that was obtained (0.17% overall) made the route impractical for the preparation of the quantities required for field tests. A shorter (5 steps) and possibly more efficient synthesis, in which the key reaction involved the intramolecular alkylation of an ω-haloalkyl-phenylthioacetate, was attempted (Fig. 16). The phenylthioacetyl derivative of geraniol (1) was obtained in quantitive yield from geraniol and phenylthioacetyl chloride. Allylic oxidation of 1 with selenium dioxide and pyridine in refluxing ethanol (Camps *et al.*, 1978) gave a crude product which was a mixture of the alcohol (2) and the corresponding aldehyde (1:1). An attempted reduction of the crude product with sodium borohydride gave a complex mixture which contained mainly the ether (27) (from complete reduction of the ester moiety), and product derived from ester interchange.



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A pair of signals in the PMR spectrum of 27 at δ 3.08 (2H, CH_2-SPh, t, J=6.0 Hz) and 3.72 (2H, CH_2-O, t, J=6.0 Hz) and the absence of the absorption at

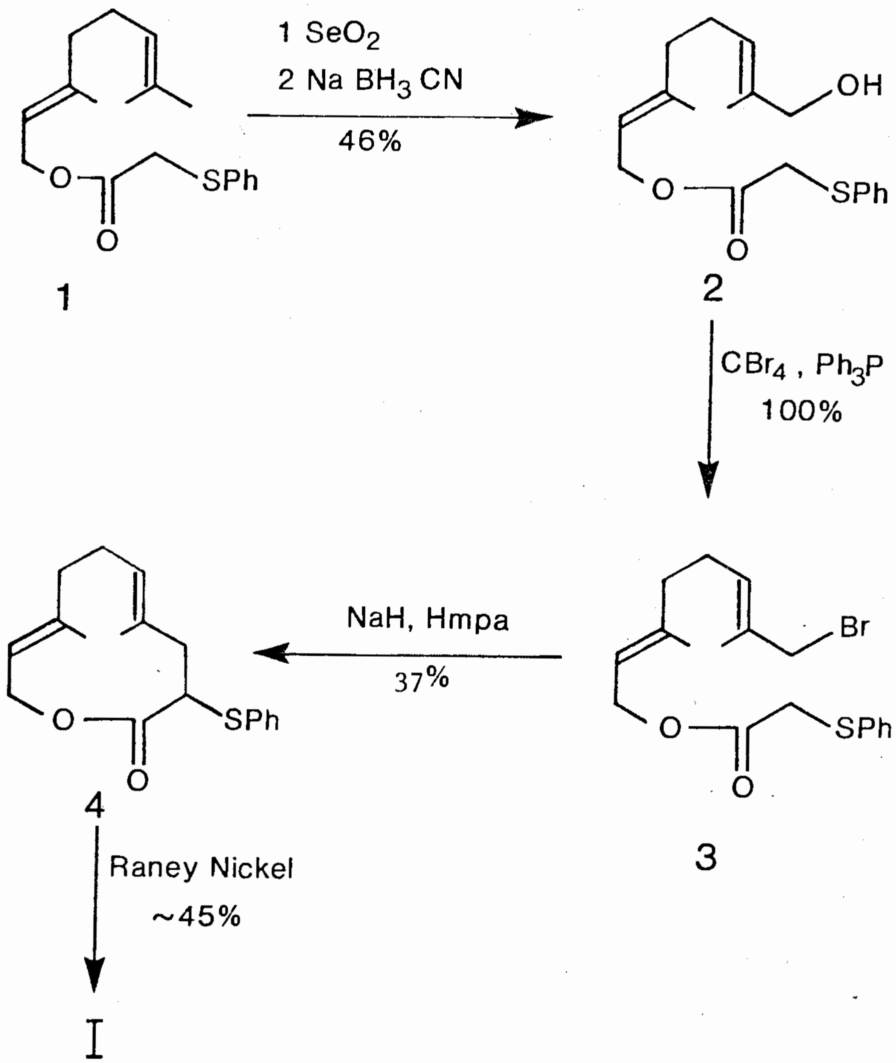
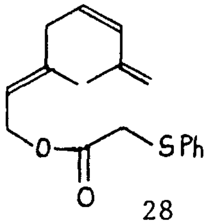


Figure 16. Attempted five-step synthesis of I via the intramolecular alkylation of an ω-haloalkyl-phenylthioacetate (3)

1735 cm^{-1} in the IR spectrum, confirmed the presence of the ether moiety β to the phenylthio group. Presumably, the proximity of the sulfur atom activated the ester carbonyl toward reduction by sodium borohydride. To circumvent this problem a milder reducing agent, sodium cyanoborohydride, was used instead of sodium borohydride. Although the reaction was slow (18 hr at 23°C) no products which corresponded to ester interchange or reduction of the ester carbonyl were detected. The yield was still only moderate (45.9% based on recovered 1) because of the formation of very polar side products. Oxidation with selenium dioxide and t-butylhydroperoxide (Umbreit and Sharpless, 1977) did not result in any improvement and gave only a 20.7% yield of the aldehyde.

Preparation of 3 was readily achieved in a quantitative yield by reaction of the alcohol with a two-fold excess of carbon tetrabromide and triphenylphosphine according to the procedure of Hooz and Gilani (1968). Problems were encountered in the ring closure of 3 according to the method of Takahashi. The reaction (Takahashi *et al.*, 1978) involved the generation of a stabilized carbanion followed by the intramolecular displacement of a primary alkyl halide to give an α-phenylthiolactone. Yields of 49-75% were reported for ten and fifteen-membered rings. Ring closure of 3 under high dilution conditions (15.8 mM) resulted in a 37% yield of 4. Only a narrow range of experimental conditions (temperature, dilution, rate of addition of 3) were suitable for the ring closure reaction. The 1,4-elimination of HBr to give the triene (28) was a major competing reaction, and in most cases 28



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was the major product. Signals in the PMR spectrum of the major product at δ 4.87 (2H, exo-methylene), 5.60 (1H, C_5, dd, J=6.5 Hz, 15.0 Hz), and 6.13 ppm (1H, C_6, d, J=15.0 Hz) confirmed the identity of 28. The 1,4-elimination of 3 was favored over nucleophilic displacement because of the relative stabilities of the two products (4 or 28). The ring strain due to transannular interactions and bond angle deformations are quite severe in medium ring compounds such as 4. The presence of the two E double bonds, the two methyl groups, and the bulky phenylthio group would be expected to contribute further to ring strain which hindered the ring closure reaction. The requirement for large volumes of HMPA made this reaction hazardous as well as impractical. The use of alternate conditions for carbanion generation (sodium hexamethyldisilazane in refluxing THF) did not result in the formation of 4. The major product which was recovered (8-bromo-3,7-dimethyloctadien-1-ol) was probably produced during the aqueous workup by the base hydrolysis of the ester moiety in 3.

