ISOLATION AND IDENTIFICATION OF AGGREGATION PHEROMONES FOR *CRYPTOLESTES PUSILLUS* (SCHÖNHERR) AND *CRYPTOLESTES TURCICUS* (GROUVELLE), AND THE SYNTHESIS OF MACROLIDE PHEROMONES FOR *CRYPTOLESTES* AND *ORYZAEPHILUS* SPECIES (COLEOPTERA:CUCUJIDAE)

by

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B.Sc. (Hons.), Simon Fraser University, 1978

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the

Department of Chemistry

SIMON FRASER UNIVERSITY

October, 1983

APPROVAL

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Degree: Doctor of Philosophy

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ABSTRACT

Aggregation pheromones were isolated from Cryptolestes pusillus (Schönherr) and C. turcicus (Grouvelle), two species of coleopteran pests of stored products. Porapak Q-captured beetle and frass volatiles were fractionated by preparative gas chromatography. The fractions were tested for biological activity with an arena olfactometer bioassay and with a two-choice pitfall bioassay.

Three biologically-active compounds eliciting aggregation behaviour from adult C. pusillus were isolated and identified by spectroscopic methods as (3Z)-dodecenolide (I), 13-methyl-(5Z)-tridecenolide (II) and (3Z,6Z)-dodecadienolide (IV). Compound I was the major constituent of the pheromone and was active by itself. Compound II was not active alone but synergized the response to I. Compound IV was active by itself at high concentrations but did not significantly increase the response when added to the most active mixture of I and II.

Two biologically-active compounds were isolated from beetle and frass volatiles of C. turcicus and identified spectroscopically. The first, 13-methyl-(5Z,8Z)-tridecadienolide (III), was active alone and was synergized by the other previously identified component, 13-methyl-(5Z)-tridecenolide (II). Compound II was not active alone.

For both C. pusillus and C. turcicus, the pheromones were shown to be male-produced but attractive to both sexes. In addition, it was found that pheromone production increased dramatically when the insects were aerated on a food source, as opposed to aeration of insects with no food source present.

Compounds I to IV are all macrocyclic lactones and were synthesized by

routes based on the intramolecular lactonization of the appropriate hydroxy-acids. The enantiomers of II and III were made via the stereo- and regiospecific ring opening of chiral methyloxiranes.

An aggregation pheromone component for the closely related coleopteran Oryzaephilus mercator (Fauvelle), isolated and identified by other workers as 11-methyl-(3Z,6Z)-undecadienolide (V), was synthesized in racemic form. Macrolide V is also produced by Cryptolestes ferrugineus (Stephens).

To my family and friends,

who never lost faith in me.

Problems worthy of attack

Prove their worth by hitting back.

Piet Hein

ACKNOWLEDGEMENTS

I would like to thank Drs. A.C. Oehlschlager, H.D. Pierce, Jr., A.M. Pierce, and J.H. Borden for assistance and guidance. Thanks also to Mr. B.D. Johnston for advice on synthetic methods.

I would like to thank all the technicians for their assistance and patience; especially Mr. G. Owen, Mrs. M. Tracey, Ms. L. Chong, Mr. A. Javar, and Mr. M. Yang. Thanks also to Ms. L. Vincent for typing the manuscript.

Thanks to Dr. A.V. Barak at the University of Minnesota for C. pusillus cultures, and thanks to the Agriculture Canada Research Station in Winnipeg for the C. turcicus culture.

Finally, I gratefully acknowledge the generous financial support of the Natural Sciences and Engineering Research Council, and of the university's C.D. Nelson Memorial Scholarship Fund.

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I. INTRODUCTION

Insect pests are responsible for considerable economic losses in a wide variety of stored products. Until recently, most monitoring techniques were based on random sampling methods or passive encounter traps (Loschiavo, 1967) which suffer the disadvantage that insect infestations tend to be localized in pockets of high density (Freeman, 1952) rather than dispersed throughout the grain mass. There is thus a definite need for a monitoring system which will actively attract pest insects to a trap. Such systems would provide a more reliable indication of insect populations. There has been considerable and wide-spread interest in and research devoted to developing pheromone-based monitoring systems for stored product insects. Recently, Zoecon Corporation[1] has introduced a commercial line of pheromone-baited traps for a variety of stored product pests, including weevils and beetles.

Two serious and wide-spread pests of stored products for which pheromones had not been identified are Cryptolestes pusillus (Schönherr), the flat grain beetle (Currie, 1967), and Cryptolestes turcicus (Grouvelle), the flour mill beetle (Chang and Loschiavo, 1971). Both of these insects have been reported in storage, processing and transportation facilities world-wide (Howe and Lefkowitz, 1957), infesting primarily whole or milled grain. They have also been found on foodstuffs as diverse as cacao, spices (Reid, 1942), fish meal (Payne, 1946), dried mushrooms, and copra (Howe and Lefkowitz, 1957). In Canada, both C. pusillus and C. turcicus are reported to be quite common, especially in grain milling and processing facilities. No accurate surveys for the individual species are available, as all Cryptolestes species are classified together as C. ferrugineus, the rusty

grain beetle (John Elvidge[2], pers. comm.). In the United States, more accurate surveys have determined C. pusillus and C. turcicus to be of major importance, especially in the grain-producing mid-western states (Barak and Harein, 1981; D.K. Mueller[3], pers. comm.).

In addition to the damage to stored grains caused by Cryptolestes species feeding, further damage is caused by the metabolic heating generated by an infestation (Freeman, 1952; Watters, 1969). The heat may cause the infested grain to cake and germinate. Fungi and moulds are usually associated with infestations, so the grain quality is considerably lowered (Anderson, 1943). Fumigation of infested grain is also required before it may be exported.

It is thus essential to detect and eradicate insect populations in stored products as soon as possible to minimize damage and prevent the spread of an infestation. Pheromone-baited traps may be the ideal detection method.

A previous study initiated under the auspices of the USDA (Quaife, 1980) indicated that male flat grain beetles produce an aggregation pheromone. Both male and female insects approximately one-week old were found to respond positively to Porapak-Q captured male volatiles in two different bioassay methods. However, no attempt was made to isolate or identify the biologically-active compounds.

To date, no work has been reported on pheromones for C. turcicus. However, as aggregation pheromones had been demonstrated in two species of the same genus, it seemed likely that C. turcicus might also utilize aggregation pheromones.

Objectives

The major objectives of this research were:

1) To isolate, identify, synthesize and bioassay aggregation pheromone components for the flat grain beetle.

2) To prove or disprove the existence and utilization of aggregation pheromones for the flour mill beetle.

3) If aggregation pheromones exist for the flour mill beetle, to isolate, identify, synthesize and bioassay their components.

In addition, research by other workers (Pierce *et al.*, 1981; 1983) on the closely related *Oryzaephilus* genus revealed the existence of aggregation pheromone components of similar or identical chemical structures to those of the *Cryptolestes* species. Thus, as part of a collaborative effort, the syntheses of some *Oryzaephilus* pheromone components were undertaken.

II. EXPERIMENTAL METHODS

A. Rearing of C. pusillus and C. turcicus

C. pusillus starter cultures were obtained from stock cultures maintained at the University of Minnesota, St. Paul, Minnesota, and from Kansas State University, Manhattan, Kansas. C. turcicus cultures were obtained from the Agriculture Canada Research Station in Winnipeg, Manitoba. Both species of insects were reared on a diet of rolled oats and brewer's yeast (95:5, w/w) in 3.8 L wide-mouthed glass jars. The cultures were maintained at 30° ±2° and >60% relative humidity in the dark, with a density of 1000-3000 insects per kg of diet. Jars were recultured every 6-8 weeks.

Insects for bioassay or aeration were sieved out of the rolled oats media with a No. 25 sieve. The residual tailings plus insects were dumped into an enamel pan and the insects were aspirated up as they walked away from the tailings. The tailings were then sifted through a standard 250 μm sieve to collect the frass (a mixture of boring dust and insect feces), which often contains insect pheromones. The collected frass was stored at -30° in sealed glass jars until needed.

Numbers of insects were estimated by weight (≈3200 insects/g). Sexually segregated beetles were obtained by a previously undescribed method. It was observed that gentle pressure on the abdomen of cold-immobilized female insects of both species resulted in reversible extrusion of the genitalia. The process did not appear to do any permanent damage to the insects. With male insects, no extrusion occurred. This method appeared to be more reliable and much easier than the previously reported methods of sexing by differences in antennae length or counting the segments of the hind tarsi (Currie, 1967).

B. Capture of Volatiles

Capture of Insect Volatiles

Insect volatiles were captured on Porapak Q by the procedure of Verigin (1980) and Wong (1982). Air was drawn by water aspirator at 2 L/min through a water bubbler and an activated charcoal scrubber tube (2.4 cm O.D. × 12 cm, 50/80 mesh charcoal). The humidified, clean air was then passed over beetles held in a 2 L Erlenmeyer flask. The exitting air was passed through a glass tube (2.4 cm O.D. × 20 cm) full of Porapak Q chromatographic packing material (ethylvinylbenzene-divinylbenzene copolymer, 50/80 mesh, Applied Science Laboratories, Inc.) to trap the insect volatiles.

Insects for aeration were sieved from high-density mixed age and sex cultures. Approximately 20,000 to 100,000 insects were aerated at a time, for a period of three days. The insects were then allowed to feed for at least three days before being aerated again. A Porapak Q trap was loaded with up to fifty million beetle-hours before extraction.

Relative amounts of beetle volatiles and extracts were calculated in units of beetle-hours (bh), where one bh equals the amount of volatiles produced by aerating one beetle for one hour.

New Porapak Q was preconditioned as follows. The beads were loaded into the glass tube used for volatiles trapping and heated to 240° for 24 h under helium. This was followed by Soxhlet extraction with ether for 24 h to remove non-polymerized material.

Volatiles were extracted from the Porapak Q by Soxhlet extraction for at least 24 hours with doubly distilled-in-glass pentane. The pentane extracts were concentrated to a couple of mL with the aid of a 30 cm Dufton

column and then made up to 10.0 mL in a volumetric flask. The extracts were transferred to glass vials with Teflon-lined screw caps and stored at -30° until used.

The pentane-extracted Porapak Q was then extracted again with ether, as described above, air-dried for several days and repacked into the trapping tube. The tube was heated at 60° for 8 h under helium to remove traces of solvent, and was then ready for re-use. The ether extract was treated as described for the pentane extract.

Capture of Frass Volatiles

Frass volatiles were captured as described by Verigin (1980) and Wong (1982). Approximately 300 g of frass were packed into a glass tube (4.8 cm O.D. × 40 cm) fitted with a tube of activated charcoal (2.4 cm O.D. × 12 cm) and a tube of Porapak Q (2.4 cm O.D. × 20 cm). Air was drawn sequentially by aspiration (≈2 L/min) through the charcoal scrubber, the frass and the Porapak Q, leaving the frass volatiles trapped on the Porapak Q. Aeration was continued for one week, after which time the frass was ground in a ceramic ball mill and re-aerated for another week. The frass volatiles were then extracted from the Porapak Q as described above. Relative amounts of frass volatiles and extracts were calculated in units of gram-hours (gh), where one gh equalled the volatiles from one gram of frass aerated for one hour.

Capture of Volatiles of Insects on Oats

To determine the effect of having a food source present on pheromone production, approximately 3000 insects were aerated on 50 g of rolled oats for a period of one week. The oats plus insects were loaded into a glass

chamber (15 cm O.D. × 20 cm), fitted with the previously described water bubbler, charcoal scrubber and Porapak Q trap, and air was drawn through the system by aspiration (≈2 L/min). The volatiles were extracted from the Porapak Q as described above.

Aeration of Sexed Insects

A scaled-down version of the insect aeration apparatus was used to aerate sexed insects. Thus, air purified by passage through a charcoal scrubber was drawn by aspiration (500 mL/min) over several hundred male or female insects, respectively, in a 125 mL Erlenmeyer flask, and the insect volatiles were trapped on a small glass tube filled with Porapak Q (1 cm I.D. × 12 cm). The volatiles were then extracted from the Porapak Q as described above.

C. Isolation of Compounds from Volatile Extracts

General Experimental Procedures

The pentane extracts of insect volatiles were subjected to analytical GLC analysis, using a Hewlett-Packard 5830A gas chromatograph equipped with a 18835B capillary inlet system and flame ionization detector, and a Hewlett-Packard 5985B coupled GC/MS system. Columns and representative running conditions are shown in Table I. A sample size of 2-5 μL was injected in the splitless mode.

For preparative GLC work, a Varian 1200 gas chromatograph fitted with a 10:1 effluent splitter, a flame ionization detector and a thermal gradient collector (Verigin, 1980) was used. In a typical separation, the 10 mL solution of insect volatiles in pentane was cooled and concentrated to approximately 50 μL under a stream of nitrogen, in a conical-bottomed

vial so that the residue could be quantitatively removed by syringe. The concentrate was injected on the column and fractions were collected in 20 cm × 1.6 mm O.D. glass tubes. The tubes were then capped with rubber cryocaps for short-term storage or sealed for long-term storage. Separated fractions were stored at -30° until needed. The purity of collected samples was determined by capillary GLC.

Preparative GLC was found to give adequate separation of all the active pheromone components I-III for C. pusillus and C. turcicus.

D. Identification of Pheromone Components

Spectroscopic Methods

Low resolution mass spectra of crude volatile extracts were obtained with a Hewlett-Packard 5985B GC/MS system, with electron impact ionization at an energy of 70 eV. GLC columns and conditions were as described in Table I.

^{1}H NMR spectra were recorded on a Bruker WM 400 MHz instrument, in either 1.2 mm or 5 mm I.D. NMR tubes, using $CDCl_3$ (99.8%-D, Merck, Sharpe and Dohme) or D_6-benzene (99.96%-D, Merck, Sharpe and Dohme) as solvents.

Fourier transform IR spectra in the gas phase of crude volatile extracts were taken by the Nicolet Corporation of Madison, Wisconsin, on a Nicolet 60SX FT-IR coupled to a Hewlett-Packard 5792A capillary gas chromatograph.

Coupled GLC-high resolution mass spectra were taken on a Varian MAT 212 GC-HRMS, using a SS 200 data system. A 25 m × 0.21 mm Carbowax 20M fused silica column was used, with temperature programming from 50-200°C at 5°/min. Spectra were taken by Varian Corporation of Palo Alto,

Instrument	Column						Running Conditions		
	Type	Liquid Phase	Solid Support	Length (m)	Diameter (mm)	Construction Material	Injector Temp. (°C)	Detector Temp. (°C)	Typical Oven Temp.
Hewlett Packard 5830A	Capillary	SP-1000		30	0.66 (ID)	Glass	260	275	70° for 2 min, 4°/min to 200°C
	Capillary	Superox-4		48	0.5 (ID)	Glass	260	275	70° for 2 min, 4°/min to 200°C
Hewlett Packard 5895 GC/MS	Capillary	Carbowax 20M		30	0.32 (ID)	Fused Silica	260		50° for 2 min, 4°/min to 200°C
	Capillary	SP-1000		30	0.32 (ID)	Fused Silica	260		50° for 2 min, 4°/min to 200°C
	Capillary	DB-1		15	0.2 (ID)	Fused Silica	260		50° for 2 min, 4°/min to 200°C
Varian 1200	Packed Column	10% SP-1000	Supelcoport, 100/120 Mesh	3	3.2 (OD)	Stainless Steel	240	275	70° for 2 min, 4°/min to 200°C

Table I. Experimental conditions for gas-liquid chromatography.

California.

Microhydrogenation of Crude Volatile Extracts

A thick-walled 2 mL vial with a tight-fitting rubber septum was loaded with a few grains of 10% palladium on carbon. Pentane (250 μL) and 50 μL of crude volatiles solution were added, the vial was pressurized to 10 p.s.i. with hydrogen and the mixture was stirred for 1 h. The mixture was then filtered through glass wool and submitted to analytical GLC and GLC/MS.

E. Bioassay Procedures

Arena Olfactometer

The arena olfactometer bioassay was essentially the same as that described by Borden et al. (1979), with minor modifications. The arena was a 15 cm × 15 cm piece of black construction paper clipped to a glass plate. An air nozzle in the middle of one side of the arena was used to create an odour plume over the arena surface. A glass tube containing a rolled up filter paper wick impregnated with the stimulus was slipped into the air nozzle and the air flow (800 mL/min) was directed towards the insect release point at the center of the arena.

Insects for bioassay were maintained in holding vials, with fifteen insects per vial, and starved for approximately 48 h in the dark. This "conditioning" procedure was modified for later bioassays, by starving the insects in a 2 L aeration flask, with an air flow of ≈2 L/min over the insects, so that any insect-produced volatiles were removed. The insects were starved for 48 h, and then counted into holding vials 1-2 h before use.

A typical bioassay was run as follows. A holding vial containing fifteen insects was inverted onto the center of the arena and left in place so that the insects could not escape. Meanwhile, a stimulus tube was inoculated with the required amount of stimulus solution and placed in the air nozzle, so that an odour plume was directed from the edge of the arena towards the insects trapped under the holding vial. The vial was then removed and the insects were allowed to walk around freely. Any insect reaching a 3 cm long × 1 cm deep area in front of the stimulus tube was counted as a positive responder, while any insect which reached the edge of the arena anywhere except at the "goal" area was removed and counted as a negative responder, as was any insect still walking around on the arena at the end of the allotted time limit of two minutes. Any dead or maimed insects were recorded and not counted in the total number of possible responders.

Each stimulus was tested with six groups of fifteen insects each. A fresh stimulus tube was used for each group and the arena paper was changed for each new stimulus. As the bioassay represented only a single choice, standard runs were done in each set of bioassays with a pentane blank and a standardized stimulus solution to ensure that the insects were responding well. On a given day, all test insects were drawn from the same population and each group of insects was only used once. Bioassays were run at room temperature under fluorescent light.

Two-choice Pitfall Olfactometer

The two-choice pitfall bioassay, developed by Pierce *et al.* (1981), was used to assay volatile stimuli *versus* a solvent control. Two 10 mL glass vials were suspended from holes cut in the bottom of a 15 cm diameter

plastic petri dish. A filter paper disk was treated with a pentane solution of the stimulus to be tested, the solvent was allowed to evaporate, and the disk was placed in one vial. A filter paper disc treated with the same volume of pure pentane was placed in the other vial. Fifteen insects were then released in the middle of the petri dish and the dish was covered. Six replicates of each stimulus were tested.

Bioassays were run for two hours in the dark, at 30° and approximately 60% humidity in a controlled environment chamber. At the end of the two-hour period, the numbers of insects in the control and stimulus vials were recorded, as well as the number of insects dead or still walking around.

After considerable experimentation to establish the conditions to obtain maximum responsiveness, the following general guidelines were established. Maximally responding insects were 4-10 week old adults of mixed sex from low density cultures of less than 1500 insects/kg diet. Insects were sifted out of cultures approximately 48 h before the bioassay and aerated in the dark in a 2 L aeration flask with clean, moist air until approximately two hours before the bioassay. Insects were then counted out into holding vials, using a hand aspirator.

After a bioassay, insects were allowed to feed for at least four days before being reused.

F. Experimental Bioassay Methods

Twelve sets of bioassays were performed with C. pusillus, as outlined in Table II (results are summarized in Tables IV-XIII and Figs. 15 and 16). The first four experiments used pentane extracts of Porapak Q captured beetle volatiles or fractions thereof. The next five assessed the attractiveness of synthetic macrolides I, II, and IV, followed by two

Table II. Description of experiments performed to investigate the response of C. pusillus to synthetic and beetle-produced pheromones.

Exp. No.	Objective	Experimental Procedures
1	To determine if attractive volatiles from C. pusillus populations could be captured on Porapak Q and extracted therefrom.	Pentane extracts of Porapak Q-captured volatiles tested for attractiveness at various concentrations in the arena olfactometer.
2	To determine whether fractions of a pentane extract of beetle volatiles were attractive to C. pusillus.	Prep. GLC fractions of a pentane extract of beetle volatiles tested in the pitfall olfactometer.
3	Retest the possibly attractive fractions from Exp. 2 above.	Prep. GLC fractions of a pentane extract of beetle volatiles and an unfractionated extract tested in the arena olfactometer.
4	To determine whether there was synergism of the attractive prep. GLC fraction of C. pusillus volatiles by any of the other fractions.	Pentane solutions of I, II, and mixtures of I + forerun, I + II, and I + afterrun tested in the arena olfactometer.

Table II (Cont'd)

Exp. No.	Objective	Experimental Procedures
5	To determine whether synthetic I alone and in combination with synthetic (±)-II was attractive to C. pusillus.	Pentane solutions of synthetic I and several ratios of synthetic I + (±)-II tested in the arena olfactometer.
6	To determine the upper and lower limits of amounts of synthetic I + (±)-II which will elicit positive attractance from C. pusillus.	Pentane solutions of ≈1:1 I:(±)-II tested over a range of concentrations from 1 ng to 20 µg in the arena olfactometer.
7	To determine the ratio of I:(±)-II which is most attractive to C. pusillus.	Pentane solutions of I and mixtures of I with (±)-II from 20:1 to 1:2 (I:(±)-II) tested in the arena olfactometer.
8	Repeat of Exp. 7 above, using different bioassay method.	Pentane solutions of I and mixtures of I with (±)-II from 20:1 to 1:2 (I:(±)-II) tested in the pitfall olfactometer.
9	To determine whether synthetic IV is attractive to C. pusillus.	Pentane solutions of IV tested in the pitfall olfactometer at several concentrations.

Table II (Cont'd)

Exp. No.	Objective	Experimental Procedures
10	To compare the attractiveness of synthetic pheromones to naturally produced pheromones.	Prep. GLC fractions of feeding *C. pusillus* volatiles, the unfractionated volatiles, combinations of the fractions, and synthetic (I + (±)-II) tested in the pitfall olfactometer.
11	To compare the attractiveness of synthetic pheromones to naturally produced pheromones.	Prep. GLC "macrolide" fraction, unfractionated volatiles, I, IV, and I + (±)-II tested in the pitfall olfactometer.
12	To test the attractiveness of synthetic (I + (±)-II) to male and female beetles.	Several concentrations of a pentane solution of synthetic I + (±)-II tested in the pitfall olfactometer.

experiments comparing synthetic and beetle-produced pheromones. The final experiment tested the attractiveness of a mixture of synthetic I plus (±)-II to male and female insects.

Ten experiments were performed with C. turcicus, as outlined in Table III. The first tested the attractiveness of clean food, beetle-infested food, live beetles, and frass, respectively. The next four experiments tested the attractiveness of various pentane extracts of beetle volatiles and fractions thereof, followed by four experiments testing responses to racemic and/or enantiomeric III and II and mixtures thereof. The final experiment tested the response of male and female insects to (±)-III.

Table III. Description of experiments performed to investigate the response of C. turcicus to synthetic and beetle-produced pheromones.

Exp. No.	Objective	Experimental Procedures
13	To determine if the odours of clean food, beetle-infested food, live beetles, or frass were attractive to C. turcicus.	Arena olfactometer; air to olfactometer outlet nozzle passed over clean food, beetle-infested food, live beetles, and frass, respectively, and tested for attractiveness to C. turcicus.
14	To determine if attractive volatiles from C. turcicus populations could be captured on Porapak Q and extracted therefrom.	Pentane extracts of Porapak Q-captured volatiles tested for attractiveness to C. turcicus at various concentrations in the arena olfactometer.
15	Repeat of Exp. 14, using different bioassay method.	Pentane extracts of Porapak Q-captured volatiles tested for attractiveness to C. turcicus at several concentrations in the pitfall olfactometer.

Table III (Cont'd)

Exp. No.	Objective	Experimental Procedures
16	To determine whether fractions of a pentane extract of beetle volatiles were attractive to *C. turcicus*.	Prep. GLC fractions of a pentane extract of beetle volatiles tested for attractiveness in the arena olfactometer.
17	To determine whether frass volatiles and volatiles from feeding beetles were attractive to *C. turcicus*.	Pentane extracts of Porapak Q-captured frass volatiles, or volatiles from feeding beetles tested for attractiveness at several concentrations in the pitfall olfactometer.
18	To determine whether synthetic (±)-III or (±)-II were attractive to *C. turcicus*.	Pentane solutions of (±)-III and (±)-II tested for attractiveness over a range of concentrations in the pitfall olfactometer.
19	To determine the most attractive ratio of the enantiomers of III to *C. turcicus*.	Pentane solutions of ratios of the enantiomers of III from pure (*R*)-III to pure (*S*)-III tested for attractiveness in the pitfall olfactometer.

Table III (Cont'd)

Exp. No.	Objective	Experimental Procedures
20	To determine the ratio of (±)-III:(±)-II which is most attractive to C. turcicus.	Pentane solutions of (±)-III and mixtures of (±)-III with (±)-II tested for attractiveness in the pitfall olfactometer.
21	To determine whether one enantiomer of II is more attractive than the other to C. turcicus in combinations with (±)-III.	Pentane solutions of mixtures of (±)-III with the enantiomers of II tested for attractiveness in the pitfall olfactometer.
22	To test the attractiveness of (±)-III to male and female C. turcicus.	Pentane solutions of (±)-III tested for attractiveness to sexed beetles at several concentrations in the pitfall olfactometer.

III. ISOLATION AND IDENTIFICATION OF COMPOUNDS I-V

A. Isolation of I-III

Isolation of (3Z)-Dodecenolide (I)

Compound I was isolated from approximately 9×10^6 beetle-hours of C. pusillus volatiles, by preparative GLC, as previously described. A purity check of the isolated material by analytical GLC on a SP-1000 capillary column (Table I) showed it to be >97% pure.

Isolation of 13-Methyl-(5Z)-tridecenolide (II)

Compound II was isolated by preparative GLC from approximately 8.4×10^4 gram-hours of C. pusillus frass volatiles. A purity check of the isolated fraction showed it to be >95% pure by analytical GLC on a SP-1000 capillary column (Table I). Compound II was also isolated by preparative GLC from 4.8×10^6 beetle-hours of C. turcicus volatiles.

Isolation of 13-Methyl-(5Z,8Z)-tridecadienolide (III)

Compound III was isolated by preparative GLC from 4.8×10^6 beetle-hours of C. turcicus volatiles. A purity check of the isolated material by capillary GLC showed it to be >96% pure. However, the actual purity was probably somewhat less, as the NMR of the isolated material showed some column bleed.

B. Identification of I-V

Identification of (3Z)-Dodecenolide (I)

Identification of I and II was considerably assisted by reference to the spectra obtained for 11-methyl-(3Z)-undecenolide, a pheromone component

of C. ferrugineus (Wong, 1982). Identification of I-IV was accomplished by chromatographic and spectrometric analysis (Figs. 1-8). Thus, preliminary examination of the crude extract of C. pusillus volatiles by GC-MS (Fig. 1-3) revealed that the major component (60% of the total volatiles) had a retention time and fragmentation pattern (Fig. 4) very similar to that of 11-methyl-(3Z)-undecenolide, indicating the strong possibility that I was a structural isomer of that compound. The highest observed peak in the mass spectrum was m/e 196, for a possible molecular formula of $C_{12}H_{20}O_2$, with three sites of unsaturation. The molecular formula was later confirmed by GC-HRMS (calculated for $C_{12}H_{20}O_2$: 196.1458; observed 196.060). GLC/FT-IR showed a carbonyl at 1735 cm^{-1}, corresponding to a non-conjugated carbonyl. Microscale hydrogenation showed the presence of one reducible olefin bond. Thus, the olefin and the carbonyl are responsible for two sites of unsaturation.

A closer examination of the mass spectrum showed the first significant fragment to be at m/e 178 (M-18), corresponding to loss of H_2O. Significantly, there was no peak at M-15, possibly indicating that there were no methyl groups. In the m/e 40-140 range there were clusters of peaks, separated by fourteen mass units, indicative of a hydrocarbon chain.

The ^1H NMR spectrum (Fig. 5) of I in $CDCl_3$ was analyzed as follows. A multiplet at δ 5.55 corresponded to two coupled, overlapping hydrogens, with a coupling of 10 Hz consistent with hydrogens on a cis double bond. A two-proton triplet (J = 5.25 Hz) at δ 4.09 was assigned to a methylene group adjacent to an ether or ester oxygen, coupled to another methylene group. A doublet (J = 6.8 Hz) with additional fine splitting, corresponding to two hydrogens, at δ 3.05, was coupled to the olefin hydrogens. The

simplicity of the splitting pattern and the chemical shift of this signal suggested it was due to an isolated methylene group between the double bond and a carbonyl (carbonyl confirmed by FT-IR). A two-hydrogen multiplet at δ 2.12, which collapsed to a triplet (J = 6.6 Hz) when decoupled from the olefin hydrogens, was identified as another allylic methylene group. Another two-hydrogen multiplet at δ 1.65 was coupled to the methylene at δ 4.09, with its chemical shift suggesting it was due to hydrogens β to an oxygen. There were ten additional methylene protons (δ 1.25-1.51) and no other high-field resonances, i.e., no methyl groups, suggesting a cyclic structure. This was supported by the remaining site of unsaturation not yet accounted for. In addition, the structure was probably one large ring with no branch points as there were no methine hydrogens or methyl groups. Thus, the macrolide structure I, shown in Fig. 7, was proposed on the basis of the evidence above. The only minor uncertainty was the geometry of the double bond.

The structure of the carbon skeleton of I was proven by hydrogenation of I to dodecanolide, which gave a mass spectrum and GLC retention times on several columns (Table I, HP 5985B) identical to a sample of synthetic dodecanolide (vide infra for synthesis).

Final confirmation of the structure of I was obtained by synthesis.

Identification of 13-Methyl-(5Z)-tridecenolide (II)

Preliminary GC-MS examination of the crude pentane extracts of C. pusillus volatiles and frass volatiles revealed a compound with a slightly longer retention time (Figs. 1-3) and similar fragmentation pattern (Fig. 6) to 11-methyl-3Z-undecenolide and to macrolide I. The highest observed peak in the mass spectrum was at m/e 224, suggesting a molecular

formula of $C_{14}H_{24}O_2$, which was later confirmed by GC-HRMS (calculated for $C_{14}H_{24}O_2$, 224.1776; observed: 224.140). This formula requires three sites of unsaturation. Closer examination of the mass spectrum revealed small but distinct peaks at m/e 209 (M-15, loss of CH_3) and at m/e 206 (M-18, loss of H_2O). In addition, the spectrum in the region m/e 40-140 exhibited clusters of peaks separated by approximately fourteen mass units, indicative of a hydrocarbon chain in the molecule.

Coupled GLC/FT-IR of II indicated a non-conjugated carbonyl (1735 cm^{-1}), accounting for one site of unsaturation.

Catalytic hydrogenation of II, followed by GC-MS analysis (Table I) of the hydrogenated products, revealed one carbon-carbon double bond, thus accounting for a second site of unsaturation. A cyclic structure was postulated to account for the third site.

The ^1H NMR spectrum ($CDCl_3$) of II isolated from C. pusillus frass volatiles (Fig. 7) showed two coupled single-hydrogen resonances at δ 5.32 and 5.38 (J = 10.5 Hz), indicating a Z disubstituted double bond. A one-hydrogen multiplet at δ 4.98 was coupled (J = 6.1 Hz) to a three-hydrogen methyl doublet at δ 1.22, suggesting a methine hydrogen on a carbon α to an oxygen, as in an ester. Two coupled (J = 15 Hz) multiplets at δ 2.43 and δ 2.22 were assigned as a methylene group α to a carbonyl. Irradiation of one of these signals (at δ 2.43) resulted in the partial collapse of a one-hydrogen multiplet at δ 1.83, locating one hydrogen β to the carbonyl. Irradiation of this signal resulted in the partial collapse of two one-hydrogen multiplets at δ 2.32 and δ 1.93. The chemical shifts of these signals suggested they were due to allylic hydrogens. This was confirmed by irradiation of the olefinic hydrogen at δ 5.30, which resulted in partial

collapse of the multiplets at δ 2.32 and δ 1.93. Thus, the double bond was confirmed to be δ,ε to the carbonyl. Two additional allylic hydrogens were located by irradiation of the signal due to the other olefinic hydrogen (δ 5.38), which partially collapsed two one-hydrogen multiplets at δ ≈2.20 and δ 1.73. The remaining eleven hydrogen resonances formed an overlapped multiplet at δ 1.10-1.65, characteristic of hydrocarbon chains. Thus, the structure of II was postulated to be a 14-membered lactone ring, with a methyl group on the carbon α to the oxygen of the ester function and a Z double bond four carbons removed from the ester carbonyl; that is, 13-methyl-(5Z)-tridecenolide (Fig. 7). The carbon skeleton was confirmed by catalytic hydrogenation, which gave a compound of molecular weight 226, with a mass spectrum identical to that of the compound 13-methyl-tridecanolide (Kaiser and Lamparsky, 1978). Final confirmation of the complete structure was obtained by synthesis (vide infra).

Identification of (3Z,6Z)-Dodecadienolide (IV)

Macrolide IV was found in C. pusillus volatiles when the insects were aerated either alone or on oats. The preliminary GLC-MS analysis of the crude volatile extract (Fig. 1) missed macrolide IV, as it was present at less than 1% of the main component, macrolide I. More careful examination of the volatiles mixture at a later date revealed the presence of IV. As IV was present in such small amounts, it was not isolated from C. pusillus volatiles. However IV had been isolated and tentatively identified from Oryzaephilus species by Drs. A.M. and H.D. Pierce (1983)[4]. Macrolide IV from both sources gave identical mass spectra (Fig. 8) and GLC retention times on three different columns (Table I). Final confirmation of the structure was obtained by synthesis (vide infra).

Figure 1

Gas-liquid chromatogram of the pentane extract of Porapak Q-trapped C. pusillus volatiles (SP-1000 capillary column). Numbers designate compounds I, II, and IV (Fig. 14).