Due to the limited quantity of 4 that was available, conditions for the reductive elimination of the phenylthio group were not optimized. However, from the experiments that were performed, it appeared that the use of a specific quantity of W-2 Raney nickel in refluxing ethanol would be successful in the removal of the phenylthio group without isomerization of the double bonds.

Because of the problems associated with the ring closure reaction, the attempted synthesis of I via the route shown in Figure 16 was abandoned. Efforts were directed toward a scheme (Fig. 17) which employed the synthetic approach initially developed by Verigin (1980) in his synthesis of I. The

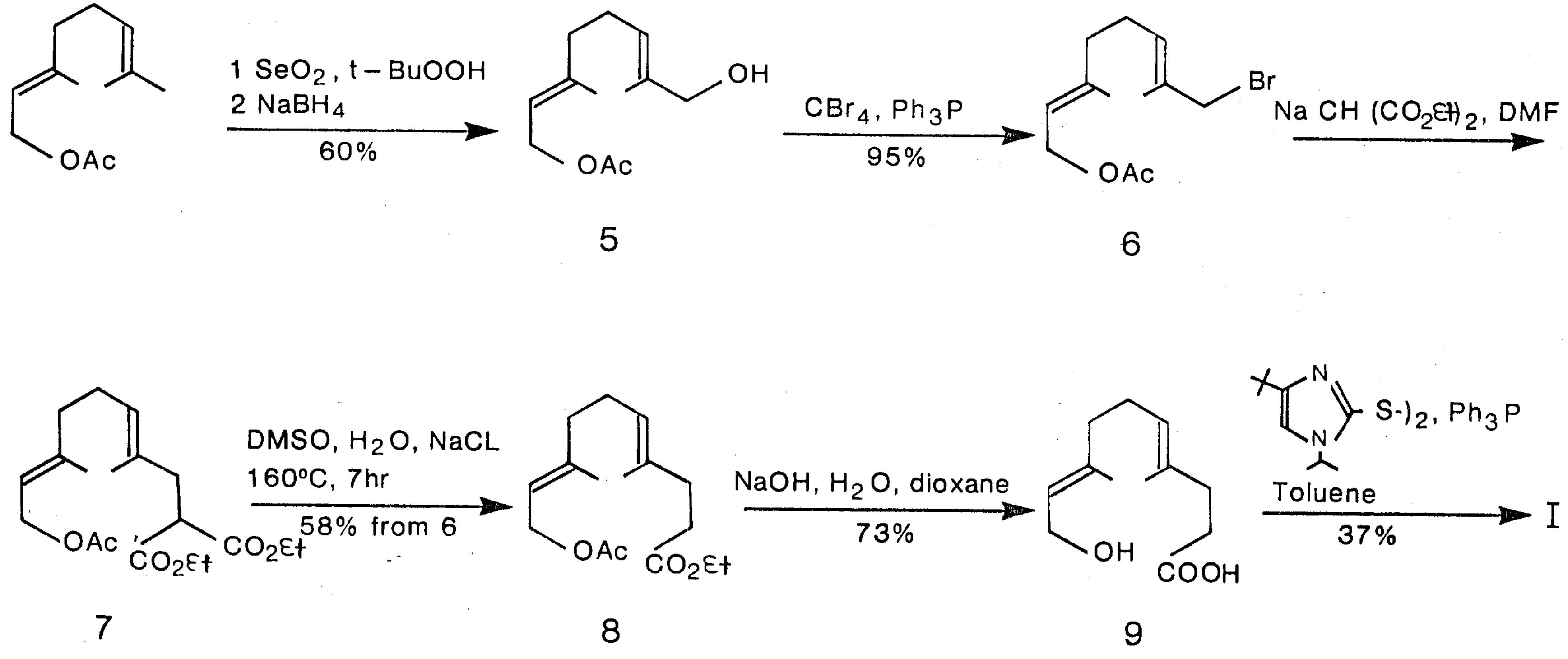
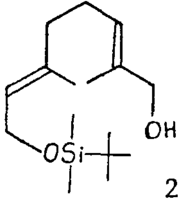


Figure 17. Synthesis of I via the intramolecular esterification of the ω-hydroxy acid (9)

major differences between the two synthetic schemes were in the choice of the alcohol protecting group and in the reagents used for the required transformations. Protection of the alcohol function of geraniol by acetylation was chosen because the lability of the acid-sensitive tetrahydropyranyl group was considered to be a contributing factor toward the low yield obtained in Verigin's synthesis. Other protecting groups such as the t-butyldimethylsilyl ether and the methoxymethyl ether were tried but were found to be unsuitable. In the case of the t-butyldimethylsilyl ether of geraniol, selenium dioxide oxidation (Catalytic procedure, olefin:selenium dioxide, 16.7:1 mol ratio, 96 hr, Umbreit and Sharpless, 1977) gave a 14.9% yield of the desired product (29) but also gave 13.2% of the undesired secondary alcohol (30). The PMR spectrum of 30 provided sufficient information for structural identification: PMR (60 MHz, $CDCl_3$) δ 0.17 (6H, CH_3-Si, s), 1.00 (9H, t-butyl-Si, s), 1.34 (1H, OH, bs), 1.72 (6H, gem-dimethyl, s), 1.78 (3H, CH_3-C_3, d, J=1.8 Hz), 2.33 (2H, C_5, bt, J=6.5 Hz), 4.07 (1H, C_4, t, J=6.5 Hz), 4.30 (2H, C_1, d, J=6.0 Hz), 5.17 (1H, C_6, t, J=6.5 Hz), 5.60 (1H, C_2, bt, J=6.0 Hz). Although methylene groups on trisubstituted double bonds are oxidized more readily than methyl groups, the oxidation of gem-dimethyl olefins usually result in the formation of primary allylic alcohols (E geometry) (Bhalerao and Rapoport, 1971; Rabjohn, 1976). The oxidation of other geraniol derivatives (1, geranyl acetate, methoxymethyl ether of geraniol) did not lead to any products with an alcohol



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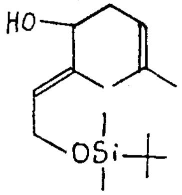

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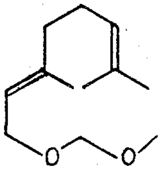
function at the C_4 position. Therefore, the t-butyldimethylsilyl group must have participated by activating the $\Delta^{2,3}$ double bond toward allylic oxidation.

The methoxymethyl derivative of geraniol (31) performed adequately in



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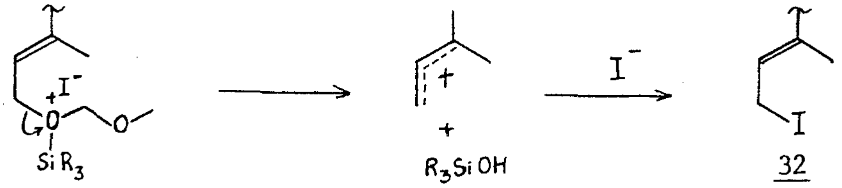
Verigin's synthetic scheme up to the penultimate reaction. A major problem was encountered in the attempted cleavage of the methoxymethyl group. In a model reaction, treatment of the methoxymethyl ether (31) with tri-alkylsilyl iodides(alkyl=methyl or n-butyl, Jung and Lyster, 1977) resulted in the formation of the iodide (32) instead of geraniol. The presence of bulky alkyl substituents on silicon (eg. n-Butyl) should have directed the



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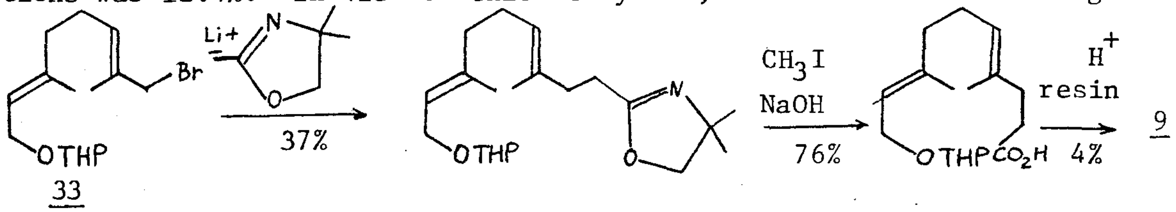
attack of the tri-alkylsilyl iodide to the least hindered oxygen. However, neither oxygen atom was hindered since the alcohol was allylic. In fact, resonance stabilization of the carbonium ion which was formed after ether cleavage favored attack at the allylic oxygen. Therefore, the methoxymethyl ether should not be used as a protecting group for allylic alcohols.

The stoichiometric selenium dioxide oxidation of geranyl acetate gave a 45% yield of 5. This yield would have been higher (∿60-65%) except that a considerable quantity of the acetate group was hydrolyzed during the sodium borohydride reduction. Combined yields of 60% (literature value, Umbreit and Sharpless, 1977) for 5 and the corresponding aldehyde were routinely obtained for smaller scale reactions (∿10 mmol) in which the reduction step was omitted. Sodium cyanoborohydride in a slightly acidic medium could probably be employed to circumvent the problem of ester interchange. However, the yield of 45-60% was a significant improvement over the 19% yield reported by Verigin for the oxidations of the tetrahydropyranyl ether of geraniol.

The bromo-acetate (6) was prepared in 94.5% yield with carbon tetrabromide and triphenylphosphine. The yield obtained by this procedure was twice that of the yield (47%) that was reported by Verigin for his bromination of the tetrahydropyranyl alcohol.

In Verigin's synthesis, conversion of the bromide(23) to the ω-hydroxy-acid (9) was carried out by Meyer's procedure (Meyers *et al.*, 1974) in three steps: 1) alkylation with the lithio salt of 2,4,4-trimethyl-2-oxazoline, 2) base hydrolysis to give the carboxylic acid, and 3) acid catalyzed cleavage of the tetrahydropyranyl group. The overall yield for these three reactions was 12.4%. In view of this low yield, the acetic acid homologation was attempted via the classical diethyl malonate route. Alkylation of 6 with the sodium salt of diethyl malonate gave the diester 7, which was decarboxylated to give the monoester-acetate (8) in a yield of 57.6%. The ω-hydroxy-acid (9) was obtained in one step by base hydrolysis. The improvements which have been discussed in this section resulted in an overall yield of 23.8% for the five steps between geranyl acetate and 9. The equivalent transformation in Verigin's synthesis gave a 1.1% yield.



Lactonization of 9 by the double activation method (Corey and Brunelle,

1976) with 2,2'-Bis-(4-t-butyl-N-isopropyl)-imidazolyl disulfide (34) gave a 19% yield of I in Verigin's synthesis (experiment 5, Table 4). The lactonization of 9 was attempted under a variety of conditions in order to optimize the yield. The results of these experiments are shown in Table 4. Conditions which resulted in an 83% yield of hexadecanolide (Corey and Brunelle, 1976) gave a 23.3% yield of I (experiment 4, Table 4). Ring strain must account for part of the large discrepancy between the yields of I and the unstrained, 17-membered hexadecanolide. The ring closure was sensitive to the temperature and rate of addition of the thioester solution. Decreased yields were obtained when the thioester was added quickly (experiment 1) and at room temperature (experiment 2). The addition of silver ion (experiment 4 and 5), which should have accelerated the rate of ring closure by making the sulfur atom a better leaving group (Nimitz and Wollenberg, 1978), caused a sharp drop in the yield of I. This deleterious effect may be explained by the complexation of the silver ions with the double bonds which would increase the already severe steric crowding and therefore decrease the rate of ring closure. Significant improvements in the yields of I were obtained when the thioester solution was diluted at once instead of by dropwise addition into refluxing benzene (experiment 6 and 7). The highest yield, 37% (experiment 7), was obtained when the thioester was prepared at low temperatures (-68^{o} to -10^{o}) prior to immediate dilution. Note that the yield of diolide was much lower under the conditions employed in experiment 7. The results tabulated in Table 4 suggested that the thioester underwent ring closure and polymerization at fairly low temperatures (-10^{o} to 23^{o}). In fact, experiment 8 using refluxing toluene did not result in any increase in the yield of I.

Table 4. Lactonization of 4,8-Dimethyl-10-hydroxy-4-E,8-E-decadienoic acid by the Double Activation Method.