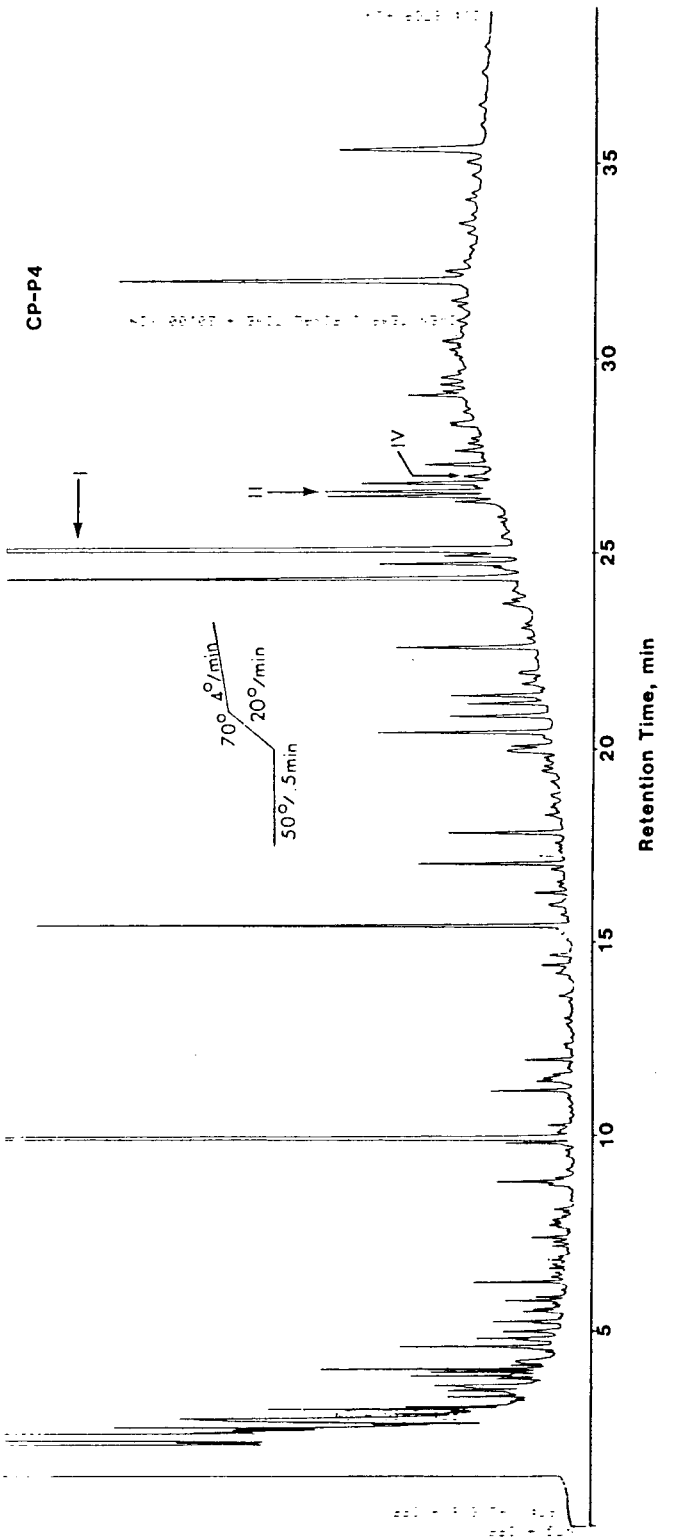

CP-P4
I
II
IV
70° 4°/min
20°/min
50°/.5min
5
10
15
20
25
30
35
Retention Time, min

Figure 2

Gas-liquid chromatogram of the pentane extract of Porapak Q-trapped C. pusillus frass volatiles (SP-1000 capillary column). Numbers designate compounds I, II, and IV (Fig. 14).

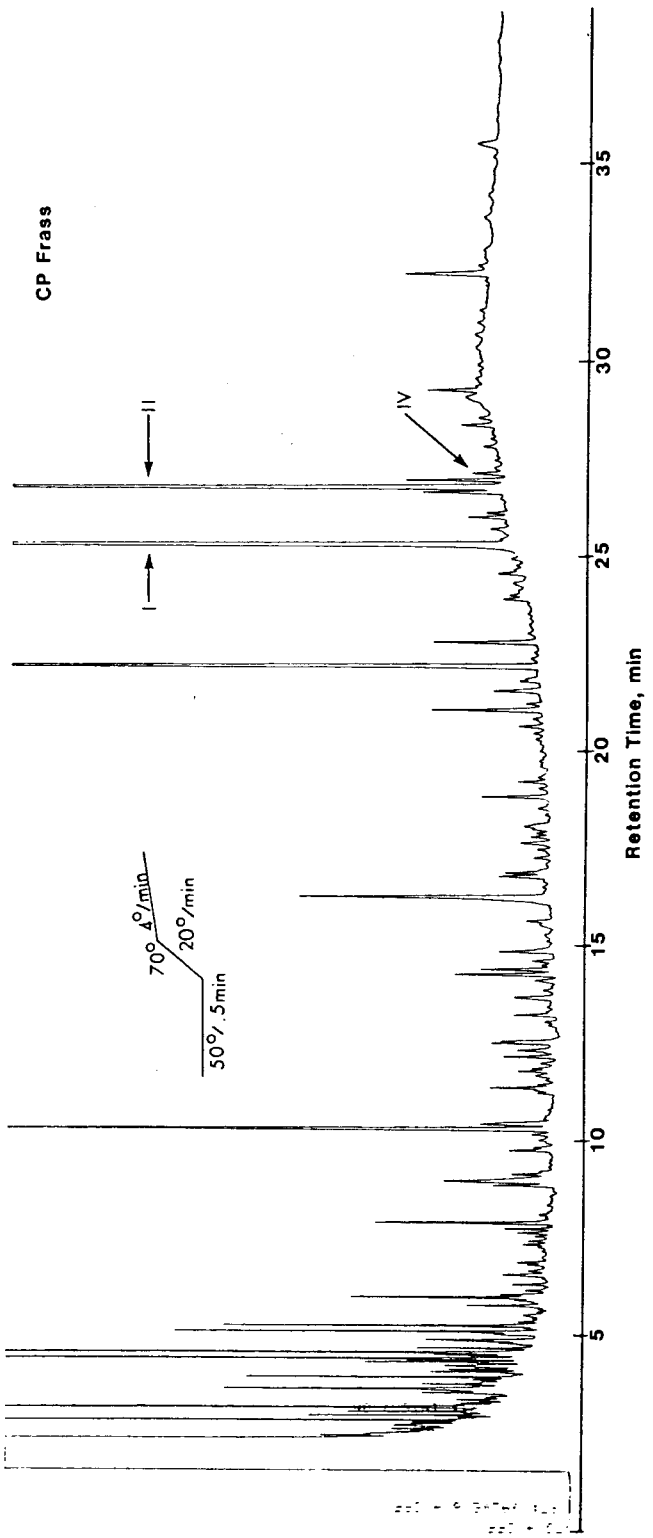

CP Frass
I
II
IV
70° 4°/min
20°/min
50°/.5min
5
10
15
20
25
30
35
Retention Time, min

Figure 3

Gas-liquid chromatogram of the pentane extract of Porapak Q-trapped volatiles from C. pusillus feeding on oats (SP-1000 capillary column). Numbers designate compounds I, II, and IV (Fig. 14).

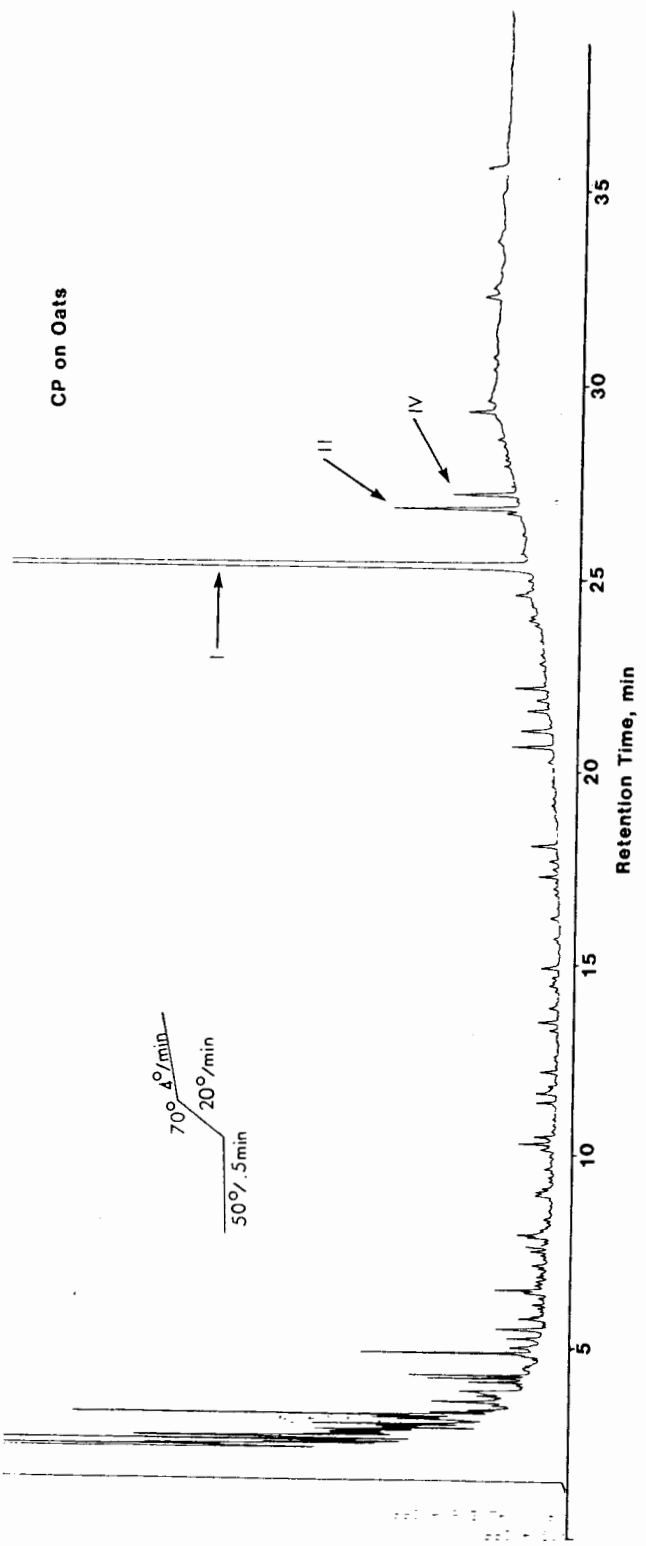

CP on Oats
70° 4°/min
20°/min
50°/.5min
I
II
IV
5
10
15
20
25
30
35
Retention Time, min

Figure 4

Unit resolution mass spectrum of I.

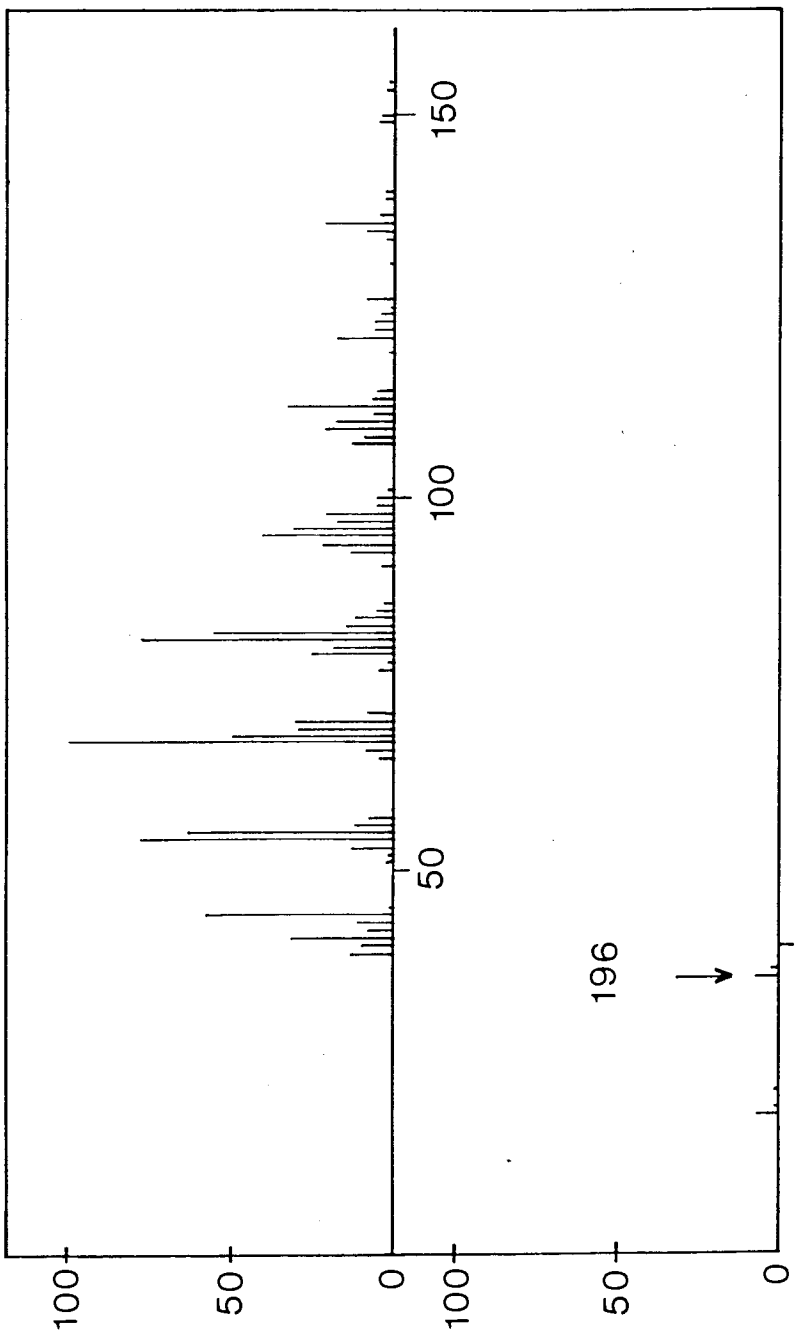

100
50
0
100
50
0
50
100
150
196

Figure 5

^{1}H NMR (400 MHz) of I in $CDCl_3$.

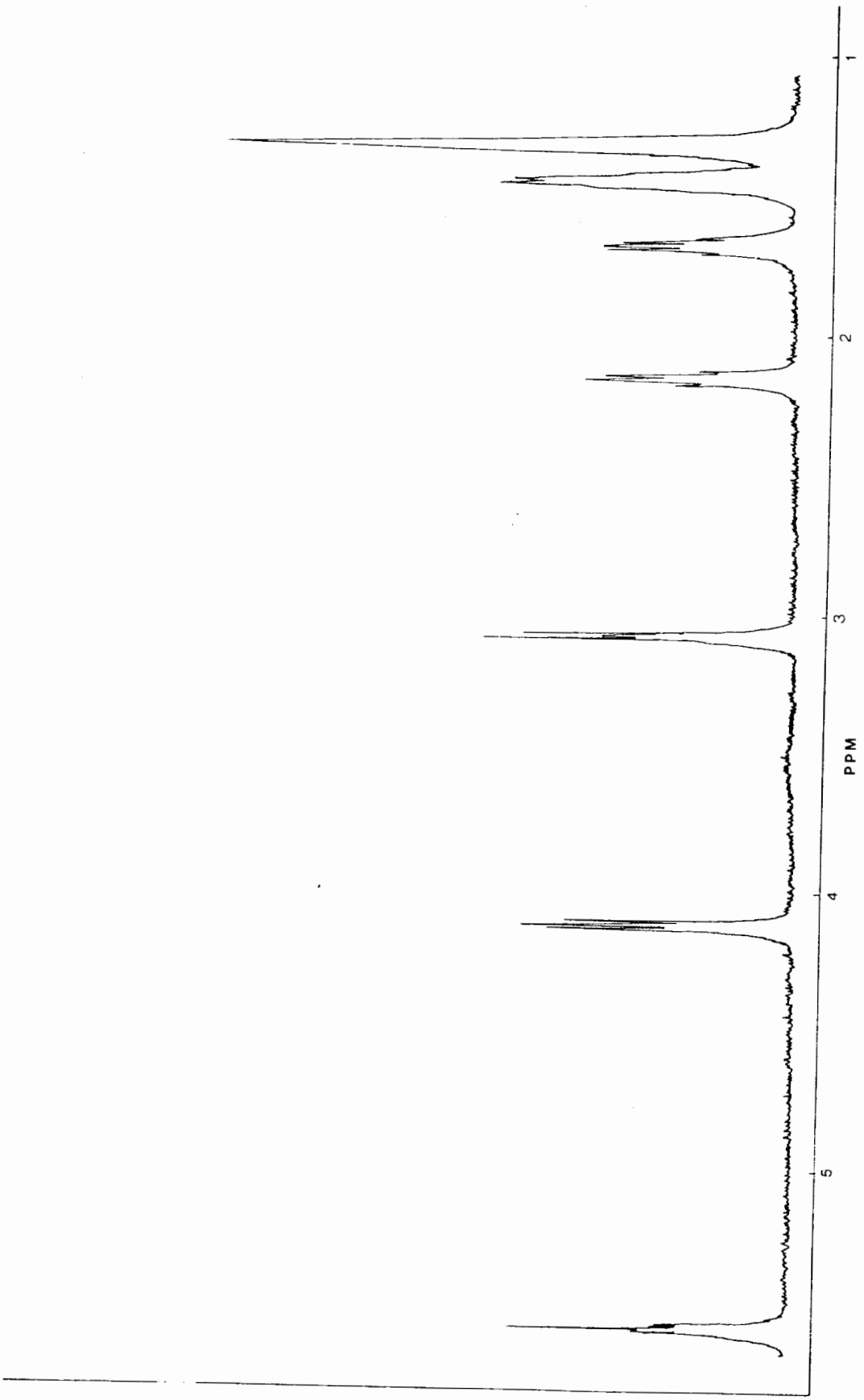

1
2
3
4
5
PPM

Figure 6

Unit resolution mass spectrum of II.

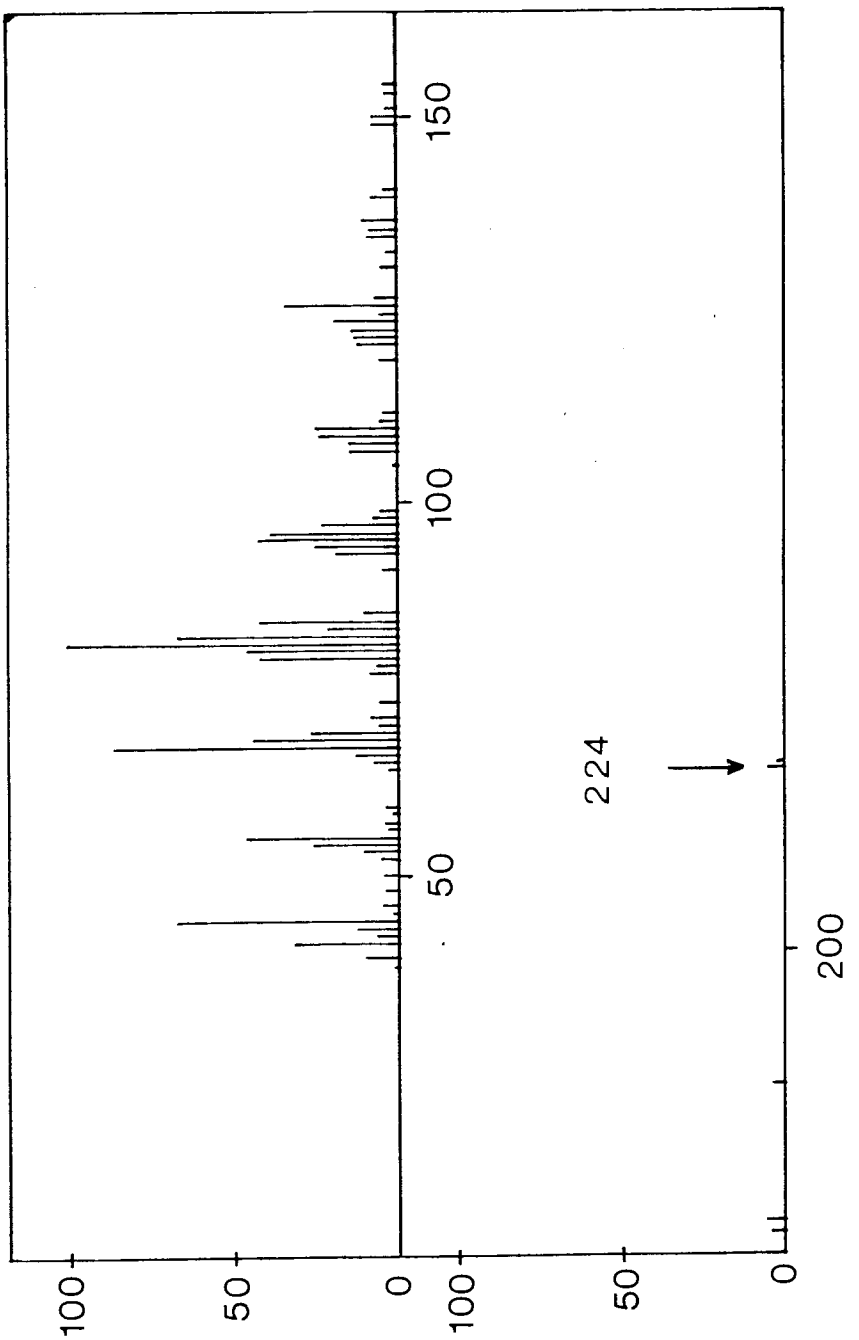

100
50
0
100
50
0
50
100
150
200
224

Figure 7

^{1}H NMR (400 MHz) of II isolated from C. pusillus in $CDCl_3$.

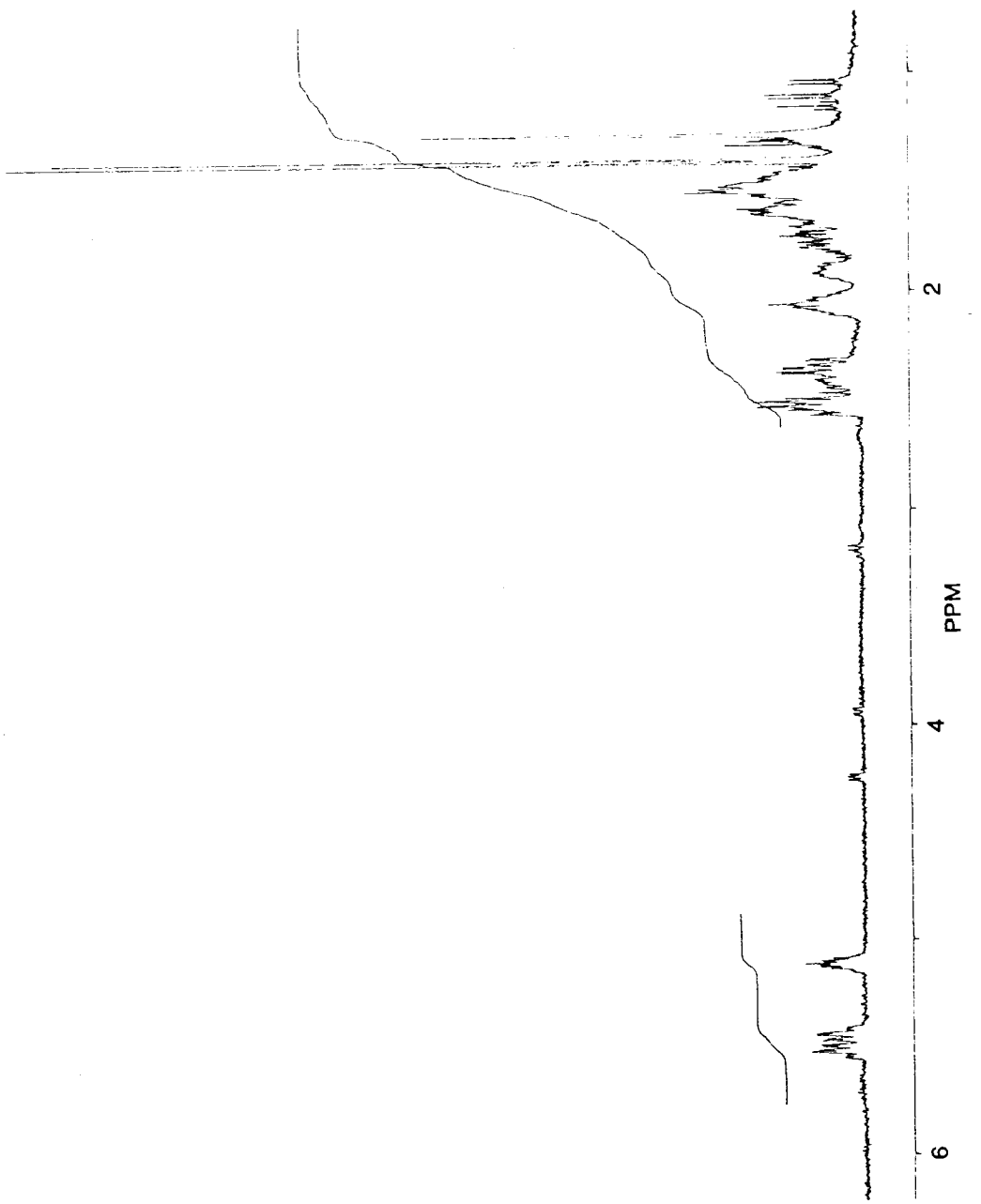
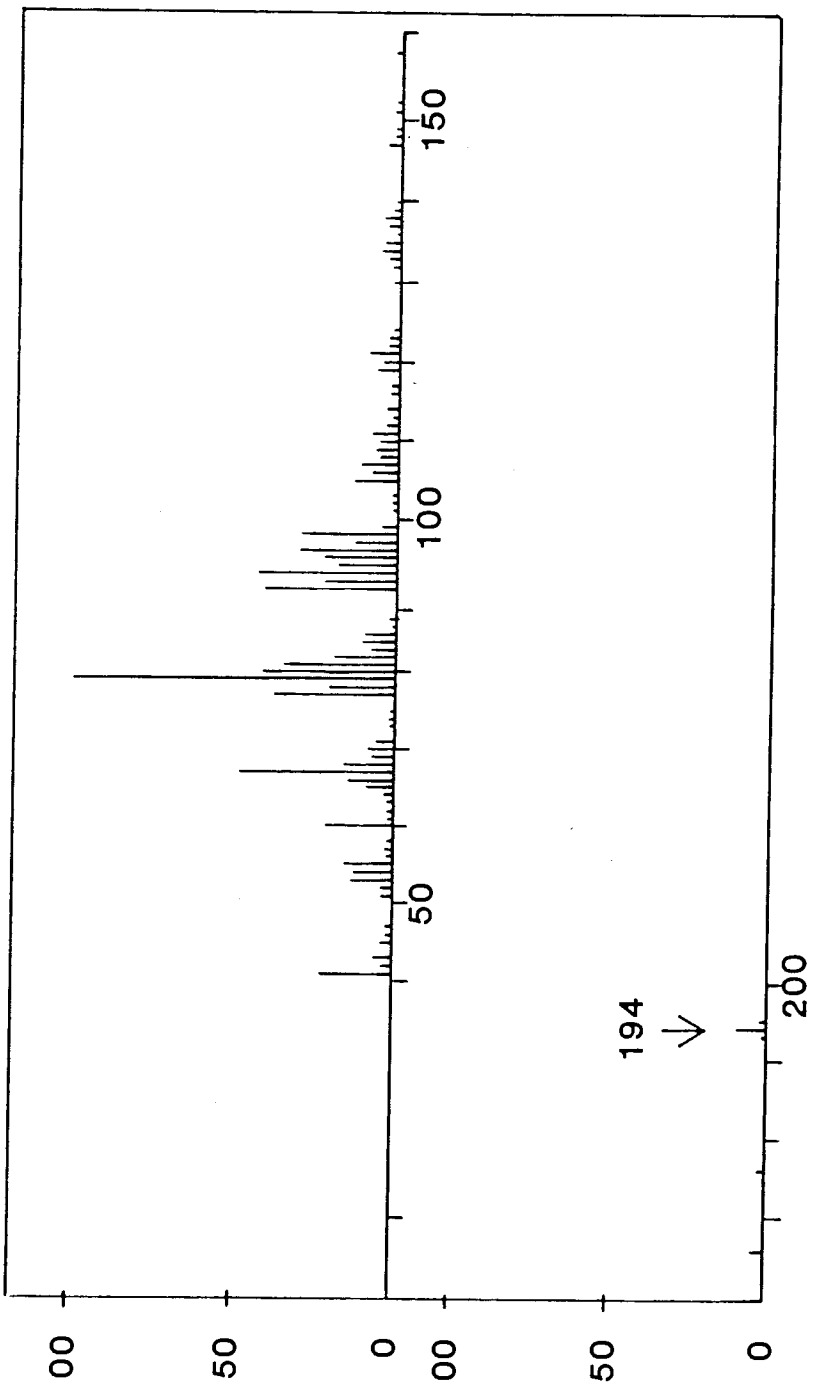

6
4
2
PPM

Figure 8

Unit resolution mass spectrum of IV.


100
50
0
100
50
0
50
100
150
200
194

Identification of 13-Methyl-(5Z,8Z)-tridecadienolide (III)

Compounds II and III from C. turcicus volatiles were identified by chromatographic and spectroscopic analysis (Figs. 9-13). Preliminary GLC-MS analysis of C. turcicus volatiles (Fig. 9), frass volatiles (Fig. 10), and volatiles from C. turcicus on oats (Fig. 11) revealed a major component, III, with retention times slightly longer than macrolide II. The highest observed mass in the mass spectrum of III was at m/e 222 (Fig. 12) and a typical macrolide-type fragmentation pattern, along with the retention time, suggested that III was analogous to II, with an additional double bond or, alternatively, a triple bond. Microscale hydrogenation gave a compound with m/e 226, with an identical mass spectrum and GLC retention time to the macrolide obtained on catalytic hydrogenation of compound II, i.e., 13-methyl-tridecanolide. This confirmed the carbon skeleton of III as a 14-membered lactone, with a methyl group attached to the carbon α to the ester oxygen. Thus, it was only necessary to identify the placement of the olefin or acetylene bonds.

The 400 MHz ^{1}H NMR spectra ($CDCl_3$) of an impure sample of III isolated from C. turcicus volatiles by preparative GLC (Fig. 13) revealed signals due to four olefinic hydrogens, one at δ 5.27 and three overlapped at δ 5.40, confirming the presence of two double bonds.

The isolated proton at δ 5.27 was coupled (J = 9.75 Hz) to one of the other vinyl hydrogen signals, confirming at least one Z double bond. There was a single-hydrogen multiplet at δ 5.03, coupled to a methyl group at δ 1.24 (J = 6.1 Hz), corresponding to the methine hydrogen of C_{13}. A one-hydrogen doublet of triplets at δ 3.15, geminally coupled (J = 15.1 Hz) to a hydrogen in a three-hydrogen multiplet at δ 2.20-2.43 and also coupled to

the olefin multiplet (J ≈ 9.75 Hz) at δ 5.40, was identified by the coupling and downfield shift as a bis-allylic hydrogen. Thus, there was a skipped diene system. The other bis-allylic hydrogen gave the geminal coupling in the δ 2.20-2.43 multiplet. The other two hydrogens in that multiplet were identified as allylic hydrogens on different carbons by partial collapse of their signals upon irradiation of the olefin multiplets. The two remaining allylic hydrogens were located at the same time, at δ 1.98, and as part of a four-hydrogen multiplet at δ 1.56-1.77.

The two coupled hydrogens (J = 14.5 Hz) of a methylene α to the carbonyl, located at δ 2.27 and 2.40, respectively, were identified by their chemical shift and splitting patterns. Irradiation of the signal at δ 2.40 resulted in partial collapse of a one-hydrogen multiplet at δ 1.83 and of part of the four-hydrogen multiplet at δ 1.56-1.77, indicating a methylene group β to the carbonyl. Finally, irradiation of the one-hydrogen signal at δ 1.83 resulted in partial collapse of signals due to two previously identified allylic hydrogens at δ 1.98 and in the multiplet at δ 2.20-2.43, thus locating the first double bond between C_5 and C_6. In addition, as irradiation of the single olefinic hydrogen signal at δ 5.27 removed a large coupling (≈9.75 Hz) from the allylic hydrogen at δ 1.48, the δ 5.27 hydrogen must be located on C_5 and previous analysis had shown this hydrogen to be on a Z disubstituted double bond. The other double bond must be between C_8 and C_9, as the discussion above had identified a single methylene between the two double bonds.

The remaining four unassigned hydrogens, on C_{11} and C_{12}, were assigned to the remainder of the four-hydrogen multiplet at δ 1.56-1.77 and to the two proton multiplet at δ 1.40.

Thus, the only unknown factor was the geometry of the double bond between C_8 and C_9, the protons of which were completely overlapped. Changing the solvent from $CDCl_3$ to C_6D_6 gave no improvement. Shift reagents could have been tried but were not, as the recovered sample had to be used for bioassays and it was thought unwise to contaminate the sample. Final confirmation that the C_8-C_9 bond geometry was *cis* was obtained by synthesis (*vide infra*).

Identification of 13-Methyl-(5Z)-tridecenolide (II) from *C. turcicus*

GLC and GLC-MS analysis of the crude pentane extract of *C. turcicus* volatiles (Fig. 9) showed a compound with the same retention times on several columns (Table I) and the same mass spectrum as macrolide II isolated from *C. pusillus* volatiles. In addition, a sample of II isolated by preparative GLC (Table I) of a *C. turcicus* volatile extract gave an identical NMR spectrum to II isolated from *C. pusillus*, proving conclusively that the compounds had the same structure. However, it should be noted that the two species may produce different enantiomers or a different ratio of the enantiomers of II.

Macrolides from *Oryzaephilus mercator*

During the course of these investigations, Drs. A.M. and H.D. Pierce[4] also isolated and spectroscopically identified compounds II, III and IV from *O. mercator* volatiles, thus adding further incentive to the synthesis of these molecules. In addition, these workers isolated, identified and bioassayed 11-methyl-(3Z,6Z)-undecadienolide (V). V was found to be highly attractive to *O. mercator* and so V was synthesized as part of a collaborative effort, to confirm the structure and provide material for bioassays.

Figure 9

Gas-liquid chromatogram of the pentane extract of Porapak Q-trapped C. turcicus volatiles (SP-1000 capillary column). Numbers designate compounds II and III (Fig. 14).