Experiment No.	Conditions					Concentration of Thioester(mM)	% Yield[b]	
	Method[a]	Addition Time,hr	Addition Temperature, °	$AgClO_4$	Solvent (Reflux time,hr)		I	Diolide
1	A	1	23	-	benzene(4)	5.7	11.5	3.6
2	A	4	23	-	benzene(1.5)	5.7	15.8	0
3	A	4	4	-	benzene(1)	4.7	23.3	0
4	A	4	4	1.lequiv.	benzene(1)	4.7	10.5	2.5
5	B	-	-	2 equiv.	toluene(5)	7.1	19.0	n.d.
6	C	-	-	-	benzene(5)	4.7	28.6	5.1
7	D	-	-	-	toluene(0)	4.7	37.0	1.9
8	E	-	-	-	toluene(5)	4.7	7.6	0

[a] Method A: Thioester was prepared by stirring 9, 34 (1.5 equiv.) and Ph_3P (1.5 equiv.) in benzene at 4°C for 18 hr. The crude thioester was diluted with benzene and adding dropwise to refluxing benzene.

Method B: Thioester was prepared as described by Verigin (1980) and then diluted all at once with toluene which contained 2 equivalents of $AgClO_4$.

Method C: Thioester was prepared as described for method A and then diluted all at once with benzene and refluxed.

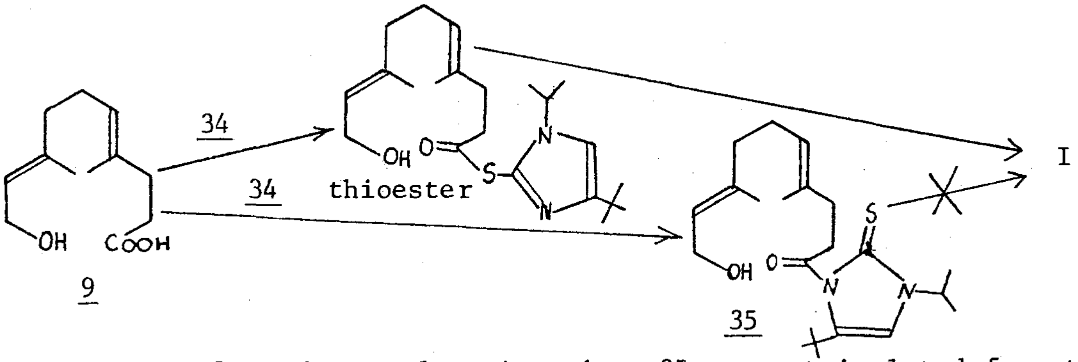
Method D: Procedure as described in section II-9-ii-f.

Method E: Procedure as described in section II-9-ii-f except with 2,2'-dipyridyl disulfide instead of 34.

[b] Reported yields were of isolated products after chromatography.

n.d.=not determined

Although a two-fold increase in the yield of I was realized by variation of experimental conditions, the overall yield was still very poor. Analysis of the crude lactonization products by TLC revealed the absence of any starting material (9). We suspect the ω-hydroxy-acid must have reacted with 34 to give the amide (35) which did not undergo ring closure.

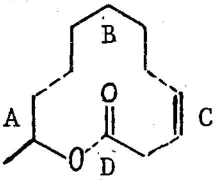


There was no proof of this explanation since 35 was not isolated from the lactonization reaction.

Other reagents for the activation of the carboxyl function of ω-hydroxy-acids for macrolide synthesis have been reported. The highest lactonization yields (85% and 99% for dodecanolide and pentadecanolide, respectively) were reported by Narasaka *et al.* (1978) for the reagent, 2-chloro-6-methyl-1,3-diphenylpyridinium tetrafluoroborate. Although an example of a medium ring macrolide was not included in Narasaka's report, lactonization of 9 should be attempted with his reagent.

ii) Synthesis of Ferrulactone II

The design of the synthesis for ferrulactone II was also based on the intramolecular esterification of an ω-hydroxy-acid (17). The structure of II can be divided into four segments (A-D) on the basis of functional groups and the chiral center. Synthesis of 17 from these segments could be accomplished in a linear (A + B + C + D) or convergent ((A + B) + (C + D)) manner. Initially, a synthesis was attempted in which the Grignard of a protected 1,5-bromopentanol (B) was coupled to propylene oxide (A) via cuprous iodide catalysis. The secondary alcohol was then protected and the primary alcohol deprotected and converted to a primary bromide. Coupling of the bromide to the dilithio dianion of 3-butynoic acid (C + D) was attempted without success. This reaction failed because the treatment of 3-butynoic acid with n-butyllithium gave an allene due to the acidity of the C_2 protons. Therefore, synthesis of II could not be achieved with a free carboxyl group (D) and alkyne (C) joined together. This problem was eliminated by the introduction of the C-D unit as 3-butyn-1-ol which was oxidized to give the carboxylic acid.



II

The synthesis of racemic II is shown in Figure 18. Coupling of the lithio salt of 10 with 1-chloro-5-iodopentane was achieved in 62% isolated yield by the addition of HMPA to a solution of the two starting materials. This novel procedure was adopted after poor to moderate yields (<50%) were obtained by the normal method in which the halide was added to the lithio-alkyne (Barve and Gunstone, 1971), or by the inverse method in which the lithio-alkyne was added to the halide. The higher yield was realized by a more complete reaction of the starting materials and the decrease of a major side product (36), which was tentatively identified by mass spectroscopy (electron impact): m/z (relative intensity) 244 (0.3), 243 (1.6), 242 (3.7),