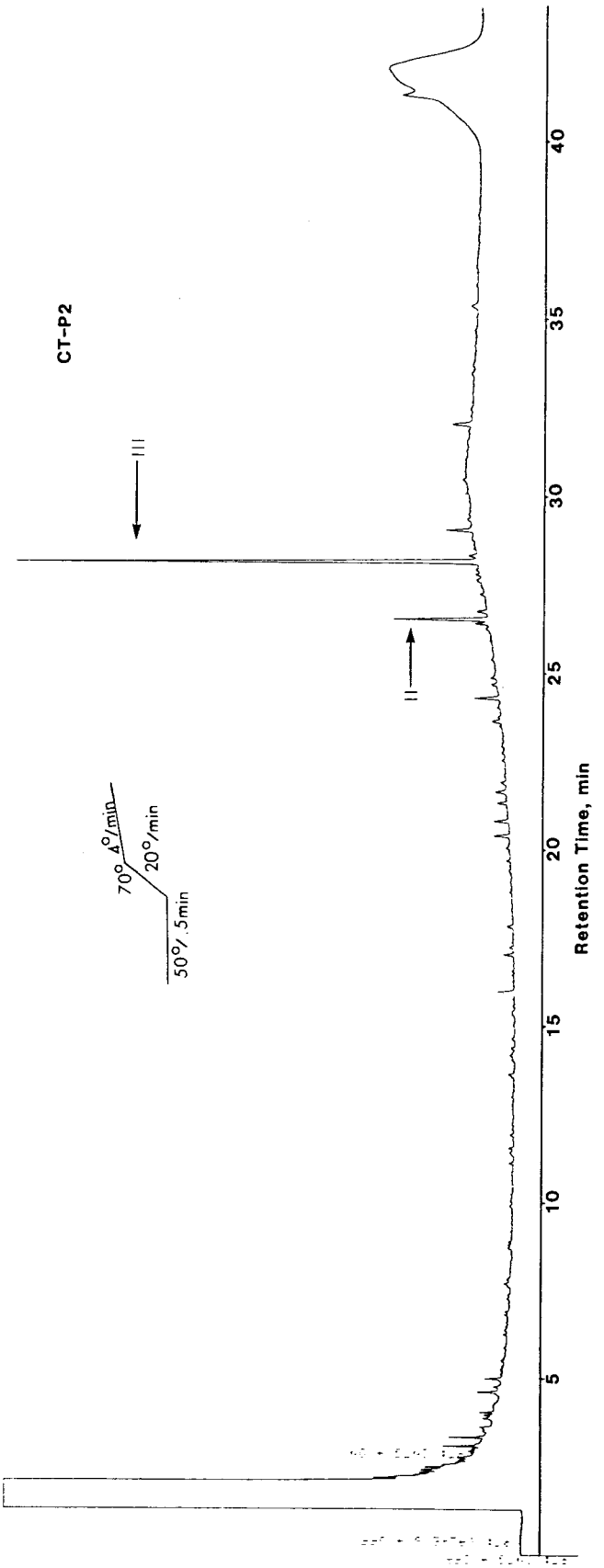

CT-P2
III
II
70° 4°/min
20°/min
50°/.5min
5
10
15
20
25
30
35
40
Retention Time, min

Figure 10

Gas-liquid chromatogram of the pentane extract of Porapak Q-trapped C. turcicus frass volatiles (SP-1000 capillary column). Numbers designate compounds II and III (Fig. 14).

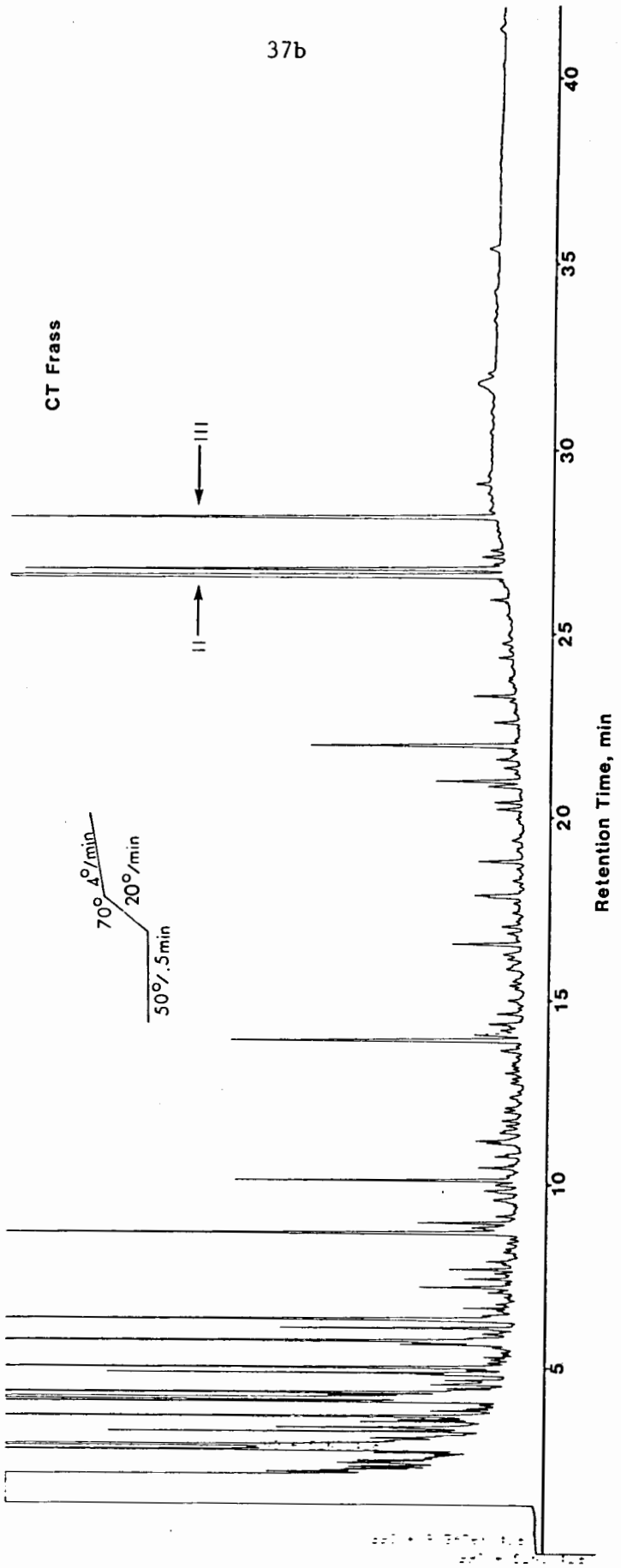

CT Frass
III
II
70° 4°/min
20°/min
50°/.5min
5
10
15
20
25
30
35
40
Retention Time, min

Figure 11

Gas-liquid chromatogram of the pentane extract of Porapak Q-trapped volatiles from C. turcicus feeding on oats (SP-1000 capillary column). Numbers designate compounds II and III (Fig. 14).

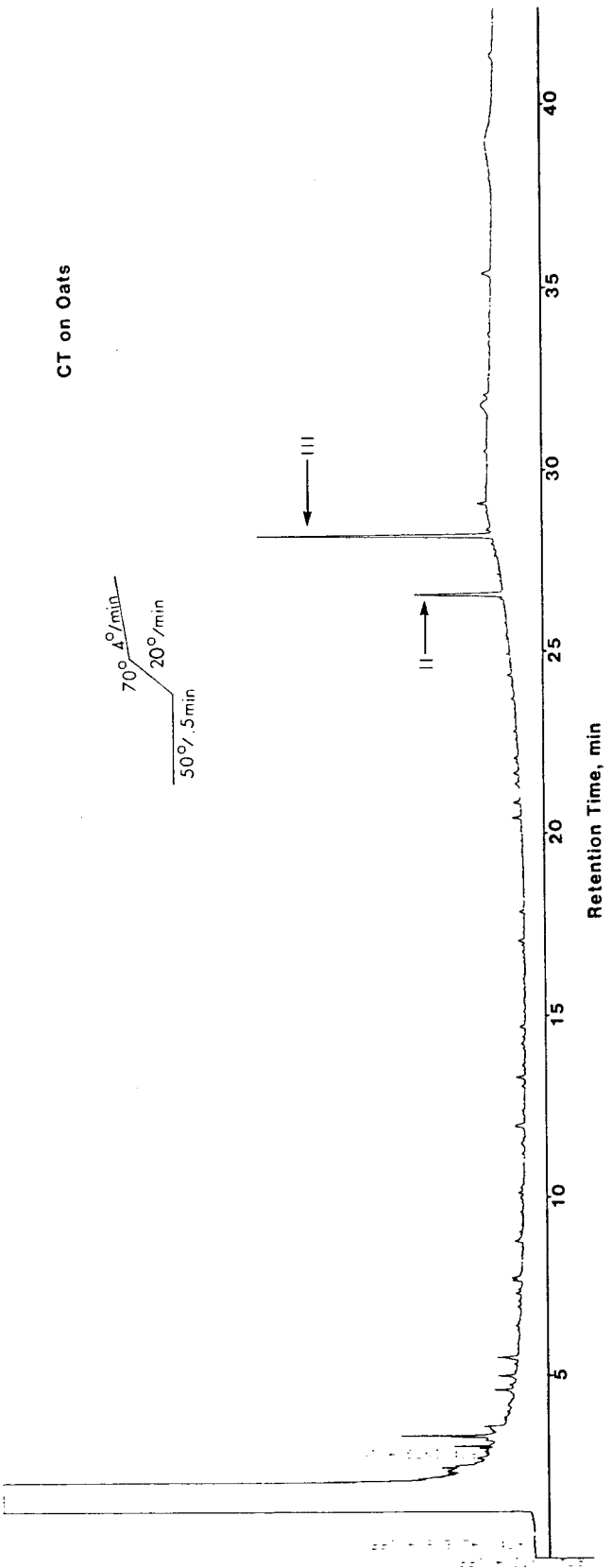

CT on Oats
70° 4°/min
20°/min
50°/.5min
II
III
5
10
15
20
25
30
35
40
Retention Time, min

Figure 12

Unit resolution mass spectrum of III.

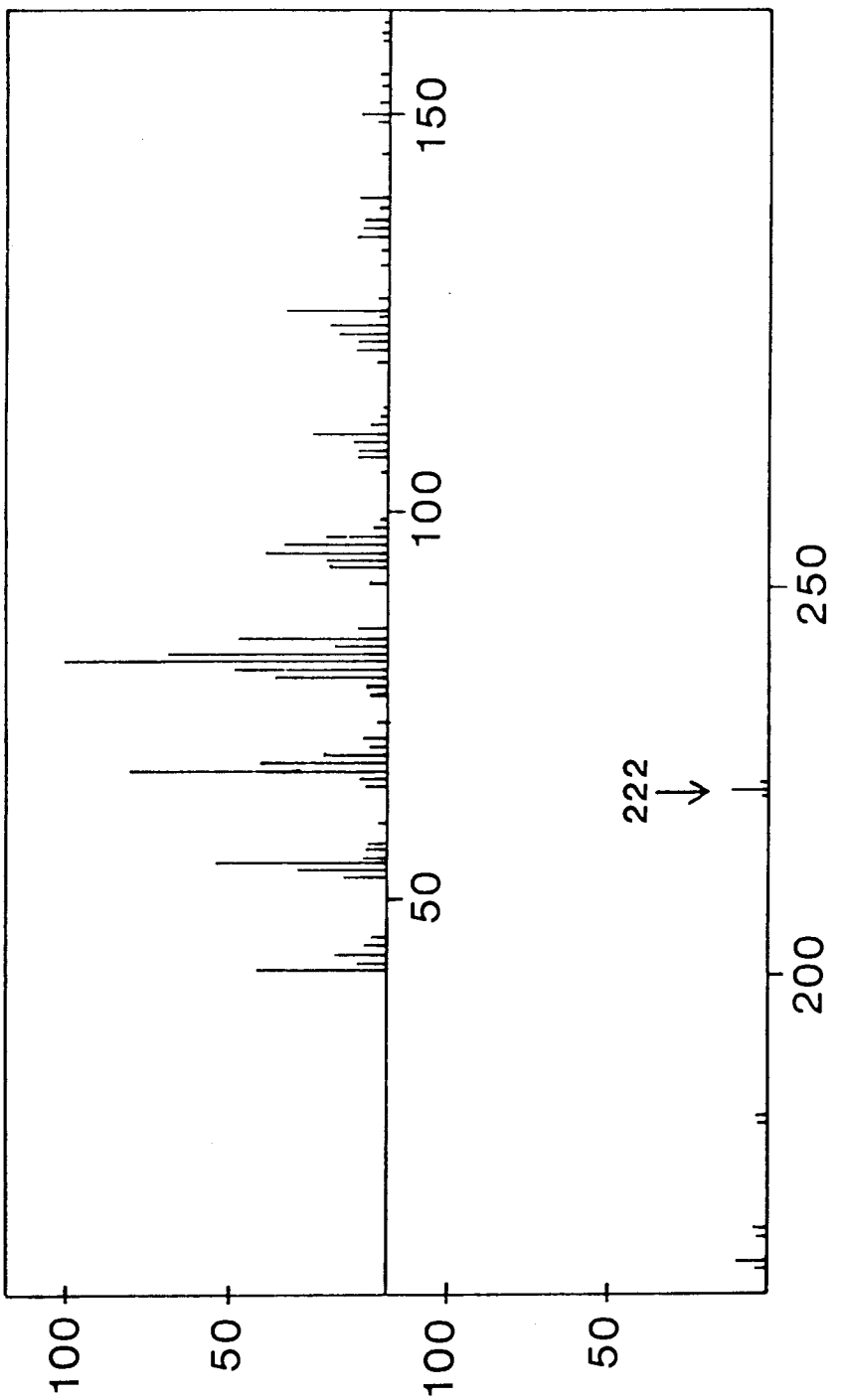

100
50
100
50
50
100
150
200
222
250

Figure 13

^{1}H NMR (400 MHz) of III in $CDCl_3$.

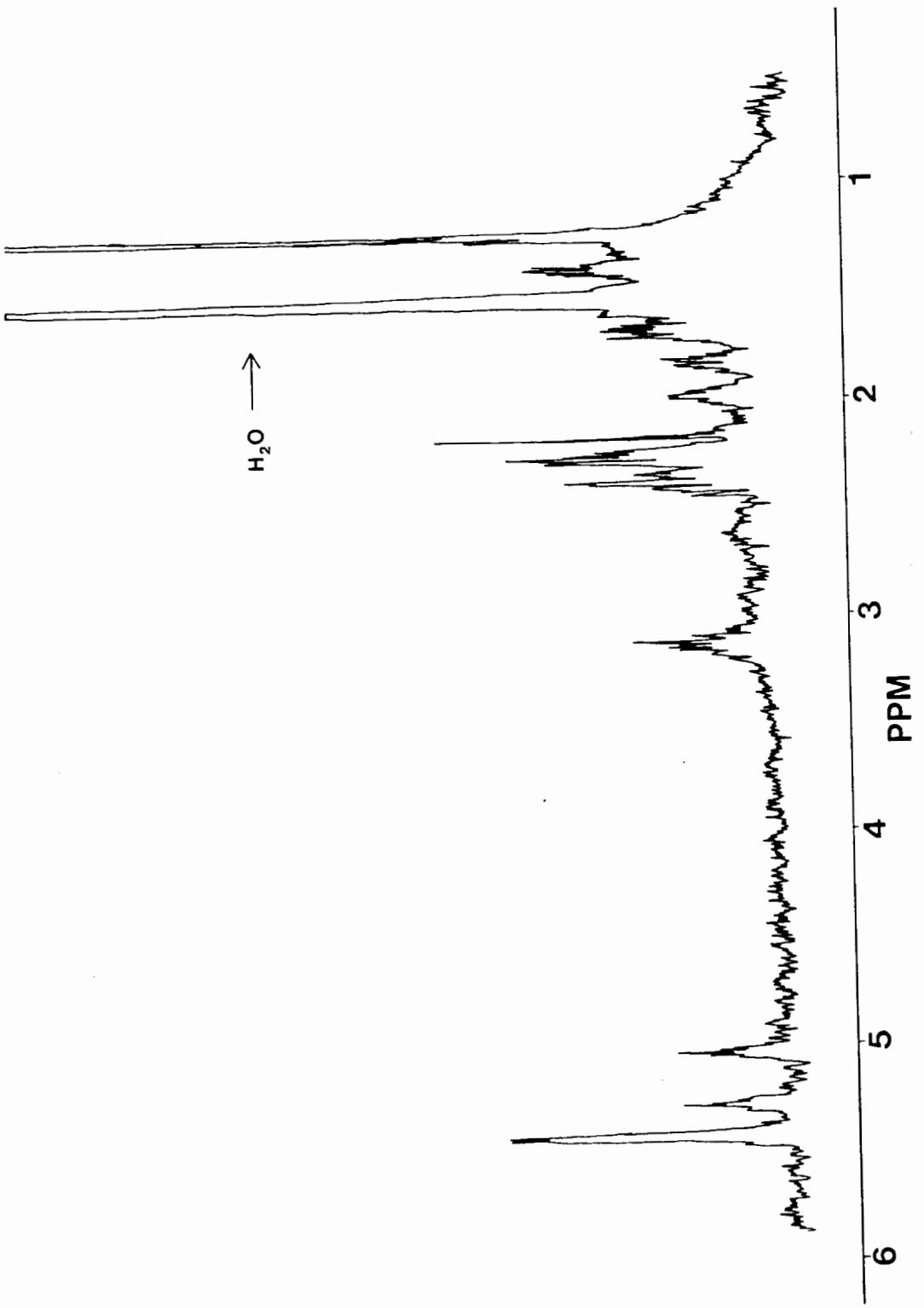
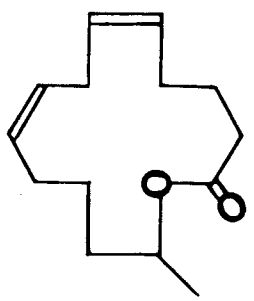
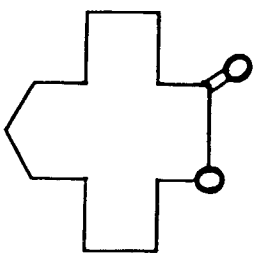

H2O
1
2
3
4
5
6
PPM

Figure 14

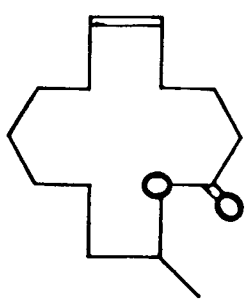
Structures of isolated and/or synthesized macrolides I-V.



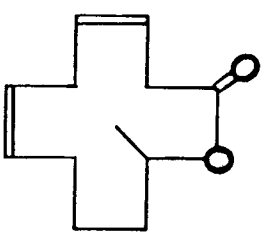
III



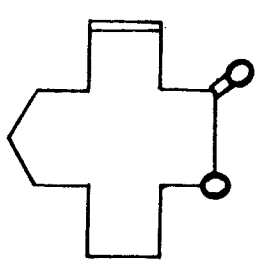
Ia



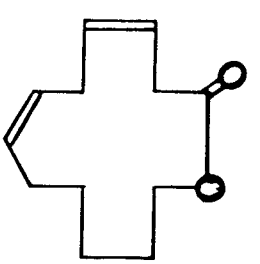
II



V



I



IV

IV. SYNTHESIS OF MACROLIDES

A. Introduction

The term macrolide is used to describe a diverse class of natural products characterized by large lactone rings. The simplest ones, with no functionality other than the ester moeity, have been used in the perfume industry for a number of years (Abe et al., 1973). A number of these, along with some unsaturated and hydroxylated macrolides, have been isolated from plants (e.g., Kerschbaum, 1927; Kaiser and Lamparsky, 1978), fungi (Versonder et al., 1971) and insects (Prestwich, 1982; Johansson et al., 1982; Wong, 1982; Moore and Brown, 1976). The highest level of complexity is represented by multiply-functionalized macrolide antibiotics, of which several hundred are now known (Back, 1977; Nicolau, 1977; Masamune et al., 1977). These include such diverse structural types as the polyene, polyoxo and nitrogen-containing cytochalasin families. They possess a wide range of useful biological properties, ranging from antifungal to antitumour activity.

A tremendous amount of effort has been expended over the last four decades in developing methods of macrolide synthesis. Reactions of general applicability using mild conditions have only been worked out in the last ten years, with the result that the body of literature on synthesis of macrolides is now expanding rapidly.

Several types of synthetic strategies have been used, depending on the complexity of the desired product. These include:

1) Baeyer-Villiger oxidation of the corresponding ketones (e.g., Kaiser and Lamparsky, 1978; Kruizinga and Kellogg, 1981). This method is

severely limited by the availability of the required macrocyclic ketones and by the severity of the reaction conditions, which preclude the presence of any double or triple bonds.

2) Fusion-bond cleavage of bicyclic compounds, such as enol ethers, where the C=C bond forms the fusion-bond of the bicyclic compound (e.g., Borowitz et al., 1968; 1972; 1973). This route is again limited by the availability of the bicyclic precursors and by the oxidative reaction conditions which preclude the presence of unsaturation elsewhere in the molecule.

3) Ring enlargement reactions, such as the thermal 1,6-sigmatropic reaction of a 1,2-bis-vinylogous compound (Clive et al., 1982). This method suffers the disadvantages of the high temperatures needed for the reaction to proceed and from limitations in the control of the geometry of the resulting cyclic olefin.

4) Cyclizations of acyclic precursors. These are by far the most versatile methods of macrolide synthesis and generally proceed under the mildest conditions. Comprehensive reviews of these and other techniques have been published (Masamune et al., 1977; Nicolau, 1977; Back, 1977; Newkome et al., 1977). The most commonly used technique utilizes simultaneous activation of both the hydroxyl and carboxyl functions of an Ω-hydroxy acid substrate, so that the ease and, consequently, the rate of internal esterification to the macrolide is greatly enhanced. This "double-activation" is achieved by formation of an intermediate such as a 2-pyridyl thioester (Corey et al., 1974; 1975a; 1975b; 1976a), wherein the pyridyl thio group increases the electrophilicity of the carbonyl, and the pyridyl nitrogen

hydrogen-bonds the hydroxyl hydrogen, increasing the nucleophilicity of the alcohol oxygen. There are a number of variations on this general theme, using different activating groups (e.g., Masumune et al., 1977; 1975a; 1975b; Mukaiyama et al., 1976; Gerlach et al., 1974; 1975; Nimitz and Wollenberg, 1978; Rastetler and Phillion, 1979; 1981; Kurihara et al., 1976).

Another similar technique involves the cyclization of Ω-halo acid salts, where high dilution conditions necessary to prevent oligomerization are achieved by the low solubility of the carboxylate salt in the reaction medium. This was the first method developed for the formation of a macrolide (Kerschbaum, 1927) and there have been several recent improvements (e.g., Kruizinga and Kellogg, 1981; Kimura and Regen, 1983).

In addition, several methods of macrolide formation via intramolecular carbon-carbon bond formation have been described. These include intramolecular Wittig reactions (Stork and Nakamura, 1979; Nicolau et al., 1979) and intramolecular displacement of a leaving group by a stabilized carbanion (e.g., Takahashi et al., 1978). However, these methods have not been widely used due to the somewhat rigorous reaction conditions needed for carbon-carbon bond formation. Previous experience in our laboratory (Wong, 1982; Verigin, 1980) with carbon-carbon bond forming macrolide closure reactions has also met with very limited success.

The synthetic goal in this work was to make several hundred milligram quantities of stereochemically and enantiomerically pure macrolides, I-V, both as a final proof of the proposed structures and to test biological activity. In the latter case, purity may be critical as the presence of the wrong enantiomer or geometrical isomer may inhibit response to the

pheromone (e.g., Borden et al., 1976). The macrolides themselves represent several structural types of varying stability. Thus, a general synthetic method must:

1) Be sufficiently flexible to accommodate a variety of ring sizes, with varying degrees of unsaturation in various positions.
2) Allow complete stereocontrol of the geometry of the double bonds.
3) Allow for introduction of a chiral center where necessary.
4) Use reaction conditions that are sufficiently mild so that no isomerization and/or racemization occurs once the double bonds and/or chiral centers are in place.

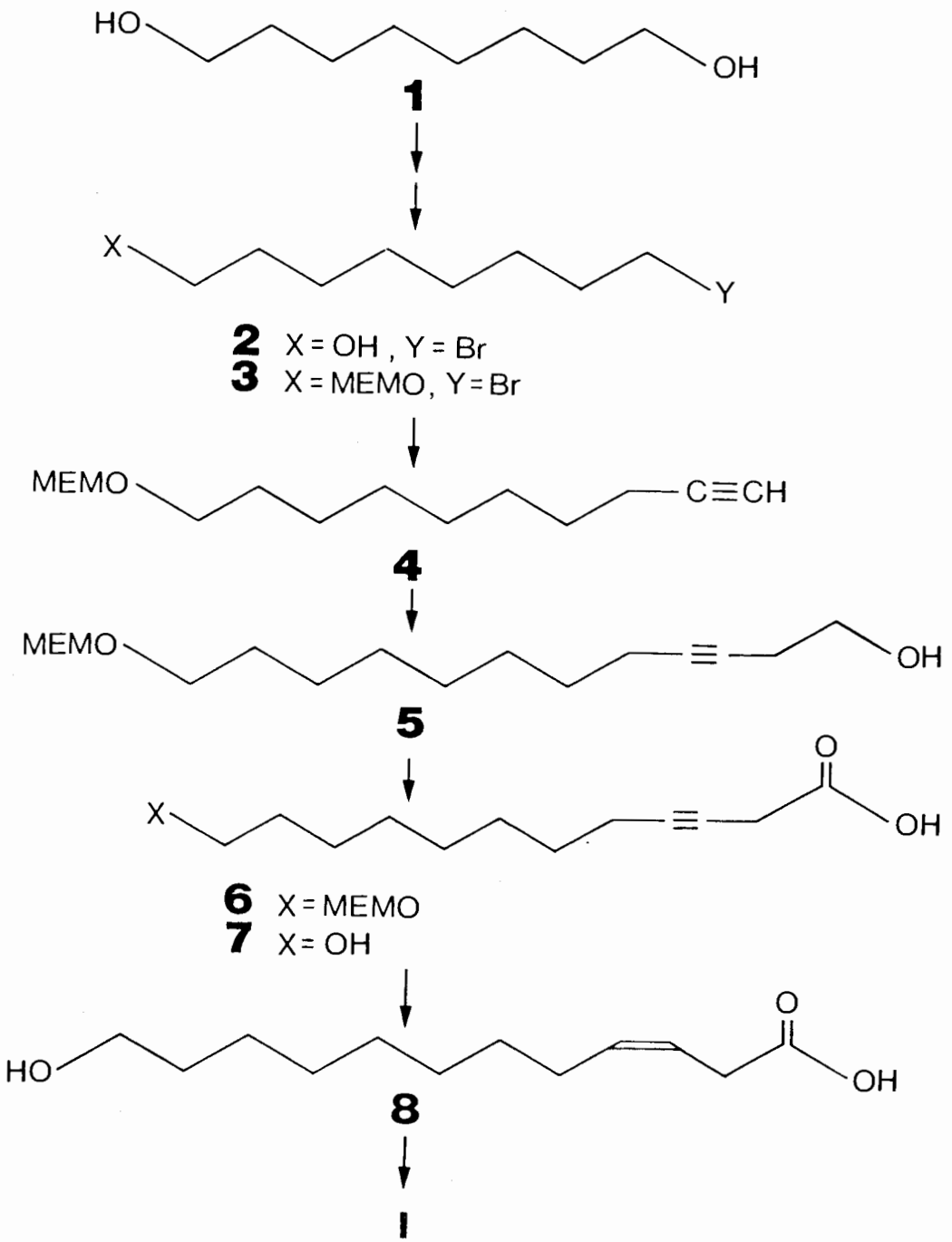
The method which best meets these criteria is the cyclization of acyclic precursors, where double bonds and functionalities can be placed at will at the required positions along the acyclic skeleton. In particular, the mild reaction conditions now available for macrocyclization of hydroxy acids seemed well suited to the constraints mentioned above. In addition, the body of literature on preparation of the acyclic precursors, i.e. singly- and multiply-unsaturated carboxylic acids, with unsaturations in various positions, is now extensive.

B. Synthetic Strategies

Synthesis of (3Z)-Dodecenolide (I)

Retrosynthetic analysis of macrolide I revealed that key steps were the lactonization of the acyclic hydroxy acid, stereoselective introduction of the Z olefin β,γ to the carboxyl and introduction of the carboxyl function. In addition, there was slight uncertainty as to the geometry of the double bond, so the synthesis of I was designed so that either E- or

Scheme I


HO
OH
1
X
Y
2 X = OH , Y = Br
3 X = MEMO, Y = Br
MEMO
C≡CH
4
MEMO
OH
5
X
O
OH
6 X = MEMO
7 X = OH
HO
O
OH
8
I

Z-unsaturation could be introduced. The synthetic route (Scheme I), as far as intermediate 5, was similar to that of Maurer and Grieder (1977), who prepared the tetrahydropyranyl ether analog of 5. Thus, 1,8-octanediol 1 was converted to 8-bromo-1-octanol, 2, by reaction with 48% aqueous HBr, with continuous extraction with hot heptane. The hydroxyl function of 2 was then protected as the β-methoxyethoxymethyl ether, by treatment with MEM chloride in diisopropylethylamine (Corey et al., 1976b).

Attempted reaction of the resulting bromide 3 with the dilithio salt of 3-butyn-1-ol (Ames et al., 1963), to give alcohol 5 directly, resulted in low yields of product, with considerable unreacted starting material. Instead, 5 was synthesized in two steps. Alkyne 4 was prepared in excellent yield from reaction of 3 with lithium acetylide in THF/HMPA (Beckmann et al., 1975), followed by reaction of the Grignard of 4 with ethylene oxide (Brandsma, 1971). Alcohol 5 proved to be surprisingly difficult to oxidize cleanly, presumably due to the proximity of the alcohol to the triple bond. This has also been noted by other workers (e.g., Kajiwara et al., 1977; Wong, 1982). Thus, attempted oxidation under neutral conditions with pyridinium dichromate in DMF (Corey and Schmidt, 1979) gave low yields of the desired β,γ-unsaturated acid 6. Stepwise oxidation via the aldehyde, using several different methods (alkaline Ag_2O; pyridinium chlorochromate in CH_2Cl_2; Ag_2CO_3 in cellite) also proved unproductive. Acid 6 was finally obtained in reasonable yield by oxidation of 5 with cold dilute Jones reagent, using the inverse addition method (Holland and Gilman, 1974), with no detectable cleavage of the relatively acid-insensitive MEM protecting group. Cleavage of the MEM ether by the normal procedure (Corey et al., 1976b), using powdered anhydrous zinc bromide in CH_2Cl_2, gave a

mixture of products, possibly due to the interaction of the Lewis acid $ZnBr_2$ with the triple bond. Instead, conditions were worked out for acid hydrolysis of the protecting group using THF:water:conc. HCl (12:2:1-8:2:1) at 20° for 24-48 h, giving hydroxy acid 7 in good yield. Stereoselective cis reduction of the triple bond in 7 was attempted by three methods. The first, using dicyclohexylborane (Zweifel and Polston, 1970), gave an intermediate borane adduct which proved to be somewhat difficult to hydrolyze. Catalytic reduction with palladium on barium sulphate, poisoned with quinoline (Lindlar's catalyst), gave a final product contaminated with a few percent of the trans isomer. Finally, catalytic reduction with P-2 nickel (Brown and Ahuja, 1973a; 1973b) gave an excellent yield of the Z olefin 8, uncontaminated by the trans isomer, conjugated isomers or products of over-reduction.

A variety of methods are available for cyclization of the hydroxy acid. The double activation method of Corey was used because it had been successfully applied to a variety of macrolide closures (Corey et al., 1976a; 1975a; 1975b; 1974) and because previous workers in our laboratory had achieved reasonable yields via this method. Thus, I was obtained in moderate yield by slow addition of a dilute solution of the 2-pyridinethiol ester of 8 to refluxing xylene.

Attempts to improve the yield in the cyclization step, including changing solvents (toluene, benzene), changing the rate of addition and the dilution factor, and changing the cyclization agent from 2,2'-dipyridyldisulfide to 2,2'-dithiobis(4-tert-butyl-1-isopropylimadazole) (Corey and Brunelle, 1976c) gave no significant increases in yield. The addition of catalytic amounts of silver salts, as recommended by Gerlach et al. (1975;

1974), also gave no improvement in yield.

There are several possible explanations for the low yields obtained.

1) Fixed rate of addition dropping funnels and high dilution distillation heads were not used. Thus, the conditions of high dilution may not have been achieved.

2) The apparatus and solvent may not have been completely dry, even though the solvents were dried by azeotropic removal of water or by distillation from calcium hydride.

3) The cyclization agents, which are hygroscopic and thermally unstable, may not have been dry, despite handling under nitrogen in a dry-box.

4) The intermediate pyridinethiol esters are thermally unstable (i.e., cyclization occurs merely by heating) and may have partially oligomerized in the dropping funnel before being added to the refluxing xylene. This is supported by the fact that some dimerized material and considerable polar residue were obtained from each cyclization.

5) The molecule may have been intrinsically difficult to cyclize, due to conformational and steric constraints in the transition state (Corey et al., 1976d). However, this is not borne out by examination of space-filling molecular models.

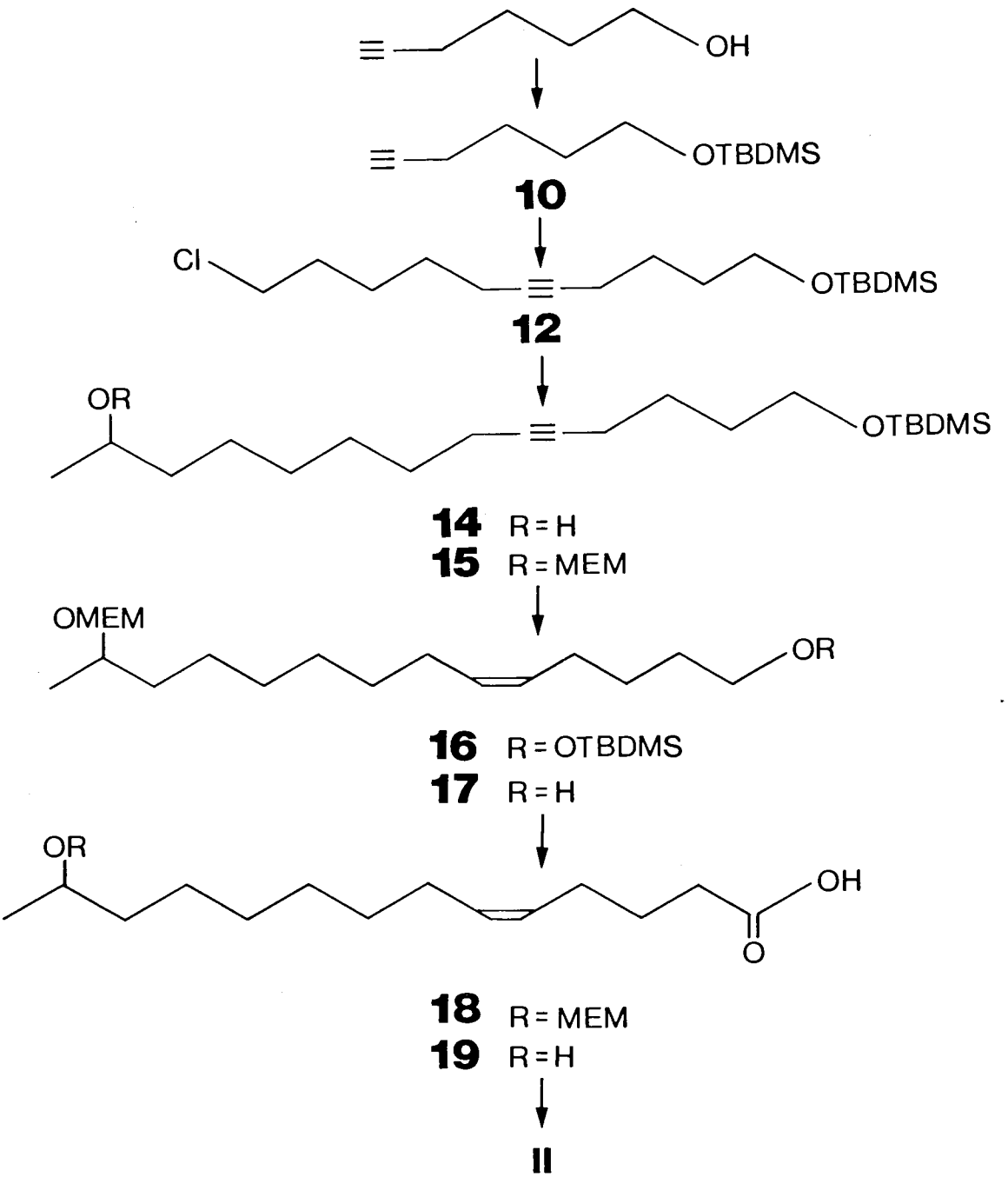
It should also be mentioned that the low yield was not due to impure precursor 8, as this compound was chromatographically pure (TLC, GLC of methyl ester (CH_2N_2)) and gave a very good carbon-hydrogen analysis.

The overall yield of I from 1,8-octanediol (1) was 10%.

Synthesis of 13-Methyl-(5Z)-tridecenolide (II)

The synthetic route to macrolide II (Scheme II) used many of the same

Scheme II


OH
OTBDMS
10
Cl
OTBDMS
12
OR
OTBDMS
14 R = H
15 R = MEM
OMEM
OR
16 R = OTBDMS
17 R = H
OR
OH
O
18 R = MEM
19 R = H
II

steps as in the synthesis of I. The only previously untried key step in this sequence was the incorporation of a chiral center. The chiral synthons chosen were the readily available (R)- and (S)-methyloxiranes 13 (Seuring and Seebach, 1977; Hillis and Ronald, 1981), which can be regio- and stereospecifically opened (Johnston and Slessor, 1979; Acker, 1977; Szabo and Lee, 1980) to give the corresponding chiral alcohols. Thus, racemic or chiral II could be made by use of the respective racemic or chiral methyloxiranes.

The synthesis of II commenced with 5-hexyn-1-ol (9), the hydroxyl of which was protected as the tert-butyldimethylsilyl ether by treatment with TBDMS chloride and imadazole in DMF (Corey and Venkateswarlu, 1972), giving 10 quantitatively. The lithium salt of 10 was alkylated with 1-chloro-5-iodopentane (11, Ahmad et al., 1948) in THF/HMPA (Beckmann et al., 1975) at -40-0° to give, selectively, the chloride 12.

Initial attempts to make the Grignard of distilled 12 were not successful due to traces of silyl alcohol in the distillate. Washing the distillate through a column of silica gel removed traces of water and/or alcohol, and the Grignard of 12 could then be formed, albeit with difficulty. Cuprous iodide catalyzed reaction of the Grignard with the appropriate methyloxirane gave alcohol 14 in reasonable yield. The alcohol was protected as the MEM ether 15 and stereoselectively reduced to the Z-olefin 16, as previously described for I. Selective deprotection of the primary hydroxyl with AcOH:THF:H_2O (3:1:1), followed by oxidation of the resulting alcohol 17 with pyridinium dichromate in DMF (Corey and Schmidt, 1979), produced acid 18 in high yield. Acid hydrolysis of the MEM protecting group, as described for I, gave hydroxy acid 19, which was cyclized with

2-chloro-1-methylpyridinium iodide (Mukaiyama et al., 1976), which also operates via the double-activation principle. Thus, an acetonitrile solution of the triethylamine salt of 19 was added slowly to a refluxing acetonitrile solution of the cyclizing agent, giving macrolide II in 33-49% yield. The overall yield from 5-hexyn-1-ol was about 10%. Racemic, (R)-, and (S)-II were made via this route.

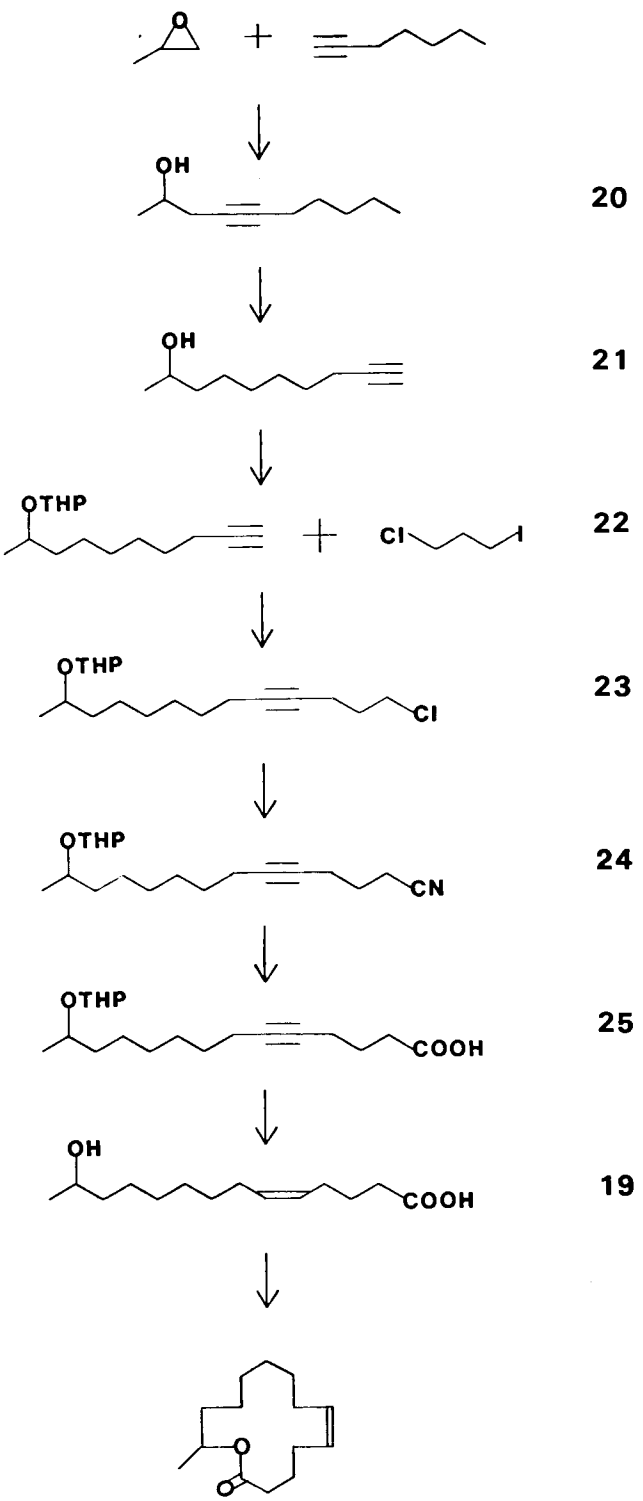
The optical purities of the enantiomers of 19, the immediate precursors to II, were checked by reaction of the methyl ester (CH_2N_2) of each enantiomer with (+)-α-methoxytrifluoromethylphenylacetyl chloride (Dale et al., 1969). The resulting diastereomers gave 400 MHz ^1H NMR spectra in which the 13-methyl groups had chemical shifts differing by 0.05 ppm. In each case, only one isomer was detectable, giving an estimated enantiomeric excess of >95%.

Experiments to determine the chiral purity of the enantiomers of II directly, using chiral NMR shift reagents, resulted in minimal resolution of the 13-methyl groups (≈2 Hz), with considerable line broadening. Thus, this procedure could not be used for determination of enantiomeric ratios of II.

Due to the difficulties encountered in the formation of the Grignard of 12 and the moderate yields of II obtained, a new synthetic route to II was developed (Scheme III), the key to which is the so-called acetylene zipper reaction (Brown and Yamashita, 1975; 1976; Lindhoudt et al., 1976) which isomerizes an internal triple bond to a terminal position. The reaction can be performed on chiral acetylenic alcohols, with no loss of alcohol chirality (Midland et al., 1981).

Thus, the lithium salt of 1-heptyne at -10° in THF was used to

Scheme III


OH
20
OH
21
OTHP
Cl
22
OTHP
Cl
23
OTHP
CN
24
OTHP
COOH
25
OH
COOH
19
II

stereo- and regioselectively open methyloxirane (Brandsma, 1971). The resulting propargyl alcohol 20 was reacted with potassium 3-aminopropylamide in excess 1,3-diaminopropane, the zipper reaction, to give the terminal alkyne 21 in excellent yield, with no loss of chirality (checked by 400 MHz ^{1}H NMR of the (+)-α-methoxytrifluoromethylphenylacetates (Dale et al., 1969)). The alcohol was protected as the tetrahydropyranyl ether 22. The lithium salt of 22 was then reacted with 1-chloro-3-iodopropane at -40-0°, in THF/HMPA, to selectively give chloride 23.

The chloride was converted to nitrile 24 by treatment with potassium cyanide in refluxing acetonitrile, via phase transfer catalysis with 18-crown-6 (Cook et al., 1974). The nitrile 24 was cleanly and quantitatively hydrolyzed to acid 25 by treatment with alkaline hydrogen peroxide (Corey et al., 1976a). Deprotection of the alcohol with AcOH:THF:H_2O (3:1:1), followed by stereoselective cis reduction with P-2 nickel, gave the previously prepared Z-hydroxy acid 19, which could be cyclized as previously described.

This route was only completely worked out for racemic II but would work equally well for the enantiomers of II. It was found to be superior to the previous route, both in terms of overall yield (15%) and convenience.

Synthesis of 13-Methyl-(5Z,8Z)-tridecadienolide (III)

The key features of III are the (5Z,8Z) diene system and the chiral center at C13. Several approaches have been used to construct so-called skipped diene systems. For instance, reactions between β,γ-unsaturated aldehydes and Wittig reagents have given skipped dienes (Bestmann and

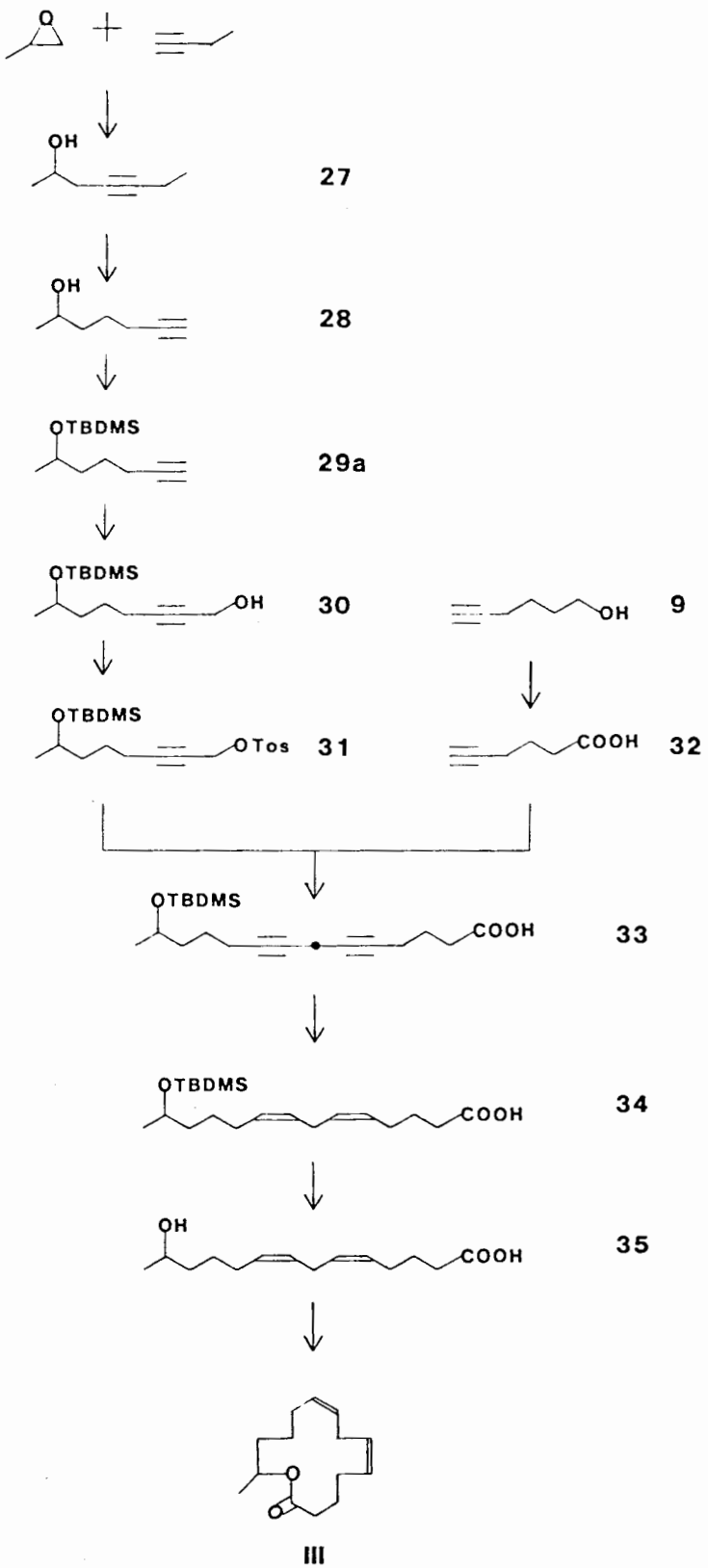
Vostrowsky, 1979); however, the stereochemical control is by no means absolute (Corey et al., 1980). Vinylic organocuprates (Alexakis et al., 1979) and organoboranes (Miyaura et al., 1980) have been coupled with allylic halides but the limitations and compatability with various functional groups have not been fully explored. The same can be said about the newly-developed method, using dibutyl-1,5-stannacyclohexadiene as the synthetic equivalent of (Z,Z)-LiCH=CH-CH_2-CH=CHLi (Kang and Corey, 1982).

The simplest and most common method of making (Z,Z) skipped dienes involves the stereoselective reduction of the appropriate diyne precursors. The diynes are made by the coupling of a propargyl halide or, better, a propargyl tosylate (Verkruijsse and Hasselaar, 1979; Mori and Ebata, 1981), with the Grignard of an alkyne, using cuprous salt catalysts. In addition, it is possible to couple the diGrignard derivative of an Ω-acetylenic acid of five carbons or more with a propargyl halide or tosylate (Osbond et al., 1961; Beerthius et al., 1971). Thus, in the case of macrolide III, the required unsaturations and the unprotected carboxyl function could be introduced in one step, giving a convergent synthesis.

The chiral center was introduced by the same method as was used in the improved synthesis of II; that is, the lithium salt of an alkyne was used to regio- and stereoselectively open chiral methyloxiranes, followed by isomerization of the triple bond of the resulting propargyl alcohol to the terminal position by way of the acetylene zipper reaction.

The complete synthetic route is shown in Scheme IV. Thus, the appropriate methyloxirane was opened stereo- and regioselectively by the lithium salt of 1-butyne in THF at -20°-20°, giving the propargyl alcohol 27. Isomerization of the alkyne to the terminal position with potassium

Scheme IV



III

3-aminopropylamide gave alkyne 28, the hydroxyl of which was protected as the t-butyldimethylsilyl ether 29a. The order of the steps was important, because if alcohol 27 was first protected, followed by the acetylene zipper reaction, a mixture of products resulted, presumably due to steric hindrance by the bulky protecting group. The lithium salt of 29a in THF was then chain extended by one carbon by reaction with dry, powdered paraformaldehyde (Brandsma, 1971), giving the propargyl alcohol 30. Tosylation of 30 with p-tosyl chloride and powdered KOH in ether at -10-0° (Brandsma, 1971) completed the synthesis of half of the carbon skeleton.

Alternatively, racemic 28 could be made by oxidation of 5-hexyn-1-ol (9) to 5-hexyn-1-al (36) with pyridinium dichromate in CH_2Cl_2 (Corey and Suggs, 1975), followed by treatment of an ether solution of the aldehyde with methyl magnesium bromide, giving alcohol 28.

The chiral purities of the enantiomers of 28 were determined by ^1H NMR of the (+)-α-methoxytrifluoromethylphenyl acetates (Dale et al., 1969), as previously described for II. Only one isomer was seen in each case, for a minimum e.e. of 95%.

The second fragment of the carbon skeleton, 5-hexynoic acid (32), was prepared by oxidation of 5-hexyn-1-ol with pyridinium dichromate in DMF (Corey and Schmidt, 1979). The two fragments 31 and 32 were then coupled by cuprous bromide catalyzed reaction of the diGrignard derivative of acetylenic acid 32 with tosylate 31, giving a good yield of crude diyne acid 33. The crude acid was then stereoselectively reduced with P-2 nickel to the (Z,Z) diene acid 34, followed by deprotection of the hydroxyl function with AcOH:H_2O:THF (3:1:1), yielding 35. Hydroxy acid 35 was then cyclized by Mukaiyama's method in moderate yield, with an overall yield of about 8%

from methyloxirane. R, S, and racemic macrolide III have been prepared by this route.

It should be mentioned that acids 33, 34 and 35 were unstable. Thus, 33 and 34 were carried through subsequent steps without purification. In addition, attempts to purify analytical samples of 33 and 34 were not successful as the acidic conditions needed to prevent ionization of the acid functions during TLC also caused some hydrolysis of the acid-labile protecting group.

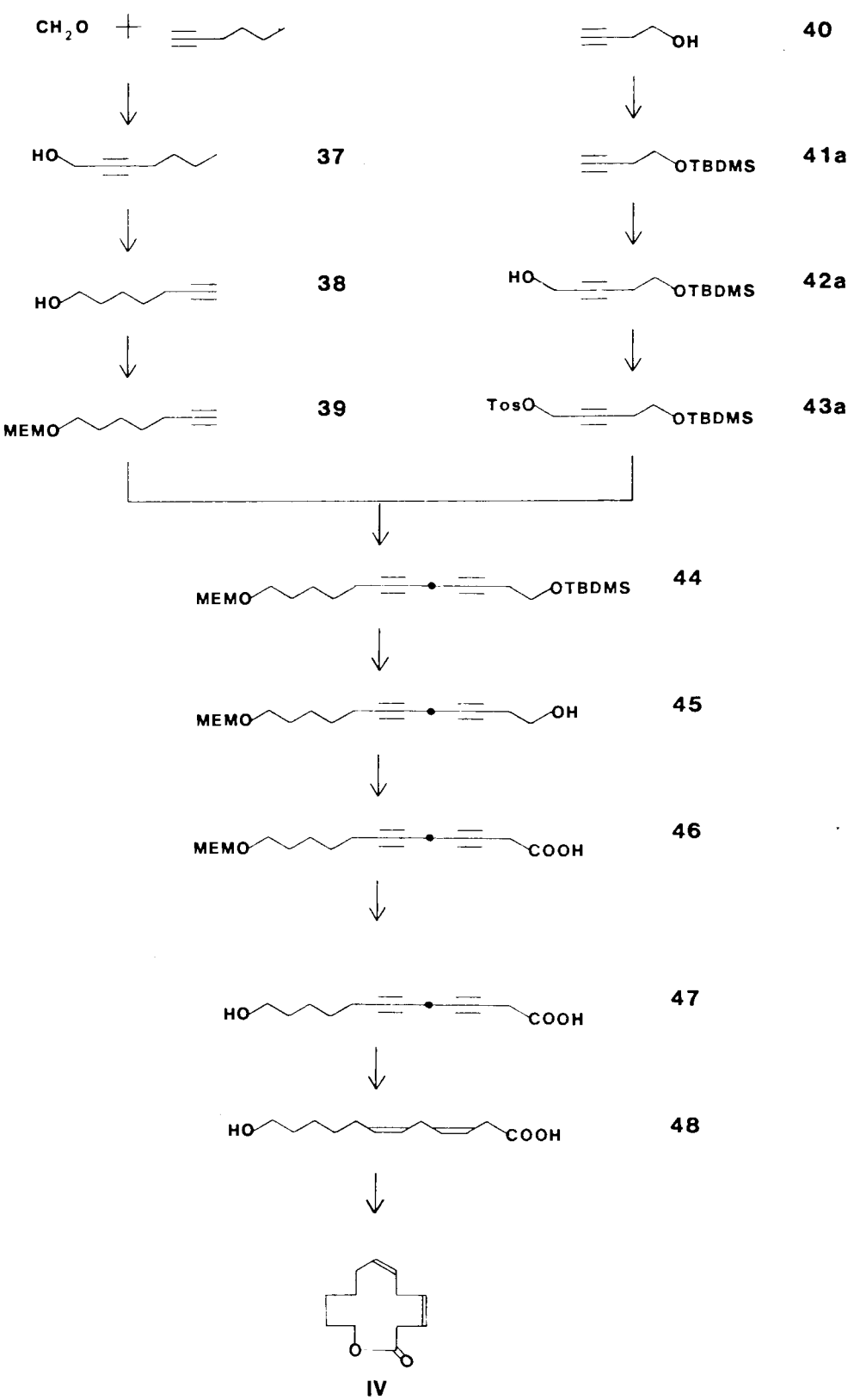
Synthesis of (3Z,6Z)-Dodecadienolide (IV)

The key feature of macrolide IV is the (Z,Z) skipped diene system. However, as the diene is β,γ to the carboxyl function, the diyne precursor cannot be made via the coupling reaction between a propargyl tosylate and the dianion of 3-butynoic acid because the protons α to the carbonyl are too acidic. Consequently, coupling occurs predominantly α to the carbonyl, instead of at the terminal alkyne. Thus, it was necessary to use a somewhat longer synthesis, involving the coupling of a propargyl tosylate with a protected alkynol, followed by deprotection and oxidation of the resulting alcohol (Scheme V).

Thus, the lithium salt of 1-hexyne in THF was chain-extended by one carbon by reaction with powdered paraformaldehyde. The resulting propargyl alcohol 37 was isomerized to the terminal alkyne 38 by treatment with potassium 3-aminopropylamide. The hydroxyl function was protected as the relatively acid-insensitive MEM ether 39 by treatment with MEM chloride and diisopropylethylamine, completing one fragment of the carbon skeleton.

The other fragment was constucted from 3-butyn-1-ol (40). The

Scheme V


CH2O
HO
37
HO
38
MEMO
39
OH
40
OTBDMS
41a
HO
OTBDMS
42a
TosO
OTBDMS
43a
MEMO
OTBDMS
44
MEMO
OH
45
MEMO
COOH
46
HO
COOH
47
HO
COOH
48
IV

hydroxyl was protected as the t-butyldimethylsilyl ether, followed by one-carbon chain extension by reaction of the lithium salt of 41a with powdered paraformaldehyde. The propargyl alcohol 42a was then tosylated with p-tosyl chloride and powdered KOH in ether at -10°-0°, yielding 43a, the second fragment of the carbon skeleton.

The diyne was then constructed by cuprous bromide catalyzed coupling of the Grignard of 39 with tosylate 43a (Verkruijsse and Hasselaar, 1979). The crude diyne was selectively deprotected with p-toluenesulphonic acid in dry MeOH. Oxidation of the resulting homopropargyl alcohol 45 with pyridinium dichromate in DMF was not successful, as was found in the synthesis of macrolide I. Instead, inverse addition of an acetone solution of 45 to cold Jones reagent (Holland and Gilman, 1974) was found to give a reasonable yield of acid 46, with no observed hydrolysis of the MEM ether. The MEM ether was then carefully hydrolyzed with THF:H_2O:conc. HCl (8:2:1) for 24 h at 20°. Stereoselective reduction of hydroxy acid 47 with P-2 nickel gave the required (Z,Z) diene 48, with a small amount of product resulting from over-reduction of the double bond β,γ to the carboxyl function. The diene hydroxy acid was then cyclized in moderate yield with 2-chloro-1-methylpyridinium iodide (Mukaiyama et al., 1976). The overall yield from 1-hexyne was about 2%.

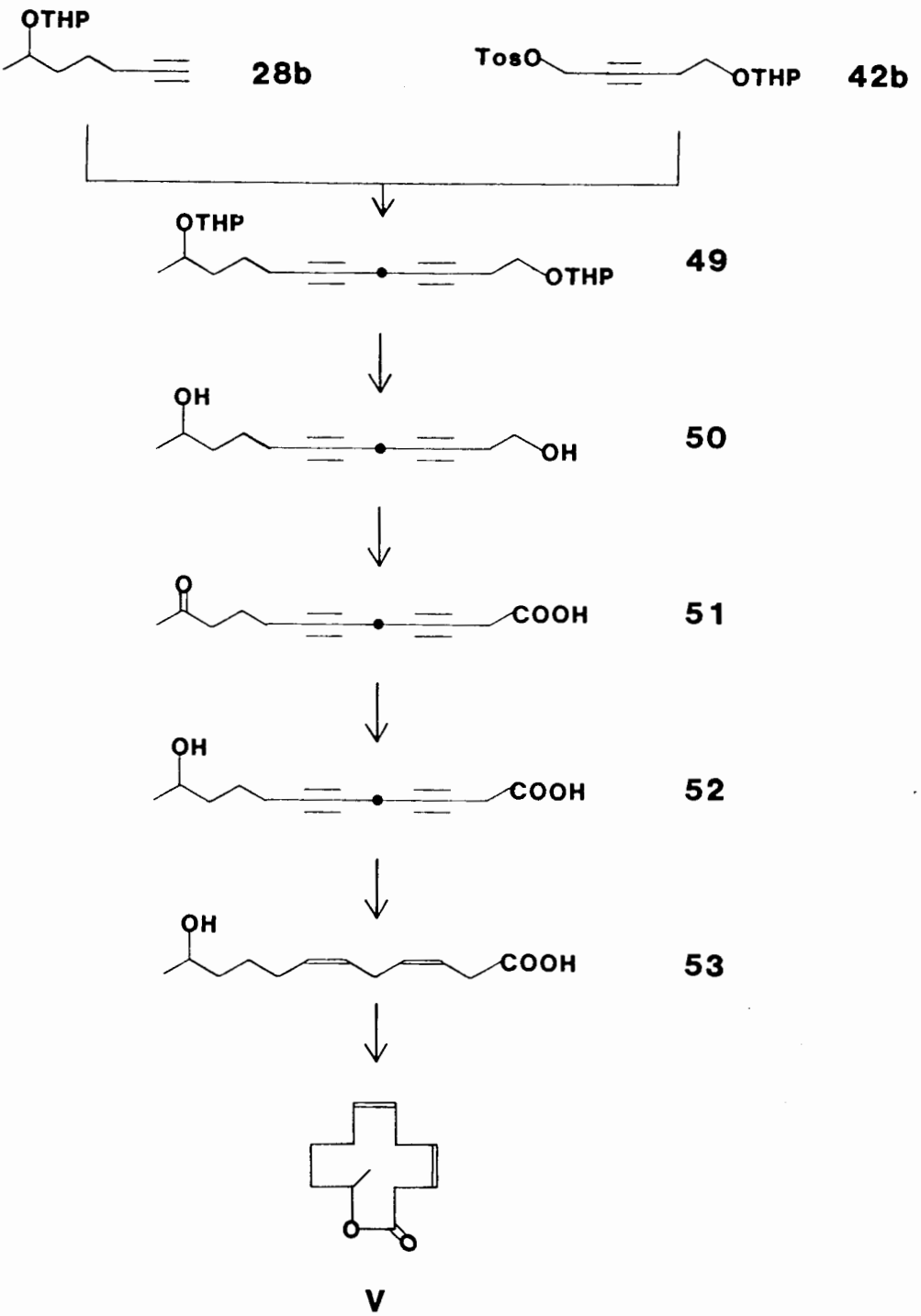
The major problem with the synthesis was the instability of the diyne intermediates 44 to 48, which rapidly discolour and decompose, even at -30°. Thus, it was necessary to work as rapidly as possible, with the minimum number of purification steps.

Synthesis of 11-Methyl-(3Z,6Z)-undecadienolide (V)

Retrosynthetic analysis of macrolide V showed that the key diyne precursor 49 (Scheme VI) could be constructed from two previously-made fragments, namely, protected alkynol 28, from the synthesis of III, and tosylate 42, from the synthesis of IV. Cuprous bromide catalyzed coupling of the Grignard of 28b and tosylate 42b gave the diyne 49. The tetrahydropyranyl ether protecting groups were hydrolyzed with p-toluenesulphonic acid in dry MeOH, yielding diol 50. Oxidation of the diol by inverse addition to cold Jones reagent gave a moderate yield of the keto acid 51. The ketone was selectively reduced by addition of $NaBH_4$ to an ethanolic solution of 51 at -10°-0°, giving hydroxy acid 52. Stereoselective reduction of the triple bonds with P-2 nickel produced the (Z,Z) diene hydroxy acid 53, with a small amount of product resulting from over-reduction of the double bond β,γ to the carboxyl, as was found for compound 48 in the synthesis of IV. Finally, cyclization of 53 with 2-chloro-1-methylpyridinium iodide (Mukaiyama et al., 1976) gave a very low yield of macrolide V (7-10%) on several attempts. It appears that the molecule is intrinsically difficult to cyclize.

Several minor variations could also be used. For instance, the t-butyldimethylsilyl ether analogs of 28b and 42b were made and carried through to the diol 50, using the conditions described for the diyne coupling reaction and the deprotection of the diol as were used for 28b and 42b. Yields were roughly the same. It was concluded that the silyl derivatives were preferred to work with because of lower boiling points and because they were not mixtures of diastereomers, whereas the THP ones were diastereomeric mixtures, due to the chiral center of the THP ring.

Scheme VI


OTHP
28b
TosO
OTHP
42b
OTHP
OTHP
49
OH
OH
50
O
COOH
51
OH
COOH
52
OH
COOH
53
V

The MEM ether analog of 28b was also synthesized and coupled with tosylate 42b (Scheme VII). The primary hydroxyl of the resulting diyne 49b was then selectively deprotected with PTSA in dry MeOH, giving 54, which was then oxidized with cold dilute Jones reagent, using the inverse addition method. The secondary alcohol of the resulting acid 55 was then deprotected with THF:H_2O:HCl (8:2:1), yielding the diyne hydroxy acid 52, as produced by the previous route, in approximately the same yield.

It was essential to leave the diyne in non-reduced form until after the oxidation of the alcohol to the carboxyl function, as all attempts to oxidize the dienol or diendiol obtained from reduction of compounds 49a or 49b resulted in intractable multi-component mixtures. This is supported by evidence of facile intramolecular cyclization of 3,6-dienals (Corey and Suggs, 1975) and by evidence that electrophilic additions to triple bonds proceed more slowly than the additions to double bonds (March, 1968).

As with the intermediates in the synthesis of IV, all the diyne intermediates were unstable, thus contributing to the low overall yields obtained.