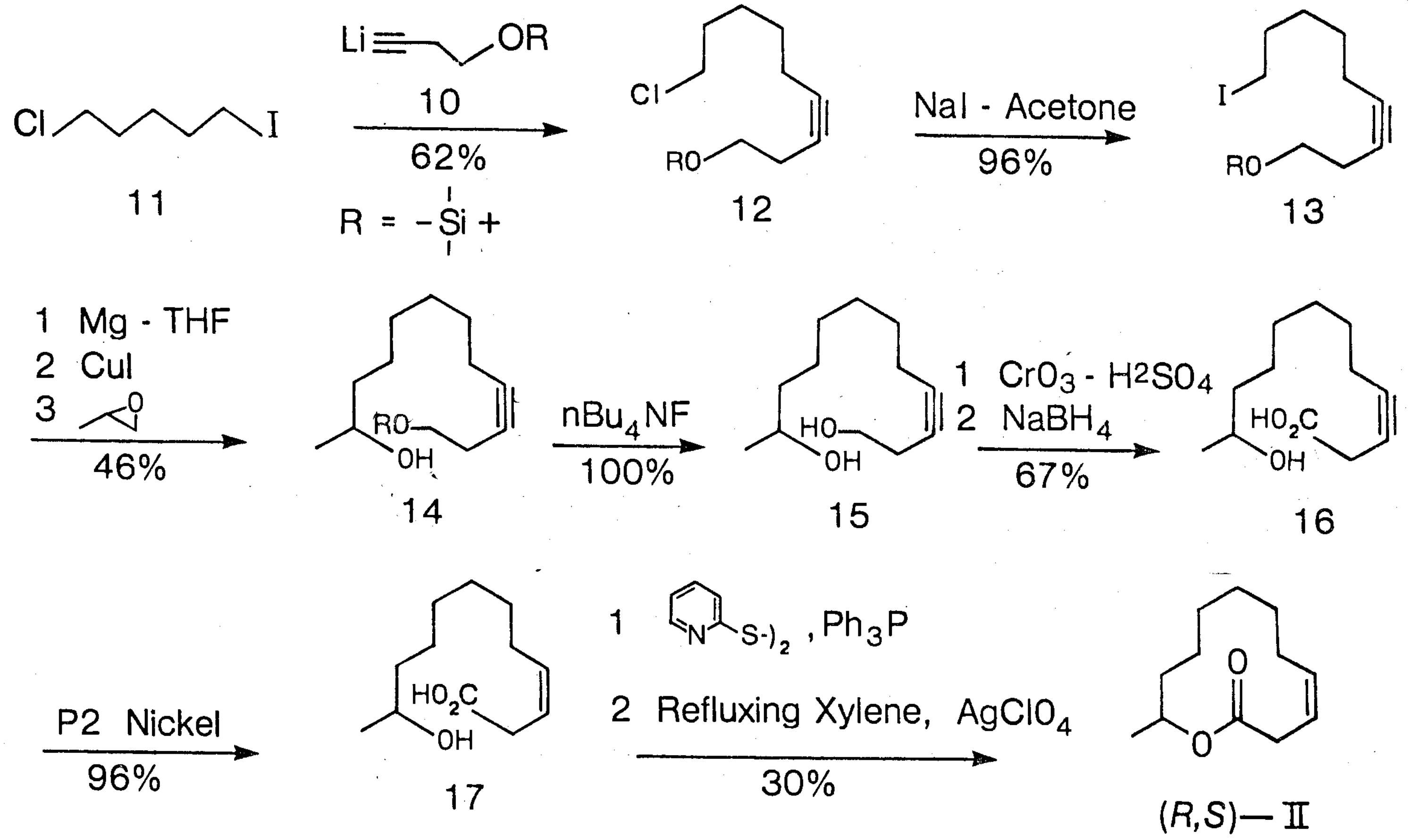


Figure 18. Synthesis of (R,S)-II via the intramolecular esterification of the ω-hydroxy acid (17)

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241, (16.2, M-57), 199 (9, M-99), 187 (9), 186 (19), 185 (100), 171 (18), 157 (23), 147 (28), 133 (19), 73 (57). Compounds which contain the t-butyldimethylsilyl group readily lose the t-butyl radical to give an M-57 ion. The presence of isotope peaks at m/z 242, 243 and 244 indicated that the m/z 241 ion contained two silicon atoms. Loss of a neutral fragment which had a molecular weight of 113 (t-butyldimethylsilyl group - two hydrogens) left the base peak (m/z 185) which only contained one silicon atom. This information led to the tentative identification of 36. The yield of 36 accounted for as much as 28% of the reaction products when the inverse addition method was employed, however, this yield decreased to 11% when HMPA was added last. In this reaction the rate increase caused by the carbanion stabilizing property of HMPA was quite dramatic, since no reaction was observed in the absence of HMPA. The regioselectivity of the nucleophilic displacement was very high (chloride 12:iodide 13, 38.5:1 by GLC quantitation).

Preparation of the iodide (13) was used to facilitate the formation of the Grignard reagent. A 96% yield of 13 was obtained by the standard sodium iodide in refluxing acetone procedure.

The nucleophilic ring opening of oxiranes by organometallic reagents is a well known reaction (Gaylord and Becker, 1951; Johnson *et al.*, 1973). Recently, the stereospecific ring opening of (R)-(+)- and (S)-(-)-propylene oxide by an allylic Grignard reagent in the presence of cuprous iodide, was used to prepare the two enantiomers of 6-methyl-5-hepten-2-ol, pheromones for *Gnathotricus* spp. (Johnston and Slessor, 1979). This reaction was attempted on 13 with 0.5 equivalents of cuprous iodide and succeeded in giving a 46% yield of the alcohol (14). The remainder of the product was mainly the alkane which resulted from hydrolysis of the unreacted Grignard. Variations in the reaction conditions such as solvent used, temperature, concentration of starting material, and quantity of cuprous iodide, did not cause any improvement in the yield of 14. No satisfactory explanation for the low yield of 14 was determined.

The diol (15), obtained in quantitative yield after the removal of the t-butyldimethylsilyl group, was oxidized with 6 equivalents of chromium trioxide in 6N sulfuric acid by the inverse addition procedure (Holland and Gilman, 1974). The inverse addition method, in which an acetone solution of the alcohol is added slowly to the chromium trioxide-sulfuric acid solution, was employed to minimize the yield of ester side products that can be derived from the reaction of intermediate oxidation products with unreacted alcohols. The resultant keto-acid was purified by crystallization and then reduced with sodium borohydride to give 16 in 67% yield from the diol.

Reduction of the alkyne in 16 to a Z olefin, with a high degree of stereospecificity, was achieved by hydrogenation with P-2 nickel catalyst modified with ethylenediamine (Brown and Ahuja, 1973). The Z:E ratio that was determined in (R,S)-II (376:1) was higher than the ratios reported by Brown and Ahuja for a series of model alkynes. However, a larger quantity of the fully reduced product (Z:fully reduced product, 376:18) was detected as

11-methylundecanolide.