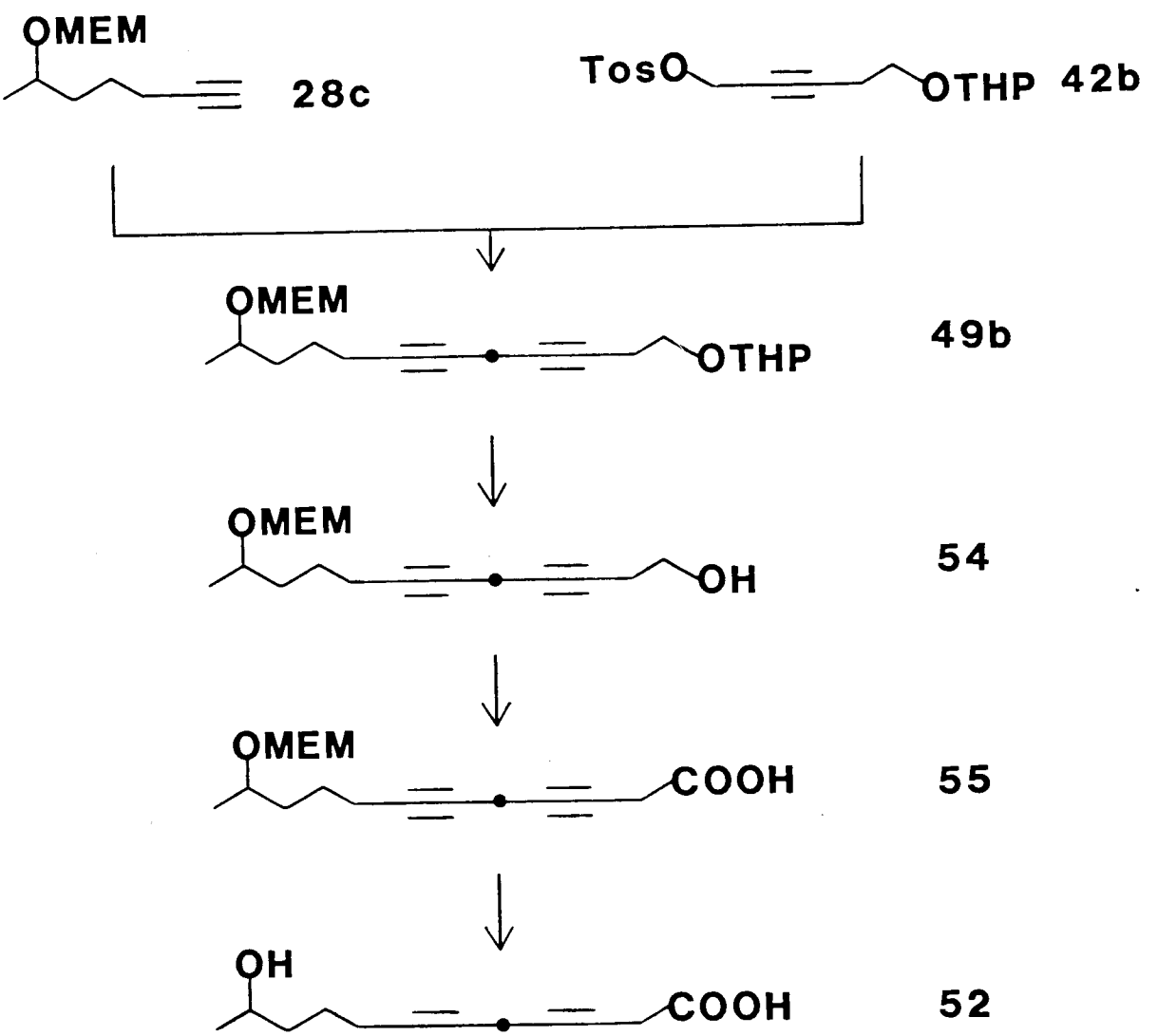
C. Experimental Procedures

General Procedures

Routine GLC analyses were run on a Hewlett-Packard 5880A gas chromatograph, with WCOT capillary columns coated with OV-101, SP-2100 or Durabond 1.

Analytical (0.25 mm) and preparative (0.75 mm) thin layer chromatography plates were prepared from silica gel GF_{254} (E. Merck, Darmstadt). Column chromatography was performed by the flash chromatography method

Scheme VII


OMEM
28c
TosO
OTHP
42b
OMEM
OTHP
49b
OMEM
OH
54
OMEM
COOH
55
OH
COOH
52

(Still et al., 1978), on silica gel (Kieselgel 60, 40-63 μm, E. Merck, Darmstadt). Chromatographic solvents were distilled before use.

IR spectra were determined on a Perkin-Elmer 599B spectrophotometer. Samples were run as a neat film on NaCl plates or as solutions in a cell with NaCl windows.

^{1}H NMR were recorded on a Varian XL-100 (100 MHz) or a Brüker 400 (400 MHz) NMR spectrometer. Chemical shifts are reported in δ units, referenced to an internal (deuterium) lock signal. Splitting patterns are described as: s, singlet; d, doublet; t, triplet; quartet; quintet; m, multiplet; and combinations thereof. Coupling constants are reported in Hertz (Hz).

Low resolution mass spectra were obtained with direct insertion probe or GLC-MS samples on a Hewlett-Packard 5985B coupled gas chromatograph-mass spectrometer. All samples were run using electron impact ionization (70 eV) unless otherwise specified. Samples run with chemical ionization (denoted by CI) were run with isobutane as the ionizing gas, unless otherwise stated. High resolution mass spectra were obtained on a DS-50 instrument at the University of British Columbia. Elemental analyses were performed by Mr. M. Yang (Department of Biological Sciences, S.F.U.) on a Perkin Elmer Model 240 elemental analyzer.

Optical rotations were measured with a Rudolph Model 70 polarimeter, using a 1 dm × 2 mm ID sample cell, or with a Perkin Elmer P_{22} spectropolarimeter, using a 0.5 dm × 5 mm ID sample cell. Concentrations are reported in g/100 mL solvent.

All reactions requiring anhydrous and/or oxygen-free conditions were run under a positive pressure of nitrogen or argon, in flame-dried

glassware. Tetrahydrofuran (THF) was freshly distilled from lithium aluminum hydride. Dimethylformamide (DMF) and hexamethyl phosphoric triamide (HMPA) were distilled from calcium hydride under reduced pressure. 1,3-Diaminopropane was distilled from barium oxide. Dry ethanol and methanol were distilled off Mg turnings.

Boiling points are uncorrected.

Synthesis of Dodecanolide (Ia)

Dodecanolide was prepared from cyclododecanone (Matheson, Coleman and Bell), as described by Kruizinga and Kellogg (1981). Thus, cyclododecanone (10.0 g, 50.5 mmol) and m-chloroperbenzoic acid (21.0 g, 105 mmol) were refluxed together for 48 h in dry $CHCl_3$ (250 mL, dried by distillation off P_2O_5). The resulting mixture was cooled to 0° and filtered to remove solid benzoic acid. The filtrate was washed with saturated sodium bisulfite solution and concentrated under reduced pressure. The residue was taken up in ether (200 mL) and washed with 1 M K_2CO_3 (2 × 200 mL) and brine. The ethereal solution was then dried ($MgSO_4$), concentrated under reduced pressure and distilled, yielding ≈9.3 g (86%) of Ia, b.p. 71° (0.5 mm Hg). ^1H NMR and mass spectra obtained agreed with literature data.

Synthesis of Macrolide I

Preparation of 8-bromo-1-octanol (2)

Bromoalcohol (4) was prepared from 1,8-octanediol (Aldrich Chemical Co., Inc.) via the method of Maurer and Grieder (1977). Thus, 1,8-octanediol (40.6 g, 278 mmol) was treated with 225 mL 48% aqueous HBr (1.8 mol) at 80° for 24 h, under continuous extraction with heptane. The cooled

heptane solution was washed sequentially with saturated $NaHCO_3$ and brine and dried ($MgSO_4$). The solution was then concentrated in vacuo and vacuum distilled through a Vigreux column to give 2, 53.6 g (91%), 77-78° (0.01 mm Hg); IR (film) 3335, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25-1.65 (m, 10H), 1.75-1.95 (m, 2H), 2.53 (br s, 1H), 3.39 (t, J=6.5 Hz, 2H), 3.52 (t, J=6.0 Hz, 2H); mass spectrum, m/e (relative intensity) 164(18), 162(19), 83(40), 69(97), 55(100).

Preparation of 1-(2-methoxyethoxymethoxy)-8-bromooctane (3)

Diisopropylethylamine (48.9 g, 380 mmol) and freshly-distilled β-methoxyethoxymethyl chloride (47.0 g, 380 mmol) were stirred in 500 mL dry methylene chloride at 0°. 8-Bromo-1-octanol (51.5 g, 246 mmol) was added dropwise over 30 min. The solution was stirred overnight at r.t. and extracted with water (2 × 300 mL). The organic layer was dried ($MgSO_4$), concentrated, and vacuum distilled to give 3, (55.7 g, 76%), b.p. 118-125° (0.35 mm Hg); ^1H NMR ($CDCl_3$) δ 1.25-1.65 (m, 10H), 1.75-1.95 (m, 2H), 3.39 (t, J=6.5 Hz, 2H), 3.39 (s, 3H), 3.45-3.74 (m, 6H), 4.69 (s, 2H); mass spectrum, m/e (relative intensity) 297(2), 295(2), 223(9), 221(10), 137(9), 135(9), 89(80).

Preparation of 1-(2-methoxyethoxymethoxy)-9-decyne (4)

Purified acetylene was bubbled into 250 mL of dry THF at 0°. With vigorous stirring, 79 mL of 1.6 M n-BuLi in hexane (126 mmol) was added dropwise. Following addition of the n-BuLi, acetylene was passed through the resulting white suspension for a further 10 min. The suspension was maintained at 15° for 30 min, then cooled to 0°. A solution of bromide 3 (32.4 g, 109 mmol), in 100 mL of dry HMPA, was added dropwise over 1 h.

The solution was warmed to 10° and maintained at 20° for 1 h. The solution was poured into cold water (400 mL) and extracted with ether (3 × 300 mL). The combined ether extracts were washed with water (2 × 200 mL) and brine (1 × 200 mL), dried ($MgSO_4$), and concentrated. Vacuum distillation yielded 4 (22.6 g, 88%), b.p. 105-111° (0.3 mm Hg); IR (film) 3305, 2130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25-1.65 (m, 12H), 1.92 (t, 1H, J=1.5 Hz), 2.16 (td, 2H, J=6, 2.5 Hz), 3.37 (s, 3H), 3.45-3.74 (m, 6H), 4.68 (s, 2H); mass spectrum, m/e (relative intensity) 241(2), 105(89), 89(100), 81(98), 59(95). Anal. calcd. for $C_{14}H_{26}O_3$: C, 69.39; H, 10.81. Found: C, 69.44; H, 10.98.

Preparation of 12-(2-methoxyethoxymethoxy)-3-dodecyn-1-ol (5)

Approximately 115 mmol of ethyl magnesium bromide were prepared from ethyl bromide (12.5 g, 115 mmol) and Mg turnings (3.65 g, 150 mmol) in 75 mL of dry THF. The resulting solution was decanted under argon into a dry 500 mL flask.

A solution of 4 (22.2 g, 92 mmol) in 25 mL THF was added dropwise, maintaining the temperature <30°. The mixture was heated to 50° for 1 h under argon, then cooled to 5°, and ethylene oxide (9 g, 155 mmol) was bubbled in over 30 min. The solution was warmed to r.t. and stirred overnight. The reaction mixture was poured into 20% aqueous NaCl (800 mL) and extracted with ether (3 × 200 mL). The combined organic layers were washed with water (150 mL) and brine (150 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Final traces of solvent were removed under high vacuum, giving a colourless oil (27.2 g, >100%). Since high vacuum distillation of a small sample resulted in extensive decomposition, crude 5 was used in the subsequent reaction. An analytical sample of 5 was purified by TLC (hexane:

EtOAc, 1:1). IR (film) 3450, 2260, 1055 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25-1.65 (m, 12H), 2.05-2.28 (m, 3H), 2.42 (tt, 2H, J=6.3, 2.5 Hz), 3.38 (s, 3H), 3.45-3.74 (m, 8H), 4.69 (s, 2H); mass spectrum, m/e (relative intensity) 227(100), 211(34), 197(45), 105(47), 89(77). Anal. calcd. for $C_{16}H_{30}O_4$: C, 67.10; H, 10.56. Found: C, 67.29; H, 10.40.

Preparation of 12-(2-methoxyethoxymethoxy)-3-dodecynoic acid (6)

A solution of chromium trioxide (6.25 g, 62.5 mmol) in 1.5 M H_2SO_4 (100 mL, 150 mmol) was maintained between 5 and 10° while adding a solution of 5 (4.76 g, 16.6 mmol) in acetone (200 mL) over 6 h. The mixture was warmed to r.t. and stirred 2 h. Ether (150 mL) was added and the mixture was extracted with brine (3 × 150 mL). The organic phase was concentrated under reduced pressure without heating, then taken up in ether (100 mL). The ethereal solution was extracted with 1 M NaOH (2 × 75 mL). The combined basic extracts were acidified with 6 M H_2SO_4 and back-extracted with ether (3 × 75 mL). These combined ether extracts were washed with water and brine, dried ($MgSO_4$), and concentrated. Final purification was accomplished by flash chromatography on silica gel (20 cm × 5 cm ID), eluting with hexane:EtOAc:AcOH, 60:40:1, yielding 3.34 g (67%) of 6, which solidified upon refrigeration. IR (film) 3500-2800, 2258, 1718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2-1.8 (m, 12H), 2.06-2.50 (m, 2H), 3.26 (t, 2H, J=2 Hz), 3.40 (s, 3H), 3.44-3.78 (m, 6H), 4.71 (s, 2H), 5.41 (br s, 1H); mass spectrum of methyl ester, (CH_2N_2), m/e (relative intensity) 255(1), 89(45), 59(100).

Preparation of 12-hydroxy-3-dodecynoic acid (7)

The MEM-protected hydroxy-acid 6 (3.4 g, 12.2 mmol) was stirred at r.t. for 48 h in a mixture of THF:H_2O:conc. HCl (12:2:1). Ether (200 mL)

was added and the mixture was extracted with brine (2 × 150 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield 2.23 g (93%) of 7 as a waxy solid, which was used without further purification. An analytical sample was purified by flash chromatography on silica gel, eluting with hexane:EtOAc:AcOH (60:40:1). IR (film) 3700-2400, 2260, 1727 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20-1.90 (m, 12H), 2.06-2.3 (m, 2H), 3.28 (dt, 2H, J=7, 2.5 Hz), 4.13 (t, 2H, J=6.5 Hz), 5-6 (br s, 2H); mass spectrum of methyl ester (CH_2N_2), m/e (relative intensity) 226(1), 194(3), 93(88), 81(90), 79(100), 67(86). High-resolution mass spectrum, calcd. for $C_{12}H_{20}O_3$, 212.1412; obsd. 212.1420.

Preparation of 12-hydroxy-(3Z)-dodecenoic acid (8)

A suspension of P-2 nickel was prepared from 1.5 g of $Ni(OAc)_2 \cdot 6H_2O$ (6 mmol) in 40 mL of 95% EtOH, 6.0 mL of 1 M ethanolic $NaBH_4$ and 1.2 mL of freshly-distilled 1,2-diaminoethane, under a hydrogen atmosphere. Hydroxy-acid 7 (2.9 g, 13.7 mmol) in 10 mL EtOH was added by syringe and the mixture was stirred under H_2 for 2 h. Charcoal was then added and the resulting black suspension was filtered with suction through a glass fiber filter paper. The filter pad was rinsed with ethanol. The ethanol was removed under reduced pressure and the resulting oil was partitioned between water (50 mL) and ether (100 mL). The mixture was acidified with 6 M H_2SO_4, the ether was decanted and the aqueous portion was extracted with ether (2 × 50 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$), and concentrated. The concentrate was purified by flash chromatography on silica gel (20 cm × 5 cm ID) eluting with hexane:EtOAc:AcOH (100:50:1), to give 2.55 g (87%) of hydroxy-acid 8, which solidified

upon refrigeration. IR (film) 3700-2300, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15-1.75 (m, 12H), 1.9-2.1 (m, 2H), 3.09 (d, 2H, J=5.5 Hz), 3.64 (t, 2H, 6.5 Hz), 5.2-5.5 (br s, 2H), 5.58 (m, 2H); mass spectrum, m/e (relative intensity) 196(19), 178(10), 81(86), 67(100), 55(86). Anal. calcd. for $C_{12}H_{22}O_2$: C, 67.26; H, 10.35. Found: C, 67.14; H, 10.43.

Preparation of (3Z)-dodecenolide (I)

All xylene used in this step was dried azeotropically and deoxygenated. The intermediate pyridyl thioester of 8 was formed by adding hydroxy acid 8 (100.5 mg, 0.47 mmol) to a stirred solution of 2,2'-dipyridyldisulfide (220 mg, 1 mmol) and triphenylphosphine (262 mg, 1 mmol) in xylene (10 mL) under argon. The yellow mixture was stirred for 1 h at r.t., then transferred to a dropping funnel and diluted with xylene (200 mL). This solution was added dropwise over 8 h to refluxing xylene (150 mL). Refluxing was continued for an hour after the addition was complete, and the xylene was removed at reduced pressure. The remaining oil was flash chromatographed on silica gel (12 cm × 2.5 cm ID) eluting with hexane:EtOAc (40:1) yielding 30.8 mg (33%) of macrolide I (98% pure by capillary GLC). This material was identical spectrally and chromatographically to I, isolated from C. pusillus volatiles. IR (film) 1735 cm^{-1}; ^1H (400 MHz) NMR ($CDCl_3$) δ 1.21-1.32 (m, 6H), 1.32-1.46 (m, 4H), 1.57-1.66 (m, 2H), 2.05-2.12 (m, 2H), 2.99-3.06 (m, 2H), 4.03-4.08 (t, 2H, J=5 Hz), 5.45-5.56 (m, 2H); mass spectrum, m/e (relative intensity) 196(6), 178(5), 81(76), 67(100), 54(91). Anal. calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27. Found: C, 73.80; H, 10.18.

Synthesis of Macrolide II

R-(+)-Methyloxirane (R-(+)-13) was made in 48% yield from S-(+)-ethyl lactate via the method of Hillis and Ronald (1981), $[\alpha]_D^{22}$ = +12.1 (neat), lit. $[\alpha]_D^{22}$ = +13.0° (neat). S-(-)-Methyloxirane (S-(-)-13) was prepared in 36% yield by the procedure of Seuring and Seebach (1977), $[\alpha]_D^{23}$ = -14.1 (neat), lit. $[\alpha]_D$ = -12.5° (neat). In the following synthetic procedure, the appropriate chiral or racemic methyloxirane was used at the appropriate step. Yields and procedures reported are for the synthesis of the S-(+)-II and were analogous for the parallel procedures leading to R-(-)-II and racemic II.

Preparation of 1-t-butyldimethylsiloxy-5-hexyne (10)

5-Hexyn-1-ol (Farchan Labs., Albany International, 12.25 g, 125 mmol) and imidazole (18.36 g, 270 mmol) were stirred in dry DMF (30 mL) at 0°. t-Butyldimethylsilyl chloride (20.3 g, 135 mmol) was added in one portion and the cooling bath was removed after 10 min. The reaction was stirred overnight. The reaction mixture separated into two layers. The top layer was decanted and water (100 mL) was added to the bottom layer. The resulting solution was extracted with ether (2 × 75 mL) and the ether extracts were added to the recovered top layer. The ether solution was then back-extracted with water (2 × 50 mL) and brine (1 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo to yield 10 (26.5 g, 100%), which was >99% pure by GLC and was used without further purification. IR (film) 3303, 2109, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.90 (s, 9H), 1.62 (m, 4H), 1.95 (t, 2H, J=2.5 Hz), 2.23 (td, 2H, J=6.75, 2.5 Hz), 3.63 (t, 2H, J=5.5 Hz); mass spectrum m/e (relative intensity), 197(0.1), 155(3), 75(100). Anal.

calcd. for $C_{12}H_{24}OSi$: C, 67.86; H, 11.34. Found: C, 67.95; H, 11.49.

Preparation of 2-(t-butyldimethylsiloxy)-11-chloro-5-undecyne (12)

To a solution of alkyne 10 (26.5 g, 125 mmol) and a few crystals of triphenylmethane in 250 mL of dry THF, cooled to -40°, were added 62 mL (130 mmol) of 2.1 M n-BuLi in hexane. The solution was allowed to warm to 0° over 20 min, then cooled again to -40°. Dry HMPA (100 mL) was added, followed by dropwise addition of 1-chloro-5-iodopentane (11, 29.3 g, 126 mmol). The solution was warmed to r.t. over 6 h, stirred overnight and then poured into water (500 mL). The product was extracted with ether (3 × 150 mL). The combined ether extracts were back-washed with 5% aqueous sodium thiosulphate, water and brine, dried ($MgSO_4$), concentrated, and vacuum distilled to give 12 (29.9 g, 76%), b.p. 115-125° (0.05 mm Hg). IR (film) 2160, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.90 (s, 9H), 1.37-1.93 (m, 10H), 2.03-2.41 (m, 4H), 3.55 (t, 2H, J=6.5 Hz), 3.63 (t, 2H, J=5.5 Hz); mass spectrum, m/e (relative intensity) 261(3), 259(11), 149(21), 107(45), 93(100). Anal. calcd. for $C_{17}H_{33}OSiCl$: C, 64.41; H, 10.49. Found: C, 64.09; H, 10.61.

Preparation of (13S)-1-(t-butyldimethylsiloxy)-5-tetradecyn-13-ol ((13S)-14)

Chloride 12 was washed through a short column of silica gel with petroleum ether immediately before use to remove traces of moisture and silyl alcohols. The resulting oil was converted to the Grignard reagent as follows. Dry THF (50 mL) and magnesium turnings (6.1 g, 250 mmol) were stirred under argon. 1,2-Dibromoethane (≈1 mL) was added and, after the exothermic reaction subsided, additional dry THF (50 mL) was added.

Chloride 12 (37.5 g, 118 mmol) was then added, first as a 5 mL aliquot and then dropwise over 4 h under reflux. Six further 1 g portions of Mg turnings were added at intervals during this time. Reflux was continued for 1 h after the addition was complete. The mixture was cooled to r.t. and the liquid portion transferred under argon to a dry flask. The remaining solids were washed with dry THF (25 mL) and the washings transferred. The resulting solution was cooled to -30°, purified CuI (Linstrumelle et al., 1976) (1.7 g, 9 mmol) was added and the solution was stirred for 15 min. A solution of (S)-(-)-methyloxirane, (S)-13, (7.54 g, 130 mmol) in dry THF (20 mL) was added dropwise over 30 min. The reaction mixture was slowly warmed to r.t. over 6 h, stirred overnight and poured into cold saturated NH_4Cl solution (400 mL). The mixture was extracted with ether (3 × 200 mL) and the combined ether extracts were washed with brine, dried ($MgSO_4$), and concentrated to yield (13S)-14 (30.3 g, 76% crude yield), which was used without further purification. An analytical sample was purified by flash chromatography on a silica gel column (15 cm × 2.5 cm ID) eluting with hexane:EtOAc (9:2). IR (film) 3340, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.90 (s, 9H), 1.18 (d, 3H, J=5.5 Hz), 1.23-1.65 (m, 14H), 1.95 (br s, 1H), 2.10-2.20 (m, 4H), 3.62 (t, 2H, J=6.5 Hz), 3.75-3.85 (m, 1H); mass spectrum, m/e (relative intensity) 283(17), 189(10), 95(78), 75(100), 45(52). High resolution mass spectrum, calcd. for $C_{20}H_{40}O_2Si$: 340.2797; obsd. 340.2785. $[\alpha]_D^{31}$ = +3.6° (c, 1.82, $CHCl_3$).

Preparation of (13S)-1-(t-butyldimethylsiloxy)-13-(2-methoxyethoxymethoxy)-5-tetradecyne ((13S)-15)

The triethylamine salt of MEM chloride (Corey et al., 1976) (14.9 g,

66 mmol) was dissolved in dry CH_3CN (100 mL) and alcohol 14 (15.0 g, 44.1 mmol) was added. The solution was refluxed overnight, during which time considerable precipitate formed. The solution was cooled to r.t., filtered and the filter cake was washed with CH_3CN (2 × 10 mL). The filtrate was concentrated *in vacuo*, poured into water (150 mL) and the resulting suspension was extracted with ether (3 × 100 mL). The combined ether extracts were washed with water and brine, dried ($MgSO_4$) and concentrated. The residue was flash chromatographed in three batches on silica gel (20 cm × 5 cm ID), eluting with hexane:EtOAc (9:1), giving (13S)-15 (14.8 g, 78%). IR (film) 1100, 1039 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.90 (s, 9H), 1.16 (d, 3H, J=5.5 Hz), 1.23-1.65 (m, 14H), 2.10-2.20 (m, 4H), 3.40 (s, 3H), 3.53-3.60 (m, 2H), 3.63 (t, 2H, J=6.5 Hz), 3.68-3.75 (m, 3H), 4.73 (d, 1H, J=7.5 Hz), 4.79 (d, 1H, J=7.5 Hz). Anal. calcd. for $C_{24}H_{48}O_4Si$: C, 67.24; H, 11.29. Found: C, 67.29; H, 11.48. $[\alpha]_D^{31}$ = +4.8° (c, 4.94, $CHCl_3$).

Preparation of (13S)-1-(t-butyldimethylsiloxy)-13-(2-methoxyethoxymethoxy)-(5Z)-tetradecene ((13S)-16)

To a solution of P-2 nickel (1 mmol) in ethanol (100 mL) was added (13S)-15 (9.85 g, 23 mmol) and the solution was stirred at r.t. under hydrogen for 6 h. The black suspension was then filtered through a 0.5 cm pad of activated charcoal and the charcoal was rinsed with ethanol (2 × 10 mL). The violet filtrate was concentrated, poured into water (100 mL) and extracted with ether (3 × 100 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$), and concentrated to yield (13S)-16 (9.9 g, 100%). The product (>98% pure by GLC) was used without further

purification. IR (film) 1100, 1039 cm^{-1}; ^1H NMR (C_6D_6) δ 0.13 (s, 6H), 1.05 (s, 9H), 1.19 (d, 2H, J=5.5 Hz), 1.26-1.70 (m, 14H), 2.09-2.19 (m, 4H), 3.2 (s, 3H), 3.33 (t, 2H, J=5.5 Hz), 3.61 (t, 2H, J=6.5 Hz), 3.70-3.80 (m, 3H), 4.74 (d, 1H, J=7.5 Hz), 4.79 (d, 1H, J=7.5 Hz), 5.5-5.55 (m, 2H); mass spectrum (CI, isobutane) m/e 431 (P+1). High resolution mass spectrum, calcd. for $C_{24}H_{50}O_4Si$: 430.3478; obsd.: 430.3460. $[\alpha]_D^{33}$ = +4.7° (c, 4.17, $CHCl_3$).

Preparation of (13S)-13-(2-methoxyethoxymethoxy)-(5Z)-tetradecen-1-ol ((13S)-17)

Crude (13S)-16 (9.4 g, 22 mmol) was stirred at r.t. for 24 h in 100 mL of AcOH:THF:H_2O (3:1:1). The solution was concentrated under reduced pressure and the silanol removed by pumping under high vacuum (0.1 mm Hg) for 12 h at 40°. The resulting (13S)-17 (6.9 g, 100%, >95% pure by GLC) was used without further purification. IR (film) 3420, 1100, 1038 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (d, 3H, J=5.5 Hz), 1.25-1.48 (m, 10H), 1.49-1.64 (m, 4H), 1.98-2.10 (m, 5H), 3.40 (s, 3H), 3.57 (t, 2H, J=5.5 Hz), 3.65 (t, 2H, J=6.5 Hz), 3.68-3.75 (m, 3H), 4.72 (d, 1H, J=7.5 Hz), 4.78 (d, 1H, J=7.5 Hz), 5.30-5.40 (m, 2H). Anal. calcd. for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.19; H, 11.29. $[\alpha]_D^{34}$ = +6.5° (c, 2.31, $CHCl_3$).

Preparation of (13S)-13-(2-methoxyethoxymethoxy)-(5Z)-tetradecenoic acid ((13S)-18)

Pyridinium dichromate (25 g, 66 mmol) was dissolved in dry DMF (75 mL) in a 250 mL flask fitted with a mechanical stirrer. Crude alcohol (13S)-17 (6.9 g, 22 mmol) was added and the solution was stirred vigorously for 16 h at r.t. The mixture was poured into water (800 mL) and extracted with

ether (4 × 200 mL). The combined ether extracts were washed with water and brine, dried ($MgSO_4$), and concentrated to yield (13S)-18 (6.9 g, 95%), purity >90% by GLC of the methyl ester (CH_2N_2). An analytical sample was further purified by flash chromatography on silica gel (15 cm × 2.5 cm ID) eluting with hexane:EtOAc:AcOH (100:20:1). IR (film) 3550-2200, 1729 cm^{-1}; ^1H NMR (C_6H_6) δ 1.18 (d, 3H, J=5.5 Hz), 1.32-1.52 (m, 10H), 1.58-1.72 (m, 4H), 2.00-2.15 (m, 4H), 2.19 (t, 2H, J=7.0 Hz), 3.20 (s, 3H), 3.43 (t, 2H, J=4.5 Hz), 3.65-3.82 (m, 3H), 4.77 (d, ^1H, J=7.5 Hz), 4.81 (d, 1H, J=7.5 Hz), 7.30 (br s, 1H). High resolution mass spectrum, calcd. for $C_{18}H_{33}O_5$ (P-1): m/e 329.2328; obsd.: 329.2335. $[\alpha]_D^{33}$ = +7.2 (c, 3.33, $CHCl_3$).

Preparation of (13S)-13-hydroxy-(5Z)-tetradecenoic acid ((13S)-19)

Crude (13S)-18 (6.37 g, ≈19 mmol) was stirred in 110 mL of THF:H_2O: conc. HCl (8:2:1) for 24 h at r.t. The solution was poured into water (300 mL) and extracted with ether (4 × 125 mL). The combined ether extracts were washed with water and brine, dried ($MgSO_4$), and concentrated. Purification was accomplished by flash chromatography on silica gel (20 cm × 5 cm ID) eluting with hexane:EtOAc:AcOH (75:25:1), yielding (13S)-19 (3.34 g, 72%) as a colourless oil. IR (film) 3600-2300, 1711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (d, 2H, J=5.5 Hz), 1.25-1.57 (m, 10H), 1.73 (quintet, 2H, J=6 Hz), 1.98-2.10 (m, 2H), 2.10-2.18 (m, 2H), 2.38 (t, 2H, J=7.0 Hz), 3.82-3.90 (m, 1H), 5.30-5.38 (m, 1H), 5.42-5.50 (m, 1H), 7.0-7.5 (br s, 2H); mass spectrum of methyl ester (CH_2N_2), m/e (relative intensity) 238(3.5), 81(100), 67(94), 55(83), 45(72). High resolution mass spectrum, calcd. for $C_{14}H_{26}O_3$: 242.1882. Obsd. 242.1880. $[\alpha]_D^{32}$ = +4.9°

(c, 4.06, $CHCl_3$).

Preparation of (13S)-13-methyl-(5Z)-tridecenolide ((13S)-II)

A solution of 2-chloro-1-methylpyridinium iodide (4.2 g, 17 mmol) in dry acetonitrile (250 mL) was heated to reflux under argon. A solution of (13S)-19 (1.0 g, 4.1 mmol) and triethylamine (3.4 g, 34 mmol) in dry acetonitrile (250 mL) was added dropwise over 6 h. The resulting solution was refluxed a further 2 h, then cooled and concentrated. The concentrate was poured into water (100 mL) and extracted with pentane (3 × 50 mL). The combined pentane extracts were washed with water, dried ($MgSO_4$), and concentrated. The resulting product was flash chromatographed on silica gel (18 cm × 2.5 cm ID) eluting with hexane:EtOAc (40:1), giving (13S)-II (494 mg, 49%). This (13S)-II was spectrally and chromatographically identical to II isolated from C. pusillus. IR (film) 1725, 1248, 1216 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (d, 2H, 5.5 Hz), 1.25-1.68 (m, 13H), 1.70-1.78 (m, 1H), 1.78-1.98 (m, 2H), 2.17-2.37 (m, 2H), 2.19-2.27 (ddd, 1H, J=2.5,9, 15 Hz), 2.40-2.48 (ddd, 1H, J=2.5,9,15 Hz), 4.94-5.03 (m, 1H), 5.33 (td, 1H, J=4.5, 10.5 Hz), 5.40 (td, 1H, J=4.5, 10.5 Hz); mass spectrum, m/e (relative intensity) 224(9.1), 81(94.5), 67(91.5), 55(77.1), 41(100). High resolution mass spectrum, calcd. for $C_{14}H_{24}O_2$: 224.1776; obsd.: 224.1776. (13S)-(+)-II, $[\alpha]_D^{32}$ = +49.6° (c, 4.62, $CHCl_3$). (13R)-(-)-II, $[\alpha]_D^{35}$ = -46.4° (c, 2.19, $CHCl_3$).

Alternate Synthesis of Macrolide II

Preparation of 4-decyn-2-ol (20)

A solution of 1-heptyne (9.6 g, 100 mmol) in THF (150 mL) was

maintained at ≈-10° in a dry ice-acetone bath under argon while adding n-BuLi in hexane (1.6 M, 75 mL, 120 mmol) dropwise over 20 min. The solution was stirred at 0° for 30 min, then cooled again to -20°. Dry HMPA (50 mL) was added in one portion, followed by dropwise addition of a solution of distilled racemic methyloxirane (7.25 g, 125 mmol) in HMPA (50 mL). The solution was warmed to 20° over 4 h and stirred an additional 12 h. The solution was then poured into aqueous NaCl (50 g NaCl in 300 mL H_2O) and the mixture was extracted with ether (4 × 150 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$), concentrated under reduced pressure and distilled *in vacuo*, yielding **20** (14.7 g, 96%), b.p. 77° (1.5 mm Hg). IR (film) 3340 cm^{-1}; mass spectrum, m/e (relative intensity) 154(1.5), 95(24), 81(51), 68(51), 54(100), 45(41); ^1H NMR ($CDCl_3$) δ 0.90 (t, 3H, C_{10}, J=7.1 Hz), 1.24 (d, 3H, C_1, J=6.1 Hz), 1.28-1.40 (m, 4H, C_8 and C_9), 1.50 (quintet, 2H, C_7, J=7.0 Hz), 1.98 (s, 1H, OH), 2.17 (tt, 2H, C_6, J=7.2, 2.5 Hz), 2.30 (dt, 2H, C_3, J=7.2, 2.5 Hz), 3.90 (m, 1H, C_2).

Preparation of 9-decyn-2-ol (21)

A 1 L three-necked flask equipped with argon flush and a glass-covered stir bar was loaded with KH in mineral oil (25% w/w, 32.0 g, 200 mmol). The oil was washed off with THF (3 × 30 mL) and last traces of THF were removed by pumping under vacuum for 30 min. Dry 1,3-diaminopropane (20 mL) was added in one portion, with vigorous stirring, and the resulting yellow suspension was stirred for 1.25 h. 4-Decyn-2-ol (10.0 g, 65 mmol) was added in one portion and the mixture was stirred at 20° for 1.5 h. The mixture was then cooled to 0° and cautiously quenched by addition of small pieces of ice (≈20 g), with vigorous stirring to suppress foaming. The

mixture was then slowly poured into salty ice-water (800 mL) and the resulting mixture was extracted with ether (4 × 150 mL). The combined ether extracts were back-washed with 2 M HCl (200 mL) and saturated $NaHCO_3$ (100 mL), dried ($MgSO_4$), concentrated under reduced pressure and distilled *in vacuo*, yielding alcohol 21 (8.0 g, 80%), b.p. 80-85° (1.2 mm Hg). IR (film) 3340, 3308, 2117 cm^{-1}; mass spectrum, m/e (relative intensity) 153(0.5), 95(32), 81(58), 67(35), 54(47), 45(100); ^1H NMR ($CDCl_3$) δ 1.22 (d, 3H, CH_3, J=6.1 Hz), 1.25-1.50 (m, 8H, C_5-C_8), 1.54 (quintet, 2H, C_4, J≈7 Hz), 1.56 (s, 1H, OH), 1.95 (t, 1H, C≡CH, J=2.5 Hz), 2.20 (td, 2H, C_3, J=7.0, 2.5 Hz), 3.79 (m, 1H, C_9). Anal. calcd. for $C_{10}H_{18}O$: C, 77.87; H, 11.76. Found: C, 77.66; H, 11.44.

Preparation of 2-[(tetrahydropyranyl)oxy]-9-decyne (22)

Alcohol 21 (4.9 g, 32 mmol) in anhydrous ether (20 mL) was cooled to 0°. A few crystals of *p*-toluenesulphonic acid were added, followed by dropwise addition of 3,4-dihydro-2H-pyran (4.0 g, 48 mmol). The solution was warmed to 20° and stirred for 16 h. The solution was then washed with saturated aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), concentrated *in vacuo* and distilled, giving 22 (7.25 g, 95%), b.p. 94-97° (0.25 mm Hg). IR (film) 3304, 2117 cm^{-1}; mass spectrum, CI, m/e 239 (M+1); ^1H NMR of the mixture of diastereomers ($CDCl_3$) δ 1.11, 1.23 (d, 3H, CH_3, J=6.1 Hz), 1.25-1.88 (m, 16H, methylenes), 1.94 (t, 1H, C≡CH, J=2.5 Hz), 2.18 (td, 2H, $CH_2C{\equiv}C$, J=7.0, 2.5 Hz), 3.45-3.53 (m, 1H, C_9), 3.67-3.97 (m, 2H, OCH_2 of THP), 4.63, 4.70 (dd, 1H, methine of THP, J≈5, 2.5 Hz). Anal. calcd. for $C_{15}H_{26}O_2$: C, 75.59; H, 10.99. Found: C, 75.57; H, 10.94.

Preparation of 1-chloro-12-[(tetrahydropyranyl)oxy]-4-tridecyne (23)

A solution of alkyne 22 (6.66 g, 28 mmol) in THF (30 mL) was cooled to 0° under argon. n-BuLi in hexane (2.3 M, 13 mL, 30 mmol) was added dropwise and the solution was stirred for 30 min at 0°. The solution was then cooled to -10° and dry HMPA (15 mL) was added, followed by dropwise addition of 3-chloro-1-iodopropane (6.14 g, 30 mmol) in THF (10 mL). The mixture was warmed to 20° over 4 h and stirred a further 12 h. The solution was poured into water (200 mL) and the mixture was extracted with ether (3 × 100 mL). The combined ether extracts were washed with water (1 × 100 mL) and brine (1 × 100 mL), dried ($MgSO_4$) and concentrated in vacuo. Distillation yielded 23 (5.41 g, 61%, 88% based on recovered alkyne 22), b.p. 141-150° (0.1 mm Hg). Mass spectrum, CI, m/e 315 and 317 (M+1); ^1H NMR of diastereomers ($CDCl_3$) δ 1.11, 1.23 (d, 3H, CH_3, J=6.1 Hz), 1.25-1.88 (m, 16H, methylenes), 1.93 (quintet, 2H, C_2, J≈6.75 Hz), 2.18 (tt, 2H, C_6, J=7.0, 2.25 Hz), 2.34 (tt, 2H, C_3, J=7.0, 2.5 Hz), 3.45-3.53 (m, 1H, C_{12}), 3.65 (t, 2H, CH_2Cl), 3.67-3.97 (m, 2H, OCH_2), 4.63, 4.70 (dd, 1H, methine of THP, J≈5.0, 2.5 Hz).

Preparation of 12-[(tetrahydropyranyl)oxy]-4-tridecynonitrile (24)

A mixture of chloride 23 (1.80 g, 5.73 mmol), powdered KCN (0.85 g, 13 mmol) and 18-Crown-6 (86 mg, 0.33 mmol) was refluxed in dry acetonitrile for 48 h. The cooled solution was then filtered and the filtrate was concentrated under reduced pressure. The residue was poured into water (20 mL) and the mixture was extracted with ether (3 × 20 mL). The combined ether layers were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was flash chromatographed on silica gel (2.5 cm ID ×

18 cm) eluting with hexane:EtOAc (4:1), yielding nitrile 14 (1.32 g, 80%). IR (film) 2238 cm^{-1}; ^1H NMR of diastereomeric mixture ($CDCl_3$) δ 1.11, 1.23 (d, 3H, CH_3, J=6.1 Hz), 1.25-1.88 (m, 16H, methylenes), 1.84 (quintet, 2H, C_2, J≈6.9 Hz), 2.18 (tt, 2H, C_6, J=7.0, 2.25 Hz), 2.34 (tt, 2H, C_3, J=7.0, 2.25 Hz), 2.48 (t, C_1, J=7.25 Hz), 3.45-3.53 (m, 1H, C_{12}), 3.67-3.97 (m, 2H, OCH_2), 4.63, 4.70 (dd, 1H, methine of THP, J≈5.25 Hz). Anal. calcd. for $C_{19}OH_{31}NO_2$: C, 74.71; H, 10.23; N, 4.59. Found: C, 74.83; H, 10.38; N, 4.62.

Preparation of 13-[(tetrahydropyranyl)oxy]-5-tetradecynoic acid (25)

To a solution of nitrile 24 (610 mg, 2.0 mmol) in 95% EtOH (5 mL) was added 30% aqueous KOH (3.57 mL, 18.6 mmol) and 30% aqueous hydrogen peroxide (1.0 mL, 8.9 mmol). The mixture was heated at 40-45° for 1 h, then refluxed while bubbling N_2 into the mixture, until the effluent gases no longer contained NH_3 (≈4 h). The mixture was cooled to 0°, saturated with NaCl, acidified with 12 M HCl and extracted with ether (4 × 20 mL). The combined ether layers were washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*, to yield 25 (0.63 g, 97%), >98% pure by GLC of the methyl ester (CH_2N_2). TLC (hexane:EtOAc, 9:1) of the methyl ester showed only one spot. IR (film) 3600-2500, 1720 cm^{-1}; mass spectrum of the methyl ester, CI, m/e 339 (M+1); ^1H NMR of diastereomeric mixture ($CDCl_3$) δ 1.12, 1.23 (d, 3H, CH_3, J=6.1 Hz), 1.27-1.88 (m, 16H, methylenes), 1.84 (quintet, 2H, C_3, J≈7 Hz), 2.18 (tt, 2H, C_7, J=7.0, 2.25 Hz), 2.34 (tt, 2H, C_4, J=6.75, 2.25 Hz), 2.48, 2.49 (t, 2H, CH_2COOH, J=7.0 Hz), 3.45-3.53 (m, 1H, C_{13}), 3.67-3.97 (m, 2H, OCH_2), 4.67, 4.70 (m, 1H, methine of THP). Anal. calcd. for $C_{19}H_{32}O_4$: C, 70.34; H, 9.94. Found: C, 70.46; H, 10.03.

Preparation of 13-hydroxy-(5Z)-tetradecenoic acid (19)

Protected hydroxy-acid 25 (0.50 g, 1.54 mmol) was stirred in ≈10 mL of a solution of AcOH:THF:H_2O (3:1:1) for 24 h at 20°. The solution was then concentrated in vacuo and the residue was dissolved in EtOH and added to a solution of P-2 nickel (0.3 mmol, prepared from 75 mg of $Ni(OAc)_2 \cdot 6H_2O$, 0.3 mL of 1 M ethanolic $NaBH_4$ solution and 0.1 mL of ethylene diamine, in 20 mL of 95% EtOH). The mixture was stirred under H_2 for 3.5 h, then filtered with suction through a 5 mm charcoal pad. The charcoal was rinsed thoroughly with acidified (6 N HCl) EtOH. The filtrate was concentrated under reduced pressure without heating. Water (25 mL) was added to the residue, the mixture was acidified with 6 M HCl and extracted with ether (4 × 20 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo, yielding 19 (373 mg, 91%), >95% pure by GLC of the methyl ester (CH_2N_2). TLC of the methyl ester (hexane:EtOAc, 3:1) also gave only one spot. IR (film) 3600-2500, 3010, 1720 cm^{-1}; mass spectrum of the methyl ester, m/e 257 (M+1); ^1H NMR ($CDCl_3$) δ 1.22 (d, 3H, CH_3, J=6.0 Hz), 1.24-1.52 (m, 12H, methylenes and OH's), 1.71 (quintet, 2H, C_3, J≈7.5 Hz), 2.01 (dt, 2H, C_7, J=7.5, 7.5 Hz), 2.11 (dt, 2H, C_4, J=7.5, 7.5 Hz), 2.38 (t, 2H, CH_2COOH, J=7.5 Hz), 3.78-3.88 (m, 1H, C_{13}), 5.32 (dtt, 1H, C_5, J=11, 7.5, 1.5 Hz), 5.41 (dtt, 1H, C_6, J=11, 7.5, 1.5 Hz). Anal. calcd. for $C_{14}H_{26}O_3$: C, 69.37; H, 10.81. Found: C, 69.55; H, 11.03.

Synthesis of Macrolide III

Preparation of 5-Hexynal (36)

5-Hexyn-1-ol (14.7 g, 150 mmol) was added to a mechanically-stirred suspension of pyridinium dichromate (85 g, 225 mmol) in dry CH_2Cl_2 (250 mL). The mixture was stirred for 24 h at 20°, filtered and concentrated to ≈100 mL under reduced pressure without heating. Ether was added to the residue and the mixture was filtered through a 1 cm pad of Fluorisil. The filtrate was washed with 2 N HCl (100 mL) and brine (100 mL), dried ($MgSO_4$), concentrated *in vacuo* and distilled, yielding aldehyde 36 (6.73 g, 47%), b.p. 55-63 (15 mm Hg). In addition, there was ≈7 g of pot residue, which was found to be mainly the overoxidized product, 5-hexynoic acid. IR (film) 3300, 2736, 2122, 1720 cm^{-1}; mass spectrum, m/e (relative intensity) 96(1), 95(18), 81(9), 68(100), 67(63); ^1H NMR ($CDCl_3$) δ 1.90 (tt, 1H, C_3, J=7.0, 6.5 Hz), 1.99 (t, 1H, C≡CH, J=2.5 Hz), 2.28 (td, 2H, C_4, J=6.5, 2.5 Hz), 2.62 (td, 2H, $\underline{CH_2}CH$, J=7.0, 1.5 Hz), 9.82 (t, 1H, CH, 1.5 Hz).

Preparation of (R,S)-6-heptyn-2-ol ((R,S)-28)

Methyl magnesium bromide in ether (2.98 M, 40 mL, 119 mmol) was diluted with dry ether (200 mL) under argon and cooled to -5°. With vigorous stirring, 5-hexynal (8.0 g, 83 mmol) in ether (50 mL) was added dropwise over 30 min, maintaining the temperature below 0°. The mixture was allowed to warm to 20°, then cooled to 0° again and quenched by cautious addition of saturated aqueous NH_4Cl (25 mL). The quenched mixture was then poured into 250 mL of saturated NH_4Cl and extracted with ether (4 × 150 mL). The

combined organic extracts were washed with brine, dried ($MgSO_4$), concentrated at reduced pressure with no heating, and distilled, yielding (R,S)-28 (5.3 g, 58%), b.p. 60-68° (2.0 mm Hg). IR (film) 3360, 3310, 2120, 1378 cm^{-1}; mass spectrum, m/e (relative intensity) 112(0.5), 97(9), 79(18), 67(33), 45(100); ^1H NMR ($CDCl_3$) δ 1.22 (d, 3H, CH_3, J=6.5 Hz), 1.57-1.73 (m, 4H, C_4 and C_5), 1.57 (s, 1H, OH), 1.96 (t, 1H, C≡CH, J=2.5 Hz), 2.23 (td, 2H, $CH_2C{\equiv}C$, J=6.75, 2.5 Hz), 3.84 (m, 1H, C_6). Anal. calcd. for $C_7H_{12}O$: C, 74.96; H, 10.78. Found: C, 74.86; H, 10.84.

Preparation of (R,S)-4-heptyn-2-ol (27)

Dry THF (40 mL) in a 250 mL three-necked flask was cooled to -40° under argon. Condensed 1-butyne (6.6 mL, 83 mmol) was added in one aliquot, followed by dropwise addition of n-BuLi in hexane (2.1 M, 21 mL, 44 mmol). The reaction mixture was warmed to 0° over 30 min, then cooled again to -20°. Dry HMPA (15 mL) was added, followed by dropwise addition over 15 min of racemic methyloxirane (2.61 g, 45 mmol) in HMPA (15 mL). The reaction was stirred at -20° for 30 min, warmed to 20° over 4 h, and stirred an additional 12 h. The mixture was then poured into ice-water (100 mL) and extracted with ether (4 × 50 mL). The combined organic extracts were backwashed with brine, dried ($MgSO_4$) and concentrated at reduced pressure with no heating. Distillation of the residue gave 29 (4.93 g, 100%), b.p. 41° (2.0 mm Hg). IR (film) 3360 cm^{-1}; mass spectrum, m/e (relative intensity) 112(0.5), 97(9), 68(81), 67(100), 53(36), 45(81); ^1H NMR ($CDCl_3$) δ 1.07 (t, 3H, $CH_2\underline{CH_3}$, J=7.5 Hz), 1.18 (d, 3H, C_1, J=6.1 Hz), 2.13 (quartet of triplets, 2H, $C{\equiv}C\underline{H_2}CH_3$, J=7.5, 2.3 Hz), 2.20-2.36 (m, 2H, C_3), 2.74 (broad d, 1H, OH), 3.65-3.94 (m, 1H, C_2). Anal. calcd. for $C_7H_{12}O$: C,

74.96; H, 10.78. Found: C, 74.91; H, 11.00.

Preparation of (S)-(+)-4-heptyn-2-ol ((S)-(+)-27) and (R)-(-)-27

The lithium salt of 1-butyne (6.7 g, 120 mmol) was reacted with (S)-(-)-methyloxirane (6.0 g, 103 mmol; $[\alpha]_D^{23} = -14.06°$, neat, prepared by the method of Seuring and Seebach, 1977), via the procedure described for the preparation of (R,S)-27, to give 10.05 g (87%) of (S)-(+)-27; $[\alpha]_D^{32} = +17.7°$ (c, 1.60, $CHCl_3$).

R-(-)-27 was prepared from the lithium salt of 1-butyne (10.8 g, 200 mmol) and (R)-(+)-methyloxirane (7.0 g, 120 mmol; $[\alpha]_D^{21} = +12.1°$, neat, prepared by the method of Hillis and Ronald, 1981), yielding (R-(-)-27 (10.8 g, 81%); $[\alpha]_D^{32} = -17.5°$ (c, 2.13, $CHCl_3$).

Preparation of (R,S)-6-heptyn-2-ol ((R,S)-28)

KH in mineral oil (25% suspension, 2.4 g, 15 mmol) was introduced into a dry flask under argon. The oil was removed by washing the KH with dry THF (2 × 5 mL) and the last traces of THF were removed by pumping under vacuum. The flask was then refilled with argon, and 1,3-diaminopropane (15 mL, dried by distillation off BaO) was added to the residue. The resulting orange suspension was stirred for 1 h, following which 4-heptyn-1-ol (0.56 g, 5 mmol) was added in one aliquot. The reaction was stirred at 20° for 1 h, then quenched by the cautious addition of crushed ice (5 g) in portions. The resulting mixture was poured into ice-water (50 mL) and extracted with ether (4 × 30 mL). The combined ether extracts were backwashed with brine, dried ($MgSO_4$), and concentrated under reduced pressure without heating. The residue was distilled in a Kugelrohr tube to yield (R,S)-28, b.p. ≈70-80° (15 mm Hg). IR (film) 3360, 3310, 2120,

1378 cm^{-1}; mass spectrum, m/e (relative intensity) 112(0.5), 97(9), 79(18), 67(33), 45(100); ^1H NMR ($CDCl_3$) δ 1.22 (d, 3H, C_1, J=6.5 Hz), 1.57-1.73 (m, 4H, C_3-C_4), 1.57 (s, 1H, OH), 1.96 (t, 1H, C_7, J=2.5 Hz), 2.23 (td, 2H, C_5, J=6.75, 2.5 Hz), 3.84 (m, 1H, C_2). Anal. calcd. for $C_7H_{12}O$: C, 74.96; H, 10.78. Found: C, 74.86; H, 10.84.

Preparation of (S)-(+)-6-heptyn-2-ol ((S)-(+)-28) and (R)-(-)-28

(S)-(+)-27 (9.5 g, 85 mmol) was subjected to the "acetylene zipper" reaction, as described for the preparation of (R,S)-28, yielding (S)-28 (5.82 g, 62%); $[\alpha]_D^{30}$ = +13.4° (c, 1.57, $CHCl_3$). (R)-(-)-27 (9.5 g, 85 mmol) was treated identically, yielding (R)-(-)-28 (7.6 g), contaminated with starting material. An analaytical sample was further purified by flash chromatography on silica gel (2 cm ID × 15 cm) eluting with hexane: EtOAc (4:1). $[\alpha]_D^{30}$ = -13.6° (c, 0.404, $CHCl_3$).

Preparation of (R,S)-6-(t-butyldimethylsiloxy)-1-heptyne ((R,S)-29a)

A solution of imidazole (4.5 g, 66 mmol) and (R,S)-28 (3.5 g, 31 mmol) in dry DMF (10 mL) was cooled to 0°. t-Butyldimethylsilyl chloride (5.0 g, 33 mmol) was added in one portion and the reaction mixture was allowed to warm to 20°. The mixture was stirred at 20° for 16 h, by which time two layers had developed. The top layer was removed and saved. Water (50 mL) was added to the bottom layer and the solution was extracted with ether (2 × 25 mL). The ether extracts were added to the saved top layer and the combined organic phase was back-washed with water (2 × 25 mL) and brine (1 × 25 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Distillation yielded (R,S)-29a (6.14 g, 88%), b.p. 105° (15 mm Hg) as a colourless oil. IR (film) 3322, 2865, 2124, 1259 cm^{-1}; mass spectrum, CI,

m/e 227 (M+1); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H, CH_3Si), 0.88 (s, 9H, $C(CH_3)_3$), 1.13 (d, 3H, CH_3, J=6.1 Hz), 1.48-1.67 (m, 4H, C_4 and C_5), 1.94 (t, 1H, C≡CH, J=2.5 Hz), 2.20 (td, 2H, $CH_2C{\equiv}C$, J=6.5, 2.5 Hz), 3.84 (sextet, 1H, C_6, J≈6 Hz). Anal. calcd. for $C_{13}H_{26}OSi$: C, 68.96; H, 11.57. Found: C, 69.16; H, 11.76.

Preparation of (S)-(+)-6-(t-butyldimethylsiloxy)-1-heptyne ((S)-(+)-29a) and (R)-(-)-29a

(S)-(+)-28 (5.6 g, 55 mmol) was silylated as described for racemic 28, yielding (S)-(+)-29a (10.85 g, 99%). This material was >98% pure by GLC and was used without further purification. $[\alpha]_D^{31}$ = +13.8° (c, 2.696, $CHCl_3$).

(R)-(-)-29a was prepared in similar yield. $[\alpha]_D^{33}$ = -14.1° (c, 3.096, $CHCl_3$).

Preparation of (R,S)-7-(t-butyldimethylsiloxy)-2-octyn-1-ol ((R,S)-30)

Racemic 29a (5.65 g, 25 mmol) was dissolved in dry THF (75 mL) under argon and the solution was cooled to -10° in an ice-salt bath. BuLi in hexane (1.3 M, 20 mL, 26 mmol) was added dropwise, maintaining the temperature below 0° and the resulting solution was stirred at 0° for 30 min. The solution was then cooled again to -10° and dry paraformaldehyde (1.13 g, 37.5 mmol) was added in one portion. The mixture was warmed to 20° over several hours and stirred at 20° for 12 h. The reaction was worked up by pouring into ice-water (100 mL) and extracting with ether (3 × 100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Distillation of the residue gave racemic 30 (4.76 g, 74%), b.p. 104-107° (0.2 mm Hg). IR (film) 3360, 2862, 2226, 1259 cm^{-1};

mass spectrum, CI, m/e 257 (M+1); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H, CH_3Si), 0.88 (s, 9H, $C(CH_3)_3$), 1.13 (d, 3H, CH_3, J=6.1 Hz), 1.48-1.67 (m, 4H, C_5 and C_6), 1.47 (t, 1H, OH, J=6.0 Hz), 2.22-2.27 (m, 2H, C_4), 3.82 (sextet, 1H, C_7, J≈6.1 Hz), 4.26 (dt, 2H, CH_2OH, J=6.4, 2.1 Hz). Anal. calcd. for $C_{14}H_{28}O_2Si$: C, 65.57; H, 11.01. Found: C, 65.72; H, 11.26.

Preparation of (S)-(+)-7-(t-butyldimethylsiloxy)-2-octyn-1-ol ((S)-(+)-30) and (R)-(-)-30

(S)-(+)-30 was produced in 77% yield via the procedure used in the preparation of racemic 30. $[\alpha]_D^{32}$ = +12.3° (c, 3.98, $CHCl_3$).

(R)-(-)-30 was produced in 67% yield. $[\alpha]_D^{31}$ = -11.0° (c, 6.62, $CHCl_3$).

Preparation of (R,S)-7-(t-butyldimethylsiloxy)-2-octyn-1-yl-p-toluenesulphonate ((R,S)-31)

Racemic 30 (40 g, 15.6 mmol) and p-toluenesulphonyl chloride (3.6 g, 18.7 mmol) were dissolved in anhydrous ether (30 mL) and cooled to -5° in an ice-salt bath. Finely powdered KOH (8.74 g, 156 mmol) was added in five equal portions at 5 min intervals, maintaining the temperature of the mixture below 0°. The mixture was then stirred at 0° for 30 min and poured into ice-water (100 mL). The organic layer was removed and the aqueous residue was extracted twice more with ether (2 × 50 mL). The combined organic extracts were backwashed with brine, dried (Na_2SO_4) and concentrated *in vacuo* without heating. Final traces of solvent were removed by pumping under high vacuum (0.1 mm Hg) for 4 h, yielding the tosylate 31 as an oil (6.2 g, 96%). Tosylate 31 gave one spot on TLC (hexane:EtOAc, 3:1) and was used without further purification. IR (film) 2862, 2244, 1601, 1375,

1259 cm^{-1}; mass spectrum, CI, m/e 411 (M+1); ^1H NMR ($CDCl_3$) δ 0.03 (s, 6H, CH_3Si), 0.86 (s, 9H, $C(CH_3)_3$), 1.09 (d, 3H, CH_3, J=6.1 Hz), 1.48-1.68 (m, 4H, C_5 and C_6), 2.03-2.10 (m, 2H, C_4), 2.43 (s, 3H, Ar-CH_3), 3.75 (sextet, 1H, C_7, J≈6.0 Hz), 4.67 (t, 2H, CH_2OSO_2, J=2.2 Hz), 7.33 (d, 2H, tosyl, J=8.0 Hz), 7.79 (d, 2H, tosyl, J=8.0 Hz).

Preparation of (S)-(+)-7-(t-butyldimethylsiloxy)-2-octyn-1-yl-p-toluenesulphonate ((S)-(+)-31) and (R)-(-)-31

(S)- and (R)-31 were prepared exactly as described for racemic 31 and used without further purification. The optical rotations of the enantiomers were too small to measure accurately (<1°).

Preparation of 5-hexynoic acid (32)

Chromium trioxide (20.0 g, 200 mmol) was dissolved in 5 M H_2SO_4 (250 mL, 1.25 mol) and the solution was cooled to 5°. 5-Hexyn-1-ol (9.8 g, 100 mmol) in reagent acetone (100 mL) was added dropwise over 2 h, maintaining the temperature at 5-10°. When addition was complete, the cooling bath was removed and the mixture was stirred for 30 min. The mixture was then concentrated to ≈250 mL under reduced pressure at 20°. The residue was extracted with ether (6 × 100 mL) and the combined ether extracts were in turn extracted with 3 M aqueous NaOH (2 × 150 mL). The combined basic solutions were cooled and acidified with 12 M HCl. The aqueous solution was then extracted with ether (6 × 60 mL) and the combined ether extracts were washed with brine, dried ($MgSO_4$), concentrated in vacuo and distilled, yielding 5-hexynoic acid (32) (7.64 g, 68%), b.p. 73-76° (0.2 mm Hg) as a colourless oil. IR (film) 3500-2500, 3292, 2118, 1705 cm^{-1}; mass spectrum, m/e (relative intensity) 112(0.2), 111(3), 97(13), 94(8), 70(100), 60(37);

^{1}H NMR ($CDCl_3$) δ 1.84 (quintet, 2H, C_3, J=7.1 Hz), 1.99 (t, 1H, C≡CH, J=2.6 Hz), 2.29 (td, 2H, C≡CCH_2, J=7.1, 2.6 Hz), 2.52 (t, 2H, CH_2COOH, J=7.1 Hz), 11.58 (s, 1H, COOH).

Preparation of (R,S)-13-(t-butyldimethylsiloxy)-5,8-tetradecadiynoic acid ((R,S)-33)

Ethyl magnesium bromide (20 mmol) was freshly prepared from Mg turnings (1.0 g, 41 mmol) and ethyl bromide (2.18 g, 20 mmol) in dry THF (≈20 mL) and transferred under argon to a dry flask under argon. The Mg residue was rinsed with dry THF (5 mL) and the rinsings were transferred into the dry flask. The EtMgBr solution was cooled to 5° and a solution of 5-hexynoic acid (1.01 g, 9.0 mmol) in dry THF (5 mL) was added dropwise over 15 min. The solution was warmed to 20° and stirred at 20° for 2 h. The solution was then cooled to 0° and freshly prepared CuBr (57 mg, 0.4 mmol) was added in one portion. The mixture was stirred at 0° for 15 min, followed by the dropwise addition of racemic tosylate 31 (3.28 g, 8.0 mmol) in THF (10 mL). The mixture was warmed to 20° over several hours, stirred at 20° for 16 h and then poured into ice-water (50 mL). The mixture was acidified to pH 3 with 6 M HCl and extracted with ether (3 × 60 mL). The combined ether extracts were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* at 20°, yielding crude racemic 33 (3.3 g, >100%) as a yellow oil. The oil was unstable and gradually decomposed to brown tars, even when stored at -30°. Consequently, the crude material was carried through to the next step without further purification. IR and ^{1}H NMR spectra of the crude material were taken to confirm that the coupling had occurred. IR (film) 3600-2500, 2238, 1713, 1256 cm^{-1}; mass spectrum of methyl ester (CH_2N_2), CI, m/e 365 (M+1); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H,

CH_3Si), 0.88 (s, 9H, $C(CH_3)_3$), 1.13 (d, 3H, CH_3, J=6.1 Hz), 1.48-1.67 (m, 4H, C_{11} and C_{12}), 1.84 (quintet, 2H, C_3, J≈7.0 Hz), 2.20 (m, 2H, C_{10}), 2.29 (m, 2H, C_4), 2.52 (t, 2H, $\underline{CH_2}COOH$, J=7.0 Hz), 3.10 (quintet, 2H, $C{\equiv}C\underline{CH_2}C{\equiv}C$, J=2.1 Hz), 3.82 (m, 1H, C_{13}).

Preparation of (S)-(+)-13-(t-butyldimethylsiloxy)-5,8-tetradecadiynoic acid ((S)-(+)-33) and (R)-(-)-33

(S)- and (R)-33 were prepared exactly as described for racemic 33. Due to the instability of these compounds, the crude products were used directly in the next step, so optical rotations were not measured. The yields of crude material were comparable to that obtained for racemic 33 and the major product in each case was chromatographically identical by TLC or GLC to the racemic material.

Preparation of (R,S)-13-(t-butyldimethylsiloxy)-(5Z,8Z)-tetradecadienoic acid ((R,S)-34)

P-2 nickel (3.0 mmol) was prepared from $Ni(OAc)_2{\cdot}6H_2O$ (0.75 g, 3.0 mmol), 1 M ethanolic $NaBH_4$ solution (3.0 mL, 3.0 mmol) and ethylene diamine (0.54 mL, 8.1 mmol), in 95% EtOH (30 mL) under H_2. Crude racemic acid 33 (2.0 g, ≈4.8 mmol) was added in one portion and the mixture was stirred for 4 h, monitoring the progress of the reaction by GLC of the methyl esters (CH_2N_2). The intermediate enynes were clearly seen. At the end of the 4 h period, the reduction was complete. The mixture was filtered through a 5 mm pad of charcoal and the charcoal was rinsed with a few mL of ethanol. The filtrate was poured into cold brine (100 mL), extracted with ether (3 × 75 mL), dried ($MgSO_4$) and concentrated in vacuo. Last traces of solvent were removed by pumping at high vacuum (0.1 mm Hg)

for 3 h, yielding crude diene (R,S)-34 (2.15 g). This was carried through immediately to the next step to minimize decomposition.

Preparation of (R,S)-13-hydroxy-(5Z,8Z)-tetradecadienoic acid ((R,S)-35)

Crude racemic acid 34 (1.90 g) was stirred at 20° for 16 h in 25 mL of AcOH:H_2O:THF (3:1:1). The solvents were removed *in vacuo* at 20° and the residue was flash chromatographed on silica gel (2.5 cm ID × 20 cm) eluting with hexane:EtOAc:AcOH (140:60:2), yielding hydroxy-acid (R,S)-35 (0.55 g, 53% from tosylate 31) as a viscous oil. IR (solution, $CHCl_3$) 3550-2500, 3020, 1711 cm^{-1}; mass spectrum of methyl ester (CH_2N_2), CI, m/e 255 (M+1); ^1H NMR ($CDCl_3$) δ 1.21 (d, 3H, CH_3, J=6.3 Hz), 1.28-1.53 (m, 4H, C_{11} and C_{12}), 1.71 (quintet, 2H, C_3, J=7.0 Hz), 2.00-2.18 (m, 4H, C_4 and C_{10}, allylic), 2.12 (s, 2H, OH), 2.37 (t, 2H, CH_2COOH, J=7.0 Hz), 2.80 (t, 2H, C_7, bisallylic, J≈6.5 Hz), 3.78-3.89 (m, 1H, C_{13}), 5.30-5.48 (m, 4H, olefin).

Preparation of (S)-(+)-13-hydroxy-(5Z,8Z)-tetradecadienoic acid ((S)-(+)-35) and (R)-(-)-35

(S)- and (R)-35 were prepared exactly as described for racemic 35. The intermediate TBDMS-protected dienes 34 were not isolated. Pure (S)-35 was recovered in 48% yield from tosylate (S)-31, while pure (R)-35 was obtained in 51% yield from (R)-31. Both gave ^1H NMR spectra identical to racemic 35.

Preparation of (R,S)-13-methyl-(5Z,8Z)-tridecadienolide (III)

A solution of hydroxy-acid 35 (0.50 g, 2.08 mmol) and dry triethylamine (distilled from P_2O_5, 3.0 g, 30 mmol) in dry acetonitrile (dried with 3 Å molecular sieve, 200 mL) was added dropwise under argon via a

high-dilution head to a refluxing solution of 2-chloro-1-methylpyridinium iodide (2.5 g, 10 mmol) in dry acetonitrile (200 mL), over a 24 h period. Reflux was continued for an additional 2 h. The mixture was then cooled to 20° and concentrated under reduced pressure. Water (150 mL) was added to the residue and the mixture was extracted with pentane (3 × 100 mL). The combined pentane extracts were washed with water (1× 50 mL), dried ($MgSO_4$), concentrated under reduced pressure and flash chromatographed on silica gel (2.5 cm ID × 17 cm) eluting with hexane:EtOAc (60:1), giving racemic macrolide III (208 mg, 47%). IR (film) 3009, 1731 cm^{-1}; mass spectrum, m/e (relative intensity) 222(8), 180(14), 140(16), 126(11), 106(19), 93(49), 79(100), 67(59), 55(26), 41(36); ^1H NMR ($CDCl_3$) δ 1.24 (d, 3H, CH_3, J=6.1 Hz), 1.33-1.77 (m, 6H, C_3, C_{10}, C_{11}, C_{11}', C_{12}, C_{12}'), 1.83 (m, 1H, C_3'), 1.98 (m, 1H, C_4), 2.20-2.43 (m, 3H, C_4', C_7, C_{10}'), 2.27 (ddd, 1H, C_2, J=14.5, 8.2, 3 Hz), 2.40 (ddd, 1H, C_2', J=14.5, 10, 3 Hz), 3.15 (ddd, C_7', J=15.1, 9.5, 9.5 Hz), 5.03 (dqd, 1H, C_{13}, J=10, 6.1, 2.8 Hz), 5.27 (m, 1H, C_5), 5.40 (m, 3H, C_6, C_8, C_9). Anal. calcd. for $C_{14}H_{22}O_2$: C, 75.65; H, 9.98. Found: C, 75.88; H, 9.97.

Preparation of (S)-(+)-13-methyl-(5Z,8Z)-tridecadienolide ((S)-(+)-III) and (R)-(-)-III

(S)-(+)-III was prepared in 37% yield, following the cyclization procedure for racemic III. $[\alpha]_D^{32}$ = +41.8° (c, 0.958, $CHCl_3$).

(R)-(-)-III was similarly prepared in 33% yield. $[\alpha]_D^{34}$ = -43.4° (c, 0.737, $CHCl_3$).