Lactonization of 17 was initially attempted using the reagents and conditions developed for the ring closure of 9. Under these conditions no product was detected even when the toluene was refluxed for several hours. The reaction was then attempted with 2,2'-dipyridyl disulfide instead of 34. Dropwise addition of the thioester mixture into refluxing toluene which contained silver perchlorate also yielded no product until xylene was added to the reaction mixture. Apparently, the higher reflux temperature was needed to force the ring closure to take place. The best yields (30-37%) were obtained by dropping the thioester into refluxing xylene (with silver perchlorate). Note that silver ion was necessary for the lactonization of 17, while it decreased the yield of I. Although II contains a 12-membered ring and can be considered a large ring macrolide, an examination of space-filling models revealed that its structure is much more rigid and strained compared to the 11-membered (medium ring) I. The presence of the Z-olefin in II is probably responsible for a large measure of the ring strain which made ring closure difficult since saturated 11- and 12- membered rings have approximately the same reactivity toward lactonization (Galli *et al.*, 1973). Recifeiolide, which is identical to II except that it has an (E)-$\Delta^{8,9}$ unsaturation and an R configuration,was prepared in 75% yield under conditions which were almost identical to those described for (R,S)-II (Gerlach *et al.*, 1976). Ring closure to form recifeiolide took place under relatively mild conditions since the reaction was performed in refluxing acetonitrite (65^{o}). Therefore, it appeared that ring strain caused by the Z-olefin was probably the major cause of the low yields obtained for the lactonization of 17. The overall yield for the synthesis of (R,S)-II was 5.3%

Syntheses of (R)-(-)- and (S)-(+)-II were carried out by the scheme shown in Figure 19. This scheme is similar to the one shown in Figure 18 except for the two steps required for the protection and deprotection of the chiral secondary alcohol. The β-methyoxy-ethoxymethyl (MEM) group was chosen for its stability toward mild acids, which was necessary to withstand the oxidation conditions (Corey, *et al.*, 1976). Preparation of the MEM ethers of (R)- and (S)-18 followed by cleavage of the t-butyldimethylsilyl group gave the primary alcohols ((R)- and (S)-19) in 72% yield overall. Oxidation under the conditions described for the preparation of 16 gave a 54% yield of the MEM-acid ((R)- and (S)-20). The yield was lower than that reported for 16 due to the partial loss of the MEM group. Difficulty was encountered in the attempted removal of the MEM group from 20. When the reaction was carried out by Corey's procedure (i.e. procedure used for preparation of (S)-16), a very low yield of the desired product was obtained. Although the starting MEM-ether had been totally consumed, GLC analysis revealed a very complex product mixture. By reducing the reaction time to 5 min (preparation of (R)-16) a yield of 49% was obtained. Another lewis acid, titanium tetrachloride, was tried without success. Because of the problems encountered in removal of the MEM group, another protecting group should be used for the secondary alcohol.

The synthesis of (R,S)-III was carried out by a route which was analogous to the one used for preparation of (R)- and (S)-II. A higher yield (70%) was obtained for the coupling of the protected alkynol (21) with 1-chloro-5-iodopentane. The extremely low yield reported for the oxidation and

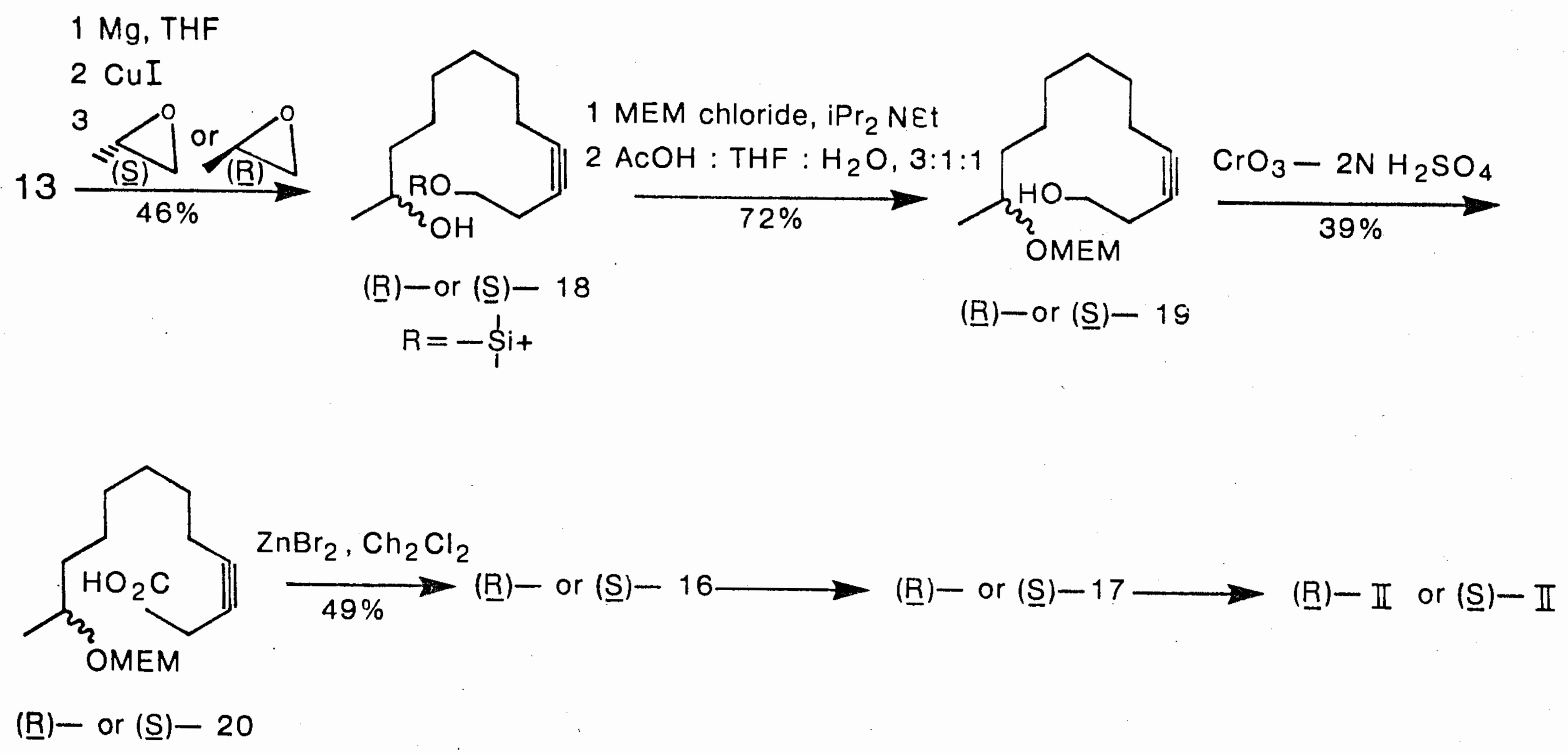

Figure 19. Syntheses of (S)-(+)- and (R)-(-)-II

the removal of the MEM group (10.8% overall) was due mainly to the MEM removal reaction since the problems discussed above were not yet recognized. Lactonization via the procedure used for the preparation of I, gave a low yield of the 14-membered lactone. Note that ring closure under these conditions was not achieved for the 11-membered II. A higher yield of (R,S)-III would have been obtained with the lactonization procedure developed for II.