Synthesis of Macrolide IV

Preparation of 2-heptyn-1-ol (37)

A solution of 1-hexyne (12.3 g, 150 mmol) in 300 mL dry THF was cooled to -10° under argon. n-BuLi in hexane (2.3 M, 65.2 mL, 150 mmol) was added dropwise over 20 min, maintaining the temperature below 0°. When the addition was complete, the solution was stirred for a further 15 min at 0° before adding in one portion dry paraformaldehyde (4.8 g, 160 mmol). The resulting suspension was slowly warmed to 20°, stirred for 12 h and poured into ice-water (200 mL). The mixture was extracted with ether (3 × 100 mL) and the combined extracts were washed with brine, dried ($MgSO_4$), concentrated under reduced pressure and distilled, yielding 37 (15.99 g, 95%), b.p. 64-66° (2.2 mm Hg; lit. 95° at 22 mm Hg). IR (film) 3330, 2220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3H, C_7, J=7.1 Hz), 1.30-1.52 (m, 4H, C_5-C_6), 1.63 (t, 1H, OH, J=6 Hz), 2.20 (tt, 2H, C_4, J=7, 2.1 Hz), 4.23 (dt, 2H, C_1, J=6, 2.1 Hz). Mass spectrum, m/e (relative intensity) 112(12), 97(15), 83(100), 79(66), 70(81), 55(73). Anal. calcd. for $C_7H_{12}O$: C, 74.96; H, 10.78. Found: C, 74.69; H, 10.42.

Preparation of 6-heptyn-1-ol (38)

A suspension of potassium hydride in mineral oil (25% w/w, 67.4 g, 420 mmol) was washed free of oil with dry THF (3 × 50 mL). The resulting powder was pumped under high vacuum for 1 h to remove all traces of THF, then covered with argon. Dry 1,3-diaminopropane (350 mL, distilled off BaO) was then added in one portion and the foamy suspension was stirred vigorously under argon at 20° for 2 h. Alkynol 37 (15.68 g, 140 mmol) was then added dropwise over 5 min and the mixture was stirred for 30 min. The

suspension was then cooled to 0°, the reaction was cautiously quenched with water (20 mL) and the mixture was poured onto ice (200 g). The resulting aqueous mixture was extracted with ether (4 × 125 mL) and the combined ether extracts were washed with 6 M HCl (50 mL) and brine. Concentration under reduced pressure and distillation gave 38 (7.19 g, 46%), b.p. 60-65° (2.2 mm Hg). IR (film) 3320, 3300, 2120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (s, 1H, OH), 1.43-1.67 (m, 6H, C_2-C_4), 1.95 (t, 1H, C_7, J=2.5 Hz), 2.22 (td, 2H, C_5, J=7, 2.5 Hz), 3.65 (t, 2H, C_1, J=6.4 Hz). Mass spectrum, m/e (relative intensity) 112(0.6), 97(7), 79(100), 77(33), 70(53), 66(33). Anal. calcd. for $C_7H_{12}O$: C, 74.96, H, 10.73. Found: C, 75.09; H, 11.00.

Preparation of 7-(2-methoxyethoxymethoxy)-1-heptyne (39)

A solution of alkynol 38 (6.72 g, 60 mmol) and diisopropylethylamine (11.6 g, 90 mmol) in CH_2Cl_2 (75 mL) was cooled to 0°. MEM chloride (11.2 g, 90 mmol) was added dropwise over 15 min and the resulting solution was warmed to 20° and stirred for 10 h. The solution was poured into water (100 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined CH_2Cl_2 extracts were washed with 1 M HCl (25 mL) and brine, dried ($MgSO_4$), concentrated under reduced pressure and distilled, to yield 39 (11.76 g, 98%), b.p. 70-73° (0.2 mm Hg). IR (film) 3295, 2118 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45-1.67 (m, 6H, C_4-C_6), 1.95 (t, 1H, C_1, J=2.5 Hz), 2.21 (tt, 2H, C_3, J=7, 2.5 Hz), 3.40 (s, 3H, $-OCH_3$), 3.57 (t, 2H, C_7, J=6.3 Hz), 3.58 (m, 2H, OCH_2CH_2O), 3.70 (m, 2H, OCH_2CH_2O), 5.72 (s, 2H, OCH_2O). Mass spectrum, CI, m/e 201 (M+1).

Preparation of 4-(t-butyldimethylsiloxy)-1-butyne (41a)

t-Butyldimethylsilyl chloride (19.63 g, 130 mmol) was added in one

portion to a solution of 3-butyn-1-ol (8.4 g, 120 mmol) and imidazole (18.0 g, 260 mmol) in dry DMF (30 mL) at 0°. After stirring at 0° for 15 min, the mixture was allowed to warm to 20° and stirred 12 h. Water (100 mL) was added and the mixture was extracted with ether (3 × 50 mL). The combined ether extracts were washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. Distillation of the residue gave 41a (20.69 g, 94%), b.p. 45-46° (2.5 mm Hg). IR (film) 3318, 2124, 1258 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.08 (s, 6H, CH_3-Si), 0.92 (s, 9H, $C(CH_3)_3$), 1.98 (t, 1H, C_1, J=2.5 Hz), 2.42 (tt, 2H, C_3, J=7, 2.5 Hz), 3.76 (t, 2H, C_4, J=7 Hz). Mass spectrum (CI) m/e 185 (M+1). Anal. calcd. for $C_{10}H_{20}OSi$: C, 65.15; H, 1.094. Found: C, 65.16; H, 11.10.

Preparation of 5-(t-butyldimethylsiloxy)-2-pentyn-1-ol (42a)

2.3 M n-BuLi in hexane (48 mL, 110 mmol) was added dropwise to a solution of alkyne 41a (20.03 g, 109 mmol) in dry THF (250 mL) at -10°. The solution was stirred for 20 min at 0°, then cooled to -10° and dry paraformaldehyde (3.6 g, 120 mmol) was added. The suspension was allowed to warm to 20° and was stirred 12 h. The reaction mixture was then poured into ice-water (200 mL) and extracted with ether (4 × 150 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$), concentrated in vacuo and distilled, yielding 42a (19.31, 83%), b.p. 80-85° (0.15 mm Hg). IR (film) 3340, 2225, 1258 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.07 (s, 6H, Si-CH_3), 0.40 (s, 9H, $C(CH_3)_3$), 1.52 (t, 1H, OH, J=6 Hz), 2.43 (tt, 2H, C_4, J=7.1, 2.1 Hz), 3.73 (t, 2H, C_5, J=7.1 Hz), 4.15 (dt, 2H, C_1, J=6, 2.1 Hz). Mass spectrum, CI, m/e 215 (M+1).

Preparation of 5-(t-butyldimethylsiloxy)-2-pentyn-1-yl p-toluenesulphonate (43a)

Powdered KOH (40 g, 710 mmol) was added in portions to a vigorously stirred solution of alcohol 42a (13.91 g, 65 mmol) and p-toluenesulphonyl chloride (13.6 g, 71.5 mmol) in dry ether (200 mL) at -10°. The mixture was stirred at 0° for 45 min and poured into ice-water (200 mL). The ether was decanted and the aqueous residue was extracted with ether (2 × 100 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo without heating. Final traces of solvent were removed by pumping under vacuum (0.1 mm Hg) for 4 h, giving a quantitative yield of the tosylate 43a (one spot on TLC, hexane:EtOAc, 4:1). IR (film) 2242, 1600, 1340, 1256 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.04 (s, 6H, CH_3-Si), 0.83 (s, 9H, $C(CH_3)_3$), 2.31 (tt, 2H, C_4, J=7.1, 2.2 Hz), 2.45 (s, 3H, $-CH_3$), 3.60 (t, 2H, C_5, J=7.1 Hz), 4.68 (t, 2H, C_1, J=2.2 Hz), 7.35 (d, 2H, tosyl, J=8), 7.80 (d, 2H, tosyl, J=8 Hz). Anal. calcd. for $C_{18}H_{28}O_4SSi$: C, 58.66; H, 7.66. Found: C, 58.70; H, 7.72.

Preparation of 12-(2-methoxyethoxymethoxy)-1-(t-butyldimethylsiloxy)-3,6-dodecadiyne (44)

Ethyl magnesium bromide (≈65 mmol) in THF was prepared from ethyl bromide (7.085 g, 65 mmol) and magnesium turnings (3.0 g, 125 mmol) and was transferred into a dry flask under argon. Alkyne 39 (11.5 g, 57.5 mmol) in dry THF (25 mL) was added dropwise over 15 min, maintaining the temperature <30°. The solution was stirred for 2.5 h at 20° and then cooled to 0°. Cuprous bromide (0.75 g, 5.2 mmol) was added and the mixture was stirred for 15 min. A solution of tosylate 43a (24 g, 65 mmol) in dry THF (50 mL)

was then added dropwise over 1 h. The resulting mixture was warmed to 20° over 3 h, stirred an additional 12 h at 20° and poured into NH_4Cl solution (10 g NH_4Cl in 150 mL H_2O). The mixture was extracted with ether (3 × 100 mL) and the combined ether extracts were washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. One gram of the crude material was removed for analysis, while the remainder was carried through directly to the next step. IR (film) 2234, 1255, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.07 (s, 6H, CH_3-Si), 0.91 (s, 9H, $(CH_3)_3C$), 1.38-1.68 (m, 6H, C_9-C_{11}), 2.16 (tt, 2H, C_8, J=7, 2.4 Hz), 2.49 (tt, 2H, C_2, J=7.4 × 2.4 Hz), 3.11 (quint, 2H, C_5, J=2.4 Hz), 3.40 (s, 3H, CH_3O), 3.53-3.60 (m, 4H, OCH_2CH_2O + C_{12}), 3.68-3.75 (m, 4H, OCH_2CH_2 + C_1), 4.72 (s, 2H, OCH_2O). Mass spectrum, CI, m/e 397 (M+1).

Preparation of 12-(2-methoxyethoxymethoxy)-3,6-dodecadiyn-1-ol (45)

Crude alkyne 44 was dissolved in dry MeOH (200 mL) and *p*-toluenesulphonic acid (200 mg) was added. The solution was stirred for 2 h at 20°, then concentrated *in vacuo* without heating. The residue was dissolved in ether (125 mL), extracted with saturated $NaHCO_3$ (25 mL), washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was flash chromatographed in three portions on silica gel (5 cm ID × 20 cm) eluting with hexane:EtOAc (1:3), to yield 45 (8.3 g, 54% from 43a). IR (film) 3420, 2218 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34-1.68 (m, 7H, C_9-C_{11} + OH), 2.17 (tt, 2H, C_8, J=7, 2.5 Hz), 2.45 (tt, 2H, C_2, J=6.1, 2.5 Hz), 3.13 (quintet, 2H, C_5, J=2.5 Hz), 3.40 (s, 2H, OCH_3), 3.48-3.65 (m, 4H, OCH_2CH_2O + C_{12}), 3.65-3.77 (m, 4H, OCH_2CH_2O + C_1), 4.72 (s, 2H, OCH_2O). Mass spectrum, CI, m/e 283 (M+1).

Preparation of 12-(2-methoxyethoxymethoxy)-dodecadiynoic acid (46)

A solution of alcohol 45 (2.20 g, 7.8 mmol) in reagent acetone (100 mL) was added dropwise over 4 h to a solution of CrO_3 (3.12 g, 31.2 mmol) in 1.5 M H_2SO_4 (50 mL, 75 mmol) at 0°. When the addition was complete, the mixture was allowed to warm to 20° and was stirred an additional 2 h. Ether (100 mL) was added and the mixture was extracted with brine (3 × 75 mL). The ethereal solution was then concentrated in vacuo. The residue was taken up in ether (75 mL) and extracted with 0.5 M $NaHCO_3$ (3 × 30 mL). The combined basic extracts were acidified with 6 M HCl to pH 2 and extracted with ether (3 × 50 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo, yielding crude 46 (1.59 g, 69% crude yield). An analytical sample was withdrawn and the remainder was submitted directly to the next step. IR (film) 3600-2500, 2239, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43-1.68 (m, 7H, C_9-C_{11} + OH), 2.18 (tt, 2H, C_8, J=6.6, 2.4 Hz), 3.16 (quintet, 2H, C_5, J=2.4 Hz), 3.30 (t, 2H, C_2, J=2.4 Hz), 3.42 (s, 3H, OCH_3), 3.58 (t, 2H, C_{12}, J=6.5 Hz), 3.53-3.63 (m, 2H, OCH_2CH_2O), 3.66-3.75 (m, 2H, OCH_2CH_2O), 4.73 (s, 2H, OCH_2O).

Preparation of 12-hydroxy-3,6-dodecadiynoic acid (47)

Crude acid 46 (1.50 g, 5.07 mmol) was stirred for 24 h at 20° in 27.5 mL of THF:H_2O:conc. HCl (8:2:1). Ether (100 mL) was added and the mixture was extracted with brine (2 × 50 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was flash chromatographed on silica gel (2.5 cm ID × 20 cm) eluting with hexane:EtOAc:AcOH (25:75:1), yielding 47 (675 mg, 64%) as a viscous oil, which crystallized on refrigeration. IR

(film) 3600-2400, 2240, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38-1.78 (m, 8H, C_9-C_{11} + 2OH), 2.16-2.27 (m, 2H, C_8), 3.15 (quintet, 2H, C_5, J=2.4 Hz), 3.29 (t, 2H, C_2, J=2.4 Hz), 3.70 (t, 2H, C_{12}, J=6 Hz). Mass spectrum of methyl ester (CH_2N_2), CI, m/e 223 (M+1).

Preparation of 12-hydroxy-(3Z,6Z)-dodecadienoic acid (48)

P-2 nickel (1 mmol) was prepared in 95% EtOH (20 mL) from $Ni(OAc)_2 \cdot 6H_2O$ (0.25 g, 1 mmol), 1 M $NaBH_4$ solution (1 mL, 1 mmol) and ethylene diamine (0.2 mL) by the standard procedure. Diyne 47 (450 mg, 2.16 mmol) was added in EtOH (10 mL) and the mixture was stirred under H_2 for 5 h. The mixture was then filtered with suction through a 0.5 cm charcoal pad and the charcoal was rinsed several times with EtOH. The filtrate was concentrated *in vacuo* and the residue was dissolved in water (20 mL) and carefully acidified to pH 3 with 3 M HCl. The aqueous mixture was then extracted with ether (4 × 25 mL) and the combined ether extracts were washed with brine, dried ($MgSO_4$) and evaporated. The resulting yellow oil was used without further purification. IR (film) 3600-2500, 3010, 1711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (m, 4H, C_9-C_{10}), 2.11 (s, 2H, OH), 1.53-1.65 (m, 2H, C_{11}), 2.02-2.11 (m, 2H, C_8), 2.83 (t, 2H, c_5, J=6.4 Hz), 3.16 (d, 2H, C_2, J=6.4 Hz), 3.67 (t, 2H, C_{12}, J-6.8 Hz), 6.34-5.46 and 5.51-5.70 (m, 4H, olefins). Mass spectrum of methyl ester (CH_2N_2), CI, m/e 227 (M+1).

Preparation of (3Z,6Z)-dodecadienolide (IV)

A solution of hydroxy-acid 48 (250 mg, 1.18 mmol) and dry triethylamine (1.19 g, 11.8 mmol) in dry acetonitrile (150 mL) was added over 6 h via a fixed rate dropping funnel and a high dilution head to a refluxing

solution of 2-chloro-1-methylpyridinium iodide (1.22 g, 5 mmol) in acetonitrile (200 mL). Reflux was continued a further 2 h. The cooled solution was concentrated under reduced pressure. Water (50 mL) was added to the residue and the mixture was extracted with ether (3 × 50 mL). The combined ether extracts were washed with water and brine, dried ($MgSO_4$), concentrated in vacuo and flash chromatographed on silica gel (2.5 cm ID × 20 cm) eluting with hexane:EtOAc (40:1). Macrolide IV was obtained in 27% yield, or 2.8%, from 1-hexyne. IR (film) 3010, 1735 cm^{-1}; ^1H NMr ($CDCl_3$) δ 1.34 (m, 2H, C_9), 1.46 (m, 2H, C_{10}), 1.71 (m, 2H, C_{11}), 2.05 (quartet, 2H, C_8, J=7.5 Hz), 2.91 (dd, 2H, C_5, J≈7.7 Hz), 3.09 (d, 2H, C_2, J=7.75 Hz), 4.11 (m, 2H, C_{12}), 5.43-5.58 (m, 4H, C_3, C_4, C_6, C_8). Mass spectrum, m/e (relative intensity) 194(9), 176(3), 93(44), 91(41), 80(41), 79(100), 67(47). Anal. calcd. for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.47; H, 9.46.

Synthesis of Macrolide V

Preparation of 6-[(tetrahydro-2H-pyran-2-yl)oxy]-1-heptyne (28b)

Alcohol 9 (7.7 g, 68.75 mmol) was dissolved in 75 mL dry ether and cooled to 0°. A few crystals of p-toluenesulphonic acid were added, followed by dropwise addition of 6.35 g (75.6 mmol) of dihydropyran. The solution was warmed to 20°, stirred for 18 h and then extracted with saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL). The solution was dried ($MgSO_4$), concentrated in vacuo and distilled, yielding 12.96 g of 28b (96%), b.p. 60-65° (0.25 mm Hg). IR (film) 3300, 2120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13, 1.25 (d, 3H, C_7, J=6.5 Hz), 1.47-1.88 (m, 10H, C_5, THP), 1.93, 1.95 (d, 1H, C_1, J=2.5 Hz), 2.15-2.27 (m, 2H, C_3), 3.45-3.53 (m, 1H, C_6), 3.72-

3.96 (m, 2H, THP), 4.64, 4.70 (d, 1H, THP, J=6.5 Hz). Mass spectrum (CI methane) 197 (M+1). Anal. calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27. Found: C, 73.80; H, 10.48.

Preparation of 4-[(tetrahydro-2H-pyran-2-yl)oxy]-1-butyne (41b)

3-Butyn-1-ol (40, 7.0 g, 100 mmol) was dissolved in 50 mL dry ether and the solution was cooled to 0°. A few crystals of p-toluenesulphonic acid were added, followed by dropwise addition of 9.24 g (110 mmol) of dihydropyran. The resulting solution was warmed to 20°, stirred for 16 h and extracted with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The solution was dried ($MgSO_4$), concentrated in vacuo, and distilled to give 41b (14.78 g, 96%), b.p. 62-65° (2.5 mm Hg). IR (film) 3297, 2120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47-1.89 (m, 6H, THP), 1.98 (t, 1H, C_1, J=2.5 Hz), 2.51 (td, 2H, C_3, J=7.1, 2.5 Hz), 3.58, 3.87 (td, 2H, C_4, J=8.5, 7.0 Hz), 3.47-3.55, 3.84-3.93 (m, 2H, THP), 4.65 (t, 1H, THP, J=6.5 Hz). Mass spectrum (CI methane) 155 (M+1). Anal. calcd. for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 69.93; H, 9.37.

Preparation of 5-[(tetrahydro-2H-pyran-2-yl)oxy]-2-pentyn-1-ol (42b)

A solution of alkyne 41b (15.4 g, 100 mmol) in dry THF (250 mL) was cooled to -10° under argon and 48 mL of 2.1 M n-BuLi in hexane (202 mmol) was added dropwise, maintaining the temperature below 0°. The solution was stirred for 20 min at 0°, and then powdered dry paraformaldehyde (3.75 g, 125 mmol of CH2O) was added in one portion. The mixture was warmed to 20° over 2 h, stirred for 16 h, poured into ice-water (100 mL) and extracted with ether (3 × 100 mL). The combined ether extracts were washed with brine (50 mL), dried ($MgSO_4$) and concentrated in vacuo. Vacuum

distillation yielded a forerun of 2.82 g of alkyne 41b, followed by 9.93 g of 41b (66% based on recovered starting material), b.p. 125-128° (0.1 mm Hg). IR (film) 3600-3100, 2225; ^{1}H NMR ($CDCl_3$) δ 1.47-1.89 (m, 6H, THP), 1.57 (t, 1H, OH, J=6.0 Hz), 2.51 (tt, 2H, C_4, J=7.1, 2.25 Hz), 3.58, 3.87 (td, 2H, C_5, J=8.5, 7.0 Hz), 3.47-3.55, 3.84-3.93 (m, 2H, THP), 4.25 (td, 2H, C_1, J=6.0, 2.25 Hz), 4.65 (t, 1H, THP, J=6.5 Hz). Mass spectrum (CI methane) 185(M+1). Anal. calcd. for $C_{10}H_{16}O_3$: C, 65.20; H, 8.75. Found: C, 65.48; H, 9.03.

Preparation of 5-[(tetrahydro-2H-pyran-2-yl)oxy]-2-pentyn-1-yl p-toluenesulphonate (43b)

A solution of alcohol 42b (6.17 g, 33.5 mmol) and p-toluenesulphonyl chloride (7.36 g, 38.5 mmol) in dry ether (80 mL) was cooled to -10°. Powdered KOH (25 g, 446 mmol) was added in 5 g portions over 20 min. The resulting suspension was stirred at 0° for 45 min, poured into ice-water (200 mL) and extracted with ether (3 × 100 mL). The combined ether solutions were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Final traces of solvent were removed by pumping for 6 h at 0.1 mm Hg, yielding 11.23 g of 43b (99%). This was used without further purification. IR (film) 2225, 1599, 1367, 1178 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.47-1.85 (m, 6H, THP), 2.40 (tt, 2H, C_4, J=7.0, 2.1 Hz), 2.45 (s, 3H, $ArCH_3$), 3.42 (td, 1H, C_5, J=9.75, 7.0 Hz), 3.45-3.53 (m, 1H, THP), 3.70 (td, 1H, C_5, J=9.75, 7.0 Hz), 3.80-3.88 (m, 2H, THP), 4.58 (m, 1H, THP), 4.68 (t, 2H, C_1, J=2.1 Hz), 7.80 (d, 2H, Ar, J=8.5 Hz), 7.33 (d, 2H, Ar, J=8.5 Hz). Mass spectrum (CI, methane) 339(M+1).

Preparation of 3,6-dodecadiyn-1,11-diol (50)

A solution of EtMgBr (38 mmol) in ≈40 mL dry THF was prepared from ethyl bromide (4.17 g, 38 mmol) and magnesium turnings (2.0 g, 82 mmol) and transferred under argon to a dry flask. Alkyne 28b (6.86 g, 35 mmol) in dry THF (20 mL) was added dropwise over 30 min, during which time the temperature rose to 30° and H_2 was evolved. The solution was stirred at 20-30° for 2 h, cooled to 0° and CuBr (400 mg, 2.8 mmol) was added. The resulting suspension was stirred for 15 min, and tosylate 43b (11.5 g, 34 mmol) in dry THF (20 mL) was added dropwise over 20 min. The mixture was warmed to 20° over 2 h, stirred for 16 h and poured into water (100 mL) containing 10 g NH_4Cl. The resulting mixture was extracted with ether (3 × 75 mL) and the combined ether extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to 12.44 g of yellow oil. 100 mg were removed for analysis and a solution of a few crystals of *p*-toluenesulphonic acid in dry MeOH (75 mL) was added to the remainder. The resulting solution was stirred at 20° for 2 h, then concentrated *in vacuo* at 0°. The residue was taken up in ether (100 mL), washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. Final purification by flash chromatography (hexane:EtOAc, 2:3) yielded 4.36 g of 50 (66%) as a yellow oil, which rapidly darkened on standing. IR (film) 3700-3050, 2220, 1045 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (t, 3H, C_{12}, J=6.0 Hz), 1.50-1.68 (m, 6H, OH, C_9, C_{10}), 2.15-2.23 (m, 2H, C_8), 2.45 (tt, 2H, C_2, J=6.0, 2.2 Hz), 3.15 (quint, 2H, C_5, J=2.2 Hz), 3.72 (t, 2H, C_1, J=6.0 Hz), 4.86 (m, 1H, C_{11}). Mass spectrum, m/e (relative intensity) 194(0.1), 103(44), 91(100), 77(51), 71(44).

Preparation of 11-oxo-3,6-dodecadiynoic acid (51)

Chromium trioxide (12.0 g, 120 mmol) was dissolved in 120 mL of 3.5 M H_2SO_4 (420 mmol) and cooled to -5°. A solution of diol 50 (4.30 g, 22.2 mmol) in acetone (250 mL) was added dropwise over 4 h. When the addition was complete, the mixture was warmed to 20° over 45 min, then poured into water (400 mL) and extracted with ether (4 × 150 mL). The combined ether extracts were washed with brine (3 × 50 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was flash chromatographed (hexane: EtOAc:AcOH, 75:75:1), yielding 1.83 g (40%) of a highly unstable oil. IR (film) 3700-2400, 2240, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.79 (quint, 2H, C_9, J=6.75 Hz), 2.12 (s, 1H, COOH), 2.17 (s, 3H, C_{12}), 2.23 (tt, 2H, C_8, J=6.75, 2.2 Hz), 2.58 (t, 2H, C_{10}, J=6.75 Hz), 3.18 (quint, 2H, C_5, J=2.2 Hz), 3.38 (t, 2H, C_2, J=2.2 Hz).

Preparation of 11-hydroxy-3,6-dodecadiynoic acid (52)

Keto-acid 51 (1.8 g, 8.7 mmol) was dissolved in 25 mL absolute EtOH, and cooled to -20°. $NaBH_4$ (0.456 g, 12 mmol) was added in one portion and the solution was warmed to 0° over 15 min, then cooled to -20° again. The reaction mixture was then slowly acidified with 3.5 N HCl, brine (25 mL) was added and the solution was extracted with ether (4 × 25 mL). The combined ether extracts were washed with brine (2 × 20 mL), dried ($MgSO_4$) and concentrated in vacuo to give an orange oil, which rapidly darkened in colour. This was used immediately, without purification, in the next step. An analytical sample of ≈50 mg was removed. IR (film) 3650-2400, 2235, 1710 cm^{-1}; ^1H NMR ($CDCl_3$), δ 1.22 (d, 3H, C_{12}, J=6.3 Hz), 1.48-1.72 (m, 4H, C_9, C_{10}), 2.10 (s, 2H, COOH, OH), 2.15-2.23 (m, 2H, C_8), 3.15

(quint, 2H, C_5, J=2.25 Hz), 3.32 (t, 2H, C_2, J=2.25 Hz), 3.87 (m, 1H, C_{11}). Mass spectrum of methyl ester (CH_2N_2), m/e (relative intensity) 222(0.2), 203(3), 129(45), 105(45), 91(100), 77(51), 45(35).

Preparation of 11-hydroxy-(3Z,6Z)-dodecadienoic acid (53)

4 mmol of P-2 nickel was made by the standard procedure in 95% EtOH (30 mL). The crude acid 52 was added and the mixture was stirred under H_2 for 5 h. The solution was then filtered with suction through 5 mm of activated charcoal, rinsing several times with EtOH. The filtrate was concentrated in vacuo at 20°. Ether (20 mL) was added and the solution was acidified to pH 3 with 2 M HCl. The ether layer was separated and the aqueous portion was extracted with ether (3 × 20 mL). The combined ether extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo, to yield 1.53 g of crude product. Flash chromatography (hexane:EtOAc:AcOH, 75:75:1) yielded 0.66 g of 53 (≈35% from 43b). IR (film) 3650-2400, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (d, 3H, C_{12}, J=6.3 Hz), 1.37-1.52 (m, 4H, C_9, C_{10}), 2.00-2.15 (m, 2H, C_8), 2.82 (t, 2H, C_5, J=6.0 Hz), 3.17 (m, 2H, C_2), 5.32-5.48 (m, 2H, C_6, C_7) 5.53-5.65 (m, 2H, C_3, C_4). Mass spectrum of methyl ester (CH_2N_2), m/e (relative intensity) 92(68), 91(69), 79(100), 74(68).

Preparation of 11-methyl-(3Z,6Z)-undecadienolide (V)

A solution of hydroxy-acid 53 (0.64 g, 3.1 mmol) and dry triethylamine (2.42 g, 24 mmol) in dry acetonitrile (200 mL) was added over 20 h via a fixed-rate addition funnel and a high dilution head to a refluxing solution of 2-chloro-1-methylpyridinium iodide (3.18 g, 12.4 mmol) in dry acetonitrile (200 mL), under argon. The resulting solution was refluxed a

further 2 h, cooled and concentrated *in vacuo*. The residue was taken up in pentane (50 mL) and washed with water (50 mL). The aqueous phase was extracted twice more with pentane (25 mL) and the combined pentane extracts were washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was flash chromatographed (hexane:EtOAc, 40:1), yielding 45 mg (7.5%) of macrolide V. IR (film) 3010, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (t, 3H, C_{12}, J=6.25 Hz), 1.33 (m, 1H, C_9), 1.51 (m, 1H, C_9), 1.67 (m, 1H, C_{10}), 1.76 (m, 1H, C_{10}), 2.05 (ddt, 1H, C_8, J=13.75, 10.5, 9, 6.5 Hz), 2.44 (dddd, 1H, C_8, J=13.75, 6.5, 6.5, 6.5 Hz), 2.62 (dddd, 1H, C_5, J=13.75, 6.75, 6.75 Hz), 2.95 (dd, 1H, C_2, J=13.0, 7.5 Hz), 3.16 (dt, 1H, C_5, J=13.75, 9.9 Hz), 3.21 (dd, 1H, C_2, J=13.0, 9.5 Hz), 4.97 (dd, quart, 1H, C_{11}, J=7.5, 6.25, 3 Hz), 5.29 (ddd, 1H, C_7, J=10.5, 6.5, 10.5 Hz), 5.49 (ddd, 2H, C_3, C_6, J=10.5, 9, 6.75 Hz), 5.63 (ddd, 1H, C_4, J=10.5, 9, 6.75 Hz). Mass spectrum, m/e (relative intensity) 194(3), 186(4), 91(42), 79(100), 77(39), 67(36). High resolution mass spectrum, calcd. for $C_{12}H_{18}O_2$: 194.1306; obsd.: 194.1306.

V. RESULTS AND DISCUSSION

A. Extracts of Insect Volatiles

Crude Extracts of Insect Volatiles

During the initial stages of isolation and identification of pheromone components from both C. pusillus and C. turcicus, insects were aerated with no food or other material available. This was done to provide volatile extracts wherein only beetle-produced compounds were present. It was found that the rate of production of pheromone components, as evidenced by the absolute amounts of pheromone produced per beetle-hour, varied for different aerations by as much as an order of magnitude. This was probably due to variations in the age of the beetles, as has been clearly demonstrated for related species (Pierce et al., 1983). When bioassays were conducted to test isolated components versus their synthetic counterparts, the solutions of natural material were calibrated by GLC against solutions of synthetic material of known concentrations, ensuring that the same absolute amounts of material were bioassayed against each other.

Comparison of frass volatiles with beetle volatile extracts (Fig. 1 vs. Fig. 2, Fig. 9 vs. Fig. 10) showed the same macrolide components to be present in each, for both C. pusillus or C. turcicus. The frass extracts also contained numerous other compounds, which is to be expected as frass is a mixture of food particles and insect feces.

When either species was aerated on oats, the rate of pheromone production increased by at least an order of magnitude (Figs. 3, 11). In both cases, the pheromone components comprised more than sixty percent of the total extract. This supports the hypothesis that the pheromones are truly

aggregation pheromones, released to attract other members of the species to a good food source. It also suggests that the insects need a food source to produce pheromones, as in many bark beetles (Borden, 1983).

Volatiles from Sexed C. pusillus

The crude volatile extracts from male and female insects were examined by GLC/MS on a SP-1000 capillary column (Table I). It was found that the males produced copious quantities of macrolide I (75% of total extract) and macrolide II (4.8% of total extract). In addition, a trace amount of an isomer of macrolide I, with a considerably shorter retention time, was detected.

The extract of female volatiles showed only a trace amount of I and no II. The amount of I detected was so small, in comparison to the males' extract, that it could have been due to a few mistakenly-sexed females or to traces of I adsorbed onto the females' exoskeleton.

Macrolide IV was not detected in either the male's or female's volatiles, although a careful search was made with selected ion monitoring. The reason for its absence is not known.

The experiment conclusively proved that both macrolides I and II are male-produced. This result confirms previous work by Quaife (1981), who determined that only male volatile extracts were attractive to either sex.

Volatiles from Sexed C. turcicus

GC/MS analysis of crude volatile extracts from male and female insects were analyzed on a SP-1000 capillary column (Table I). The extract of females' volatiles showed no trace of macrolide II or III, even with selected

ion monitoring. The extract of male volatiles, however, contained small but definite amounts of II and III, thus indicating again that the pheromones are male-produced. It is not known why C. turcicus males produced so much less pheromone than the closely related C. pusillus.

B. Bioassay Results

The bioassays for both C. pusillus and C. turcicus caused many problems, as the insects had to be "conditioned" before reasonably consistent and reproducible results could be obtained. It was found by trial and error that to obtain the best responses to stimuli, insects should be approximately 6-10 weeks old and raised in a low-density culture (<1500 insects/kg medium) (A.M. Pierce[4], pers. comm.). Insects starved for 48 h in the dark responded better than those starved for only 24 h. In addition, if insect-produced volatiles were removed by flushing air through the flask in which the insects were being starved, the response improved considerably (A.M. Pierce[4], pers. comm.).

Thus, in the preliminary bioassays on crude extracts, the overall response obtained was rather low, but the difference in responses to stimuli and solvent controls was still highly significant.

Response of C. pusillus to Beetle-Produced Volatiles

Previous experiments (Quaife, 1981) had demonstrated the production of and aggregation in response to a C. pusillus pheromone. Preliminary arena olfactometer bioassays confirmed that there was a definite positive response to pentane extracts of beetle volatiles (Table IV). The response at 27 bh is comparable to threshold sensitivites for other insects (Borden et al., 1979; Wong et al., 1983), and indicates a potent pheromone in C.

Table IV. Response of C. pusillus of mixed sex and age to pentane extracts of beetle volatiles in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 1

Experimental Stimulus	Stimulus Dose	% Response†
Pentane	25 µL	11.0a
Crude volatile extract	27 bh	43.0b
	300 "	78.0c
	3,000 "	92.0c
	13,200 "	87.0c
	26,400 "	93.0c

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

pusillus. A very high response was still obtained at a concentration three orders of magnitude larger.

Four preparative GLC fractions of the crude volatiles extract, consisting of 1) all compounds eluting before macrolide I (forerun), 2) macrolide I (I), 3) macrolide II (II), and 4) all compounds eluting after II (afterrun) were bioassayed in the pitfall olfactometer. Only I exhibited attraction comparable to the crude extract, while the forerun appeared to be repellent (Fig. 15). When the possibly attractive fractions were bioassayed with the arena olfactometer (Table V), I was confirmed to be highly attractive, and again was comparable to the unfractionated extract. The afterrun and II were also significantly attractive compared to the solvent control.

Thus, most of the activity in the crude extract was due to I, its major component. To investigate possible synergism, combinations of II with the other fractions were tested at a low stimulus concentration, so that any enhancement of response to I could be clearly seen. The combination of I and II produced the highest response, which was not, however, significantly higher than to I alone (Table VI).

The bioassays thus far were independently corroborated by A.V. Barak[5] (pers. comm.), who confirmed that I was highly attractive to beetles of both sexes, using a suspended disc bioassay. In addition, the forerun, II, and afterrun fractions were found by him to be inactive when tested alone.

Bioassay of Synthesized C. pusillus Macrolides

The first bioassays of synthetic I were done by A.V. Barak[5], and demonstrated that synthetic I was indeed highly attractive to both sexes of

Figure 15

Response of C. pusillus to preparative GLC fractions of C. pusillus volatiles in the two-choice pitfall bioassay.

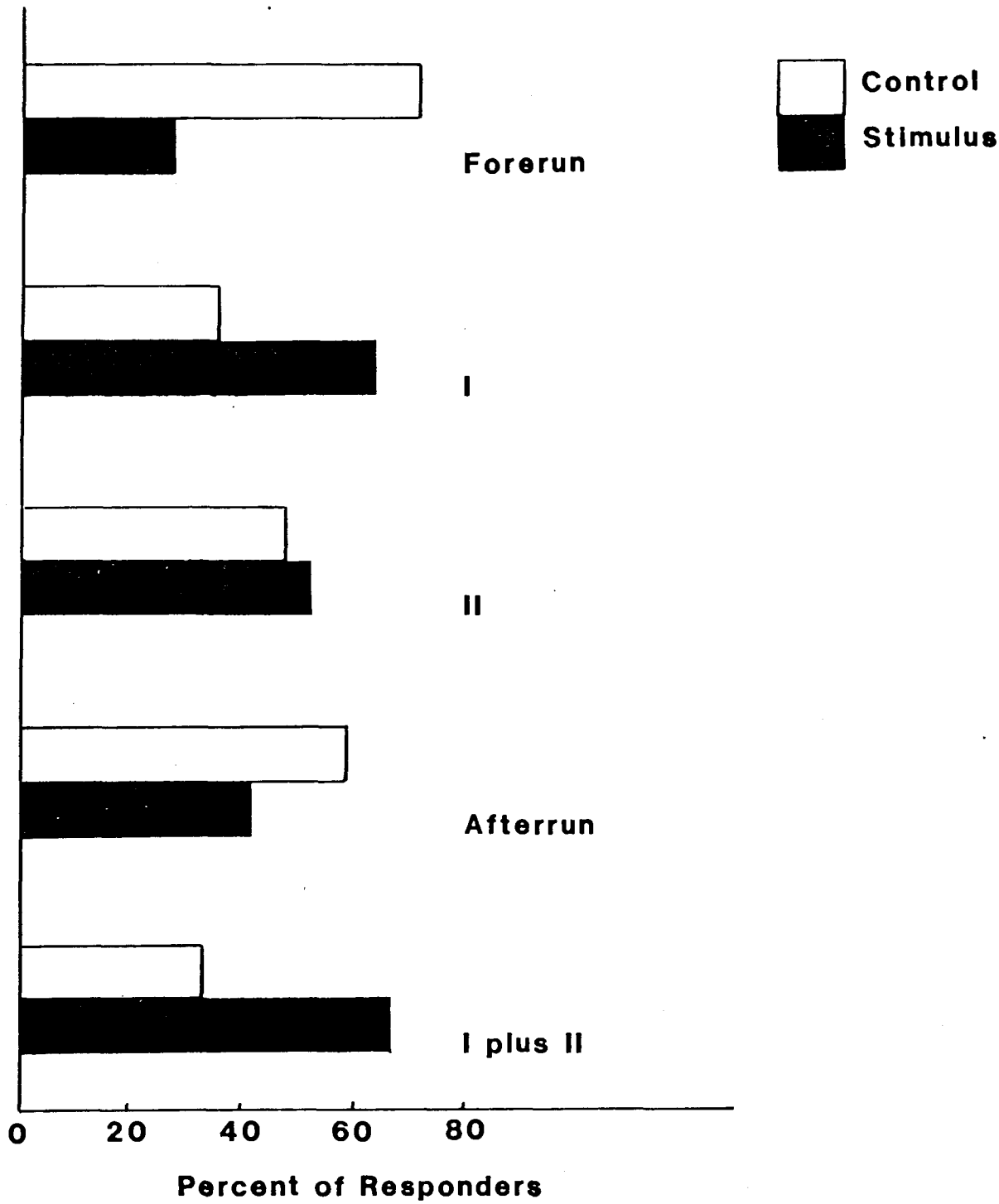

Control
Stimulus
Forerun
I
II
Afterrun
I plus II
0
20
40
60
80
Percent of Responders

Table V. Response of C. pusillus of mixed sex and age to preparative GLC fractions of a pentane extract of beetle volatiles in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 3

Experimental Stimulus	Stimulus Dose	% Response†
Pentane	20 µL	11.0a
I	3400 bh	81.0c
II	"	46.0b
Afterrun	"	39.0b
Crude volatile extract	"	92.0c

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

Table VI. Response of C. pusillus of mixed sex and age to single and combined preparative GLC fractions of beetle volatiles in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 4

Experimental Stimulus	Stimulus Dose	% Response†
Pentane	20 µL	4.0a
I	1550 bh	32.0cd
I + Forerun	"	18.0bc
I + II	"	44.0d
I + Afterrun	"	20.0bc
II	"	9.0ab

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

C. pusillus, confirming its identification as an aggregation pheromone. All further bioassays were carried out at Simon Fraser University. These bioassays verified the activity of I beyond question (Tables VII-XIII).

Experiments with mixtures of I and (±)-II showed a significant synergism of I by (±)-II (Table VII). This result also indicates that if the insects do indeed produce one enantiomer of II preferentially, their response is not inhibited by the presence of the other enantiomer.

A dose-response experiment for the attractive mixture of ≈1:1 I:(±)-II in the arena olfactometer determined that the threshold concentration for significant activity was between 1 and 4 ng of each of I and racemic II (Fig. 16). The response then increased steadily, to reach a maximum between 4 and 20 µg. At 20 µg, the response decreased significantly from that at 4 µg, suggesting that sensory adaptation (Seabrook, 1977), disorientation due to atmospheric permeation (Nara et al., 1981) or arrestment downwind of the stimulus source (Wood et al., 1966) occurred.

A systematic test of the ratio of I to (±)-II in both types of bioassay disclosed that the best ratio approached the naturally produced ratio of 20:1 I:(±)-II (Table VIII, IX). The responses with the two bioassay methods differed; with the enclosed chamber of the pitfall bioassay, the highest concentration of II was inhibitory.

Despite numerous repetitions of bioassays with both methods, it was not possible to demonstrate that the response to I is synergized more by one enantiomer of II than the other. This suggests that the insects use either enantiomer equally well. As it is not known which enantiomer or enantiomeric ratio is produced by the insects, two response categories have to be considered (Silverstein, 1979):

Table VII. Response of C. pusillus of mixed sex and age to synthetic I and combinations of I with synthetic (±)-II in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 5

Experimental Stimulus	Respective Stimulus Dose (μg)	% Response†
I	2	22.0a
I + II	2, 2.3	44.0b
I + II	2, 4.7	53.0b
I + II	2, 9.4	51.0b

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

Figure 16

Dose response curve of C. pusillus to mixtures of I + racemic II (1:1.2) in the arena olfactometer. Stimulus concentration = weight of I used. Ninety insects of mixed sex and age tested per stimulus. A significant response versus a solvent control is indicated by * ($P<0.01$).

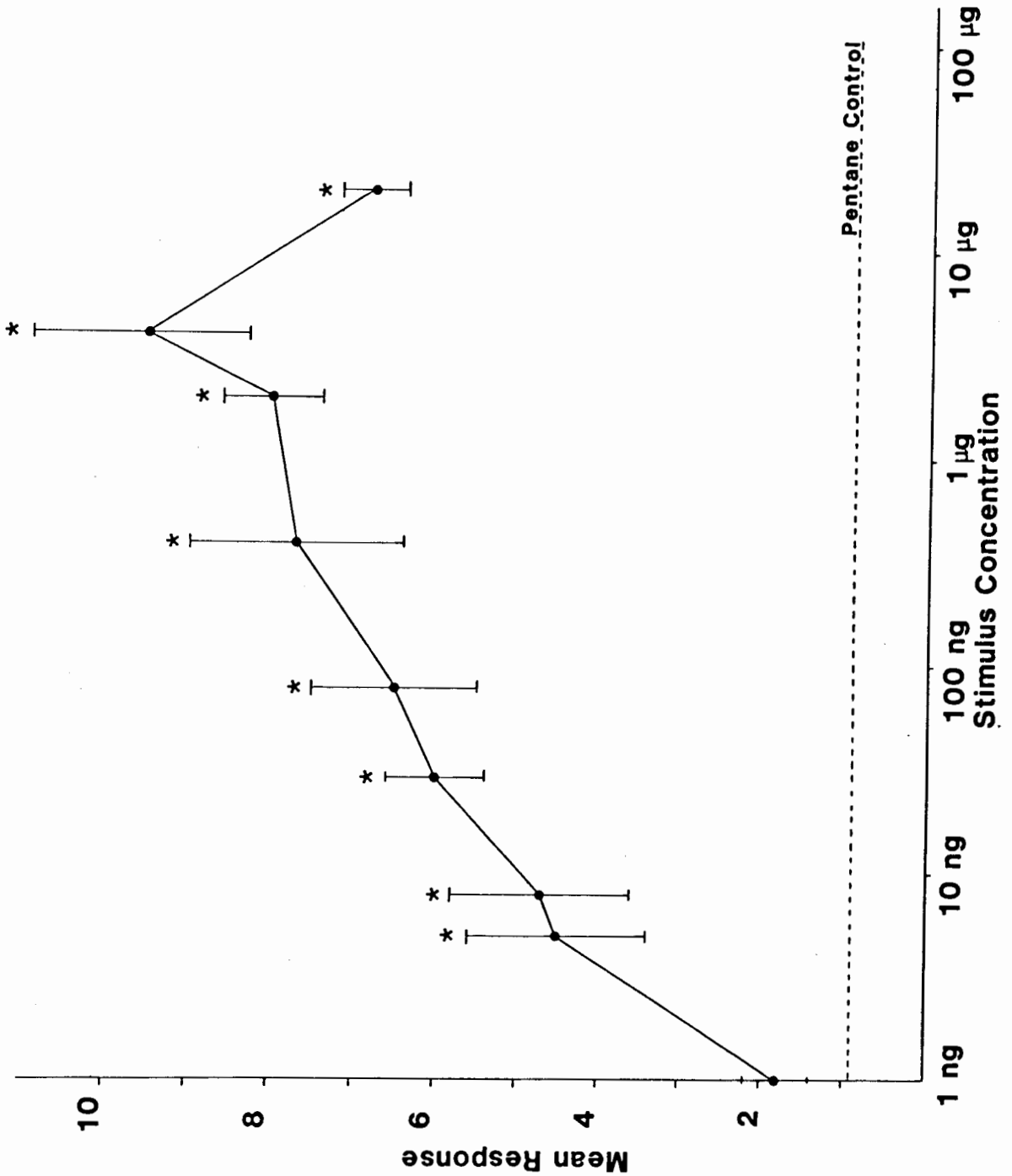

Mean Response
10
8
6
4
2
1 ng
10 ng
100 ng
1 µg
10 µg
100 µg
Stimulus Concentration
Pentane Control
*

Table VIII. Response of C. pusillus of mixed sex and age to synthetic I and combinations of I with synthetic (±)-II in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 7

Experimental Stimulus	Respective Stimulus Dose	% Response†
Pentane	20 µL	6.0a
I	4.0 µg	44.0b
I + (±)-II	4.0 µg, 0.2 µg	69.0c
	" , 0.4 µg	54.0b
	" , 0.8 µg	51.0b
	" , 1.6 µg	52.0b
	" , 4 µg	46.0b
	" , 8 µg	49.0b

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

Table IX. Response of C. pusillus of mixed age and sex to synthetic I and combinations of I with synthetic (±)-II in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 8

Experimental Stimulus	Respective Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Blank Control
I	2.0 µg	5.7 ±1.4	1.2 ±0.5 *
I + (±)-II	" , 0.1 µg	7.3 ±1.1	1.5 ±0.6 **
" "	" , 0.2 µg	6.7 ±1.7	2.0 ±0.7 *
" "	" , 0.4 µg	8.2 ±1.2	2.2 ±1.0 *
" "	" , 0.8 µg	5.7 ±0.4	1.3 ±0.4 ***
" "	" , 2 µg	6.0 ±0.6	3.3 ±0.7 *
" "	" , 4 µg	4.2 ±0.8	1.3 ±0.3 **
" "	" , 8 µg	4.2 ±0.9	3.0 ±0.6 NS

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

1) The insect produces a single enantiomer, but cannot distinguish between it and the other enantiomer, or

2) The insect produces both enantiomers and responds equally to each enantiomer.

It is also significant that neither enantiomer of II is repellent, indicating that species specificity of response could not be maintained by repellency of C. pusillus by either enantiomer of II produced by another species.

Macrolide IV was identified in C. pusillus volatile extracts in a GC-MS survey of all compounds present in the extract. As it had been implicated as an aggregation pheromone for O. mercator (Pierce et al., 1983), and as it is very similar in structure to I, it was tested for attraction. Significant response to IV was obtained at 1 and 10 μg, but not at 100 ng, indicating that the threshold for response was about 100× greater than for I (Table X). In addition, when IV was tested at a concentration equal to that of a highly active unfractionated extract, there was absolutely no significant activity (Table XII). This result suggests two possibilities:

1) The insects were responding to small amounts of impurities in the solution of IV. As IV is unstable, it is almost impossible to avoid trace impurities.

2) Because of its close structural similarity to I, IV may act as an analogue of I at high concentrations.

Tests for synergism of I by IV revealed no consistent significant synergism, over a range of ratios of I:IV of 100:1 through 10:1 (the natural ratio is ≈75:1).

Table X. Response of C. pusillus of mixed age and sex to synthetic IV at several concentrations in pitfall olfactometer bioassays.
N = 6 replicates, 15 insects/replicate.

Experiment 9

Experimental Stimulus	Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Blank Control
IV	10 ng	7.2 ±0.5	5.8 ±0.8 NS
IV	100 ng	6.0 ±1.1	3.8 ±1.1 NS
IV	1,000 ng	7.0 ±0.8	4.2 ±1.2 **
IV	10,000 ng	9.5 ±1.5	2.0 ±0.4 **

†Significant response (t-test) to experimental stimulus indicated by:
** P <0.01, NS = not significant.

Bioassays of synthetic compounds and preparative GLC fractions (a forerun, a fraction containing I, II and IV, and an afterrun) of feeding C. pusillus were conducted (Table XI). Three pertinent phenomena were demonstrated:

1) All the activity in the crude extract was recovered in the recombined fractions, indicating that no active compounds are destroyed by passage through the GLC column.

2) The forerun (mainly food volatiles) induced a response comparable to that induced by the macrolides fraction, while the afterrun fraction was inactive. Combination of the forerun and macrolide fractions resulted in total recovery of activity, at a very high significance level. Thus, food volatiles are attractive, and they enhance the response to the pheromone fraction.

3) Synthetic I and (±)-II at the same concentrations as the macrolide fraction exhibited activity comparable to the isolated macrolide fraction.

The latter finding was corroborated by another series of bioassays (Table XII), in which the isolated macrolides, synthetic I, and a mixture of synthetic I and (±)-II all had similar levels of activity. The unfractionated extract was the most attractive stimulus, due to the presence of macrolides plus food volatiles, while IV at natural concentrations was totally inactive. In this case, there was no significant synergism of I by (±)-II detected.

Response of Sexed C. pusillus to (I + (±)-II)

Both male and female beetles responded to several concentrations of a 10:1 mixture of I:(±)-II (Table XIII), indicating that they are true

aggregation pheromones. The males started responding at a lower stimulus concentration, and had a consistently higher level of overall response than females over the three concentrations tested. This result is rather curious, as I and II are male-produced (vide supra), and the opposite sex is usually preferentially attracted to aggregation pheromones in cases where the pheromone is produced by one sex (Borden, 1983). However, when the responses of both sexes to the pheromones plus the host volatiles is fully elaborated, the differences between sexes may be slight.

Table XI. Response of C. pusillus of mixed age and sex to preparative GLC fractions of a beetle volatiles extracts to combinations thereof, and to a mixture of synthetic I + (±)-II in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 10

Experimental Stimulus	Stimulus Dose	% Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Blank Control
Crude volatile extract of C. pusillus on Oats	660 bh	8.8 ±1.0	1.8 ±0.6 **
Forerun fraction	660 bh	8.7 ±1.4	2.8 ±0.8 *
Macrolides fraction	660 bh	7.8 ±1.1	3.8 ±0.9 *
Afterrun fraction	660 bh	5.2 ±0.8	4.8 ±0.5 NS
Forerun + macrolides	660 bh	11.5 ±1.0	1.5 ±0.6 ***
Recombined fractions	660 bh	9.0 ±0.6	2.2 ±0.5 ***
Synthetic I + (±)-II	660 bh††	8.3 ±1.5	2.3 ±0.7 *

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

††Calculated relative to the crude volatile extract of C. pusillus on oats. In absolute amounts, this was approximtely 850 ng of I + 43 ng of (±)-II.

Table XII. Response of C. pusillus of mixed age and sex to a crude extract of C. pusillus on oats, to the macrolide fraction thereof, and to synthetic I, I + (±)-II, and IV in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 11

Experimental Stimulus	Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Blank Control
C. pusillus on oats, crude extract	660 bh	12.5 ±0.5	1.8 ±0.6 ***
Synthetic I	660 bh††	8.0 ±0.6	4.7 ±0.8 **
Synthetic I + (±)-II	660 bh††	8.5 ±0.9	3.8 ±0.8 *
Synthetic IV	660 bh††	4.5 ±1.0	4.8 ±0.5 NS
Macrolides fraction	660 bh	7.5 ±0.7	2.5 ±0.7 **

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

††Concentrations of synthetic I, (±)-II, and IV are calibrated relative to the crude extract of C. pusillus on oats. In absolute terms, this represents 850 ng of I, 43 ng of (±)-II, and 11 ng of IV.

Table XIII. Response of male and female C. pusillus of mixed age to a mixture of I and (±)-II at several concentrations in pitfall olfactometer bioassays. N = 6 replicates, 10 insects/replicate.

Experiment 12

Sex	Experimental Stimulus	Respective Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
			Experimental Stimulus	Blank Control
♂♂	I + (±)-II	400 ng, 40 ng	4.8 ±0.7	0.8 ±0.3 **
	"	2 µg, 200 ng	6.2 ±0.6	0.7 ±0.3 ***
	"	10 µg, 1 µg	7.0 ±0.6	0.8 ±0.3 ***
♀♀	I + (±)-II	400 ng, 40 ng	2.5 ±0.7	1.0 ±0.4 NS
	"	2 µg, 200 ng	5.2 ±0.9	2.0 ±0.3 *
	"	10 µg, 1 µg	6.5 ±0.7	1.8 ±0.4 **

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

Bioassay of C. turcicus Volatiles

There had been no previous investigation of semiochemicals, either pheromones or host volatiles, for C. turcicus. Therefore, the first series of bioassays was designed to ascertain whether or not this species employed aggregation pheromones, as do C. ferrugineus (Wong et al., 1983) and C. pusillus (Tables IV-XIII). There was no detectable response to non-infested food volatiles, while there was a definite attraction to the odour of infested media, live beetles, and frass (Table XIV). This response was evidently due to attractive compounds produced by the beetles or by organisms growing in conjunction with them.

In further experiments, a pentane extract of Porapak Q-captured volatiles was found to be attractive to C. turcicus over a range of concentrations, in both the arena (Table XV) and the pitfall (Table XVI) olfactometers. The minimum concentration required to elicit a significant response was determined to be between 500 and 1000 bh, approximately an order of magnitude higher than for C. pusillus.

Bioassays of seven preparative GLC fractions of the crude volatile extract revealed that only fraction 5, consisting primarily of 13-methyl-(5Z,8Z)-tridecadienolide (III), showed any activity by itself (Table XVII). Fraction 4, consisting of 13-methyl-tridecenolide (II), was not active by itself, despite being the second largest peak in the extract (Fig. 9).

Pitfall bioassays showed crude extracts of frass volatiles to be highly attractive at lower concentrations (Table XVIII), while at high concentrations, the response was not significantly different from the pentane control, suggesting sensory adaptation, disorientation, or arrestment.

Table XIV. Response of C. turcicus of mixed age and sex to air passed over clean food, beetle-infested food, live beetles, or frass in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 13

Experimental Stimulus	% Response†
Pure air	6.0a
13.0 g mixed oats and cracked wheat (≈1:1)	6.0a
13.0 g used culture media, oats and cracked wheat	37.0b
4.1 g live beetles, mixed sex and age (≈12000 insects)	23.0b
5.6 g beetle frass	37.0b

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

Table XV. Response of C. turcicus of mixed age and sex to a pentane extract of Porapak Q-captured beetle volatiles in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 14

Experimental Stimulus	Stimulus Dose	% Response†
Pentane	20 µL	5.0a
Pentane extract of beetle volatiles	2,000 bh	24.0b
"	5,000 "	28.0bc
"	10,000 "	41.0c
"	20,000 "	46.0c

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

Table XVI. Response of C. turcicus of mixed age and sex to a pentane extract of Porapak Q-captured beetle volatiles in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 15

Experimental Stimulus	Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Blank Control
Pentane extract of C. turcicus volatiles	500 bh	6.3 ±1.0	8.0 ±1.1 NS
	1,000 "	9.5 ±1.2	3.8 ±0.9 *
	2,000 "	10.0 ±0.5	4.5 ±0.6 **

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

Table XVII. Response of C. turcicus of mixed age and sex to preparative GLC fractions of a beetle volatile extract in arena olfactometer bioassays. Ninety insects tested per stimulus.

Experiment 16

Experimental Stimulus	Stimulus Dose	% Response†
Pentane	20 µL	9.0a
Fraction 1	≈20,000 bh	5.0a
2	"	4.0a
3	"	7.0a
4 (Compound II)	"	8.0a
5 (Compound III)	"	35.0b
6	"	5.0a
7	"	7.0a

†Percentages followed by the same letter are not significantly different, Neuman-Keuls test modified for testing proportions ($P < 0.05$).

Table XVIII. Response of C. turcicus of mixed age and sex to extracts of volatiles of feeding beetles or of frass in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 17

Experimental Stimulus	Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Pentane Control
C. turcicus frass volatiles	3150 gh††	7.2 ±1.7	4.7 ±1.8 NS
	315 gh	10.2 ±0.7	1.8 ±0.5 ***
	31.5 gh	10.5 ±0.6	1.3 ±0.2 ****
C. turcicus on oats extract	6200 bh, 84 gh†††	9.7 ±0.6	1.7 ±0.8 ***
	620 bh, 8.4 gh	9.3 ±0.6	3.0 ±0.4 ***
	62 bh, 0.84 gh	5.7 ±1.3	4.0 ±1.2 NS

†Significant response (t-test) to experimental stimulus indicated by: **** P <0.0001, *** P <0.001, NS = not significant.

††1 gh = volatiles from 1 g of frass aerated for one hour.

†††bh = beetle-hours of beetle volatiles; gh = gram-hours of oat volatiles

Coupled GLC-MS analysis of the frass volatiles extract showed that major components of the extract were II and III, as in the beetle volatiles extract (Figs. 10, 11), so it is reasonable that the frass extract should exhibit comparable biological activity.

In a similar fashion, bioassays of an extract of volatiles of C. turcicus feeding on oats also elicited a strong positive response (Table XVIII), with the threshold for response being between 62 and 620 bh. Comparison of the GLC traces of this extract with the extract of beetle volatiles (Figs. 9, 11) showed that the major components were II and III, in approximately equal proportions. The lower response threshold (Table XVIII) than to beetle volatiles (Table XVI) may reflect the larger absolute amounts of chemicals produced per bh of aeration by insects feeding as compared to starved insects.

Bioassays of Synthesized C. turcicus Macrolides

The synthetic racemic forms of the major components of C. turcicus volatiles, III and II, were first bioassayed individually. Racemic II was totally inactive by itself (Table XIX), corroborating the data on naturally produced II (Table XVII). The threshold significant response level for racemic III was determined to be between 240 ng and 2.4 μg (Table XIX), considerably higher than expected as compared to the response of C. pusillus to I (Tables VII-IX). This result is possibly due to:

1) the necessity for other components to be present to elicit maximal response,

2) a difference in enantiomeric composition between natural III and the racemic synthetic III tested, or

3) a relatively low sensitivity by C. turcicus to its pheromone.

Table XIX. Response of C. turcicus of mixed age and sex to synthetic (±)-III and (±)-II in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 18

Experimental Stimulus	Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Blank Control
Synthetic (±)-III	240 ng	5.3 ±0.6	5.5 ±0.6 NS
	2,400 ng	6.7 ±0.8	4.0 ±0.6 *
	24,000 ng	9.8 ±0.9	2.5 ±0.6 **
Synthetic (±)-II	200 ng	4.2 ±0.5	3.2 ±0.8 NS
	2,000 ng	5.3 ±1.6	3.5 ±1.0 NS
	20,000 ng	3.3 ±0.7	4.2 ±0.9 NS

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

Bioassay of a series of solutions ranging from pure (R)- to pure (S)-III showed that C. turcicus did not respond to pure (R)- or pure (S)-III at the 5 µg level (Table XX). However, there were significant levels of activity at all but one of the enantiomeric ratios tested (5:95, R:S), indicating a synergism between enantiomers. This is the first evidence of enantiomeric synergism outside of the Scolytidae, in which it occurs in Gnathotrichus sulcatus (Borden et al., 1976) and Ips pini (Lanier et al., 1980).

The minimal attractiveness of the pure enantiomers of III was confirmed on a separate occasion, when pitfall bioassay of either enantiomer at concentrations of 10 ng, 100 ng, 1 µg, and 10 µg produced no significant response.

A test for synergism of racemic III by racemic II over a range of ratios (25:1 to 1:2) showed that all mixtures used were attractive (Table XXI), and that ratios of 3:1 and 3:2 of III:II were significantly more attractive than III alone (Neuman-Keuls test, $P < 0.05$), indicating that II does synergize III when used in approximately the natural ratio. The fact that all the stimuli tested were highly attractive suggested that the range of ratios exhibiting significant synergism could probably be extended by testing at lower concentrations. However, this hypothesis was not confirmed in bioassays of combinations of racemic III with the enantiomers of II, using two ratios of III:II at two concentrations (Table XXII). When 5 µg of III was used, all mixtures of III with the enantiomers of II were equally attractive (Neuman-Keuls test, $P<0.05$). When the concentration of III was reduced to 1 µg, only the mixture containing 670 ng of (R)-II showed any significant synergism. Thus, it is suggested

Table XX. Response of C. turcicus of mixed age and sex to pure (R)-III, pure (S)-III, and ratios thereof in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 19

Experimental Stimulus	Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Pentane Control
(R)-III	5 µg††	4.5 ±1.5	1.5 ±0.4 NS
(R)-III/(S)-III, 95/ 5	"	9.7 ±1.2	1.8 ±0.7 **
" 90/10	"	6.7 ±1.0	2.0 ±0.4 **
" 80/20	"	4.5 ±1.1	0.3 ±0.2 **
" 60/40	"	3.8 ±1.2	0.8 ±0.5 *
" 40/60	"	8.2 ±1.3	1.3 ±0.4 **
" 20/80	"	8.0 ±0.8	3.0 ±0.7 **
" 10/90	"	5.0 ±1.0	2.0 ±0.8 *
" 5/95	"	6.7 ±1.1	3.7 ±1.4 NS
(S)-III	"	4.8 ±0.7	2.8 ±1.2 NS

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

††Total weight of (R)-III + (S)-III.

Table XXI. Response of C. turcicus of mixed age and sex to synthetic (±)-III and to mixtures of (±)-III with (±)-II in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 20

Experimental Stimulus	Respective Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Pentane Control
Synthetic (±)-III	10 µg	4.5 ±0.8	1.0 ±0.7 **
Synthetic (±)-III, (±)-II	10 µg, 400 ng	7.7 ±1.3	1.8 ±0.3 **
"	" 800 ng	7.3 ±1.5	0.8 ±0.7 **
"	" 1.67 µg	7.0 ±1.3	2.7 ±0.7 **
"	" 3.33 µg††	10.3 ±0.8	1.8 ±0.9 **
"	" 6.67 µg	10.5 ±1.6	0.83 ±0.4 **
"	" 10 µg	8.3 ±0.8	1.7 ±0.5 ***
"	" 20 µg	8.7 ±0.6	2.1 ±0.7 ***

†Significant response (t-test) to experimental stimulus indicated by: *** P <0.001, ** P <0.01.

††Ratio occurring in beetle-produced volatiles.

Table XXII. Response of C. turcicus of mixed age and sex to mixtures of (±)-III and (R)- or (S)-II in pitfall olfactometer bioassays. N = 6 replicates, 15 insects/replicate.

Experiment 21

Experimental Stimulus	Respective Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
		Experimental Stimulus	Pentane Control
(±)-III	5 µg	7.3 ±1.3	3.0 ±0.4 **
(±)-III, (R)-II	5 µg, 1.67 µg	7.1 ±1.6	0.7 ±0.2 **
" (S)-II	" 1.67 µg	8.3 ±1.3	2.0 ±0.4 **
" (R)-II	" 3.33 µg	8.5 ±1.5	1.0 ±0.4 **
" (S)-II	" 3.33 µg	4.5 ±0.9	2.2 ±0.4 *
(±)-III	1 µg	5.0 ±1.2	4.8 ±0.7 NS
(±)-III, (R)-II	1 µg, 0.33 µg	3.5 ±0.7	2.7 ±0.9 NS
" (S)-II	" 0.33 µg	6.7 ±1.5	2.7 ±0.8 NS
" (R)-II	" 0.67 µg	7.5 ±1.4	2.5 ±0.6 *
" (S)-II	" 0.67 µg	4.7 ±1.4	3.5 ±0.4 NS

†Significant response (t-test) to experimental stimulus indicated by:

** $P < 0.01$, * $P < 0.05$, NS = not significant.

that (R)-II, or a mixture of enantiomers of II of predominantly the R isomer, synergizes III.

Response of Sexed C. turcicus to Synthetic (±)-III

Both sexes of C. turcicus responded to (±)-III (Table XXIII), although the response of males was not significant at the 1 μg level. However, as the bioassay was run with relatively few insects from a poor culture, this bioassay needs repeating, testing a wider concentration range and more insects per stimulus. The test did show that (±)-III is attractive to both sexes, and is thus a true aggregation pheromone.

Table XXIII. Response of male and female C. turcicus of mixed age to synthetic (±)-III in pitfall olfactometer bioassays. N = 6 replicates, 10 insects/replicate.

Experiment 22

Sex	Experimental Stimulus	Stimulus Dose	Response ($\bar{X}$ + S.E.)†	
			Experimental Stimulus	Pentane Control
♂♂	(±)-III	10 µg	3.7 ±0.5	1.0 ±0.4 **
	"	2.5 µg	3.0 ±0.7	0.2 ±0.2 **
	"	1 µg	1.7 ±0.4	1.3 ±0.4 NS
♀♀	(±)-III	10 µg	4.3 ±0.7	1.2 ±0.6 *
	"	1 µg	2.2 ±0.3	0.8 ±0.2 *

†Significant response (t-test) to experimental stimulus indicated by:

** $P < 0.01$, * $P < 0.05$, NS = not significant.

VI. CONCLUSIONS

Macrolide I is an aggregation pheromone for C. pusillus. The response to I is slightly synergized by racemic II, although it is not yet known which enantiomer or mixture of enantiomers of II is produced by the insects. The attractive compounds are male-produced.

C. turcicus utilize aggregation pheromones, which can be isolated from frass or beetle volatiles. The major attractive component is III, which is probably synergized by II. It is not yet known which enantiomer or mixture of enantiomers of II and III are produced by the beetles. II and III are male-produced. In laboratory bioassays, the beetles are attracted to synthetic racemic III, and the response is synergized by racemic II. In addition, beetles do not respond to pure (R)- or pure (S)-III, but do respond to mixtures thereof, in the first known case of enantiomeric synergism in Cucujidae.

Macrolides I-V can be synthesized in low to moderate yield from acyclic precursors. The enantiomers of II and III are readily available via stereo- and regiospecific ring-opening of chiral methyloxiranes, followed by further elaboration of the resulting alcohols. V is particularly difficult to synthesize due to its strained twelve-membered ring.

Further efforts should be directed towards identifying the enantiomeric composition of naturally produced II and III, and a rigorous investigation of pheromone production and utilization as a function of age and culturing conditions, for both C. turcicus and C. pusillus.

Although the first macrolide pheromones, 4,8-dimethyl-(4E,8E)-decadienolide and 11-methyl-(3Z)-undecenolide, were given the trivial names ferrulactones I and II, respectively, after the species C. ferrugineus from

which they were identified (Wong et al., 1983), it is now evident that these and related macrolides comprise a class of pheromones known only for the Cucujidae. Therefore, it is proposed that these pheromones be given the collective trivial name of cucujolides. The designation of each pheromone would be as follows: cucujolide I, 4,8-dimethyl-(4E,8E)-decadienolide; cucujolide II, 11-methyl-(3Z)-undecenolide; cucujolide III, 11-methyl-(3Z,6Z)-undecadienolide; cucujolide IV, (3Z)-dodecenolide; cucujolide V, (3Z,6Z)-dodecadienolide; cucujolide VI, 13-methyl-(3Z)-tridecenolide; cucujolide VII, 13-methyl-(3Z,6Z)-tridecadienolide.

LIST OF FOOTNOTES

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