IV. CONCLUSION

16) Ferrulactones I and II, First Macrolide Insect Pheromones

The identification of ferrulactones I and II as the aggregation pheromone for *C. ferrugineus* revealed the first example of macrolide insect pheromones. Other structurally similar macrolide natural products have been isolated from a variety of organisms including insects. In fact, compounds II and V for which no function has been assigned in *C. ferrugineus*, have been identified from the beetle volatiles of other *Cryptolestes* spp. Compound III was a minor constituent of *C. pusillus* volatiles while both III and V were found in *C. turcicus* volatiles. Their role as pheromones is currently under investigation (J. Millar, personal communication)[1]. Interspecific communication between *C. ferrugineus* and the other *Cryptolestes* spp. would be possible if III and IV were attractive to their emitters.

The metasternal gland secretion of a cerambycid beetle, *Phoracantha synonyma* (Newman) contains several macrolides (Moore and Brown, 1976) which are structurally similar to the compounds isolated from *C. ferrugineus*. Ten, twelve and fourteen-membered lactones, each bearing a methyl group adjacent to the alkyl oxygen of the ester, were identified. The 12-membered lactone which Moore and Brown (1976) discovered, (Z)-5-dodecen-11-olide, differs from II only in the position of the double bond. They also report the identification of 5-tetradecen-13-olide (III) and 5,8-tetradecadien-12-olide which may be the same as V. The macrolides produced by *P. synonyma* have not been implicated as pheromones.

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[1] J. Millar, Chemistry Department, Simon Fraser University.

A 14-membered lactone, 13-(1-methylpropyl)tridecanolide, has been isolated from the total extract of the argentine ant, *Iridomyrmex humilus* (Cavill *et al.*,1979). The biological function of this macrolide has not yet been determined.

Recifeiolide, (8E,11R)-dodecen-11-olide, is a fungal metabolite which was isolated from the growth medium of *Cephalosporium recifei* (Vesonder *et al.*, 1971). The only differences between recifeiolide and II are the position and geometry of the double bond and the absolute configuration of the chiral center. A number of studies have been directed toward the synthesis of recifeiolide (Gerlach *et al.*, 1976; Utimoto *et al.*, 1977; Trost and Verhoeven, 1978; Shreiber, 1980).

Saturated 13, 14, 15 and 16-membered lactones which have a methyl side chain next to the ester alkyl oxygen, have been isolated from a *Galbanum* absolute (Kaiser and Lamparsky, 1978)

γ-Nonalactone is a microbial product which as been detected in beer (Tressl *et al.*, 1978). The role of γ-nonalactone as a pheromone of *C. ferrugineus* has not been determined.

17) Potential Use of Ferrulactones I and II in Detection, Survey and Control of *C. ferrugineus*

Results from the preliminary field tests of ferrulactone I and II (section III-14) have indicated that pheromone-baited traps are more effective than unbaited traps at capturing *C. ferrugineus* in grain storage bins. Further studies concerned with trap location, density, and pheromone release rates are required to determine the most effective procedure for rusty grain beetle detection. Studies should be carried out in an enclosed, grain-filled bin which has been sealed to prevent insect entry or exit. For example, to determine the best trap locations, baited traps should be placed at regular intervals into grain in which a known number of beetles has been released and allowed to disperse. The results from this experiment should indicate whether beetles are found preferentially near the top, bottom or corners of a bin and therefore provide some idea as to where survey traps should be placed. Trap density depends on the range of the pheromone concentration. At a given pheromone release rate (1, 2, 3, ... etc. fibres), range can be determined by introducing a specific number of insects between a control trap and a baited trap. The distance between the two traps could be increased until there is no significant difference between the numbers of beetles caught by the traps in a given period of time. The efficiency of a trap utilizing a specific pheromone release rate can be measured by the percentages of the total number of released beetles that are captured. For example, if a single fibre trap captures 50 out of 100 released beetles within a 152 cm radius, then the trap can be considered to be 50% efficient for the 152 cm range. All of the information that could be obtained from these proposed experiments would be valuable for setting up a monitoring program. Once the range and efficiency of a baited trap is known, survey of an insect population becomes possible. An order of events for the control of a stored-products pest utilizing pheromone-baited traps has been proposed by Levinson and Levinson (1979). Initially a few baited traps are placed in locations which are likely to be infested. The discovery of insects in these detection traps would be the signal for placing more baited traps into the grain for the purpose of estimating the size of the infestation. Once the insect population is known a decision can be made concerning the application of pesticides or other control procedures. The choice of control procedure would depend on whether the economic threshold has been reached (Burkholder, 1981). The pheromone-baited trap may be useful for control as a mass-trapping tool, or utilized with pesticides or insect pathogens. Population suppression by the dissemination of a protozoan pathogen has been carried out successfully with a pheromone-baited trap for *Trogoderma glabrum* (Shapas *et al.*, 1977).

18) Future Directions

The results presented in this study have firmly established the identity and function of ferrulactones I and II as the aggregation pheromone for *C. ferrugineus.* Thus the objectives set out in the introduction have all been reached. Preliminary field tests suggested that the ferrulactones could be useful in the detection of *C. ferrugineus* in grain storage areas. Proposals for future studies which could add to the knowledge gained in this study are presented below.

i) Fungal Attractants for *C. ferrugineus*

The existence of fungal attractants for *C. ferrugineus* was invoked to account for the difference in activity between frass volatiles and a mixture of I and II (section III-11-ii). In nature, *C. ferrugineus* may use fungal volatiles to locate a pocket of grain which is moist and otherwise suitable for infestation. The ability to locate the source of fungal volatiles would give an advantage to the beetles since infestations are easier to start in mouldy grain due to the warmth, moisture, and additional food source. The possibility that a readily available fungal compound could enhance the attractiveness of the ferrulactones and therefore decrease the quantity of I and II that are necessary for trap baits, should provide incentive for a search for fungal attractants. For example, the fungal compound 1-octen-3-ol, is attractive to *Oryzaephilus mercator* at a dose of 100 picograms (A.M. Pierce, personal communication)[1]. Fungal volatiles could be obtained by the aeration of mycelial mats from still cultures of pure strains of fungi which are usually associated with stored wheat. *Nigrospora sphaerica* would be a prime target since kernels of wheat infected with *N. sphaerica* were attractive to *C. ferrugineus* (Loschiavo and Sinha, 1966). The fungal volatiles could then be tested for attraction, alone and in combination with the ferrulactones, in the pitfall olfactometer. Once an attractive extract has been identified, it can then be put through the fractionation procedure used for the isolation of the pheromones. If readily available attractants are isolated from fungal volatiles, they may be used in conjunction with the ferrulactones.

ii) Biosynthesis of the *C. ferrugineus* Macrolides

Very little research has been done on the biosynthesis of beetle pheromones. The information that could be obtained from a study of *in vivo* pheromone synthesis may be of importance in several ways: 1) an insect pest might be specifically controlled by the interruption of its pheromone

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[1] A.M. Pierce, Chemistry Department, Simon Fraser University.

synthesis, 2) one could determine the extent to which insects depend on host plant products for pheromone synthesis, and 3) the study of pheromone biosynthesis could reveal information about the evolution of chemical communication in insects.

The primary objectives of initial biosynthetic studies on the ferrulactones would be to determine the precursors, intermediates, and mode of synthesis. Precursor-product relationships can usually be established by the administration of heavy atom or radio-labelled precursors followed by the isolation and analysis of the products, the structures of the *C. ferrugineus* macrolides suggest that they could all be derived from fatty acids except for I which appears to be terpenoid. Radio-labelled acetate could be fed to the beetles (impregnated on wheat germ) to determine if *de novo* biosynthesis of the macrolides occur. For example, the pheromones of *Musca domestica* (Dillwith *et al.*, 1981) and *Trichoplusia ni* (Jones and Berger, 1978) are synthesized *de novo* from acetate. The terpenoid origin of I could be established by the administration of labelled mevalonate. Failure to incorporate label from simple precursors may indicate that pheromone synthesis takes place by the degradation of host products. Ferrulactone I may be derived from E,E-farnesol by an oxygenase mediated cleavage of the terminal double bond followed by ring closure (Fig. 20). Farnesol labelled with deuterium or tritium at the C_1 position could be used to determine if this takes place. The position ($C_{9,10}$ from the terminal carbon) and geometry (Z) of the double bond in II and III suggest that they could be derived from oleic acid. Two or three cycles of β-oxidation would be required to generate the 12- and 14 carbon acids. The stereospecific oxidation of the penultimate

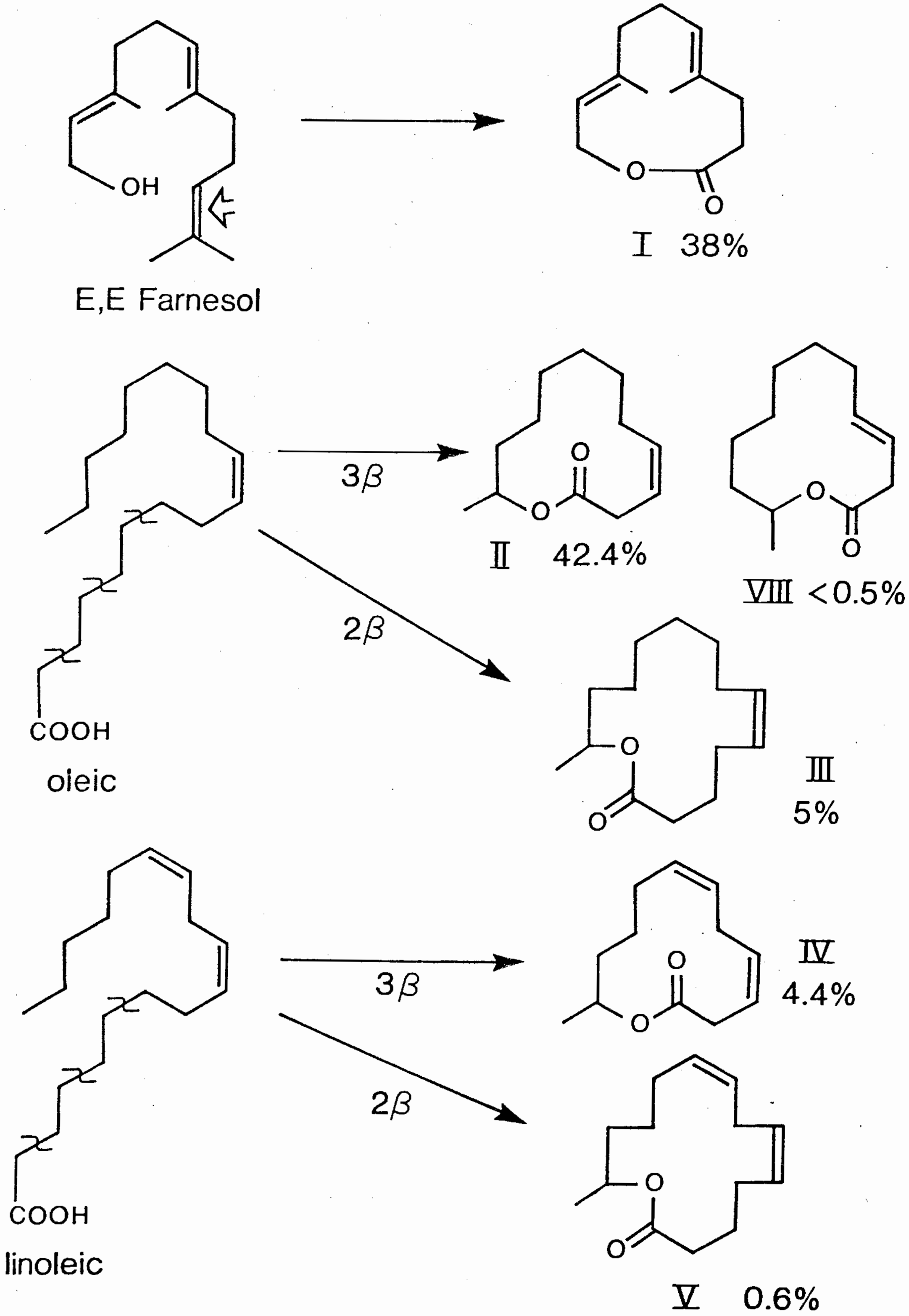

Figure 20. Biosynthetic scheme for *C. ferrugineus* macrolides

carbon, which is required to introduce the secondary alcohol, has been observed in a *Torulopsis* sp. (Heinz *et al.*, 1970). To determine if dietary oleic acid undergoes cleavage and oxidation, [9, 10-^{3}H]-oleic acid could be administered together with [1-^{14}C]-oleic acid. Label would be incorporated only from the [9, 10-^{3}H]-oleic acid since the ^{14}C label would be lost in the first cycle of β-oxidation. Compounds IV and V could be derived in an analogous manner from linoleic acid. Example of pheromones which are derived from a dietary constituent are n-nonanal and n-undecanal, sex pheromones for *Galleria mellonella*, which were shown to be degradation products of dietary oleic acid (Schmidt and Monroe, 1976).

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